STUDIES ON THE CHEMISTRY OF TAXOL

BY

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in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In

Chemistry

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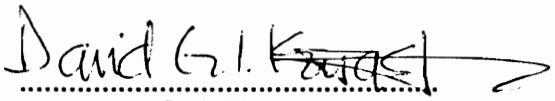

D. G. I. Kingston

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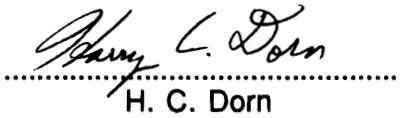

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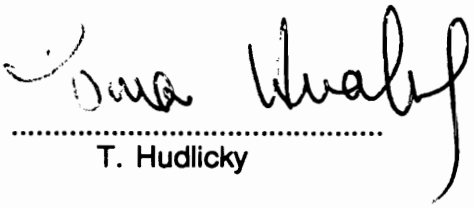

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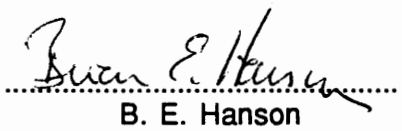

B. E. Hanson

May. 1990
Blacksburg, Virginia

ABSTRACT

The novel diterpenoid taxol isolated from the western yew *Taxus brevifolia* is one of the most important lead compounds to emerge from the search for anticancer agents from plants. It shows consistent clinical activity against ovarian cancer and may also be active against other cancers. In this study, the preparation of various taxol derivatives was investigated, with the objective of better understanding the structural requirements for activity in the taxol series. The 7-hydroxyl group of taxol was derivatized with a photoaffinity label and other reagents as a beginning of the project to understand the interaction of taxol and tubulin, and the activity of all the derivatives in a tubulin assay was determined. A study of the deacylation and reacylation reactions of baccatin III was carried out in order to find conditions suitable for the preparation of 2-debenzoylbaccatin III , and thus 2-debenzoyltaxol. Finally, the reactions of taxol with various electrophilic reagents were investigated, and the structures of products with an opened oxetane ring and/or contracted ring A were determined. Biological assay results are reported on many of the compounds in this investigation.

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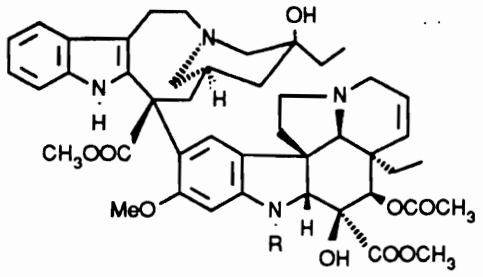
1.0 INTRODUCTION

1.1 Anticancer Drugs From Plants

The use of plant derived materials as potential anticancer agents dates back at least to the Ebers papyrus in 1550 B.C,[1] but it is only in the last 30 years[2] that a serious scientific study of natural products as anticancer agents has been undertaken. Many natural products thus discovered are now used in cancer treatment or are used as templates to synthesize more potent analogs.

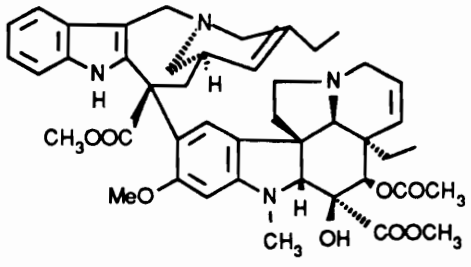
Illustrative examples among such agents are the vinca alkaloids[3] (**1**) from the plant *Catharanthus roseus* (commonly known as periwinkle), and the podophyllotoxin (**2b**) derivatives etoposide (**2c**) and teniposide (**2d**).[3,2] Podophyllotoxin (**2b**) is isolated from the plant *Podophyllum peltatum* (American mandrake or May apple) and related Indian species, and its derivatives represent the most useful non-alkaloid plant products in cancer chemotherapy.

A number of microorganism-derived compounds, mostly antibiotics, also play a significant role in chemotherapy. Adriamycin (**3a**) and daunorubicin (**3b**) belonging to the anthracycline[4] class are clinically used anticancer agents. Bleomycin (**4**), a mixture of two glycopeptides,[5] bleomycin A_2 (**4a**) and bleomycin B_2 (**4b**), has been used since 1968 for cancer treatment.

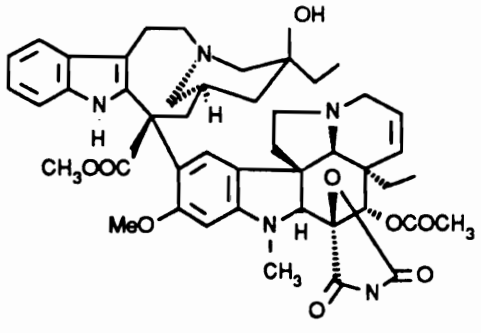


1a, R = CH_3, vinblastine

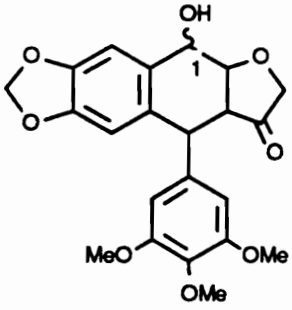
1b, R = CHO, vincristine



1c, nevelbine

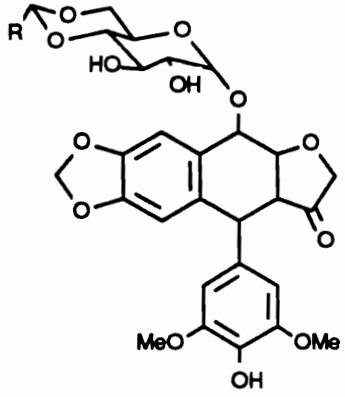


1d, vinzolidine



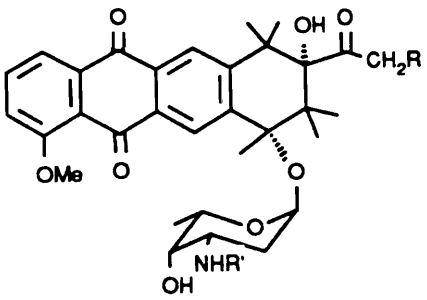
2a, 1β-OH, podophyllotoxin

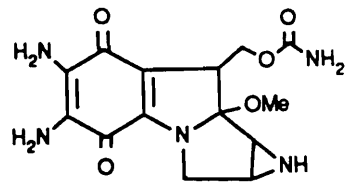
2b, 1α-OH, epi-podophyllotoxin



2c, R = CH_3, etoposide, (vp-16-213)

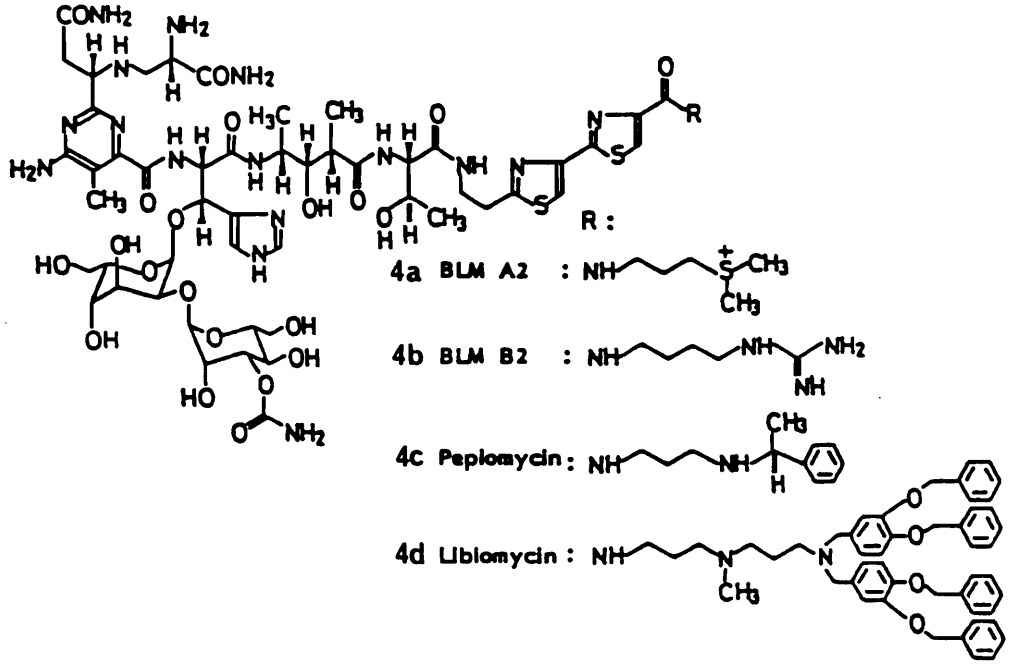
2d, R =  teniposide, (VM-26)



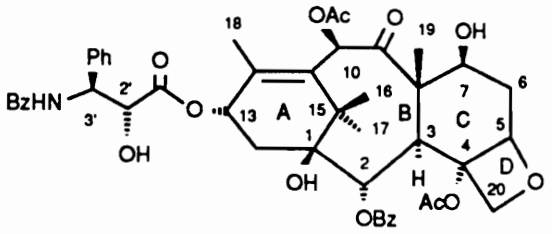


7, Mytomycin

	R'	R	
3a	H	H	Adriamycin
3b	H	OH	Daunorubicin
3c	CF_3CO	$CH_3(CH_2)_3CO$	AD32
3d	CF_3CO	$CO(CH_2)_4COOH$	AD 143

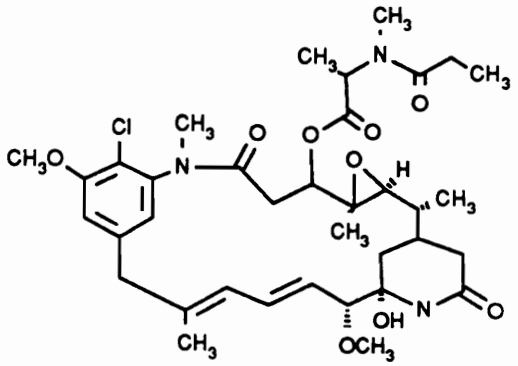


The most recent member to join this select family of clinically useful natural anticancer agents is taxol (**5**). A diterpene amide that was isolated from the Pacific yew plant, *Taxus brevifolia*,[6] it shows potent antitumor and antileukemic activity in several animal cell systems.[7] It entered Phase II clinical trials in 1987, and these trials demonstrated its effectiveness in treating advanced ovarian cancers.[8]



5

Over 1400 compounds were listed[9] by 1981 as antitumor compounds from plants. It is however very rare that a compound that shows activity in preliminary assays makes it all the way into a therapeutic drug. Even drugs that advance to Phase II clinical trials may not show any therapeutic value, as shown by maytansin[10](**6**); which was inactive in Phase II trials after showing promising activity in earlier assays.



6

Developing an anticancer compound into a clinically useful drug thus involves a large amount of luck in addition to an enormous scientific endeavor. In the scientific area fundamental studies on the molecular level play an important part. Thus a knowledge of the mechanism of drug action, its structure-activity relationships, and the synthesis of analogs of the natural product in order to enhance its activity and/or suppress its adverse properties are essential in evaluating the full potential of the drug. Often these categories are interrelated. Many of the compounds mentioned previously, especially ones that are clinically used, are being studied continuously. The following discussion will highlight some recent developments in this area.

Anticancer drugs may be categorized into four groups.

1 Alkylation agents	Alkylate DNA
2. Antibiotics	Various mechanisms of action including DNA cleavage
3. Antimetabolites	Interfere with DNA synthesis
4. Antimitotics	Stop cell division

Natural anticancer agents fall primarily into classes 2 and 4. The vinca alkaloids (**1**), and taxol (**5**) are antimitotics, whereas the anthracyclines (**3**) and bleomycins (**4**) are antibiotics.[11] The podophyllotoxin derivatives etoposide (**2c**) and teniposide (**2d**) appear to act by inhibiting topoisomerase, an enzyme crucial to DNA replication and thus to cell division.

A number of derivatives of these drugs are currently under investigation. Phase I studies of vinzolidine (**1d**), an orally administered semi-synthetic vinca alkaloid analog, has been reported recently;[12] it has been shown that vinzolidine (**1d**) is more active than vinblastine (**1a**) in certain type of cancers. Nevelbine (**1e**), a semi-synthetic 5'-nor-anhydro-vinblastine, was found to be as active as vincristine (**1b**) in Phase I clinical trails;[13] these trails also indicated the drug's rapid distribution, slow elimination properties and a remarkable patient tolerance for it.

The natural product epipodophyllotoxin (**2a**) as previously noted is not used as a drug due to it extreme toxicity. However, its two derivatives VP-16 (**2c**) and VM-26 (**2d**) are currently clinically used drugs. Interestingly enough these two derivatives showed different modes of action from that of the parent compound, *epi*-podophyllotoxin (**2a**). The parent compound is a microtubule assembly inhibitor, but these two derivatives act by causing single and double strand breaks[2] in DNA by inhibition of topoisomerase. Investigation of DNA cleavage with analogs of these podophyllotoxin derivatives has confirmed that the 4'-oxygen in ring E is a requirement for DNA cleavage and cytotoxicity;[14] summary of other results on this subject is published in a recent review.[15]

The bleomycins (**4**) deserve some discussion due to their widespread use and some understanding of their unusual mechanism of action. The bleomycins are known to cleave the N-glycidic bonds of DNA, releasing nucleotide bases; as a result of subsequent reactions DNA strand scission occurs.[16] Pepleomycin (**4c**), a biosynthetic bleomycin analog which is more potent and has less pulmonary toxicity is used clinically in Japan.[5]

Liblomycin (**4d**), a semisynthetic analogue, is anticipated to have minimal pulmonary toxicity.[17] Chemically bleomycins are unique cytotoxic compounds. For example, in bleomycin A_2 (**4a**), the right hand side bithiazole part is capable of inter-chelating DNA and the left hand portion chelates with metals such as Fe, Zn, etc. Fe(II) complex has a structure of the type given in Figure 1;[11] it is apparent that the Fe(II)-chelated intermediate is the actual active species, pointing to an O_2 mediated free radical mechanism for DNA cleavage.[5]

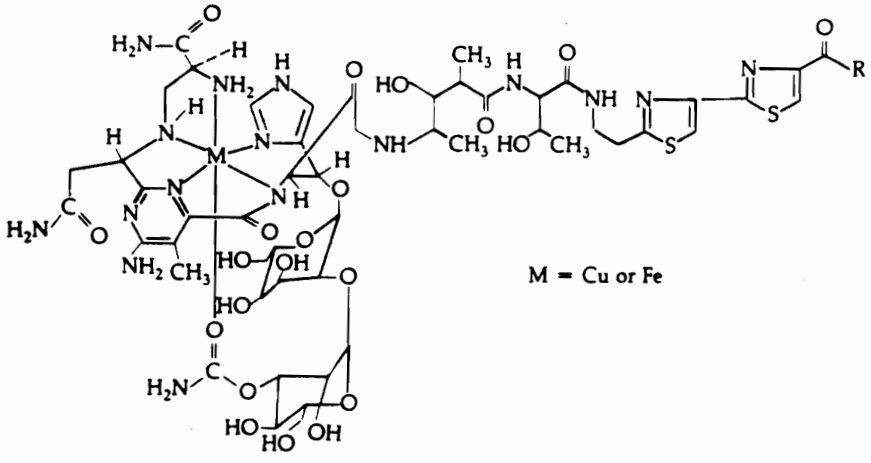


Figure 1 Bleomycin-metal complex

New anthracycline derivatives AD 32 (**3c**) and AD 143 (**3d**) are emerging as superior drugs compared to other anthracyclines[18] such as adriamycin (**3a**) and daunorubicin (**3b**) and AD 32 is especially noted for its patient response and water solubility.

Mitomycin C (**7**), isolated from *Streptomyces caespitosus*,[19] is the only natural compound which falls into the class of alkylating agents. Three required structural features for antineoplastic activity of this compound are suspected to be the aziridine ring, the C-10 carbamate and the quinone unit.[20]

The general conclusions emerging from the above discussion can be summarized as follows:

(1) The activity of natural anticancer agents can often be improved by careful structure modifications.

(2) Functional group modifications to get desired properties can be done without much consideration of the steric bulk of the group to be introduced.
eg. bleomycin (**4a, 4b**) to liblomycin (**4d**)

(3) Significant skeletal modifications do not always impair the activity of the product. eg. vinblastine (**1a**), to Nevelbine (**1e**)

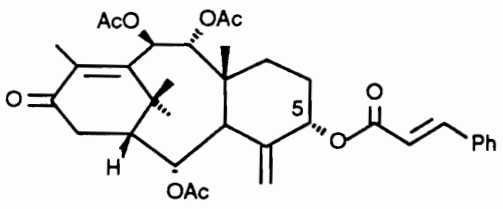
(4) Even the mode of action can be altered for the better through chemical modification (eg. *epi*-podophyllotoxin (**2a**) to teniposide (**2d**) and etoposide (**2c**))

1.2 Occurrence of Taxane Diterpenoids

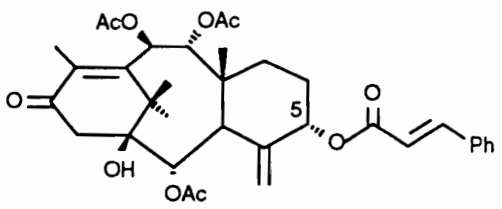
As mentioned in the previous section, taxol (**5**) has potent anticancer and antileukemic properties in several cell systems,[6,7] and has confirmed clinical activity against ovarian cancer.[8,21] It has also shown an exciting activity against non-small-cell lung cancer in preliminary clinical trails with a curative effect in at least one case.[22] This compound has become one of the most important lead compounds to emerge from plant screening.

Taxol (**5**) is a member of a broad class of compounds called taxanes, a group of diterpenes characterized by a bicyclo(5.3.1)undecane system (rings A and B) fused to a six-membered ring C. The source of these compounds are yew plants, (*Taxus* spp.) which are known to be extremely toxic. Many compounds isolated from various *Taxus* species were reviewed by Miller in 1980[23] and Potier et. al. in 1987.[24]

In historical perspective, Lucas[25] isolated a mixture of alkaloids from *Taxus baccata* in 1856. This mixture of compounds was not identified, and it was not until 1967, after many years of work by Lythgoe and his collaborators, that the first two structures were finally established.[26] These first structures were of O-cinnamoyl-taxicin-I (**8a**) and O-cinnamoyltaxicin-II (**8e**);



8a



8e

Many other taxanes have now been isolated and had their structure determined. All the compounds isolated to date can be included in four main structural groups[24] (Figure 2).

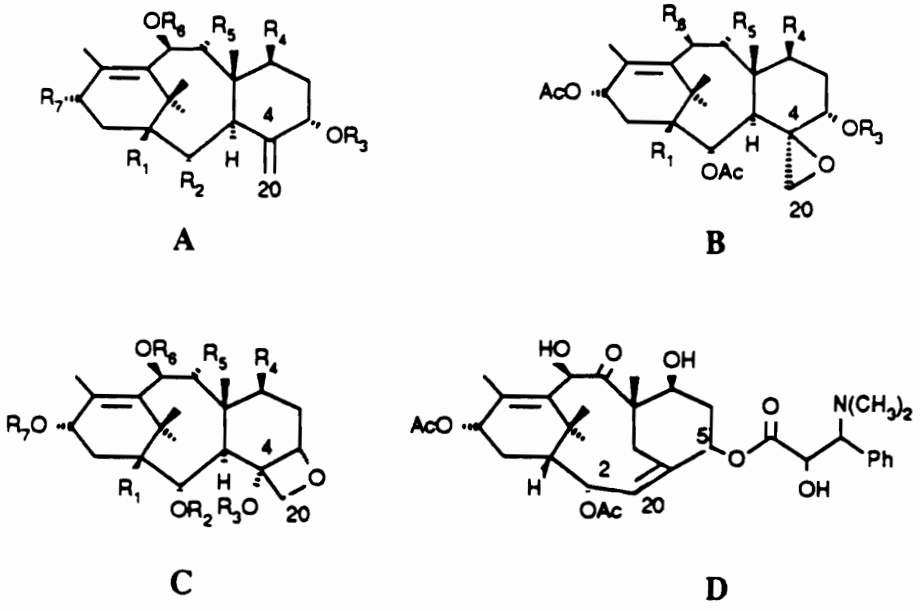
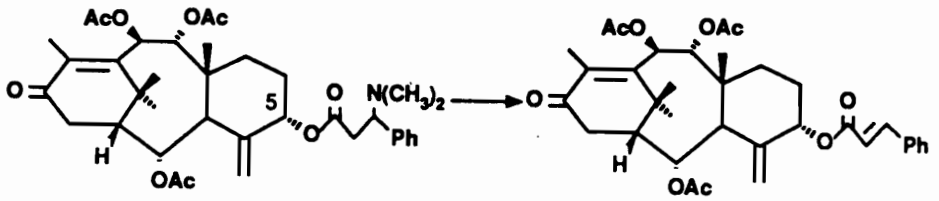


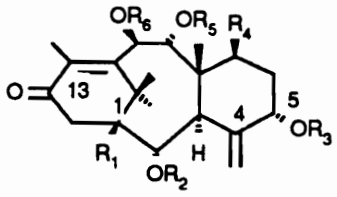
Figure 2 Four main structural groups of taxanes

Compounds corresponding to type A are summarized in Table 1. Common features of this type are a C-4 exocyclic methylene group and different oxygen functions at C-13; compounds having no oxygen function at C-13 are also known.[27] The most common substituent at the C-5 position is cinnamate, derived by chemical conversion of the natural ester at this position. The stereostructure of several compounds of this class have been confirmed by X-ray crystallography.[28,29,30] These compounds are not present as such in *T.baccata*, but are formed from the natural alkaloids by chemical conversions during the isolation process. As described by Lythgoe,[32] the natural substituent at C-5 is 3-dimethylamino-3-phenylpropionic acid. Since these natural amino compounds are unstable, they were often deliberately deaminated to yield the more stable cinnamate. As an example, the compound **20** decomposes to O-cinnamoyltaxicin-II (**8e**).



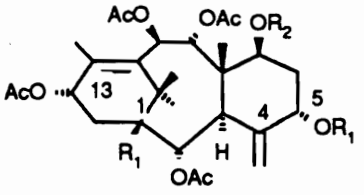
20 **8e**

Table 1 **Group A taxanes**



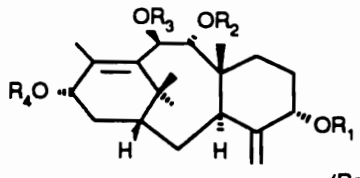
(Ref 24)

	R_1	R_2	R_3	R_4	R_5	R_6
8a	H	Ac	COCH=CHPh	H	Ac	Ac
8b	H	Ac	H	H	Ac	Ac
8c	H	Ac	COCH=CHPh	OAc	Ac	Ac
8d	H	Ac	Ac	H	Ac	Ac
8e	OH	Ac	COCH=CHPh	H	Ac	Ac
8f	OH	H	H	H	H	H



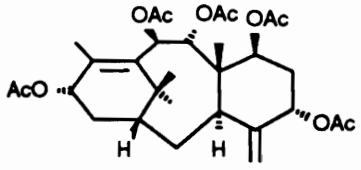
(Ref 24)

	R_1	R_2
9a	COCH=CHPh	H
9b	COCH=CHPh	OAc
9c	Ac	H
9d	Ac	OAc
9e	H	OAc

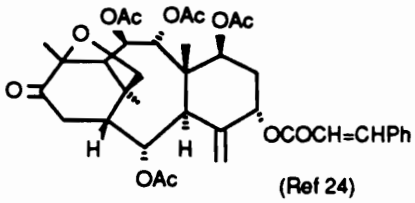


(Ref 24)

	R_1	R_2	R_3	R_4
10a	H	Ac	Ac	H
10b	H	Ac	Ac	Ac
10c	Ac	Ac	Ac	Ac

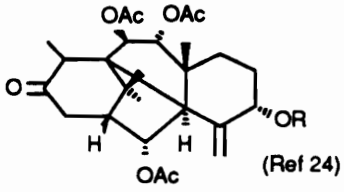


11 (Ref 24)



(Ref 24)

12

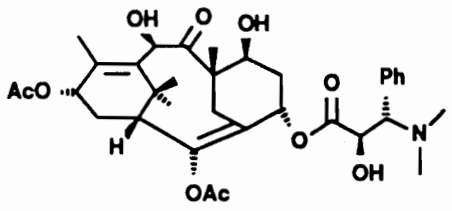


(Ref 24)

13a R = H

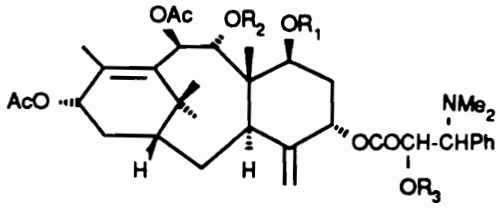
13b R = Ac

Recently Potier and co-workers were able to isolated several new taxane derivatives with a nitrogen-containing side-chain from *Austrotaxus spicata*.[24,32] These are shown in Table 2. Taxine A (**21a**), isolated from *Taxus baccata* has a similar structure.[34]



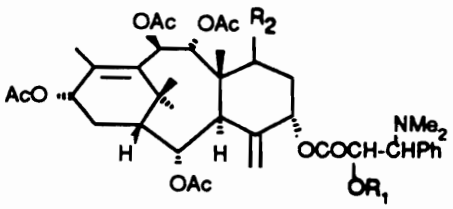
21a

Table 2 **Group A taxanes with 5-(3-dimethylamino)-3-phenylpropionyl group and its derivatives as the substituent**

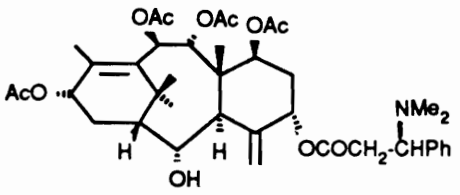


(Ref 32)

	R_1	R_2	R_3
15a	Ac	Ac	H
15b	Ac	Ac	Ac
15c	H	Ac	Ac
15d	H	H	Ac
15e	H	H	OH

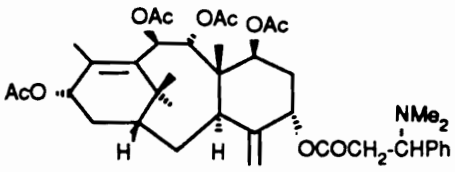


	R_1	R_2	
14a	H	H	(Ref 24)
14b	Ac	H	(Ref 24)
14c	H	OAc	(Ref 32)

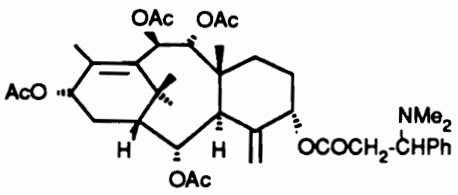


(Ref 32)

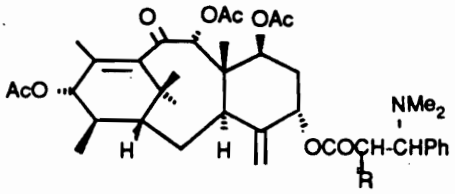
17



16 (Ref 32)



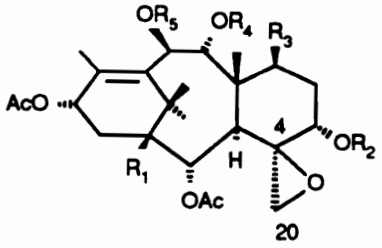
18 (Ref 32)



	R	
19a	OAc	(Ref 32)
19b	H	
19c	OH	

Group B Compounds are characterized by having a C-4(20) oxirane ring. All the known compounds are listed in Table 3. Biogenetically the oxirane compound can arise from epoxidation of the C4-(20) double bond of the group A compounds.

Table 3 Group B taxanes



(Ref 24)

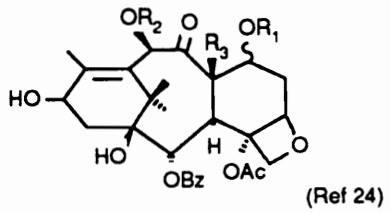
	R_1	R_2	R_3	R_4	R_5
21b	H	Ac	OAc	Ac	Ac
21c	H	H	OAc	Ac	Ac
21d	OH	Ac	OAc	Ac	Ac
21e	OH	Ac	OH	Ac	Ac
21f	H	COCH=CHPh	H	H	Ac
21g	H	$COCH_2CH(NMe_2)Ph$	H	H	Ac
21h	H	$COCH_2CH(NMe_2)Ph$	H	Ac	H

Group C taxanes (Table 4) are identified by a C(4)-5 oxetane ring, an unusual structural feature in a natural product. Taxol (**5**) and its C-13 deacyl derivative baccatin III (**23**) are included in this group. Cephalomannine (**25**), which is also an antitumor agent, differs from taxol (**5**) only by the substituent on the side-chain nitrogen atom;[35] taxol (**5**) contains a benzoyl group whereas cephalomannine contains a 2-methylbutenoyl moiety. Chemical studies of taxanes, inspired by their biological activity, are now largely focused on this group of compounds. Thus Potier and co-workers have recently developed a method to convert 10-deacetylbaccatin III (**22c**) into the natural isomer of taxol.[36]

Potier and his co-workers have also noted that the C-4 acetoxy group of baccatin III (**23**) is spatially vary close to the C-13 hydroxyl group. This allows the C-13 hydroxyl group to be strongly hydrogen bonded to the C-4 acetate group[37] (Figure 3).

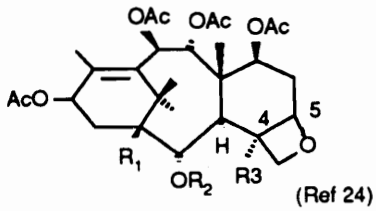
Figure 3 Stereo drawing of baccatin III (**23**)

Table 4 Group C taxanes



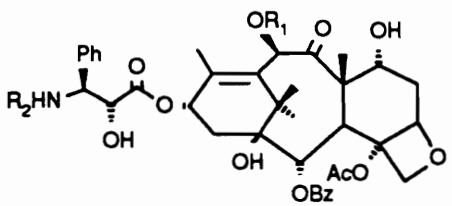
(Ref 24)

	R_1	R_2	R_3
22a	OH (7α)	Ac	CH_3
22b	OH (7β)	H	CH_3
22c	OH (7β)	H	CH_2OH
23	OH (7β)	Ac	CH_3

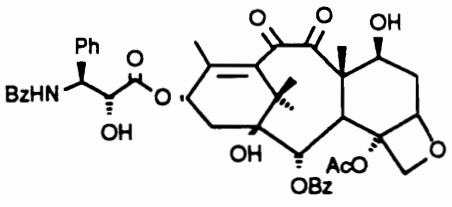


(Ref 24)

	R_1	R_2	R_3
24a	OH	Ac	Ac
24b	H	Ac	Ac
24c	OH	OBz	Ac
24d	OH	COC_5H_{11}	Ac



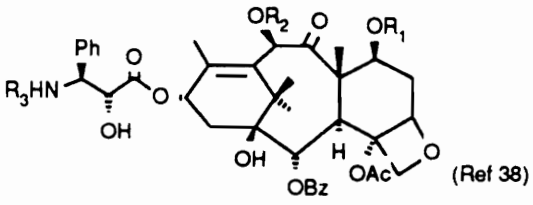
	R_1	R_2	
27a	Ac	Bz	(Ref 34)
27b	H	Bz	(Ref 94)
27c	H	$CH_3CH=C(CH_3)_2$	(Ref 94)



26

(Ref 39)

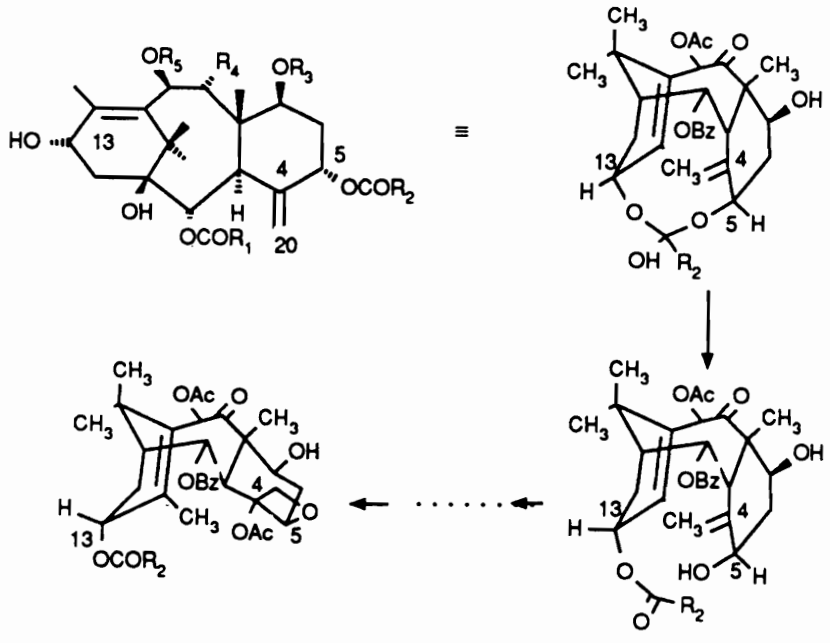
Table 4 Cont'd



(Ref 38)

	R_1	R_2	R_3
5	H	Ac	PhCO
25	H	Ac	$CH_3CH=C(CH_3)CO$
26a	β-xylose	H	PhCO
26b	,,	H	$CH_3CH=C(CH_3)CO$
26c	,,	H	$C_5H_{11}CO$
26d	,,	Ac	PhCO
26e	,,	Ac	$CH_3CH=C(CH_3)CO$
26f	,,	Ac	$C_5H_{11}CO$
26g	H	H	PhCO
26h	H	H	$CH_3CH=C(CH_3)CO$
26i	H	$COCH_2CH(OH)CH_3$	PhCO
26j	H	$COCH_2CH(OH)CH_3$	$CH_3CH=C(CH_3)CO$
26k	β-xylose (OAc)	Ac	$CH_3CH=C(CH_3)CO$

Based on this observation, they have proposed[24] a biogenetic origin of the complex taxol (**5**) side-chain (Scheme 1). This scheme is supported by the fact that some congeners in Group A have a C-5 3-dimethylamino-3-phenylpropionate (see Table 3) moiety which could transfer to the C-13 hydroxyl group and could give rise to the taxol (**5**) side-chain by further biosynthetic modification.

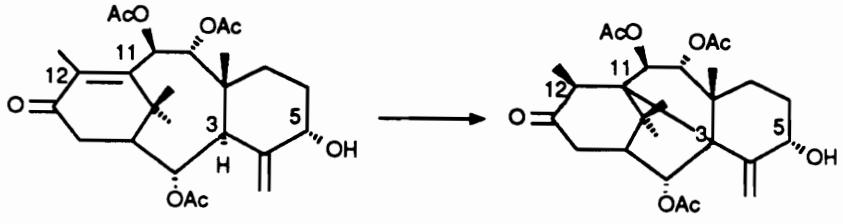


Scheme 1 Biogenetic hypothesis for taxol side-chain

1.3 Chemistry of Taxol and Other Taxanes

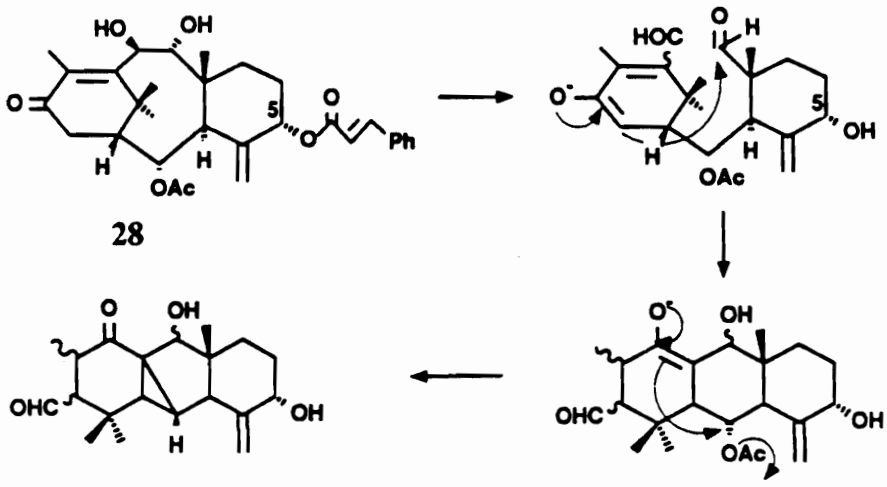
1.3.1 Skeletal Rearrangements of Taxinins

The taxinine derivative **13a** isolated from *Taxus cuspidata* (Japanese yew) is also available through a photochemical reaction of taxinine compound **8b**.[40] When **8b** is irradiated with UV light in dioxane it undergoes a transannular proton abstraction which effects the transfer of the C-3 proton to the C-11(12) double bond yielding **13a**.



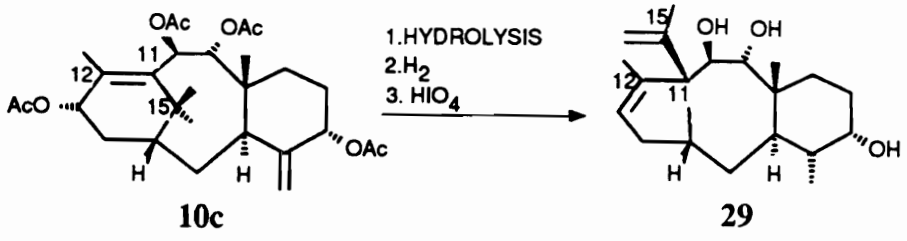
8b **13a**

Another interesting transformation of the taxinine derivative **28** has been observed in basic medium and the mechanism for this reaction has been proposed as outlined in the Scheme 2.[41]



Scheme 2 A rearrangement of taxinine

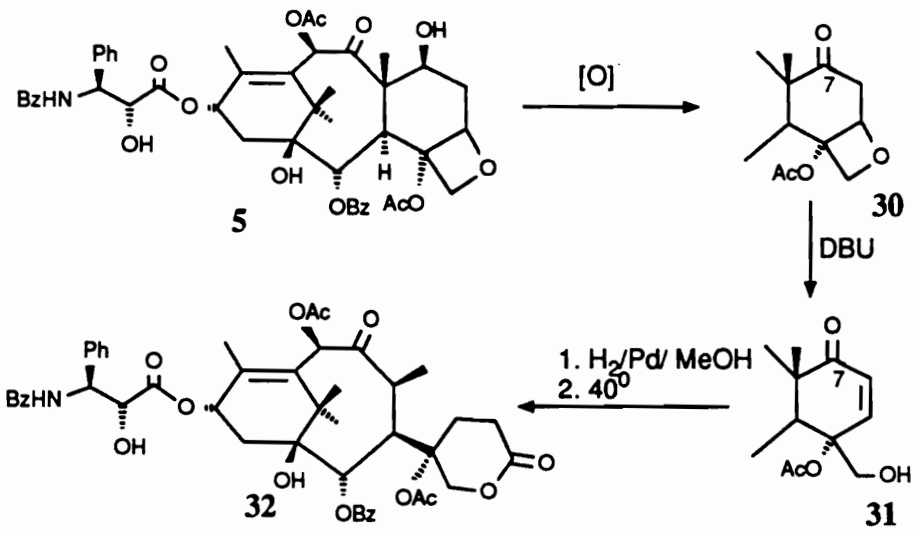
The tetraol, derived from **10c**, undergoes elimination of water involving the allylic hydroxyl group with concurrent migration of the C-11(12) double bond and the C1(15) bond; the structure of the product (**29**) was determined by X-ray crystallography.[42]



The types of rearrangements mentioned above are not possible for taxol (**5**). They depend on the presence of C-13 hydroxyl group or carbonyl group, and taxol (**5**) does not have a free oxygen substituent at C-13.

1.3.2 Skeletal Rearrangements of Taxol

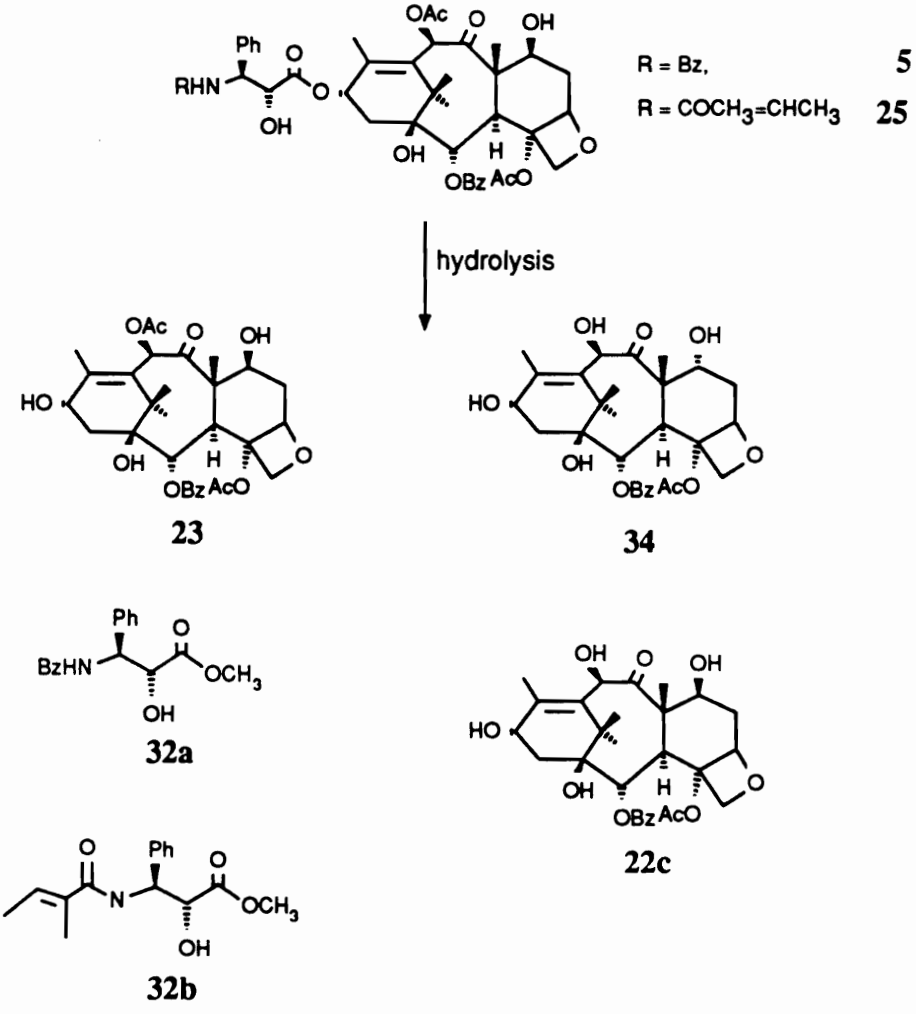
Very limited skeletal rearrangements for taxol (**5**) are reported by Kingston and coworkers during their study of the importance of various structural units of taxol to its bioactivity. Oxidation of taxol with Jones' reagent yielded 7-oxotaxol (**30**) which was converted to the compound **31** on treatment with diazabicycloundecene (DBU). Hydrogenation and then mild heat converted it to the lactone (**32**);[43] this final rearrangement involved a retro-Claisen reaction followed by lactonization as shown in Scheme 3.



Scheme 3 Oxidation products of taxol

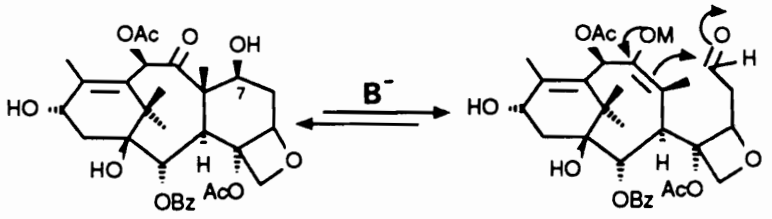
1.3.3 Reactivity of Taxol

One of the first reactions carried out on taxol (**5**) was hydrolysis of its many acyl groups. Base catalyzed methanolysis[6] of taxol yielded the side-chain methyl ester **32a** and 10-deacetylbaccatin III (**22c**).[6] However, reaction of methanolic sodium bicarbonate on cephalomannine (**25**)[35] yielded 10-deacetyl-7-*epi*-baccatin III (**34**), and 7-*epi*-baccatin III (**22a**) along with the side-chain methyl ester **32b** in addition to baccatin III (**23**) and 10-deacetylbaccatin III (**22c**) (Scheme 4).



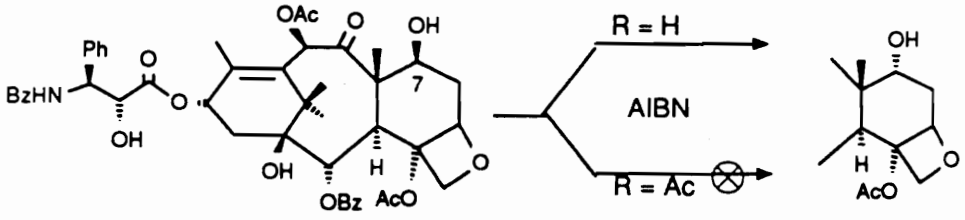
Scheme 4 Hydrolysis of taxol (**5**) and cephalomannine (**25**)

Epimerization of the C-7 hydroxyl can occur during hydrolysis through a retro-aldol reaction (Scheme 5). 7-*Epi*- derivatives of taxol and cephalomannine (**27a** and **27c** in Table 4) also occur naturally in *T.wallichiana*[38] and, taxol has been converted to 7-*epi*-taxol (**27a**) on treatment with AIBN (azobis(isobutyronitrile)).[39]



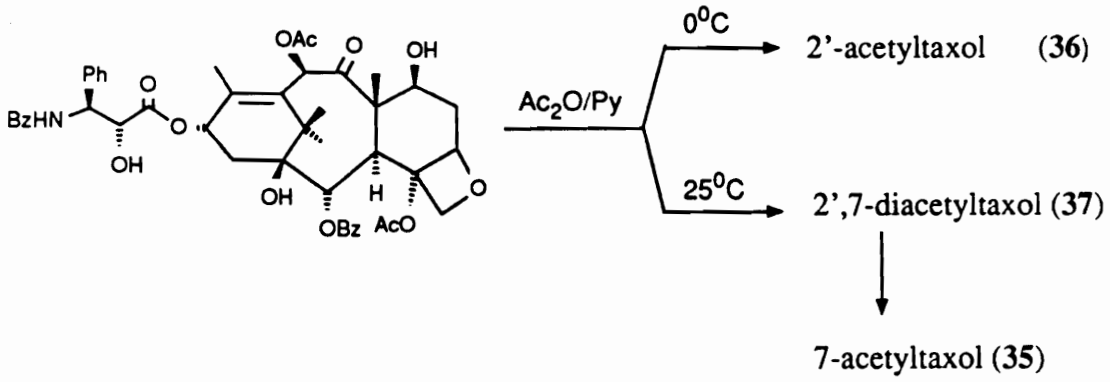
Scheme 5 Epimerization at C-7 of taxanes

It is apparent that the 7-*epi* series is thermodynamically more stable presumably due to the known hydrogen bonding[44] between a 7-α-hydroxyl group and the 4-α-acetoxy group. This hypothesis was supported by the fact that 7-acetyltaxol (**35**) failed to undergo any epimerization with AIBN owing to its inability to form hydrogen bonding,[36] (Scheme 6) although other interpretations of these results are also possible.



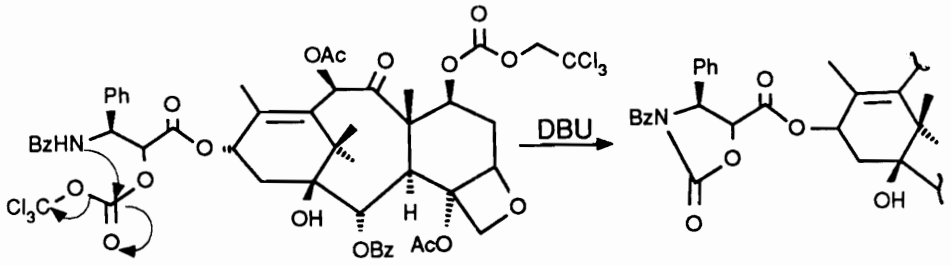
Scheme 6 AIBN-mediated C-7 epimerization of taxol

Taxol (**5**) and cephalomannine (**25**) both contain two secondary hydroxyl groups at C-2' and C-7 and one tertiary hydroxyl group at C-1. The C-1 hydroxyl group is highly hindered and unreactive. Selective acetylation[45] at low temperature provided 2'-acetyltaxol (**36**) while 2,7-diacetyltaxol (**37**) was obtained at room temperature. 7-Acetyltaxol (**35**) was obtained by selective deacylation of **37** (Scheme 7).



Scheme 7 Acetylation of taxol

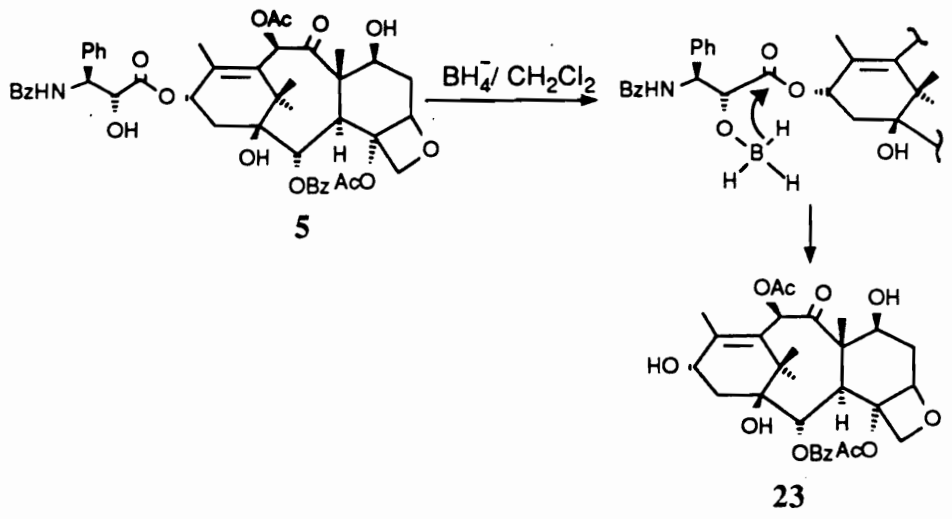
Other protecting groups such as trichloroethyloxycarbony (Troc)[46] and (t-butyldimethyl)silyl[47] groups have been used with taxol (**5**) and baccatin III (**23**) derivatives. The troc protecting group was found unsuitable as a protecting group at C-2' of taxol under basic conditions. When treated with DBU, 2,7-ditroctaxol (**38**) underwent a cyclization reaction leading to the oxazolone **39**[47] (Scheme 8).



38 **39**

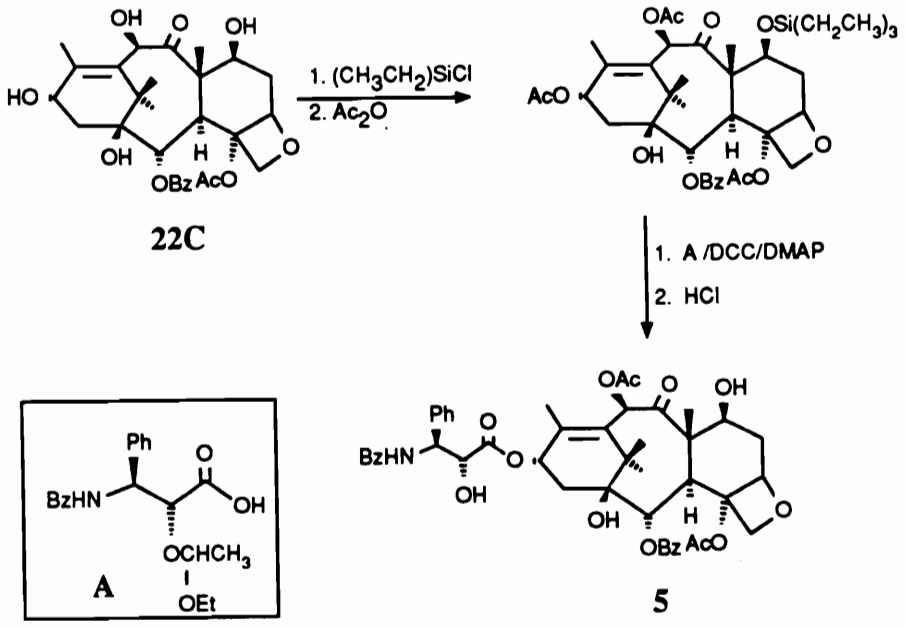
Scheme 8 Formation of oxazolone

The taxol (**5**) side-chain can be selectively cleaved by the method described by Kingston and coworkers.[48] Treatment of taxol with tetrabutylammonium borohydride reductively cleaves the side-chain. Formation of the intermediate **40** followed by intramolecular hydride transfer to the ester carbonyl is proposed as the mechanism of this reaction (Scheme 9).



Scheme 9 Preparation of baccatin III from taxol

Green and coworkers have synthesized the taxol side-chain in enantiomerically pure form.[49] Coupling of the side-chain to baccatin III (**23**) would supply a very practical route to taxol. This is significant because taxol occurs in the bark of *T. brevifolia* in very minute quantities and *T. brevifolia* itself is a relatively rare and slow-growing tree. The supply of taxol from *T. brevifolia* is thus inadequate to meet the anticipated demand for its use as an anticancer agent. On the other hand 10-deacetylbaccatin III (**22c**) can be obtained from the leaves of *T. baccata* and the leaves can of course be harvested without destruction of the plant. Acylation of baccatin III (**23**) at the C-13 position turned out to be very difficult, and a procedure for doing this was only reported recently[36] (Scheme 10). A more recent and improved procedure for the synthesis of taxol from baccatin III (**23**) has been reported by Holton.[50]



22C

5

Scheme 10 Hemi-synthesis route to taxol

1.3.4 Physical Properties of Taxol

a. Stereostructure of taxol

Hydrolysis of taxol (**5**) yielded the C-13 side-chain ester (**32**) and 10-deacetyl-baccatin III (**22c**). X-ray analysis of these individual components led Wall and Wani[6] to the structure of taxol. They did not however supply a stereo drawing of these components in their original communication and a full paper on their work has never appeared. The closest stereo-structure published is thus for baccatin V (**22a**, Figure 4) by Castellano and Hodder.[44] According this work the 6-membered A ring is in a boat form, somewhat distorted by the presence of the double bond. The ring C is a very distorted chair form with C-4, C-5, C-7 and C-8 being nearly planar as required by the planarity of the oxetane ring. The same stereo structure is thus assumed for the taxol (**5**) molecule. Recently a reference to an X-ray structure of a baccatin III derivative has been made,[51] but the X-ray structure itself has not been published.

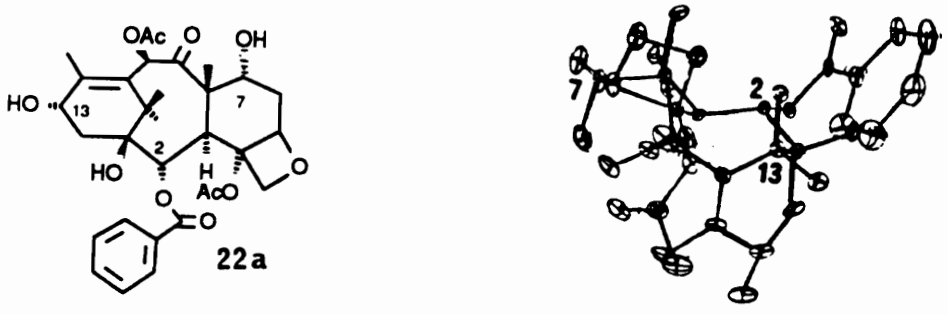


Figure 4 X-ray structure of baccatin V (**22a**)

b. 1H NMR Spectrum of Taxol

Elegant ^1H NMR analysis of many taxinine has been published by Nakanishi et al.[52] For taxol (**5**) a collection of ^1H NMR data has not been published since the review in 1980 by Miller.[23] There are several characteristic features in the ^1H NMR spectrum of taxol (Figure 5). The four methyl groups are furthest up field. The singlet for the C-18 methyl is much broader than those of the others, because of long range coupling to the C-13 proton. The two methylene groups (C-14 and C-6) present in taxol and its derivatives are usually buried in the methyl region, and most of the time are overlapped with each other. The C-20 protons always appear as an AB quartet. The signal for the C-5 proton is a double doublet but often it couples strongly only to one of the C-6 protons, with a very weak coupling to the other. For this reason it sometimes may appear as a broad doublet. The presence of two benzoyl groups can be identified by their 2' and 6' proton multiplets, appearing farthest down field of all the protons in the spectrum.

c. 13C NMR of taxol

The ^{13}C NMR spectrum of baccatin III (**23**) has been well interpreted.[53] The spectrum for taxol (Figure 6) has been interpreted (Table 5) by comparison with baccatin III (**23**), INEPT and selective decoupling experiments.[54] Some ambiguities exist in the assignments of the aromatic and the olefinic regions, however, and It is not possible to assign all the 18 aromatic carbons which are present as overlapping signals.

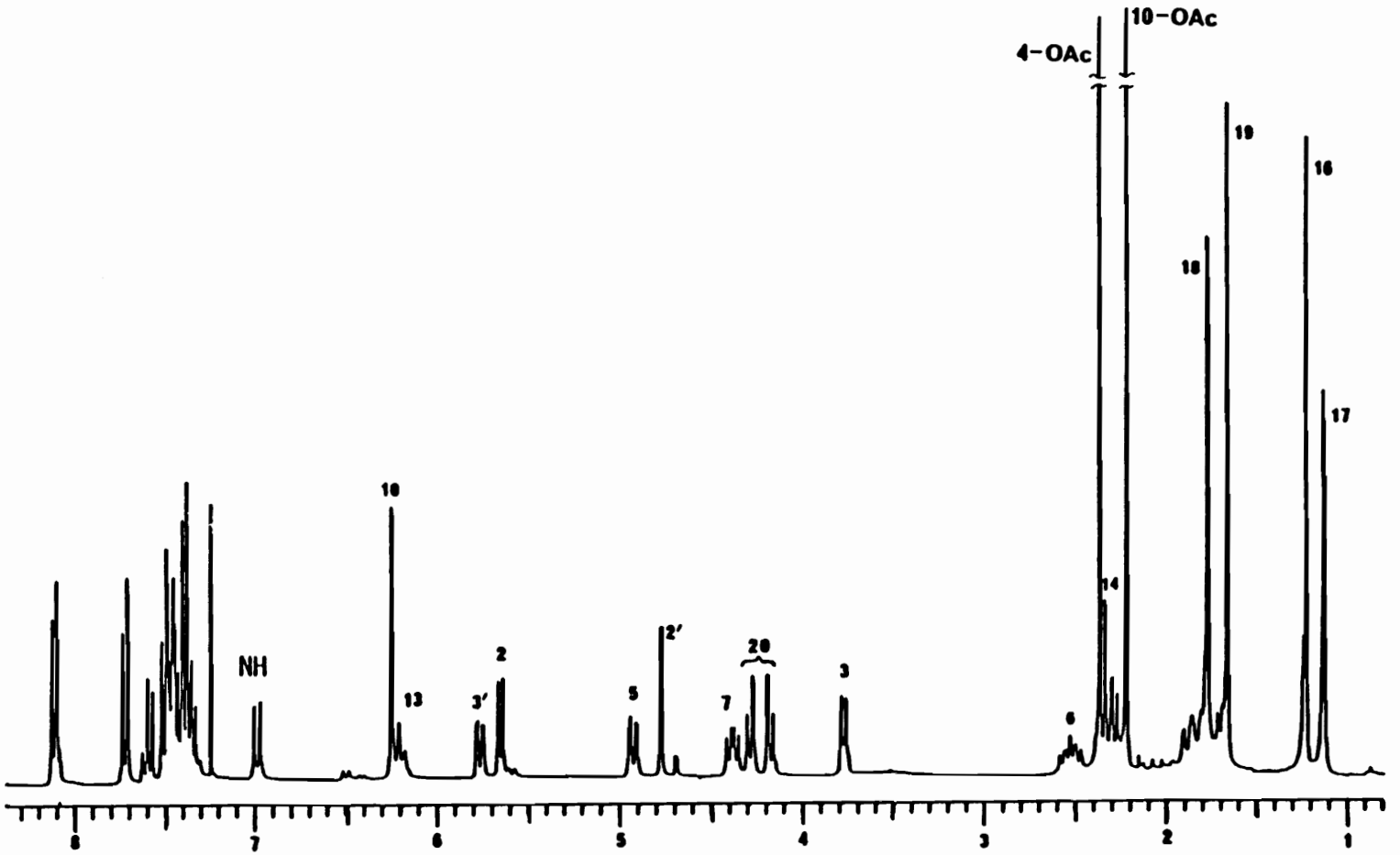
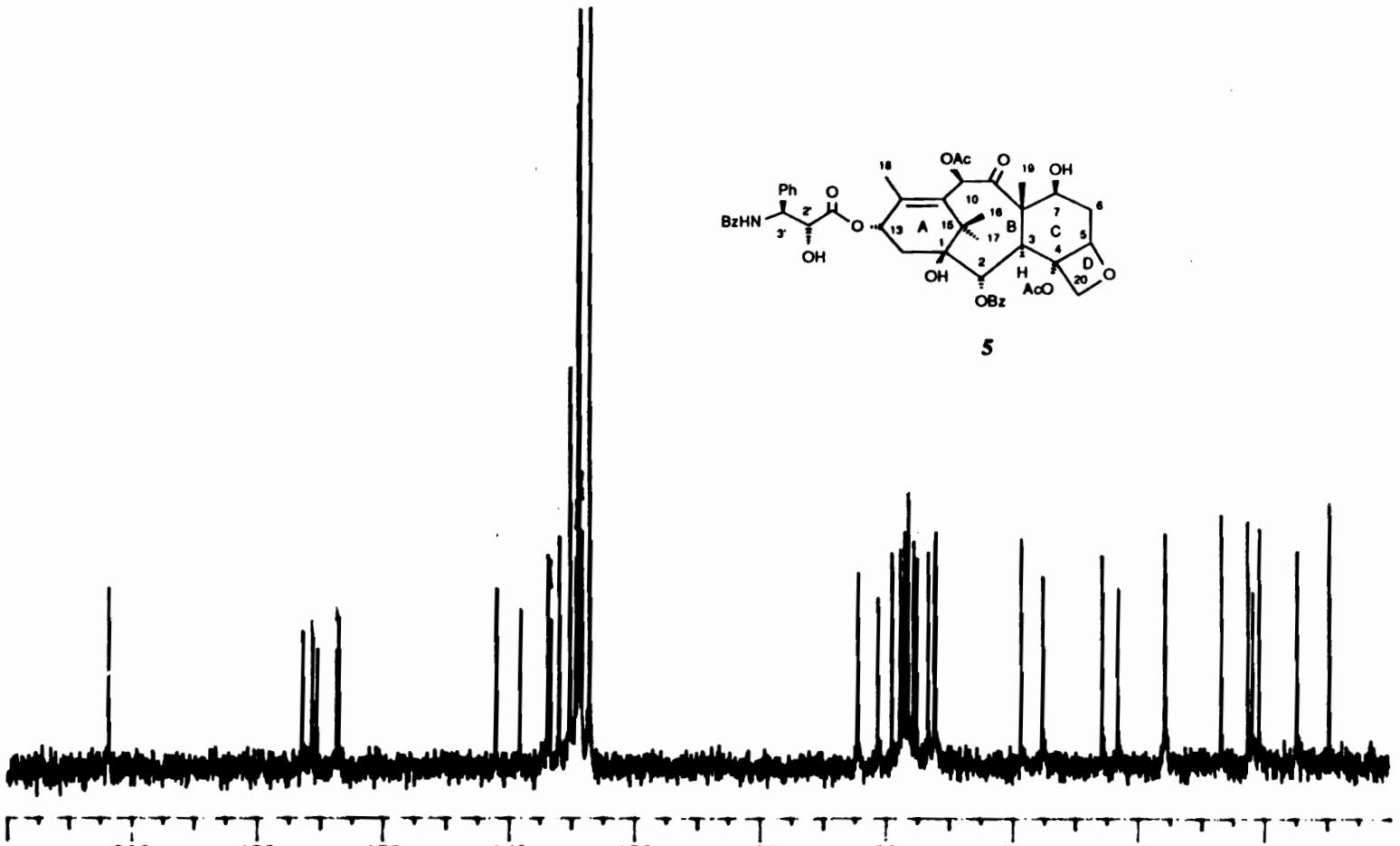


Figure 5 ^{1}H NMR of taxol (5)



5

Figure 6 ^{13}C NMR of taxol (5)

Table 5 **^{13}C NMR assignment of taxol (5)**

Carbon	δ(ppm)	Type by INEPT	Assignment method
1	78.79	q	b
2	75.47	t	a
3	45.73	t	a
4	81.04	q	b
5	73.20	t	a
6	35.64	s	b
7	76.33	t	a
8	58.39	q	b
9	203.39	q	b
10	75.15	t	a
11	133.74	q	b
12	41.66	q	b
13	75.47	t	a
14	35.64	s	b
15	43.05	q	b
16	26.55	p	a
17	21.33	p	a
18	14.6	p	a
19	9.2	p	a
20	71.8	s	a
1'	172.48[c]	q	b
2'	84.25	t	a
3'	54.81	t	a
C_4-OAc	1.92	p	a
C_{10}-OAc	0.54	p	a
$C_{3'}$-NBz			
-CO	166.68[c]	q	b
-para	133.13	t	b
C_2-OBz			
-CO	167.22[c]	q	b
-para	133.39	t	b
Acetates CO	170.19	q	b
	170.79	q	b
$C_{3'}$-Ph			
$-C_1$	138.12	q	b
Other aromatics	128.130	-	-

a selective proton decoupling
b chemical shift arguments, and comparison with Baccatin III
c may be interchangeable

1.4 Biological Activity of Taxol

1.4.1 Clinical studies

Some data on the antileukemic and anticancer activities of taxol (**5**) is given in Table 6. It completed its phase I clinical trails in 1987;[21] in these trials the evaluation of maximum tolerable dose, toxicities, and best schedule of drug administration were performed. Trial doses for Phase II clinical studies were determined to be 250mg/m^2. Phase II clinical trails, now in progress, will determined efficacy against specific cancers and chronic and cumulative toxicities. Available data indicate that it has strong activity against advanced ovarian cancers.[8] The importance of taxol (**5**) in cancer chemotherapy is evident by the following quotation from the authors, Rowinsky et. al., reporting the preliminary results of phase II clinical trials[8] " During Phase I study, a dramatic prolonged response was noted in a heavily pretreated and cisplatin-refractory patient with advanced progressive ovarian cancer. Because responses in drug refractory ovarian cancer are extremely uncommon, this unusual response was the basis for initiating a Phase II trial".

1.4.2 Mechanism of Action

Taxol was found to be a mitotic poison (i.e. inhibiting cell division) by Fuchs and Johnson in 1978.[55] Cell division is a very important phase of the life cycle of the cell. In this phase the nuclear membrane breaks down and the nuclear materials (chromosomes) are released. Chromosome doublets are separated into individual components (chromatids).

Table 6 **Antitumor activity of taxol at National Cancer Institute**

(from reference 7)

Tumor system	T/C at optimal dose	Evaluation
B16 melanoma	263	++
CX-1 colon xenograft	12	+
L1210 leukemia	131	+
LX-1 lung xenograft	13	+
P388 leukemia	170	+
P1534 leukemia	300	+
MX-1 mammary xenograft	-67	++

As shown in Figure 7 this whole process is associated with a cellular structure called the mitotic apparatus. This apparatus is made up of microtubules, a macromolecular protein. Microtubules can be compared to a system of cables; They radiate from two opposite poles near the nucleus and some of them run pole to pole. Some are connected to each chromatid. During the cell division the microtubules pull the chromatids towards the poles.[56] These chromatids become nuclear materials for two newly forming daughter cells.

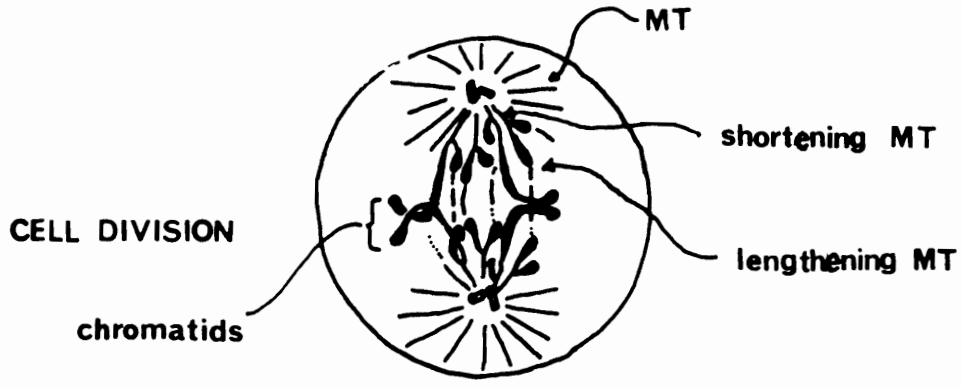


Figure 7 Cell division with mitotic apparatus

Microtubules are formed by the polymerization of tubulin, a macromolecular protein made up of two monomer units called α and β tubulin (Figure 8). Tubulin and microtubules are in a dynamic equilibrium with each other.

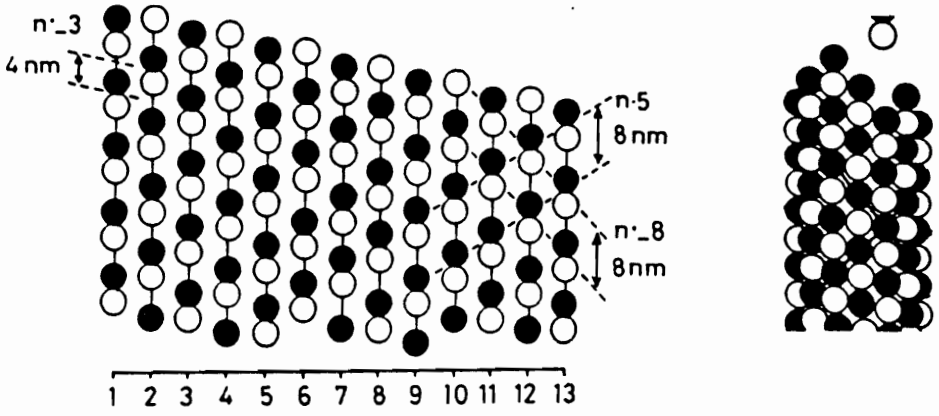


Figure 8 Tubulin-microtubule structure: black and white dots represent α and β tubulins, the hollow tubular structure shown in the right hand side is made up of the thirteen microfilaments shown in the open form

in vitro studies of tubulin and microtubule equilibrium by Gaskin et. al.[57] let them propose the equilibrium equation for the polymerization reaction (Eq 1).

Eq 1 $$\text{Tubulin} + \text{Microtubule} \xrightarrow{K_p} \text{Microtubules}$$

K_p = apparent equilibrium constant

The mechanism for assembly and disassembly of tubulin can be explained by a model which was first proposed by Wagner.[58] This model accounts for the involvement of GTP in polymerization, and it also explains the unidirectional flux of subunits at steady state. As can be seen from Figure 9 both ends of the microtubule are capable of assembly and disassembly; the + end enjoys net assembly while the - end experiences net disassembly, leading to a net flow of subunits. Thus is mechanism by which microtubules perform the transportation of chromatids during

cell division. A very comprehensive discussion on the kinetic mechanism of tubulin assembly is found in a review by Cote et. al.[59]

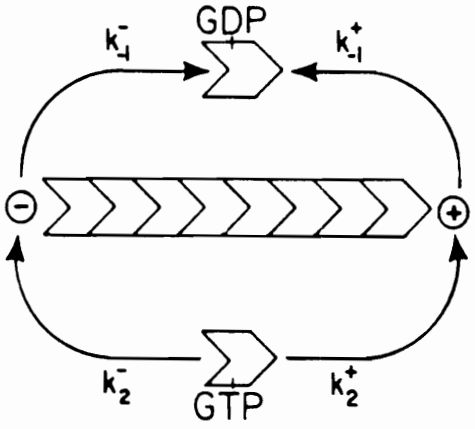
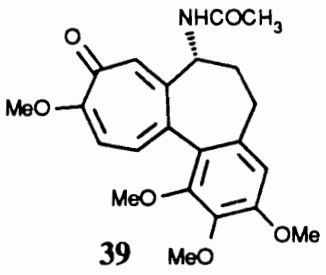


Figure 9 Mechanism of tubulin assembly and disassembly

Taxol (**5**) promotes the assembly of tubulin into microtubules *in vitro* and *in vivo* as shown by Schiff and co-workers;[60,61] this occurs even in the absence of exogenous GTP.[62] This mechanism is in contrast to that of other mitotic poisons such as podophyllotoxin (**2b**) and the vinca alkaloids (**1**) which inhibit microtubule assembly. Taxol (**5**) is thus unique in its mechanism of action.

In vivo studies indicate that taxol blocks assembly of the normal mitotic apparatus (made up of microtubules) but causes the appearance of many unorganized, look-alike structures in the cell. This impairs the cell's ability to undergo mitosis, thus killing the cell. The binding site for taxol is assumed to be in the microtubule polymer. Taxol binds to microtubules with an apparent binding constant of $8.7X10^{-7}$ in a near stoichiometric ratio (0.6 taxol bound/Mol. tubulin dimer).[63] The actual binding site for taxol has not been determined, but it has been shown that this

site does not overlap with the binding sites of other tubulin binding compounds such as colchicine (**39**), *epi*-podophyllotoxin (**2a**), and vinblastine (**1a**).[64]



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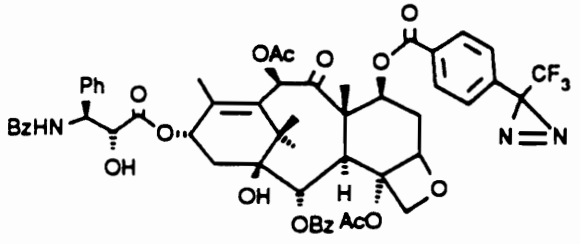
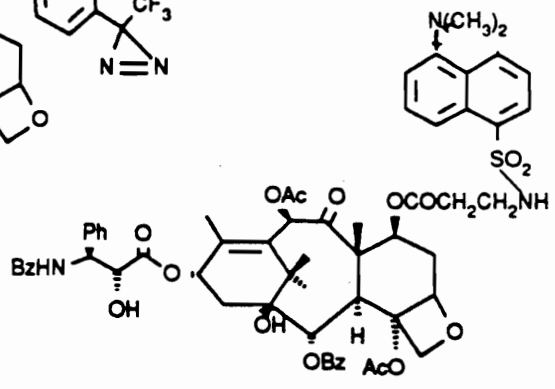
2. RESULTS AND DISCUSSION

2.1 Functionalization of Taxol to Obtain Special Purpose Analogs

As pointed out in the introduction, functional group modification is a standard technique in drug chemistry. The primary object of functional group manipulation is to obtain a drug with better therapeutic value, but another use is to obtain special purpose analogs for further studies of the drug without losing the original activity of the parent compound. The chemistry described here involves the preparation of three taxol derivatives, a photolabeled taxol (**42**), a fluorescent taxol (**43**) and 7-trimethoxybenzoyltaxol (**44**). The reasons for the selection of these analogs will be apparent during the discussion. Attempts to modify the C-2 position of taxol (**5**) will also be discussed.



42



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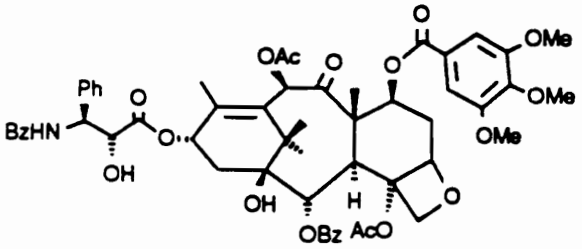

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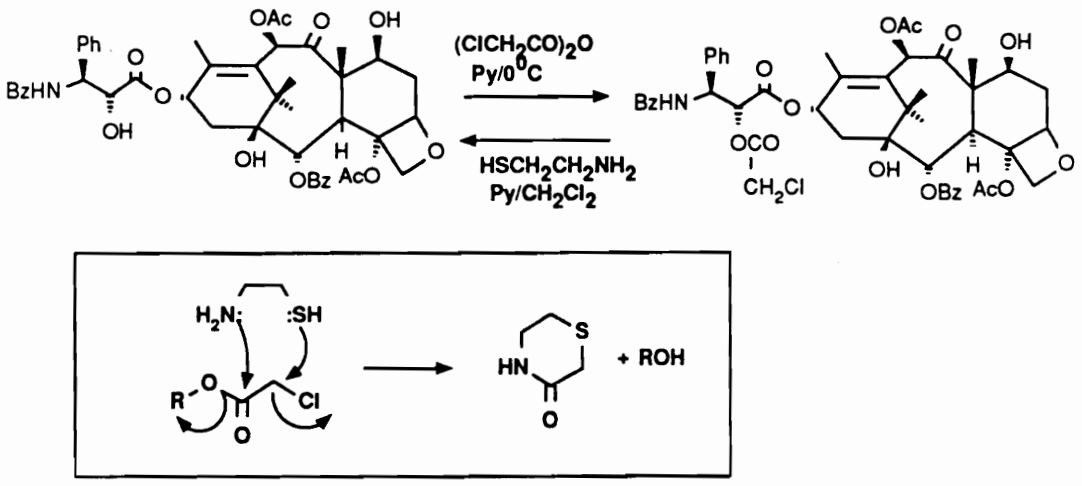
2.1.1 Selection of Functional Groups for Modification

Any scheme to prepare taxol (**5**) derivatives for bioassay purposes can most easily start with the readily available hydroxyl groups of taxol. As outlined in Scheme 7, Kingston and coworkers[45] showed that the 2'-OH is the most reactive of the two secondary hydroxyls groups present. Preparation of 2'-acetyltaxol (**36**) and 7-acetyltaxol (**35**) followed by determination of the activity of these compounds led to the conclusion that the addition of an acetyl moiety at the 2'-OH group results in loss of *in vitro* activity but not *in vivo* activity. This is presumably due to the ready lability of the 2'-substituent, which might thus undergo *in vivo* hydrolysis (see Section 2.3.4, Structure-Activity Relationships for details). The activity of 7-acetyltaxol (**35**) however, is similar to that of taxol and this consideration led us to select the C-7 hydroxyl group as the appropriate position for modifications.

2.1.2 General Method for Derivatization of C-7 Hydroxyl Group

The original preparation of 7-acetyltaxol involved the selective deacylation of 2,7-diacetyltaxol (Scheme 7),[45] but It was accompanied by some hydrolysis of the C-13 side-chain. For this reason we looked for a protecting group which could be introduced at the 2'-OH position under mild conditions and which could also be removed under mild conditions. We found that the chloroacetyl protecting group[65] meets all the above requirements.

Taxol (**5**) was treated with chloroacetic anhydride in pyridine at 0°C to obtain 2'-chloroacetyltaxol (**45**) in 98% yield (Scheme 11). The presence of the two inner peaks of an AB quartet at δ4.78 (the outer peaks are rarely observed) indicated



Scheme 11 Protection and deprotection of 7-hydroxyl group of taxol

the presence of the chloromethylene protons. The signal for the C-2' methine proton was shifted to δ5.50 and a MH^+ peak at *m/z* 930 in the FAB mass spectrum of the derivative was consistent with the assigned structure **45**.

The 2'-chloroacetyl group is much more labile than the 2-acetyl group. If the acylation reaction was run in pyridine for an extensive period of time, some de-protection of the product was detected. Also prolonged contact with nucleophilic solvents such as methanol, specially in the presence of silica gel, cause deprotection to occur, and hence use of any nucleophilic solvents should be avoided. Dilution is important in this reaction; in concentrated solutions, more 2',7-diacyltaxol will be formed, whereas in dilute solutions more anhydride and a longer reaction time are required, but the formation of diacylated product is minimal.

The chloroacetyl group was removed with 2-aminoethanethiol in pyridine. The mechanism of this reaction involves an initial attack by the thiol group at the chloromethylene carbon and then intra-molecular aminolysis of the ester (Scheme 11). No other product except taxol was detected in the reaction mixture.

2.1.2.1 A Photoaffinity Labeled Taxol Derivative

Taxol derivatives apparently exert their biological effects by binding to polymerized tubulin in microtubules. If the three-dimensional nature of the binding site of taxol on polymerized tubulin were known, it would greatly facilitate the understanding of the minimal structural requirements of taxol for its biological activity. It would thus point to a simpler analog which would have all the biological activity of taxol, but which might well be more synthetically accessible than taxol. The method of choice for doing this type of research is that of photoaffinity labeling which can lead to the delineation of the amino acids present in the binding site of taxol on polymerized tubulin. This technique, coupled with other information about the three-dimensional structure of tubulin (for example, from X-ray or molecular modeling studies) can lead in principle to a complete description of the nature of the binding site.

a. Basic Photoaffinity Labeling

The technique of photoaffinity labeling was developed initially by Westheimer and his co-workers,[66] and has since been improved and used by many other investigators.[67-71] The basic concept of the technique is that a drug or other small mole-

cule is labeled in some way with a photoreactive ligand which does not interfere with the biological activity of the parent drug. Incubation of the labeled drug with its natural macromolecular receptor (whether enzyme, other protein or DNA), followed by irradiation, will yield a reactive species which will then bind irreversibly to the receptor molecule. Degradation of the labeled receptor then enable the location of the binding site to be determined. Taxol, like many other guest molecules interacting with a macromolecular protein, binds reversibly to tubulin. Synthesis of a photo-labeled taxol derivative will thus provide a method of labeling the taxol-binding region of polymerized tubulin.

As shown in the schematic diagram below (Figure 10) the taxol labeled tubulin can be broken into smaller fragments by proteolysis and the fragment(s) containing label can be isolated and sequenced. Since the amino acid sequence of several different tubulins are known,[72] it would be possible to reconstruct the binding site from this information.

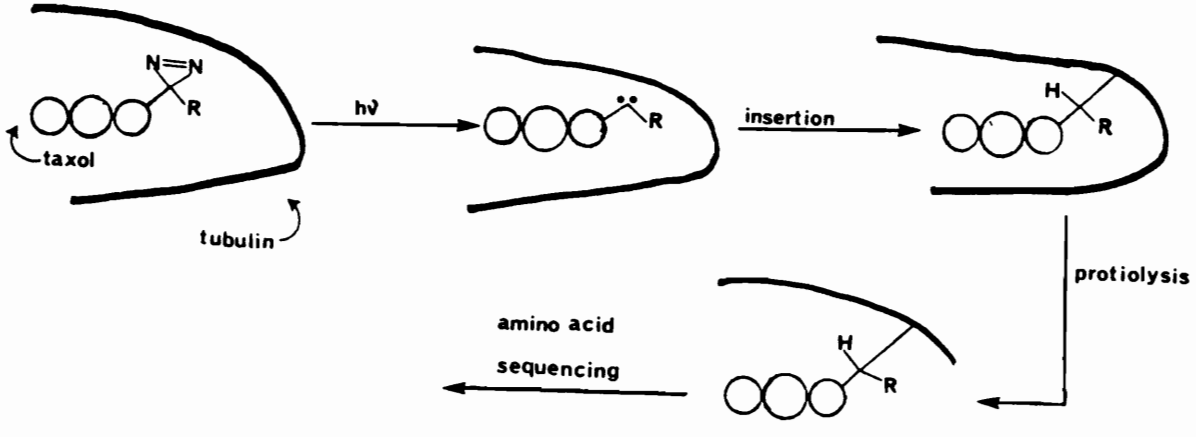
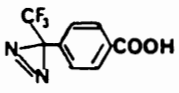
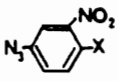
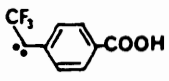
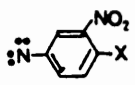


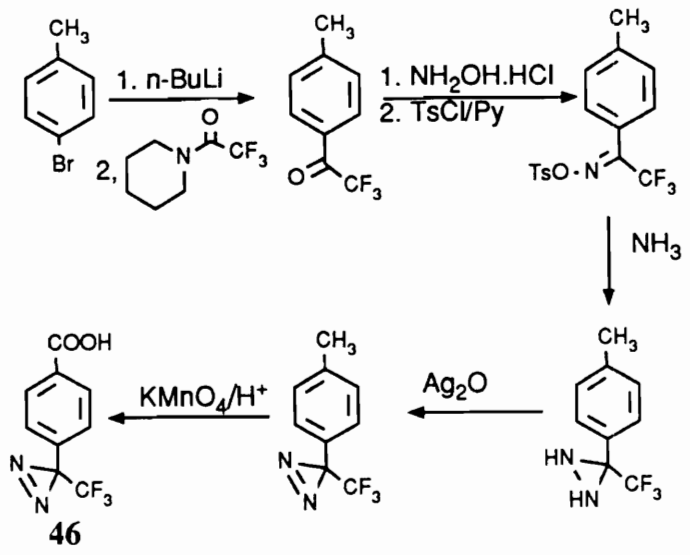
Figure 10 Photoaffinity labeling

Several different types of photolabels have been used in studies of photoaffinity labeling. Aromatic azides have been widely used as photolabels, but they suffer some disadvantages[67] as shown in Table 7. On the other hand aromatic diaziridines are a convenient[73] way around the unfavorable properties of aryl azides. They are easy to handle, they do not decompose much under ambient light and their irradiation wavelength at 353nm is such that sensitive macromolecules like tubulin are not decomposed. Most importantly, they yield carbenes on irradiation; this is significant since carbenes give primarily the desired C-H insertion reactions rather

Table 7 Properties of aryl azides and aryl diazirines

	Aryl diazirine	Aryl azide
Example		
Irradiation product		
Disadvantages	photolability at $\lambda > 300$nm means must handled in dark	C-H bond insertion is slow Reduced to amines by thiols present in buffers Rearrange to electrophilic species

than the various rearrangements undergone by nitrenes.[67] For these reasons we selected the aryl diaziridine **46** as the photolabel for taxol. The label was synthesized according to the procedure described by Nasaal[74] as outlined in Scheme 12.

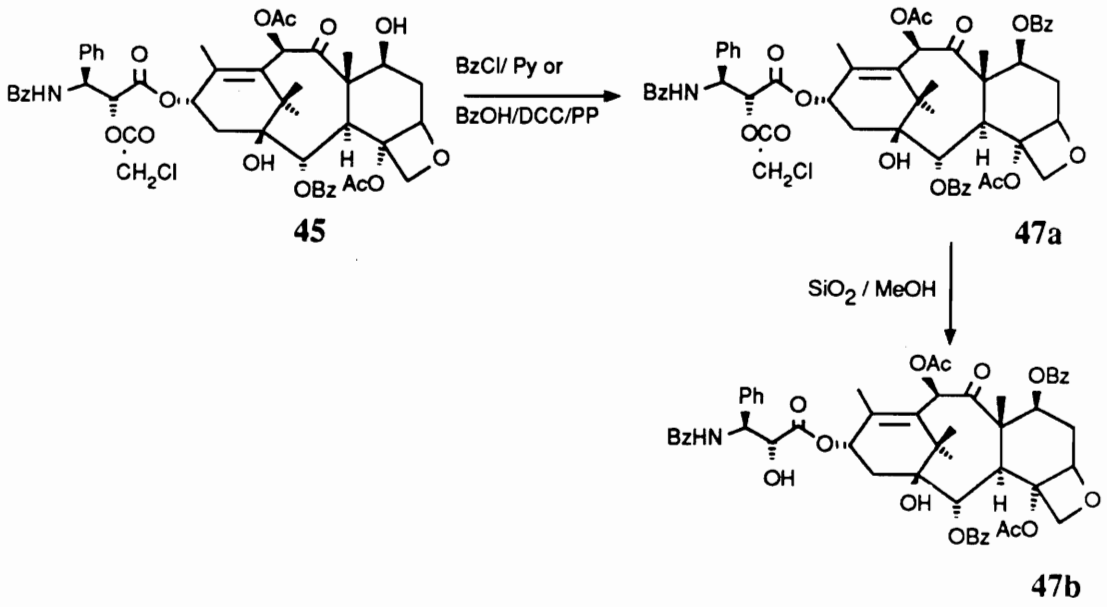


46

Scheme 12 Synthesis of the photolable

b. Preparation of 7-benzoyltaxol Prior to the synthesis of the labeled taxol derivative we were interested in preparing a model compound to see whether it is possible to derivatize the 7-OH with a bulky acyl group and retain tubulin assembly activity in the resulting product. The logical model was 7-benzoyltaxol (**47b**).

Treatment of 2'-chloroacetyltaxol (**45**) with benzoyl chloride in pyridine yielded 2'-chloroacetyl-7-benzoyltaxol (**47a**) in 52% yield (Scheme 13). The compound was identified by its ^{1}H NMR spectrum. A new aromatic signal at δ7.74 for two protons (2',6' of the C-7 benzoyl group) indicated the presence of an additional benzoyl moiety in the molecule. The signal for the C-7 methine proton was shifted to δ5.75 from its position at δ4.33 in the taxol (**5**) spectrum (Table 8).



45 47a 47b

Scheme 13 Preparation of 7-benzoyltaxol (**47b**)

Benzoylation was later carried out with dicyclohexylcarbodiimide (DCC)[75] with a catalytic amount of pyrolidinopyridine (PP), and benzoic acid. This method is faster and more convenient and gives higher yields. When using the DCC method to acylate the 7-position, three equivalents of acid should usually be used to make the reaction complete in a reasonable time. The 2-position was deprotected to give 7-benzoyltaxol (**47b**) in 64% yield.

The *in vitro* activity of 7-benzoyltaxol (**47b**) turned out to be in the same order as that of taxol (**5**), as described later (section 2.3.3, Table 17). With this information it was appropriate to synthesize 7-azibenzoyltaxol (**42**).

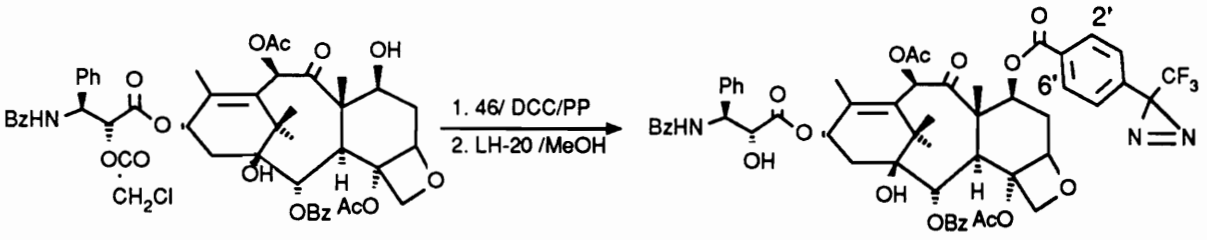
Table 8 **^{1}H NMR assignment of 2'-chloroacetyltaxol (45), 7-benzoyl-2'-chloroacetyltaxol (47a), 7-benzoyltaxol (47b) and 7-azibenzoyltaxol (42)**

Proton on	45	47b	47a	42
C-2	5.70 (d,5)	5.72[a] (m)	5.75[a] (m)	5.70[a] (m)
C-3	3.80 (d,5)	4.00 (d,7)	4.05 (d,8)	4.00 (d,7)
C-5	4.90 (d,8)	5.00 (d,8)	5.02 (d,8)	4.98 (d,8)
C-6	2.40 (m)	2.77 (m)	2.80 (m)	2.75 (m)
C-7	4.41 (br m)	5.72[a] (m)	5.75[a] (m)	5.70[a] (m)
C-10	6.25[a] (s)	6.32 (s)	6.40 (s)	6.30 (s)
C-13	6.25[a] (m)	6.17 (t,12)	6.25 (t,8)	6.18 (m)
C-14	2.55 (m)	2.17 (m)	2.32 (m)	2.35 (m)
C-16,17,18	1.12 (s) 1.22 (s) 1.64 (s)	1.17 (s, 6H) 1.94[a] (s)	1.20 (s, 6H) 1.95[a] (s)	1.18 (s) 1.20 (s) 1.85 (s)
C-18	1.90 (s)	1.94 (s)[a]	1.95[a] (s)	1.92 (s)
C-20	4.20 (ABq,8 $\Delta\nu_{AB}$=31)	4.27 (ABq,7 $\Delta\nu_{AB}$=29)	4.30 (ABq,8 $\Delta\nu_{AB}$=29)	4.28 (ABq,7 $\Delta\nu_{AB}$=28)
C-2'	5.50 (d, 3)	4.80 (br s)	5.60 (d,3)	4.80 (d,3)
C-3'	6.00 (dd,3,8)	5.80 (br d, 8)	6.05 (dd, 3,7)	5.80 (dd, 3,7)
3'-NH-	6.95 (d,8)	7.70 (d,8)	6.95 (d,7)	7.02 (d,7)
OAc 2'-$OCOCH_2Cl$	2.20 (s) 2.40 (s) 4.20 (d,3)	2.14 (s) 2.40 (s) -	2.05 (s) 2.48 (s) 4.18 (d,3)	2.00 (s) 2.40 (s) -
2-OBz(ortho)	7.70 (m,2H)	7.75 (m,2H)	7.74 (m,2H)	7.75 (m,2H)
3'-NBz(ortho)	8.15 (m,2H)	8.12 (m,2H)	8.14 (m,2H)	8.10 (m,2H)
7-OBz(ortho)		7.92 (m,2H)	7.92 (m,2H)	
7-Azi-benzoyl				7.92 (m,2H) 7.20 (m,2H)
all other aromatic H	7.3-7.6 (m)	7.3-7.6 (m)	7.3-7.6 (m)	7.3-7.6 (m)

a. overlapping peaks

c. Synthesis of Photolabeled Taxol; 7-(4-(1-Azi-2,2,2,-Trifluoromethylethyl)-benzoyl)Taxol (7-Azibenzoyltaxol, 42)

2'-Chloroacetyltaxol (**45**) was acylated with azibenzoic acid (**46**) under the DCC/PP conditions described above. The excess acid was removed by chromatography on Sephadex (LH-20); during the chromatography the 2'-chloroacetyl group was partially removed by the eluent methanol (Scheme 14).



45 **42**

Scheme 14 Synthesis of 7-azibenzoyltaxol (**42**)

Due to this partial deprotection of the 2'-protecting group, the purification was not very efficient. The final purification was done by silica gel column chromatography with ethyl acetate and hexane, and this latter purification method is recommended for any repetition of this experiment to avoid the problems in the gel chromatography step.

The ^{1}NMR spectrum of the product **42** showed (Table 8) the presence of the two additional 2' and 6' protons of the azibenzoyl moiety at δ7.92 and the downfield C-7 methine proton at δ5.70. The FAB mass spectrum of the product had an MH^+

peak at *m/z* 1066 which is consistent with composition $C_{56}H_{54}N_3O_{15}F_3$ for 7-azibenzoyltaxol (**42**). In its IR spectrum the N=N stretching absorption appeared at 1606 cm^{-1} and its UV spectrum absorption corresponding to the benzoyl functionality appeared at 350nm. These data confirmed the structure **42** for 7-azibenzoyltaxol. The tubulin assay data on this compound will be discussed in Section 2.3.4 below.

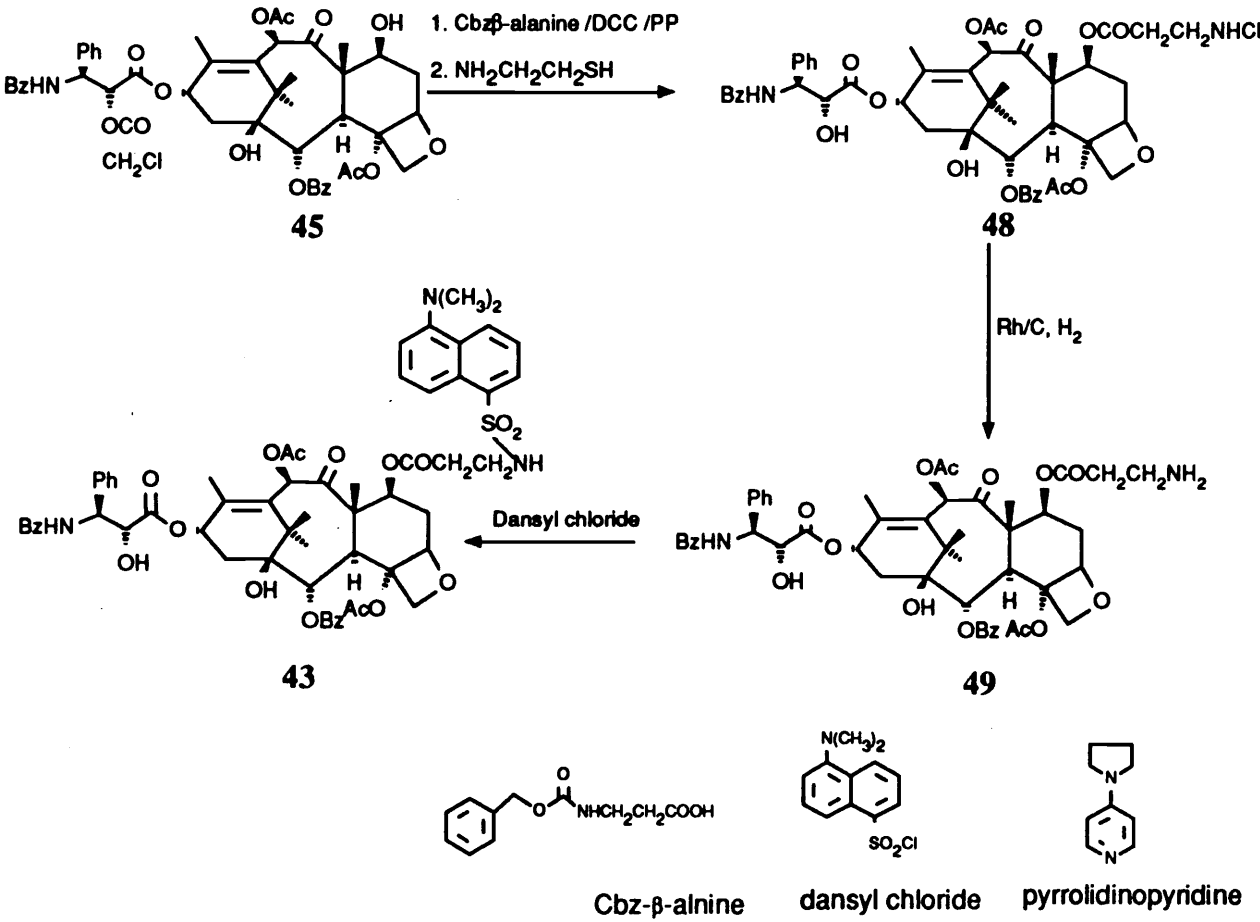
2.1.2.2 Fluorescent Labeled Taxol

In addition to its importance as an anticancer drug, taxol is also an important tool for cell biologists and other scientists interested in the structure and function of microtubules in the cell.[76,77] Since microtubules can be observed under a microscope, the availability of a fluorescent "tag" that would selectively label taxol-bound micrtotubules would be of significant assistance to this work. In principle, a fluorescent taxol derivative could fulfill this function, and could in addition be of assistance in tracking drug transportation in cells and *in vivo*.

For this reason we elected to prepare a fluorescent taxol derivative and we selected the dimethylaminonaphthalene sulfonyl (dansyl) group as our label of choice. This label has been widely used in biochemistry, especially for labeling amino acids.

Since the dansyl group shows its best fluorescence when it is linked to an amino group we chose to link it to taxol through a β-alanyl linker unit. Selection of β-alanine (Scheme 15) as the spacer group had an additional advantage, since it yielded an intermediate amino compound (**49**) which could be converted into a salt form which would be more water soluble than taxol. Taxol is virtually insoluble in

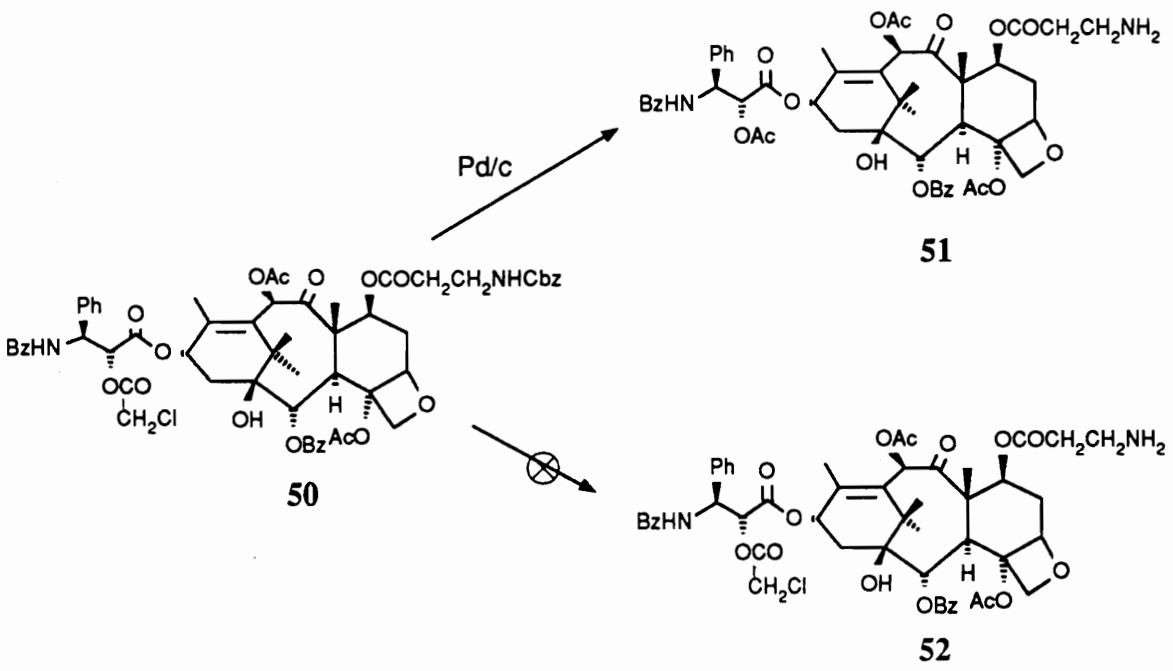
aqueous media and this insolubility causes difficulty in drug formulation in clinical trials, so a water-soluble form would be very useful. Previous work suggested that derivatization of the 7-position of taxol would yield an active compound and thus the introduction of the dansyl group was carried out by the reactions shown in Scheme 15.



Scheme 15 synthesis of fluorescent taxol (43)

The 7-OH group was derivatized with N-carbobenzyloxy-β-alanine (N-cbz-β-ala) by the DCC/PP route to give 2'-chloroacetyl-7-(N-cbz-β-alanyl)taxol (**50**) in near quantitative yield. The presence of the N-cbz-β-alanyl moiety was evident from its ^{1}H NMR data (Table 9); the signal for the oxymethylene protons of the cbz protecting group appeared at δ5.17. The methylene protons α and β to the carbonyl group appeared at δ3.45 and 2.50 respectively. The chloromethylene protons of the 2'-chloroacetyl group appeared at δ4.13.

At this point the 2'-choroacetyl group had to be removed since it was not compatible with the next hydrogenation step for removing the N-cbz group; the 2'-chloroacetyl group readily undergoes hydrogenolysis to an acetyl group. Thus when **50** was subjected to hydrogenolysis over 10% Pd/C, 2'-acetyl-7-(β-alanyl)taxol (**51**) was obtained (Scheme 16). The presence of an additional acetoxy methyl signal in its ^{1}H NMR supported the proposed structure. The mass spectrum of this compound showed a molecular ion peak MH^+ at *m/z* 967 which also was consistent with the structure **51**.



Scheme 16 Hydrogenation of 2'-chloroacetyl-7-(N-cbz-β-alanyl)taxol (**50**)

Although this experiment did not yield the desired 2'-chloroacetyl-7-β-alanyl-taxol (**52**), it was particularly helpful as a pilot experiment since it indicated that hydrogenolysis is compatible with the other functional groups of the molecule. In addition, the acetate **51** afforded a reliable mass spectrum and a ^{1}H NMR spectrum.

Table 9. **^{1}H NMR Assignments of 2'-chloroacetyl-7-(N-cbz-β-alanyl)taxol, (50) 7-(N-cbz-β-alanyl)taxol (48), 2'-acetyl-7-(β-alanyl)taxol (51) and 7-(N-dansyl-β-alanyl)taxol (43)**

Proton on	50	48	51	43
C-2	5.65[b] (m)	5.66[b] (m)	5.67 (m)[b]	5.55 (d,7)
C-3	3.92 (d,8)	3.91 (d,8)	3.92 (d,7)	3.88 (d,7)
C-5	4.92 (d,8)	4.90 (d,8)	4.95 (d,8)	4.90 (d,10)
C-6	a	2.50[b] (m)	a	a
C-7	5.65[b] (m)	5.66[b] (m)	5.67 (m)[b]	5.22 (dd, 7,10)
C-10	6.25 (s)	6.23 (s)	6.30 (s)[b]	6.20[b] (s)
C-13	6.20 (t,10)	6.17 (t,8)	6.30 (m)[b]	6.20[b] (m)
C-14	a	2.32 (m)	a	2.32 (m)
C-16,17,19	1.10 (s) 1.20 (s) 1.77 (s)	1.15 (s) 1.20 (s) 1.66 (m)	1.12 (s) 1.22 (s) 1.82 (s)	1.14 (s) 1.20 (s) 1.72 (s)
C-18	2.05 (s)	1.82 (s)	1.95 (s)	1.80 (s)
C-20	4.02 (ABq,8 $\Delta\nu_{AB}$=41)	4.24 (ABq,8 $\Delta\nu_{AB}$=35)	4.22 (ABq,8 $\Delta\nu_{AB}$=43)	4.12 (ABq,7 $\Delta\nu_{AB}$=32)
C-2'	5.55 (d,3)	4.78 (br s)	5.55 (d,3)	4.80 (d,3)
C-3'	6.00 (dd, 3,8)	5.79 (dd, 3,9)	5.92 (dd, 3,11)	5.80 (dd, 3,10)
3'-NH-	6.90 (d,8)	7.06 (d,9)	6.95 (d,11)	7.04 (d,10)
OAc	2.05 (s) 2.42 (s)	2.02 (s) 2.35 (s)	2.17 (s) 2.45 (s) 2.23 (s)	2.16 (s) 2.34 (s)
C-2'-OCOCH$_2$Cl	4.13 (d,3)			
2-OBz (ortho)	7.70 (m,2H)	7.75 (m,2H)	7.75 (m,2H)	7.74 (m,2H)
3'-NBz (ortho)	8.10 (m,2H)	8.10 (m,2H)	8.12 (m,2H)	8.12 (m,2H)

cont'd

Table 9 (cont'd)

Proton on	**50**	**48**	**51**	**43**
all other aromatic-H	7.3-7.6 (m)	7.3-7.6 (m)	7.3-7.6 (m)	7.3-7.6 (m)
-CH_2Ph	5.17 (br s)	5.09 (d,2)		
-CH_2NH (β-ala)	2.50 (m)	2.50[b] (m)	2.70 (m)	3.20 (m)
-CH_2CO-	3.45 (m)	3.46 (m)	3.32 (m)	2.40 (m)
NH (β-ala)		5.57 (dd, 7,10)		5.92 (br t, 7)
Napthalene- C-2				8.52 (m)
C-4				8.32 (m)
C-6				8.24 (m)
C-8				7.16 (m)
NMe_2				2.80 (s)

[a] hidden under other peaks
[b] overlapping peaks

The treatment of 2'-chloroacetyl-7-(N-cbz-β-alanyl)taxol (**50**) with 2-ethanothiolamine yielded 7-(N-cbz-β-alanyl)taxol (**48**) in 77% yield. The compound was characterized by its ^{1}H NMR spectrum (Figure 11) and its mass spectrum; the absence a two proton doublet at δ4.13 and the presence of an upfield signal for the 2'-methine proton at δ4.78 (see Table 9) indicated the loss of the chloroacetyl group. The 7-(N-cbz-β-alanyl) group was intact as shown by the signals for benzylic methylene protons at δ5.09, the proton on the carbamate nitrogen at δ5.57, and the methylene protons α and β to the carbamate nitrogen at δ2.5 and δ3.46 respectively. The C-7 proton appeared at the same place as in the spectrum of **50** (Table 9). A molecular ion peak MH^+ at *m/z* 1059 in the FAB mass spectrum of the compound was consistent with the molecular formula, $C_{58}H_{62}N_2O_{17}$ thus confirming the assigned structure.

Attempts to remove the cbz group from **48** by hydrogenolysis over Pd/C under varying conditions gave poor yields, presumably due to catalyst poisoning by the free amine group of the product. The use of rhodium as a catalyst, which is less sensitive to poisoning, led to the formation of 7-(β-alanyl)taxol (**49**) in 58% yield.

The ^{1}H NMR spectrum of the partially purified product lacked the signal for the oxymethylene protons of the cbz moiety, indicating the loss of the cbz group. A mass spectrum of the compound could not be recorded, however, bacause the compound proved to be very unstable. This is not surprising when the presence of free amino group is considered. The attempt to prepare its hydrochloric salt with ethereal hydrochloric acid failed; the best way to get its salt would probably be to carry out the hydrogenolysis in an appropriate acid.

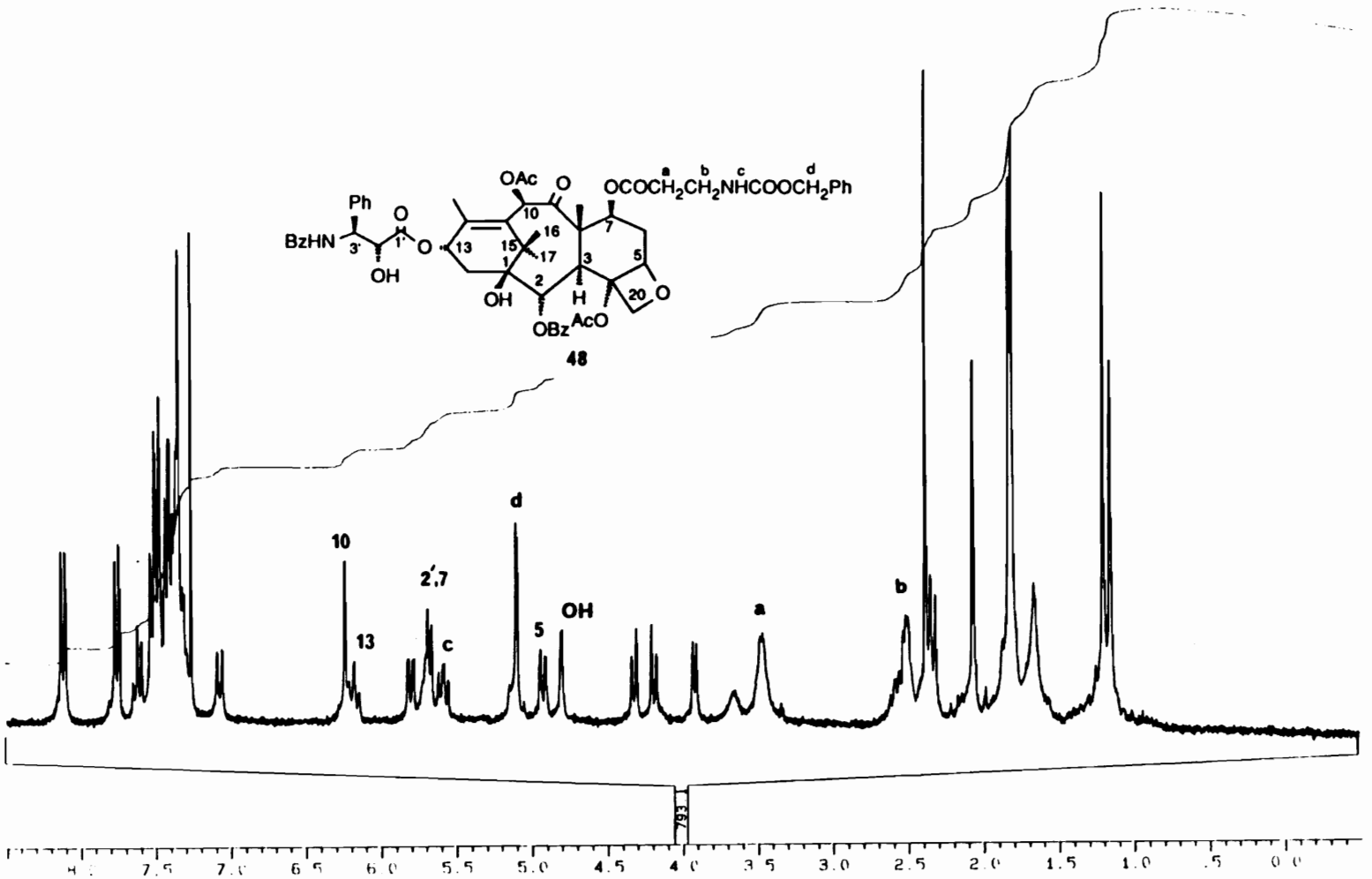


Figure 11 ^{1}H NMR of 7-(N-Cbz-β-alanyl)taxol (48)

Because of its instability as noted above, the hydrogenolysis product **49** isolated after filtration of the catalyst and evaporating the solvent was used without further purification. It was immediately reacted with dansyl chloride for 15 min, to obtain 7-(N-dansyl- β-alanyl)taxol (**43**) in 40% overall yield from **48**.

The FAB mass spectrum of **43** showed MNa^+ at *m/z* 1180, consistent with the composition $C_{62}H_{67}N_3O_{17}S$. Its ^1H NMR spectrum (Figure 12) was interpreted by comparison with those of taxol (**5**) and N-dansyl-β-alanine, and on the basis of selective irradiation experiments.

In the ^1H NMR spectrum of 7-dansyl-β-alanine (Figure 12) protons A through F were assigned by chemical shift arguments and by consideration of multiplicities. As expected signals for protons B and C appeared as two overlapping quartets; when proton F was irradiated one of the quartets collapsed to a doublet, confirming the peak assignment.

In the ^1H NMR spectrum (Figure 13) of 7-(N-dansyl-β-alanyl)taxol (**43**) the aromatic proton signals appeared at the same relative positions as in the combined spectra of taxol (Figure 5) and dansyl-β-alanine (Figure 12). The N,N-dimethyl protons appeared at δ2.80 as a singlet, and the sulfonamide proton triplet situated at δ5.9 collapsed to a singlet when the two proton multiplet at δ3.2 was irradiated (Figure 13). The same irradiation caused the multiplet at δ2.45 to collapse into a singlet, indicating that the δ3.2 and δ2.45 multiplets belong respectively to the protons α and β to the alanyl-carbonyl group. The rest of the proton signals were consistent with those of any other 7-derivatized taxol compound (Table 9).

The biological activity of 7-(N-dansyl-β-alanyl)taxol (**43**) was tested in the tubulin binding assay, as described in section 2.3.4, and its activity was found to be

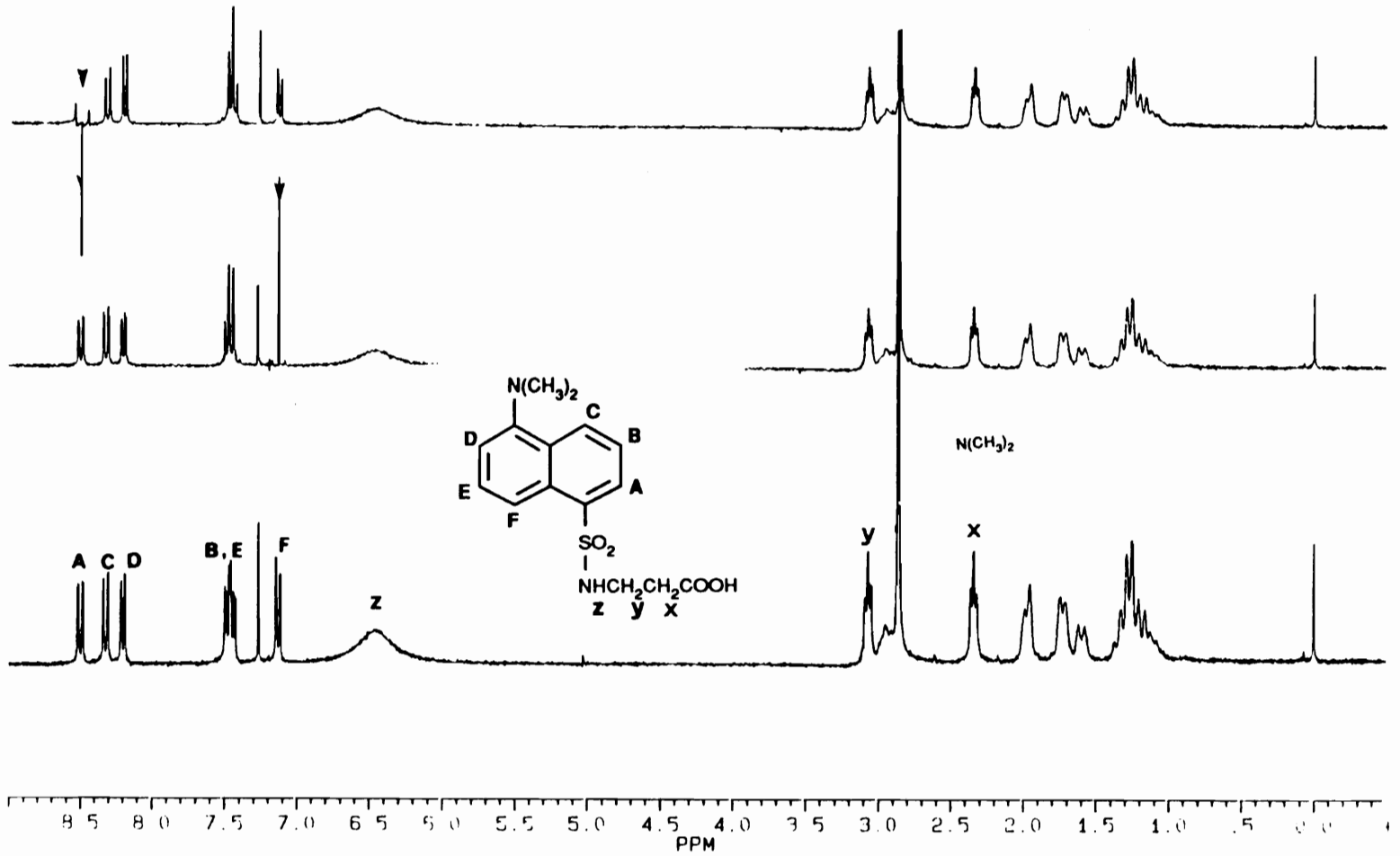


Figure 12 ^{1}H NMR N-dansyl- β-alanine

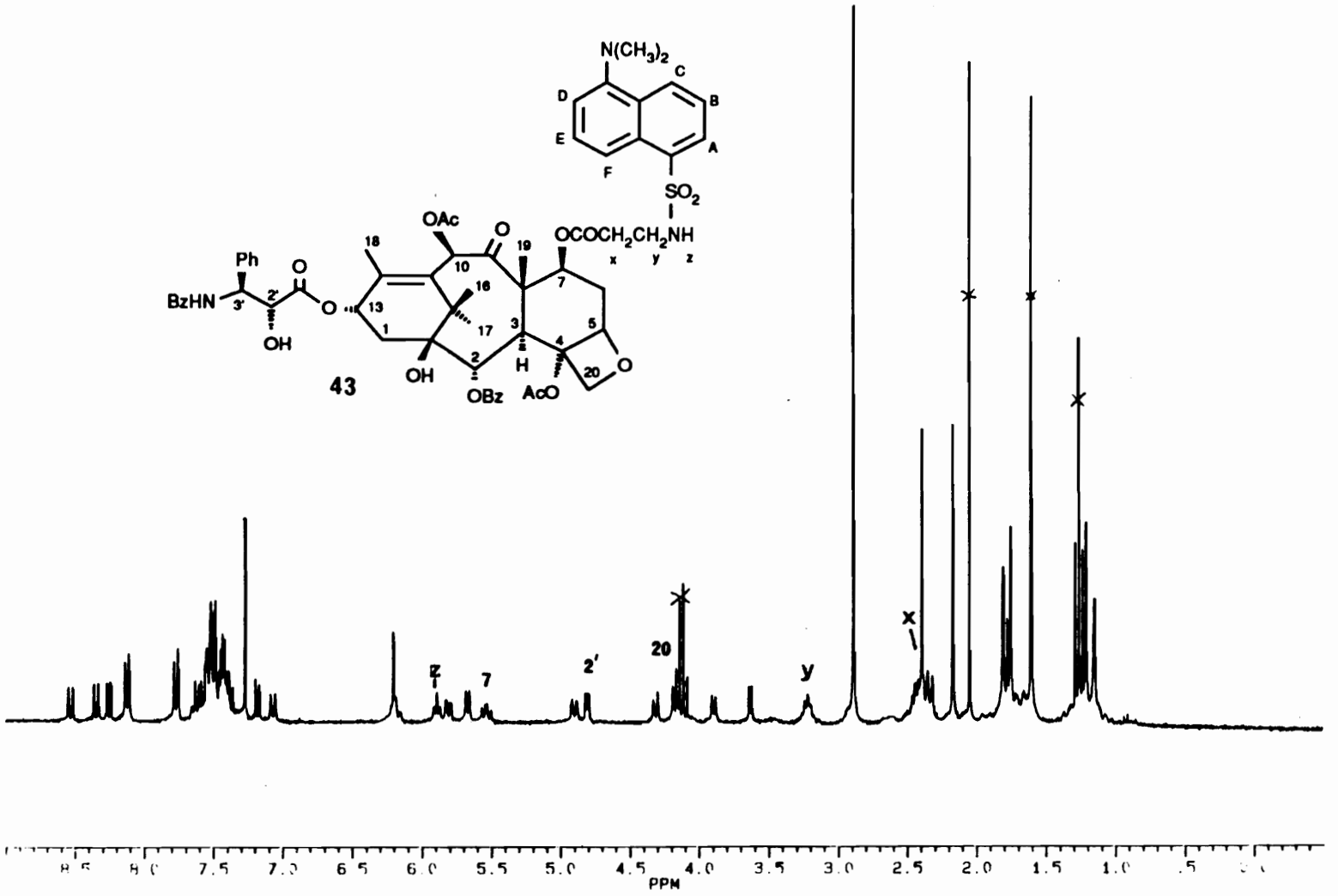


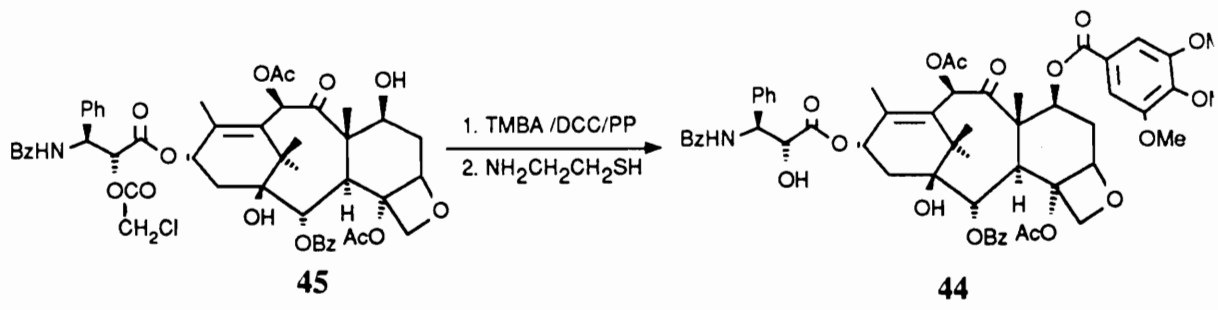
Figure 13 ^{1}H NMR spectrum of 7-(N-dansyl-β-alanyl)taxol (**43**)

identical with that of taxol. It showed a strong greenish fluorescence at 514 nm, indicating that the compound is suitable for use as a fluorescent taxol derivative.

2.1.2.3 7-Trimethoxybenzoyl Taxol

Methoxybenzene units are found in many natural antibiotics such as the anthracyclines and in some antimitotic natural compounds such as podophyllotoxin (**2b**), colchicine (**39**) and vinblastine (**1a**). It thus seemed possible that addition of a methoxybenzoyl group to the taxol molecule might yield a more active drug. The experience that a bulky aryl substituent such as the azibenzoyl group did not destroy the biological activity of taxol, prompted us to prepare the 7-trimethoxybenzoyl derivative of taxol.

2'-Chloroacetyltaxol (**45**) was treated with 3,4,5-trimethoxybenzoic acid in the presence of DCC and PP at room temperature as previously described. Deprotection of the 2'-chloroacetyl group under the usual conditions afforded 7-(3,4,5-trimethoxy)benzoyltaxol (**44**) in 88% overall yield (Scheme 17).



45 **44**

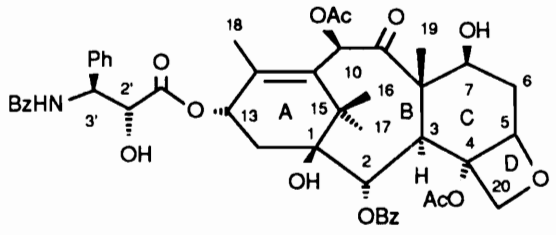
Scheme 17 Preparation of 7-trimethoxybenzoyl taxol (**44**)

The identification of the compound was straightforward from its ^1H NMR and mass spectra. The presence of a nine proton singlet at δ3.9 for three methoxy groups and the observation of the MNa^+ peak at *m/z* 1070 in its FAB mass spectrum confirmed the identity of the compound. The biological activity of the compound is almost same as that of taxol in the tubulin binding assay; more extensive testing has not been carried out due to lack of material.

2.1.3 The C-2 Hydroxyl Group as a Modification Site

2.1.3.1 A Strategy for Freeing the C-2 Hydroxyl Group of Baccatin III

The C-2 hydroxyl group in taxol (**5**) occurs as a benzoate, and thus cannot be derivatized without first removing the benzoyl group. Derivatization of the C-2 position is an important objective, both for the preparation of taxol analogs modified at the C-2 position and for the preparation of photoaffinity labeled taxol derivatives. Since the benzoate group cannot be removed selectively in the presence of other ester groups of taxol it was necessary to develop a method to hydrolyse the 2-benzoyl group and then reacylate selectively.



5

Mild hydrolysis of cephalomannine (**25**) yielded a complex mixture of products (see Scheme 4) including baccatin III (**23**) and 7-*epi* products formed by epimerization at C-7 through a retro-aldol reaction. In that study, debenzoylation at C-2 was not observed, which is understandable on the basis of the lower reactivity of benzoates.

The complications which arise from the presence of the taxol side-chain do not exist if baccatin III (**23**) is used. Therefore we elected to prepare 2-debenzoyl-baccatin III (**53**), with the concept that the C-13 ester side-chain would subsequently be attached (Figure 14) to obtain 2-debenzoyltaxol (**54**).

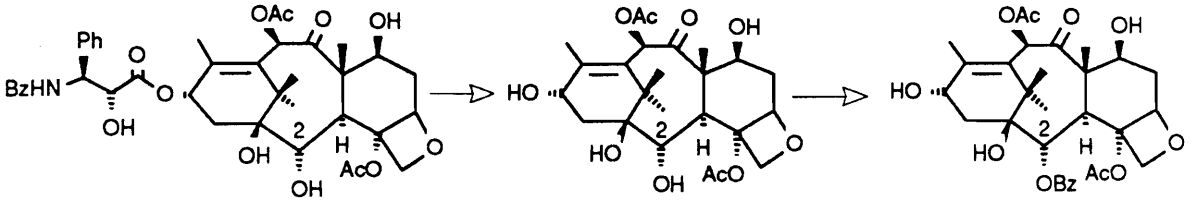
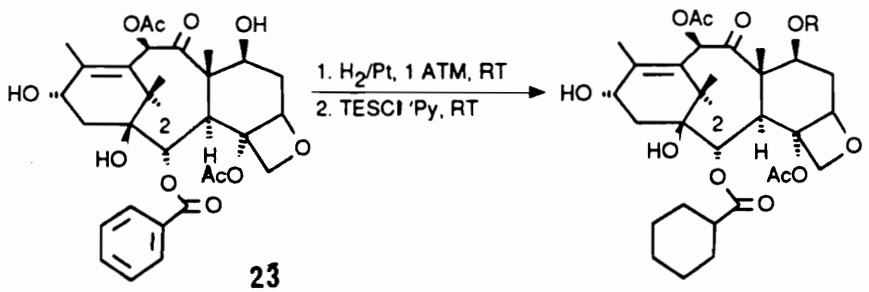


Figure 14 Proposed route to 2-debenzoyl taxol (**54**)

The possibility of using the C-1 hydroxyl group as a directing group in a BH_4 reductive cleavage of 2-benzoyl group of baccatin III was investigated first. This type of reactivity was observed by Kingston and Magri in their side-chain cleavage of taxol[48] (see Scheme 9). In this case, however, the reaction was unsuccessful, even under vigorous conditions.

Previous work in our group[54] had shown that the C-2 benzoyl group can be hydrogenated, using Pt as catalyst, to a cylohexane carboxylate group. This reaction was selective for the benzene ring, and reduction of the highly hindered C11-(12) double bond was not observed (Scheme 18). It was anticipated that the cyclo-hexane carboxylate, being an aliphatic ester, would undergo hydrolysis as readily as the acetate groups in the molecule. Hydrogenation of baccatin III (**23**) over 5% Pt/C



23

R = H, **55**

R = TES, **56**

Scheme 18 Hydrogenation of Baccatin III (**23**)

for 20 h yielded hexahydrobaccatin III (**55**) in quantitative yield. The 7-hydroxyl group was protected at this point to prevent it from epimerization in the following basic methanolysis step. Treatment of the hydrogenated product **55** with triethylsilyl chloride (TESCl) yielded the 7-TES-hexahydrobaccatin III (**56**) in 75% yield. Reaction work-up as given in the experimental section was crucial in isolating the product in good yield.

The ^{1}H NMR spectra of baccatin III (**23**) and hexahydrobaccatin III (**55**) are compared in Figure 15. The aromatic proton signals in the baccatin III (**23**) spectrum in the δ7.4-8.4 region are completely absent in the spectrum of **55**, indicating the complete hydrogenation of the benzene ring. The other assignments are as shown in the diagram (see Table 10 for data). The addition of six hydrogens to baccatin III was confirmed by the MNa^+ peak at *m/z* 615 in the FAB mass spectrum of the hydrogenated product **55**.

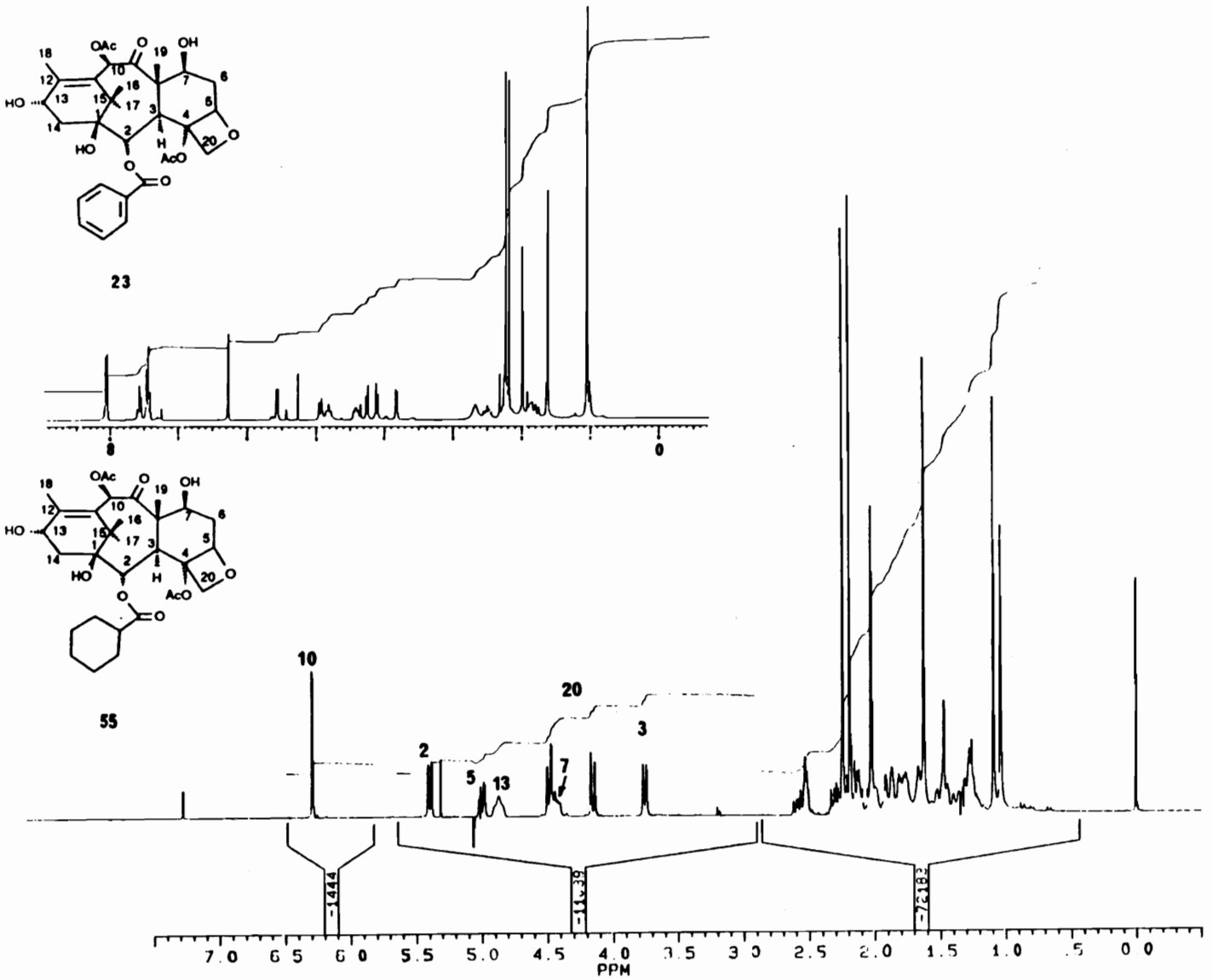


Figure 15 ^{1}H NMR spectra of baccatin III (**23**) and hexahydrobaccatin III (**55**)

Table 10 **^{1}H NMR assignment of hexahydrobaccatin III (55), 7-TES-hexahydrobaccatin III (56) and baccatin III (23)**

Proton on	55	56	23
C-2	5.40(d,7)	5.40 (d,7)	5.58 (d,7)
C-3	3.73 (d,7)	3.73 (d,7)	3.84 (d,7)
C-5	5.00 (br d,11)	4.94 (br d,8)	4.94 (dd, 2,8)
C-6	a	a	2.6 (m), 2.3 (m)
C-7	4.42 (m)	4.44 (m)	4.42 (m)
C-10	6.23 (s)	6.41 (s)	6.28 (s)
C-13	4.83 (m)	4.80 (m)	4.82 (br t,9)
C-14	a	a	2.3 (m)
C-16,17	1.02 (s) 1.06 (s)	1.00 (s) 1.11 (s)	1.04 (s) 1.04 (s)
C-18	2.00 (s)	2.14 (s)	1.98 (s)
C-19	1.60 (s)	1.60 (s)	1.62 (s)
C-20	4.30 (ABq,7, $\Delta\nu_{AB}$=72)	4.30 (ABq, 8, $\Delta\nu_{AB}$=70)	4.18 (ABq, 8, $\Delta\nu_{AB}$=42)
OAc	2.22 (s) 2.16 (s)	2.14 (s) 2.15 (s)	2.20 (s) 2.24 (s)
TES		0.90 (9H, t,7) 0.56 (6H,m)	
2-OBz (ortho)			8.50 (m)
other aromatic-H			7.46 (m)

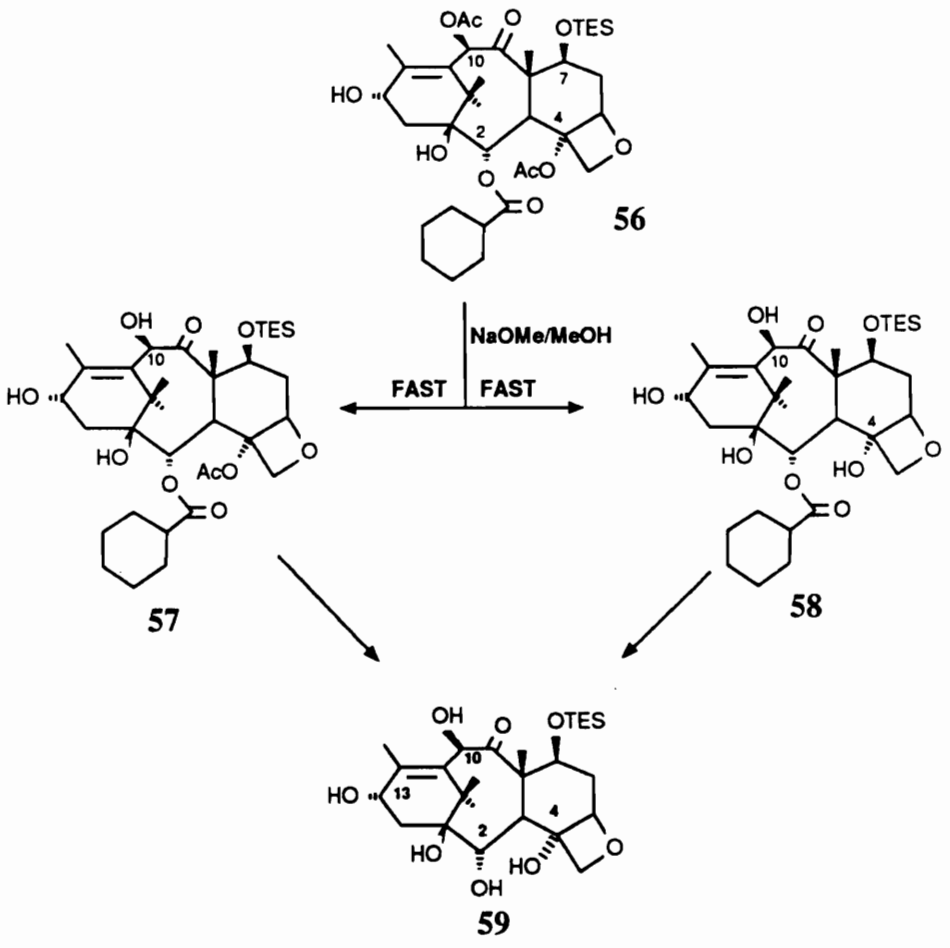
a. These protons are hidden under cyclohexane proton envelope

In the ^1H NMR spectrum of the triethylsilyl derivative **56**, the triethylsilyl protons appeared in the range of δ0.5 to 0.9 (Table 10); the six methylene protons appeared as a multiplet at δ0.56 and the three methyl groups appeared as a triplet at δ0.90. These protons signal always appear away from the other methyl signals. Triethylsilylation did not cause any significant chemical shift change in the C-7 proton signal.

2.1.3.2 Methanolysis of 7-TES-hexahydrobaccatin III (56)

Treatment of the compound **56** with aqueous methanolic sodium bicarbonate failed to produce any hydrolysed product even after a prolonged reaction time (two days). Treatment with sodium methoxide in methanol, however, yielded a mixture of products with various degrees of acylations. As shown in Scheme 19, all the intermediate products were eventually converted to a single product, 7-TES-2,4,10-tris(deacyl)baccatin III (**59**) in 69% yield.

All the intermediate products were isolated in a controlled methanolysis experiment. Two products which were more polar than the starting material appeared after 30 minutes of reaction time. The least polar of the two was found to be the 10-deacetyl compound **57** (7-TES-10-deacetyl-hexahydrobaccatin III). In the ^1H NMR spectrum of this compound, the C-10 methine proton appeared shifted to δ5.1 from δ6.4 in the starting compound **56** (Table 11), indicating deacetylation at C-10.



Scheme 19 Hydrolysis of 7-TES-hexahydrobaccatin III (**56**)

The C-4 acetoxy methyl singlet appeared at δ2.17. A molecular ion peak MH^+ at *m/z* 665 in its FAB mass spectrum is consistent with the composition $C_{35}H_{56}O_{10}Si$.

Surprisingly, the second compound was identified as the 4,10-deacetylated product **58** (7-TES-4,10-bis(deacetyl)-hexahydrobaccatin III); a tertiary and sterically very hindered C-4 acetate is solvolysed in the presence of a secondary acyl group (cyclohexanecarboxylate) at C-2. The compound was identified by comparison of its

^{1}H NMR data (Table 11) with those of the 10-deacetyl compound (**57**). In the 10-deacetyl compound there was only one acetoxy group present. The compound **58** did not have the signal due to this acetoxy methyl group (Figure 16), clearly indicating that deacetylation at C-4 had occurred. The AB quartet for the diastereotopic C-20 protons shows a small chemical shift difference compared to that for compounds with an intact C-4 acetoxy group; this small difference was indicated by a very close AB pattern appearing at δ4.45. The $\Delta\nu_{AB}$ value of 19Hz contrasts with the normal value of about 80Hz. The most remarkable shift was shown for the C-7 methine proton which appeared at δ3.95, shifted from δ4.45 ($\Delta\delta$ = 0.42ppm) in the spectrum of the 10-deacetyl compound (**57**).

In fact all the protons on the concave face of the molecule, namely C-3, C-5, C-10 protons, showed upfield shifts of approximately 0.2ppm compared with those of the starting material **56**. The C-2 and C-20 protons on the convex face did not show any appreciable shifts. These shifts are probably due to the removal of the magnetic field exerted by the C-4 acetyl group or to the release of steric compression.[78]

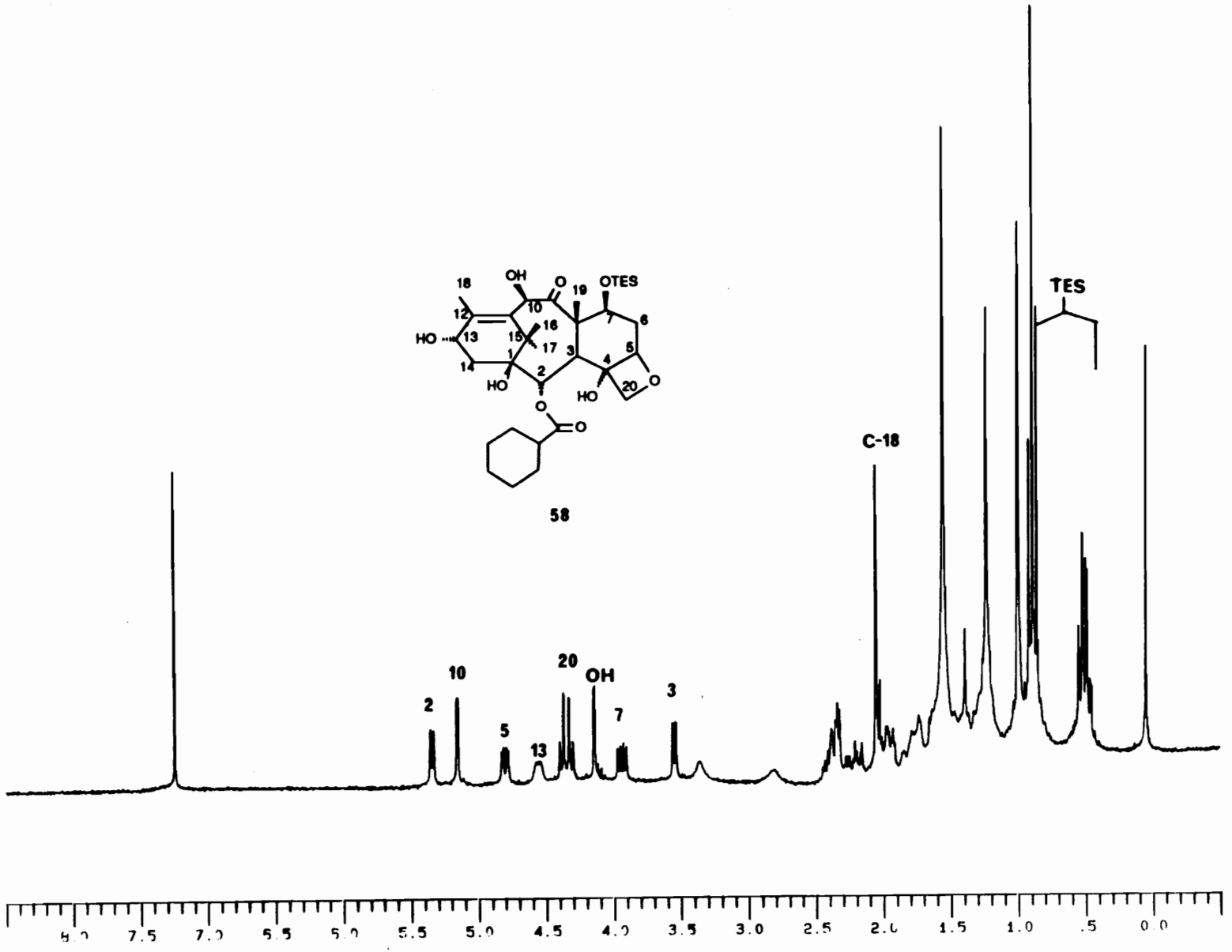


Figure 16 ^{1}H NMR of 7-TES-4,10-bis(deacetyl)-hexahydrobaccatin III (58)

Table 11 **^{1}H NMR assignment of 7-TES-10-deacetyl-hexahydrobaccatin III (57), 7-TES-4,10-bis(deacetyl)-hexahydrobaccatin III (58) and 7-TES-2,4,10-tris(deacyl)baccatin III (59) and comparison with 7-TES-hexahydrobaccatin III (56)**

Proton on	56	57	58	59
C-2	5.40(d,7)	5.32 (d,7)	5.35 (d,7)	3.77 (d,7)
C-3	3.73 (d,7)	3.80 (d,7)	3.55 (d,7)	3.25 (d,7)
C-5	4.94 (br d,8)	4.95 (br d,7)	4.82 (dd,3,8)	4.76 (dd, 3,8)
C-6	a	a	a	2.40 (m), 1.97 (m)
C-7	4.44 (m)	4.37 (dd,7,10)	3.95 (dd, 7,13)	3.94 (dd, 5,11)
C-10	6.41 (s)	5.10 (s)	5.15 (s)	5.10 (s)
C-13	4.80 (m)	4.80 (br t,7)	4.55 (br t,7)	4.55 (br d,8)
C-14	a	a	a	2.08(m), 2.40 (m)
C-16,17	1.00 (s) 1.11 (s)	1.40 (s) 1.00 (s)	1.00 (6H,s) 1.05 (s)	0.97 (6H,s)
C-18	2.14 (s)	2.03 (s)	2.04 (s)	2.12 (s)
C-19	1.60 (s)	1.70 (s)	1.62 (s)	1.59 (s)
C-20	4.30 (ABq,8, $\Delta\nu_{AB}$=70)	4.30 (ABq, 7 $\Delta\nu_{AB}$=83)	4.36 (ABq, 7 $\Delta\nu_{AB}$=19)	4.55 (ABq, 8 $\Delta\nu_{AB}$=89)
OAc	2.14 (s) 2.15 (s)		2.16 (s)	
TES	0.90 (9H, t,7) 0.56 (6H,m)	0.92 (9H, t,7) 0.52 (6H,m)	0.90 (9H, t,7) 0.51 (6H,m)	0.91 (9H, t,7) 0.51 (6H,m)

a These protons are hidden under the cyclohexane proton envelope

The data presented in Table 12 suggest that, as a general trend, removal of α substituents from **56** leads to progressive upfield shift of the protons on the α-face of the molecule while it does not have a significant effect on protons on β-face (Figure 17, only the protons of interest are shown).

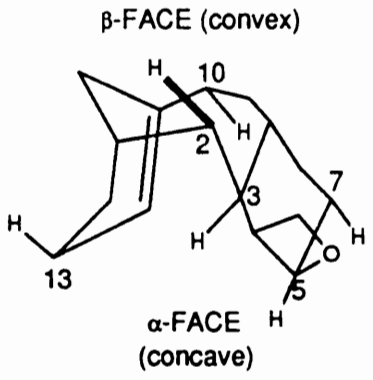


Figure 17 Protons on the α and β face of the baccatin III derivatives

After five hours, both products **57** and **58** were converted to the fully deacetylated compound 7-TES-2,4,10-tris(deacetyl)baccatin III (**59**). The MNa^+ peak at *m/z* 535 in its mass spectrum was consistent with its expected molecular mass of 512. The ^1H NMR of this compound (Figure 18) was similar to that of the 4,10-deacetyl compound **58** except that the C-2 methine proton had the appropriate upfield deacylation shift. The C-5 and C-7 protons were assigned by selective decoupling and by chemical shift arguments. The unusual broad doublet shape of the C-13 proton and the well resolved C-14 methylene proton signals which occurred as a an ABX pattern at δ2.3 were not observed in other baccatin derivatives. The signal for the C-13 proton was however assigned to the broad doublet at δ4.76 by a selective

decoupling experiment; irradiation of this peak collapsed a portion of the multiplet at δ2.3 to an AB quartet (see Figure 18). This observation is possible only for the 13-H, 14-H spin system. As discussed later the acetylation shift of the resonance at δ4.76 to δ5.85 in the acetyl derivative **63** further supported the assignment.

The C-5 and C-7 protons of **59** (at δ4.8 and 3.9 respectively) were distinguished by a decoupling experiment as presented below. Both of these protons are coupled to the C-6 methylene protons. In baccatin III derivatives, however, the C-5 proton shows a strong coupling to only one of the C-6 protons; the other coupling constant is very small. It was therefore, possible to identify the weakly coupling C-6 proton; when this was irradiated, the C-5 proton collapsed to a broad singlet while the C-7 only collapsed to a doublet. This experiment identified the C-5 proton as the doublet of doublets at δ4.8.

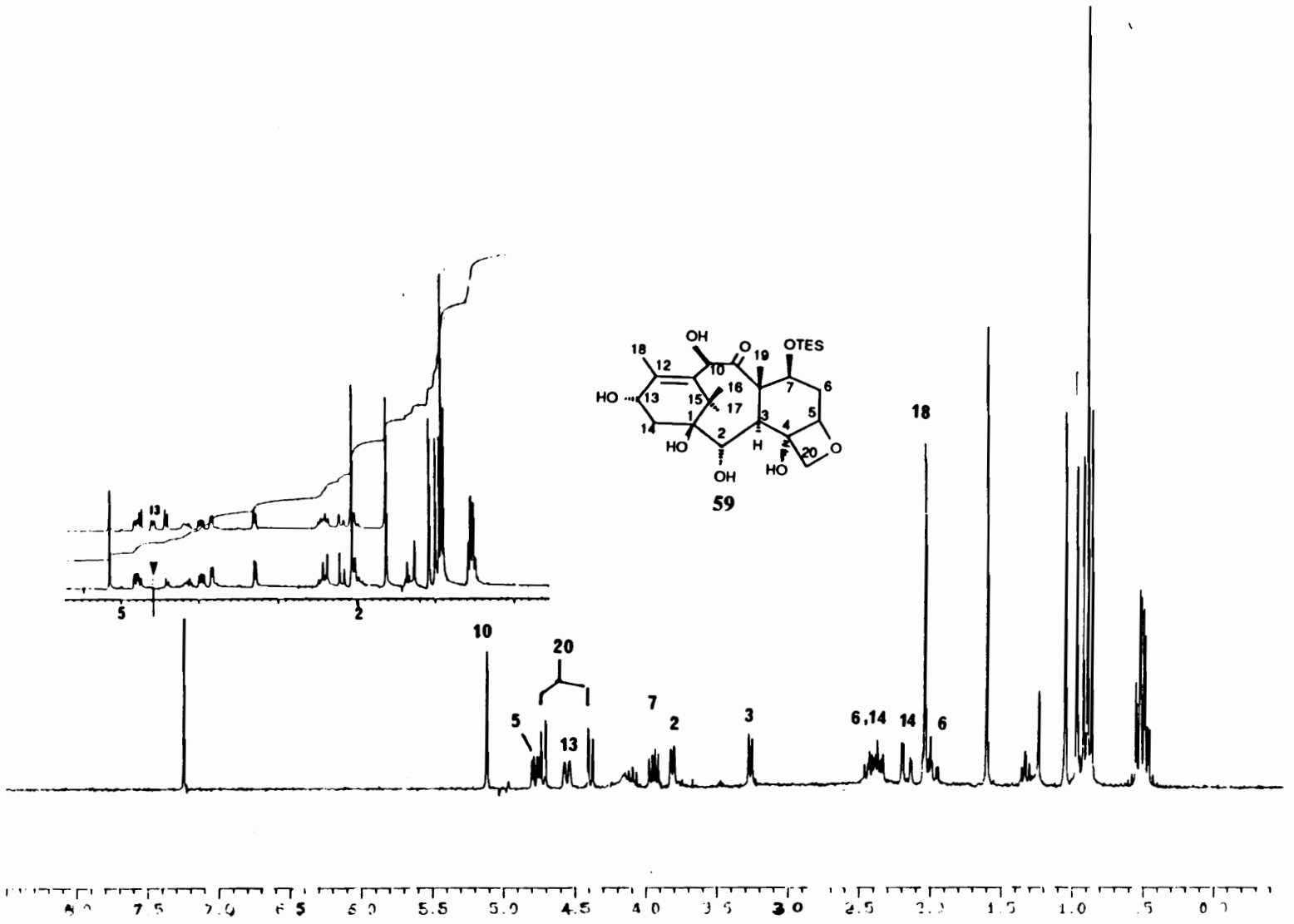
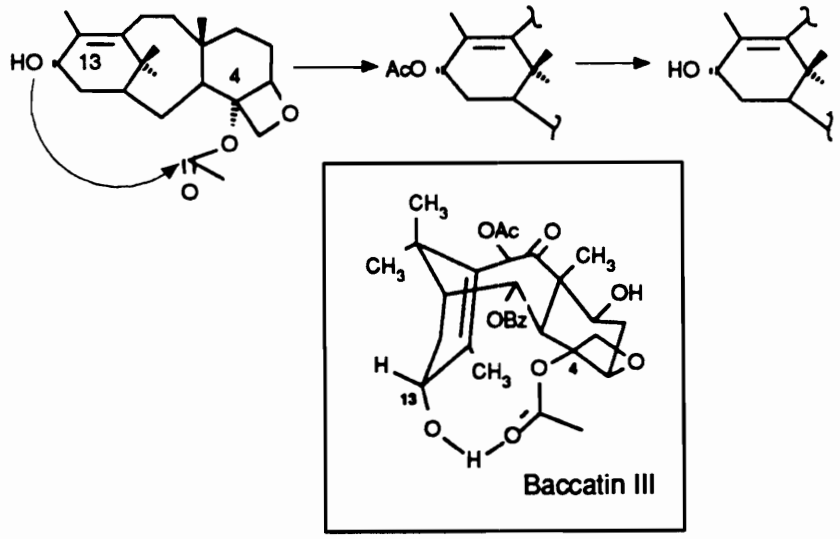


Figure 18 ^{1}H NMR spectrum of 7-TES-2,4,10-tris(deacyl)baccatin III (59)

2.1.3.3 Methanolysis of 7,13-Bis(TES)baccatin III

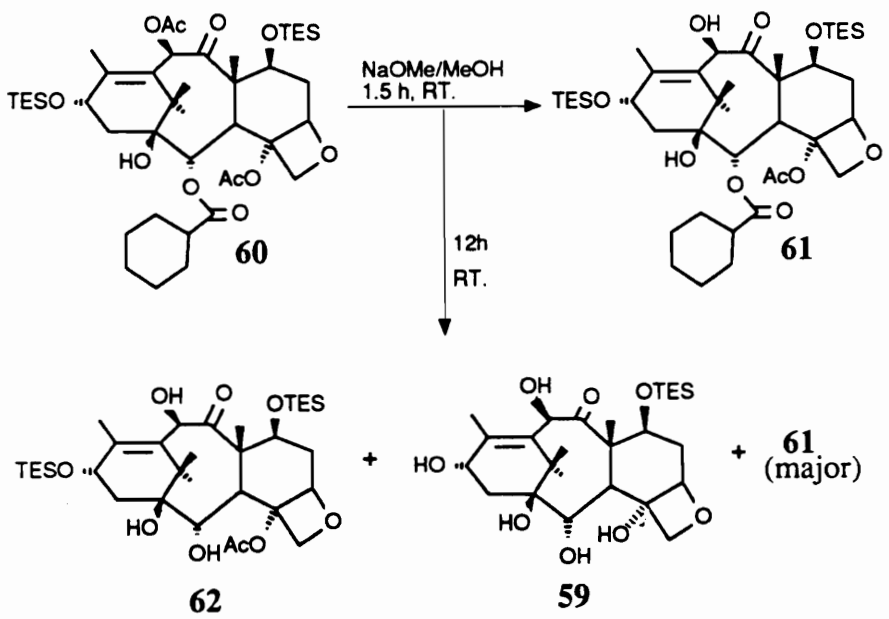
The anomalous reactivity of the acyl groups of 7-TES-hexahydrobaccatin III (**56**) deserves some explanation. As we discussed previously, the C-4 tertiary acetoxy group undergoes hydrolysis more readily than the secondary C-2 cyclohexanecarboxylate group. This might well be due to acetyl transfer from the C-4 hydroxyl group to the C-13 hydroxyl group. Once at C-13, the acetoxy group can undergo ready solvolysis (Scheme 20). The conformation of the baccatin III (**23**) molecule is such that those two functional groups are in very close proximity (see Figure 3). To verify this hypothesis we prepared a C-13 protected baccatin derivative for solvolysis. 7,13-Bis(TES)-hexahydrobaccatin III (**60**) was prepared from hexahydrobaccatin III (**55**) with triethylsilylchloride and imidazole in THF. This



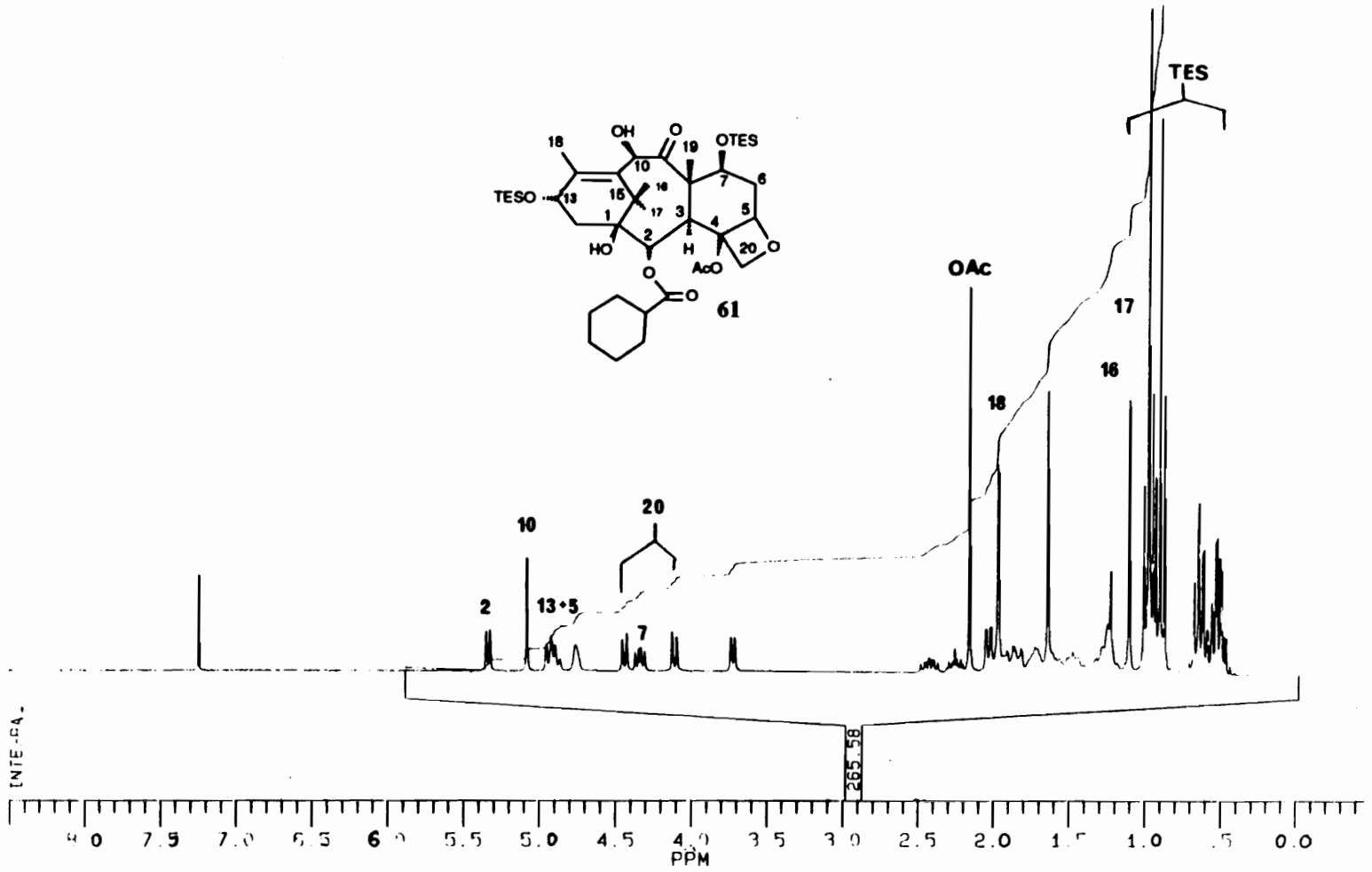
Scheme 20 Acetyl transfer in baccatin III derivatives

compound was subjected to solvolysis with methanolic NaOMe (Scheme 21). A sample of 7-TES-hexahydrobaccatin III (**56**) was subjected to solvolysis as a control. After 1.5 hours, the product observed in the reaction mixture containing **60** was exclusively the 10-deacetyl compound **61** which was identified by its ^{1}H NMR (Figure 19), whereas all three deacylated products (**57**-**59**, Scheme 19) were present in the control with no starting material.

The diprotected product **60** was allowed to react for a period of 12 h to determine whether or not any other products were formed. The major product isolated was still the 10 deacetyl compound **61** (42%), with only a small amount of



Scheme 21 Methanolysis of 7,13-bis(TES)baccatin III (**60**)



61

Figure 19 1H NMR of 10-deacetyl-7,13-bis(TES)-baccatin III (61)

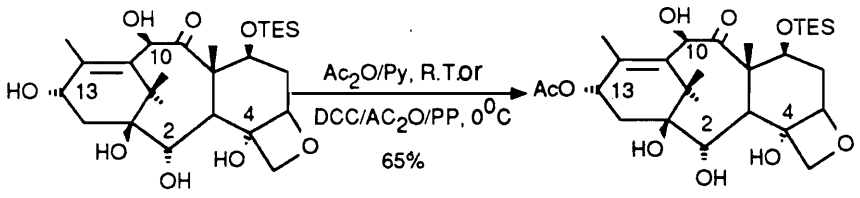
a complex mixture of other compounds detected by TLC. This complex mixture was separated to determine its major components. Complete purification of the mixture was not possible due to the minute amounts isolated, and the ^{1}H NMR spectra of the partially purified samples were recorded. The least polar compound had lost its C-2 substituent as judged by the upfield shift of its C-2 methine proton and the C-4 acetoxy methyl appeared as a singlet indicating, it is the desired 2,10-bis(deacyl) product (**62**). The most polar compound had lost the C-13 TES protecting group, in addition to all its acyl groups and was therefore assigned the structure of the fully deacylated compound 7-TES-2,4,10-tris(deacyl)baccatin III (**59**). Identical ^{1}H NMR spectra of the two compounds confirmed the assigned structure.

This experiment supported our acetyl transfer hypothesis and also apparently solved the C-4 acetate solvolysis problem. However, hydrolysis of **60** to the 2,10-deacylated product (**62**) is extremely slow and produces a complex mixture and therefore, is not a practical method for obtaining the desired 2,10-deacylated baccatin III derivatives. Nevertheless this experiment suggests that it may be possible to produce the desired product under some reaction conditions.

2.1.3.4. Acylation of 7-TES-2,4,10-Tris(deacyl)-hexahydrobaccatin III and its Derivatives

The ultimate goal of the aforementioned deacylation experiment was to prepare 2-debenzoylbaccatin III (**53**) which could be converted into 2-debenzoyltaxol (**54**). This would give us a free hydroxyl group at C-2 for further derivatization. As

discussed above, the tertiary acetate group at C-4 undergoes methanolysis before the secondary acyl group at C-2, leading to the problem of selective acetylation of the C-4 hydroxyl group. Selective acetylation of the C-10 hydroxyl group is not a problem since this group readily undergoes acetylation.[36] We therefore initiated a study of the reacylation of 7-TES-2,4,10-tris(deacyl)baccatin III (**59**). Under mild acetylation conditions (0°C/DCC/PP/Ac_2O/0.5 h or RT/Py/Ac_2O/22 h) the only product obtained was the C-13 acetyl product **63** (Scheme 22) in 65% yield.



59 **63**

Scheme 22 Formation of 13-acetyl-7-TES-2,4,10-tris(deacyl)baccatin III (**63**)

The presence of the 13-OAc group was clear by its ^{1}H NMR spectrum (Figure 20). The acetoxy methyl gave a signal at δ2.12 and the C-13 proton was shifted downfield to δ5.84; irradiation of the signal for the C-13 proton led to collapse of the multiplet for the C-14 protons at δ2.26, confirming that the signal irradiated is indeed of the C-13 proton. The rest of the ^{1}H NMR data (Table 12) were similar to those of the starting material **59**. A FAB mass spectrum yielded the MH^+ peak at *m/z* 555 corresponding to the molecular mass of the protonated compound.

This finding of the ready acetylation of the C-13 position contrasts strongly with that of the acetylation of baccatin III (**23**) itself, where the C-13 position is less reactive even than the very hindered C-7-position.[36] The reason for this lack of reactivity at C-13 in baccatin III (**23**) has been attributed to hydrogen bonding between the C-4 acetoxy group and the C-13 hydroxyl group[37] (see Figure 3) and this new finding supports this hypothesis.

Acetylation (Scheme 23) of the other acetate groups of **63** was monitored by TLC. After one hour a mixture of products was detected by TLC and no selectivity was observed in the formation of these products. Three fractions were isolated from the reaction mixture. The most polar compound was found to be a 1:2 mixture of the 2,13-diacetyl compound (**64**) and the 2,10,13-triacetyl compound (**65**) by its ^{1}H NMR spectrum (see experimental). Two MH^+ peaks at *m/z* 639 and 597 in the FAB mass spectrum of the mixture were consistent with the presence of these two compounds. The fraction of medium polarity was a complex mixture and was not identified.

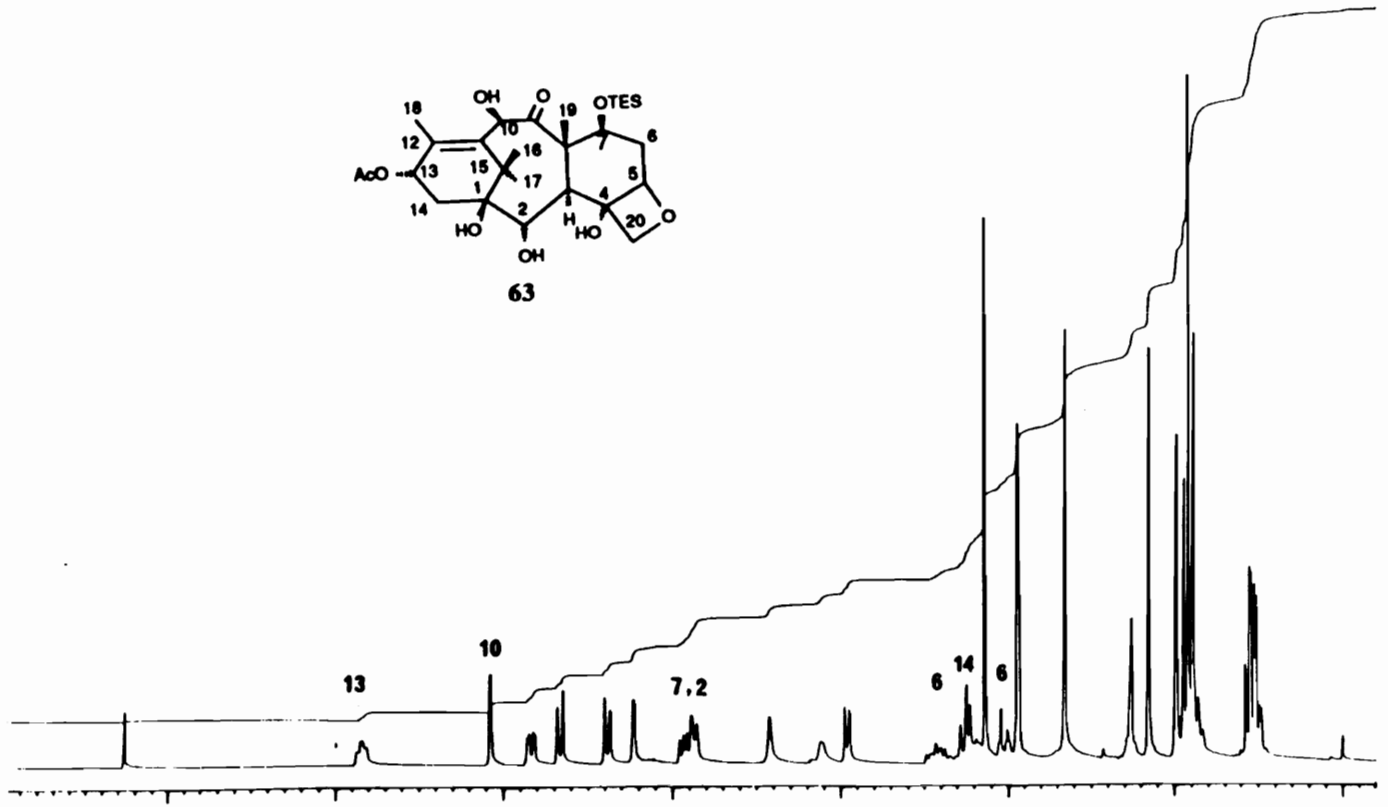
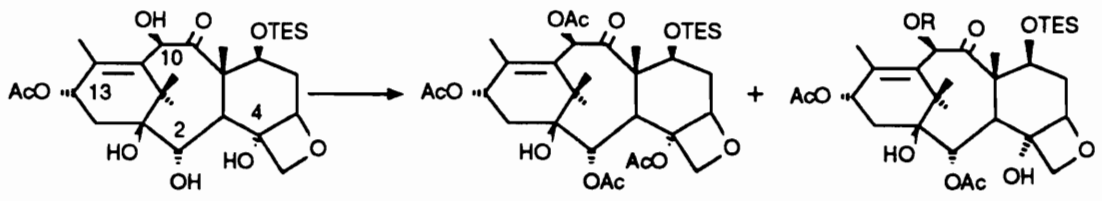


Figure 20 ^{1}H NMR 0f 13-acetyl-7-TES-2,4,10-tris(deacyl)baccatin III (**63**)

Table 12 **^{1}H NMR assignment of 13-acetyl-7-TES-2,4,10-tris(deacyl)baccatin III (63) and 2-debenzoyl-7-TES-2,13-diacetylbaccatin III (66) and comparison with 7-TES-2,4,10-tris(deacyl)baccatin III (59)**

Proton on	63	66	59
C-2	3.89 (d,7)	5.40 (d,7)	3.77 (d,7)
C-3	2.97 (d,7)	3.69 (d,9)	3.25 (d,7)
C-5	4.85 (dd,4,10)	4.95 (d,8)	4.76 (dd,3,8)
C-6	2.02 (m), 2.44 (m)	a	2.40 (m) 1.97 (m)
C-7	3.92 (dd,7,12)	4.47 (dd,7,9)	3.94 (dd,5,11)
C-10	5.06 (s)	6.43 (s)	5.10 (s)
C-13	5.84 (br t,8)	6.12 (br t,7)	4.55 (br d,8)
C-14	2.44 (m)	a	2.08 (m) 2.40 (m)
C-16,17	0.96 (s) 1.14 (s)	1.15 (s) 1.22 (s)	0.97 (6H,s)
C-18	1.91 (s)	2.05 (s)	2.12 (s)
C-19	1.66 (s)	1.62 (s)	1.59 (s)
C-20	4.52 (ABq,8, $\Delta\nu_{AB}$=59)	4.35 (ABq,8 $\Delta\nu_{AB}$=78)	4.55 (ABq,8 $\Delta\nu_{AB}$=89)
OAc	2.12 (s)	2.22 (3H,s) 2.15 (6H,s)	
TES	0.90 (9H, t, 7) 0.52 (6H,m)	0.92 (9H, t, 7) 0.57 (6H,m)	0.91 (9H, t, 7) 0.51 (6H,m)

a. not determined



63 **66** R = H, **64**
R = Ac, **65**

Scheme 23 Acetylation of 13-acetyl-7-TES-2,4,10-tris(deacyl)baccatin III (**63**)

Most importantly, the least polar compound was a tetracetate, indicating that the C-4 tertiary hydroxyl group was also acetylated along with the C-2 and the C-10 hydroxyl groups. The mass spectrum of this compound showed a MH^+ peak at *m/z* 681 which corresponds to the protonated molecular mass of 680 expected for the 2-debenzoyl-7-TES-2,13-diacetylbaccatin III (**66**). In its ^1H NMR spectrum the C-2 and C-10 protons showed appropriate down field shifts (Table 12), and the C-7 proton returned to δ4.4, its original shift found in the spectrum of 7-TES-hexahydrobaccatin III (**56**).

Some important conclusions from the above experiments can be summarized as follows:

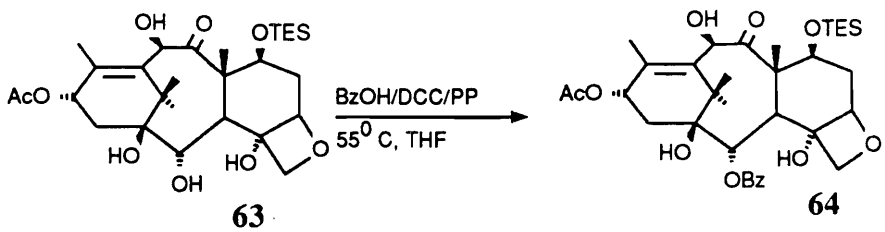
1. The C-13 is the most reactive hydroxyl group in 2-debenzoyl-4,10-bis(deacetyl)-7-TES-baccatin III (**59**). This result contrasts with the well-observed difficulty[36] of the acylation at this position in baccatin III (**23**). We can rationalize this newly acquired reactivity of the C-13 hydroxyl group as

stemming from the loss of hydrogen bonding to the C-4 acetoxy group.

2. The C-2 hydroxyl group showed a 2:1 selectivity over the C-10 hydroxyl group on acylation. This selectivity will be increased if a bulky acyl group is used.
3. Acetylation at C-4 would be possible under forcing conditions.
4. A possible synthetic route to a C-2 modified taxol would begin by first introducing the protected side-chain selectively at the C-13 hydroxyl group of the fully deacylated baccatin III derivative (**55**). Subsequent introduction of the desired acyl group at C-2, followed by acetylation of the C-10 and C-4 hydroxyl groups would yield the desired compound.

Benzoylation of **63** would verify the second point stated in the previous paragraph, since the benzoyl group is a fairly bulky acyl group. Then, If we could in addition acetylate the C-4 and C-10 hydroxyl groups of the benzoyl derivative **67**, it would prove the correctness of the third point. At the same time, we would form a derivative of natural baccatin III, as an ultimate proof of all the structural assignments made so far.

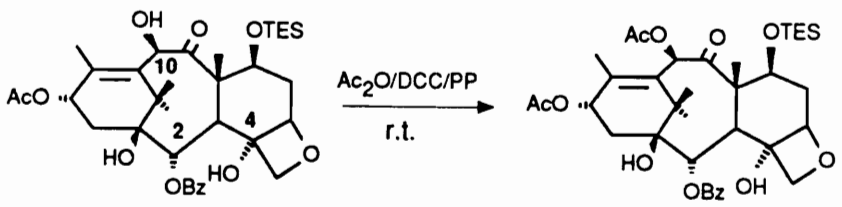
Benzoylation of **63** at room temperature with DCC/PP/BzOH was not successful, but heating at 55^{0}C for 72 h with the same reagents yielded the 2-benzoyl derivative, 7-TES-13-acetyl-4,10-bis(deacetyl)baccatin III (**67**) in 14% yield (Scheme 24).



Scheme 24 Benzoylation of 13-acetyl-7-TES-2,4,10-tris(deacyl)baccatin III (**63**)

The ^{1}H NMR spectrum of the benzoyl derivative (**67**) is shown in Figure 21. The presence of the 2-benzoyl group is evident by the identical set of peaks appearing between δ7.4 and 8.2, and by the downfield shift of the C-2 proton to δ5.6, due to the benzoylation. Note that the C-7 proton is still positioned up field at δ3.9; this indicates that the C-4 hydroxyl group is still free. The other proton assignments and are shown in Table 13.

Further acetylation (Scheme 25) of **67** at elevated temperature yielded only the C-10 acetylated derivative 7-TES-13-acetyl-4-deacetylbaccatin III (**68**) and, failed to effect any C-4 acetylation. The ^{1}H NMR spectrum of **68** is compared with 7-TES-13-acetylbaccatin III **69** (prepared from an authentic sample of baccatin III) in Figure 22; The C-7 proton shifted upfield when compared to that of **69** and the different peak shape of C-13 proton indicate that the C-4 hydroxyl in **68** still free. A comparison of ^{1}H NMR data for the compounds **63, 67, 68, 69** are given in the Table 13.



67 68

Scheme 25 Acetylation of 7-TES-13-acetyl-4,10-bis(deacetyl)baccatin III (**67**)

Conclusions

The C-2 benzoyl group of baccatin III (**23**) can be removed without any skeletal rearrangement occurring, but only with concomitant deacetylation at C-4 and C-10. The reacetylation aptitudes of the hydroxyl groups were found to be in the order 13-OH > 2-OH > 10-OH. Unfortunately, the final acetylation of 4-hydroxyl was unsuccessful under the conditions studied. Further studies on this specific problem are necessary.

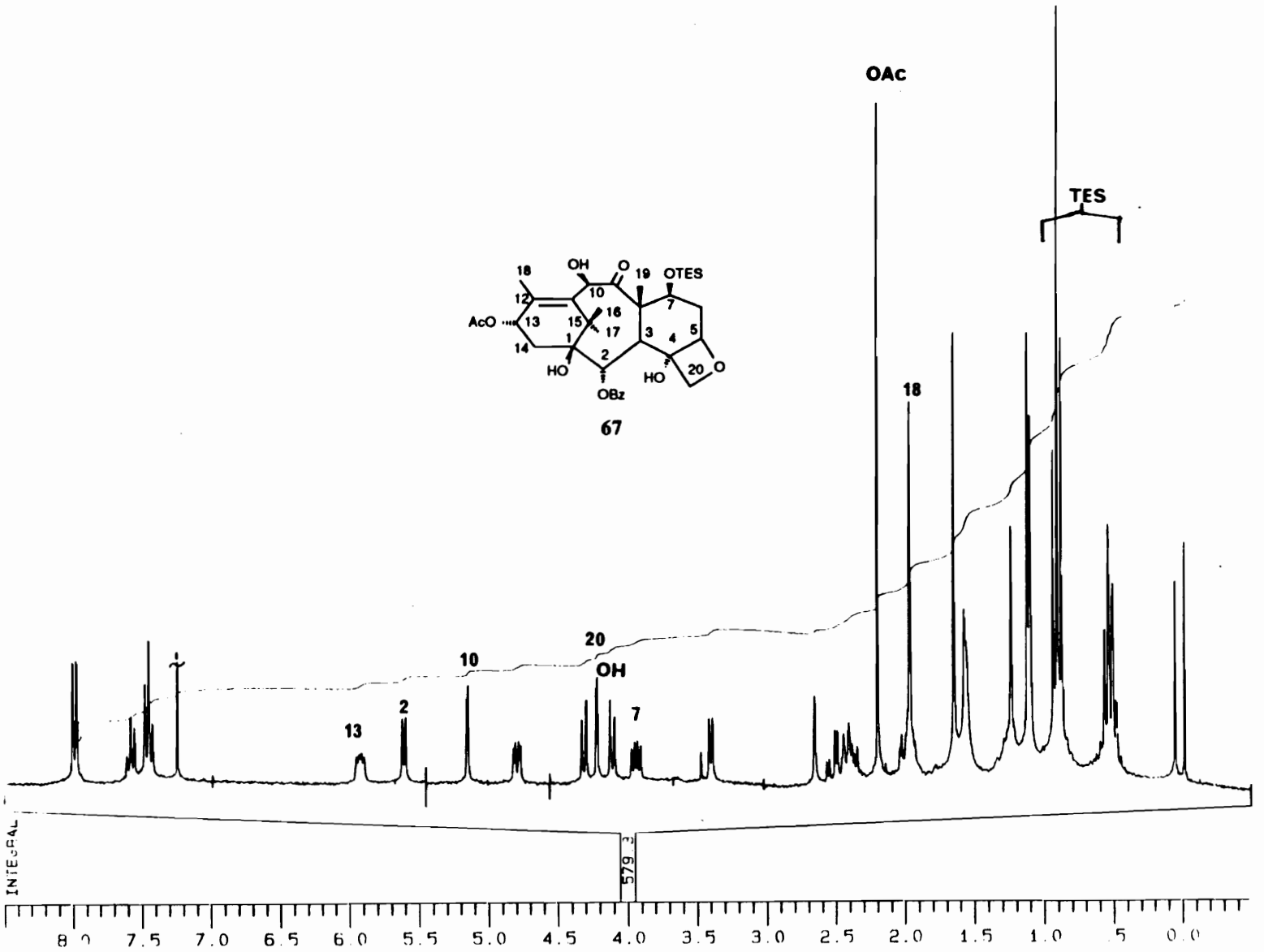


Figure 21 ^{1}H NMR spectrum of 7-TES-13-acetyl-4,10 -bis(deacetyl)baccatin III (**67**)

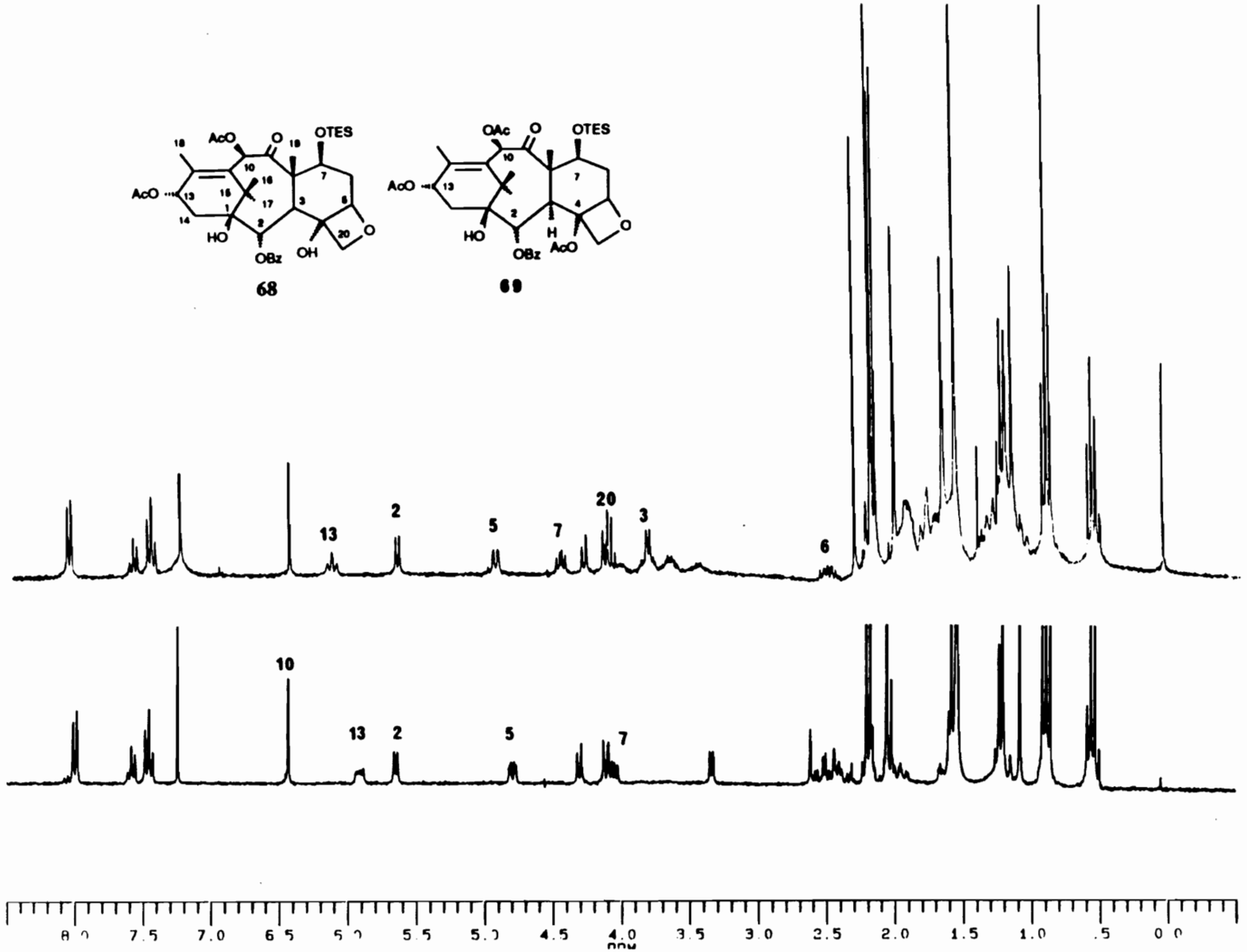


Figure 22 Comparison of ^{1}H NMR spectra of 7-TES-13-acetyl-4-deacetylbaccatin III (**68**) and 7-TES-13-acetylbaccatin III **69**

Table 13 **^{1}H NMR assignment of 13-acetyl-7-TES-2,4,10-tris(deacyl)baccatin III (63), 7-TES-13-acetyl-4,10 -bis(deacetyl)baccatin III (67), 7-TES-13-acetyl-4-deacetylbaccatin III (68) and 7-TES-13-acetylbaccatin III (69)**

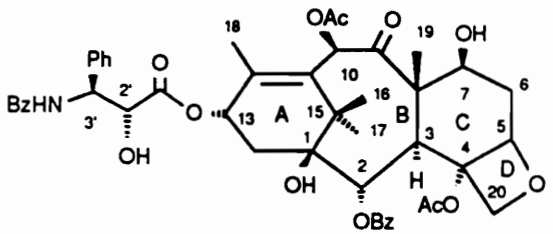
Proton on	63	67	68	69
C-2	3.89 (d,7)	5.63 (d,5)	5.89 (d,6)	5.66 (d,6)
C-3	2.97 (d,7)	3.42 (d,5)	3.34 (d,6)	3.83 (d,6)
C-5	4.85 (dd,4,10)	4.81 (dd,4.10)	4.79 (dd,4,10)	4.95 (br d,8)
C-6	2.02 (m) 2.44 (m)	2.42 (m)	1.97 (m) 2.38 (m)	1.80 (m) 2.51 (m)
C-7	3.92 (dd,7,12)	3.96 (dd,5,11)	4.07 (dd,4,10)	4.47 (dd,7,11)
C-10	5.06 (s)	5.17 (d,2)	6.43 (s)	6.46 (s)
C-13	5.84 (br t, 8)	5.93 (dd,5,8)	5.88 (m)	6.15 (br t, 9)
C-14	2.44 (m)	2.42 (m)	2.38 (m)	a
C-16,17	0.96 (s) 1.14 (s)	0.92 (s) 0.95 (s)	1.08 (s) 1.21 (s)	1.22 (s) 1.17 (s)
C-18	1.91 (s)	2.05 (d,3)	2.02 (d,1)	2.03 (d,1)
C-19	1.66 (s)	1.67 (s)	1.60 (s)	1.68 (s)
C-20	4.52 (ABq,8 $\Delta\nu_{AB}$=59)	4.23 (ABq,8 $\Delta\nu_{AB}$=53)	4.21 (ABq,8 $\Delta\nu_{AB}$=51)	4.22 (ABq,8 $\Delta\nu_{AB}$=43)
OAc	2.12 (s)	2.22 (s)	2.18 (s) 2.21 (s)	2.20 (s) 2.21(s) 2.33 (s)
2-OBz-ortho		7.98 (m)	8.00 (m)	8.09 (m)
-other		7.57 (m)	7.51 (m)	7.57 (m)
TES	0.90 (9H,t,7) 0.52 (6H,m)	0.89 (9H,t,7) 0.52 (6H,m)	0.91 (9H,t,7) 0.55 (6H,m)	0.92 (9H,t,7) 0.56 (6H,m)

a. not determined

2.2 Skeletal Modifications of Taxol

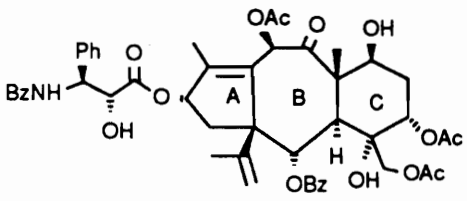
Functional group modifications of taxol and their importance were discussed in the first part of this thesis. This second part is devoted to the discussion of taxol derivatives in which the ring skeleton has undergone modifications, with a view to identifying essential ring features for the biological activity of taxol. There are no modified compounds of this type reported in literature.

One of the unusual features of the taxol structure (**5**) is the oxetane ring at the C-4, C-5 positions. A study of Dreiding models indicated to us that the taxane skeleton of taxol is very rigid and inflexible, but that opening of the oxetane ring makes it relatively flexible. It thus seemed that the oxetane ring might play a key role in the binding of taxol to the presumed receptor on the polymerized tubulin.

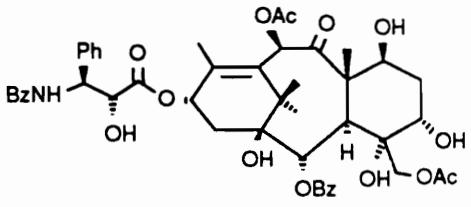


5

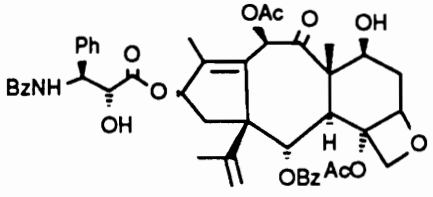
The oxetane ring is susceptible to ring opening by electrophilic reagents. We found that the oxetane ring of taxol can be opened with electrophiles such as triethyloxonium tetrafluoroborate (Meerwein's reagent) and an acetyl chloride. These studies led to two taxol derivatives a D-*seco*-taxol (**70**) and acetyl chloride product (**71**) in which the oxetane ring is opened. One of these products (**71**) showed concurrent A ring contraction. This chemistry led us to convert taxol (**5**) to A-*nor*-taxol (**72**). These derivatives thus provided information on the importance not only of an intact oxetane ring but also of an intact ring A to the biological activity of taxol.



71



70

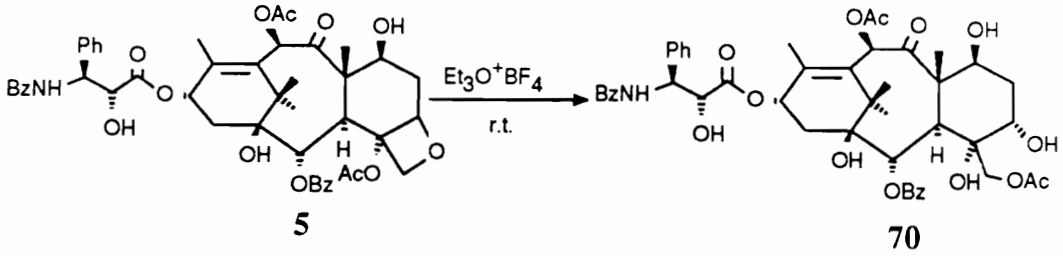


72

2.2.1 The Reaction of Taxol With Meerwein's Reagent

2.2.1.1 Formation of an Oxetane-Ring-Opened taxol

Treatment of taxol (**5**) with excess of Meerwein's reagent at 0°C and aqueous acidic work up yielded largely a single product (**70**) in 51% yield, as previously described[54] (Scheme 26). This product gave a mass spectrum which showed a molecular ion at *m/z* 871, corresponding to taxol+H_2O. Despite the fact that Meerwein's reagent is a strong ethylating agent, no ethyl groups were observed in the ^{1}H NMR spectrum of **70** (Figure 23).



5 70

Scheme 26 Formation of D-*seco*-taxol (**70**)

The major changes in the ^{1}H NMR spectrum of **70** as compared to that of taxol (**5**) occurred at the C-5 and C-20 protons and at one acetoxy methyl group. The C-5 proton changes from δ4.92 in taxol (**5**) to δ3.70 in **70**, consistent with the opening of the oxetane ring, while the chemical shift of the C-20 protons changed from δ4.17 in the taxol (**5**) spectrum to δ3.85 (Table 14), consistent with ring-opening and acetylation.[79] A very sharp peak which appeared at δ1.65 was

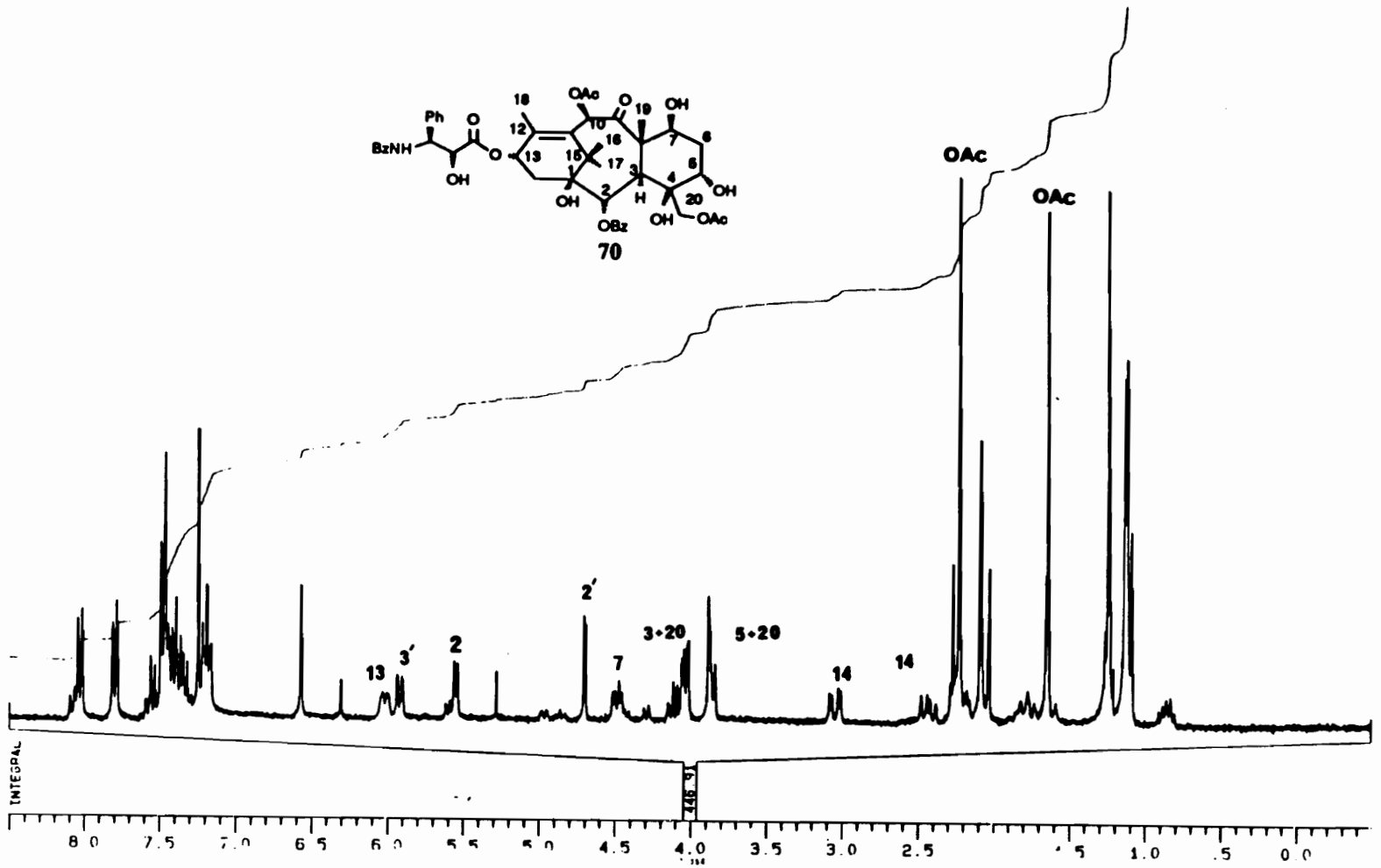


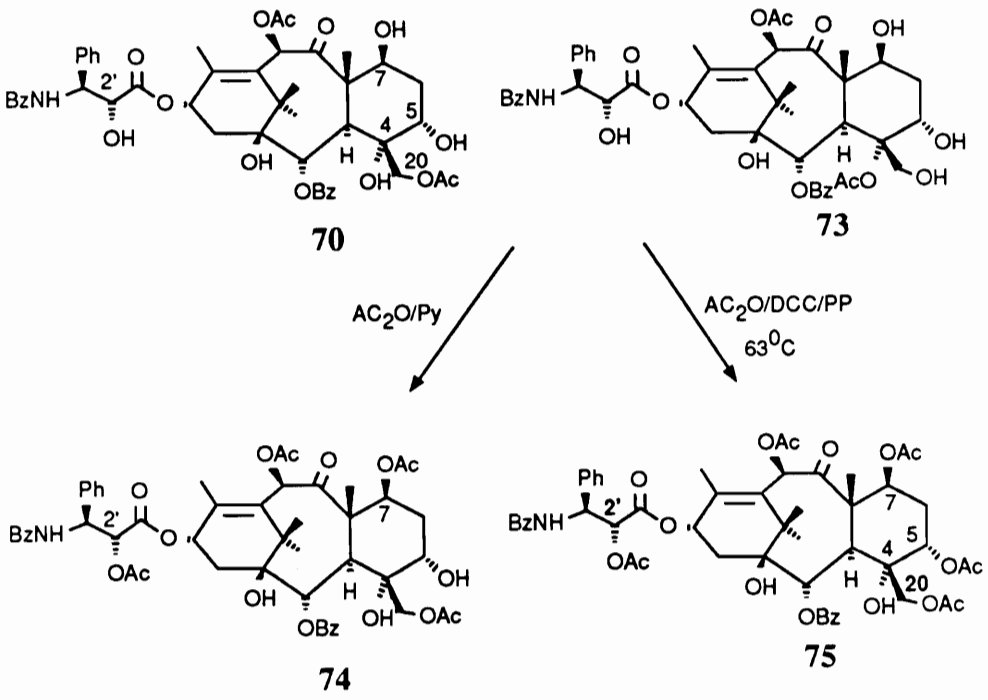
Figure 23 ^{1}H NMR spectrum of the D-*seco*-taxol (**70**)

assigned to an acetoxy methyl, although it occurs at an unusual position for such a group. However, it was assign to the C-20 acetate group, suggesting that it may be situated in the shielding region of the C-2 phenyl group. The C-14 geminal protons, which appear as a multiplet at δ2.31 in the spectrum of taxol (**5**), were well resolved into two doublets in the spectrum of **70**, appearing at δ2.45 and δ3.05.

Two structures **70** and **73** (Scheme 26) are possible on the basis of this evidence, and chemical shift arguments alone cannot be used to distinguish one from the other. As noted in the previous chapter some chemical shift changes in the taxane skeleton are not at all predictable. The protons of this molecule are subjected to strong anisotropic effects even from very remote constituents, and the question is complicated further by the fact that opening of the oxetane ring relieves the strain of the molecule causing possible hybridation changes. These facts contribute to the observed chemical shift changes. In fact the alternative structure (**73**) was considered at one time as the structure of the product.[80]

Mild acetylation (Scheme 27) of the D-*seco*-taxol did not produce any C-20 acetate, instead, only the 2,7-diacetate (**74**) was obtained. The expected acetylation shifts of the C-2, and C-7 methine protons were observed in its ^{1}H NMR spectrum (Table 14). This experiment tentatively ruled out the structure **73**, since the primary hydroxyl group of this compound would be expected to undergo ready acetylation.

The secondary hydroxyl group at C-5 of the D-seco-taxol (**70**) proved surprisingly difficult to acetylate. The reaction was finally achieved by the use of



Scheme 27 Acetylation of D-*seco*-taxol (**70**)

Ac_2O/DCC/PP at 63^0, and the 2',5,7-triacetate (**75**) was obtained. Desorption chemical ionization mass spectrometry gave the expected MH^+ peak at 998amu, thus supporting the composition of the triacetate.

This acetylation greatly simplified the ^1H NMR spectrum (Figure 24). The C-5 proton, which was buried in the C-20 AB quartet in the spectrum of the D-*seco*-taxol, shifted to δ5.26, clarifying the AB quartet and the C-3 proton doublet, and these data established the structure as **75**.

Table 14. **^{1}H NMR assignment of taxol (5), D-*seco*-taxol (70), triacetate (75) of D-*seco*-taxol and acetonide (76) of D-*seco*-taxol**

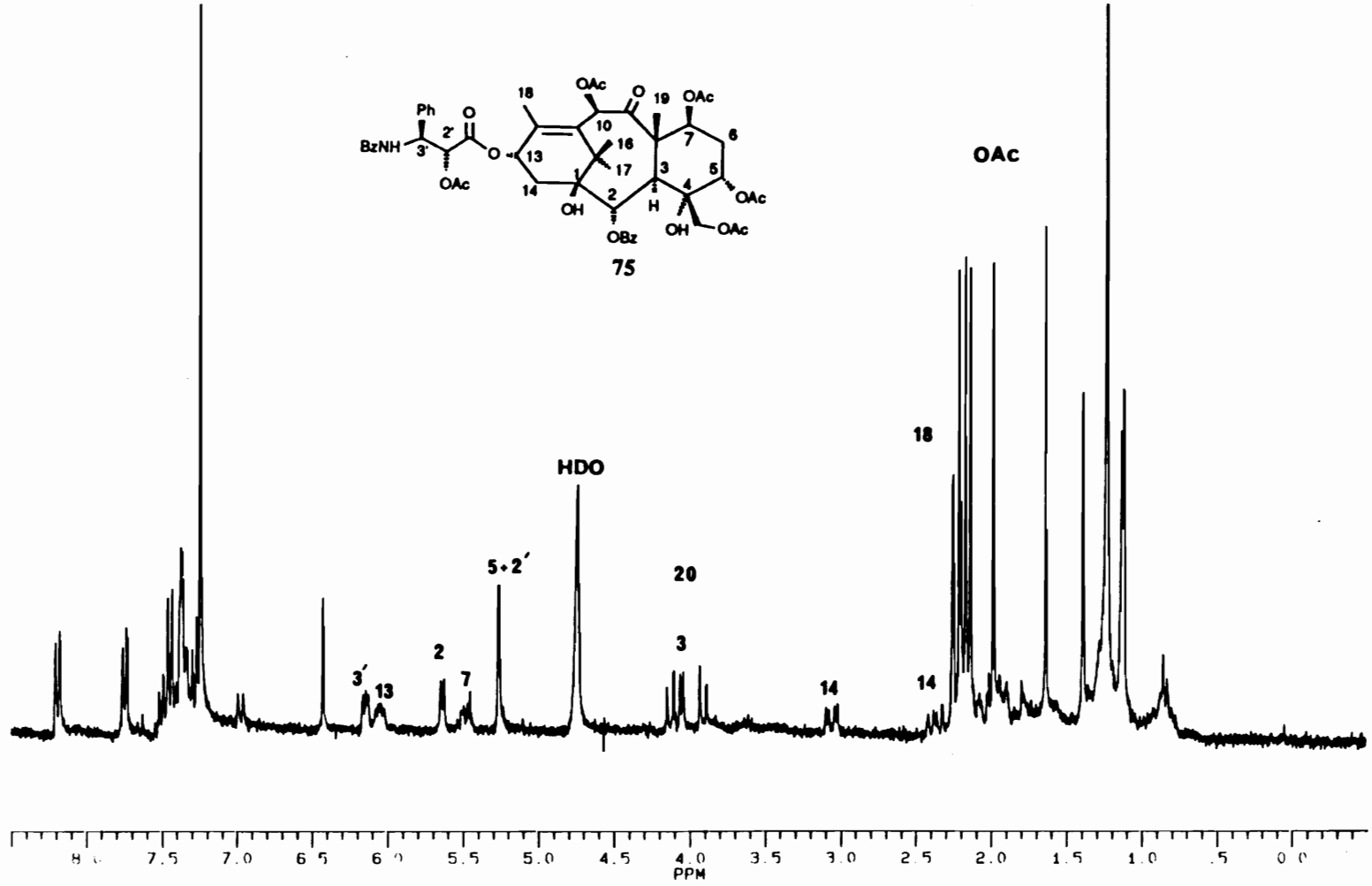
Protons on Carbon	**5**	**70**	**75**	**76**
C_2	5.62 (d,7)	5.56 (d,6)	5.63 (d,5)	5.34 (d, 8)
C_3	3.80 (d,7)	4.03 (d,6)	4.04 (d,5)	3.06 (d, 8)
C_5	4.92 (dd,2,8)	3.70 (br s)	5.26[c] (m)	4.34 (m)
C_6	a	a	a	6_{ax} 1.95 (ddd, 5, 11,15)[b] 6_{eq} 2.36 (brdd, 2,5,15)[b]
C_7	4.33 (m)	4.49 (dd,4,11)	5.43 (br d, 9)	4.48(dd,5,11)
C_{10}	6.26 (s)	6.57 (s)	6.42(s)	6.38(d, 2)
C_{13}	6.15 (t, 8)	6.01 (br dd, 4,11)	6.03 (m)	5.68 (m)
C_{14}	2.50 (m)	2.45 (dd,11,16) 3.08 (dd,4,16)	2.40 (dd,9,15) 3.07 (dd,5,15)	2.57 (AB part of ABX, 9,14, $\Delta\nu$=62)
C_{16}	1.25 (s)	1.12[c] (s)	1.13 (s)	4.67 (s), 4.75 (s)
C_{17}	1.14 (s)	1.12[c] (s)	1.11 (s)	1.63 (s)
C_{18}	1.78 (s)	2.10 (s)[d]	2.24 (s)	1.67[b] (s)
C_{19}	1.67 (s)	1.22 (s)	1.38 (s)	1.62 (s)
C_{20}	4.17 (ABq, 8 $\Delta\nu_{AB}$= 31)	3.85 (ABq, 11 $\Delta\nu_{AB}$=86)	4.01 (ABq, 12, $\Delta\nu_{AB}$=57)	4.15 (ABq, 12, $\Delta\nu_{AB}$=26)
$C_{2'}$	4.71 (d,8)	4.70 (br s)	5.26[c] (m)	4.50 (d,3)
$C_{3'}$	5.72 (dd,3,9)	5.92 (dd,2,9)	6.12 (dd,3,10)	5.60 (dd,3,8)
NH	7.00 (d,9)	7.19 (d,9)	7.20 (br d,9)	6.98 (d,8)

cont'd

Table 14 cont'd

Protons on Carbon	**5**	**70**	**75**	**76**
OAC	2.23 (s) 2.38 (s)	1.65 (s) 2.25 (s)	1.98 (s), 2.13 (s) 2.16 (s), 2.19 (s), 2.20 (s)	1.83 (s), 2.17 (s)
2-OBz (ortho)	8.11 (m)	8.03 (m)	8.20(m)	8.10 (m)
3'-NBz (ortho)	7.70 (m)	7.80 (m)	7.80 (m)	7.73 (m)
3'-ph	7.4-7.6 (m)	7.3-7.6 (m)	7.2-7.6 (m)	7.3-7.4 (m)
all other aromatics	7.3-7.6 (m)	7.3-7.6 (m)	7.2-7.6 (m)	7.34-7.45 (m)
OH	3.91 (s)			
OTHER				1.30 (s)[e] 1.33 (s)

a. hidden under methyl envelope not determined
b. determined by decoupling experiment
c. overlapping peaks
d. determined by COSY experiment
e. acetonide methyls



75

Figure 24 ^{1}H NMR spectrum of 2',5,7-triacetate (75)

The difficulty of acetylation of the C-5 hydroxyl group is probably due to the presence of strong intramolecular hydrogen bonding with the C-20 acetoxy group and possibly with the C-4 hydroxyl group (Figure 25). In support of this conclusion the ^{1}H NMR spectrum of the D-seco-taxol (**70**) shows a large chemical shift difference between the C-20 protons($\Delta\nu_{AB}$=86Hz), suggestive of a restricted conformation. The acetonide (**76**), which lacks a hydroxyl group to form a hydrogen bond with the C-20 acetoxy group, shows very similar chemical shifts ($\Delta\nu_{AB}$=26Hz) for these protons. All the protons of this molecule were assigned by a COSY experiment as shown in Figure 26.

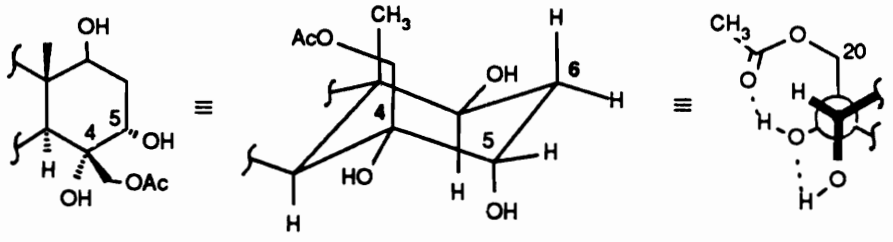


Figure 25 Hydrogen bonding in D-*seco*-taxol (**70**)

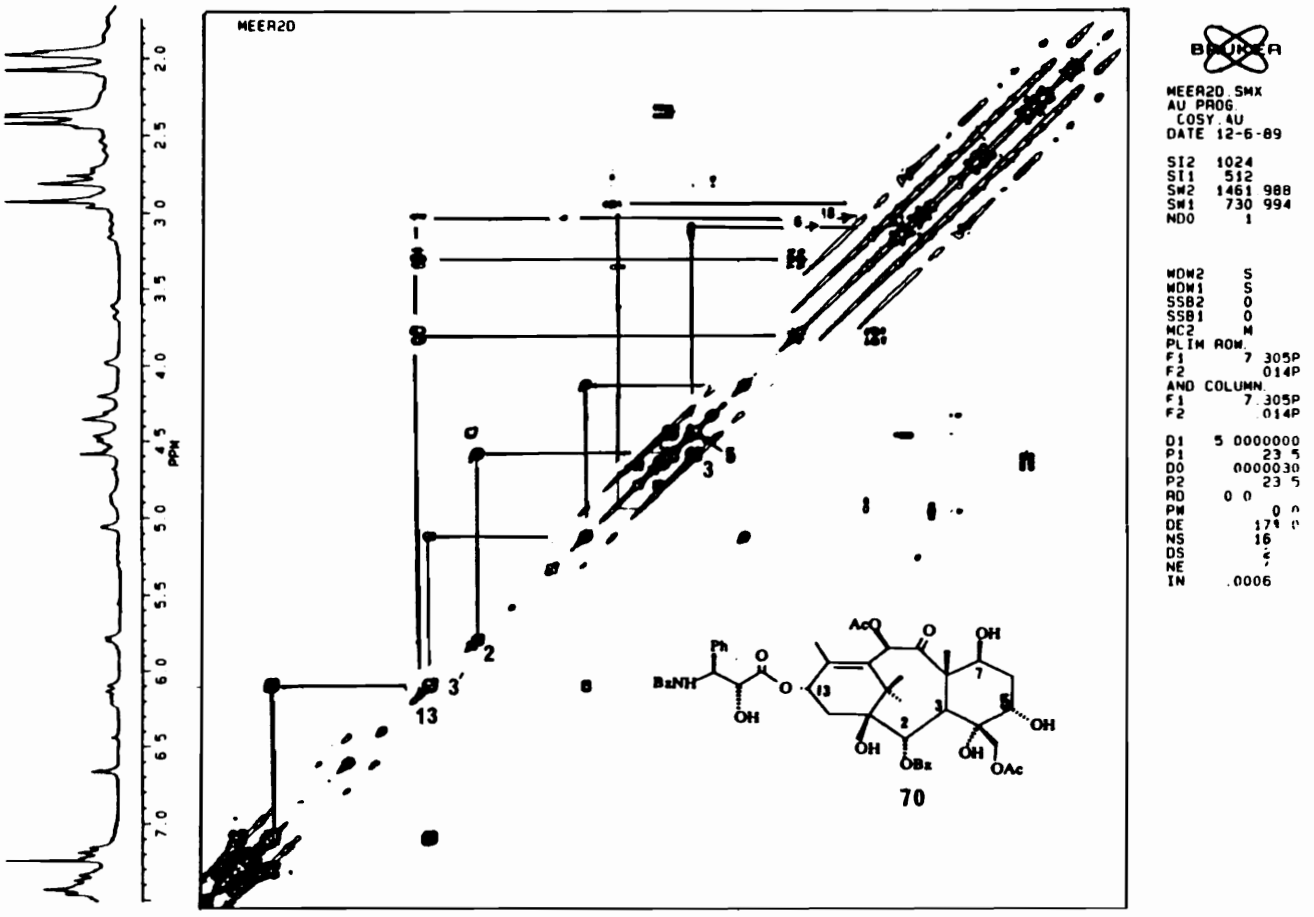
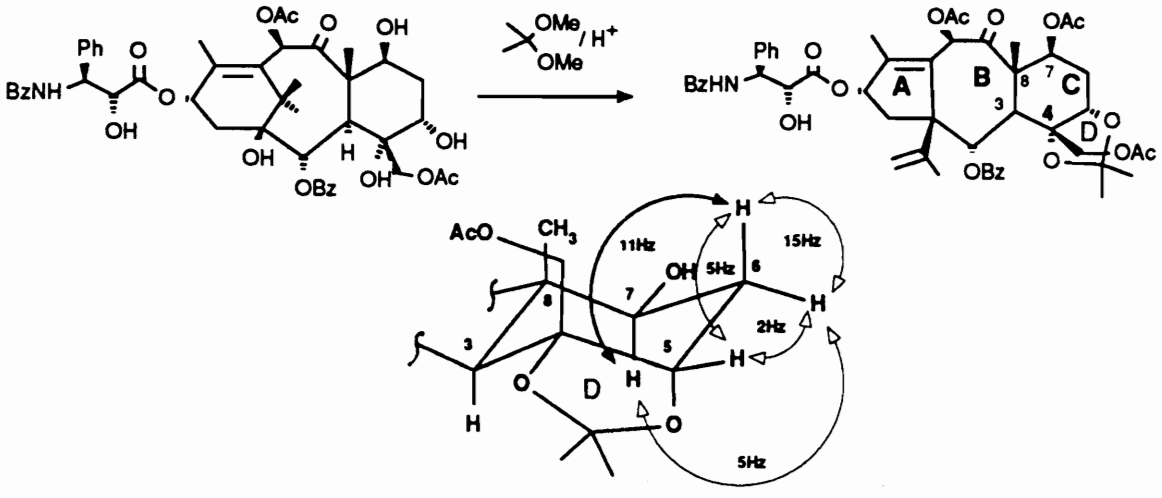


Figure 26 COSY spectrum of D-*seco*-taxol (**70**)

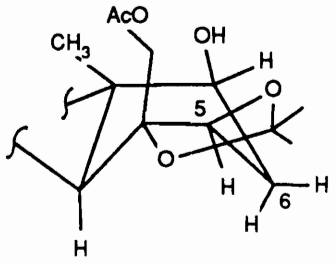
2.2.1.2 The Stereochemistry of Ring Opening

The stereochemistry of the C-5 hydroxyl group of **70** was established as α by conversion of **70** to the acetonide **76** (Scheme 28). This conversion also caused a rearrangement of ring A to occur; this rearrangement will be discussed in detail below. The coupling constants of all the protons in ring C were determined by specific proton decoupling and are shown in Scheme 28.



Scheme 28 Preparation of acetonide (**76**) and the coupling constants of its ring-C protons

The small coupling constant of 5Hz and 2Hz between the C-5 proton and the two C-6 protons established that the C-5 proton is equatorial and that the ring-C has the chair conformation. An alternate structure (**77**) in which the ring-C is in the boat conformation would require a large anticoplaner coupling between C-5 and one of the C-6 protons.



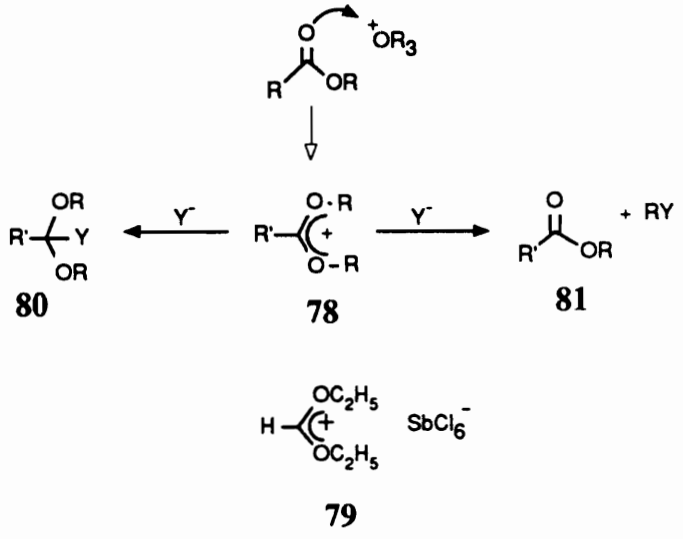
77

Molecular mechanics calculations on the taxane skeleton (without the oxetane ring) indicated[81] that the minimum energy conformation contains a C-ring chair conformation. Therefore, it is unlikely that it reacts in the boat conformation. The observed coupling constants also established that the C-7 hydroxy group retains its β-configuration.

One additional point concerning the D-*seco*-taxol **70** deserves mention. As noted earlier, a Dreiding model of taxol shows that the ring system is essentially rigid, locked into one conformation by the geometry of the tetracyclic system. The opening of the oxetane ring changes the conformation of the ring system, as evident by significant differences in coupling constants of the A ring protons from the corresponding protons of taxol (**5**). The C-13 proton in **70**, for example, appears as a broad doublet of doublets (J=11,4 Hz); in taxol (**5**) this proton appears as a triplet with J=8Hz (Table 14).

2.2.1.3 A Mechanism for the Formation of the D-*seco*-taxol

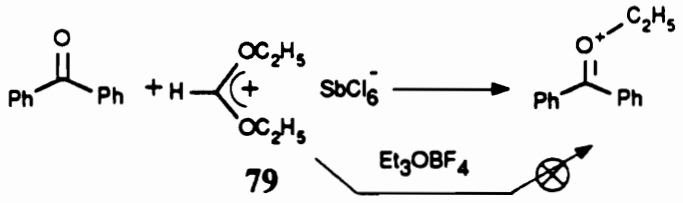
Meerwein's reagent is capable of making dialkoxycarbonium ions (**78**) from esters (Scheme 29). These are very stable cations which can be isolated in salt forms such as **79**.



Scheme 29 Generation of dialkyloxonium cations

Kabuss[82] studied these cations in alkylation reactions. In a kinetically controlled reaction, an addition product **80** is obtained, while under thermodynamically controlled conditions, the alkylation product of the nucleophile (**81**) is observed.[82] The reactions are very fast and often quantitative.

According to Kabuss, dialkoxycarbonium ions are stronger alkylating agents than trialkyloxonium salts such as Meerwein's reagent. As an example, benzophenone, a very weak nucleophile, could be alkylated with dialkoxycarbocation **79** in 91% yield to obtain the alkyl derivative **82** as a crystalline solid (Scheme 30) Trialkyloxonium reagents are incapable of carrying out this alkylation.



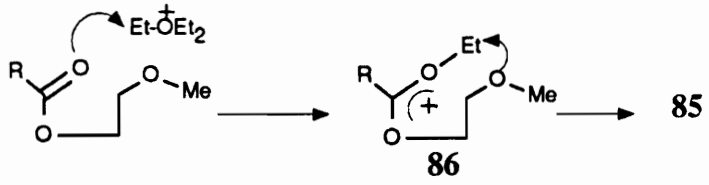
Scheme 30 Alkylation with dialkoxycarbocations

The formation of 1,3-dioxolanes (**83**) from methoxymethylcarboxylates (**84**) has been described by Raber et. al.[83] The proposed mechanism of this reaction is given in the Scheme 31.

Scheme 31 Formation of 1,3-dioxolanes with Meerwein reagent

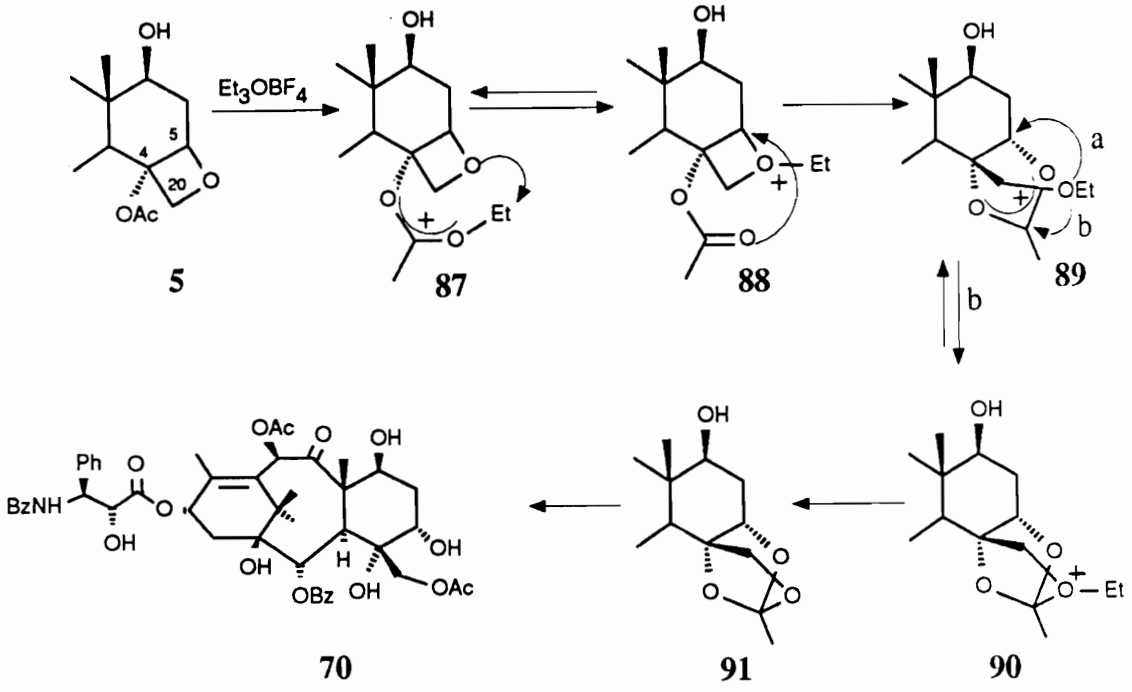
However, according to the above discussion, it is more plausible that the dialkoxycarbonium ion **86** (Scheme 32) is formed first rather than the trialkyloxonium ion **85**. This dialkoxycarbonium ion, being a better alkylating agent, alkylates the

methoxy group giving the trialkyloxonium ion **85**. The rest of the mechanism should be as shown in Scheme 32.



Scheme 32 Mechanism of the formation of dioxolanes

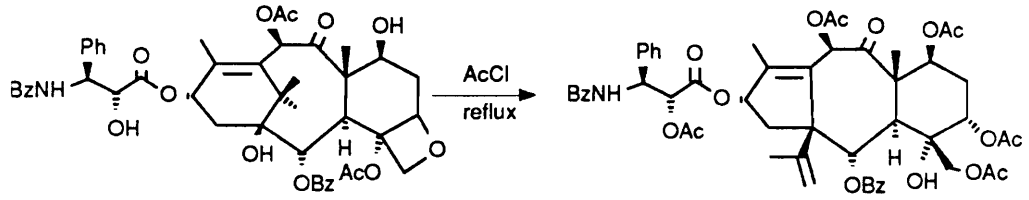
This discussion is helpful in rationalizing the speculative mechanism (Scheme 33) for the formation of the D-*seco*-taxol (**70**). The C-4 acetate is alkylated with Meerwein's reagent to yield the dialkoxycarbonium ion **87**; alkylation of the ether oxygen of the oxetane ring by this cation (**87**) would lead to the trialkyloxonium intermediate **88**. This intermediate would undergo ring opening assisted by the C-4 acetoxy group to yield the dialkoxycarbonium ion intermediate **89** which can undergo the reactions given in Scheme 29, namely alkylation or addition. Alkylation will yield the starting cation **88** (path a) while addition would yield the unstrained trialkyloxonium salt **90** (path b). The driving force of the reaction would be the opening of the oxetane ring favoring the reaction path a. The intermediate **90** would then form the orthoester **91**, upon addition of water. The orthoester would be hydrolysed further to the stable final product **70** under acidic work-up conditions.



Scheme 33 A speculative mechanism for the formation of D-*seco*-taxol (**70**)

2.2.2 The Reaction of Taxol With Acetyl Chloride; The Oxetane Ring Opened and the Ring-A Contracted Taxol Through A Novel Rearrangement of the Taxane Skeleton

The second elecrophilic reagent studied was acetyl chloride. This has been shown to give an oxetane ring opened product in other cases[84] but reaction of taxol (**5**) (Scheme 120a) with refluxing acetyl chloride yielded a product (**71**) which had undergone more extensive changes than simple oxetane ring opening.



5 **71**

Scheme 34 Formation of acetyl chloride product (**71**)

The ^{1}H NMR spectrum of the product **71** (Figure 27) showed the presence of two new vinylic protons at δ4.69 and δ4.82, and the ^{13}C NMR INEPT spectrum (Figure 28) showed the presence of two additional vinyl carbons at δ112.6 and δ144.27; the signal at δ112.6 was a CH_2 fragment indicating the presence of a terminal methylene group. As expected the C-2' and C-7 hydroxyl groups were acetylated, and signals for two additional acetates groups were observed. The FAb mass spectrum of **71** showed that it had a molecular weight of 979, corresponding to taxol (**5**) plus three acetates.

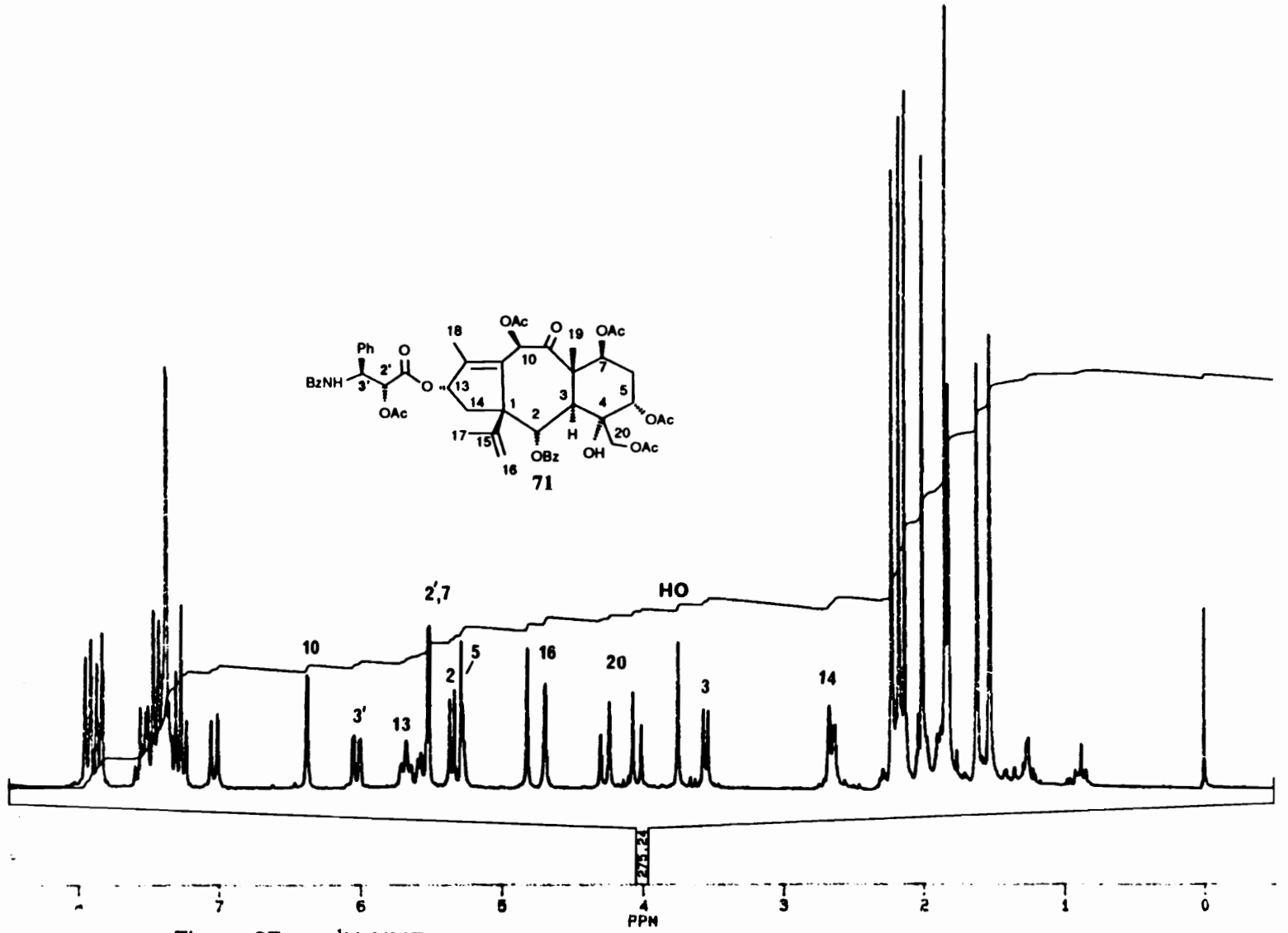


Figure 27 ^{1}H NMR spectrum of acetyl chloride product (71)

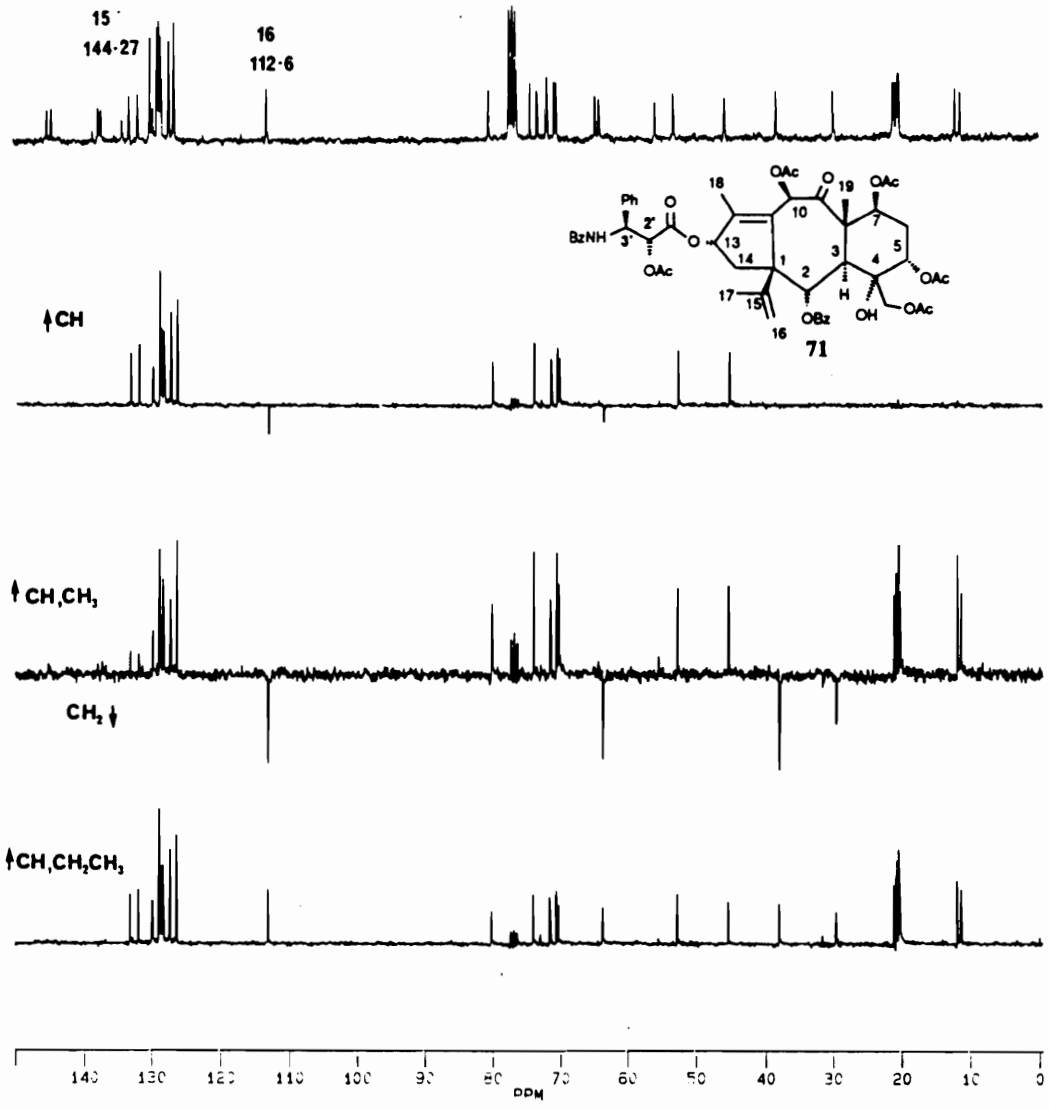


Figure 28 INEPT spectra of acetyl chloride product (**71**)

Important additional evidence for the structure of **71** came from our study of the D-*seco*-taxol (**70**). Acetylation of **70** in the presence of acetyl chloride and excess of triethyl amine yielded a single product identical with the acetyl chloride product **71**. Since the D-*seco*-taxol is formed by an overall hydration of taxol (**5**), this implies that the conversion of **70** to **71** must involve overall dehydration to yield a methylene group. Figure 29 summarizes the available data that led to this conclusion.

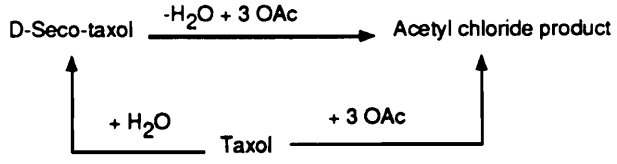
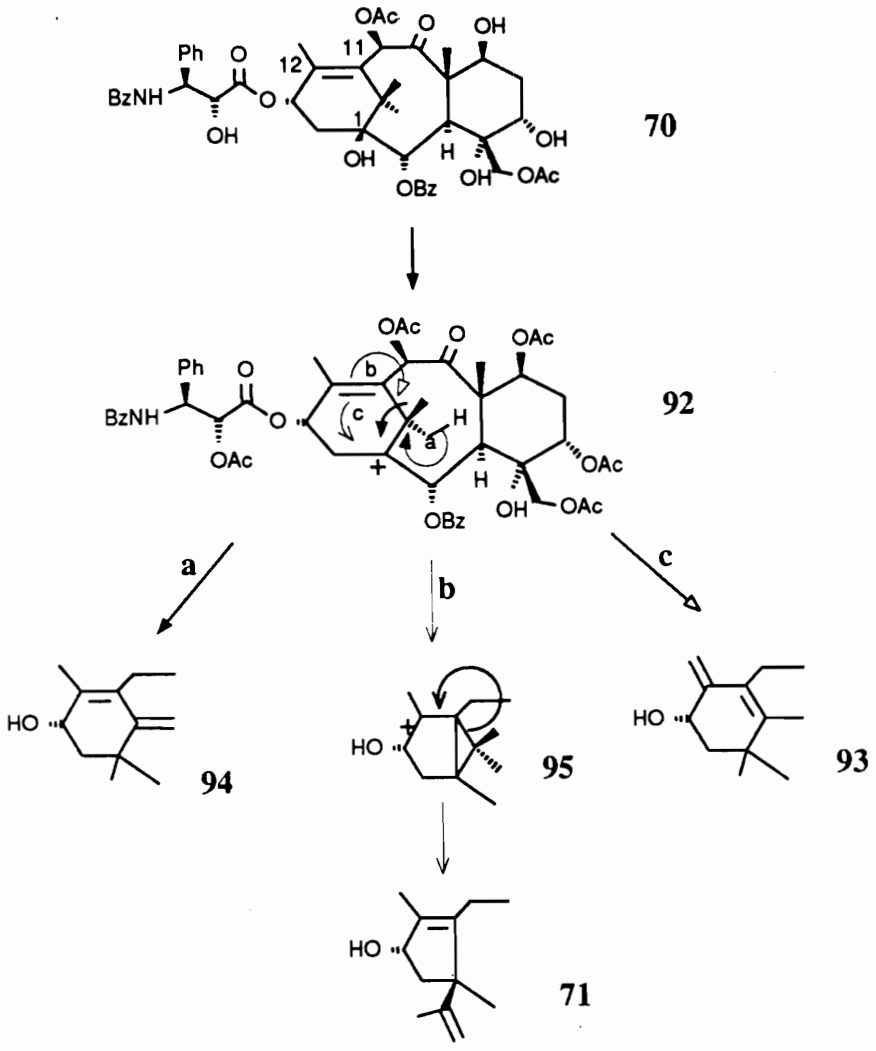


Figure 29 Summary of data on acetyl chloride product (**71**)

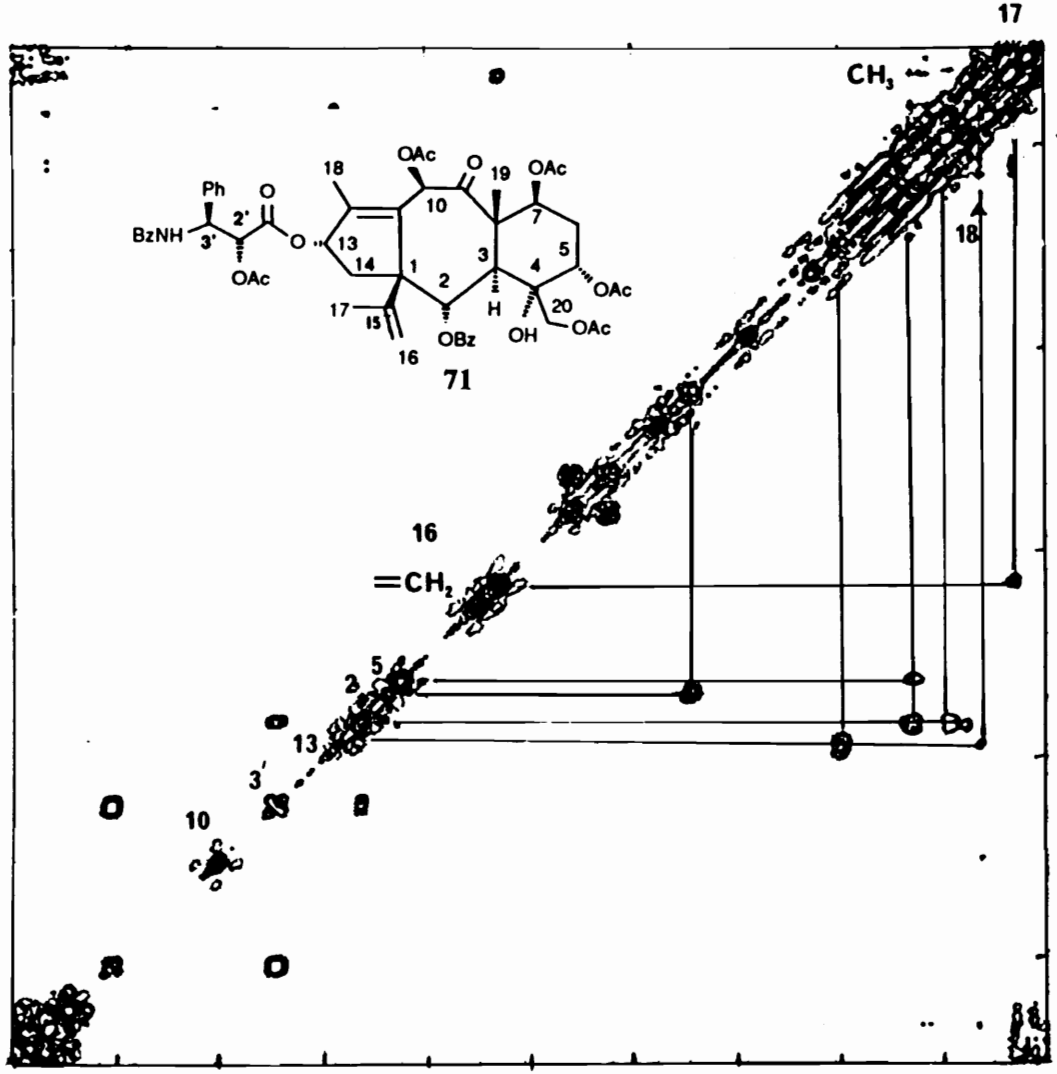
Three possible products of such dehydration of the D-*seco*-taxol (**70**) are shown in Scheme 35. It would yield the intermediate **75** (See Scheme 27) on acetylation of the C-2',C-7, and C-5 hydroxyl groups. The loss of the C-1 hydroxyl group (probably after acetylation) would yield the cation **92** which could then rearrange via a 1,2 methyl migration followed by loss of a proton to give either of the exocyclic methylene derivatives **93** and **94**. Alternatively, participation of the 11(12)-double bond followed by opening of the resulting cyclopropylcarbinyl cation **95** would lead to the product **71**.

A distinction between the possible rearranged products **93**, **94** and **71** was made on the basis of a COSY spectrum (Figure 30). The spectrum showed a clear

long-range coupling between the C-13 proton and a vinyl methyl group, which must therefore be the C-18 methyl group; this evidence exclude the structure **93**. Additionally, the vinyl protons at δ4.69 and 4.82 showed a long range coupling to a vinyl methyl group which is not C-18, and must thus be C-16; this evidence is only consistent with the structure **71**. All the protons and corresponding carbons were assigned by a Hetero-COSY (Figure 31) experiment.



Scheme 35 A speculative mechanism for the conversion of D-*seco*-taxol (**70**) to the acetyl chloride product (**71**)



BRUKER

GAM.SMX
AU PROG:
COSY.AU
DATE 4-12-89

SI2 512
SI1 256
SW2 1237.624
SW1 618.812
ND0 1

WDW2 S
WDW1 G
LB1 0.0
GB1 0.0
SSB2 0
MC2 M
PLIM ROW:
F1 -.692P
F2 -6.852P
AND COLUMN:
F1 -.692P
F2 -6.852P

D1 5.0000000
P1 23.5
D0 .0000030
P2 23.5
AD 0.0
PW 0.0
DE 202.0
NS 16
DS 2
NE 128
IN .0008080

Figure 30 COSY spectrum of acetyl chloride product (**71**)

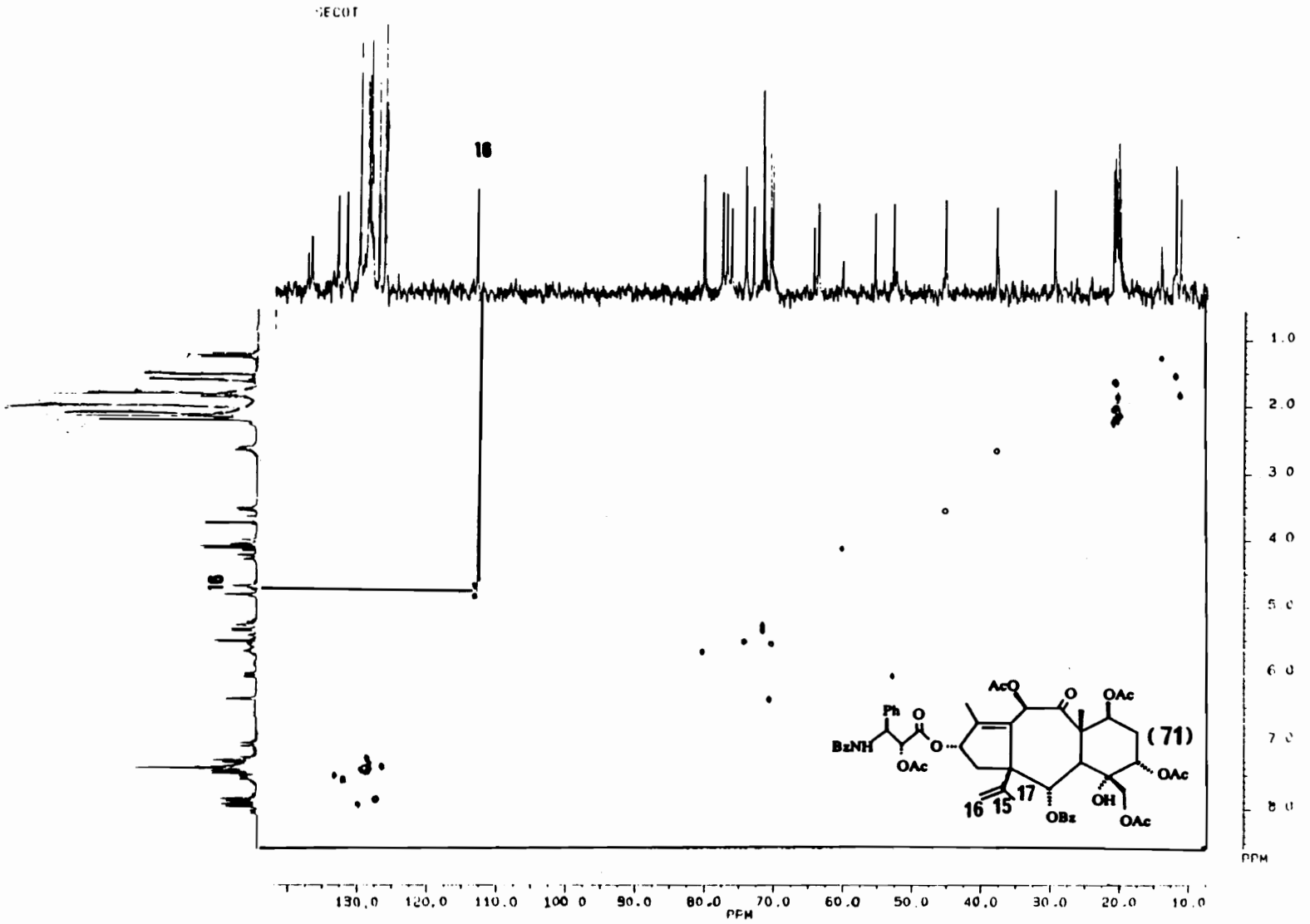
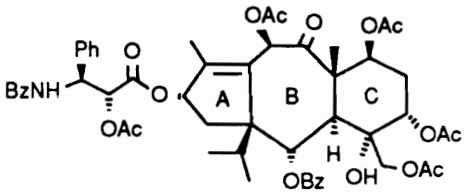


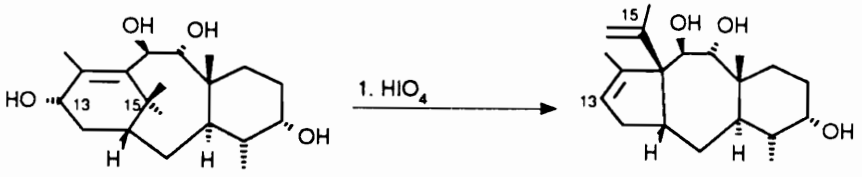
Figure 31 Het-COSY spectrum of acetyl chloride product (71)

Final support for the structure **71** was obtained by hydrogenation (Pt/H_2), which converted it to the dihydro product **96**. The ^1H NMR spectrum of **96** (Figure 32) showed two new methyl doublets at δ0.76 and 0.78 and a methine proton multiplet at δ1.62; these assignments were confirmed by selective proton decoupling experiments. Comparison of ^1H NMR data of **96** with those of Acetyl chloride product **71**)is given in Table 15.



96

A rearrangement, similar to the one we discussed here, was reported by Chen and coworkers; as shown in Scheme 36, the tetrol **97** was rearranged to **98** on treatment with acid. The labile allylic hydroxyl group is well suited for acid catalyzed elimination assisted by the 11(12) double bond. The rearrangement that we found in the formation of the acetyl chloride product (**71**) is unprecedented.



97 **29**

Scheme 36 Ring A rearrangement in taxinine

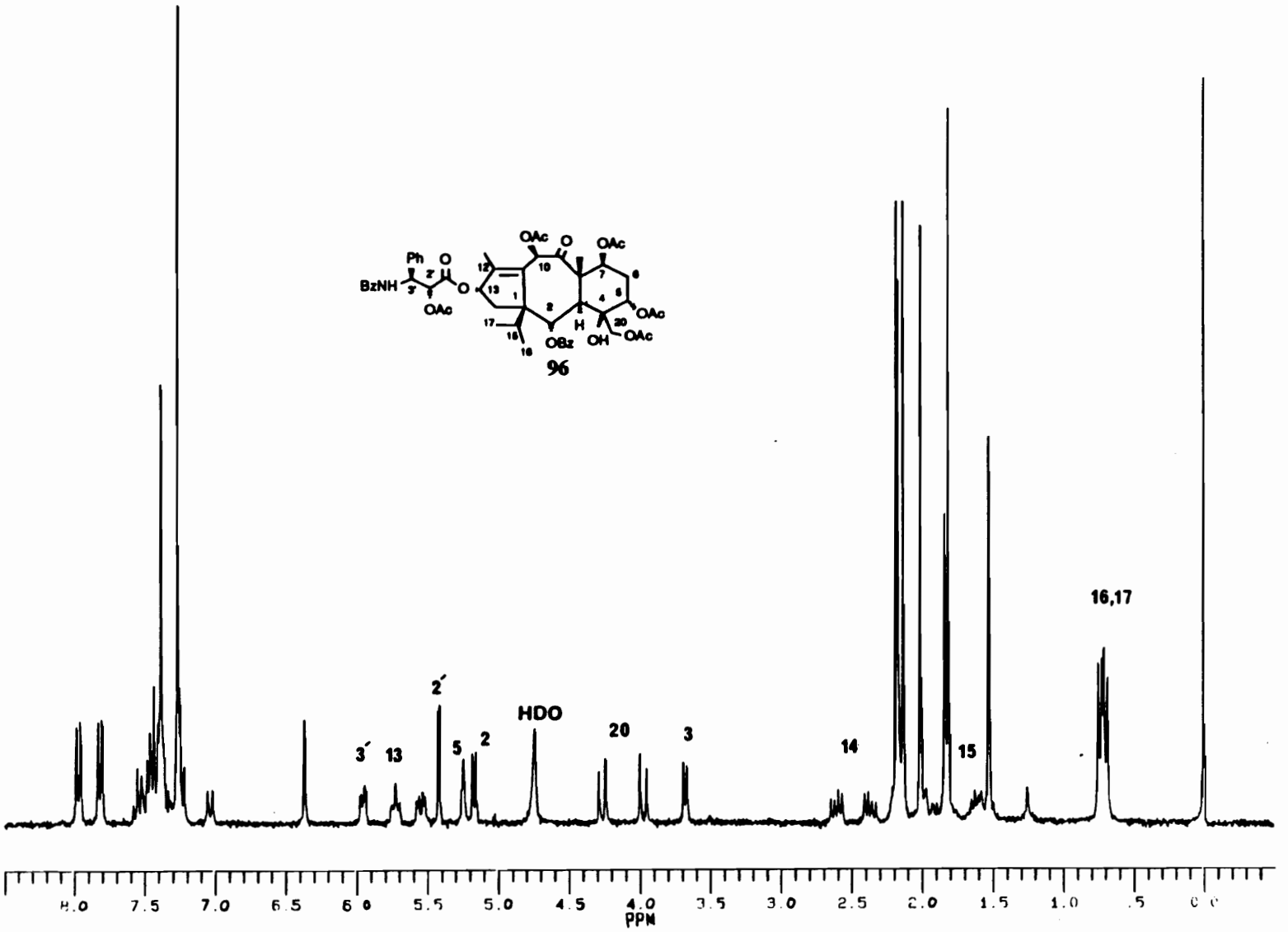


Figure 32 ^{1}H NMR spectrum of 96

Table 15. **^{1}H NMR assignment of acetyl chloride product (71) and dihydro acetyl chloride product (96)**

Protons on Carbon	**71**	**96**
C_2	5.35 (d, 7)	5.17 (d, 8)
C_3	3.54 (d, 7)	3.68 (d, 8)
C_5	5.28 (br s)	4.94 (br d, 11)
C_6	a	a
C_7	5.54 (dd, 4,13)	5.54 (dd, 5,11)
C_{10}	6.38 (s)	6.36 (s)
C_{13}	5.67 (t, 7)	5.72 (m)
C_{14}	2.64 (m)	2.48 (AB part of ABX 14,16, $\Delta\nu_{AB}$= 65)
C_{15}		1.60 (m)
C_{16}	4.69 (s), 4.82 (s)	0.76 (d, 7)
C_{17}	1.62 (s)	0.78 (d, 7)
C_{18}	1.82 (s)	1.83 (s)
C_{19}	1.53 (s)	1.53 (s)
C_{20}	4.15 (ABq, 12 ν_{AB}= 55)	4.11 (ABq, 11 $\Delta\nu_{AB}$=78
$C_{2'}$	5.52 (d, 2)	5.40 (d, 3)
$C_{3'}$	6.02 (dd, 2,9)	5.95 (dd, 3,8)
NH	7.03 (d, 9)	7.02 (d, 8)

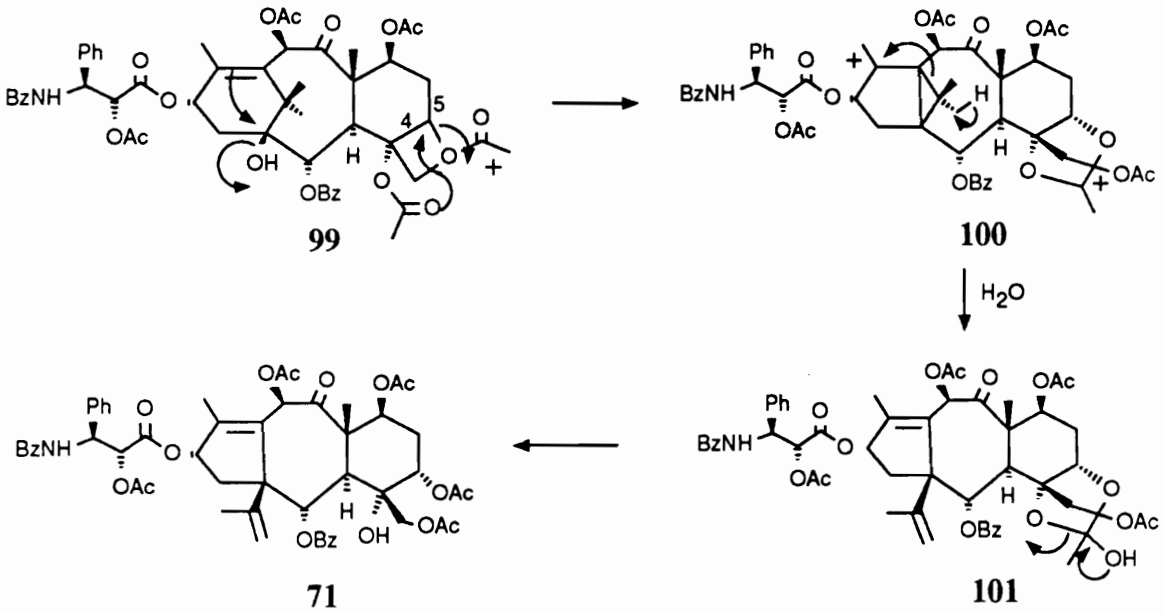
cont'd

Table 15 cont'd

Protons on Carbon	**71**	**96**
OAC	1.85 (s) 2.00 (s) 2.13 (s) 2.17 (s) 2.22 (s)	1.17 (s) 1.18 (s) 2.00 (s) 2.13 (s) 2.17 (s)
2-OBz (Ortho)	7.93 (m),	7.91 (m)
3'-NBz (Ortho)	7.85 (m)	7.83 (m)
3'-ph	7.2-7.9 (m)	7.2-7.6 (m)
OH	3.75 (s)	
other aromatic H	7.2-7.9 (m)	7.2-7.6 (m)

[a] hidden under methyl envelop not determined

The mechanism of the overall transformation of taxol (**5**) to the acetyl chloride product **71** presumably involves separate reactions of acetyl chloride with the oxetane ring and with ring A. One possible sequence is shown in Scheme 37. The oxetane ring opening is presumed to occur through the acyl cation **99** and the tetrahedral intermediate **100**. Concurrent ionization of the C-1 hydroxyl group

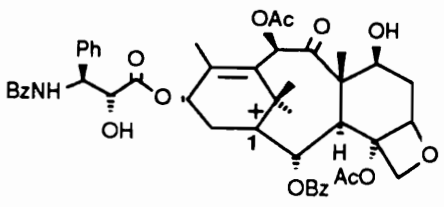


Scheme 37 A speculative mechanism for the formation of the acetyl chloride product (**71**)

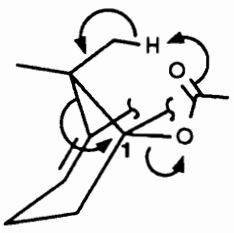
assisted by the 11(12)-double bond would lead to the cyclopropyl carbinyl cation at ring A. As discussed before, the cyclopropyl carbinyl cation would undergo ring

opening to yield the isopropenyl moiety of the product. The opening of the oxetane ring would follow a mechanism parallel to one discussed for the formation of the D-*seco*-taxol (**70**)

Elimination of the C-1 hydroxyl group deserves some comments due to its unusual nature. The hydroxyl group can leave as a water molecule after protonation by hydrochloric acid present in the medium. There are two mechanisms available for the elimination. If it went through an E_1 type mechanism, a carbonium ion **102** should have formed at C-1. This is impossible, because it is like introducing a double bond (i.e. sp^2 hybridization) at the second available bridge head of the bicyclic system. If reaction proceeds through a concerted displacement by the double bond, the incipient cation is formed at C-11, which is already sp^2 hybridized. Therefore, the rearrangement should occur through the cyclopropyl carbinyl intermediate **100** (Scheme 37). Alterrnatively the rearrangement could occur through the C-1 acetete as indicated in the partial structure **103.**



102



103

The acetonide derivative **76** prepared as described earlier from the D-*seco*-taxol (**70**) was found to have undergone the same ring A rearrangement as the acetyl chloride product **71**, as evidenced by its ^1H NMR spectrum (Figure 33), particularly by the appearance of vinyl proton signals at δ4.67 and 4.75. It is

noteworthy that the D-*seco*-taxol underwent rearrangement to **76** under relatively mild conditions (p-toluenesulfonic acid, 1 h room temp.). Identical conditions had no effect on taxol, and taxol also survived oxidation with Jones's reagent[43] without any rearrangement occurring. This evidence tend to support a carbocation mechanism, since presumably the oxetane ring is holding taxol in a conformation that does not permit the formation of a cyclopropyl carbinyl cation **100**, while the more flexible derivative **70** can form the same intermediate relatively easily. Based on these observations we can argue that in the formation of the acetyl chloride product **71**, the opening of the oxetane ring must occur prior to the ring-A rearrangement.

The fact that the tertiary C-4 hydroxyl groups of the D-*seco*-taxol **70** and the acetyl chloride product **71** are stable to the relatively harsh conditions of the rearrangement is attributed to their equatorial orientation (Figure 34).

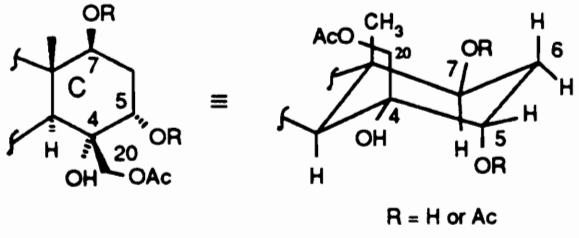


Figure 34 Stereochemistry of the C-4 hydroxyl group of D-*seco*-taxol (**70**) and the acetyl chloride product (**71**)

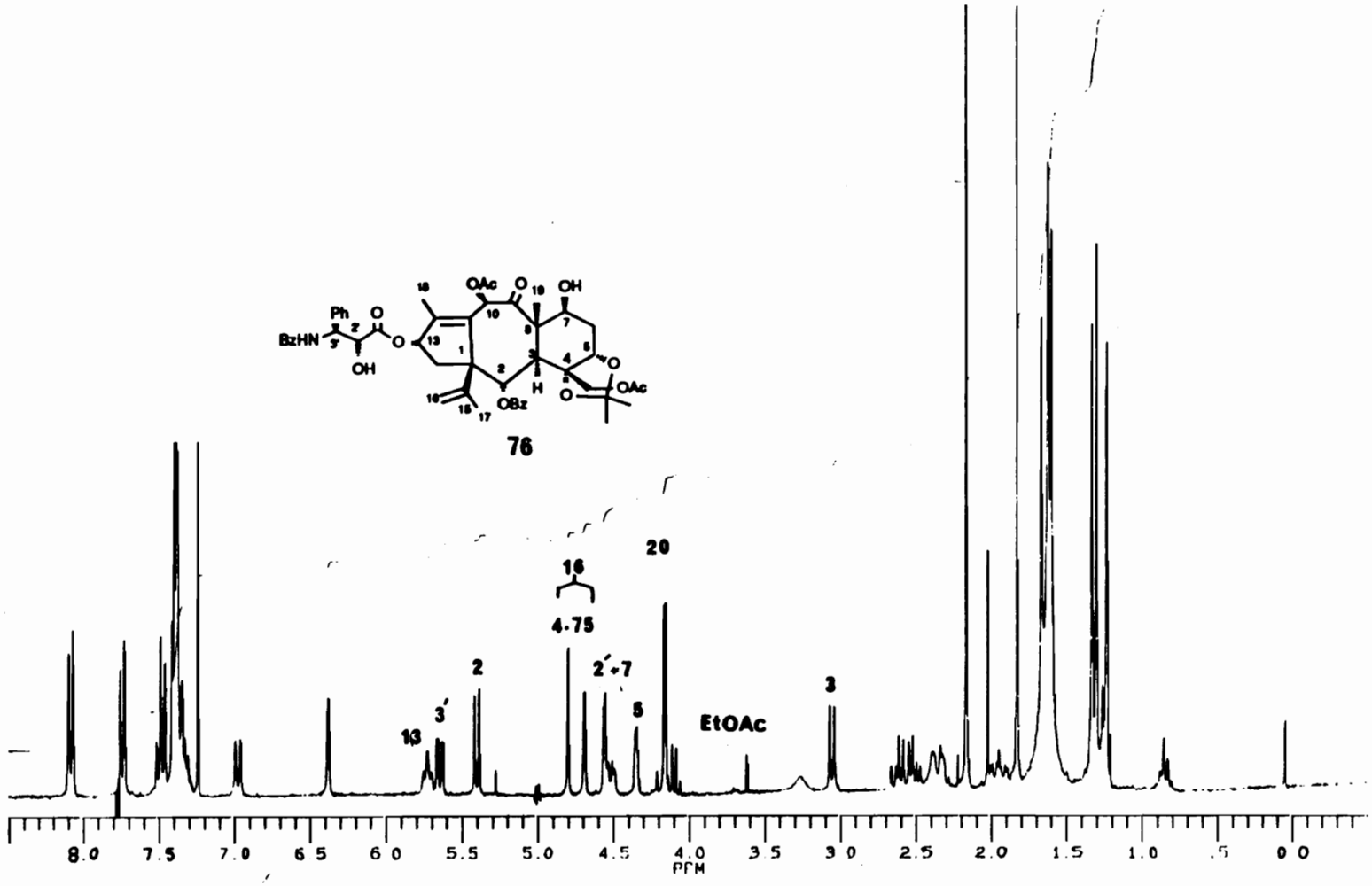


Figure 33 ^{1}H NMR of the acetonide (76)

2.2.3 Synthesis of a Ring-A Contracted Taxol: A-*nor*-taxol

Although as noted above the rearrangement of D-*seco*-taxol (**70**) occurred under relatively mild conditions, the presence of acid raises the possibility that some deep-seated rearrangement is occurring and that the product might therefore not have the structure **71** proposed for the acetyl chloride product.[a] Most importantly, if we could effect ring A rearrangement in taxol (**5**) without causing the oxetane ring to open, we would then have a compound which could be tested to determine the importance of ring A for the biological activity of taxol. We thus elected to carry out rearrangement of ring A under basic conditions so as to eliminate the possibility of a deep-seated rearrangement.

Reaction of taxol (**5**) with triethylsilylchloride in the presence of imidazole yielded 2',7-bis(triethylsilyl)taxol (**104**). Treatment of this with methane sulfonyl chloride[85] and triethylamine, in dichloromethane at -15° to 0°, followed by a quench with aqueous triethylamine yielded the rearranged derivative **106** as the major non-polar product (Scheme 38) presumably formed via mesylate **105**. Isolation of the mesylate **105** was not possible even though is was detected as a much less polar compound than **106** on TLC.

The structure of the rearranged product **106** was confirmed by its ^{1}H NMR data (Table 16) which showed resonances for two vinyl protons at δ4.66 and 4.75 for the terminal methylene protons, and by its mass spectrum (MH^+ *m/z* 1064), corresponding to the overall loss of water from the silyl taxol **104**. Attempts to deprotect

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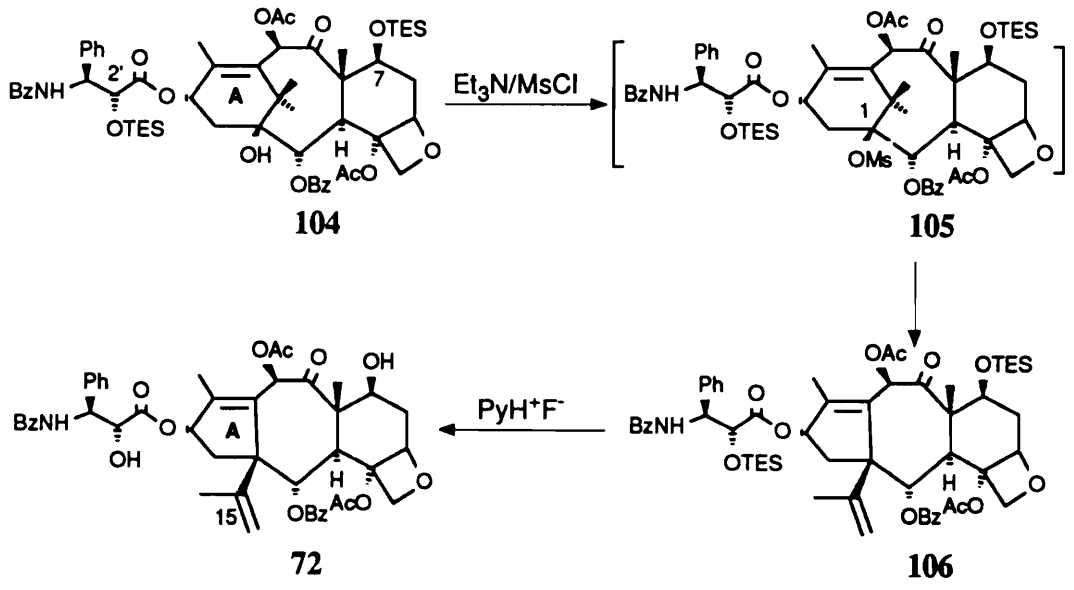
[a] We thank Professor R. M. Coates for this suggestion



Scheme 38 Synthesis of A-*nor*-taxol (**72**)

the silyl groups with tetrabutylammonium fluoride yielded a mixture of products, but pyridinium hydrofluoride[86] at 0° under extremely anhydrous conditions converted **106** to A-*nor*-taxol (**72**).

The structure of the compound was confirmed by its ^{1}H NMR spectrum (Figure 35). The presence of only five methyls accounted for the C-4 and C-10 acetoxy methyls, two vinyl nethyls, and the quaternary methyl on C-8. The signals at δ4.77 and 4.69 were assigned to the olefinic proton on C-16. The other signals are similar to those of taxol (**5**). The FAB mass spectrum of the compound indicated the appropriate MH^+ at *m/z* 836. We tested A-*nor*-taxol (**72**) in an *in vitro* bioassay, and the results are discussed in the next section.

Table 16. **^{1}H NMR Assignment of 2',7-bis(triethylsilyl)taxol (104), rearranged silyl taxol (106) and A-*nor*-taxol (72)**

Protons on Carbon	104	106	72
C_2	5.71 (m)	5.54 (d, 8)	5.49 (d,8)
C_3	3.88 (d, 6)	3.53 (d, 8)	3.48 (d,8)
C_5	4.94 (br d, 11)	5.02 (d, 8)	5.04 (d,8)
C_6	a	1.90 (m)	1.86 (dd, 11,15) 2.59 (ddd, 8,9,15)
C_7	4.48 (dd, 6,14)	4.53 (dd, 8,5)	4.63 (dd, 9,11)
C_{10}	6.44 (s)	6.39 (s)	6.32 (s)
C_{13}	6.20 (m)	5.81 (br t, 7)	5.71 (m)
C_{14}	a	2.40 (m) 2.60 (m)	2.04 (dd, 8,13) 2.42 (dd, 13,6)
C_{16}	1.22 (s)	4.66 (s), 4.75 (s)	4.69 (br s) 4.76 (br s)
C_{17}	1.18 (s)	1.65 (s)	1.59 (s)
C_{18}	2.02 (s)	1.62 (s)	1.61 (s)
C_{19}	1.70 (s)	1.73 (s)	1.64 (s)
C_{20}	4.27 (ABq, 11, $\Delta\nu_{AB}$=63)	4.15 (ABq, 12, $\Delta\nu_{AB}$=26)	4.24 (ABq, 8 $\Delta\nu_{AB}$=34)
$C_{2'}$	4.71 (d, 3)	4.60 (d, 2)	4.67 (d,2)
$C_{3'}$	5.71 (m)	5.64 (dd, 2,11)	5.71 (dd, 11,2)
NH	7.12 (d, 9)	7.15 (d, 11)	6.89 (d,11)

cont'd

Table 16 cont'd

Protons on Carbon	**104**	**106**	**72**
OAc	2.17 (s) 2.55 (s)	2.02 (s) 2.40 (s)	2.17 (s) 2.36 (s)
2-OBz (ortho)	8.12 (m)	8.01 (m)	8.10 (m)
3'-NBz (Ortho)	7.79 (m)	7.70 (m)	7.66 (m)
3'-Ph	7.26-7.55 (m)	7.2-7.5 (m)	7.2-7.4 (m)
other aromatic-H	7.2-7.5(m)	7.2-7.5(m)	7.2-7.5 (m)
TES groups	0.48 (6H, m) 0.57 (6H, m) 0.81 (9H, t, 8) 0.91 (9H, t, 8)	0.38 (5H, m) 0.60 (6H, m) 0.75 (9H, t, 8) 0.90 (9H, t, 8)	

a hidden under methyl envelop, not determined

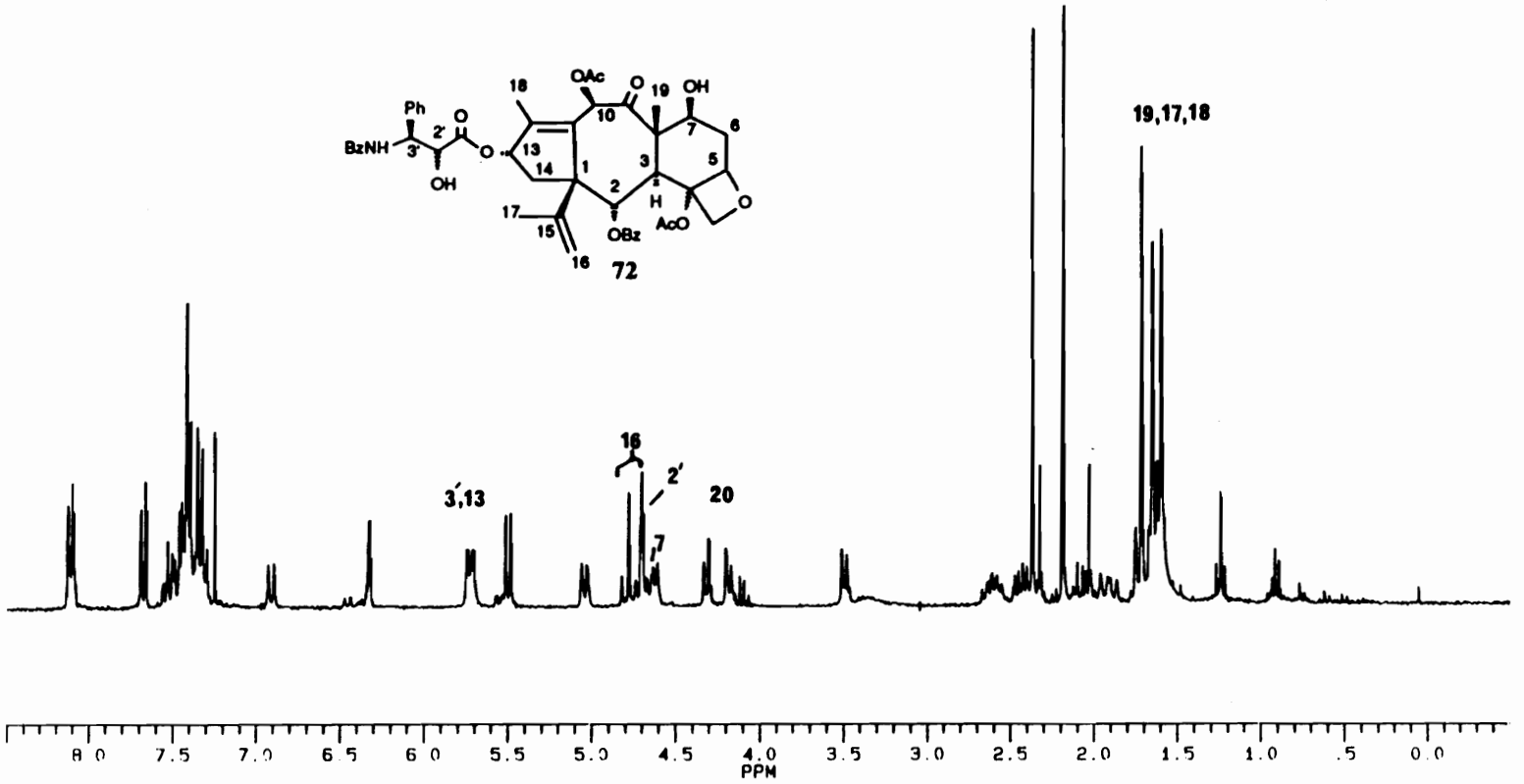


Figure 35 ^{1}H NMR spectrum of A-N*or*-taxol (**72**)

2.3 Bioassay of Taxol Derivatives and Related Compounds

2.3.1 Bioassay Systems

Two different assay systems were used for preliminary evaluation of modified taxols as anticancer agent: KB cell culture, an *in vivo* assay, and the *in vitro* tubulin assembly assay. The KB cell culture assay measures the ability of the drug to inhibit the growth of cancer cells. In this assay drugs at varying concentrations are incubated with cancer cells to determine the concentration of the drug that is sufficient to inhibit the growth by 50% of control. The results of this assay are a reasonably good indicator of activity in the taxol area, since they correlate well with results from animal-based assays such as the P-388 lymphocytic leukemia mouse assay. The disadvantages of this assay system are the possibility of drug modification by enzymes of cells, the fact that toxic drugs will show good activity, and the requirement of several milligrams of the drug.

The tubulin assay is more convenient, requiring only microgram quantities of the drug. This assay is based on the following properties of tubulin. Tubulin assembles (polymerizes) to microtubules in the presence of GTP at 37^0; this process is reversible, and thus formed microtubules disassemble when subjected to cold or Ca2+.[57] Horwitz and coworkers demonstrated[61,62] that taxol (**5**) can promote the assembly of tubulin to microtubules even in the absence of GTP, and that such assembled microtubules are resistant to disassembly. This property of tubulin is used to evaluate cytotoxicity of taxol derivatives.[87]

2.3.2 Isolation of Tubulin From Cow Brain

Tubulin was isolated from cow brains according to procedure of Williams and Lee.[88] This isolation method is based on the dynamic equilibrium between tubulin and its polymeric form, microtubules.[89]

The cow brains used were obtained immediately after slaughter and were transported to the laboratory on ice. Meninges and superficial blood vessels were removed at 0^0. The remaining mass was minced with scissors and then homogenized in a Waring blender for intermittent short periods of time at pH 7.6. The homogenate was subjected to centrifugation at 4^0. At this temperature tubulin is in its soluble monomeric form, and this step thus removed all the insoluble tissue. The clear supernatant containing tubulin was collected and was then incubated at 34^0; at this temperature tubulin polymerizes to insoluble microtubules. The progress of polymerization was evident by the increasing viscosity and turbidity of the liquid. The microtubules thus formed were separated by centrifugation at 35,000 rpm.

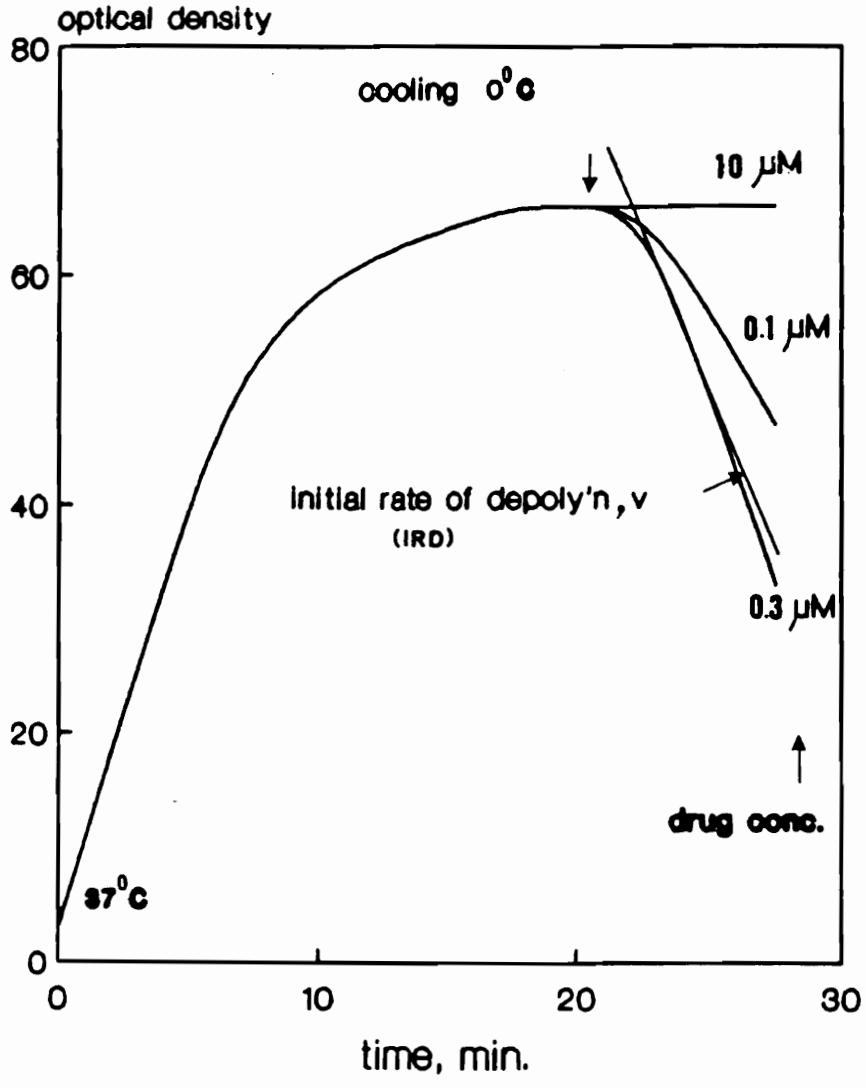
This cycle of assembly and disassembly was carried out once more before storing the tubulin at -80^0. Tubulin frozen at this temperature is known[90] to retained its activity even after a period of six years.

The purity of the isolated tubulin was found to be 85% as determined by 7.5% SDS-polyacrylamide gel electrophoresis.[91] Total protein content was determined to be 2 mg/ml by the Coomassie Brilliant Blue G-250 assay.[92] Although the tubulin obtained by this method was not completely pure, it was of adequate purity for use in the tubulin assembly assay described below.

2.3.3 General Method for the Tubulin Assay and Presentation of activity Activity Data for Taxol Derivatives

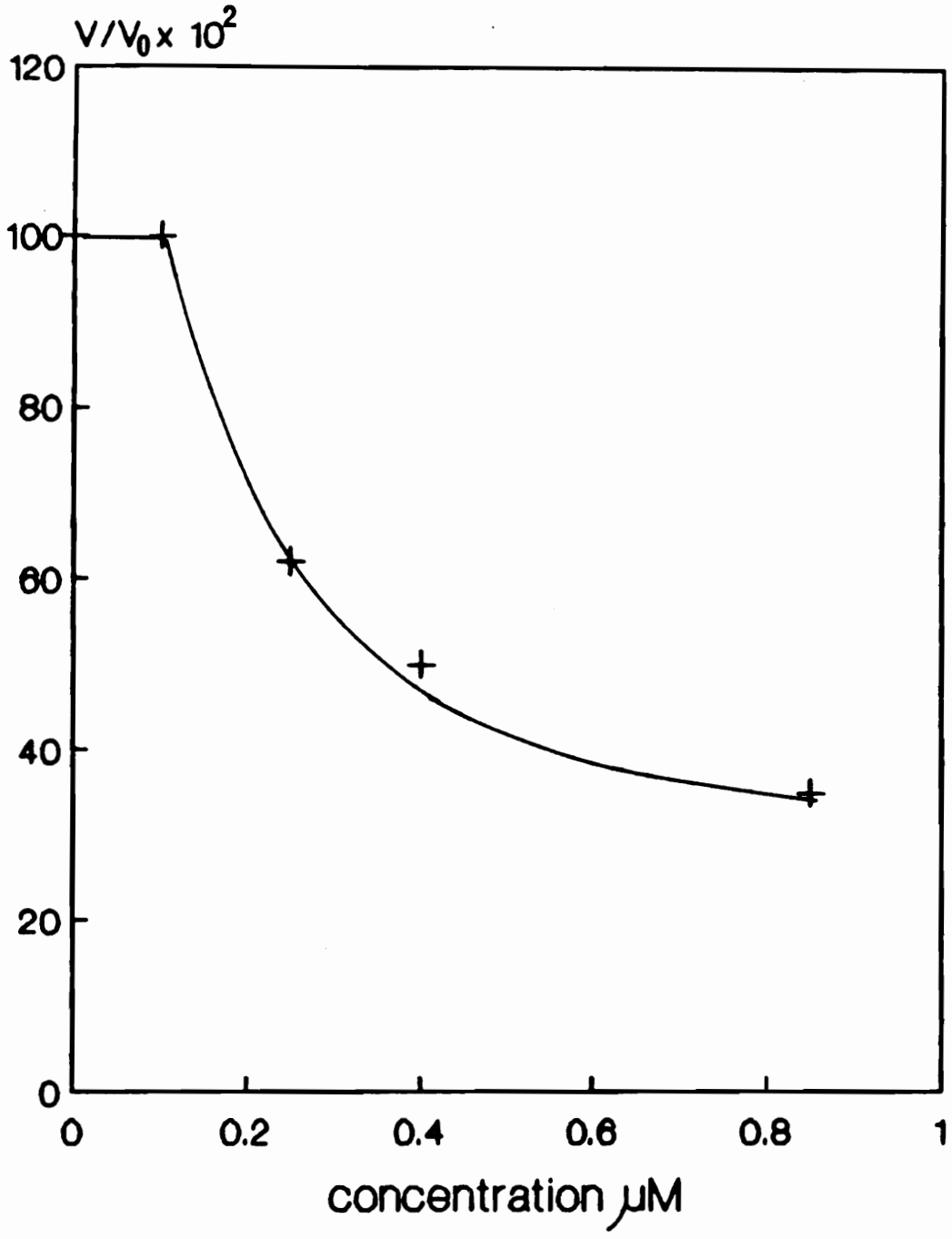
Tubulin prepared as previously described was subjected to one additional assembly-disassembly cycle immediately prior to the bioassay. A UV-Vis spectrophotometer was used to monitor the turbidity, using 350 nm light. Tubulin was measured into a UV cuvette and diluted with Mes buffer (0.1 M 2-(N-morphilino)-ethanesulfonic acid, 1 mM EGTA, 0.5 mM $MgSO_4$, 1 mM GTP, pH 6.6 at 27°)[87] to a tubulin concentration of 1 mg/ml and GTP was added to 0.5 μM. The cuvette was placed in the sample compartment which was thermostated at 37°. The turbidity was measured at 350nm against time. After approximately 15 min the system attained a steady state which was indicated by the horizontal portion of the curve (Figure 36). At this point, the sample was cooled to 0° allowing the microtubules to disassemble, and the disassembly was monitored by the decrease in absorption at 350nm. The slope of the disassembly portion of the curve represents the initial rate of depolymerization (IRD). In the presence of taxol (**5**) or an active taxol derivative, the IRD is decreased by the drug, and thus measurement of IRD values in the presence of varying amounts of drug enables the constructions of a dose-response curve (Figure 37). In order to quantify the activity of compounds a quantity called Inhibition Dose (ID_{50}) is defined as the concentration of drug that is sufficient to reduce the initial rate of disassembly of tubulin by 50%. This quantity is calculated by determining IRD values for several different concentrations of a compound and plotting these values against concentration. The values are normalized to the IRD of tubulin only

(taken as 100). The ID_{50} is determined from the graph as shown in Figure 37. ID_{50} values for several different compounds that we have studied are listed in Table 17.



optical density vs. time

Figure 36 Depolymerization curve for taxol-tubulin assay (schematic)



initial relative rate vs. conc.

Figure 37 Dose response curve for taxol derivatives (schematic)

Table 17 **ID_{50} for taxol derivatives**

Compound	Structure	Tubulin Assay ID_{50} (μM)	KB cell assay ED_{50} (μg/ml)
Taxol	(5)	0.3	$0.31x10^{-5}$
7-Benzoyltaxol	(47b)	0.2	..
7-Azibenzoyltaxol	(42)	0.3	..
D-*seco*-taxol	(70)	>6.3	0.2
7-(2,3,5-Trimethoxybenzoyl)taxol	(44)	0.8	..
A-*nor*-taxol	(72)	0.9	2
7-(N-dansyl-β-alanyl)taxol	(43)	0.3	..
Acetyl chloride product	(71)	0	2.5

2.3.4 Structure-Activity Relationships of Taxol Derivatives

A recent review by Kingston and coworkers[93] summarizes the essential structural features of taxol (**5**) derivatives for their biological activity.

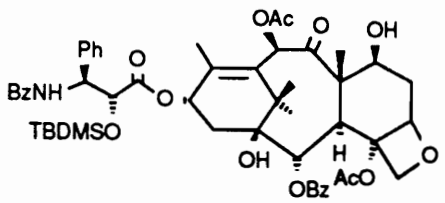
1. The C-13 ester side-chain is absolutely required for cytotoxicity. Thus taxol and cephalomannine(**25**)[94] are both active, while compounds lacking the side chain such as baccatin III (**23**)[31] decinnamoyltaxinine J (**9c**)[31] and 10-deacetyl-baccatin III (**22c**)[95] are essentially inactive.

2. N-Acyl substituents on the C-13 side-chain appear not to be that important. Thus although the ED_{50} values for taxol (**5**) vary somewhat when determined in different laboratories, both taxol and cephalomannine show comparable values when determined in the same laboratory.

3. The stereochemistry of the C-7 hydroxyl group does not make a large difference in activity. Thus both taxol (**5**) and 7-*epi*-taxol (**27a**)[39] show comparable activity.

4. C-2' substituted taxols are not active in *vitro* assays but show cytotoxicity in the KB assay. It was suggested[45] that possibly 2'-acyl derivatives are intra-cellularly converted into an active form, either taxol or some other unknown active taxol metabolite. This hypothesis is supported by recent work by Zalkow, which indicates that 2'-succinyl salts[47] (**108**) and more complex

succinyl and glutaryl derivatives have excellent *in vivo* activity.[96] Hydrolytically stable 2'-(t-butyldimethylsilyl)taxol (**107**)[47] showed essentially no activity, indicating the need for a free hydroxyl group at the 2' position for activity.



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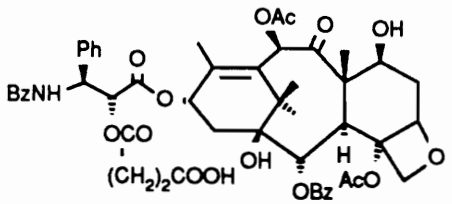

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5. Derivatization of the C-7 hydroxyl group does not eliminate activity, as observed for 7-acetyltaxol (**35**)[45,95] and 7-xylosyltaxol (**26d**)[87] which are comparably active to taxol (**5**) *in vivo* and *in vitro*.

These observations agree with the new data presented in the Table 17. The C-7 derivatives 7-(2,3,5-trimethoxybenzoyl)taxol (**44**), 7-benzoyltaxol (**47b**), 7-azibenzoyltaxol (**42**) and 7-(N-dansyl-β-alanyl)taxol (**43**) have *in vitro* activity comparable to taxol (**5**) as determined by *in vitro* tubulin assay. The reduced activity shown by the trimethoxybenzoyl derivative may be due to the steric bulk of the substituent.

The acetyl chloride product (**71**) and the D-*seco*-taxol (**70**) have much reduced activity as compared whit that of taxol (**5**), in both *in vivo* and *in vitro* assays. The acetyl chloride product has essentially no activity with an ED_{50} of 2.5 μg/ml, while the D-*seco*-taxol has an ED_{50} of 0.2 μg/ml. Both products lack the oxetane ring, and the acetyl chloride product has also undergone ring A rearrangement.

The data for the D-*seco*-taxol indicate the requirement of an intact oxetane ring for the biological activity of taxol derivatives; the oxetane ring may be associated with the reactivity of the taxol molecule with tubulin or it may be holding the molecule in required conformation for the formation of microtubule-taxol complex.

The inactivity of the acetyl chloride product is also attributed to the opened oxetane ring, but its reduced activity as compared with the Meerwein product, D-*seco*-taxol, may be due to changes in the ring-A as well. We expected to resolve this ambiguity by testing the biological activity of A-*nor*-taxol (**72**) which has an intact oxetane ring but a contracted ring A. In an *in vitro* tubulin assay A-*nor*-taxol showed the same order of activity (ID_{50} = 0.9 μM) as taxol (**5**) (0.3 μM). This indicated to us that the ring A of taxol is not a requirement for tubulin assembly activity. However, the *in vivo* KB bioassay showed that this compound is essentially inactive suggesting that an intact ring A is a requirement for cytotoxicity. The difference between the *in vitro* and *in vivo* assay results in unusual but not unprecedented, and may be due to the instability of A-nor-taxol under the assay conditions.

3. EXPERIMENTAL SECTION

General

Melting points were recorded on a hot stage apparatus and are uncorrected. Proton and ^{13}C NMR spectra were recorded on a Bruker WP 270SY spectrometer and the two-dimensional spectra were recorded on a Bruker WP 200 spectrometer. Chemical shift values are given in parts per million (ppm) from TMS resonance. Spectra were recorded in $CDCl_3$ at ambient temperature. Infrared spectra were recorded on a Perkin Elmer 710 B spectrophotometer. UV spectra were recorded on a Beckman DU-50 single beam apparatus. Low resolution FAB mass spectra were recorded on a VG 7070 HF mass spectrometer with DTE of glycerol or nitrobenzyl alcohol as the matrix. High resolution FAB spectra were recorded on a Kratos MS50 at the Midwest Center for Mass spectrometry at Nebraska.

Analytical thin layer chromatography was performed on silica gel 60 F_{254} (0.2 mm) aluminum-back plates available from E. Merck. Preparative TLC was carried out on 20x20 GF_{254} plates from Analtech. Silica gel of 230-400 mesh was used in all column chromatography.

Preparation of 2'-chloroacetyltaxol (45)

Taxol (**5**) (1.117 g, 1.31 mmol) was dissolved in freshly distilled dichloromethane (2 mL) and pyridine (400 μL) and then the solution was cooled to 0^0 in ice under N_2 atmosphere. Chloroacetic anhydride (269 mg, 1.2 eq) dissolved in CH_2Cl_2 (5-7 mL) was added to the solution during 30 min. After 65 min. the reaction was stopped by adding water (5 mL) and the mixture was stirred for 15 min. The organic layer was washed with water several times and dried over $MgSO_4$. Evaporation of the solvent yielded a crude solid which was purified by flash column chromatography with 50% EtOAc/Hexane to yield pure 2'-chloroacetyltaxol (**45**) (1.19g, 98%): mp 210^0-212^0; IR(KBr), 1750(s), 1660(s); MS(FAB) *m/z* (rel.int.) 930(MH^+, 100), 931(56), 932(33); ^1H NMR, see Table 8.

Deprotection of 2'-chloroacetyltaxol (45) to taxol

2'-chloroacetyltaxol (**45**) (5 mg, 0.005 mmol), Et_3N (30 μL) and 2-aminoethanethiol (1 mg) were dissolved in pyridine (200 μL) under N_2 atmosphere. After 30 min the reaction was complete. Solvent was evaporated *in vacuo*; the last traces of solvent were co-evaporated with n-heptane. The residue was dissolved in ethyl acetate and the ethyl acetate layer was washed several times with water. Evaporation of solvent yielded a product which was shown to be taxol (**5**) by the comparison of the ^1H NMR spectrum and TLC of the product with those of an authentic sample of taxol.

2'-Chloroacetyl-7-benzoyltaxol (47a)

2'-Chloroacetyltaxol (**45**) (30 mg, 0.032 mmol) was dissolved in dry dichloromethane (0.5 mL) and pyridine (10 μL) under an inert atmosphere. Benzoylchloride (5 μL) was added to the solution which was stirred at room temperature for one hour. The solvent was then removed *in vacuo* and the traces of pyridine remaining were evaporated with n-heptane. The residue was purified by PTLC with 50% EtOAc/hexane. The product isolated was recrystallized as platlets from hexane/dichloromethane (19 mg, 52%). In this work up procedure, the hydrolysis of excess benzoyl chloride is largely prevented. If aqueous work-up is used the separation of benzoic acid from the product is very difficult: mp 140-144°; ^{1}H NMR, see Table 8. Attempt at determination of the mass spectrum of this compound by FAB failed to yield an identifiable molecular ion.

7-Benzoyltaxol (47b)

A solution of **47a** (19 mg, 0.020 mmol) in methanol : dichloromethane (9 : 1) was treated with 50 mg of silica gel (GF_{50}). After stirring for 24 h under an N_2 atmosphere at room temperature, the solvent was evaporated, the residue was dissolved in 5% methanol in chloroform, and the solution was filtered through a cotton plug. The residue obtained after evaporation of the solvent was purified by PTLC with 50% EtOAc/hexane as the eluent. Compound **47b** was obtained as a white solid (8.2 mg 47%): MS(FAB) *m/z* (rel.int.) 958(MH^+, 100), 899(MH^+-OAc, 80), 673 (MH^+-Side-chain-H, 10), 880(MH^+-AcOH-H_2O, 20), 613(673-HOAc, 40), 553(613-AcOH, 100), 369(100), 185(100); ^{1}H NMR, see Table 9.

7-(Azibenzoyl)taxol (42)

2'-Chloroacetyltaxol (**45**) (200 mg, 0.215 mmol), DDC (150 mg, 3.3 eq) and a catalytic amount of 4-pyrrolidinopyridine (1mg) were dissolved in dry dichloromethane (3 mL). A solution of azibenzoic acid (**46**) (3 eq) in dichloromethane (300 μL) was added to the above solution stirred at room temperature under N_2 atmosphere. After one hour, the solution was washed with 5% aqueous acetic acid and then water (30 mLx2). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The crude residue (113 mg) was purified on a Sephadex column (LH-20, 25-100μ, 2x18cm bed, 3 mL/min) to yield a partially pure product (52 mg). This fraction was further purified on a flash column. The pure product isolated was recrystallized from CH_2Cl_2/hexane to yield homogeneous material (12 mg, 5%). The ^1H NMR spectrum of the product indicated it to be 7-(azibenzoyl)taxol (**42**): mp 205° (decompose); IR ($CHCl_3$) 1732(s) 1660(w), 1606(w); UV λ_{max} (MeOH) nm(ε) 350(1050), 280(5960), 231(43000); MS(FAB) *m/z* (rel.int.) 1066(MH^+, 72), 1047(M-H^+-F, 5), 1066(MH^+-HOAc, 18), 981(MH^+-CF_3-Me, 32), 953(MH^+-CF_3N_2+CH3, 100); ^1H NMR, see Table 8.

2'-Chloroacetyl-7-(N-cbz-β-alanyl)taxol (50)

Taxol (**5**) (323 mg, 0.347 mmol) dissolved in dichloromethane (6 mL) was added dropwise to a dichloromethane solution of N-cbz-β-alanine (158 mg, 2.2eq), pyrrolidinopyridine (1 mg, cat.) and DCC (146 mg, 2.2 eq), under an inert atmosphere and at room temperature. After 1 h the solvent was evaporated and the residue was resuspended in chloroform and passed through a short silica gel column. The column was washed with ethyl acetate/hexane (50%, 100 mL).

Evaporation of the eluent yielded a amorphous white solid (390 mg, 99%) which could not be recrystallized: IR ($CDCl_3$) 1732(s), 714(s); ^1H NMR, see Table 9. Attempt at determination of the mass spectrum of this compound by FAB failed to yield an identifiable molecular ion.

Hydrogenolysis of 2'-chloroacetyl-7-(N-cbz-β-alanyl)taxol (50); 2'-acetyl-7-(β-alanyl)taxol (51)

Palladium catalyst (10% Pd/C, 14 mg, 20% w/w) was weighed into a round bottom flask and the flask was connected to the hydrogenator. After filling the system with hydrogen, the starting material (88 mg, 0.077 mmol) dissolved in 95% ethanol (5 mL) was injected into the flask. The mixture was stirred under atmospheric pressure and at room temperature for 3 h. The catalyst was filtered off and the solution was concentrated *in vacuo* to yield a crude solid which was purified PTLC with 10% MeOH/$CHCl_3$ as the eluent. The white solid thus isolated was recrystallized from EtOAc/hexane (60 mg, 78%): mp 188-190^0; ^1H NMR, Table 9; MS(FAB) *m/z* (rel.int.); 967(MH^+, 2), 989(MNa^+, 1); 907(MH^+-HOAc, 0.5), 598(MNa^+-side-chain - H - $NH_2CH_2CH_2COOH$ - H_2O, 60), 268(20)

Deprotection of 2'-chloroacetyl-7-(N-cbz-β-alanyl)taxol (50) with 2-aminoethanethiol; 7-(N-cbz-β-alanyl)taxol (48)

2'-Chloroacetyl-7-(N-cbz-β-alanyl)taxol (**50**) (390 mg, 0.343 mmol) was dissolved in dry dichloromethane (6 mL) under an inert (N_2) atmosphere. Solid 2-aminoethanethiol (58 mg, 2.2 eq) was added to the above solution and stirred at room temperature. After 1.5 h the reaction mixture was concentrated and passed

through a short silica gel column (4X3 cm) with 50% EtOAc/hexane. Evaporation of the solvent yielded a white solid. Recrystallization of the solid from dichloro-methane/hexane yielded white flakes (330 mg, 92%): mp 135°-138°; IR(KBr) 2820(m), 1740(s), 1720(s), 1625(m), 1500(m), 225(s), 960(m); λ_{max} (MeOH) nm(ε) 272 (4300), 225 (49900); MS(FAB) *m/z* (rel.int.) 1081(MNa^+-HOAC, 2), 796(MNa^+- side-chain-H, 20), 738(10), 452(MNa^+- side-chain-HOAc, 20), 177(100); ^1H NMR, see Table 9.

7-(N-Dansyl-β-alanyl)taxol (43)

7-(N-Cbz-β-alanyl)taxol (**48**) (30 mg, 0.028 mmol) was dissolved in absolute ethanol and hydrogenated over 5% Rh/C (96 mg) for 3.5 h, at atmospheric pressure and at room temperature. The reaction mixture was filtered through a short Avicel (crystalline cellulose) column and the column was washed with NH_3/methanol (0.1%). The combined eluents were evaporated to dryness to yield material homogeneous on TLC (14 mg, 53%). This material (0.015 mmol) was immediately dissolved in dry pyridine (1 mL) under argon and dansyl chloride (4 mg, 0.015 mmol) was added in solid form to the stirred solution. After 15 min, pyridine was evaporated *in vacuo* and the resulting solid was dissolved in ethyl acetate; the ethyl acetate layer was acidified with 3N HCl and washed several times with water. Evaporation of the solvent yielded a crude brown solid; this solid was further purified by column chromatography to yield the greenish yellow compound **43** (6 mg, 34%) as an amorphous solid which could not be crystallized: IR(KBr) 2590(m), 1740(s), 1640(m) 180(w), 1240(s); λ_{max} (MeOH) nm(ε) 336(8360), 257(sh, 34000), 221(133000); Fluorescent spectrum, λ_{max} (MeOH) nm(rel. fluorescent intensity, excitation at

340 nm) 514(67); MS(FAB) *m/z* (rel.int.) 1159(MH^+, 100), 1158(90); ^1H NMR, see Table 9.

7-(3,4,5-Trimethoxybenzoyl)taxol (44)

2'-Chloroacetyltaxol (**45**) (25 mg, 0.027 mmol) was dissolved in dry dichloromethane (700 μL); DCC (20 mg, 4 eq), 3,4,5-trimethoxy benzoic acid (67 mg, 3.2 eq) and a catalytic amount (6 mg, 3 eq) of pyrrolidinopyridine were added in solid form to the above solution and the solution was stirred for 1 h. Since no products were observed at this point 500 μL of pyridine were added and the solution was stirred an additional 2h until the reaction was complete. The solution was then acidified with 6N HCl and washed with water several times. The solid recovered by evaporation of the organic layer was partitioned between 5% aqueous sodium bicarbonate and ethyl acetate to remove trimethoxybenzoic acid. Evaporation of ethyl acetate yielded impure 2'-chloroacetyl-7-(3,4,5-trimethoxybenzoyl)taxol (20 mg). Without further purification this material was used for the deprotection step. The impure material (20 mg) was dissolved in dry dichloromethane (300 μL) and treated with 2-aminoethanethiol (100 mg) at room temperature. After 1.5 h the reaction mixture was diluted with ethyl acetate, acidified with aq. HCl (1N) and then the EtOAc layer was washed with water. The organic layer was evaporated and the residue was purified by PTLC to yield trimethoxybenzoyl taxol (**44**) as a white solid (25 mg, 88% overall): mp 172-174° (recrystallized from EtOAc/Hexane); IR(KBr) 1740(s), 1725(s), 1660(m), 1640(m), 1590(m), 1340(m), 1215(5); UV λ_{max} (MeOH) nm(ε) 264(10300), 212(44300); ^1H NMR ($CDCl_3$, 270MHz) δ(ppm); 1.25 , 1.26 (each 3H, s, 16-H & 17-H), 1.86 (3H s, 19-H), 1.94 (3H, s, 18-H), 2.02 & 2.40 (each 3H,

s, acetoxy methyls), 2.36 (2H, m, 14-H), 2.76 (1H, d, 7, 2-H), 2.82 (2H, m, 6-H), 3.88 (3H, s) & 3.90 (6H, s) for three OMe groups, 4.01 (1H, d, 7, 3-H), 4.30 (2H, ABq, 8, $\Delta\nu_{AB}$=32, 20-H), 4.82 (1H, d, 3, 2-H'), 5.00 (1H, br d, 12, 5-H), 5.62 (1H, dd, 7, 10, 7-H), 5.80 (1H, dd, 3, 8, 3-H'), 6.20 (1H, br t, 12, 13-H), 6.34 (1H, s, 10-H), 7.17 (2H, s, o-proton of trimethoxybenzoyl group), 7.04 (1H, d, 8, NH), 7.76 (2H, m, o-protons), 7.3-7.6 (all other aromatic protons); MS(FAB) *m/z* (rel.int.)1070(MNa^+, 4), 1010(MNa^+-HOAc, 0.5), 785(MNa^+-side-chain-H, 11), 725(MNa^+- OAc, 3), 195(100).

Hexahydrobaccatin III (55)

Baccatin III (**23**) (219 mg, 0.374 mmol) was dissolved in ethyl acetate (14 mL) was hydrogenated over Pt/C at atmospheric pressure for 10 h. The catalyst was filtered off through silica gel with additional ethyl acetate. Crystallization of the crude material from ethyl acetate/hexane yielded pure hexahydrobaccatin III (200 mg, 90%): mp 150-154°; IR (KBr) 3500(s), 2950(s) 1720(s): ^{1}H NMR, see Table 10; MS (FAB) *m/z* (rel.int.) 615(MNa^+, 10), 555(MNa^+-HOAc, 3), 461(2) 487(MNa^+-cyclohexanecarboxylic acid), 3).

7-Triethylsilyl-hexahydrobaccatin III (56)

Triethylsilyl chloride (674 µL, 10 eq) was added dropwise to a solution of hexahydrobaccatin III (238 mg, 0.402 mmol) in pyridine (10 mL) at room temperature. The reaction was quenched after 24 h by addition of water and stirring for 10 min. The reaction mixture was cooled in ice water, diluted with ethyl acetate, and carefully neutralized with HCl (3N) to pH 6. The ethyl acetate layer was then washed with water followed by brine, and dried with anhydrous Na_2SO_4. Evaporation

of the solvent yielded a gummy solid which was purified by column chromatography to yield the pure compound **86** (241 mg, 85%). Since the triethylsilyl group was found to be sensitive to chloride ion it was necessary to cool the reaction mixture during the acidification step. The best result was obtained using ethyl acetate (distilled and filtered through basic alumina or anhydrous K_2CO_3) and hexane for the column chromatography: mp 144-146^0 (recrystallized from ethyl acetate/hexane); IR (KBr) 3500(s), 2900(s), 1740(s), 1640(w), 1380(s), 1240(s), 840(s); ^{1}H NMR, see Table 10; ^{13}C NMR($CDCl_3$, 67.5 MHz) δ(ppm) 9.39(19), 15.39(18), 20.83(2xOAc), 22.46(17), 25.19(3xSi$\underline{C}H_2$), 25.62(3xSiCH$_2\underline{C}H_3$), 25.78(cyclohex.3',5'), 27.04((16), 28.48(cyclohex. 2',6'), 29.38(cyclohex. 4'), 35.65(6), 38.45(14), 42.78(15), 43.65(cyclohex. 1'), 46.04(3), 58.86(8), 68.06(13), 72.24(7), 74.46(20), 76.23(10,2), 79.19(1), 80.77(4), 84.66(5), 132.50(11) 146.25(12), 170.63 and 171.25 (2xOAc), 188.43 (cyclohexane carbonyl), 209.02(9); MS(FAB) *m/z* (rel.int.) 707(MH^+, 45), 689(MH^+-H_2O, 10), 648(MH^+-OAc, 70), 630(648-H_2O, 10), 570(630-HOAc, 10), 600(630-CH_2O, 20), 371(100).

Methanolysis of 7-triethylsilyl-hexahydrobaccatin III (56) for an extended period of time: 7-triethylsilyl-2,4,10-tris(deacyl)baccatin III (59)

7-Triethylsilyl-hexahydrobaccatin III (**56**) (10 mg, 0.014 mmol) was dissolved in 0.5 mM NaOMe in methanol (1 mL) and the solution was stirred at room temperature for 5 h. Then the solution was cooled in ice water and neutralized with 1% methanolic HCl using multiindicator pH papers to pH 7.0. The resulting mixture was concentrated *in vacuo* and then extracted with EtOAc. The impure solid obtained after evaporation of the solvent was purified by column chromatography to yield a

pure compound **59** (5 mg, 69%): mp 132-134°; IR(KBr) 3450(s), 2950(s), 1710(s), 1630(s), 820(s); ^{1}H NMR, see Table 11; ^{13}C NMR($CDCl_3$, 67.5MHz) δ(ppm) 5.36, 6.68, 9.76, 17.03, 17.87, 29.45, 37.70, 38.26, 41.72, 51.36, 58.33, 69.14, 73.40, 73.63, 75.37, 81.14, 85.47, 137.90, 139.82, 210.4; MS(FAB) *m/z* (rel.int.) 577(MNa^+, 22), 517(MNa^+-ACOH, 100), 487(517-CH_2O, 20), 327(11)

Controlled methanolysis of 7-triethylsilyl-hexahydrobaccatin III (56): 7-triethylsilyl-10-deacetyl-hexahydrobaccatin III (57) and 7-triethylsilyl-4,10-bis(deacetyl)–hexahydrobaccatin III (58)

7-Triethylsilyl-hexahydrobaccatin III (**56**) (85 mg, 0.12 mmol) was dissolved in 0.5 NaOMe in methanol and the solution was stirred at room temperature under argon. After 2 h the reaction was quenched by neutralizing the solution with 1% methanolic HCl using multi-indicator pH papers. The mixture was concentrated and purified by PTLC; three pure compounds were isolated and identified as: (**a**). **7-triethylsilyl-10-deacetyl-hexahydrobaccatin III (57)** (6mg, 8%): amorphous solid; IR 3500(s), 2955(s), 1720(s), 1735(s); ^{1}H NMR, see Table 11; MS(FAB) *m/z* (rel.int.) 665(MH^+, 65), 647(MH^+-H_2O, 45), 635(MH^+-CH_2O, 10), 617(635-H_2O, 10), 605(MH^+-HOAc, 45), 587(605-CH_2O-H_2O, 25), 569(605-2x H_2O, 25), 495(587-$C_6H_{11}COOH$, 100); (**b**). **7-triethylsilyl-4,10-bis(deacetyl)hexahydrobacctin III (58)** (3mg, 4%): amorphous solid; IR(KBr) 3450(s), 2900(s), 1745(s), 1740(m), 1700(s), 1669(w), 1240(m), 840(w); ^{1}H NMR, see Table 11; (**c**). the previously isolated **7-triethylsilyl-2,4,10-tris(deacetyl)baccatin III** (**59**) (17 mg, 28%); Physical data as previously reported.

7,13-Bis(triethylsilyl)-hexahydrobaccatin III (60)

7-Triethylsilyl-hexahydrobaccatin III (**55**) (30 mg, 0.042 mmol) and imidazole (14 mg, 5 eq) were dissolved in DMF (500 μL), treated with triethylsilylchloride (100 μL, 14 eq), and the solution was heated at 60°. After 18 h the reaction was quenched by addition of brine and diluted with EtOAc. The aqueous layer was extracted several times (10 mLX4) with EtOAc and the combined organic layer was washed with brine (5 mLx5) and dried with Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography to yield 7,13-bis(triethylsilyl)-hexahydrobaccatin III (**60**) as a amorphous white solid which colud not be crystallised (36 mg, 91%): IR (KBr) 2975(s), 1750(s), 1740(s), 1240(s), 840(m); ^{1}H NMR, see Table 18; MS(FAB) *m/z* (rel.int.) 843(MNa^+, 100), 786(MNa^+-AcOH, 15).

Hydrolysis of 7,13-bis(triethylsilyl)-hexahydrobaccatin III (60)

7,13-Bis(triethylsilyl)-hexahydrobaccatin III (**60**) (30 mg, 0.023 mmol) was dissolved in 0.5 M NaOMe in methanol and the solution was stirred at room temperature. TLC of the reaction mixture, after 1.5 h, showed that all the starting material was converted to a less polar product which was identified as 10-deacetyl-7,13-bis(triethylsilyl)-hexahydrobaccatin III (**61**) as described below. After 10h the solution was directly purified by PTLC without prior work-up, and the major compound isolated was **10-deacetyl-7,13-bis(triethylsilyl)-hexahydrobaccatin III** (**61**, 12 mg, 58%): ^{1}H NMR, see Table 18. The medium polarity fraction was a mixture (0.6 mg) and contained the 2,10-bis(deacetyl) compound as the major constituent: ^{1}H NMR($CDCl_3$, 270MHz) δ(ppm); 0.50, 0.60, 0.9 (all multiplets for triethylsilyl groups), 1.95 (s, 18-H), 5.15 (s, C-4 OAc) 3.3 (d, 3-H), 4.1 (ABq, 20-H), 4.9 (d, 2-H),

Table 18 **^{1}H NMR assignment of 7,13-bis(triethylsilyl)-hexahydrobaccatin III (60) and 10-deacetyl-7,13-bis(triethylsilyl)-hexahydrobaccatin III (61)**

Proton on	**60**	**61**
C-2	5.39 (d,7)	5.33 (d,7)
C-3	3.69 (d,7)	3.72 (d,7)
C-5	4.95 (d,8)	4.94 (d,8)
C-6	2.5 (m) 1.8 (m)	2.4 (m) 1.8 (m)
C-7	4.42 (dd, 6,11)	4.33 (dd, 5,8)
C-10	6.42 (s)	5.07 (s)
C-13	4.87 (br t, 9)	4.88 (br t, 9)
C-14	2.3 (m) 2.1 (m)	2.3 (m) 2.0 (m)
C-16,17	1.13 (s) 1.10 (s)	0.97 (s) 1.10 (s)
C-18	2.10 (s)	1.97 (s)
C-19	1.63 (s)	1.64 (s)
C-20	3.49 (ABq, 8, $\Delta\nu_{AB}$=81)	4.27 (ABq, 8, $\Delta\nu_{AB}$=88)
OAc	2.20 (s) 2.17 (s)	2.15 (s)
TES	0.97 (9H, t, 7) 0.52 (6H,m)	0.92 (9H, t, 7) 0.60 (6H,m)
	0.88 (9H, t, 5) 0.55 (6H,m)	1.02 (9H, t, 5) 0.67 (6H,m)

5.0(d, 5-H), other peaks were not assigned. The most polar compound was identified as **7-triethylsilyl-2,4,10-tris(deacetyl)baccatin III (59)** by comparison of its ^{1}H NMR spectrum with that of an authentic sample.

7-Triethylsilyl-13-acetyl-2,4,10-tris(deacyl)baccatin III (63)

7-Triethylsilyl-2,4,10-tris(deacyl)baccatin III (**59**) (17 mg, 0.030 mmol) was dissolved in dry dichloromethane and DCC (3.7 mg, 0.6 eq), pyrrolidinopyridine (0.04 mg, 0.01 eq) and Ac_2O (2 μL, 0.7 eq) were added to the above solution at 0°. The reaction was stopped after 45 min by addition of water and stirring for 10 min. The resulting mixture was diluted with ethyl acetate and the ethyl acetate layer was washed several times with water. The ethyl acetate layer was dried with anhydrous Na_2SO_4 and evaporated to dryness; the crude solid obtained was purified by PTLC to yield 7-TES-13-acetyl-2,4,10-tris(deacyl)baccatin III (**63**) (12 mg, 72%) as an amorphous solid which could not be crystallized: IR (KBr) 3350(s), 2950(s), 1740(s), 1710(m), 1640(s), 1240(s); ^{1}H NMR, see Table 12. Attempts at determination of the mass spectrum of this compound by FAB failed to yield an identifiable molecular ion.

Acetylation of 7-triethylsilyl-13-acetyl-2,4,10-tris(deacyl)baccatin III (63)

A solution of 7-triethylsilyl-13-acetyl-2,4,10-tris(deacyl)baccatin III (**63**) (12 mg, 0.02 mmol) in dichloromethane (1 mL) treated with DCC (5 mg, 1 eq), Ac_2O (6 μL, 3 eq) and a catalytic amount of pyrrolidinopyridine at room temperature. The reaction was stopped after 3 h, when all the starting material had disappeared, by addition of aqueous acetic acid (6N) until the reaction mixture was slightly acidic. The reaction mixture was extracted with EtOAc and the EtOAc layer was washed with

water. The solid recovered from the EtOAc layer was purified by PTLC (3% MeOH in CHCl3); three bands were isolated were identified as 7-triethylsilyl-2,4,10-triacetyl-2-debenzoylbaccatin III (3.3 mg), a 1:2 mixture (3.1 mg) of the 2,13-diacetyl compound and the 2,10,13-triacetyl compound, and some other unidentified material (1.1 mg) (**a**). **2-debenzoyl-7-triethylsilyl-2,13-diacetylbaccatin III (66)** (3.3 mg); amorphous solid; ^{1}H NMR ($CDCl_3$, 270MHz) δ(ppm); 0.92 (9H, t, 5) and 0.57 (6H, m) for 7-triethylsilyl group, 1.22 and 1.15 (each 3H, s, 16-H & 17-H), 1.62 (3H, s, 19-H), 2.05 (3H, s, 18-H), 2.22 (3H, s) & 2.15 (6H, s) for three acetate groups, 3.69 (d, 7, 3-H), 4.47 (1H, dd, 7,9, 7-H), 4.95 (1H, d, 9, 5-H), 5.40 (d, 7, 2-H), 6.12 (1H, br t, 7, 13-H), 6.43 (1H, s, 10-H); MS(FAB) *m/z* (rel.int.) 681(MH^+,40), 639(MH^+-Ac, 40), 621(MH^+-ACOH, 50), 603(621-H_2O, 55) 561(621-HOAc, 30), 531(561-CH_2O, 20), 501(561-HOAc, 40), 459(501-Ac, 60),441(501-60, 60), 371(100); (**b**) **2,13-diacetate (64) and 2,10,13-triacetate (65) mixture** (1:2, 3.1 mg); amorphous solid; ^{1}H NMR ($CDCl_3$, 270MHz) δ(ppm) 0.55 and 0.90 (m, triethylsilyl groups), 1.03, 1.01 and 1.05, 1.02(s, 16-H and 17-H), 1.60 and 1.52(s, 19-H). 1.95 and 2.02(s, 18-H), 2.05, 2.12 and 2.14(s all acetoxy methyls), 3.22 and 3.30(d, 3-H), 3.92 and 4.05(dd, 7-H), 4.32(ABq, 20-H), 4.85(dd, 5-H), 5.12 and 6.30(d, 10-H), 5.37(two overlapping doublets, 2-H), 5.65(m, 13-H for both cpds.) MS(FAB) *m/z* (rel.int.) 639(MH^+,10, for triacetate), 597 (MH^+, 3, triacetate), 580(MH^+-OH, 60, for diacetate), 562(580-H_2O, 60).

Benzoylation of 7-triethylsilyl-13-acetyl-2,4,10-tris(deacyl)baccatin III (63); 7-triethylsilyl-13-acetyl-4,10-bis(deacetyl)baccatin III (67)

7-Triethylsilyl-13-acetyl-2,4,10-tris(deacyl)baccatin III (**63**) (12 mg, 0.022 mmol) dissolved in dry THF (1 mL) under argon was treated with DCC (7 mg, 1.5 eq), benzoic acid (3 mg, 1.1 eq), and a catalytic amount of pyrrolidinopyridine and heated at 55^0 for 72 h. The solvent was blown off with a stream of argon and ethyl acetate was added to the reaction mixture. The precipitated urea derivative was filtered off and the filtrate was washed with HCl (1N, 1 mL). The organic layer thus obtained was washed with 5% aqueous $NaHCO_3$ (1 mL x2) followed by water and brine, and the evaporation of the solvent yielded a crude solid which was purified by PTLC to yield the pure compound, 7-triethylsilyl-13-acetyl-4,10-bis(deacetyl)baccatin III (2 mg, 14%): amorphous solid; ^{1}H NMR, see Table 13; MS(FAB) *m/z* (rel.int.) 659(MH^+, 10), 641(MH^+-H_2O, 100), 623(641-H_2O, 90), 605(623-H_2O).

Acetylation of 7-triethylsilyl-13-acetyl-4,10-bis(deacetyl)baccatin III (67): 7-triethylsilyl-13-acetyl-4-deacetylbaccatin III (68):

7-Triethylsilyl-13-acetyl-4,10-bis(deacetyl)baccatin III (**67**) (5.5 mg, 0.0084 mmol) dissolved in dry THF was treated, under argon, with DCC (5.21 mg, 3 eq), Ac_2O (50 µL, 63 eq) and a catalytic amount of pyrrolidinopyridine. The stirred reaction was heated at 55^0 for 4.5 h; a single product was detected by TLC at the expense of all the starting material. The reaction mixture was maintained, at the same temperature for an additional 8 h; no other product was detected by this time. The solvent was evaporated and dissolved in EtOAc and the precipitated urea derivative was filtered. The concentrated filtrate was purified by PTLC with 10%

isopropyl alcohol/n-heptane to yield 7-triethylsilyl-13-acetyl-4-deacetylbaccatin III (**68**) (5 mg, 89%) as an amorphous solid which could not be crystallized: IR (KBr) 3500(s), 2930(s), 1760(s), 1740(s), 1660(w), 1240(s), 840(m); ^{1}H NMR, see Table 13; MS(FAB) *m/z* (rel.int.) 683(MH^+-H_2O, 10), 665(683-H_2O, 5), 641(MH^+-AcOH, 25), 623(683-AcOH, 25), 611(641-CH_2O, 5), 592(623-CH_2O, 5), 581(641-AcOH, 30), 562(581-H_2O, 10), 501(623-BzOH, 40), 235 (100), 371(60).

7-Triethylsilyl-13-acetylbaccatin III (69)

7-Triethylsilyl-baccatin III (10 mg, prepared by the treatment of baccatin III (**23**) with Et_3SiCl (24 eq) in pyridine (25 mL/ mmol) at room temperature for 20 h) was acetylated by the DCC/pyrrolidinopyridine/Ac_2O method to get 7-triethylsilyl-13-acetylbaccatin III (8.6 mg, 81%) as an amorphous solid: ^{1}H NMR, see Table 13.

Reaction of taxol (5) with Meerwein's reagent; D-*seco*-taxol (70).

To a cooled (0^0) and stirred solution of taxol (**5**) (100 mg, 0.117 mmol) in dry dichloromethane, triethyloxonium tetrafluoroborate (200 μL, 1 M in CH_2Cl_2) from a freshly opened bottle, was added dropwise. After 30 min the reaction was quenched with ethereal HCl (3 mL of 1:2 mixture of 1N HCl:ether) the mixture stirred for 10 min. and organic materials extracted with ethyl acetate. The ethyl acetate layer was washed with water, dried over Na_2SO_4 and evaporated to dryness, and the crude solid was further purified by flash chromatography and PTLC to yield 53 mg (51%) pure material: mp 160-166^0 (amorphous solid); IR (KBr) 1745(s), 1760(s), 1535(w), 1505(w), 1447(s), 1395(m), 1120(m), 1080(m), 1060(m); UV λ_{max} (MeOH) nm(ε) 272(3318), 226(60754); ^{1}H NMR see Table 14; ^{13}C NMR(HetJRES, 50Mz,

$CDCl_3$) δ(ppm) 10.16(q,19), 15.77(q,18), 18.41, 19.46, 20.23(each q, 10-OAc, 20-OAc & 17), 27.42(q,16), 31.04, 34.66(each t,14 & 6), 42.08(s,15), 45.34(d,3), 54.03(d,3'), 60.01(s,8), 63.66(t,20), 68.20(d,13), 71.41, 72.42, 73.16, 74.18, 75.14(each d,-2,7,5,10,2'), 74.14(s,1), 126.19-132.67(aromatics), 133.79(s,11), 134.68(s,1'of Ph at 3'), 138.36(s,1'of NBz), 139.89(s,12), 209.15 (s, 9); MS(FAB) *m/z* (rel.int.) 872(MH^+, 100), 854(MH^+-H_2O, 10), 916(MNa^+, 100), 832(MNa^+-C_3H_5, 50); *m/z* 872.3463 (MH^+; $C_{47}H_{54}O_{15}N$ requires 872.3493).

Acetylation of 70 under mild conditions: 2',7-diacetyl-D-*seco*-taxol (74).

D-*seco*-taxol (**70**) (5 mg, 0.0057 mmol) was dissolved in pyridine (100 μL) and excess acetyl chloride (300 μL) was added to the solution. After 30 min at room temperature, the solution was warmed to 60° for 1 h. The reaction was quenched by addition of water and then usual workup yielded a crude material which was purified on PTLC to yield 3 mg (55%) of pure 2',7-diacetyl-D-*seco*-taxol (**74**) as an amorphous white solid which could not be crystallized: ^1H NMR, see Table 14. Attempt at determination of the mass spectrum of this compound by FAB failed to yield an identifiable molecular ion.

Acetylation of 2',7-diacetyl-D-*seco*-taxol (74): 2',5,7-triacetyl-D-*seco*-taxol (75).

2',7-Diacetyl-D-*seco*-taxol (**74**) (8 mg, 0.008 mmol) was dissolved in THF (750 μL) and to this solution dicyclohexylcarbodiimide (5 mg, 2.5 eq), acetic anhydride (4 μL, 5 eq) and a catalytic amount of pyrrolidinopyridine were added. The stirred solution was heated (60°) for 7.5 h, and the solvent was then evaporated and

the residue extracted into ethyl acetate. The ethyl acetate layer was washed with 1N HCl and water and dried with Na_2SO_4; evaporation of the solvent yielded a crude mixture which was purified by PTLC with 1% MeOH/$CHCl_3$ to yield 3 mg (38% yield at 63% conversion) of 2',5,7-triacetyl-D-*seco*-taxol (**75**): amorphous solid; IR 1740(s) 1720(s), 1675(m), 1625(s), 1225(s); UV λ_{max} (MeOH) nm(ε) 270(3970), 223(18500), ^{1}H NMR, see Table 14; MS(FAB) *m/z* (rel.int.) 998 (MH^+, 13), 980(MH^+-H_2O, 12), 936(6), 848(23), 650(100).

Acetonide of D-*seco*-taxol (76).

A mixture of D-*seco*-taxol (**74**) (6 mg, 0.007 mmol) and 2,2-dimethoxypropane (200 μL) dissolved in dry dichloromethane (500 μL) was treated with a catalytic amount of p-toluenesulfonic acid and stirred for one hour; complete conversion of starting material to the product was observed. The organic layer, with added EtOAc, was washed with water and dried over Na_2SO_4. Evaporation of the solvent yielded a crude product which was further purified by PTLC to obtain 6 mg (95%) of pure acetonide (**76**) as an amorphous solid which could not be crystallized: ^{1}H NMR, see Table 14; UV λ_{max} (MeOH) nm(ε) 270(4850), 227(29200); MS(FAB) *m/z* (rel.int), 916-(MNa^+, 100), 832(MNa^+-C_3H_5, 50), 855(MNa^+-AcOH-H, 25), 832(MNa^+-Ac-C_3H_5, 50), 761(40).

Acetyl chloride product (71).

Taxol (**5**) (200 mg, 0.23 mmol) was dissolved in acetyl chloride (2 mL) and the solution refluxed for one hour. The reaction was quenched with ice water and ethyl acetate and stirred for 30 min, and the ethyl acetate layer was washed with

water and then dried with Na_2SO_4. Evaporation of the solvent yielded a white solid. Recrystallization of this solid from ethyl acetate and hexanes yielded the acetyl chloride product **70** as white needles (150 mg, 68%): mp 140-142°; IR($CHCl_3$) 1750(s), 1660(m), 1606(m), 1372(m), 1282(m), 1156(m); UV λ_{max} (MeOH) nm(ε) 268(3760), 229(44900); ^{1}H NMR, see Table 15; ^{13}C NMR[b] (67.5MHz, $CDCl_3$) δ(ppm) 11.28, 11.29(18 & 19), 20.09-21.05(five OAc methyls & 16), 29.65(6), 38.01(14), 45.37(3), 52.85(3'), 55.59(8), 63.79(1), 64.71(20), 70.30(2), 70.37(10), 70.50(2'), 71.71 & 71.84(7 & 3'), 73.19(4), 74.26(2), 80.36(16), 112.84(16), 126-130(aromatics), 131.92(p-NBz), 133.85(p-OBz), 136.92(12), 137.45(1'of 3'-Ph), 144.27(15), 145.29-(11), 165.99, 167.16, 167.63, 169.29. 169.60, 170.07 (ester carbonyls), 201.26(9); MS(FAB) *m/z* (rel.int.) 1002(MNa^+, 35), 676(MNa^+-side-chain, 15), 616(676-AcOH, 30), 554(676-PhCOOH, 20), 494(616-PhCOOH, 30), 411(24), 372(24), 177(100); *m/z* 1002.3557 (MNa^+; $C_{53}H_{57}NO_{17}Na$ requires 1002.3524)

Formation of 71 from D-*seco*-taxol (74).

D-*seco*-taxol (**74**) (50 mg, 0.057 mmol) was dissolved in chloroform and a catalytic amount of pyrrolidinopyridine (3 mg) and excess acetyl chloride (5 eq) were added; excess Et_3N was then added dropwise to the stirred solution at room temperature. A red color developed which disappeared when more acetyl chloride was added; Et_3N and acetyl chloride were added until a single major product was obtained. The reaction was stopped after a total reaction time of 5 h, by addition of 3 mL of water and stirring for 30 min. The mixture was extracted into ethyl acetate,

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[b] peaks were assigned by INEPT and HetCOSY experiments

the ethyl acetate layer was washed with 3 N HCl and again with water, dried (Na_2SO_4) and evaporated to dryness. The mixture of products obtained was subjected to PTLC to obtain the major component as a white solid (10 mg, 18%). This compound was identical (^{1}H NMR, MS and IR) to the acetyl chloride product **71**.

Hydrogenation of acetyl chloride product 71 to its dihydro derivative 96.

The acetyl chloride product **71** (24 mg, 0.023 mmol) was dissolved in ethyl acetate (2.5 mL) and hydrogenated over Pd/H_2. After 24 h the catalyst was filtered off and the solvent which evaporated to yield a crude solid which consisted of product and unreacted starting material, which was not separable from the product. The crude product was dissolved in dichloromethane and treated with m-chloroperbenzoic acid (58%, 5 mg) at room temperature for 3 h to convert the starting material to its separable epoxide. The solvent was evaporated and the residue subjected to PTLC with 4% MeOH/$CHCl_3$ to yield pure dihydro compound (8 mg, 35%) along with a mixture of diastereomeric epoxides (11 mg). Compound **96** was recrystallized from ethyl acetate and hexanes: mp 148-150^0; IR(KBr) 1740(s), 1720(m), 1640(m), 1220(m), 910(m); UV λ_{max} (MeOH) nm(ε) 268(9060), 224(59700); ^{1}H NMR, see Table 15; MS(FAB) *m/z*,(rel.int) 1004(MNa^+,100), 962-(MNa^+-C_3H_6, 10), 944(MNa^+-AcOH, 15).

2',7-Bis(triethylsilyl)-taxol (104).

To a solution of taxol (**5**) (200 mg, 0.234 mmol) in DMF (2.5 mL) under argon was added solid imidazole (238 mg, 10 eq). Triethylsilylchloride (196 μL, 10 eq) was added to the stirred solution at room temperature in one portion and the

solution was warmed to 45-50°. Reaction was complete after 2 h, and the solution was diluted with water and extracted with ethyl acetate. The crude solid obtained after evaporation of the solvent was purified on a silica gel flash column to yield 242 mg (96%) of pure 2',7-bis(triethylsilyl)taxol (**104**): mp 122-123°; IR (KBr) 1740(s), 1740(s), 1660(s), 1640(m), 1240(s), 810(m); ^{1}H NMR, see Table 15; MS(FAB) *m/z* (rel.int) 1104(MNa^+-H, 100), 1004(30), 982(MNa^+-PhCOO, 10).

2',7-Bis(triethylsilyl)-A-*nor*-taxol (106).

A solution of silyl derivative **104** (30 mg, 0.028 mmol) in dry dichloromethane (3 mL) was cooled to -15° in ethylene glycol and treated with triethyl amine (600 μL, 154 eq), followed by mesyl chloride (300 μL, 138 eq) in dichloromethane (1 mL) during 5 min, under Argon. The system was allowed to warm to -5- 0° and was maintained at this temperature for a total reaction time of 2.5 h; 50% conversion of the starting material was observed at this point. The solution was cooled again to -15° and additional amounts of Et_3N (1 mL) and MsCl (500 μL) were added. This procedure was repeated one additional time and the reaction was then stopped by adding 2 mL Et_3N, water (5 mL) and EtOAc (5 mL). The aqueous layer was extracted several times with EtOAc and the combined organic layer was washed with water. Evaporation of the solvent yielded a crude material which was purified by PTLC to yield 6 mg (20%) of 2',7-bis(triethylsilyl)-A-*nor*-taxol (**106**) as an amorphous solid which could not be crystsallized, along with some starting material (2 mg). Some deprotection of the 2'-silyl group occurred during the reaction: ^{1}H NMR, see Table 16; MS(FAB) *m/z* (rel.int.) 1086(MNa^+, 45), 1064(MH^+, 75), 1005(MH^+-OAc, 25),

975(MH^+-OAc-CH_2O, 15), 963(MH^+-OAc-C_3H_6, 15), 820(MH^+-PhCOOH-$PhCONH_2$-H, -100).

Deprotection of 2',7-bis(triethylsilyl)-A-*nor*-taxol (106): A-*nor*-taxol (72)

A sample of the protected A-*not-taxol (*106) (67 mg, 0.07 mmol) of was dissolved in dry THF (1 mL) under argon. The solution was cooled to 0^0 and treated with pyridinium hydrofluoride (70% in pyridine, 100 μL). After 3 h the cooling bath was removed, and the reaction was allowed to proceed for an additional 45 h at room temperature. Then the reaction was quenched with aqueous pyridine (10% v/v pyridine, 2 mL) and the resulting mixture was extracted into ethyl acetate. The ethyl acetate layer was washed with water followed by brine and dried over anhydrous Na_2SO_4. A crude solid isolated after the evaporation of ethyl acetate was purified by PTLC with 8% MeOH/$CHCl_3$ as the eluent. A-*nor*-taxol (**72**) was obtained (29 mg, 55%) as a amorphous white solid which could not be crystallized: IR (KBr) 3450(s), 2950(s), 1740(s), 1735(s), 1640(s), 1600(w), 1235(s), 1100(s), 980(w), 860(w), 710(s); UV λ_{max} (MeOH) nm(ε) 272(28500), 272(2710); ^1H NMR, see Table 16; MS(FAB) *m/z* (rel.int.) 836(MH^+, 100) , 776(MH^+-AcOH, 30) 551(MH^+-side-chain-H, 10,); *m/z* 836.3272 (MH^+; $C_{47}H_{50}NO_{13}$ requires 836.3282)

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APENDIX

Table A. Biological activity of some taxanes

Compound	Structure number	ED_{50} (μg/mL) in KB cell culture [a]
Taxol	**5**	1×10^{-5}, 1.2×10^{-3} [b]
Baccatin III	**23**	2.0
Decinnamoyltaxinine J	**9c**	2.0
10-Deacetylbaccatin III	**22c**	1.0
7-*epi*taxol	**27a**	3×10^{-5}
Cephalomannine	**25**	3.8×10^{-3}

[a] data from reference 93

Table B. **Biological activity of acyl and ether derivatives of taxol.**

Compound	Structure	ED_{50} (μg/mL) in number KB cell culture [a]
Taxol	**5**	1×10^{-5}
2′-Acetyltaxol	**36**	2×10^{-5}
2′,7-Diacetyltaxol	**37**	2×10^{-1}
7-Acetyltaxol	**35**	4×10^{-3}
2′-succinyltaxol	**108**	1×10^{-2}
2′-(t-Butyldimethylsilyl)taxol	**107**	3×10^{-1}
10-Deacetyltaxol	**26h**	2.7×10^{-2} [b]

[a] Data from reference 47.
[b] Data estimated from reference 31.

VITA

Gamini Samaranayake was born in March 20, 1955 in Sri Lanka. He obtained his B.S. degree in 1979 from the University of Peradeniya, Sri Lanka. In 1985 he received an M.Phil. degree from the same university in Marine Natural Product Chemistry. Between Spring 1984 and Fall 1985 he worked as a research assistant in the Peradeniya Center of the WHO Research Program, after which he enrolled in the Ph.D. program in chemistry at Virginia Polytechnic and State University (U.S.A.) in 1985. He was awarded the Ph.D. degree in May 1990. He was married to Deepani Kotalawala on August 22, 1985. Presently he is working as a Research Associate in the Department of Wood Science and Forest Products at Virginia Polytechnic and State University.