Chapter 20

Chemistry and Antitumor Activity of 9(*R*)-Dihydrotaxanes

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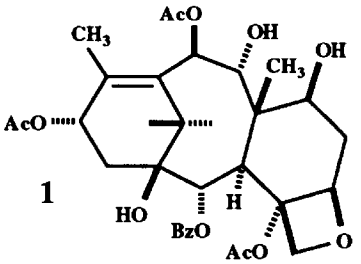
The 9(R)-dihydrotaxanes are a new family of semisynthetic antitumor agents which show great promise as a second generation class of antimicrotubule agents. These compounds have increased water solubility and stability as compared to taxol and also exhibit excellent activity in tumor models. Other advantages of the 9(R)-hydroxyl group can be found in its use as an additional site for chemical modification towards the preparation of new derivatives. Furthermore, its effect on the surrounding functionalities allows for access to novel chemistry and ring systems from this taxane template.

The discovery of new analogs of the antitumor agent taxol which exhibit broader spectrum, enhanced in vivo activity, or improved water solubility will be important in the continued evolution of this class of agents. The 9(R)-dihydrotaxanes show great promise as a second generation class of agents in that they have greater solubility and stability and also show excellent in vivo activity in several solid tumor models.



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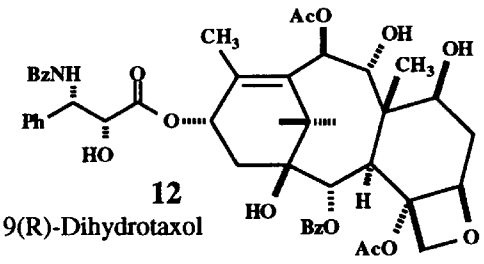

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9(R)-Dihydrotaxol

The isolation of the novel component, 13-acetyl-9(R)-dihydrobaccatin III (**1**) was reported by several laboratories in 1992 (*1-3*). This component differs in structure from taxol in two respects: 1) the C-13 phenyl isoserinate sidechain which is required for activity is replaced by an acetate; 2) the C-9 carbonyl is replaced by an α-hydroxyl group. It is found as a constituent in *Taxus canadensis*, a bush common to the northeastern United States and southern Quebec (*4*), but has also been found in other species such as *Taxus chinensis* in China. Since **1** exists primarily in the needles, access to this component is quite similar to that of baccatin III from *Taxus baccata* in that the source is a cultivatable and renewable resource. The quantity of **1** available from this plant varies from 0.08-0.3% (dried needles); however, a simple extractive workup and recrystallization can give access to this component without the need for any chromatography (Gunawardana, G., Abbott Labs., unpublished data).

NOTE: Paclitaxel is the generic name for Taxol, which is now a registered trademark.

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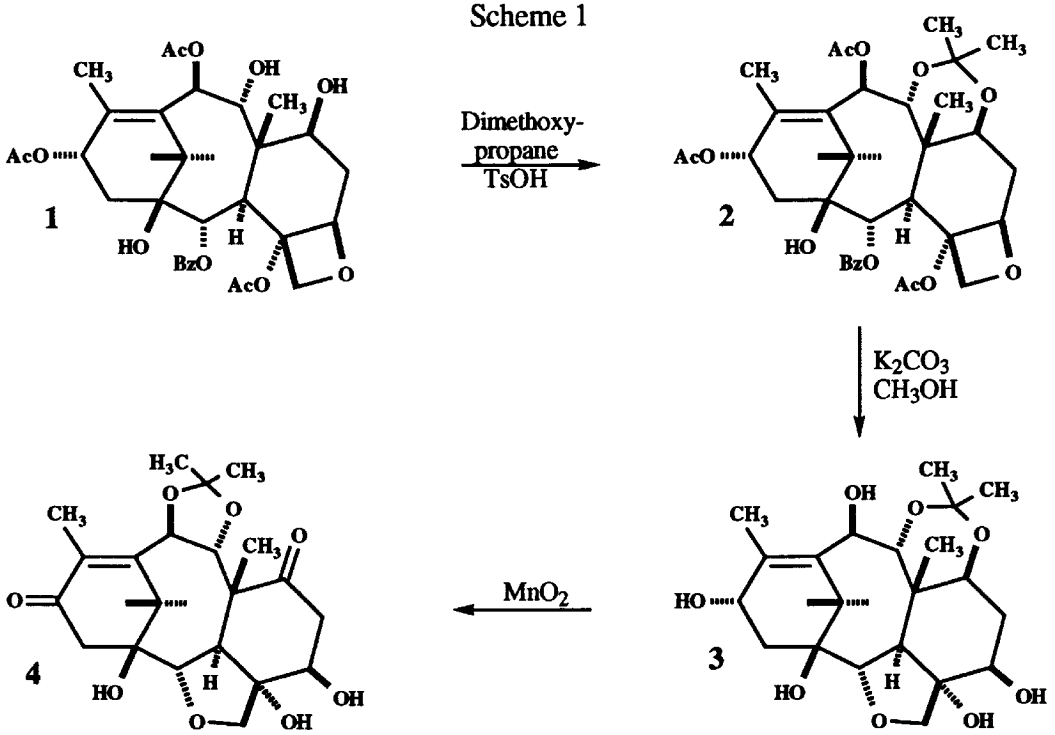
The x-ray structure of **1** shows exceptional overlap with that of the corresponding baccatin III derivatives due to the fact that the C-9 carbonyl in the latter series bisects the hydrogen/hydroxyl sp3 center in 1 (*1*). Several advantages and opportunities afforded by this novel structure are apparent: 1) the presence of the C-9 hydroxyl serves as an additional site for modifications; 2) the presence of this hydroxyl serves to increase the water solubility of these analogs; 3) the lack of a C-9 carbonyl serves to stabilize the system relative to the base-catalyzed C-7 epimerization (*5*).

Chemistry of 13-Acetyl-9(R)-dihydrobaccatin III (1)

In order to ascertain the effect of the C-9 hydroxyl on the antitumor activity of this system, we needed to remove the C-13 acetyl group present in **1** and reacylate this center with an appropriate sidechain. While many ways of conducting the latter reaction have been reported, no method for selective removal of the C-13 acetate was known prior to our work.

Selective Deacetylation at C-13. Initial deacetylation attempts involving hydrolytic deacylation succeeded in rapid and selective removal of C-10 acetate in good yield; however, further hydrolysis afforded only complicated mixtures involving non-selective deacylation of C-2, C-4, and C-13 acyl groups. Deacetylation of the 7,9-acetonide **2** obtained under standard conditions (Scheme 1) only slowed the C-10 deacylation but failed to enhance selectivity at the C-13 center. Under more vigorous conditions the C-2 benzoate

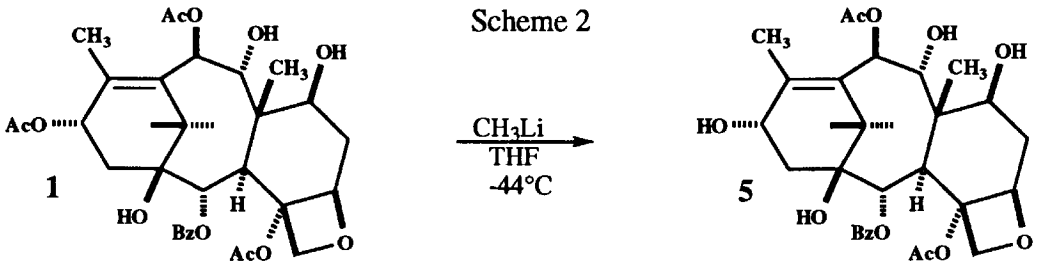
Scheme 1



also undergoes hydrolysis followed by facile ring opening of the oxetane ring with concomitant formation of the tetrahydrofuran sideproduct **3**. This product was reported in the C-9 carbonyl series and has been shown to lead to inactive compounds (*6,7*). In this case, structure **3** was actually identified via characterization of its oxidation product **4** obtained by treatment of **3** with manganese dioxide (acetone, reflux). Under these conditions, this oxidation of the C-13 hydroxyl group takes place in conjunction with transfer of the 7,9-acetonide to the 9,10-position occurs thus allowing further oxidation

In contrast to hydrolytic conditions, the use of carbanion reagents such as alkyl lithiums, Grignard reagents, and hydride reagents such as lithium triethylborohydride produced a different reaction profile such that the desired C-13 deacetylation product **5** predominated (*8*). Methyl lithium was found to be the reagent of choice giving >80% yield of the desired product from **1** without the need for protecting groups (Scheme 2) (Klein, L.L., Abbott Labs, unpublished data). This selective deacylation was found to be of general use, also applicable to the C-9 carbon Perhaps one reason for this selectivity can be related to another experiment which involved treatment of the acetonide **2** with lithium hexamethyldisilazide and allyl bromide (Scheme 3). The major product was the 13-(4-pentenoyl) analog **6** which arose from the enolization of the C-

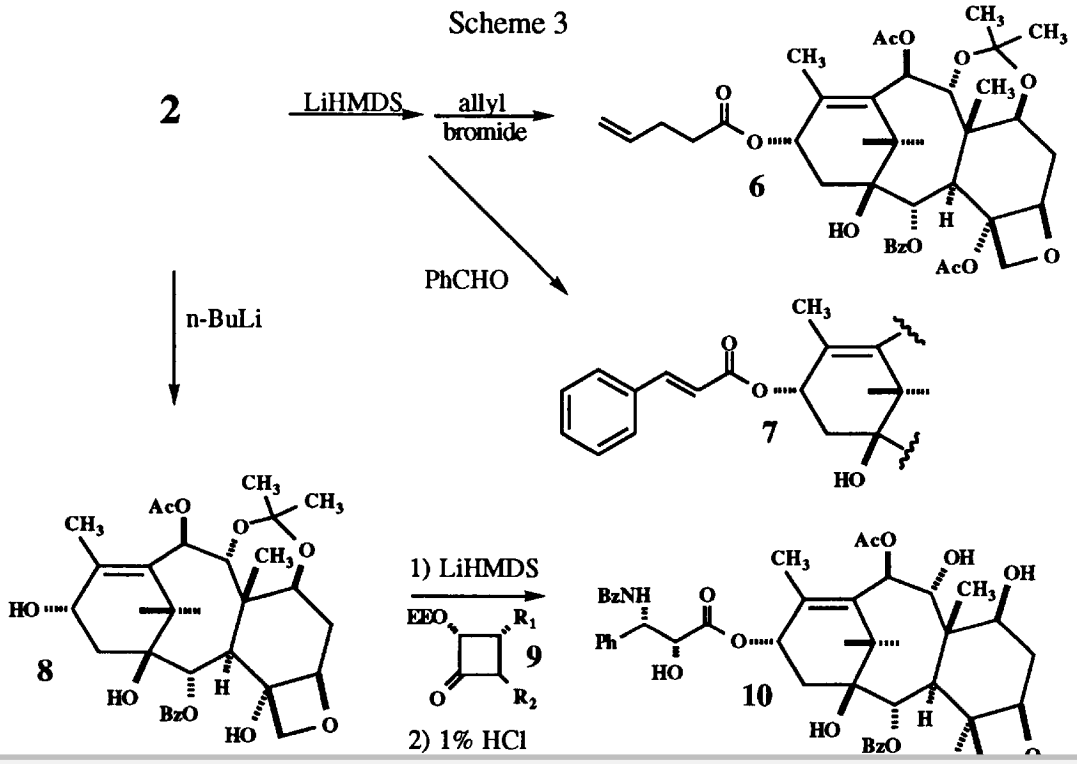
Scheme 2



13 acetyl group. This result established that: 1) condensation of the C-13 enolate with electrophiles was viable. A similar reaction of this enolate with benzaldehyde led, after elimination, to the styryl derivative **7** containing the carbon skeleton of the taxol sidechain without requiring removal of this acetate; 2) decomposition of this enolate via a ketene elimination provides a mechanistic explanation for the deacetylation; however, it is still not clear why this presumably less accessible ester is the site for deprotonation.

One minor product isolated from the deacetylation of the acetonide **2** was the 4,13-dideacetyl analog **8**. The sidechain was attached to **8** under standard conditions which involved low temperature deprotonation of the C-13 hydroxyl group with lithium hexamethyldisilazide followed by treatment with the appropriate 4-substituted-3-(2-ethoxy)ethoxy-N-acyl-2-azetidinone **9** (*9*) giving good yields of the adduct (*10*).

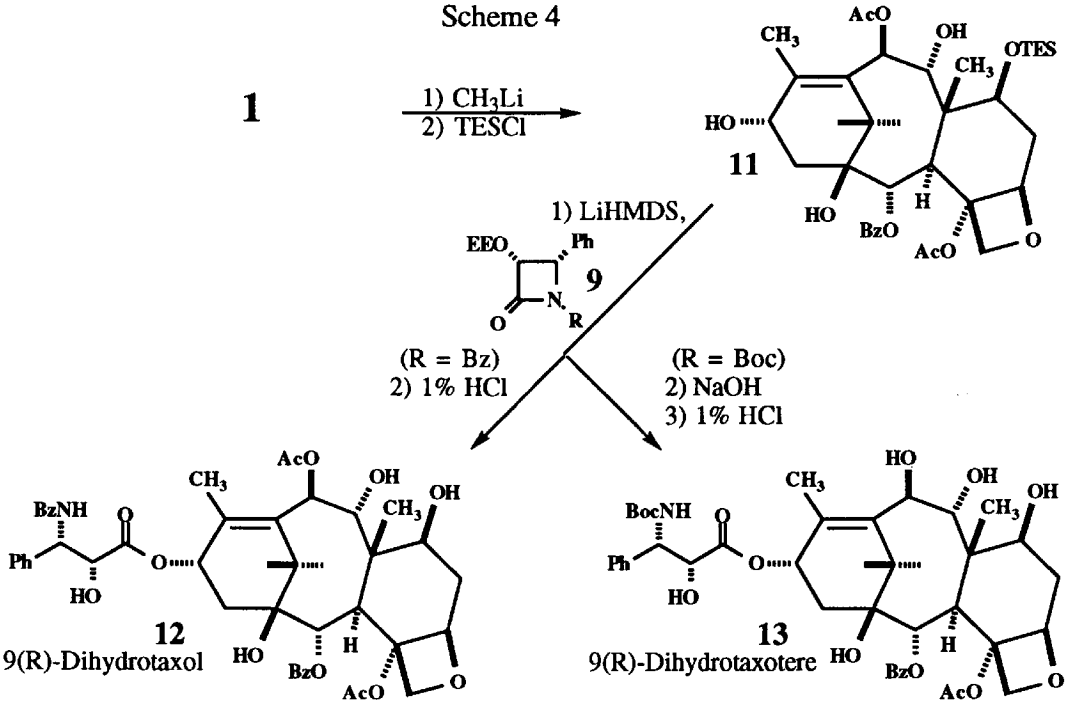
Scheme 3



Deprotection of the ethoxyethyl protecting group and the acetonide afforded 4-deacetyl-9(R)-dihydrotaxol (**10**). This compound proved to be practically devoid of activity (Table I) in the tubulin assembly assay (*11*) and against the four tumor cell lines (*12*) which made up our routine testing protocol; thus, the C-4 position appeared to be very sensitive to change.

Synthesis of 9(R)-Dihydrotaxol (9-DHT). For the preparation of 9(R)-dihydrotaxol, the C-13 deacetylation was performed on isolate **1** and was followed by standard protection (triethylsilyl ether) of the C-7 hydroxyl group to give intermediate **11** (Scheme 4). Either sodium hydride at 25°C or lithium hexamethyldisilazide (LiHMDS) at -44°C was used to form the oxyanion of **11** which was reacted with lactam **9** (R_1 = Ph; R_2 = Bz). After deprotection under acidic conditions, 9(R)-dihydrotaxol (**12**) was produced. This synthetic sequence also allows for the preparation of the more soluble 10-deacetyl analogs in this series. Toward that end reaction of **11** with modified lactam **9** (R_1 = Ph; R_2 = Boc) in a similar manner produces an adduct which first undergoes quantitative basic hydrolysis followed by acidic deprotection. In this way 9(R)-dihydrotaxotere (**13**) was easily produced. The facile deacetylation of the C-10 acetate is not trivial in the C-9 carbonyl series and reflects the greater stability of the 9(R)-dihydro series. These products were shown to have excellent tubulin assembly activity and similar *in vitro* activity as compared to taxol and taxotere; therefore, these preliminary results establish that the C-9 carbonyl is not required for activity.

Scheme 4



9(R)-Dihydrotaxol exhibited good efficacy versus the murine M109 solid tumor model (IP), delaying tumor growth to a greater extent than taxol. Compound **13** also showed good efficacy *in vivo* and was found to have 100-fold greater water solubility than taxol (**13**, 226 μg/mL; taxol 1-3 μg/mL). This allowed for a decrease in the amount of Cremophor EL vehicle required in the administrative solutions.

Table I. Tumor Cell Cytotoxicity of 9(R)-Dihydrotaxol Analogs

Compound	Tumor cell lines, IC_{50} (ng/mL)				Tubulin (μM)
	A549	*HT-29*	*B16F10*	*P388*	ED_{50} / ED_{50} taxol
Taxol	2.5-4.3	1.8-3.5	3.4-6.3	8.8-11	1.00
Taxotere	0.18	0.21	0.6	1.5	0.7
4-Deacetyl-9-dihydrotaxol (10)	>1000	8800	7900	>1000	>10
9-Dihydrotaxol (12)	16-22	6.4-9.6	25	49-57	0.86
9-Dihydrotaxotere (13)	0.26	0.65	0.4	2.8	1.31
10-Deacetyl-9-dihydrotaxol	11	1.9	39	140	0.83

Synthesis of 9DHT Analogs

Encouraged by the discovery that the 9(R)-dihydrotaxanes define an active template, the preparation of derivatives having increased solubility and/or potency was pursued. Modifications to both the sidechain and to the ring system were studied.

C-3' Side Chain Analogs. Analog work involved variation of the sidechain nitrogen substituent at C-3' via formation of amides, ureas, and carbamates; however,

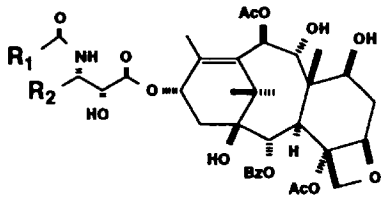


Table II. Tumor Cell Cytotoxicity of C-3' Alkyl Analogs

Entry	Compound R_1	Compound R_2	Tumor cell lines, IC_{50} (ng/mL) *A549*	*HT-29*	*B16F10*	*P388*
1.	Ph	Ph	16-22	6.4-9.6	25	49-57
	9(R)-DHT (12)					
2.	Ph	CH_3OCH_2	>100	79	>100	>100
3.	t-BuO		>100	>100	86	>100
4.	t-BuO		>100	>100	>100	>100
5.	t-BuO	CH_3	15	8.3	19	43
6.	t-BuO	$PhCH_2$	>100	>100	>100	>100
7.	t-BuO	Et	0.83	1.4	3.2	11
8.	t-BuO	CH_2=CH	1.1	1.8	4.4	16
9.	t-BuO	c-C_6H_{12}	1.2	5	5.7	14

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