			69849.5		2750.91							
1	46	10.00	16603.4	.49	1597.90	1.58	545.	1				
			59991.7		2646.30							
10	47	10.00	16754.7	.49	1621.10	1.57	547.	1				
			60761.5		2686.67							
1	48	10.00	15341.0	.51	1471.10	1.64	547.	1				
			55700.3		2435.34							
10	49	10.00	16059.6	.50	1577.80	1.59	544.	1				
			58218.2		2618.46							
1	50	10.00	15068.4	.51	1213.30	1.81	548.	1				
			54260.6		2000.23							
1	51	10.00	15549.0	.51	199.80	4.37	546.	1				
			56878.9		289.47							
10	52	10.00	15816.7	.50	574.60	2.62	545.	1				
			57837.6		922.29							
1	53	10.00	15180.4	.51	1145.30	1.86	547.	1				
			54882.5		1886.68							
10	54	10.00	15774.1	.50	1401.00	1.68	545.	1				
			57123.9		2318.80							
1	55	10.00	15900.1	.50	1509.00	1.62	545.	1				
			57567.7		2501.44							
1	56	10.00	16502.8	.49	1574.50	1.59	543.	1				
			59946.6		2612.55							
10	57	10.00	16387.2	.49	1575.30	1.59	545.	1				
			59239.5		2611.10							
1	58	10.00	16200.5	.50	1510.50	1.62	548.	1				
			58151.9		2497.36							
10	59	10.00	15843.5	.50	1512.80	1.62	545.	1				
			57283.5		2507.21							
1	60	10.00	16033.5	.50	1514.70	1.62	547.	1				
			57707.8		2506.81							
1	61	10.00	16912.0	.49	1388.70	1.69	547.	1				
			61069.5		2292.19							

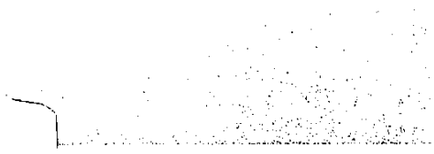
	S#	TIME	CPMA/K DPM1/K	%DEV	CPMB/K DPM2/K	%DEV	QIP	FLAGS	SCR	MIN		
10	52	10.00	15816.7 57837.6	.50	574.60 922.29	2.62	545.	1	.036 -3 .016	567	.32	66 I
1	53	10.00	15180.4 54882.5	.51	1145.30 1886.68	1.86	547.	1	.075 -4 .034	578	.73	21 I
10	54	10.00	15774.1 57123.9	.50	1401.00 2318.80	1.68	545.	1	.089 -5 .041	589	.89	4 I
1	55	10.00	15900.1 57567.7	.50	1509.00 2501.44	1.62	545.	1	.095 -6 .043	600	.93	101C
1	56	10.00	16502.8 59946.6	.49	1574.50 2612.55	1.59	543.	1	.095 -7 .044	611	.95	103C
10	57	10.00	16387.2 59239.5	.49	1575.30 2611.10	1.59	545.	1	.096 -8 .044	622	.95	103C
1	58	10.00	16200.5 58151.9	.50	1510.50 2497.36	1.62	548.	1	.093 -7 .043	633	.93	101C
10	59	10.00	15843.5 57283.5	.50	1512.80 2507.21	1.62	545.	1	.095 -8 .044	643	.95	103C
1	60	10.00	16033.5 57707.8	.50	1514.70 2506.81	1.62	547.	1	.094 .043	654		
1	61	10.00	16912.0 61069.5	.49	1388.70 2292.19	1.69	547.	1	.082 .038	665		

(RN 30485 64-948/Wa)

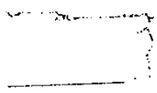
(RN 30448 64-936/Wa)

64-727 Dma centred

236



5



237

DATE 12/6/84 SOLVENT DMA

1) Compactin (24291) 12/6/84 E.A. % DA

10-1	02	4	96
10-2	02	4	96
10-3	01	18	82
10-4	33	64	36
10-5	61	98	2
10-6	68		
10-7			
10-8			

DRUG INHIBITION STUDY FOR SANDOZ CONTRACT

Sandoz unknowns were dissolved in DMA (Dimethylacetamide from Sigma), and Buffer A and DMSO: 0.1 M NaOH.

2) 62-326/86-4 (24444) 12/4 Dilution of each compound gave the concentrations indicated in the results.

10-1	02	4	96
10-2	04	8	92
10-3	04	8	92
10-4	57	110	
10-5			
10-6			
10-7			
10-8			

Microsomes were prepared from male Sprague-Dawley rats (~180g) in Buffer A with 10 mM DTT and frozen at -80°C until thawed and used for experiment. 200 µl Aliquots of microsomal suspension (1/12-1:30 mg/ml) plus 10 µl of drug dilution were assayed for HMG-CoA reductase activity.

3) 63-346 (25467) (SAP) 12/4 Compactin in DMA at various concentrations was assayed for inhibition and indicated in the results. Buffer A, and DMA: DMSO:0.1M NaOH were also assayed by adding 10 µl of each to 31 200 µl of microsomal suspensions and they showed not significant inhibition of HMG-CoA reductase:

10-1	92	184	-
10-2	95	190	-
10-3	96	192	-
10-4	95	190	-
10-5			
10-6			
10-7			
10-8			

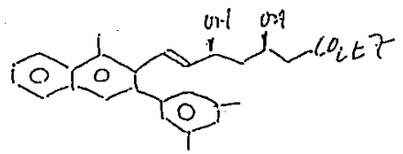
4) 63-346 (25467) 12/4

NOTE: That compound marked (SAP) was saponified in a 50° waterbath for 2 hr.

10-1	50	100	
10-2	65	126	-
10-3	59	114	-
10-4	53	102	-
10-5	3	6	-
10-6	1	2	-
10-7			
10-8			

RECEIVED DEC 20 1984

COMPOUND	DATE	SOLVENT	S.A.	% OF CONTROL	IC_{50}	% OF INHIBITION	REMA
1) Compactin (24291)	12/4/84	DMA					
1mM			.02	4		96	
10^{-1}			.02	4		96	
10^{-2}			.09	18	1.78	82	
10^{-3}			.33	64		36	
10^{-4}			.51	98		2	
10^{-5}			.58	112		-	
10^{-6}			.61	118		-	
10^{-7}			--	--		-	
10^{-8}			.53	102		-	
2) 62-320/Na-4 (~~24291~~) 23531	12/4/84	DMA					
10^{-2}			.02	4		96	
10^{-3}			.04	8		92	
10^{-4}			.10	20		80	
10^{-5}			.18	34		66	
10^{-6}			.54	104	0.006	-	
10^{-7}			.59	114		-	
10^{-8}			.57	110		-	
3) 63-346(25467)(SAP)	12-4-84	DMSO:0.1M NaOH					
10^{-2}			.61	69		31	
10^{-3}			.85	96		4	
10^{-4}			.94	106		-	
10^{-5}			.92	104	>10	-	
10^{-6}			.95	108		-	
10^{-7}			.98	111		-	
10^{-8}			.97	110		-	
4) 63-346 (25467)	12/4/84	DMA					
10^{-2}			.50	96		4	
10^{-3}			.63	122		-	
10^{-4}			.65	126		-	
10^{-5}			.59	114	>10	-	
10^{-6}			.53	102		-	
10^{-7}			.60	116		-	
10^{-8}			.58	112		-	

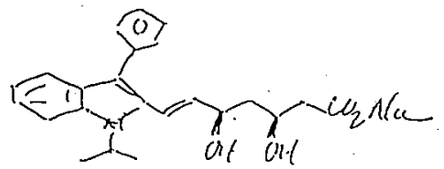


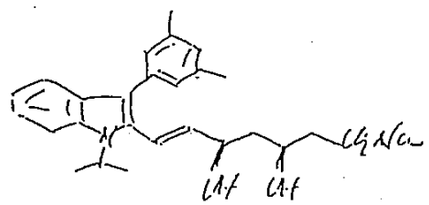
238

COMPOUND	DATE	SOLVENT	S.A.	% OF CONTROL		% OF INHIBITION	REMA
5) 63-347/Na(25468)	12/4/84	Buffer A					
10^{-2}			.84	83		17	
10^{-3}			1.02	101	>10	-	
10^{-4}			1.02	101		-	
10^{-5}			.98	97		3	
10^{-6}			1.07	106		-	
10^{-7}			1.00	99		1	
10^{-8}			1.02	101		-	
6) 63-352/Na(25475)	12/4/84	DMA					
10^{-2}			.05	10		90	
10^{-3}			.25	48		52	
10^{-4}			.51	98	1.11	2	
10^{-5}			.56	108		-	
10^{-6}			.57	110		-	
10^{-7}			.60	116		-	
10^{-8}			.65	126		-	
7) 63-353 (25476)	12/4/84	DMA					
10^{-2}			.04	8		92	
10^{-3}			.20	38	0.77	62	
10^{-4}			.46	90		10	
10^{-5}			.56	108		-	
10^{-6}			.59	114		-	
10^{-7}			.57	110		-	
10^{-8}			.58	112		-	
8) 63-265/3 (25488)	12/4/84	DMA					
10^{-2}			.01	2		98	
10^{-3}			.03	6		94	
10^{-4}			.06	12	0.004	88	
10^{-5}			.13	26		74	
10^{-6}			.39	76		24	
10^{-7}			.55	106		-	
10^{-8}			.58	112		-	

239

COMPOUND	DATE	SOLVENT	S.A.	% OF CONTROL		% OF INHIBITION	REMAR
9) Compactin (24291)	12/12/84	DMA					
1mM			.00	-		100	
10^{-1}			.01	1		99	
10^{-2}			.10	9	0.85	91	
10^{-3}			.55	50		50	
10^{-4}			.90	83		17	
10^{-5}			1.01	93		7	
10^{-6}			1.12	103		-	
10^{-7}			1.06	97		3	
10^{-8}			1.04	96		4	
10) 62-320/Na-4 (24531)	12/12/84	DMA					
10^{-2}			.00	-		100	
10^{-3}			.04	3		97	
10^{-4}			.16	15	0.004	85	
10^{-5}			.37	34		66	
10^{-6}			-	-		-	
10^{-7}			1.03	95		5	
10^{-8}			1.03	95		5	
11) 63-361/Na (25481)	12/12/84	DMA					
10^{-2}			.00	-		100	
10^{-3}			.07	7		93	
10^{-4}			.31	28	0.016	72	
10^{-5}			.54	50		50	
10^{-6}			.98	91		9	
10^{-7}			1.01	93		7	
10^{-8}			1.02	94		6	
12) 62-562/Na(24908)	12/12/84	DMA					
10^{-2}			.00	-		100	
10^{-3}			.01	1		99	
10^{-4}			.08	8		92	
10^{-5}			.26	24	0.005	76	
10^{-6}			.94	86		14	
10^{-7}			.97	90		10	
10^{-8}			.99	91		9	



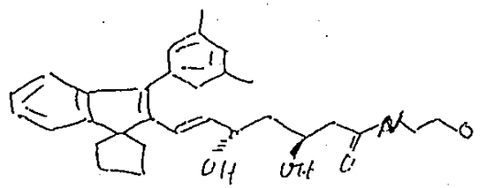


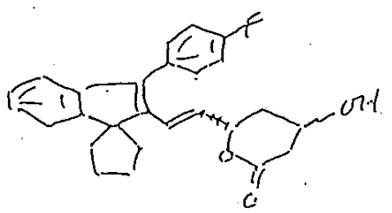
240

COMPOUND	DATE	SOLVENT	S.A.	% OF CONTROL		% OF INHIBITION	REMA
13) 63-354 (25477)	12/12/84	DMA					
10^{-2}			.09	9		91	
10^{-3}			.48	44		56	
10^{-4}			.89	82	0.73	18	
10^{-5}			1.01	93		7	
10^{-6}			1.03	95		5	
10^{-7}			1.03	95		5	
10^{-8}			.93	85		15	
14) 63-355 (25474)	12/12/84	DMA					
10^{-2}			.26	24		76	
10^{-3}			.57	52		48	
10^{-4}			.94	86	1.35	14	
10^{-5}			.97	90		10	
10^{-6}			1.01	93		7	
10^{-7}			1.02	94		6	
10^{-8}			.99	91		9	
15) 63-356 (25480)	12/12/84	DMA					
10^{-2}			.00	-		100	
10^{-3}			.06	5		95	
10^{-4}			.26	24		76	
10^{-5}			.44	40	0.009	60	
10^{-6}			.92	85		15	
10^{-7}			.96	89		11	
10^{-8}			.98	91		9	
16) 62-265/3 (25488)	12/12/84	DMA					
10^{-2}			.00	-		100	
10^{-3}			.02	2		98	
10^{-4}			.11	10		90	
10^{-5}			.22	21	0.004	79	
10^{-6}			.84	78		22	
10^{-7}			.96	89		11	
10^{-8}			.96	89		11	

RESULTS

COMPOUND	DATE	SOLVENT	S.A.	% OF CONTROL		% OF INHIBITION	REMARKS
17) Compactin (24291)	12/13/84	DMA					
1mM			.00	-		100	
10^{-2}			.02	2		98	
10^{-3}			.10	10		90	
10^{-4}			.43	45	0.72	55	
10^{-5}			.75	80		20	
10^{-6}			.06	91		9	
10^{-7}			.91	97		3	
10^{-8}			.89	95		5	
			.92	98		2	
18) 62-320/Na-4(~~24291~~ 23531)	12/13/84	DMA					
10^{-2}			.01	1		99	
10^{-3}			.04	4		96	
10^{-4}			.13	14	0.007	86	
10^{-5}			.25	27		73	
10^{-6}			.84	90		10	
10^{-7}			.92	98		2	
10^{-8}			.90	96		4	
19) 63-364 (24489)	12/13/84	DMA					
10^{-2}			.71	76		24	
10^{-3}			.86	91		9	
10^{-4}			.89	95	> 10	5	
10^{-5}			.90	96		4	
10^{-6}			.84	89		11	
10^{-7}			.86	91		9	
10^{-8}			.82	87		13	
20) 63-365 (25490)	12/13/84	DMA					
10^{-2}			.02	2		98	
10^{-3}			.08	8		92	
10^{-4}			.16	17	0.017	83	
10^{-5}			.58	62		38	
10^{-6}			.84	89		11	
10^{-7}			.85	90		10	
10^{-8}			.87	92		8	



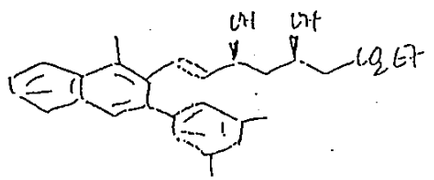


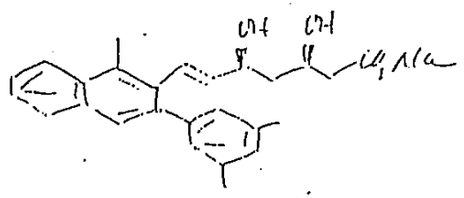
242

COMPOUND	DATE	SOLVENT	S.A.	% OF CONTROL		% OF INHIBITION	REMARK
21) 63-366 (25496)	12/13/84	DMA					
10^{-2}			.21	22		78	
10^{-3}			.57	61		39	
10^{-4}			.81	86	1.58	14	
10^{-5}			.80	94		6	
10^{-6}			.90	96		4	
10^{-7}			.88	94		6	
10^{-8}			.87	92		8	
22) 63-369 (25512)	12/12/84	DMA					
10^{-2}			.02	2		98	
10^{-3}			.14	15		85	
10^{-4}			.44	46	0.035	54	
10^{-5}			.62	66		34	
x 10^{-6}			.72	77		23	
10^{-7}			.87	92		8	
10^{-8}			.88	94		6	
23) 63-162/3 (25500)	12/13/84	DMA					
10^{-2}			.01	1		99	
10^{-3}			.02	2		98	
10^{-4}			.11	11	0.007	89	
x 10^{-5}			.19	20		80	
10^{-6}			.65	97		3	
10^{-7}			.81	86		14	
10^{-8}			.78	83		17	
24) 63-270/2	12/13/84	DMA					
10^{-2}			.01	1		99	
10^{-3}			.05	5		95	
10^{-4}			.09	9	0.004	91	
x 10^{-5}			.28	30		70	
10^{-6}			.71	76		24	
10^{-7}			.77	82		18	
10^{-8}			.79	84		16	

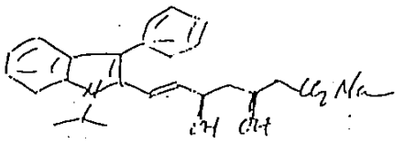
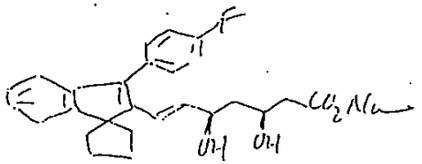
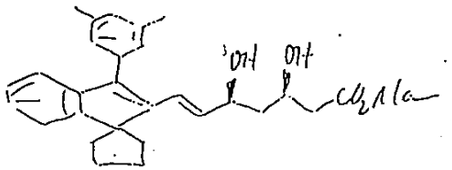
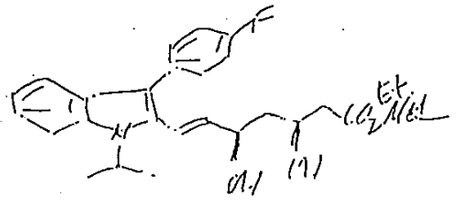
243

COMPOUND	DATE	SOLVENT	S.A.	% OF CONTROL		% OF INHIBITION	REMA
25) Compactin (24291)	12/14/84	DMA					
1mM			.00	-		100	
10^{-1}			.00	-		100	
10^{-2}			.02	3		97	
10^{-3}			.38	46	0.87	54	
10^{-4}			.78	93		7	
10^{-5}			.85	101		-	
10^{-6}			.93	111		-	
10^{-7}			.89	107		-	
10^{-8}			.89	107		-	
26) 62-320/Na-4	12/14/84	DMA					
10^{-2}			.00	-		100	
10^{-3}			.00	-		100	
10^{-4}			.08	10	0.007	90	
10^{-5}			.21	25		75	
10^{-6}			.78	93		7	
10^{-7}			.88	106		-	
10^{-8}			.80	96		4	
27) 63-346 (25467)	12/14/84	DMSO:0.1M NaOH					
10^{-2}			.59	89		31	
10^{-3}			.89	104		-	
10^{-4}			.85	99	>10	1	
10^{-5}			.81	95		5	
10^{-6}			.85	99		1	
10^{-7}			.80	93		7	
10^{-8}			.83	97		3	
28) 63-347 (25468)	12/14/84	DMA					
10^{-2}			.76	82		18	
10^{-3}			.97	105		-	
10^{-4}			.97	105	>10	-	
10^{-5}			1.02	109		-	
10^{-6}			.97	105		-	
10^{-7}			.94	100		-	
10^{-8}			.96	103		-	





COMPOUND	DATE	SOLVENT	S.A.	% OF CONTROL		% OF INHIBITION	REP
29) 63-352 (25475)	12/14/84	DMA					
10^{-2}			.08	10		90	
10^{-3}			.29	35	0.72	65	
10^{-4}			.73	88		12	
10^{-5}			.83	100		-	
10^{-6}			.79	94		6	
10^{-7}			.80	96		4	
10^{-8}			.89	107		-	
30) Compactin (24291)	12/17/84	DMA					
1mM			.01	1		99	
10^{-1}			.03	3		97	
10^{-2}			.14	13		87	
10^{-3}			.61	55		45	
10^{-4}			1.00	90		10	
10^{-5}			1.09	98	1.17	2	
10^{-6}			1.22	109		-	
10^{-7}			1.18	105		-	
10^{-8}			1.23	110		-	
31) 62-320/Na-1(25480)	12/12/84	DMA					
10^{-2}			.01	1		99	
10^{-3}			.05	5		95	
10^{-4}			.18	17	0.012	83	
10^{-5}			.47	42		58	
10^{-6}			1.13	102		-	
10^{-7}			1.12	104		-	
10^{-8}			1.18	106		-	
32) 62-562/Na-2(25488)	12/17/84	DMA					
10^{-2}			.01	1		99	
10^{-3}			.04	4		96	
10^{-4}			.09	8	0.005	92	
10^{-5}			.25	22		78	
10^{-6}			.98	88		12	
10^{-7}			1.06	95		5	
10^{-8}			1.06	95		5	

COMPOUND	DATE	SOLVENT	S.I.	CONTROL		I	II
33) 63-361 (25485)	12/17/84	DMA					
10^{-2}			.01	1			99
10^{-3}			.08	7			93
10^{-4}			.31	28	0.017		72
10^{-5}			.50	45			55
10^{-6}			1.01	91			9
10^{-7}			1.10	99			1
10^{-8}			1.11	100			-
34) 63-162/3 (25500)	12/17/84	DMA					
10^{-2}			.01	1			99
10^{-3}			.04	4			96
10^{-4}			.14	13	0.005		87
10^{-5}			.25	22			78
10^{-6}			.96	86			14
10^{-7}			1.02	92			8
10^{-8}			1.01	91			9
35) 63-270/2 (25501)	12/17/84	DMA					
10^{-2}			.02	2			98
10^{-3}			.07	6			94
10^{-4}			.12	11	0.008		89
10^{-5}			.47	42			58
10^{-6}			.97	87			13
10^{-7}			1.04	94			6
10^{-8}			1.05	94			6
36) 62-265/3 (25488)	12/17/84	DMA					
10^{-2}			.01	1			99
10^{-3}			.01	1			99
10^{-4}			.07	6	0.001		94
10^{-5}			.18	17			83
10^{-6}			.55	50			50
10^{-7}			.99	89			11
10^{-8}			1.01	91			9

June 27, 1985

DRUG INHIBITION STUDY FOR SANDOZ CONTRACT

Sandoz unknowns were dissolved in DMA (Dimethylacetamide from Sigma), and Buffer A and DMSO: 0.1 M NaOH. Dilution of each compound gave the concentrations indicated in the results.

Microsomes were prepared from male Sprague-Dawley rats (163 g) in Buffer A with 10 mM DTT and frozen at -80°C until thawed and used for experiment. 200 µl Aliquots of microsomal suspension (.97 - 1.11 mg/ml) plus 10 µl of drug dilution were assayed for HMG-CoA reductase activity.

Compactin in DMA at various concentrations was assayed for inhibition also and is indicated in the results. Buffer A, and DMA were also assayed by adding 10 µl of each to 200 µl of microsomal suspension and they showed no significant inhibition of HMG-CoA reductase.

242

RESULTS

COMPOUND	DATE	SOLVENT	S.A.	% OF CONTROL	IC50 (mM)	% OF INHIBITION	REMARKS
1) Compactin (24291)	6/13/85	DMA					
1mM			.00	-		100	
10^{-1}			.02	4		96	
10^{-2}			.06	12	1.041	88	
10^{-3}			.24	52		48	
10^{-4}			.41	88		12	
10^{-5}			.47	100		-	
10^{-6}			.47	100		-	
10^{-7}			.43	92		8	
10^{-8}			.49	104		-	
2) 62-320/Na-4(23531)	6/13/85	DMA					
10^{-2}			.00	-		100	
10^{-3}			.02	4		96	
10^{-4}			.09	20		80	
10^{-5}			.15	32	0.0038	68	
10^{-6}			.32	68		32	
10^{-7}			.45	96		4	
10^{-8}			.47	100		-	
3) 63-518/2(RN 26020)	6/13/85	DMA					
10^{-2}			.37	80		20	
10^{-3}			.45	96		4	
10^{-4}			.49	104	>10	-	
10^{-5}			.45	96		4	
10^{-6}			.51	108		-	
10^{-7}			.49	104		-	
10^{-8}			.49	104		-	
4) 63-537/Na(RN 26039)	6/13/85	DMA					
10^{-2}			.02	4		96	
10^{-3}			.09	20		80	
10^{-4}			.32	68	0.169	32	
10^{-5}			.39	84		16	
10^{-6}			.45	96		4	
10^{-7}			.47	100		-	
10^{-8}			.47	100		-	

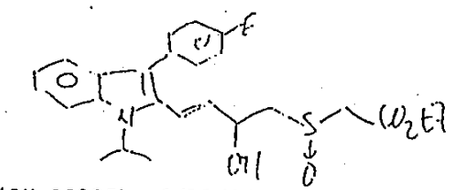
842

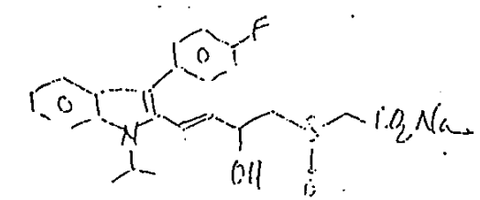
COMPOUND	DATE	SOLVENT	RESULTS S.A.	% OF CONTROL		% OF INHIBITION	REMARKS
5) 63-547(RN 26075)	6/13/85	DMA					
10^{-2}			.00	–		100	
10^{-3}			.04	8		92	
10^{-4}			.13	28	0.017	72	
10^{-5}			.22	48		52	
10^{-6}			.41	88		12	
10^{-7}			.47	100		–	
10^{-8}			.47	100		–	
6) 63-548(RN 26080)	6/13/85	DMA					
10^{-2}			.13	28		72	
10^{-3}			.37	80		20	
10^{-4}			.47	100	3.775	–	
10^{-5}			.47	100		–	
10^{-6}			.45	96		4	
10^{-7}			.47	100		–	
10^{-8}			.45	96		4	
7) 63-549(RN 26082)	6/13/85	DMA					
10^{-2}			.21	44		56	
10^{-3}			.41	88		12	
10^{-4}			.47	100		–	
10^{-5}			.47	100	7.31	–	
10^{-6}			.47	100		–	
10^{-7}			.45	96		4	
10^{-8}			.47	100		–	
8) 63-550(RN 26083)	6/13/85	DMA					
10^{-2}			.07	16		84	
10^{-3}			.28	60		40	
10^{-4}			.41	88		12	
10^{-5}			.47	100	1.348	–	
10^{-6}			.47	100		–	
10^{-7}			.47	100		–	
10^{-8}			.47	100		–	

x2582A

247

COMPOUND	DATE	SOLVENT	RESULTS S.A.	% OF CONTROL		% OF INHIBITION	REMAR
9) Compactin (24291)	6/18/85	DMA					
1nM			.02	2		98	
10^{-1}			.02	2		98	
10^{-2}			.14	14		86	
10^{-3}			.54	52	0.978	48	
10^{-4}			.86	83		17	
10^{-5}			.94	91		9	
10^{-6}			.98	95		5	
10^{-7}			1.04	101		–	
10^{-8}			1.10	107		–	
10) 62-320/Na-4(23531)	6/18/85	DMA					
10^{-2}			.02	2		98	
10^{-3}			.04	4		96	
10^{-4}			.16	16		84	
10^{-5}			.38	37	0.0081	63	
10^{-6}			.90	87		13	
10^{-7}			1.00	97		3	
10^{-8}			.98	95		5	
11) 63-551(RN 26084)	6/18/85	DMA					
10^{-2}			.78	76		24	
10^{-3}			.96	93		7	
10^{-4}			1.00	97	>10	3	
10^{-5}			1.04	101		–	
10^{-6}			1.02	99		1	
10^{-7}			.98	95		5	
10^{-8}			1.00	97		3	
12) 63-552/Na(RN 26085)	6/18/85	DMA					
10^{-2}			.82	80		20	
10^{-3}			1.04	101		–	
10^{-4}			1.00	97	>10	3	
10^{-5}			1.00	97		3	
10^{-6}			.98	95		5	
10^{-7}			1.02	99		1	
10^{-8}			.98	95		5	





250

COMPOUND	DATE	SOLVENT	RESULTS S.A.	% OF CONTROL		% OF INHIBITION	REMAI
13) 63-553(RN26086)	6/18/85	DMA					
10-2			.36	35		65	
10-3			.80	78		22	
10-4			.94	91	4.479	9	
10-5			1.02	99		1	
10-6			1.00	97		3	
10-7			1.04	101		-	
10-8			1.00	97		3	
14) 63-554/Na(RN 26087)	6/18/85	DMA					
10-2			.18	17		83	
10-3			.64	62		38	
10-4			.92	89	1.466	11	
10-5			1.00	97		3	
10-6			1.02	99		1	
10-7			1.02	99		1	
10-8			.98	95		5	
15) Compactin (24291)	6/19/85	DMA					
1mM			.04	4		96	
10-1			.04	4		96	
10-2			.14	15	1.247	85	
10-3			.51	53		47	
10-4			.90	93		7	
10-5			.98	101		-	
10-6			1.00	103		-	
10-7			1.06	109		-	
10-8			1.00	103		-	
16) 62-320/Na-4(23531)	6/19/85	DMA					
10-2			.04	4		96	
10-3			.08	8		92	
10-4			.20	21	.0059	79	
10-5			.45	46		54	
10-6			.65	67		33	
10-7			1.02	105		-	
10-8			1.04	107		-	

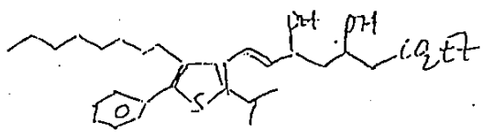
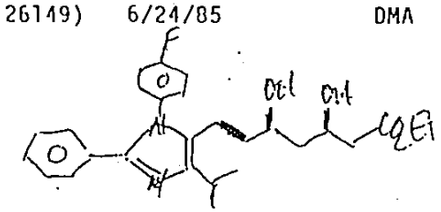
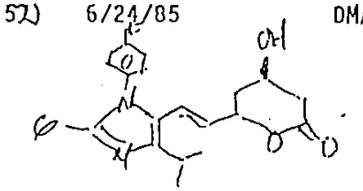
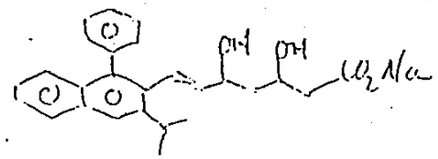
152

COMPOUND	DATE	SOLVENT	RESULTS S.A.	% OF CONTROL		% OF INHIBITION	REMAR
17) 63-558/Na(RN 26098)	6/19/85	DMA					
10^{-2}			.06	6		94	
10^{-3}			.31	32		68	
10^{-4}			.73	76	0.454	24	
10^{-5}			.96	99		1	
10^{-6}			1.02	105		–	
10^{-7}			.96	99		1	
10^{-8}			.98	101		–	
18) 63-559(RN 26106)	6/19/85	DMA					
10^{-2}			.12	13		87	
10^{-3}			.49	51		49	
10^{-4}			.90	93	1.144	7	
10^{-5}			.98	101		–	
10^{-6}			1.00	103		–	
10^{-7}			1.00	103		–	
10^{-8}			.92	95		5	
19) 63-550/2 Na(RN 26108)	6/19/85	DMA					
10^{-2}			.12	13		87	
10^{-3}			.53	55		45	
10^{-4}			.94	97	1.315	3	
10^{-5}			1.00	103		–	
10^{-6}			1.00	105		–	
10^{-7}			1.02	105		–	
10^{-8}			1.04	107		–	
20) 63-563(RN 26127)	6/19/85	DMA					
10^{-2}			.31	32		68	
10^{-3}			.80	82	4.365	18	
10^{-4}			.96	99		1	
10^{-5}			1.00	103		–	
10^{-6}			1.00	103		–	
10^{-7}			1.02	105		–	

252

COMPOUND	DATE	SOLVENT	RESULTS S.A.	% OF CONTROL		% OF INHIBITION	REMA
21) 63-564/Na(26129)	6/19/85	DMA					
10^{-2}			.20	21		79	
10^{-3}			.67	69	2.488	31	
10^{-4}			.96	99		1	
10^{-5}			1.02	105		-	
10^{-6}			1.00	103		-	
10^{-7}			1.04	107		-	
10^{-8}			1.04	107		-	
22) 63-565(RN 26128)	6/19/85	DMA					
10^{-2}			.10	11		89	
10^{-3}			.33	34		66	
10^{-4}			.78	80	0.573	20	
10^{-5}			.96	99		1	
10^{-6}			1.00	103		-	
10^{-7}			.96	99		1	
10^{-8}			.98	101		-	
23) Compactin (24291)	6/24/85	DMA					
1mM			.03	3		97	
10^{-1}			.03	3		97	
10^{-2}			.13	15	1.536	85	
10^{-3}			.50	61		39	
10^{-4}			.81	97		3	
10^{-5}			.91	109		-	
10^{-6}			.96	115		-	
10^{-7}			.91	109		-	
10^{-8}			.88	106		-	
24) 62-320/Na-4(23531)	6/24/85	DMA					
10^{-2}			.00	-		100	
10^{-3}			.05	6		94	
10^{-4}			.15	18	0.01	82	
10^{-5}			.33	39		61	
10^{-6}			.78	94		6	
10^{-7}			.86	103		-	
10^{-8}			.88	106		-	

253

	COMPOUND	DATE	SOLVENT	RESULTS S.A.	% OF CONTROL		% OF INHIBITION	REMA
25)	63-566(RN 26148)	6/24/85	DMA					
	10^{-2}			.73	88		12	
	10^{-3}			.86	103	>10	-	
	10^{-4}			.91	109		-	
	10^{-5}			.91	109		-	
	10^{-6}			.83	100		-	
	10^{-7}			.86	103		-	
	10^{-8}			.91	109		-	
26)	63-567(RN 26149)	6/24/85	DMA					
	10^{-2}			.23	27		73	
	10^{-3}			.63	76		24	
	10^{-4}			.70	85	2.734	15	
	10^{-5}			.76	91		9	
	10^{-6}			.78	94		6	
	10^{-7}			.78	94		6	
	10^{-8}			.76	91		9	
27)	63-568(RN 26152)	6/24/85	DMA					
	10^{-2}			.03	3		97	
	10^{-3}			.13	15		85	
	10^{-4}			.28	33	0.086	67	
	10^{-5}			.78	94		6	
	10^{-6}			.81	97		3	
	10^{-7}			.78	94		6	
	10^{-8}			.78	94		6	
28)	63-560(RN 26107)	6/24/85	Buffer A					
	10^{-2}			.08	9		91	
	10^{-3}			.43	52	0.981	48	
	10^{-4}			.73	88		12	
	10^{-5}			.81	97		3	
	10^{-6}			.81	97		3	
	10^{-7}			.88	106		-	
	10^{-8}			.86	103		-	

254

COMPOUND	DATE	SOLVENT	RESULTS S.A.	% OF CONTROL		% OF INHIBITION	REMA
29) 63-555(RN 26088)	6/24/85	DMA					
10^{-2}			.00	-		100	
10^{-3}			.05	6		94	
10^{-4}			.20	24	0.014	76	
10^{-5}			.40	48		52	
10^{-6}			.70	85		15	
10^{-7}			.78	94		6	
10^{-8}			.78	94		6	
30) Compactin (24291)	6/26/85	DMA					
1mM			.00	-		100	
10^{-1}			.02	2		98	
10^{-2}			.11	12		88	
10^{-3}			.44	49	0.899	51	
10^{-4}			.74	84		16	
10^{-5}			.87	99		1	
10^{-6}			.90	101		-	
10^{-7}			.83	94		6	
10^{-8}			.87	99		1	
31) 62-320/Na-4(23531)	6/26/85	DMA					
10^{-2}			.00	-		100	
10^{-3}			.04	5		95	
10^{-4}			.13	15	0.008	85	
10^{-5}			.28	32		68	
10^{-6}			.81	91		9	
10^{-7}			.85	96		4	
10^{-8}			.90	101		-	
32) 63-556 (RN 26093)	6/26/85	DMA					
10^{-2}			.11	12		88	
10^{-3}			.44	49	0.753	51	
10^{-4}			.68	77		23	
10^{-5}			.83	94		6	
10^{-6}			.87	99		1	
10^{-7}			.90	101		-	
10^{-8}			.85	96		4	

255

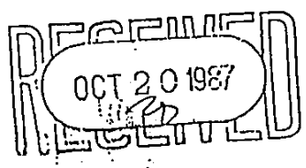
OCTOBER 8, 1987

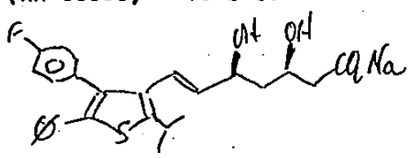
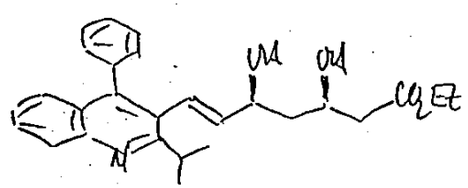
DRUG INHIBITION STUDY FOR SANDOZ CONTRACT

Sandoz unknowns were dissolved in DMA (Dimethylacetamide from Sigma), and Buffer A
Dilution of each compound gave the concentrations indicated in the results.

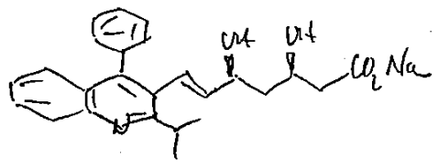
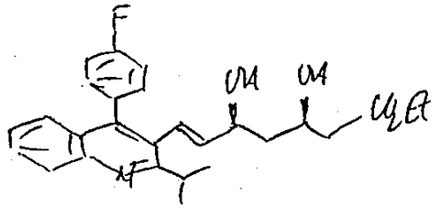
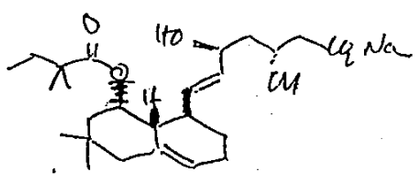
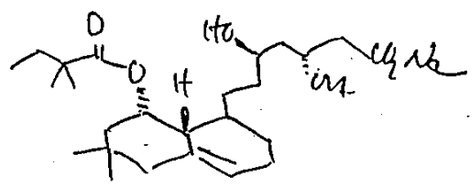
Microsomes were prepared from male Sprague-Dawley rats (150 g) in Buffer A with 10 mM DTT and frozen at -80°C until thawed and used for experiment. 200 µl Aliquots of microsomal suspension (0.91 mg/ml) plus 10 µl of drug dilution were assayed for HMG-CoA reductase activity.

Compactin in DMA at various concentrations was assayed for inhibition also and is indicated in the results. Buffer A, and DMA were also assayed by adding 10 µl of each to 200 µl of microsomal suspension and they showed no significant inhibition of HMG-CoA reductase.

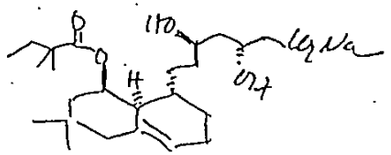


	COMPOUND	DATE	SOLVENT	S.A.	% OF CONTROL		% OF INHIBITION	REMAR
)	Compactin (29299)	10/8/87	DMA					
	1mM			.01	1		99	
	10^{-1}			.04	3	1.37	97	
	10^{-2}			.18	17		83	
	10^{-3}			.62	61		39	
	10^{-4}			.88	86		14	
	10^{-5}			1.04	102		-	
	10^{-6}			1.04	102		-	
	10^{-7}			1.02	100		-	
	10^{-8}			1.04	102		-	
:)	62-320 (24135)	10/8/87	DMA					
	10^{-2}			.01	1		99	
	10^{-3}			.06	6	0.007	94	
	10^{-4}			.20	20		80	
	10^{-5}			.36	36		64	
	10^{-6}			.83	82		18	
	10^{-7}			1.02	100		-	
	10^{-8}			1.02	100		-	
3)	64-906 (RN 30393)	10-8-87	DMA					
	10^{-2}		CHIRAL	.01	1		99	
	10^{-3}			.01	1		99	
	10^{-4}			.11	10	0.0012	90	
	10^{-5}			.27	26		74	
	10^{-6}			.55	54		46	
	10^{-7}			1.02	100		-	
	10^{-8}			1.02	100		-	
1)	64-933 (RN 30441)	10-8-87	DMA					
	10^{-2}			.20	20		80	
	10^{-3}			.69	68	2.37	32	
	10^{-4}			.99	98		2	
	10^{-5}			1.04	102		-	
	10^{-6}			.99	98		2	
	10^{-7}			1.04	102		-	
	10^{-8}			.99	98		2	

257

COMPOUND	DATE	SOLVENT	S.A.	% OF CONTROL		% OF INHIBITION	REMARKS
5) 64-934/Na(RN 30442)	10/8/87	DMA					
10^{-2}			.22	22		78	
10^{-3}			.71	70	2.61	30	
10^{-4}			.99	98		2	
10^{-5}			1.04	102		-	
10^{-6}			1.04	102		-	
10^{-7}			1.02	100		-	
10^{-8}			1.23	121		-	
6) 64-935 (RN 30447)	10/8/87	DMA					
10^{-2}			.13	13		87	
10^{-3}			.32	31		69	
10^{-4}			.74	72	0.413	28	
10^{-5}			.92	91		9	
10^{-6}			.95	93		7	
10^{-7}			.97	95		5	
10^{-8}			1.02	100		-	
7) 64-942/Na(RN 30461)	10/8/87	DMA					
10^{-2}			.71	70		30	Unable to weigh out
10^{-3}			.99	98		2	compound-assuming
10^{-4}			.99	98	>10	2	exactly 0.6mg in via
10^{-5}			.97	95		5	sent from Sandoz,
10^{-6}			1.02	100		-	dilution calculated
10^{-7}			1.02	100		-	and made directly in
10^{-8}			1.02	100		-	vial.
8) 64-727/Na(RN 30024)	10/8/87	DMA					
1 mM			.06	6		94	
10^{-1}			.39	38		62	
10^{-2}			.90	89		11	
10^{-3}			1.02	100	0.73	-	
10^{-4}			1.06	105		-	
10^{-5}			.99	98		2	
10^{-6}			.99	98		2	

COMPOUND	DATE	SOLVENT	S.A.	% br CONTROL		% INHIBITION	REMARI
) 64-948/Na(RN 30485)	10/8/87	DMA					
10-2			.99	98		2	
10-3			.99	98	>10	2	
10-4			1.02	100		-	
10-5			.97	95		5	
10-6			.99	98		2	
10-7			.99	98		2	
10-8			.95	93		7	



259

OCTOBER 15, 1987

DRUG INHIBITION STUDY FOR SANDOZ CONTRACT

Sandoz unknowns were dissolved in DMA (Dimethylacetamide from Sigma), and Buffer A
Dilution of each compound gave the concentrations indicated in the results.

Microsomes were prepared from male Sprague-Dawley rats (150 g) in Buffer A with 10 mM DTT and frozen at -80°C until thawed and used for experiment. 200 µl Aliquots of microsomal suspension (0.96 mg/ml) plus 10 µl of drug dilution were assayed for HMG-CoA reductase activity.

Compactin in DMA at various concentrations was assayed for inhibition also and is indicated in the results. Buffer A, and DMA were also assayed by adding 10 µl of each to 200 µl of microsomal suspension and they showed no significant inhibition of HMG-CoA reductase.

COMPOUND	DATE	SOLVENT	S.A.	% OF CONTROL	% OF INHIBITION
1) Compactin (29299)	10-13-87	DMA			
1mM			.02	2	98
10^{-1}			.02	2	98
10^{-2}			.18	20	80
10^{-3}			.64	69	31
10^{-4}			.84	91	9
10^{-5}			.95	103	-
10^{-6}			1.02	110	-
10^{-7}			.98	106	-
10^{-8}			.98	106	-
2) 62-320 (24135)	10-13-87	DMA			
10^{-2}			.02	2	98
10^{-3}			.05	5	95
10^{-4}			.18	20	80
10^{-5}			.30	32	68
10^{-6}			.86	93	7
10^{-7}			.98	106	-
10^{-8}			.95	103	-
3) 64-942/Na (30461)	10-13-87	DMA			
10^{-2}			.73	79	21
10^{-3}			.95	103	-
10^{-4}			1.05	113	-
10^{-5}			.91	98	2
10^{-6}			.93	101	-
10^{-7}			1.00	108	-
10^{-8}			.98	106	-
4) 62-526/Na (29724)	10-13-87	DMA			
10^{-2}			.02	2	98
10^{-3}			.11	12	88
10^{-4}			.46	50	50
10^{-5}			.80	86	14
10^{-6}			.93	101	-
10^{-7}			.98	106	-
10^{-8}			.98	106	-

COMPOUND	DATE	SOLVENT	S.A.	CONTROL	INHIBITION
5) 64-727 (RN 30024)	10-13-87	DMA			
1mM			.05	5	95
10^{-1}			.34	37	63
10^{-2}			.82	88	12
10^{-3}			.93	101	-
10^{-4}			.95	103	-
10^{-5}			.98	106	-
10^{-6}			.93	101	-
10^{-7}			.93	101	-
10^{-8}			.95	103	-
6) 64-948/Na(RA 30485)	10-13-87	DMA			
10^{-2}			.95	103	-
10^{-3}			1.00	108	-
10^{-4}			.95	103	-
10^{-5}			.98	106	-
10^{-6}			.95	103	-
10^{-7}			.98	106	-
7) 64-936/Na (RN 30448)	10-13-87	DMA			
10^{-2}			.07	7	93
10^{-3}			.32	34	66
10^{-4}			.73	79	21
10^{-5}			.89	96	4
10^{-6}			.93	101	-
10^{-7}			.95	103	-
10^{-8}			.95	103	-

Exhibit F

VOLUME III

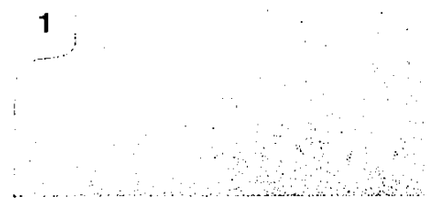
Interference No. 102,648 - #66

Interference No. 102,975 - #8

WATTANASIN Consolidated

Affidavit Testimony

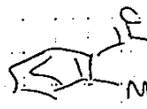
and Exhibits

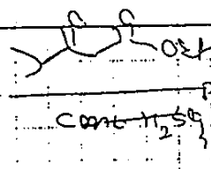

1

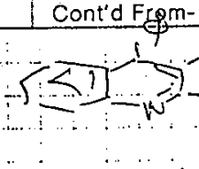
Title-

Date 6-1-4 Proj.

Cont'd From-







263

5233-24 120G-129-18

319.44

$C_{21}H_{21}NO_2$

23324 (120G-129-18) = 11.5 g (0.04930 mole)

(08980) 1582 = 11.93 ml (0.07395 81 mM) - 5 equiv.

EtOH = 10 ml + 5 ml

~~Conc H_2SO_4~~ = ~~2.5 ml~~

Ref : 120G-92

Above misc. was heated to reflux stirred at r.t. overnight

Ranaap to dryness to yellow oil basified with NH_4OH, extracted with Et_2O, washed with H_2O, brine, dried, filtered, washed, rotavap. gave 16.21 g orange yellow solids (120G-130-27)

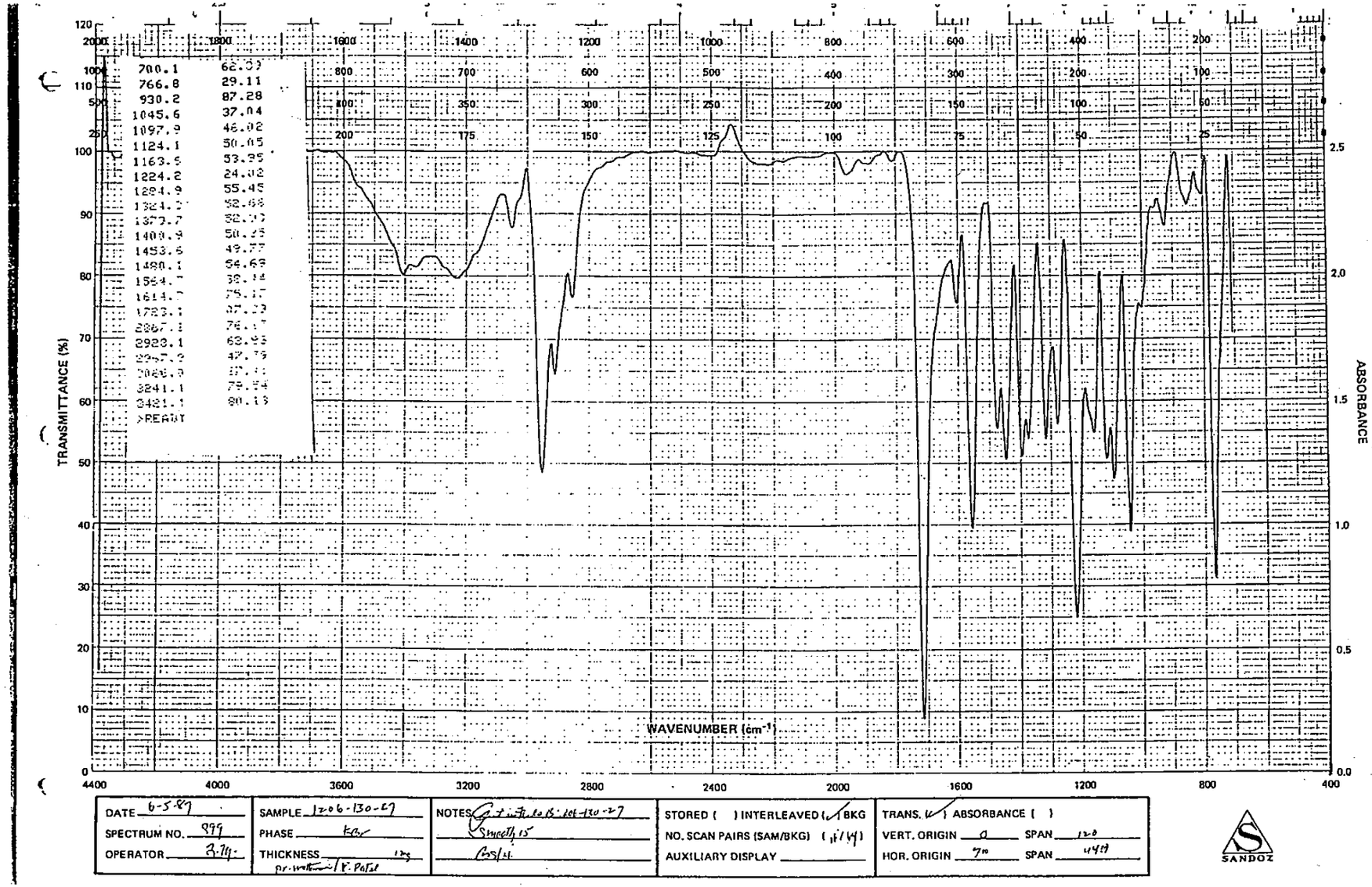
nmr, ir, MS → ~~no 213 cm⁻¹~~ mm⁺ = 320

Theory: 15.748 g, % = 64.86

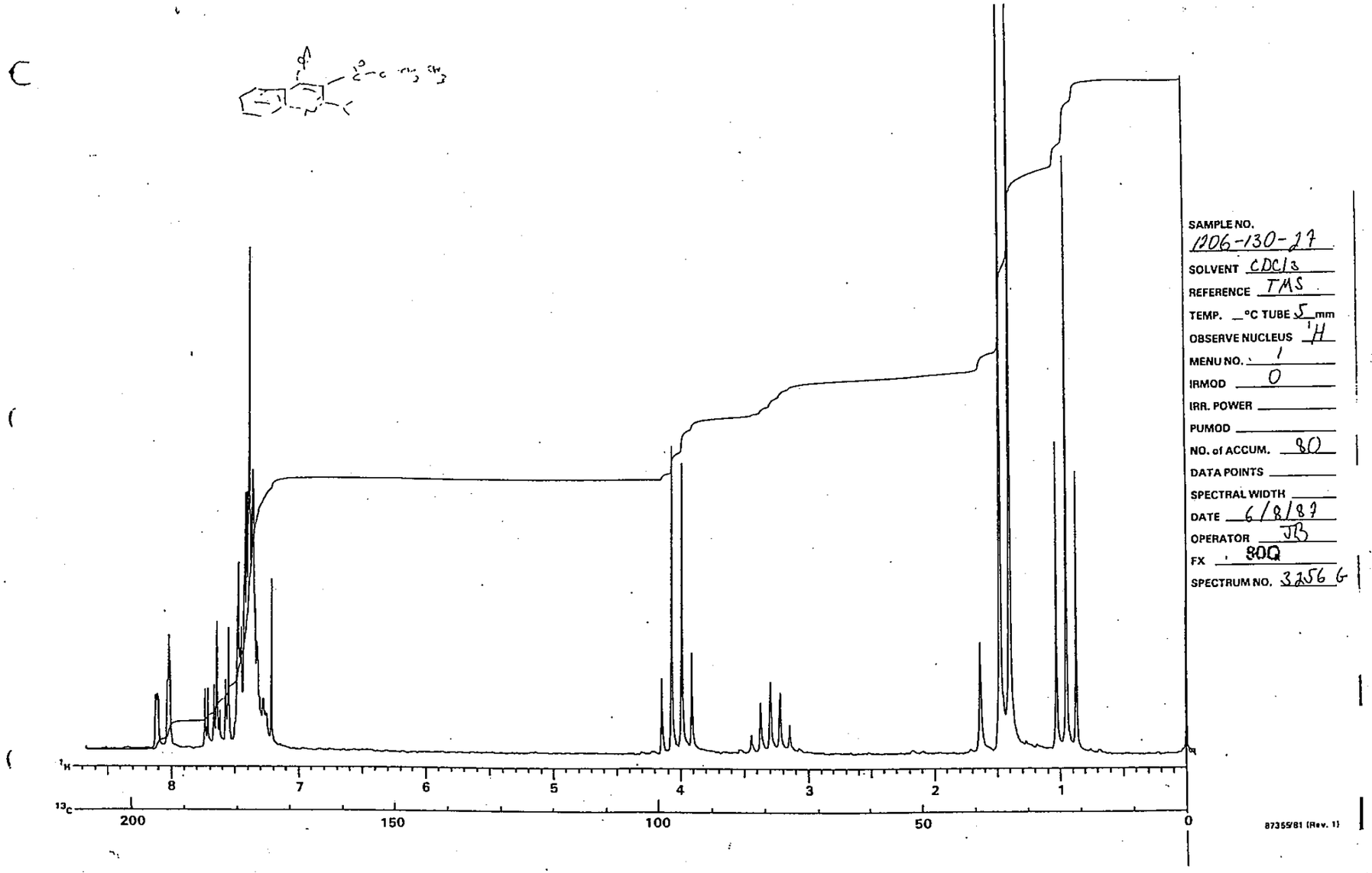
Performed by- Raj Patel 6-5-4.

Witness- Siwanthami

Cont'd to-



264

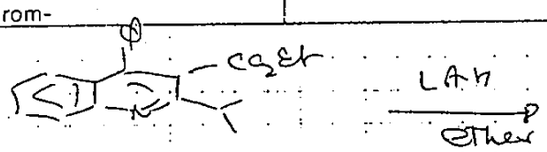


265

Date 6-9-87 Proj. Title-

Cont'd From-

266

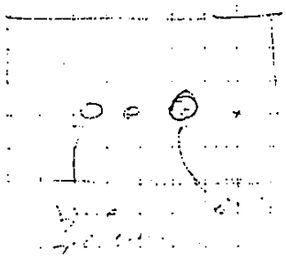


319.44
120G-130-27

277.44
$C_{21}H_{21}NO_2$

(319.44) 120G-130-27 = 10.21g (0.0319621 mole)
(38) LAH = 2.43g (0.0632421 mole)
dry ether = 100ml
Ref: 120G-96

To 120G-130-27 in dry ether with cooling was added LAH portionwise, exothermic, foaming, stirred at r.t. for 3hr. C9 31-12



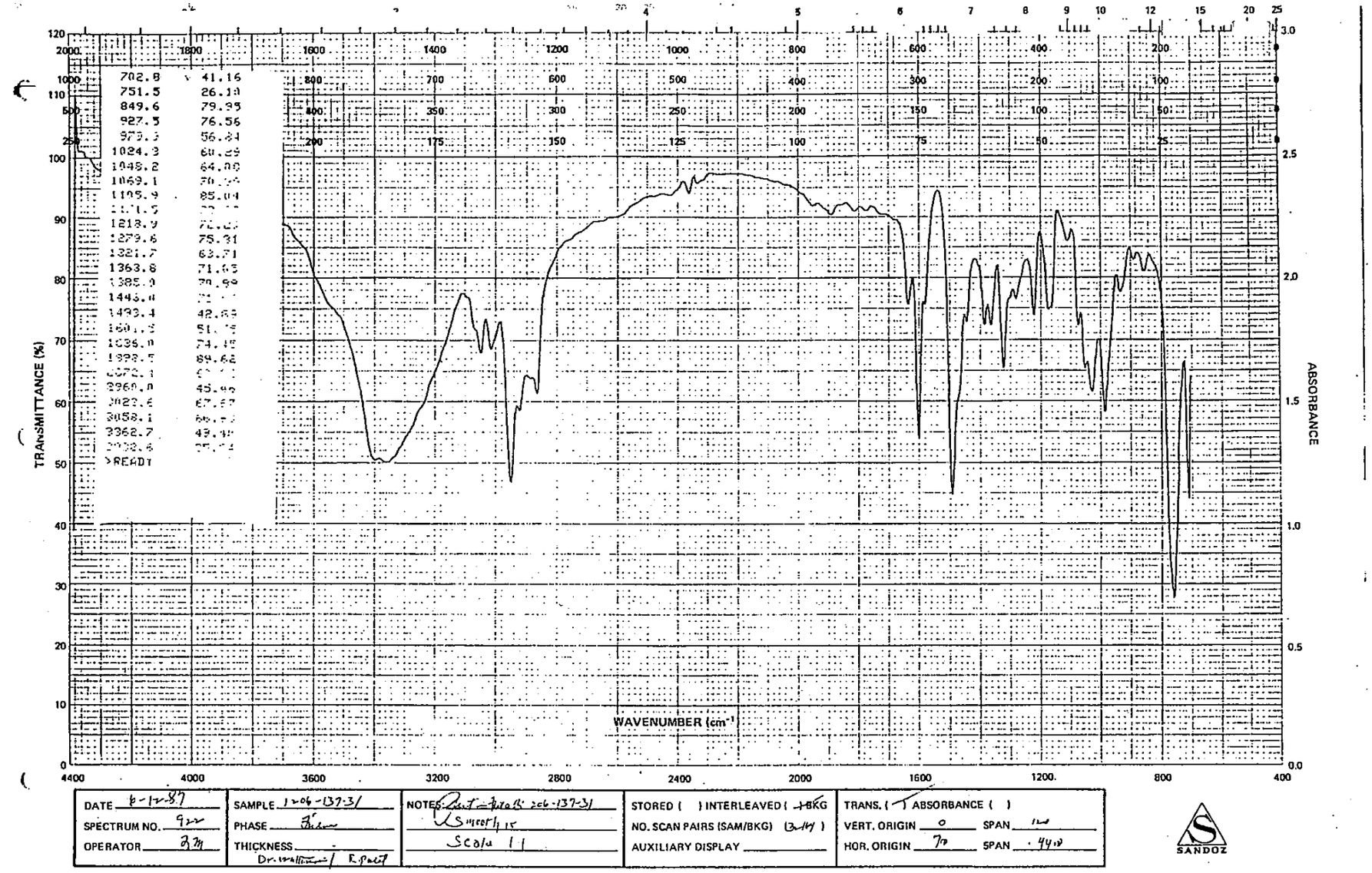
Rx mix. poured in ice H_2O (exothermic, strong Rx) extracted with ether, washed with H_2O brine, dried, filtered, washed, rotavap gave yellow solids wt = 8.5g (120G-137-B1) mmt, in MS

Theory: 8.86g (95.8%)

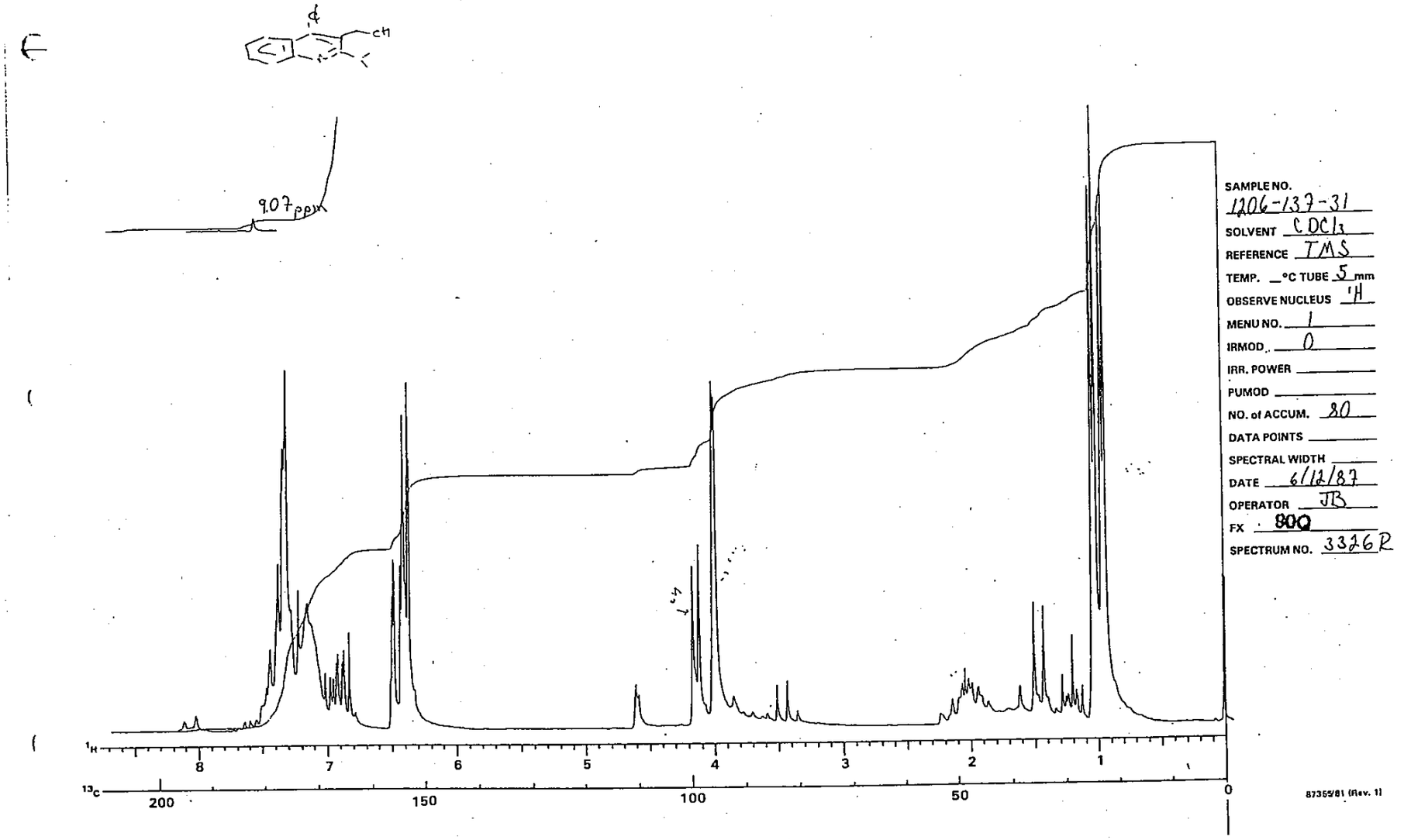
Performed by- Raj Patel 7-2-87

Witness- S. Wattanasin

Cont'd to-



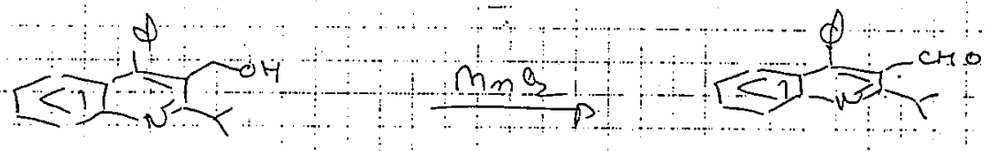
267



Date 6-17-87 Proj. Title-

Cont'd From-

269



1206-137-31
277.4

275.0
$C_{19}H_{17}NO$

277.4 1206-137-31 = ~~8.0~~ 8.0 g (0.02889 2mole)
MnO2 = 16.0 g
toluene = 150 mL

To 1206-137-31 in toluene was added MnO2 → heated to reflux (111° - 45° - 2P)

filtered thru pad of* silica gel, washed with toluene, rotovap to dryness, gave yellow solids = 2.6518 g (1206-145-25) nmr, tlc, ms MH+ = 276 desired pt
orange solids = 4.6463 g (1206-145-26) nmr, tlc, ms MH+ = 278 S.M.

- During filtration, separated two bands, which was filtered separately & rotovap

Theory: 7.91 g (74.52%)

Total yield: 2.6518 g + 3.269 = 5.91 g
(1206-145-25) (1206-148-33)

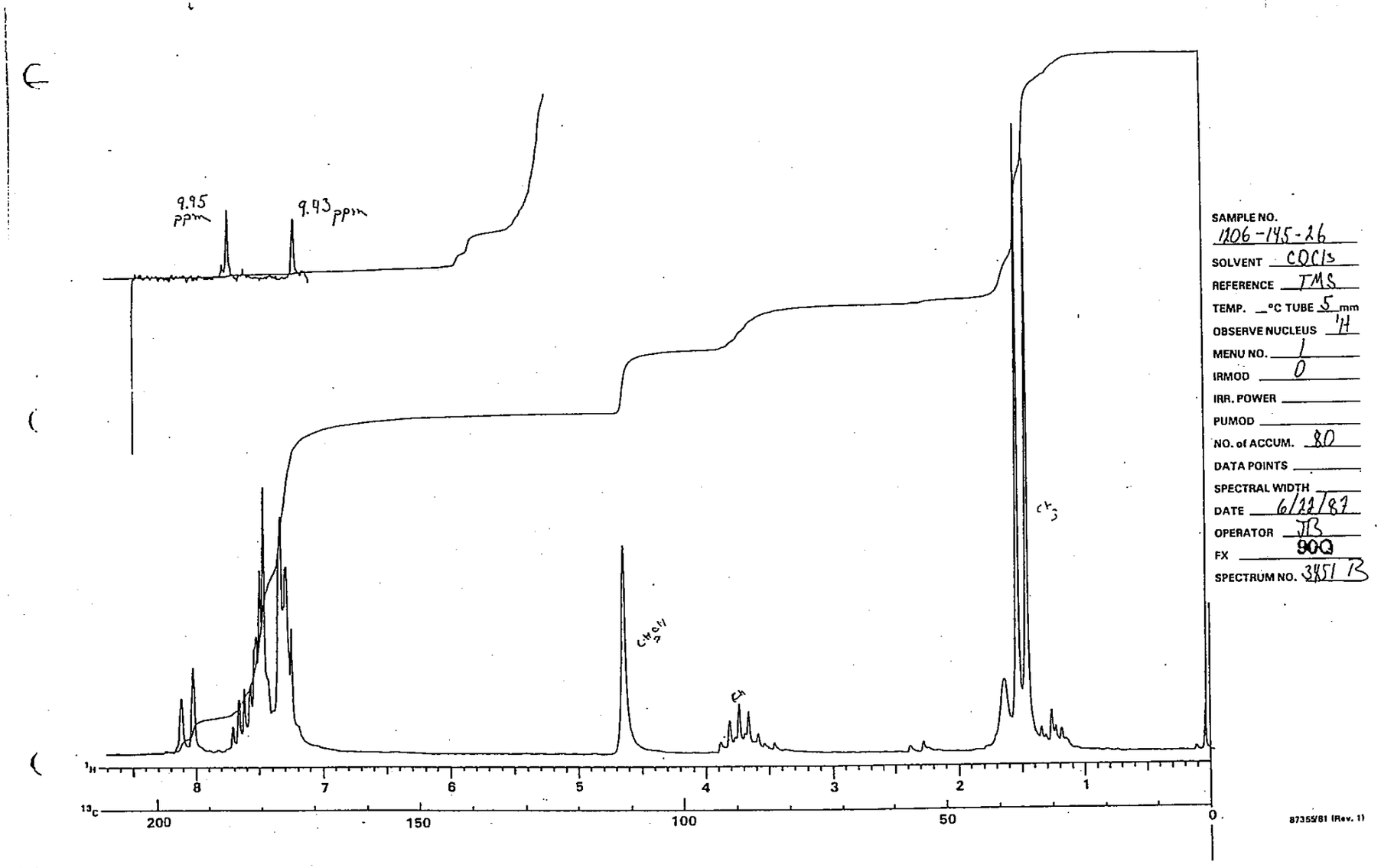
Performed by-

Witness- S. Wattanasin

Cont'd to- 148

SAMPLE NO. 1206-145-25
SOLVENT CDCl3
REFERENCE TMS
TEMP. °C TUBE 5mm
OBSERVE NUCLEUS 1H
MENU NO. 1
IRMOD 0
IRR. POWER
PUMOD
NO. of ACCUM. 80
DATA POINTS
SPECTRAL WIDTH
DATE 6/21/87
OPERATOR YB
FX 90a
SPECTRUM NO. 3450
8735/81 (Rev. 1)
1H
13C

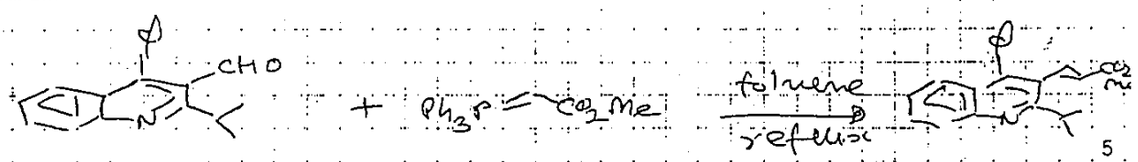
270


9.95 ppm
9.93 ppm
CH2Cl2
CH
CH3
SAMPLE NO. 1206-145-26
SOLVENT CDCl3
REFERENCE TMS
TEMP. °C TUBE 5 mm
OBSERVE NUCLEUS 1H
MENU NO. 1
IRMOD 0
IRR. POWER
PUMOD
NO. of ACCUM. 80
DATA POINTS
SPECTRAL WIDTH
DATE 6/22/87
OPERATOR JB
FX 90Q
SPECTRUM NO. 3451 B
1H
8
7
6
5
4
3
2
1
13C
200
150
100
50
0
87355/81 (Rev. 1)

142

Date 6. 30-87 Proj. Title-

Cont'd From-



275 331

(275) { 1206-145-25 / 1206-148-33 } = 2-65+326 = 5.915(0.02149 mole)

~~MeOH~~ = ~~11.83 g~~

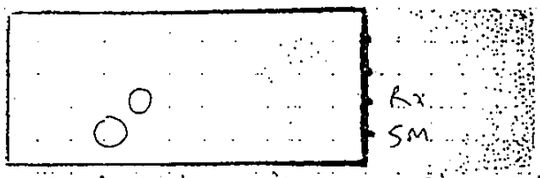
toluene = 85 ml + 20 ml

(334) $Ph_3P{=}CO_2Me$ = 8.6135 g (0.025789 mole) 1.2 eq

Ref : 1206-146

Above mix. was heated to reflux (Yellow heterogenous before heating) for 1½ hrs. Stirred at r.t. overnight

7-1-87



Diluted with 50% Et2O/Pet ether filtered thru' pad of silicagel washed Rotavap to dryness to give yellow crystalline solid 8.6 g : Triturate with MeOH gave off white solids (Theory : 7.113 g) wt = 5.5198 g (1206-153-31) 77.6%

nmr dry ms mp = 332

Rotavap mother liquor to dryness to yellow oil wt = 2.7593 g (1206-153-34)

7-6-87 Trituration with MeOH gave 761.6 mg light yellow solids (1206-153-37) nmr ms mp 332

Rotavap mother liquor to dryness to yellow solid (1206-153-38) ms

Total yield = 5.5198 + 0.7616 (1206-153-

7-9-87 m.p = 128°-130°C

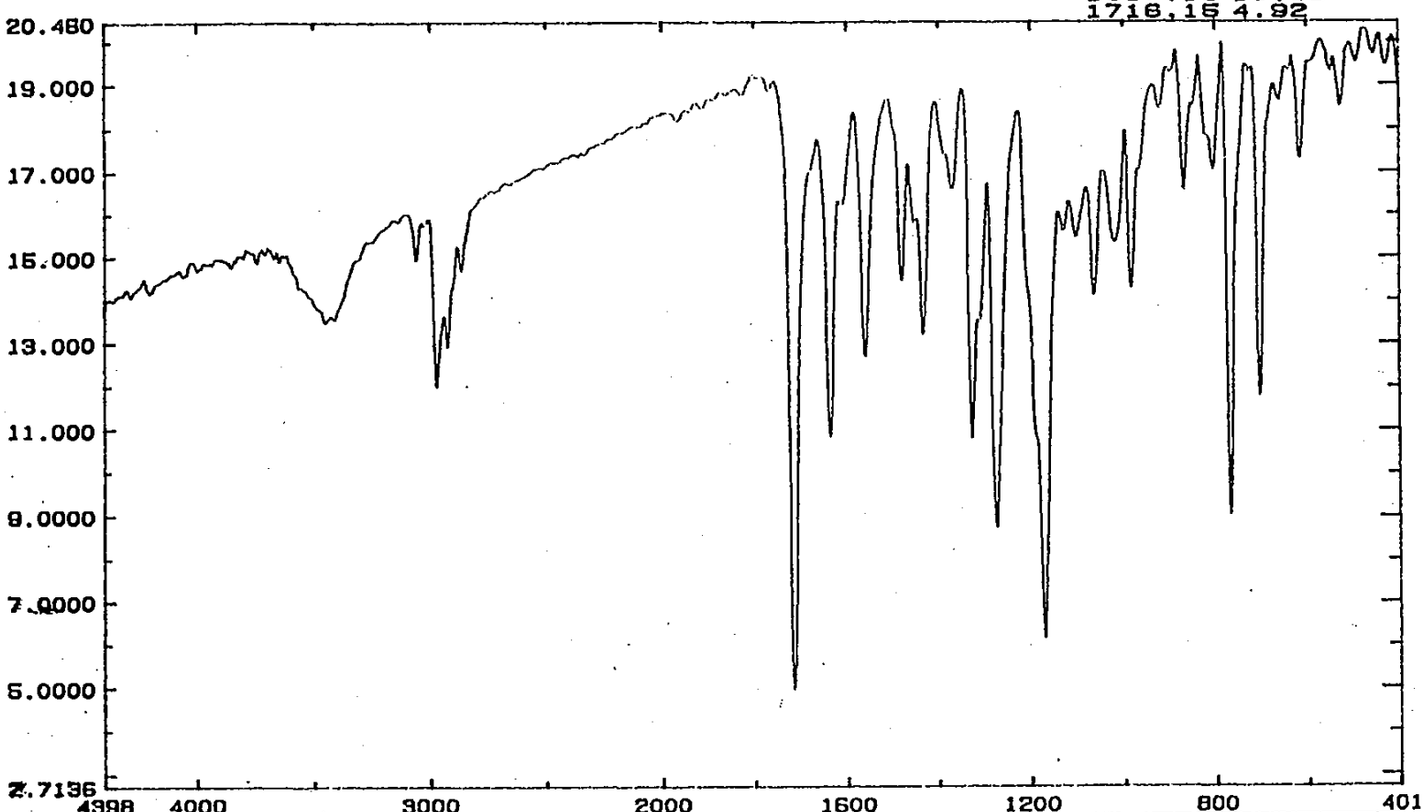
	C	H	N
	77.3	6.38	4.37
	74.65	4.63	
	75.52	6.42	3.75

Performed by- Raj Patel 7-6-87

Witness- S Wattanasin Cont'd to-

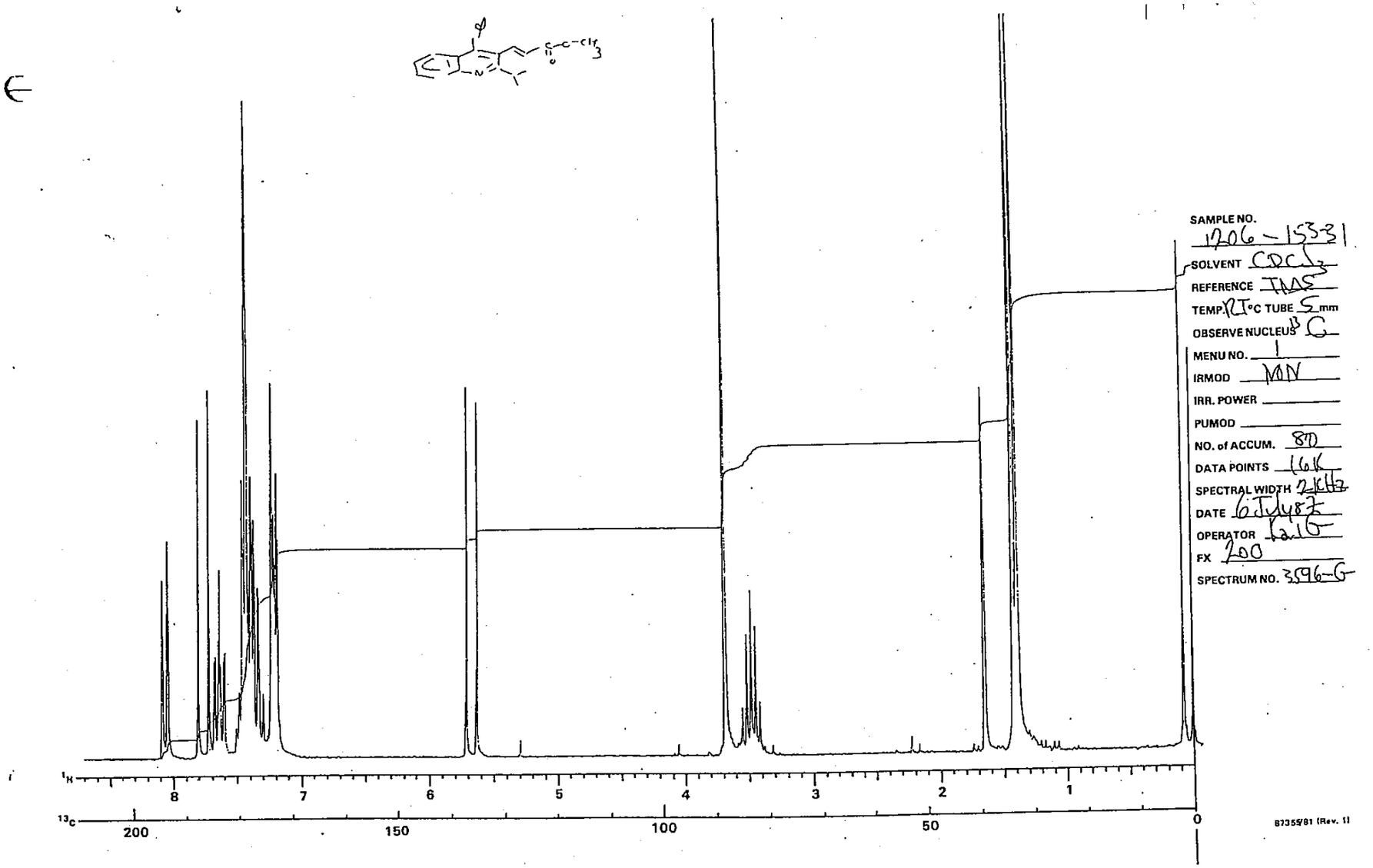
L

CM-1	%T	CM-1	%T
2927.38	12.93	704.39	11.79
2973.07	12.02	759.73	9.00
3413.38	13.56	1065.62	14.10
3452.89	13.49	1172.68	8.14
4207.45	14.18	1276.44	8.70
4289.61	14.06	1328.08	10.79
		1436.50	13.20
		1661.36	12.69
		1897.15	10.85
		1716.15	4.92

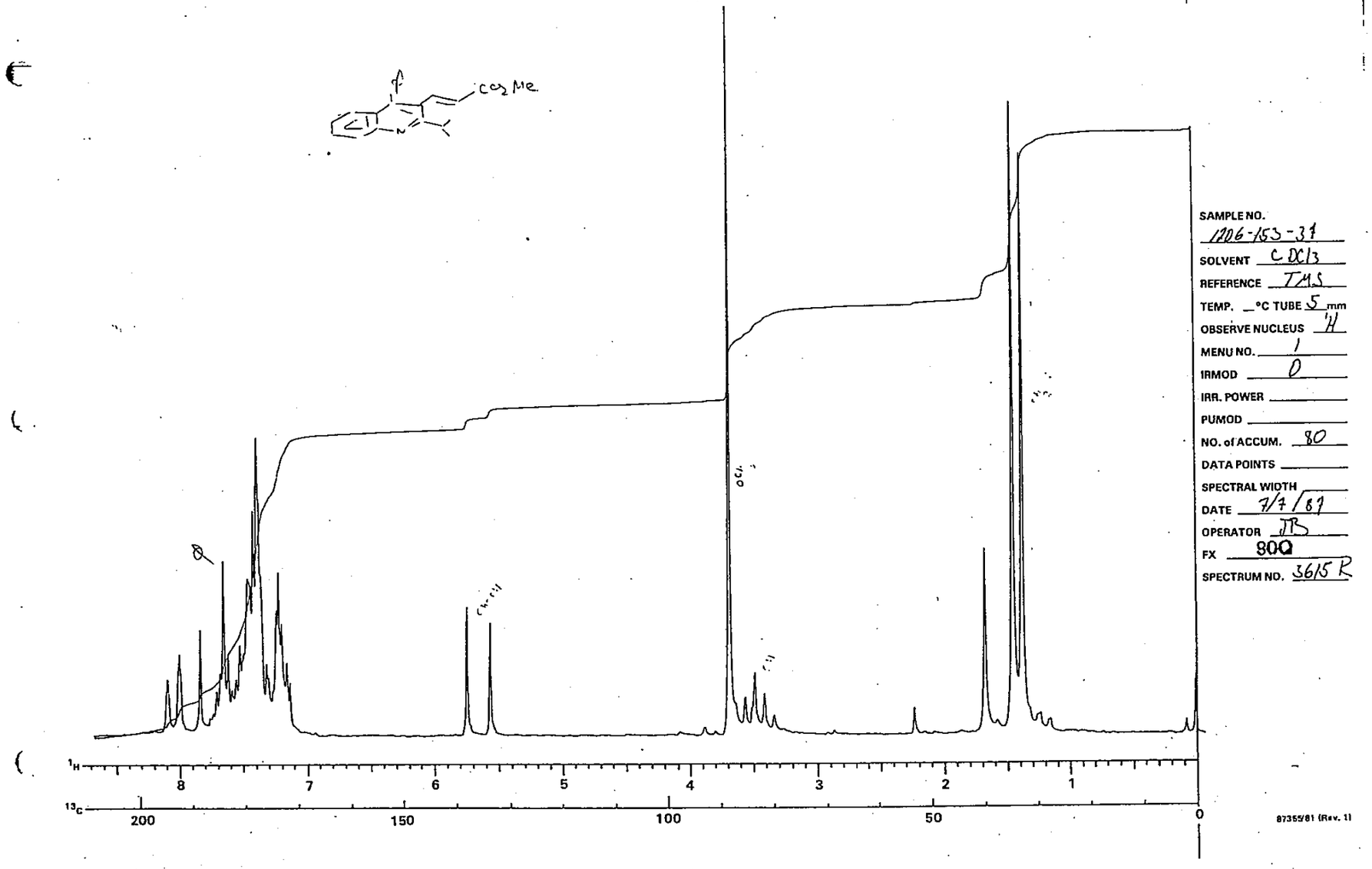


FILE NAME	: 1206-153-34 #1007	GAIN : 2	ANALECT FX-6160
#SCANS	: 64	DET : TGS	ORD : %T
#BKG	: 64	RES : 4 CM-1	ABSC: WAVENUMBER
APOD	: HAPP-GENZEL	DATE : 07/15/87	TIME: 11:46:59
COMMENT	: kbr wattasin/r.p.lab358 pcc		

273

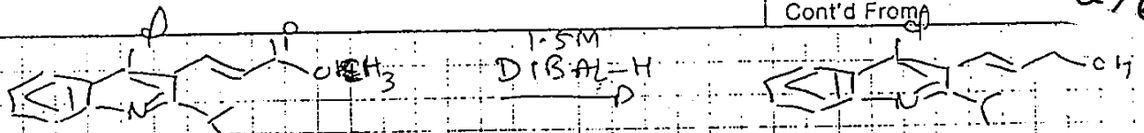


274



158 Title- Date 7-7-87 Proj 276

Cont'd From

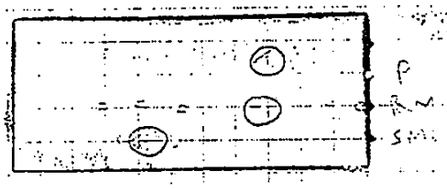


331 302 ($C_{21}H_{21}NO$)

1206-153-40 = 6.25g (0.0188821 mole)
1.5M DIBAL-H/Toluene = 25.18 ml (0.0377642 mole) 2eqiv
CH_2Cl_2 = 75 ml

Ref: 1206-155, 87

To soln of 1206-153-40 in CH_2Cl_2 was added at -78°C 1.5M DIBAL-H/toluene, stirred at -78°C for 3 hrs (13% - 37%)



C	H	N	O
83.13	6.98	4.62	5.27
82.65	6.81	3.9	
82.08	6.89	3.89	

quenched with 12.5 ml 2N NaOH, diluted with Etoac stirred at r.t. overnight → lots of white gel solids came out.

7-8-87

filtered thru' pad of silica gel, washed with Etoac, washed org. layer with H_2O, brine dried rotavap to dryness gave off white solid: 5.42g (1206-158-35) Dissolved solids in Et_2O insolubles (white) (aluminium oxide) was filtered thru' sintered glass funnel rotavap to dryness gave white-yellow solids 5.229 (1206-158-5) Theory: 5.79g 73.7%

Dissolved solids in Et_2O, insoluble (aluminium oxide) was filtered rotavap to dryness gave yellowish solids = 4.211g (1206-158-41) nmr, ir, mts, mm = 304 micro

m.p.: 119°-121°C

Performed by- Ray Patel 7.17.87

Witness- S. Wattanasin Cont'd to-

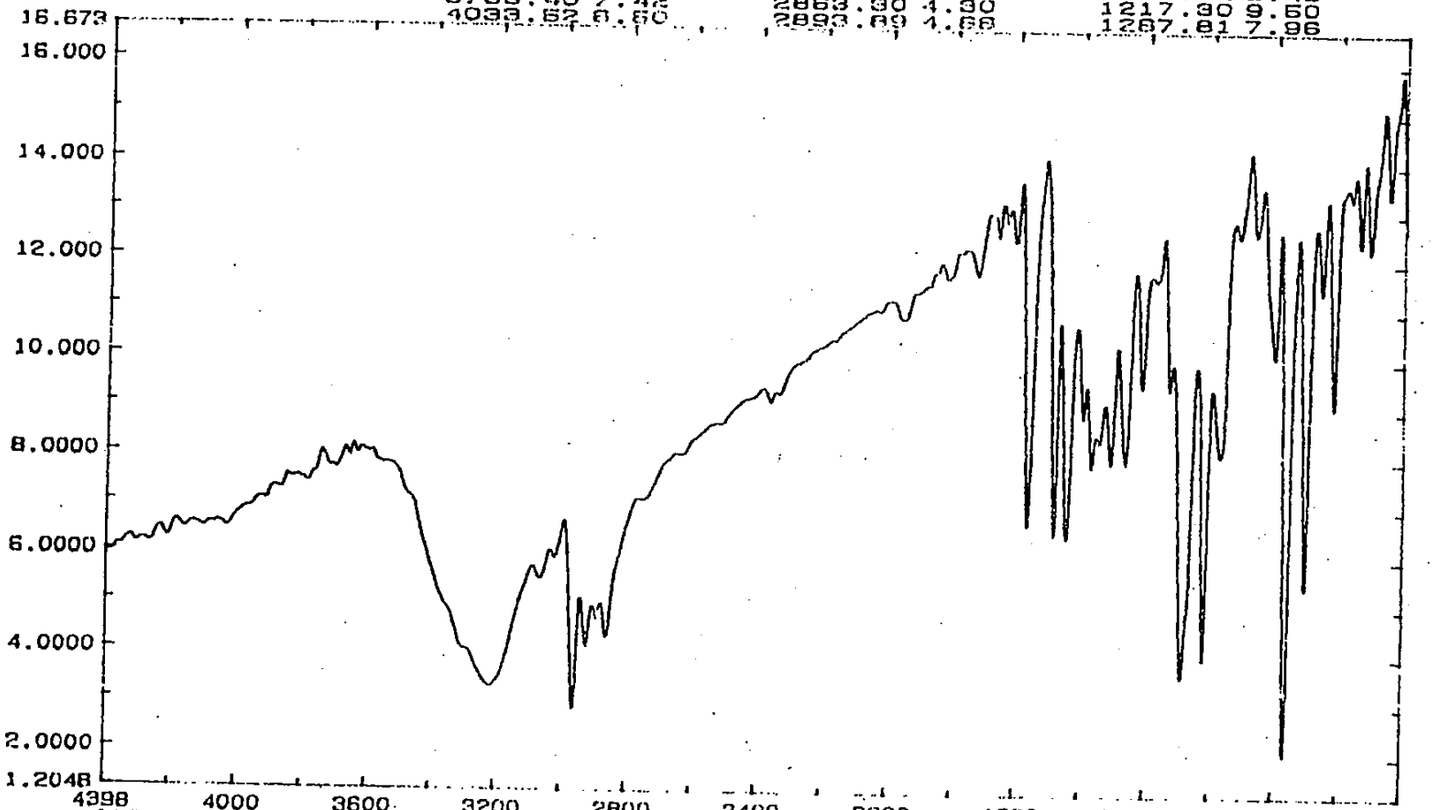
L

CM-1	%T
4104.80	6.48
4161.02	6.46
4214.36	6.27
4308.29	6.15

CM-1	%T
2926.08	4.13
2866.19	2.86
3026.13	6.93
3066.88	6.55
3217.77	3.30
3535.92	8.01
3661.66	7.97
3703.60	7.71
3789.40	7.42
4099.62	6.60

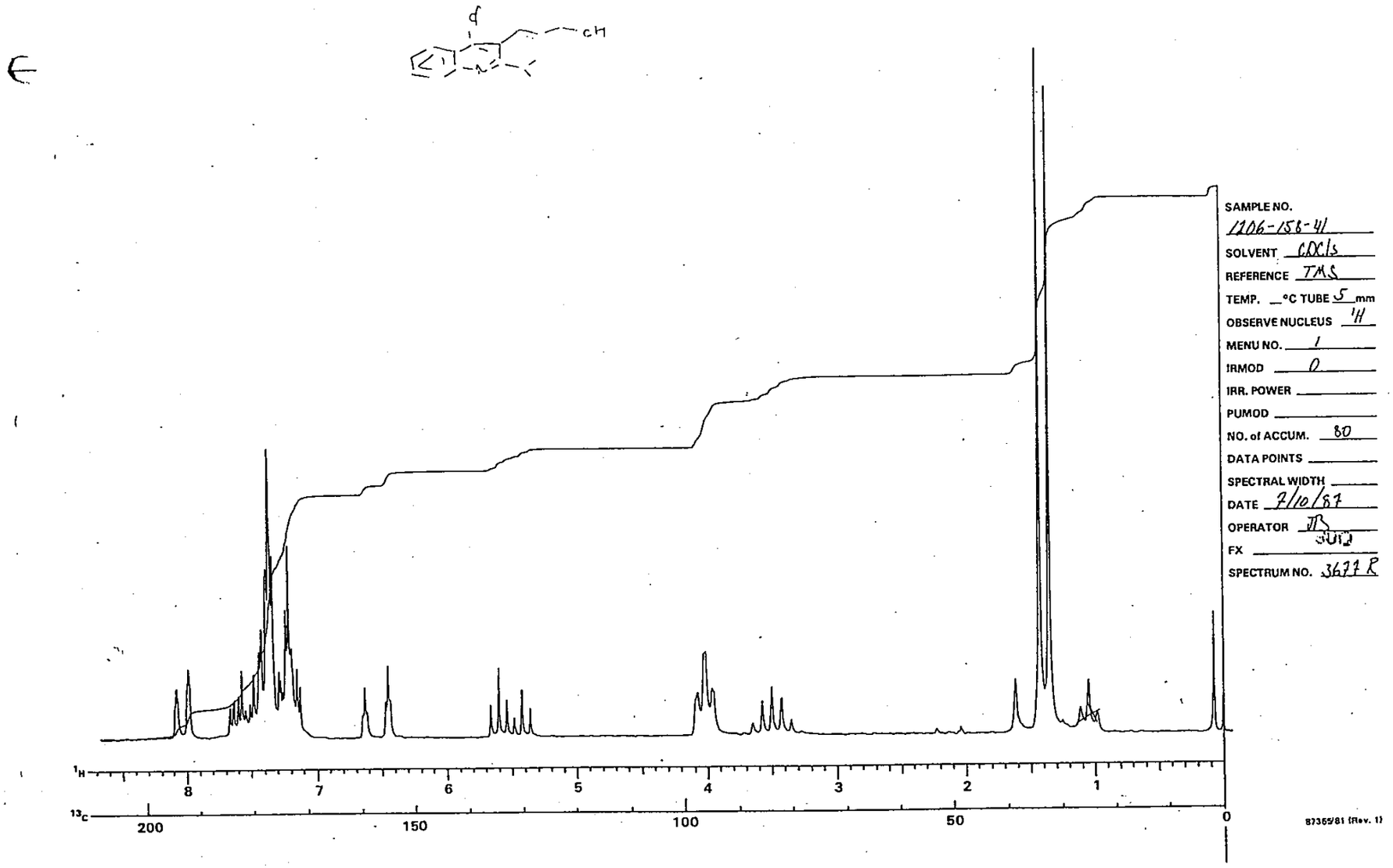
CM-1	%T
1311.78	7.94
1344.55	8.37
1372.03	8.88
1397.90	8.85
1446.43	6.44
1484.95	6.48
1567.10	6.69
2855.78	9.13
2863.30	4.30
2893.69	4.68

CM-1	%T
819.97	9.10
705.86	5.45
765.24	2.08
803.62	10.13
973.00	8.12
1021.66	3.96
1088.47	3.80
1131.72	9.43
1217.90	9.50
1267.81	7.96



FILE NAME	: 1206-158-41 #1037	GAIN : 2	ANALECT FX-6160
#SCANS	: 64	DET : TGS	ORD : %T
#BKG	: 64	RES : 4 CM-1	ABSC: WAVENUMBER
APOD	: HAPP-GENZEL	DATE : 07/21/87	TIME: 15:00:01
COMMENT	: Kbr wattanasin/r.p.lab358 pcr		

247

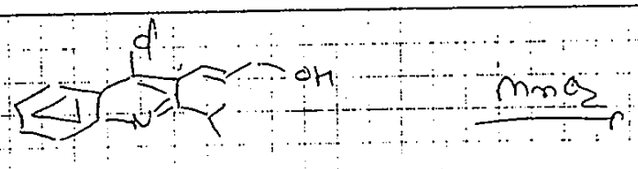


278

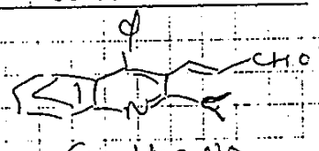
166 Title-

Date 7-15-87 Proj

Cont'd From- 279



303

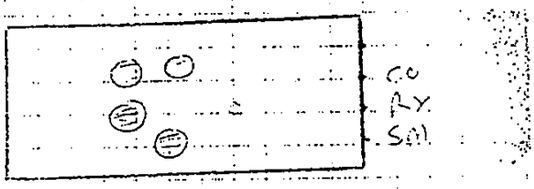


$C_{21}H_{19}NO$
301

1206-158-41 = 4.0g (0.0132013 mole
MnO_2 = 8.0g
toluene = 50 ml

Ref: 1206-164

To 1206-158-41 in toluene added MnO_2 & heated to reflux (2hr - 3hr), stirred at r.t. overnight



7-16-87

filtered thru pad of silica gel, washed pad with ether, rotavac to dryness. gave 3.4946g yellow crystaline material (1206-166-30)

IR ✓ nmr ✓ ms ✓ $m/e^+ = 302$

Theory: 3.97369 (88%)

7-28-87 micro

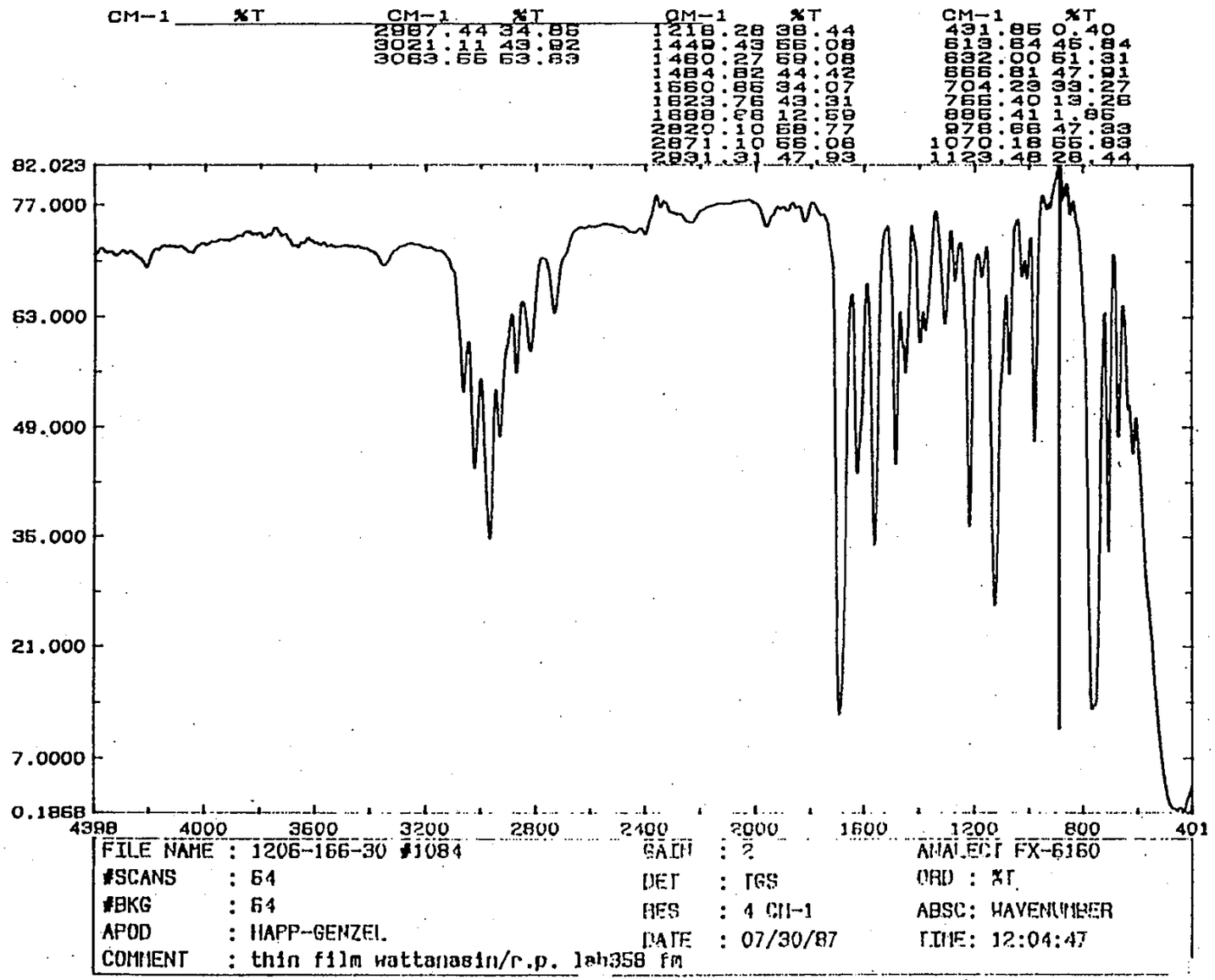
	C	H
Found		
Calcd		

7-30-87 hi-res mass obs. mass = 302.15404 m.p. = 98-101°
calc. mass = 302.15448

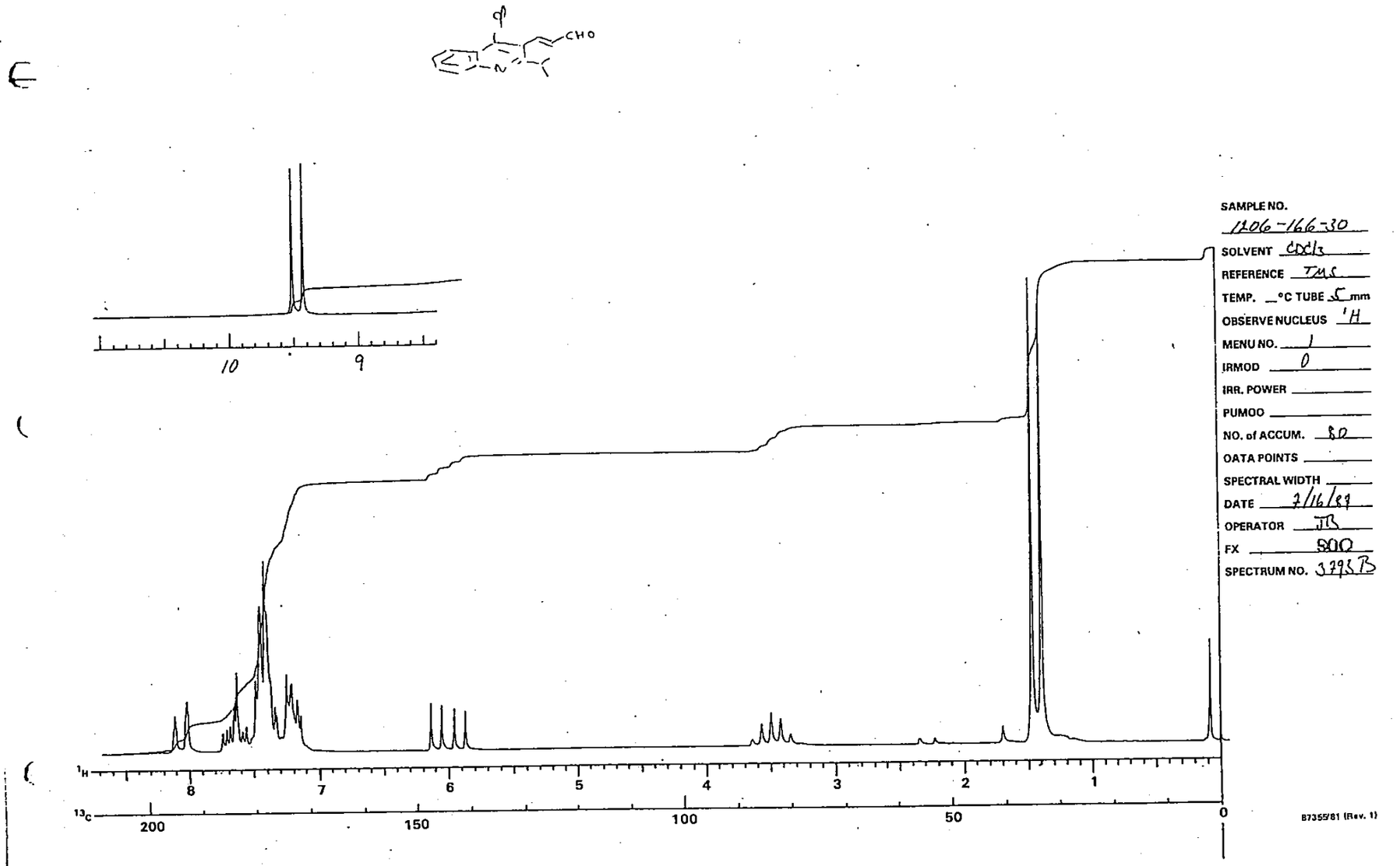
Performed by- Roy Rabel : 7-20-87

Witness-

Cont'd to-


CM-1 %T CM-1 %T CM-1 %T CM-1 %T
2987.44 34.85 1218.28 38.44 431.85 0.40
3021.11 43.92 1449.43 56.08 613.64 46.84
3063.55 53.89 1480.27 59.08 832.00 51.31
1484.82 44.42 866.81 47.91
1660.85 34.07 704.23 39.27
1823.76 43.31 765.40 13.26
1888.98 12.59 885.41 1.86
2820.10 58.77 978.68 47.33
2871.10 56.06 1070.18 55.83
2931.31 47.93 1123.48 28.44
82.023
77.000
63.000
49.000
35.000
21.000
7.0000
0.1868
4398 4000 3600 3200 2800 2400 2000 1600 1200 800 401
FILE NAME : 1206-166-30 #1084
#SCANS : 64
#BKG : 64
APOD : HAPP-GENZEL
COMMENT : thin film wattanasin/r.p. lab358 fm
GAIN : 2
DET : TGS
RES : 4 CM-1
DATE : 07/30/87
ANALECT FX-6160
ORD : %T
ABSC: WAVENUMBER
TIME: 12:04:47

280

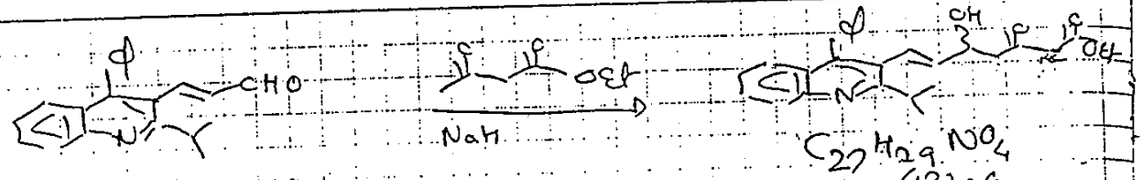


182

Title-

Date 7-20-87 Proj 282

Cont'd From-



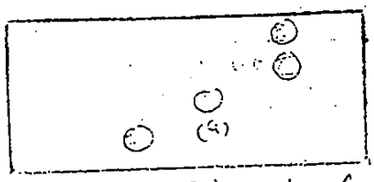
301

301 1206-166-30 = 3.5g (0.011629 mole)
130.14, 1.021 Ethyl acetoacetate = 5 ml (0.04 mole)
24 60% NaH =
1.6M n-Buli/Hex = 27 ml
THF = 60ml + 40ml

To a soln of 1206-166-30 in dry THF (40ml) at -5° to -10°C was added a soln of dianion (11ml + 27ml)(38 ml), prepared as described previously

Dianion (got from Dr. Som)

To soln of 5 ml Ethyl acetoacetate in 60ml dry THF was added 1.9 g 50% NaH at -5° to 0°C, stirred for 15 min (foaming H_2 evolved) At -10° - -15°C was added 27 ml 1.6M n-Buli/Hex, stirred for 20 min at -10°C → yellow homogeneous soln
Total vol = 92 ml (0.04 mole Used 48.38 ml dianion
= 0.01652 mole (1.4 equiv.) → color changed from yellow to orange to dark red
TLC (50% Et2O/Pet) after 15 min → complete rxn



Rx was stirred for 30 min. quenched with sat. NH_4Cl (saturated with Et2O), washed with H_2O brine, dried, filtered, rotavaped gave yellow oil 5.9188 g (1206-172-41)
Theory: 5.01 g (67.8%)

Performed by- Raj Patel 7-21-87

Witness-

Cont'd to- 72 c 6 - 175

Date 7-22-87 Proj. Title-

Cont'd From- 120G-172

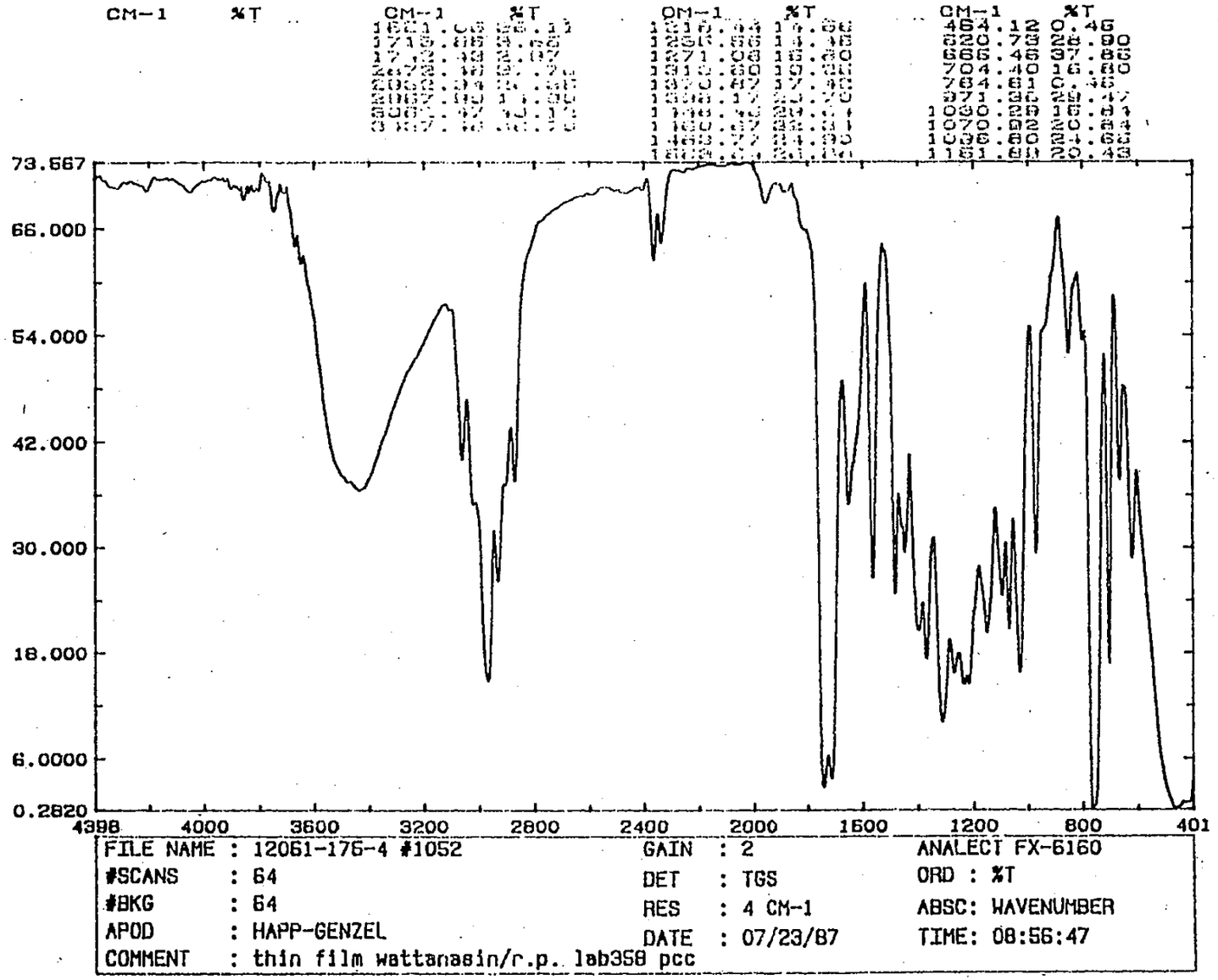
Flash chromatography (5% 25% EtOAc/Hex) gave
(a) yellow solids = 3.4004 g CR0G-175-A7 micro, IR, MS
m.p. = 84-87°C 68% yield

	C	H	N	O		
Calc.	74.4	6.2		14.5		
Found	74.2	6.1		14.3		

Performed by- Raj Patel 8-5-87

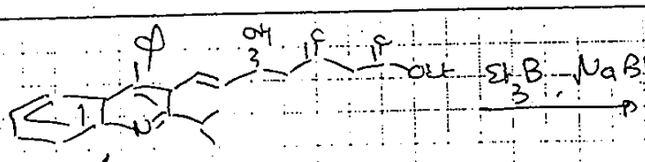
Witness- S. Wattanasin

Cont'd to-

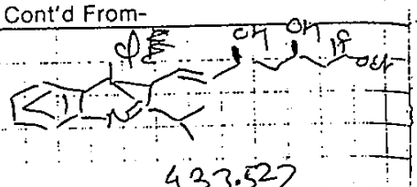

CM-1 %T
73.667
66.000
54.000
42.000
30.000
18.000
6.0000
0.2820
4398 4000 3600 3200 2800 2400 2000 1600 1200 800 401
FILE NAME : 12061-176-4 #1052
#SCANS : 64
#BKG : 64
APOD : HAPP-GENZEL
COMMENT : thin film wattanasin/r.p. lab358 pcc
GAIN : 2
DET : TGS
RES : 4 CM-1
DATE : 07/23/87
ANALECT FX-6160
ORD : %T
ABSC: WAVENUMBER
TIME: 08:56:47

285

SAMPLE NO. 1206-125-4
SOLVENT CDCl3
REFERENCE TMS
TEMP. RT °C TUBE 5 mm
OBSERVE NUCLEUS 1H
MENU NO. VAN
IRMOD
IRR. POWER
PUMOD
NO. of ACCUM. 16
DATA POINTS 16K
SPECTRAL WIDTH 2KHz
DATE 25 JUN 87
OPERATOR Paul C
FX 200
SPECTRUM NO. 3874-5
8735/81 (Rev. 1)
1H
13C
0
1
2
3
4
5
6
7
8
50
100
150
200



431



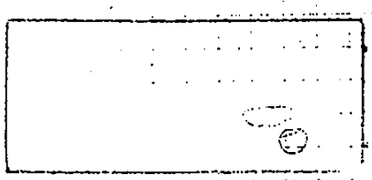
433.527

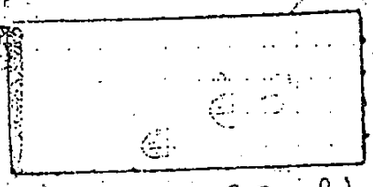
$C_{27}H_{31}NO_4$

(431) 1206-175-4 = 1.0 g (0.002320 mole)
1m Et_3B / THF = 3.5 ml (0.003480 mole) 1.5 eq.
dry THF = 10 ml
CH_3CN = 3.5 ml
37.8 $NaBH_4$ = 0.1315 g (0.003480 mole) 1.5

Ref: 1206-140 (chemiopeneers)

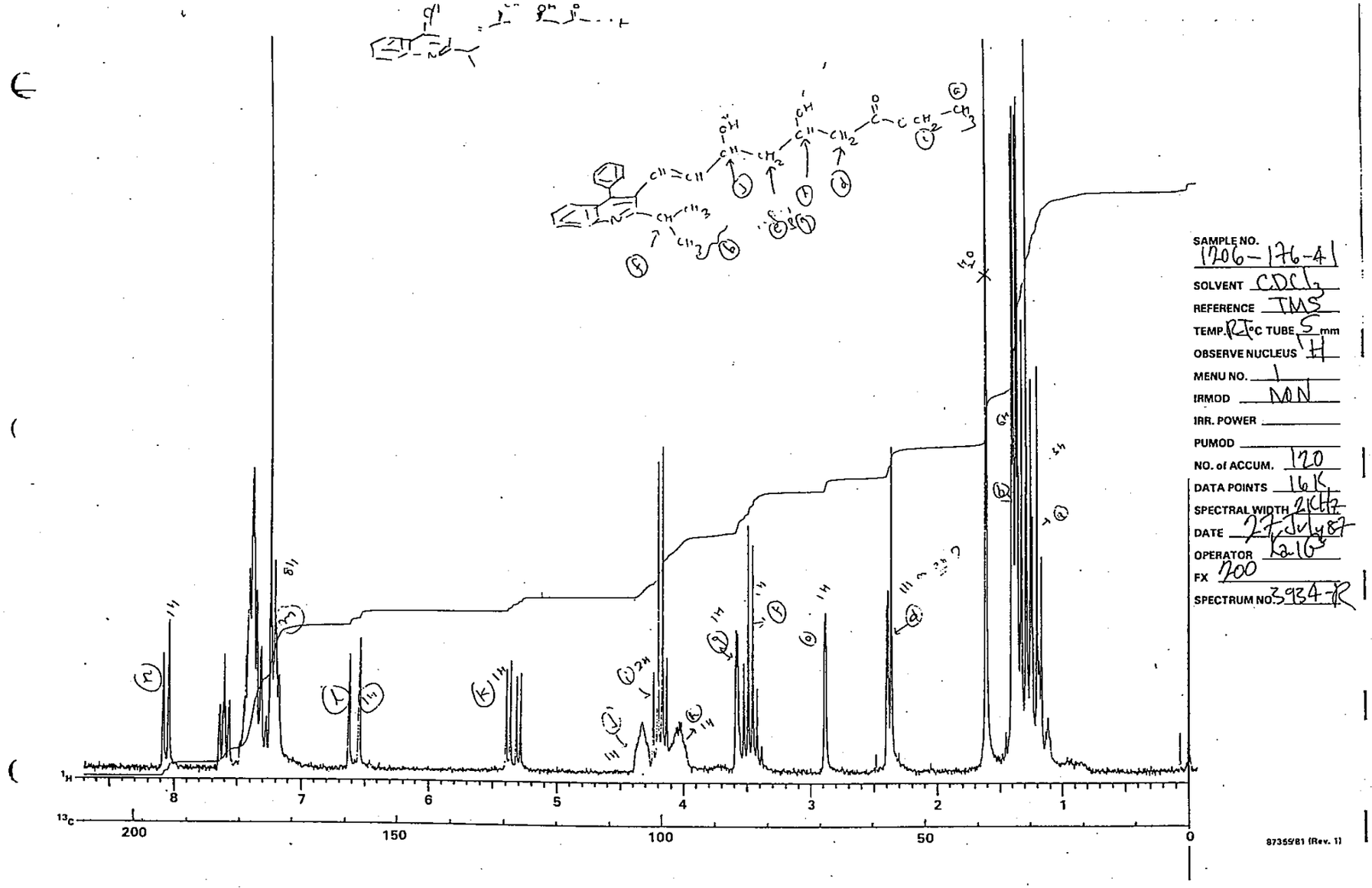
To 1206-175-4 in THF / MeOH added 1m Et_3B / THF at r.t., stirred for 1 hr (9^{45} - 10^{45}). The solution was cooled to -78°C, $NaBH_4$ was added portionwise. The rx. was stirred at -78°C for (11^{00} - 3^{00}) 4 hrs.



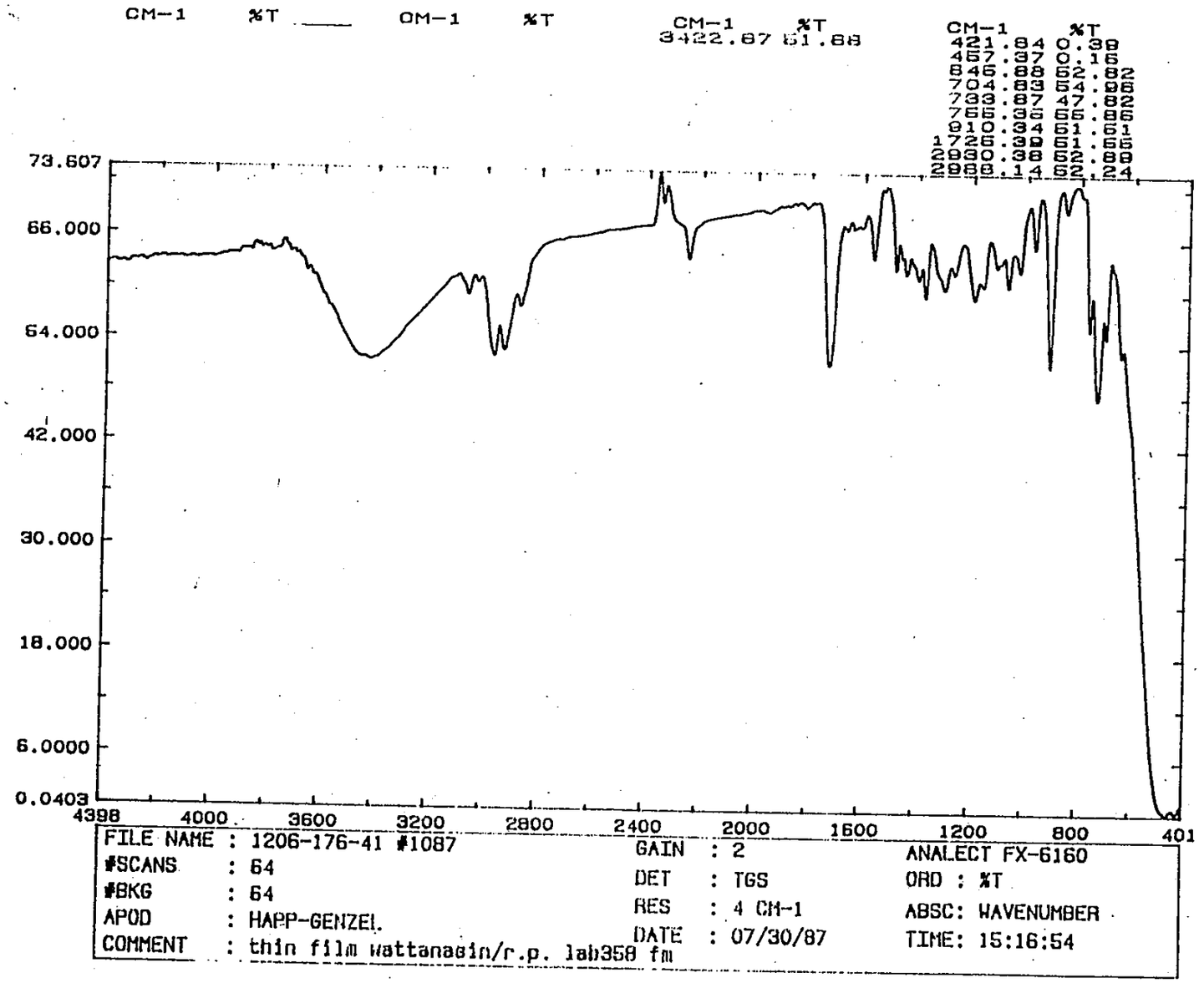


The rx. was quenched with MeOH (5 ml) at -78°C. Ethyl acetoacetate was added & let it warm upto r.t. Org. layer was washed with sat. $NaHCO_3$, H_2O, brine, dried & filtered. Residue was redissolved in MeOH evaporated to dryness. This evaporation process (in MeOH) was repeated until TLC showed desired product.
Wt. of orange oil = 1.0914 g (1206-176-39) ✓ m.p. = 104-106° ✓ exact mass 13
Flash column (60:40 Hex/EtOAc) gave yellow oil (91) F_{4-6} = 0.4043 g (1206-176-41) ✓ nmr, ✓ ms mh^+ = 434.2 HPLC (98.3%) 13
F_{7-13} = 0.510 g (1206-176-43) ✓ nmr, ✓ ms mh^+ = 434.2 HPLC (93.2%)

Performed by- [illegible] (Rej Patel 8-5-87)
Witness- [signature] Cont'd to-



287



288

[redacted]

TOTAL 31
RESOL14653E -4 HZ
EXREF 77.0000PPM
OBS 1756.9331 HZ
NGAIN 0

NO	FREQ(HZ)	PPM	INTS
1	9655.91	172.405	295
2	9256.59	165.327	257
3	7816.10	139.587	814
4	7728.16	138.055	195
5	7615.73	136.434	1816
6	7532.54	134.171	1137
7	7479.75	129.128	562
8	7451.05	128.505	667
9	7428.58	129.098	2305
10	7387.47	127.291	961
11	7348.52	126.346	1135
12	7314.21	125.921	632
13	7282.48	125.597	816
14	4164.56	82.985	-155
15	3895.41	77.642	8388
16	3874.43	77.284	408
17	3864.17	77.988	9358
18	3831.93	76.357	9553
19	3623.86	72.211	933
20	3485.39	67.898	1224
21	3052.25	60.824	921
22	2121.92	42.283	1971
23	2082.35	41.495	1192
24	1729.21	34.458	178
25	1559.74	33.086	991
26	1178.51	21.933	1494
27	1097.58	21.375	1448
28	756.41	15.276	149
29	713.13	14.195	991
30	187.11	3.126	-148
31	-262.14	-5.221	-147

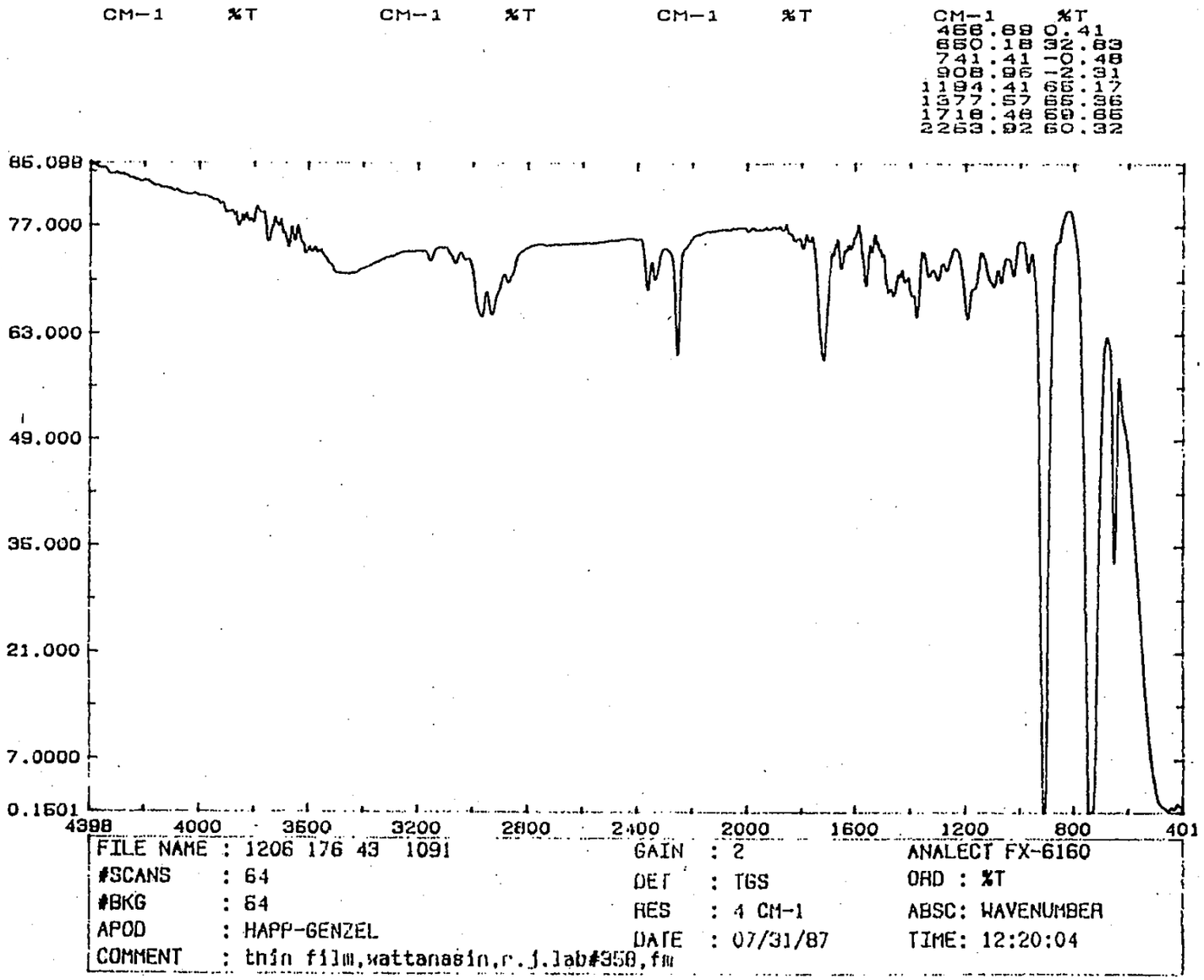
[redacted]

SAMPLE NO. 1406-176-41
SOLVENT $CDCl_3$
REFERENCE $CDCl_3$
TEMP. RT °C TUBE 5 mm
OBSERVE NUCLEUS ^{13}C
MENU NO. #22
IRMOD COM
IRR. POWER
PUMOD
NO. of ACCUM. 30667
DATA POINTS #16K
SPECTRAL WIDTH 17KH
DATE 29 July 87
OPERATOR
FX 200
SPECTRUM NO. 03934

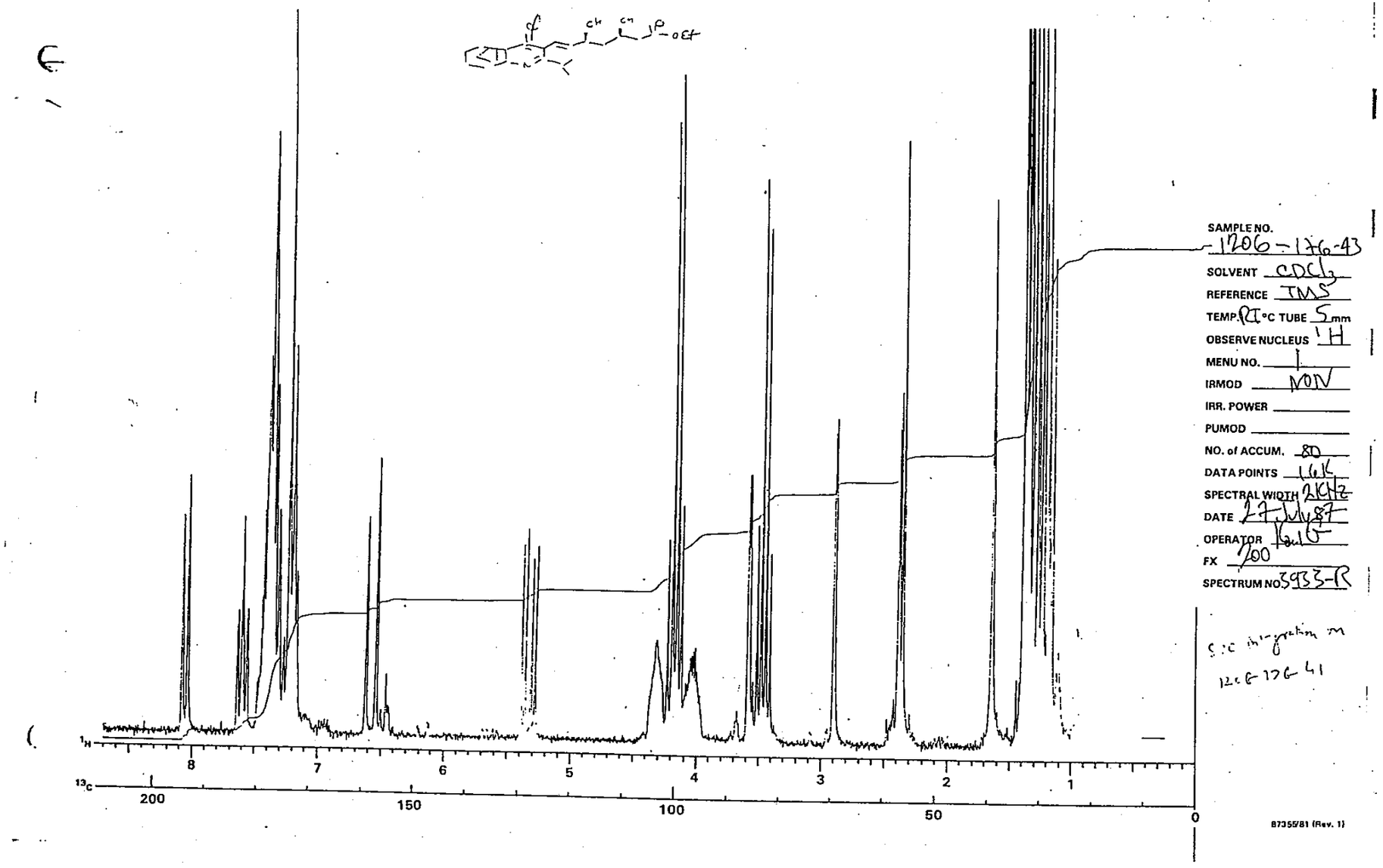
^1H 8 7 6 5 4 3 2 1
^{13}C 200 150 100 50 0

87355/81 (Rev. 1)

282

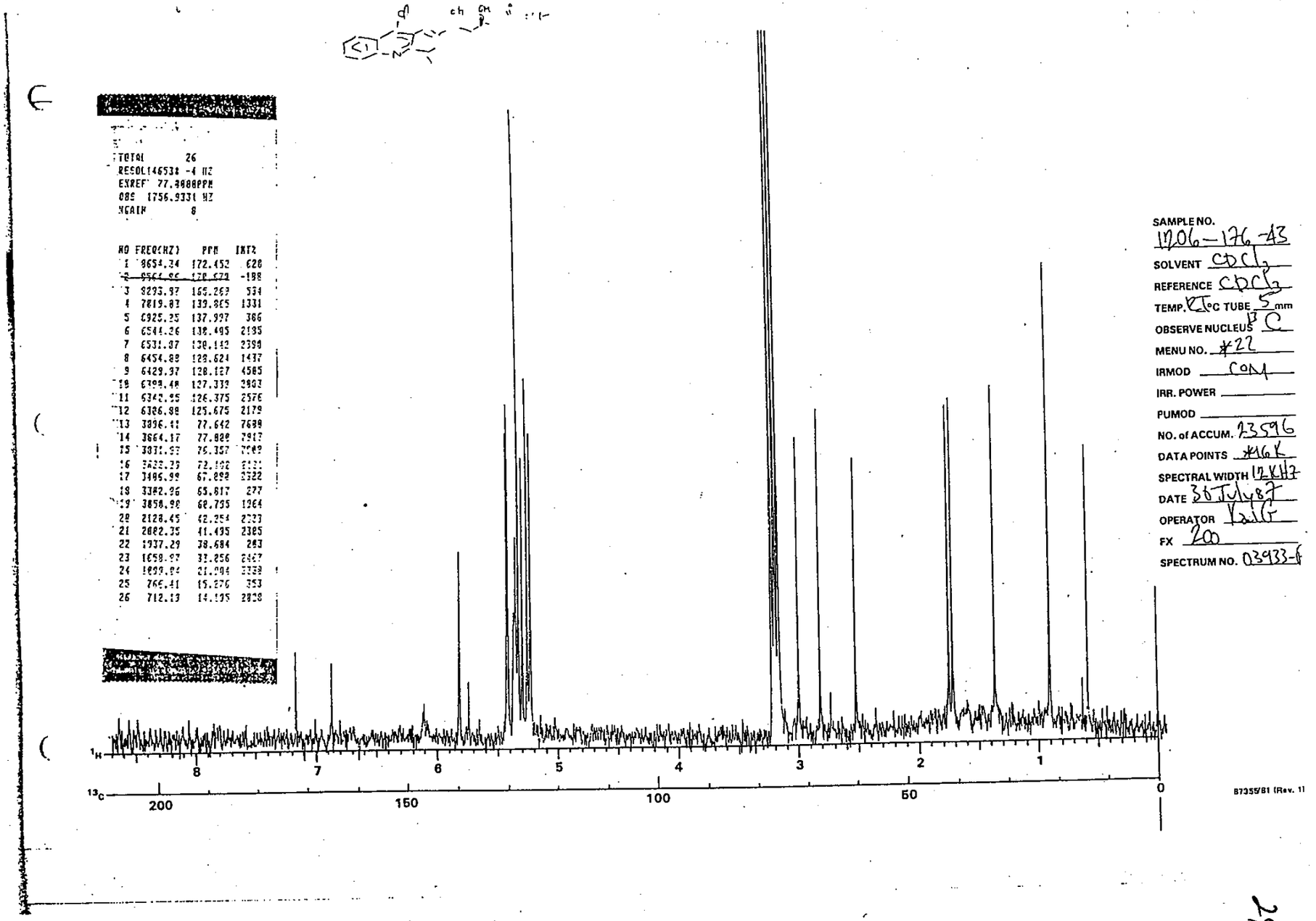

CM-1 %T
CM-1 %T
CM-1 %T
CM-1 %T
456.69 0.41
650.18 32.83
741.41 -0.48
908.96 -2.31
1194.41 66.17
1377.57 65.36
1718.48 69.65
2263.92 60.32
86.088
77.000
63.000
49.000
36.000
21.000
7.0000
0.1601
4398 4000 3600 3200 2800 2400 2000 1600 1200 800 401
FILE NAME : 1206 176 43 1091
#SCANS : 64
#BKG : 64
APOD : HAPP-GENZEL
COMMENT : thin film,wattanasin,r.j.lab#358,fm
GAIN : 2
DET : TGS
RES : 4 CM-1
DATE : 07/31/87
ANALECT FX-6160
ORD : %T
ABSC: WAVENUMBER
TIME: 12:20:04

290



S:C integration in 1206-176-41

172

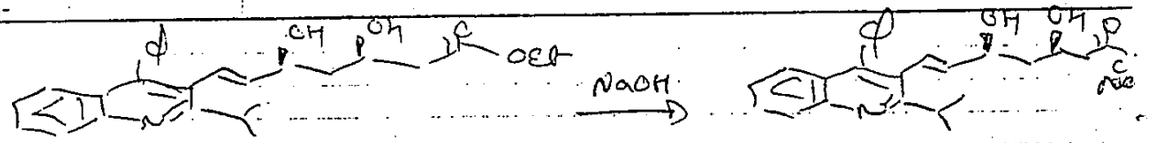

TOTAL 26
RESOL 14653 -4 HZ
EXREF 77.0000PPM
OBS 1756.9331 HZ
NGAIN 8
NO FREQ(HZ) PPM INT
1 9654.34 172.452 628
2 9564.96 170.854 -198
3 9293.97 166.269 534
4 7819.83 139.665 1331
5 7925.25 137.997 386
6 7544.26 138.495 2195
7 7531.87 138.142 2398
8 6454.88 128.624 1437
9 6429.97 128.127 4585
10 6399.48 127.319 3903
11 6342.55 126.375 2576
12 6326.98 125.675 2179
13 3896.11 77.642 7699
14 3864.17 77.888 7917
15 3831.93 76.357 7069
16 3622.29 72.192 6131
17 3496.99 67.898 5322
18 3382.96 65.817 277
19 3858.98 68.795 1264
20 2128.45 42.254 2223
21 2082.35 41.495 2385
22 1937.29 38.684 283
23 1658.97 33.256 6467
24 1099.84 21.994 3738
25 769.41 15.276 353
26 712.13 14.135 2838
SAMPLE NO. 1706-176-43
SOLVENT CDCl3
REFERENCE CDCl3
TEMP. RT °C TUBE 5 mm
OBSERVE NUCLEUS 13C
MENU NO. #22
IRMOD COM
IRR. POWER
PUMOD
NO. of ACCUM. 23596
DATA POINTS #16K
SPECTRAL WIDTH 17KHZ
DATE 30 July 87
OPERATOR
FX 200
SPECTRUM NO. 03933-6
1H
8
7
6
5
4
3
2
1
0
13C
200
150
100
50
87355/81 (Rev. 11)

292

293 179

Date 7-28-87 Proj. Title-

Cont'd From-

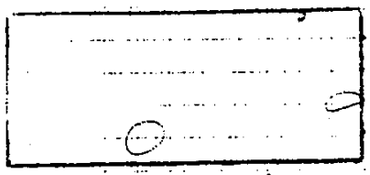


433 427-467

$C_{25}H_{26}NO_4Na$

(433 1206-176-41 = 200.0mg (0.461893mmole)
0.5N NaOH = 439 ul (0.438799mmole)
abs. etoH = 5 ml + 439ml 95%

To 1206-176-43 in abs. etoH, was added at 0°c 0.5N NaOH stirred at 0°c for 1hr. (12°-17°) → yellow oil



Diluted with ether Rotavap to dryness to yellow oil, diluted with ether Lots of solids came out of sol'n, washed with ether, decant out ether dried yellow solids under vac.
Wt: 178.8 mg (1206-179-30)
nmr, ir, uv, micro m.p.: Shrinked at 187°c, does not melt up to 210°c

Rotavap ether layer to dryness to yellow solids (1206-179-34)

Theory: 197.2 mg (90.6%)

10-6-87 submitted for (2mg) Solubility test

(6-7-87) Solubility = 0.0809 mg/ml

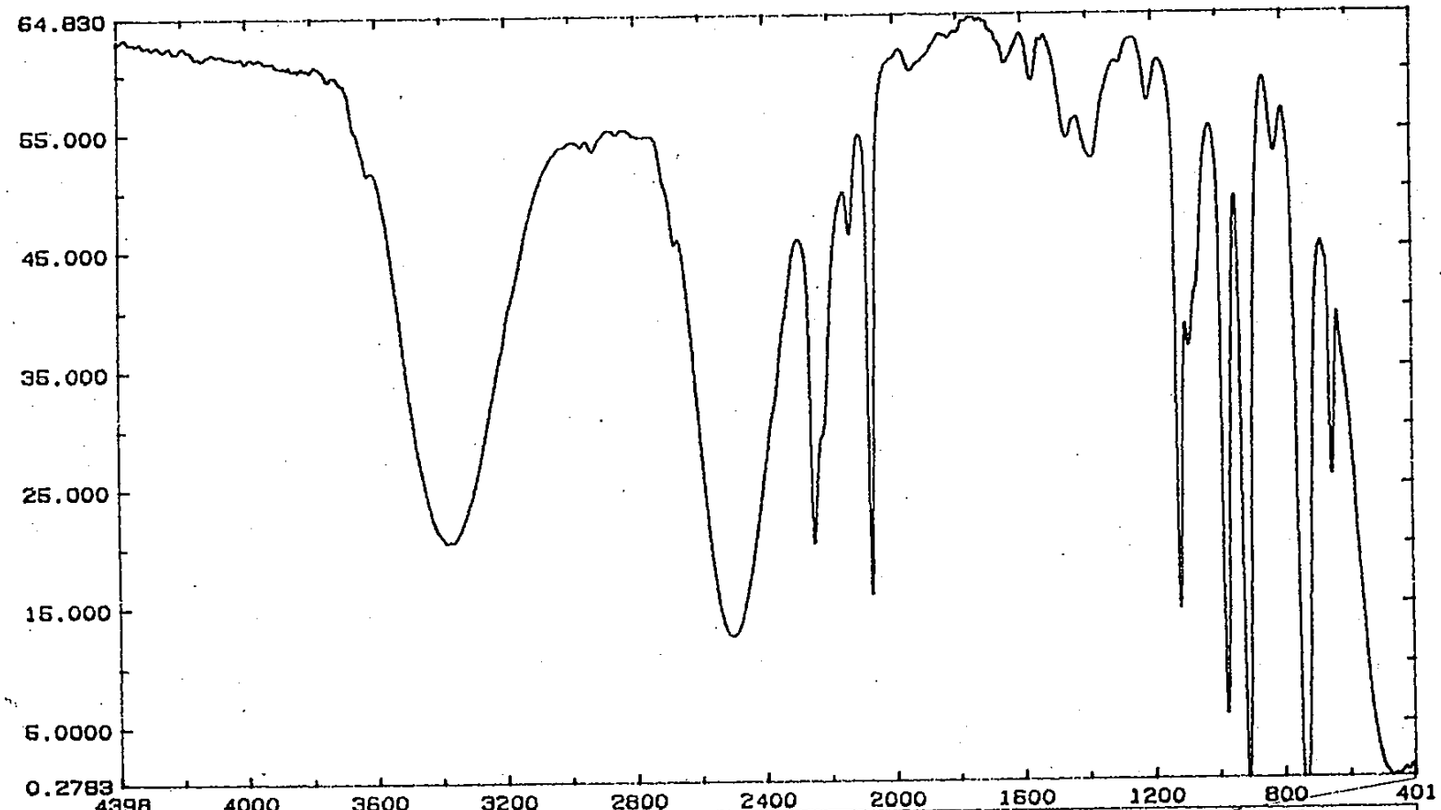
	C	H	N	O		
Calc.						
Found						

Performed by- Ray Patel 8-5-87

Witness- A Perez

Cont'd to-

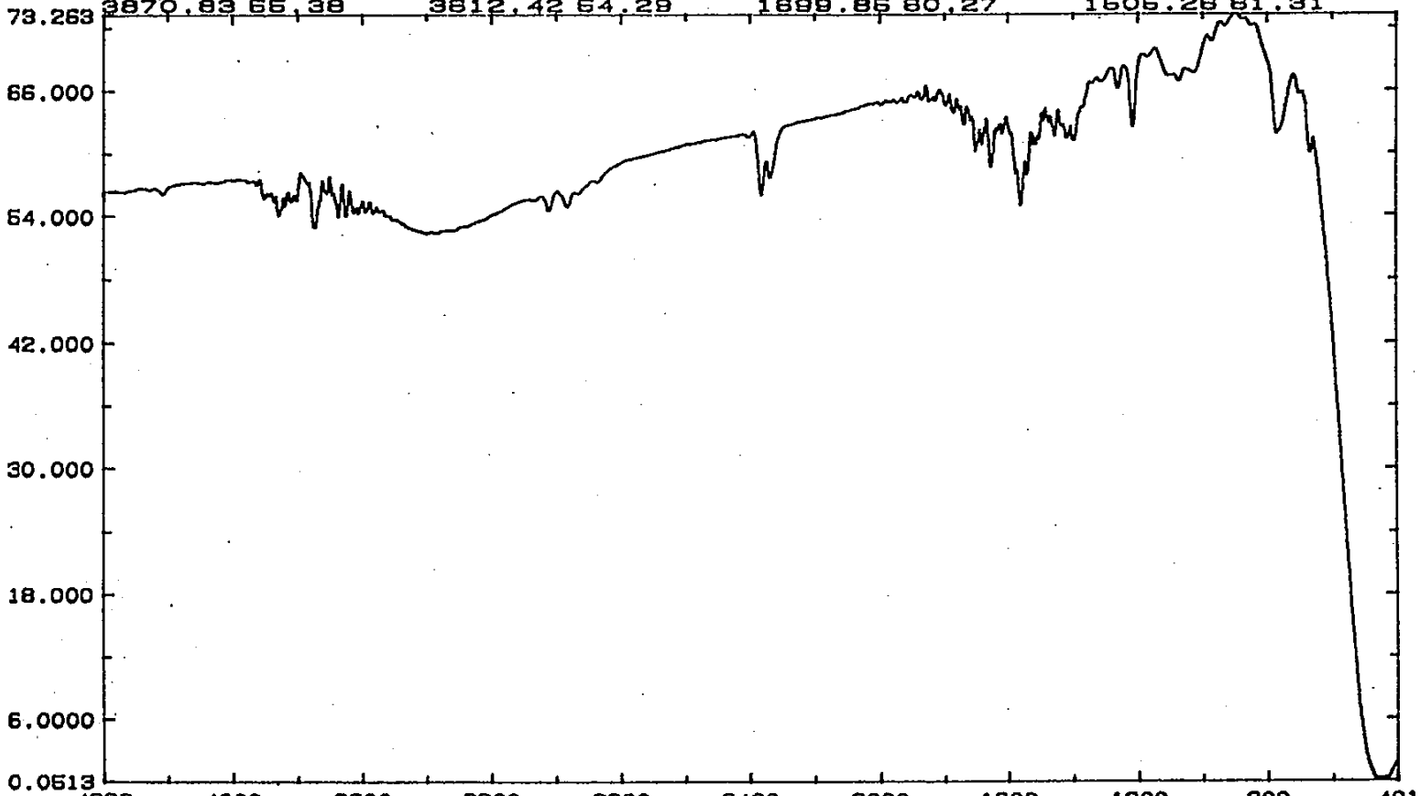
CM-1	%T	CM-1	%T	CM-1	%T
468.45	0.45				
739.00	-0.98				
910.64	-0.24				
976.43	5.67				
2601.90	12.69				



FILE NAME : 1206-179-30 1093	GAIN : 2	ANALECT FX-6160
#SCANS : 64	DET : JGS	ORD : %T
#BKG : 64	RES : 4 CM-1	ABSC: WAVENUMBER
APOD : HAPP-GENZEL	DATE : 07/31/87	TIME: 14:09:38
COMMENT : thin film;wattanasin,r.j. lab#358 fm		

294

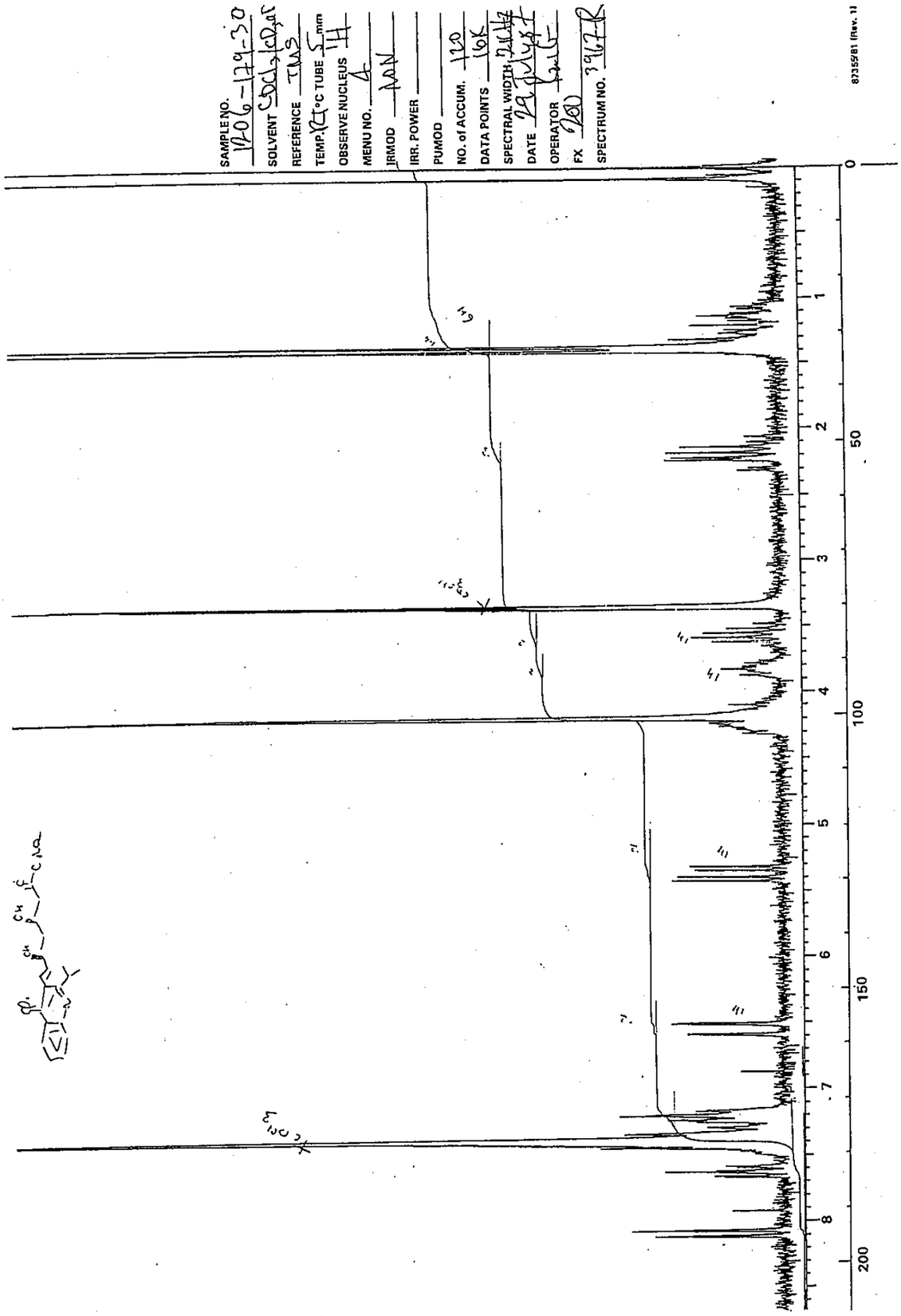
CM-1	%T	CM-1	%T	CM-1	%T	CM-1	%T
3825.31	54.37	1715.99	63.13	1513.88	60.82	448.81	0.23
3650.43	54.05	1735.68	62.83	1523.11	60.75	666.02	60.02
3672.89	53.98	2336.04	57.66	1541.93	57.93	768.38	61.89
3709.60	56.21	2362.44	56.01	1660.71	54.99	1215.79	62.57
3745.57	52.96	2864.39	54.94	1675.14	57.99	1386.97	61.26
3801.68	55.53	3020.54	54.58	1618.32	61.95	1419.62	61.43
3817.22	55.39	3387.92	52.35	1652.48	58.89	1456.21	61.67
3837.12	54.98	3585.71	54.32	1889.61	82.00	1473.60	62.98
3855.46	54.10	3589.90	54.44	1678.17	60.85	1490.37	63.16
3870.83	55.38	3812.42	54.29	1699.85	60.27	1505.28	61.31



FILE NAME : 1206-179-30/1085		GAIN : 2	ANALECT FX-6160
#SCANS : 64		DET : TGS	ORD : %T
#BKG : 64		RES : 4 CM-1	ABSC: WAVENUMBER
APOD : HAPP-GENZEL		DATE : 07/30/87	TIME: 12:27:50
COMMENT : thin film wattanasin/r.p.lab#358 fm			

Small Sample

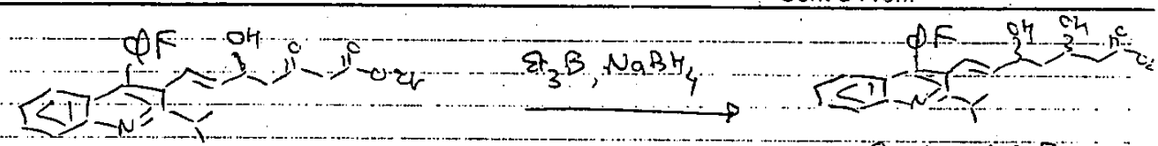
295


SAMPLE NO. 1206-179-30
SOLVENT CDCl3/CD3OD
REFERENCE TMS
TEMP. °C TUBE 5mm
OBSERVE NUCLEUS 1H
MENU NO. 4
IRMOD NN
IRR. POWER
PUMOD
NO. of ACCUM. 120
DATA POINTS 16K
SPECTRAL WIDTH 2.5kHz
DATE 29 JUL 97
OPERATOR
FX 270
SPECTRUM NO. 9717-12
8235/81 (Rev. 1)
0
1
2
3
4
5
6
7
8
50
100
150
200

190 Title-

Date 8-10-87 Proj.

Cont'd From-



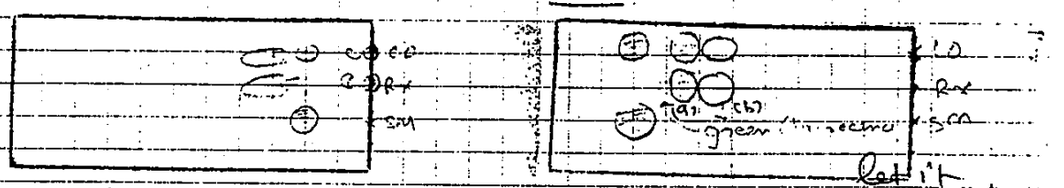
449 $C_{27}H_{20}NO_4F$ 451

449 1206-187-14 = 400 mg (0.890868 5 mmole)
1M Et_3B / THF = 133.6 ml (1.336302 mmole) 1.5 eqv.
dry THF = 5 ml
HPLC grade CH_3OH = 1.25 ml
37.8 $NaBH_4$ = 50.5 mg (1.336302 mmole) 1.5 eqv.

Ref: 1206-176

To 1206-187-14 in THF & MeOH was added 1M Et_3B/THF, stirred at r.t. for 1hr (yellow homogeneous) Cooled to -78°C, added 51 mg $NaBH_4$ stirred at -78°C for (12 hr T -3°)

after



let it

quenched with 2.5 ml AcOH added EtOAc (warmed up to r.t., extracted with EtOAc washed with Satd. $NaHCO_3$, H_2O, brine, dried, filtered washed, added MeOH 8-11-87 evaporated to dryness to give yellow oil washed with 5% MeOH gave yellow oil = 414 mg (1206-190-35) mg mmt=45
8-12-87 Flash column (80% Et_2O/Pet) gave mix of (a) & (b) : again seperated on flash (20% acetone/pet ether) gave mmt=452
yellow-orange oil (a) = 228 mg (1206-190-38) nmr, ir, ms, exact mass
green fluorescine (b) = 139.2 mg (1206-190-39) nmr, ms

Dried (a) over high vac gave 206.6 mg solid to oil (1206-190-
nmr ^{13}C
obs. mass = 452.223566 Theory: 401.78 mg (51.4%)
Calc = 452.22371

Performed by- Rajeshvari D. Patel 9-18-87

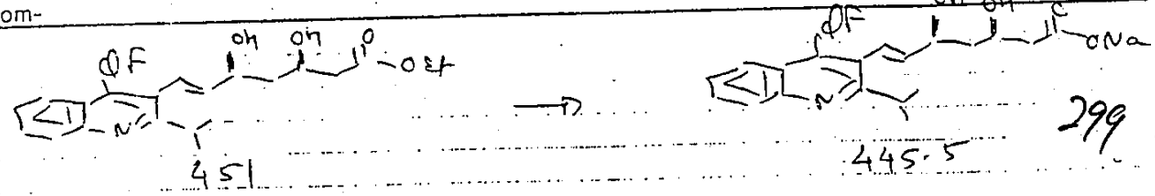
Witness- L Perez

Cont'd to-

CM-1 %T
106.86
103.00
91.000
79.000
67.000
55.000
43.000
30.933
4000 3600 3300 3000 2700 2400 2100 1800 1500 1200 900 600
FILE NAME : 1206-190-38 1176
#SCANS : 16
#BKG : 64
APOD : HAPP-GENZEL
COMMENT : CAST FILM(CDCL3) WATTANASIN/R.P.LAB#358
GAIN : 2
DET : TGS
RES : 4 CM-1
DATE : 08/20/87
ANALECT FX-6260
ORD : %T
ABSC: WAVENUMBER
TIME: 08:37:25

Date 8-25-87 Proj. Title-

Cont'd From-

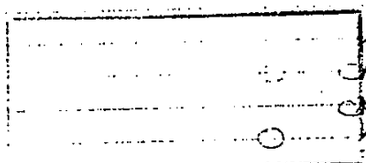


451 445.5 299

451 1206-190-41 = 100mg (0.221294 mmole)
1N NaOH = 217.3 ml (0.217294 mmole) 98%
abs. eton = 3ml + 2ml

Ref: 1206-179

To 1206-190-41 in abs. eton at 0°C with stirring was added dropwise 1N NaOH. The mix was stirred at 0°C (TLC 3c 3c) → yellow oil



Diluted with ether, rotavap to dryness to yellow oil added ether ppts (yellow) came out filtered, washed, dried gave 86.4 mg yellow solid (1206-201- nmr, MS, mixmp → not too good BL MH+=445)

Theory: 98.7 mg (87.5%)

Doesn't melt up to 225° C

10-6-87 Submitted for solubility study (20 mg) to minzkib

10-7-87 Solubility = 0.0958 mg/ml

Performed by- Roy Patel 9-1-87

Witness- L Perez

Cont'd to-

300
1206-201-30 / CD30D / 43776
BRUKER
HFRI 4377
DATE 28-8-87
SF 500.137
SY 83.0
D1 10790.527
SI 65536
TD 65536
SW 10000.000
HZ/PT .305
PW 6.6
RD 0.0
AQ 3.277
RG 64
NS 197
TE 297
FM 12500
D2 5142.005
DP 63L PO
LB -.300
GB .300
CX 37.50
CY 0.0
F1 9.540P
F2 .165P
HZ/CM 125.033
PPM/CM .250
SR 7393.06
PPM

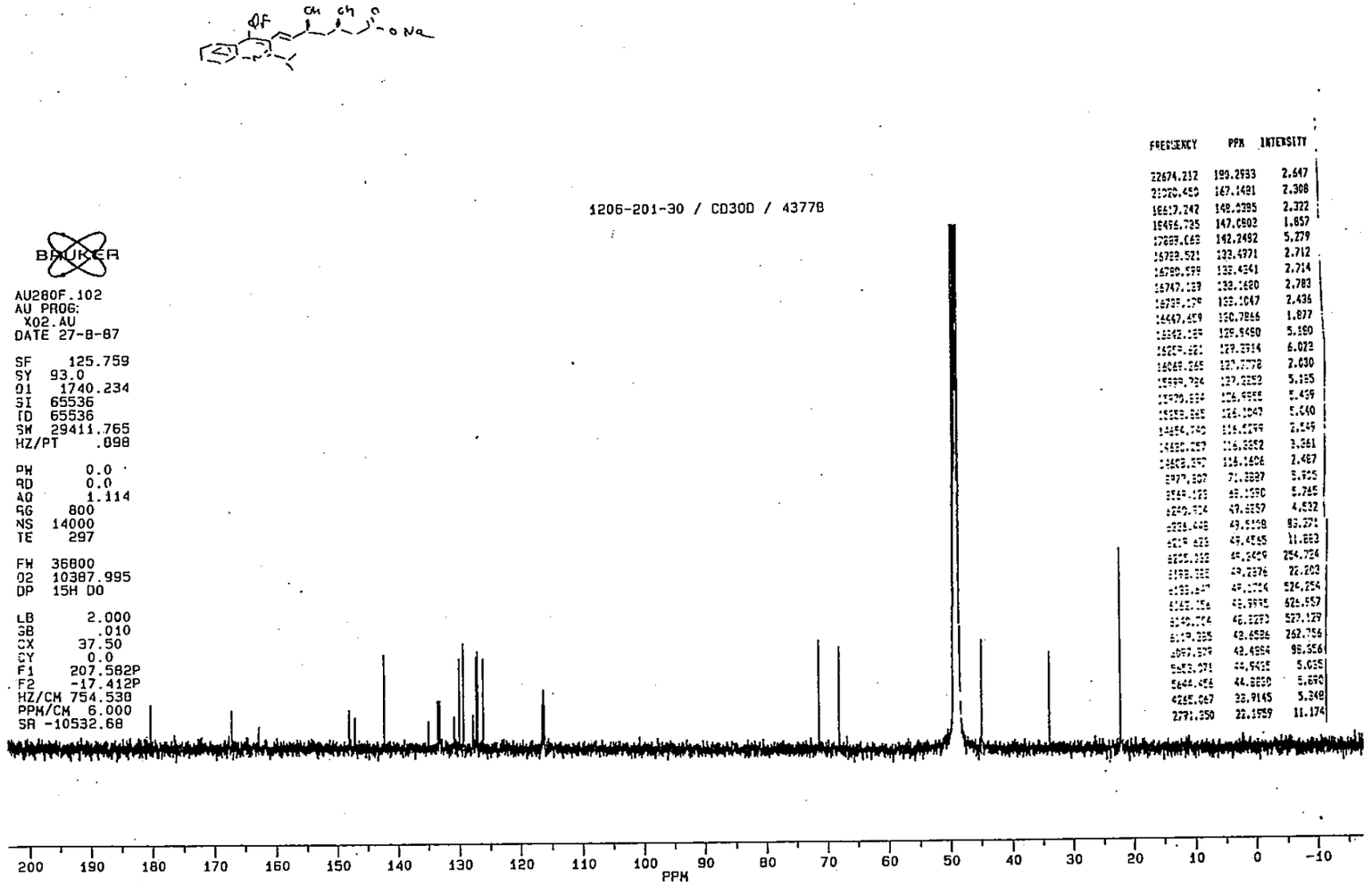
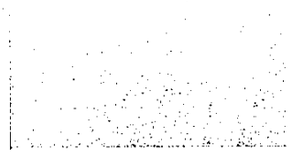
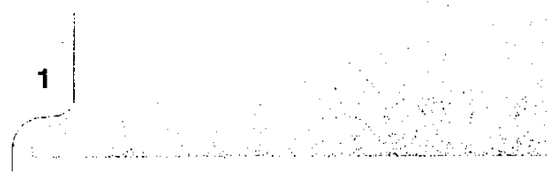
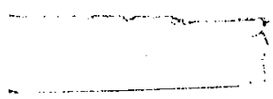


Exhibit G




1



	SAMPLE #	BOOK #	DATE
	1351	1040-237-23	MAY 25 1984
	1352	977-228-17	MAY 25 1984
	1353	1040-238-24	MAY 25 1984
OR	1354	1054-246-24	MAY 25 1984
	1355	1065-12-32	MAY 25 1984
	1356	1066-45-5	MAY 29 1984
	1357	1060-143-26	MAY 29 1984
	1358	1064-89-21	MAY 29 1984
	1359	1049-232-37	MAY 29 1984
	1360	978-171-37	MAY 29 1984
	1361	1023-292-38	MAY 29 1984
	1362	1040-239-36	MAY 29 1984
	1363	1067-40-31	MAY 29 1984
	1364	1017-249-37	MAY 29 1984
	1365	1061-77-12	MAY 29 1984
UV-VIS	1366	1039-246-26	MAY 30 1984
OR	1367	1054-229-38	MAY 30 1984
	1368	972-248-44	MAY 30 1984
UV-VIS	1369	1061-77-12	MAY 30 1984
	1370	1049-237-19	MAY 30 1984
	1371	1061-77-12	MAY 30 1984
	1372	1065-13-27	MAY 30 1984
	1373	1033-129-24	MAY 30 1984
	1374	1023-291-18	MAY 30 1984
	1375	1060-146-24	MAY 31 1984
	1376	1060-147-25	MAY 31 1984
	1377	1049-237-27	MAY 31 1984
	1378	1049-240-36	MAY 31 1984
OR	1379	1054-252-43	MAY 31 1984
OR	1380	1054-259-24	MAY 31 1984
OR	1381	1054-256-11	MAY 31 1984
	1382	981-258-35	MAY 31 1984
	1383	1013-228-14	MAY 31 1984
	1384	1024-233-5	MAY 31 1984
	1385	1024-231-22	MAY 31 1984
	1386	1024-231-21	MAY 31 1984
	1387	1024-231-19	MAY 31 1984
	1388	1070-19-37	MAY 31 1984
	1389	1013-226-42	MAY 31 1984
	1390	1013-226-31	MAY 31 1984
	1391	000-185-19	MAY 31 1984
	1392	1040-240-27	JUN. 1 1984
	1393	1068-014-39	JUN. 1 1984
	1394	1066-48-18	JUN. 1 1984
	1395	1048-42-27	JUN. 1 1984
	1396	1066-46-19	JUN. 1 1984
	1397	1064-92-29	JUN. 1 1984
	1398	1070-21-40	JUN. 1 1984
	1399	1023-297-5	JUN. 1 1984
	1400	1060-152-25	JUN. 4 1984

SAMPLE #	BOOK #	DATE
2001	1060-220-25	AUG. 9 . 1984
2002	1080-8-32	AUG. 9 . 1984
2003	1060-221-25	AUG. 9 . 1984
2004	1021-212-27	AUG. 9 . 1984
2005	1024-271-22	AUG. 10 1984
2006	1040-299-28	AUG. 10 1984
2007	1064-170-22	AUG. 10 1984
2008	1049-257-29	AUG. 10 1984
2009	1079-22-28	AUG. 10 1984
2010	1033-229-35	AUG. 10 1984
2011	1084-5-33	AUG. 13 1984
2012	1061-136-36	AUG. 13 1984
2013	1084-3-33	AUG. 13 1984
2014	1061-133-29	AUG. 13 1984
OR 2015	1061-136-36	AUG. 13 1984
OR 2016	1057-53-4	AUG. 13 1984
OR 2017	1057-45-38	AUG. 13 1984
OR 2018	1057-55-31	AUG. 13 1984
2019	1021-215-26	AUG. 13 1984
2020	1080-13-34	AUG. 13 1984
2021	1063-130-26	AUG. 13 1984
2022	1021-215-39	AUG. 13 1984
NV 2023	1030-138-2	AUG. 14 1984
NV 2024	1030-138-5	AUG. 14 1984
2025	1084-6-35	AUG. 14 1984
OR 2026	1057-56-30	AUG. 14 1984
2027	1079-13-42	AUG. 14 1984
2028	977-277-12	AUG. 14 1984
2029	1079-27-25	AUG. 14 1984
OR 2030	000-132-29	AUG. 15 1984
2031	1075-42-35	AUG. 15 1984
OR 2032	1055-204-35	AUG. 15 1984
OR 2033	1057-61-24	AUG. 15 1984
2034	1024-275-34	AUG. 15 1984
2035	1057-23-27	AUG. 15 1984
2036	1063-132-28	AUG. 15 1984
2037	1063-119-28	AUG. 15 1984
2038	1084-2-39	AUG. 15 1984
2039	1017-292-14	AUG. 15 1984
OR 2040	1051-16-28	AUG. 16 1984
2041	1080-18-32	AUG. 16 1984
2042	1038-221-6	AUG. 16 1984
2043	1038-223-39	AUG. 16 1984
2044	1061-138-23	AUG. 16 1984
OR 2045	998-90-18	AUG. 16 1984
OR 2046	998-90-24	AUG. 16 1984
OR 2047	998-90-21	AUG. 16 1984
2048	977-277-24	AUG. 16 1984
2049	1080-21-22	AUG. 16 1984
OR 2050	1033-229-35	AUG. 17 1984

SAMPLE #	BOOK #	DATE
2501	1062-221-24	NOV. 8 1984
OR 2502	1838-132-13	NOV. 8 1984
OR 2503	1038-130-9	NOV. 8 1984
2504	1085-53-44	NOV. 8 1984
OR 2505	1078-18-4	NOV. 8 1984
OR 2506	1078-20.09	NOV. 8 1984
2507	1079-101-25	NOV. 8 1984
2508	1085-49-30	NOV. 8 1984
2509	1079-101-28	NOV. 8 1984
2510	972-296-31	NOV. 8 1984
2511	1077-85-39	NOV. 8 1984
2512	1060-289-39	NOV. 9 1984
2513	1060-289-36	NOV. 9 1984
2514	1079-105-35	NOV. 9 1984
2515	1063-18229	NOV. 9 1984
2516	1075-107-5	NOV. 1984
2517	1075-106-43	NOV. 1984
2518	1080-89-37	NOV. 12 1984
2519	1063-183-27	NOV. 12 1984
2520	1063-184-37	NOV. 12 1984
2521	1080-86-28	NOV. 12 1984
2522	972-295-40	NOV. 12 1984
2523	1062-222-35	NOV. 12 1984
2524	1090-7-30	NOV. 12 1984
2525	1090-9-24	NOV. 12 1984

IK

SAMPLE #	BOOK #	DATE
OR 2526	993-83-12	NOV. 1 3 198
2527	1075-163-24	NOV. 1 3 198
2528	1037-300-35	NOV. 1 3 198
2529	1063-184-32	NOV. 1 3 198
2530	1075-109-36	NOV. 1 3 198
2531	1060-291-23	NOV. 1 3 1984
2532	1057-205-14	NOV. 1 3 19
2533	1060-285-30	NOV. 1 3 19
2534	1063-185-29	NOV. 1 3 19
2535	1080-94-22	NOV. 1 3 19
2536	1063-187-31	NOV. 1 4 1984
2537	1079-99-29	NOV. 1 4 198
2538	1079-107-29	NOV. 1 4 198
2539	1065-69-35	NOV. 1 4 1984
2540	1058-42-36	NOV. 1 4 198
2541	1057-213-28	NOV. 1 4 1
2542	1080-80-31	NOV. 1 4 1
2543	1063-189-18	NOV. 1 4
2544	1080-92-22	NOV. 1 5 19
2545	1063-189-25	NOV. 1 5 19
2546	1063-188-25	NOV. 1 5 19
CDcl 2547 Sgl	1059-221-8	NOV. 1 5 19
2548	1100-13-40	NOV. 1 5 19
2549	1084-41-39	NOV. 1 5 19
2550	1064-221-30	NOV. 1 5 19

304 LE

SAMPLE #	BOOK #	DATE
2551	1060-29-23	NOV. 15 1984
2552	1060-296-42	NOV. 16 1984
2553	1063-190	NOV. 16 1984
2554	1058-50-33	NOV. 16 1984
2555	1058-50-20	NOV. 16 1984
2556	1059-221-24	NOV. 16 1984
2557	1059-221-27	NOV. 16 1984
2558	1060-297-39	NOV. 16 1984
2559	1024-263-16	NOV. 16 1984
2560	1084-63-33	NOV. 19 1984
2561	1060-297-31	NOV. 16 1984
2562	1058-51-23	NOV. 19 1984
2563	1058-43-32	NOV. 16 1984
OR 2564	1037-296-30	NOV. 16 1984
2565	1080-96-28	NOV. 19 1984
2566	1061-213-3	NOV. 19 1984
2567	1062-224-38	NOV. 19 1984
2568	1080-90-Fu, S	NOV. 16 1984
2569	1063-192-29	NOV. 20 1984
2570	1063-188-30	NOV. 20 1984
2571	1063-188-32	NOV. 20 1984
2572	1075-113-3	NOV. 20 1984
2573	1060-295-36	NOV. 20 1984
2574	1084-65-33	NOV. 20 1984
2575	1034-173-27	NOV. 20 1984

SAMPLE #	BOOK #	DATE
2576	1058-58-21	NOV. 20 1984
2577	1061-212-20	NOV. 20 1984
2578	1060-299-9	NOV. 20 1984
2579	1063-193-27	NOV. 20 1984
2580	1063-194-28	NOV. 20 1984
2581	1080-95-F5-8	NOV. 21 1984
2582	1040-286-32	NOV. 21 1984
2583	1079-112-23	NOV. 21 1984
2584	1004-207-30	NOV. 21 1984
2585	972-294-37	NOV. 21 1984
2586	997-247-36	NOV. 21 1984
2587	1080-100-26	NOV. 21 1984
2588	1080-98-32	NOV. 21 1984
2589	1079-111-19	NOV. 21 1984
2590	1063-198-25	NOV. 26 1984
2591	1063-198-23	NOV. 26 1984
2592	1063-199-25	NOV. 26 1984
2593	1063-197-23	NOV. 26 1984
2594	1063-196-28	NOV. 26 1984
2595	1063-175-18	NOV. 26 1984
2596	1062-229-32	NOV. 26 1984
2597	1058-62-10	NOV. 26 1984
2598	1079-113-25	NOV. 26 1984
2599	1064-225-33	NOV. 26 1984
2600	1075-119-23	NOV. 26 1984

SAMPLE #	BOOK #	DATE
UV 1001	978-189-44	
1002	1123-3-13	MAY 3 1985
1003	1116-55-41	MAY 3 1985
1004	1126-29-34	MAY 3 1985
1005	1108-44-30	May 3-1985
1006	1108-43-44	May 3, 1985
1007	1126-30-15	MAY 6 1985
1008	1116-76-14	MAY 6 1985
1009	1092-204-34	MAY 6 1985
1010	1127-8-26	MAY 6 1985
1011	1127-7-28	MAY 6 1985
1012	1127-5-23	MAY 6 1985
1013	1053-86-31	MAY 6 1985
1014	1053-88-31	MAY 6 1985
1015	1053-83-35	MAY 6 1985
1016	1053-86-33	MAY 6 1985
UV 1017	555-146-22	MAY 6 1985
1018	1100-103-20	MAY 7 1985
UV 1019	555-146-22	MAY 1985
1020	1114-42-10	MAY 7 1985
1021	1119-59-31	MAY 8 1985
1022	1119-56-32	MAY 8 1985
1023	1119-61-31	MAY 8 1985
1024	1092-209-27	MAY 8 1985
1025	1080-285-24	MAY 8 1985

SAMPLE #	BOOK # 306	DATE
UV 1026	993-170-2	MAY 8 1985
1027	1053-90-35	MAY 8 1985
1028	1053-89-30	MAY 8 1985
1029	1108-47-44	MAY 8 1985
OR 1030	1068-138-35	MAY 8 1985
1031	1092-210-35	MAY 9 1985
1032	1092-213-7	MAY 9 1985
1033	SC-3-30	MAY 9 1985
1034	1080-281-41	MAY 9 1985
1035	998-154-36	MAY 9 1985
1036	62-562 Step 2 By-prod	MAY 9 1985
1037	1080-282-22	MAY 9 1985
OR 1038	1068-138-35	MAY 9 1985
1039	1126-35-35	MAY 10 1985
1040	SC-4-25	MAY 10 1985
1041	1126-37-33	MAY 10 1985
1042	1095-43-16	MAY 10 1985
1043	1100-107-21	MAY 10 1985
1044	1092-214-30	MAY 13 1985
1045	1123-41-21	MAY 13 1985
1046	1079-264-28	MAY 13 1985
1047	1075-271-41	MAY 13 1985
1048	1123-42-38	[illegible]
1049	1127-17-30	MAY 13 1985
R Active 1050	1125-58-42	MAY 13 1985

SAMPLE #	BOOK #	DATE
1051	602-320 NA	MAY 13 1985
OR 1052	1085-142-29	MAY 14 1985
OR 1053	1080-301	MAY 14 1985
1054	1127-17-32	MAY 14 1985
1055	1080-290-26	MAY 14 1985
1056	1080-289-22	MAY 14 1985
1057	1080-301	MAY 14 1985
1058	1092-215-33	MAY 14 1985
1059	1123-43-40	MAY 14 1985
1060	1116-105-14	MAY 14 1985
1061	969-266-24	MAY 14 1985
1062	1092-216-37	MAY 14 1985
1063	1112-38-27	MAY 14 1985
1064	1119-65-36	MAY 15 1985
OR 1065	969-266-19	MAY 15 1985
1066	969-266-19	MAY 15 1985
1067	1110-134-19	MAY 15 1985
1068	1108-48-40	MAY 15 1985
1069	1119-70-29	MAY 15 1985
1070	1110-130-32	MAY 15 1985
UV 1071	1128-4-12	MAY 15 1985
UV 1072	1128-4-10	MAY 15 1985
UV 1073	1128-4-9	MAY 15 1985
1074	1092-223-30	MAY 15 1985
1075	SC-7-25	MAY 15 1985
1076	SC-7-28	MAY 15 1985
1077	1092-217-35	MAY 15 1985
1078	1080-291-F6	MAY 16 1985
1079	1119-68-30	MAY 16 1985
1080	1127-24-27	MAY 16 1985
1081	998-155-40	MAY 16 1985
1082	1058-215-44	MAY 16 1985
1083	1080-292-32	MAY 16 1985
1084	1080-287-22	MAY 16 1985
1085	1085-144-21	MAY 16 1985
1086	1085-144-24	MAY 16 1985
UV 1087	1045-295-14	MAY 16 1985
1088	1080-294-22	MAY 17 1985
1089	1092-227-31	MAY 17 1985
1090	1092-218-24	MAY 17 1985
1091	1100-109-14	MAY 17 1985
1092	1100-109-16	MAY 17 1985
1093	1119-71-28	MAY 17 1985
1094	1127-11-34	MAY 17 1985
1095	1127-11-37	MAY 17 1985
1096	1095-48-24	MAY 17 1985
1097	1058-215-31	MAY 17 1985
1098	1056-111-19	MAY 17 1985
UV 1099	1045-201-14	MAY 17 1985
1100	1110-141-25	MAY 17 1985

SAMPLE #	BOOK #	DATE
851	1190-266-39	MAY 26 1987
852	1205-28-35	MAY 26 1987
853	1191-253-18	MAY 26 1987
854	000-122-25	MAY 27 1987
855	000-124-29	MAY 27 1987
856	000-104-27	MAY 27 1987
857	000-104-29	MAY 27 1987
858	1224-29-40	MAY 27 1987
859	121-119-30	MAY 27 1987
860	1230-114-31	MAY 27 1987
OR 861	1230-111-24	MAY 28 1987
862	121-104-22	MAY 28 1987
863	117-104-24	MAY 28 1987
864	1230-115-32	MAY 28 1987
865	121-108-32	MAY 28 1987
866	1205-36-36	MAY 28 1987
867	121-122-21	MAY 28 1987
868	1211-120-21	MAY 28 1987
OR 869	1138-193-06	MAY 29 1987
870	205-39.7 EPI-51-B1	MAY 29 1987
871	1230-120-26	MAY 29 1987
872	1230-121-28	MAY 29 1987
873	1191-119-19	MAY 29 1987
OR 874	1230-117-34	MAY 29 1987
875	000-126-28	JUN 1 1987

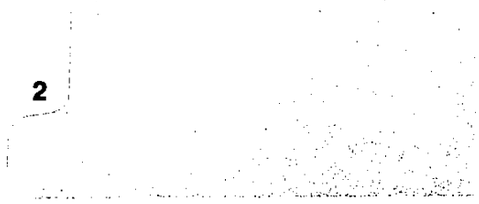
SAMPLE #	BOOK #	DATE
876	000-126-24	JUN. 1 1987
877	000-126-26	JUN. 1 1987
878	1161-234-8	JUN. 1 1987
879	1190-273-37	JUN. 1 1987
880	1205-33-20	JUN. 1 1987
881	1206-128-39	JUN. 2 1987
882	1206-129-18	JUN. 2 1987
883	1216-37-39	JUN. 2 1987
884	1190-272-41	JUN. 2 1987
885	000-119-20	JUN. 2 1987
886	000-111-10	JUN. 2 1987
887	1206-132-42	JUN. 2 1987
888	1211-122-28	JUN. 2 1987
889	JS-6 SC	JUN. 3 1987
890	1211-124-23	JUN. 3 1987
891	1224-30-1	JUN. 3 1987
892	1190-272-41	JUN. 4 1987
893	1208-118-22	JUN. 5 1987
894	000-127-28	JUN. 5 1987
895	1205-43-27	JUN. 5 1987
896	1133-198-28	JUN. 5 1987
897	1133-173-35	JUN. 5 1987
898	1190-270-33	JUN. 5 1987
899	1206-130-27	JUN. 5 1987
900	1206-124-26	JUN. 6 1987

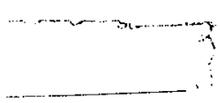
SAMPLE #	BOOK #	DATE
901	1206-133-38	JUN. 5 1987
902	1216-53	JUN. 8 1987
903	1216-44	JUN. 8 1987
904	1215-102-27	JUN. 8 1987
905	1239-2-21	JUN. 8 1987
906	1208-132-16	JUN. 9 1987
907	1230-129-26	
908	1215-115-25	JUN. 9 1987
909	1190-278-29	JUN. 10 1987
910	1206-131-43	JUN. 10 1987
911	JS-6833	JUN. 10 1987
912	JS-682B	JUN. 10 1987
913	JS-684A	JUN. 10 1987
914	1225-5-6	JUN. 10 1987
915	1230-122-30	JUN. 10 1987
916	1197-92-37	JUN. 10 1987
917	000-130-25	JUN. 11 1987
918	000-130-27	JUN. 11 1987
919	1208-133-13	JUN. 11 1987
920	1208-134-19	JUN. 11 1987
921	1216-57-28	JUN. 11 1987
922	1206-137-31	JUN. 12 1987
923	1208-135-15	JUN. 12 1987
924	1230-131-28	JUN. 15 1987
RA 925	1172-29-26	JUN. 16 1987

SAMPLE #	BOOK # 309	DATE
STD 926	STD 1172-29	JUN. 16 1987
927	1208-137-17	JUN. 16 1987
928	1216-59-27	JUN. 16 1987
929	1216-59-31	JUN. 16 1987
930	1183-234-13	JUN. 16 1987
931	1206-141-31	JUN. 17 1987
932	1216-55	JUN. 17 1987
933	1216-63-31	JUN. 17 1987
934	1225-15-29	JUN. 17 1987
935	1225-13-11	JUN. 17 1987
936	1215-127-30	JUN. 17 1987
937	1216-58-37	JUN. 18 1987
938	1190-289-32	JUN. 18 1987
939	1211-130-28	JUN. 18 1987
940	1216-62-30	JUN. 19 1987
941	1195-115-35	JUN. 22 1987
942	1224-38-35	JUN. 22 1987
OR 943	1230-135-f14-3	JUN. 24 1987
OR 944	1230-135-f3	JUN. 24 1987
945	1224-36-40	JUN. 25 1987
OR 946	1206-135-32	JUN. 25 1987
OR 947	1230-148-26	JUN. 29 1987
OR 948	1206-149-39	JUN. 30 1987
949	1215-130-21	JUL. 13 1987
950	1142-106-15	JUL. 13 1987

SAMPLE #	BOOK #	DATE	SAMPLE #	BOOK # 310	DATE
1001	1190-297-27	JUL 15 1987	1026	1230-158-①	JUL 21 1987
1002	1216-73-33	JUL 15 1987	1027	1230-163-30	JUL 21 1987
1003	1216-72-35	JUL 15 1987	1028	1230-159-37	JUL 21 1987
1004	000-134-29	JUL 15 1987	1029	1230-168-30	JUL 21 1987
1005	000-134-27	JUL 15 1987	1030	1230-165-26	JUL 21 1987
1006	1230-157-27	JUL 15 1987	1031	1230-159-28	JUL 21 1987
1007	1206-153-34	JUL 15 1987	1032	1206-160-39	JUL 21 1987
OR 1008	1230-158①	JUL 15 1987	1033	1206-157-39	JUL 21 1987
1009	000-137-29	JUL 16 1987	1034	1206-154-40	JUL 21 1987
1010	000-135-21	JUL 16 1987	1035	1206-158-41	JUL 21 1987
1011	1224-48-15	JUL 16 1987	1036	1206-154-40	JUL 21 1987
1012	1224-51-35	JUL 16 1987	1037	1206-158-41	JUL 21 1987
KBIR 1013	HG-61-26	JUL 17 1987	1038	1225-38-17	JUL 22 1987
CHCl2 1014	HG-61-26	JUL 17 1987	OR 1039	1215-157-28	JUL 22 1987
KBI2 1015	HG-61-29	JUL 17 1987	1040	1216-783/	JUL 22 1987
CHCl2 1016	HG-61-29	JUL 17 1987	1041	1216-77-26	JUL 22 1987
1017	1225-35-29	JUL 20 1987	1042	000-136-25	JUL 22 1987
OR 1018	1197-132-38	JUL 20 1987	1043	000-140-28	JUL 22 1987
OR 1019	1197-141-24	JUL 20 1987	1044	000-140-26	JUL 22 1987
1020	1225-34-37	JUL 20 1987	1045	000-141-27	JUL 22 1987
1021	1205-55-30	JUL 20 1987	1046	1205-59-17	JUL 22 1987
1022	1225-31-9	JUL 20 1987	1047	1205-59-15	JUL 22 1987
RA 1023	1204127-28R2	JUL 20 1987	1048	1205-59-13	JUL 22 1987
STD 1024	Lot 4 To 23 1204-137-36	JUL 20 1987	1049	1205-59-11	JUL 22 1987
OR 1025	1197-143-10	JUL 20 1987	1050	1230-172-2	JUL 23 1987

SAMPLE #	BOOK #	DATE
1051	1230-171(2)	JUL 23 1987
1052	1206-175-4	JUL 23 1987
1053	1215-158-36	JUL 23 1987
1054	HG-62-19	JUL 23 1987
1055	HG-62-21	JUL 23 1987
1056	000-142-23	JUL 23 1987
1057	HG-63-24	JUL 23 1987
1058	1211-163-25	JUL 23 1987
1059	1206-173-39	JUL 24 1987
OR 1060	1138-229-15	JUL 24 1987
1061	HG-64-28	JUL 24 1987
1062	1215-159-38	JUL 24 1987
OR 1063	1138-229-15	JUL 24 1987
1064	1245-6-39	JUL 24 1987
1065	1230-174-34	JUL 24 1987
1066	1230-175-25	JUL 24 1987
OR 1067	1230-176-22	JUL 27 1987
1068	1152-43-27	JUL 27 1987
1069	1216-80	JUL 27 1987
1070	1237-101-20	JUL 27 1987
1071	1237-100-24	JUL 27 1987
1072	1211-166-20	JUL 27 1987
OR 1073	1138-232-21	JUL 27 1987
1074	1224-55-39	JUL 28 1987
1075	1215-166-27	JUL 28 1987
OR 1076	1138-234-34	JUL 28 1987
1077	1138-232-21	JUL 28 1987
1078	1224-53-20	JUL 28 1987
1079	1205-63-36	JUL 28 1987
1080	HG-65-28	JUL 28 1987
OR 1081	1138-234-34	JUL 29 1987
1082	1205-63-38	JUL 29 1987
1083	1245-8-35	JUL 29 1987
1084	1206-166-30	JUL 30 1987
1085	1206-179-30	JUL 30 1987
1086	1206-177-33	JUL 30 1987
1087	1206-176-41	JUL 30 1987
1088	1197-149-8	JUL 30 1987
1089	1225-40-41	JUL 30 1987
1090	1208-178-19	JUL 31 1987
1091	1206-176-43	JUL 31 1987
1092	1206-180-39	JUL 31 1987
1093	1206-179-30	JUL 31 1987
1094	1215-159-38	JUL 31 1987
OR 1095	1181-232-38	AUG. 3 1987
OR 1096	1181-228-38	AUG. 3 1987
1097	1215-168-28	AUG. 3 1987
1098	1215-163-21	AUG. 3 1987
1099	1224-63-34	AUG. 3 1987
OR 1100	1225-45-34	AUG. 3 1987


2



E #	BOOK #	DATE	SAMPLE #	BOOK #	DATE
501	1195-122-38	JUL. 01 1987	526	000-105-23	JUL. 16 1987
502	1219-63-20	JUL. 06 1987	527	1213-123-30	JUL. 17 1987
503	1219-61-27	JUL. 06 1987	528	1169-263-31	JUL. 17 1987
504	1203-101-35	[illegible] 1987	529	1169-265-34	[illegible] 1987
505	1190-279-29	JUL. 06 1987	UV 530	1225-34-37	[illegible] 1987
506	1142-163-6	JUL. 06 1987	UV 531	1225-25-29	JUL. 17 1987
507	1224-45-38	JUL. 06 1987	532	1204-165-10	JUL. 22 1987
508	1206-146-37	JUL. 07 1987	533	1219-72-30	JUL. 22 1987
509	1169-258-30	JUL. 07 1987	534	1219-68-24	JUL. 22 1987
510	1214-41-42	JUL. 07 1987	535	1219-67-32	JUL. 22 1987
511	1214-41-22	JUL. 07 1987	536	1213-146-26	JUL. 22 1987
512	1203-104-39	JUL. 07 1987	537	1213-131-25	JUL. 22 1987
513	1169-259-26	[illegible]	UV 538	1204-130-2	JUL. 22 1987
514	1190-297-27	JUL. 08 1987	UV 539	1120-192-12	JUL. 22 1987
515	1224-48-15	JUL. 08 1987	540	1245-1-22	JUL. 22 1987
516	1183-251-32	JUL. 08 1987	541	1203-115-35	JUL. 23 1987
517	1216-73-34	JUL. 08 1987	542	H6-62-19	JUL. 23 1987
518	1206-153-31	JUL. 08 1987	543	H6-62-21	JUL. 23
519	1230-159-28	JUL. 09 1987	544	1230-171-(2)	JUL. 23 1987
520	000-135-21	JUL. 10 1987	545	1206-175-4	JUL. 23 1987
521	1230-159-37	JUL. 13 1987	546	1230-172-20	JUL. 23 1987
522	1223-46-29	JUL. 14 1987	UV 547	1228-111-11	JUL. 23 1987
523	1230-158-(1)	JUL. 14 1987	548	H6-63-24	JUL. 24 1987
524	1206-158-41	JUL. 15 1987	549	1223-81-22	JUL. 24 1987
525	1224-51-35	[illegible] 1987	550	000-142-23	[illegible] 1987

T #	BOOK #	DATE	SAMPLE #	BOOK #	DATE
551	H6-64-28	JUL. 24 1987	576	1169-275-5	AUG. 03 1987
552	1206-173-39	JUL. 24 1987	577	1213-153-29	AUG. 03 1987
553	1230-175-25	JUL. 24 1987	578	1204-178-7	AUG. 03 1987
554	1010-63-2	JUL. 27 1987	579	1204-182-9	AUG. 03 1987
555	1219-77-20	JUL. 27 1987	UV 580	1157-290-12	AUG. 04 1987
556	1213-153-23	JUL. 28 1987	581	1224-63-34	AUG. 05 1987
557	H6-65-28	JUL. 28 1987	582	1181-234-38	AUG. 05 1987
558	1206-177-33	JUL. 28 1987	583	1181-233-38	AUG. 05 1987
559	1207-1222	JUL. 28 1987	584	1213-121-34	AUG. 05 1987
560	1216-166-30	JUL. 28 1987	585	1213-153-32	AUG. 05 1987
561	1224-55-39	JUL. 28 1987	586	1206-185-31	AUG. 05 1987
562	1213-149-27	JUL. 28 1987	587	1152-55-22	AUG. 05 1987
563	1206-179-30	JUL. 28 1987	588	1169-276-22	AUG. 06 1987
564	1219-79-24	JUL. 28 1987	589	1247-27-35	AUG. 06 1987
565	1206-180-39	JUL. 28 1987	590	1158-254-34	AUG. 07 1987
566	63-370	JUL. 29 1987	591	1206-187-15	AUG. 10 1987
567	1239-40-12	JUL. 30 1987	592	1224-62-40	AUG. 10 1987
568	1239-40-20	JUL. 30 1987	593	000-147-27	AUG. 10 1987
569	1239-40-26	JUL. 30 1987	594	1215-166-27	AUG. 11 1987
570	1169-268-41	JUL. 31 1987	595	1211-172-26	AUG. 11 1987
571	000-144-22	JUL. 31 1987	596	1245-15-35	AUG. 11 1987
572	1224-59-16	JUL. 31 1987	597	1213-153-39	AUG. 11 1987
573	045-11-31	JUL. 31 1987	598	1181-242-36	AUG. 11 1987
574	1181-228-38	AUG. 03 1987	599	1245-16-40	AUG. 12 1987
575	1181-232-38	AUG. 03 1987	600	1205-68-22	AUG. 12 1987

11/1200

BOOK #	DATE	SAMPLE #	BOOK #	DATE
1245-21-37	AUG. 12 1987	626	1245-21-42	AUG. 21 1987
000-149-21	AUG. 12 1987	627	1245-40-39	AUG. 21 1987
Povidone RTA 378	AUG. 13 1987	628	1245-21-42	AUG. 21 1987
1213-171-27	AUG. 13 1987	629	1220-76-41	AUG. 24 1987
000-150-20	AUG. 13 1987	630	1219-102-19	AUG. 24 1987
1205-25-37	AUG. 13 1987	631	Povidone RTA 378	AUG. 25 1987
1158-256-29	AUG. 13 1987	632	Crospovidone RTA 370	AUG. 25 1987
824-64-35	AUG. 14 1987	633	1152-72-140	AUG. 25 1987
1158-259-15	AUG. 14 1987	634	1206-201-30	AUG. 26 1987
1237-117-9	AUG. 14 1987	635	000-152-9	AUG. 27 1987
1237-116-7	AUG. 17 1987	636	000-144-29	AUG. 27 1987
219-94-25	AUG. 17 1987	pKa 637	1158-173-10	AUG. 27 1987
945-299-35	AUG. 18 1987	638	1169-287-38	AUG. 28 1987
996-94-18	AUG. 18 1987	639	000-151-29	AUG. 28 1987
1141-295-39	AUG. 18 1987	640	1211-177-31	AUG. 28 1987
1152-65-38	AUG. 18 1987	641	1211-189-23	AUG. 28 1987
000-151-23	AUG. 18 1987	642	1169-290-22	AUG. 28 1987
000-151-24	AUG. 18 1987	643	010-6-25	AUG. 31 1987
1169-279-30	AUG. 19 1987	644	1237-134-25	AUG. 31 1987
1152-69-8	AUG. 19 1987	UV 645	1228-84-32	AUG. 31 1987
1219-100-24	AUG. 20 1987	646	1245-57-40	SEP. 01 1987
1219-100-31	AUG. 20 1987	647	1213-193-22	SEP. 02 1987
1-68-35	AUG. 21 1987	648	62-320 Lot 5	SEP. 03 1987
203-127-32	AUG. 21 1987	649	62-320 Lot 11	SEP. 03 1987
203-122-34	AUG. 21 1987		1219-112-16	SEP. 03 1987

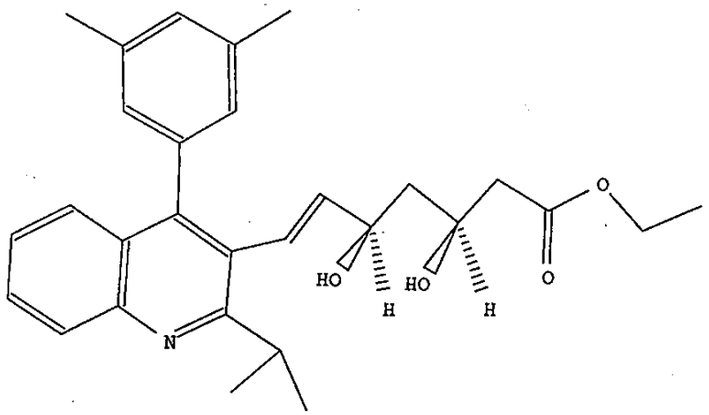
650

LOT #	BOOK #	DATE
801	844-17	MAY 29 1984
802	844-19	MAY 29 1984
803	1040-221-24	MAY 29 1984
804	972-248-44	MAY 30 1984
805	1013-228-4	MAY 30 1984
806	1017-249-34	MAY 30 1984
807	1017-254-6	MAY 30 1984
808	1033-179-24	MAY 30 1984
809	1036-124-39	MAY 30 1984
810	1036-124-38	MAY 30 1984
811	1036-124-37	MAY 30 1984
812	000-123-27	MAY 31 1984
813	1049-237-27	MAY 31 1984
814	981-255-20	MAY 31 1984
815	1024-231-22	MAY 31 1984
816	981-258-35	MAY 31 1984
817	1060-141-30	MAY 31 1984
818	1070-19-37	MAY 31 1984
819	1040-240-27	MAY 31 1984
820	1033-178-37	MAY 31 1984
821	1013-228-16	MAY 31 1984
822	844-19	MAY 31 1984
823	844-14	MAY 31 1984
824	1040-192-37	MAY 31 1984
825	1040-146-24	MAY 31 1984
826	1060-152-38	MAY 1984
827	1013-230-38	JUN.
828	1064-96-21	JUN. 4 1984
829	1067-51-2	JUN. 6 1984
830	990-286-39	JUN. 6 1984
831	981-261-25	JUN. 6 1984
832	981-259-21	JUN. 6 1984
833	1049-246-28	JUN. 6 1984
834	921-220-42	JUN. 7 1984
835	945-239-28	JUN. 7 1984
836	1070-24-35	JUN. 7 1984
837	1040-244-38	JUN. 7 1984
838	978-176-40	JUN. 7 1984
839	1013-231-04	JUN. 8 1984
840	1024-239-04	JUN. 8 1984
841	604-59-15	JUN. 8 1984
842	1064-97-24	JUN. 8 1984
843	978-175-41	JUN. 8 1984
844	1013-231-24	JUN. 8 1984
845	1062-100-15	JUN. 8 1984
846	1067-54-15	JUN. 8 1984
847	1040-251-29	JUN. 8 1984
848	1040-250-31	JUN. 11 1984
849	1070-26-35	JUN. 11 1984
850	1060-160-7	JUN. 11 1984

3/5

Exhibit H

INT.REG.NO	SAH.NO	SALTCODE	CHEM.NO	SUBMITTED	UNIT	CHEMISTS	DISCL
25496	SAH-063366		1079-111-19	11-26-84	KATH	KATHAWALA WATTANASIN	299-84



KNOWN? N | L&D. NO

MP

BP

PRESSURE

OTHER.PHYS.DATA

OIL

SOL. CODE D OR E OR C

DETAILS DMA OR EtOH OR CMC SUSPENSION

SCREENS

CSI

NOTES SEE LONGNOTE

KEEP REFRIGERATE
ERYTHRO:THREO=95:5

FORM DESIGNED BY BARCZA

C29 H35 N O4	MW 461.606	SAH

COMPARE WITH 58-512

MADE. FROM. REGNO 25495.0 A)ETHYLACETOACETATE/LDA B)Et3B/NaBH4 C)CH3OH

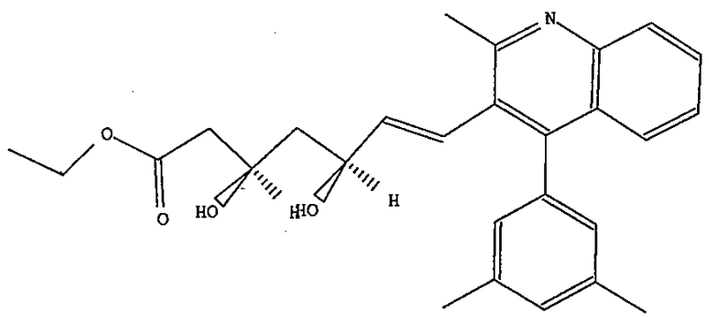
AMOUNTS, mg

0.0
14.5 - 0.0 SCALLEN

SUM. SYNTH 25491+25492--->25493--->25494--->25495--->25496

3/6

INT.REG.NO	SAH.NO	SALTCODE	CHEM.NO	SUBMITTED	UNIT	CHEMISTS	DISCL
26080	SAH-063548		1127-011-34	05-17-85	KATH	KATHAWALA WATTANASIN	299-84



FORM DESIGNED BY BARCZA

KNOWN? N | L&D. NO 13329.0

MP

BP

PRESSURE

OTHER.PHYS.DATA

OIL

SOL. CODE C OR D OR E

DETAILS CMC OR DMA OR EtOH

SCREENS

CSI, CSTC, CSTV

NOTES SEE LONGNOTE

PURE ERYTHRO COMPOUND
KEEP REFRIGERATE

C27 H31 N O4	MW 433.552	SAH

COMPARE WITH 58-512

MADE. FROM. REGNO 26079.0 - 24540.0 A) LDA B) 24540 C) n-Bu4NF

AMOUNTS,mg

4.8
2.0 - 4.8 SCALLEN

SUM. SYNTH 26076--->26077--->26078--->26079+24540--->26080

3/2

INT.REG.NO	SAH.NO	SALTCODE	CHEM.NO	SUBMITTED	UNIT
26082	SAH-063549		1127-011-37	05-17-85	KATH

CHEMISTS: KATHAWALA WATTANASIN

DISCL: 299-84

KNOWN? N

L&D. NO: 13329.0

MP:

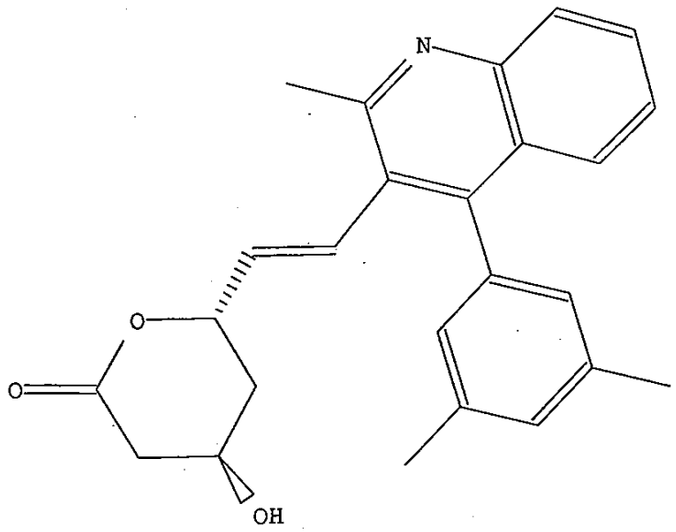
BP:

PRESSURE:

OTHER.PHYS.DATA: OIL

SOL. CODE: C OR D OR E

DETAILS: CMC SUSPENSION OR DMA OR EtOH



FORM DESIGNED BY BARCZA

SCREENS

CSI

NOTES: SEE LONGNOTE

PURE TRANS LACTONE
CONTAINS SOME IMPURITIES
KEEP REFRIGERATE

	MW	
C25 H25 N O3	387.483	SAH

COMPARE WITH: 58-512

MADE. FROM. REGNO: 26081.0 A)Bu4NF,THF B)CHROMATOGRAPHY

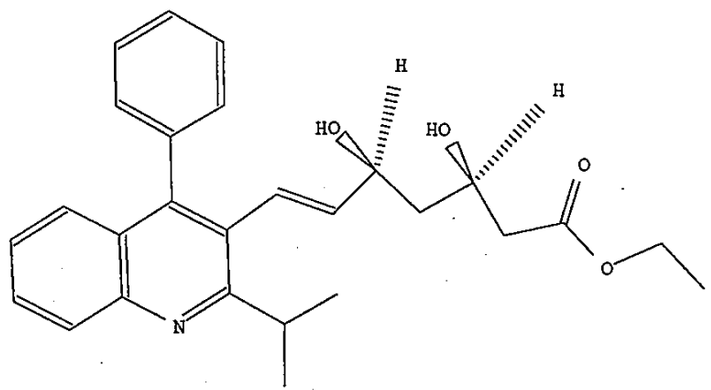
AMOUNTS,mg

2.0 - 0.0 SCALLEN

SUM. SYNTH: 26081--->26082

3/8

INT.REG.NO	SAH.NO	SALTCODE	CHEM.NO	SUBMITTED	UNIT	CHEMISTS	DISCL
30441	SAH-064933		1206-176-43	09-21-87	WATT	PATEL WATTANASIN	299-84



FORM DESIGNED BY BARCZA

KNOWN?	L&D. NO
N	

MP

BP

PRESSURE

OTHER.PHYS.DATA

SOL. CODE D OR E OR C

DETAILS DMA,EtOH,CMC SUSPENSION

SCREENS

CSI CSIC CSIV

NOTES SEE LONGNOTE

ERYTHRO:THREO > 95:5
REFRIGERATE

	MW	
C27 H31 N O4	433.552	SAH

COMPARE WITH 62-320

MADE. FROM. REGNO 30440.0 A) ETHYLACETOACETATE B) Et3B,MeOH/THF C) HOAc/MeOH

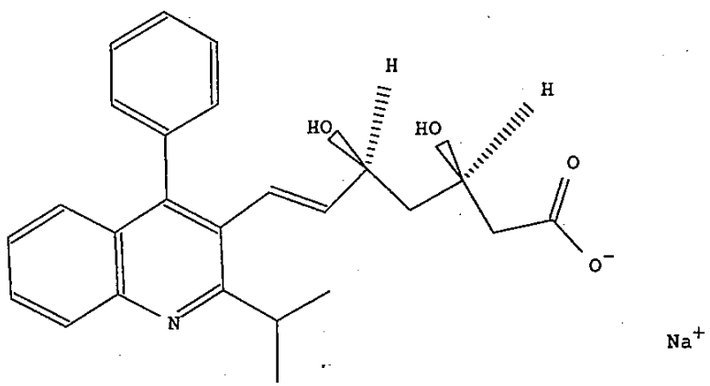
SUM. SYNTH 30437+24214---> 30438---> 30439---> 30440---> 30441

AMOUNTS,mg

50.0
50.0 SCALLEN

3/9

INT.REG.NO	SAH.NO	SALTCODE	CHEM.NO	SUBMITTED	UNIT	CHEMISTS	DISCL
30442	SAH-064934	NA	1206-179-30	09-21-87	WATT	PATEL WATTANASIN	299-84



FORM DESIGNED BY BARCZA

KNOWN? N

L&D. NO

MP 210.0 PLUS

BP

PRESSURE

OTHER.PHYS.DATA

SOL. CODE D OR E OR C

DETAILS DMA,EtOH,CMC SUSPENSION

SCREENS
CSI CSIC CSIV

NOTES SEE LONGNOTE

ERYTHRO: THREO > 95:5
REFRIGERATE

	MW	
C25 H26 N Na O4	427.48	SAH

COMPARE WITH 62-320

MADE. FROM. REGNO 30441.0 NaOH, 0 DEG.

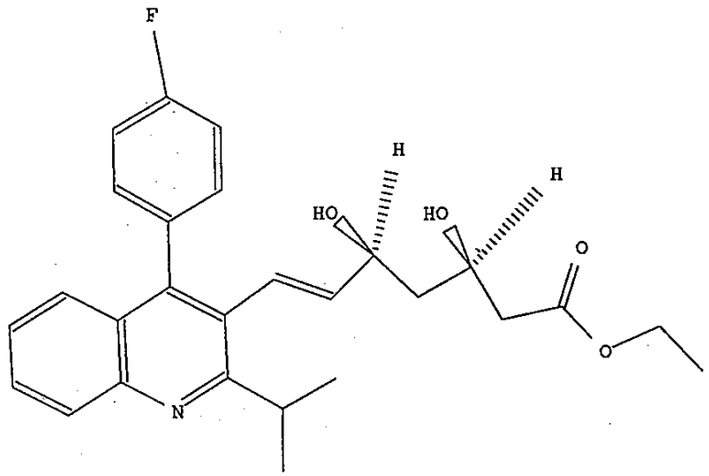
AMOUNTS,mg

50.0
50.0 SCALLEN

SUM. SYNTH 30441---> 30442

320

INT.REG.NO	SAH.NO	SALTCODE	CHEM.NO	SUBMITTED	UNIT	CHEMISTS	DISCL
30447	SAH-064935		1206-190-41	09-21-87	WATT	PATEL WATTANASIN	299-84



FORM DESIGNED BY BARCZA

KNOWN?	L&D. NO
N	

MP

BP

PRESSURE

OTHER.PHYS.DATA

SOL. CODE D OR E OR C

DETAILS DMA,EtOH,CMC SUSPENSION

SCREENS

CSI CSIC CSIV

NOTES SEE LONGNOTE

ERTHRO: THREO > 95:5
REFRIGERATE

	MW	
C27 H30 F N O4	451.543	SAH

COMPARE WITH 62-320

MADE. FROM. REGNO 30446.0 A) ETHYLACETOACETATE,NaH/BuLi B) Et3B,CH3OH/THF C)HOAc/CH3OH

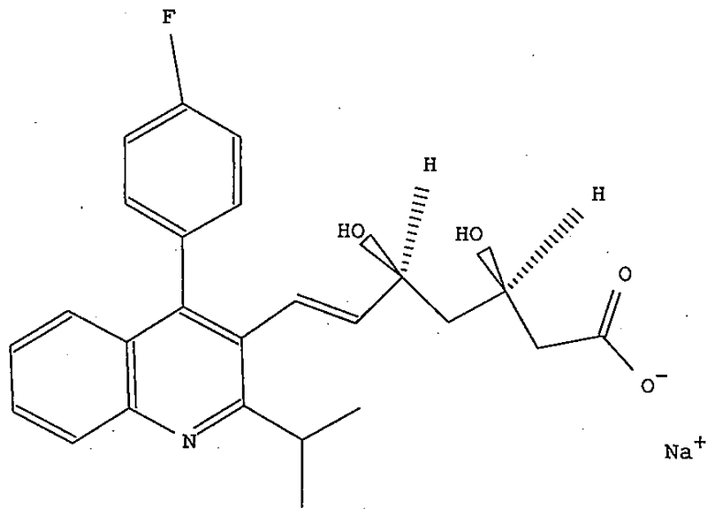
AMOUNTS,mg

20.0
20.0 SCALLEN

SUM. SYNTH 30443+24214---> 30444---> 30445---> 30446---> 30447

321

INT.REG.NO	SAH.NO	SALTCODE	CHEM.NO	SUBMITTED	UNIT	CHEMISTS	DISCL
30448	SAH-064936	NA	1206-201-30	09-22-87	WATT	PATEL WATTANASIN	299-84



FORM DESIGNED BY BARCZA

KNOWN?	L&D. NO
N	

MP

BP 225.0 PLUS

PRESSURE

OTHER.PHYS.DATA

SOL. CODE D OR E OR C

DETAILS DMA,EtOH,CMC SUSPENSION

SCREENS

CSI CSIC CSIV

NOTES SEE LONGNOTE

ERYTHRO: THREO > 95:5
REFRIGERATE

C25 H25 F N Na O4	MW 445.47	SAH

COMPARE WITH 62-320

MADE. FROM. REGNO 30447.0 NaOH, 0 DEG.

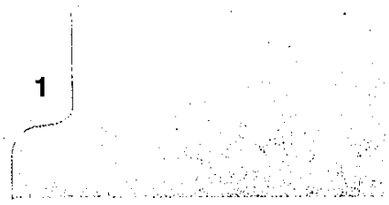
SUM. SYNTH 30447---> 30448

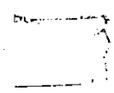
AMOUNTS,mg

20.0
20.0 SCALLEN

322

Exhibit I


1



SANDOZ, INC.
E. HANOVER, N.J.

323

FROM: Dr. R. Damon DATE: Dec. 3, 1984

TO: Prof. T. Scallen

PURPOSE: HMG CoA REDUCTASE SCREENING

COMPOUND No.	QUANTITY	PRECAUTIONS &/OR SPECIAL INSTRUCTIONS & SOLVENTS
#63-364(25489)	6mg	CMC, DMA, EtOH (Refrigerate)
63-365(25490)	10mg	CMC, DMA, EtOH (Refrigerate)
63-366(25496)	14.5mg	CMC, DMA, EtOH (Refrigerate)
63-369(25512)	3.9mg	DMA
#63-162/3(25500)	10mg	DMA, Ethanol (Refrigerate)
63-270/2(25501)	10mg	CMC (Refrigerate)

FOR LABORATORY USE ONLY

H. Lukas

82374/80 (Rev. 1)

SANDOZ, INC.
E. HANOVER, N.J.

324

FROM: Dr. R. Damon DATE: June 3, 1985

TO: Prof. T. Scallen

PURPOSE: HMG CoA REDUCTASE SCREENING

COMPOUND No.	QUANTITY	PRECAUTIONS &/OR SPECIAL INSTRUCTIONS & SOLVENTS
#63-518/2(RN 26020)	7.5mg	CMC, DMA, EtOH (Refrigerate)
63-537/Na(RN 26039)	19mg	50/D
63-547(RN 26075)	6.0mg	DMA
✓ 63-548(RN 26080)	2.0mg	CMC, DMA, EtOH (Refrigerate)
✓ 63-549(RN 26082)	2.0mg	CMC, DMA, EtOH (Refrigerate)
63-550/Na(RN 26083)	5.2mg	DMA
63-551(RN 26084)	20mg	CMC, DMA, EtOH (Refrigerate)
63-552/Na(RN 26085)	22mg	" " " "
63-553(RN 26086)	20mg	" " " "
63-554/Na(RN 26087)	24mg	" " " "
63-555/Na(RN 26088)	5.0mg	DMA (Refrigerate)
63-556(RN 26093)	14mg	CMC, DMA, EtOH (Refrigerate)
63-558/Na(RN 26098)	5.2mg	DMA
63-559 (RN 26106)	5mg	DMA
63-550/2-Na(RN 26-108)	0.6mg	DMA
63-563 (RN 26127)	10mg	DMA
63-564/Na(RN 26129)	10mg	DMA
63-565 (RN 26128)	10mg	DMA
63-566(RN 26148)	10mg	DMA
63-567 (RN 26149)	2.7mg	DMA
63-568 (RN 26157)	25mg	DMA
63-560 (RN 26107)	5mg	Water

FOR LABORATORY USE ONLY

82374/80 (Rev. 1)

H. Lukas

SANDOZ, INC.
E. HANOVER, N.J.

325

FROM: Dr. R. Damon

DATE: Oct. 2, 1987

TO: Prof. T. Scallen

PURPOSE: HMG CoA REDUCTASE SCREEN

COMPOUND No.	QUANTITY	PRECAUTIONS &/OR SPECIAL INSTRUCTIONS & SOLVENTS
#64-906/Na (RN 30393)	80.0 mg	50/W
64-933 (RN 30441)	50.0 mg	CMC, DMA, EtOH (Refrigerate)
64-934/Na (RN 30442)	50.0 mg	" " " "
64-935 (RN 30447)	20.0 mg	" " " "
64-936/Na (RN 30448)	20.0 mg	" " " "
64-942/Na (RN 30461)	0.6 mg	CMC, DMA (Compare with #62-526 & #64-727)
64-948/Na (RN 30485)	1.5 mg	" " (Refrigerate) Compare with 62-526 Or 58-512 and 64-727

FOR LABORATORY USE ONLY

82374/80 (Rev 1)

H. Lukas

326

SANDOZ .RMACEUTICALS CORP. | MAIL SERVICES REQUEST | ADMINISTRATIVE SERVICES

FROM: HONORA LUKAS | DEPT: PRECLIN RES. | BLDG. # 403 | DATE: 10/2/97 | EXT: .7654

IDENTIFICATION (JOB) NUMBER (5): 61529

COST UNIT (6 7 8 9): 8 4 0 2

TYPE OF MAIL (CHECK DESIRED BLOCKS):

- ☐ APPROXIMATE ARRIVAL DATE SHOULD BE _____
- ☐ MOST ECONOMICAL WAY
- ☐ FIRST CLASS MAIL
- ☐ AIR MAIL
- ☐ REGISTERED MAIL
- ☐ CERTIFIED MAIL
- ☐ RETURN RECEIPT
- ☒ OVERNIGHT SERVICE
- ☐ UNITED PARCEL

MAILING DATE REQUESTED: _____ ◀ STATE REALISTIC DATE NOT RUSH, ASAP, ETC.

INSURANCE NEEDED ☐ YES ☒ NO | VALUE:

ADDRESSEE:

Prof. T. Scallen
Dept. Of Biochemistry
School of Medicine
University of New Mexico
Albuquerque, New Mexico
87131

ITEM DESCRIPTION & QUANTITY OF EACH: (SPECIAL INSTRUCTIONS)

7 --- Hanover samples

FOR LABORATORY USE ONLY!

ITEMS FOR MAILING WILL BE RECD. FROM: | DATE: 10/2/97

SANDOZ

_____ SALES REPRESENTATIVES
_____ REGIONAL MANAGERS
_____ AREA SALES MANAGERS
_____ TECHNICAL REPRESENTATIVES
_____ MANAGERS, GOVERNMENT AFFAIRS
_____ MEDICAL SCIENCES LIAISON MANAGERS
_____ MEDICAL SCIENCES LIAISON
_____ HOSPITAL REPRESENTATIVES
_____ AREA HOSPITAL SALES MANAGERS
_____ IN-HOUSE DISTRIBUTION

DORSEY

_____
_____
_____
_____
_____

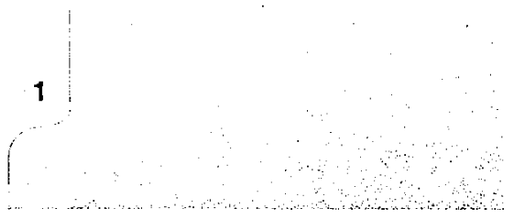
MISCELLANEOUS INFORMATION

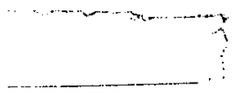
DATE RECEIVED WORK ORDER 10/3/97
DATE RECEIVED MATERIAL _____
DATE DISPATCHED ✓
SUPPLY COST 25
WEIGHT 1
TOTAL PIECES 1
PREPARATION TIME 5
TOTAL POSTAGE F.E.X.

82100/83-1 (Rev. 7)

White — Mailroom; Canary — Returned to Originator when job is completed.

Exhibit J


1



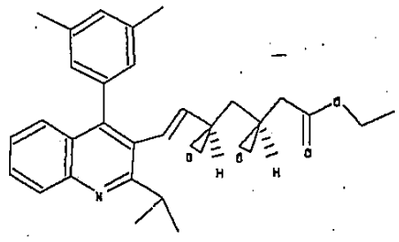
Date Proj.

Cont'd From-

Title- 63366 1069-113 327

PATENT AND TRADEMARK DEPT.
FEB 27 1990
JMG

SAH-063366 | 25496



CHEM.NO. 1079-111-19 | TESTS2

DATE 11-26-84 | MOL.WEIGHT 461.606

DISCL.NO. 299-84 | CHEMISTS KATHAWALA WATTANASIN

TESTS1 CSI | AMOUNTS 0.0 14.5 - 0.0 SCALLEN

MICROSOMAL ASSAY (SCALLEN)

CONC. (UM)	% INHIBITION DATE 12/13/84 SOLVENT DMA	DATE SOLVENT	DATE SOLVENT	DATE SOLVENT
1000	∞			
100	∞			
10	78 2			
1	39 2			
0.1	14 2			
0.01	6			
0.001	4			
0.0001	6			
IC50	1.58			

Performed by-

Witness-

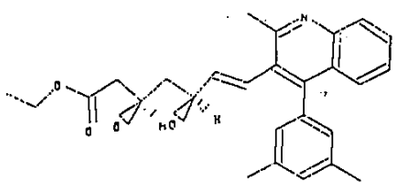
Cont'd to-

Title- **198**

Date Proj.

Cont'd From-

SAH-063548 | 26080



CHEM.NO. 1127-011-34	TESTS2
DATE 05-17-85	MOL.WEIGHT 433.552
DISCL.NO. 299-84	CHEMISTS KATHAWALA WATTANASIN
TESTS1 CSI, CSTC, CSTV	AMOUNTS 4.8 2.0 - 4.8 SCALLEN

MICROSOMAL ASSAY (SCALLEN)

CONC. (UM)	% INHIBITION			
	DATE 6/13/85	DATE	DATE	DATE
	SOLVENT DMA	SOLVENT	SOLVENT	SOLVENT
1000	0			
100	X			
10	72 R			
1	20 R			
0.1	0 R			
0.01	0			
0.001				
0.0001				
IC50	3.775			

Performed by- R. Dunn

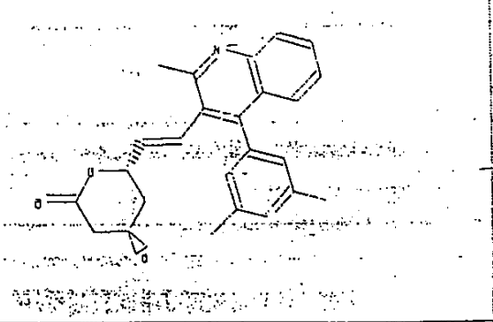
Witness- S. Wattanasin

Cont'd to-

Date | Proj. | Title- | 197

Cont'd From- | 329

SAH-063549 | 26082



CHEM.NO. 1127-011-37 | TESTS2

DATE 05-17-85 | MOL.WEIGHT 387.483

DISCL.NO. 299-84 | CHEMISTS KATHAWALA WATTANASIN

TESTS1 CSI | AMOUNTS 2.0 - 0.0 SCALLEN

MICROSOMAL ASSAY (SCALLEN)

CONC. (UM)	% INHIBITION DATE 6/18/85 SOLVENT DMA	DATE	DATE	DATE
		SOLVENT	SOLVENT	SOLVENT
1000	>>			
100	>>			
10	56 R			
1	12 R			
0.1	0			
0.01	0			
0.001	0			
0.0001				
IC50	7.31			

Performed by- R. Dawson

Witness- S. Watson

Cont'd to-

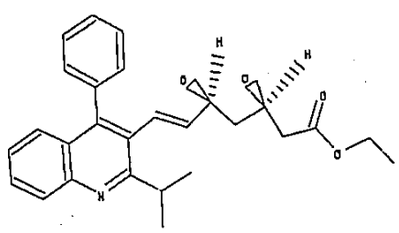
Date Proj. Title-

Cont'd From-

13

330

SAH-064933 | 30441



CHEM.NO. 1206-176-43 | TESTS2

DATE 09-21-87 | MOL.WEIGHT 433.552

DISCL.NO. 299-84 | CHEMISTS PATEL WATTANASIN

TESTS1 CSI CSIC CSIV | AMOUNTS 50.0 50.0 SCALLEN

MICROSOMAL ASSAY (SCALLEN)

CONC. (UM)	% INHIBITION DATE 10/5/87 SOLVENT DMSO	DATE SOLVENT	DATE SOLVENT	DATE SOLVENT
1000	x			
100	x			
10	50 ½			
1	32 ½			
0.1	2 ½			
0.01	(-2.)			
0.001				
0.0001				
IC50	2.37			

Performed by- R. Patel

Witness- S. Wattanasin

Cont'd to-

14

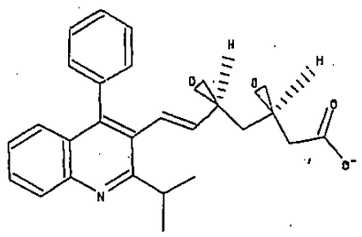
Title-

Date

Proj. 331

Cont'd From-

SAH-064934	30442



CHEM.NO. 1206-179-30	TESTS2
DATE 09-21-87	MOL.WEIGHT 427.48
DISCL.NO. 299-84	CHEMISTS PATEL WATTANASIN
TESTS1 CSI CSIC CSIV	AMOUNTS 50.0 50.0 SCALLEN

MICROSOMAL ASSAY (SCALLEN)

CONC. (UM)	% INHIBITION DATE 10/5/87 SOLVENT DMSO	DATE SOLVENT	DATE SOLVENT	DATE SOLVENT
1000	b			
100	b			
10	78 k.			
1	30. i.			
0.1	2 i			
0.01	(-2)			
0.001				
0.0001				
IC50	2.61			

Performed by-

Witness-

Cont'd to-

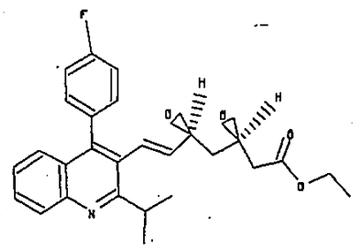
Date Proj. Title-

Cont'd From-

15

332

SAH-064935 | 30447



CHEM.NO. 1206-190-41	TESTS2
DATE 09-21-87	MOL.WEIGHT 451.543
DISCL.NO. 299-84	CHEMISTS PATEL WATTANASIN
TESTS1 CSI CSIC CSIV	AMOUNTS 20.0 20.0 SCALLEN

MICROSOMAL ASSAY (SCALLEN)

CONC. (UM)	% INHIBITION DATE 10/8/87 SOLVENT DMA	DATE SOLVENT	DATE SOLVENT	DATE SOLVENT
1000	X			
100	X			
10	87			
1	69			
0.1	28			
0.01	9			
0.001	7			
0.0001	5			
IC50	0.413			

Performed by- R. Patel

Witness- [illegible]

Cont'd to-

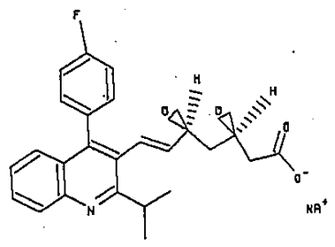
16 Title-

Date Proj.

Cont'd From-

333

SAH-064936 | 30448



CHEM.NO.	TESTS2
1206-201-30	
DATE 09-22-87	MOL.WEIGHT 445.47
DISCL.NO. 299-84	CHEMISTS PATEL WATTANASIN
TESTS1 CSI CSIC CSIV	AMOUNTS 20.0 20.0 SCALLEN

MICROSOMAL ASSAY (SCALLEN)

CONC. (UM)	% INHIBITION DATE 10/13/87 SOLVENT DMA	DATE SOLVENT	DATE SOLVENT	DATE SOLVENT
1000	b			
100	b			
10	93			
1	66 12			
0.1	21 12			
0.01	4 12			
0.001				
0.0001				
IC50	0.53			

Performed by- R. Dam

Witness- S. Walthall

Cont'd to-

Exhibit K

10/22/87 Proj. H318

Cont'd From- 134

Title- Cholesterol Synthesis Inhibition Screen

133

334

CHOLESTEROL BIOSYNTHESIS INHIBITION SCREEN

LIPID METABOLISM DEPARTMENT
HMGR SCREENING UNIT
Sandoz Research Institute
To: Dr. D. Weinstein, Departmenthead
Mr. R. Slaughter, Responsible Technician
From: Mr. R. Engstrom, Responsible Investigator
CC: D.N., M.L.R., ARC

STUDY #: H318
STUDY ON 10/22/87
BK.REF. 917-83
APPROVAL
DATE 10/21/87
GEN. ARC#86-006

Title: In vivo single dose assay to test for inhibition of biosynthesis by compounds: 63-748, 64-844, 64-936

Purpose: Determine the invivo effects of test compounds in rats on cholesterol biosynthesis.

Experimental design: IN VIVO CHOLESTEROL BIOSYNTHESIS INHIBITION DT0065 Invivo single dose assay or inhibition of Reference/method: 740/001. Stock solutions and dilutions prepared in 0.5% CMC, administered p.o. at 1ml/100gm weight. Rats bled via carotic incision using hexobarbital anesthesia. Animal use will be in compliance with ARC regulations. Duration = 1 hr. No/group = 6. No of groups = 14. WCR rats.

RATs	COMPOUND	REGNO	DOSE mg/kg	STOCK mg/20ml	WORKING SOLUTION ml stock q.s. to 15ml
1-6	Control	------	------	------	----------
7-12	63-748	26688	1	2	UNDILUTED
13-18	"	"	0.3	-	4.5
19-24	"	"	0.1	-	1.5
25-30	64-844	30280	0.3	2	4.5
31-36	"	"	0.1	-	1.5
37-42	"	"	0.03	-	0.45
43-48	64-936	30488	1	2	UNDILUTED
49-54	"	"	0.3	-	4.5
55-60	"	"	0.1	-	1.5
61-66	62-320	30859	0.3	2	4.5
67-72	"	"	0.1	-	1.5
73-78	"	"	0.03	-	0.45
79-84	Control	------	------	------	----------

Performed by- Tony R. Slaughter

Witness- R Engstrom

Cont'd to- 135

134

Title- Cholesterol Synthesis Inhibition Screen

Date 10/22/88 Prot. 143

Cont'd From- 133

335

IN VIVO CHOLESTEROL SYNTHESIS INHIBITION SCREEN H318

RAT	COMPOUND	REGNO	DOSE mg/kg	mCi/dl	STATISTICS	
	BLANK	30280		ST.		132.6
	14C-STANDARD			20178	% EFFIC=	.99
1	CONTROL			493		
2	CONTROL			677	MEAN =	537.7
3	CONTROL			590	STD =	128.6
4	CONTROL			455	SE =	37.1
5	CONTROL			490		
6	CONTROL			365		
79	CONTROL			462		
80	CONTROL			318		
81	CONTROL			599		
82	CONTROL			650		
83	CONTROL			610		
84	CONTROL			745		
8	63-748	26688	1.00	170	MEAN =	155.9
9	63-748	26688	1.00	278	STD =	73.1
10	63-748	26688	1.00	113	SE =	32.7
11	63-748	26688	1.00	113	t =	7.7
12	63-748	26688	1.00	106	p	<.01
7	63-748	26688	1.00	528*	%CHG =	-71
13	63-748	26688	.300	396	MEAN =	319.3
14	63-748	26688	.300	356	STD =	68.3
16	63-748	26688	.300	391	SE =	39.5
17	63-748	26688	.300	199	t =	4.0
18	63-748	26688	.300	253	p	<.01
15	63-748	26688	.300	794*	%CHG =	-40.6
19	63-748	26688	.100	348	MEAN =	458.7
20	63-748	26688	.100	728	STD =	213.5
21	63-748	26688	.100	310	SE =	87.2
22	63-748	26688	.100	650	t =	0.8
23	63-748	26688	.100	538	p	N.S.
24	63-748	26688	.100	178	%CHG =	-14.7
25	64-844	30280	.300	268	MEAN =	165.8
26	64-844	30280	.300	170	STD =	57.3
27	64-844	30280	.300	165	SE =	23.4
28	64-844	30280	.300	126	t =	6.5
29	64-844	30280	.300	174	p	<.01
30	64-844	30280	.300	101	%CHG =	-69.2
31	64-844	30280	.100	308	MEAN =	219.8
32	64-844	30280	.100	273	STD =	66.9
33	64-844	30280	.100	195	SE =	29.9
35	64-844	30280	.100	157	t =	6.7
36	64-844	30280	.100	166	p	<.01
34	64-844	30280	.100	698*	%CHG =	-59.1

Performed by- Todd A. Shaughnessy

Witness- R Eastman

Cont'd to- 135

Date 10/22/87 Proj# 3.8

Cont'd From- 134

Title- Cholesterol Synthesis Inhibition Screen

135

336

INVIVO CHOLESTEROL SYNTHESIS INHIBITION SCREEN H318

RAT	COMPOUND	REGNO	DOSE mg/kg	nCi/dl	STATISTICS	
37	64-844	30280	.030	354	MEAN =	419.7
38	64-844	30280	.030	518	STD =	138.6
39	64-844	30280	.030	639	SE =	56.6
40	64-844	30280	.030	248	t =	1.7
41	64-844	30280	.030	356	p	N.S.
42	64-844	30280	.030	402	%CHG =	-21.9
43	64-936	30488	1.00	580	MEAN =	489.4
44	64-936	30488	1.00	642	STD =	132.8
45	64-936	30488	1.00	280	SE =	54.2
46	64-936	30488	1.00	388	t =	0.7
47	64-936	30488	1.00	532	p	N.S.
48	64-936	30488	1.00	513	%CHG =	-9.0
49	64-936	30488	.300	167	MEAN =	326.7
50	64-936	30488	.300	232	STD =	165.0
51	64-936	30488	.300	586	SE =	67.4
52	64-936	30488	.300	278	t =	2.7
53	64-936	30488	.300	223	p	<.02
54	64-936	30488	.300	473	%CHG =	-39.2
55	64-936	30488	.100	485	MEAN =	416.5
56	64-936	30488	.100	181	STD =	166.8
57	64-936	30488	.100	339	SE =	68.9
58	64-936	30488	.100	686	t =	1.6
59	64-936	30488	.100	367	p	N.S.
60	64-936	30488	.100	439	%CHG =	-22.5
61	62-320	30559	.300	72	MEAN =	67.5
62	62-320	30559	.300	89	STD =	13.1
63	62-320	30559	.300	72	SE =	5.4
64	62-320	30559	.300	53	t =	12.5
65	62-320	30559	.300	64	p	<.01
66	62-320	30559	.300	55	%CHG =	-87.5
67	62-320	30559	.100	135	MEAN =	165.3
68	62-320	30559	.100	238	STD =	51.1
70	62-320	30559	.100	198	SE =	22.8
71	62-320	30559	.100	109	t =	8.5
69	62-320	30559	.100	149	p	<.01
72	62-320	30559	.100	138	%CHG =	-69.3
73	62-320	30559	.030	333	MEAN =	351.2
74	62-320	30559	.030	360	STD =	173.3
75	62-320	30559	.030	77	SE =	70.8
76	62-320	30559	.030	579	t =	2.3
77	62-320	30559	.030	483	p	<.05
78	62-320	30559	.030	277	%CHG =	-34.7

* = rejected by "Q" test Computed 12-09-87

^ =LACK OF SAMPLE

Performed by- Rod K. Slaughter

Witness- P Emerson

Cont'd to-

136 Title- Cholesterol Synthesis Inhibition Screen

Date 10/29/87 Proj: 319

Cont'd From-

337

CHOLESTEROL BIOSYNTHESIS INHIBITION SCREEN

LIPID METABOLISM DEPARTMENT
HMGR SCREENING UNIT
Sandoz Research Institute
To: Dr. D. Weinstein, Departmenthead
Mr. R. Slaughter, Responsible Technician
From: Mr. R. Engstrom, Responsible Investigator
CC: D.N., M.L.R., ARC

STUDY # H319
STUDY ON 10/29/87
BK.REF. 917-136
APPROVAL
DATE 10/29/87
GEN. ARC#88-006

Title: In vivo single dose assay to test for inhibition of biosynthesis by compounds: 64-298, 64-933, 63-935

Purpose: Determine the invivo effects of test compounds in rats on cholesterol biosynthesis.

Experimental design: IN VIVO CHOLESTEROL BIOSYNTHESIS INHIBITION DT0065 Invivo single dose assay of inhibition of Reference method: 740/001. Stock solutions and dilutions prepared in 0.5% CMC, administered p.o. at 1ml/100gm weight. Rats bled via carotid incision using hexobarbital anesthesia. Animal use will be in compliance with ARC regulations. Duration = 1 hr, No/group = 6, No of groups = 14, WCR rats.

RAT#	COMPOUND	REGNO	DOSE mg/kg	STOCK mg/20ml	WORKING SOLUTION ml stock q.s. to 15ml
1-6	Control	------	------	------	------
7-12	64-298	29277	1	2	UNDILUTED
13-18	"	"	0.3	-	4.5
19-24	"	"	0.1	-	1.5
25-30	64-933	30447	1	2	UNDILUTED
31-36	"	"	0.3	-	4.5
37-42	"	"	0.1	-	1.5
43-48	64-935	30441	1	2	UNDILUTED
49-54	"	"	0.3	-	4.5
55-60	"	"	0.1	-	1.5
61-66	82-320	30558	0.3	2	4.5
67-72	"	"	0.1	-	1.5
73-78	"	"	0.03	-	0.45
79-84	Control	------	------	------	------

Performed by- R. Slaughter

Witness- R Engstrom

Cont'd to- 337

338

Date 10/27/71 Proj.

Cont'd From-

Title- [illegible]

137

INVIVO CHOLESTEROL SYNTHESIS INHIBITION SCREEN H319

RAT	COMPOUND	REGNO	DOSE mg/kg	nCi/dl	STATISTICS	
	BLANK			7		
	14C-STANDARD			20176	% EFFIC=	99
1	CONTROL			983		
2	CONTROL			515	MEAN =	671.8
3	CONTROL			646	STD =	211.0
4	CONTROL			578	SE =	60.9
5	CONTROL			934		
6	CONTROL			354		
79	CONTROL			756		
80	CONTROL			347		
81	CONTROL			814		
82	CONTROL			548		
83	CONTROL			872		
84	CONTROL			714		
7	64-298	28277	1.00	203	MEAN =	151.7
8	64-298	28277	1.00	361	STD =	113.6
9	64-298	28277	1.00	82	SE =	46.4
10	64-298	28277	1.00	78	t =	6.8
11	64-298	28277	1.00	71	p	<.01
12	64-298	28277	1.00	115	%CHG =	-77
13	64-298	28277	.300	311	MEAN =	235.1
14	64-298	28277	.300	264	STD =	81.4
15	64-298	28277	.300	257	SE =	33.2
16	64-298	28277	.300	307	t =	6.3
17	64-298	28277	.300	114	p	<.01
18	64-298	28277	.300	157	%CHG =	-65.0
19	64-298	28277	.100	381	MEAN =	388.7
20	64-298	28277	.100	387	STD =	81.5
21	64-298	28277	.100	248	SE =	33.3
22	64-298	28277	.100	392	t =	4.1
23	64-298	28277	.100	499	p	<.01
24	64-298	28277	.100	426	%CHG =	-42.1
25	64-933	30447	1.00	838	MEAN =	428.1
26	64-933	30447	1.00	275	STD =	253.4
27	64-933	30447	1.00	136	SE =	103.5
28	64-933	30447	1.00	594	t =	2.0
29	64-933	30447	1.00	288	p	N.S.
30	64-933	30447	1.00	447	%CHG =	-36.3
31	64-933	30447	.300	530	MEAN =	557.4
32	64-933	30447	.300	646	STD =	100.5
33	64-933	30447	.300	525	SE =	41.0
34	64-933	30447	.300	596	t =	1.6
35	64-933	30447	.300	368	p	N.S.
36	64-933	30447	.300	618	%CHG =	-17.0

Performed by-

138 Title-

Date 12/28/87 Proj.-

Cont'd From- T37

INVIVO CHOLESTEROL SYNTHESIS INHIBITION SCREEN H319

RAT	COMPOUND	REGNO	DOSE mg/kg	nCi/dl	STATISTICS	
37	64-933	30447	.100	556	MEAN =	547.0
38	64-933	30447	.100	735	STD =	147.2
39	64-933	30447	.100	370	SE =	60.1
40	64-933	30447	.100	378	t =	1.5
41	64-933	30447	.100	591	p	N.S.
42	64-933	30447	.100	652	%CHG =	-18.6
43	64-935	30441	1.00	182	MEAN =	230.0
44	64-935	30441	1.00	307	STD =	78.2
45	64-935	30441	1.00	186	SE =	31.9
46	64-935	30441	1.00	321	t =	6.4
47	64-935	30441	1.00	124	p	<.01
48	64-935	30441	1.00	281	%CHG =	-65.8
49	64-935	30441	.300	776	MEAN =	472.2
50	64-935	30441	.300	282	STD =	179.5
51	64-935	30441	.300	520	SE =	73.3
52	64-935	30441	.300	413	t =	2.1
53	64-935	30441	.300	344	p	N.S.
54	64-935	30441	.300	438	%CHG =	-28.7
55	64-935	30441	.100	411	MEAN =	428.2
56	64-935	30441	.100	320	STD =	119.1
57	64-935	30441	.100	296	SE =	48.6
58	64-935	30441	.100	426	t =	3.1
59	64-935	30441	.100	621	p	<.02
60	64-935	30441	.100	495	%CHG =	-36.3
61	62-320	30559	.300	60	MEAN =	165.6
62	62-320	30559	.300	107	STD =	107.1
63	62-320	30559	.300	222	SE =	43.7
64	62-320	30559	.300	60	t =	6.8
65	62-320	30559	.300	217	p	<.01
66	62-320	30559	.300	327	%CHG =	-75.3
67	62-320	30559	.100	262	MEAN =	331.7
68	62-320	30559	.100	434	STD =	165.7
70	62-320	30559	.100	569	SE =	74.1
71	62-320	30559	.100	168	t =	3.5
69	62-320	30559	.100	226	p	<.01
72	62-320	30559	.100	604	%CHG =	-50.6
73	62-320	30559	.030	421	MEAN =	445.1
74	62-320	30559	.030	472	STD =	94.1
75	62-320	30559	.030	571	SE =	38.4
76	62-320	30559	.030	374	t =	3.1
77	62-320	30559	.030	517	p	<.01
78	62-320	30559	.030	315	%CHG =	-33.8
					Computed	12-09-87

Performed by-

Witness- R Blackburn

Cont'd to-

340

64568	29651	280-85	>	1	09-JUN-87	917-065
64569	29652	280-85	=	.16	15-JUN-87	917-081
64602	29743	101-85	>	.3	05-MAY-87	917-050
64602	29743	101-85	>	.3	05-MAY-87	917-050
64604	29744	101-85	>	.3	05-MAY-87	917-051
64604	29744	101-85	>	.3	05-MAY-87	917-051
64604	29745	101-85	=	.48	14-JUL-87	917-086
64608	29756	298-85	>	7.5	13-MAY-87	917-056
64638	29835	570-83		.34	09-DEC-87	917-140
64639	29836	570-83	>	1	09-JUN-87	917-066
64640	29839	367-86	>	1	09-JUN-87	917-068
64641	29840	367-86	>	1	09-JUN-87	917-068
64642	29841	367-86	>	1	09-JUN-87	917-089
64673	29904	280-85	=	2.6	18-SEP-87	917-111
64686	29927	387-85	>	10	18-SEP-87	917-113
64691	29942	366-86		.58	16-DEC-87	917-141
64722	30004	280-85	=	.2	23-OCT-87	917-126
64723	30627	100-85	=	.16	19-FEB-88	917-159
64723	30877	100-85	=	.09	19-FEB-88	917-159

SAHNUM	REGNO	PATENT	R	ED50	EDATE	REF
64723	30766	100-85	=	.22	19-FEB-88	917-159
64723	30009	100-85	=	.36	18-SEP-87	917-107
64744	30059	295-84	>	.1	14-JUL-87	917-090
64745	30765	295-84	=	.016	19-FEB-88	917-154
64745	30060	295-84	=	.016	20-OCT-87	917-127
64747	30067	298-84	=	.11	01-JUL-87	917-087
64748	30068	298-84	=	.04	19-FEB-88	917-165
64792	30146	260-85	=	.74	13-OCT-87	917-123
64816	30199	295-84	=	.1	12-OCT-87	917-119
64844	30280	384-85	=	.07	09-DEC-87	917-135
64844	30769	384-85	=	.08	19-FEB-88	917-167
64896	30378	366-87	>	.3	06-OCT-87	917-119
64897	30379	366-87	>	.3	06-OCT-87	917-120
64906	30393	280-85	=	.045	05-JAN-88	917-150
64906	30772	280-85	=	.1	15-JAN-88	917-155
64933	30441	299-84	>	1	09-DEC-87	917-138
64935	30447	299-84	=	.49	09-DEC-87	917-138
64936	30488	299-84	>	1	09-DEC-87	917-135
64999	30623	298-84	=	.1	19-FEB-88	917-168
65002	30629	101-85	=	.76	05-JAN-88	917-144
65003	30630	101-85	=	.09	19-FEB-88	917-159

SAHNUM	REGNO	PATENT	R	ED50	EDATE	REF
65003	30902	101-85	=	.06	19-FEB-88	917-170
86665	25887	102-82	>	10	06-MAY-87	917-056
87469	26362	101-82	>	10	06-MAY-87	917-056
89826	29587	101-82	>	10	06-MAY-87	917-057
317223	24022		>	16	20-MAR-84	812-183
880349	29591	102-82	>	10	18-AUG-87	917-098
880586	29588	102-82	>	10	18-AUG-87	917-098
880820	29589	102-82	>	10	18-AUG-87	917-099

140 records selected.

SQL>

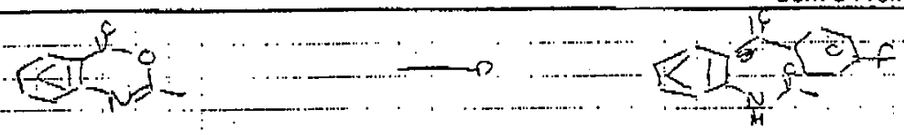
Exhibit L


1

Title-

Date 4-13-87 Proj.

Cont'd From- 341



$C_{17}H_{17}NO_2$ (161) 257

Benzoxazine $C_{15}H_{12}Cl_2NF$

1206-66-14

Ref: K. Suzuki et al JOC, 1981, 2239, 2241

(161.61) 1206-66-14 = 9.4 g (0.05816833 mole)

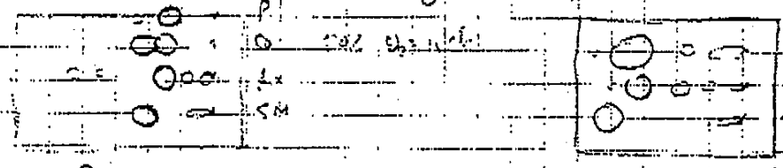
dry Benzene = 216 ml

(199.2) 2-oM F-C6H4-MgBr = 29.28 ml (0.05855585)

in ether

Benzoxazine in 216 ml benzene + 10 ml Et2O was cooled to 0°C (ice salt) via addition funnel added 2-oM F-C6H4-MgBr dropwise over 45 min. at 0°–10°C → yellow heterogeneous mix. Stirred for 1 hr at this temp then warmed up to r.t. stirred at r.t. overnight (1 hr – overnight)

4-14-87 2 PM → yellow orange heterogeneous mix quenched in 2 N HCl extracted with Et2O washed with brine dried filtered rotovap gave brown oil = 16.41 g (1206-86-27)



Sent for Prep LC Theory: 14.94 g

4-21-87 f8-9 = 9.63 g (1206-86-86) nmr, ir, ms mp: 258

Sample

5-4-87 micro 5-8-87

	C	H	N	O
Cal	70.02	4.70	5.44	12.84
found	70.1	4.46	5.40	

% = 64.45%

m.p. = 95°–97°C

Performed by- Roy Patel 4-14-87

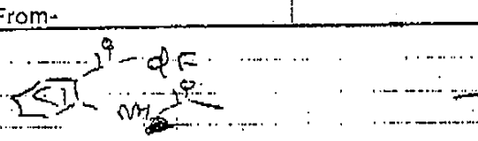
Witness- A Perez

Cont'd to-

ate 4-28-87 Proj. Title-

Cont'd From-

342



(257) (251) $C_3H_{11}NOFCl$

120C-99-26

257 (120C-86-36) = 9.5g (0.0369649 mole)

dry HCl

etOH ÷ 200ml

To 120C-86-36 in 200ml etOH (homogeneous light yellow) passed HCl gas for 15 min, He → dark yellow sol^n (homogeneous) Heated to reflux (1H - 2 hrs) → brownish homogeneous sol^n

Concentrated to dryness to give pinkish solids; diluted with ether filtered, washed with ether gives 8.5g pinkish solids (120C-99-26)

5-4-87 nmr, iv micro

m.p. = 172-175°C

	C	H	N	Cl
Calc	62.03	4-40	5.56	14.08
Found	61.91	4.24	5.54	14.32

Theory : 9.2g

% : 92.4%

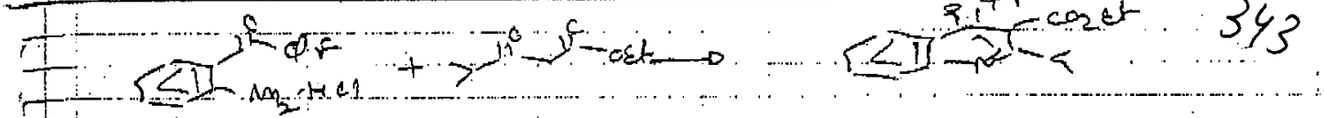
Performed by- Ray Patel 5-5-87

Witness- L Perez

Cont'd to-

Date 5-4-87 Proj. Title-

Cont'd From-



343

1206-99-26 237.2

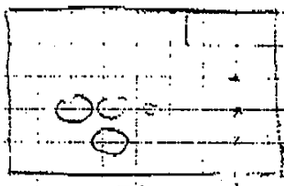
(25%)

(25%) 1206-99-26 = 7.5g (0.02968)

1582, d=0.981 = 7.25ml (0.04482 06) 1.5 eq

abs. ethyl = 5ml

Above mix was heated to reflux (9 45 A - 11 PM



5-5-87 Shaved out v.t.

5-5-87 Concentrated basified with NH_4OH, extracted with ether, washed with H_2O, brine, dried, filtered

5-11-87 Rotavap to dryness to give 8.00 g yellow oil (1206-103-28) hmw, ms mnt=338 solidified on standing.

Theory = 10.07 % yield = 79.5%

Performed by- Raj Patel 5-5-87

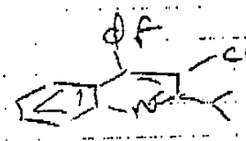
Witness- L Perez

Cont'd to-

Date 5-20-87 Proj. Title-

Cont'd From-

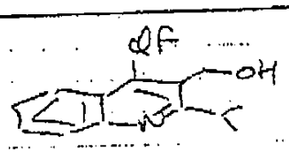
344



337.2

120G 103-28

LAH / ether



295.35

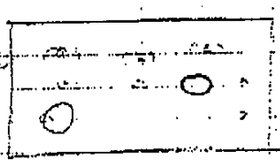
$C_{19}H_{18}NOF$

337.2 — 120G 103-28 = 8.0 g (0.023724 mole)

38 — LAH = 1.8 g (0.04744 mole) 2 eqv

ether = 90 ml

To 120G-103-28 in ether (yellow homogeneous) with cooling was added 1.8 g LAH portionwise at 15-20°C (exothermic). Stirred at r.t. (9 h - 12 h)



Added in cold H_2O, extracted with ether, washed with H_2O, brine, dried, filtered, washed, rotavap gave 8.0 g yellow solid (120G-119-26)

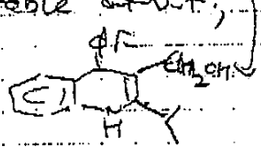
yellow solid (a) = 180.2 mg (120G-119-28)

orange yellow solid (b) = 294.8 mg (120G-119-29)

beige solid (c) = 5.0883 g (120G-119-30) mp, ir, ms, mass mh 296

turned to orange on standing (may be unstable amine, neat, Hmm. Store in refrigerator)

Theory: 7.0 g % = 72.5%



	C	H	N	O		
Calc	77.26	6.14	4.74			
Found	75.77	7.41	4.51			

Performed by- Ray Patel 5-27-87

Witness- L Perez

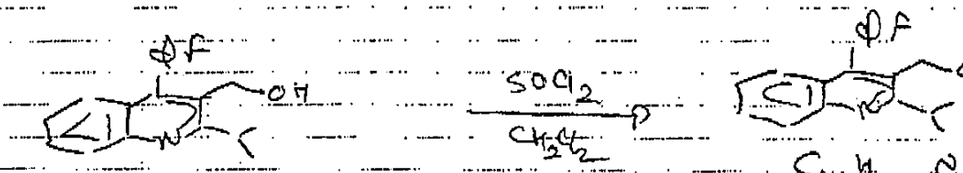
Cont'd to-

Title-

Date 5-26-87 Proj.

Cont'd From-

345



1206-119-30
(295)

$C_{19}H_{17}NFCl$
313

295 1206-119-30 = 5.0 g (0.0169491 mole)
$SOCl_2$ = 5 ml
anhy CH_2Cl_2 = 50 ml

Ref: 1206-110

1206-119-30 in anhy CH_2Cl_2 was cooled in ice bath (orange homogeneous sol'n) to 5°C. $SOCl_2$ at 15°-20°C with cooling → dark brown homogeneous sol'n was stirred at r.t. overnight.

5-22-87 Rotovap to dryness to give yellow foam (1206-124-64-87) basified with satd $NaHCO_3$ extracted with Et_2O was with H_2O, brine dried filtered washed, rotovap gave yellow solids wt: 4.25g (1206-124-26) mw 1½, ms m

Theory: 5.3g

% : 80.2%

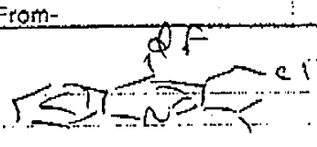
Performed by- Ray Peterson 5-22-87

Witness- L Perez

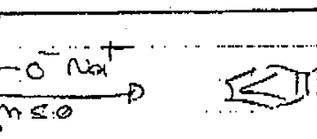
Cont'd to-

Date 7-15-87 Proj. Title-

Cont'd From-



313



399

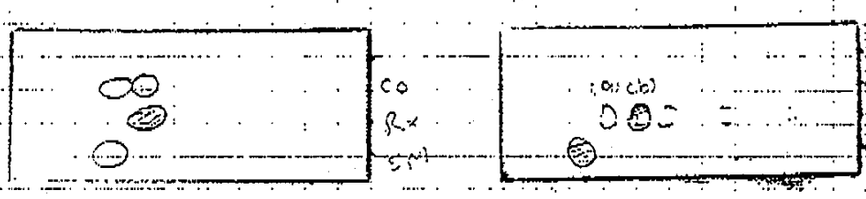
1206-124-26 = 3.7g (0.011821 mol)

Ph-C(=O)-C⁻ Na⁺ = 3.2g

DMSO = 75 ml

Ref: 1206-162

Above mixture was heated to 100°C (oil bath temp) for 1 hr.



quenched with H_2O, extracted with Et_2O, washed org. layer with H_2O, brine, dried, filtered, washed

7-17-87 rotavap gave yellow foam wt: 6.0206g (1206-167-2)

on drying at : 5.7503g mmr, ms

Theory: 4.7165g (98.6%) mmr

7-20-87

flash column (10% ethyl acetate/hex) gave

Mix. of (a)+(b) { F_{3-11} yellow foam = 3.48g (1206-167-35) MH^+ = 400

f_{12-31} yellow oil/foam = 630.4mg (1206-167-37) ms mmr

only (b) f_{32-76} yellow foam = 541.2mg (1206-167-39) ms mmr

Total: 4.65g (1206-167-41)

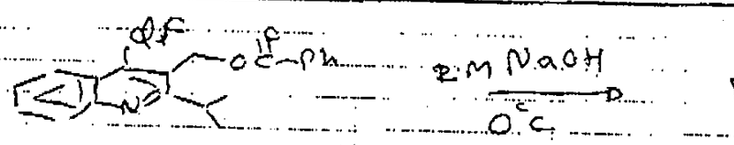
Performed by- Ray Patel 7-20-87

Witness- K Pierce

Cont'd to-

Date 7-28-87 Proj. Title- 173

Cont'd From-



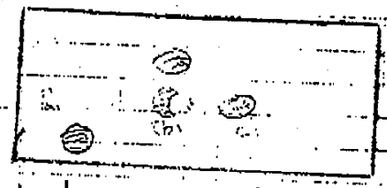
399 295 $C_{19}H_{18}NOF$

1206-167-48 = ~~5.75~~ 4.05g (0.01165 mole)
2M NaOH = 5.8ml (0.01165 mole)
Abs. EtOH = 60 ml + 11 ml

Ref: 1206-165

To 1206-167-48 in abs. EtOH at 0°C was added 5.8ml 2M NaOH, stirred at 0°C for 3 hrs (C_9 ~~at~~ - 12) → C yellow heterogeneous mix, lots of white solids came out on add^n of NaOH, ? most of which on stirring went in to sol^n)

Rohan



	C	H	N	O		
Calc.						
Found						

Concentrated to yellow solids, extracted with ether, washed with H_2O, brine, dried, filtered, rotavap gave yellow foam: 3.12g

3-23-87

Flash chromatography (25% Sh/Pet) gave

Theory: 3.49g (45%)
162 yellow solid = 1.5186g (1206-173-38) mw 328 = mH$^+$, ms
mmw
162 yellow foam = 2.1394g (1206-173-41) mH$^+$ = 296
+ oil (red) mmw ms mH$^+$ = 328

m.p.: 114°–116°C

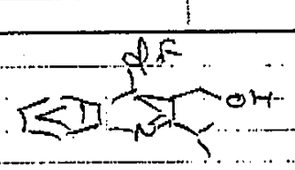
Performed by- Roy Patel 8-5-87

Witness- L Perez

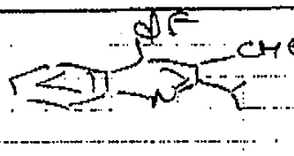
Cont'd to-

ate 7-24-87 Proj. Title-

ont'd From-



MnO_2



295

293

$C_9H_{17}NOF$

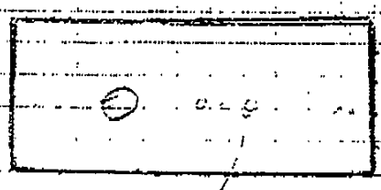
295 1206-173-39 = 105 mg (0.338 mmole)

MnO_2 = 200 mg

toluene = 2 ml

Ref: 1206-148

To 1206-173-39 in toluene was added MnO_2 & heated to reflux (115° - 5hr) Stirred at r.t. over weekend



C	H	O
77.2	8.15	
73.17	8.17	3.83
73.2	8.2	

Filtered thr' pad of silica gel, washed with toluene, ether, ratever gave yellow crystalline solids wt: 88mg (1206-173-33) mole mmr=293, mmr

Theory: 99 mg (89.6%) UV micro

m.p. = 115°-119°

Performed by- Raj Patel 8-5-87

Witness- A Perne

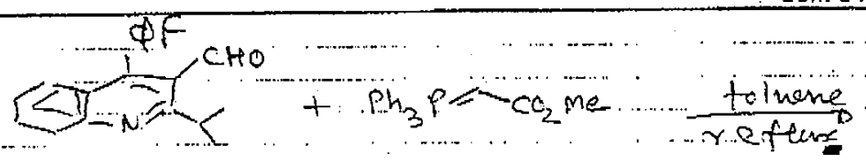
TOTAL P.05

180

Title-

Date 7-28-87 Proj.

Cont'd From-

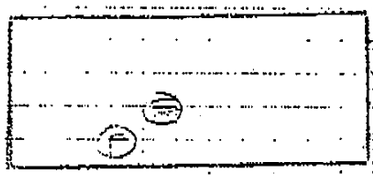


293

(293) 1206-177-33 = 75 mg (0.2559726 mmole)
(334) $Ph_3P{=}CO_2Me$ = 102.6 mg (0.3071671 mmole) 1.2eq
toluene = 2 ml EtOH

Ref: 1206-153

Above mix was heated to reflux (110°) for 1 hr.



	C	H	N	O	
Calc.					
Found					

Diluted with ~25 ml 50% EtOAc/Pet ether, filtered thru pad of silica gel (to remove phosphine oxide), washed with 50% EtOAc/Pet ether, Rotovap to dryness gave yellow solids which on trituration with MeOH gave 58.2 mg white solids (1206-180-34) mm, ms M_H^+ = 350

Theory: 89.3 mg (65%)

Rotovap MeOH layer triturate again with MeOH gave 12.7 mg yellow solids (1206-180-39) ms M_H^+ = 350
mp: (145-147°C dec) mins mm

3rd crop = 6.0 mg (1206-180-41) ms M_H^+ = 350

total: 58.2+12.7 = 70.9 mg (79.4%) (1206-180-42)

Performed by- Raj Patel 8-5-87

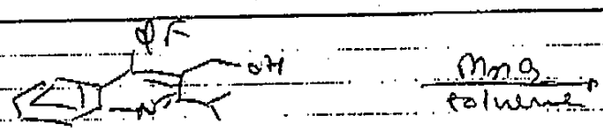
Witness- J Perez

Cont'd to-

178 Title-

Date 7-27-87 Proj.

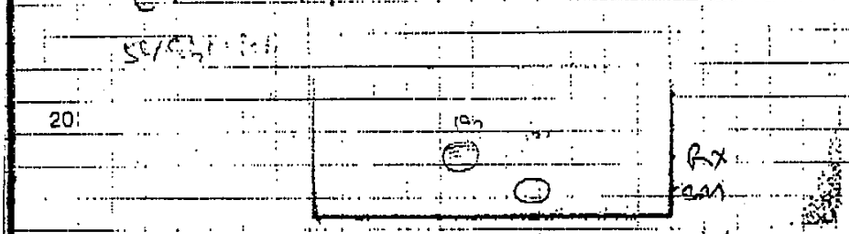
Cont'd From



295 293 $C_9H_{16}NOF$

(295) 120E 123-39 = 1.41 g (0.004796 mole)
MnO_2 = 2.82 g
toluene = 25 ml

Above mix was heated to reflux



stirred at r.t. overnight

7-28-87

Filtered thr' pad of silica gel washed with toluene & ether collected washings in two portions, Rotavap to dryness to give

(a) yellow crystalline solids = 930.8 mg (120G-178-31) mixture for yellow oil = 230 mg) nmr, ms
exact mass mH=294
obs. mass = 294.13008
theory: 1.4 g (66.4%) cal. mass = 294.12941

Seperated 210 mg oil on flash (25% Et_2O / hex) chrom gave

(a) yellow oil = 60.08 mg (120G-178-39) ms mH+=294
(b) white foam = 170 mg (120G-178-40) ms mH+=296

S.M.

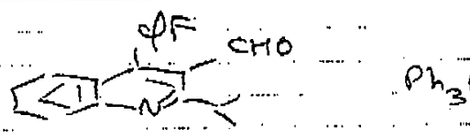
Performed by- Ray Patel 8.5-87

Witness- R Perez

Cont'd to-

7-298) Proj. Title- 351

d From-



293 $C_{25}H_{20}NO_2F$ 349

293 { 1206-178-31 = 910.8 mg } 3.313583G mmole
1206-178-39 = 60-08 }
334 $Ph_3P=CO_2Me$ = 1.328 gm (3.976300 3m mole)

toluene = 15ml

Ref: 1206-153, 180

Above mix. was heated to reflux
C16 3c A (12a)
TLC ⇒ No S.M. only P

Diluted with 5% EtOAc/Pet filtered thru pad of silica gel, washed with 5% EtOAc Pet, Rotavap to dryness, gave yellow solid which on trituration with MeOH gave light yellow solid
Wt = 1.1608 g (130C-181-26) mm, MS mm⁺ = 350

Theory = 1.1564 g

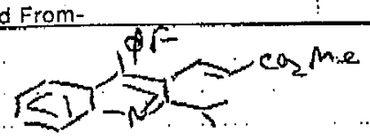
Performed by- Raj Patel 8-5-8

Witness- L Perez

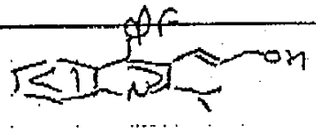
Cont'd to-

Date 8-3-87 Proj. Title-

Cont'd From-



1.5 M DIBAL-H Toluene

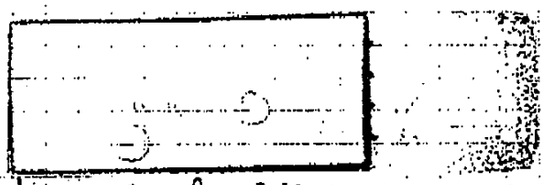


349 321

349 1206-181-26 = 1.1608 (0.003326 mole)
1.5 M DIBAL-H/Toluene = 4.4 ml (0.006652 mole)
CH_2Cl_2 = 20 ml

Ref: 1206-182

To 1206-182-26 in CH_2Cl_2 at -78°C was added 4.4 ml 1.5 M DIBAL-H (R_f 1/2 - 10/1)



Added 2.5 ml 2M NaOH warmed up to r.t., added EtOAc, dried over $MgSO_4$, filtered, washed Rotavap to dryness gave white foam wt: 1.1651 g. Flash chromatography (50 25:1 2:1 EtOAc/Hex) gave 1.0419 g yellow oil (1206-183-31) in mm Hg, MS m/e^+ = 322 exact mass

Theory: 1.0676 g m.p. = 79-84°C

8-6-87 exact mass: Obs'd mass: 321.1533
Calcd mass: 321.1528

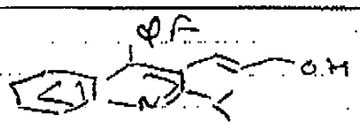
Performed by- Roy Patel 8-5-87

Witness- L Perez

Cont'd to-

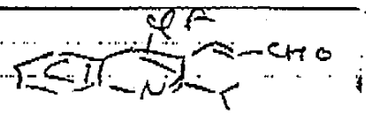
ate 8-4-87 Proj. Title-

Cont'd From-



321

MnO_2 / toluene

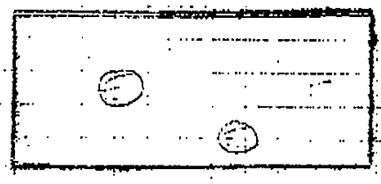


319.35
$C_{21}H_{18}NOF$

(321) 1206-183-31 = 1.019 g (0.0031484 mole)
MnO_2 = 2.02 g
toluene = 15 ml

Above mix. was heated to reflux for ½ hr. T.L.C. (50% EtOAc/Hex) ⇒ only desired product

50% EtOAc/Hex



	C	H	N	O	F	
calc.	78.48	5.69	4.36	4.98	5.96	
found	78.42	5.67				
	78.61	5.78	4.19			

Combined 1206-184. Cooled to r.t. filtered thru' pad of silica gel, washed with toluene, rotavap to dryness to give yellow solids

8-5-87 Wt.: 536 mg (1206-185-31) nmr, ms, micro IV

Theory: 1.00 g (53.6%) m.p. = 123-126°C

* By mistake combine 100 mg 1206-185-31 with 1206-174-40 ∴ Seperated on prep TLC (50% EtOAc/Hex)

(1) yellow oil: 45.5 mg (1206-185-40) ms, mp 123-125
(2) white solids: 43 mg (1206-185-41) ms, mp 320
(3) ← 1206-174-40

Performed by-

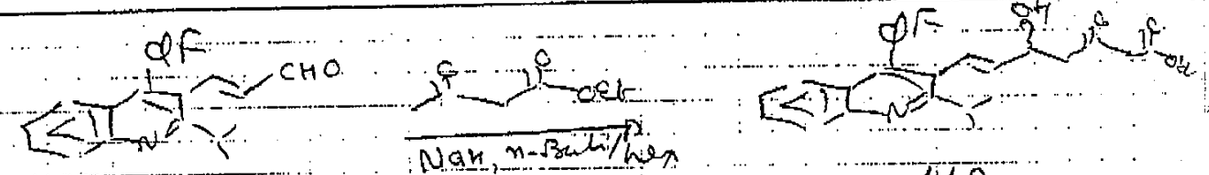
Witness- L Perez

Cont'd to-

186 Title-

Date 6-5-87 Proj.

Cont'd From-



319

449

$C_{27}H_{28}N_4F$

319 1206-185-31 = 450 mg (1.4106583 mmole)
(102.1, 130.14 Ethyl acetoacetate = 799.4 ml (6.269.5924 mmole)
24 50% NaH = 301 mg (" ")
1.6M n-Buli/hex = 4.18 ml (" " ")
THF = 1 amt 30 ml

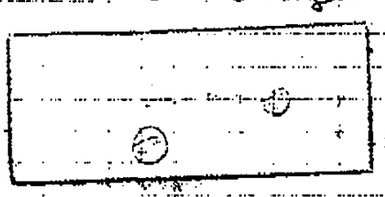
Ref: 1206-172

To a sol^n of 1206-185-31 in 30 ml THF was added at -20° to -15°C a sol^n of dianion total = 9.8 ml (*2.5 equiv.) prepared as below:

Dianion (* Need to use only ~~1.5 equiv~~ 2 equiv. by TLC.)

To sol^n of 799.4 ml Ethyl acetoacetate in 10 ml dry THF was added 301 mg 50% NaH at once at -15° to -10°C (foaming, H_2 gas evolved) → clear homogeneous sol^n stirred for 15 min. At -10° → -15°C was added 4.18 ml 1.5M n-Buli/Hex dropwise sol^n changed color to yellow homogeneous sol^n total vol. = 15.2 ml

Used in portions to get complete rxn by TLC color changed from light yellow to dark yellow to orange to dark yellow



Stirred at -20° to -15°C for 30 min. quenched with sat'd NH_4Cl & warmed up to r.t. extracted with Et$_2$O, washed with H_2O, brine, dried over $MgSO_4$, filtered, washed

Performed by- Raj Patel 8-5-87

Witness- R Perez

Cont'd to- 1206-187

Date 8-5-87 Proj. Title-

Cont'd From- C120G-186

rotavap to dryness gave yellow oil = 918 mg
C120G-187-21

(Theory: 633.38 mg) (74%)

Added ether to oil for flash chromatography (25% ether/Hex) → some solids crystalized out filtered solids washed with ether gave yellow crystals = 90.7 mg C120G-187-11 ms mp 150 mmu

Rotavap mother liquor to dryness to yellow oil flash chromatography of mother liquor (50% C2H5OAc/Hex) gave yellow solids = 378 mg C120G-187-15 ms in mmu mp 98 mmu mp = 101-103°C

total yield: 90.7 + 378 = 468.7 mg (120G-187-18)

	C	H	N	O	F	
Calc.	72.11	6.23	3.12	14.24	4.22	
Found	71.9	6.16	3.02			
	72.06	6.16	2.97			

obs. mass = 450.20831
calc. " = 450.20806

Performed by- Rajeshwari D. Patel 9-1-87

Witness

TOTAL P.14

49-111-0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	
	:	INTERFERENCE NO.: 102,648
V.		102,975
	:	EXAMINER-IN-CHIEF:
FUJIKAWA ET AL	:	MICHAEL SOFOCLEOUS

REQUEST FOR EXTENSION OF TIME

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

ATTENTION: EXAMINER-IN-CHIEF: URYNOWICZ
BOX INTERFERENCE

SIR:

Pursuant to the provision of Rules 635 and 645, Fujikawa hereby requests an extension of time in which to take cross-examination of Declarants in the above-captioned patent Interferences. The Junior Party has presented testimony with respect to priority in the above-captioned Interferences. The time for cross-examination expires December 15, 1992, and the parties have been unable to schedule a time convenient to complete cross-examination. The parties are in agreement that the cross-examination may run concurrently with the rebuttal testimony of the Senior Party, as well as the Senior Party's period for affidavit testimony, set to close February 25, 1993. Further, the parties

have agreed on a tentative date for cross-examination to begin of January 12, 1993. Accordingly, this extension of time is sought on good cause, will not require the rescheduling of any of the dates set in Paper No. 59 in Interference 102,648, or Paper No. 5 in Interference 102,975, and will facilitate timely completion of testimony.

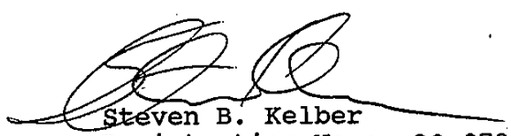
Counsel for Junior Party Wattanasin has discussed this Motion with undersigned Counsel, and the parties join in requesting this extension of time.

In the absence of EIC Sofocleous, the above proposal was discussed with EIC Urynowicz. The EIC indicated that on the above-stated grounds, this Motion would be granted. The assistance and cooperation of the EIC Urynowicz is deeply appreciated.

Accordingly, grant of this Motion, extending the time to take cross-examination testimony of the Junior Party's Affiants to February 25, 1993 is respectfully requested.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Steven B. Kelber
Registration No.: 30,073
Attorney for Fujikawa et al

CERTIFICATE OF SERVICE

I hereby certify that true copies of:

1. **REQUEST FOR EXTENSION OF TIME**

2. **CERTIFICATE OF SERVICE**

were served upon Counsel for Wattanasin as follows:

Diane E. Furman
SANDOZ CORP.
59 Route 10
E. Hanover, New Jersey 07936

via first-class mail, postage prepaid, this 10th day of December, 1992.

STEVEN B. KELBER

#68

Docket Number: 49-[illegible]

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	INTERFERENCE NUMBER: 102,648
		and
	:	INTERFERENCE NUMBER: 102,975
V.	:	EXAMINER-IN-CHIEF:
FUJIKAWA ET AL	:	MICHAEL SOFOCLEOUS

FUJIKAWA ET AL REQUEST FOR CROSS-EXAMINATION OF DECLARANT WATTANASIN

RECEIVED
DEC 7 1992
BOARD OF PATENT APPEALS
AND INTERFERENCES

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231
BOX INTERFERENCE

Sir:

Pursuant to the Decision of the EIC (Paper Number 59 in the '648 Interference, Paper Number 5 in the '975 Interference) counsel for Fujikawa et al hereby files its *pro forma* Request for Cross-Examination of Declarant Wattanasin, submitted pursuant to the provisions of Rule 672.

Undersigned counsel has already talked to counsel for the Junior Party, and has agreed that the deposition may be conducted at headquarters of the assignee in interest, East Hanover, New Jersey.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Norman F. Oblon
Attorney of Record
Registration Number 24,618

Steven B. Kelber
Attorney of Record
Registration Number 30,073

Fourth Floor
1755 Jefferson Davis Highway
Arlington, Virginia 22202
(703) 521-5940

CERTIFICATE OF SERVICE

I hereby certify that true copies of:

1. **FUJIKAWA REQUEST FOR CROSS-EXAMINATION OF DECLARANT WATTANASIN**

2. **CERTIFICATE OF SERVICE**

were served upon Counsel for Wattanasin as follows:

Diane E. Furman
SANDOZ CORP.
59 Route 10
E. Hanover, New Jersey 07936

via first-class mail, postage prepaid, this 7th day of December, 1992.

STEVEN B. KELBER

49-111-0

#69

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	
	:	INTERFERENCE NO.: 102,648
V.	:	EXAMINER-IN-CHIEF:
FUJIKAWA ET AL	:	MICHAEL SOFOCLEOUS

**FUJIKAWA NOTICE OF INTENT
TO ARGUE ABANDONMENT, SUPPRESSION OR CONCEALMENT -
37 CFR §1.632**

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231
BOX INTERFERENCE

SIR:

Pursuant to the provisions of the above Rule, Fujikawa hereby serves notice it intends to argue that Wattanasin, Junior Party, has abandoned, suppressed or concealed whatever actual reduction to practice of the Count of the above Interference is made out by the priority evidence submitted by Wattanasin.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Steven B. Kelber
Registration No.: 30,073
Attorney for Fujikawa et al

Fourth Floor
1755 South Jefferson Davis Highway
Arlington, Virginia 22202
703-521-5940

CERTIFICATE OF SERVICE

I hereby certify that true copies of:

1. **FUJIKAWA NOTICE OF INTENT TO ARGUE ABANDONMENT, SUPPRESSION OR CONCEALMENT - 37 CFR §1.632**

2. **CERTIFICATE OF SERVICE**

were served upon Counsel for Wattanasin as follows:

Diane E. Furman
SANDOZ CORP.
59 Route 10
E. Hanover, New Jersey 07936

via first-class mail, postage prepaid, this 15th day of December, 1992.

STEVEN B. KELBER

Case No. [illegible]0-7101/CONT/Int.
Patent

#70

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN

v.

FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

NOTICE OF DEPOSITION PURSUANT TO 37 CFR §§1.672(b), 1.673(e)

The party Wattanasin hereby serves notice that the party Fujikawa shall take cross-examination by oral deposition of the following affiant for the party Wattanasin on the date and at the place below-indicated:

Affiant:	Sompong Wattanasin, Ph.D.
Date:	Tuesday, January 12, 1993
Location:	Sandoz Pharmaceuticals Corporation Patent and Trademark Department 25 Hanover Road Building b Florham Park, NJ 07936

Undersigned counsel for Wattanasin certifies that the above represents the mutual agreement of the parties reached in oral conference.

Respectfully submitted,

Diane Furman

Diane E. Furman
Attorney for the Party Wattanasin
Registration No. 31,104
201-503-7332

SANDOZ CORPORATION
59 Route 10
East Hanover, NJ 07936

DEF:rmf

December 11, 1992

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on December 11, 1992
(Date of Deposit)
Diane E. Furman
Name of applicant, assignee, or Registered Representative
Diane Furman
Signature
December 11, 1992
Date of Signature

CERTIFICATE OF SERVICE

It is hereby certified that a true copy of the paper entitled:

NOTICE OF DEPOSITION PURSUANT TO 37 CFR §§1.672(b), 1.673(e)

was served on counsel for the party Fujikawa et al., this 11th day of December, 1992, by postage pre-paid first-class mail addressed to the following:

Oblon, Spivak, McClelland, Maier & Neustadt, P.C.
Attn: Steven B. Kelber, Esq.
1755 South Jefferson Davis Highway
Crystal Square 5, Ste. 400
Arlington, VA 22202

Diane Furman 12/11/92
Diane E. Furman

No. 102648
Vol. (III)
pps. 71-

Sofocleous
EXAMINER IN CHIEF

INTERFERENCE

Vol. III
pps. 71-

Wattanasin S.N. 07/498,301

v.

Picard et al. P.N. 4,761,419

v.

Fujikawa et al. S.N. 07/233,752

Quinoline Type Mevalonolac-
Tones

Group 1201

PTO-257

102648
VOL. (III)
PPS. 71

102648

ATTORNEYS

Wattanasin v. Picard et al. v. Fujikawa et al.
(Patentee)

DECLARATION, MOTIONS DUE

1 .....
2 .....
3 .....
4 .....
5 .....
6 .....
7 .....
8 .....
9 .....
10 .....
11 .....
12 .....
13 .....
14 .....
15 .....
16 .....
17 .....
18 .....
19 .....
20 .....
21 .....
22 .....
23 .....
24 .....
25 .....
26 .....
27 .....
28 .....
29 .....
30 .....

★ U.S. GOVERNMENT PRINTING OFFICE 1992-327-206

#71

49-111-0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN :

: INTERFERENCE NO.: 102,648
: 102,975

V. : EXAMINER-IN-CHIEF:

FUJIKAWA ET AL : MICHAEL SOFOCLEOUS

APPROVED

DEC 21 1992

By ..... Examiner-in-Chief

REQUEST FOR EXTENSION OF TIME

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

ATTENTION: EXAMINER-IN-CHIEF: URYNOWICZ
BOX INTERFERENCE

SIR:

Pursuant to the provision of Rules 635 and 645, Fujikawa hereby requests an extension of time in which to take cross-examination of Declarants in the above-captioned patent Interferences. The Junior Party has presented testimony with respect to priority in the above-captioned Interferences. The time for cross-examination expires December 15, 1992, and the parties have been unable to schedule a time convenient to complete cross-examination. The parties are in agreement that the cross-examination may run concurrently with the rebuttal testimony of the Senior Party, as well as the Senior Party's period for affidavit testimony, set to close February 25, 1993. Further, the parties

#72

Case No. 000-7101/CONT/INT.
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

WATTANASIN MOTION FOR LEAVE TO PRESENT ADDITIONAL TESTIMONY
37 CFR §1.635, §1.651(c)(4)

In response to the Fujikawa "Notice of Intent to Argue Abandonment, Suppression or Concealment - 37 CFR §1.632" dated December 15, 1992, in the above-captioned interferences, the party Wattanasin hereby requests leave to present additional testimony in connection with the allegations set forth in said notices.

Specifically, Wattanasin respectfully moves for designation of a testimony period for Wattanasin to present evidence by deposition or affidavit going to the absence of abandonment, suppression or concealment of the Wattanasin invention. In particular, the period of January 4, 1993 to February 1, 1993 is suggested.

REMARKS

The status of the above-captioned interferences is as follows:

Testimony-in-chief of the party Wattanasin, originally set to close December 15, 1992, has been extended for purposes of cross-examination to February 25, 1993 (See Paper No. 71, Int. No. 102,648; Paper No. 16, Int. No. 102,975).

Therefore, by prior agreement of the parties and with the approval of the EIC, the period for the Wattanasin testimony-in-chief is already set to run concurrently with the Fujikawa et al. rebuttal and affidavit testimony period, i.e. to February 25, 1993.

The party Wattanasin has presented its testimony with respect to the issue of priority during the Wattanasin affidavit testimony period, which closed November 15, 1992.

The EIC will note that Wattanasin, as junior party, has adduced for the record, for priority purposes, documentation of activities relating to an actual reduction to practice of the Wattanasin invention from prior to the Fujikawa priority date of August 20, 1987 up to a date of about December 9, 1987, which is approximately 15 months prior to the filing of the Wattanasin Rule 60 parent application on March 3, 1989. (See Record, pp. 110; 340).

Fujikawa et al. in their Rule 632 notices have now raised for the first time in these interferences an allegation of abandonment, suppression or concealment of the Wattanasin invention.

As a first matter, these Fujikawa notifications are wholly devoid of specificity or particularity as to the basis for the allegation of abandonment, suppression or concealment.

Therefore, Wattanasin requests, first of all, that the party Fujikawa indicate with specificity the basis for its allegation of abandonment, suppression or concealment.

Secondly, Wattanasin respectfully requests an opportunity to defend against the allegation of abandonment, supression or concealment of the Wattanasin invention by taking additional testimony in connection therewith. (Preferably, the substantive basis for said allegation will be sufficiently defined by Fujikawa et al. on a timely basis to permit Wattanasin to present testimony responsive thereto.)

Under the circumstances, it is believed consistent with the purpose of 37 CFR §1.632 -- that is, to foster full and fair adjudication of the issue of abandonment, suppression or concealment (see MPEP 2332)-- to afford Wattanasin an opportunity at this time to present such additional evidence.

Furthermore, given the fact that the period of the Wattanasin testimony-in-chief has already been extended to February 25, 1993 for purposes of cross-examination, it is not believed that designation of an additional Wattanasin testimony period to run from January 4, 1993 to February 1, 1993 would require rescheduling of either the above date of February 25, 1993, or of any of the other dates set forth in Paper No. 59 in Interference No. 102,648 or Paper No. 5 in Interference No. 102,975.

Therefore, in the interest of justice in permitting Wattanasin to adequately respond to the charge of abandonment, etc. now being raised for the first time by Fujikawa et al., and without foreseeably affecting the testimony periods already set and agreed to by the parties, it is respectfully requested that the EIC designate a period for Wattanasin testimony on the issue of abandonment, suppression or concealment of the Wattanasin invention.

Undersigned counsel for Wattanasin has today conferred with Mr. Steven Kelber, counsel for Fujikawa et al., who has indicated that the party Fujikawa will oppose this motion. However, there is agreement by counsel for the parties that the cross-examination of Dr. Sompong Wattanasin, now set for January 12, 1993, may be rescheduled for another time in the Wattanasin testimony period depending on the disposition of this motion.

Accordingly, grant of this motion to set a period for additional Wattanasin testimony on the issue of abandonment, suppression or concealment of the Wattanasin invention, preferably to run from January 4, 1993 to February 1, 1993, is respectfully requested.

Respectfully submitted,

Diane Furman

Diane E. Furman
Attorney for the Party Wattanasin
Registration No. 31,104
201-503-7332

SANDOZ CORPORATION
59 Route 10
East Hanover, NJ 07936
DEF:rmf
December 31, 1992

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on December 31, 1992
(Date of Deposit)
Diane E. Furman
Name of applicant, assignee, or Registered Representative
Diane Furman
Signature
Dec. 31, 1992

CERTIFICATE OF SERVICE

It is hereby certified that a true copy of the paper entitled:

WATTANASIN MOTION FOR LEAVE TO PRESENT ADDITIONAL TESTIMONY
37 CFR §1.635, §1.651(c)(4)

was served on counsel for the party Fujikawa et al., this 31st day of December 1992, by postage pre-paid first-class mail addressed to the following:

Oblon, Spivak, McClelland, Maier & Neustadt, P.C.
Attn: Steven B. Kelber, Esq.
1755 South Jefferson Davis Highway
Crystal Square 5, Ste. 400
Arlington, VA 22202

Diane Furman
Diane E. Furman

RECEPTION OK

TN #	5440
CONNECTION TEL	9
CONNECTION ID	G3
START TIME	01/08 12:21
USAGE TIME	03' 53
PAGES	6

Case No. 600-7101/CONT/INT.
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

AFFIRMATION OF FILING AND SERVICE

I hereby certify that on December 31, 1992 the below-indicated paper:

WATTANASIN MOTION TO PRESENT ADDITIONAL TESTIMONY
37 CFR §1.635, §1.651(c)(4)

a copy of which is attached hereto, was deposited with the United States Postal Service as first-class mail, in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, Box Interference.

I also certify that on December 31, 1992, a copy of the above paper was also served on counsel for the party Fujikawa et al., by postage pre-paid first-class mail directed to the following address:

Oblon, Spivak, McClelland, Maier & Neustadt, P.C.
Attn: Steven B. Kelber, Esq.
1755 South Jefferson Davis Highway
Crystal Square 5, Ste. 400
Arlington, VA 22202

This paper and its attachment are being telefaxed this 8th day of January 1993, to:

The U.S. Patent Office, Box Interference, (703) 557-8642, attention M. Sofocleous (EIC); and

Counsel for the party Fujikawa et al., at (703) 413-2220.

Respectfully submitted,

Diane Furman

Diane E. Furman
Attorney for the Party Wattanasin
Registration No. 31,104
201-503-7332

SANDOZ CORP.
59 Route 10
E. Hanover, NJ 07936
Att: 5 pages
January 8, 1993

49-111-0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	
	:	INTERFERENCE NO.: 102,648
V.		INTERFERENCE NO.: 102,975
	:	EXAMINER-IN-CHIEF:
FUJIKAWA ET AL	:	MICHAEL SOFOCLEOUS

FUJIKAWA ET AL OPPOSITION TO
WATTANASIN'S MOTION FOR LEAVE TO PRESENT
ADDITIONAL TESTIMONY

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

BOX INTERFERENCE

SIR:

Fujikawa opposes Wattanasin's Motion for a new testimony period, in which to present additional testimony, apparently related to the issues of abandonment, suppression or concealment. It is respectfully submitted that the Wattanasin Motion, presented pursuant to the provisions of 37 CFR §1.651(c)(4) is procedurally inadequate, and substantively in error. Accordingly, the Motion must be dismissed, or in the alternative, denied. Each of these

arguments is developed, sequentially, below.

I. FACTS

In Paper No. 59 (Interference 102,648) and Paper No. 5 (Interference 102,975), both mailed September 22, 1992, Junior Party Wattanasin was given a two and one-half month testimony period for its case-in-chief. That period closed December 15, 1992.

Prior to December 15, 1992, Wattanasin presented its testimony in the form of Declarations, as to which Fujikawa requested the opportunity for cross-examination. The parties have agreed to extend the period for cross-examination. The parties have not agreed to extend Wattanasin's period for testimony-in-chief.

On December 15, 1992, in accordance with the provisions of 37 CFR §1.632, Fujikawa filed Notice of its Intention to Argue Abandonment, Suppression or Concealment at final hearing, based on the testimony-in-chief presented by the Junior Party. Wattanasin does not complain that the Notice is in any way in error, or procedurally improper.

Apparently, on December 31, 1992, Wattanasin filed a Motion for Leave to Present Additional Testimony. That Motion is alleged

to be responsive to the Notice of Intention to Argue Abandonment, Suppression or Concealment filed December 15, 1992. The Motion was not received by undersigned Counsel until January 7, 1993, in response to a call made by undersigned Counsel to Counsel for Wattanasin, inquiring as to the status of the Motion proposed in an earlier teleconference.

II. ARGUMENT

A. The Wattanasin Motion is Procedurally Inadequate

Wattanasin's Motion is respectfully submitted to fail to meet the standards of the Rules. Specifically, although Wattanasin requests an additional testimony period, Wattanasin fails to describe the evidence it desires to present during that additional testimony period, save to describe it as "going to the absence of abandonment, suppression or concealment of the Wattanasin invention." See the Motion, page 1. Wattanasin does not indicate what type of testimony it will present, nor the particulars of that testimony. Fujikawa respectfully submits that not only is identification of the specific testimony sought to be presented by Wattanasin a prerequisite to the relief sought, but support for the ability of Wattanasin to present such testimony, confirmed by

appropriate Declaration, would be desirable.

It is well established that all Interference Motions, including those presented pursuant to 37 CFR §1.635, must set forth both a statement of the material facts in support of the Motion, and a full statement of the reasons why the relief requested should be granted. Specificity and particularity is important in proceeding pursuant to 37 CFR §1.637(a), which is specifically cross-referenced in Rule 635. The requirement for specificity is substantial. Jacobs v. Moriarity, 6 USPQ 2d 1799, 1801-1802 (PBAI 1988). A review of the Wattanasin Motion reveals it to be devoid as to any details of the testimony sought to be presented. It is not clear whether the testimony will be presented via deposition or affidavit. Indeed, the Motion requires both. See page 1. If presented via affidavit, it will require additional time in which to take cross-examination. Moreover, and of greater importance, the Motion fails to indicate what facts Wattanasin will attempt to prove. Indeed, the Motion is devoid of even a bare assertion that Wattanasin can adduce any evidence responsive to the issue of abandonment, suppression or concealment. Surely, such is a prerequisite prior to the extraordinary testimony period sought by Wattanasin.

It is respectfully submitted that it has long been the case

that in order to secure an extraordinary testimony period, beyond that originally provided for proof of priority, the movant should set forth the facts to be proven, and desirably accompany the Motion by affidavit sufficient to establish the movant's ability to prove the same. Revise & Caesar, Interference Law and Practice, Section 458, page 1962 (1947). This long-standing directive finds contemporary echoes in the decision Hanagan v. Kimura, 16 USPQ 2d 1791 (Comm. of Pats. 1990). Specifically, like Wattanasin herein, the party Kimura filed a Motion for permission to take testimony in a period the movant would not otherwise be entitled to. In the Motion, Kimura explained, in some detail, the nature of the testimony sought to be presented. 16 USPQ at 1792. Although the Motion was decided pursuant to the provisions of 37 CFR §1.639(c), there does not appear to be grounds for applying a different standard to Rule 639 and Rule 651. Indeed, Rule 651 has a "good cause" requirement not present in Rule 639, which presumably would require a higher standard. Note the petition for a testimony period was denied in Hanagan, for, inter alia, failure to describe the facts to be presented, identify the individuals to be called, and the absence of any declaration stating the factual testimony of the individuals to be presented. 16 USPQ 2d at 1794.

For failure to meet the simple standard of proof required of

a Motion, Fujikawa respectfully submits the Wattanasin Motion for an Additional Testimony Period must be dismissed.

B. If not Dismissed, the Wattanasin Motion must be Denied

As the sole "good cause" for an additional testimony period, Wattanasin appears to be urging that the filing of a Notice under Rule 632 automatically gives Wattanasin an opportunity to present additional testimony. The sole authority Wattanasin relies on is M.P.E.P. 2332. Neither that section, nor any other statute, regulation or case decision supports the conclusion that the appropriate response to the Notice required by 37 CFR §1.632 is the reopening of testimony. Specifically, testimony should be reopened only where the issue of abandonment, suppression of concealment comes as a surprise to the Junior Party. Nothing of the sort has been demonstrated in the current Interference.

Indeed, Wattanasin's Motion makes it quite clear that prior to the close of Wattanasin's testimony period, Counsel for Wattanasin was aware that Wattanasin's proof of priority ended approximately fifteen months prior to the filing of the Wattanasin effective filing date of March 3, 1989. See the Wattanasin Motion, page 2. Accordingly, Wattanasin was on notice, prior to the close of its

testimony period, that there was a fifteen-month delay between its proof of reduction to practice and the date for filing of a patent application. Well prior to Wattanasin's testimony in this case, it had been established that a fifteen-month delay is not per se reasonable, absent mitigating facts. Engelhard Corp. v. M.C. Canfield Sons, 13 USPQ 2d 1561 (DC NJ 1989).

M.P.E.P. 2332 indicates that Rule 632 was instituted to avoid surprise at the briefing stage. Indeed, section 2332 makes it clear that under prior practices, the Junior Party would not be aware of arguments relative to abandonment, suppression or concealment until receipt of the Senior Party's brief, a point in time at which it would be too late for the Junior Party to contest the issue. Suh v. Hoefle, 23 USPQ 2d 1321 (PBAI 1992). Wattanasin does not even allege the presence of surprise in this case, which might warrant the reopening of testimony addressed in the M.P.E.P. section referred to.

Rather, Wattanasin appears to be in the position of the party seeking a reopening of testimony in Issidorides v. Lay, 4 USPQ 2d 1854, 1859 (PBAI 1987). Specifically, Wattanasin was aware of the large hole in its proof, but decided to take the risk that Fujikawa would either not see that hole, or not take the appropriate action. Having rested its evidence with knowledge of a fifteen-month

hiatus, and electing to run the risk with respect thereto, Wattanasin cannot now be heard to reopen testimony for the purpose of presenting evidence that is not even fairly described in the Wattanasin Motion. Quite simply, there is no support in the rules or law for this repeated attempt at a bite at the apple.

Wattanasin can hardly be ignorant of the requirement that a party attempting to rely on an earlier conception and reduction to practice, such as Wattanasin, must prove that earlier invention was by one "who had not abandoned, suppressed or concealed it." 35 U.S.C. §102(g), first sentence. Thus, Wattanasin knew the task it had to meet, and quite simply elected to risk the silence of its proof as to its extended hiatus with regard to the invention in question, apparently in hopes that Fujikawa would not raise the same as an issue. While Wattanasin now suggests that it can present the necessary proofs, the type of proof to be presented is not even hinted at in the Motion. It would be highly inappropriate to present such evidence in reply to this opposition, as the provisions of 37 CFR §1.637 must be met in the motion itself, not the reply.

The requirement of presentation of good cause to reopen testimony period is hardly new. See Turner v. Bensinger, 1903 CD 53, 102 OG 1552 (Comm. 1902) and Brill v. Ubelades, 1902 CD 220, 99

OG 2966 (Comm. 1902). That requirement is codified in 37 CFR §1.651(c). Wattanasin ignores it.

While the precise issue of whether or not a filing of a notice pursuant to Rule 632 automatically gives the opponent the right to an additional testimony period does not appear to have been addressed, the legislative history of the rules, and prior case law is instructive. It was the intention of the drafter of Rule 632 that:

> Early notice will eliminate the need for the party moving to reopen the testimony period.

49 FR 48416 (December 12, 1984). This is true even though it is clear that a notice under Rule 632 is timely even if filed ten days after the period for testimony closes. 57 FR 2698. Quite clearly, both sides are on notice, absent some surprise not alleged in the Wattanasin Motion, that in those cases where abandonment, suppression or concealment may be proved by the absence of any activity on the part of the Junior Party for a substantial period of time, that abandonment may be an issue if appropriately raised pursuant to Rule 632.

As noted above, Wattanasin does not indicate the nature of the

proof it intends to submit, much less whether or not it was available during Wattanasin's original testimony period. It is respectfully submitted that prior case law indicates that if the material could have been presented during the original testimony period, the excuse of surprise, that the party seeking a new testimony period did not realize it would be required, is insufficient. Weber v. Kunz, 209 USPQ 864, 866 (POBI 1980). To the same effect Weber v. Kunz, 211 USPQ 637, 638-639 (POBI 1980) holding that the party's original showing should be as complete as possible.

The Interference decision in Rexroth v. Gunther, 202 USPQ 837, 838 (POBAI 1978) specifically deals with a party's request to present evidence responsive to the issue of abandonment, suppression of concealment. Specifically, the opportunity to respond by the presentation of evidence is not granted where the Junior Party had knowledge that the issue might be raised. Clearly, Wattanasin, having recognized the substantial hiatus in its own evidence without any indication of the same from Fujikawa, was aware that the issue might be raised. Further, the burden was on Wattanasin to explain this hiatus initially, as the burden is always on the inventor to explain an unreasonable or excessive delay. Horwath v. Lee, 195 USPQ 701 (CCPA 1977). As a general

matter, in this and other cases, additional testimony of the type sought to be presented by Wattanasin is permitted only where all possible steps have been taken to make sure that such testimony is presented in the original period provided for. Davis v. Reddy, 191 USPQ 866, 867 (POBI 1976).

Accordingly, Wattanasin was on notice during its original testimony period that it had the burden to explain the substantial, and per se, unreasonable delay between its alleged reduction to practice, and its effective filing date. Wattanasin does not indicate it was unaware of that burden, or of the hiatus in the proof offered. Wattanasin does not make any showing that it attempted to prove activity during the period in question, and was unable to, or indeed even assert that the testimony it now seeks to present was unavailable during its period for testimony-in-chief. Having failed to described with particularity the testimony Wattanasin now seeks to present, and failed to present good cause as to why it could not have earlier been presented, Wattanasin's Motion for a new testimony period must be denied. The same is respectfully requested.

C. Requiring Wattanasin to Specify its Argument is Improper

Apparently, Wattanasin finds in the rules a requirement for a

Senior Party seeking to preserve its opportunity to argument abandonment, suppression or concealment not self-evident from 37 CFR §1.632. Specifically, pages 2-3 of its Motion, Wattanasin indicates that the burden is on Fujikawa to provide the necessary:

> Specificity or particularity as to the basis for the allegation of abandonment, suppression or concealment.
>
> Therefor, Wattanasin requests, first of all that the party Fujikawa indicate with specificity the basis for its allegation of abandonment, suppression or concealment.
>
> Secondly, Wattanasin respectfully requests an opportunity to defend against the allegation of abandonment, suppression or concealment of the Wattanasin invention by taking additional testimony in connection therewith (preferably the substantive basis for said allegation will be sufficiently defined by Fujikawa et al on a timely basis to permit Wattanasin to present

testimony responsive thereto.

The final sentence of the above quotation is a non sequitur. Wattanasin is seeking a testimony period, yet it does not even know what the testimony it seeks to present is! This, in itself, is grounds for denying the Wattanasin Motion. In any event, there is absolutely no support, any where, for the argument that Fujikawa must provide additional specificity to support its Notice under Rule 632. Indeed, Rule 632 is just that, a "notice" provision, to avoid surprise. As noted above, the burden rests on Wattanasin to present a full proof in accordance with the provisions of 35 U.S.C. §102(g), either the first or second sentence. Fujikawa is obligated only to give notice that it takes issue with the adequacy of Wattanasin's proof in this regard, and Wattanasin concedes that Fujikawa has indeed done so. More is not required of Fujikawa.

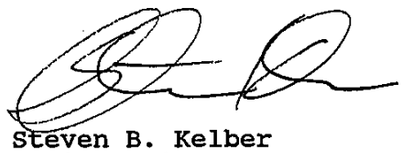
D. Summary

Having failed to specify, with any particularity at all, what type of evidence Wattanasin seeks to present, having failed to establish that Wattanasin could not have presented the evidence it now seeks to present during its testimony-in-chief, having conceded that it was aware of the fifteen-month gap in proof offered in its

testimony-in-chief, and having conceded that it is not even yet aware of what type of proof it will offer (see Section C above), Wattanasin has failed to present the good cause and compelling argument required by the rules for an additional testimony period. Accordingly, the Motion must be dismissed, or in the alternative, denied.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Steven B. Kelber
Registration No.: 30,073
Attorney for Fujikawa et al

Fourth Floor
1755 South Jefferson Davis Highway
Arlington, Virginia 22202
703-521-5940

CERTIFICATE OF SERVICE

I hereby certify that true copies of:

1. **FUJIKAWA ET AL OPPOSITION TO WATTANASIN'S MOTION FOR LEAVE TO PRESENT ADDITIONAL TESTIMONY**

2. **CERTIFICATE OF SERVICE**

were served upon Counsel for Wattanasin as follows:

Diane E. Furman
SANDOZ CORP.
59 Route 10
E. Hanover, New Jersey 07936

via first-class mail, postage prepaid, this 13TH day of JANUARY, 1993.

STEVEN B. KELBER

Attorney Docket No.: 49-111-0
49-125-0 DIV

#74

49-111-0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	
	:	INTERFERENCE NO.: 102,648
V.	:	EXAMINER-IN-CHIEF:
FUJIKAWA ET AL	:	MICHAEL SOFOCLEOUS

NOTICE, 37 CFR §1.671(a)

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

BOX INTERFERENCE

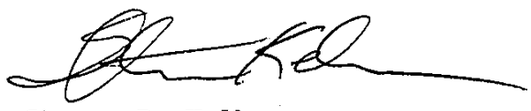
SIR:

Pursuant to the provisions of the above-captioned Rules, Fujikawa hereby serves notice of its intention to rely on the Affidavit of Masaki Kitahara - Patentably Distinct Subject Matter, and the Supplemental Declaration of Kitahara, filed and served June 11 and August 11, 1992, respectively. As copies of both Declarations have been served, the Declarations are deemed filed

pursuant to 137 CFR §1.672(b).

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Steven B. Kelber
Registration No.: 30,073
Attorney for Fujikawa et al

Fourth Floor
1755 South Jefferson Davis Highway
Arlington, Virginia 22202
703-413-3000

CERTIFICATE OF SERVICE

I hereby certify that true copies of:

1. NOTICE, 37 CFR §1.671(a)

2. NOTICE, 37 CFR §1.682 WITH REFERENCES IDENTIFIED BELOW:

1. Medicinal Research Reviews, Vol. 11, No. 2, 121-146 (1991)
2. J Med. Chem. 1990, 33, No. 1, 21-31
3. J Med. Chem. 1990, 33, No. 1, 31-38
4. J Med. Chem. 1990, 33, No. 1, 52-60
5. J Med. Chem. 1990, 33, No. 1, 61-70
6. J Med. Chem. 1990, 33, No. 2, 758-765
7. J Med. Chem. 1991, 34, No. 1, 357-366
8. J Med. Chem. 1991, 34, No. 1, 367-373
9. J Med. Chem. 1991, 34, No. 9, 2804-2815

3. CERTIFICATE OF SERVICE

were served upon Counsel for Wattanasin as follows:

Diane E. Furman
SANDOZ CORP.
59 Route 10
E. Hanover, New Jersey 07936

via first-class mail, postage prepaid, this 1ST day of FEBRUARY, 1993.

STEVEN B. KELBER

#75

49-111-0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	
	:	**INTERFERENCE NO.: 102,648**
V.		
	:	**EXAMINER-IN-CHIEF:**
FUJIKAWA ET AL	:	**MICHAEL SOFOCLEOUS**

NOTICE, 37 CFR §1.682

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

BOX INTERFERENCE

SIR:

Fujikawa et al hereby serves notice pursuant to the provisions of 37 CFR §1.682 that the following printed publications are introduced into evidence:

1. Medicinal Research Reviews, Vol. 11, No. 2, 121-146 (1991)
2. J Med. Chem. 1990, 33, No. 1, 21-31
3. J Med. Chem. 1990, 33, No. 1, 31-38
4. J Med. Chem. 1990, 33, No. 1, 52-60
5. J Med. Chem. 1990, 33, No. 1, 61-70
6. J Med. Chem. 1990, 33, No. 2, 758-765

7. J Med. Chem. 1991, 34, No. 1, 357-366

8. J Med. Chem. 1991, 34, No. 1, 367-373

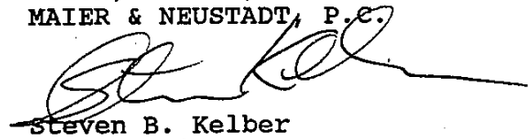
9. J Med. Chem. 1991, 34, No. 9, 2804-2815

The publications 1-9 referenced above are relevant to the issue of actual reduction to practice, and conception, of the subject matter of the Count in the above-captioned Interference. Specifically, these publications relate to measurements of the activity of specific HMG-CoA reductase inhibitors, the demonstration of which is a prerequisite to demonstration of an actual reduction to practice, purportedly shown by the Junior Party in the above-captioned Interference.

Pursuant to the provisions of Rule 682(a)(4) and Rule 682(b), copies of the publications identified above accompany this notice, and have been served on the Junior Party.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Steven B. Kelber
Registration No.: 30,073
Attorney for Fujikawa et al

Fourth Floor
1755 South Jefferson Davis Highway
Arlington, Virginia 22202
703-413-3000

(B⁺); IR (KBr) 3600–3000 (NH_2, OH), 1750, 1600 cm^{-1} (C≡C, C=N); UV λ_{max} 253 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 11.05–10.95 (s, 1 H, 7-OH, D_2O exchangeable), 7.10–6.90 (br, 2 H, NH_2, D_2O exchangeable), 4.95–4.80 (m, 1 H, H-1′), 4.70–4.50 (br, 1 H, CH_2OH, D_2O exchangeable), 3.50–3.40 (d, 2 H, CH_2OH), 2.32–1.55 (m, 7 H, H-4′, CH_2CH_2, CHH′). Anal. ($C_{10}H_{14}N_6O_2 \cdot 1.25H_2O$) C, H, N.

(±)-*cis*-[4-(5,7-Diamino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)-2-cyclopentenyl]carbinol (11a). Compound 9a (267 mg, 1 mmol) was processed as described for compound 6a with a reaction time of 20 h at 60 °C. The residual mixture was absorbed onto silica gel (2 g); it was packed into a column (2.0 × 10 cm) and eluted by $CHCl_3$–MeOH (15:1) to yield 11a as white crystals, 204 mg (83%). The crude product was recrystallized from ethanol–water (2:1) to yield 11a: mp 240–242 °C dec; MS (30 eV, 240 °C) *m/e* 247 (M^+), 229 ($M^+ - 18$), 217 ($M^+ - 30$), 151 (B^+); IR (KBr) 3600–3100 (NH_2, OH), 1700, 1650, 1600 cm^{-1} (C=O, C=C, C=N); UV λ_{max} 253, 283 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 7.80–7.20 (br, 2 H, NH_2, D_2O exchangeable), 6.50–6.30 (s, 2 H, NH_2, D_2O exchangeable), 6.15–6.10 and 5.95–5.90 (dd, 2 H, CH=CH vinyl, J = 5.0 Hz), 5.65–5.55 (m, 1 H, H-1′), 4.75–4.65 (t, 1 H, CH_2OH, D_2O exchangeable), 3.55–3.40 (m, 2 H, CH_2OH), 2.95–2.85 (m, 1 H, H-4′), 2.65–2.55 (m, 1 H, CHH′), 1.90–1.80 (m, 1 H, CHH′). Anal. ($C_{10}H_{13}N_7O \cdot H_2O$) C, H, N.

(±)-*cis*-[3-(5,7-Diamino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)cyclopentyl]carbinol (11b). Compound 9b (268 mg, 1 mmol) was processed as described for 9a to yield 220 mg of 11b (88%), which was recrystallized from ethanol–water (1:2) to afford pink-white crystals: mp 223–225 °C; MS (30 eV, 250 °C) *m/e* 249 (M^+), 218 ($M^+ - 31$), 151 (B^+); IR (KBr) 3600–3100 (NH_2, OH), 1700, 1600 cm^{-1} (C=C, C=N); UV λ_{max} 253, 283 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 7.85–7.25 (br, 2 H, NH_2, D_2O exchangeable), 6.50–6.30 (s, 2 H, NH_2, D_2O exchangeable), 4.95–4.85 (m, 1 H, H-1′), 4.65–4.60 (t, 1 H, CH_2OH, D_2O exchangeable), 3.50–3.40 (d, 2 H, CH_2OH), 2.35–1.60 (m, 7 H, H-4′, CH_2CH_2, CHH′). Anal. ($C_{10}H_{15}N_7O$) C, H, N.

Acknowledgment. This work was supported by Public Health Service Grant CA23263 from the National Cancer Institute. We gratefully acknowledge the valuable assistance of Jay Brownell.

Registry No. 1a, 61865-50-7; 1b, 65898-98-8; 2a, 122624-72-0; 2b, 78795-20-7; 3a, 122624-73-1; 3b, 122624-74-2; 4a, 122624-75-3; 4b, 122624-76-4; 5a, 122624-77-5; 5b, 122624-78-6; 6a, 118237-87-9; 6b, 118237-86-8; 7a, 118353-05-2; 7b, 112915-00-1; 8a, 118237-88-0; 8b, 120330-36-1; 9a, 122624-79-7; 9b, 122624-80-0; 10a, 122624-81-1; 10b, 122624-82-2; 11a, 122624-83-3; 11b, 122624-71-9; 2-amino-4,6-dichloropyrimidine, 56-05-3; *p*-chloroaniline, 106-47-8.

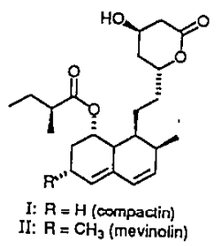
Inhibitors of Cholesterol Biosynthesis. 1. *trans*-6-(2-Pyrrol-1-ylethyl)-4-hydroxypyran-2-ones, a Novel Series of HMG-CoA Reductase Inhibitors. 1. Effects of Structural Modifications at the 2- and 5-Positions of the Pyrrole Nucleus

B. D. Roth,* D. F. Ortwine,* M. L. Hoefle, C. D. Stratton, D. R. Sliskovic, M. W. Wilson, and R. S. Newton

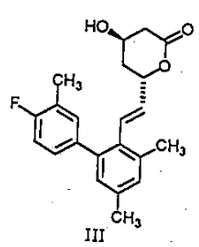
Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105. *Received January 25, 1989*

A novel series of *trans*-6-(2-pyrrol-1-ylethyl)-4-hydroxypyran-2-ones and their dihydroxy acid derivatives were prepared and evaluated for their ability to inhibit the enzyme HMG-CoA reductase in vitro. A systematic study of substitution at the 2- and 5-positions of the pyrrole ring revealed that optimum potency was realized with the 2-(4-fluorophenyl)-5-isopropyl derivative 8x (Table III), which possessed 30% of the in vitro activity of the potent fungal metabolite compactin (I). A molecular modeling analysis led to the description of a pharmacophore model characterized by (A) length limits of 5.9 and 3.3 Å for the 2- and 5-substituents, respectively, as well as an overall width limit of 10.6 Å across the pyrrole ring from the 2- to the 5-substituent and (B) an orientation of the ethyl(ene) bridge to the 4-hydroxypyran-2-one ring nearly perpendicular to the planes of the parent pyrrole, hexahydronaphthalene, and phenyl rings of the structures examined (Figure 3, θ = 80–110°). Attempts to more closely mimic compactin's polar isobutyric ester side chain with the synthesis of 2-phenylpyrroles containing polar phenyl substituents resulted in analogues (Table III, 8m–p) with equal or slightly reduced potencies when compared to the 2-[(unsubstituted or 4-fluoro)phenyl]pyrroles, supporting the hypothesis that inhibitory potency is relatively insensitive to side-chain polarity or charge distribution in this area.

The discovery that the fungal metabolites compactin (I)[1] and mevinolin (II)[2] are not only potent inhibitors of the enzyme HMG-CoA reductase (HMGR), the rate-limiting enzyme in cholesterol biosynthesis, but are also effective hypocholesterolemic agents in man[3] has led to a plethora of publications describing synthetic and biological studies of close structural analogues.[4]



I: R = H (compactin)
II: R = CH_3 (mevinolin)



III

The disclosure of a series of very potent 6-(*o*-biphenylyl)-substituted 4-hydroxypyran-2-ones (III) by Willard et al.[5] led us to hypothesize that the key structural

(1) (a) Endo, A.; Kuroda, M.; Tsujita, Y. *J. Antibiot.* 1976, 1346–8. (b) Endo, A.; Kuroda, Y.; Tanzawa, K. *FEBS Lett.* 1976, *72*(2), 323–6. (c) Brown, A. G.; Smale, T. C.; King, T. J.; Hassenkamp, R.; Thompson, R. H. *J. Chem. Soc., Perkin Trans. 1* 1976, 1165–9.

(2) (a) Endo, A. *J. Antibiot.* 1979, *32*, 852. (b) Alberts, A.; Chen, J.; Kuron, G.; Hunt, V.; Huff, J.; Hoffman, C.; Rothrock, J.; Lopez, M.; Joshua, H.; Harris, E.; Pachett, A.; Monaghan, R.; Currie, S.; Stapley, E.; Albers-Schonberg, G.; Hensens, O.; Hirshfield, J.; Hoogsteen, K.; Liesch, J.; Springer, J. *Proc. Natl. Acad. Sci. U.S.A.* 1980, *77*(7), 3957–61.

(3) (a) Therapeutic response to Lovastatin (Mevinolin) in Non-Familial Hypercholesterolemia. *J. Am. Med. Assoc.* 1986, *256*, 2829. (b) Vega, L.; Grundy, S. *J. Am. Med. Assoc.* 1987, *257*(1), 33–38 and references contained therein.

(4) For a review, see: Rosen, T.; Heathcock, C. *Tetrahedron* 1986, *42* (18), 4909–51.

0022-2623/90/1833-0021$02.50/0

Scheme I[a]

Method A

$$R_1COCH{=}CH_2 + R_2CHO \xrightarrow{a} R_1COCH_2CH_2COR_2$$
1 2 3

Method B

$$R_2COCH_2CO_2CH_3 \xrightarrow{b,c} 3$$

[a] (a) 3-Benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride, Et_3N, 70 °C. (b) NaH, R_1COCH_2Br. (c) NaOH, CH_3OH.

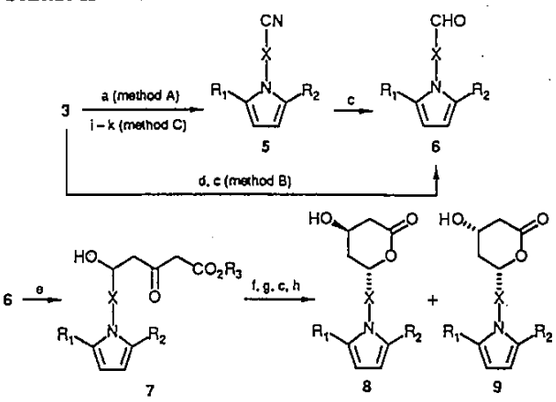
feature possessed by all of these agents was a large lipophilic group held in a particular spatial relationship with respect to the 4-hydroxypyran-2-one moiety. Indeed, examination of CPK models of these inhibitors suggested that the ortho phenyl ring might occupy the same space as the isobutyric ester moiety of compactin and mevinolin. This hypothesis is supported by the 100-fold loss in potency found on hydrolysis of the isobutyric ester group,[6] as well as the suggestion by Nakamura and Abeles that this portion of mevinolin fits into a lipophilic pocket in the active site of HMGR normally occupied by coenzyme A.[7] If this were true, then any connecting group that served to hold the lactone and the lipophilic moiety in the correct spatial relationship might be sufficient for potent inhibition. To investigate this, we selected the pyrrole ring as the anchor for various connecting groups, since there appeared to be sufficient synthetic methodology to allow for the simultaneous introduction of a variety of 2- and 5-substituents. By varying the steric and electronic properties of these substituents, modifying the connecting group, and employing a molecular modeling analysis, we hoped to discern, at least in part, the optimal spatial relationship between the lipophilic group and the 4-hydroxypyran-2-one moiety and use this information in the design of potent HMGR inhibitors.

We herein present our initial investigations into this series of inhibitors that define the structure–activity relationships at the 2- and 5-positions of the pyrrole nucleus and in the connecting group to the lactone ring. Also reported is the molecular modeling study and associated pharmacophore model, which describe conformational requirements of the side chain and steric requirements at the 2- and 5-positions of the pyrrole ring.

Chemistry

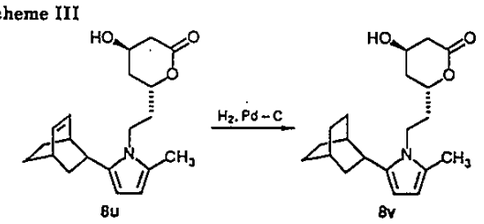
Our general synthetic strategy entailed the preparation of a suitable 1,4-diketone (3, Table I), either by the thiazolium salt chemistry developed by Stetter (Scheme I, method A)[8] or by alkylation of a β-keto ester with an α-halo ketone followed by hydrolysis and decarboxylation (method B). The Stetter reaction proved to be the more versatile and generally higher yielding of the two. Paal–Knorr cyclization with 3-aminopropionitrile or an ω-amino acetal provided the pyrroles in good yield (Scheme II). The one exception was 1-(4-fluorophenyl)-5,5-dimethyl-

Scheme II[a]

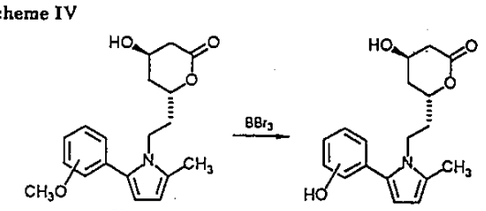


[a] (a) H_2N–X–CN, HOAc, reflux. (b) DIBAL-H, toluene, −78 °C. (c) aqueous HCl. (d) H_2N–X–$CH(OEt)_2$, toluene, cat. *p*-TSA, reflux. (e) $^-CH_2CO^-CHCH_3CH_3$, THF, −78 °C. (f) *n*-Bu_3B, $NaBH_4$, −78 °C. (g) H_2O_2, OH^-. (h) Toluene, reflux. (i) H_2N–X–OH, HOAc. (j) CH_3SO_2Cl, pyr. (k) KCN, DMF–H_2O, 100 °C.

Scheme III



Scheme IV



hexane-1,4-dione (3q), which was extremely resistant to cyclization. After considerable experimentation, it was found that treatment with ethanolamine in acetic acid resulted in an exothermic reaction from which the pyrrole was isolated in 84% yield. Mesylation and displacement with potassium cyanide in DMF/H_2O afforded the requisite nitrile. Reduction of the nitriles 5 with DIBAL-H produced the desired aldehydes 6 in good yields (Table II). Condensation of 6 with the dianion of methyl or ethyl acetoacetate under the conditions of Weiler[9] afforded the corresponding alcohols 7. Sih et al.[10] reported the reduction of a related δ-hydroxy-β-keto ester in their synthesis of compactin in which little stereoselectivity (2:1 erythro:threo) was found employing either sodium or zinc borohydride. We, and others,[5b] have found excellent selectivity (>10:1 erythro:threo) employing the procedure of Narasaka and Pai,[11] in which 7 was complexed with a trialkylborane prior to treatment with borohydride at low temperature. The resultant boronate was hydrolyzed with

(5) (a) Willard, A.; Novello, F.; Hoffman, W.; Cragoe, E. USP 4459422. (b) Stokker, G.; Hoffman, W.; Alberts, A.; Cragoe, E.; Deana, A.; Gilfillan, J.; Huff, J.; Novello, F.; Prugh, J.; Smith, R.; Willard, A. *J. Med. Chem.* 1985, *28*, 347–358. (c) Stokker, G. E.; Alberts, A. W.; Anderson, P. S.; Cragoe, E. J.; Deana, A. A.; Gilfillan, J. L.; Hirshfield, J.; Holtz, W. J.; Hoffman, W. F.; Huff, J. W.; Lee, T. J.; Novello, F. C.; Prugh, J. D.; Rooney, C. S.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* 1986, *29*, 170–181.

(6) Endo, A. *J. Med. Chem.* 1985, *28*, 401–5.

(7) Nakamura, C.; Abeles, R. *Biochemistry* 1985, *24*, 1364–76.

(8) (a) Stetter, H. *Angew. Chem., Int. Ed. Engl.* 1976, *15*, 639. (b) Stetter, H.; Kuhlmann, H. *Chem. Ber.* 1976, *109*, 2890. (c) Stetter, H.; Schreckenberg, M. *Chem. Ber.* 1974, *107*, 2453. (d) Stetter, H.; Kuhlmann, H. *Synthesis* 1975, 379.

(9) Huckin, S. N.; Weiler, L. *J. Am. Chem. Soc.* 1974, *96*, 1082–1087.

(10) Wang, N. Y.; Hsu, C. T.; Sih, C. J. *J. Am. Chem. Soc.* 1981, *103*, 6538–6539.

(11) (a) Narasaka, K.; Pai, H. C. *Chem. Lett.* 1980, 1415–1418. (b) Ibid. *Tetrahedron* 1984, *40*, 2233–2238.

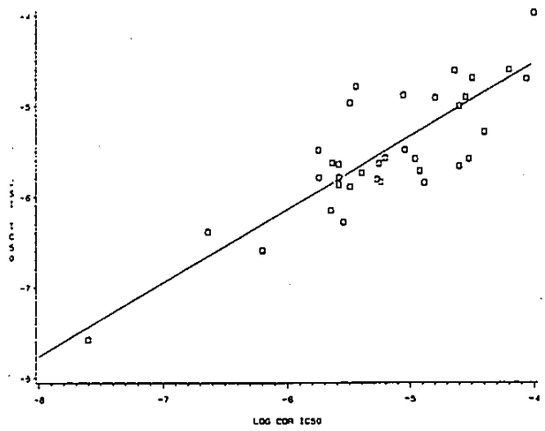


Figure 1. Correlation between CSI and COR IC_{50}'s.

Table I. Substituted 1,4-Diketones

$R_1COCH_2CH_2COR_2$
3

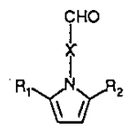
no.	R_1	R_2	bp (mmHg), °C	% yield[a] (procedure)
3a[5b]	Ph	CH_3	100 (0.1)	80 (A)
3b	4-*F*C_6H_4	CH_3	46–8	66 (A)
3c	4-PhC_6H_4	CH_3	109–112	73 (A)
3d[5c]	4-ClC_6H_4	CH_3	116–8 (1.0)	44 (A)
3e[5a]	4-$CH_3OC_6H_4$	CH_3	*b*	57 (A)
3f	3-$F_3CC_6H_4$	CH_3	*b*	38 (A)
3g	3-$CH_3OC_6H_4$	CH_3	143–5 (0.2)	80 (A)
3h	2-$CH_3OC_6H_4$	CH_3	133–5 (1.0)	51 (A)
3i	2-naphthyl	CH_3	87–8	55 (A)
3j	1-naphthyl	CH_3	105 (0.1)	83 (A)
3k		CH_3	114–6 (1.0)	76 (A)
3l		CH_3	*b*	98 (A)
3m[5d]	cyclohexyl	CH_3	110 (4)	88 (A)
3n	Ph_2CH	CH_3	*b*	61 (A)
3o	4-FC_6H_4	C_2H_5	*b*	89 (A)–55 (B)
3p	4-FC_6H_4	$CH(CH_3)_2$	133–5 (1.0)	58 (A)
3q	4-FC_6H_4	$C(CH_3)_3$	108–9 (0.2)	56 (A)
3r	4-FC_6H_4	$CH(C_2H_5)_2$	132–3 (0.2)	54 (A)
3s	4-FC_6H_4	cyclopropyl	*b*	75 (A)
3t	4-FC_6H_4	cyclobutyl	132–5 (1.0)	65 (A)
3u	4-FC_6H_4	cyclohexyl	150–5 (0.1)	51 (A)
3v	4-FC_6H_4	CF_3	*b*	25 (B)
3w	$CH(C_2H_5)_2$	$CH(C_2H_5)_2$	79–83 (0.2)	53 (A)
3x	3-FC_6H_4	$CH(CH_3)_2$	*b*	90 (B)
3y	2-FC_6H_4	$CH(CH_3)_2$	*b*	95 (A)
3z	2,4-$F_2C_6H_3$	$CH(CH_3)_2$	*b*	77 (A)
3aa	2-$CH_3OC_6H_4$	$CH(CH_3)_2$	138–141 (0.2)	71 (A)
3bb	2,6-$(CH_3O)_2C_6H_3$	$CH(CH_3)_2$	160–2 (2)	68 (B)

[a] All spectral data were consistent with assigned structures. [b] Purified by silica gel chromatography.

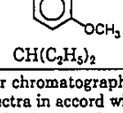
aqueous peroxide and base.[12] The dihydroxy acids were then lactonized by refluxing in toluene with azeotropic removal of water. Generally, the lactones were crystalline, such that the small amounts of the cis lactone stereoisomer 9 present were easily removed by recrystallization, providing >95% of the racemic trans stereoisomer (8). The conversion of 8u to 8v was accomplished by hydrogenation over Pd–C at 1 atm (Scheme III). Finally, the phenol analogues 8k, 8h, and 8p were prepared from the corresponding methyl ethers 8i, 8m, and 8o by BBr_3-mediated demethylation (Scheme IV).[13]

(12) A detailed examination of this reaction has appeared: Kathawala, F.; Prager, B.; Prasad, K.; Repic, O.; Shapiro, M.; Stabler, R.; Widler, L. *Helv. Chim. Acta* 1986, *69*, 803–5.

Table II. 2,5-Disubstituted Pyrrol-1-yl Carbox- or Benzaldehydes



6

no.	X	R_1	R_2	% yield[a,b] (method)
6a		4-FC_6H_4	CH_3	63 (A)
6b		4-FC_6H_4	CH_3	56 (A)
6c		4-FC_6H_4	CH_3	35 (A)
6d	$-CH_2CH_2CH_2-$	4-FC_6H_4	CH_3	65 (A)
6e	$-CH(CH_3)CH_2-$	4-FC_6H_4	$CH(CH_3)_2$	34 (C)
6f	$-CH_2CH_2-$	4-FC_6H_4	CH_3	45 (A)
6g	$-CH_2CH_2-$	Ph	CH_3	27 (A)
6h	$-CH_2CH_2-$	4-PhC_6H_4	CH_3	60 (A)
6i	$-CH_2CH_2-$	4-$CH_3OC_6H_4$	CH_3	32 (A)
6j	$-CH_2CH_2-$	4-ClC_6H_4	CH_3	56 (A)[c]
6k	$-CH_2CH_2-$	3-$F_3C_6H_4$	CH_3	37 (A)
6l	$-CH_2CH_2-$	3-$CH_3OC_6H_4$	CH_3	68 (A)
6m	$-CH_2CH_2-$	2-$CH_3OC_6H_4$	CH_3	58 (A)
6n	$-CH_2CH_2-$	2-naphthyl	CH_3	50 (A)
6o	$-CH_2CH_2-$	1-naphthyl	CH_3	23 (A)
6p	$-CH_2CH_2-$	cyclohexyl	CH_3	60 (A)
6q	$-CH_2CH_2-$		CH_3	63 (A)
6r	$-CH_2CH_2-$		CH_3	22 (A)
6s	$-CH_2CH_2-$	Ph_2CH	CH_3	32 (A)
6t	$-CH_2CH_2-$	4-FC_6H_4	$CH(CH_3)_2$	92 (A)
6u	$-CH_2CH_2-$	4-FC_6H_4	$C(CH_3)_3$	42 (C)
6v	$-CH_2CH_2-$	4-FC_6H_4	$CH(C_2H_5)_2$	46 (A)
6w	$-CH_2CH_2-$	4-FC_6H_4	cyclopropyl	25 (A)
6x	$-CH_2CH_2-$	4-FC_6H_4	cyclobutyl	34 (A)
6y	$-CH_2CH_2-$	4-FC_6H_4	cyclohexyl	22 (A)[d]
6z	$-CH_2CH_2-$	4-FC_6H_4	CF_3	55 (A)
6aa	$-CH_2CH_2-$	3-FC_6H_4	$CH(CH_3)_2$	29 (A)
6bb	$-CH_2CH_2-$	2-FC_6H_4	$CH(CH_3)_2$	17 (A)
6cc	$-CH_2CH_2-$	2,4-$F_2C_6H_3$	$CH(CH_3)_2$	20 (A)
6dd	$-CH_2CH_2-$	2-$CH_3OC_6H_4$	$CH(CH_3)_2$	42 (A)
6ee	$-CH_2CH_2-$	2,6-$(CH_3O)_2C_6H_3$	$CH(CH_3)_2$	36 (A)[e]
6ff	$-CH_2CH_2-$	2,5-$(CH_3)_2C_6H_3$	$CH(CH_3)_2$	43 (A)
6gg	$-CH_2CH_2-$	2-[$(CH_3)_2CHO$]C_6H_4	$CH(CH_3)_2$	79 (A)
6hh	$-CH_2CH_2-$	2-ClC_6H_4	$CH(CH_3)_2$	46 (A)
6ii	$-CH_2CH_2-$		$CH(CH_3)_2$	41 (C)
6jj	$-CH_2CH_2-$	$CH(C_2H_5)_2$	$CH(C_2H_5)_2$	60 (A)

[a] Isolated yields after chromatography on silica gel. [b] All compounds possessed ^1H NMR spectra in accord with assigned structure (aldehydic proton, singlet, δ 8.95–9.65). [c] Mp 70–3 °C. [d] Mp 104–6 °C. Anal. C, H, N. [e] Mp 105–7 °C. Anal. C, H, N.

Biological Results

The target lactones (8, Table III) were saponified and tested for their ability to inhibit HMGR employing two protocols. Method I[14] (cholesterol synthesis inhibition screen, or CSI) measured the rate of conversion of [^{14}C]-

(13) McOmie, J.; Watts, M.; West, D. *Tetrahedron* 1968, *24*, 2289.
(14) Dugan, R.; Slakey, L.; Briedis, A.; Porter, J. *Arch. Biochim. Biophys.* 1972, *152*, 21–7.

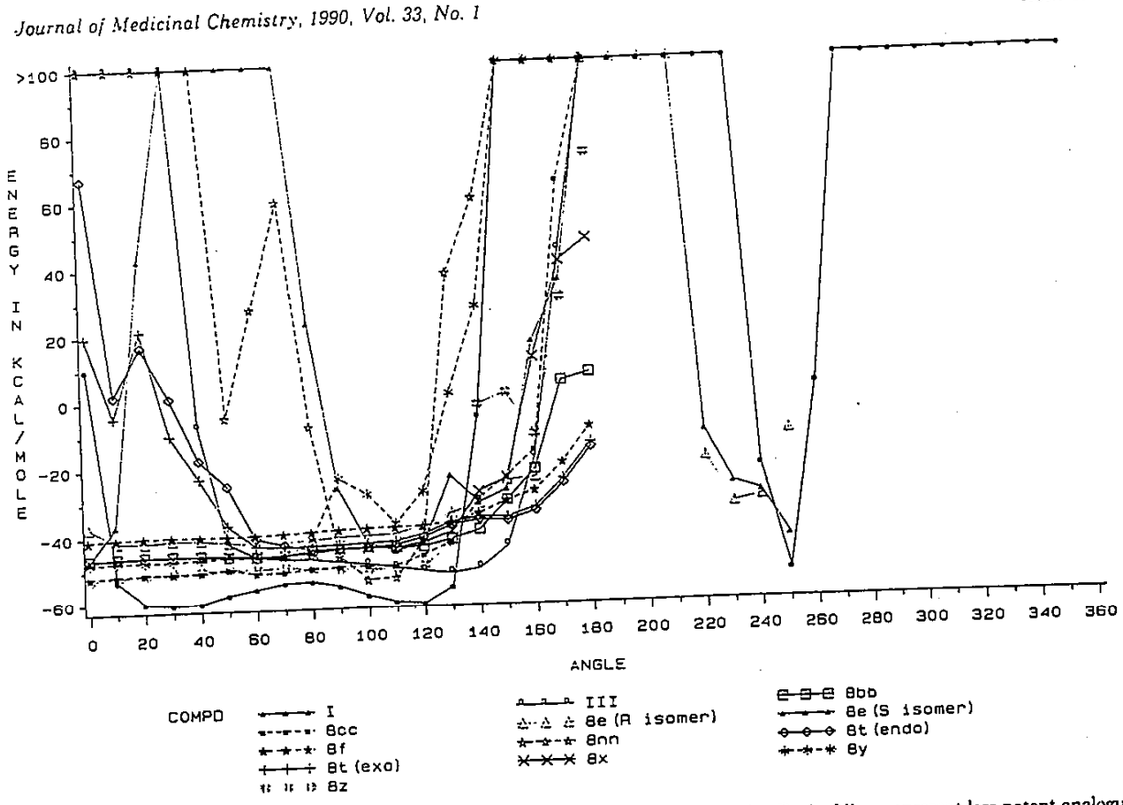


Figure 2. CAMSEQ-II energies calculated for comparable orientations of the lactone side chain. Dashed lines represent less potent analogues (8j, 8z, 8bb, 8cc, and 8nn; CSI $IC_{50} > 5$ μM).

acetate to cholesterol employing a crude liver homogenate derived from rats fed a chow diet containing 5% cholestyramine. Method II[15] (CoA reductase inhibition screen, or COR) was a more specific screen employing a partially purified microsomal enzyme preparation to measure the direct conversion of D,L-[^{14}C]HMG-CoA to mevalonic acid. The biological activities are reported as IC_{50} values and as a ratio to compactin, which was employed as the internal standard in each testing protocol. Compactin consistently displayed an IC_{50} between 0.02 and 0.03 μM. The IC_{50} values from the two assays were moderately correlated (eq 1,[16] Figure 1).

$$\log (IC_{50}, \text{COR}) = 0.81\ (\pm 0.09) \log (IC_{50}, \text{CSI}) - 1.32 \quad (1)$$

$$n = 36,\ r^2 = 0.70,\ F = 81,\ s = 0.39$$

Structure–Activity Relationships

As very little was known about heterocycle-containing inhibitors at the outset of this study, our strategy was to systematically examine each portion of the structure, keeping the 4-hydroxypyran-2-one ring intact. Initially, the optimum chain length between the lactone and the pyrrole ring was determined. A two-carbon bridge (8f) was superior to either a three-carbon (8d) or aryl spacer (8a–c) (Table III). This is consistent with the findings of Stokker et al.[5b]

Holding the bridge constant as ethyl, the structure–activity relationships of the 2 and 5 pyrrole substituents were explored. With 5-methyl substitution (8f–w), high potency was conferred by bulky cycloalkyl 2-substitutents (8s–v). Among 2-(substituted-phenyl)-5-methyl derivatives (8f–r), aside from a length limitation of the 2-substituent (see the molecular modeling section below), no obvious structure–activity relationships could be discerned. Optimum potency resided in the 4-fluorophenyl analogue, 8f. With 2-substitution held constant as the optimal 4-fluorophenyl, potency increased with increasing length of the 5-substituent from methyl (8f) through cyclopentyl (8aa) to a maximum with isopropyl (8x) (length = 2.5 Å; see modeling section below). Potency decreased thereafter to a low of >100 μM with 5-cyclohexyl substitution (8cc).

With 5-substitution held constant as the optimal isopropyl, additional variation of the 2-phenyl substituents, now keeping within the length limit of 5.9 Å suggested by the modeling analysis (8ee–mm), failed to improve the potency over the 2-(4-fluorophenyl)-5-isopropyl derivative, 8x. Indeed, an additional "front-to-back" width limitation (Figure 3) may be apparent with 8ii and 8mm, which project significantly greater bulk in these directions than the other analogs. Finally, of interest is the 2-(4-fluorophenyl)-5-trifluoromethyl analogue 8dd, whose high potency may be due in part to stabilization of the pyrrole ring by the electron-withdrawing trifluoromethyl group, an aspect to be addressed in future communications.

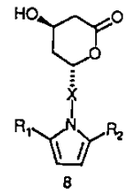
These results, combined with results from the molecular modeling study, confirmed our belief that 8x possessed the optimum substitution pattern, since structural modifications at the 2- and 5-positions, as well as variation of the bridge to the lactone ring, led to decreased potency. A similar conclusion can be inferred from the examination of other 5-membered ring heterocycles reported in the patent literature.[17]

(15) Kita, T.; Brown, M.; Goldstein, J. *J. Clin. Invest.* 1980, *66*, 1094–1100.

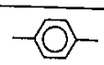
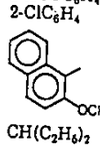
(16) Compounds 8c and 8cc were assigned IC_{50} values of 100 μM so they could be included in the correlation.

(17) Kathawala, F. G. WIPO Patent WO 84/02131, 1984.

Table III. *trans*-6-(2-Pyrrol-1-ylalkyl or -aryl)-4-hydroxypyran-2-ones



8

no.	X	R_1	R_2	mp, °C	% yield	formula[a]	IC_{50},[b,c] μM, CSI	log IC_{50}, CSI	relative potency,[d] CSI	IC_{50},[c,e] μM, COR	log IC_{50}, COR
8a		4-FC_6H_4	CH_3	155–7	32	$C_{22}H_{20}FNO_3$	20	−4.7	0.10	–	–
8b		4-FC_6H_4	CH_3	54–7	29	$C_{22}H_{20}FNO_3$	24	−4.6	0.01	63	−4.2
8c		4-FC_6H_4	CH_3	142–5	21	$C_{22}H_{20}FNO_3$	>100	−4.0	<0.01	>100	−4.0
8d	$-CH_2CH_2CH_2-$	4-FC_6H_4	CH_3	oil	41	$C_{19}H_{21}FNO_3$	53	−4.3	0.02	–	–
8e	$-CH(CH_3)CH_2-$	4-FC_6H_4	$CH(CH_3)_2$	167–9	30	$C_{21}H_{26}FNO_3$	5.0	−5.3	0.50	40	−4.4
8f	$-CH_2CH_2-$	4-FC_6H_4	CH_3	oil	32	$C_{18}H_{20}FNO_3$	0.51	−6.3	0.90	2.8	−5.6
8g	$-CH_2CH_2-$	Ph	CH_3	89–91	29	$C_{18}H_{21}NO_3$	1.4	−5.9	0.40	13	−4.9
8h	$-CH_2CH_2-$	4-PhC_6H_4	CH_3	104–7	35	$C_{24}H_{25}NO_3$	23	−4.6	0.10	23	−4.6
8i	$-CH_2CH_2-$	4-$MeOC_6H_4$	CH_3	95–96	50	$C_{19}H_{23}NO_4$	12	−4.9	0.10	28	−4.6
8j	$-CH_2CH_2-$	4-ClC_6H_4	CH_3	118–121	28	$C_{18}H_{20}ClNO_3$	10	−5.0	0.20	3.2	−5.5
8k	$-CH_2CH_2-$	4-HOC_6H_4	CH_3	161–2	–	$C_{18}H_{21}NO_4$	2.6	−5.6	1.0	6.3	−5.2
8l	$-CH_2CH_2-$	3-$F_3CC_6H_4$	CH_3	oil	65	$C_{19}H_{20}F_3NO_3$	1.5	−5.8	0.30	5.4	−5.3
8m	$-CH_2CH_2-$	3-$MeOC_6H_4$	CH_3	106–9	21	$C_{19}H_{23}NO_4$	2.5	−5.6	0.80	11	−5.0
8n	$-CH_2CH_2-$	3-HOC_6H_4	CH_3	144–5	–	$C_{18}H_{21}NO_4$	1.9	−5.7	1.40	12	−5.0
8o	$-CH_2CH_2-$	2-$MeOC_6H_4$	CH_3	112–3	38	$C_{19}H_{23}NO_4$	2.1	−5.7	0.90	25	−4.6
8p	$-CH_2CH_2-$	2-HOC_6H_4	CH_3	140–2	–	$C_{18}H_{21}NO_4$	2.5	−5.6	1.10	30	−4.5
8q	$-CH_2CH_2-$	2-naphthyl	CH_3	foam	30	$C_{22}H_{23}NO_3$[f]	16	−4.8	0.10	3.6	−5.4
8r	$-CH_2CH_2-$	1-naphthyl	CH_3	137–8	21	$C_{22}H_{23}NO_3$	1.8	−5.8	0.70	4.0	−5.4
8s	$-CH_2CH_2-$	cyclohexyl	CH_3	129–130	25	$C_{18}H_{27}NO_3$	0.69	−6.2	0.50	2.2	−5.6
8t	$-CH_2CH_2-$		CH_3	125–6	20	$C_{19}H_{25}NO_3$	1.4	−5.8	1.10	5.8	−5.2
8u	$-CH_2CH_2-$		CH_3	135–8	13	$C_{20}H_{27}NO_3$[g]	1.3	−5.9	1.60	3.2	−5.5
8v	$-CH_2CH_2-$		CH_3	135–9	68	$C_{20}H_{29}NO_3$	2.3	−5.6	1.10	2.3	−5.6
8w	$-CH_2CH_2-$	Ph_2CH	CH_3	129–132	33	$C_{25}H_{27}NO_3$	13	−4.9	0.10	8.9	−5.4
8x	$-CH_2CH_2-$	4-FC_6H_4	$CH(CH_3)_2$	105–6	34	$C_{20}H_{24}FNO_3$	0.40	−6.4	30.2	0.23	−6.6
8y	$-CH_2CH_2-$	4-FC_6H_4	$C(CH_3)_3$	117–8	24	$C_{21}H_{26}FNO_3$	1.6	−5.8	1.70	1.8	−5.7
8z	$-CH_2CH_2-$	4-FC_6H_4	$CH(C_2H_5)_2$	107–8	36	$C_{22}H_{28}FNO_3$	20	−4.7	0.10	32	−4.5
8aa	$-CH_2CH_2-$	4-FC_6H_4	cyclopropyl	foam	22	$C_{20}H_{22}FNO_3$	2.2	−5.7	1.30	2.6	−5.6
8bb	$-CH_2CH_2-$	4-FC_6H_4	cyclobutyl	88–9	5	$C_{21}H_{24}FNO_3$	17	−4.8	0.20	–	–
8cc	$-CH_2CH_2-$	4-FC_6H_4	cyclohexyl	64–6	30	$C_{23}H_{28}FNO_3$	>100	−4.0	<0.01	>100	−4.0
8dd	$-CH_2CH_2-$	4-FC_6H_4	CF_3	oil	58	$C_{18}H_{17}F_4NO_3$	0.25	−6.6	8.0	0.63	−6.2
8ee	$-CH_2CH_2-$	3-FC_6H_4	$CH(CH_3)_2$	87–9	40	$C_{20}H_{24}FNO_3$	1.3	−5.9	1.8	2.6	−5.6
8ff	$-CH_2CH_2-$	2-FC_6H_4	$CH(CH_3)_2$	oil	9	$C_{20}H_{24}FNO_3$[h]	3.2	−5.5	0.9	1.8	−5.8
8gg	$-CH_2CH_2-$	2,4-$F_2C_6H_3$	$CH(CH_3)_2$	75–7	8	$C_{20}H_{23}F_2NO_3$	1.6	−5.8	1.5	2.6	−5.2
8hh	$-CH_2CH_2-$	2-$MeOC_6H_4$	$CH(CH_3)_2$	oil	16	$C_{21}H_{27}NO_4$	2.2	−5.6	1.0	5.6	5.2
8ii	$-CH_2CH_2-$	2,6-$(MeO)_2C_6H_3$	$CH(CH_3)_2$	foam	36	$C_{22}H_{29}NO_5$	19	−4.7	0.2	87	−4.1
8jj	$-CH_2CH_2-$	2,5-$Me_2C_6H_3$	$CH(CH_3)_2$	oil	25	$C_{22}H_{29}NO_3$[i]	12	−4.9	0.2	16	−4.8
8kk	$-CH_2CH_2-$	2-$iPrOC_6H_4$	$CH(CH_3)_2$	oil	12	$C_{23}H_{31}NO_4$[j]	3.2	−5.5	0.9	–	–
8ll	$-CH_2CH_2-$	2-ClC_6H_4	$CH(CH_3)_2$	foam	25	$C_{20}H_{24}ClNO_3$	3.2	−5.5	0.5	9.1	−5.0
8mm	$-CH_2CH_2-$		$CH(CH_3)_2$	oil	34	$C_{25}H_{29}NO_4$[k]	9.6	−5.0	0.2	25	−4.6
8nn	$-CH_2CH_2-$	$CH(C_2H_6)_2$	$CH(C_2H_5)_2$	oil	20	$C_{21}H_{33}NO_3$	>100	−4.0	<0.01	–	–
I	compactin						0.026	−7.6	100	0.025	−7.6

[a] Analytical results are within ±0.4% of theoretical values unless otherwise noted. [b] Cholesterol synthesis inhibition screen; a measure of the rate of conversion of [^{14}C]acetate to cholesterol employing a crude liver homogenate. [c] IC_{50} values were determined with four dose levels of each inhibitor in the assay systems described in ref 14 (CSI) and 15 (COR). [d] Calculated as follows: (IC_{50} of test compound)/(IC_{50} of compactin determined simultaneously) × 100. [e] CoA reductase inhibition screen; a measure of the direct conversion of D,L-[^{14}C]HMG-CoA to mevalonic acid employing a partially purified microsomal enzyme preparation. [f] C: calcd, 75.62; found, 75.12. [g] C: calcd, 72.92; found, 72.50. [h] C: calcd, 69.54; found, 71.37; H: calcd, 7.01; found, 7.54. [i] C: calcd, 74.33; found, 74.78. [j] C: calcd, 71.66; found, 72.09. [k] C: calcd, 73.69; found, 72.09.

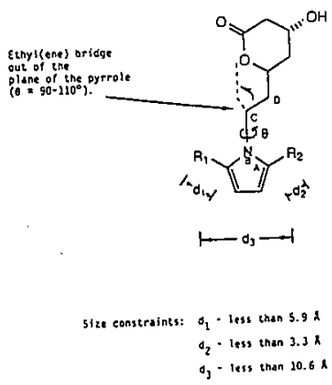


Figure 3. Summary of conclusions from the molecular modeling study.

Molecular Modeling

In order to identify the required spatial relationship between the lipophilic group (represented by the substituted pyrrole, phenyl, and hexahydronaphthalene ring systems) and the 4-hydroxypyran-2-one moiety, quantify steric tolerances across the pyrrole ring, and evaluate the relationship between potency and the polarity (charge distribution) of the side chains, selected analogues from Table III, compactin (I), and the potent biphenyl inhibitor III were modeled by using the CAMSEQ-II program package[18,19] (Table IV; see the Experimental Section). Conformational preferences of the ethyl (or ethylene) bridge to the lactone ring, size of the R_1 and R_2 substituents (Table IV), and charge distribution were compared to potency in the CSI screen (at the outset of this study, affinities in the COR screen were unavailable for the majority of the analogues studied) in order to develop a pharmacophore model for HMGR inhibition.

Lactone Side Chain Conformations. For reference purposes, calculated energies for the 0°, 90°, 180°, and lowest energy conformations of θ are summarized in Table IV. Figure 2 depicts the calculated energies for individual conformations. From Figure 2, all of the modeled compounds, including compactin (I), the biphenyl analogue III, and the less potent analogues **8z**, **8bb**, **8cc**, and **8nn**, can adopt an enereticaly favorable conformation where the ethyl(ene) bridge is nearly perpendicular to the parent pyrrole, benzene, or hexahydronaphthalene ring systems. Indeed, for the potent derivatives 8t and III, the calculations show that the out of plane ($\theta \approx 80$–$110°$) orientation is the only one allowed. In addition, the reduced potency of the *tert*-butyl (8y) over the isopropyl (**8x**) analogue may be explained by the fact that the out of plane conformation ($\theta = 110°$) of 8y is calculated to be energetically disfavored over the in-plane ($\theta = 0$–$70°$) orientations.

Thus, it is concluded that a conformation of the ethyl(ene) bridge to the 4-hydroxypyran-2-one ring out of the plane (90–120°) of the parent ring systems is consistent with increased potency as a HMGR inhibitor. Interestingly, this corresponds to the calculated minimum energy and not the X-ray conformation[1b] of compactin. The X-ray conformation represents a secondary minimum at θ = 24.6°, 1.2 kcal/mol higher in energy, probably due to packing interactions.

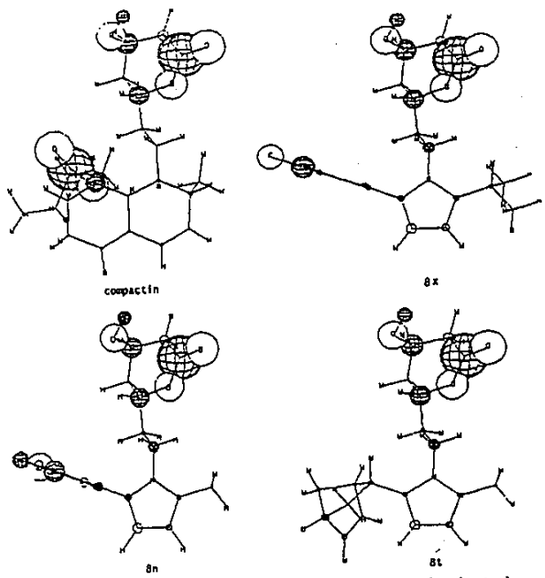


Figure 4. Charge distribution of compactin and selected analogues. Hatched and open spheres represent positive and negative charges, respectively. Sphere size is proportional to the magnitude of the atomic charge.

Steric Tolerances. In determining steric tolerances, the substituents were somewhat arbitrarily assigned. Larger substituents such as substituted phenyl, norbornenyl, and the isobutyric ester on compactin were placed at R_1 (Table IV); small alkyl groups were assigned to R_2. Changing the assignment would affect the conclusions regarding these tolerances. Low-energy, extended conformations of the substituents were used in the distance calculations; other orientations of flexible groups such as $CH(C_2H_5)_2$ could produce different distances.

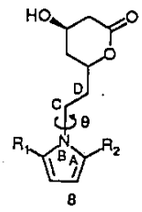
The maximum lengths of R_1 and R_2 and the overall width of the molecule across the parent ring system from R_1 to R_2 are given in Table IV. The calculations show a clear dependence of CSI potency on all three distances summarized in Figure 3. High potency ($IC_{50} < 1.6$ μM) is observed only for those analogues whose (a) maximum length of R_1 (Figure 3, d_1) is <5.9 Å (Table IV: compare 8f and 8j), (b) maximum length of R_2 (Figure 3, d_2) is <3.3 Å (compare 8x and 8z or **8nn**), and (c) overall width (Figure 3, d_3) is <10.6 Å (compare 8y and **8bb**). Other analogues not included in Table IV reinforce the length constraints at R_1: the 2-naphthyl analogue 8q ($d_1 = 6.40$ Å) is less potent than the 1-naphthyl ($d_1 = 4.20$ Å), and the para-substituted derivatives 8h and 8i possess reduced potency.

Charge Distribution. Initially, it was hypothesized that the spatial orientation of polar regions with relatively large partial charges within the molecule might be connected to CSI potency. Compactin contains two distinct regions of relatively large partial charges corresponding to the 4-hydroxypyran-2-one ring and the isobutyric ester side chain (Figure 4). The potent inhibitors 8f and 8x also present relatively large partial charges, albeit weaker in strength, in roughly the same region as this side chain. However, attempts to increase potency by more closely mimicking the polar regions associated with the isobutyric ester of compactin with the more polar 2- and 3-(methoxy and hydroxy)phenyl analogues **8m–p** resulted in equipo-

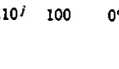
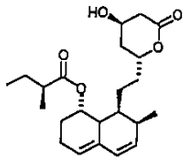
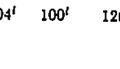
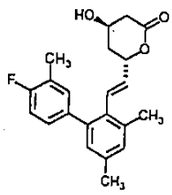
(18) (a) Potenzone, R., Jr.; Cavicchi, E.; Weintraub, H. J. R.; Hopfinger, A. J. *Comput. Chem.* 1977, *1*, 187. (b) Potenzone, R., Jr.; Hopfinger, A. J. *A Demonstration of the CAMSEQ-II Software System* In DHEW Publ. (FDA) (U.S.), Issue FDA 78-1046, Structural Correlations of Carcinogenesis and Mutagenesis, 1978, pp 102–103.

(19) In-house conversion of the program to run on an IBM 3033 under MVS/TSO (J. W. Vinson, unpublished work).

Table IV. Results of Modeling Studies on Compactin and Substituted Pyrroles



8

no.	R_1	R_2	IC_{50},[a] μM	lactone side chain rotations, CAMSEQ energies[b] 0°	90°	180°	min en conf	maximum overall width, Å (R_1 to R_2)	maximum lengths, Å R_1	R_2	other rotations[c]
8e	4-FC_6H_4 (α-Me)[d]	$CH(CH_3)_2$	5.0	−37.10[e]	−41.43[e]	100[e]	60°, −42.92[e]	10.12	5.58	2.48	; also bond from α-Me to lactone side chain from 0° to 60° by 20°
8e	4-FC_6H_4 (α-Me)[f]	$CH(CH_3)_2$	5.0	−46.93[e]	−27.09[e,g]	100[e]	0°, −46.93[j]	10.12	5.58	2.48	as above
8f	4-FC_6H_4	CH_3	0.51	−40.92	−39.27	−10.03	0°, −40.92	7.66	5.58	1.50	methyl group (R_2) from 0° to 60° by 10°
8j	4-ClC_6H_4	CH_3	10					9.33	5.89	1.50	as above
8t[h]		CH_3	1.4	67.11	−44.98	−16.40	90°, −44.98	7.22	3.64	1.50	bond from R_1 to pyrrole from 0° to 360° by 20°
8t[i]		CH_3	1.4	19.63	−43.65	−15.01	70°, −44.65	7.87	4.27	1.50	as above
8x	4-FC_6H_4	$CH(CH_3)_2$	0.40	−46.64	−45.06	46.29	0°, −46.64	10.12	5.58	2.48	
8y	4-FC_6H_4	$C(CH_3)_3$	1.6	−47.77	−24.10[j]	100	0°, −47.77	10.20	5.58	2.48	 ; all bonds from 0° to 60° by 20°
8z	4-FC_6H_4	$CH(C_2H_5)_2$	20	−52.35	−50.97	100	0°, −52.35	10.99	5.58	3.74	; terminal methyls set to a staggered conformation
8bb	4-FC_6H_4	cyclobutyl	17	−46.46	−44.82	6.01	60°, −46.64	10.62	5.58	3.35	bond from R_2 to pyrrole from 0° to 360° by 20°
8cc	4-FC_6H_4	cyclohexyl	100	−51.76	−50.31	100	0°, −51.76	11.92	5.58	4.33	bond from R_2 to pyrrole from 0° to 360° by 20°
8nn	$CH(C_2H_5)_2$	$CH(C_2H_5)_2$	100	100	−47.28	100	100°, −54.31	9.41	3.74	3.74	see compound 8z above
I			0.026	10.17[f]	−56.04[f]	100[f]	120°, −61.74[f]	8.81	5.66	1.50	 ; terminal alkyl groups set to a staggered conformation
III			0.01	100	−48.89	100	130°, −52.92	8.74	5.52	1.50	bond from R_2 (Me) to phenyl from 0° to 60° by 20°; bond from R1 (4-F,3-MeC_6H_3) to phenyl from 0° (biphenyl coplanar) to 90° by 15°

[a] CSI screen (see Table III). [b] Counterclockwise rotation of θ from 0 to 180° by 10°, unless otherwise noted, starting from the in-plane conformation shown (atoms A, B, C, D in a cis orientation). Steric and electrostatic (using charges calculated via the CNDO/2 method) terms were used. Energies are in kilocalories/mole. [c] At each conformation of the lactone side chain, rotations were performed on the marked bonds from 0° to 180° by 20°, unless otherwise indicated. Substituted phenyl rings at R_1 were held perpendicular to the pyrrole. [d] *R* stereoisomer. [e] θ was scanned from 0° to 250° by 10°. [f] *S* stereoisomer. [g] θ = 110° conformer, −46.09 kcal/mol. [h] Endo isomer. [i] Exo isomer. [j] θ = 70° conformer, −46.93 kcal/mol. [k] Chair form; equatorial attachment to pyrrole. [l] θ was scanned from 0° to 350° by 10°.

tent, not more potent, analogues. In addition, compounds containing bicyclo moieties at R_1 (8t–v) demonstrated that a polar substituent in this area (or an aryl ring, for that matter) was not required for CSI potency at the 1 μM level. Thus, it is concluded that CSI potency is relatively insensitive to the polarity of the group at R_1.

Conclusions

A series of 6-(2-pyrrol-1-ylethyl)-4-hydroxypyran-2-ones (8) has been identified as inhibiting the enzyme HMG-CoA

reductase (HMGR). By measuring the inhibition of HMGR in vitro, the 2- and 5-substituents on the pyrrole ring have been optimized, thus obtaining a compound (8x) that possesses 30% of the in vitro potency of the potent fungal metabolite compactin.

From a molecular modeling study, it was determined that so long as the 2- and 5-substituents did not interfere with the ability of the ethyl bridge to the lactone ring to attain an out-of-plane conformation (θ = 90–110°), and the substituents were within the distance contraints given in Figure 3, one could expect to achieve potency at the 1 μm level in the CSI screen. Attempts to enhance potency by mimicking partial charges in the polar isobutyric ester side chain in compactin failed. It is concluded that there are no strong electronic requirements for binding in this area.

In addition, the reduced potency of 8w, 8ii, and 8mm relative to other substituted phenyl derivatives suggests a steric intolerance off of one of the ortho phenyl positions of the R_1 substituent. One other noteworthy observation is that substitution of the 5-isopropyl with trifluoromethyl produced an analogue, 8dd, of essentially equal potency, (Table III: compare 8dd with 8f and 8x). This suggests the desirability of an electron-deficient pyrrole ring and a possible direction for future exploration. Efforts to further optimize the inhibitory potency of this series will be reported in subsequent publications from these laboratories.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. THF was distilled from sodium and benzophenone. All organic extracts were dried over $MgSO_4$ except where otherwise noted. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were determined on a Nicolet MX-1 FT-IR spectrophotometer. NMR spectra were determined on either a Varian EM-390 spectrophotometer or a Varian XL-200 instrument. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. Elemental analyses for carbon, hydrogen, and nitrogen were determined on a Perkin-Elmer Model 240C elemental analyzer and are within 0.4% of theory unless noted otherwise. HPLC analyses were performed with a Varian 5500 unit equipped with a Reodyne 7126 loop injector, a Dupont variable wavelength detector, and an octadecylsilane column (Alltech C18 600RP, CH_3CN–H_2O eluant, 60:40, v/v) interfaced to Varian 402 data system for computation of peak areas. All starting materials were commercially available unless indicated otherwise.

Preparation of 1-(4-Fluorophenyl)-5-methyl-1,4-hexanedione (3p). Method A. 1-(4-Fluorophenyl)-2-propen-1-one (43.0 g, 287 mmol) was mixed with 31.2 mL (344 mmol) of isobutyraldehyde, 28 mL (200 mmol) of triethylamine, and 14.5 g (58 mmol) of 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride. The mixture was stirred at 70 °C under nitrogen for 12 h, cooled to room temperature, and partitioned between ether (500 mL) and water (100 mL). The aqueous layer was further extracted with ether (300 mL). The combined ether extracts were washed successively with water (200 mL), 2 M HCl (2 × 100 mL), and brine (100 mL) and dried. Filtration and concentration to dryness in vacuo provided an oil which was distilled (bp 115–120 °C, 0.2 mmHg) to provide 36.7 g (58%) of the title compound which solidified on standing: 90-MHz NMR ($CDCl_3$) δ 1.15 (d, 6 H, J = 7 Hz), 2.7 (septet, 1 H, J = 7 Hz), 2.8 (m, 2 H), 3.05 (m, 2 H), 7.12 (t, 3 H), 7.95 (m, 2 H). An analytical sample could be obtained by recrystallization from hexane, mp 51–3 °C. Anal. ($C_{13}H_{15}FO_2$) C, H, N.

Alternate Synthesis of 3p. A mixture of 2-methyl-4-penten-4-one[8d] (2.0 g, 20 mmol), 4-fluorobenzaldehyde (2.4 g, 20 mmol), 2 mL (14 mmol) of triethylamine, and 1.0 g (4 mmol) of 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride was stirred under nitrogen for 5 h at 70 °C, cooled to room temperature, and partitioned between ether (200 mL) and water (50 mL). The water layer was extracted with ether (200 mL). The ether extracts were combined, washed successively with water (50 mL), 2 M HCl (50 mL), and brine (50 mL), and dried. After concentration to dryness in vacuo, the residue was flash chromatographed on silica gel with hexane–ethyl acetate (20:1 v/v) as eluant, affording 2.6 g of 3p, mp 47–49 °C.

Method B. To a suspension of hexane-washed NaH (6.5 g, 270 mmol) in dry DMF (300 mL) at 0 °C under dry nitrogen was added a solution of methyl 4-methyl-3-oxopentanoate (37.5 g, 260 mmol) in 100 mL of dry DMF. When gas evolution had subsided, a solution of 2-bromo-4′-fluoroacetophenone (260 mmol) in dry DMF (100 mL) was added dropwise over 60 min. The mixture was allowed to warm to 25 °C overnight, poured into ice-cold 2 M HCl (300 mL), and extracted with ether (2 × 200 mL). The organic layer was washed with water (3 × 50 mL) and brine (50 mL) and concentrated to dryness in vacuo. The crude product was dissolved in 800 mL of 3:1 THF–water and treated with NaOH (24 g, 600 mmol), and the mixture was stirred overnight. The solution was made acidic with 6 N HCl and extracted with ether (2 × 300 mL). The ether extracts were washed with water (50 mL), bicarbonate (50 mL), and brine (50 mL) and dried. Distillation provided 40 g (69%) of 3p.

Preparation of 2-[2-(4-Fluorophenyl)-5-(1-methylethyl)-1*H*-pyrrol-1-yl]-1-cyanoethane (5, R_1 = 4-FPh, R_2 = $CH(CH_3)_2$, X = $-CH_2CH_2-$). A mixture of 3p (365 g, 1.65 mol), 3-aminopropionitrile $^1/_2$-fumarate (234 g, 1.83 mol), and 1 g of p-TSA in glacial acetic acid (1800 mL) was stirred and heated at reflux for 8 h. After cooling to room temperature, the solution was poured into ice water (3 L). The solid that formed was isolated by suction filtration and recrystallized from isopropyl ether and hexane (212 g, mp 75–78 °C). The filtrate was extracted with ether (2 × 1 L). The combined ether extracts were washed with water (1 L), saturated aqueous sodium bicarbonate (until gas evolution ceased), and brine (500 mL) and dried. Filtration and concentration to dryness in vacuo afforded a solid which was recrystallized from isopropyl ether to provide a further 98 g of the title compound (310 g total, 73%): IR (KBr) 2990, 2249, 1566, 1522, 1484, 1219, 1162, 847, 782 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.30 (d, 6 H, J = 7 Hz), 2.32 (t, 2 H, J = 7 Hz), 2.92 (septet, 1 H, J = 7 Hz), 4.22 (t, 2 H, J = 7 Hz), 6.00 (d, 1 H, J = 3.5 Hz), 6.10 (d, 1 H, J = 3.5 Hz), 7.0–7.4 (m, 4 H). Anal. ($C_{16}H_{17}FN_2$) C, H, N.

Preparation of 3-[2-(4-Fluorophenyl)-5-(1-methylethyl)-1*H*-pyrrol-1-yl]propanal (6t). A stirred solution of the above intermediate (200 g, 780 mmol) in 1500 mL of CH_2Cl_2 at ambient temperature under nitrogen was treated dropwise with 936 mL of a 1.0 M solution of diisobutylaluminum hydride (DIBAL-H) in CH_2Cl_2 over 4 h. The resulting mixture was stirred overnight at room temperature, and then the excess hydride was destroyed by cautious addition of methanol. When gas evolution was complete, the solution was carefully poured into 1500 mL of vigorously stirred ice-cold 2 M HCl (exothermic). The emulsion that resulted was extracted with ether (2 × 1 L), and the combined ether extracts were washed successively with water (500 mL), saturated aqueous sodium bicarbonate (2 × 500 mL), and brine (500 mL) and dried. The solvents were removed in vacuo, and the residue was flash chromatographed over silica gel, eluting with hexane–ethyl acetate (10:1, v/v) to provide 6t (187 g, 92%) as a colorless oil: IR (film) 2930, 1720, cm^{-1}; 90-MHz NMR ($CDCl_3$) δ 1.25 (d, 6 H, J = 7 Hz), 2.50 (t, 2 H, J = 7 Hz), 2.85 (septet, 1 H, J = 7 Hz), 4.20 (t, 2 H, J = 7 Hz), 5.90 (d, 1 H, J = 2.5 Hz), 6.03 (d, 1 H, J = 2.5 Hz), 6.0–7.3 (m, 4 H), 9.45 (s, 1 H).

Preparation of Methyl 7-[2-(4-Fluorophenyl)-5-(1-methylethyl)-1*H*-pyrrol-1-yl]-5-hydroxy-3-oxoheptanoate (7, R_1 4-FPh, R_2 = $CH(CH_3)_2$, X = $-CH_2CH_2-$). A stirred suspension of hexane-washed NaH (2.17 g, 91 mmol) in anhydrous THF (200 mL) at 0 °C under nitrogen was treated dropwise with a solution of methyl acetoacetate (8.9 mL, 82 mmol) in anhydrous THF (150 mL) over 30 min. When gas evolution was complete, n-butyllithium (39 mL of a 2.1 M solution in hexane) was added dropwise. The resulting solution was stirred for 30 min and then treated dropwise over 30 min with a solution of 6t (19.4 g, 74.9 mmol) in anhydrous THF (150 mL). The solution was stirred for an additional 1 h and the reaction was quenched with saturated aqueous NH_4Cl (100 mL), followed by 2 M HCl (100 mL).

The resulting mixture was partitioned between ether (500 mL) and water (100 mL). The water layer was separated and extracted

with ether (300 mL). The ether extracts were combined, washed with brine (50 mL), and dried. The solvents were removed in vacuo, and the residue was flash chromatographed on silica gel, eluting with hexane–ethyl acetate (5:1, v/v) to yield 19.9 g (64%) of the title compound as a colorless oil: 200-MHz NMR ($CDCl_3$) δ 1.28 (d, 6 H, J = 7 Hz), 1.55 (m, 2 H), 2.45 (m, 2 H), 2.6 (br s, 1 H, J = 2.5 Hz), 7.0–7.4 (m, 4 H); IR (film) 3520, 2966, 2873, 1749, 1716, 1518, 1223, 1159, 845, 815, 767 cm^{-1}.

Preparation of *trans*-6-[2-[2-(4-Fluorophenyl)-5-(1-methylethyl)-1*H*-pyrrol-1-yl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (8x). Air (30 mL) was bubbled by syringe through a stirred solution of n-Bu_3B (58 mL of a 1 M THF solution) in dry THF (50 mL) containing 19.9 g (53 mmol) of the above intermediate at room temperature. The solution was stirred for 18 h at room temperature and cooled to −78 °C, and sodium borohydride (2.27 g, 60 mmol) was added in one portion. The mixture was stirred for 60 min at −78 °C and warmed to 0 °C for 90 min. A mixture of water (10 mL) and methanol (10 mL) was carefully added (gas evolution). NaOH (3 M, 60 mL) and 30% H_2O_2 (30 mL) were added simultaneously to the mixture from separate dropping funnels. The vigorously stirred mixture was held at 0 °C for 60 min and then at room temperature for 2 h.

The mixture was partitioned between water (300 mL) and ether (300 mL). The ether layer was extracted with 10% aqueous NaOH (50 mL). The aqueous layers were combined, acidified with concentrated HCl, and extracted with ethyl acetate (2 × 500 mL). The ethyl acetate extracts were combined, washed twice with brine (100 mL), and dried. Removal of the solvents in vacuo yielded 12.5 g of an oil which was dissolved in toluene (500 mL) and heated at reflux with azeotropic removal of water (Dean–Stark trap). The cooled solution was concentrated and the residue flash chromatographed on silica gel, eluting with hexane–ethyl acetate (5:1 v/v) to yield 11 g of a colorless solid. Recrystallization from isopropyl ether yielded 9.5 g (52%) of 8x, mp 104–105 °C, which was a 97:3 mixture of diastereomers by HPLC: 200-MHz NMR ($CDCl_3$) δ 1.30 (d, 6 H, J = 7 Hz), 1.5–1.9 (m, 4 H), 2.60 (m, 2 H), 2.98 (septet, 1 H, J = 7 Hz), 4.0–4.3 (m, 3 H), 4.45 (m, 1 H), 5.98 (d, 1 H, J = 2.5 Hz), 6.08 (d, 1 H, J = 2.5 Hz), 7.10 (m, 2 H), 7.33 (m, 2 H); IR (KBr) 3440, 2966, 2870, 1690, 1518, 1268, 1223, 1075, 837, 773 cm^{-1}. Anal. ($C_{20}H_{24}FNO_3$) C, H, N.

Preparation of 2-[2-(4-Fluorophenyl)-5-(1,1-dimethylethyl)-1*H*-pyrrol-1-yl]-1-cyanoethane (5, R_1 = 4-FPh, R_2 = $C(CH_3)_3$, X = $-CH_2CH_2-$). Glacial acetic acid (125 mL) was added in one portion to a stirred solution of 3q (66 mmol) and ethanolamine (27 mL) at ambient temperature. A vigorous exothermic reaction ensued (the internal temperature rose to 95 °C). When the exotherm had subsided (TLC indicated reaction almost complete), the solution was stirred and heated at reflux for 30 min (TLC indicated all starting material was consumed, but a new high-R_f spot had appeared). The reaction mixture was cooled to room temperature and poured into ice water (200 mL). The aqueous mixture was extracted with ether (2 × 500 mL). The combined ether extracts were washed with water (2 × 200 mL), saturated aqueous bicarbonate (2 × 200 mL), and brine (100 mL), dried, and concentrated to dryness in vacuo. Flash chromatography of the residue on silica gel, eluting the ethyl acetate–hexane (10:1 v/v) provided 10.7 g of 2-[2-(4-fluorophenyl)-5-(1,1-dimethylethyl)-1*H*-pyrrol-1-yl]-2-ethanol product (62%) and 5 g of a high-R_f material which appeared to be the corresponding *O*-acetate by NMR (3 H, s, δ 2.05). The high-R_f fraction was stirred with NaOH (2 g) in CH_3OH (50 mL) and water (10 mL) for 2 h. The solution was concentrated, diluted with water (20 mL), and extracted with ethyl acetate (2 × 200 mL). The ethyl acetate extracts were washed with brine (50 mL) and dried. Filtration and concentration to dryness in vacuo provided a further 3.7 g of the above alcohol (14.4 g total, 84%).

Mesyl chloride (1.93 mL, 25 mmol) was added dropwise to a stirred solution of the above alcohol (5 g, 19.1 mmol) in pyridine (15 mL) cooled in an ice bath. The mixture was stirred for 2.5 h at 0 °C, warmed to room temperature, poured into water (300 mL), and extracted with ether (2 × 300 mL). The combined ether extracts were washed with water (50 mL), 2 M HCl (50 mL), bicarbonate (2 × 50 mL), and brine (50 mL), dried, and concentrated to dryness in vacuo. The crude mesylate was used without further purification.

A solution of KCN (1.54 g, 23.6 mmol) and KI (1.16 g, 10 mmol) in water (12 mL) was added dropwise to a stirred, 70 °C solution of the mesylate (4.0 g, 18 mmol) in DMF (36 mL). The resulting solution was heated under reflux for 24 h, cooled, and poured into ice water. The mixture was extracted with ether (2 × 200 mL). The combined ether extracts were washed with water (50 mL), 2 M HCl (25 mL), bicarbonate (2 × 50 mL), and brine (25 mL), dried, and concentrated to dryness in vacuo. Flash chromatography of the residue on silica gel, eluting with hexane–ethyl acetate (20:1, v/v), provided 2.8 g (88%) of the title compound: 90-MHz NMR ($CDCl_3$) δ 1.42 (s, 9 H), 2.20 (t, 2 H), J = 2 Hz), 4.30 (t, 2 H, J = 7 Hz), 5.90 (d, 1 H, J = 4 Hz), 6.00 (d, 2 H, J = 4 Hz), 6.9–7.4 (m, 4 H).

Prepartion of 6-[2-(2-Bicyclo[2.2.2]oct-2-yl-5-methyl-1*H*-pyrrol-1-yl)ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (8v). To a solution of 8u (0.3 g, 0.91 mmol) in ethyl acetate (10 mL) was added 0.03 g of 10% Pd–C. The mixture was evacuated, placed under a balloon of hydrogen (1 atm) at room temperature, and stirred overnight. The suspension was filtered through Celite and concentrated to dryness in vacuo, and the solid residue was recrystallized from isopropyl ether to afford 0.21 g of 8v (68%), mp 135–139 °C. Anal. ($C_{20}H_{29}NO_3$) C, H, N.

General Demethylation Procedure (Preparation of 8n). BBr_3 (11 mmol) was dissolved in 8 mL of CH_2Cl_2 and added dropwise to a solution of 8m (1.2 g, 3.64 mmol) in 100 mL of CH_2Cl_2 at −20 °C under dry nitrogen. The mixture was stirred for 2 h, and then a further 2 mmol of BBr_3 was added. The solution was allowed to warm slowly to 0 °C, poured into saturated aqueous bicarbonate (500 mL), and extracted with ethyl acetate (2 × 200 mL). The combined organic extracts were washed with 10% aqueous bisulfite (50 mL), saturated aqueous bicarbonate (30 mL), and brine (30 mL), dried, and concentrated to dryness in vacuo. Flash chromatography of the residue provided 450 mg of impure phenol. Two recrystallizations from isopropyl ether provided pure 8n, mp 110–111.5 °C. Anal. ($C_{18}H_{21}NO_4$) C, H, N.

HMG-CoA Reductase Inhibition Assay 1: The Cholesterol Synthesis Inhibition Screen (CSI). The procedure is a modification of the protocol developed by Dugan et al.[14] Male rats (type CD from Charles River) weighing 300–400 g were kept in-house for at least 1 week before the day of the experiment. For 3 consecutive days before being used, they were fed a diet of 5% cholestyramine (by weight) in normal ground chow. On the day of the assay, the rats were anesthetized with ether and sacrificed. Their livers were removed, weighed, and placed on Saran Wrap on ice. The entire livers were minced and diluted with 2 volumes of ice-cold pH 7.4 homogenizing buffer (0.1 M KPO_4, 0.004 M $MgCl_2 \cdot 6H_2O$, 0.001 M EDTA, and 0.01 M 2-mercaptoethanol).

Liver homogenates were prepared by use of five to six passes of a Teflon pestle in a 50-mL glass homogenizer. The homogenates were pooled and centrifuged at 5000*g* for 10 min at 4 °C. Initial supernatants were pooled and centrifuged at 20000*g* for 15 min at 4 °C. Final supernatants were carefully drawn off, avoiding the loose pellet and lipid layer, pooled, and kept on ice. One-milliliter aliquots of this crude microsomal preparation were used for the assay.

Compounds were dissolved in 2 mL of toluene and sonicated if not fully soluble. The mixture was treated with 2 mL of 0.1 N NaOH and stirred constantly for 2 h in a water bath at 45–50 °C. Any remaining toluene was blown off under a stream of N_2. Approximately 6 mL of 0.1 N NaOH was added and the saponified drug placed on ice immediately. If the salt had crystallized, it was sonicated to achieve as uniform a suspension as possible. The pH was adjusted to 7.4 with HCl and the volume brought to 10 mL with H_2O. One-milliliter aliquots were frozen in dry ice–acetone and stored at −70 °C.

On the day of the screen, drugs were dissolved in 1 mL of 0.1 N KOH and diluted with 11 mL of homogenizing buffer to make a 2 mM stock solution. If necessary, sonication was used to achieve a solution, or in some cases, a suspension of drug. The 2 mM stock was diluted 1:1 with a mixture of 1 mL of 0.1 N KOH and 11 mL of homogenizing buffer. The resulting 1 mM solution was further diluted with homogenizing buffer alone to produce a series of 10 × stocks from 10^{-6} to 10^{-3} M. The sodium salt of compactin was used as a reference compound in every assay in a concentration range of 10^{-9} to 10^{-6} M.

Assay Conditions. The assay was carried out in duplicate in 16 × 125 mm screw-capped tubes. The reaction mixture contained the following, on ice (initial concentrations): 0.1 mL of 20 mM NAD, 0.1 mL of 20 mM NADP, 0.1 mL of 200 mM glucose 6-phosphate, 0.5 mL of 0.12 mM niacinamide, and 0.2 mL of the 10 × drug stocks. Controls were also run with 0.2 mL of a mixture of 1 mL of 0.1 N KOH, plus 11 mL of homogenizing buffer in place of drug. One milliliter of the crude microsomal preparation was added immediately after the drugs, to give a total volume of 2 mL. Final drug concentrations were 10^{-4} to 10^{-7} M, or in the case of compactin, 10^{-6} to 10^{-9} M. The samples were warmed at 37 °C for 5 min before adding the radioactive precursor. [1-^{14}C]Acetate was used in the amount of 2.88 μCi per sample, plus 98 μmol of sodium acetate as cold carrier. When [^{3}H]-mevalonate was used, the amount of 0.5 μCi per sample with cold carrier was added to make a total of 0.2 μmol per sample. Volume of radiolabel per sample was 100 μL. After receiving radiolabel, samples were incubated at 37 °C for 1 h and treated with 2.5 mL of 10% KOH in ethanol, and the saponification was carried out at 70 °C for 2 h in a water bath. After cooling to room temperature, the nonsaponifiable lipids (cholesterol accounts for approximately 80% of nonsaponifiable lipids; the remainder are methyl sterols) were extracted by shaking the samples with 4.2 mL of hexane for 10 min. After phase separation, 2 mL of the hexane layer was diluted with 8 mL of Handifluor and counted.

Percent inhibition was calculated as follows: 1.0 − (drug cpm/control cpm). Control refers to the samples that received buffer only. From a plot of percent inhibition versus the log of the drug concentration, the IC_{50} was determined. Every assay yielded an IC_{50} for the reference compound, compactin, thus providing a comparison for the other compounds as well as a standard to check for consistency between assays.

HMG CoA Reductase Inhibition Assay 2: Co-A Reductase Inhibition Screen (COR). This procedure is a modification of that reported by Kita et al.[15] Male Charles River (CD) rats weighing 200–300 g were fed a chow diet containing cholestyramine (5%) for 3 days in order to increase levels of liver microsomal HMG–CoA reductase. Between 9 a.m. and 10 a.m., fed animals were anesthetized with ether prior to a midline incision to open the abdomen. Traverse cuts were made to the left and right of abdominal cavity exposing the hepatic portal vein. A syringe with a 22-gauge needle containing 10 mL of exsanguinating buffer (40 mM Tris, 0.25 M sucrose, 0.3 mM EDTA, 5 mM dithiothreitol (DTT), pH 7.2) was injected into the portal vein after cutting the inferior vena cava. Prior to excision, the liver was cleared of blood by perfusion with exsanguinating buffer. Immediately after excision, the liver was added to ice-cold (4 °C) pH 7.4 buffer (0.3 M sucrose, 5 mM DTT, 50 mM leupeptin, 5 mM EGTA, 1 mM PMSF). Approximately 1 g samples were taken from the largest lobe and homogenized with 10 strokes of a tight-fitting Potter-Elvehjem homogenizer. Each homogenate was centrifuged for 15 min at 12000*g* in a Servall refrigerated-automatic centrifuge (SM-34 rotor). The supernatant was decanted and respun under the same conditions. The resulting supernatant was removed via pipet, with special care being taken not to remove any of the mitochondrial-rich pellet. The supernatants were then pooled and centrifuged with a 50 Ti or 60 Ti rotor in a Beckman L8-80 ultracentrifuge. After ultracentrifugation, the pellet was mixed with ice-cold KH_2PO_4 buffer (0.2 M, pH 7.4), homogenized, and stored in liquid nitrogen at 10 mg/mL microsomal protein. Microsomes maintained in liquid nitrogen retained HMG-CoA reductase activity for up to 1 year. Each pellet was resuspended in a solution of 0.3 M sucrose and 10 mM 2-mercaptoethanol and frozen immediately in liquid nitrogen. The aliquoted samples (500 μL) were then stored at −70 °C for no more than 1 month. For each microsomal isolation, an activity/microgram of microsomal protein curve was determined so that the amount of microsomal protein utilized in each assay was in the linear part of the activity curve.

Assay Conditions. Frozen microsomes (see above) were allowed to slowly thaw on ice. Assay solutions were prepared as follows:

A. Resuspension buffer: 0.2 M KH_2PO_4 buffer, pH 7.4.

B. Incubation buffer: 0.2 M KH_2PO_4 buffer (stock, 3 M KH_2PO_4·$3H_2O$, 1 M KH_2PO_4, final 2 M); 0.01 M EDTA, 12 mM dithiothreitol; 40 mM glucose 6-phosphate; 4 mM NADPH; 0.45 μM DL-3-hydroxymethylglutaryl-coenzyme A (glutaryl-3-^{14}C) (stock, 7.4 μM unlabeled; HMG-CoA + 0.68 μM [^{14}C]HMG-CoA (4.5 μCi/μmol); final concentration 8.9 μM).

Resuspension buffer (70 μL) + microsomal solution (20 μL; 100 μg protein) + drug (10 μL) = 100 μL.

Incubation buffer (90 μL) + [^{14}C]HMG-CoA (10 μL) (final addition) = 100 μL.

Total volume of assay mix = 100 μL + 100 μL = 200 μL.

The assay solution was vortexed and incubated in a shaking water bath at 37 °C for 60 min. Termination of the reaction was accomplished with 30 μL of concentrated HCl. Conversion of the [^{14}C]mevalonic acid to the lactone form occurred in a water bath for 30 min at 37 °C. Conversion of [^{14}C]mevalonic acid to the lactone form occurred during refrigeration overnight. To each reaction tube was added DL-[2-^{3}H]mevalonic acid lactone (10000–15000 cpm + 200 μg of unlabeled mevalonolactone) as an internal standard to correct for incomplete recovery of [^{14}C]-mevalonate. After vortexing, an aliquot (50 μL) from the assay mix in each tube was put over a AG 1-X8 (200–400 mesh) formate form anion exchange resin column. The mevalonate was eluted with 3 × 750 μL of water into scintillation vials. Scintillation cocktail (Beckman Readi-Solv, 10 mL) was then added to each vial. The vials were vortexed and allowed to equilibrate for 1 h. Standards for the [^{14}C]HMG-CoA, [^{3}H]mevalonolactone, and acid-inactivated microsomes (blank) were also isolated by column separation in a Hewlett-Packard Model 3320 Tricarb scintillation spectrometer set for double label counting at maximum efficiency. Standards for [^{14}C]HMG-CoA, [^{3}H]mevalonolactone, and acid-inactivated microsomes (blank) were also isolated by TLC, scraped, and counted. Calculations were performed in the usual manner taking into consideration crossover of ^{3}H into the ^{14}C channel and visa versa, as well as dilution factors and specific activity of [^{14}C]HMG-CoA used. Reductase activity was expressed as picomole of [^{14}C]HMG-CoA converted to [^{14}C]mevalonic acid lactone/milligram of microsomal protein per minute. Compactin was used as a reference compound at concentrations of 10^{-9} and 10^{-7} M to determine the concentration at 50% inhibition from control value. Drugs were tested for their inhibitory characteristics at four concentrations run in triplicate. Statistical significance from control values was determined by using Dunnett's *t* test.

Molecular Modeling. Selected analogues were modeled by using an in-house modified version[17] of CAMSEQ-II[18] operating on an IBM 3083 machine. The structure of compactin was obtained from published[1b] X-ray data; the structure of pyrrole came from a compendium[20] of minimized structures. Coordinates for other groups were extracted from the library of fragments within CAMSEQ-II. Structures III and 8 were built to attaching the side chain containing the 4-hydroxypyran-2-one ring (coordinates for which were copied from the X-ray structure of compactin) to the benzene and pyrrole rings, respectively, and adding the other substituents. Side chains were rotated to remove steric contacts.

After CNDO/2 was employed to generate atomic charges, counterclockwise rotations (unless otherwise noted, from 0° to 180° by 10°) were performed using the SCAN module about θ, starting from the in-plane conformation shown in the structure at the top of Table IV (atoms A–B–C–D coplanar). The conformation of the 4-hydroxypyran-2-one ring was held fixed throughout these calculations. Steric and electrostatic energy terms were used. At each conformation of θ, the conformational flexibility of the 2- and 5-substituents was investigated (Table IV; column headed by "other rotations"), including energy evaluation, to insure that a low-energy conformer of θ was selected. Both the endo and exo isomers of tbe norbornenyl analogue 8t as well as the *R* and *S* isomers of 8e were modeled. The axial-attached isomer of 8cc proved to be sterically hindered and was not included. Figures 1 and 2 were generated by using the SAS-GRAPH program package.[21] In eq 1, the number in parentheses is the standard error of the regression coefficient, *n* is the number of compounds, *r* is the correlation coefficient, *F* is a significance test, and *s* is the standard error.

(20) SYBYL Standard Fragment Library, generously supplied by Tripos Associates, St. Louis, MO.

(21) SAS Institute, Inc. SAS/GRAPH User's Guide, Version 5 Edition; SAS Institute, Inc., Cary, NC, 1985.

Acknowledgment. We are indebted to E. H. Ferguson and C. S. Sekerke for conducting the enzyme inhibition assays, to Dr. S. Brennan, T. Hurley, and D. Sherwood for HPLC analyses, to Dr. F. A. MacKellar and staff for analytical and spectral determinations, and to P. Carr and D. Sandy for manuscript preparation.

Registry No. 1 (R_1 = Ph), 768-03-6; 1 (R_1 = 4-F-C_6H_4), 51594-59-3; 1 (R_1 = 4-Ph-C_6H_4), 42575-11-1; 1 (R_1 = 4-Cl-C_6H_4), 7448-87-5; 1 (R_1 = 4-CH_3O-C_6H_4), 7448-86-4; 1 (R_1 = 3-F_3C-C_6H_4), 123184-14-5; 1 (R_1 = 3-CH_3O-C_6H_4), 51594-60-6; 1 (R_1 = 2-CH_3O-C_6H_4), 77942-10-0; 1 (R_1 = 2-naphthyl), 4452-06-6; 1 (R_1 = 1-naphthyl), 22422-69-1; 1 (R_1 = bicyclo[2.2.1]-hept-5-en-2-yl), 100234-78-4; 1 (R_1 = bicyclo[2.2.2]-oct-5-en-2-yl), 123184-15-6; 1 (R_1 = cyclohexyl), 2177-34-6; 1 (R_1 = Ph_2CH), 93021-71-7; 1 (R_1 = $CH(C_2H_5)_2$), 123184-16-7; 1 (R_1 = 2-F-C_6H_4), 89638-21-1; 1 (R_1 = 2,4-F_2-C_6H_3), 123184-17-8; 1 (R_1 = $CH(CH_3)_2$), 1606-47-9; 2 (R_2 = CH_3), 75-07-0; 2 (R_2 = $CH(CH_3)_2$), 78-84-2; 2 (R_2 = $CH(C_2H_5)_2$), 97-96-1; 2 (R_2 = cyclopropyl), 1489-69-6; 2 (R_2 = cyclobutyl), 2987-17-9; 2 (R_2 = cyclohexyl), 2043-61-0; 2 (R_2 = $C(CH_3)_3$), 630-19-3; 2 (R_2 = 4-F-C_6H_4), 459-57-4; 2 (R_2 = C_2H_5), 123-38-6; 3a, 583-05-1; 3b, 123183-95-9; 3c, 63472-37-7; 3d, 53842-12-9; 3e, 2108-54-5; 3f, 123183-96-0; 3g, 123183-97-1; 3h, 104562-48-3; 3i, 123183-98-2; 3j, 123263-79-6; 3k, 70353-45-6; 3l, 123183-99-3; 3m, 61771-79-7; 3n, 123184-00-9; 3o, 123184-01-0; 3p, 104568-68-5; 3q, 123184-02-1; 3r, 123184-03-2; 3s, 123184-04-3; 3t, 123184-05-4; 3u, 123184-06-5; 3v, 123184-07-6; 3w, 123184-08-7; 3x, 123184-09-8; 3y, 123184-10-1; 3z, 123184-11-2; 3aa, 123184-12-3; 3bb, 123184-13-4; 5a, 123184-20-3; 5b, 123184-21-4; 5c, 123184-22-5; 5d, 123184-23-6; 5e, 123184-89-4; 5f, 123184-24-7; 5g, 123184-25-8; 5h, 123184-26-9; 5i, 123184-27-0; 5j, 123184-28-1; 5k, 123184-29-2; 5l, 123184-30-5; 5m, 123184-31-6; 5n, 123184-32-7; 5o, 123184-33-8; 5p, 123184-34-9; 5q, 123184-35-0; 5r, 123184-36-1; 5s, 123184-37-2; 5t, 104568-69-6; 5u, 123184-88-3; 5v, 123184-38-3; 5w, 123184-39-4; 5x, 123184-40-7; 5y, 123184-41-8; 5z, 104568-91-4; 5aa, 104568-69-6; 5bb, 123184-42-9; 5cc, 123184-43-0; 5dd, 123184-44-1; 5ee, 123184-45-2; 5ff, 123184-46-3; 5gg, 123184-47-4; 5hh, 123184-48-5; 5ii, 123184-49-6; 5jj, 123184-50-9; 6a, 123184-51-0; 6b, 123184-52-1; 6c, 123184-53-2; 6d, 123184-54-3; 6e, 123184-55-4; 6f, 123184-56-5; 6g, 123184-57-6; 6h, 123184-58-7; 6i, 123184-59-8; 6j, 123184-60-1; 6k, 123184-61-2; 6l, 123184-62-3; 6m, 123184-63-4; 6n, 123184-64-5; 6o, 123184-65-6; 6p, 123184-66-7; 6q, 123184-67-8; 6r, 123184-68-9; 6s, 123184-69-0; 6t, 104568-70-9; 6u, 123184-70-3; 6v, 123184-71-4; 6w, 123184-72-5; 6x, 123184-73-6; 6y, 123184-74-7; 6z, 123184-75-8; 6aa, 123184-76-9; 6bb, 123184-77-0; 6cc, 123184-78-1; 6dd, 123184-79-2; 6ee, 123184-80-5; 6ff, 123184-81-6; 6gg, 123184-82-7; 6hh, 123184-83-8; 6ii, 123184-84-9; 6jj, 123184-85-0; 7a, 123184-90-7; 7b, 123184-91-8; 7c, 123184-92-9; 7d, 123184-93-0; 7e, 123184-94-1; 7f, 123184-95-2; 7g, 123184-96-3; 7h, 123184-97-4; 7i, 123184-98-5; 7j, 123184-99-6; 7l, 123185-00-2; 7m, 123185-01-3; 7o, 123185-02-4; 7q, 123185-03-5; 7r, 123185-04-6; 7s, 123185-05-7; 7t, 123185-06-8; 7u, 123185-07-9; 7w, 123185-08-0; 7x, 104568-71-0; 7y, 123185-09-1; 7z, 123185-10-4; 7aa, 123185-11-5; 7bb, 123185-12-6; 7cc, 123185-13-7; 7dd, 123185-14-8; 7ee, 123185-15-9; 7ff, 123185-16-0; 7gg, 123185-17-1; 7hh, 123185-18-2; 7ii, 123185-19-3; 7jj, 123185-20-6; 7kk, 123185-21-7; 7ll, 123185-22-8; 7mm, 123185-23-9; 7nn, 123185-24-0; 8a, 123185-25-1; 8b, 123185-26-2; 8c, 123185-27-3; 8d, 123185-28-4; 8e (stereoisomer 1), 123185-29-5; 8e (stereoisomer 2), 123185-49-9; 8f, 104568-74-3; 8g, 105356-37-4; 8h, 104568-81-2; 8i, 104568-78-7; 8j, 123185-30-8; 8k, 123185-31-9; 8l, 104568-80-1; 8m, 123185-32-0; 8n, 123185-33-1; 8o, 104568-77-6; 8p, 123185-34-2; 8q, 104568-83-4; 8r, 104568-82-3; 8s, 104568-79-8; 8t (stereoisomer 1), 123355-04-4; 8t (stereoisomer 2), 123283-97-6; 8u, 123185-35-3; 8v, 123185-36-4; 8w, 104568-85-6; 8x, 104568-73-2; 8y, 104568-76-5; 8z, 123185-37-5; 8aa, 104568-75-4; 8bb, 123185-38-6; 8cc, 123185-39-7; 8dd, 104568-92-5; 8ee, 123185-40-0; 8ff, 123185-41-1; 8gg, 123185-42-2; 8hh, 105356-38-5; 8ii, 123185-43-3; 8jj, 123185-44-4; 8kk, 123185-45-5; 8ll, 123185-46-6; 8mm, 123185-47-7; 8nn, 123185-48-8; $EtCOCH_2CO_2Me$, 30414-53-0; $CF_3COCH_2CO_2Me$, 83643-84-9; m-$FC_6H_4COCH_2Br$, 53631-18-8; $(CH_3)_2CHCH_2CO_2Me$, 42558-54-3; p-$FC_6H_4COCH_2Br$, 403-29-2; 2,6-$(MeO)_2C_6H_3COCH_2Br$, 123184-19-0; 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride, 4568-71-2; 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride, 123184-18-9; 3-aminopropionitrile $^1/_2$-fumarate, 2079-89-2; 2-[2-(4-fluorophenyl)-5-(1,1-dimethylethyl)-1*H*-pyrrol-1-yl]-2-ethanol, 123184-86-1; 2-[2-(4-fluorophenyl)-5-(1,1-dimethylethyl)-1*H*-pyrrol-1-yl]-2-ethyl methanesulfonate, 123184-87-2; methyl acetoacetate, 105-45-3; cholesterol, 57-88-5.

Supplementary Material Available: CAMSEQ-II energies calculated for individual conformations of θ for compounds appearing in Table IV. The data are plotted in Figure 2. Also, a description of the format of a CAMSEQ-II MOL file, followed by MOL files giving x, y, z coordinates for the conformations of compounds I, III, and 8x used in the pharmacophore model (7 pages). Ordering information is given on any current masthead page.

Inhibitors of Cholesterol Biosynthesis. 2. 1,3,5-Trisubstituted [2-(Tetrahydro-4-hydroxy-2-oxopyran-6-yl)ethyl]pyrazoles

D. R. Sliskovic,* B. D. Roth, M. W. Wilson, M. L. Hoefle, and R. S. Newton

Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105. *Received March 16, 1989*

A series of 1,3,5-trisubstituted pyrazole mevalonolactones were prepared and evaluated for their ability to inhibit the enzyme HMG-CoA reductase in vitro. Since previous studies suggested that the 5-(4-fluorophenyl) and 3-(1-methylethyl) substituents afforded optimum potency, attention was focused on variations in position 1 of the pyrazole ring. Biological evaluation of analogues bearing a variety of 1-substituents suggested that, although most substituents were tolerated, none afforded an advantage over phenyl, which exhibited potency comparable to that of compactin in vitro.

We previously described a series of 2,5-disubstituted pyrrole mevalonolactones whose 3,5-dihydroxyheptanoic acid derivatives were shown to possess varying degrees of intrinsic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity in vitro.[1] Structure–activity relationships (SAR) for this series of compounds were determined, and the preferred substituents in the 2- and 5-positions of the pyrrole nucleus were found to be 4-fluorophenyl and 1-methylethyl, respectively. This paper describes the synthesis and biological activity of a series of 1,3,5-trisubstituted pyrazole mevalonolactones[2] with

(1) Roth, B. D.; Hoefle, M. L.; Stratton, C. D.; Sliskovic, D. R.; Wilson, M. W.; Newton, R. S. Submitted to *J. Med. Chem.*

(2) During the course of this study, a series of trisubstituted pyrazole mevalonolactones were reported to inhibit HMG-CoA reductase by J. R. Wareing at Sandoz Pharmaceuticals Corp. U.S. Patent. 4613610.

0022-2623/90/1833-0031$02.50/0

Acknowledgment. We are indebted to E. H. Ferguson and C. S. Sekerke for conducting the enzyme inhibition assays, to Dr. S. Brennan, T. Hurley, and D. Sherwood for HPLC analyses, to Dr. F. A. MacKellar and staff for analytical and spectral determinations, and to P. Carr and D. Sandy for manuscript preparation.

Registry No. 1 (R_1 = Ph), 768-03-6; 1 (R_1 = 4-F-C_6H_4), 51594-59-3; 1 (R_1 = 4-Ph-C_6H_4), 42575-11-1; 1 (R_1 = 4-Cl-C_6H_4), 7448-87-5; 1 (R_1 = 4-CH_3O-C_6H_4), 7448-86-4; 1 (R_1 = 3-F_3C-C_6H_4), 123184-14-5; 1 (R_1 = 3-CH_3O-C_6H_4), 51594-60-6; 1 (R_1 = 2-CH_3O-C_6H_4), 77942-10-0; 1 (R_1 = 2-naphthyl), 4452-06-6; 1 (R_1 = 1-naphthyl), 22422-69-1; 1 (R_1 = bicyclo[2.2.1]-hept-5-en-2-yl), 100234-78-4; 1 (R_1 = bicyclo[2.2.2]-oct-5-en-2-yl), 123184-15-6; 1 (R_1 = cyclohexyl), 2177-34-6; 1 (R_1 = Ph_2CH), 93021-71-7; 1 (R_1 = $CH(C_2H_5)_2$), 123184-16-7; 1 (R_1 = 2-F-C_6H_4), 89638-21-1; 1 (R_1 = 2,4-F_2-C_6H_3), 123184-17-8; 1 (R_1 = $CH(CH_3)_2$), 1606-47-9; 2 (R_2 = CH_3), 75-07-0; 2 (R_2 = $CH(CH_3)_2$), 78-84-2; 2 (R_2 = $CH(C_2H_5)_2$), 97-96-1; 2 (R_2 = cyclopropyl), 1489-69-6; 2 (R_2 = cyclobutyl), 2987-17-9; 2 (R_2 = cyclohexyl), 2043-61-0; 2 (R_2 = $C(CH_3)_3$), 630-19-3; 2 (R_2 = 4-F-C_6H_4), 459-57-4; 2 (R_2 = C_2H_5), 123-38-6; **3a**, 583-05-1; **3b**, 123183-95-9; **3c**, 63472-37-7; **3d**, 53842-12-9; **3e**, 2108-54-5; **3f**, 123183-96-0; **3g**, 123183-97-1; **3h**, 104562-48-3; **3i**, 123183-98-2; **3j**, 123263-79-6; **3k**, 70353-45-6; **3l**, 123183-99-3; **3m**, 61771-79-7; **3n**, 123184-00-9; **3o**, 123184-01-0; **3p**, 104568-68-5; **3q**, 123184-02-1; **3r**, 123184-03-2; **3s**, 123184-04-3; **3t**, 123184-05-4; **3u**, 123184-06-5; **3v**, 123184-07-6; **3w**, 123184-08-7; **3x**, 123184-09-8; **3y**, 123184-10-1; **3z**, 123184-11-2; **3aa**, 123184-12-3; **3bb**, 123184-13-4; **5a**, 123184-20-3; **5b**, 123184-21-4; **5c**, 123184-22-5; **5d**, 123184-23-6; **5e**, 123184-89-4; **5f**, 123184-24-7; **5g**, 123184-25-8; **5h**, 123184-26-9; **5i**, 123184-27-0; **5j**, 123184-28-1; **5k**, 123184-29-2; **5l**, 123184-30-5; **5m**, 123184-31-6; **5n**, 123184-32-7; **5o**, 123184-33-8; **5p**, 123184-34-9; **5q**, 123184-35-0; **5r**, 123184-36-1; **5s**, 123184-37-2; **5t**, 104568-69-6; **5u**, 123184-88-3; **5v**, 123184-38-3; **5w**, 123184-39-4; **5x**, 123184-40-7; **5y**, 123184-41-8; **5z**, 104568-91-4; **5aa**, 104568-69-6; **5bb**, 123184-42-9; **5cc**, 123184-43-0; **5dd**, 123184-44-1; **5ee**, 123184-45-2; **5ff**, 123184-46-3; **5gg**, 123184-47-4; **5hh**, 123184-48-5; **5ii**, 123184-49-6; **5jj**, 123184-50-9; **6a**, 123184-51-0; **6b**, 123184-52-1; **6c**, 123184-53-2; **6d**, 123184-54-3; **6e**, 123184-55-4; **6f**, 123184-56-5; **6g**, 123184-57-6; **6h**, 123184-58-7; **6i**, 123184-59-8; **6j**, 123184-60-1; **6k**, 123184-61-2; **6l**, 123184-62-3; **6m**, 123184-63-4; **6n**, 123184-64-5; **6o**, 123184-65-6; **6p**, 123184-66-7; **6q**, 123184-67-8; **6r**, 123184-68-9; **6s**, 123184-69-0; **6t**, 104568-70-9; **6u**, 123184-70-3; **6v**, 123184-71-4; **6w**, 123184-72-5; **6x**, 123184-73-6; **6y**, 123184-74-7; **6z**, 123184-75-8; **6aa**, 123184-76-9; **6bb**, 123184-77-0; **6cc**, 123184-78-1; **6dd**, 123184-79-2; **6ee**, 123184-80-5; **6ff**, 123184-81-6; **6gg**, 123184-82-7; **6hh**, 123184-83-8; **6ii**, 123184-84-9; **6jj**, 123184-85-0; **7a**, 123184-90-7; **7b**, 123184-91-8; **7c**, 123184-92-9; **7d**, 123184-93-0; **7e**, 123184-94-1; **7f**, 123184-95-2; **7g**, 123184-96-3; **7h**, 123184-97-4; **7i**, 123184-98-5; **7j**, 123184-99-6; **7l**, 123185-00-2; **7m**, 123185-01-3; **7o**, 123185-02-4; **7q**, 123185-03-5; **7r**, 123185-04-6; **7s**, 123185-05-7; **7t**, 123185-06-8; **7u**, 123185-07-9; **7w**, 123185-08-0; **7x**, 104568-71-0; **7y**, 123185-09-1; **7z**, 123185-10-4; **7aa**, 123185-11-5; **7bb**, 123185-12-6; **7cc**, 123185-13-7; **7dd**, 123185-14-8; **7ee**, 123185-15-9; **7ff**, 123185-16-0; **7gg**, 123185-17-1; **7hh**, 123185-18-2; **7ii**, 123185-19-3; **7jj**, 123185-20-6; **7kk**, 123185-21-7; **7ll**, 123185-22-8; **7mm**, 123185-23-9; **7nn**, 123185-24-0; **8a**, 123185-25-1; **8b**, 123185-26-2; **8c**, 123185-27-3; **8d**, 123185-28-4; **8e** (stereoisomer 1), 123185-29-5; **8e** (stereoisomer 2), 123185-49-9; **8f**, 104568-74-3; **8g**, 105356-37-4; **8h**, 104568-81-2; **8i**, 104568-78-7; **8j**, 123185-30-8; **8k**, 123185-31-9; **8l**, 104568-80-1; **8m**, 123185-32-0; **8n**, 123185-33-1; **8o**, 104568-77-6; **8p**, 123185-34-2; **8q**, 104568-83-4; **8r**, 104568-82-3; **8s**, 104568-79-8; **8t** (stereoisomer 1), 123355-04-4; **8t** (stereoisomer 2), 123283-97-6; **8u**, 123185-35-3; **8v**, 123185-36-4; **8w**, 104568-85-6; **8x**, 104568-73-2; **8y**, 104568-76-5; **8z**, 123185-37-5; **8aa**, 104568-75-4; **8bb**, 123185-38-6; **8cc**, 123185-39-7; **8dd**, 104568-92-5; **8ee**, 123185-40-0; **8ff**, 123185-41-1; **8gg**, 123185-42-2; **8hh**, 105356-38-5; **8ii**, 123185-43-3; **8jj**, 123185-44-4; **8kk**, 123185-45-5; **8ll**, 123185-46-6; **8mm**, 123185-47-7; **8nn**, 123185-48-8; $EtCOCH_2CO_2Me$, 30414-53-0; $CF_3COCH_2CO_2Me$, 83643-84-9; *m*-$FC_6H_4COCH_2Br$, 53631-18-8; $(CH_3)_2CHCH_2CO_2Me$, 42558-54-3; *p*-$FC_6H_4COCH_2Br$, 403-29-2; 2,6-$(MeO)_2C_6H_3COCH_2Br$, 123184-19-0; 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride, 4568-71-2; 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride, 123184-18-9; 3-aminopropionitrile $^1/_2$-fumarate, 2079-89-2; 2-[2-(4-fluorophenyl)-5-(1,1-dimethylethyl)-1*H*-pyrrol-1-yl]-2-ethanol, 123184-86-1; 2-[2-(4-fluorophenyl)-5-(1,1-dimethylethyl)-1*H*-pyrrol-1-yl]-2-ethyl methanesulfonate, 123184-87-2; methyl acetoacetate, 105-45-3; cholesterol, 57-88-5.

Supplementary Material Available: CAMSEQ-II energies calculated for individual conformations of θ for compounds appearing in Table IV. The data are plotted in Figure 2. Also, a description of the format of a CAMSEQ-II MOL file, followed by MOL files giving x, y, z coordinates for the conformations of compounds I, III, and 8x used in the pharmacophore model (7 pages). Ordering information is given on any current masthead page.

Inhibitors of Cholesterol Biosynthesis. 2. 1,3,5-Trisubstituted [2-(Tetrahydro-4-hydroxy-2-oxopyran-6-yl)ethyl]pyrazoles

D. R. Sliskovic,* B. D. Roth, M. W. Wilson, M. L. Hoefle, and R. S. Newton

Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105. *Received March 16, 1989*

A series of 1,3,5-trisubstituted pyrazole mevalonolactones were prepared and evaluated for their ability to inhibit the enzyme HMG-CoA reductase in vitro. Since previous studies suggested that the 5-(4-fluorophenyl) and 3-(1-methylethyl) substituents afforded optimum potency, attention was focused on variations in position 1 of the pyrazole ring. Biological evaluation of analogues bearing a variety of 1-substituents suggested that, although most substituents were tolerated, none afforded an advantage over phenyl, which exhibited potency comparable to that of compactin in vitro.

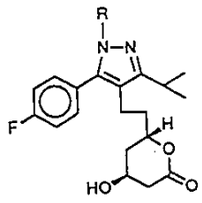
We previously described a series of 2,5-disubstituted pyrrole mevalonolactones whose 3,5-dihydroxyheptanoic acid derivatives were shown to possess varying degrees of intrinsic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity in vitro.[1] Structure–activity relationships (SAR) for this series of compounds were determined, and the preferred substituents in the 2- and 5-positions of the pyrrole nucleus were found to be 4-fluorophenyl and 1-methylethyl, respectively. This paper describes the synthesis and biological activity of a series of 1,3,5-trisubstituted pyrazole mevalonolactones[2] with

(1) Roth, B. D.; Hoefle, M. L.; Stratton, C. D.; Sliskovic, D. R.; Wilson, M. W.; Newton, R. S. Submitted to *J. Med. Chem.*

(2) During the course of this study, a series of trisubstituted pyrazole mevalonolactones were reported to inhibit HMG-CoA reductase by J. R. Wareing at Sandoz Pharmaceuticals Corp. U.S. Patent. 4613610.

0022-2623/90/1833-0031$02.50/0

Table I. Physical Properties and in Vitro HMG-CoA Reductase Inhibitory Actives of Pyrazole Mevalonolactones I

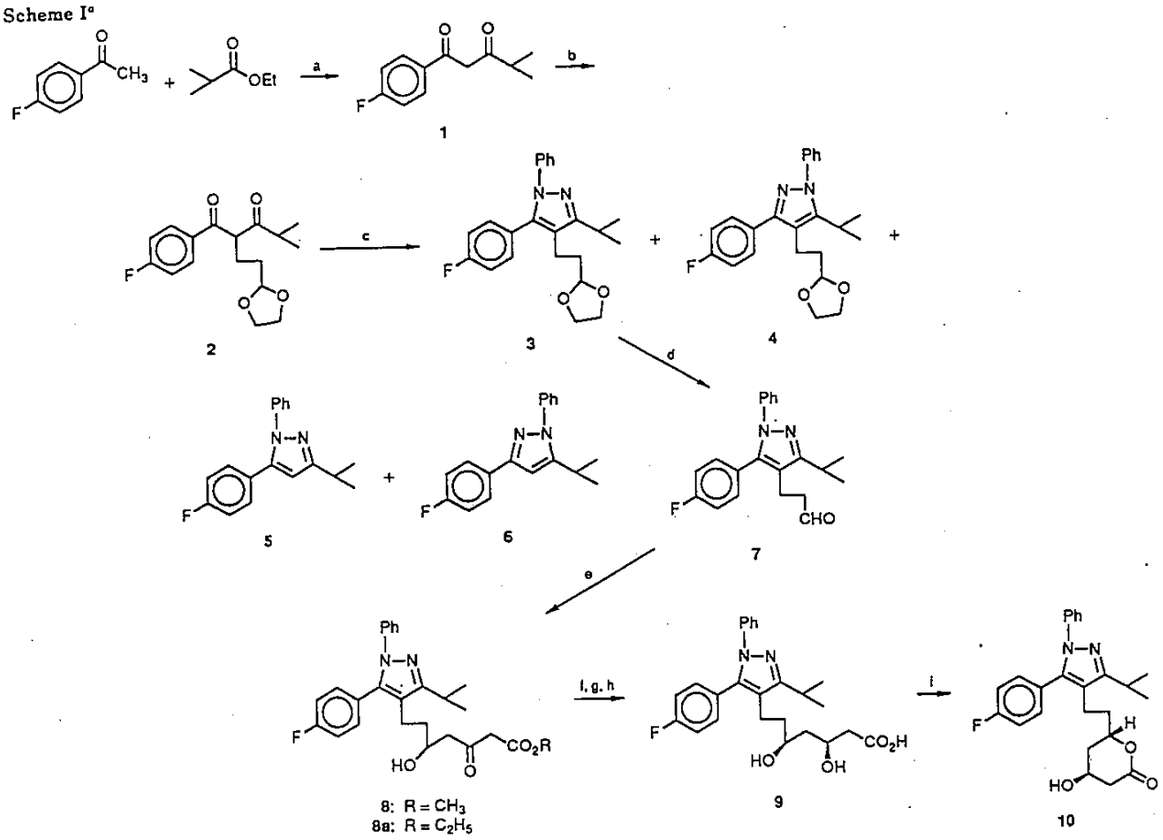


I

no.	R	mp, °C	formula[a]	method of prep	CSI IC_{50},[f,g] μM	rel (CSI) potency[b]
10	Ph	165–167	$C_{25}H_{27}FN_2O_3$	A, B	0.035	83.0
25	4-fluorophenyl	138–142	$C_{25}H_{26}F_2N_2O_3$	A	0.032	62.0
26	4-methylphenyl	152–153	$C_{26}H_{29}FN_2O_3$	A	0.040	49.0
27	4-tolylsulfonyl	foam	$C_{26}H_{29}FN_2O_5S$	B	0.660	4.5
28	4-methoxyphenyl	134–139	$C_{26}H_{29}FN_2O_4$[c]	A	0.039	75.8
29	benzyl	145–148	$C_{26}H_{29}FN_2O_3$[d]	A	0.158	12.6
30	1-naphthyl	75–81	$C_{29}H_{29}FN_2O_3$[e]	B	0.234	19.6

[a] Analytical results are within ±0.4% of the theoretical values unless otherwise noted. [b] Potency of compactin arbitrarily assigned a value of 100, and the IC_{50} value of the test compound was compared with that of compactin determined simultaneously. [c] Anal. Calcd: C, 69.01. Found: C, 68.30. >98% pure by HPLC. [d] Anal. Calcd: H, 6.70. Found: H, 7.22, Calcd: N, 6.42. Found: N, 5.85. >98% pure by HPLC. [e] Anal. Calcd: C, 73.21. Found: C, 72.46. >98% pure by HPLC. [f] Cholesterol synthesis inhibition (CSI). Assays of each inhibitor concentration were performed in triplicate and the precision for compactin was 37%. See ref 1. [g] All compounds tested had a diastereometric purity of >95% of the trans diastereomer as determined by HPLC and/or 200-MHz NMR.

Scheme I[a]



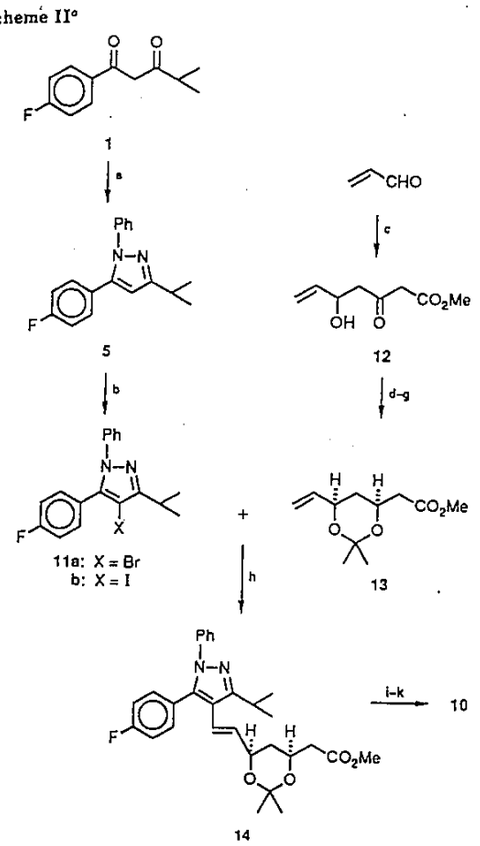
[a] (a) NaH, DMF, 80 °C; (b) NaH, DMF, NaI, $BrCH_2CH_2\overline{CHO(CH_2)O}$; (c) $PhNHNH_2$, AcOH, room temperature; (d) 70% aqueous AcOH, Δ; (e) $^-CH_2CO^-CHCO_2R$; (f) BR_3, air; (g) $NaBH_4$, −78 °C; (h) H_2O_2, ^-OH; (i) tol, Δ.

improved inhibitory potencies compared to the pyrrole mevalonolactones.

Chemistry

The target lactones, listed in Table I, were prepared by the general synthetic routes outlined in Schemes I and II. The general method (method A) employed for the construction of the pyrazole nucleus was condensation of a 1,3-dicarbonyl compound with a suitably substituted hydrazine. Two regioisomers can theoretically arise, but by

Scheme II[a]



[a] (a) $PhNHNH_2$, AcOH, room temperature; (b) NBS or NIS, DMF, 0 °C; (c) $^-CH_2CO^-CHCO_2Et$; (d) Bu_3B, air; (e) $NaBH_4$; (f) H_2O_2/OH^-, (g) $(CH_3)_2C(OCH_3)_2$, CSA, acetone; (h) $(Ph_3P)_2PdCl_2$, Et_3N, DMF, 70 °C; (i) H_2, Pd/C; (j) HCl, NaOH; (k) Tol, Δ.

judicial choice of solvent and reaction temperature, one regioisomer can predominate. Initial studies began with the incorporation of the preferred substituents (4-fluorophenyl and isopropyl) discovered in the SAR of the pyrrole mevalonolactones.[1] The requisite 1,3-diketone 1 was synthesized by a Claisen type acylation of 4-fluoroacetophenone with ethyl isobutyrate.[3] This product, which was almost completely enolized (86% by NMR), was alkylated with 2-(2-bromoethyl)-1,3-dioxolane[4] to give the C-alkylated 1,3-diketone 2 in 58% yield, together with a small amount of material presumed to be the O-alkylated product. Condensation with phenylhydrazine in acetic acid at room temperature afforded predominantly one regioisomer (~90%), tentatively assigned structure 3 in which the aryl groups exist in a 1,5-relationship (rather than 1,3). NMR studies[5] on 1,3- and 1,5-diphenylpyrazoles have shown that the chemical shifts of phenyl groups in the 1,3-regioisomer extend from δ 7.0 to 8.1 ppm. In our case, downfield resonances at δ 8.0 ppm were barely discernible. The majority of the aryl proton resonances were found in the region from δ 7.0 to 7.3 ppm which was in accordance with resonances published for 1,5-diphenylpyrazole. This regiochemistry was confirmed by an X-ray crystallographic analysis of the eventual target lactone derived from 3 (vide supra).[6] An ORTEP drawing of the solid-state conformation of compound 10 is shown in Figure 1. Increased amounts of the 1,3-regioisomer 4 were obtained by changing the reaction solvent to absolute ethanol or by raising the reaction temperature (regardless of solvent choice). Using either (4-chlorophenyl)hydrazine or (4-fluorophenyl)-hydrazine in absolute ethanol at reflux, the regioisomer ratio of pyrazoles obtained was 5:1 (1,5:1,3), this ratio was improved (~10:1) by changing solvent to acetic acid. Also isolated from this reaction was an oil later identified by NMR and independent synthesis[7] as a 5:1 mixture of pyrazole regioisomers 5 and 6 which was presumably derived from the O-alkylated material present from the previous reaction.

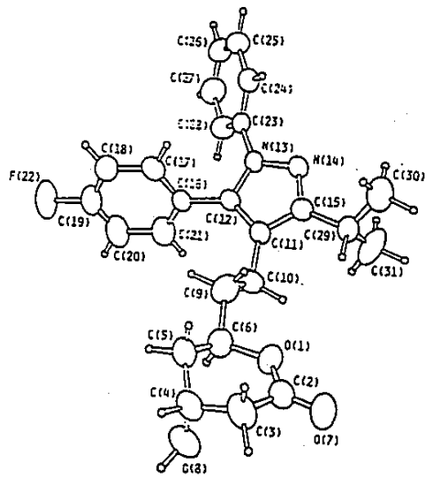


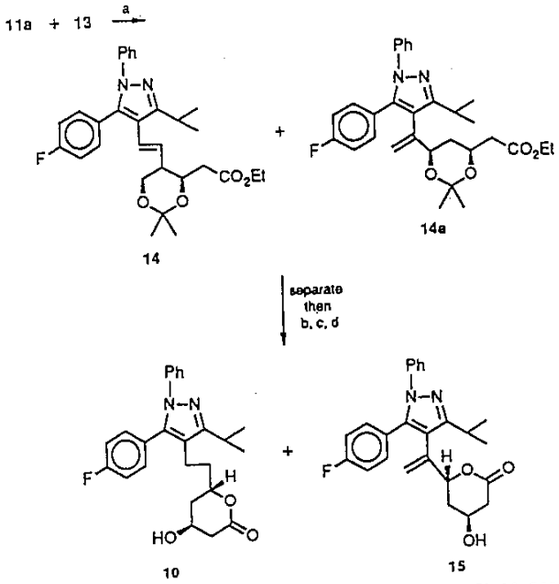
Figure 1. ORTEP view of lactone 10. Solid-state conformation and crystallographic atom numbering scheme; small circles denote hydrogen atoms.

Acidic hydrolysis of the acetal 3 provided aldehyde 7, which was condensed with the dianion of methyl acetoacetate.[8] Reduction of the resulting δ-hydroxy-β-keto ester 8 was achieved by the boron chelation method of Narasaka and Pai.[9] Thus, compound 8 was complexed with tri-*n*-butylborane prior to treatment with sodium borohydride. The resulting boronate ester was hydrolyzed with 30% hydrogen peroxide and base to give a mixture of syn (9) and anti 1,3-dihydroxy acids, which were lactonised in refluxing toluene with azeotropic removal of water to give predominantly the trans lactone 10 in good yield. HPLC analysis of the lactone 10 showed that the stereoselectivity achieved (3.3:1 trans:cis diastereomers) was not as high as that achieved in the pyrrole series (10:1 trans:cis).[1] No improvement in stereoselectivity was found on addition of an extra equivalent of n-Bu_3B, ruling out the possibility of competitive chelation with the pyrazole free nitrogen atom; thus the reason for this lack of stereoselectivity in the pyrazole series remains unclear. Excellent stereoselectivity (>20:1 trans:cis) was achieved by employing triethylborane as chelating agent with pivalic acid catalysis and methanol as cosolvent.[10]

An alternative route (Scheme II) was devised in which the key step was the palladium-catalyzed vinylation of a

(3) Levine, R.; Conroy, J. A.; Adams, J. T.; Hauser, C. R. *J. Am. Chem. Soc.* **1945**, *67*, 1516.
(4) Buchi, G.; Wüest, H. *J. Org. Chem.* **1969**, *34*, 1122.
(5) Ruu, T.; LeStrat, G. *Bull. Soc. Chem. Fr.* **1975**, *5–6*, 1375.
(6) Prof. A. T. McPhail. Personal communication.
(7) Katritzky, A. R.; Rees, C. W. *Comprehensive Heterocyclic Chemistry*; Pergamon: Elmsford, NY, 1984; Vol. 5, p 277.
(8) Huckin, S. N.; Weiler, L. *J. Am. Chem. Soc.* **1981**, *96*, 1082.
(9) (a) Narasaka, K.; Pai, H. C. *Chem. Lett.* **1980**, 1415. (b) Ibid. *Tetrahedron* **1984**, *40*, 2233.
(10) Verhoeven, T. R. *Eur. Pat.* 0164, 049, 1985.

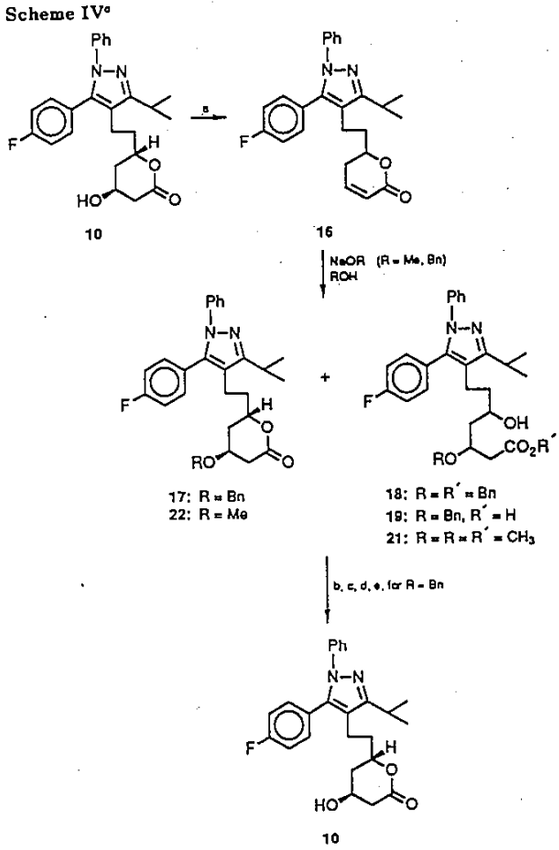
Scheme III



[a] (a) $(PPh_3)_2PdCl_2$, DMF, Et_3N; (b) H_2, Pd/C; (c) HCl, NaOH; (d) Tol, Δ, $-H_2O$.

halopyrazole (11a,b) with the intact lactone side chain (13).[11] This route had the advantages of being convergent and providing products of satisfactory stereochemical purity (method B). The heterocyclic halides 11a,b were prepared by condensation of 1,3-diketone 1 with phenylhydrazine in acetic acid at room temperature followed by halogenation of the resulting pyrazole 5 with either NBS or NIS in DMF at 0 °C. The alkene portion (13) was constructed via aldol condensation of acrolein with the dianion of methyl (or ethyl) acetoacetate,[12] reduction as before gave the diol, which was protected as the acetonide 13 (25:1 trans:cis diastereomers). Although treatment of 11a with 13 under the standard conditions described by Heck[11] did in fact provide a modest (50%) yield of 14, this reaction proved capricious. A variety of catalysts were employed (e.g., $(Ph_3P)_2PdCl_2$, $Pd(OAc)_2$, 10% Pd/C, polymer-supported catalysts, etc.), and it was concluded that 2–6 mol % of $(Ph_3P)_2PdCl_2$ was the preferred catalyst. A number of bases (e.g., tri-*n*-butylamine, diisopropylethylamine, and triethylamine) and solvents (e.g., DMF and acetonitrile) were examined, and the best yields were obtained with triethylamine and DMF as solvents. Changing the heterocyclic halide from bromide (11a) to iodide (11b) gave increased amounts of the dehalogenated pyrazole 5. Although it has been reported that use of a more hindered phosphine ligand on the catalyst reduces this side reaction, replacement of $(Ph_3P)_2PdCl_2$ with $[(o\text{-}CH_3Ph)_3P]_2PdCl_2$ provided no improvement in yield.[11] The 200-MHz NMR showed the formation of predominantly the trans alkene 14 (J_{trans} = 15 Hz). A minor product was produced by addition to the more substituted carbon atom of the double bond (Scheme III), giving the olefin 14a. This structure was confirmed by HETCOR NMR[13] on the resulting lactone 15. Catalytic reduction of olefin 14, removal of the protecting groups, and lactonization afforded lactone 10 as a mixture of diastereomers (64:1 trans:cis).

Scheme IV[a]

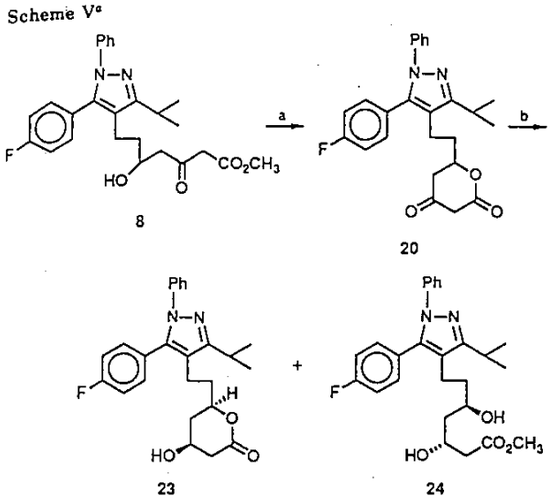


[a] (a) Ac_2O, DBU, CH_2Cl_2; (b) NaOH, (c) H^+; (d) H_2, Pd/C, EtOAc; (e) Tol, Δ.

In order to avoid the very low temperature reduction of compound 8 in Scheme I and the capricious nature of the Heck reaction shown in Scheme II, an alternative synthesis was devised in which the required 1,3-asymmetry was introduced by the stereospecific 1,4-conjugate addition of an alkoxide.[14] Thus, elimination of water from the mixture of lactone diastereomers 10 produced by borohydride reduction or from the cis lactone 23 obtained from the catalytic reduction of compound 20 produced the $\Delta^{\alpha,\beta}$-unsaturated lactone 16 in 68% yield (Scheme IV). Addition of sodium benzylate in benzyl alcohol afforded a mixture of products thought to consist mainly of compounds 17 and 18. After base hydrolysis the mixture was acidified to predominantly hydroxy acid 19. This material was then hydrogenated over 10% Pd/C and the resulting material lactonized to give compound 10 as a mixture of diastereomers (8:1 trans:cis by HPLC). In a similar fashion, sodium methoxide was added to lactone 16 to give, after base hydrolysis, acidification, and lactonization, the 4-methoxy lactone 22 as a mixture of diastereomers (7.4:1 trans:cis by HPLC). The cis diastereomer 23 was obtained as the predominant product by catalytic hydrogenation of ketone 20, which was prepared by base hydrolysis of compound 8 (Scheme V). Catalytic reduction of compound 20 gave, after chromatography, a mixture of ester 24 and lactone 23 (4:1 cis:trans diastereomers).

(11) Heck, R. F. *Org. React.* (*N.Y.*) 1982, *27*, 345.
(12) Brussani, G.; Ley, S. V.; Wright, J. L.; Williams, D. J. *J. Chem. Soc., Perkin Trans. 1* 1986, 303.
(13) Benn, R.; Günther, H. *Angew. Chem., Int. Ed. Engl.* 1983, *22*, 350.
(14) Roth, B. D.; Roark, W. H. *Tetrahedron Lett.* 1988, 1255–58.

Scheme V[a]



[a] (a) NaOH then H^+; (b) 10% Ru–C, H_2, MeOH, room temperature.

Table II. In Vitro Inhibitory Potencies against HMG-CoA Reductase

no.	CSI IC_{50},[a,c] μM	rel potency[b]
15	17.8	0.17
20	10.0	0.32
22	3.16	1.00
23	0.7	4.40

[a] Cholesterol synthesis inhibition (CSI). Assays of each inhibitor concentration were performed in triplicate and the precision for compactin was 37%. See ref 1. [b] Potency of compactin arbitrarily assigned a value of 100, and the IC_{50} value of the test compound was compared with that of compactin determined simultaneously. See ref 1. [c] The diastereomeric purities of compounds 22 and 23 are indicated in the Experimental Section. Compound 15 had a diastereomeric purity of >95% of the trans diastereomer as indicated by 200-MHz NMR.

Biological Results

The target lactones and related compounds listed in Tables I and II were saponified to the hydroxy acids and tested for their ability to inhibit the enzyme HMG-CoA reductase by employing a crude liver homogenate derived from rats fed a chow diet containing 5% cholestyramine.[1,15] This screen was designated CSI (cholesterol synthesis inhibition screen). The biological activities are displayed in Tables I and II as an IC_{50} (i.e., the concentration needed to inhibit enzyme activity by 50%). Compactin was employed as the internal standard in each testing protocol.

The optimum distance between the lactone and the heterocyclic ring in the pyrrole series was achieved by a two-carbon bridging unit.[1] This feature was incorporated in all the pyrazole derivatives described here apart from compound 15, in which the pyrazole and lactone portions are separated by only one carbon atom. This compound is relatively inactive.

Modification of the lactone portion generally decreases the activity and confirms the strict structural requirements found by others.[16] Methyl ether 22 exhibited about $^1/_{100}$ potency of compactin whereas the racemic hydroxy compound 10 was nearly equipotent; if resolved, this compound would be expected to be more potent than compactin. The keto analogue 20 also exhibited low potency.[17] The cis lactone stereoisomer 23 (a 4:1 mixture of cis:trans diastereomers by HPLC) also displayed significantly reduced biological activity.[16] The residual biological activity was probably due to the presence of the trans diastereomer.

As previous studies suggested that the 5-(4-fluorophenyl) and 3-(1-methylethyl) substituents afforded optimum potency, we focused our attention on variations in position 1 of the pyrazole ring. A number of (para-substituted phenyl)hydrazines were employed, and it was demonstrated that in the limited series of compounds prepared, varying the electronic distribution in the phenyl ring did not, in general, have deleterious effects on in vitro potency. Electron-withdrawing, e.g., 25, and electron-donating, e.g., 26 and 28, groups were equally tolerated; however, compound 27, which has a hydrophilic electron-withdrawing group present, was considerably less potent. Replacement by naphthyl (e.g., 30) caused a significant decrease in potency as did replacement by an alkyl group, e.g., 29.

Conclusion

A small series of pyrazole mevalonolactones were prepared and evaluated for their ability to inhibit the enzyme HMG-CoA reductase in vitro. By focusing on compounds possessing the 5-(4-fluorophenyl)-3-(1-methylethyl) substitution found to be optimum in previous studies, a compound (10) was rapidly identified that was almost equipotent to compactin. Additional modification of the 1-phenyl ring of 10 did not improve activity in vitro.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Tetrahydrofuran (THF) was distilled from sodium and benzophenone. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were determined on a Nicolet MX-1 FT-IR spectrophotometer. Nuclear magnetic resonance spectra were determined on either a Varian EM-390 or a Varian XL-200 spectrometer. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. Elemental analyses were determined on a Perkin-Elmer 240C elemental analyzer. HPLC analyses were performed on a Varian 5500 HPLC with a UV 200 detector (wavelength was 251 nm). The detailed protocol of the biological assay is described in ref 1.

1-(4-Fluorophenyl)-4-methyl-1,3-pentanedione (1). A mixture of 4-fluoroacetophenone (150 g, 1.09 mol) and ethyl isobutyrate (126 g, 1.09 mol) in dioxane (1.5 L) was added dropwise under a nitrogen atmosphere to a vigorously stirred suspension of hexane-washed sodium hydride (133 g, 58.8% NaH, 3.25 mol) in dioxane (3.0 L). Vigorous evolution of gas ensued, after which the mixture was heated to 80–90 °C for 4 h. The mixture was then allowed to cool to room temperature, after which it was poured into ice-cold 2 M hydrochloric acid (6 L) with vigorous stirring and extracted with ethyl acetate (4 × 1 L). The combined ethyl acetate extracts were washed with water (2 × 500 mL) and brine (2 × 500 mL) and dried ($MgSO_4$). The solution was filtered and the filtrate concentrated under vacuum. Distillation of the residue yielded compound 1: bp 100–110 °C/1 mm (116 g, 50%); ^1H NMR ($CDCl_3$) δ 1.25 (s, 3 H), 1.30 (s, 3 H), 2.60 (m, 1 H, J = 7 Hz), 6.1 (s, 1 H), 7.15 (m, 2 H), 7.9 (m, 2 H), and 16.2 (br s, 1 H) ppm. IR (thin film) 2973, 2825, 1653, 1603, 1578, 1509, 1462, 1240, 1160, 1089, 851, and 793 cm^{-1}. Anal. ($C_{12}H_{13}FO_2$) C, H, F.

2-[2-(1,3-Dioxolan-2-yl)ethyl]-1-(4-fluorophenyl)-4-methyl-1,3-pentanedione (2). To a suspension of hexane-washed sodium hydride (22.8 g, 58% NaH, 0.56 mol) in anhydrous dimethylformamide (DMF) (750 mL) was added dropwise, with

(15) Dugan, R.; Slakey, L. L.; Briedis, A. V.; Porter, J. W. *Arch. Biochem. Biophys.* 1972, *152*.
(16) Stokker, G. E.; Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J.; Deana, A. A.; Gilfillan, J. L.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* 1985, *28*, 347.

(17) One possible explanation for this lack of activity may have been that during the biological assay procedure, base treatment of compound 20 may not have produced the open acid form. We thank the reviewer for this suggestion.

vigorous stirring under a nitrogen atmosphere, a solution of 1 (116 g, 0.56 mol) in anhydrous DMF (450 mL). Vigorous effervescence ensued. When gas evolution had ceased, sodium iodide (21.0 g, 0.14 mol) was added, followed by the dropwise addition of 2-(2-bromoethyl)-1,3-dioxolane[4] (100.9 g, 0.56 mol) in anhydrous DMF (450 mL). The resulting mixture was heated at 80–90 °C for 36 h after which it was cooled to room temperature and poured into ice–water (2 L). This was extracted with ethyl acetate (4 × 1 L), and the combined organic extracts were washed successively with water (500 mL) and brine (500 mL) and dried ($MgSO_4$). The solution was filtered and the filtrate was concentrated under vacuum. The residue was flash chromatographed on silica gel, eluting with 25% ethyl acetate–hexane to yield 2 (100 g, 58%); ^1H NMR ($CDCl_3$) δ 1.1 (s, 3 H), 1.15 (s, 3 H), 1.7 (m, 2 H), 2.2 (m, 2 H), 2.8 (m, 1 H), 3.9 (m, 4 H), 4.7 (t, 1 H), 4.9 (t, 1 H), 7.2 (m, 2 H), and 8.1 (m, 2 H) ppm; IR (thin film) 2972, 1723, 1676, 1600, 1509, 1411, 1237, 1160, and 1037 cm^{-1}. Anal. ($C_{17}H_{21}FO_4$) C, H, F.

4-[2-(1,3-Dioxolan-2-yl)ethyl]-5-(4-fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazole (3). To solution of 2 (104.75 g, 0.34 mol) in absolute ethanol under nitrogen (1 L) was added dropwise, with stirring, phenylhydrazine (40.45 g, 0.374 mol). When addition was complete, the solution was heated under reflux for 5 days[18] and then cooled to room temperature. The solution was concentrated under vacuum and chromatographed on silica gel. Elution with 15% ethyl acetate–hexane gave a yellow oil (9.7 g, R_f 0.55 (15% EtOAc–hexane)) identified by NMR and synthesis as a 5:1 mixture of regioisomers 5 and 6. Further elution gave a 10:1 regioisomer mixture of pyrazoles 3 and 4 (NMR shows two sets of isopropyl methyl groups at δ 1.4 and 1.2 ppm in a 10:1 ratio). This mixture solidified and was recrystallized (hexane) to give 3: mp 98–100 °C (hexane) (50.85 g, 40%); ^1H NMR ($CDCl_3$) δ 1.4 (s, 3 H), 1.35 (s, 3 H), 1.8 (m, 2 H), 2.7 (m, 2 H), 3.1 (t, 1 H), 3.9 (m, 4 H), 4.8 (t, 1 H), and 7.2 (m, 9 H) ppm; IR (KBr) 2950, 2900, 1596, 1566, 1511, 1440, 1377, 1227, 1158, 1143, 1058, 970, and 842 cm^{-1}. Anal. ($C_{23}H_{25}FN_2O_2$) C, H, N.

3(or 5)-(4-Fluorophenyl)-5(or 3)-(1-methylethyl)-1-phenyl-1*H*-pyrazoles (5 and 6). To a solution of 1 (1 g, 0.0048 mol) in absolute ethanol (10 mL) was added via a syringe, with stirring, phenylhydrazine (0.52 mL, 0.0053 mol). The solution was heated to reflux for 24 h and then cooled to room temperature. The solution was concentrated under vacuum and then chromatographed on silica gel. Elution with 5% ethyl acetate–hexane gave a yellow oil (1.1 g, R_f 0.24 (5% EtOAc–hexane)) identified by NMR as a 5:1 regioisomer mixture of 5 and 6. The oil solidified and was recrystallized (hexane) to give a 5:1 mixture of regioisomers: mp 67–70 °C (0.5 g, 37%); ^1H NMR ($CDCl_3$) δ 1.2 (d, 6 H, $(CH_3)_2CH$, regioisomer (6) (ht = 1)), 1.3 (d, 6 H, $(CH_3)_2CH$, regioisomer (5) (ht = 5), 3.1 (m, 1 H), 6.35 (s, 1 H, 4 H regioisomer (5) (ht = 5)), 6.5 (s, 1 H, 4 H regioisomer (6) (ht = 1)) and 6.9–7.4 (m, 9H) ppm; IR (KBr) 3450, 3053, 2964, 1594, 1510, 1440, 1374, 1302, 1222, 1164, 996, and 849 cm^{-1}. Anal. ($C_{18}H_{17}FN_2$) C, H, N.

5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazole-4-propanal (7). A solution of 3 (50.85 g, 0.134 mol) in 70% aqueous acetic acid (1.0 L) was heated under reflux for 48 h with stirring. The solution was then cooled to room temperature and partitioned between ethyl acetate (1.0 L) and water (1.0 L). The phases were separated, and the aqueous phase was reextracted with ethyl acetate (1.0 L). The combined organic layer was washed successively with saturated sodium bicarbonate solution (250 mL), water (250 mL), and brine (250 mL). The ethyl acetate solution was dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was flash chromatographed on silica gel, eluting with 15% ethyl acetate–hexane. The eluted material solidified and was recrystallized (hexane) to give 7: mp 86–88 °C (hexane) (29.0 g, 65%); ^1H NMR ($CDCl_3$) δ 1.3 (s, 3 H), 1.35 (s, 3 H), 2.4 (t, 2 H), 2.7 (t, 2 H), 3.05 (m, 1 H), 7.2–7.6 (m, 9 H), and 9.6 (s, 1 H) ppm. IR (KBr) 2961, 2869, 1728, 1609, 1598, 1498, 1439, 1376, 1334, 1224, 1159, 971, 840, and 767 cm^{-1}. Anal. ($C_{15}H_{17}FN_2O$) H, N; C: calcd, 69.21; found, 68.51.

(±)-Methyl 5-(4-Fluorophenyl)-δ-hydroxy-3-(1-methylethyl)-β-oxo-1-phenyl-1*H*-pyrazole-4-heptanoate (8). Methyl acetoacetate (11.48 mL, 0.106 mol) in anhydrous THF (100 mL) was added dropwise to a stirred suspension of sodium hydride (58.8% oil suspension, 4.56 g, 0.116 mol) in anhydrous THF (100 mL) at 0 °C under an N_2 atmosphere. When gas evolution was complete, a 2.6 M solution (40.9 mL, 0.106 mol) of *n*-butyllithium in hexane was added over 30 min. The resulting solution was stirred for an additional 60 min at 0 °C and then cooled to −78 °C (dry ice/acetone). This was then treated with a solution of 7 (23.8 g, 0.0709 mol) in anhydrous THF (100 mL) added dropwise over 60 min. The resulting orange solution was stirred 30 min at −78 °C and then at 0 °C for an additional 30 min before quenching with glacial acetic acid (35 mL) and 2 M aqueous HCl (70 mL) with vigorous stirring. The resulting mixture was then partitioned between diethyl ether (750 mL) and water (250 mL). After separation of phases, the aqueous layer was reextracted with diethyl ether (200 mL), and the combined organic extracts were washed successively with 0.2 M HCl (200 mL), water (200 mL), saturated sodium bicarbonate solution (3 × 150 mL), and brine (200 mL). The ether solution was dried ($MgSO_4$), filtered, and concentrated in vacuo to yield a yellow oil, which was then flash chromatographed on silica gel. Elution with 40% ethyl acetate gave 8 (32.3 g, 84%): ^1H NMR ($CDCl_3$) δ 1.3 (s, 3 H), 1.4 (s, 2 H), 1.45 (m, 2 H), 2.47 (d, 2 H), 2.7 (m, 2 H), 3.1 (m, 1 H), 3.6 (s, 3 H), 3.38 (s, 2 H), 3.9 (m, 1 H), and 6.8–7.2 (m, 9 H) ppm. The ethyl ester 8a was also synthesized in comparable yield with ethyl acetoacetate: ^1H NMR ($CDCl_3$) δ 1.27 (t, 3 H), 1.36 (s, 3 H), 1.40 (s, 3 H), 1.45 (m, 2 H), 2.6 (d, 2 H), 2.4–2.7 (m, 2 H), 3.1 (m, 1 H), 3.4 (s, 2 H), 3.9 (m, 1 H), 4.2 (q, 2 H), and 7.0–7.2 (m, 9 H) ppm; IR (thin film) 2965, 1743, 1714, 1654, 1599, 1559, 1512, 1500, 1374, 1227, 1160, and 844 cm^{-1}; HPLC indicated, 100% purity (retention time 23.2 min). Anal. ($C_{26}H_{29}FN_2O_4$) C, H; N: calcd, 6.19; found, 5.73.

(±)-*trans*-6-[2-[5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (10). **(i) Use of Tri-*n*-butylborane and Air Activation.** Through a THF (150 mL) solution of tri-*n*-butylborane (76.5 mL, 1 M, 0.076 mol) and 8 (31.48 g, 0.070 mol) was bubbled air (125 mL), and the solution was stirred at room temperature under a nitrogen atmosphere for 24 h. The solution was then cooled to −78 °C, and sodium borohydride (3.15 g, 0.0835 mol) was added in one portion. The mixture was allowed to warm to −20 °C over 2 h and then to 0 °C where it was stirred for 1 h. The reaction was then quenched by the addition of glacial acetic acid (14.6 mL, 0.205 mol) and water (17 mL). When gas evolution had ceased, 2 N sodium hydroxide (167 mL) was added followed by the dropwise addition of 30% hydrogen peroxide (25.7 mL, 0.25 mol) over 1 h. The resulting mixture was allowed to warm to room temperature overnight and then partitioned between ether (500 mL) and water (500 mL). The aqueous layer was separated and the ether layer was washed with 3 N NaOH (2 × 200 mL). The combined aqueous layers were then cooled to 0 °C and acidified with ice-cold 6 N HCl. This was then extracted with ethyl acetate (4 × 200 mL). The combined organic extracts were then washed with water (200 mL) and brine (2 × 200 mL), dried ($MgSO_4$), filtered, and concentrated under vacuum to yield 9 (30 g, 95%) as a mixture of 3*R*,5*R*/3*S*,5*S* and 3*S*,5*R*/3*R*,5*S* racemates. This material was dissolved in toluene (500 mL) and heated under reflux with azeotropic removal of water for 3 h. The mixture was cooled to room temperature and concentrated in vacuo. The residue was flash chromatographed on silica gel, eluting with 75% ethyl acetate–hexane to produce 10 (16.6 g, 60%) as a colorless solid: mp 157–159 °C (5:1 cyclohexane:chloroform).

^1H NMR ($CDCl_3$) δ 1.3 (s, 3 H), 1.4 (s, 3 H), 1.6–1.9 (m, 4 H), 2.2 (br s, 1 H), 2.5–2.8 (m, 4 H), 3.1 (m, 1 H), 4.3 (m, 1 H), 4.6 (m, 1 H), and 7.0–7.3 (m, 9 H) ppm; IR (KBr) 3400, 2962, 2868, 1707, 1598, 1511, 1440, 1376, 1252, 1225, 1052, 972, 843, and 767 cm^{-1}.

HPLC (stationary phase, Altex C 18 column; mobile phase, 50:50 0.05 M citric acid (pH = 4.0)/CH_3CN) indicated a 3.3:1 mixture of trans (t_R = 13.1 min)/cis (t_R = 12.0 min) diastereomers. Anal. ($C_{25}H_{27}FN_2O_3$) C, H, N. The cis diastereomer was visible by NMR; the H6 and H4 protons appeared as a broad multiplet at δ 4.1 ppm.

(ii) Use of Triethylborane with Pivalic Acid Catalysis. To a room temperature solution of triethylborane (2.5 mL of a 1 M THF solution (0.002 14 mol)) under a nitrogen atmosphere

(18) Use of acetic acid as solvent greatly reduces reaction times.

was added, with stirring, a catalytic amount of pivalic acid (0.022 g, 0.000 21 mol). The resulting solution was stirred at room temperature for 1 h before a THF (7 mL) solution of 8a (1 g, 0.002 14 mol) was added dropwise. The resulting solution was stirred at room temperature for a further 1 h before cooling to −78 °C. Methanol (1 mL) was added followed by the addition of sodium borohydride (0.0893 g, 0.002 36 mol) in one portion. Vigorous gas evolution ensued. This mixture was stirred at −78 °C for 2.5 h. It was then poured into an excess of ice-cold 30% hydrogen peroxide (10 mL) and extracted with ethyl acetate. The organic layer was then washed extensively with water and brine, dried ($MgSO_4$), filtered, and evaporated to yield 1.0 g of the corresponding 1,3-diol (quantitative) as a 23:1 mixture of 3*R*,5*R*/3*S*,5*S*; and 3*S*,5*R*/3*R*,5*S* racemates. (HPLC indicated that the 3*R*,5*R*/3*S*,5*S* racemate had a retention time of 13.5 min and the 3*R*,5*R*/3*R*,5*S* racemate had a retention time of 11.7 min.)

5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazole (5). To a solution of 1 (10.6 g, 0.0509 mol) in glacial acetic acid (100 mL) was added at room temperature phenylhydrazine (6.04 g, 0.0559 mol). The mixture was stirred overnight at room temperature and then poured into ice-cold saturated aqueous sodium bicarbonate (200 mL). An oil precipitated, which then crystallized. These crystals were collected and redissolved in hexane. The hexane solution was washed with water (100 mL) and brine (100 mL) and then dried ($MgSO_4$). The solution was then concentrated to one-quarter of its original volume and cooled to yield 5 as colorless crystals: mp 70–72 °C (hexane) (12.0 g, 84%); ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 3 H), 1.38 (s, 3 H), 3.1 (m, 1 H), 6.3 (s, 1 H), 6.9–7.3 (m, 9 H) ppm; IR (KBr) 3052, 2964, 1594, 1510, 1440, 1374, 1302, 1222, 1164, 1089, 995, and 849 cm^{-1}. Anal. ($C_{18}H_{17}FN_2$) C, H, N.

4-Bromo-5-(4-fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazole (11a). *N*-Bromosuccinimide (6.21 g, 0.0348 mol) was added to a solution of 5 (11.3 g, 0.0348 mol) in DMF (130 mL) at 0 °C under a nitrogen atmosphere. After 1 h, a solid was deposited, which was filtered and washed extensively with water. This solid was recrystallized from toluene to yield 11a: mp 126–128 °C (toluene) (8.1 g, 56%); ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 3 H), 1.42 (s, 3 H), 3.1 (m, 1 H), 7.0–7.3 (m, 9 H); IR (KBr) 1593, 1551, 1496, 1376, 1304, 1227, 1160, 1109, 1036, 968, and 843 cm^{-1}. Anal. ($C_{18}H_{16}BrFN_2$) C, H, N.

5-(4-Fluorophenyl)-4-iodo-3-(1-methylethyl)-1-phenyl-1*H*-pyrazole (11b). *N*-Iodosuccinimide (4.81 g, 0.0214 mol) was added in one portion to a stirred solution of 5 (5.0 g, 0.0178 mol) in DMF (100 mL) cooled to 0 °C under a dry nitrogen atmosphere. The mixture was allowed to warm to room temperature overnight and then recooled to 0 °C before more *N*-iodosuccinimide (0.24 g, 0.0011 mol) was added. This was then allowed to warm to room temperature and then poured into water (500 mL). This aqueous mixture was extracted with diethyl ether (2 × 250 mL). The ether extracts were diluted with hexane (200 mL) and washed with water (100 mL), 10% aqueous sodium bisulfite (100 mL), and brine (100 mL) and dried ($MgSO_4$). Filtration and concentration afforded 11b (6.8 g, 94%) as orange/tan needles (mp 141–143 °C) (hexane): ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 3 H), 1.42 (s, 3 H), 3.1 (m, 1 H), and 7.0–7.3 (m, 9 H) ppm; IR (KBr) 2929, 1600, 1542, 1500, 1460, 1427, 1373, 1298, 1229, 1159, 1028, 968, and 845 cm^{-1}. Anal. ($C_{18}H_{16}FIN_2$) C, H, N.

Methyl 5-hydroxy-3-oxo-6-heptenoate (12) was prepared as described by Ley et al.[12] Ethyl 5-hydroxy-3-oxo-6-heptenoate was prepared similarly in 94% yield: 12: ^{1}H NMR ($CDCl_3$) δ 1.2 (tr, 3 H), 2.78 (d, 2 H, 4-H, *J* = 6.3 Hz), 3.4 (s, 2 H, 2-H), 4.2 (q, 2 H), 4.6 (dt, 1 H, 5-H, *J* = 6.0, 6.3 Hz), 5.07–5.35 (m, 2 H, 7-H), and 5.88 (ddd, 1 H, 6-H, *J* = 16.3, 10.0, 6.0 Hz) ppm.

Methyl 6-Ethenyl-2,2-dimethyl-1,3-dioxane-4-acetate (13). Air (20 mL) was bubbled through a solution of triethylborane (64 mL, 1 M THF, 0.064 mol) and 12 (10 g, 0.058 mol) in anhydrous THF (50 mL) under a nitrogen atmosphere. The resulting solution was stirred overnight at room temperature and then cooled to −78 °C. Sodium borohydride (2.64 g, 0.0696 mol) was added in one portion, and the vigorously stirred suspension was allowed to warm slowly to 0 °C over 2 h. (Vigorous gas evolution was noticed at −50 °C.) The reaction was quenched by the dropwise addition of glacial acetic acid (15 mL) followed by addition of water (20 mL) and methanol (20 mL). After all the solid had been consumed, saturated aqueous sodium bicarbonate solution (50 mL) was added carefully, followed by the dropwise addition of 30% hydrogen peroxide (19.2 mL). This solution was stirred for 1 h and then poured into ether (800 mL). The organic phase was washed with water (2 × 160 mL) and brine (100 mL). It was dried ($MgSO_4$), filtered, and evaporated. The residue was flash chromatographed on silica gel, eluting with ethyl acetate–hexane (50:50), to give methyl 3,5-dihydroxy-6-heptenoate (7.05 g, 69%) as a mixture of 3*R*,5*R*/3*S*,5*S* and 3*S*,5*R*/3*R*,5*S* racemates, which was used in the subsequent step without further purification. This crude mixture (7.0 g, 0.04 mol) was dissolved in a mixture of dichloromethane (100 mL) and 2,2-dimethoxypropane (20 mL, 0.162 mol). A catalytic amount of camphorsulfonic acid (0.05 g) was added and the solution was stirred overnight at room temperature. Concentration and flash chromatography on silica gel (eluting with 25% ethyl acetate–hexane) of the resulting residue gave 13 (4.25 g, 50%) as a 25:1 mixture of 3*R*,5*R*/3*S*,5*S* and 3*S*,5*R*/3*R*,5*S* racemates (HPLC indicated that the 3*R*,5*R*/3*S*,5*S* racemate had a retention time of 8.5 min and the 3*S*,5*R*/3*R*,5*S* racemate had a retention time of 8.4 min): ^{1}H NMR ($CDCl_3$) δ 1.2–1.3 (m, 1 H, 5-H), 1.38 (s, 3 H), 1.45 (s, 3 H), 1.60 (m, 1 H, 5-H'), 2.36 (dd, 1 H, *J* = 14, 6 Hz), 2.56 (dd, 1 H, *J* = 14, 6 Hz), 3.6 (s, 3 H), 4.3–4.5 (m, 2 H, 4-H, 6-H), 5.1–5.3 (m, 2 H), 5.8 (m, 1 H) ppm; IR (thin film) 2994, 1743, 1439, 1382, 1316, 1261, 1203, 1170, 1099, 1001, and 926 cm^{-1}. Anal. ($C_{11}H_{18}O_4$) H; C: calcd, 61.66; found, 60.12.

(*E*)-Methyl 6-[2-[5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethenyl]-2,2-dimethyl-1,3-dioxane-4-acetate (14). A solution of 11a (1.07 g, 0.003 mol), 13 (1.1 g, 0.0051 mol), and bis(triphenylphopshine)palladium(II) chloride (0.042 g, 0.00006 mol, 2 mol %) in 6 mL of a 50:50 mixture of triethylamine and DMF was stirred and heated at reflux overnight under a nitrogen atmosphere. The solution was cooled to room temperature and diluted with ether (100 mL) and washed with water (100 mL), 2 M hydrochloric acid (50 mL), water (100 mL), saturated aqueous sodium bicarbonate (100 mL), and brine (500 mL). The organic extracts were dried ($MgSO_4$), filtered, and evaporated. The residue was flash chromatographed on silica gel, eluting with 10% ethyl acetate–hexane, to give 14 (0.74 g, 50%) as yellow crystals, mp 136–137 °C, together with small amounts of 5: ^{1}H NMR ($CDCl_3$) δ 1.25–1.6 (m, 14 H), 2.36 (dd, 1 H, *J* = 14, 6 Hz), 2.56 (dd, 1 H, *J* = 14, 6 Hz), 3.20 (m, 1 H), 3.7 (s, 3 H), 4.3 (m, 2 H), 5.7 (dd, 1 H, *J* = 15 Hz, 7 Hz), 6.23 (d, 1 H, *J* = 15 Hz), and 7.0–7.3 (m, 9 H) ppm; IR (KBr) 2914, 1739, 1663, 1597, 1546, 1510, 1441, 1379, 1276, 1225, 1160, 1078, 974, and 841 cm^{-1}; HPLC indicated a 59:1 mixture of 4*R*,6*R*/4*S*,6*S* and 4*S*,6*R*/4*R*,6*S* racemates (the 4*R*,6*R*/4*S*,6*S* racemate had a retention time of 12.57 min, and the 4*S*,6*R*/4*R*,6*S* racemate had a retention time of 13.87 min). Anal. ($C_{29}H_{33}FN_2O_4$) C, H, N.

(±)-*trans*-6-[2-[5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (10). A solution of 14 (0.63 g, 0.001 28 mol) in ethyl acetate (10 mL) was hydrogenated under a balloon of hydrogen gas with 10% palladium on charcoal as catalyst at 25 °C for 2 days. The catalyst was then removed by filtration through Celite, and the filtrate was concentrated and redissolved in 50:50 THF/1 M HCl (30 mL). This was stirred for 5 h at room temperature, and then 25% sodium hydroxide was added until the solution was basic (pH ~10). After stirring for 30 min, the mixture was diluted with water and extracted with ether. The aqueous solution was then acidified with 2 M hydrochloric acid and extracted with ethyl acetate. The organic extracts were then washed with brine and dried ($MgSO_4$). Filtration and concentration provided the crude dihydroxy acid, which was lactonized with azeotropic removal of water by refluxing in toluene for 3 h. The cooled solution was concentrated to ca. 10 mL and allowed to stand. Pure lactone 10 crystallized as a white solid (0.35 g, 65%) (mp 163–165 °C, 2× 165–167 °C): HPLC indicated a 64:1 mixture of trans (t_R = 13.4 min)/cis (t_R = 12.3 min) diastereomers. Anal. ($C_{25}H_{27}FN_2O_3$) C, H, N.

(±)-*trans*-6-[1-[5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethenyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (15). A mixture of crude 14 (34 g, 0.067 mol) and 10% Pd/C (1 g) in absolute EtOH (100 mL) was hydrogenated for 2 days at atmospheric pressure and room temperature. The catalyst was removed by filtration through Celite. After concentration, the filtrate residue was dissolved in 3:2:1 THF–2 M

HCl–MeOH (600 mL) and the mixture stirred for 3 days at room temperature. This was made alkaline (25% aqueous NaOH) and partitioned between ether and water. The aqueous layer was then acidified (2 M HCl) and extracted with ethyl acetate (2 × 250 mL). The combined organic extracts were then washed with brine (100 mL), dried ($MgSO_4$), filtered, and evaporated. The residue was dissolved in toluene and refluxed with azeotropic removal of water for 2 h. Concentration and flash chromatography on silica gel provided a first fraction identified as 15 (1.5 g, 5.3%; mp 157–158 °C) and a second fraction of 10 (6 g, 22%; mp 156–157 °C): ^1H NMR ($CDCl_3$) δ 1.3 (s, 6 H), 1.5 (m, 1 H), 1.7 (m, 1 H), 2.1 (br s, 1 H), 2.4 (m, 1 H), 2.7 (m, 1 H), 3.1 (m, 1 H), 4.1 (m, 1 H), 4.9 (dd, 1 H), 5.4 (d, 1 H), 5.7 (d, 1 H), and 7.0–7.4 (m, 9 H) ppm; IR (KBr) 2931, 1725, 1642, 1598, 1546, 1510, 1438, 1379, 1229, 1159, 1071, 1045, 975, 845, and 766 cm^{-1}. Anal. ($C_{25}H_{25}FN_2O_3$) C, H, N.

6-[2-[5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethyl]-5,6-dihydro-2*H*-pyran-2-one (16). A solution of 10 (3.3:1 mixture of trans:cis isomers) (20 g, 0.0473 mol) was dissolved in anhydrous dichloromethane (50 mL) under a nitrogen atmosphere. Acetic anhydride (5.3 g, 0.052 mol) and DBU (15.8 g, 0.104 mol) were added dropwise to the solution. The reaction mixture was stirred overnight and then diluted with ether (150 mL) and washed with 2 M HCl (100 mL), saturated aqueous sodium bicarbonate solution (100 mL), and brine (100 mL), and dried ($MgSO_4$). Filtration and concentration gave a residue (17 g), which was passed through silica gel. Elution with hexane gave 16 (13 g, 68%) as a white solid (mp 89 °C (hexane)): ^1H NMR ($CDCl_3$) δ 1.36 (d, 6 H), 1.6–1.9 (m, 2 H), 2.2 (m, 2 H), 2.7 (m, 2 H), 3.0 (m, 1H), 4.3 (m, 1 H), 6.0 (dd, 1 H), 6.8 (m, 1 H), and 7.0–7.3 (m, 9 H) ppm; IR (KBr) 2961, 2868, 1723, 1596, 1562, 1511, 1439, 1376, 1336, 1248, 1159, 1094, 1043, 970, and 844 cm^{-1}. Anal. ($C_{25}H_{25}FN_2O_2$) C, H, N.

6-[2-[5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethyl]dihydro-2*H*-pyran-2,4(3*H*)-dione (20). Ethyl acetoacetate (1.14 mL, 0.0089 mol) in anhydrous THF (15 mL) was added dropwise to a stirred suspension of hexane-washed sodium hydride (58.8% oil suspension) (0.225 g) in anhydrous THF (20 mL) at 0 °C under an N_2 atmosphere. When gas evolution was complete, a solution of *n*-butyllithium in hexane (3.9 mL, 0.0089 mol, 2.3 M) was added over 30 min. The resulting solution was stirred an additional 30 min at 0 °C and then cooled to −78 °C. This was then treated with a solution of 7 (2.0 g, 0.0059 mol) in anhydrous THF (15 mL). The resulting solution was stirred at −78 °C for an additional 40 min and then at 0 °C for 30 min. This was then poured into 25% aqueous NaOH (50 mL). The resulting mixture was then washed with ether (to remove starting aldehyde) and then acidified with ice-cold 6 M HCl. This was then extracted with ethyl acetate, the organic extract was washed with water and brine, dried ($MgSO_4$), filtered, and evaporated. Recrystallization from Et_2O–hexane (1:10) provided 20 (1.62 g, 65%): mp 141–143 °C; ^1H NMR ($CDCl_3$) δ 1.3 (d, 6 H), 1.6–1.9 (m, 2 H), 2.4 (m, 2 H), 2.8 (m, 2 H), 3.1 (m, 1 H), 3.3 (d, 2 H), 4.5 (m, 1 H), 7.1–7.3 (m, 9 H) ppm; IR (KBr) 2900, 1599, 1511, 1440, 1376, 1273, 1226, 1159, 842, and 766 cm^{-1}. Anal. ($C_{25}H_{25}N_2O_3F$) H, N; C: calcd, 71.41; found, 70.93.

Addition of Benzyl Alcohol to Compound 16. To a solution of 16 (6 g, 0.0148 mol) in benzyl alcohol (45 mL) at 0 °C was added sodium benzylate in benzyl alcohol (5.9 mL, 0.5 M). The reaction was allowed to warm to room temperature and then stirred for 24 h. The solution was then diluted with methanol and made alkaline (0.02 mol, 3 M NaOH). The resulting aqueous layer was washed with ether, acidified with 2 M HCl, and extracted with ethyl acetate. The organic extracts were washed with water and brine and dried ($MgSO_4$). Filtration and concentration yielded a crude mixture of products (7.8 g) consisting mainly of the benzyl ether dihydroxy acid 19 and a small amount of lactone 17. This material was dissolved in ethyl acetate (30 mL) and 10% Pd/C (0.5 g) added. This was then hydrogenated at 1 atm of pressure for 2 days. The catalyst was then removed by filtration and the filtrate concentrated. The residue was dissolved in toluene (50 mL) and heated to reflux with azeotropic removal of water. The solution was cooled and the product (10) crystallized (3.8 g, 60%). HPLC showed a 8:1 trans:cis mixture of diastereomers.

Addition of Methanol to Compound 16. To a solution of compound 16 (1.1 g, 0.0027 mol) in methanol (25 mL) at room temperature under a nitrogen atmosphere was added sodium methoxide (0.017 g, 0.0003 mol). Reaction was almost instantaneous. TLC showed the formation of two products, the main product was presumably the ring opened methyl ether 21, the minor product was the lactone 22. This was then made alkaline with 25% NaOH and concentrated in vacuo. The residue was extracted with hexane and the remaining aqueous solution was acidified (0 °C, 12 N, HCl). The solution was then extracted with ethyl acetate and the organic solution was washed with water and brine and dried ($MgSO_4$). Filtration and concentration yielded crude product (1.1 g). This was dissolved in toluene (100 mL) and heated under reflux with azeotropic removal of water for 4 h. Flash chromatography on silica gel eluting with 40% ethyl acetate–hexane gave 6-[2-[5-(4-fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethyl]tetrahydro-4-methoxy-2*H*-pyran-2-one (22) (0.89 g, 75%): mp 86–88 °C; HPLC indicated a 7.4:1 mixture of trans (t_R = 23.9 min):cis (t_R = 21.8 min) diastereomers; ^1H NMR ($CDCl_3$) δ 1.25 (d, 6 H), 1.4–1.9 (m, 4 H), 2.4–2.6 (m, 4 H), 3.0 (m, 1 H), 3.2 (s, 3 H), 3.6 (m, 1 H), 4.3 (m, 1 H), 6.9–7.1 (m, 9 H) ppm; IR (KBr) 2958, 1744, 1595, 1565, 1511, 1439, 1376, 1253, 1224, 1157, 1098, 1071, and 840 cm^{-1}. Anal. ($C_{26}H_{29}FN_2O_3$) C, H, N.

(±)-*cis*-6-[2-[5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (23). A methanolic solution (25 mL) of 20 (1 g, 0.0024 mol) was hydrogenated at atmospheric pressure and room temperature using 10% Ru/C as catalyst. This was stirred at room temperature for 5 days, filtered, and concentrated to yield 1.3 g of crude material. Flash chromatography on silica gel, eluting with 40% ethyl acetate–hexane provided a first fraction identified as 24 (0.55 g, 51%): mp 92–94 °C; ^1H NMR ($CDCl_3$) δ 1.37 (d, 6 H), 1.5 (m, 4 H), 2.4–2.7 (m, 4 H), 3.1 (m, 1 H), 3.7 (s, 3 H), 3.8 (m, 1 H), 4.2 (m, 1 H), and 7.0–7.2 (m, 9 H) ppm; IR (KBr) 2958, 2867, 1735, 1595, 1562, 1511, 1439, 1325, 1337, 1222, 1159, 1093, 983, and 840 cm^{-1}. Anal. ($C_{26}H_{31}FN_2O_4$) C, H, N.

A second fraction gave material identified as 23 (0.13 g, 13%): mp 145–147 °C; HPLC indicated a 4:1 mixture of cis (t_R = 10.51 min):trans (t_R = 11.41 min) diastereomers; ^1H NMR ($CDCl_3$) δ 1.3 (d, 6 H), 1.4–2.0 (m, 4 H), 2.3–2.9 (m, 4 H), 3.1 (m, 1 H), 4.1 (m, 2 H), and 7.0–7.2 (m, 4 H) ppm. Anal. ($C_{25}H_{27}FN_2O_3$) C, H, N.

The other diastereomer exhibits peaks at δ 4.5 ppm (H6′) and 4.3 ppm (H4′); IR (KBr) 3400, 2950, 1700, 1605, 1511, 1376, and 845 cm^{-1}.

Acknowledgment. We thank Prof. Andrew T. McPhail of Duke University for performing the initial X-ray structure determination of lactone 10, E. H. Ferguson for conducting the enzyme inhibition assays, Dr. S. Brennan T. Hurley, and D. Sherwood for HPLC analyses and Dr F. A. MacKellar and staff for analytical and spectral determinations.

Supplementary Material Available: Preliminary X-ray crystallographic data for lactone 10 (4 pages). Ordering information is given on any current masthead page.

mol) of 6 and 10 mL of HCOOH was heated at reflux for 14 h. Then, 200 mL of water was added and the solution was made basic (pH 9) by addition of sodium carbonate. The resulting solution was extracted with benzene (2 × 150 mL); the organic extracts were dried (Na_2SO_4) and evaporated to give a residue, which crystallized as yellow needles from acetone–hexane. 11: ^{1}H NMR (DMSO) 9.22 (1 H, s, C1-H), 8.97 (1 H, ex, t, N*H*CH_2), 8.40 (2 H, t, C10-H and C7-H), 8.00 (1 H, d, J = 8.6, C3-H), 7.92 (1 H, t, C9-H), 7.59 (1 H, t, C8-H), 6.83 (1 H, d, J = 9.0, C4-H), 3.46 (2 H, qu*, –NHC*H*$_2$CH_2–), 2.62 (2 H, t, $CH_2CH_2NMe_2$), 2.28 (6 H, s, N(CH_3)$_2$).

Compounds 12, 13, and 16–22 were obtained in an analogous manner. Compound 14 required a refluxing time of 28 h.

(b) 5-[[2-(Dimethylamino)ethyl]amino]-1-octylimidazo-[4,5,1-*de*]acridin-6-one (15). A mixture of 1.48 g (0.004 mol) of hydrochloride 6, 8 mL (0.045 mol) of nonanoic acid, and 10 mL of bromobenzene was heated at reflux for 12 h. After cooling, the solution was diluted with $CHCl_3$ (100 mL) and extracted with 5% aqueous HCl. The aqueous extracts were made basic with NaOH and extracted with benzene. The organic extracts, dried with $CaCl_2$, were evaporated to dryness, and the crude product was crystallized from benzene–heptane. 15: ^{1}H NMR (CD_3OD) 8.56 (1 H, d, C7-H), 8.20 (1 H, d, C10-H), 7.92 (1 H, t, C9-H), 7.88 (1 H, d, J = 8.8, C3-H), 7.59 (1 H, t, C8-H), 6.84 (1 H, d, J = 8.9, C4-H), 3.66–0.88 (27 H, m, series of overlapping signals relative to the aliphatic moieties).

Acknowledgment. This work was supported by Polish Project CPBR 11.5 and by Italian Ministero della Pubblica Instruzione (Fondi 60%). We thank E. Augustin for the determination of cytotoxic activity against $HeLaS_3$ cells in tissue culture, K. Maturska for skillful technical assistance in animal experiments, and F. Lupidi for NMR spectra.

Registry No. 3, 99139-99-8; 3·HCl, 123381-64-6; 3·$MeSO_3H$, 99140-00-8; 4, 99140-23-5; 4·HCl, 123381-65-7; 4·$MeSO_3H$, 99140-24-6; 5, 123381-83-9; 5·HCl, 123381-66-8; 6, 123381-84-0; 6·2HCl, 123381-67-9; 7, 123381-85-1; 7·2HCl, 123381-68-0; 8, 123381-86-2; 8·2HCl, 123381-69-1; 9, 123381-87-3; 9·2HCl, 123381-70-4; 10, 123381-88-4; 10·2HCl, 123381-71-5; 11, 123381-89-5; 11·2HCl, 123381-72-6; 12, 123381-90-8; 12·2HCl, 123381-73-7; 13, 123381-91-9; 13·2HCl, 123381-74-8; 14, 123381-92-0; 14·2HCl, 123381-75-9; 15, 123381-93-1; 15·2HCl, 123381-76-0; 16, 123381-94-2; 16·2HCl, 123381-77-1; 17, 123381-95-3; 17·2HCl, 123381-78-2; 18, 123381-96-4; 18·2HCl, 123411-29-0; 19, 123381-97-5; 19·2HCl, 123381-79-3; 20, 123381-98-6; 20·2HCl, 123381-80-6; 21, 123381-99-7; 21·2HCl, 123381-81-7; 22, 123382-00-3; 22·2HCl, 123381-82-8; $Me_2N(CH_2)_2NH_2$, 108-00-9; $Me_2N(CH_2)_3NH_2$, 109-55-7; $Me_2N(CH_2)_5NH_2$, 3209-46-9; $EtCO_2H$, 79-09-4; $PrCO_2H$, 107-92-6; Me_2CHCO_2H, 79-31-2; $PhCO_2H$, 65-85-0; 1-chloro-4-nitroacridin-9(10*H*)-one, 20621-51-6; nonanoic acid, 112-05-0.

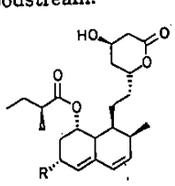
Synthesis and Biological Activity of New HMG-CoA Reductase Inhibitors. 1. Lactones of Pyridine- and Pyrimidine-Substituted 3,5-Dihydroxy-6-heptenoic (-heptanoic) Acids

G. Beck, K. Kesseler, E. Baader, W. Bartmann,* A. Bergmann, E. Granzer, H. Jendralla, B. v. Kerekjarto, R. Krause, E. Paulus, W. Schubert, and G. Wess

Hoechst AG, Postfach 80 03 20, 6230 Frankfurt/M. 80, West Germany. *Received October 24, 1988*

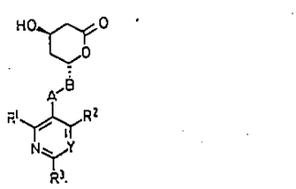
Lactones of pyridine- and pyrimidine-substituted 3,5-dihydroxy-6-heptenoic (-heptanoic) acids 2–4 have been synthesized. Extensive exploration of structure-activity relationships led to several compounds exceeding the inhibitory activity of mevinolin (1b) on HMG-CoA reductase, both in vitro and in vivo. First clinical trials with 2i (HR 780) are in preparation.

Only a few years after the discovery of the LDL receptor by Brown and Goldstein in 1973,[1] the fungal metabolites compactin (1a)[2,3] and mevinolin (1b)[4,5] have been isolated. Both compounds are potent inhibitors of cholesterol biosynthesis at the level of the major rate-limiting enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase). Through a feedback mechanism, inhibition of HMG-CoA reductase results in an increase of LDL-receptor synthesis with subsequent removal of LDL from the bloodstream.[6]



1a: R = H

1b: R = CH_3



2: A–B = (E)–CH=CH; Y = CH, N

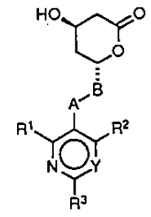
3: A–B = (Z)–CH=CH; Y = CH, N

4: A–B = CH_2–CH_2 ; Y = CH, N

(1) Goldstein, J. L.; Brown, M. S. *Proc. Natl. Acad. Sci. U.S.A.* 1973, *70*, 2804.
(2) (a) Endo, A.; Kuroda, M.; Tsujita, Y. J. *Antibiotics* 1976, *29*, 1346. (b) Endo, A.; Kuroda, M.; Tanzawa, K. *FEBS Lett.* 1976, *72*, 323. (c) Brown, A. G.; Smale, T. C.; King, T. J.; Hasenkamp, R.; Thompson, R. H. *J. Chem. Soc. Perkin Trans. 1* 1976, 1165.
(3) Alberts, A. W.; Chen, J.; Kuron, G.; Hunt, V.; Huff, J.; Hoffmann, C.; Rothrock, J.; Lopez, M.; Joshua, H.; Harris, E.; Patchett, A.; Monaghan, R.; Currie, S.; Stapley, E.; Albers-Schonberg, G.; Hensens, O.; Hirshfield, J.; Hoogsteen, K.; Liesch, J.; Springer, J. *Proc. Natl. Acad. Sci. U.S.A.* 1980, *77*, 3957.
(4) (a) Hoffmann, W. F.; Alberts, A. W.; Anderson, P. S.; Chen, J. S.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* 1986, *29*, 849. (b) Mol, M. J. T. M.; Erkelens, D. W.; Gevers Leuven, J. A.; Schouten, J. A. *Lancet* 1986, 936.
(5) (a) Serizawa, N.; Nakagawa, K.; Hamano, K.; Tsujita, Y.; Terahara, A.; Kuwano, H. *J. Antibiotics* 1983, *36*, 5. (b) *Drugs Future* 1987, *12*, 437.
(6) Brown, M. S.; Goldstein, J. L. *Sci. Am.* 1984, 52.

0022-2623/90/1833-0052$02.50/0

Table I. Physical Properties and Inhibitory Activities of Lactones 2–4



2: A–B = (*E*)-CH=CH
3: A–B = (*Z*)-CH=CH
4: A–B = CH_2CH_2

no.	Y	R^1	R^2	R^3	purificn[a]	% yield[b]	formula	mp, °C	anal.[c]	IC_{50},[d] nM
1b	–	–	–	–	–	–	–	–	–	8
2a	CH	CH_3	4-FC_6H_4	CH_3	A	16	$C_{20}H_{20}FNO_3$	205	C, H, F, N	260
2b	CH	CH_3	4-ClC_6H_4	CH_3	A	15	$C_{20}H_{20}ClNO_3$	oil	C, H, Cl, N	94
2c	CH	CH_3	4-FC_6H_4	C_6H_5	B	13	$C_{25}H_{22}FNO_3$	149	C, H, F, N	38
2d	CH	C_2H_5	4-FC_6H_4	C_6H_5	C	13	$C_{25}H_{24}FNO_3$	oil	C, H, F, N	40
2e	CH	*i*-C_3H_7	4-FC_6H_4	CH_3	C	23	$C_{22}H_{24}FNO_3$	oil	C, H, F, N	9
2f	CH	*i*-C_3H_7	4-FC_6H_4	*i*-C_3H_7	C	28	$C_{24}H_{28}FNO_3$	137–140	C, H, F, N	3
2g	CH	*i*-C_3H_7	4-FC_6H_4	*t*-C_4H_9	C	16	$C_{25}H_{30}FNO_3$	158–160[e]	C, H, F, N	1
2h	CH	*i*-C_3H_7	4-FC_6H_4	*c*-C_6H_{11}	C	13	$C_{27}H_{32}FNO_3$	135–138	C, H, F, N	4
2i	CH	*i*-C_3H_7	4-FC_6H_4	C_6H_5	C	24	$C_{27}H_{26}FNO_3$	141[f,g]	C, H, F, N	3
2j	CH	*i*-C_3H_7	4-FC_6H_4	4-FC_6H_4	C	22	$C_{27}H_{25}F_2NO_3$	oil	C, H, F, N	2
2k	CH	*i*-C_3H_7	4-FC_6H_4	2,5-$(CH_3)_2C_6H_3$	C	28	$C_{29}H_{30}FNO_3$	oil	C, H, F, N	5
2l	CH	*i*-C_3H_7	4-FC_6H_4	3,5-$(CH_3)_2C_6H_3$	C	26	$C_{29}H_{30}FNO_3$	80	C, H, F, N	8
2m	CH	*i*-C_3H_7	4-$CH_3OC_6H_4$	C_6H_5	C	30	$C_{28}H_{29}NO_4$	oil	C, H, N	13
2n	CH	*i*-C_3H_7	4-$CF_3C_6H_4$	C_6H_5	C	21	$C_{28}H_{26}F_3NO_3$	oil	C, H, F, N	36
2o	CH	*t*-C_4H_9	4-FC_6H_4	C_6H_5	C	19	$C_{28}H_{28}FNO_3$	oil	C, H, F, N	18
2p	CH	*c*-C_6H_{11}	4-FC_6H_4	C_6H_5	C	11	$C_{30}H_{30}FNO_3$	196–198	C, H, F, N	30
2q	CH	4-FC_6H_4	*i*-C_3H_7	C_6H_5	C	25	$C_{27}H_{26}FNO_3$	oil	C, H, F, N	4
2r	N	CH_3	4-FC_6H_4	CH_3	D	18	$C_{19}H_{19}FN_2O_3$	174–176[h]	C, H, F, N	500
2s	N	CH_3	4-ClC_6H_4	CH_3	D	20	$C_{19}H_{19}ClN_2O_3$	oil	C, H, Cl, N	600
2t	N	*i*-C_3H_7	4-FC_6H_4	*i*-C_3H_7	E	13	$C_{23}H_{27}FN_2O_3$	oil	C, H, F, N	3
2u	N	*i*-C_3H_7	4-FC_6H_4	*c*-C_6H_{11}	C	19	$C_{26}H_{31}FN_2O_3$	128	C, H, F, N	1
2v	N	*i*-C_3H_7	4-FC_6H_4	C_6H_5	D	18	$C_{26}H_{25}FN_2O_3$	164–166[i]	C, H, F, N	3
2w	N	*i*-C_3H_7	4-FC_6H_4	4-FC_6H_4	C	22	$C_{26}H_{24}F_2N_2O_3$	138–140	C, H, F, N	1
3a	CH	CH_3	4-FC_6H_4	CH_3	A	8	$C_{20}H_{20}FNO_3$	188	C, H, F, N	>1000
3c	CH	CH_3	4-FC_6H_4	C_6H_5	B	8	$C_{25}H_{22}FNO_3$	216	C, H, F, N	100
3s	N	CH_3	4-ClC_6H_4	CH_3	D	18	$C_{19}H_{19}ClN_2O_3$	165–166	C, H, Cl, N	>1000
4d	CH	C_2H_5	4-FC_6H_4	C_6H_5	–	17	$C_{26}H_{26}FNO_3$	53–55	C, H, F, N	3
4i	CH	*i*-C_3H_7	4-FC_6H_4	C_6H_5	–	22	$C_{27}H_{28}FNO_3$	oil	C, H, F, N	19
4r	N	CH_3	4-FC_6H_4	CH_3	–	18	$C_{19}H_{21}FN_2O_3$	170–172	C, H, F, N	1000

[a] Purified by flash chromatography on silica using the following eluents: A ethyl acetate/methanol 10:1, B cyclohexane/ethyl acetate 1:4, C cyclohexane/ethyl acetate 2:1, D ethyl acetate, E cyclohexane/ethyl acetate 1:1. [b] Represents overall yield for purified material from Wittig reaction of 6. [c] Analytical results for purified material were within ±0.4% of the theoretical values. [d] Tested in the ring-opened potassium dihydroxycarboxylate form, for assay protocol see the Experimental Section. [e] $[\alpha]^{20}_D = +26°$ (c = 1, methanol). [f] $[\alpha]^{20}_D = +25°$ (c = 1, methanol). [g] Obtained as an oil, which crystallized on standing for several weeks; melting point determined after recrystallization from diisopropyl ether/ethyl acetate 2:1. [h] $[\alpha]^{20}_D = +21°$ (c = 1, methanol). [i] $[\alpha]^{20}_D = +14°$ (c = 1, methanol).

Recent reports by Merck Sharp & Dohme,[7] Sandoz,[8] and Warner-Lambert[9] have described natural products and synthetic analogues related to mevinolin (1b). In our laboratories structurally simplified HMG-CoA reductase inhibitors have been synthesized as well.[10,11] Structure–activity relationships (SAR) in previous series[7,10,11] revealed that the chiral lactone moiety in mevinolin (1b) is essential for strong biological activity, whereas the hexahydronaphthalene moiety allows more structural variations. In the present paper we describe the synthesis and biological activity of new HMG-CoA reductase inhibitors 2–4, which contain for the first time monocyclic,[12] six-membered

(7) (a) Stokker, G. E.; Hoffmann, W. F.; Alberts, A. W.; Cragoe, E. J., Jr.; Deana, A. A.; Gilfillan, J. L.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* 1985, *28*, 347. (b) Hoffmann, W. F.; Alberts, A. W.; Cragoe, E. J., Jr.; Deana, A. A.; Evans, B. E.; Gilfillan, J. L.; Gould, N. P.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Rittle, K. E.; Smith, R. L.; Stokker, G. E.; Willard, A. K. *J. Med. Chem.* 1986, *29*, 159. (c) Stokker, G. E.; Alberts, A. W.; Anderson, P. S.; Cragoe, E. J., Jr.; Deana, A. A.; Gilfillan, J. L.; Hirshfield, J.; Holtz, W. F.; Hoffmann, W. F.; Huff, J. W.; Lee, T. J.; Novello, F. C.; Prugh, J. D.; Rooney, C. S.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* 1986, *29*, 170. (d) Hoffmann, W. F.; Alberts, A. W.; Anderson, P. S.; Chen, J. S.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* 1986, *29*, 849. (e) Stokker, G. E.; Alberts, A. W.; Gilfillan, J. L.; Huff, J. W.; Smith, R. L. *J. Med. Chem.* 1986, *29*, 852.

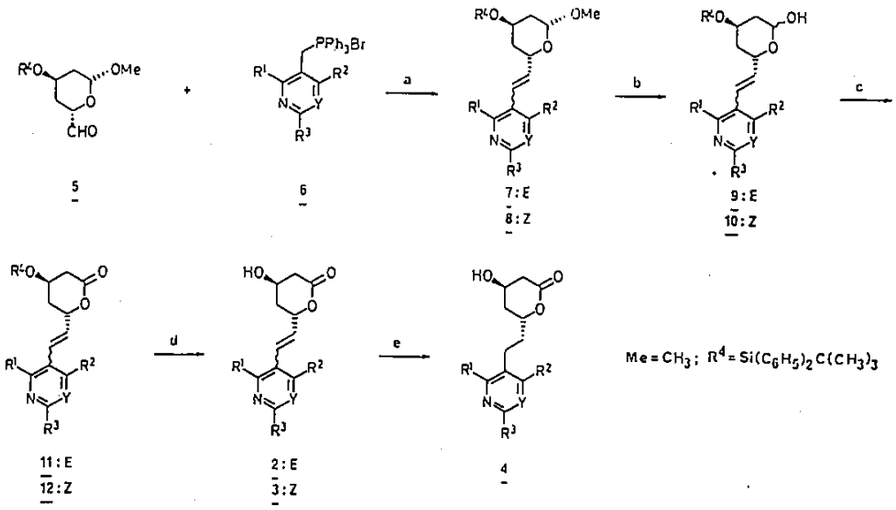
(8) (a) Sandoz, patent WO 84/02131, 1984. (b) Sandoz, patent WO 86/00307, 1986. (c) Sandoz, patent WO 86/07054, 1986. (d) Sandoz, European Application EP-A-0221025, 1987.

(9) Warner-Lambert, European Application EP-A-0179559, 1986.

(10) Baader, E.; Bartmann, W.; Beck, G.; Bergmann, A.; Granzer, E.; Jendralla, H.; v. Kerekjarto, B.; Kesseler, K.; Krause, R.; Paulus, E. F.; Schubert, W.; Wess, G.; 4th. International Conference of Chemistry and Biotechnology of Biologically Active Natural Products, Budapest, August 10–14, 1987, submitted for publication (Raven Press, New York).

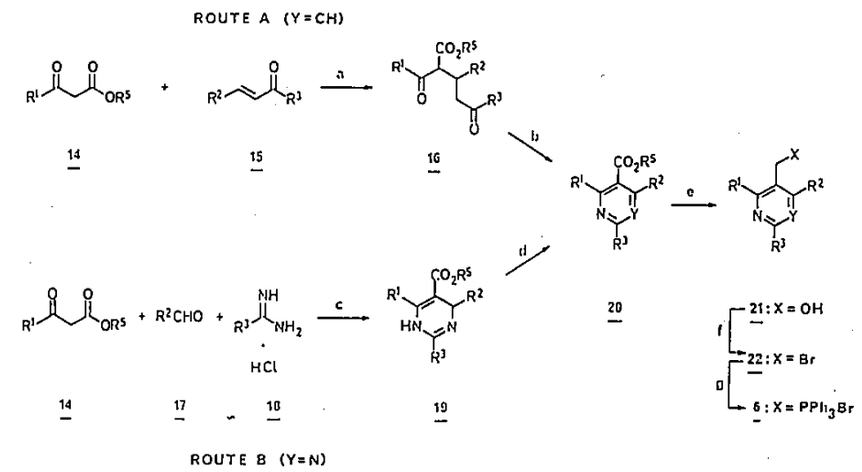
(11) (a) Bartmann, W.; Beck, G.; Granzer, E.; Jendralla, H.; v. Kerekjarto, B.; Wess, G. *Tetrahedron Lett.* 1986, *27*, 4709. (b) Baader, E.; Bartmann, W.; Beck, G.; Bergmann, A.; Jendralla, H.; Kesseler, K.; Wess, G.; Schubert, W.; Granzer, E.; v. Kerekjarto, B.; Krause, R. *Tetrahedron Lett.* 1988, *29*, 929.

Scheme I[a]



[a] (a) *n*-BuLi, THF, 0–20 °C, (b) HOAc, H_2O, THF, reflux, (c) NIS, TBAI, CH_2Cl_2, 20 °C, (d) TBAF, HOAc, THF, 20 °C, (e) H_2, Pd/C cat., MeOH, EtOAc, 20 °C.

Scheme II[a]



[a] (a) KO-*t*-Bu cat., *i*-Pr_2O, 20 °C, (b) NH_4OAc, $FeCl_3 \cdot 6H_2O$, HOAc, reflux, (c) KOAc, PhMe, reflux, (d) DDQ, PhMe, reflux, (e) $LiAlH_4$, THF, 20 °C, (f) PBr_3, CH_2Cl_2, 20 °C, (g) PPh_3, PhMe, reflux.

heteroaromatic groups with basic properties.

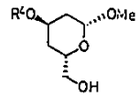
Chemistry

The new compounds 2–4 were synthesized in optically pure form by the general method shown in Scheme I and are listed in Table I. Compounds 2 were obtained through Wittig reaction with the chiral aldehyde 5 and ylides generated from the phosphonium salts 6, followed by cleavage of the lactol ether moiety of 7, oxidation of 9 to lactones 11, and desilylation. *Z*-configurated analogues 3 were prepared through the general sequence 8 → 10 → 12 → 3.

The Wittig reaction proceeded with high stereoselectivity, leading predominantly to the biologically more potent *E* isomers. Double-bond geometry was assigned on the basis of the ^1H NMR coupling constants of the olefinic protons (*E* isomers, J = 16 Hz; *Z* isomers, J = 11 Hz).

The saturated analogues 4 were synthesized by catalytic hydrogenation of compounds 2 or 3.

In all cases, the configuration of the lactone moiety results from synthesis via the optically pure 4*R*,6*S* aldehyde 5.[13] Compound 5 was easily prepared through Swern oxidation[14] of the corresponding alcohol 13,[13] obtained stereoselectively from glucose.



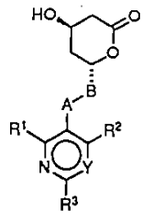
13

For compound 2i the assigned relative configuration has been additionally confirmed by X-ray crystallographic analysis.

(12) Quinoline-containing HMG-CoA reductase inhibitors have recently been produced by Warner-Lambert, U.S. Patent 4761419 A, 1988.

(13) Yang, Y. L.; Falck, J. R. *Tetrahedron Lett.* 1982, *23*, 4305.
(14) Swern, D.; Manusco, A.; Huany, S. *J. Org. Chem.* 1978, *43*, 2480.

Table II. Inhibitory Effect of Compounds 2–4 on the de Novo Cholesterol Biosynthesis of HEP-G2 Cell Cultures[a]



2: A–B = (*E*)-CH=CH
3: A–B = (*Z*)-CH=CH
4: A–B = CH_2CH_2

no.	Y	R^1	R^2	R^3	IC_{50}, nM	rel potency[b]
1b	–	–	–	–	50	1.00
2a	CH	CH_3	4-FC_6H_4	CH_3	2000	0.03
2c	CH	CH_3	4-FC_6H_4	C_6H_5	90	0.56
2e	CH	*i*-C_3H_7	4-FC_6H_4	CH_3	50	1.00
2g	CH	*i*-C_3H_7	4-FC_6H_4	*t*-C_4H_9	20	2.50
2h	CH	*i*-C_3H_7	4-FC_6H_4	*c*-C_6H_{11}	9.5	5.26
2i	CH	*i*-C_3H_7	4-FC_6H_4	C_6H_5	5.0	10.00
2j	CH	*i*-C_3H_7	4-FC_6H_4	4-FC_6H_4	7.5	6.67
2k	CH	*i*-C_3H_7	4-FC_6H_4	2,5-$(CH_3)_2$-C_6H_3	20	2.50
2m	CH	*i*-C_3H_7	4-$CH_3OC_6H_4$	C_6H_5	150	0.33
2p	CH	*c*-C_6H_{11}	4-FC_6H_4	C_6H_5	>5000	>0.01
2q	CH	4-FC_6H_4	*i*-C_3H_7	C_6H_5	10	5.00
2t	N	*i*-C_3H_7	4-FC_6H_4	*i*-C_3H_7	4.8	10.42
2u	N	*i*-C_3H_7	4-FC_6H_4	*c*-C_6H_{11}	26	1.92
2y	N	*i*-C_3H_7	4-FC_6H_4	C_6H_5	5	10.00
2w	N	*i*-C_3H_7	4-FC_6H_4	4-FC_6H_4	18	2.78
3c	CH	CH_3	4-FC_6H_4	C_6H_5	5000	0.08
4i	CH	*i*-C_3H_7	4-FC_6H_4	C_6H_5	370	0.14

[a] For assay protocol, see the Experimental Section. [b] Potency of mevinolin (1b) was arbitrarily assigned a value of 1.00.

The synthesis of phosphonium salts 6, via esters 20, is outlined in Scheme II. Pyridine esters 20 (Y = CH) were obtained through Michael addition[15] of keto esters 14[16] and enones 15,[17] followed by oxidative cyclization[18] of the intermediate 1,5-diketones 16 (route A, see Table III). Pyrimidine esters 20 (Y = N) were synthesized through condensation of 14 with aldehydes 17 and amidinium salts 18,[19] followed by oxidation of the resulting 1,4-dihydropyrimidines 19 by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ; route B, see Table III). In all cases, esters 20 were transformed to phosphonium salts 6 in three steps via reduction, halogenation of the resulting alcohols 21, and finally reaction of bromides 22 with triphenyl phosphine (see Table IV).

Biological Results and Discussion

The new pyridine and pyrimidine analogues 2–4 (Table I) were evaluated for their ability to inhibit solubilized, partially purified rat liver HMG-CoA reductase in vitro. Compounds 2–4 were also investigated for inhibition of cellular HMG-CoA reductase in cultures of hepatic cells (HEP G2, a human hepatoma cell line), determined by decreased incorporation of sodium [^{14}C]acetate into cholesterol (Table II). Selected compounds were further evaluated for their ability to inhibit hepatic cholesterol synthesis and to decrease cholesterol levels in several animal species upon po administration.[20]

All biological experiments were performed with optically pure 1b as reference for direct comparison.

In general, the structure–activity relationships of pyrimidines (2r–w) are comparable to those of the corresponding pyridines (2a–q) (e.g. 2i vs 2v, 2a vs 2r, 2j vs 2w; Table I). The inhibitory potency strongly depends on the substitution pattern of the heteroaromatic ring. We[10–12] and others[7] have recently shown that substitution in 2-, 4-, and 6-position of the central aromatic ring leads to strong biological activity.

However, through appropriate choice of substituents, the inhibitory potency of the compounds can be further increased by 3 orders of magnitude.

The biological activity of compounds 2 reaches a maximum if an isopropyl group is introduced in position 2 of the central heteroaromatic ring (e.g. 2i vs 2o, 2p, 2d, and 2a). Polar substituents in position 4, which seem to mimic the polar ester moiety of mevinolin, have previously been shown to result in compounds with high activity.[7]

In our series 4-(chlorophenyl)- and 4-(fluorophenyl)-substituted analogues are equally potent inhibitors (e.g. 2a vs 2b, 2r vs 2s). 4-(Methoxyphenyl) or 4-[(trifluoromethyl)phenyl] substitution leads to significant loss of activity (2m, 2n, vs 2i).

Substitution in position 6 turns out to be the most critical for optimal biological activity. Marked increase of potency is obtained not only by introduction of bulky alkyl groups (e.g. 2f, 2g, 2h, 2t vs 2e, 2s) but also by the use of phenyl moieties (e.g. 2i, 2j, 2k, 2v, 2w).

In order to further understand the structure–activity relationships, inhibitor 2i was compared with mevinolin (1b) by using computer-assisted methods.

For both compounds a conformational analysis was carried out in order to determine their low-energy conformations. Structure 2i was fitted to 1b by reorienting it as a whole and allowing groups to move independently (for details, see the Experimental Section).

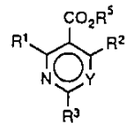
A graphical representation of the fit of 2i against 1b is shown in Figure 1. If the lactone moieties are oriented the same way in both conformers, the isopropyl group of 2i occupies partly the region of the hexahydronaphthalene system of 1b. At the same time the 4-fluorophenyl group of 2i occupies most of the space of the ester group of 1b. The phenyl ring of 2i, however, completely extends beyond the volume of 1b.

Since 2i and all other compounds bearing bulky substituents as R^3 (e.g. 2f, 2h, 2j, 2n, 2t, 2w) are more potent than mevinolin, one might speculate that R^3 serves as an additional anchor, interacting with a second hydrophobic region of the enzyme and thus increases binding. A final explanation might be expected by the elucidation of the tertiary structure of the HMG-CoA reductase. All *Z* double bond isomers 3 showed only weak in vitro activity (e.g. 3a, 3c, 3r). Also hydrogenation of *E* isomers 2 in most cases significantly decreased inhibitory potency (e.g. 2i vs 4i, 2r vs 4r). However, rather unexpectedly, 4d was 10 times more active in vitro than 2d. This points to a delicate balance[21] between the length of the carbon bridge and the steric bulk of R^1 with regard to adaptation of the inhibitor to the active site of the enzyme.

(15) Connor, R.; Andrews, D. B. *J. Am. Chem. Soc.* 1934, *56*, 2713.
(16) Jackman, M.; Klenk, M.; Fishburn, B.; Tullar, B. F.; Archer, S. *J. Am. Chem. Soc.* 1948, *70*, 2884.
(17) (a) Drake, N. L.; Allen, P. In *Organic Synthesis*; John Wiley & Sons, Inc. New York, 1932; Collect. Vol. I, p 77. (b) Kohler, E. L.; Chadwell, H. M. *Ibid.* p 78.
(18) Rehberg, R.; Kroehnke, F. *Justus Liebigs Ann. Chem.* 1968, *717*, 91.
(19) (a) Dox, A. W. In *Organic Synthesis*; Wiley and Sons Inc., New York, 1932; Collect. Vol. I, p 5. (b) Brown, D. J.; Lan, S.; Mori, K. *Aust. J. Chem.* 1984, *37*, 2093. (c) Hagemeyer, J. H.; Gammans, W. J. U.S. 3, 402, 193 (Eastman Kodak Co.); *Chem. Abstr.* 1960, *70*, 3316.

(20) Results will be published separately.

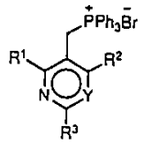
Table III. Physical Properties of Esters 20



no.	Y	R^1	R^2	R^3	R^5	purificn[a]	% yield[b]	formula	mp, °C	anal.[c]
20a	CH	CH_3	4-FC_6H_4	CH_3	CH_3	A	66	$C_{15}H_{14}FNO_2$	oil	C, H, F, N
20b	CH	CH_3	4-ClC_6H_4	CH_3	CH_3	B	73	$C_{15}H_{14}ClNO_2$	oil	C, H, Cl, N
20c	CH	CH_3	4-FC_6H_4	C_6H_5	C_2H_5	B	69	$C_{21}H_{18}FNO_2$	oil	C, H, F, N
20d	CH	C_2H_5	4-FC_6H_4	C_6H_5	C_2H_5	C	28	$C_{22}H_{20}FNO_2$	oil	C, H, F, N
20e	CH	i-C_3H_7	4-FC_6H_4	CH_3	C_2H_5	D	58	$C_{18}H_{20}FNO_2$	oil	C, H, F, N
20f	CH	i-C_3H_7	4-FC_6H_4	i-C_3H_7	C_2H_5	C	68	$C_{20}H_{24}FNO_2$	oil	C, H, F, N
20g	CH	i-C_3H_7	4-FC_6H_4	t-C_4H_9	C_2H_5	E	46	$C_{21}H_{26}FNO_2$	oil	C, H, F, N
20h	CH	i-C_3H_7	4-FC_6H_4	c-C_6H_{11}	C_2H_5	E	45	$C_{23}H_{28}FNO_2$	oil	C, H, F, N
20i	CH	i-C_3H_7	4-FC_6H_4	C_6H_5	C_2H_5	D	66	$C_{23}H_{22}FNO_2$	oil	C, H, F, N
20j	CH	i-C_3H_7	4-FC_6H_4	4-FC_6H_4	C_2H_5	E	55	$C_{23}H_{21}F_2NO_2$	109–111	C, H, F, N
20k	CH	i-C_3H_7	4-FC_6H_4	2,5-$(CH_3)_2C_6H_3$	C_2H_5	E	79	$C_{25}H_{26}FNO_2$	oil	C, H, F, N
20l	CH	i-C_3H_7	4-FC_6H_4	3,5-$(CH_3)_2C_6H_3$	C_2H_5	E	61	$C_{25}H_{26}FNO_2$	oil	C, H, F, N
20m	CH	i-C_3H_7	4-$CH_3OC_6H_4$	C_6H_5	C_2H_5	E	66	$C_{24}H_{25}NO_3$	70–74	C, H, N
20n	CH	i-C_3H_7	4-$CF_3C_6H_4$	C_6H_5	C_2H_5	E	71	$C_{24}H_{22}F_3NO_2$	oil	C, H, F, N
20o	CH	t-C_4H_9	4-FC_6H_4	C_6H_5	C_2H_5	C	22	$C_{24}H_{24}FNO_2$	oil	C, H, F, N
20p	CH	c-C_6H_{11}	4-FC_6H_4	C_6H_5	C_2H_5	C	55	$C_{26}H_{26}FNO_2$	oil	C, H, F, N
20q	CH	4-FC_6H_4	i-C_3H_7	C_6H_5	CH_3	D	52	$C_{22}H_{20}FNO_2$	114	C, H, F, N
20r	N	CH_3	4-FC_6H_4	CH_3	C_2H_5	F	43	$C_{15}H_{15}FN_2O_2$	oil	C, H, F, N
20s	N	CH_3	4-ClC_6H_4	CH_3	C_2H_5	F	47	$C_{15}H_{15}ClN_2O_2$	oil	C, H, Cl, N
20t	N	i-C_3H_7	4-FC_6H_4	i-C_3H_7	C_2H_5	A	33	$C_{19}H_{23}FN_2O_2$	141	C, H, F, N
20u	N	i-C_3H_7	4-FC_6H_4	c-C_6H_{11}	C_2H_5	B	47	$C_{22}H_{27}FN_2O_2$	oil	C, H, F, N
20v	N	i-C_3H_7	4-FC_6H_4	C_6H_5	C_2H_5	C	51	$C_{22}H_{21}FN_2O_2$	105	C, H, F, N
20w	N	i-C_3H_7	4-FC_6H_4	4-FC_6H_4	C_2H_5	C	73	$C_{22}H_{20}F_2N_2O_2$	105–108	C, H, F, N

[a] Purified by flash chromatography on silica using the following eluents: A cyclohexane/ethyl acetate 2:1, B cyclohexane/ethyl acetate 1:1, C cyclohexane/ethyl acetate 4:1, D cyclohexane/ethyl acetate 3:1, E cyclohexane/ethyl acetate 8:1, F cyclohexane/methanol 9:1. [b] Represents overall yield from Michael reaction of keto esters 14. [c] Analytical results were within ±0.4% of the theoretical values.

Table IV. Physical Properties of Phosphonium Salts 6



no.	Y	R^1	R^2	R^3	% yield[a]	formula	mp, °C	anal.[b]
6a	CH	CH_3	4-FC_6H_4	CH_3	65	$C_{32}H_{28}BrFNP$	218–220	C, H, Br, F, N, P
6b	CH	CH_3	4-ClC_6H_4	CH_3	32	$C_{32}H_{28}BrClNP$	oil	C, H, Br, Cl, N, P
6c	CH	CH_3	4-FC_6H_4	C_6H_5	64	$C_{37}H_{30}BrFNP$	230–232	C, H, Br, F, N, P
6d	CH	C_2H_5	4-FC_6H_4	C_6H_5	91	$C_{38}H_{32}BrFNP$	218–220	C, H, Br, F, N, P
6e	CH	i-C_3H_7	4-FC_6H_4	CH_3	29	$C_{34}H_{32}BrFNP$	209	C, H, Br, F, N, P
6f	CH	i-C_3H_7	4-FC_6H_4	i-C_3H_7	60	$C_{36}H_{36}BrFNP$	100[c]	C, H, Br, F, N, P
6g	CH	i-C_3H_7	4-FC_6H_4	t-C_4H_9	63	$C_{37}H_{38}BrFNP$	100[c]	C, H, Br, F, N, P
6h	CH	i-C_3H_7	4-FC_6H_4	c-C_6H_{11}	64	$C_{39}H_{40}BrFNP$	223–226	C, H, Br, F, N, P
6i	CH	i-C_3H_7	4-FC_6H_4	C_6H_5	34	$C_{39}H_{34}BrFNP$	268–274	C, H, Br, F, N, P
6j	CH	i-C_3H_7	4-FC_6H_4	4-FC_6H_4	42	$C_{39}H_{33}BrF_2NP$	235–239	C, H, Br, F, N, P
6k	CH	i-C_3H_7	4-FC_6H_4	2,5-$(CH_3)_2C_6H_3$	54	$C_{41}H_{38}BrFNP$	250	C, H, Br, F, N, P
6l	CH	i-C_3H_7	4-FC_6H_4	3,5-$(CH_3)_2C_6H_3$	58	$C_{41}H_{38}BrFNP$	250	C, H, Br, F, N, P
6m	CH	i-C_3H_7	4-$CH_3OC_6H_4$	C_6H_5	67	$C_{40}H_{37}BrNOP$	270–275	C, H, Br, N, P
6n	CH	i-C_3H_7	4-$CF_3C_6H_4$	C_6H_5	82	$C_{40}H_{34}BrF_3NP$	250	C, H, Br, F, N, P
6o	CH	t-C_4H_9	4-FC_6H_4	C_6H_5	55	$C_{40}H_{36}BrFNP$	250	C, H, Br, F, N, P
6p	CH	c-C_6H_{11}	4-FC_6H_4	C_6H_5	70	$C_{42}H_{38}BrFNP$	270[c]	C, H, Br, F, N, P
6q	CH	4-FC_6H_4	i-C_3H_7	C_6H_5	41	$C_{39}H_{34}BrFNP$	254	C, H, Br, F, N, P
6r	N	CH_3	4-FC_6H_4	CH_3	45	$C_{31}H_{27}BrFN_2P$	232–236	C, H, Br, F, N, P
6s	N	CH_3	4-ClC_6H_4	CH_3	56	$C_{31}H_{27}BrClN_2P$	217–219	C, H, Br, Cl, N, P
6t	N	i-C_3H_7	4-FC_6H_4	i-C_3H_7	40	$C_{35}H_{33}BrFN_2P$	166–169	C, H, Br, F, N, P
6u	N	i-C_3H_7	4-FC_6H_4	c-C_6H_{11}	42	$C_{38}H_{39}BrFN_2P$	oil	C, H, Br, F, N, P
6v	N	i-C_3H_7	4-FC_6H_4	C_6H_5	69	$C_{38}H_{33}BrFN_2P$	272–274	C, H, Br, F, N, P
6w	N	i-C_3H_7	4-FC_6H_4	4-FC_6H_4	70	$C_{38}H_{32}BrF_2N_2P$	210–214	C, H, Br, F, N, P

[a] Represents overall yield from reduction of esters 20. [b] Analytical results were within ±0.4% of the theoretical values. [c] Decomposition.

In HEP G2 cells, lactones 2–4 show comparable structure–activity relationships (SAR) as indicated above for their sodium salts in the enzyme test (Table II). A series of compounds (e.g. 2g–k, 2v, 2w) are more potent in HEP G2 cells than mevinolin.

Inhibition of hepatic cholesterol "de novo" synthesis in vivo after oral administration to rats for selected compounds 2 also exceeds that of mevinolin.[20] Several compounds (e.g. 2i and 2t) were also investigated in normolipemic rabbits. Analogue 2i (10 mg/kg) after oral ad-

(21) Although these results are somewhat conflicting, they are in line with observations made in a series of HMG-CoA reductase inhibitors containing a central phenyl moiety.[7] Depending on the substitution pattern of the aromatic ring, saturation of the ethylenic bridge in some cases decreased activity,[7c] whereas in other cases it increased activity.[7a,b]

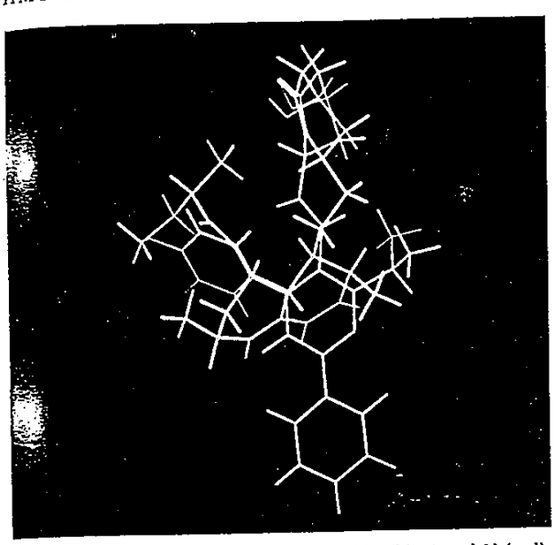


Figure 1. Superposition of structures of 1b (blue) and 2i (red). Except for the phenyl ring, 2i occupies the same regions of space as 1b.

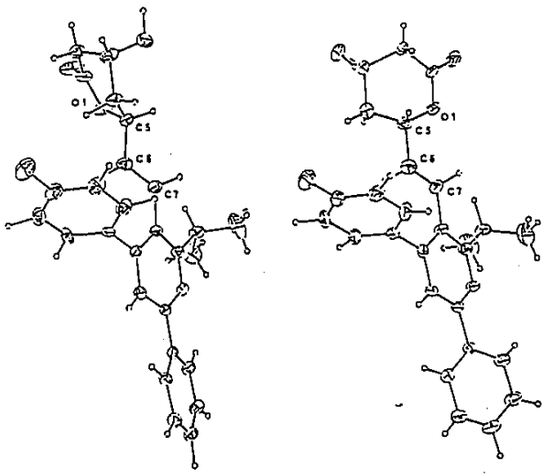


Figure 2. Computer-generated ORTEP drawings of conformers A (left) and B (right) of compound 2i forming an asymmetrical unit within the unit cell.

ministration for 19 days decreased serum total and LDL-cholesterol levels by 35% and 53%, respectively (mevinolin at 10 mg/kg for 19 days: total cholesterol −17%, LDL-cholesterol −30%). Oral treatment with 2t (5 mg/kg) for 10 days resulted in a 30% decrease of total cholesterol.

X-ray Crystallography for 2i

The X-ray structure analysis of 2i resulted in two distinct molecules forming an asymmetric unit, which show quite different conformations (Figure 2). The lactone ring of molecule A adopts a boat conformation; that of molecule B is in the chair conformation. Further, large differences in the torsion angles O1–C5–C6–C7 (43.4° and 130.4°, respectively) were detected. There are no substantial differences in bond lengths or bond angles; all the different planar groups of atoms are not coplanar, because otherwise the steric hindrance would be too large. The dihedral angles between the central pyridine ring and the ethylene bridge, the fluorophenyl, and the phenyl group are 50.8°, 83.2°, and 18.3° (conformer A) and 51.4°, 71.5°, and 17.6° (conformer B). The congruency of the parameters of the two molecules was not optimal, because of the unsatisfactory crystal quality usually obtained when two molecules of different conformation are crystallizing together.

Conclusion

The pyridine and pyrimidine analogues 2–4 synthesized for this study are potent inhibitors toward HMG-CoA reductase. SAR studies showed that a similar 2,4,6-substitution pattern of the pyridine and pyrimidine ring was necessary for optimal biological activity. Different from SAR studies in other series,[7] we showed that bulky lipophilic substituents in position 6 of the central aromatic ring add significantly to the biological activity of synthetic HMG-CoA reductase inhibitors. A series of compounds 2 and 4 exceeded the activity of mevinolin in HEP G2 cells, as well as in the reduction of plasma cholesterol levels in normolipemic rabbits. Some of these compounds are currently being evaluated for development as antiarteriosclerotic drugs. With the pyridine analogue 2i (HR 780) toxicological studies in rats and monkeys have already been performed.[20] The first clinical trials with this compound are in preparation.

Experimental Section

Reaction with materials sensitive to air or moisture were run in dry-glass apparatus under an argon atmosphere with absolute solvents. All reactions were monitored by TLC. Unless noted otherwise, reaction mixtures were worked up by quenching with water, separation of the organic layer, and extraction of the aqueous phase with ether. The combined organic extracts were washed with water or brine, dried over $MgSO_4$, and evaporated on a rotary evaporator. Melting points were determined on a Büchi capillary melting point apparatus (according to Dr. Tottoli) and are uncorrected. ^1H NMR spectra were recorded on a Bruker WP60 or WM270 spectrometer using $CDCl_3$ as solvent. Chemical shifts are given in ppm relative to tetramethylsilane as an internal standard. Mass spectra were recorded on a Kratos MS 9 (FAB) or MS 80 (CI) mass spectrometer. Optical rotations were determined on a Perkin-Elmer 141 polarimeter.

β-Keto Esters 14. These compounds were synthesized according to the method of Jackman.[16]

Enones 15. These compounds were prepared according to literature methods.[17]

Amidinium Hydrochlorides 18. These compounds were prepared according to literature,[19] if not commercially available.

General Procedure for the Synthesis of Pyridine- and Pyrimidine-3-carboxylic Acid Esters 20a–w (Table III). 3-(4-Fluorophenyl)-2-(1-oxoethyl)-5-oxohexanoic Acid Methyl Ester (16a). A solution of 4-(4-fluorophenyl)but-3-en-2-one (15a; 41.0 g, 0.25 mol) in ether (600 mL) was added dropwise to a mixture of methyl acetoacetate (14a; 58.1 g, 0.50 mol), potassium hydroxide (1.2 g), and ethanol (12 mL). During the addition, the reaction temperature was kept below 30 °C. The resulting solution was allowed to stand for 3 h, was acidified (pH 5) by addition of acetic acid, and successively shaken with water and saturated $NaHCO_3$ solution. Usual workup gave 50.6 g (72%) of 16a as a yellow oil, which was used in the next step without purification: ^1H NMR δ 0.8–1.0 (6 H, m), 1.9 (3 H, s), 2.2–2.9 (2 H, m), 3.1–4.1 (7 H, m), 7.0–7.8 (4 H, m).

1,4-Dihydro-4-(4-fluorophenyl)-2-isopropyl-6-phenylpyrimidine-3-carboxylic Acid Ethyl Ester (19v). To a suspension of benzamidine hydrochloride (18b; 102.2 g, 0.85 mol) and potassium acetate (90.7 g, 0.94 mol) in 1.5 L of toluene were added 4-methyl-3-oxopentanoic acid ethyl ester (98.6 g, 0.62 mol) and 4-fluorobenzaldehyde (17a; 77.0 g, 0.62 mol); the mixture was stirred for 24 h under reflux, with a Dean–Stark trap, until no more water separated. The reaction mixture was cooled and worked up in the usual manner. The residual oil was chroma-

tographed on silica gel. Elution with cyclohexane/ethyl acetate 4:1 provided 19v (110 g, 50%) as a viscous, yellow oil: ^{1}H NMR δ 1.2 (3 H, t, J = 7 Hz), 1.3 (6 H, d, J = 7 Hz), 4.0–4.5 (3 H, m), 5.8 (1 H, s), 7.0–7.9 (10 H, m). Anal. ($C_{22}H_{23}FN_2O_2$) C, H, F, N.

2,6-Dimethyl-4-(4-fluorophenyl)pyridine-3-carboxylic Acid Methyl Ester (20a). A suspension of 16a (28.0 g, 100 mmol), ammonium acetate (120 g), and iron(III) chloride hexahydrate (120 g) in acetic acid (1000 mL) was refluxed for 4 h with continuous stirring. The resulting deep red mixture was cooled and filtered. After washing of the remaining solid with toluene and ethanol, the filtrates were combined and evaporated. The residue was suspended in water, neutralized by addition of solid $NaHCO_3$, and worked up as usual. Chromatography gave 20a (23.6 g, 91%) as a white solid: mp 89–90 °C; ^{1}H NMR δ 2.6 (6 H, s), 3.7 (3 H, s), 7.0–7.5 (5 H, m); MS $C_{15}H_{14}FNO_2$ m/e = 259 (M^+). Anal. ($C_{15}H_{14}FNO_2$) C, H, F, N.

4-(4-Fluorophenyl)-2-isopropyl-6-phenylpyrimidine-3-carboxylic Acid Ethyl Ester (20v). To a solution of 19v (24.2 g, 66 mmol) in toluene (300 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ; 18.0 g, 79 mmol), and the mixture was stirred for 3 h at 50 °C. The reaction mixture was cooled, the solvent was evaporated, and the dark residual oil was extracted five times with cyclohexane/ethyl acetate 4:1 (100 mL). The organic extracts were evaporated and the brown, residual oil was chromatographed on silica gel. Elution with cyclohexane/ethyl acetate 4:1 provided 20v (19.9 g, 82%): mp 105–107 °C; ^{1}H NMR δ 1.1 (3 H, t, J = 7 Hz), 1.4 (6 H, d, J = 7 Hz), 3.2 (1 H, h, J = 7 Hz), 4.2 (2 H, q, J = 7 Hz), 7.0–8.0 (7 H, m), 8.5–8.8 (2 H, m). Anal. ($C_{22}H_{21}FN_2O_2$) C, H, F, N.

General Procedure for the Synthesis of Pyridine and Pyrimidine Phosphonium Salts 6a–w (Table IV). [2,6-Dimethyl-4-(4-fluorophenyl)pyridin-3-yl]methanol (21a). A 1.0 M solution of $LiAlH_4$ in THF (30 mL, 30 mmol) was added to a solution of 20a (7.80 g, 30.1 mmol) in THF (40 mL). The resulting reaction mixture was stirred at room temperature for 1.5 h and poured onto water. After usual workup, the crystalline residue was washed with a 1:1 mixture of cyclohexane and ethyl acetate, which gave 21a (6.5 g, 93%) as a white solid: mp 124 °C; ^{1}H NMR δ 2.0 (1 H, s), 2.5 (3 H, s), 2.7 (3 H, s), 4.6 (2 H, s), 6.9 (1 H, s), 7.0–7.5 (4 H, m); MS $C_{14}H_{14}FNO$ m/e = 231 (M^+). Anal. ($C_{14}H_{14}FNO$) C, H, F, N.

Bromo[2,6-dimethyl-4-(4-fluorophenyl)pyridin-3-yl]methane (22a). A solution of 21a (6.4 g, 27.7 mmol) and phosphorous tribromide (5.3 mL, 54.4 mmol) in a mixture of toluene (50 mL) and dichloromethane (25 mL) was stirred at room temperature for 1 h. The resulting mixture was poured onto saturated $NaHCO_3$ solution and worked up as usual to yield essentially pure 22a (6.4 g, 79%) as a pale yellow solid, mp 86–87 °C, which was used in the next step without purification: ^{1}H NMR δ 2.5 (3 H, s), 2.7 (3 H, s), 4.4 (2 H, s), 6.9 (1 H, s), 7.0–7.5 (4 H, m); MS $C_{14}H_{13}BrFN$ m/e = 295, 293 (M^+). Anal. ($C_{14}H_{13}BrFN$) C, H, F, N.

[2,6-Dimethyl-4-(4-fluorophenyl)pyridin-3-yl]methyltriphenylphosphonium Bromide (6a). A solution of 22a (6.4 g, 22.5 mmol) and triphenylphosphine (6.2 g, 23 mmol) in toluene (200 mL) was refluxed for 5 h. Upon cooling, a white precipitate formed, which was collected on a Büchner funnel, washed with ether, and dried in vacuo to yield analytically pure 6a (6.4 g, 89%): mp 218–220 °C; ^{1}H NMR δ 2.3 (3 H, d, J = 2 Hz), 2.5 (3 H, d, J = 3 Hz), 6.5 (2 H; d, J = 16 Hz), 6.8–7.9 (20 H, m); MS $C_{32}H_{28}BrFNP$ m/e = 476 (M^+). Anal. ($C_{32}H_{28}BrFNP$) C, H, Br, F, N, P.

General Procedure for the Synthesis of Lactones 2–4 (Table I). (E)- and (Z)-4(R)-[(*tert*-Butyldiphenylsilyl)oxy]-6(S)-[2-[2,6-dimethyl-4-(4-fluorophenyl)pyridin-3-yl]ethenyl]-2(R)-methoxy-3,4,5,6-tetrahydro-2H-pyrans (7a and 8a). A 1.6 M solution of *n*-butyllithium in hexane (12 mL, 19.2 mmol) was added dropwise to a solution of 6a (9.70 g, 17.5 mmol) in THF (100 mL) at 0 °C. The resulting reaction mixture was stirred for 0.5 h, then a solution of 5 (7.29 g, 18.4 mmol) in THF (40 mL) was added, and the stirring was continued for 1 h. The solution was poured onto water, acidified (pH 5–6) by addition of acetic acid, and extracted several times with ether. The combined organic layers were shaken with saturated $NaHCO_3$ solution and further worked up as usual. The remaining oil was chromatographed to provide 7a (4.99 g, 48%) as an oil and the corresponding Z isomer 8a (2.36 g, 22%) as a white solid. 7a: ^{1}H NMR δ 1.1 (9 H, s), 1.1–1.9 (4 H, m), 2.5 (3 H, s), 2.6 (3 H, s), 3.5 (3 H, s), 4.2 (1 H, mc), 4.5 (1 H, mc), 4.9 (1 H, mc), 5.5 (1 H, dd, J = 16 Hz, 6 Hz), 6.4 (1 H, d, J = 16 Hz), 6.9–7.7 (15 H, m); MS $C_{37}H_{42}FNO_3Si$ m/e = 596 ($M + 1$)$^+$. Anal. ($C_{37}H_{42}FNO_3Si$) C, H, F, N. 8a: mp 111–113 °C; ^{1}H NMR δ 0.9 (9 H, s), 1.0–1.8 (4 H, m), 2.6 (6 H, s), 3.3 (3 H, s), 4.2 (1 H, mc), 4.3 (1 H, mc), 4.5 (1 H, m), 5.5 (1 H, mc), 6.3 (1 H, d, J = 10 Hz), 6.9–7.8 (15 H, m); MS $C_{37}H_{42}FNO_3Si$ m/e = 596 ($M + 1$)$^+$. Anal. ($C_{37}H_{42}FNO_3Si$) C, H, F, N.

(E)- and (Z)-4(R)-[(*tert*-Butyldiphenylsilyl)oxy]-6(S)-[2-[2,6-dimethyl-4-(4-fluorophenyl)pyridin-3-yl]ethenyl]-2-hydroxy-3,4,5,6-tetrahydro-2H-pyrans (9a and 10a). A solution of 7a (4.93 g, 8.4 mmol) in THF (60 mL), water (60 mL), and acetic acid (100 mL) was refluxed for 48 h. Toluene (150 mL) was added and the resulting mixture was evaporated. The residue was shaken with saturated $NaHCO_3$ solution and worked up as usual. Chromatography (silica gel, cyclohexane/ethyl acetate 1:1) gave 9a (3.14 g, 63%): mp 119 °C; ^{1}H NMR δ 1.1 (9 H, s), 1.2–2.0 (4 H, m), 2.5 (3 H, s), 2.6 (3 H, s), 3.9–5.0 (3 H, m), 5.1–5.6 (2 H, m), 6.4 (1 H, d, J = 16 Hz), 6.9–7.8 (15 H, m); MS $C_{36}H_{40}FNO_3Si$ m/e = 581 (M^+). Anal. ($C_{36}H_{40}FNO_3Si$) C, H, F, N.

The corresponding Z isomer 10a was prepared by the same procedure in 60% yield: mp 147–149 °C; ^{1}H NMR δ 0.9 (9 H, s), 1.0–1.9 (4 H, m), 2.5 (6 H, s), 4.0–4.4 (2 H, m), 4.8–6.5 (3 H, m), 6.9–7.6 (15 H, m); MS $C_{36}H_{40}FNO_3Si$ m/e = 581 (M^+). Anal. ($C_{36}H_{40}FNO_3Si$) C, H, F, N.

(E)- and (Z)-4(R)-[(*tert*-Butyldiphenylsilyl)oxy]-6(S)-[2-[2,6-dimethyl-4-(4-fluorophenyl)pyridin-3-yl]ethenyl]-3,4,5,6-tetrahydro-2H-pyran-2-ones (11a and 12a). A solution of 9a (3.00 g, 5.18 mmol), *N*-iodosuccinimide (5.82 g, 25.9 mmol), and tetra-*n*-butylammonium iodide (1.91 g, 5.18 mmol) in dichloromethane (70 mL) was stirred for 2 h at room temperature, poured into a saturated $Na_2S_2O_3$ solution, and worked up in the usual manner. The remaining oil was treated with diisopropyl ether and filtered. After evaporation, the oily residue was chromatographed (silica gel, deactivated with 10% water; cyclohexane/ethyl acetate 1:1) to yield pure 11a (2.45 g, 76%) as an oil: ^{1}H NMR δ 1.1 (9 H, s), 1.3–1.7 (2 H, m), 2.4–2.6 (8 H, m), 4.2 (1 H, mc), 5.2 (1 H, mc), 5.4 (1 H, mc), 6.5 (1 H, d, J = 16 Hz), 6.9–7.7 (15 H, m); MS $C_{36}H_{38}FNO_3Si$ m/e = 580 ($M + 1$)$^+$. Anal. ($C_{36}H_{38}FNO_3Si$) C, H, F, N.

In a similar run, the corresponding Z isomer 12a was obtained from 10a in 76% yield: mp 188 °C; ^{1}H NMR δ 0.9 (9 H, s), 1.3–1.7 (2 H, m), 2.4 (2 H, mc), 2.6 (6 H, s), 4.2 (1 H, mc), 5.0 (1 H, mc), 5.6 (1 H, mc), 6.5 (1 H, d, J = 11 Hz), 6.9–7.5 (15 H, mc); MS $C_{36}H_{38}FNO_3Si$ m/e = 580 ($M + 1$)$^+$. Anal. ($C_{36}H_{38}FNO_3Si$) C, H, F, N.

(E)- and (Z)-6(S)-[2-[2,6-Dimethyl-4-(4-fluorophenyl)-pyridin-3-yl]ethenyl]-4(R)-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-ones (2a and 3a). Tetra-*n*-butylammonium fluoride trihydrate (3.42 g, 10.8 mmol) was added to a solution of 11a (2.10 g, 3.64 mmol) and acetic acid (8.3 mL, 14.5 mmol) in THF (35 mL). The resulting solution was stirred at room temperature for 15 h and then quenched with saturated $NaHCO_3$ solution. After usual workup, the crude product was purified by chromatography (silica gel, desactivated with 10% water; ethyl acetate/methanol 10:1) to give 2a (0.97 g, 78%) as a white solid: mp 205 °C; ^{1}H NMR δ 1.6–1.9 (3 H, m), 2.5 (3 H, s), 2.6 (3 H, s), 2.6–2.8 (2 H, m), 4.3 (1 H, mc), 5.3 (1 H, mc), 5.5 (1 H, mc), 6.6 (1 H, d, J = 16 Hz), 6.9 (1 H, s), 7.0–7.3 (4 H, m); MS $C_{20}H_{20}FNO_3$ m/e = 341 (M^+). Anal. ($C_{20}H_{20}FNO_3$) C, H, F, N.

The corresponding Z isomer 3a was prepared from 12a analogously in 75% yield: mp 188 °C; ^{1}H NMR δ 1.5 (1 H, mc), 1.8–2.2 (2 H, m), 2.4–2.6 (8 H, m), 4.2 (1 H, mc), 4.8 (1 H, mc), 5.6 (1 H, mc), 6.5 (1 H, mc), 6.9 (1 H, s), 7.0–7.4 (4 H, m); MS $C_{20}H_{20}FNO_3$ m/e = 341 (M^+). Anal. ($C_{20}H_{20}FNO_3$) C, H, F, N.

6(R)-[2-[4-(4-Fluorophenyl)-2-(1-methylethyl)-6-phenylpyridin-3-yl]ethyl]-4(R)-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one (4i). A mixture of 2i (1.00 g, 2.3 mmol), triethyl amine (50 μL), methanol (10 mL), and ethyl acetate (10 mL) was shaken under an hydrogen atmosphere, until no more hydrogen was consumed. This mixture was filtered through a pad of Celite and evaporated to give 4i (0.91 g, 91%) as an oil: ^{1}H NMR δ 1.3–1.8 (11 H, m), 2.3–2.8 (4 H, m), 3.4 (1 H, h, J = 7 Hz), 4.2 (1 H, mc), 4.5 (1 H, mc), 7.1 (2 H, mc), 7.3–7.5 (6 H, mc), 8.1 (2

H, mc); MS $C_{27}H_{28}FNO_3$ m/e = 433 (M^+). Anal. ($C_{27}H_{28}FNO_3$) C, H, F, N.

Biological Assays. HMG-CoA Reductase Inhibition Assay. The inhibitory activity of compounds 2–4 on rat liver HMG-CoA reductase was estimated with soluble-enzyme preparations obtained from the microsomal fraction.[22] The test was performed according to the method described by Avigan.[23] The complete assay medium contained the following in a total volume of 0.2 mL: Tris, 6mM; EDTA, 2.5 mM; DTT 2.5, mM; NADP, 50 mM; glucose 6-phosphate, 50 mM; glucose 6-phosphate dehydrogenase, 2.8 units; HMG-CoA, 0.91 mM containing 100 nCi (3.7 kBq) of [^{14}C] HMG-CoA (New England Nuclear); partially purified enzyme stock solution, 50 μL. Test compounds 2–4 as well as 1b (after conversion to their corresponding potassium 3(*R*),5(*S*)-dihydroxy carboxylates through reaction with 1 equiv of potassium hydroxide in ethanol) were added to the assay system in 10-μL volumes at multiconcentration levels. The complete assay was incubated at 37 °C with shaking during 20 min and the reaction was stopped by addition of 75 μL of 2 N $HClO_4$. After 1 h at room temperature and 10 min in an ice bath, 75 μL of 3 N potassium acetate and 150 μL of water were added, and the precipitate was centrifuged. The supernatant (250 μL) was applicated to an 0.6 × 8.0 cm column containing 100–200-mesh AG 1×8, Cl form (Bio-Rad). Mevalonolactone was eluted with 3.5 mL of Milli-Q water and 0.5-mL portions of the eluate were mixed with 10 mL of Quickscint 212 (Zinsser) for measurement in a Beckman scintillation counter. The assay was carried out in triplicate; the average of six values was calculated for the percentage inhibition. IC_{50} values were obtained by plotting the percentage inhibition against test compound concentration.

Inhibition of Acetate Incorporation in Cholesterol in Cultures of HEP G2 Cells. Monolayers of HEP G2 cells in RPMI 1640 medium (Flow) with 10% delipidated fetal calf serum were preincubated for 1 h with suitable concentrations of the test compounds 2, 3, or 4. After addition of ^{14}C-labeled sodium acetate, the incubation was continued for 3 h. [^{3}H] Cholesterol was added as an internal standard and an aliquot of the cells was saponified with alkali. The lipids were extracted with chloroform/methanol 2:1. After addition of carrier cholesterol, the lipid mixture was separated preparatively on TLC plates using chloroform/acetone 9:1. The cholesterol zone was visualized with iodine vapor and a TLC radioscanner and scraped out. The amount of [^{14}C] cholesterol was determined scintigraphically. With another aliquot of cell monolayers, cell proteins were determined for calculation of [^{14}C] cholesterol biosynthesis per milligram of cell protein. The same procedure was done at three different inhibitor concentrations, using cells of the same culture, and additionally without preincubation with a test compound (solvent control).

For each compound, IC_{50} values were calculated by plotting the ratio between the relative amount of [^{14}C] cholesterol synthesized in inhibitor-treated cells and in solvent controls against inhibitor concentrations. Relative potencies were calculated on the basis of 1b as external standard.

Hypocholesterolemic Activity in Rabbits. Normolipemic male white New Zealand rabbits (3–5.5 kg) in groups of four to six animals received the compounds, suspended in 1% aqueous (carboxymethyl)cellulose (Tylose) daily in the morning by stomach tube; the control groups were given only Tylose. In samples of venous blood, taken every 3–4 days 20 h after the oral administration, serum total cholesterol was enzymatically determined by test combination of Boehringer-Mannheim (CHOD-PAP high-performance method). The serum cholesterol level of drug-treated groups was compared with that of control groups. After the time of "administration" a time of "withdrawal" followed.

Conformational Analysis and Structural Comparison of Compounds 1b and 2i. A computer-assisted conformational analysis of 1b and 2i was carried out using a commercially available program[24] in order to determine their low-energy conformations.

An initial conformation of 1b was modeled from the conformation of compactin (1a) as determined by X-ray crystallography.[25,26] A systematic conformational search with rotatable bonds

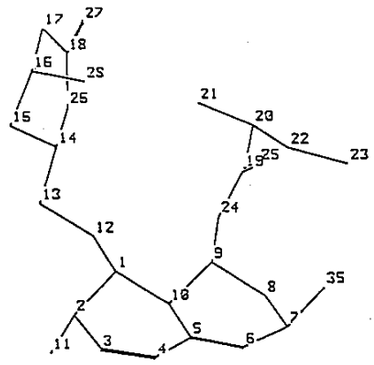


Figure 3. Low-energy conformation of 1b as determined by computer-assisted analysis (hydrogen atoms omitted).

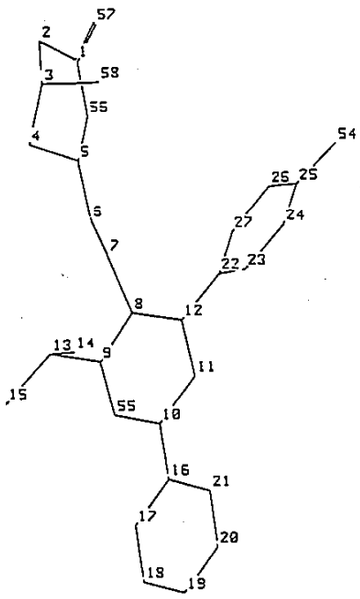


Figure 4. Low-energy conformation of 2i as determined by computer-assisted analysis (hydrogen atoms omitted).

13–14, 12–13, 1–12, 9–24, 19–24, 19–20, and 20–22 (see Figure 3) being varied in 30° steps over a range of 360° led to 13 669 conformations. Atom number 14 was the anchor atom. Scale factors for the van der Waals radii of 0.85 for 1,5 and greater interactions, 0.75 for 1,4 interactions, and 0.55 for H-bond interactions were specified in order to make sure that the initial conformation was contained in the set of generated conformations. A set of 1605 conformations were within 5.0 kcal/mol of the energy minimum. The minimum was located at the starting conformation with an energy of −9.8 kcal/mol (Figure 3). All energy values did not include Coulombic interactions.

A systematic conformational search was carried out in order to also determine the low-energy conformations of 2i. The initial conformation was taken from the crystal structure (see Figure 2). Since there are two conformations present in the crystal, the one which has the lactone in almost the same conformation as 1b (conformer B) was chosen. The energy of this conformation could be minimized[24] from 262.7 to 5.0 kcal/mol. Although the

(22) Philippi, B. W.; Shapiro, D. J. *J. Lipid Res.* 1979, *20*, 588.
(23) Avigan, J.; Bathena, S. J.; Schreiner, M. E. *J. Lipid Res.* 1975, *16*, 151.
(24) SYBYL 3.3, Tripos Associates, St. Louis, MI 63117.
(25) Brown, A. G.; Smale, T. C. *J. Chem. Soc. Perkin Trans. 1* 1976, 1165.
(26) Since the crystal structure of 1b is not known, 1a was used for analysis. Compactin differs from 1b by just one methyl group, suggesting that the conformational energies of both compounds should be similar.

energy decreased substantially, the original and minimized structure showed a standard deviation of only 0.15 Å. The high energy of the crystal structure is due to terminal hydrogens being slightly displaced. The systematic conformational search[24] yielded 1056 conformations. The rotatable bonds 5–6, 6–7, 7–8, 12–22, 10–16, and 9–13 (see Figure 4) were varied in steps of 30°, 180°, 30°, 30°, and 30° over ranges of 360°, 360°, 360°, 180°, 180°, and 360°, respectively. Atom number 5 was chosen to be the anchor atom. The van der Waals radii were scaled by 0.9 for 1,5 and greater interactions, 0.8 for 1,4 interactions, and 0.6 for H-bond interactions. With these scale factors the initial conformation was contained in the set of generated conformations.

From the 1056 conformations generated, 348 were within 5.0 kcal/mol of the minimum of 3.5 kcal/mol found. The energies did not contain Coulombic interactions. With use of computer graphics, these conformations were oriented in space such that the lactone moiety approximately fitted the lactone of **1b** and the fluorophenyl group qualitatively matched the ester group of **1b**. The structure of **2i** thus selected was then subjected to a flexible fit[24] against **1b**.

The conformation of **2i** chosen graphically differs from its crystal structure. However, with an energy value of 4.0 kcal/mol, it still is one of the low-energy conformations. For the flexible fit a force constant of 100.0 kcal/mol Å^2 was specified among the oxygen atoms 56, 57, and 58 of **2i** and 26, 27, and 28 of **1b**. A force constant of 20.0 kcal/mol Å^2 was given for atom pairs 8 and 27 of **2i** and 1 and 24 of **1b**. The fit energy of 16.0 kcal/mol was counterbalanced by an energy of 17.4 kcal/mol of **2i**. The standard deviation of the specified atoms was calculated to be 0.217 Å. When the fitted structure was relaxed, its energy is lowered to 8.1 kcal/mol, which appeared to be mainly due to releasing angle strain. The structure underwent only slight changes as indicated by standard deviation of atoms of 0.066 Å.

X-ray Structural Analysis of 2i. Compound **2i** (60 mg) was recrystallized from a mixture of 1 mL of diisopropyl ether and 0.5 mL of ethyl acetate. The crystal used for X-ray analysis was 0.55 × 0.35 × 0.13 mm, sealed in a Lindeman glass capillary: 25 reflections for cell refinement, Mo-Kα radiation, Nicolet R3 computer-controlled diffractometer, monoclinic, $C2$, $Z = 8$, $a = 34.99$ (2) Å, $b = 8.201$ (4) Å, $c = 16.66$ (1) Å, $\beta = 104.98$ (3)°, $V = 4618.2$ Å^3, $D = 1.241$ g/cm^3, $\mu = 0.8$ mm^{-1}, Ω scan, $2\vartheta_{max} = 56°$, 3° ϑ/min, 1 standard reflection (8 0 0), variation 2.8%; 6421 reflections measured, 4616 of the 5942 unique reflections had $I > \sigma(I)$ and were used for the structure analysis, $-46 < h < 2$, $0 < k < 10$, $-21 < l < 21$, no corrections for absorption or extinction. The phase problem could not be solved by the usual direct methods, but it was solved by the random-start multisolution program SHELXS-86;[27] in the final refinement all hydrogens were also refined, partly found in a difference electron density synthesis and partly calculated by using a model with idealized geometry (C–H 0.96 Å); other atoms were refined anisotropically; least-squares refinement on F with 4609 data, 720 parameters: $w = 1/\sigma(F)$, $R(1) = 0.108$, $R(2) = R(w) = 0.045$, $S = 1.7$ max $\Delta/\sigma = 0.1$; 10 largest peaks in final difference electron density synthesis between 0.27 and 0.31 e Å^{-3}; calculations were performed with a Nova 3/12 computer and SHELXTL scattering factors and f', f'' from *International Tables for X-ray Crystallography* (1974).

Supplementary Material Available: Analytical and spectral data for compounds **2a–w**, **3a,c,r**, and **4d,i,r** and analysis data for **6a–w** and **20a–w** (10 pages). Ordering information is given on any current masthead page.

(27) Sheldrick, G. M. In *Crystallographic Computing 3*, Sheldrick, G. M., Krueger, C., Goddard, R., Eds.; Oxford University Press, 1985; p 175.

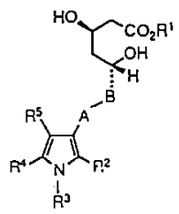
Synthesis and Biological Activity of New HMG-CoA Reductase Inhibitors. 2. Derivatives of 7-(1*H*-Pyrrol-3-yl)-substituted-3,5-dihydroxyhept-6(*E*)-enoic (-heptanoic) Acids

H. Jendralla, E. Baader, W. Bartmann,* G. Beck, A. Bergmann, E. Granzer, B. v. Kerekjarto, K. Kesseler, R. Krause, W. Schubert, and G. Wess

Hoechst AG, Postfach 800320, 6230 Frankfurt (Main) 80, West Germany. *Received October 24, 1988*

A series of 7-(1*H*-pyrrol-3-yl)-substituted-3,5-dihydroxyhept-6(*E*)-enoates (-heptanoates) 1 and 2 have been prepared and tested for inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. The most potent compounds exceeded mevinolin's activity in vitro and in vivo.

In continuation of our work on HMG-CoA reductase inhibitors with a central heterocyclic ring containing nitrogen atoms,[1] we report here on analogues 1 and 2 with a 1*H*-pyrrol-3-yl central moiety.



1 and 2: R^1 = CH_3, H, Na;
A–B = (*E*)—CH=CH (1), CH_2CH_2 (2);
R^2–R^5 = see Table I

Chemistry

Compounds 1 cannot be obtained in reasonable yield by utilizing the glucose-derived "compactin aldehyde" 3. This difference in behavior compared with pyridine and pyrimidine analogues[1] stems from the instability of pyrroles against acid-catalyzed hydrolysis. Instead, compounds 1 and 2, respectively, were prepared from the appropriate aldehydes 4 (Scheme I). Compounds 4 were converted with >95% *E* selectivity to the corresponding α,β-unsaturated aldehydes 6, by utilizing *cis*-(2-ethoxyvinyl)lithium according to Wollenberg.[2] Alternatively, some aldehydes 4 were converted by Emmons–Horner coupling with diisopropyl (cyanomethyl)phosphonate to the α,β-unsaturated nitriles 5. Compounds 5 were reduced and then hydrolyzed to aldehydes 6. Addition of the dianion of methyl acetoacetate gave the racemic β-keto-δ-hydroxy esters 7. Highly stereoselective reduction of the keto group[3,4] was conducted with triethylborane and sodium borohydride to give methyl β,δ-dihydroxy carboxylates 1, R^1 = CH_3.

Catalytic hydrogenation of 1 led to 2. Saponification of the methyl esters 1 and 2 gave the corresponding sodium salts 1 and 2 (R^1 = Na), respectively.

Selected examples of these racemic sodium salts 2 were also synthesized in optically active form 13, having the biologically active configuration 3*R*,5*R* (Scheme II). It should be emphasized that 2 and 13 are structurally identical, except for the ratio of the two enantiomers. They have been assigned different numbers for the sake of unambiguous differentiation in tables with biological results.

Aldehydes 6 were subjected to a highly stereoselective aldol reaction,[5,6] using the dianion 8 (generated from (*S*)-(−)-phenyl 2-hydroxy-2,2-diphenylacetate[7] and 2 equiv of LDA) to give 9. In all cases, the indicated 3(*S*)-hydroxy isomer 9 exceeded its undesired 3*R* diastereomer by more than 96:4 (HPLC). Compound 9 was transformed into the corresponding methyl ester 10 with sodium in methanol. Reaction of 10 with 4 equiv of the enolate of *tert*-butyl acetate yielded the *tert*-butyl β-keto-δ-(*S*)-hydroxy carboxylate 11, which was transformed to 3(*R*),5(*R*)-dihydroxyheptanoate 13 (R^1 = *t*-Bu) in analogy to the racemic ester 7 described above.

As shown by the HPLC analysis, 13 exceeded its undesired 3*S*,5*R* diastereomer by more than 96:4. Additionally according to ^{1}H NMR (Eu(hfc)$_3$) analyses, 13 had an optical purity of more than 92% ee. Saponification of the *tert*-butyl ester 13 gave the corresponding sodium salt (13, R^1 = Na).

The sodium salts of the olefins 1 (A–B = (*E*)-HC=CH) are acid sensitive while the hydrogenated analogues 2 (A–B = CH_2CH_2) are perfectly stable. When the olefinic methyl esters 1 (R^1 = CH_3) or their precursors 7 were dissolved in $CDCl_3$ that had not been filtered through basic alumina immediately before use, they decomposed very quickly, while 2 was stable. Likewise, the olefinic compounds 1 and 7 decomposed when chromatographed through silica gel in the absence of triethylamine, while the saturated analogue 2 was stable. Protolytic removal of the 5-hydroxy group of 1 leads to a cation that has a highly stabilizing resonance structure with a positively charged tetravalent nitrogen when A–B = HC=CH, but not when A–B = CH_2CH_2.

Aldehydes 4 were prepared following several synthetic routes as outlined in Schemes III–VI.

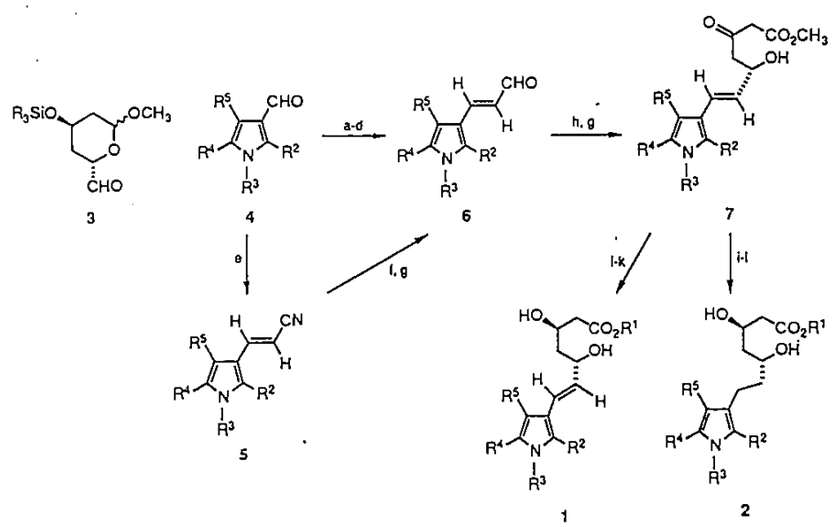
On the basis of the work of Gómez-Sanchez et al.,[8] substituted nitroethenes 15 were reacted with 2 equiv of β-keto esters 16[9] to give the hydroxylamines 17. Upon heating 17 with primary amines, especially anilines, the pyrrolecarboxylic acid esters 18 were obtained; they gave aldehydes 4 after reduction/oxidation (Scheme III).

According to H. Meyer[10] pyrrole esters 18 or 21 could also be prepared by cyclocondensation of nitroethenes 15

(1) Beck, G.; Kesseler, K.; Baader, E.; Bartmann, W.; Bergmann, A.; Granzer, E.; Jendralla, H.; von Kerekjarto, B.; Krause, R.; Paulus, E.; Schubert, W.; Wess, G. *J. Med. Chem.* Preceding paper in this issue.
(2) Wollenberg, R. H.; Albizati, K. F.; Peries, R. *J. Am. Chem. Soc.* 1977, *99*, 7365.
(3) Kathawala, F. G.; Prager, B.; Prasad, K.; Repič, O.; Shapiro, M. J.; Stabler, R. S.; Widler, L. *Helv. Chim. Acta* 1986, *69*, 803.
(4) Narasaka, K.; Pai, F.-C. *Tetrahedron* 1984, *40*, 2233.
(5) Braun, M.; Devant, R. *Tetrahedron Lett.* 1984, *25*, 5031.
(6) Devant, R.; Mahler, U.; Braun, M. *Chem. Ber.* 1988, *121*, 397.
(7) Commercially available as (*S*)-(−)-HYTRA from Merck-Schuchhardt, West-Germany.
(8) Gómez-Sanchez, A.; Stiefel, B. M.; Fernández, R.; Pascual, C.; Bellanato, J. *J. Chem. Soc., Perkin Trans. 1* 1982, 441.
(9) Jackman, M.; Klenk, M.; Fishburn, B.; Tullar, B. F.; Archer, S. *J. Am. Chem. Soc.* 1948, *70*, 2884.
(10) Meyer, H. *Liebigs Ann. Chem.* 1981, 1534.

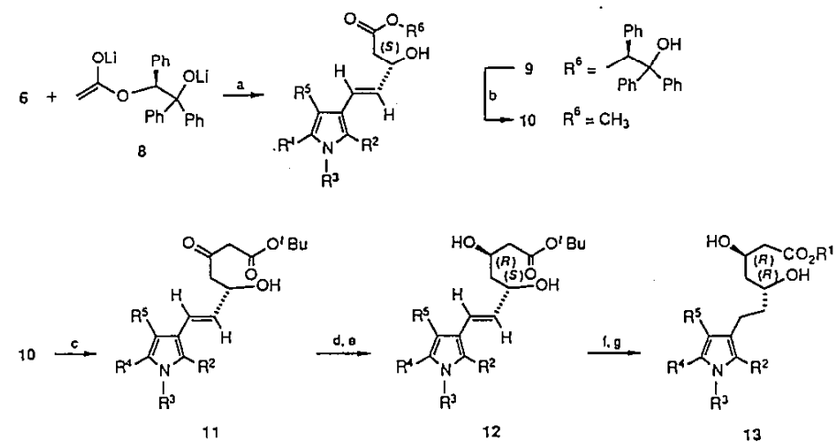
0022-2623/90/1833-0061$02.50/0

Scheme I[a]



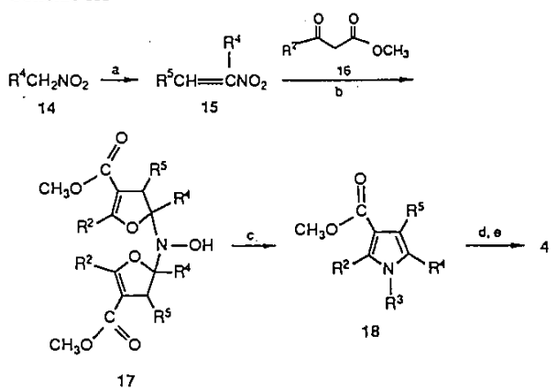
[a] (a) EtOCH=CHSn(*n*-Bu)$_3$;[2] (b) *n*-BuLi/−70 °C; (c) NH_4Cl/H_2O; (d) $TsOH/H_2O$; (e) $NCCH_2PO(O\text{-}i\text{-}Pr)_2/NaH$/0 °C; (f) $(i\text{-}Bu)_2AlH$; (g) NaH_2PO_4/H_2O; (h) $CH_3COCH_2CO_2CH_3/NaH/n\text{-}BuLi$/−15 °C; (i) Et_3B; (j) $NaBH_4$/−75 °C; (k) $NaOH/H_2O/CH_3OH$; (l) $Pd/C/H_2$.

Scheme II[a]



[a] (a) THF/−80 to −90 °C, 2 h; (b) 0.5 equiv of $NaOCH_3/CH_3OH$/23 °C; (c) 4 equiv of $CH_3CO_2{}^tBu$/4 equiv of LDA, −30 °C; (d) 1.05 equiv of Et_3B/24 equiv of CH_3OH in THF/−70 °C; (e) (1) 1.3 equiv of $NaBH_4$/−70 °C, (2) CH_3OH/25 °C; (f) $Pd/C/H_2$; (g) $NaOH/H_2O/CH_3OH$/12 h.

Scheme III[a]

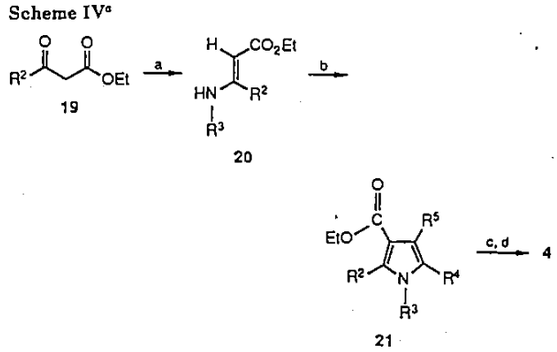


[a] (a) R^5CHO; (b) $NaOCH_3$; (c) R^3NH_2/Δ; (d) $LiAlH_4$; (e) MnO_2.

with enamino esters 20 (Scheme IV). When substituent R^2 was not sterically demanding (e.g. $R^2 = CH_3$), 20 were easily obtained by addition of 1 equiv of amine to the β-keto ester 19 under acid catalysis.

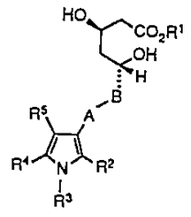
However, when R^2 was bulky (e.g. R^2 = isopropyl), amines R^3NH_2 (especially anilines) attacked the ester

Scheme IV[a]



[a] (a) $R^3NH_2/AcOH/-H_2O$; (b) 15/Δ; (c) $LiAlH_4$; (d) MnO_2.

Table I. Inhibition of Solubilized Rat Liver HMG-CoA Reductase in Vitro[a] for Compounds of the General Structure 1,[c] 2,[c] and 13[d]

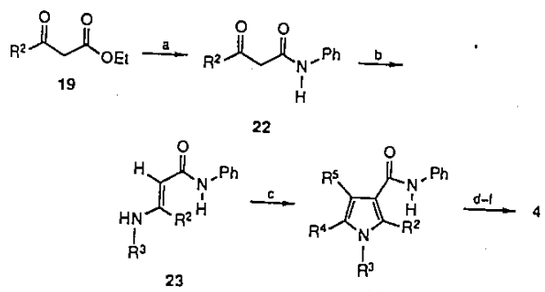


1[c], 2[c], and 13[d]

no.	R^1	R^2	R^3	R^4	R^5	A–B	formula	anal.[e]	IC_{50},[f] nM	rel[g] pot.
1a	Na	CH_3	Ph	H	*p*-C_6H_4F	CH=CH	$C_{24}H_{23}FNO_4Na$	C, H, N	65	12
1b	Na	*i*-Pr	Ph	H	*p*-C_6H_4F	CH=CH	$C_{26}H_{27}FNO_4Na$	C, H, N	7	110
2b	Na	*i*-Pr	Ph	H	*p*-C_6H_4F	CH_2CH_2	$C_{26}H_{29}FNO_4Na$	C, H, N	6	128
13b	Na	*i*-Pr	Ph	H	*p*-C_6H_4F	CH_2CH_2	$C_{26}H_{29}FNO_4Na$	C, H, N	3	257
1c	Na	CH_3	Ph	CH_3	*p*-C_6H_4F	CH=CH	$C_{25}H_{25}FNO_4Na$	C, H, N	250	3
2c	Na	CH_3	Ph	CH_3	*p*-C_6H_4F	CH_2CH_2	$C_{25}H_{27}FNO_4Na$	C, H, N	70	11
1d	Na	CH_3	*i*-Pr	H	*p*-C_6H_4F	CH=CH	$C_{21}H_{25}FNO_4Na$	C, H, N	330	2
2d	Na	CH_3	*i*-Pr	H	*p*-C_6H_4F	CH_2CH_2	$C_{21}H_{27}FNO_4Na$	C, H, N	100	9
1e	Na	*i*-Pr	*i*-Pr	H	*p*-C_6H_4F	CH=CH	$C_{23}H_{29}FNO_4Na$	C, H, N	117	6
2e	Na	*i*-Pr	*i*-Pr	H	*p*-C_6H_4F	CH_2CH_2	$C_{23}H_{31}FNO_4Na$	C, H, N	18	42
13e	Na	*i*-Pr	*i*-Pr	H	*p*-C_6H_4F	CH_2CH_2	$C_{23}H_{31}FNO_4Na$	C, H, N	9	85
1f	Na	*i*-Pr		H	*p*-C_6H_4F	CH=CH	$C_{26}H_{33}FNO_4Na$	C, H, N	69	12
2f	Na	*i*-Pr		H	*p*-C_6H_4F	CH_2CH_2	$C_{26}H_{33}FNO_4Na$	C, H, N	9	92
1g	Na	*i*-Pr	Ph	CH_3	*p*-C_6H_4F	CH=CH	$C_{27}H_{29}FNO_4Na$	C, H, N	6	125
2g	Na	*i*-Pr	Ph	CH_3	*p*-C_6H_4F	CH_2CH_2	$C_{27}H_{31}FNO_4Na$	C, H, N	5	149
13g	Na	*i*-Pr	Ph	CH_3	*p*-C_6H_4F	CH_2CH_2	$C_{27}H_{31}FNO_4Na$	C, H, N	2.5	300
mevinolin							$C_{24}H_{37}O_6Na$		8	100

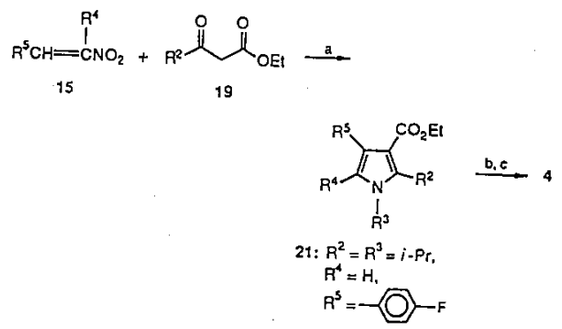
[a] The assay system described in ref 1 was used. [b] Ring-opened sodium dihydroxy carboxylate form, optically pure. [c] Racemic. [d] Optically active 3*R*,5*R* configuration. [e] Analytical results were within ±0.4% of the theoretical value. [f] IC_{50} values were determined by using four or five concentrations of each inhibitor. [g] For estimation of relative inhibitory potencies, mevinolin was assigned a value of 100. The IC_{50} value of test compound was compared with that of mevinolin, corrected for the somewhat different molecular weight.

Scheme V[a]



[a] (a) $PhNH_2$/AcOH; (b) R^3NH_2/AcOH/$-H_2O$; (c) 15/Δ; (d) NaH/CH_3I/toluene/Δ; (e) $LiAlH_4$/Δ; (f) CrO_3/pyridine.

Scheme VI[a]



[a] (a) R^3NH_2/CH_3OH/25 °C/1 day; (b) $LiAlH_4$; (c) 4 equiv of  /0.02 equiv of $(Ph_3P)_3RuCl_2$.

functionality much faster than the keto group of 19. In this case, it was necessary to preform the anilides 22 (Scheme V). Addition of aliphatic or aromatic primary amines R^3NH_2 to 22 under acid catalysis gave 23, which were cyclocondensed with nitroethenes 15 to give 3-pyrrolecarbanilides 24. While amides on LAH reduction usually lead to the corresponding amines, carbanilides 24 could be reduced to the corresponding aldehydes 4 via N-methylation, LAH treatment, and subsequent oxidation.

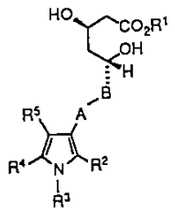
A new three-component coupling reaction allowed a one-pot synthesis of ethyl 1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxylate (21, Scheme VI). When a methanolic solution of β-nitro-*p*-fluorostyrene (15: R^4 = H, R^5 = *p*-C_6H_4F), β-keto ester 19 (R^2 = *i*-Pr), and isopropylamine was stirred at ambient temperature, the pyrrole ester 21 was obtained in 50% yield. LAH reduction followed by ruthenium(II)-catalyzed oxidation of the alcohol with *N*-methylmorpholine-*N*-oxide[11] gave the corresponding aldehyde 4. This convenient three-component coupling may also be applicable for the syntheses of pyrrole esters 21 with other substitution patterns for R^2–R^5.

Biological Results and Discussion

The racemic sodium salts (1 and 2, R^1 = Na) as well as the optically active sodium salts 13 (R^1 = Na) were evaluated for their ability to inhibit solubilized, partially purified rat liver HMG-CoA reductase in vitro (Table I) and

(11) Sharpless, K. B.; Akashi, K.; Oshima, K. *Tetrahedron Lett.* 1976, *29*, 2503.

Table II. Inhibition of Cellular HMG-CoA Reductase in Cultures of HEP G2 Cells[a] for Sodium Salts of the General Formula 1[c] and 2[c]



1[c] and 2[c]

	IC_{50},[d] μM	relative[e] potency
mevinolin[b]	0.05	100
1a	0.83	6
1b	0.014	350
2b	<0.01	>500
1c	5.0	1
2c	0.57	9
1d	6.0	1
2d	0.27	19
1e	0.05	100
2e	0.002	2500
1f	0.106	47
2f	0.018	275

[a] Assay described in the preceding paper.[1] [b] Ring-opened sodium dihydroxy carboxylate form, optically pure. [c] Racemic. For definition of R^1–R^5 and A–B see Table I. [d] IC_{50} values varied somewhat for different batches of cells. Mevinolin sodium salt averaged $IC_{50} = 5 \times 10^{-8}$ M and was used in every run as an internal standard. The measured IC's for test compounds 1 and 2 were corrected for deviations of mevinolin's IC from its average value. [e] Mevinolin was assigned a value of 100. Potencies were obtained by comparison of racemic test compounds 1 or 2 with the internal standard mevinolin.

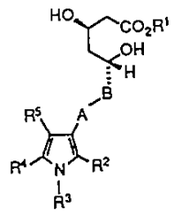
to inhibit *cellular* HMG-CoA reductase in cultures of hepatic cells (HEP G2, a human hepatoma cell line), as determined by the inhibition of the incorporation of sodium [^{14}C]acetate into cholesterol (Table II).

Selected compounds were evaluated for their ability to inhibit hepatic cholesterol "de novo" synthesis in male rats after po administration, as determined by the inhibition of the incorporation of sodium [^{14}C]octanoate[12] into hepatic cholesterol (Table III).

Selected compounds were further evaluated for their ability to decrease plasma cholesterol levels in normolipemic rabbits and dogs after po administration.

All tests were also conducted under the same experimental conditions with optically pure mevinolin. The respective results are included in Tables I–III. For substitution patterns "b", "e", and "g", we prepared and tested the racemic 2 as well as the optically active 3*R*, 5*R* sodium salt 13. Optically active compounds 13 proved to have twice the potency in HMG-CoA reductase inhibition than the structurally identical but racemic 2 (Table I). This result was expected, since the antipode of the configuration drawn for 1, 2, and 13, is biologically inactive.[13]

Table III. Inhibition of Hepatic Cholesterol "De Novo" Synthesis in Vivo (Rat, Orally)[a]



1 and 2

	% cholesterol "de novo" synthesis	relative potency
no drug	100	
mevinolin[b]	14	100
1b[c]	5.5	255
2b[c]	5.6	250
2e[c]	9	156
2f[c]	6.0	233

[a] Assay described in ref 16. [b] Lactone form, optically pure, 5 mg/kg bw. [c] Racemic sodium salts, 10 mg/kg bw. For definition of R^1–R^5 and A–B see Table I.

For better comparison of structure–activity relationships in 1 and 2 as well as with extensive work on analogues of the phenolic type (isocyclic central aromatic, A = oxygen, B = CH_2),[14,15] R^5 was kept constant as *p*-fluorophenyl.

The work on analogues of the phenolic type[14,15] has shown that alkyl substitution of the second ortho position is essential and leads to optimal biological activity for an isopropyl substituent.

We concentrated on R^2 = methyl or isopropyl, since ortho substituents smaller (methyl, ethyl, longer *n*-alkyl) or larger (cyclopentyl, *tert*-butyl) than the isopropyl group decreased activity in analogues of the phenolic type[14,15] and since halogen substituents (Cl, Br) led to good activity but increased toxicity.

Table I shows that the isopropyl derivatives were more potent than the methyl derivatives by a factor of 10–40 (e.g. 1b vs 1a, 1g vs 1c, 2g vs 2c).

There is much tolerance concerning R^3. Variation of R^3 (Ph, *i*-Pr, cyclohexyl) led to only small activity changes (e.g. 2b vs 2e vs 2f, 1b vs 1e vs 1f, 1a vs 1d).

Substitution of R^4 = hydrogen for a methyl group either slightly decreased (e.g. 1a vs 1c) or slightly increased (2b vs 2g and 1b vs 1g) activity, depending on the nature of the other substituents. Hydrogenation of the trans olefinic bridge (A–B = (*E*)-HC=CH) had little influence on the biological activity of 1 in vitro (e.g. 1b vs 2b, 1c vs 2c, 1d vs 2d, 1e vs 2e, 1f vs 2f, 1g vs 2g); however, the hydrogenated derivatives 2 were much less acid sensitive (vide supra) and much more active in vivo.

In the HEP G2 cell-test (Table II) the racemic compounds 1b, 2b, and 2e are 3.5, 5.0, and 25 times, respectively, more active than optically pure mevinolin sodium salt of the same concentration. General trends in Tables I and II are comparable. The superiority of 1b, 2b, and

(12) Dietschy, J. M.; McGarry, J. D. *J. Biol. Chem.* 1974, *249*, 52. Andersen, J. M.; Dietschy, J. M. *J. Lipid Res.* 1979, *20*, 740. Stange, E. F.; Dietschy, J. M. *J. Lipid Res.* 1983, *24*, 72.

(13) 3-epi, 5-epi, and 3,5-bis epi isomers of compactin and mevinolin have been reported to be biologically inactive: Heathcock, C. H.; Hadley, C. R.; Rosen, T.; Theisen, P. D.; Hecker, S. J. *J. Med. Chem.* 1987, *30*, 1858. Stokker, G. E.; Rooney, C. S.; Wiggins, J. M.; Hirshfield, J. J. *J. Org. Chem.* 1986, *51*, 4931. The biological inactivity of synthetic compactin analogues with 3*S* configuration has also been reported: Lee, T.-J. *Trends Pharmacol. Sci.* 1987, *8*, 442 and references cited therein.

(14) Baader, E.; Bartmann, W.; Beck, G.; Bergmann, A.; Granzer, E.; Jendralla, H.; von Kerekjarto, B.; Kesseler, K.; Krause, R.; Wess, G. International Symposium on Cholesterol Control And Cardiovascular Diseases: Prevention And Therapy, Milan (Italy) July 7–9, 1987; Abstract book page 133.

(15) European Application 0216 127, 1987.

(16) Dietschy, J. M.; Spady, D. K. *J. Lipid Res.* 1984, *25*, 1469. Alfin-Slater, R. B.; Deuel, H. J., Jr.; Scholtz, M. C.; Shimoda, F. K. Group Report No. N3, 1950; University of Southern California, Consolidated Eng. Corp.

especially 2e compared with mevinolin is more pronounced in the cell test. Inhibition of hepatic cholesterol "de novo" synthesis in vivo by oral 1b or 2b is about 2.5 times stronger than that for mevinolin (Table III). In normally fed rabbits (n = 6), 20 mg/kg racemic 2b decreased total plasma cholesterol levels by 34% after oral administration for 10 days (optically pure mevinolin at 10 mg/kg for 10 days, 25%), while 1b was totally inactive under the same conditions. The reason for the lack of activity of 1b in the rabbit experiment is currently not known.[22] The chemically demonstrated acid sensitivity of 1b (vide supra) would suggest that, contrary to 2b, 1b may not survive the stomach passage. However this view is not consistent with the comparable activity of 1b and 2b to inhibit hepatic cholesterol "de novo" synthesis in rats after po administration (Table III). In normally fed rabbits (n = 4), 10 mg/kg racemic 2e decreased total plasma cholesterol levels by 42% after oral administration for 6 days (optically pure mevinolin at 10 mg/kg for 6 days, 25%).[17,18]

In normally fed male beagle dogs (n = 4), 20 mg/kg racemic 2b decreased LDL-cholesterol levels by 48% and increased HDL-cholesterol levels by 14% after oral administration for 14 days (optically pure mevinolin at 10 mg/kg for 19 days: LDL-cholesterol −18%, HDL-cholesterol +2%).[18]

In conclusion, some compounds of general formula 2 exceeded mevinolin in their ability to inhibit HMG-CoA reductase in vitro and to inhibit cholesterol biosynthesis in vivo. They are promising candidates for development as antiarterosclerotic agents.

Experimental Section

For general remarks see the preceding paper in this issue.[1] ^{1}H NMR spectra were recorded in $CDCl_3$, unless noted otherwise. All starting materials were commercially available unless indicated otherwise.

1-(p-Fluorophenyl)-2-nitropropene (15). A solution of p-fluorobenzaldehyde (84 g), nitroethene (69.4 g), and n-butylamine (4 mL) in xylol (110 mL) was refluxed for 20 h under a Dean–Stark trap. On cooling to 0 °C, 21.7 g of the product crystallized (mp 64–65 °C). To the filtrate were added nitroethene (41.4 g) and n-butylamine (3 mL), and the solution was refluxed for 14 h under a Dean–Stark trap. The solution was evaporated in vacuo and the residue was digerated with methanol at 0 °C, until crystallization occurred. The crystals were collected and washed with cold methanol (53.8 g, mp 65–66 °C). Anal. ($C_9H_8FNO_2$) C, H, F, N.

Ethyl 3-(Phenylamino)-but-2(*E*)-enoate (20). A solution of aniline (45.5 mL, 0.5 mol), ethyl acetoacetate (63.5 mL, 0.5 mol), and glacial acetic acid (1 mL) in toluene (100 mL) was refluxed for 4 h under a Dean–Stark trap. The solvent was evaporated and the residue was distilled to give 57.9 g of colorless oil: bp 118–120 °C (1.5 mm); MS $C_{12}H_{15}NO_2$ m/e = 205 (M^+). Anal. ($C_{12}H_{15}NO_2$) C, H, N.

***N*,*N*-Bis[3-(4-fluorophenyl)-4-(methoxycarbonyl)-5-methyl-2,3-dihydrofuran-2-yl]hydroxylamine (17).** To a stirred solution of sodium methanolate (2.92 g, 54 mmol) in methanol (54 ml) was added methyl acetoacetate (20.9 g, 180 mmol) dropwise at 0 °C followed by 4-fluoro-β-nitrostyrene[19] (30.1 g, 180 mmol). After 15 min, a thick mash formed that was allowed to stand for 2 h at 0 °C. The solid was collected by suction, washed with ice-cold methanol, and dried over P_4O_{10} in vacuo to give 22.0 g of colorless solid: mp 139–141 °C; 7.0 g of product were obtained from the mother liquor; NMR δ 2.25 (6 H, s), 3.32 (3 H, s), 3.50 (3 H, s), 4.30 (2 H, dd), 5.40 (2 H, d), 7.16 (8 H, d), 8.72 (1 H, s); MS $C_{26}H_{25}F_2NO_7$ FAB m/e = 502 ($M + H^+$), 458, 235. Anal. ($C_{26}H_{25}F_2NO_7$) C, H, F, N.

1-Phenyl-2-methyl-3-(methoxycarbonyl)-4-(4-fluorophenyl)-1*H*-pyrrole (18a). Aniline (5.59 g, 60 mmol) was added to a solution of hydroxylamino compound 17 (15 g, 30 mmol) in ethanol (600 mL). The mixture was refluxed for 24 h. Aniline (1.1 g) was added and the mixture was refluxed for 16 h. The solvent was removed in vacuo and the residue was distributed between dichloromethane and 1 N hydrochloric acid. The organic layer was washed with saturated sodium bicarbonate solution and then with brine, dried, and concentrated. The residue was chromatographed with n-hexane/ether/dichloromethane (16:3.5:0.5) over silica, giving 4.0 g of reddish, thick oil: NMR δ 2.43 (3 H, s), 3.70 (3 H, s), 6.70 (1 H, s), 6.87–7.66 (9 H, m); MS $C_{19}H_{16}FNO_2$ m/e = 309 (M^+), 278, 248. Anal. ($C_{19}H_{16}FNO_2$) C, H, F, N.

1-Isopropyl-2-methyl-3-(methoxycarbonyl)-4-(4-fluorophenyl)-1*H*-pyrrole (18d). Isopropylamine (3.6 g, 60 mmol) was added to a suspension of hydroxylamino compound 17 (15 g, 30 mmol) in methanol (500 mL). The suspension was heated for 2 h at 40 °C and for 5 h at 50 °C, changing to a clear solution. The solvent was removed in vacuo and the residue was chromatographed with n-hexane/ether (4:1) over silica to yield 7.3 g of pale reddish crystals: mp 97–99 °C; NMR δ 1.42 (6 H, d), 2.53 (3 H, s), 3.65 (3 H, s), 4.37 (1 H, sept.), 6.60 (1 H, s), 6.80–7.46 (4 H, m); MS $C_{16}H_{16}FNO_2$ m/e = 275 (M^+), 244, 202, 201. Anal. ($C_{16}H_{16}FNO_2$) C, H, F, N.

Ethyl 1-Phenyl-2,5-dimethyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxylate (21c). A solution of 20 (23.1 g, 113 mmol) and 15 (20.5 g, 113 mmol) in ethanol (250 mL) was refluxed for 30 h. The solvent was evaporated in vacuo and the residue was chromatographed over silica (1 kg) with cyclohexane/ethyl acetate (95:5) to give 26.0 of a colorless oil: NMR δ 1.05 (3 H, t), 1.85 (3 H, s), 2.3 (3 H, s), 4.1 (2 H, q), 6.9–7.6 (9 H, m); MS $C_{21}H_{20}FNO_2$ m/e = 337 (M^+), 308, 292. Anal. ($C_{21}H_{20}FNO_2$) C, H, F, N.

Preparation of Substituted 1*H*-Pyrrole-3-carboxaldehydes 4 from Substituted 3-(Alkoxycarbonyl)-1*H*-pyrroles 18 or 21. General Procedure. A solution of ester 18 or 21 (82 mmol) in ether (150 mL) was added dropwise at 0–5 °C to the stirred suspension of lithium aluminum hydride (7.8 g, 200 mmol) in ether (300 mL). The suspension was stirred for 1 h at 0 °C and then for 2 h at room temperature. At 0 °C, 35 mL of ethyl acetate and then 16 mL of water followed by 24 mL of 2 N aqueous sodium hydroxide were added dropwise. The suspension was stirred for 30 min at room temperature and filtered. The filtrate was concentrated in vacuo and the residue was chromatographed over 1 kg of silica with cyclohexane/ethyl acetate (2:1) containing 0.2% triethylamine (yield 85–95%).

To a solution of the substituted 3-(hydroxymethyl)pyrrole (70 mmol) in ether (1.2 L) and triethylamine (12 mL) was added activated manganese dioxide (182.5 g). The suspension was stirred at room temperature under nitrogen. After 24 h, the same amount of manganese dioxide was added. After 24 h the solid was removed and washed with ether. The filtrates were concentrated in vacuo; the residue was chromatographed over silica with cyclohexane/ethyl acetate (6:1) containing 0.1% triethylamine (yield 65–85%).

1-Phenyl-2,5-dimethyl-3-(hydroxymethyl)-4-(4-fluorophenyl)-1*H*-pyrrole: colorless oil, crystallizing on standing; NMR δ 1.3 (1 H, br s), 2.0 (3 H, s), 2.1 (3 H, s), 4.55 (2 H, s), 6.9–7.65 (9 H, m); MS $C_{19}H_{18}FNO$ m/e = 295 (M^+), 278 (M^+ – OH). Anal. ($C_{19}H_{18}FNO$) C, H, F, N.

1-Phenyl-2-methyl-3-(hydroxymethyl)-4-(4-fluorophenyl)-1*H*-pyrrole: pale yellow, resinous solid; NMR δ 1.5 (1 H, br s), 2.26 (3 H, s), 4.63 (2 H, s), 6.87 (1 H, s), 6.93–7.70 (9 H, m); MS $C_{18}H_{16}FNO$ m/e = 281 (M^+), 264 (M^+ – OH). Anal. ($C_{18}H_{16}FNO$) C, H, F, N.

1-Isopropyl-2-methyl-3-(hydroxymethyl)-4-(fluorophenyl)-1*H*-pyrrole: colorless oil; MS $C_{15}H_{18}FNO$ m/e = 247 (M^+ – OH), 188. Anal. ($C_{15}H_{18}FNO$) C, H, F, N.

1-Phenyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxaldehyde (4a): yellow, resinous solid; NMR δ 2.50 (3 H, s), 6.80 (1 H, s), 6.85–7.70 (9 H, m), 10.03 (1 H, s); MS $C_{18}H_{14}FNO$ m/e = 279 (M^+), 278 (M^+ – H). Anal. ($C_{18}H_{14}FNO$) C, H, F, N.

1-Phenyl-2,5-dimethyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxaldehyde (4c): yellow solid; NMR δ 1.94 (3 H, s), 2.35 (3 H, s), 6.95–7.7 (9 H, m), 9.85 (1 H, s); MS $C_{19}H_{16}FNO$ m/e =

(17) Hypocholesterolemic activity in rabbits was tested following the protocol described in ref 1.
(18) Hypocholesterolemic activity in animal studies will be described in detail in a future publication.
(19) Gattermann-Wieland *Die Praxis des Organischen Chemikers*, 43rd ed.; W. de Gruyter: Berlin, 1982; p 361.

293 (M^+). Anal. ($C_{19}H_{16}FNO$) C, H, F, N.

1-Isopropyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxaldehyde (4d): colorless oil; NMR δ 1.43 (6 H, d), 2.60 (3 H, s), 4.30 (1 H, sept), 6.68 (1 H, s), 6.9–7.56 (4 H, m), 9.92 (1 H, s); MS $C_{15}H_{16}FNO$ m/e = 245 (M^+), 202. Anal. ($C_{15}H_{16}FNO$) C, H, F, N.

3-Oxo-4-methylpentanoic Acid Anilide (22). A solution of ethyl 3-oxo-4-methylpentanoate[9] (47.4 g, 0.3 mol), aniline (27.93 g, 27.3 mL, 0.3 mol), and acetic acid (0.6 mL) in toluene (360 mL) was refluxed for 4 h with a Dean–Stark trap. The cold mixture was washed twice with 0.5 N hydrochloric acid, twice with saturated sodium bicarbonate solution, once with brine, dried, concentrated, and chromatographed with toluene/ethyl acetate (10:1) over 1 kg of silica, giving 40.5 g (66% yield) of a pale pink oil: NMR δ 1.2 (6 H, d), 2.8 (1 H, sept), 3.65 (2 H, s), 7.0–7.75 (5 H, m), 9.1–9.4 (1 H, br s); MS $C_{21}H_{15}NO_2$ m/e = 205 (M^+), 93. Anal. ($C_{21}H_{15}NO_2$) C, H, F, N.

3-(Phenylamino)-4-methylpent-2(*E*)-enoic Acid Anilide (23b). A solution of ethyl 3-oxo-4-methylpentanoate[9] (31 mL, 0.2 mol), aniline (37 mL, 0.41 mol), and acetic acid (1.0 mL) in toluene (50 mL) was refluxed for 6 h with a Dean–Stark trap. The solvent was removed in vacuo. On cooling the residue crystallized. It was recrystallized from toluene/petroleum ether (80–110 °C) (2:1) to yield 38.7 g of colorless solid: mp 147–148 °C; a second crop of crystals can be obtained from the mother liquor; NMR δ 1.1 (7 H, d + m), 2.9 (1 H, sept), 4.75 (1 H, s), 6.8–7.6 (10 H, m), 11.1 (1 H, br s). Anal. ($C_{18}H_{20}N_2O$) C, H, N.

3-(Isopropylamino)-4-methylpent-2(*E*)-enoic Acid Anilide (23e). To a solution of anilide 22 (35.7 g, 174 mmol) and acetic acid (0.6 mmol) in toluene (600 ml), refluxing under a Dean–Stark trap, was added isopropylamine (20.6 g, 348 mmol) dropwise over 3 h. The mixture was refluxed for 16 h, concentrated in vacuo, and cooled, leading to crystallization. The solid was digerated with diisopropyl ether/petroleum ether (1:1), collected with suction filtration, and washed with petroleum ether, giving 28.9 g of colorless solid: mp 152–153 °C; NMR δ 1.1 (6 H, d), 1.25 (6 H, d), 2.73 (1 H, sept), 3.8 (1 H, m), 4.43 (1 H, s), 6.7 (1 H, s), 6.9–7.6 (5 H, m), 9.1–9.6 (1 H, br s); MS $C_{15}H_{22}N_2O$ CI m/e = 247 (M + H^+), 154. Anal. ($C_{15}H_{22}N_2O$) C, H, N.

3-(Cyclohexylamino)-4-methylpent-2(*E*)-enoic Acid Anilide (23f). A solution of anilide 22 (31.6 g, 154 mmol), acetic acid (1.5 mL), and cyclohexylamine (30.55 g, 308 mmol) in toluene (750 mL) was refluxed for 20 h under a Dean–Stark trap. The solvent was removed in vacuo, the residue was swirled with 150 mL of diisopropyl ether, collected with suction filtration, and washed with petroleum ether to give 27.1 g of a colorless solid (an addition 8.9 g came from the mother liquor): yield 82%; mp 123–132 °C; NMR δ 1.15 (6 H, d), 1.0–2.1 (10 H, m), 2.7 (1 H, sept), 3.45 (1 H, m), 4.4 (1 H, s), 6.55 (1 H, m), 6.9–7.6 (5 H, m), 9.5 (1 H, br s) MS $C_{18}H_{26}N_2O$ m/e = 286 (M^+), 194, 93. Anal. ($C_{18}H_{26}N_2O$) C, H, N.

Preparation of Substituted 1*H*-Pyrrole-3-carboxanilides 24 from Enamino Anilides 23. General Procedure. A solution of the nitro olefin 15 (95 mmol) and enamino carboxanilide 23 (100 mmol) in ethanol (300 mL) was refluxed for 12 h under nitrogen. Most of the solvent was removed in vacuo. Cooling of the residue in an ice bath gave crystals that were swirled in cyclohexane/ethyl acetate (200 mL), collected, and recrystallized.

1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxanilide (24b): yield 78%; mp 192–194 °C (from methanol); NMR δ 1.30 (6 H, d), 3.14 (1 H, sept), 6.73 (1 H, s), 7.00–7.70 (10 H, m). Anal. ($C_{26}H_{23}FN_2O$) C, H, F, N.

1,2-Diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxanilide (24e): yield 50%; mp 131–133 °C (not recryst); NMR δ 1.45 (6 H, d), 1.55 (6 H, d), 3.75 (1 H, sept), 4.6 (1 H, sept), 6.7 (1 H, s), 6.7–7.6 (10 H, m); MS $C_{23}H_{25}FN_2O$ m/e = 364 (M^+), 272, 230. Anal. ($C_{23}H_{25}FN_2O$) C, H, F, N.

1-Cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxanilide (24f): yield 52%; mp 215–216 °C (not recryst); NMR δ 0.9–2.2 (16 H, d + m), 3.5–4.3 (2 H, m), 6.65 (1 H, s), 6.8–7.6 (10 H, m) MS $C_{26}H_{29}FN_2O$ CI m/e = 405 (M + H^+), 312, 230. Anal. ($C_{26}H_{29}FN_2O$) C, H, F, N.

1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-5-methyl-1*H*-pyrrole-3-carboxanilide (24g): yield 80%; mp 190–192 °C (from cyclohexane/ethyl acetate); NMR δ 1.3 (6 H, d), 1.83 (3 H, s), 3.2 (1 H, sept), 6.8–7.6 (15 H, m); MS $C_{27}H_{25}FN_2O$ m/e = 412 (M^+), 320 (M^+ – PhNH). Anal. ($C_{27}H_{25}FN_2O$) C, H, F, N.

Preparation of Substituted 1*H*-Pyrrole-3-carboxaldehyde 4 from Substituted 1*H*-Pyrrole-3-carboxanilides 24. General Procedure. (a) **N-Methylation.** To a mechanically stirre solution of anilide 24 (55 mmol) in toluene (300 mL) was adde a 50% dispersion of NaH in mineral oil (5.5 g, 115 mmol) at 2 °C under a nitrogen atmosphere. The suspension was warme for 30 min at 60 °C and for 10 min at 100 °C. The suspensio was cooled to 20 °C and methyl iodide (62.5 g, 440 mmol) wa added. It was refluxed (bath at 75 °C) for 4–16 h, depending o steric hindrance (TLC control). With external cooling with dr ice/methanol, first water (80 mL) was added dropwise, followe by ether (400 mL). The organic phase was separated, washed wit brine, dried, and concentrated in vacuo. The residues ofte crystallized when swirled with *n*-hexane or diisopropyl ether t a colorless to pale yellow solid. Oily products were purified b chromatography with cyclohexane/ethyl acetate/triethylamin (8:2:0.01) over silica.

1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-*N*-methyl-1*H*-pyrrole-3-carboxanilide: yield 94%; mp 126–127 °C (not re cryst); MS $C_{27}H_{25}FN_2O$ m/e = 412 (M^+), 306, 262. Anal. ($C_{27}H_{25}FN_2O$) C, H, F, N.

1,2-Diisopropyl-4-(4-fluorophenyl)-*N*-methyl-1*H*-pyrrole-3-carboxanilide: yield 73%; oil; NMR δ 1.40 (12 H, d) 3.23 (4 H, s + sept), 4.40 (1 H, sept), 6.50 (1 H, s), 6.5–7.5 (9 H m); MS $C_{24}H_{27}FN_2O$ m/e = 378 (M^+), 272, 91. Anal. ($C_{24}H_{27}FN_2O$) C, H, F, N.

1-Cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-*N*-methyl-1*H*-pyrrole-3-carboxanilide: yield 98%; mp 102–105 °C (not recryst); NMR δ 1.35 (3 H, d), 1.50 (3 H, d), 1.1–2.2 (11 H, m). 3.25 (3 H, br s) 3.95 (1 H, m), 6.4–7.4 (10 H, m); MS $C_{27}H_{31}FN_2O$ CI m/e = 419 (M + H^+), 312. Anal. ($C_{27}H_{31}FN_2O$) C, H, F, N.

1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-5-methyl-*N*-methyl-1*H*-pyrrole-3-carboxanilide: yield 84%; mp 62–63 °C (not recryst); NMR δ 1.2 (3 H, d), 1.3 (3 H, d), 1.8 (3 H, s), 2.8 (1 H, sept), 3.17 (3 H, s), 6.5–7.5 (14 H, m); MS $C_{28}H_{27}FN_2O$ m/e = 426 (M^+), 320 (M^+ – $PhNCH_3$). Anal. ($C_{28}H_{27}FN_2O$) C, H, F, N.

(b) **Reduction.** To a suspension of lithium aluminum hydride (60 mmol) in dry THF (120 mL) under nitrogen was added dropwise a solution of *N*-methylanilides (29 mmol) in THF (120 mL). The mixture was refluxed for 20 h and then cooled to 0 °C. Ethyl acetate (15 mL) and then water (5 mL) followed by 2 N sodium hydroxide solution (10 mL) were added dropwise. The mixture was stirred for 30 min at 25 °C. The solids were removed and washed with ether.

The filtrate was concentrated in vacuo. The residues often crystallized when swirled with *n*-pentane. Oily products were purified by chromatography with toluene/ethyl acetate/triethylamine (20:1:0.01) over silica.

1-Phenyl-2-isopropyl-3-(hydroxymethyl)-4-(4-fluorophenyl)-1*H*-pyrrole: yield 92%; oil; NMR δ 1.28 (7 H, d + m), 3.03 (1 H, sept), 4.70 (2 H, s), 6.73 (1 H, s), 6.90–7.70 (9 H, m); MS $C_{20}H_{20}FNO$ m/e = 309 (M^+), 294, 276. Anal. ($C_{20}H_{20}FNO$) C, H, F, N.

1,2-Diisopropyl-3-(hydroxymethyl)-4-(4-fluorophenyl)-1*H*-pyrrole: yield 75%; pale yellow oil that slowly crystallized; NMR δ 1.2–1.6 (12 H, m), 2.35 (1 H, br s), 3.33 (1 H, sept), 4.40 (2 H, s), 4.50 (1 H, sept), 6.70 (1 H, s), 6.8–7.65 (4 H, m); MS $C_{17}H_{22}FNO$ CI m/e = 275 (M^+), 258, 242, 200. Anal. ($C_{17}H_{22}FNO$) C, H, F, N.

1-Cyclohexyl-2-isopropyl-3-(hydroxymethyl)-4-(4-fluorophenyl)-1*H*-pyrrole: yield 67%; mp 114–116 °C (not recryst); NMR δ 1.37 (6 H, d), 1.2–2.1 (10 H, m), 3.30 (1 H, sept), 3.96 (1 H, m), 4.38 (2 H, s), 6.70 (1 H, s), 6.95 (2 H, m), 7.47 (2 H, m); MS $C_{20}H_{26}FNO$ m/e = 315 (M^+), 300, 282, 200. Anal. ($C_{20}H_{26}FNO$) C, H, F, N.

1-Phenyl-2-isopropyl-3-(hydroxymethyl)-4-(4-fluorophenyl)-5-methyl-1*H*-pyrrole: yield 63%; colorless solid; NMR δ 1.25 (6 H, d), 1.9 (3 H, s), 2.8 (1 H, m), 4.35 (1 H, s), 4.55 (2 H, s), 6.85–7.75 (9 H, m); MS $C_{21}H_{22}FNO$ m/e = 323 (M^+), 308 (M^+ – CH_3), 290 (M^+ – CH_3 – H_2O). Anal. ($C_{21}H_{22}FNO$) C, H, F, N.

(c) **Oxidation. Variant A.** To a mechanically stirred suspension of Celite (50 g) and finely powdered CrO_3 (25 g, 250 mmol) in dry dichloromethane (250 mL) at 15 °C was added dropwise a solution of dry pyridine (39.5 g, 500 mmol) in CH_2Cl_2 (250 mL).

After stirring at room temperature (20 min), a solution of the substituted (hydroxymethyl)pyrrole (25 mmol) in CH_2Cl_2 (250 mL) was added dropwise but quickly. The reaction temperature was kept between 20 and 24 °C. After 15 min cyclohexane (500 mL) was added. The solid was suction filtered and washed with dichloromethane/cyclohexane (3:7). The filtrate was concentrated and chromatographed with cyclohexane/ethyl acetate/triethylamine (4:1:0.01) over 500 g of silica.

Variant B.[11] To a solution of *N*-methylmorpholine *N*-oxide (46.8 g, 400 mmol) in acetone (400 mL, dried over K_2CO_3) was added tris(triphenylphosphine)ruthenium(II) dichloride (3.8 g, 4.0 mmol). The mixture was stirred 20 min at 20 °C. A solution of the substituted (hydroxymethyl)pyrrole (100 mmol) in dry acetone (600 mL) was added dropwise. The mixture was stirred for 10–20 h at room temperature. After complete reaction (TLC, cyclohexane/ethyl acetate/triethylamine 4:1:0.1), the mixture was filtered through a short, thick silica pad. The pad was washed with ether (3 L); the filtrate was concentrated in vacuo. The residue, pure 4, usually crystallized, when digerated with *n*-pentane at 0 °C.

1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxaldehyde (4b): yield (variant A) 35%, (variant B) 87%; pale yellow solid; mp 119–120 °C; NMR δ 1.36 (6 H, d), 3.16 (1 H, sept), 6.65 (1 H, s), 7.0–7.7 (9 H, m), 10.1 (1 H, s); MS $C_{20}H_{18}FNO$ m/e = 307 (M^+), 292. Anal. ($C_{20}H_{18}FNO$) C, H, F, N.

1,2-Diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxaldehyde (4e): yield (variant B) 87%; yellow oil; NMR δ 1.43 (6 H, d), 1.47 (6 H, d), 3.80 (1 H, sept), 4.57 (1 H, sept), 6.62 (1 H, s), 7.06 (2 H, m), 7.37 (2 H, m), 9.89 (1 H, s); MS $C_{17}H_{20}FNO$ m/e = 273 (M^+), 258, 244. Anal. ($C_{17}H_{20}FNO$) C, H, F, N.

1-Cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxaldehyde (4f): yield (variant B) 98%; colorless crystals; mp 134–135 °C; NMR δ 1.45 (6 H, d), 1.1–2.2 (10 H, m), 3.55–4.35 (2 H, m + sept), 6.65 (1 H, s), 6.9–7.6 (4 H, m), 9.95 (1 H, s); MS $C_{20}H_{24}FNO$ m/e = 313 (M^+), 298, 231, 216. Anal. ($C_{20}H_{24}FNO$) C, H, F, N.

1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-5-methyl-1*H*-pyrrole-3-carboxaldehyde (4g): yield (variant A) 45%; pale yellow solid; NMR δ 1.3 (6 H, d), 2.1 (3 H, s), 3.1 (1 H, sept), 6.9–7.6 (9 H, m), 10.0 (1 H, s); MS $C_{21}H_{20}FNO$ m/e = 321 (M^+). Anal. ($C_{21}H_{20}FNO$) C, H, F, N.

Synthesis of 1,2-Diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxaldehyde (4e) via Three-Component Coupling Reaction According to Scheme VI. (a) Three-Component Coupling. **Ethyl 1,2-Diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxylate (21e).** To a suspension of 4-fluoro-β-nitrostyrene[19] (209 g, 1.25 mol) in absolute methanol (500 mL) was added ethyl 3-oxo-4-methylpentanoate[9] (214 g, 1.35 mol) under ice cooling followed by isopropylamine (128 mL, 1.50 mol), both in one portion. Absolute methanol (1 L) was added, the ice bath was removed, and the reaction mixture was stirred for 48 h in a tightly stoppered flask. Volatile components were removed in vacuo. The brown, viscous oil was filtered with toluene/0.1% triethylamine through 5 kg of silica gel (70–200 μm) to give 197 g (49.7% yield) of a yellow solid: mp 72–74 °C; NMR (CD_2Cl_2) δ 1.07 (3 H, t), 1.36 (6 H, d), 1.42 (6 H, d), 3.73 (1 H, sept), 4.06 (2 H, q), 4.50 (1 H, sept), 6.60 (1 H, s), 6.80–7.40 (4 H, m); MS (DCI, posit, isobutane) $C_{19}H_{24}FNO_2$ m/e = 318 ($M + H^+$), 317, 302. Anal. ($C_{19}H_{24}FNO_2$) C, H, F, N.

(b) Reduction. 1,2-Diisopropyl-3-(hydroxymethyl)-4-(4-fluorophenyl)-1*H*-pyrrole. A solution of the ethyl ester (197 g, 0.62 mol) in ether (750 mL) was added dropwise at 0 °C to a suspension of lithium aluminum hydride (47.2 g, 1.24 mol) in ether (1.5 L). The reaction mixture was stirred for 1 h at 0 °C and for 1 h at 20 °C. At 0–10 °C ethyl acetate (150 mL) was added dropwise, and then water (38 mL) followed by 2 N sodium hydroxide solution (75 mL) was added. The mixture was stirred for 15 min at room temperature. The inorganic solids were removed by suction filtration and washed thoroughly with ether.

Triethylamine (1 mL) was added to the combined filtrate and washings and the solvent was removed in vacuo to give a yellow solid (131 g, 77% yield) that had spectra identical with those of the authentic material described above.

(c) Oxidation was performed as described above to give 4e as a yellow solid in 92% yield.

Pyrrole-Substituted Acrylonitriles 5. General Procedure. At 0 °C a solution of diisopropyl (cyanomethyl)phosphonate (13.5 g, 66.0 mmol) in dry THF (200 mL) was added dropwise to a suspension of sodium hydride (3.78 g of a 50% dispersion in mineral oil, 78.7 mmol) in dry THF (700 mL). After 40 min at 0 °C, a solution of aldehyde 4 (44.0 mmol) in THF (100 mL) was added dropwise. The mixture was stirred for 2 h at room temperature. The reaction mixture was poured into 1 L of brine. The organic phase was separated and the aqueous phase was extracted with ether. The combined organic phases were dried and concentrated in vacuo. The residue was chromatographed over silica with cyclohexane/ethyl acetate (6:1), containing 0.1% triethylamine.

β-[1-Phenyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-acrylonitrile (5a): yield 78%; pale yellow solid; NMR δ 2.30 (3 H, s), 5.23 (1 H, d), 6.73 (1 H, s), 7.0–7.6 (10 H, m); MS $C_{20}H_{15}FN_2$ m/e = 302 (M^+). Anal. ($C_{20}H_{15}FN_2$) C, H, F, N.

β-[1,2-Diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-acrylonitrile (5e): yield 91%; crystals; mp 121–123 °C (not recryst); NMR δ 1.43 (12 H, 2 × d), 3.30 (1 H, sept), 4.50 (1 H, sept), 4.93 (1 H, d), 6.60 (1 H, s), 6.9–7.4 (4 H, m), 7.53 (1 H, d); MS $C_{19}H_{21}FN_2$ m/e = 296 (M^+), 281, 256, 239. Anal. ($C_{19}H_{21}FN_2$) C, H, F, N.

β-[1-Cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-acrylonitrile (5f): yield 96%; pale yellow solid; mp 130–132 °C (not recryst); NMR δ 1.40 (6 H, d), 1.2–2.1 (10 H, m), 3.30 (1 H, sept), 4.00 (1 H, m), 4.95 (1 H, d), 6.60 (1 H, s), 6.9–7.4 (4 H, m), 7.55 (1 H, d); MS $C_{22}H_{25}FN_2$ m/e = 336 (M^+), 321, 239. Anal. ($C_{22}H_{25}FN_2$) C, H, F, N.

Preparation of Pyrrole-Substituted Acroleins 6 from Acrylonitriles 5. General Procedure. To a solution of nitrile 5 (24 mmol) in dry THF (200 mL) was added dropwise 60 mL (72 mmol) of a 1.2 M solution of diisobutylaluminum hydride in toluene at 0 °C. The mixture was stirred for 1 h at 0 °C and then for 1.5 h at room temperature. At 0 °C, saturated aqueous sodium dihydrogen phosphate solution (100 mL) and then water (200 mL) were added dropwise. The mixture was stirred for 1 h at room temperature and then saturated with sodium chloride and extracted with ether. The combined organic phases were washed with saturated aqueous sodium bicarbonate and then dried and concentrated in vacuo. The residue was chromatographed over silica with cyclohexane/ethyl acetate (5:1), containing 0.1% triethylamine.

3-[1-Phenyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-propenal (6a): yield 70%; pale yellow solid; NMR δ 2.36 (3 H, s), 6.26 (1 H, dd), 6.97 (1 H, d), 7.15–7.70 (10 H, m), 9.54 (d, 1 H); MS $C_{20}H_{16}FNO$ m/e = 305 (M^+), 290, 276, 264. Anal. ($C_{20}H_{16}FNO$) C, H, F, N.

3-[1,2-Diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-propenal (6e): yield 70%; crystals; mp 119–121 °C; NMR δ 1.45 (12 H, 2 × d), 3.45 (1 H, sept), 4.53 (1 H, sept), 6.00 (1 H, d), 6.65 (1 H, s), 6.9–7.5 (4 H, m), 7.63 (1 H, d), 9.45 (1 H, d); MS $C_{19}H_{22}FNO$ m/e = 299 (M^+), 256, 214. Anal. ($C_{19}H_{22}FNO$) C, H, F, N.

3-[1-Cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-propenal (6f): yield 81%; pale yellow crystals; mp 124 °C (not recryst); NMR δ 1.46 (6 H, d), 1.3–2.2 (10 H, m), 3.50 (1 H, sept), 4.00 (1 H, m), 6.05 (1 H, dd), 6.65 (1 H, s), 6.9–7.5 (4 H, m), 7.65 (1 H, d), 9.50 (1 H, d); MS $C_{22}H_{26}FNO$ m/e = 339 (M^+), 296, 214. Anal. ($C_{22}H_{26}FNO$) C, H, F, N.

Synthesis of Pyrrole-Substituted Acroleins 6 from Aldehydes 4 with the Wollenberg Reagent. General Procedure. To a solution of 1-ethoxy-2-(tri-*n*-butylstannyl)ethylene[20] (3.46 g, 9.6 mmol) in dry THF (110 mL) was added a solution of *n*-butyllithium in *n*-hexane (6.25 mL of a 1.6 M solution, 10 mmol) at −70 °C under nitrogen. After 2 h at −73 °C, a solution of the aldehyde 4 (8 mmol) in THF (12 mL) was added dropwise. During this operation, the reaction temperature rose to −66 °C. After 2 h at −73 °C and 10 min at −50 °C, a saturated aqueous ammonium chloride solution (18.6 mL) was added dropwise at −40 °C. The mixture was allowed to warm to room temperature. The organic layer was separated; the aqueous layer was extracted twice

(20) Leusink, A. J.; Budding, H. A.; Drenth, W. *J. Organomet. Chem.* 1967, *9*, 285.

with ether. The combined organic layers were washed with brine and then dried and concentrated in vacuo. The residue was taken up in THF (93 mL) and water containing *p*-toluenesulfonic acid (18 mL) and stirred for 1 h at room temperature. The organic layer was separated and the aqueous layer was extracted with ether. The combined organic layers were washed with brine and then dried and concentrated. The residue was chromatographed with cyclohexane/ethyl acetate/triethylamine (3:1:0.1) over 450 g of silica.

3-[1-Phenyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-propenal (6a): yield 98%; spectra, see above.

3-[1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-propenal (6b): yield 50% (46% recovered starting material); NMR δ 1.35 (6 H, d), 3.16 (1 H, sept), 6.05 (1 H, dd), 6.63 (1 H, s), 7.0–7.5 (9 H, m), 7.75 (1 H, d), 9.50 (1 H, d); MS $C_{22}H_{20}FNO$ DCI m/e = 334 (M + H$^+$), 290. Anal. ($C_{22}H_{20}FNO$) C, H, F, N.

3-[1-Phenyl-2,5-dimethyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-propenal (6c): yield 88%; amorphous solid; NMR δ 1.9 (3 H, s), 2.2 (3 H, s), 6.07 (1 H, dd), 6.9–7.7 (10 H, m), 9.45 (1 H, d); MS $C_{21}H_{18}FNO$ m/e = 319 (M$^+$), 290 (M$^+$ – CHO). Anal. ($C_{21}H_{18}FNO$) C, H, F, N.

3-[1-Isopropyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-propenal (6d): yield 94%; colorless solid; NMR δ 1.47 (6 H, d), 2.43 (3 H, s), 4.42 (1 H, sept), 6.20 (1 H, dd), 6.72 (1 H, s), 6.9–7.5 (4 H, m), 7.50 (1 H, d), 9.48 (1 H, d); MS $C_{17}H_{18}FNO$ m/e = 271 (M$^+$), 256, 242, 200. Anal. ($C_{17}H_{18}FNO$) C, H, F, N.

3-[1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-5-methyl-1*H*-pyrrol-3-yl]-(*E*)-propenal (6g): yield 91%; yellow solid; NMR δ 1.3 (6 H, d), 2.0 (3 H, s), 3.1 (1 H, sept), 6.1 (1 H, dd), 7.0–7.8 (10 H, m), 9.5 (1 H, d); MS $C_{23}H_{22}FNO$ DCI m/e = 348 (M + H$^+$). Anal. ($C_{23}H_{22}FNO$) C, H, F, N.

β-Keto-δ-hydroxy Esters 7. General Procedure. To a suspension of sodium hydride (12.7 mmol) in THF (86 mL) was added dropwise a solution of methyl acetoacetate (1.43 g, 12.33 mmol) in THF (10 mL) at −15 °C during 5 min. The solution was stirred for 50 min at −15 °C. A solution of *n*-butyllithium in hexane (7.68 mL of a 1.6 M solution, 12.26 mmol) was added during 10 min. The reaction mixture was stirred for 20 min at −15 °C. A solution of aldehyde 6 (7.0 mmol) in THF (25 mL) was added during 10 min. The reaction mixture was stirred for 45 min at −15 °C. At −10 °C, a saturated sodium dihydrogen phosphate solution (13 mL) was added dropwise. After 5 min at 0 °C, the mixture was distributed between ether and brine. The organic layer was separated and the aqueous layer was extracted with ether. The combined organic layers were washed with brine, dried, concentrated, and chromatographed with cyclohexane/ethyl acetate/triethylamine (2:1:0.1) over silica, giving a pale yellow oil (76–85% yield).

Methyl 5(*RS*)-hydroxy-3-oxo-7-[1-phenyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (7a): NMR δ 2.27 (3 H, s), 2.55 (1 H, br), 2.80 (2 H, m), 3.50 (2 H, s), 3.74 (3 H, s), 4.69 (1 H, q), 5.65 (1 H, dd), 6.60 (1 H, d), 6.76 (1 H, s), 7.00–7.12 (4 H, m), 7.30–7.52 (5 H, m); MS $C_{25}H_{24}FNO_4$ m/e = 421 (M$^+$), 403, 345, 302. Anal. ($C_{25}H_{24}FNO_4$) C, H, F, N.

Methyl 5(*RS*)-hydroxy-3-oxo-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (7b): MS $C_{27}H_{28}FNO_4$ m/e = 449 (M$^+$), 432, 373, 334, 290. Anal. ($C_{27}H_{28}FNO_4$) C, H, F, N.

Methyl 5(*RS*)-hydroxy-3-oxo-7-[1-phenyl-2,5-dimethyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (7c): NMR δ 1.6 (1 H, s), 1.9 (3 H, s), 2.13 (3 H, s), 2.36 (2 H, s), 3.57 (2 H, AB), 3.73 (3 H, s), 5.99 (1 H, d), 6.16 (1 H, dd), 6.94 (1 H, d), 7.08–7.33 (5 H, m), 7.44–7.58 (4 H, m); MS $C_{26}H_{26}FNO_4$ m/e = 435 (M$^+$), 417, 320, 319, 316, 290. Anal. ($C_{26}H_{26}FNO_4$) C, H, F, N.

Methyl 5(*RS*)-hydroxy-3-oxo-7-[1-isopropyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (7d): NMR δ 1.44 (8 H, d + m), 1.58 (1 H, br s), 2.37 (3 H, s), 3.58 (2 H, s), 3.75 (3 H, s), 4.35 (1 H, sept), 6.02 (1 H, d), 6.27 (1 H, dd), 6.67 (1 H, s), 7.06 (2 H, m), 7.28 (2 H, m); MS $C_{22}H_{26}FNO_4$ m/e = 387 (M$^+$), 369, 272. Anal. ($C_{22}H_{26}FNO_4$) C, H, F, N.

Methyl 5(*RS*)-hydroxy-3-oxo-7-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (7e): NMR (CD_2Cl_2) δ 1.36 (6 H, d), 1.42 (6 H, d), 2.37 (1 H, d), 2.68 (2 H, m), 3.30 (1 H, sept), 3.48 (2 H, s), 3.70 (3 H, s), 4.44 (1 H, sept), 4.59 (1 H, m), 5.32 (1 H, dd), 6.62 (1 H, d), 7.00 (2 H, m), 7.30 (2 H, m); MS $C_{24}H_{30}FNO_4$ m/e = 415 (M$^+$), 397, 300, 256. Anal. ($C_{24}H_{30}FNO_4$) C, H, F, N.

Methyl 5(*RS*)-hydroxy-3-oxo-7-[1-cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (7f): NMR (CD_2Cl_2) δ 1.35 (6 H, d), 1.3–2.3 (10 H, m), 2.35 (1 H, d), 2.65 (2 H, d), 3.30 (1 H, sept), 3.50 (2 H, s), 3.70 (3 H, s), 4.00 (1 H, m), 4.60 (1 H, m), 5.35 (1 H, dd), 6.65 (1 H, s), 6.65 (1 H, d), 6.85–7.50 (4 H, m); MS $C_{27}H_{34}FNO_4$ m/e = 455 (M$^+$), 437, 340, 296, 214. Anal. ($C_{27}H_{34}FNO_4$) C, H, F, N.

Methyl 5(*RS*)-hydroxy-3-oxo-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-5-methyl-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (7g): MS $C_{28}H_{30}FNO_4$ m/e = 463 (M$^+$), 446. Anal. ($C_{28}H_{30}FNO_4$) C, H, F, N.

β,δ-Dihydroxy Esters 1 (R^1 = CH$_3$). General Procedure. To a solution of β-keto-δ-hydroxy ester 7 (5 mmol) in dry THF (70 mL) was added dropwise a solution of triethylborane in THF (6 mL of a 1 M solution, 6 mmol) during 5 min. After 20 min at 20 °C, 14 mL of dry air was bubbled through the solution with a syringe. After 2 h at 20 °C, the reaction mixture was cooled to −75 °C. Sodium borohydride (246 mg, 6.5 mmol) was added at once. After 12 h at −75 °C under nitrogen, the mixture was allowed to warm to −10 °C and saturated sodium dihydrogen phosphate solution (35 mL) was added dropwise. The reaction mixture was partitioned between ether and brine. The organic layer was washed with brine, dried, and concentrated. The residue was stirred for 3 h with dry methanol (300 mL). The solvent was evaporated and the residue was chromatographed with cyclohexane/ethyl acetate/triethylamine (1:1:0.1) through silica to yield 60–85% of a thick, pale yellow oil.

Methyl 3(*RS*),5(*SR*)-dihydroxy-7-[1-phenyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1a): NMR δ 2.12 (2 H, m), 2.24 (3 H, s), 2.37 (2 H, s), 2.54 (1 H, dd), 2.75 (1 H, dd), 3.72 (3 H, s), 4.26 (1 H, m), 5.32 (1 H, m), 5.75–5.85 (2 H, m), 6.78 (1 H, s), 7.00–7.10 (2 H, m), 7.28–7.50 (7 H, m); MS $C_{25}H_{26}FNO_4$ m/e = 423 (M$^+$), 306, 264. Anal. ($C_{25}H_{26}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*SR*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1b): NMR (C_6D_6) δ 1.30 (7 H, d + m), 1.57 (1 H, dt), 2.03 (1 H, dd), 2.18 (1 H, dd), 2.70 (1 H, br s), 3.09 (1 H, sept), 3.27 (3 H, s), 3.45 (1 H, br s), 4.03 (1 H, m), 4.34 (1 H, m), 5.67 (1 H, dd), 6.50 (1 H, s), 6.87–7.15 (8 H, m), 7.45 (2 H, dd); MS $C_{27}H_{30}FNO_4$ m/e = 451 (M$^+$), 433, 334, 292, 290, 276. Anal. ($C_{27}H_{30}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*SR*)-dihydroxy-7-[1-phenyl-2,5-dimethyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1c): NMR (C_6D_6) δ 1.37 (1 H, dt), 1.67 (1 H, dt), 1.90 (3 H, s), 2.08 (3 H, s), 2.05–2.12 (1 H, dd), 2.26 (1 H, dd), 2.40 (1 H, d), 3.26 (3 H, s), 3.48 (1 H, d), 4.11 (1 H, m), 4.30 (1 H, m), 5.72 (1 H, dd), 6.72 (1 H, d), 6.85–6.91 (2 H, m), 6.95–7.17 (5 H, m), 7.32–7.40 (2 H, m); MS $C_{25}H_{28}FNO_4$ m/e = 437 (M$^+$), 419, 320, 302, 278. Anal. ($C_{26}H_{28}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*SR*)-dihydroxy-7-[1-isopropyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1d): NMR (C_6D_6) δ 0.98 (6 H, d), 1.40 (1 H, dt), 1.68 (1 H, dt), 2.05 (3 H, s), 2.09 (1 H, dd), 2.27 (1 H, dd), 3.27 (3 H, s), 3.73 (1 H, sept), 4.14 (1 H, m), 4.34 (1 H, m), 5.72 (1 H, dd), 6.50 (1 H, s), 6.73 (1 H, d), 6.98 (2 H, m), 7.43 (2 H, m); MS $C_{22}H_{28}FNO_4$ m/e = 389 (M$^+$), 272, 230. Anal. ($C_{22}H_{28}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*SR*)-dihydroxy-7-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1e): NMR (CD_2Cl_2) δ 1.35 (6 H, d), 1.42 (6 H, d), 1.50–1.70 (2 H, m), 2.45 (2 H, d), 2.62 (1 H, br s), 3.31 (1 H, sept), 3.54 (1 H, d), 3.68 (3 H, s), 4.22 (1 H, m), 4.33–4.52 (2 H, sept + m), 5.32 (1 H, d), 6.58 (1 H, d), 6.62 (1 H, s), 7.00 (2 H, m), 7.31 (2 H, m); MS $C_{24}H_{32}FNO_4$ m/e = 417 (M$^+$), 399 (M$^+$ – H_2O), 300, 258, 212. Anal. ($C_{24}H_{32}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*SR*)-dihydroxy-7-[1-cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1f): NMR (CD_2Cl_2) δ 1.25–2.05 (12 H, m), 1.34 (6 H, d), 2.45 (2 H, d), 2.62 (1 H, d), 3.30 (1 H, sept), 3.55 (1 H, d), 3.69 (3 H, s), 3.95 (1 H, tt), 4.20 (1 H, m), 4.38 (1 H, m), 5.33 (1 H, dd), 6.58 (1 H, d), 6.62 (1 H, s), 7.00 (2 H, m), 7.30 (2 H, m); MS $C_{27}H_{36}FNO_4$ m/e = 457 (M$^+$), 439 (M$^+$ – H_2O), 421 (M$^+$ – 2H_2O), 366, 340, 298, 212. Anal. ($C_{27}H_{36}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*SR*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-5-methyl-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1g): NMR (C_6D_6) δ 1.3 (7 H, d + m), 1.6 (1 H, m), 1.95 (3 H, s), 2.0–2.3 (2 H, m), 2.5 (1 H, br s), 3.1 (1 H, sept), 3.3 (3 H, s), 3.5 (1 H, s), 4.1 (1 H, m), 4.3 (1 H, m), 5.7 (1 H, dd), 6.8–7.5 (10 H, m); MS $C_{28}H_{32}FNO_4$ *m/e* = 465 (M^+), 447 ($M^+ - H_2O$). Anal. ($C_{28}H_{32}FNO_4$) C, H, F, N.

Hydrogenated β,δ-Dihydroxy Esters 2 ($R^1 = CH_3$). General Procedure. Ten percent palladium on charcoal (2.2 g) was added under nitrogen to a solution of the olefinic β,δ-dihydroxy ester 1 ($R^1 = CH_3$) (70 mmol) in methanol (1.3 L) and triethylamine (13 mL). The mixture was shaken for 20 min in a hydrogen atmosphere at atmospheric pressure and room temperature. H_2 (1240 mL) was taken up (theoretical 1570 mL). The catalyst was filtered off and washed with methanol. The filtrate was concentrated in vacuo. The residue was chromatographed with cyclohexane/ethyl acetate (5:3), containing 0.1% triethylamine, through 1.3 kg of silica. The first compound eluted was the pure product 2 (yield 75–80%, pale yellow thick oil). Shortly thereafter a diastereomer of 2 (yield 8%) was eluted that stemmed either from incomplete stereoselectivity during the borane-catalyzed reduction of keto ester 6 (steps i, j) or from some isomerization during the catalytic hydrogenation. As a last fraction, the lactonized form of 2 (yield 4–5%) was obtained, containing some diastereomers. TLC (cyclohexane/ethyl acetate 1:1, silica) R_f values: 1 (starting material), 0.26; 2, 0.29; diastereomer of 2, 0.25; δ-lactone of 2, 0.19.

Methyl 3(*RS*),5(*RS*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (2b): NMR (C_6D_6) δ 1.03 (1 H, dt), 1.28–1.43 (1 H, m), 1.32 (3 H, d), 1.33 (3 H, d), 1.60–1.85 (2 H, m), 1.94 (1 H, dd), 2.12 (1 H, dd), 2.90–3.02 (1 H, m), 3.03–3.22 (3 H, m), 3.24 (3 H, s), 3.43 (1 H, br s), 3.75 (1 H, m), 3.88 (1 H, m), 6.58 (1 H, s), 6.94 (2 H, m), 7.03–7.15 (5 H, m), 7.42 (2 H, m); MS $C_{27}H_{32}FNO_4$ FAB *m/e* = 454 (M + H^+), 292. Anal. ($C_{27}H_{32}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*RS*)-dihydroxy-7-[1-phenyl-2,5-dimethyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (2c): NMR (C_6D_6) δ 1.10 (1 H, dt), 1.38 (1 H, dt), 1.50–1.76 (2 H, m), 1.97 (3 H, s), 2.01 (1 H, dd), 2.08 (3 H, s), 2.17 (1 H, dd), 2.77 (2 H, m), 2.86 (1 H, d), 3.27 (3 H, s), 3.50 (1 H, d), 3.72 (1 H, m), 3.95 (1 H, m), 6.90–7.13 (7 H, m), 7.28–7.36 (2 H, m); MS $C_{26}H_{30}FNO_4$ *m/e* = 439 (M^+), 407, 279. Anal. ($C_{26}H_{30}FN$) C, H, F, N.

Methyl 3(*RS*),5(*RS*)-dihydroxy-7-[1-isopropyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (2d): NMR (C_6D_6) δ 1.02 (6 H, 2 × d), 1.38 (2 H, dt), 1.50–1.75 (2 H, m), 1.97 (1 H, dd), 2.10 (3 H, s), 2.15 (1 H, dd), 2.82 (2 H, m), 3.27 (3 H, s), 3.70 (1 H, m), 3.78 (1 H, sept), 3.93 (1 H, m), 6.58 (1 H, s), 6.98 (2 H, m), 7.39 (2 H, m); MS $C_{22}H_{30}FNO_4$ DCI *m/e* = 392 (M + H^+), 391, 360, 331, 230. Anal. ($C_{22}H_{30}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*RS*)-dihydroxy-7-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (2e): NMR (CD_2Cl_2) δ 1.36 (6 H, d), 1.42 (6 H, d), 1.4–1.55 (4 H, m), 2.40 (2 H, d), 2.50–2.76 (2 H, m), 2.87 (1 H, br s), 3.22 (1 H, sept), 3.60 (1 H, br d), 3.68 (3 H, s), 3.76 (1 H, qui), 4.12 (1 H, qui), 4.43 (1 H, sept), 6.62 (1 H, s), 7.03 (2 H, m), 7.32 (2 H, m); MS $C_{24}H_{34}FNO_4$ DCI *m/e* = 420 (M + H^+), 419 (M^+), 259. Anal. ($C_{24}H_{34}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*RS*)-dihydroxy-7-[1-cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (2f): NMR (CD_2Cl_2) δ 1.36 (6 H, d), 1.3–1.8 (10 H, m), 1.32–2.05 (4 H, m), 2.39 (2 H, d), 2.50–2.72 (2 H, m), 2.88 (1 H, br s), 3.22 (1 H, sept), 3.61 (1 H, br d), 3.67 (3 H, s), 3.76 (1 H, qui), 3.94 (1 H, tt), 4.12 (1 H, qui), 6.61 (1 H, s), 7.02 (2 H, m), 7.31 (2 H, m); MS $C_{27}H_{38}FNO_4$ *m/e* = 459 (M^+), 427 ($M^+ - CH_3OH$), 299, 298, 256. Anal. ($C_{27}H_{38}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*RS*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-5-methyl-1*H*-pyrrol-3-yl]heptanoate (2g): NMR (C_6D_6) δ 1.1–1.5 (2 H, m), 1.3 (6 H, d), 1.6–2.2 (7 H, m + s), 2.9–3.2 (4 H, m), 3.3 (3 H, s), 3.45 (1 H, br s), 3.8–4.1 (2 H, m), 6.8–7.5 (9 H, m); MS $C_{28}H_{34}FNO_4$ FAB *m/e* = 468 (M + H^+). Anal. ($C_{28}H_{34}FNO_4$) C, H, F, N.

Optically Active HMG-CoA Reductase Inhibitors of General Formula 13 via Asymmetric Synthesis According to Scheme II. (a) Diastereoselective Aldol Reaction of Enolate 8 with Aldehydes 6. General Procedure. To a solution of diisopropylamine (97 mL, 70.0 g, 692 mmol) in dry THF (500 mL), cooled with dry ice, was added a 1.6 M solution of *n*-butyllithium in hexane (430 mL, 688 mmol) via a Flex-needle.[21] The mixture was stirred for 30 min at 0 °C under nitrogen. Another 4-L-four-necked flask, equipped with a mechanical stirrer, low-temperature thermometer, dropping funnel with cooling finger, and nitrogen inlet/mercury bubbler, was charged with (*S*)-(−)-phenyl 2-hydroxy-2,2-diphenylacetate[7] (104.7 g, 315 mmol) and dry THF (1 L). The suspension was cooled with dry ice.

A LDA-solution (vide supra) was transferred via a Flex-needle through a septum into the dropping funnel and added to the stirred suspension at such a rate that the reaction temperature stayed below −20 °C. The mixture was stirred for 30 min at 0 °C and became a reddish-brown, clear solution. A precooled solution of aldehyde 6 (300 mmol) in dry THF (300 mL) was added to this solution of dianion 8 at −90 °C. The reaction mixture was stirred for 1–2 h (TLC control) at this temperature. The cold mixture was poured into the mechanically stirred saturated aqueous solution of ammonium chloride (2 L) and stirred for 20 min (pH 8, 0 °C). The organic layer was separated and the aqueous layer was extracted with ether. The combined organic layers were washed with brine, dried, and filtered, and the solvent was evaporated in vacuo to give a pale yellow solid that according to TLC consisted mostly of aldol product 9 with small amounts of unreacted chiral acetate and traces of unreacted aldehyde 6. For purification, the crude solid was shaken with hot toluene/ethyl acetate (2 L, 6:4 + 0.1% triethylamine). After the suspension had come to room temperature it was filtered, and the solid after washing with toluene was discarded. Combined filtrate and washings were evaporated in vacuo, and the remaining solid residue was stirred with *n*-pentane (2 × 1 L). The resulting suspension was suction filtered. Colorless solid 9, obtained in 95–98% yield, was pure by TLC. The pentane solution contained unreacted aldehyde 6.

The diastereomeric excess (de) of the desired 3*S* isomer of 9 was 95–96% according to HPLC analysis (LiChrosorb SI 60 Merck 506487, 40 °C, 1.2 mL/min *n*-hexane/methyl *tert*-butyl ether 3:1).

(*S*)-(−)-2-Hydroxy-1,2,2-triphenylethyl (3*S*)-hydroxy-5-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-pent-4(*E*)-enoate (9b): mp 188–190 °C; NMR (CD_2Cl_2) δ 1.22 (6 H, 2 × d), 1.53 (1 H, s), 1.57 (1 H, d), 2.38 (2 H, d), 3.00 (1 H, hept), 4.37 (1 H, m), 5.28 (1 H, dd, *J* = 16 and 7 Hz), 6.59 (1 H, s), 6.67 (1 H, dd, *J* = 16 and 1.5 Hz), 6.69 (1 H, s), 6.93–7.58 (24 H, m); MS (DCI, posit, isobutane) $C_{44}H_{40}FNO_4$ *m/e* = 665 (M^+), 648 (M^+ − OH), 376, 334. Anal. ($C_{44}H_{40}FNO_4$) C, H, F, N.

(*S*)-(−)-2-Hydroxy-1,2,2-triphenylethyl (3*S*)-hydroxy-5-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]pent-4-(*E*)-enoate (9e): mp 194 °C; NMR (CD_2Cl_2) δ 1.32 (6 H, d), 1.43 (6 H, d), 2.10 (1 H, d), 2.38 (2 H, d), 2.98 (1 H, s), 3.27 (1 H, sept), 4.37 (1 H, m), 4.43 (1 H, sept), 5.23 (1 H, dd, *J* = 16 and 7 Hz), 6.53 (1 H, dd, *J* = 16 and 1.5 Hz), 6.62 (1 H, s), 6.68 (1 H, s), 6.93–7.01 (2 H, m), 7.05–7.37 (15 H, m), 7.50–7.60 (2 H, m); MS (FAB, NBA/LiI) $C_{41}H_{42}FNO_4$ *m/e* = 638 (M + Li^+), 631 (M^+), 614 (M^+ − OH), 358, 342, 300. Anal. ($C_{41}H_{42}FNO_4$) C, H, F, N.

(b) Transesterification of 9 to Optically Active Methyl Esters 10. General Procedure. To a suspension of ester 9 (178 mmol) in absolute methanol (1.4 L) was added dropwise a solution of sodium (2.0 g, 89 mmol) in absolute methanol (200 mL) at 20 °C. The mixture was stirred for 3 h at room temperature. At <10 °C, the mixture was neutralized by dropwise addition of the solution of acetic acid (5.1 mL, 89 mmol) in methanol (15 mL). Triethylamine (0.5 mL) was added, and the solvent was evaporated

(21) Commercially available from Aldrich Chemical Co., Milwaukee, WI.

(22) The oral activity in the rat is an *acute* experiment, in which the hepatic cholesterol biosynthesis inhibition is measured within 3 h after po administration. Oral activities in the rabbit and dog are *chronic* experiments, in which decrease of serum cholesterol is measured. The decrease of serum cholesterol should be coupled to the hepatic cholesterol biosynthesis inhibition, but only via a long, complex chain of biochemical reactions. It seems possible that pronounced differences of the two compounds in metabolic stability and pharmacokinetics are responsible for the lack of oral activity of the unsaturated compound 1b in the rabbit model.

at <20 °C in vacuo. The solid residue was taken up in ether and half-concentrated brine. The ether phase was washed with sodium bicarbonate and then with brine. The solvent was removed in vacuo. The liberated diol was removed from methyl ester 10 by filtration with diisopropyl ether/cyclohexane (1:1) through 2 kg of silica: yield 94–100% 10; pale-yellow oil.

Methyl esters 10 decomposed quickly in solution at room temperature, especially on air contact.

Methyl (3*S*)-hydroxy-5-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]pent-4(*E*)-enoate (10b): NMR (CD_2Cl_2) δ 1.26 (6 H, d), 2.48 (2 H, AB of AB X), 3.03 (1 H, hept), 3.60–3.71 (1 H, m), 3.67 (3 H, s), 4.53 (1 H, br s), 5.37 (1 H, dd), 6.58 (1 H, s), 6.72 (1 H, dd), 7.00 (2 H, m), 7.27–7.49 (7 H, m). Anal. ($C_{25}H_{26}FNO_3$) C, H, F, N.

Methyl (3*S*)-hydroxy-5-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]pent-4(*E*)-enoate (10e): MS (DCI, posit, isobutane) $C_{22}H_{28}FNO_3$ m/e = 373 (M^+), 356 (M^+ – OH). Anal. ($C_{22}H_{28}FNO_3$) C, H, F, N.

(c) Transformation of δ-Hydroxy Methyl Esters 10 to β-Keto-δ-hydroxy *tert*-Butyl Esters 11. General Procedure. *tert*-Butyl acetate (81.3 g, 94 mL, 700 mmol) was added dropwise at –75 °C under N_2 to a solution of LDA (730 mmol) in THF/hexane (1:1, 1 L). After 40 min at –70 °C, the solution of methyl ester 10 (178 mmol) in THF (100 mL) was added dropwise. The mixture was stirred for 10 min at –70 °C and then for 1 h at –30 °C. The cold solution was poured into mechanically stirred, half-saturated ammonium chloride solution (2 L). After 10 min, the organic phases were washed twice with sodium bicarbonate solution and then with brine, dried, filtered, and evaporated. Toluene (100 mL) was added and then evaporated at 20 °C (to remove the excess *tert*-butyl acetate). Residual volatile components were removed in high vacuo (24 h). *tert*-Butyl esters 11 were obtained as yellow, very viscous oils in 95–100% yield.

***tert*-Butyl (5*S*)-hydroxy-3-oxo-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (11b):** NMR (CD_2Cl_2) δ 1.25 (6 H, d), 1.46 (9 H, s), 2.68 (2 H, d), 3.03 (1 H, hept), 3.37 (2 H, s), 3.68 (1 H, m), 4.60 (1 H, m), 5.37 (1 H, dd), 6.60 (1 H, s), 6.74 (1 H, dd), 7.03 (2 H, m), 7.30–7.52 (7 H, m); MS (DCI, posit, isobutane) $C_{30}H_{34}FNO_4$ m/e = 491 (M^+), 474 (M^+ – OH), 418 (M^+ – isobutene), 390 (M^+ – CO_2tBu), 334. Anal. ($C_{30}H_{34}FNO_4$) C, H, F, N.

***tert*-Butyl (5*S*)-hydroxy-3-oxo-7-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (11e):** NMR (CD_2Cl_2) δ 1.36 (6 H, d), 1.40–1.48 (15 H, s + 2 × d), 1.57 (1 H, d), 2.67 (2 H, d), 3.32 (1 H, hept), 3.36 (2 H, s), 4.45 (1 H, hept), 4.57 (1 H, m), 5.32 (1 H, dd, J = 16 and 7 Hz), 6.62 (1 H, dd, J = 16 and 1.5 Hz), 6.63 (1 H, s), 7.00 (2 H, m), 7.30 (2 H, m); MS (DCI, posit, isobutane) $C_{27}H_{36}FNO_4$ m/e = 457 (M^+), 440 (M^+ – OH), 397. Anal. ($C_{27}H_{36}FNO_4$) C, H, F, N.

(d) Diastereoselective Reduction of β-Keto-δ-hydroxy *tert*-Butyl Esters 11 to β,δ-Dihydroxy *tert*-Butyl Esters 12. General Procedure. Triethylborane (185 mL of a 1 M solution in THF) was added dropwise at 20 °C to a solution of 130 mL of absolute methanol in 510 mL of dry THF. A solution of crude *tert*-butyl ester 11 (177 mmol) in THF (150 mL) was added dropwise. The mixture was stirred for 1 h at –70 °C. Sodium borohydride (8.73 g, 231 mmol) was added at once. The mixture was stirred for 1.5 h at –70 °C and then poured into half-concentrated ammonium chloride solution (2 L). The mixture was stirred for 15 min and the organic phase was separated. The aqueous phase was extracted twice with ether. The combined organic layers were washed with brine, and the solvent was evaporated in vacuo. The residue was taken up several times in wet methanol and this solvent was evaporated in vacuo at <20 °C. TLC (100% diisopropyl ether) indicated the successful conversion of the unpolar boron ester of the diol (R_f ~0.57) to free diol 12 (R_f ~0.19). Pure 12 was obtained after chromatography through silica (2 kg, 70–200 μm) with diisopropyl ether as a colorless solid (yield 70–80%).

***tert*-Butyl 3(*R*),5(*S*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (12b):** mp 107–110 °C; NMR (CD_2Cl_2) δ 1.26 (6 H, d), 1.48 (9 H, s), 1.55 (2 H, m), 2.38 (2 H, d), 2.87 (1 H, t), 3.03 (1 H, hept), 3.63 (1 H, br s), 4.16 (1 H, m), 4.39 (1 H, m), 5.37 (1 H, dd), 6.60 (1 H, s), 6.71 (1 H, dd), 7.03 (2 H, m), 7.30–7.52 (7 H, m); MS (DCI, posit, isobutane) $C_{30}H_{36}FNO_4$ m/e = 493 (M^+), 476 (M^+ – OH), 458 (M^+ – OH – H_2O). Anal. ($C_{30}H_{36}FNO_4$) C, H, F, N.

***tert*-Butyl 3(*R*),5(*S*)-dihydroxy-7-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (12e):** MS (DCI, posit, isobutane) $C_{27}H_{38}FNO_4$ m/e = 459 (M^+). Anal. ($C_{27}H_{38}FNO_4$) C, H, F, N.

(e) Catalytic hydrogenations of *tert*-butyl esters 12 were performed in analogy to that of the corresponding methyl esters 2 (vide supra), yield 75–82%.

***tert*-Butyl 3(*R*),5(*R*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate:** mp 108–110 °C; NMR (CD_2Cl_2) δ 1.25 (6 H, d), 1.46 (9 H, s), 1.40–1.57 (4 H, m), 2.33 (2 H, m), 2.63–2.91 (2 H, m), 3.02 (1 H, hept), 3.13 (1 H, br s), 3.67 (1 H, br s), 3.79 (1 H, qui), 4.11 (1 H, br qui), 6.62 (1 H, s), 7.05 (2 H, m), 7.30–7.50 (7 H, m); MS (DCI, posit, isobutane) $C_{30}H_{38}FNO_4$ 496 (M + H^+), 495 (M^+), 440 (M + H^+ – isobutene), 293. Anal. ($C_{30}H_{38}FNO_4$) C, H, F, N.

***tert*-Butyl 3(*R*),5(*R*)-dihydroxy-7-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate:** mp 128–130 °C; MS (DCI, posit, isobutane) $C_{27}H_{40}FNO_4$ m/e = 462 (M + H^+), 461 (M^+), 406 (M + H^+ – isobutene), 259. Anal. ($C_{27}H_{40}FNO_4$) C, H, F, N.

β,δ-Dihydroxy Sodium Carboxylates 1 or 2 (R¹ = Na). General Procedure. To a solution of methyl ester 1 or 2 (R¹ = CH_3, 48 mmol) in methanol (500 mL) was added dropwise 1 N aqueous sodium hydroxide solution (50 mL, 50 mmol) during 1 h at 0–10 °C. The mixture was stirred for 1 h at 0 °C and for 1 h at room temperature. The mixture was filtered and the filtrate was evaporated in vacuo. The residue was taken up in ethanol (100 mL), evaporated in vacuo, and dried in high vacuo. The residue was stirred with ether (300 mL). The solid was collected by suction filtration, washed with pentane, and dried for 4 h in vacuo in a desiccator over phosphorous pentoxide and potassium hydroxide; pale yellow solid, yield 64%. The ethereal mother liquor was evaporated in vacuo and treated as described above to give a solid with the same melting point and ^{1}H NMR; yield 31%, combined yield 95%.

Sodium 3(*RS*),5(*SR*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1b): mp 232–234 °C dec; NMR (DMSO-d_6) δ 1.20 (6 H, d), 1.25–1.62 (2 H, m), 1.80–2.11 (2 H, m), 2.98 (1 H, sept), 3.72 (1 H, m), 4.20 (1 H, m), 4.83 (1 H, br s), 5.37 (1 H, dd), 6.52 (1 H, d), 6.80 (1 H, s), 7.14 (2 H, t), 7.30 (1 H, br s), 7.40–7.60 (8 H, m). Anal. ($C_{26}H_{27}FNO_4Na$) C, H, N.

Sodium 3(*RS*),5(*RS*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (2b) mp 231–233 °C dec. Anal. ($C_{26}H_{29}FNO_4Na$) C, H, N.

Sodium 3(*R*),5(*R*)-Dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (13b). The corresponding *tert*-butyl ester (48 g, 97 mmol) was suspended in ethanol (250 mL) at 5 °C. Sodium hydroxide (1 N, 98.8 mL) was added dropwise. The suspension was stirred for 20 h at room temperature, becoming a clear solution. Solvents were removed in vacuo. The residue was washed with ether and then with pentane to give 44.6 g (yield 99.8%) of a colorless solid: mp 252–254 °C dec; NMR (DMSO-d_6) δ 1.22 (6 H, d), 1.20–1.50 (4 H, m), 1.83 (1 H, dd, J = 15 and 8 Hz), 2.04 (1 H, dd, J = 15 and 4 Hz), 2.50–2.67 (1 H, m), 2.71–2.87 (1 H, m), 2.96 (1 H, hept), 3.61 (1 H, br s), 3.74 (1 H, m), 4.70 (1 H, br s), 6.77 (1 H, s), 7.10–7.21 (2 H, m), 7.32–7.57 (7 H, m). Anal. ($C_{26}H_{29}FNO_4Na$) C, H, N.

Sodium 3(*R*),5(*R*)-dihydroxy-7-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (13e) was obtained from the corresponding *tert*-butyl ester in analogy to the method for 13b (vide supra) to give a colorless solid: mp 255 °C dec; NMR (DMSO-d_6) δ 1.30 (6 H, d), 1.37 (6 H, d), 1.82 (1 H, dd, J = 15 and 8 Hz), 2.03 (1 H, dd, J = 15 and 4 Hz), 2.32–2.48 (1 H, m), 2.52–2.67 (1 H, m), 3.18 (1 H, hept), 3.57 (1 H, br s), 3.76 (1 H, hept), 4.41 (1 H, hept), 4.57 (1 H, br s), 6.80 (1 H, s), 7.12 (2 H, m), 7.33 (2 H, m). Anal. ($C_{23}H_{31}FNO_4Na$) C, H, N.

Biological assays: see the preceding paper in this issue

were prepared and frozen at −30 °C until used. Bovine and human tissues were used in some assays (see below). Freshly dissected (or frozen) tissue was homogenized (Polytron setting 6 for 20 s) in 30 volumes of ice-cold buffer containing 50 mM Tris·HCl (pH 7.4 at 37 °C; pH 8.0 at 4 °C), 0.5 mM Na_2EDTA, and 10 mM $MgSO_4$, and centrifuged at 30000*g* for 15 min. The supernatant was discarded; the pellet was resuspended and preincubated for 15 min at 37 °C. The homogenate membranes were washed twice by centrifugation and resuspension. The final assay buffer contained 10 μM parglyine, and 0.1% ascorbate was added last to the incubation medium. Protein determinations were made by the Lowry method.

5-HT_{1A} sites were labeled with 0.1 nM [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]OH-DPAT) (157 Ci/mmol; New England Nuclear) and 4 mg wet weight of rat hippocampal tissue. 8-OH-DPAT (1 μM) was used to determine nonspecific binding. The 5-HT_{1B} receptor was labeled with 2.0 nM [^{3}H]-5-HT (28.3 Ci/mmol; New England Nuclear) and 8 mg of rat striatal membrane homogenate. 5-HT (10^{-6} M) was used to define nonspecific binding, and 10^{-7} M 8-OH-DPAT and mesulergine were included to block 5-HT_{1A} and 5-HT_{1C} receptors, respectively. 5-HT_{1C} sites were labeled with 1 nM [^{3}H]-5-HT and 10 mg of rat frontal cortical tissue homogenate; 20 nM spiperone was used to mask 5-HT_2 sites. 5-HT_{1D} sites were labeled with 10 nM [^{3}H]-5-HT and 10 mg of bovine caudate homogenate; 1 μM pindolol was used to block 5-HT_{1A} and 5-HT_{1B} istes, and 100 nM mesulergine was used to block 5-HT_{1C} sites. 5-HT_{1E} sites were labeled with 2 nM [^{3}H]-5-HT and 10 mg of human cortical homogenate in the presence of 100 nM 5-carboxamidotryptamine to block any 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} sites and 100 nM mesulergine was used to block 5-HT_{1C} 5-HT_1 sites. 5-HT_2 binding studies were conducted as previously reported.[3]

Eleven concentrations of nonradioactive competing drugs were made fresh daily in assay buffer, and assays were performed in (at least) triplicate. Following incubation with membranes and radioligand at 37 °C for 30 min, samples were rapidly filtered over glass-fiber filters (Schleicher and Schuell) and were washed with 10 mL of ice-cold 50 mM Tris·HCl buffer. Individual filters were inserted into vials and equilibrated with 5 mL of scintillation fluid (Scinti-Verse, Fisher) for 6 h before counting at 50% efficiency in a Beckman 3801 counter. Results were analyzed with an updated version of the program EBDA[21] in order to determine IC_{50}, K_i, and Hill values.

Acknowledgment. This work was supported in part by US PHS Grant NS 23523.

Registry No. 2, 304-52-9; 3, 78263-90-8; 4, 6260-79-3; 5·HCl, 1453-99-2; 6, 18658-09-8; 7, 124224-49-3; 5-(benzyloxy)-3-(2-nitropropenyl)indole, 101731-72-0; oxalyl chloride, 79-37-8; 5-(benzyloxy)-2-methylindole, 124224-50-6; 5-methoxy-2-methylindole, 1076-74-0.

(21) Macpherson, G. A. *Comput. Programs Biomed.* 1983, *17*, 107.

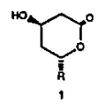
3-Hydroxy-3-methylglutaryl-coenzyme A Reductase Inhibitors. 6.[1] *trans*-6-[2-(Substituted-1-naphthyl)ethyl(or ethenyl)]-3,4,5,6-tetrahydro-4-hydroxy-2*H*-pyran-2-ones

John D. Prugh,* Alfred W. Alberts,† Albert A. Deana, James L. Gilfillian,† Jesse W. Huff,† Robert L. Smith, and J. Mark Wiggins

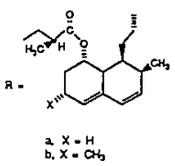
Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486, and Rahway, New Jersey 07065.
Received July 10, 1989

A variety of *trans*-6-[2-(substituted-1-naphthyl)ethyl(or ethenyl)]-3,4,5,6-tetrahydro-4-hydroxy-2*H*-pyran-2-ones were prepared and, upon conversion to their 3,5-dihydroxy carboxylates, were found to have good inhibitory activity against the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the rate-determining enzyme in cholesterogenesis. The most active compounds are 2,4,6- and 2,4,7-trichloro derivatives and would be expected to display about the same potency as the standard compactin (1a) upon resolution.

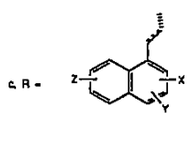
The enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase catalyzes the rate-determining step and point of natural regulation of cholesterogenesis. Potent inhibitors of this enzyme (e.g. 1a) have been shown to lower cholesterol blood levels in animals and man by about 30%.[2] The results of the Lipid Research Clinics Coronary Primary Prevention Trial showed that reduction in blood cholesterol by even a modest 10% results in significantly diminished risk of coronary heart disease.[3] Thus cholesterol blood level lowering by a 1a and similar inhibitors can be expected to significantly reduce the risk of coronary heart disease. In pursuit of this goal, we wanted to prepare wholly synthetic analogues of 1a and 1b without the complex stereochemistry. We began with some simple probes with modest activity.[4] Nonetheless these probes pointed the way to classes of compounds which after further ex-



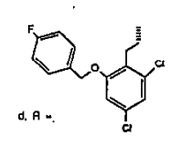
1



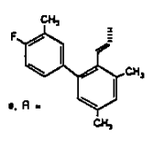
a, R = X = H
b, R = X = CH_3



c, R = Z = X = Y = Cl



d, R = F, Cl, Cl



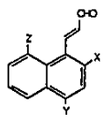
e, R = F, CH_3, CH_3, CH_3

[†] Rahway, NJ.

(1) Part 5: Stokker, G. E; Alberts, A. W.; Gilfillian, J. L.; Huff, J. W.; Smith, R. L. *J. Med. Chem.* 1986, *29*, 852.
(2) (a) Tobert, J. A.; Hitzenberger, G.; Kukovetz, W. R.; Holmes, I. B.; Jones, K. H. *Atherosclerosis (Shannon, Irel.)* 1982, *41*, 61. (b) Tobert, J. A.; Bell, G. D.; Birtwell, J.; James, I.; Kukovetz, W. R.; Pryor, J. S.; Buntinx, A.; Holmes, I. B.; Chao, Y.-S.; Bolognese, J. A. *J. Clin. Invest.* 1982, *69*, 913.
(3) (a) LRC-CPPT, *JAMA, J. Am. Med. Assoc.* 1984, *251*, 351. (b) LRC-CPPT, *JAMA, J. Am. Med. Assoc.* 1984, *251*, 365.
(4) Stokker, G. E.; Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J., Jr.; Deana, A. A.; Gilfillan, J. L.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* 1985, *28*, 347.

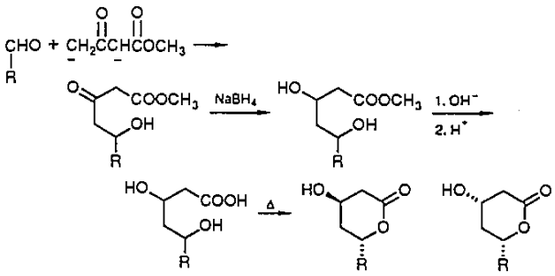
0022-2623/90/1833-0758$02.50/0

Table I. Physical Properties of 1-Naphthylpropenals

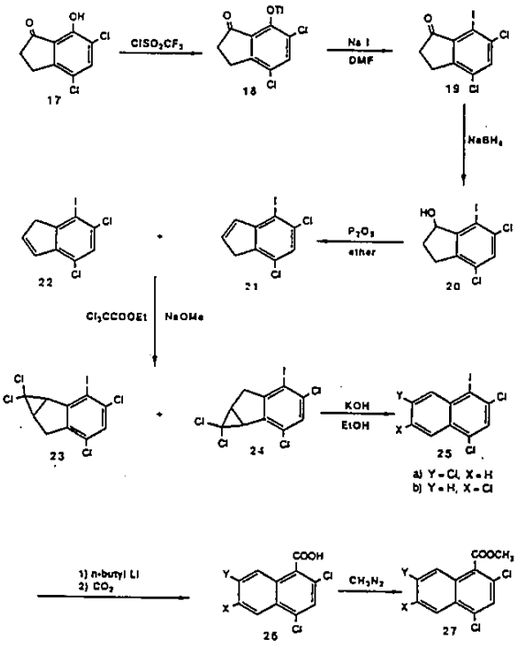


no.	X	Y	Z	recryst solvent	% yield	mp, °C	formula	anal.
14	Cl	H	H	EtOH/H_2O	44	82–84	$C_{13}H_9ClO$	C, H
15	H	Br	H	*n*-C_4H_9Cl	29	134–137	$C_{13}H_9BrO$	C, H
16	H	H	Br	hexane	25	96–98	$C_{13}H_9BrO$	

Scheme I



Scheme II



ploration gave benzyl ethers 1d, which display an interesting order of activity,[5] and biphenyls 1e, which are highly active.[6] Initial results[4] with the probe compound 1c (*x* = Y = Z = H) showed sufficient activity to merit more extensive investigation. We report herein the results of further study in the 1c series which afforded substituted naphthalene derivatives, some of which display activity similar to that of 1b.

Chemistry

The known aldehydes 2-chloro-1-naphthaldehyde[7] and 4-bromo-1-naphthaldehyde[8] were converted to propenal intermediates 14 and 15 by the method of Baker[9] (Table I), and the lactone ring was introduced with the known chemistry[4] of Scheme I to give, respectively, 2-chloro derivative 5 and 4-bromo derivative 3. The synthesis of 8-bromo propenal intermediate 16 (Table I) was accomplished by using the general method of Newman[10] and the lactone ring was introduced by using Scheme I technology. The double bond of 3 was hydrogenated with rhodium-on-carbon catalyst[11] to give 4.

Applying Parham methodology[12] produced the intermediate 2,4,6- and 2,4,7-trichloronaphthalene methyl ester derivatives 26 as outlined in Scheme II. Attempts to substitute the triflate of 18 with basic nucleophiles such as cyanide in DMF[13] were unsuccessful and gave only black tar. However, in a novel step the neutral nucleophile iodide ion smoothly displaced the triflate in DMF solvent. The remaining chemistry proceeded in a straightforward fashion to give esters 27, which were separated by HPLC and assigned structures on the basis of ^1H NMR NOE experiments (the Experimental Section). Esters 27 were converted to the intermediate propanals 31 as outlined in Scheme III. Thus treatment of chloromethyl compounds 29 with imine carbanion 30[14] followed by hydrolysis gave the desired aldehydes 31a and 31b. Introduction of the lactone ring via Scheme I technology gave final products 7 and 8.

In the chromatographic purification of 31b, an impurity (33) was isolated. Loss of the chlorine in the 2-position must have occurred during $LiAlH_4$ reduction, probably via intramolecular hydride delivery from an oxyaluminum hydride intermediate to give 32 after workup, which was then carried through the sequence undetected until the aldehyde stage. Compound 33 was then converted to 9 by using the method of Scheme I. Friedel–Crafts chemistry

(5) Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J., Jr.; Deana, A. A.; Evans, B. E.; Gilfillan, J. L.; Gould, N. P.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Rittle, K. E.; Smith, R. L.; Stokker, G. E.; Willard, A. K. *J. Med. Chem.* 1986, *29*, 159.

(6) Stokker, G. E.; Alberts, A. W.; Anderson, P. S.; Cragoe, E. J., Jr.; Deana, A. A.; Gilfillan, J. L.; Hirshfield, J.; Holtz, W. J.; Hoffman, W. F.; Huff, J. W.; Lee, T. J.; Novello, F. C.; Prugh, J. D.; Rooney, C. S.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* 1986, *29*, 170.

(7) Shoesmith, J. B.; Mackie, A. *J. Chem. Soc.* 1930, 1584.

(8) Moyer, F.; Sieglitz, A. *Ber. Dtsch. Chem. Ges.* 1922, *55*, 1835.

(9) Baker, B. R.; Janson, E. E.; Vermeulea, M. J. *J. Med. Chem.* 1969, *12*, 898.

(10) Newman, H. *J. Org. Chem.* 1973, *38*, 2254.

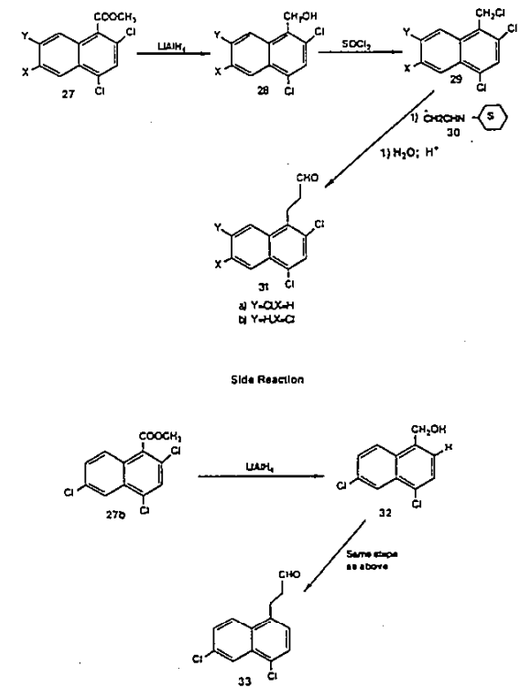
(11) Breitner, E.; Roginski, E.; Rylander, P. N. *J. Org. Chem.* 1959, *24*, 1855.

(12) Parham, W. E.; Reiff, H. E.; Swartzentruber, P. *J. Am. Chem. Soc.* 1956, *78*, 1437.

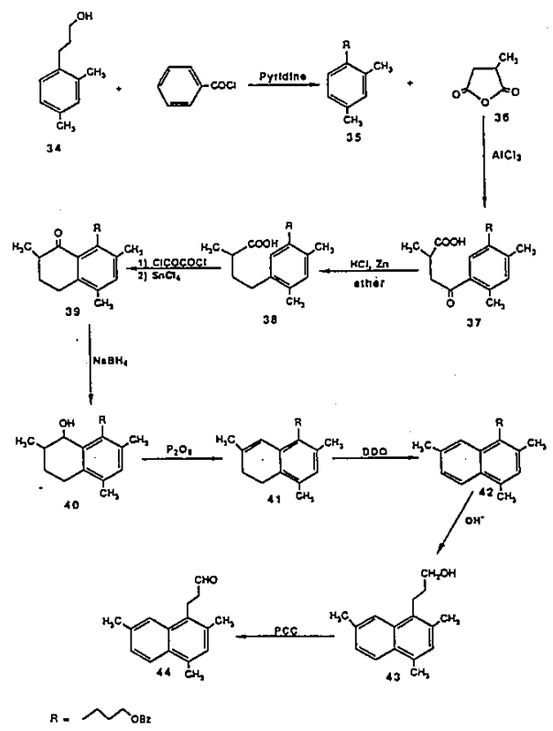
(13) Williams, H. W. R.; Rooney, C. S.; Bicking, J. B.; Robb, C. M.; de Solms, S. J.; Woltersdorf, O. W.; Cragoe, E. J., Jr. *J. Org. Chem.* 1979, *44*, 4060.

(14) (a) Wittig, G.; Hesse, A. *Organic Synthesis*; Breslow, R., Ed.; Wiley: New York, 1970, Vol. *50*, p 66. (b) Buchi, von G.; Wuest, H. *Helv. Chim. Acta*, 1967, *50*, 2440.

Scheme III

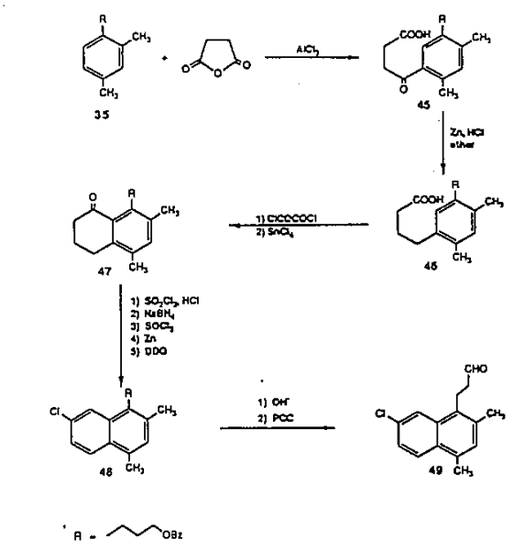


Scheme IV

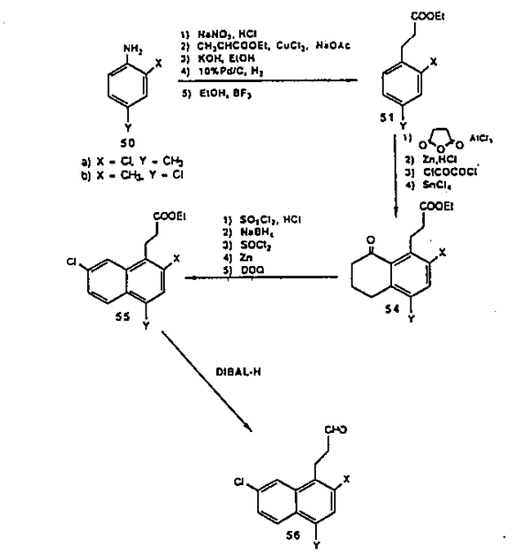


was used to construct the methyl-substituted naphthalene ring via tetralones.[15] The synthesis of the needed intermediate aldehyde 44 is outlined in Scheme IV. Friedel–Crafts acylation of benzoate 35 with anhydride 36 gave 37 with high regioselectivity.[15a] The remainder of the synthesis was straightforward, giving the naphthalene aldehyde 44, which, when carried through the lactone elaboration steps of Scheme I, gave final product 10.

Scheme V



Scheme VI

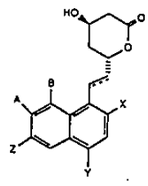


Synthesis of intermediate 49, wherein the 7-methyl group has been replaced with chlorine, is outlined in Scheme V. The novel part of this scheme, the unambiguous introduction of the 7-chloro substituent to form 48 and 55 (Scheme VI) beginning with tetralones, has been reported[16] and briefly involved gem dichlorination α to the ketone using sulfuryl chloride, reduction of the ketone to the alcohol with $NaBH_4$, followed by conversion of the alcohol to chloride with thionyl chloride. After adjacent chlorines were removed with activated Zn to give a vinyl chloride, aromatization was completed with DDQ.

(15) (a) Peto, A. G.; Reactions of Anhydrides. Friedel–Crafts and Related Reactions; Olah, G. A., Ed.; Coll. Vol. III, Part I, p 550, (b) Sethna, S. Cycliacylation. *Ibid.* Part 2, p 911.

(16) Prugh, J. D.; Deana, A. A.; Wiggins, J. M. *Synthesis* 1989, 554.

Table II. Physical Properties and in Vitro HMG-CoA Reductase Inhibitory Activities



no.	A	B	X	Y	Z	bridge	recryst solvent	mp, °C	formula	IC_{50}, μm	relative[e] potency
2	H	H	H	H	H	sat.	none	glass	$C_{17}H_{18}O_3 \cdot {}^1/_2H_2O$	81	0.043
3	H	H	H	Br	H	ene	*a*	177–179	$C_{17}H_{15}BrO_3$[c]	4	0.96
4	H	H	H	Br	H	sat.	*a*	141–143	$C_{17}H_{17}BrO_3$	23.3	0.15
5	H	H	Cl	H	H	ene	butyl chloride	88–91	$C_{17}H_{15}ClO_3$	1.51	2.3
6	H	Br	H	H	H	ene	*a*	128–129	$C_{17}H_{15}BrO_3$	4.12	0.72
7	Cl	H	Cl	Cl	H	sat.	*b*	111–115	$C_{17}H_{15}Cl_3O_3$	0.032	47
8	H	H	Cl	Cl	Cl	sat.	*b*	123–125	$C_{17}H_{15}Cl_3O_3$	0.033	46
9	H	H	H	Cl	Cl	sat.	none	glass	$C_{17}H_{16}Cl_2O_3$	7.0	0.3
10	CH_3	H	CH_3	CH_3	H	sat.	*b*	118–120	$C_{20}H_{24}O_3$	0.36	5
11	Cl	H	CH_3	CH_3	H	sat.	none	glass	$C_{19}H_{21}ClO_3$	0.2	7
12	Cl	H	CH_3	Cl	H	sat.	*a*	111–114	$C_{18}H_{18}Cl_2O_3$[d]	0.06	30
13	Cl	H	Cl	CH_3	H	sat.	*b*	126–128	$C_{18}H_{18}Cl_2O_3$	0.13	15

[a] Acetone/hexane. [b] Ether/hexane. [c] 0.05 C_6H_{14}. [d] 0.25 Et_2O. [e] Relative to compactin = 100.

We next prepared the isomeric dichloro compounds 12 and 13. Synthesis of the intermediate aldehydes required for the straightforward elaboration of both compounds is outlined in Scheme VI. Aldehydes 56 were then transformed into target structures 12 and 13 by the chemistry shown in Scheme I.

Biological Results and Discussion

The target compounds presented in Table II as the lactones were tested as the corresponding ring-opened dihydroxy carboxylate sodium salts, the active form, in aqueous solution by using the in vitro procedure reported earlier.[4] Our investigation was limited to halogen and methyl substituents on the naphthalene ring and a brief study of the saturated or unsaturated two-carbon bridge. When comparing the bridge ene in 3 versus the saturated ethyl bridge in 4, the activities show strong enhancement with the double bond as in the biphenyl series.[6] We reported previously[4] that the two-carbon bridge between the naphthalene ring and the lactone is optimal in a series where zero, two, and three methylene units were prepared with the naphthalene ring unsubstituted.

Halogens in the 2- and 4-positions were activity enhancing as they were in the benzyl ether[5] and the biphenyl[6] series. A halogen in the 8-position also was useful. The combination of 2,4,8-trihalo substitution is an obvious objective; however, this pattern was not readily accessible synthetically. We opted rather for the more accessible 2,4,6- and 2,4,7-trichloro compounds 7 and 8, whose activity turned out to be outstanding and of a useful order of magnitude since they are racemates and, if resolved, would have activity comparable to compactin (1b). The importance of the 2-substituent was reemphasized with the nearly total loss of activity of compound 9 when compared to 8. The synthesis of these compounds was however long and inefficient. Therefore, we next prepared all-methyl compound 10, where the more readily executed Friedel–Crafts chemistry could be used. To our dismay it had very little activity. This result is contrary to the biphenyl series,[6] where replacement of chlorines with methyls was permissible. We concluded that at least one of the chlorines was needed. Accordingly, we replaced the 7-methyl substituent with chlorine, which gave only a small increase in activity. Clearly replacement of another chlorine was necessary, so we prepared both of the remaining chlorine substitutions at the 2- and 4-positions, leaving the chlorine in the 7-position (compounds 12 and 13). Although most of the activity was restored, the activity of 12 and 13 is not high enough to warrant further biological evaluation.

Conclusions

A useful order of activity has been achieved in the two trichlorinated naphthalene derivatives 7 and 8. All the permutations of a methyl substituent were not made, but those that were prepared indicate that all three chlorines are needed for a useful order of activity. The protracted and tedious chemistry of the trichlorinated compounds coupled with the inability to use Friedel–Crafts chemistry in the presence of two inactivating chlorine substituents led us to terminate this work.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^1H NMR spectra were recorded in $CDCl_3$ (unless otherwise noted) on a Varian T-60, EM-390, XL-300, or NT 360 spectrometer. Chemical shifts are reported in parts per million relative to Me_4Si as the internal standard. Elemental analysis for carbon, hydrogen, and nitrogen were determined with a Perkin-Elmer Model 240 elemental analyzer and are within ±0.4% of the theoretical values unless noted otherwise. All starting materials were commercially available and used as received unless so indicated.

4,6-Dichloro-7-[[(trifluoromethyl)sulfonyl]oxy]indan-1-one (18). 4,6-Dichloro-7-hydroxyindan-1-one[17] (21.71 g, 0.1 mol) was dissolved in DMF (80 mL) in a dry apparatus under nitrogen. Trifluoromethanesulfonyl chloride (21.60 g, 0.128 mol) was added with stirring, slowly, dropwise over a 20-min period with occasional cooling to keep the internal temperature below 30 °C. After the addition was complete, the reaction mixture was stirred at room temperature for 30 min and then poured into ice–water with swirling. The green crystals were collected, washed with water, sucked dry, and then dried in a vacuum oven at 50 °C to give 32.7 g of product. mp: 96–100 °C. Recrystallization from hexanes gave 22.4 g. mp: 96–98 °C. A sublimed sample had the following. mp: 90–96 °C. Anal. ($C_{10}H_5Cl_2F_3O_4S$): C, H.

4,6-Dichloro-7-iodoindan-1-one (19). 4,6-Dichloro-7-[[(trifluoromethyl)sulfonyl]oxy]indan-1-one (56.0 g, 0.160 mol), sodium iodide (133.1 g, 0.8 mol), and DMF (320 mL) in a dry apparatus were stirred under nitrogen at a bath temperature of 130 °C for 4 days, cooled to room temperature, and poured into 1 L of ice–water. The crystals were collected, washed with water, dried

(17) Hokema, T.; Traxler, J. T. U.S. 4,322,414, 1982.

overnight in a vacuum oven at 50 °C, and then sublimed at 170–190 °C at 0.05 mm to give 38.3 g of crude product, which was recrystallized from toluene to give 31.8 g of product. mp 170–172 °C; ^{1}H NMR: δ 2.7–3.2 (4 H, m), 7.6 (1 H, s). Anal. ($C_9H_5Cl_2IO$): C, H.

4,6-Dichloro-7-iodoindan-1-ol (20). 4,6-Dichloro-7-iodoindan-1-one (14.71 g, 45 mmoles) was suspended and partially dissolved in ethanol (140 mL). Sodium borohydride (1.70 g, 45 mmol) was added and the mixture was stirred for 50 min. Aqueous sodium hydroxide 20% (w/v) (40 mL) was added and stirred for 10 min. The reaction mixture was poured into 700 mL of ice–water with vigorous stirring. The crystals were collected, washed with water, sucked dry, and dried in a vacuum oven at 50 °C overnight to give 14.08 g of the title compound, mp 95–100 °C. Recrystallization from acetonitrile gave material with the following data. mp: 99–102 °C. ^{1}H NMR: δ 2.1–3.3 (4 H, m), 5.2 (1 H, m), 7.3 (1 H, s). Anal. ($C_9H_7Cl_2IO$): C, H.

4,6-Dichloro-7-iodo-1-indene and 4,6-Dichloro-7-iodo-2-indene (21 and 22). 4,6-Dichloro-7-iodo-1-indanol (13.98 g, 42.50 mmol) was dissolved in ether (350 mL) and the solution was stirred mechanically. Phosphorus pentoxide (6.03 g 42.50 mmol) was added and the sealed reaction mixture was stirred vigorously overnight. The addition of phosphorus pentoxide (6.03 g, 42.5 mmol) and stirring overnight was repeated three times. The ether containing the product was decanted, washed with aqueous $NaHCO_3$ solution, dried ($MgSO_4$), and filtered, and the solvent was evaporated to leave 10.76 g of a mixture of the title compounds. mp: 89–96 °C. Recrystallization from hexane gave material with the following data. mp: 95–97 °C. ^{1}H NMR: δ 3.5 (2 H, m), 6.5–6.9 (2 H, m), 7.25 (1 H, s). Anal. ($C_9H_5Cl_2I$): C, H.

1,1,3,5-Tetrachloro-1a,6a-dihydro-2-iodocycloprop[*a*]indene and 1,1,2,3-Tetrachloro-1a,6a-dihydro-5-iodocycloprop[*a*]indene (23 and 24). To a solution of a mixture of 4,6-dichloro-7-iodo-1-indene and 4,6-dichloro-7-iodo-2-indene (3.11 g, 10 mmol) and ethyl trichloroacetate (17.2 g, 12.5 mL, 90 mmol) in dry toluene (20 mL) cooled in an ice bath and stirred under nitrogen was added, in divided portions, fresh sodium methoxide (5.4 g, 100 mmol). After the addition was complete, the reaction was stirred for 2.5 h in an ice bath. When the reaction was complete, the mixture was diluted with ether and extracted with water. The ether layer was dried ($MgSO_4$) and filtered, and the solvent was evaporated in vacuo to leave 8.1 g of crude product. The product was triturated with hexanes and filtered, and the solvent was evaporated in vacuo from the hexane-soluble product. This crude product was chromatographed on silica gel (500 g) eluting with hexanes to give, after evaporation of the solvent, in vacuo, 1.4 g of the mixture of compounds as an oil. ^{1}H NMR: δ 2.2–2.55 (1 H, m), 3.15–3.6 (3 H, m), 7.2 (1 H, s).

2,4,7-Trichloro-1-iodonaphthalene and 2,4,6-Trichloro-1-iodonaphthalene (25). A mixture of 1,1,3,5-tetrachloro-1a,6a-dihydro-2-iodocycloprop[*a*]indene and 1,1,2,4-tetrachloro-1a,6a-dihydro-5-iodocycloprop[*a*]indene (4.54 g, 11.5 mmol) was refluxed in 10% (w/v) KOH in ethanol (100 mL) for 1.5 h and cooled and approximately 80% of the ethanol was evaporated in vacuo. The remainder was dissolved in ether and extracted with water, dried ($MgSO_4$), and filtered, and the solvent was evaporated in vacuo to leave 3.4 g of crude product, which was flash chromatographed on a silica gel column (60 × 150 mm) by elution with hexane to give, after evaporation of the solvent in vacuo, 2.85 g of the product mixture. mp: 45–50 °C. Ratio of the two naphthalenes is 4:5 or 5:4. ^{1}H NMR: δ 7.0–7.9 (4 H, m).

2,4,6-Trichloro-1-naphthoic Acid and 2,4,7-Trichloro-1-naphthoic Acid (Ratio 5:4 or 4:5) (26). The mixture of 2,4,7-trichloro-1-iodonaphthalene and 2,4,6-trichloro-1-iodonaphthalene (7.79 g, 21.8 mmol) was dissolved in dry ether (200 mL) and cooled under nitrogen to an internal temperature of −50 °C with stirring. Butyl lithium (17.7 mL of a 1.48 M solution in hexane, 26.2 mmol) was added dropwise over about 5 min. The reaction was stirred for 30 min at −78 °C. The −78 °C reaction mixture was poured onto powdered dry ice (excess) covered with ether. The excess CO_2 was allowed to evaporate and the ether warmed to room temperature. The ether was extracted with water once and four times with dilute aqueous $NaHCO_3$ solution. The combined aqueous extracts were acidified with concentrated HCl, the product was extracted with ether four times, dried ($MgSO_4$), and filtered, and the solvent was evaporated to leave 4.0 g of the product mixture. mp: 182–200 °C. Anal. ($C_{11}H_5Cl_3O_2$): C, H.

Methyl 2,4,6-Trichloro-1-naphthoate and Methyl 2,4,7-Trichloro-1-naphthoate (27): Preparation and Separation. The mixture of 2,4,6-trichloro-1-naphthoic acid and 2,4,7-trichloro-1-naphthoic acid (3.63 g, 13.2 mmol) was dissolved in ether and cooled to 5 °C. Diazomethane, in ether (generated from 3.40 g of *N*-nitroso-*N*-methylurea and base in 50 mL of ether at 5 °C), was added dropwise to maintain the internal temperature below 5 °C. An excess was noted by the persistence of a yellow color. The reaction mixture was stirred a few minutes and the excess diazomethane was blown off with nitrogen, and the solvent was evaporated in vacuo to leave 3.7 g of the product mixture.

The two isomers were separated by preperative HPLC (Waters 500) using 5% methylene chloride in hexane. The solvent from the first isomer to emerge from the column was evaporated in vacuo to leave 1.4 g of methyl 2,4,7-trichloro-1-naphthoate (27a).[18] mp: 113–115 °C. ^{1}H NMR: δ 4.09 (3 H, s), 7.25–8.25 (4 H, m). Anal. ($C_{12}H_7Cl_3O_2$): C, H.

The solvent containing the second isomer from the column was evaporated in vacuo to leave 1.1 g of methyl 2,4,6-trichloro-1-naphthoate (27b).[18] mp: 110–112 °C. ^{1}H NMR: δ 4.07 (3 H, s), 7.25–8.3 (4 H, m). Anal. ($C_{12}H_7Cl_3O_2$): C, H.

(2,4,7-Trichloronaphthalen-1-yl)methanol (28a). A solution of methyl 2,4,7-trichloro-1-naphthoate (1.3 g, 4.5 mmol) in ether (50 mL) was added dropwise (15 min) to a well-stirred suspension of lithium aluminum hydride (0.25 g, 6 mmol) in ether (25 mL). After stirring at room temperature for 17 h, the reaction mixture was treated with an additional 0.25 g of lithium aluminum hydride. The mixture was stirred for 3 h, cooled in an ice bath, and treated dropwise with 0.5 mL of water, 1.5 mL of 20% (w/v) of aqueous NaOH solution, and 0.5 mL of water. After filtration, the solid was extracted with ether. The combined ether solutions were dried ($MgSO_4$), filtered, and concentrated in vacuo to give 1.0 g of the product. mp: 107–112 °C. ^{1}H NMR: δ 5.23 (2 H, d), 7.55–8.28 (4 H, m).

1-(Chloromethyl)-2,4,7-trichloronaphthalene (29a). (2,4,7-Trichloronaphthalen-1-yl)methanol (1.0 g, 3.8 mmol) was added portionwise to thionyl chloride (10 mL) with cooling (ice bath). The reaction mixture was stirred at room temperature for 30 min at a reflux for 2 h and then concentrated to dryness in vacuo. The oily residue was taken up in methylene chloride and the solution was dried over $MgSO_4$. The solution was filtered and concentrated in vacuo to give 1.0 g of the product. ^{1}H NMR: δ 5.12 (2 H, s), 7.58–8.27 (4 H, m).

3-(2,4,7-Trichloronaphthalen-1-yl)propanal (31a). A solution of *n*-butyllithium in hexane (3.2 mL, 4.3 mmol) was added dropwise (3 min) to a solution of diisopropylamine (0.45 g, 4.5 mmol) in dry tetrahydrofuran (10 mL) with cooling (ice bath). After stirring under nitrogen for 15 min, ethylidenecyclohexylamine (0.55 g, 4.3 mmol) was added dropwise (5 min) at 0 °C. The mixture was stirred for 15 min and then the ice bath was replaced by a dry ice–acetone bath. A solution of 1-(chloromethyl)-2,4,7-trichloronaphthalene (1.0 g, 3.8 mmol) in tetrahydrofuran (15 mL) was added (5 min) at −75 °C. The reaction mixture was stirred at −70 °C for 30 min and at room temperature overnight (20 h) and then concentrated to dryness in vacuo. The residual oil was taken up in ether (100 mL) and 5% aqueous oxalic acid (100 mL) and the mixture was stirred at room temperature for 3.5 h. The layers were separated, and the aqueous phase was extracted (2×) with ether. The ether extracts were combined, washed with cold water and brine, and dried over $MgSO_4$. The solution was filtered and concentrated in vacuo to give a red-brown oil (1.1 g). This material was chromatographed with a 50-mm flash column containing 150 g of silica gel (230–400 mesh) eluting with 30% methylene chloride in hexane (v/v) to give 0.21 g of the product as a pale yellow solid. ^{1}H NMR: δ 2.81 (2 H, m),

(18) One of the compounds gave a 3% NOE of the proton in the 8-position when the methyl protons of the ester was irradiated. This compound was assigned structure 27a because it has no adjacent hydrogen for relaxation of the NOE. The other compound did not show an NOE. Further, when ester 27a is reduced to hydroxy methylene, the proton in the 8-position exhibits a 10% NOE when the methylene hydrogens are irradiated.

3.48 (2 H, m), 7.53–8.27 (4 H, m), 9.92 (1 H, b s).

3-(2,4,6-Trichloronaphthalen-1-yl)propanol (31b). With essentially the same chemistry with the other isomeric methyl 2,4,6-trichloro-1-naphthoate, there was obtained via essentially the same three steps the isomeric propanol (31b). ^{1}H NMR: δ 2.81 (2 H, t), 3.50 (2 H, t), 7.57 (1 H, dd), 7.63 (1 H, s), 7.93 (1 H, d), 8.28 (1 H, d), 9.91 (1 H, s). Anal. ($C_{13}H_9Cl_3O$): C, H.

3-(4,6-Dichloronaphthalen-1-yl)propanal (33). A small amount of a second product isolated by the chromatographic purification of 3-(2,4,6-trichloronaphthalen-1-yl)propanal was identified as 3-(4,6-dichloronaphthalen-1-yl)propanal by its ^{1}H NMR and by its conversion to 9. ^{1}H NMR: δ 2.78 (2 H, t), 3.34 (2 H, t), 7.14–8.2 (5 H, m), 9.5 (1 H, s). This reduction probably took place at the reduction of the ester methyl 2,4,6-trichloro-1-naphthoate via a six-membered intramolecular hydride transfer from an intermediate oxyaluminum hydride complex and was carried through the reaction sequence.

3-(2,4-Dimethylphenyl)propyl Benzoate (35). Benzoyl chloride (21.0 g, 0.15 mol) dissolved in dry pyridine (10 mL) was added slowly dropwise (15 min) to a well-stirred solution of 3-(2,4-dimethylphenyl)propanol (21.7 g, 0.132 mol) in dry pyridine (40 mL) with cooling in an ice-water bath. The reaction was then stirred overnight and then poured into ice–water (300 mL) and the excess pyridine was removed by azeotropic evaporation of solvent in vacuo. The remainder was partitioned between ether and water. The ether layer was washed successively with water, aqueous $NaHCO_3$, and brine, and then dried ($MgSO_4$) and filtered, and the solvent was evaporated in vacuo to leave 39 g of crude product, which was distilled in vacuo to give 35.1 g of pure product. bp1.5 mm:. 176–182 °C. Anal. ($C_{18}H_{20}O_2$): C, H.

4-[2,4-Dimethyl-5-[3-(benzoyloxy)propyl]phenyl]-4-oxo-2-methylbutyric Acid (37). Aluminum chloride (4.6 g, 34 mmol) was added in divided portions (5 min) to a well-stirred solution of 3-(2,4-dimethylphenyl)propyl benzoate (2.7 g, 10 mmol) and methylsuccinic anhydride (1.2 g, 10.5 mmol) in anhydrous nitroethane (15 mL), which was cooled in an ice-water bath. After the addition was complete, the ice bath was removed and the reaction stirred at ambient temperature for 2 h and then poured into ice–water (150 mL) containing 2 mL of concentrated HCl. The product was extracted (2×) with ether, and the combined ether extracts were washed with cold water and then brine, dried ($MgSO_4$), and filtered, and the solvent was evaporated in vacuo to leave 3.8 g of crude product, which is pure enough for the next step but may be purified with silica gel flash chromatography (60 × 150 mm), eluting with methylene chloride and then a mixture of acetic acid (0.5%), acetone (4.5%), and methylene chloride (95%). After evaporation of the fractions containing the product, there remained 3.1 g of product as an oil. Anal. ($C_{23}H_{26}O_5$): C, H.

Activated Zinc Dust. Zinc dust (24 g) was stirred with 2% aqueous HCl (150 mL) for 5 min, filtered by suction, and washed with water until the washings were neutral. The zinc was then washed successively with ethanol (75 mL), acetone (150 mL), and ether and then dried in a vacuum oven at 90 °C for 15 min and then used promptly in the following reaction.

4-[2,4-Dimethyl-5-[3-(benzoyloxy)propyl]phenyl]-2-methylbutyric Acid (38). Dry gaseous HCl was bubbled vigorously into a solution of 4-[2,4-dimethyl-5-[3-(benzoyloxy)-propyl]phenyl]-4-oxo-2-methylbutyric acid (8.0 g, 20 mmol) in dry ether (360 mL) for 15 min while being cooled in an ice-water cooling bath. Activated zinc dust was added in small portions with cooling in an ice-water bath so as to keep the internal temperature below 80 °C. After the addition, the reaction was cooled with an ice–water bath and stirred for 1 h. The reaction mixture was diluted with ether and then passed onto ice-water (350 mL) containing a little HCl (2 mL) and extracted with ether (2×). The combined ether extracts were washed with water and brine, dried ($MgSO_4$), and filtered, and the solvent was evaporated to leave 7.3 g of crude oily product, which was pure enough for the next step. A 0.2-g sample was purified by silica gel flash chromatography on a 20 × 150 mm Still column after eluting with methylene chloride and then with a mixture of 0.5% acetic acid, 4.5% acetone, and 95% methylene chloride. The fractions containing the product were combined, and the solvent was evaporated in vacuo to give 0.11 g of pure product as an oil. Anal. ($C_{23}H_{28}O_4$): C, H.

3-(5,6,7,8-Tetrahydro-2,4,7-trimethyl-8-oxonaphthalen-1-yl)propyl Benzoate (39). A solution of 4-[2,4-dimethyl-5-[3-(benzoyloxy)propyl]phenyl]-2-methylbutyric acid (7.4 g, 20 mmol) in methylene chloride (20 mL) was added dropwise in 10 min to oxalyl chloride (20 mL) with stirring and cooling in an ice-water bath. After the addition, the reaction was stirred at room temperature for 30 min and then warmed slowly to a bath temperature of 65 °C when the reaction refluxed. The refluxing was continued with stirring for 2 h. The reaction was then cooled, and the excess oxalyl chloride and solvent were evaporated in vacuo to leave 4-[2,4-dimethyl-5-[3-(benzoyloxy)propyl]phenyl]-2-methylbutyryl chloride as an oil which was dissolved in dry methylene chloride (20 mL) and cooled in an ice-water bath. To this was added a solution of stannic chloride (20 mL) in dry methylene chloride (20 mL) at a rapid drip (10 min). The reaction was stirred at room temperature for 30 min and poured into ice–water (300 mL), containing concentrated HCl (20 mL). The mixture was extracted with ether (3×). The combined ether extracts were washed successively with water twice, aqueous sodium bicarbonate, water, and brine, dried ($MgSO_4$), and filtered and the solvent was evaporated to leave 7.2 g of crude product, which was purified by silica gel flash chromatography using an 80 × 160 mm Still column eluting with methylene chloride for 35 × 125 mL fractions and then 2% acetone in methylene chloride for 20 × 125 mL fractions. The fractions containing the product were combined, and the solvent was evaporated in vacuo to leave 3.3 g of oil product. ^{1}H NMR: δ 1.22 (3 H, d), 1.82 (1 H, m), 2.00 (1 H, m) 2.1–2.3 (2 H, m), 2.23 (3 H, s), 2.34 (3 H, s), 2.66 (1 H, m), 2.80 (1 H, m), 2.90 (1 H, m), 3.04 (2 H, t), 4.47 (2 H, t), 7.14 (1 H, s), 7.45 (2 H, t), 7.56 (1 H, t), 8.10 (2 H, d). Anal. ($C_{23}H_{26}O_3$): C, H.

***cis*- and *trans*-3-(5,6,7,8-Tetrahydro-8-hydroxy-2,4,7-trimethylnaphthalenyl)propyl Benzoate (40).** Sodium borohydride (0.50 g, 13 mmol) was added in divided portions to a stirred solution of 3-(5,6,7,8-tetrahydro-2,4,7-trimethyl-8-oxonaphthalen-1-yl)propyl benzoate (2.65 g, 7.5 mmol) in ethanol (40 mL) and then stirred at room temperature for 7 h (reaction complete by TLC; 1% acetone in methylene chloride–silica gel). The clear reaction was poured into ice water, acidified with dilute HCl, and extracted with ether (3×). The combined ether extracts were washed successively with cold water and brine, dried ($MgSO_4$), filtered, and the solvent was evaporated in vacuo to leave 2.7 g of the product.

3-(5,6-Dihydro-2,4,7-trimethylnaphthalen-1-yl)propyl Benzoate (41). *cis*- and *trans*-3-(5,6,7,8-tetrahydro-8-hydroxy-2,4,7-trimethylnaphthalen-1-yl)propyl benzoate (2.7 g, 7.7 mmol) were dissolved in dry ether (200 mL), to this was added powdered phosphorus pentoxide (5 g), and the sealed reaction mixture was stirred overnight. The addition of phosphorus pentoxide and stirring overnight was repeated once. When TLC (1% acetone in methylene chloride/silica gel) showed the reaction to be complete. The ether was decanted and the residue was washed with ether by decantation. The phosphorus residue was treated with ice–water and extracted with ether. The combined ether decantations and washings were washed successively with water, aqueous sodium bicarbonate, and brine, dried ($MgSO_4$), and filtered, and the solvent was evaporated to leave 2.8 g of crude product. This product was purified by flash chromatography on a 50 × 160 mm Still column eluting with 50% hexane in methylene chloride. The fractions containing the product were combined, and the solvent was evaporated in vacuo to give 1.6 g of oil product. ^{1}H NMR: δ 1.66–2.2 (2 H, m), 1.9 (3 H, s), 2.2 (3 H, s), 2.25 (3 H, s), 2.6–3.0 (6 H, m), 4.2 (2 H, s), 6.45 (1 H, s), 6.8 (1 (H, s), 7.2–7.6 (3 H, m), 7.9–8.1 (2 H, m).

3-(2,4,7-Trimethylnaphthalen-1-yl)propyl Benzoate (42). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ); 0.95 g, 42 mmol) was added to a solution of 3-(5,6-dihydro-2,4,7-trimethylnaphthalen-1-yl)propyl benzoate (1.25 g, 37 mmol) in toluene (60 mL) and stirred at room temperature for 1 h. The reaction mixture was filtered and the solvent was evaporated in vacuo to leave crude product. This product was purified by flash chromatography on a 50 × 150 mm Still silica column eluting with 50% hexane in methylene chloride. The fractions containing the product were combined, and the solvent was evaporated in vacuo to leave 0.78 g of oil product. ^{1}H NMR: δ 2.12 (2 H, m), 2.47 (3 H, s), 2.49 (3 H, s), 2.62 (3 H, s), 3.22 (2 H, t), 4.52 (2 H, 5), 7.19

(1 H, s), 7.29 (1 H, d), 7.48 (2 H, t), 7.59 (1 H, t), 7.83 (1 H, s), 7.87 (1 H, d), 8.12 (2 H, d). Anal. ($C_{23}H_{24}O_2$): C, H.

3-(2,4,7-Trimethylnaphthalen-1-yl)propanol (43). A solution of 3-(2,4,7-trimethylnaphthalen-1-yl)propyl benzoate (0.75 g, 2.3 mmol) was added to a solution of potassium hydroxide (0.5 g, 7 mmol) in ethanol (50 mL) and stirred at room temperature for 4 h. Most of the ethanol was evaporated in vacuo and the residue was partitioned between ether and water. The ether was washed with water twice, dried ($MgSO_4$), and filtered, and the solvent was evaporated in vacuo to leave 0.53 g of product.

3-(2,4,7-Trimethylnaphthalen-1-yl)propanol (44). 3-(2,4,7-Trimethylnaphthalen-1-yl)propanol (0.68 g, 3 mmol) was added to a suspension of pyridinium chlorochromate (1.28 g, 6 mmol) in methylene chloride (20 mL). The reaction mixture was stirred at room temperature for 2 h and then diluted with ether (10 mL) and the solvent was decanted. The black solids were washed with ether by decantation twice. The combined organic extracts were filtered through a pad of Florisil, and the solvent was evaporated in acid to leave 0.53 g of product. Recrystallization from petroleum ether gave a white crystalline solid. mp: 79–83 °C. ^{1}H NMR: δ 2.44 (3 H, s), 2.54 (3 H, s), 2.62 (3 H, s), 2.77 (2 H, t), 3.36 (2 H, t), 7.09 (1 H, s), 7.31 (1 H, d), 7.69 (1 H, s), 7.88 (1 H, d), 9.93 (1 H, t). Anal. ($C_{16}H_{18}O$): C, H.

3-(5,6,7,8-Tetrahydro-2,4-dimethyl-8-oxonaphthalen-1-yl)propyl Benzoate. Following the experimental method of the 2,4,7-trimethyl analogue but substituting succinic anhydride for 3-methyl succinic anhydride, there was obtained in succession the following.

4-[2,4-Dimethyl-5-[3-(benzoyloxy)propyl]phenyl]-4-oxobutyric acid (45) as an oil (3.68 g, 78%). Anal. ($C_{22}H_{25}O_5$): C, H.

4-[2,4-Dimethyl-5-[3-(benzoyloxy)propyl]phenyl]butyric Acid (46).

3-(5,6,7,8-Tetrahydro-2,4-dimethyl-8-oxonaphthalen-1-yl)propyl Benzoate (47). TLC: R_f = 0.33, 1% acetone/CH_2Cl_2. ^{1}H NMR: δ 1.75–2.42 (4 H, m), 2.42 (3 H, s), 2.50 (3 H, s), 2.50–2.95 (4 H, m), 2.95–3.32 (2 H, m), 4.72 (2 H, t), 7.15 (1 H, s), 7.3–7.55 (3 H, m), 7.92–8.15 (2 H, m).

7-Chloro-2,4-dimethyl-1-(3-hydroxypropyl)naphthalene. 1-[3-(Benzoyloxy)propyl]-7-chloro-2,4-dimethylnaphthalene (48) (2.60 g, 7.37 mmol) was suspended in ethanol (30 mL) and potassium hydroxide (1.65 g, 29.5 mmol) added and stirred at room temperature for 2 h then at 60–65 °C bath temperature for 1 h. The reaction mixture was cooled in an ice bath, filtered from sodium benzoate, and washed thoroughly with ethanol. The combined filtrates were dissolved in ether and extracted with water. The water was extracted with ether three times. The combined ether extracts were washed with water three times and then with brine, dried ($MgSO_4$) and filtered, and the solvent was evaporated to leave 1.80 g (98%) of the product. A sublimed sample [100 °C bath temp (0.1 mm)] had mp 104–105 °C. Exact mass calcd for $C_{15}H_{17}ClO$: 248.0968. Found: 248.0968. ^{1}H NMR: δ 1.60–2.20 (4 H, m), 2.44 (3 H, s), 2.56 (3 H, s), 3.05 (2 H, q), 3.74 (2 H, t), 7.0–7.95 (4 H, m).

3-(7-Chloro-2,4-dimethylnaphthalen-1-yl)propanal (49). Pyridinium chlorochromate (3.12 g, 14.47 mmol) and powdered 3-Å molecular sieves (3.6 g) were suspended in methylene chloride (25 mL), and 7-chloro-2,4-dimethyl-1-(3-hydroxypropyl)-naphthalene (1.70 g, 6.83 mmol) dissolved in methylene chloride (25 mL) was added all at once and stirred for 2 h. The reaction mixture was worked up by diluting with ether (50 mL) and filtering through a silica gel pad. The pad was washed with ether and the solvent was evaporated in vacuo to give 1.24 g (73%) of product. Exact mass calcd for $C_{15}H_{15}ClO$: 246.0811. found: 246.0813. ^{1}H NMR: δ 2.3–2.95 (2 H, m), 2.40 (3 H, s), 2.55 (3 H, s), 3.25 (2 H, t), 7.02–8.0 (4 H, m). TLC: R_f = 0.36 (50% CH_2Cl_2–hexane/silica gel).

Ethyl 3-(4-Chloro-2-methylphenyl)propionate (51h). Boron trifluoride etherate (1.5 mL, 0.012 mol) was added dropwise to a solution of 3-(4-chloro-2-methylphenyl)propionic acid (1.99 g, 0.01 mol) in absolute ethanol (14 mL). The reaction mixture was heated at reflux for 6.5 h, cooled, and concentrated in vacuo to remove the solvent, and the residual oil was taken up in ether. The ether solution was washed with aqueous Na_2CO_3 and cold water, dried, and evaporated to give an orange oil, which was distilled at about 1.5 mm to give the product as an oil (1.5 g, 66%). bp: 126–131 °C. Anal. ($C_{12}H_{15}ClO_2$): C, H.

4-[2′-Chloro-4′-methyl-5′-[2-(ethoxycarbonyl)ethyl]-phenyl]-4-oxobutyric Acid (52b). Aluminum chloride (5.87 g, 0.044 mol) was added portionwise (5 min) to a mixture of succinic anhydride (1.1 g, 0.011 mol) and ethyl 3-(4-chloro-2-methylphenyl)propionate (2.27 g, 0.01 mol) in CH_2Cl_2 (20 mL) with cooling (ice bath). The reaction mixture was stirred at room temperature for 24 h, poured into ice and 10 mL of concentrated HCl, and extracted with ether. The ether solution was dried and evaporated to give a yellow brown oil, which was purified by flash column chromatography (silica gel and 2% HOAc–10% acetone–90% CH_2Cl_2) to give the product as a yellow oil (3.0 g, 92% yield). Anal. ($C_{16}H_{19}ClO_5$): C, H.

4-[2′-Chloro-4′-methyl-5′-[2-(ethoxycarbonyl)ethyl]-phenyl]butyric Acid (53b). Gaseous HCl was bubbled into a well-stirred solution of 4-[2′-chloro-4′-methyl-5′-[(ethoxycarbonyl)ethyl]phenyl]-4-oxobutyric acid (3.27 g, 0.01 mol) in acetic anhydride (60 mL) for 20 min with cooling (ice–acetone bath). Activated zinc dust (13.11 g 6.2 mol) was added portionwise (15 min) to keep the temperature below 0 °C. The reaction mixture was stirred at about 0 °C for 7 h, filtered (glass wool) into ice and water and extracted with ether. The ether solution was dried and evaporated to give a brown oil, which was purified by flash column chromatography (silica gel and 0.5% HOAc–4.5% acetone–95% CH_2Cl_2) to yield the product as a viscous yellow oil (2.17 g, 69%).

Ethyl 3-(4-Chloro-2-methyl-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl)propanoate (54b). Oxalyl chloride (23.5 mL) was added dropwise to a well-stirred solution of 4-[2′-chloro-4′-methyl-5′-[(ethoxycarbonyl)ethyl]phenyl]butyric acid (10.38 g, 0.033 mol) in toluene (50 mL). The reaction mixture was stirred at room temperature for 18 h, heated at reflux for 4 h, cooled, and concentrated to dryness, and the residual oil was taken up in CH_2Cl_2 (50 mL). After addition of stannic chloride (31.5 mL) with cooling (ice bath), the reaction mixture was stirred at room temperature for 5 days and then poured into ice and concentrated HCl (20 mL) and extracted with ether. The ether solution was dried and evaporated to give a viscous brown oil, which after silica gel chromatography eluting with 15% ethyl acetate in hexane gave the product as a gum. Exact mass calcd for $C_{16}H_{19}ClO_3$: 284.1021. Found: 294.1019. ^{1}H NMR: δ 1.27 (3 H, t, CH_2CH_3), 2.10 (2 H, p, CH_2), 2.34 (3 H, s, $ArCH_3$), 2.57 (2 H, t, CH_2), 2.65 (2 H, t, CH_2), 2.99 (2 H, t, CH_2), 3.21 (2 H, t, CH_2), 4.16 (2 H, q, CH_2CH_3), 7.36 (1 H, s, Ar).

3-(4,7-Dichloro-2-methylnaphthalen-1-yl)propanal (56b). Ethyl 3-(4,7-dichloro-2-methylnaphthalen-1-yl)propanoate (1.583 g, 5.087 mmol) was dissolved in dry toluene (25 mL) under nitrogen with syringe cap attached to flask. The solution was cooled to −78 °C in dry ice–acetone bath and diisobutylaluminum hydride (3.62 mL of a 1.5 M solution in toluene, 5.443 mmol) was added dropwise slowly by syringe. Stirring was continued at −78 °C for 1 h. Then while still at −78 °C, the reaction was poured quickly into an aqueous NH_4Cl solution with stirring. This mixture was extracted two times with ether. The combined ether extracts were extracted successively with NH_4Cl solution, water, and brine, and then dried ($MgSO_4$), and filtered, and the solvent was evaporated to leave a solid. This solid was triturated with a little ether in hexane to give 0.701 g of pure solid product (mp: 104–106 °C). The solvent was stripped from the mother liquor to give 0.681 g of impure product. This impure product was flash chromatographed on a 20 × 200 mm silical column eluting with 70% CH_2Cl_2 in hexane to give 0.45 g of pure solid product. mp: 103–105 °C. Combining the two samples of pure solid product gave 1.15 g of pure product (mp: 104–106 °C) after drying. ^{1}H NMR: δ 2.47 (3 H, s, CH_3), 2.76 (2 H, t, CH_2), 3.28 (2 H, t, CH_2), 7.42 (1 H, s, Ar), 7.49 (1 H, dd, Ar), 7.90 (1 H, d, Ar), 8.22 (1 H, d, Ar), 9.92 (1 H, s, *CHO*). Anal. ($C_{14}H_{12}Cl_2O$) C, H.

With the above experimental procedures but substituting 3-(2-chloro-4-methylphenyl)propionic acid for 3-(4-chloro-2-methylphenyl)propionic acid there was obtained in succession the following.

Ethyl 3-(2-Chloro-4-methylphenyl)propanoate (51a). Bp: 104–107 °C. Anal. ($C_{12}H_{15}ClO_2$): C, H.

4-[4-Chloro-2-methyl-5-[2-(ethoxycarbonyl)ethyl]-phenyl]-4-oxobutyric Acid (52a). Mp: 72–74 °C. Anal. ($C_{16}H_{19}ClO_5$): C, H.

4-[4-Chloro-2-methyl-5-[2-(ethoxycarbonyl)ethyl]-phenyl]butyric Acid (53a). Mp: 50–52 °C. Anal. ($C_{16}H_{21}ClO_4$): C, H.

Ethyl 3-(2-Chloro-4-methyl-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl) (54a). Mp: 63–65 °C. Anal. ($C_{16}H_{19}ClO_3$): C, H.

3-(2,7-Dichloro-4-methylnaphthalen-1-yl)propanal (56a). mp: 103–105 °C. ^1H NMR: δ 2.66 (3 H, s), 2.82 (2 H, t), 3.49 (2 H, t), 7.34 (1 H, s), 7.52 (1 H, d), 7.95 (2 H, m), 9.95 (1 H, s). Anal. ($C_{14}H_{12}Cl_2O$): C, H.

Registry No. 2, 124243-86-3; 2·Na, 124244-18-4; 3, 124243-87-4; 3·Na, 124244-19-5; 4, 124243-88-5; 4·Na, 124244-20-8; 5, 124243-89-6; 5·Na, 124244-21-9; 6, 124243-90-9; 6·Na, 124244-22-0; 7, 124243-91-0; 7·Na, 124244-23-1; 8, 124243-92-1; 8·Na, 124244-24-2; 9, 124243-93-2; 9·Na, 124244-25-3; 10, 124243-94-3; 10·Na, 124244-26-4; 11, 124243-95-4; 11·Na, 124244-27-5; 12, 108579-26-6; 12·Na, 124244-28-6; 13, 108579-36-8; 13·Na, 124244-29-7; 14, 124243-96-5; 15, 124243-97-6; 16, 124243-98-7; 17, 81945-11-1; 18, 108578-92-3; 19, 108578-93-4; 20, 10578-94-5; 21, 108578-95-6; 22, 108578-96-7; 23, 108578-97-8; 24, 108578-98-9; 25a, 108578-99-0; 25b, 108579-00-6; 26a, 108579-02-8; 26b, 108579-01-7; 27a, 108579-04-0; 27b, 108579-03-9; 28a, 108579-05-1; 28b, 124244-13-9; 29a, 108579-06-2; 29b, 124244-14-0; 31a, 108579-07-3; 31b, 108579-11-9; 33, 124243-99-8; 34, 27650-80-2; 35, 124244-00-4; 37, 124244-01-5; 38, 124244-02-6; 39, 124244-03-7; *cis*-40, 124244-04-8; *trans*-40, 124244-17-3; 41, 124244-05-9; 42, 124244-06-0; 43, 124244-07-1; 44, 124244-08-2; 45, 124266-46-2; 46, 124244-09-3; 47, 124244-10-6; 48, 124244-11-7; 49, 124244-12-8; 50a, 615-65-6; 50b, 95-69-2; 51a, 108579-27-7; 51b, 108579-13-1; 52a, 108579-28-8; 52h, 108579-14-2; 53a, 108579-29-9; 53b, 108579-15-3; 54a, 108579-30-2; 54b, 108579-16-4; 55a, 108579-34-6; 55b, 108579-22-2; 56a, 108579-35-7; 56b, 108579-23-3; Cl_3CCO_2Et, 515-84-4; $H_2^-CCOC^-HCO_2Me$, 30568-00-4; 3-hydroxy-3-methylglutaryl-coenzyme A, 1553-55-5; *N*-ethylidenecyclohexylamine, 1193-93-7; methylsuccinic anhydride, 4100-80-5; succinic anhydride, 108-30-5; 7-chloro-2,4-dimethyl-1-(3-hydroxypropyl)naphthalene, 124244-15-1; 3-(4-chloro-2-methylphenyl)propionic acid, 879-75-4; 3-(2-chloro-4-methylphenyl)propionic acid, 124244-16-2.

Lipophilic 1,3-Xylyl-21-crown-6 Macrocyclic Polyether 2-Carboxylic Acids as Biological Mimics of the Ionophore Antibiotics

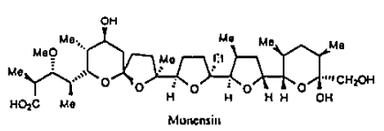
Frank J. Urban,* Larry R. Chappel, Arthur E. Girard, Banavara L. Mylari, and Ian J. Pimblett[†]

Pfizer Central Research, Eastern Point Road, Groton, Connecticut 06340, and Ramsgate Road, Sandwich, Kent CT9 13NJ, United Kingdom. *Received June 15, 1989*

Twelve lipophilic 1,3-xylyl-21-crown-6 macrocyclic polyether 2-carboxylic acids (9a–9l), two lariat ether 1,3-xylyl-21-crown-6 macrocyclic polyether 2-carboxylic acids (21 and 22), and two 1,3-xylyl-28-crown-8 macrocyclic polyether 2-carboxylic acids (10a and 10b) were synthesized and tested for in vitro antibacterial activity, in vitro stimulation of rumen propionic acid production, and in vivo anticoccidial activity in chickens. These are biological screens relevant to animal health areas where the ionophore antibiotics such as monensin have found application. While the parent structure 1 without lipophilic substituents was biologically inactive, the lipophilic macrocycles were active in the two in vitro tests but not against chicken coccidiosis. One compound (9f) was tested in cattle and was found to increase levels of propionic acid in the rumen fermentation. This effect is considered an important factor for increasing the efficiency of feed utilization in cattle exhibited by the ionophore antibiotic monensin. The alkali ion salts of these lipophilic macrocyclic polyether carboxylic acids are very soluble in organic solvents and insoluble in water. These compounds are proposed to act as ion-transport agents and functional mimics of the ionophore antibiotics in the biological systems described above.

The ionophore antibiotics with their fascinating array of complex structures have provided a continuing challenge to organic chemists.[1] These compounds exhibit unique activity in many biological systems via a mechanism of action which is deceptively simple: the exchange of alkali ions for protons across biological membranes.[2] Synthetic molecules which try to mimic the physical properties of the natural antibiotics have been described,[3] but only marginal success was achieved in demonstrating biological activity and no in vivo activity in either animal health area where the ionophores have made a major impact, coccidiosis control in chickens or cattle performance enhancement, has been reported. In this paper, we describe our efforts in the synthesis of polyether mimics of natural ionophores with in vivo activity in cattle and in vitro antibacterial activity.

In 1967, monensin was the first polyether antibiotic to have its structure[4] and potent biological activities,[5] such as inhibition of alkali metal cation transport in mitochondria and broad-spectrum anticoccidial activity, disclosed. It was approved for commercial use as a poultry anticoccidial in 1971 and as a cattle performance enhancer in 1975. The structure of the silver salt of monensin,[4] which is typical for the entire class, has a lipophilic exterior and a hydrophilic central cavity lined with oxygen atoms which serve as ligands for encapsulated alkali ions; the molecule as a whole is therefore neutral and lipophilic. When the carboxylate is protonated, at an interface, either biological or in solvent, the complexation of the ion, while still possible in dry, organic solvents, is weaker by several orders of magnitude[6] and the alkali ion is readily given up to the acidic aqueous layer. It is this large difference in complexation constant for alkali ions between the carboxylic acid and the carboxylate forms of the ionophore



Monensin

[†]Sandwich, Kent, United Kingdom.

(1) Westley, J. W. Ed. *Polyether Antibiotics: Naturally Occurring Acid Ionophores, Vol. 2: Chemistry*; Marcek Dekker, Inc.; New York, 1982. Evans, D. A.; Bender, S. L.; Morris, J. *J. Am. Chem. Soc.* 1988, *110*, 2506 and reference therein.
(2) Reed, P. W. In *Polyether Antibiotics: Naturally Occurring Acid Ionophores*; Westley, J. W. Ed.; Marcel Dekker, Inc.: New York, 1982; Vol. 1, Chapter 5.
(3) (a) Gardner, J. O.; Beard, C. C. *J. Med. Chem.* 1978, *21*, 357. (b) Brown, G. R.; Foubister, A. J. *J. Med. Chem.* 1979, *22*, 997. (c) Brown, G. R.; Foubister, A. J. *J. Med. Chem.* 1983, *26*, 590.
(4) Agtarap, A.; Chamberlin, J. W.; Pinkerton, M.; Stienrauf, L. *J. Am. Chem. Soc.* 1967, *89*, 5737.
(5) Shumard, R. F.; Callender, M. E.; *Antimicrob. Agents Chemother.* 1967, 369.
(6) Hoogerheide, J. G.; Popov, A. I. *J. Solution Chem.* 1979, *8*, 83.

0022-2623/90/1833-0765$02.50/0

The solution was washed with saturated $NaHCO_3$ and evaporated to dryness. The residue was purified by chromatography on silica gel ($CHCl_3$–MeOH) to give the title compound (387 mg, 24%) after crystallization from petroleum ether: ^1H NMR ($CDCl_3$) δ 0.06 (s, 6 H, Me_2Si), 0.89 (s, 9 H, Me_3C), 3.66, 3.77 (A_2B_2, 4 H, $SiOCH_2CH_2O$), 5.25 (s, 2 H, NCH_2O), 5.27 [dd, J = 10.9, 1.1 Hz, 1 H, CH=C*H*(*Z*)H(*E*)], 5.98 [dd, J = 17.6, 1.1 Hz, 1 H, CH=CH(*Z*)*H*(*E*)], 6.42 (dd, J = 17.6, 10.9 Hz, 1 H, C*H*=CH_2), 7.41 (s, 1 H, 6-H), 9.59 (br, 1 H, NH).

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-6-(phenylthio)-5-vinyluracil. Following the general procedure for the preparation of 17–19, the title compound was prepared from the above compound with diphenyl disulfide as an electrophile: yield 46%; ^1H NMR ($CDCl_3$) δ 0.01 (s, 6 H, Me_2Si), 0.84 (s, 9 H, Me_3C), 3.63 (s, 4 H, $SiOCH_2CH_2O$), 5.33 [dd, J = 11.8, 2.0 Hz, 1 H, CH=C*H*(*Z*)H(*E*)], 5.61 (s, 2 H, NCH_2O), 6.33 [dd, J = 16.8, 2.0 Hz, 1 H, CH=CH(*Z*)*H*(*E*)], 6.71 (dd, J = 16.8, 11.8 Hz, 1 H, C*H*=CH_2), 7.15–7.30 (m, 5 H, SPh), 10.15 (br, 1 H, NH).

Following method A, **55** was prepared from the above compound.

1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)-5-vinyluracil (55): yield 41%; mp 100–103 °C (EtOAc–petroleum ether); UV (MeOH) λ_{max} 306 (ϵ 7600), 243 nm (ϵ 14 000); MS m/z 320 (M^+); ^1H NMR (Me_2SO-d_6) δ 3.35–3.52 (m, 4 H, $HOCH_2CH_2O$), 4.62 (t, J = 5.4 Hz, 1 H, OH), 5.22 [dd, J = 11.3, 2.2 Hz, 1 H, CH=C*H*(*Z*)H(*E*)], 5.48 (s, 2 H, NCH_2O), 6.21 [dd, J = 16.4, 2.2 Hz, 1 H, CH=CH(*Z*)*H*(*E*)], 6.63 (dd, J = 16.4, 11.3 Hz, 1 H, C*H*=CH_2), 7.23–7.40 (m, 5 H, SPh), 11.75 (br, 1 H, NH). Anal. ($C_{15}H_{16}N_2O_4S \cdot {}^1/_2H_2O$) C, H, N.

Antiviral Assay Procedures. The anti-HIV assays were based on the inhibition of the virus-induced cytopathic effect in MT-4 cells as previously described.[32] Briefly, MT-4 cells were suspended in culture medium at 2.5×10^5 cells/mL and infected with 1000 $CCID_{50}$ (50% cell culture infective dose) of HIV. Immediately after virus infection, 100 μL of the cell suspension was brought into each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After a 4 (Table II) or 5 (Table I) day incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[33] Cytotoxicity of the compounds was assessed in parallel with their antiviral activity. It was based on the viability of mock-infected host cells as determined by the MTT method.[33]

(32) Pauwels, R.; De Clercq, E.; Desmyter, J.; Balzarini, J.; Goubau, P.; Herdewijn, P.; Vanderhaeghe, H.; Vandeputte, M. *J. Virol. Methods* 1987, *16*, 171–185.
(33) Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* 1988, *20*, 309–322.

Inhibitors of Cholesterol Biosynthesis. 3. Tetrahydro-4-hydroxy-6-[2-(1*H*-pyrrol-1-yl)ethyl]-2*H*-pyran-2-one Inhibitors of HMG-CoA Reductase. 2. Effects of Introducing Substituents at Positions Three and Four of the Pyrrole Nucleus

B. D. Roth,*[†] C. J. Blankley,[†] A. W. Chucholowski,[†] E. Ferguson,[‡] M. L. Hoefle,[†] D. F. Ortwine,[†] R. S. Newton,[‡] C. S. Sekerke,[‡] D. R. Sliskovic,[†] C. D. Stratton,[†] and M. W. Wilson[†]

Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105.
Received June 26, 1990

A series of *trans*-tetrahydro-4-hydroxy-6-[2-(2,3,4,5-substituted-1*H*-pyrrol-1-yl)ethyl]-2*H*-pyran-2-ones and their dihydroxy acids were prepared and tested for their ability to inhibit the enzyme HMG-CoA reductase in vitro. Inhibitory potency was found to increase substantially when substituents were introduced into positions three and four of the pyrrole ring. A systematic exploration of structure–activity relationships at these two positions led to the identification of a compound ((+)-33, (+)-(4*R*)-trans-2-(4-fluorophenyl)-5-(1-methylethyl)-*N*,3-diphenyl-1-[(tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl)ethyl]-1*H*-pyrrole-4-carboxamide) with five times the inhibitory potency of the fungal metabolite compactin.

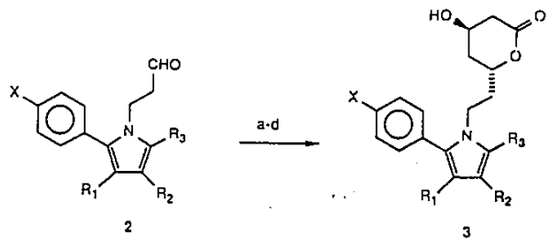
Inhibition of HMG-CoA reductase (HMGR), the rate-limiting enzyme in cholesterol biosynthesis, has proven to be an effective means for lowering total and low-density lipoprotein (LDL) cholesterol in animal models and man.[1,2] The early reports describing the activity of the fungal metabolites compactin (mevastatin)[3] and mevinolin (lovastatin)[4] have been followed by a host of publications describing a large variety of natural[5] and synthetic inhibitors.[6] Previously, we disclosed a series of 1,2,5-trisubstituted-pyrrol-1-ylethylmevalonolactones which were found to be moderately potent inhibitors of HMGR in vitro.[7] By systematically altering the 2 and 5 substituents, maximal potency was obtained with the 2-(4-fluorophenyl)-5-isopropyl analogue (1). On the basis of those results, a molecular-modeling analysis led to the description of a pharmacophore model which characterized the size of the substituents at positions 2 and 5 and the conformation of the side chain. We have now discovered

[†] Department of Chemistry.
[‡] Department of Pharmacology.

(1) Roth, B. D.; Sliskovic, D. R.; Trivedi, B. K. *Ann. Rep. Med. Chem.* 1989, *24*, 147–56.
(2) Grundy, S. M. *N. Engl. J. Med.* 1988, *319*, 24–32.
(3) (a) Endo, A.; Kuroda, M.; Tsujita, Y. J. *Antibiotics* 1976, 1346–8. (b) Endo, A.; Kuroda, Y.; Tanzawa, K. *FEBS Lett.* 1976, *72*, 323–6. (c) Brown, A. G.; Smale, T. C.; King, T. J.; Hasenkamp, R.; Thompson, R. H. *J. Chem. Soc., Perkin Trans. 1* 1976, 1165–9.
(4) (a) Endo, A. *J. Antibiot.* 1979, *32*, 852. (b) Alberts, A.; Chen, J.; Kuron, G.; Hunt, V.; Huff, J.; Hoffman, C.; Rothrock, J.; Lopez, M.; Joshua, H.; Harris, E.; Patchett, A.; Monaghan, R.; Currie, S.; Stapley, E.; Albers-Schonberg, G.; Hensens, O.; Hirshfield, J.; Hoogsteen, K.; Liesch, J.; Springer, J. *Proc. Natl. Acad. Sci. U.S.A.* 1980, *77*, 3957–61.
(5) Tsujita, Y.; Kuroda, M.; Shimada, Y.; Tanzawa, K.; Arai, M.; Kaneko, I.; Tanaka, M.; Masuda, H.; Tarumi, C.; Watanabe, Y.; Fujii, S. *Biochim. Biophys. Acta.* 1986, *877*, 50–60.

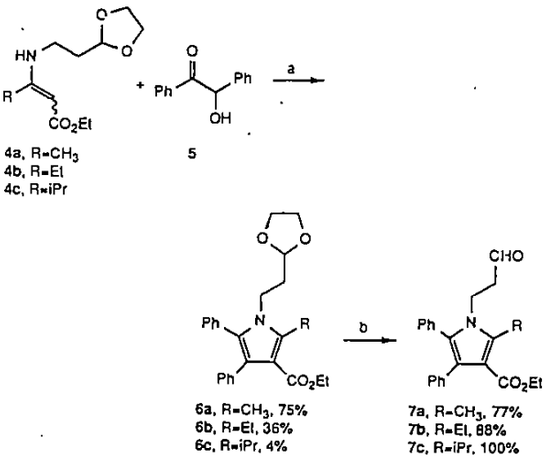
0022-2623/91/1834-0357$02.50/0

Scheme I[a]



[a] (a) $\bar{C}H_2C\bar{O}\bar{C}HCO_2ET$, THF, −78 °C; (b) *n*-$Bu_3B$/$NaBH_4$, −78 °C; (c) H_2O_2, NaOH; (d) toluene, reflux.

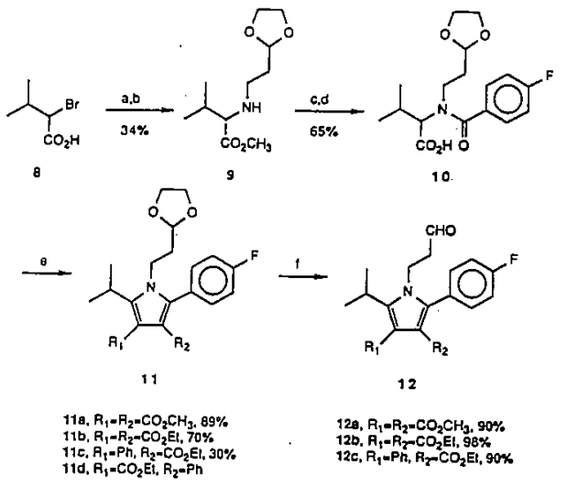
Scheme II. Method A[a]

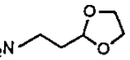


[a] (a) $ZnCl_2$, EtOH, reflux; (b) *p*-TSA, acetone–water, reflux.

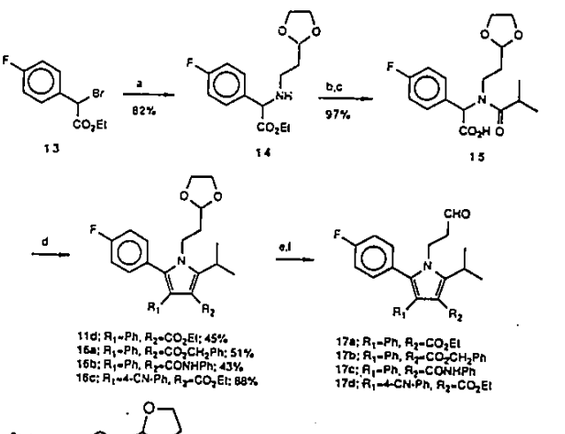
that the introduction of substituents into the 3 and 4 positions of the pyrrole ring results in significant improvements in potency at inhibiting HMGR in vitro. The results of these studies are described in this report.

Scheme III. Method B[a]

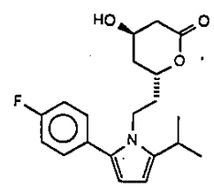


[a] (a) CH_3OH,DCC, DMAP; (b) H_2N  Et_3N, CH_3CN, reflux; (c) 4-F-Ph-COCl, Et_3N; (d) NaOH; (e) R_1—≡—R_2, Ac_2O, 90 °C; (f) *p*-TSA, acetone–water, reflux.

Scheme IV. Method C[a]



[a] (a) H_2N Et_3N, CH_3CN, 25 °C; (b) $(CH_3)_2CHOCl$, Et_3N, CH_2Cl_2, 0 °C; (c) NaOH; (d) Ac_2O, R_1—≡—R_2, 90 °C; (e) HCl, EtOH, reflux; (f) *p*-TSA, acetone–water, reflux.



i

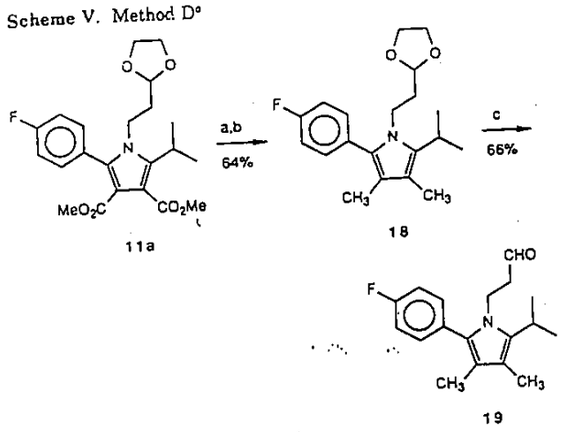
Chemistry

The general synthetic strategy employed was identical with that employed previously.[7] Thus, the pyrrole-3-propionaldehydes 2 were converted to the racemic, trans

(6) (a) Lee, T.-J.; Holtz, W. J.; Smith, R. L. *J. Org. Chem.* 1982, *47*, 4750–7. (b) Stokker, G. E.; Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J.; Deana, A. A.; Gilfillan, J. L.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* 1985, *28*, 347–58. (c) Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J.; Deana, A. A.; Evans, B. E.; Gilfillan, J. L.; Gould, N. P.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Rittle, K. E.; Smith, R. L.; Stokker, G. E.; Willard, A. K. *J. Med. Chem.* 1986, *29*, 159–69. (d) Stokker, G. E.; Alberts, A. W.; Anderson, P. S.; Cragoe, E. J.; Deana, A. A.; Gilfillan, J. L.; Hirshfield, J.; Holtz, W. J.; Hoffman, W. F.; Huff, J. W.; Lee, T. J.; Novello, F. C.; Prugh, J. D.; Rooney, C. S.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* 1986, *29*, 170–81. (e) Hoffman, W. F.; Alberts, A. W.; Anderson, P. S.; Chen, J. S.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* 1986, *29*, 849–52. (f) Prugh, J. D.; Alberts, A. W.; Deana, A. A.; Gilfillan, J. L.; Huff, J. W.; Smith, R. L.; Wiggins, J. M. *J. Med. Chem.* 1990, *33*, 758–65. (g) Balasubramanian, N.; Brown, P. J.; Catt, J. D.; Han, W. T.; Parker, R. A.; Sit, S. Y.; Wright, J. J. *J. Med. Chem.* 1989, *32*, 2038–41. (h) Bartmann, W.; Beck, G.; Granzer, E.; Jendralla, H.; Kerekjarto, B. v.; Wess, G. *Tetrahedron Lett.* 1986, 4709–12. (i) Sliskovic, D. R.; Roth, B. D.; Wilson, M. W.; Hoefle, M. L.; Newton, R. S. *J. Med. Chem.* 1990, *33*, 31–8. (j) Beck, G.; Kesseler, K.; Baader, E.; Bartmann, W.; Beck, G.; Bergmann, A.; Granzer, E.; Jendralla, H.; Kerekjarto, B. v.; Kesseler, K.; Krause, R.; Paulus, E.; Schubert, W.; Wess, G. *J. Med. Chem.* 1990, *33*, 52–60. (k) Baader, E.; Bartmann, W.; Beck, G.; Bergmann, A.; Granzer, E.; Jendralla, H.; Kerekjarto, B. v.; Kesseler, K.; Krause, R.; Schubert, W.; Wess, G. *J. Med. Chem.* 1990, *33*, 61–70.

(7) Roth, B. D.; Ortwine, D. F.; Hoefle, M. L.; Stratton, C. D.; Sliskovic, D. R.; Wilson, M. W.; Newton, R. S. *J. Med. Chem.* 1990, *33*, 21–31.

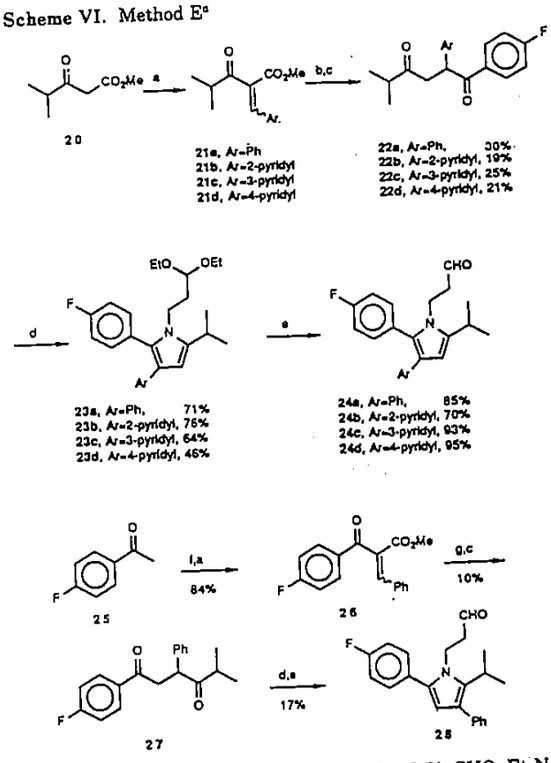
Scheme V. Method D[a]



[a] (a) $LiAlH_4$, ether–dichloromethane, reflux; (b) Et_3SiH, TFA–CH_2Cl_2, 0 °C; (c) *p*-TSA, acetone–water.

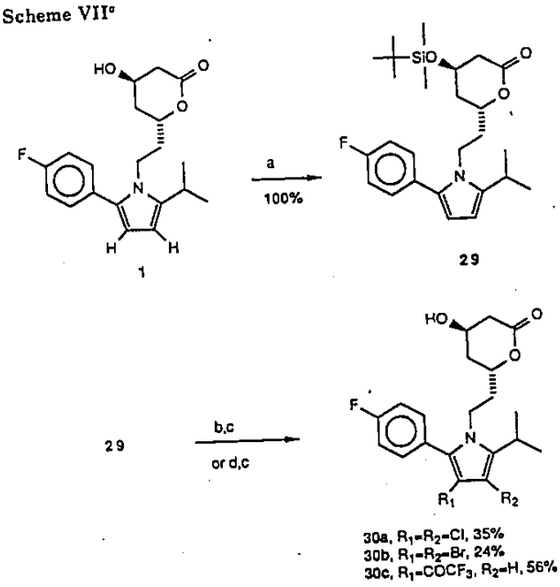
lactone stereoisomers **3** by (1) Weiler dianion condensation with ethyl acetoacetate, (2) stereoselective reduction to the syn-1,3-diol with tributylborane and sodium borohydride, (3) base hydrolysis, and (4) lactonization by refluxing in toluene with azeotropic removal of water (Scheme I). The requisite propionaldehydes **2** were prepared by several different synthetic routes. The less sterically hindered pentasubstituted pyrrole-3-propionaldehydes (**7a**, R = CH_3; **7b**, R = Et, Scheme II) could be prepared by $ZnCl_2$-catalyzed condensation of enamines **4a** and **4b** (prepared from 2-(2-aminoethyl)-1,3-dioxolane[8] and the requisite β-keto ester) with benzoin **5** (method A).[9] This reaction proved ineffective for the more sterically hindered pyrrole **7c**, containing the preferred 5-isopropyl substituent. The 5-isopropylpyrroles could be prepared in good yields, however, by the regioselective [3 + 2] cycloaddition of acetylenes with the amido acids **10** or **15** (Schemes III and IV).[10] Thus, reaction of ethyl phenylpropiolate with amido acid **10** in hot acetic anhydride afforded a 4:1 mixture of **11c** and **11d** (Scheme III, method B) from which **11c** crystallized in 30% yield. The reaction of **15** under identical conditions was regiospecific, producing **11d** as the sole product (Scheme IV, method C). The regiochemistry of compounds **11c** and **11d** were determined by comparison of their proton NMRs with that of the closely related **6c** ($(CH_3)_2CH$, occurs at δ 3.50 ppm in both **6c** and **11d**, but at δ 3.00 ppm in **11c**). As expected, the yield in this cycloaddition reaction was improved when more electron-deficient acetylenes were employed (compare **11a**, **11b**, and **16c** vs **11d** Scheme IV). The 3,4-dimethylpyrrole analogue **19** was prepared by reduction of diester **11a** to the corresponding diol with lithium aluminum hydride, followed by deoxygenation with triethylsilane and trifluoroacetic acid (Scheme V, method D).[11] The regioisomeric 3- and 4-arylpyrrole-3-propionaldehyde isomers **24a–d** and **28** were prepared by a Stetter reaction[12] of the appropriate aldehydes with the complementary α-benzylidene-β-keto esters (4-fluorobenzaldehyde with **21** and isobutyraldehyde with **26**, Scheme VI), followed by Paal–Knorr cyclization[13] with 3,3-diethoxy-1-amino-

Scheme VI. Method E[a]



[a] (a) ArCHO, *p*-TSA, toluene, reflux; (b) 4-F-Ph-CHO, Et_3N, 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride; (c) NaOH, CH_3OH, 25 °C; (d) $H_2NCH_2CH_2CH(OEt)_2$, *p*-TSA, toluene, reflux; (e) H_3O^+, (f) NaH, $(CH_3O)_2CO$; (g) $(CH_3)_2CHCHO$, Et_3N, 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride.

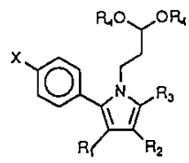
Scheme VII[a]



[a] (a) *t*-$BuMe_2SiCl$, imidazole, DMF, 25 °C, 18 h; (b) 2 equiv *N*-halosuccinimide, DMF, 0 °C; (c) *n*-Bu_4NF, HOAc, THF, 25 °C; (d) $(CF_3CO)_2O$, DMF, 0 °C.

(8) Crumbie, R. L.; Nimitz, J. S.; Mosher, H. S. *J. Org. Chem.* 1982, *47*, 4040–5.
(9) McKinnon, D. M. *Can. J. Chem.* 1965, *43*, 2628–31.
(10) (a) An extensive review of mesoionic heterocycles has appeared: Newton, C. G.; Ramsden, C. A. *Tetrahedron* 1982, *38*, 2965–3011. (b) An analogous synthesis can be found: Anderson, W. K.; Halat, M. J. *J. Med. Chem.* 1979, *22*, 977–80.
(11) Doyle, M. P.; DeBruyn, D. J.; Kooistra, D. A. *J. Am. Chem. Soc.* 1972, *94*, 3659–61.
(12) Stetter, H. *Ang. Chem., Int. Ed. Engl.* 1976, *15*, 639–47.

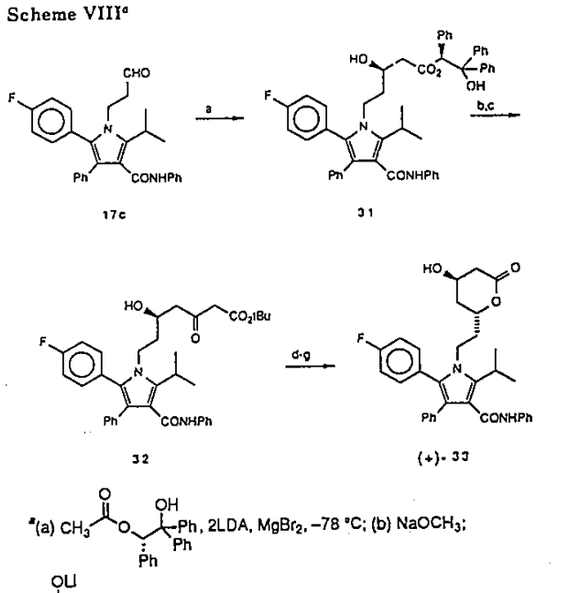
Table I



no.	X	R_1	R_2	R_3	R_4	% yield (method)	mp,[a,b] °C
6a	H	Ph	CO_2Et	CH_3	$-CH_2CH_2-$	75 (A)	oil[c]
6b	H	Ph	CO_2Et	Et	$-CH_2CH_2-$	36 (A)	oil[c]
6c	H	Ph	CO_2Et	*i*-Pr	$-CH_2CH_2-$	4 (A)	oil[c]
11a	F	CO_2CH_3	CO_2CH_3	*i*-Pr	$-CH_2CH_2-$	65 (B)	143–6
11b	F	CO_2Et	CO_2Et	*i*-Pr	$-CH_2CH_2-$	70 (B)	oil[c]
11c	F	CO_2Et	Ph	*i*-Pr	$-CH_2CH_2-$	30 (B)	146–8
16a	F	Ph	CO_2Et	*i*-Pr	$-CH_2CH_2-$	45 (C)	158–9
16b	F	Ph	CO_2CH_2Ph	*i*-Pr	$-CH_2CH_2-$	51 (C)	oil[c]
16c	F	Ph	CONHPh	*i*-Pr	$-CH_2CH_2-$	43 (C)	161–3
16d	F	4-CNPh	CO_2Et	*i*-Pr	$-CH_2CH_2-$	88 (C)	oil[c]
18	F	CH_3	CH_3	*i*-Pr	$-CH_2CH_2-$	64 (D)	oil[c]
23a	F	Ph	H	*i*-Pr	Et	71 (E)	84–7
23b	F	2-pyridyl	H	*i*-Pr	Et	76 (E)	84–6
23c	F	3-pyridyl	H	*i*-Pr	Et	64 (E)	96–8
23d	F	4-pyridyl	H	*i*-Pr	Et	46 (E)	123–5

[a] All compounds possess ^1H NMR spectra consistent with assigned structure. [b] Combustion analyses within ±0.4% of theoretical unless otherwise noted. [c] This compound was purified, but not analyzed before use in the next step.

Scheme VIII[a]

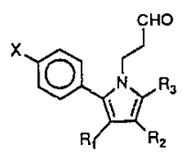


[a] (a) CH_3, 2LDA, $MgBr_2$, −78 °C; (b) $NaOCH_3$; (c) OLi, $OtBu$ (d) Et_3B, $NaBH_4$; (e) H_2O_2, CH_3OH; (f) NaOH; (g) $PhCH_3$, reflux.

propane[14] and deprotection (Scheme VI, method E). Finally, the 3,4-dichloro, 3,4-dibromo, and 3-trifluoroacetyl analogues (30a–c) were prepared from 1 by protection of the 4′-hydroxyl as the *tert*-butyldimethylsilyl ether, followed by electrophilic substitution on the pyrrole ring[15] and deprotection with *n*-Bu_4NF buffered with acetic acid (Scheme VII). The assignment of the regiochemistry of 30c was made in a manner analogous to 11c and 11d.

Chiral lactone (+)-33 was prepared by application of the asymmetric aldol procedure developed by Braun (Scheme VIII).[16] Thus, reaction of aldehyde 17c with the magnesium enolate of (*S*)-(+)-2-acetoxy-1,1,2-triphenylethanol afforded alcohol 31 in 60% yield and 97% ee. Transesterification ($NaOCH_3$, CH_3OH) followed by Claisen condensation with excess lithio *tert*-butylacetate produced δ-hydroxy-β-keto ester 32 in 75% yield. After reduction with Et_3B and $NaBH_4$, base hydrolysis, and lactonization, (+)-33 was isolated as a 98:2 mixture of stereoisomers. Fortuitously, the *d,l* pair selectively crystallized from ethyl acetate–hexanes and pure (+)-33 ($[\alpha]^{23}_D$ = +24.53°, 0.53% in $CHCl_3$) could then be isolated from the mother liquors as a foamy solid.[17]

Table II

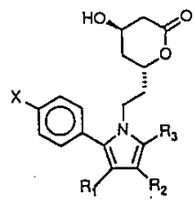


no.	X	R_1	R_2	R_3	% yield	mp,[a,b] °C
7a	H	Ph	CO_2Et	CH_3	77	100–1
7b	H	Ph	CO_2Et	Et	88	oil[c]
7c	H	Ph	CO_2Et	*i*-Pr	100	oil[c]
12a	F	CO_2CH_3	CO_2CH_3	*i*-Pr	90	oil[c]
12b	F	CO_2Et	CO_2Et	*i*-Pr	95	oil[c]
12c	F	CO_2Et	Ph	*i*-Pr	90	oil[c]
17a	F	Ph	CO_2Et	*i*-Pr	81	127–8
17b	F	Ph	CO_2CH_2Ph	*i*-Pr	60	oil[c]
17c	F	Ph	CONHPh	*i*-Pr	86	164–5
17d	F	4-CNPh	CO_2Et	*i*-Pr	75	oil[c]
19	F	CH_3	CH_3	*i*-Pr	66	oil[c]
24a	F	Ph	H	*i*-Pr	85	oil[c]
24b	F	2-pyridyl	H	*i*-Pr	70	120–2
24c	F	3-pyridyl	H	*i*-Pr	93	oil[c]
24d	F	4-pyridyl	H	*i*-Pr	95	oil[c]
28	F	H	Ph	*i*-Pr	90	oil[c]

[a] All compounds possessed ^1H NMR and IR spectra consistent with assigned structure. [b] Combustion analyes within ±0.4% of theoretical unless otherwise noted. [c] This compound was purified by chromatography, but not analyzed before use in the next step.

(13) Broadbent, H. S.; Burnham, W. S.; Olsen, R. K.; Sheeley, R. M. *J. Heterocycl. Chem.* 1968, *5*, 757–67.
(14) Suzuki, E.; Inone, S.; Goto, T. *Chem. Pharm. Bull.* 1968, *16*, 933–8.
(15) Aiello, E.; Dattolo, G.; Cirrincione, G.; Almerico, A. M.; D'Asdia, I. *J. Heterocycl. Chem.* 1982, *19*, 977–9.
(16) Braun, M.; Devant, R. *Tetrahedron Lett.* 1984, 5031–4.

Table III



no.	X	R_1	R_2	R_3	mp, °C	formula[a]	IC_{50},[b] μM	relative potency[c]
1	F	H	H	*i*-Pr	105–6	$C_{20}H_{24}FNO_3$	0.23	10.9
3a	H	Ph	CO_2Et	CH_3	oil	$C_{27}H_{29}NO_5$	4.0	0.6
3b	H	Ph	CO_2Et	Et	65–8	$C_{28}H_{31}NO_5$	0.89	6.3
3c	H	Ph	CO_2Et	*i*-Pr	157–9	$C_{29}H_{33}NO_5$	0.17	23.5
3d	F	CO_2CH_3	CO_2CH_3	*i*-Pr	169–170	$C_{24}H_{28}FNO_7$	0.180	14.3
3e	F	CO_2Et	CO_2Et	*i*-Pr	121–3	$C_{26}H_{32}FNO_7$	0.35	2.8
3f	F	CO_2Et	Ph	*i*-Pr	158–9	$C_{29}H_{32}FNO_5$	0.050	100
3g	F	Ph	CO_2Et	*i*-Pr	159–160	$C_{29}H_{32}FNO_5$	0.20	35.5
3h	F	Ph	CO_2CH_2Ph	*i*-Pr	174–5	$C_{34}H_{34}FNO_5$	0.040	24.0
(±)-3i	F	Ph	CONHPh	*i*-Pr	104–110	$C_{33}H_{33}FN_2O_4$	0.025	81.4
3j	F	4-CN-Ph	CO_2Et	*i*-Pr	oil	$C_{30}H_{31}FN_2O_5$	0.280	16.2
3k	F	CH_3	CH_3	*i*-Pr	oil	$C_{24}H_{28}FNO_3$	0.140	16.0
3l	F	Ph	H	*i*-Pr	oil	$C_{26}H_{28}FNO_3$	0.347	12.5
3m	F	2-pyridyl	H	*i*-Pr	186–7	$C_{25}H_{27}FN_2O_3$	0.046	76
3n	F	3-pyridyl	H	*i*-Pr	70–4	$C_{25}H_{27}FN_2O_3$	0.071	9.4
3o	F	4-pyridyl	H	*i*-Pr	174–6	$C_{25}H_{27}FN_2O_3$	0.310	2.1
3p	F	H	Ph	*i*-Pr	135–6	$C_{26}H_{28}FNO_3$	0.120	36.3
30a	F	Cl	Cl	*i*-Pr	129–131	$C_{20}H_{22}Cl_2FNO_3$	0.028	78.6
30b	F	Br	Br	*i*-Pr	141.2	$C_{20}H_{22}Br_2FNO_3$	0.028	78.6
30c	F	$COCF_3$	H	*i*-Pr	oil	$C_{22}H_{23}F_4NO_4$	0.800	8.8
(+)-33	F	Ph	CONHPh	*i*-Pr	foam	$C_{33}H_{33}FN_2O_4$	0.007	500
(−)-33	F	Ph	CONHPh	*i*-Pr	foam	$C_{33}H_{33}FN_2O_4$	0.440	13.9
		compactin					0.030	100

[a] Analytical results are within ±0.4% of theoretical values except where otherwise noted. [b] CoA reductase inhibition (COR) screen; a measure of the direct conversion of D,L-[^{14}C]HMG-CoA to mevalonic acid. Assays of each inhibitor were performed at four concentrations in triplicate. The precision for compactin was 37%. See ref 7 for experimental details. [c] Calculated as follows: (IC_{50} of compactin/IC_{50} of test compound determined simultaneously) × 100. Compactin arbitrarily assigned a value of 100.

Alternatively, relatively pure (+)- and (−)-33 could be obtained by preparation of the corresponding diastereomeric (*R*)-α-methylbenzylamides, separation by preparative HPLC, hydrolysis, and relactonization.[6b] This process afforded 94.6% pure (+)-33 ($[\alpha]^{23}_D$ = +25.5°, 0.51% in $CHCl_3$) and 97.8% pure (−)-33 ($[\alpha]^{23}_D$ = −24.8°, 0.51% in $CHCl_3$).

Biological Results and Discussion

The compounds listed in Table III were all hydrolyzed to the corresponding dihydroxy acid sodium salts and evaluated for their ability to inhibit a partially purified preparation of rat liver HMG-CoA reductase.[3] Two conclusions were readily apparent. The first was the confirmation of the 5-isopropyl as the preferred substituent (compare 3c with 3a and 3b). The second was the significant increase in in vitro potency found with the introduction of certain lipophilic electron-withdrawing groups into the 3 and 4 positions of the pyrrole ring (e.g., Cl or Br, compare 1 with 30a and 30b), such that, these compounds displayed potency equivalent to compactin. This effect did not hold for the esters or ketones (CO_2Me, CO_2Et, $COCF_3$, compounds 3d, 3e, 30c), except when combined with a phenyl (compounds 3f, 3h, and 3i). There also appeared to be a positional effect, since the 3-carbethoxy-4-phenyl analogue (3f) was 4 times more potent than the 3-phenyl-4-carbethoxy analogue (3g). In vitro activity for the 3-phenyl analogues were improved significantly by increasing the size of the 4-substituent (compare 3h, 3i, and 3g with 3l). Potency was also increased when the 3-phenyl was replaced with a 3-(2-pyridyl) moiety (compound 3m). The 3-(3- and 4-pyridyl) isomers (3n and 3o) were equipotent to phenyl (3l). Introduction of the electron-withdrawing cyano group into the 4-position of the 3-phenyl (3j) led to a slight reduction in potency. Finally, as others have reported, in the case of 3i essentially all of the biological activity was contained in the dextrorotatory stereoisomer ((+)-33 vs 3i).[6b] We speculate that the activity found in (−)-33 (97.8% pure) is derived from the 2% contamination with (+)-33.

An attempt was made to confirm these observations with a quantitative structure–reactivity relationship (QSAR) analysis. In the early stages of the development of the series, there was an indication that size, as parameterized by MR of the combined 3- and 4-substituents, as well as electronic-withdrawing character might be possible contributors to activity and this preliminary analysis partially guided further synthesis. Synthetic constraints precluded the preparation of an optimally designed set, however, and the set of compounds described in this paper did not ultimately support the derivation of a significant Hansch equation including these parameters. Furthermore, available parameters for electronic and lipophilic effects of these highly hindered functional groups are likely to be seriously inaccurate. Nevertheless, the trends observed from plots and single parameter correlations supported the observation that a size benefit exists, but derives mainly from the 4-substituent, as opposed to the 3-substituent. Polar functionality can be tolerated in this region, although there is a suggestion that lipophilicity may ultimately play

(17) A similar sequence was employed by Lynch et al.: Lynch, J. E.; Volante, R. P.; Wattley, R. V.; Shinkai, I. *Tetrahedron Lett.* 1987, 1385–8.

the dominant role among the simple parameterized effects, since $Pi_{3,4}$ has one of the best single parameter correlations with activity ($r = 0.46$). Clearly, other factors not readily parameterized have equal or larger influence on relative activity in this series. The activity of polar-substituted analogues is enhanced when the polar group is "insulated" from the enzyme as in **3m** vs **3n** and **3o**. Similarly, the better activity of **3f** over **3g** may derive from the better shielding of the polar ester group in the former compound by the flanking phenyl groups as opposed to a phenyl and isopropyl group in the latter. The activity of the halogenated analogues **30a** and **30b** is better accommodated by a lipophilicity effect, rather that a size or dispersion effect reflected in MR. Other QSAR analyses of synthetic HMG-CoA reductase inhibitors have reached similar conclusions about structural variations in this region of related molecules.[18,19]

In conclusion, although it is still most critical in this type to have the optimal substituents flanking the dihydroxyglutarate side chain, i.e., 4-fluorophenyl and isopropyl,[7] this work shows that further modulation and improvement in potency at inhibiting HMG-CoA reductase may be obtained with a variety of additional substituents capable of interacting with an apparently fairly spacious hydrophobic region distal from the side-chain location. The importance of this interaction is further supported by the potent inhibition evidenced by other inhibitors which possess substituents in this region.[1] Preparation of the optically pure *R,R*-isomer ((+)-**33**) of the most potent compound in this series (**3i**) resulted in a compound which was 5 times more potent than the fungal metabolite compactin in vitro. Further in vivo studies with (+)-**33** will be described in subsequent papers from this laboratory.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. THF was distilled from sodium and benzophenone. All organic extracts were dried over $MgSO_4$ except when otherwise noted. Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. Infrared spectra were determined on a Nicolet MX-1 FT-IR spectrophotometer. NMR spectra were determined on either a Varian EM-390 spectrometer, or a Varian XL-200 or Bruker 250 MHz instrument. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. Elemental analyses for carbon, hydrogen, and nitrogen were determined on a Perkin-Elmer Model 240C elemental analyzer and are within 0.4% of theory unless noted otherwise. Optical rotations were determined with use of a Perkin-Elmer 241 polarimeter. Routine HPLC analyses were performed with use of a Varian 5500 unit equipped with a Reodyne 7126 loop injector, a Dupont variable wavelength detector, and an octadecylsilane (Alltech C18 600RP, CH_3CN-H_2O eluant, 60:40, v/v) or silica gel column (Beckman Altex Ultrasphere 5 μm) interfaced to Varian 402 data system for computation of peak areas. Chiral HPLC analyses were performed with use of a Chiracel of 10-μm column (Diacel Chem. Ind., LTD).

Method A. Ethyl 3-[2-(1,3-Dioxolan-2-yl)ethyl]amino-2-pentenoate (4b). A solution of methyl propionylacetate (12.55 mL, 100 mmol), 2-(2-aminoethyl)-1,3-dioxolane[8] (12.3 g, 105 mmol) and one drop of glacial acetic acid was stirred and heated in refluxing toluene (200 mL) for 2 h with azeotropic removal of water. The cooled solution was concentrated to provide 24 g of pure 4b, which was used without further purification.

Ethyl 2-Ethyl-1-[2-(1,3-dioxolan-2-yl)ethyl]-4,5-diphenyl-1*H*-pyrrole-3-carboxylate (6b). A mixture of benzoin (4.25 g, 20 mmol), 4b (5.44 g, 22 mmol), and $ZnCl_2$ (6 g, 44 mmol) in 50 mL of absolute ethanol was stirred and heated at reflux for 48 h. The cooled solution was diluted with ether (500 mL), washed with water (50 mL), 2 *M* HCl (2 × 50 mL), saturated aqueous bicarbonate (50 mL), and brine (50 mL), and dried. Flash chromatography (silica gel, 10:1 v/v hexane–ethyl acetate) provided 3 g (36%) of 6b: 90-MHz NMR ($CDCl_3$) δ 0.98 (t, 3 H, J = 7 Hz), 1.34 (t, 3 H, J = 7 Hz), 1.85 (m, 2 H), 3.08 (q, 2 H, J = 7 Hz), 3.7–4.1 (m, 8 H), 4.60 (t, 1 H, J = 4 Hz), 7.1 (s, 5 H), 7.22 (s, 5 H) ppm.

Ethyl 2-Ethyl-1-[1-(3-oxopropyl)]-4,5-diphenyl-1*H*-pyrrole-3-carboxylate (7b). A solution of 6b (2.4 g, 5.7 mmol) in 100 mL of absolute ethanol containing 1 drop of concentrated HCl was stirred and heated at reflux for 24 h. The cooled solution was concentrated and dissolved in 125 mL of 4:1 acetone–water, and 1 g of *p*-TSA–H_2O was added. The resulting solution was stirred and heated at reflux for 24 h. The cooled solution was concentrated and partitioned between ether and water. The ether layer was then washed with saturated aqueous bicarbonate and brine and dried. Filtration and concentration afforded 1.9 g of 7b (88%): 90-MHz NMR ($CDCl_3$) δ 1.0 (t, 3 H, J = 7 Hz), 1.28 (t, 3 H, J = 7 Hz), 2.58 (m, 2 H), 3.10 (q 2 H, J = 7 Hz), 4.05 (q, 2 H, J = 7 Hz), 4.2 (m, 2 H), 7.05 (s, 5 H), 7.1–7.4 (m, 5 H), 9.50 (s, 1 H) ppm.

Ethyl 3-[[2-(1,3-Dioxolan-2-yl)ethyl]amino]-4-methyl-2-pentanoate (4c). A solution of ethyl isobutyrylacetate (6 g, 42 mmol) and 2-(2-aminoethyl)-1,3-dioxolane (5.4 g, 46.7 mmol) in toluene (50 mL) containing 2 drops of glacial acetic acid was stirred and heated at reflux with azeotropic removal of water for 2 h. Concentration provided crude 4c which was used without further purification.

Ethyl 1-[2-(1,3-Dioxolan-2-yl)ethyl]-2-(1-methylethyl)-4,5-diphenyl-1*H*-pyrrole-3-carboxylate (6c). A mixture of 4c (17 g, 80 mmol), benzoin acetate (75 mmol, 19 g), and $ZnCl_2$ (20 g, 147 mmol) in 100 mL of ethanol was stirred and heated at reflux for 2 days. The mixture was cooled to room temperature, poured into ether (1 L), washed with water (200 mL), 2 M HCl (100 mL), H_2O (100 mL), and brine, and dried. Flash chromatography (silica gel, 10:1 v/v hexane–ethyl acetate) provided 1.2 g of 6c: 90-MHz NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz), 1.45 (d, 6 H, J = 7 Hz), 1.90 (m, 2 H), 3.45 (septet, 1 H, J = 7 Hz), 3.8–4.1 (m, 8 H), 4.60 (t, 1 H, J = 4 Hz), 7.0 (s, 5 H), 7.0–7.3 (m, 5 H) ppm.

Ethyl 1-(3-Oxopropyl)-5-(1-methylethyl)-4,5-diphenyl-1*H*-pyrrole-3-carboxylate (7c). A solution of 6c (1.3 g, 3 mmol) and *p*-TSA–H_2O (0.6 g, 3 mmol) in 50 mL of 4:1 acetone–water was stirred and heated at reflux overnight. The cooled mixture was poured into ether (200 mL), washed with saturated aqueous bicarbonate (2 × 50 mL), water (50 mL), and brine (50 mL), and dried. Filtration and concentration provided 1.0 g (100%) of pure 7c which was used without further purification: 90-MHz NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz), 1.40 (d, 6 H, J = 7 Hz), 2.55 (m, 2 H), 3.44 (septet, 1 H, J = 7 Hz), 3.95 (q, 2 H, J = 7 Hz), 4.15 (m, 2 H), 7.0 (s, 5 H), 7–7.3 (m, 5 H), 9.43 (s, 1 H) ppm.

Method B. *N*-[2-(1,3-Dioxolan-2-yl)ethyl]-DL-valine, Methyl Ester (9). A solution of the methyl 2-bromo-3-methylbutyrate (4.6 g, 23.6 mmol), 2-(2-aminoethyl)-1,3-dioxolane (2.9 g, 25 mmol), and triethylamine (3.5 mL, 25 mmol) in 25 mL of acetonitrile was stirred and heated at reflux for 20 h. The cooled solution was poured into ether (500 mL) and extracted with 2 M HCl (2 × 50 mL). The aqueous layer was made alkaline with 25% aqueous NaOH and extracted with ethyl acetate (2 × 100 mL). The combined ethyl acetate extracts were washed with brine and dried. Filtration and concentration provided 3 g (55%) of 9 as a yellow oil: 90-MHz NMR ($CDCl_3$) δ 0.93 (d, J = 7 Hz, 6H), 1.70 (br s, 1 H, 4NH), 1.86 (m, 2 H), 2.60 (m, 3 H), 2.94 (d, J = 6 Hz, 1 H), 3.68 (s, 3 H), 3.85 (m, 4 H), 4.89 (t, J = 4 Hz, 1 H) ppm.

***N*-[2-(1,3-Dioxolan-2-yl)ethyl]-*N*-(4-fluorobenzoyl)-DL-valine (10).** To a stirred solution of 9 (3 g, 13 mmol) and triethylamine (3.6 mL, 26 mmol) in 20 mL of CH_2Cl_2, cooled to 0 °C, was added a solution of 4-fluorobenzoyl chloride (1.65 mL, 14 mmol) in 10 mL of CH_2Cl_2. The solution was stirred 50 min at 0 °C and 60 min at room temperature. It was then poured into ether (200 mL), washed with water (2 × 50 mL), saturated aqueous bicarbonate (50 mL), and brine (50 mL), and dried. Flash chromatography (silica gel, 1:1 v/v hexane–ethyl acetate) provided 3 g (65%) of crude (±)-methyl *N*-(4-fluorobenzoyl)-*N*-[2-(2-ethyl)-1,3-dioxolanyl]valine: 90-MHz NMR ($CDCl_3$) δ 0.90, (br

(18) Aggarwal, D.; Saha, R. N.; Gupta, J. K.; Gupta, S. P. *J. Pharmacobio-Dyn.* **1988**, *11*, 591.
(19) Prabhakar, Y. S.; Saxena, A. K.; Doss, M. J. *Drug Des. Deliv.* **1989**, *4*, 97.

d, J = 7 Hz, 6 H), 1.8–2.5 (m, 3 H), 3.45 (br dd, J = 6, 8 Hz, 1 H), 3.72 (s, 3 H), 3.80 (m, 6 H), 4.80 (m, 1 H), 6.9–7.5 (m, 4 H) ppm.

A solution of this methyl ester (1 g, 2.83 mmol) and NaOH (0.4 g, 10 mmol) in 10 mL of 4:1 methanol–water was stirred and heated at reflux for 3 h. The cooled solution was diluted with water and extracted with ether. The aqueous layer was acidified with 6 M HCl and extracted with ethyl acetate (2×). The combined ethyl acetate extracts were washed with brine and dried. Filtration and concentration provided 0.96 g (2.8 mmol) of 10 as a gum: 90-MHz NMR ($CDCl_3$) δ 0.85 (m, 6 H), 1.8 (m, 2 H), 2.5 (m, 1 H), 3.3–3.9 (m, 7 H), 4.6 (m, 1 H), 6.8–7.4 (m, 4 H) ppm.

Dimethyl 1-[2-(1,3-Dioxolan-2-yl)ethyl]-2-(4-fluorophenyl)-5-(1-methylethyl)-1*H*-pyrrole-3,4-dicarboxylate (11a). Dimethyl acetylenedicarboxylate (1.3 mL, 10.6 mmol) was added to a solution of 10 (1.8 g, 5.28 mmol) in 10 mL of acetic anhydride at room temperature. Carbon dioxide evolution began immediately. The solution was stirred a further 2 h, concentrated to remove excess dimethyl acetylenedicarboxylate and solvent, and then filtered through silica gel. This provided 2 g (89%) of 11a as a colorless solid. Recrystallization from isopropyl ether–hexane afforded colorless crystals: mp 143–146 °C; IR (KBr) 1719, 1449, 1241, 1209, 1178, 945 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.35 (d, J = 7 Hz, 6 H), 1.80 (m, 2 H), 3.18 (septet, J = 7 Hz, 1 H), 3.56 (s, 3 H), 3.7 to 4.0 (m, 6 H), 3.83 (s, 3 H), 4.64 (t, J = 4 Hz, 1 H), 7–7.3 (m, 4 H) ppm. Anal. C, H, N.

Dimethyl 2-(4-Fluorophenyl)-5-(1-methylethyl)-1-(3-oxopropyl)-1*H*-pyrrole-3,4-dicarboxylate (12a). A solution of 11a (0.5 g, 1.18 mmol) and *p*-TSA–H_2O (0.23 g, 1.2 mmol) in 12 mL of 5:1 acetone–water was stirred and heated at reflux for 48 h. The cooled solution was concentrated, diluted with ether (200 mL), washed with saturated aqueous bicarbonate (2 × 50 mL) and brine (50 mL), and dried. Flash chromatography on silica gel (4:1 v/v hexane–ethyl acetate) provided 0.4 g (90%) of pure 12a: 90-MHz NMR ($CDCl_3$) δ 1.35 (d, J = 7 Hz, 6 H), 2.61 (t, J = 7 Hz, 2 H), 3.18 (septet, J = 7 Hz, 1 H), 3.53 (s, 3 H), 3.81 (s, 3 H), 4.03 (t, J = 7 Hz, 2 H), 6.9–7.3 (m, 4 H), 9.45 (s, 1 H) ppm.

Ethyl 1-[2-(1,3-Dioxolan-2-yl)ethyl]-2-(4-fluorophenyl)-5-(1-methylethyl)-4-phenyl-1*H*-pyrrole-3-carboxylate (11c). A mixture of 10 (3.0 g, 8.8 mmol), acetic anhydride (15 mL), and ethyl phenylpropiolate (3.0 g, 17.6 mmol) was stirred at 110 °C for 5 h. The solution was then cooled and the excess acetic anhydride removed under vacuum. The residual dark oil was purified by flash chromatography on silica gel (4:1 v/v ethyl acetate–hexane). The product solidified on standing and was recrystallized from ether–hexane. The first crop gave 2.2 g (30%) of pure 11c: 90-MHz NMR ($CDCl_3$) δ 0.65 (t, 3 H, J = 7 Hz), 1.10 (d, 6 H, J = 7 Hz), 1.7–2.0 (m, 2 H), 3.00 (septet, 1 H, J = 7 Hz), 3.6–4.0 (m, 8 H), 4.60 (t, 1 H, J = 4 Hz), 6.9–7.4 (m, 9 H) ppm.

Method C. Ethyl α-[[2-(1,3-Dioxolan-2-yl)ethyl]amino]-4-fluorobenzeneacetate (14). A solution of 26 g (220 mmol) of 2-(2-aminoethyl)-1,3-dioxolane in 50 mL of acetonitrile was added at room temperature with stirring to a solution of 52 g (200 mmol) of ethyl α-bromo-4-fluorobenzeneacetate[20] and 42 mL (300 mmol) of triethylamine in 350 mL of acetonitrile. The resulting mixture was stirred at room temperature overnight and then poured into ether (500 mL). The suspension which resulted was washed with water (300 mL) and 2 M HCl (2 × 300 mL). The combined acidic extracts were made alkaline with 25% aqueous NaOH and extracted with ethyl acetate (2 × 500 mL). The ethyl acetate extracts were combined, washed successively with water and brine, and dried. Filtration and concentration yielded 49.5 g (82.5%) of 14 as an oil: 90-MHz NMR ($CDCl_3$) δ 1.18 (t, 3 H, J = 7 Hz), 1.85 (m, 2 H), 2.20 (br s, 1 H), 2.6 (m, 2 H), 3.85 (m, 4 H), 4.1 (q, 2 H, J = 7 Hz), 4.22 (s, 1 H), 4.83 (t, 1 H, J = 4.5 Hz), 6.8–7.3 (m, 4 H) ppm.

α-[[2-(1,3-Dioxolan-2-yl)ethyl](2-methyl-1-oxopropyl)amino]-4-fluorobenzeneacetic Acid (15). 14 (30 g, 100 mmol) was dissolved in 200 mL of CH_2Cl_2 with 28.6 mL (205 mmol) of triethylamine. The resulting mixture was cooled to 0 °C under dry nitrogen. A solution of 11 mL (105 mmol) of isobutyryl chloride in 50 mL of CH_2Cl_2 was slowly added with stirring. After addition was complete, the mixture was stirred for an additional 1 h and then poured into 100 mL of ether. The ether solution was washed successively with water (25 mL), 2 M HCl (25 mL), saturated aqueous bicarbonate (25 mL), and brine (25 mL), and dried. Filtration and evaporation of the solvents yielded 35 g of α-[[2-(1,3-dioxolan-2-yl)ethyl](2-methyl-1-oxopropyl)amino]-4-fluorobenzeneacetic acid, ethyl ester: 90-MHz NMR ($CDCl_3$) δ 1.2 (m, 9 H), 1.7 (m, 2 H), 2.85 (m, 1 H), 3.35 (m, 2 H), 3.80 (m, 4 H), 4.20 (q, 2 H, J = 7 Hz), 4.60 (t, 1 H, J = 4.5 Hz), 5.81 (s, 1 H), 6.8–7.3 (m, 4 H) ppm.

A solution of this ester (35 g) and 12 g (300 mmol) of NaOH in 480 mL of 5:1 methanol–water was stirred and heated at reflux for 2 h. The solution was cooled to room temperature, concentrated, and diluted with 500 mL of water. The resulting solution was extracted with ether. The aqueous layer was then acidified with ice-cold 6 M HCl and extracted with ethyl acetate (2 × 300 mL).

The combined ethyl acetate extracts were washed with brine, dried, filtered, and evaporated to yield 30 g of crude 15 as a gum which was used without further purification: 90-MHz NMR ($CDCl_3$) δ 1.11 (d, 6 H, J = 7 Hz), 1.4–1.9 (m, 2 H), 2.85 (m, 1 H), 3.32 (m, 2 H), 3.75 (m, 4 H), 4.52 (t, 1 H, J = 4.5 Hz), 5.73 (s, 1 H), 6.8–7.3 (m, 4 H) ppm.

1-[2-(1,3-Dioxolan-2-yl)ethyl]-5-(4-fluorophenyl)-2-(1-methylethyl)-*N*,4-diphenyl-1*H*-pyrrole-3-carboxamide (16b). A solution of 95 g (280 mmol) of 15 and 98 g (439 mmol) of *N*,3-diphenylpropynamide[21] in acetic anhydride (200 mL) was heated at 90 °C with stirring for 4 h (vigorous gas evolution). The mixture was then cooled to room temperature, concentrated, and chromatographed twice on silica gel (4:1 v/v hexane–ethyl acetate) to separate the product (R_f = 0.35, 4:1 hexane–ethyl acetate) from the *N*,3-diphenylpropynamide (R_f = 0.5). Recrystallization of the product from isopropyl ether provided 59.5 g (119 mmol) of 16b as colorless crystals: mp 159–162 °C; 200-MHz NMR ($CDCl_3$) δ 1.54 (d, 6 H, J = 7 Hz), 1.91 (m, 2 H), 3.60 (septet, 1 H, J = 7 Hz), 3.7–4.1 (m, 6 H), 4.74 (t, 1 H, J = 4.3 Hz), 7.0–7.3 (m, 15 H); IR (KBr) 3400, 1658, 1596, 1530 cm^{-1}. Anal. C, H, N.

5-(4-Fluorophenyl)-2-(1-methylethyl)-1-(3-oxopropyl)-*N*,4-diphenyl-1*H*-pyrrole-3-carboxamide (17c). A solution of 59 g (118 mmol) of 16c and 0.4 mL of concentrated HCl in 1200 mL of absolute ethanol was heated under reflux with stirring for 24 h. The mixture was cooled to room temperature and concentrated and the residue taken up in 3:1 acetone–water (1200 mL). *p*-TSA–H_2O (5 g) was added. This mixture was heated under reflux with stirring for 2 days, cooled to room temperature, and partitioned between ether (1000 mL) and brine (200 mL). The organic layer was separated, washed successively with saturated aqueous bicarbonate (2 × 200 mL) and brine (100 mL), dried, filtered, and concentrated. The resulting oil was dissolved in the minimum amount of hot isopropyl ether, and the crystals which formed upon cooling were collected by filtration to yield 36.8 g (81 mmol) of 17c, mp 164–5 °C. A further crop of 9.8 g was obtained from the mother liquor: 200-MHz NMR ($CDCl_3$) δ 1.52 (d, 6 H, J = 7 Hz), 2.68 (br t, 2 H, J = 4 Hz), 3.63 (septet, 1 H, J = 7 Hz), 4.27 (br t, 2 H, J = 4 Hz), 6.86 (br s, 1 H), 7.0–7.2 (m, 14 H), 9.60 (s, 1 H); IR (KBr) 3400, 2966, 1720, 1673, 1596, 1511 cm^{-1}. Anal. C, H, N.

Methyl 7-[2-(4-Fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrol-1-yl]-3-hydroxy-5-oxo-1-heptanoate. A solution of methyl acetoacetate (26.4 mL, 243 mmol) in 250 mL of anhydrous THF was added dropwise to a stirred suspension of hexane-washed sodium hydride (6.4 g, 267 mmol) in 200 mL of THF at 0 °C. When gas evolution was complete, 97.2 mL of a 2.5 M solution of *n*-butyllithium in hexanes was added dropwise over 1 h.

The resulting solution was stirred for 30 min at 0 °C and cooled to −78 °C, and a solution of 36.8 g (81 mmol) of 17c in 100 mL of THF was added over a period of 30 min. The resulting solution was stirred for 30 min at −78 °C, then warmed to 0 °C, and held for an additional 1 h.

(20) Epstein, J. W.; Brabander, H. J.; Fanshawe, W. J.; Hofmann, C. M.; McKenzie, T. C.; Safir, S. R.; Osterberg, A. C.; Cosulich, D. B.; Lovell, F. M. *J. Med. Chem.* 1981, *24*, 481–90.

(21) Cabre, J.; Palomo, A. L. *Synthesis* 1984, 413–7.

The mixture was then acidified by the dropwise addition of 300 mL of ice-cold 3 M HCl, diluted with ether, washed with water and brine, dried, filtered, and evaporated. Flash chromatography on silica gel (3:1 v/v hexane–ethyl acetate) yielded 37.9 g of methyl 7-[2-(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrol-1-yl]-5-hydroxy-3-oxo-1-heptanoate: 90-MHz NMR ($CDCl_3$) δ 1.50 (d, 6 H, J = 7 Hz), 1.8 (m, 2 H), 2.45 (d, 2 H, J = 7 Hz), 2.8 (br s, 1 H), 3.33 (s, 2 H), 3.5 (m, 1 H), 3.67 (s, 3 H), 3.8–4.0 (m, 2 H), 6.8–7.3 (m, 14 H) ppm.

(±)-*trans*-5-(4-Fluorophenyl)-2-(1-methylethyl)-*N*,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-6-yl)ethyl]-1*H*-pyrrole-3-carboxamide (3i). Air (60 mL) was bubbled via a syringe through a solution of methyl 7-[2-(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)-carbonyl]-1*H*-pyrrol-1-yl]-5-hydroxy-3-oxo-1-heptanoate (48 g, 84 mmol) and 92.5 mL of a 1 M THF solution of tributylborane in 100 mL of anhydrous THF. The mixture was stirred overnight at room temperature and then cooled to −78 °C. Sodium borohydride (3.85 g, 102 mmol) was added to the cooled mixture in one portion. The vigorously stirred suspension was allowed to warm slowly to 0 °C over 3 h (vigorous gas evolution ensued).

The dry ice–acetone bath cooling the reaction vessel was replaced by an ice bath and 18.3 mL of glacial acetic acid was added dropwise, followed by 204 mL of 3 N NaOH and 30.5 mL of 30% aqueous H_2O_2.

The mixture was vigorously stirred and allowed to warm to room temperature overnight. The mixture was partitioned between ether and water. The aqueous layer was separated, acidified, and extracted with ethyl acetate (2×).

The ethyl acetate extracts were washed with brine, dried, and evaporated to yield crude (*R**,*R**)-3,5-dihydroxy-7-[(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)-carbonyl]-1*H*-pyrrol-1-yl]-1-heptanoic acid which was used without further purification.

The crude acid was taken up in toluene and heated at reflux for 6 h with azeotropic removal of water. Chromatography (silica gel, 1:1 v/v hexane–ethyl acetate) provided 30 g of 3i as a foamy solid, mp 90–97 °C.

This material was found by HPLC analysis to be a 9:1 mixture cis and trans isomers. Recrystallization from toluene–ethyl acetate yielded essentially pure trans 3i: mp 148–9 °C; 200-MHz NMR ($CDCl_3$) δ 1.52 (m, 6 H), 1.6–2.0 (m, 4 H), 2.48 (br s, 1 H), 2.51 (m, 2 H), 3.55 (septet, 1 H, J = 7 Hz), 4.0–4.2 (m, 2 H), 4.29 (m, 1 H), 4.52 (m, 1 H), 6.90 (br s, 1 H), 7.0–7.3 (m, 14 H) ppm; IR (KBr) 3400, 1734, 1654, 1597, 1511 cm^{-1}. Anal. C, H, N.

Phenylmethyl 1-[2-(1,3-Dioxolan-2-yl)ethyl]-5-(4-fluorophenyl)-2-(1-methylethyl)-4-phenyl-1*H*-pyrrole-3-carboxylate (16a). A solution of 15 (10 g, 29 mmol) and benzyl phenylpropiolate (7.7 g, 44 mmol) was stirred and heated in 30 mL of acetic anhydride at 90 °C for 6 h. After cooling to room temperature, the solution was concentrated, diluted with ether, washed with water, saturated aqueous bicarbonate, and brine, and dried. Flash chromatography on silica gel (10:1 v/v hexane–ethyl acetate) provided 5.9 g (45%) of crude 16a. Recrystallization from isopropyl ether provided 4.8 g of colorless 16a: mp 158–9 °C; IR (KBr) 1683 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 0.93 (t, 3 H, J = 7 Hz), 1.48 (d, 6 H, J = 7 Hz), 1.93 (m, 2 H), 3.50 (septet, 1 H, J = 7 Hz), 3.7–4.1 (m, 8 H), 4.71 (t, 1 H, J = 4.4 Hz), 6.95–7.2 (m, 9 H) ppm. Anal. C, H, N.

Method D. 1-[2-(1,3-Dioxolan-2-yl)ethyl]-2-(4-fluorophenyl)-3,4-dimethyl-5-(1-methylethyl)-1*H*-pyrrole (18). A solution of 11a (1.0 g, 2.37 mmol) in 5 mL of CH_2Cl_2 was added dropwise to a stirred suspension of lithium aluminum hydride (0.3 g, 7.4 mmol) in 20 mL of ether at room temperature. When addition was complete, the mixture was heated to reflux for 30 min, cooled to room temperature, and quenched by dropwise addition of water (0.3 mL), 25% aqueous NaOH (0.2 mL), and water (0.9 mL). After stirring vigorously for 30 min, the mixture was filtered and washed well with CH_2Cl_2. The filtrated was dried, filtered, and concentrated, providing 0.78 g (90%) of pure diol.

Trifluoroacetic acid (5.2 mL, 67 mmol) was added to a stirred solution of the diol (1.23 g, 3.4 mmol) and triethylsilane (1.2 mL, 7.5 mmol) in 10 mL of CH_2Cl_2 cooled to 0 °C under dry nitrogen. The solution was stirred for 2 h at 0 °C before warming to room temperature for 1 h. It was then poured into 300 mL of 50:50 ether–hexane and washed with saturated aqueous bicarbonate (3 × 50 mL) and brine (50 mL), and dried. Flash chromatography on silica gel (10:1 v/v hexane–ethyl acetate) provided 0.80 g (71%) of 18 as an oil: 90-MHz NMR ($CDCl_3$) δ 1.32 (d, 6 H, J = 7 Hz), 1.7–1.9 (m, 2 H), 1.86 (s, 3 H), 2.07 (s, 3 H), 3.10 (septet, 1 H, J = 7 Hz), 3.7–4.0 (m, 6 H), 4.58 (t, 1 H, J = 4 Hz), 6.9–7.3 (m, 4 H) ppm.

Method E. Methyl 4-Methyl-3-oxo-2-(phenylmethylene)pentanoate (21a). A mixture of methyl isobutyrylacetate (144 g, 1 mol), benzaldehyde (116 g, 1.1 mol), piperidine (4 mL), and HOAc (12 mL) in 200 mL of toluene was stirred and heated at reflux with azeotropic removal of water for 3 h. The solution was cooled, poured into ether (1 L), washed with 1 M HCl (200 mL), saturated aqueous bicarbonate (200 mL), and brine, and dried. Concentration and distillation (bp 127–130 °C/1 mmHg) provided 186.6 g (80%) of 21a as a mixture of diastereomers (isomer 1, major ~70%): 90-MHz NMR ($CDCl_3$) δ 0.98 (d, 6 H, J = 7 Hz), 2.58 (septet, 1 H, J = 7 Hz), 3.70 (s, 3 H), 7.28 (s, 5 H), 7.68 (s, 1 H) ppm. Isomer 2: 90-MHz NMR ($CDCl_3$) δ 1.14 (d, 6 H, J = 7 Hz), 3.14 (septet, 1 H, J = 7 Hz), 3.70 (s, 3 H), 7.80 (s, 5 H), 7.48 (s, 1 H) ppm.

1-(4-Fluorophenyl)-5-methyl-2-phenyl-1,4-hexanedione (22a). To a solution of 21a (376 g, 1.62 mol), 4-fluorobenzaldehyde (201 g, 1.62 mol), and Et_3N (158 mL) in a 3-L three-neck round-bottom flask with an air-driven stirrer was added 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride (65.5 g, 243 mmol). The mixture was stirred and heated at 70 °C for 24 h. After cooling to room temperature, the mixture was diluted with ether (3 L), washed with water, dilute HCl, saturated aqueous bicarbonate, and brine, and dried. The crude oil which remained after filtration and concentration was dissolved in THF (1500 mL) and added to a solution of NaOH (130 g) in 750 mL of water. The mixture was vigorously stirred overnight, acidified (pH 5) with 6 N HCl, and extracted with ether. The ether layer was washed several times with 3 N NaOH and water (to remove a low R_f, base soluble material) and brine and dried. The crude material was filtered through silica gel (100 g) and concentrated. It was then Kugelrohr distilled in two portions to afford 314 g (66%) of 22a: bp 145 °C (0.3 mmHg) IR (film) 1711, 1684, 1600 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.08 (d, 3 H, J = 7 Hz), 1.13 (d, 3 H, J = 7 Hz), 2.65 (septet, 1 H, J = 7 Hz), 2.77 (dd, 1 H, J = 18, 4 Hz), 3.63 (dd, 1 H, J = 18, 10 Hz), 5.07 (dd, 1 H, J = 10, 4 Hz), 7.10 (m, 2 H), 7.27 (m, 5 H), 7.98 (m, 2 H) ppm.

1-(3,3-Diethoxypropyl)-2-(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-1*H*-pyrrole (23a). To a solution of 22a (230 g, 0.77 mol) in 1 L of toluene was added 3,3-diethoxy-1-aminopropane[19] (176 g, 1.2 mol) at room temperature. The mixture solidified, but dissolution occurred on adding *p*-TSA–H_2O and heating to reflux (Dean–Stark) for 24 h. To the cooled solution was added 100 mL of absolute ethanol and the mixture concentrated and filtered through silica gel. The residue on concentration was dissolved in the minimum amount of isopropyl ether and allowed to crystallize. A first crop of 89 g (mp 84–7 °C) was isolated. A further 145 g were isolated as an oil: IR (KBr) 2973, 1603, 1511 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.11 (t, 3 H, J = 7 Hz), 1.35 (d, 6 H, J = 7 Hz), 1.75 (m, 2 H), 3.04 (septet, 1 H, J = 7 Hz), 3.2–3.6 (m, 4 H), 3.91 (m, 2 H), 4.27 (t, 1 H, J = 4.4 Hz), 6.20 (s, 1 H), 7.0–7.4 (m, 9 H) ppm. Anal. C, H, N.

Methyl 3-(4-Fluorophenyl)-3-oxopropanoate. To a suspension of dimethyl carbonate (195 g, 2.17 mmol) and hexane-washed NaH (72g, 3.0 mol) in dry THF (600 mL) at 60 °C was added 164 g (1.2 mol) of *p*-fluoroacetophenone dropwise. The reaction was maintained at gentle reflux by adjusting the temperature and addition rate (exothermic). After the addition was complete, the reaction was heated at reflux for 4 h, then cooled to room temperature.

The reaction was poured carefully into ice cold acetic acid (183 mL, 3.2 mol) and water (400 mL). The product was extracted with ether (2×), and the combined ether layers were washed with saturated aqueous bicarbonate, brine and dried. Distillation provided 204 g (96%) of desired product (bp 91 °C/0.5 mmHg): 90-MHz NMR ($CDCl_3$) δ 3.65 (s, 3 H), 3.92 (s, 2 H), 6.82–7.20 (m, 2 H), 7.57–8.01 (m, 2 H), 12.45 (singlet, 1 H) ppm.

Methyl 3-(4-Fluorophenyl)-3-oxo-2-(phenylmethylene)-propanoate (26). A mixture of methyl, 3-(4-fluorophenyl)-3-oxopropionate (100 g, 510 mmol), benzaldehyde (59.5 g, 561 mmol), piperidine (2 mL), and acetic acid (6 mL) in toluene (100 mL)

was stirred and heated at reflux with azeotropic removal of water for 4 h. The solution was cooled and filtered through silica gel (600 g) with toluene as eluant. Concentration afforded 127.2 g (88%) of 26 as a mixture of *E*- and *Z*-isomers: 90-MHz NMR ($CDCl_3$) δ 2.22 (s, 3 H, isomer 1), 3.62 (s, 3 H, isomer 2), 6.80–8.11 (m, 10 H) ppm.

1-(4-Fluorophenyl)-3-phenyl-5-methylhexane-1,4-dione (27). A mixture of 26 (130 g, 454 mmol), isobutyraldehyde (41 mL, 454 mmol), Et_3N (33 mL), and 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride (24 g, 91 mmol) was stirred and heated at 70 °C for 18 h. Additional isobutyraldehyde (6 g) was added and stirring continued for a further 6 h. After cooling to room temperature, the mixture was diluted with ether, washed with 2 M HCl (2×), saturated aqueous bicarbonate, and brine, and dried. The crude product was used without further purification.

To a solution of the crude diketo ester (31 g, 86.9 mmol) in 5:1 THF–H_2O (500 mL) was added NaOH (8 g, 200 mmol) in one portion. A small amount of methanol was added to ensure homogeneity. The reaction was stirred overnight at room temperature. The solvent was removed on the rotary evaporator, and the residue was dissolved in ether. This was then washed with 2 M HCl and brine and dried. Purification by flash chromatography (9:1 v/v ethyl acetate–hexane) gave 9.0 g (35%) of 27 as an oil: 90-MHz NMR ($CDCl_3$) δ 0.8 (d, 3 H, J = 7 Hz), 1.2 (d, 3 H, J = 7 Hz), 2.4–3.0 (m, 1 H), 3.6–4.0 (m, 1 H), 4.4–4.55 (m, 1 H), 6.8–7.3 (m, 7 H), 7.7–7.9 (m, 2 H) ppm.

5-(4-Fluorophenyl)-2-(1-methylethyl)-3-phenyl-1*H*-pyrrole-1-propanal (28). To a solution of 17 (9.0 g, 30.2 mmol) and 3,3-diethoxy-1-aminopropane (6.6 g, 45.3 mmol) in toluene (150 mL) was added a catalytic amount of *p*-TSA–H_2O. The resulting mixture was heated to reflux with azeotropic removal of water (Dean–Stark) overnight.

The solution was cooled and concentrated, and the residue was purified by flash chromatography on silica gel (10:1 v/v ethyl acetate–hexane). This provided 2.4 g (19%) of the pyrrole acetal as an oil and 7.1 g of recovered 27. The pyrrole acetal was taken up in 5:1 acetone–water. Camphorsulfonic acid (0.2 g) was added and the solution refluxed for 18 h. The cooled solution was concentrated, diluted with ether, washed with water, bicarbonate, and brine, and dried. Flash chromatography on silica gel (9:1 v/v hexane–ethyl acetate) afforded 1.9 g of 28 as an oil: 90-MHz NMR ($CDCl_3$) δ 1.3 (d, 6 H, J = 7 Hz), 2.56 (m, 2 H), 3.22 (septet, 1 H, J = 7 Hz), 4.37 (m, 2 H), 6.1 (s, 1 H), 6.9–7.5 (m, 9 H), 9.5 (s, 1 H) ppm.

(2*R*)-*trans*-4-[[(1,1-Dimethylethyl)silyl]oxy]-6-[2-[2-(4-fluorophenyl)-5-(1-methylethyl)-1*H*-pyrrol-1-yl]ethyl]-tetrahydro-2*H*-pyran-2-one (29). To a solution of 1 (0.52 g, 1.5 mmol) and *tert*-butyldimethylchlorosilane (0.27 g, 1.8 mmol) in 5 mL of dry DMF was added imidazole (0.31 g, 4.5 mmol) in one portion. The solution was stirred overnight at room temperature before partitioning between hexane (100 mL) and water (50 mL). The aqueous layer was extracted with two 50-mL portions of hexane. The combined hexane extracts were washed with water (2 × 25 mL) and brine (25 mL) and dried. Filtration through silica gel and concentration provided 0.7 g (100%) of 29 as a colorless oil: 90-MHz NMR ($CDCl_3$) δ 0.10 (s, 6 H), 0.90 (s, 9 H), 1.30 (d, J = Hz, 6 H), 1.4–1.8 (m, 4 H), 2.48 (m, 2 H), 2.95 (m, 1 H), 3.9–4.3 (m, 3 H), 5.85 (d, J = 2 Hz, 1 H), 6.02 (d, J = 2 Hz, 1 H), 6.8–7.3 (m, 4 H).

(2*R*)-*trans*-6-[2-[3,4-Dichloro-2-(4-fluorophenyl)-5-(1-methylethyl)-1*H*-pyrrol-1-yl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (30a). *N*-Chlorosuccinimide (6.48 mmol, 0.87 g) was added in one portion to a stirred solution of 29 (1.49 g, 3.24 mmol) in dry DMF (10 mL) cooled to 0 °C under dry nitrogen. The solution was stirred for 1 h at 0 °C then warmed to room temperature over 3 h. This was then diluted with water (50 mL) and extracted with ether (2 × 100 mL). The ether extracts were diluted with 100 mL of hexane, washed with water (50 mL), saturated aqueous bicarbonate (50 mL), 10% aqueous $NaHSO_3$ (50 mL), and brine (50 mL), and dried. After filtration and concentration, the crude product was dissolved in THF (15 mL) and treated with glacial acetic acid (0.75 mL, 13 mmol) and n-Bu_4F (9.72 mL of 1 M THF solution). The solution was stirred for 5 h, diluted with ethyl acetate (100 mL), washed with saturated aqueous bicarbonate (2 × 50 mL) and brine (25 mL), and dried. The residue which remained after filtration and concentration was flash chromatographed on silica gel (2:1 v/v hexane–ethyl acetate). This provided 0.50 g (35%) of 30a as a colorless solid. Recrystallization from ether–hexane provided colorless crystals: mp 129–131 °C; IR (KBr) ν 3550, 2990, 1711, 1518, 1225, 1160, 1055, 851, 816 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.44 (d, J = 7 Hz, 6 H), 1.8 (m, 4 H), 2.12 (d, J = 3 Hz, 1 H, OH), 2.55 (m, 2 H), 3.10 (m, 1 H), 4.0 (m, 2 H), 4.30 (m, 1 H), 4.45 (m, 1 H), 7.0–7.4 (m, 4 H) ppm. Anal. C, H, N.

(2*R*)-*trans*-6-[2-[2-(4-Fluorophenyl)-5-(1-methylethyl)-3-(trifluoroacetyl)-1*H*-pyrrol-1-yl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (30c). Trifluoroacetic anhydride (0.17 mL, 1.2 mmol) was added dropwise to a stirred solution of 29 (0.50 g, 1.09 mmol) in 2 mL of DMF cooled to 0 °C under nitrogen. The light yellow solution was stirred for 1 h at 0 °C, diluted with 150 mL of 50:50 ether–hexane, washed with saturated aqueous bicarbonate (3 × 50 mL), and brine, and dried. Filtration and concentration provided a single product which was dissolved in 5 mL of anhydrous THF and stirred overnight at room temperature with 4 equiv of glacial acetic acid and 3 equiv of n-Bu_4NF. The mixture was then diluted with ether, washed with 2 M HCl and brine, and dried. Flash chromatography on silica gel (2:1 v/v hexane–ethyl acetate) provided 0.25 g of 30c as an oil: IR (KBr) 3450, 1687, 1609 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.31 (d, 6 H, J = 7 Hz), 1.4–2.0 (m, 5 H), 2.6 (m, 2 H), 3.00 (septet, 1 H, J = 7 Hz), 3.9–4.1 (m, 2 H), 4.33 (m, 1 H), 4.49 (m, 1 H), 6.48 (q, 1 H, J = 2.1 Hz), 7.0–7.4 (m, 4 H) ppm. Anal. C, H, N.

[*S*-(*R,*S**)]-5-[2-(4-Fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrol-1-yl]-3-hydroxy-1-pentanoic Acid, 2-Hydroxy-1,2,2-triphenylethyl Ester (31).** *n*-Butyllithium in hexane (285 mL, 2.2 M) was added dropwise with stirring to diisopropylamine (92 mL) in THF (300 mL) at −50 to −60 °C in a 1000-mL one-neck flask via a dropping funnel under nitrogen. The yellow solution was allowed to warm to approximately −20 °C, then cannulated into a suspension of 99 g of (*S*)-(+)-2-acetoxy-1,1,2-triphenylethanol[16] in 500 mL of anhydrous THF at −70 °C. When addition was complete, the reaction mixture was allowed to warm to −10 °C over a period of 2 h. Meanwhile, a suspension of 0.63 mol of $MgBr_2$ was prepared by addition of 564 mL (0.63 mol) of bromine dropwise into a suspension of 15.3 g of magnesium (0.63 mol) in 500 mL of THF in a 3-L flask equipped with reflux condenser and mechanical stirrer. The $MgBr_2$ suspension was cooled to −78 °C and the enolate solution cannulated into the suspension over 30 min. Stirring was continued for 1 h at −78 °C. 17c (150 g) in 800 mL of THF was then added dropwise over 30 min. The solution was stirred for 1.5 at −78 °C and then quenched with 200 mL of glacial acetic acid at −78 °C. After warming to 0 °C, 500 mL of water were added and the mixture concentrated in vacuo at 40–50 °C. 1:1 ethyl acetate–heptane (500 mL) was added to the yellow slurry, which was then filtered. The filtrate was washed extensively with 0.5 N HCl, then several times with water, and finally with cold (−20 °C) ethyl acetate–heptane (3:1). The light brown crystalline product was dried in vacuo at 40 °C, affording 194 g of crude aldol product. Recrystallization from ethyl acetate at −10 °C yielded 100 g of 31 (mp 229–230 °C) which analyzed as a 97.4:2.2 mixture of the *R,S*-:*S,S*-isomers by HPLC: IR (KBr) 3400, 2961, 1716, 1663, 1595, 1511, 701 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.44 (d, 6 H, J = 7 Hz), 1.5 (m, 2 H), 2.12 (m, 2 H), 2.39 (br s, 1 H) 3.40 (septet, 1 H, J = 7 Hz), 3.62 (m, 1 H), 3.81 (m, 1 H), 4.07 (m, 1 H), 6.63 (s, 1 H), 6.8–7.5 (m, 29 H) ppm. Anal. C, H, N.

Methyl (*R*)-(+)-5-[2-(4-Fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrol-1-yl]-3-hydroxy-1-pentanoate. To a suspension of 162 g (0.206 M) of the triphenylethanediol ester prepared above in 800 mL methanol–THF (5:3) cooled to 0 °C was added 11.7 g of sodium methoxide. The mixture was stirred until dissolution occurred and then put in the freezer overnight. The reaction mixture was then allowed to warm to room temperature, quenched with 15 mL of glacial acetic acid and concentrated in vacuo at 40 °C to obtain an oil, which was partitioned between water (500 mL) and ethyl acetate (2 × 300 mL). The combined organic extracts were washed with saturated aqueous bicarbonate and brine, dried, and filtered and the solvent evaporated. The residue was chromatographed on silica gel (1:4 v/v, ethyl acetate–heptane) to yield 109 g of the methyl ester as a colorless oil which solidified on

standing. Recrystallization from ether–heptane yielded 73.9 g of colorless crystals: mp 125–6 °C; $[\alpha]^{20}_D$ = 4.23° (1.17 M, CH_3OH); IR (KBr) 3400, 2960, 1720, 1646, 1511, 1160, 755 cm^{-1}; 250-MHz NMR ($CDCl_3$) δ 1.53 (d, 6 H, J = 7 Hz) 1.6–1.7 (m, 2 H), 2.30 (d, 2 H, J = 6 Hz), 2.88 (br s, 1 H), 3.57 (septet, 1 H, J = 7 Hz), 3.67 (s, 3 H), 3.85 (m, 1 H), 3.97 (m, 1 H), 4.15 (m, 1 H), 6.85 (s, 1 H), 6.95–7.25 (m, 14 H) ppm. Anal. C, H, N.

1,1-Dimethylethyl (*R*)-7-[2-(4-Fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrol-1-yl]-5-hydroxy-3-oxo-1-heptanoate (32). Diisopropylamine (75 mL, 550 mmol) was dissolved in THF (250 mL) in a 2000-mL three-neck flask equipped with thermometer and dropping funnel under nitrogen. The mixture was cooled to −42 °C and then 200 mL of 2.2 M *n*-butyllithium in hexane was added dropwise over 20 min. After stirring for 20 min, 62 mL (460 mmol) of *tert*-butyl acetate dissolved in THF (200 mL) was added over 30 min. This mixture was stirred for 30 min at −40 °C, then a further 140 mL of 2.2 M *n*-butyllithium was added over 20 min. When addition was complete, 81 g (153 mmol) of methyl (*R*)-(+)-5-[2-(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrol-1-yl]-3-hydroxy-1-pentanoate in anhydrous THF (500 mL) was added as quickly as possible without allowing the temperature to rise above −40 °C. Stirring was continued for 4 h at −70 °C. The reaction mixture was quenched with glacial acetic acid (69 mL) and allowed to warm to room temperature. It was then concentrated in vacuo and the residue taken up in ethyl acetate, washed extensively with water, saturated aqueous NH_4Cl, saturated aqueous bicarbonate, and brine. The organic layer was dried and filtered and the solvent evaporated to produce 73 g of 32: IR (KBr) 3400, 2933, 1700, 1665, 1511, 1151 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.45 (s, 9 H), 1.53 (dd, 6 H, J = 7.1 Hz), 1.6 (m, 2 H), 2.51 (s, 1 H), 2.53 (d, 1 H, J = 2 Hz), 2.80 (d, 1 H, J = 2 Hz, O*H*), 3.31 (s, 2 H), 3.60 (septet, 1 H, J = 7 Hz), 3.9–4.0 (m, 2 H), 4.09–4.22 (m, 1 H), 6.85 (s, 1 H), 6.95–7.2 (m, 14 H) ppm. Anal. C, H, N.

(+)-(4*R*)-*trans*-2-(4-Fluorophenyl)-5-(1-methylethyl)-*N*,3-diphenyl-1-[(tetrahydro-4-hydroxy-2-oxo-2*H*-pyran-6-yl)ethyl]-1*H*-pyrrole-4-carboxamide ((+)-33). To a solution of 73 g (119 mmol) of 32 in THF (500 mL) was added triethylborane (120 mL of a 1 M THF solution) and pivalic acid (0.7 g). The mixture was stirred for 10 min and cooled to −78 °C and methanol (70 mL) was added, followed by $NaBH_4$ (4.5 g, 119 mmol). The mixture was stirred at −78 °C for 6 h, then poured slowly into a 4:1:1 mixture of ice–30% aqueous H_2O_2–water. This mixture was stirred overnight and then allowed to warm to room temperature. Chloroform (400 mL) was added and the mixture partitioned between chloroform and water. The aqueous layer was further extracted with chloroform. The organic extracts were combined and washed extensively with water until a test for peroxide was negative. The organic layer was dried, filtered, and evaporated. The residue was flash chromatographed on silica gel (1:3 v/v ethyl acetate–hexane) to yield 51 g of crude dihydroxy ester which was dissolved in THF–methanol and 1 N NaOH (100 mL) was added with stirring at room temperature. After 4 h, the solution was concentrated, water (100 mL) was added, and it was extracted with ether (2 × 100 mL). The aqueous layer was acidified with 1 N HCl and extracted with ethyl acetate (3 × 200 mL). The combined organic layers were washed with water. The organic layer was dried, filtered and evaporated. The residue was taken up in toluene (2 L) and heated to reflux (Dean–Stark) for 20 min. After cooling, the procedure above was repeated. The reaction was left at room temperature for 10 days and then concentrated to yield 51 g of crude (+)-33 as a colorless foam. This was dissolved in the minimum amount of chloroform and chromatographed on silica gel (1:1 v/v ethyl acetate–heptane) to yield 23 g of impure (+)-33. Further chromatography on silica gel (98.5:1.5 v/v chloroform–propanol) yielded 13.2 g of (+)-33 as a crude solid.

Recrystallization from ethyl acetate–hexane produced 8.2 g of crystals shown to be a mixture of isomers by HPLC. Concentration of the mother liquors yielded 4.6 g of an oil which was shown to be 100% of pure (+)-33 by HPLC. Chromatography (silica gel, 98:2 v/v chloroform–2-propanol) afforded 4.18 g of (+)-33 as colorless foam, $[\alpha]^{23}_D$ = +24.53° (0.53% in $CHCl_3$).

α-Methylbenzeneacetamides. A solution of **3i** (30 g, 55.5 mmoL) in (*R*)-(+)-α-methylbenzylamine (575 mL, 4.45 mol, 98% Aldrich) was stirred overnight at room temperature. The resulting solution was diluted with ether (2 L) and washed exhaustively with 2 M HCl (4 × 500 mL), water (2 × 500 mL), and brine (2 × 500 mL). The organic extract was dried, filtered, and concentrated in vacuo to yield 28.2 g of the diastereomeric α-methylbenzylamides as a white solid, mp 174–7 °C. The α-methylbenzylamides were separated by dissolving 1.5 g of the mixture in 1.5 mL of 98:1.9:0.1 chloroform–methanol–NH_4OH and injecting onto a preparative HPLC column (silica gel, 300 mm × 41.4 mm i.d.) by a gas-tight syringe and eluting with the above solvent mixture. Diastereomer 1 eluted at 41 min. Diastereomer 2 eluted at 49 min. Center cut fractions were collected. This procedure was repeated 3 times and the like fractions combined and concentrated. Examination of each by analytical HPLC indicated that diastereomer 1 was 99.84% pure and diastereomer 2 was 96.53% pure. Each isomer was taken on separately.

(+)-(4*R*)-*trans*-2-(4-Fluorophenyl)-5-(1-methylethyl)-*N*,3-diphenyl-1-[(tetrahydro-4-hydroxy-2-oxo-2*H*-pyran-6-yl)ethyl]-1*H*-pyrrole-4-carboxamide ((+)-33). To an ethanolic solution (50 mL) of diastereomer 1, [3*R*-[3*R**,5*R**]]-7-[2-(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)-carbonyl]-1*H*-pyrrol-1-yl]-3,5-dihydroxy-*N*-[(*R*)-1-phenylethyl]-1-heptanamide, (1 g, 1.5 mmol) was added 1 N NaOH (3.0 mL, 3 mmol). The resulting solution was heated to reflux for 48 h.

The solution was cooled to room temperature and concentrated in vacuo. The residue was resuspended in water and carefully acidified with 6 N HCl. The resulting acidic solution was extracted with ethyl acetate. The organic extract was washed with water and brine, dried, filtered, and concentrated in vacuo. This residue was redissolved in toluene (100 mL) and heated to reflux with azeotropic removal of water for 3 h. This was cooled to room temperature and concentrated in vacuo to yield 1.2 g of a yellow semisolid. Flash chromatography on silica gel (2:3 v/v ethyl acetate–hexane) afforded 0.42 g of a white solid which still contained some impurities. This was rechromatographed (same system) to produce 0.1 g of essentially pure (+)-33, as a white foam. HPLC showed this material to be 94.6% chemically pure ($[\alpha]^{23}_D$ = +25.5° (0.51% in $CHCl_3$). The peak with a retention time of 53.46 min was tentatively assigned to an unknown diastereomer resulting from the 2% (*S*)-(−)-α-methylbenzylamine present in the Aldrich α-methylbenzylamine.

Preparation of (−)-(4*S*)-*trans*-2-(4-Fluorophenyl)-5-(1-methylethyl)-*N*,3-diphenyl-1-[(tetrahydro-4-hydroxy-2-oxo-2*H*-pyran-6-yl)ethyl]-1*H*-pyrrole-4-carboxamide ((−)-33). Carrying out the procedure described above on diastereomer 2 afforded 0.6 g of a foamy solid which was flash chromatographed on silica gel (1:1 v/v ethyl acetate–hexane) to afford 0.46 g of essentially pure (−)-33, as a white foam. HPLC showed this material to be 97.83% chemically pure, $[\alpha]^{23}_D$ = −24.8% (0.51% in $CHCl_3$).

Acknowledgment. We are indebted to Dr. S. Brennan, Mr. T. Hurley, and Mr. D. Sherwood for HPLC analyses and the separation of the α-methylbenzylamides derived from 3i, Dr. G. McClusky, Ms. S. Uhlendorf, and staff for spectral and analytical data, and Ms. P. Elka for manuscript preparation.

Inhibitors of Cholesterol Biosynthesis. 4. *trans*-6-[2-(Substituted-quinolinyl)ethenyl/ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-ones, a Novel Series of HMG-CoA Reductase Inhibitors[1]

D. R. Sliskovic,* J. A. Picard, W. H. Roark, B. D. Roth, E. Ferguson, B. R. Krause, R. S. Newton, C. Sekerke, and M. K. Shaw

Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105.
Received June 8, 1990

A series of substituted quinoline mevalonolactones were prepared and evaluated for their ability to inhibit the enzyme HMG-CoA reductase both in vitro and (cholesterol biosynthesis) in vivo. Since previous studies suggested that the 4-(4-fluorophenyl) and 2-(1-methylethyl) substituents afforded optimum potency, attention was focused on variations at position 6 of the quinoline ring. Biological evaluation of a small number of analogues bearing a variety of 6-substituents showed that modification at this position had little effect on potency. Several compounds (8b, 8e, and 11) were identified that showed comparable potency to compactin and mevinolin in both the in vitro and in vivo assays.

We have previously described two series of novel HMG-CoA reductase inhibitors. In each series the structurally complex hexahydronaphthalene ring system common to the naturally occurring fungal metabolites compactin and mevinolin was replaced by a five-membered monocyclic heteroaromatic system, such as the nonbasic pyrrole[2] and pyrazole[3] ring systems. Inhibitors containing basic six-membered monocyclic heteroaromatic[4] and nonbasic[5,6] heteroaromatic ring systems have been reported.

This report describes the synthesis and biological activity of a series of quinoline mevalonolactones, the first HMG-CoA reductase inhibitors to contain a basic bicyclic heteroaromatic ring system.

In addition, many of the compounds described herein exhibit improved in vitro potency when compared to both the pyrrole and pyrazole mevalonolactones previously reported.

Chemistry

Most potent inhibitors of HMG-CoA reductase have the 4-hydroxy-2*H*-pyran-2-one moiety flanked by a bulky lipophilic group and an alkyl group, where both of these groups are anchored in the correct spatial arrangement by various carbocyclic and heterocyclic structures.[7]

We initially investigated the synthesis of quinoline-containing mevalonolactones in which the lactone moiety was connected to position 3 of the quinoline nucleus via a two-carbon spacer and was flanked at positions 2 and 4 by an alkyl group and a 4-fluorophenyl group, respectively.[2,3] By attaching the lactone moiety at position 4 of the quinoline nucleus and employing an alkyl flanking group at position 3 we were able to investigate whether the "benzenoid" ring of the quinoline nucleus could replace the 4-fluorophenyl flanking group and give a compound which retained biological activity. Our general synthetic strategy to the quinolin-3-ylmevalonolactones employed the Friedlander reaction between a suitably substituted benzophenone derivative and an active methylene compound to construct the target quinoline nucleus (Scheme I).

Acid-catalyzed condensation of the requisite 2-aminobenzophenones[8] with various β-keto esters produced esters 1a–e. Reduction to alcohols 2a–e followed by Swern oxidation afforded the corresponding aldehydes 3a–e, which were converted, with >95% *E* selectivity, to α,β-unsaturated esters 4a–e by reaction with carbomethoxymethylenetriphenylphosphorane. DIBAL-H reduction afforded alcohols 5a–e, which were oxidized to aldehydes 6a–e by employing either MnO_2 or the Swern procedure. Condensation with the dianion of ethyl acetoacetate[9] then gave δ-hydroxy-β-keto esters 7a–e. Stereoselective reduction employing the boron-chelation method of Narasaka and Pai[10] gave, after hydrolysis, a mixture of *erythro*- and *threo*-1,3-dihydroxy acids (>12:1) which were lactonized by refluxing in toluene with azeotropic removal of water. Generally, the lactones were crystalline, such that the small amount of the cis stereoisomer present was easily removed by recrystallization, providing almost exclusively the racemic trans stereoisomers 8a–e.

Compounds containing a saturated bridging unit were readily available from 4 via catalytic hydrogenation to give 9. The same sequence of steps utilized for the synthesis of lactones 8a–e was then employed to convert 9 to lactone 10.

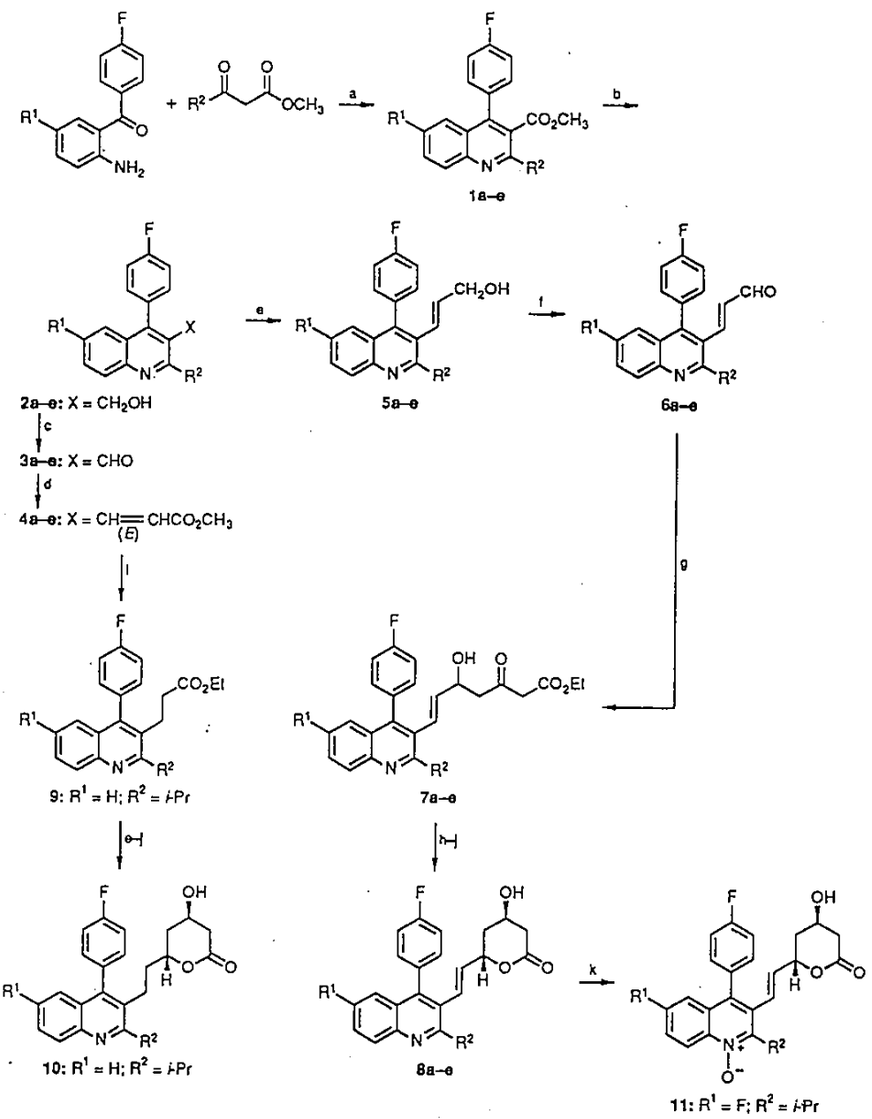
Treatment of lactone 8d with *m*-chloroperbenzoic acid in refluxing dichloromethane produced *N*-oxide 11, which was expected to exhibit very different physicochemical properties than the parent quinoline (vide supra).

Lactone 8d was also synthesized as the pure, biologically active 3*R*,5*S* stereoisomer employing Heathcock's β-ketophosphonate lactone synthon[11] (Scheme II). Thus, β-ketophosphonates 12 and 13 (prepared as an 8:1 mixture of diastereomers employing the literature procedure[12]) were

(1) A preliminary report of this work was presented at the 198th Meeting of the ACS, Miami, FL, September 10–15, 1989, MEDI 73. Following this report workers at Bayer AG presented data on a similar series of compounds at the 10th International Symposium on Drugs Affecting Lipid Metabolism, Houston, TX, November 8–11, 1989, Abstracts 510, 511.
(2) Roth, B. D.; Ortwine, D. F.; Stratton, C. D.; Sliskovic, D. R.; Wilson, M. W.; Newton, R. S. *J. Med. Chem.* **1990**, *33*, 21 and references contained therein.
(3) Sliskovic, D. R.; Roth, B. D.; Wilson, M. W.; Hoefle, M. L.; Newton, R. S. *J. Med. Chem.* **1990**, *33*, 31.
(4) Beck, G.; Kesseler, K.; Baader, E.; Bartmann, W.; Bergmann, E.; Granzer, H.; Jendralla, B.; v. Kerekjarto, B.; Krause, R.; Paulus, E.; Schubert, W.; Wess, G. *J. Med. Chem.* **1990**, *33*, 52.
(5) Coppola, G. M.; Scallen, T. J.; DelPrete, A.; Montano, R. *Heterocycles* **1989**, *29*, 1497.
(6) Kathawala, F. G.; Scallen, T. J.; Engstom, R. G.; Weinstein, D. B.; Schuster, H.; Stabler, R. *Abstracts of Papers*, 194th National Meeting of the American Chemical Society, New Orleans, August 30–September 4, 1987; American Chemical Society: Washington, DC, 1987; MEDI 79.
(7) Roth, B. D.; Sliskovic, D. R.; Trivedi, B. K. *Annu. Rep. Med. Chem.* **1989**, *24*, 147.
(8) Walsh, D. A. *Synthesis* **1980**, 677 and references contained therein.
(9) Huckin, S. N.; Weiler, L. *J. Am. Chem. Soc.* **1981**, *96*, 1082.
(10) (a) Narasaka, K.; Pai, H. C. *Chem. Lett.* **1980**, 1415. (b) Ibid. *Tetrahedron* **1984**, *40*, 2233.
(11) Heathcock, C. H.; Hadley, C. R.; Rosen, T.; Theisen, P. D.; Hecker, S. J. *J. Med. Chem.* **1987**, *30*, 1858.

0022-2623/91/1834-0367$02.50/0

Scheme I[a]



[a] (a) pTSA, toluene, Δ; (b) DIBAL-H, CH_2Cl_2, −78 °C; (c) $(COCl)_2$, DMSO, TEA, −78 °C; (d) $Ph_3P{=}CHCO_2CH_3$; (e) DIBAL-H, CH_2Cl_2, −78 °C; (f) Swern or MnO_2, toluene, Δ; (g) $^-CH_2CO{-}CHCO_2Et$; (h) $B(Et)_3$, $NaBH_4$, $(CH_3)_3CCO_2H$ then H_2O_2; (i) NaOH then HCl; (j) toluene, Δ; (k) *m*CPBA, CH_2Cl_2, Δ; (l) 10% Pd/C, H_2, MeOH.

coupled with aldehyde 3, employing the conditions developed by Roush and Masamune[13] (LiCl, DBU, CH_2Cl_2), in 64% yield. This yield represents the best achieved.[14] The resulting enones (14 and 15) were deprotected and stereoselectively reduced (Et_3B, $NaBH_4$) to give a mixture of *erythro*- (16) and *threo*-1,3-dihydroxy esters. Saponification followed by lactonization and chromatography gave predominantly *trans*-lactone (+)-17 (trans:cis = 26:1). HPLC analysis of the corresponding (*R*)-(+)-α-methylbenzamide derivatives indicated an enantiomeric purity of 89% ee.

In an attempt to increase the aqueous solubility of these compounds (and thereby improve absorption in vivo), a dimethylamino group was incorporated into position 2 of the quinoline ring in place of the isopropyl group (Scheme III).

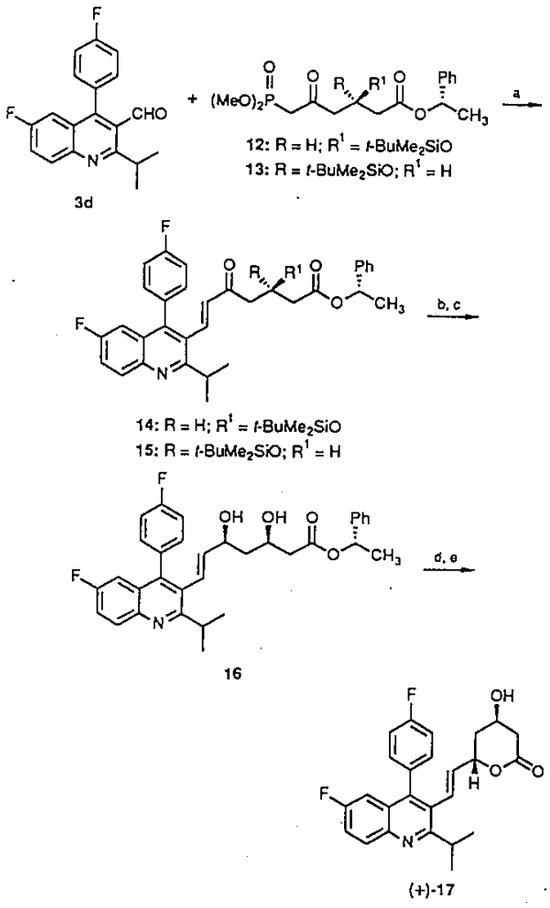
Treatment of benzophenone 18 with ethyl malonyl chloride and silica gel gave 1,2-dihydroquinoline 19 in 88% yield. Chlorination using phosphoryl chloride gave ester 20, which was then reduced and reoxidized to aldehyde 21. Nucleophilic substitution of the chloride with dimethylamine gas in toluene at 130 °C (autoclave) gave dimethylamino aldehyde 22. Aldehyde 22 was then con-

(12) This ratio of diastereomers may be improved to 22:1 by employing (*R*)-1-(1′-naphthyl)ethanol as chiral auxilliary; see: Theisen, P. D.; Heathcock, C. H. *J. Org. Chem.* 1988, *53*, 2374.

(13) Blanchette, M. A.; Choy, C. O.; Davis, J. T.; Essenfield, A. P.; Masamune, S.; Roush, W. R. *Tetrahedron Lett.* 1984, *25*, 2183.

(14) A variety of other conditions were examined, e.g., K_2CO_3/18-crown-6/toluene, $(NH_4)_2CO_3$/toluene, and NaH/THF, however, all of these led to β-elimination products derived from both the starting materials (12 and 13) and products (14 and 15). See: Rosen, T.; Heathcock, C. H. *J. Am. Chem. Soc.* 1985, *107*, 3731.

Scheme II[a]



[a] (a) LiCl, DBU, CH_2Cl_2, −10 °C; (b) HF, CH_3CN; (c) $B(Et)_3$, $NaBH_4$, $(CH_3)_3CCO_2H$ then H_2O_2; (d) NaOH then HCl; (e) toluene, Δ.

verted to the desired lactone 26 by employing the chemistry described previously.

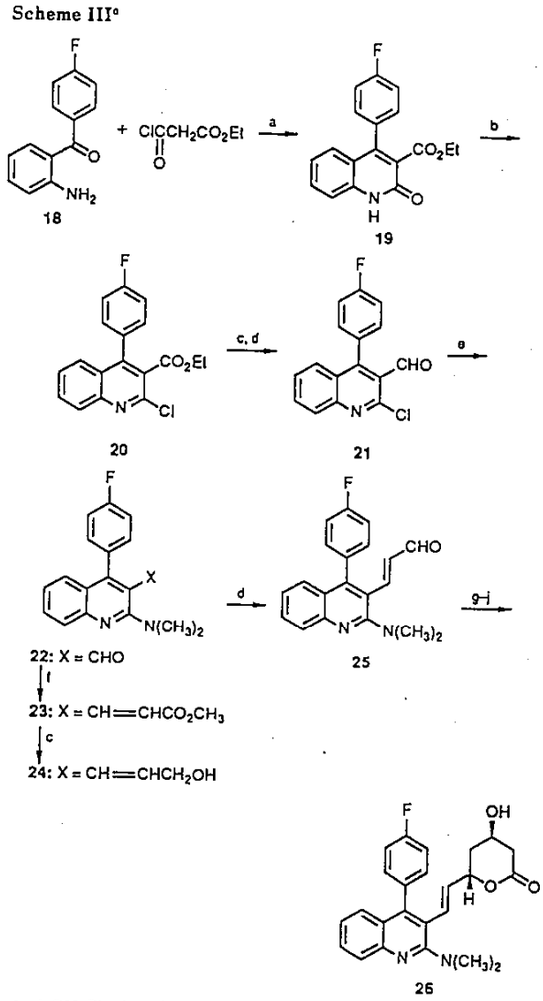
Quinolin-4-ylmevalonolactone 34 was synthesized as shown in Scheme IV. Methyl 3-methyl-4-quinolinecarboxylate[15] (27) was reduced to alcohol 28 and then oxidized under Swern conditions to aldehyde 29. α,β-Unsaturated aldehyde 32 was constructed in an entirely analogous manner to that depicted in Scheme I and was subsequently treated with the dianion of ethyl acetoacetate to yield 33, which was converted to the target lactone 34 (trans:cis = 23:1).

Biological Results

The lactones listed in Table I were saponified to the 3,5-dihydroxy acids and tested for their ability to inhibit the enzyme HMG-CoA reductase, employing two protocols.[2] Method I (cholesterol synthesis inhibition screen or CSI) measured the rate of conversion of [^{14}C]acetate to cholesterol by employing a crude liver homogenate derived from rats fed a chow diet containing 5% cholestyramine. Method II (HMG-CoA reductase inhibition screen or COR) was a more specific screen employing a partially purified microsomal enzyme preparation to measure the direct conversion of [^{14}C]HMG-CoA to mevalonic acid. The

(15) Lindberg, U. H.; Ulff, B.; Yeoman, G. *Acta. Pharm. Suec.* 1968, *5*, 441.

Scheme III[a]



[a] (a) CH_2Cl_2 then SiO_2; (b) $POCl_3$, Δ; (c) DIBAL-H, CH_2Cl_2, −78 °C; (d) $(COCl)_2$, DMSO, TEA, −78 °C; (e) $HN(CH_3)_2$, toluene, autoclave, 130 °C; (f) $Ph_3P{=}CHCO_2CH_3$, CH_2Cl_2; (g) $^-CH_2CO^-CHCO_2Et$; (h) $B(Et)_3$, $NaBH_4$, $(CH_3)_3CCO_2H$ then H_2O_2; (i) NaOH then HCl; (j) toluene, Δ.

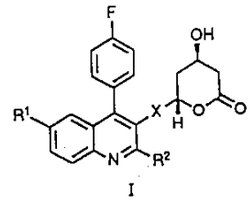
biological activities are displayed in Table I as an IC_{50} (i.e., the concentration needed to inhibit enzyme activity by 50%). Compactin was employed as the internal standard in each testing protocol. The compounds were also evaluated for their ability to inhibit cholesterol biosynthesis in male rats, as determined by the inhibition of the incorporation of sodium [1-^{14}C]acetate into plasma [^{14}C]-cholesterol after po administration of the test substance.[16] This screen was designated the AICS (acute inhibition of cholesterol synthesis) screen.

Most of the compounds tested were more potent than compactin in the in vivo screen and 8b–e exhibited both in vitro and in vivo potencies comparable to those of mevinolin.

As expected, an isopropyl group at position 2 of the quinolinyl-3-mevalonolactones produced a compound, 8b,

(16) Alberts, A. W.; Chen, J.; Kuron, J.; Hunt, V.; Huff, J.; Hoffman, C.; Rothrock, J.; Lopez, M.; Joshua, H.; Harris, E.; Patchett, A.; Monaghan, R.; Currie, S.; Stapley, E.; Albers-Schonberg, G.; Hensens, O.; Hoogsteen, K.; Liesch, J.; Springer, J. *Proc. Natl. Acad. Sci. U.S.A.* 1980, *77*, 3997.

Table I. Physical Properties and in Vitro and in Vivo HMG-CoA Reductase Inhibitory Activities of Quinoline Mevalonolactones I

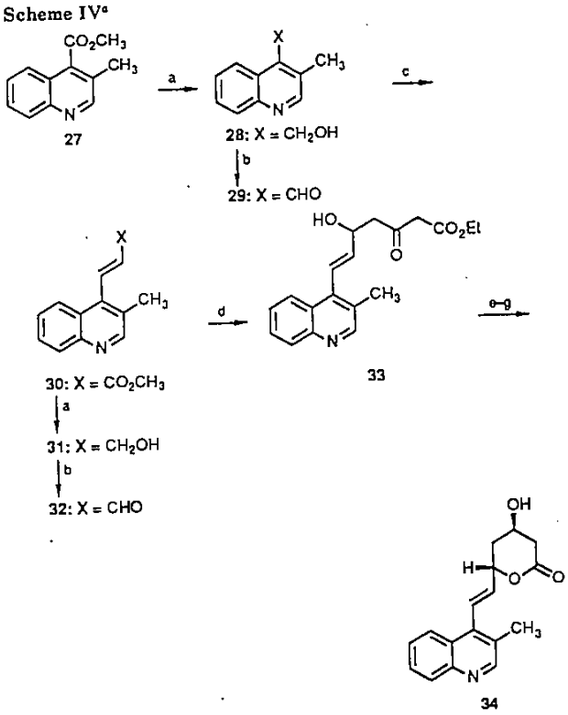


I

no.	R^1	R^2	X	mp, °C	formula[a]	CSI[b,c] IC_{50}, μM	rel (CSI)[d] potency	COR[c,h] IC_{50}, μM	AICS[e] (% inhibn)
compactin						0.030		0.025	36
mevinolin						0.025	118	0.028	72
8a	Cl	CH_3	$-CH=CH-$	188–190	$C_{23}H_{19}ClFNO_3$	0.4	6.3	0.72	18 (1.5)
8b	Cl	$CH(CH_3)_2$	$-CH=CH-$	173–175	$C_{25}H_{23}ClFNO_3$	0.032	100	0.025	61 (1.5)
8c	H	$CH(CH_3)_2$	$-CH=CH-$	168–170	$C_{25}H_{24}FNO_3$	0.042	75.8	0.032	70
10	H	$CH(CH_3)_2$	$-CH_2CH_2-$	199–202	$C_{25}H_{26}FNO_3$	>1.0	<1	–	–
8d	F	$CH(CH_3)_2$	$-CH=CH-$	174–176	$C_{25}H_{23}F_2NO_3$	0.05	77.6	0.20	68
17	F	$CH(CH_3)_2$	$-CH=CH-$	foam	$C_{25}H_{23}F_2NO_3 \cdot 0.25C_4H_8O_2$	ND[g]	ND[g]	–	69
11 (*N*-oxide)	F	$CH(CH_3)_2$	$-CH=CH-$	235–238	$C_{25}H_{23}F_2NO_4$	0.018	112	0.079	47
8e	OCH_3	$CH(CH_3)_2$	$-CH=CH-$	foam	$C_{26}H_{26}FNO_4$	0.013	100	0.053	60
26	H	$N(CH_3)_2$	$-CH=CH-$	150–152	$C_{24}H_{23}FN_2O_3 \cdot 0.5C_4H_8O_2$	0.047	13.2	0.35	52
34				198–200	$C_{17}H_{17}NO_3 \cdot 0.25C_4H_8O_2$	>1.0	<1	–	42

[a] Analytical results are within ±0.4% of the theoretical values unless otherwise noted. [b] Cholesterol synthesis inhibition (CSI). Assays of each inhibitor concentration were performed in triplicate, and the precision for compactin was 37%. [c] All compounds tested had a diastereomeric purity of >95% of the trans diastereomer as determined by HPLC and/or 200-MHz NMR. [d] Potency of compactin arbitrarily assigned a value of 100 and the IC_{50} value of the test compound was compared with that of compactin determined simultaneously. [e] All compounds were dosed in DMA/PEG solution of 1.0 mg/kg unless otherwise indicated in parentheses. [f] Anal. Calcd: C, 71.70. Found: C, 70.67. >98% pure by HPLC. [g] Not determined. [h] HMG-CoA reductase inhibition (COR). Assays of each inhibitor concentration were performed in triplicate, and the precision for compactin was 37%.

Scheme IV[a]



[a] (a) DIBAL-H, CH_2Cl_2, −78 °C; (b) $(COCl)_2$, DMSO, TEA, −78 °C; (c) $Ph_3P{=}CHCO_2CH_3$, CH_2Cl_2; (d) $^-CH_2CO^-CHCO_2Et$; (e) $B(Et)_3$, $NaBH_4$, $(CH_3)_3CCO_2H$ then H_2O_2; (f) $NaOH^-$ then HCl; (g) toluene, Δ.

significantly more potent both in vitro and in vivo than the corresponding 2-methyl compound 8a. Compound 10, which has a saturated two-carbon bridging unit between the quinoline moiety and the mevalonolactone, was considerably less potent than the corresponding unsaturated bridge containing compound 8c.

As previous studies suggested that the 4-(4-fluorophenyl) and 2-(1-methylethyl) substitution afforded optimum potency, attention was focused on variations at position 6 of the quinoline ring. From the limited number of compounds prepared (i.e., 8b–e), it can be seen that varying the substitution at position 6 did not significantly effect either in vivo or in vitro potencies. The dimethylamino-containing compound 26 retained in vivo potency when compared to the corresponding isopropyl-containing compound 8c, but was somewhat less potent in vitro.

N-Oxide 11 was as potent in vitro as compactin and mevinolin and more potent than the corresponding free base but was slightly less potent in vivo.

Quinolin-4-ylmevalonolactone 34 was considerably less potent than either compactin or mevinolin in vitro, however it was comparable to compactin when tested in vivo. The source of the in vivo activity for 34, despite its lack of in vitro activity, is unclear.

Conclusion

A series of quinoline mevalonolactones was prepared and evaluated for their ability to inhibit the enzyme HMG-CoA reductase in vitro and cholesterol biosynthesis in vivo. By focusing on compounds possessing the 4-(4-fluorophenyl) and 2-(1-methylethyl) substituents found to be optimum in previous studies, several compounds, i.e., 8b, 8e, and 11, were identified that were of comparable potency to compactin and mevinolin both in vitro and in vivo. Modifications at position 6 of the quinoline ring had little effect on potency.

In conclusion it has been shown that the quinoline nucleus can be used as a suitable replacement for the hexahydronaphthalene ring present in the fungal metabolites compactin and mevinolin. Compounds have been described which are equipotent to both naturally occurring HMG-CoA reductase inhibitors under the conditions studied.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Tetrahydrofuran (THF) was distilled from sodium and benzophenone. All organic extracts were dried over $MgSO_4$ except where otherwise noted. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were determined on a Nicolet MX-1 FT-IR spectrometer. Nuclear magnetic resonance spectra were determined on either a Varian EM-390 or a Varian XL-200 spectrometer. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. Elemental analyses were determined on a Perkin-Elmer 240C elemental analyzer. HPLC analyses were performed on a Varian 5500 HPLC with a UV 200 detector (wavelength was 251 nm) and an octadecylsilane column [Alltech Econisil C18; mobile phase, 50:50 0.05 M citric acid (pH = 4.0)–CH_3CN]. Optical rotations were performed on a Perkin-Elmer 241 polarimeter. The detailed protocols of the in vitro biological assays are described in ref 2.

Methyl 4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinecarboxylate (1c). A solution of methyl 4-methyl-3-oxopentanoate (14.7 g, 0.102 mol), (2-aminophenyl)-4-(fluorophenyl)methanone[15] (18.34 g, 0.085 mol), and a small amount of *p*-TSA in toluene (400 mL) was heated under reflux with azeotropic removal of water for 5 h. The solution was then cooled and concentrated in vacuo. Flash chromatography of the residue, eluting with 10% ethyl acetate–hexane, gave 1c (7.66 g, 28%): ^{1}H NMR ($CDCl_3$) δ 8.05 (d, 1 H), 7.72–6.95 (m, 7 H), 3.52 (s, 3 H), 3.16 (heptet, 1 H), 1.40 (d, 6 H) ppm. Anal. ($C_{20}H_{18}FNO_2$) C, H, N.

4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinemethanol (2c). To a solution of 1c (7.66 g, 0.024 mol) in dichloromethane (100 mL) at −78 °C under an atmosphere of nitrogen was added 55 mL of a 1.0 M solution of DIBAL-H. The resulting solution was stirred for 3 h before quenching with saturated aqueous sodium sulfate (20 mL). After warming to room temperature, the solution was filtered through Celite and the resulting filtrate dried and concentrated in vacuo to yield 6.61 g (94%) of 2c: ^{1}H NMR ($CDCl_3$) δ 7.97 (d, 1 H), 7.57–6.93 (m, 7 H), 4.52 (bs, 2 H), 3.62 (heptet, 1 H), 1.9 (bs, 1 H), 1.43 (d, 6 H) ppm. Anal. ($C_{19}H_{18}FN_2O$) C, H, N.

4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinecarboxaldehyde (3c). To a solution of oxalyl chloride (2.3 mL, 0.027 mol) in anhydrous dichloromethane (50 mL), at −78 °C under an atmosphere of nitrogen, was added dimethyl sulfoxide (3.8 mL, 0.053 mol). After complete addition the resulting solution was stirred for 15 min at −78 °C and then a solution of 2c (6.05 g, 0.02 mol) in dichloromethane (50 mL) was added dropwise. This was stirred for a further 1 h at −78 °C and then quenched by the addition of triethylamine (14.3 mL, 0.103 mol) and saturated aqueous ammonium chloride solution (15 mL). The organic layer was separated and the aqueous layer was extracted with additional dichloromethane. The combined organic layers were dried, filtered, and concentrated in vacuo to yield 3c (6.38 g, quant.) as a pale yellow solid: mp 119–121 °C; ^{1}H NMR ($CDCl_3$) δ 9.92 (s, 1 H), 8.02 (d, 1 H), 7.72–7.52 (m, 1 H), 7.37–6.98 (m, 6 H), 3.94 (heptet, 1 H), 1.38 (d, 6 H) ppm. Anal. ($C_{19}H_{16}FNO$) C, H, N.

Methyl (*E*)-3-[4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]-2-propenoate (4c). Methyl (triphenylphosphoranylidene)acetate (7.5 g, 0.024 mol) and 3c (6.38 g, 0.021 mol) in dichloromethane (100 mL) were stirred at room temperature under nitrogen for 72 h. The solution was then concentrated in vacuo. Flash chromatography on silica gel, eluting with hexanes–ethyl acetate, gave 4c (5.62 g, 74%) as a pale orange solid: mp 147–149 °C; ^{1}H NMR ($CDCl_3$) δ 7.96 (d, 1 H), 7.72–7.04 (m, 8 H), 5.58 (d, 1 H), 3.63 (s, 3 H), 3.38 (heptet, 1 H), 1.35 (d, 6 H) ppm. Anal. ($C_{22}H_{20}FNO_2$) C, H, N.

(*E*)-3-[4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]-2-propen-1-ol (5c). To a solution of 4c (5.62 g, 0.016 mol) in dichloromethane (100 mL) at −78 °C under an atmosphere of nitrogen was added 37.7 mL of a 1.0 M solution of DIBAL-H. The resulting solution was stirred for 2 h at −78 °C and then quenched by addition of saturated aqueous sodium sulfate (15 mL). After warming to room temperature, the solution was filtered through Celite. The resulting filtrate was dried and concentrated in vacuo. The residue was flash chromatographed, eluting with 10% ethyl acetate–hexanes, to yield 5c (4.7 g, 91%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.99 (d, 1 H), 7.60–6.97 (m, 7 H), 6.48 (d, 1 H), 5.45 (dt, 1 H), 4.00 (bs, 2 H), 3.48 (heptet, 1 H), 2.05 (bs, 1 H), 1.38 (d, 6 H) ppm.

(*E*)-3-[4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]-2-propenal (6c). To a solution of oxalyl chloride (1.66 mL, 0.019 mol) in anhydrous dichloromethane (25 mL), at −78 °C under an atmosphere of nitrogen, was added dimethyl sulfoxide (2.75 mL, 0.038 mol) in dichloromethane (25 mL). The resulting solution was stirred for 15 min at −78 °C and then a solution of 5c (4.7 g, 0.015 mol) in dichloromethane (50 mL) was added dropwise. This was stirred for 1 h and then quenched by the addition of triethylamine (10.2 mL, 0.073 mol) and saturated aqueous ammonium chloride solution (15 mL). The organic layer was separated and the aqueous layer was extracted with additional dichloromethane. The combined organic layers were dried, filtered, and concentrated in vacuo to yield 6c (4.37 g, 94%): ^{1}H NMR ($CDCl_3$) δ 9.36 (d, 1 H), 7.96 (d, 1 H), 7.63–7.00 (m, 8 H), 5.90 (dd, 1 H), 3.4 (heptet, 1 H), 1.4 (d, 6 H) ppm.

Ethyl (*E*)-7-[4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]-5-hydroxy-3-oxo-6-heptenoate (7c). Ethyl acetoacetate (2.25 g, 0.017 mol) in anhydrous THF (25 mL) was added dropwise to a stirred suspension of sodium hydride (60% oil suspension, 0.74 g, 0.018 mol) in anhydrous THF (25 mL) at 0 °C under a nitrogen atmosphere. When gas evolution was complete, a 2.4 M solution (7.2 mL, 0.017 mol) of *n*-butyllithium in hexanes was added over 30 min. This was then treated with a solution of 6c (3.68 g, 0.011 mol) in anhydrous THF added dropwise over 30 min. The resulting solution was stirred for 1 h at −78 °C and then quenched by the addition of glacial acetic acid (15 mL) with vigorous stirring. The resulting mixture was then partitioned between diethyl ether and water. After separation of the phases, the aqueous layer was reextracted with diethyl ether, and the combined organic extracts were washed with saturated aqueous sodium bicarbonate and dried. The solvents were removed in vacuo, and the residue was flash chromatographed with hexanes–ethyl acetate as eluant to yield 5.1 g (95%) of the title compound 7c as an orange oil: ^{1}H NMR ($CDCl_3$) δ 8.07 (d, 1 H), 7.64–7.17 (m, 7 H), 6.62 (d, 1 H), 5.34 (dd, 1 H), 4.59 (m, 1 H), 4.21 (q, 2 H), 3.48 (heptet, 1 H), 3.41 (s, 2 H), 2.44 (d, 2 H), 1.38 (d, 6 H), 1.29 (t, 3 H) ppm.

[4α,6β(*E*)]-6-[2-[4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]ethenyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (8c). To a room temperature solution of triethylborane (7.2 mL of a 1 M THF solution; 0.007 mol) under a dry-air atmosphere was added, with stirring, a catalytic amount of pivalic acid (0.7 g, 0.0007 mol). The resulting solution was stirred at room temperature for 10 min before a THF (25 mL) solution of 7c (3.0 g, 0.007 mol) was added dropwise. The resulting solution was stirred at room temperature for a further 15 min before cooling to −78 °C. Methanol (5 mL) was added followed by the addition of sodium borohydride (0.28 g, 0.007 mol) in one portion. Vigorous effervescence ensued. This mixture was stirred at −78 °C for 6 h. It was then quenched by pouring into ice-cold 30% hydrogen peroxide (10 mL). The mixture was allowed to warm slowly to room temperature and then was partitioned between chloroform and water. The organic layer was washed extensively with water, dried, and concentrated in vacuo to yield 3.07 g of the corresponding 1,3-diols as a mixture of erythro and threo diastereomers which were used without any further purification.

This residue was then redissolved in THF (50 mL) and methanol (5 mL) and treated with 1 N aqueous sodium hydroxide (6.7 mL). The resulting solution was stirred at room temperature for 2 h and then concentrated to dryness. The residue was then partitioned between water and ether. The ether layer was extracted with 1 N aqueous NaOH. The aqueous layers were combined, acidified with concentrated HCl, and extracted with ethyl acetate. The ethyl acetate extracts were combined, washed with water, and dried. Removal of the solvents in vacuo yielded a yellow foam which was dissolved in toluene (100 mL) and heated for 3 h at reflux with azeotropic removal of water. The cooled solution was concentrated and the residue flash chromatographed on silica gel, eluting with 50% hexanes–ethyl acetate to yield 8c (1.26 g, 56%) as a white solid, which was shown to be a 97:3 mixture of trans and cis diastereomers by HPLC: mp 168–170

°C; ^{1}H NMR ($CDCl_3$) δ 8.02 (d, 1 H), 7.71 (dt, 1 H), 7.51–7.28 (m, 6 H), 6.69 (d, 1 H), 5.48 (dd, 1 H), 5.24 (bs, 1 H), 5.10–5.00 (m, 1 H), 4.0 (bs, 1 H), 3.48 (heptet, 1 H), 2.67–2.31 (m, 2 H), 1.57–1.42 (m, 2 H), 1.33 (d, 6 H) ppm; IR (KBr) 3430, 2967, 1715, 1514, 1256, 1224, 1160, 1067, 974 cm^{-1}. Anal. ($C_{25}H_{24}FNO_3$) C, H, N.

Compounds 8a–e were synthesized by the general method outlined in Scheme I and exemplified for compound 8c; their physical and biological properties are listed in Table I.

[4α,6β(*E*)]-6-[2-[6-Fluoro-4-(4-fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]ethenyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one, *N*-Oxide (11). A dichloromethane solution (100 mL) of 8d and *m*-CPBA was heated under reflux for 6 h under an atmosphere of nitrogen. The solution was then cooled and washed with saturated aqueous sodium bicarbonate solution. The organic layer was then dried, filtered, and concentrated in vacuo to yield an orange foam (1.24 g), which was flash chromatographed (eluant, 30% ethyl acetate–hexanes) to yield 11 (0.77 g, 74%) as a white solid: mp 235–238 °C; ^{1}H NMR ($CDCl_3$) δ 8.81 (dd, 1 H), 7.49–7.41 (m, 1 H), 7.20 (d, 4 H), 7.01 (dd, 1 H), 6.53 (d, 1 H), 5.44 (dd, 1 H), 5.18–5.13 (m, 1 H), 5.02 (bs, 1 H), 4.15–4.09 (m, 1 H), 3.74 (m, 1 H), 2.79 (bs, 2 H), 2.60 (d, 2 H), 1.55 (d, 6 H) ppm; IR (KBr) 3430, 3260, 1730, 1624, 1513, 1303, 1248, 1218, 1049, 831 cm^{-1}. Anal. ($C_{25}H_{23}F_2NO_4$) C, H, N.

The compounds bearing a saturated two-carbon spacer between the quinoline nucleus and the lactone moiety can be synthesized in an entirely similar manner to that of lactones 8a–e. The experimental details for the key reduction of the α,β-unsaturated esters 4 is exemplified below for the preparation of compound 9.

Methyl 3-[4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]propanoate (9). Compound 4c (10.0 g, 0.029 mol) and 10% Pd/C (0.75 g) were stirred in methanol (250 mL) at room temperature under 50 psi of hydrogen gas. After 5 h, the suspension was filtered and the filtrate concentrated in vacuo to yield 10.14 g of an orange oil. Trituration with hexanes afforded 6.06 g (60%) of 9 as an off-white solid: mp 117–119 °C; ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 1 H), 7.62 (t, 1 H), 7.33 (t, 1 H), 7.29–7.16 (m, 5 H), 3.64 (s, 3 H), 3.44 (heptet, 1 H), 2.96 (t, 2 H), 2.39 (t, 2 H), 1.44 (d, 6 H) ppm. Anal. ($C_{22}H_{22}FNO_2$) C, H, N.

[*R*-(*R,*R**)]-1-Phenylethyl 3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-7-[6-fluoro-4-(4-fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]-5-oxo-6-heptenoate (14).** To a solution of 3d (0.6 g, 0.002 mol) and β-ketophosphonates (12–13, 8:1 mixture of diastereomers) (1.35 g, 0.003 mol) in dichloromethane (10 mL) at −10 °C under a nitrogen atmosphere was added a small amount of LiCl and DBU (2.85 mL, 0.019 mol). The resulting orange solution was stirred at −10 °C for 1.5 h and then quenched by addition of ice-cold phosphoric acid (0.5 M). The organic layer was separated, washed with water, dried, filtered, and concentrated in vacuo to yield a yellow oil (1.65 g). Flash chromatography on silica gel, eluting with 10% ethyl acetate–hexanes gave recovered aldehyde 3d (0.29 g, 0.0009 mol, 48%), 14–15 (0.42 g, 0.0006 mol, 33%), and recovered β-ketophosphonate 12–13: ^{1}H NMR ($CDCl_3$) δ 7.98 (dd, 1 H), 7.51 (d, 1 H), 7.33–6.84 (m, 11 H), 5.89 (d, 1 H), 5.77 (q, 1 H), 4.45 (m, 1 H), 3.34 (heptet, 1 H), 2.59 (d, 2 H), 2.40 (d, 2 H), 1.48 (d, 3 H), 1.33 (d, 6 H), 0.78 (s, 9 H), 0.01 (s, 6 H) ppm.

[4*R*-[4α,6β(*E*)]]-6-[2-[6-Fluoro-4-(4-fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]ethenyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (17). A solution of 48% aqueous HF (0.36 mL, 0.0007 mol) in acetonitrile (3 mL) was added to a solution of 14–15 (0.42 g, 0.0006 mol) in acetonitrile (3 mL). The resulting solution was stirred at room temperature for 1.5 h. It was then diluted with diethyl ether (20 mL) and washed with saturated aqueous sodium bicarbonate solution. The organic layer was dried and concentrated in vacuo to give the desilylated compound (0.31 g, 0.0006 mol, 89%) as a colorless oil, which was used in the next step without any further purification: ^{1}H NMR ($CDCl_3$) δ 8.02 (dd, 1 H), 7.58 (d, 1 H), 7.39–6.83 (m, 11 H), 5.93 (d, 1 H), 5.85 (q, 1 H), 4.34 (m, 1 H), 3.34 (heptet, 1 H), 2.59 (d, 2 H), 2.48 (d, 2 H), 1.52 (d, 3 H), 1.37 (d, 6 H) ppm.

The alcohols were then dissolved in anhydrous THF (5 mL) containing pivalic acid (0.006 g, 0.00006 mol) under a dry-air atmosphere at room temperature. To this solution was added triethylborane (0.63 mL of a 1 M THF solution; 0.0006 mol). The resulting solution was stirred at room temperature for 10 min before cooling to −78 °C. Methanol (1 mL) was added, followed by sodium borohydride (0.024 g, 0.0006 mol) in one portion. Vigorous effervescence ensued. This mixture was stirred at −78 °C for 6 h and then quenched by pouring into ice-cold 30% hydrogen peroxide (1 mL). The mixture was allowed to warm slowly to room temperature and then partitioned between chloroform and water. The organic layer was washed extensively with water, dried, and concentrated in vacuo to yield a foam (0.25 g) which contained compound 16 as its major component.

The crude product was then dissolved in THF (5 mL) and methanol (0.5 mL) and treated with 1 N aqueous sodium hydroxide (0.46 mL). This solution was stirred at room temperature for 3 h, and then all solvents were removed in vacuo. The residue was partitioned between diethyl ether and water. The aqueous layer was acidified with 1 N hydrochloric acid, extracted with ethyl acetate, dried, filtered, and concentrated in vacuo to yield a yellow foam, which was redissolved in toluene (60 mL) and heated for 6 h at reflux with azeotropic removal of water. The cooled solution was concentrated and the residue flash chromatographed on silica gel, eluting with 30% ethyl acetate–hexanes, to give 17 (0.035 g, 18%) as a white foam: $[\alpha]_D = +3.4°$ ($c = 0.235$, $CHCl_3$); HPLC analysis of the corresponding (*R*)-(+)-α-methylbenzylamide derivative indicated an enantiomeric purity of 89% ee; ^{1}H NMR ($CDCl_3$) δ 8.09 (dd, 1 H), 7.47–7.37 (m, 1 H), 7.27–7.18 (m, 4 H), 6.99 (dd, 1 H), 6.68 (d, 1 H), 5.38 (dd, 1 H), 5.20–5.10 (m, 1 H), 4.25–4.19 (m, 1 H), 3.46 (heptet, 1 H), 2.77–2.52 (m, 2 H), 1.83–1.26 (m, 9 H) ppm. Anal. ($C_{25}H_{23}F_2NO_3 \cdot 0.25C_4H_8O_2$) C, H, N.

Ethyl 4-(4-Fluorophenyl)-1,2-dihydro-2-oxo-3-quinolinecarboxylate (19). Ethyl malonyl chloride (125 g, 0.84 mol) was added in portions to a solution of 18[17] in dichloromethane (1 L) at 0 °C under an atmosphere of nitrogen. The reaction mixture was warmed slowly (~1 h) to room temperature, dried, and concentrated to an approximate volume of 600 mL. Silica gel (50 g) was then added. The resulting suspension was stirred overnight at room temperature, and filtered, and the silica gel was washed extensively with ethyl acetate. The filtrate was then concentrated and the residue triturated with hexanes to yield 19 (192 g, 88%) as a white solid: mp 204–206 °C; ^{1}H NMR ($CDCl_3$) δ 12.60 (bs, 1 H), 7.60–7.10 (m, 8 H), 4.17 (q, 2 H), 1.04 (t, 3 H) ppm. Anal. ($C_{18}H_{14}FNO_3$) C, H, N.

Ethyl 2-Chloro-4-(4-fluorophenyl)-3-quinolinecarboxylate (20). A solution of 19 (12.8 g, 0.041 mol) in phosphorus oxychloride (40 mL) was heated to reflux under an atmosphere of nitrogen for 1 h. It was then cooled and concentrated in vacuo and the resulting residue neutralized by the careful addition of cold 1 N sodium hydroxide solution. This was then extracted with ethyl acetate; the organic solution was filtered through a small bed of silica gel to yield 20 (13.2 g, 98%) as a white solid: mp 113–114 °C; ^{1}H NMR ($CDCl_3$) δ 8.02 (d, 1 H), 7.75–7.70 (m, 1 H), 7.52–7.43 (m, 2 H), 7.34–7.28 (m, 2 H), 7.20–7.12 (m, 2 H), 4.14–4.07 (q, 2 H), 1.02 (t, 3 H) ppm. Anal. ($C_{18}H_{13}ClFNO_2$) H, N, Cl, F; C: calcd, 65.56; found, 66.17.

2-Chloro-4-(4-fluorophenyl)-3-quinolinecarboxaldehyde (21). Compound 20 was reduced to the corresponding alcohol, 2-chloro-4-(4-fluorophenyl)-3-quinolinemethanol, in 83% yield in a manner analogous to the reduction of compounds 1a–e to compounds 2a–e in Scheme I: mp 159–160 °C; ^{1}H NMR ($CDCl_3$) δ 8.07 (d, 1 H), 7.79–7.70 (m, 1 H), 7.53–7.22 (m, 6 H), 4.67 (d, 2 H), 2.24 (t, 1 H) ppm. Anal. ($C_{16}H_{11}ClFNO$) C, H, N.

This compound was then oxidized to 21 in a manner analogous to the oxidation of compounds 2a–e to compounds 3a–e in Scheme I: mp 168–169.5 °C; yield 90%; ^{1}H NMR ($CDCl_3$) δ 10.25 (s, 1 H), 8.12 (d, 1 H), 7.91–7.83 (m, 1 H), 7.57–7.53 (m, 2 H), 7.36–7.22 (m, 4 H) ppm. Anal. ($C_{16}H_9ClFNO$) C, H, N.

2-(Dimethylamino)-4-(4-fluorophenyl)-3-quinolinecarboxaldehyde (22). A solution of 21 (5.28 g, 0.019 mol) and dimethylamine (15 mL) in toluene (75 mL) was heated in an autoclave at 123–126 °C for 14 h. It was then cooled and concentrated in vacuo. The residue was partitioned between ethyl acetate and saturated aqueous potassium carbonate solution. The organic layer was dried, filtered, and concentrated in vacuo. The residue was flash chromatographed on silica gel, eluting with 10%

(17) Suzuki, K.; Weisburger, E. K.; Weisburger, J. H. *J. Org. Chem.* 1961, *26*, 2239.

ethyl acetate–hexanes, to yield 22 (4.2 g, 77%) as an orange solid: ^{1}H NMR ($CDCl_3$) δ 9.73 (s, 1 H), 7.78–6.96 (m, 8 H), 3.10 (s, 6 H) ppm. Anal. ($C_{18}H_{15}FN_2O$) C, H, N.

Methyl (*E*)-3-[2-(dimethylamino)-4-(4-fluorophenyl)-3-quinolinyl]-2-propenoate (23) was prepared analogously to compounds 4a–e in Scheme I: yield 92%; ^{1}H NMR ($CDCl_3$) δ 7.78–6.87 (m, 9 H), 5.98 (d, 1 H), 3.60 (s, 3 H), 2.95 (s, 6 H) ppm. Anal. ($C_{21}H_{19}FN_2O_2$) C, H, N.

(*E*)-3-[2-(Dimethylamino)-4-(4-fluorophenyl)-3-quinolinyl]-2-propen-1-ol (24) was prepared analogously to compounds 5a–e in Scheme I: yield 98%; ^{1}H NMR ($CDCl_3$) δ 7.72 (d, 1 H), 7.50–7.30 (m, 1 H), 7.20–6.98 (m, 6 H), 6.31 (d, 1 H), 5.72 (dt, 1 H), 3.99 (bd, 2 H), 2.96 (s, 6 H), 1.54 (bs, 1 H) ppm. Anal. ($C_{20}H_{19}FN_2O$) H; C: calcd, 74.51; found, 72.52; N: calcd, 8.69; found, 7.84.

(*E*)-3-[2-(Dimethylamino)-4-(4-fluorophenyl)-3-quinolinyl]-2-propenal (25) was prepared analogously to compounds 6a–e in Scheme I: yield 92%; ^{1}H NMR ($CDCl_3$) δ 9.35 (d, 1 H), 7.75 (d, 1 H), 7.58–6.98 (m, 8 H), 6.32 (dd, 1 H), 2.99 (s, 6 H) ppm. Anal. ($C_{20}H_{17}FN_2O$) H, N; C: calcd, 74.98; found, 72.85.

[4α,6β(*E*)]-6-[2-[2-(Dimethylamino)-4-(4-fluorophenyl)-3-quinolinyl]ethenyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (26) was prepared in 29% overall yield from compound 25 in an analogous manner to the preparation of lactones 8a–e from aldehydes 6a–e: mp 150–152 °C; ^{1}H NMR ($CDCl_3$) δ 7.83 (d, 1 H), 7.57–7.50 (m, 1 H), 7.26–7.16 (m, 6 H), 6.49 (d, 1 H), 5.66 (dd, 1 H), 5.16–5.06 (m, 1 H), 4.28–4.25 (m, 1 H), 3.01 (s, 6 H), 2.75–2.60 (q, 2 H), 2.07 (bs, 1 H), 1.82–1.51 (m, 1 H) ppm. Anal. ($C_{24}H_{23}FN_2O_3 \cdot 0.5C_4H_8O_2$) C, H, N.

3-Methyl-4-quinolinemethanol (28) was prepared in 73% yield via a DIBAL-H reduction of 27:[15] ^{1}H NMR ($CDCl_3$) δ 8.55 (s, 1 H), 8.17–7.90 (m, 2 H), 7.68–7.42 (m, 2 H), 5.05 (s, 2 H), 2.46 (s, 3 H), 2.20 (bs, 1 H) ppm.

3-Methyl-4-quinolinecarboxaldehyde (29) was prepared in 70% yield from 28 via a Swern oxidation: ^{1}H NMR ($CDCl_3$) δ 10.77 (s, 1 H), 8.68 (s, 1 H), 8.52–8.41 (m, 1 H), 8.03–7.87 (m, 1 H), 7.67–7.34 (m, 2 H), 2.67 (s, 3 H) ppm.

Methyl (*E*)-3-(3-methyl-4-quinolinyl)-2-propenoate (30) was prepared in 76% yield via treatment of 29 with methyl (triphenylphosphoranylidene)acetate in an analogous manner to the preparation of compounds 4a–e in Scheme I: ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1 H), 8.10–7.34 (m, 5 H), 6.21 (d, 1 H), 3.80 (s, 3 H), 2.42 (s, 3 H) ppm. Anal. ($C_{14}H_{13}NO_2$) C, H, N.

(*E*)-3-(3-Methyl-4-quinolinyl)-2-propen-1-ol (31) was prepared in 71% yield from 30 via DIBAL-H reduction: ^{1}H NMR ($CDCl_3$) δ 8.65 (s, 1 H), 8.10–7.85 (m, 2 H), 7.66–7.33 (m, 2 H), 6.92 (d, 1 H), 6.11 (dt, 1 H), 4.35 (bs, 3 H), 2.46 (s, 3 H) ppm.

(*E*)-3-(3-Methyl-4-quinolinyl)-2-propenal (32) was prepared in 71% yield from 31 via a Swern oxidation. ^{1}H NMR ($CDCl_3$) δ 9.75 (d, 1 H), 8.63 (s, 1 H), 8.02–7.14 (m, 5 H), 6.38 (dd, 1 H), 2.41 (s, 3 H) ppm.

[4α,6β(*E*)]-6-[2-(3-Methyl-4-quinolinyl)ethenyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (34) was prepared in 10% overall yield from aldehyde 32. The low yield is due to inefficient extraction of the dihydroxy acid from the aqueous phase during the acidification procedure: mp 198–200 °C; ^{1}H NMR ($CDCl_3$) δ 8.61 (s, 1 H), 7.94–7.87 (m, 2 H), 7.55–7.34 (m, 2 H), 6.87 (d, 1 H), 5.92 (dd, 1 H), 5.46–5.37 (m, 1 H), 4.90 (bs, 1 H), 4.26 (bs, 1 H), 2.62 (d, 2 H), 2.33 (s, 3 H), 2.15–2.03 (m, 1 H), 1.89–1.76 (m, 1 H) ppm.

In Vivo Acute Inhibition of Cholesterol Synthesis Assay (AICS). Male Sprague–Dawley rats (250 g body weight), previously fed 2.5% cholestyramine for 3 days, were randomly divided into groups (N = 5/group) and given a single dose of vehicle (controls) or compound by an oral gavage at the indicated doses. One hour after drug dosing, all rats were injected intraperitoneally with sodium [1-^{14}C]acetate (20.0 μCi/rat in 0.3 mL of saline). After 50 min, blood samples were taken, plasma was obtained by centrifugation, and plasma [^{14}C]cholesterol was measured after saponification and extraction.

Acknowledgment. We thank Dr. F. A. MacKellar and staff for analytical and spectral determinations, and last but not least Ms. Patty Elka for manuscript preparation.

Disubstituted Tetrahydrofurans and Dioxolanes as PAF Antagonists

Javier Bartroli, Elena Carceller, Manuel Merlos, Julián García-Rafanell, and Javier Forn*

Chemistry Laboratories and Pharmacology Laboratories, Research Center, J. Uriach & Cía.S.A., Degà Bahí 59-67, 08026 Barcelona, Spain. *Received August 4, 1989*

A new series of disubstituted tetrahydrofuran and dioxolane derivatives were prepared and evaluated for their PAF antagonist activity in the PAF-induced in vitro platelet-aggregation and in vivo hypotension tests. Several of these compounds exhibited more potent activity than the structurally related 2-[*N*-acetyl-*N*-[[[[2-methoxy-3-[(octadecylcarbamoyl)oxy]propoxy]carbonyl]amino]methyl]-1-ethylpyridinium chloride (CV-6209, 3) in the in vitro assay, whereas all showed less potency in the in vivo test. The role of both the substituent nature and the placement and number of oxygen atoms in the ring are discussed. A qualitative SAR study was carried out on these nuclei.

Platelet activating factor (PAF, 1) is a naturally occurring phospholipid first described in 1972.[1] It is produced by stimulated basophils, neutrophils, platelets, macrophages, endothelial cells, and IgE-sensitized bone marrow cells.[2] PAF is involved in a wide range of biological actions such as stimulation of platelets and leukocytes, bronchoconstriction, hypotension, negative inotropic cardiac effects, and increase in vascular permeability.[3-5] In vivo experiments have demonstrated PAF's role in several pathological conditions,[6] such as asthma,[7] inflammation,[8] anaphylactic shock,[9] gastric ulceration,[10] and

(1) Benveniste, J.; Henson, P. M.; Cochrane, C. G. *J. Exp. Med.* 1972, *136*, 1356.
(2) Vargaftig, B. B.; Benveniste, J. *Trends Pharmacol. Sci.* 1983, *4*, 341.
(3) Braquet, P.; Vargaftig, B. B. *Transplant. Proc.* 1986, *18* (*Suppl. 4*), 10.
(4) Morley, J. *Agents Actions* 1986, *5*, 107.
(5) (a) Snyder, F. *Med. Res. Rev.* 1985, *5*, 107. (b) Venuti, M. C. *Annu. Rep. Med. Chem.* 1985, *20*, 193.
(6) (a) *New Horizons in Platelet Activating Factor Research*; Winslow, C. M., Lee, M. L., Eds.; Wiley: New York, 1987. (b) Braquet, P.; Touqui, L.; Shen, T. Y.; Vargaftig, B. B. *Pharmacol. Rev.* 1987, *39*, 97.
(7) (a) Page, C. P. *Developments in Asthma. A View of Current Research*; PJB Publications: Richmond, Surrey, England, 1987. (b) Mencia Huerta, J. M.; Benhamou, M. In *Asthma. Clinical Pharmacology and Therapeutic Progress*; Kay, A. B., Ed.; Blackwell Scientific: Oxford, 1986; pp 237–250. (c) Patterson, R.; Bernstein, P. R.; Harris, K. E.; Krell, R. D. *J. Lab. Clin. Med.* 1984, *104*, 340. (d) Morley, J.; Page, C. P.; Sanjar, S. *Int. Arch. Allergy Appl. Immunol.* 1985, *77*, 73. (e) Page, C. P.; Archer, C. B.; Paul, W.; Morley, J. *Trends Pharmacol. Sci.* 1984, *5*, 239.

0022-2623/91/1834-0373$02.50/0

J. Med. Chem. 1991, *34*, 2804–2815

Phosphorus-Containing Inhibitors of HMG-CoA Reductase. 2.[1] Synthesis and Biological Activities of a Series of Substituted Pyridines Containing a Hydroxyphosphinyl Moiety[2]

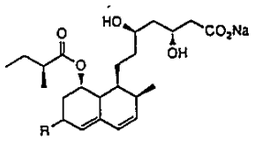
Jeffrey A. Robl,* Laurelee A. Duncan, Jelka Pluscec, Donald S. Karanewsky, Eric M. Gordon, Carl P. Ciosek, Jr., Lois C. Rich, Viviane C. Dehmel, Dorothy A. Slusarchyk, Thomas W. Harrity, and Kelly A. Obrien

The Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543-4000.
Received February 22, 1991

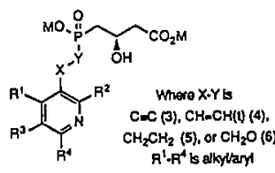
A series of 2,3,4,(5),6-substituted pyridines containing a hydroxyphosphinyl functionality have been prepared and were evaluated for their ability to inhibit the enzyme HMG-CoA reductase. Systematic substitution of both R^1–R^4 and X–Y led to compounds of type 3–6 with in vitro potency greater than that of mevinolin (Na salt).

Introduction

High serum cholesterol levels have been linked to the development of atherosclerosis and coronary heart disease (CHD).[3] A major constituent of serum cholesterol, low density lipoprotein (LDL), is widely believed to be atherogenic upon oxidative modification in vivo,[4] and therefore methods to reduce circulating levels of LDL are highly desirable. Mevinolin (1) and pravastatin (2), two closely



1, R = α-Me (Mevinolin Na)
2, R = β-OH (Pravastatin)

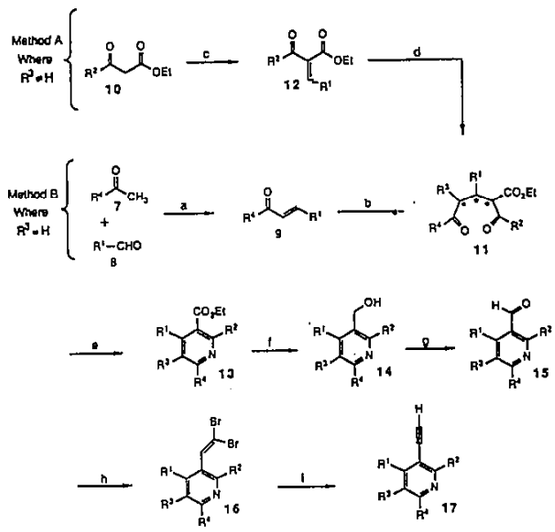


Where X-Y is C=C (3), CH=CH(t) (4), CH_2CH_2 (5), or CH_2O (6). R^1-R^4 is alkyl/aryl and M is Na or U

related natural products, are currently finding use as therapeutic agents in the treatment of hypercholesterolemia.[5] These compounds act as HMG-CoA reductase (HMGR) inhibitors. Through a complex sequence of regulatory mechanisms, they serve to increase hepatic LDL receptor levels, thereby lowering LDL concentration in the plasma.[6] Inhibition of HMGR, the rate-limiting enzyme in the biosynthesis of cholesterol, is therefore a proven approach to the treatment of hypercholesterolemia.

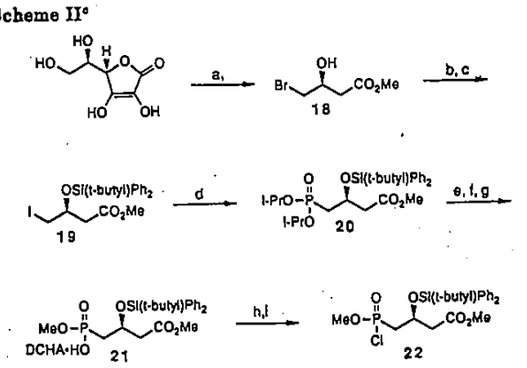
In an attempt to design better, more potent reductase inhibitors, much effort has been expended on replacing the complex decalin portion of the mevinic acids (i.e. 1 or 2) with structurally simpler, achiral aromatic surrogates.[7] In most cases, the 3,5-dihydroxyheptanoic acid portion of the molecule, the pharmacophore that interacts with the 3-

Scheme I[a]



[a] (a) EtONa, EtOH, room temperature; (b) 10, EtONa, EtOH, room temperature; (c) 8, piperidine, HOAc, PhH, reflux, $-H_2O$; (d) $R^4COCH_2R^3$, $LiN(TMS)_2$, THF, −78 °C; (e) NH_4OAc, $Cu(OAc)_2$, HOAc, reflux; (f) $LiAlH_4$, THF; (g) Dess–Martin periodinane, *tert*-butyl alcohol, CH_2Cl_2, room temperature, *or* $(CO)_2Cl_2$, DMSO, CH_2Cl_2, −78 °C then TEA *or* TPAP, 4-methylmorpholine *N*-oxide, 4A molecular sieves, CH_2Cl_2, room temperature; (h) CBr_4, PPh_3, $CH_2Cl_2(CH_3CN)$; (i) *n*-BuLi (2.2 equiv), THF, −78 °C, then saturated NH_4Cl quench.

Scheme II[a]



[a] (a) See ref 12; (b) (*t*-Bu)Ph_2SiCl, DMAP, imidazole, DMF; (c) NaI, MEK, reflux; (d) $(i\text{-PrO})_3P$, 160 °C; (e) TMSBr, BSTFA, CH_2Cl_2; (f) MeOH, DCC, pyridine; (g) dicyclohexylamine, Et_2O; (h) 5% $KHSO_4$, then TMSDEA, CH_2Cl_2; (i) $(CO)_2Cl_2$, catalytic DMF, CH_2Cl_2.

(1) For part 1 in this series, see: Karanewsky, D. S.; Badia, M. C.; Ciosek, C. P., Jr.; Robl, J. A.; Sofia, M. J.; Simpkins, L. M.; DeLange, B.; Harrity, T. W.; Biller, S. A.; Gordon, E. M. Phosphorus-Containing Inhibitors of HMG-CoA Reductase. 1. 4-[(2-Arylethyl)hydroxyphosphinyl]-3-hydroxybutanoic Acids: A New Class of Cell Selective Inhibitors of Cholesterol Biosynthesis. *J. Med. Chem.* 1990, *33*, 2952–2956.

(2) Presented in part at the 199th Meeting of the American Chemical Society, Boston, MA, April 1990, Abstract MEDI 128.

(3) Endo, A. Compactin (ML-236B) and Related Compounds as Potential Cholesterol-Lowering Agents That Inhibit HMG-CoA Reductase. *J. Med. Chem.* 1985, *28*, 401–405 and references therein.

(4) Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. Modifications of Low-Density Lipoprotein That Increase Its Atherogenicity. *N. Eng. J. Med.* 1989, *320*, 915–924.

(5) (a) Hoeg, J. M.; Brewer, H. B., Jr. 3-Hydroxy-3-Methylglutaryl-CoEnzyme A Reductase Inhibitors in the Treatment of Hypercholesterolemia. *J. Am. Med. Assoc.* 1987, *258*(24), 3532–3536. (b) Grundy, S. M. HMG-CoA Reductase Inhibitors for Treatment of Hypercholesterolemia. *N. Eng. J. Med.* 1988, *319*, 24–31.

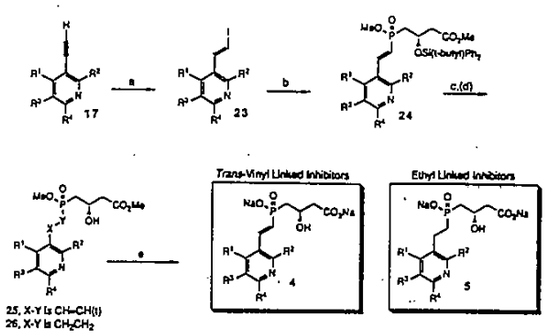
(6) Brown, M. S.; Goldstein, J. L. A Receptor-Mediated Pathway for Cholesterol Homeostasis. *Science* 1986, *232*, 34–47.

0022-2623/91/1834-2804$02.50/0

hydroxy-3-methylglutaryl (HMG) binding domain of the enzyme,[8] has been retained. In our previous communication,[1] we described a rationale for the design of a new class of HMGR inhibitors that utilizes a hydroxyphosphinyl functionality in place of the commonly exploited C-5 hydroxy functionality present in the 3,5-dihydroxyheptanoic acid pharmacophore. The hydroxyphosphinyl group was designed to bind to the protonated form of the catalytic group, which serves to activate substrate carbonyl groups toward delivery of a hydride ion in the enzymatic reduction of HMG-CoA to mevalonic acid.

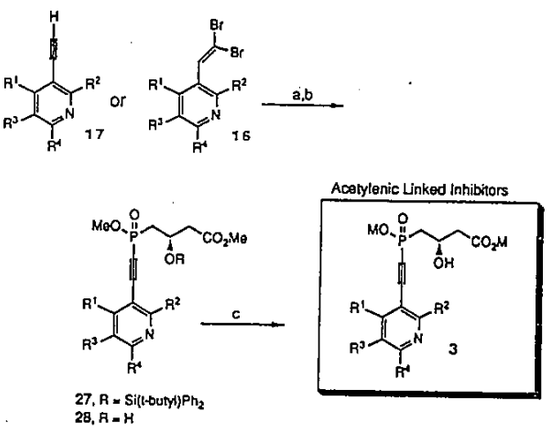
We have prepared hydroxyphosphinyl-containing HMGR inhibitors utilizing a wide variety of aromatic hydrophobic binding domain surrogates. In this paper, we

Scheme III[a]



[a] (a) Bu_3SnH, cat. AIBN, 140 °C, then I_2, Et_2O; (b) *t*-BuLi, THF, −78 °C, then **22**, THF, −100 °C; (c) TBAF, HOAc, THF; (d) H_2, Pd/C, MeOH; (e) NaOH, H_2O, dioxane, Δ.

Scheme IV[a]



[a] (a) *n*-BuLi (1.1 equiv for **17**, 2.2 equiv for **16**), THF, −78 °C, then **22**, THF, −78 °C; (b) TBAF, HOAc, THF, then CH_2N_2, Et_2O; (c) NaOH or LiOH, H_2O, dioxane, 50 °C.

describe the utilization of substituted pyridines[2,9] in the synthesis of hydroxyphosphinyl containing inhibitors **3**–**6**, in which both the "linker" portion (X–Y) of the molecule and the substituents on the pyridine "anchor" have been widely varied.

Chemistry

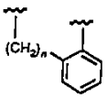
Methods for the synthesis of the requisite pyridine nuclei are depicted in Scheme I. Claisen–Schmidt condensation of methyl ketone **7** with aldehyde **8** provided *trans*-enone **9**. Ethoxide-catalyzed Michael addition of β-keto ester **10** to **9** gave the desired adducts **11**, usually as a 1:1 mixture of diastereomers. Method B provides **11**

(7) (a) Stokker, G. E.; Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J.; Deana, A. A.; Gilfillan, J. L.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Smith, R. L.; Willard, A. K. 3-Hydroxy-3-methylglutaryl-coenzyme A Reductase Inhibitors. 1. Structural Modification of 5-Substituted 3,5-dihydroxypentanoic Acids and Their Lactone Derivatives. *J. Med. Chem.* 1985, *28*, 347. (b) Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J.; Deana, A. A.; Evans, B. E.; Gilfillan, J. L.; Gould, N. P.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Rittle, K. E.; Smith, R. L.; Stokker, G. E.; Willard, A. K. 3-Hydroxy-3-methylglutaryl-coenzyme A Reductase Inhibitors. 2. Structural Modification of 7-(substituted aryl)-3,5-dihydroxy-6-heptenoic Acids and Their Lactone Derivatives. *J. Med. Chem.* 1986, *29*, 159–169. (c) Stokker, G. E.; Alberts, A. W.; Anderson, P. S.; Cragoe, E. J.; Deana, A. A.; Gilfillan, J. L.; Hirshfield, J.; Holtz, W. J.; Hoffman, W. F.; Huff, J. W.; Lee, T. J.; Novello, F. C.; Prugh, J. D.; Rooney, C. S.; Smith, R. L.; Willard, A. K. 3-Hydroxy-3-methylglutaryl-coenzyme A Reductase Inhibitors. 3. 7-(3,5-Disubstituted [1,1'-biphenyl]-2-yl)-3,5-dihydroxy-6-heptenoic Acids and Their Lactone Derivatives. *J. Med. Chem.* 1986, *29*, 170–181. (d) Stokker, G. E.; Alberts, A. W.; Gilfillan, J. L.; Huff, J. W.; Smith, R. L. 3-Hydroxy-3-methylglutaryl-coenzyme A Reductase Inhibitors. 5. 6-(Fluoren-9-yl)- and 6-(Fluoren-9-ylidenyl)-3,5-dihydroxyheptanoic Acids and Their Lactone Derivatives. *J. Med. Chem.* 1986, *29*, 852–855. (e) Balasubramanian, N.; Brown, P. L.; Catt, J. D.; Han, W. T.; Parker, R. A.; Sit, S. Y.; Wright, J. J. A Potent, Tissue-Selective, Synthetic Inhibitor of HMG-CoA Reductase. *J. Med. Chem.* 1989, *32*, 2038–2041. (f) Roth, B. D.; Ortwine, D. F.; Hoefle, M. L.; Stratton, C. D.; Sliskovic, D. R.; Wilson, M. W.; Newton, R. S. Inhibitors of Cholesterol Biosynthesis. 1. *trans*-6-(2-Pyrrol-1-ylethyl)-4-hydroxypyran-2-ones, a Novel Series of HMG-CoA Reductase Inhibitors. 1. Effects of Structural Modifications at the 2- and 5-Positions of the Pyrrole Nucleus. *J. Med. Chem.* 1990, *33*, 21–31. (g) Sliskovic, D. R.; Roth, B. D.; Hoefle, M. L.; Wilson, M. W.; Newton, R. S. Inhibitors of Cholesterol Biosynthesis. 2. 1,3,5-Trisubstituted [2-(Tetrahydro-4-hydroxy-2-oxopyran-6-yl)ethyl]pyrazoles. *J. Med. Chem.* 1990, *33*, 31–38. (h) Jendralla, H.; Baader, E.; Bartmann, W.; Beck, G.; Bergmann, A.; Granzer, E.; Kerekjarto, B. v.; Kesseler, K.; Krause, R.; Schubert, W.; Wess, G. Synthesis and Biological Activity of New HMG-CoA Reductase Inhibitors. 2. Derivatives of 7-(1*H*-Pyrrol-3-yl)-substituted-3,5-dihydroxyhept-6(*E*)-enoic(-heptanoic) Acids. *J. Med. Chem.* 1990, *33*, 61–70. (i) Roth, B. D.; Blankley, C. J.; Chucholowski, A. W.; Ferguson, E.; Hoefle, M. L.; Ortwine, D. F.; Newton, R. S.; Sekerke, C. S.; Sliskovic, D. R.; Stratton, C. D.; Wilson, M. W. Inhibitors of Cholesterol Biosynthesis. 3. Tetrahydro-4-hydroxy-6-[2-(1*H*-pyrrol-1-yl)ethyl]-2*H*-pyran-2-one Inhibitors of HMG-CoA Reductase. 2. Effects of Introducing Substituents at Positions Three and Four of the Pyrrole Nucleus. *J. Med. Chem.* 1991, *34*, 357–366. (j) Sliskovic, D. R.; Picard, J. A.; Roark, W. H.; Roth, B. D.; Ferguson, E.; Krause, B. R.; Newton, R. S.; Sekerke, C. S.; Shaw, M. K. Inhibitors of Cholesterol Biosynthesis. 4. *trans*-6-[2-(Substituted quinolinyl)ethenyl/ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-ones, a Novel Series of HMG-CoA Reductase Inhibitors. *J. Med. Chem.* 1991, *34*, 367–373.

(8) Abeles, R. H.; Nakamura, C. E. Mode of Interaction of β-Hydroxy-β-methylglutaryl CoEnzyme A Reductase with Strong Binding Inhibitors: Compactin and Related Compounds. *Biochemistry* 1985, *24*, 1364–1376.

(9) During the course of this work, others have disclosed their efforts on pyridine based 3,5-dihydroxyheptanoic acid containing HMGR inhibitors; see (a) Beck, G.; Kesseler, K.; Baader, E.; Bartmann, W.; Bergmann, A.; Granzer, E.; Jendralla, H.; Kerekjarto, B. v.; Krause, R.; Paulus, E.; Schubert, W.; Wess, G. Synthesis and Biological Activity of New HMG-CoA Reductase Inhibitors. 1. Lactones of Pyridine- and Pyrimidine-Substituted 3,5-Dihydroxy-6-heptenoic(-heptanoic) Acids. *J. Med. Chem.* 1990, *33*, 52–60. (b) Angerbauer, R.; Fey, P.; Hubsch, W.; Phillips, T.; Bischoff, H.; Petzinna, D.; Schmidt, D.; Thomas, G. European Patent Application EP-A-0325130. (c) Chucholowski, A. W.; Roth, B. D.; Creswell, M. W.; Sliskovic, D. R. European Patent Application EP-A-0306929.

Table I. Pyridyl Alcohols 14

no.	R^1	R^2	R^3	R^4	mp, °C	% yield[a] (method)	formula	anal.[b]
14a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	167–169	82 (B)	$C_{21}H_{20}FNO$	C, H, N
14b	4-FC_6H_4	i-C_3H_7	H	2-MeC_6H_4	114–115	65 (B)	$C_{22}H_{22}FNO$	C, H, F, N
14c	4-FC_6H_4	i-C_3H_7	H	2-($C_6H_5CH_2$)C_6H_4	122–124	40 (B)	$C_{28}H_{26}FNO$	C, H, F, N
14d	4-FC_6H_4	i-C_3H_7	H	1-naphthyl	73–75	30 (B)	$C_{25}H_{22}FNO$	C, H, F, N
14e	4-FC_6H_4	i-C_3H_7	H	2,3,5,6-$(F)_4C_6H_1$	130–132	60 (B)	$C_{21}H_{16}F_4NO$	c
14f	4-FC_6H_4	i-C_3H_7	H	2-thienyl	151–153	37 (B)	$C_{19}H_{18}FNOS$	C, H, F, N, S
14g	4-FC_6H_4	i-C_3H_7	H	CH_3	154–155	22 (B)	$C_{16}H_{18}FNO$	C, H, F, N
14h	4-FC_6H_4	i-C_3H_7	H	i-C_3H_7	88–90	57 (B)	$C_{18}H_{22}FNO$	C, H, F, N
14i	4-FC_6H_4	i-C_3H_7	H	c-C_3H_5	94–95	24 (B)	$C_{18}H_{20}FNO$	C, H, F, N
14j	4-FC_6H_4	i-C_3H_7	H	$(C_6H_5)_2CH$	139–140	13 (B)	$C_{28}H_{26}FNO$	C, H, F, N
14k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	112–113	49 (B)	$C_{19}H_{24}FNO$	C, H, F, N
14l	4-FC_6H_4	i-C_3H_7	H	c-C_6H_{11}	101–104	40 (B)	$C_{21}H_{28}FNO$	C, H, F, N
14m	4-FC_6H_4	i-C_3H_7	H	1-adamantyl	143–145	56 (B)	$C_{25}H_{30}FNO$	C, H, F, N
14n	4-FC_6H_4	i-C_3H_7	H		114–115	42 (B)	$C_{22}H_{20}FNO_3$	C, H, F, N
14o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	182–184	68 (A)	$C_{22}H_{22}FNO$	C, H, F, N
14p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	228–230	53 (A)	$C_{23}H_{24}FNO$	C, H, F, N
14q	4-FC_6H_4	i-C_3H_7	i-C_3H_7	C_6H_6	244–246	21 (A)	$C_{24}H_{26}FNO$	C, H, F, N
14r	4-FC_6H_4	i-C_3H_7	C_6H_5	C_6H_5	169–171	52 (A)	$C_{27}H_{24}FNO$	C, H, F, N
14s	4-FC_6H_4	i-C_3H_7	F	C_6H_6	163–165	8 (A)	$C_{21}H_{19}F_2NO$	c
14t	4-FC_6H_4	i-C_3H_7	$n = 1$		166–167	13 (A)	$C_{22}H_{20}FNO$[d]	C, H, F, N
14u	4-FC_6H_4	i-C_3H_7	$n = 2$		138–139	41 (A)	$C_{23}H_{22}FNO$	C, H, F, N
14v	4-FC_6H_4	i-C_3H_7	$n = 3$		161–162	68 (A)	$C_{24}H_{24}FNO$	C, H, F, N
14w	4-FC_6H_4	t-C_4H_9	H	C_6H_5	oil	20 (B)	$C_{23}H_{24}FNO$	c
14x	4-FC_6H_4	c-C_3H_5	H	C_6H_5	176–177	62 (B)	$C_{21}H_{18}FNO$	C, H, F, N
14y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	140–142	71 (A)	$C_{22}H_{20}FNO$	C, H, F, N
14z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	180–181	61 (A)	$C_{21}H_{20}FNO$	c
14aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	178–180	72 (A)	$C_{20}H_{16}FNO$	C, H, F, N
14bb	i-C_3H_7	4-FC_6H_4	H	C_6H_5	172–173	31 (B)	$C_{21}H_{20}FNO$	C, H, F, N
14cc	4-F-3-MeC_6H_3	i-C_3H_7	H	C_6H_5	159–160	60 (B)	$C_{22}H_{22}FNO$	C, H, F, N
14dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	134–135	66 (B)	$C_{22}H_{22}FNO$	C, H, F, N

[a] Represents overall yield from 12 (method A) or from 9 (method B). [b] Analytical results were within ±0.4% of the theoretical value. [c] Microanalysis was not performed. Compound possessed ^1H NMR and MS in accord with assigned structure. [d] Anal. Calcd: C, 79.25. Found: C, 78.74.

in generally good yields in the cases where R^3 = H but was unsatisfactory in cases where R^3 was alkyl or aryl. In these cases, introduction of the R^3 substituent was best carried out utilizing method A. β-Keto α,β-unsaturated ester 12, generated by Knoevenagel condensation of β-keto ester 10 with aldehyde 8, readily underwent Michael addition with lithium enolate $R^4C(OLi)=CHR^3$ to give 11 as a complex mixture of diastereomers. Treatment of 1,5-diketone 11 with NH_4OAc in hot HOAc afforded the intermediate dihydropyridine, which underwent $Cu(OAc)_2$ oxidation[10] in situ, affording pyridyl ester 13. Utilization of either method A or method B allowed for the rapid and convenient generation of tetra- and pentasubstituted pyridines 13, in which the substituents R^1–R^4 could be independently selected from a variety of alkyl or aryl groups. Simple $LiAlH_4$ reduction of 13 gave pyridyl alcohols 14 (Table I). Alcohols 14 provided an entry to phosphonic acid based inhibitors 6 (see Scheme V), but a one-carbon homologation was necessary for generation of the phosphinic acid class of compounds (see Schemes III and IV). Oxidation of 14 could be effected under a variety of conditions to give the corresponding aldehydes 15. Reaction of 15 with CBr_4/PPh_3 provided the vinyl dibromides[11] (Table II) in generally excellent yields. Treatment of 15 with n-BuLi in THF at −78 °C generated the corresponding acetylenic anions in situ. The anions could be utilized in carbon–phosphorus bond formation directly or quenched with a proton source to give acetylenes 17.

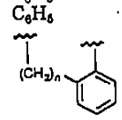
The routes we have developed[1] for the synthesis of both the phosphinic and phosphonic acid based inhibitors utilize phosphonochloridate 22 as a synthon for the introduction of the 3-hydroxy-4-(hydroxyphosphinyl)butanoic side chain. The S enantiomer of compound 22 was prepared by a multistep route (outlined in Scheme II) from isoascorbic acid via known[12] bromohydrin ester 18. Silylation of 18 followed by Finkelstein reaction on the silylated bromide provided 19 in 74% overall yield. Arbuzov reaction of 19 was best effected with triisopropyl phosphite to give 20 in 75% yield. Phosphorus deesterification with TMSBr followed by reesterification with MeOH/DCC in pyridine gave the corresponding phosphonic acid monomethyl ester, which was conveniently isolated and stored in stable form as its dicyclohexylamine salt 21. Regeneration of the free acid followed by subsequent treatment with TMSDEA and oxalyl chloride thus provided phos-

(10) Bell, T. W.; Rothenberger, S. D. Synthesis of Annelated Pyridines from 1,5-Diketone Equivalents using Cupric Acetate and Ammonium Acetate. *Tetrahedron Lett.* 1987, 4817–4820.

(11) Corey, E. J.; Fuchs, P. L. A Synthetic Method for Formyl–Ethynyl Conversion (RCHO – RC≡CH or RC≡CR'). *Tetrahedron Lett.* 1972, 3769–3772.

(12) (a) Bock, K.; Lundt, I.; Pedersen, C. Synthesis of (S)- and (R)-4-Amino-3-hydroxybutyric Acid (GABOB) and (S)- and (R)-Carnitine from Arabinose or *Ascorbic Acid. *Acta Chem. Scand., B* 1983, *37*, 341–344. (b) Isbell, H. S.; Frush, H. L. Oxidation of L-ascorbic acid by hydrogen peroxide: preparation of L-threonic acid. *Carbohydr. Res.* 1979, *72*, 301–304.

Table II. Pyridyl Vinyl Dibromides 16

no.[a]	R^1	R^2	R^3	R^4	mp, °C	% yield[b] (method)[c]
16a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	oil	88 (C)
16b	4-FC_6H_4	i-C_3H_7	H	2-MeC_6H_4	108–110	68 (D)
16c	4-FC_6H_4	i-C_3H_7	H	2-($C_6H_5CH_2$)C_6H_4	foam	62 (D)
16d	4-FC_6H_4	i-C_3H_7	H	1-naphthyl	foam	74 (D)
16e	4-FC_6H_4	i-C_3H_7	H	2,3,5,6-$(F)_4C_6H_1$	77	62 (D)
16f	4-FC_6H_4	i-C_3H_7	H	2-thienyl	107–108	86 (D)
16g	4-FC_6H_4	i-C_3H_7	H	CH_3	oil	94 (D)
16h	4-FC_6H_4	i-C_3H_7	H	i-C_3H_7	52–53	71 (D)
16i	4-FC_6H_4	i-C_3H_7	H	c-C_3H_5	oil	71 (D)
16j	4-FC_6H_4	i-C_3H_7	H	$(C_6H_5)_2CH$	141–142	86 (D)
16k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	98–100	68 (D)
16l	4-FC_6H_4	i-C_3H_7	H	c-C_6H_{11}	98–100	72 (D)
16m	4-FC_6H_4	i-C_3H_7	H	1-adamantyl	176–177	69 (D)
16n	4-FC_6H_4	i-C_3H_7	H		129–131	74 (D)
16o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	169–170	85 (D)
16p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	155–157	82 (D)
16q	4-FC_6H_4	i-C_3H_7	i-C_3H_7	C_6H_5	foam	83 (D)
16r	4-FC_6H_4	i-C_3H_7	C_6H_5	C_6H_5	155–158	88 (D)
16s	4-FC_6H_4	i-C_3H_7	F	C_6H_5	105–107	76 (D)
16t	4-FC_6H_4	i-C_3H_7	$n = 1$		foam	58 (D)[d]
16u	4-FC_6H_4	i-C_3H_7	$n = 2$		121–122	76 (E)
16v	4-FC_6H_4	i-C_3H_7	$n = 3$		173–175	83 (D)
16w	4-FC_6H_4	t-C_4H_9	H	C_6H_5	oil	58 (D)
16x	4-FC_6H_4	c-C_3H_5	H	C_6H_5	170–172	69 (E)
16y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	155–157	77 (E)
16z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	137–138	72 (E)
16aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	141–143	73 (E)
16bb	i-C_3H_7	4-FC_6H_4	H	C_6H_5	124–126	84 (C)
16cc	4-F-3-MeC_6H_3	i-C_3H_7	H	C_6H_5	102–104	89 (D)
16dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	128–129	75 (D)

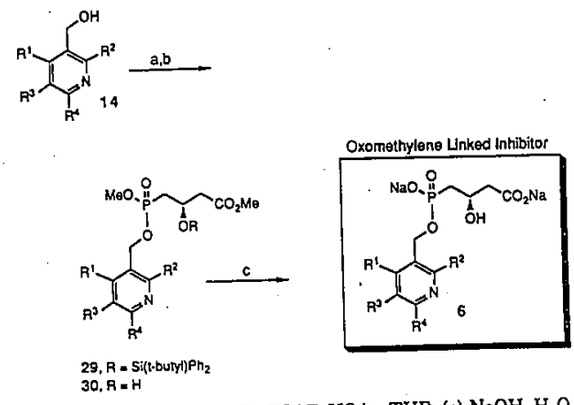
[a] All spectral data were consistent with assigned structures. [b] Represents overall yield from 14. [c] Represents method of oxidation. Method C: Dess–Martin periodinane, *tert*-butyl alcohol, CH_2Cl_2, room temperature. Method D: $(CO)_2Cl_2$, DMSO, CH_2Cl_2, −78 °C, then TEA. Method E: TPAP, 4-methylmorpholine *N*-oxide, 4A molecular sieves, CH_2Cl_2, room temperature. [d] CH_3CN used as solvent in the formation of 16w from 15w.

phonochloridate 22. Silylation of the free acid of 21 prior to treatment with oxalyl chloride generates TMSCl rather than HCl as a byproduct of the reaction, allowing the *tert*-butyldiphenylsilyl protecting group to remain intact.

Scheme III outlines the route developed for the synthesis of *trans*-vinyl (X–Y = CH=CH(t)) and ethyl (X–Y = CH_2CH_2) linked inhibitors 4 and 5. Hydrostannylation of acetylene 17 with tributyltin hydride under free-radical conditions[13] followed by treatment of the intermediate *trans*-vinylstannane with iodine stereospecifically provided the *trans*-vinyl iodides 23 in good yields. Metallation of 23 with *tert*-butyllithium generated the corresponding vinyl anion, which was subsequently coupled with phosphonochloridate 22 at −100 °C to give 24 in yields averaging 55%. Higher reaction temperatures led to a substantial diminution in product yield. Desilylation with buffered fluoride provided 25, which was saponified to give *trans*-vinyl-linked inhibitors 4, or, was subjected to catalytic hydrogenation followed by saponification to give ethyl linked inhibitors 5.

Synthesis of ethynyl (X–Y = C≡C) linked inhibitors 3 was, in general, more straight forward (Scheme IV). The lithium anion of 17, generated by the reaction of either 16 or 17 with *n*-butyllithium, smoothly underwent coupling with phosphonochloridate 22 at −78 °C to give 27, usually in 65–80% yields. Desilylation followed by saponification thus provided diacids 3. In the case of the ethynyl-linked compounds, cleavage of the silyl ether of 27 with fluoride ion also led to partial deesterification at the methyl phosphinate ester. Reesterification with diazomethane was

Scheme V[a]



[a] (a) 22, pyridine, 4 °C; (b) TBAF, HOAc, THF; (c) NaOH, H_2O, dioxane, 55 °C.

(13) Tolstilkov, G. A.; Miftakhov, M. S.; Danilova, N. A.; Vel'der, Y. L. Regio- and Stereoselective Hydrostannylation of 3-Hydroxy-4-phenoxy-1-butyne: Effective Approach to Intermediates in the Total Synthesis of ω-Aryloxyprostaglandins. *Synthesis* 1986, 496–499.

necessary in order to obtain the desired products, 28, in consistently good yields.

Phosphonic acid based inhibitors 6 were generated as shown in Scheme V. Reaction of pyridyl alcohols 14 with phosphonochloridate 22 in pyridine gave 29, which were subsequently desilylated and saponified to give inhibitors of type 6. Treatment of diesters 30 with base led to a mixture of both 6 and 14, resulting from competing hydrolysis of the methyl and pyridylmethyl phosphonic esters.

Biological Results

Compounds 3–6 were tested for inhibition of the conversion of ^{14}C-HMG-CoA to [^{14}C]mevalonic acid by partially purified HMG-CoA reductase (Table III). Activities are expressed as concentration of drug producing 50% inhibition of the enzyme (I_{50} value). The I_{50}'s of the sodium salts of mevinolin (1) and pravastatin (2) are shown for comparison. Structure–activity relationships were studied by (i) varying the nature of the substituents ortho to the binding domain pharmacophore, (ii) varying the substituents at carbons C-5 and C-6 (R^3 and R^4) on the pyridine ring, (iii) varying the nature of the "linking" group X–Y, and (iv) fusing the C-5 and C-6 positions of the pyridine ring with cycloalkylbenzo substituents.

Workers at Merck had previously shown[7b] in a dihydroxyheptanoic acid based inhibitor series that, for optimal inhibitory potency, an aryl and an alkyl group must flank the HMGR binding domain pharmacophore. Early in our studies, we found that placement of the alkyl substituent (preferably isopropyl) at R^2 and the aryl substituent (preferably 4-fluorophenyl) at R^1 lead to compounds of higher potency relative to their regioisomers (compare 3a and 4a with 3bb and 4bb). Subsequent studies were carried out utilizing this substitution pattern. It is apparent that the enzyme is able to accommodate a wide variety of substituents at C-6 (R^4) of the pyridine nucleus. Very large groups such as naphthyl (3d), 2-benzylphenyl (3c), and adamantyl (3m) are well tolerated. In general, the presence of sterically demanding groups such as diphenylmethyl (3j) and *tert*-butyl (3k) is preferred over smaller substituents such as methyl and isopropyl. A notable exception is seen in the case where R^4 is cyclopropyl (3i). This compound was found to be 20-fold more active than its isopropyl counterpart (3h).

Substitution at C-5 (R^3) of the pyridine nucleus with an alkyl or aryl group dramatically increases intrinsic potency (compare compounds 3o–r with 3a). The effect is greatest with methyl and decreases with increasing steric bulk (i.e. for R^3, methyl > ethyl > isopropyl > phenyl) with R^4 as phenyl. It is believed that this effect is due to a favorable skewing of the R^4 phenyl group out of the plane of the pyridine ring. In order to test this hypothesis, a series of conformationally restricted cycloalkylbenzo-fused pyridines were evaluated (compounds 3t–v). Cyclopentyl- and cyclohexylbenzo-fused pyridines 3t and 3u were essentially equipotent to their nonfused counterpart 3a, whereas cycloheptylbenzo-fused pyridine 3v was 4–5-fold more active. The propylene bridge in 3v necessarily holds the fused phenyl group out of the plane with the pyridine ring.[14] The converse is true with methylene or ethylene bridging units. As proposed above, deviation of planarity of the R^4 phenyl substituent leads to optimal inhibitory potency.

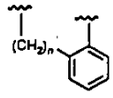
In order to study the relationship between activity, the linker group X–Y, and the alkyl substituent at R^2, a variety of inhibitors were synthesized in which the R^2 group (R^2 = methyl, ethyl, cyclopropyl, and isopropyl) as well as the linker X–Y (C≡C, CH=CH(t), CH_2CH_2, and CH_2O) were varied. These studies show there is a strong interdependence between R^2 and X–Y. Where R^2 is isopropyl or cyclopropyl (e.g 3–6a,k,o,p,v,y), the general order of activity with respect to X–Y is CH=CH(t) > CH_2O ≥ C≡C > CH_2CH_2. In general, compounds possessing the *trans*-vinyl group are 2–32-fold more active than their acetylenic or methylene ether counterparts and 5–95-fold more potent than their ethyl-linked counterparts. A reversal in activity occurs when R^2 is methyl. In this case (e.g. **3aa**, **5aa**, and 6aa), the order of activity is $CH_2CH_2 \gg C{\equiv}C \approx CH_2O$. As expected, ethyl substitution at R^2 (e.g. 3–6z) exhibits activity that is intermediate between that of isopropyl and methyl substitution (i.e. $CH_2CH_2 \approx C{\equiv}C$ for X–Y). In essentially all cases studied, the *trans*-vinyl group was found to be the superior linking functionality, regardless of the substitution pattern at R^1 and R^2. The SAR of the phosphonic acid based inhibitors 6 (X–Y is CH_2O) more closely parallels that of the inhibitors possessing the acetylenic or *trans*-vinyl linkers, rather than the isosteric ethylene linkers. These data indicate that the alkyl R^2 group must be tailored to the appropriate linker X–Y in order to optimize inhibitory potency. On the basis of these SAR, the most potent compounds possess either an isopropyl or a cyclopropyl group at R^2, a *trans*-vinyl or oxomethylene linker for X–Y, a 4-fluorophenyl group at R^1, and substitution at both R^3 and R^4. Indeed, most of the compounds that possess low to subnanomolar activity against HMGR (i.e. 4o, 4p, 4v, 6v, and 6y) fulfill these criteria.

Since the main site of both LDL synthesis and expression of LDL receptors is in the liver, inhibition of cholesterol biosynthesis in extrahepatic tissue may lead to undesirable side effects. We therefore felt it would be advantageous to develop HMGR inhibitors that would be selective for hepatic cells over extrahepatic cells.[15] Consequently, the phosphorus-based inhibitors were evaluated for their ability to inhibit cholesterol synthesis from [^{14}C]acetate in both hepatic and nonhepatic cells (Table IV). For comparison, mevinolin (1) and pravastatin (2) were also evaluated. One striking difference between pravastatin and mevinolin is exhibited in their ability to inhibit cholesterol synthesis in whole cells. Pravastatin shows inhibition in freshly isolated rat hepatocytes com-

(14) For a study on the conformational analysis of bridged biphenyls and 2,2′-bipyridines, see: Jaime, C.; Font, J. Conformational Analysis of Bridged Biphenyls and 2,2′-Bipyridines Empirical Force Field Calculations (MM2-V4). *J. Org. Chem.* 1990, *55*, 2637–2644.

(15) For papers concerning cell and tissue selectivity of HMGR inhibitors, see: (a) Tsujita, Y.; Kuroda, M.; Shimada, Y.; Tanzawa, K.; Arai, M.; Kaneko, I.; Tanaka, M.; Masuda, H.; Tarumi, C.; Watanabe, Y.; Fiji, S. CS-514, A Competitive Inhibitor of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase: Tissue Selective Inhibition of Sterol Synthesis and Hypolipidemic Effect on Various Animal Species. *Biochim. Biophys. Acta* 1986, *877*, 50–60. (b) Reference 7e. (c) Germershausen, J. I.; Hunt, V. M.; Bostedor, R. G.; Bailey, P. J.; Karkas, J. D.; Alberts, A. W. Tissue Selectivity of the Cholesterol-Lowering Agents Lovastatin, Simvastatin, and Pravastatin in Rats in Vivo. *Biochem. Biophys. Res. Commun.* 1989, *158*, 667–675. (d) Roth, B. D.; Bocan, T. M. A.; Blankley, C. J.; Chucholowski, A. W.; Creger, P. L.; Creswell, M. W.; Ferguson, E.; Newton, R. S.; O'Brien, P.; Picard, J. A.; Roark, W. H.; Sekerke, C. S.; Sliskovic, D. R.; Wilson, M. W. Relationship between Tissue Selectivity and Lipophilicity for Inhibitors of HMG-CoA Reductase. *J. Med. Chem.* 1991, *34*, 463–466. (e) Shaw, M. K.; Newton, R. S.; Sliskovic, D. R.; Roth, B. D.; Ferguson, E.; Krause, B. R. HEP-G2 Cells and Primary Rat Hepatocytes Differ In Their Response To Inhibitors Of HMG-CoA Reductase. *Biochem. Biophys. Res. Commun.* 1990, *170*, 726–734.

Table III. Inhibition of HMG-CoA Reductase in Vitro for Compounds 1–6

no.	R^1	R^2	R^3	R^4	X–Y	M	$[\alpha]_D$, deg (c, MeOH)	% yield[a] (method)[b]	formula[c]	I_{50},[d] nM
1[e]	–	–	–	–	–	–	–	–	–	4.0
2	–	–	–	–	–	–	–	–	–	,24.0
3a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	C≡C	Li	+4.8 (0.72)	59 (I)	$C_{28}H_{23}FNO_6PLi_2 \cdot 0.80H_2O$	62.0
4a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	CH=CH(t)	Li	+6.2 (0.56)	36 (F)	$C_{28}H_{25}FNO_6PLi_2 \cdot 1.30H_2O$[f]	1.9
5a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	CH_2CH_2	Li	–3.3 (0.45)	10[g]	$C_{28}H_{27}FNO_6PLi_2 \cdot 1.76H_2O$	181
6a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	CH_2O	Na	–2.4 (0.47)	34 (J)	$C_{28}H_{25}FNO_6PNa_2 \cdot H_2O$	12.7
3b	4-FC_6H_4	i-C_3H_7	H	2-MeC_6H_4	C≡C	Li	+4.0 (0.59)	15 (H)	$C_{27}H_{25}FNO_6PLi_2 \cdot 1.10H_2O$	32.6
3c	4-FC_6H_4	i-C_3H_7	H	2-($C_6H_5CH_2$)C_6H_4	C≡C	Na	+3.4 (0.53)	55 (H)	$C_{33}H_{29}FNO_6PNa_2 \cdot 1.24H_2O$[h]	62.6
3d	4-FC_6H_4	i-C_3H_7	H	1-naphthyl	C≡C	Na	+4.9 (0.80)	33 (H)	$C_{32}H_{25}FNO_6PNa_2 \cdot 1.22H_2O$	28.7
3e	4-FC_6H_4	i-C_3H_7	H	2,3,4,5-$F_4C_6H_1$	C≡C	Na	+2.7 (0.48)	28 (H)	$C_{26}H_{19}F_6NO_6PNa_2 \cdot 4.60H_2O$[f]	8.9
3f	4-FC_6H_4	i-C_3H_7	H	2-thienyl	C≡C	Na	+7.6 (0.95)	33 (H)	$C_{24}H_{21}FNO_6PSNa_2 \cdot 1.55H_2O$	14.6
3g	4-FC_6H_4	i-C_3H_7	H	CH_3	C≡C	Na	+4.7 (0.62)	22 (H)	$C_{21}H_{21}FNO_6PNa_2 \cdot 3.0H_2O$	231
3h	4-FC_6H_4	i-C_3H_7	H	i-C_3H_7	C≡C	Na	+5.6 (0.78)	16 (H)	$C_{25}H_{25}FNO_6PNa_2 \cdot 1.40H_2O$	80.9
3i	4-FC_6H_4	i-C_3H_7	H	c-C_3H_5	C≡C	Na	+5.1 (0.74)	28 (H)	$C_{25}H_{23}FNO_6PNa_2 \cdot 2.50H_2O$	4.2
3j	4-FC_6H_4	i-C_3H_7	H	$(C_6H_5)_2CH$	C≡C	Na	+4.2 (0.38)	17 (I)	$C_{33}H_{29}FNO_6PNa_2 \cdot 2.0H_2O$	14.9
3k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	C≡C	Na	+6.5 (0.77)	33 (H)	$C_{24}H_{27}FNO_6PNa_2 \cdot 1.17H_2O$	6.1
4k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	CH=CH(t)	Na	+3.3 (0.60)	16 (F)	$C_{24}H_{29}FNO_6PNa_2 \cdot 2.10H_2O$	3.2
5k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	CH_2CH_2	Na	+0.9 (0.68)	28 (G)	$C_{24}H_{31}FNO_6PNa_2 \cdot 2.0H_2O$	17.2
6k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	CH_2O	Na	–1.6 (0.43)	32 (J)	$C_{23}H_{29}FNO_6PNa_2 \cdot 1.50H_2O$	1.4
3l	4-FC_6H_4	i-C_3H_7	H	c-C_6H_{11}	C≡C	Na	+4.4 (0.45)	34 (H)	$C_{28}H_{29}FNO_6PNa_2 \cdot 1.60H_2O$	48.4
3m	4-FC_6H_4	i-C_3H_7	H	1-adamantyl	C≡C	Na	+5.8 (0.72)	15 (H)	$C_{30}H_{33}FNO_6PNa_2 \cdot 2.0H_2O$	43.8
3n	4-FC_6H_4	i-C_3H_7	H		C≡C	Li	+6.9 (0.72)	13 (H)	$C_{27}H_{23}FNO_7PLi_2 \cdot 1.40H_2O$	84.6
3o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	C≡C	Li	+9.4 (0.36)	46 (H)	$C_{27}H_{25}FNO_6PLi_2 \cdot 1.06H_2O$	4.5
4o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	CH=CH(t)	Na	+10.0 (0.50)	47 (F)	$C_{27}H_{27}FNO_6PNa_2 \cdot 1.2H_2O$	1.2
5o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	CH_2CH_2	Na	+0.8 (0.49)	65 (G)	$C_{27}H_{29}FNO_6PNa_2 \cdot 3.69H_2O$	9.2
6o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	CH_2O	Na	–2.0 (0.50)	34 (J)	$C_{26}H_{27}FNO_6PNa_2 \cdot 0.94H_2O$	5.1
3p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	C≡C	Na	+11.1 (0.45)	53 (H)	$C_{28}H_{27}FNO_6PNa_2 \cdot 1.89H_2O$	5.6
4p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	CH=CH(t)	Na	+10.9 (0.52)	36 (F)	$C_{28}H_{29}FNO_6PNa_2 \cdot 3.85H_2O$	0.55
5p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	CH_2CH_2	Na	+1.0 (0.48)	68 (G)	$C_{28}H_{31}FNO_6PNa_2 \cdot 3.33H_2O$	19.1
6p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	CH_2O	Na	–0.1 (0.82)	33 (J)	$C_{27}H_{29}FNO_6PNa_2 \cdot H_2O$	2.5
3q	4-FC_6H_4	i-C_3H_7	i-C_3H_7	C_6H_5	C≡C	Na	+11.0 (0.39)	40 (H)	$C_{29}H_{29}FNO_6PNa_2 \cdot 2.78H_2O$	9.8
3r	4-FC_6H_4	i-C_3H_7	C_6H_5	C_6H_5	C≡C	Na	+12.1 (0.52)	49 (H)	$C_{32}H_{27}FNO_6PNa_2 \cdot 1.22H_2O$	15.6
3s	4-FC_6H_4	i-C_3H_7	F	C_6H_5	C≡C	Na	+5.8 (0.48)	40 (H)	$C_{26}H_{22}FNO_6PNa_2 \cdot 1.79H_2O$	44.9
3t	4-FC_6H_4	i-C_3H_7	$n = 1$		C≡C	Li	+14.1 (0.46)	18 (H)	$C_{27}H_{23}FNO_6PLi_2 \cdot 1.31H_2O$	75.6
3u	4-FC_6H_4	i-C_3H_7	$n = 2$		C≡C	Na	+11.4 (0.40)	18 (H)	$C_{28}H_{25}FNO_6PNa_2 \cdot 2.25H_2O$	52.5
3v	4-FC_6H_4	i-C_3H_7	$n = 3$		C≡C	Na	+11.2 (0.60)	66 (H)	$C_{29}H_{27}FNO_6PNa_2 \cdot 0.80H_2O$	14.4
4v	4-FC_6H_4	i-C_3H_7	$n = 3$		CH=CH(t)	Na	+12.2 (0.45)	21 (F)	$C_{29}H_{29}FNO_6PNa_2 \cdot 2.50H_2O$	1.3
5v	4-FC_6H_4	i-C_3H_7	$n = 3$		CH_2CH_2	Na	+1.3 (0.38)	80 (G)	$C_{29}H_{31}FNO_6PNa_2 \cdot 2.04H_2O$	18.6
6v	4-FC_6H_4	i-C_3H_7	$n = 3$		CH_2O	Na	–0.3 (0.34)	28 (J)	$C_{28}H_{29}FNO_6PNa_2 \cdot 2.0H_2O$	1.2
3w	4-FC_6H_4	t-C_4H_9	H	C_6H_5	C≡C	Li	+13.1 (0.42)	17 (H)	$C_{27}H_{25}FNO_6PLi_2 \cdot 1.01H_2O$	68.4
3x	4-FC_6H_4	c-C_3H_5	H	C_6H_5	C≡C	Na	+5.6 (0.81)	59 (H)	$C_{28}H_{21}FNO_6PNa_2 \cdot 2.0H_2O$	90.9
6x	4-FC_6H_4	c-C_3H_5	H	C_6H_5	CH_2O	Na	–1.2 (0.50)	35 (J)	$C_{25}H_{23}FNO_6PNa_2 \cdot 2.50H_2O$	29
3y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	C≡C	Na	+8.8 (0.62)	57 (H)	$C_{27}H_{23}FNO_6PNa_2 \cdot 1.25H_2O$	4.6
4y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	CH=CH(t)	Na	+6.7 (0.50)	30 (F)	$C_{27}H_{25}FNO_6PNa_2 \cdot 1.33H_2O$	2.5
5y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	CH_2CH_2	Na	–0.3 (0.32)	76 (G)	$C_{27}H_{27}FNO_6PNa_2 \cdot 1.25H_2O$	9.2
6y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	CH_2O	Na	–2.4 (0.41)	32 (J)	$C_{26}H_{25}FNO_6PNa_2 \cdot 1.75H_2O$	1.3
3z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	C≡C	Na	+8.1 (0.35)	21 (H)	$C_{26}H_{23}FNO_6PNa_2 \cdot 2.0H_2O$	109.9
4z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	CH=CH(t)	Na	+11.8 (0.48)	13 (F)	$C_{28}H_{25}FNO_6PNa_2 \cdot 1.75H_2O$	4.7
5z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	CH_2CH_2	Na	0 (0.33)	46 (G)	$C_{28}H_{27}FNO_6PNa_2 \cdot 1.25H_2O$	79.8
6z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	CH_2O	Na	–0.7 (0.45)	22 (J)	$C_{25}H_{25}FNO_6PNa_2 \cdot 3.13H_2O$	19.4
3aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	C≡C	Na	+7.5 (0.4)	19 (H)	$C_{28}H_{21}FNO_6PNa_2 \cdot 2.75H_2O$	1300
5aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	CH_2CH_2	Na	0 (0.44)[j]	44[g]	$C_{25}H_{25}FNO_6PNa_2 \cdot 2.05H_2O$	235
6aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	CH_2O	Na	–0.7 (0.58)	29 (J)	$C_{24}H_{23}FNO_6PNa_2 \cdot 1.84H_2O$	1300
3bb	i-C_3H_7	4-FC_6H_4	H	C_6H_5	C≡C	Li	+7.5 (0.85)	41 (I)	$C_{28}H_{25}FNO_6PLi_2 \cdot H_2O$	420
4bb	i-C_3H_7	4-FC_6H_4	H	C_6H_5	CH=CH(t)	Li	+1.8 (0.45)	12 (F)	$C_{28}H_{25}FNO_6PLi_2 \cdot 2.25H_2O$	45.3
3cc	4-F-3-MeC_6H_3	i-C_3H_7	H	C_6H_5	C≡C	Li	+9.5 (0.78)	53 (H)	$C_{27}H_{25}FNO_6PLi_2 \cdot 1.11H_2O$	22.0
3dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	C≡C	Na	+8.8 (0.38)	33 (I)	$C_{27}H_{25}FNO_6PNa_2 \cdot 3.0H_2O$	156
4dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	CH=CH(t)	Na	+1.6 (0.41)	12 (F)	$C_{27}H_{27}FNO_6PNa_2 \cdot 2.2H_2O$	3.9
5dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	CH_2CH_2	Na	–1.9 (0.43)	63 (G)	$C_{27}H_{29}FNO_6PNa_2 \cdot 1.75H_2O$	115

[a] Represents overall yield from vinyl dibromide 16 or acetylene 17. [b] Method F (Scheme III, steps a–c, e). Method G (Scheme III, steps d, e from 25). Method H (Scheme IV, steps a–c from 16). Method I (Scheme IV, steps a–c from 17). Method J (Scheme V, steps a–c). [c] Analyzed for C, H, N, F, and P. Results were within ±0.4% of theory unless otherwise noted. [d] Compounds were assayed against rat microsomal reductase using 100 μM R,S-HMG-CoA and 2.7 mM NADPH. See the Experimental Section for a description of this assay. Reductase enzyme I_{50} values and 95% confidence intervals were calculated from the linear segments of composite log dose versus percent inhibition regressions from 2–5 experiments. The average 95% confidence interval for I_{50} values reported was ±18.4% with a range of 8–40%. [e] Dihydroxy acid form, sodium salt. [f] Anal. Calcd: H, 5.36. Found: H, 5.82. [g] Obtained via hydrogenation of 27 rather than 25. [h] Not analyzed for phosphorus. [i] Anal. Calcd: H, 4.18. Found: H, 3.68. [j] $[\alpha]^{Hg}_{365} = -3.9°$ (MeOH, c = 0.44).

parable to mevinolin but is a much weaker inhibitor in human skin fibroblasts (31-fold). In fact, mevinolin is 7.7-fold more potent in fibroblasts than in hepatocytes. In contrast, our phosphorus-containing inhibitors exhibit a 4–140-fold selectivity for inhibition in hepatocytes versus fibroblasts, with 3o being the most selective. This selectivity is directly related to the presence of the phosphinic acid functionality. The corresponding dihydroxyheptanoic

Table IV. Inhibition of Cholesterol Synthesis from [^{14}C]Acetate in Hepatocytes and Fibroblasts and Inhibition of Cholesterol Biosynthesis from [^{14}C]Acetate in Rats on Intravenous (iv) and Oral (po) Administration[a]

no.	reductase (I_{50}, nM)	hepatocytes (I_{50}, nM)	fibroblasts[b] (I_{50}, nM)	selectivity[c]	in vivo testing (ED_{50}, mpk) iv	po
1[d]	4.0	.146	18.8	0.13	0.033	0.40[e]
2	24.0	100	3080	31	0.053	0.75
3a	59	197	9300	47	0.47	3.9
4a	1.9	77	2000	26	0.22	21.4
3o	4.5	81	11300	140	0.13	3.1
3k	6.1	556	2400	4.3	0.7	3.5
4o	1.2	260	2000	7.7	0.1	0.46
3p	5.6	519	6750	13	ND[f]	4.5
4p	0.55	241	4700	19.5	0.2[e]	>10

[a] The average 95% confidence intervals for the reported reductase, hepatocyte, and fibroblast I_{50} values were ±18.4, 40.9, and 56.9%, respectively. The average 95% confidence intervals for the iv and po ED_{50} values were 33.8 and 37.6%, respectively. All compounds were tested in 2–5 experiments. [b] Human skin fibroblasts. [c] Selectivity is measured as a ratio of I_{50} fibroblasts/I_{50} hepatocytes. [d] Tested as the dihydroxy acid form, sodium salt. [e] Tested po as the corresponding δ-lactone form. [f] Not determined.

acid[16] of 4a (where the P(O)OH group in 4a is replaced by (S)-OH) is 69-fold more potent in fibroblast (I_{50} = 2.6 nM) than in hepatocytes (I_{50} = 180 nM). These and other examples[1] indicate that hepatocyte selectivity is a general phenomenon in the phosphinic and phosphonic acid class of reductase inhibitors.

Also listed in Table IV are data obtained for the inhibition of cholesterol biosynthesis from [^{14}C]acetate in rats for a selected number of inhibitors. In general, these phosphinic acids are not as effective as the mevinic acids 1 and 2 upon intravenous (iv) or oral (po) administration. An exception is compound 4o, which shows in vivo activity comparable to that of both 1 and 2. The oral activity of these phosphorus-containing HMGR inhibitors shows no direct correlation with either in vivo reductase inhibitory potency or with in vivo activity after intravenous administration. However, there does appear to be a correlation between iv in vivo activity and activity in isolated rat hepatocytes. For example, despite the fact that 3o and 3k are nearly equipotent against HMGR, 3k is a 7-fold weaker inhibitor of cholesterol biosynthesis in hepatocytes. This is mirrored in a 5-fold loss in potency relative to 3o upon iv administration. However, 3o is still 4-fold less active than mevinolin (1) on iv administration despite equivalent intrinsic potency against reductase. This suggests that the poor in vivo activity of these compounds may be due in part to poor bioavailability to the liver, the target organ. Differences in oral activity (e.g., compare 3o and 4o) are probably due to poor oral absorption. The reasons for the lack of correlation between the in vitro and in vivo potencies of these compounds are currently under investigation.

Conclusion

A potent series of phosphorus-containing reductase inhibitors has been synthesized based on the utilization of highly substituted pyridine nuclei as hydrophobic anchor groups. By proper selection of both the pyridine anchor group and linker X–Y, compounds with enzyme inhibitory activities comparable to or greater than mevinolin (Na salt) have been attained. As determined with rat hepatocytes and human skin fibroblasts, these compounds also show a degree of hepatocyte selectivity not generally exhibited in the dihydroxyheptanoic acid class of inhibitors. In these studies, compound 4o exhibited acute in vivo activity in rats comparable to the clinically proven agents 1 and 2. Inhibitor 4o has been studied for cholesterol-lowering activity in other animal species such as rabbits, dogs, and monkeys. The results of these studies will be presented separately. In addition, an extension of this work to other aromatic and heteroaromatic hydrophobic anchor systems will also be the subject of future disclosures.

Experimental Section

All reactions were carried out under a static atmosphere of argon and stirred magnetically unless otherwise noted. All reagents used were of commercial quality and were obtained from Aldrich Chemical Co. Dry THF and Et_2O were obtained by distillation from the sodium ketyl of benzophenone under nitrogen. Dry CH_2Cl_2 was obtained by distillation from CaH_2 under nitrogen. Pyridine and dioxane were obtained from American Burdick and Jackson and were stored over 4A molecular sieves. Boiling points are uncorrected. Melting points were obtained on a Hoover Uni-melt melting point apparatus and are uncorrected. Infrared spectra were recorded on a Mattson Sirius 100-FTIR spectrophotometer. ^{1}H NMR spectra were recorded on a JEOL JNM-GX270 spectrometer using Me_4Si as an internal standard. Optical rotations were measured in a 1-dm cell on a Perkin-Elmer 241 polarimeter and c is expressed in g/100 mL. All flash chromatographic separations were performed using E. Merck silica gel (60, particle size, 0.040–0.063 mm). MCI Gel CHP-20P is a highly porous polystyrene–divinylbenzene copolymer resin (75–150 μM) supplied by Mitsubishi Chemical Industries Ltd. Reactions were monitored by TLC using 0.25 mm E. Merck silica gel plates (60 F_{254}) and were visualized with UV light, 5% phosphomolybdic acid in 95% EtOH, or p-anisaldehyde in EtOH/H_2SO_4/HOAc.

General Procedure for the Synthesis of 1,5-Diketones 11. Method A. 2-[(4-Fluorophenyl)methylene]-4-methyl-3-oxopentanoic Acid, Ethyl Ester (12, R^1 = 4-FC$_6$H$_4$, R^2 = i-C$_3$H$_7$). A mixture of 4-fluorobenzaldehyde (3.00 g, 24 mmol), ethyl isobutyrylacetate (3.82 g, 24 mmol), piperidine (240 μL), and HOAc (42 μL) was refluxed in benzene (15 mL) with removal of water (Dean–Stark trap) for 22 h. The cooled mixture was diluted with Et_2O, washed successively with 2% HCl, saturated $NaHCO_3$, H_2O, and brine, dried (Na_2SO_4), filtered, and stripped to yield an oil. Distillation of the oil (bp 110–113 °C (0.25 mmHg)) afforded 12 (R^1 = 4-FC$_6$H$_4$, R^2 = i-C$_3$H$_7$, 5.32 g, 83%) as a pale yellow liquid. The compound was obtained as a 1:1 mixture of E and Z isomers (a and b): TLC R_f 0.35 (20% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$) δ 1.07 (d, J = 7.2 Hz, 6 H$_a$), 1.18 (d, J = 7.2 Hz, 6 H$_b$), 1.25–1.35 (m, 6 H$_{a\&b}$), 2.70 (m, 1 H$_a$), 3.14 (m, 1 H$_b$), 4.25–4.37 (m, 4 H$_{a\&b}$), 7.01–7.09 (m, 4 H$_{a\&b}$), 7.34–7.49 (m, 4 H$_{a\&b}$), 7.53 (s, 1 H$_b$), 7.72 (s, 1 H$_a$); IR (neat) 1722, 1699, 1605, 1510, 1239 cm^{-1}. Anal. ($C_{15}H_{17}FO_3$) C, H, F. In the same manner, ethyl 3-cyclopropyl-3-oxopropionate[17] (R^2 = c-C$_3$H$_5$), methyl propionylacetate (R^2 = CH_2CH_3), and ethyl acetoacetate (R^2 = CH_3) were reacted with 4-fluorobenzaldehyde to give the corresponding Knoevenagel condensation products 12 in 82%, 70%, and 68% yields, respectively.

(16) The corresponding dihydroxyheptanoic acid (Li salt) of 4a was prepared in racemic form from 15a utilizing methods similar to that described in ref 7c.

(17) Jackman, M.; Bergman, A. J.; Archer, S. The Preparation of Some 6-Substituted-2-thiouracils. *J. Am. Chem. Soc.* 1948, *70*, 497–500.

β-(4-Fluorophenyl)-α-(2-methyl-1-oxopropyl)-δ-oxobenzenepentanoic Acid, Ethyl Ester (11o). A −78 °C solution of $LiN(TMS)_2$ (1.0 M in THF, 14.1 mL, 14.1 mmol) in dry THF (15 mL) was treated with a solution of propiophenone (1.900 g, 14.2 mmol) in THF (1.5 mL) over a 5-min period. After 1 h, a solution of compound 12 (R^1 = 4-FC_6H_4, R^2 = i-C_3H_7, 3.717 g, 14.1 mmol) in THF (3 mL) was added dropwise to the above solution. After 1.5 h, the mixture was quenched with saturated NH_4Cl and warmed to room temperature. The mixture was diluted with H_2O and subsequently extracted twice with Et_2O. The combined Et_2O extracts were washed with brine, dried (Na_2SO_4), filtered, and stripped to give an oil. Flash chromatography (15% EtOAc in hexane as eluant) afforded Michael adduct 11o (4.755 g, 85%) as a complex mixture of three diastereomers. The mixture was used directly in the next reaction: TLC R_f 0.34–0.31 (20% EtOAc in hexanes); IR ($CHCl_3$) 2974, 1740, 1713, 1682, 1510, 1224 cm^{-1}. In most cases, an excess of ketone $R^4COCH_2R^3$ (1.2 equiv) and $LiN(TMS)_2$ (1.2 equiv) relative to 12 were used for the formation of compound 11. The crude adducts were used directly in the next reaction prior to removal of the volatiles by vacuum distillation (0.2 mmHg at 80 °C).

Method B. 3-(4-Fluoro-3-methylphenyl)-1-phenyl-2-propen-1-one (9, R^1 = 4-F, 3-MeC_6H_3, R^4 = C_6H_5). A mixture of 4-fluoro-3-methylbenzaldehyde 8 (16.000 g, 115.8 mmol) and acetophenone (13.920 g, 115.8 mmol) in absolute EtOH (120 mL) was treated with a solution of EtONa in EtOH (21% wt solution, 4.3 mL, 11.6 mmol). A precipitate soon fell out of solution. After stirring at room temperature for 16 h, the mixture was cooled to −10 °C and the precipitate was collected by filtration. The solid was washed with cold EtOH and dried in vacuo to yield enone 9 (R^1 = 4-F, 3-MeC_6H_3, R^4 = C_6H_5, 23.560 g, 85%) as a pale yellow solid: mp 100–101 °C; TLC R_f 0.42 (20% EtOAc in hexane); ^1H NMR ($CDCl_3$) δ 2.32 (s, 3 H), 7.04 (t, J = 8.8 Hz, 1 H), 7.40–7.62 (m, 6 H), 7.75 (d, J = 15.8 Hz, 1 H), 7.97–8.06 (m, 2 H); IR (KBr) 1659, 1600, 1587, 1501, 1247 cm^{-1}. Anal. ($C_{16}H_{13}FO$) C, H, F.

β-(4-Fluoro-3-methylphenyl)-α-(2-methyl-1-oxopropyl)-δ-oxo-δ-phenylpentanoic Acid, Ethyl Ester (11cc). A slurry of enone 9 (R^1 = 4-F, 3-MeC_6H_3, R^4 = C_6H_5, 23.165 g, 96.5 mmol) and ethyl isobutyrylacetate (22.88 g, 144.6 mmol) in absolute EtOH (400 mL) was treated with a solution of EtONa in EtOH (21% wt solution, 5.4 mL, 14.5 mmol). After being stirred at room temperature for 4.5 h, the solution was concentrated to 200 mL and partitioned between 50% saturated NH_4Cl and EtOAc. The layers were separated, and the EtOAc layer was washed with H_2O (2×) and brine (2×), dried (Na_2SO_4), filtered, and stripped to yield an oil. The oil was taken up in warm hexane and cooled to produce a solid. The solid was boiled in hexanes and cooled to give Michael adduct 11cc (30.815 g, 80%), a 1:1 mixture of diastereomers, as a white amorphous solid: TLC R_f 0.34 and 0.30 (20% EtOAc in hexanes); ^1H NMR ($CDCl_3$, 270 MHz, integration values are relative) δ 0.70 (d, J = 6.6 Hz, 3 H), 0.94–1.05 (m, 6 H), 1.07–1.13 (m, 6 H), 1.24 (t, J = 7.2 Hz, 3 H), 2.18 (s, 6 H), 2.39 (m, 1 H), 2.76 (m, 1 H), 3.20–3.52 (m, 4 H), 3.93 (q, J = 7.2 Hz, 2 H), 4.06–4.23 (m, 6 H), 6.83 (pseudo t, 2 H), 7.01 (m, 4 H), 7.38–7.57 (m, 6 H), 7.87 (m, 4 H); IR (KBr) 1738, 1711, 1683, 1503, 1245 cm^{-1}. Anal. ($C_{24}H_{27}FO_4$) C, H, F.

General Procedure for the Synthesis of Pyridyl Alcohols 14 (Table I). 4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinecarboxylic Acid, Ethyl Ester (13o). A mixture of 11o (4.730 g, 11.87 mmol), NH_4OAc (2.745 g, 35.6 mmol), and $Cu(OAc)_2$ (5.935 g, 29.7 mmol) in glacial HOAc (30 mL) was gently refluxed for 24 h. The solution was cooled to room temperature and subsequently poured into an ice-cold mixture of concentrated NH_4OH (50 mL) in H_2O (100 mL). The mixture was extracted twice with Et_2O, and the pooled Et_2O extracts were washed with H_2O and brine, dried (Na_2SO_4), filtered, and stripped to yield an oil. The oil was flash chromatographed (20% EtOAc in hexanes as eluant) to give pyridyl ester 13o as an oil (3.916 g, 87%), which slowly solidified on standing: mp 84–88 °C; TLC R_f 0.47 (20% EtOAc in hexanes); ^1H NMR ($CDCl_3$) δ 1.00 (t, J = 7.0 Hz, 3 H), 1.33 (d, J = 6.5 Hz, 6 H), 2.04 (s, 3 H), 3.12 (m, 1 H), 4.01 (q, J = 7.0 Hz, 2 H), 7.05–7.59 (m, 9 H); IR (KBr) 1718, 1510, 1270 cm^{-1}. Anal. ($C_{24}H_{24}FNO_2$) C, H, F, N.

4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinemethanol (14o). An ice-cold slurry of $LiAlH_4$ (1.49 g, 39.3 mmol) in dry THF (50 mL) was treated with a solution of ester 13o (4.571 g, 12.11 mmol) in dry THF (20 mL). Ten minutes after the addition, the cooling bath was removed and the mixture was stirred at room temperature for 4 h. Additional $LiAlH_4$ (500 mg) was added, and stirring was continued for 2 more h. The solution was recooled to 0 °C and quenched in succession with H_2O (2 mL), 10% NaOH (2.5 mL), and H_2O (6 mL). The solution was filtered, and the salts were washed with EtOAc. The filtrate was washed with H_2O and brine and then dried (Na_2SO_4). Filtration and removal of the solvent afforded a solid. The solid was recrystallized from EtOAc/hexane to provide compound 14o (3.729 g, 92%) as white crystals: mp 182–184 °C; TLC R_f 0.20 (20% EtOAc in hexanes); ^1H NMR ($CDCl_3$) δ 1.29 (t, J = 5.3 Hz, 1 H, OH), 1.36 (d, J = 7.0 Hz, 6 H), 1.96 (s, 3 H), 3.50 (m, 1 H), 4.44 (d, J = 5.3 Hz, 2 H), 7.12–7.26 (m, 4 H), 7.33–7.47 (m, 3 H), 7.54–7.60 (m, 2 H); IR (KBr) 3420, 1509, 1218 cm^{-1}. Anal. ($C_{22}H_{22}FNO$) C, H, N, F.

General Procedure for the Synthesis of Pyridyl Vinyl Dibromides 16 (Table II). Oxidation with Dess–Martin Periodinane.[18] 4-(4-Fluorophenyl)-2-(1-methylethyl)-6-phenyl-3-pyridinecarboxaldehyde (15a). A slurry of Dess–Martin periodinane (8.60 g, 20.3 mmol) in CH_2Cl_2 (100 mL) was treated with *tert*-butyl alcohol (1.9 mL, 1.49 g, 20.2 mmol), and the mixture was stirred at room temperature for 15 min. A solution of alcohol 14a (5.011 g, 15.6 mmol) in CH_2Cl_2 (85 mL) was then added over a 5-min period. After 30 min, the mixture was diluted with Et_2O and 1 N NaOH and stirred rapidly for 10 min. The organic layer was separated and washed in succession with 1 N NaOH, H_2O, and brine, dried (Na_2SO_4), filtered, and stripped. The solid residue was flash chromatographed (10% EtOAc in hexanes as eluant) to give aldehyde 15a (4.314 g, 87%) as a white solid: mp 105–107 °C (hexane); TLC R_f (0.50 (20% EtOAc in hexanes); ^1H NMR ($CDCl_3$) δ 1.41 (d, J = 6.6 Hz, 6 H), 3.98 (m, 1 H), 7.16 (m, 2 H), 7.33–7.53 (m, 5 H), 7.57 (s, 1 H), 8.17 (m, 2 H), 10.07 (s, 1 H); IR (KBr) 1688, 1573, 1508, 1233 cm^{-1}. Anal. ($C_{21}H_{18}FNO$) C, H, F, N.

Oxidation with TPAP/NMO.[19] 6-(Cyclopropyl)-4-(4-fluorophenyl)-5-methyl-2-(1-methylethyl)-3-pyridinecarboxaldehyde (15y). A solution of 4-methylmorpholine *N*-oxide (4.002 g, 34.2 mmol) in CH_2Cl_2 (130 mL) was dried over $MgSO_4$ for 15 min. The solution was filtered directly into a 500-mL flask, using approximately 30 mL of CH_2Cl_2 to effect the transfer. The flask was then charged with dry 4A molecular sieves (16 g), alcohol 14y (5.686 g, 17.05 mmol), and tetrapropylammonium perruthenate (TPAP, 301 mg, 0.86 mmol). After being stirred at room temperature for 30 min, the black solution was diluted with Et_2O (200 mL), stirred for 5 min, and then filtered through a plug of silica gel (65 × 30 mm), washing with Et_2O. The filtrate was stripped to give a pale yellow solid. The solid was recrystallized from EtOAc/hexane to give aldehyde 15y (3.982 g) as white crystals. Flash chromatography of the mother liquor (20% EtOAc in hexane as eluant) gave additional product, which was recrystallized from hexane (499 mg). Total pooled solids, 4.481 g (79%): mp 137–139 °C; TLC R_f 0.50 (20% EtOAc in hexane); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.00 (m, 2 H), 1.24 (m, 2 H), 2.00 (s, 3 H), 3.16 (m, 1 H), 7.14–7.26 (m, 4 H), 7.39–7.58 (m, 5 H), 9.88 (s, 1 H); IR (KBr) 1686, 1545, 1508, 1223 cm^{-1}. Anal. ($C_{22}H_{18}FNO$) C, H, F, N.

Oxidation with Oxalyl Chloride/DMSO.[20] 4-(4-Fluorophenyl)-6,7-dihydro-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridine-3-carboxaldehyde (15v). A −78 °C solution of oxalyl chloride (630 μL, 917 mg, 7.2 mmol) in CH_2Cl_2 (40 mL) was treated dropwise with a solution of dry DMSO (1.10 mL, 1.21 g, 15.5 mmol) in CH_2Cl_2 (1 mL). After 10 min, a solution

(18) Dess, D. B.; Martin, J. C. Readily Accessible 12-I-5′ Oxidant for the Conversion of Primary and Secondary Alcohols to Aldehydes and Ketones. *J. Org. Chem.* 1983, *48*, 4155–4156.
(19) Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. Preparation and Use of Tetra-*n*-butylammonium Perruthenate (TBAP reagent) and Tetra-*n*-propylammonium Perruthenate (TPAP reagent) as New Catalytic Oxidants for Alcohols. *J. Chem. Soc., Chem. Commun.* 1987, 1625–1627.
(20) Mancuso, A. J.; Huang, S.-L.; Swern, D. Oxidation of Long-Chain and Related Alcohols to Carbonyls by Dimethyl Sulfoxide "Activated" by Oxalyl Chloride. *J. Org. Chem.* 1978, *43*, 2480–2482.

of alcohol 14v (2.000 g, 5.5 mmol) in THF (5 mL) was added dropwise to the above mixture. Fifteen minutes after the addition, TEA (4.6 mL) was added and the mixture was stirred at −78 °C for 5 min and then warmed to room temperature. The mixture was diluted with Et_2O and washed twice with H_2O and once with brine. The organic layer was dried (Na_2SO_4), filtered, and stripped to give a yellow oil, which produced a solid upon cooling to −78 °C in hexane. The mixture was crystallized from hexane to give aldehyde 15v (1.775 g, 89%) as white needles: mp 132–134 °C; TLC R_f 0.54 (20% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.37 (d, J = 7.0 Hz, 6 H), 2.06 (m, 2 H), 2.18 (m, 2 H), 2.62 (m, 2 H), 3.96 (m, 1 H), 7.11–7.48 (m, 7 H), 7.89 (d, J = 8.0 Hz, 1 H), 9.90 (s, 1 H); IR (KBr) 1693, 1546, 1507, 1223 cm^{-1}. Anal. ($C_{24}H_{22}FNO$) H, F, N; C: calcd 80.20, found 79.58.

3-(2,2-Dibromoethenyl)-4-(4-fluorophenyl)-6,7-dihydro-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridine (16v). A solution of carbon tetrabromide (2.336 g, 7.0 mmol) in CH_2Cl_2 (6 mL) was added over a 7-min period to a cold (0 °C) solution of aldehyde 15v (1.688 g, 4.7 mmol) and triphenylphosphine (3.698 g, 14.1 mmol) in CH_2Cl_2 (20 mL). After the addition was complete, the cooling bath was removed and the mixture was stirred at room temperature for 25 min. The solution was quenched with saturated $NaHCO_3$ and extracted twice with CH_2Cl_2. The organic layers were dried (Na_2SO_4), filtered, and concentrated. The concentrate was flash chromatographed (40% CH_2Cl_2 in hexane as eluant) to give vinyl dibromide 16v as a solid. Recrystallization of the material from EtOAc/hexane provided pure 16v (2.257 g, 93%) as a white solid: mp 173–175 °C; TLC R_f 0.44 (10% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.33 (broad, 6 H), 2.06 (m, 2 H), 2.18 (m, 2 H), 2.61 (m, 2 H), 3.19 (m, 1 H), 7.03–7.43 (m, 8 H), 7.84 (d, J = 8.4 Hz, 1 H); IR (KBr) 2950, 2920, 1603, 1508, 1222 cm^{-1}. Anal. ($C_{25}H_{22}Br_2FN$) C, H, Br, F, N.

(*S*)-4-Iodo-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (19). A solution of bromohydrin 18 (4.00 g, 20.4 mmol), imidazole (6.94 g, 102 mmol), and DMAP (12 mg) in dry DMF (40 mL) was treated with *tert*-butylchlorodiphenylsilane (5.84 mL, 6.17 g, 22.5 mmol), and the homogeneous mixture was stirred at room temperature overnight. The mixture was partitioned between 5% $KHSO_4$ and EtOAc, and the organic phase was washed with H_2O and brine, dried (Na_2SO_4), filtered, and stripped to give 9.32 g (100%) of the crude silyl ether (TLC R_f 0.75 (25% EtOAc in hexanes)). A solution of the silyl ether (9.32 g, 20.1 mmol) in dry methyl ethyl ketone (MEK, 60 mL) was treated with sodium iodide (15.06 g, 100.5 mmol), and the yellow suspension was refluxed for 5 h. The mixture was cooled, diluted with EtOAc, and filtered, and the filtrate was washed with dilute $NaHSO_3$ and brine. The organic layer was dried (Na_2SO_4), filtered, and stripped to give a yellow oil. Flash chromatography (25% CH_2Cl_2 in hexanes as eluant) afforded iodide 19 (7.69 g, 74% from 18) as a colorless oil: TLC R_f 0.75 (25% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.05 (s, 9 H), 2.67 (m, 2 H), 3.20 (m, 2 H), 3.58 (s, 3 H), 3.95 (m, 1 H), 7.28–7.72 (m, 10 H).

(*S*)-4-[Bis(isopropyloxy)phosphinyl]-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (20). Freshly distilled triisopropyl phosphite (113.4 mL, 93.92 gm, 451 mmol) was added in one portion to iodide 19 (21.70 g, 45.1 mmol), and the mixture was heated at 155 °C for 16.5 h. The mixture was cooled to room temperature, and the excess triisopropyl phosphite and volatile reaction products were removed by short path distillation (10 mmHg) followed by Kugelrohr distillation (100 °C, 8 h at 0.5 mmHg). The product was further purified by flash chromatography (6:3:1 hexanes–acetone–toluene as eluant) to afford 20 (17.68 g, 75%) as a clear viscous oil: TLC R_f 0.32 (6:3:1 hexanes–acetone–toluene); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.01 (s, 9 H), 1.12 and 1.19 (2 d, J = 6.3 Hz each, 12 H), 1.87–2.24 (m, 2 H), 2.60 and 2.65 (2 d, J = 7.4 Hz each, 1 H), 2.88 and 2.94 (2 d, J = 3.7 Hz each, 1 H), 3.59 (s, 3 H), 4.44–4.57 (m, 3 H), 7.35–7.45 (m, 6 H), 7.65–7.70 (m, 4 H).

(*S*)-4-(Hydroxymethoxyphosphinyl)-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester, Dicyclohexylamine (1:1) Salt (21). A solution of compound 20 (10.66 g, 30.5 mmol) in dry CH_2Cl_2 (80 mL) was treated dropwise (5 minutes) with bis(trimethylsilyl)trifluoroacetamide (BSTFA, 8.71 mL, 8.44 g, 32.8 mmol), followed by dropwise addition (10 min) of trimethylsilyl bromide (TMSBr, 6.75 mL, 7.84 g, 51.3 mmol). After stirring at room temperature for 20 h, the reaction mixture was quenched with 200 mL of 5% $KHSO_4$ and stirred vigorously for 15 min. The aqueous layer was extract with EtOAc (3×), and the pooled organic layers were washed with brine, dried (Na_2SO_4), filtered, and stripped. The residue was azeotroped twice with 50 mL of toluene. The precipitate that formed was suspended in toluene and removed by filtration. The filtrate was concentrated, and the azeotrope/filter process was repeated to give a viscous, clear oil. The oil was dissolved in pyridine (50 mL) and subsequently treated with dicyclohexylcarbodiimide (DCC, 4.65 g, 22.6 mmol) followed by methanol (1.67 mL, 1.31 g, 41 mmol). After being stirred at room temperature for 20 h, the mixture was filtered through a pad of Celite, which was subsequently washed with EtOAc. The filtrate was stripped, redissolved in EtOAc, and washed with 5% $KHSO_4$ (2×) and brine. The EtOAc solution was dried (Na_2SO_4), filtered, and stripped, and the residue was azeotroped twice with toluene. The residue was suspended in toluene and filtered. The filtrate was again concentrated, taken up in toluene, filtered, stripped, and placed under high vacuum to give the corresponding phosphonate monoester (10.2 g, >100%, TLC R_f 0.50 (7:2:1 *n*-PrOH–NH_4OH–H_2O)) as a clear, viscous oil. The monoester (1.16 g, 2.57 mmol) was dissolved in dry Et_2O (10 mL) and treated with dicyclohexylamine (0.528 mL, 0.481 g, 2.65 mmol). The resulting homogeneous solution was stored at room temperature for 7 h and at −20 °C for 16 h. The solid/liquid auspension was warmed to room temperature and filtered, and the solid was washed with cold Et_2O and dried in vacuo to give 21 (1.25 g, 77% yield) as a white powdery solid: mp 155–156 °C; TLC R_f 0.57 (20% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.00 (s, 9 H), 1.08–1.92 (m, 22 H), 2.56–2.62 (m, 1 H), 2.64–2.77 (m, 2 H), 3.11 (d, J = 11.0 Hz, 3 H), 3.22 and 3.28 (2 m, 1 H), 3.52 (s, 3 H), 4.02 (m, 1 H), 7.32–7.40 (m, 6 H), 7.65–7.71 (m, 4 H); IR (KBr) 1736 cm^{-1}; $[\alpha]_D$ = −16.0° (MeOH, c = 3.57). Anal. ($C_{22}H_{31}O_6PSi \cdot C_{12}H_{23}N$) C, H, N.

General Procedure for the Synthesis of Acetylenic Linked Phosphinic Acids 3. (*S*)-4-[[[4-(4-Fluorophenyl)-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridin-3-yl]-ethynyl]methoxyphosphinyl]-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (27v). DCHA salt 21 (3.682 g, 5.83 mmol) was partitioned between EtOAc and 5% $KHSO_4$. The EtOAc layer was washed three times with 5% $KHSO_4$ and then with brine, dried (Na_2SO_4), filtered, and stripped to give a colorless oil (phosphonic acid monomethyl ester). The oil was dissolved in dry CH_2Cl_2 (10 mL) and treated with diethyl(trimethylsilyl)amine (2.10 mL, 1.61 g, 11.1 mmol). After the mixture was stirred at room temperature for 1 h, the solvent was removed in vacuo and the residue was azeotroped with dry toluene (15 mL). The residue was redissolved in dry CH_2Cl_2 (15 mL), cooled to 0 °C, and treated with 2 drops of DMF and oxalyl chloride (620 μL, 902 mg, 7.1 mmol). After 15 min, the solution was warmed to room temperature and stirred for an additional 45 min. The solvent was stripped, and the yellow residue (phosphonochloridate 22) was azeotroped with toluene (15 mL) and dried in vacuo (oil pump) for 1 h.

Meanwhile, a solution of vinyl dibromide 16v (2.000 g, 3.88 mmol) in THF (10 mL) at −78 °C was treated with *n*-BuLi (2.5 M in hexane, 3.3 mL, 8.2 mmol) over a 1-min period, and the resulting clear green solution was stirred at −78 °C for 50 min. The acetylenic anion solution was added dropwise via canula over a 10-min period to a −78 °C solution of the above prepared phosphonochloridate 22 in THF (12 mL). The resulting mixture was stirred at −78 °C for 30 min and then quenched with 50% saturated NH_4Cl. The solution was warmed to 0 °C and poured into saturated $NaHCO_3$. The aqueous phase was extracted once with Et_2O. The Et_2O layer was washed with brine, dried (Na_2SO_4), filtered, and stripped to give an oil. The residue was flash chromatographed (40% EtOAc in hexanes as eluant) to afford compound 27v, a mixture of diastereomers, as a colorless foam (2.517 g, 82%): TLC R_f 0.31 (40% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.02 (s, 9 H), 1.31 and 1.35 (2 d, J = 6.6 Hz each, 6 H), 2.00–2.38 (m, 6 H), 2.47–2.81 (m, 4 H), 3.30 and 3.37 (2 d, $J_{H,P}$ = 12.6 Hz each, 3 H), 3.54 (m, 1 H), 3.58 (s, 3 H), 4.51 (m, 1 H), 6.99–7.46 (m, 13 H), 7.58–7.72 (m, 4 H), 7.83 (d, J = 7.2 Hz, 1 H); IR (KBr) 2168, 1740, 1508, 1224, 1036 cm^{-1}. In the case where acetylene 17 is used in the coupling reaction, 1.1 equiv

of *n*-BuLi is added to a solution of the acetylene in 17 in THF at −78 °C. After 20 min, the acetylenic anion solution is then coupled to 22 as described above.

(*S*)-4-[[[4-(4-Fluorophenyl)-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridin-3-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic Acid, Methyl Ester (28v). A mixture of compound 27v (2.487 g, 3.15 mmol) and HOAc (810 μL, 850 mg, 14.1 mmol) in THF (40 mL) was treated with tetra-*n*-butylammonium fluoride (1.0 M in THF, 11.0 mL, 11.0 mmol). After stirring at room temperature for 18 h, the solution was diluted with EtOAc and washed with 5% $KHSO_4$ (3×) and once with brine. The EtOAc layer was dried (Na_2SO_4), filtered, and stripped to afford a yellow oil. The oil was dissolved in Et_2O, cooled to 0 °C, and treated with excess diazomethane for 10 min. The excess diazomethane was destroyed by the addition of HOAc, and the solvent was removed in vacuo. The residue was flash chromatographed (40% acetone in hexanes as eluant) to afford compound 28v (1.534 g, 89%) as a colorless foam: TLC R_f 0.38 (1:1 acetone–hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.40 (d, *J* = 6.6 Hz, 6 H), 1.94–2.15 (m, 4 H), 2.15–2.28 (m, 2 H), 3.53–3.67 (m, 4 H), 3.59 (d, $J_{H,P}$ = 12.6 Hz, 3 H), 3.57–3.70 (m, 2 H, C*H*-$(CH_3)_2$ and O*H*), 3.73 (s, 3 H), 4.36 (m, 1 H), 7.12–7.48 (m, 7 H), 7.85 (d, *J* = 6.6 Hz, 1 H); IR (KBr) 2170, 1737, 1508, 1223, 1035 cm^{-1}.

(*S*)-4-[[[4-(4-Fluorophenyl)-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridin-3-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic Acid, Disodium Salt (3v). A solution of compound 27v (780 mg, 1.42 mmol) in dioxane (7 mL) was treated with 1 N NaOH (5.0 mL, 5.0 mmol), and the mixture was stirred at room temperature for 18 h. The solvent was evaporated, and the residue was chromatographed on CHP-20P (25 mm × 90 mm), eluting in succession with H_2O (200 mL), 50% MeOH in H_2O (200 mL), and MeOH (100 mL). The desired fractions were pooled and evaporated, and the residue was taken up in H_2O and lyophilized to give 3v (744 mg, 90%) as a white solid: TLC R_f 0.17 (8:1:1 CH_2Cl_2–HOAc–MeOH); ^{1}H NMR (CD_3OD, 270 MHz) δ 1.36 (d, *J* = 7.0 Hz, 6 H), 1.55–1.72 (m, 2 H), 2.01–2.20 (m, 4 H), 2.26 (dd, *J* = 7.8, 15.0 Hz, 1 H), 2.40 (dd, *J* = 4.2, 15.0 Hz, 1 H), 2.59 (m, 2 H), 3.83 (m, 1 H), 4.19 (m, 1 H), 7.16–7.42 (m, 7 H), 7.72 (m, 1 H); IR (KBr) 2164, 1634, 1508, 1213, 1184, 1058 cm^{-1}. Anal. ($C_{29}H_{27}FNNa_2O_5P\cdot0.80H_2O$) C, H, F, N, P.

General Procedure for the Synthesis of *trans*-Vinyl- and Ethyl-Linked Phosphinic Acids 4 and 5. 3-(1-Ethynyl)-4-(4-fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenylpyridine (17o). To a solution of *n*-BuLi (2.5 M in hexanes, 4.00 mL, 10.0 mmol) in dry THF (8 mL) at −78 °C was added a solution of vinyl dibromide 16o (2.267 g, 4.63 mmol) in dry THF (8 mL) over a 5-min period. After being stirred at −78 °C for 1 h, the pale green solution was quenched with saturated NH_4Cl and warmed to room temperature. The mixture was diluted with H_2O and extracted with Et_2O, and the Et_2O extract was washed with brine, dried (Na_2SO_4), filtered, and stripped to yield a solid. The residue was recrystallized from EtOAc/hexane to afford acetylene 17o (1.420 g, 93%, 2 crops) as a white solid: mp 178.0–178.5 °C; TLC R_f 0.43 (10% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.34 (d, *J* = 7.0 Hz, 6 H), 2.04 (s, 3 H), 3.18 (s, 1 H), 3.69 (m, 1 H), 7.15 (m, 2 H), 7.27 (m, 2 H), 7.36–7.48 (m, 3 H), 7.60 (m, 2 H); IR (KBr) 3165, 2099, 1509, 1213 cm^{-1}. Anal. ($C_{23}H_{20}FN$) C, H, F, N.

(*E*)-4-(4-Fluorophenyl)-3-(2-iodoethenyl)-5-methyl-2-(1-methylethyl)-6-phenylpyridine (23o). A mixture of acetylene 17o (1.355 g, 4.1 mmol) and AIBN (20 mg) in tri-*n*-butyltin hydride (2.0 mL) was rapidly heated to 120 °C. After 4 min of heating, the mixture was treated with additional Bu_3SnH (0.6 mL) and the temperature of the reaction was raised to 140 °C. Approximately 20 mg of AIBN was added to the reaction mixture 1 and 2 h after heating was initiated. After 3 h, the mixture was cooled to room temperature, diluted with Et_2O (50 mL), and treated with solid I_2 (3.50 g, 13.8 mmol). The dark reaction mixture was stirred for 45 min and then poured into a 50% saturated $NaHCO_3$ solution containing 6.7 g of $Na_2S_2O_3$. The layers were shaken and separated. The ethereal layer was washed successively with H_2O, 1.7 M NH_4OH, and brine, dried (Na_2SO_4), filtered, and stripped to yield a wet solid. The solid was taken up in Et_2O, filtered through Celite, and stripped. The residue was recrystallized from hexane to give compound 23o (1.335 g) as white crystals. The mother liquor was flash chromatographed (5% EtOAc in hexanes as eluant), and the desired fractions were pooled, stripped, recrystallized, and pooled with the above solid to give a total of 1.637 g (87%) of *trans*-vinyl iodide 23o: mp 148.5–150.0 °C; TLC R_f 0.13 (2% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.29 (d, *J* = 7.0 Hz, 6 H), 2.00 (s, 3 H); 3.31 (m, 1 H), 6.03 (d, *J* = 15.2 Hz, 1 H), 7.05–7.22 (m, 5 H), 7.34–7.49 (m, 3 H), 7.59 (m, 2 H); IR (KBr) 2961, 1508, 1221, 841 cm^{-1}. Anal. ($C_{23}H_{21}FIN$) C, H, F, I, N.

(*E*),(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethenyl]methoxyphosphinyl]-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (24o). A solution of *trans*-vinyl iodide 23o (1.400 g, 3.06 mmol) in THF (6 mL) was added over a 5-min period to a −100 °C solution of fresh *tert*-butyllithium (1.7 M in pentane, 3.70 mL, 6.3 mmol) in THF (8 mL). The resulting deep red solution was stirred at −100 °C for 25 min and then added via canula over an 8-min period to a −100 °C solution of phosphonochloridate 22 (prepared as in the example for compound 27v from 3.288 g 21) in THF (15 mL). The resulting yellow mixture was stirred at −100 °C for 5 min and at −78 °C for 25 min and then quenched with 50% saturated NH_4Cl. The solution was warmed to room temperature, diluted with H_2O, and poured into saturated $NaHCO_3$. The aqueous phase was extracted twice with Et_2O. The combined Et_2O layers were washed with brine, dried (Na_2SO_4), filtered, and stripped. The resulting yellow oil was flash chromatographed (50% EtOAc in hexanes as eluant) to afford adduct 24o, a 1:1 mixture of diastereomers, as an off-white foam (1.541 g, 66%): TLC R_f 0.22 (40% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.01 and 1.03 (2 s, 9 H), 1.20–1.31 (m, 7 H), 1.78 (m, 1 H), 1.98 and 2.00 (2 s, 3 H), 2.56 (m, 1 H), 2.81 (m, 1 H), 3.19 (pseudo t, $J_{H,P}$ = 11.5 Hz, 3 H), 3.21 (m, 1 H), 3.59 and 3.61 (2 s, 3 H), 4.38 and 4.52 (2 m, 1 H), 5.01 (dd, *J* = 17.9, 24.8 Hz, 0.5 H), 5.26 (dd, *J* = 17.9, 24.3 Hz, 0.5 H), 6.89–7.72 (m, 20 H); IR ($CHCl_3$) 2959, 1740, 1605, 1508, 1223, 1036 cm^{-1}.

(*E*),(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethenyl]methoxyphosphinyl]-3-hydroxybutanoic Acid, Methyl Ester (25o). A solution of compound 24o (1.519 g, 1.98 mmol) in THF (15 mL) was treated with HOAc (640 μL, 671 mg, 11.2 mmol) followed by tetra-*n*-butylammonium fluoride (1.0 M in THF, 10.0 mL, 10.0 mmol). After being stirred at room temperature for 19 h, the solution was poured into saturated $NaHCO_3$ and extracted with EtOAc. The EtOAc extract was washed with brine, dried (Na_2SO_4), filtered, and stripped to give an oil that was subsequently flash chromatographed (40–60% acetone in hexanes as eluant). Compound 25o (978 mg, 94%) was obtained as a white foam: TLC R_f 0.34 (1:1 acetone–hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.30 (d, *J* = 7.0 Hz, 6 H), 1.68–1.93 (m, 2 H), 2.00 (s, 3 H), 2.57 (m, 2 H), 3.30 (m, 1 H), 3.43 and 3.47 (2 d, $J_{H,P}$ = 4.7 and 4.1 Hz, 3 H), 3.66 and 3.79 (2 d, *J* = 2.4 Hz each, 1 H, O*H*), 3.72 (s, 3 H), 4.19 and 4.31 (2 m, 1 H), 5.51 (dd, *J* = 17.6, 24.6 Hz, 0.5 H), 5.52 (dd, *J* = 17.6, 24.3 Hz, 0.5 H), 7.10–7.65 (m, 10 H); IR ($CHCl_3$) 2961, 1736, 1605, 1510, 1221, 1034 cm^{-1}.

(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethyl]methoxyphosphinyl]-3-hydroxybutanoic Acid, Methyl Ester (26o). A mixture of compound 25o (494 mg, 0.94 mmol) and 10% Pd on carbon (110 mg) in MeOH (20 mL) was shaken under 50 psi of H_2 for 3 days. The solution was filtered through Celite, stripped, and flash chromatographed (50% acetone in hexanes) to give compound 26o (419 mg, 85%) as a colorless oil: TLC R_f 0.36 (1:1 acetone–hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.33 (d, *J* = 6.6 Hz, 6 H), 1.57–1.91 (m, 4 H), 1.92 (s, 3 H), 2.42–2.59 (m, 2 H), 2.60–2.74 (m, 2 H), 3.25 (m, 1 H), 3.55 and 3.57 (2 d, $J_{H,P}$ = 10.8 Hz each, 3 H), 3.72 (s, 3 H), 3.78 and 3.87 (2 d, *J* = 3.0 Hz each, 1 H, O*H*), 4.25 and 4.40 (2 m, 1 H), 7.11–7.25 (m, 4 H), 7.33–7.47 (m, 3 H), 7.56 (m, 2 H); IR ($CHCl_3$) 1734, 1509, 1221, 1179, 1040 cm^{-1}.

(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic Acid, Disodium Salt (4o). A solution of compound 25o (461 mg, 0.88 mmol) in dioxane (5 mL) was treated with 1 N NaOH (3.2 mL, 3.2 mmol), and the mixture was stirred at 60 °C for 1.5 h. The solvent was evaporated, and the residue was dissolved in H_2O and chromatographed on CHP-20P (25 mm

× 80 mm), eluting in succession with H_2O (150 mL) and 50% MeOH in H_2O (200 mL). The desired fractions were pooled and evaporated, and the residue was taken up in H_2O and lyophilized to give 4o (430 mg, 87%) as a white solid: TLC R_f 0.10 (8:1:1 CH_2Cl_2–HOAc–MeOH); ^1H NMR (CD_3OD, 400 MHz) δ 1.27 (d, J = 7.0 Hz, 6 H), 1.54 (dd, J = 7.2, 14.5 Hz, 2 H), 1.93 (s, 3 H), 2.33 (m, 2 H), 3.57 (m, 1 H), 4.10 (m, 1 H), 5.85 (dd, J = 18.0, 19.8 Hz, 1 H), 7.07 (pseudo t, J = 18.0 Hz, 1 H), 7.19 (d, J = 7.0 Hz, 4 H), 7.37–7.54 (m, 5 H); MS (FAB) [M – 2 Na + 3 H]$^+$ 498. Anal. ($C_{27}H_{27}FNNa_2O_5P\cdot1.2H_2O$) C, H, F, N, P.

(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic Acid, Disodium Salt (5o). Saponification of ethyl linked phosphinate 26o was similar to that of *trans*-vinyl-linked phosphinate 25o to give 5o in 77% yield: TLC R_f 0.10 (8:1:1 CH_2Cl_2–HOAc–MeOH); ^1H NMR (CD_3OD, 270 MHz) δ 1.41 (d, J = 7.0 Hz, 6 H), 1.49 (dd, J = 6.0, 12.6 Hz, 2 H), 1.71 (m, 2 H), 1.93 (s, 3 H), 2.35 (m, 2 H), 2.78 (m, 2 H), 3.58 (m, 1 H), 4.25 (m, 1 H), 7.20–7.60 (m, 9 H); IR (KBr) 2961, 1579, 1509, 1405, 1157 cm^{-1}. Anal. ($C_{27}H_{29}FNNa_2O_5P\cdot3.69H_2O$) C, H, F, N, P.

General Procedure for the Synthesis of Phosphonic Monoesters 6. (*S*)-4-[[[5-Ethyl-4-(4-fluorophenyl)-2-(1-methylethyl)-6-phenyl-3-pyridinyl]methoxy]methoxyphosphinyl]-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (29p). A 0 °C solution of phosphonochloridate 22 (from 2.89 g, 4.57 mmol DCHA salt 21) in pyridine (20 mL) was treated with a solution of alcohol 14p (888 mg, 2.54 mmol) in dry pyridine (7.0 mL). The resulting mixture was stirred at 0 °C for 16 h, diluted with EtOAc, and washed with 50% saturated NH_4Cl. The organic layer was then washed with H_2O followed by brine, dried (Na_2SO_4), filtered, and stripped. The amber residue was subject to flash chromatography (30% EtOAc in hexane) to give adduct 29p (1.104 gm, 56%) as a yellow oil: TLC R_f 0.53 (45% EtOAc in hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 0.70 (m, 3 H), 1.00 (s, 9 H), 1.22–1.38 (m, 8 H), 1.90 and 2.12 (2 m, 1 H), 2.37 (m, 2 H), 2.55 and 2.81 (2 m, 1 H), 3.29–3.39 (m, 4 H), 3.58 (s, 3 H), 4.43 (m, 1 H), 4.59 and 4.71 (2 m, 2 H), 7.02–7.70 (m, 9 H); IR (CH_2Cl_2) 2954, 1740, 1511, 1223, 1015 cm^{-1}.

(*S*)-4-[[[5-Ethyl-4-(4-fluorophenyl)-2-(1-methylethyl)-6-phenyl-3-pyridinyl]methoxy]methoxyphosphinyl]-3-hydroxybutanoic Acid, Metbyl Ester (30p). The silyl protecting group on 29p was removed via the same procedure as that described for compound 24o to give 30p in 90% yield: TLC R_f 0.59 (1:1 acetone–hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 0.70 (t, J = 6.8 Hz, 3 H), 1.34 (d, J = 7.0 Hz, 6 H), 1.92 (m, 2 H), 2.39 (q, J = 6.8 Hz, 2 H), 2.57 (d, J = 7.2 Hz, 2 H), 3.43 (m, 1 H), 3.63 (d, $J_{H,P}$ = 10.8 Hz, 3 H), 3.72 (s, 3 H), 4.31 (m, 1 H), 4.85 (m, 2 H), 7.12–7.28 (m, 5 H), 7.39–7.56 (m, 4 H); IR (CH_2Cl_2) 1734, 1636, 1510, 1221 cm^{-1}.

(*S*)-4-[[[5-Ethyl-4-(4-fluorophenyl)-2-(1-methylethyl)-6-phenyl-3-pyridinyl]methoxy]hydroxyphosphinyl]-3-hydroxybutanoic Acid, Disodium Salt (6p). A solution of compound 30p (650 mg, 1.20 mmol) in dioxane (10 mL) was treated with 1 N NaOH (3.7 mL, 3.7 mmol), and the mixture was stirred at 55 °C for 3 h. The solvent was evaporated to give a white solid. The residue was slurried in warm H_2O and chromatographed on CHP-20P (25 mm × 100 mm) eluting in succession with H_2O (200 mL) and 50% MeOH in H_2O (400 mL). The desired fractions were pooled and evaporated, and the residue was taken up in H_2O and lyophilized to give 6p (435 mg, 65%) as a white solid: TLC R_f 0.31 (8:1:1 CH_2Cl_2–HOAc–MeOH); ^1H NMR (CD_3OD, 270 MHz) δ 0.65 (t, J = 6.8 Hz, 3 H), 1.30 (d, J = 7.0 Hz, 6 H), 1.48 (dd, J = 7.6, 16.0 Hz, 2 H), 2.28 (q, J = 6.8 Hz, 2 H), 2.37 (m, 2 H), 3.66 (m, 1 H), 4.19 (m, 1 H), 4.64 (m, 2 H), 7.18–7.50 (m, 9 H); IR (KBr) 2935, 1581, 1510, 1404, 1222, 1020 cm^{-1}. Anal. ($C_{27}H_{28}FNNa_2O_6P\cdot H_2O$) C, H, F, N, P.

Biological Assays. Rat Hepatic HMG-CoA Reductase Inhibition. Rat hepatic HMG-CoA reductase activity is measured using a modification of the method described by Edwards.[21] Rat hepatic microsomes are used as a source of enzyme, and the enzyme activity is determined by measuring the conversion of the ^{14}C-HMG-CoA substrate to [^{14}C]mevalonic acid. Livers are removed from 2–4 cholestyramine-fed, decapitated, Sprague–Dawley rats, and homogenized in phosphate buffer A (potassium phosphate, 0.04 M, pH 7.2; KCl, 0.05 M; sucrose, 0.1 M; EDTA, 0.03 M, aprotinin, 500 KI units/mL). The homogenate is spun at 16000*g* for 15 min at 4 °C. The supernatant is removed and recentrifuged under the same conditions a second time. The second 16000*g* supernatant is spun at 100000*g* for 70 min at 4 °C. Pelleted microsomes are resuspended in a minimum volume of buffer A (3–5 mL per liver) and homogenized in a glass homogenizer. Dithiothreitol is added (10 mM), and the preparation is aliquoted, quick frozen in acetone/dry ice, and stored at −80 °C. The specific activity of a typical microsomal preparation is 0.68 nmol of mevalonic acid/mg of protein per minute. The reductase is assayed in 0.25 mL, which contains the following components at the indicated final concentrations: 0.04 M potassium phosphate, pH 7.2; 0.05 M KCl; 0.10 M sucrose; 0.03 M EDTA; 0.01 M dithiothreitol; 3.5 mM NaCl; 1% dimethyl sulfoxide; 50–200 μg of microsomal protein; 100 μM of ^{14}C-[D,L]-HMG-CoA (0.05 μCi, 30–60 mCi/mmol); 2.7 mM NADPH. Reaction mixtures are incubated at 37 °C. Under conditions described, enzyme activity increases linearly up to 300 μg of microsomal protein per reaction mixture and is linear with respect to incubation time up to 30 min. The standard incubation time chosen for drug studies is 20 min, which results in 12–15% conversion of HMG-CoA substrate to the mevalonic acid product. [D,L]HMG-CoA substrate is used as 100 μM, twice the concentration needed to saturate the enzyme under the conditions described. NADPH is used in excess at a level 2.7 times the concentration required to achieve maximum enzyme velocity. Standardized assays for the evaluation of inhibitors are conducted according to the following procedure. Microsomal enzyme is incubated in the presence of NADPH at 37 °C for 15 min. DMSO vehicle with or without test compound is added, and the mixture further incubated for 15 min at 37 °C. The enzyme assay is initiated by adding ^{14}C-HMG-CoA substrate. After 20 min of incubation at 37 °C, the reaction is stopped by the addition of 25 μL of 33% KOH. [^3H]Mevalonic acid (0.05 μCi) is added, and the reaction mixture allowed to stand at room temperature for 30 min. Fifty microliters of 5 N HCl is added to lactonize the mevalonic acid. Bromophenol blue is added as a pH indicator to monitor an adequate drop in pH. Lactonization is allowed to proceed for 30 minutes at room temperature. Reaction mixtures are layered onto 2 g of AG 1-X8 anion exchange resin (Biorad, formate form), poured in 0.7 cm (i.d.) glass columns, and eluted with 2.5 mL of H_2O. The first 0.5 mL is discarded, and the next 2.0 mL is collected and counted for both tritium and carbon-14 in 10.0 mL of Opti-fluor (Packard) scintillation fluid. Results are calculated as nanomoles mevalonic acid produced per 20 min and are corrected to 100% recovery of tritium. Drug effects are expressed as I_{50} values (concentration of drug producing 50% inhibition of enzyme activity) derived from composite dose response data from 2–5 experiments.

Inhibition of Cholesterol Synthesis in Freshly Isolated Rat Hepatocytes. Inhibitors of HMG-CoA reductase are evaluated for their ability to inhibit [^{14}C]acetate incorporation into cholesterol in freshly isolated rat hepatocyte suspensions using a modification of the methods originally described by Capuzzi.[22] Sprague–Dawley rats (180–220 g) are anesthetized with Nembutal (50 mg/kg). The abdomen is opened, and the first branch of the portal vein is tied closed. Two closing sutures are placed on the distal section of the portal vein, and the portal vein is canulated between the sutures and the first branching vein. The liver is perfused at a rate of 20 mL/min with prewarmed (37 °C) oxygenated buffer A ((HBSS, Hanks' Balanced Salt Solution) without calcium or magnesium containing 0.05% EDTA) after severing the vena cava to allow drainage of the effluent. The liver is additionally perfused with 200 mL of prewarmed oxygenated buffer B (HBSS containing 0.05% bacterial collagenase). Following perfusion with buffer B, the liver is excised and decapsulated in 50 mL of Waymouth's medium, allowing free cells to

(21) Edwards, P. A.; Lemongello, D.; Fogelmann, A. M. Improved Methods for the Solubilization and Assay of Hepatic 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase. *J. Lipid Res.* 1979, *20*, 40–46.

(22) Capuzzi, D. M.; Margolis, S. Metabolic Studies in Isolated Rat Liver Cells: 1. Lipid Synthesis. *Lipids* 1971, *6*, 601–607.

disperse into the medium. Hepatocytes are isolated either by low-speed centrifugation for 3 min at 50g at room temperature or by unit gravity sedimentation at 4 °C for 30–45 min. Pelleted hepatocytes are washed once in Waymouth's medium, counted, and assayed for viability by trypan blue exclusion. These hepatocyte enriched cell suspensions routinely show 70–90% viability. Hepatocytes are resuspended at 5×10^6 cells per 2.0 mL in incubation medium (IM) [0.02 M Tris-HCl (pH 7.4), 0.1 M KCl, 0.33 mM $MgCl_2$, 0.01 mM $MnCl_2$, 0.001 mM sodium succinate, 0.003 mM Coenzyme A, 0.33 mM sodium citrate, 0.67 mM nicotinamide, 0.23 mM NADP, 1.7 mM glucose-6-phosphate]. Test compounds are routinely dissolved in H_2O, DMSO, or DMSO–H_2O (1:3) and added to the IM. Final DMSO concentration in the IM is ≤1.0% and has no significant effect on cholesterol synthesis. Incubation is initiated by adding [^{14}C]acetate (58 mCi/mmol, 2 μCi/mL) and placing the cell suspensions (2.0 mL) in 35-mm tissue culture dishes at 37 °C for 2.0 h. Following incubation, cell suspensions are transferred to glass centrifuge tubes and spun at 50g for 3 min at room temperature. Cell pellets are resuspended and lysed in 1.0 mL of H_2O. Lipids are extracted essentially as described by Bligh and Dyer.[23] Following extraction, the lower organic phase is removed and dried under a stream of nitrogen and the residue resuspended in 100 μL $CHCl_3$–MeOH (2:1). The total sample is spotted on silica gel (LK6D) thin-layer plates and developed in CH_2Cl_2–acetone (60:1). Plates are scanned and counted using a BioScan automated scanning system. Radiolabel in the cholesterol peak (R_f 0.28) is determined and expressed as total counts per peak and as a percent of the label in the total lipid extract. Cholesterol peaks in control cultures routinely contain 5000–20000 dpm, and are approximately 30% of the label present in the total lipid extract. Drug effects (percent inhibition of cholesterol synthesis) are determined by comparing the percent of label in the cholesterol peak for control and drug treated cultures. Dose response curves are constructed from composite data from two or more studies and results are expressed as I_{50} values (concentration of drug which inhibits cholesterol synthesis 50%).

Inhibition of Cholesterol Synthesis in Human Skin Fibroblasts. Human skin fibroblasts (passage 7-27) are grown in minimal essential medium (MEM, Gibco) containing 10% fetal calf serum. For each experiment, stock cultures are trypsinized to disperse the cell monolayer, counted, and plated in 35-mm tissue culture wells (5×10^5 cells/2.0 mL). Cultures are incubated for 18 h at 37 °C in 5% CO_2/95% humidified room air. Cholesterol biosynthetic enzymes are induced by removing the serum containing medium, washing the cell monolayers with MEM, adding 1.0 mL of MEM containing 1.0% fatty acid free bovine serum albumin, and incubating the cultures an additional 24 h. Test compounds are dissolved in H_2O, DMSO, or DMSO–EM (1:3) (final DMSO concentration in cell cultures ≤1.0%) and added to the cultures, and the cultures are preincubated for 30 min at 37 °C in 5% CO_2/95% humidified room air. Following preincubation with drugs, sodium [1-^{14}C]acetate (2.0 μCi/mL, 58 mCi/mmol) is added, and the cultures are reincubated for 4 h. After incubation, the culture medium is removed and the cell monolayer is scraped into 1.0 mL of H_2O. Lipids in the lysed cell suspension are extracted as described for hepatocyte suspensions. The organic phase is dried under nitrogen, and the residue is resuspended and analyzed as described for hepatocytes. Cholesterol peaks in control cultures routinely contain 8000–12000 dpm and are approximately 15% of the label present in the total lipid extract.

Inhibition of cholesterol synthesis is determined as described for hepatocytes. Results are expressed as I_{50} values and are derived from composite dose response curves from two or more experiments.

In Vivo Cholesterol Biosynthesis Inhibition in Rats. The methods used for intravenous (iv) and oral (po) drug testing were adapted from a procedure originally described by Sandoz.[24] Male Sprague–Dawley rats (200–300 g) were adapted to a reverse light cycle for 7–10 days and fed Purina rat chow (no. 5001) ad libitum. In order to measure cholesterol synthesis, sodium [1-^{14}C]acetate (1–3 mCi/mmol) (25 μCi/100 g of body weight) was injected intraperitoneally (ip) 2 h before the mid-dark point in the diurnal cycle. Two hours after the mid-dark point animals were anesthetized ip with ketamine/xylazine and bled into EDTA-treated centrifuge tubes from the abdominal aorta. Plasma was obtained by centrifugation at 1100g for 10 min. One-milliliter plasma samples were aliquoted and either processed directly or frozen at −20 °C. For iv testing, the salt forms of test compounds were routinely dissolved in saline and injected iv into the tail vein 5 min before [^{14}C]acetate injection. For po testing, drugs were dissolved in saline and given by gavage 30 min before [^{14}C]acetate injection. Cholesterol synthesis was measured by determining the level of ^{14}C-labeled nonsaponifiable lipid present in 1 mL of plasma; the method used is a modification of the method described by Dugan.[25] One milliliter physiological saline was added to 1 mL of plasma, followed by the addition of 5.0 mL of 10% KOH in absolute ethanol. Samples were mixed and saponified at 75 °C for 1 h. After cooling, approximately 0.02 μCi (44,000 dpm) [1,2-^{3}H]cholesterol (40–60 Ci/mmol) was added to each sample. Samples were extracted once with 5 mL of petroleum ether, and the organic phase was backwashed with 5 mL of saline. This extraction procedure resulted in 50–90% recovery of the added [^{3}H]cholesterol internal standard. The extracts were dried in glass vials, and the residue resuspended in 0.5 mL of $CHCl_3$–MeOH (2:1). Samples were counted for both ^{3}H and ^{14}C in 10 mL of Optifluor scintillation fluid. The [^{3}H]cholesterol internal standard recovery value from each sample was used to correct each sample to 100% recovery of [^{14}C]cholesterol. In early experiments, sample extract residues were redissolved in 100 mL of $CHCl_3$–MeOH (2:1) and chromatographed on silica gel (Whatman LK6D) thin-layer plates using either hexanes–Et_2O–HOAc (75:25:1) or CH_2Cl_2–acetone (60:1). Using either chromatographic system, greater than 90% of the ^{14}C-label cochromatographed with authentic cholesterol. Thus, to simplify the method, the TLC step was omitted in subsequent experiments and results were calculated as ^{14}C-labeled nonsaponifiable plasma lipid values, of which, greater than 90% of the ^{14}C-label is authentic cholesterol. The percent inhibition of cholesterol synthesis was derived by comparing ^{14}C-labeled nonsaponifiable plasma lipid values per milliliter of plasma from control and drug-treated animal groups (4–5 rats/group). Percent inhibition is plotted relative to the log drug dose and a linear best fit regression line is determined for each experiment. Mean ED_{50} values (level of drug required to suppress cholesterol synthesis in vivo by 50%) were calculated from two or more experiments.

(23) Bligh, E. G.; Dyer, W. J. A Rapid Method of Total Lipid Extraction and Purification. *Can. J. Biochem. Physiol.* 1959, *37*, 911–917.

(24) Wareing, J., U.S. Patent 4,613,610 and PCT Int. Appl., WO 86/00367.

(25) Dugan, R. E.; Slakey, L. L.; Briedis, A. V.; Porter, J. W. Factors affecting the Diurnal Variation in the Level of β-Hydroxy-β-methylglutaryl Coenzyme A Reductase and Cholesterol Synthesizing Activity in Rat Liver. *Arch. Biochem. Biophys.* 1972, *152*, 21–27.

HMG-CoA Reductase Inhibitors: An Exciting Development in the Treatment of Hyperlipoproteinemia

F. G. Kathawala

Preclinical Research Department, Sandoz Research Institute, Route 10, East Hanover, New Jersey 07936

I. INTRODUCTION

Coronary heart disease (CHD) continues to be one of the major health problems in all the developed countries of the world. A considerable body of clinical and epidemiological data has emerged over the years linking elevated blood levels of total cholesterol, Low Density Lipoprotein Cholesterol (LDL-C), and Very Low Density Lipoprotein Cholesterol (VLDL-C) as important risk factors for the development of coronary heart disease.[1]

For the treatment of elevated LDL-C and VLDL-C, a judicious diet, low in cholesterol and fat with saturated fatty acids replaced by polyunsaturated fatty acids, is the recommended choice. However, for patients nonresponsive

Medicinal Research Reviews, Vol. 11, No. 2, 121–146 (1991)

CCC 0198-6325/91/020121-26$04.00

to dietary intervention, the development of effective and safe therapeutic agents for the treatment of hyperlipoproteinemia remains an important need. This need has gained considerable support as a result of two important events: (1) the results of the Lipid Research Clinic's Coronary Primary Prevention Trial (LRC-CPPT), a multicenter, randomized, double-blind study involving 3806 asymptomatic middle-aged men in the United States with type II hyperlipoproteinemia, that demonstrated that a statistically significant reduction of 19% in the rate of fatal plus nonfatal coronary heart disease was associated with a 9% decrease in blood cholesterol levels,[2] and (2) the recommendation to treat individuals with blood cholesterol above the 75th percentile, which emerged from the consensus panel of the December, 1984 NIH Consensus Development Conference on the lowering of blood cholesterol to prevent coronary heart disease.[3]

In recent years, to achieve this goal of finding effective and safe therapeutic agents to lower LDL-cholesterol, great interest has focused on potent inhibitors of the enzyme β-Hydroxy-β-Methyl-Glutaryl-CoA reductase (HMG-CoA reductase, EC 1.1.1.34), which controls a key step in the endogenous synthesis of cholesterol. Several studies, both in animals and humans, have been reported with HMG-CoA reductase inhibitors: compactin (Mevastatin), CS-514 (Pravastatin, Mevalotin®, Pravachol®), mevinolin (Lovastatin, Mevacor®) and Synvinolin (Simvastatin, Zocor®),[4] which are structurally very closely related to one another. In order to assess fully the potential of HMG-CoA reductase inhibitors as an effective therapeutic intervention for the treatment of hyperlipoproteinemia, it is thus desirable to study in humans a variety of these inhibitors derived from different structural prototypes which can be distinguished in their overall biological profile from one another. This conceptual framework formed the basis for initiating efforts at the Sandoz Research Institute to develop and study a variety of HMG-CoA reductase inhibitors with chemical structures different in several respects from compactin, pravastatin (a hydroxy analog of compactin), lovastatin (a methyl analog of compactin), and simvastatin (a dimethyl analog of compactin), and has led to fluvastatin (XU 62-320), the first totally synthetic HMG-CoA reductase inhibitor currently in Phase III human clinical trials (Fig. 1).

II. DESIGN ASPECT FOR HMG-CoA REDUCTASE INHIBITORS AT SANDOZ RESEARCH INSTITUTE LEADING TO FLUVASTATIN (XU 62-320)

Investigations by Akira Endo with compactin[4] have to be largely credited for the resurgence of the research on cholesterol biosynthesis and the renewed interest in HMG-CoA reductase inhibitors, a field now almost three decades

---

F. G. Kathawala *obtained his M.Sc. from the University of Bombay, India, and his Ph.D. in 1961 from Technische Hochschule Braunschweig, West Germany (Prof. H. H. Inhoffen), in Synthetic Organic Chemistry. After a few years of postdoctoral work at Harvard (Prof. R. B. Woodward), Wisconsin (Prof. H. Muxfeldt), and Göttingen (Prof. F. Cramer), he joined Sandoz in East Hanover, New Jersey, as a Senior Scientist, in 1969. Currently, he is the Director of Medicinal Chemistry in the area of Lipoprotein Metabolism/Atherosclerosis. His research interests in Medicinal Chemistry are focused towards the discovery of agents affecting lipoprotein metabolism/atherosclerosis.*

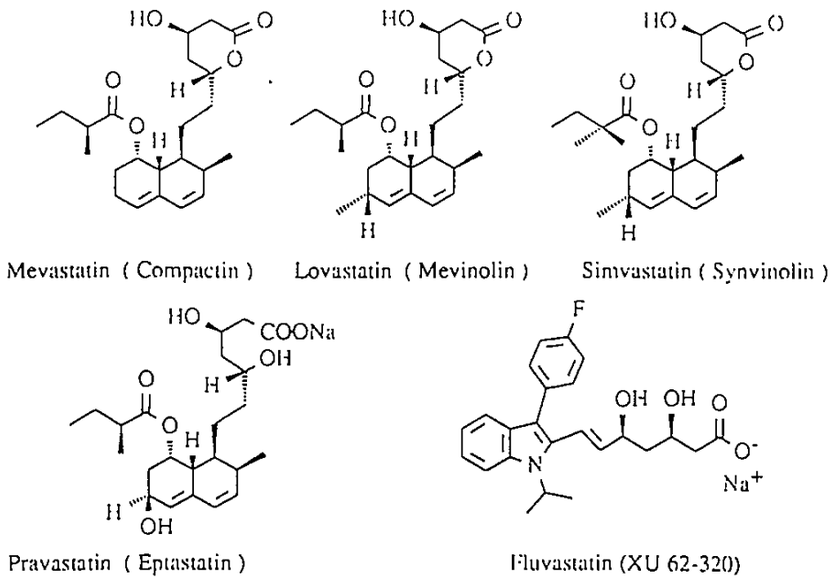


Figure 1

old. While all intensive studies hitherto conducted have been with closely related metabolites, such as compactin, mevinolin, and CS-514 (pravastatin), derived from fungal broths, efforts at the Sandoz Research Institute towards the development of new HMG-CoA reductase inhibitors have been based on synthesis, guided by the following assumptions:

(a) There are two regions at the active site of the enzyme: one with high specific recognition of a 5-carbon unit (C-1 to C-5 as shown below) of the β-OH-β-Methyl-Glutaryl portion, and the other of CoA moiety present in HMG-CoA (Fig. 2).

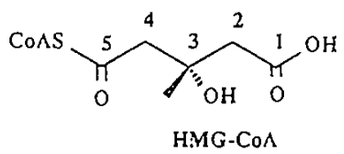


Figure 2

(b) Compactin (R = H, Fig. 3), a known inhibitor of the enzyme, may be regarded as a transition state analog, when in the open dihydroxy acid form.

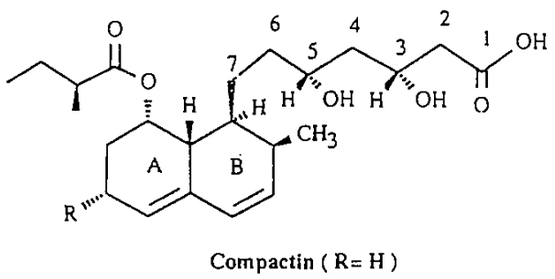


Figure 3

The 5-carbon unit of the side chain present in compactin (Fig. 3) probably occupies the same region as the 5-carbon unit in HMG-CoA (Fig. 2); the bicyclic A-B-ring system, with its substituents in compactin (Fig. 3), possibly sits in the same region or very close to the same region the CoA portion of the substrate HMG-CoA occupies at the active site of the enzyme. However, it is difficult to see any similarity in structure between the bicyclic-ring system of compactin and CoA, when one examines the structure of CoA shown in Fig. 4.

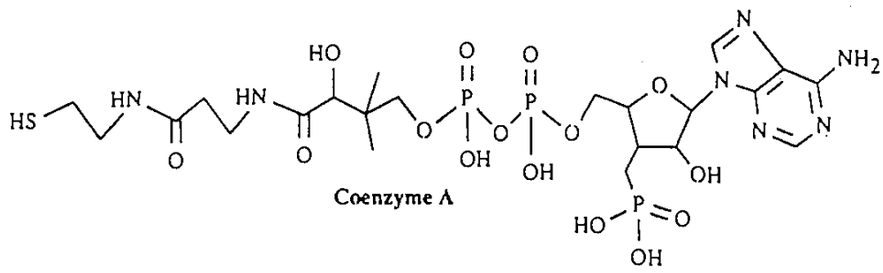


Figure 4

In light of (a) and (b) above, one hoped that it might possible to prepare interesting synthetic inhibitors of HMG-CoA reductase with a very general structure as shown in Fig. 5, with the 5-carbon unit (C-1 to C-5) preferably possessing the absolute configurations of C-3-OH and C-5-OH as present in compactin.

Choice of R and R_1 in Fig. 5 has depended on:

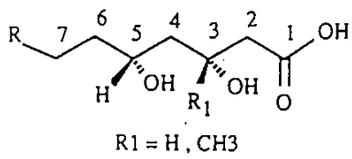


Figure 5

(a) Consideration of the elements of structure of CoA.

(b) Considerations of the overall shape and assumptions of the importance of substituents on Ring A-B of compactin (Fig. 3), first with molecular models and later with computer modelling.

(c) Exploiting the knowledge gained in structure activity relationships with our own Sandoz Research Institute compounds or being reported in literature by outside investigators.

Efforts with the above considerations in mind have led to the development of a variety of novel HMG-CoA reductase inhibitors. Synthesis and Structure Activity Relationships (SAR) of some of these novel inhibitors are discussed below with emphasis on the Phase III candidate, fluvastatin (XU 62-320): [R*,S*-(E)]-(±)-Sodium-3,5-dihydroxy-7-[3-(4-fluorophenyl)-1-(1-methyl-ethyl-1H-indol-2-yl]-hept-6-enoate (Fig. 1), a mevalonic acid analog more potent than compactin and lovastatin.

III. GENERAL CHEMISTRY APPROACH

Guided by the conviction that the C-3, C-5 dihydroxy acid fragment was the key pharmacophore necessary for the inhibition of HMG-CoA reductase,

our synthetic approach towards the synthesis of compounds of generic structure (Fig. 5) involved:

(a) A convergent synthesis coupling chiral Synthon 1 or racemic or chiral (3R, 5S) C-3, C-5-dihydroxy ester Synthon 2 with a variety of aryl or alkyl fragments 3 (Fig. 6), or

(b) A linear synthesis of the C-3, C-5 dihydroxy acid derivatives wherein the aldehyde 4 is reacted with acetoacetate 5 (Fig. 7) to provide a hydroxyketo ester intermediate, which, with subsequent steps, gives the desired final products of Fig. 5.

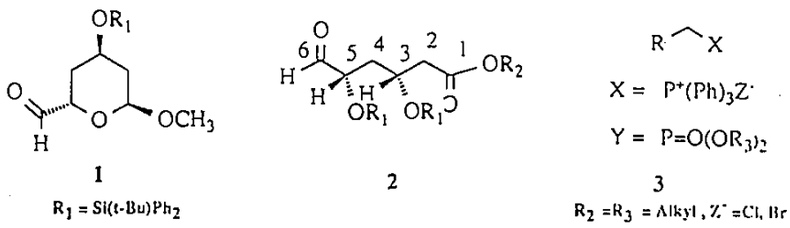


Figure 6

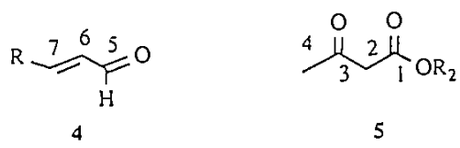
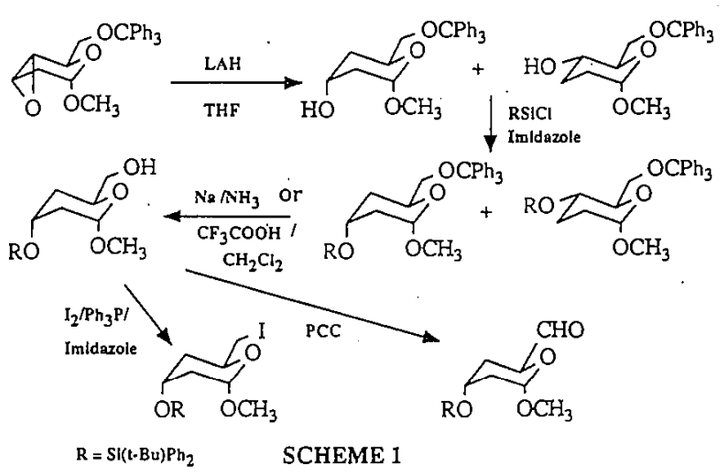


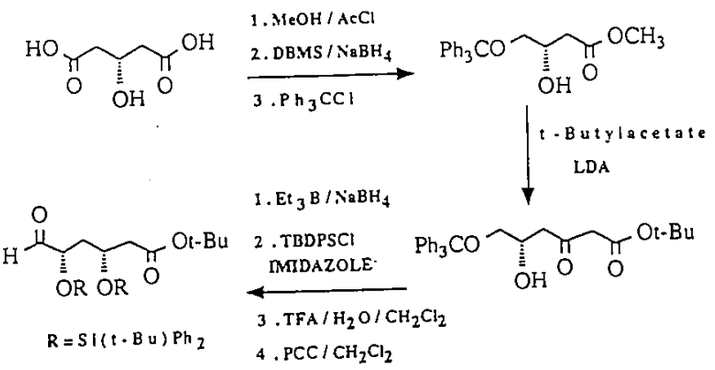
Figure 7

A. Synthesis of Synthon 1 and 2, Fig. 6 (Scheme 1 and Scheme 2)

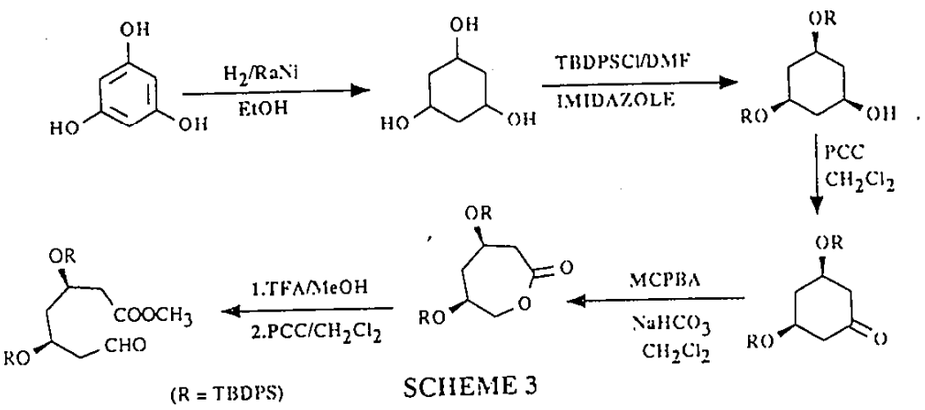
Synthon 1 has been synthesized starting from D-glucose via the key lithium aluminum hydride reductive opening of the epoxide as depicted in Scheme 1.[5] The desired axial alcohol could be separated from the equatorial isomer by preparation of the silyl derivatives. The protected axial alcohol on PCC oxidation gave the desired lactol aldehyde.



SCHEME 1



SCHEME 2



SCHEME 3

Synthesis of chiral Synthon 2 has been accomplished starting from S-malic acid in excellent yields via an eight-step reaction as illustrated in Scheme 2.[6]

On the other hand, an efficient route was developed for the preparation of racemic Synthon 2 starting from 1,3,5-trihydydroxy benzene through a five-step reaction sequence shown in Scheme 3.[7]

B. Choice of R and Synthesis of Intermediates 3, Fig. 6, and 4, Fig. 7

Our initial efforts at the synthesis, and the biological results of C-3, C-5-dihydroxy acid derivatives (Fig. 5) wherein choice of R was based on elements of substructures of coenzyme A (Fig. 4) or the decalin ring structure of compactin (Fig. 3) were not promising.[8] This led us to question the importance and the necessity of the complex stereochemistry and the substituents present in the decalin ring of compactin and turn our attention towards the preparation of C-3, C-5-dihydroxy acid derivatives (Fig. 5) wherein R was a naphthalene ring. During these ongoing efforts, we were being encouraged and helped by two important publications[9] describing mevalonolactone derivatives of the general structure 6 and 7 as inhibitors of HMG-CoA reductase (Fig. 8).

Further exploration of R in Fig. 5 led to the first interesting indolyl derivative (Fig. 9) comparable to compactin in its inhibitory activity against HMG-CoA reductase.[10(a)]

An extensive and rapid analog program allowed the choice of XU 62-320

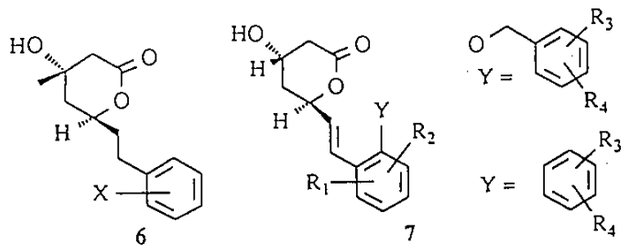


Figure 8

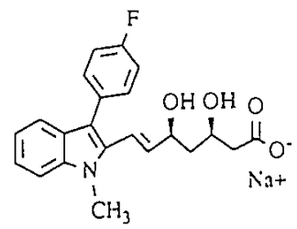


Figure 9

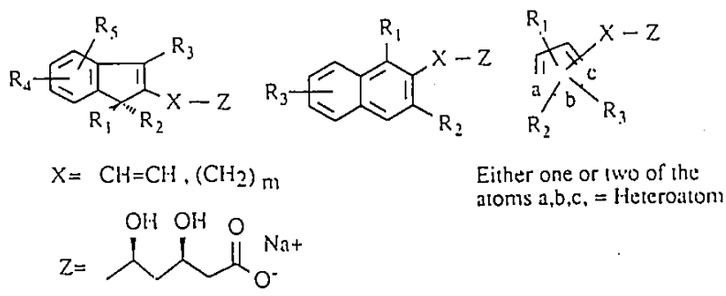
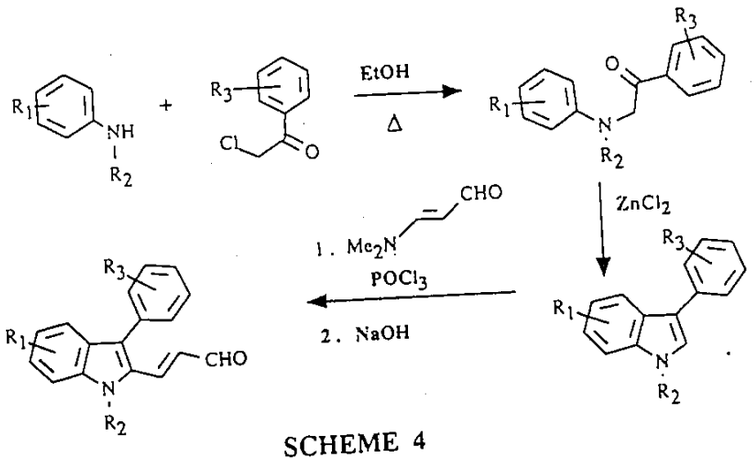


Figure 10

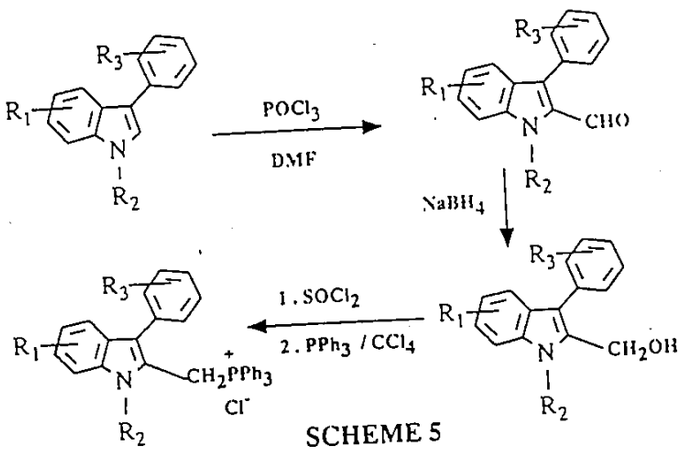
(Fig. 1) as a candidate for extensive biological testing. Currently, fluvastatin (XU 62-320) is in clinical Phase III trials.

With the discovery of XU 62-320, the stage was set for a large number of variations of R in Fig. 5. Extensive work at the Sandoz Research Institute has led to many novel HMG-CoA reductase inhibitors, some of which are discussed in this paper as shown in Fig. 10,[10] and Figs. 12–14.[21–23]

Synthesis of the many interesting fragments 3 (Fig. 6) and 4 (Fig. 7) needed for synthesis of final HMG-CoA-R inhibitors are described in Schemes 4–12 below.[10] Since the appearance of Merck & Co., Inc. and Sandoz patents and publications,[5,9,10(a)] extensive efforts have followed in many laboratories worldwide with semi-synthetic and totally synthetic HMG-CoA reductase inhibitors. A brief overview of these reported activities is presented in Section VIII. It is no wonder that in such a feverish pursuit of finding patentable HMG-CoA reductase inhibitors, review of patent and published literature presents overlapping activities in the laboratories of competing pharmaceutical research companies.



SCHEME 4



SCHEME 5

C. Synthesis of Indole Intermediates

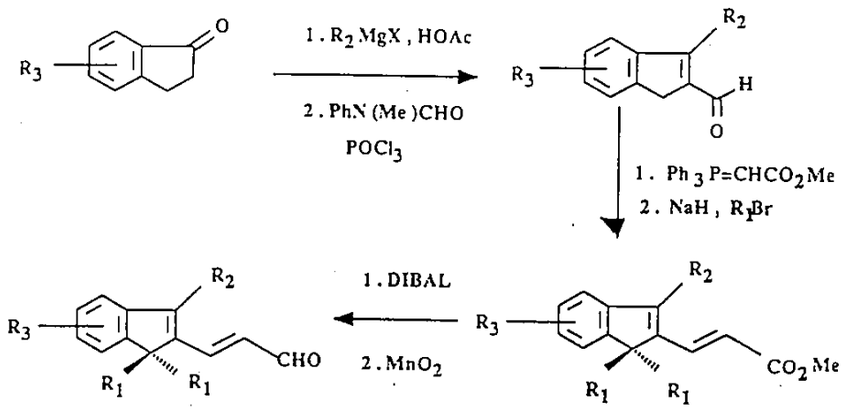
Scheme 4 describes the preparation of α,β-unsaturated aldehydes readily obtained from a variety of 3-phenyl substituted indoles using dimethylamino-acrolein and phosphorous oxychloride, while the triphenyl phosphonium salts of indolyl derivatives are prepared via the 2-formyl and 2-hydroxymethyl indoles using standard procedures (Scheme 5).[10(a)]

D. Synthesis of Indene Intermediates

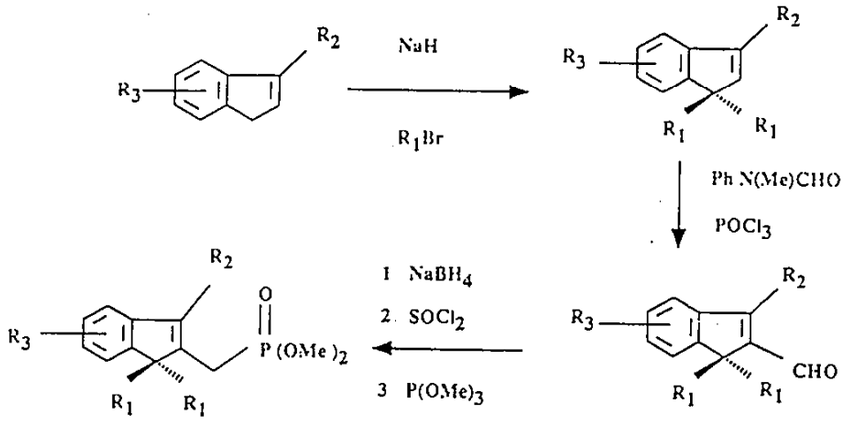
A variety of indenyl-α, β-unsaturated aldehydes and phosphonates have been synthesized via a six-step reaction sequence as depicted in Schemes 6 and 7. The synthesis of these derivatives involves the preparation of the desired indenes from the respective indanones followed by either formylation at C-2 and subsequent alkylations at C-1 or vice versa, and then processing the formyl group through standard reaction sequences to the desired intermediates.[10(b)]

E. Synthesis of Naphthalene Intermediates

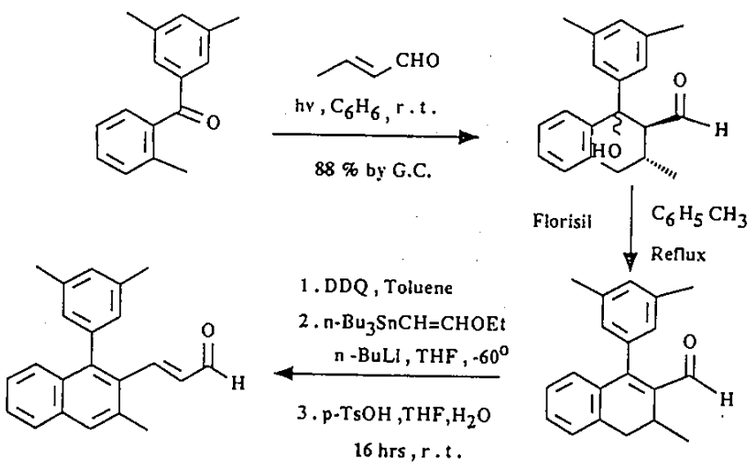
For the preparation of naphthalene derivatives, a novel photochemical route[11] was exploited to give the key hydroxy aldehyde, which on dehydration provides the ene aldehyde. Dehydrogenation of the ene aldehyde and chain



SCHEME 6



SCHEME 7

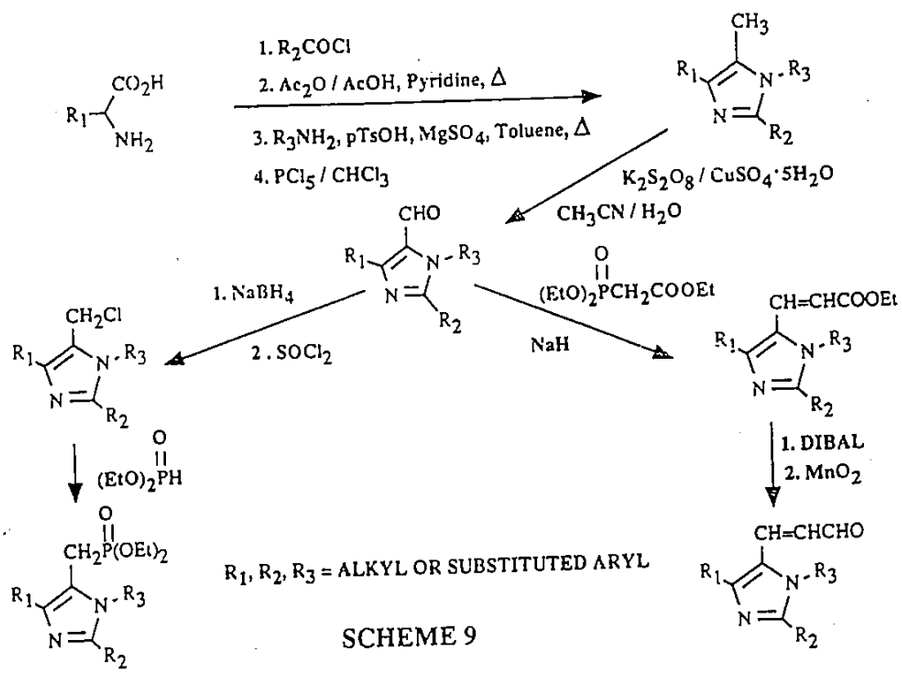


SCHEME 8

extension of the formyl group then leads to the desired α,β-unsaturated aldehydes[10(c)] (Scheme 8).

F. Synthesis of Imidazole Intermediates

Highly substituted imidazole derivatives with the desired functional group at the desired C- or hetero- atom are not well described in the literature. Synthesis of the required imidazole intermediates was best accomplished starting from the respective glycine derivatives as shown in Scheme 9. The key step in the synthetic pathway involves oxidation of the methyl group with potassium persulfate to give the 5-formyl imidazole derivatives, which through standard reaction sequences give the needed α,β-unsaturated aldehydes or the phosphonates.[10(d)]



SCHEME 9

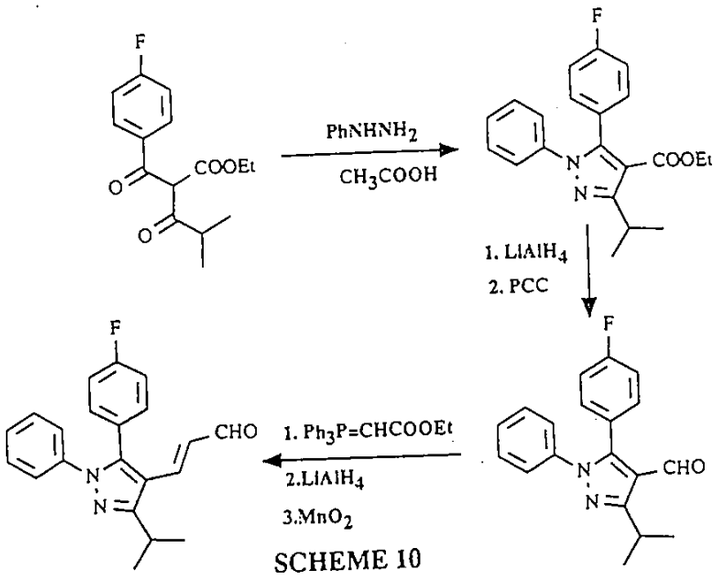
G. Synthesis of Pyrazole Derivatives

A number of pyrazole intermediates have been prepared via procedures dependent on whether one needs the 1,5 (Scheme 10), the 1,3 (Scheme 11), or the 3,4 (Scheme 12) disubstituted pyrazole intermediates. 2,3-disubstituted pyrazole derivatives are obtained through the reaction of the appropriate diketoesters with aryl-hydrazines, requiring separation from the concomitant formation of the corresponding 1,3 isomer (Scheme 10).[10(e)]

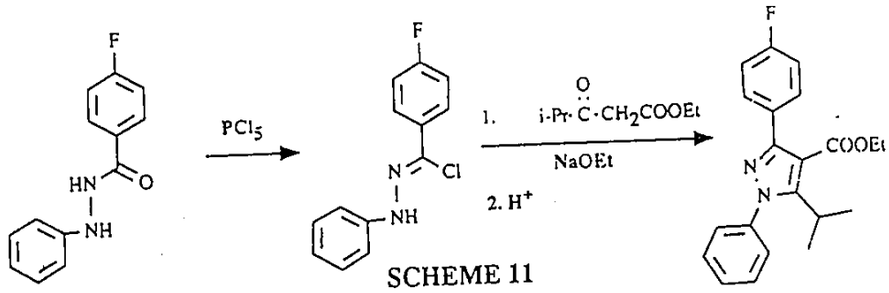
1,3-disubstituted pyrazoles can be best synthesized from the imide chloride on reaction with the acetoacetate derivatives (Scheme 11), while the ring closure of arylhydrazones give the desired 3,4 diaryl pyrazole intermediates (Scheme 12).

H. Synthesis of HMG-CoA-R Inhibitors

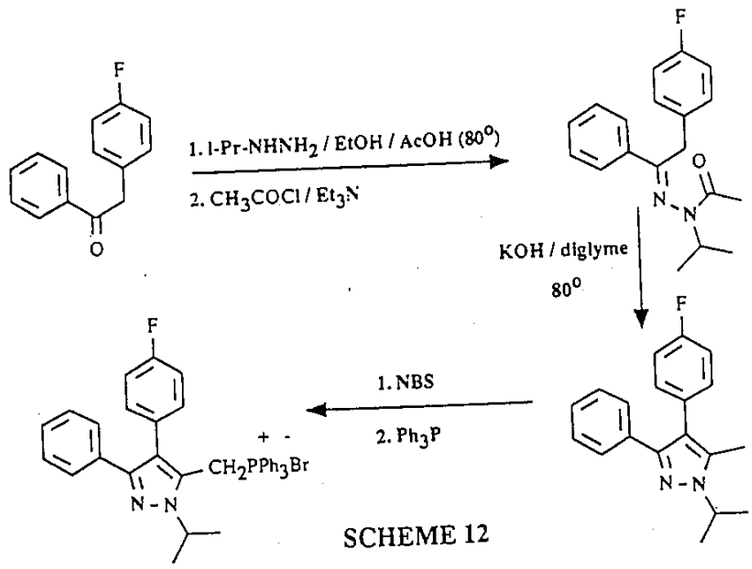
All of the intermediates of the many different prototypes described above in Schemes 4–12 could be converted to the final HMG-CoA reductase inhib-



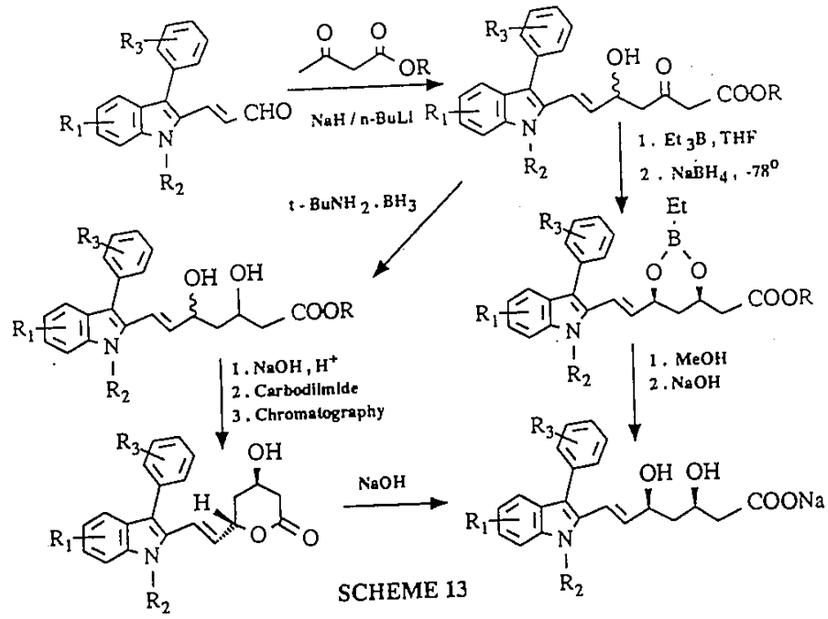
SCHEME 10



SCHEME 11



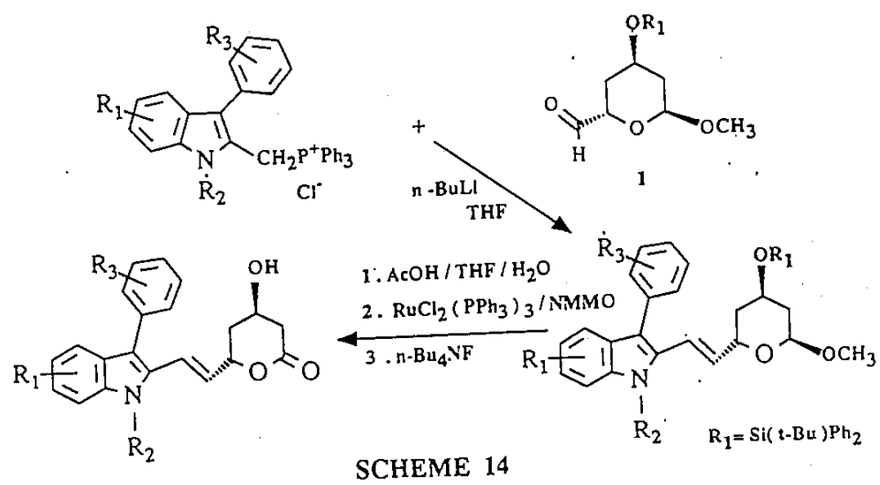
SCHEME 12



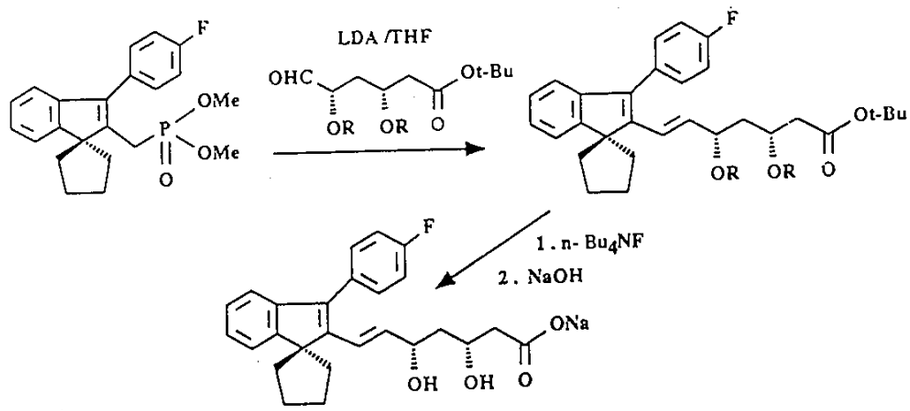
SCHEME 13

itors either using the linear route involving the "dianion chemistry," or the coupling of the respective phosphonates or phosphonium salts with the chiral Synthons 1 and 2 (Fig. 6) or with the racemic Synthon 3 (Fig. 6).

1. *Linear Route.* Synthesis using the linear route is illustrated in Scheme 13 for the preparation of the indolyl HMG-CoA reductase inhibitors. The key step involves the reduction of the hydroxyketoester using trialkylborane/THF/MeOH with sodium borohydride at −78° (Ref. 12) to give the mixture of desired erythro and threo isomers in the ratio of 95–98:5–2%, respectively. In some cases, the boronic esters can be crystallized, which on methanolysis and subsequent hydrolysis with sodium hydroxide provide the desired sodium salts. Nonstereoselective reduction of hydroxyketoester with borane t-butylamine complex has been used to prepare a mixture of *cis* and *trans* lactones separable on flash chromatography.[10(a)]



SCHEME 14



R = Si (t-Bu) Ph_2 SCHEME 15

2. *Convergent Route.* For illustrative purposes, a convergent route for the preparation of chiral indolyl HMG-CoA reductase inhibitors using the silyl protected Synthon 1 is depicted in Scheme 14. The crucial step in this reaction pathway is the oxidation of lactol with $RuCl_2(PPh_3)_3$/NMMO.[10(f)]

Scheme 15 shows the use of silyl-protected aldehyde Synthon 2 (derived from malic acid) for the synthesis of indenyl HMG-CoA reductase inhibitors.[13]

IV. BIOLOGICAL RESULTS AND DISCUSSION

A. Results in *in vitro* HMG-CoA Reductase Microsomal Assay and in *in vivo* Cholesterol Biosynthesis Assay

All initial studies to assess the inhibitory potency of various compounds against HMG-CoA reductase were conducted with rat liver microsomal suspensions, freshly prepared from male Sprague-Dawley rats, using an assay for HMG-CoA reductase activity as described in Ref. 14. The potency of each compound is expressed as IC_{50} (in μmoles, the concentration which inhibits to the extent of 50% conversion of the substrate HMG-CoA to mevalonate) and for structure activity relationship compared either to compactin = 1 or to XU 62-320 = 1. Tables I–XII summarize the most salient features of structure activity relationships for a few of the varied structural prototypes as HMG-CoA reductase inhibitors being currently studied at the Sandoz Research Institute. In Tables X–XIII, the Relative Potency column is derived from the IC_{50} values of each compound vs. compactin in the *in vitro* rat microsomal HMG-CoA reductase assay.

B. SAR of Fluvastatin (XU 62-320) Analogs

Table I compares the *in vitro* inhibitory activity against HMG-CoA reductase of XU 62-320 with compactin and lovastatin and as their corresponding sodium salts. XU 62-320 is 146- and 52-fold more active than compactin and Lovastatin, respectively. As compared to the respective sodium salts of compactin and Lovastatin, XU 62-320 is 22- and 10-fold more potent in inhibiting HMG-CoA reductase. It is important to note that current clinical studies are being conducted with XU 62-320, which is a dihydroxy acid sodium salt. In contrast,

Table I
Comparison of Microsomal HMG-CoA Reductase Inhibitory Activity

Compound	IC_{50} (μM)	Relative Potency*
XU 62-320	0.0069	146.1
Compactin	1.011	1.0
Lovastatin	0.352	2.8
Na Salt Compactin	0.154	6.5
Na Salt Lovastatin	0.068	14.8

*As compared to Compactin = 1

compactin used in clinical studies and Lovastatin (Mevacor®), now marketed, both exist as the lactone forms (Fig. 1).

Features of the side chain are very important for maximal inhibitory activity as shown in Table II. Erythro configuration, as well as the double-bond configuration, are very important [anti-isomer 17-fold less active and dramatic loss of activity for one (Z) diene isomer]. The dihydro derivative, as well as the ester and the lactone forms, are considerably less active. Maximal inhibitory activity resides in the 3R, 5S antipode.

The importance of the features of the side chain described in Table II for the indole series holds true as well for all the prototypes to be described later and hence, during the discussion of SAR of these prototypes, these aspects will not be reemphasized. HMG-CoA, the substrate for the HMG-CoA reductase, has at C-3 a methyl group. It was important to determine if an analog of XU 62-320 carrying a methyl group at C-3 would be more potent. Surprisingly, introduction of methyl group at C-3 in either of syn- or anti-configuration was considerably less active (Table III).

Studies of the effects of the substituents in the 3-phenyl ring of the indole moiety are given in Table IV. Either electron-withdrawing or electron-donating substituents in the 3-phenyl ring tend to decrease the potency, which is unaffected by the presence of alkyl groups.

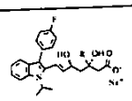
Electron-donating or electron-withdrawing substituents (not shown in Table IV) or bulky alkyl groups at C-5 of the indole moiety led to decrease of potency. However, alkyl or alkoxy groups at C-4 and C-6 tend to maintain or enhance the potency slightly (Table V).

Table II
SAR of Variations in the Side Chain

Compound	IC_{50} (μM)	Relative Potency*
XU 62-320	0.0069	1.0
3R, 5S	0.0024	2.8
3S, 5R	0.08	0.086
Na Salt, ANTI	0.12	0.057
Methyl Ester, SYN	0.052	0.13
Trans Lactone	0.029	0.23
CIS(Z) Double Bond	0.62	0.011
Dihydro (Reduced Double Bond)	0.114	0.06

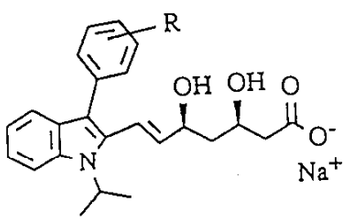
*As compared to XU 62-320 = 1.

Table III
Comparative Activity of XU with the 3-Methyl Analogs

Compound	IC_{50} (μM)	Relative Potency*
		
XU 62-320	0.0069	1.0
R = CH_3, SYN	0.14	0.049
R = CH_3, ANTI	0.51	0.013

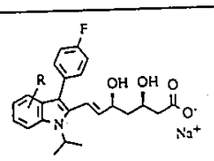
*As compared to XU 62-320 = 1

Table IV
SAR for the Substituents of the 3-Phenyl Ring

R	IC_{50} (μM)	Relative Potency*
		
4-F	0.0069	1
2-Me	0.14	0.049
2-Me, 4-F	0.004	1.7
3-Me, 4-F	0.009	0.76
3,5-diMe, 4-F	0.02	0.345
3,5-diMe	0.005	1.38
H	0.017	0.40
4-CF_3	0.09	0.076
4-SCH_3	1.152	0.006
4-COONa	>10.0	

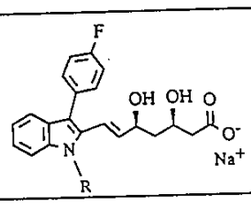
*As compared to XU 62-320 = 1

Table V
SAR for the Substituents of the Benzenoid Indole Ring

R	IC_{50} (μM)	Relative Potency*
		
H (62-320)	0.0069	1.0
4,6-diMe	0.011	0.62
4,6-dii-Pr	0.005	1.38
5-C_6H_{11}	24.0	0.0022
6-OCH_2Ph	0.0026	2.65

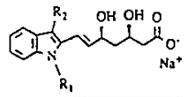
*As compared to XU 62-320 = 1

Table VI
SAR for the Substituents of Indolyl-Nitrogen

R	IC_{50} (μM)	Relative Potency*
		
i-Pr (62-320)	0.0069	1.0
CH_3	0.62	0.011
C_2H_5	0.096	0.071
C_6H_{11}	50	0.0001
CH_2CH_2Ph	49.4	0.0001
$CH_2CH(CH_3)_2$	0.245	0.028

*As compared to XU 62-320 = 1

Table VII
SAR for Reversing Substituents at 1 and 3 Positions

	R_1	R_2	IC_{50} (μM)	Relative Potency*
	i-Pr (62-320)	4-FC_6H_4, syn	0.0069	1.0
	4-FC_6H_4	i-Pr, syn	0.0016	4.3
	i-Pr	4-FC_6H_4, anti	0.12	0.057

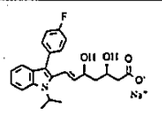
*As compared to XU 62-320 = 1

Most sensitive to the activity is the substituent on the nitrogen of the indole moiety (Table VI). Optimal activity is provided by the isopropyl group, while marked loss in potency results with either bulky alkyl or phenethyl groups.

Reversing the substituents on N-1 and C-3 of the indole moiety to give (Table VII) 3-isopropyl-N-p-fluorophenyl analog of XU 62-320 gives a 4-fold increase in potency.

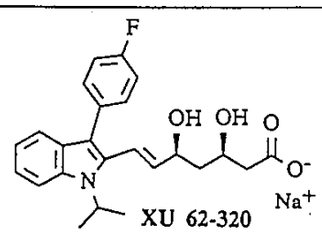
Most of the substances with a reasonable level of activity against HMG-CoA reductase in *in vitro* microsomal assay were studied *in vivo* for their effects on inhibition of sterol biosynthesis. Results are expressed as ED_{50} (mg/kg), effective concentration which inhibits to the extent of 50% incorporation of C^{14} acetate into sterols in rats when administered as appropriate doses of drug substances as compared to controls receiving vehicle alone. Table VIII shows that *in vivo* XU 62-320 is about 40- and 4.5-fold more potent than compactin and Lovastatin, respectively, in inhibiting endogenous cholesterol synthesis in rats. For most substances, although not for all, the relative

Table VIII
Relative Potency for Inhibition of Cholesterol Biosynthesis

	Compound	ED_{50} (mg/kg)	Relative Potency*
	XU 62-320	0.093	37.6
	Compactin	3.5	1.0
	Lovastatin (Monacolin)	0.414	8.4

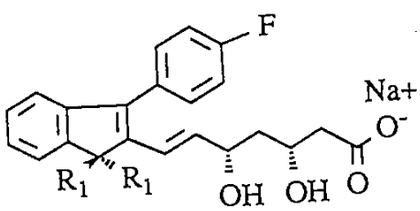
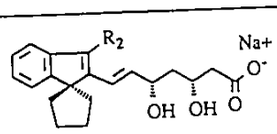
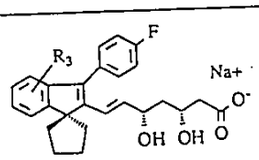
*As compared to Compactin = 1

Table IX
SAR for Cholesterol Biosynthesis Inhibition

	Compound	ED_{50} (mg/kg)	Relative Potency*	
	XU 62-320	0.093	1.0	1.0
	3R, 5S	0.056	1.66	2.8
	3S, 5R	>0.5		0.08
	Na Salt, Anti	1.37	0.067	0.057
	Methyl ester, Syn	0.40	0.23	0.13
	Trans Lactone	0.33	0.28	0.23
	Dihydro (Reduced Double Bond)	1.23	0.075	0.06

*As compared to XU 62-320 = 1

Table X
SAR of Indene Derivatives

	R_1	Relative Potency*
	$(CH_2)_4$	202
	(Racemic) $(CH_2)_4$	337
	(3R, 5S) $(CH_2)_2$	38
	$(CH_2)_5$	1.5†
	CH_2CH_3	<.2
	CH_3	2
	H,iPr	8
	R_2	
	Phenyl	88†
	3,5-Dimethylphenyl	146
	iPr	<0.5
	Cyclohexyl	16.5
	R_3	
	4-Me	114
	6-Me	181
	7-Me	24
	6-OMe	130
	4,6-$(OMe)_2$	60

*As compared to Compactin = 1
†As its Ethyl Ester

potency determined in *in vitro* microsomal assay against HMG-CoA reductase parallels the *in vivo* activity in rats for the inhibition of ^{14}C-acetate into sterols.

As an example, comparison of Tables II and IX reveals the relative potency of several analogs of XU 62-320 when compared in *in vitro* and in *in vivo*. Thus, as compared to XU 62-320, the anti-isomer is ~ 17- (Table II) and ~ 15-fold (Table IX) less active than XU 62-320 in *in vitro* and in *in vivo* assays, respectively. Similarly, close parallelism prevails for the ester (less active ~ 7.5-fold, *in vitro* vs. 4.3-fold, *in vivo*), *trans*-lactone (less active 4.2-fold, *in vitro* vs. 3.5, *in vivo*) and the dihydro derivative (less active 16.5-fold, *in vitro* vs. 13-fold *in vivo*).

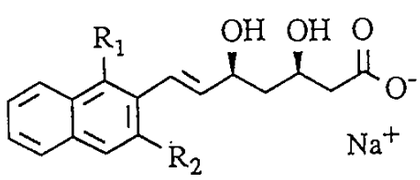
C. SAR of Indene Derivatives

The structure activity relationships for the indene derivatives can be best summarized as follows: Maximal activity is obtained with a spiro cyclopentyl group at C-1, again emphasizing the importance of the bulky group in the vicinity of the dihydroxy acid side chain. At C-3 the best substituent is 4-F-phenyl, while the optimal substituent for the benzenoid portion of the indene moiety is hydrogen (see Table X).

D. SAR of Naphthalene Derivatives

The most interesting part of the structure activity relationships for this group of compounds is the difference observed in the potency of 1-(4-F-

Table XI
SAR of Napthalene Derivatives



R_1	R_2	Relative Potency*
4-F-Ph	H	0.10
4-F-Ph	CH_3	8
4-F-Ph	Et	19
4-F-Ph	i-Pr	22
3,5-diMe-Ph	CH_3	56
Ph	CH_3	2
i-Pr	4-F-Ph	337
i-Pr	Ph	144

*As compared to Compactin = 1

phenyl)-3-isopropyl derivative vs. 1-isopropyl-3-(4-F-phenyl) compound (22 times more potent vs. 337 as compared to compactin) (see Table XI).

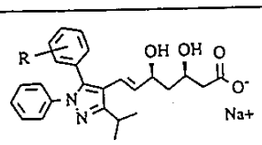
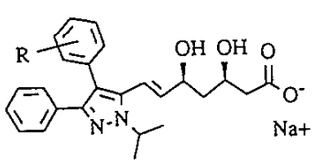
E. SAR of Pyrazole Derivatives

Table XII illustrates the structure activity relationships for a few of the many pyrazole derivatives prepared. Here, too, the optimal substituents are the 4-F-phenyl and isopropyl group adjacent to the dihydroxy acid side chain. The dihydro and the 5-keto derivatives are substantially less potent. 1,3-diaryl-substituted pyrazole derivatives show decreased inhibitory activity (not shown in the table) in contrast to the 1,5 and 3,4-diaryl-substituted compounds, which tend to have comparable potency.

F. SAR of Imidazole Derivatives

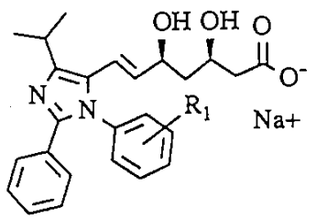
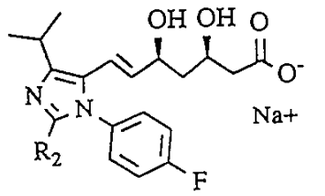
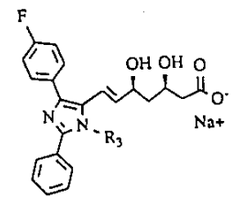
To emphasize the most salient features of the structure activity relationships for the imidazole derivatives, only a few of the derivatives prepared are tabulated in Table XIII. Optimal activity is obtained with 1,2-diaryl derivatives

Table XII
SAR of Pyrazole Derivatives

	R	Relative Potency*
	4-F	60
	4-F (6,7 Dihydro)	5.9
	4-F (5 Keto)	3.5
	H	5.6
	3,5 Dimethyl	4.1
	4-F	30

*As compared to Compactin = 1

Table XIII
SAR of Imidazole Derivatives

		Relative Potency*
	R_1	
	4-F (Racemic)	337
	4-F (3R, 5S)	532
	p-Cl	84
	p-Br	20
	3,5-Di-Me	7
	3,5-Di-Cl	10
	R_2	
	i-Pr	4.4
	t-Butyl	4.4
	cyclohexyl	4.8
	2-Thienyl	202
	1,4-Biphenylyl	35
	p-Dimethylamino-phenyl	56
	p-Nitro-phenyl	72
	R_3	
	i-Pr	1.1
	4-F-Phenyl	<0.1

*As compared to Compactin = 1

with the 4-F substituent preferred in the phenyl ring on nitrogen and H atom being the preferred substituent for the phenyl ring at C-2. Alkyl substituents at C-2 tend to lead to considerable loss of activity. The 1,3-diaryl-substituted imidazole derivatives suffer a dramatic loss of activity when compared to the very potent 1,2-diaryl compounds.

V. EFFECTS OF FLUVASTATIN (XU 62-320) ON PLASMA LIPOPROTEIN LEVELS

Fluvastatin (XU 62-320) has been studied in several species for its effects on serum lipoprotein levels.

Significant and sustained reductions of *rat* serum VLDL + LDL-cholesterol have been observed after treatment of rats with XU 62-320. However, these lipoprotein changes are not observed after chronic dosing of normolipemic rats either with compactin or lovastatin.

In the beagle dog, after three weeks of administration, fluvastatin lowers serum LDL + VLDL-cholesterol to the extent of ~ 47% either at 2 mg/kg/day given once a day or 1 mg/kg/day given twice a day. A comparable effect on VLDL + LDL-cholesterol is observed with compactin at a dose of 20 mg/kg/day

given once a day. In the Rhesus monkey, a reduction of 30% in serum VLDL + LDL-cholesterol is achieved with fluvastatin at a dose of 30 mg/kg/day at the end of three weeks of daily administration.[15]

VI. TOXICOLOGICAL, DRUG METABOLISM, AND PHARMACOKINETIC STUDIES OF FLUVASTATIN (XU 62-320)

The safety, drug metabolism, and pharmacokinetic evaluation of fluvastatin (XU 62-320) has been extensively carried out in acute, subchronic, and chronic rat, dog, monkey, and mouse studies at Sandoz Research Institute. These studies have allowed extensive clinical trials with the first totally synthetic HMG-CoA reductase inhibitor.[16]

VII. HUMAN STUDIES WITH FLUVASTATIN (XU 62-320)

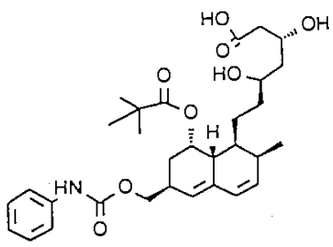
Through completion of Phase II multi-center dose-response and dose-frequency trials, in all 658 subjects have been randomized to treatment with fluvastatin (XU 62-320) in double-blind safety and efficacy trials with another 269 placebo subjects serving as controls. Fluvastatin (XU 62-320) was well tolerated at all doses studied and was free from serious or unexpected adverse effects. Dose-dependent mean reductions of 11% to 21% in total plasma cholesterol and 15% to 28% in LDL-cholesterol were achieved on 5 to 40 mg QPM of fluvastatin. Dose-dependent mean reductions of triglycerides and a drug-related increase in HDL-cholesterol were also observed. Equivalent reductions of LDL-C (22% vs. 23%) were produced by 20 mg per day of fluvastatin when given as a single dose or divided into a BID regimen. A dose of 20 mg once a day at bedtime gave LDL-cholesterol reductions similar in magnitude to that of the marketed agent lovastatin (Mevacor®).

VIII. OVERVIEW OF PUBLISHED LITERATURE ON HMG-CoA REDUCTASE INHIBITORS

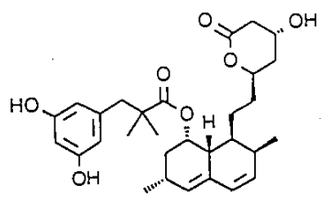
A very large number of reviews have described the importance of HMG-CoA reductase inhibitors for the treatment of elevated serum total cholesterol and LDL + VLDL-cholesterol.[4,17] Also, extensive information is available on the pharmacology and clinical efficacy of lovastatin (Mevacor®, MSD), marketed in the United States,[4,18] simvastatin (Zocor®, MSD),[19] marketed in several European countries but not yet available in the United States, and pravastatin (Mevalotin®, Pravachol®, Sankyo, Squibb), yet marketed only in Japan.[20] However, in this section, an overview is presented (Figs. 11–19), describing the attempts in many laboratories towards the discovery of new HMG-CoA reductase inhibitors since the discovery of compactin lovastatin, simvastatin, pravastatin, and fluvastatin. In Figures 11–19, only one specific representative structure is depicted to describe the varied structural prototypes reported in the literature as HMG-CoA reductase inhibitors.

- Scientists at Merck & Co. continue the derivatization efforts towards semisynthetic derivatives using lovastatin as starting material (Fig. 11).[21] Very many wide variants in the acyloxy side chain at C-8 of mevinolin have been executed. Elegant "Barton-type" chemistry has allowed the functionalization of 6-Methyl group in ring A of mevinolin leading to a large number of derivatives with many functional groups at C-6.

MERCK & Co.



EP 251625



EP 245990

Figure 11

• At Sandoz Research Institute, besides the fluvastatin, indenyl, naphthyl, imidazolyl, and pyrazolyl analogs discussed in this paper, a variety of other HMG-CoA reductase inhibitors have been synthesized varying the heterocyclic hydrophobic domain. These derivatives are described in Figs. 12–14.[22–24] The overlapping reports from other companies on similar derivatives are shown as well in Figs. 12 and 13.[22,23]

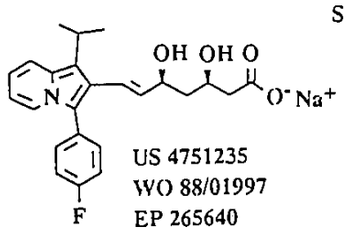
• In addition to the HMG-CoA reductase inhibitors described above, scientists at Hoechst, Baeyer, Warner-Lambert, May & Baker, Rorer, Bristol-Myers, Squibb, and Pfizer have published their efforts and their results in this exciting area (Figs. 15–17).[25–27]

• A set of novel structural prototypes as HMG-CoA reductase inhibitors have been claimed by Pan Medica (Fig. 18).[28] One of the Pan Medica candidates is currently in clinical trials.

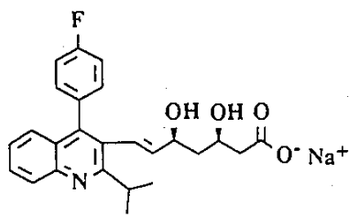
• Two groups have focused their efforts towards the development of "regulators of HMG-CoA reductase" rather than towards the development of competitive inhibitors.

• Schroepfer *et al.* have studied extensively Cholest-8(14)-en-15-one as a very interesting hypolipoproteinemic agent. This agent is being studied in

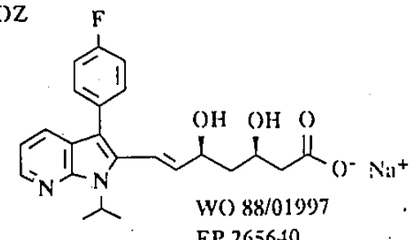
SANDOZ



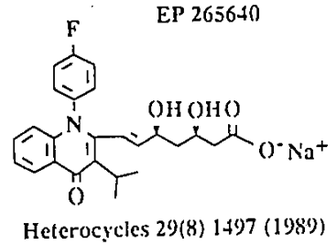
US 4751235
WO 88/01997
EP 265640



Sandoz Patent Pending
W.Lambert US 4761419
Nissan EP 304063A



WO 88/01997
EP 265640



Heterocycles 29(8) 1497 (1989)

Figure 12

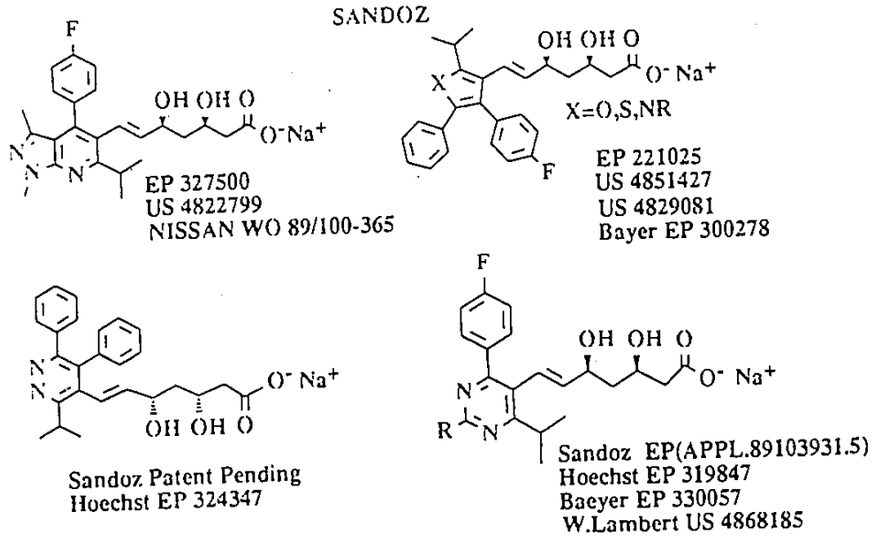


Figure 13

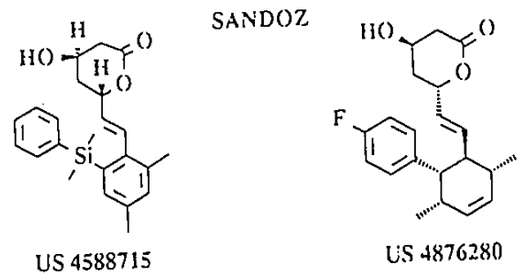


Figure 14

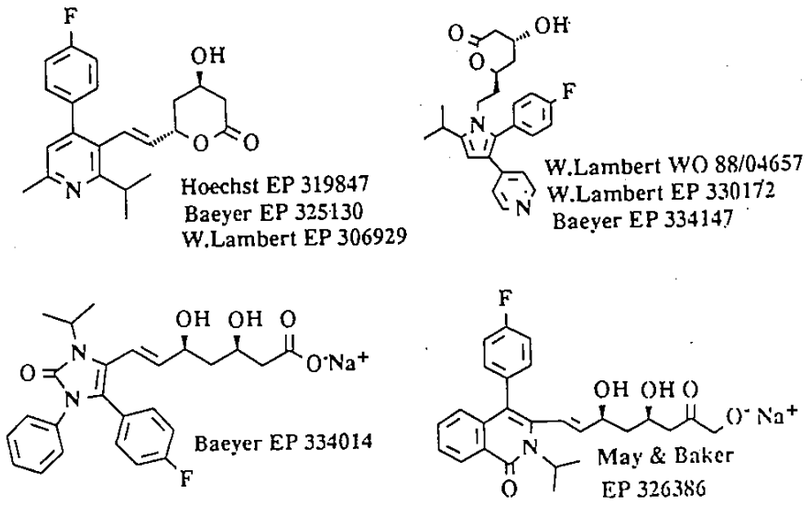


Figure 15

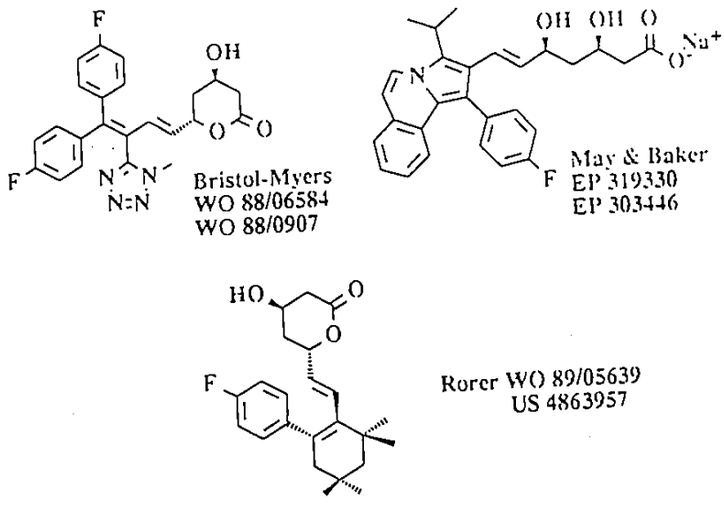


Figure 16

the clinic (Lederle Labs). Taylor *et al.* (DuPont) have attempted to develop inhibitors of HMG-CoA reductase via inhibition of lanosta-8, 24-dien-3 beta-ol-14 alpha-methyldemethylase (Fig. 19).[29]

IX. CONCLUSION

During the discussion on cholesterol biosynthesis inhibitor, Sabine commented, "The development of an effective agent that will lower, and/or prevent a rise in man's level of plasma cholesterol, without accompanying any undesirable side effects, is a pharmacological rainbow at the end of which is an immense pot of gold. Hence, the search for such an agent is conducted with a great deal of vigor, skill, imagination, and money. I myself certainly hope that the attainment of this therapeutic ideal is indeed not a rainbow, but that the possible existence of such an agent is in fact a solid reality and not just a pleasant illusion of light and color."[30]

Since Sabine's remark, HMG-CoA reductase inhibitors have indeed emerged as solid realities and have not remained mere pleasant illusions of light and color. Mevacor®, Zocor®, and Mevalotin® are marketed products showing remarkable efficacy in lowering LDL-cholesterol without serious side effects. Fluvastatin (XU 62-320), being studied intensely in Phase III clinical trials, has shown very good efficacy with no serious adverse effects. Future work will certainly shed more experience not only with Mevacor®, Zocor®, Mevalotin®, and Fluvastatin, but possibly with a host of other HMG-CoA reductase inhibitors reviewed in this paper. Also, in 1989 the worldwide sales of Merck's Mevacor® (launched in September, 1987), being $535 M, speak to the HMG-CoA reductase inhibitor as being the pharmacological rainbow at the end of which is an immense pot of gold.

Excitement has been added to the fascinating story of the development of HMG-CoA reductase inhibitors by the elegant and outstanding work in the laboratories of Nobel laureates Brown and Goldstein, to explain the mechanism of action of these inhibitors. The HMG-CoA reductase inhibitors lower

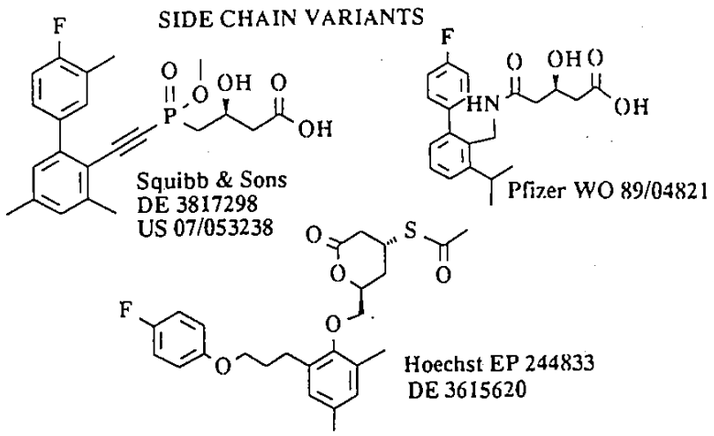


Figure 17

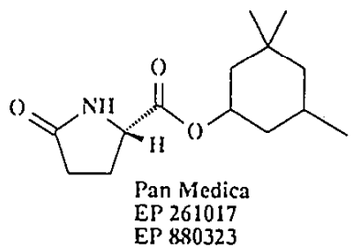


Figure 18

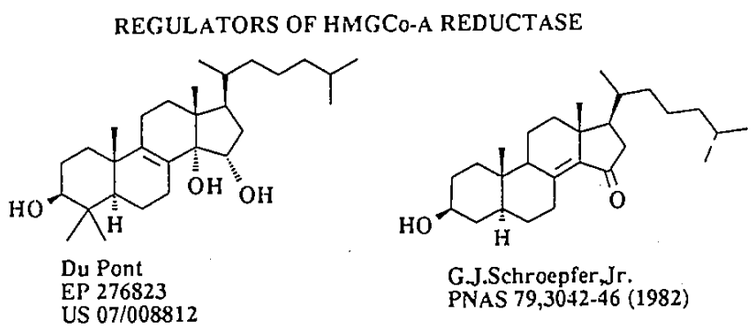


Figure 19

serum lipoprotein levels by up-regulating the lower LDL-receptors. But how do the many different HMG-CoA reductase inhibitors, described in this paper, affect the HMG-CoA reductase activity precisely at the detailed molecular level? The elegant molecular biology efforts in the laboratories of Brown and Goldstein have given us the amino acid sequence of HMG-CoA reductase of several species, but little is known of the detailed tertiary structure of the enzyme. What are the precise conformations of the many HMG-CoA reductase inhibitors, described in this paper, when bound to the active site domain of HMG-CoA reductase? What is the topography of the amino acid residues at the active site of HMG-CoA reductase when one or the other HMG-CoA reductase inhibitors is bound to it? What precise details of molecular recog-

nition are involved and need to be understood to explain the rank-order potency of many of the described analogs of HMG-CoA reductase inhibitors? Fascinating work remains to be done to provide answers to the many interesting unanswered questions in the exciting field of HMG-CoA reductase inhibitors.

ACKNOWLEDGMENTS

I wish to acknowledge the publication of the schemes and tables describing the SAR of the Sandoz compounds by Elsevier in their book *Trends in Medicinal Chemistry '88* (edited by H. van der Goot *et al.*). The extensive work at Sandoz Research Institute on HMG-CoA reductase inhibitors described in part in this paper is truly an outcome of a cohesive team effort of a very large number of dedicated and creative individuals. Most important original contributors to be recognized are: In the Medicinal Chemistry Department, for indole derivatives: H. F. Schuster, R. Stabler, J. Kratunis; for indene derivatives: S. Wattanasin, R. Patel; for naphthalene derivatives: P. L. Anderson, S. W. Meyers, N. A. Paolella; for pyrazole derivatives: J. R. Wareing, M. Martin, C. F. Jewell, Jr., R. Stabler; for imidazole derivatives: J. R. Wareing, J. M. Leginus, J. Linder, G. T. Lee, R. Stabler, M. Martin, L. Widler; for chiral synthon from D-glucose: J. R. Wareing, C. E. Fuller; for synthesis of chiral derivatives using chiral synthon from D-glucose: J. R. Wareing, C. F. Jewell, L. Widler; for coordination of the project: R. E. Damon; in the Process Research and Chemical Development Department, for the chiral synthon from S-malic acid and its use: P. Kapa, K. M. Chen, O. Repic and G. E. Hardtmann; for the racemic synthon and its use: P. Kapa and O. Repic; for large scale preparation and many important improvements of the processes for intermediates and final products: R. E. Walkup, S. Palermo, J. Linder, G. T. Lee, M. Thiede; in the Pharmacology Department, for in *in vivo* testing: R. G. Engstrom, D. B. Weinstein, J. B. Eskesen, M. L. Rucker, R. Miserendino. The success of this work is, in large part, due to our collaboration with Prof. T. Scallen, Department of Biochemistry, University of New Mexico, Albuquerque, New Mexico, who has carried out all the *in vitro* studies. Finally, many thanks are extended for the efforts of J. Birch and P. Schaefer for the preparation of this manuscript.

REFERENCES

1. R. I. Levy, *Circulation*, 72, 686 (1985).
2. *JAMA*, 251, 351–374 (1984).
3. *JAMA*, 253, 2080 (1985).
4. S. M. Grundy, *N. Engl. J. Med.*, 319 (1), 24–33 (1988) and references cited therein.
5. J. R. Wareing, U. S. 4474971; U. S. 4625039.
6. K. M. Chen, G. E. Hardtmann, K. Prasad, and O. Repic, "Efficient synthesis of chiral synthon for compactin analogs," 192nd ACS National Meeting, Annaheim, California, Abstract No. 31, September, 1986; U. S. 4870199; P. Kapa, U. S. 4571428; U.S. 4841071.
7. P. Kapa and O. Repic, *Tetrahedron Lett.*, 25 (23), 2435–2438 (1984).
8. S. Wattanasin, S. Barcza, R. Damon, F. G. Kathawala, G.R. Marshall, R. K. Boeckman, Jr., and T. Scallen, in *Proceedings VIII International Symposium on Medicinal Chemistry, Vol. 2, 327–329 (1985).*
9. A. Sato, et al. *Chem. Pharm. Bull*, 28 (5), 1509–1525 (1980); Merck & Co., Inc. EP 0024348 (1981); W. F. Hoff, et al., *J. Med. Chem.*, 29, 159–181 (1986).

10. (a) F. H. Kathawala, (Sandoz), U. S. 4739073; WO 84/02131; (b) F. G. Kathawala, S. Wattanasin, (Sandoz), WO 86/03488; (c) P. L. Anderson, (Sandoz) DE 3525256; (d) J. R. Wareing (Sandoz) U. S. 4755606; WO 86/07054; (e) J. R. Wareing (Sandoz) U. S. 4613610; WO 86/00307.
11. R. Stevenson and E. Block, *J. Chem. Soc. Perkin, Trans.*, 1, 308 (1973).
12. K. Narasaka and F. C. Pai, *Chem. Lett.*, 1415 (1980); *Tetrahedron*, 40, 2233 (1984).
13. S. Wattanasin et al., Paper presented at the 196th ACS National Meeting, Los Angeles, Sept. 25–30, 1988.
14. M. E. Ackerman et al., *J. Lipid Res.*, 18, 408 (1977).
15. (a) F. G. Kathawala, "Efforts towards the Development of New HMG-CoA Reductase Inhibitors," invited lecture at the Gordon Research Conference on Medicinal Chemistry (1986); (b) F. G. Kathawala, et al., "XU 62-320, an HMG-CoA Reductase Inhibitor, More Potent than Compactin," IX International Symposium on Drugs Affecting Lipid Metabolism, Florence (1986); VIII International Symposium on Atherosclerosis, Rome (1988); (c) R. G. Engstrom, et al., "XU 62-320: Hypolipoproteinemic Effects of a Potent HMG-CoA Reductase Inhibitor," International Symposium on Drugs Affecting Lipid Metabolism, Florence (1986); VIII International Symposium on Atherosclerosis, Rome (1988).
16. (a) R. E. Stoll, et al., "Subchronic Target Organ Toxicity of XU 62-320 in the Rat, Mouse, Dog, and Monkey," VIII International Symposium on Atherosclerosis, Rome (1988); (b) F. H. Ballard, et al., "Pharmacokinetics and Drug Metabolism of XU 62-320 in Animals and Men, VIII International Symposium on Atherosclerosis, Rome (1988); (c) F. L. S. Tse, et al., "Disposition of Fluvastatin in Mouse, Rat, Dog, and Monkey, "*Biopharmaceutics and Drug Disposition*, in press.
17. W. H. Frishman, *J. Clin. Pharmacol.*, 29 (11), 975–82 (1989); J. L. Witztum, *Circulation*, 80 (5), 1101–1114 (1989); G. Mantell, *Clin. Exp. Hypertens. [A]*, 11 (5-6), 927–941 (1989).
18. W. H. Frishman, et al., *Med. Clin. North Am.*, 73 (2), 437–448 (1989); J. A. Tobert, *Am. J. Cardiol.*, 62 (15), 28J–34J (1988); A. W. Alberts, *Am. J. Cardiol.*, 62 (15), 10J–15J (1988).
19. J. F. Walker, *Am. J. Med.*, 87 (4A), 44S–46S (1989); R. J. Gerson, et al., *Am. J. Med.*, 87 (4A), 28S–38S (1989).
20. Y. Saito, et al., *Atherosclerosis*, 72 (2–3), 205–211 (1988). See also: *Drugs Affecting Lipid Metabolism*, R. Paoletti, et al., eds., Springer-Vertag, pp. 247, 255–277; *Atherosclerosis VIII*, G. Crepoldi, et al., eds., Excerpta Medica, Amsterdam, 1989, pp. 757–760.
21. Merck & Co., EP 245990; EP 245004; U. S. 4766145; EP 251625.
22. Aza Indoles: P. L. Anderson, et al. (Sandoz), EP 265640; U.S. 4751235. Quinolines: J. A. Picard, et al., (Warner Lambert), U. S. 4761419; S. Wattanasin, et al., Paper presented at the 5th SCI-RSC Medicinal Chemistry Symposium, Cambridge, 10–13 September 1989; G. M. Coppola, *Heterocycles*, 29 (8), 1497–1515 (1989).
23. Pyrazolopyridine: (Sandoz) F. G. Kathawala, U. S. 4822799; EP 327500. Pyrroles: (Sandoz) J. R. Wareing, U. S. 4851427; EP 221025; (Hoechst) H. Jendralla, et al., *J. Med. Chem.*, 33, 61–70 (1990). Thiophenes: (Sandoz) R. E. Damon, J. R. Wareing, U. S. 4829081. Furans: (Sandoz) R. E. Damon, J. R. Wareing, EP 221025. Pyridazines: (Sandoz) F. G. Kathawala, Patent pending; Hoechst, EP 324347. Pyrimidines: (Sandoz) F. G. Kathawala, EP (APPL. 89/103931.5); (Hoechst), EP 319847; G. Beck, et al., *J. Med. Chem.*, 33 (1), 52–60 (1990); (Baeyer), EP 330057; (Warner Lambert), U. S. 4868185.
24. Silyl Derivatives: (Sandoz) R. E. Damon, U. S. 4588715. "Cyclohexene" Derivatives: (Sandoz) R. E. Damon, U. S. 4876280.
25. Pyridine: (Hoechst), EP 319847; G. Beck, et al., *J. Med. Chem.*, 33 (1), 52–60 (1990); (Baeyer), Ep 325130; (Warner-Lambert) EP 306929. Pyrrole: (Warner-Lambert), WO 88/04657; B. D. Roth, et al., *J. Med. Chem.*, 33, 21–31 (1990). Imidazoline: (Baeyer), EP 334014. Isoquinoline: (May & Baker), EP 326386.
26. Tetrazoles: (Bristol-Myers), WO 88/06584; N. Balasubramanian, et al., *J. Med. Chem.*, 32 (9), 2038–2041 (1989). "Tricyclic": (May & Baker), EP 319330; 303446. "Cyclohexene": (Rorer), WO 89/05639; U. S. 4,863,957.
27. "Phosphoryl Side Chain": (Squibb), D. S. Karanewsky, et al., DE 3817298. "Amide Side Chain": (Pfizer), WO 89/04821A. "3-Thioaryl": (Hoechst), EP 244833.
28. (Pan Medica) EP 261017.
29. "Lanosterol" Derivatives (DuPont), J. L. Gaylor, et al., EP 276823. Cholest-8(14)-en-15-one: G. J. Schroepfer, Jr., *PNAS*, 79, 3042–3046 (1982).
30. J. R. Sabine, *Cholesterol*, Marcel Dekker, Inc., 1977.

#76

Case No. 600-7101/CONT/INT FYI
Patent

FEB 1- 1993

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

RECEIVED IN BOX INTERFERENCE

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

WATTANASIN REPLY TO FUJIKAWA OPPOSITION TO WATTANASIN MOTION FOR LEAVE TO PRESENT ADDITIONAL TESTIMONY

I. BACKGROUND

By paper dated December 15, 1992, Fujikawa et al. served on Wattanasin a notification pursuant to 37 CFR §1.632 in the above interferences, indicating an intention to raise an affirmative defense of abandonment, suppression or concealment.

In response, the party Wattanasin on December 31, 1992 filed and served a motion for leave to present additional testimony going to the absence of abandonment, suppression or concealment of the Wattanasin invention.

The testimony in question would be presented in affidavit form, and relates primarily to activity of the inventor, Dr. Wattanasin, showing the absence of abandonment, suppression and concealment, and to attorney activities over a period of about fifteen months prior to the filing of the Wattanasin application on March 3, 1989.

Fujikawa have now opposed the Wattanasin motion (Paper of January 13, 1993).

Fujikawa in their Opposition have made certain arguments with respect to the substantive requirements of Rule 632, as well as the formal sufficiency of the Wattanasin motion, to which Wattanasin replies as follows:

II. 37 CFR §1.632

37 CFR §1.632, which became effective on February 11, 1985, as part of the revised interference rules, has no predecessor section in the prior interference rules.

The related commentary of the Patent and Trademark Office makes clear that Rule 632, as a newly created rule, was specifically intended to address situations developing in the case law where the issue of abandonment, concealment or suppression was not raised by a party until the briefing stage or at final hearing, thereby depriving the opposer of a fair opportunity to present relevant testimony thereon, except by way of a re-opened testimony period well beyond the interlocutory stage[1].

---

1. The commentary refers to Klug v. Wood, 212 USPQ 767, 771, n. 2 (Bd. Pat. Int. 1981) wherein the senior party apparently raised the defense suppression and concealment in the final brief. The Board's denial of the junior party's motion to re-open its testimony period to admit evidence to rebut the accusation turned on the belatedness of that motion, which was not made until after final hearing

The commentary on Rule 632 states in part as follows (the sentences being separated into numbered paragraphs for convenience):

> "[1] Under current practice where notice is not required, it is possible that a party may learn for the first time that abandonment, suppression, or concealment is an issue when the party receives an opponent's brief at final hearing. See Klug v. Wood, 212 USPQ 767, 771, n.2 (Bd. Pat. Int. 1981). At that point it is often too late to reopen proceedings in the interference. The purpose of requiring the notice under §1.632 is to make the parties and the Board aware during the interlocutory stage of an interference that abandonment, suppression, or concealment may be an issue in the interference.
>
> [2] Early notice will permit the parties to ask for and the examiner-in-chief to set appropriate testimony periods for a party to present evidence related to abandonment, suppression or concealment, particularly in cases where long unexplained delays tend to prove the allegation of suppression or concealment." [emphasis supplied]
>
> "[3] Early notice will also eliminate the need for the party moving to reopen the testimony period. Klug v. Wood, supra".

1062 OG 219 (January 7, 1986)

First of all, paragraph [2] makes clear that the drafters of Rule 632 contemplated that parties will be permitted to ask for, and the EIC to set, testimony periods for evidence to be presented going to the abandonment issue during the interlocutory period.

(Footnote 1 continued from previous page)
(evidently some six months after submission of briefs).

It is noted that paragraph [3] -- isolated from context and lacking the citation of the 1981 Klug opinion -- was relied on by Fujikawa in their Opposition at p. 9 as a blanket assertion by the drafters that early notice would eliminate the need for a party to reopen the testimony period.

On the contrary, when paragraph [3] is read in context, i.e. sequentially after paragraph [2], and with the reference to Klug restored, it obviously reflects an intention by the drafters not that testimony periods never be reopened, which would surely be at variance with the prior paragraph, but that recurrence of another Klug-type situation be prevented.

Thus it is evident that the drafters did intend that reopened testimony periods, if seasonably requested, be permitted in response to a Rule 632 Notification. Moreover, given that Rule 632 permits Notification to be made even up to 10 days beyond the opposing party's testimony-in-chief, it must typically be the case that any reopened testimony period of the opposer would extend well beyond the period originally set.

The rationale of Rule 632 is clearly to facilitate an orderly presentation of testimony on all issues prior to submission of briefs and final hearing.

However, notwithstanding the clear directive contained in the PTO commentary, Fujikawa further argue that the receiving party of a Rule 632 Notification must meet some additional threshold element of "surprise" in order to be granted leave to present additional testimony going to abandonment, etc. (Opp. at

p. 9).[2]

On this rationale, Fujikawa sieze upon Wattanasin's statements concerning the substance of its proofs as to priority already made of record -- which were made for the convenience of the EIC in evaluating Wattanasin's motion -- as some sort of admission that Wattanasin lacks the requisite mental state of "surprise" to be granted a reopened testimony period.

The fact is, no such element of "surprise" is envisaged by the commentary in relation to practice under the new Rule 632.

---

2. Fujikawa cite various pre-1985 and post-1984 cases, none of which is considered on point:

Suh v. Hoefle, 23 USPQ2d 1321 (BPAI 1992), turns on whether a belated motion for judgment based on unpatentability, made some 34 months after close of the preliminary motions period, met the good cause requirement of 37 CFR §1.655(b)(3). Hanagan v. Kimura, 16 USPQ2d 1791 (Comm. Pat. 1990), concerns the sufficiency of a Rule 639 motion to take testimony. At issue in Jacobs v. Moriarity, 6 USPQ2d 1799 (BPAI 1988), is the sufficiency of a preliminary motion for judgment on the ground of unpatentability. Issidorides v. Ley, 4 USPQ2d 1854 (BPAI 1987), concerns a belated motion after final hearing to reopen the testimony period to retake deposition testimony invalidated by formal deficiencies, where the movant had already been given at least 3 "bites at the apple," including leave to take testimony after final hearing.

With respect to the pre-1985 cases:

Rexroth v. Gunther, 202 USPQ 837 (BPAI 1978), is an example of the confusion arising under the old interference rules concerning notification of intent to argue abandonment. In that case the Board ruled that the senior party had in effect given notice by requesting additional discovery in relation thereto, making the junior party aware of the issue prior to the times for taking testimony. Horwath v. Lee, 195 USPQ 701 (CCPA 1977), also referred to in the commentary to Rule 632, simply stands for the proposition that suppression or concealment issues must be considered on a case-by-case basis. In Horwath, a nearly 6-year delay between reduction to practice and filing was found prima facie unreasonable under the circumstances but rebuttable (even though not found rebutted) by the evidence.

Quite to the contrary, paragraph [2], above, specifically states that it is "particularly" instances where "long, unexplained delays" raise a prima facie case of abandonment, etc., that the rule was intended to address.[3]

While Wattanasin does not believe that the period of time at issue, i.e. about 15 months, raises a prima facie case of abandonment, paragraph [2] obviously indicates the drafters' intent, even in cases where the delay does rise to such level, that there should be no restriction on reopening of testimony to complete the record in this regard. Far from being "extraordinary," as Fujikawa persist in alleging (Opp. at p. 4-5), the Wattanasin motion is fully countenanced by the PTO commentary on Rule 632, as evident above.

Furthermore, in order to harmonize the commentary on Rule 632 with the other involved interference rules, it has to be inferred that a Rule 632 Notification, in itself, provides sufficient "good cause" under 37 CFR §1.651 for reopening the testimony period.

Wattanasin also takes issue with the Fujikawa characterization of the time period at issue as being either "not per se reasonable (Opp., p.7), or alternatively, "per se unreasonable" (Opp. at p. 11), neither of which terms to Wattanasin's knowledge has a recognized legal meaning. Fujikawa's citation to Engelhard Corp. v. M.C. Canfield Sons, 13 USPQ2d 1561

3. Alternatively, Fujikawa can hardly be saying that only a party who is "unaware" or "surprised" by either the content of its own proofs and/or the law concerning 35 USC 102(g) would receive the benefit of a reopened testimony period!

(DNJ 1989), is ironic given that the district court in that case acknowledged the virtual unanimity of the case law on the point that "delays of less than two years are reasonable," 13 USPQ2d at 1564-1565.

Of course, when an affirmative defense of abandonment is raised, the issue turns not on whether a period of inactivity is "not per se" reasonable or "per se unreasonable"; but, rather, whether it is "prima facie" unreasonable. And even when a prima facie case has been established, it can be overcome by submission of proofs that it is not unreasonable.

Lacking any real support in either the PTO commentary on Rule 632 or the relevant case law for challenging the substantive basis of the Wattanasin motion, Fujikawa refer to a litany of alleged formal deficiencies in the motion.

However, Wattanasin submits that its motion was both seasonably presented and had ample specificity, in that it referred to the Fujikawa Rule 632 notification, presented the status of the subject interferences, and described Wattanasin's requested relief in the form of an additional testimony period to present evidence going to the absence of abandonment, suppression and concealment of the Wattanasin invention.

III. CONCLUSION

The arguments of Fujikawa are contradicted by the clear language of the PTO commentary on Rule 632. Rule 632 is intended precisely to permit a party on notice of an affirmative defense of abandonment, suppression or concealment to seasonably request and

present evidence going to the absence thereof, in order to facilitate a full briefing on the issues before the Board and avoid belated presentation of testimony.

In the present circumstances, where in fact the relevant period of time before the filing of the Wattanasin application is not considered prima facie unreasonable under the prevailing law, it is appropriate and entirely consistent with the commentary surrounding Rule 632, that the Wattanasin motion be granted.

It is noted that undersigned counsel for Wattanasin in the prior motion inadvertently expressed a preference that a reopened Wattanasin testimony period run from January 4, 1993 to February 1, 1993, in erroneous disregard of the need to account for periods for filing opposition and replies on the Wattanasin motion. Therefore, Wattanasin hereby amends its motion to the extent of requesting that any such re-opened testimony period preferably run for a period of about two to three weeks from the date of the EIC decision thereon.

Grant of the Wattanasin motion would not be seem to impinge on the PTO interest in expediting resolution of the underlying interferences: Since Fujikawa et al. are relying on their Japanese priority documents as a constructive reduction to practice, it is expected that the interlocutory period will be effectively completed in relatively short time, i.e. as soon as Fujikawa have completed cross-examination of the Wattanasin testimony.

It is further noted that Mr. Kelber, counsel for Fujikawa et al. has indicated to the undersigned that he will be unavailable and out of the country during the period of February 2

to February 13, 1993; and therefore the scheduling of a re-opened Wattanasin testimony period overlapping at least with this period would not seem to be particularly disruptive to Fujikawa et al.

Accordingly, grant of the Wattanasin motion for leave to present additional testimony is respectfully requested.

Respectfully submitted,

Diane Furman

Diane E. Furman
Attorney for the Party Wattanasin
Registration No. 31,104
201-503-7332

SANDOZ CORPORATION
59 Route 10
East Hanover, NJ 07936

DEF:rmf
January 28, 1993

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on Jan. 28, 1993
(Date of Deposit)
Diane E. Furman
Name of applicant, assignee, or Registered Representative
Diane Furman
Signature
1/28/93
Date of Signature

CERTIFICATE OF SERVICE

It is hereby certified that a true copy of the paper entitled:

**WATTANASIN REPLY TO
FUJIKAWA OPPOSITION TO
WATTANASIN MOTION FOR LEAVE TO PRESENT ADDITIONAL TESTIMONY**

was served on counsel for the party Fujikawa et al., this 28th day of January 1993, by postage pre-paid first-class mail addressed to the following:

Oblon, Spivak, McClelland, Maier & Neustadt, P.C.
Attn: Steven B. Kelber, Esq.
1755 South Jefferson Davis Highway
Crystal Square 5, Ste. 400
Arlington, VA 22202

Diane Furman 1/28/93
Diane E. Furman

Paper No. 77

All communications respecting this case should identify it by number and names of parties.

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: BOX INTERFERENCE
Commissioner of Patents and Trademarks
Washington, D.C. 20231

Telephone: (703)557-4007
Facsimile: (703)557-8642

Interference No. 102,648

Wattanasin et al.

v.

Fujikawa et al.

Receipt is acknowledged of the motion for leave to present additional testimony, filed on January 6, 1993 by Wattanasin et al. (Paper No. 72). An opposition and a reply thereto have been filed.

For the reasons stated therein and in the reply to the opposition, the motion is granted. It is the practice of the Board to permit a party to reopen its testimony for the purpose of presenting additional evidence where an opponent files a notice under 37 CFR 1.632 raising the issue of abandonment, suppression or concealment.

Accordingly, the times are reset as follows:

Testimony-in-chief of the junior party Wattanasin for deposition testimony, including cross-examination of witnesses, to close February 25, 1993.

Testimony-in-chief of the junior party Wattanasin for affidavit testimony (affidavits pursuant to 37 CFR 1.671(e) and 1.672(b) must be filed) to close February 20, 1993.

Cross-examination of any junior party's affiants to close February 25, 1993.

Interference No. 102,648

Since the parties have agreed to have the rebuttal testimony of the senior party Fujikawa et al. run concurrently with any cross-examination of the junior party witnesses, the EIC does not perceive of any reason to reset the rebuttal testimony period.

The time for filing and serving the record and the briefs remains as set in Paper No. 59

Michael Sofocleous
Examiner-in-Chief
(703) 557-4066

gjh

49-111-0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	
	:	INTERFERENCE NO.: 102,648
V.	:	EXAMINER-IN-CHIEF:
FUJIKAWA ET AL	:	MICHAEL SOFOCLEOUS

MOTION FOR EXTENSION OF TIME,
37 CFR §1.645, §1.635

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

BOX INTERFERENCE

SIR:

Responsive to the Decisions of the EIC in the above-captioned Interference and related Interference (Paper No. 77 in Interference 102,648 and Paper No. 22 in Interference 102,975), Fujikawa et al hereby move all pending dates for action subsequent to the date for completion of testimony-in-chief by the Junior Party be extended one month. Accordingly, cross-examination of any Junior Party affiant, and the date for completion of any Senior Party rebuttal testimony, including any cross-examination, would close March 25,

1993. Other dates would be extended, as set forth below.

As grounds for this request, it is respectfully submitted that the reopening of the Junior Party testimony period for leave to present new testimony related to the issue of abandonment, suppression or concealment ordered does not provide sufficient time for cross-examination of the Junior Party affiants, followed by the submission of rebuttal testimony, if necessary. Specifically, the testimony of the Junior Party will not be completed until February 20, 1993 (actually filed and served February 22, 1993). The current date for cross-examination of such witnesses to close, and the date for presentation of rebuttal testimony by the Senior Party, is February 25, 1993. It is unlikely that undersigned Counsel will receive the testimony of the Junior Party, much less be in a position to cross-examine with respect to the same, or present rebuttal testimony, by February 25, 1993.

Accordingly, Counsel for the Junior Party and undersigned Counsel have discussed the situation, and are in agreement that all dates in Interferences 102,648 and 102,975 subsequent to the closing date for testimony-in-chief of the Junior Party be extended one month. This will provide sufficient time for cross-examination of the Junior Party affiants, as well as the presentation of rebuttal testimony, which should be completed by March 25, 1993.

If granted, this Motion will extend the established times as follows:

--Testimony-in-chief of the Junior Party for Affidavit Testimony to close February 20, 1993.

--Cross-examination of any Junior Party's affiants to close March 25, 1993.

--Rebuttal testimony for the Senior Party, including affidavit testimony and cross-examination as well as deposition testimony, to close March 25, 1993.

--Filing and serving of the record, April 25, 1993.

--Junior Party's Opening Brief due May 25, 1993.

--Senior Party's Brief due June 25, 1993.

--Junior Party's Reply Brief due July 15, 1993.

EIC Sofocleous was contacted on the morning of February 18, 1993, and indicated that on the above grounds, this Motion would be

granted. The cooperation and assistance of the EIC is deeply appreciated.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Steven B. Kelber
Registration No.: 30,073
Attorney for Fujikawa et al

CERTIFICATE OF SERVICE

I hereby certify that true copies of:

1. **MOTION FOR EXTENSION OF TIME, 37 CFR §1.645, §1.635**

2. **CERTIFICATE OF SERVICE**

were served upon Counsel for Wattanasin as follows:

Diane E. Furman
SANDOZ CORP.
59 Route 10
E. Hanover, New Jersey 07936

via first-class mail, postage prepaid, this 18th day of FEBRUARY, 1993.

STEVEN B. KELBER

[redacted] BOARD OF PATENT [redacted] & INTERFERENCES

MAILED

FEB 19 1993

PAT. & T.M. OFFICE BOARD OF PATENT APPEALS & INTERFERENCES

FEB 18 1993 #79

49-111-0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	
	:	**INTERFERENCE NO.: 102,648**
V.	:	**EXAMINER-IN-CHIEF:**
FUJIKAWA ET AL	:	**MICHAEL SOFOCLEOUS**

APPROVED

FEB 19 1993

By ..... Examiner-In-Chief

MOTION FOR EXTENSION OF TIME,
37 CFR §1.645, §1.635

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

BOX INTERFERENCE

SIR:

Responsive to the Decisions of the EIC in the above-captioned Interference and related Interference (Paper No. 77 in Interference 102,648 and Paper No. 22 in Interference 102,975), Fujikawa et al hereby move all pending dates for action subsequent to the date for completion of testimony-in-chief by the Junior Party be extended one month. Accordingly, cross-examination of any Junior Party affiant, and the date for completion of any Senior Party rebuttal testimony, including any cross-examination, would close March 25,

49-111-0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	
	:	INTERFERENCE NO.: 102,648
V.	:	EXAMINER-IN-CHIEF:
FUJIKAWA ET AL	:	MICHAEL SOFOCLEOUS

FUJIKAWA ET AL REQUEST FOR
CROSS-EXAMINATION

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

BOX INTERFERENCE

SIR:

Responsive to the filing of Wattanasin Consolidated Affidavit Testimony (Volume IV) bearing a filing date of February 22, 1993, Fujikawa hereby requests cross-examination of the following Affiants:

1. Sompong Wattanasin
2. Melvyn M. Kassenoff
3. Joanne M. Giesser

4. Linda Rothwell

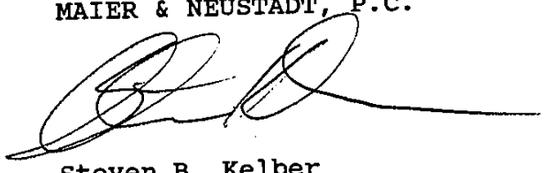
5. Lorraine M. Chesley

The cross-examination of Robert G. Engstrom will not be required.

The cross-examination will be as to all Declarations submitted by Sompong Wattanasin in this Interference. The remaining declarants are believed confined to Volume IV.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Steven B. Kelber
Registration No.: 30,073
Attorney for Fujikawa et al

CERTIFICATE OF SERVICE

I hereby certify that true copies of:

1. **FUJIKAWA ET AL REQUEST FOR CROSS-EXAMINATION**

2. **CERTIFICATE OF SERVICE**

were served upon Counsel for Wattanasin as follows:

Diane E. Furman
SANDOZ CORP.
59 Route 10
E. Hanover, New Jersey 07936

via first-class mail, postage prepaid, this 25th day of FEBRUARY, 1993.

STEVEN B. KELBER

#81

49-111-0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	
	:	INTERFERENCE NO.: 102,648
V.	:	EXAMINER-IN-CHIEF:
FUJIKAWA ET AL	:	MICHAEL SOFOCLEOUS

NOTICE OF DEPOSITION

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

BOX INTERFERENCE

SIR:

Pursuant to 37 CFR §1.673(a), Fujikawa et al hereby serve notice of the deposition of Dr. Chester E. Holmlund to be held at the offices of undersigned Counsel on March 12, 1993, beginning at 10:00 AM, and continuing from time-to-time until done. It is not expected that the deposition will last beyond a single day, but in the event it does, the deposition will be resumed March 15, 1993.

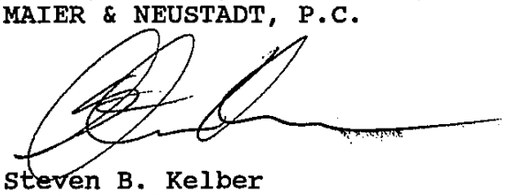
The current address for Dr. Holmlund is 9200 Edwards Way, Apartment 516, Adelphi, Maryland. The witness is expected to

testify in a rebuttal capacity, as to the adequacy of the proof of the Junior Party with respect to conception and actual reduction to practice.

Undersigned Counsel, prior to the service of this notice, contacted Counsel for the Junior Party, Diane Furman, to establish a mutually acceptable time and place for conducting the deposition. Counsel for the Junior Party indicated that she could not at the time agree to any date in the period provided in the approved Motion for Extension of Time, mailed February 19, 1993, due to unspecified contingencies. If Counsel for the Junior Party indicates the designated time is unacceptable, undersigned Counsel shall initiate a conference call with the EIC.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Steven B. Kelber
Registration No.: 30,073
Attorney for Fujikawa et al

CERTIFICATE OF SERVICE

I hereby certify that true copies of:

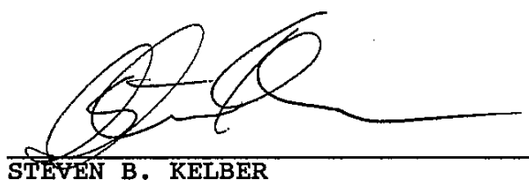
1. **NOTICE OF DEPOSITION**

2. **CERTIFICATE OF SERVICE**

were served upon Counsel for Wattanasin as follows:

Diane E. Furman
SANDOZ CORP.
59 Route 10
E. Hanover, New Jersey 07936

via facsimile and via first-class mail, postage prepaid, this 1ST day of MARCH, 1993.



STEVEN B. KELBER

102648 - #82

102975 - #27

WATT'N SUPP.

FYI

FEB 24 1993

RECEIVED IN
BOX INTERFERENCE

VOLUME IV

Interference No. 102,648

Interference No. 102,975

WATTANASIN Consolidated

Affidavit Testimony

and Exhibits

Case No. 600-7101/CONT/INT.(1)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

SUPPLEMENTAL DECLARATION OF SOMPONG WATTANASIN, PH.D. PURSUANT TO 37 CFR 1.'672

I, Sompong Wattanasin, do hereby declare as follows:

1. All of the below-indicated activities took place in the United States.

BACKGROUND

2. Since about 1981, Sandoz Research Institute (SRI) has been engaged in a concerted research effort to develop compounds having utility as HMG-CoA reductase inhibitors for treatment of hypercholesterolemia.

3. Much of this research has focused on compounds which comprise heterocyclic analogs of mevalonolactone and the open chain derivatives thereof.

4. For example, since 1981 SRI has prepared indenyl, indolyl, indolizinyl, imidazolyl, pyrazolopyridinyl, pyrrole, as well as quinolinyl, and other analogs of mevalonolactone and derivatives thereof.

5. The Sandoz research effort culminated in 1992 in the completion of an NDA filing on fluvastatin, i.e. (E)-(±)-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2- yl]-3,5-dihydroxy-6-heptenoic acid, sodium salt, which compound is a member of a family of indole analogs of mevalonolactone and the open chain analogs thereof.

6. My laboratory was only one of six laboratories devoted virtually exclusively to the synthesis of HMG-CoA reductase inhibitors. By way of illustration of the large number of HMG-CoA compounds being synthesized at Sandoz, I note that during the period of July 1985 to July 1987, my laboratory alone prepared 60 such compounds. This is evidence of Sandoz' high level of interest in the project and intention since 1981, and including the period of July 1985 to July 1987, to pursue its basic research project in the HMG-CoA reductase area and the inventive concept behind it.

SANDOZ QUINOLINE COMPOUNDS

7. In late March of 1987, I submitted Patent Disclosure 299/84 direct to quinoline analogs of HMG-CoA reductase inhibitors (**Exhibit A-3** hereto) to the Sandoz Patent and Trademark Department.

8. I understand that between April and November of 1987, this disclosure was presented for rating on four occasions at the regular Sandoz patent committee meetings. On each of these occasions, PD 299/84 was rated either "B" or "X", indicating that further information was needed in order to file a patent application thereon (**Exhibits M-1 - M-4** hereto).

9. In the period between July and December 1987, additional compounds of the invention were synthesized under my direction, and they were tested for activity in vitro and in vivo as HMG-CoA reductase inhibitors.

(The synthesis and testing of these compounds are further described in my Declaration of November 13, 1992; the Declaration and Supplemental Declaration of Rajeshvari Patel dated November 13 and 16, 1992; the Declaration of Dr. Terence Scallen dated November 13, 1992; and the Declarations of Robert G. Engstrom and Rodney Slaughter dated November 13, 1992.)

10. I learned shortly after the January 1988 Patent Committee Meeting that my Patent Disclosure 299/84 was rated for filing.

11. 2. On or about February 29, 1988, I sent certain information to Melvyn M. Kassenoff of the Patent Department relating to PD 299/84.

Exhibit O hereto comprises a true copy of the following material which I sent to the Patent Department:

(1) a "post-it" stating "sent to M. Kassenoff. 2/29/88" which is in my handwriting'

(2) 4 pages comprising handwritten reaction schemes and notes bearing my name and a date in my handwriting of February 29, 1988 on the first page (see also **Exhibit P-1**);

12. Additional material which I sent to the Patent Department comprises the following:

Exhibit P-2: 7 pages of computer printouts of specific compounds containing my handwritten notations of the Notebook pages on which they were prepared and relevant physical properties; and

Exhibit P-3: 9 laboratory notebook pages numbered 130, 137, 145, 153, 158, 166, 172, 175 and 176.

13. On November 1, 1988, I printed out the Sandoz database containing the structures of the quinoline compounds of PD 299/84. I subsequently consulted with Robert G. Enstrom about the IC_{50} and ED_{50} values for these compounds, which I wrote on the printout. I sent this printout to the Patent Department. Since the cover page is dated January 4, 1989 in my handwriting, I would have mailed it on or about that date.

Exhibit Y-2 comprises a true copy of the printout bearing my handwritten notations.

14. On or before November 8, 1988, I sent to Mrs. Joanne M. Giesser of the Patent Department a handwritten memorandum outlining a synthesis of the quinoline compounds of my invention according to the procedure identified as "Route I" in my patent disclosure.

Exhibit U-2 comprises a true copy of this memorandum. The front page bears my initials and the date of November 7, 1988 in my handwriting.

15. I received a memorandum dated December 14, 1988 from Mrs. Giesser enclosing a first draft of the patent application on PD 299/84.

Exhibit W comprises a true copy of the memorandum I received.

16. I made handwritten corrections on pages of the draft application and returned them to the Patent Department on or about December 22, 1988.

Exhibit X comprises a true copy of these pages bearing my corrections and my handwritten date of December 22, 1988.

17. On or about January 4, 1989, I returned to Mrs. Giesser a handwritten memorandum and other material in connection with the patent application draft for case 600-7101.

Exhibit Y hereto comprises a true copy of this material, i.e.:

Y-1: 6 pages of handwritten notes on the first draft and a handwritten synthesis step;

Y-2: the computer printout I received from Biology, which I dated January 4, 1989.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this ____ day of February, 1993.

S. Wattanasin 2/9/93.

Sompong Wattanasin, Ph.D.

Case No. 600-7101/CONT/INT.(2)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN

v. Interference Nos. 102,648, 102,975

FUJIKAWA et al. Examiner-in-Chief: M. Sofocleous

DECLARATION OF MELVYN M. KASSENOFF PURSUANT TO 37 CFR §1.672

I, Melvyn M. Kassenoff, do hereby declare as follows:

1. All of the below-indicated activities took place in the United States.

2. I have been employed by Sandoz Corporation in the Patent and Trademark Department since 1972. My current position is Director, Patent and Trademark Affairs. I am an associate counsel of record in these interferences.

3. I have had responsibility for the filing and prosecution of Sandoz patent applications in the HMG-CoA reductase inhibitor area since 1982. However, this area was only a very small portion of my total workload, the bulk of which comprised prosecuting applications in the azo dye area originating from research done by Sandoz AG in Basle, Switzerland.

Since about 1981, Sandoz Research Institute has been engaged in a research effort to develop compounds having utility as HMG-CoA reductase inhibitors for use in the treatment of hypercholesterolemia. This project resulted in numerous patent disclosures being submitted to the Patent Department, including Patent Disclosure 299/84 of Dr. Wattanasin.

Prior to approximately April 1987, when he took permanent leave for health reasons, Mr. Fred Weinfeldt, a senior patent attorney in the Sandoz Patent Department, shared the responsibility of filing of patent applications in the HMG-CoA reductase inhibitor area. In August 1987, Mrs. Joanne M. Giesser joined the Department as a patent attorney and took over a portion of Mr. Weinfeldt's docket of patent disclosures to be filed.

4. Within a week or two following the January 27, 1988 Patent Committee meeting, I was aware that Patent Disclosure 299/84 of Sompong Wattanasin had received an "A" rating. It was my intention that the case would be filed by Mrs. Giesser or myself depending on who was available after existing filing priorities had been completed, inasmuch as following Mr. Weinfeldt's departure, a backlog in unfiled HMG-CoA reductase disclosures had been developing.

5. It is noted that the Sandoz U.S. filings in the HMG-CoA reductase area commenced in about 1982 and continued into 1991. For example, a representative list of Sandoz original (including CIP) U.S. patent application filings in the HMG-CoA reductase inhibitor area comprises the following:

Case 600-6951 filed Nov. 22, 1982 (abandoned)
Case 600-6951/B filed Nov. 4, 1983 (R60 of which) issued as U.S. 4,739,073 (1988)
Case 600-6951/C filed Nov. 22, 1982 (pending)
Case 600-7013 filed June 4, 1984 now U.S. 4,588,715 (1986)
Case 600-7015 filed June 22, 1984 (abandoned)
Case 600-7022 filed Dec. 4, 1984 (abandoned)
Case 600-7025 filed Apr. 12, 1985 (abandoned)
Case 600-7028 filed May 22, 1985 now U.S. 4,668,794 (1988)

Case 600-7015/B filed June 6, 1985 now U.S. 4,613,610 (1986)
Case 600-7035 filed Oct. 25, 1985 (abandoned)
Case 600-7022/B filed Mar. 7, 1986 (abandoned)
Case 600-7041 filed Apr. 30, 1986 (abandoned)
Case 600-7028/B filed May 14, 1986 (R60 of which) issued as U.S. 4,755,606 (1988)
Case 600-7035/B filed Oct. 15, 1986 now U.S. 4,851,427 (1989)
Case 600-7050 filed Dec. 23, 1986 now U.S. 4,751,235 (1988)
Case 600-7025/ CIP filed May 5, 1987 (abandoned)
Case 600-7022/C filed Jul. 1, 1988 now U.S. 5,001,255 (1991)
Case 600-7025/ CIP/CIP/CIP filed Oct. 6, 1988 (abandoned)
Case 600-7025/ CIP/CIP/CIP/ CIP filed Jan. 16, 1990 (pending)
Case 600-7041/ CIP filed Mar. 6, 1987 (abandoned for R60)
Case 600-7064 filed Jan. 27, 1988 now U.S. 4,822,799 (1989)
Case 600-7041/ CIP/CIP filed Mar. 10, 1988 (abandoned)
Case 600-6955/ XN//B/CONT/X filed Mar. 10, 1988 now U.S. 4,876,1989 (1989)
Case 600-7087 filed Oct. 13, 1988 (abandoned)
Case 600-7101 filed Mar. 3, 1989 (abandoned for R60 cont.)
Case 600-7087/B filed May 8, 1989 (abandoned)
Case 600-7104 filed May 22, 1989 (abandoned)
Case 600-7041/ CIP/CIP/II filed Jul. 13, 1989 now U.S. 4,870,199
Case 600-7104/ CIP filed Feb. 20, 1990 (pending)
CAse 600-7087/C filed Sept. 5, 1990 (abandoned)
Case 600-7087/D filed Feb. 26, 1991 (pending)

Appendix Z hereto contains copies of the cover sheets of some of the above-indicated U.S. patents which issued on the above cases.

6. It is my best recollection that in February of 1988, I was in communication with Dr. Wattanasin concerning information

which was needed by the Patent Department in order to prepare an application based PD 299/84. (The application that was subsequently filed was designated as, and is referred to herein as, "Case 600-7101".)

Exhibit N hereto comprises a true copy of a page containing my handwritten notations concerning Case 600-7101 and a handwritten date of February 12, 1988.

These notes comprise a checklist of information items which needed to be developed or confirmed in order to draft Case 600-7101. The fact that these notes were made on the reverse side of the second attachment page to PD 299/84; and furthermore, that paragraph 2 discusses the scope of the disclosure and in sub-paragraph (c), refers to "other substitu [sic] on the quinoline ring," indicates their pertinence to the involved Wattanasin application.

These notes further indicate that I spoke with Sompong Wattanasin ("S.W.") on February 12, 1988 concerning his quinoline compounds and requested that he provide me with certain information.

7. On or about March 1, 1988, I received from Dr. Wattanasin certain reaction schemes which were to be included in case 600-7101.

Exhibit O comprises a copy of material which I received from Dr. Wattanasin for the preparation of Case 600-7101. This shows two different reaction routes to preparing quinoline compounds of the case.

8. It was my practice to request the Sandoz Biology Department to send me IC_{50} and ED_{50} values for compounds I was planning to cover in a patent application, as well as other biological information necessary to properly draft a patent application directed to a pharmaceutical.

Exhibit Q hereto comprises a Biological Data Report and computer printout which I received from the Sandoz Biology Department. The Wattanasin disclosure number, i.e. "299/84" is written in my handwriting on the front page, and the compounds of Patent Disclosure 295/84 as well as PD 299/84 are included in the printout.

The printout bears a date of May 23, 1988.

9. On July 1, 1988 I filed Case 600-7022/C based on PD 295/84, which was indicated for filing ahead of PD 299/84.

Exhibit R hereto comprises a copy of the front page of U.S. Patent No. 5,001,255, which issued on Case 600-7022/C, and indicates a filing date of July 1, 1988.

10. With reference to **Exhibit Y-2**: page 2 of this computer printout bears a date of January 11, 1989 written in my handwriting.

11. At no time subsequent to the "A" rating of Patent Disclosure 299/84 did I or, insofar as I am aware, any other member of the Patent and Trademark Department of Sandoz Corporation, ever have any intention not to file a United States patent application on the quinoline compounds of said patent disclosure in due course.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this 19th day of February, 1993.

Melvyn M. Kassenoff

MELVYN M. KASSENOFF

Case No. 600-7101/CONT/INT.(3)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN

v.

FUJIKANA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

DECLARATION OF JOANNE M. GIESSER PURSUANT TO 37 CFR §1.672

I, Joanne M. Giesser, do hereby declare as follows:

1. All of the below-indicated activities took place in the United States.

2. I was employed by Sandoz Corporation as a patent attorney from August 16, 1987 to November 6, 1992, and during the time periods referred to herein was a member of the Patent and Trademark Department located in East Hanover, New Jersey. (On September 1, 1992, I transferred to the patent department of the Sandoz Crop Protection affiliate of Sandoz Corp. in Palo Alto, California.) I am currently employed as a patent attorney for Amoco Corporation in Naperville, Illinois.

3. I filed the involved Wattanasin continuation application, and I also drafted and filed the parent application thereof, Serial No. 07/318,773 filed on March 3, 1989. As of its filing date, the '773 application received internal docketing number 600-7101, and is hereinafter referred to as "Case 600-7101".

4. Case 600-7101 is based on Patent Disclosure No. 299/84 of Dr. Sompong Wattanasin.

5. At the January 27, 1988 meeting of the Sandoz Corporation Patent Committee, said PD 299/84 was rated "A" for filing. I would have received a copy of the Minutes of the meeting sometime in February 1988.

6. PD 299/84 was assigned to me, although Mr. Kassenoff of the Patent Department and I intended that the case would be filed by either one of us depending on who was available after existing filing priorities had been completed.

7. I received certain materials from Dr. Wattanasin in connection with the filing of Case 600-7101.

Exhibit P comprises a copy of material which the Patent Department received which related to the preparation of Case 600-7101. These materials comprise:

P-1: 4 pages containing handwritten reaction schemes and notes bearing the handwritten name of "S. Wattanasin" and a date of February 29, 1988 on the first page;

P-2: 7 pages of computer printouts of specific compounds containing handwritten notations of the Notebook pages on which they were prepared and relevant physical properties; and

P-3: 9 laboratory notebook pages numbered 130, 137, 145, 153, 158, 166, 172, 175 and 176.

8. When I received the pages which comprise **Exhibit P**, I made handwritten annotations on some of the pages, which appear on the pages of the Exhibits.

9. It will be noted that in the calendar year 1988, I compiled an airline travel mileage of approximately 75,000 miles. My travel and entertainment expense reports for the period of February 1, 1988 to March 3, 1989, indicate that I was required to be out of the office on business on at least the following dates:

February 21-26.
March 1, 15-16, 20 and 28-31.
April 20-22.
May 2
June 15-16, 24
July 12
August 29-31
September 1, 10-14
October 9-11, 16-17, 27-28
December 6-8
January 8-12
February 21, 28
March 1-2

Exhibit S hereto comprises true copies of travel and entertainment expense reports which I filled out and submitted to the Sandoz Travel Department to obtain reimbursement of my business travel expenses. Each of these reports is in my handwriting and bears my true signature.

10. No later than October 1988, I would have started writing a draft of Case 600-7101.

11. On November 6, 1988, I filed continuation-in-part application, Case 600-7025/CIP/CIP (Serial No. 07/466,083), which was indicated for filing ahead of PD 299/84.

Exhibit T hereto comprises a copy of the filing receipt for Case 600-7025/CIP/CIP/.

12. In early November of 1988, my secretary, Ms. Lorraine M. Chesley, began typing a draft of Case 600-7101.

Exhibit U-1 hereto appears to comprise a copy of the label of the computer disc on which this application is stored, which indicates a starting date of November 3, 1988 and a mailing date of March 3, 1989.

13. Also in about November of 1988, I received a memorandum from Dr. Wattanasin which outlined certain synthesis steps for preparing compounds of Case 600-7101.

Exhibit U-2 comprises a memorandum received from Dr. Wattanasin by the Patent Department, which comprises a cover page and 8 pages containing synthesis steps for preparing compounds covered by PD 299/84.

This memorandum bears a handwritten date of November 7, 1988 and was date stamped November 8, 1988 by the Patent Deparment.

14. On or before November 8, 1988, I requested Mr. Siegfied S. Warhman of Sandoz Information Services to provide correct nomenclature for various compounds of PD 299/84 and starting materials used in their synthesis.

Exhibit V-1 comprises a true copy of my handwritten request, which became the cover page of a responding memorandum from Mr. Henry Mah, also of Sandoz Information Services. The return memorandum is dated November 8, 1988; and the Patent Department date stamp on my request memo indicates that it was received by the Patent Department on November 9, 1988.

372

Exhibit V-2 is another memorandum which was received by the ınt Department from Mr. Henry Mah which bears a date of mber 14, 1988 and is also date stamped November 14, 1988, h provides further nomenclature of the quinoline compounds of PD 299/84 and their reaction intermediates.

15. On or about December 14, 1988, I sent a first draft of 600-7101 to Dr. Wattanasin for his review.

Exhibit W comprises a true copy of the cover letter for the application which I sent to Dr. Wattanasin.

15. Further information related to Case 600-7101 which is in ɔssession of the Patent Department comprises:

Exhibit X: which comprises four pages of reaction diagrams .ning notations some of which are written in my handwriting, ıe handwritten date of December 22, 1988.

xhibit Y-1: a handwritten memorandum of changes in a draft e 600-7101 bearing a date of January 4, 1989;

hibit Y-2: a computer printout of the structures of the ıds of PD 299/84, with handwritten IC50 and/or ED50 values ıandwritten date of January 4, 1989.

On March 3, 1989, I filed Case 600-7101, the parent tion of the involved Wattanasin application.

FE

FEB 19 '93 16:24 SANDOZ CORP. PAT. AND TM

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this 19 day of February, 1993.

Joanne M. Giesser

JOANNE M. GIESSER

Case No. 600-7101/CONT/INT.(4)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

DECLARATION OF LINDA ROTHWELL PURSUANT TO 37 CFR §1.672

I, Linda Rothwell, do hereby declare as follows:

All of the below-indicated activities took place in the United States.

1. I have been employed by Sandoz Pharmaceuticals Corporation continuously since 1968 to the present. My position, both currently and during the time periods indicated below, has been Patent Administrator of the Sandoz Patent Department.

2. One of my responsibilities as Patent Administrator has been to type or supervise the typing of the Minutes of each Sandoz Pharmaceutical Corp. Patent Committee Meeting based on notes taken at the meeting by the attending attorney(s). The Minutes serve as the official record for the Sandoz Patent Department of decisions and recommendations made at each Patent Committee Meeting (PCM).

3. Since prior to April 1987, another of my responsibilities as Patent Administrator has been to docket patent disclosures as soon as they are received by the Patent and Trademark Department, for consideration at the following scheduled PCM.

4. Patent Disclosure 299/84 was docketed for initial consideration by the Sandoz Pharmaceuticals Corp. Patent Committee at its April 29, 1987 Meeting.

5. According to Sandoz policy which has been in effect since prior to April 29, 1987, a disclosure which is considered by the Patent Committee and is rated "B", is deferred for reconsideration by the Patent Committee within three months' time. An "X"- rated disclosure is deferred for reconsideration by the Patent Committee within one month's time. A "B" or "X" rating is given when further information is needed before making a decision whether to file a patent application. An "A"- rated disclosure represents a decision to file a patent application on the subject matter of the patent disclosure.

Section 5 of the Minutes is devoted to the rating of newly submitted Patent Disclosures or the re-rating of previously rated Patent Disclosures.

6. **Exhibits M-1** to **M-5** appended hereto comprise copies of pages of Patent Committee Minutes prepared in the ordinary course of business by me or under my supervision. Confidential material unrelated to PD 299/84 has been masked. These are true copies with respect to the unmasked material.

The Minutes are maintained under my supervision and control in the files of the Sandoz Patent and Trademark Department in the ordinary course of my employment.

Exhibit M-1 is a masked copy of page 2 of the minutes of the Sandoz Pharmaceuticals Corp. PCM held on Wednesday, April 29, 1987. This page shows that Patent Disclosure 299/84 was rated "B," and was assigned to Frederick H. Weinfeldt ("FHW"), a senior patent attorney in the Sandoz Patent Department.

Exhibit M-2 is a masked copy of page 3 of the minutes of the PCM held on Wednesday, July 29, 1987. This page shows that PD 299/84 was re-rated "B".

Exhibit M-3 is a masked copy of page 3 of the minutes of the PCM held on October 28, 1987. This page shows that PD 299/84 was rated "X".

Exhibit M-4 is a masked copy of page 2 of the minutes of the PCM held on Wednesday, November 25, 1987. This page shows that PD 299/84 was rated "X".

Exhibit M-5 is a masked copy of page 4 of the minutes of the PCM held on Wednesday, January 27, 1988. This page shows that PD 299/84 was rated "A," and was re-assigned to Mrs. Joanne M. Giesser, a patent attorney in the Sandoz Patent Department.

The Patent Department records indicate that no later than about April 1987, Mr. Weinfeldt had taken permanent disability leave (and is now deceased). In August of 1987, Mrs. Giesser joined the Patent Department and assumed a part of Mr. Weinfeldt's docket.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing DECLARATION this 19th day of February, 1993.

Linda Rothwell

LINDA ROTHWELL

Case No. 600-7101/CONT/INT.(5)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

SUPPLEMENTAL DECLARATION OF ROBERT G.ENGSTROM PURSUANT TO 37 CFR §1.672

I, Robert G. Engstrom, do hereby declare as follows:

All of the below-indicated activities took place in the United States.

Exhibit Q comprises a true copy of a Biological Activity Data Report dated May 24, 1988 which I sent to the Patent Department concerning the compounds of PD 299/84, together with a computer printout of the Sandoz database dated May 23, 1988. The printout contains IC_{50} and some ED_{50} values for compounds of Patent Disclosure 295/84 and compounds of the subject Patent Disclosure 299/84.

(I note that I became aware of a computer entry error comprising the inadvertent "switching" of the ED_{50} data for compounds 64-933 and 64-935. The corrections on the printout are in my handwriting and would have been made on or about May 23, 1988.)

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful

false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this 19 day of February, 1993.

Robert Engstrom

Robert Engstrom

Case No. 600-7101/CONT/INT.(6)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

DECLARATION OF LORRAINE M. CHESLEY PURSUANT TO 37 CFR §1.672

I, Lorraine M. Chesley, do hereby declare as follows:

1. All of the below-indicated activities took place in the United States.

2. I have been employed as a secretary in the Patent and Trademark Department of Sandoz Corporation since August 6, 1984 to the present. My current position is Senior Administrative Secretary.

2. I was Mrs. Joanne Giesser's secretary from 1987 to 1991.

3. **Exhibit U-1** hereto comprises a true copy of a computer disc label which is written in my handwriting, indicating that I started typing Case No. 600-7101 on November 3, 1988 and that the case was mailed to the Patent and Trademark Office on March 3, 1989.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing DECLARATION this 19th day of February, 1993.

Lorraine M. Chesley

LORRAINE M. CHESLEY

Exhibit M

DR. D. CORNISH	DR. J. NADELSON	MR. J. BOROVIAN	MR. T. MC GOVERN
DR. J. FOLEY	DR. L. SALANS	MR. T. DOYLE	MRS. L. ROTHWELL
DR. G. HARDTMANN	DR. R. SAUNDERS	MR. R. HONOR	MR. G. SHARKIN
DR. W. HOULIHAN	DR. D. WEINSTEIN(2)	MR. W. JEWELL	MR. R. VILA
DR. F. KATHAWALA	DR. D. WINTER	MR. M. KASSENOFF	MR. F. WEINFELDT
BASLE (2)			

MINUTES

PATENT COMMITTEE MEETING

HELD WEDNESDAY, APRIL 29, 1987

* *

3. NOTICES OF ALLOWANCE:

3.1 Th
a
13
in-part;

3.2 Th
th
65
respecting

4. FINAL REJECTIONS:

4.1 T

5. DISCLOSURES:

5.1 The following disclosure has been rated "A":

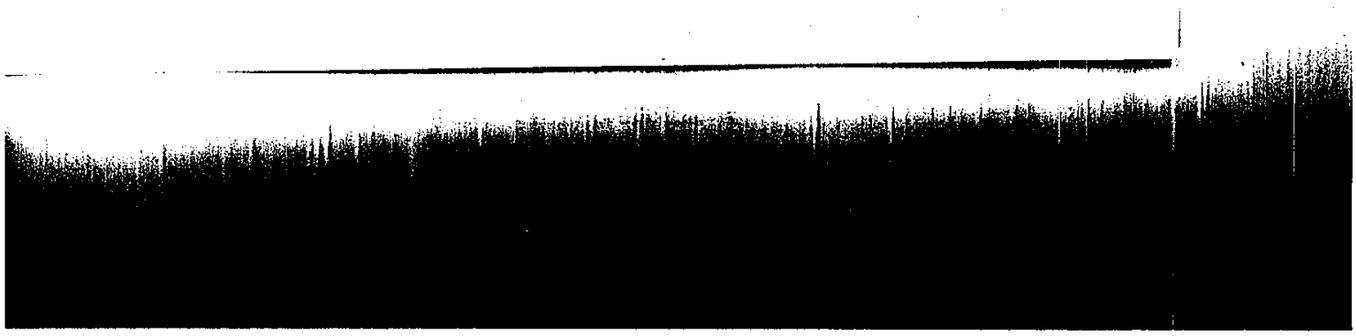
5.2
"A" and a
matter from a
U.S. patent
considering
tent
a separate
ter in due

5.3 The following disclosures have been rated "X":

5.4 The following disclosures have been rated "B":

299/84 WATTANASIN FHW

5.5



DR. D. CORNISH	DR. L. OSTBERG	MR. J. BOROVIAN	MR. T. MC GOVERN
DR. J. FOLEY	DR. L. SALANS	MR. T. DOYLE	MRS. L. ROTHWELL
DR. G. HARDTMANN	DR. R. SAUNDERS	MR. R. HONOR	MR. G. SHARKIN
DR. W. HOULIHAN	DR. D. WEINSTEIN(2)	MR. W. JEWELL	MR. R. VILA
DR. F. KATHAWALA	DR. D. WINTER	MR. M. KASSENOFF	MR. F. WEINFELDT
DR. J. NADELSON	BASLE (2)		

MINUTES

PATENT COMMITTEE MEETING

HELD WEDNESDAY, JULY 29, 1987

* *

5. DISCLOSURES:

5.1 The following disclosures are rated "X":

5.2 The following disclosures are rated "B":

299/84 WARRANASIN FHW

. D. CORNISH	DR. L. OSTBERG	MR. J. BOROVIAN	MR. T. MCGOVERN	
. J. FOLEY	DR. L. SALANS	MR. T. DOYLE	MRS. L. ROTHWELL	
. G. HARDTMANN	DR. R. SAUNDERS	MRS. J. GIESSER	MR. G. SHARKIN	
. W. HOULIHAN	DR. D. WEINSTEIN(2)	MR. R. HONOR	MR. R. VILA	
. F. KATHAWALA	DR. D. WINTER	MR. W. JEWELL	MR. F. WEINFELDT	
. J. NADELSON	BASLE (2)	MR. M. KASSENOFF		

MINUTES OF THE

PATENT COMMITTEE MEETING

HELD WEDNESDAY, OCTOBER 28, 1987

* *

5.3 The following disclosures are rated X.

299/84 WATTANASIN FHW

5.4 The following disclosures are rated B.

FHV:lmc
:./6/87

DR. D. CORNISH	DR. L. OSTBERG	MR. J. BOROVIAN	MR. M. KASSENOFF
DR. J. FOLEY	DR. L. SALANS	MR. T. DOYLE	MR. T. MC GOVERN
DR. G. HARDTMANN	DR. R. SAUNDERS	MRS.J. GIESSER	MRS.L. ROTHWELL
DR. W. HOULIHAN	DR. D. WEINSTEIN(2)	MR. R. HONOR	MR. G. SHARKIN
DR. F. KATHAWALA	DR. D. WINTER	MR. W. JEWELL	MR. R. VILA
DR. J. NADELSON	BASLE (2)		

MINUTES

PATENT COMMITTEE MEETING

HELD WEDNESDAY, NOVEMBER 25, 1987

* *

1A. FOREIGN FILINGS:

---TINGS. (Cont.)

5. DISCLOSURES:

5.1 The following disclosures are rated "X":

	299/84	WATTANASIN	FHW
	6		
	1		
	8		
	9		

DR.	D. CORNISH	DR.	L. OSTBERG	MR.	J. BOROVIAN	MR.	T. MCGOVERN
DR.	J. FOLEY	DR.	L. SALANS	MR.	T. DOYLE	MRS.	L. ROTHWELL
DR.	G. HARDTMANN	DR.	R. SAUNDERS	MRS.	J. GIESSER	MR.	G. SHARKIN
DR.	W. HOULIHAN	DR.	D. WEINSTEIN(2)	MR.	R. HONOR	MR.	R. VILA
DR.	F. KATHAWALA	DR.	D. WINTER	MR.	W. JEWELL		
DR.	J. NADELSON	BASLE (2)		MR.	M. KASSENOFF		

MINUTES OF THE

PATENT COMMITTEE MEETING

HELD WEDNESDAY, JANUARY 27, 1988

* *

1.A. FOREIGN FILINGS:

5. DISCLOSURES:

5.1 The following disclosures are rated A.

.lity search
formed).

.ction
only).

299/84 WATTANASIN JMG

REV:lmc

Exhibit N

want:

1) Typical example or lab notebook pages
2) Scope
   a) R_1 $R_{1'}$ R_2
   b) (E)-CH=CH- replacements
   c) other substituents on quinoline ring
3) Which compds are known
4) Process condns for AA-AD
5) Unusual condns for std rxns
6) Anything unusual - Lab, Standard rxns that didn't work
7) Complete list of end products + NMR (tabulated) spectrum, mp. and isomeric compn of each

Scope same as naphthalene

X = (Z) or (E)-CH=CH $-CH_2CH_2-$, $-CH_2-$, $-(CH_2)_3-$

Substituents on alkyl
alkoxy
OH
OCH_2O
CF_3
halo

Spoke with S.W. 2-12-88; Requested info will be sent

Route II

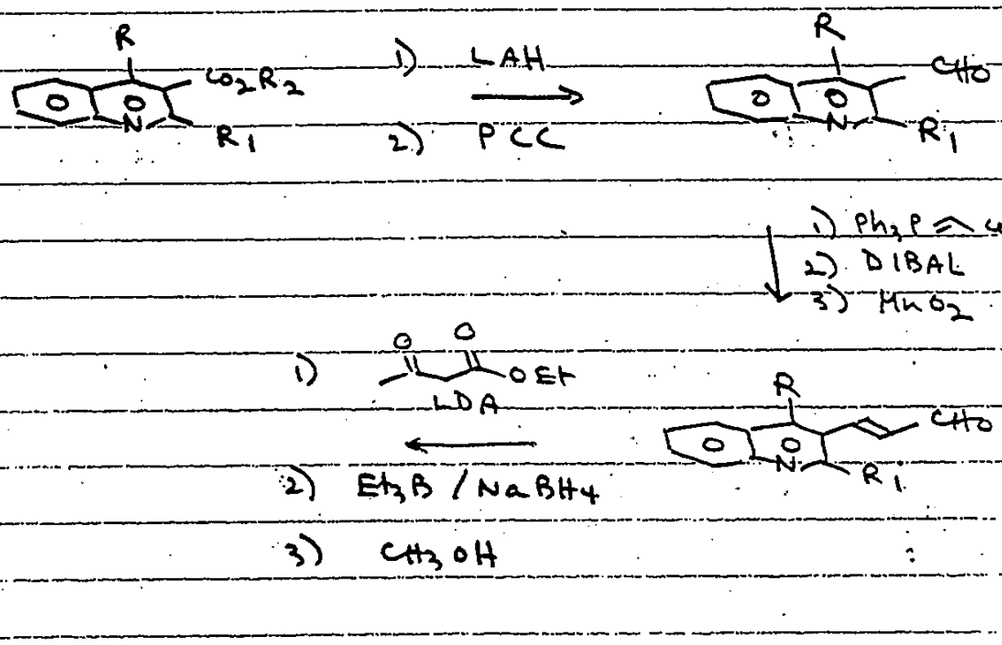

R
CO_2R_2
R_1
1) LAH
2) PCC
CHO
1) $Ph_3P=CO_2CH_3$
2) DIBAL
3) MnO_2
1)
OEt
LDA
2) Et_3B / $NaBH_4$
3) CH_3OH
I

Exhibit O

sent to

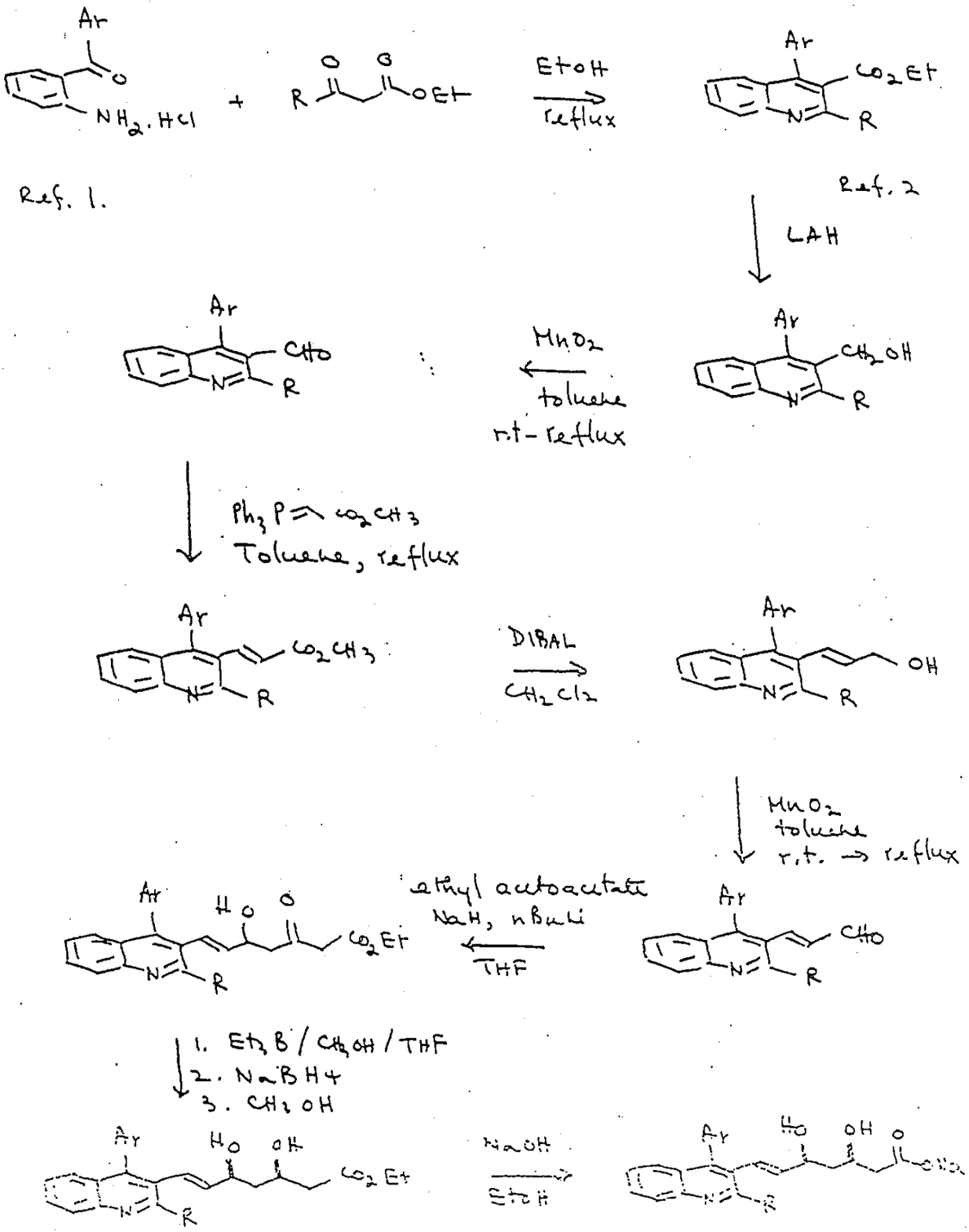
M. Kasenoff.

2/29/88.

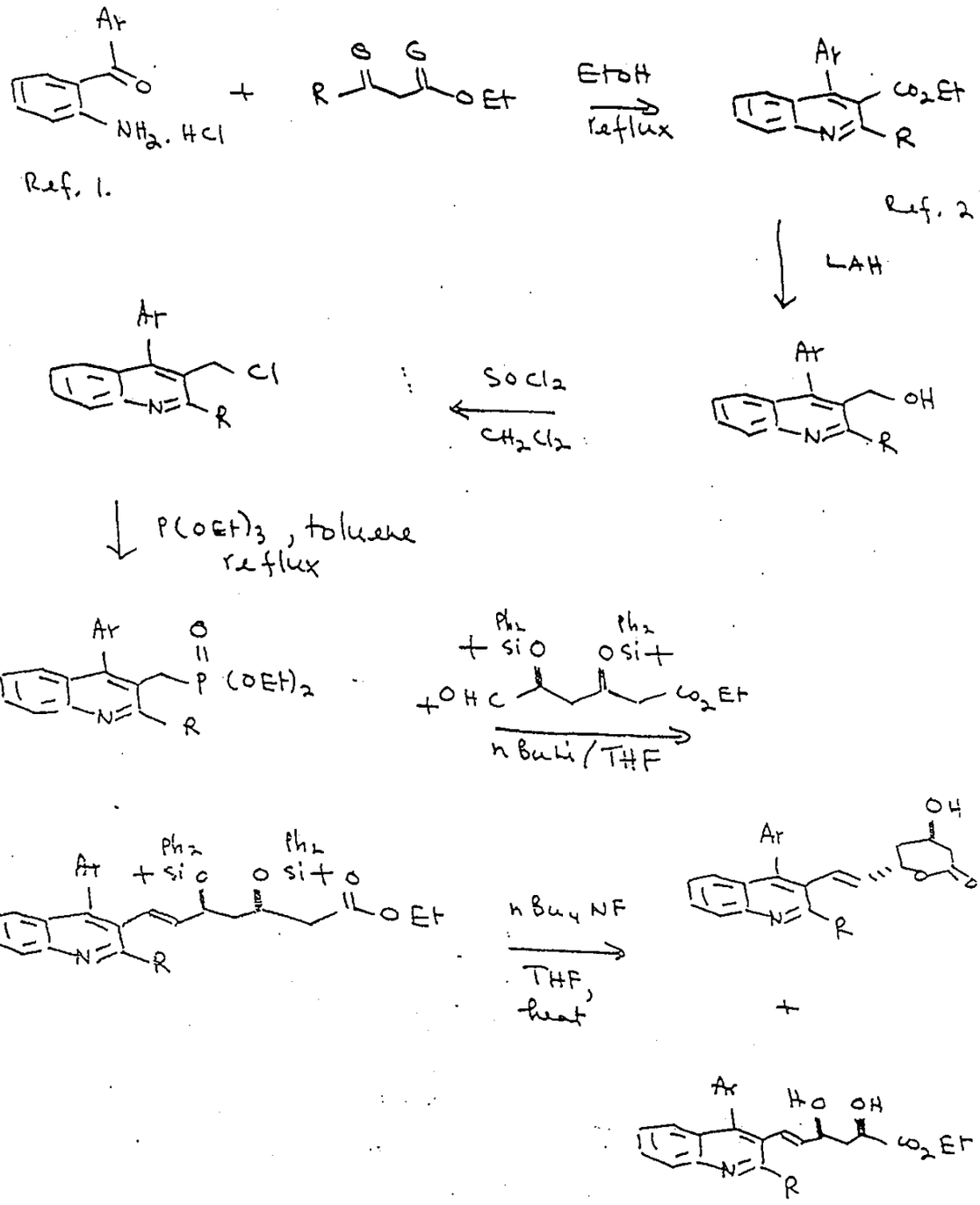
S. Wattanasin.
2/29/88

394

SCHEME I



SCHEME 2

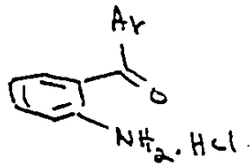

Ar
NH2. HCl
R
OEt
EtOH
reflux
CO2Et
N
R
Ref. 1.
Ref. 2
LAH
OH
SOCl2
CH2Cl2
Cl
P(OEt)3, toluene
reflux
P (OEt)2
Ph2
Si
OHC
nBuLi / THF
nBu4NF
THF,
heat

References + Notes

1. A. Morrison and T.P. C. Mulholland, J. Chem. Soc. 2702 (1958)

2. E.A. Fehnel J. Heterocyclic Chem. 4, 565 (1968).

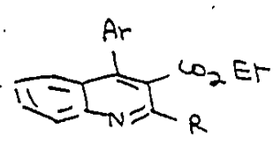
3. The starting aminoketones 1 are known compounds and prepared according to



1 Ar = Phenyl
= 3,5-dimethylphenyl
= p-Fluorophenyl

a procedure described in ref. 1.

4. The quinolines 2 were prepared by a modified procedure of ref. 2.



2

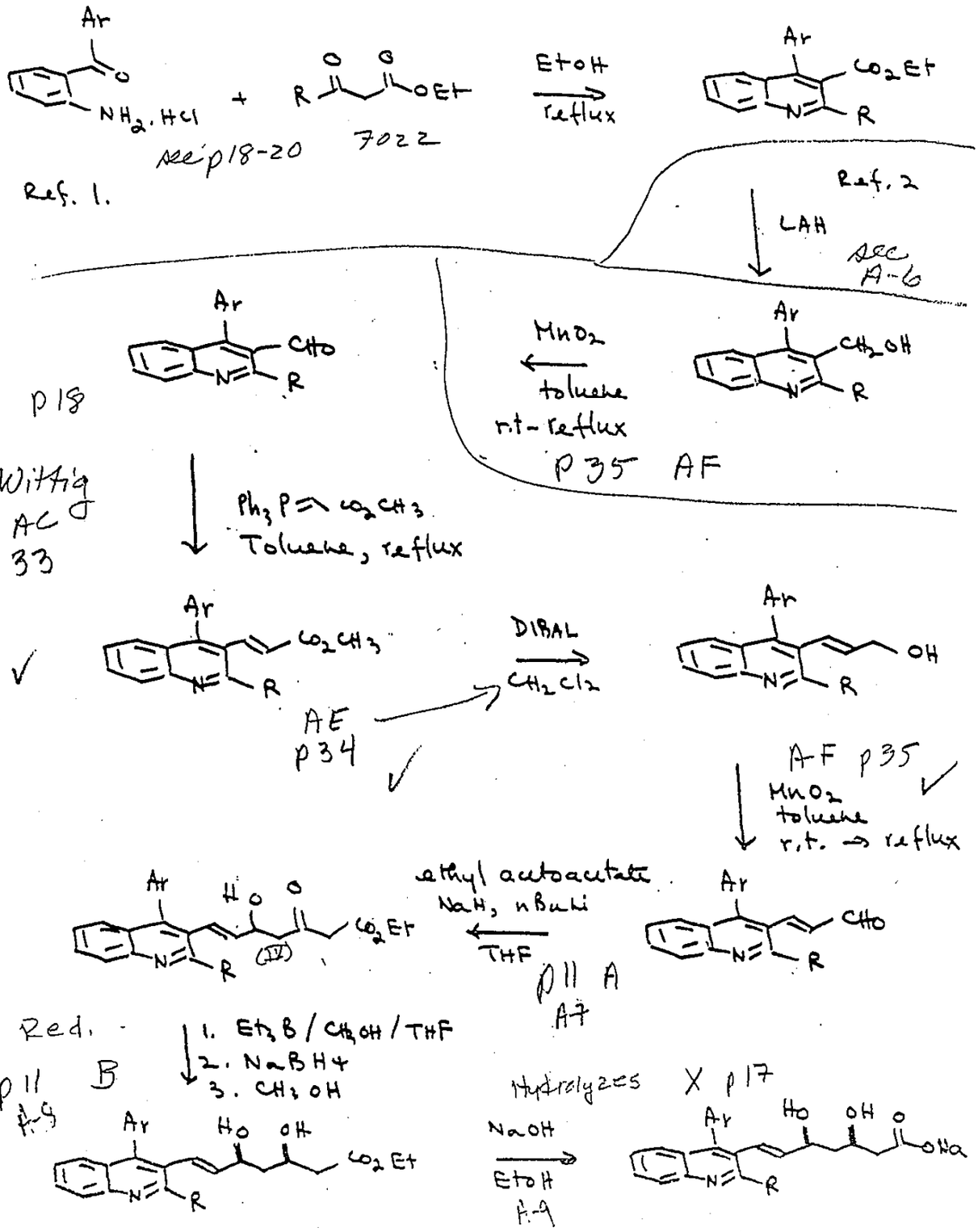
5. According to a search, only quinoline 2 where Ar = Ph and R = CH_3 is known

Exhibit P

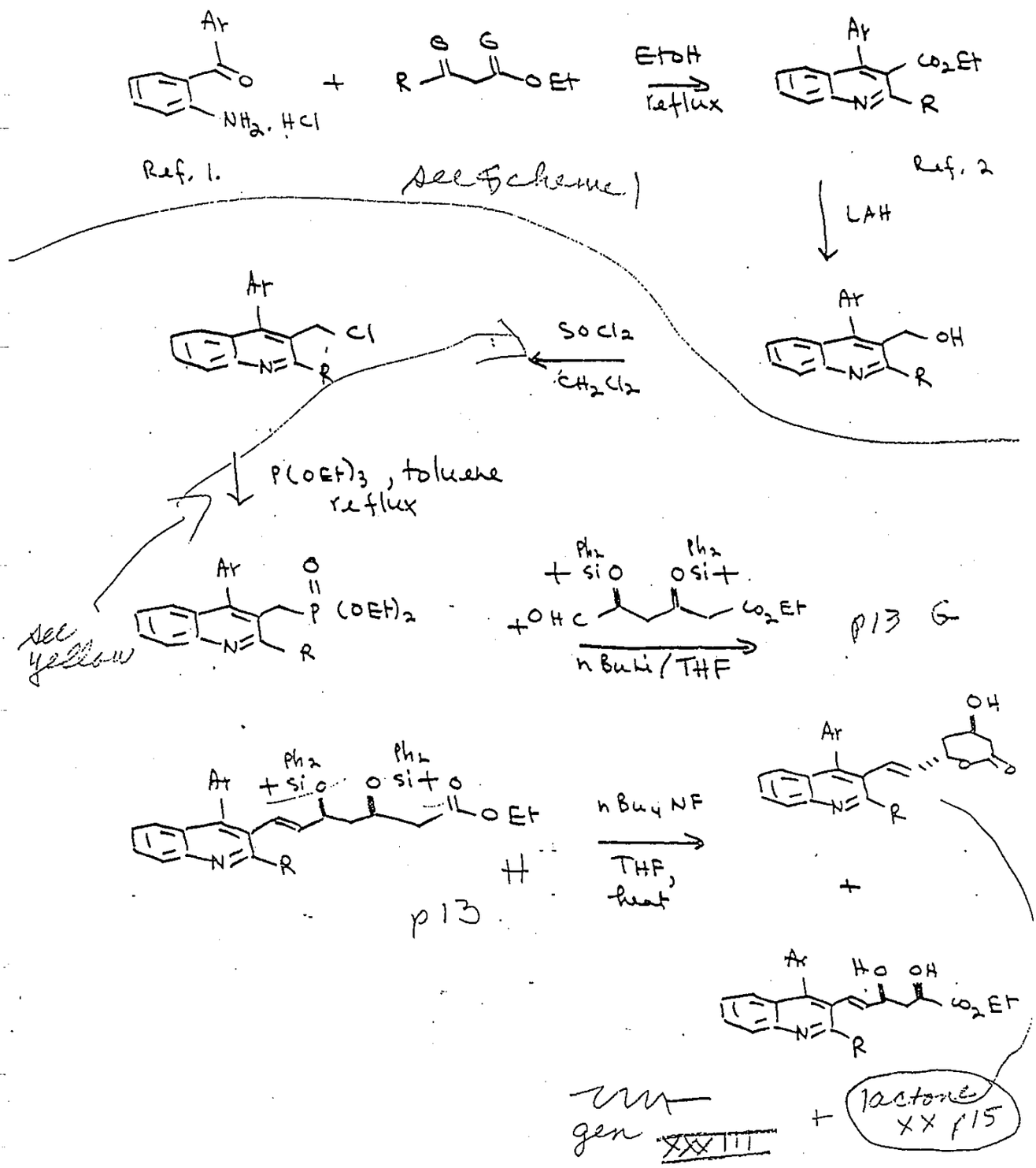
see 7022 C

S. Wattanasin, 2/29/88. 398

SCHEME I



SCHEME 2

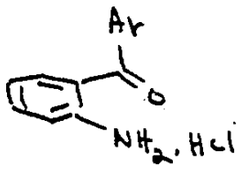

Ar
NH2. HCl
R
OEt
EtOH
reflux
CO2Et
Ref. 1.
(see Scheme 1)
Ref. 2
LAH
OH
Cl
SOCl2
CH2Cl2
P(OEt)3, toluene
reflux
P(OEt)2
see yellow
Ph2
Si
nBuLi / THF
p13 G
nBu4NF
THF, heat
p13
lactone
XX p15
gen XXIII

References + Notes

1. A. Morrison and T.P. C. Mulholland, J. Chem. Soc. 2702 (1958)

2. E.A. Fehnel J. Heterocyclic Chem. 4, 565 (1968).

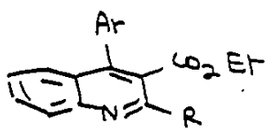
3. The starting aminoketones 1 are known compounds and prepared according to



1 Ar = Phenyl
= 3,5-dimethylphenyl
= p-Fluorophenyl

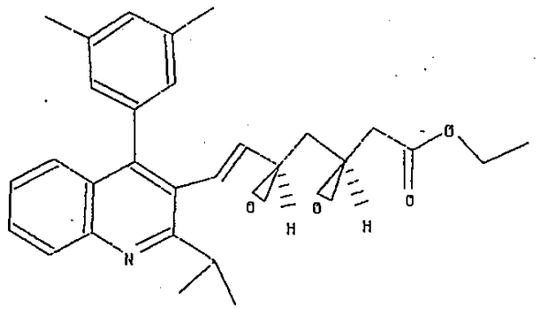
a procedure described in ref. 1.

4. The quinolines 2 were prepared by a modified procedure of ref. 2.



2

5. According to a search, only quinoline 2 where Ar = Ph and R = CH_3 is known.

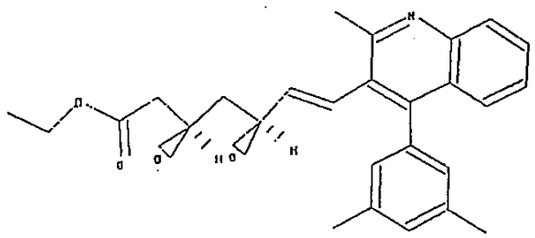
STRUCTURE	DATA
	– Notebook # 1079 – 111 – 19 – oil – erythro : threo ≈ 95 : 5 – nmr 0.9 (t, 3H) 1.6 (d , 6H) 2.1 (m , 2H) 3.7 (m, 1H) 3.7 – 4.0 (m, 3H) 4.2 (m, 1H) 5.4 (q , 1H) 6.6 – 7.7 (m, 8H) 8.4 (d, 1H) – prepared according to SCHEME 1.

SAH.NO	REG.NO	MW	CHEMIST	DATE
SAH-063366	25496	461.606	KATHAWALA WATTANASIN	11-26-84

Ex BM 3A

402

STRUCTURE



DATA

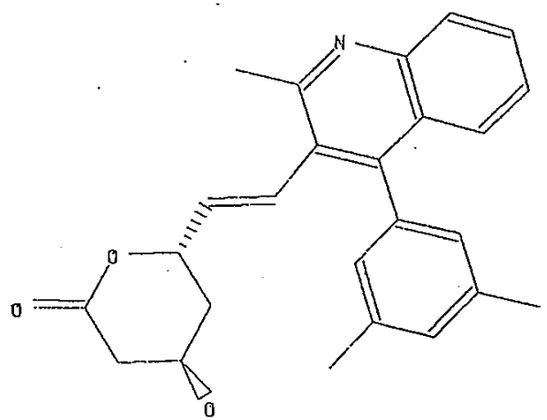
- Notebook # 1127-11-34
- oil
- erythro: threo ~ 95 : 5
- nmr
  - 1.3 (t, 3H)
  - 2.4 (m, 5H)
  - 4.1 (m, 1H)
  - 4.2 (q, 2H)
  - 4.4 (m, 1H)
  - 5.5 (q, 1H)
  - 6.5 (d, 1H)
  - 6.7 - 8 (m, 7H)
- prepared according to SCHEME2

SAH.NO	REG.NO	MW	CHEMIST	DATE
SAH-063548	26080	433.552	KATHAWALA WATTANASIN	05-17-85

Ex 3E

403

STRUCTURE



DATA

- Notebook # 1127-11-37
- oil
- cis : trans lactone ~ 5 : 95
- nmr

2.3 (s, 1H)
2.5-2.9 (m, 4H)
4.1 (m, 1H)
5.1 (m, 1H)
5.5 (q, 1H)
6.6 (d, 1H)
6.8-8.0 (m, 7H)

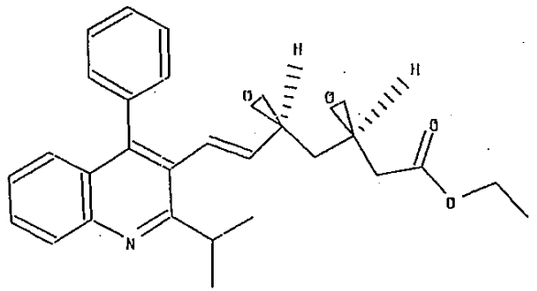
- prepared according to SCHEME 2.

SAH.NO	REG.NO	MW	CHEMIST	DATE
SAH-063519	26082	387.483	KATHAWALA WATTANASIN	05-17-85

Ex 2

404

STRUCTURE



DATA

- Note book # 1206-176-43
- m.p. 104-106°C
- erythro : threo ≥ 95:5
- nmr
  1.3 (t, 3H)
  1.35 (d, 6H)
  2.35 (m, 1H)
  2.9 (d, 1H)
  3.6 (d, 1H)
  3.5 (m, 1H)
  4.0 (m, 1H)
  4.12 (q, 2H)
  4.35 (m, 1H)
  5.35 (q, 1H)
  6.6 (d, 1H)
  7.1 - 7.7 (m, 8H)
  8.1 # (d, 1H)
- prepared according to SCHEME 1. *

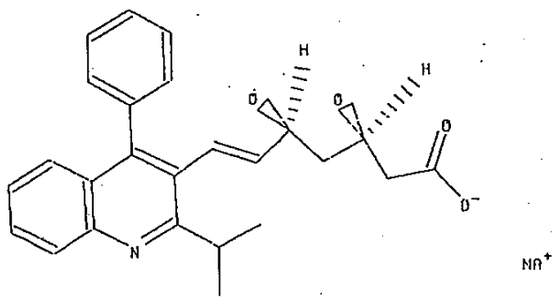
SAH.NO	REG.NO	MW	CHEMIST	DATE
SAH-064933	30441	433.552	PATEL WATTANASIN	09-21-87

* A copy of the note book of all steps is attached.

Ex 1

405

STRUCTURE



DATA

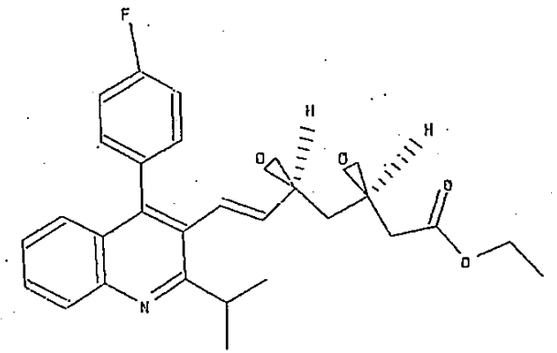
- Notebook # 1206-179-30
- m.p. > 216°C
- erythro : threo ≥ 95:5
- nmr
  - 1.4 (d, 6H)
  - 2.2 (m, 2H)
  - 3.6 (m, 1H)
  - 3.8 (m, 1H)
  - 4.2 (m, 1H)
  - 5.4 (q, 1H)
  - 6.6 (d, 1H)
  - 7.1-7.7 (m, 8H)
  - 8.1 (d, 1H)
- prepared according to SCHEME I.

SAH.NO	REG.NO	MW	CHEMIST	DATE
SAH-064934	30442	427.48	PATEL WATTANASIN	09-21-87

Ex 3B

406

STRUCTURE



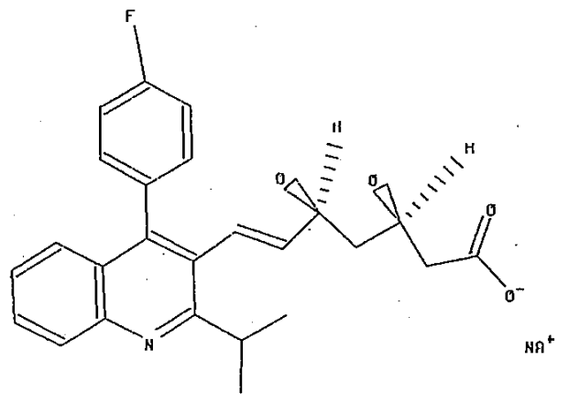
DATA

- Notebook # 1206 - 190 - 41
- oil
- erythro : threo ~ 95 : 5
- nmr
  1.3 (t, 3H)
  1.4 (dd, 6H)
  2.4 (m, 2H)
  3.1 (d, 1H)
  3.5 (m, 1H)
  3.6 (m, 1H)
  4.1 (m, 1H)
  4.2 (q, 2H)
  4.4 (m, 1H)
  5.4 (q, 1H)
  6.6 (d, 1H)
  7.0 - 7.4 (m, 7H)
  7.6 (m, 1H), 8.1 (d, 1H)
- prepared according to SCHEME 1.

SAH.NO	REG.NO	MW	CHEMIST	DATE
SAH-064935	30447	451.543	PATEL WATTANASIN	09-21-87

Ex. 3C

407

STRUCTURE	DATA
	— Notebook # 1206-201-30 — mp > 225°C. — erythro : threo ≥ 95 : 5 — nmr 1.3 (d, 6H) 2.2 (m, 2H) 3.6 (m; 1H) 3.8 (m, 1H) 4.25 (m, 1H) 5.5 (q, 1H) 6.6 (d, 1H) 7.3–7.4 (m, 7H) 7.6 (m, 1H) 8.1 (d, 1H) — prepared according to SCHEME 1.

SAH. NO	REG. NO	MW	CHEMIST	DATE
SAH-064936	30448	445.47	PATEL WATTANASIN	09-22-87

EX 3D

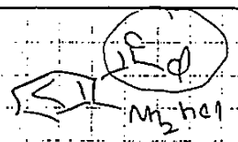
408

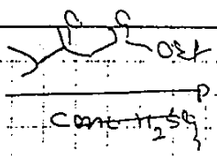
Title-

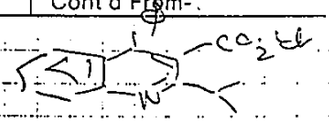
Date 6-15 Proj.

Cont'd From-

409







5233-24 120G-129-18

319.44
$C_{21}H_{21}NO_2$

233.24 (120G-129-18) = 11.5 g (0.04930 mole)

(0.0980) 1.582) = 11.93 ml (0.07395 81 mM) - Equiv.

Etoh = 10 ml + 5 ml

Conc H_2SO_4 = 2.5 ml

Ref : 120G-92

Above mixt was heated to reflux (10.7 - 4pm) stirred at r.t overnight

Ex

Evap

Evaporated to dryness to yellow oil basified with NH_4OH, extracted with Et_2O, washed with H_2O, brine, dried, filtered, washed, rotavap gave 10.21 g Orange-Yellow solids (120G-130-22)

mmole, 'n', wt g → mmt = 320

Theory: 15.748 g , % = 64.86

Performed by- Roy Patel 6-5-15

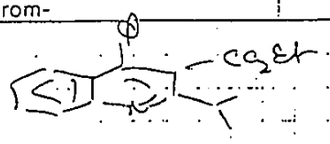
Witness- S. Wattamwar

Cont'd to-

Date 6-9-87 Proj. Title-

Cont'd From-

410

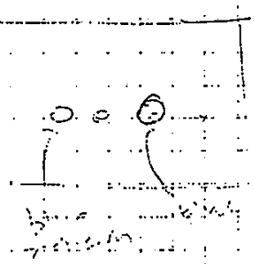


319.44
120G-130-27

277.44
$C_{21}H_{21}NO_2$

(319.44) 120G-130-27 = 10.21g (0.0319621 mole)
(38) LAH = 2.43g (0.0639242 mole)
Tol dry ether = 100ml
Ref: 120G-96

To 120G-130-27 in dry ether with cooling was added LAH portionwise, exothermic foaming, stirred at r.t. for 3hr C9 -12



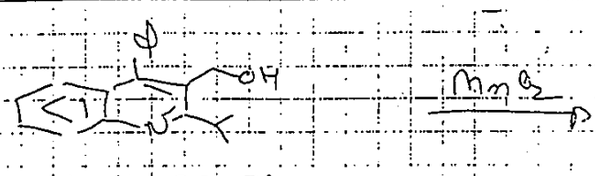
Rx mix. poured in ice H_2O (exothermic, strong Rx) Extracted with ether, washed with H_2O brine, dried, filtered, washed, rotavap to give yellow solids wt = 5.5g C120G-137-B1) mmt, tlc, ms

Theory: 8.86g (95.8%)

Performed by- Raj Patel 9-2-87

Witness- S. Wathen

Cont'd to-



1206-137-31
277.4

275.0
$C_{19}H_{17}NO$

277.4 1206-137-31 = ~~8.0~~ g (0.02889 2mM)
MnO_2 = 16.0 g
toluene = 150 ml

To 1206-137-31 in toluene was added MnO_2
→ heated to reflux (111° - 45° - 24°)

○	1.0	
○	x	Rf
○	x	SM

filtered thru pad of* silicagel, washed with toluene, rotovap to dryness, gave pale yellow solids: 2.6518 g (1206-145-25) nmr, ir, ms mH+ = 276 cleaved Rf
orange solids: 4.6463 g (1206-145-26) nmr, ir, ms mH+ = 278 S.M.

* During filtration, separated two bands, which was filtered separately & rotovap

Theory: 7.91 g (74.52%)

Total yield = 2.6518 g + 3.26 g = 5.91 g
(1206-145-25) (1206-148-33)

*

Performed by-

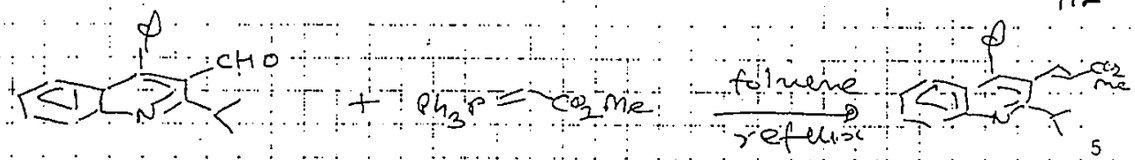
Witness- S. [illegible]

Cont'd to- 145

Date 6-30-0 / Proj. | Title-

Cont'd From-

412



275 331

(275) {1206-145-25, 1206-148-33} = 2.65+3.26 = 5.915g (0.021490 mole)

~~MeOH~~ = ~~11.8 g~~

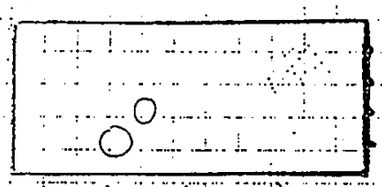
toluene = 85 ml + 20 ml

(334) $Ph_3P{=}CO_2Me$ = 8.6135g (0.025789 mole) 1.2 eq

Ref.: 1206-146

Above mix. was heated to reflux (yellow heterogeneous before heating) for 1½ hrs. stirred at r.t. overnight

7-1-87



Diluted with 50% Et2O/Pet ether filtered thru pad of silica gel

7-2-87 washed Rotavap to dryness to give yellow crystaline solid 8.60g: Triturate with MeOH gave off white solids (Theory: 7.113 g) wt = 5.5198 g (1206-153-31) 77.6%

nmr ir ms mp = 332

Rotavap mother liquor to dryness to yellow oil wt = 2.7593g (1206-153-34)

7-6-87 Trituration with MeOH gave 761.6mg light yellow solids (1206-153-37) nmr ms mp 332

Rotavap mother liquor to dryness to yellow solid (1206-153-38) ms

Total yield = 5.5198 + 0.7616 (1206-153-40)

7-9-87 m.p. = 128°-130°C

	C	H	N
373	6.98	4.37	
374	6.55	4.03	
375	6.42	3.75	

Performed by- Raj Patel 7-6-87

Witness- S. Wattanasin

Cont'd to-

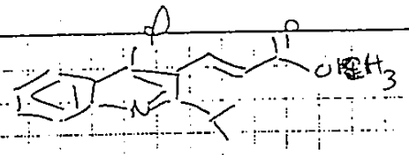
158

Title-

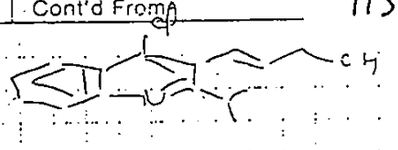
Date 7-7-87 Proj

Cont'd From

413



1.5M DIBAL-H



331

303

($C_{21}H_{21}NO$)

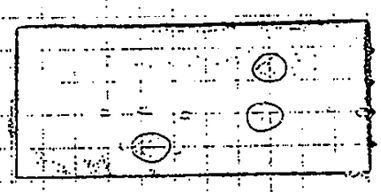
1206-153-40 = 6.259 g (0.0188821 mole)

1.5M DIBAL-H/toluene = 25.18 ml (0.0377642 mole) 2eqiv

CH_2Cl_2 = 75 ml

Ref: 1206-155, 87

To soln of 1206-153-40 in CH_2Cl_2 was added at -78°C 1.5M DIBAL-H/toluene, stirred at -78°C for 3 hrs (1hr - 3hr)



C	H	N	O
83.13 (84.625)			
82.05	6.81	3.9	
82.08	6.89	3.89	

quenched with 12.5 ml 2N NaOH, diluted with etoac stirred at r.t. overnight → lots of white (gel) solids came out.

7-8-87

filtered thru' pad of silica gel, washed with etoac, washed org. layer with H_2O (brine) dried rotavap to dryness gave off white solid: 5.429 (1206-158-35) Dissolved solids in Et_2O insolubles (white) (aluminium oxide) was filtered thru' sintered glass funnel rotavap to dryness gave white-yellow solids 5.229 (1206-158-37)

Theory: 5.729 g 73.7%

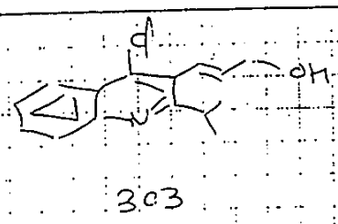
Dissolved solids in Et_2O, insoluble (aluminium oxide) was filtered rotavap to dryness gave yellowish solids = 4.211 g (1206-158-41) nmr, ir, ms, tlc nmr = 304 micro

m.p. = 119°-121°C

Performed by- Raj Patel 7-17-87

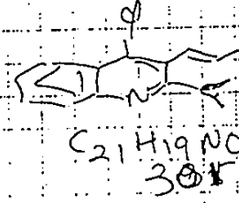
Witness-

Cont'd to-



303

MnO2

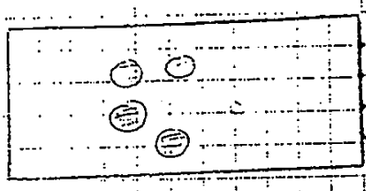


$C_{21}H_{19}NO$
301

1206-158-41 = 4.0g (0.0132013 mole
MnO2 = 8.0g
toluene = 50 ml

Ref: 1206-164

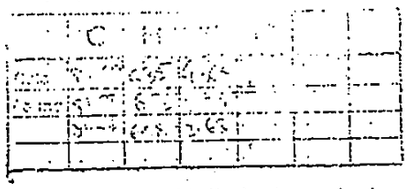
To 1206-158-41 in toluene added MnO2 & heated to reflux (2hr - 3hr), stirred at r.t. overnight



7-16-87

filtered thr' pad of silica gel, washed pad with ether, rotavap to dryness, gave 3.4946g yellow crystaline material (1206-166-30)
UV nmr ms mnt=302
Theory: 3.9736g (88%)

7-28-87 micro



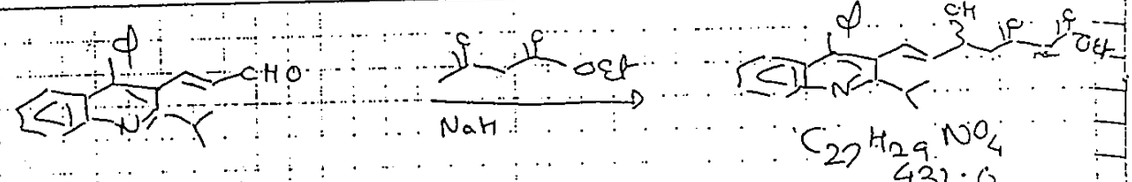
7-30-87 exact mass obs. mass = 302.15404 m.p. = 98-101°
Calc. mass = 302.15448

Performed by- Raheel 2209
Witness-
Cont'd to-

Title-

Date 1-2-88 Proj. 415

Cont'd From-



301

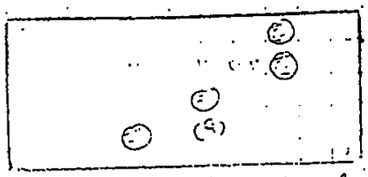
$C_{27}H_{29}NO_4$
431.6

301 1206-166-30 = 3.5g (0.011629 mole)
130.14, 1.021 Ethyl acetoacetate = 5ml (0.04 mole)
24 60% NaH =
1.6M n-Buli/Hex = 27ml
THF = 60ml + 40ml

To a soln of 1206-166-30 in dry THF (40ml) at -5° to -10°C was added a soln of dianion (11ml + 27ml)(38ml), prepared as described previously

Dianion (got from Dr. Som)

To soln of 5 ml Ethyl acetoacetate in 60ml dry THF was added 1.9 g 50% NaH at -5° to 0°C, stirred for 15 min (foaming H_2 evolved). At -10° - -15°C was added 27 ml 1.6M n-Buli/Hex, stirred for 20 min at -10°C → yellow homogeneous soln
Total vol = 92 ml (0.04 mole Used 48 38 ml dianion
= 0.01652 mole (1.4 equiv.) → color changed from yellow to orange to dark red
TLC (50% Et_2O/Pet) after 15 min ⟹ complete rxn



Rx was stirred for 30 min, quenched with NH_4Cl, extracted with EtOAc, washed with H_2O, brine, dried, filtered, rotavap gave yellow oil 5.9188 g (1206-172-41)
Theory: 5.01 g (67.8%)

Performed by- Raj Patel 2-2-88

Witness- S. Wattamwar

Cont'd to- 1206-175

Date 7/22/01 Proj. | Title-

Cont'd From- 120G-102

416

Flash chromatography (25% EtOAc/Hex) gave

(a) Yellow solids = 3.4004 g CR0G-175-43 mmm, mp 43

micro

m.p. = 84-87°C 68% yield.

	C	H	N	O		
Calc.						
Found						

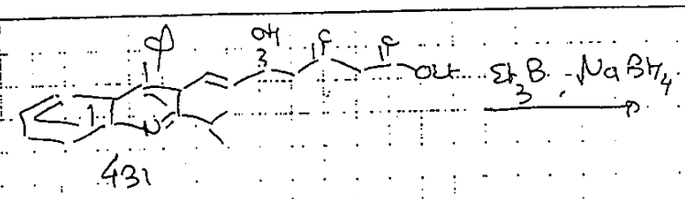
Performed by- Raj Patel 8-5-01

Witness- S. Wattanasin

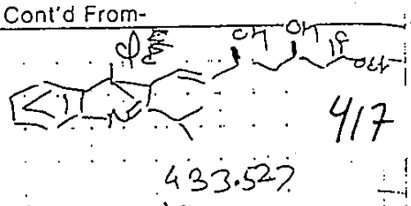
Cont'd to-

Title-

Cont'd From-



431



417

433.527

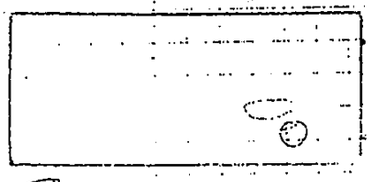
$C_{27}H_{31}NO_4$

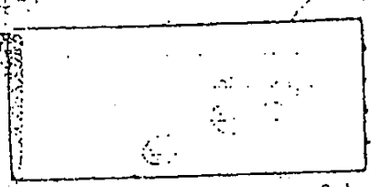
(431) 1206-175-4 = 1.0 g (0.002320 mole)
1m Et_3B / THF = 3.5 ml (0.003480 mole) 1.5eq.
dry THF = 10 ml
CH_3OH = 2.5 ml
37.8 $NaBH_4$ = 0.13159 g (0.003480 mole) 1.5

Ref: 1206-140

(Chemiequivalents)

To 1206-175-4 in THF / MeOH added 1m Et_3B / THF at r.t. stirred for 1 hr (9:45 - 10:45). The solution was cooled to -78°C, $NaBH_4$ was added portionwise. The rxn was stirred at -78°C for (11:30 - 3:00) 4 hrs.





The rxn was quenched with MeOH (5 ml) at -78°C. Ethyl acetoacetate was added & let it warm up to r.t. Org. layer was washed with sat. $NaHCO_3$, H_2O, brine, dried, filtered. Residue was redissolved in MeOH evaporated to dryness. This evaporation process (in MeOH) was repeated until TLC showed desired product.

Wt. of orange oil = 1.0914 g (1206-176-39)

Flash column (80:20 Hex/EtOAc) gave ✓ m.p. = 104-106° exact mass 13 1

yellow oil (a) F_{4-6} = 0.4043g (1206-176-41) IR nmr, MS MH^+ = 434 c
solid HPLC (95.3%) 13

F_{7-13} = 0.510 g (1206-176-43) IR nmr, MS MH^+ = 434 c
HPLC (93.2%)

for Patel. 8-5-8

Performed by-

Witness- S. Waterman

Cont'd to-

Exhibit Q

BIOLOGICAL ACTIVITY DATA REPORT (FOR PATENT DEPT.)

INVENTOR: S. Wattanasin DISCL. NO.: 299-84 4/8

ATTORNEY: M. Kassenoff DATE: May 24, 1988

1. ACTIVITY TO BE DISCLOSED:
Inhibition of cholesterol biosynthesis, antihypercholesteremic, antiatherosclerotic

2. IF ANY COMPOUNDS COVERED BY ABOVE-NOTED DISCLOSURE HAVE MORE THAN ONE ACTIVITY, INDICATE TOTAL NUMBER OF ACTIVITIES AND PREPARE A SEPARATE B.A.D.R. SHEET FOR EACH. TOTAL NO. OF ACTIVITIES: 1

3.a) TEST METHODS USED TO ESTABLISH ACTIVITY:
HMG-CoA reductase inhibition in rat liver microsomes (DT 64)
Cholesterol synthesis inhibition invivo in rats (DT 65)

b) DOSAGE RANGES BASED ON ACTUAL DOSES USED IN TEST PROCEDURE:
0.050 - 1.5 mg/kg

4. COMPOUNDS TESTED WITHIN DISCLOSURE WHICH EXHIBIT WEAK OR GREATER ACTIVITY:
64-935, 64-933

5. DOSAGE SCHEDULE - Broad Ranges:

a) Large / small animals:	.10	to	1.0	mg/kg.
b) Large animals:	20	to	200	mg/day.

6. MOST PREFERED COMPOUND FOR ACTIVITY DESIGNATED:

64-935

7. OTHER PREFERRED OR POTENTIALLY PREFERRED COMPOUNDS FOR DESIGNATED ACTIVITY:
64-936, 63-366, 64-933, 64-934

8. ED50 FOR THE PREFERRED COMPOUND IN EACH OF THE TEST METHODS INDICATED IN 3a) FOR THE DESIGNATED ACTIVITY:

COMPOUND	IC50 uM DT64	ED50 mg.kg DT65	Potency x Mevinolin*
Compactin	1.01	3.5	0.11
Mevinolin	0.14	0.41	1 (standard)
64-935	0.41	0.49	0.3
64-936	0.53	>1.0	
64-933	2.37	2.40	

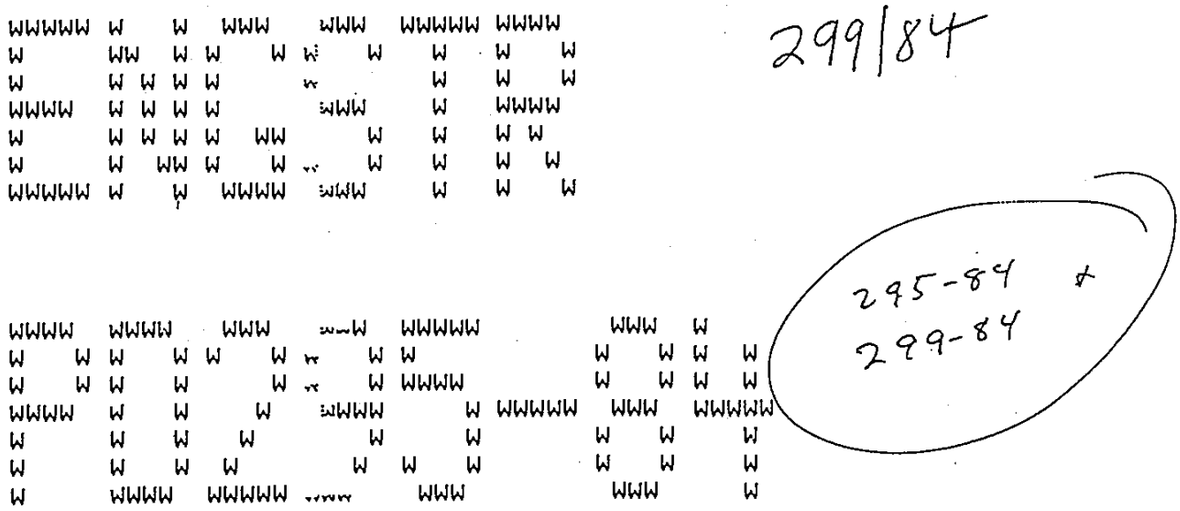
* Clinical dose of mevinolin (Lovasatin) = 20-80 mg/day

User: STR -at pr0

<USER02>ENGSTR>IC5 TA>PD295-84

419

299/84



295-84 &
299-84

Label: PRT002 -form

Pathname: <USER02>ENGSTR>IC50DATA>PD295-84
File last modified: 88-05-23.08:25:36.Mon

Spooled: 88-05-23 08:50:36.Mon [Spooler rev 19.4.6]
Started: 88-05-23 08:50:40.Mon on: PR0 by: PR0

IC50 TABLE RAT MICROSOMAL ASSAY (CSI-DT64)

THIS FILE IS A CALCULATED ESTIMATE OF THE IC50 (CONCENTRATION WHICH REDUCES THE CONVERSION OF HMG-CoA TO MEVALONATE BY 50%) USING ALL THE STUDIES ON THE RELEVANT COMPOUNDS UP TO THE SORT DATE.

LAST UPDATE: 02-04-88 SORT BY: DISCLNO

COMPOUND	REGNO	DISCL	IC50 uM	DATE	REF	COMMENTS
SAH-062977	24162	295-84	25.0000	02-07-84	1014-248	
SAH-062978	24163	295-84	0.0180	02-07-84	1014-249	
SAH-063033	24315	295-84	0.0450	04-18-84	1014-257	SAPONIFIED
SAH-063033	24315	295-84	0.5250	02-29-84	1014-257	
SAH-063034	24316	295-84	0.3630	02-22-84	1014-258	
SAH-063035	24317	295-84	0.0400	02-22-84	1014-259	
SAH-063074	24446	295-84	0.4000	05-23-84	1014-277	
SAH-063074	24446	295-84	0.6900	03-26-84	1014-277	
SAH-063075	24448	295-84	0.5300	04-18-84	1014-278	SAPONIFIED
SAH-063075	24448	295-84	0.9040	03-26-84	1014-278	
SAH-063076	24449	295-84	0.5800	06-12-84	1014-279	
SAH-063076	24449	295-84	0.6400	05-23-84	1014-279	
SAH-063076	24449	295-84	0.9000	03-26-84	1014-279	
SAH-063083	24511	295-84	1.9100	03-28-84	1014-281	
SAH-063083	24511	295-84	2.3200	03-28-84	1014-281	
SAH-063084	24512	295-84	3.1600	06-12-84	1014-282	
SAH-063084	24512	295-84	6.3200	03-28-84	1014-282	
SAH-063144	24750	295-84	1.1600	05-10-84	1014-294	SAPONIFIED
SAH-063144	24750	295-84	2.0200	05-10-84	1014-294	
SAH-063145	24755	295-84	>10.0000	05-07-84	1014-295	SAPONIFIED
SAH-063145	24755	295-84	>10.0000	05-10-84	1014-295	
SAH-063146	24756	295-84	>10.0000	05-07-84	1014-296	
SAH-063158	24809	295-84	0.1000	06-04-84	1069-002	SAPONIFIED
SAH-063158	24809	295-84	0.3430	06-04-84	1069-002	
SAH-063159	24810	295-84	0.2250	06-12-84	1069-003	
SAH-063159	24810	295-84	0.2630	06-04-84	1069-003	
SAH-063160	24811	295-84	0.1110	06-04-84	1069-004	SAPONIFIED
SAH-063160	24811	295-84	1.5600	06-04-84	1069-004	
SAH-063161	24821	295-84	0.0020	06-04-84	1069-005	
SAH-063161	24821	295-84	0.0020	06-12-84	1069-005	
SAH-063162	24822	295-84	0.0030	06-04-84	1069-006	
SAH-063162	24822	295-84	0.0035	06-12-84	1069-006	
SAH-063174	24865	295-84	0.0140	06-06-84	1069-013	SAPONIFIED
SAH-063174	24865	295-84	0.0190	06-06-84	1069-013	
SAH-063175	24866	295-84	0.0260	06-06-84	1069-014	
SAH-063229	25075	295-84	>10.0000	08-04-84	1069-036	
SAH-063230	25078	295-84	0.0042	08-01-84	1069-037	
SAH-063231	25079	295-84	0.0058	08-04-84	1069-038	
SAH-063269	25205	295-84	0.0030	09-10-84	1069-053	SAPONIFIED
SAH-063269	25205	295-84	0.0440	09-12-84	1069-053	
SAH-063270	25206	295-84	0.0080	09-05-84	1069-054	
SAH-063271	25208	295-84	0.0320	09-10-84	1069-055	SAPONIFIED
SAH-063271	25208	295-84	0.1450	09-12-84	1069-055	

SAH-064484	F	29413	E95-84	0.0320	11-24-86	1149-227
SAH-064744	E	30059	E95-84	0.0320	05-01-87	1149-293
AH-064745	S	30060	E95-84	0.0030	05-01-87	1149-294
AH-064745	S	30060	E95-84	0.0030	07-07-87	1149-297
SAH-064815	E	30198	E95-84	0.0220	07-07-87	1238-001
AH-064816	S	30199	E95-84	0.0450	07-07-87	1238-002
AH-063162	S	30203	E95-84	0.0080	07-07-87	1238-003
SAH-064745		30765	E95-84	0.0020	01-12-88	1238-030
SAH-063366		25496	E99-84	1.5800	12-13-84	1069-113
SAH-063549		26082	E99-84	7.3100	06-13-84	1069-197
AH-063548		26080	E99-84	3.7750	06-13-84	1069-198
AH-064933	E	30441	E99-84	2.3700	10-08-87	1238-013
SAH-064934	S	30442	E99-84	2.6100	10-08-87	1238-014
AH-064935	E	30447	E99-84	0.4130	10-08-87	1238-015
AH-064936	S	30448	E99-84	0.5300	10-13-87	1238-016

ED50 TABLE RAT INVIVO ACETATE INCORPORATION (CSIV-DT65)

THIS FILE IS A CALCULATED ESTIMATE OF THE ED50 (DOSE WHICH REDUCES THE INCORPORATION OF 14C-ACETATE INTO CHOLESTEROL BY 50%) USING ALL THE STUDIES ON THE RELEVANT COMPOUNDS UP TO THE SORT DATE.

LAST UPDATE: 1-06-88 SORT BY: REGNO

COMPOUND	REGNO	CISCL		ED50 mg/kg	DATE mm-dd-yy	REF bk-pg	COMMENTS
SAH-064745	30060	E95-84	=	0.016	10-20-87	917-127	N=9
SAH-064745	30765	E95-84	=	0.016	02-19-88	917-154	N=3 BS BATCH
SAH-064745	ALL	E95-84	=	0.016	02-19-88	917-154	N=12 2BATCHES
SAH-063162	25500	E95-84	=	0.019	09-18-87	917-101	N=10
SAH-063162	ALL	E95-84	=	0.040	09-18-87		N=19 3BATCHES
SAH-063162	25085	E95-84	=	0.079	10-11-84	812-266	N=8
SAH-064119	27563	E95-84	=	0.08	05-16-86	869-228	N=6
SAH-064744	30059	E95-84	>	0.10	07-14-87	917-090	N=3 -21% @.10
SAH-064816	30199	E95-84	=	0.10	10-12-87	917-119	N=6
SAH-064483	29412	E95-84	=	0.13	02-06-87	917-024	N=3
SAH-064063	27424	E95-84	=	0.19	04-17-86	869-211	N=3
SAH-064309	28718	E95-84	=	0.19	11-03-86	869-283	N=3
SAH-063231	25079	E95-84	>	0.25	08-30-84	812-250	
SAH-064393	29163	E95-84	=	0.25	02-25-87	917-031	N=6
SAH-063161	24821	E95-84	>	0.250	11-29-84	812-293	-12@0.25
SAH-063989	27237	E95-84	=	0.28	04-04-86	869-195	N=6
SAH-063425	25687	E95-84	>	0.3	03-20-85	869-046	N=3
SAH-064305	28701	E95-84	>	0.3	11-03-86	869-280	N=3 -34% @.3
SAH-064480	29404	E95-84	>	0.3	02-06-87	917-023	N=3 +3% @.3
SAH-063270	ALL	E95-84	=	0.308	02-07-85		N=11 2BATCHES
SAH-063270	25206	E95-84	=	0.33	10-11-84	812-267	
SAH-063270	25501	E95-84	=	0.362	01-21-85	869-018	
SAH-064307	28705	E95-84	=	0.47	02-06-87	917-020	N=6
SAH-063159	24810	E95-84	>	0.5	06-19-84	812-219	

422

SAH-063162	24822	E95-84	<	0.5	06-19-84	812-219	N=1 -87@0.5
SAH-063175	24866	E95-84	<	0.5	06-19-84	812-220	
SAH-063230	25078	E95-84	>	0.500	11-29-84	812-294	
SAH-064391	29161	E95-84	=	0.51	10-30-86	917-011	N=3
SAH-063035	24317	E95-84	>	0.6	05-07-84	812-201	
SAH-063145	24755	E95-84	>	0.6	05-18-84	812-208	
SAH-063146	24756	E95-84	>	0.6	05-18-84	812-208	
SAH-063174	24865	E95-84	=	0.706	06-19-84	812-220	
SAH-064481	29406	E95-84	>	1.0	02-06-87	917-024	N=3 -28% @1.0
SAH-064482	29411	E95-84	>	1.0	03-18-87	917-041	N=3 -41% @1.0
SAH-064064	27433	E95-84	=	1.05	07-17-86	869-263	N=6
SAH-064204	27793	E95-84	=	1.21	10-02-86	869-298	N=6
SAH-064141	27630	E95-84	>	1.25	02-24-87	917-029	N=6 -24% @1.25
SAH-064308	28717	E95-84	>	1.5	11-03-86	869-283	N=3 -16% @1.5
SAH-064193	27760	E95-84	>	2.4	07-24-86	869-269	N=3 -24% @2.4
SAH-063076	24449	E95-84	<	2.5	05-14-84	812-204	
SAH-063084	24512	E95-84	>	2.5	05-07-84	812-201	
SAH-064933	30441	E99-84	=	~~0.49~~ ≥1	12-09-87	917-138	N=3 -36 %@1.0
SAH-064935	30447	E99-84	≈	~~1=0.49~~	12-09-87	917-138	N=3

Exhibit R

423

600-7022/C

United States Patent [19]

Kathawala et al.

[11] Patent Number: 5,001,255

[45] Date of Patent: Mar. 19, 1991

[54] IDENE ANALOGS OF MEVALONOLACTONE AND DERIVATIVES THEREOF

[75] Inventors: Faizulla G. Kathawala, Mountain Lakes; Sompong Wattanasin, Hopatcong, both of N.J.

[73] Assignee: Sandoz Pharm. Corp., E. Hanover, N.J.

[21] Appl. No.: 214,560

[22] Filed: Jul. 1, 1988

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 837,479, Mar. 7, 1986, abandoned, which is a continuation-in-part of Ser. No. 677,917, Dec. 4, 1984, abandoned.

[51] Int. Cl.[5] C07C 69/76

[52] U.S. Cl. 560/56; 560/53; 556/441; 549/264; 549/291; 562/462; 562/466

[58] Field of Search 560/56, 53; 549/264, 549/291; 562/462, 466; 514/530, 569

[56] References Cited

U.S. PATENT DOCUMENTS

3,207,779	9/1965	Cutler	560/56
3,532,752	10/1970	Shen	560/56
3,668,241	6/1972	Cragoe et al.	560/56
3,983,140	9/1976	Endo et al.	260/343.5
4,006,180	2/1977	Cragoe et al.	560/56
4,012,524	3/1977	Cragoe et al.	560/56
4,057,573	11/1977	Haas et al.	560/56
4,070,539	1/1978	Cragoe et al.	560/56
4,125,731	11/1978	Sugie et al.	560/56
4,137,322	1/1979	Endo et al.	424/273
4,198,425	4/1980	Mitsui et al.	424/279
4,248,889	2/1981	Oka et al.	424/308
4,255,444	3/1981	Oka et al.	424/279
4,308,378	12/1981	Stokker	542/441
4,361,515	11/1982	Terahara et al.	549/292
4,375,475	3/1983	Willard et al.	424/279
4,474,971	10/1984	Wareing	549/214
4,588,715	5/1986	Damon	514/63
4,613,610	9/1986	Wareing	514/406
4,647,576	3/1987	Hoefle	514/422
4,654,363	3/1987	Prugh	560/56

FOREIGN PATENT DOCUMENTS

142146	5/1985	European Pat. Off. .
84/02131	6/1984	PCT Int'l Appl. .
84/02903	8/1984	PCT Int'l Appl. .
86/03488	6/1986	PCT Int'l Appl. .

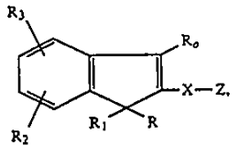
OTHER PUBLICATIONS

Hulcher, Arch. Biochem. Biophys. 146, 422–427 (1971).
Sato et al., Chem. Pharm. Bull. 28, 1509–1525 (1980).
Singer et al., Proc. Soc. Exp. Biol. Med. 102, 370–373 (1959).

Primary Examiner—Paul J. Killos
Attorney, Agent, or Firm—Gerald D. Sharkin; Richard E. Vila; Melvyn M. Kassenoff

[57] ABSTRACT

Compounds of the formula



the use thereof for inhibiting cholesterol biosynthesis and lowering the blood cholesterol level and, therefore, in the treatment of hyperlipoproteinemia and atherosclerosis, pharmaceutical compositions comprising such compounds and processes for and intermediates in the synthesis of such compounds.

27 Claims, No Drawings

Exhibit S

NAME: Joanne M. Giesser
BASE CITY: E. Hanover
CAR NO.: REGION NO.

05854
PERIOD COVERED

	MO.	DAY	YEAR
FROM	2	21	88
TO	2	26	88

COMMENTS:

SANDOZ PHARMACEUTICALS
TRAVEL & ENTERTAINMENT EXPENSE REPORT

LINE NO.	DATE	NATURE OF EXPENSE	TRANSPORTATION EXPENSES: AIR & RAIL (1)	GAS & OIL (2)	PARKING & TOLLS (3)	SUNDRY (4)	OTHER EXPENSES: LODGING (5)	BUSINESS MEALS (6)	BUSINESS ENTERTAIN. (7)	SUNDRY (8)	AUTO REPAIRS ON FLEET CARS (9)	CITY VISITED & PURPOSE OF TRIP (10)
1		Airplane tickets										visited Northrup King (Minneapolis), Rogers Brothers (Boise) and Zoecon (Palo Alto)
2		Newark - Minneapolis - Boise - San Francisco - Newark	939 00 ✓									
3												
4												
5	2/21	Hotel - Minneapolis					116 60 ✓			19 99 ✓		
6	2/23	Shuttle bus to airport				15 00 ✓						
7	2/23							14 02 ✓				
8	2/23	Hotel - Boise					122 10 ✓			14 25 ✓		
9												
10	2/25	Hotel - Palo Alto					115 50 ✓					
11		Rental Car				49 67 ✓						
12												
13	2/26	Airport parking			2 00 ✓							
14												
15												
16												
17												
18												
19												
20												
21							X					
22			✓	✓	✓	✓	✓	✓	✓	✓	✓	
23												
		TOTAL EXPENSES	939 00 ✓		2 00 ✓	64 67 ✓	355 20 ✓	14 02 ✓		34 24 ✓		TOTAL EXPENSES (COLUMNS 1-9) $ 1409 13

RECEIVED
Finance Division
MAR 4 1988
Travel Expense Section

TRANSFER OF EXPENSES APPROVED BY MANAGER (FULL SIGNATURE) C.U.

OFFICE USE ONLY: CW 354.20 Adj ded 1.00 col. 5

TOTAL PAID BY CO. AIR & RAIL (Col. 1) → $ 939 00 ✓

DUE EMPLOYEE → $ 470 13

EMPLOYEE'S FULL SIGNATURE: Joanne M. Giesser

APPROVED BY: FULL SIGNATURE Richard C. Wilke

OFFICE USE	ODOMETER READING	COMPANY FLEET CARS – MILEAGE
AUDITED BY: MM	END.	PERSONAL →
	BEG.	BUSINESS →
	DIFF.	EXPENSE DAYS

FOR OFFICE USE

Sawai Ex 1005
Page 1172 of 4322

NAME: Joanne M. Giesser

BASE CITY: E. Hanover

CAR NO.: _____ REGION NO. _____

PLC NUM: 05854

PERIOD COVERED

	MO.	DAY	YEAR
FROM	3	1	88
TO	3	1	88

COMMENTS:

TEAMSPIRIT

SANDOZ PHARMACEUTICALS

TRAVEL & ENTERTAINMENT EXPENSE REPORT

LINE NO.	DATE	NATURE OF EXPENSE	TRANSPORTATION EXPENSES: AIR & RAIL (1)	GAS & OIL (2)	PARKING & TOLLS (3)	SUNDRY (4)	OTHER EXPENSES: LODGING (5)	BUSINESS: MEALS (6)	BUSINESS: ENTERTAIN. (7)	SUNDRY (8)	AUTO REPAIRS ON FLEET CARS (9)	CITY VISITED & PURPOSE OF TRIP (10)
1	3/1	Airplane tickets	158 00									Washington D.C., attended NACA patent committee meeting
2												
3		Subway tickets				3 60						
4												
5		Lunch + Dinner						19 50				
6												
7		Airport parking			4 00 ✓							
8												
9												
10												
11												RECEIVED Finance Division
12												
13												MAR 4 1988
14												
15												Travel Expense Section
16												
17												
18												
19												
20												
21												
22												
23												
		TOTAL EXPENSES	158 00		4 00	3 60		19 50				TOTAL EXPENSES (COLUMNS 1-9) $ 195 10

TRANSFER OF EXPENSES APPROVED BY MANAGER (FULL SIGNATURE) _____ C.U. _____

OFFICE USE: ✓

TOTAL PAID BY CO. AIR & RAIL (Col. 1) → $ ✓ 158 00

DUE EMPLOYEE → $ 27 10

EMPLOYEE'S FULL SIGNATURE: Joanne M. Giesser

APPROVED BY: FULL SIGNATURE Raymond F. Ulm

OFFICE USE — AUDITED BY: MM

ODOMETER READING		COMPANY FLEET CARS – MILEAGE	
END.		PERSONAL →	
BEG.		BUSINESS →	
DIFF.		EXPENSE DAYS	

FOR OFFICE USE: 3-2-88 45

APLC NUM

NAME: Joanne M. Giesser

BASE CITY: E. Hanover

CAR NO.: — REGION NO.

05854

PERIOD COVERED

	MO.	DAY	YEAR
FROM	3	15	88
TO	4	15	88

TEAMSPIRIT

Sandoz Pharmaceuticals Corporation

SANDOZ PHARMACEUTICALS

TRAVEL & ENTERTAINMENT EXPENSE REPORT

COMMENTS:

LINE NO.	DATE	NATURE OF EXPENSE	TRANSPORTATION EXPENSES: AIR & RAIL (1)	GAS & OIL (2)	PARKING & TOLLS (3)	SUNDRY (4)	OTHER EXPENSES: LODGING (5)	BUSINESS MEALS (6)	BUSINESS ENTERTAIN. (7)	SUNDRY (8)	AUTO REPAIRS ON FLEET CARS (9)	CITY VISITED & PURPOSE OF TRIP (10)
1	3/15	Plane tickets	~~165.75~~									Boston- visit
2		Taxi	179.00			10.00						Repligen-Sandoz Res.Corp
3	3/16	Hotel					148.09	17.55		7.35		
4												
5												
6	3/28	Plane tickets EMP	690.00									Palo Alto, CA. visit
7		Rental car				127.99						Sandoz Crop Protection
8		Hotel (3 nights)					364.00	16.59		23.99		
9								10.03				
10								14.74				
11	3/31	Airport parking			2.00							
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												TOTAL EXPENSES (COLUMNS 1-9)
		TOTAL EXPENSES	~~360.175~~		2.00	132.99	644.08	59.21		31.34		$ ~~1748.62~~ 1738.62

TRANSFER OF EXPENSES APPROVED BY MANAGER (FULL SIGNATURE) C.U.

OFFICE USE 869.00

TOTAL PAID BY CO. AIR & RAIL (Col. 1) → $ ~~869.00~~ 869.00

DUE EMPLOYEE → $ ~~879.62~~ 869.62

EMPLOYEE'S FULL SIGNATURE: Joanne M. Giesser

APPROVED BY: [redacted] SIGNATURE

OFFICE USE — AUDITED BY:

ODOMETER READING: END. / BEG. / DIFF.

COMPANY FLEET CARS – MILEAGE: PERSONAL → / BUSINESS → / EXPENSE DAYS

APR 20 1988

FOR OFFICE USE: 1738.62 − ~~869.00~~ 17. = ~~869.62~~ 1559.62

926

EMPLOYEE NUMBER: 05854

NAME: Joanne M. Giesser

BASE CITY: E. Hanover

REGION NO.

CAR NO.:

PERIOD COVERED

	MO.	DAY	YEAR
FROM	4	20	88
TO	4	22	88

COMMENTS:

SANDOZ PHARMACEUTICALS

TEAMSPIRIT

TRAVEL & ENTERTAINMENT EXPENSE REPORT

LINE NO.	DATE	NATURE OF EXPENSE	TRANSPORTATION EXPENSES: AIR & RAIL (1)	GAS & OIL (2)	PARKING & TOLLS (3)	SUNDRY (4)	OTHER EXPENSES: LODGING (5)	BUSINESS: MEALS (6)	BUSINESS: ENTERTAIN. (7)	SUNDRY (8)	AUTO REPAIRS ON FLEET CARS (9)	CITY VISITED & PURPOSE OF TRIP (10)
1	4/20	Plane tickets	325 00									Des Plaines Ill
2	4/22											Visit with Seed
3												Committee &
4							88 80					Crop Protection
5	4/20-	Hotel (2 nights)					~~177 60~~			22 11		
6	4/22						88 80					
7												
8		Taxi (Newark-Morristown)				40 00	✓					
9												
10		Parking			10							
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
		TOTAL EXPENSES	325 00		10 00	41 00	177 60			22 11		TOTAL EXPENSES (COLUMNS 1-9) $ 575 71

TRANSFER OF EXPENSES APPROVED BY MANAGER (FULL SIGNATURE) C.U.

OFFICE USE

TOTAL PAID BY CO. AIR & RAIL (Col. 1) → $ 325 00

DUE EMPLOYEE → $ 250 71

EMPLOYEE'S FULL SIGNATURE: Joanne M. Giesser

APPROVED BY: FULL SIGNATURE Richard E. Vila 4/28/88

OFFICE USE — AUDITED BY:

ODOMETER READING: END. / BEG. / DIFF.

COMPANY FLEET CARS – MILEAGE: PERSONAL → / BUSINESS → / EXPENSE DAYS

FOR OFFICE USE

MAY 13 1988

427

90222/86

EMPLOYEE NUMBER: 05854

NAME: Joanne M. Giesser

BASE CITY: E. Hanover

CAR NO.: REGION NO.

PERIOD COVERED — FROM: MO. 5 DAY 2 YEAR 88 — TO: 6 27 88

COMMENTS:

SANDOZ PHARMACEUTICALS

TEAMSPIRIT

TRAVEL & ENTERTAINMENT EXPENSE REPORT

LINE NO.	DATE	NATURE OF EXPENSE	TRANSPORTATION EXPENSES: AIR & RAIL (1)	GAS & OIL (2)	PARKING & TOLLS (3)	SUNDRY (4)	OTHER EXPENSES: LODGING (5)	BUSINESS MEALS (6)	BUSINESS ENTERTAIN. (7)	SUNDRY (8)	AUTO REPAIRS ON FLEET CARS (9)	CITY VISITED & PURPOSE OF TRIP (10)
1	5/7/88	Plane Tickets	178 00	✓								Wash, DC
2		Taxi			3 00	3 25						IBA meeting
3												
4	6/15	Plane Tickets	690 00	✓								Palo Alto - Visit
5		Hotel					242 00	25 05		32 67*		* Phone Sandoz Crop Protect
6	6/16							18 25				
7		Airport parking			5 80							
8		Rental Car				95 27						
9												
10	6/24	Plane Tickets	178 00	✓	3 00							Wash, DC
11		Metro Ticket				1 00						IBA Meeting
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												TOTAL EXPENSES (COLUMNS 1-9)
		TOTAL EXPENSES	1,046 00	✓	11 00	99 52	242 00	43 30		32 67		$ 1,474 49
		TOTAL PAID BY CO. AIR & RAIL (Col. 1) →										$ 1046 00
		DUE EMPLOYEE →										$ 428 49

TRANSFER OF EXPENSES APPROVED BY MANAGER (FULL SIGNATURE) C.U.

OFFICE USE

EMPLOYEE'S FULL SIGNATURE: Joanne M. Giesser

APPROVED BY: FULL SIGNATURE

OFFICE USE — AUDITED BY:

ODOMETER READING — END. / BEG. / DIFF.

COMPANY FLEET CARS – MILEAGE — PERSONAL → / BUSINESS → / EXPENSE DAYS

FOR OFFICE USE

428

/86 EMPLOYEE NUMBER

NAME: Joanne M. Giesser

BASE CITY:

CAR NO.: REGION NO.

05854

PERIOD COVERED

	MO.	DAY	YEAR
FROM	07	12	88
TO	07	14	88

COMMENTS:

Sandoz Pharmaceutical Corporation

TEAMSPIRIT

SANDOZ PHARMACEUTICALS

TRAVEL & ENTERTAINMENT EXPENSE REPORT

LINE NO.	DATE	NATURE OF EXPENSE	TRANSPORTATION EXPENSES: AIR & RAIL (1)	GAS & OIL (2)	PARKING & TOLLS (3)	SUNDRY (4)	OTHER EXPENSES: LODGING (5)	BUSINESS MEALS (6)	BUSINESS ENTERTAIN. (7)	SUNDRY (8)	AUTO REPAIRS ON FLEET CARS (9)	CITY VISITED & PURPOSE OF TRIP (10)
1	7/12	Airline tickets	253 00									Des Plaines, Ill.
2		Cab (E. Hanover - Newark)				38						Sandoz Crop Protection
3		Cab to hotel				11 00						Patent Committee Meeting
4		Hotel					75 60	11 72		5 60		
5								2 50				(no receipt)
6		Cab (Newark - Morristown)				35 00						
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
		TOTAL EXPENSES	253 00			84 00	75 60	14 22		5 60		TOTAL EXPENSES (COLUMNS 1-9) $ 432 42

RECEIVED
Finance Division
JUL 19 1988
Travel Expense Section

TRANSFER OF EXPENSES APPROVED BY MANAGER (FULL SIGNATURE) C.U.

OFFICE USE

TOTAL PAID BY CO. AIR & RAIL (Col. 1) → $ 253

DUE EMPLOYEE → $ 179

EMPLOYEE'S FULL SIGNATURE: Joanne M. Giesser

APPROVED BY: FULL SIGNATURE Richard E. Vela Atty

OFFICE USE — AUDITED BY:

ODOMETER READING: END. / BEG. / DIFF.

COMPANY FLEET CARS – MILEAGE: PERSONAL → / BUSINESS → / EXPENSE DAYS

FOR OFFICE US

JL 22 [illegible]

427

Sandoz Pharmaceuticals Corporation

SANDOZ PHARMACEUTICALS

TRAVEL & ENTERTAINMENT EXPENSE REPORT

'86 EMPL E NU R 05854

NAME: Joanne M. Giesser

BASE CITY: E. Hanover

CAR NO.: REGION NO.

PERIOD COVERED

	MO.	DAY	YEAR
FROM	08	20	88
TO	09	20	88

COMMENTS: Swiss franc exchange rate - 1 Fr = $0.66.

LINE NO.	DATE	NATURE OF EXPENSE	TRANSPORTATION EXPENSES: AIR & RAIL (1)	GAS & OIL (2)	PARKING & TOLLS (3)	SUNDRY (4)	OTHER EXPENSES: LODGING (5)	BUSINESS MEALS (6)	BUSINESS ENTERTAIN. (7)	SUNDRY (8)	AUTO REPAIRS ON FLEET CARS (9)	CITY VISITED & PURPOSE OF TRIP (10)
1	8/29	Plane tickets	690 00					12 34		23 15		Palo Alto - Patent
2	8/29-9/1	hotel room					115 50					Policy meeting w/
3												Dechert Price & Rhoads
4		Airport parking			15 00		115 50					Crop Protection
5							115 50					
6												
7	9/6	Passport								67 00		Basle - Patent policy
8		Passport photo (no receipt)								5 00		(seeds) meeting
9	9/10	Plane tickets	208 00									(Authorization attached
10		Limo (Morristown - JFK)				91 20						
11	9/11-14	Hotel						6 60				
12		Limo (JFK - Morristown - shared)				54 50	59 40					
13							59 40					
14							59 40					
15							59 40					
16												
17												
18												
19												
20												
21												
22												
23												
		TOTAL EXPENSES	2789 00		15 00	145 70	584 10	18 94		95 15		

RECEIVED
Finance Division
SEP 20 1988
Travel Expense

TRANSFER OF EXPENSES APPROVED BY MANAGER (FULL SIGNATURE) C.U.

OFFICE USE

TOTAL PAID BY CO. AIR & RAIL (Col. 1) → $

DUE EMPLOYEE → $

TOTAL EXPENSES (COLUMNS 1-9) $

EMPLOYEE'S FULL SIGNATURE: Joanne M. Giesser

APPROVED BY: FULL SIGNATURE

OFFICE USE	ODOMETER READING	COMPANY FLEET CARS – MILEAGE	FOR OFFICE USE
AUDITED BY: KH	ENO.	PERSONAL →	3647.
	BEG.	BUSINESS → SEP 23 1988	2789.
	DIFF.	EXPENSE DAYS KH	858.8

Sawai Ex 1005
Page 1178 of 4322

NAME: Joanne M. Giesser

BASE CITY: E. Hanover

CAR NO.: _____ REGION NO.

EMPLOYEE NO.: 05854

PERIOD COVERED: FROM MO. 9 DAY 20 YEAR 88 — TO 10 20 88

COMMENTS:

SANDOZ PHARMACEUTICALS

TRAVEL & ENTERTAINMENT EXPENSE REPORT

LINE NO.	DATE	NATURE OF EXPENSE	TRANSPORTATION EXPENSES: AIR & RAIL (1)	GAS & OIL (2)	PARKING & TOLLS (3)	SUNDRY (4)	OTHER EXPENSES: LODGING (5)	BUSINESS MEALS (6)	BUSINESS ENTERTAIN. (7)	SUNDRY (8)	AUTO REPAIRS ON FLEET CARS (9)	CITY VISITED & PURPOSE OF TRIP (10)
1	10/9	Plane tickets	690 00									Palo Alto - Sandoz
2	10/7	Car rental			[illegible]	56 80						Crop Protection Patent
3	10/9-11	Hotel					~~147 60~~					Committee Meeting
4		Phone					121 00			21 80		
5		Food					121 00	6 72				
6	10/11	Taxi (Newark - Morristown)				35 00						
7												
8												
9	10/16	Plane Tickets	549 00									Madison, WI - visit
10	10/16-17	Hotel					~~141 12~~					Agrigenetics, return
11		Phone					70 56			4 77		via Wash. D.C.
12		Food					70 56	27 07				
13		Parking			18 00							
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
		TOTAL EXPENSES	1239 00		18 00	91 80	383 12	33 79		26 57		

RECEIVED
Finance Division
OCT 21 1988
Travel Expense Section

TOTAL EXPENSES (COLUMNS 1-9): $ 1792 28

TOTAL PAID BY CO. AIR & RAIL (Col. 1) → $ 1239 ✓ 00

DUE EMPLOYEE → $ 553 28

TRANSFER OF EXPENSES APPROVED BY MANAGER (FULL SIGNATURE) C.U.

OFFICE USE

EMPLOYEE'S FULL SIGNATURE: Joanne M. Giesser

APPROVED BY: FULL SIGNATURE Richard E. Vila

OFFICE USE — AUDITED BY:

ODOMETER READING: END. ____ BEG. ____ DIFF. ____

COMPANY FLEET CARS – MILEAGE: PERSONAL → ____ BUSINESS → ____ EXPENSE DAYS ____

FOR OFFICE USE

KH

OCT 24 1988

431

NAME: Joanne M. Giesser | 05034 | PERIOD COVERED

BASE CITY: E. Hanover

CAR NO.: | REGION NO.

FROM: MO. 10 DAY 20 YEAR 88

TO: 11 20 88

COMMENTS:

TEAMSPIRIT

SANDOZ PHARMACEUTICALS

TRAVEL & ENTERTAINMENT EXPENSE REPORT

LINE NO.	DATE	NATURE OF EXPENSE	TRANSPORTATION EXPENSES: AIR & RAIL (1)	GAS & OIL (2)	PARKING & TOLLS (3)	SUNDRY (4)	OTHER EXPENSES: LODGING (5)	BUSINESS: MEALS (6)	BUSINESS: ENTERTAIN. (7)	SUNDRY (8)	AUTO REPAIRS ON FLEET CARS (9)	CITY VISITED & PURPOSE OF TRIP (10)
1	10/27	Plane tickets	218 00									Boulder, CO - inspect
2	10/27	Morristown - Newark				45 54						patent files of
3	10/27	Hotel					49 19					Agrigenetics
4		Phone								2 11		
5	10/28	Rental car				51 07						
6	10/28	Newark - Morristown				57 54						
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
		TOTAL EXPENSES	218 00	+	+	154 15	49 19	+	+	2 11		TOTAL EXPENSES (COLUMNS 1-9) $ 423 45

RECEIVED
Finance Division
NOV 02 1988
Travel Expense Section

TRANSFER OF EXPENSES APPROVED BY MANAGER (FULL SIGNATURE) C.U.

OFFICE USE

TOTAL PAID BY CO. AIR & RAIL (Col. 1) → $ 218 00

DUE EMPLOYEE → $ 205 45

EMPLOYEE'S FULL SIGNATURE: Joanne M. Giesser

APPROVED BY: FULL SIGNATURE Richard T. Wilson

OFFICE USE: AUDITED BY:

ODOMETER READING: END. | BEG. | DIFF.

COMPANY FLEET CARS – MILEAGE: PERSONAL → | BUSINESS → | EXPENSE DAYS

FOR OFFICE USE

NOV 14 1988

432

:2/86

EMPLOYEE NUMBER: 05854

NAME: Joanne M. Giesser

BASE CITY: E. Hanover

CAR NO.: REGION NO.

PERIOD COVERED

	MO.	DAY	YEAR
FROM	12	1	88
TO	12	31	88

COMMENTS:

Sandoz Pharmaceuticals Corporation

SANDOZ PHARMACEUTICALS

TRAVEL & ENTERTAINMENT EXPENSE REPORT

LINE NO.	DATE	NATURE OF EXPENSE	TRANSPORTATION EXPENSES: AIR & RAIL (1)	GAS & OIL (2)	PARKING & TOLLS (3)	SUNORY (4)	OTHER EXPENSES: LODGING (5)	BUSINESS MEALS (6)	BUSINESS ENTERTAIN. (7)	SUNORY (8)	AUTO REPAIRS ON FLEET CARS (9)	CITY VISITED & PURPOSE OF TRIP (10)
1	12/62	Airline tickets	595 00									*Chicago- delivered
2	12/83											patent lecture to
3	12/6	Hotel					157 36	7 44				Northrup King group
4	12/5									24 50		phone charges
5	12/6	cab to hotel				12 50						
6	12/8	van service to airport				9 75						no receipt available
7								4 80				
8	12/8	airport parking			15 00							
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
		TOTAL EXPENSES	595 00		15 00	22 25	157 36	12 24		24 80		TOTAL EXPENSES (COLUMNS 1-9) $ 826 65

DEC 20

TRANSFER OF EXPENSES APPROVED BY MANAGER (FULL SIGNATURE) C.U.

OFFICE USE

TOTAL PAID BY CO. AIR & RAIL (Col. 1) → $ 595 00

DUE EMPLOYEE → $ 231 65

EMPLOYEE'S FULL SIGNATURE: Joanne M. Giesser

APPROVED BY: FULL SIGNATURE Richard E. Vida

OFFICE USE — AUDITED BY:

ODOMETER READING		COMPANY FLEET CARS – MILEAGE	
END.		PERSONAL →	
BEG.		BUSINESS →	
DIFF.		EXPENSE DAYS	

FOR OFFICE USE

JAN 18

433

_____/86

NAME: Joanne M. Giesser

BASE CITY: E. Hanover

CAR NO.: _____ REGION NO.

EMPL. E NI R: 05854

PERIOD COVERED

	MO.	DAY	YEAR
FROM	1	1	89
TO	1	20	89

COMMENTS:

TEAMSPIRIT

Sandoz Pharmaceutical Corp

SANDOZ PHARMACEUTICALS

TRAVEL & ENTERTAINMENT EXPENSE REPORT

LINE NO.	DATE	NATURE OF EXPENSE	TRANSPORTATION EXPENSES: AIR & RAIL (1)	GAS & OIL (2)	PARKING & TOLLS (3)	SUNDRY (4)	OTHER EXPENSES: LODGING (5)	BUSINESS MEALS (6)	BUSINESS ENTERTAIN. (7)	SUNDRY (8)	AUTO REPAIRS ON FLEET CARS (9)	CITY VISITED & PURPOSE OF TRIP (10)
1	1/4	Plane tickets	1078.00									Minneapolis (Northrup King Patent Committee)
2												Palo Alto (Zoecon Patent Committee)
3												
4												
5												
6	1/8	Taxi (Airport - Hotel)				30.00						
7	1/9	Taxi (Hotel - Northrup King)				6.00						
8	1/9	Hotel					60.42			8.52		(phone)
9	1/11	Rental Car				92.69						
10	1/11	Hotel					~~212.00~~	7.06		24.27		(phone)
11	1/12	Parking at Airport			2.00	✓	+21.00					no receipt
12						✓	121.00					
13												
14												
15												
16												
17												
18												
19												
20												
21												
22			✓									
23												
		TOTAL EXPENSES	1078.00		2.00	128.69	302.42	7.06		32.79		TOTAL EXPENSES (COLUMNS 1-9) $ 1550

TRANSFER OF EXPENSES APPROVED BY MANAGER (FULL SIGNATURE) C.U.

OFFICE USE

TOTAL PAID BY CO. AIR & RAIL (Col. 1) → $ ✓1078

DUE EMPLOYEE → $ 472

EMPLOYEE'S FULL SIGNATURE: Joanne M. Giesser

APPROVED BY: FULL SIGNATURE Richard E. Vila ✓

OFFICE USE — AUDITED BY: ✓

ODOMETER READING: END. _____ BEG. _____ DIFF. _____

COMPANY FLEET CARS — MILEAGE: PERSONAL → _____ BUSINESS → _____ EXPENSE DAYS _____

FOR OFFICE USE: JAN 24 1989

434

NAME: Joanne M. Giesser

BASE CITY: E. Hanover

CAR NO.: _____ REGION NO. _____

EMPLOYEE NUMBER: 05854

PERIOD COVERED

	MO.	DAY	YEAR
FROM	2	1	89
TO	2	28	89

COMMENTS:

SANDOZ PHARMACEUTICALS

TRAVEL & ENTERTAINMENT EXPENSE REPORT

LINE NO.	DATE	NATURE OF EXPENSE	TRANSPORTATION EXPENSES: AIR & RAIL (1)	GAS & OIL (2)	PARKING & TOLLS (3)	SUNDRY (4)	OTHER EXPENSES: LODGING (5)	BUSINESS: MEALS (6)	BUSINESS: ENTERTAIN. (7)	SUNDRY (8)	AUTO REPAIRS ON FLEET CARS (9)	CITY VISITED & PURPOSE OF TRIP (10)
1	2/21	Plane tickets	844 00 ✓									Boise, Id. Patent
2												Lecture to Rogers Bros
3	2/28-	Hotel					84 40	35 82		6 39		phone
4	3/2						44 40	6 67				
5		Airport parking			2 00							No receipt available
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
		TOTAL EXPENSES	844 00 ✓		2 00		88 80	42 49		6 39		

Finance Division
MAR 01 1989
Travel Expense

TOTAL EXPENSES (COLUMNS 1-9): $ ~~1,133~~ 983

TRANSFER OF EXPENSES APPROVED BY MANAGER (FULL SIGNATURE) _____ C.U. _____

OFFICE USE

TOTAL PAID BY CO. AIR & RAIL (Col. 1) → $ 844

DUE EMPLOYEE → $ 139

985 ✓

EMPLOYEE'S FULL SIGNATURE: Joanne M. Giesser

APPROVED BY: FULL SIGNATURE _____

OFFICE USE — AUDITED BY: _____

ODOMETER READING: END. _____ BEG. _____ DIFF. _____

COMPANY FLEET CARS – MILEAGE: PERSONAL → _____ BUSINESS → _____ EXPENSE DAYS _____

FOR OFFICE USE: MAR 20 1989 BB

Sandoz Pharmaceuticals Corporation

SANDOZ PHARMACEUTICALS

TRAVEL & ENTERTAINMENT EXPENSE REPORT

NAME: Joanne M. Giesser

BASE CITY: E. Hanover

CAR NO.: —

REGION NO.

EMPLOYEE NUMBER: 05854

PERIOD COVERED

FROM: MO. 03 DAY 2 YEAR 89

TO: 03 31 89

COMMENTS:

LINE NO.	DATE	NATURE OF EXPENSE	TRANSPORTATION EXPENSES: AIR & RAIL (1)	GAS & OIL (2)	PARKING & TOLLS (3)	SUNDRY (4)	OTHER EXPENSES: LODGING (5)	BUSINESS MEALS (6)	BUSINESS ENTERTAIN. (7)	SUNDRY (8)	AUTO REPAIRS ON FLEET CARS (9)	CITY VISITED & PURPOSE OF TRIP (10)
1	3/20	Plane tickets	422 00									Stanton, MN Northrup King Research Mgmt Comm. Meeting
2												
3	3/20	Car rental				94 45						
4												
5	3/20	Hotel					61 04	2 70		7 90		Phone
6								2 50				no receipt
7												
8	3/31	Parking			15 45							NYC - Judges Dinner
9		Tolls			4 00							
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
		TOTAL EXPENSES	422 00		19 45	94 45	61 04	5 20		7 90		TOTAL EXPENSES (COLUMNS 1-9)

RECEIVED

APR 1 3 1989

TOTAL EXPENSES (COLUMNS 1-9)	$ 610 04
TOTAL PAID BY CO. AIR & RAIL (Col. 1) →	$ 422 00
DUE EMPLOYEE →	$ 188 04

TRANSFER OF EXPENSES APPROVED BY MANAGER (FULL SIGNATURE) C.U.

OFFICE USE

EMPLOYEE'S FULL SIGNATURE: Joanne M. Giesser

APPROVED BY: FULL SIGNATURE Richard E. Vila

OFFICE USE — AUDITED BY:

ODOMETER READING: END. / BEG. / DIFF.

COMPANY FLEET CARS – MILEAGE: PERSONAL → / BUSINESS → / EXPENSE DAYS

FOR OFFICE USE

APR 13 1989

85

436

Exhibit T

PTO-103X
(Rev. 11-86)

FILING RECEIPT

RE CORRECTED

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

437

SERIAL NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTORNEY DOCKET NO.	DRWGS	TOT CL	IND CL
07/254,514	10/06/88	121	$ 450.00	600-7025/CIP/KIP	0	14	1

GERALD D. SHARKIN
SANDOZ CORPORATION
59 ROUTE 10
EAST HANOVER, NJ 07936

PATENT AND
TRADEMARK DEPT.

MAY 7 - 1990

Receipt is acknowledged of the patent application identified herein. It will be considered in its order and you will be notified as to the examination thereof. Be sure to give the U.S. SERIAL NUMBER, DATE OF FILING, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this transmittal.

Applicant(s)

FAIZULLA G. KATHAWALA, MOUNTAIN LAKES, NJ.

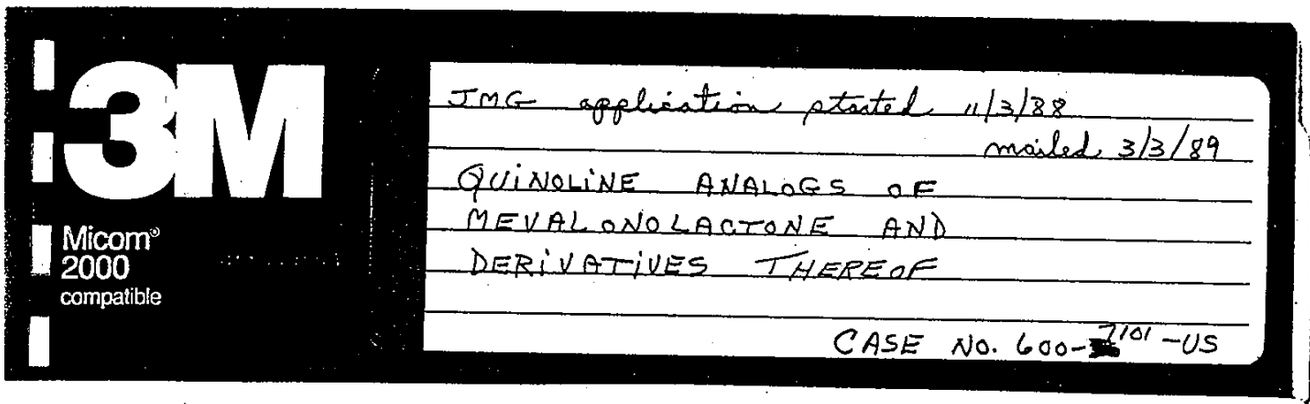
CONTINUING DATA AS CLAIMED BY APPLICANT-
THIS APPLN IS A CIP OF. 07/047,358 05/11/88
WHICH IS A CIP OF 06/722,829 04/12/85 ABAN

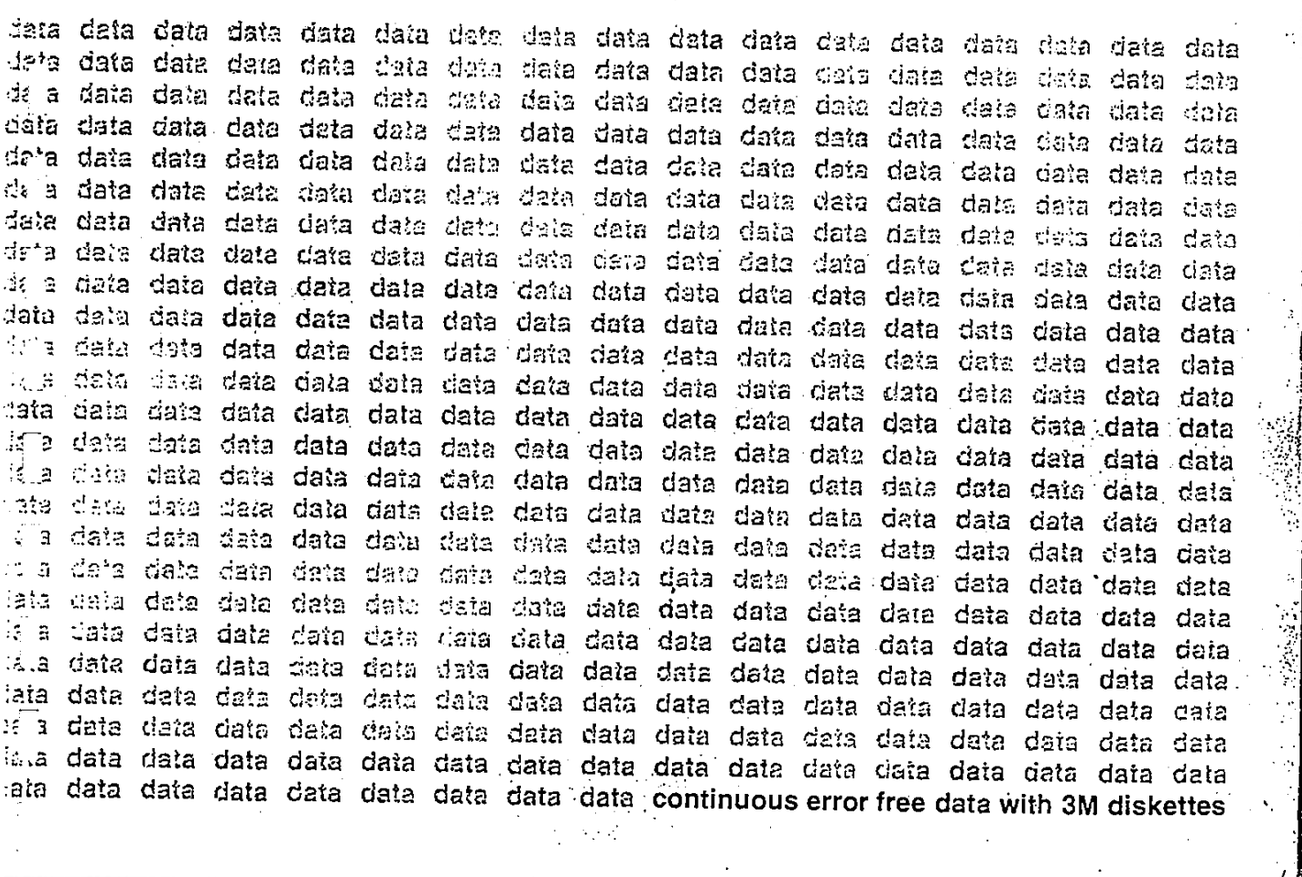
FOREIGN FILING LICENSE GRANTED 12/29/88
TITLE
PYRIMIDINE DERIVATIVES

PRELIMINARY CLASS: 514

(see reverse)

Exhibit U


3M
Micom®
2000
compatible
IMG application ptasted 11/3/88
mailed 3/3/89
QUINOLINE ANALOGS OF
MEVALONOLACTONE AND
DERIVATIVES THEREOF
CASE No. 600-7101-US


data
continuous error free data with 3M diskettes

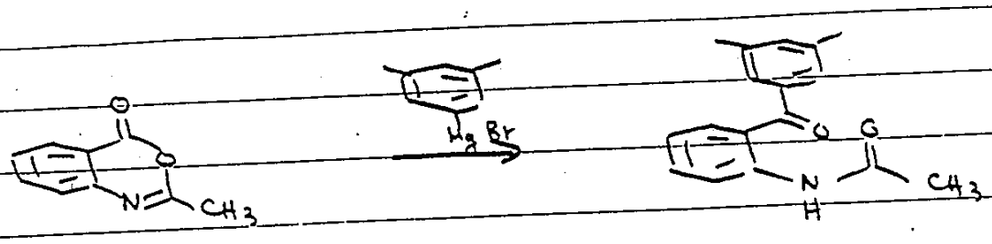
PATENT AND TRADEMARK DEPT.
NOV 8 - 1988
JMG

1 439

To: J. Giesser
From: S. Wattanasin

Attached is the written procedures for the synthesis of quinoline analogs according to Route I in the disclosure of invention # 299/84.

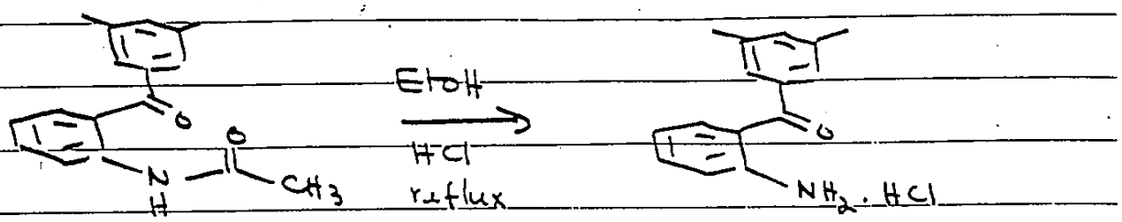
Som
11/7/88



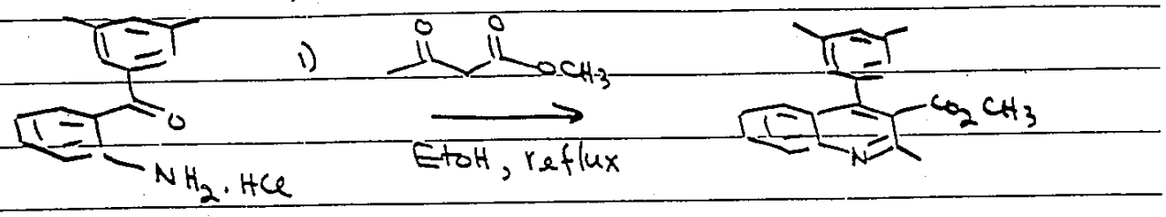
A solution of the benzoxazine* (10g, 0.062 mol) in THF (50 ml) was added dropwise over a 30 min period to a solution of 3,5-dimethylphenyl-magnesium bromide [prepared from 5-bromo-m-xylene (17.2 g, 0.093 l), magnesium (2.33 g, 0.0931 mol) and a trace of iodine in THF and 1,2-dibromo-ethane in diethyl ether (40 ml)] stirred at room temperature under nitrogen. The reaction mixture was stirred at room temperature for 1 h, and quenched with saturated ammonium chloride solution and extracted with ethyl acetate. The extracts were dried (Na_2SO_4) and evaporated at reduced pressure, and the residual oil (10 g) was chromatographed on a silica gel column to obtain the product as an oil (6 g).

* prepared according for a literature procedure. Morrison and Mullholland JCS 2700 (1958

441

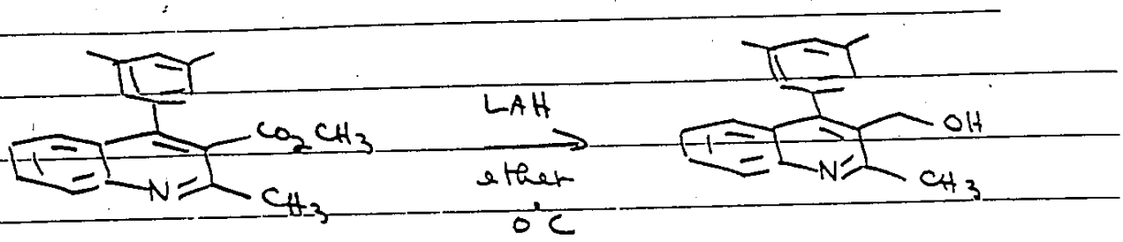


A mixture of the keto amide (3.8 g, 0.01428 mol) and 12 N hydrochloric acid (1.19 ml, 0.01428 mol) in absolute ethanol (20 ml) was stirred and heated at reflux for 3 h. The mixture was cooled and diluted with diethyl ether. The resulting solid was collected by filtration, washed with diethyl ether and vacuum dried to afford the product as a pale yellow solid (2.85 g), m.p. 193–195°C.



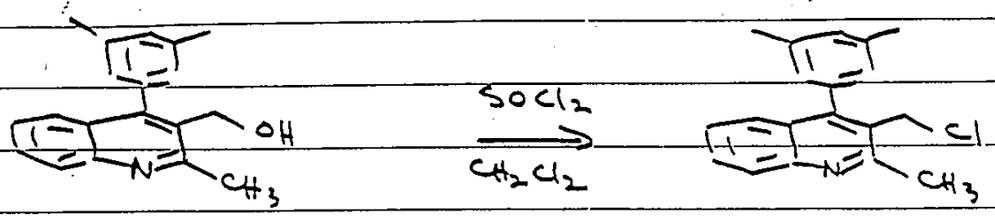
A mixture of the ketone hydrochloride (0.8 g, 0.003059 mol), methyl acetoacetate (0.33 ml, 0.00306 mol) in absolute ethanol (20 ml) was stirred at reflux for 3h. The mixture was slowly cooled to 10° C and diluted with diethyl ether. The precipitating white solid is collected by filtration and dried to obtain the quinoline hydrochloride (930 mg), m.p 209 – 211° C.

A mixture of the above hydrochloride salt (620 mg and diisopropylamine (2 ml) in dry diethylether (10 ml) was stirred at room temperature for 1h. The mixture was diluted with diethyl ether ~~The~~ diisopropylamine ~~diisopropyl~~ hydrochloride was removed by filtration. The filtrate was evaporated at reduced pressure ~~to obtain the~~ and the obtained colorless oil was crystallized from petrol to give the product as a colorless solid (600 mg), mp 88 – 90° C.

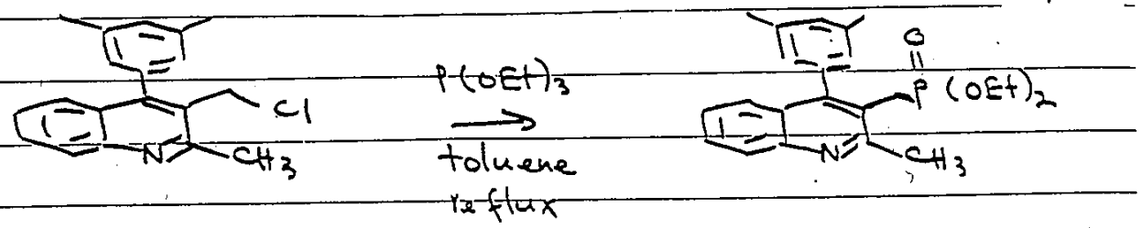


To a solution of the ester (486 mg, 0.00189 mol) in dry diethyl ether (9 ml) was added lithium aluminium hydride (~~74~~ 148 mg) at 0°C. The reaction mixture was stirred at 0°C for 3.5 h. The reaction mixture was cautiously poured into cold water and extracted with ethyl acetate. The extracts were dried (Na_2SO_4) and filtered. The filtrate was concentrated at reduced pressure to give a ~~colorless~~ solid (~~213 mg~~). Recrystallization (ether–petrol) gave the product as a colorless ~~solid~~ solid (213 mg), m.p. 194–195°C.

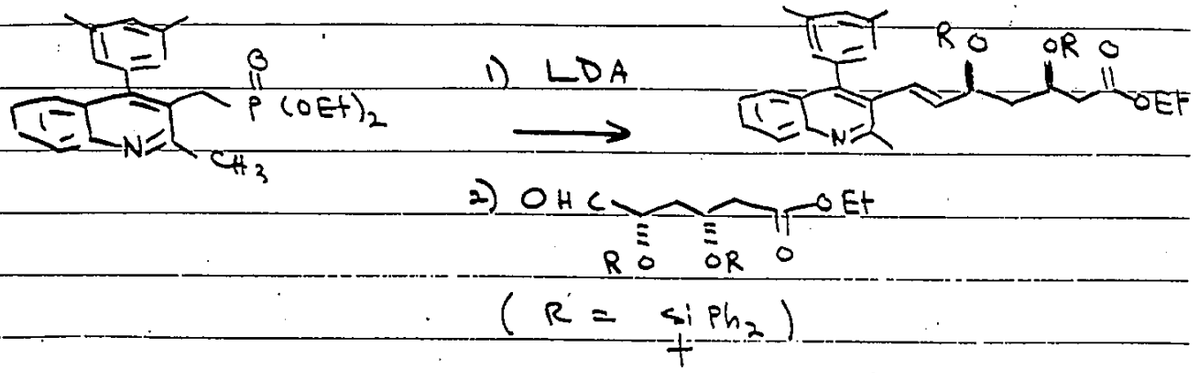
444



To a solution of the quinoline alcohol (190 mg, 0.0006859 mol) in CH_2Cl_2 (5 ml) at room temperature was added thionyl chloride (0.1 ml, 0.00137 mol). The reaction solution was stirred at room temperature for 4 h. The solvent was removed at reduced pressure. The crude oil was purified by Prep TLC (ether–petrol, 1:1) to give the product as a white solid (160 mg).



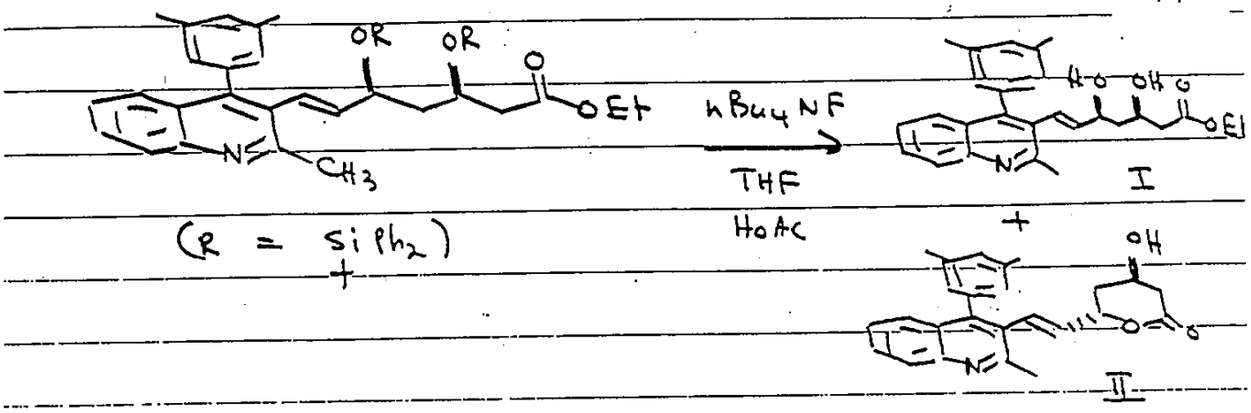
A mixture of the chloride (150 mg, 0.000508 mol) and triethyl phosphite (0.8 ml) in toluene (2ml) was stirred at reflux under nitrogen for 20 h. Evaporation at ~~of~~ reduced pressure gave an oily product which solidified on standing (160 mg), m.p. 105-107°



(150 mg, 0.00037 mol)

To a solution of the diethyl phosphonate in THF (3 ml) at −55°C was added a solution of lithium diisopropylamide monotetrahydrofuran / cyclohexane (1.7 M, 0.27 ml). The reaction mixture was stirred at −55 → −60°C for 10 min, a solution of the aldehyde (293 mg, 0.0004534 mol) in THF (2 ml) was added dropwise with stirring at −55°C. The reaction mixture was stirred at −55°C for 20 min. Acetic acid (0.5 ml) and 10% HCl solution were added, and the mixture was extracted with ethyl acetate. The extracts were combined, washed with water, ~~sat~~ saturated sodium bicarbonate, water and brine. Dried (Na_2SO_4), filtered and evaporate at reduced pressure gave the crude product as a yellow oil. Preparative TLC (ether–petrol, 1:1) gave the product as a pale yellow oil (100 mg).

447



To a solution of the silyl ether (90 mg, 0.0001012 mol) and glacial acetic acid (0.03 ml, 0.0005 mol) in THF (2 ml) at room temperature was added a solution of tetra-n-butylammonium fluoride (tetrahydrofuran (1M, 0.61 ml, 0.000607 mol). The reaction mixture was stirred at ~~50°c~~ 50-60°c for 40 h. The mixture was evaporated at reduced pressure to give the crude product as a brown oil. The crude product was purified by preparative chromatography (ether : ethyl acetate, 1:1) to obtain the product I as an oil (10 mg) and product II as an oil (10 mg).

Exhibit W

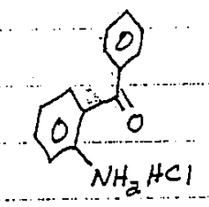
TRADEMARK DEPT.
NOV 9 - 1993
JMG

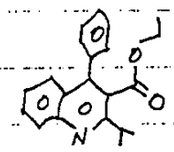
448

LIDH 15994

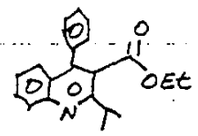
To: Ziggy Wahrman
From: Jody Giesser

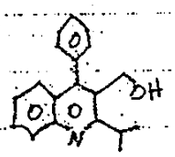
Please provide the names for the following compounds. Thanks.

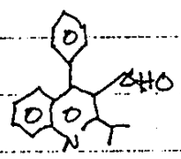
1) 

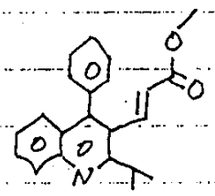
2) 

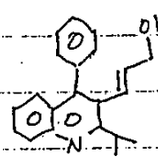
same?

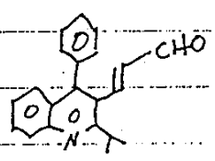
3) 

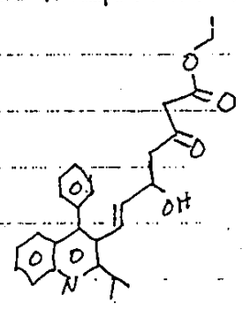
4) 

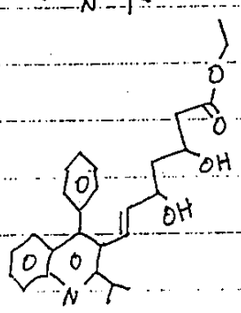
5) 

6) 

7) 

8) 

9) 

10) 

SANDOZ RESEARCH INSTITUTE

East Hanover, New Jersey

To: Jody Giesser

From: Henry Mah

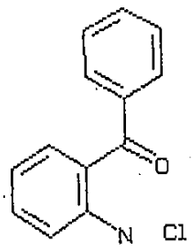
Date: November 8, 1988

Subject: Nomenclature on the following

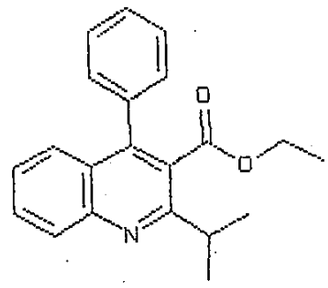
L&D # 15994

PLEASE NOTE THE CHANGE IN THE NUMBERING OF THE COMPOUNDS. COMPOUNDS (2) AND (3) ON THE ORIGINAL SHEET ARE IDENTICAL.

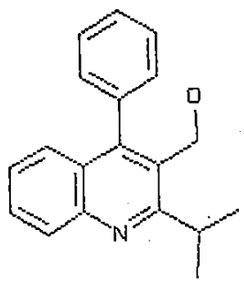
(1)



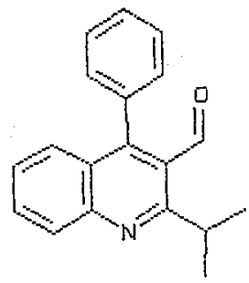
(2)



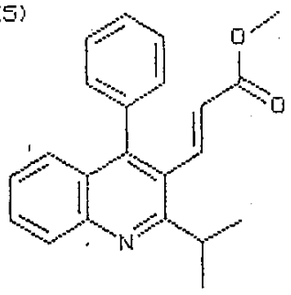
(3)



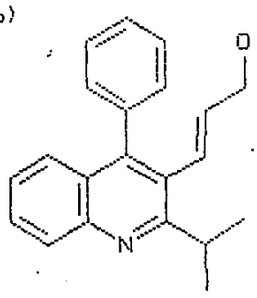
(4)



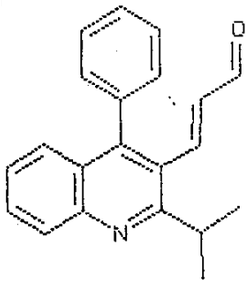
(5)



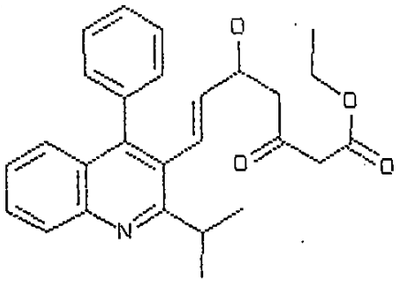
(6)



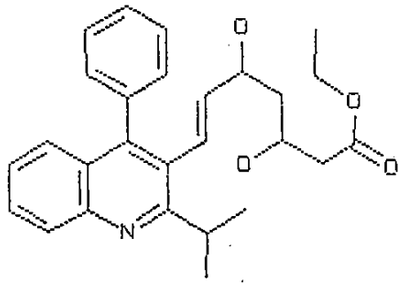
(7)



(8)



(9)



(1) Methanone, (2-aminophenyl)phenyl-
hydrochloride

(2) 3-Quinolinecarboxylic acid, 2-(1-methylethyl)-4-phenyl-
ethyl ester

(3) 3-Quinolinemethanol, 2-(1-methylethyl)-4-phenyl-

(4) 3-Quinolinecarboxaldehyde, 2-(1-methylethyl)-4-phenyl-

(5) 2-Propenoic acid, 3-[2-(1-methylethyl)-4-phenylquinolin-3-yl]-
methyl ester, (E)-

(6) 2-Propenol, 3-[2-(1-methylethyl)-4-phenylquinolin-3-yl]-
(E)-

(7) 2-Propenal, 3-[2-(1-methylethyl)-4-phenylquinolin-3-yl]-
(E)-

(8) 6-Heptenoic acid, 5-hydroxy-7-[2-(1-methylethyl)-4-phenylquinolin-
3-yl]-3-oxo-
ethyl ester, (E)-

(9)6-Heptenoic acid, 3,5-dihydroxy-7-[2-(1-methylethyl)-4-
phenylquinolin-3-yl]-
ethyl ester, (E)-

451

SANDOZ RESEARCH INSTITUTE

East Hanover, New Jersey

To: Ms. Jody Giesser

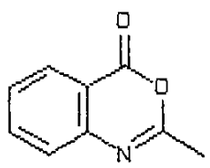
From: Henry Mah

Date: November 14, 1988

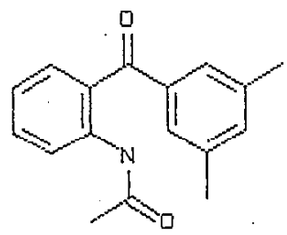
Subject: Nomenclature on the following

L&D # 16008

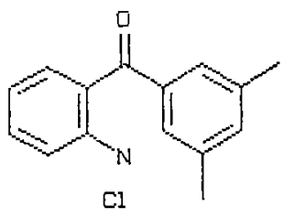
(1)



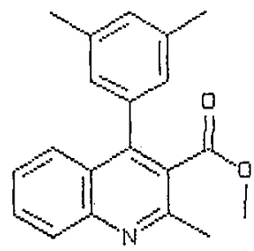
(2)



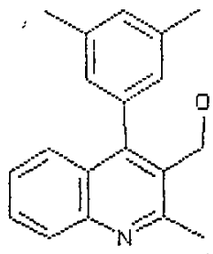
(3)



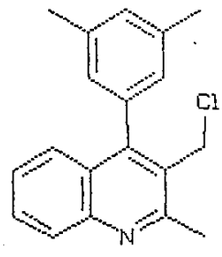
(4)



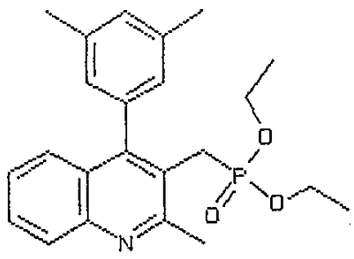
(5)



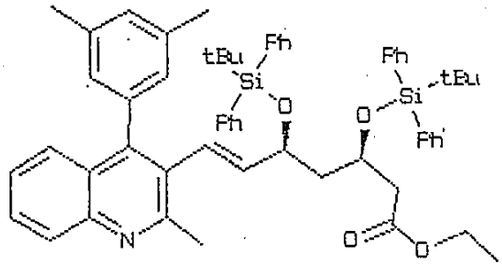
(6)



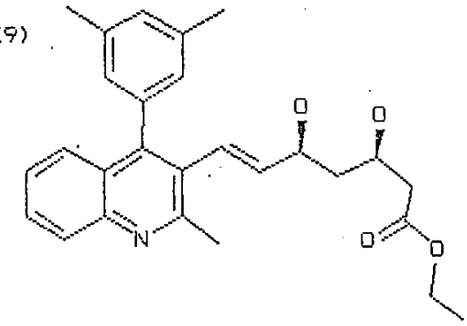
(7)



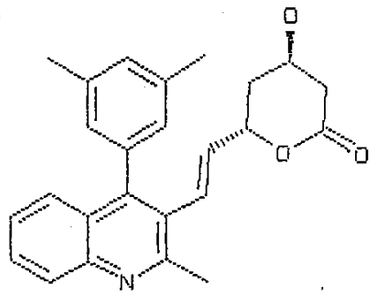
(8)



(9)



(10)



(1) 4H-3,1-Benzoxazine-4-one, 2-methyl-

(2) Acetamide, N-[2-(3,5-dimethylbenzoyl)phenyl]-

(3) Methanone, (2-aminophenyl)(3,5-dimethylphenyl)-
hydrochloride

(4) 3-Quinolinecarboxylic acid, 4-(3,5-dimethylphenyl)-2-methyl-
methyl ester

(5) 3-Quinolinemethanol, 4-(3,5-dimethylphenyl)-2-methyl-

(6) Quinoline, 3-chloromethyl-4-(3,5-dimethylphenyl)-2-methyl-

(7) Phosphonic acid, [[4-(3,5-dimethylphenyl)-2-methylquinolin-3-yl]methyl]-
diethyl ester

(8) 6-Heptenoic acid, 3,5-bis[[(1,1-dimethylethyl)diphenylsilyl]oxy]-7-[4-(3,5-dimethylphenyl)-2-methylquinolin-3-yl]-
ethyl ester, [(R*,S*)-(E)]-, (+,-)-

(9) 6-Heptenoic acid, 7-[4-(3,5-dimethylphenyl)-2-methylquinolin-3-yl]-3,5-dihydroxy-
ethyl ester, [(R*,S*)-(E)]-, (+,-)-

(10) 2H-Pyran-2-one, 6-[2-[4-(3,5-dimethylphenyl)-2-methylquinolin-3-yl]ethenyl]tetrahydro-
[trans-(E)]-, (+,-)-

Henry Mehl
9/5

Exhibit W

454 V

SANDOZ

PATENT AND TRADEMARK DEPARTMENT
Telex 240867
Telefax (201) 503-8807

DATE: December 14, 1988

TO: Dr. Sompong Wattanasin

FROM: Joanne M. Giesser

SUBJECT: Quinoline Analogs of Mevalonolactone and Derivatives Thereof

Enclosed is the first draft of the patent application entitled "Quinoline Analogs of Mevalonolactone and Derivatives Thereof". Please feel free to make any changes you think are appropriate and call me at X8420 so we can discuss the next draft.

Regards,

JMG:lmc
Enc.

85330/81A (Rev. 2)

Exhibit X

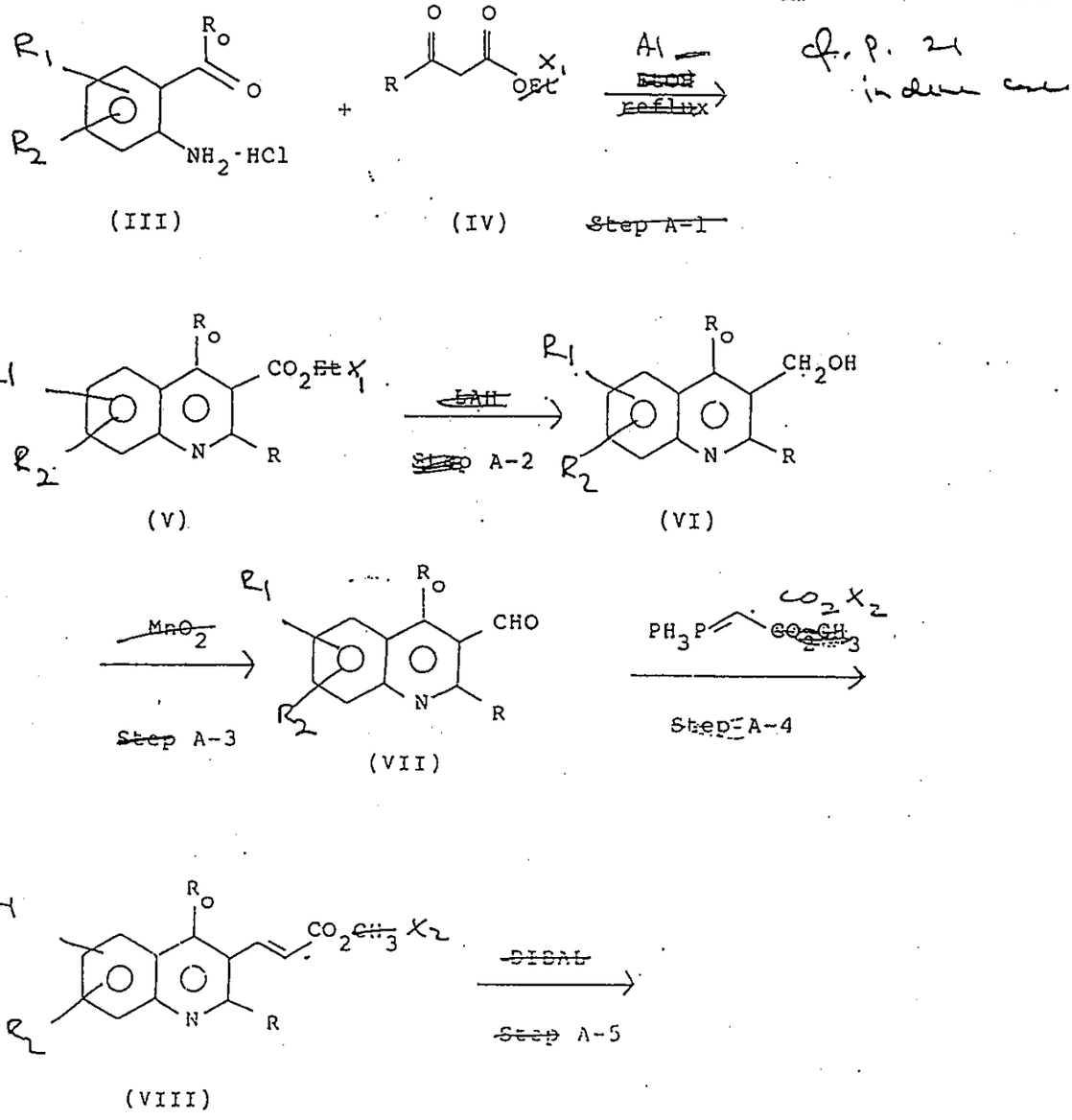
12/22/88

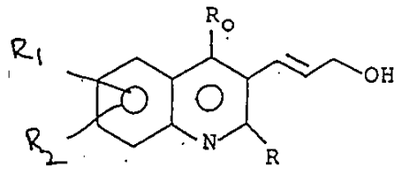
455

The compounds of both Formula I may be prepared according to the following Reaction Scheme A.

GENERAL REACTION SCHEME A

- general rx

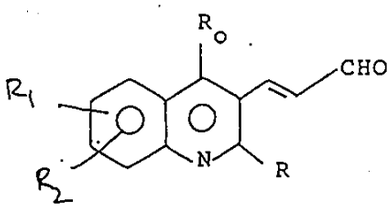




(IX)

MnO_2

Step A-6



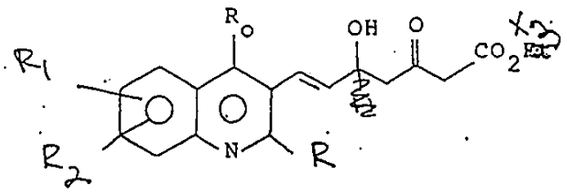
(X)

ethyl aceto acetate (Xa)

base

?

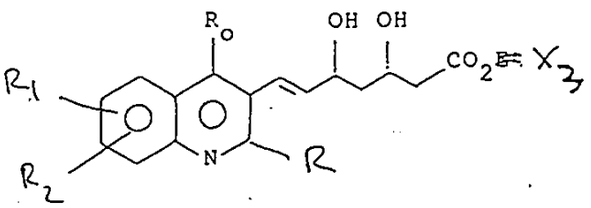
Step A-7



(XI)

reducing agent

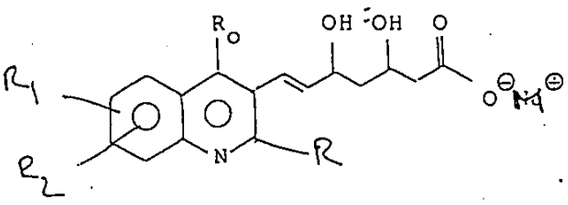
Step A-8



(XII)

NaOH

Step A-9

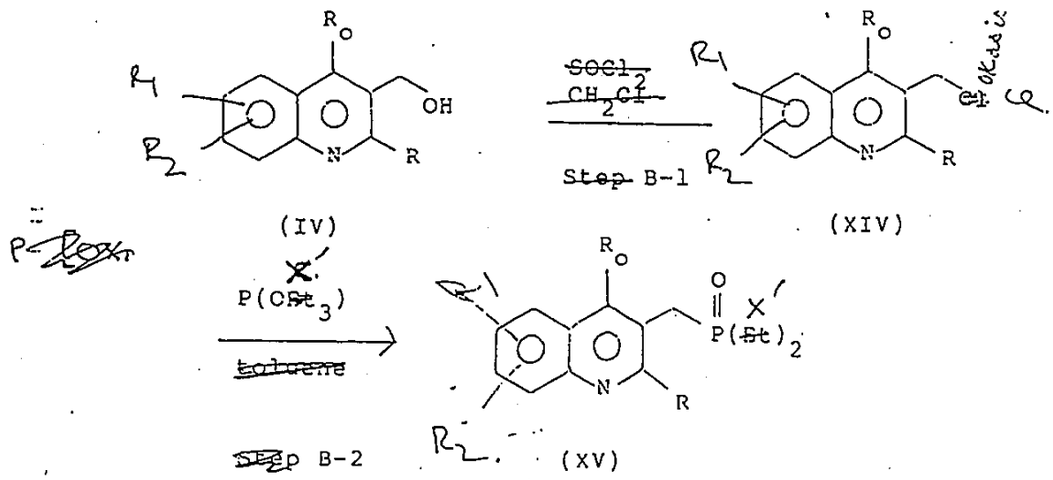


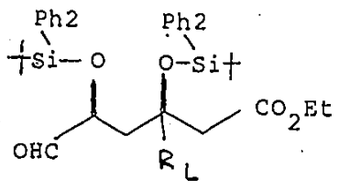
(XIII)

Starting material III is known and can be obtained by methods described by Morrison and Mulholland, 1958, J. Chem. Soc. p. 2702, which is hereby incorporated by reference. Next, V is reduced with lithium aluminum hydride, (LAH) to give VI. This reaction has also been described by Fehnel, 1968. J. Heterocyclic Chem 4:565, which is also hereby incorporated by reference. In Step A-3, VI is oxidized to VII. Step A-4 is a Wittig reaction producing VIII. Compound VIII is then reduced using diisobutylaluminum hydride (DIBAL) to IX. In Step A-6, IX is oxidized to X. The aldehyde X is then reacted with ethyl acetoacetate in Step A-7 to give XI. Compound XI is reduced to give XII. Next, in Step A-9, XII is hydrolyzed to the salt form XIII.

Compounds of both Formula I and II may be made according to Reaction Scheme B. Starting material for Reaction Scheme B is Compound VI from Reaction Scheme A.

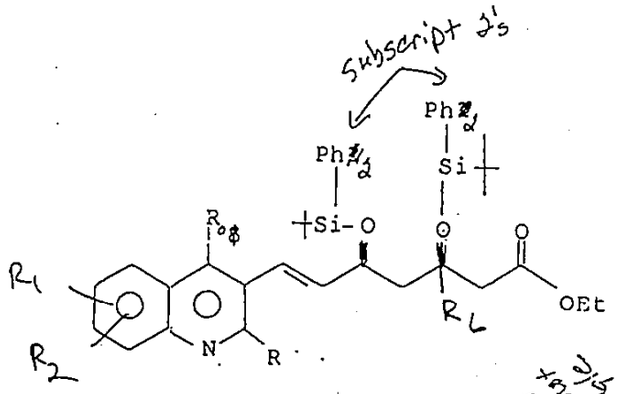
REACTION SCHEME B





~~nBu4NF/THF~~

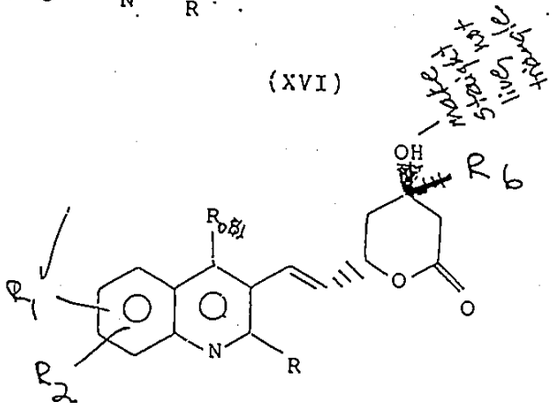
~~Step~~ B-3



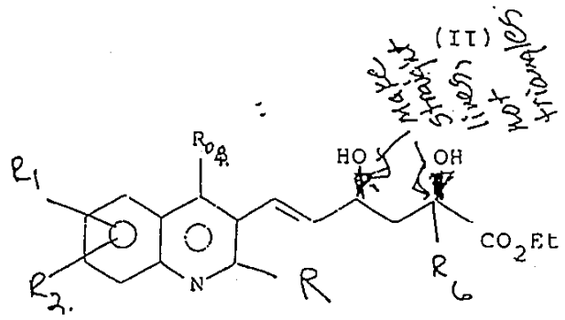
(XVI)

~~nBu4NF~~

~~Step~~ B-4



and



(I)

Exhibit Y

To: J. Glasser

From: S. Wattanasin

CASE 299/84

KW.

1/4/89.

459

The following changes are suggested:

1) On page 2, structure (b), change H (on C-3) to R6

2) On page 3,

- change H to R6 (as above)
- R like 19 and 20, R and R6 should be ~~independ~~ independently a C_{1-6} alkyl, and more preferably isopropyl and methyl or ring A
- Ring A preferably 3,4-dimethyl phenyl or 4-fluorophenyl

- ~~Preferably~~ Preferred definition for

  R_1 & R_2

  R_7

  H

- Page 4-7, reaction scheme A and B shld change to general schemes with general structures for starting materials, reagents and conditions (cf. P. 21 of the ~~Index~~ case; Cm # 600-2026). The specific reaction schemes can be shown as examples

later, preferably before the experimental procedures.

3) Page 8
- line 1 : delete "reduction and"
- line 3 : change "withig to Coupling"

4) Page 6
- line 1 : delete "is known and"
- line 4 : add "a reducing agent such as" after with
- line 9 : delete using diisobutylaluminum hydride (DIBAL)
- line 11 : change ~~ethyl~~ to alkyl
- change structure IV to VI

5) Page 7
- add R to structure I

6) Page 11 a ~~4~~.
- A-~~3~~1 :
- A-~~3~~2 :
- A-~~3~~3 :
- A-5 :
- ~~A-4~~ :

} See attached.

7) Page 14

- A-9
- B-2
- B-3
- add B-4

} see attached.

8) Page 15

- should show IC_{50} of ~~Ex~~ Sah - 64935 and 64936 with proper numbering referring numbers. (see attached list of IC_{50} - ED_{50})

9) Page 16

- should show ED_{50} of Sah - 63548 and 64935 with proper referring numbers.

10) Page 17

- The preferred compounds of the invention are 63548 and 64935 (see structures in the above list)

11) Page 24

- structure of the reagent in step A should be

- Add the hydrolysis procedure (see attached)

before example 2

12. Page 29

— add ethyl group to the 2-position of the structure of product.

13. Page 33

— No. 4 should be deleted or changed to cover ~~salt 63548~~ our key structures such as salt - 63548, 64935, 63366 and their sodium salts.

i.e.

R & R_0 is independently CH_3 and i-Pr or 3,5-dimethylphenyl and 4-fluorophenyl

14. Page 35

— in the lactone structure change H to R6.

B-3 (Coupling Reaction).

1) 1 – 1.2 moles strong base, pref. n-butyl lithium or lithium diisopropylamide per mole XV

-78 – 0° C

10 – 90 min

THF

2) 1 – 1.2 moles aldehyde per mole XV

-78 – 0° C

10 – 90 min

solvent same as step 1.

3) Quenched with, eg. acetic acid

-78 – 25° C

1 – 5 min

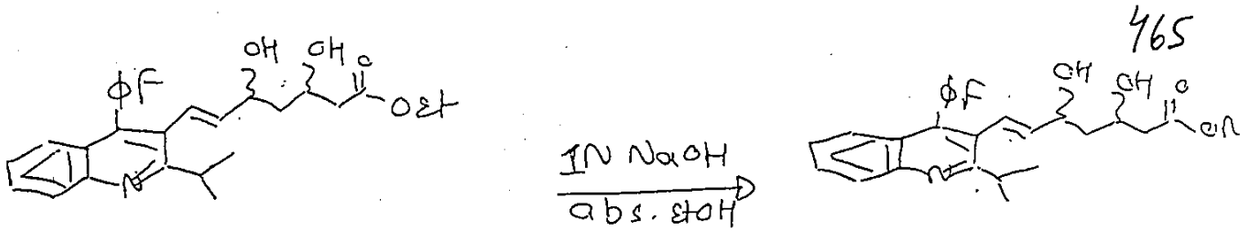
B-4 (Deprotection)

2 - 15 moles, pref. 4-10 moles, fluoride reagent, esp. tetra-n-butylammonium fluoride per mole XXII and 0.5 - 2 mole, pref. ~~1.2~~ 1.0 - 1.5 moles, glacial acetic acid per mole fluoride reagent

20 - 60° C

2 - 120 hrs.

Also, eg. ES, pref. THF, or mixture of ES, pref. THF, and acetonitrile.



To the sol^{n} of 100mg (0.00022172 mole) diol ester in 3 ml abs. EtOH was added 0.2173 ml (0.00021794 mole) 1 N NaOH dropwise at 0°C. After stirring at 0°C for 3 hrs, the reaction mixture was diluted with ether and evaporated in vacuo leaving yellow oil. On addition of ether, yellow solids came out which was then filtered, washed with ether and on drying gave 86.4 mg (87.5%) yellow solids M.P. > 225°C. NMR (CD_3OD, 500 MH_2) : 1.39, m, 1H; 1.35, d, 6H; 1.5, m, 1H; 2.13–2.3, m, 1H; 3.65, q, 1H; 3.75, m, 1H; 4.25, m, 1H; 5.45, dd, 1H; 6.59, d, 1H; 7.21, m, 5H, 7.36, m, 1H; 7.62, m, 1H; 8.05, d, 1H

EDD from Bob Eyestone
1/4/1989.

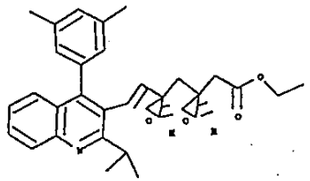
WATTANASIN_S

MET002

SPOOLED: 11/01/88 11:53 AM

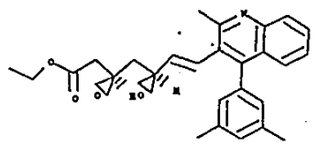
994

IC50 1.58 μM.



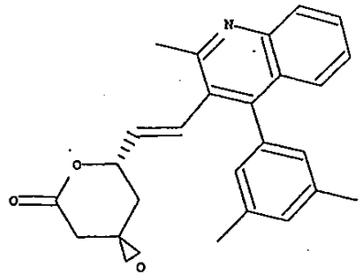
25496 (SAH-063366) SAH

IC50 3.77 μM.



26080 (SAH-063548) SAH

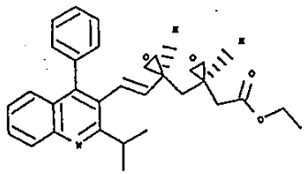
IC50 7.3 μM.



26082 (SAH-063549) SAH

No EH_{50} 2.576
acid to RE
1-11-89

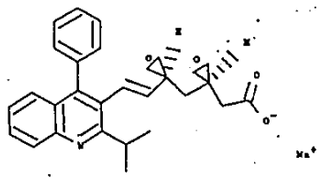
IC50 2.32 μM



> 1.0
30% @ 1.0 μM

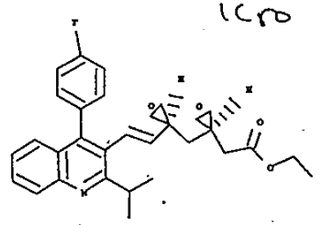
30441 (SAH-064933) SAH

IC50 2.61 μM.



30442 (SAH-064934) SAH

IC50 0.41 μM.



CD 60 0.49

30447 (SAH-064935) SAH

30448 (SAH-064936) SAH

>1.0

٩٪ ١,٥

١١٤٢ ٥.٤٣.

Exhibit Z

469

600-7013-C

United States Patent [19]

Damon, II

[11] Patent Number: 4,588,715

[45] Date of Patent: May 13, 1986

[54] HEPTENOIC ACID DERIVATIVES

[75] Inventor: Robert E. Damon, II, Wharton, N.J.

[73] Assignee: Sandoz, Inc., E. Hanover, N.J.

[21] Appl. No.: 616,720

[22] Filed: Jun. 4, 1984

[51] Int. Cl.[4] C07F 7/08; A61K 31/695

[52] U.S. Cl. 514/63; 549/214; 549/292; 556/441

[58] Field of Search 549/214, 292; 556/441; 424/184; 560/56; 514/63

[56] References Cited

U.S. PATENT DOCUMENTS

4,000,265	12/1976	Quilichini	424/184
4,255,444	3/1981	Oka et al.	549/292
4,262,013	4/1981	Mistui et al.	549/292
4,293,496	10/1981	Willard	549/292
4,361,515	11/1982	Terahara et al.	549/292
4,375,475	3/1983	Willard et al.	549/292
4,420,475	12/1983	Damon, II	424/184
4,472,426	9/1984	Hoffman et al.	549/292

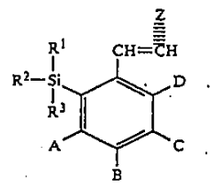
FOREIGN PATENT DOCUMENTS

7713317	5/1979	France	424/184

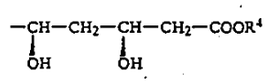
Primary Examiner—Alton D. Rollins
Assistant Examiner—D. L. Dinner
Attorney, Agent, or Firm—Gerald D. Sharkin; Richard E. Vila; Frederick H. Weinfeldt

[57] ABSTRACT

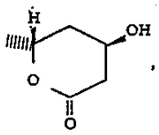
Compounds of the formula



wherein R^1, R^2 and R^3 are alkyl or aryl groups, A, B, C and D are non-reactive substituents or two are joined to form an additional ring, and Z is either of the formula Z':



wherein R^4 is H, lower alkyl or a cation; or a -6-oxotetrahydropyran-2-yl ring of the formula Z":



e.g. 4-hydroxy-6-{2-[2-(methyldiphenylsilyl)phenyl]ethenyl]ethyenyl}-tetrahydro-2H-pyran-2-one, (trans, trans). The compounds inhibit cholesterol biosynthesis and are useful as anti-atherosclerotic agents.

22 Claims, No Drawings

GDC-1013 470

United States Patent [19]

Wareing

[11] Patent Number: 4,613,610

[45] Date of Patent: Sep. 23, 1986

[54] CHOLESTEROL BIOSYNTHESIS INHIBITING PYRAZOLE ANALOGS OF MEVALONOLACTONE AND ITS DERIVATIVES

[75] Inventor: James R. Wareing, Randolph, N.J.

[73] Assignee: Sandoz Pharmaceuticals Corp., E. Hanover, N.J.

[21] Appl. No.: 741,903

[22] Filed: Jun. 6, 1985

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 623,393, Jun. 22, 1984, abandoned.

[51] Int. Cl.[4] A61K 31/415; C07D 231/12; C07D 405/06

[52] U.S. Cl. 514/406; 548/374; 548/378

[58] Field of Search 548/374, 378; 514/406

[56] References Cited

U.S. PATENT DOCUMENTS

3,983,140	9/1976	Endo et al.	549/292
4,198,425	4/1980	Mitsui et al.	549/292
4,248,889	2/1981	Oka et al.	560/56
4,255,444	3/1981	Oka et al.	549/292
4,308,378	12/1981	Stokker	549/292
4,351,844	9/1982	Patchett et al.	549/292
4,361,515	11/1982	Terahara et al.	549/292
4,375,475	3/1983	Willard et al.	549/292
4,376,863	3/1983	Lam	549/292
4,387,242	6/1983	Lam	560/119
4,440,927	4/1984	Prugh	549/292
4,474,971	10/1984	Wareing	549/214
4,503,072	3/1985	Hoffman et al.	514/529

FOREIGN PATENT DOCUMENTS

895445	4/1983	Belgium	549/292
38061	10/1981	European Pat. Off.	549/292
68038	1/1983	European Pat. Off.	549/292
56-7775	1/1981	Japan	549/292
WO84/02131	6/1984	PCT Int'l Appl.	548/467
WO84/02903	8/1984	PCT Int'l Appl.	549/292

OTHER PUBLICATIONS

Hulcher, Arch. Biochem. Biophys. 146, 422–427 (1971).

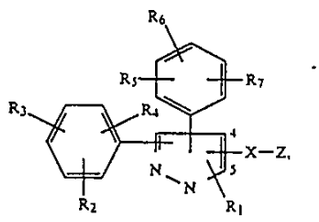
Sato et al., Chem. Pharm. Bull. 28, 1509–1525 (1980).

Singer et al., Proc. Soc. Exp. Biol. Med. 102, 370–373 (1959).

Primary Examiner—Richard A. Schwartz
Assistant Examiner—Kurt G. Briscoe
Attorney, Agent, or Firm—Gerald D. Sharkin; Richard E. Vila; Melvyn M. Kassenoff

[57] ABSTRACT

Compounds of the formula



wherein

R_1 is C_{1-6}alkyl not containing an asymmetric carbon atom,

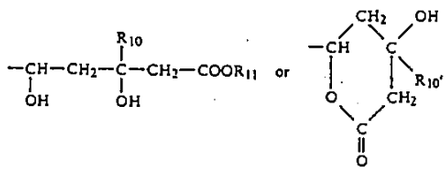
each of R_2 and R_5 is independently hydrogen, C_{1-3}alkyl, n-butyl, i-butyl, t-butyl, C_{1-3}alkoxy, n-butoxy, i-butoxy, trifluoromethyl, fluoro, chloro, phenyl, phenoxy or benzyloxy,

each of R_3 and R_6 is independently hydrogen, C_{1-3}alkyl, C_{1-3}alkoxy, trifluoromethyl, fluoro, chloro, phenoxy or benzyloxy,

each of R_4 and R_7 is independently hydrogen, C_{1-2}alkyl, C_{1-2}alkoxy, fluoro or chloro, with the provisos that not more than one of R_2 and R_3 is trifluoromethyl, not more than one of R_2 and R_3 is phenoxy, not more than one of R_2 and R_3 is benzyloxy, not more than one of R_5 and R_6 is trifluoromethyl, not more than one of R_5 and R_6 is phenoxy, and not more than one of R_5 and R_6 is benzyloxy,

X is $-(CH_2)_m-$, $-CH=CH-$, $-CH=CH-CH_2-$ or $-CH_2-CH=CH-$, wherein m is 0, 1, 2 or 3, and

Z is



wherein R_{10} is hydrogen or C_{1-3}alkyl, wherein R_{12} is a physiologically acceptable and hydrolyzable ester group, and

M is a pharmaceutically acceptable cation,

with the provisos that (i) the —X—Z group is in the 4- or 5-position of the pyrazole ring, and (ii) the R_1 group and the —X—Z group are ortho to each other,

the use thereof for inhibiting cholesterol biosynthesis and lowering the blood cholesterol level and, therefore, in the treatment of hyperlipoproteinemia and atherosclerosis, pharmaceutical compositions comprising such compounds and processes for and intermediates in the synthesis of such compounds.

27 Claims, No Drawings

471

600-6951/B/cont-US

United States Patent [19]

Kathawala

[11] Patent Number: 4,739,073

[45] Date of Patent: Apr. 19, 1988

[54] INTERMEDIATES IN THE SYNTHESIS OF INDOLE ANALOGS OF MEVALONOLACTONE AND DERIVATIVES THEREOF

[75] Inventor: Faizulla G. Kathawala, Mountain Lakes, N.J.

[73] Assignee: Sandoz Pharmaceuticals Corp., E. Hanover, N.J.

[21] Appl. No.: 707,854

[22] Filed: Mar. 4, 1985

Related U.S. Application Data

[63] Continuation of Ser. No. 548,850, Nov. 4, 1983, which is a continuation-in-part of Ser. No. 443,668, Nov. 22, 1982.

[51] Int. Cl.[4] C07D 405/06; C07D 209/12

[52] U.S. Cl. 548/406; 548/414; 548/494

[58] Field of Search 548/465, 467, 494, 468, 548/414, 406

[56] References Cited

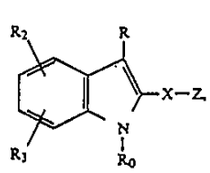
U.S. PATENT DOCUMENTS

3,259,633	7/1966	Metlesics et al.	548/493
4,248,889	2/1981	Oka et al.	514/532
4,255,444	3/1981	Oka et al.	514/460
4,272,533	6/1981	Gradient et al.	514/212
4,375,475	3/1983	Willard et al.	514/460
4,474,971	10/1984	Wareing	549/214

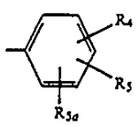
Primary Examiner—Donald G. Daus
Assistant Examiner—William A. Teoli, Jr.
Attorney, Agent, or Firm—Gerald D. Sharkin; Richard E. Vila; Melvyn M. Kassenoff

[57] ABSTRACT

Compounds of the formula



wherein one of R and R_o is



and the other is primary or secondary C_{1-6}alkyl not containing an asymmetric carbon atom, C_{3-6}cycloalkyl or phenyl$(CH_2)_m$—, wherein

R_4 is hydrogen, C_{1-3}alkyl, n-butyl, i-butyl, t-butyl, C_{1-3}alkoxy, n-butoxy, i-butoxy, trifluoromethyl, fluoro, chloro, phenoxy or benzyloxy,

R_5 is hydrogen, C_{1-3}alkyl, C_{1-3}alkoxy, trifluoromethyl, fluoro, chloro, phenoxy or benzyloxy,

R_{5a} is hydrogen, C_{1-2}alkyl, C_{1-2}alkoxy, fluoro or chloro, and

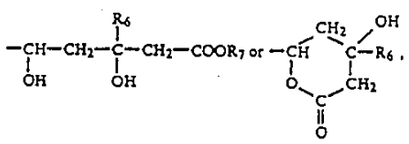
m is 1, 2 or 3, with the provisos that both R_5 and R_{5a} must be hydrogen when R_4 is hydrogen, R_{5a} must be hydrogen when R_5 is hydrogen, not more than one of R_4 and R_5 is trifluoromethyl, not more than one of R_4 and R_5 is phenoxy, and not more than one of R_4 and R_5 is benzyloxy,

R_2 is hydrogen, C_{1-3}alkyl, n-butyl, i-butyl, t-butyl, C_{3-6}cycloalkyl, C_{1-3}alkoxy, n-butoxy, i-butoxy, trifluoromethyl, fluoro, chloro, phenoxy or benzyloxy,

R_3 is hydrogen, C_{1-3}alkyl, C_{1-3}alkoxy, trifluoromethyl, fluoro, chloro, phenoxy or benzyloxy, with the provisos that R_3 must be hydrogen when R_2 is hydrogen, not more than one of R_2 and R_3 is trifluoromethyl, not more than one of R_2 and R_3 is phenoxy, and not more than one of R_2 and R_3 is benzyloxy,

X is $—(CH_2)_n—$ or —CH=CH—, wherein n is 0, 1, 2 or 3, and

Z is



wherein

R_6 is hydrogen or C_{1-3}alkyl, and

R_7 is hydrogen, C_{1-3}alkyl, n-butyl, i-butyl, t-butyl, benzyl or M, wherein M is a pharmaceutically acceptable cation,

the use thereof for inhibiting cholesterol biosynthesis and lowering the blood cholesterol level, and, therefore, in the treatment of hyperlipoproteinemia and atherosclerosis, pharmaceutical compositions comprising such compounds and processes for and intermediates in the synthesis of such compounds.

20 Claims, No Drawings

600-7050-US
472

United States Patent [19]

Anderson

[11] Patent Number: 4,751,235

[45] Date of Patent: Jun. 14, 1988

[54] ANTI-ATHEROSCLEROTIC INDOLIZINE DERIVATIVES

[75] Inventor: Paul L. Anderson, Randolph, N.J.

[73] Assignee: Sandoz Pharm. Corp., East Hanover, N.J.

[21] Appl. No.: 945,750

[22] Filed: Dec. 23, 1986

[51] Int. Cl.[4] A61K 31/435; C07D 471/04

[52] U.S. Cl. 514/299; 546/112

[58] Field of Search 546/112; 514/299

[56] References Cited

U.S. PATENT DOCUMENTS

4,248,889	2/1981	Oka et al.	424/308
4,255,444	3/1981	Oka et al.	424/279
4,375,475	3/1983	Willard et al.	549/292
4,474,971	10/1984	Wareing	549/214
4,520,026	5/1985	Rosseels et al.	546/112

FOREIGN PATENT DOCUMENTS

8402131	6/1984	PCT Int'l Appl. .
8402903	8/1984	PCT Int'l Appl. .
8600307	1/1986	PCT Int'l Appl. .
8603488	6/1986	PCT Int'l Appl. .

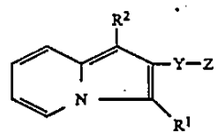
Primary Examiner—Richard A. Schwartz

Assistant Examiner—Bernard I. Dentz

Attorney, Agent, or Firm—Gerald D. Sharkin; Richard E. Vila; Melvyn M. Kassenoff

[57] ABSTRACT

7-(indolizin-2-yl)hept-6-enoic acids of the formula I:



I

wherein each of R^1 and R^2 is, independently, H, alkyl, cycloalkyl, aralkyl or aryl,

Y is $-CH=CH-$, or $-CH_2-CH_2-$; and

Z is $CH(OH)-CH_2-CH(OH)-CH_2COOR^8$

in which R^8 is H, an ester residue or cation; or the lactone thereof. The compounds are useful as hypocholesteremic agents.

20 Claims, No Drawings

473

600-7628/B/CONT

United States Patent [19]

Wareing

[11] Patent Number: 4,755,606

[45] Date of Patent: Jul. 5, 1988

[54] IMIDAZOLYL-3,5-DI-(DIPHENYL-BUTYL-SILYLOXY) CARBOXYLIC ACID ESTER INTERMEDIATES

[75] Inventor: James R. Wareing, Randolph, N.J.

[73] Assignee: Sandoz Pharm. Corp., E. Hanover, N.J.

[21] Appl. No.: 79,194

[22] Filed: Jul. 29, 1987

Related U.S. Application Data

[60] Division of Ser. No. 863,267, May 14, 1986, abandoned, which is a continuation-in-part of Ser. No. 736,679, May 22, 1985, Pat. No. 4,668,794.

[51] Int. Cl.[4] C07D 7/18

[52] U.S. Cl. 548/110

[58] Field of Search 548/110

[56] References Cited

U.S. PATENT DOCUMENTS

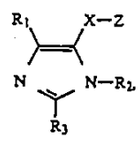
4,028,343	6/1977	Amort et al.	548/110 X
4,530,922	7/1985	Moberg	548/110 X
4,650,890	3/1987	Jewell et al.	556/446
4,677,211	6/1987	Jewell et al.	548/491

Primary Examiner—Richard A. Schwartz

Attorney, Agent, or Firm—Gerald D. Sharkin; Richard E. Vila; Melvyn M. Kassenoff

[57] ABSTRACT

Compounds of the formula



and the pharmaceutically acceptable acid addition salts thereof, wherein the various substituents are defined herein below,

the use thereof for inhibiting cholesterol biosynthesis and lowering the blood cholesterol level and, therefore, in the treatment of hyperlipoproteinemia and atherosclerosis, pharmaceutical compositions comprising such compounds and processes for and intermediates in the synthesis of such compounds.

12 Claims, No Drawings

474

600-7064-43

United States Patent [19]

Kathawala

[11] Patent Number: 4,822,799

[45] Date of Patent: Apr. 18, 1989

[54] PYRAZOLOPYRIDINE ANALOGS OF MEVALONOLACTONE AND DERIVATIVES THEREOF USEFUL FOR INHIBITING CHOLESTEROL BIOSYNTHESIS IN MAMMALS

[75] Inventor: Faizulla G. Kathawala, Mountain Lakes, N.J.

[73] Assignee: Sandoz Pharm. Corp., E. Honover, N.J.

[21] Appl. No.: 149,232

[22] Filed: Jan. 27, 1988

[51] Int. Cl.[4] C07D 471/04; A61K 31/395

[52] U.S. Cl. 514/303; 546/119

[58] Field of Search 546/119; 514/303

[56] References Cited

U.S. PATENT DOCUMENTS

4,248,889	2/1981	Oka et al.	424/308
4,255,444	3/1981	Oka et al.	424/279
4,293,496	10/1981	Willard	260/343.5
4,459,422	7/1984	Willard et al.	560/59
4,479,965	10/1984	Terahara et al.	424/279
4,571,428	2/1986	Kapa	556/437
4,588,715	5/1986	Damon	514/63
4,613,610	9/1986	Wareing	514/406
4,647,576	3/1987	Hoefle et al.	514/422
4,751,235	6/1988	Anderson	514/299
4,755,606	7/1988	Wareing	548/110

FOREIGN PATENT DOCUMENTS

84/02131 6/1984 World Int. Prop. O. .

87/02662 5/1987 World Int. Prop. O. .

OTHER PUBLICATIONS

Hulcher, Arch. Biochem. Biophys. 146, 422–427 (1971).

Sato et al., Chem. Pharm. Bull. (Tokyo) 28, 1509–1525 (1980).

Singer et al., Proc. Soc. Exp. Biol. Med. 102, 370–373 (1959).

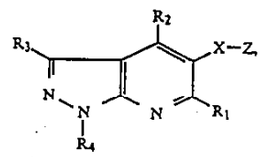
Primary Examiner—Mary C. Lee

Assistant Examiner—John A. H. Russell

Attorney, Agent, or Firm—Gerald D. Sharkin; Richard E. Vila; Melvyn M. Kassenoff

[57] ABSTRACT

Compounds of the formula



and processes for and intermediates in the synthesis thereof, pharmaceutical compositions comprising such a compound and the use of such compounds for inhibiting cholesterol biosynthesis and lowering the blood cholesterol level and, therefore, in the treatment of hyperlipoproteinemia and atherosclerosis.

20 Claims, No Drawings

475

600-7035/B

United States Patent [19]

Wareing

[11] Patent Number: 4,851,427

[45] Date of Patent: Jul. 25, 1989

[54] PYRROLE ANALOGS OF MEVALONOLACTONE, DERIVATIVES THEREOF AND PHARMACEUTICAL USE

[75] Inventor: James R. Wareing, Randolph, N.J.

[73] Assignee: Sandoz Pharm. Corp., E. Hanover, N.J.

[21] Appl. No.: 919,275

[22] Filed: Oct. 15, 1986

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 791,198, Oct. 25, 1985, abandoned.

[51] Int. Cl.[4] A61K 31/40; C07D 707/337; C07D 405/04; C07D 405/05

[52] U.S. Cl. 514/422; 514/427; 548/517; 548/562

[58] Field of Search 548/517, 562; 514/422, 514/427

[56] References Cited

U.S. PATENT DOCUMENTS

4,198,425	4/1980	Mitsui et al.	424/279
4,255,444	3/1981	Oka et al.	424/279
4,459,422	7/1984	Willard et al.	560/59
4,472,426	9/1984	Hoffman et al.	424/279
4,474,971	10/1984	Wareing	549/214
4,588,715	5/1986	Damon	514/63
4,613,610	9/1986	Wareing	514/406
4,647,576	3/1987	Hoefle et al.	514/422
4,681,893	7/1987	Roth	514/422

FOREIGN PATENT DOCUMENTS

11928	6/1980	European Pat. Off. .
179559	4/1986	European Pat. Off. .
2261965	6/1973	Fed. Rep. of Germany .
84/02131	6/1984	PCT Int'l Appl. .
84/02903	8/1984	PCT Int'l Appl. .

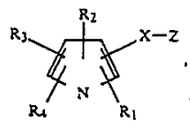
OTHER PUBLICATIONS

Hoffman et al., J. Med. Chem., 29, 159–169, (1986).
Hulcher, Arch. Biochem. Biophys., 146, 422–427, (1971).
Sato et al., Chem. Pharm. Bull., (Tokyo), 28, 1509–1525, (1980).
Singer et al., Proc. Soc. Exper. Biol. Med., 102, 370–373, (1959).
Stokker et al., J. Med. Chem., 28, 347–358, (1985).
Stokker et al., J. Med. Chem., 29, 170–181, (1986).

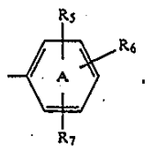
Primary Examiner—Richard L. Raymond
Attorney, Agent, or Firm—Gerald D. Sharkin; Richard E. Vila; Melvyn M. Kassenoff

[57] ABSTRACT

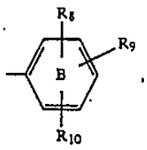
Compounds of the formula



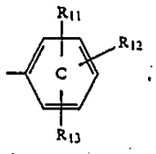
wherein R_1 is C_{1-6}alkyl not containing an asymmetric carbon atom, C_{3-7}cycloalkyl or



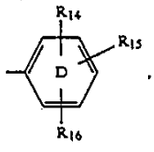
wherein R_5, R_6 and R_7 are as defined below,
R_2 is C_{1-6}alkyl not containing an asymmetric carbon atom, C_{3-7}cycloalkyl or



wherein R_8, R_9 and R_{10} are as defined below.
R_3 is hydrogen, C_{1-6}alkyl not containing an asymmetric carbon atom, C_{3-7}cycloalkyl or



wherein R_{11}, R_{12} and R_{13} are as defined below,
R_4 is hydrogen, C_{1-6}alkyl not containing an asymmetric carbon atom, C_{7-7}cycloalkyl or



wherein R_{14}, R_{15} and R_{16} are as defined below,
X is $-(CH_2)_m-$, $-CH=CH-$, $-CH=CH-CH_2-$ or $-CH_2-CH=CH-$, wherein m is 0, 1, 2 or 3, and
Z is

(Abstract continued on next page.)

476

600-6955/XN/B/cont# X

United States Patent [19]

Damon, II

[11] Patent Number: 4,876,280

[45] Date of Patent: Oct. 24, 1989

[54] ARYLCYCLOHEXANE AND ARYLCYCLOHEXENE ANALOGS OF MEVALONOLACTONE DERIVATIVES AND THEIR USE

[75] Inventor: Robert E. Damon, II, Wharton, N.J.

[73] Assignee: Sandoz Pharm. Corp., E. Hanover, N.J.

[21] Appl. No.: 166,356

[22] Filed: Mar. 10, 1988

[51] Int. Cl.[4] A61K 31/19; A61K 31/215

[52] U.S. Cl. 514/510; 549/292; 514/460; 514/532; 562/469

[58] Field of Search 549/292; 562/469

[56] References Cited

U.S. PATENT DOCUMENTS

3,983,140	9/1976	Endo et al.	260/343.5
4,248,889	2/1981	Oka et al.	424/308
4,375,475	3/1983	Willard et al.	424/279
4,474,971	10/1984	Wareing	549/214
4,499,289	2/1985	Baran et al.	549/292
4,650,890	3/1987	Jewell et al.	556/446
4,772,626	9/1988	Smith et al.	549/292

FOREIGN PATENT DOCUMENTS

86/03488	6/1986	PCT Int'l Appl. .	
1325056	8/1973	United Kingdom	562/469

OTHER PUBLICATIONS

Hulcher, Arch. Biochem. Biophys. 146, 422–427 (1971).

Singer et al., Proc. Soc. Exp. Biol. Med. 102, 370–373 (1959).

Stokker et al., J. Med. Chem. 28, 347–358 (1985).

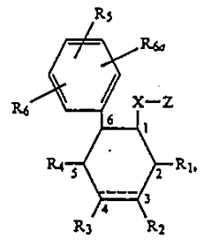
Primary Examiner—Robert T. Bond

Assistant Examiner—B. A. Trinh

Attorney, Agent, or Firm—Gerald D. Sharkin; Richard E. Vila; Melvyn M. Kassenoff

[57] ABSTRACT

Compounds of the formula



wherein R_1 is hydrogen, C_{1-3}alkyl, n-butyl, i-butyl or t-butyl,

R_2 is hydrogen or C_{1-3}alkyl,

R_3 is hydrogen or C_{1-3}alkyl,

R_4 is hydrogen, C_{1-3}alkyl, n-butyl, i-butyl or t-butyl,

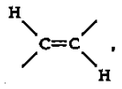
R_5 is hydrogen, C_{1-3}alkyl, n-butyl, i-butyl, t-butyl, C_{1-3}alkoxy, n-butoxy, i-butoxy, fluoro, chloro, trifluoromethyl, phenoxy or benzyloxy,

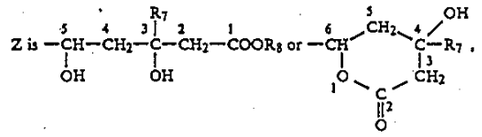
R_6 is hydrogen, C_{1-3}alkyl, C_{1-3}alkoxy, fluoro, chloro, trifluoromethyl, phenoxy or benzyloxy, with the provisos that not more than one of R_5 and R_6 is trifluoromethyl, not more than one of R_5 and R_6 is phenoxy, and not more than one of R_5 and R_6 is benzyloxy, or

R_5 and R_6 are attaohed to adjacent carbon atoms and taken together form a radical of the formula —CH=CH—CH=CH—,

R_{6a} is hydrogen, C_{1-2}alkyl, fluoro or chloro,

X is —CH_2CH_2— or





wherein R_7 is hydrogen or C_{1-3}alkyl, and

R_8 is hydrogen, R_9 or M, wherein R_9 is a physiologically acceptable ester group, and M is a pharmaceutically acceptable cation, and the broken line represents a double (π) bond or two hydrogen atoms (one on each carbon atom), the use thereof for inhibiting cholesterol biosynthesis and lowering the blood cholesterol level and, therefore, in the treatment of hyperlipoproteinemia and atherosclerosis, pharmaceutical compositions comprising such compounds and processes for and intermediates in the synthesis of such compounds.

13 Claims, No Drawings

600-7022/C

477

United States Patent [19]

Kathawala et al.

[11] Patent Number: 5,001,255

[45] Date of Patent: Mar. 19, 1991

[54] IDENE ANALOGS OF MEVALONOLACTONE AND DERIVATIVES THEREOF

[75] Inventors: Faizulla G. Kathawala, Mountain Lakes; Sompong Wattanasin, Hopatcong, both of N.J.

[73] Assignee: Sandoz Pharm. Corp., E. Hanover, N.J.

[21] Appl. No.: 214,560

[22] Filed: Jul. 1, 1988

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 837,479, Mar. 7, 1986, abandoned, which is a continuation-in-part of Ser. No. 677,917, Dec. 4, 1984, abandoned.

[51] Int. Cl.[5] C07C 69/76

[52] U.S. Cl. 560/56; 560/53; 556/441; 549/264; 549/291; 562/462; 562/466

[58] Field of Search 560/56, 53; 549/264, 549/291; 562/462, 466; 514/530, 569

[56] References Cited

U.S. PATENT DOCUMENTS

3,207,779	9/1965	Cutler	560/56
3,532,752	10/1970	Shen	560/56
3,668,241	6/1972	Cragoe et al.	560/56
3,983,140	9/1976	Endo et al.	260/343.5
4,006,180	2/1977	Cragoe et al.	560/56
4,012,524	3/1977	Cragoe et al.	560/56
4,057,573	11/1977	Haas et al.	560/56
4,070,539	1/1978	Cragoe et al.	560/56
4,125,731	11/1978	Sugie et al.	560/56
4,137,322	1/1979	Endo et al.	424/273
4,198,425	4/1980	Mitsui et al.	424/279
4,248,889	2/1981	Oka et al.	424/308
4,255,444	3/1981	Oka et al.	424/279
4,308,378	12/1981	Stokker	542/441
4,361,515	11/1982	Terahara et al.	549/292
4,375,475	3/1983	Willard et al.	424/279
4,474,971	10/1984	Wareing	549/214
4,588,715	5/1986	Damon	514/63
4,613,610	9/1986	Wareing	514/406
4,647,576	3/1987	Hoefle	514/422
4,654,363	3/1987	Prugh	560/56

FOREIGN PATENT DOCUMENTS

142146	5/1985	European Pat. Off. .
84/02131	6/1984	PCT Int'l Appl. .
84/02903	8/1984	PCT Int'l Appl. .
86/03488	6/1986	PCT Int'l Appl. .

OTHER PUBLICATIONS

Hulcher, Arch. Biochem. Biophys. 146, 422–427 (1971).

Sato et al., Chem. Pharm. Bull. 28, 1509–1525 (1980).

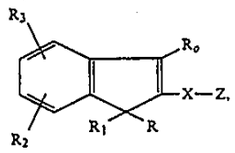
Singer et al., Proc. Soc. Exp. Biol. Med. 102, 370–373 (1959).

Primary Examiner—Paul J. Killos

Attorney, Agent, or Firm—Gerald D. Sharkin; Richard E. Vila; Melvyn M. Kassenoff

[57] ABSTRACT

Compounds of the formula



the use thereof for inhibiting cholesterol biosynthesis and lowering the blood cholesterol level and, therefore, in the treatment of hyperlipoproteinemia and atherosclerosis, pharmaceutical compositions comprising such compounds and processes for and intermediates in the synthesis of such compounds.

27 Claims, No Drawings

Case No. 600-7101/CONT/Int.
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference No. 102,648 — #82
Examiner-in-Chief: M. Sofocleous

WATTANASIN
v.
FUJIKAWA et al.
v.
FUJIKAWA et al.

Interference No. 102,975 — #27
Examiner-in-Chief: M. Sofocleous

FYI

FEB 24 1993

RECEIVED IN
BOX INTERFERENCE

NOTICE OF THE FILING OF WATTANASIN CONSOLIDATED AFFIDAVIT TESTIMONY (VOL. IV) PURSUANT TO 37 CFR 1.672

Appended is Volume IV of the consolidated affidavit testimony of the party Wattanasin for the above-numbered interferences.

These papers are being filed pursuant to the EIC decision dated February 5, 1993 in the above-numbered interferences (Int. No. 102,648, Paper No. 77; Int. No. 102,975, Paper No. 22).

Respectfully submitted,

Diane Furman

Diane E. Furman
Attorney for the Party Wattanasin
Registration No. 31,104
201-503-7332

SANDOZ CORPORATION
59 Route 10
E. Hanover, NJ 07936

DEF:rmf
February 22, 1993

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on February 22, 1993
(Date of Deposit)
Diane E. Furman
Name of applicant, assignee, or Registered Representative
Diane Furman
Signature
February 22, 1993
Date of Signature

(cont'd)

Enclosures: Volume IV (pages 356-477)

(pages 356-381)
Supp. Declaration of S. Wattanasin
Declaration of M. Kassenoff
Declaration of J. Giesser
Declaration of L. Rothwell
Supp. Declaration of R. Engstrom
Declaration of L. Chesley

(pages 382-477)
Exhibits M-1, M-2, M-3, M-4 and M-5
Exhibit N
Exhibit O
Exhibits P-1, P-2, P-3
Exhibit Q
Exhibit R
Exhibit S
Exhibit T
Exhibits U-1, U-2
Exhibits V-1,V-2
Exhibit W
Exhibit X
Exhibits Y-1, Y-2
Exhibit Z

. . .

CERTIFICATE OF SERVICE

It is hereby certified that a true copy of the paper entitled:

NOTICE OF THE FILING OF WATTANASIN CONSOLIDATED AFFIDAVIT TESTIMONY (VOL. IV) PURSUANT TO 37 CFR 1.672

together with the declarations and exhibits appended to said paper, were served on counsel for the party Fujikawa et al., this 22nd day of February, 1993 by postage pre-paid first-class mail addressed to the following:

Oblon, Spivak, McClelland, Maier & Neustadt, P.C.
Attn: Steven B. Kelber, Esq.
1755 South Jefferson Davis Highway
Crystal Square 5, Ste. 400
Arlington, VA 22202

Diane Furman

Diane E. Furman

Feb. 22, 1993

ERRATA SHEET

Name of case: Wattanasin v. Fujikawa et al.
Deposition of: Sompong Wattanasin
Date taken: March 22, 1993
Page 1/1

PAGE	LINE	CHANGE	REASON
33	16	Change "hardware" to "pathway".	My best recollection is that I actually spoke the term "pathway," and not "hardware". Also, I would not have said "hardware" because it makes no sense in this context.
46, 46	13 15	Change "and" to "an".	This is an obvious typographical error. The proper word is obviously "an"; the word "and" makes no sense in this context.
38,	5	Change "1988" to "1985".	I believe that the question actually referred to "1985", and that the date of "1988" appears to be a typographical error, since it refers to the date of 5/7/85 on page 37, l. 22.

Sompong Wattanasin,
SOMPONG WATTANASIN
4/20/93

SUBSCRIBED AND SWORN TO BEFORE ME
This 20th day of April, 1993

Antoinette Lombardi
A Notary Public

ANTOINETTE LOMBARDI
Notary Public of New Jersey
My Commission Expires April 3, 1994

49-111-0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	
	:	INTERFERENCE NO.: 102,648
V.	:	EXAMINER-IN-CHIEF:
FUJIKAWA ET AL	:	MICHAEL SOFOCLEOUS

FUJIKAWA ET AL REQUEST FOR
CROSS-EXAMINATION

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

BOX INTERFERENCE

SIR:

Responsive to the filing of Wattanasin Consolidated Affidavit Testimony (Volume IV) bearing a filing date of February 22, 1993, Fujikawa hereby requests cross-examination of the following Affiants:

1. Sompong Wattanasin
2. Melvyn M. Kassenoff
3. Joanne M. Giesser

4. Linda Rothwell

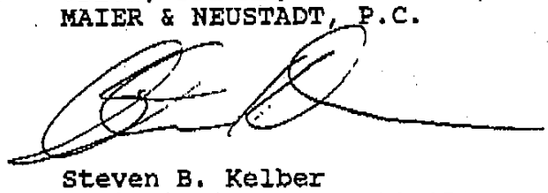
5. Lorraine M. Chesley

The cross-examination of Robert G. Engstrom will not be required.

The cross-examination will be as to all Declarations submitted by Sompong Wattanasin in this Interference. The remaining declarants are believed confined to Volume IV.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Steven B. Kelber
Registration No.: 30,073
Attorney for Fujikawa et al

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN

v.

FUJIKAWA et al.

Interference Nos. 102,648, 102,975

#28

Examiner in Chief: M. Sofocleous

APPROVED

3/19 1993

Examiner-in-Chief

JOINT REQUEST FOR EXTENSION OF TIME

The parties Wattanasin and Fujikawa et al. jointly request an extension of time in which to complete taking of cross-examination and rebuttal testimony, as well as an extension of the dates currently set for taking subsequent action, in the above interferences.

The EIC and the parties have been in agreement that cross-examination of the junior party Wattanasin's affiants may run concurrently with the rebuttal testimony of senior party Fujikawa. The current closing date for cross-examination and rebuttal is set for March 25, 1993.

Fujikawa et al. have noticed five Wattanasin affiants for cross-examination, and will also take rebuttal testimony from one non-party witness.

However, owing to other commitments of the involved parties and their witnesses, it has been necessary to tentatively defer the dates for taking rebuttal testimony and certain of the cross-examination until after the current closing date of March 25, 1993[1], pending decision on this motion.

Therefore, the parties now jointly move to reset the relevant dates in the above interferences as follows:

Cross-examination of Wattanasin affiants to close April 15, 1993.
Rebuttal testimony for Fujikawa ..... to close April 15, 1993.
Filing and serving of the record due ..... May 15, 1993.
Wattanasin opening brief due ..... June 15, 1993.
Fujikawa brief due ..... July 15, 1993.
Wattanasin reply brief due ..... August 4, 1993.

Undersigned counsel for the party Wattanasin has discussed this matter with EIC Sofocleous, who indicated he would be agreeable to resetting the dates as set forth above. The courtesy of the EIC is gratefully acknowledged.

---

1. The rebuttal testimony of Dr. Holmlund is tentatively set for March 26, 1993, and cross-examination of Joanne M. Giesser, Esq. is tentatively scheduled for April 9, 1993. The cross-examination of the other Wattanasin affiants will be held on March 22, 1993.

Accordingly, grant of this joint motion is respectfully requested.

Respectfully submitted,

Diane Furman 3/17/93

Diane E. Furman
Attorney for the party Wattanasin
Registration No. 31,104
201-503-7332

Steven B. Kelber
Attorney for the party Fujikawa et al.
Registration No. 30,073
(703) 413-3000

Attorney Docket No. - 600-7101-US

07 3187

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

Transmitted herewith is a patent application

of: Sompong Wattanasin

For Quinoline Analogs of Mevalonolactone and Derivatives Thereof

Also enclosed are a return postcard and

A. ☐ ____ Sheets of drawing included with the application.

B. ☐ A check in the amount of $_____, to cover the filing fee, calculated as follows:

Basic Filing Fee		=	$ 340
Multiple Dependent Claim Fee	$110	=	0
Total Number of Claims	10 -20 x $12	=	0
Total Number of Independent Claims	1 - 3 x $34	=	0
	TOTAL FILING FEE		$ 340.

The Commissioner is hereby authorized to charge to Deposit Account No. 19-0134

1. ☒ $340 Basic filing fee, if not enclosed herewith.

2. ☒ $340 Basic filing fee plus fee for all other claims submitted on filing, if not enclosed herewith.

3. ☒ The issue fee, but only when a signed issue fee transmittal form is filed, if not covered by a valid check.

4. ☒ All other required fees not referred to in 1, 2 and 3 above, e.g., fees for claims added during prosecution, for extensions of time, for petitions, and for appeals, if not covered by a valid check.

The Commissioner is likewise authorized to credit any overpayment to said Deposit Account. A duplicate of this sheet is appended.

Respectfully submitted,

Joanne M. Giesser

Joanne M. Giesser
Attorney/Agent of Record
(201) ~~XXX~~ 503-8420
Registration No. 32,838

SANDOZ CORP.
59 Route 10
E. Hanover, NJ 07936

Enclosure: As Noted

DATE: March 3, 1989

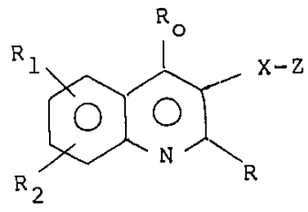
JMG:lmc

SUBMITTED IN DUPLICATE

601

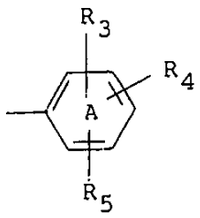
QUINOLINE ANALOGS OF MEVALONOLACTONE AND DERIVATIVES THEREOF

This invention relates to compounds of the formula



(A)

wherein each of R and R_o is, independently C_{1-6}alkyl (primary, secondary or tertiary), C_{3-7}cycloalkyl or ring A

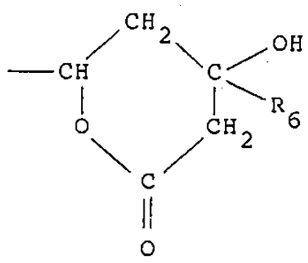


each of R_1, R_2, R_3, R_4 and R_5 is, independently hydrogen, C_{1-4}alkyl, C_{1-4}alkoxy, trifluoromethyl, fluoro, chloro, phenoxy, benzyloxy or hydroxy; with the provisos that not more than one of R_1 and R_2 is trifluoromethyl, not more than one of R_1 and R_2 is phenoxy, not more than one of R_1 and R_2 is benzyloxy, not more than one of R_1 and R_2 is hydroxy, not more than one of R_3-R_5 is the trifluoromethyl, not more than one of R_3-R_5 is phenoxy, not more than one of R_3-R_5 is benzyloxy and not more than one of R_3-R_5 is hydroxy;

X is $-(CH_2)_2-$ or $-CH=CH-$ (cis and/or trans);

Z is $-Q-CH_2-\underset{\underset{OH}{|}}{\overset{\overset{R_6}{|}}{C}}-CH_2-CO_2R_7$ (a)

or



(b) ;

wherein Q is $-\overset{\overset{O}{\|}}{C}-$ or $-\overset{\overset{OH}{|}}{CH}-$

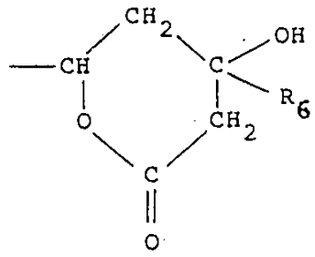
with the proviso that Q may be $-\overset{\overset{O}{\|}}{C}-$ only when X is -CH=CH

and/or R_6 is C_{1-3}alkyl;

R_6 is hydrogen or C_{1-3}alkyl;
R_7 is hydrogen, R_8 or M;
R_8 is a physiologically acceptable and hydrolyzable ester group; and
M is a pharmaceutically acceptable cation.

The term "pharmaceutically acceptable and hydrolyzable ester group" means a group which, together with the -COO- radical to which it is attached, forms an ester group which is physiologically acceptable and hydrolyzable under physiological conditions to yield a compound of Formula A wherein R_7 is hydrogen and an alcohol which itself physiologically acceptable, i.e. non-toxic at the desired dosage level, and which, preferably, is free of centers of asymmetry. Examples of such groups are C_{1-3}alkyl, n-butyl, i-butyl, t-butyl, and benzyl, collectively referred to as R_{8a}.

Compounds of this invention may conveniently be categorized into two groups, depending on the value of Z. Compounds where Z is $-CH(OH)-CH_2-CH(OH)-CH_2-COOR_7$ will be referred to as compounds of Formula I. Compounds where Z is



will be referred to as compounds of Formula II.

The compounds of the present invention have two centers of asymmetry (the two carbon atoms bearing the hydroxy groups when Z is (a), and the carbon atom bearing the hydroxy group and the carbon atom having the free valence when Z is (b) provided that R_7 is free of centers of asymmetry). Thus there are four stereoisomeric forms (enantiomers) of each compound (two racemates or pairs of diastereoisomers). The four stereoisomers may be designated as the R,R, R,S, S,R, and S,S enantiomers, all four stereoisomers being within the scope of this invention. When R_7 contains one or more centers of asymmetry, there are eight or more stereoisomers. When Q is -CH(OH)-, then each compound has one center of asymmetry (the carbon atom bearing the hydroxy group and R_6), and therefore, there are two enantiomers of each compound, provided that R_7 does not contain any center of asymmetry. The two stereoisomers may be designated as the 3R and 3S isomers. If R_7 contains one or more centers of asymmetry, then there are four or more centers of asymmetry.

As between otherwise identical compounds of Formula A, those where the Z group is a) are generally preferred over those where the Z group is b). For compounds where Z is a),

the erythro isomers are generally preferred over the threo isomers, erythro and threo referring to the relative positions of the hydroxy groups in the 3- and 5- positions of the group a). When Z is b), the trans lactones are generally preferred over the cis lactones, cis and trans referring to the relative positions of R_6 and the hydrogen atom in the 6- position of the group b) (adjacent to the O in the ring).

The preferred stereoisomers of the compounds having only two centers of asymmetry wherein X is -CH=CH- and Z is a) are the 3R, 5S and 3R,5R isomers and the racemate of which each is a constituent, i.e. the 3R,5S-3S,5R (erythro) and 3R,5R-3S,5S (threo) racemates, with the 3R,5S isomer and the racemate of which it is a constituent being more preferred and the 3R,5S isomer being most preferred.

The preferred stereoisomers of the compounds of Formula I having only two centers of asymmetry wherein X is b) are the 4R,6R and 4R,6S isomers and the racemate of which each is a constituent, i.e., the 4R,6R-4S,6S (trans lactone) and the 4R,6S-4S,6R (cis lactone) racemates, with the 4R,6R isomer and the racemate of which it is a constituent being more preferred and the 4R,6R isomer being most preferred.

These preferences also apply to compounds having more than two centers of asymmetry and represent the preferred configurations of the indicated positions.

Preferred compounds of this invention are the following.

R_1 and R_2 are preferably hydrogen;

one of R and R_o is preferably C_{1-6}alkyl, more preferably isopropyl or methyl, and the other is preferably Ring A, more preferably phenyl, 4-fluorophenyl or 3,5-dimethylphenyl; most preferably R is the alkyl group and R_o is Ring A;

X is preferably -CH=CH-, most preferably (E)-CH=CH- ;

Z is preferably (a) wherein Q is -CH(OH)-

or (b), most preferably the former,

Q is preferably -CH(OH)- ;

R_6 is preferably hydrogen;

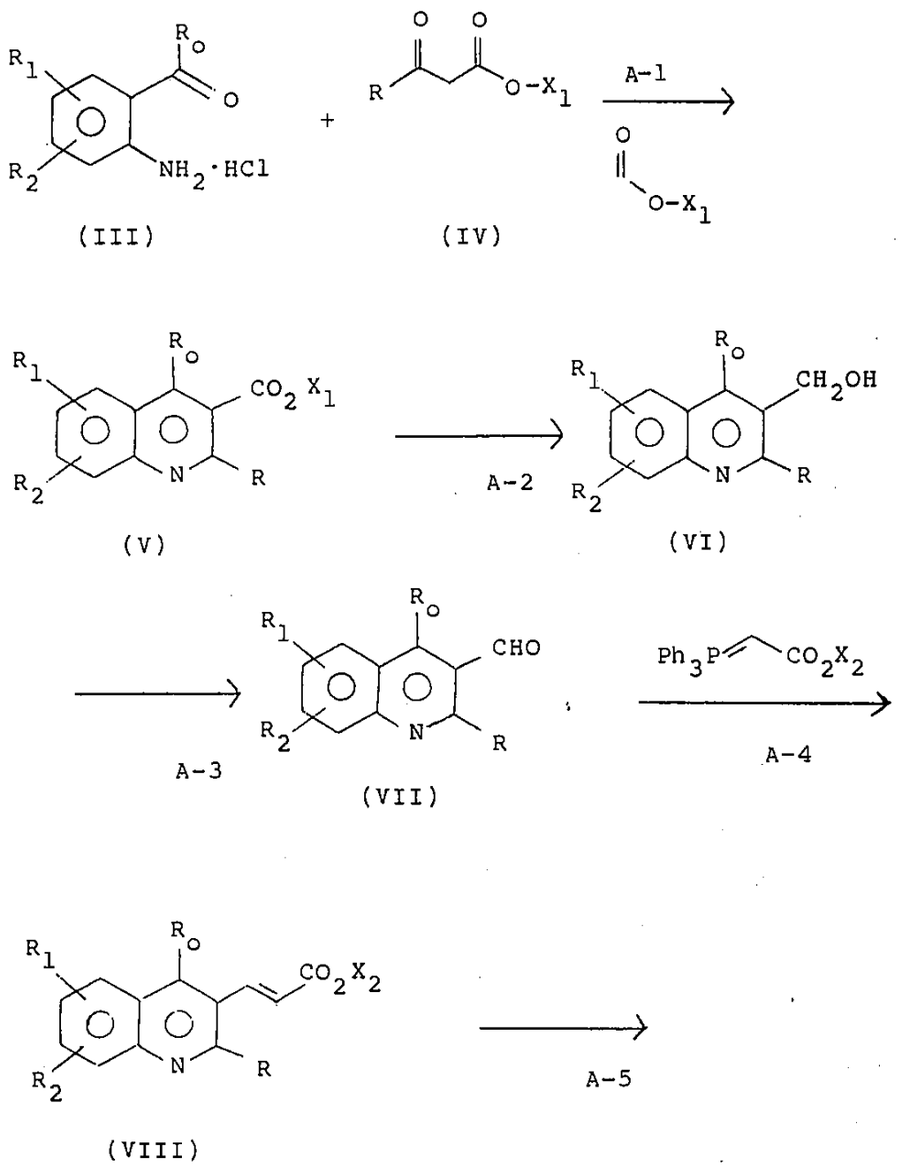
R_7 is preferably hydrogen, M or C_{1-2}alkyl; most preferably M or C_{1-2}alkyl;

R_8 is preferably methyl or ethyl;

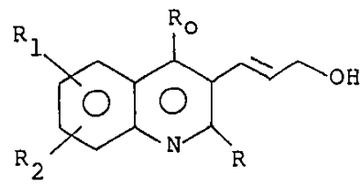
M is preferably Na^+, K^+ or NH_4^+, most preferably Na^+.

Specific compounds of Formula I may be prepared according to the following preferred Reaction Scheme A. It should be noted in the following Reaction Schemes, that if any compound of Formula A contains a hydroxy group as R_1-R_5, then the hydroxy group should be protected by e.g. a diphenyl-t-butyl silyl group (in compounds of formula III-XI and XIV-XVI). The group is cleaved at the end of the synthesis by Reaction B-4 (detailed below).

REACTION SCHEME A

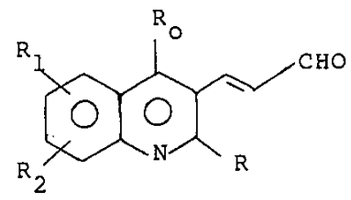


Case No. 600-7101-US



(IX)

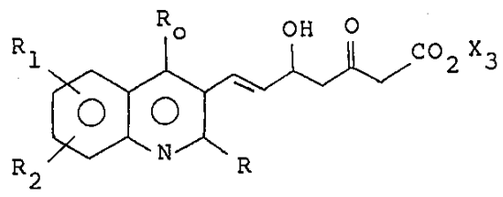
A-6



(X)

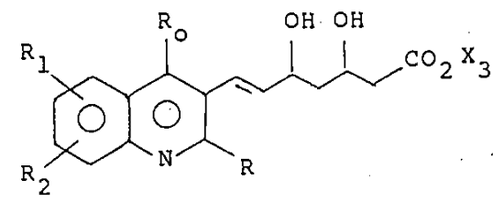
Xa

A-7



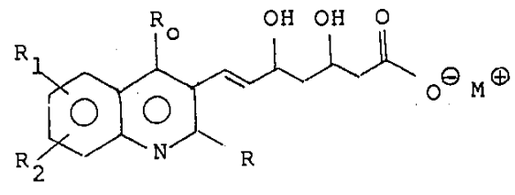
(XI)

A-8



(XII)

A-9

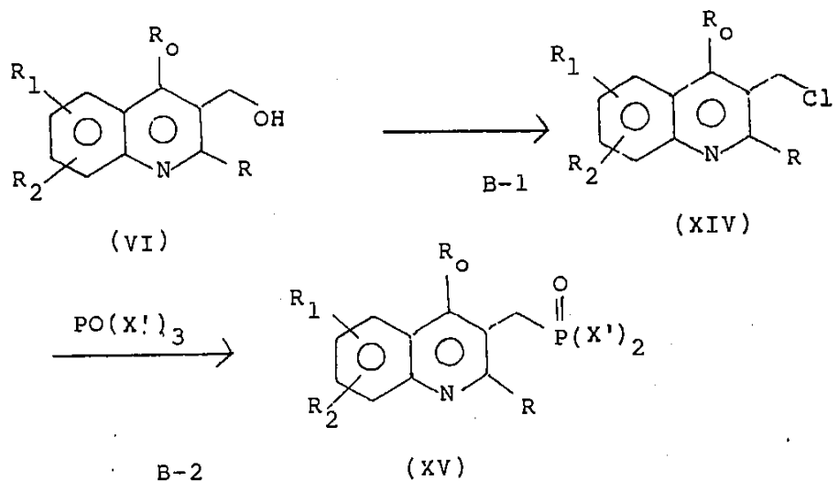


(XIII)

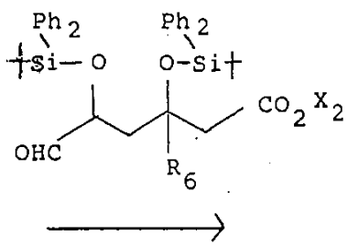
Starting material III can be obtained by methods described by Morrison and Mulholland, 1958, J. Chem. Soc. p. 2702, which is hereby incorporated by reference. Next, V is reduced to give VI. This reaction has also been described by Fehnel, 1968. J. Heterocyclic Chem 4:565, which is also hereby incorporated by reference. In Step A-3, VI is oxidized to VII. Step A-4 is a Wittig reaction producing VIII. Compound VIII is then reduced to IX. In Step A-6, IX is oxidized to X. The aldehyde X is then reacted with an acetoacetate in Step A-7 to give XI. Compound XI is reduced to give XII. Next, in Step A-9, XII is hydrolyzed to the salt form XIII.

Compounds of both Formula I and II may be made according to Reaction Scheme B. Starting material for Reaction Scheme B is Compound VI from Reaction Scheme A.

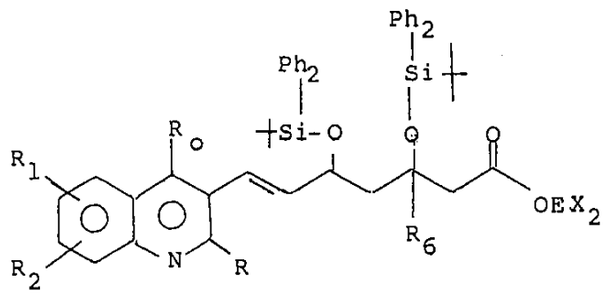
REACTION SCHEME B



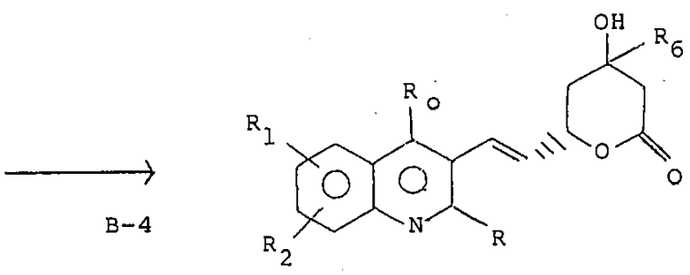
Case No. 600-7101-US



B-3



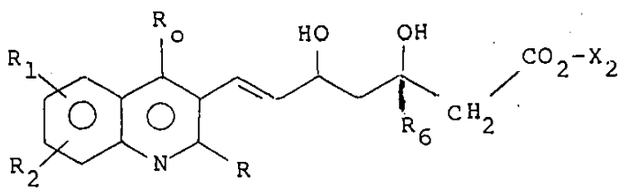
(XVI)



B-4

and

(II)

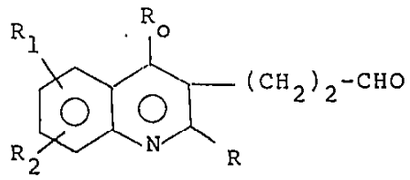


(I)

Step B-1 is a chlorination of Compound IV to yield XIV. Next, the phosphonate (XV) is made. In Step B-3, a coupling reaction forms Compound XVI. This product is then deprotected in Step B-4 to yield Compounds of Formulae I and II.

REACTION SCHEME C

Compounds of formula A wherein X is $-(CH_2)_2-$ and Z is (a) where R_6 is hydrogen may be synthesized by the following reactions:

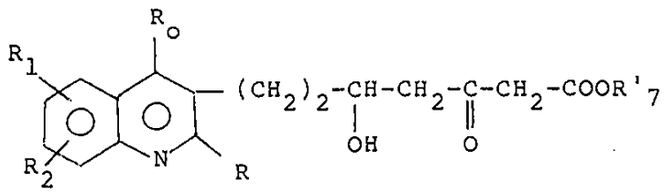


XVII

C-1

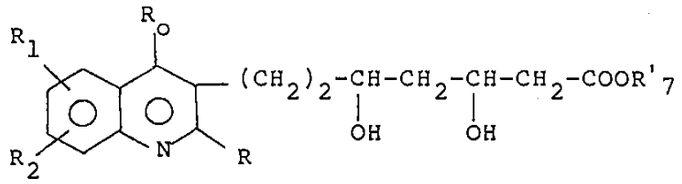
1) base + $CH_3-CO-CH_2-COOR'_{12}$ (XVIII)

2) aldehyde of XVII



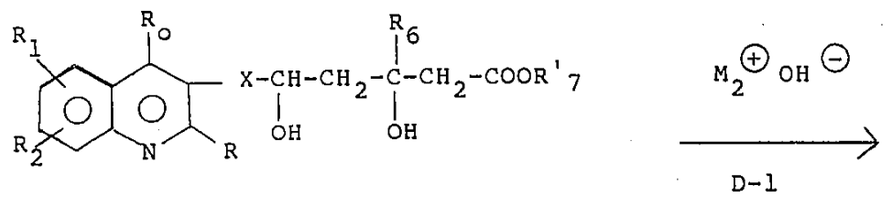
XIX

C-2

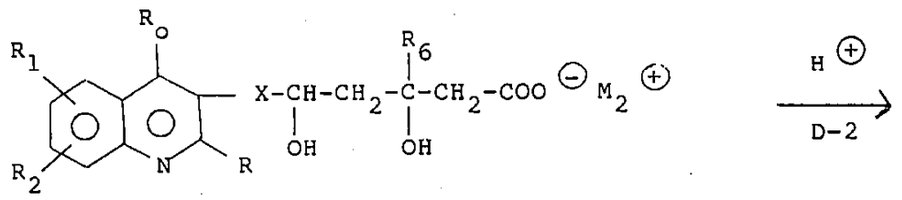


XX

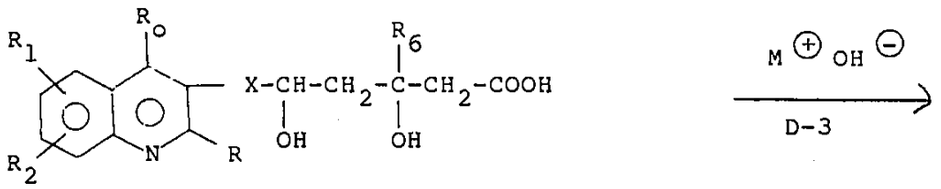
REACTION SCHEME D



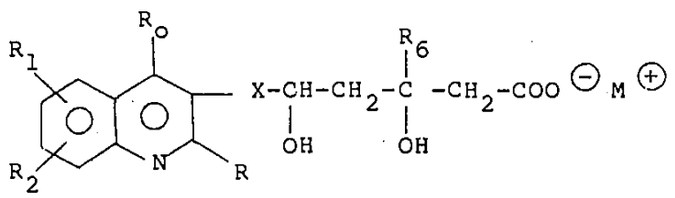
(XXI)



(XXII)



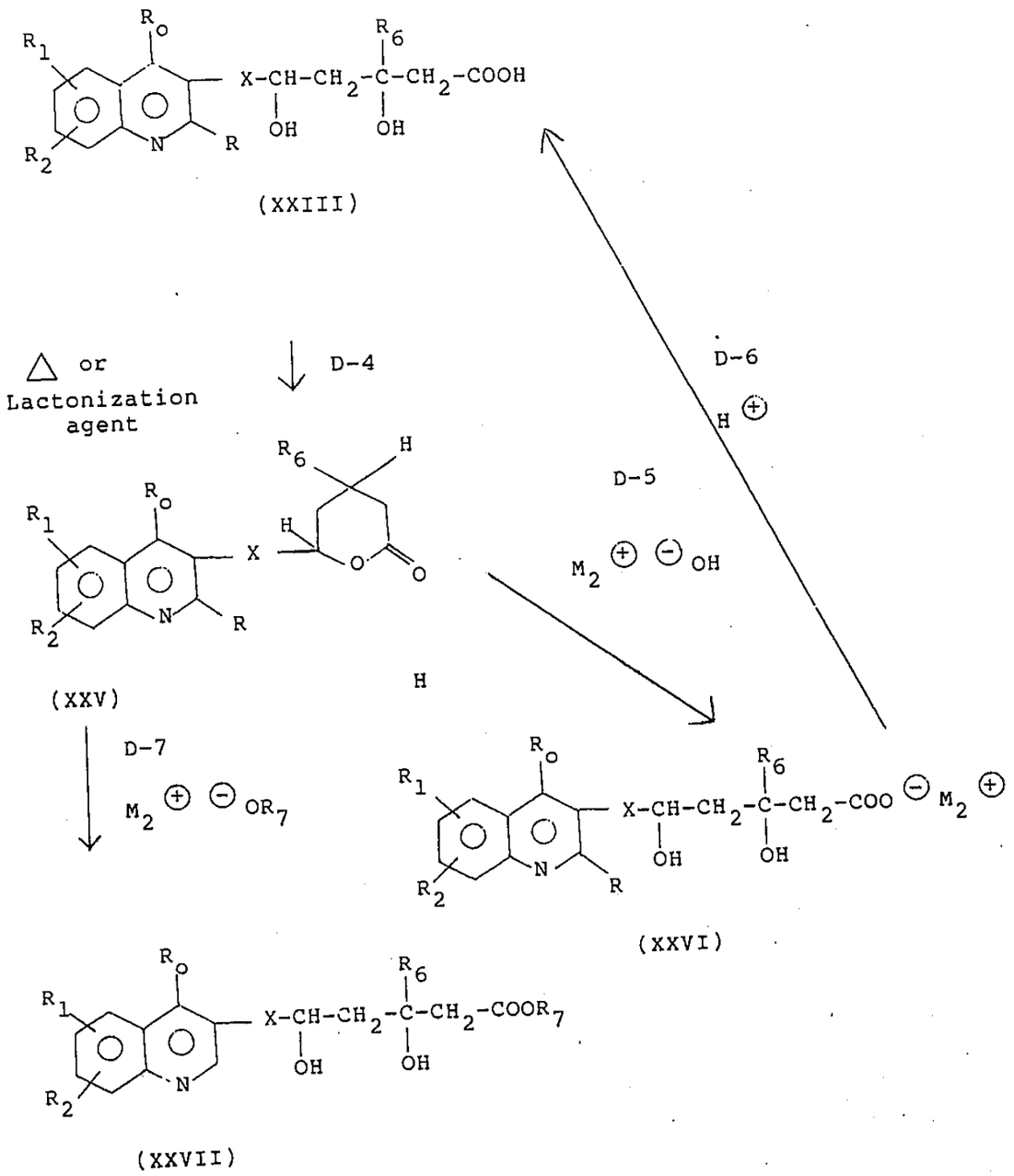
(XXIII)



(XXIV)

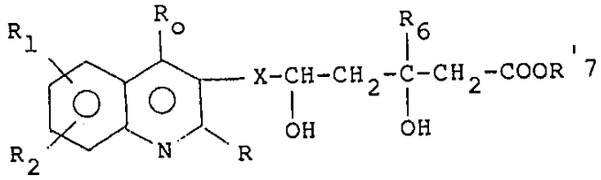
Case No. 600-7101-US

REACTION SCHEME D (continued)



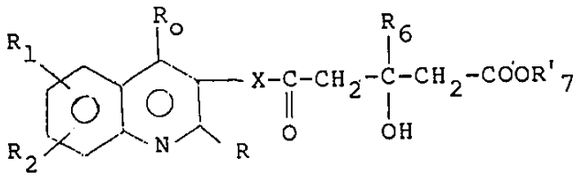
Case No. 600-7101-US

REACTION SCHEME E



(XXI)

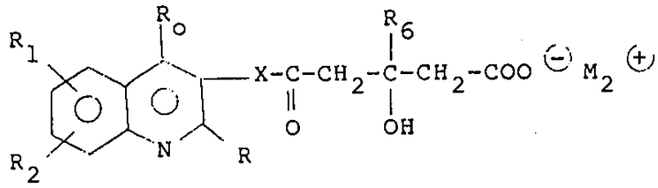
E-1



(XXVIII)

$M_2 \oplus \ominus OH$

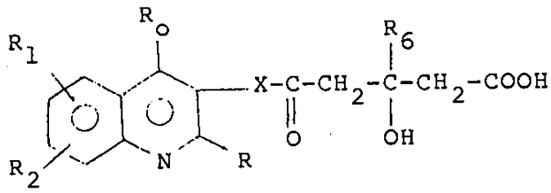
E-2



(XXIX)

$H \oplus$

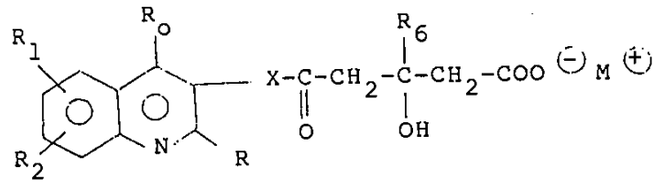
E-3



(XXX)

$M \oplus \ominus OH$

E-4



(XXXI)

In the foregoing reaction schemes,

X_1 may be any alkyl group, especially C_{1-2}alkyl;

X_2 may be C_{1-3} alkyl, n-butyl, i-butyl, t-butyl or benzyl;

X_3 may be any alkyl group, preferably X_2 and most preferably C_{1-2}alkyl;

X' may be ethyl or methyl;

X_a is an acetoacetate, alkyl or benzyl ester, preferably ethyl acetoacetate; and

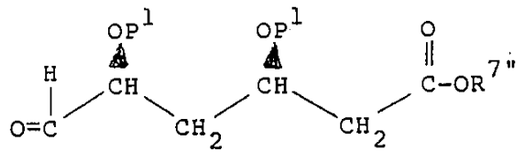
R_6 may be as defined above.

R'_7 is C_{1-3}alkyl, n-butyl, i-butyl, t-butyl or benzyl, more preferably C_{1-3}alkyl, and most preferably C_{1-2}alkyl, especially ethyl.

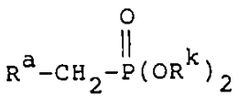
Particular reaction conditions for Reaction Schemes A and B are presented below. In this table, the following abbreviations are used:

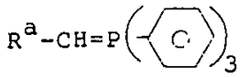
AIO = anhydrous inert organic solvent
ES = ether solvent, for example, diethyl ether, 1,2-diethoxyethane, 1,2-dimethoxyethane, tetrahydrofuran and mixtures thereof
esp. = especially
HC = hydrocarbon solvent, for example, benzene, toluene, xylene and mixtures thereof
HLA = halogenated lower alkane solvent, for example, carbon tetrachloride, chloroform, 1,1-dichloroethane, 1,2-dichloroethane, methylene chloride and 1,1,2-trichloroethane, usually preferably methylene chloride
hr. (hrs.) = hour(s)
IO = inert organic solvent
min. = minutes
pref. = preferably, preferred
THF = tetrahydrofuran

Compounds of high optical purity are obtainable by a multi-step procedure involving carrying out a Wittig reaction between a 1) 3R,5S-dihydroxy-diprotected aldehyde of the formula W1:

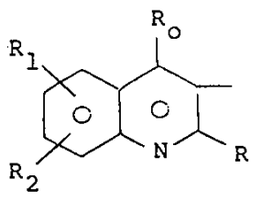
W1 

in which $R^{7"}$ is a C_{1-3} alkyl, n-butyl, i-butyl, t-butyl or benzyl, preferably methyl or ethyl, and P' is a protective group, i.e. a trisubstituted silyl radical in which the substituents are bulky groups, e.g. aryl or tertiary-aryl, such as diphenyl-tert.-butyl-silyl, and 2) a Wittig reagent of the formula W2 or W3:

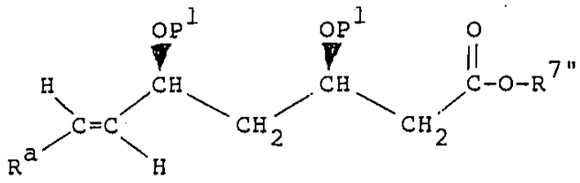
 W2 or

 W3 or

where R^a is a quinolinyl moiety of the formula



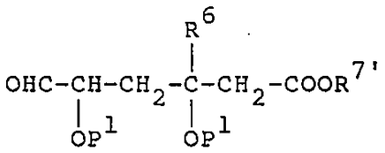
where R_a, R_o, R_1 and R_2 are as defined above and R^k is methyl or ethyl, to obtain a corresponding intermediate of the formula W4:



W4

in which R^a, $R^{7"}$, and P^1 are as defined above; and deprotecting the resulting compound W4 to obtain the corresponding quinoline.

The process may also be employed to obtain all the compounds of Formula I where Q is $-CH(OH)-$, and X is $-CH=CH-$ by reacting appropriate compounds of the formula W1':



W1'

with a reagent of formula W2 or W3. The former has a tendency to give the trans olefin exclusively although some cis olefin may be obtained, whereas the latter gives a mixture of cis and trans olefins, but predominantly the trans olefin. Compounds of the formula W1' are disclosed in United States Patent 4,613,610. The obtained olefinic compounds may be hydrogenated analogously to the hydrogenation reactions of said patent to obtain the corresponding compounds of Formula I where X is $-CH_2-CH_2-$.

Most of the molar amounts (ratios) given in the following table are merely exemplary and may be varied, as is evident to one of ordinary skill in the art. For example, in a reaction of two compounds one of which is readily available and one of which isn't, an excess of the readily available compound may be used to drive the reaction further towards completion (unless the use of an excess would increase the synthesis of an undesired compound).

Likewise, most of the temperature ranges given in the following table are merely exemplary, and it is within the ability of one of ordinary skill in the art to vary those that are not critical.

The reaction times set forth in the following table are also merely exemplary and may be varied. As is well-known, the reaction time is often inversely related to the reaction temperature. Generally, each reaction is monitored by, for example, thin layer chromatography and is terminated when at least one starting material is no longer present, when it appears that no more of the desired product is being formed, etc.

Conventional work-up procedures have generally been omitted from the table.

As utilized in the following table, the term "solvent" embraces mixtures of solvents and implies that the reaction medium is a liquid at the desired reaction temperature. It should, therefore, be understood that not all of the solvents listed for a particular reaction may be utilized for the entire recited temperature range. It should also be understood that the solvent must be at least substantially inert to the reactants employed, intermediates generated and end products under the reaction conditions utilized.

The term "inert atmosphere", as utilized in the following table, means an atmosphere that does not react with any of the reactants, intermediates or end products or

otherwise interfere with the reaction. While a carbon dioxide atmosphere is suitable for certain reactions, the inert atmosphere is usually dry nitrogen, helium, neon, argon or krypton, or a mixture thereof, and most often dry nitrogen, to maintain anhydrous conditions. Most reactions, including those where the use of an inert atmosphere is not specified, are carried out under an inert atmosphere, usually dry nitrogen, for convenience.

In the preceding table, n-butyllithium is preferably employed as a 1.3-1.7M. solution in hexane, and lithium diisopropylamide is preferably prepared *in situ* from n-butyllithium and diisoipropylamine.

Reaction Step	Reagents, Molar Ratios and Comments	Temperature	Time	Solvent	Inert Atmospher
A-1 Condensation with a β-Keto ester	EtOH X_1 is any alkyl group 1-1.2 moles IV per mole III	80-100°C	1-5 hrs.	Lower alkanol, pref. ethanol	Yes
A-2 reduction	Strong metal hydride reducing agent, e.g. lithium aluminum hydride or diiso-butylaluminum hydride; at least 2 equivalents, pref. 2.5-5 equivalents, of transferable hydride per mole V, e.g. at least 0.5 mole, pref. 1-1.25 moles, lithium aluminum hydride or at least 2 moles, pref. 2.5-5 moles diisobutylaluminum hydride per mole V.	20 to -80°C	0.3-4 hrs.	AIO, pref. ES, eg. THF, or diethyl ether, HLA, esp. methylene chloride, or mixture of HLA and toluene	Yes
A-3 oxidation	5-50 moles, pref. 7-25 moles manganese dioxide (pref. activated) per mole VI	20°-120°C., pref. 110°	2-72 hrs., pref. 3-5 hrs.	IO, pref. HLA, esp. methylene chloride or HC, esp. toluene	-
A-4 Wittig	1-1.3 moles $Ph_3P=CH-COOX_2$, preferably $Ph_3P=CH-COOCH_3$, per VII	50°C.-reflux, pref. 60°-115°C., esp. 90°-115°C.	3-8 hrs., pref. 4-8 hrs.	AIO, pref. E.S. THF, or HC esp. toluene	Yes Yes
A-5 Reduction	Same as A-2				

Reaction Step	Reagents, Molar Ratios and Comments	Temperature	Time	Solvent	Inert Atmosphere
A-6 Oxidation	5-50 moles, pref. 7-25 moles manganese dioxide (pref. activated) per mole IX	20°-80°C., pref. pref. 20°-25°C.	2-72 hrs., pref. 12-48 hrs.	IO, pref. HLA, esp. methylene chloride or HC, esp. toluene	-
A-7	1) Generation of dianion of Xa; 1 mole Xa and 2-2.2 equivalents strong base, pref. 1-1.1 moles sodium hydride then 1-1.1 moles n-butyl-lithium or 2-2.2 moles lithium di-isopropylamide.	-50°-10°C., pref. -30°-5°C.	0.3-1.5 hrs.	AIO, e.g., ES, pref. THF	Yes
	2) 1-2.5 moles, pref. 1.2-2.2 moles, more pref. 1.3-2.0 moles, of dianion of Xa (assuming 100% conversion of Xa to its dianion) per mole X. Product (XI) is racemic.	-80°-0°C., pref. -60°-0°C., more pref. -30° - -10°C.	0-3-4 hrs., pref. 0.3-2 hrs.	Same as Step 1	Yes
	3) Quench with, for example, ammonium chloride solution or 1N. hydro-chloric acid	-80°-25°C.	1-5 min.	Same as Step 1	-

Reaction Step	Reagents, Molar Ratios and Comments	Temperature	Time	Solvent	Inert Atmosphere
A-8	a) Non-stereoselective: 1-4, pref. 2-4, equivalents of transferable hydride per mole XI, pref. sodium borohydride or complex of t-butylamine and borane. When a racemic XI is utilized, product XII is a mixture of all four possible stereo-isomers (the erythro and threo racemates) wherein the ratio of the erythro stereo-isomers to the threo stereo-isomers ranges from 3:2 to 2:3.	-10^{o}-30^{o}C.	1-8 hrs.	IO, e.g., lower alkanol, esp. ethanol	Yes
	b)1) 1-1.3 moles, pref. 1.02-1.3 moles, tri-(primary or secondary C_{2-4} alkyl)borane, pref. triethyl-borane, and, pref., 0.3-8 liters, e.g., 0.75-6.5 liters, air (at 25°C. and 760 mm. Hg.) per mole XI	0^{o}-50^{o}C., pref. 0^{o}-25°C.	0.5-6 hrs., pref. 1-3.5 hrs.	AIO, pref. ES, esp. THF, or pref., mixture of THF and methanol, more pref. a 3-4:1 mixture	Yes

Reaction/Type	Reagents, Molar Ratios and Comments	Temperature	Time	Solvent	Inert Atmosphere
A-8 (Reduction) (Cont'd)	2) 0.4-3.5 moles, pref. 1.5-2.5 moles, sodium borohydride per mole IX. After the reaction, quench the reaction mixture with, for example, 1N. hydrochloric acid at -78°- -20°C. and isolate the crude product by extracting with a suitable inert organic solvent (e.g., diethyl ether) and evaporating the solvent at reduced pressure. It is pref. to crystallize the cyclic boron ester, if possible. If the reaction mixture is quenched with water instead of acid, product of this step may be a mixture containing the boron ester and a compound of Formula XXII.	-100°- -40°C., pref. -100°- -70°C.	2-48 hrs., pref. 16-48 hrs.	Same as Step 1	Yes
	3) large excess of anhydrous methanol, e.g., 50-500 moles per mole IX, or a mixture of methanol (e.g., 10-20 l. per mole IX), hydrogen peroxide (e.g., 4-8 l. of 30% aqueous hydrogen peroxide per mole IX), and a pH 7-7.2 aqueous phosphate buffer (pref. 6-10 l. of a pH 7 aqueous phosphate buffer (e.g., 0.054M. sodium, 0.024M. potassium and 0.047M. phosphate) per mole IX). The amount of buffer must be sufficient to maintain a pH of 7-7.2. Dissolve product of Step 2 in methanol and add buffer and aqueous hydrogen peroxide. See Narasaka et al., Tetrahedron 40, 2233-2238 (1984).	20°-40°C., pref. 20°-25°C., with methanol alone and -30°- 25°C., pref. -10°-10°C., with a mixture of methanol, hydrogen peroxide and buffer	0.7-60 hrs., pref. 4-60 hrs., with methanol alone and 0.5-2 hrs. with a mixture of methanol, hydrogen peroxide and buffer	Neat	-

Reaction Step	Reagents, Molar Ratios and Comments	Temperature	Time	Solvent	Inert Atmosphere
A-9 Hydrolysis	0.95-1.05 equivalent, pref. 0.96-0.98 equivalent NaOH per mole XII when XIII is the desired end product	0^{o}-75^{o}C., pref. 20^{o}-25^{o}C	0.5-3 hrs.	Inert aqueous organic eg. mixture of water and lower alkanol, pref. a mixture of water and methanol or esp. ethanol	-
B-1 Haolgenation	1-2 moles, pref. 1.3-1.8 moles of $SOCl_2$ per mole VI	-10^{o}-80^{o}C	2-18 hrs.	AIO, pref. ES, eg. diethyl ether or THF, HLA, eg. methylene chloride or HC, e.g. benzene	-
B-2	a) 1-1.1 moles $PO(X')_3$ e.g. $P(OEt)_3$ per mole XIV. Can use excess P (OEt_3) as solvent. b) Phosphenium variation	20^{o}-140^{o}C, usually 100^{o}-140^{o}C	6-24 hrs., usually 10-16 hrs.	HC, eg. benzene toluene, or xylene or neat (excess is solvent) $P(OEt_3)$	Yes
B-3 Coupling reaction	1) 1-1.2 moles strong base, pref. n-butyl lithium or lithium diiso-propylamide per mole XV. 2) 1-1.2 moles aldehyde per mole XV 3) Quenched with, e.g. acetic acid	-78-0^{o}C -78-0^{o}C -78-25^{o}C	10-90 min. 10-90 min. 1-5 min.	THF THF -	
B-4 deprotection	2-15 moles, pref. 4-10 moles, fluoride reagent, esp. tetra-n-butylammonium fluoride, per mole XVI and 0.5-2 moles, pref. 1.0-1.5 moles glacial acetic acid per mole fluoride reagent	20-60^{o}C	2-120 hrs.	AIO, e.g. ES, pref. THF or mixture of ES, pref. THF, and acetonitrile	

Reaction/Type	Reagents, Molar Ratios and Comments	Temperature	Time	Solvent	Inert Atmos-phere
C-1	1) Generation of dianon of XVII: 1 mole XVIII and 2-2.2 equivalents strong base, pref. 1-1.1 moles sodium hydride then 1-1.1 moles n-butyllithium or 2-2.2 moles lithium diisopropylamide.	-50°C-10°C, pref. -30-5°C	0.3-1.5 hours	AIO, e.g. ES, pref. THF	Yes
	2) 1-2.5 moles, pref. 1.2-2.2 moles, more pref. 1.3-2.0 moles of dianion of XVIII (assuming 100% conversion of XVIII to its dianion) per mole of XVII. Product XIX is racemic.	-80°-0°C, pref. -60°-0°C, more pref. -30 - -10°C.	0.3-4 hrs. pref. 0.3-2 hours	Same as Step 1	Yes
	3) Quench with, e.g. ammonium chloride solution or 1N HCl.	-80°-25°C	1-5 min.	Same as Step 1.	-
C-2 Reduction	a) non-stereoselective: 1-4, pref. 2-4 equivalents of transferable hydride per mole XIX, pref. sodium borohydride or complex of t-butylamine and borane. When racemic XIX is used, XX is a mixture of all four stereoisomers, with ratio of erythro to threo stereoisomers ranging from 3:2 to 2:3.	-10-30°C	1-8 hours	IO, eg. lower alkanol esp ethanol	Yes
	b) Stereoselective: 1) 1-1.3 moles, pref. 1.02-1.3 moles, tri (primary or secondary C_{2-4}alkyl)-borane, pref. triethylborane, and pref. 0.3-8 liters, eg. 0.75-6.5 liters, air (at 25°C and 760 mm Hg) per mole XIX.				

Reaction/Type	Reagents, Molar Ratios and Comments	Temperature	Time	Solvent	Inert Atmosphere
C (Reduction) (continued)	2) 0.4-3.5 moles, pref. 1.5-2.5 moles, sodium borohydride per mole XIX. After the reaction, quench the reaction mixture with, for example, 1N. hydrochloric acid at -78° - -20°C. and isolate the crude product by extracting with a suitable inert organic solvent (e.g., diethyl ether) and evaporating the solvent at reduced pressure. It is pref. to crystallize the cyclic boron ester, if possible.	-100° - -40°C., pref. -100° - -70°C.	2-48 hours, pref. 16-48 hours.	Same as Step 1	Yes
	3) large excess of anhydrous methanol, e.g., 50-500 moles per mole XIX, or a mixture of methanol (e.g., 10-20 l. per mole XIX), hydrogen peroxide (e.g., 4-8 l. of 30 aqueous hydrogen peroxide per mole XIX), and a pH 7-7.2 aqueous phosphate buffer (pref. 6-10 l. of a pH 7 aqueous phosphate buffer (e.g., 0.054M. sodium, 0.024M. potassium and 0.047M. phosphate) per mole XIX). The amount of buffer must be sufficient to maintain a pH of 7-7.2. Dissolve product of Step 2 in methanol and add buffer and aqueous hydrogen peroxide. See Narasaka et al., Tetrahedron 40, 2233-2238 (1984).	20°-40°C., pref. 20°-25°C., with methanol alone and -30° - 25°C., pref. -10°-10°C., with a mixture of methanol, hydrogen peroxide and buffer	0.7-60 hrs., pref. 4-60 hrs., with methanol alone and 0.5-2 hrs. with a mixture of methanol, hydrogen peroxide and buffer	Neat	-

Reaction/Type	Reagents, Molar Ratios and Comments	Temperature	Time	Solvent	Inert Atmos-phere
C (Reduction) (Cont'd)	c) Alternative Stereoselective: 1) 1-5 moles zinc borohydride (pref. as 0.1-0.2M. solution in anhydrous diethyl ether produced as described in Gensler et al., J. Am. Chem. Soc. 32, 6074-6081 (1960)) per mole XIX.	-80°- -50°C., pref. -80°- -70°C.	0.5-5 hrs., pref. 1-4 hrs.	AIO, pref. ES, esp. diethyl ether or mixture of diethyl ether with another ES	Yes
	2) Add excess methanol (e.g., 10-100 moles per mole IX) and allow to slowly warm to 20°-25°C.	-80°- -50°C., pref. -80°- -70°C., ⟶ 20°-25°C.	1-2 hrs.	Same as Step 1	-
	3) Add excess dilute aqueous acetic acid to quench the reaction mixture. Can also add the dilute acetic acid at -80°- -50°C. and then allow to warm to 20°-25°C.	20°-25°C.	-	Same as Step 1	-
	When a racemic IX is utilized in Alternative b or c, product (XII) is a mixture of the four possible stereoisomers wherein the ratio of the erythro isomers (racemate) to the threo isomers (racemate) is about 4-20:1, usually 5-15:1, except as noted below. Repeated recrystallization of the cyclic boron ester produced in Step 2 of Alternative b, if a solid, may raise the ratio or even yield pure erythro racemate and mother liquors enriched with threo racemate. When, however, the solvent in Step 1 of Alternative b is a mixture of THF and methanol, said ratio may be as high as 50-100:1.				

Reaction/Type	Reagents, Molar Ratios and Comments	Temperature	Time	Solvent	Inert Atmosphere
D-1 hydrolysis	1-1.3 equivalents of M_2OH per mole XXI or if it is desired to isolate XXII, 0.95-0.995 equivalent M_2OH per mole XXI.	0°C-reflux, pref. 0-75°C, esp. 0-25°C	0.5-4 hrs.	Inert aqueous organic, e.g. mixture of water and lower alkanol, pref. water and methanol, or esp. ethanol.	-
D-2 acidification	At least 1 equivalent, e.g. 1-1.25 equivalents, acid e.g. 2N HCl per mole XXII.	0-25°C	1-5 min.	Water or mixture of water and water missible inert organic solvent e.g. methanol, ethanol, diethyl ether or THF.	-
D-3 (Neutralization)	0.95-0.99 equivalent, pref. 0.96-0.98 equivalent, $M^{\oplus}OH^{\ominus}$ per mole XXIII.	0°-25°C., pref. 20-25°C.	2-10 min.	Same as D-1.	-

Case No. 600-7101-US

Reaction/Type	Reagents, Molar Ratios and Comments	Temperature	Time	Solvent	Inert Atmos-phere
D-4 (Lactonization)	a) Use of catalytic amount of a strong acid such as p-toluenesulfonic acid monohydrate is optional but usually omit. Use of Dean-Stark trap is pref. if solvent forms azeotrope with water.	75°C.-reflux, pref. 75°-150°C., esp. 80°-120°C.	3-18 hrs., pref. 4-7 hrs.	AIO, pref. HC, e.g., benzene, toluene or xylene or mixture thereof.	-
	b) 1-1.5 moles of a lactonization agent, e.g., a carbodiimide, pref. a water-soluble carbodiimide such as N-cyclohexyl-N'-[2'-(N"-methylmorpholinium)-ethyl]-carbodiimide p-toluenesulfonate, per mole XXIII.	10°-35°C., pref. 20°-25°C.	2-8 hrs., pref. 3-4 hours.	AIO, pref. HLA, esp. methylene chloride.	-
	Alternative b often results in higher yields of XXV than Alternative a. Racemic erythro XXIII yields racemic trans (lactone) XXV, racemic threo XXIII yields racemic cis (lactone) XXV, mixture of racemic erythro and threo XXIII yields mixture of racemic trans and cis (lactones) XXV, and single enantiomer of XXIII yields single enantiomer of XXV, e.g., 3R,5S erythro XXIII yields 4R,6S trans XXV.				

Reaction/Type	Reagents, Molar Ratios and Comments	Temperature	Time	Solvent	Inert Atmos-phere
D-5 (Hydrolysis)	1-1.3 equivalents $M_2^{\oplus}OH^{\ominus}$ per mole XXV or, if it is desired to isolate XXVI, 0.94-1 equivalent, preferably 0.97-0.99 equivalent $M_2^{\oplus}OH^{\ominus}$ per mole XXV. Racemic trans (lactone) XXV yields racemic erythro XXVI, racemic cis (lactone) XXV yields racemic threo XXVI, mixture of racemic trans and cis (lactones) XXV yields mixture of racemic erythro and threo XXVI, and single enantiomer of XXV yields single enantiomer of XXVI, e.g., 4R,6S trans XXV yields 3R,5S erythro XXVI.	0°C.-reflux, pref. 0°-75°C., more pref. 20°-75°C.	0.5-6 hrs., pref. 1-4 hours	Same as D-3	-
D-6 acidification	Same as D-2.	0-25°C	1-5 min.	Water or mixture of water and water missible inert organic solvent e.g. methanol, ethanol, diethyl ether or THF	-

Reaction/Type	Reagents, Molar Ratios and Comments	Temperature	Time	Solvent	Inert Atmos-phere
D-7 (Esterification)	At least 2 moles, e.g., 2-10 moles, pref. 2.05-2.5 moles, $M_2^{\oplus}$ OR_7 per mole XXV. Racemic trans (lactone) XXV yields racemic erythro XXVII, racemic cis (lactone) XXV yields racemic threo XXVII, mixture of racemic trans and cis (lactones) XXV yields mixture of racemic erythro and threo XXII, and single enantiomer of XXV yields single enantiomer of XXVII, e.g., 4R,6S trans XXV yields 3R,5S erythro XXVII.	0°-70°C., pref. 0°-25°C. when R_7 is primary alkyl	1-12 hrs., pref. 1-3 hrs. when R_7 is primary alkyl	AIO, e.g., ES such as THF or alcohol of the formula R_7-OH (R_7 must be same as in $M_2^{\oplus}$ $OR_7^{\ominus}$, if a liquid.	-

Reaction/Type	Reagents, Molar Ratios and Comments	Temperature	Time	Solvent	Inert Atmosphere
E-1 Oxidation	X is -CH=CH- 5-50 moles manganese dioxide (pref. activated) per mole XXI	20°-80°C, pref. 40°-80°C	1-4 days	AIO, pref. ES or HC, esp. toluene	Yes
	when X is $-(CH_2)_2$:				
	1) Prepare Swern's Reagent: 0.9596 l oxalyl chloride and 1.561 l dimethyl sulfoxide per mole XXI to be used in Step 2.	-20-0°C	5-15 min.	neat	Yes
	2) Swern's reagent from Step 1 and 6.969 l triethylamine per mole XXI.	-60 to -40°C, pref. -50°C	1-6 hrs.	methylene chloride	Yes
E-2 Hydrolysis	1-1.3 equivalents $M_2^{\oplus}\,^{\ominus}OH$ per mole XXVIII, or if it is desired to isolate XXIX, 0.95-0.995 equivalent $M_2^{\oplus}\,^{\ominus}OH$ per mole XXVIII	0°C to reflux, pref. 0-75°C esp. 0-25°C	0.5-4 hrs.	Inert aqueous organic, e.g. mixture of water and lower alkanol, pref. mixture of water and methanol or esp. ethanol	-
E-3 Acidification	At least 1 equivalent, e.g. 1-1.25 equivalents, acid, e.g. 2N HCl per mole XXIX.	0-25°C	1-5 min.	Water or mixture of water and water-miscible inert organic solvent e.g. methanol, ethanol, diethyl ether or THF.	-

Reaction/Type	Reagents, Molar Ratios and Comments	Temperature	Time	Solvent	Inert Atmos-phere
E-4 Neutralization	0.95-0.99 equivalent, pref. 0.96-0.98 equivalent M $\oplus$ $\ominus$ OH per mole XXX.	0-25°C., pref. 20-25°C	2-10 min.	Inert aqueous organic, e.g. mixture of water and lower alkanol, pref. mixture of water and methanol or esp. ethanol.	-

UTILITY

The compounds of formulae I and II, ie in lactone, ester, free acid or salt form, exhibit pharmacological activity and are therefore useful as pharmaceuticals, e.g. for therapy.

In particular the compounds show activity in the following tests:

Test A. In Vitro Microsomal Assay of HMG-CoA Reductase Inhibition:

200 µl. aliquots (1.08-1.50 mg./ml.) of rat liver microsomal suspensions, freshly prepared from male Sprague-Dawley rats (150-225 g. body weight), in Buffer A with 10 mmol. dithiothreitol are incubated with 10 µl. of a solution of the test substance in dimethylacetamide and assayed for HMG-CoA reductase activity as described in Ackerman et al., J. Lipid Res. 18, 408-413 (1977). In the assay the microsomes are the source of the HMG-CoA reductase enzyme which catalyzes the reduction of HMG-CoA to mevalonate. The assay employs a chloroform extraction or a Dowex® 1X8 (200-400 mesh, formate form) ion exchange column to separate the product, [^{14}C]mevalono-lactone, formed by the HMG-CoA reductase reduction of the substrate, [^{14}C]HMG-CoA. [^{3}H]mevalonolactone is added as an internal reference. Inhibition of HMG-CoA reductase is calculated from the decrease in specific activity ([^{14}C/^{3}H]mevalonate) of test groups compared to controls.

The following results were obtained by test A:

Product of Example 3C IC_{50} = 0.41 µ molar.
Product of Example 4 IC_{50} = 0.53 µmolar.
Compactin IC_{50} = 1.01 µ molar.
Mevinolin IC_{50} = 0.14 µ molar.

IC_{50} is the concentration of the test substance in the assay system calculated to produce a 50% inhibition of

HMG-CoA reductase activity. The tests are run at concentrations of test substance between 0.05 and 1000 μ molar.

Test B. In Vivo Cholesterol Biosynthesis Inhibition Test: In vivo studies utilize male Wistar Royal Hart rats weighing 150 ± 20 g. which have been kept for 7-10 days on an altered light cycle (6:30 A.M. - 6:30 P.M. dark) housed two per cage and fed powdered Purina Rat Chow and water ad libitum. Three hours before the diurnal maximum of cholesterol synthesis at mid-dark, the rats are administered the test substance (e.g., 0.01-20 mg./kg. body weight) dissolved or as a suspension in 0.5% carboxymethylcellulose in a volume of 1 ml./100 g. body weight. Controls receive vehicle alone. One hour after receiving the test substance, the rats are injected intraperitoneally with about 25 μCi/100 g. body weight of sodium [1-^{14}C]acetate 1-3 mCi/mmol. Two hours after mid-dark, blood samples are obtained under sodium hexobarbital anesthesia, and the serum is separated by centrifugation.

Serum samples are saponified, neutralized, and the 3β-hydroxysterols are precipitated with digitonin basically as described in Sperry et al., J. Biol. Chem. 187, 97 (1950). The [^{14}C]digitonides are then counted by liquid scintillation spectrometry. After correcting for efficiencies, the results are calculated in nCi (nanocuries) of 3β-hydroxysterol formed per 100 ml. of serum. Inhibition of 3β-hydroxysterol synthesis is calculated from the reduction in the nCi of 3β-hydroxysterols formed from test groups compared to controls.

The following results were obtained by Test B:

Example 3C ED_{50} = 0.49 mg/kg.
Example 4 ED_{50} = >1.0 mg/kg.
Compactin ED_{50} = 3.5 mg/kg.
Mevinolin ED_{50} = 0.41 mg/kg.

The above presented test data indicate that the compounds of Formulae I and II are competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in cholesterol biosynthesis, and, therefore, they are inhibitors of cholesterol biosynthesis. Consequently, they are useful for lowering the blood cholesterol level in animals, e.g., mammals, especially larger primates, and, therefore, as hypolipoproteinemic and anti-atherosclerotic agents. For these indications, the exact dosage will of course vary depending upon the compound employed, mode of administration and treatment desired. For the larger primates, e.g. humans, an indicated daily dosage is in the range from about 1 mg to about 500 mg, preferably from about 10 to 80 mg of a compound of formula I conveniently administered, for example, in divided doses 2 to 4 times a day in unit dosage form containing for example from about 0.25 mg to about 250 mg, preferably in unit dosages of from about 0.25 to 25 mg, of the compound or in sustained release form.

The preferred compounds of the invention are the products of Examples 3C and 4.

The compounds of Formulae I and II may be administered in lactone, ester or free acid form or in pharmaceutically acceptable salt form. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free acid form. The present invention also provides a pharmaceutical composition comprising a compound of Formula I or II in any of its forms in association with pharmaceutically acceptable solid or liquid carrier or diluent. Such compositions may be formulated in conventional manner.

The compounds may be administered by any conventional route in particular enterally, preferably orally, e.g., in the form of tablets or capsules, or parenterally, e.g., in the form of injectable solutions or suspensions.

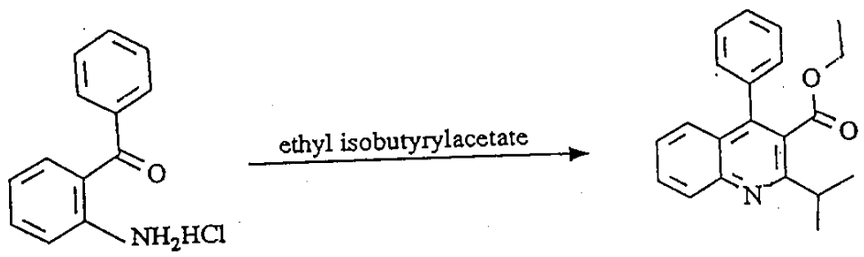
Salts may be prepared in conventional manner from free acids, lactones and esters and vice-versa. Whilst all salts are covered by the invention pharmaceutically acceptable salts especially sodium, potassium and ammonium, particularly sodium salts are preferred.

The following non-limiting Examples illustrate the invention. Thus another aspect of this invention is a method of inhibiting cholesterol biosynthesis comprising administering a cholesterol biosynthesis-reducing amount of the compounds of either formula I or II.

Example 1

Preparation of 6-Heptenoic acid, 3,5-dihydroxy-7-[2-(1-methylethyl)-4-phenylquinolin-3-yl]- ethyl ester, (E)-

Step A: Preparation of 3-Quinolinecarboxylic acid, 2-(1-methylethyl)-4-phenyl- ethyl ester

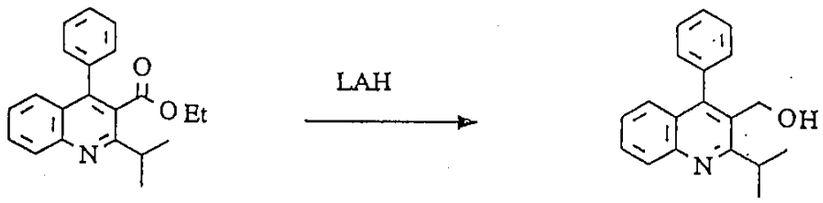


A solution of 11.5 g (0.0493 mole) α-aminobenzophenone hydrochloride and 11.93 ml ethyl isobutyrylacetate (0.07395 mole) in 150 ml abs. ethanol is refluxed for 6 hrs. After the reaction is complete, the solvent is removed under

reduced pressure. The residue is basified with NH_4OH and product is isolated by extraction with ether. The combined ether extracts are washed with H_2O and brine. The organic phase is dried ($MgSO_4$) and concentrated under reduced pressure giving 10.21 g (65%) orange yellow solids.

M.P.: 77°-80°. Anal. Calcd. for $C_{21}H_{21}NO_2$; C, 78.97; H, 6.63; N, 4.39. Found: C, 78.97; H, 6.63; N, 4.39. NMR(90 MH_z): δ0.9,t,3H; 1.4,d,6H; 3.2,m,1H; 4.0,q,2H; 7.3-7.7,m,8H; 8.2,d,1H.

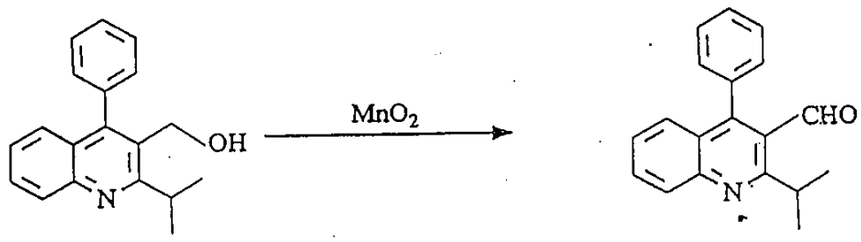
Step B: Preparation of 3-Quinolinemethanol, 2-(1-methyl-ethyl)-4-phenyl-



To the solution of 10.21 g (.03196 mole) quinoline ester in 100 ml anhydrous ether is added 2.43 g (.063242 mole) $LiAlH_4$ portionwise. After 3 hr. stirring at R.T., the reaction mixture is quenched by pouring it into cold water and is then extracted with ether. The dry ether layer is evaporated in vacuo leaving 8.5 g (96%) of alcohol as yellow solids.

NMR(90 MH_z,$CDCl_3$): δ1.0,d,6H; 2.0,m,1H 4.0,s,2H; 4.1,s,1H; 6.3-7.5,m,9H.

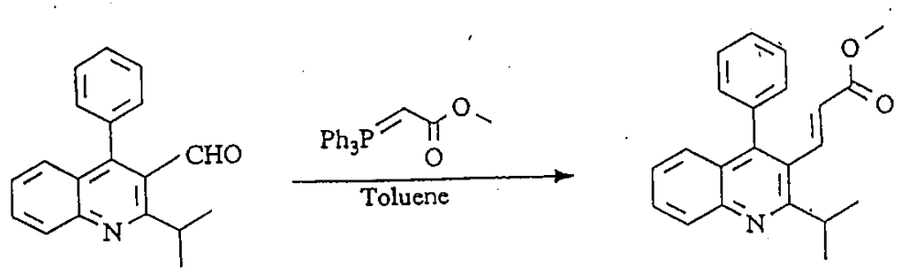
Step C: Preparation of 3-Quinolinecarboxaldehyde, 2-(1-methylethyl)-4-phenyl-



A mixture of 8.0 g alcohol from Step B and 16 g of activated manganese dioxide in 150 ml toluene is refluxed for 4 hrs. and is filtered through a pad of silica gel. The evaporation of solvent yields 5.91 g (75%) aldehyde as yellow solids.

The crude product is purified by flash chromatography (elution with 20% ether/pet. ether) M.P.: 82°-85°C. Anal. Calcd. for $C_{19}H_{17}NO$: C, 82.88; H, 6.22; N, 5.09; Found: C, 82.48; H, 6.43; N, 4.72. NMR(200 MH_Z,$CDCl_3$): δ1.35,d,6H; 4.1,m,1H; 7.3-7.7,m,8H; 8.1,d,1H; 10.0,s,1H.

Step D: Preparation of 2-Propenoic acid, 3-[2-(1-methyl-ethyl)-4-phenylquinolin-3-yl] methyl ester, (E)-

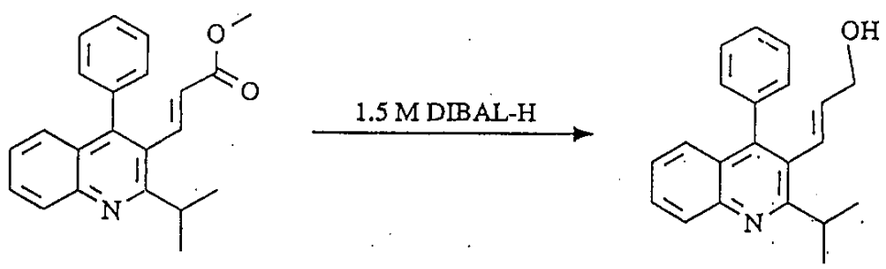


Case No. 600-7101-US

A solution of 5.91 g (0.02149 mole) aldehyde and 8.6 g (0.02578 mole) methyl(triphenyl phosphoranylidene)acetate in 100 ml toluene is refluxed for 1.5 hrs. and is stirred at R.T. overnight. The reaction mixture is diluted with 50% ether/pet. ether and filtered through pad of silica gel. The solvent is removed under reduced pressure. The resulting crystalline residue is triturated with MeOH to give 5.5 g (77.6%) off-white solids.

M.P.: 128°-130°C. Anal. Calcd. for $C_{22}H_{21}NO_2$: C, 79.73; H, 6.38; N, 4.37; Found: C, 78.74,H, 6.55; N,4.03. NMR(90 MH_z,$CDCl_3$): δ1.4,d,6H; 3.5,m,1H; 3.7,s,3H; 5.5-5.75,d,2H; 7.1-7.7,m,8H; 8.1,d,1H.

Step E: Preparation of 2-Propenol, 3-[2-(1-methylethyl)-4-phenylquinolin-3-yl]-(E)-

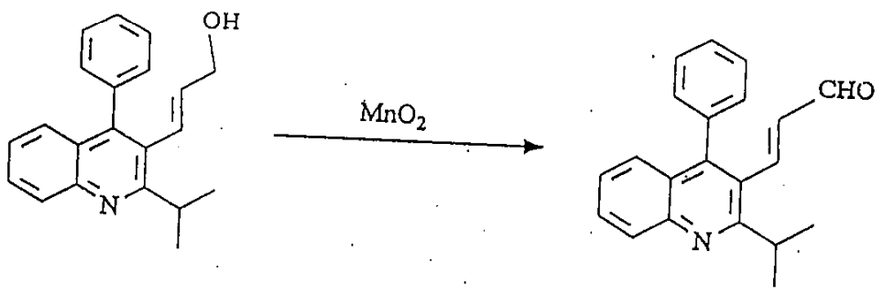


To a cold solution (-78°C) of 6.25 g (0.01888 mole) α,β-unsaturated ester in 75 ml CH_2Cl_2 is slowly added 25.2 ml (0.037764 mole) 1.5M diisobutylaluminum hydride in toluene. After the addition is complete, the reaction mixture is stirred at -78°C for an additional 3 hrs., at which time it is quenched by the addition of 12 ml of 2N NaOH and diluted with ethyl acetate. The mixture is filtered through a pad of silica gel and is washed exhaustively with ethyl acetate. The combined dry organic layers are concentrated in vacuo, yielding 5.42 g crude

alcohol as off-white solids. The solids are dissolved in ether and insolubles (aluminum oxides) are filtered. The solvents are evaporated under reduced pressure to give 4.2 g (73.4%) pure alcohol as yellow solids.

M.P.: 119°-121°C. Anal. Calcd. for $C_{21}H_{21}NO$: C, 83.13; H, 6.98; N, 4.62. Found: C, 82.05; H, 6.86; N, 3.9. NMR(90 MH_z,$CDCl_3$): δ1.4,d,6H; 3.5,m,1H; 4.0,t,2H; 5.3-5.7, pair of t,1H; 6.4-6.6, pair of t,1H; 7.1-7.7,m,8H; 8.1,d,1H.

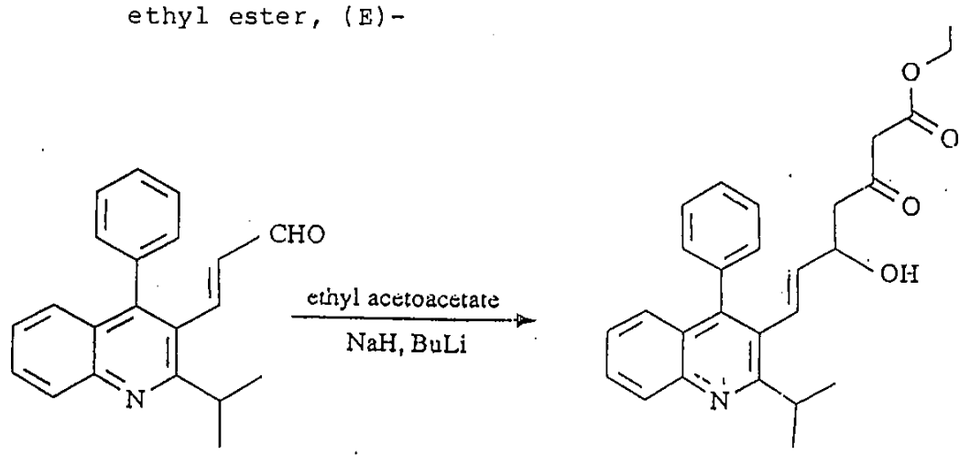
Step F: Preparation of 2-Propenal, 3-[2-(1-methylethyl)-4-phenylquinolin-3-yl]-(E)-



A mixture of 4.0 g (0.0132013 mole) α,β-unsaturated quinoline alcohol and 8.0 g of activated manganese dioxide in 50 ml toluene is heated to reflux for 1 hr. and filtered through a pad of silica gel. Evaporation of the solvent yields 3.5 g (88%) of yellow crystalline solids.

M.P.: 98°-101°C. Calcd. exact mass: 302.15448; Obsd. exact mass: 302.15404. NMR(90 MH_z): α1.4,d,6H; 3.5,m,1H; 5.9,d,1H; 6.1,d,1H; 7.1-77,m,8H; 8.1,d,1H; 9.5,d,1H.

Step G: Preparation of 6-Heptenoic acid, 5-hydroxy-7-[2-(1-methylethyl)-4-phenylquinolin-3-yl]-3-oxo-ethyl ester, (E)-



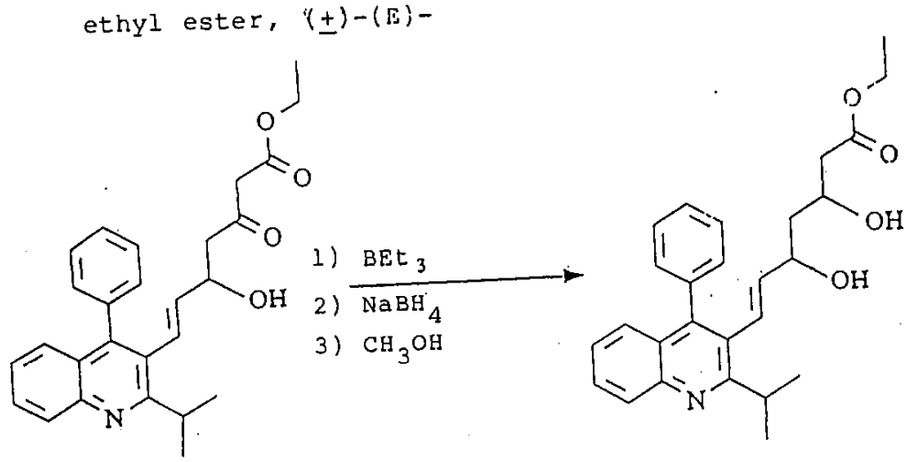
To a solution of 5 ml (0.04 mole) ethyl acetoacetate in 50 ml dry THF is added at -5° to -10°C, 1.9 g 50% NaH in mineral oil. After stirring for 15 min. at this temperature, 27 ml. 1.6M BuLi/hex. is added at -10° to -13°C. Continued stirring at -10°C for 20 min. gives 92 ml (0.04 mole) of dianion of ethyl acetoacetate as a yellow homogeneous solution.

To a solution of 3.5 g (0.0116 mole) of α,β-unsaturated quinoline aldehyde in 40 ml dry THF is added at -5° to -10°C 38 ml (0.0165 mole, 1.2 equiv.) of the above dianion solution, freshly prepared. After 1/2 hr. stirring at this temperature, the reaction mixture is quenched with saturated NH_4Cl, extracted with ethyl acetate and washed with water and brine. The ethyl acetate layer is dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude product is chromatographed on silica gel. Elution with 25% ether/pet. ether gives 3.4 g. (67.8%) α,β-unsaturated hydroxy keto ester as yellow solids.

M.P.: 84°-87°C. Anal. Calcd. for $C_{27}H_{29}NO_4$: C, 75.15; H, 677; N, 3.25. Found: C, 74.99; H, 7.04; N, 2.98. NMR(200 MH_z,$CDCl_3$): α 1.3,t,3H; 1.35, pair of d,6H; 2.3,m,2H;

2.5,d,1H; 3.35,s,1H; 3.5,m,1H; 4.2,q,2H; 4.5, broad s,1H; 5.25,q,1H; 6.55,d,1H; 7.1-7.7,m,8H; 8.1,d,1H.

Step H: Preparation of 6-Heptenoic acid, 3,5-dihydroxy-7-[2-(1-methylethyl)-4-phenylquinolin-3-yl]-ethyl ester, (±)-(E)-

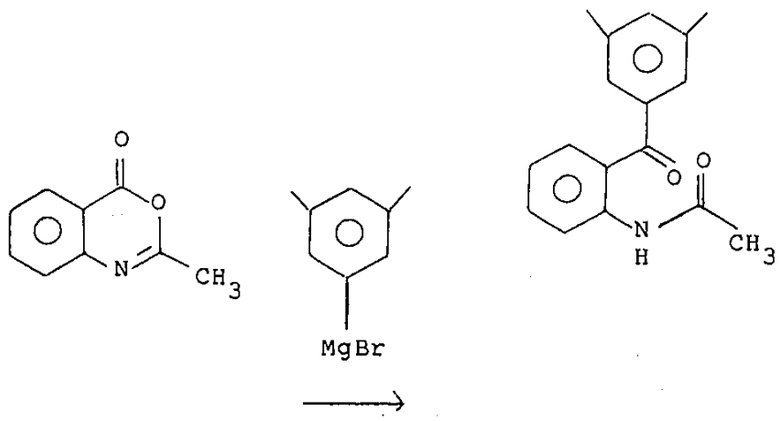


A solution of 1.0 g (0.0023201 mole) hydroxy keto ester and 3.5 ml (0.0034801 mole) 1M Et_3B/THF in 2.5 ml/10 ml MeOH/THF is stirred at R.T. for 1 hr. Then, 0.1315 g (0.0034801 mole) $NaBH_4$ is added at -78°C portionwise. After stirring for 4 hrs. at -78°C, 5 ml acetic acid is added, followed by the addition of ethyl acetate at R.T. The ethyl acetate extracts are combined, washed with saturated sodium bicarbonate, water, and brine and are dried over anhydrous $MgSO_4$. Removal of solvent <u>in vacuo</u> yields a crude product which is redissolved in methanol and concentrated. This procedure is repeated until a boron complex disappears from a thin layer chromatograph (using 50% ether/petroleum ether) and only main product appears. The crude product (1.0914 g), an orange oil, is chromatographed on silica gel. Elution with 80% ether/pet. ether gives 0.91 g (90.5%) yellow solids.

M.P.: 104°-106°C. NMR (200 MH_Z,$CDCl_3$): δ1.3,t,3H; 1.35,d,6H; 2.35,m,1H; 2.9,d,1H; 3.6,d,1H; 3.5,m,1H; 4.0,m,1H; 4.2,q,2H; 4.35,m,1H; 5.35,q,1H; 6.6,d,1H; 7.1-7.7,m,8H; 8.1,d,1H.

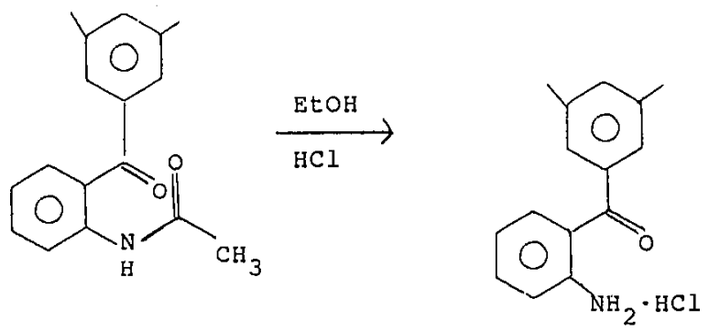
Example 2

Step A: Preparation of Acetamide, N-[2-(3,5-dimethyl-benzoyl)phenyl]-



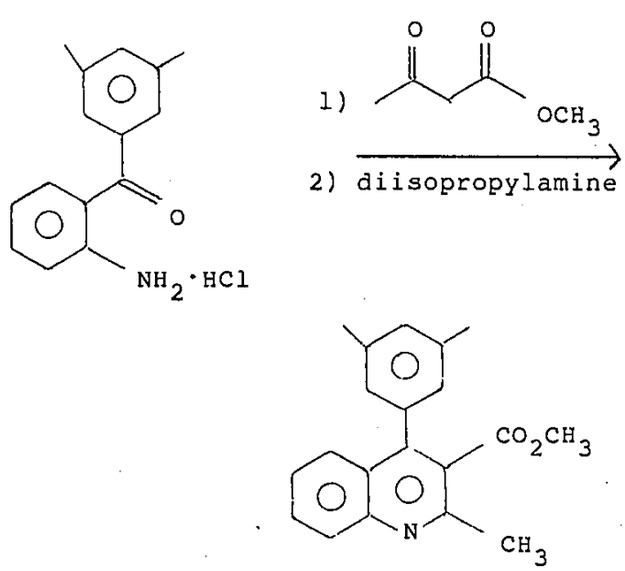
4H-3,1-benzoxazine-4-one, 2-methyl- is prepared according to Morrison and Mullholland, 1958, J. Chem. Soc. p. 2702, and 10 g (0.0621 mol) in THF (50 ml) is added dropwise to a solution of 3,5-dimethylphenyl-magnesium bromide (which is prepared from 17.2 g (0.0931 mole) 5-bromo-m-xylene, 2.33 g(0.0931 mole) magnesium, a trace of iodine, and 1,2-dibromoethan in 40 ml diethyl ether). The resultant mixture is stirred at room temperature under nitrogen, then quenched with a saturated ammonium chloride solution, and is extracted with ethyl acetate. The extracts are dried (Na_2SO_4) and evaporated at a reduced pressure. The resulting oil (10 g) is chromatographed on a silica gel column to obtain the product as an oil (6 g).

Step B: Preparation of Methanone, (2-aminophenyl)(3,5-dimethylphenyl)-hydrochloride



A mixture of the keto amide of Step A (3.8 g., 0.01428 mol) and 12 N hydrochloric acid (1.19 ml, 0.01428 mol) in 20 ml absolute ethanol is stirred and is heated at reflux for 3 hrs. The mixture is then cooled and diluted with diethyl ether. The resulting solid is collected by filtration, washed with diethyl ether and vacuum dried to yield 2.85 g of a pale yellow solid, m.p. 193-195°C.

Step C: Preparation of 3-Quinolinecarboxylic acid, 4-(3,5-dimethylphenyl)-2-methyl- methyl ester

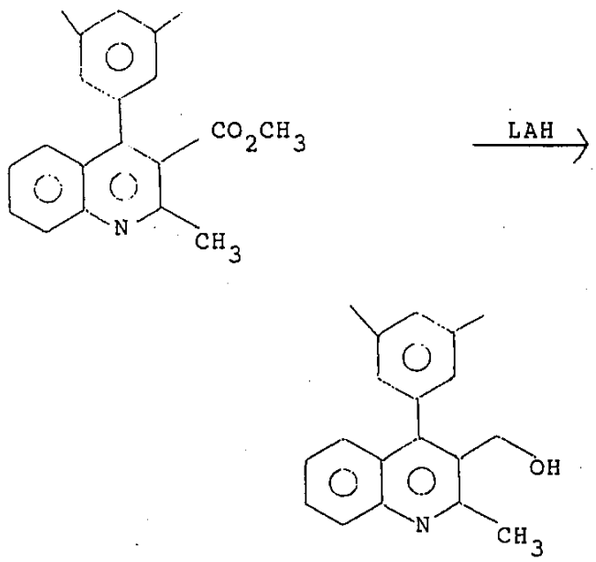


Case No. 600-7101-US

A mixture of the ketone hydrochloride of Step B (0.8 g, 0.003059 mol), and 0.33 ml., (0.00306 mol) methyl acetoacetate is stirred in 20 ml ethanol at reflux for 3 hrs. The mixture is slowly cooled to 10°C and diluted with diethyl ether. The precipitating white solid is collected by filtration and dried to obtain 930 mg of the quinoline hydrochloride, m.p. 209-211°C.

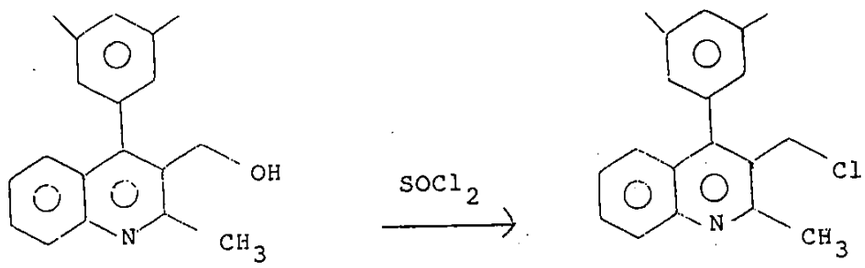
A mixture of 620 mg of the above hydrochloride salt and 2 ml diisopropylamine in 10 ml dry diethylether is stirred at room temperature for 1 hr. The mixture is diluted with diethyl ether, and the diisopropylamine hydrochloride is removed by filtration. The remaining filtrate is evaporated at reduced pressure, and a colorless oil results. The product, a colorless solid, is crystallized from petroleum ether. Yield is 600 mg., m.p. 88-90°C.

Step D: Preparation of 3-Quinolinemethanol, 4-(3,5-dimethylphenyl)-2-methyl-



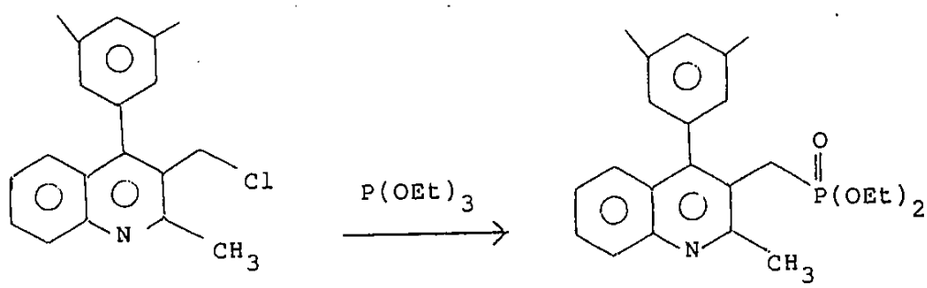
148 mg of lithium aluminum hydride is added to a solution of 486 mg (0.00189 mol) of the ester of Step C in 9 ml dry diethyl ether at 0°C, and stirred at 0°C for 3.5 hrs. The reaction mixture is poured into cold water and extracted with ethyl acetate. The extracts are dried (Na_2SO_4) and are filtered. The filtrate is concentrated at a reduced pressure to give solids. This product is recrystallized in petroleum ether to yield 213 mg of a colorless solid, m.p. 194-195°C.

Step E: Preparation of Quinoline, 3-chloromethyl-4-(3,5-dimethylphenyl)-2-methyl-



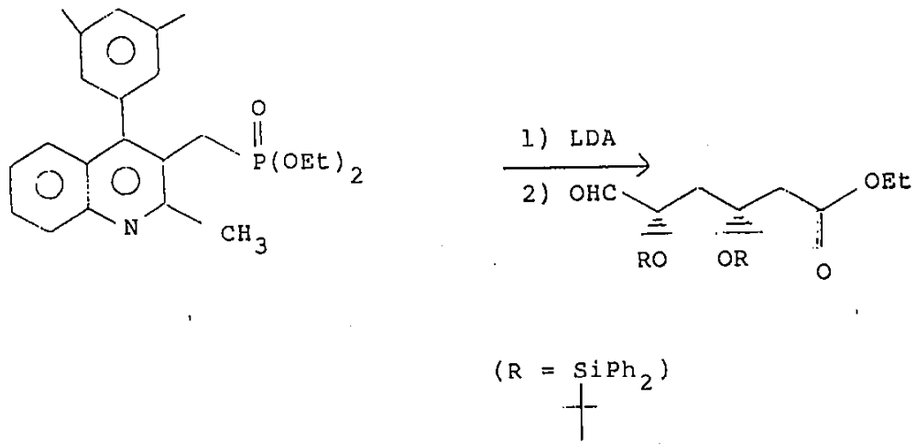
0.1 ml (0.00137 mol) thionyl chloride is added to a solution of 190 mg (0.0006859 mol) of the quinoline alcohol of Step D in 5 ml CH_2Cl_2 at room temperature. This solution is stirred at room temperature for 4 hours, after which the solvent is removed at reduced pressure. The resulting oil is purified by Prep TLC (ether-petroleum 1:1) to yield 160 mg of a white solid.

Step F: Preparation of Phosphonic acid [[4-(3,5-dimethyl-phenyl)-2-methylquinolin-3-yl]methyl]-diethyl ester



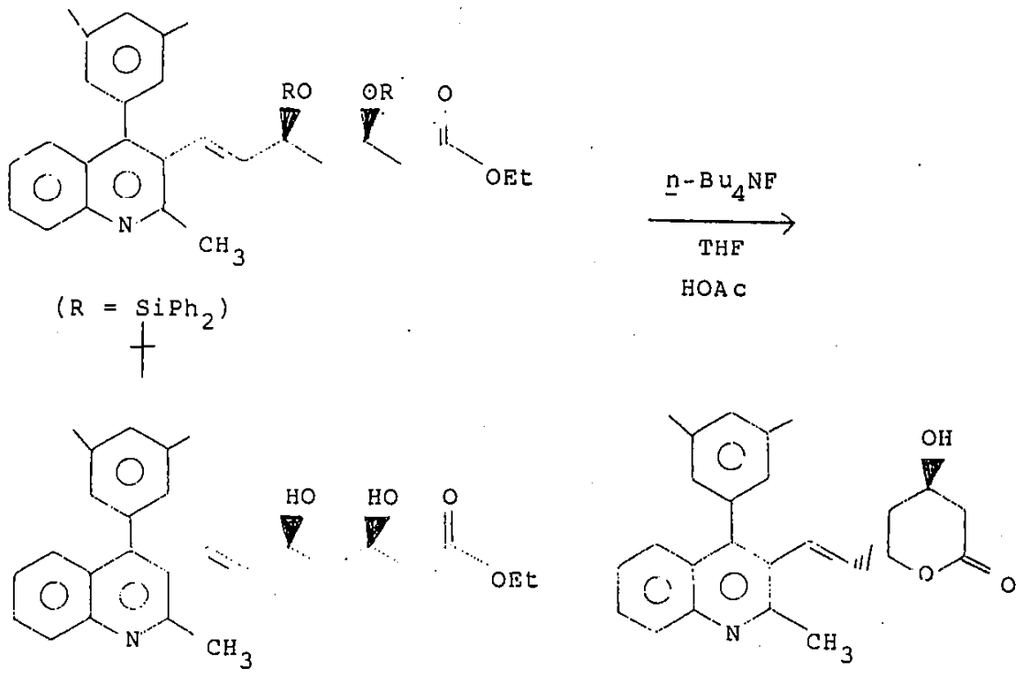
150 mg (0.000508 mol) of the chloride of Step E is mixed with 0.8 ml triethyl phosphite in 2 ml toluene, and then is stirred at reflux under nitrogen for 20 hours. The result is evaporated under reduced pressure to give an oily product which solidifies upon standing to yield 160 mg of product, m.p. 105-107°C.

Step G: Preparation of 6-Heptenoic acid, 3,5-bis[[(1,1-dimethylethyl)diphenylsilyl]oxy]-7-[4-(3,5-dimethylphenyl)-2-methylquinolin-3-yl]- ethylester [(R*,S*)-(E)]-(+,-)-



A solution of 0.27 ml (1.7M) lithium diisopropylamide monotetrahydrofuran/cyclohexane is added to 150 mg (0.000378 mol) of the diethyl phosphonate of Step F in 3 ml THF, at -55°C. The mixture is stirred at -55° to -60°C for 10 min., then a solution of the above aldehyde (293 mg, 0.0004534 mol) in 2 ml THF is added dropwise at -55°C. The reaction mixture is stirred at -55°C for 20 min. Next, 0.5 ml acetic acid and 10% HCl are added, and the mixture is extracted with ethyl acetate. The extracts are combined, washed with water, saturated sodium bicarbonate, water, and brine, then dried (Na_2SO_4), filtered, and evaporated at a reduced pressure to give the crude product as a yellow oil. Preparative TLC (ether:petroleum 1:1) yields 100 mg of a pale yellow oil.

Step H: Preparation of 6-Heptenoic acid, 7-[4-(3,5-dimethylphenyl)-2-methylquinolin-3-yl]-3,5-dihydroxy- ethyl ester [(R*,S*)-(E)]-, (+,-)- and 2H-Pyran-2-one, 6-[2-[4-(3,5-dimethylphenyl)-2-methylquinolin-3-yl]-ethenyl]tetrahydro-[trans-(E)]-, (+,-)-



(I) and (II)

To a solution of the silyl ether of Step G (90 mg, 0.0001012 mol) and glacial acetic acid (0.03 ml, 0.0005 mol) in 2 ml THF at room temperature is added a solution of 1M, 0.61 ml, 0.000607 mol of tetra-n-butyl ammonium fluoride/ tetrahydrofuran. The reaction mixture is stirred at 50-60°C for 40 hr. The mixture is then evaporated at a reduced pressure to give the crude product as a brown oil. This is purified by preparative chromatography (ether: ethyl acetate 1:1) to obtain product I as an oil (10 mg) and product II as an oil (10 mg).

The Formula I compound is in an erythro:threo ratio of approximately 95:5. Nmr analysis yields the following:

1.3 (t, 3H); 2.4 (m, 5H); 4.1 (m, 1H); 4.2 (q, 2H); 4.4 (m, 1H); 5.5 (q, 1H); 6.5 (d, 1H); 6.7-8 (m, 7H).

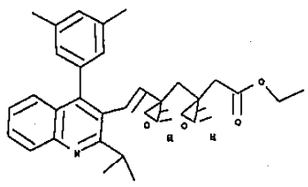
The Formula II compound is in a cis:trans ratio of approximately 5:95. Nmr analysis yields the following:

2.3 (s, 1H); 2.5-2.9 (m, 4H); 4.1 (m, 1H); 5.1 (m, 1H); 5.5 (q, 1H); 6.6 (d, 1H); 6.8-8.0 (m, 7H).

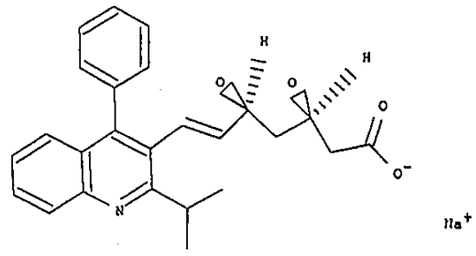
Example 3

Following procedures analogous to those described in Examples 1 and 4 the following compounds are made:

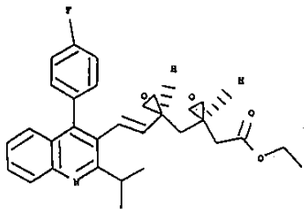
Example 3A



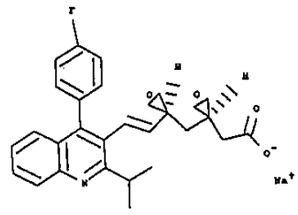
Example 3B



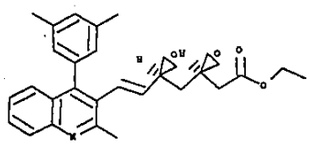
Example 3C



Example 3D



Example 3E

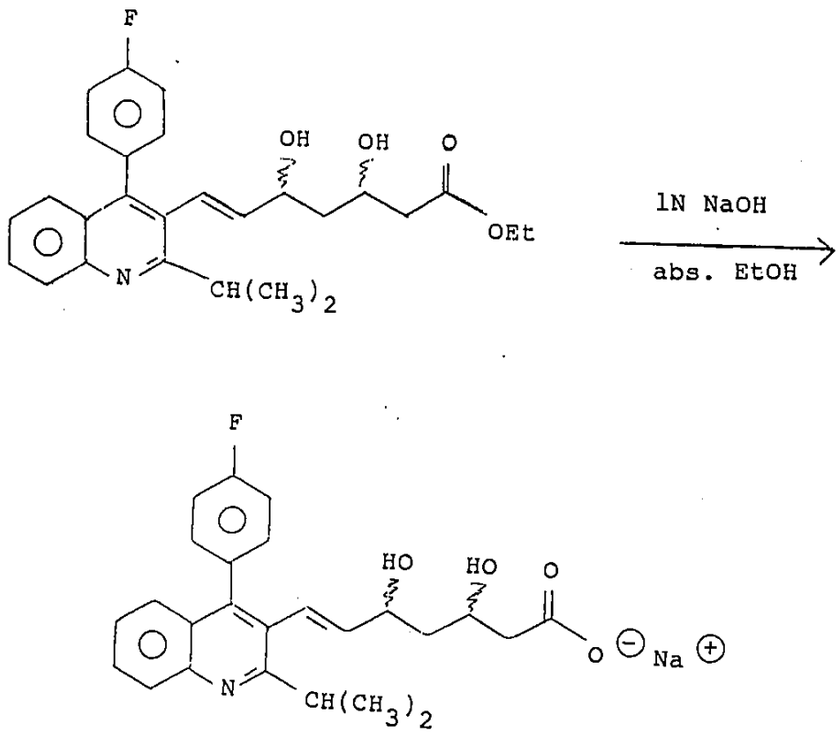


NMR analysis of the above compounds gives the following results:

Ex	NMR spectra
3A	0.9 (t, 3H); 1.6 (d, 6H); 2.1 (m, 2H); 3.7 (m, 1H); 3.7-4.0 (m, 3H); 4.2 (m, 1H); 5.4 (q, 1H); 6.6-7.7 (m, 8H); 8.4 (d, 1H)
3B	1.4 (d, 6H); 2.2 (m, 2H); 3.6 (m, 1H); 3.8 (m, 1H); 4.2 (m, 1H); 5.4 (q, 1H); 6.6 (d, 1H); 7.1-7.7 (m, 8H); 8.1 (d, 1H)
3C	1.3 (t, 3H); 1.4 (dd, 6H); 2.4 (m, 2H); 3.1 (d, 1H); 3.5 (m, 1H); 3.6 (m, 1H); 4.1 (m, 1H); 4.2 (q, 2H); 4.4 (m, 1H); 5.4 (q, 1H); 6.6 (d, 1H); 7.0-7.4 (m, 7H); 7.6 (m, 1H); 8.1 (d, 1H)
3D	1.3 (d, 6H); 2.2 (m, 2H); 3.6 (m, 1H); 3.8 (m, 1H); 4.25 (m, 1H); 5.5 (q, 1H); 6.6 (d, 1H); 7.3-7.4 (m, 7H); 7.6 (m, 1H); 8.1 (d, 1H)
3E	1.3 (t, 3H); 2.4 (m, 5H); 4.1 (m, 1H); 4.2 (q, 2H); 4.4 (m, 1H); 5.5 (q, 1H); 6.5 (d, 1H); 6.7-8 (m, 7H)

d = doublet; dd = doublet of a doublet;
m = multiplet; q = quartet, t = triplet

Example 4

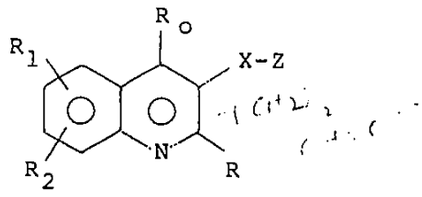


To a solution of 100 mg (0.00022172 mole) of the diol ester of Example 1 in 3 ml absolute ethanol is added 0.2173 ml (0.000217294 mole) 1N NaOH dropwise at 0°C. The mixture is stirred for 3 hours at 0°C, then diluted with ether and is evaporated in vacuo, leaving a yellow oil. Upon the addition of ether, yellow solids are precipitated out, which are then filtered, washed with ether and dried (86.4 mg), m.p. > 225°C.

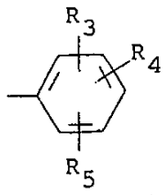
NMR (CD_3OD, 500 MH_2): 1.39 (m, 1H); 1.35 (d, 6H); 1.5 (m, 1H); 2.13-2.3 (m, 1H); 3.65 (q, 1H); 3.75 (m, 1H); 4.25 (m, 1H); 5.45 (dd, 1H); 6.59 (d, 1H); 7.21 (m, 5H); 7.36 (m, 1H); 7.62 (m, 1H); 8.05 (d, 1H).

What is claimed is:

1. A compound of the formula



wherein each of R and R_o is, independently, C_{1-6}alkyl, C_{3-7}cycloalkyl or

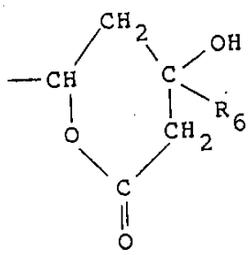


each of R_1, R_2, R_3, R_4 and R_5 is, independently, hydrogen, C_{1-4}alkyl, C_{1-4}alkoxy, trifluoromethyl, fluoro, chloro, phenoxy, benzyloxy or hydroxy;

with the provisos that not more than one of R_1 and R_2 is trifluoromethyl, not more than one of R_1 and R_2 is phenoxy, not more than one of R_1 and R_2 is benzyloxy, not more than one of R_1 and R_2 is hydroxy, not more than one of R_3-R_5 is trifluoromethyl, not more than one of R_3-R_5 is phenoxy, not more than one of R_3-R_5 is benzyloxy, and not more than one of R_3-R_5 is hydroxy;

X is $-(CH_2)_2-$ or $-CH=CH-$;

Z is $-Q-CH_2-\underset{\underset{OH}{|}}{\overset{\overset{R_6}{|}}{C}}-CH_2-CO_2R_7$ (a) or



(b)

wherein Q is $-\overset{\overset{O}{\|}}{C}-$ or $-\overset{\overset{OH}{|}}{CH}-$;

with the proviso that Q may be $-\overset{\overset{O}{\|}}{C}-$ only when X is -CH=CH

and/or R_6 is C_{1-3}alkyl;

R_6 is hydrogen or C_{1-3}alkyl;
R_7 is hydrogen, R_8 or M;
R_8 is a physiologically acceptable and hydrolyzable ester group; and
M is a pharmaceutically acceptable cation.

2. A compound according to claim 1, wherein Z is (a) and Q is $-\overset{\overset{OH}{|}}{C}-$.

3. A compound according to claim 2 which is a 3R,5S isomer.

4. A compound according to claim 2 wherein R and R_o are independently CH_3, isopropyl, phenyl, 3,5-dimethylphenyl or 4-fluorophenyl.

5. A compound according to claim 1 which is (E)-6-heptenoic acid, 3,5-dihydroxy-7-[2-(1-methylethyl)-4-phenylquinolin-3-yl]-ethyl ester, or its sodium salt.

6. A compound according to claim 1 which is 7-[4-(3,5-dimethylphenyl)-2-methylquinolin-3-yl-3,5-dihydroxy-ethyl ester, or its sodium salt.

7. A compound according to claim 1 which is 6-[2-[4-(3,5-dimethylphenyl)-2-methylquinolin-3-yl]-ethenyl]tetrahydro-2H-pyran-2-one, or its sodium salt.

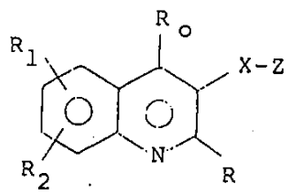
8. A method of inhibiting cholesterol biosynthesis comprising administering to a mammal in need of such treatment a cholesterol-biosynthesis-inhibiting amount of a compound of claim 1.

9. A method of treating atherosclerosis comprising administering to a mammal in need of such treatment an effective amount of a compound according to claim 1, said effective amount being an amount effective for the treatment of atherosclerosis.

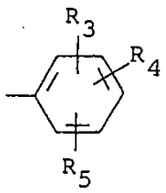
10. A pharmaceutical composition comprising a cholesterol-biosynthesis inhibiting amount of a compound according to claim 1 and a pharmaceutically acceptable carrier.

ABSTRACT

Quinoline analogs of mevalonolactone of the following formula are useful as anti-cholesterol synthesis agents:



where R and R_o are each, independently C_{1-6}alkyl, C_{3-7}cycloalkyl, or

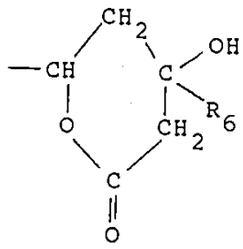


;

each of R_1, R_2, R_3, R_4, and R_5 is independently hydrogen, C_{1-4}alkyl, C_{1-4}alkoxy, trifluoromethyl, fluoro, chloro, phenoxy, benzyloxy or hydroxy;

X is $-(CH_2)_2-$ or $-CH=CH-$;

Z is $-Q-CH_2-\underset{\underset{OH}{|}}{\overset{\overset{R_6}{|}}{C}}-CH_2-CO_2R_7$ or



Q is $-\overset{\overset{O}{\|}}{C}-$ or $-\overset{\overset{OH}{|}}{C}-$;

R_6 is hydrogen or C_{1-3}alkyl; R_7 is hydrogen, R_8 or M; R_8 is a physiologically acceptable and hydrolyzable ester group; and M is a pharmaceutically acceptable cation.

Case No. 600-7101-US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :

SOMPONG WATTANASIN :

Serial No. : Art Unit: Unassigned

Filed: Herewith : Examiner: Unassigned

For: QUINOLINE ANALOGS OF MEVALONOLACTONE AND DERIVATIVES THEREOF :

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on March 3, 1989
(Date of Deposit)
Joanne M. Giesser
Name of applicant, assignee, or Registered Representative
Joanne M. Giesser
Signature
March 3, 1989
Date of Signature

REQUEST FOR INTERFERENCE WITH PATENT UNDER 37 CFR 1.607

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Dear Sir:

Applicant hereby seeks to have an interference declared between the instant application and the following unexpired patent under the provisions of 37 CFR 1.607:

U.S. Patent 4,761,419

Issued August 2, 1988

Filed December 7, 1987

Inventors: Picard, et al.

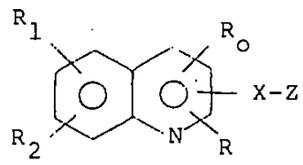
Assignee: Warner-Lambert Company
Morris Plains, New Jersey.

A copy of this patent accompanies this Request.

Three proposed counts for the interference are as follows:

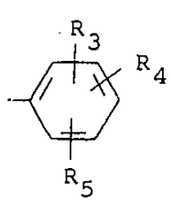
Proposed Count 1

1. A compound of the formula



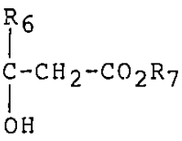
wherein each of R_1 and R_2 is, independently, hydrogen, C_{1-6}alkyl, C_{1-4}alkoxy, trifluoromethyl, fluoro-, chloro-, bromo-, phenoxy-, benzyloxy, hydroxy, cyclopropyl, cyano, nitro, amino, acetylamino, aminomethyl, phenyl, phenyl substituted with: fluorine, chlorine, bromine, hydroxy, trifluoromethyl, C_{1-4}alkyl or C_{1-4}alkoxy; phenylmethyl, phenylmethyl substituted with: fluorine, chlorine, bromine, hydroxy, trifluoromethyl or C_{1-4}alkyl;

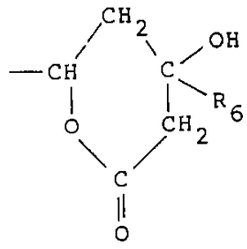
each of R and R_o is, independently, hydrogen C_{1-6}alkyl, trifluoromethyl, C_{3-7}cycloalkyl, cyclohexylmethyl, phenylmethyl, phenylmethyl substituted with: fluorine, chlorine, bromine, hydroxy, trifluoromethyl, C_{1-4}alkyl, or C_{1-4}alkoxy; 2-, 3-, or 4-pyridinyl, 2-, 4-, or 5-pyrimidinyl, or

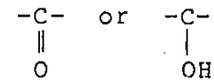


wherein each of R_3, R_4 and R_5 is, independently, hydrogen, C_{1-4}alkyl, C_{1-4}alkoxy, trifluoromethyl, fluoro-, chloro-, bromo-, phenoxy-, benzyloxy or hydroxy;

X is $-(CH_2)_2-$ or $-CH=CH-$;

Z is  (a) or

 (b)

wherein Q is  ;

R_6 is hydrogen or C_{1-3}alkyl;

R_7 is hydrogen, R_8 or M;

R_8 is a physiologically acceptable and hydrolyzable ester group; and

M is a pharmaceutically acceptable cation.

Proposed Count 2

2. A pharmaceutical composition for inhibiting cholesterol biosynthesis comprising an effective amount of a compound of Count 1 in combination with a pharmaceutically acceptable carrier.

Proposed Count 3

3. A method of inhibiting cholesterol biosynthesis in a patient in need of such treatment comprising administering a cholesterol biosynthesis inhibiting amount of a compound of Count 1 in combination with a pharmaceutically acceptable carrier.

Claims which correspond to the above proposed Counts are as follows:

	Proposed Count 1	Count 2	Count 3
Picard, et al.	1-13	14	15
Wattanasin	1	10	8

Respectfully submitted,

Joanne M. Giesser

Joanne M. Giesser
Attorney for SOMPONG WATTANASIN
Registration No. 32,838
(201) 503-8420

JMG:lmc

SANDOZ CORPORATION
59 Route 10
E. Hanover, N.J. 07936

March 3, 1989

Enclosures: U.S. Patent 4,761,419;
Postcard; COM Stamp

Case No. 600-7101/CONT/INT.(1)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

SUPPLEMENTAL DECLARATION OF SOMPONG WATTANASIN, PH.D.
PURSUANT TO 37 CFR 1.'672

I, Sompong Wattanasin, do hereby declare as follows:

1. All of the below-indicated activities took place in the United States.

BACKGROUND

2. Since about 1981, Sandoz Research Institute (SRI) has been engaged in a concerted research effort to develop compounds having utility as HMG-CoA reductase inhibitors for treatment of hypercholesterolemia.

3. Much of this research has focused on compounds which comprise heterocyclic analogs of mevalonolactone and the open chain derivatives thereof.

4. For example, since 1981 SRI has prepared indenyl, indolyl, indolizinyl, imidazolyl, pyrazolopyridinyl, pyrrole, as well as quinolinyl, and other analogs of mevalonolactone and derivatives thereof.

5. The Sandoz research effort culminated in 1992 in the completion of an NDA filing on fluvastatin, i.e. (E)-(±)-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2- yl]-3,5-dihydroxy-6-heptenoic acid, sodium salt, which compound is a member of a family of indole analogs of mevalonolactone and the open chain analogs thereof.

6. My laboratory was only one of six laboratories devoted virtually exclusively to the synthesis of HMG-CoA reductase inhibitors. By way of illustration of the large number of HMG-CoA compounds being synthesized at Sandoz, I note that during the period of July 1985 to July 1987, my laboratory alone prepared 60 such compounds. This is evidence of Sandoz' high level of interest in the project and intention since 1981, and including the period of July 1985 to July 1987, to pursue its basic research project in the HMG-CoA reductase area and the inventive concept behind it.

SANDOZ QUINOLINE COMPOUNDS

7. In late March of 1987, I submitted Patent Disclosure 299/84 direct to quinoline analogs of HMG-CoA reductase inhibitors (Exhibit A-3 hereto) to the Sandoz Patent and Trademark Department.

8. I understand that between April and November of 1987, this disclosure was presented for rating on four occasions at the regular Sandoz patent committee meetings. On each of these occasions, PD 299/84 was rated either "B" or "X", indicating that further information was needed in order to file a patent application thereon (Exhibits M-1 - M-4 hereto).

9. In the period between July and December 1987, additional compounds of the invention were synthesized under my direction, and they were tested for activity in vitro and in vivo as HMG-CoA reductase inhibitors.

(The synthesis and testing of these compounds are further described in my Declaration of November 13, 1992; the Declaration and Supplemental Declaration of Rajeshvari Patel dated November 13 and 16, 1992; the Declaration of Dr. Terence Scallen dated November 13, 1992; and the Declarations of Robert G. Engstrom and Rodney Slaughter dated November 13, 1992.)

10. I learned shortly after the January 1988 Patent Committee Meeting that my Patent Disclosure 299/84 was rated for filing.

11. 2. On or about February 29, 1988, I sent certain information to Melvyn M. Kassenoff of the Patent Department relating to PD 299/84.

Exhibit O hereto comprises a true copy of the following material which I sent to the Patent Department:

(1) a "post-it" stating "sent to M. Kassenoff. 2/29/88" which is in my handwriting'

(2) 4 pages comprising handwritten reaction schemes and notes bearing my name and a date in my handwriting of February 29, 1988 on the first page (see also **Exhibit P-1**);

12. Additional material which I sent to the Patent Department comprises the following:

Exhibit P-2: 7 pages of computer printouts of specific compounds containing my handwritten notations of the Notebook pages on which they were prepared and relevant physical properties; and

Exhibit P-3: 9 laboratory notebook pages numbered 130, 137, 145, 153, 158, 166, 172, 175 and 176.

13. On November 1, 1988, I printed out the Sandoz database containing the structures of the quinoline compounds of PD 299/84. I subsequently consulted with Robert G. Enstrom about the IC_{50} and ED_{50} values for these compounds, which I wrote on the printout. I sent this printout to the Patent Department. Since the cover page is dated January 4, 1989 in my handwriting, I would have mailed it on or about that date.

Exhibit Y-2 comprises a true copy of the printout bearing my handwritten notations.

14. On or before November 8, 1988, I sent to Mrs. Joanne M. Giesser of the Patent Department a handwritten memorandum outlining a synthesis of the quinoline compounds of my invention according to the procedure identified as "Route I" in my patent disclosure.

Exhibit U-2 comprises a true copy of this memorandum. The front page bears my initials and the date of November 7, 1988 in my handwriting.

15. I received a memorandum dated December 14, 1988 from Mrs. Giesser enclosing a first draft of the patent application on PD 299/84.

Exhibit W comprises a true copy of the memorandum I received.

16. I made handwritten corrections on pages of the draft application and returned them to the Patent Department on or about December 22, 1988.

Exhibit X comprises a true copy of these pages bearing my corrections and my handwritten date of December 22, 1988.

17. On or about January 4, 1989, I returned to Mrs. Giesser a handwritten memorandum and other material in connection with the patent application draft for case 600-7101.

Exhibit Y hereto comprises a true copy of this material, i.e.:

Y-1: 6 pages of handwritten notes on the first draft and a handwritten synthesis step;

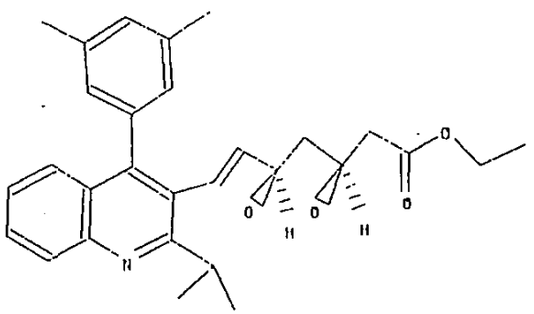
Y-2: the computer printout I received from Biology, which I dated January 4, 1989.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this ____ day of February, 1993.

S. Wattanasin 2/19/93.

Sompong Wattanasin, Ph.D.

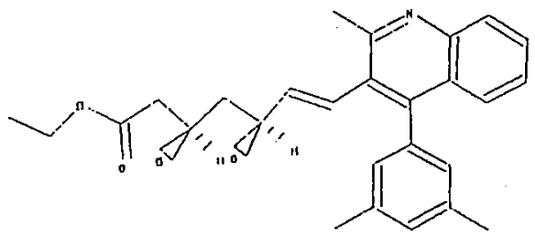
STRUCTURE	DATA
	- Notebook # 1079 - 111 - 19 - oil - erythro : threo ≈ 95 : 5 - nmr 0.9 (t, 3H) 1.6 (d, 6H) 2.1 (m, 2H) 3.7 (m, 1H) 3.7 - 4.0 (m, 3H) 4.2 (m, 1H) 5.4 (q, 1H) 6.6 - 7.7 (m, 8H) 8.4 (d, 1H) - prepared according to SCHEME 1.

SAH.NO	REG.NO	MW	CHEMIST	DATE
SAH-063366	25496	461.606	KATHAWALA WATTANASIN	11-26-84

Ex Exhibit 3A

Exhibit P2 402

STRUCTURE



DATA

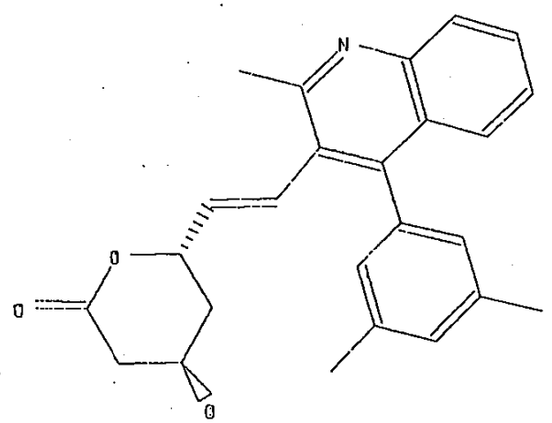
- Notebook # 1127-11-34
- oil
- erythro: threo ~ 95 : 5
- nmr 1.3 (t, 3H)
  2.4 (m, 5H)
  4.1 (m, 1H)
  4.2 (q, 2H)
  4.4 (m, 1H)
  5.5 (q, 1H)
  6.5 (d, 1H)
  6.7-8 (m, 7H)
- prepared according to SCHEME2

SAH.NO	REG.NO	MW	CHEMIST	DATE
SAH-063548	26080	433.552	KATHAWALA WATTANASIN	05-17-85

Ex 3E

403

STRUCTURE



DATA

- Notebook # 1127-11-37
- oil
- cis : trans lactone ~ 5 : 95
- nmr

2.3 (s, 1H)
2.5-2.9 (m, 4H)
4.1 (m, 1H)
5.1 (m, 1H)
5.5 (q, 1H)
6.6 (d, 1H)
6.8-8.0 (m, 7H)

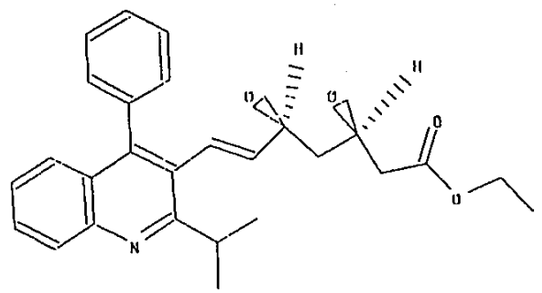
- prepared according to SCHEME 2.

SAH.NO	REG.NO	MW	CHEMIST	DATE
SAH-063549	26082	387.483	KATHAWALA HATTANASIN	05-17-85

Ex 2

404

STRUCTURE



DATA

- Notebook # 1206-176-43
- m.p. 104-106°C
- erythro : threo ≥ 95:5
- nmr
  - 1.3 (t, 3H)
  - 1.35 (d, 6H)
  - 2.35 (m, 1H)
  - 2.9 (d, 1H)
  - 3.6 (d, 1H)
  - 3.5 (m, 1H)
  - 4.0 (m, 1H)
  - 4.2 (q, 2H)
  - 4.35 (m, 1H)
  - 5.35 (q, 1H)
  - 6.6 (d, 1H)
  - 7.1 - 7.7 (m, 8H)
  - 8.1 # (d, 1H)
- prepared according to SCHEME 1. *

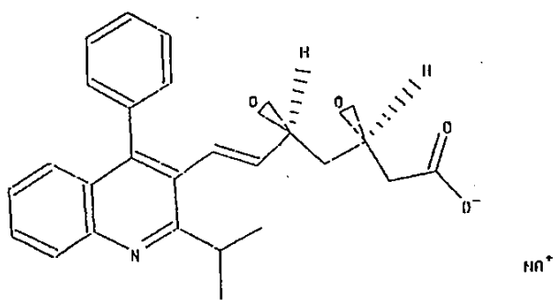
SAH.NO	REG.NO	MW	CHEMIST	DATE
SAH-064933	30441	433.552	PATEL WATTANASIN	09-21-87

* A copy of the notebook of all steps is attached.

Ex 1

405

STRUCTURE



DATA

- Note book # 1206-179-30
- m.p. > 210°C
- erythro : threo ≥ 95 : 5
- nmr

1.4	(d, 6H)
2.2	(m, 2H)
3.6	(m, 1H)
3.8	(m, 1H)
4.2	(m, 1H)
5.4	(q, 1H)
6.6	(d, 1H)
7.1-7.7	(m, 8H)
8.1	(d, 1H)

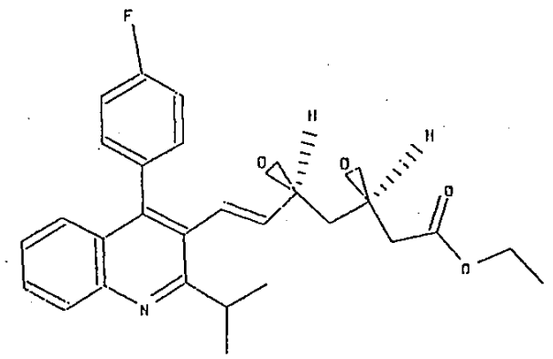
- prepared according to SCHEME 1.

SAH. NO	REG. NO	MW	CHEMIST	DATE
SAH-064934	30442	427.48	PATEL WATTANASIN	09-21-87

Ex 3B

906

STRUCTURE



DATA

- Notebook # 1206-190-41
- oil
- erythro : threo ~ 95 : 5
- nmr

1.3 (t, 3H)
1.4 (dd, 6H)
2.4 (m, 2H)
3.1 (d, 1H)
3.5 (m, 1H)
3.6 (m, 1H)
4.1 (m, 1H)
4.2 (q, 2H)
4.4 (m, 1H)
5.4 (q, 1H)
6.6 (d, 1H)
7.0-7.4 (m, 7H)
7.6 (m, 1H), 8.1 (d, 1H)

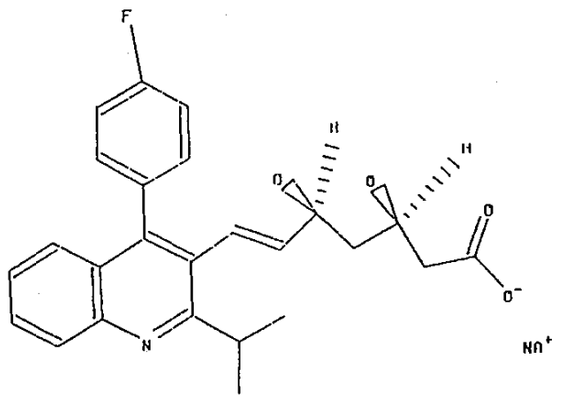
- prepared according to SCHEME 1.

SAH.NO	REG.NO	MW	CHEMIST	DATE
SAH-064935	30447	451.543	PATEL WATTANASIN	09-21-87

Ex. 3C

407

STRUCTURE



DATA

- Notebook # 1206-201-30
- mp > 225°C
- erythro : threo ≥ 95 : 5
- nmr
  - 1.3 (d, 6H)
  - 2.2 (m, 2H)
  - 3.6 (m, 1H)
  - 3.8 (m, 1H)
  - 4.25 (m, 1H)
  - 5.5 (q, 1H)
  - 6.6 (d, 1H)
  - 7.3-7.4 (m, 7H)
  - 7.6 (m, 1H)
  - 8.1 (d, 1H)
- prepared according to SCHEME 1.

SAH. NO	REG. NO	MW	CHEMIST	DATE
SAH-064936	30448	445.47	PATEL WATTANASIN	09-22-87

Ex 3D

408

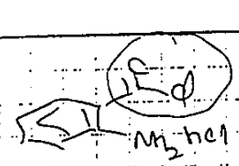
Title-

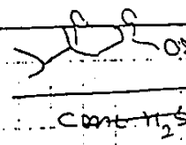
Date 6-14

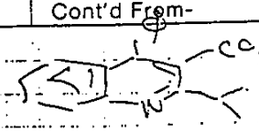
Exhibit P3 Proj.

Cont'd From-

409







5233-24 120G-129-18

319.44
$C_{21}H_{21}NO_2$

233.24 (120G-129-18) = 11.5 g (0.04930 mole)

(0.09859) 15.2 = 11.93 ml (0.07395 81 mM) 1-equiv.

= 10 ml + 5 ml

~~conc H_2SO_4~~ = ~~2.5 ml~~

Ref: 120G-92

Above mixt was heated to reflux (107 - 4 hr) stirred at rt overnight

Ranap to dryness to yellow oil

~~2G 4M~~ basified with NH_4OH, extracted with Et_2O, washed with H_2O, brine, dried, filtered, washed, rotavap gave 16.21g orange-yellow solids (120G-130-27)

nmr, ir, ms → ~~no~~ mwt = 320

Theory: 15.748g, % = 64.86

Performed by- Raj Patel 6-15-94

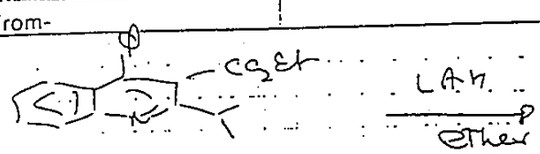
Witness- S. Swaminathan

Cont'd to-

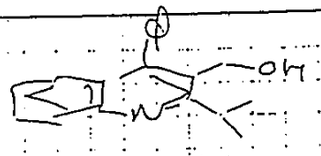
Date 6-7-87 Proj. Title-

Cont'd From-

410



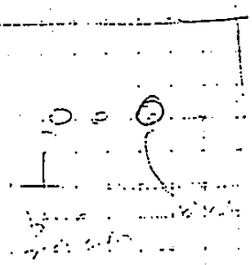
319.44
120G-130-27



277.44
$C_{21}H_{21}NO_2$

(319.44) 120G-130-27 = 10.21 g (0.0319621 mole)
(38) LAH = 2.43 g (0.0639242 mole)
To dry ether = 100 ml
Ref: 120G-96

To 120G-130-27 in dry ether with cooling was added LAH portionwise, exothermic foaming, stirred at r.t. for 3 hr. CG 3c -12 3c



Rx mix. poured in ice H_2O (exothermic, strong Rx) extracted with ether, washed with H_2O, brine, dried, filtered, washed, rotavap gave yellow solids wt = 8.5 g (120G-137-31) mmc, ir uns

Theory: 8.86 g (95.8%)

Performed by- Raj Patel 9-2-87

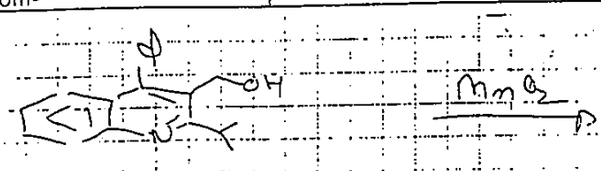
Witness- S. Wattanasin

Cont'd to-

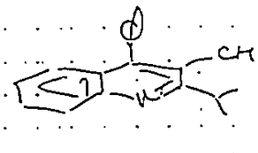
Date 6-17-97 Proj. Title-

Cont'd From-

411



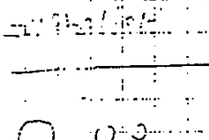
1206-137-31
277.4



275.0
$C_{19}H_{17}NO$

277.4 1206-137-31 = ~~8.0 g~~ 8.0 g (0.02889 2mole)
MnO_2 = 16.0 g
toluene = 150 ml

To 1206-137-31 in toluene was added MnO_2
→ heated to reflux (111°C - 27°C)



filtered thru pad of* silica gel, washed with toluene, rotovap to dryness, gave yellow
solids = 2.6518 g (1206-145-25) nmr, ir, ms MH+ = 276 desired pdt
orange solids = 4.6463 g (1206-145-26) nmr, ir, ms MH+ = 278 S.M.

+ During filtration, separated two bands, which was filtered separately & rotovap

Theory: 7.91 g (74.52%)

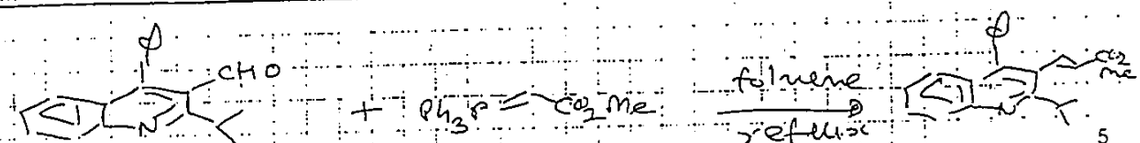
Total yield: 2.6518 g + 3.269 = 5.91 g
(1206-145-25) (1206-143-33)

*

Performed by-

Witness- S. Wathen

Cont'd to- 145



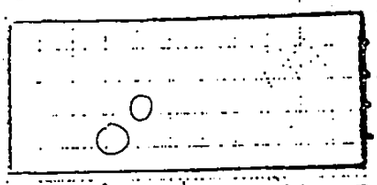
275 331

(275) {1206-145-25, 1206-148-33} = 2.65+3.26 = 5.915g (0.02149 mole)
toluene = 85 ml + 20 ml
(334) $Ph_3P=CO_2Me$ = 8.61359g (0.02578 mole) (1.2 eq.)

Ref.: 1206-146

Above mix. was heated to reflux (yellow heterogeneous before heating) for 1½ hrs. stirred at r.t. overnight.

7-1-87



7-2-87 Diluted with 50% Et_2O/Pet ether filtered thru' pad of silicage washed Rotavap to dryness to give yellow crystaline solid 8.6g : Triturate with MeOH gave offwhite solids (Theory: 7.113 g) wt = 5.5198g (1206-153-31) 77.6%
nmr, ir, ms, mp = 332

Rotavap mother liquor to dryness to yellow oil wt = 2.7593g (1206-153-34)

7-6-87 Trituration with MeOH gave 761.6mg light yellow solids (1206-153-37) nmr, ms, mp 332
Rotavap mother liquor to dryness to yellow solid (1206-153-38) ms

Total yield = 5.5198 + 0.7616 (1206-153-

7-9-87 m.p = 128°–130°C.

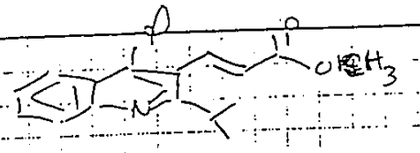
C	H	N
73.63	6.32	4.37
74.65	5.43	4.03
73.52	6.42	3.75

Performed by- Raj Patel 7-6-87
Witness- S. Wattanasin
Cont'd to-

Title-

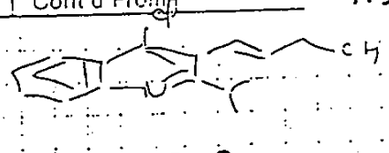
Date 7-7-87 Proj

Cont'd From



331

1.5M DIBAL-H



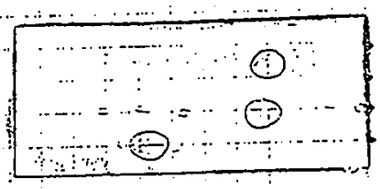
303

($C_{21}H_{21}NO$)

1206-153-40 = 6.259 (0.0185821 mole)
1.5M DIBAL-H/Toluene = 25.18 ml (0.0377642 mole) 2eq
CH_2Cl_2 = 75 ml

Ref: 1206-155, 87

To soln of 1206-153-40 in CH_2Cl_2 was added at -78°C 1.5M DIBAL-H/toluene, stirred at -78°C for 3 hrs (12th - 3rd)



C	H	N	O
83.13	6.98	4.62	5.27
82.65	6.86	3.9	
82.68	6.89	3.89	

quenched with 12.5 ml 2N NaOH, diluted with Etoac stirred at r.t. overnight → lots of white solids came out.

7-8-87 (gel)

filtered thru' pad of silica gel, washed with Etoac, washed org. layer with H_2O, (brine) dried rotavap to dryness gave off white solid = 5.429 (1206-158-35) Dissolved solids in Et_2O insolubles (white) (aluminium oxide) was filtered thru' sintered glass funnel rotavap to dryness gave white-yellow solids = 5.229 (1206-158-36)

Theory = 5.729g 73.7%

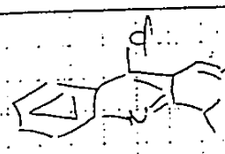
Dissolved solids in Et_2O insoluble (aluminium oxide) was filtered rotavap to dryness gave yellowish solids = 4.217g (1206-158-41) nmr, ir, mts, mwt = 304 micro

m.p. = 119°-121°C.

Performed by- Raj Patel 7.17.87

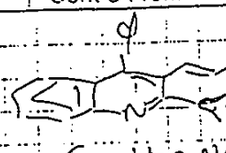
Witness- S. Wattamwar

Cont'd to-



303

MnO2

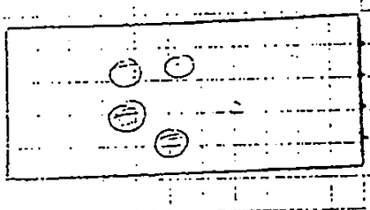


$C_{21}H_{19}NO$
308

1206-158-41 = 4.0g (0.0132013 mole
MnO2 = 8.0g
toluene = 50 ml

Ref: 1206-164

To 1206-158-41 in toluene added MnO2 & heated to reflux (2nd – 3rd), stirred at r.t. overnight

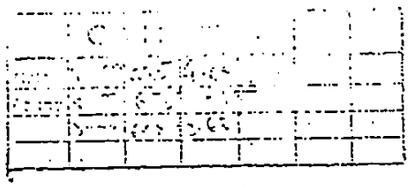


7.168?

filtered thr' pad of silica gel, washed pad with ether, rotavap to dryness, gave 3.4946g, yellow crystaline material (1206-166-30)
IR nmr ms mnt=302
Theory: 3.9736g (88%)

7.28?

micro

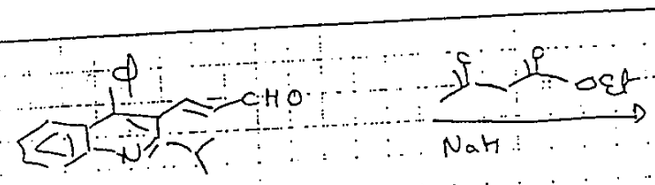


7.3c.d

exact mass obs. mass = 302.15404 m.p. = 98–10
Calc. mass = 302.15448

Performed by- Raghavi 2207
Witness-
Cont'd to-

172 Title- Date 1-20-87 Proj. 415



301

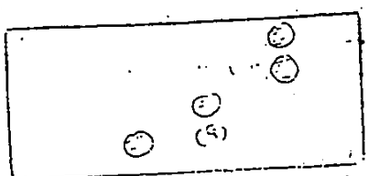
$C_{27}H_{29}NO_4$
431.0

301 1206-166-30 = 3.5g (0.0116279 mole)
~~4 ccmg~~ ~~(0.0133225 mmole)~~
130.14, 1.021 Ethyl acetoacetate = 5ml (0.04 mole)
24 60% NaH = 2 ml
1.6M n-Buli/Hex = 27ml
THF = 60ml + 40ml

To a soln of 1206-166-30 in dry THF (40ml) at -5° to -10°C was added a soln of dianion (11ml + 27ml)(38ml), prepared as described previously

Dianion (got from Dr. Som)

To soln of 5 ml Ethyl acetoacetate in 60ml dry THF was added 1.9 g 50% NaH at -5° to 0°C, stirred for 15 min (foaming H_2 evolved.) At -10 - -15°C was added 27 ml 1.6 M n-Buli/Hex, stirred for 20 min at -10°C → yellow homogeneous soln
Total vol = 92 ml (0.04 mole Used 48 38 ml dianion
= 0.01652 mole (1.4 equiv.) → color changed from yellow to cream to dark red
TLC (50% Et2O/Pet) after 15 min → complete rxn



Rx was stirred for 30 min, quenched with NH4Cl, extracted with Et2O, washed with H2O brine, dried, filtered, rotavap gave yellow oil 5.9188 g (1206-172-41)
Theory: 5.017 (67.89%)

Performed by- Raj Patel 7-21-87
Witness
Cont'd to- 72 c 6 - 175

Date 1-22-10 Proj. | Title- | 416

Cont'd From- 120G-172

Flash chromatography (25% EtOAc/Hex) gave

(a) Yellow solids = 3.4004 g CR06-175-42 micro

m.p. = 84-87°C 68% yield

	C	H	N	O		
Calc.						
Found						

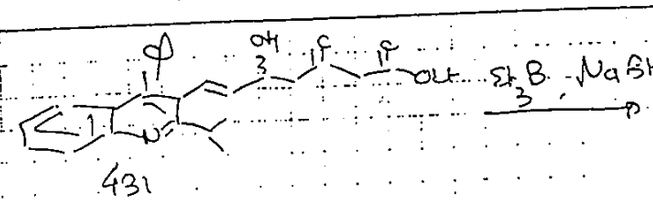
Performed by- Raj Patel 8-5-09

Witness- S. Wattamwar

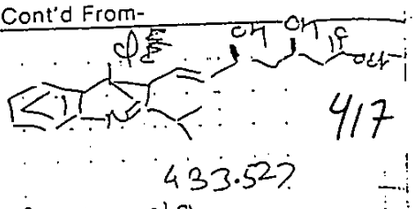
Cont'd to-

Title-

Cont'd From-



431



417

433.527

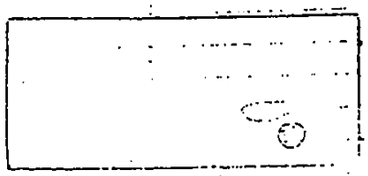
$C_{27}H_{31}NO_4$

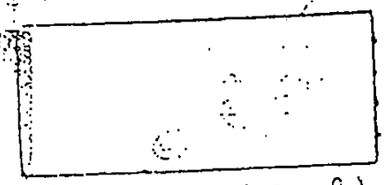
(431) 1206-175-4 = 1.0 g (0.002320 mole)
1m Et_3B / THF = 3.5 ml (0.003480 mole) 1.5 eq.
dry THF = 10 ml
CH_3OH = 2.5 ml
37.8 $NaBH_4$ = 0.1315 g (0.003480 mole) 1.5

Ref: 1206-140

(chemiquemais)

To 1206-175-4 in THF / MeOH added 1m Et_3B / THF at r.t. stirred for 1 hr (9^{45}_{PM} – 10^{45}) The solution was cooled to -78°C, $NaBH_4$ cubes added portionwise. The rxn was stirred at -78°C for (11^{00}_{PM} – 3^{00}_{PM}) & 4 hrs.





The rxn was quenched with MeOH (5 ml) at -78°C Ethyl acetoacetate was added & let it warm upto r.t. org. layer was washed with sat. NH_4Cl, H_2O, brine dried & filtered. Residue was redissolved in MeOH evaporated to dryness. This evaporation process (in MeOH) was repeated until TLC showed desired product
wt. of orange oil = 1.0914 g (1206-176-39)
flash column (silica gel) gave
yellow oil (9) F_{4-6} = 0.4043 g (1206-176-41) ir nmr, ms mh+ = 434 c
solid F_{7-13} = 0.510 g (1206-176-43) ir nmr, ms mh+ = 434 c
m.p. = 104-106° exact mass
HPLC (96.3%) 13
HPLC (93.2%)

Performed by- Raj Patel. 8-5-8

Witness- S. Wattanasin

Cont'd to-

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	
	:	INTERFERENCE 102,648
V.	:	EXAMINER-IN-CHIEF:
	:	MICHAEL SOFOCLEOUS
PICARD ET AL	:	
	:	
V.	:	
	:	
FUJIKAWA ET AL	:	

DECLARATION--PATENTABLY DISTINCT
SUBJECT MATTER

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, DC 20231
BOX INTERFERENCE

SIR:

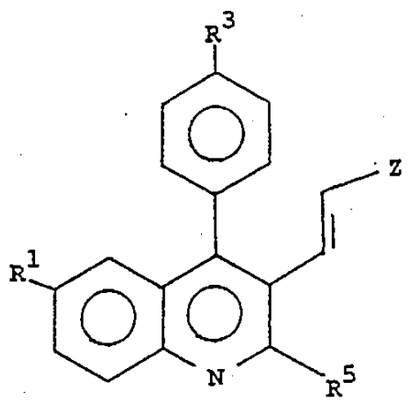
I, MASAKI KITAHARA, do hereby declare and state that:

1. I am a citizen and resident of Japan, and a named co-inventor in U.S. Patent Application 07/233,752, involved in the above-captioned patent Interference.

2. To demonstrate the unpredicted improvement in inhibition of cholesterol biosynthesis obtained when making specific election

for the substituents of the subject matter of the Count of the above Interference, the tests described below were conducted by me, or under my direct supervision.

3. Tests were conducted to determine the impact of specific substituents on compounds of the following formula:



wherein R^1 = H

R^3 = F

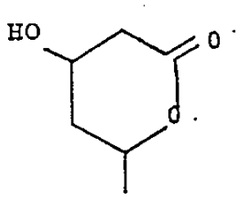
R^5 = cyclopropyl (c-Pr) and Z is selected from the group consisting of

$-CH(OH)-CH_2-CH(OH)-CH_2-COOH$ (carboxylic acid),

$-CH(OH)-CH_2-CH(OH)-CH_2-COONa$ (sodium salt),

$-CH(OH)-CH_2-CH(OH)-CH_2COO\frac{1}{2}Ca$ (calcium salt),

$-CH(OH)-CH_2-CH(OH)-CH_2COOR$, wherein R is C_{1-3} alkyl and



(lactone)

In compounds of the above formula, where R^5 is cyclopropyl, unpredictably enhanced inhibition of cholesterol biosynthesis, as tested both <u>in vitro</u> and <u>in vivo</u> (culture cell) is obtained. This unexpected improvement is maintained even when contrasted with identical compounds save for the identity of R^5, wherein R^5 is isopropyl or n-propyl. This is true even if the identity of R^5 is of larger size, such as a C_6 substituent.

4. In the test described above, inhibition of cholesterol

biosynthesis was determined according to two tests, A and B, as set forth in the specification of U.S. Patent Application 07/233,752, involved in the above-captioned Interference. These tests are set forth and identified as tests A and B on pages 28-30 of the specification. The results of the tests are set forth in the Tables attached to this Declaration. In the tables presented, the IC_{50} values are given, thus indicating higher activity in compounds giving lower IC_{50} values.

5. The superior activity of compounds bearing a R^5 cyclopropyl substituent could not, on the basis of my personal knowledge and experience, be predicted on the basis of chemical structure alone. There is nothing in the art that would lead one of skill, having the approximate level of a graduate chemist with several years of experience in the field, to conclude, on the basis of structural comparison alone, that the cyclopropyl substituent at R^5 would confer superior activity in the inhibition of cholesterol biosynthesis.

I hereby declare that all statements made herein of my own knowledge are true, and all statements made on information and belief are believed true. Further, I am aware that willful false

statements and the like are punishable by fine, imprisonment or both, 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of U.S. Patent Application 07/233,752, any patent issued thereon, as well the rights of the party Fujikawa et al in the above-captioned Interference.

DATE: June 1, 1992

Masaki Kitahara
MASAKI KITAHARA

(1) Test A: Inhibition of cholesterol biosynthesis from acetate in vitro

This test was carried out as described on pages 28-29 of the specification. The numerical values indicate IC_{50} (nanomolar concentration i.e. mol x 10^{-9}).

(a) Sodium salt

R^5	carbon number		1	2	3	6
	structure	normal	71.0	15.0	93.1(n-Pr)	>1000
		iso	X	X	10.0(i-Pr)	-
		cyclic	X	X	4.2(c-Pr)	51

(b) Calcium salt

R^5	carbon number		1	2	3	6
	structure	normal	-	-	-	-
		iso	X	X	23.0(i-Pr)	-
		cyclic	X	X	4.4(c-Pr)	-

(c) Ethyl ester

R^5	carbon number		1	2	3	6
	structure	normal	-	24.3	39.9(n-Pr)	>1000
		iso	X	X	-	-
		cyclic	X	X	2.8(c-Pr)	96

(d) Lactone

R^5	carbon number		1	2	3	6
	structure	normal	-	-	-	-
		iso	X	X	25.9(i-Pr)	-
		cyclic	X	X	6.8(c-Pr)	-

X: Not existing

-: Not tested

(2) Test B: Inhibition of cholesterol biosynthesis in culture cells

This test was carried out as described on pages 29 to 30 of the specification. The numerical values indicate IC_{50} (nanomolar concentration i.e. mol x 10^{-9}).

(a) Sodium salt

R^5	carbon number		1	2	3	6
	structure	normal	-	1050	733(n-Pr)	>10000
		iso	X	X	100(i-Pr)	-
		cyclic	X	X	17.5(c-Pr)	394

(b) Calcium salt

R^5	carbon number		1	2	3	6
	structure	normal	-	-	-	-
		iso	X	X	105(i-Pr)	-
		cyclic	X	X	35.0(c-Pr)	-

(c) Ethyl ester

R^5	carbon number		1	2	3	6
	structure	normal	-	797	501(n-Pr)	>10000
		iso	X	X	-	-
		cyclic	X	X	39.1(c-Pr)	4000

X: Not existing

-: Not tested

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	INTERFERENCE 102,648
	:	EXAMINER-IN-CHIEF:
V.	:	MICHAEL SOFOCLEOUS
	:	
PICARD ET AL	:	
	:	
V.	:	
	:	
FUJIKAWA ET AL	:	

COPY

SUPPLEMENTAL DECLARATION - PATENTABLY DISTINCT SUBJECT MATTER

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, DC 20231
BOX INTERFERENCE

SIR:

I, MASAKI KITAHARA, do hereby declare and state that:

1. I am a citizen and resident of Japan, and a named co-inventor in U.S. Patent Application Serial No. 07/233,752, involved in the above-captioned Interference. Further, I am the same Masaki Kitahara executing the Declaration dated June 1, 1992 entitled "Patentably Distinct Subject Matter" in the above-captioned

Interference.

2. In my prior Declaration dated June 1, 1992, data for the lactone species identified, as determined by test B, the inhibition of cholesterol biosynthesis in culture cells, carried out pursuant to the description on pages 29-30 of U.S. Patent Application Serial No. 07/233,752, was not included, as it was not available at that time. I have now obtained such data, and the same is reproduced in the table attached to this Declaration.

3. As can be readily confirmed by the comparison between the IC_{50} value reported for the isopropyl and cyclopropyl isomers, that subject matter wherein Z is of the lactone structure and R^5 is cyclopropyl exhibits unobvious superiority, when compared with the closely related isopropyl isomer of the same compound. Thus, all compounds within the scope of the formula set forth in paragraph 3 of my Declaration dated June 1, 1992, uniformly demonstrate unobvious superiority when R^5 is cyclopropyl, as opposed to closely related isomeric structures.

The observations in paragraphs 4 and 5 of my Declaration of June 1, 1992 remain accurate.

I hereby declare that all statements made herein of my own knowledge are true, and all statements made on information and belief are believed true. Further, I am aware that willful false statements and the like are punishable by fine, imprisonment or both, 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of U.S. Patent Application 07/233,752, any patents issued thereon, as well as the rights of the party Fujikawa et al in the above-captioned Interference.

DATE: July 6, 1992 Masaki Kitahara

MASAKI KITAHARA

Test B: Inhibition of cholesterol biosynthesis in culture cells

This test was carried out as described on pages 29 to 30 of the specification. The numerial values indicate IC_{50} (nanomolar concentration i.e. mol x 10^{-9}).

R^5	carbon number		1	2	3	6
	structure	normal	-	-		-
		iso	x	x	123.8(i-pr)	-
		cyclic	x	x	47.5(c-pr)	-

Date 5/7/85 Proj.

Title- Book # 1127

Cont'd From-

cf. 1079-97

164

SiR_3 : Ph, Si+, Ph

(889)

4.33 / $C_{27}H_{31}O_4N$

1127-9-33	=	90 mg	(0.0001012 mol)
^1H BuNF	=	0.61 ml.	(0.000607 mol) 1Q
H_2O_2	=	0.03 ml.	(0.0005 mol) 5.
THF	=	2 ml.	

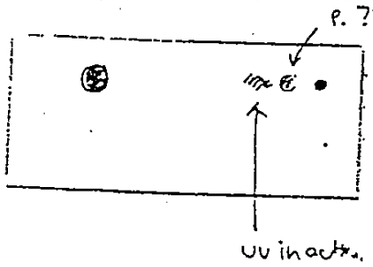
δ 0.04 ; 1.09

The mixt. was stirred at r.t.

9.00 am:

- BuNF	0.6
- "	0.6
- H_2O_2	0.05 ml

TLC 5/8/85 9.00 am.

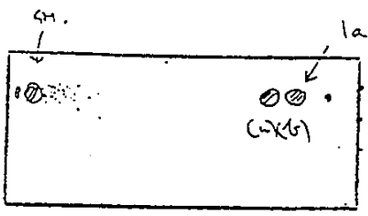


TLC 5/13/85 : 8.30 am ⇒ a mixt of SM + P(s)

The soln was heated at 50°C 9.00 am:

TLC 11.00 pm : mixt. of 2 spots.

stop 5:30 pm



concentrated on the crude oil was purified by prep TLC (ether : CH2Cl2)

(a) : colorless oil = 10 mg (1127-11-36)

nmr MS / HP 433 /

(b) : oil = 10 mg (1127-11-37)

nmr MS / HP 387 / $C_{24}H_{30}O_3N$

5/17/85. 1127-11-34 { 2 mg CSI, 48 mg CSTU, CSTC

1127-11-37 { 2 mg CSI

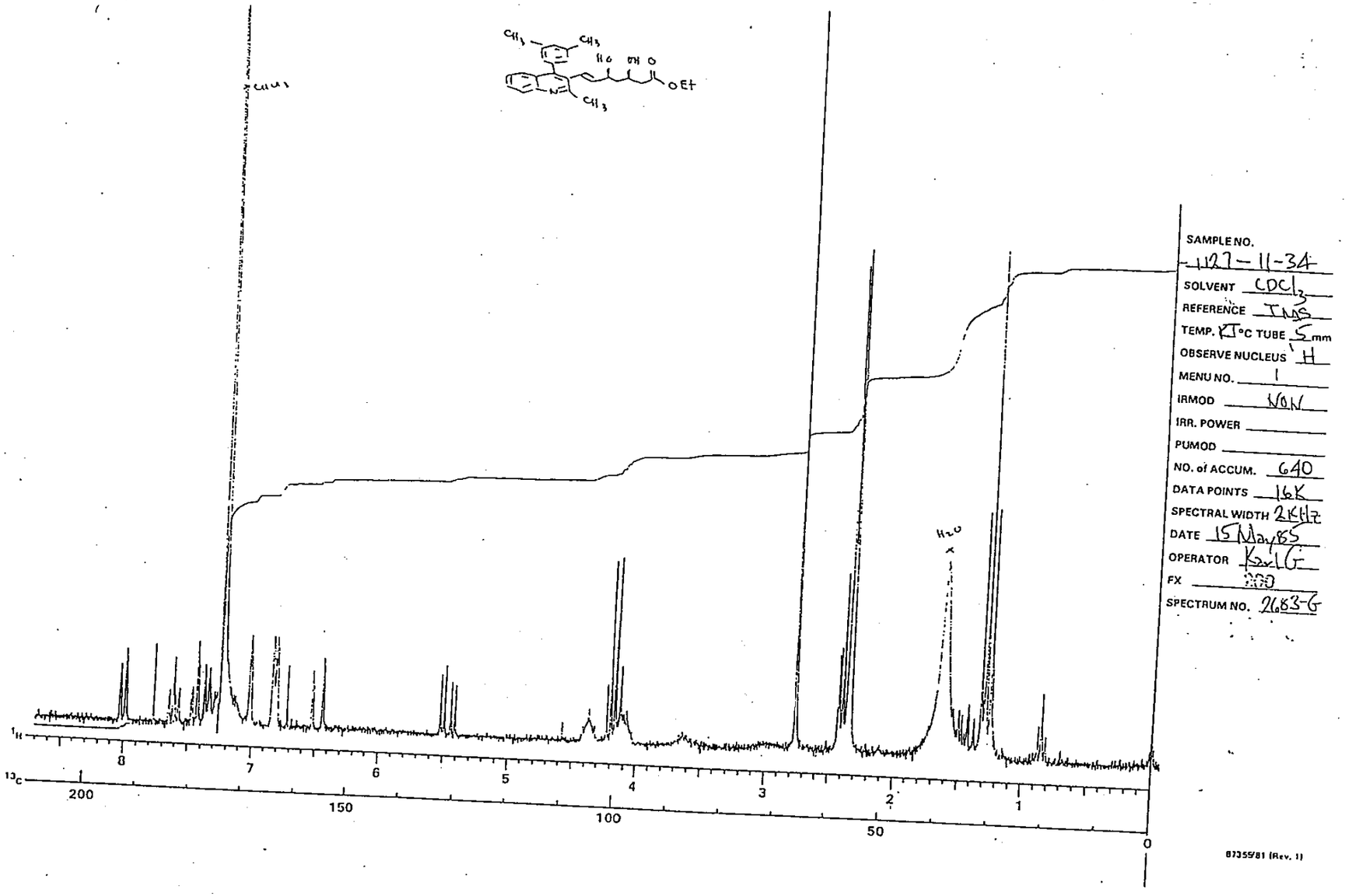
Performed by- S. Wattanasin

Witness-

Cont'd to-

165

ABSORBANCE
TRANSMITTANCE (%)
WAVENUMBER (cm⁻¹)
SANDOZ
DATE 5-12-22
SPECTRUM NO. 1024
OPERATOR B.Raj
SAMPLE 12-7-11-34
PHASE CDCl3
THICKNESS Thin Soln
Dr. Widdemann
NOTE Sol: 12-7-11-34
Soln 1.8
Expected Sample shown
STORED ( ) INTERLEAVED ( )
NO. SCAN PAIRS (SAM/BKG)
AUXILIARY DISPLAY
TRANS. ABSORBANCE ( )
VERT. ORIGIN SPAN
HOR. ORIGIN SPAN


SAMPLE NO. 1127-11-34
SOLVENT CDCl3
REFERENCE TMS
TEMP. RT °C TUBE 5mm
OBSERVE NUCLEUS 1H
MENU NO. 1
IRMOD VAN
IRR. POWER
PUMOD
NO. of ACCUM. 640
DATA POINTS 16K
SPECTRAL WIDTH 2KHz
DATE 15 May 85
OPERATOR KaxLG
FX 200
SPECTRUM NO. 2683-G
H2O
1H
13C
8
7
6
5
4
3
2
1
200
150
100
50
0
87355/81 (Rev. 1)

9/6

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

#83

WATTANASIN

v.

Interference Nos. 102,648, 102,975

FUJIKAWA et al.

Examiner in Chief: M. Sofocleous

JOINT REQUEST FOR EXTENSION OF TIME

The parties Wattanasin and Fujikawa et al. jointly request an extension of time in which to complete taking of cross-examination and rebuttal testimony, as well as an extension of the dates currently set for taking subsequent action, in the above interferences.

The EIC and the parties have been in agreement that cross-examination of the junior party Wattanasin's affiants may run concurrently with the rebuttal testimony of senior party Fujikawa. The current closing date for cross-examination and rebuttal is set for March 25, 1993.

Fujikawa et al. have noticed five Wattanasin affiants for cross-examination, and will also take rebuttal testimony from one non-party witness.

However, owing to other commitments of the involved parties and their witnesses, it has been necessary to tentatively defer the dates for taking rebuttal testimony and certain of the cross-examination until after the current closing date of March 25, 1993[1], pending decision on this motion.

Therefore, the parties now jointly move to reset the relevant dates in the above interferences as follows:

Cross-examination of Wattanasin affiants to close April 15, 1993.
Rebuttal testimony for Fujikawa ..... to close April 15, 1993.
Filing and serving of the record due ..... May 15, 1993.
Wattanasin opening brief due ..... June 15, 1993.
Fujikawa brief due ..... July 15, 1993.
Wattanasin reply brief due ..... August 4, 1993.

Undersigned counsel for the party Wattanasin has discussed this matter with EIC Sofocleous, who indicated he would be agreeable to resetting the dates as set forth above. The courtesy of the EIC is gratefully acknowledged.

---

1. The rebuttal testimony of Dr. Holmlund is tentatively set for March 26, 1993, and cross-examination of Joanne M. Giesser, Esq. is tentatively scheduled for April 9, 1993. The cross-examination of the other Wattanasin affiants will be held on March 22, 1993.

Accordingly, grant of this joint motion is respectfully requested.

Respectfully submitted,

Diane Furman 3/17/93

Diane E. Furman
Attorney for the party Wattanasin
Registration No. 31,104
201-503-7332

Steven B. Kelber
Attorney for the party Fujikawa et al.
Registration No. 30,073
(703) 413-3000

CERTIFICATE OF SERVICE

I hereby certify that true copies of:

1. **JOINT REQUEST FOR EXTENSION OF TIME (EXECUTED)**

2. **CERTIFICATE OF SERVICE**

were served upon Counsel for Wattanasin as follows:

Diane E. Furman
SANDOZ CORP.
59 Route 10
E. Hanover, New Jersey 07936

via first-class mail, postage prepaid, this 19TH day of MARCH, 1993.

STEVEN B. KELBER

Attorney Docket No.: 49-111-0
49-125-0 DIV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

BOARD OF PATENT APPEALS & INTERFERENCES
MAR 19 1993

WATTANASIN

v.

FUJIKAWA et al.

MAILED
MAR 19 1993
PATENT AND TRADEMARK OFFICE
BOARD OF PATENT APPEALS AND INTERFERENCES

#84

Interference Nos. 102,648, 102,975

Examiner in Chief: M. Sofocleous

APPROVED
MAR 19 1993
Examiner-in-Chief

JOINT REQUEST FOR EXTENSION OF TIME

The parties Wattanasin and Fujikawa et al. jointly request an extension of time in which to complete taking of cross-examination and rebuttal testimony, as well as an extension of the dates currently set for taking subsequent action, in the above interferences.

The EIC and the parties have been in agreement that cross-examination of the junior party Wattanasin's affiants may run concurrently with the rebuttal testimony of senior party Fujikawa. The current closing date for cross-examination and rebuttal is set for March 25, 1993.

Fujikawa et al. have noticed five Wattanasin affiants for cross-examination, and will also take rebuttal testimony from one non-party witness.

49-111-0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	
	:	INTERFERENCE NO.: 102,648
V.	:	EXAMINER-IN-CHIEF:
FUJIKAWA ET AL	:	MICHAEL SOFOCLEOUS

NOTICE OF DEPOSITION

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

BOX INTERFERENCE

SIR:

Pursuant to 37 CFR §1.673(a), Fujikawa et al hereby serve notice of the deposition of Dr. Chester E. Holmlund to be held at the offices of undersigned Counsel on March 26, 1993, beginning at 10:00 AM, and continuing from time-to-time until done. It is not expected that the deposition will last beyond a single day, but in the event it does, the deposition will be resumed March 29, 1993.

The current address for Dr. Holmlund is 9200 Edwards Way, Apartment 516, Adelphi, Maryland. The witness is expected to testify in a rebuttal capacity, as to the adequacy of the proof of the Junior Party with respect to conception and actual reduction to practice.

A true copy of the foregoing Notice of Deposition was served, by hand, on Diane Furman, Sandoz Corporation, on March 26, 1993, agreement as to the date of deposition and manner of notice having been earlier agreed upon.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Steven B. Kelber
Registration No.: 30,073
Attorney for Fujikawa et al

Case No. 600-7101/CONT/INT.
Patent

#86

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN

v.

FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

WATTANASIN NOTICE OF CROSS-EXAMINATION DEPOSITION 37 CFR §1.673(e)

By agreement of the parties, the cross-examination deposition of Joanne M. Giesser will be held on Friday, April 9, 1993 at the following address:

Amoco Corp.
55 Shuman Boulevard
"N Building"
Suite 600
Naperville, IL 60563

The starting time will be 12 noon.

Respectfully submitted,

Diane Furman

Diane E. Furman
Attorney for the Party Wattanasin
Registration No. 31,104
201-503-7332

SANDOZ CORPORATION
59 Route 10
East Hanover, NJ 07936

DEF:rmf
April 5, 1993
Encs: OVERVIEW MAP AND LOCAL MAPS A,B AND C

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on April 5, 1993
(Date of Deposit)
Diane E. Furman
Name of applicant, assignee, or Registered Representative
Diane Furman
Signature
4/5/93
Date of Signature

CERTIFICATE OF SERVICE

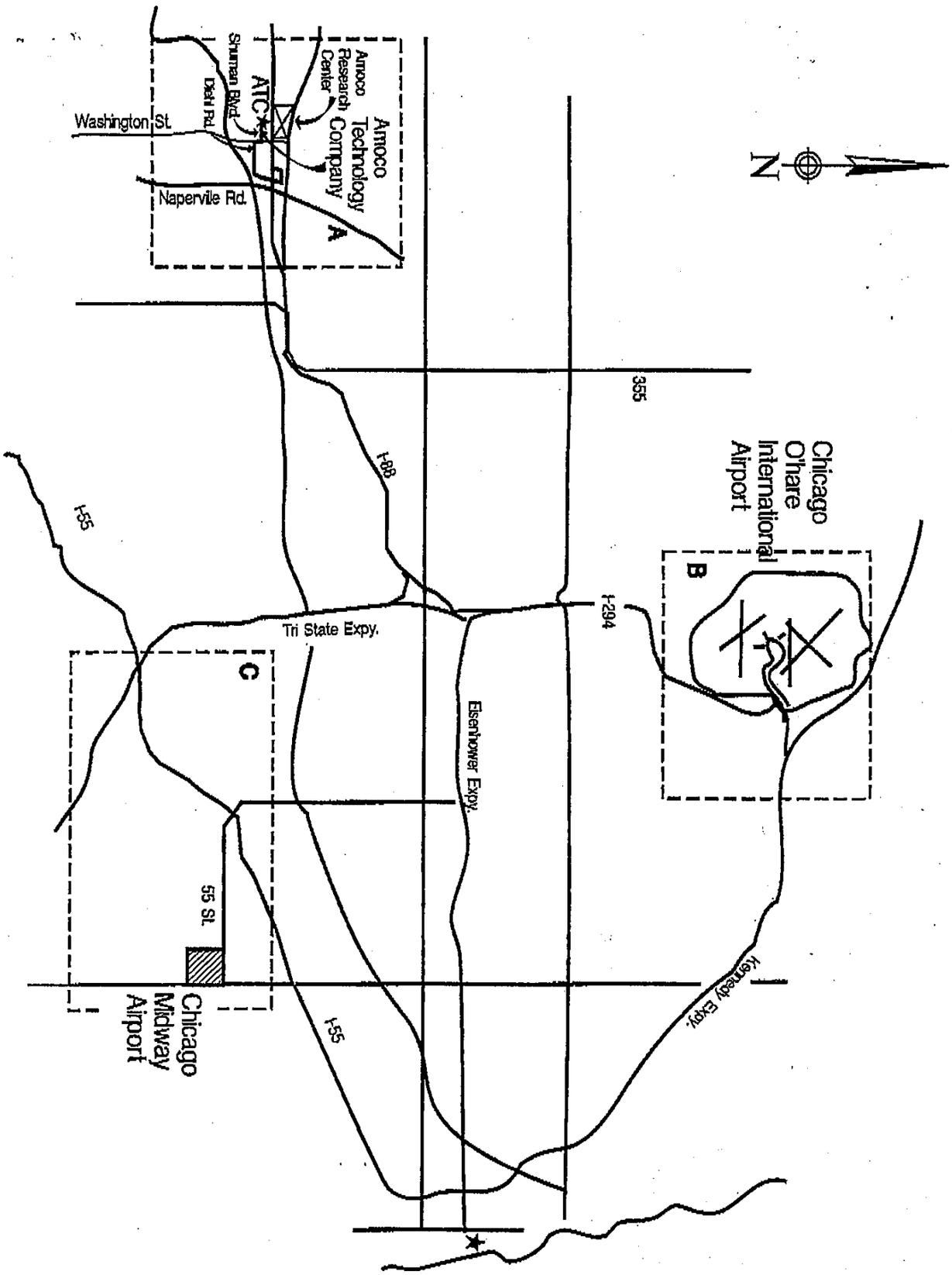
It is hereby certified that a true copy of the paper entitled:

WATTANASIN NOTICE OF CROSS-EXAMINATION DEPOSITION 37 CFR §1.673(e)

was served on counsel for the party Fujikawa et al., this 5th day of April 1993, by facsimile and by postage pre-paid first-class mail addressed to the following:

Oblon, Spivak, McClelland, Maier & Neustadt, P.C.
Attn: Steven B. Kelber, Esq.
1755 South Jefferson Davis Highway
Crystal Square 5, Ste. 400
Arlington, VA 22202
FAX: (703) 413-2220

Diane Furman
Diane E. Furman


Amoco Technology Company
Amoco Research Center
ATC
Shuman Blvd.
Diehl Rd.
Washington St.
Naperville Rd.
A
355
I-88
I-55
Chicago O'hare International Airport
B
I-294
Tri State Expy.
C
Eisenhower Expy.
55 St.
Chicago Midway Airport
I-55
Kennedy Expy.
N

55 Shuman Blvd
"The N Building"
Suite 600
Naperville, IL 60563

EXPRESS MAIL
NEXT DAY SERVICE
POST OFFICE TO ADDRESSEE
ORIGIN
ACCEPTANCE
DELIVERY WAS ATTEMPTED
Waiver of Signature and Indemnity (Domestic Only)
ADDRESSEE'S COPY
TO:
US PATENT & TRADEMARK OFFICE
DEPARTMENT OF COMMERCE
WASHINGTON DC 20231-0000
BOX Interference
FROM:
SANOFI CORPORATION
PATENT DEPARTMENT
59 RT 10
EAST HANOVER NJ 07936-1080
For Postal Use
For Customer Use

#87

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Express Mail Mailing Label Number TB216846399US

Date of Mailing April 22, 1993 Interference Nos. 102,648, 102,975

I hereby certify that on the date indicated above, these materials, comprising the original transcripts of the depositions of Sompong Wattanasin, Melvyn M. Kassenoff, Esq., and Linda Rothwell in Interference Nos. 102,648 and 102,975, are being deposited with the United States Postal Service as Post Office to Addressee Express Mail addressed to the Commissioner of Patents and Trademarks, Box Interference, Washington, D.C. 20231.

FYI

Antoinette Lombardi
Signature of Person Mailing the Materials

APR 29 1993

RECEIVED IN
BOX INTERFERENCE

Antoinette Lombardi
Printed or Typed Name of Person Mailing the Materials

ORIGINAL

102648 - #87
102975 - #32

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
INTERFERENCE NOS. 102,648
102,975

WATTANASIN,

vs.

FUJIKAWA, et al.

DEPOSITION OF:
LINDA ROTHWELL

Monday, March 22, 1993
Florham Park, New Jersey

FYI

APR 22 1993

RECEIVED IN
BOX INTERFERENCE

A P P E A R A N C E S:

RICHARD E. VILA, ESQ.,
-and-
DIANE E. FURMAN, ESQ.,
Sandoz Corporation
59 Route 10
East Hanover, New Jersey 07936
(201) 503-7332
Attorneys for Wattanasin.

MESSRS. OBLON, SPIVAK, MC CLELLAND,
MAIER & NEUSTADT
Fourth Floor
1755 Jefferson Davis Highway
Arlington, Virginia 22202
(703) 413-3000
BY: STEVEN B. KELBER, ESQ.,
Attorneys for Fujikawa.

Reporting Services Arranged Through
ROBERTS, WALSH & COMPANY
425 Eagle Rock Avenue
Roseland, New Jersey 07068
(201) 228-9280

CERTIFIED TRANSCRIPT FOR Fujikawa

I N D E X

E X H I B I T S

(Before Gary M. Talpins, a Certified Shorthand Reporter and Notary Public of the State of New Jersey, held at the offices of Sandoz Corporation, Patent and Trademark Affairs Department, 25 Hanover Road, Florham Park, New Jersey, on Monday, March 22, 1993, commencing at 2:35 p.m.)

- - - - - - - - - - - - - - - - - -

L I N D A R O T H W E L L, 2 Rambling Woods Drive, Morris Township, New Jersey 07960, Sworn.

CROSS EXAMINATION BY MR. KELBER:

Q. Good afternoon, Linda.

A. Hello.

Q. I'm going to have the reporter mark as an Exhibit F-9, a document, and after he marks it and hands it to you, if you would review it briefly.

(Whereupon the document was received and marked F-9 for identification.)

A. Okay.

Q. Is that your signature on page four?

A. Yes, it is.

Q. And did you review this document prior to signing it?

A. Yes.

Q. Miss Rothwell, are you a patent attorney or agent?

A. No, administrator.

Q. If you would turn to page one of that document, F-9, you describe a couple of the responsibilities you have as patent administrator. I would like to focus on the one described in paragraph three, the responsibility to docket patent disclosures. Can you elaborate on that? What is involved in docketing the patent disclosures?

A. Once it's been rated, if it's been rated "A", then it's docketed for three weeks for filing and that's what the docketing procedure is. They get little blue cards.

Q. And after you have docketed it for three weeks, do you have follow-up responsibility?

A. Yes.

Q. Can you describe that?

A. I just go in and check with the attorney.

Rothwell - cross

Q. And if the application has not been prepared, what happens? Let's suppose, I will give you a hypothetical, you docket it for three weeks and do you go in and discuss with the attorney, and the application hasn't been prepared for lack of sufficient information from the inventor, is any further date set for docketing review?

A. No. I would just move it maybe another three weeks or two weeks, if he knows when he is going to get more information.

Q. If he doesn't have any idea when he is going to get more information, is a further date set?

A. No, I would just go back in a couple of weeks.

Q. And do you keep on checking until --

A. Yes.

Q. Do you keep on checking until the application is filed?

A. Yes.

Q. At paragraph four on page one of F-9, you make reference to 299/84. Did you have responsibility for docketing that disclosure for filing after it had been rated "A"?

A. I believe so, yes.

Q. Do you recall checking, as you have just described, with the attorney responsible after the first three weeks in that disclosure?

A. To the best that I can remember, yes.

Q. Do you know who that attorney was?

A. I think at the time, it was Fred Weinfeldt, unless it had already been turned over.

Q. Do you recall checking with any other attorney besides Mr. Weinfeldt with regard to 299/84?

A. It would have to be whoever took over the disclosure.

Q. You don't have a recollection as to who that was?

A. No.

Q. Is there anybody else in the Sandoz Patent Department with responsibility for docketing applications for filing?

A. No.

Q. Just yourself. You mentioned a three week date. Is that generally given all applications?

A. Just if it's rated "A" at the meeting.

Rothwell - cross

Q. I see. So in the course of performing those responsibilities with regard to docketing, have you developed an approximation of on average how long it takes from the time a disclosure is rated "A" to the time an application is filed? Do you have a feeling for that?

A. Not really because some of them are filed quick and others take a little longer for one reason or another.

Q. Would a year be an unusually long time?

A. Yes.

Q. If you are familiar with the procedure, when a disclosure is rated "B" and supplemental information is provided, is it provided to you?

A. No. I would just automatically bring it up at the next meeting.

MR. KELBER: Thank you very much. I appreciate it. I have no further questions. Diane?

MS. FURMAN: I have no questions.

REDIRECT EXAMINATION BY MR. VILA:

Q. You were asked a question with regard to essentially the average time that it would take to file a patent application from the time of an

Rothwell - redirect

"A" rating to disclosure. Would that vary in pattern as you might recognize it among different attorneys in the department?

A. Yes.

Q. With regard to Mr. Kassenoff, would you say that he filed in the average time slower than average, faster than average?

A. Some he would do real quick and others, he would just get held up by some of the inventors.

Q. Were there other reasons for him to be --

A. Not that I would know of.

Q. But in some cases, it would be a longer than average time?

A. Yes.

Q. With regard to Jody Giesser, concerning pharmaceutical patent applications that had been assigned to her, would you have ever had an opportunity to form a judgment there?

A. No.

MR. VILA: Thank you very much.

THE WITNESS: Okay, thank you.

(Time noted is 2:45 p.m.)

Linda Rothwell
LINDA ROTHWELL

Subscribed and Sworn to before me
This 20th day of April, 1993

Antoinette Lombardi
A Notary Public

ANTOINETTE LOMBARDI
Notary Public of New Jersey
My Commission Expires April 3, 1994

C E R T I F I C A T E

I, GARY M. TALPINS, a Notary Public and Certified Shorthand Reporter of the State of New Jersey, do hereby certify that prior to the commencement of the examination, LINDA ROTHWELL was duly sworn by me to testify the truth, the whole truth and nothing but the truth.

I DO FURTHER CERTIFY that the foregoing is a true and accurate transcript of the testimony as taken stenographically by and before me at the time, place and on the date hereinbefore set forth, to the best of my ability.

I DO FURTHER CERTIFY that I am neither a relative nor employee nor attorney nor agent of any of the parties to this action, and that I am neither a relative nor employee of such attorney or counsel, and that I am not interested directly or indirectly in the interference either as counsel, attorney, agent or otherwise.

Gary M. Talpins, C.S.R.
License No. XI00561

49-111-0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN	:	
	:	INTERFERENCE NO.: 102,648
V.	:	EXAMINER-IN-CHIEF:
FUJIKAWA ET AL	:	MICHAEL SOFOCLEOUS

FUJIKAWA ET AL REQUEST FOR
CROSS-EXAMINATION

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

BOX INTERFERENCE

SIR:

Responsive to the filing of Wattanasin Consolidated Affidavit Testimony (Volume IV) bearing a filing date of February 22, 1993, Fujikawa hereby requests cross-examination of the following Affiants:

1. Sompong Wattanasin
2. Melvyn M. Kassenoff
3. Joanne M. Giesser

4. Linda Rothwell

5. Lorraine M. Chesley

The cross-examination of Robert G. Engstrom will not be required.

The cross-examination will be as to all Declarations submitted by Sompong Wattanasin in this Interference. The remaining declarants are believed confined to Volume IV.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Steven B. Kelber
Registration No.: 30,073
Attorney for Fujikawa et al

[redacted] [redacted]

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

BOARD OF PATENT APPEALS & INTERFERENCES

MAR 19 1993

WATTANASIN

v.

Interference Nos. 102,648, 102,975

#28

FUJIKAWA et al.

Examiner in Chief: M. Sofocleous

APPROVED

3/19 1993

Examiner-in-Chief

JOINT REQUEST FOR EXTENSION OF TIME

The parties Wattanasin and Fujikawa et al. jointly request an extension of time in which to complete taking of cross-examination and rebuttal testimony, as well as an extension of the dates currently set for taking subsequent action, in the above interferences.

The EIC and the parties have been in agreement that cross-examination of the junior party Wattanasin's affiants may run concurrently with the rebuttal testimony of senior party Fujikawa. The current closing date for cross-examination and rebuttal is set for March 25, 1993.

Fujikawa et al. have noticed five Wattanasin affiants for cross-examination, and will also take rebuttal testimony from one non-party witness.

However, owing to other commitments of the involved parties and their witnesses, it has been necessary to tentatively defer the dates for taking rebuttal testimony and certain of the cross-examination until after the current closing date of March 25, 1993[1], pending decision on this motion.

Therefore, the parties now jointly move to reset the relevant dates in the above interferences as follows:

Cross-examination of Wattanasin affiants to close April 15, 1993.
Rebuttal testimony for Fujikawa ..... to close April 15, 1993.
Filing and serving of the record due ..... May 15, 1993.
Wattanasin opening brief due ..... June 15, 1993.
Fujikawa brief due ..... July 15, 1993.
Wattanasin reply brief due ..... August 4, 1993.

Undersigned counsel for the party Wattanasin has discussed this matter with EIC Sofocleous, who indicated he would be agreeable to resetting the dates as set forth above. The courtesy of the EIC is gratefully acknowledged.

---

1. The rebuttal testimony of Dr. Holmlund is tentatively set for March 26, 1993, and cross-examination of Joanne M. Giesser, Esq. is tentatively scheduled for April 9, 1993. The cross-examination of the other Wattanasin affiants will be held on March 22, 1993.

Accordingly, grant of this joint motion is respectfully requested.

Respectfully submitted,

Diane Furman 3/17/93

Diane E. Furman
Attorney for the party Wattanasin
Registration No. 31,104
201-503-7332

Steven B. Kelber
Attorney for the party Fujikawa et al.
Registration No. 30,073
(703) 413-3000

37

Case No. 600-7101/CONT/INT.(4)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

DECLARATION OF LINDA ROTHWELL PURSUANT TO 37 CFR §1.672

I, Linda Rothwell, do hereby declare as follows:

All of the below-indicated activities took place in the United States.

1. I have been employed by Sandoz Pharmaceuticals Corporation continuously since 1968 to the present. My position, both currently and during the time periods indicated below, has been Patent Administrator of the Sandoz Patent Department.

2. One of my responsibilities as Patent Administrator has been to type or supervise the typing of the Minutes of each Sandoz Pharmaceutical Corp. Patent Committee Meeting based on notes taken at the meeting by the attending attorney(s). The Minutes serve as the official record for the Sandoz Patent Department of decisions and recommendations made at each Patent Committee Meeting (PCM).

3. Since prior to April 1987, another of my responsibilities as Patent Administrator has been to docket patent disclosures as soon as they are received by the Patent and Trademark Department, for consideration at the following scheduled PCM.

4. Patent Disclosure 299/84 was docketed for initial consideration by the Sandoz Pharmaceuticals Corp. Patent Committee at its April 29, 1987 Meeting.

5. According to Sandoz policy which has been in effect since prior to April 29, 1987, a disclosure which is considered by the Patent Committee and is rated "B", is deferred for reconsideration by the Patent Committee within three months' time. An "X"- rated disclosure is deferred for reconsideration by the Patent Committee within one month's time. A "B" or "X" rating is given when further information is needed before making a decision whether to file a patent application. An "A"- rated disclosure represents a decision to file a patent application on the subject matter of the patent disclosure.

Section 5 of the Minutes is devoted to the rating of newly submitted Patent Disclosures or the re-rating of previously rated Patent Disclosures.

6. **Exhibits M-1 to M-5** appended hereto comprise copies of pages of Patent Committee Minutes prepared in the ordinary course of business by me or under my supervision. Confidential material unrelated to PD 299/84 has been masked. These are true copies with respect to the unmasked material.

The Minutes are maintained under my supervision and control in the files of the Sandoz Patent and Trademark Department in the ordinary course of my employment.

Exhibit M-1 is a masked copy of page 2 of the minutes of the Sandoz Pharmaceuticals Corp. PCM held on Wednesday, April 29, 1987. This page shows that Patent Disclosure 299/84 was rated "B," and was assigned to Frederick H. Weinfeldt ("FHW"), a senior patent attorney in the Sandoz Patent Department.

Exhibit M-2 is a masked copy of page 3 of the minutes of the PCM held on Wednesday, July 29, 1987. This page shows that PD 299/84 was re-rated "B".

Exhibit M-3 is a masked copy of page 3 of the minutes of the PCM held on October 28, 1987. This page shows that PD 299/84 was rated "X".

Exhibit M-4 is a masked copy of page 2 of the minutes of the PCM held on Wednesday, November 25, 1987. This page shows that PD 299/84 was rated "X".

Exhibit M-5 is a masked copy of page 4 of the minutes of the PCM held on Wednesday, January 27, 1988. This page shows that PD 299/84 was rated "A," and was re-assigned to Mrs. Joanne M. Giesser, a patent attorney in the Sandoz Patent Department.

The Patent Department records indicate that no later than about April 1987, Mr. Weinfeldt had taken permanent disability leave (and is now deceased). In August of 1987, Mrs. Giesser joined the Patent Department and assumed a part of Mr. Weinfeldt's docket.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing DECLARATION this 19th day of February, 1993.

Linda Rothwell

LINDA ROTHWELL

ORIGINAL

102648-#87
102975-#32

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
INTERFERENCE NOS. 102,648
102,975

WATTANASIN,	:	
	:	
vs.	:	DEPOSITION OF:
	:	SOMPONG WATTANASIN
FUJIKAWA, et al.	:	
	:	

Monday, March 22, 1993
Florham Park, New Jersey

FYI

APR 22 1993

RECEIVED IN
BOX INTERFERENCE

A P P E A R A N C E S:

RICHARD E. VILA, ESQ.,
-and-
DIANE E. FURMAN, ESQ.,
Sandoz Corporation
59 Route 10
East Hanover, New Jersey 07936
(201) 503-7332
Attorneys for Wattanasin.

MESSRS. OBLON, SPIVAK, MC CLELLAND,
MAIER & NEUSTADT
Fourth Floor
1755 Jefferson Davis Highway
Arlington, Virginia 22202
(703) 413-3000
BY: STEVEN B. KELBER, ESQ.,
Attorneys for Fujikawa.

Reporting Services Arranged Through
ROBERTS, WALSH & COMPANY
425 Eagle Rock Avenue
Roseland, New Jersey 07068
(201) 228-9280

CERTIFIED TRANSCRIPT FOR Fujikawa

I N D E X

WITNESS	DIRECT	CROSS	REDIR	RECR
SOMPONG WATTANASIN				
By Mr. Kelber		3		60
By Ms. Furman			31,64	

E X H I B I T S

FOR IDENT.	DESCRIPTION	PAGE
F-4	Patent application	3
F-5	Request for interference with patent under 37 CFR 1.607	7
F-6	Supplemental declaration of Sompong Wattanasin	12
F-7	Document dated 11-26-84 and attachments	22
F-8	Pages 409 to 417	23
W-2	Document entitled "Declaration - Patentably Distinct Subject Matter"	37
W-3	Pages 164, 165 and 166	37

(Before Gary M. Talpins, a Certified Shorthand Reporter and Notary Public of the State of New Jersey, held at the offices of Sandoz Corporation, Patent and Trademark Affairs Department, 25 Hanover Road, Florham Park, New Jersey, on Monday, March 22, 1993, commencing at 11:55 a.m.)

- - - - - - - - - - - - - - - - -

S O M P O N G W A T T A N A S I N, 11 DiVito Trail, Hopatcong, New Jersey, Sworn.

MR. VILA: Dr. Wattanasin, speak up so everyone here can hear you.

CROSS EXAMINATION BY MR. KELBER:

Q. Doctor, I'm going to hand you a multi-paged document which you can feel free to disassemble as necessary.

MR. KELBER: I would ask the reporter first to mark it as Exhibit F-4, I believe.

(Whereupon the document was received and marked F-4 for identification.)

Q. If you would take a moment to review

the document.

Q. Dr. Wattanasin, do you recognize the document that has been identified as Exhibit F-4?

A. That's something that I have to check because I don't think I remember all of the numbers and so on.

Q. Do you recall seeing a document like this?

A. Oh, yes, definitely, yes.

Q. And can you identify it for me?

MS. FURMAN: By subject matter.

Q. Dr. Wattanasin, is this a patent application prepared by Sandoz?

A. Yes.

Q. And to your recollection, does it name you as an inventor?

A. Yes.

Q. Would you turn to page 54 of F-4.

A. Okay.

Q. Do you see the rather lengthy written passage numbered one there? It continues on to the next page of the document.

A. Yes.

Q. And do you see that that passage, which

begins with the number one, describes a certain genus of compounds?

A. Yes.

Q. Doctor, when did you first learn that another company had filed for United States patent protection on compounds similar to those set forth in the passage numbered one?

A. From my recollection, I think I saw a patent maybe at the end of '88 from I think Warner-Lambert.

Q. Did you receive an initial draft of the document that's been identified as F-4 prior to its completion in the form it's been presented to you?

A. I believe so.

Q. Do you recall if you became aware of the patent, I believe you identified it as Warner-Lambert patent before you received that draft copy of the application?

A. I don't think so.

Q. Do you recall who first brought the Warner-Lambert patent to your attention?

A. I think my supervisor, I believe so, because we have review, you know, it's a routine process in the department that we review the patent

applications not only from Warner-Lambert, from other companies that work on HMG-CoA reductase inhibitor at that time.

Q. Do you know whose responsibility it was to secure those patents of other companies?

A. As I say, it's routine practice in our department to circulate abstracts.

Q. Did you draw the existence of the Warner-Lambert patent, did you draw the attention of anybody in the Patent Department at Sandoz to the fact that the Warner-Lambert patent had issued?

A. I may or may not have called someone in the Patent Department saying that okay, this is the patent from Warner-Lambert similar to our case. From a scientific point, I really have no interest in the Warner-Lambert patent.

Q. Do you have any recollection as to what attorney in the Patent Department of Sandoz prepared --

A. At that time, maybe Jody Giesser, I believe, Jody Giesser.

Q. Do you recall at all discussing the Warner-Lambert patent with her?

A. I believe probably just mentioned that

this is the patent from Warner-Lambert, that's all.

Q. Doctor, I'm going to hand you an exhibit that I would like identified as F-5. It's paper number two from the file, the request for declaration of interference.

(Whereupon the document was received and marked F-5 for identification.)

Q. If you would take just a minute to look at that, doctor.

MR. VILA: Pardon me, can we go off the record.

(Whereupon a discussion took place off the record.)

Q. Doctor, I obtained the document that's been identified as F-5 from the records of the United States Patent and Trademark Office in an application 318773, which identifies you as an inventor, and my question to you is do you recall seeing F-5 prior to this day?

A. I don't think so.

Q. You never saw it prior to today, to the best of your recollection?

A. Yes.

Wattanasin - cross

Q. Do you recall, doctor, at any time discussing the need to bring the Warner-Lambert patent to the attention of the United States Patent and Trademark Office in connection with your application?

A. Yes, I did discuss it sometime, yes, at some point.

Q. Do you recall whether that discussion was before or after the application was filed?

A. Which application?

Q. The original application that is embodied in Exhibit F-4.

A. I did not recall.

Q. Could you take a look at page one of F-5, doctor, the very first page. Do you see the date stamp circle at the very top of the left-hand corner of that page?

A. Yes.

Q. What is that? Can you make out the date that's in there, doctor?

A. March 3, 1989?

Q. Doctor, do you have any knowledge as to whether any patent application besides the application involved in this interference naming

Wattanasin - cross

you as an inventor has ever been involved in an interference in the United States Patent and Trademark Office?

A. Yes.

Q. Would that other application and other interference have occurred prior to the interference that you are testifying in today?

A. Excuse me? I didn't quite understand.

MS. FURMAN: Off the record.

(Whereupon a discussion took place off the record.)

Q. Doctor, has any application for patent been filed by Sandoz Corporation naming you as an inventor other than the application involved in today's interference of --

A. Yes.

Q. Any of those other applications filed naming you as an inventor by Sandoz, of those aplications, to the best of your knowledge, has any been involved in an interference before the United States Patent and Trademark Office?

A. No, I don't think so.

Q. Do you have any recollection of discussing with Ms. Giesser the need for an

Wattanasin - cross

interference in connection with the application involved in today's proceeding prior to its actual filing?

A. Maybe. I cannot say for sure. Maybe, yes, because -- yes.

Q. It's the only interference you have ever been involved in. Is that correct?

A. Yes.

Q. Are you familiar with the nature of an interference, what an interference is?

A. I'm not fully familiar with the legal process.

Q. Did you ever discuss with Ms. Giesser the need to establish a date of invention prior to the Warner-Lambert patent filing date?

A. Yes, I think so.

Q. Do you recall whether that discussion was prior to March 3, 1989?

A. That I don't recall.

Q. Would you flip back to page 54 of F-4, doctor. Do you see the third text line, the second line after the initial formula on that page, where it says, "C_{3-7}cycloalkyl or"? Do you see that line?

A. C_3 --

Q. I'm sorry, counting from the Arabic numeral one on page 54, the third line of text.

A. Okay.

Q. Do you see the recitation C_{3-7}?

A. Yes.

Q. Do you recall having an understanding of what you meant by C_{3-7} at the time this application was originally filed?

A. I believe so, yes.

Q. What was that understanding, doctor?

A. What understanding, can be anything, anything that contains cyclics, having carbon 3 to carbon 7 in it.

Q. That would be five compounds, actually, wouldn't it, doctor, independent of substitutions, that would be five?

A. Yes.

Q. Can you name those compounds for me, what five basic compounds are encompassed by that group C_3 to C_7 cycloalkyl?

A. The name?

Q. The name of the compound.

A. It should be cyclopropane, cyclobutane,

Wattanasin - cross

cyclopentane, cyclohexane and cycloheptane.

Q. Thank you, doctor. Do you have any knowledge as to the level of skill that an initial, an entry category researcher would have in the field of HMG-CoA reductase, what kind, in general, of educational level would be required of such a researcher? By that I mean -- go ahead.

A. I would say it depends on -- I would say at least a Bachelor's degree.

Q. In chemistry?

A. In chemistry, yes.

Q. Would such an individual understand that C_3, in your opinion, that C_3-C_7cycloalkyl included those five basic compounds?

A. Yes.

Q. Thank you, doctor. Doctor, I'm going to hand you a declaration -- sorry, a paper that I would like identified as F-6 and ask you to review that. This one is of record in volume four.

(Whereupon the document was received and marked F-6 for identification.)

MR. VILA: What record page number is that?

MS. FURMAN: Which is it?

Wattanasin - cross

MR. KELBER: Here is my copy.

MS. FURMAN: His declaration.

MR. KELBER: I prefer we not identify what the document is until the witness has a chance to identify it.

MS. FURMAN: Fine.

Q. Doctor, do you recognize this document that's been marked F-6?

A. Yes.

Q. Can you recall the first circumstances under which you saw this document?

A. This is the application that had been filed.

Q. In fact, this document was prepared in connection with this interference, wasn't it, doctor?

A. Yes.

Q. I should say interferences. By interference, I mean Interference 102,648 and 102,975.

A. Right.

Q. Doctor, how many applications, if you know, have been filed by Sandoz naming you as an inventor or co-inventor directed to the field of

HMG-CoA reductase?

A. At least three including the quinoline case.

Q. Let me turn your attention, doctor, to paragraph seven, page two of Exhibit F-6. Why did you submit patent disclosure 299/84 in late March of 1987?

A. Because I believe that at that time, we felt that we should be able to complete most of the key compounds involved in the quinoline cases.

Q. I'm sorry, doctor, I didn't catch your full response. You thought that you could --

A. At that time, we felt that we should be able to finish making most of the key compounds involved in this case.

Q. In general, why do you file a patent disclosure, submit a patent disclosure to the Patent Department? What criteria do you use to determine when to file a patent disclosure?

A. When we feel that we have a class of compound that we can use --

Q. I'm sorry, if you could continue the answer. When you feel you have a class of compounds that can be used?

A. For this particular objective in our department to find inhibitor of HMG-CoA reductase.

Q. Does that represent a determination by you that these compounds are new?

A. Yes.

Q. Does it represent a determination to you that these compounds may be valuable to the corporation?

A. Yes, that's right.

Q. Did any event subsequent to March of 1987 indicate to you that your decision that the compounds identified in 299/84 were not either new or valuable to Sandoz Corporation?

A. I don't think so.

Q. Let me turn your direction to paragraph eight, doctor. Do you know why during the period April through November of 1987, the Sandoz disclosures were rated, let's take the rating "B" first -- not the Sandoz disclosure, your disclosure, PD 299/84, was rated "B" by the Patent Committee?

A. I'm not in the Patent Committee but I understand it bears on the factor that further information on this case would be needed before the

application can be filed so more work needs to be done, I think that's the bottom line.

Q. Did you receive notification that the disclosure had been rated "B"?

A. This is by oral, by verbally.

Q. But you did receive that notification?

A. Yes.

Q. What type of extra work needed to be done?

A. Basically, we have to complete the whole set of compounds that need to be prepared.

Q. And why was that, doctor, why did you have to complete the whole set?

A. I think the objective of making, working on any class of compound is to insure that we come up with an optimum structure. In this particular case, we just making only partially part of the set, we are not complete the whole set yet.

Q. Did you expect to find in the set, part of the set that had not been completed a difference, qualitative difference in the compounds in terms of their value to Sandoz Corporation? In other words, you had completed some of the compounds but not all of the compounds of the set.

Wattanasin - cross

A. True.

Q. Did you have a personal expectation as to the activity you anticipated from the rest of the compounds?

A. Yes.

Q. And what was that expectation, doctor?

A. My expectation is I expect that I may come up with some compounds that show better activity.

Q. Did you expect that some of the compounds in the set to be completed might have worse activity?

A. Yes, that can be the case.

Q. Did, in fact, you come up with compounds subsequent to March of 1987 that had better activity than the compounds identified in the disclosure?

A. Yes. That's normal.

Q. Did you come up with compounds that were worse?

A. Oh, yes, I come up with a compound worse and compound better.

Q. Let's turn now to the "X" rating. When you received notification that your disclosure has

Wattanasin - cross

been rated "X", what does that mean to you, what does "X" indicate?

A. I think it indicates the same thing to me. I mean as I say, I'm not the one who made this thing but it indicates the same thing, more information will be needed to complete, to complete the whole application process of this case.

Q. Was the information needed in response to an "X" rating different, in your opinion, than the information needed for a "B" rating?

A. No, I don't think so.

Q. Do you see the reference in paragraph nine to additional synthesis and testing between July and December of 1987?

A. Yes.

Q. Was that additional synthesis and testing done responsive to the "B" or "X" rating that your disclosure received?

A. No.

Q. You would have done that, anyway?

A. I would have done that, anyway, yes.

Q. Thank you, doctor. If the disclosure had been rated "A", would you have continued that testing that's referred to in paragraph nine?

A. Yes, I believe so.

Q. Thank you, doctor. In the other applications naming you as an inventor completed by or on behalf of Sandoz Corporation, do you have a recollection as to how long it took between the time you learned that the disclosure had been rated "A" and the time you received the first draft of that application? Do you have any idea?

A. No, I cannot give you that honestly.

Q. Can you tell me was it more than six months?

A. I would say about six months, yes.

Q. About six months?

A. About six months.

Q. Do you know does Sandoz have a written policy regarding responding to questions from the Patent Department for additional information?

A. Yes.

Q. It does have a written policy?

A. Yes, policy as to you have to comply with requests from the Patent Department.

Q. There is such a written policy, you think?

A. I think so, yes.

Q. If there is such a policy, can you send us a copy to the extent it's not privileged?

THE WITNESS: I --

Q. That's okay, they will get a chance to ask you all about it in not too long a period of time.

Do you have an appreciation based on the experiences of other researchers at Sandoz as to the time it takes for the preparation of a draft application from the time a disclosure is rated "A"? Do you have a general idea?

A. No, no idea.

Q. Let me turn your attention to paragraph 11 on page three of F-6, doctor. Why did you send certain information to Melvyn Kassenoff about February 29 of 1988?

A. I believe that I was requested by Mr. Kassenoff for subsequent information.

Q. You already knew that your disclosure had been rated "A". Is that correct?

A. At that time, yes.

Q. Was it your understanding that the material you sent to Mr. Kassenoff was required or requested -- I'm sorry, requested for the purposes

of preparing that application?

A. Yes.

Q. Did you have occasion, do you recall, to speak with anybody in the Patent Department between February 29 of 1988 and the end of May 1988 regarding the patent application to be prepared on your disclosure?

A. Yes, I think so.

Q. Do you recall who you spoke with?

A. Either Mel Kassenoff or Jody Giesser.

Q. Do you recall the substance of those discussions?

A. Mostly related to specific information as far as the compound, you know, included in the patent.

Q. Did you at any time ask when you might expect a patent application to be prepared?

A. I don't think so.

Q. Let me turn your attention to paragraph 12, pages three and four of F-6. Why did you send that information to the Patent Department?

A. Again, I was requested from the Patent Department for some information.

Q. Do you recall when you sent that

Wattanasin - cross

information?

A. I don't recall when I received the actual copy of the thing I sent to the Patent Department. Generally I would know what date I sent it on the copy.

MS. FURMAN: Could you repeat your last sentence, please.

THE WITNESS: In general, I don't exactly remember the date that I sent any material to anyone but in general, before I send something to someone, I would note the page, I would date the page.

MS. FURMAN: You would date the page.

Q. Let's take them one at a time. I'm going to hand the reporter a document I would like identified as F-7. I will ask you to review that document briefly, doctor.

(Whereupon the document was received and marked F-7 for identification.)

Q. Doctor, does your review of P-2 enable you to determine in any way about when you might have sent that material to the Patent Department?

A. I can tell you that this is after February 29, 1988.

Q. Do you know would it have been before May of 1988?

A. No.

Q. Did you send it in response to a request from the Patent Department?

A. Yes.

Q. Do you recall who the request came from?

A. I think this is from Mel Kassenoff.

Q. Let me hand you a document, P-3, for identification as Exhibit F-8.

(Whereupon the document was received and marked F-8 for identification.)

Q. Does F-8 contain documents that were sent to the Patent Department as described in paragraph 12 of your declaration?

A. Yes, I think so, yes.

Q. Does review of that document enable you in any way to fix the time you sent those documents to the Patent Department?

A. No.

Q. But you know they were before February of 1988 -- I'm sorry, after February of 1988?

A. After, yes, definitely, yes.

Wattanasin - cross

Q. And you know you sent them in response to a request by Mr. Kassenoff?

A. Yes.

Q. After submission of those documents, but prior to November of 1988, do you recall having any further written or oral communications with the attorneys in the Patent Department at Sandoz regarding your disclosure 299/84?

A. Yes, I think so, yes.

Q. Do you have an actual recollection of it?

A. No, I don't have actual recollection.

Q. Do you have an actual recollection of anything that might have been said or written at that time?

A. Mostly anything that related to the draft or something on it, I see something where they have seen some question that needs to be clarified, I think in general.

Q. But you did not see a draft until November of 1988. Isn't that correct?

A. Yes.

Q. In fact, you didn't see the draft until December of 1988. Is that correct, doctor?

Wattanasin - cross

A. Yes.

Q. Isn't it correct, doctor, that you didn't receive the draft declaration until after you had learned of the existence of a Warner-Lambert patent?

MR. VILA: The declaration?

MR. KELBER: I'm sorry.

Q. Isn't it correct, doctor, that you had received the draft memorandum of your patent application after you had learned of the existence of the Warner-Lambert patent?

A. Let me check the date again. That may be the case, yes.

Q. Do you recall exchanging in writing any communications with Ms. Giesser concerning the Warner-Lambert patent?

A. In writing, no, I don't think so.

Q. Anybody else at the Patent Department, did you exchange correspondence concerning the Warner-Lambert patent?

A. No.

Q. Do you recall publishing the subject matter at item one of page 54-55 of your application, the document that's been marked F-4,

prior to March of 1989?

A. I don't think so.

Q. You had completed the initial set of compounds back in March of 1987. Is that correct?

A. Can you repeat that again?

Q. You had completed the initial set of compounds to which PD 299/84 and subsequently, your application document F-4, pertained, you had completed that initial set of compounds by March of 1987. Is that correct?

A. By March, yes.

Q. And you didn't publish information regarding those compounds until after March of 1989. Is that correct?

A. Yes.

Q. Compounds were interesting to you?

A. Compounds were interesting to me, of course, yes.

Q. Do you think the compounds would have been interesting to other researchers in the field?

A. Of course.

Q. Was there any reason for not publishing that information until after March of 1989?

A. There is no particular reason, I don't

think so.

Q. When did you become aware that Nissan Chemical Corporation had filed for U.S. patent protection on compounds similar to those identified at item one of page 54 of your application?

A. I don't remember the date exactly but I think it happened after we already, you know, talking about a patent application of this case.

Q. So after the application was filed or before?

A. I don't recall the date. I cannot give you the definite time.

Q. Do you recall having discussed the existence of the Nissan Chemical Company's request for patent protection with Ms. Giesser?

A. Yes.

Q. Subsequent to the classification of your disclosure as "A" in January of 1988, did you at any time express any concern to anyone about the progress made in preparing the application corresponding to that disclosure?

A. No, I don't think so.

Q. In your experience at Sandoz Corporation, the period of January of 1988 till

March of 1989, is it customary to take that 14 months for preparation of the patent application?

A. That is unusual. That is unusual.

Q. During that time period, were any other applications naming you as an inventor or co-inventor filed by Sandoz Corporation, January of '88 through March of 1989? Do you recall were any other applications naming you as an inventor or co-inventor filed?

A. There are a couple -- I would say there are two other patents involving HMG-CoA reductase inhibitor but I don't recall the exact date.

Q. Have those, either of those patent applications been issued as a U.S. patent?

A. Yes.

Q. Do you know the number offhand?

A. We are in one of four.

MR. KASSENOFF: Off the record.

(Whereupon a discussion took place off the record.)

Q. I want to return just to one subject and that's the question of the information needed in response to a "B" or "X" classification by the Patent Committee. We talked about the need to

provide more information in response to a "B" classification. What specific type of information is necessary? The synthesis of the compounds, is that required?

A. I think at this time, let me say when you set up on any class of compound, you want to make a few of the compound to find optimum structure and I think at that point in time, we know we are not complete the whole set of compound yet and I think until then, I think we still need further information.

Q. So synthesis and testing of the compound would be required?

A. Synthesis and testing of the compound.

Q. Any of the compounds that are identified in the original disclosure, PD 299/84, did any of those compounds show the type of activity that suggested they might have utility as HMG-CoA reductase inhibitors?

A. Certainly.

Q. Did anything occur between March of 1987 and March of 1989 that suggested that that might not be true, they did not have sufficient activity?

Wattanasin - cross

A. No, I don't think so.

MR. KELBER: Doctor, I really appreciate your patience with me in being here this morning. I have no more questions at this time.

THE WITNESS: Thank you.

MR. VILA: Do you want to take lunch break?

MR. KASSENOFF: Let me ask one question on redirect.

MR. KELBER: I have no objection -- I have discomfort with a witness crossing.

MR. VILA: We will take that question up later.

MR. KELBER: Okay.

MR. VILA: It's a matter of clarifying some things.

MR. KELBER: My only concern is keeping the good doctor longer than we need to. If you have got a lot --

MR. VILA: He is invited to lunch.

MR. KELBER: I kind of hoped you would feed him.

(Whereupon the luncheon recess was taken.)

Wattanasin - redirect

REDIRECT EXAMINATION BY MS. FURMAN:

Q. Dr. Wattanasin, referring to your testimony concerning the C_3 to C_7 cycloalkyl substituents on the quinoline ring, you testified that that would include, among others, cyclopropyl and you testified that a person of skill in the art would recognize it to include cyclopropyl. Do you think that a person of skill in the art would regard cyclopropyl as being obvious as that structure being obvious in view of isopropyl?

MR. KELBER: Objection. I don't know that the witness -- I don't know how you are using the term "obvious" but I don't know that the witness has demonstrated a knowledge under the 103 sense, if you could rephrase it.

Q. Dr. Wattanasin, do you understand what the term "obvious" means under the patent law or can you give me your definition of the term "obvious"?

A. The obvious, in my term, in the medicinal chemistry term, is a kind of, what do you call it, kind of group that you like to make to cover your hypothesis.

Q. Do you think that someone knowing about

an isopropyl substituted compound, based on that information alone, would be led to prepare a cyclopropyl compound?

A. That's what I mean by obvious because in medicinal chemistry, cyclopropyl would be an obvious analogue of cyclopropyl group. If you look at some of the --

Q. Excuse me, I didn't understand you. Cyclopropyl would what?

A. Cyclopropyl, cyclopropane group would be obvious analogue of cyclopropyl group. Do you understand the word analogue?

Q. Yes. If someone in your lab knew about an isopropyl compound, do you think based on that information, they would be led to prepare a cyclopropyl compound?

MR. KELBER: Objection. You are now asking his opinion as to what others in the laboratory would do.

Q. Would you be led to prepare a cyclopropyl compound?

A. Yes, definitely.

Q. Why would you be led to prepare a cyclopropyl compound?

Wattanasin - redirect

A. Because of the, this is according to scientific, basically, when you put the group on any structure, you are looking for two things, two things you are looking for, two properties of that group, sterically and electronically and in this case, cyclopropyl, and cyclopropyl are very similar.

Q. I am talking about cyclopropyl versus isopropyl. Is cyclopropyl similar in chemistry to isopropyl sterically?

A. What I'm saying is sterically and electronically, cyclopropyl group would be very similar to isopropyl group and not only that, you can see from the scheme of the chemistry, chemistry scheme, we have the hardware that can make both compounds quite easily.

MS. FURMAN: I would like to put into evidence as W-2 the declarations that were submitted in this interference of Mr. Kitahara.

MR. KELBER: I will wait until your question but I would object to the extent they would go to anything in the nature of direct questioning.

Q. Dr. Wattanasin, do you recognize the

Wattanasin - redirect

structure on page two of the Kitahara declaration?

A. Yes, I do.

Q. What is that structure?

A. This is the structure of isoquinoline derivative.

Q. The R-5 substituent, what is the R-5 substituent?

A. In this case, R-5 can be cyclopropyl or isobutyl.

Q. Going to the test on that declaration, which compares the activity of cyclopropyl with the isopropyl compound, what is your opinion of this activity information?

MR. KELBER: Before you answer, doctor, I'm going to object to that on the grounds that this is in the nature of direct testimony and if you had wanted to submit it, it should have been submitted together with the remainder of your direct testimony. As far as I'm aware, this is our cross on direct and you have not requested rebuttal response or the opportunity to cross our own declarant. I can't stop you from asking your questions but I do definitely object to further questions on this issue.

Wattanasin - redirect

MR. VILA: Can I conference with you outside?

MS. FURMAN: Yes.

(Whereupon a brief recess was taken.)

MS. FURMAN: I will go on to a different line of questioning.

BY MS. FURMAN:

Q. Dr. Wattanasin, the patent disclosure on your quinoline compound is numbered 299/84. Do you know how this number was assigned to your patent disclosure?

A. I think this number was assigned on an annual basis, I believe. Before the end of the year, one of the secretaries here send you the patent disclosure form for the next year.

Q. A blank patent disclosure --

A. A blank patent disclosure.

Q. With the number appearing at the top?

A. Yes.

Q. And that was sent to you when?

A. Around the end of the year, in December.

Q. In December of --

A. Of '83.

Q. Of '83. You synthesized at least one compound by the end of 1984. Is that correct?

A. Yes.

MR. KELBER: Just for clarification, we are talking about the compounds of the disclosure or compounds in general or what?

THE WITNESS: The first compound we are making, one of the first compounds we are making in this case.

Q. After that compound was synthesized, what additional work was done in relation to your quinoline patent disclosure? After the synthesis of 63366, what compounds did you synthesize?

A. There are a number of compounds we synthesized during that period. At that time, we were still working on basically all of them. All of them are HMG-CoA reductase inhibitors and two more compounds, two more compounds were synthesized, the number I believe is 64548 and 64549.

MS. FURMAN: I would like to put into evidence as Exhibit W-3 pages --

MR. KELBER: We started to talk about

W-2 but we never did get around to marking it. Do you want to have W-2 in or do you want to just mark those as W-2?

MS. FURMAN: Yes, let's put W-2 in.

(Whereupon the document was received and marked W-2 for identification.)

MS. FURMAN: I would like to put into the record as W-3 pages 164 through 166 of the Wattanasin testimony.

(Whereupon the document was received and marked W-3 for identification.)

Q. Dr. Wattanasin, do you recognize those pages?

A. Yes, I do.

Q. Can you describe them?

A. This is reaction, this is a notebook, from my notebook, the synthesis of one of the compounds that later on is designated as 64548.

Q. And what is the date at the top of the page?

A. 5/7/85.

Q. What would the date at the top of the page signify?

A. This is the date that I start doing

this particular reaction that leads to the synthesis of this particular compound 64548.

Q. So you had synthesized 64548 sometime on or after May 7, 1988?

A. Yes.

Q. Is there an additional compound that you synthesized around that time?

A. The next compound we synthesized is the compound 64549.

Q. Was that also synthesized --

A. Around this date.

Q. Around May of 1985?

A. '85, yes.

Q. Your patent disclosure, which is numbered 299/84, when was that submitted to the Patent Committee by you?

A. I think by March, in March '88.

Q. March of --

A. March of 1988.

Q. Submitted to the Patent --

A. I'm sorry, March of 1987.

Q. What made you submit the patent disclosure in March of '87? Why did you not submit the patent disclosure after you made 64548 or 49?

Wattanasin - redirect

A. I think the reason for that is because of we are not complete the whole set of this class of compound yet.

Q. Why had you not completed the whole set?

A. The reason is because, I think one of the key reasons is because of a lack of manpower at that time because I'm the only one working at that time on the HMG-CoA reductase in this lab.

Q. Your lab was the only lab synthesizing quinoline compounds?

A. Yes.

Q. When did you realize you lacked manpower to proceed with the whole series?

A. Actually, at that time, actually 1985 because we are dealing with different classes of HMG-CoA reductase inhibitor compound, quinoline is not the only compound we are making. We are making other, different kind of heterocyclics, as well.

MR. KELBER: I don't know that it raises to the level of an objection, Diane, but to what part of the cross does this line of questioning go to?

MS. FURMAN: I believe you did ask him

about his activities in that time period.

MR. KELBER: I asked him if anything occurred with regard to the period between the submission and the "A" rating, I asked him if anything occurred to change his mind.

MS. FURMAN: You were discussing the initial set of compounds, you asked whether they were completed by March of 1987 and I was trying to develop that testimony.

MR. KELBER: Okay.

Q. When did you realize there was a manpower shortage?

A. I think around this time, I think sometime in 1985.

Q. How long did it take you to find somebody to fill that position or positions?

A. Normally to get someone, you have got to have approval from your boss and then subsequently, you have got to get approval by your department head and then it also depends on whether or not the opening is available at that time and when you got the actual head count, the opening, then you have got to get approval from your boss, from your department head and then from the head

of -- from the president of SRI. And then you have to recruit the person. It takes a long time, actually.

Q. How long did it take?

A. You have an opening, after you have an opening, then you have to place an ad and looking for someone, I would say at least six months.

MR. KELBER: I'm going to object because I'm not sure but I don't think the answer was responsive to the question. I think the answer was general and you had a very specific question.

Q. Can you answer the question more specifically. How long did it take you in this case to find somebody?

A. In this case, a whole year.

Q. When did you ultimately find somebody?

A. I got someone to join my lab in January 1987.

Q. What was the name of that person?

A. Miss Patel.

Q. Can you spell out the full name?

A. Rajeshvari Patel, R-a-j-e-s-h-v-a-r-i P-a-t-e-l.

Q. Was she assigned to your lab exclusively?

Wattanasin - redirect

A. Yes.

Q. Did you supervise her work?

A. Yes.

MS. FURMAN: Do you want to continue with questioning or do you want to leave it open?

MR. VILA: Are you finished completely or do you want to come back later?

MS. FURMAN: I'm going to come back later.

BY MR. VILA:

Q. Was there any relationship or significance to the timing of the submission of the patent disclosure to the Patent Department relative to this lack of manpower that you mentioned?

A. Yes, because --

Q. What would that be?

A. Because if I did have the manpower before 1987, some key compounds should have been synthesized before that date, before March 3, 1987.

Q. You mentioned this Miss Patel and she was hired in January of --

A. 1987.

Wattanasin - redirect

Q. -- '87. Do you recall what assignments she was given when she was hired?

A. There were a number of assignments given to her, key projects were given to her and this quinoline project is one of them.

Q. From the start, she --

A. From the start, yes.

Q. -- she was assigned this?

A. Yes.

Q. Having not submitted that disclosure previously, why would you have at that particular time submitted the disclosure?

MR. KELBER: I think that has been asked and answered.

THE WITNESS: Yes.

Q. You can answer it. Go ahead.

A. Because at that time, with additional manpower, I felt that we should be able to complete the whole set of this quinoline case, that's why I file the patent disclosure at that time.

Q. You had testified in response to questions on cross examination with regard to publication of the subject matter of this patent disclosure in this patent application. Would you

have published on this subject matter prior to March of '89 when the patent application was actually filed?

A. No, I wouldn't.

Q. Why would you have not done that?

MR. KELBER: I'm going to object just to the form. Is the question did he or would he have? I don't understand the subjective tense of the question.

MR. VILA: Would he have. I believe he testified before that he could have --

MR. KELBER: If he didn't, he wouldn't. I mean I don't understand the nature of what -- is there a difference between did and would?

MR. VILA: Yes.

MR. KELBER: Are you asking for a hypothetical situation? We know what he would have done, he did it in this situation. Are you asking --

MR. VILA: Let's go off the record a second.

(Whereupon a discussion took place off the record.)

Wattanasin - redirect

Q. I will simply ask you did you make any publication on the subject matter of that patent application prior to its filing?

A. No.

Q. Can you explain why you did not make a publication on that subject matter?

A. If I understand, you cannot disclose the information related to the patent disclosure until it was approved by the Patent Department, until it be cleared by the Patent Department.

Q. I believe you testified on cross examination that there was a written policy or you thought there was a written policy with regard to communications with the Patent Office and in particular, responding to requests by the Patent Department.

A. Yes.

Q. Have you ever seen such a written policy?

A. What I meant in that time is this is part of what you call the job description, that you are supposed to comply with all of the requests, information related to the patent application of your discovery.

Wattanasin - redirect

BY MS. FURMAN:

Q. You testified concerning the activity of the compounds in the quinoline series. In response to questioning, you indicated that after you did the earliest work, you would have expected some compounds would come up with better activity or worse activity. Is that true?

A. I cannot predict that but it can be seen from the IC_{50} of one of the first compounds, I believe 63366, the IC_{50} of 1.5 micromolar. That, in my judgment, that is comparable to IC_{50} of Compactin and established HMG-CoA reductase inhibitor.

Q. And established HMG-CoA reductase inhibitor?

A. Yes.

Q. So based on the first compound you made, what was the likelihood that the later compounds would have activity in vitro as an HMG-CoA reductase inhibitor?

MR. KELBER: Objection. What later compounds?

MS. FURMAN: 64933, 934, 935 and 936.

A. I cannot predict activity of those

Wattanasin - redirect

compounds before I make them. However, based on the information, we have learned from closely related analogue of this quinoline compound, I would say that we would have very good chance of being active and as you can see from the IC_{50} of those compounds, again, they are comparable again to Compactin and as you know, going back to the in vivo, as you know, Compactin has a good potency, not only in vitro but also in vivo, as well. So when some of those compounds have IC_{50} similar to Compactin, one would predict that to have a good activity in vivo, as well.

Q. Predicted?

A. One would expect that.

Q. Expect it?

A. Yes.

Q. What level of assurance would you have? How high would be your expectation?

A. Actually, I would say it I would be very certain that the compound should have activity in vivo, as well.

BY MR. VILA:

Q. Would that statement that you just made

apply to 63933, which is part of your mention on page 27 of your original declaration, results on page 27 of the record? Do you know the structure of the compound I referred to as 63933?

A. Yes, I do.

Q. Would that statement apply to that compound?

A. I'm not quite sure. That's project 933, 64933. If I recall, IC_{50} of 64933 is somewhat less active than the first compound I made. However, the statement would apply to the later compound, the number is 64935, which we have better IC_{50} and also have very good potency based on ED_{50} based on in vivo testing.

Q. We know the IC_{50}'s now, I think we are going back to the point when you prepared these compounds and before they were tested, you said that you would have a very high degree of confidence that they would exhibit activity. We don't know the level of the activity.

A. Yes.

Q. Would that high degree of confidence apply to 63933?

A. Yes, I think so.

Q. And the compound -- I'm sorry, is that --

A. 64933.

Q. I'm sorry, I beg your pardon, correct the record, I'm referring to 64933, correct?

A. Yes.

Q. And that's a compound you know the structure of?

A. Yes.

Q. It's in the record. I would ask the same question with regard to compound 64934. Do you know the structure of that compound?

A. Yes.

Q. Would you have or not have that same degree of confidence as to the activity of that compound at the time it was prepared and before you tested it?

A. I would have the same degree of confidence.

Q. And 64935?

A. Yes.

Q. The same?

A. Same degree of confidence.

Q. In the record that I have observed

here, the compound 64933 and 64934 allegedly were prepared --

A. In August, I believe.

Q. -- sometime in July or August of '89.

A. No, '87.

Q. '87, I'm sorry. Yet they were not sent for testing at that point.

MR. KELBER: Objection, assuming facts not in the record of today's deposition. The fact that you may have submitted them elsewhere doesn't make them of record here.

MS. FURMAN: Off the record.

(Whereupon a discussion took place off the record.)

BY MR. VILA:

Q. You testified those compounds were prepared sometime in August of '87 from your recollection.

A. Yes.

Q. Do you recall when they were submitted for testing?

A. I think it's in one of these exhibits. It's definitely. I do recall, yes. I believe it

Wattanasin - redirect

was submitted for testing on October 2nd, 1987.

Q. And by submitted for testing, what does that mean to you, October 2 of '87?

A. What do you mean this means to me?

Q. You say they were submitted for testing and I asked you what do you mean by submitting, what event took place on October 2, 1987?

A. On October 2, 1987, the compound was shipped to Professor Terry, T-e-r-r-y, Scallen, S-c-a-l-l-e-n.

Q. These compounds were prepared in August, as you say, and they were sent in October. Why weren't they submitted earlier? Do you have a recollection on why they were not submitted earlier to Dr. Scallen?

A. There are basically two key reasons. First of all, doing the process, the compound has to be made and the -- doing the process of the compound being synthesized and purification and characterization, I went to a meeting in New Orleans for over a week and when I came back, I was aware that the next shipment would be on October 2nd and so even though these last three compounds were made before that October 2nd, I would like all

of these compounds to ship for testing together so I can have a better comparison of the potency in the same study.

Q. When you say all of these compounds, you are referring to which ones?

A. 933, 64933, 64934 and 64935 and 64936, as well.

Q. Could you tell me whether you had any particular procedures or arrangements for sending compounds to Dr. Scallen?

A. Yes. Normally after you finish the synthesis and the compound has been purified and the compound had been submitted to different measurements in the physical chemistry department to identify the identity of the compound, then we would, we, I mean the chemists in my lab would then submit the compound to the drug room and then there would be one person responsible for registering the compound into the system and then after the compound had been registered into the system, there would be another person who would be responsible for collecting all of this compound and ship it, ship them for testing.

MR. KELBER: I'm going to renew my

Wattanasin - redirect

objection to this line of questioning at this time. I know I didn't go into anything regarding in vivo testing and the procedures therefor on direct.

MR. VILA: I believe you have been into the questions of abandonment and diligence in this area and I think --

MR. KELBER: Certainly not diligence, never. With respect to abandonment, suppression, concealment, that's an issue but it's hardly anything that gives rise to a free-for-all in determining what kind of activities. My understanding of the rules provide that you can ask in areas developed on redirect that were initially explored on cross. I just want to make my objection for the record because the rule requires it to be made now rather than later.

MR. VILA: All right. I think that we are probably finished with that line.

BY MR. VILA:

Q. In January of 1988, your disclosure 299/84 was rated "A" by the Patent Committee, I believe you have testified to that. As a result of

that rating, what would have been your expectancy with regard to the subject matter in that disclosure?

MR. KELBER: The witness can answer if he can but I admit, I'm totally confused by your question. What is his expectation with regard to this subject matter?

Q. What did that rating mean to you?

A. I think I already answered that question this morning, that the rating doesn't mean to me, it's only my intention to complete the synthesis of one of the key compounds in the quinoline case.

Q. I believe it was also testified this morning that the "A" rating would signal the filing of a patent application.

A. Yes, you are right.

Q. And I would ask you whether that created a certain expectancy in your mind with regard to that filing of a patent application?

A. Yes.

Q. And what would that expectancy be?

A. The expectation would be that the compound should be finished as soon as possible.

Wattanasin - redirect

Q. I'm referring to the "A" rating of the decision to file a patent application, whether that decision created a certain expectancy in your mind. Would you have expected that a patent application would have been filed as a result of that "A" rating?

A. Yes.

Q. I would ask you, then, from the period January of 1988, when that was rated "A", and March of 1989, when the patent application was actually filed, whether anything occurred that would have changed your expectancy that a patent application would have been filed?

A. Nothing.

Q. Do you want to verbalize the answer.

A. Can you repeat the question? I'm not quite really understanding the point. Can you repeat the question again, please?

MR. VILA: Do you want to read him the question.

(Whereupon the record was read.)

A. Nothing.

MR. VILA: Let's go off the record for a minute.

Wattanasin - redirect

(Whereupon a discussion took place off the record.)

Q. You just testified that you expected a patent application to file. Are you aware of any activities on the part of anybody else that may have indicated any kind of a decision not to file a patent application on that disclosure which had been rated "A" in January of --

A. I was not aware of any.

BY MS. FURMAN:

Q. Did either Mel Kassenoff or Jody Giesser ever indicate to you an intention not to file a patent application?

A. No, definitely not.

Q. You testified earlier that you spoke with Jody Giesser about the Warner-Lambert patent and possibly about the Nissan application. Is that correct?

A. Yes.

Q. I want to ask you again whether you can remember exactly when you spoke to her about those publications. Do you remember for certain that you spoke with her before the filing of the patent

Wattanasin - redirect

application?

A. I believe so, yes.

Q. Do you remember exactly when that was?

A. I don't remember exactly when.

Q. Did you arrive at any conclusion based on your talk with her about that?

A. Conclusion about what?

Q. The Warner-Lambert patent. Had you been working on the patent application already when you spoke with her about the Warner-Lambert?

A. Yes.

Q. You were working with her on the draft before you spoke with her about the Warner-Lambert?

A. Yes.

Q. You received a draft of the application in, I believe, December of 1988.

A. December or November.

Q. November of 1988.

A. Yes.

Q. Were you in communication with Jody Giesser before that date concerning the patent application?

A. Yes.

Wattanasin - redirect

Q. Were you in communication with her between February and November at any time?

A. Of what year?

Q. 1988.

MR. KELBER: Asked and answered. He said before that day.

MS. FURMAN: More specifically, between February and November.

A. Yes.

Q. Were those communications oral or written?

A. Mostly I believe oral, over the phone.

Q. Dr. Wattanasin, is English your first language?

A. No.

Q. What is your first language?

A. Thai.

Q. Thai?

A. Yes.

Q. Did Jody Giesser ever have trouble understanding you?

A. I don't think so.

Q. You don't think so.

MS. FURMAN: That's about it.

MR. VILA: I just have one final question.

BY MR. VILA:

Q. During the period sometime in 1985, after you had made the three compounds, the first three compounds, those being, according to the record, 63366, 63548, 63549, that synthesis ending sometime in 1985, and early 1987, when the activities resumed on this quinoline series, was it ever your intention that that earlier work would be considered abandoned in your mind in the sense that it would be no longer of interest?

A. No, definitely not.

Q. And how would you describe the interest that you had in those compounds during that period?

A. My interest in those compounds, I would say very high but as I stated before, that the reason that the gap is somewhat apart is because of two reasons. The first one is because of the manpower that I mentioned before. I think the second thing is because of the priority and the priority is sometimes set by me and most of the time set by my supervisors.

MR. VILA: I don't think I have any more questions.

MR. KELBER: I have just a few, doctor. I'm sorry to belabor you but I do understand you clearly, I don't think there is a problem there.

RECROSS EXAMINATION BY MR. KELBER:

Q. The very last answer you gave had to do with the manpower shortage and the priority being set on things. Did you set the priority with regard to the compounds in question that you just testified to?

A. The priority was set either by myself or my boss.

Q. In this particular case, do you recall who set the priority?

A. In this particular case, I think -- actually both, I will say both. You see, I mentioned before this is not the only compound, only class of compound we are working with. We are working on different classes of compounds during the HMG-CoA reductase and probably as you have seen from the patent, as well, we have two key

Wattanasin - recross

compounds, very important compounds, indole and indene.

Q. Did those projects receive a higher priority than the project in question?

A. Yes, according to my supervisor, yes.

Q. You also mentioned the kind of arduous process that anybody with supervisory authority is involved with hiring somebody new and you couldn't find anybody for over a year. Is that correct?

A. No, what I'm saying is the process, because of, first of all, before you can hire anyone, you have got to get approval from different people first and once you got approval for hiring someone, then it would take at least six months before you actually get someone to join your lab.

Q. Understood. This manpower shortage, if you will, that was a fairly big problem for you in connection with this?

A. Big problem because I'm the only one working in the lab on a number of compounds, on a number of projects.

Q. Did you speak to anybody in the chain of command, your boss or above, regarding expediting the process of bringing in somebody?

Wattanasin - recross

A. Yes, I did speak many times with my bosses about this issue, yes.

Q. To the best of your knowledge, did anybody do anything to expedite it?

A. As I say, the decision not only depend on my boss.

Q. But the decision also included those above your boss?

A. Yes.

Q. And do you recall today making a decision to expedite the search for manpower in this particular case? Did they move faster than the regular procedure in the case that was eventually satisfied by Dr. Patel?

A. That I don't have information to tell you.

Q. Did you ever submit a disclosure relevant to the quinoline derivatives that we have been talking about today for clearance by the Patent Department?

A. Beside quinoline cases?

Q. Besides the patent application and patent disclosure itself, I'm sorry, let me go backwards, during redirect, you spoke that a

Wattanasin - recross

disclosure outside of a patent application can't be released until it's cleared by the Patent Department. Do you recall that testimony?

A. Yes.

Q. Did you, yourself, ever submit a publication for clearance by the Patent Department relative to the subject matter of the application involved?

A. Yes, I prepared some, yes.

Q. And that would have been prior to the filing date?

A. After the filing dates.

Q. You did not submit a disclosure prior to the filing date?

A. That I'm not quite sure. I have to check my record before I can answer to you definitely.

MR. KELBER: Can we ask you to check those records and get back to us.

Q. You testified, doctor, that on the basis of your initial work reflected in the patent disclosure, you had a reasonably high expectation as to the issue of whether the compounds later prepared would exhibit activity.