HMG-CoA Reductase Inhibitors

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Selectively reviewing the literature published up to October 1992

1 Introduction

Extensive epidemiological studies performed in many countries have shown that increased blood cholesterol levels, or, more specifically, increased levels of low density lipoprotein (LDL) cholesterol, are a major cause of coronary heart disease. There is also substantial evidence that lowering total and LDL cholesterol levels will reduce the incidence of coronary heart disease.[1]

In 1976, Endo *et al.* reported the isolation of mevastatin (formerly called ML 236B or compactin) (1) as a potent inhibitor of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in endogenous cholesterol synthesis.[2,3] They elucidated biochemical mechanisms for the action of mevastatin[4–8] and by 1980, had shown that mevastatin strikingly lowers total and LDL cholesterol in patients with hypercholesterolemia.[9] These findings stimulated the worldwide development of mevastatin analogues in the 1980s and by 1990, three drugs, lovastatin (formerly called mevinolin[10] or monacolin K[11,12]) (2), simvastatin[13] (3) and pravastatin[14] (4), had been marketed in many countries.[15,16] In addition to these, many other mevastatin analogues have been synthesized, some of which are now under clinical development.

2 Biochemical and Pharmacological Mechanisms

2.1 HMG-CoA Reductase

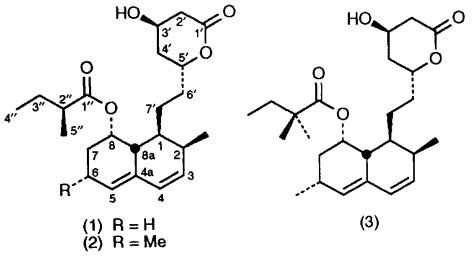
HMG-CoA reductase, a 97 kDa glycoprotein,[17] catalyses the reductive deacylation of HMG-CoA to mevalonate in a two-step reaction (Scheme 1).

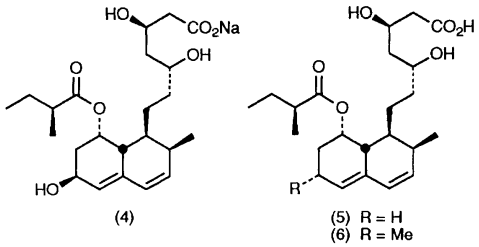
The stereospecificity of HMG-CoA reductase is illustrated in Scheme 1. Only the 3*S* isomer of HMG-CoA is utilized in the reaction.[18–20] Each hydride transfer involves the *pro-R* or 'A' side of the NADPH pyridine ring,[21,22] the first forming the 3S, 5R-thiohemiacetal, and the second incorporating the hydrogen into the 5-*pro-S* position of (3*S*)-mevalonate.[19,23,24]

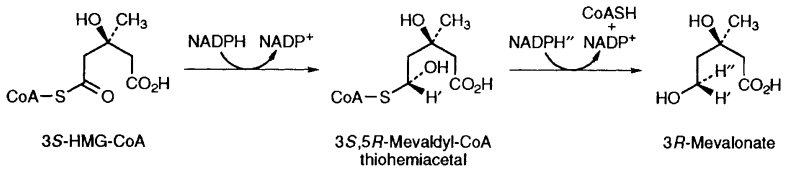
Mevastatin analogues are reversible competitive inhibitors of HMG-CoA reductase.[3,13] The K_m value for mammalian HMG-CoA reductase is $\sim 10\ \mu$M, while the K_i value for the ring-opened acids of mevastatin (5) and lovastatin (6) are in the range of 0.2—1 nM.[25] Thus, the affinity of HMG-CoA reductase for mevastatin analogues is 10000-fold or more than its affinity for the natural substrate, HMG–CoA. The 3,5-dihydroxyheptanoic acid portion of these compounds resembles the HMG portion of HMG-CoA (Scheme 1).

The 3,5-dihydroxyheptanoic acid chain of mevastatin interacts with the HMG binding domain of the enzyme's active site. It has been postulated that the tight binding of mevastatin is the result of its ability to simultaneously interact with both the HMG binding domain and an adjacent hydrophobic pocket which is not utilized in substrate binding.[26]

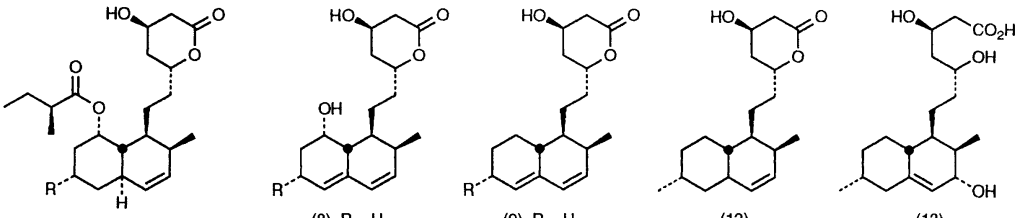
Kinetic studies have shown that the enzymatic reaction is consistent with the general chemical mechanism postulated for







Scheme 1



dehydrogenase catalysis in assisting direct transfer of a hydride ion between nucleotide and substrate.[27] The p*Ka* of this catalytic group is dependent on whether reduced or oxidized cofactor (NADPH or $NADP^+$, respectively) is bound at the active site. Mevastatin (1) apparently owes its inhibitory activity to the ability to mimic the half-reduced substrate mevalonate hemithioacetal. Therefore, by analogy, the 5-hydroxy group of mevastatin must interact with the unprotonated form of this catalytic group.

Mevastatin analogues inhibit cholesterol biosynthesis in a variety of mammalian cell cultures at nM concentrations.[5,6,25] In human skin fibroblast cultures, sterol synthesis from [^{14}C]acetate is inhibited by 50% at ~ 1 nM. When mevastatin analogues are given orally to rats, sterol synthesis in the liver, the major organ of sterol synthesis, is acutely inhibited.[4]

Mevastatin and lovastatin do not lower plasma cholesterol levels in rats and mice,[28] but these agents are highly effective in reducing plasma cholesterol in dogs,[29,30] monkeys,[31] rabbits[32,33] and humans.[9,15,16,34]

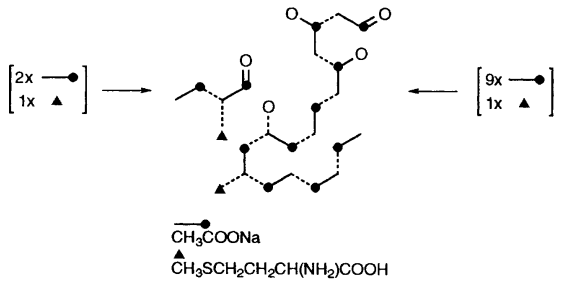
2.2 Plasma Cholesterol

Cholesterol is transported in plasma in the form of lipoproteins. By means of ultracentrifugation six distinct classes of lipoprotein can be isolated from plasma. Most of the cholesterol in humans is carried by LDL.

Mammalian cells from normal subjects possesses LDL receptors on the cell surface which bind LDL with a high-affinity.[35] The bound LDL is incorporated into the cells and undergoes lysosomal digestion, leading to hydrolysis of cholesterol esters. The free cholesterol that is released serves to control the rate of cholesterol synthesis within the cells by down-regulating HMG-CoA reductase.[17] The chief role of the LDL receptor is to provide a constantly available source of cholesterol throughout the body. Mutations of the gene encoding the LDL receptor results in impaired degradation of LDL and thus cause familial hypercholesterolemia (FH).[36]

Endogenous cholesterol synthesis is decreased by exposing cells to LDL which facilitates delivery of exogenous cholesterol and thus down-regulates HMG-CoA reductase.[35]

The co-ordinate regulation of LDL receptor expression and HMG-CoA reductase activity provides a homeostatic mechanism for ensuring an adequate supply of cholesterol to cells such as hepatocytes which metabolize large amounts of LDL each day.[36] HMG-CoA reductase inhibitors typified by mevastatin (1) block endogenous cholesterol synthesis, especially in the liver which requires cholesterol as a substrate for bile acid synthesis.[4] To overcome the short-fall, hepatocytes express a greater number of LDL receptors and thereby promote influx of LDL cholesterol from plasma.[36,37] The net result is a decrease in plasma LDL cholesterol. Lovastatin (2) and simvastatin (3) are both lactones and inactive until metabolized in the liver to the open-ring hydroxy acids.



Scheme 2

3 Mevinic Acids of Fungal Origin

3.1 Isolation

Mevastatin (1), a metabolite of *Penicillium citrinum*, was isolated in 1973, filed for patent in 1974, and first described in the literature in 1976.[2,3] This compound was independently isolated from *P. brevicompactum* as an antibiotic by Brown *et al.*[38] Subsequently, lovastatin (2) was isolated from *Monascus ruber*[11,12] and *Aspergillus terreus*,[10] respectively. Lovastatin is slightly more potent than mevastatin in inhibiting HMG-CoA reductase. These compounds can be easily converted to the respective open-chain dihydroxy acids (5) and (6).

Along with mevastatin, dihydrocompactin (7),[39] ML-236A (8),[2] and ML-236C (9)[2] have been isolated from *P. citrinum*. Monacolin J (10),[40] monacolin L (11),[40] dihydromonacolin L (12),[41] and 3α-hydroxy-3,5-dihydromonacolin L acid (13)[42] are minor metabolites of *M. ruber*. Dihydromevinolin (14) is a product of *A. terreus*.[43]

The class of compounds mentioned above, distinguished by a highly functionalized hexalin or octalin unit and a β-hydroxy-δ-lactone portion linked by an ethylene bridge, are collectively referred to as mevinic acids.[44]

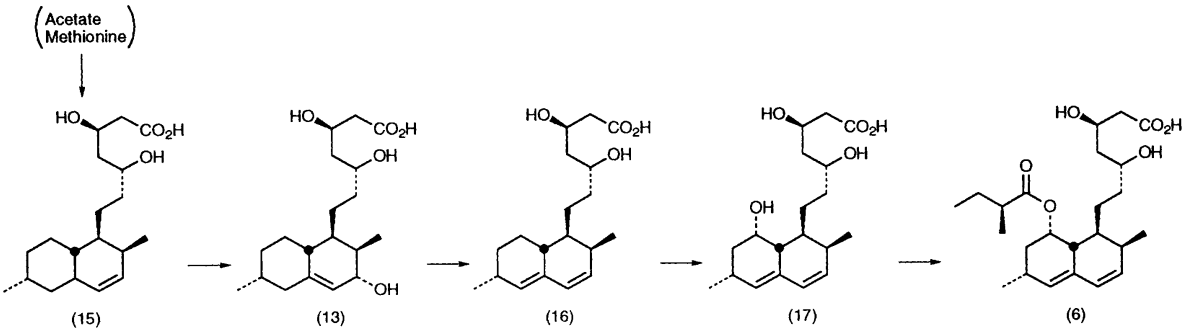
Dihydrocompactin (7) and dihydromevinolin (14) are comparable to mevastatin and lovastatin, respectively, in inhibiting HMG-CoA reductase, while other metabolites that lack the side chain ester are far less active.[25]

3.2 Biosynthesis

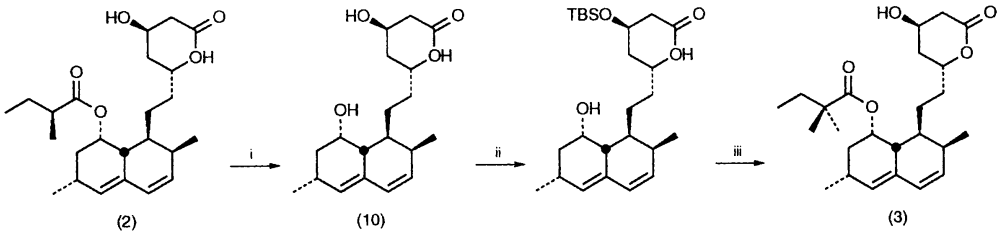
[^{13}C]Acetate, [methyl-^{13}C]methionine and $^{18}O_2$ are incorporated into mevastatin and lovastatin in cultures of *P. citrinum*, *M. ruber* and *A. terreus*, and the ^{1}H and ^{13}C NMR spectra of the two products have been fully assigned by a combination of spectral analyses. Both compounds are formed by the head-to-tail coupling of two polyketide chains (C_4 and C_{18}) each derived from acetate units. The C_4 chain has one methionine derived methyl group, and a methyl group at C-6 in the bicyclic ring system of lovastatin is also derived from methionine (Scheme 2).[45–47]

The above data suggest involvement of a biological Diels–Alder cyclization to generate the correct ring stereochemistry in a single step. Such ring forming processes have been suggested for a number of reduced polyketide metabolites.

Mevinic acids isolated from *M. ruber* by Endo and co-workers include dihydromonacolin L (12), 3α-hydroxy-3,5-



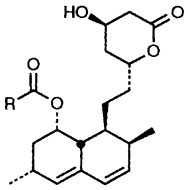
Scheme 3

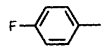


Reagents: i, LiOH, H_2O, 100 °C; H_3O^+, toluene, 110°C; ii, TBSCl; iii, 2,2-dimethylbutyryl chloride; Bu^nNF, HOAc

Scheme 4

Table 1 Effects of modification of the side chain ester moiety of lovastatin.



R	Relative potency[a]
	254
CH_3	2.1
$CH_3(CH_2)_3$	64
$CH_3(CH_2)_8$	1.9
$CF_3CH(CH_3)_2$	157
$C_2H_5C(CH_3)_3$ (3)	622
$(C_2H_5)_2C(CH_3)$	394
$C_3H_7(C_2H_5)_2$	294
	15

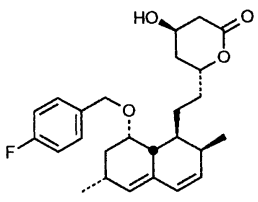
[a] Potency of mevastatin is assigned a value of 100.

dihydromonacolin L acid (13), monacolin L (11), monacolin J (10) and monacolin K (lovastatin) (2). They have shown that dihydromonacolin L acid (15) is converted to 3α-hydroxy-3,5-dihydromonacolin L acid (13) by *M. ruber* in the presence of molecular oxygen.[42] The latter can be spontaneously dehydrated to monacolin L acid (16), although conversion of exogenously added 3α-hydroxy-3,5-dihydromonacolin L acid to monacolin L acid by *M. ruber* has not been successful. Monacolin L acid is hydroxylated to monacolin J acid (17) by the action of a monooxygenase.[48] The end-product monacolin K acid (6) can be derived from monacolin J acid (17) by *M. ruber*.[49] One possible mechanism for this conversion is the esterification of monacolin J acid with α-methylbutyryl-CoA to monacolin K acid. These results are summarized in Scheme 3.

3.3 Modification

The 2*S*-methylbutyryl ester of mevastatin and lovastatin, gave ML-236A and monacolin J, respectively, by hydrolysis with either alkali or carboxylesterase of the fungus *Emericella unguis*.[50,51] Hoffman *et al.* at Merck, Sharp & Dohme (MSD) synthesized a series of the side chain ester analogues of lovastatin from monacolin J (Scheme 4).[13] A systematic exploration of the structure-activity relationships showed that the introduction of an additional aliphatic group on the carbon α to the carbonyl group increased potency (Table 1).[13] This observation led to the synthesis of simvastatin (3) (Scheme 4), which has about 2.5 times the intrinsic inhibitory activity of lovastatin (2).[13] A process involving enolization/methylation of the 2*S*-methylbutyrate side chain of lovastatin also affords simvastatin highly efficiently.[52] Simvastatin has been marketed since 1988.

Side chain ether analogues of lovastatin are weaker inhibitors of HMG-CoA reductase than the corresponding side chain ester analogues. Of the ether analogues prepared by Lee *et al.*, the 4-fluorobenzyl ether analogue (18) proved to enhance the potency.[53]



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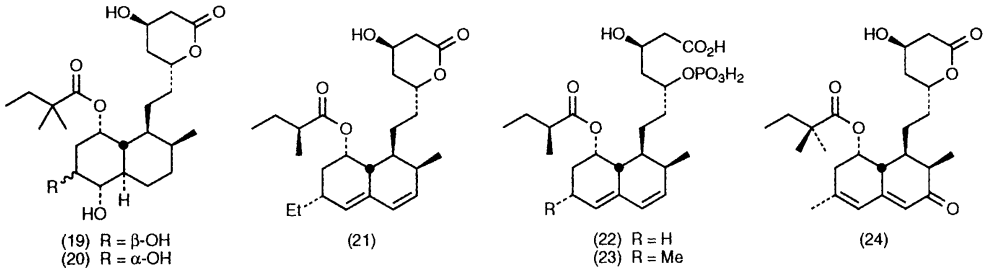
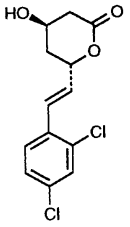


(19) R = β-OH
(20) R = α-OH

(21)

(22) R = H
(23) R = Me

(24)



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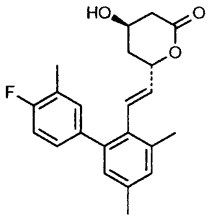

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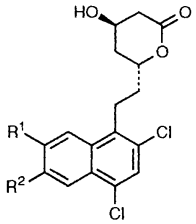


(27) R^1 = Cl, R^2 = H
(28) R^1 = H, R^2 = Cl

Modification of the hexahydronaphthalene ring 6-position in simvastatin (3) *via* oxygenation and oxa replacement by Duggan *et al.* led to the selection of (19) and (20) for pharmacological evaluation.[54] These two compounds proved to be orally active as hypocholesterolemic agents in cholestyramine-primed dogs and also exhibit low peripheral plasma drug activity levels, which may minimize pharmacologically related side effects since the major site of cholesterol synthesis is the liver. (+)-6-Ethylmevastatin (21) is comparable to lovastatin in inhibitory potency.[55]

Pravastatin (4)[14] is prepared by microbial transformation.[56,57] The enzyme cytochrome $P\text{-}450_{sca}$ is responsible for this conversion by *Streptomyces carbophilus*.[58] Pravastatin is structurally different from lovastatin in that it contains a hydroxyl group in the hexahydronaphthalene ring, making pravastatin more hydrophilic than lovastatin. Pravastatin is comparable to mevastatin and lovastatin in inhibiting HMG-CoA reductase and in lowering plasma cholesterol. It has been on the market since 1989.

The phosphorylated derivatives (22) and (23) are produced by the action of several fungal strains.[59] These derivatives are converted to the respective parent compounds (5) and (6) in the liver, when administered to rats.

Compound L-669262 (24) is derived from simvastatin (3) by microbial conversion, and is 6–7 times more active than simvastatin in inhibiting HMG-CoA reductase.[60]

4 Synthetic Analogues

Since the mid 1980s a plethora of work has been directed towards the preparation of synthetic analogues of mevastatin (1) and lovastatin (2) by many pharmaceutical companies. Of these studies, initial investigations at MSD served to delineate key structure-activity relationship for mevastatin-like mimics and afforded a series of moderately effective HMG-CoA reductase inhibitors bearing a monocyclic substituent, of which the ring-opened form of lactone (25) was the most potent.[61,62]

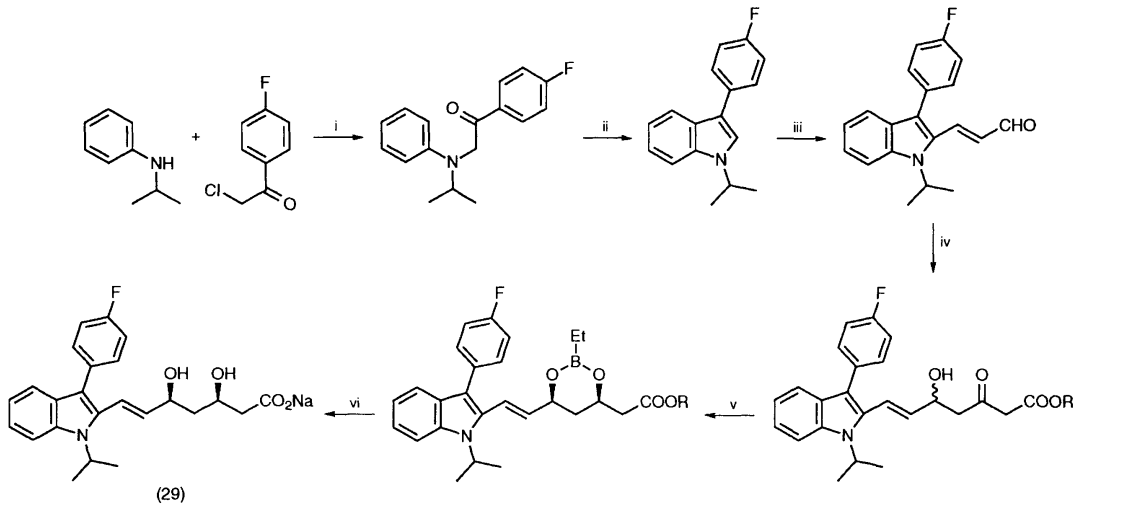
In general, unless the hydroxy groups remain unsubstituted in an *erythro* relationship, inhibitory activity is greatly reduced. Furthermore, only one enantiomer of the ring-opened form of the lactone possesses the activity displayed by the racemate.[61] These findings reveal that the chiral lactone moiety is essential for biological activity.

Insertion of a bridging unit other than ethyl or (*E*)-ethenyl between the 5-carbinol moiety and an appropriate lipophilic moiety (*e.g.* 2,4-dichlorphenyl) attenuates activity.[61]

Further studies of a series of substituted derivatives of (25) at MSD provided a series of 7-[3,5-disubstituted(1,1′-biphenyl)-2-yl]-3,5-dihydroxy-6-heptanoic acids, of which (26) possessed 2.8 times the inhibitory activity of mevastatin.[63] X-ray crystallography studies on compound (26) showed it to possess the same chirality in the lactone ring as mevastatin. Potent inhibitory activity was not retained without concomitant substitution at the 3- and 5-positions of the central phenyl ring of the biphenyl moiety with methyl or chloro groups.[63] The type and position of substituents on the external phenyl ring is critical. An electron-donating group (CH_3 or CH_3O) in the 4′-position is detrimental, whereas a halogen (Cl or F) in this position is beneficial. Ring fusion of substituted biphenyls afforded products that were substantially less active, indicating that the dihedral angle between phenyl rings must be greater than 0° to maintain a high level of inhibitory potency.[64] Substituted naphthalene derivatives, (27) and (28), display about the same potency as mevastatin.[65]

Investigations at the Sandoz Research Institute further extended the findings obtained at MSD. The researchers chose indolyl derivatives by considering structures and molecular shapes of both coenzyme A and mevastatin. An extensive and rapid analogue program led to the choice of fluvastatin (XU62-320) (29) (Scheme 5) as a candidate for extensive biological testing.[66] As compared to the respective sodium salts of mevastatin and lovastatin, fluvastatin is 22- and 10-fold more potent in inhibiting HMG-CoA reductase. However, fluvastatin is comparable to mevastatin in cholesterol-lowering activity in patients.[67] This drug is now under development and expected to be marketed in 1993.

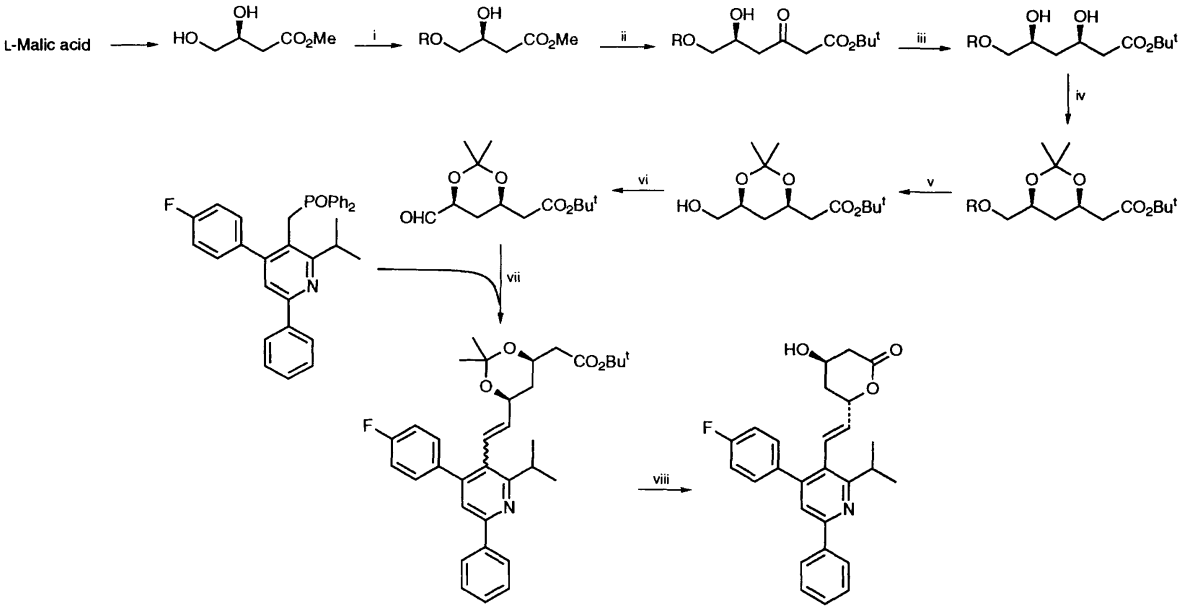
Many synthetic analogues of mevastatin have been prepared at Hoechst AG. Baader *et al.* prepared compound (30), which is comparable to lovastatin with respect to inhibition of HMG-CoA reductase.[68] However, the cholesterol-lowering activity of (30) in rabbits is slightly lower when compared to that of lovastatin. The same group prepared the pyridine analogue HR 780 (31) (Scheme 6),[69,70] which exceeded the activity of



(29)

Reagents: i, EtOH, Δ, ii, $ZnCl_2$; iii, $Me_2NCH{=}CHCHO$, $POCl_3$; NaOH; iv, NaH, Bu^nLi; v, Et_3B, THF; $NaBH_4$, −78 °C; vi, MeOH, NaOH.

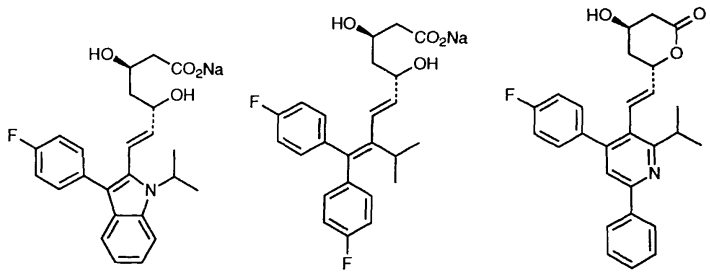
Scheme 5



(31)

Reagents: i, Bu^tPh_2SiCl; imidozole; ii, $CH_3CO_2Bu^t$, LDA; iii, Et_3B, $NaBH_4$; iv, $MeC(OMe)_2H$, H^+; v, Bu_4NF; vi, Swern oxidation; vii, base; viii, CF_3CO_2H

Scheme 6



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