APPROACHES TO THE SYNTHESIS OF MODIFIED TAXOLS

by

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(Abstract)

Investigation on the synthesis of the C-13 side chain of taxol was carried out in order to prepare modified taxol derivatives by coupling of the side chain acid chloride to a suitably protected baccatin III. The side chain was prepared by the Darzens condensation. Acylation of baccatin III was performed with simple acylating agents and extensive studies of the ^{1}H NMR and ^{13}C NMR spectra of various acylbaccatins III were carried out aided by homonuclear and heteronuclear COSY experiments. This work led to the unambiguous assignment of the ^{1}H NMR and ^{13}C NMR spectra of these compounds. Coupling of more bulky side chains to 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III was difficult and yields were poor. Conventional methods, using triethylamine or pyridine with 4-dimethylaminopyridine in the coupling reaction of 3-phenylpropanoyl chloride and 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III led to the desired coupled product in low yield together with two coupled compounds possessing more than one phenylpropanoyl group on the C-13 side chain. When the coupling reaction was performed in the presence of silver cyanide in refluxing toluene, only 13-(3-phenylpropanoyl) baccatin III was obtained. However, these two

methods were not successful in the coupling reaction of 2-acetyl-3-phenyllactyl chloride with 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III. Preliminary studies on the cleavage of the N-acyl group at the C-3′ position of taxol and cephalomannine were performed. Taxol reacted with zinc bromide in chloroform-methanol solution to produce 10-deacetyl-7-epitaxol and 10-deacetyltaxol. No cleavage of the N-acyl group was detected in this case and in other reactions in which taxol was treated with various selective reagents. Other attempts involved the conversion of cephalomannine to its ozonolysis products with a pyruvyl group at the 3′-NH group. A method of cleavage of the N-pyruvyl group has not yet been found, however.

ACKNOWLEDGEMENT

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Finally, I would like to express my sincere appreciation to my parents, my wife, Charumal, and my family for their support and understanding during my graduate studies and also to Judy Duston for typing this manuscript.

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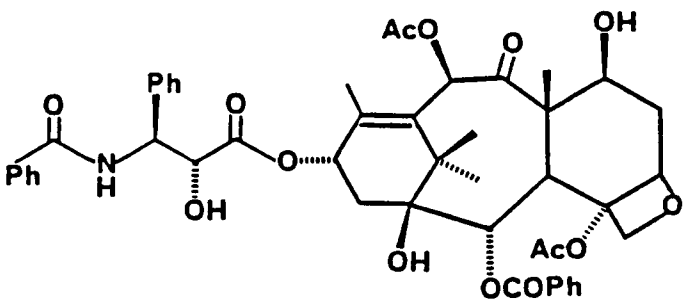
1.0 INTRODUCTION

1.1 Purpose

The purpose of this research is to establish the structure-activity relationships of the anti-cancer agent taxol (1). Modified taxol from structural modification of the C-13 ester side chain and of other positions of taxol will be synthesized and their biological activities determined in order to establish partial structure-activity correlations for this important compound.



Taxol (1)

1.2 Natural Products In Cancer Chemotherapy

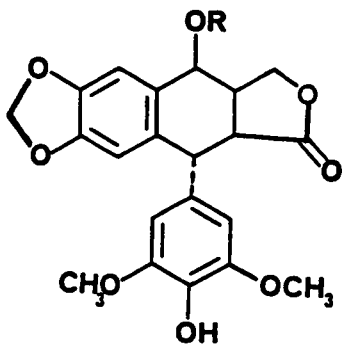
Drugs from plants and animals have played important roles in the treatment of various diseases for several hundred years. Some one-

fourth of useful drugs currently used in Western medicine are derived from plants, for example, aloe, belladonna, cinchona, colchicum, digitalis, ergot, periwinkle and rauwolfia,[1] are among a large number of very useful and classic plant drugs.[2] The National Cancer Institute Program for discovery of new and clinically useful anticancer drugs from plant has demonstrated that 3-4% of plant species produced a great variety of anticancer agents of very diverse structural types.[3,4,5,6]

Anticancer drugs isolated from plants have been more and more important in cancer chemotherapy. In 1982 the curative treatment of cancer patients by chemotherapy, with plant-derived drugs, reached nearly 50,000 cases.[7] Major advances are now being made in the cancer chemotherapeutic treatment of solid tumors such as bladder, cervical, esophageal, head and neck, lung, and ovarian cancers.

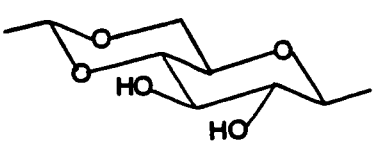
Historically, plant species of the Berberidaceae family have been used for treatment of warts and solid tumors in China and India for at least 1,700 years. In the United States, an American species, Podophyllum peltatum L. has been used for analogous purposes for over 200 years. In 1950, Hartwell found that the active constituent from P. peltatum, podophyllotoxin (2), was active against the sarcoma 37 test system.[8] Because of drug formulation problems due to its low solubility and a low therapeutic index, the clinical study was not pursued. However, two especially useful derivatives of podophyllotoxin known as VP-16-213 (3) and VM-26 (4) were prepared and found to be important anticancer drugs.[9] VP-16-213 led to a 40% response rate in patients with small cell lung cancer and was also found useful in many other types of cancer.

Many other anticancer drugs from plants and microorganisms, for example vincristine (5), vinblastine (6), adriamycin (7), and daunomycin (8), are also in widespread use.[10]

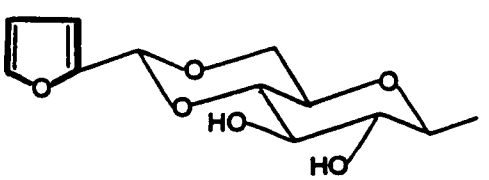


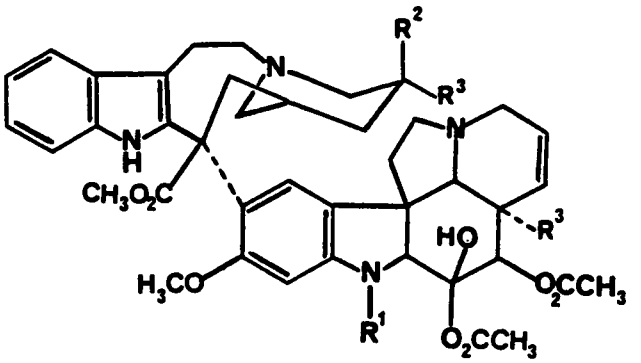
Podophyllotoxin (2), R = H

VP-16-213 (3), R =



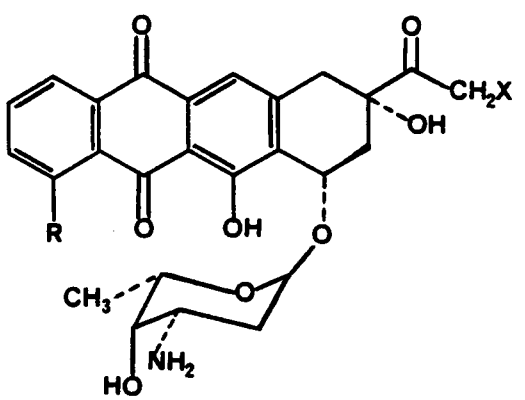
VM-26 (4), R =





Vincristine (**5**), R^1 = CHO; R^2 = OH; R^3 = C_2H_5

Vinblastine (**6**), R^1 = CH_3; R^2 = OH; R^3 = C_2H_5



Adriamycin (**7**), R = OCH_3; X = OH

Daunomycin (**8**), R = OCH_3; X = H

With the continuing discovery of new antineoplastic and/or cytotoxic drugs from plants, microorganisms, and animals, it is probable that new and improved clinically effective drugs will also continue to be developed.

1.3 Review of the Literature

1.3.1 Taxol and Taxane Derivatives

The compounds of plants in the Taxaceae have been studied for over a hundred years. In 1856, Lucas[11] reported the isolation of an alkaloid called taxine from the needles and other parts of the English yew, Taxus Baccata L. It was later found to be a mixture of many alkaloids.[12,13,14] In 1963, Taylor reported the isolation of a compound called baccatin from the heartwood of T.baccata.[15] Taylor's compound was later named baccatin I by Halsall[16] who himself isolated baccatin II, baccatin III, and baccatin IV.

Between 1967 and 1970, many taxane derivatives were isolated from various yew plants.[17] Among these, only the baccatin-type compounds have the unique oxetane ring at C-4 and 5, other derivatives either have an exocyclic double bond at C-4 or a methylene at this position.

In 1971, Wani and co-workers,[18] guided by a biological activity test, isolated taxol (1) from the stem bark of the Pacific yew, T. brevifolia. Taxol is also found in other Taxus species, such as T. baccata and T. cuspidata.[18] It possesses important antileukemic and antitumor activities and is currently in Phase II clinical trial at the National Institutes of Health. The structure of taxol, numbered according to the IUPAC system is shown in Figure 1.

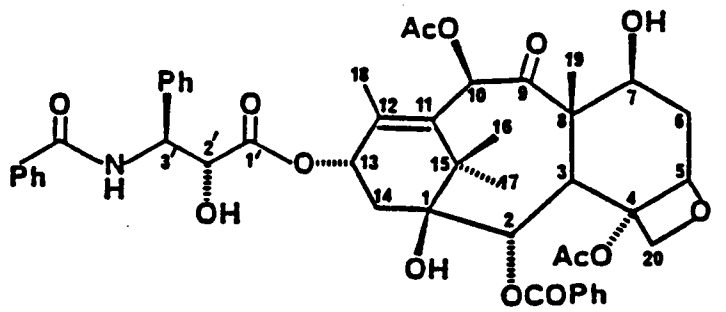
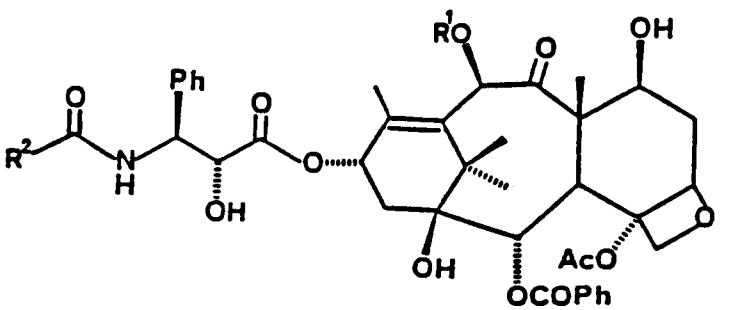


Figure 1. Structure of Taxol (1)

In 1979, a new antitumor alkaloid called cephalomannine (9), was isolated from Taxus wallichiana by Powell et al.[19,20] and was found to be cytotoxic in KB cell culture and also showed potent inhibition of PS leukemia in mice. Two new taxane derivatives, 10-deacetyltaxol (10) and 10-deacetylcephalomannine (11) were also isolated from Taxus wallichiana by McLaughlin et al.[21]



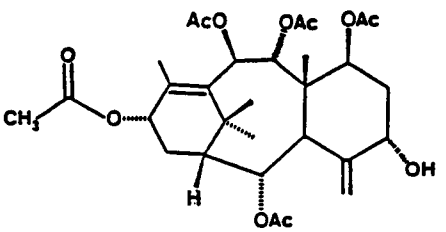
Cephalomannine (9), R^1 = $COCH_3$; R^2 = $CH_3CH{=}C(CH_3)-$

10-Deacetyltaxol (10), R^1 = H; R^2 = Ph

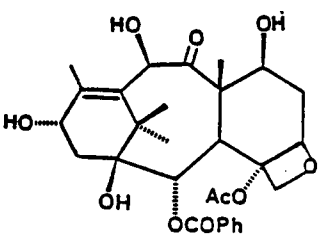
10-Deacetylcephalomannine (11), R^1 = H; R^2 = $CH_3CH{=}C(CH_3)-$

10-Deacetyltaxol and 10-deacetylcephalomannine were reported to be especially labile, each forming equilibrium mixtures with their epimers at C-7.

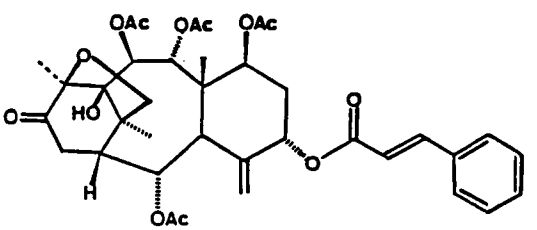
In 1982, Kingston and co-workers [22] isolated two new taxane derivatives form Taxus brevifolia. They were decinnamoyltaxinine J (12) and 10-deacetylbaccatin III (13). Another new taxane derivatives, taxagifine (14), was isolated from the ethanolic extracts of the leaves of Taxus baccata L. by Chauviere et al.[23] Taxagifine possesses a unique structure among these taxane derivatives by having an O-bridge between C-12 and C-16, and in addition it lacks the oxetane ring at C-20 and C-5. It was less active than taxol.



Decinnamoyltaxinine J (12)



10-Deacetylbaccatin III (13)



Taxagifine (14)

In 1984, Senilh et al [24] isolated taxol, cephalomannine and several new taxane derivatives from the trunk bark of Taxus baccata L. These new compounds all have a xylose unit at C-7.

Due to the important antileukemic and cytotoxic activity and the unique structure of taxol, several research groups have been engaged in synthetic approaches to this compound.[25-43] Semisynthesis of some taxane derivatives has recently been reported.[44]

1.3.2 Structure of the Taxane Skeleton

Taxol and other taxane derivatives have a relatively complex structure. It was established by a combination of chemical, spectroscopic studies, and x-ray crystallographic techniques.[18,45]

Taxol has a complex caged structure with the six membered ring A existing as a distorted boat form, cis-fused to the eight membered ring B which has a boat-chair conformation. The six-membered ring C has a boat conformation and is trans-fused to ring B. The oxetane ring D is essentially planar and sits on the top face of the cage structure. Ring A and C which fold back together are almost perpendicular to the plane of ring B. The three dimensional structure of the taxane skeleton is shown in Figure 2.

Senilh et al [44] reported the formation of intramolecular hydrogen bonding between the carbonyl oxygen of the acetate at C-4 and the hydrogen atom of the hydroxyl group at C-13. The distance was found to be 2.50 A° by the molecular mechanics method.

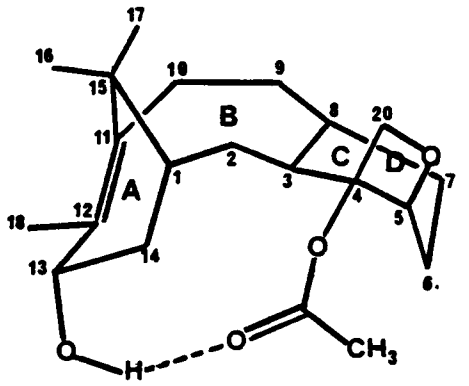


Figure 2. Three-Dimensional Structure of the Taxane Skeleton

1.3.3 Nuclear Magnetic Resonance Spectra of Taxol and Taxane Derivatives

Considering the complex structure of taxol and other taxane derivatives, their proton NMR spectra are relatively simple and can be easily recognized. Most signals are well spread over the region from 1.0 to 8.2 ppm. Their spectra can be generally divided into three regions: the first region between 1.0 and 2.5 ppm consists of strong three-proton singlets of the methyl and acetate groups, together with complex multiplets for certain methylene groups. In the second region between 2.5 and 7.0 ppm, the signals of most of the protons on the taxane skeleton and the side chain(s) are observed. The aromatic protons of the C-2 benzoate, C-3′ phenyl and C-3′ benzamide groups appear between 7.0 and 8.3 ppm. The 270 MHz proton NMR of taxol is shown in Figure 3

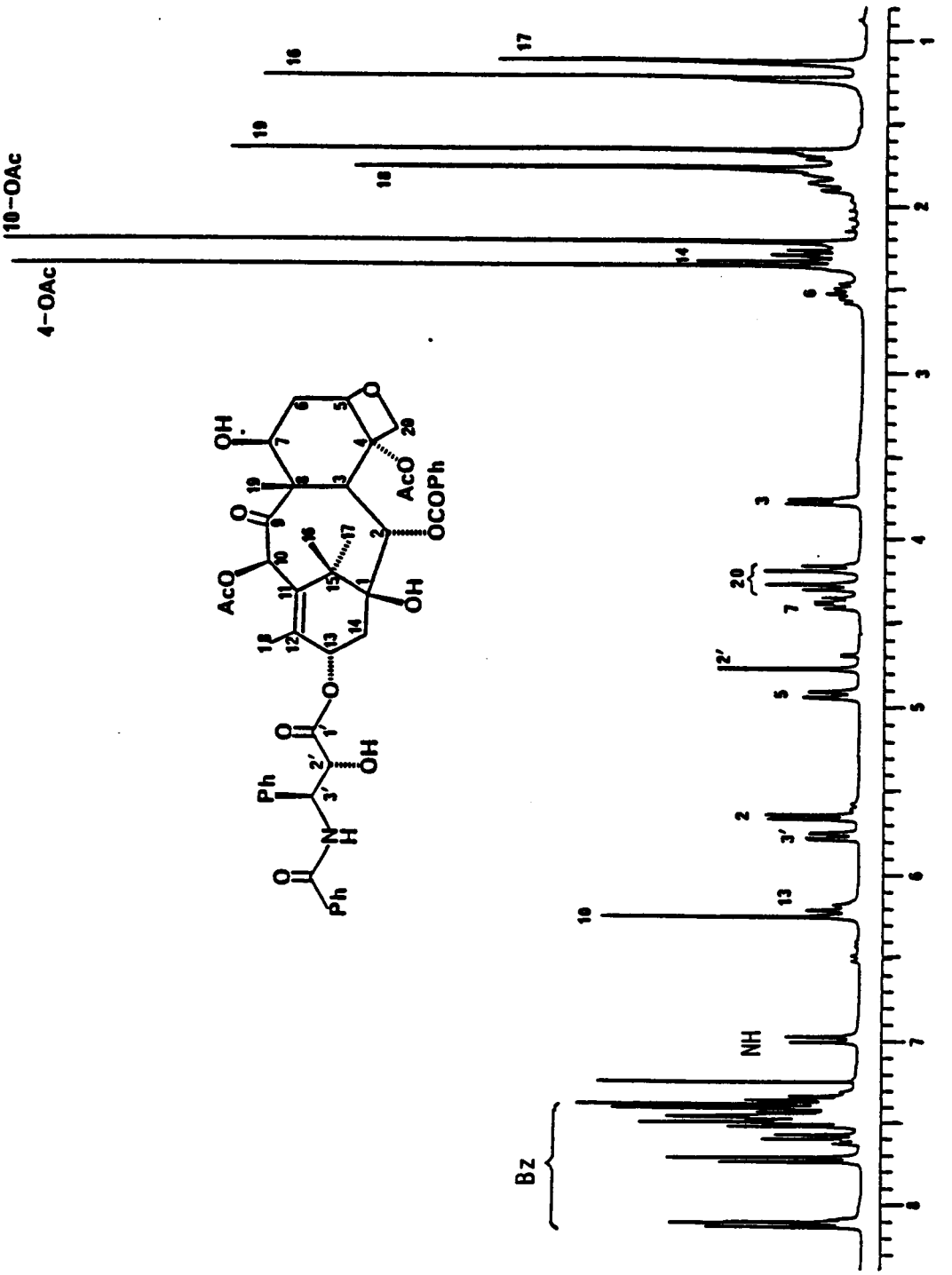


Figure 3. ^{1}H NMR Spectrum of Taxol (1)

Peak shapes and chemical shifts are always good criteria for the structure of taxane derivatives. In taxol, sharp intense singlets belong to the methyl of the acetate groups at the C-4 and C-10. Signals of other methyl groups are generally less intense. Long-range coupling of the C-18 methyl protons to the C-13 proton causes the C-18 singlet to be broader than the C-19 signal.[45] Similarly, the C-17 signal is also broader than the C-16 methyl singlet. The other singlet belongs to the C-10 proton which appears at 6.25 ppm. The C-2 and C-3 proton signals are seen as doublets at 5.65 and 3.75 ppm respectively. The other doublet at 4.71 ppm is assigned to the C-2′ proton of the C-13 side chain with 3 Hz coupling constant. The fourth doublet represents the C-3′ NH signal and is seen at 7.0 ppm.

The signals for the C-3′ and C-7 protons are both seen as doublets of doublets at 5.8 and 4.4 ppm respectively. The only AB quartet belongs to the C-20 of the oxetane ring with the coupling constant of 8-9 Hz. When this oxetane ring is cleaved the coupling constant generally increases to about 12 Hz while the peak shape is unchanged. The β-proton of C-13 is seen as a broad triplet at 6.15 ppm, its long-range coupling with the C-18 methyl protons can be observed by its 2 Hz coupling constant. The chemical shift of this triplet is a good criterion for the presence of an ester group at the C-13 hydroxyl group of baccatin III or a 7-protected baccatin III. This is also true for the esterification of the C-2′ and C-7 hydroxyl groups, where their doublets will shift downfield.

Only a few ^{13}C NMR spectra of taxane derivatives have been reported.[21,46] In the course of our investigation, the ^{13}C NMR spectra of taxane derivatives are always recorded where sample quantities

permit. Peak assignments are possible with the aid of the INEPT experiment, and in some cases with two-dimensional NMR experiments.

1.3.4 Mass Spectra and Other Physical Data

Mass spectral data of taxane derivatives have been reported in most recent publications. Fast Atom Bombardment (FAB) mass spectrometry is especially useful in revealing the molecular ion of these high molecular weight compounds.

In the mass spectra of taxol or other taxane derivatives with the C-13 ester side chains, intense peaks result from the cleavage of the C-O ester linkage are always observed. Fragments corresponding to subsequent cleavages of other ester functional groups on the taxane skeleton are also seen.

Other physical data such as infrared and ultraviolet spectra as well as specific rotation are usually reported for the taxane derivatives, although they are generally less informative than the NMR and MS data.

1.4 Mechanism of Action of Taxol

Taxol possesses confirmed activity in the L-1210, P-388, and P-1534 leukemia, the B-16 melanoma, and the Walker 256 carcinosarcoma *in vivo* assays and also shows strong cytotoxicity in KB cell culture [18,3] In 1978, Fuchs, and Johnson reported that taxol has antimitotic activity similar to that observed with vinca alkaloids and maytansine. [47] Schiff et al. showed that taxol induces microtubule assembly *in vitro* and in living cells.[48,49]

Taxol acts as the promoter for microtubule formation by decreasing

the lag time for microtubule assembly and shifting the equilibrium in favor of the microtubule.[48] The rate and extent of polymerization was increased and the microtubules were relatively resistant to depolymerization by cold and calcium chloride. In living cells, taxol was reported to be a potent inhibitor of HeLa and mouse fibroblast replication.[49] It was proposed that the inability of the cells to form mitotic spindles in the presence of taxol could be due to the fact that the cells were unable to depolymerize their microtubule cytoskeletons. The optimal effect of taxol on in vitro polymerization and stabilization of microtubules were observed near stoichiometric equivalent of tubulin dimers.

De Brabander et al[50] observed that taxol apparently induced the assembly of free microtubules in the cytoplasm, not attached to the centrosomes or kinetochores. They found that those preexisting microtubules, attached to the organizing-centers, were not stabilized and gradually disappeared. The centrosomes and kinetochores largely lost their capacity to organize microtubule assembly, this is evident from the disappearance of the cytoplasmic microtubule complex and the mitotic spindles. Taxol, therefore, apparently blocks the organizing capacity of microtubule-organizing centers by decreasing the critical tubulin concentration.

It was later found that taxol can induce microtubule assembly in the absence of microtubule-associated proteins.[51,52] Again, the optimum assembly occurred at approximately equal concentrations of tubulin and taxol. In the binding experiment, no competition was observed between

taxol and microtubule-associated proteins or other tubulin binding drugs such as colchicine, podophyllotoxin, and vinblastine, and it is concluded that taxol must bind to another site not shared by these drugs.

Due to its important biological activity and unique mechanism of action, taxol and its derivatives have been subjected to extensive investigation by several research groups. It can be expected that many interesting results will be reported soon.

1.5 Biological Activity of Taxol and Related Compounds

Taxol and several related compounds possess biological activities in various assays. Three important assay systems, the P-388 *in vivo* lymphocytic leukemia system, the 9KB cell culture system, and the microtubule assembly and binding assays, are usually performed to investigate the activity of these compounds. The last assay systems are the best assays of the action of taxol at the cellular level because they give the information about the intrinsic activity of taxol and related compounds.

Previous investigations by several research groups [18,20,22,53,55] have established some general trends in the structure-activity relationships of taxol and related compounds. It is agreed that activity in all systems requires the presence of the C-13 ester side chain. The intact taxane ring alone is not sufficient for cytotoxic and *in vitro* microtubule assembly activity since baccatin III **(15)** and its 9-hydroxy derivative are inactive both *in vitro* and *in vivo*.

Kingston et al[54] have shown that the presence of the acetyl group

at C-10 has some effect on the P-388 activity but compounds lacking this group still possess *in vivo* activity at higher doses and also show microtubule assembly and binding activity. The hydroxyl group at C-2′can be acetylated and the product still possesses P-388 *in vivo* activity at higher dose than taxol itself, but it loses the *in vitro* activity. Acetylation at both C-2′ and C-7 gave a product which lost its *in vitro* activity but not cytotoxicity. It was later reported that the properties of 7-acetyltaxol in its effects on cell replication and on *in vitro* microtubule polymerization are similar to those of taxol, and therefore the free hydroxyl group at C-7 is not required for the *in vitro* activity and might be subjected to structural modifications.[55]

The fact that both taxol and cephalomannine, which differ only at the C-3′ aminoacyl functional group, show comparable activities both *in vivo* and *in vitro* indicates that the C-13 ester side chain can be modified to some extent. It was the major purpose of this research to make a series of compounds with different acyl group at the C-3′ amino group. It is proposed that modification at the C-3′ aminoacyl function will cause significant activity changes in taxol and related compounds and may lead to the better understanding of the structure-activity relationships of these important anticancer drugs.

2.0 SYNTHESIS OF 3-PHENYLISOSERINE

2.1 Introduction

In order to accomplish the goal of this reseach which is the determination of the structure-activity relationships of taxol, a series of modified taxol must be obtained. Since taxol is a rather complex molecule with relatively high functionalities, sites of modification must be considered first.

The two major modification possibilities are acylation of C-7 or modification of the C-13 ester side chain of taxol. This is the result of earlier studies which showed that acetylation at the 2′-position or at the 2′ and 7-positions gave inactive acetates using tubulin assembly promotion assay, while acetylation at C-7 hydroxyl group yielded an active acetate.[55] These results indicated that modification at C-7 is a possible route, but that modification at C-2′ would be less likely to yield active derivatives unless the derivatives were to be readily hydrolysable *in vivo*.

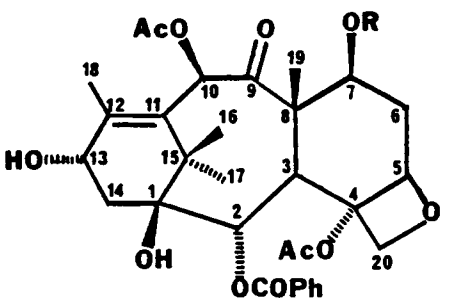
It was shown from the study of the activity of various taxol derivatives that the C-13 ester side chain is necessary for the *in vivo* activity of taxol. It has been suggested that the activity of taxol may be due to the easily-cleaved allylic α-hydroxyester at C-13 which may act as a leaving group during the physiological process.[18]

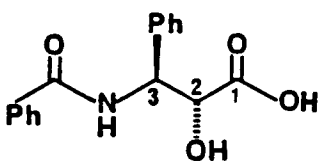
Modification of the C-13 ester side chain of taxol can be done at one of several sites. First, the C-2′ hydroxyl group can be selectively acylated and it has already been reported that 2′-acetyltaxol still possesses cytotoxicity at a somewhat lesser extent than taxol itself.

Several 2′-acyltaxols were synthesized and were shown to be active,[55] presumably because 2′-acyltaxols are readily hydrolysed to taxol under mild conditions.

An even more attractive structural modification of the C-13 ester side-chain involves the C-3′ acylamino group. Since both taxol and cephalomannine, which differ only in their acylamino groups at C-3′, are comparably active, it follows that the nature of this group is not totally significant for the activitiy of modified taxol compounds and certain acyl groups might lead to more active taxol-like compounds.

The overall strategy thus consists of the synthesis of (2R,3S)-3-phenylisoserine (**17**), its acyation with various acylating agents to yield the N-acyl derivatives, and its çoupling with a suitably protected baccatin III (**16**) to yield a protected taxol derivatives which can then





(17)

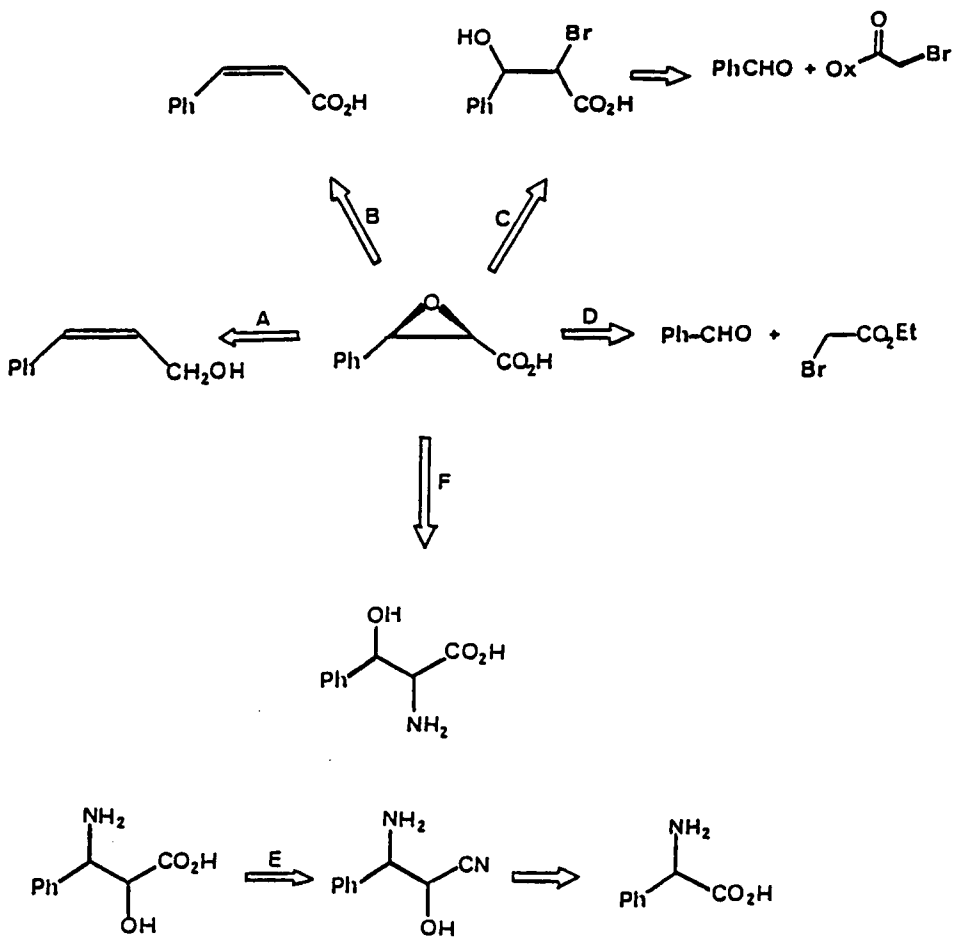
Baccatin III, **15**, **R** = **H**

16, **R** = protecting group

be deprotected to yield either taxol itself or a taxol derivatives. One advantage of this strategy is that only one chiral amino acid (**17**)

needs to be synthesized, since it can be acylated with various acid chlorides at will.

This chapter will address the attempted syntheses of (2R,3S)-3-phenylisoserine (17) by various method (scheme 1). There are five possible pathways to cis-phenylglycidic acid with correct stereochemistry. Five of these six routes have the same epoxy carboxylic acid as a key intermediate.



Scheme 1

Route A involves the chiral Sharpless epoxidation method[56-58] in the conversion of cis-cinnamyl alcohol to the corresponding epoxide. Oxidation of the alcohol to the acid and treatment of the acid with ammonium hydroxide will open the epoxide ring to yield 3-phenylisoserine with correct stereochemistry.

Epoxidation of trans-cinnamic acid by potassium peroxomonosulfate (or potassium caroate) in acetone was reported by Edwards et al.[59] This method will also be discussed as shown as route B in scheme 1.

The modified aldol condensation of benzaldehyde and chiral α-haloimidate in the presence of dialkyl boron triflate or tin (II) triflate was studied by Evans[60] and also reported by Lantos.[61] It is also shown in Scheme 1 as route C and will be discussed in detail.

The Darzens condensation[62] which gives racemic ethyl cis-phenyglycidate was used in preparing the C-13 side chain acid. The recemic acid can be readily resolved by chiral resolving reagent such as ephedrine (see route D in scheme 1).

Route E in scheme 1 represents the conversion of phenylglycine to the aldehyde. Umezawa[63] reported the synthesis of (2R)-N-carbobenzoxy-(-)-phenylglycine aldehyde in 9% yield by lithium aluminum hydride reduction of N-carbobenzoxy -(-)-phenylglycine-3,5-dimethylpyrazolide.

A brief discussion on the preparation of the quaternary ammonium salt of threo-β-phenyl-L-serine[65] and subsequent attempted ring closure to the epoxide will also be presented in the next section.

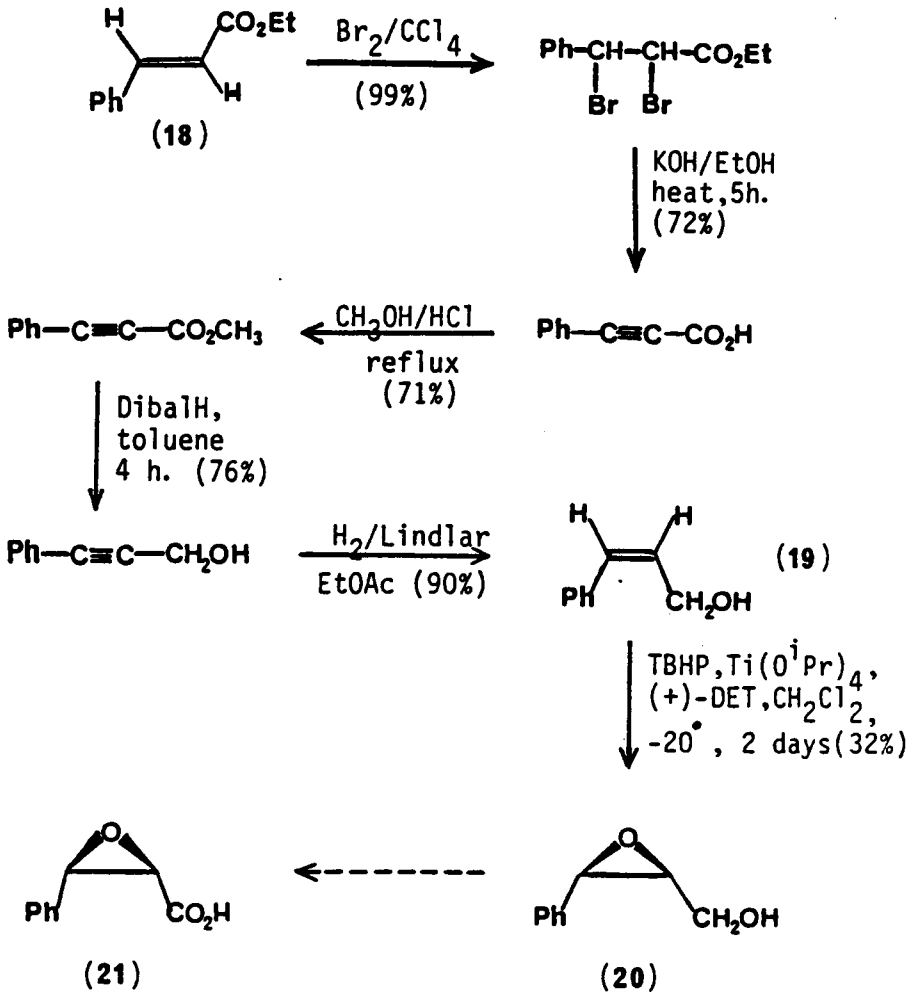
2.2 Results and Discussion

2.2.1 Synthesis of 3-Phenylisoserine via the Sharpless Epoxidation

In the preparation of the modified side chain of taxol, the key intermediate in several routes is (2R,3R)-cis-phenylglycidic acid **(21)**. Our first attempt to synthesize this compound utilized the asymmetric epoxidation discovered by Sharpless and co-workers.[56-58] Scheme 2 shows the reaction scheme in detail.

(2S,3R)-cinnamyl alcohol epoxide **(20)** was prepared in 6 steps from trans-ethyl cinnamate. Trans-ethyl cinnamate was first brominated in quantitative yield to ethyl α,β-dibromo-β-phenylpropiolate.[66] Dehydrobromination of the dibromo compound yielded phenylpropiolic acid in 72% yield.[67] Reduction of methyl phenylpropiolate by diisobutyl aluminum hydride gave phenylpropagyl alcohol in 76% yield.[68] This alcohol was then hydrogenated over Lindlar's catalyst to cis-cinnamyl alcohol **(79)** in 90% yield.[69]

Epoxidation of cis-cinnamyl alcohol by the Sharpless procedure[56-58] using (+)-diethyl tartrate, tert-butyl hydroperoxide (TBHP), and titanium tetra-isopropoxide gave (2S,3R)-cinnamyl alcohol epoxide **(20)** in 32% yield. The percent enantiomeric excess of the epoxide was determined by preparing the Mosher ester of the chiral epoxy alcohol **(20)** and also of the racemic cis-epoxy alcohol.[70] The proton NMR spectra of the Mosher esters of the chiral and racemic cis-epoxy alcohols showed different chemical shifts in the 3.3 to 3.4 ppm region and in the 3.7 to 4.1 ppm region. Signals in the 3.3-3.4 ppm region were well resolved and showed larger intensity difference, therefore they were used in the calculation of the percent enantiomeric excess, which was found to be 78.4 percents (see figure 4).



Scheme 2

Synthesis of (2R,3R)-cis-Phenylglycidic Acid (21)

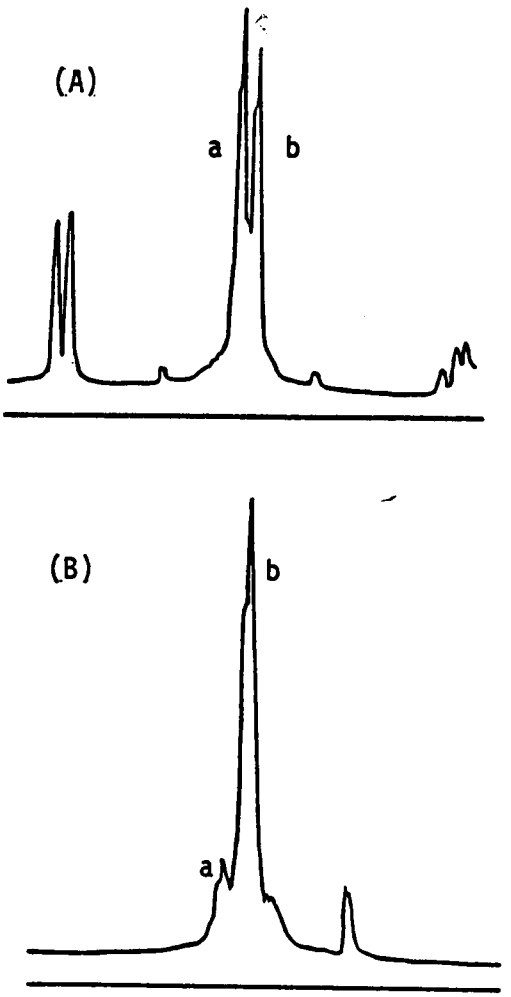


Figure 4

^{1}H NMR Spectra of the Mosher Esters of Racemic Cis-Cinnamyl Alcohol Epoxide and (2S,3R)-Cinnamyl Alcohol Epoxide

(A) Racemic cis-Cinnamyl Alcohol Epoxide

(B) Chiral cis Cinnamyl Alcohol Epoxide

(a) Methoxy signal of (2R,3S)-Form

(b) Methoxy signal of (2S,3R) Form

The oxidation of cis-cinnamyl alcohol epoxide (20) to the corresponding acid (21) with various oxidizing agents was unsuccessful. The following reagents were attempted: $RuCl_3$-$NaIO_4$ in $CH_3CN/CCl_4/H_2O$,[71-72] aqueous $KMnO_4$,CrO_3-AcOH, CrO_3-pyridine, Tollens' reagent, AgO, $KMnO_4$-AcOH, N-chlorosuccinimide-dimethyl sulfide, and the Swern oxidation. The failure of the oxidation may be due to the sensitivity of this molecule to oxidation. Benzoic acid was isolated in several cases along with a large number of side products and no desired epoxy acid was observed at any time by TLC or proton NMR techniques. It has been reported that the epoxy acid was unstable at room temperature and readily decomposed to pheylacetaldehyde.[62]

Another attempt to make the epoxy acid was made by converting the epoxy alcohol to the aldehyde and subjected this to mild oxidation. Racemic trans-cinnamyl alcohol epoxide was prepared and converted to the corresponding aldehyde in 43% yield by treatment with N-bromosuccinimide and methyl sulfide.[73] This aldehyde then served as the model for the oxidation step. Again, oxidation to the corresponding acid was unsuccessful by the following reagents: $KMnO_4$ in acetone, $KMnO_4$ in pyridine, CrO_3 in acetic acid, $K_2Cr_2O_7$ in acetic acid, Tollens' reagent, AgO-KCN, seloxette in various solvents, and pyridinium dichromate. Benzaldehyde was used as the model compound in this oxidation.

2.2.2 Attempted Preparation of β-Phenylisoserine via Phenylglycine

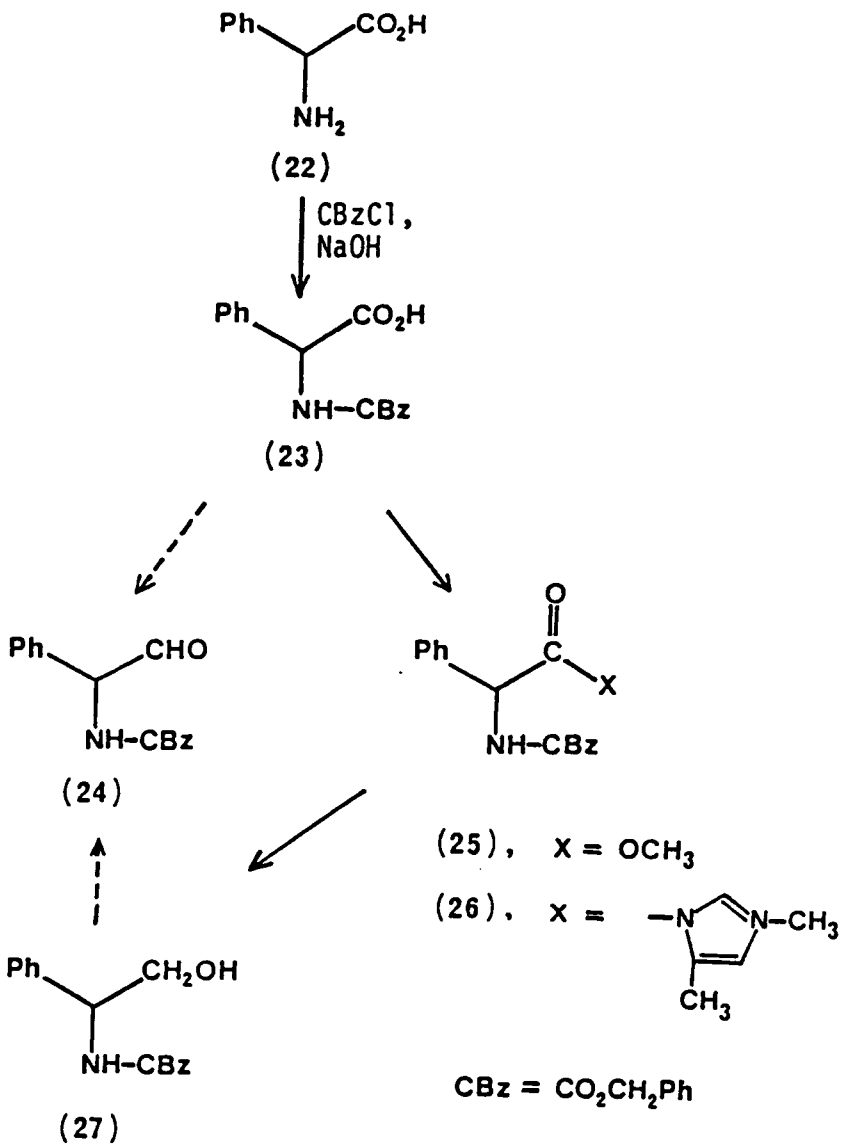
The synthesis of the side chain acid was reported by Umezawa in 1977.[63] The compound was obtained in low yield when N-benzyloxycarbonyl-α-phenylglycine-3,5-dimethylpyrazolide (26) was reduced by lithium aluminum hydride. Our attempts are to use less

reactive reducing agents to reduce either the protected phenylglycine itself or its proper derivatives to the corresponding aldehyde (24). The detailed methods are shown in Scheme 3.

R-(-)-α-Phenylglycine (22) was first protected with the benzyloxy-carbonyl group by the Schotten-Baumann method to give N-benzyloxy-carbonyl -(-)-α- phenylglycine (23) in 68% yield.[74] It was then converted to the methyl ester (25) and 3,5-dimethylpyrazolide (26).[63] The methyl ester was also reduced by diisobutyl aluminum hydride to the alcohol, but the conversion to its corresponding aldehyde by various reagents such as Moffatt's reagent,[75] pyridinium dichromate,[76] and N-bromosuccinimide-methyl sulfide,[73] were not successful.

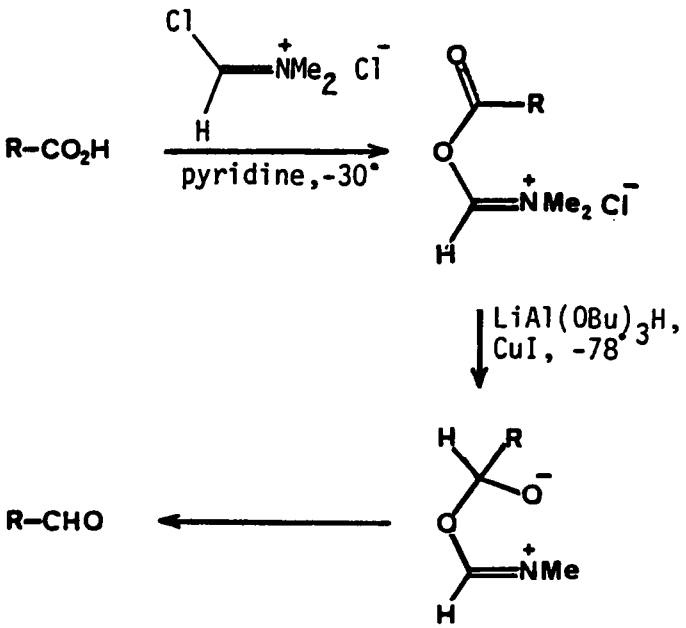
The methyl ester of N-benzyloxycarbonyl-(-)-α-phenylglycine (25) could not be converted to the aldehyde when treated with diisobutyl aluminum hydride. The 3,5-dimethylpyrazolide (28) was subjected to reduction with the following reagents: lithium aluminum hydride, lithium tris-tert-butoxy aluminum hydride,[77] and lithium bis-ethoxy aluminum hydride,[78] but no aldehyde could be detected by TLC or proton NMR techniques.

It was reported that several chloromethylene iminium chlorides, formed by reaction of carboxylic acids with phosgene and dimethyl formamide, could be converted to their corresponding aldehyde by reacting with lithium tris-tert-butoxy aluminum hydride[77] (see Scheme 4) in relatively good yield.



Scheme 3

Synthesis of 3-Phenylisoserine via α-Phenylglycine



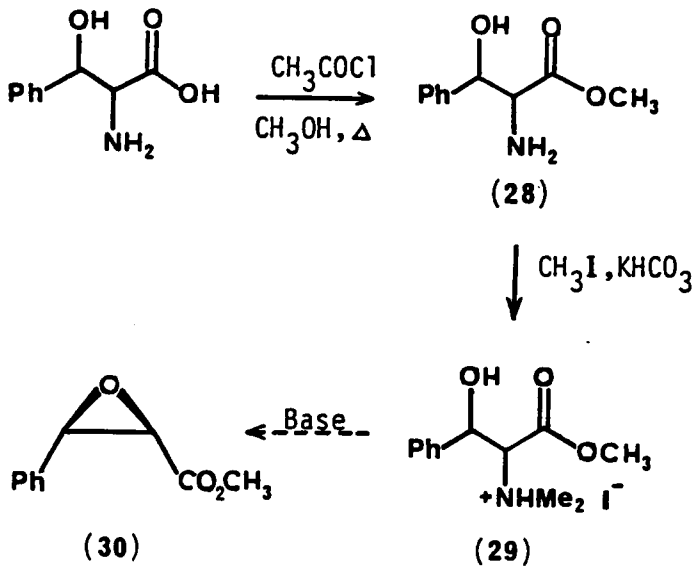
Scheme 4

Conversion of Carboxylic Acid to Aldehyde via the Chloromethylene iminium Chloride

In our case, N-benzyloxycarbonyl-(-)-α-phenylglycine and N-benzoyl-(-)-α-phenylglycine were used under the literature conditions, but no aldehyde was detected. The methyl ester of N-benzoyl-(-)-α-phenylglycine was also prepared and treated with reducing agents, but as in the case of N-benzyloxycarbonyl analogs the aldehyde was not observed.

2.2.3 Attempted Synthesis of the Epoxy Acid via the Quaternary Ammonium Salt of β-Phenyl-L-Serine

Another approach to the synthesis of the C-13 side chain acid involved the formation of the quaternary ammonium salt of β-phenyl-L-serine (29), then subsequent ring cyclization to phenylglycidate ester (30). β-Phenyl-L-serine was first converted to the corresponding methyl ester (28) (Scheme 5). It was then treated with methyl iodide and potassium bicarbonate at room temperature.[65]

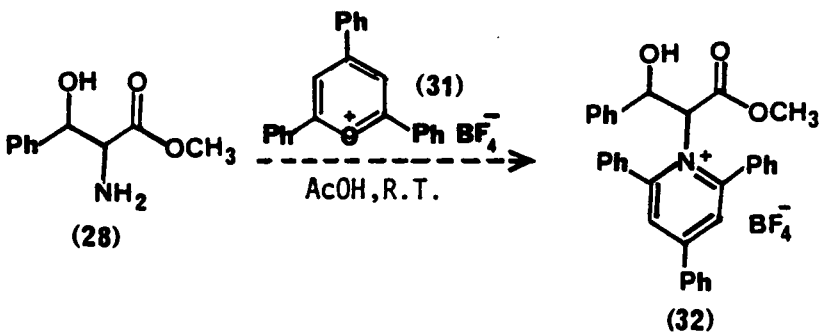


Scheme 5

Attempted Synthesis of 3-Phenylisoserine via 3-Phenyl-L-serine

From the proton NMR spectrum and thin layer chromatography, no quaternary ammonium salt of β-phenyl-L-serine was detected. It might be possible that this salt was initially formed but subsequently cyclized to the unstable phenylglycidic acid which immediately decomposed.

Katritzky et al has reported that certain types of pyrylium salts can form good leaving groups when they are converted to pyridinium salts.[79] 2,4,6-Triphenylpyrylium tetrafluoroborate (31) was made by the method of Dimroth et al[80] and was reacted with β-phenylserine methyl ester (28) and glacial acetic acid in dichloromethane at room temperature. After working up, some solid was obtained but did not show the molecular ion of the correct compound in the mass spectrum.



Scheme 6

Attempted Synthesis of the Pyrylium Salt

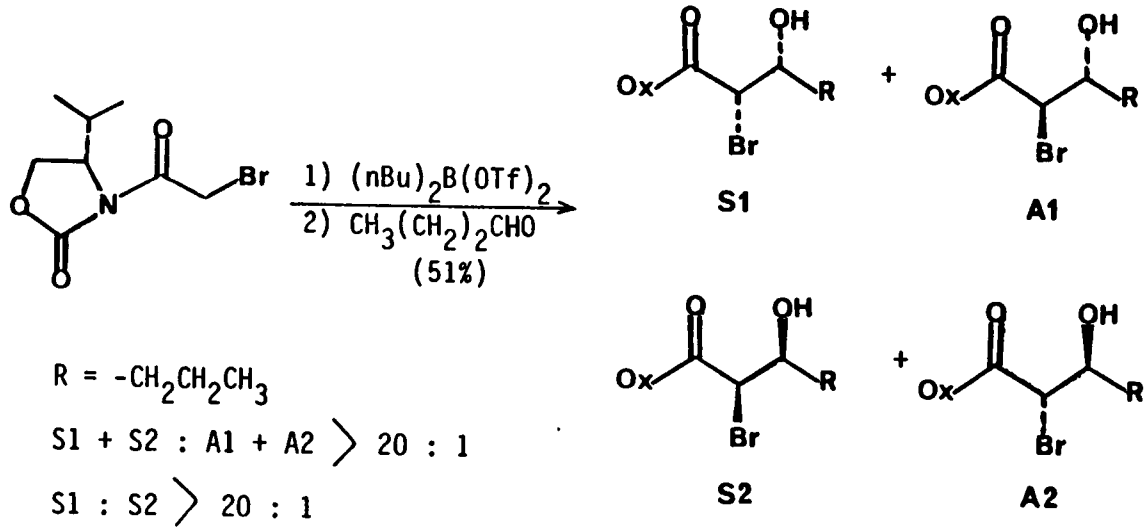
It can be envisaged that if the pyridinium salt of β-phenylserine can be obtained by some other means, it might be readily cyclized to the epoxide compound. We could not obtain the pyridinium salt (32) in the first attempt in which acetic acid was used, probably due to some steric hindrance at the reaction center. There are still several other reagents such as triethylamine or other bases to be tried and also some more vigorous conditions.

2.2.4 Epoxidation of Cinnamic Acid with Potassium Peroxomonosulfate

Edwards et al[59] has reported the epoxidation of cinnamic acid by potassium peroxomonosulfate, $KHSO_5$, in acetone in good yield. It was briefly attempted in this project, but only a very low yield of epoxy acid could be detected. Since the method involves careful control of the pH of the solution by a pH stat, the failure of the reaction might be due to the lack of the correct pH throughout the reaction.

2.2.5 Attempted Synthesis of the Side Chain Acid by the Modified Aldol Condensation of Chiral α-haloimidates with Benzaldehyde in the Presence of Tin (II) Triflate

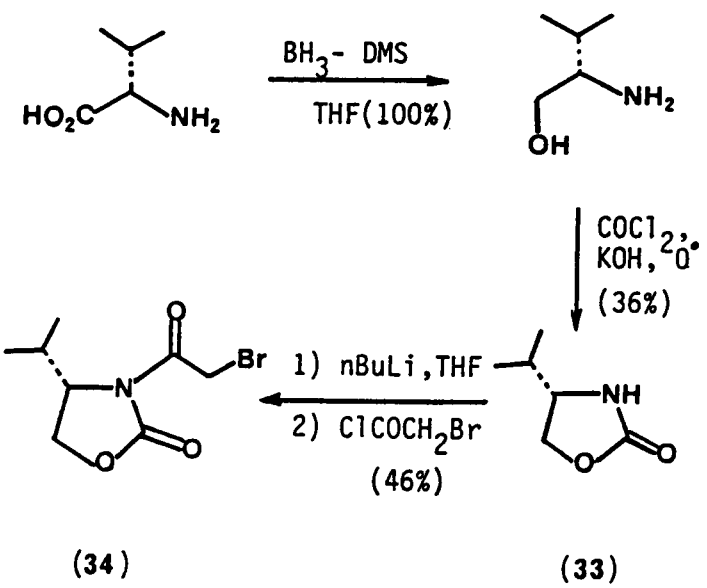
The chiral phenylglycidic acid might be prepared by the modified aldol condensation as shown in Scheme 7. Previous studies[60,61] showed that the chiral α-haloimidate (33) will effectively serve as an auxiliary group in subsequent aldol condensation with aldehydes and give a relatively high erythro-diastereoface selectivity. Another advantage is the ability to remove and recycle this group without any significant racemization of the substrate.



Scheme 7

Reaction of Metal Enolate with Aldehyde

In order to prepare the α-bromoimidate, L-valinol was prepared quantitatively by reduction of valine with borane-methyl sulfide.[81] L-Valinol was then treated with phosgene in the presence of potassium hydroxide to yield 4-isopropyloxazolidone (**34**) in 36% yield.[60,82] N-Bromoacetyl-4-isopropyloxazolidone (**35**) was subsequently obtained in 46% yield from the reaction of the monoanion of the oxazolidone with bromoacetyl chloride.[60a] (see Scheme 8)

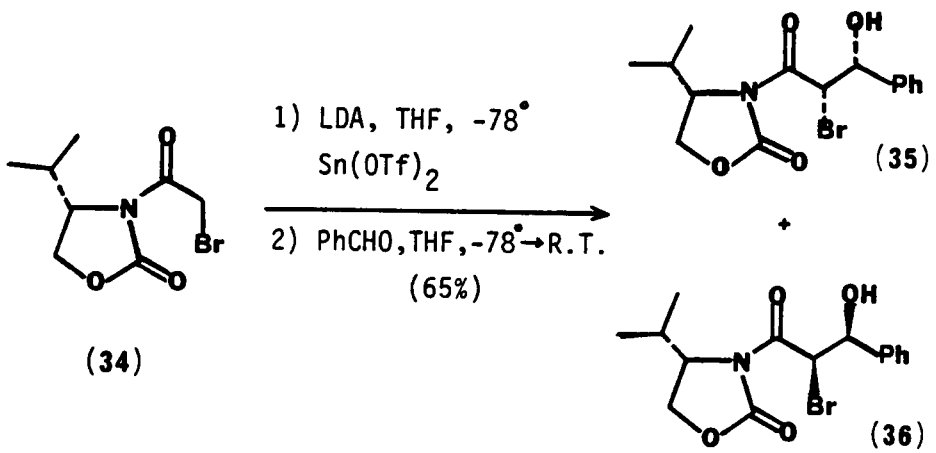


Scheme 8

Preparation of N-Bromoacetyl-4-Isopropyloxazolidone (**34**)

First, an attempt was made to react the α-bromoimidate (34) with benzaldehyde in the presence of di n-butyl boron triflate. The expected 1,2-bromohydrin was not detected, probably due to failure to generate the boron enolate in the first stage of the reaction.

Anhydrous tin (II) triflate was thus prepared from the reaction of stannous chloride and trifluoromethanesulfonic acid[83] and was used in the condensation of N-bromoacetyl-4-isopropyloxazolidone with benzaldehyde.[61] Two products were obtained and isolated by flash column chromatography.

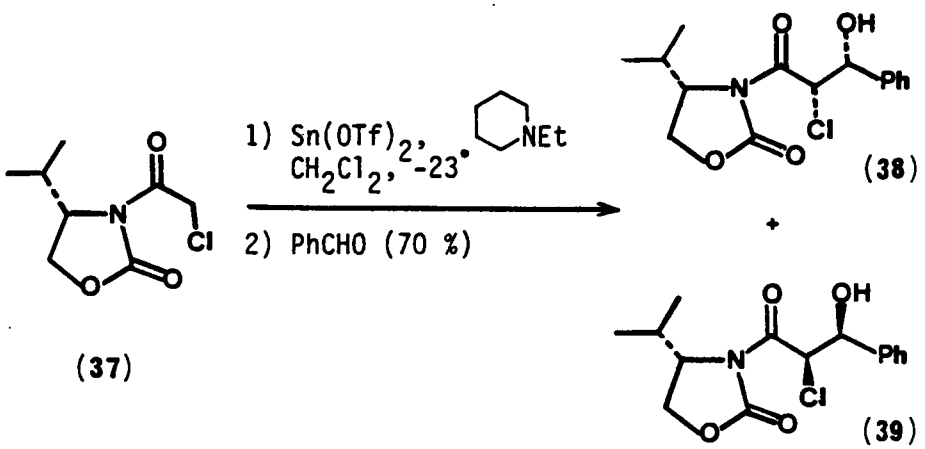


Ratio of **35** : **36** = 3.5 : 1

Scheme 9

Reaction of α-Bromoimidate with PhCHO in the Presence of $Sn(OTf)_2$

Lantos[61] in his work on N-chloroacetyl-4-isopropyloxazolidone (37) with benzaldehyde in the presence of tin (II) triflate isolated two erythro products (38,39) in the ratio of 3 to 4 (see Scheme 10).



Ratio of **38** : **39** = 3 : 4

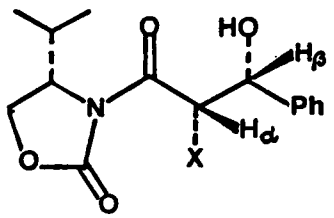
Scheme 10

Reaction of α-Chloroimidate with

PhCHO in the Presence of Tin (II) Triflate

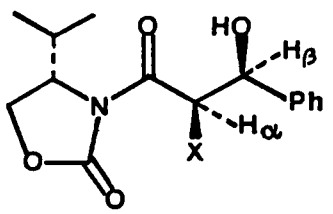
In Lantos' case, the diastereofacial selectivity was not great, and the ratio of the diastereomers was nearly 1 to 1, with the desired product (38) being the less abundant one. In our case, with the more bulky bromine atom in the metal enolate, the reaction was clearly more selective and the desire diastereomer was obtained.

The identification of the diastereomers were based on their proton NMR spectra, the infrared spectra, and also their mass spectra, comparing them with spectra of the two diastereomeric chlorohydrins obtained by Lantos.[61] Figure 2 shows the chemical shifts of these four compounds.



38, X = Cl ; H_α = 6.00 ppm, J = 6.0 Hz
H_β = 5.20 ppm, J = 6.0 Hz
OH = 3.15 ppm

35, X = Br ; H_α = 5.95 ppm, J = 6.0 Hz
H_β = 5.12 ppm, J = 6.0 Hz
OH = 3.60 ppm



39, X = Cl ; H_α = 5.85 ppm, J = 12.0 Hz
H_β = 5.01 ppm, J = 12.0 Hz
OH = 3.70 ppm

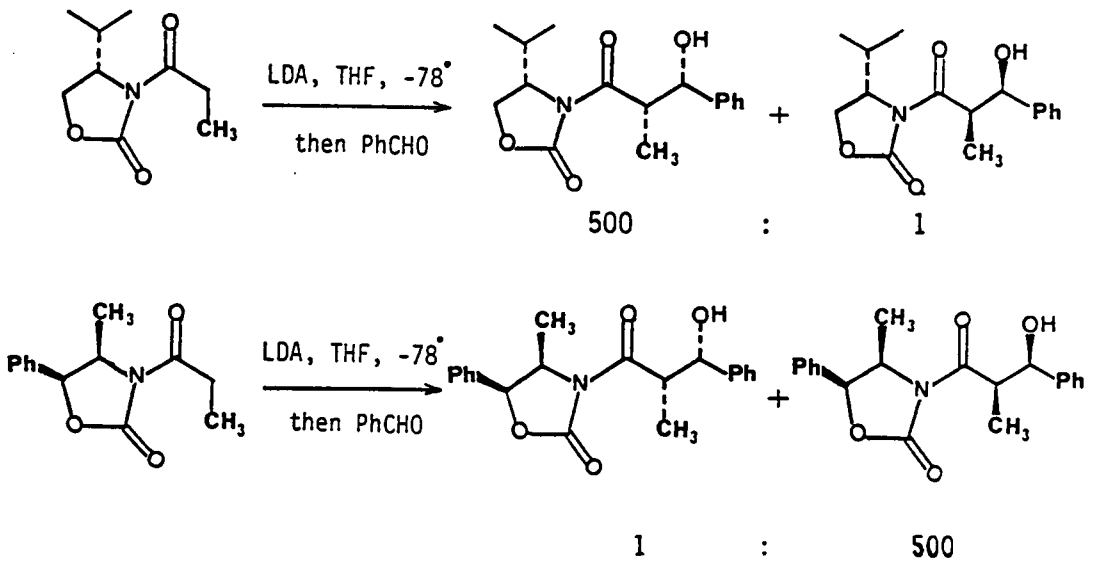
36, X = Br ; H_α = 5.85 ppm, J = 8.4 Hz
H_β = 5.03 ppm, J = 8.4 Hz
OH = 3.40 ppm

Figure 5

The Proton NMR Spectral Data of the Condensation Products

Stereoselectivity of the Crossed Aldol Condensation of α-Bromoimidate with Benzaldehyde

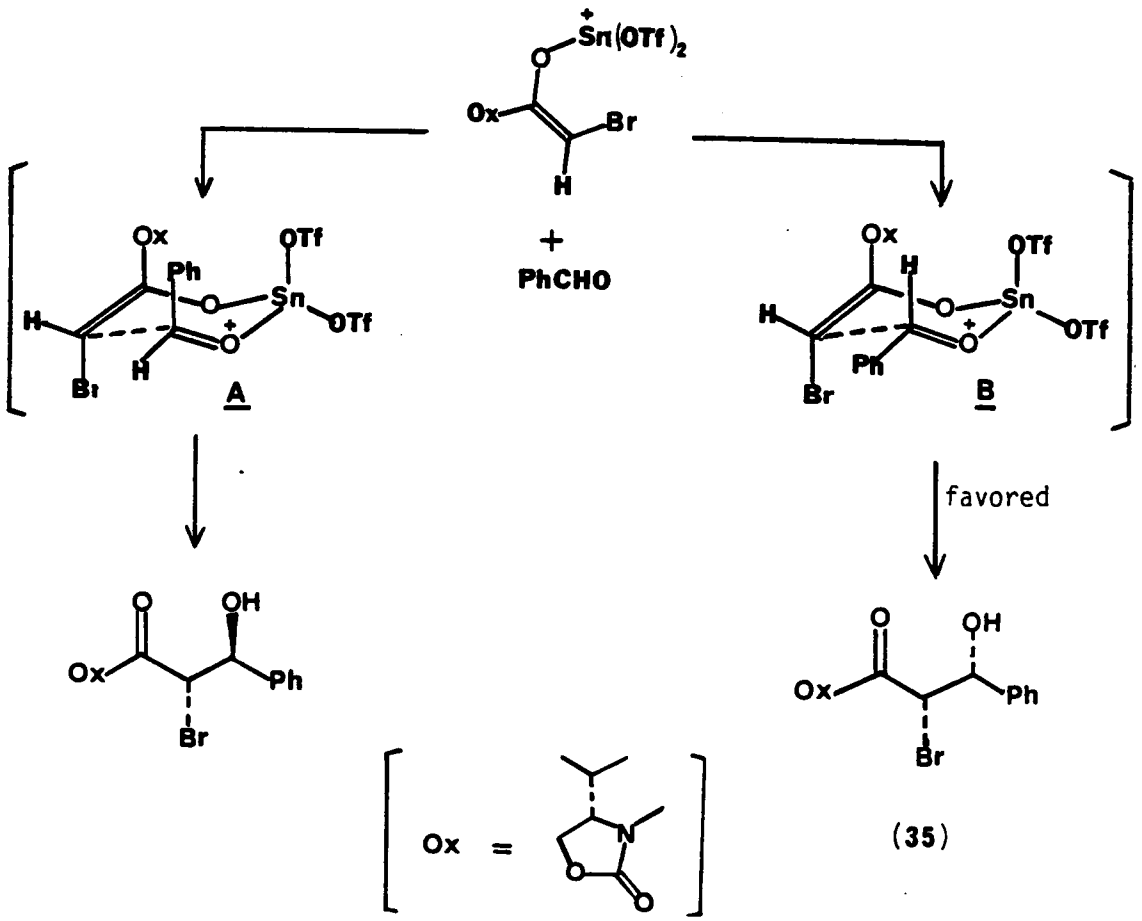
Evans et al[60] have studied the crossed aldol condensation of the enolate of α-haloimidates with various aldehydes and reported the highly diastereoselective and enantioselective formation of products when the chiral 2-oxazolidones are used as recyclable auxiliary groups. As one example in Scheme 11, the products were both highly diastereoselective and enantioselective and were dependent on the stereochemistry of the 4-alkyl group of the 2-oxazolidone ring.



Scheme 11

Stereoselectivity of the Crossed Aldol Condensation of α-Bromoimidate with Benzaldehyde

In our case, the Z-enolate was kinetically generated with LDA (THF,-78°C) and simultaneously treated with $Sn(OTf)_2$, followed by benzaldehyde to yield two products in 3.5 to 1 ratio (see Scheme 9). The chairlike transition state of the reaction between the Z-enolate and benzaldehyde which involved cooperative metal ion ligation of both the enolate and carbonyl substrates was proposed as shown in Scheme 12.[60b,c]

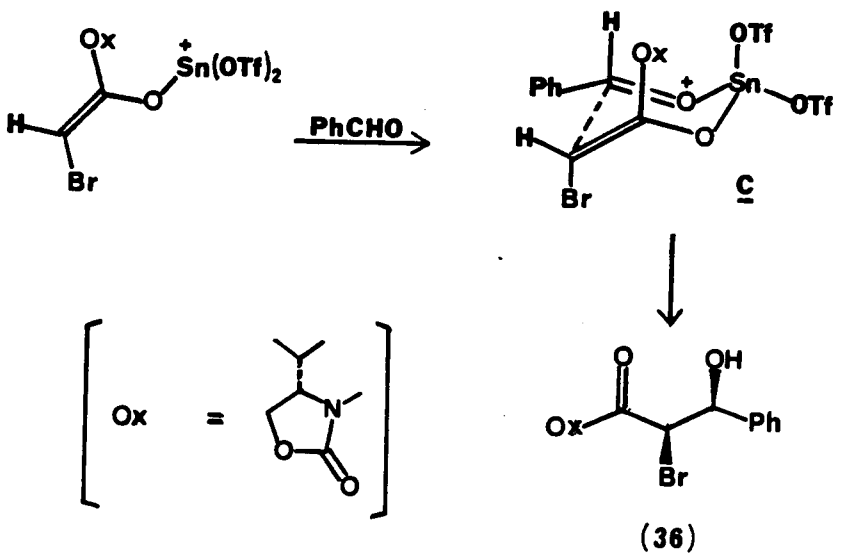


Scheme 12

Proposed Transition State of the Crossed Aldol Reaction between α-Bromoimidate and Benzaldehyde in $LDA/Sn(OTf)_2$

The transition state B which led to the erythro product (35) was prefereably formed than A because of the 1,3— interaction between the phenyl, 2-oxazolidone ring, and the metal ligand in A.

Attack of benzaldehyde from the opposite diastereoface of the Z-enolate theoretically gave the other erythro product (36) as shown in Scheme 13.



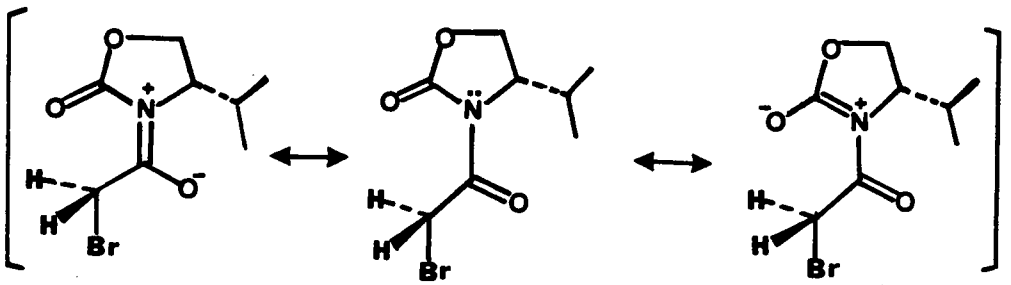
Scheme 13

Formation of the Minor Erythro Product

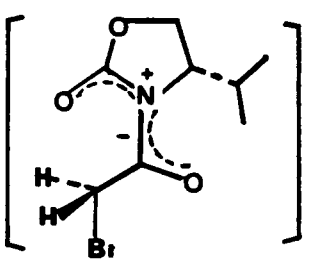
The stereochemical feature of the chiral auxiliary group, (S)-4-isopropyloxazolidone, played an important role in determining the stereochemistry of the products (35 and 36). Consideration of the resonance forms of α-Bromoimidate (34), the most stable form is expected to be the one which has the farthest separation of the negative charges (see Scheme 14). Due to the amide structure on both sides of the nitrogen atom, we expect that the five atoms on the amide backbone will lie on the same plane (as shown as thick lines in (40)) This will also be true when one of the hydrogen atoms on the α-methylene group of the bromoacetyl group was abstracted by base to form the Z-enolate. The 4-isopropyl group will be forced to lie on the same side of the carbonyl group of the bromoacetyl group. The isopropyl group will partially block the incoming benzaldehyde when the attack occurs from the back-side of the Z-enolate while no such interaction will be observed in the front side attack.

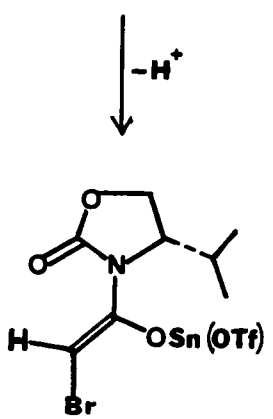
As a result, the transition state C was less favored than B, therefore compound (35) will be preferentially formed as observed in the reaction.

Lantos and co-workers,[61] reported the conversion of the chlorohydrin (38) to the epoxide by treatment with lithium benzyloxide in tetrahydrofuran. The isolated yield was low and the reaction was accompanied by epimerization of the α-center and also retroaldol reactions.



(40)





Scheme 14

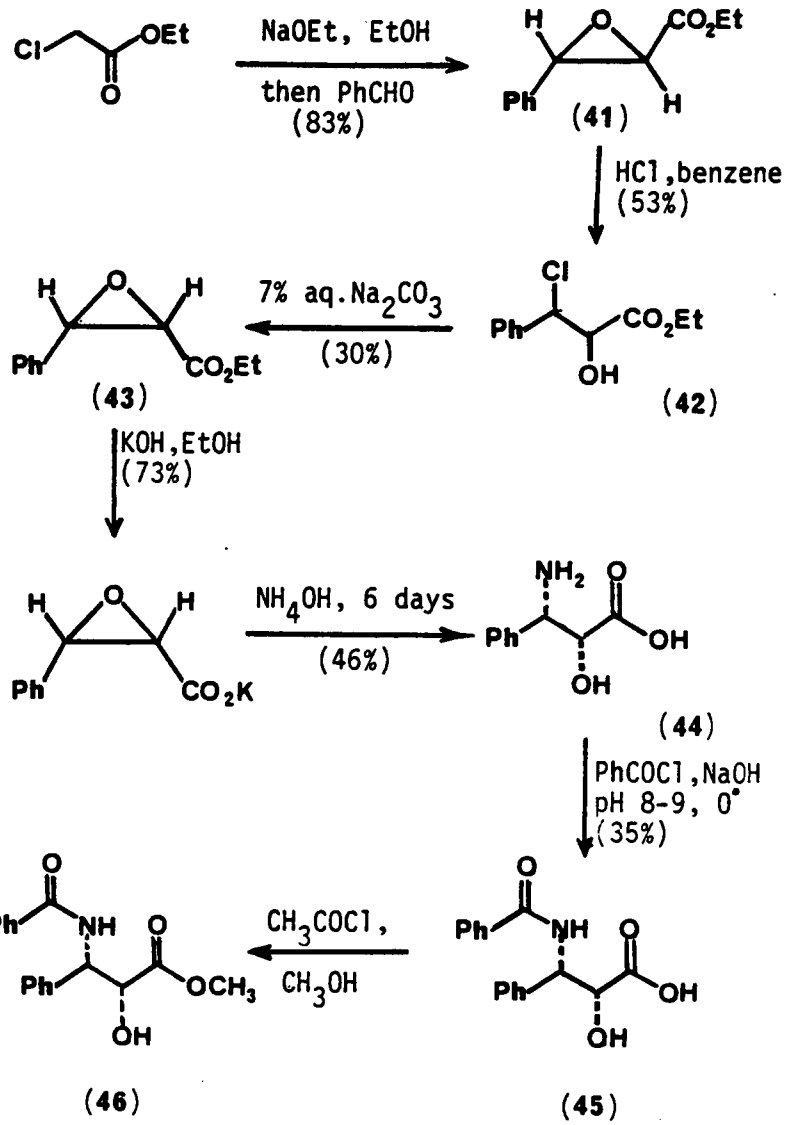
Formation of the Stable Enolate of α-Bromoimidate

Our attempts at conversion of the erythro-bromohydrin (35) to the corresponding epoxide were carried out by treatment with various bases such as sodium hydride, potassium hydride, and lithium benzyloxide. No epoxide was detected by thin layer chromatography and proton NMR spectroscopy in any of these attempts.

2.2.6. Synthesis of 3-Phenylisoserine by the Darzens Condensation[62]

The C-13 side chain acid of taxol can be synthesized by the Darzens condensation as shown in Scheme 15. This method finally yielded racemic-cis-ethyl-3-phenylglycidate in relatively good yield in 3 steps. To avoid the stability problem of phenylglycidic acid, the epoxy ester was converted to the corresponding potassium salt, then treated with concentrated ammonium hydroxide at room temperature for 6 days to yield racemic 3-phenylisoserine.

As shown in Scheme 15, the reaction of benzaldehyde with ethyl chloroacetate in the presence of sodium ethoxide gave racemic trans-ethyl-3-phenylglycidate (41) in 83% yield. From the proton NMR spectrum, the crude product contained a mixture of trans-and-cis-ethyl-3-phenylglycidate in 4.5 to 1 ratio. The β-proton of trans-ethyl-3-phenylglycidate appears at δ 4.03 ppm (J = 2.0 Hz) while the cis-isomer has δ 4.20 ppm (J = 4.5 Hz). The α-proton of the trans-ester was seen at 3.44 ppm as a doublet with 2.0 Hz coupling constant, while the cis-isomer resonated at 3.74 ppm (doublet, J = 4.5 Hz). The proton NMR chemical shifts of both trans- and cis-ethyl-3-phenylglycidate were consistent with those previously reported data.[84]



Scheme 15

Synthesis of N-Benzoyl-β-Phenylisoserine via Darzens Condensation

The crude ester was therefore purified by distillation under reduced pressure to yield pure racemic trans-ethyl-3-phenylglycidate (41) which was then treated with hydrogen chloride gas to yield threo ethyl-3-chloro-2-hydroxy-3-phenylpropanoate (42) in 53% yield. The chlorohydrin's melting point and ^{1}H NMR spectrum were consistent with the literature data.[62b]

Racemic threo-ethyl-3-bromo-2-hydroxy-3-phenylpropanoate (47) was also prepared by bubbling hydrogen bromide gas into the benzene solution of racemic trans ethyl-3-phenylglycidate (41) at room temperature. The crude product was recrystallized from ether as yellow needle crystals which were contaminated with some impurities and were difficult to remove. The proton NMR data was similar to the chloro compound.

The threo chloroalcohol (42) was converted to cis-ethyl-3-phenylglycidate (43) in 30% isolated yield by treating with 7% aqueous sodium carbonate solution.[62] The crude product was purified by flash chromatography and gave the cis-isomer having the correct proton NMR spectrum.

The racemic cis-ester was treated with a solution of potassium hydroxide in absolute ethanol at 0°C to give potassium cis-3-phenylglycidate in 73% yield. Preparation of 3-phenylisoserine was achieved by stirring a mixture of potassium cis-3-phenylglycidate with concentrated ammonium hydroxide at room temperature for 6 days. The compound was recrystallized from water to yield colorless needle crystals in 46.5% yield. It was then reacted with benzoyl chloride in 1N sodium hydroxide while the pH of the solution was adjusted to 8-9

throughout the reaction by simultaneous additions of the acid chloride and sodium hydroxide solutions. The crude product was recrystallized from ethanol-water to give N-benzoyl-3-phenylisorerine (**45**) in 35% yield. The isolated yield of (**45**) was low due to the formation of dibenzoylated product which was isolated when crude benzoylated product was converted to the corresponding ester.

2.3. Experimental

A. General

General methods employed in this chapter are also valid in all other chapters unless specifically noted.

All melting points were determined on a hot stage apparatus and were uncorrected. Solvents for anhydrous reactions were dried according to procedures in Loewenthal.[85]

B. Spectra

^{1}H NMR spectra were recorded on an EM 390 90 MHz spectrometer or a Bruker WP 270SY 270 MHz spectrometer. ^{13}C NMR spectra were taken on a Bruker WP 270SY 270 MHz or a Bruker WP 200 200-MHz spectrometer. Two-dimensional NMR spectra were obtained on a Bruker WP200 200 MHz spectrometer.

Chemical shifts were recorded in parts per million (ppm) downfield from TMS in the case of proton NMR, while ^{13}C NMR chemical shifts were based on the chloroform chemical shift at 77.0 ppm. Spectra were generally recorded in $CDCl_3$ solution at ambient temperature.

Infrared (IR) spectra were recorded on a Perkin Elmer 710 B infrared spectrophotometer. Ultraviolet (UV) spectra were taken on a

Perkin-Elmer 330 UV-visible spectrophotometer. Mass spectra (MS) were obtained by the fast atom bombardment (FAB) method on a Kratos MS 50 instrument at the Midwest Center for Mass Spectrometry at the University of Nebraska. The EI (electron impact) mass spectra were taken on a VG 7070 HF or MAT-1125 mass spectrometer in the Biochemistry Department, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

C. Chromatography

High-performance liquid chromatography (HPLC) separations were performed on a system composed of a Waters Associates M 6000A pump, a Valco injection valve and a Waters Associates Model 441 absorbance detector operating at 254 nm. Analytical HPLC was carried out with a Resolve-C8 Radial-Pak cartridge (Waters). Preparative HPLC was performed on a Dynamax Macro HPLC C18 column, 8μm, 250 x 10 mm. (Rainin).

Analytical thin-layer chromatography (TLC) was carried out on Silica gel 60 F_{254} (0.2 mm thickness) (E. Merck). Preparative TLC was performed on silica gel GF plates, 20 x 20 cm x 1000 μm thick (Analtech) or on silica gel GF tapered plates, 20 x 20 cm x 1000 μm thick (Analtech).

Flash chromatography was performed using silica gel 60, 0.040 - 0.063 mm (230-400 mesh) particle size. Column sizes were varied and were specified in the pertinent experimental sections.

2.3.1. Attempted Synthesis of 3-Phenylisoserine via Sharpless Epoxidation

Ethyl α,β-Dibromo-β-Phenylpropiolate.[66] Ethyl α,β-dibromo-β-phenylpropiolate was obtained in nearly quantitative yield from the reaction of ethyl cinnamate and bromine in carbon tetrachloride solution at 0°C, mp 60-69°C (lit.[66] mp 65-71°C).

Phenylpropiolic Acid.[67] Preparation of phenylpropiolic acid was achieved by refluxing the mixture of ethyl α, β-dibromo-β-phenylpropiolate (31.3 g,0.09 mol) and potassium hydroxide (25 g) in absolute ethanol (120 mL) at 40-50°C for 5 h. Recrystallization of the crude product from carbon tetrachloride gave colorless needle crystals of phenylpropiolic acid, 9.7 g, mp 132-133°C (lit.[67] mp 135-136°C), in 72% yield.

Methyl Phenylpropiolate. Phenylpropiolic acid (69 g,0.47 mol) in methanol (270 mL) and acetyl chloride (15 mL) was refluxed for 2 h, the crude product was then purified by distillation under reduced pressure to yield a colorless liquid, 53.4 g (71% yield) bp 88-95°C (2mm Hg). ^1H NMR (90 MHz, $CDCl_3$/TMS) δ 3.80 (s, 3H, OCH_3), 7.30 (s, 5H, aromatic); IR (neat) ν 1740, 1510, 1465, 1320, 1220, 1030, 910, 780, 710 cm^{-1}.

Phenylpropargyl Alcohol.[68] Methyl phenylpropiolate (26.6 g,0.17 mol) in toluene (400 mL) was treated with diisobutyl aluminum hydride (71.3g) (3 equivalents) under nitrogen over 2 h. The mixture was stirred at room temperature for 4 h, and methanol in toluene solution followed by water was then added. Stirring was continued until gas evolution subsided and the solution attained room temperature. Solid aluminum salts were filtered of and the organic layer washed several

times with water. After evaporation of the solvent, the residual yellow liquid was distilled under reduced pressure to afford phenylpropargyl alcohol (17.1g,76% yield) as a colorless liquid, bp 87.5-90.5°C (1mm Hg). ^{1}H NMR (90 MHz, $CDCl_3$/TMS) δ 2.95 (s, 1H, OH), 4.45 (s, 2H, CH_2), 7.25 (m, 5H, aromatic); IR (neat) ν 3400, 1630, 1520, 1470, 1285, 1095, 980, 780, 715 cm^{-1}.

cis-Cinnamyl Alcohol(19).[69] Phenylpropargyl alcohol (22.0 g,0.17 mol) in ethyl acetate (1.2 L) was hydrogenated using Lindlar's catalyst. The crude product was purified by flash-chromatography, eluted with 20% ethyl acetate in hexane, to yield a pale yellow liquid (20.1 g, 90%). ^{1}H NMR (90 MHz, $CDCl_3$/TMS) δ 3.50 (s, 1H, H-2), 4.38 (s, 2H, H-3, -OH), 5.65-6.55 (m, 2H, H-1), 6.90-7.40 (m, 5H, aromatic); IR (neat) ν 3400, 2950, 1498, 1460, 1040, 892, 740, 698 cm^{-1}; EIMS m/z (relative intensity) 134 (M^+, 1.7), 131 (28), 115 (9), 103 (20), 91 (6.5), 77 (24), 43 (20), 28 (73), 18 (100).

(2S,3R)-2,3-Epoxy-3-Phenylpropanol (20).[56-58] Dry dichloromethane (250 mL) was charged into a 500 mL round-bottom flask cooled in a dry-ice-CCl_4 bath (-20°C). Titanium tetraisopropoxide (17.8 mL, 60 mmol) was added via a syringe under a nitrogen atmosphere. After 5 min, (+)-diethyl tartrate (13.0 mL, 75 mmol) in dichloromethane (25 mL) was added. The solution was stirred for 5 min and a solution of cis-cinnamyl alcohol (19, 6.7 g, 50 mmol) in dichloromethane (50 mL) was added. After another 10 min, tert-butyl hydroperoxide (TBHP, 24.1 mL, 100 mmol) was added and the solution was kept in the refrigerator freezer for 2 days. A saturated solution of sodium sulfate (40 mL) and THF (80 mL) were then added and the solution was stirred at room

temperature for 3 h. The mixture was filtered through a celite column and the residue which was left after removal of the organic solvent was taken up in ether (100 mL). The solution was stirred with a 10% solution of sodium hydroxide in saturated brine (100 mL) for 1 h. The organic layer was collected, washed with a small volume of water and dried over anhydrous magnesium sulfate. The solvent was removed and the yellow liquid residue was subjected to flash chromatography, eluted with 20% ethyl acetate in hexane. (2S,3R)-2,3-Epoxy-3-phenylpropanol **(20)** was obtained as a pale yellow liquid, 2.4 g (32% yield). ^{1}H NMR (200 MHz, $CDCl_3$/TMS) δ 3.27-3.51 (m, 3H, H-2, H-1), 3.82 (broad s, 1H, -OH), 4.05-4.12 (d, 1H, H-3, J = 3.6 Hz), 7.25 (s, 3H, aromatic); IR (neat) ν 3400,1480, 1050, 920, 750 cm^{-1}; EIMS m/z (relative intensity) 150 (M^+, 6), 132 (32), 119 (28), 107 (100), 105 (36), 104 (40), 92 (60),91 (72), 90 (56), 80 (40), 78 (34).

Racemic cis-2,3-Epoxy-3-Phenylpropanol.[57] cis-Cinnamyl alcohol **(19)** (1.10 g,8.2 mmol) was added into a stirred solution of vanadyl acetylacetonate (20 mg) in CH_2Cl_2 (50 mL) at 0°C under nitrogen. Tert-butyl hydroperoxide (TBHP) (3.6 g, 16.0 mmol) was added into the previous solution after five min and the mixture was stirred at 0°C for another 4 h. The reaction mixture was extracted with dichloromethane, washed with water and brine, then dried over anhydrous magnesium sulfate. After removal of the solvent, the residual liquid was purified by flash chromatography, eluted with 20% ethyl acetate in hexane. Racemic cis-2,3-epoxy-3-phenylpropanol was obtained as a pale yellow liquid (26% yield). ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.32-3.52 (m, 4H, H-1, H-2, -OH), 4.11-4.13 (d, 1H, H-3, J = 3.4 Hz), 7.28 (s, 5H, aromatic).

(2'S, 3'R)-2'3'-Epoxy-3'-Phenylpropyl-(R)-2-Methoxy-2-Trifluoromethyl Phenylacetate.[70] 2,3-Epoxy-3-phenylpropanol (20) (35.8 mg, 0.24 mmol) was dissolved in dichloromethane (3.5 mL). Into the above solution were added 4-dimethylaminopyridine (2 mg), triethylamine (0.1 mL), and (R)-2-methoxy-2-trifluoromethyl phenylacetyl chloride[70b] (0.1 g, prepared by refluxing a mixture of the corresponding acid, thionyl-chloride, and sodium chloride for 50 h, then distilling the crude product in a Kugelrohr distillation apparatus under reduced pressure). The mixture was stirred for 1.5 h and the solvent was distilled off to yield a yellow liquid which was purified by flash chromatography to afford (2'S, 3'R)-2',3'-epoxy-3'-phenylpropyl-(R)-2-methoxy-2-trifluoromethyl phenylacetate (79.6 mg, 100% yield) as a colorless liquid. ^{1}H NMR (200 MHz, benzene-d_6) δ 2.98-3.05 (m, 1H, H-2), 3.36 (m, 3H, -OCH_3), 3.62-3.64 (d, 1H, H-3), 3.84-3.90 (m, 2H, H-1), 7.00-7.16 (m, 8H, aromatic), 7.57-7.61 (m, 2H, aromatic); IR (neat) ν 2990, 1770, 1515, 1470, 1280, 1190, 1135, 1030 cm^{-1}.

Racemic cis-2',3'-Epoxy-3'-Phenylpropyl-(R)-2-Methoxy-2-trifluoromethyl Phenylacetate.[70] The Mosher ester of racemic-cis-2,3,-epoxy-3-phenylpropanol was obtained by the same procedure in 100% yield as a colorless liquid. ^{1}H NMR (200 MHz, benzene-d_6) δ 2.99-3.05 (m, 1H, H-2), 3.36-3.40 (m, 3H, -OCH_3), 3.64-3.66 (d, 1H, H-3), 3.79-4.00 (m, 2H, H-1), 7.01-7.16 (m, 8H, aromatic), 7.57-7.62 (m, 2H, aromatic); IR (neat) ν 3000, 1770, 1520, 1475, 1280, 1190, 1140, 1040 cm^{-1}.

Racemic-trans-2,3-Epoxy-3-Phenylpropanol.[57] Racemic-trans-2,3-epoxy-3-phenylpropanol was prepared by reaction of vanadyl

acetylacetonate with trans-cinnamyl alcohol at 0°C in dichloromethane. Tert-butyl hydroperoxide in dichloromethane was then added and the mixture stirred for 2 h. Work up as in the racemic cis-epoxyalcohol preparation and purification by flash chromatography gave a pale yellow liquid in 60% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.1-3.2 (m, 1H, H-3), 3.30-3.45 (s, 1H, -OH), 3.65 (m, 3H, H-1, H-2), 7.25 (s, 5H, aromatiic); IR (neat) ν 3450, 1520, 1480, 1080, 1040, 900, 785, 705 cm^{-1}.

Racemic trans-2,3-Epoxy-3-Phenylpropanal.[73] To a stirred solution of N-bromosuccinimide (1.09 g, 6.0 mmol) in toluene (20 mL) was added dimethyl sulfide (0.6 mL, 8.2 mmol) at 0°C under nitrogen. The mixture was then cooled to -25°C (carbon tetrachloride-dry ice bath) and a solution of 0.45 g of racemic trans-2,3-epoxy-3-phenylpropanol in toluene (4 mL) was added dropwise. Stirring was continued for 4 h at this temperature. Then a solution of triethylamine (0.84 mL) in toluene (1 mL) was added dropwise. The cooling bath was removed after 5 min and dichloromethane (40 mL) was added. The organic layer was washed with water (10 mL), dried over magnesium sulfate, and the solvent was removed to give a pale yellow liquid which was purified by flash-chromatography, eluted with 20% ethyl acetate in hexane. Racemic trans-2,3-epoxy-3-phenylpropanal was obtained as a pale yellow liquid in 43% yield (192.0 mg). ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.37-3.42 (dd, 1H, J = 1.8, 6.3 Hz, H-2), 4.11-4.13 (d, 1H, H-3, J= 1.8 Hz), 7.25-7.37 (m, 5H, aromatic), 8.35 (d, 1H, -CHO, J = 6.3 Hz); IR (neat) ν 3500, 1750, 1480, 1080, 770, 710, cm^{-1}. EIMS m/z (relative intensity) 148 (M^+, 21), 147 (18.5), 132 (14.7), 131 (21), 120 (15.8), 119 (21), 105 (21), 103 (16), 92 (16), 91 (100), 90 (21), 89 (24), 77 (13), 76 (29).

2.3.2 Attempted Synthesis of β-Phenylisoserine via α-Phenylglycine N-Benzyloxycarbonyl-(R)-(-)-α-Phenylglycine(23).[63] Benzyl chloroformate (2.5 g, 14.65 mmole) and 1 N. NaOH were simultaneously added dropwise into an ice-cooled solution of R-(-)-α-phenylglycine (2.2 g, 14.55 mmol) in 1 N NaOH (14.6 mL), the rate of addition being such that the pH of solution was 8-9 throughout. The solution of pH 8 was then stirred at 0°C for 30 min, then for another 30 min while it attained room temperature. The solution was then washed with 2 x 15 mL of ether, cooled to 0°C and added slowly with stirring to an ice-cooled 4 N HCl (1.5 mL). The white solid was filtered by suction filtration, washed with cold water (15 mL). Recrystallization from ethanol-water (3:1) gave N-benzyloxycarbonyl-R-(-)-α-phenylylycine (3.2 g, 68%), mp 130-133°C (lit.[63] mp 130-130.5°C). ^{1}H NMR (90 MHz, DMSO-d_6) δ 5.07 (s, 2H, -OCH_2), 5.23 (d, 1H, -C<u>H</u>-NH), 7.32 (s, 10H, aromatic), 8.03 (broad d, 1H, -NH). IR (nujol) ν 3450, 1770, 1690, 1555, 1270, 1195, 1070, 735 cm^{-1}.

N-Benzyloxycarbonyl-R-(-)-α-Phenylglycine Methyl Ester (25). N-Benzyloxy-carbonyl-R-(-)-α-phenylglycine (23) (1.36 g, 4.77 mmol), methanol (7 mL), and acetyl chloride (0.1 mL) were refluxed for 4 h. The solvent was distilled off to yield a white solid which was recrystallized from methanol-water (4:1) to give colorless needles (1.32 g, 92%), mp 62-65°C (lit.mp 76-77°C). ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.65 (s, 3H, -OCH_3), 5.04 (s, 2H, -<u>CH_2</u>Ph), 5.24-5.44 (d, 1H, -<u>CH_2</u>CO), 5.70-594 (d, 1H, NH), 7.27 (s, 10H, aromatic). IR (nujol) ν 3390, 1760, 1710, 1550, 1370, 1340, 1270, 1230, 1070, 1015, 740 cm^{-1}.

N-Benzyloxycarbonyl-R-(-)-α-Phenylglycine-3,5-Dimethyl Pyrazolide (26).[63] N-Benzyloxycarbonyl-R-(-)-α-phenylglycine (23) (1.0 g, 3.5 mmol) and 3,5-dimethylpyrazole (0.40 g, 4.2 mmol) were treated with dicyclohexylcarbodiimide (0.72 g, 3.5 mmol) in chloroform (70 mL) at -7 to -10°C for 1/2 h, and then allowed to stand overnight at room temperature. N,N'-Dicyclohexylurea was removed by suction filtration and the solvent was evaporated off in a rotary evaporator. The oily residue was dissolved in ethyl acetate (20 mL) and washed with 1N HCl and water. The organic layer was dried over anhydrous magnesium sulfate and concentrated to a yellow oil. The crude product was purified by flash chromatography eluted with 40% ethyl acetate in hexane to yield a pale yellow oil (0.77 g, 60%). ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.18 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 5.07 (s, 2H, $-OCH_2$), 5.85 (s, 1H, =CH), 6.02 (d, 1H, NH), 6.70 (d, 1H, -N-CH), 7.28 (m, 10H aromatic). IR (neat) ν 3420, 1740, 1520, 1400, 1380, 1250, 1070, 980, 770, 710 cm^{-1}.

N-Benzoyl-R-(-)-α-Phenylglycine. N-Benzoyl-R-(-)-α-phenylglycine was similarly prepared by the Schotten-Baumann procedure. Benzoyl chloride was reacted with R-(-)-α-phenylglycine at pH 8-9 and the crude product was recrystallized from ethanol to yield a white crystalline compound in 100% yield, mp 188-194°C (lit. mp 187-188°C). ^{1}H NMR (90 MHz, DMSO-d_6) δ 5.65 (d, 1H, CH), 7.20-7.95 (m, 10H, aromatic), 8.10 (d, 1H, NH). IR (nujol) ν 3480, 1750, 1650, 1550, 1240, 730 cm^{-1}. EIMS m/z (relative intensity) 255 (M^+, 0.7), 237 (5.6), 211 (16), 150 (12), 133 (35), 105 (100), 104 (21), 77 (45).

N-Benzoyl-S-(+)-α-Phenylglycine Methyl Ester. N-Benzoyl-S-(+)-α-phenylglycine (2.0 g, 7.84 mmol) and acetyl chloride (0.5 mL) in methanol (20 mL) were refluxed for 2 h. The solvent was removed and the crude product was recrystallized from methanol-water to a white crystalline compound (1.50g, 71%), mp 91.5-93°C. ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.74 (s, 3H, OCH_3), 5.76 (d, 1H, C<u>H</u>-NH), 7.20-7.90 (m, 11H, aromatic and -NH). IR ($CDCl_3$) ν 3725, 3670, 3475, 1760, 1680, 1620, 1600, 1530, 1500, 1460 cm^{-1}.

N-Benzoyl-R-(-)-α-Phenylglycine -3,5-Dimethylpyrazolide. N-Benzoyl-R-(-)-α-phenylglycine-3,5-dimethylpyrazolide was similarly prepared as for N-benzyloxycarbonyl-R-(-)-α-phenylglycine-3,5-dimethylpyrazolide. The yield was 85% after recrystallizaion of the crude product from methanol-water as white needle crystals, mp 142.5-144.0°C. ^{1}H NMR (90 MHz, $CDCl_3$), 2.20 (s, 3H, CH_3), 2.54 (s,3H,CH_3), 5.90 (s, 1H, CH_3-C=C<u>H</u>), 7.00-7.85 (m, 12H, -C<u>H</u>, N<u>H</u>, and aromatic). IR (nujol) ν 3375, 1770, 1660, 1600 cm^{-1}. EIMS m/z (relative intensity) 333 (M^+, 1), 237 (3), 224 (4), 210 (18), 193 (8), 173 (38), 143 (6), 105 (100), 91 (22), 77 (42), 55 (41).

Attempted Reduction of N-Benzyloxycarbonyl-R-(-)-α-Phenylglycine Methyl Ester (25) with Diisobutyl Aluminum Hydride.

N-Benzyloxycarbonyl-R-(-)-α-phenylglycine methyl ester (25) was treated with diisobutyl aluminum hydride (1.5 M in toluene solution, 1.2 equivalent) at -78°C. The solution was stirred for 2 h and the mixture was treated with saturated ammonium chloride solution. After filtering off some solid, the mixture was extracted with ether, dried over anhydrous magnesium sulfate and the organic layer concentrated to a

yellow oil. Proton NMR did not show any aldehyde proton signal and TLC usually showed a complicated mixture of products. Similar results were obtained when the stirring was extended to 3 or 4 h.

N-Benzyloxycarbonyl-R-(-)-α-Phenylglycinol (27). N-Benzyloxycarbonyl-R-(-)-α-phenylglycine methyl ester (100 mg 0.33 mmol) (25) in toluene (2 mL) was treated with a solution of diisobutyl aluminum hydride in toluene (3.0 equivalents,0.94 mL of 1.5 solution in toluene) at room temperature with stirring under nitrogen atmosphere. The reaction mixture was stirred for 4 h. A solution of methanol in toluene (1:5, 2.4 mL) was added, followed by water. The solution was stirred for 10 min, then the white solid was filtered off and washed several times with methanol. Evaporation of the solvent gave a pale yellow oil which was purified by flash chromatography to a pale yellow solid (55.0 mg, 61% yield). ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.28 (broad s, 1H, -OH), 3.75 (d, 2H, $-CH_2O$), 4.75 (m, 1H, C<u>H</u>-NH), 5.04 (s, 2H, CH_2Ph), 5.60 (broad s, 1H, NH), 7.28 (s, 10H, aromatic).

Attempted Oxidation of N-Benzyloxycarbonyl-R-(-)-α-Phenylglycinol to the Corresponding Aldehyde by Various Reagents.

(a) Moffatt's Reagent. The alcohol in dimethyl sulfoxide was treated with dicyclohexylcarbodiimide, DCC), pyridine, and trifluoroacetic acid. The mixture was stirred at room temperature and worked up by the literature procedure. No aldehyde was detected by thin layer chromatography and proton NMR.

(b) Pyridinium Dichromate Reagent. N-Benzyloxycarbonyl-R-(-)-α-phenylglycinol (27) in dichloromethane was stirred with pyridinium dichromate reagent (1.5 equivalents) at room temperature overnight.

After work-up by the literature procedures, no aldehyde could be detected by thin layer chromatography and proton NMR.

(c) N-Bromosuccinimide-Dimethyl Sulfide. N-Benzyloxycarbonyl-R-(-)-α-phenylglycinol (**27**) was subjected to oxidation by N-bromosuccinimide-dimethyl sulfide as in previous experiments. No aldehyde was detected by TLC or ^{1}H NMR spectrum.

Attempted Reduction of N-Benzyloxycarbonyl-R-(-)-α-Phenylglycine-3,5-Dimethylpyrazolide with Lithium Aluminum Hydride.[63]

A solution of N-benzyloxycarbonyl-R-(-)-α-phenylglycine-3,5-dimethylpyrazolide (0.2262 g, 0.62 mmol) in dry THF (5 mL) was added slowly into a stirred suspension of $LiAlH_4$ (50 mg, 1.30 mmol) in THF (6 mL) at -20°C over 10 min under nitrogen atmosphere. Stirring was continued for 1 h and the reaction was then quenched with 1 N. HCl (2.5 mL). The white precipitate was removed by centrifugation and the organic solvent was distilled off in a rotary evaporator. The residue was dissolved in ether, washed with water and dried over anhydrous $MgSO_4$. Evaporation of the solvent yielded a pale yellow liquid which showed a complex TLC pattern. Proton ^{1}NMR did not reveal any aldehydic proton signal.

Similar procedures were used when lithium tris-tert-butoxy aluminum hydride or lithium bis-ethoxy aluminum hydride were employed as the reducing agents. No aldehyde could be detected in either experiment.

Bis-(triphenylphosphine) copper tetrahydroborate, $(Ph_3P)_2$ $CuBH_4$, was prepared by a literature procedure[64] and was then reacted with 3,5-dimethylpyrazolide. No aldehyde was detected.

Attempted Conversion of N-Benzyloxycarbonyl-R-(-)-α-Phenylglycine to the Corresponding Aldehyde via the Chloromethylene Iminium Chloride and Lithium tris-tert-Butoxy Aluminum Hydride.[77]

The iminium salt of the acid was formed from the reaction of oxalyl chloride (0.25 g) with DMF (0.07 mL) in dichloromethane (1.5 mL), then treated with N-benzyloxycarbonyl-R-(-)-α-phenylglycine (285 mg, 1.0 mmol and pyridine (0.08 mL) in THF at -30°C. After stirring for 1 h, the mixture was added into a suspension of copper (I) iodide (2 mg, 1.0 mmol) and $LiAlH(OBu^t)_3$ in THF (1.30 mL of 1.54 M THF solution) at -78°C. The reaction mixture was stirred for 10 min then worked up as in the $LiAlH_4$ reaction. The crude residue gave a very complicated TLC pattern and did not show any aldehyde proton signal in the proton NMR spectrum.

N-Benzoyl-R-(-)-α-phenylglycine derivatives were also employed in these reactions, but no aldehyde could be observed in these cases either.

2.3.3 Attempted Synthesis of the Epoxy Acid via β-Phenylserine

β-Phenyl-DL-Serine Methyl Ester (28).

β-Phenyl-DL-serine (10 g, 0.06 mmol) was refluxed with acetyl-chloride (11.7 mL) in methanol (350 mL) for 4.5 h. The solvent was removed and the aqueous solution of the residue was treated with excess potassium carbonate. The solution was extracted with ether and dried over $MgSO_4$. Evaporation of the ether gave a yellow liquid in 29% yield. ^1H NMR (90 MHz, $CDCl_3$) δ 2.63 (broad s, 3H, -OH, NH_2), 3.35-3.40 (d, 1H, $C\underline{H}NH_2$), 3.54 (s, 3H, OCH_3), 4.80 (d, 1H, $C\underline{H}$-Ph), 7.24 (s, 5H, aromatic). IR (neat) ν 3410, 1760, 1605, 1480, 1290, 1070, 720 cm^{-1}.

Attempted Reaction of β-Phenyl-DL-Serine Methyl Ester with 2,4,6-Triphenylpyrylium Tetrafluoroborate. 2,4,6-Triphenylpyrylium tetrafluoroborate was prepared by the method of Dimroth et al[80] in 62.5% yield. It was then reacted with β-phenyl-DL-serine methyl ester and acetic acid at room temperature overnight. The reaction mixture was extracted with ether. The residual solid was obtained after removal of ether. It did not give the molecular ion of the expected quaternary ammonium salt in the EIMS.

β-Phenyl-DL-serine methyl ester was also reacted with methyl iodide and potassium bicarbonate in methanol. No quaternary ammonium iodide of phenylserine was detected by ^1H NMR spectroscopy.

2.3.4 Attempted Epoxidation of Cinnamic Acid with Potassium Peroxomonosulfate.[59] A solution of trans-cinnamic acid in water and acetone at 2°C was treated with a solution of potassium peroxomonosulfate (oxone) (2.5 equivalents) and EDTA in water dropwise over a period of 1.5 h. The pH was kept at 7.5 by adding a 0.5 N KOH solution. The solution was then acidified with 5% HCl solution, extracted with ether and then washed with water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated off to a white solid. The proton NMR showed the presence of about 40% of trans-2,3-epoxy cinnamic acid together with unreacted cinnamic acid.

2.3.5 Attempted Synthesis of the Side Chain Acid by the Modified Aldol Condensation of α-Haloimidate with Benzaldehyde in the Presence of Tin (II) Triflate.

Tin (II) triflate was prepared from the reaction of stannous chloride and trifluoromethanesulfonic acid.[83] Stannous chloride was

weighed in a dry box, and all filtration and working of tin (II) triflate were conducted in an argon atmosphere using the assembly suggested by Ciruna and Robinson.[83a]

L-Valinol. L-Valinol was prepared by treating a solution of L-valine in THF with a solution of borane-dimethyl sulfide solution in THF (1.4 equivlent) at room temperature under nitrogen atmosphere.[81] L-Valinol was obtained as a colorless liquid in quantitative yield. It was identical with an authentic sample and was used without further purification.

(S)-4-Isopropyloxazolidone (33).[60,82] (S)-4-Isopropyloxazolidone (33) was prepared by treating L-valinol solution with KOH and then with phosgene at 0°C. The organic layer was collected and evaporated to yield a white solid which was recrystallized from ether to a colorless crystal in 36% yield, mp 70.5-71.5°C (lit. mp 71.0-72.0°C). ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.95 (m, 6H,-CH($C\underline{H}_3$)$_2$), 1.73 (m, 1H, $C\underline{H}(CH_3)_2$), 3.65 (m, 1H, $C\underline{H}$-NH), 4.25 (m, 2H, -$C\underline{H}_2$O), 7.33 (s, broad, 1H, NH). IR ($CHCl_3$) ν 3500, 3275, 3000, 2425, 1740, 1480, 1405, 1020 cm^{-1}.

N-Bromoacetyl-4-Isopropyloxazolidone (34).[60a] 4-Isopropyloxazolidone (33, 2.96 g, 22.5 mmol) in dry THF (30 mL) was treated with n-butyl lithium (9.60 mL of 2.6 M of benzene solution, 24.8 mmol) during 15 min at -78°C under nitrogen atmosphere. After additional stirring for 15 min, bromoacetyl chloride (2.0 mL, 23.25 mmol) was added dropwise at this temperature. The mixture was stirred for 30 min, then allowed to attain room temperature. Water (40 mL) was added, the aqueous layer was extracted with ether (40 mL) and the combined organic layer was dried over anhydrous $MgSO_4$. Evaporation of

the solvent gave a dark brown liquid which was purified by flash chromatography eluted with 15% ethyl acetate in hexane to a pale yellow liquid (2.65 g, 46%). ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.91 (m, 6H, $2CH_3$), 2.37 (m, 1H, C<u>H</u>$(CH_3)_2$), 4.20-4.58 (m, 4H, -CH_2O,-NC<u>H</u>, C<u>H</u> Br), 4.70 (s, 1H, -C<u>H</u> Br). IR (neat) ν 3000, 1785, 1725, 1385, 1345, 1220, 1120, 1030, cm^{-1}. EIMS m/z (relative intensity) 249 (M+, 0.7), 208 (4), 206 (4), 162 (14), 86 (100), 85 (35), 77 (10), 69 (9), 68 (27), 43 (22), 42 (31), 41 (23).

<u>Modified Crossed Aldol Condensation of N-Bromoacetyl-4-Isopropyl-oxazolidone (34) with Benzaldehyde using Lithium Diisopropylamide in the Presence of Tin (II) Triflate.</u>[61] A solution of N-bromoacetyl-4-isopropyloxazolidone (1.5 g, 6.0 mmol) in dry THF (6.0 mL) was added slowly into an LDA solution (14.2 mL of freshly prepared 1.0 M solution) at -78°C under argon atmosphere. After stirring for 30 min, tin (II) triflate suspension (2.8 g, 6.6 mmol) in THF (10 mL) was added slowly into the previous solution during 10 min. Benzaldehyde (0.9 mL, 7.2 mmol) in dry THF (5.0 mL) was added dropwise and the solution was stirred for 30 min at -78°C, then for another 1.5 h at room temperature. Phosphate buffer (pH 7, 50 mL) was added into the dark red solution with ether (200 mL) and the ethereal layer then washed with brine, and then stirred for 20 min. The solution was extracted with water, and dried over anhydrous Na_2SO_4. Evaporation of the ether gave a dark red liquid which showed five spots on TLC. The crude product was purified by flash chromatography, eluted with 25% ethyl acetate in hexane and gave two compounds in 3.5 to 1 ratio (65% total isolated yield). The major product (35) was identified as the (2S,3R)-adduct while the minor

product (36) was (2R,3S)-compound.

Compound 35 (0.80 g., white solid, mp 84-86° (dec.)). ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.87 (m, 6H, $CH(C\underline{H}_3)_2$), 2.31 (m, 1H, $C\underline{H}(CH_3)_2$), 3.60 (broad s, 1H, -OH), 4.02 (m, 3H, CH_2), 5.12 (d, 1H, -C<u>H</u>OH, J = 6 Hz), 5.95 (d, 1H, C<u>H</u>Br, J = 6 Hz), 7.32 (s, 5H, aromatic). IR ($CHCl_3$) ν 3740, 3675, 1860, 1725, 1535, 1440, 1400, 1050, 935 cm^{-1}. EIMS m/z (relative intensity) 277 (3), 276 (2), 260 (3), 259 (3), 205 (32), 206 (65), 148 (49), 132 (36), 131 (42), 127 (30), 107 (64), 105 (50), 86 (73), 85 (73), 79 (55), 77 (75), 69 (35), 68 (100).

Compound 36 (0.23 g of colorless liquid). ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.90 (m, 6H, $CH(C\underline{H}_3)_2$), 3.40 (s broad, 1H, -OH), 4.30 (m, 3H, CH_2), 5.03 (d, 1H, -C<u>H</u>OH, J = 8.4 Hz), 5.85 (d, 1H, -C<u>H</u> Br, J = 8.4 Hz), 7.33 (s, 5H, aromatic). IR ($CDCl_3$) ν 1800, 1720, 1535, 1440, 1400, 1050, 935cm^{-1}. EIMS m/z (relative intensity) 278 (M^+-C_6H_6, 3), 277 (9), 260 (9), 208 (10.7), 206 (28), 148 (18), 132 (100), 131 (23), 127 (10), 107 (28), 105 (29), 103 (30), 86 (22), 85 (30), 79 (24), 77 (47), 69 (16), 68 (41).

2.3.6 Synthesis of Racemic-threo-N-Benzoyl Phenylisoserine via the Darzens Condensation. Racemic ethyl-3-phenylglycidate (41). A 0.1 N sodium ethoxide solution was freshly prepared from sodium (2.3 g, 0.1 mol) and dry ethanol (50 mL). It was then added dropwise over 45 min into a solution of benzaldehyde (10.1 mL, 0.1 mol) and ethyl chloroacetate (15.1 mL, 0.1 mol) in dry ethanol (10 mL). The reaction mixture was stirred for 3 h at room temperature, then allowed to stand overnight in the refrigerator. It was then poured into water (40 mL), and extracted twice with ether (600 mL). The ethereal layer was washed

until the washing was neutral to litmus, then dried over anhydrous Na_2SO_4. Concentration *in vacuo* yielded a pale yellow liquid which was distilled under reduced pressure, bp 110-116°C (2 mm. Hg), 16.0 g (83%). The distillate was found, by ^1H, NMR to be composed of trans to cis-isomer in 4.5 to 1 ratio. ^1H NMR (90 MHz, $CDCl_3$) δ 0.94 (t, 3H, CH_3 of cis-isomer), 1.27 (t, 3H, CH_3 of trans), 3.44 (d, 1H, H-2 of trans), 4.03 (d, 1H benzylic proton of trans), 4.20 (q, 2H, H-3 of cis-), 7.27 (s, 10H, aromatic of both trans and cis).

Racemic threo-Ethyl-3-Chloro-2-Hydroxy-3-Phenylpropionate (42).[62b] Anhydrous HCl gas was bubbled into a solution of ethyl 3-phenylglycidate (41, 7.30 g, 38.02 mmol) in benzene (250 mL) at room temperature during 3 h. The solvent was removed to yield an oil which solidified to white solid. The crude product was recrystallized from ether-hexane (1:2, 70 mL) to colorless needles, 4.55 g (53%), mp 83-86°C (lit. mp 85-86°C). ^1H NMR (90 MHz, $CDCl_3$) δ 1.33 (t, 3H, CH_3, J = 8.5-9.0 Hz), 3.26 (d, 1H,-OH), 4.28 (q, 2H, CH_2, J = 8.5-9.0 Hz), 4.37-4.57 (dd, 1H, -C<u>H</u>OH, J = 3.0 Hz), 5.27 (d, 1H, -C<u>H</u>Cl, J = 3.0 Hz), 7.20-7.60 (m, 5H, aromatic). IR (CHC_3) ν 3740, 3675, 3580, 1760, 1505, 1440, 1130, 940, 880 cm^{-1}.

Racemic cis-Ethyl-3-Phenylglycidate (43).[62b] Threo-ethyl-3-chloro-2-hydroxy-3-phenylpropionate (1.50g, 6.56 mmol) was heated with 7% sodium carbonate solution (15.2 mL, 10.0 mmol) at 48-50°C for 2 h. The mixture was saturated with sodium chloride and extracted with ether (110 mL), dried with anhydrous $MgSO_4$ and concentrated *in vacuo* to yield a liquid which solidified on standing. The crude product was purified by flash chromatography eluted with 20% ethyl acetate in hexane to a colorless liquid, 0.36 g,(29%). ^1H NMR (90 MHz, $CDCl_3$) δ 1.02 (t, 3H,

CH_3, J = 7 Hz), 3.44 (d, 1H, H-2, J = 4.5 Hz), 3.95 (q, 2H, CH_2, J = 7 Hz), 4.20 (d, 1H, H-3, J = 4.5 Hz), 7.30 (m, 5H, aromatic). IR (neat) ν 3025, 1760, 1300, 1210, 1035 cm^{-1}.

Racemic Potassium cis-3-Phenylglycidate.[62a] Ethyl-cis-3-phenylglycidate (3.20 g, 16.6 mmol) in absolute ethanol (6.5 mL) was added slowly into a solution of potassium hydroxide (1.6 g, 28.5 mmol) in absolute ethanol (9.5 mL) at 0°C. Stirring was continued for 3 h after which the white precipitate was filtered off and washed repeatedly with ethanol. The yield was 2.45 g, 73%, mp 196-199°C (lit. mp 191-193°C).

Racemic threo-3-Phenylisoserine (44). Racemic potassium cis-3-phenylglycidate (2.40 g, 11.9 mmol), was treated with concentrated NH_4OH (86 mL). The solution was allowed to stand at room temperature for 6 days and then evaporated to dryness. The white solid was dissolved in water (180 mL) and the pH was adjusted to 6 with 6 N. HCl. The white precipitate was recrystallized from water (240 mL) as colorless needles (1.0 g, 46%), mp 262-268°C (dec). IR (nujol) ν 3530, 1680, 1600, 1550, 1420, 1370, 1290, 1250, 1090, 965 cm^{-1}.

Racemic threo-N-Benzoyl-3-Phenylisoserine (45). Racemic threo-3-phenylisoserine **(44)** was benzoylated by the Schotten-Baumann procedure.[63] The crude product was recrystallized from ethanol-water (1:2, 50 mL) as a white solid (0.20 g, 25%), mp 174-177°C (dec). ^1H NMR(90 MHz, $CDCl_3$) δ 4.43 (d, 1H, H-2), 5.60 (d,1H,-OH), 5.70 (m, 1H, H-3), 7.22-8.0 (m, 10H, aromatic), 8.15 (broad s, 1H, NH). IR (nujol) ν 3580, 3400, 1725, 1640, 1540, 1280, 1130 cm^{-1}. EIMS m/z (relative intensity) 267 ($M^+ - H_2O$, 2), 222 (8), 210 (17), 193 (9), 106 (10), 105 (100), 103 (11), 91 (9) 89 (9), 77 (60), 76 (9) 51 (26), 50 (11).

3.0 COUPLING REACTION OF THE SIDE CHAIN TO THE TAXANE NUCLEUS

3.1 Introduction

In this chapter the research work involving attempts to attach the C-13 side chain to the suitably protected taxane nucleus will be presented. The ultimate goal is to obtain a series of modified taxols with structurally different side chains which will be used in an investigation of structure-activity relationships.

As shown in Figure 2 (p.9), the C-13 hydroxyl group of baccatin (III) **(15)** exists in the α-position and is very sterically hindered due to the cup-shape structure of taxane nucleus. In addition, the presence of the C-4 α-acetoxyl group provides a carbonyl group capable of hydrogen bonding to the C-13 hydoxyl group, thus reducing its reactivity still further.

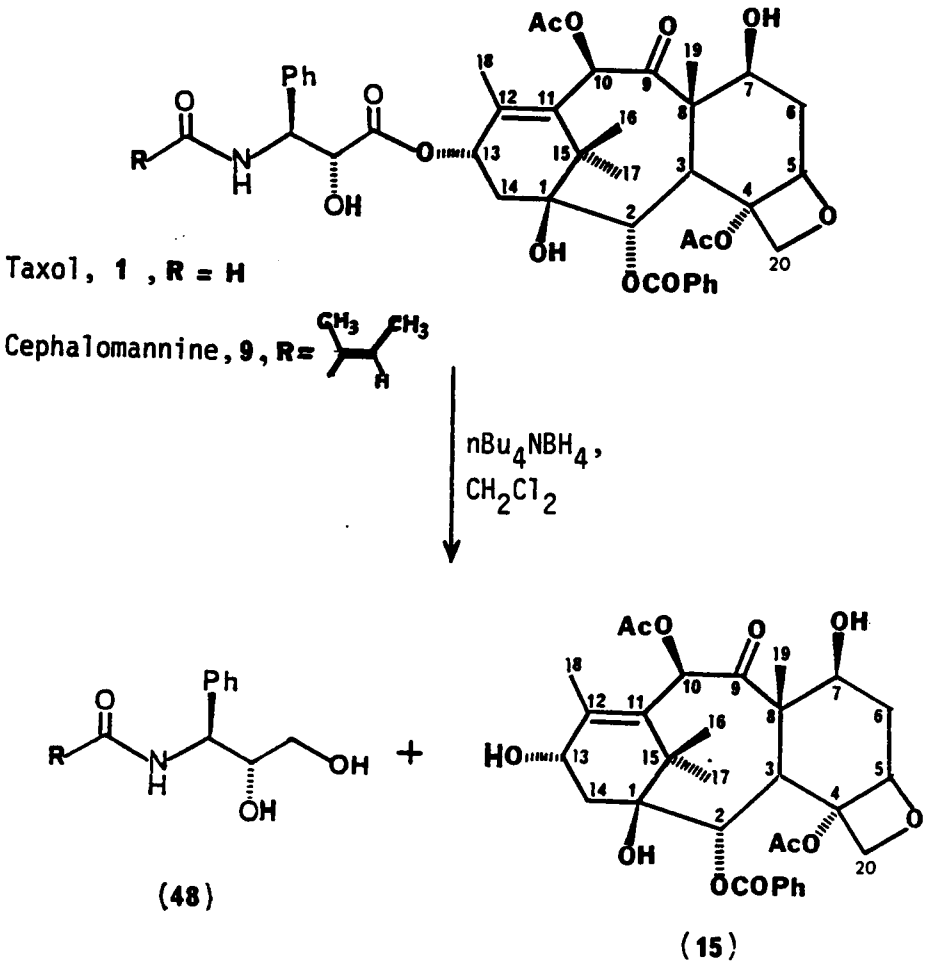
Several groups have been engaged in the investigation of coupling reaction of the C-13 side chain to the taxane nucleus,[44,86] and some acetylbaccatin IV derivatives had been prepared in this laboratory by Magri,[87] but not completely characterized.

3.2 Results and Discussion

3.2.1 Preparation and Protection of Baccatin III

In the investigation of the coupling reaction of the C-13 side chain to taxane nucleus, baccatin III **(15)** was chosen for the taxane nucleus because of its closely related structure to taxol. In fact, it is structurally a taxol molecule without the C-13 ester side chain. Any reaction which couples different side chains to it will produce modified taxol compounds. Baccatin III (15) can be obtained in good yield from the reaction of tetra-butyl ammonium borohydride on the mixture of taxol

and cephalomannine.[45]



Scheme 16

Preparation of Baccatin III (15)

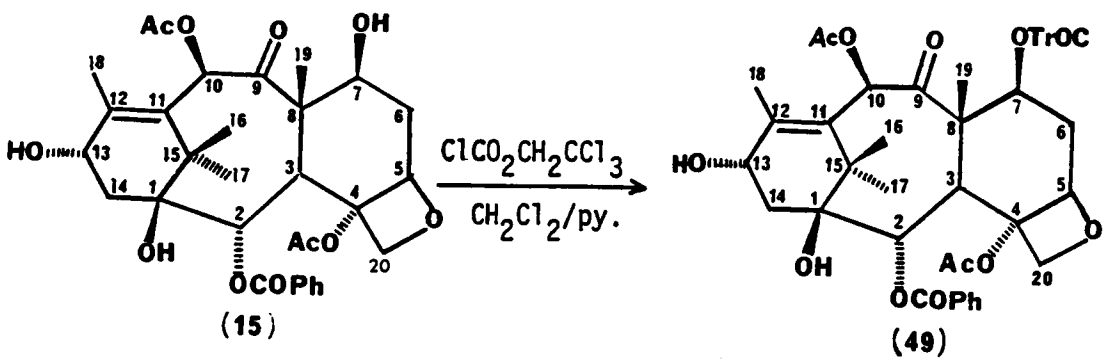
Baccatin III was obtained in 95% yield along with the reduced side-chain alcohol (48). The cleavage was proved to be reductive and the free 2'-hydroxyl group is essential for this reaction.[55] Characterization of the product was achieved by comparison of its ^1H NMR

spectrum to the reported data.

3.2.1.1 7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (49)

In a molecule of baccatin III (15) there are three hydroxyl groups. The one at C-1 is a tertiary type and is very hindered while the other two at C-7 an C-13 are secondary hydroxyl groups. No reaction at the C-1 hydroxyl group has been observed. The hydroxyl group at C-7 is more readily attacked by various reagents than that at C-13, since acetylation by acetic anhydride and pyridine at room temperature for a few hours afforded mostly 7-acetylbaccatin III.[87] For this reason the C-7 hydroxyl must be protected in order to prepare 13-acylbaccatins III.

The 2,2,2-trichloroethyloxycarbonyl group has been widely used as a protecting group in taxol research. Since it can be easily removed by treatment with zinc in methanol or acetic acid.[88] 7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (49) was prepared in 100% yield



(15) (49)

Scheme 17

Preparation of 7-(2,2,2-Trichloroethyl-oxycarbonyl) baccatin III (49)

by stirring a solution of baccatin III (15) in CH_2Cl_2 with 2,2,2-trichloroethyloxycarbonyl chloride and pyridine at room temperature for 45 min.

The ^1H NMR of the product showed a signal at 5.60 ppm as a doublet of doublets which was assigned to the C-7 proton, which is in contrast with the C-7 proton of baccatin III at 4.42 ppm. The methylene protons of the protecting group were seen as a well separated doublet of doublets at 4.62 and 5.02 ppm (J = 12 Hz) (Figure 6 and Table 1). The downfield shift of the C-7 proton and the presence of the trichloroethyloxycarbonyl group thus indicated that this compound is 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (49). The structure was confirmed by an FABMS which showed intense peaks at 761 mass units (MH^+), and 701 (MH^+- AcOH) indicating a molecular weight of 760.

The homonuclear COSY spectrum of 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (49) is shown in Figure 7, and expected couplings between protons were observed. Although the trichloroethyloxycarbonyl group appeared only as a large spot on the diagonal axis, the proton couplings between C-5 and C-6, and C-13 and C-14 could be seen by spots which showed the connection between each pair of protons. The COSY spectrum also revealed the chemical shifts of the C-6 methylene protons at 2.3 and 2.6 ppm.

The decoupled ^{13}C NMR spectrum of 7-(2,2,2-trichloroethyl-oxycarbonyl) baccatin III (49) was recorded and was shown in Figure 8. Peak assignments were aided by the INEPT spectrum (Figure 9) and the heteronuclear COSY spectrum (Figure 10 and Table 2). Rojas et al have reported the ^{13}C spectral data of several taxane-type compounds,

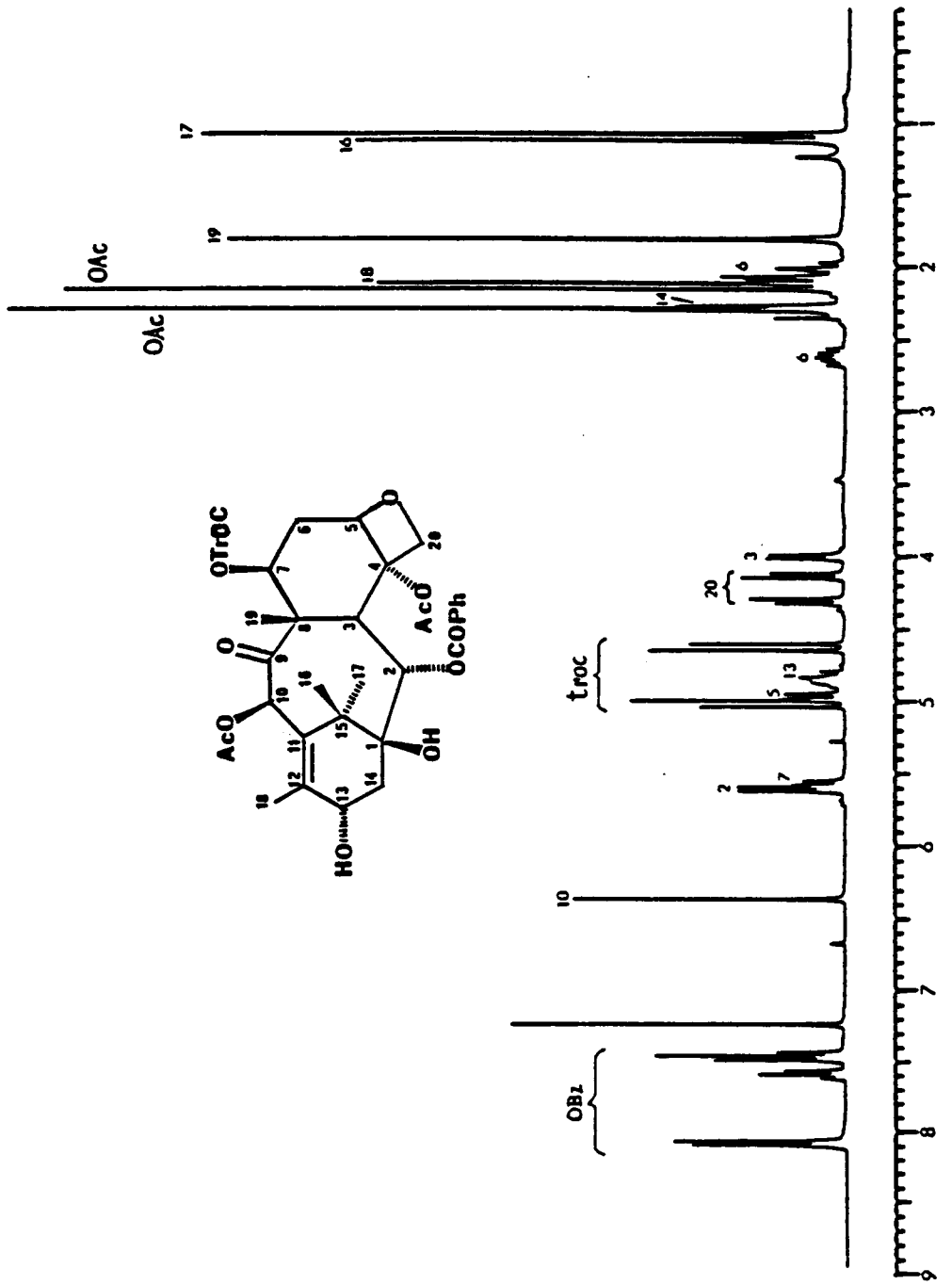


Figure 6. ^{1}H NMR of 7-(2,2,2-trichloroethyloxycarbonyl)baccatin III(49)

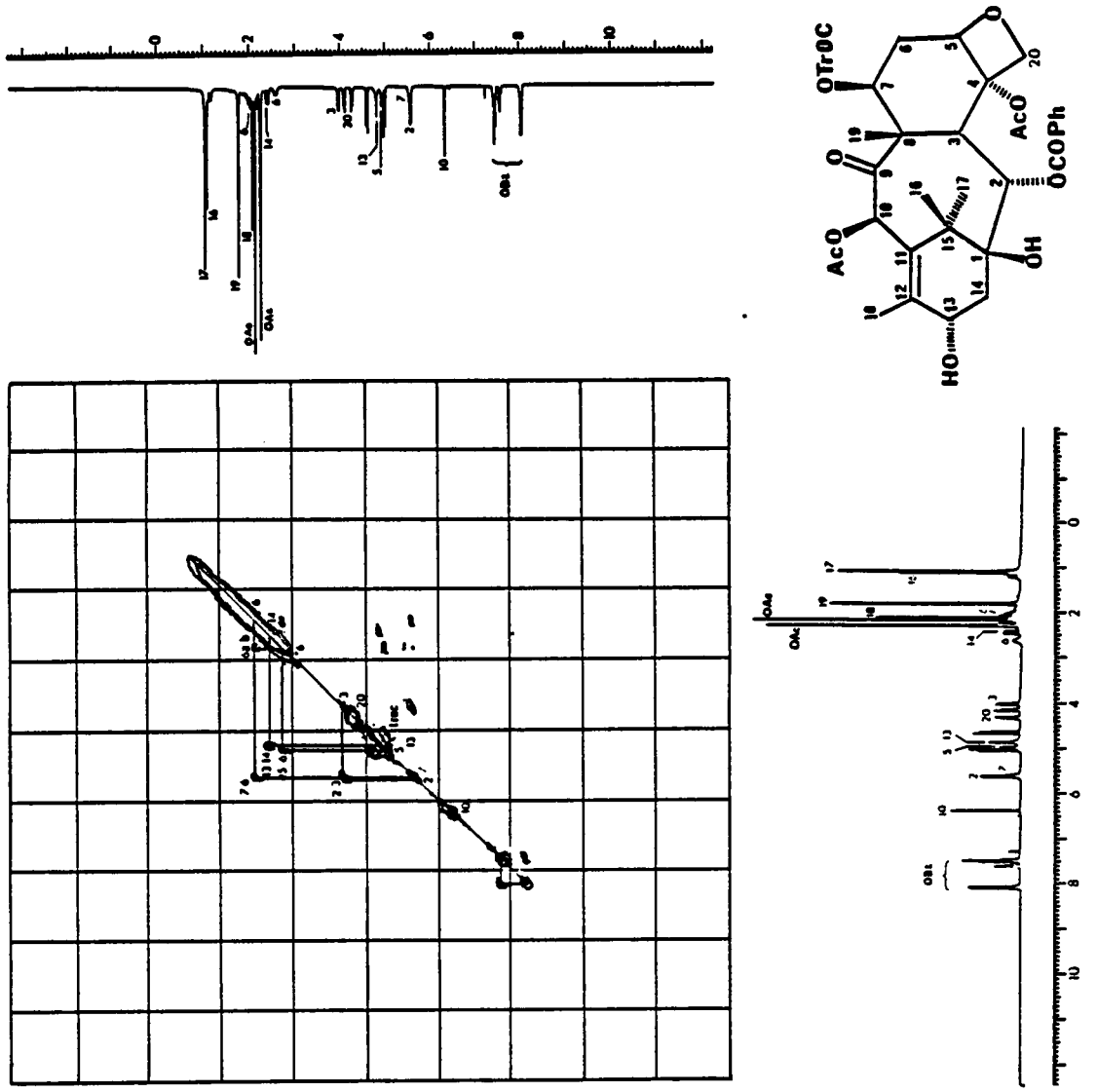


Figure 7. Homonuclear COSY spectrum of 7-(2,2,2-trichloroethyl-oxycarbonyl)baccatin III (49)

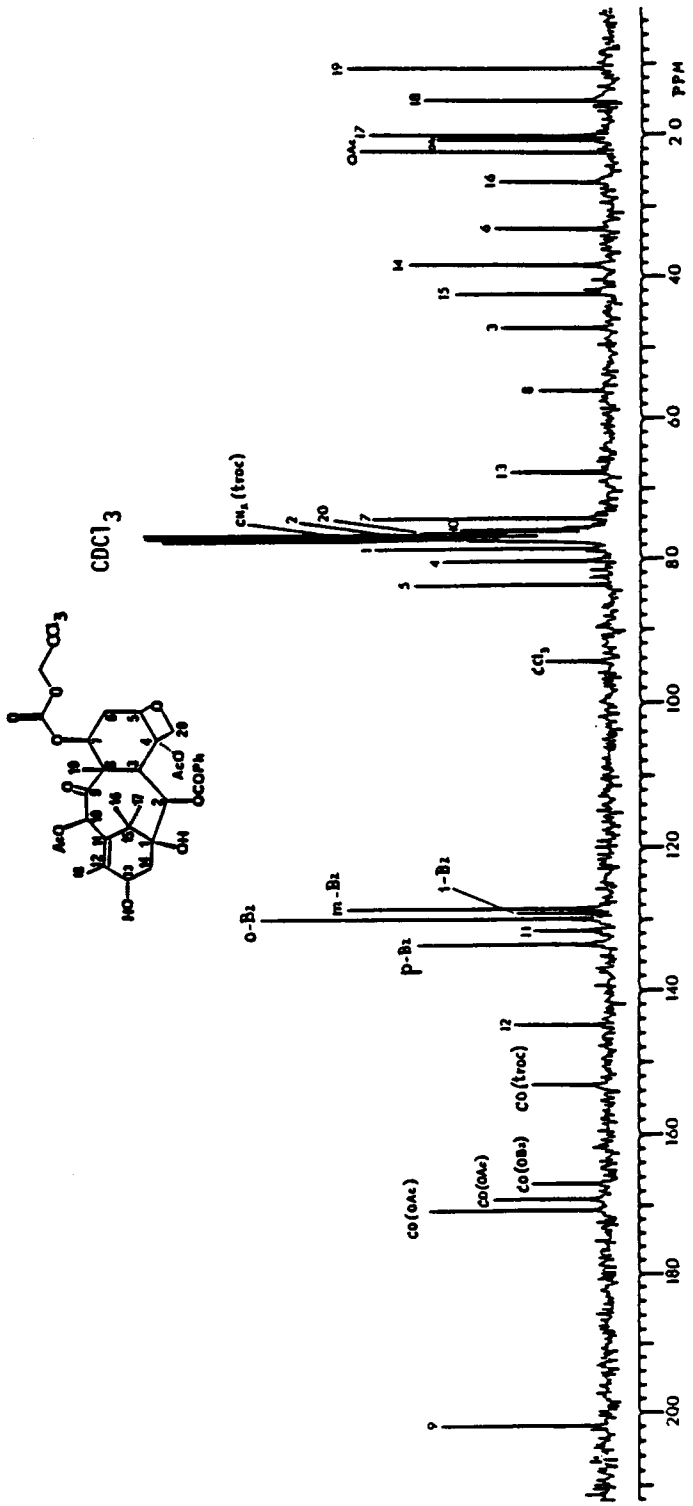


Figure 8. Proton-decoupled ^{13}C NMR spectrum of 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (49)

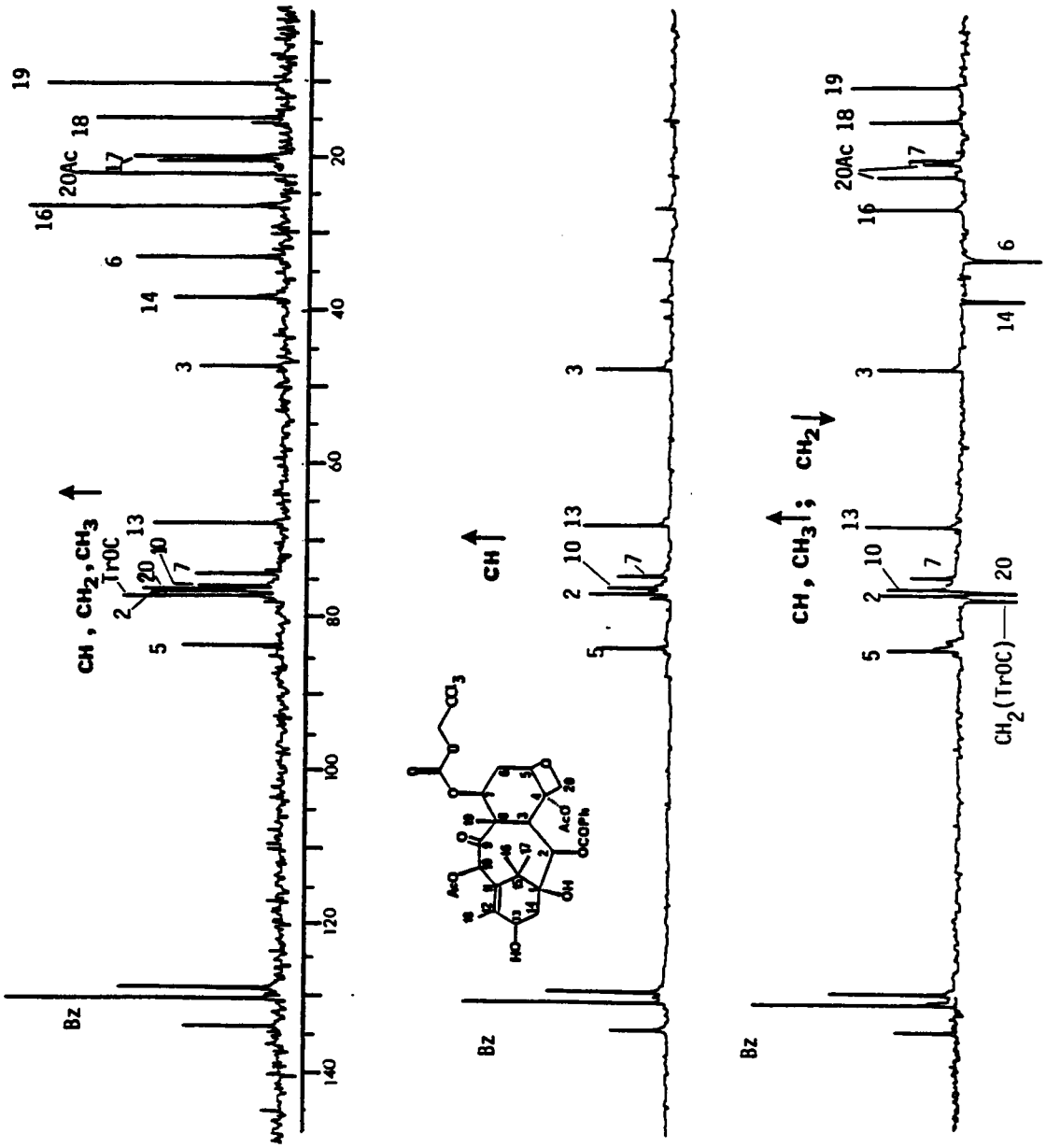


Figure 9. The INEPT Spectrum of 7-(2,2,2-trichloroethyl-oxycarbonyl)baccatin III((49)

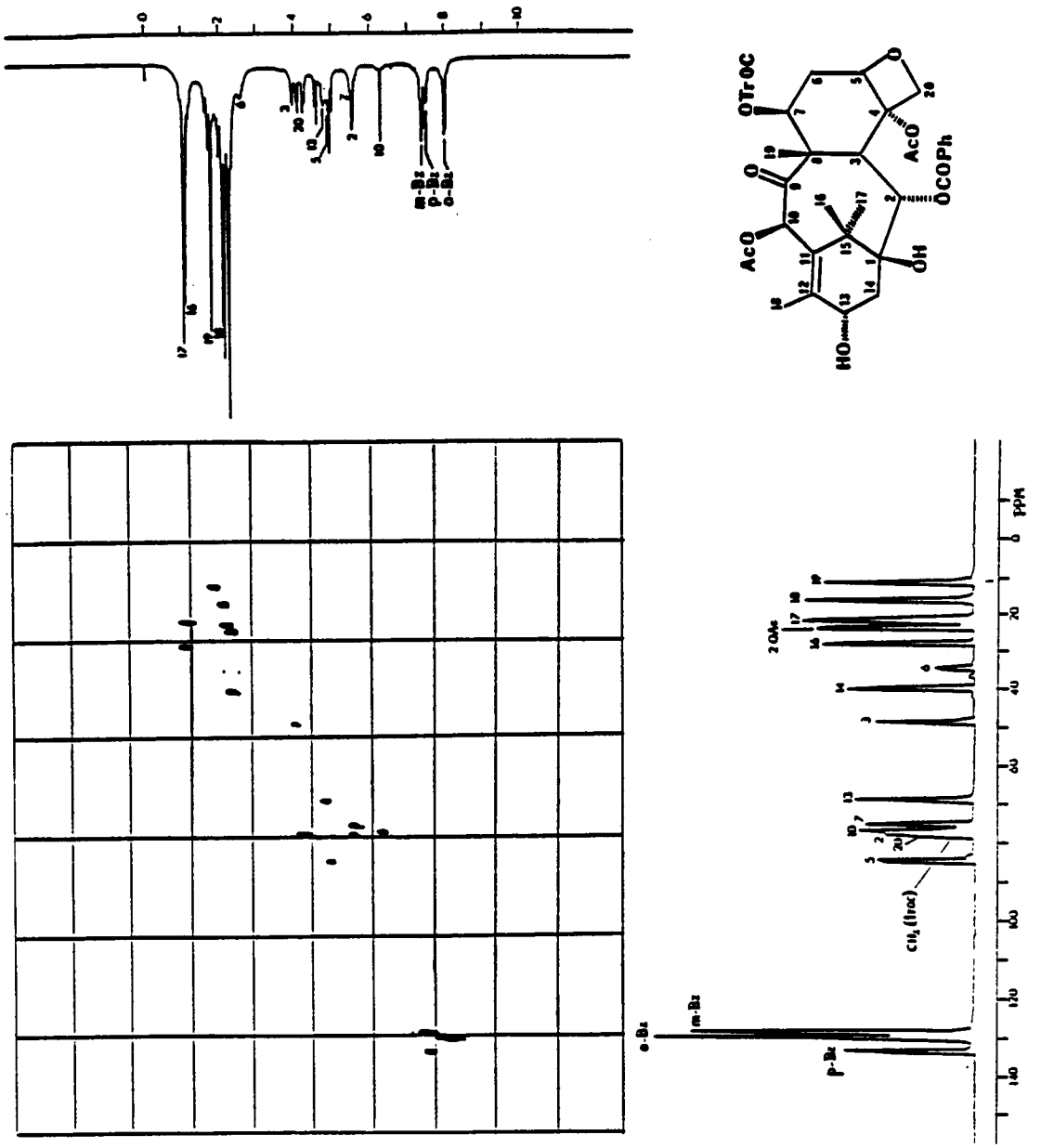
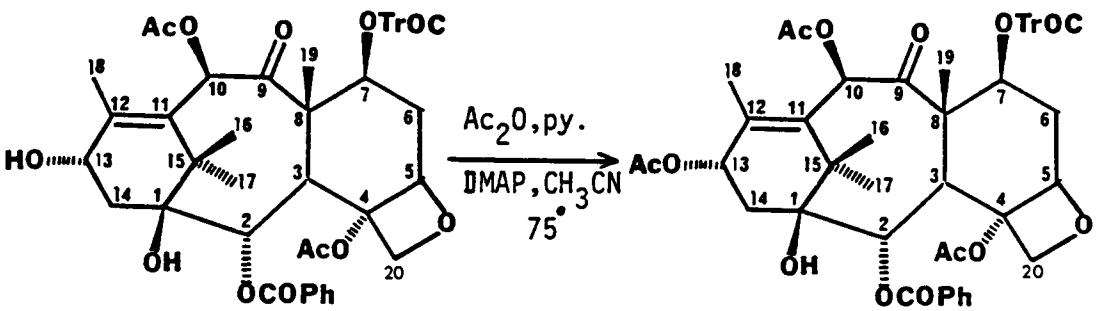


Figure 10. Heteronuclear COSY spectrum of 7-(2,2,2-trichloroethyloxycarbonyl)baccatin III (49)

including baccatin III and baccatin IV.[89] From the COSY spectrum the C-7 signal appeared at 74.4 ppm, compared with 72.3 ppm for the C-7 of baccatin III, which is in agreement with the fact that the former was deshielded by the presence of an ester functional group. Esterification at C-7 also caused a shielding of about 2 ppm on C-6 and C-8, and similar shielding was observed for C-12 and C-14 when C-13 was esterfied (next section). The assignment of C-6 and C-14 by Rojas et al must be reversed in order to be consistent with these facts and the heteronuclear COSY assignment, but their other assignments were in agreement with our assignments.

3.2.1.2 13-Acetyl-7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (50)

The preparation of 13-acetyl-7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (50) was achieved by heating a mixture of 7-(2,2,2-



(49) (50)

Scheme 18

Preparation of 13-Acetyl-7-(2,2,2-Trichloro-ethyloxycarbonyl)Baccatin III (50)

trichloroethyloxycarbonyl) baccatin III (49), 4-dimethylaminopyridine and pyridine in dry acetonitrile at 75° for 6 h (Scheme 18). The crude product was purified by preparative TLC.

The ^{1}H NMR spectrum of the compound is shown in Figure 11 and the chemical shifts are shown in Table 1. The signal at 6.18 ppm (broad triplet, J = 8 Hz) was assigned to the C-13 proton. Its downfield shift by 1.4 ppm from the original chemical shift of 4.82 ppm in baccatin III was due to the transformation of the free hydroxyl group to the acetate group; the broad triplet is characteristic for the C-13 proton. The signal of the new acetate methyl group was found at 2.35 ppm as a singlet indicating that one more hydroxyl group was converted to the ester. All other protons could be accounted for as shown in Table 1. The methylene protons of the trichloroethyloxycarbonyl group were at 4.65 and 5.05 ppm (two well separated doublets). The FABMS showed peaks at m/z 803 and 805 mass units for MH^{+} indicating a molecular weight of 802 [based on ^{35}Cl]. Thus, all data indicated that the product was 13-acetyl-7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (50).

The decoupled ^{13}C NMR spectrum of this compound is shown in Figure 12 and peak assignments are shown in Table 2. The assignments were achieved by comparing with the acetylbaccatin III derivatives and literature spectra.[87,89] The C-7 signal was assigned at 74.3 ppm which agreed with that of 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (49) which appeared at 74.4 ppm. The C-6 and C-8 carbons were shielded and their signals were found at 33.2 and 56.1 ppm respectively. The C-13 carbon was assigned to the peak at 69.5 ppm, 1.5 ppm downfield from

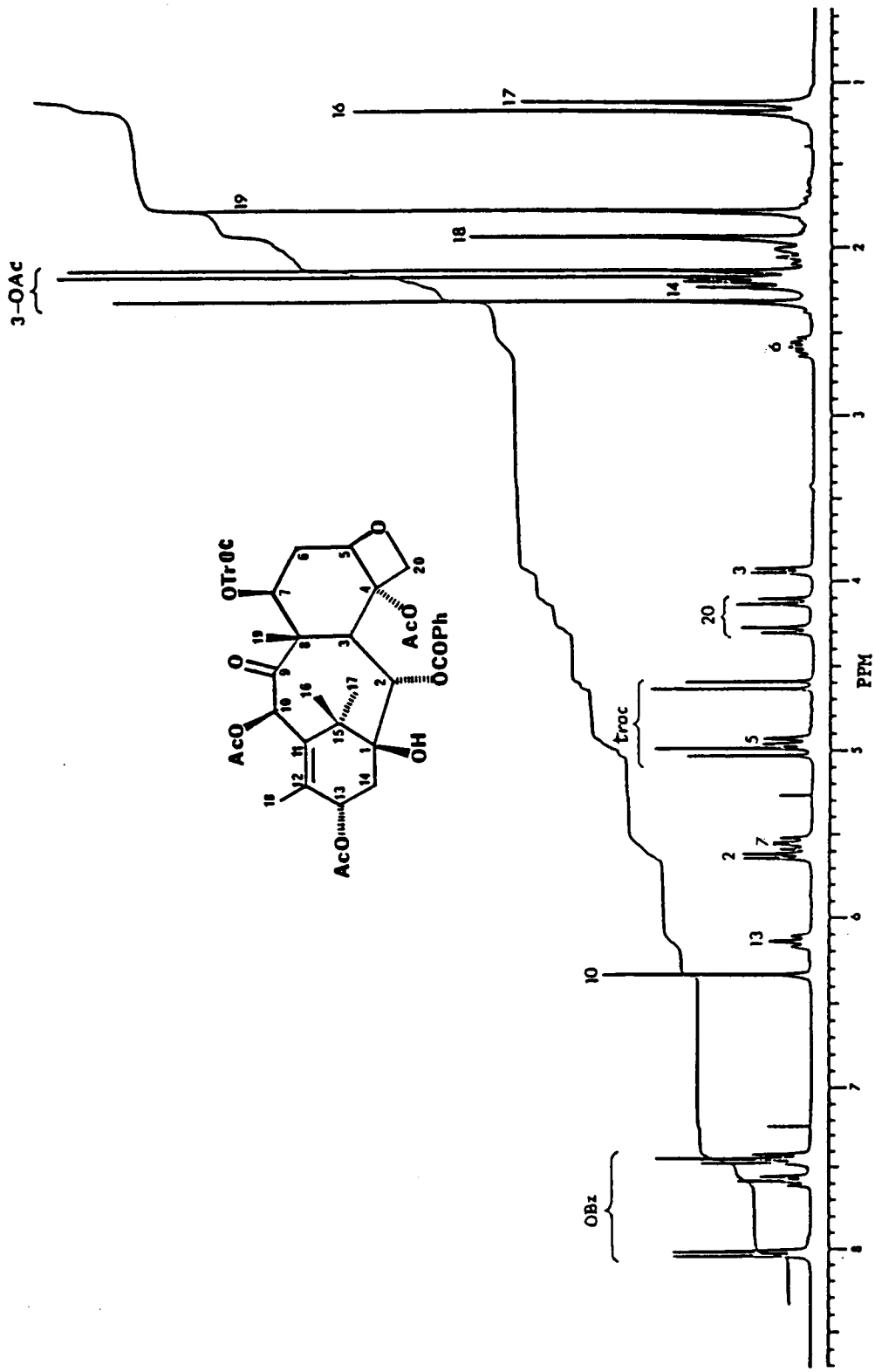


Figure 11. ^{1}H NMR of 13-Acetyl-7-(2,2,2-trichloroethyloxycarbonyl)baccatin III(50)

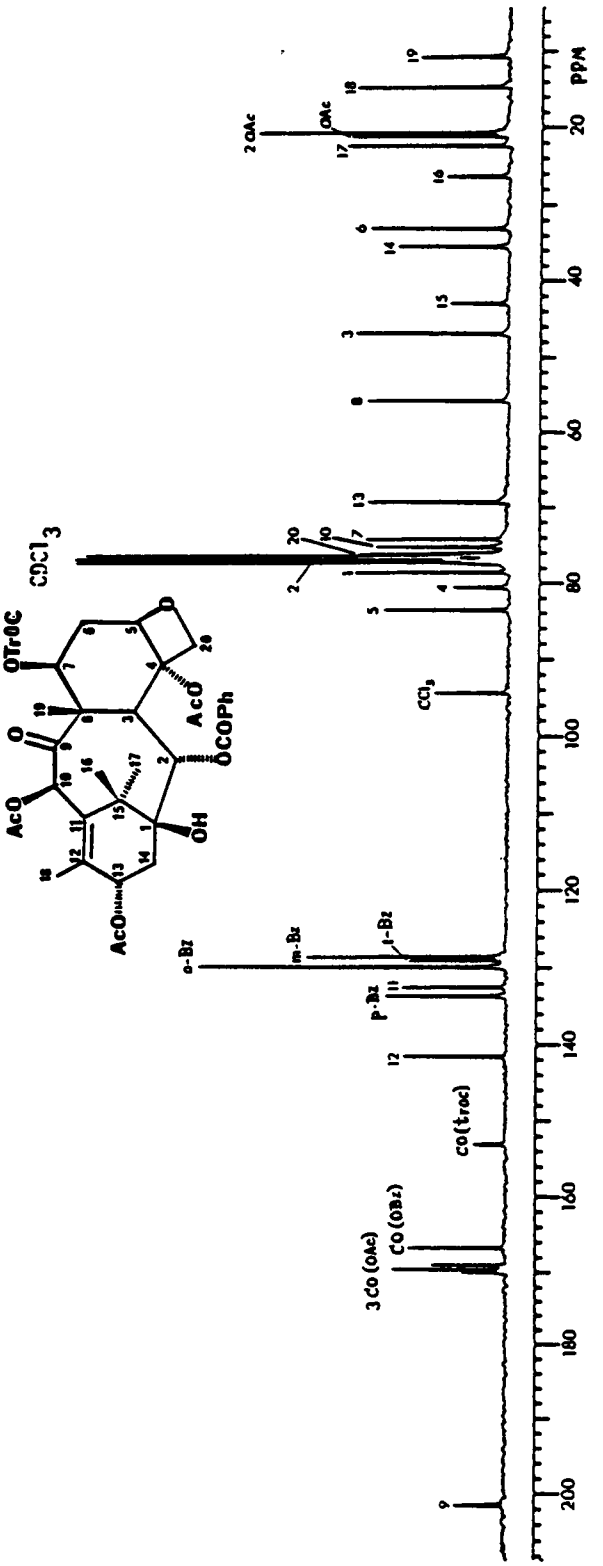
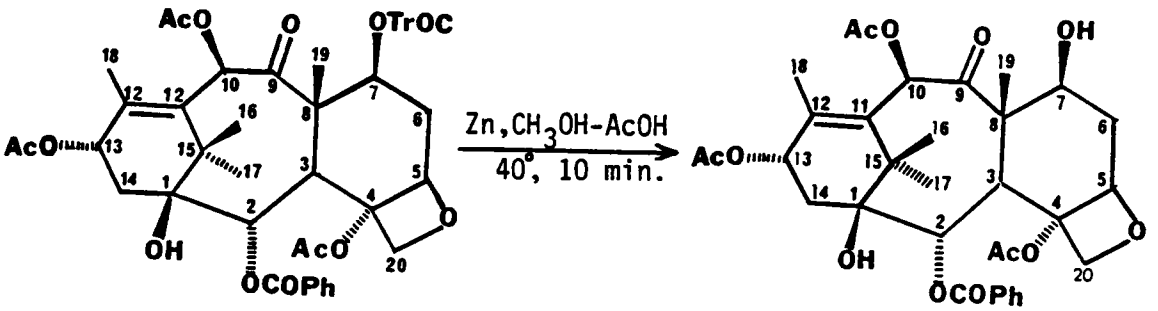


Figure 12 ^{13}NMR spectrum of 13-Acetyl-7-(2,2,2-trichloroethyloxycarbonyl)baccatin III (5a)

C-13 of baccatin III which was at 68.0 ppm. The C-12 and C-14 carbons were also shielded by about 3 ppm by the ester at C-13.

3.2.1.3 13-Acetylbaccatin III (51)

13-Acetylbaccatin III (51) was obtained by treating 13-acetyl-7-(2,2,2-trichloroethyloxycarbonyl baccatin III (50) with zinc in methanol and acetic acid at 40° for 10 min. The crude product was purified by preparative TLC to give 51 in 83% yield.



(50) (51)

Scheme 19

Preparation of 13-Acetylbaccatin III (51)

The ^{1}H NMR spectrum of the product is shown in Figure 13 and the chemical shifts are shown in Table 1. The C-13 proton was seen as a broad triplet at 6.15 ppm while the C-7 proton signal was assigned to a

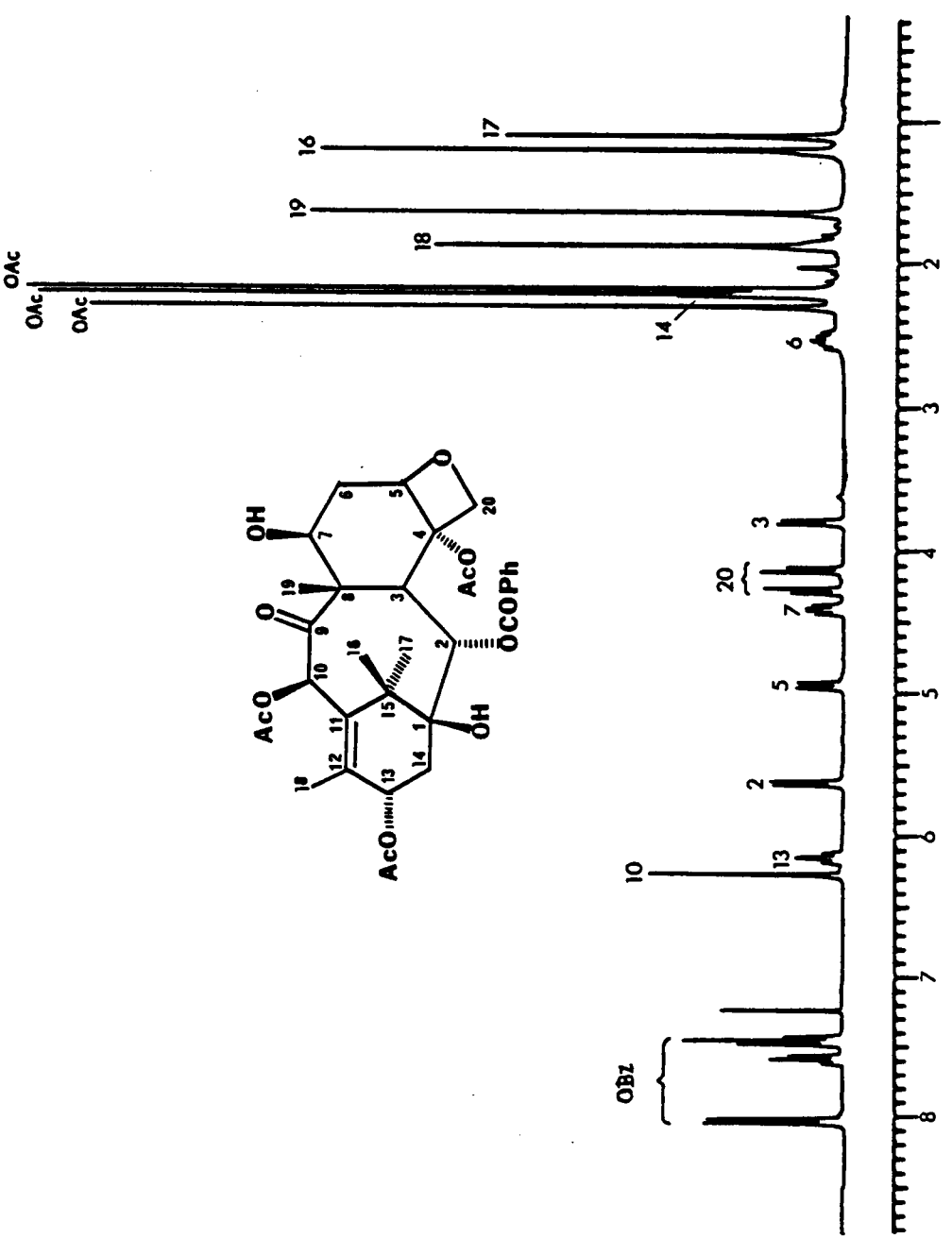


Figure 13. ^{1}H NMR spectrum of 13-Acetylbaccatin III (51)

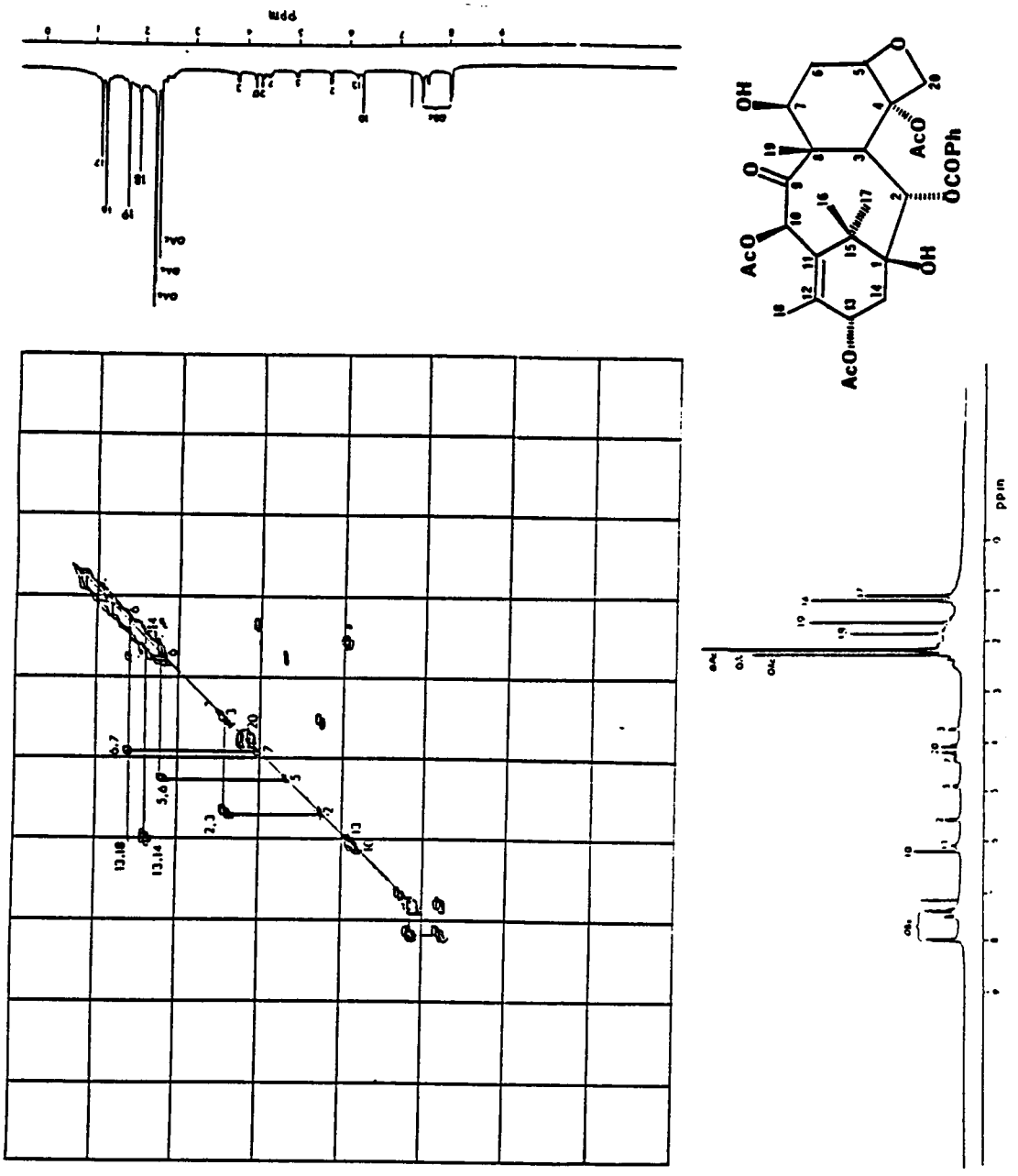


Figure 14. Homonuclear COSY spectrum of 13-Acetylbaccatin III (51)

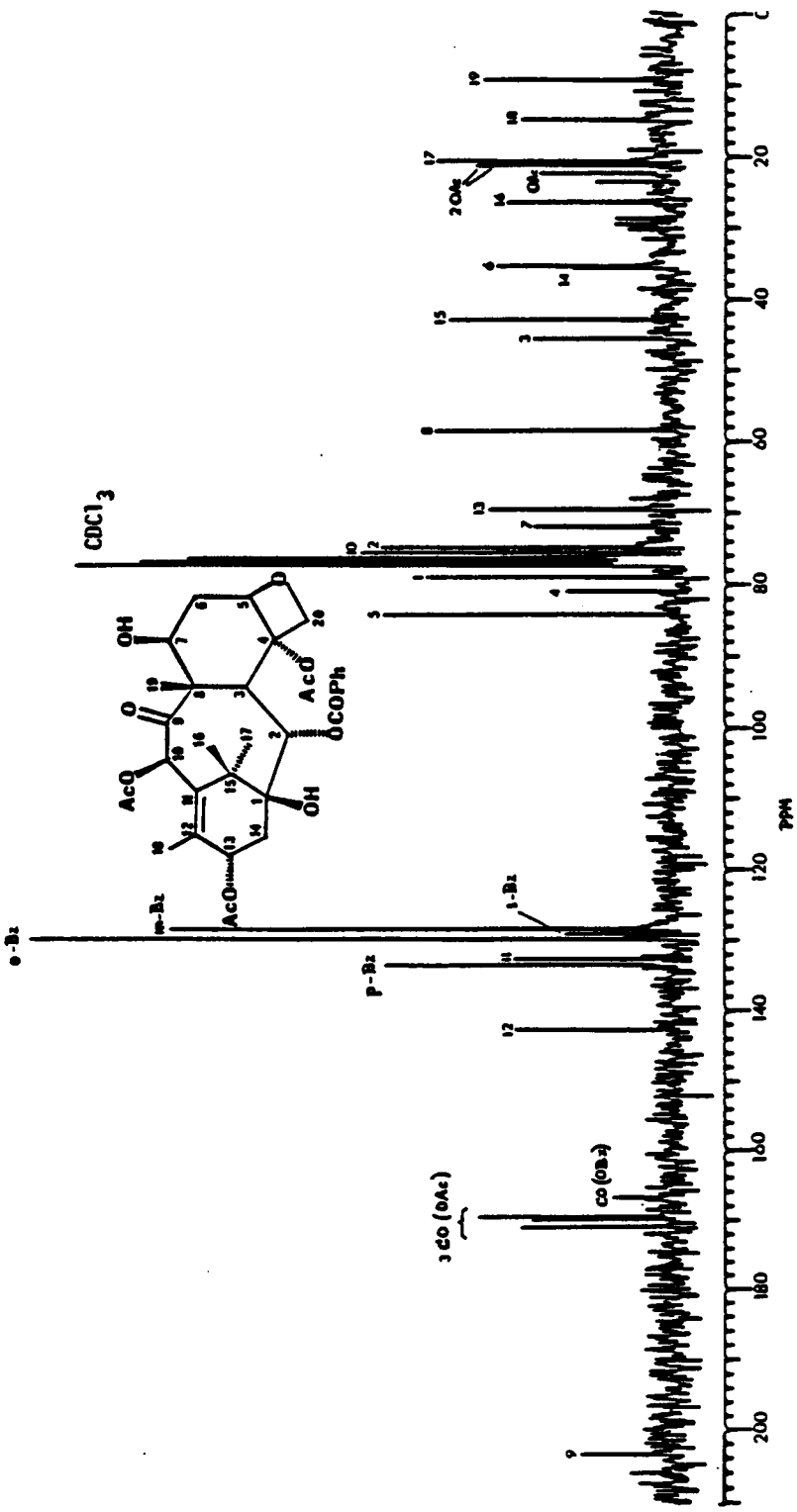


Figure 15. Proton-decoupled ^{13}C NMR spectrum of 13-Acetylbaccatin III (51)

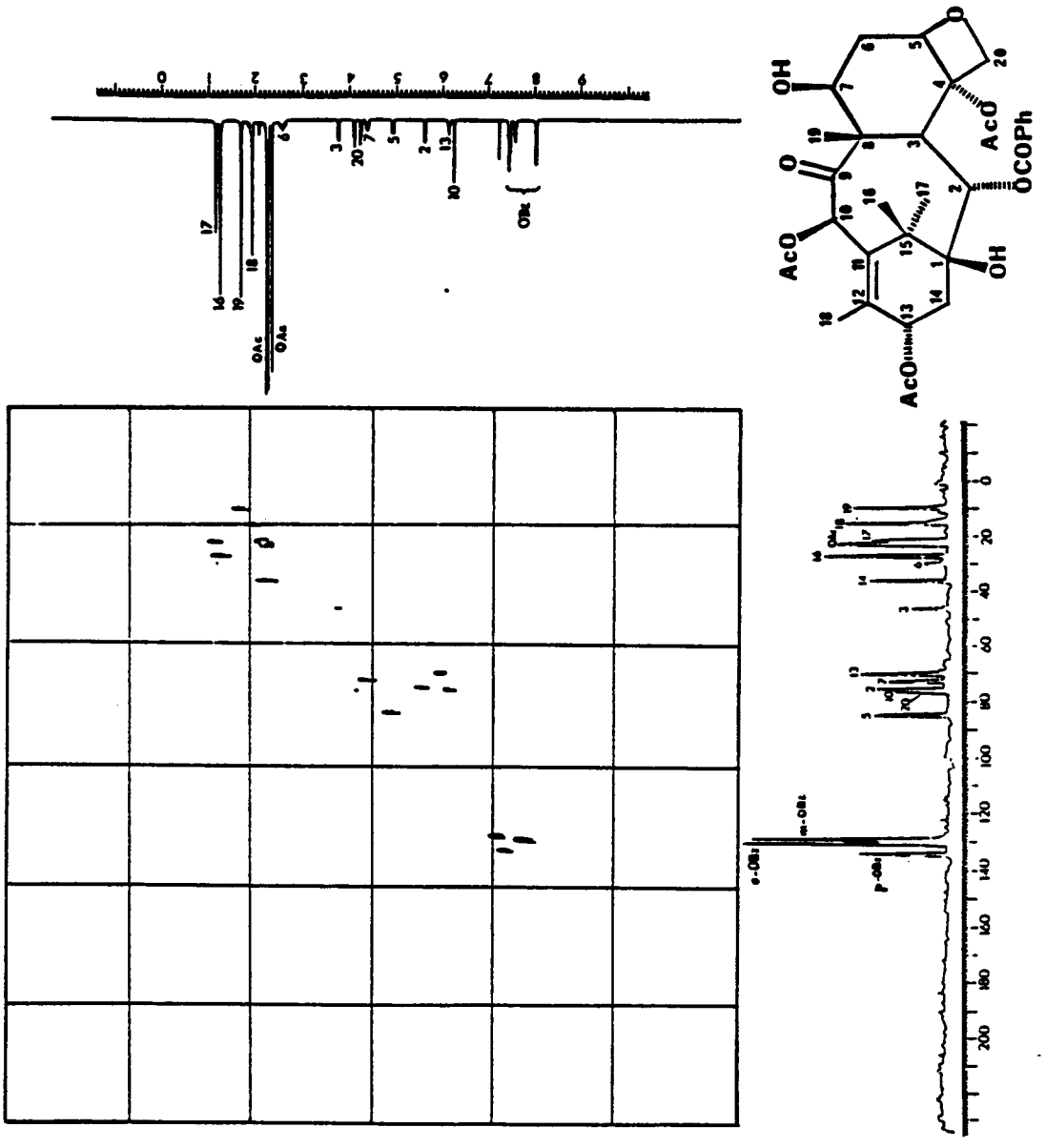


Figure 16 Heteronuclear COSY spectrum of 13-Acetyl-baccatin III (**51**)

broad triplet at 4.42 ppm indicating the presence of a free hydroxyl group. The removal of the trichloroethyloxycarbonyl group was confirmed by the absence of the signals at 4.6 and 5.05 ppm. The homonuclear COSY spectrum of **51** is shown in Figure 14. The couplings between protons C-2 and C-3, C-13 and C-14, C-5 and C-6, and C-6 anc C-7 were observed. In addition, a small coupling between the C-13 and C-18 protons could be seen. The FABMS of the product showed peaks at m/z 651 (MNa^+), and 629 (MH^+) indicating a molecular weight of 628 and the fragmentation pattern was similar to that of 7-acetylbaccatin III. Taken together, these facts contain the structure of **51** as 13-acetylbaccatin III.

The decoupled ^{13}C NMR and the heteronuclear COSY spectra are shown in Figures 15 and 16. The C-7 signal was seen at 72.1 ppm indicating the presence of a free hydroxyl group at C-7. The C-13 signal was found at 69.6 ppm, about 1.5 ppm downfield compared to the C-13 of baccatin III, indicating that the C-13 acetate was actually formed. The C-12 and C-14 carbons were also shielded by the C-13 acetate while the C-6 and C-8 carbons remained unchanged.

3.2.1.4. 7-Acetylbaccatin III (52)

7-Acetylbaccatin III **(52)** was prepared by stirring a mixture of baccatin III **(15)**, acetic anhydride, and pyridine at room temperature for 4 h. The crude product was purified by preparative TLC to yield a major product and some unreacted starting material.

The ^1H NMR spectrum (Figure 17) showed a doublet of doublets at 5.8 ppm (J = 2,11 Hz) which was assigned to the C-7 proton. It was deshielded by about 1.5 ppm from that of the C-7 proton of baccatin III indicating the C-7 acetate was formed. An additional methyl singlet

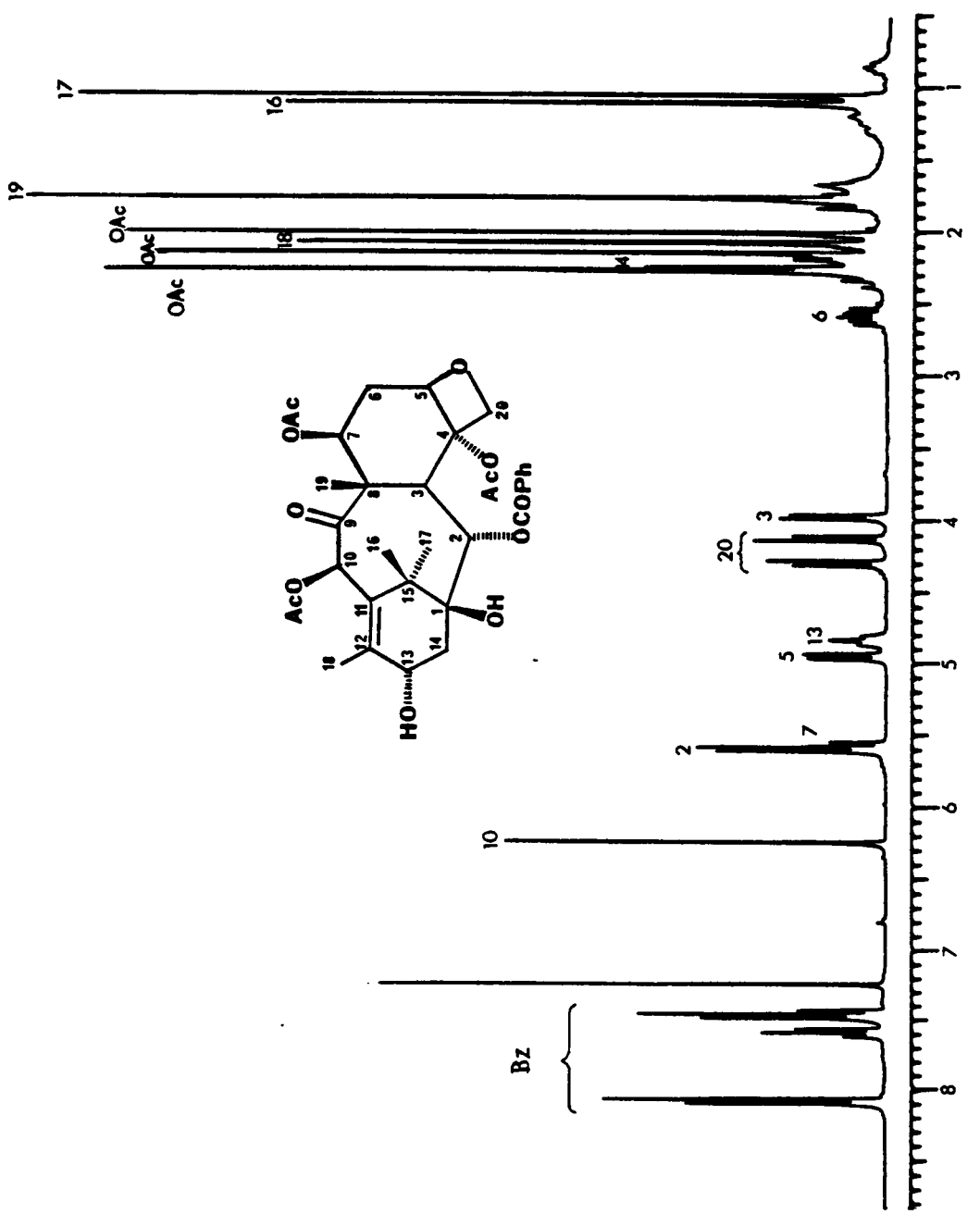


Figure 17. ^{1}H NMR Spectrum of 7-Acetylbaccatin III (52)

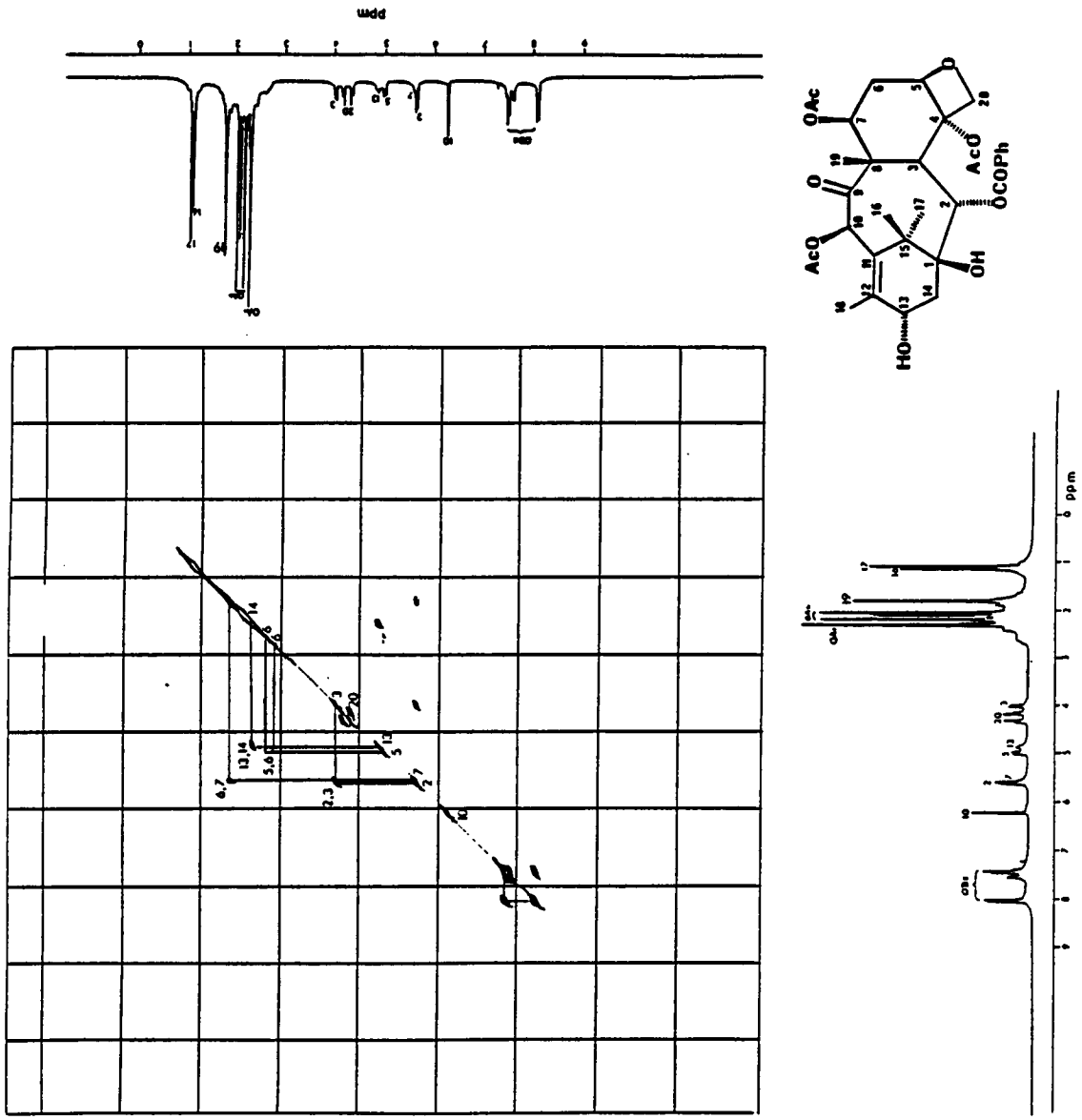
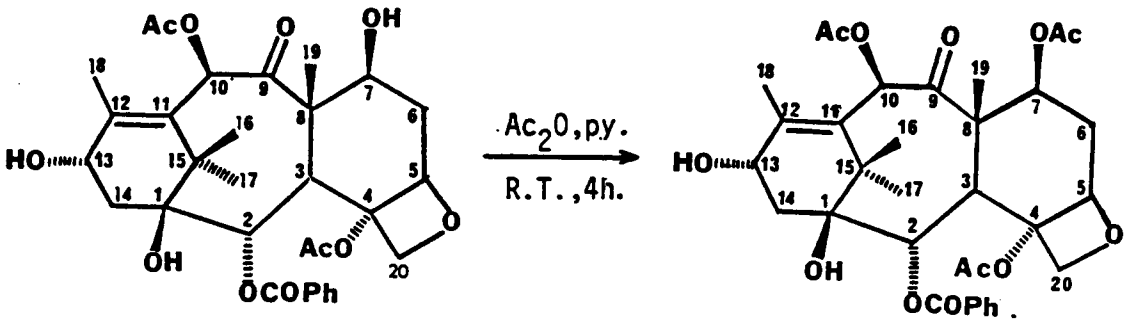


Figure 18. Homonuclear COSY spectrum of 7-Acetylbaccatin III (52)



(15) (52)

Scheme 20

Preparation of 7-Acetylbaccatin III (52)

found at 2.00 ppm confirmed this structure. The homonuclear COSY spectrum (Figure 18) showed coupling between the protons of C-2 and C-3, C-5 and C-6, C-6 and C-7, and C-13 and C-14.

The decoupled ^{13}C NMR spectrum and the heteronuclear COSY spectrum of 7-acetyl baccatin III are shown in Figures 19 and 20 respectively. The ^{13}C NMR chemical shifts are shown in Table 2. The C-7 signal was found at 71.6 ppm, about 0.7 ppm upfield from the C-7 of baccatin III. This was unusually high for the carbon with the acetate which was seen at about 74.3-74.5 ppm in 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III and 13-acetyl-7-(2,2,2-trichloroethyloxycarbonyl) baccatin III. However, a similar chemical shift was seen for the C-7 of 7, 13-

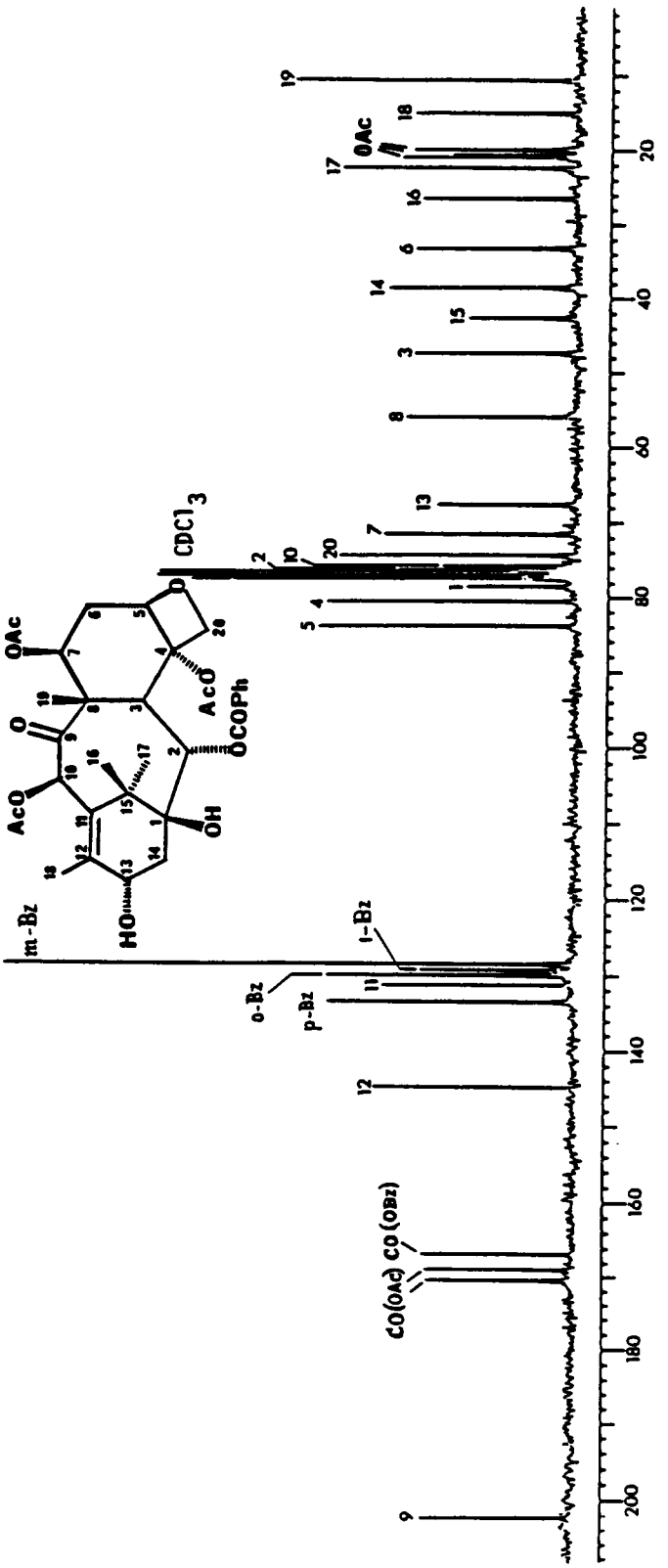


Figure 19. Proton-decoupled ^{13}C NMRspectrum of 7-Acetylbaccatin III (52)

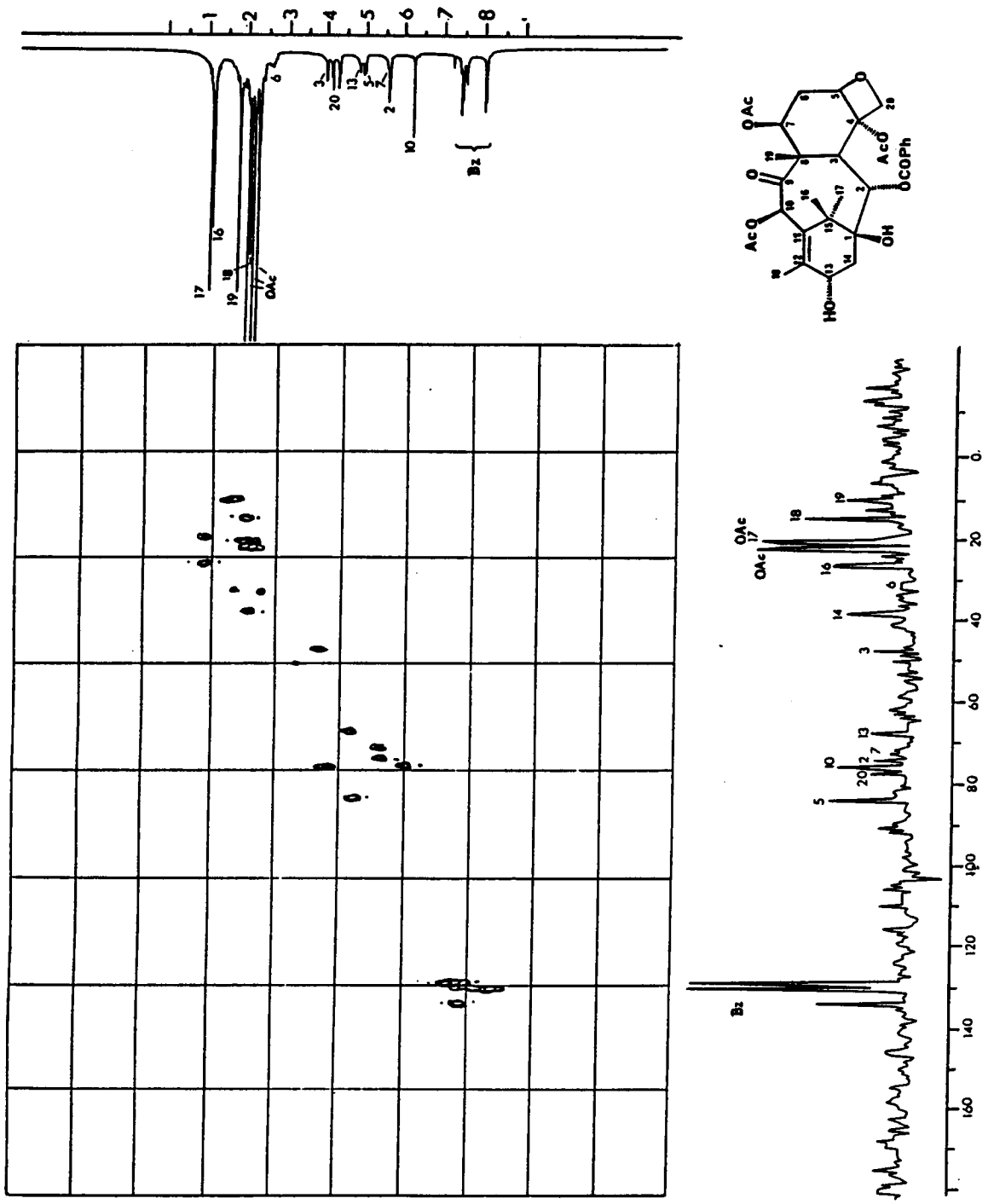


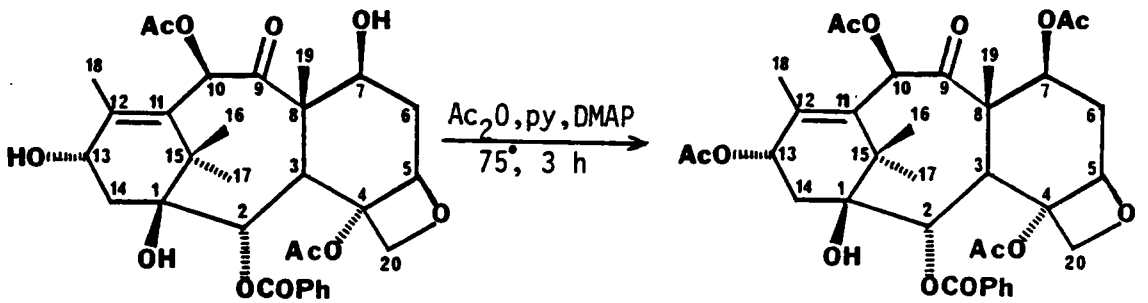
Figure 20. Heteronuclear COSY spectrum of 7-Acetyl-baccatin III (**52**)

diacetyl baccatin III (53), which was found at 71.4 ppm.

The FABMS of 7-acetylbaccatin III (52) showed peaks at m/z 651 (MNa^+), 649 (MNa^+-H_2), and 629 (MH^+) indicating a molecular weight of 628. The infrared spectrum displayed strong absorption bands at 1770, 1745, and 1700 cm^{-1}.

3.2.1.5 7,13-Diacetylbaccatin III (53)

7,13-Diacetylbaccatin III (53) was prepared by heating baccatin III (15) with excess acetic anhydride, 4-dimethylaminopyridine, and pyridine at 75° for 3 h. The crude product was purified by preparative TLC and obtained in 49% yield.



(15) (53)

Scheme 21

Preparation of 7,13-Acetylbaccatin III (53)

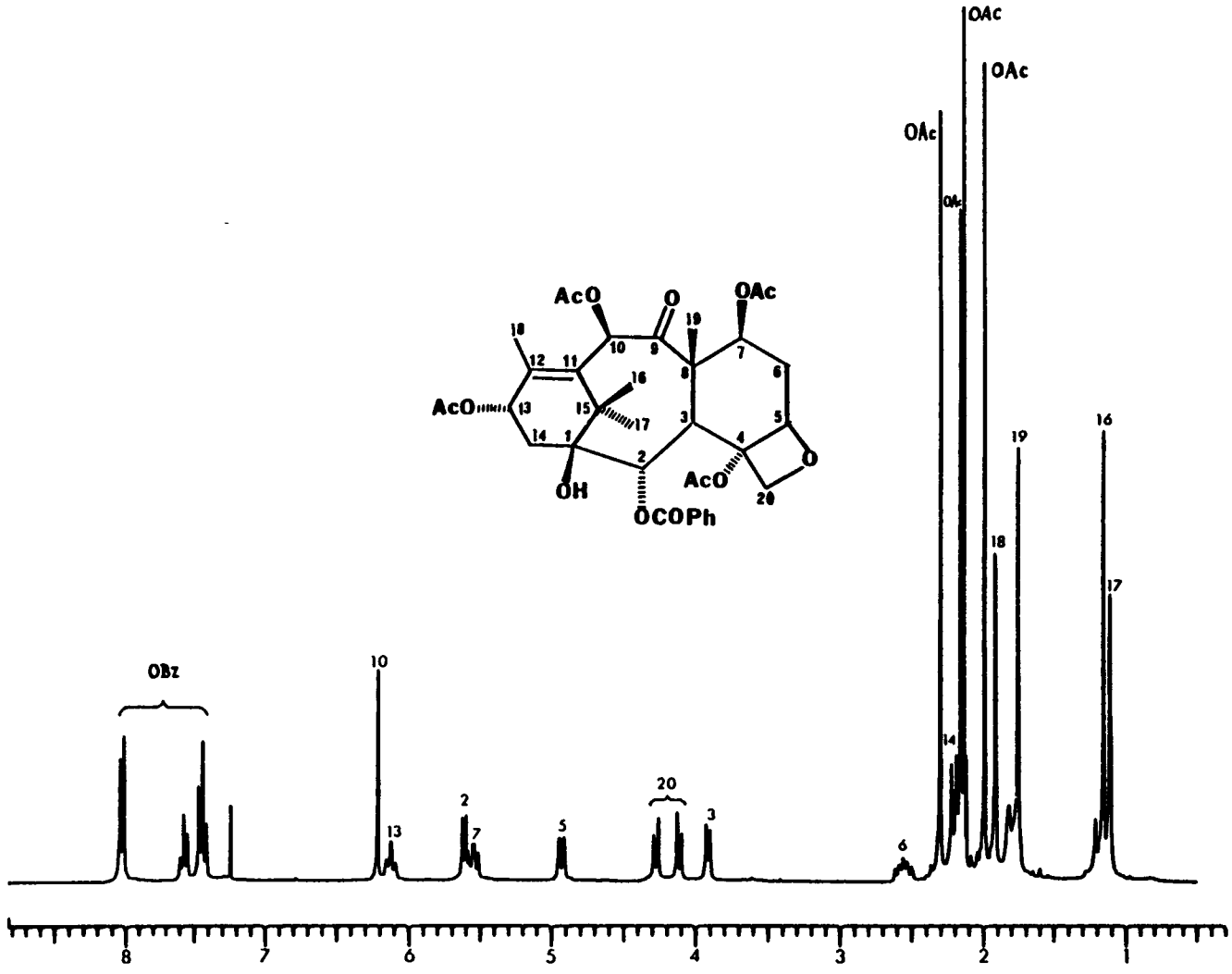


Figure 21. ^{1}H NMR spectrum of 7,13-Diacetylbaccatin III (**53**)

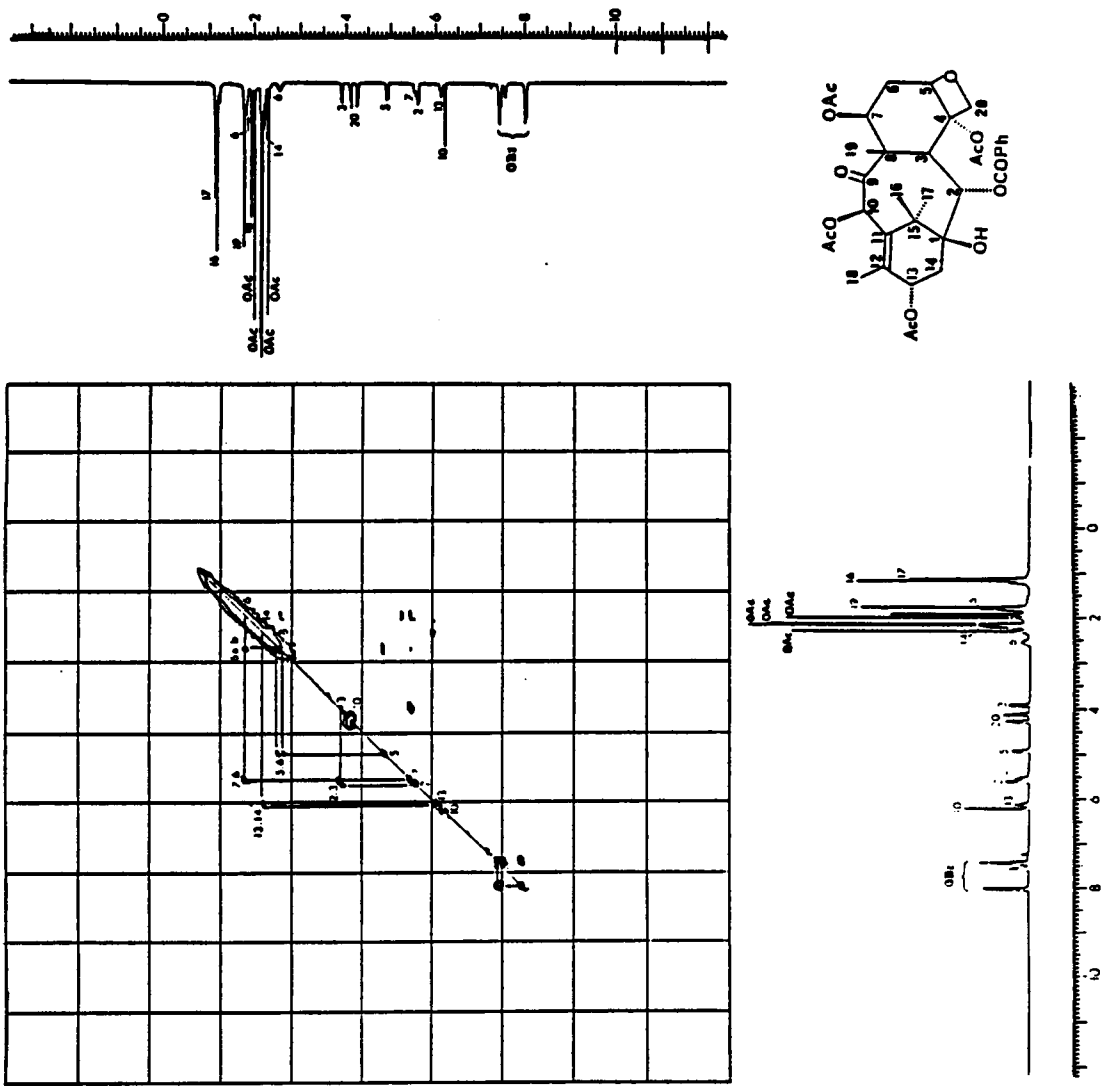


Figure 22. Homonuclear COSY spectrum of 7,13-Diacetyl-baccatin III (53)

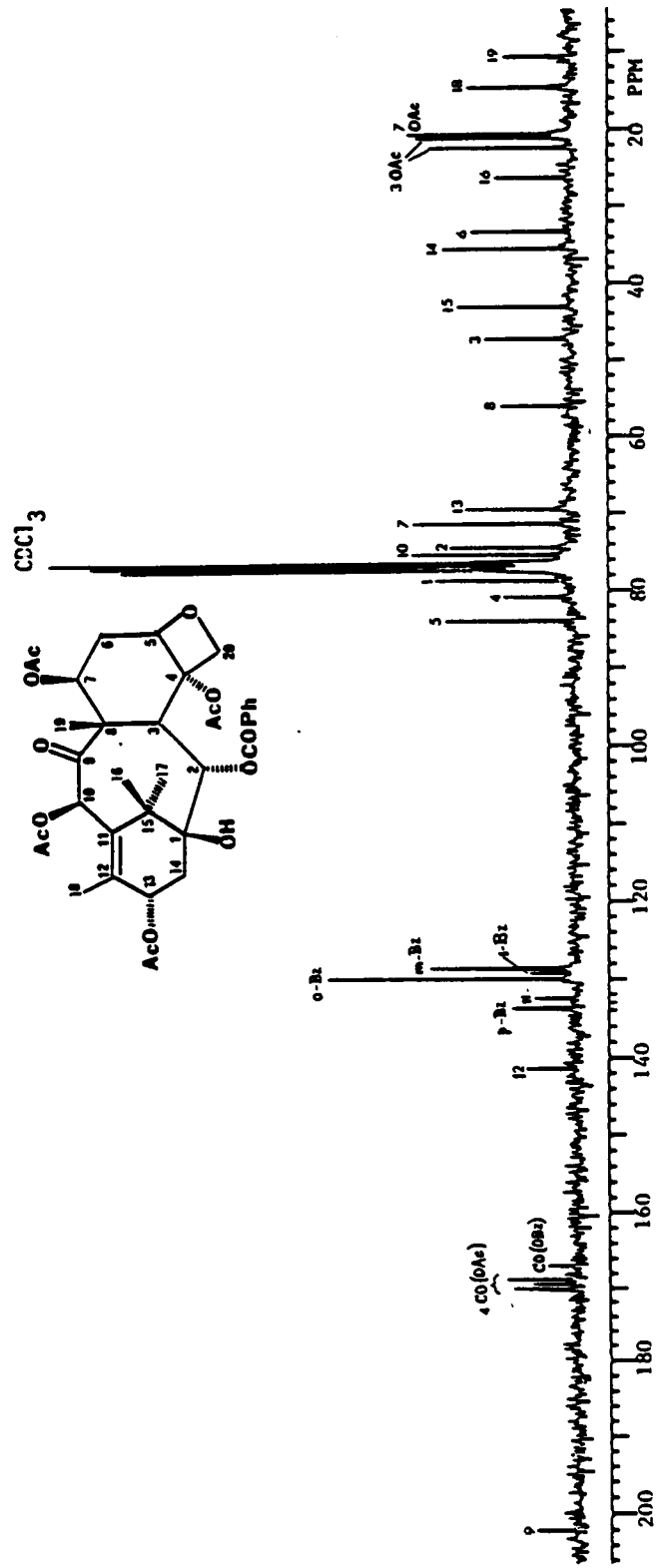


Figure 23. Proton-decoupled ^{13}C NMR spectrum of 7,13-Diacetylbaccatin III (53)

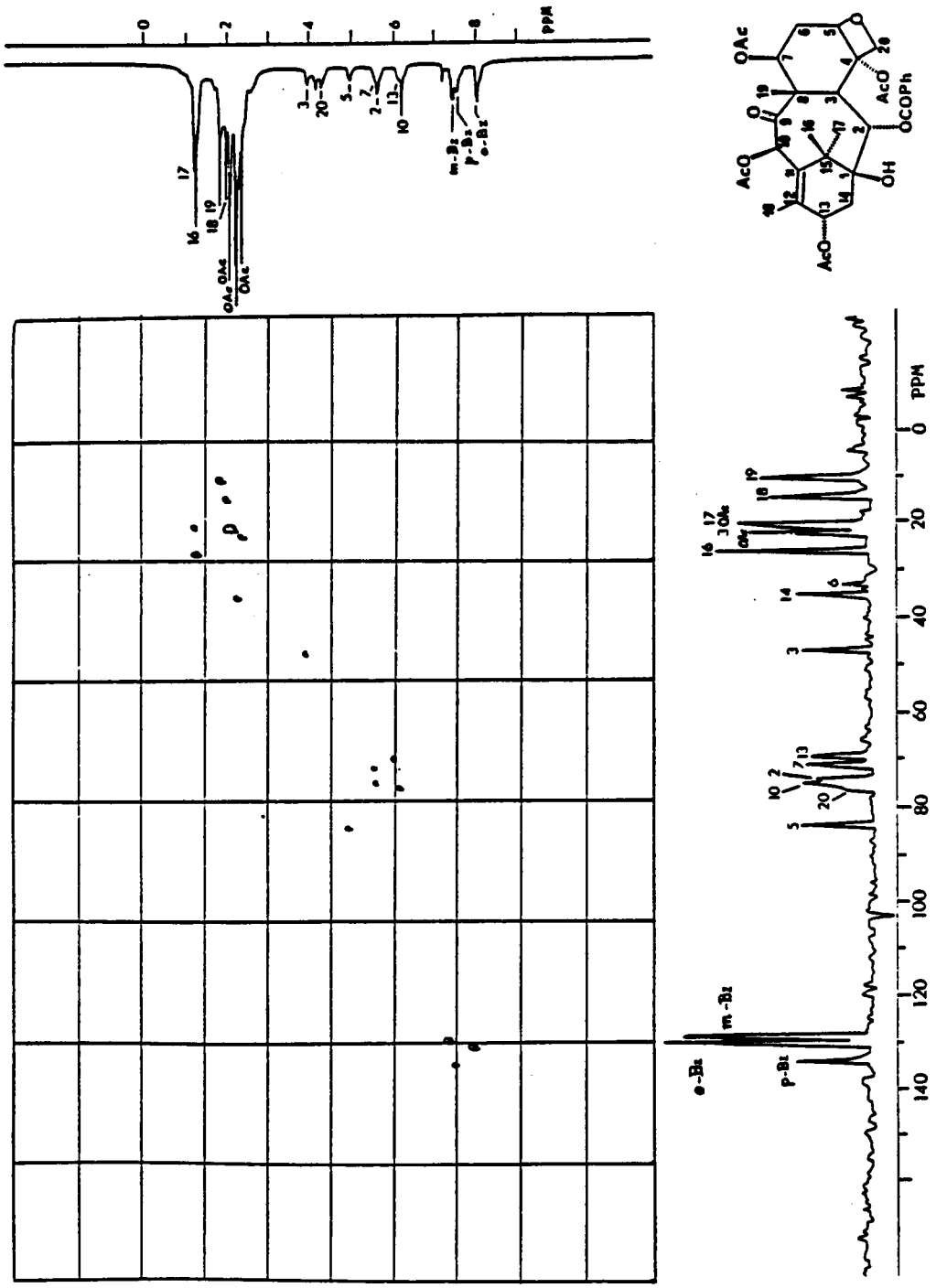


Figure 24. Heteronuclear COSY spectrum Of 7,13-Diacetyl-baccatin III (**53**)

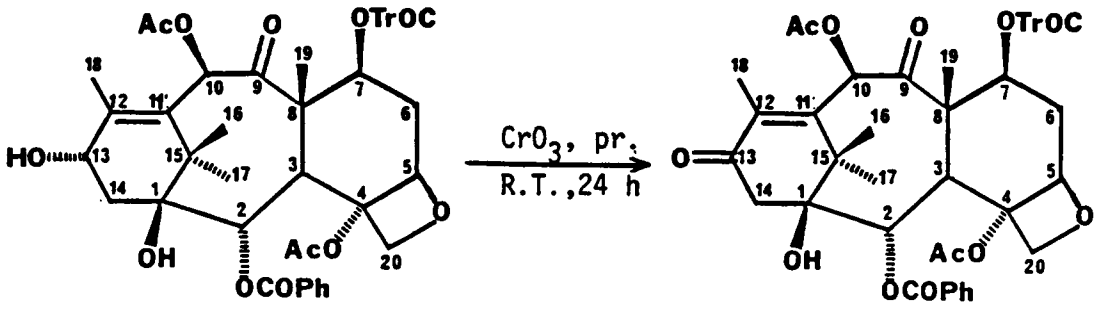
The ^{1}H NMR spectrum (Figure 21) shows the C-7 proton chemical shift at 5.60 ppm as a doublet of doublets and the C-13 proton at 6.18 ppm as a broad triplet, both shifted downfield from similar protons in baccatin III. All other signals were similar to that of 7-acetylbaccatin III. The homonuclear COSY spectrum is shown in Figure 22; it shows all expected couplings of 7,13-diacetylbaccatin III **(53)**.

The decoupled ^{13}C NMR spectrum and the heteronuclear COSY spectrum were recorded and are shown in Figures 23 and 24 respectively. The C-13 signal was found at 69.5 ppm, 1.5 ppm downfield from that of the C-13 of baccatin III. The C-7 carbon was assigned to a peak at 71.4 ppm, similar to C-7 of 7-acetylbaccatin III **(52)**, although this assignment does not take into account the expected deshielding effect by the ester group. The explanation for both cases is not obvious but might result from the steric compression of the taxane nucleus which is always sensitive to any transformation of the atoms or groups on the taxane skeleton.

The mass spectrum obtained by the FAB technique showed peaks at m/z 693 (MNa^{+}) , and 671 (MH^{+}) indicating a molecular weight of 670. This result together with the previously discussed results confirmed the structure of 7,13-diacetylbaccatin III **(53)**.

3.2.1.6 13-Oxo-7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (54).

13-Oxo-7-(2,2,2-trichloroethyloxycarbonyl) baccatin III **(54)** was obtained by oxidation of 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III **(49)** with chromic oxide in pyridine. The crude product was purified by preparative TLC to a pure compound in 62% yield.



(49) (54)

Scheme 22

Preparation of 13-Oxo-7-(2,2,2-Trichloroethyloxy-carbonyl) baccatin III (54)

The ^1H NMR and homonuclear COSY spectra of the compound 54 are shown in Figures 25 and 26. The ^1H NMR spectrum was similar to that of 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (49) except for the absence of signals for the C-13 proton. As expected, the spectrum did not show the typical broad triplet signal of the C-13 proton, and the C-14 protons were seen as two doublets at 2.66 and 2.94 ppm (J = 20 Hz). The C-10 proton was still a singlet but shifted about 0.2 ppm downfield due to the formation of the extended unsaturated carbonyl system which further increased the deshielding effect. The signal for the C-18 methyl protons also shifted downfield about 0.2 ppm by the same effect.

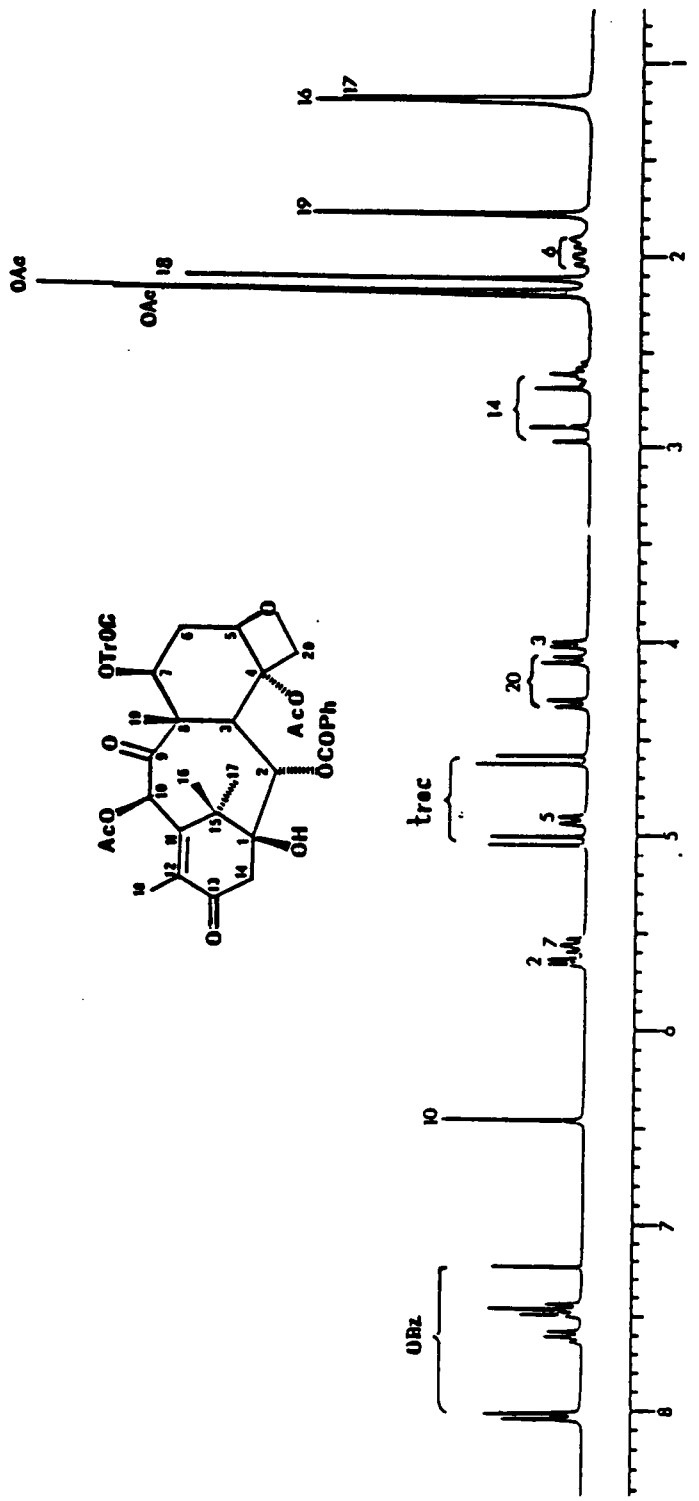


Figure 25. ^{1}H NMR spectrum of 13-Oxo-7-(2,2,2-trichloroethyloxycarbonyl)-baccatin III (54)

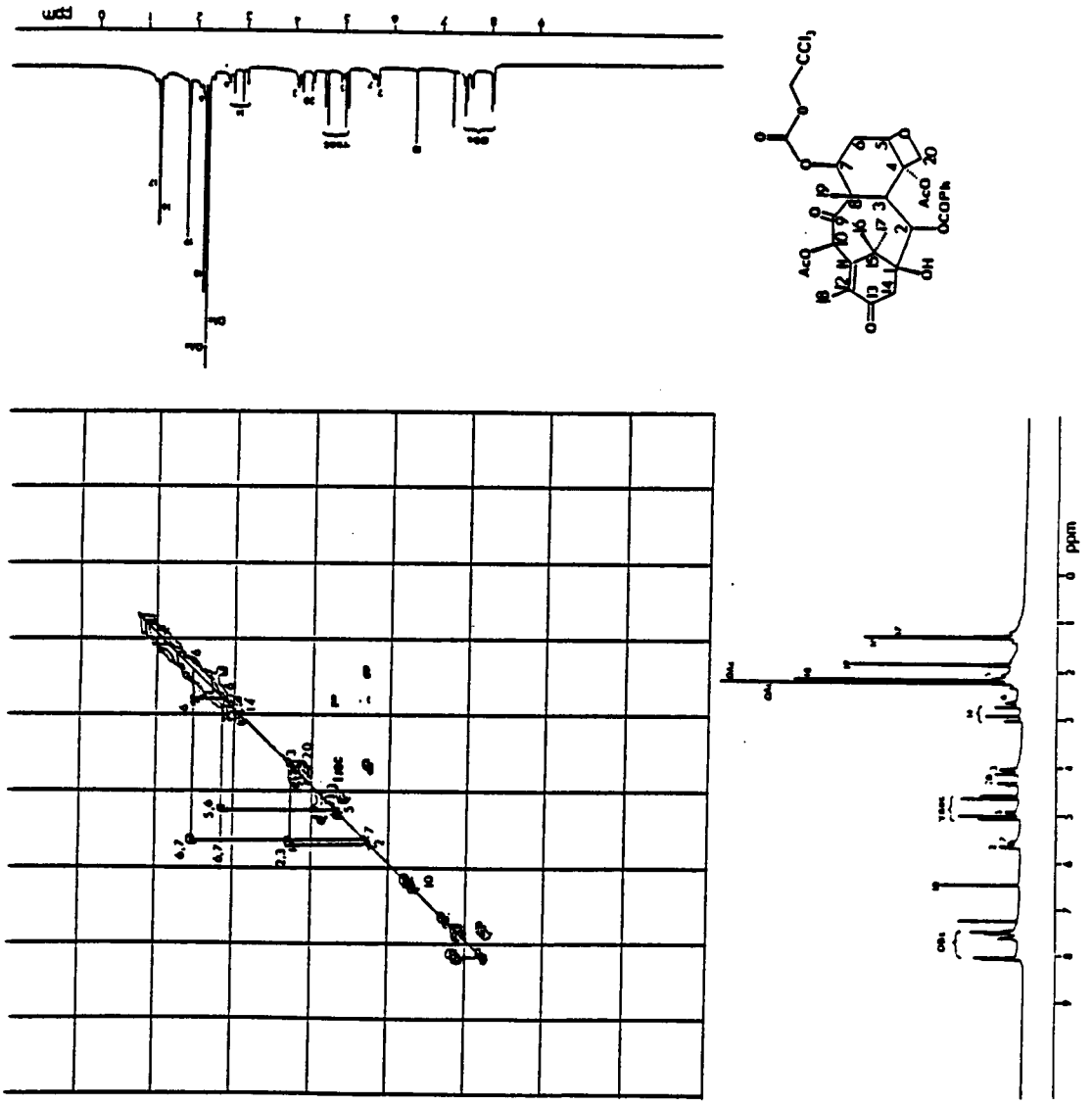


Figure 26. Homonuclear COSY spectrum of 13-Oxo-7-(tri-chloroethyloxycarbonyl)baccatin III (S4)

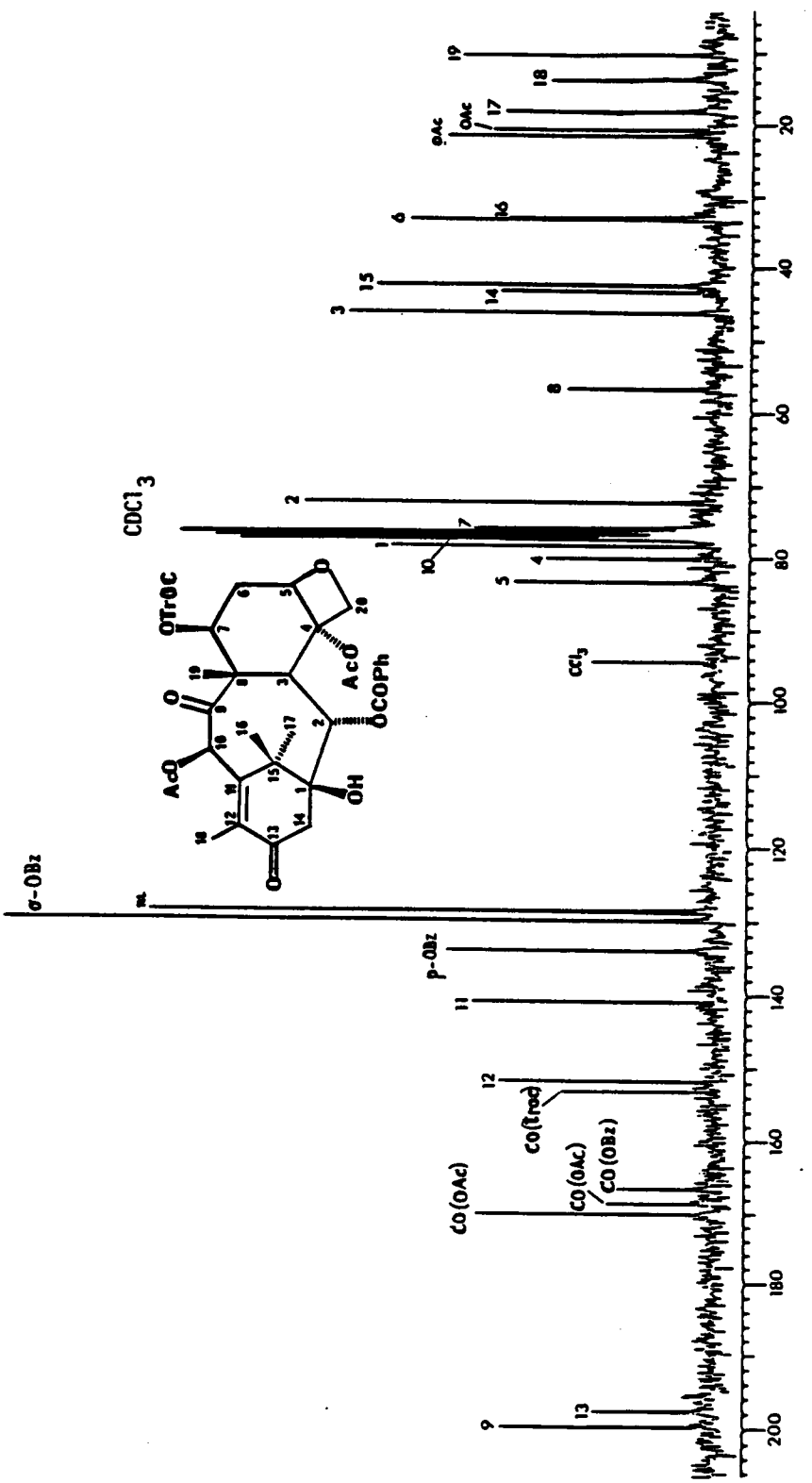


Figure 27. Proton-decoupled ^{13}C NMR spectrum of 13-Oxo-7-(trichloroethyloxycarbonyl)-baccatin III (54)

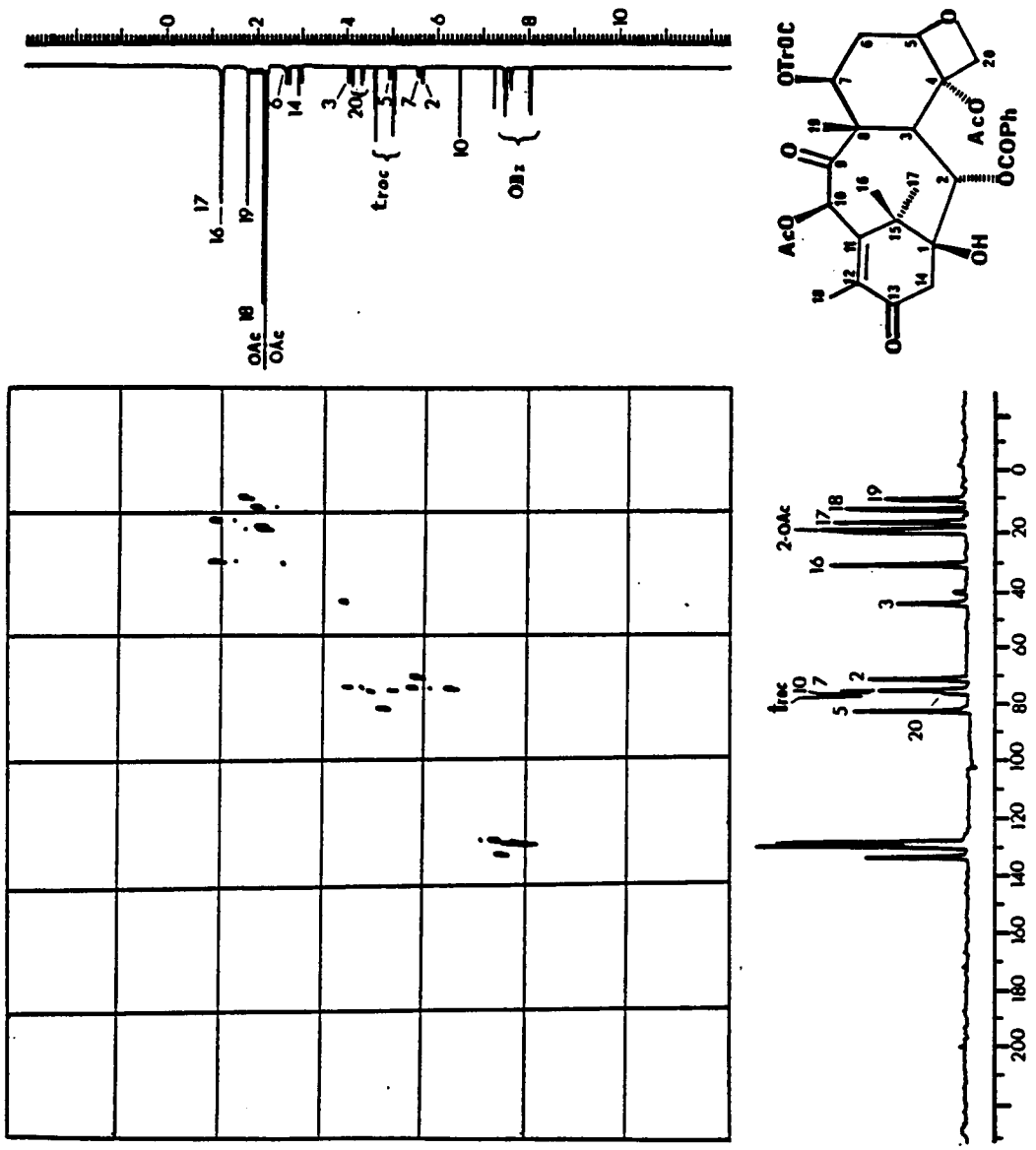


Figure 28. Heteronuclear COSY spectrum of 13-Oxo-7-(2,2,2-trichloroethyloxycarbonyl)baccatin III (**54**)

Small downfield shifts were also observed for the signals of the C-2 and C-3 protons.

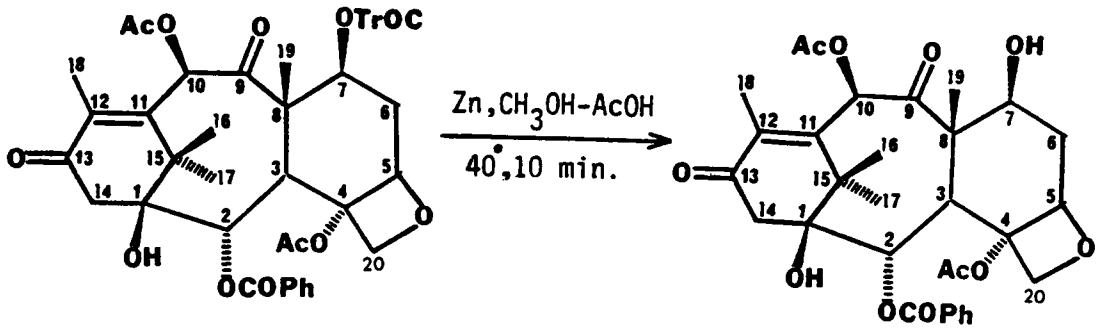
The homonuclear COSY spectrum (Figure 26) showed all the expected couplings. The off diagonal signals displayed the proton couplings between protons of C-2 and C-3, C-5 and C-6, C-6 and C-7, and also the geminal coupling between the two protons of C-6. There was no off-diagonal signal for the protons of C-14 except for a possible small signal assignable to geminal coupling, confirming the absence of the C-13 proton.

The ^{13}C NMR spectrum is shown in Figure 27 and Table 2. Peak assignments were aided by its heteronuclear COSY spectrum (Figure 28) and also by analogy with the previously assigned ^{13}C NMR spectra of baccatin III derivaties.[87] The obvious absence of the C-13 signal at about 67-69 ppm was observed, and a new carbonyl carbon signal at 197.8 ppm was assigned to C-13. The C-14 carbon appears at 43.4 ppm, about 8 ppm downfield from the original chemical shift found in baccatin III due to the deshielding effect of the C-13 carbonyl group. The C-11 and C-12 carbons were also shifted about 10 ppm downfield due to the same deshielding effect. The conversion of a hydroxyl group to a carbonyl at C-13 also effected significant changes of chemical shifts at C-16, C-17, and C-18. The C-16 signal shifted downfield by about 6 ppm whereas the C-17 and C-18 both shifted upfield. All other signals were seen at similar positions as in the other baccatin III derivatives.

3.2.1.7 13 -Oxobaccatin III (55)

13-Oxobaccatin III **(55)** was obtained by treating 13-oxo-7-(2,2,2-trichloroethyoxycarbonyl) baccatin III **(54)** with zinc dust in acetic

acid-methanol at 40° for 10 min. After removal of the solid by filtration and concentration of the solution in vacuo, pure compound (55) was obtained without further purification.



(54) (55)

Scheme 23

The ^{1}H NMR of 55 is shown in Figure 29. As expected, signals of the methylene protons of the 2,2,2-trichloroethyloxycarbonyl group were not observed and the signal of the C-7 proton was seen shifted upfield by 1.21 ppm. This result confirmed the deprotection of the 7-hydroxyl group. All other signals were similar to that of 13-oxo-7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (54).

The ^{13}C NMR spectrum of 13-oxobaccatin III is shown in Figure 30. Peak assignments were achieved by comparison with its 7-(2,2,2-trichloroethyloxycarbonyl) analogs. The chemical shifts of C-7 and C-13 were assigned to peaks at 7.31 and 197.9 ppm respectively and they agreed with the fact that the C-7 signal became shielded when the protected group was removed from C-7. Other signals also were consistent with previous assignments.

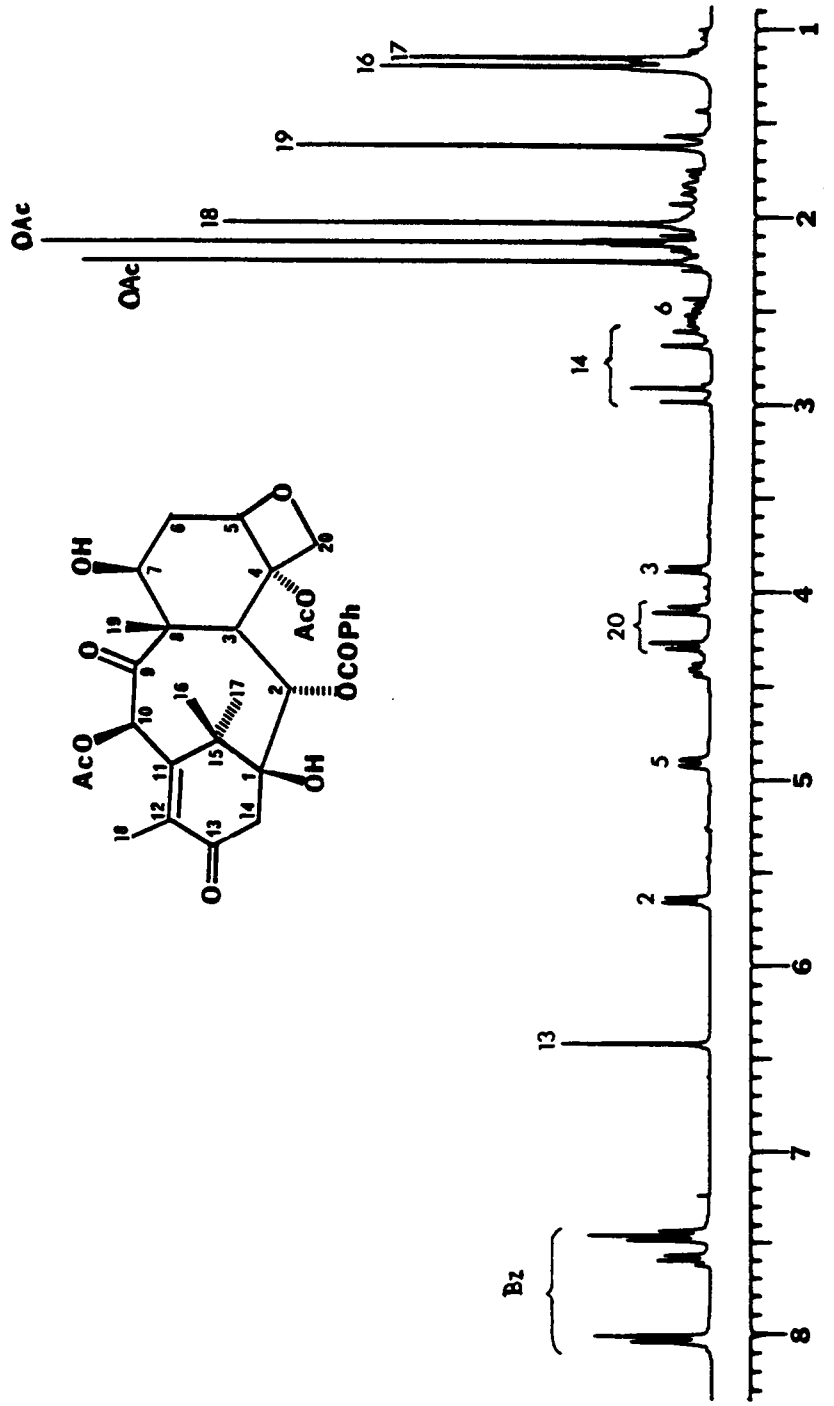


Figure 29. ^{1}H NMR spectrum of 13-Oxobaccatin III (54)

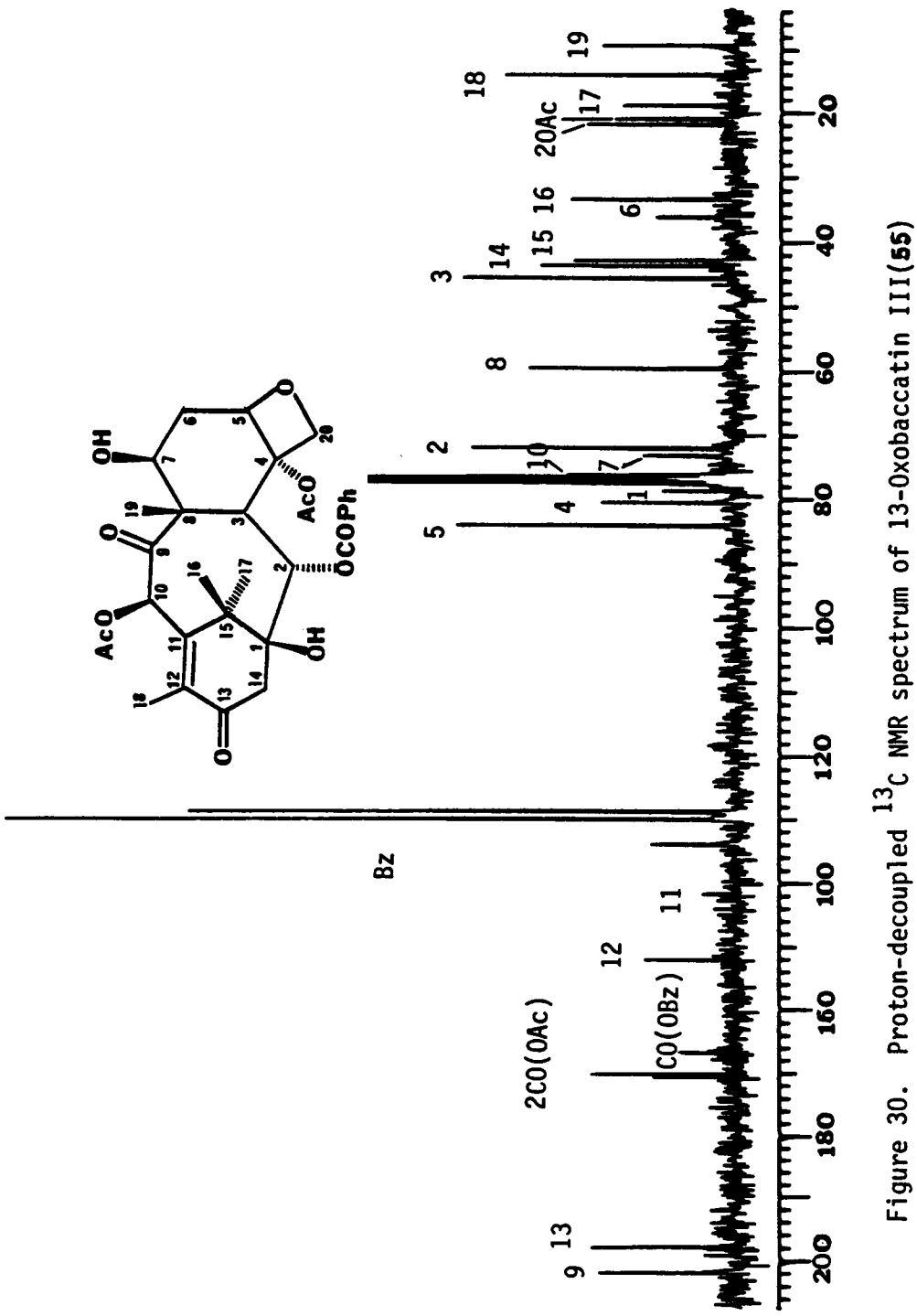


Figure 30. Proton-decoupled ^{13}C NMR spectrum of 13-Oxobaccatin III(55)

3.2.2 Acylation of 7-(2,2,2,-Trichloroethyloxycarbonyl) baccatin III at C-13 With Various Acids

3.2.2.1 Introduction

In the first part of this chapter, the acetylation of 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (**49**) was discussed. Selective acetylation could be achieved by using different reaction conditions. However, further investigation is still needed on the coupling of side chains with different size and functionalities in order to establish the feasibility of the coupling process. Since the C-13 hydroxyl group is more hindered than the C-7 hydroxyl group, 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (**49**) was used and the protecting group was removed later.

3-Phenylpropanoyl chloride (**56**) was used in the first coupling reaction. It has the same chain length as the phenylisoserine side chain but lacks both the amino and hydroxyl groups. 3-Phenyllactyl chloride (57) was the next side chain to be used as a more functionalized model for the coupling reaction.

Recently the partial synthesis of taxol-related compounds involving the coupling of side chains to the taxane nucleus has been reported.[44,86]

Various acylation methods for 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III have been used. Simple acylation using acyl chloride with pyridine or triethylamine in the presence of 4-dimethylaminopyridine (DMAP), and in some cases also with dicyclohexylcarbodiimide (DCC) was extensively investigated. In addition, various methods to increase the nucleophilicity of the C-13 hydroxyl group by converting it to the alkoxide ion under mild, non-basic, conditions were investigated. These methods include treatment of a 2,2,2-trichloroethyloxycarbonyl derivative with metallic zinc or a Zn(Cu) alloy, treatment of a 7-protected baccatin III with sodium hydride, and attempted formation of a 13-(trialkylsilyl) baccatin III for desilylations by fluoride ion. Acylation by the mixed anhydride method[90] was also investigated.

3.2.2.2 Reaction of 3-Phenylpropanoyl Chloride with 7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (49) in the Presence of 4-Dimethylaminopyridine and Triethylamine.

The esterification of 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (49) with 3-phenylpropanoyl chloride was first carried out in the presence of triethylamine and 4-dimethylaminopyridine (DMAP) in dichloromethane. The reaction mixture was refluxed for 14 h and yielded a crude product which showed a rather complex TLC pattern. It was then purified by preparative TLC to give two major fractions. The less polar fraction was showed by analytical HPLC and ^{1}H NMR spectrum to be composed of two compounds in a 1 to 5 ratio.Two triplets at 5.70 and 5.88 ppm clearly represented the protons of C-13 of the two coupling products. If the reaction was kept at room temperature for 36 h this ratio would become 2 to 3 approximately.

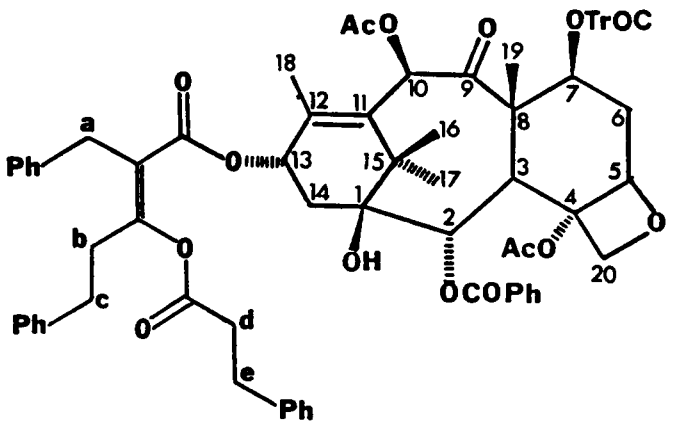
This mixture could not be separated on preparative HPLC, therefore it was purified by analytical HPLC to yield two compounds **58** and **59**.

The more polar fraction from preparative TLC was pure and was identified by ^{1}H NMR, and FABMS as 13-(3-phenylpropanoyl)-7- (2,2,2-trichloroethyloxycarbonyl) baccatin III **(60)**. The characterization of this compound will be discussed in the next section.

3.2.2.2(a) Compound **58**

The ^{1}H NMR of **58** was shown in Figure 31 and Table 2. The broad triplet at 5.70 ppm indicated that a C-13 ester was formed, although the signal appeared at about 0.5 ppm upfield from those usually observed in taxol or baccatin III derivatives. The integration of the aromatic and the methylene protons of the side chains (between 2.5 and 4.0 ppm) indicated that more than one unit of 3-phenylpropanoyl group was attached to the baccatin III nucleus. Twenty protons were calculated from the aromatic region, thus there were three phenylpropanoyl groups added in the esterification process. Ten additional protons were observed in the methylene region which also supported the presence of three phenylpropanoyl groups. Eight protons were seen in two multiplets at 2.6 to 3.0 ppm, but the remaining two protons were seen as two well-separated doublets at 3.75 and 4.02 ppm. All other protons could be found at positions similar to those of baccatin III derivatives.

Structure **58**, proposed for this product, explains the ^{1}H NMR data for this product. Protons at **a** should appear at a lower field than protons on **b**, **c**, **d**, and **e** because protons on **a** are both allylic and benzylic whereas the others either allylic or benzylic. Therefore,



(58)

the methylene protons on a were assigned to signals at 3.74 and 4.02 ppm. The other protons appeared as multiplets at 2.6 to 3.0 ppm.

The FABMS of **58** showed intense peaks at 1159 and 1157 (MH^+); the M+2 peak (1159) was due to the ^{37}Cl isotope of the three chlorine atoms. The fragmentation pattern due to these three chlorine atoms were observed. Therefore, the mass spectrum supported the structure of **58**.

3.2.2.2(b) Compound 59

Compound **59** had a very similar ^1H NMR spectrum to **58**, and this is shown in Figure 32 and Table 2. The C-13 proton was seen as a triplet at 5.88 ppm indicating the formation of an ester at this hydroxyl group. The methylene protons of the phenylpropanoyl side chains were seen as multiplets at 2.6 to 3.0 ppm and two well-separated doublets at 3.52 and 3.68 ppm. The integration indicated ten protons for these two groups of signals and four phenyl groups in the molecule.

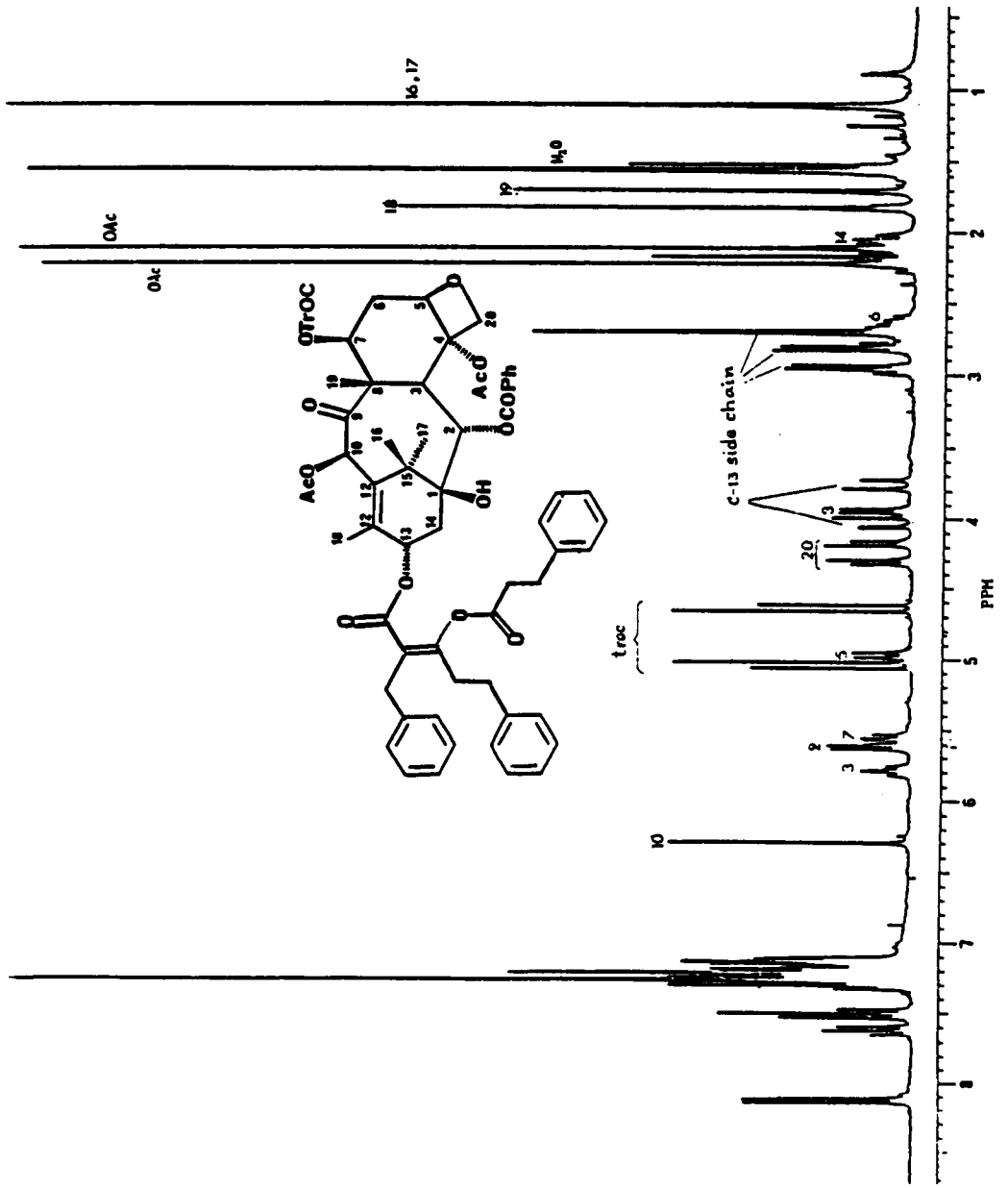


Figure 31. ^{1}H NMR spectrum of (58)

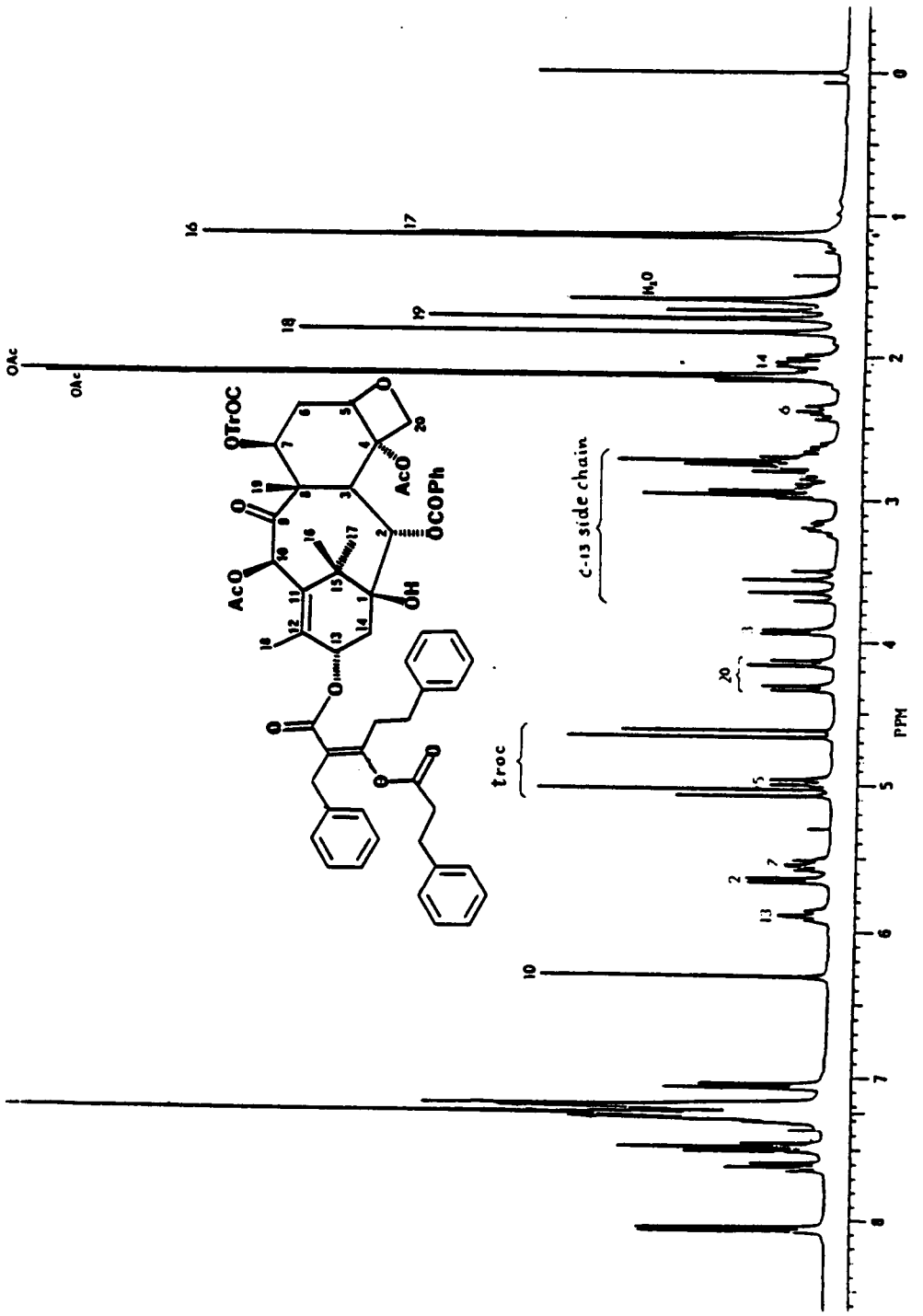


Figure 32. ^1H NMR spectrum of(59)

There were no significant changes for other chemical shifts of protons on the taxane skeleton, indicating that there was no conformational change in its skeleton.

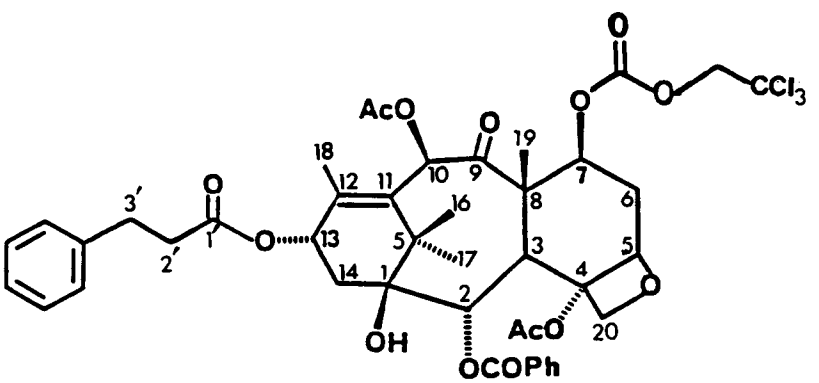
By a selective decoupling experiment, the protons of C-6 were assigned to signals at 2.00 and 2.65 ppm whereas protons of C-14 were at 2.00 and 2.35 ppm respectively.

The FABMS spectrum showed intense peaks at 1159 m/z (MH^++2) and 1157 (MH^+) indicated the molecular weight of 1156 which agreed with the presence of three phenylpropanoyl groups on the C-13 side chain. The typical fragmentation pattern of three chlorine atom was also seen.

The structure of this compound was proposed as the geometric isomer of compound 58.

3.2.2.2(c) 13-(3-Phenylpropanoyl)-7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (60).

13-(3-Phenylpropanoyl)-7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (60) was obtained as the major product in this reaction by preparative TLC purification.



(60)

The ^{1}H NMR spectrum of **60** was shown in Figure 33 and Table 3. The C-13 proton was seen as a broad triplet at 6.21 ppm, typical for taxol-like compounds. The aromatic region was composed of ten protons indicating that one phenylpropanoyl group was incorporated into the baccatin III nucleus. The four methylene protons of the side chain were seen as multiplets at 2.75 and 3.05 ppm and confirmed the structure of **60**. All other protons showed similar chemical shifts to those of taxol and baccatin III derivatives.

The homonuclear COSY spectrum of **60** is shown in Figure 34. The proton couplings between C-2 and C-3, C-5 and C-6, C-6 and C-7, and C-13 to C-14 were seen.

The FABMS spectrum showed peaks at m/z 896 and 894. The peak at 894 probably represented MH^{+}_{2} whereas the peak at m/z 896 was the $MH_{2}^{+}+2$ resulting from the presence of three chlorine atoms in the molecule. The mass spectrum thus confirmed the molecule of **60**.

The ^{13}C NMR spectrum of 13-(3-phenylpropanoyl)-7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (**60**) was shown in Figure 35 and Table 4. Peak assignments were again aided by the assistance of a heteronuclear COSY spectrum (Figure 36) and also by comparison with those of baccatin III derivatives (Table 2). Most peaks matched very well with the previous assignments of baccatin III derivatives.

The C-7 signal appeared at rather lower field than other baccatin III derivatives previously assigned. The C-6 and C-8 chemical shifts were consistent with the shielding effect by the C-7 ester. The chemical shift of C-13 was 69.5 ppm which also agreed with other C-13 esters in baccatin III derivatives. The shielding effect on the C-12

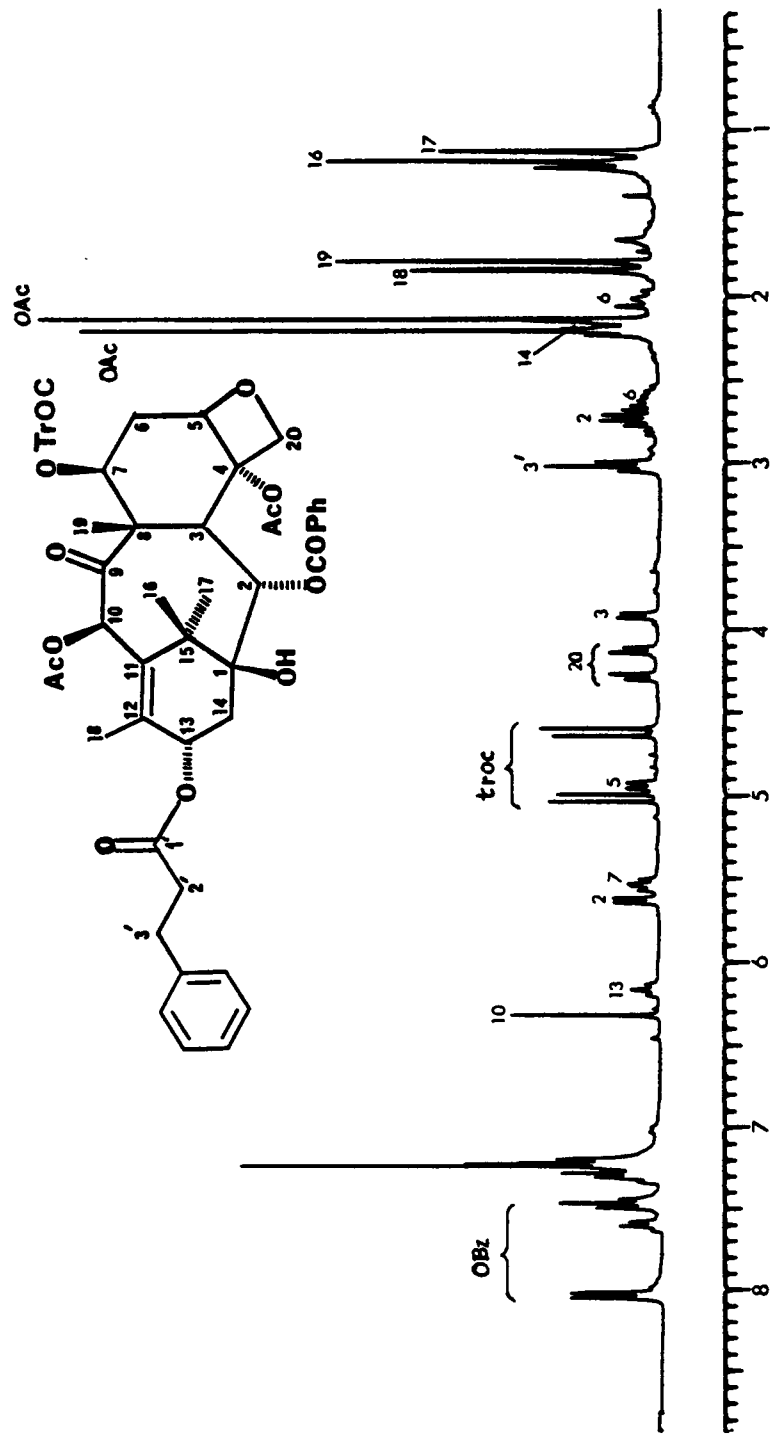


Figure 33. ^{1}H NMR spectrum of 13-(3-phenylpropanoyl)-7-(2,2,2-trichloroethyloxycarbonyl)baccatin III (**60**)

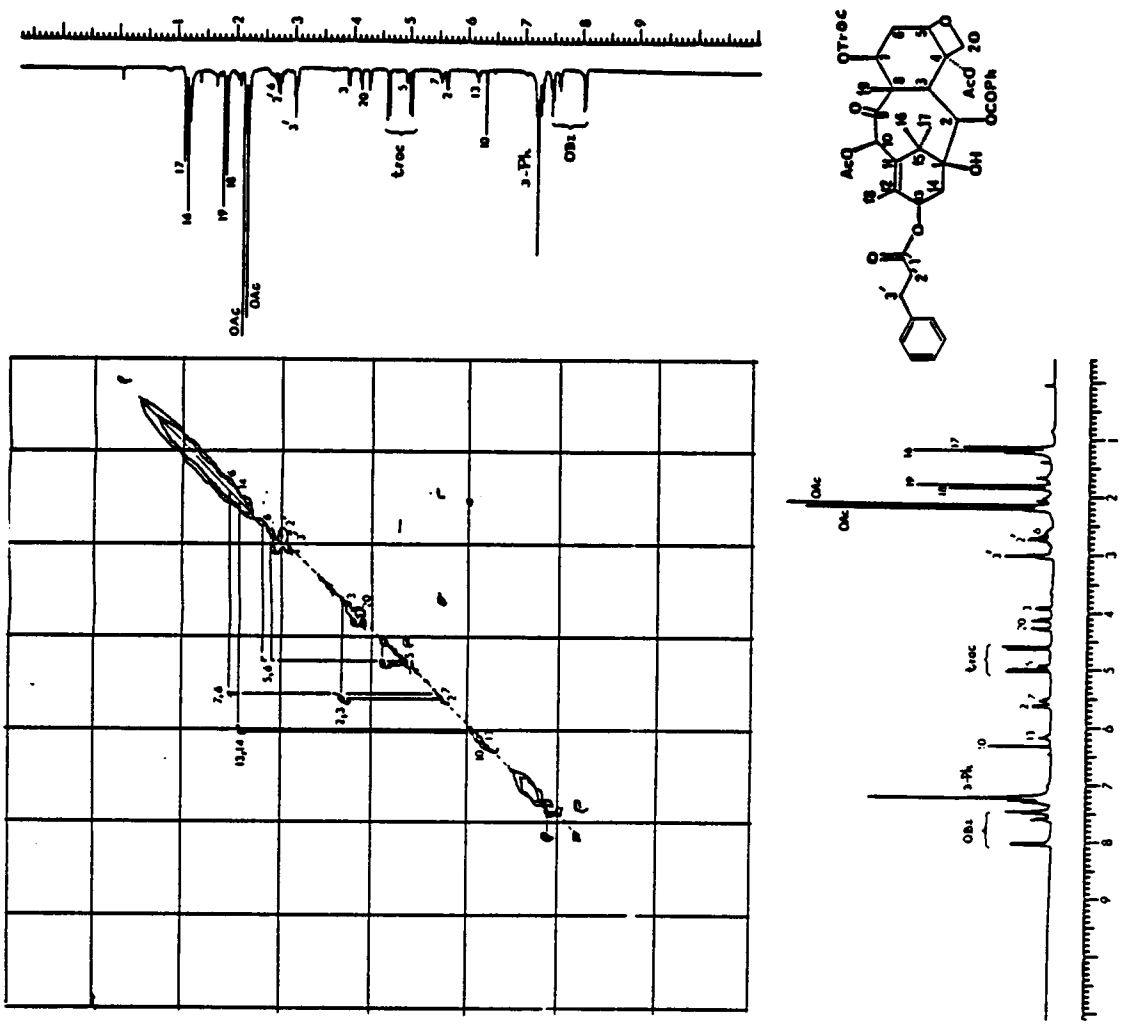


Figure 34. Homonuclear COSY spectrum of 13-(3-phenylpropanoyl)-7-(2,2,2-trichloroethyloxycarnyl)baccatin III (**60**)

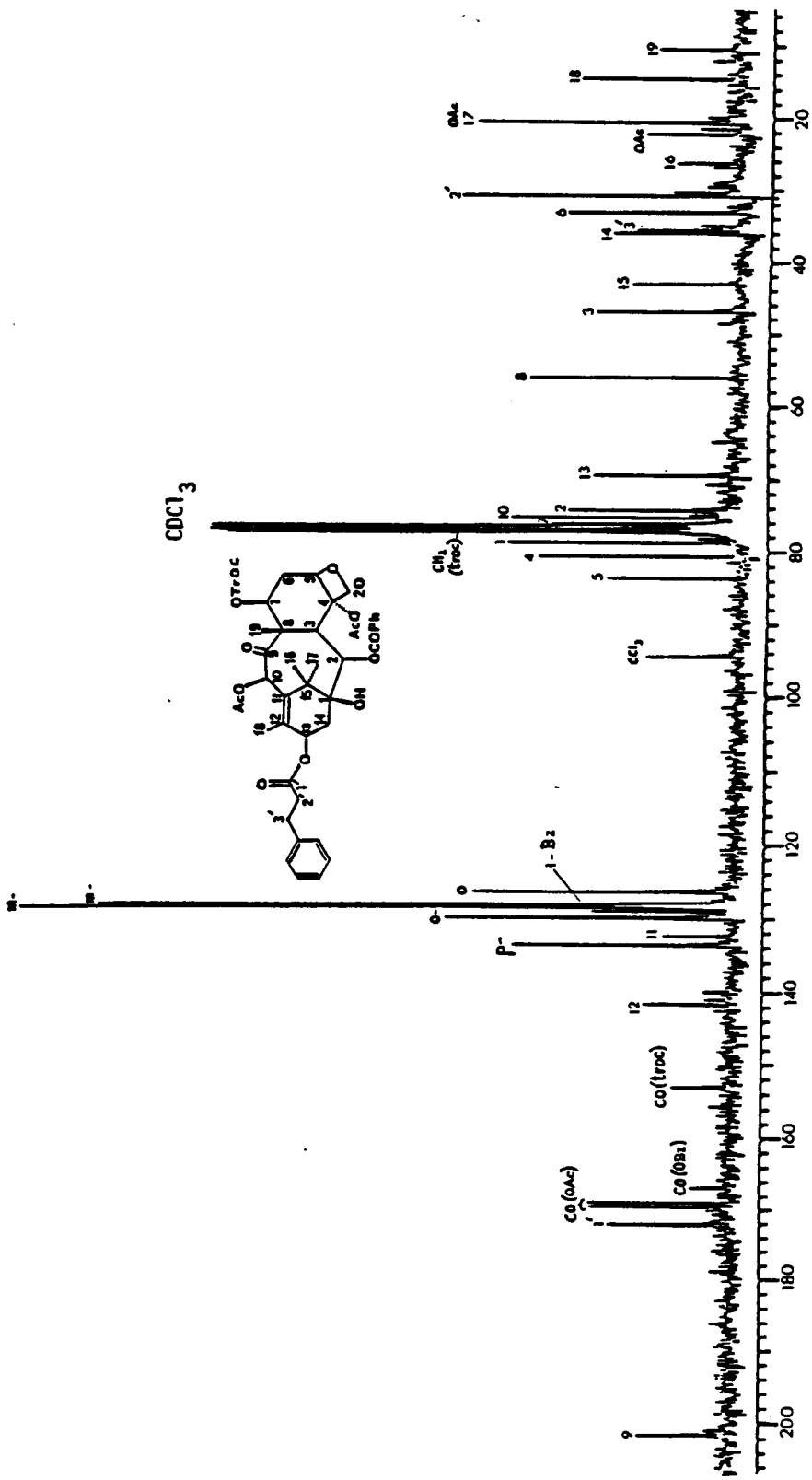


Figure 35. Proton-decoupled ^{13}C NMR spectrum of 13-(3-phenylpropanoyl)-7-(2,2,2-trichloroethyloxycarbonyl)baccatin III (**60**)

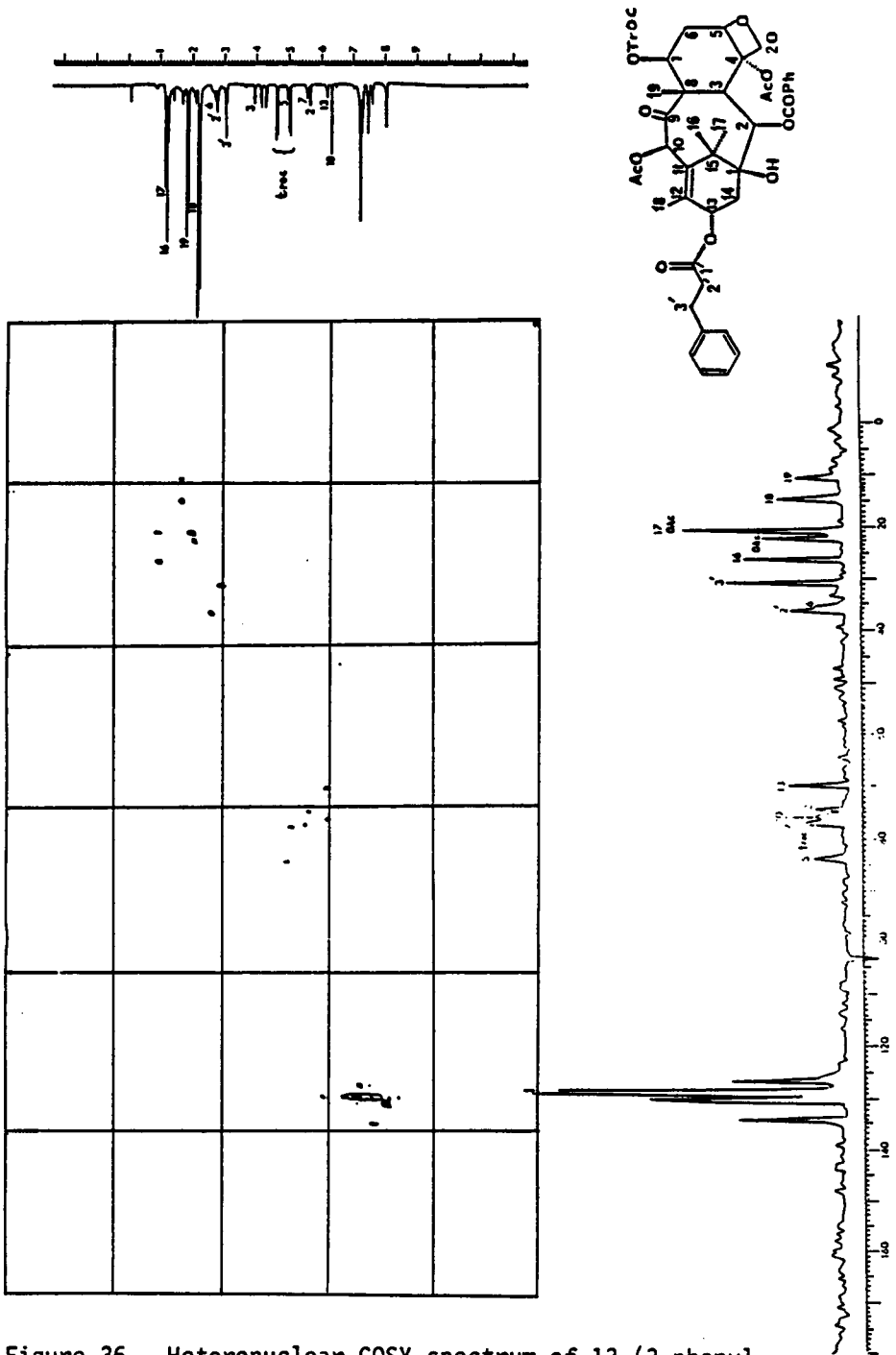
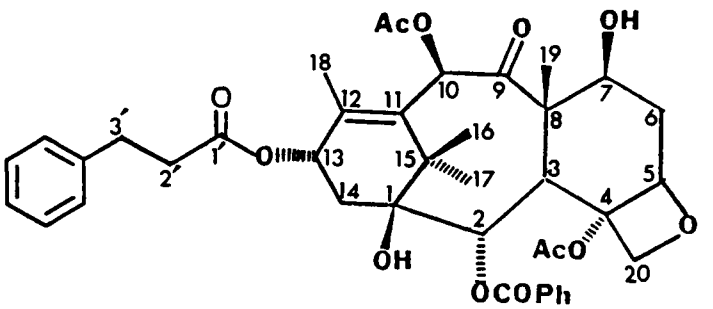


Figure 36. Heteronuclear COSY spectrum of 13-(3-phenyl-propanoyl)-7-(2,2,2-trichloroethyloxycarbonyl)-baccatin III (**60**)

and C-14 was also observed. Four carbonyl carbon signals were seen in the proton-decoupled ^{13}C NMR spectrum. The C-9 was seen at the lowest field at 201.5 ppm whereas the C-1′ carbonyl carbon was assigned to a peak at 172.2 ppm. The two carbonyl carbons of the acetate groups were seen at 169.0 and 169.6 ppm. All other signals were consistent with the data of baccatin III derivatives although the aromatic carbons could not be clearly assigned.

3.2.2.2(d) 13-(3-Phenylpropanoyl) baccatin III (61)

13-(3-Phenylpropanoyl) baccatin III **(61)** was obtained in 47% yield upon removal of the 2,2,2-trichloroethyloxycarbonyl group from compound **60** by treatment with zinc in acetic acid-methanol. In addition, a minor product **(62)** was also obtained in 12% yield.



(61)

The ^{1}H NMR of **61** was shown in Figure 34 and Table 1. The disappearance of signals of the methylene protons of the 2,2,2-trichloroethyloxycarbonyl group and the upfield shift of the C-7 proton signal from 5.57 ppm in **60** to 4.40 ppm in **61** clearly indicated the

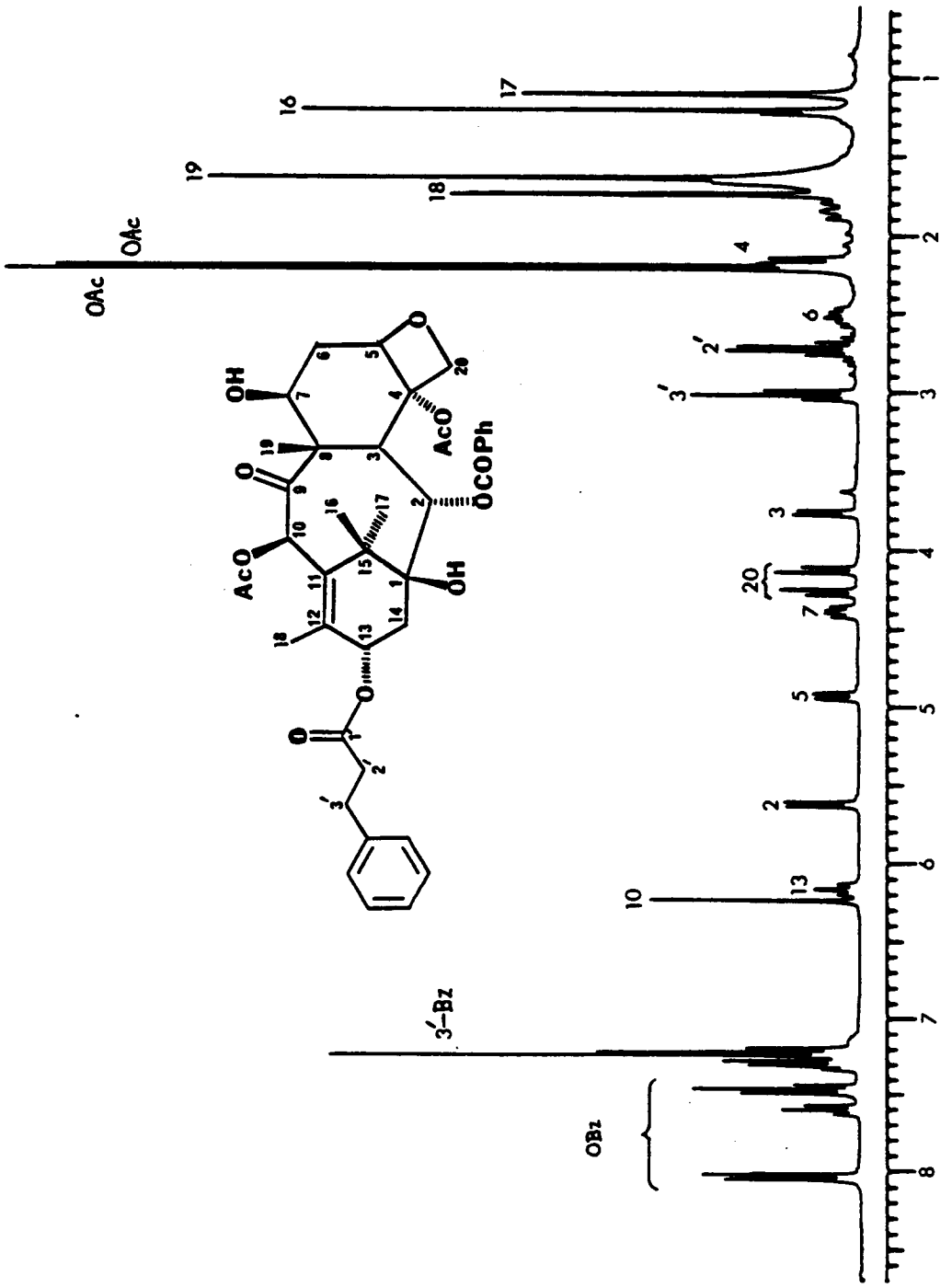


Figure 37. ^{1}H NMR Spectrum of 13-(3-Phenylpropanoyl)baccatin III (61)

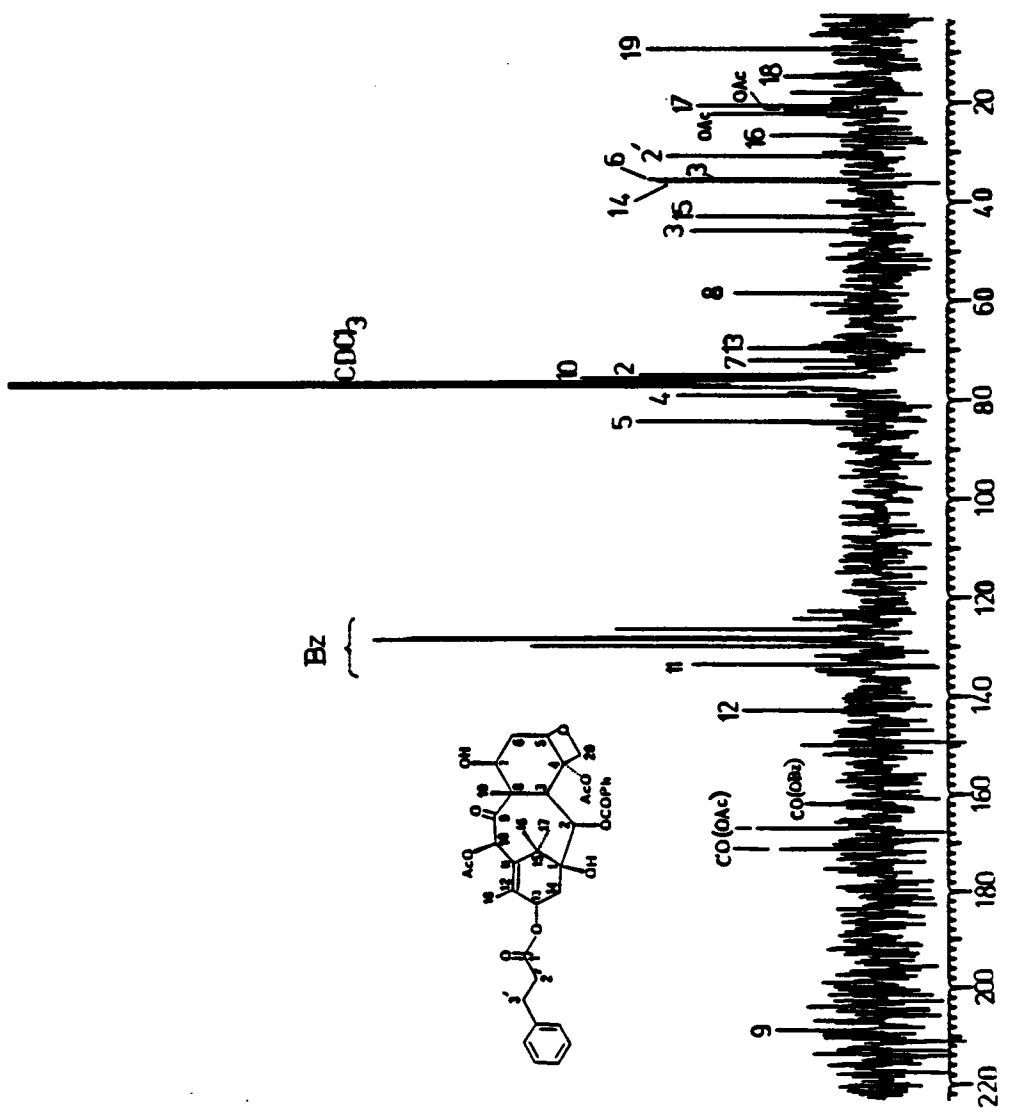


Figure 38. Proton-decoupled ^{13}C NMR Spectrum of 13-(3-phenylpropanoyl)-baccatin III (61)

structure of **61**. Signals of protons of C-2′ and C-3′ of both **60** and **61** were seen as two multiplets at 2.73 and 3.05 ppm. All other signals were very similar to compound **60**.

Peak assignments of the ^{13}C NMR spectrum of **61** (Figure 39) were made by comparison with those of **60**. The C-7 signal was assigned to a peak at 72.2 ppm, about 4.0 ppm upfield from the C-7 signal of the corresponding 7-(2,2,2-trichloroethyloxycarbonyl) compound (**60**). The C-6 and C-8 signals were also influenced by shielding effect, each peak shifted upfield by about 2.4 ppm. Other signals were consistent with those of compound **60**.

3.2.2.2(e) Reaction of Crude-3-Phenylpropanoyl Chloride with 7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (**49**) in the Presence of Pyridine and 4-Dimethylaminopyridine.

In one instance, crude 3-phenylpropanoyl chloride was used in the coupling reaction in the presence of pyridine and 4-dimethylamino-pyridine. After stirring for 18 h at room temparature the crude product showed one major component on the TLC. It was purified by preparative TLC to a pure compound (**62**).

The ^{1}H NMR spectrum of **62** was shown in Figure 39. The broad triplet at 4.70 ppm indicated that no ester was formed at C-13. Only one methyl signal of the acetate group was seen at 2.15 ppm. The signals of the C-16 and C-17 protons moved nearly 1 ppm downfield compared with those of taxol and baccatin III derivatives. Two singlets

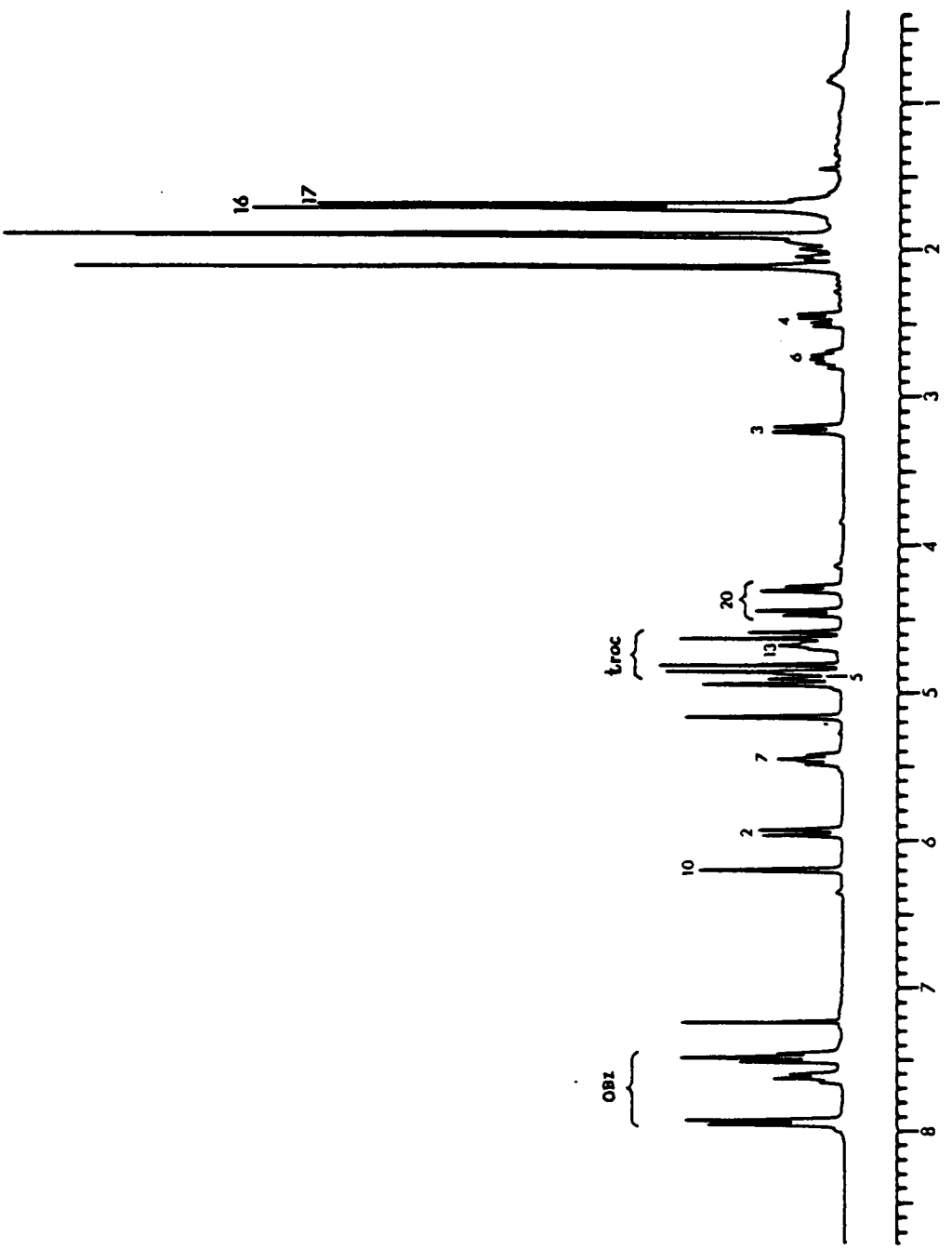


Figure 39. ^{1}H NMR spectrum of (62)

of one proton each were seen at 5.20 and 4.95 ppm respectively, probably vinylic-type protons. The signals of C-18 and C-19 were also seen shifted downfield by approximately 0.1 to 0.2 ppm indicating a possible change in the conformation of the taxane ring.

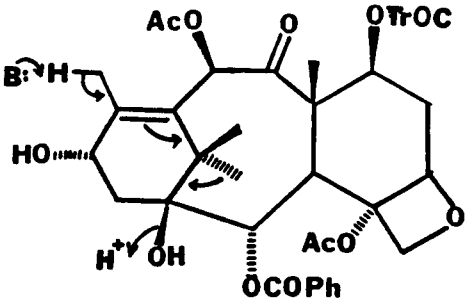
Significant changes were also observed for the chemical shifts of protons on C-2 and C-3. The proton of C-2 shifted about 0.3 to 0.4 ppm downfield whereas the C-3 proton shifted upfield nearly 0.7 ppm. This was also another evidence for the conformational change of the taxane skeleton.

Selective decoupling experiments showed that the protons of C-6 and C-4 were at 2.78 and 2.50 ppm respectively. The protons of C-5 and C-7 resonated at 4.94 and 5.50 ppm and signal at 4.95 was coupled to that of 5.20 ppm.

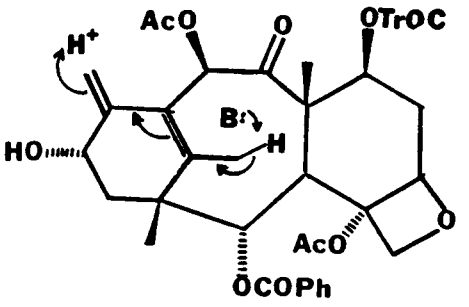
The FABMS showed intense peaks at 765, 763, 761 and 703. The peak at 765 is assumed to correspond to the MNa^+ species, and hence the molecular weight of the compound **62** is 742. Scheme 23 shows the proposed mechanism for the generation of **62**.

Structures **62** and **63** have the molecular weight of 742 which agrees with the mass spectral data. In terms of its ^1H NMR spectrum, structure **62** is somewhat different from 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (**49**) and would cause a great deal of conformational change whereas structure **63** still possesses mostly the same conformation as a whole except the double bond at C-15 and C-16.

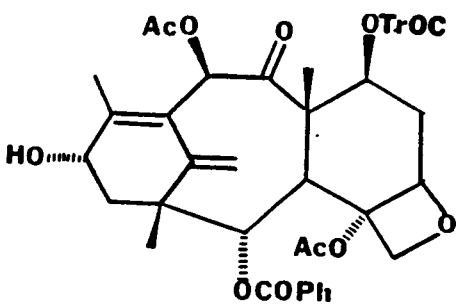
It would be possible to confirm the structure by conversion of the product to its 13-oxo derivatives and determine its ultraviolet spectrum; structure **63** would absorb at higher wavelength than structure



(49)



(62)



(63)

Scheme 24

62 because of the conjugated nature of the molecule. Unfortunately compound 62 (or 63) was not stable even at about 4°C and we have been unable to obtain more product when the reaction was repeated.

3.2.2.3 Reaction of 3-Phenylpropanoyl Chloride with 7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (49) in the Presence of Silver Cyanide.

Silver cyanide has been found to be a very effective reagent in the preparation of sterically hindered esters from the corresponding acid chloride and alcohol. It was reported to be superior to the conventional acid chloride-alcohol-pyridine method as regard to yield and the rapidity of reactions. The reaction is generally carried out at either room temperature or at 80°C in benzene, HMPA, or toluene. Another advantage of this method is the more convenient work-up procedure which involves only the filtration off the silver salt and subsequent purification of the product.

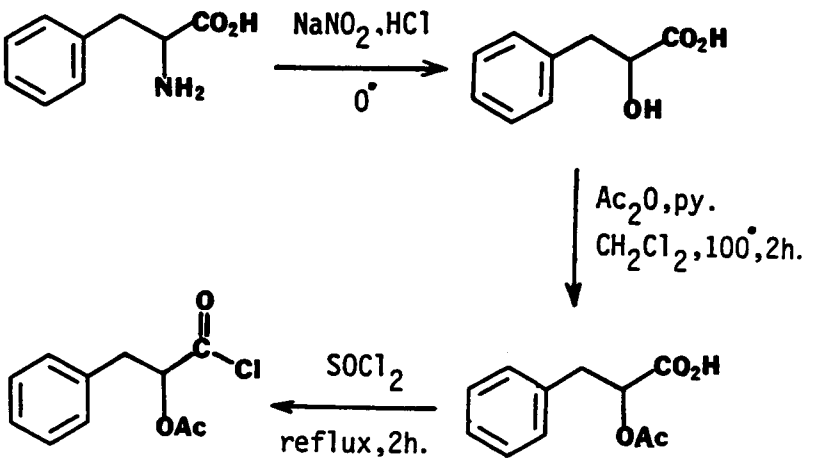
7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (49), 3-phenylpropanoyl chloride, and silver cyanide in tolune were heated at 80°C for 18 h. Analytical TLC showed mostly one less-polar spot which was purified by preparative TLC to a pure compound in 31% yield.

The ^{1}H NMR spectrum and the melting point of this product were agreed with those of 60.

3.2.2.4 Attempted Coupling Reaction of 3-Phenyllactyl Chloride with 7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (49)

In this section, attempted coupling reaction of 3-phenyllactyl chloride (57) with 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (49) will be discussed. 3-Phenyllactic acid was prepared from phenylalanine

in 35% yield by the method of Inouye et al.[91] The ^1H NMR spectrum and



(64)

Scheme 25

melting point are consistent with the literature data. The configuration of the asymmetric carbon atom was retained in this reaction.

2-tert-Butyl dimethylsilyl-3-phenyllactic acid was prepared by treating 3-phenyllactic acid with tert-butyl dimethylsilyl chloride and imidazole in dimethyl formamide. The corresponding acid chloride was then made by refluxing the protected acid with thionyl chloride. Attempts to couple this acid chloride with 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III **(49)** were made but no coupled product was detected by ^{1}H NMR technique.

2-Acetyl-3-phenyllactic acid **(64)** was prepared by heating the mixture of the acid with acetic anhydride and pyridine in dichloromethane at 100°C for 2 h. The crude product was purified by distillation under reduced pressure in a Kugelhrohr distillation apparatus. The acid chloride was then prepared by refluxing with thionyl chloride and then purified by distillation under reduced pressure. The formation of the acid chloride was indicated by the strong absorption band at 1820 cm^{-1} whereas the carbonyl absorption of the ester appeared at 1760 cm^{-1}.

The attempt to couple 2-acetyl-3-phenyllactyl chloride with 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III in the presence of either triethylamine (or pyridine) and 4-dimethylaminopyridine or silver cyanide did not give any coupling product. The failure may result from the steric nature of the C-13 hydroxyl group since smaller acid chloride molecules can be coupled to this hydroxyl group as discussed in the acetylation of baccatin III and also in the case of 3-phenylpropanoyl chloride.

3.3 Experimental

3.3.1 Baccatin III (15)

Baccatin III (15) was prepared from a crude mixture of taxol and cephalomannine by the method developed by Magri.[46] The crude product was purified by flash-column chromatography eluted with CH_2Cl_2-MeOH (94:6 v/v) and baccatin III was obtained in 40% yield.

3.2.2. 7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (49).

Baccatin III (15, 77 mg, 1.31 mmol) in dry dichloromethane (5.0 mL) was cooled in an acetone-dry ice bath at -20°C. A cold solution of 2,2,2-trichloroethyloxycarbonyl chloride (0.98 mL, 5.6 mmol) was added dropwise into the previous solution. The resulting solution was then treated with pyridine (0.98 mL, 3.5 mmol) slowly at the same temperature. The reaction was monitored by TLC and was complete in 30 min. Water (5 mL) was added and the solution acidified with 1 N. HCl, washed with 5% $NaHCO_3$ and then water until the washing was neutral to litmus. The organic layer was dried over anhydrous $MgSO_4$ and then concentrated to a pale yellow liquid which was purified by flash chromatography, eluted with 70% ethyl acetate in hexane to yield a white solid, mp 211-213°C. FABMS m/z 761 (MH^+, 66), 701 (MH^+-ACOH, 58), 683 (20), 625 (7), 105 (100); IR (KBr) 1770, 1730, 1660, 1550, 1385, 1290, 1260, 1115, 1160, 980, 720 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR see Table 2.

3.3.3 13-Acetyl-7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (50).

7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (49) (200 mg, 0.26 mmol), 4-dimethylaminopyridine (15 mg), and acetic anhydride (0.4 mL) in dry acetonitrile (4.0 mL) was heated to 75°C and kept at this temperature for 6 h. Usual work-up and purification by preparative

TLC (hexane: ethyl acetate, 4:1) yielded **50** as a white solid, 0.15 g (71%); mp 239.5-241°C (dec); FABMS, m/z 803/805 (MH^+); IR, 1770, 1740 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2.

3.3.4 13-Acetylbaccatin III (51)

13-Acetyl-7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (**50**) (150 mg, 0.19 mmol) was treated with Zn dust (250 mg) in CH_3OH-AcOH, 1:1 (15 mL) at 40° for 10 min. The reaction was worked up by filtering off the solid residue and evaporating the salts *in vacuo*. The product was obtained after preparative TLC and recrystallization from MeOH-H_2O as a white solid (83%), mp 222.5-225°C. FABMS, m/z 651 (MNa^+, 7) 629 (MNH^+, 22), 611 (MH^+-H_2O, 2), 569 (MH^+-AcOH, 11), 551 (MH^+-AcOH-H_2O), 509 (MH^+-2AcOH), 135 (17), 119 (25) 105 (100); IR (KBr) 1760, 1740, 1725, 1670, 1570, 1390, 1295, 1120, 1090, 1060, 900, 720 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2.

3.3.5 7-Acetylbaccatin III (52).

A mixture of baccatin III (**15**, 280 mg, 0.48 mmol) and acetic anhydride (3.0 mL) was treated with pyridine (0.5 mL). The mixture was stirred at room temperature for 4 h and worked up as usual. The residual liquid was purified by preparative TLC (hexane: ethyl acetate, 1:1) to yield 150 mg (50%) of white solid, mp 228-231°C (CH_3OH-H_2O). FABMS, m/z 651 (MNa^+, 14), 649 (MNa^+-H_2, 6), 629 (MH^+, 60), 627 (MH^+-H_2, 43), 569 (MH^+-AcOH, 49), 567 (MH^+-AcOH-H_2, 37), 551 (MH^+-AcOH-H_2O, 28), 509 (MH^+-2AcOH, 15), 155 (37), 152 (23), 135 (28), 119 (74), 105 (100); IR 1770, 1745, 1700, 1395, 1260, 1200, 1145, 980, 730 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR see Table 2.

3.3.6 7,13-Diacetylbaccatin III (53).

A mixture of baccatin III (150 mg, 0.51 mmol), acetic anhydride (1.5 mL), 4-dimethylaminopyridine (8 mg) and pyridine (0.125 mL) was heated at 75°C for 3 h. The mixture was worked up by the standard procedure and purified by preparative TLC to give 53, 85 mg (49%), mp 234-236°C. FABMS, m/z 693 (MNa^+, 25), 671 (MH^+, 15) 611 (MH^+-AcOH, 12), 593 (MH^+-AcOH-H_2O, 3), 551 (MH^+-2AcOH, 10), 533 (4), 525 (6), 517 (17), 459 (90), 433 (100); IR 1750, 1765 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2.

3.3.7 13-Oxo-7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (54).

7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (49) (110 mg, 0.145 mmol) in pyridine (0.5 mL) was added into a cooled slurry of CrO_3 (48 mg) in pyridine (0.5 mL) in one portion with stirring. The mixture was stirred at this temperature for 30 min, then left overnight at room temperature. Water was added and the solution was extracted with ether and then washed with 1 N HCl, 5% $NaHCO_3$, and water. The crude white solid was purified by preparative TLC (hexane:ethyl acetate, 1:1) to a pale yellow solid (68 mg, 62%). ^1H NMR, see Table 1; ^{13}C NMR, see Table 2.

3.3.8 13-Oxobaccatin III (55).

13-Oxo-7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (54) (6 mg) in CH_3OH-AcOH (1:1, 1.5 mL) was heated to 40°C with zinc (50 mg) for 10 min. The product obtained after filtration and evaporation of the solvent was homogeneous on TLC,mp 108.0-110.0°C, IR 1745, 1700, 1395, 1260, 750 cm^{-1}. ^1H NMR, see Table 1; ^{13}C NMR, see Table 2.

3.3.9 3-Phenylpropanoyl Chloride (56).

3-Phenylpropanoic acid was refluxed with excess thionyl chloride for 45 min. Excess of thionyl chloride was removed by distillation *in vacuo* and the orange liquid was distilled in a Kugelrohr apparatus under reduced pressure.

3.3.10 Coupling of 7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (49) with 3-Phenylpropanoyl Chloride (56) in the Presence of Triethylamine and DMAP.

7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (**49**, 200 mg, 0.25 mmol), DMAP (3 mg, 0.024 mmol), and triethylamine (0.3 mL) in dry dichloromethane (6 mL) was treated with a solution of 3-phenylpropanoyl chloride (1.7 mmol) in dichloromethane (0.5 mL) at room temperature. The mixture was refluxed for 14 h, water was added and the mixture acidified with 1 N HCl and then extracted with CH_2Cl_2 (100 mL). The organic layer was washed with water, dried (anhydrous $MgSO_4$) and concentrated to a yellow liquid. Analytical TLC showed two major spots with several other minor spots, one of which was the starting material. Preparative TLC (ethylacetate-hexane, 1:1) yielded two major bands. From the ^1H NMR spectrum the less polar fraction was a mixture of two coupled products as evidenced by observing two triplets at 5.75 and 5.87 ppm at about 1 to 5 ratio, these product could not be separated by preparative TLC but could be separated by HPLC on an analytical Resolve - C 8 Radial-Pak cartridge (Waters), CH_3CN-H_2O, 85:15 v/v to give compounds **58** and **59**, respectively.

Compound **58**. ^1H NMR, see Table 3. FABMS, m/z 1159, 1157, 949, 685, 683, 503, 397, 369, 327, 309.

Compound 59. ^{1}H NMR see Table 3. FABMS, m/z 1159, 1157, 949, 685, 683, 503, 397, 369, 327, 309.

The more polar fraction was pure and was also the coupled product with a triplet at 6.15 ppm. The compound was identified as 13-(3-phenylpropanoyl)-7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (60), mp 218.5-220°C; FABMS m/z 896, 894, 836, 834, 818, 686, 684. IR 1765, 1750, 1680, 1400, 1275, cm^{-1}. ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 4.

3.3.11 13-(3-Phenylpropanoyl) baccatin III (61) 13-(3-Phenylpropanoyl)-7-(2,2,2-trichloroethyloxycarbonyl) baccatin III was heated to 40°C with zinc in acetic acid-methanol (1:1) for 10 min. After working up as in previous cases, the crude product was purified by preparative TLC to give two compounds. The major product was identified as 13-(3-phenylpropanoyl) baccatin III (61) by its ^{1}H NMR spectrum, ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 4.

3.3.12 Reaction of Crude 3-Phenylpropanoyl Chloride with 7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (49) in the Presence of Pyridine and DMAP.

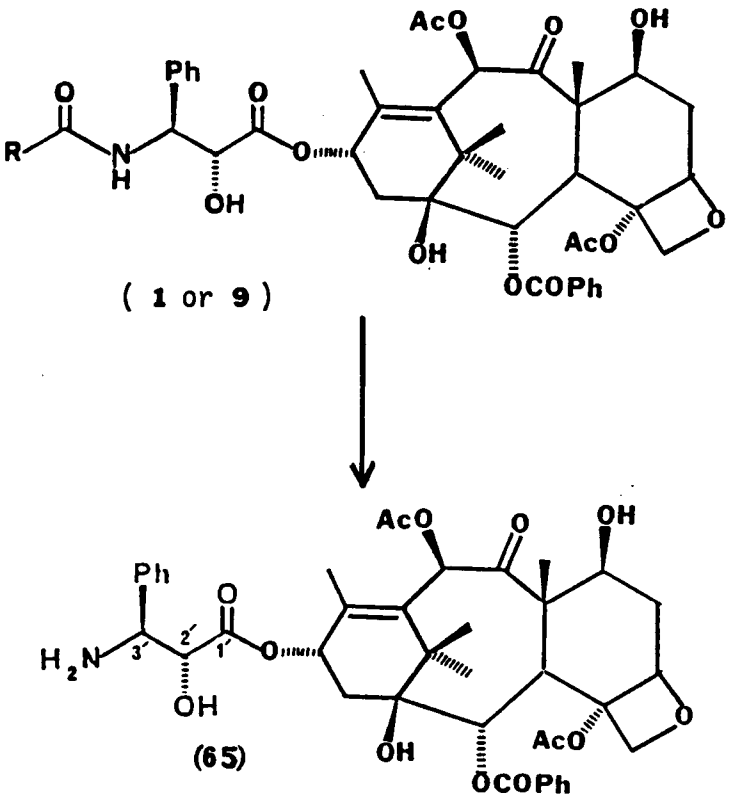
Instead of triethylamine, pyridine was used in the reaction of 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (49) (100 mg, 0.12 mmol) with 3-phenypropanoyl chloride (125 mg) in the presence of DMAP. The mixture in dry dichloromethane (2 mL) was stirred at room temperature for 18 h and worked up as before. Preparative TLC (hexane-ethyl acetate, 7:3) gave a single pure product (62) in 34% yield. ^{1}H NMR, see Table 3. FABMS, m/z 765 (MNa^{+}), 761, 762, 703, 667, 640, 596, 551, 523, 503.

3.3.13 Coupling of 3-Phenylpropanoyl Chloride with 7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III (49)in the Presence of Silver Cyanide in Toluene.

A mixture of 7-(2,2,2-trichloroethyloxycarbonyl) baccatin III (49) (50 mg. 0.066 mmol), 3-phenylpropanoyl chloride (0.03 mL, 0.3 mmol), and silver cyanide (30 mg) in toluene (5 mL) was heated to 85-90°C in an oil bath. The heating was kept at this temperature for 18 h until analytical TLC showed the completion of the reaction. The solid residue was filtered through a short celite column. After evaporating off the solvent, chloroform (40 mL) was added, washed with 5% $NaHCO_3$, brine and water. The organic solution was concentrated in vacuo to a white residue which was purified by preparative TLC to a white solid, 18.3 mg (31%), mp 220-221.5°C; ^1H NMR, see Table 3, ^{13}C NMR, see Table 4, FABMS, identical to compound **60.**

4.0 CLEAVAGE OF THE N-ACYL GROUP OF TAXOL AND CEPHALOMANNINE

A second approach to the preparation of taxol derivatives may be possible by removal of the N-Acyl group from taxol (1) or cephalomannine (9) to give compound 65, followed by reacylation with different acyl groups.

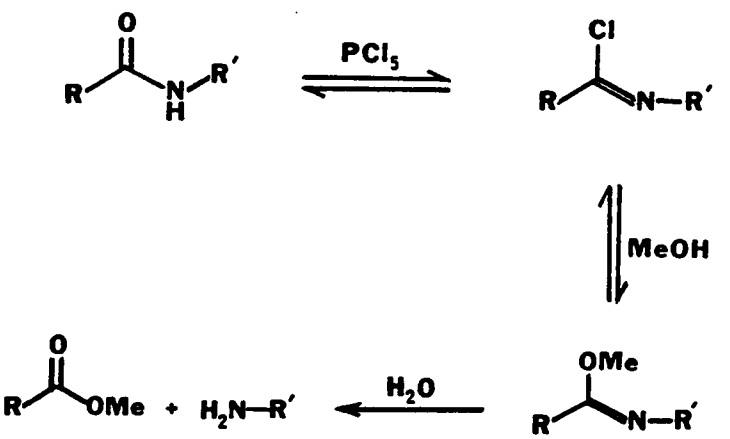


Since taxol (1) and cephalomannine (9) are highly functionalized molecules, possessing both ester and amide groups, an oxetane ring, a β-hydroxy ketone, and also an allylic ester at C-13, carefully-selected conditions must be used in order to selectively cleave the amide linkage without causing any undesired change at other parts of the molecule.

Alkaline hydrolysis of the amide linkage in taxol or cephalomannine is not favored since it will cause several problems such as ester hydrolysis, cleavage of the C-13 ester side chain, and epimerization of hydroxyl groups at C-2′ and C-7.[21] In this chapter, attempts to cleave the N-acyl group from taxol or cephalomannine by various reagents will be discussed.

4.1 Cleavage of the N-Benzoyl Group of Taxol

Phosphorus pentachloride has been used in removal of the benzoyl group from cephalosporin derivatives.[92] The amide reacted with phosphorus pentachloride and pyridine in dry benzene to form imino chlorides in good yield, and methanol was then added to react with the imino chloride to form the imido ester which readily hydrolyzed with water to yield the free amino compound (Scheme 25).



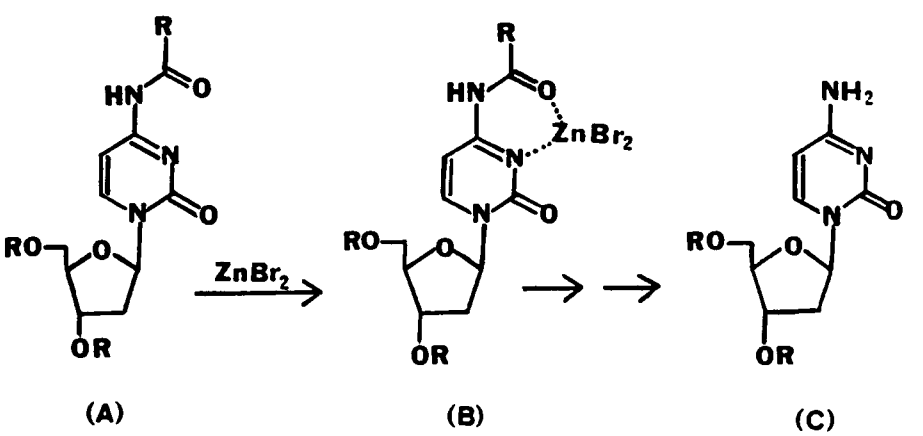
Scheme 25

2',7-Diacetyltaxol prepared from the reaction of taxol, acetic anhydride, and pyridine, was treated with PCl_5 and pyridine at -20°C. The reaction mixture was then treated with methanol and worked up as cited in the literature, TLC showed complicated patterns and no single major product was detected.

Taxol was also treated with α-chymotrypsin, an enzyme which was reported to cleave some N-acyl group from natural products such as cephalosporin, in a pH 7 medium at room temperature. No change in the compound was observed after 24 h.

4.1.1 Reaction of Taxol with Zinc Bromide in CH_3OH-$CHCl_3$

Kierzek et al reported deacylation as a side reaction in the attempt to deblock the di-p-anisyl phenylmethyl group from nucleoside derivatives using $ZnBr_2$ in protic solvents such as methanol-chloroform, 4:1 (v/v).[91] A mechanism was proposed which involved the bidentate chelated intermediate B which was subsequently attacked by methanol as shown in Scheme 26.



Scheme 26

Eventhough taxol lacks the second nitrogen atom for the second site of chelation, it does have a hydroxyl group which might fulfill the same function, and we expected the cleavage of the N-benzoyl group to occur to some extent. Taxol was therefore treated with $ZnBr_2$ in methanol-chloroform (4:1 v/v) and the solution was kept at room temperature for 30 h. The crude product was purified by preparative TLC to two compound **66** and **67** in about a 2 to 1 ratio. The major product was less polar than taxol and had a different ^1H NMR spectrum from taxol.

The ^1H NMR and the homonuclear COSY spectra of the major product **66** are shown in Figures 40 and 41. The obvious feature of these spectra is the absence of the doublet of doublets of the C-20 protons which are usually found at about 4.10 and 4.30 ppm, and the absence of the C-10 proton singlet at about 6.30 ppm. By comparison with the ^1H NMR spectrum of taxol (Figure 3), the broad triplet at 6.25 ppm was assigned to the proton of C-13, the doublet of doublets at 5.78 ppm to the C-3′ proton, and the doublet at 5.72 ppm to the proton of C-2. The multiplet at 4.90 ppm was assigned to the proton of C-5 whereas the doublet at 4.78 ppm was assigned to the C-2′ proton. The one-proton doublet at 3.90 ppm thus represented the proton of C-3. All of these assignments were confirmed by the couplings displayed in the Homonuclear COSY spectrum (Figure 41).

Based on the integration and the Homonuclear COSY spectrum, the singlet at 5.40 ppm was assigned to the proton of C-10. Another intense broad singlet appeared at 4.38 ppm and was assigned to protons of C-20. A broad doublet of doublets appeared at 3.68 ppm and was assigned to the proton of C-7. This signal was shifted about 0.7 ppm upfield as

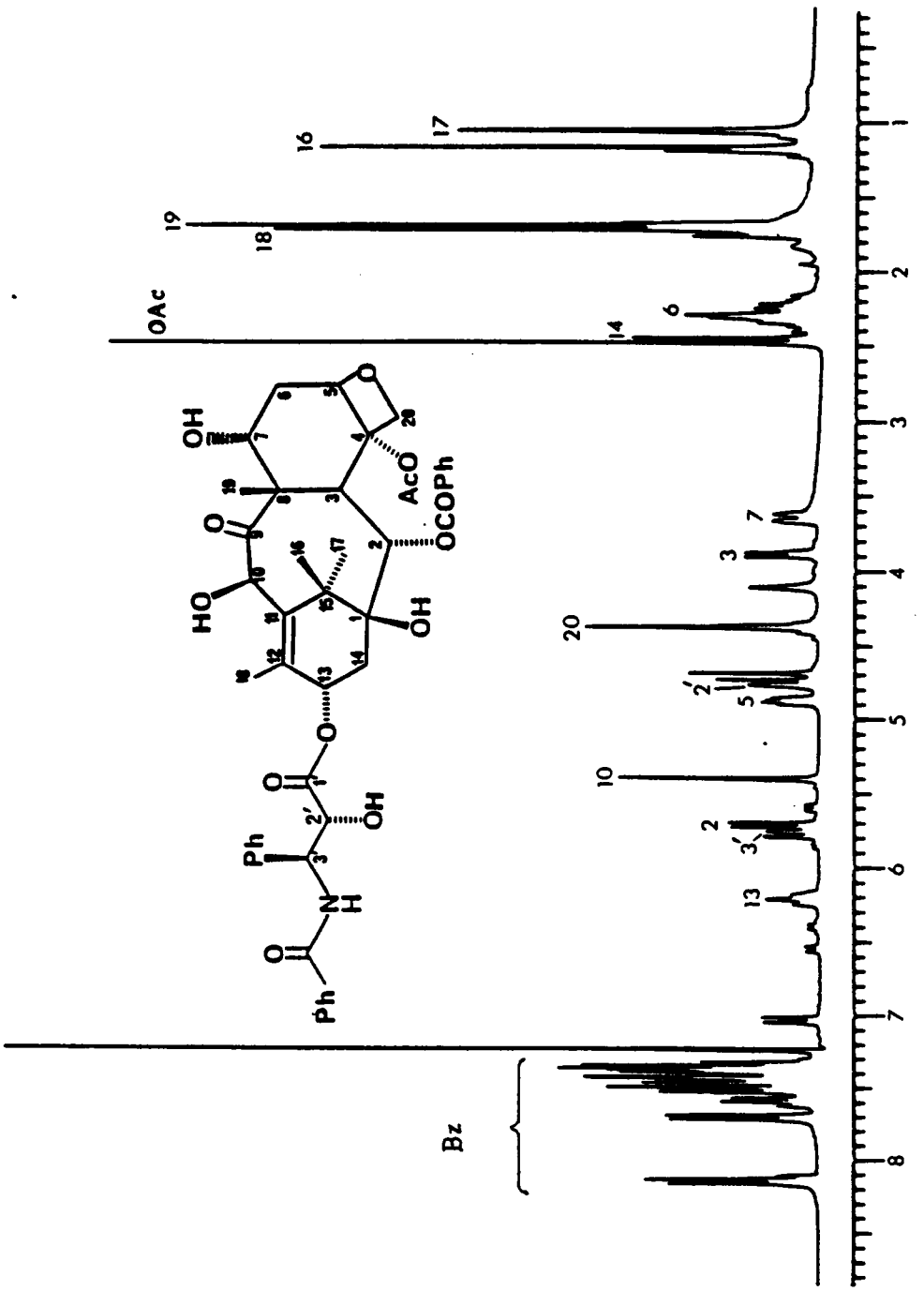


Figure 40. ^{1}H NMR Spectrum of 10-Deacetyl-7-epitaxol (**66**)

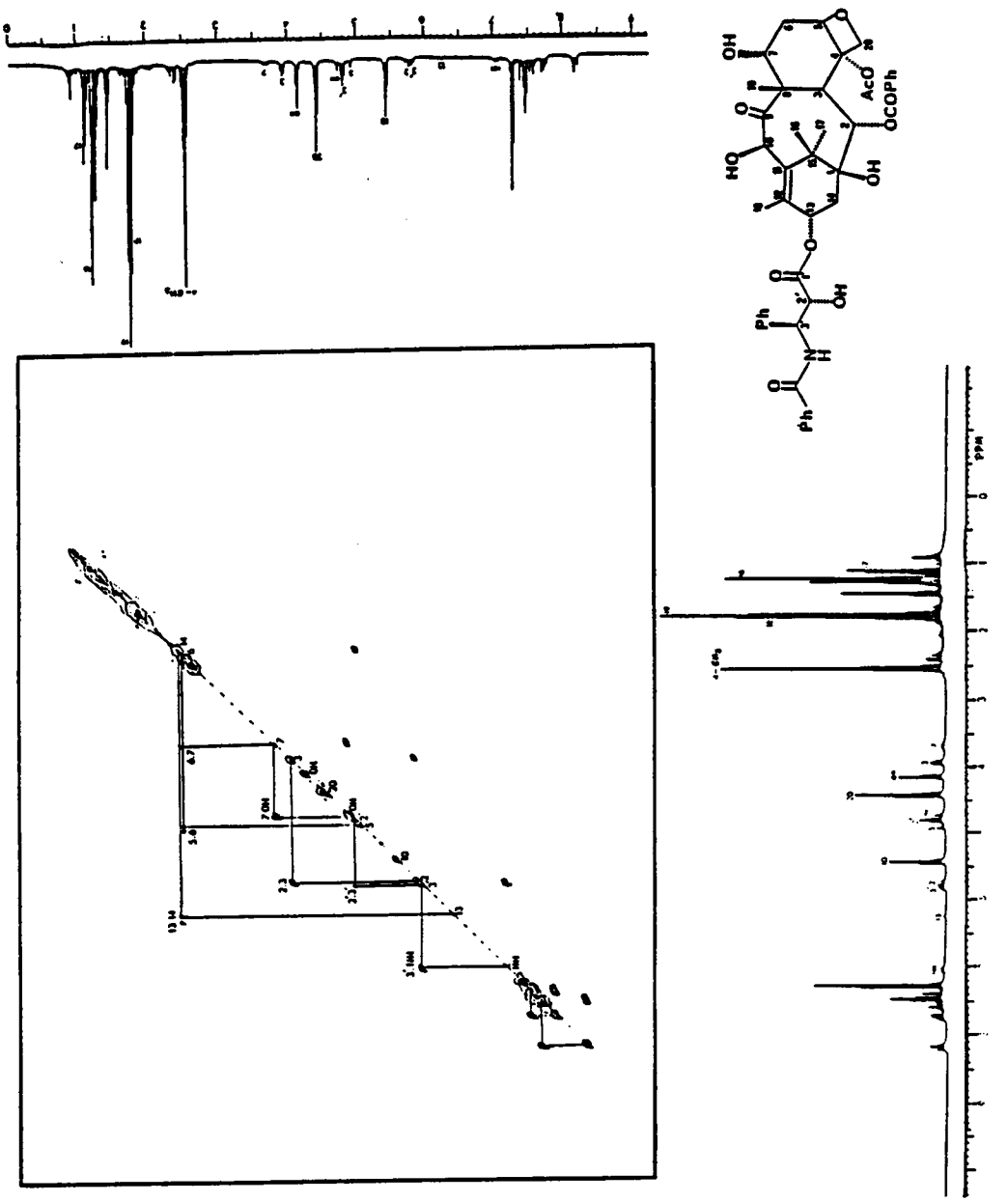
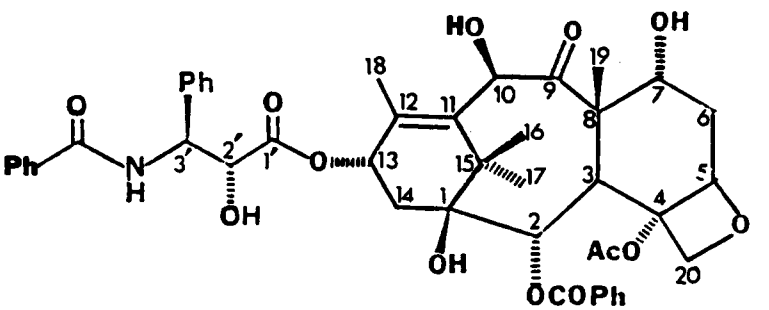


Figure 41. Homonuclear COSY spectrum of 10-Deacetyl--7-epitaxol (66)

compared with taxol, and this shift is characteristic of taxols epimerized at the C-7 position. The conformational change due to this phenomenon accounts for the upfield shift of the C-7 protons and also for the drastic change in peak shape of the C-20 protons. Two other broad singlets at 4.12 and 4.72 ppm were observed which probably represent the C-7 and C-2′ hydroxyl protons. Deacetylation of the C-10 acetate was also detected by the absence of a three-proton singlet at 2.2 ppm. The ^{1}H NMR data thus indicated that compound **66** was 10-deacetyl-7-epitaxol and this was in agreement with the literature data.[21]



(66)

The FABMS showed peaks at 812 (MH^{+}) and 834 (MNa^{+}) which indicated a molecular weight of 811, consistent with the strucure of 10-deacetyl-7-epitaxol **(66)**. An intense peak at 286 representing the side chain acid fragment was also observed.

The ^{13}C NMR and heteronuclear COSY spectra of **66** are shown in Figures 42 and 43. The ^{13}C NMR spectrum was similar to that of taxol except for three peaks which were later assigned to carbons of 3-, 7- and 10- positions with the aid of the heteronuclear COSY spectrum. The C-3 peak of 10-deacetyl-7-epitaxol (**66**) was seen at 40.3 ppm, about 6 ppm higher field than in other taxane compounds. The C-7 signal shifted downfield by 4 ppm whereas the C-13 signal was observed at 72.4 ppm which was about 3 ppm downfield than most 13-acylbaccatin III derivatives.

The ^{1}H NMR and homonuclear COSY spectra of the minor product **67** are shown in Figures 44 and 45. The singlet at 4.21 ppm was assigned to proton of C-10 indicating that deacetylation had occurred in this case also. Integration showed that no cleavage occurred at the benzamide group since there were fifteen protons in the aromatic region. The proton of C-13 was seen as a broad triplet at 6.18 ppm. The protons of C-2 and C-3 were assigned to two doublet at 5.68 and 3.90 ppm respectively. The protons of C-2′ and C-3′ were found at 4.80 and 5.77 ppm as a broad triplet and a doublet of doublets respectively. The broad doublet at 4.94 ppm was assigned to the proton of C-5 whereas the C-20 protons were located at 4.21 and 4.31 ppm as a doublet of doublets. The C-7 proton signal was partially hidden under one of the doublets of the C-20 protons. Since there were no changes in chemical shifts and peak shapes of the C-20 and C-7 protons it was assumed that there were neither changes at the oxetane ring nor epimerization of the C-7 hydroxyl group. The homonuclear COSY showed all the expected coupling

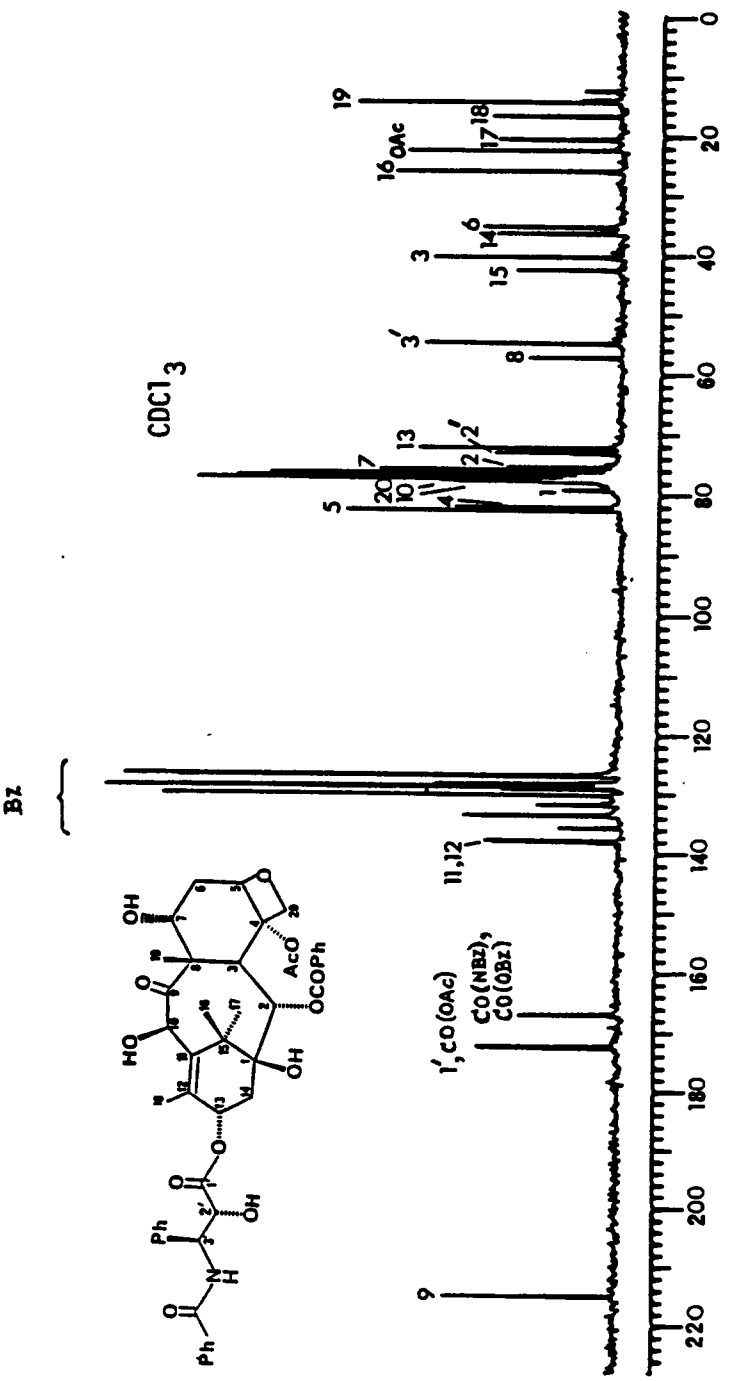


Figure 42. Proton-decoupled ^{13}C NMR spectrum of 10-Deacetyl-7-epitaxol (66)

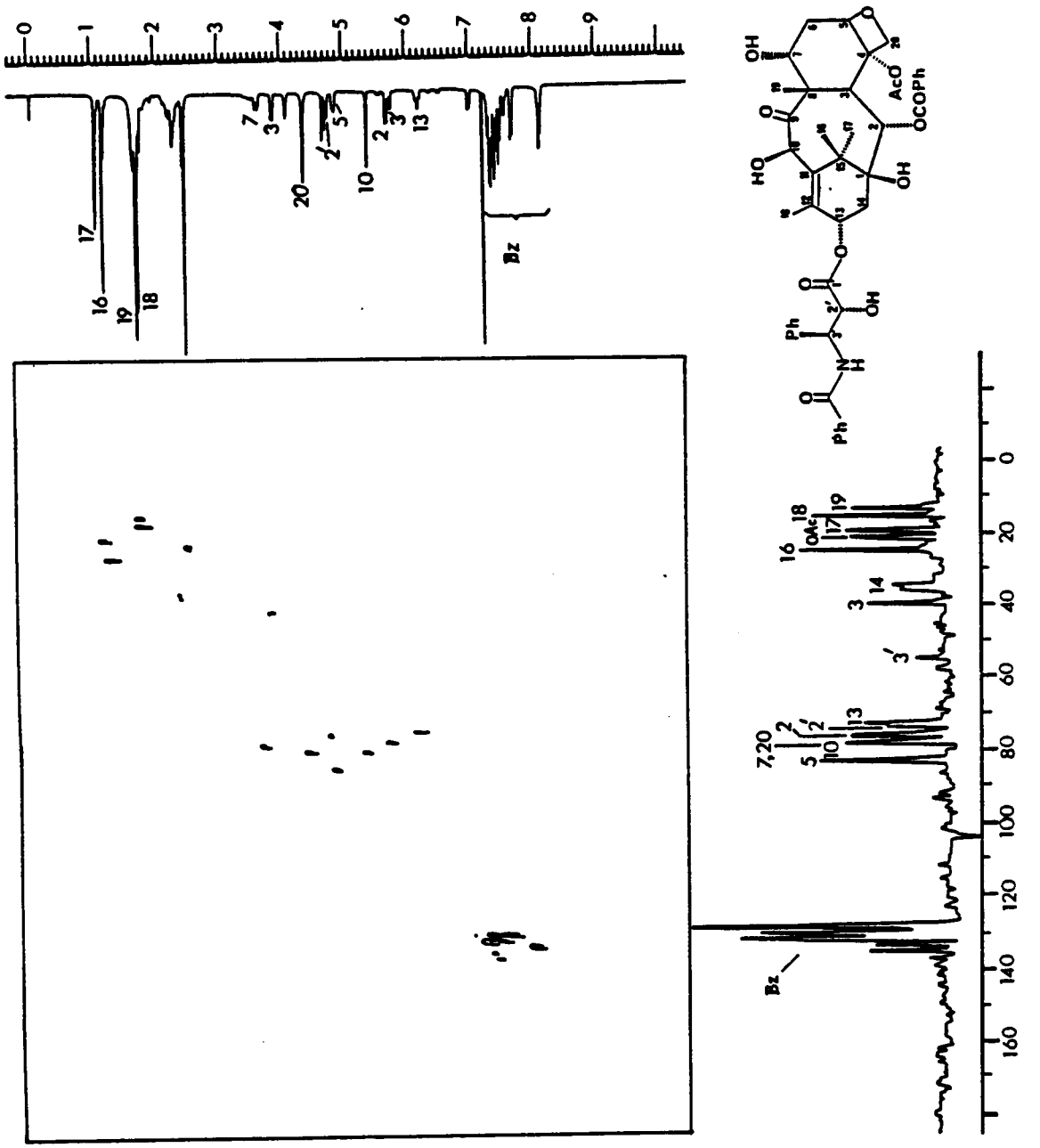


Figure 43. Heteronuclear COSY spectrum of 10-Deacetyl-
-7-epitaxol (**66**)

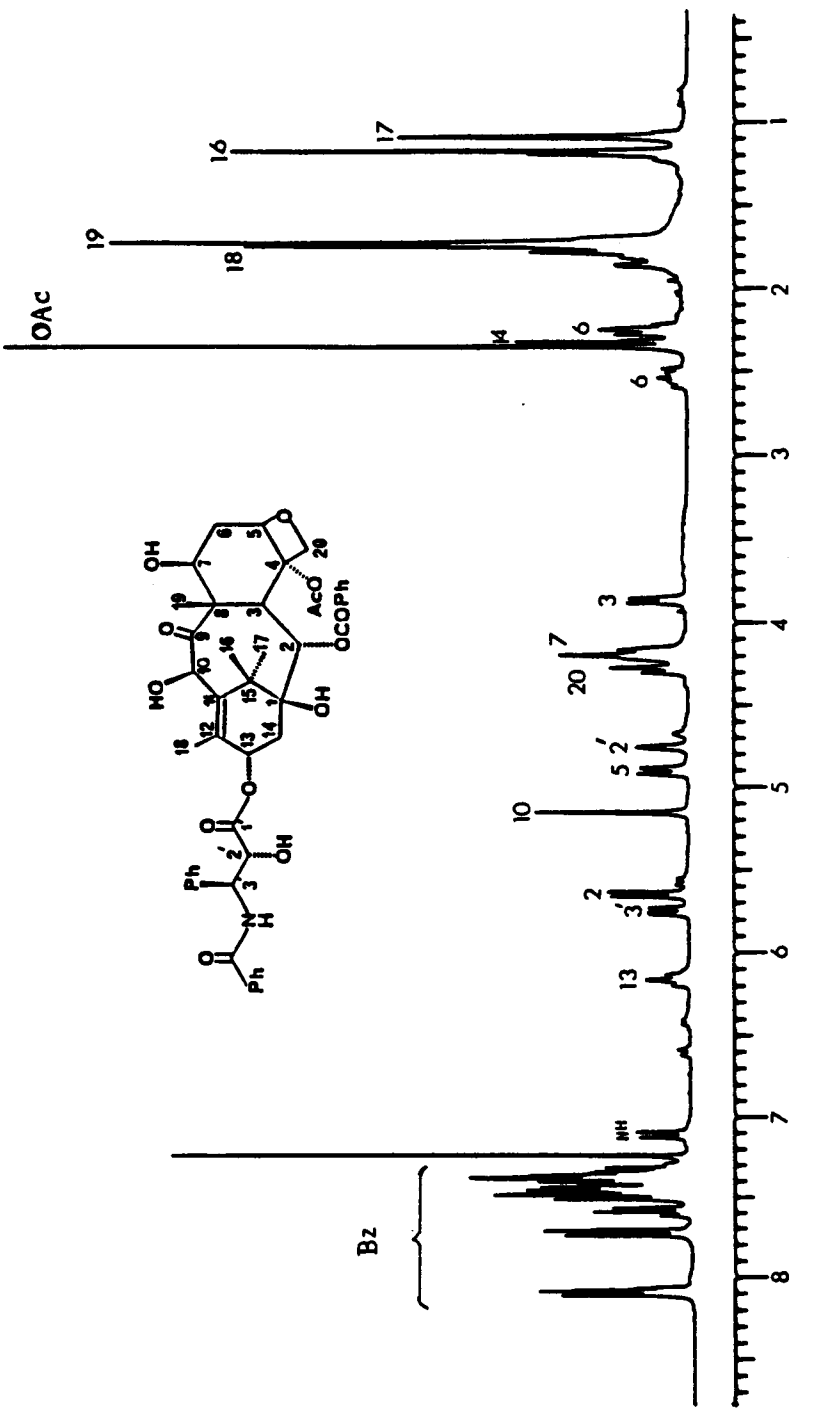


Figure 44. ^{1}H NMR Spectrum of 10-Deacetyltaxol (67)

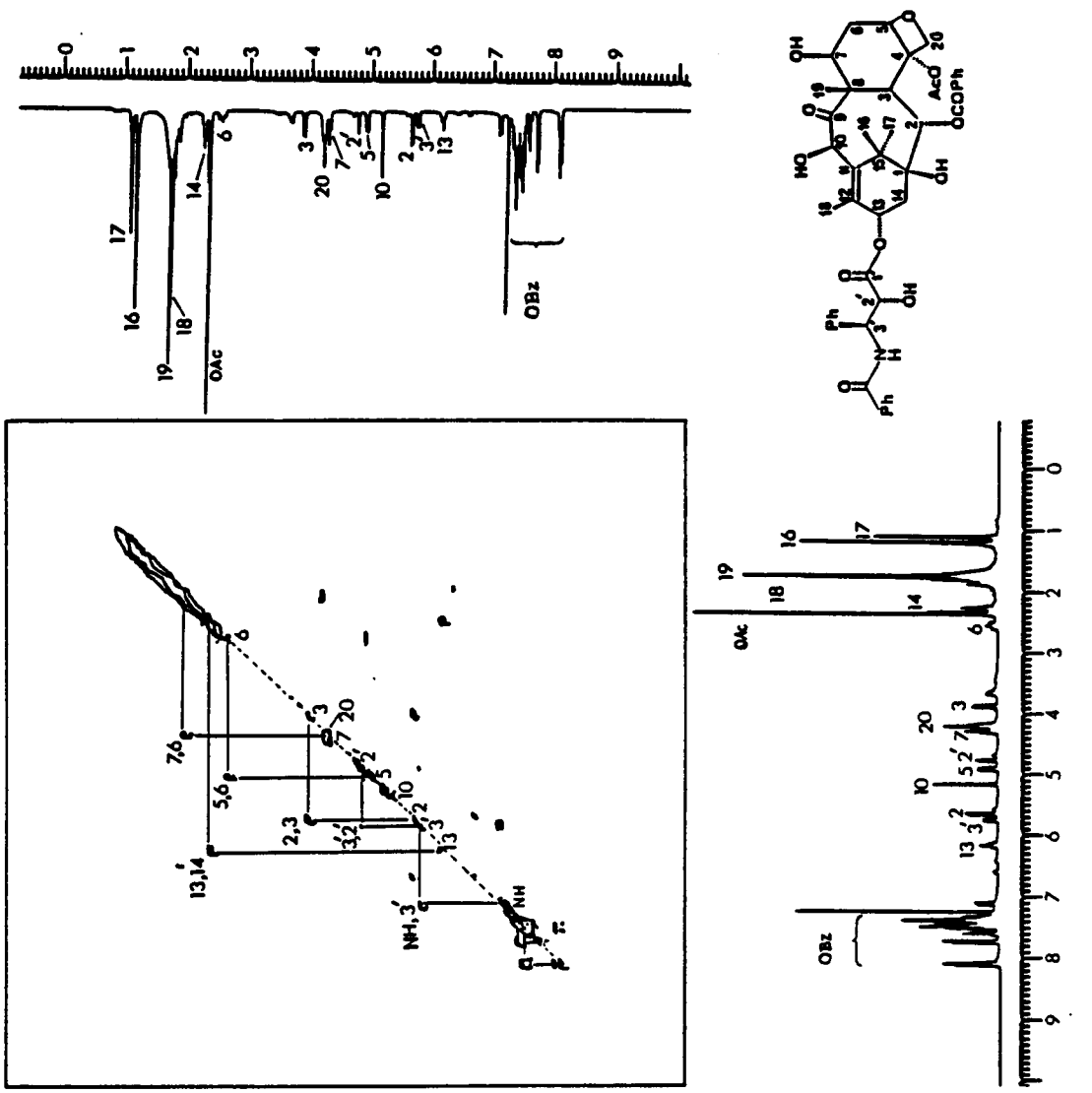
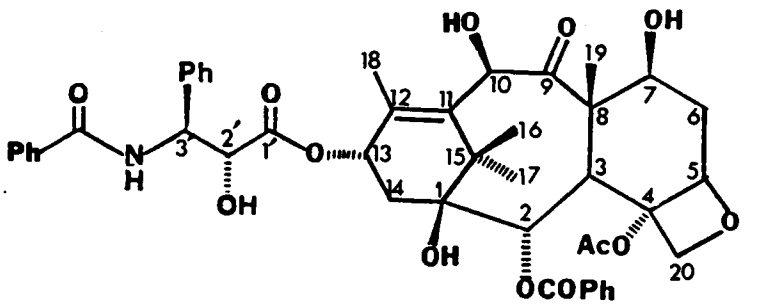


Figure 45. Homonuclear COSY spectrum of 10-Deacetyltaxol (**67**)

connectivities, and therefore the compound was identified as 10-deacetyltaxol (67). The ^{1}H NMR data were consistent with the literature data.[21]



(67)

The decoupled ^{13}C NMR and heteronuclear COSY spectra of compound 67 are shown in Figures 46 and 47. The C-10 signal was found at 74.8 ppm, about 1.0 ppm upfield from those compounds with 10-acetyl groups. The C-7 signal was found at 72.3 ppm, similar to other compounds wiht a β-hydroxyl at this carbon.

The FABMS showed peaks at 812 (MH^+), 834 (MNa^+) and 850 (MK^+) indicating a molecular weight of 811, which was in agreement with the structure of 10-deacetyltaxol. An intense peak at 286 represented the fragment of the side chain acid at C-13.

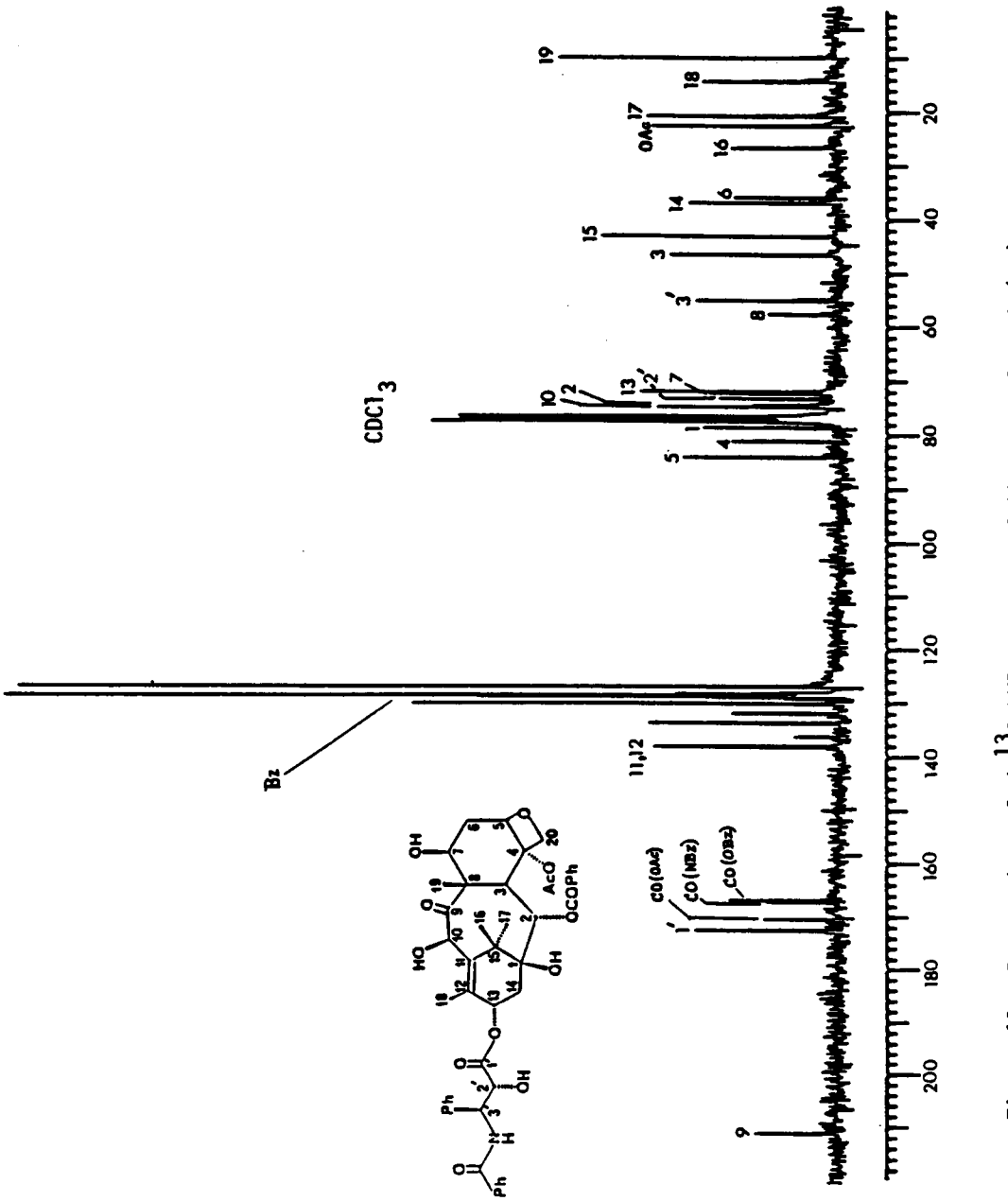


Figure 46. Proton-decoupled ^{13}C NMR spectrum of 10-Deacetyltaxol (67)

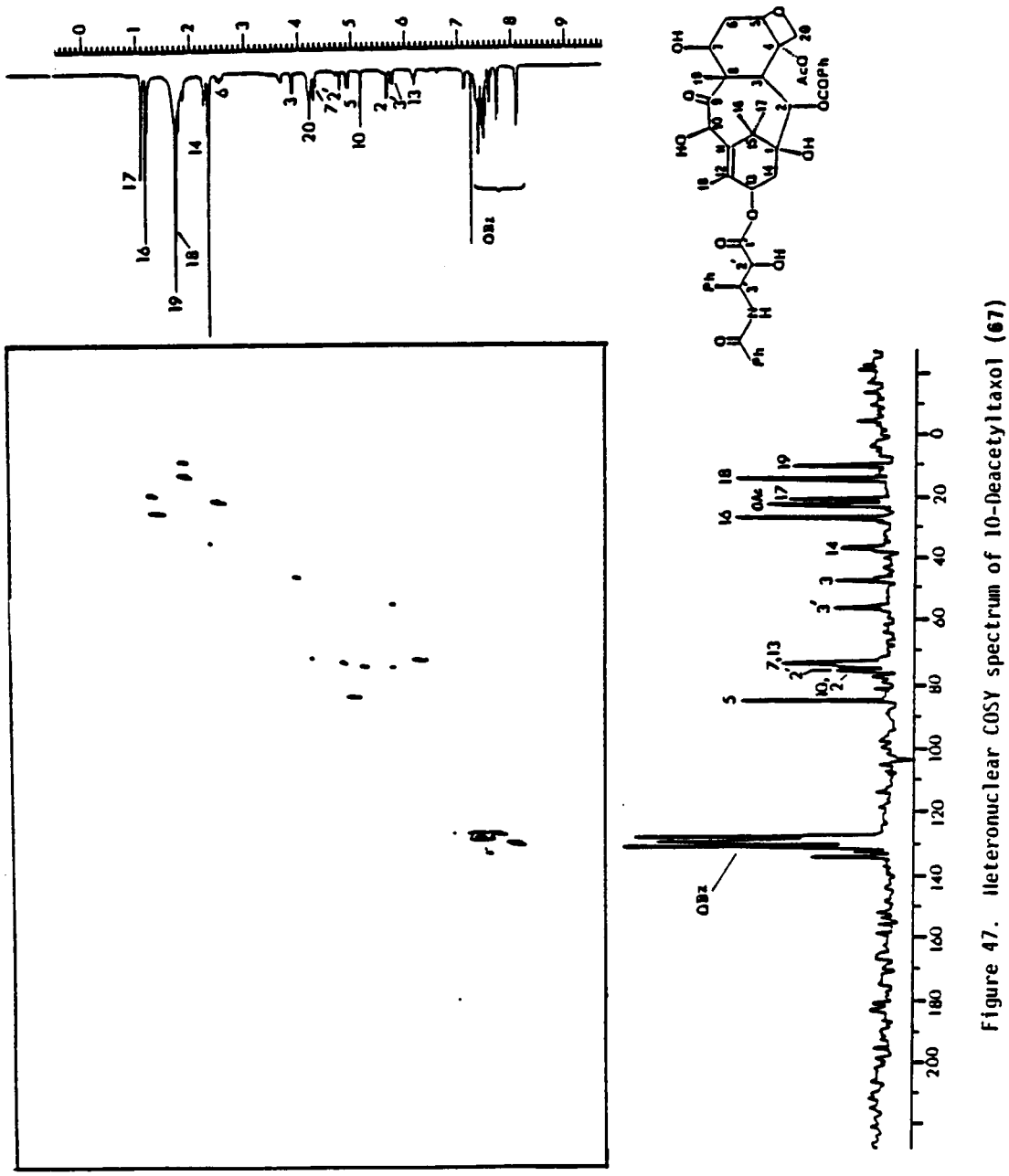


Figure 47. Heteronuclear COSY spectrum of 10-Deacetyltaxol (67)

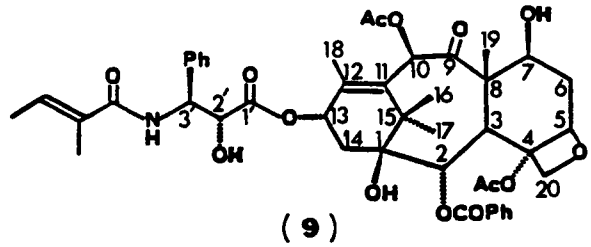
It was therefore concluded that the reaction of $ZnBr_2$ in methanol-chloroform with taxol at room temperature did not give an N-benzoyl cleavage product. When the reaction was carried out at 50°C for 24 h the ratio of compound **66** to **67** was about 4 to 1, and no other product was detected. The absence of the second nitrogen atom to form the second chelate bond might be the cause of the failure of this reaction.

4.2 Attempted Cleavage of N-Tigloyl Group of Cephalomannine .

Another approach to obtain modified taxols is to remove the N-tigloyl group from cephalomannine (**9**) to give the desired aminotaxol **65**. A promising route is to convert the alkene part of the tigloyl group to the more easily cleaved, 1,2-dicarbonyl, by ozonolysis of cephalomannine. The ozonolysis product will then be subjected to various reactions in order to cleave the 1,2-dicarbonyl group.

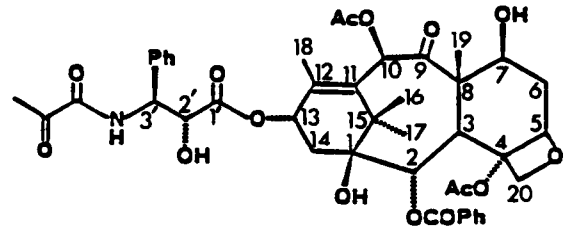
4.2.1 Ozonolysis of Cephalomannine

Cephalomannine (**9**) was ozonized in dichloromethane at -78°C. The mixture was hydrogenated using palladium on carbon as catalyst. The crude product showed a spot with very similar Rf to cephalomannine but analytical HPLC showed a very complex product mixture with one major product in about 50% yield. The crude product was purified on a preparative HPLC column in 30% yield.



(9)

1) O_3,CH_2Cl_2,-78°
2) H_2,Pd/C



(68)

The ^1H NMR and homonuclear COSY of cephalomannine (9) are shown in Figures 48 and 49. Cephalomannine differs from taxol in that it has a tigloyl group instead of a benzoyl group at 3′-NH. The proton of C-3′′ will be seen as doublet of doublet at 6.43 ppm whereas the C-2′′ and C-4′′ methyls are seen as two three-proton doublets at 1.68 and 1.75 ppm respectively. Other protons have very similar chemical shifts to those of taxol and are confirmed by the couplings displayed in this homonuclear COSY spectrum.

The FABMS of cephalomannine showed peaks at 832 (MH^+),

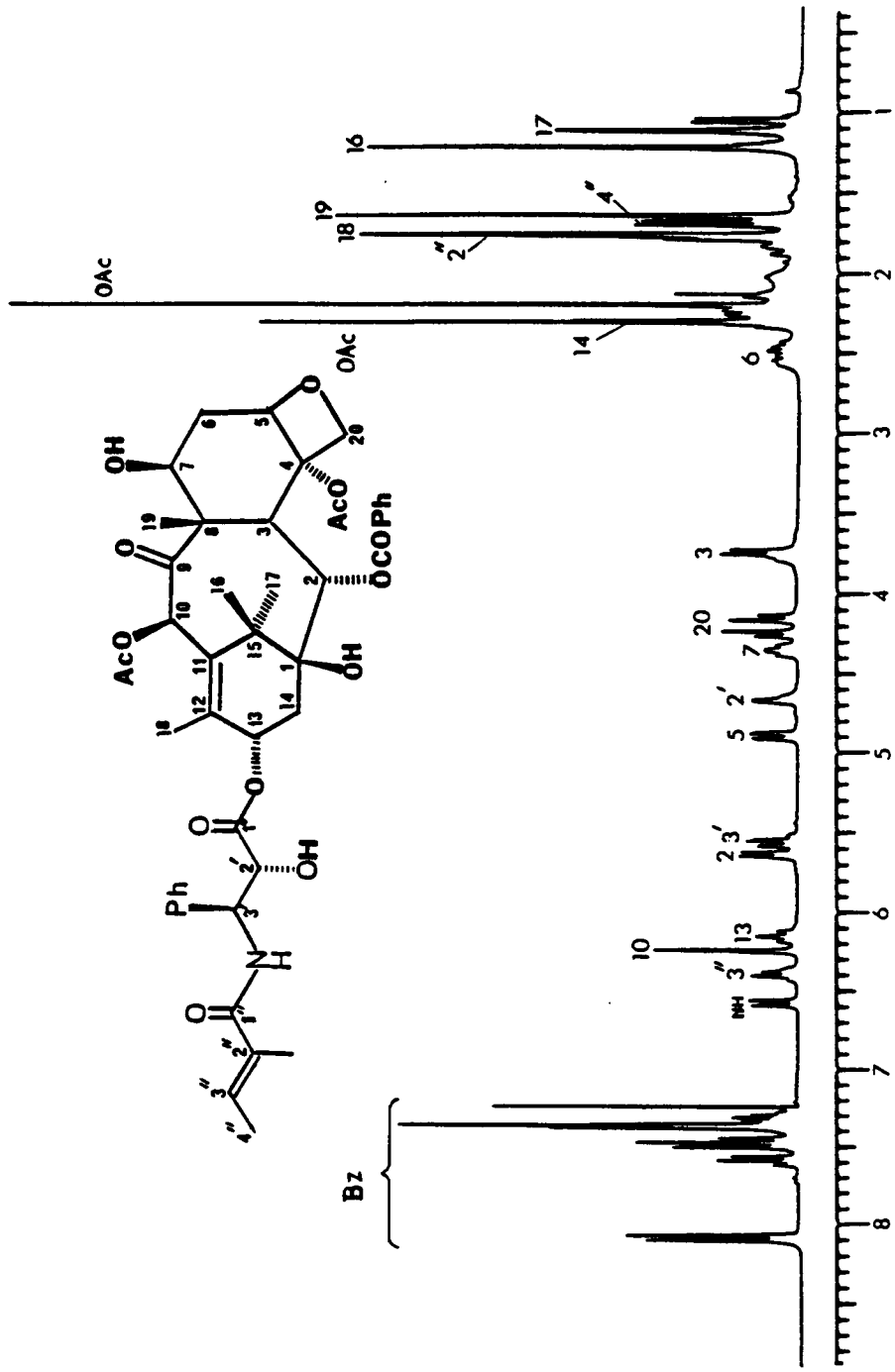


Figure 48. ^{1}H NMR Spectrum of Cephalomannine (**9**)

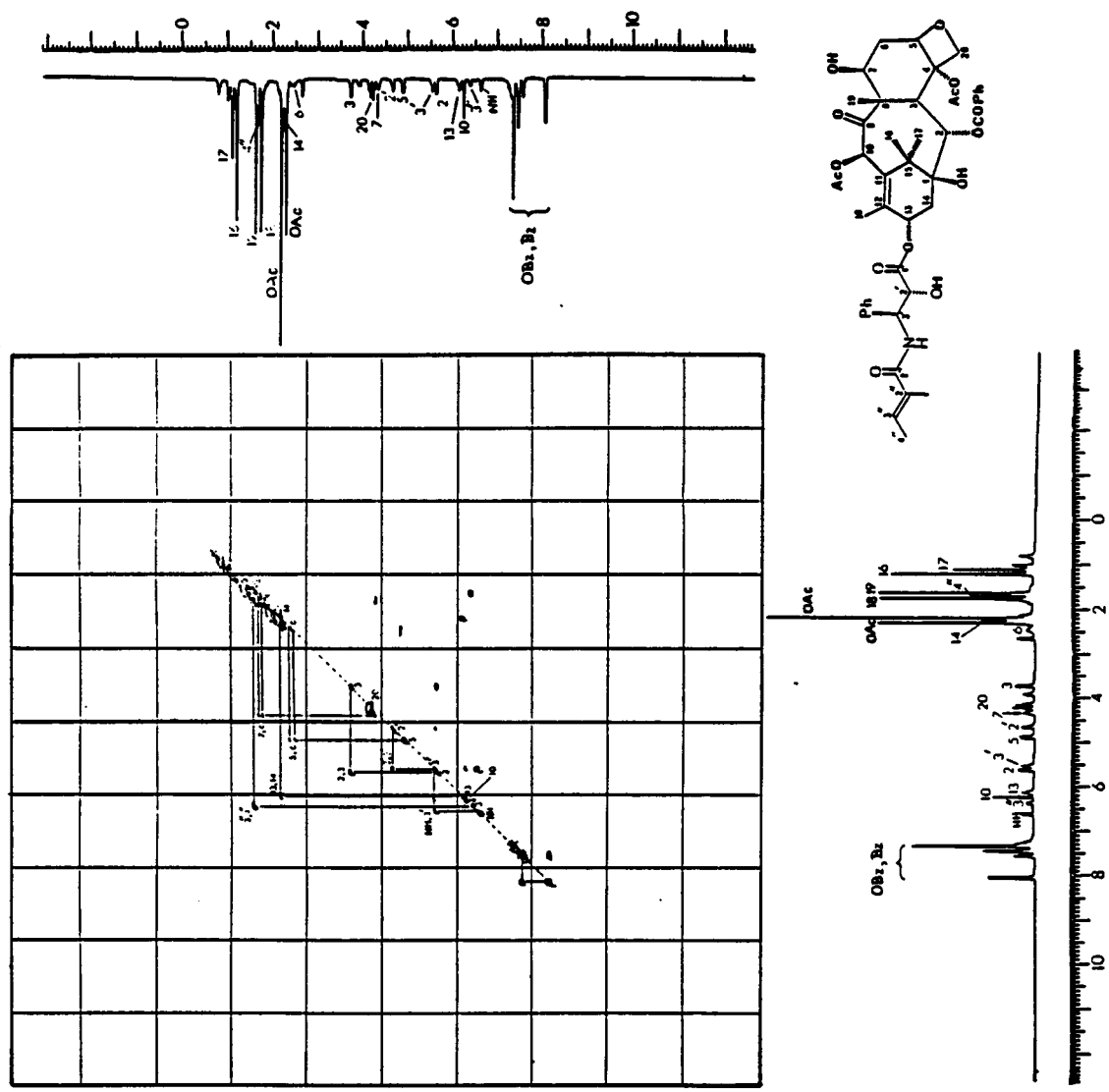


Figure 49. Homonuclear COSY spectrum of Cephalomannine (9)

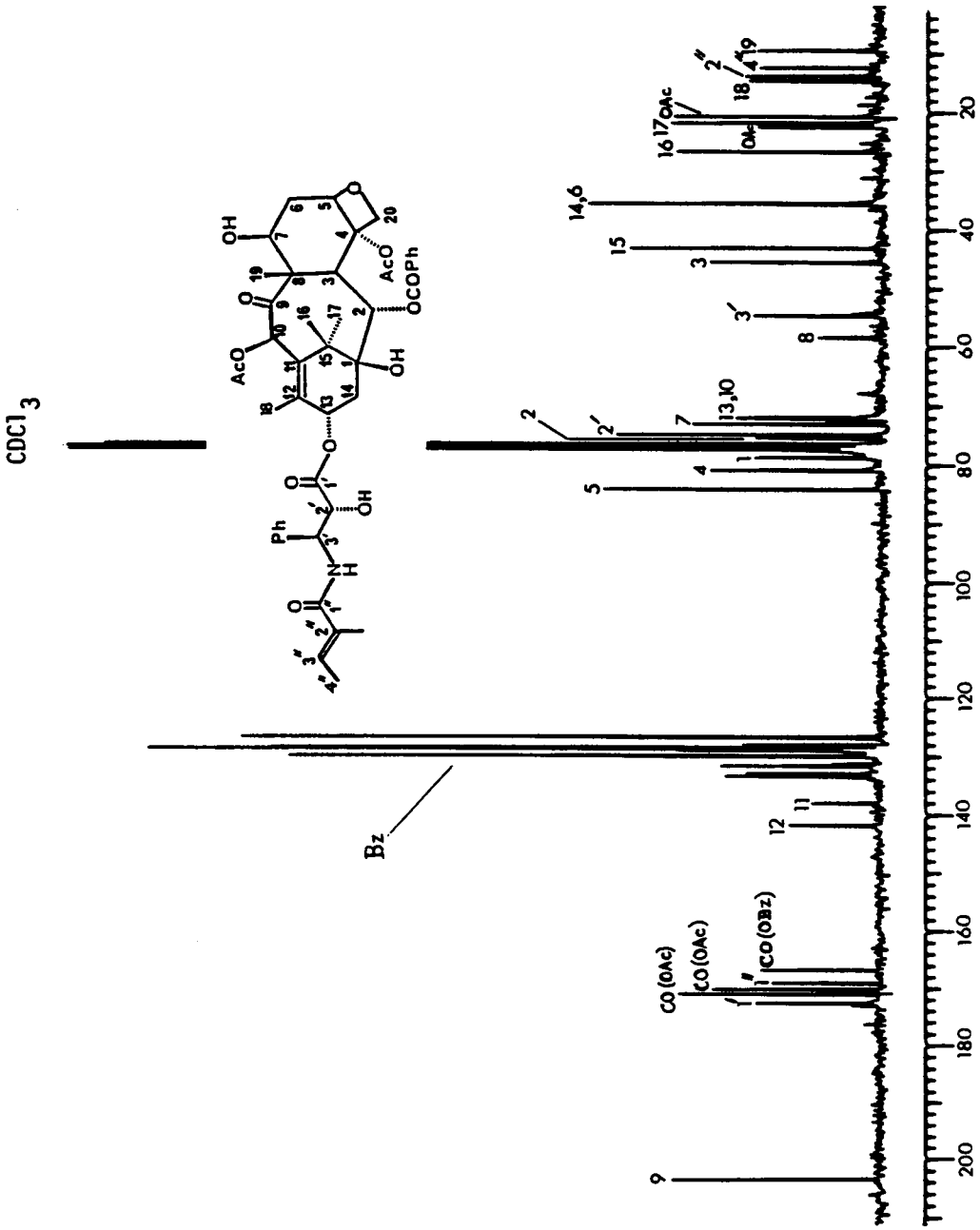


Figure 50. ^{13}C Broad Band Decoupled Spectrum of Cephalomannine (9)

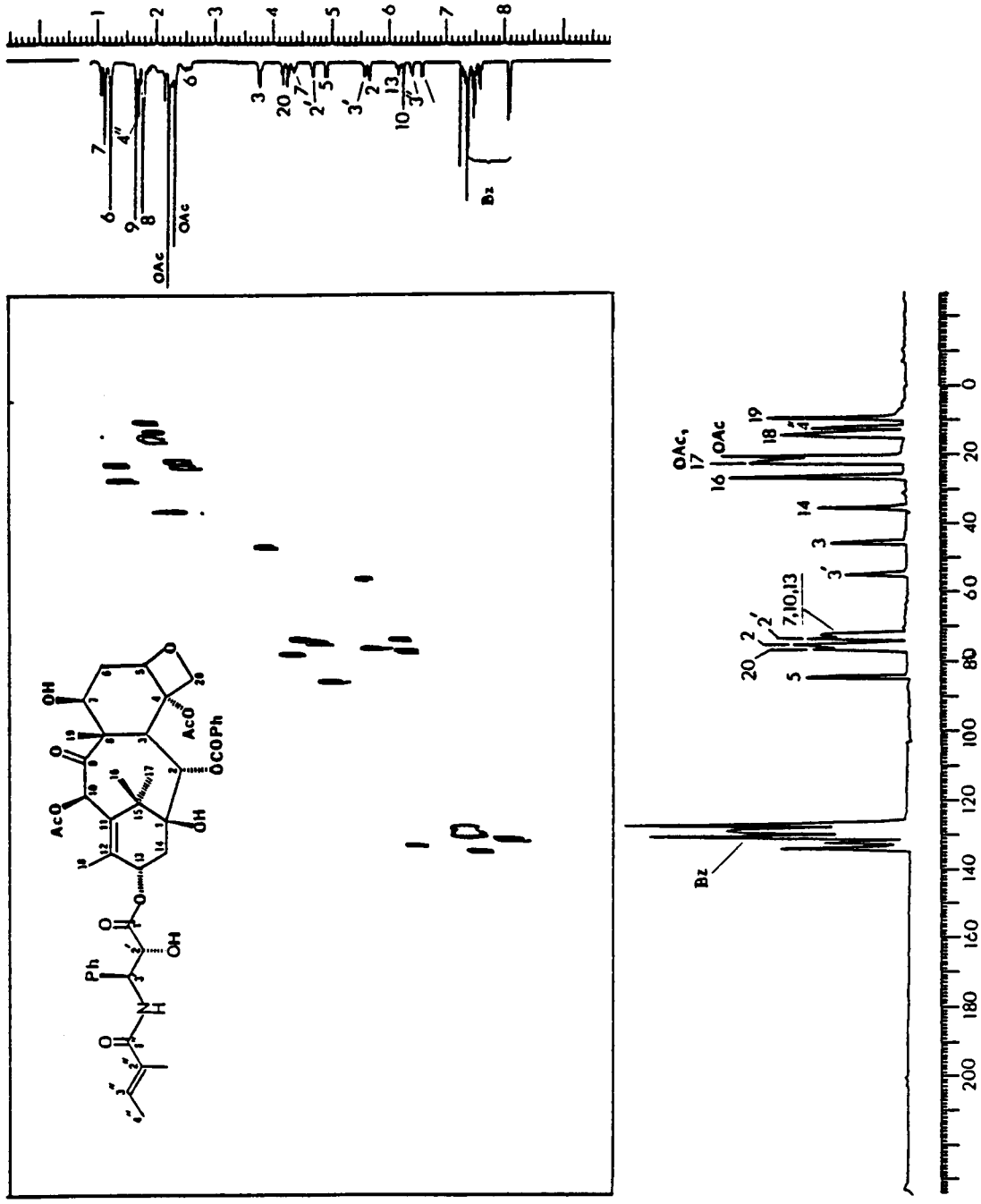


Figure 51. Heteronuclear COSY spectrum of Cephalomannine(**9**)

569 (M^+-side chain at C-13), and 509 (M^+-side chain at C-13-CH_3COOH).

The ^{13}C NMR of cephalomannine and its heteronuclear COSY were also taken and are shown in Figures 50 and 51.

The ^1H NMR and homonuclear COSY spectra of the ozonolysis product **(68)** are shown in Figures 52 and 53. The absence of signals for the C-3'' and C-4'' protons clearly showed that the 2'', 3''-double bond was ozonized. The proton of C-3' moved upfield from 5.57 to 5.49 ppm whereas the N-H signal moved downfield by about 1.3 ppm. Other signals were similar to those of cephalomannine. The methyl protons of the pyruvyl group were assigned to the intense singlet at 2.39 ppm, about 1.0 ppm downfield from its original signal in cephalomanine, and thus confirming the formation of the pyruvyl group in **68**.

The homonuclear COSY spectrum (Figure 53) showed all of the expected coupling connectivities for the ozonolysis product **(68)**. The ^{13}C NMR of compound **68** is shown in Figure 54. The peak assignments were aided by its heteronuclear COSY spectrum shown in Figure 55.

The FABMS showed peaks at 820 (MH^+), 760 (MH^+-CH_3COOH), 570 (MH^+-side chain at C-13), and 510 (MH^+-side chain at C-13 - CH_3COOH), confirming the structure of compound **68**.

Preliminary efforts have been made to cleave the N-pyruvyl group of compound **68**. The 2',7-diprotected derivative of this compound was first treated with sodium periodate solution at room temperature. No reaction was detected by TLC after 24 h. When the temperture was raised to 50°C and was kept for 16 h, no product was observed.

The ozonolysis product **68** was also treated with o-aminophenol in methanol-water. The free amino compound **(63)** was expected from a

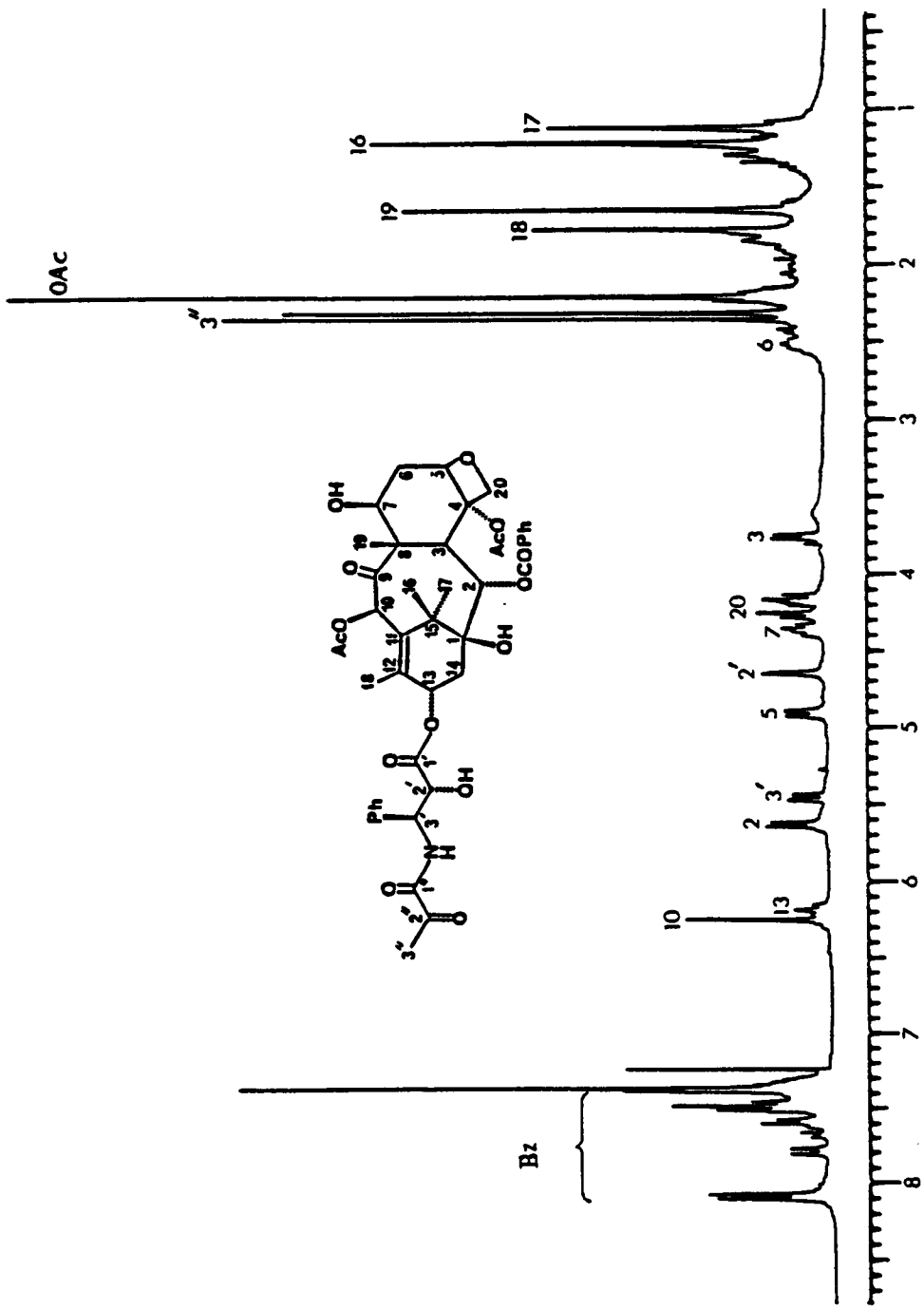


Figure 52. ^{1}H NMR Spectrum of the Ozonolysis Product (68)

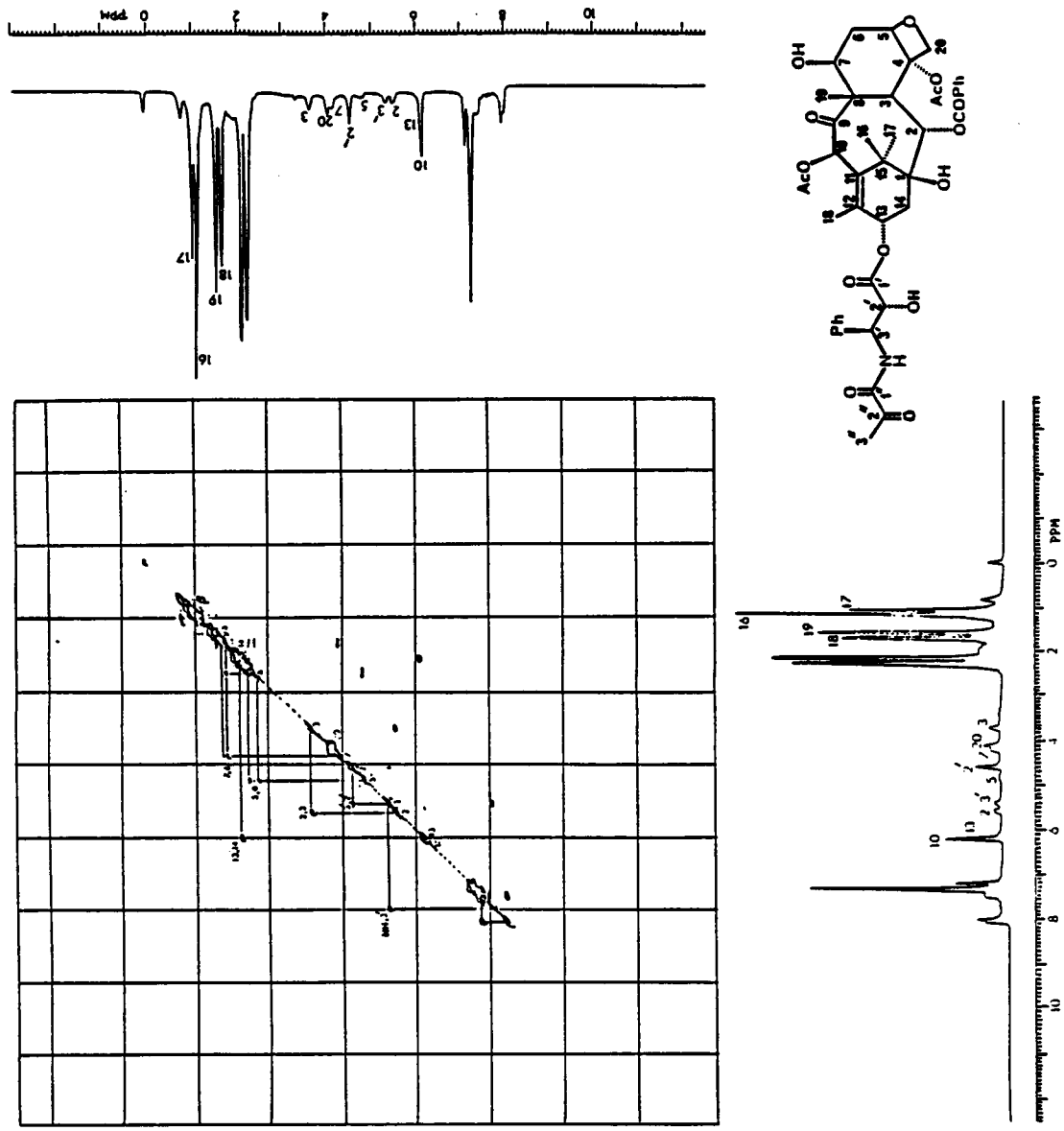


Figure 53. Homonuclear COSY spectrum of (**68**)

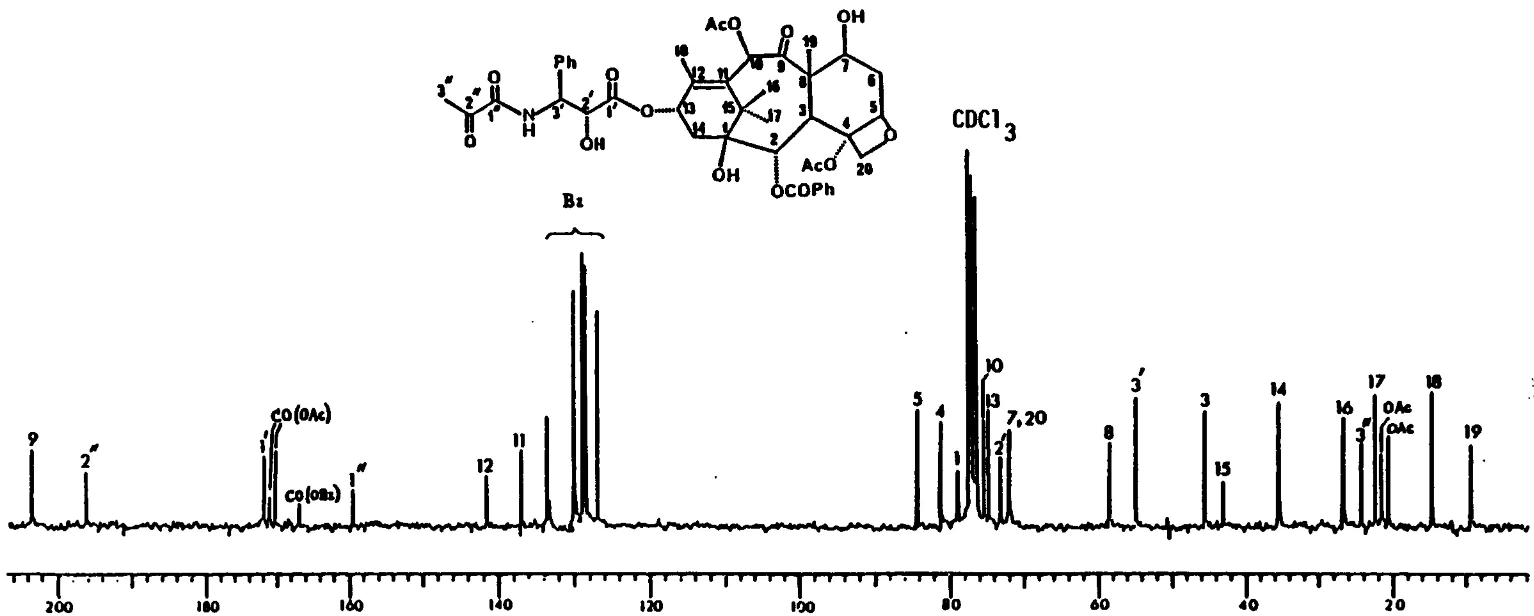


Figure 54. Proton-decoupled ^{13}C NMR spectrum of (68)

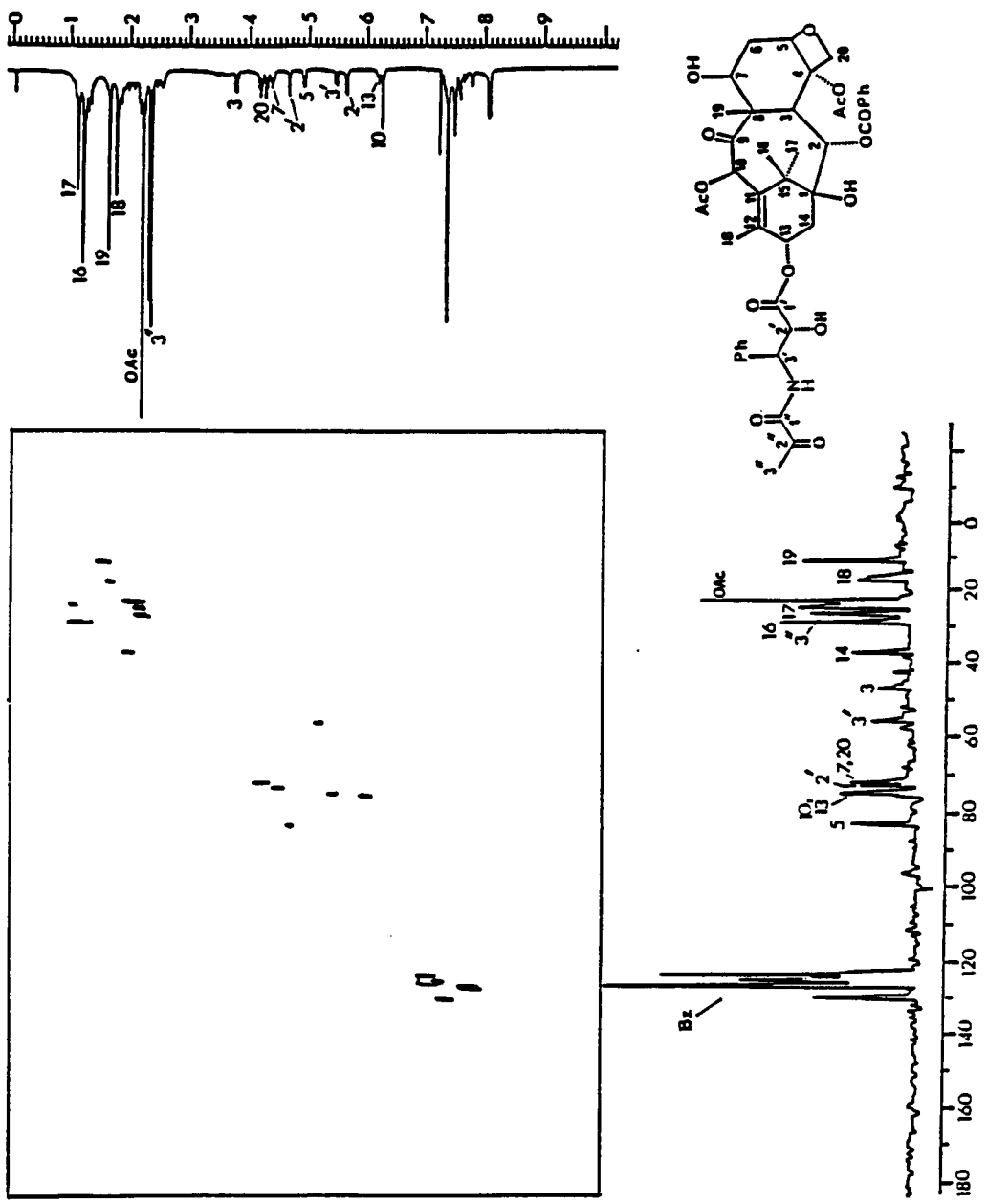
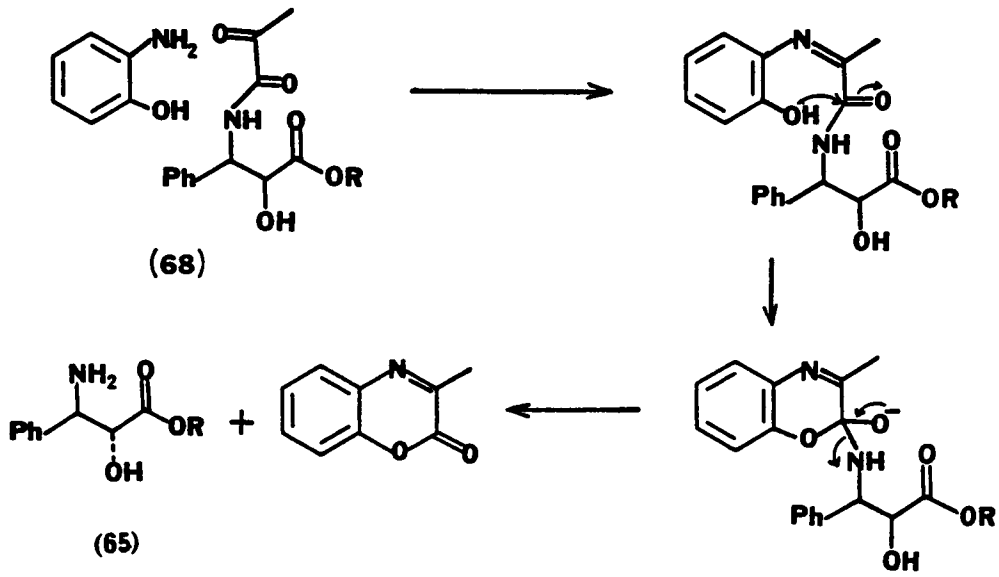


Figure 55. Heteronuclear COSY spectrum of (68)

reaction which was proposed to occur by the following mechanism:



Scheme 27

The first attempt was performed by stirring the mixture of compound **68** and o-aminophenol in methanol-water at room temperature for 9 h. The mixture showed a complex TLC pattern with no significant product and no further purification was carried out.

Similar treatment of **68** with o-phenylenediamine also yielded no desired cleavage product.

4.3 Experimental

4.3.1 Attempted Cleavage of the N-Benzoyl Group of Taxol by Zinc Bromide in Chloroform-Methanol.

A mixture of taxol (100 mg, 0.117 mmol) and $ZnBr_2$ (3.3 g, 14.6 mmol) in $CHCl_3$-CH_3OH (1/4 v/v, 10 mL) was stirred for 30 h at room

temperature. Water (25 mL) was added and the mixture was extracted with dichloromethane and the organic layer was dried over anhydrous $MgSO_4$. A white solid was obtained after removal of the solvent and was then purified by preparative TLC (EtOAc-Hexane 1:1) to yield two products (**66** and **67**) and a small amount of taxol. In a separate experiment the mixture of taxol and $ZnBr_2$ in $CHCl_3$-CH_3OH was heated to 40°C for 24 h. The reaction was complete and the ratio of **(66)** to **(67)** was 4 to 1 (61% total yield).

The major product **(66)** was identified as 10-deacetyl-7-epitaxol, mp 167.0-169.0°C. FABMS, m/z 834 (MNa^+), 812 (MH^+), 286. IR (KBr) 1740, 1680, 1275, 1120, 1080, 720 cm^{-1}. ^1H NMR, see Table 5; ^{13}C NMR, see Table 6.

The minor product **(67)** was characterized as 10-deacetyltaxol, mp 169.0-171.5°C. FABMS, m/z 850 (MK^+) 834 (MNa^+) IR (KBr) 1740, 1675, 1390, 1280, 1100, 1000, 725 cm^{-1} ^1H NMR, see Table 5; ^{13}C NMR, see Table 6.

4.3.2 Ozonolysis of Cephalomannine. Cephalomannine (300 mg, 0.36 mmol) in dichloromethane (20 mL) was treated with a saturated solution of ozone in dichloromethane at -78°C. The clear blue solution was stirred at -20°C for 2 h afterwhich methanol (0.5 mL) was added and the solution was hydrogenated over palladium at room temperature for 1 h. The solid was filtered off and the solvent was removed in vacuo. Analytical TLC showed one spot with a similar Rf to that of cephalomannine in EtOAc-hexane, 1:1. Analytical HPLC showed about 50% of the major product among several other compounds. The crude product was purified by preparative HPLC to yield a pure compound **(68)** in 29% yields,

mp 161.0-162.5°C. FABMS, m/z 820 (MH^+), 760 (MH^+-AcOH), 570 (MH^+-AcOH,), 552, (MH^+-AcOH-side chain at C-13-H_2O), 510 (MH^+-AcOH), IR (KBr) 1740, 1385, 1260, 1080, 720 cm^{-1}. ^1H NMR, see Table 7, ^{13}C NMR, see Table 6.

4.3.3 2', 7-Bis-(2,2,2-Trichloroethyloxycarbonyl) cephalomannine (69).

Cephalomannine (200 mg, 0.24 mmol) in dry dichloromethane (5 mL) was treated with a cold solution of 2,2,2-trichloroethyloxycarbonyl chloride (0.15 mL, 1:1 mmol) in dry dichloromethane (0.5 mL) at -25°C. Pyridine (0.5 mL) was added dropwise into the previous solution and the mixture was stirred for 2.5 h at -25°C. Water (2 mL) was added and the organic layer washed with 0.1 N HCl, 5% $NaHCO_3$, and water until the washing was neutral to litmus. The organic layer was dried over anhydrous $MgSO_4$ and then evaporated in vacuo to a yellow liquid which solidified on standing. TLC indicated that the reaction was complete and only one product was produced. The crude product was purified by flash chromatography (EtOAc-hexane, 1:1) to a white solid (137.0 mg, 48% yield), mp 153.0-154.5°C. IR (KBr) 1780, 1750, 1690, 1400, 1280, 1080, 725 cm^{-1}. ^1H NMR, see Table 7.

4.3.4 Attempted Cleavage of the N-Pyruvyl Group of the Ozonolysis Product of Cephalomannine (68).

(a) 1,2-Phenylenediamine. The ozonolysis product (68) (50 mg, 0.06 mmol) in water (1.0 mL) and methanol (0.2 mL) was stirred with 1,2-phenylenediamine (15 mg, 2.2 equivalent) at room temperature for 2 h. TLC showed that the major product had a very similar Rf value to the starting material. Preparative TLC gave the major product which showed a taxol-like structure but with the N-pyruvyl group still intact.

(b) o-Aminophenol. The ozonolysis product **(68)** (25 mg, 0.03 mmol) in water-methanol (5:1, 0.6 mL) was stirred with O-aminophenol (10 mg) at room temperature for 10 h. The solution was concentrated in vacuo, extracted with CH_2Cl_2 (30 mL) and washed with water. TLC of the residue showed a very complex pattern and no further work was carried out.

4.3.5 Attempted Ozonolysis of 2',7-bis (2,2,2-Trichloroethyloxycarbonyl Cephalomanine (69). 2',7-Bis-(2,2,2-trichloroethyloxycarbonyl) cephalomannine **(69)** was treated with a saturated solution of ozone in dichloromethane until the solution was blue. It was stirred at -78°C for 3 h and then hydrogenated over palladium as in case of the ozonolysis of cephalomannine. After usual work-up, TLC showed 3 major spots and the crude product was purified by preparative TLC. The ^1H NMR spectra of the three fractions from these separation showed very complex spectra and characterization was not possible without further purification.

4.3.6 Attempted Cleavage of N-Pyruvyl Group of the Ozonolysis Product (70) by Sodium Periodate.

2',7-Bis (2,2,2-trichloroethyloxycarbonyl) derivative of the ozonolysis product **(70)** (2 mg, 0.0024 mmol) in CH_3OH (0.2 mL) was treated with $NaIO_4$ solution (0.02 mL, prepared from $NaIO_4$ (18 mg) and 1 mL of 1 N H_2SO_4. The mixture was stirred at room temperature and was monitored by TLC. After 24 h, no new compound was detected.

CONCLUSION

Several attempted syntheses of the C-13 ester side chain of taxol were carried out in this project. The unsuccessful result of the method initially attempted is probably due to the inherent instability of the cis-phenylglycidic acid, but the desired acid was finally obtained in racemic form by a modification of a known method.

Coupling of the side chain to the C-13 hydroxyl group was difficult. Acetylbaccatin III can be produced in relatively good yield but coupling of larger side chains is more sluggish. 13-(3-phenylpropanoyl) baccatin III can be obtained by using either triethylamine or pyridine and 4-dimethylaminopyridine or by refluxing with silver cyanide in toluene. 2-Acetyl-3-phenyllactyl chloride did not couple to the 7-protected baccatin III by either method. These results indicate that the C-13 hydroxyl group is very hindered.

Attempted cleavage of the N-acyl group of taxol and cephalomannine have not yet succeeded. In the case of taxol the C-10 acetate group is sensitive to zinc bromide reagent and 10-deacetyl-7-epitaxol and 10-deacetyltaxol were produced without any cleavage of the N-acyl group. Attempts to form the iminium chloride of taxol were made but no cleavage of the N-benzoyl group was detected.

The ozonolysis product of cephalomannine was produced and preliminary investigations of the cleavage of the N-pyruvyl group have been carried out but no cleavage of the amide linkage has yet been detected.

APPENDIX A

Table 1. ^{1}H NMR Spectra of Baccatin III and Its Derivatives [a]

Protons on	15	49	52	50
C-2	5.58(d,7)	5.62(d,7)	5.60(d,7)	5.69 (d,7)
C-3	3.84(d,7)	4.15(d,7)	3.98(d,7)	3.98(d,7)
C-5	4.94(dd,2,8)	4.97(br d,9)	4.95(br d,9)	4.99(br d,9)
C-6	2.6(m),2.3(m)	2.6(m),2.3(m)	1.8,2.6(m)	2.63(m),2.3(m)
C-7	4.42(m)	5.60(dd,7,10)	5.58(dd,2,11)	5.60(dd,7,10)
C-10	6.28(s)	6.37(s)	6.24(s)	6.38(s)
C-13	4.82(br t,9)	4.82(br t,7)	4.83(br t,8)	6.18(br t,8)
C-14	2.3(m)	2.0-2.3(m)	2.2(m)	2.2-2.3(m)
C-16	1.04(s)	1.11(s)	1.11(s)	1.21(s)
C-17	1.04(s)	1.07(s)	1.05(s)	1.17(s)
C-18	1.98(s)	2.10(s)	2.03(s)	1.98(s)
C-19	1.62(s)	1.80(s)	1.77(s)	1.83(s)
C-20	4.10(d,8)	4.00(d,8)	4.14(d,9)	4.17(d,8)
	4.26(d,8)	4.31(d,8)	4.31(d,9)	4.34(d,8)
OAc	2.20(s)	2.28(s)	2.27(s)	2.16(s)
	2.24(s)	2.14(s)	2.13(s)	2.21(s)
			2.00(s)	2.35(s)
2-OBz	8.05(dd,2,8)	8.08(d,8)	8.09(d,7)	8.08(d,8)
	7.46(m)	7.60(t,8)	7.59(t,7)	7.62(t,8)
		7.47(t,8)	7.46(t,7)	7.49(t,8)
		4.62(d,12)[b]		4.65(d,12)[b]
		5.02(d,12)[b]		5.05(d,12)[b]

[a] Multiplicity and coupling constants (in Hertz) in parentheses.
[b] CH_2 protons of the 2,2,2-trichlorocthyloxycarbonyl group.
All ^{2}spectra obtained in $CDCl_3$

Table 1 (continued)

Protons on	51	53	54	55
C-2	5.64(d,7)	5.65(d,7)	5.79(d,7)	5.65(dd,1,7)
C-3	3.81(d,7)	3.95(d,7)	4.05(d,7)	3.89(dd,2,10)
C-5	4.95(dd,2,9)	4.98(d,9)	4.96(br d,9)	4.91(dd)
C-6	1.8,2.6(m)	1.8,2.6(m)	2.10,1.9(m)	2.52(m)
C-7	4.42(br t,8)	5.60(dd,7,10)	5.60(dd,11)	4.41(dd,7,11)
C-10	6.28(s)	6.25(s)	6.50(s)	6.42(s)
C-13	6.16(t d,8,1)	6.18(br t,2,9)		
C-14	2.2(m)	2.25(m)	2.7(d),2.9(d,20)	2.66,2.96(d,21
C-16	1.21(s)	1.20(s)	1.25(s)	1.20(s)
C-17	1.10(s)	1.17(s)	1.23(s)	1.16(s)
C-18	1.89(br s)	1.97(s)	2.15(s)	2.04(s)
C-19	1.65(s)	1.81(s)	1.80(s)	1.63(s)
C-20	4.14(d,8)	4.15(d,9)	4.13(d,9)	4.10(d,9)
	4.28(d,8)	4.33(d,9)	4.35(d,9)	4.30(d,9)
OAc	2.30(s)	2.04(s)	2.23(s)	2.13(s)
	2.22(s)	2.18(s)	2.24(s)	2.25/s)
	2.18(s)	2.20(s)		
		2.35(s)		
2-OBz	8.05(d,8)	8.08(t)	8.05(m)	8.05
	7.40(t,8)	7.62(m)	7.63(m)	7.61
	7.47(t,8)	7.48(m)	7.50(m)	7.47
			4.64(d,12)[b]	
			5.06(d,12)[b]	

Table 2. ^{13}C NMR Spectra of Baccatin III Derivatives

	15 (a)	49	52	50
C-1	75.3	78.7	78.5	78.7
C-2	79.2	76.7*	76.3	77.2
C-3	46.3	47.4*	47.4	47.1
C-4	81.0	80.6	80.6	80.4
C-5	84.7	83.8*	83.9	83.7
C-6	38.8	33.2	33.3	33.2
C-7	72.3	74.4*	71.6	74.3
C-8	58.8	56.2	56.0	56.1
C-9	204.4[b]	201.9	202.4	201.9
C-10	76.6[b]	75.9*	75.8	75.4
C-11	132.1	131.7	131.4	132.6
C-12	146.6	144.9	144.9	141.6
C-13	68.0	67.9*	67.6	69.5
C-14	35.7	38.5*	38.6	35.6
C-15	42.8	42.7	42.7	43.1
C-16	27.0	26.6*	26.6	26.3
C-17	20.9	20.1*	22.4	22.4
C-18	15.6	15.1*	15.1	14.6
C-19	9.5[b]	10.6*	10.6	10.6
C-20	76.4[b]	76.3*	74.4	76.2
(C=O) of	170.9	169.1	168.9	169.0
OAC	171.6	170.7	170.5	169.7
			170.5	170.1
CH_3 of OAC	20.9	22.4*	21.0	21.1
	22.6	20.7*	20.7	21.1
			20.0	20.7
(C=O) of OBz	167.3	166.9	166.8	166.8
1-benzoyl	129.6	129.2	129.3	129.1
O-benzoyl	128.8(2)	130.0(2)	130.0(2)	130.0(2)
m-benzoyl	130.3(2)	128.6(2)	128.6(2)	128.6(2)
p-benzoyl	133.9	133.7	133.6	133.7
(C=O) of TROC		153.2		153.1
$CH_2\underline{C}Cl_3$		94.5		94.5
$\underline{C}H_2CCl_3$		77.3		c

a Rojas et al. Org. Magn. Reson.1983, 21, 257-260,[89]
b Assignments can be interchanged
c Peak concealed by signal of $^{13}CDCl_3$
* Assignments confirmed by heteronuclear COSY assignment

Table 2 (continued)

	51	53	54	55
C-1	79.1	78.8	78.4	78.9
C-2	74.9*	74.5*	72.4*	72.0
C-3	45.7*	47.3*	46.4*	45.4
C-4	81.0	80.9	80.2	80.6
C-5	84.3	84.0*	83.5*	84.2
C-6	35.5	33.4	33.1	35.9
C-7	72.1*	71.4*	75.9*	73.1
C-8	58.5	56.1	56.8	59.4
C-9	203.6	202.0	199.8	201.9
C-10	75.7*	75.4*	77.2*	76.1
C-11	132.8	132.5	141.0	141.7
C-12	142.9	141.4	151.8	152.1
C-13	69.6*	69.5*	197.8*	197.9
C-14	35.7	35.6*	43.4	43.5
C-15	43.0	43.2	42.5	42.5
C-16	26.6*	26.4*	32.9	33.2
C-17	20.7	20.7*	18.1*	18.7
C-18	14.9*	14.7*	13.7*	13.8
C-19	9.4*	10.8*	10.2*	9.2
C-20	76.1*	76.5	C	C
(C=O) of	169.7	170.2	168.6*	170.1
OAC	170.1	170.2	170.1*	170.6*
	171.3	169.5		
		168.7		
CH_3 of OAC	22.5	22.2*	20.6*	20.8
	21.5	22.4	21.5*	21.7
	21.2	20.7*		
		20.7*		
(C=O) of OBz	167.0	167.0	166.6	166.7
1-benzoyl	129.2	129.3	130.0	130.0
O-benzoyl	130.1(2)	130.0(2)	130.0	130.0
m-benzoyl	128.7(2)	128.6(2)	128.7	128.8
p-benzoyl	133.7	133.7	134.0	134.0
(C=O) of TROC			153.2	
$CH_2\underline{C}Cl_3$			94.5	
$\underline{C}H_2CCl_3$			C	

a Rojas et al. Org. Magn. Reson. **1983**, 21, 257-260,[89]
b Assignments can be interchanged
c Peak concealed by signal of $^{13}CDCl_3$
* Assignments confirmed by heteronuclear COSY assignment

Table 3. ^{1}H NMR Spectra of The Coupling Products

Proton on Carbon	1	59	58
2	5.62 (d,7)	5.65 (d,7)	5.62 (d,7)
3	3.80 (d,7)	3.92 (d,6)	3.94 (d,7)
5	4.92 (dd,2,8)	4.98 (dd,8)	4.96 (dd,2,8
6	2.0 (m)	2.00,2.65(m)	2.65 (m)
7	4.33 (m)	5.55 (dd)	5.57 (dd)
10	6.26 (s)	6.30 (s)	6.29 (s)
13	6.15 (t)	5.88 (t)	5.70 (t)
14	2.50 (m)	2.00,1.35(m)	2.10 (m)
16	1.25 (s)	1.16 (s)	1.10 (s)
17	1.14 (s)	1.14 (s)	1.10 (s)
18	1.78 (s)	1.81 (s)	1.82 (s)
19	1.67 (s)	1.74 (s)	1.70 (s)
20	4.17 (d,8)	4.14 (d,8)	4.18 (d,8)
	4.27 (d,8)	4.32 (d,8)	4.22 (d,8)
OAC	2.16 (s)	2.10 (s)	2.10 (s)
	2.23 (s)	2.12 (s)	2.20 (s)
2-OBz	8.11 (dd)	8.06 (m)	8.12 (m)
	7.40 (m)	7.62 (m) 7.50(m)	7.62 (m) 7.52(m)
2′	4.71 (d,3)	2.60-3.0(m)	
3′	5.72 (dd,3,9)	3.60 (m)	2.70-2.90(m)
3′pH	7.40 (m)	7.25 (m)	7.20 (m)
3′-NH	7.0 (d,9)		
3′-NBz	7.70 (dd)		
	7.70 (m)		
CH_2 (TROC)		4.63 (d,12)	4.65 (d,12)
		5.05 (d,12)	5.05 (d,12)

Table 3 (continued)

Proton on Carbon	62	60	61
2	5.98 (d,10)	5.67 (d,7)	5.62 (d,7)
3	3.25 (d,10)	3.93 (d,7)	3.78 (d,7)
5	4.94 (d,9)	4.97 (dd,9)	4.94 (dd,2,9
6	2.10,2.78(m)	2.0,2.65(m)	2.53 (m)
7	5.50 (dd)	5.57 (dd 7,10)	4.40 (dd 7,
10	6.24 (s)	6.35 (s)	6.24 (s)
13	4.73 (t)	6.21 (t,8)	6.17 (br t,2
14	2.10,2.50(m)	2.20, (m)	2.17 (m)
16	1.75 (s)	1.22 (s)	1.20 (s)
17	1.73 (s)	1.17 (s)	1.17 (s)
18	1.94 (s)	1.87 (s)	1.75 (s)
19	1.92 (s)	1.81 (s)	1.64 (s)
20	4.13 (d,9)	4.15 (d,8)	4.12 (d,8)
	4.50 (d,9)	4.32 (d,8)	4.28 (d,8)
OAC	2.15 (s)	2.15 (s)	2.19 (s)
		2.22 (s)	2.21 (s)
2-OBz	7.98 (m)	8.10 (dd,1,8)	8.04 (dd)
	7.68 (m)	7.67 (m)	7.60 (m)
	7.52 (m)	7.51(m)	7.47(m)
2'		2.73 (m)	2.73 (m)
3'		3.05 (m)	3.02 (m)
3'pH		7.27 (m)	7.25 (m)
3'-NH			
3'-NBz			
CH_2 (TROC)	4.65 (d,11)	4.64 (d,12)	
	4.84 (d,11)	5.05 (d,12)	

Table 4. ^{13}C NMR Spectra of The Coupling Products

	(60)	(61)
C-1	78.8	78.1
C-2	74.3*	75.0
C-3	47.0*	45.8
C-4	80.7*	79.3
C-5	83.7*	84.4
C-6	33.2*	35.6
C-7	76.2*	72.2
C-8	56.1	58.6
C-9	201.5	
C-10	75.4*	75.7
C-11	132.5	133.7
C-12	141.7	143.0
C-13	69.5*	69.3
C-14	36.0*	36.1
C-15	43.1	43.1
C-16	26.3*	26.8
C-17	20.7*	20.8
C-18	14.6*	14.5
C-19	10.6*	9.5
C-20	a	76.2
CO(OAc)	169.0	
	169.6	
CH_3(OAc)	20.7*	21.5
	22.2*	22.5
CO(OBz)	166.5	167.0
1-benzoyl	126.5, 128.2	126.6,128.3
O-benzoyl	128.6,129.1*	128.7,130.1
m-benzoyl	130.0*	
p-benzoyl	133.7	above
1′	172.2	171.2
2′	30.9*	31.0
3′	35.6	35.7
3′-Ph	--above	above
3′-NH	--	--
3′-NBz	--	--
CO(TROC)	153.2	
$CH_2\underline{C}Cl_3$	94.5	
$\underline{CH}_2CCl_3$	77.2*	

* Peak assignments by Heteronuclear COSY assignments

a Peaks concealed by $CHCl_3$ peaks

Table 5. ^1H NMR Spectra of Products from Taxol and $ZnBr_2$ Reactions

Proton on Carbon	(66)	(67)
2	5.75 (d, 5)	5.68 (d, 7)
3	3.92 (d, 5)	3.90 (d, 7)
5	4.90 (dd, 4, 8)	4.94 (dd, 2, 9)
6	2.3 (m)	
7	3.68 (dt)	4.25 (m)
10	5.40 (s)	5.21 (s)
13	6.25 (t, 8)	6.18 (t, 9)
14	2.35 (m)	
16	1.19 (s)	1.18 (s)
17	1.08 (s)	1.00 (s)
18	1.74 (s)	1.78 (s)
19	1.72 (s)	1.72 (s)
20	4.40 (s)	4.21 (d, 9)
		4.31 (d, 9)
OAC	2.5 (s)	2.38 (s)
2-OBz	8.20 (dd)	8.14 (dd)
	7.75 (m)	7.75 (m)
	7.50 (m)	7.50 (m)
2′	4.80 (d, 2)	4.80 (d, 3)
3′	5.80 (dd, 2, 8)	5.77 (dd, 3, 9)
3′pH	7.40 (m)	7.40 (m)
3′-NH	7.08 (d, 8)	7.18 (br s)
3′-NBz	7.60 (m)	7.60 (m)
	7.40 (m)	7.40 (m)

Table 6. ^{13}C NMR Spectra of Products from Attempted Cleavage Reaction

	(1)	(66)	(67)	(9)	(68)
C-1	78.9	79.2	78.6	79.0	78.9
C-2	75.5	75.4*	74.5*	75.6*	**
C-3	45.7	40.3*	46.4*	45.7*	45.7*
C-4	81.0	82.1	81.1	81.2	81.2
C-5	73.2	82.6*	84.1*	84.4*	84.3*
C-6	35.6	35.3*	35.9*	35.7	35.5
C-7	76.3	75.9*	72.3*	73.3*	72.2*
C-8	58.4	57.3	57.6	58.6	58.5
C-9	203.4	215.0	211.2	203.7	203.4
C-10	75.2	77.8*	74.8*	72.3*	75.5*
C-11	133.7	137.8	137.9	138.0	137.1
C-12	141.7	137.8	138.1	142.1	141.8
C-13	75.5	72.4*	71.9*	72.2*	74.9*
C-14	35.6	36.3	36.8	35.7*	35.5*
C-15	43.1	42.5	43.0	43.2	43.1
C-16	26.6	26.0*	26.5*	26.9*	26.8*
C-17	21.3	20.6*	20.6*	21.9*	22.5*
C-18	14.6	16.7*	14.2*	14.8*	14.8*
C-19	9.2	14.3*	9.8*	9.6*	9.5*
C-20	71.8	77.7	**	76.0*	72.1
CO(OAc)	170.2	172.4	170.4	170.4	170.3
	170.8			171.2	171.1
CH_3(OAc)	20.5	22.4*	22.5*	20.8*	20.7*
	21.9			22.6*	21.7*
CO(OBz)	167.2	167.1	166.9	167.0	167.1
CO(NBz)		167.1	167.2	167.0	167.1
1-OBz	138.1				
O-OBz					
M-OBz		126.8-135.7	127.0-133.6	127.0	126.9-133.7
p-OBz	133.4				
C1′	172.5	172.6	172.6	171.2	171.9
C2′	84.3	73.2*	73.2*	75.0*	73.2*
C3′	54.8	54.9*	55.0	54.9*	54.9**
1′′				167.0	159.8
2′′				133.7	196.2
2′′CH_3				13.9	
3′′				133.9	24.4

* Peak assignments by Heteronuclear COSY

** under $CHCl_3$ signal

Table 7. ^{1}H NMR Spectra of Products of Cephalomannine

Proton	(9)	(68)	(69)
2	5.65(d,7)	5.68(d,7)	5.70(d,7)
3	3.76(d,7)	3.78(d,7)	3.97(d,7)
5	4.91(dd,2,9)	4.93(dd,2,9)	4.97(dd,2,9)
6	2.25(m),2.50(m)	2.53(m)	
7	4.37(m)	4.40(dd,7,11)	5.58(dd,8,11)
10	6.25	6.28(s)	6.36(s)
13	6.18(br t,8)	6.21(t,9)	6.25(br t,9)
14	2.52(m)		2.62(m)
16	1.22(s)	1.25(s)	1.24(s)
17	1.12(s)	1.15(s)	1.18(s)
18	1.77(br s)	1.80(s)	1.98(s)
19	1.65(s)	1.68(s)	1.84(s)
20	4.17(d,8)	4.17(d,9)	4.18(d,8)
	4.27(d,8)	4.30(d,9)	4.32(d,8)
OAc	2.22(s)	2.25(s)	2.16(s)
	2.34(s)	2.35(s)	2.46(s)
2-OBz	8.12(dd,1,7)	8.10(dd,2,8)	8.12(m)
	7.55(m)	7.62(t)	7.62(m)
		7.50(t)	7.52(m)
2′	4.69(dd,3,5)	4.68(d,5)	5.47(d,3)
3′	5.57(dd,3,9)	5.49(dd,12,5)	5.90(dd,3,10)
3′-Ph	7.38(m)	7.40(m)	7.38(m)
3′-NH	6.50(d,9)	7.80(d,9)	6.50(d,10)
3′-NBz			
2′′	1.68(s,CH_3)		1.82(s,CH_3)
3′′	6.40(dd,1,7)	2.39(s,CH_3)	6.43(dd,2,7)
4′′	1.75(d,1,7)		1.74(d,7)
CH_2 (TROC)			4.64(d,12)
			5.04(d,12)
			4.78(dd,12,14)

APPENDIX B
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