THE CHEMISTRY OF CEPHALOMANNINE

by

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Dissertation submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Chemistry

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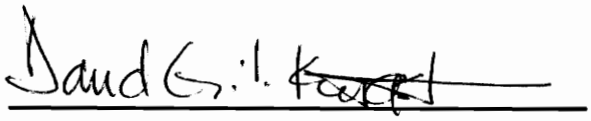

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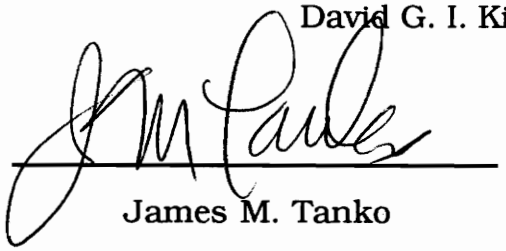

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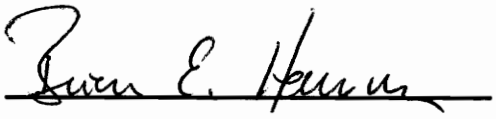

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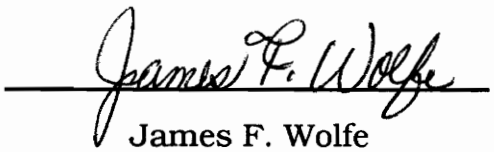

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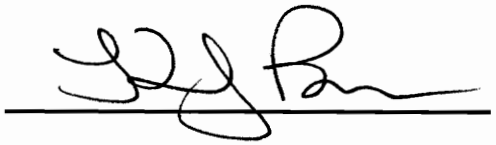

Karen Brewer

June, 1995

Blacksburg, Virginia

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Department of Chemistry

(ABSTRACT)

Cephalomannine is a naturally occurring taxane diterpenoid closely related to the potent anticancer agent Taxol. Three aspects of its chemistry were examined. First, cephalomannine was converted to Taxol. This conversion was accomplished by the reaction of a 2'-benzoyl-7-Troc cephalomannine/Taxol mixture with oxalyl chloride to generate a common oxamic acid intermediate. Treatment of this intermediate with diphenylcarbodiimide cleaved the N-oxalyl group which resulted in a spontaneous transfer of the 2'-benzoyl group to the 3'-N position. Deprotection of the 7-Troc group afforded Taxol. Second, a number of 3'-N-acyl cephalomannine and Taxol analogs were prepared and their biological activity determined. The N-tigloyl group of cephalomannine was modified by oxygenation and halogenation to yield several cephalomannine derivatives. The Taxol analogs were prepared by coupling a protected side chain to baccatin III, deprotecting, and acylating the resulting free amine. This methodology was used to prepare several oxalyl and halogenated analogs as well as N-(phenylglyoxyl) and N-crotonyl derivatives. One

derivative in particular, N-debenzoyl-N-(2"-bromopropenoyl)taxol, was found to be significantly more active than Taxol. Third, Taxotere, 10-acetyltaxotere, N-debenzoyl-N-(phenoxyacetyl)taxol, and the cephalomannine diol were synthetically prepared for testing in several tubulin polymerization systems. Earlier studies had shown that some Taxol analogs had the ability to stabilize tubulin polymers to cold, but failed to induce assembly as does Taxol. The compounds prepared were used to investigate the differences and this led to the conclusion that the hypernucleation of tubulin assembly and polymer stabilization observed with Taxol represent two distinct properties of the drug.

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to my advisor, Dr. David G. I. Kingston, for his support, encouragement, and wise advice during this work. I am especially thankful for his patience throughout the process. I would also like to thank each of my committee members for their ideas, suggestions, and corrections.

I thank Bristol-Myers Squibb for the cephalomannine sample used in this work and for their financial support. I gratefully acknowledge the assistance of the staff of the Midwest Center for Mass Spectrometry for mass spectral determinations.

I am very grateful for the assistance of Dr. John Rimoldi and Dr. Mahindra Chordia in obtaining last-minute spectra. I also wish to thank Dr. Leslie Gunatilaka, Dr. Milind Gharpure, Dr. Ashok Chaudhary, and Dr. Carl Heltzel for helpful discussions during the course of this work.

Special thanks are due to my parents, Dr. and Mrs. Donald P. Molinero, for their support and guidance. Finally, but not least, I owe a great deal to my wife, Rhonda, and my kids, Amy, Christine, David, and Peter, for their continual encouragement, support, and prayers throughout this time.

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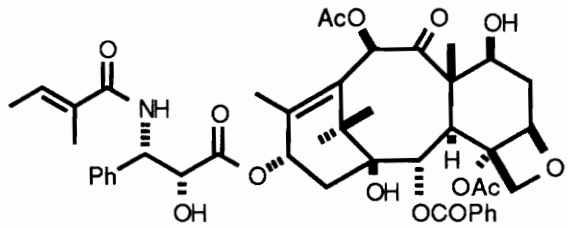
1. Introduction

In 1979 Powell and coworkers were involved in a project designed to isolate new antitumor agents from plant sources. From the roots and stem of a coniferous tree found in the Shillong Forest of India, they isolated a new alkaloid which they called cephalomannine (**1.1**).[1,2] Using a combination of spectral, chemical, and X-ray crystallographic methods its structure was determined and cephalomannine was found to belong to a class of natural products known as the taxane diterpenoids. At the time of its discovery, cephalomannine was noted to possess very potent cytotoxic and antileukemic properties. Interestingly, in spite of its activity, cephalomannine has remained just another "novel compound."

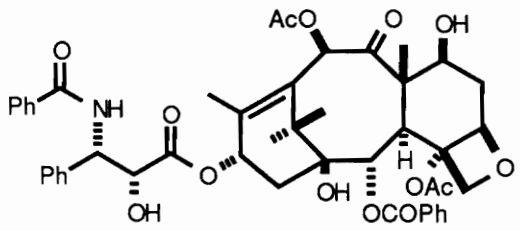


Cephalomannine (**1.1**)

The primary reason for the lack of interest in cephalomannine is the fact that Taxol®* (**1.2**), a closely related and significantly more active

* The name Taxol has recently become a registered trademark. The generic name is now paclitaxel. However, the name Taxol has been in common use for over twenty years and is used herein for purposes of clarity.

taxane, was reported eight years earlier.[3] Structurally these two compounds are almost identical. A detailed examination will reveal that their structures differ only in the nature of the 3'-N-acyl group. At this position, cephalomannine has a N-tigloyl group while Taxol has an N-benzoyl group. Although this difference would seem minor, Taxol is almost twice as active as cephalomannine in test systems, and as a result, research in this area has focused almost exclusively on Taxol.



Taxol (**1.2**)

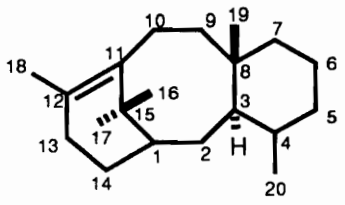
An examination of the literature will show very little has been published on the chemistry of cephalomannine. In contrast, the remarkable potential of Taxol as an anticancer agent has stimulated an intense effort around the world to uncover the secrets of its activity. A large number of structure-activity studies have been published[4-10] while an even larger number remain unpublished due to proprietary reasons. These studies have defined much of the chemistry of Taxol and because of the structural similarity, the chemistry of cephalomannine.

The one area of the chemistry of cephalomannine that remains largely untouched is that of the 3'-N-tigloyl group. Although at first, the chemistry of the tigloyl group may seem insignificant, further thought brings rise to three very interesting questions. First, is there a simple

way to cleave the tigloyl group and introduce a benzoyl group? If this could be achieved, then cephalomannine could quickly be converted to Taxol. Second, is there any simple way to modify the tigloyl in a manner that would create a taxane diterpenoid even more potent than Taxol? Third and finally, how does the tigloyl group affect the mode of action of the "Taxol-like" anticancer agents? In light of the fact that cephalomannine is less active that Taxol, the 3'-N-acyl group must play some significant role.

1.1. Historical Background

The taxane diterpenoids comprise a novel group of natural products that with only a few exceptions share the tricyclic skeleton (**1.3**).[11-12] In recent years, the number of taxane diterpenoids has grown



1.3

to over 100 and almost without exception they are found in the various members of the genus *Taxus* or closely related genera.[13] Shrubs and trees belonging to the *Taxus* genus are evergreen Gymnosperms of the order *Taxales* and family *Taxaceae*. Better known as yews (the scientific name, *Taxus*, being the Latin word for yew), they can be found

throughout the northern temperate zones of Asia, India, Europe, North Africa, and North America.[14]

Among trees, yews are noted for their unique combination of traits.[14] They are very slow growing evergreens with extremely long life spans. One tree is thought to be over 3,000 years old.[14,15] Careful examination will reveal that the individual trees are either male or female. Although the bark is paper thin, the wood is a very pliable, workable hardwood. The entire tree is known to be poisonous except for the fleshy red aril or "berry" surrounding the seed. Because of these characteristics, the yew has a rich and varied history.

Almost from the beginning of civilization yew wood has been prized for its use in making weapons, tools or other critical implements.[14] Greek, Roman, and European archers sought the wood of the yew to make their best and strongest bows. Even the bow of the famous hero Robin Hood was said to be made of yew wood.[14] On the other side of the world, Indians of the Pacific Northwest for millennia used yew wood to create salmon spears, whale harpoons, bows to hunt elk and deer, as well as basic digging sticks and pry bars to harvest shell fish at low tide. Legends from many other countries indicate a tremendous demand for yew wood.

Interest in the constituents of the yew are equally as old. The juice or oil extracted from the crushed leaves, bark, or seeds of the yew was prized as a deadly poison, almost rivaling hemlock.[16] Perhaps the earliest recorded case of human poisoning by yew is found in Caesar's *De Bello Gallico* where he notes that Catavolcus, king of Eburones,

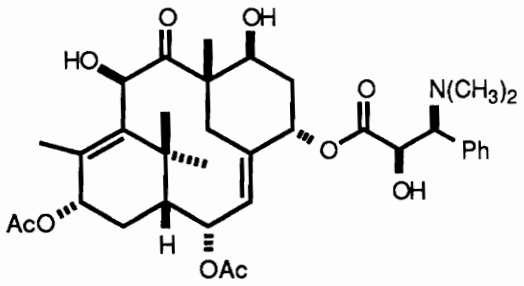
committed suicide after some disgrace by drinking yew juice.[13,17] Other interesting accounts of its deadly nature include Virgil's belief that Corsican honey was either tainted or poisoned by yew pollen[16] and Pliny's description of how the juice which he referred to as *toxica* was used to tip arrows in order to make them deadly.[14]

In light of the value placed on the wood as well as the poisonous nature of the tree, it is not surprising that over the centuries a poetic and mythic image of the yew grew.[14] Because of its extreme longevity and its use for weapons and poison, the yew became associated with death and the after life for many European cultures. The literature is replete with references to the yew. Upon reading its history the myth and legend surrounding the yew create a tremendous sense of awe at the tree on which man has placed so much value over the ages.

Although wood for bows and juice for poison had captivated interest for centuries, it was not until 1856 that Lucas undertook the first chemical study of the species.[18] His investigation resulted in the isolation of an alkaloidal substance from *Taxus baccata* (English yew) which he named taxine. Over the next several decades, German, French, Italian, English, Swiss, and Japanese scientists all spent time working with this very mysterious and unstable substance.[13,19] In spite of their efforts, the techniques of the day were simply not sophisticated enough to solve the problem of structure determination. The most significant work published during this period was that performed by Winterstein and his colleagues. Winterstein *et. al.* assigned the empirical formula

$C_{37}H_{51}NO_{10}$ to taxine[20] and determined the structure of a fragment isolated after acidic hydrolysis of taxine.[21]

Interestingly, it appears to have been exactly 100 years after Lucas's initial discovery that Graf and coworkers first recognized that taxine was not a pure substance, but actually a mixture of alkaloids.[12,22] Graf identified three components which he called taxine A, taxine B, and taxine C. Although it took over twenty years, eventually the structures (**1.4**) and (**1.5**) were assigned to taxine A and taxine B, respectively.[23,24] The structure of (**1.5**) was later revised to (**1.6**).[25]



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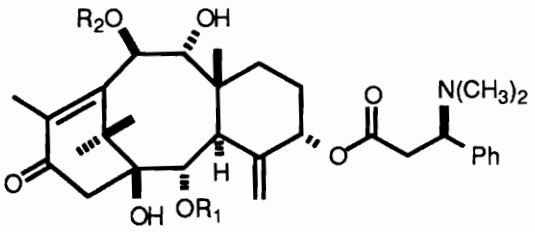
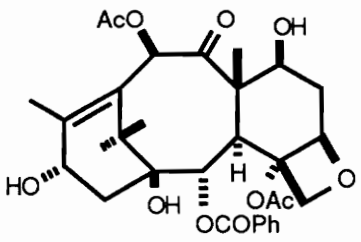


1.5 R_1 = Ac, R_2 = H

1.6 R_1 = H, R_2 = Ac

During the 1960's isolation and identification of taxanes from various yew species blossomed. Lythgoe and his collaborators published a series of ten papers describing their work on taxine I and taxine II, as well as other taxane derivatives. This work eventually lead to the structural assignments of several taxane diterpenoids which were summarized in a 1968 review article on *Taxus* alkaloids.[26]

Taylor[27] and Halsall,[28] both working with the heartwood of *T. baccata*, contributed the baccatin series of which baccatin III (**1.7**) is the most well-known. During the same time period, Japanese workers using *T. cuspidata* (Japanese yew) isolated and identified several other taxanes.



1.7

Even though the taxanes possess an unusual skeleton which in itself generates considerable interest, it is interesting to speculate where this area of natural products might have gone had it not been for Wani and Wall's discovery in the late 1960's. Working in collaboration with the National Cancer Institute, Wani and Wall at the Research Triangle Institute detected cytotoxic activity in a sample of crude alcoholic extract from the stem bark of the Pacific yew, *Taxus brevifolia*. In spite of the difficulties encountered due to the low yield, the active component was eventually isolated and its structure elucidated.[3] In retrospect, discovery of this compound, now known as Taxol, ranks as one of the most important discoveries ever made in the field of naturally occurring anticancer agents.

After Taxol's discovery research in the area of taxane diterpenoids has literally erupted. Numerous groups continue to isolate and identify new taxane diterpenoids while over 36 groups world wide have taken on

the challenge of the total synthesis of this group of compounds. Holton's synthesis of (-)-taxusin (enantiomer of the natural product) in 1988 was the first milestone in this area.[29] For almost two decades, there have been attempts at the total synthesis of Taxol, but the structural complexity and the magnitude of the synthetic challenge thwarted all efforts toward this goal until recently. This challenge has finally been met by both Holton[30] and Nicolaou.[31] A considerable number of other groups have and are continuing to pursue the basic chemistry of the taxanes, especially Taxol.[4] Reviews that cover the isolation[13], synthesis,[11, 32] clinical pharmacology,[33, 34] biochemistry,[35] and chemistry of Taxol,[4, 5, 6] or the taxane diterpenoids[13, 36] in general have appeared recently. Research in the area of taxane diterpenoids is likely to continue to be of intense interest for a number of years to come.

1.2. Taxol

Although Taxol (**1.2**) has been hailed as one of the most significant advances in cancer chemotherapy[30] and is the motivating force behind the intense research in the area of taxane diterpenoids, its beginning was not quite so spectacular. Isolated in 1971 from the western yew, *T. brevifolia*, by Wani and Wall, its structure was established as the N-benzoylphenylisoserine ester of baccatin III using a combination of chemical and X-ray crystallographic techniques.[3] Initial assays indicated Taxol had potent anticancer and antileukemic properties, showing good activity in P-388 and P-1534 leukemias as well as being highly active as

an inhibitor of WM-256 carcinosarcoma.[3] In addition, it showed considerable cytotoxicity in the 9KB assay. Wall considered it one of the most important samples they had had in a long time, but NCI felt it was simply too hard to work with and too difficult to get.[37]

In 1977 the situation changed and Taxol was finally selected for clinical development. Although biological data had been accumulating since its discovery, the decision to further develop Taxol was based primarily on the impressive activity it showed against murine B16 melanoma and MX-1 mammary tumor xenographs in nude mice.[38, 39]

Taxol received another major boost in 1979 when Horwitz and coworkers, working at the Albert Einstein College of Medicine, discovered Taxol exhibited a unique mechanism of action.[40] Their work revealed that Taxol functioned as an antimitotic agent, but in a manner distinctly different than other known antimitotic agents and by a mechanism previously unknown. This discovery led to the selection of Taxol as a new lead structure for further pharmacological studies and ultimately opened the door for clinical investigations.

Phase I clinical trials began in 1983, but were prematurely discontinued because of a high incidence of acute hypersensitivity reactions in several trials. After protocols were developed to minimize these reactions, clinical trials were continued on a variety of schedules in a effort to evaluate Taxol's effect on solid tumors and adult leukemia. The results from some of the early trials showed antineoplastic activity in several tumor types including melanoma, adenocarcinoma of unknown origin, refractory ovarian carcinoma, non-small cell lung carcinoma,

gastric, colon, and head and neck carcinomas.[32, 41-47] The studies determined the optimal dose and protocol for Phase II trials and revealed the primary dose-limiting toxicity to be neutropenia.[32]

Although severe shortages of Taxol prevented conventional, broad-scale Phase II trials, limited trials began in 1985. These early Phase II trials focused on specific neoplasms chosen on the basis of antitumor activity in preclinical and Phase I trials. The results of these trials created a great deal of excitement and catapulted Taxol to the forefront of interest as an anticancer drug. The most exciting results observed were by McGuire *et. al.*[48] in a clinical trial of 40 patients suffering from drug-refractory epithelial ovarian cancer. In this study, 11 partial responses, one pathological complete response, and seven minor responses were observed, an impressive 40% overall response rate. The fact that these responses were observed in patients that had been heavily pretreated with radiation and chemotherapy as well as patients who were considered to be resistant to cisplatin made the results particularly encouraging. The trials to date indicated that Taxol had definite clinical activity against ovarian cancer[48] and breast cancer,[49] and possible activity against non-small cell lung cancer and head and neck cancers.[32]

In spite of the current excitement generated by its clinical activity and unique mechanism of action, Taxol has historically suffered from two major problems. The seriousness of these problems almost led to its demise as a clinically useful anticancer agent.

The first and major problem is that of supply. The primary source of Taxol since its discovery has been the bark of the western yew. Found

mainly in the rain forests of the Pacific Northwest, the yew is a slow growing understory tree that has paper thin bark. As a result, a typical 200-year old tree may be only 5 to 10 inches in diameter and will yield only around 6-10 lbs. of bark. Current isolation figures indicate that this 6-10 lbs. of bark will produce only 300 milligrams of Taxol.[4] Using this information plus the fact that the normal course of treatment requires about two grams, it can be calculated that the bark of 6 to 10 trees is required to get enough Taxol to treat a single patient. Unfortunately, current estimates indicate that there is only around four million yews and only 35 percent of these are 5 inches or greater in diameter.[14] Considering the number of cancer patients and the fact that stripping the bark is fatal to the tree, it is relatively obvious that yew bark will never be a permanent solution to the supply problem. To make matters even worse, access to the yews may soon be restricted. The majority of the Pacific yews are located in a region that is likely to be declared off limits to logging because it is the habitat of the Northern Spotted Owl, an endangered species.

The second problem is Taxol's poor aqueous solubility. This lack of solubility has made clinical formulation difficult. Current protocol is to administer Taxol as an emulsion, using Cremophor EL (polyethoxylated castor oil) as the vehicle, but this is certainly not ideal. In early Phase I trials severe allergic reactions were observed in some patients[42] and although it is not completely clear, it is thought that these reactions were primarily due to the Cremophor component rather than Taxol. Thus, the regimen became hospitalization, a pretreatment with antihistamines and

steroids to prevent hypersensitivity, and then a 24-hour infusion. While things have changed recently, clearly a more soluble analog of Taxol which would allow a simpler formulation (saline or other similar media) would be highly desirable.

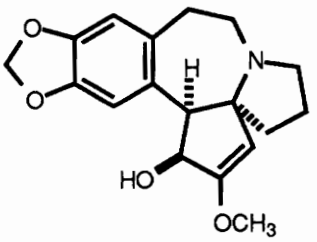
Research efforts designed to address these problems have been intense over the past few years and this intensity is likely to continue. In light of the clinical results observed so far and Taxol's potential as a anticancer agent, permanent solutions to these problems need to be found.

1.3. Cephalomannine

Cephalomannine (**1.1**) is a novel diterpenoid that is structurally identical to Taxol, except for the 3'-N-acyl group. At this location Taxol has a benzoyl group while cephalomannine has a tigloyl group. At the time of its discovery, cephalomannine, like Taxol, was found to possess potent antileukemic properties,[1] yet, in spite of this, it is not currently, or ever likely to be, a clinically useful anticancer agent. The discovery of Taxol eight years earlier plus the fact that it is significantly more active than cephalomannine in all test systems[39] has made Taxol the compound of choice. Thus, current interest in cephalomannine stems not from its own properties, but rather its structural similarity to Taxol. Furthermore, studies over the last decade have shown that cephalomannine almost always co-occurs with Taxol. These two features

make cephalomannine unique and give it the potential to solve some of the problems associated with Taxol.

Cephalomannine has an interesting history. It was first isolated in 1979 by Powell *et. al.* during a search for new antitumor agents from plant sources.[1] During the course of their investigation, Powell and coworkers encountered KB and PS activity in the alcoholic extracts of the stems and roots of a coniferous tree native to India that had been identified and shipped to them under the name *Cephalotaxus mannii.* When the active component was isolated and identified as a new taxane diterpenoid, it was somewhat of a surprise. Prior to this discovery, the taxanes had been found only in the genus *Taxus*, while other known antitumor alkaloids of *Cephalotaxus* had all been derivatives of cephalotaxine (**1.8**). In light of this novel discovery, this new taxane was

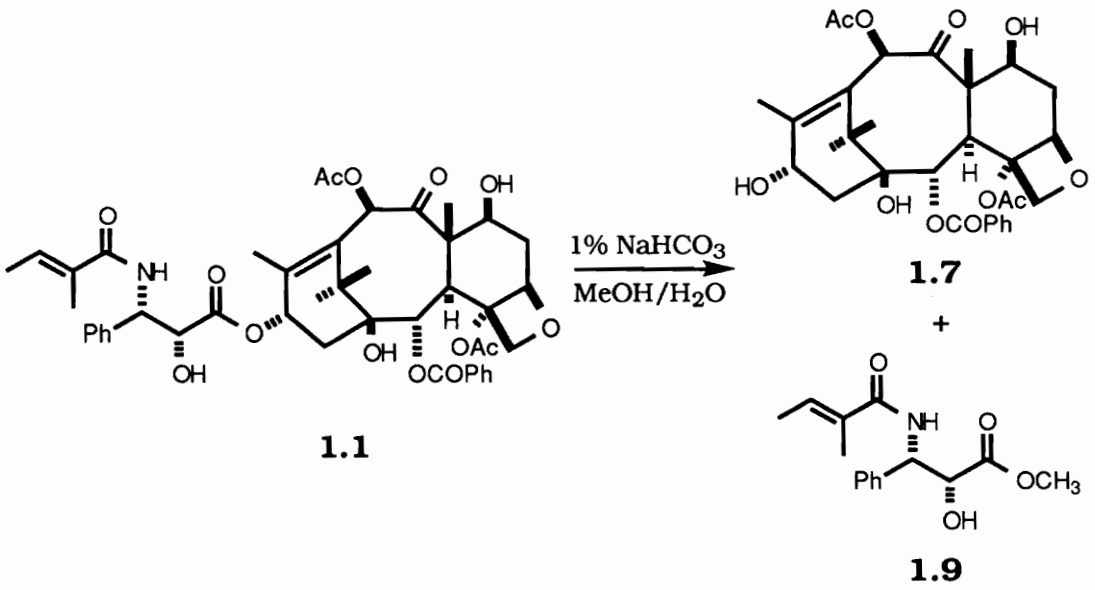


1.8

given the name cephalomannine. However, as work continued, Powell and coworkers recognized that the putative *C. mannii* contained none of the alkaloids typically characteristic of this species and, as a result, the plant was sent for reidentification and found actually to be *Taxus wallichiana.*[2] The initial misidentification was rather unfortunate since it led to a name which obscures cephalomannine's close relationship to

Taxol and also erroneously implies a relationship to the authentic *Cephalotaxus* alkaloids.[4] Although other names have been suggested,[50] the original name of cephalomannine remains the most commonly used.

The structure of cephalomannine was initially identified by spectroscopic methods and then confirmed by mild methanolysis which led to a mixture of five products, two of which were identified as baccatin III (**1.7**) and the methyl ester (**1.9**) (*Scheme* 1.1).[2] The structure of the methyl ester was established using a combination of spectroscopic methods and X-ray crystallography. The three remaining products were identified by NMR and mass spectroscopy and found to be 10-deacetylbaccatin III, 7-*epi*-baccatin III, and 10-deacetyl-7-*epi*-baccatin III.



Scheme 1.1. Methanolysis of cephalomannine.

In addition to cephalomannine, Powell and coworkers also isolated Taxol, although in lesser amounts.[2] Over the past decade, it has become readily apparent that cephalomannine almost always co-occurs with Taxol. A recent study by Wheeler *et. al.*[51] which was designed to determine the distribution and magnitude of genetic and non-genetic sources of variation in taxane content in the genus *Taxus* is of particular interest. The results of this study revealed not only that all taxa examined contained Taxol (**1.2**), cephalomannine (**1.1**), and baccatin III (**1.7**) (Table 1.1), but also that although there was great variability, on average, the cephalomannine content was greater than Taxol. Considering the scarcity of Taxol, this is a interesting discovery.

Table 1.1. Survey of Taxane Content.[a,b]

Species	Taxol	Cephalomannine	Baccatin III
	X+SE(Range)	X+SE(Range)	X+SE(Range)
Taxus baccata	0.0088± 0.0023	0.0246±0.0049	0.0285±0.0045
(*n* = 27)	(0.0008-0.049)	(0.0033-0.1237)	(0.0005-0.0899)
Taxus brevifolia	0.0081±0.0014	0.0093±0.0021	0.0466±0.0122
(*n* = 17-33)	(0.0005-0.0333)	(0.0008-0.0305)	(0.0004-0.2036)
Taxus cuspidata	0.0077±0.0015	0.0237±0.0038	0.0322±0.0050
(*n* = 40)	(0.0003-0.0390)	(0.0013-0.1146)	(0.0001-0.1418)
Taxus media[c]	0.0056±0.0008	0.0242±0.0036	0.0243±0.0026
(*n* = 90)	(0.0000-0.0513)	(0.0007-0.2225)	(0.0002-0.0963)

[a]From Reference 51. [b]Mean, standard error, and range of taxane concentration (% dry wt) for *Taxus* spp. based on fall shoot collections. [c]*Taxus baccata* by *Taxus cuspidata* hybrids.

1.4. The NMR Spectra of Cephalomannine and Taxol

Although interest in the last decade has centered on the ^1H NMR spectrum of Taxol, the proton assignments for both cephalomannine[2] and Taxol are well established.[2, 12, 52] Cephalomannine's spectrum is very similar to Taxol's and both are easy to work with since the majority of the proton signals are dispersed over a wide range of chemical shifts with only a few overlapping in the 1.5-2.5 ppm region. The assignments are shown in Fig. 1.1 and the ^1H NMR spectrum of cephalomannine is reproduced in Fig. 1.2. Although a number of investigators have reported assignments for the proton signals in Taxol, a recent publication

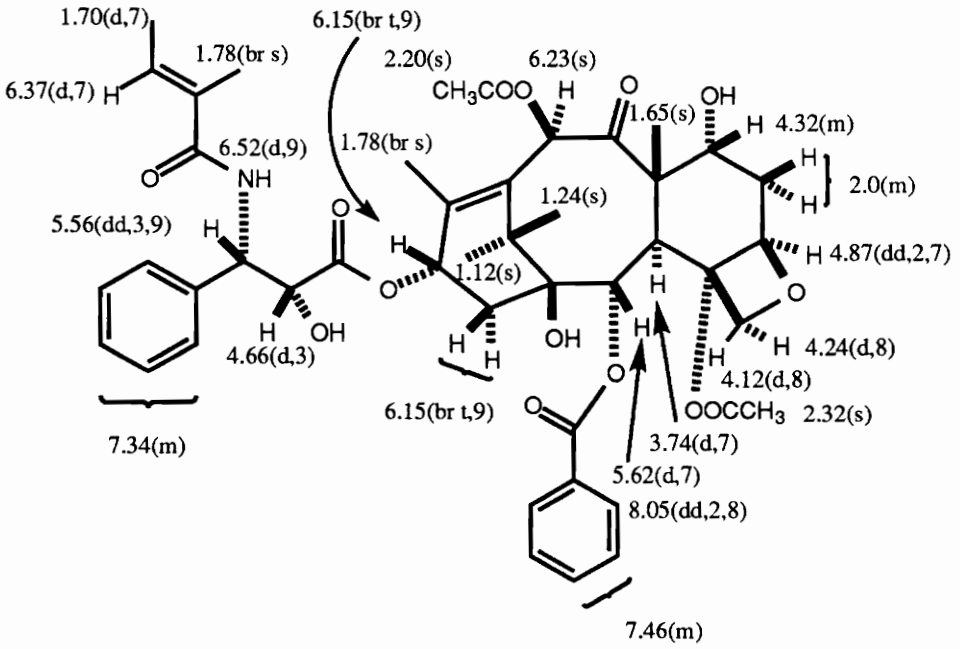


Figure 1.1. ^1H NMR assignments for cephalomannine.

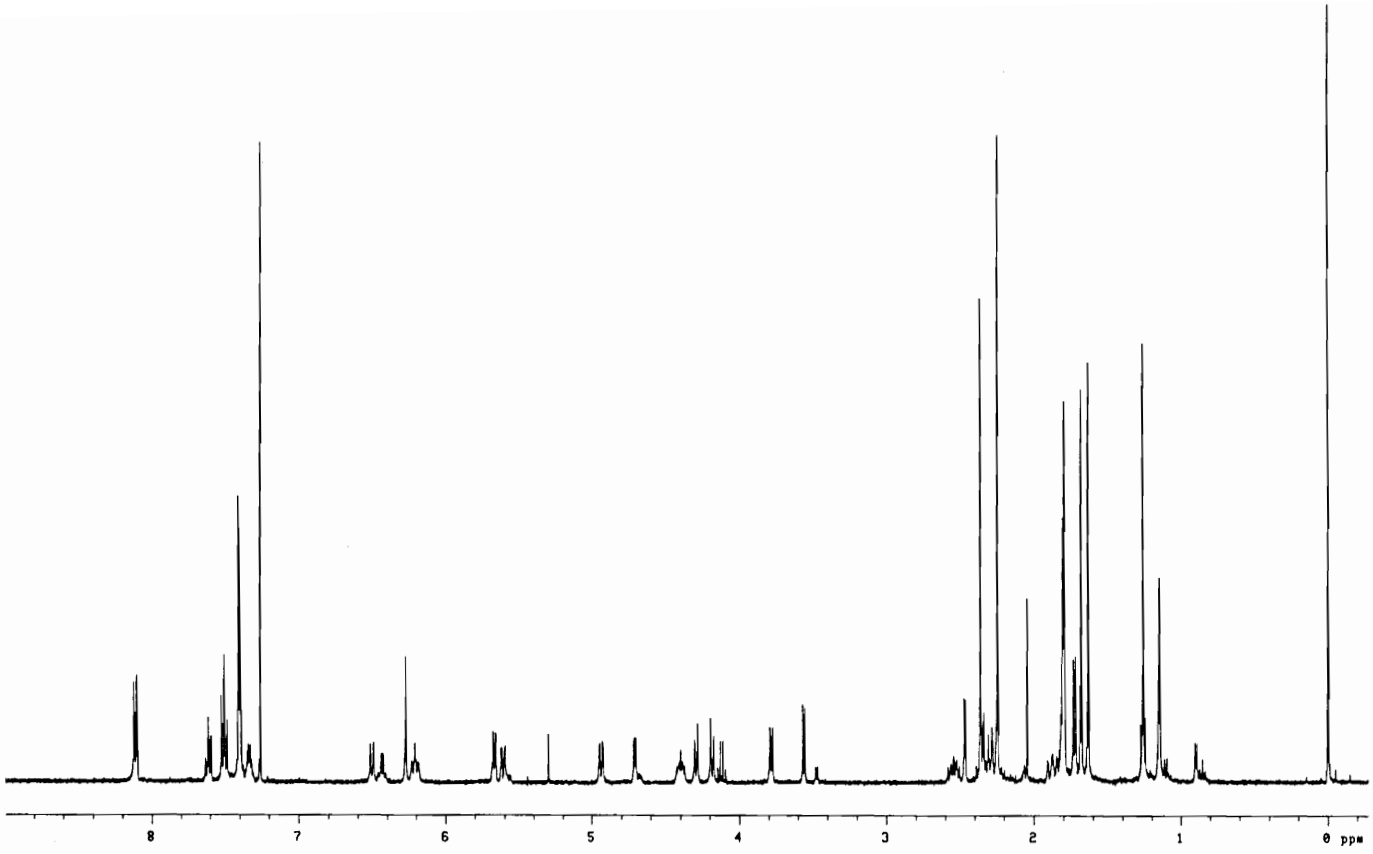


Figure 1.2. 400 MHz ^{1}H NMR Spectrum of cephalomannine in $CDCl_3$.

by Beutler *et. al.*[53] provides the most extensive analysis of the spectrum. Beutler and coworkers assignments were based on 1D, COSY, and TOCSY spectra obtained at 500 MHz. The assignments were found to be consistent with those previously made at lower fields except for a reversal in assignment of the 16-Me and 17-Me. The change was based on NOE measurements. If this is the case, then a similar reversal would be expected for cephalomannine. As a comparison, Taxol's assignments are shown in Fig. 1.3 and the ^{1}H NMR spectrum is reproduced in Fig. 1.4.

The ^{1}H NMR spectra of chemically modified or other related compounds can generally be assigned by comparison with Taxol's or cephalomannine's spectrum.

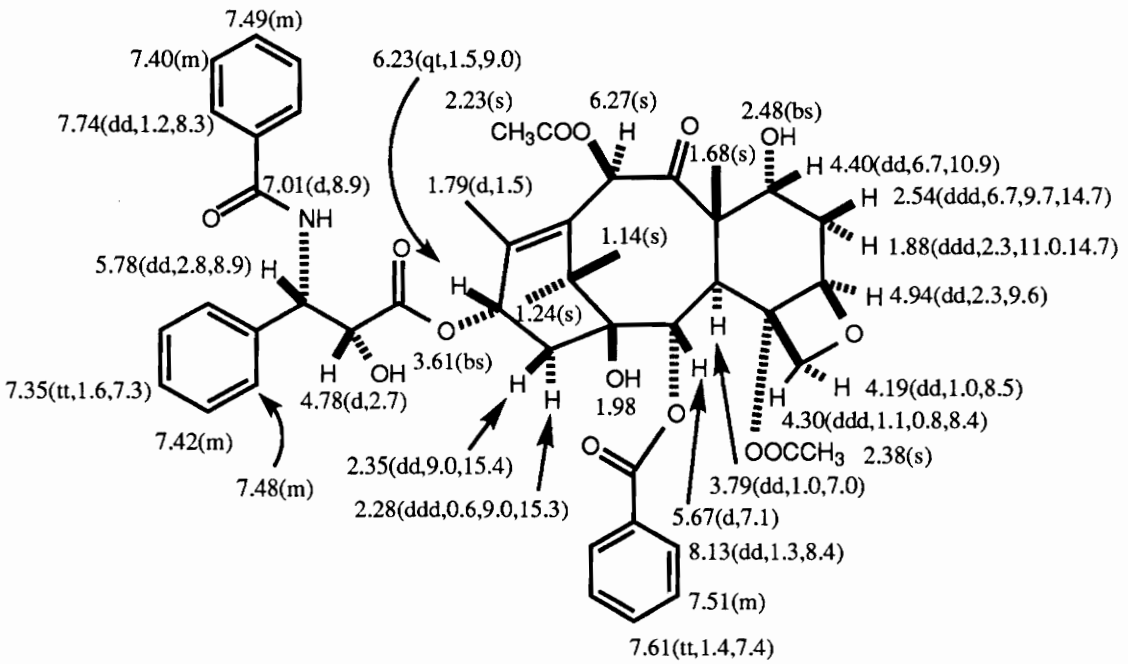


Figure 1.3. ^{1}H NMR assignments for Taxol.

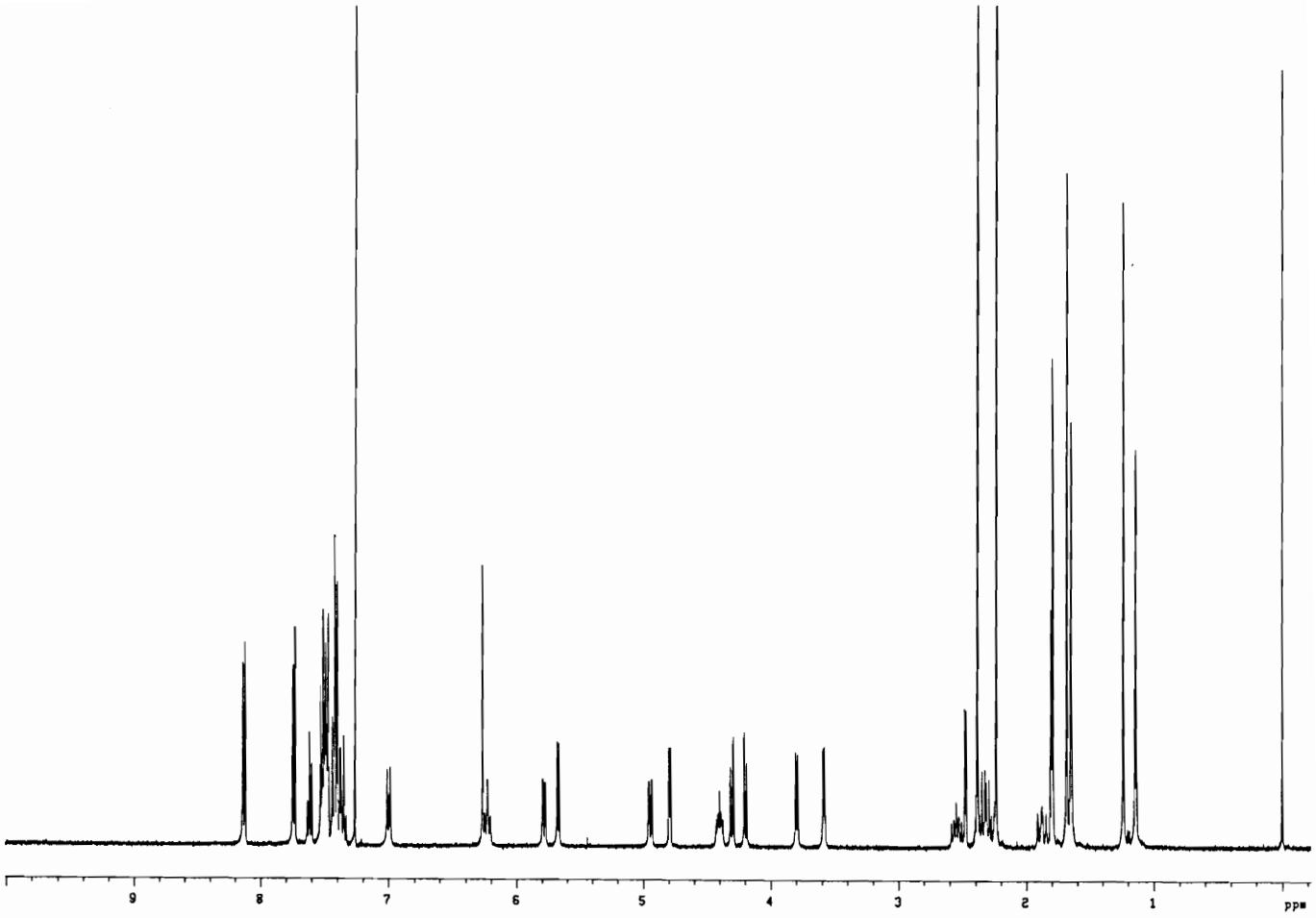


Figure 1.4. 400 MHz ^{1}H NMR Spectrum of Taxol in $CDCl_3$.

The ^{13}C NMR spectra of both are also well established.[53] The assignments for cephalomannine are shown in Fig. 1.5 and Taxol's is shown in Fig. 1.6.

1.5. The Chemistry of Cephalomannine

The biological activity of Taxol has been the driving force behind studies to determine the chemistry of the taxane diterpenoids including cephalomannine. Over the last decade, the chemistry of Taxol has been extensively studied and although some of what is known remains unpublished due to proprietary reasons, that which has been published is summed up in several recent reviews.[4, 13] Because cephalomannine and Taxol share the same skeleton, and in fact the same side chain with exception of the 3'-N-acyl group, the chemistry of Taxol is also the chemistry of cephalomannine. Since interest here is in cephalomannine and its difference from Taxol, the chemistry that they have in common will not be included. Instead, the discussion will focus on the chemistry of the 3'-N-tigloyl group since this is where the two compounds differ.

The presence of the simple double bond of the tigloyl group provides an opportunity for selective modification of cephalomannine in the presence of Taxol. Kingston *et. al.* took advantage of this by reacting a mixture of cephalomannine and Taxol with OsO_4 under mild conditions which led to a diastereomeric mixture of *cis*-diols (**1.11**) and unreacted Taxol[54] (*Scheme* 1.2). There was little worry about the Δ^{11} double bond

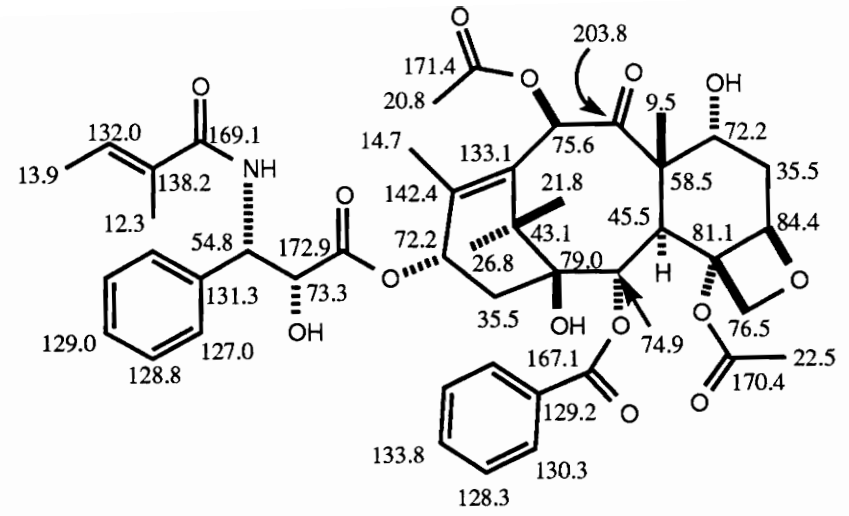


Figure 1.5. ^{13}C NMR assignments for cephalomannine.

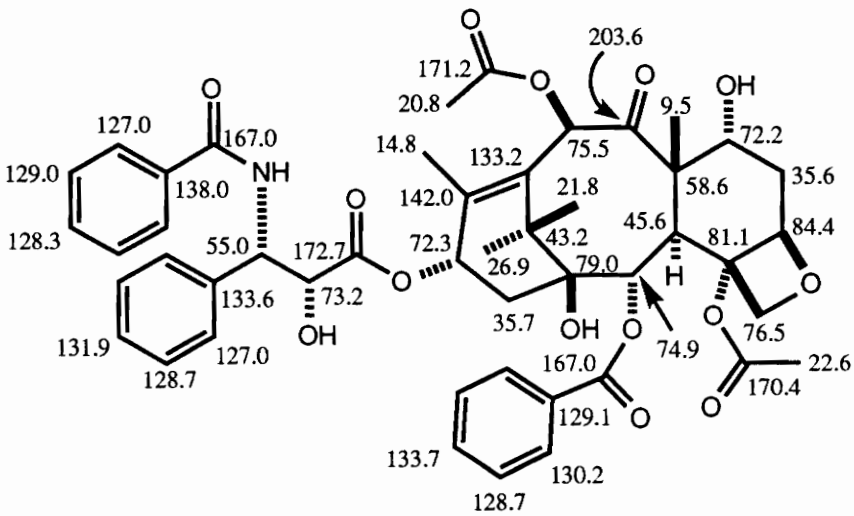
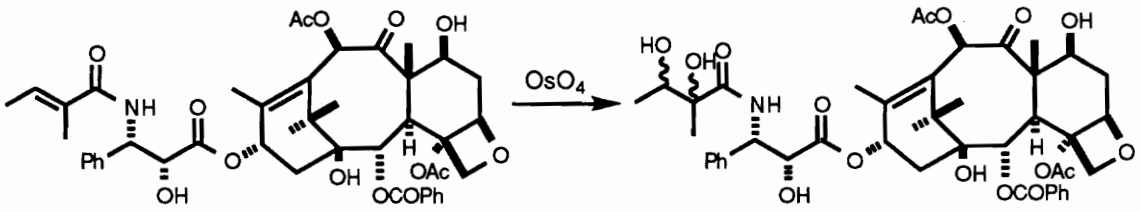


Figure 1.6. ^{13}C NMR assignments for Taxol.

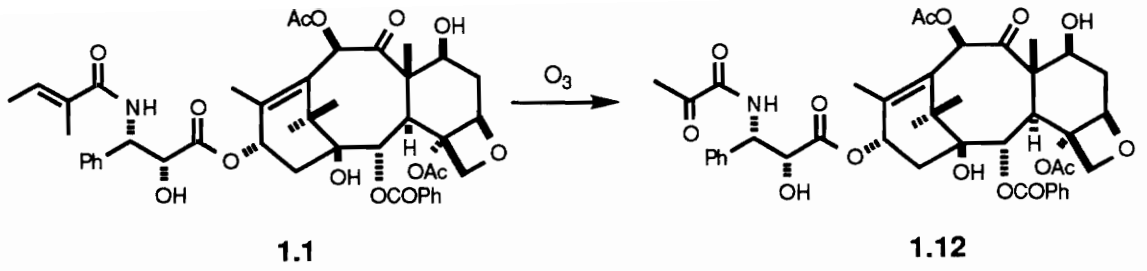
of both Taxol and cephalomannine since it is sterically hindered and therefore inaccessible to most reagents. The value of this reaction is that the resulting mixture of Taxol and cephalomannine diols could be readily separated by flash chromatography (Si gel) on large or small scale. In the past, separation was difficult, requiring either very careful chromatography under low resolution conditions or separation by HPLC. This reaction could also be run by using a catalytic amount of OsO_4 in the presence of *t*-BuOOH and $(C_2H_5)_4N(O_2CCH_3)$.



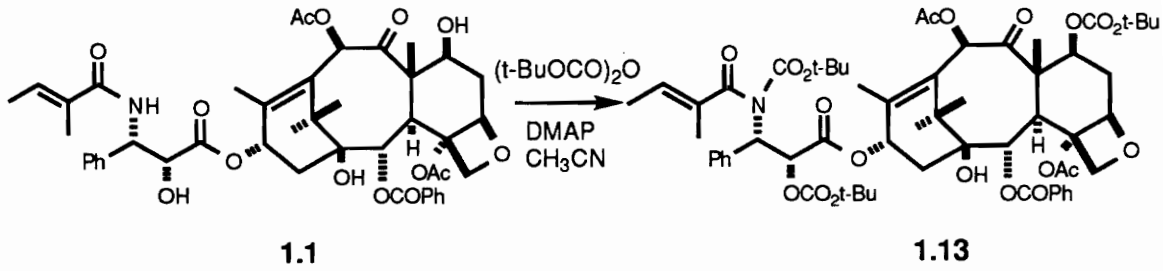
Scheme 1.2. Cephalomannine *cis*-diols.

As part of an attempt to convert cephalomannine to Taxol, Jitrangsi prepared ketoamide (**1.12**) by ozonolysis[55] (*Scheme* 1.3). This same compound could be prepared by formation of the cephalomannine diols (**1.11**) followed by oxidative cleavage using $NaIO_4$.

During the course of an investigation aimed at cleaving the 3'-N-tigloyl group, Gunatilaka discovered that tri-*t*-Boc cephalomannine (**1.13**) could be prepared using di-*tert*-butyl dicarbonate[56] (*Scheme* 1.4).

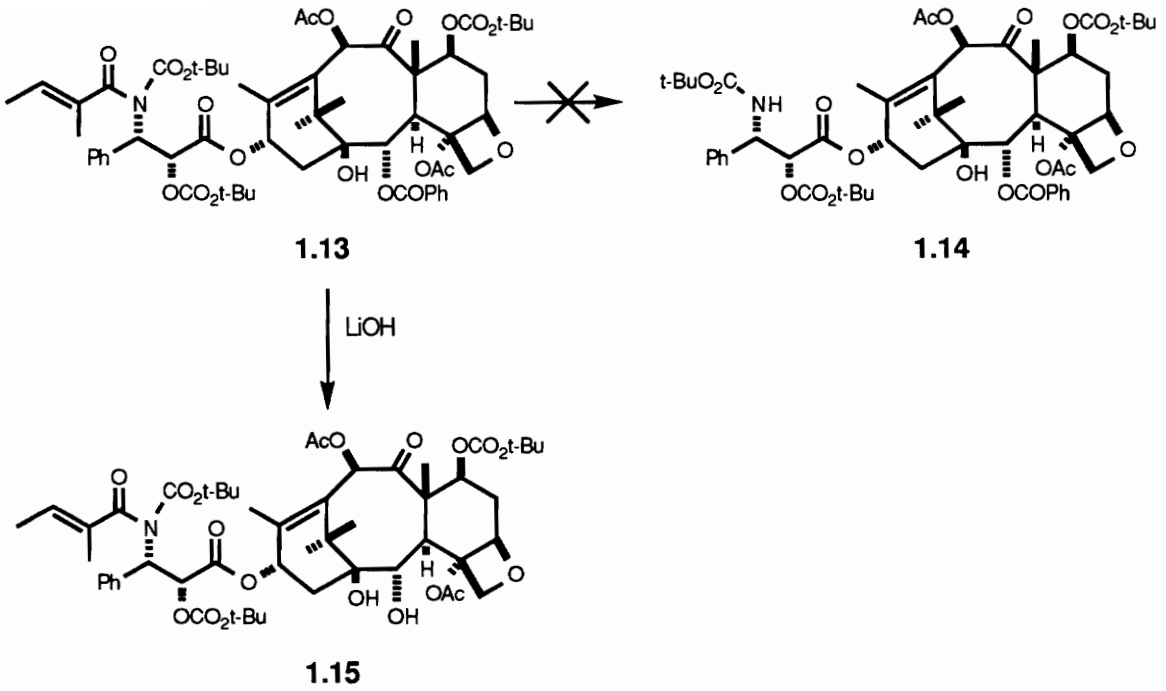


Scheme 1.3. Ozonolysis of cephalomannine.



Scheme 1.4. Formation of tri-*t*-Boc cephalomannine.

Although the reaction at the 2' and 7 positions took place in several hours, the reaction required extended time periods (7 days) since the formation of the N-*t*-Boc derivative was extremely slow. An attempt to cleave the N-tigloyl group using lithium hydroxide failed to give the desired product (**1.14**), but led to 2-debenzoyltri-*t*-Boc cephalomannine (**1.15**) instead (*Scheme* 1.5).



Scheme 1.5. Attempted hydrolysis of tri-*t*-Boc cephalomannine.

1.6. Structure-Activity Relationships of C-13 Side Chain Analogs

Since Taxol is the most active of the taxane diterpenoids, it is the standard and each new analog, whether naturally occurring or synthetic, can be viewed as a "Taxol" analog, including cephalomannine. As with its chemistry, Taxol's structure-activity relationships have been extensively studied and have recently been reviewed.[4, 6] Although hundreds of analogs, encompassing almost every position, have been prepared, proprietary reasons have kept much of this information unpublished and therefore, unavailable. Even so, much is known and several important conclusions can be drawn. Since interest here is on

cephalomannine and cephalomannine differs from Taxol only by the structure of the side chain, the discussion will focus primarily on side chain analogs. The structure-activity studies of skeletal analogs will be briefly mentioned, but any reader interested in these analogs is referred to the previously cited reviews for complete details.

Any discussion of structure-activity relationships is complicated by the fact that various investigators have used a variety of different assays to assess the biological activity of the analogs. In addition, even when the same assay is used the numerical value obtained can vary depending on the exact conditions of the assay. Because of this, the bioactivity data presented herein is the activity relative to Taxol. The different assays used can be grouped into three major classes: microtuble assembly assays, mammalian cell culture toxicity, and assays in the mouse. Of these three, the first two are more common and therefore, the third class will not be included here.

Microtuble assembly assays are based on the fact that Taxol is known to promote the assembly of tubulin to microtubules and then stabilize them against depolymerization. This process is unique to Taxol, since other antimitotic agents such as colchicine, podophyllotoxin, and the vinca alkaloids are known to inhibit microtubule assembly. These type of assays are performed either by measuring the initial rate of microtubule assembly or by the initial rate of disassembly.

The cytotoxicity of Taxol derivatives in mammalian cell cultures has generally been determined using KB cells (human carcinoma of the

nasopharynx), J774.2 cells (a mouse macrophage-like cell line), or P388 cells (a mouse lymphocytic leukemia cell line).

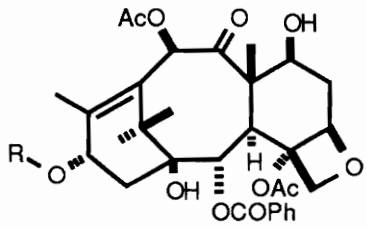
1.6.1. Structure-Activity Relationships of the Side Chain.

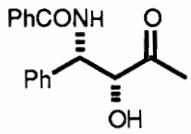
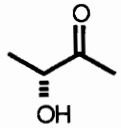
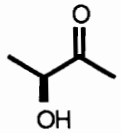
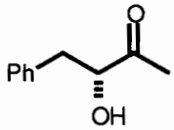
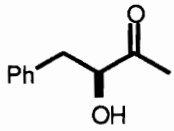
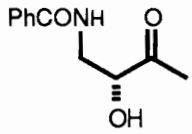
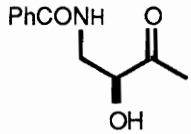
Early on it was established that the C-13 side chain was essential for activity. Thus compounds such as Taxol and cephalomannine are active, while those lacking the side chain such as baccatin III and decinnamoyl taxinine J are inactive. Exactly why this is true and what role the side chain plays is less clear, but since the side chain is relatively easy to work with there have been several investigations that focus on this question.

1.6.1.1. Swindell's Deletion Analogs

One of the most interesting studies in this area was published by Swindell *et. al.* in 1991.[7] This report described the preparation and biological evaluation of six side chain analogs in which one or more functional groups had been deleted (Table 1.2). The results indicated that even with major structural changes some activity was still observed, but certain features definitely enhanced activity. The most notable deletion was the phenyl substituent at the terminus. Analogs that lacked this phenyl substituent show the greatest decrease in activity. The results also indicated that the 3'-amide was not required for activity, but did play a significant role when present. Interestingly there was little dependence of activity on the stereochemistry of the 2'-hydroxyl group

Table 1.2. Swindell's Deletion Analog Study.[7]



Name	R	Bioactivity: Cytotoxicity[a] EC_{50}^{rel}	Bioactivity: Tubulin Assembly[b]
Taxol (**1.2**)		1	100
Baccatin III 13-(R-lactate) (**1.16**)		300	5.6
Baccatin III 13-(S-lactate) (**1.17**)		322	6.4
Baccatin III 13-(R-3-phenyllactate) (**1.18**)		39	13.2
Baccatin III 13-(S-3-phenyllactate) (**1.19**)		38	15.7
Baccatin III 13-(R-N-benzoyl-*iso*-serinate) (**1.20**)		222	5.6
Baccatin III 13-(S-N-benzoyl-*iso*-serinate) (**1.21**)		>500	Negligible

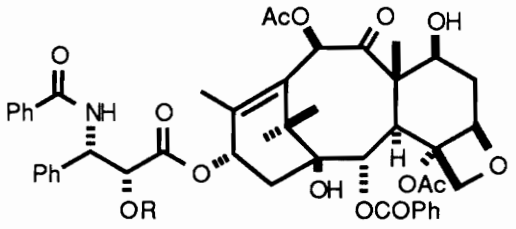
[a] EC_{50} = concentration leading to 50% inhibition in the amount of cell growth. EC_{50}^{rel} = EC_{50}/EC_{50}(Taxol) in the J774.2 cell assay. EC_{50}(Taxol) = 0.09 μM.
[b]Relative initial slope. Relative initial slope = (initial slope analog/initial slope Taxol) x 100. Initial slope (in AU/min X 10^{-2}) is measured for mammalian tubulin assembly at 35°C in the presence of 15 μM drug.

unless the 3'-amide was present. When the 3'-amide was present, the natural R configuration was strongly preferred over the unnatural S configuration. Swindell and coworkers came to the conclusion that the Taxol recognition site on microtubles must possess a hydrophobic cleft designed to accept a side chain with the functionality preorganized by stereochemistry and hydrogen bonding.

1.6.1.2. C-2' Analogs

One of the earliest positions to be studied was the C-2' hydroxyl. Although only a few analogs have been published, it is quite clear that a free 2'-hydroxyl is necessary for maximal activity.[6,57] Thus, 2'-TBDMS taxol (**1.22**) is significantly less active than Taxol[58] (Table 1.3). Interestingly, the 2'-acetyl derivative (**1.23**) is 30 times less active than Taxol in the tubulin disassembly assay, but only 2 times less cytotoxic.[57] This apparent discrepancy is almost certainly explained by the fact that the tubulin disassembly assay is performed under conditions where hydrolysis of the 2'-acetyl group is slow or nonexistent, while the longer time periods required for the cytotoxicity assay plus the presence of living cells promotes deacylation to a significant extent.[6] These results seem to support Swindell's notion that hydrogen bonding plays an important role in preorganizing the side chain in a conformation that produces maximal activity. Thus, when the 2'-hydroxyl is blocked so hydrogen bonding is not possible, activity is lost because the side chain is no longer organized in the most efficient manner.

Table 1.3. Structure-Activity Relationships at the C-2' Position.



Compound	R	Cytotoxicity[a] EC_{50}^{rel}	Ref.
Taxol (**1.2**)	H	1	
1.22	$SiBu^tMe_2$	3×10^4	58
1.23	Ac	2[b,c]	57

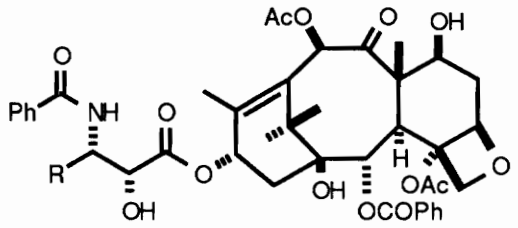
[a]EC_{50} = concentration leading to 50% inhibition of cell growth in the KB cell assay. EC_{50}^{rel} = EC_{50}(analog)/EC_{50}(Taxol). [b]This analog has an EC_{50}^{rel} = 4.3 in the J774.2 cell line. [c]This analog has an IC_{50}^{rel} of 30 in the tubulin disassembly assay. IC_{50} = concentration of drug leading to 50% inhibition of the rate of mammalian microtubule disassembly. IC_{50}^{rel} = IC_{50}(analog)/ IC_{50}(Taxol).

1.6.1.3. C-3' Phenyl Analogs

Although a large number of 3'-phenyl analogs have been made, proprietary reasons have kept the complete details of much of this data unavailable.[59-62] Introduction of substituents on the C-3' phenyl group, such as the *p*-methyl,[59, 63] *p*-hydroxy,[64] or *p*-chloro,[65,66] led to analogs with activities comparable to Taxol in the tubulin assembly assay, but which were slightly less cytotoxic (Table 1.4). When the phenyl group was completely removed, the activity drops dramatically.[7] Although complete data is not available, a recent report states that heteroaromatic C-3' Taxol analogs, such as thienyl and furyl, also possess good to

excellent activity.[59] The conclusion that can be drawn from this work appears to be that the presence of the 3'-phenyl is essential for bioactivity, but that the introduction of substituents or modification of the aromatic ring is not only tolerated, but possibly beneficial.[59]

Table 1.4. Structure-Activity Relationships at the C-3' Phenyl.



Compound	R	Tubulin Assembly[a]	Cytotoxicity[d] EC_{50}^{rel}	Ref.
Taxol (**1.2**)	C_6H_5	1	1	
1.20	H	*b*	222[e]	7
1.24	*p*HOC_6H_5	0.8	9.8[f]	64
1.25	*p*ClC_6H_5	*c*	2.2[g]	65,66

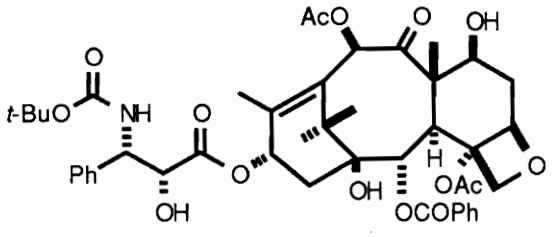
[a]Numbers are IC_{50}^{rel} values for disassembly inhibition. IC_{50} = concentration of drug leading to a 50% inhibition of the rate of mammalian microtubule disassembly. IC_{50}^{rel} = IC_{50}(analog)/IC_{50}(Taxol). [b]The initial slope was measured at 35°C in the presence of 15μM drug. The relative slope is the (initial slope analog/initial slope Taxol) x 100. The relative slope of this analog was 5.6. [c]An EC_{50}^{rel} for assembly promotion was reported. EC_{50} = concentration leading to 50% maximum absorbance increase at 350 nm in the presence of 5 μM drug. EC_{50}^{rel} = EC_{50}(analog)/EC_{50}(Taxol). EC_{50}^{rel} = 1.9. [d]EC_{50} = concentration leading to 50% inhibition of cell growth in the KB cell assay. EC_{50}^{rel} = EC_{50}(analog)/EC_{50}(Taxol). [e]Determined in the J774.2 cell line. [f]Determined in the L1210 cell line. [g]Determined in the B16-melanoma cell line.

1.6.1.4. 3'-N-Acyl Analogs

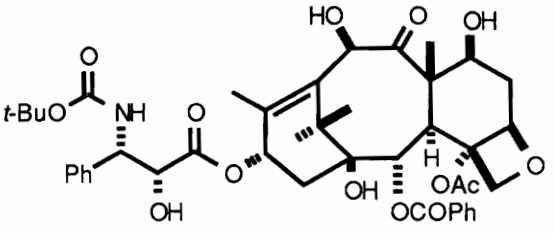
One of the most extensively studied positions has been the 3'-N-acyl group (Table 1.5). The general conclusion that has emerged is that the exact nature of the N-acyl group does not appear to be crucial. Thus, cephalomannine (**1.1**) is only slightly less active than Taxol[2, 67, 68], while N-*t*-Boc derivative (**1.28**), a synthetic analog prepared by Potier and coworkers,[69] is slightly more active.[70]

A closer examination of the data gives rise to a couple of other interesting observations. First, even though the exact nature of the N-acyl group does not seem to be really important, its removal to give the free amine (**1.26**) has a very negative effect.[70] Second, it is interesting to note that while the N-*t*-Boc analog (**1.28**) is slightly more active than Taxol, the closely related N-pivaloyl analog[71] (**1.31**) is much less active.

It should also be noted at this point, that even though the conclusion remains that the N-acyl group does not have a major bearing on the activity, the most promising synthetic analog to date fits in this category. In 1989, Potier and colleagues prepared the N-*t*-Boc taxol derivative (**1.28**) and the N-*t*-Boc-10-deacetyl analog (**1.32**). Both

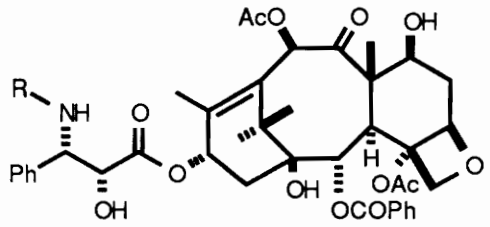


1.28



1.32

Table 1.5. Structure-Activity Relationships at the 3'-N-Acyl Group.



Cmpd	R	Tubulin Assembly		Cytotoxicity[c] EC_{50}^{rel}	Ref.
		Disassembly Inhibition[a]	Assembly Promotion[b]		
Taxol (**1.2**)	C_6H_5CO	1	1	1	
1.1	$CH_3CH{=}C(CH_3)CO$	1.5	-	1.4[d]	68
1.26	H	44	-	-	70
1.27	*p*-ClC_6H_5CO	2.4	-	1.5[e]	65,66
1.28	*t*-BuOCO	0.5	-	0.4[d]	70
1.29	$(CH_3)_2CHCH_2CO$	-	2.65	1.18[e]	71
1.30	$(CH_3)_3CH_2CO$	-	3.13	0.74[e]	71
1.31	$(CH_3)_3CCO$	-	22.4	2.61[e]	71

[a]Numbers are IC_{50}^{rel} values. IC_{50} = concentration of drug leading to a 50% inhibition of the rate of mammalian microtubule disassembly. IC_{50}^{rel} = IC_{50}(analog)/IC_{50}(Taxol). IC_{50}(Taxol) = 0.5μM. [b]ED_{50} = concentration which causes polymerization of 50% of the tubulin present in 15 min at 37°C. ED_{50}^{rel} = ED_{50}(analog)/ED_{50}(Taxol). [c]EC_{50} = concentration leading to 50% inhibition of cell growth. EC_{50}^{rel} = EC_{50}(analog)/EC_{50}(Taxol) in the indicated cell line. [d]Determined in the J774.2 cell line. [e]Determined in the B16-melanoma cell line.

derivatives showed activity slightly better than Taxol,[70] but the N-*t*-Boc-10-deacetyl analog (**1.32**), now known as Taxotere® (or RP56976), was selected for further studies because of its greater water solubility. Today, Taxotere has moved in to Phase II clinical trials[72] and has shown very promising results.[73-76] As with Taxol, the name Taxotere is a trademark

name and the USAN name for this analog is docetaxel.[77] In spite of this name change, it is still commonly referred to as Taxotere and therefore this name will be used in this document. A recent review gives an excellent description of the history of Taxotere.[5]

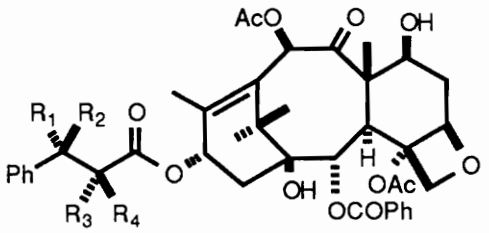
1.6.1.5. C-2'-C-3' Analogs

The focus of the work in this area has been to determine the affect of changes in the regiochemistry and stereochemistry of the 2'-OH and 3'-N-acyl group on the activity (Table 1.6). Derivatives (**1.33**) through (**1.35**) were designed to examine these changes for Taxol while derivatives (**1.36**) through (**1.38**) provide a similar comparison for 10-acetyltaxotere (**1.28**).[69, 70] The major conclusion to emerge from the data for C-2'-C-3' derivatives is that the natural regiochemistry and stereochemistry (i.e. 2'R, 3'S) results in the greatest activity. Any alteration in the regio- or stereochemistry of these positions causes a significant reduction in activity with the worst case being a change in both (Table 1.6, entries **1.35** and **1.38**). Complete removal of the 3'-N-acyl (Table 1.6, entries **1.18** and **1.19**) also has a detrimental effect.[70] It is also interesting to note that a change in the stereochemistry of the 3'-N-acyl group seems to have a bigger impact on 10-acetyltaxotere than it does on Taxol (Table 1.6, entries **1.33** and **1.36**).

1.6.1.6. Miscellaneous Side Chain Analogs

A considerable number of analogs have been made that do not fit

Table 1.6. Structure-Activity Relationships of C-2'-C3' Analogs.



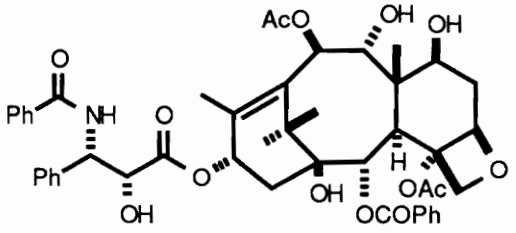
Cmpd	R_1	R_2	R_3	R_4	Tubulin Assembly[a] IC_{50}^{rel}	Cyto-toxicity[b] EC_{50}^{rel}	Ref.
1.2	PhCONH	H	OH	H	1	1	
1.18	H	H	H	OH	-	39	7
1.19	H	H	OH	H	-	38	7
1.33	H	PhCONH	H	OH	4.5	26[c]	70
1.34	OH	H	PhCONH	H	10	22[c]	70
1.35	H	OH	H	PhCONH	110	-	70
1.28	Bu^{t}OCONH	H	OH	H	0.5	0.4	70
1.36	H	Bu^{t}OCONH	H	OH	30	-	70
1.37	OH	H	Bu^{t}OCONH	H	10	-	70
1.38	H	OH	H	Bu^{t}OCONH	108	-	70

[a] IC_{50} = concentration of drug leading to a 50% inhibition of the rate of mammalian microtubule disassembly. IC_{50}^{rel} = IC_{50}(analog)/IC_{50}(Taxol). IC_{50}(Taxol) = 0.5μM. [b] EC_{50} = concentration leading to 50% inhibition of cell growth. EC_{50}^{rel} = EC_{50}(analog)/EC_{50}(Taxol) in the J774.2 cell line. [c] In the P388 cell line.

into any of the previous categories. In some cases more than one change has been made giving a series of unique compounds not easily compared with Taxol while in others side chains analogs have been prepared on ring modified derivatives.

Commerçon and colleagues recently reported the preparation of seventeen 3'-phenyl analogs of Taxotere (**1.32**).[78] The results of this study led the to the conclusion that electronic effects are not involved in the modulation of the biological activity while sterically bulky substituents such as a *p*-iodo can drastically reduce activity. Furthermore, it appeared that substituents on the ortho or para positions are better tolerated than on the meta position.

Thomas and coworkers examined a series of C-3' modified compounds using 9-dihydrotaxol (**1.39**).[79] They used a variety of different groups at the C-3' including various alkyl, alkoxy, and heteroaromatic groups. Their work revealed that for 9-dihydrotaxol



1.39

heteroatoms at the C-3' position were not tolerated, but heteroaromatic rings were acceptable. Lipophilic alkyl and alkenyl groups are allowed with increasing activity with increasing size. The best analog appeared to be a C-3' isobutyl derivative. In an M109 solid tumor trial it showed a much greater delay in tumor growth, a higher than 3-fold inhibition of tumor weight, and a significantly lower toxicity compared to Taxol.

Grampovnik *et al.* used the 9-dihydrotaxol ring system to prepare a series of 3'-N-acyl analogs.[80] In this case, they found that a variety of N-acyl groups were acceptable, although alkyl carbamates showed the best results with the *t*-butyl carbamate being the best.

Jayasinghe and coworkers prepared Taxol and Taxotere analogs with one carbon homologated side chains.[81] These analogs were found to be at least 27 times less active than Taxol in the microtubule assembly assay. It was suggested that the reduction in activity might be due to unfavorable solution conformations that ultimately prevented the necessary interactions for binding on microtubules.

A variety of other analogs that are derivatives of 10-deacetyltaxol have been prepared and the results are summarized in a recent review.[4] The conclusions that can be drawn from these analogs support the previous findings.

1.6.1.7. Solution Conformations

Although the exact binding site on microtubules and the bound-state conformation of Taxol is unknown, several investigators have examined the solution conformation in an effort to obtain information about the potentially important features of this binding site. The results of these studies indicated that in nonpolar solvents such as chloroform[53,82-84] and dichloromethane[85] the conformation appeared to be determined by intramolecular hydrogen bonding between the 1'-carbonyl, the 2'-hydroxyl, and the 3'-amide group. These interactions seemed to orient the side chain in a particular manner relative to the

rigid taxane ring system. This information contributed to the idea that the side chain was "preorganized" in this conformation before binding to the microtubules. Furthermore, the solution conformation observed for Taxol was in close agreement with the solid state conformation found in the crystal structure of Taxotere.

The picture was dramatically altered when the results of NMR and molecular modeling studies, revealed in two 1993 publications, suggested that the conformation in more polar solutions (Taxotere in methanol[86] and Taxol in DMSO/water[87]) is drastically different. Vander Velde *et. al.*[88] presented additional NMR data in support of this hypothesis and further suggested the data was consistent with a conformation where the hydrophobic clustering of the 2-benzoyl, 3'-phenyl, and 4-acetoxy groups occurred. Furthermore, they also believed that the 3'-amide group of the side chain was not involved. These drastic changes in conformation were thought to be a result of "hydrophobic collapse" and loss of hydrogen bonding in polar solvents. As a result it has been proposed that the taxane ring system is recognized first followed by additional binding interactions from the side chain, possibly by a conformation that is adopted only after the initial binding and conceivably different from any yet described.

1.6.2. Structure-Activity Relationships of the Diterpenoid Moiety.

The unique taxane diterpene skeleton possesses a plethora of functionality that provides a major challenge for those studying the structure-activity relationships of Taxol. Information in this area has

slowly been accumulating over the last decade and a clear picture is finally beginning to emerge.

One of the most interesting and earliest structural features to be studied is the oxetane ring. Kingston and colleagues found that opening the oxetane ring drastically reduced cytoxicity and tubulin polymerization activity.[89] This, plus the fact that of the over 100 known naturally occurring taxanes none that lack the oxetane are active, suggests it is required for maximal activity. It has recently been proposed that the oxetane moiety serves as a conformational lock for the taxane diterpene skeleton and the C-13 side chain.[59]

Rearrangement products of both Taxol and Taxotere which have an A-ring and B-ring contracted diterpene skeleton have been reported.[89, 90] These analogs were found to be almost as active as Taxol in the microtubule disassembly assay, but surprisingly were not cytotoxic. This finding was particular interesting since it represents a serious dichotomy between tubulin assembly activity and cytotoxicity. Other known A-ring modified analogs were found to be significantly less active.[91]

Work by Chen *et. al.* at the Bristol-Myers Squibb Pharmaceutical Research Institute indicates that the C-2 benzoyl group is essential. Thus, when 2-debenzoyloxytaxol was prepared, its ability to polymerize tubulin *in vitro* was below measurable levels while it showed only minimal *in vitro* cytotoxicity in a human colon cancer cell line.[92] In contrast, a Taxol metabolite possessing a *meta*-hydroxylated C-2 benzoyl group showed good activity in the microtubule assay.[64]

The preparation of analogs at the C-7 position have led to the conclusion that the C-7 hydroxyl group is not essential for activity. The synthesis of 7-acetyltaxol,[57, 93] 7-glutaryltaxol,[70] 7-*epi*-taxol,[94, 95] 7-deoxytaxol,[96] or analogs with a carbohydrate moiety at this position[93] resulted in derivatives with activities which were comparable to Taxol.

A recent publication by Klein describes the synthesis of 9α-dihydrotaxol.[97] Biological evaluation in the tubulin assembly assay indicated that its activity was comparable to Taxol. This, combined with the side chain analogs previously mentioned that use the 9-dihydrotaxol ring system[79, 80], seem to indicate that the 9-keto group is not essential.

At the C-10 position, it is interesting to note that removal of the acetate reduces the activity of Taxol, but Taxotere which lacks the C-10 acetate is slightly more active.[70] Deoxygenation at this position seems to have little affect on Taxol[97,98] while it appears to enhance the activity of Taxotere.[97] Although not completely clear, these data suggest that the C-10 acetate does not play a major role.

Although a large number of analogs have been prepared, much work still remains to be done in this area. One of the more interesting conclusions that seems to be emerging from these structure-activity relationship studies is that since the 7, 9, and 10 positions all tolerate a wide variety of substituents, the functionality on the upper half of the taxane ring system may not play a crucial role in the microtuble binding.[59] When this is combined with the studies dealing with solution conformations, a very interesting picture begins to appear.

1.7. The Mechanism of Action of Taxol

As previously mentioned, Horwitz and coworkers working at the Albert Einstein College of Medicine gave Taxol a much needed boost in the late 70's when they uncovered its mechanism of action.[40] Up until the time of this discovery, Taxol's potent activity and promise as a new anticancer agent had been overshadowed by its scarcity and lack of water solubility. The discovery made by Horwitz *et. al.* that radically changed this situation is simply that Taxol functioned as an antimitotic agent by interacting with subcellular organelles called microtubules in a very unique way and by a method previously unknown.[40]

Microtubules are ubiquitous cellular constituents which are known to play a key role in cell division.[100] The mitotic spindle apparatus, which forms in the cytoplasm at prophase, is composed primarily of these proteinaceous organelles. Microtubules have also been linked with other important cellular functions such as cell shape, cellular motility and attachment, and intracellular transport. Structurally, microtubules are small hollow tubes composed of strands (usually 13) called protofilaments, each one a linear assembly of subunits.[100] The basic subunits are dimers composed of two similar, but structurally distinct globular proteins, α- and β-tubulin, each with an approximate molecular weight of 50,000 daltons. Microtubules are in dynamic equilibrium with these tubulin dimers and the direction of this equilibrium appears to be determined by signals generated by intracellular regulators like calcium and guanosine triphosphate (GTP) during specific cell cycle phases[32, 100] (Fig. 1.7).

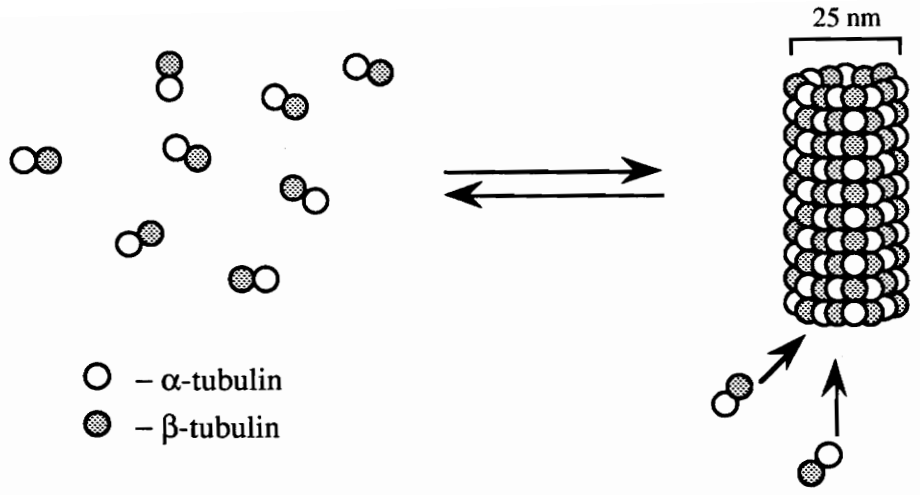


Figure 1.7. Tubulin-microtubule equilibrium.

Even though Taxol's mechanism of action is considered unique, the tubulin-microtubule equilibrium is the target for a number of chemotherapeutic agents. Colchicine, podophyllotoxin, and the vinca alkaloids, vincristine and vinblastine, are all known to interact with microtubules. These antimitotic agents have frequently been referred to as spindle poisons because they disrupt the normal formation of the mitotic spindle apparatus. Taxol stands unique among this group. Unlike all other antimitotic agents, which are known to inhibit the formation of microtubules, Taxol actually promotes assembly and then stabilizes the microtubules toward disassembly by shifting the dynamic equilibrium.[40, 101-112]

The initial work by Horwitz and coworkers revealed that both the rate and extent of microtubule formation were enhanced by shifting the equilibrium in favor of the microtubules.[40] In essence, Taxol seemed to decrease the critical concentration of tubulin required for microtubule

assembly in the presence or absence of factors which are normally required for this function such as GTP or microtubule-associated proteins.[32, 102, 113] Furthermore, when microtubules polymerized in the presence of Taxol were exposed to calcium or low temperatures, conditions which normally promoted disassembly, they were found to be stable.[40, 101, 114] Continued work revealed that Taxol binds preferentially to microtubules rather than to tubulin dimers[103] and that the binding site was distinct from the binding sites for exchangeable GTP and for colchicine, podophyllotoxin, and vinblastine.[102, 104] The binding is non-covalent and reversible[103, 105] and the stoichiometry approaches 1 mole of Taxol to 1 mole of tubulin dimer.[103]

Using electron microscopy and indirect immunoflorescence, two distinct morphological effects on microtubules in cells have been observed when clinically relevant concentrations of Taxol are used.[32] First, abundant arrays of disorganized microtubules (often aligned in parallel bundles) are observed when cells are treated with Taxol.[101, 109, 115-120] Interestingly, these arrays appear to form during all phases of the cell cycle. Second, Taxol appears to promote the formation of abnormal spindle asters during mitosis.[109, 120-125] Normally, centrioles will migrate to opposite poles of the cell where each will form a spindle aster that assists in the separation of duplicated chromosomal material before cell division. In contrast, when mitotic cells are treated with Taxol an over abundance of abnormal asters is observed and they do not require centrioles for nucleation.[120, 122]

Almost all the evidence to date indicates that the anticancer activity of Taxol is related to its ability to interact with microtubules.[13] The best supported explanation for the observed activity is connected to Taxol's ability to paralyze the mitotic spindle.[126] Normally microtubules disassemble at the kinetochore, the site of attachment of the chromosomes to the mitotic spindle, during mitotic anaphase.[127] Taxol appears to block disassembly by kinetically stabilizing the spindle microtubules.[128] However, this is not the only proposed explanation. It has also been suggested that Taxol's activity may be linked to calcium regulation in the cell.[129] If altered, it could also disrupt the mitotic spindle formation since calcium is known to be involved in the tubulin-microtubule equilibrium. Furthermore, it is also possible that Taxol's activity is due to some as yet unknown effect. The only thing that can truthfully be stated at this point is that Taxol's ability to promote the assembly of tubulin seems to be linked in some fashion to its anticancer activity, but the details of just how this translates into its observed activity is still a mystery.[13]

2. The Conversion of Cephalomannine to Taxol

Taxol is currently considered the most exciting lead in cancer chemotherapy.[130] It's clinically demonstrated efficiency against ovarian and breast cancer have literally catapulted it to the forefront of interest.[32, 48] In spite of this tremendous interest, the size and number of clinical trials have been limited.[32, 126] The major problem preventing the widespread use of Taxol has been that of supply. Although things have changed recently, at the onset of this project, the sole source of Taxol was the bark of the western yew, *Taxus brevifolia*.[130] Unfortunately, yields from the bark are poor, often as low as 0.007%.[54] This problem is compounded by the fact that the supply of bark is restricted because the yew is indigenous to the ecologically threatened old growth forests of the Pacific Northwest.[4] The supply problem has stirred great interest and a number of solutions have been proposed, including partial synthesis from baccatin III[131, 132], total synthesis[29, 30], and cell tissue culture.[133] Although these approaches for Taxol production are promising for the future, until they are fully developed the production is likely to stay linked to extraction from the bark of the yew or from an alternate source such as *Taxus* needles.[4]

The isolation of Taxol from the bark of the Pacific yew or comparable biological sources is complicated not only by the poor yields,

but also by the fact that the closely related diterpenoid cephalomannine frequently co-occurs with Taxol.[54] Although content can vary considerably, surveys over the past decade have shown that cephalomannine is almost always present.[4] One recent study by Wheeler *et al.*[51], even indicated that the cephalomannine content was often greater than the amount of Taxol. Under normal circumstances this fact would be nothing but an interesting piece of trivia. However, early isolation work revealed that the close structural similarity of the two compounds made the chromatographic separation of cephalomannine and Taxol very difficult. For almost a decade, the only practical methods reported required very careful chromatography under low resolution conditions or separation by HPLC.[54] Either process adds significant cost to the preparation of Taxol on a large scale.

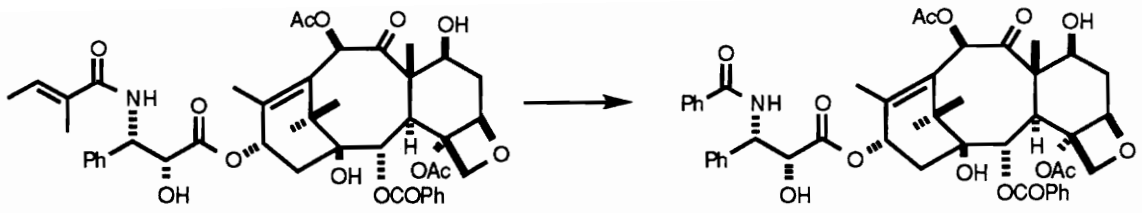
An excellent solution to the separation problem was described by Kingston and coworkers in a 1992 publication.[54] In this report, separation was achieved by selectively converting cephalomannine to a diastereomeric mixture of diols using osmium tetroxide, thus creating a mixture from which pure Taxol could readily be obtained by flash chromatography. Although this procedure gives a clean separation, it does little to address the supply problem and the diol is a useless side product.

Considering the scarcity of Taxol, it would seem that the development of a sequence to convert a crude Taxol/cephalomannine mixture, or a cephalomannine enriched mixture, to pure Taxol would be highly desirable. Such an approach would not only simplify the

purification process, thus reducing the cost, but it would also enhance the Taxol supply. Considering the limited number of yew trees, it must be recognized that ultimately the source of Taxol cannot stay linked to yew bark and consequently this approach would not be a long term solution to the supply problem. Nevertheless, as long as the source of Taxol is linked to isolation from natural sources, a method to convert cephalomannine to Taxol would offer a quick, convenient, and practical solution.

2.1. Background

Cephalomannine differs from Taxol only by the nature of the 3'-N-acyl group; cephalomannine has a tigloyl group at this position, while Taxol has a benzoyl group. Thus, the most direct route to convert cephalomannine (**2.1**) to Taxol (**2.2**) would require the cleavage of the 3'-N-tigloyl group to give the free amine followed by reacylation to introduce the benzoyl group (*Scheme* 2.1). Cleavage of the 3'-N-tigloyl provides a complex problem because of the highly functionalized nature of these molecules. In addition to the 3'-amides, both cephalomannine and Taxol possess a wide variety of functional groups including several esters, an oxetane ring, a β-hydroxy ketone, and an allylic ester at the C-13 position. Considering the number and spectrum of functional groups present, it is readily obvious that the conditions must be carefully chosen if selective cleavage of the amide linkage is to occur without causing any undesired changes in the rest of the molecule.



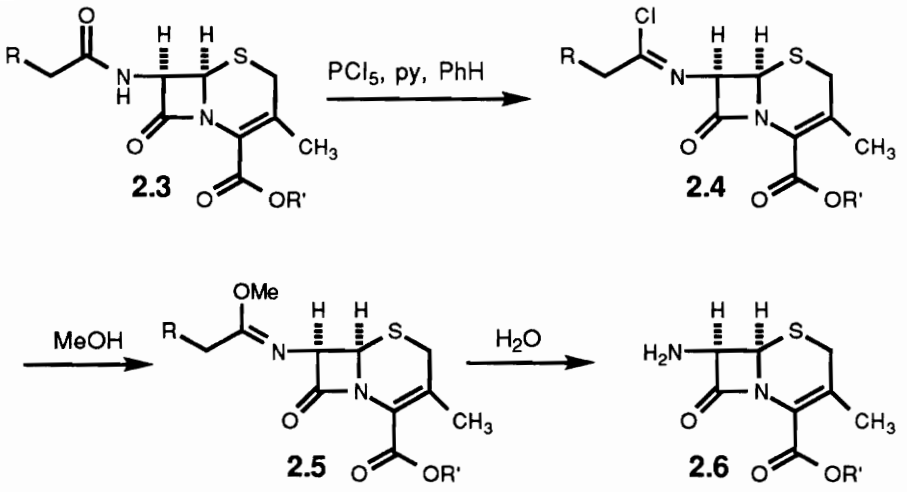
Scheme 2.1. The conversion of cephalomannine to Taxol.

Although the goal here is a direct route, it should be acknowledged that this conversion could also be achieved by an indirect method. Magri *et. al.*[134] reported that Taxol could be converted to baccatin III by treatment with tetrabutylammonium borohydride, a process which works equally well with cephalomannine. Baccatin III could then be converted to Taxol using one of any number of published routes.[131, 135-138] The major drawback of this approach is that it requires the synthesis of the β-phenylisoserine side chain of Taxol in an enantiomerically pure form, a challenge in itself. Furthermore, once the side chain has been synthesized, it must be coupled to baccatin III and this has proven to be difficult. A direct route would circumvent these problems and, hence, if one could be found it would be highly desirable.

When considering possible conversion sequences, it should be recognized that both mild conditions and a method selective for amides is required for the initial cleavage. Simple acid or base catalyzed hydrolysis of the amide linkage would not be a favorable process. The harsh conditions typically required to hydrolyze amides would also readily cleave esters. The C-13 ester side chain of Taxol and cephalomannine is

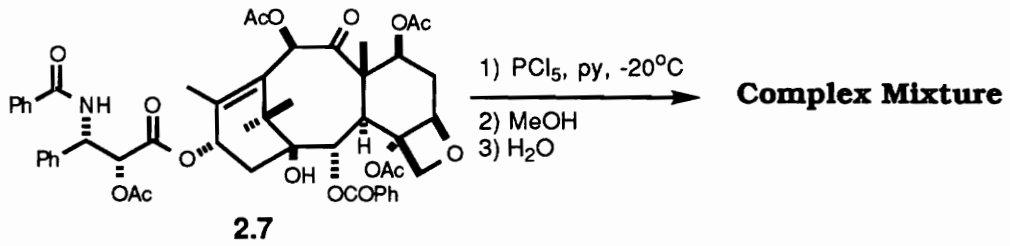
known to be particularly sensitive[1-3] and thus would be cleaved long before the 3'-amide. In addition to ester hydrolysis, problems with the epimerization of the C-7 hydroxyl would likely arise.[1-3] Thus, classical hydrolysis methods are likely to prove problematic and cleavage will require specialized conditions.

Dr. Jitrangsri[55], working in the laboratories of D. G. I. Kingston at Virginia Polytechnic Institute & State University, was the first to report the results of an investigation that focused on the attempt to cleave the N-acyl group of Taxol and/or cephalomannine. He described an attempt to cleave the N-benzoyl group of Taxol using phosphorus pentachloride, a method that has proven successful on cephalosporin derivatives. This methodology was initially developed in the early 60's by Rusting and coworkers to cleave the α-aminoadipoyl side chain of cephalosporin.[139] The methodology has been frequently applied to the chemistry of β-lactam antibiotics[140-141] and a number of modifications have been reported.[142-146] One modification of particular interest was described by Chauvette and colleagues in the early 70's.[142] Their report described the reaction of a cephalosporin derivative possessing an amide side chain (**2.3**) with phosphorus pentachloride and pyridine in dry benzene to form an imino chloride (**2.4**) (*Scheme* 2.2). The resulting imino chloride was found to react with methanol at room temperature to form an imido ester (**2.5**) which hydrolyzed instantly on contact with water to give the free amine (**2.6**). Although this route appears to be selective for amides and reasonably mild, when Jitrangsri applied this methodology to 2', 7-



Scheme 2.2. Phosphorus pentachloride cleavage.[142]

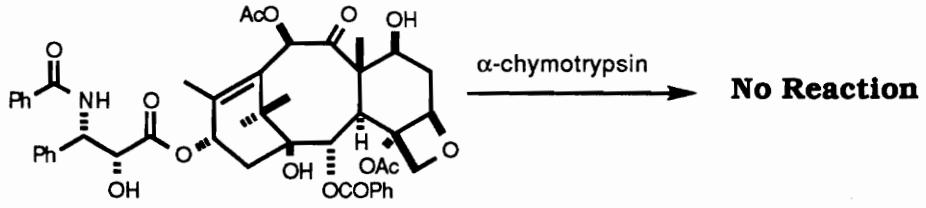
diacetyltaxol (**2.7**), a Taxol analog he prepared by the acetylation of Taxol using acetic anhydride in pyridine, the result was a complex mixture (*Scheme* 2.3).[55]



Scheme 2.3. N-benzoyl cleavage using phosphorus pentachloride.

Jitrangsri also attempted to cleave the N-benzoyl group of Taxol (**2.2**) using α-chymotrypsin (*Scheme* 2.4).[55] The use of this enzyme to cleave N-acyl groups on other natural products such as cephalosporin

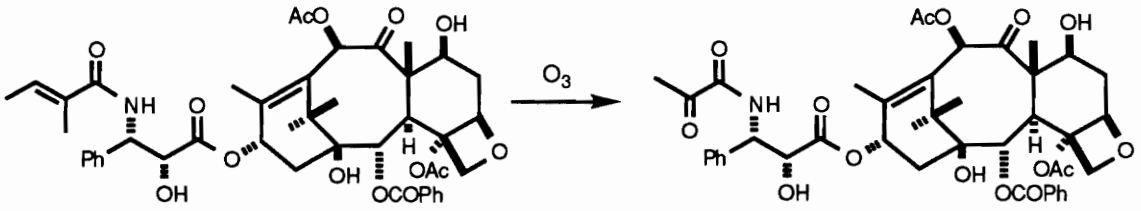
had been reported to occur in a pH 7 medium at room temperature.[55,147] When Taxol was treated with α-chymotrypsin no reaction was observed after a 24 hr period.



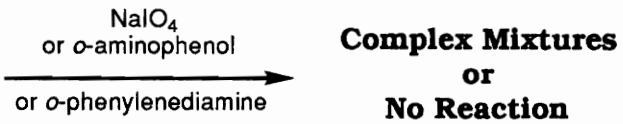
2.2

Scheme 2.4. Attempted cleavage using α-chymotrypsin.

Jitrangsri also explored the possibility of converting cephalomannine (**2.1**) to ketoamide (**2.8**) and then cleaving the N-pyruvyl group to give the free amine (*Scheme* 2.5).[55] Ketoamide (**2.8**) was prepared by ozonolysis of cephalomannine (**2.1**). Jitrangsri investigated



2.1 **2.8**



Scheme 2.5. Attempted cleavage using the ketoamide.

the use of sodium periodate, *o*-aminophenol, and *o*-phenylenediamine to cleave the N-pyruvyl group, but all of these reagents failed to produce the desired product.

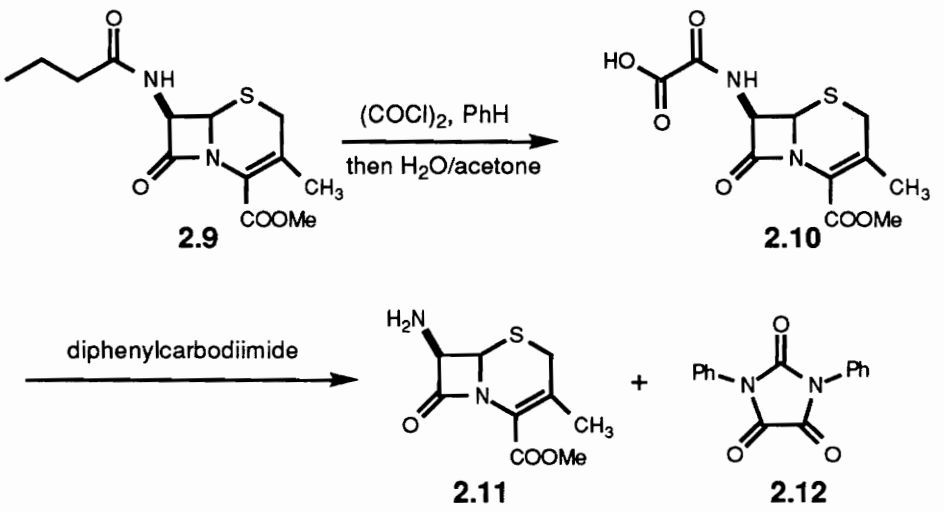
Dr. Gunatilaka, also of the Kingston laboratories, extended the scope of this investigation.[56] Dr. Gunatilaka's work focused on the preparation of tri-*t*-Boc taxol. Flynn *et. al.*[148] had reported that N-*t*-Boc derivatives of amides underwent regioselective hydrolysis using lithium hydroxide or methanolysis to yield the corresponding *t*-Boc protected amine. Dr. Gunatilaka successfully prepared the tri-*t*-Boc derivative, but when he attempted to cleave the N-benzoyl group of Taxol using lithium hydroxide, surprisingly the result was cleavage of the 2-benzoyl group rather than the 3'-amide.

2.2. Results and Discussion

The initial course of the investigation focused on the reexamination of much of the work described by Jitrangsri and Gunatilaka.[55-56] Although considerable time and effort was spent on the reinvestigation of Chauvette's phosphorus pentachloride methodology[142] and Flynn's tri-*t*-Boc route[148] as well as a number of modifications of these methods, the results obtained were not any more encouraging than those previously observed. It wasn't until a communication published by Shiozaki *et. al.*[149] was discovered that a conversion route began to look promising. Although this procedure ultimately led to the conversion of cephalomannine to Taxol, there were also some very puzzling results. In

light of the unusual final outcome, the discussion presented here will be given in a historical fashion and several of the model studies and analogs prepared will be included.

In 1977, Shiozaki reported a new method for the cleavage of the 7-amide group of cephalosporins.[149] His procedure involved the reaction of N-monosubstituted amides such as cephalosporin (**2.9**) or its derivatives with oxalyl chloride followed by a water quench to give the corresponding oxamic acid derivative (**2.10**) (*Scheme* 2.6). This derivative was then treated with diphenylcarbodiimide to yield the free amine (**2.11**) and the imidazolidine (**2.12**). This method was reported to be selective for amides and the conditions appeared to be mild.



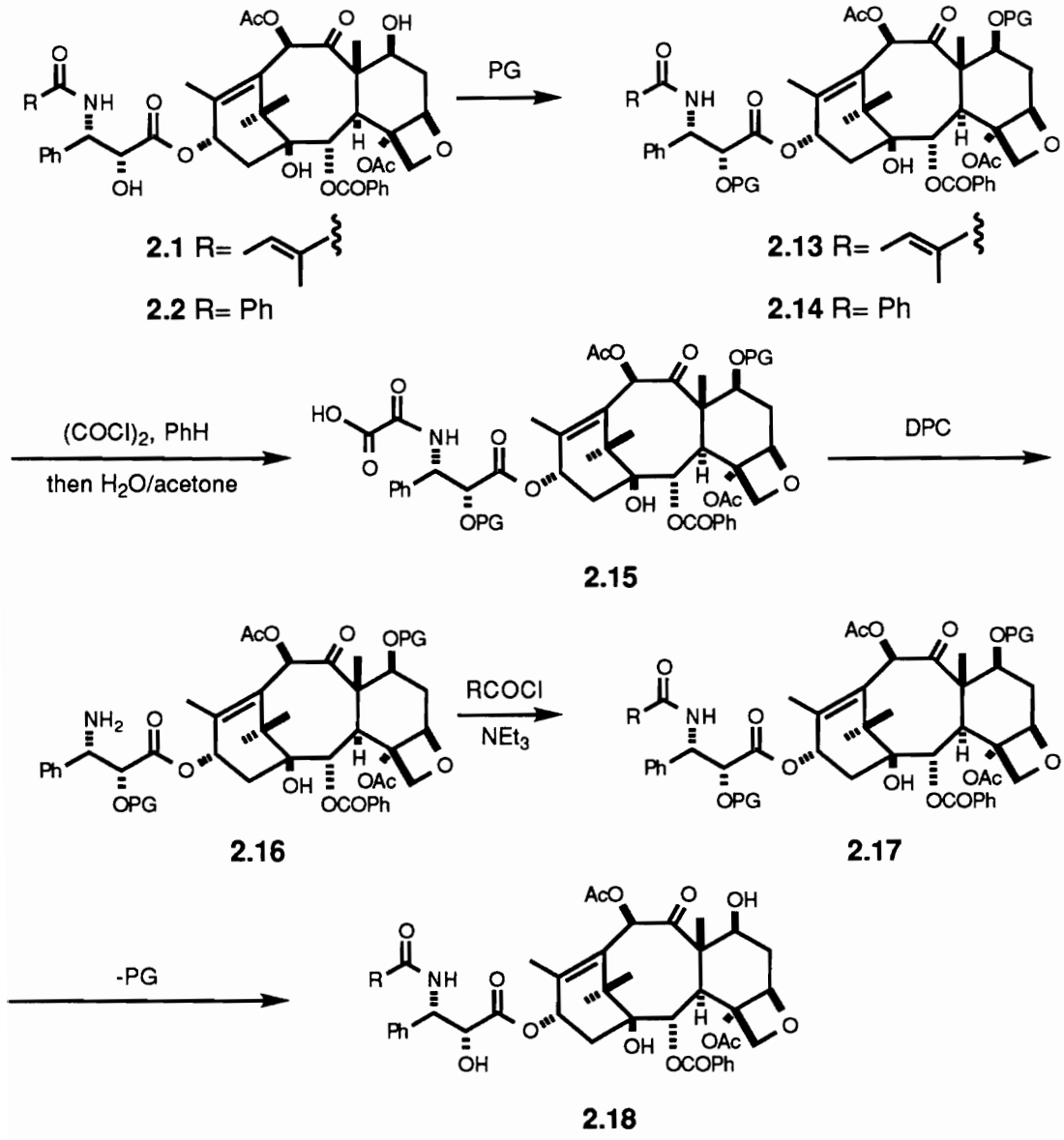
Scheme 2.6. Shiozaki's amide cleavage methodology.

In addition to a conversion process, Shiozaki's amide cleavage was also of interest because it held the possibility of the preparation of a

number of 3'-N-acyl analogs. As previously stated, the primary goal of this investigation was to convert cephalomannine into Taxol, but from the beginning it was also hoped that the conversion route would be versatile enough to prepare any 3'-N-acyl analog. The preparation of Taxotere[5] and the deletion analog studies by Swindell *et. al.*[7] suggest that the 3'-amide substituent plays an important role in the preorganization of Taxol for binding to microtubules. Hence, a route that would allow not only the conversion of cephalomannine to Taxol, but also the preparation of any 3'-N-acyl analog, would be of significant value.

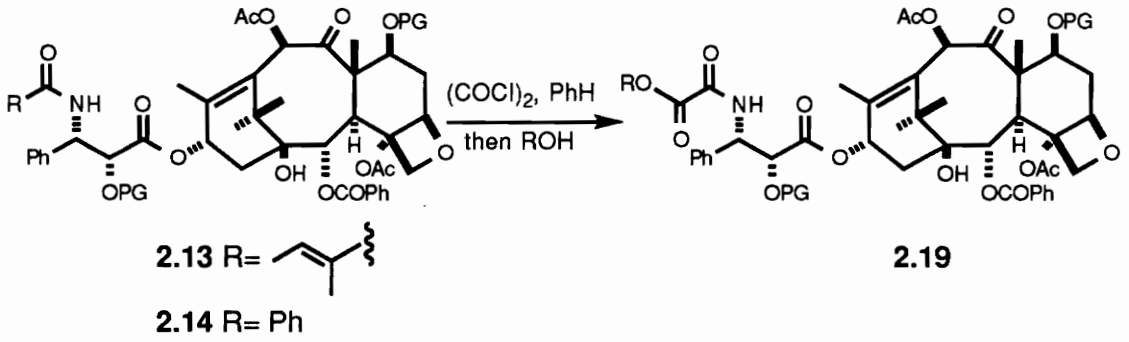
The conversion route envisioned is outlined in *Scheme* 2.7. A mixture of cephalomannine (**2.1**) and Taxol (**2.2**) would be converted to a 2', 7-diprotected cephalomannine (**2.13**)/Taxol (**2.14**) mixture. Treatment of the diprotected mixture with oxalyl chloride followed by a water quench would generate the key oxamic acid intermediate (**2.15**). Since the N-acyl group of both Taxol and cephalomannine would be cleaved at this point, the product would be a single stereoisomer. Thus, prior separation of Taxol and cephalomannine would be unnecessary. The next part of the sequence would be the reaction of the oxamic acid (**2.15**) with diphenylcarbodiimide[150] to cleave the oxalyl group and give the free amine (**2.16**). After removal of the oxalyl group the free amine could then be acylated with the desired group to produce the diprotected analog (**2.17**). The final step would simply be removal of the protecting groups to yield Taxol or a new 3'-N-acyl analog (**2.18**).

Alternatively, this methodology could be used to create a set of



Scheme 2.7. Envisioned cephalomannine to Taxol conversion.

unique oxalyl analogs (**2.19**). This could readily be accomplished by simply quenching the oxalyl chloride reaction with an alcohol instead of water (*Scheme* 2.8).



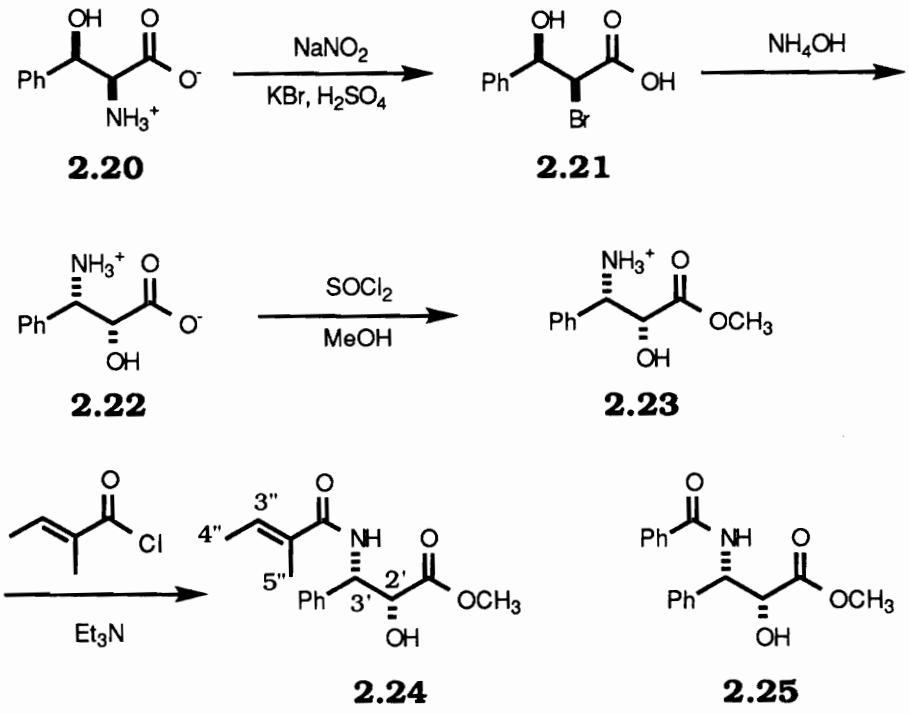
Scheme 2.8. Envisioned preparation of oxalyl analogs.

The key step of this envisioned sequence was considered to be the conversion of the diprotected Taxol/cephalomannine mixture to the oxamic acid intermediate (**2.15**). Early work on the chemistry of Taxol had established a variety of protection-deprotection schemes[58, 70, 151], therefore these steps were not expected to pose a problem. Although the use of diphenylcarbodiimide (DPC) in the cleavage of the oxamic acid intermediate would be new, dicyclohexylcarbodiimide (DCC) has frequently been used on Taxol without difficulty.[152] Hence, the use of diphenylcarbodiimide was not expected to create any serious problems either. Consequently, the only real question that remained was whether the reaction of Taxol and cephalomannine with oxalyl chloride would give the desired product without any serious side reactions.

When this investigation began, pure cephalomannine was unavailable. As was previously mentioned, Taxol and cephalomannine almost always co-occur and chromatographic separation is difficult. Typically, attempts to purify the two by chromatography results in fractions containing pure Taxol and fractions with varying mixtures of Taxol and cephalomannine. Although reasonably pure cephalomannine could be obtained at the expense of considerable time and effort, limitations on the Taxol/cephalomannine supply made this approach impractical. The use of a Taxol/cephalomannine mixture was also briefly considered, but it was felt that difficulties in characterization of early intermediates (which would be mixtures) might complicate the evaluation of the sequence. Thus, the decision was made to investigate Shiozaki's amide cleavage methodology using the cephalomannine side chain methyl ester as a model.

The cephalomannine side chain was prepared by a procedure reported by DeAmicis (*Scheme* 2.9).[153] Although this route led to a racemic mixture rather than the enantiomerically pure cephalomannine side chain, this mixture was considered suitable for the planned model studies. Thus, DL-*threo*-3-phenylserine (**2.20**) was treated with $NaNO_2$ in the presence of KBr and 2.5 N H_2SO_4 to yield 2-bromo-3-hydroxy-3-phenylpropionic acid (**2.21**). The conversion of this acid (**2.21**) to DL-*threo*-3-phenylisoserine (**2.22**) was achieved by stirring it in concentrated NH_4OH at room temperature for seven days After purification by ion exchange chromatography, reaction of (**2.22**) with thionyl chloride in methanol gave methyl ester (**2.23**). The final step was acylation of the

amine using tigloyl chloride and triethylamine to yield the methyl ester of the cephalomannine side chain (**2.24**). The ^{1}H NMR data for the side chain methyl ester is given in Table 2.1. This side chain methyl ester was used in all the model studies.



Scheme 2.9. Synthesis of the cephalomannine side chain methyl ester.

The Taxol side chain methyl ester (**2.25**) was prepared in a similar fashion except that intermediate (**2.23**) was acylated using benzoyl chloride. As a comparison the ^{1}H NMR data for the Taxol side chain methyl ester is also given in Table 2.1.

With the cephalomannine side chain methyl ester in hand, the

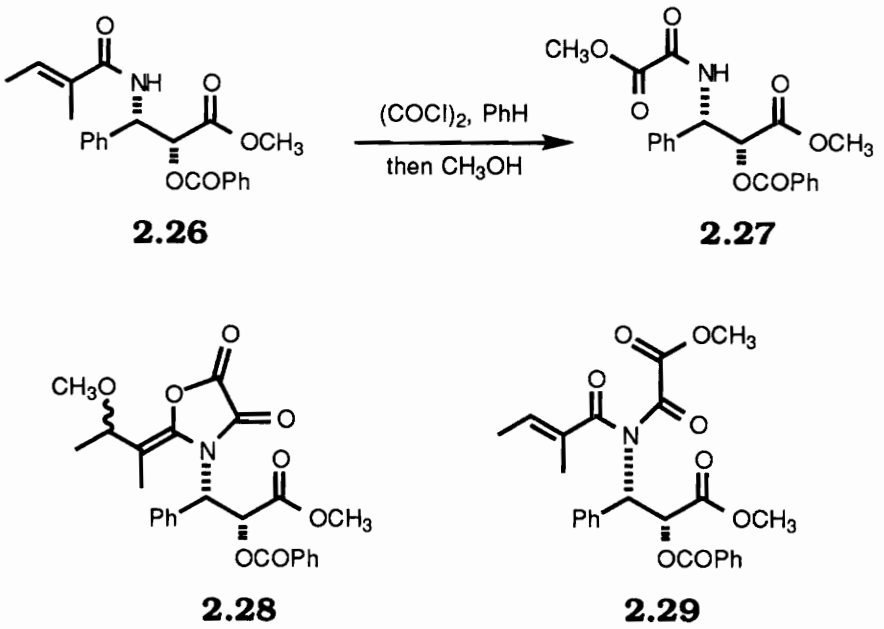
Table 2.1 ^{1}H NMR Spectra of the Side Chain Methyl Esters.[a, b]

Protons on	**2.24**	**2.25**
2'	4.55 (br s)	4.64 (br s)
3'	5.57 (dd, 9.1, 2.0)	5.75 (dd, 9.1, 1.8)
NH	6.57 (d, 9.0)	6.98 (d, 8.6)
OMe	3.81 (s)	3.85 (s)
Ph	7.26-7.40 (m)	7.30-7.60 (m)
3"	6.43 (qd, 6.9, 1.2)	
4"	1.74(dd, 6.9, 0.9)	
2"-Me	1.83 (br s)	
PhCO		7.30-7.78 (m)

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz.
[b]Multiplicity: s=singlet, d=doublet, q=quartet, m=multplet, br=broad.

focus became the evaluation of the key step, the conversion of the cephalomannine side chain to the oxamic acid. Protection of the 2'-hydroxyl was required since oxalyl chloride readily reacts with alcohols. The choice of protecting groups was made based on a report by Denis and coworkers[154-155] which described an enantioselective synthesis of the Taxol side chain. The last step in their synthesis employed an O→N benzoyl transfer. It was hoped that this same O→N transfer could be applied to the conversion at hand. Consequently, the 2'-benzoyl protected cephalomannine side chain methyl ester (**2.26**) was prepared

by reaction of the side chain with benzoyl chloride and diisopropylethylamine in methylene chloride. This 2'-protected side chain was treated with oxalyl chloride in benzene for 3 hours and then quenched with methanol (*Scheme* 2.10). Although water would be used as the quench in the actual conversion sequence, methanol was used here to generate the oxamate ester, which would simplify purification and, hence, evaluation of this key step. A TLC of the reaction mixture indicated that the starting material was consumed and two major products had been formed.



Scheme 2.10. Reaction of the cephalomannine side chain with $(COCl)_2$.

Characterization of these two products by ^1H NMR established that one, compound (**2.27**), was the desired product (Table 2.2). Spectral evidence that supported this was the disappearance of peaks due to the

tigloyl group and the presence of a singlet at 3.92 ppm due to the methyl group of the oxamic acid methyl ester. Furthermore, there was a downfield shift of the amide doublet from 6.81 ppm to 8.03 ppm, a shift characteristic of oxamate esters.[149, 156]

Table 2.2 ^{1}H NMR Spectra of the Side Chain Methyl Oxalates.[a, b]

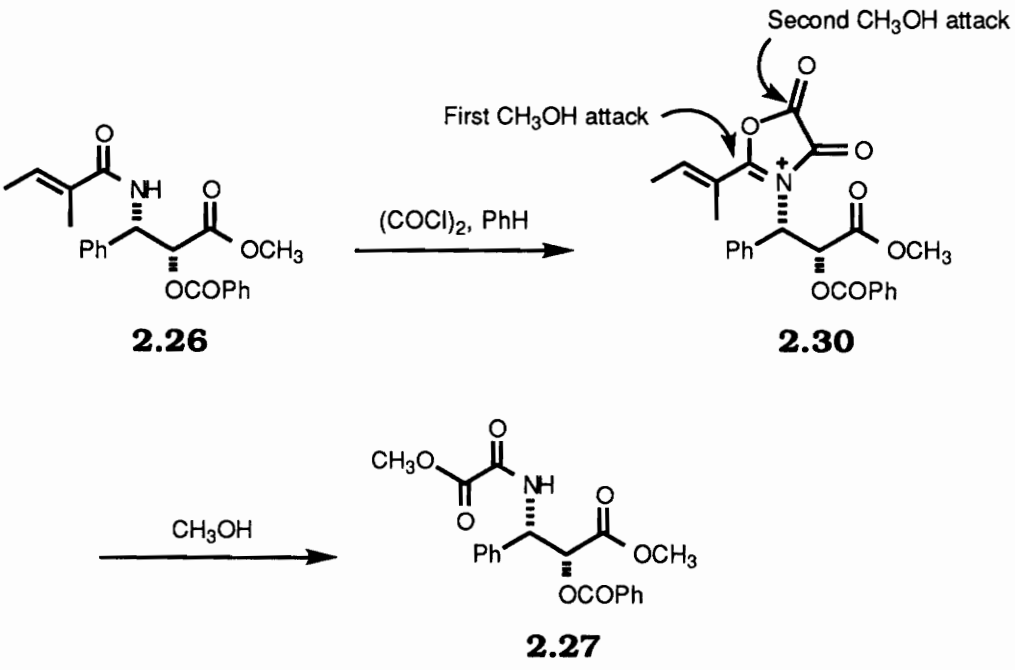
Protons on	**2.26**	**2.27**	**2.29**
2'	5.57 (d, 3.1)	5.61 (d, 3.1)	6.13 (d, 10.7)
3'	5.82 (dd, 8.9, 3.0)	5.76 (dd, 9.1, 3.1)	6.36 (d, 10.7)
NH	6.81 (d, 8.9)	8.03[c]	
OMe	3.75 (s)	3.77 (s)	3.53 (s)
Ph	7.25-8.20 (m)	7.26-8.01 (m)	7.30-8.02 (m)
3"	6.50 (q, 6.9)		5.99 (qd, 6.9, 1.3)
4"	1.74 (d, 6.9)		1.53 (d, 6.9)
5"-Me	1.84 (br s)		1.76 (s)
MeCOCO		3.92 (s)	3.66 (s)

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, q=quartet, m=multiplet, br=broad. [c]Partially hidden under the aromatic region.

The second compound was initially thought to be the unusual oxazolidine-4,5-dione derivative (**2.28**). This was suggested by the presence of the tigloyl methyl protons and the disappearance of the amide doublet. In retrospect, however, the spectral evidence seems to fit the 3'-N-diacyl analog (**2.29**) slightly better. Although it is not completely

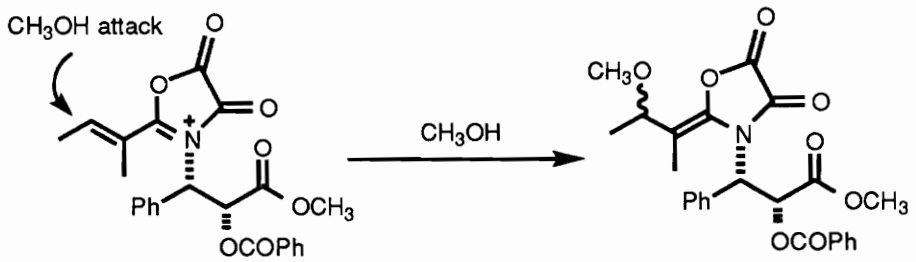
unambiguous, two pieces of evidence favor (**2.29**) over (**2.28**). First, if the cyclic derivative (**2.29**) had been formed, it would be a mixture of four diastereomers (two pairs of enantiomers) and consequently, a significantly more complex ^{1}H NMR would be expected. Second, all the signals due to the tigloyl group are intact (splitting pattern and coupling constants) (see Table 2.2) suggesting that it has not been altered.

From a mechanistic standpoint, either product is possible. Shiozaki and coworkers proposed that this reaction took place by the mechanism shown in *Scheme* 2.11.[149, 156-157] Their mechanistic proposal was based on a number of intermediates observed in their work[149, 156] and well as several studies on the reaction of oxalyl chloride



Scheme 2.11. Shiozaki's proposed mechanism.

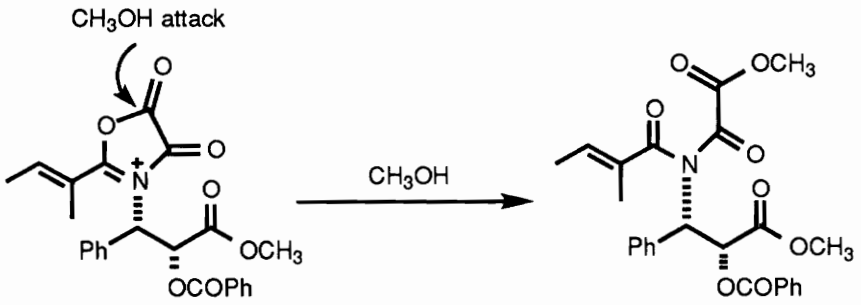
with secondary amides performed by Speziale[158] in the early 60's. Their mechanism suggests that when oxalyl chloride reacts with a secondary amide, in this case the tigloyl group of the cephalomannine side chain (**2.26**), it forms cyclic intermediate (**2.30**). When the reaction is quenched with an alcohol the cyclic intermediate is cleaved to yield the oxamate ester (**2.27**). Assuming this mechanism is correct, product (**2.28**) would arise from a Michael type addition of methanol to cyclic



2.30 **2.28**

Scheme 2.12. Formation of (**2.28**) via Michael-type attack.

intermediate (**2.30**) (*Scheme* 2.12) while derivative (**2.29**) would result from the initial attack at the position shown in *Scheme* 2.13.

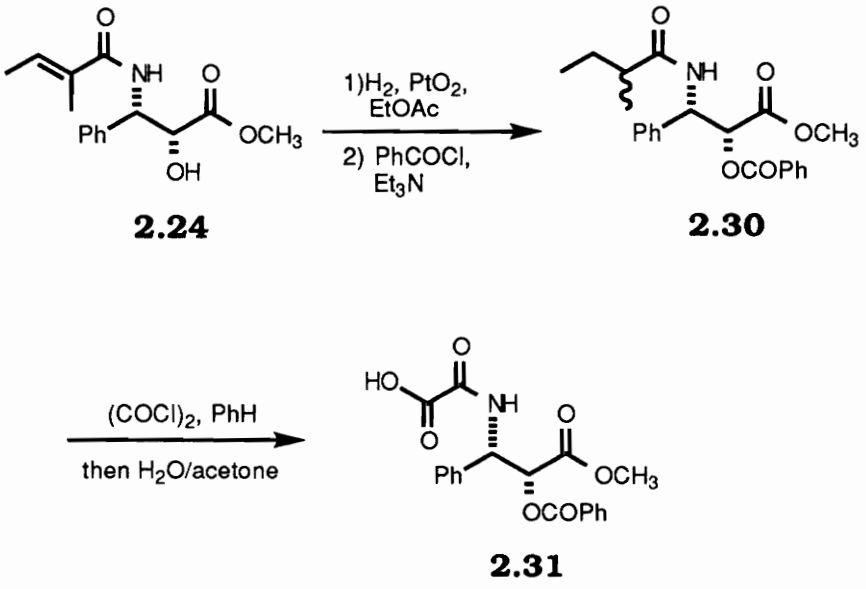


2.30 **2.29**

Scheme 2.13. Formation of (**2.29**).

Even though two products were obtained in this initial test, it established the feasibility of this sequence. Thus the next goal was to carry out the entire procedure on the side chain model.

In order to circumvent any problems that might arise due to the double bond of the tigloyl group, it was decided to hydrogenate the side chain prior to the reaction with oxalyl chloride. Although this would create four diastereomers (two pairs of enantiomers) of the side chain, or two diastereomers in the actual case of cephalomannine, it is of little consequence since the tigloyl group is ultimately removed. Thus, the cephalomannine side chain methyl ester (**2.24**) was hydrogenated at room temperature using Adam's catalyst in ethyl acetate and then benzoylated to give 2'-benzoyldihydrocephalomannine side chain methyl ester (**2.30**) (*Scheme* 2.14). This protected side chain was then treated



2.24 **2.30** **2.31**

Scheme 2.14. Preparation of the oxamic acid (**2.31**).

with oxalyl chloride in benzene and after quenching with a 50/50 water/acetone solution the crude product was isolated. As expected, purification proved to be difficult due to the highly polar nature of the product. Eventually, it was purified using Sephadex LH-20 with methylene chloride/acetone in a 9:1 ratio as the mobile phase. Characterization by ^{1}H NMR indicated the product was the desired oxamic acid derivative (**2.31**). Key spectral evidence included the disappearance of all peaks for the tigloyl protons and the characteristic

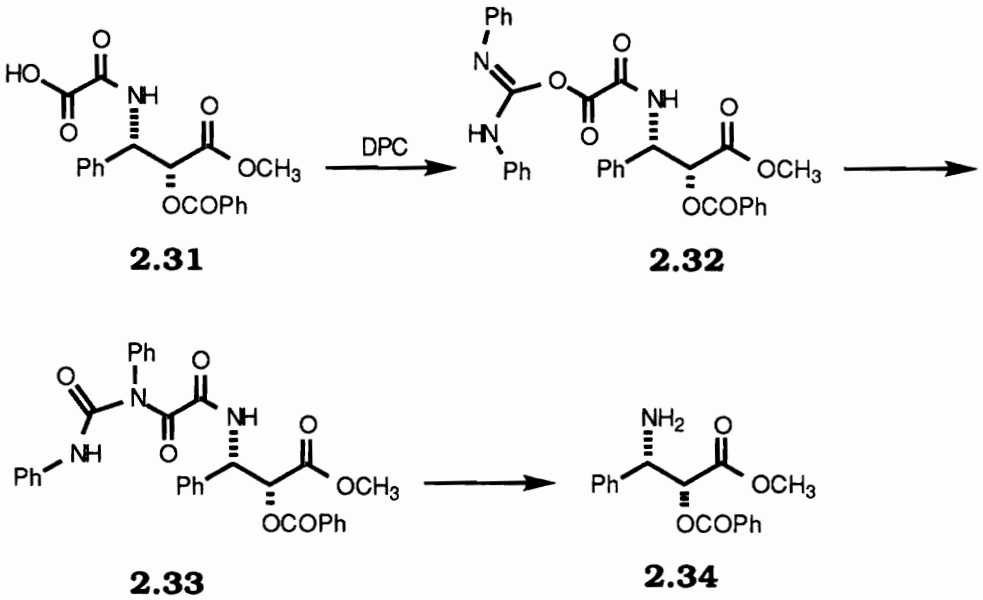
Table 2.3 ^{1}H NMR Spectra of the Oxamic Acid Side Chain.[a, b]

Protons on	**2.30**[c]	**2.31**
2'	5.56 (d, 3.2)	5.63 (d, 3.4)
3'	5.83 (m)	5.71 (dd, 9.1, 3.3)
NH	6.59 (d, 9.1)	8.39 (d, 8.7)
OMe	3.73 (s)	3.71 (s)
Ph	7.26-7.57 (m)	7.26-7.60 (m)
2"	2.24 (m)	
3"	1.44 (m)	
	1.69 (m)	
4"	0.89 (m)	
5"-Me	1.14 (m)	
PhCO	7.26-8.00 (m)	7.26-8.02 (m)

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz.
[b]Multiplicity: s=singlet, d=doublet, q=quartet, m=multiplet.
[c]Mixture of diastereomers. Many of the signals appear as complex multiplets due to the diastereomeric mixture..

downfield shift of the amide doublet from 6.59 ppm to 8.39 ppm.

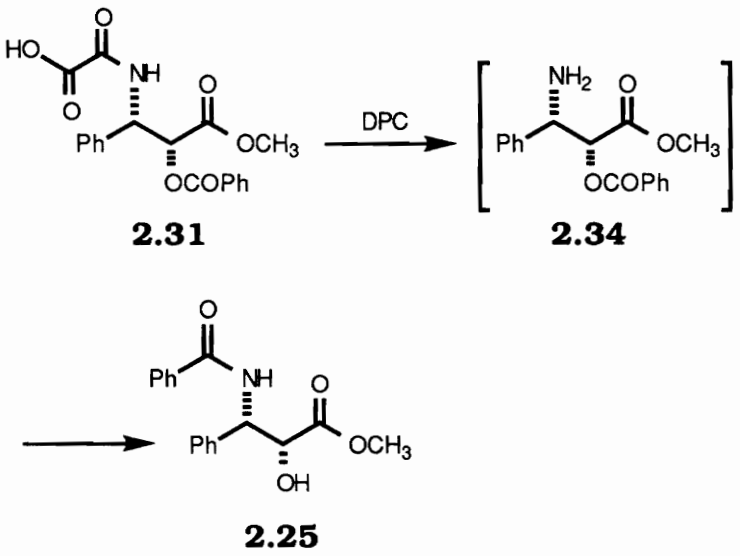
With oxamic acid (**2.31**) in hand, all that remained was the final step, the reaction of the oxamic acid with diphenylcarbodiimide (DPC). The reaction of DPC with oxamic acids to yield the free amine is a unique one. Shiozaki proposed the mechanism shown in *Scheme* 2.15 (shown here on the oxamic acid of the cephalomannine side chain).[149, 156] When an oxamic acid such as (**2.31**) is treated with DPC it reacts in a fashion typical of any carbodiimide to give intermediate (**2.32**). Once (**2.32**) forms a rapid O→N acyl shift occurs, presumably via a four-membered ring transition state, to yield (**2.33**) which is followed by cyclization to produce the free amine (**2.34**). This O→N acyl shift is unique to aromatic carbodiimides. When Shiozaki attempted to perform



Scheme 2.15. Shiozaki's proposed DPC mechanism.

this reaction with DCC this acyl shift failed to occur resulting in a variety of different products rather than the desired free amine.[149]

When oxamic acid (**2.31**) was treated with freshly prepared DPC in methylene chloride, two less polar spots were immediately observed by TLC (*Scheme* 2.16). Over the period of several days, these two TLC spots slowly converted to a single spot which had an R_f that matched with an authentic sample of the Taxol side chain (**2.25**). The ^1H NMR spectrum of this product was identical in all respects with that of a sample of the Taxol side chain methyl ester prepared by DeAmicis' methodology.[153] The success of this final step not only established the feasibility of the route, but also indicated that, as hoped, once the free amine (**2.34**) was formed by reaction of the oxamic acid with DPC, the 2'-benzoyl group would spontaneously transfer to it.

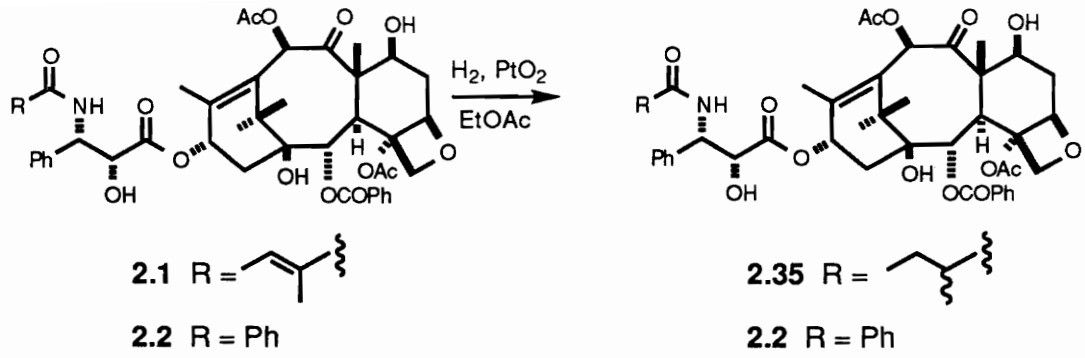


2.31 **2.34** **2.25**

Scheme 2.16. Conversion to the Taxol side chain (**2.25**).

With the success of the cephalomannine to Taxol conversion on the cephalomannine side chain model, the next step was cephalomannine itself. Although the best approach would have been to test the sequence on pure cephalomannine, the difficulty in obtaining a pure cephalomannine sample prompted the next step of the investigation to be performed on a Taxol/cephalomannine mixture. Although this posed a problem in terms of the complete characterization of some of the early intermediates, it did have the advantage that it would quickly evaluate the practical application of this route to a mixture. Therefore, the initial goal was to determine the feasibility of this route on a mixture with the intent being to reinvestigate this route on pure cephalomannine at a later date. The mixture chosen for use in this study was determined by ^{1}H NMR to be approximately 50% cephalomannine (**2.1**) and 50% Taxol (**2.2**). Although the sample was a mixture, the proton spectrum was not as complex as might be expected. Since cephalomannine differs from Taxol only in the nature of the 3'-N-acyl group the majority of their signals are identical. The spectra of the two differ only in the side chain protons and these can be readily distinguished.

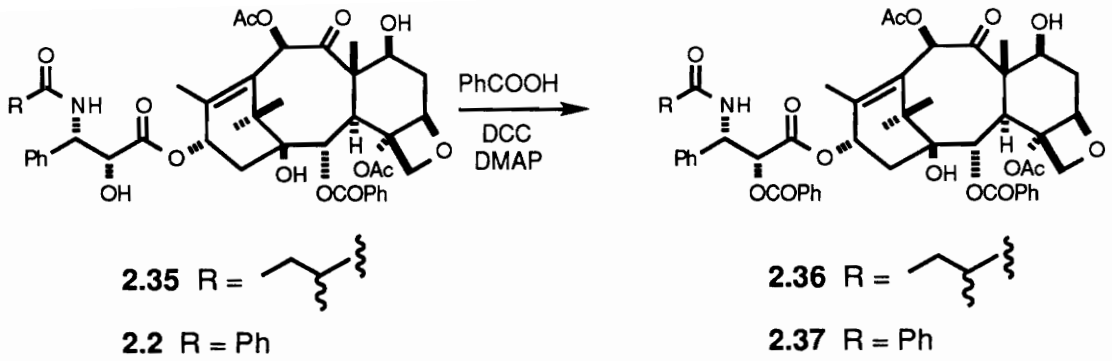
The sequence began with the hydrogenation of a Taxol/cephalomannine mixture (*Scheme* 2.17). Hydrogenation at room temperature using Adam's catalyst in ethyl acetate produced a mixture of Taxol (**2.2**) and two dihydrocephalomannine diastereomers (**2.35**) in quantitative yield. The A-ring double bond does not pose a problem since it is extremely hindered and hence is not touched. This conveniently



Scheme 2.17. Hydrogenation of Taxol/cephalomannine mixture.

allows the selective hydrogenation of the N-tigloyl group on cephalomannine. Key changes observed in the ^1H NMR are the increased complexity of the tigloyl methyl groups at 0.85 ppm and 1.1 ppm due to the formation of diastereomers, the disappearance of the tigloyl vinylic proton at 6.37 ppm, and the upfield shift of the amide proton on cephalomannine from 6.52 ppm to 6.26 ppm. Unfortunately, this upfield shift of the amide proton puts the signal on top of the C-13 proton and consequently the signal is partially hidden. The spectrum of Taxol remains unchanged. The assignments are shown Table 2.4.

After hydrogenation, protection of the 2'-hydroxyl group with the benzoyl group was accomplished by reaction of the mixture with benzoic acid, dicyclohexylcarbodiimide (DCC), and a catalytic amount of 4-dimethylaminopyridine (DMAP) (*Scheme* 2.18). The reaction proceeded smoothly to produce a 2'-benzoyl taxol (**2.36**)/dihydrocephalomannine

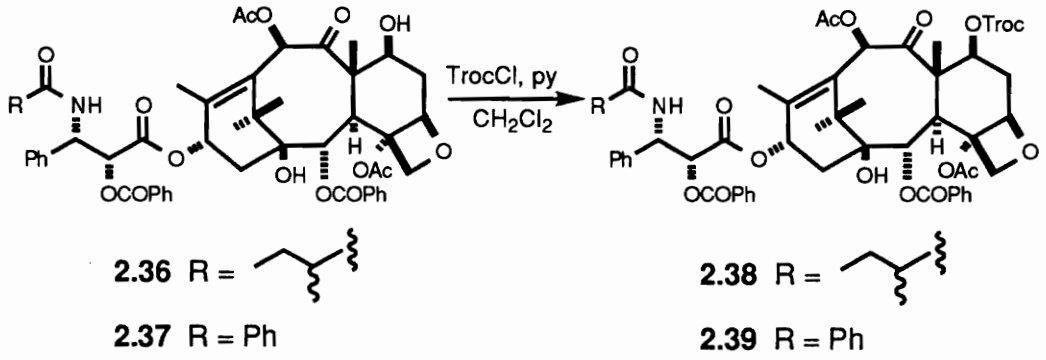


Scheme 2.18. Protection of the 2'-hydroxyl group.

(**2.37**) mixture in 95% yield. The choice of the benzoyl group as the protecting group, here again, was based on the hope that the benzoyl would spontaneously transfer when the free amine was generated. The spectral changes observed upon benzoylation include the downfield shift of the 2' proton of both Taxol and cephalomannine. As was observed in the hydrogenation step, the changes in chemical shifts that occurred made the signals difficult to see. The 2' proton on Taxol shifts from 4.79 ppm to 5.69 ppm, which falls right on top of the C-2 proton, while the 2' of cephalomannine shifts from 4.69 ppm to approximately 5.6 ppm, which is under the C-7 proton. The downfield shift in the C-3' protons for both is much smaller, but these protons are more clearly visible.

The next step of the conversion sequence was the protection of the C-7 hydroxyl. Although this step was not performed on the side chain model (since there was no C-7), protection protocols have been well established.[58, 69] The choice for a protecting group here was the 2,2,2-trichloroethylcarbonate, or Troc, group. This has frequently been used

on Taxol and has been found to go on in good yield[58] and is easily removed using zinc in acetic acid.[69] Thus, when the 2'-benzoyl protected Taxol/dihydrocephalomannine mixture was treated with 2,2,2-trichloroethyl chloroformate and pyridine in methylene the 7-Troc protected derivatives of both dihydrocephalomannine (**2.38**) and Taxol (**2.39**) were produced in 85% yield (*Scheme* 2.19). The ^{1}H NMR spectrum showed the characteristic downfield shift of the C-7 proton, spectroscopically identical for both cephalomannine and Taxol, from 4.40 ppm to 5.59 ppm and the appearance of two doublets at 4.65 ppm and 5.02 ppm which are due to the diastereotopic protons of the Troc group.



Scheme 2.19. Protection of the C-7 hydroxyl group.

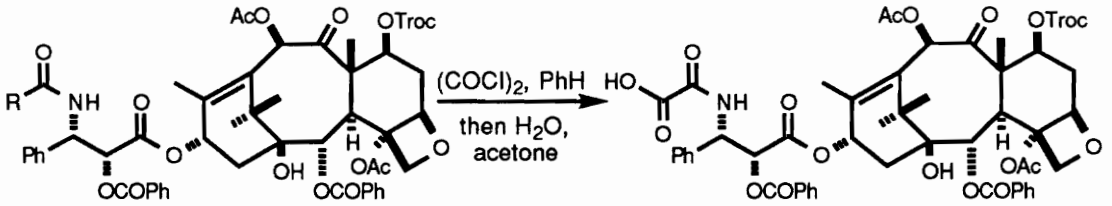
The preparation of this diprotected mixture set the stage for the key conversion to the oxamic acid intermediate (**2.40**) (*Scheme* 2.20). The 2'-benzoyl-7-Troc taxol/dihydrocephalomannine mixture was dissolved in HPLC grade benzene under argon and then treated with 6

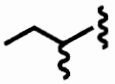
Table 2.4. ^{1}H NMR of Derivatives of Taxol/Cephalomannine Mixtures.[a, b]

Protons on	Mixture of **2.2** and **2.35**	Mixture of **2.38** and **2.39**
C-2	5.68 (d, 7)	5.70[d]
C-3	3.78 (m)	3.95 (d, 8)
C-5	4.94 (d, 10)	4.98 (d, 11)
C-6	*c*	*c*
C-7	4.40 (m)	5.59 (m)
C-10	6.28 (s)	6.38 (s)
C-13	6.23 (t, 9)	6.26 (br t)
C-14 (α, β)	*c*	*c*
C-16-CH_3	1.15 (s)	1.16 (s)
C-17-CH_3	1.25 (s)	1.21 (s)
C-18-CH_3	1.79 (s)	1.83 (s)
C-19-CH_3	1.66 (s)	1.66 (s)
C-20	4.19 (d, 6)	4.19 (d, 8)
	4.30 (d, 6)	4.33 (d, 8)
C-2'	(T) 4.79 (dd)[f]	5.69[d]
	(C) 4.69 (m)	*c*
C-3'	(T) 5.80 (dd, 7,1)[f]	(T) 6.05[f]
	(C) 5.58 (m)	(C) 5.90
3'-NH	(T) 7.05 (d, 8)[f]	(T) 7.05 (d, 8)[f]
	c	(C) 6.26
2-OBz, 3'-NBz, 3'Ph	7.35-8.15	7.38-8.12
4-OAc	2.48 (s)	2.45 (s)
10-OAc	2.24 (s)	2.15 (s)
2'-OBz		7.38-8.01
7-$OCOOCH_2CCl_3$		4.65 (d, 12)
		5.02 (d, 12)
$CH_3CH_2CH(CH_3)CO$	0.90-2.50[e]	0.90-2.50[e]

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, t=triplet, m=multiplet. [c]Signal hidden under other peaks. [d]Difficult to determine exact chemical shift due to overlapping peaks. [e]The exact chemical shifts of the hydrogenated tigloyl group are difficult to determine, not only because they overlap with other peaks, but also because hydrogenation creates a new chiral center which leads to two diastereomers resulting in a complex pattern. [f](T)=Taxol, (C)=cephalomannine.

equivalents of oxalyl chloride over a 12 hour period. After quenching with water in acetone the crude product was isolated. A single highly polar spot was detected by TLC. All attempts to purify the crude product, including the use of Sephadex LH-20 which had worked so well on the side chain model, proved unsuccessful. The yield of the crude material was 65%.



2.38 R = 

2.39 R = Ph

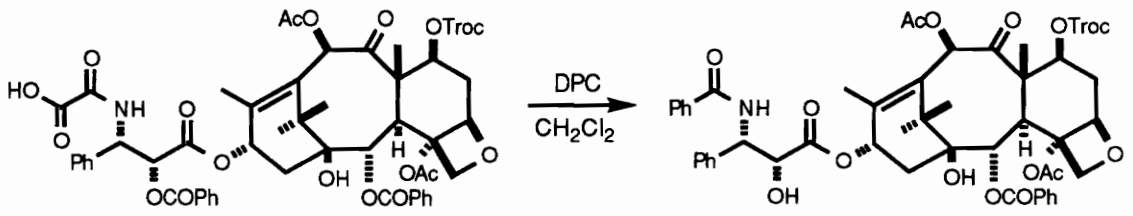
2.40

Scheme 2.20. Preparation of the oxamic acid derivative (**2.40**).

Even though purification was not possible, the crude material could be characterized by NMR. The major impurities which would be expected from the reaction are benzoic acid (N-benzoyl group from Taxol) and 2-methylbutanoic acid (hydrogenated tigloyl group from cephalomannine). Although it was not completely unambiguous, the ^{1}H NMR of the crude product provided significant evidence that supported the formation of the oxamic acid (**2.40**). The most obvious change in the proton spectrum is the disappearance of the two doublets at 6.25 ppm and 7.05 ppm which correspond to the amide protons of cephalomannine and Taxol, respectively. Formation of the oxamic acid (**2.40**), which is a

common intermediate, should result in the formation of a single doublet around 8.00-8.20 ppm. Unfortunately, the presence of aromatic protons in this region made identification of this new doublet impossible. The second noticeable change was the disappearance of the doublet of doublets at 6.05 ppm corresponding to the C-3' proton of Taxol and the two doublets of doublets at 5.90 ppm for the C-3' protons of the two diastereomeric dihydrocephalomannine molecules. These protons, which like the amide protons should become identical, apparently shift upfield slightly, but this is again difficult to see because they overlap with the C-2 and C-7 protons. The final piece of supporting evidence is the disappearance of a doublet at 7.77 ppm. This doublet has been assigned to the aromatic protons of the N-benzoyl group of Taxol. This disappearance would be consistent with the cleavage of the N-benzoyl group.

Next, the crude oxamic acid (**2.40**) was dissolved in dry methylene chloride containing diphenylcarbodiimide (DPC) and stirred at room temperature (*Scheme* 2.21). After 96 hours the reaction mixture was worked up and the crude product purified by preparative TLC.



2.40 **2.41**

Scheme 2.21. Synthesis of 7-Troctaxol.

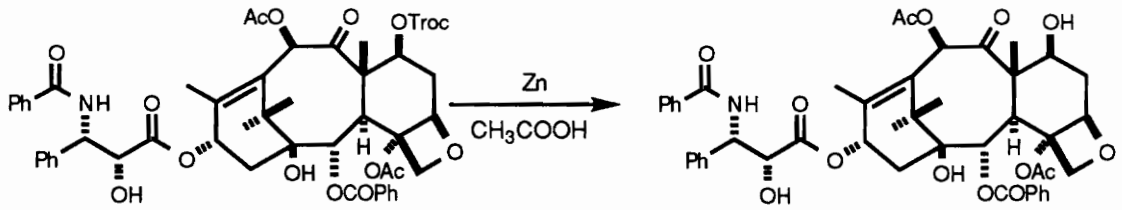
Characterization by ^1H NMR clearly indicated a single compound that was identified as 7-Troctaxol (**2.41**). The ^1H NMR spectrum of this compound was identical with an authentic sample of 7-Troctaxol.

Table 2.5. ^1H NMR Spectra of the Conversion Intermediates.[a, b]

Protons on	**2.40**	**2.41**
C-2	5.65[d]	5.68 (d, 6.9)
C-3	3.95 (d, 7)	3.95 (d, 6.9)
C-5	4.95 (d, 10)	4.95 (d, 9.8)
C-6α	*c*	2.60 (m)
C-6β		1.90 (m)
C-7	5.65[d]	5.54 (m)
C-10	6.35 (s)	6.24 (s)
C-13	6.17 (t, 9)	6.20 (t, 8.2)
C-14α	*c*	2.35[d]
C-14β		2.32[d]
C-16-CH_3	1.14 (s)	1.17 (s)
C-17-CH_3	1.21 (s)	1.25 (s)
C-18-CH_3	1.96 (s)	1.85 (s)
C-19-CH_3	1.81 (s)	1.84 (s)
C-20α	4.34 (d, 8)	4.34 (d, 8.2)
C-20β	4.17 (d, 8)	4.18 (d, 8.2)
C-2'	5.65[d]	4.79 (d, 2.5)
C-3'	5.65[d]	5.78 (dd, 8.9, 2.5)
3'-NH	8.15 (d, 80	7.05 (d, 8.9)
2-OBz, 3'-Ph	7.35-8.20	7.39-8.13
4-OAc	2.35 (s)	2.39 (s)
10-OAc	2.17 (s)	2.17 (s)
2'-OBz	7.35-8.02	
7-OCOOC$\underline{H}_2$CCl$_3$	4.67 (d, 12)[e]	4.64 (d, 12.0)
	5.05 (d, 12)	5.03 (d, 12.0)
3'-NBz		7.39-7.77

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, t=triplet, m=multiplet. [c]Signal hidden under other peaks. [d]Difficult to determine exact chemical shift or coupling constants due to overlapping peaks. [e]The two protons on the Troc group are diastereotopic.

The final step of the sequence is removal of the 7-Troc protecting group to give Taxol (*Scheme* 2.22). Protocols for this cleavage are well established. Consequently, treatment of 7-Troctaxol (**2.41**) with zinc in acetic acid led to Taxol (**2.2**). The ^{1}H NMR spectrum of this compound was identical with an authentic sample of Taxol (Table 2.6).



2.41 **2.2**

Scheme 2.22. Conversion to Taxol.

The isolation of pure Taxol completed the sequence and demonstrated the feasibility of the route. Unfortunately, the overall yield at this point was a disappointing 28 %. The two low yielding steps in the sequence were the conversion to the oxamic acid (65% yield) and the DPC cleavage of the oxamic acid (50%), but it should be kept in mind that these steps have not been optimized.

In light of the fact that the overall yield was less than the original amount of Taxol present in the original mixture, the question might arise as to whether any cephalomannine was actually converted to Taxol or whether the Taxol in the mixture was simply "carried through" the sequence. Since all attempts to purify the key oxamic acid intermediate

Table 2.6. ^{1}H NMR Spectrum of Taxol Prepared from Cephalomannine.[a,b]

Protons on	Taxol (Prepared)	Taxol (Literature)
C-2	5.67 (d, 7.0)	5.67 (d, 7.1)
C-3	3.80 (d, 7.0)	3.79 (dd, 7.1, 1.0)
C-5	4.94 (d, 7.6)	4.94 (ddt, 9.6, 6.7, 4.3)
C-6α	2.54 (m)	2.54 (ddd, 14.8, 9.7, 6.7)
C-6β	1.88 (m)	1.88 (ddd, 17.7, 11.0, 2.3)
C-7	4.40 (m)	4.40 (ddd, 10.9, 6.7, 4.3)
C-10	6.27 (s)	6.27 (s)
C-13	6.23 (t, 8)	6.23 (tq, 8.0, 1.5)
C-14α	2.35 (m)	2.35 (dd, 15.4, 9.0)
C-14β	2.28 (m)	2.28 (ddd, 15.3, 9.0, 0.6)
C-16-CH_3	1.14 (s)	1.14 (s)
C-17-CH_3	1.25 (s)	1.24 (s)
C-18-CH_3	1.79 (s)	1.79 (s)
C-19-CH_3	1.68 (s)	1.68 (s)
C-20α	4.30 (d, 8.4)	4.30 (ddd, 8.4, 1.1, 0.8)
C-20β	4.19 (d, 8.3)	4.19 (dd, 8.5, 1.0)
C-2'	4.79 (br s)	4.78 (d, 2.7)
C-3'	5.79 (dd, 8.8, 2.6)	5.78 (dd, 8.9, 2.8)
3'-NH	6.99 (d, 8.8)	7.01 (d, 8.9)
4-OAc	2.39 (s)	2.38 (s)
10-OAc	2.24 (s)	2.23 (s)
2-OBz, 3'-NBz, 3'-Ph	7.32-8.15	7.35-8.13

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet.

(2.40) failed, some uncertainty is introduced. However, several facts argue strongly in favor of the success of the conversion. First, the conversion process clearly worked on the side chain model. Since pure cephalomannine side chain was used in the model study, there would be no Taxol side chain to "carry through"; thus, the process is viable. Second, while the oxamic acid intermediate could not be purified,

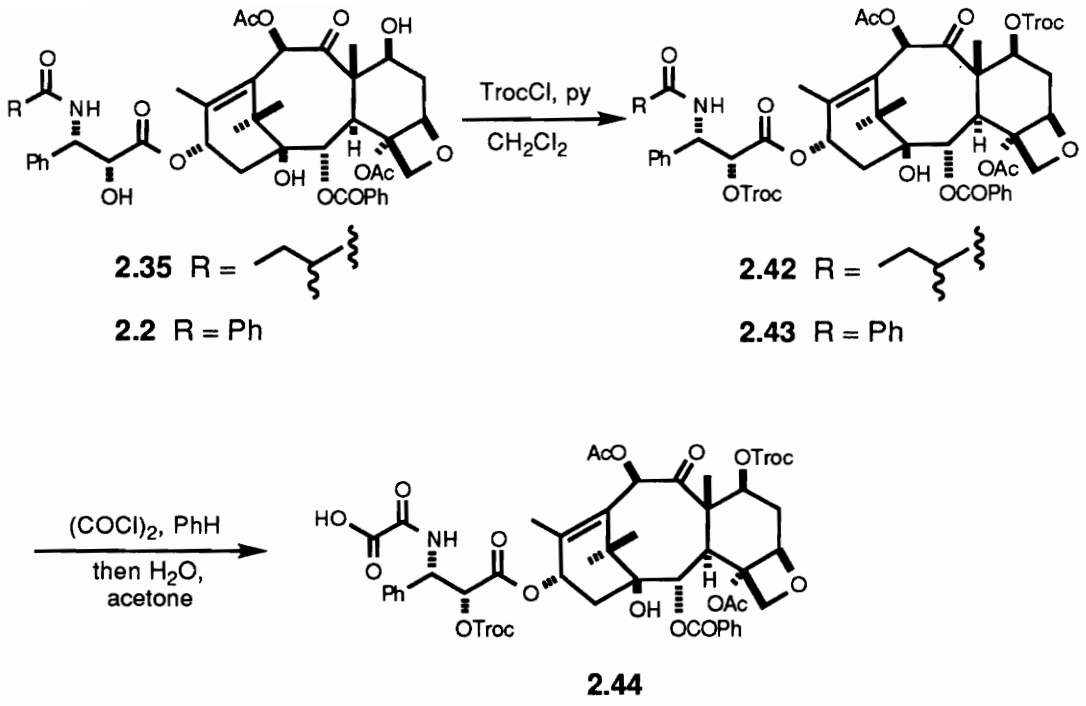
characterization of the crude product supported the presence of intermediate (**2.40**) and the absence of Taxol. Key evidence included the fact that the crude product was a single highly polar spot by TLC, analysis of the ^{1}H NMR spectrum (as was discussed earlier), and confirmation by FABMS and HRFABMS of a molecular weight consistent with (**2.40**). Moreover, the low resolution FAB mass spectrum did not contain any peaks that would support the presence of unreacted Taxol from the original mixture. Third and finally, if Taxol was simply "carried through", hydrolysis of the 2'-benzoate would have had to occur in either the oxalyl chloride reaction or in the subsequent treatment with diphenylcarbodiimide (DPC). Model studies (discussed in Section 3.2.1) clearly established the stability of the 2'-benzoate in the oxalyl chloride reaction and carbodiimides are noted for their ability to create esters, not cleave them, thus, cleavage of the 2'-benzoate is highly unlikely. When these facts are considered, the reasonable conclusion is that the conversion did take place.

Concurrently with the investigation of the sequence to convert cephalomannine to Taxol, the related goal of being able to create any N-acyl analog was examined. This goal was approached from two directions. First, the attempt was made to find a protecting group that could be used on both the 2' and 7 hydroxyls that would allow the preparation of the free amine upon cleavage of the oxamic acid with DPC. Acylation with an acid chloride and tertiary amine would then yield any desired N-acyl analog. Second, the use of different alcohols and amines

as quenches for the oxalyl chloride reaction was explored. This would allow the preparation of a series of N-oxalyl analogs.

Studies that focused on the preparation of the free amine led to the investigation of a number of protecting groups. Model studies on the cephalomannine side chain methyl ester (**2.24**) revealed that the triethylsilyl (TES) and 2,2,2-trichloroethoxymethyl (TCEM) protecting groups would not survive the oxalyl chloride reaction conditions. The benzyloxycarbonyl (Cbz) group gave some hopeful indications in the model studies, but all attempts to prepare the 2', 7-diCbz derivative of Taxol or cephalomannine proved unsuccessful. It was found that the Cbz group readily went on the 2' hydroxyl of either molecule, but the 7 hydroxyl was unreactive. Attempts to introduce the benzyl (Bn) group were similarly unsuccessful. The most productive work in this area was obtained using the 2,2,2-trichloroethoxycarbonyl (Troc) group.

The preparation of the 2', 7-(2,2,2-trichloroethoxycarbonyl) derivative of Taxol has previously been reported.[58] As was done in the conversion studies, a mixture of Taxol/dihydrocephalomannine was used here to prepare the 2', 7-diTroc derivatives. Thus, exposure of the Taxol (**2.2**)/dihydrocephalomannine (**2.35**) mixture to 2,2,2-trichloroethyl chloroformate (TrocCl) in methylene chloride in the presence of pyridine led to the desired 2', 7-diTroc analogs (**2.42** and **2.43**) (*Scheme* 2.23). This diTroc analog mixture was then treated with 6 equivalents of oxalyl chloride over a 24 hour period and the reaction monitored by TLC. After a water/acetone quench, a single highly polar spot was detected. Unlike



Scheme 2.23. Preparation of the 2',7-diTroc oxamic acid analog.

the 2'-benzoyl analog, purification of the crude product was accomplished with Sephadex LH-20 with methylene chloride as the mobile phase.

Characterization of the resulting product by ^1H NMR clearly indicated the formation of the 2', 7-diTroc oxamic acid derivative (**2.44**). Spectral evidence included the presence of the amide doublet at 8.05 ppm and the disappearance to the protons associated with the N-benzoyl and N-tigloyl groups of Taxol and cephalomannine.

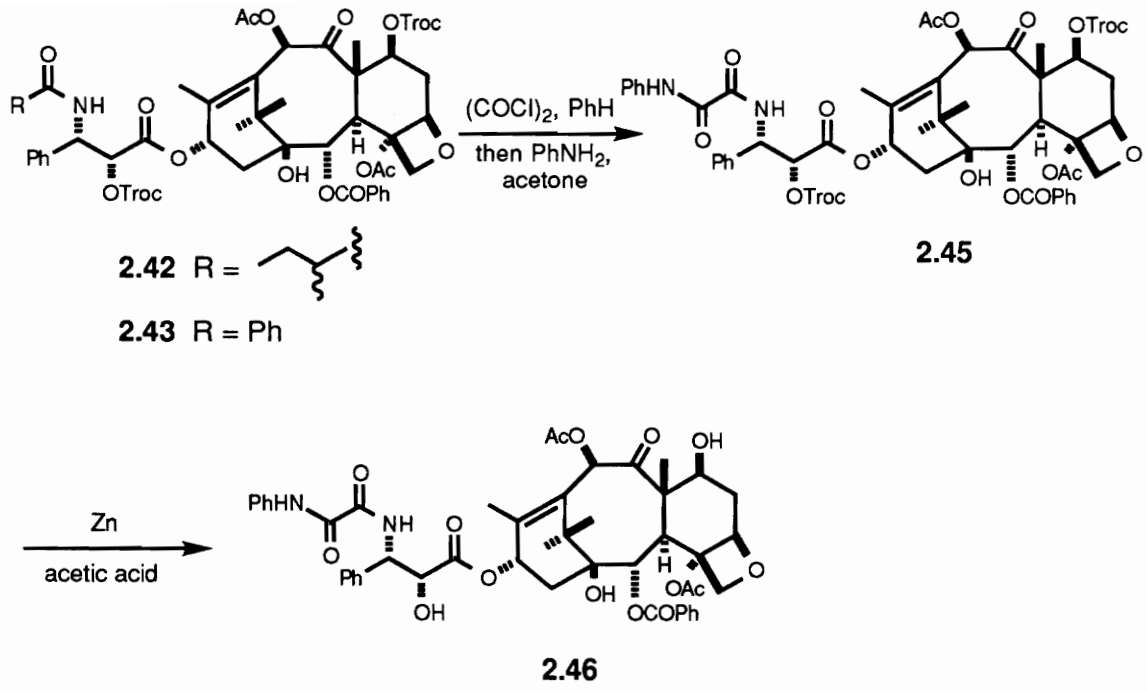
Although the 2' 7-diTroc analog mixture (**2.42** and **2.43**) was readily converted to the oxamic acid analog (**2.44**), all efforts to form the free amine from this derivative proved unsuccessful. When derivative

(**2.44**) was treated with diphenylcarbodiimide in methylene chloride the result was a complex mixture (>10 TLC spots) and a major product could not be isolated.

While a considerable amount of information was gained about the use of protecting groups on Taxol, the failure to find one suitable for generation of the free amine prompted a change in the course of research on the synthesis of analogs. Attention was next turned to the investigation of different alcohols and amines as quenches for the oxalyl chloride reaction.

Studies aimed at the preparation of N-oxalyl analogs by quenching the oxalyl chloride reaction with alcohols and amines proved slightly more successful. The 2', 7-diTroc derivatives of the Taxol (**2.43**)/ dihydrocephalomannine (**2.42**) mixture was again prepared using standard conditions. When this diprotected mixture were exposed to oxalyl chloride in HPLC grade benzene and then quenched with aniline a single product was detected by TLC. After purification by preparative TLC, characterization by ^{1}H NMR indicated the product was 2', 7-diTroc-N-debenzoyl-N-(phenyloxamido)taxol (**2.45**) (*Scheme* 2.24). Deprotection using zinc in acetic acid led to N-debenzoyl-N-(phenyloxamido)taxol (**2.46**). Characterization by ^{1}H NMR, ^{13}C NMR, and mass spectroscopy confirmed the identity of the product (see Table 2.7 for ^{1}H NMR assignments).

With the initial success of an amine for a quench, the efforts in this area were expanded. The results of this work will be discussed in detail in the next section 3.2.1.



Scheme 2.24. Synthesis of N-debenzoyl-N-(phenyloxamido)taxol.

These studies had clearly demonstrated the utility of Shiozaki's amide cleavage methodology in the preparation of N-acyl analogs of Taxol. The remaining goal was the test of this procedure on pure cephalomannine and optimization of the reaction conditions. A little over a year after the initial experiment, a mixture containing 90% cephalomannine was made available by Bristol-Myers Squibb and as a result, the pursuit of the final goal began.

The 2'-benzoyl-7-Trocdihydrocephalomannine analog (**2.38**) was prepared as described for the mixture. When this derivative was treated with oxalyl chloride and then quenched with water a single highly polar

Table 2.7. ^{1}H NMR Spectra of N-Oxalyl Taxol Analogs.[a, b]

Protons on	**2.44**	**2.46**
C-2	5.68 (d, 7.0)[c]	5.64 (d, 7.1)
C-3	3.93 (d, 6.7)	3.79 (d, 6.8)
C-5	4.97 (d, 8.0)	4.93 (d, 7.7)
C-6α	2.63 (m)	2.50 (m)
C-6β	*d*	1.82 (m)
C-7	5.57 (m)	4.39 (br t)
C-10	6.35 (s)	6.25 (s)
C-13	6.18 (t, 8)	6.25 (t, 10.4)
C-14α	*d*	2.30 (m)
C-14β	*d*	2.18 (m)
C-16-CH_3	1.16 (s)	1.12 (s)
C-17-CH_3	1.23 (s)	1.22 (s)
C-18-CH_3	1.92 (s)	1.87 (s)
C-19-CH_3	1.82 (s)	1.67 (s)
C-20α	4.34 (d, 8.4)	4.29 (d, 8.3)
C-20β	4.16 (d, 8.0)	4.17 (d, 8.5)
C-2'	5.43 (d, 3.9)	4.71 (dd, 4.9, 2.6)
C-3'	5.65[c]	5.57 (dd, 9.6, 2.6)
3'-NH	8.01 (d, 9.5)	8.37 (d, 9.7)
4-OAc	2.41 (s)	2.37 (s)
10-OAc	2.17 (s)	2.22 (s)
2-OBz, 3'-Ph	7.36-8.12	7.28-8.10
2'-$OCOOCH_2CCl_3$	4.85 (d, 11.9)	
	4.74 (d, 11.9)	
7-$OCOOCH_2CCl_3$	5.00 (d, 11.9)	
	4.64 (d, 11.9)	
oxalyl-NH		8.98 (s)
N-Ph		7.24-7.61

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet. [c]The C-2 and C-3' overlap so the exact chemical shifts and coupling constants cannot be determined. [d]Difficult to determine exact chemical shifts since the signals are partially hidden under methyl of acetate signals.

spot was observed by TLC. Again attempts to purify this compound failed. When the crude material was treated with DPC a complex mixture (>12 TLC spots) was obtain. Efforts to reproduce this and much of the earlier work led to the discovery that the reaction of oxalyl chloride with the protected substrates to created the key oxamic acid or oxamate ester intermediates had become erratic rarely giving any of the desired products. Puzzlingly, it was found that identical conditions could be used several times in a row and each time different results would be obtained.

Substantial time and effort was spent trying to identify the problem. Unfortunately, the original bottle of oxalyl chloride, the original solvent, and the original Taxol/cephalomannine mixture were no longer available. Numerous studies which focused on the solvent, time of reaction, temperature, and substrate failed to reestablish the success of the reaction. This was quite surprising since the reaction had been run successfully over 35 times using several substrates (e.g. cephalomannine side chain methyl ester, Taxol side chain methyl ester, and the Taxol/cephalomannine mixture), with several different solvents (e.g. benzene, methylene chloride, and THF), at varying lengths of time (3-24 hours), and at different temperatures (5°, 20°, and 35°C) over a 18 month period.

The earlier success of the oxalyl chloride reaction under a variety of conditions and with several substrates coupled with the later failure under all conditions with all substrates seemed to indicate the problem was with the oxalyl chloride. Although several different bottles and even

brands of oxalyl chloride were used, conditions that would establish the consistency of the original work were not found.

Although the sudden inability to reproduce the success of the earlier oxalyl chloride reactions casts shadows on the conversion sequence, the reliability of the original work is not in question. As was previously mentioned, the conversion of the cephalomannine side chain model to the Taxol side chain clearly occurred while the evidence strongly suggests the same conversion happened with the Taxol/cephalomannine mixture. Furthermore, the preparation of the diTroc oxamic acid derivative (**2.44**) and the N-(phenyloximido) analog (**2.46**) also corroborates the success of the oxalyl chloride reaction and conversion sequence.

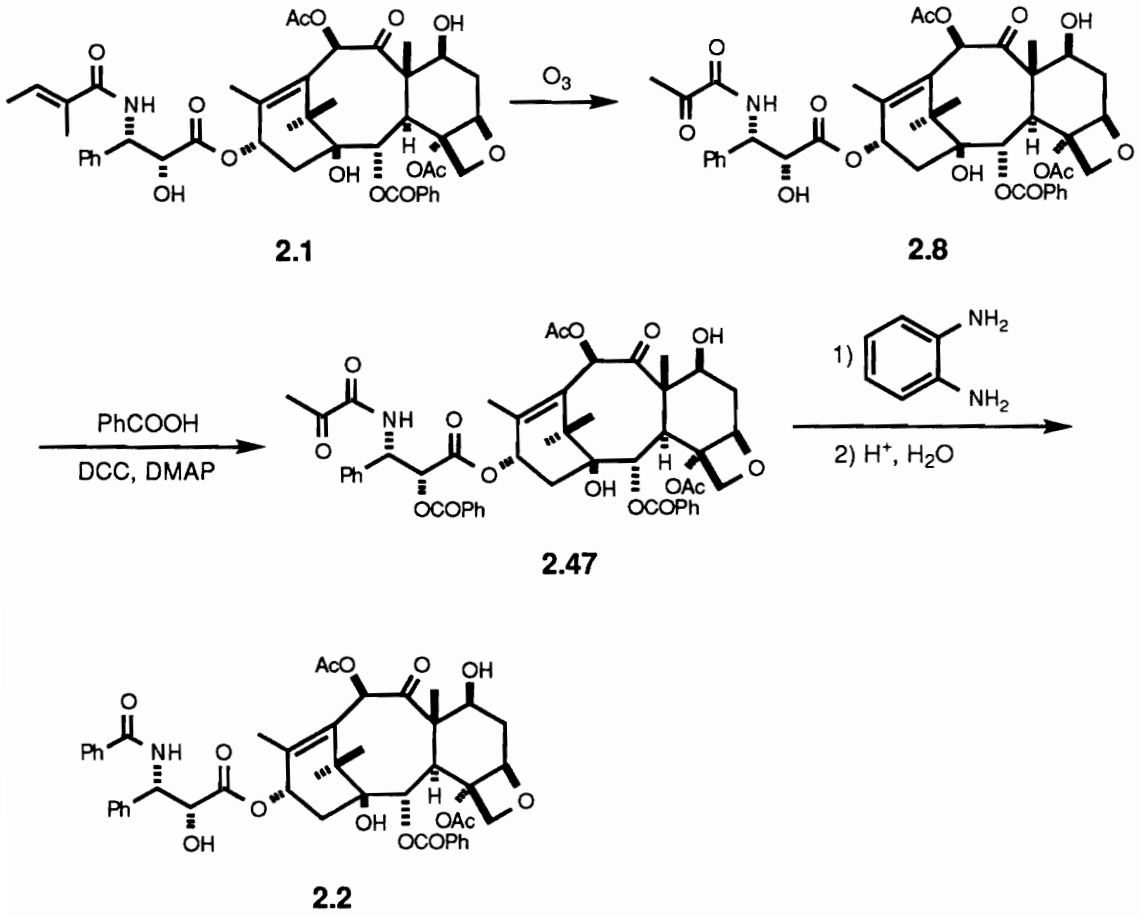
2.3. Conclusions

When the conversion sequence became erratic it created an entirely new problem. It was recognized that a considerable amount of time and effort would be required (in addition to that already spent) in order to uncover the problem. Although continued pursuit of this chemistry was of interest from an academic standpoint, the results in hand and new developments in the area of the Taxol chemistry made this course of action appear unprofitable for several reasons.

First, it should be kept in mind that the goal was to convert cephalomannine to Taxol. While this goal was achieved, the yield was disappointingly low. Consequently, the amount of Taxol produced

turned out to be less than what was in the original mixture. Although it is possible that the yields could be improved if consistency of the reaction were to be reestablished, it is not likely that they would be quantitative, which would be necessary if this route were to be practical.

Second, and perhaps even more significantly, Dr. Leslie Gunatilaka discovered a new and more efficient route.[56] Dr. Gunatilaka's route was based on work originally performed by Jitrangsri and is outlined in *Scheme* 2.25. This sequence began with the ozonolysis of



Scheme 2.25. Gunatilaka's cephalomannine to Taxol conversion.

cephalomannine (**2.1**) to generate the diketo analog (**2.22**). This analog was then benzoylated using DCC/DMAP to give (**2.47**). This set the stage for the key step. When (**2.47**) was treated with 1, 2-phenylenediamine in dry benzene and a catalytic amount of acid, the N-pyruvyl group was cleaved. Under the reaction conditions, the 2'-benzoyl group spontaneously transferred to yield Taxol (**2.2**). In contrast to the conversion route described herein, this sequence consisted of only three steps, each high yielding.

Finally, intense efforts by other research groups have uncovered new sources of Taxol.[159] Recent work found that significant amounts of baccatin III could be obtained from yew needles.[51] The application of one of the growing number of side chain syntheses and coupling methods allows for the hemisynthesis of Taxol or Taxotere.[131, 135-138] Since the needles are a renewable source, this approach has attracted considerable attention and seems to be the direction for the future.

2.4. Experimental

GENERAL EXPERIMENTAL PROCEDURES.- All nonaqueous reactions were performed in oven dried glassware. Those noted were performed under an inert atmosphere. Tetrahydrofuran was distilled over sodium with benzophenone ketyl. Benzene, toluene, and methylene chloride were distilled over CaH_2. The ^1H NMR spectra were recorded on either a Bruker WP-270 spectrometer operating at 270 MHz or a Varian Unity 400 spectrometer operating at 400 MHz. All ^{13}C NMR spectra were

obtained on a Varian Unity 400 spectrometer operating at 100.57 MHz. Analytical TLC was performed on aluminum-backed Kieselgel 60 F_{254} plates (E. Merck) with a 0.2-mm layer. Preparative TLC was performed on silica gel GF plates (20 x 20 cm) that were either 500 or 1000 μm thick (Analtech). The compounds were extracted from the silica gel using either acetone or ethyl acetate and the solvent was removed *in vacuo*. Taxol derivatives were visualized on TLC plates by spraying with an anisaldehyde/H_2SO_4 solution and then heating.

CEPHALOMANNINE SIDE CHAIN METHYL ESTER (**2.24**)- The cephalomannine side chain methyl ester was prepared according to the methodology of DeAmicis.[153] A 1.3690 g (27% overall yield) quantity of the cephalomannine side chain methyl ester (**2.24**) was obtained as a pale yellow oil that slowly crystallized over a long period of time: ^{1}H NMR δ 7.26-7.40 (overlapping m, 5H, 3'-Ph), 6.57 (d, J=9.0, 1H, NH), 6.43 (qd, J=6.9, 0.9, 1H, C-3"H), 5.57 (dd, J=9.1, 2.0, 1H, C-3'H), 4.55 (br s, 1H, C-2'H), 3.81 (s, 3H, OMe), 1.83 (br s, 3H, C-5"H_3), 1.74 (dd, J=6.9, 0.9, 3H, C-4"H_3); EIMS *m/z* (rel. int) $[M]^+$ 278 (1), 259 (2) 231 (2), 200 (5), 188 (20), 83 (100).

TAXOL SIDE CHAIN METHYL ESTER (**2.25**)- The Taxol side chain methyl ester was prepared according to the methodology of DeAmicis.[153] A 0.7675 g (22% overall yield) quantity of the Taxol side chain methyl ester (**2.25**) was obtained as a white solid: mp 160-162°C (lit.[55] mp 178-180°C); ^{1}H NMR δ 7.30-7.78 (overlapping m, 10H, 3'-Ph, PhCO), 6.98 (d,

J=8.6, 1H, NH), 5.75 (dd, J=9.1, 1.8, 1H, C-3'H), 4.64 (br s, 1H, C-2'H), 3.85 (s, 3H, OMe).

2'-BENZOYLCEPHALOMANNINE SIDE CHAIN METHYL ESTER (**2.26**)- A 100 mg (0.36 mmoles) quantity of (**2.24**) was dissolved in 2 mL of dry methylene chloride. The solution was cooled in an ice bath and then 46 μL (0.397 mmoles, 1.1 eq.) of benzoyl chloride and 75 μL (0.54 mmoles, 1.5 eq.) of triethylamine were added. The reaction was warmed to room temperature and stirred for 1 hour. At this point, an additional 46 μL of benzoyl chloride was added. The solution was stirred for another hour and then diluted with 10 mL of CH_2Cl_2 and washed with 2 N HCl (1 x 20 mL), 0.5 N NaOH (1 x 20 mL), and water (1 x 20 mL). After drying ($MgSO_4$) the solvent was removed *in vacuo*. The crude product was purified by flash chromatography using 230-400 mesh silica gel 60 (10:10:1 chloroform/hexane/acetone) to give 99 mg (72%) of the 2'-benzoylcephalomannine side chain methyl ester (**2.26**) as a clear oil: ^{1}H NMR δ 7.25-8.20 (overlapping m, 10H, 3'-Ph), 6.81 (d, J=8.9, 1H, NH), 6.50 (q, J=6.9, 1H, C-3"H), 5.82 (dd, J=8.9, 3.0, 1H, C-3'H), 5.57 (d, J=3.1, 1H, C-2'H), 3.75 (s, 3H, OMe), 1.84 (br s, 3H, C-5"H_3), 1.74 (d, J=6.9, 3H, C-4"H_3).

REACTION OF THE 2'-BENZOYLCEPHALOMANNINE SIDE CHAIN METHYL ESTER (**2.26**) WITH OXALYL CHLORIDE AND A METHANOL QUENCH- A 99 mg (0.26 mmoles) quantity of (**2.26**) was dissolved in 3 mL of benzene (Aldrich HPLC grade) and cooled in an ice bath. To this solution 45 μL

(0.52 mmoles, 2 eq.) of oxalyl chloride was added. The solution was stirred at 0-5°C for 30 minutes and then warmed to room temperature. After 90 minutes, the solution was again cooled in an ice bath and 45 µL (0.52 mmoles, 2 eq.) of oxalyl chloride was again added. After stirring 30 minutes, the reaction was quenched by adding 3 mL of methanol. The resulting mixture was stirred for 30 minutes at 0-5°C and then warmed to room temperature. After 4 hours, the solvent was removed under vacuum. The crude product was purified by flash chromatography using 230-400 mesh silica gel 60 (10:9:1 hexane/chloroform/acetone) to yield 36 mg (36%) of (**2.27**) as a clear oil: ^{1}H NMR δ 7.25-8.20 (overlapping m, 10H, 3'-Ph, 2'-OCOPh), 8.03 (overlapping d, NH), 5.76 (dd, J=9.1, 3.1, 1H, C-3'H), 5.61 (d, J=3.1, 1H, C-2'H), 3.92 (s, 3H, COCOMe), 3.77 (s, 3H, OMe); and 51 mg (42%) (**2.29**) as a crystalline solid; ^{1}H NMR δ 7.25-8.02 (overlapping m, 10H, 3'-Ph, 2'-OCOPh), 6.36 (d, J=10.7, 1H, C-3'H), 6.13 (d, J=10.7, 1H, C-2'H), 5.99 (qd, J=6.9, 1.3, 1H, C-3"H), 3.66 (s, 3H, COCOMe), 3.53 (s, 3H, OMe), 1.76 (s, 3H, C-5"H_3), 1.53 (d, J=6.9, 3H, C-4"H_3).

2'-BENZOYLDIHYDROCEPHALOMANNINE SIDE CHAIN METHYL ESTER (**2.30**)- A 200 mg (0.721 mmoles) quantity of (**2.24**) was dissolved in 5 mL of EtOAc and 24 mg of PtO_2 was added. The flask was attached to a hydrogenation apparatus and flushed with H_2 gas. After 2 hours, the flask was removed and the solution was filtered through Celite to remove the catalyst, then evaporated to dryness. The result was 200.9 mg (99.8%) of dihydrocephalomannine: ^{1}H NMR δ 7.26-7.38 (overlapping m,

5H, 3'-Ph), 6.39 (d, J=8.7, 1H, NH), 5.56 (dd, J=9.2, 2.0, 1H, C-3'H), 4.53 (br s, 1H, C-2'H), 3.80 (s, 3H, OMe), 2.15 (m, 1H, C-2"H), 1.62 and 1.40 (m, 2H, C-3"H_2), 1.10 (m, 3H, C-5"H_3), 0.89 (m, 3H, C-4"H_3). This product was used in the next step without further purification.

The sample was dissolved in 5 mL of CH_2Cl_2 and cooled in an ice bath. To this solution 83 μL (0.72 mmoles, 1.0 eq) of benzoyl chloride and 100 μL (0.72, 1.0 eq.) of Et_3N were added. The mixture was stirred at 1°C for 1 hour and then warmed to room temperature. After an additional hour of stirring the solution was diluted with 15 mL of CH_2Cl_2 and washed with 2 N HCl (1 x 20 mL) and water (1 x 20 mL). After drying ($MgSO_4$), the solvent was removed under vacuum. The crude product was purified by flash chromatography using 230-400 mesh silica gel 60 (10:9:1 hexane/chloroform/acetone) to yield 247.3 mg (90%) of the 2'-benzoyldihydrocephalomannine side chain methyl ester (**2.30**): ^1H NMR δ 7.26-8.00 (overlapping m, 10H, 3'-Ph, 2-OCOPh), 6.59 (d, J=9.1, 1H, NH), 5.83 (m, 1H, C-3'H), 5.56 (d, J=3.2, 1H, C-2'H), 3.73 (s, 3H, OMe), 2.24 (m, 1H, C-2"H), 1.69 and 1.44 (m, 2H, C-3"H_2), 1.14 (m, 3H, C-5"H_3), 0.89 (m, 3H, C-4"H_3).

2'-BENZOYL-N-DETIGLOYL-N-OXALYLCEPHALOMANNINE SIDE CHAIN METHYL ESTER (**2.31**)- A 247.3 mg (0.645 mmole) sample of (**2.30**) was dissolved in 5 mL of HPLC grade benzene (Aldrich sureseal) in a flask that had been flushed with argon. The solution was cooled in an ice bath and then 113 μL (1.29 mmoles, 2 eq.) of oxalyl chloride was added. The solution was stirred at 0°C for 30 minutes and then warmed to room

temperature. After 2 hours, the solution was again cooled in an ice bath and an additional 56 μL of oxalyl chloride was added. Approximately 30 minutes after this addition, the reaction was quenched with 5 mL of acetone and 2 mL of water and stirred at 0°C for 2 hours. The solution was then diluted with 5 mL of EtOAc and washed with 40 mL of 1 N HCl. After drying ($MgSO_4$) the solvent was removed under vacuum. The crude product was purified by column chromatography using Sephadex LH-20 (9:1 chloroform/acetone). The result was 197.1 mg (82%) of oxamic acid (**2.31**): ^{1}H NMR δ 8.39 (d, J=8.7, 1H, NH), 7.30-7.78 (overlapping m, 10H, 3'-Ph, PhCO), 5.71 (dd, J=9.1, 3.3, 1H, C-3'H), 5.63 (d, J=3.4, 1H, C-2'H), 3.71 (s, 3H, OMe).

TAXOL SIDE CHAIN METHYL ESTER (**2.25**)- A 46.0 mg (0.13 mmole) quantity of (**2.31**) was dissolved in 1 mL of dry CH_2Cl_2 and cooled to 1°C in an ice bath. To this solution 0.7 mL of a 0.20 M solution of diphenyl-carbodiimide was added. The solution was stirred at 0-5°C for 5 hours and then an additional 0.1 mL aliquot of DPC was added. After stirring overnight, the solution was warmed to room temperature and stirred for an additional 2 days. The solution was diluted with 10 mL of EtOAc and washed with saturated $NaHCO_3$ (1 x 10 mL), water (1 x 10 mL), and brine (1 x 10 mL). After drying ($Mg SO_4$), the solvent was removed *in vacuo*. The crude product was purified by flash chromatography using 230-400 mesh silica gel 60 (1:1 ethyl acetate/hexane) to afford 15.9 mg (46%) of the Taxol side chain methyl ester (**2.25**) which was spectroscopically identical to an authentic sample: ^{1}H NMR δ 7.30-7.78 (overlapping m,

10H, 3'-Ph, PhCO), 6.98 (d, J=8.6, 1H, NH), 5.75 (dd, J=9.1, 1.8, 1H, 3'), 4.64 (br s, 1H, 2'), 3.85 (s, 3H, OMe); CIMS *m/z* $[MH]^+$ 300 (100), 282 (8), 210 (25), 122 (40), 105 (18).

HYDROGENATION OF CEPHALOMANNINE (**2.1**)/TAXOL (**2.2**) MIXTURE- A 262 mg quantity of an approximately 50/50 mixture of Taxol and cephalomannine was dissolved in 10 mL of ethyl acetate in a 100 mL round bottom flask. Then 20 mg of PtO_2 was added and the flask was attached to the hydrogenation apparatus. After flushing the flask five times with hydrogen gas, the solution was allowed to stir at room temperature. After 1 hour, the flask was removed from the hydrogenation apparatus and the solution filtered through Celite to remove the catalyst. The resulting solution was then evaporated to dryness under vacuum. The result was 262 mg (100%) of a mixture of Taxol (**2.2**) and dihydrocephalomannine (**2.35**): ^1H NMR see Table 2.4. The product was used in the next step without further purification.

2'-BENZOYL DIHYDROCEPHALOMANNINE (**2.36**)/TAXOL (**2.37**)- A 251 mg quantity of a Taxol and dihydrocephalomannine mixture was dissolved in 10 mL of dry acetonitrile in a 50 mL round bottom flask that had been flushed with argon and equipped with magnetic stirring. To this solution, 41 mg of benzoic acid and 94 mg of dicyclohexylcarbodiimide were added. The solution was stirred at room temperature, and the reaction was monitored by TLC (Kieselgel 60 F_{254}, 6:4 ethyl acetate/hexane). After five hours, the reaction was stopped by

evaporating the solvent under vacuum. The crude product was purified by flash chromatography using 230-400 mesh silica gel 60 (250 mm x 25 mm bed with a 9:1 chloroform/acetone eluent). The result was 268 mg (95%) of 2'-benzoyl dihydrocephalomannine (**2.36**)/Taxol (**2.37**). Because this sample was a mixture of two cephphalomannine diastereomers and Taxol complete characterization was not attempted. A ^{1}H NMR spectrum consistent with the structure was obtained, but the multiple overlapping peaks made it difficult to unambiguously assign the signals.

2'-BENZOYL-7-(2,2,2-TRICHLOROETHYLOXYCARBONYL) DIHYDROCEPHALOMANNINE (**2.38**)/TAXOL (**2.39**)- A 244 mg quantity of a 50/50 mixture of 2'-benzoyltaxol (**2.37**) and 2'-benzoyldihydrocephalomannine (**2.36**) was dissolved in 10 mL of dry methylene chloride in a 50 mL round bottom flask which had been flushed with argon. Then 54 μL of 2,2,2-trichloroethylchloroformate and 32 mL of dry pyridine were added. The solution was stirred at room temperature using magnetic stirring. The reaction was monitored by TLC (Kieselgel 60 F254, 9:1 chloroform/acetone). After 24 hours, the solution was diluted with 50 mL of ethyl acetate and washed with 2 N HCl (2 x 50 mL), water (2 x 50 mL), and brine (1 x 50 mL). After drying with magnesium sulfate, the solvent was removed under vacuum. The crude product was purified by flash chromatography using 230-400 mesh silica gel 60 (250 mm x 25 mm bed with a 9:3:1 chloroform/hexane/acetone eluent). The result was

246 mg (85%) of 2'-benzoyl-7-(2, 2, 2-trichloroethyloxycarbonyl) dihydrocephalomannine (**2.38**)/Taxol (**2.39**): ^{1}H NMR see Table 2.4.

2'-BENZOYL-7-(2, 2, 2-TRICHLOROETHYLOXYCARBONYL)-N-DEBENZOYL-N-OXALYLTAXOL (**2.40**)- A 228 mg quantity of 2'-benzoyl-7-(2, 2, 2-trichloroethyloxycarbonyl) dihydrocephalomannine (**2.38**)/Taxol (**2.39**) was dissolved in 5 mL of HPLC grade benzene (Aldrich sureseal) in a 25 mL round bottom flask that had been flushed with argon and equipped with magnetic stirring. To this solution, 54 μL of oxalyl chloride was added and the resulting solution was stirred at room temperature. After 5 hours, an additional 54 μL of oxalyl chloride was added. After 12 hours, the reaction was quenched by the addition of 5 mL of acetone and 1 mL of water. The solution was stirred for an additional hour, and then the solvent was removed under vacuum. The crude product was purified by chromatography using a preswelled Sephadex LH-20 column (250 mm x 25 mm bed). The column was eluted with 50 mL of methylene chloride followed by 100 mL of 9:1 methylene chloride/acetone. The result was 147 mg (65%) of 2'-benzoyl-7-Troc-N-debenzoyl-N-oxalyltaxol (**2.40**): ^{1}H NMR see Table 2.5; FABMS *m/z* (rel. int.) $[M-H+2Na]^+$ 1146 (25), 825 (65), 685 (60); HRFABMS *m/z* $[M-H+2Na]^+$ 1144.1904 ($C_{52}H_{51}NO_{19}{}^{35}Cl_3Na_2$ requires 1144.1916).

7-(2, 2, 2-TRICHLOROETHYLOXYCARBONYL)TAXOL (**2.41**)- A 13.7 mg quantity of diphenylcarbodiimide was dissolved in 2 mL of methylene chloride in a 15 mL round bottom flask that had been flushed with

argon. To this solution, 52 mg of (**2.40**) was added. The reaction was stirred at room temperature and monitored by TLC (Kieselgel 60 F_{254}, 6:4 ethyl acetate/hexane). After 96 hours the reaction was stopped by removing the solvent under vacuum. The crude product was purified by preparative TLC (Analtech taperplate, 63:30:7 methylene chloride/hexane/acetone). The result was 24.5 mg (50%) of 7-(2, 2, 2-trichloroethyloxycarbonyl)taxol (**2.41**) which was spectroscopically identical to an authentic sample: ^{1}H NMR see Table 2.5.

TAXOL (**2.2**)- A 24.5 mg quantity of (**2.41**) was dissolved in 2 mL of acetic acid and 20 mg of activated zinc dust was added. The resulting heterogeneous mixture was stirred at 40°C. After 2 hours, the solution was filtered to remove the zinc and diluted with 20 mL of ethyl acetate. It was then washed with saturated sodium bicarbonate (3 x 20 mL) and water (2 x 10 mL). The organic layer was dried over magnesium sulfate and the solvent removed under vacuum. The crude product was purified by flash chromatography using 230-400 mesh silica gel 60 (320 mm x 15 mm bed with a 1:1 acetone/hexane eluent). The result was 9.5 mg (47%) of (**2.2**) which was spectroscopically identical to an authentic Taxol sample: ^{1}H NMR see Table 2.6.

2', 7-DI(2, 2, 2-TRICHLOROETHYLOXYCARBONYL)-N-DEBENZOYL-N-OXALYLTAXOL (**2.44**)- A 55.5 mg sample of 2',7-diTroc dihydro-cephalomannine (**2.42**)/Taxol (**2.43**), prepared as described by Magri and Kingston[58], was dissolved in 2 mL of HPLC grade benzene (Aldrich

sureseal) and cooled in an ice bath. To this solution 20 μL of oxalyl chloride was added and then the solution was allowed to warm to room temperature. After 2 hours, an additional 20 mL aliquot of oxalyl chloride was added and stirring continued. After stirring overnight another 10 μL aliquot of oxalyl chloride was added to drive the reaction to completion. One hour after this final addition, a 1.5 mL portion of the reaction solution was quenched with 2 mL of acetone and 100 μL of water. After 1 hour the solvent was removed under vacuum. The crude product was purified by column chromatography using Sephadex LH-20 (9:1 methylene chloride/acetone) to give 21.1 mg (52%) of 2', 7-di(2, 2, 2-trichloroethyloxycarbonyl)-N-debenzoyl-N-oxalyltaxol (**2.44**): ^{1}H NMR see Table 2.6.

2', 7-Di(2, 2, 2-Trichloroethyloxycarbonyl)-N-Debenzoyl-N-(Phenyloxamido)taxol (**2.45**)- A 55.5 mg quantity of the 2', 7-diTroc dihydrocephalomannine (**2.42**)/Taxol (**2.43**) mixture was dissolved in 2 mL of HPLC grade benzene in a round bottom flask that had been flushed with argon. To this solution 20 μL of oxalyl chloride was added and the resulting solution was stirred at room temperature. After 2 hours, an additional 20 μL of oxalyl chloride was added. When 18 hours had passed, another 10 μL of oxalyl chloride was added to drive the reaction to completion. At approximately 20 hours, 0.5 mL of the reaction mixture was quenched by the addition of 2 mL of acetone and 25 μL of aniline. Approximately 1 hour after addition of the acetone-aniline quench, the reaction was stopped by removal of the solvent under

vacuum. The crude product was purified by flash chromatography using 230-400 mesh silica gel 60 (320 mm x 15 mm bed with a 10:9:1 hexane/methylene chloride/acetone eluent). The result was a yield of 12.3 mg (85%) of 2', 7-di(2, 2, 2-trichloroethyloxycarbonyl)-N-debenzoyl-N-(phenyloxamido)taxol (**2.45**): ^{1}H NMR d 9.00 (s, 1H, oxalyl-NH), 8.33 (d, J=9.8, 1H, NH), 7.12-8.17 (overlapping m, 15H, 3'-Ph, 2-OCOPh, oxalyl-Ph), 6.35 (s, 1H, C-10H), 6.21 (t, J=8.3, 1H, C-13H), 5.74 (dd, J=9.8, 3.7, 1H, C-3'H), 5.68 (d, J=6.9, 1H, C-2H), 5.55 (m, 1H, C-7H), 5.48 (d, J=3.7, 1H, C-2'H), 5.04 and 4.64 (ABq, J=12.0, 2H, 7-$OCO_2CH_2CCl_3$), 4.90 (d, J=8.0, 1H, C-5H), 4.86 and 4.72 (ABq, J=10.3, 2H, 2'-$OCO_2CH_2CCl_3$), 4.32 and 4.16 (ABq, J=8.4, 2H, C-20H_2), 3.94 (d, J=7.1, 1H, C-3H), 2.54 (s, 3H, 4-$OCOCH_3$), 2.17 (s, 3H, 10-$OCOCH_3$), 1.91 (s, 3H, C-18H_3), 1.81 (s, 3H, C-19H_3), 1.22 (s, 3H, C-17H_3), 1.14 (s, 3H, C-16H_3).

N-DEBENZOYL-N-(PHENYLOXAMIDO)TAXOL (**2.46**)- A 8.0 mg sample of (**2.45**) was dissolved in 1 mL of HPLC grade acetic acid and 30 mg of freshly activated zinc was added. After stirring at room temperature for 6 hours, the solution was diluted with 20 mL of EtOAc and washed with saturated Na_2CO_3 (2 x 15 mL), water (2 x 15 mL), and brine (1 x 15 mL). After drying ($MgSO_4$), the solvent was removed under vacuum. The crude product was purified by preparative TLC (3:2 ethyl acetate/hexane) or give 5.0 mg (87%) of N-debenzoyl-N-(phenyloxamido)taxol (**2.46**): mp 180-183°C; $[\alpha]^{23}_D$ = -69.4° (*c*=1.08, $CHCl_3$); ^{1}H NMR see Table 2.6; FABMS *m/z* (rel. int.) $[MH]^+$ 897.3 (2),

509.2 (4), 283.1 (10), 197 (20), 119.0 (100); HRFABMS m/z $[MH]^+$ 897.3428 ($C_{48}H_{53}N_2O_{15}$ requires 897.3431).

3. The Preparation of 3'-N-Acyl Analogs.

The search for new anticancer agents using bioassay-guided fractionation of plant extracts has led to the discovery of a significant number of novel active compounds. Once a new lead structure has been isolated and identified, the standard practice in drug research is to investigate the structure-activity relationships to determine the structural features essential for activity. Identification of the essential features opens the door for the preparation of derivatives with improved pharmacological properties (e.g. reduced toxicity, elimination of side effects, increased water solubility, and enhanced activity).[160] This approach has already proven successful with several anticancer agents including podophyllotoxin[161], the vinca alkaloids[162], and camptothecin.[163]

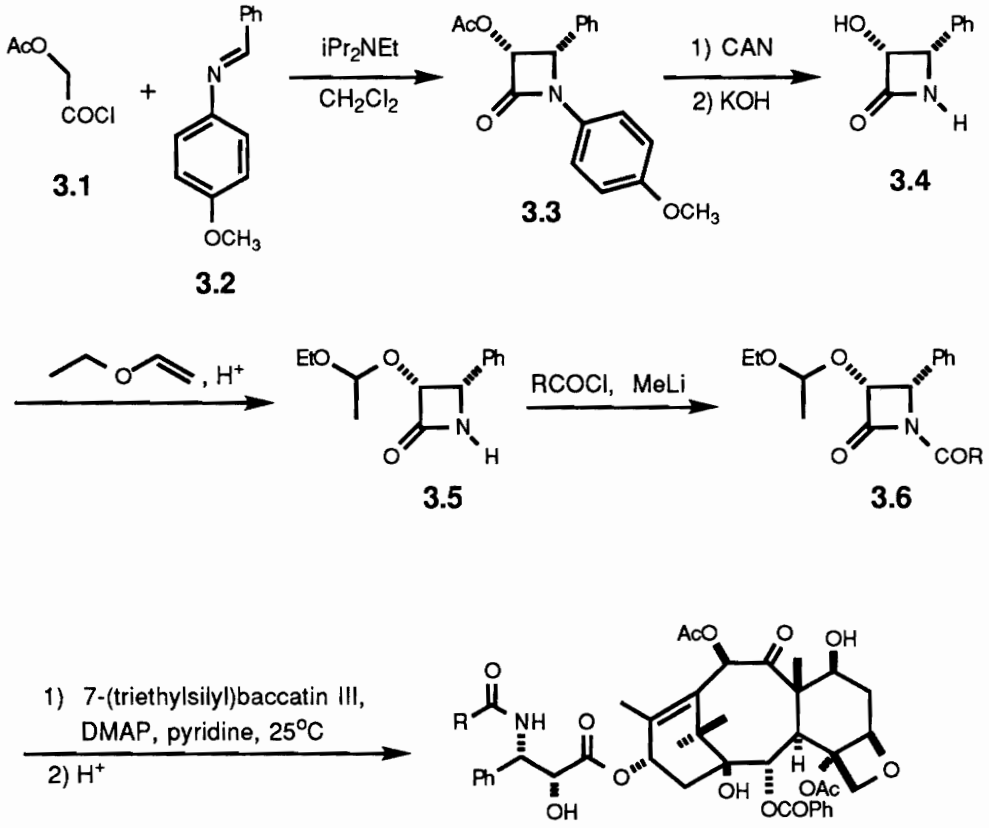
The exciting results observed in clinical trials plus Taxol's unique mechanism of action have stimulated an intense effort to determine the structural features required for its activity. The wide array of functional groups present as well as the unique chemistry of Taxol makes this a formidable challenge. A variety of derivatives have already been prepared[4, 59, 71, 89, 98 164-165] and structure-activity information is slowly beginning to accumulate[4], but perhaps the most significant discovery to come out of this work has been Taxotere, a semisynthetic 3'-

N-acyl analog.[5, 166] Taxotere, prepared by Potier and colleagues,[135] was found to possess slightly better activity than Taxol and consequently was selected for further development. Subsequent studies revealed that in addition to its enhanced activity, it also had better water solubility and, consequently, better bioavailability. These encouraging results, plus the development of an efficient semisynthesis process using a natural precursor (10-deacetylbaccatin III) isolated from a renewable source (yew needles), led to broader preclinical testing and finally to clinical trials.

The success of Taxotere fueled continued interest in the preparation of Taxol analogs, especially 3'-N-acyl analogs. During the course of an ongoing effort by Kingston's group to convert cephalomannine to Taxol, it was recognized that the N-tigloyl group of cephalomannine could readily be modified to create a host of derivatives. As previously mentioned, cephalomannine differs from Taxol only by the presence of the N-tigloyl group at the 3' position instead of a N-benzoyl group and thus, any modification of the tigloyl group would lead to a new 3'-N-acyl analog. Since cephalomannine almost always co-occurs with Taxol, it is readily available and even though it is slightly less active, the possibility exists that a simple modification could result in a derivative that is significantly more active or that has other pharmacological properties which have been improved. Thus, this section describes the results of an investigation which focused on the preparation of a variety of 3'-N-acyl analogs primarily through modification of the N-tigloyl group.

3.1. Background

The majority of 3'-N-acyl analogs which have been reported to date have been prepared by synthesizing a modified side chain and then coupling it to baccatin III. Holton and coworkers at Florida State have worked extensively in this area and have prepared a large number of 3'-N-acyl analogs using their β-lactam methodology (*Scheme* 3.1).[4] Holton's β-lactam methodology begins by coupling commercially available acetoxy acetyl chloride (**3.1**) with imine (**3.2**) to give β-lactam (**3.3**). Oxidative cleavage of the *p*-methoxyphenyl (PMP) protecting group with ceric ammonium nitrate (CAN) followed by hydrolysis of the acetate led to

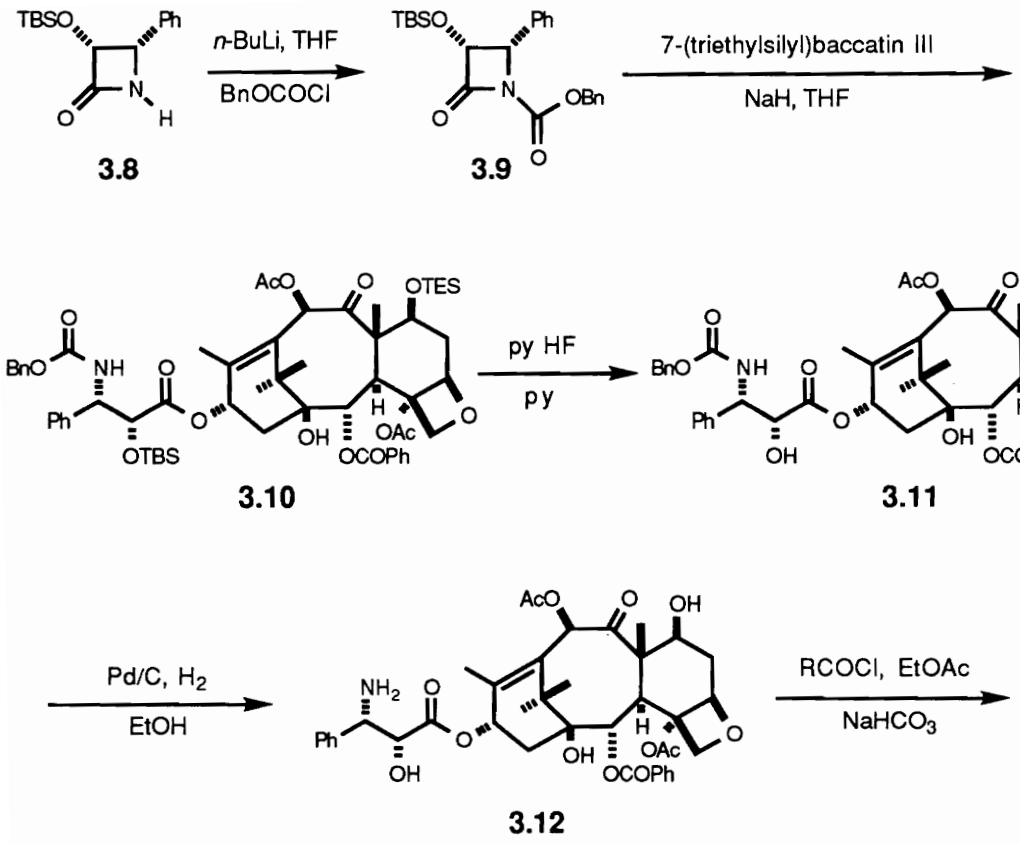


Scheme 3.1. Holton's route to 3'-N-acyl analogs.

(**3.4**). Reprotection of the hydroxyl with ethyl vinyl ether produced the key intermediate, (**3.5**), from which a variety of 3'-N-acyl analogs can be prepared. Thus, acylation of (**3.5**) to give side chain precursor (**3.6**), followed by coupling to 7-TES protected baccatin III and deprotection yields the desired analog (**3.7**). Unfortunately, proprietary reasons have kept information about the specific derivatives prepared and bioactivity data unavailable.

Georg *et. al.* have also reported the preparation of several 3'-N-acyl analogs using a β-lactam intermediate.[71] Their methodology focused on the enantioselective synthesis of β-lactam (**3.8**) as the key precursor to the N-benzoylphenylisoserine side chain of Taxol (*Scheme* 3.2). This 2-azetidinone intermediate (**3.8**), prepared using a chiral ester-enolate imine cyclocondensation, was then protected using benzyl chloroformate to give (**3.9**). Acylation of the allylic hydroxyl moiety of 7-(triethylsilyl)baccatin III with (**3.9**) produced (**3.10**) which was then deprotected using pyridinium HF to yield the Taxol analog (**3.11**). Hydrogenolysis of (**3.11**) led to free amine (**3.12**) which was then acylated with various acid chlorides under Schotten-Baumann conditions to yield 3'-N-acyl analogs (**3.13**), (**3.14**), and (**3.15**). Similar results were obtained using the *t*-butyloxycarbonate protecting group in place of the benzyloxycarbonate group. Interestingly, analogs (**3.13**) and (**3.14**) displayed activity comparable to Taxol's in the microtubule assembly assay, but were slightly less active in a B16 melanoma cell line assay.

A plethora of other side chain syntheses have been published and

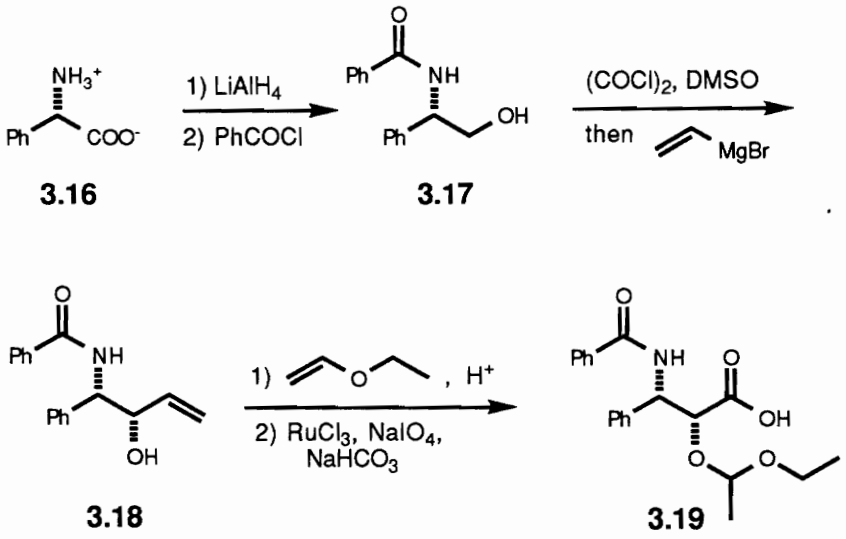


Scheme 3.2. Georg's route to 3'-N-acyl analogs.

most are versatile enough to allow for the preparation of 3'-N-acyl derivatives.[71, 130-132, 135-138, 167-171] The major problem many of these routes suffer from is the ability to couple the side chain to baccatin III. Early studies indicated that the C-13 hydroxyl group of baccatin III was very resistant to acylation and consequently, simple methods such as dicyclohexylcarbodiimide/4-dimethylaminopyridine coupling of the side chain acid are not very effective. Holton and Georg solved this problem by the use of β-lactam intermediates while others have attempted to solve it through the use of special protecting groups. Of all the published methods for the synthesis of the Taxol side chain, two stand out and are of particular interest here, even though neither has apparently been used to prepare 3'-N-acyl analogs.

A 1991 publication[168] by Denis *et. al.* describes the first route of interest. In this approach to the Taxol side chain, readily available (S)-(+)-phenylglycine (**3.16**) is used as the starting material (*Scheme* 3.3). Reduction using lithium aluminum hydride followed by benzoylation led to alcohol (**3.17**). Swern oxidation of (**3.17**) to give the aldehyde sets the stage for the enantioselective Grignard reaction to yield the allylic alcohol (**3.18**). Intermediate (**3.18**) was then protected with ethyl vinyl ether and oxidized to produce protected acid (**3.19**). The overall yield of the enantiomerically pure acid was reported to be 30% and a corresponding process gave the Taxotere side chain in 34% yield.

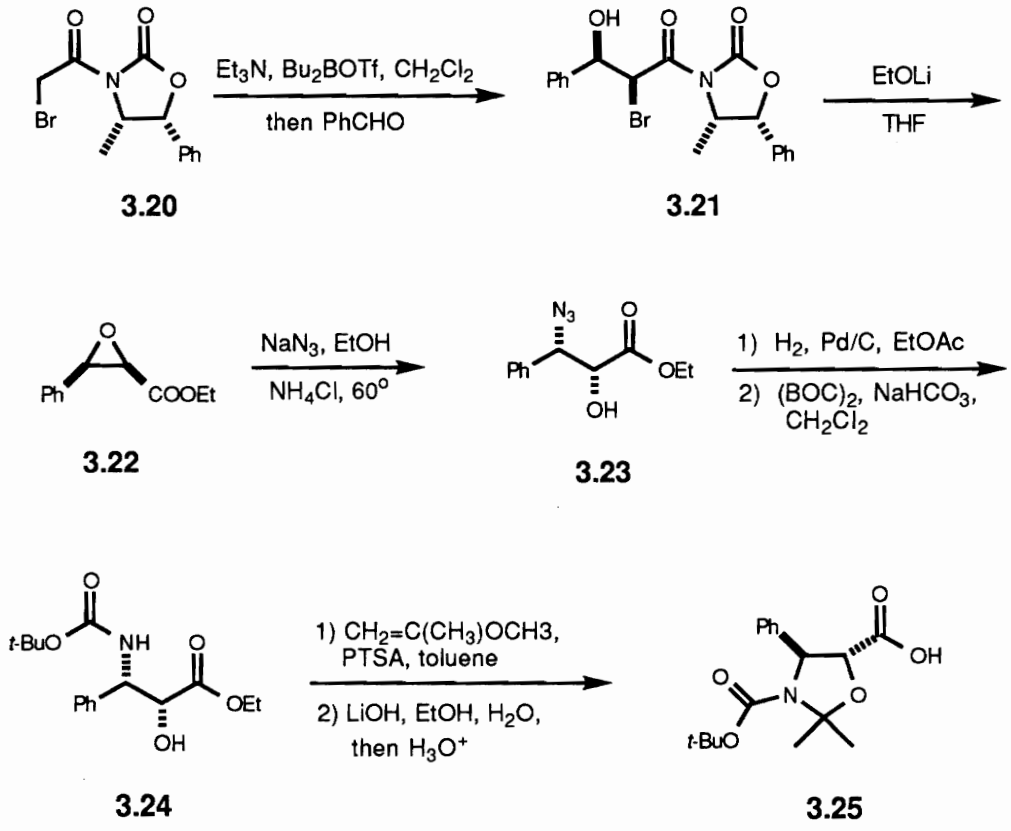
The second route of interest was disclosed in a recent publication by Commerçon and coworkers.[137] This route began with the



Scheme 3.3. Denis and coworker's side chain synthesis.

condensation of the boron enolate of oxazolidinone (**3.20**) with benzaldehyde to give hydroxy bromide (**3.21**) (*Scheme* 3.4). Reaction of (**3.21**) with lithium ethoxide in THF led to epoxide (**3.22**) which was then converted to hydroxy azide (**3.23**). Hydrogenation followed by protection of the amine with di-*tert*-butyl dicarbonate gave N-*t*-Boc phenylisoserine (**3.24**). The key N, O protected acid (**3.25**) was prepared by cyclic protection using methoxypropene in the presence of a catalytic amount of pyridinium paratoluenesulfonate (PTSP) followed by hydrolysis of the ester. Acid (**3.25**) proved to be highly stable compared to the linear analog (**3.19**). Commerçon and colleagues then coupled (**3.25**) to 7-Troc baccatin III (**3.26**) to give ester (**3.27**) (*Scheme* 3.5). This could then be converted to Taxol (**3.28**) by a deprotection, acylation, deprotection

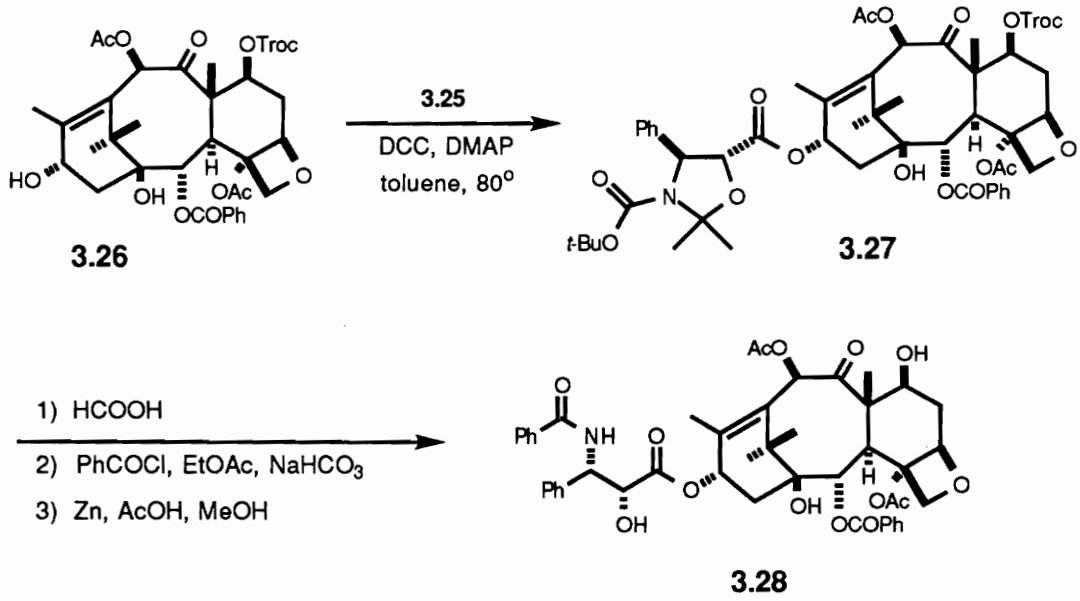
sequence. The advantage of this route was the high yield (99%) of the side chain coupling step, a step which caused problems in the past.



3.20 **3.21** **3.22** **3.23** **3.24** **3.25**

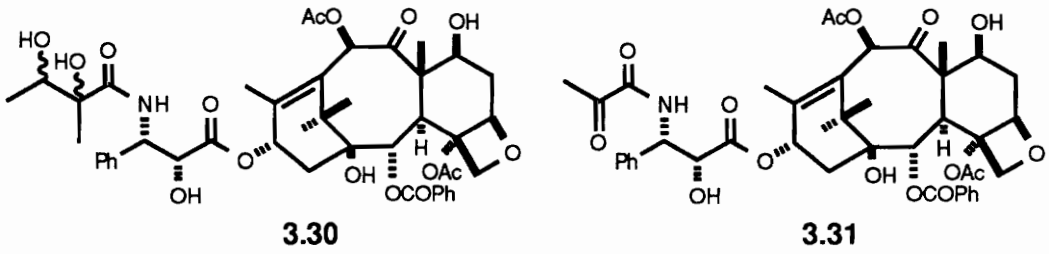
Scheme 3.4. Commerçon and coworker's side chain synthesis.

Although a large number of 3'-N-acyl Taxol analogs have been synthesized, the preparation of these derivatives by modification of the N-tigloyl group of cephalomannine has not been previously explored. Jitrangsri[55] did synthesize two cephalomannine analogs, the diol (**3.30**) and the ketoamide (**3.31**), during the course of his studies on the



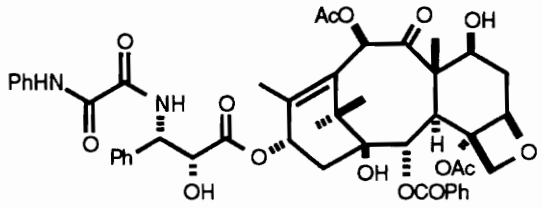
Scheme 3.5. Side chain coupling to baccatin III.

conversion of cephalomannine to Taxol, but his intent was not to create new analogs and hence neither the diol (**3.30**) nor the ketoamide (**3.31**) were ever tested for bioactivity.



3.2. Results and Discussion

The initial course of this investigation was inspired by the cephalomannine to Taxol conversion studies. The successful preparation of the N-(phenyloximido) derivative (**3.32**) using Shiozaki's amide cleavage methodology,[156] described in the previous section, opened the door for the preparation of a series of these analogs. Jitrangsri's *cis*-diol (**3.30**) and ketoamide (**3.31**), analogs which had been prepared during his attempt to convert cephalomannine to Taxol, also held the possibility of leading to a more potent/less toxic Taxol analog.



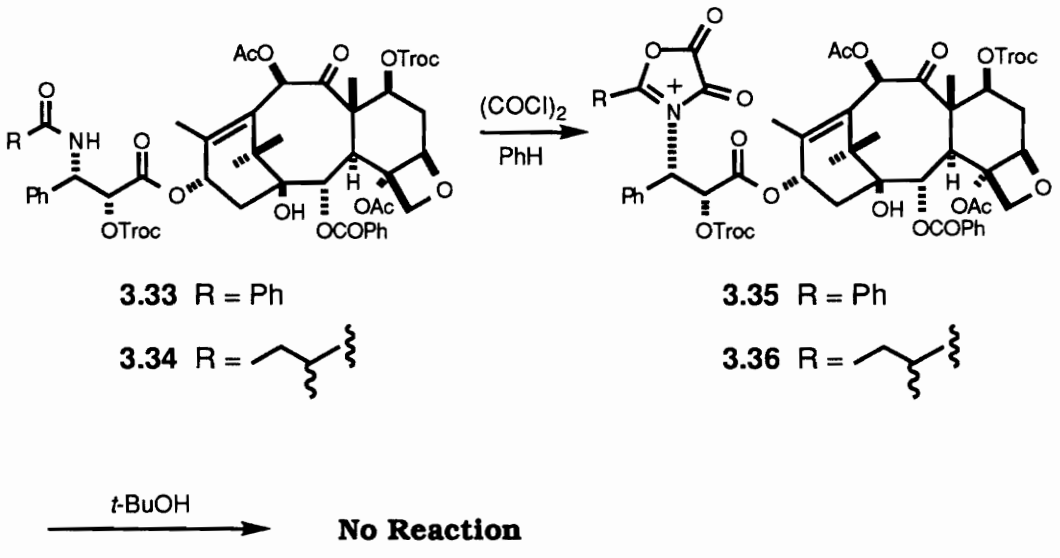
3.32

3.3.1, Oxalyl Analogs

After the preparation of the N-(phenyloxamido) analog (**3.32**), several attempts were made to synthesize other derivatives using Shiozaki's amide cleavage methodology. Unfortunately, the success that was enjoyed in the initial application of this methodology was not observed in subsequent studies.

The second attempt to create an N-oxalyl derivative involved the use of *tert*-butyl alcohol as a quench. The choice of *tert*-butyl alcohol was made in the hopes of generating a "Taxotere-like" analog even though it was recognized that the bulkiness of this tertiary alcohol might hinder the reaction. As was successful in the initial application of this

methodology, a 2', 7-diTroc protected Taxol (**3.33**)/dihydrocephalomannine (**3.34**) mixture was treated with 6 equivalents of oxalyl chloride for an 18 hour period and then quenched with *tert*-butyl alcohol (*Scheme* 3.6). The reaction was monitored by TLC and as was previously observed, the diprotected Taxol/dihydrocephalomannine mixture was converted to a highly polar material, presumably the key cyclic intermediates (**3.35**) and (**3.36**), although no attempt was made to isolate and identify this intermediate. Unfortunately, when quenched, there was no apparent reaction. Although disappointing, this was not completely unexpected. On the chance that *tert*-butyl amine might be more reactive, it was tested, but similar results were observed.

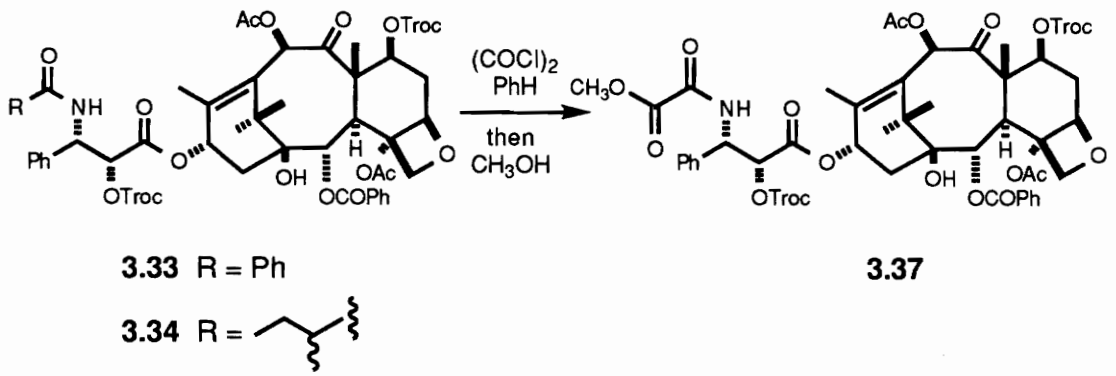


Scheme 3.6. Attempted preparation of the N-(*t*-butyloxalyl) analog.

The failure of *tert*-butyl alcohol and *tert*-butyl amine to react turned the course of the investigation toward the use of less hindered

alcohols and amines. The next two attempts focused on the use of benzyl alcohol and benzyl amine as quenches. The success of aniline as a quench in the initial test suggested that the use of a primary alcohol or amine might be more likely to produce positive results. Consequently, when these too failed to react it was a complete surprise.

The only success using Shiozaki's methodology beyond the first, and this being minor, was the preparation of the methyl oxamate analog (**3.37**) (*Scheme* 3.7). The reaction of a 2', 7-diTroc protected Taxol (**3.33**)/dihydrocephalomannine (**3.34**) mixture with oxalyl chloride for an 18 hour period followed by a methanol quench led to the desired analog (**3.37**), but the yield was extremely low and sufficient compound was not obtained so that complete characterization and biological testing could be carried out.



3.33 R = Ph

3.34 R =

3.37

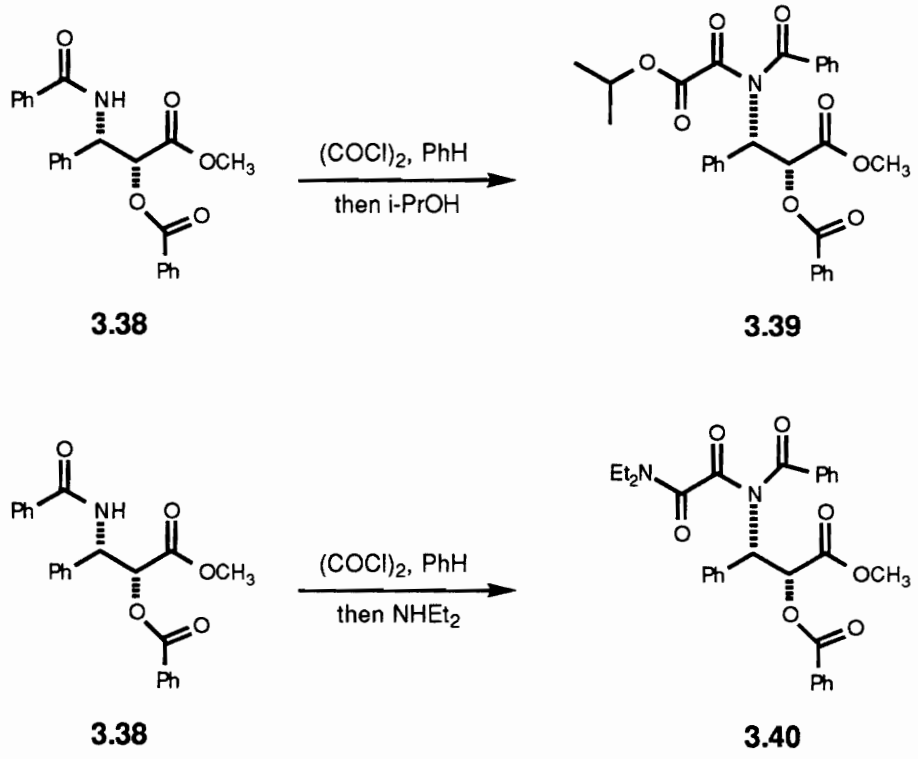
Scheme 3.7. Preparation of the N-(methyloxalyl) analog (**3.37**).

In light of the lack of success observed and the limited supply of Taxol/cephalomannine mixture available, the decision was made to continue the investigation using a side chain model. It was hoped that

optimal reaction conditions could be established using a model compound as well as indications of which amines and alcohols would be the best choices for the quench. Although the 2'-Troc dihydrocephalomannine side chain methyl ester would have been the best choice as a model, it was unavailable at the time and as a result the initial model studies were performed using the 2'-benzoyl taxol side chain methyl ester. The choice of this compound was not expected to create any new problems since 2'-benzoyl-7-Troctaxol had been a component in the mixture which was first used to test the oxalyl chloride reaction and had successfully undergone reaction.

The first model study focused on the question of which alcohols and amines would be reactive enough to generate the desired oxamate analogs. Since previous attempts with *tert*-butyl alcohol and *tert*-butyl amine indicated that tertiary alcohols and amines were not effective, it was decided to test a secondary alcohol and a secondary amine. Thus, an experiment was set up where two samples of the 2'-benzoyl taxol side chain methyl ester (**3.38**) were treated with oxalyl chloride in benzene and then quenched, one with isopropanol and one with diethylamine (*Scheme* 3.8). The reactions were monitored by TLC and both quenches appeared to react readily. Surprisingly, upon isolation and characterization by ^{1}H NMR it was determined that the two products obtained were not the expected oxamate analogs, but instead were the N-diacyl derivatives (**3.39**) and (**3.40**).

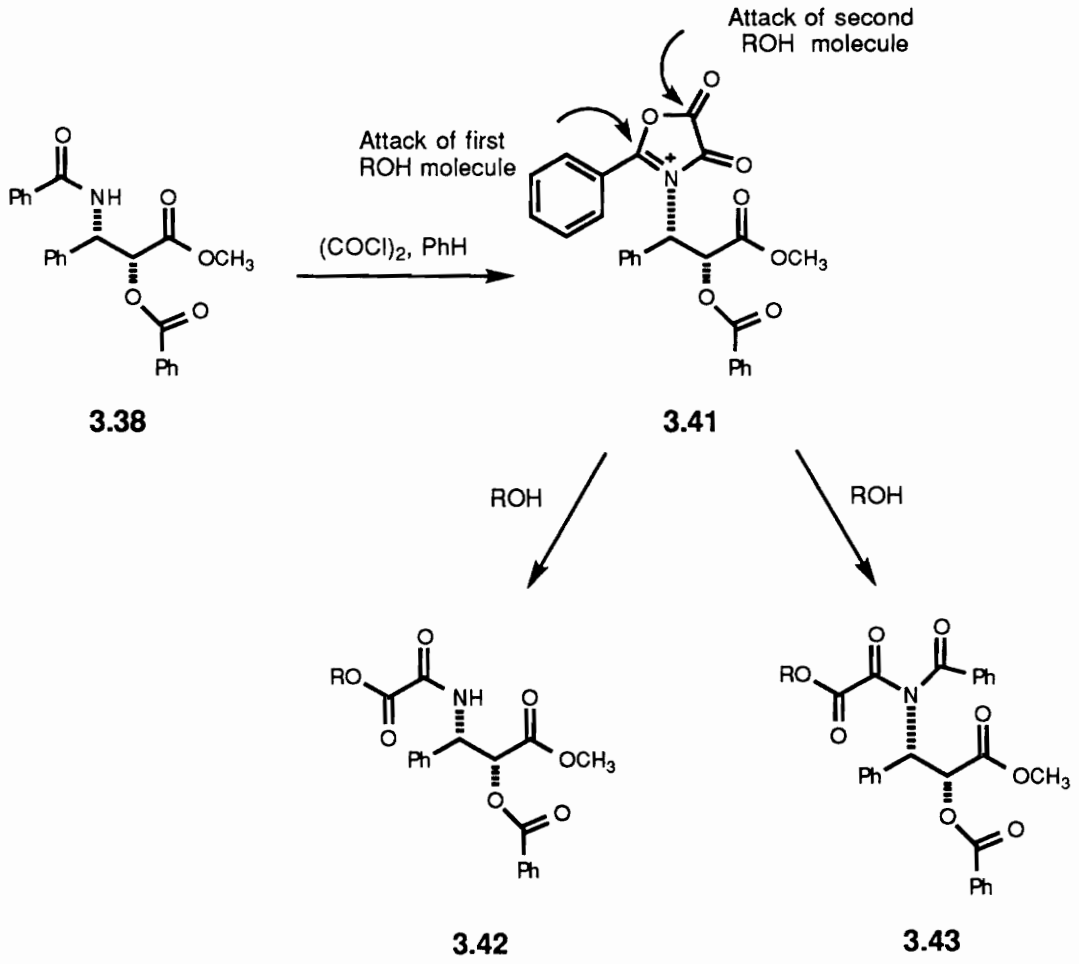
Although unexpected, the formation of these N-diacyl derivatives is



Scheme 3.8. Formation of N-diacyl analogs (**3.39**) and (**3.40**).

readily explained (*Scheme* 3.9). From a mechanistic standpoint, if the normal course of the reaction is followed then formation of cyclic intermediate (**3.41**) should occur when side chain model (**3.38**) is treated with oxalyl chloride. When quenched, this intermediate should react sequentially with two molecules of the alcohol or amine (shown in *Scheme* 3.9 with the alcohol). If formation of the desired N-oxalyl analog is to occur, then initial attack must take place at the iminium ion. Once this has happened then a second molecule can attack the carbonyl resulting in cleavage of the N-acyl group to yield the desired product (**3.42**). However, if attack of the alcohol or amine occurs at the carbonyl

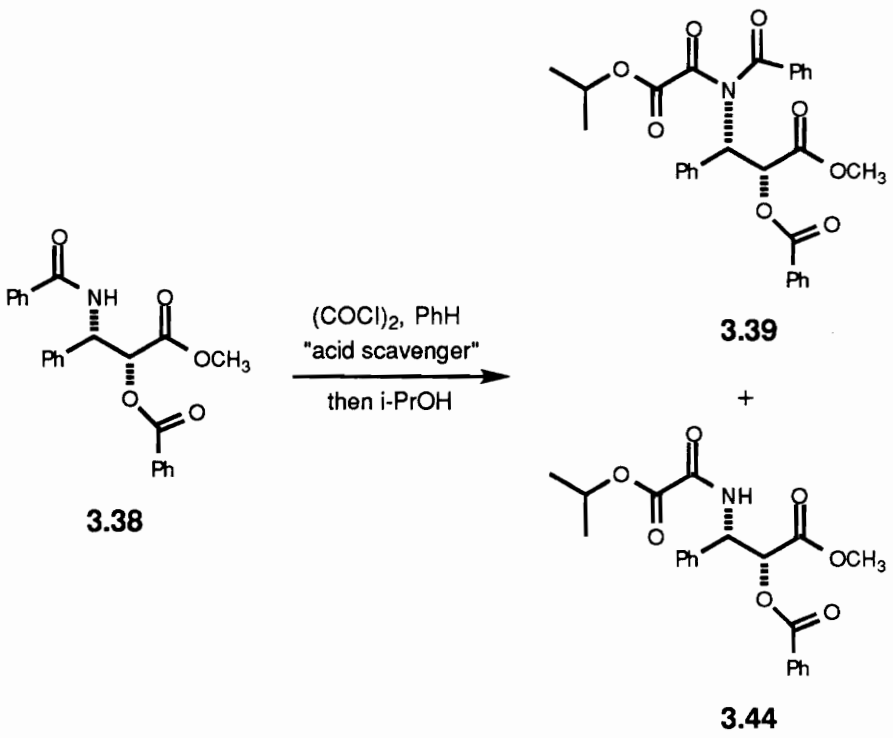
first, then the oxazolone ring opens without cleavage of the N-acyl group to yield the N-diacyl product (**3.43**).



Scheme 3.9. Mechanism of formation of the N-diacyl analogs.

Shiozaki and coworkers had observed minor amounts of these N-diacyl compounds during the course of their investigations on cephamycins and reported that they could be prevented using acid scavengers.[156] Consequently, the next model study was designed to

determine the affect of an acid scavenger on the course of the reaction. Four samples of the 2'-benzoyl taxol side chain methyl ester (**3.38**) were dissolved in benzene and treated with oxalyl chloride in the presence of an acid scavenger (*Scheme* 3.10). The acid scavengers used were pyridine, imidazole, sodium carbonate, and Reilex 402 (a polyvinylpyridine). After 18 hours each reaction was quenched using isopropanol. Upon isolation and identification it was found that both the N-diacyl adduct (**3.39**) and the desired oxamate ester (**3.44**) were present, but in all cases, the N-diacyl compound was the major product by a 3:2 ratio.

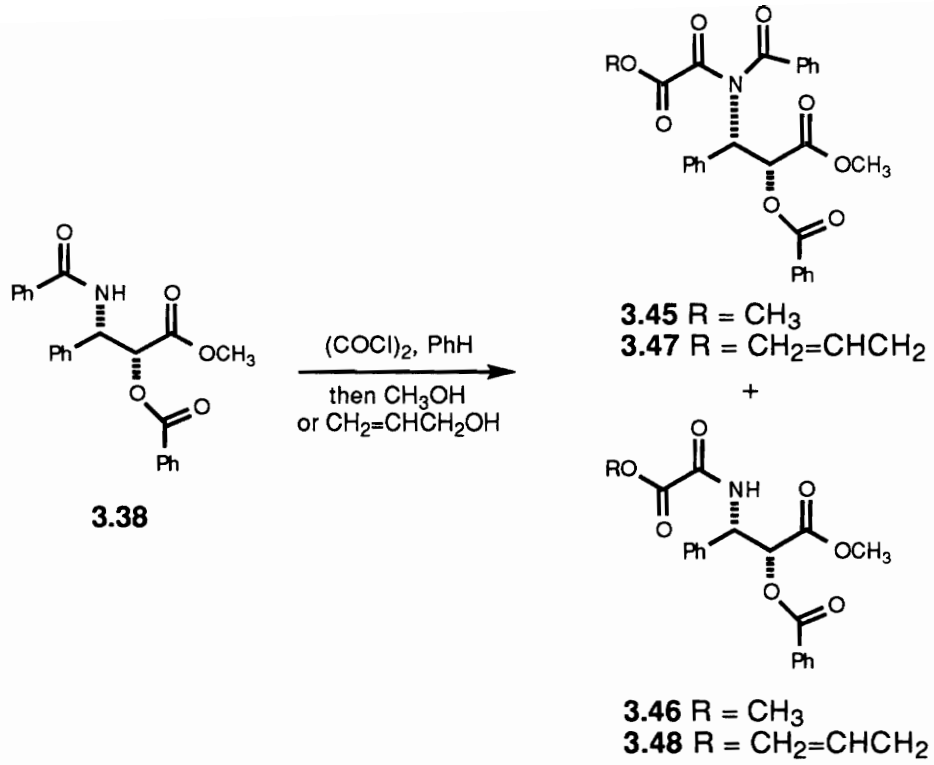


Scheme 3.10. Reaction of the side chain using an acid scavenger.

Several additional model studies were performed to determine the effect of time, temperature, and solvents on the course of the reaction. Similar results to those observed with the acid scavengers were obtained in each case.

There are two possible explanations for the dominance of the N-diacyl product in the case of the taxol side chain. First, it may be that secondary alcohols or amines, while unhindered enough to react, steer the course of reaction in favor of the N-diacyl product. The second possibility is that the N-benzoyl group of the taxol side chain plays a significant role in the outcome of the reaction. An examination of the assumed intermediate (**3.41**) shows that the iminium ion is resonance stabilized by the adjacent phenyl group (*Scheme* 3.9). Under these circumstances, the relative reactivity of the carbonyl group may be greater thus leading to the N-diacyl product. This would not be the case if the dihydrocephalomannine side chain were used.

In order to establish the role of the reagent used to quench the reaction the reaction sequence was again repeated using two primary alcohols. The alcohols chosen were methanol and allyl alcohol. When side chain model (**3.38**) was treated with oxalyl chloride and then quenched with methanol a 1:1 mixture of N-diacyl product (**3.45**) and the N-oxalyl analog (**3.46**) was obtained (*Scheme* 3.11). When allyl alcohol was used as a quench a 2:3 mixture of the N-diacyl product (**3.47**) and N-oxalyl derivative (**3.48**) was found. These results suggest that the nature of the quench used does in fact play a significant role, but apparently the N-acyl group does too.

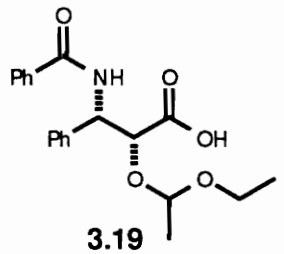


Scheme 3.11. Oxalyl chloride reaction using primary alcohols.

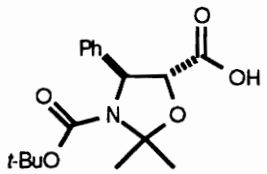
Although the logical course of action was to repeat these experiments using the 2'-benzoyl dihydrocephalomannine side chain methyl ester to determine the effect of the N-acyl group, it was recognized that the goal of this work was to obtain a few oxalyl analogs for bioactivity studies. Consequently, it was decided that the amount of time and effort required to synthesize the cephalomannine side chain and repeat these studies might be better spent exploring an alternate route.

Rimoldi and Chaudhary, working in the Kingston group, had pioneered a method to make side chain analogs using the traditional approach of side chain synthesis followed by coupling to baccatin III.[172] They initially investigated the use of the side chain synthesis of Denis *et.*

al.[168], but found that the protected acid (**3.19**) was extremely unstable. The route developed by Commerçon *et. al.*[137] was briefly considered because of the stability and efficient coupling of the N, O protected acid (**3.25**), but the length of the route did not make this approach favorable. In an effort to circumvent the problems associated with each approach, Rimoldi and Chaudhary combined the simplicity of Denis and coworker's route with the protecting group methodology used by the Commerçon group.



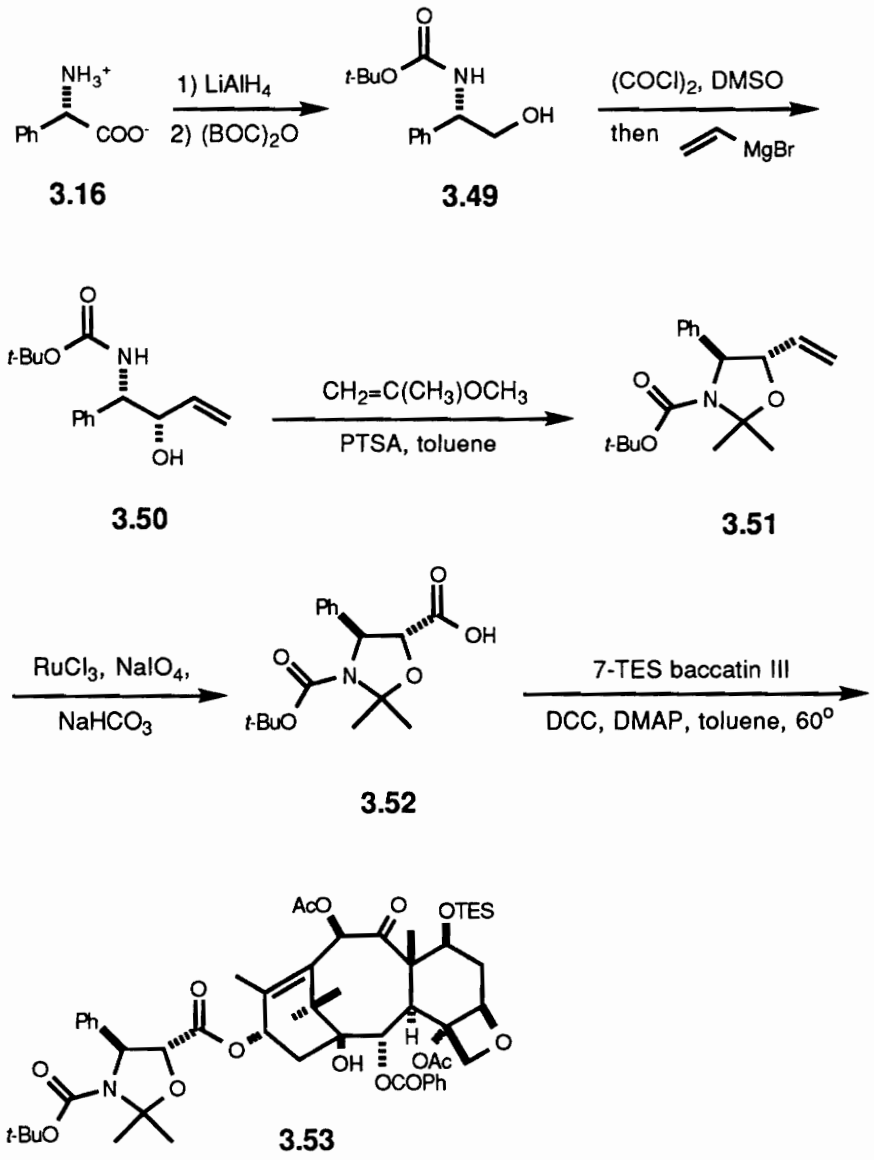
3.19



3.25

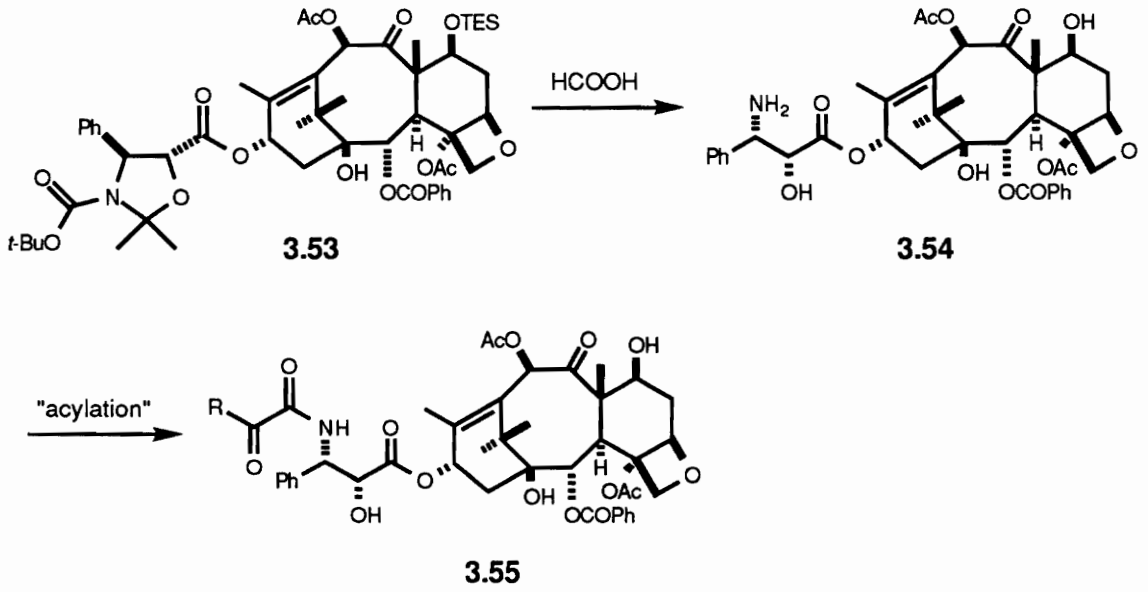
The synthetic sequence which finally emerged from this combination is outlined in *Scheme* 3.12. It began with the reduction of (+)-phenylglycine (**3.16**) using lithium aluminum hydride. This reaction was quenched with di-*tert*-butyl dicarbonate rather than benzoyl chloride to afford alcohol (**3.49**). Swern oxidation to give the aldehyde and subsequent exposure to vinyl magnesium bromide transformed alcohol (**3.49**) into (**3.50**). The N, O cyclic protection methodology of Commerçon *et al.* was introduced at this point by treatment of (**3.50**) with methoxypropene in the presence of a catalytic amount of pyridinium paratoluenesulfonate (PTSP) to produced cyclic intermediate (**3.51**). The final step of the side chain synthesis was oxidation of (**3.51**) to give protected acid (**3.52**). As expected, this cyclic acid was found to be

exceptionally stable and readily coupled to 7-(triethylsilyl)baccatin III to give key intermediate (**3.53**) in high yield. The spectroscopic data of this compound was consistent with the literature values.[172]



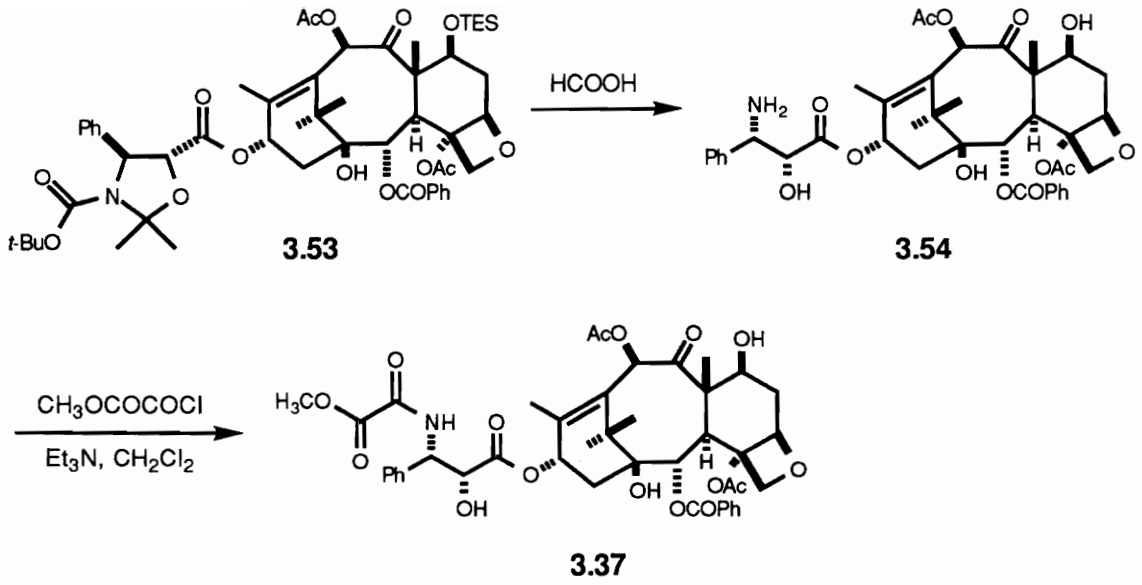
Scheme 3.12. Synthesis of key intermediate (**3.53**).

Once key intermediate (**3.53**) was obtained, a simple two step protocol could be used to prepare any desired 3'-N-acyl analog. This two step protocol, shown in *Scheme* 3.13, involved the deprotection of (**3.53**) using formic acid to give N-debenzoyltaxol (**3.54**) followed by acylation with the desired group to afford the new analog (**3.55**).



Scheme 3.13. Synthesis of N-oxalyl analogs.

Using this methodology, key intermediate (**3.53**) was prepared. With this in hand, the stage was set for the synthesis of the N-oxalyl derivatives. The initial focus was on the preparation of N-(methyloxalyl) analog (**3.37**) (*Scheme* 3.14). Treatment of (**3.53**) with 99% formic acid for 3 hours resulted in complete deprotection to yield N-debenzoyltaxol

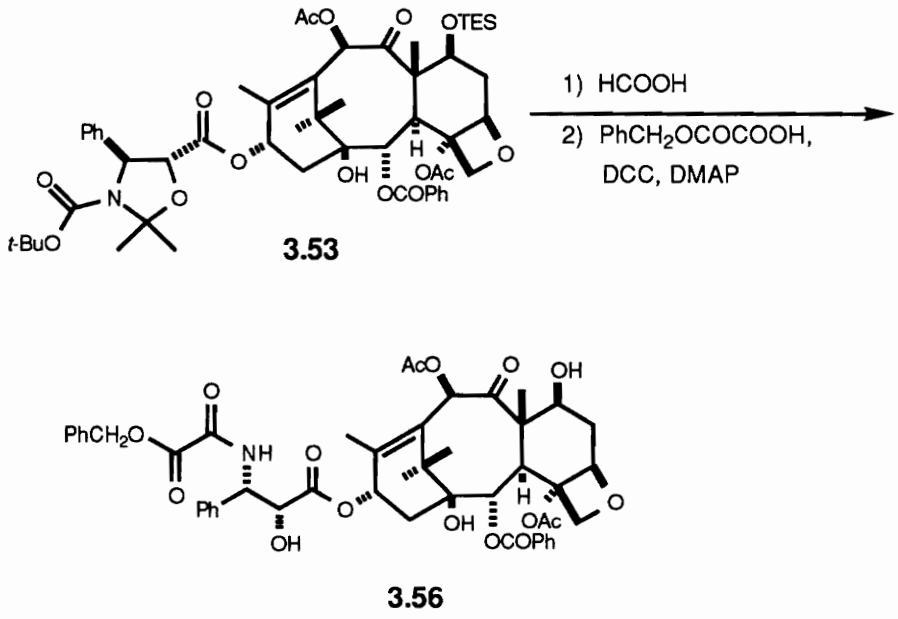


Scheme 3.14. Synthesis of N-(methyloxalyl) analog (**3.37**).

(**3.54**) after basic work up. Previous work by Rimoldi and Chaudhary had shown that under these conditions N-debenzoyl taxol was not particularly stable and any attempt to purify it generally resulted in loss of the compound.[173] Consequently, the crude material was used without purification. Acylation using methoxalyl chloride and pyridine proved to be the most effective, although similar results could be achieved using Schotten-Baumann conditions. Characterization by ^{1}H NMR confirmed its identity as (**3.37**). Key spectral evidence included the methyl peak for the oxamate ester at 3.87 ppm and the downfield shift of the amide proton from 7.0 ppm for Taxol to 7.89 ppm, which is characteristic of oxamate esters.[149]

Methyl oxalyl chloride was the only commercially available reagent of this type and as a result the options for the preparation of these analogs was limited. Furthermore, a literature search revealed that in general oxalic acid monoesters or acid chlorides are unstable and not easily prepared.[174] The one exception was monobenzyl oxalate which could be prepared by adding benzyl alcohol to a large excess of oxalyl chloride and then quenching the reaction with water.[175] After isolation, recrystallization from hexanes yielded the product in a reasonably pure form.

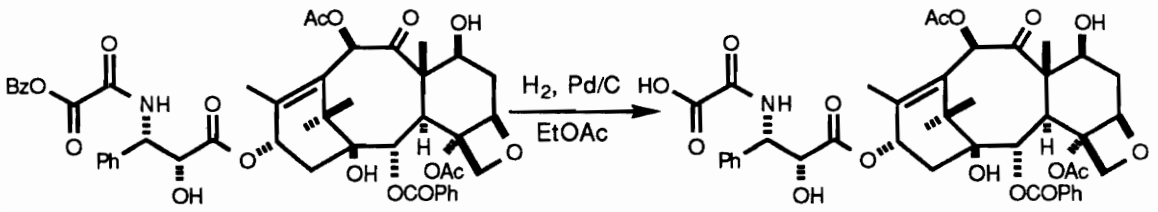
Preparation of N-(benzyloxalyl) analog (**3.56**) was accomplished using the same two step protocol as previously described. Thus, key intermediate (**3.53**) was completely deprotected to give the free amine (*Scheme* 3.15). As before, the crude amine was used immediately



Scheme 3.15. Preparation of the N-(benzyloxalyl) analog (**3.56**).

without purification. Since benzyl oxalyl chloride was expected to be unstable and difficult to prepare, acylation of the amine was carried out by coupling monobenzyl oxalate to amine (**3.54**) using dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-dimethylaminopyridine (DMAP). This produced the desired analog in 65% yield.

Several attempts were also made to prepare the oxamic acid analog (**3.57**), which was of particular interest. If this derivative possessed activity comparable to that of Taxol, then it would open the door for the preparation of water soluble salts of this analog. In light of the water solubility problems with Taxol, this would be of great value. This compound could readily be prepared in high yield from the N-(benzyloxalyl) analog (**3.56**) by hydrogenation with 10% Pd/C in ethyl acetate (*Scheme* 3.16). In theory, hydrogenation of pure (**3.56**) would lead to pure (**3.57**). This was particularly attractive since previous work had shown that the oxamic acid was difficult to purify. In practice this turned out not to be true. Analysis of the ^{1}H NMR clearly



3.56 **3.57**

Scheme 3.16. Synthesis of the N-oxalyl analog (**3.57**).

established that the oxamic acid analog (**3.57**) had been formed, but it also indicated the presence of a small amount of impurities. Several attempts to purify this compound using silica gel, reverse phase, or Sephadex chromatography all resulted in loss of the compound. As a result this analog could not be obtain in sufficient purity to fully characterize it or send it for biological testing.

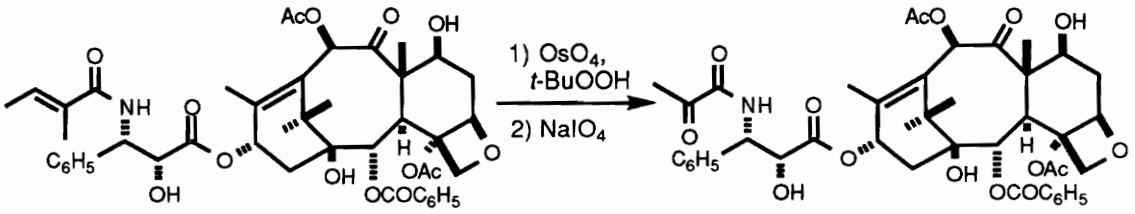
Table 3.1. ^{1}H NMR Spectra of N-Oxalyl Analogs.[a, b]

Protons on	**3.37**	**3.56**	**3.57**
C-2	5.68 (d, 7.0)	5.66 (d, 7.0)	5.65 (d, 7.0)
C-3	3.78 (d, 7.0)	3.77 (d, 7.0)	3.77 (d, 7.0)
C-5	4.94 (dd, 9.6, 1.9)	4.92 (dd, 9.4, 1.8)	4.95 (br d)
C-6α	2.55 (m)	2.55 (m)	2.52
C-6β	1.85 (m)	1.87 (m)	*c*
C-7	4.39 (m)	4.38 (m)	4.37 (m)
C-10	6.28 (s)	6.27 (s)	6.27 (s)
C-13	6.22 (t, 8.8)	6.21 (br t)	6.18 (br t)
C-14 (α, β)	*c*	*c*	*c*
C-16-CH_3	1.16 (s)	1.15 (s)	1.14 (s)
C-17-CH_3	1.25 (s)	1.24 (s)	1.22 (s)
C-18-CH_3	1.80 (s)	1.77 (s)	1.81 (s)
C-19-CH_3	1.69 (s)	1.68 (s)	1.67 (s)
C-20α	4.30 (d, 8.4)	4.28 (d, 8.3)	4.28 (d, 8.4)
C-20β	4.20 (d, 8.4)	4.19 (d, 8.3)	4.17 (d, 8.4)
C-2'	4.71 (dd, 5.2, 2.5)	4.70 (dd, 5.2, 2.6)	4.70 (br s)
C-3'	5.54 (dd, 9.3, 2.5)	5.53 (dd, 9.3, 2.6)	5.45 (br d, 9.1)
3'-NH	7.89 (d, 9.3)	7.92 (d, 9.3))	8.05 (br d)
ArH	7.26-8.11 (m)	7.30-8.10 (m)	7.30-8.10 (m)
4-OAc	2.31 (s)	2.30 (s)	2.30 (s)
10-OAc	2.25 (s)	2.24 (s)	2.25 (s)
COCOOMe	3.87 (s)		
$PhC\underline{H}_2O$		5.26 (m)	

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. [c]Difficult to determine exact chemical shifts since the signals are partially hidden under the methyl or acetate signals.

3.3.2. N-Pyruvyl Analogs

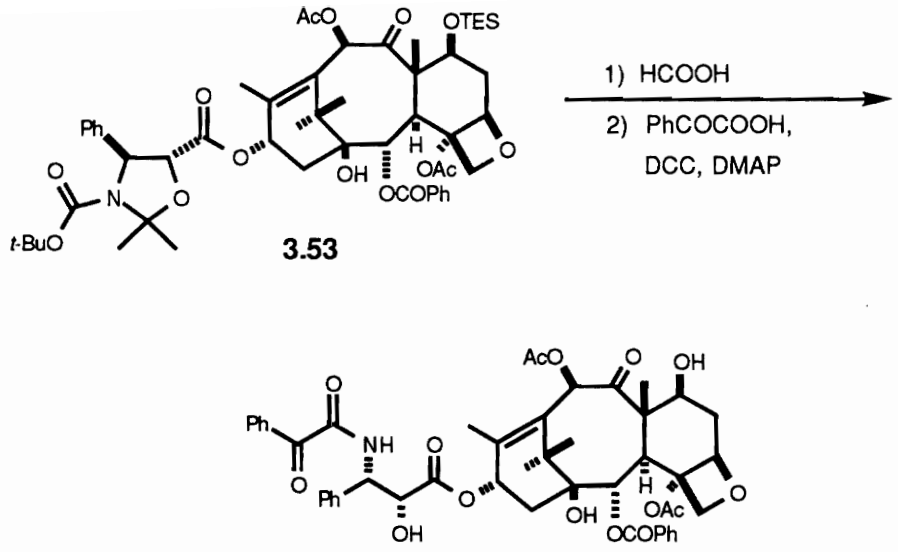
As previously mentioned, Jitrangsri prepared the ketoamide analog (**3.31**), but its biological activity was never determined. The structural similarity of this analog to the N-oxalyl analogs makes it of interest. Using established protocols[56], ketoamide analog (**3.31**) was prepared (*Scheme* 3.17). Reaction of cephalomannine (**3.58**) with osmium tetroxide to give the *cis*-diol followed by oxidative cleavage using sodium periodate led to the ketoamide analog (**3.31**) in 76% yield. The spectral data was consistent with that reported by Jitrangsri.[55]



3.58 **3.31**

Scheme 3.17. Preparation of the ketoamide (**3.31**).

As a comparison, the N-(phenylglyoxyl) derivative (**3.59**) was also prepared (*Scheme* 3.18). Structurally, this derivative is similar to Taxol except for the second carbonyl of the N-acyl group. Preparation of this analog would clearly establish the impact of the diketo moiety on the activity. This derivative was prepared using the same methodology as was used to make the N-oxalyl analogs. Exposure of acetonide (**3.53**) to



Scheme 3.18. Synthesis of the N-(phenylglyoxyl) analog (**3.59**).

formic acid for 2.5 hours at room temperature produced the free amine which was then acylated using phenylglyoxylic acid in the presence of dicyclohexylcarbodiimide and a catalytic amount of 4-dimethyl-aminopyridine. Analysis of the ^{1}H NMR spectrum confirmed the presence of the N-(phenylglyoxyl) group by the appearance of additional aromatic protons as well as the downfield shift of the amide proton.

The ^{1}H NMR assignments for (**3.31**) and (**3.59**) are given in Table 3.2.

3.3.3. Cephalomannine cis-diol Analogs

Like the ketoamide (**3.31**), the cephalomannine diol (**3.30**) had

Table 3.2. ^{1}H NMR Spectra of N-Diketo Analogs.[a, b]

Protons on	**3.31**[c]	**3.59**
C-2	5.66 (d, 7.0)	5.67 (d, 6.8)
C-3	3.78 (d, 7.0)	3.81 (d, 6.9)
C-5	4.94 (dd, 9.3, 1.7)	4.94 (dd, 9.4, 1.7)
C-6α	2.55 (m)	2.51 (m)
C-6β	*d*	*d*
C-7	4.38 (m)	4.40 (m)
C-10	6.28 (s)	6.27 (s)
C-13	6.21 (br t, 8.9)	6.26 (br t)
C-14 (α, β)	*d*	*d*
C-16-CH_3	1.15 (s)	1.14 (s)
C-17-CH_3	1.25 (s)	1.24 (s)
C-18-CH_3	1.80 (s)	1.83 (s)
C-19-CH_3	1.68 (s)	1.69 (s)
C-20α	4.29 (d, 8.4)	4.31 (d, 8.5)
C-20β	4.18 (d, 8.4)	4.19 (d, 8.5)
C-2'	4.67 (dd, 5.3, 2.9)	4.75 (dd, 5.3, 2.6)
C-3'	5.48 (dd, 9.4, 2.9)	5.65 (dd, 9.4, 2.5)
3'-NH	7.83 (d, 9.4)	7.97 (d, 9.4)
3'-Ph, COCOPh, 2-COPh	7.33-8.12 (m)	7.29-8.25 (m)
4-OAc	2.34 (s)	2.40 (s)
10-OAc	2.24 (s)	2.24 (s)
COCOMe	2.38 (s)	

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. [c]Originally prepared by Jitrangsri, Reference 55. [d]Difficult to determine exact chemical shifts since the signals are partially hidden under the methyl or acetate signals.

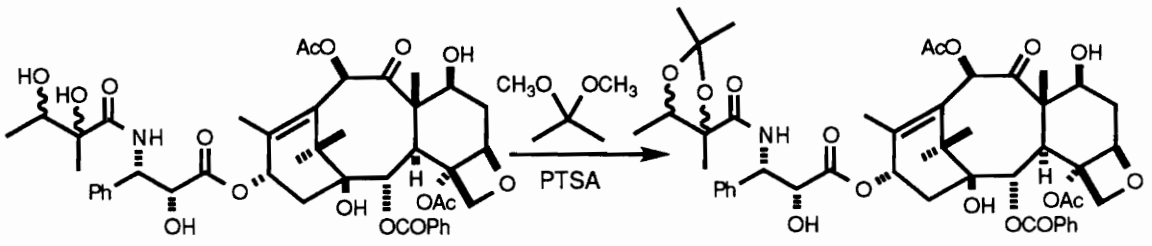
been prepared, but had not been tested for biological activity. Since interest in the diol was primarily as a means to separate cephalomannine from Taxol. In terms of activity, it is not likely that the diol would be bioactive since structure-activity studies to date seem to suggest that a hydrophobic group, as exemplified by the N-*t*-Boc group of Taxotere, is needed at this site for optimal activity.[7] However, considering the limited number of analogs reported, this conclusion remains to be firmly

established. Testing of the diol would be useful as another data point to either confirm or contradict this conclusion.

The cephalomannine diol (**3.30**) was prepared by treating cephalomannine (**3.58**) with a catalytic amount of osmium tetroxide in the presence of *t*-butylhydroperoxide and tetraethyl ammonium acetate.[54] The product exhibited spectral characteristics in accord with an authentic sample.

In an effort to probe even further, two analogs were created using the diol. The intent was to increase the hydrophobic nature of the derivative by blocking the diol.

The first derivative made was the acetonide (**3.60**). This was conveniently prepared by treating the cephalomannine diol (**3.30**) with excess 2, 2-dimethoxypropane in the presence of a catalytic amount of *p*-toluenesulfonic acid (*Scheme* 3.19). In light of the fact that the diol is a diastereomeric mixture of *cis*-diols, a diastereomeric mixture of acetonides was produced. Unfortunately, the acetonides, like the diols,

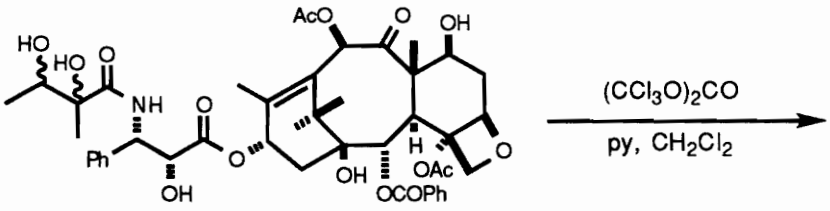


3.30 **3.60**

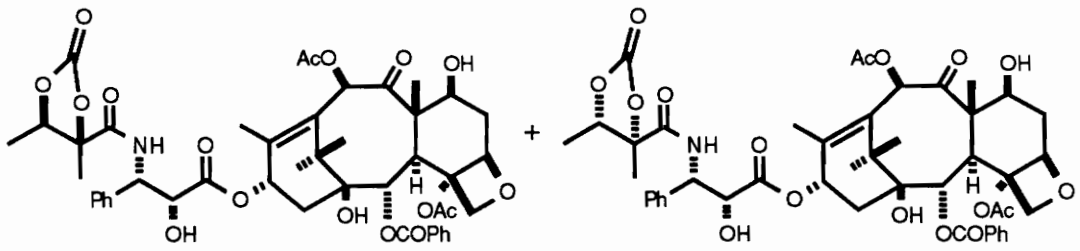
Scheme 3.19. Synthesis of the cephalomannine acetonide (**3.60**).

were chromatographically inseparable. The complexity of the ^{1}H NMR spectrum clearly indicated the diastereomeric nature of the product, while high and low resolution FABMS were in accord with the molecular composition of the acetonide.

The second diol analog prepared was the mixture of carbonates (**3.61**) and (**3.62**). The synthesis was accomplished by reaction of the cephalomannine diol (**3.30**) with 3 equivalents of triphosgene in methylene chloride in the presence of pyridine (*Scheme* 3.20). After 1 hour, the reaction was complete and TLC indicated the formation of two products in approximately a 1:1 ratio. After purification by preparative thin-layer chromatography (PTLC), the two products were characterized by ^{1}H NMR. A peak by peak comparison of the two spectra found that



3.30



3.61 **3.62**

Scheme 3.20. Preparation of the carbonate analogs (**3.61**) and (**3.62**).

Table 3.3. ^{1}H NMR Spectra of Diol Analogs.[a, b]

Protons on	**3.60**[c]	**3.61**[d]	**3.62**[d]
C-2	5.67 (m)[e]	5.66 (d, 7.1)	5.66 (d, 7.0)
C-3	3.79 (m)[e]	3.78 (d, 7.1)	3.79 (d, 7.0)
C-5	4.94 (br d, 8.2)	4.94 (d, 7.6)	4.94 (dd, 9.3, 1.7)
C-6α	2.54 (m)	2.55 (m)	2.55 (m)
C-6β	*f*	1.87 (m)	1.85 (m)
C-7	4.39 (m)	4.40 (m)	4.40 (m)
C-10	6.29 (s) & 6.28 (s)	6.27 (s)	6.28 (s)
C-13	6.17 (t, 9.0)	6.15 (t, 8.9)	6.19 (br t)
C-14 (α, β)	*f*	*f*	*f*
C-16-CH_3	1.15 (s)	1.15 (s)	1.16 (s)
C-17-CH_3	1.23 (s)	1.25 (s)	1.26 (s)
C-18-CH_3	1.84 (s) & 1.79 (s)	1.81 (s)	1.81 (s)
C-19-CH_3	1.68 (s)	1.67 (s)	1.68 (s)
C-20α	4.30 (m)[e]	4.30 (d, 8.4)	4.30 (d, 8.3)
C-20β	4.18 (d, 8.2)	4.17 (d, 8.4)	4.17 (d, 8.3)
C-2'	4.66 (m)[e]	4.65 (dd, 5.3, 2.5)	4.66 (dd, 5.4, 2.6)
C-3'	5.50 (m)[e]	5.49 (dd, 9.2, 2.5)	5.51 (dd, 9.2, 2.6)
3'-NH	*g*	*g*	*g*
ArH	7.26-8.11 (m)	7.34-8.12 (m)	7.30-8.11 (m)
4-OAc	2.38 (s)	2.34 (s)	2.34 (s)
10-OAc	2.25 (s)	2.24 (s)	2.25 (s)
C-3"	4.05 (m)[e]	4.76 (q, 6.6)	4.78 (q, 6.5)
C-4"-CH_3	1.26 (s)	1.31 (d, 6.6)	1.37 (d, 6.5)
C-5"-CH_3	1.46 (s)	1.48 (s)	1.51 (s)

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. [c]Mixture of diastereomers. [d]These may be interchanged. [e] Splitting pattern and coupling constants cannot be determined due to the multiple signals for the same proton because of the diastereomers. [f]Difficult to determine exact chemical shifts since the signals are partially hidden under the methyl or acetate signals. [g]Hidden in the aromatic region.

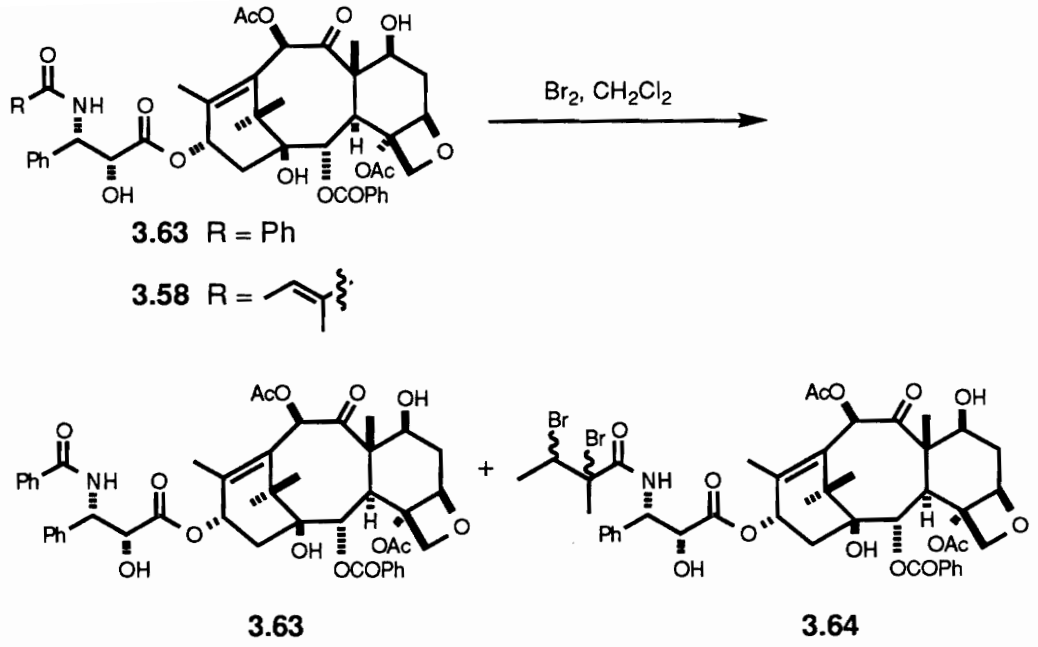
they were almost identical except for the chemical shift of the 2'-hydroxyl. This suggested that upon formation of the carbonate analog the diastereomers become chromatographically separable. This

conclusion was further substantiated by mass spectral analysis where both compounds were found to have the molecular formula $C_{46}H_{53}NO_{17}$ as established by HRFABMS.

3.3.4. Halogenated Analogs

The idea of treating a Taxol/cephalomannine mixture with bromine in order to create a chromatographically separable mixture had been discussed at length in the Kingston group. General feeling was that bromine was too harsh and that Taxol was not likely to survive the reaction conditions. Consequently, it was quite surprising when J. Rimoldi treated a Taxol (**3.63**)/cephalomannine (**3.58**) mixture with 10 equivalents of bromine in methylene chloride and observed by TLC only two spots, one of which corresponded to the starting mixture (*Scheme* 3.21).[173] Isolation and characterization by ^{1}H NMR of the substance thought to be the starting mixture clearly established that it was in fact pure Taxol (**3.63**). The second and less polar substance was tentatively identified by ^{1}H NMR to be a diastereomeric mixture of dibromides (**3.64**). Since Rimoldi's interest was in Taxol, characterization of (**3.64**) remained incomplete and the identity unconfirmed.

An authentic sample of (**3.64**) was obtained and characterization completed. The ^{1}H NMR spectrum was very clean considering (**3.64**) was a diastereomeric mixture. Analysis of the spectrum confirmed the reaction site as the tigloyl double bond by an upfield shift of the 3" proton. The diastereomeric nature of (**3.64**) was suggested by the fact



Scheme 3.21. Reaction of a Taxol/cephalomannine with bromine.

that the 3" proton appeared as two overlapping quartets. In addition, the 3' proton, which is normally a doublet of doublets, appeared to be a triplet of doublets. This unusual pattern is readily explained if the signal is considered to be two partially overlapping doublets of doublets. High and low resolution FABMS also supported the identity of (**3.64**) as a dibromide. The expected M+2 and M+4 peaks due to the ^{81}Br isotope were clearly present.

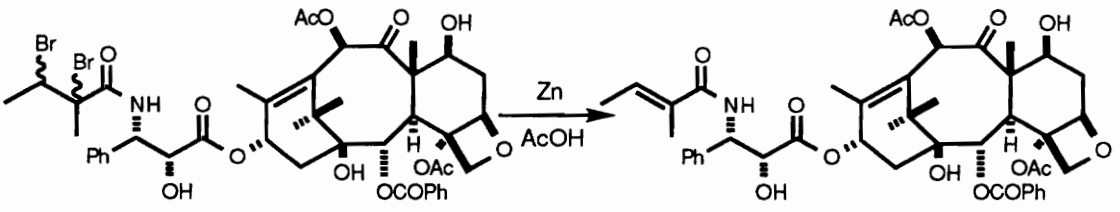
The success of this reaction generated considerable interest since it provided a very quick and efficient method to separate Taxol from cephalomannine. The speed (3-5 minutes) and selectivity of the reaction plus the fact that the separation of the dibromide by flash

chromatography could be accomplished without having to vary the composition of the mobile phase made it superior to the previously reported method which employed osmium tetroxide.[54] The one drawback was the inconsistency of the reaction. Dr.'s M. Gharpure and M. Chordia found the reaction to be extremely time dependent.[176] Considerable decomposition of Taxol was observed if the reaction was allowed to run more than a few minutes.

In an effort to optimize reaction conditions, the use of pyridinium bromide perbromide[177] and tetrabutylammonium tribromide[178] as brominating reagents was explored. Both reagents proved effective and gave good yields, although the reaction times were slightly longer. Tetrabutylammonium tribromide produced an 83% yield of the dibromide (**3.64**) in one hour, while pyridinium perbromide proved even better, giving an 93% yield in 30 minutes. Meanwhile, Dr.'s M. Gharpure and M. Chordia, who continued to pursue the use of elemental bromine, found the use of 10 equivalents of bromine in chloroform for 10 minutes to be optimal conditions for that approach.[176]

In addition to being a means to obtain pure Taxol, this route also provides a way to acquire pure cephalomannine. Treatment of the diastereomeric mixture of dibromides (**3.64**) with freshly activated zinc dust in acetic acid readily transformed the dibromides back to cephalomannine (**3.58**) in good yield (*Scheme* 3.22).[180] This is particularly significant since in the past, pure cephalomannine has been difficult to procure because the established chromatographic methods do not give a clean separation. As previously mentioned, early

chromatographic fractions provided pure Taxol, but later fractions were generally mixtures of Taxol and cephalomannine in various amounts. This route alleviates this problem.



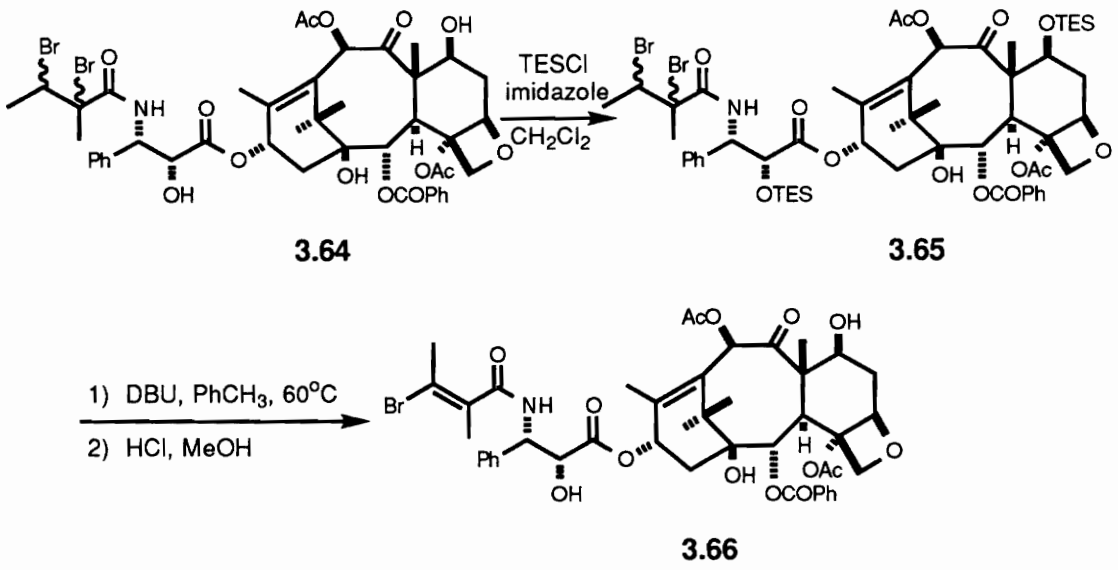
3.64 **3.58**

Scheme 3.22. Conversion of the dibromo analog to cephalomannine.

Although the cephalomannine dibromide had arisen as a result of an effort to establish a simple and efficient procedure for the isolation of Taxol in pure form from Taxol/cephalomannine mixtures, the picture radically changed when early bioassay results indicated that it was significantly more active than Taxol. This result suggested that the preparation of a series of halogenated analogs would be a profitable line of investigation.

The next halogenated derivative pursued was the monobrominated analog (**3.66**). Initial attempts to prepare this analog by treatment of the dibromide (**3.64**) with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were unsuccessful due to epimerization at the C-7 position. In order to circumvent these problems, dibromide (**3.64**) was converted to the 2', 7-diTES derivative (**3.65**) using standard conditions. Subsequent reaction of the 2', 7-diTES cephalomannine dibromide derivative (**3.65**) with DBU

in toluene at 60°C followed by deprotection of the TES groups using HCl/MeOH gave rise to a single product.



3.64 **3.65** **3.66**

Scheme 3.23. Preparation of monobromide (**3.66**).

Characterization of the product confirmed the identity as the 3"-bromo analog (**3.66**). Mass spectral analysis gave a molecular weight of 910, plus the expected M+2 peak, verifying the presence of a single bromine atom. Examination of the ^1H NMR spectrum showed a distinct downfield shift of the 4" and 5" methyls as well as the absence of a 3" proton. This supported the location of the bromine at the 3" position. Stereochemistry around the double bond of the N-tigloyl group was not unambiguously established, but was assumed to be E on the basis of the *trans* addition of bromine to the double bond followed by a *trans* elimination.

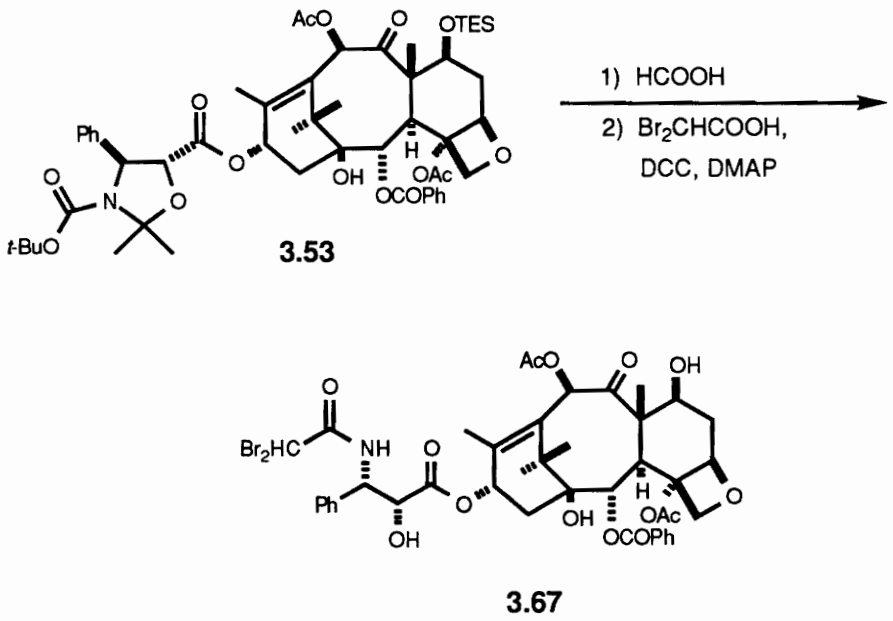
Table 3.4. ^{1}H NMR Spectra of Brominated Cephalomannine Analogs.[a, b]

Protons on	**3.64**[c]	**3.66**
C-2	5.66 (d, 7.0)	5.68 (d, 7.0)
C-3	3.77 (d, 7.0)	3.79 (d, 7.0)
C-5	4.93 (br d, 9.4)	4.93 (dd, 9.5, 1.9)
C-6α	2.54 (m)	2.55 (m)
C-6β	1.87 (m)	1.85 (m)
C-7	4.39 (m)	4.39 (m)
C-10	6.28 (s)	6.28 (s)
C-13	6.20 (t, 8.8)	6.21 (t, 9.0)
C-14 (α, β)	*d*	*d*
C-16-CH_3	1.15 (s)	1.15 (s)
C-17-CH_3	1.23 (s) & 1.25 (s)	1.26 (s)
C-18-CH_3	1.80 (s)	1.81 (s)
C-19-CH_3	1.67 (s)	1.68 (s)
C-20α	4.29 (d, 8.4)	4.30 (d, 8.3)
C-20β	4.18 (d, 8.4)	4.17 (d, 8.3)
C-2'	4.72 (m)	4.68 (dd, 5.2, 2.3)
C-3'	*e*	5.58 (dd, 9.0, 2.3)
3'-NH	*f*	6.30[g]
ArH	7.30-8.12 (m)	7.30-8.11 (m)
4-OAc	2.34 (s) & 2.35 (s)	2.32 (s)
10-OAc	2.25 (s)	2.25 (s)
C-3"	4.61 (q, 6.6)[h]	
C-4"-CH_3	1.74 (m)	2.36 (d, 1.5)
C-5"-CH_3	1.98 (s) & 1.99 (s)	1.98 (d, 1.5)

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. [c]Mixture of diastereomers some protons appear as two signals. [d]Difficult to determine exact chemical shifts since the signals are partially hidden under the methyl or acetate signals. [e]Appears as a triplet of doublets, but is actually two over lapping doublet of doublets at 5.57 and 5.53 (J=8.9, 2.3). [f]Hidden in the aromatic region. [g]Partially hidden by C-10. [h]Appears as a quartet of doublets, but is actually two closely spaced quartets.

In order to provide a clearer picture of the effect of bromination on activity, the synthesis of two additional analogs was pursued. The first of the two was N-debenzoyl-N-(dibromoacetyl)taxol (**3.67**). This could

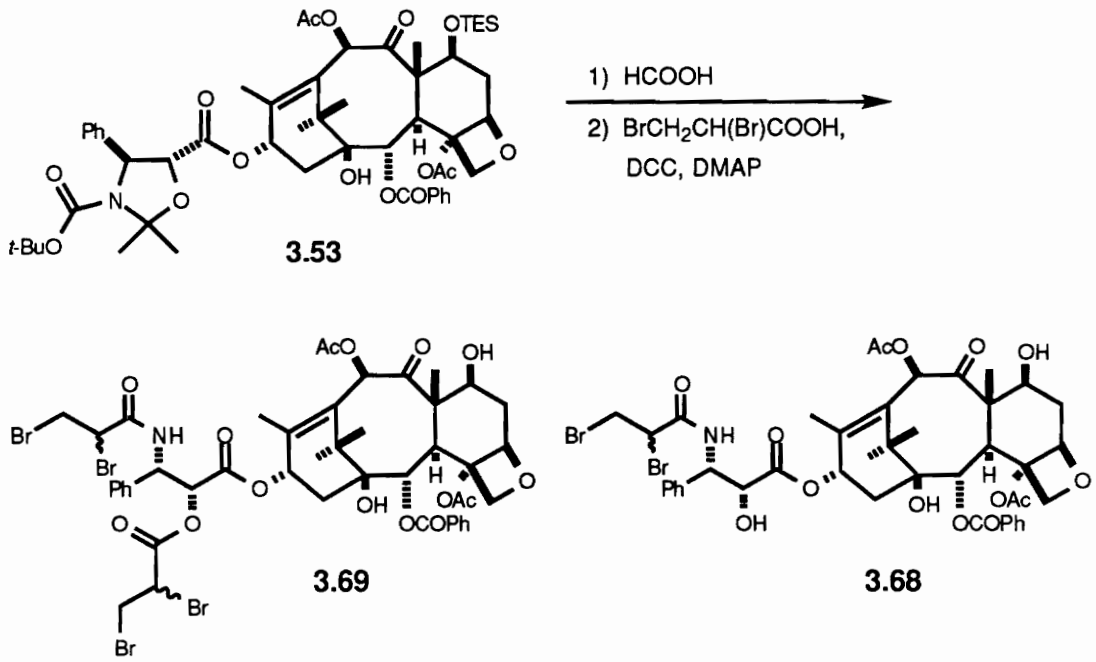
readily be prepared by treatment of acetonide (**3.53**) with formic acid for 3 hours to generate the free amine, followed by acylation using dibromoacetic acid in methylene chloride in the presence of DCC and DMAP. The structure was confirmed by ^{1}H NMR and mass spectral analysis. The appearance of a singlet in the ^{1}H NMR spectrum at 5.78 ppm integrating for one proton supported the presence of the N-(dibromoacetyl) group while mass spectral analysis gave a molecular mass consistent with the structure of (**3.67**). Moreover, the presence of M+2 and M+4 peaks in the spectrum clearly indicated the presence of two bromine atoms.



Scheme 3.24. Preparation of the N-(dibromoacetyl) analog (**3.67**).

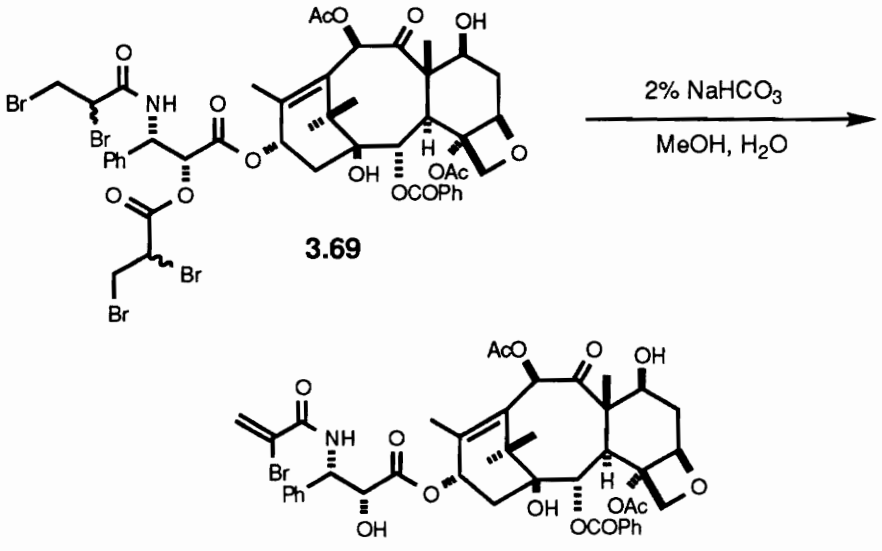
The second derivative pursued was the N-(2", 3"-dibromo) analog (**3.68**). Surprisingly, application of the same deprotection-acylation

sequence used to create the N-(dibromoacetyl) analog (**3.67**) did not lead to (**3.68**). Although formic acid deprotection of (**3.53**) did proceed as anticipated, several attempts to acylate the resulting crude free amine all led to the N, O-diacyl adduct (**3.69**) (*Scheme* 3.25). This was completely unexpected since all prior work in this area had shown that little, if any, N, O-diacyl products were formed, even when reaction times were as long as two hours. When minor amounts of the N, O-diacyl product were observed, N-acylation was almost complete before O-acylation occurred. However, in this case the N, O-diacyl analog (**3.69**) was detected within minutes and seemed to appear almost immediately after the N-acyl product formed.



Scheme 3.25 Attempted preparation of (**3.68**).

Early structure-activity studies had firmly established that a free 2'-hydroxyl was required for activity,[6] thus, the N, O-diacyl analog (**3.69**) was not expected to be of any value. Acyl groups at the 2' position have been found to be reasonably labile and consequently (**3.69**) was treated with 2% $NaHCO_3$ in MeOH-H_2O (4:1) in hopes of selectively cleaving the acyl group at this position (*Scheme* 3.26). The reaction was monitored by TLC over the course of 1 hour 20 minutes and a single more polar product was observed. After isolation and purification by preparative TLC, identification by ^1H NMR revealed that cleavage of the O-acyl group had occurred as hoped, but the presence of a doublet at 6.01 ppm and another at 6.91 ppm suggested that the N-(2", 3"-dibromopropanoyl) group had also undergone elimination to give (**3.70**). Although this was



Scheme 3.26. Hydrolysis of (**3.69**).

not anticipated, in retrospect, it is not surprising considering the relative acidity of the 2" proton. The direction of the elimination was confirmed from the coupling constants of the two vinylic protons. Vicinal protons in unsaturated systems, whether *cis* or *trans*, typically have coupling

Table 3.5. ^{1}H NMR Spectra of Brominated Taxol Analogs.[a, b]

Protons on	**3.67**	**3.70**
C-2	5.66 (d, 7.1)	5.676 (d, 7.0)
C-3	3.78 (d, 7.1)	3.79 (d, 7.0)
C-5	4.93 (dd, 9.5, 1.7)	4.94 (dd, 9.5, 1.9)
C-6α	2.55 (m)	2.55 (m)
C-6β	1.88 (m)	1.84 (m)
C-7	4.40 (m)	4.40 (m)
C-10	6.28 (s)	6.27 (s)
C-13	6.25 (t, 9.5)	6.26 (t, 8.7)
C-14 (α, β)	*c*	*c*
C-16-CH_3	1.13 (s)	1.15 (s)
C-17-CH_3	1.25 (s)	1.25 (s)
C-18-CH_3	1.80 (s)	1.78 (s)
C-19-CH_3	1.69 (s)	1.68 (s)
C-20α	4.29 (d, 8.5)	4.39 (d, 8.4)
C-20β	4.19 (d, 8.5)	4.18 (d, 8.4)
C-2'	4.75 (dd, 4.3, 2.4)	4.71 (dd, 4.9, 2.6)
C-3'	5.48 (dd, 9.4, 2.9)	5.59 (dd, 9.0, 2.6)
3'-NH	*d*	*d*
3'-Ph, COCOPh, 2-COPh	7.35-8.12 (m)	7.38-8.13 (m)
4-OAc	2.35 (s)	2.47 (s)
10-OAc	2.25 (s)	2.25 (s)
Br_2CHCO	5.78 (s)	
CH_2=CBrCO		6.91 (d, 1.6) 6.01 (d, 1.6)

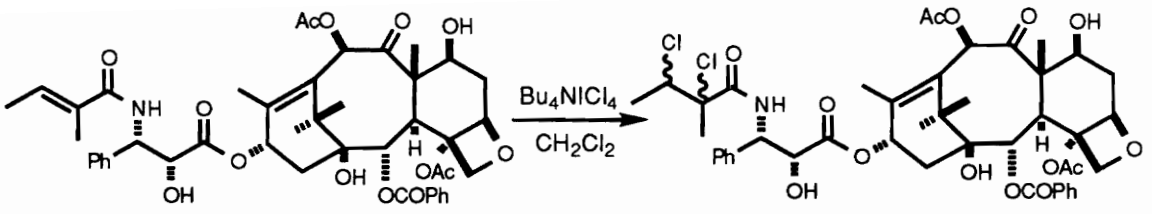
[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. [c]Difficult to determine exact chemical shifts since the signals are partially hidden under the methyl or acetate signals. [d]Hidden in the aromatic region.

constant around 6-12 Hz while geminal protons are normally 0-3 Hz. The coupling constant in the case at hand was 1.6 Hz, thus suggesting the geminal relationship of the two protons.

The specific halogen incorporated into the structure can have a dramatic affect on the activity. For example, 5-fluorouracil is a drug used in treating cancer while 5-bromouracil is a potent mutagenic agent.[181] In order to establish the effect of the halogen used in this case, the course of the investigation turned toward the synthesis of halogenated analogs containing chlorine or iodine. The maximum amount of information could be obtained only if the chlorinated or iodinated analogs were analogous to the brominated ones previously prepared, thus this was the goal.

Work in this area initially focused on the preparation of 2", 3"-dichlorocephalomannine. Tetrabutylammonium iodotetrachloride[182] was chosen as the chlorinating agent rather than molecular chlorine primarily because of the ease of handling and mildness of the reagent. In addition, tetrabutylammonium iodotetrachloride is also known to give stereospecific *trans*-addition under conditions where molecular chlorine gives mixtures of *cis*- and *trans*-addition. Treatment of cephalomannine (**3.58**) with tetrabutylammonium iodotetrachloride led to an inseparable diastereomeric mixture of dichlorides (**3.71**) in 86% yield (*Scheme* 3.27).

Analysis of the ^{1}H NMR spectrum clearly indicated the diastereomeric nature of the product. The upfield shift the of vinylic proton of cephalomannine from 6.67 ppm to 4.97 ppm confirmed the

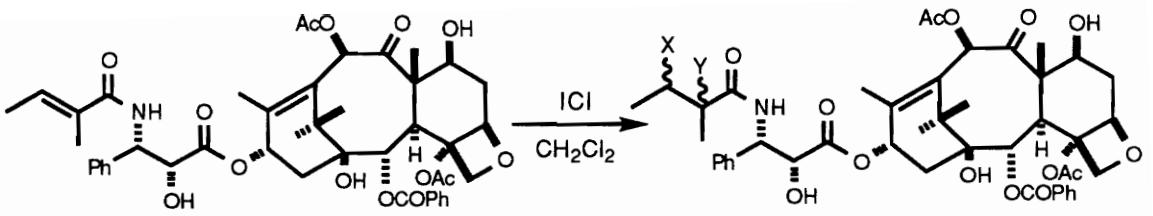


3.58 **3.71**

Scheme 3.27. Synthesis of 2", 3"-dichlorocephalomannine (**3.71**).

reaction site as the tigloyl double bond. The appearance of three doublets from 7.70-7.90 ppm corresponding to the amide proton as well as the complexity of several other signals suggested that the addition was not exclusively *trans* and that some *cis* product may be present, although this could not be unambiguously established. The FABMS showed an $(MH)^+$ of 902 as well as (M +2) and (M + 4) peaks confirming the presence of two chlorine atoms.

With the dichloride in hand, efforts were turned toward the preparation of a 3"-chloro or 3"-iodo analog which would be comparable with 3"-bromocephalomannine (**3.65**). Thus, cephalomannine (**3.58**) was treated with iodine monochloride[183] with the intent of eliminating the resulting product to create the 3"-monochloride or 3"-monoiodide, depending on the orientation of the addition (*Scheme* 3.28). The polarity of the α, β-unsaturated amide is such that the expected product would be (**3.72**) rather than (**3.73**), but iodine monochloride is known to be somewhat unpredictable.[184] It was anticipated that the orientation of addition could be established upon elimination.

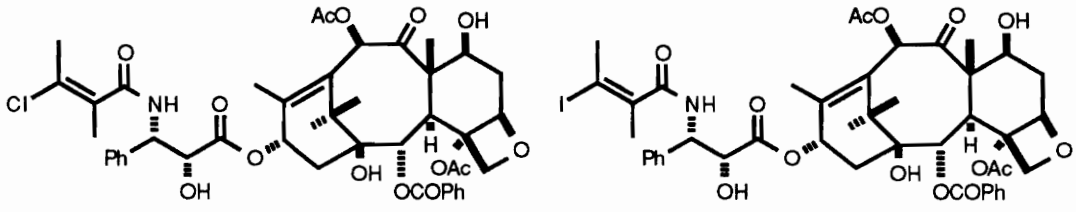


3.58

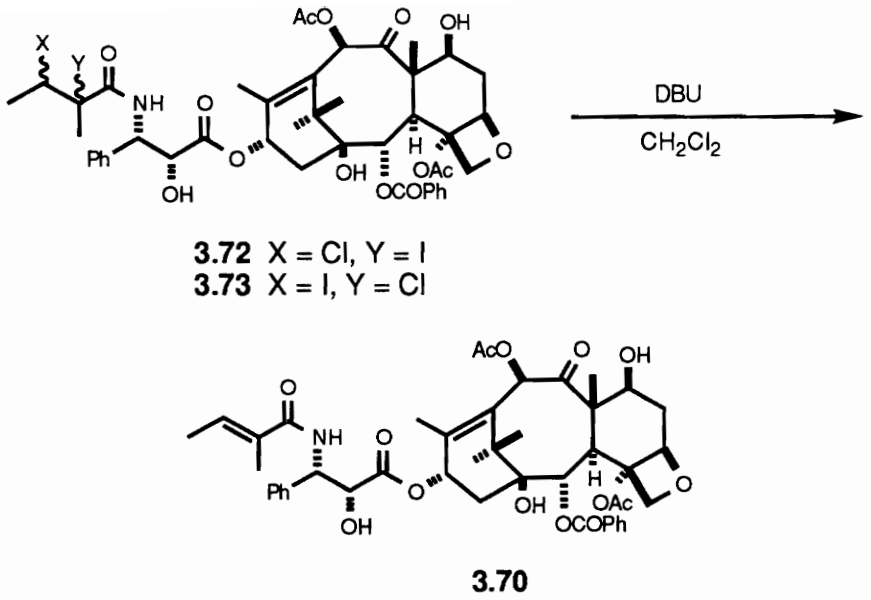
3.72 X = Cl, Y = I
3.73 X = I, Y = Cl

Scheme 3.28. Reaction of cephalomannine with iodine monochloride.

Reaction of cephalomannine (**3.58**) with iodine monochloride gave rise to a diastereomeric mixture of iodochlorides as expected. Surprisingly, an attempt to eliminate HX from this product using 1, 8-diazabicyclo[5.4.0] undec-7-ene (DBU) did not lead to the 3"-monochloride (**3.74**) or the 3"-monoiodide (**3.75**), but resulted instead in the regeneration of cephalomannine (**3.58**) (*Scheme* 3.29). A repetition of this sequence but using 2% bicarbonate instead of DBU led to the same result.



3.74 **3.75**



Scheme 3.29. Elimination of iodochloride product.

3.3.5. Miscellaneous Analogs

Two additional analogs were attempted which do not conveniently fit into any of the previous categories, although both were a direct result of the halogenation work discussed above.

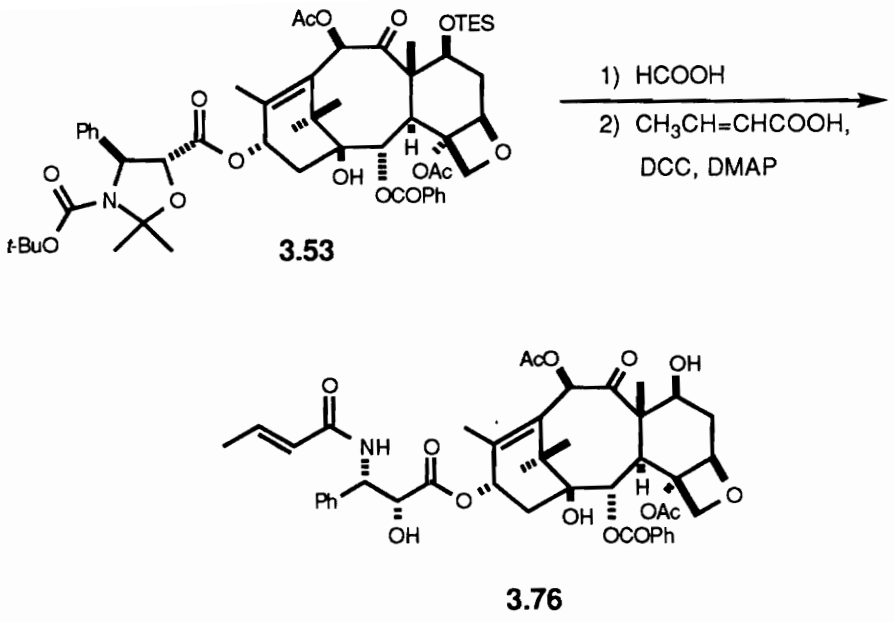
The first of the two analogs was the N-crotonyl derivative (**3.76**) (*Scheme* 3.30). This was prepared by treatment of the acetonide (**3.53**) with formic acid to afford the free amine which was then acylated with crotonic acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). The identity of the product was confirmed by ^{1}H NMR (Table 3.7) and FABMS.

The original reason for the preparation of this derivative was to

Table 3.6. ^{1}H NMR Spectra of Chlorinated Cephalomannine Analogs.[a, b]

Protons on	**3.71**[c]	**3.72/3.73**[c]
C-2	5.66 (d, 7.0)	5.67
C-3	3.78 (d, 7.0)	3.82 (d, 7.0)
C-5	4.9 (br d, 9.3)	4.94 (d, 9.3)
C-6α	2.54 (m)	2.55 (m)
C-6β	1.87 (m)	1.87 (m)
C-7	4.39 (m)	4.39 (m)
C-10	6.28 (s)	6.28 (s)
C-13	6.19 (m)	6.21 (br t)
C-14 (α, β)	*d*	*d*
C-16-CH_3	1.15 (s)	1.15 (s)
C-17-CH_3	1.24 (s)	1.25 (s)
C-18-CH_3	1.80 (s)	1.80 (s)
C-19-CH_3	1.68 (s)	1.68 (s)
C-20α	4.30 (d, 8.4)	4.30 (d, 8.4)
C-20β	4.19 (m)	4.20 (m)
C-2'	4.67 (m)	4.79 (m)
C-3'	5.50 (m)	5.67
3'-NH	7.85 (d, 9.0)[e]	7.06 (d, 9.0)
ArH	7.33-8.13 (m)	7.30-8.13 (m)
4-OAc	*f*	2.36 (s)
10-OAc	2.25 (s)	2.25 (s)
C-3"	4.97 (m)	4.59 (m)
C-4"-CH_3	1.54 (m)[g]	2.14[i]
C-5"-CH_3	1.80 (s)[h]	1.68[i]

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. [c]Several of the signals appear as multiplets due to the fact that this is a diastereomeric mixture. [d]Difficult to determine exact chemical shifts since the signals are partially hidden under the methyl or acetate signals. [e]Appears as two overlapping doublets. [f]Possibly three peaks centered at 2.35 ppm. [g]Possibly three doublets in this region. [h]Possibly two singlets around 1.80 ppm. [i]Possibly interchanged.



3.53

3.76

Scheme 3.30. Synthesis of the N-crotonyl analog (**3.76**).

generate another 2", 3"-dibromo analog. It was thought that the problems associated with the preparation of the N-(2", 3"-dibromopropanoyl) analog (**3.68**) could be circumvented by introduction of the unsaturated acyl group first and then brominating it. The bromination of the N-crotonyl analog (**3.76**) never materialized due to the bioassay results of the brominated derivatives described earlier. Nevertheless, this derivative, which could be considered to be cephalomannine (**3.58**) minus the 5"-methyl group, turned out to be interesting in itself.

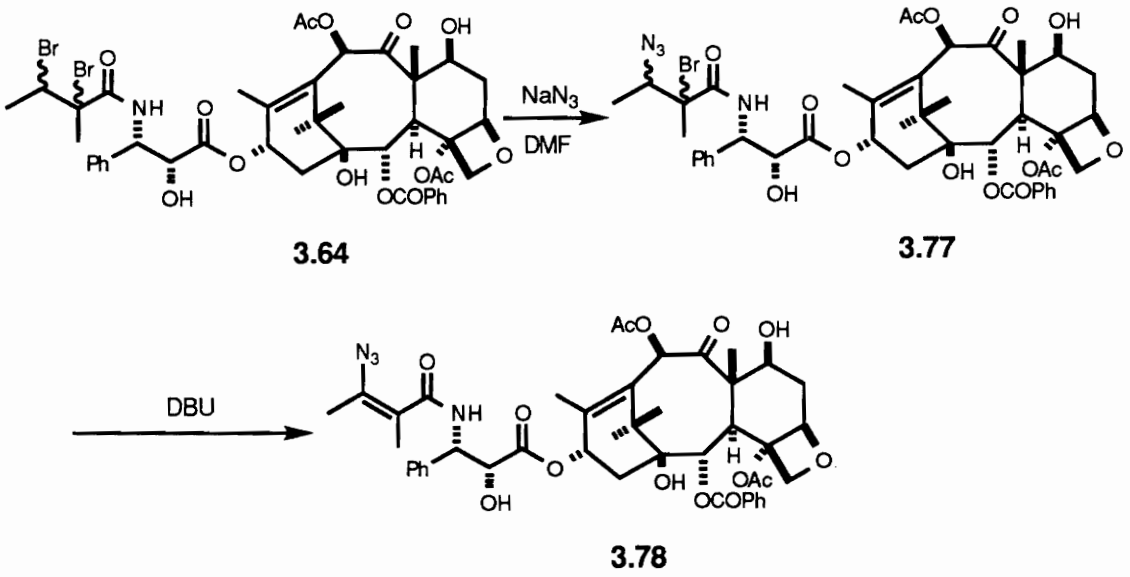
The second derivative attempted was the 3"-azido analog (**3.78**). Azido groups are generally introduced by the nucleophilic substitution of

Table 3.7. ^{1}H NMR Spectrum of N-Crotonyl Analog (**3.76**).[a, b]

Protons on	**3.76**
C-2	5.68 (d, 7.0)
C-3	3.79 (d, 7.0)
C-5	4.94 (dd, 9.5, 1.8)
C-6α	2.54 (m)
C-6β	1.87 (m)
C-7	4.39 (m)
C-10	6.28 (s)
C-13	6.22 (br t, 9.0)
C-14 (α, β)	*c*
C-16-CH_3	1.15 (s)
C-17-CH_3	1.26 (s)
C-18-CH_3	1.82 (s)
C-19-CH_3	1.68 (s)
C-20α	4.30 (d, 8.3)
C-20β	4.19 (d, 8.3)
C-2'	4.71 (br s)
C-3'	5.61 (dd, 8.8, 2.7)
3'-NH	*d*
3'-Ph, COCOPh, 2-COPh	7.27-8.13 (m)
4-OAc	2.35 (s)
10-OAc	2.25 (s)
$CH_3CH{=}C\underline{H}CO$	5.83 (dd, 15.2, 1.7)
$CH_3C\underline{H}{=}CHCO$	6.79 (m)
$\underline{CH_3}CH{=}CHCO$	1.67 (s)

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. [c]Difficult to determine exact chemical shifts since the signals are partially hidden under the methyl or acetate signals. [d]Hidden under the C-10 and C-13.

alkyl halides. Typical reaction conditions usually involve heating an alkyl halide with sodium azide in DMF or DMSO.[185] Consequently, the route envisioned for the preparation of (**3.78**) was simply the reaction of 2", 3"-dibromocephalomannine (**3.64**) with sodium azide (*Scheme* 3.31).



3.64 **3.77**

3.78

Scheme 3.31. Envisioned route to 3"-azidocephalomannine (**3.78**).

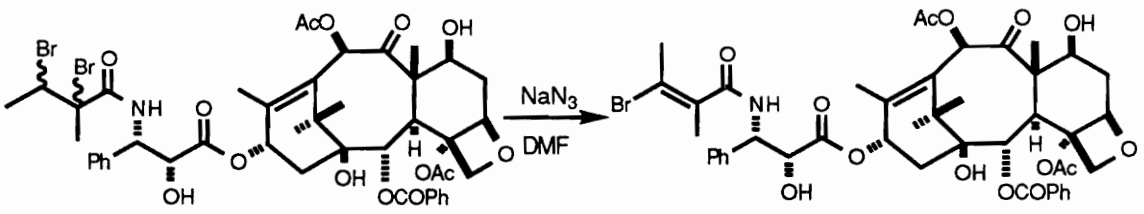
In theory, this should lead to 3"-azido-2"-bromocephalomannine (**3.77**) which could then be converted to (**3.78**) by exposure to a hindered base such as DBU. However, the fact that (**3.64**) is a dibromide introduces some uncertainty, especially in light of a 1985 study by Chong and Sharpless.[186] As part of a continuing effort to expand the synthetic utility of 2, 3-epoxy alcohols, carboxylic acids, and amides, Chong and Sharpless examined the nucleophilic opening of a variety of these compounds. Their results clearly indicated the preferred site of

nucleophilic attack on secondary 2, 3-epoxy amides and carboxylic acids was the C-2 position. Although the scope of their investigation was limited to 2, 3-epoxy carboxylic acids and amides, their results suggest that the C-2 position is the most reactive site. If extended to nucleophilic displacements in general, then displacement of a halide at C-2" would be preferred over the C-3" position. In the case at hand, the picture is complicated by the fact that the C-2" carbon is a tertiary center rather than secondary and therefore nucleophilic displacement would be disfavored (at least by an S_N2 process). Consequently, the actual outcome of the reaction was uncertain.

The 2", 3"-dibromide (**3.64**) was treated with sodium azide in DMF at 60°C for 5 hours. An examination of the reaction by TLC showed only a single spot which corresponded to starting material. Since little change in polarity was expected even if the azide had formed, the reaction was worked up and the material isolated. Analysis of the ^1H NMR spectrum confirmed that a reaction had taken place, but the identity of the product was not clear. The amide proton, which is hidden in the aromatic region for the 2", 3"-dibromide, was now clearly evident at 6.37 ppm and an upfield shift of the 3" proton from 4.59 ppm to 4.12 ppm had taken place. These two changes suggested the reaction site was at either the 2" or 3" position. The complexity of the 2' and 3' side chain protons suggested the presence of diastereomers or two different compounds.

The initial thinking was that the reaction was not complete and consequently it was repeated. Identical conditions were used, except reaction time was extended to 24 hours (*Scheme* 3.32). The product was

isolated as before and purified by preparative TLC. The ^1H NMR indicated the product was a single compound rather than a diastereomeric mixture as expected. Furthermore, the spectrum was found to be almost identical to that of 3"-bromocephalomannine (**3.66**).



3.64 **3.66**

Scheme 3.32. Reaction of 2", 3"-dibromo analog (**3.64**) with NaN_3.

The only difference in the two spectra was a slight difference in chemical shift of the amide proton; it was observed as a doublet centered at 6.32 ppm and overlapping the C-10 in the spectrum of (**3.66**), while in the case at hand it was slightly down field at 6.37 ppm and clearly separated from the C-10. From the ^1H NMR spectrum it was quite clear that the dibromide had undergone elimination to give a 3" substituted analog. Whether elimination had occurred before or after substitution was not readily apparent. Consequently, it was possible that the product was either the 3"-bromo analog (**3.66**) or the desired azido derivative (**3.78**). The question was answered when the low resolution FAB mass spectral analysis gave the characteristic pattern for a single bromine atom and high resolution FABMS supported a molecular formula of $C_{45}H_{52}NO_{14}Br$.

This confirmed the identity of the product as the 3"-bromo analog (**3.66**). Since the stereochemistry around the double bond was not unambiguously established, it is possible it is the opposite of that of (**3.65**). However, with the only difference in the ^{1}H NMR being a slight shift in the amide proton, possibly due to a concentration effect, it is likely that the dibromo analog (**3.64**) simply underwent anti elimination to give (**3.66**).

3.3.6. Biological Studies

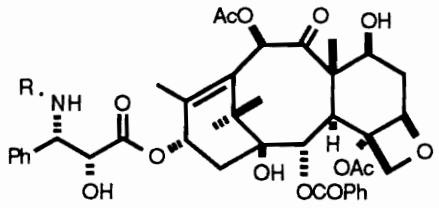
The analogs prepared were sent to Dr. William Lichter at the University of Miami School of Medicine for biological evaluation. All samples were tested for *in vitro* cytotoxicity using P-388 cells (a mouse lyphocytic leukemia cell line). The results of the bioassays are shown in Table 3.8-3.10, which are categorized according to the type of analog. The activity of each analog is given relative to Taxol ($ED_{50}/ED_{50(Taxol)}$) and cephalomannine (**3.58**) is included as a reference. Using this system, compounds which have a value of less than 1 are more active than Taxol while those with values greater than 1 are less active.

A careful examination of the biological data obtained for the analogs reveal some interesting patterns.

All of the N-oxalyl and diketo analogs were less active than Taxol (Table 3.8). Although in some cases the decrease in activity was small, it is clear that the addition of an extra carbonyl group has a negative impact on the activity. Even though the data available is limited, it also

seems to suggest that any extension in the length of the 3'-N-acyl group causes an decrease in activity (compare (**3.28**), (**3.59**), and (**3.56**)).

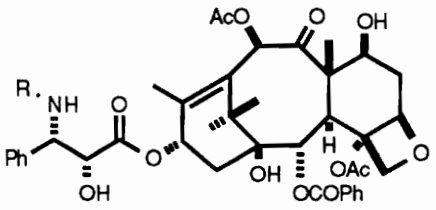
Table 3.8. Bioactivity of N-Oxalyl and Diketo Analogs.

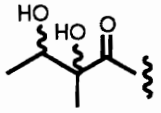
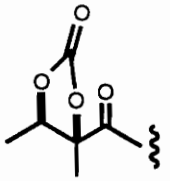
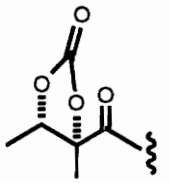
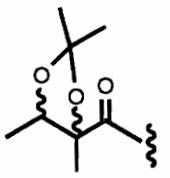


Compound	R	ED_{50}^{rel}
3.28	PhCO	1
3.58	$CH_3CH=C(CH_3)CO$	1.4
3.56	$PhCH_2OCOCO$	97
3.32	PhNHCOCO	7
3.37	CH_3OCOCO	20
3.31	CH_3COCO	14
3.59	PhCOCO	11

As with the N-oxalyl and diketo analogs, all the diol analogs were less active than Taxol and cephalomannine (Table 3.9). The cephalomannine diol (**3.30**) was found to be only slightly less active, while the carbonates (**3.61** and **3.62**) and acetonide (**3.60**) showed significant drops in activity. Since the prevailing thoughts have been

Table 3.9. Bioactivity of Diol Analogs.



Compound	R	ED_{50}^{rel}
3.28	PhCO	1
3.58	$CH_3CH=C(CH_3)CO$	1.4
3.30		10
3.61		125
3.62		143
3.60		433

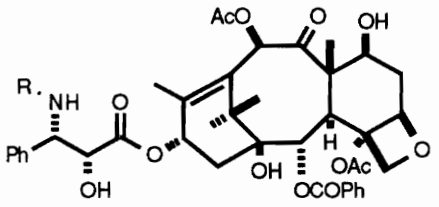
that the 3'-N-acyl group should be hydrophobic, it was interesting to note that the cephalomannine diol (**3.30**) showed only a moderate decrease in activity. Moreover, it was somewhat surprising to find that the

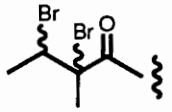
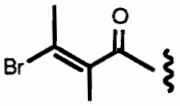
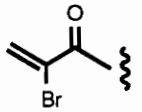
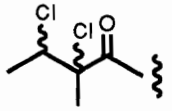
stereochemistry of the carbonates (**3.61** and **3.62**) seemed to be of little importance. If the diol analogs are examined for a general trend, it becomes readily apparent that the decrease in activity parallels an increase in size.

The role of the 3'-N-acyl group has been extensively debated. Dubois *et al.*[86] proposed that hydrophobic interactions occurring between the benzoate group at C-2 and the amino substituents at C-3' set the side chain in such a position that the C-2' hydroxyl and the C-3' phenyl can interact with tubulin residues leading to a stabilization of the drug receptor binding. In contrast, Vander Velde and coworkers[88] have argued that the crucial hydrophobic interactions are between the C-2 benzoate, the C-3' phenyl, and the C-4 acetyl group, and the C-3' amide is not involved. Alternatively, Swindell and colleagues[7] have suggested that the "taxol recognition site on microtubules possesses a hydrophobic cleft designed to accept a side chain with an array of functionality preorganized by stereochemistry and hydrogen bonding." While the data presented here does not directly support any one of these ideas, the trend observed is consistent with all of them. An increase in size of the 3'-N-acyl group could easily alter the solution conformation, thus preventing the optimal conformation for binding. This could still be true even if the 3'-N-acyl group was not directly responsible for the solution conformation. Moreover, if the activity was dependent on the acyl group fitting in a hydrophobic cleft, then an increase in size would be expected to hinder the binding.

The halogenated analogs proved to be the most interesting (Table 3.10). Both the 2", 3"-dibromide (**3.64**) and the 2"-bromopropenoyl (**3.70**) analogs were more active than Taxol while the rest were only moderately less active. The 2"-bromopropenoyl analog (**3.70**) turned out

Table 3.10. Bioactivity of Halogenated Analogs.



Compound	R	ED_{50}^{rel}
3.28	PhCO	1
3.58	$CH_3CH=C(CH_3)CO$	1.4
3.64		0.01
3.66		2.8
3.70		0.002
3.31		16
3.67	Br_2CHCO	18
3.76	$CH_3CH=CHCO$	0.05

to be the major surprise when the bioactivity data indicated *it was roughly 500 times more active than Taxol.* Surprisingly, both the 2", 3"-dibromo derivative (**3.64**) and the crotonyl analog (**3.76**) also showed a moderate increase in activity.

The encouraging results observed in the initial bioassays prompted further testing of a couple of these analogs. The 2"-bromopropenoyl analog (**3.70**) was examined in a tubulin disassembly assay and was found to be slightly more active than Taxol (IC_{50} values, 2.6 μM and 2.9 μM for (**3.70**) and Taxol). However, when tested in an *in vivo* assay using the HCT116 cell line it turned out to be slightly less cytotoxic (EC_{50} values, 40 nM and 2.3 nM for (**3.70**) and Taxol). Interestingly, in another assay using a Taxol resistant cell line (HCT/VM46) it was moderately more cytotoxic (C_{50} values, 133 nM and 327 nM for (**3.70**) and Taxol). Although the 2", 3"- dibromo analog (**3.64**) initially looked promising, subsequent biological studies have not supported the initial results.

If the biological data is evaluated as a whole, several important conclusions seem to be emerging:

1. A wide variety of acyl groups are tolerated at the 3'-N position.

2. If the groups become too lengthy or big, activity drastically decreases.

3. The acyl groups that consistently perform the best contain an α, β unsaturation.

Of the three conclusions, the third is the most debatable. Yet, in light of the data presented here, it seems warranted. Cephalomannine (**3.58**), 3"-bromocephalomannine (**3.65**), the 2"-bromopropenoyl analog (**3.70**), and the crotonyl analog (**3.76**) all showed comparable or enhanced activity (compared to Taxol) while those analogs that were saturated all proved to be less active. Continued investigation along these lines may prove fruitful.

3.3. Conclusion

Studies on the solution conformation of Taxotere by Vander Velde *et. al.*[88] led them to propose that hydrophobic interactions between the C-2 benzoate, the C-3' phenyl, and the C-4 acetyl group were responsible for the observed (and presumably the binding) conformation, and that the C-3' amide group was not involved. While it is true that the 3' position will tolerate a wide variety of N-acyl groups, there appear to be limits which if transgressed have a negative impact on the activity. Furthermore, the fact that the analogs herein range from 500 times more active to roughly 500 times less active clearly seems to indicate that even if the 3'-N-acyl group does not determine the solution conformation, it does play a significant role in the biological activity.

When thinking about a future course of action, the dramatic increase in activity shown by (**3.70**) supports continued investigations along these lines. It would be interesting to prepare a series of analogs

similar to (**3.70**), but with a different halogen such as chlorine or fluorine. Moreover, a hydrogen or methyl at the 2" position might also prove interesting.

Finally, the repreparation and biological testing of oxamic analog (**3.57**) is certainly worth pursuing. Should this analog show activity even comparable to that of Taxol, it would be worthwhile. A salt of this analog would readily solve the solubility problems associated with Taxol.

3.4. Experimental

GENERAL EXPERIMENTAL PROCEDURES- The general experimental procedures used were as described in Section 2.4. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. All optical rotations (*c*, mg/mL) were determined on a Perkin-Elmer Model 241 polarimeter using a 10 cm path length cell.

Cephalomannine, Taxol and their derivatives typically form glassy solids when the solvent is removed under vacuum. Consequently, after pure compounds were isolated they were dissolved in a small quantity of CH_2Cl_2 and hexane was added to precipitate them.

2'-BENZOYL TAXOL SIDE CHAIN METHYL ESTER (**3.38**)- A 200 mg (0.71 mmoles) of the Taxol side chain (**2.25**) was dissolved in 5 mL of dry CH_2Cl_2 in a 50 mL round bottom flask. The solution was cooled in an ice bath and then treated with 91 μL (0.78 mmoles, 1.1 eq.) of benzoyl chloride and 149 μL (1.1 mmoles, 1.5 eq.) of pyridine. The mixture was

stirred for 1 hour at 0°C and then warmed to room temperature and stirred and additional hour. The solution was then diluted with 15 mL of CH_2Cl_2 and washed with 2 N HCl (1 x 20 mL), dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by flash chromatography to give 247 mg (90%) of (**3.38**) as a clear oil: ^{1}H NMR δ 7.31-8.01 (overlapping m, 15H, 3'-Ph, PhCO, 2'-PhCO), 7.27 (d, J=8.5, 1H, NH), 5.94 (dd, J=8.8, 3.1, 1H, C-3'H), 5.65 (d, J=3.2 1H, C-2'H), 3.77 (s, 3H, OMe).

REACTION OF 2'-BENZOYL TAXOL SIDE CHAIN METHYL ESTER (**3.38**) WITH OXALYL CHLORIDE AND ISOPROPANOL QUENCH- A 30 mg (74.4 μmoles) quantity of (**3.38**) was dissolved in 1 mL of dry benzene under argon. To this solution 14 μL (166 μmoles, 2.2 eq.) of oxalyl chloride was added. The resulting mixture was stirred at room temperature. After 24 hours, a second 14 μL aliquot of oxalyl chloride was added and then stirred for an additional 24 hours. The reaction was cooled in an ice bath and a solution consisting of 0.5 mL of CH_2Cl_2 and 0.5 mL of *i*-PrOH was slowly added. After 3 hours, the solvent was removed under vacuum. The crude product was purified by preparative TLC (10:9:1 hexane/methylene chloride/acetone) to afford 19.1 mg (49.6%) of (**3.39**) as a viscous oil: ^{1}H NMR δ 7.31-8.01 (overlapping m, 15H, 3'-Ph, PhCO, 2'-PhCO), 6.44 (d, J=10.6, 1H, C-3'H), 6.19 (d, J=10.6 1H, C-2'H), 4.43 (m, 1H, OC<u>H</u>$(CH_3)_2$), 3.77 (s, 3H, OMe), 0.89 (d, J=6.3, 3H, OCH($\underline{CH_3}$)$_2$), 0.84 (d, J=6.3, 3H, OCH($\underline{CH_3}$)$_2$); CIMS *m/z* (rel. int.) $[MH]^+$ 518 (20), 414 (65), 220 (45), 105 (100).

REACTION OF 2'-BENZOYL TAXOL SIDE CHAIN METHYL ESTER (**3.38**) WITH OXALYL CHLORIDE AND DIETHYLAMINE QUENCH- A 30 mg (74.4 μmoles) quantity of (**3.38**) was dissolved in 1 mL of dry benzene under argon. To this solution 14 μL (166 μmoles, 2.2 eq.) of oxalyl chloride was added. The resulting mixture was stirred at room temperature. After 24 hours, a second 14 μL aliquot of oxalyl chloride was added and then stirred for an additional 24 hours. The reaction was cooled in an ice bath and a solution consisting of 0.5 mL of CH_2Cl_2 and 0.5 mL of Et_2NH was slowly added. After 3 hours, the solution was diluted with 20 mL of EtOAc and washed with 1.0 N HCl (2 x 20 mL), water (1 x 20 mL), an brine (1x 20 mL). After drying ($MgSO_4$) the solvent was removed *in vacuo*. The crude product was purified by preparative TLC (10:9:1 hexane/methylene chloride/acetone) to afford 29.5 mg (74.7%) of (**3.40**) as a viscous oil: ^{1}H NMR δ 7.31-8.01 (overlapping m, 15H, 3'-Ph, PhCO, 2'-PhCO), 6.39 (d, J=10.6, 1H, C-3'H), 6.19 (d, J=10.6 1H, C-2'H), 3.55 (s, 3H, OMe), 3.00 (overlapping q, $N(\underline{CH_2}CH_3)_2$) 1.15 (t, 3H, $OCH(CH_2\underline{CH_3})_2$), 0.54 (t, 3H, $N(CH_2\underline{CH_3})_2$); CIMS *m/z* (rel. int.) $[MH]^+$ 531 (15), 283 (100), 105 (32), 100 (25).

GENERAL PROCEDURE FOR ACID SCAVENGERS- A 30 mg (74 μmoles) sample of (**3.38**) was dissolved in 1 mL of dry benzene. To this solution 14 μL (166 μmoles, 2.2 eq.) of oxalyl chloride was added and 4 equivalents of the acid scavenger (Reilex 402, pyridine, imidazole, or sodium carbonate). The resulting mixture was stirred at room

temperature. After 3 hours, the reaction was quenched with 0.5 mL of *i*-PrOH and stirred overnight. The solutions were analyzed by TLC and all looked identical. One of the solutions was worked up and the products purified to evaluate the product ratio. The ratio was determined to be 3:2 diacylated (**3.39**)/monoacylated (**3.44**).

(**3.39**): see page 158.

(**3.44**): ^{1}H NMR δ 8.07 (d, J=9.5, 1H, NH), 7.29-8.01 (overlapping m, 10H, 3'-Ph, 2'-PhCO), 5.74 (dd, J=9.5, 3.1, 1H, C-3'H), 5.60 (d, J=3.2 1H, C-2'H), 5.16 (m, 1H, OC<u>H</u>$(CH_3)_2$), 3.79 (s, 3H, OMe), 1.38 (m, 6H, OCH(C<u>H</u>$_3)_2$).

REACTION OF 2'-BENZOYL TAXOL SIDE CHAIN METHYL ESTER (**3.38**) WITH OXALYL CHLORIDE AND METHANOL QUENCH- A solution of 30 mg (74 μmoles) of 2'-benzoyl taxol side chain methyl ester (**3.38**) in 1 mL of dry benzene was treated with 14 μL (160 μmoles, 2.2 eq.) of oxalyl chloride. The mixture was stirred at room temperature for 3 hours and then an additional 14 μL aliquot of oxalyl chloride was added. After stirring for a total of six hours, the reaction was quenched by adding 0.5 mL of methanol. The resulting solution was stirred for 1 hour at room temperature and then the solvent was removed under vacuum. The crude product was purified by preparative TLC (10:9:1 hexane/methylene chloride/acetone) to yield 12.3 mg (34%) of (**3.45**) and 10.1 mg (35%) of (**3.46**) as viscous oils.

(**3.45**): ^{1}H NMR δ 7.31-8.05 (overlapping m, 15H, 3'-Ph, NCOPh, 2'-COPh), 6.44 (d, J=10.6, 1H, C-3'H), 6.23 (d, J=10.6 1H, C-2'H), 3.55 (s,

3H, OMe), 3.24 (s, 3H, COCOOMe); CIMS *m/z* $[MH]^+$ 490 (10), 404 (5), 386 (5), 283 (20), 123 (38), 105 (100).

(**3.46**): ^1H NMR δ 8.06 (d, J=9.5, 1H, NH), 7.31-8.01 (overlapping m, 10H, 3'-Ph, 2'-PhCO), 5.76 (dd, J=9.5, 3.1, 1H, C-3'H), 5.60 (d, J=3.2 1H, C-2'H), 3.90 (s, 3H, COCOOMe), 3.78 (s, 3H, OMe); CIMS *m/z* $[MH]^+$ 386 (100), 301 (18), 283 (20), 192 (38), 123 (48), 105 (60).

REACTION OF 2'-BENZOYL TAXOL SIDE CHAIN METHYL ESTER (**3.38**) WITH OXALYL CHLORIDE AND ALLYL ALCOHOL QUENCH- A solution of 30 mg (74 μmoles) of 2'-benzoyl taxol side chain methyl ester (**3.38**) in 1 mL of dry benzene was treated with 14 μL (160, μmoles, 2.2 eq.) of oxalyl chloride. The mixture was stirred at room temperature for 3 hours and then an additional 14 μL aliquot of oxalyl chloride was added. After stirring for a total of six hours, the reaction was quenched by adding 0.5 mL of allyl alcohol. The resulting solution was stirred overnight. The mixture was worked up by diluting with 20 mL of EtOAc and washing with water (2 x 20 mL) and brine (1 x 20 mL). After drying ($MgSO_4$), the solvent was removed under vacuum. The crude product was purified by preparative TLC (10:9:1 hexane/methylene chloride/acetone) to yield 12.4 mg (32%) of (**3.47**) and 14.1 mg (46%) of (**3.48**) as viscous oils.

(**3.47**): ^1H NMR δ 7.30-7.78 (overlapping m, 15H, 3'-Ph, 2'-OCOPh, NCOPh), 6.44 (d, J=10.5, 1H, C-3'H), 6.22 (d, J=10.5 1H, C-2'H), 5.97 (m, 1H, $OCH_2C\underline{H}=CH_2$), 5.07 and 4.77 (m, 2H, $OCH_2CH=C\underline{H_2}$), 4.05 (m, 1H, $OC\underline{H_2}CH=CH_2$), 3.55 (s, 3H, OMe); CIMS *m/z* $[MH]^+$ 516 (18), 440 (18), 412 (100), 283 (20), 218 (38), 105 (35).

(**3.48**): ^{1}H NMR δ 8.05 (d, J=9.1, 1H, NH), 7.30-8.01 (overlapping m, 10H, 3'-Ph, PhCO), 5.97 (m, 1H, $OCH_2C\underline{H}=CH_2$), 5.75 (dd, J=9.5, 3.1, 1H, C-3'H), 5.60 (d, J=3.1 1H, C-2'H), 5.42 and 5.33 (m, 2H, $OCH_2CH=C\underline{H}_2$), 4.80 (m, 1H, $OC\underline{H}_2CH=CH_2$), 3.78 (s, 3H, OMe); CIMS *m/z* $[MH]^+$ 412 (88), 290 (10), 283 (15), 218 (33), 123 (100), 105 (98).

SYNTHESIS OF THE (4S,5R)-N-BOC-2, 2-DIMETHYL-4-PHENYL-5-OXAZOLIDINE CARBOXYLIC ACID (**3.52**)- Protected side chain (**3.52**) was prepared as described by J. Rimoldi.[172] This procedure was a modification of the methodology of Denis *et. al.*[168] and A. Commerçon *et. al.*[137]: mp 135-138°C (lit. 137°C), ^{1}H NMR δ 7.34 (m, 5H, 3'-Ph), 5.11 (br d, 1H, C-3'H), 4.52 (d, J=5.7, 1H, C-2'H), 1.79 (s, 3H, acetal CH_3), 1.74 (s, 3H, acetal CH_3), 1.19 (br s, 9H, $C(CH_3)_3$).

7-(TRIETHYLSILYL)BACCATIN III- A 92 mg (0.16 mmoles) quantity of baccatin III was dissolved in 1.5 mL of dry methylene chloride and then treated with 79 mL (0.71 mmoles, 3 eq.) of triethylsilyl chloride and 32 mg (0.47 mmoles, 3 eq.) of imidazole. The solution was stirred at room temerature for 3 hours. Purification by preparative TLC (6:4 hexane/ethyl acetate) yielded 80 mg (72%) of 7-(triethylsilyl)baccatin III: ^{1}H NMR δ 7.40-8.11 (m, 5H, C-2-OCOPh), 6.46 (s, 1H, C-10H), 5.63 (d, J=7.0, 1H, C-2H), 4.96 (br d, 1H, C-5H), 4.83 (br m, 1H, C-13H), 4.49 (m, 1H, C-7), 4.30 and 4.10 (ABq, 2H, C-20H_2), 3.88 (d, J=7.0, 1H, C-2H), 2.25 (s, 3H, 4-$OCOCH_3$), 2.18 (s, 3H, 10-$OCOCH_3$), 1.80 (s, 3H,), 1.65 (s,

3H,), 1.20 (s, 3H,), 1.04 (s, 3H,), 0.93 (t, J=8.0, 9H, $Si(CH_2\underline{CH_3})_3$), 0.58 (q, J=8.0, 6H, $Si(\underline{CH_2}CH_3)_3$)).

COUPLING OF SIDE CHAIN (**3.52**) WITH 7-(TRIETHYLSILYL)BACCATIN III- A solution of 124 mg (0.18 mmoles) of 7-(triethylsilyl)baccatin III and 85 mg (0.27 mmoles, 1.5 eq.) of side chain (**3.52**) in 1.5 mL of toluene was treated with 55 mg (0.27 mmoles, 1.5 eq.) of DCC and a catalytic amount of DMAP. The mixture was stirred at 60°C for 45 minutes. Purification on preparative TLC afforded 166 mg (94%) of coupled product (**3.53**) as a glassy solid: mp 148-150°C; ^{1}H NMR δ 7.31-8.05 (m, 10H, 2-OCOPh, 3'-Ph), 6.45 (s, 1H, C-10H), 6.24 (br t, J=8.4, 1H, C-13H), 5.65 (d, J=7.1, 1H, C-2H), 5.06 (br s, 1H, C-3'H), 4.87 (br d, J=8.0, 1H, C-5H), 4.47 (d, J=6.7, 1H, C-2'H), 4.45 (m, 1H, C-7H), 4.24 and 4.09 (ABq, J=8.3, C-$20H_2$), 3.77 (d, J=7.0, 1H, C-3H), 2.54 (m, 1H, C-6H), 2.18 (s, 3H, 4-$OCOCH_3$), 1.84 (s, 3H, acetonide CH_3), 1.81 (s, 3H, acetonide CH_3), 1.76 (s, 3H, C-$18H_3$), 1.66 (s, 3H, C-$19H_3$), 1.26 (s, 3H, C-$17H_3$), 1.20 (s, 3H, C-$16H_3$), 1.13 (br s, 9H, $OC(CH_3)_3$), 0.91 (t, J=8.3, 6H, $Si(CH_2C\underline{H_3})_3$), 0.55 (q, J=8.3, 6H, $Si(C\underline{H_2}CH_3)_3$); FABMS *m/z* (rel. int.) $[MH]^+$ 1004 (3), 902 (1), 844 (1), 276 (5), 176 (95), 105 (100).

N-DEBENZOYL-N-(METHYLOXALYL)TAXOL (**3.37**)- A 24 mg (24 μmoles) quantity of coupled product (**3.53**) was dissolved in 0.5 mL of 99% formic acid and stirred at room temperature for 3 hours. The mixture was then diluted with 10 mL of EtOAc and washed with saturated $NaHCO_3$ (2 x 10 mL), water (1 x 10 mL), and brine (1 x 10 mL). After drying ($MgSO_4$), the

solvent was removed *in vacuo*. Without further purification, the sample was dissolved in 0.5 mL of EtOAc and then 0.5 mL of H_2O and 8 mg of $NaHCO_3$ To this heterogeneous mixture, 4 μL of methoxalyl chloride was added. Two additional 2 μL portions of methyl oxalyl chloride were added at 30 minute intervals. After 75 minutes (total time), the mixture was evaporated to dryness. Purification by preparative TLC (3:2 ethyl acetate/hexane) produced 12.5 mg (61%) of (**3.37**) as a glassy solid: $[\alpha]^{23}_D$ = -76.7° (*c*=5.4, $CHCl_3$) ^1H NMR see Table 3.1; FABMS *m/z* (rel. int.) $[M+Na]^+$ 858 (12), 533 (10), 459 (19), 401 (25), 327 (50), 281 (58), 207 (78), 147 (100); HRFABMS *m/z* $[M+Na]^+$ 858.2929 ($C_{43}H_{49}NO_{16}Na$ requires 858.2949).

N-DEBENZOYL-N-(BENZYLOXALYL)TAXOL (**3.56**)- A 60 mg (60 μmole) sample of (**3.53**) was treated with 99% formic acid for 3 hours at room temperature. The mixture was then diluted with 15 mL EtOAc and washed with saturated $NaHCO_3$ (2 x 10 mL), water (1 x 10 mL), and brine (1 x 10 mL). After drying over $MgSO_4$, the solvent was removed under vacuum to give the crude free amine. This was used without further purification.

To a solution of the free amine in 0.5 mL of dry CH_2Cl_2 which had been cooled in an ice bath, 10.8 mg (60 μmoles, 0.95 eq) of PhCOCOOH, 12.3 mg (60 μmoles, 0.95 eq.) of DCC, and a catalytic amount of DMAP were added. After 30 minutes, the solution was streaked directly on a preparative TLC plate (3:2 ethyl acetate/hexane) for purification. The result was 12.5 mg (23%) of N-debenzoyl-N-(benzyloxalyl)taxol (**3.56**): mp

144-146°C; $[\alpha]^{23}_D$ = -79.4° (*c*=12.5, $CHCl_3$); ^1H NMR see Table 3.1; FABMS *m/z* (rel. int.) $[MH]^+$ 912 (60), 834 (15), 551 (50), 509 (80), 449 (35); HRFABMS *m/z* $[MH]^+$ 912.3431 ($C_{49}H_{54}NO_{16}$ requires 912.3443).

N-DEBENZOYL-N-OXALYLTAXOL (**3.57**)- A 13 mg (14 μmoles) quantity of N-benzyloxalyl-N-debenzoyltaxol (**3.56**) was dissolved in 1 mL of EtOAc and 3 mg of 10% Pd/C was added. The flask was attached to the hydrogenation apparatus and flushed 5 times with H_2 gas. The heterogeneous mixture was stirred for 1 hour and then filtered through Celite to remove the catalyst. The solvent was removed under vacuum to give 6.0 mg (51%) of N-debenzoyl-N-oxalyltaxol (**3.57**): ^1H NMR see Table 3.1.

N-DEBENZOYL-N-PYRUVYLTAXOL (**3.31**)- The cephalomannine diols (**3.30**) according to the catalytic procedure described by Kingston *et. al.*[54]

A 51 mg (59 μmoles) sample of the cephalomannine diol was dissolved in 0.6 mL of methanol. To this solution, 150 μL of water and 30 mg of $NaIO_4$ was added with stirring. After 3 hours, the solution was diluted with 10 mL of EtOAc and washed with water (1 x 10 mL) and brine (1 x 10 mL). After drying over $MgSO_4$, the solvent was removed under vacuum. Purification by preparative TLC (3:2 ethyl acetate/hexane) afforded 37 mg (76%) of (**3.31**): mp 164-168°C (lit. 161.0-162.5°C); ^1H NMR see Table 3.2; ; FABMS *m/z* (rel. int.) $[MH]^+$ 820 (3), 742 (1), 509 (5), 154 (90), 105 (100).

N-DEBENZOYL-N-(PHENYLGLYOXYL)TAXOL (**3.59**)- A 61 mg (61 μmoles) of (**3.53**) was dissolved in 0.5 mL of 99% formic acid and stirred at room temperature for 150 minutes. The mixture was then diluted with 10 mL of EtOAc and washed with saturated $NaHCO_3$ (2 x 10 mL), water (1 x 10 mL), and brine (1 x 10 mL). After drying ($MgSO_4$), the solution was evaporated to dryness.

The crude amine was dissolved in 0.5 mL of 1 mL of dry CH_2Cl_2 and then cooled in an ice bath. To this solution, 9.5 mg (63 μmoles, 1 eq.) of phenylglyoxalic acid, 13 mg (63 μmoles, 1 eq.) of DCC, and a catalytic amount of DMAP were added. The mixture was stirred at 0°C for 30 minutes. Purification by preparative TLC (3:2 ethyl acetate/hexane) led to 11 mg (20%) of N-debenzoyl-N-(phenylglyoxyl)taxol (**3.59**): mp 166-170°C; $[\alpha]^{23}_D$ = -44.9° (*c*=1.38, $CHCl_3$); ^1H NMR see Table 3.2; FABMS *m/z* (rel. int.) $[MH]^+$ 882 (2), 509 (5), 309 (5), 268 (10), 105 (100); HRFABMS *m/z* $[MH]^+$ 882.3346 ($C_{48}H_{52}NO_{15}$ requires 882.3337).

CEPHALOMANNINE DIOL (**3.30**)- The cephalomannine diol (**3.30**) was prepared according to the catalytic method described by Kingston *et. al.*[54] All spectral data was in accord with an authentic sample.

CEPHALOMANNINE ACETONIDES (**3.60**)- A solution of 50 mg (58 μmoles) of the cephalomannine diol (**3.30**) in 0.5 mL of toluene was prepared and then 75 mL (600 μmoles, 10 eq.) of 2, 2-dimethoxypropane and a catalytic amount of tosic acid was added. The mixture was stirred at room temperature for 1 hour. Purification by preparative TLC (3:2

ethyl acetate/hexane) yielded 33 mg (64%) of an inseparable diastereomeric mixture of acetonides (**3.60**): ^{1}H NMR see Table 3.3; FABMS *m/z* (rel. int.) $[MH]^+$ 906 (25), 846 (4), 569 (5), 509 (22), 445 (5); HRFABMS *m/z* $[MH]^+$ 906.3896 ($C_{48}H_{60}NO_{16}$ requires 906.3912).

CEPHALOMANNINE CARBONATES (**3.61**) AND (**3.62**)- Two reactions were run side by side. An oven dried vial that had been flushed with argon was charged with 30 mg (35 μmoles) of the cephalomannine diol (**3.30**) and 0.5 mL of dry CH_2Cl_2. To this vial, 36 μL of a 1.93 M solution of phosgene (Fluka; 20% solution in toluene) and 100 μL of pyridine. A second vial was prepared in the same fashion except it contained 20 mg (23 μmoles) of diol (**3.30**), 7 mg (23 μmoles, 3 eq.) of triphosgene, and 20 μL of pyridine. The solutions were stirred at room temperature for 1 hour. Analysis by TLC (3:2 ethyl acetate/hexane) showed the reaction profiles were identical. Each reaction was worked up by diluting with 10 mL of EtOAc and then washing with 1 N HCl (1 x 10 mL), water (1 x 10 mL), and brine (1 x 10 mL). After drying ($MgSO_4$), the solvent was removed *in vacuo*. Purification by preparative TLC (1:1 ethyl acetate/hexane) produced a combined yield of 21.3 mg (35%) of (**3.61**) and 24.3 mg (40%) of (**3.62**). (Note: The absolute stereochemistry was not determined. The numbers (**3.61**) and (**3.62**) have been arbitrarily assigned. Sample (**3.61**) is slightly less polar on TLC.)

(**3.61**): mp 174-178°C; $[\alpha]^{23}_D$ = -126.4° (*c*=2.69, $CHCl_3$); ^{1}H NMR see Table 3.3; FABMS *m/z* (rel. int.) $[MH]^+$ 892 (5), 509 (5), 387 (5), 278.1

(15), 105 (100); HRFABMS *m/z* $[MH]^+$ 892.3381 ($C_{46}H_{54}NO_{17}$ requires 892.3392).

(**3.62**): mp 180-184°C; $[\alpha]^{23}_D$ = -131.9° (*c*=4.79, $CHCl_3$); ^1H NMR see Table 3.3; FABMS *m/z* (rel. int.) $[MH]^+$ 892 (3), 509 (5), 387 (4), 278.1 (15), 105 (100); HRFABMS *m/z* $[MH]^+$ 892.3415 ($C_{46}H_{54}NO_{17}$ requires 892.3392).

2", 3"-DIBROMOCEPHALOMANNINE (**3.64**)- A 30 mg quantity of a 90% cephalomannine mixture (determined by HPLC) was dissolved in 1 mL of $CHCl_3$ and then 58 mg of pyridinium perbromide was added. The reaction was monitored by TLC (3:2 ethyl acetate/hexane). After 30 minutes the solution was streaked directly on a preparative TLC plate for purification (3:2 ethyl acetate/hexane). This led to 26.5 mg (93%) of a diastereomeric mixture of dibromides (**3.64**): ^1H NMR see Table 3.4; FABMS *m/z* (rel. int.) $[MH]^+$ 994 (10), 992 (20), 990 (10), 914 (5), 509 (50), 460 (40); HRFABMS *m/z* $[MH]^+$ 992.1929 ($C_{45}H_{54}{}^{79}Br^{81}BrNO_{14}$ requires 992.1991).

ALTERNATE PROCEDURE FOR THE PREPARATION OF 2", 3"-DIBROMOCEPHALOMANNINE (**3.64**)- A 30 mg sample of 90% cephalomannine (determined by HPLC) was dissolved in 1 mL of $CHCl_3$ and then 88 mg of n-Bu_4NBr_3 was added. The reaction was monitored by TLC (3:2 ethyl acetate/hexane). After 1 hour the solution was streaked directly on a preparative TLC plate for purification (3:2 ethyl acetate/hexane). This

led to 24 mg (83%) of a diastereomeric mixture of dibromides (**3.64**): see above.

DEHALOGENATION OF 2", 3"-DIBROMOCEPHALOMANNINE (**3.64**)- A 30 mg sample of dibromide (**3.64**) was dissolved in 0.5 mL of glacial acetic acid and treated with 38 mg of freshly activated zinc. This heterogeneous mixture was stirred at room temperature for two hours. The solution was then diluted with 10 mL of EtOAc and washed with saturated $NaHCO_3$ (2 x10 mL), water (1 x10 mL), and brine (1 x10 mL). After drying over $MgSO_4$, the solvent was removed under vacuum. Purification by preparative TLC (3:2 ethyl acetate/hexane) afforded 22 mg (88 %) yield of cephalomannine (**3.58**): ^{1}H NMR δ 7.30-8.12 (m, 10H, 2-OCOPh, 3'-Ph), 6.51 (d, J=8.9, 1H, NH), 6.44 (qd, J=7.0, 1.2, C-3"H), 6.27 (s, 1H, C-10H), 6.21 (br t, J=8.4, 1H, C-13H), 5.67 (d, J=7.0, 1H, C-2H), 5.61 (dd, J=8.8, 2.7, 1H, C-3'H), 4.93 (br d, J=7.7, 1H, C-5H), 4.41 (dd, J=5.3, 2.7, 1H, C-2'H), 4.40 (m, 1H, C-7H), 4.29 and 4.18 (ABq, J=8.4, 2H, C-20H_2), 3.78 (d, J=7.0, 1H, C-3H), 2.54 (m, 1H, C-6H), 2.36 (s, 3H, 4-OCOCH_3), 2.24 (s, 3H, 10-OCOCH_3), 1.80 (s, 3H, C-5"H_3), 1.79 (s, 3H, C-18H_3), 1.72 (d, J=7.0, C-4"H_3), 1.68 (s, 3H, C-19H_3), 1.26 (s, 3H, C-17H_3), 1.15 (s, 3H, C-16H_3).

2', 7-DITES-2", 3"-DIBROMOCEPHALOMANNINE (**3.65**)- A solution of 54 mg (55 μmoles) of 2", 3"-dibromocephalomannine (**3.64**) in 1 mL of dry CH_2Cl_2 was treated with 92 μL (545 μmoles, 10 eq.) of TESCl and 37 mg (545 μmoles, 10 eq.) of imidazole. The mixture was stirred at room

temperature for two hours. After filtering through a cotton plug to remove the imidazole hydrochloride, the solution was streaked directly on a preparative TLC plate for purification (5:4:1 methylene chloride/hexane/acetone). The result was 52 mg (77%) of (**3.65**). The product was characterized by ^{1}H NMR, but the spectrum was very complex due to the presence of the two diastereomers. Most protons gave two signals that were closely spaced. Several of the methyl signals cannot be clearly assigned. The approximate assignments are: ^{1}H NMR δ 7.30-8.12 (m, 11H, 2-OCOPh, 3'-Ph, NH), 6.50 (s, 1H, C-10H), 6.25 (m, 1H, C-13H), 5.67 (d, J=7.0, 1H, C-2H), 5.50 and 5.35 (br d, 1H, C-3'H), 4.93 (br d, J=7.7, 1H, C-5H), 4.61 (d, 1H, C-2'H), 4.61 (m, 1H, C-3"H), 4.45 (m, 1H, C-7H), 4.30 and 4.20 (ABq, J=8.4, 2H, C-20H_2), 3.80 (d, J=7.0, 1H, C-3H), 2.54 (m, 1H, C-6H), 2.48 (s, 3H, 4-OCOCH_3), 2.21 (s, 3H, 10-OCOCH_3), 1.98 (s, 3H, C-5"H_3), 1.79 (d, 3H, C-4"H_3), 1.72 (s, 3H, C-18H_3), 1.68 (s, 3H, C-19H_3), 1.26 (s, 3H, C-17H_3), 1.17 (s, 3H, C-16H_3), 0.92 and 0.83 (m, 18H, 2' and 7- Si($CH_2C\underline{H}_3$)$_3$), 0.58 and 0.48 (m, 12H, 2' and 7- Si($C\underline{H}_2CH_3$)$_3$).

3"-BROMOCEPHALOMANNINE (**3.66**)- A 9 μL (60 μmoles, 2.4 eq.) of DBU was added to a solution of 30 mg (25 μmoles) of (**3.65**) in 0.5 mL of dry toluene. The solution was heated at 60°C for 4 hours and then evaporated to dryness. The crude solid was redissolved in 1 mL of a 5% HCl/MeOH solution. After stirring for 15 minutes, the solution was diluted with 10 mL of EtOAc and washed with saturated $NaHCO_3$ (2 x 10 mL), water (1 x 10 mL), and brine (1 x 10 mL). After drying ($MgSO_4$), the

solvent was removed under vacuum. Purification by preparative TLC (3:2 ethyl acetate/hexane) yielded 9.9 mg (30%) of 3"-bromocephalomannine (**3.66**): mp 166-168°C; $[\alpha]^{23}_D$ = -119.1° (*c*=4.3, $CHCl_3$); ^1H NMR see Table 3.4; FABMS *m/z* (rel. int.) $[MH]^+$ 910 (15), 852 (5), 621 (12), 569 (30), 509 (90); HRFABMS *m/z* $[MH]^+$ 912.2600 ($C_{45}H_{53}{}^{81}BrNO_{14}$ requires 912.2629).

N-DEBENZOYL-N-(DIBROMOACETYL)TAXOL (**3.67**)- A 0.5 mL quantity of 99% formic acid was added to a 45 mg (45 μmoles) of (**3.53**) and the resulting solution was stirred at room temperature for 3 hours. The mixture was then diluted with 10 mL of EtOAc and washed with saturated $NaHCO_3$ (2 x 10 mL), water (1 x 10 mL),and brine (1 x 10 mL). After drying over $MgSO_4$, the solvent was removed *in vacuo*.

The crude product was redissolved in 0.5 mL of CH_2Cl_2 and then 10 mg of dibromoacetic acid, 9 mg of DCC, and a catalytic amount of DMAP were added. The solution was stirred at room temperature for 15 minutes. Purification by preparative TLC (3:2 ethyl acetate/hexane) afforded 12.3 mg (29%) of N-debenzoyl-N-(dibromoacetyl)taxol (**3.67**): mp 159-162°C; $[\alpha]^{23}_D$ = -115.3° (*c*=3.0, $CHCl_3$); ^1H NMR see Table 3.5; FABMS *m/z* (rel. int.) $[MH]^+$ 952 (2), 950 (4), 948 (2), 551 (4), 509 (5), 225 (90), 105 (100); HRFABMS *m/z* $[MH]^+$ 948.1455 ($C_{42}H_{48}{}^{79}Br_2NO_{14}$ requires 948.1442).

N-DEBENZOYL-N-(2"-BROMOPROPENOYL)TAXOL (**3.70**)- A 59 mg (58 μmoles) sample of (**3.53**) was dissolved in 0.5 mL of formic acid and

stirred at room temperature for 150 minutes. The mixture was diluted with 10 mL of EtOAc and washed with saturated $NaHCO_3$ (2 x 10 mL), water (1 x 10 mL),and brine (1 x 10 mL). After drying ($MgSO_4$), the solution was evaporated to dryness.

The crude product was redissolved in 0.5 mL of dry CH_2Cl_2 and then 13.5 mg (58 μmoles, 1 eq.) of 2, 3 dibromopropionic acid, 12 mg (58 μmoles, 1 eq.) of DCC, and a catalytic amount of DMAP was added. The reaction was monitored by TLC (Keiselgel 60 F_{254}, 3:2 ethyl acetate/ hexane). After 1 hour, the solution was streaked directly on a preparative TLC plate for purification (3:2 ethyl acetate/ hexane). The result was 21 mg (30%) of the diacyl adduct (**3.69**) which is a mixture of 4 diastereomers.

This mixture was dissolved in 400 μL of MeOH and then 100 μL of water and 10 mg of $NaHCO_3$ was added. After 80 minutes, the solution was diluted with 10 mL of EtOAc and washed with saturated $NaHCO_3$ (2 x 10 mL), water (1 x 10 mL),and brine (1 x 10 mL). After drying over $MgSO_4$, the solvent was removed under vacuum. The crude product was purified by preparative TLC (3:2 ethyl acetate/ hexane). This produced 14.4 mg (93%) of (**3.70**): mp 162-165°C; $[\alpha]^{23}_D$ = -68.6°(*c*=5.4, $CHCl_3$); ^1H NMR see Table 3.5; FABMS *m/z* (rel. int.) $[MH]^+$ 884 (4), 882 (3), 804 (1), 509 (4), 309 (5), 119 (100); HRFABMS *m/z* $[MH]^+$ 882.2329 ($C_{43}H_{49}{}^{79}BrNO_{14}$ requires 882.2336).

2", 3"-DICHLOROCEPHALOMANNINE (**3.71**)- A 30 mg (36 μmoles) of a cephalomannine mixture (90% cephalomannine) was dissolved in 1 mL of

$CHCl_3$ and then 92 mg (180 μmoles, 5 eq.) of Bu_4NICl_4 was added. The resulting solution was stirred for 1 hour at room temperature. Purification by preparative TLC (3:2 ethyl acetate/ hexane) afforded 28 mg (86%) of a diastereomeric mixture of 2", 3"-dichlorocephalomannine (**3.71**): ^1H NMR see Table 3.6; FABMS *m/z* (rel. int.) $[MH]^+$ 906 (8), 904 (32), 902 (40), 509 (100), 387 (60); HRFABMS *m/z* $[MH]^+$ 902.2937 ($C_{45}H_{54}{}^{35}Cl_2NO_{14}$ requires 902.2921).

REACTION OF CEPHALOMANNINE (**3.58**) WITH IODINE MONOCHLORIDE- A 30 mg (33 μmoles) quantity of a cephalomannine mixture (90% cephalomannine) was dissolved in 0.5 mL of $CHCl_3$ and an excess of ICl was added. The mixture was stirred at room temperature for 20 minutes and diluted with 15 mL of EtOAc. The resulting solution was washed with 5% $Na_2S_2O_3$ (1 x 15 mL), water (1 x 15 mL), and brine (1 x 15 mL). After drying over $MgSO_4$, the sample was evaporated to dryness. Purification by preparative TLC (3:2 ethyl acetate/hexane) yielded 13 mg (41%) of a diastereomeric mixture of iodochlorides (**3.72**) or (**3.73**): ^1H NMR see Table 3.6.

ATTEMPTED ELIMINATION OF THE IODOCHORIDES (**3.72**) OR (**3.73**)- A 17 mg (17 μmoles) of the iodochlorides (**3.72**) or (**3.73**) were dissolved in 0.5 mL of dry CH_2Cl_2 and 10 μL of DBU was added. The resulting solution was stirred at room temperature for 1 hour. The solution was streaked directly on a preparative TLC plate for purification (3:2 ethyl acetate/hexane). The result was 13 mg (92%) of cephalomannine (**3.58**):

^{1}H NMR δ 7.30-8.12 (m, 10H, 2-OCOPh, 3'-Ph), 6.51 (d, J=8.9, 1H, NH), 6.44 (qd, J=7.0, 1.2, C-3"H), 6.27 (s, 1H, C-10H), 6.21 (br t, J=8.4, 1H, C-13H), 5.67 (d, J=7.0, 1H, C-2H), 5.61 (dd, J=8.8, 2.7, 1H, C-3'H), 4.93 (br d, J=7.7, 1H, C-5H), 4.41 (dd, J=5.3, 2.7, 1H, C-2'H), 4.40 (m, 1H, C-7H), 4.29 and 4.18 (ABq, J=8.4, 2H, C-20H_2), 3.78 (d, J=7.0, 1H, C-3H), 2.54 (m, 1H, C-6H), 2.36 (s, 3H, 4-OCOCH_3), 2.24 (s, 3H, 10-OCOCH_3), 1.80 (s, 3H, C-5"H_3), 1.79 (s, 3H, C-18H_3), 1.72 (d, J=7.0, C-4"H_3), 1.68 (s, 3H, C-19H_3), 1.26 (s, 3H, C-17H_3), 1.15 (s, 3H, C-16H_3).

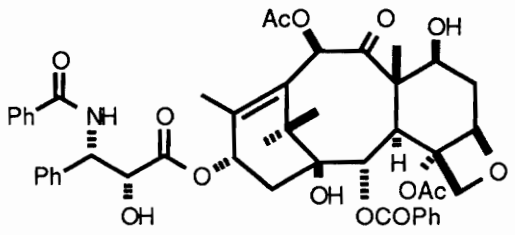
N-DEBENZOYL-N-CROTONYLTAXOL (**3.76**)- A 80 mg (80 μmoles) quantity of (**3.53**) was dissolved in 1 mL of 99% formic acid and stirred at room temperature for 3 hours. The solution was diluted with 15 mL of ethyl acetate and washed with saturated $NaHCO_3$ (2 x 10 mL), water (1 x 10 mL),and brine (1 x 10 mL). After drying over $MgSO_4$, the solvent was removed under vacuum. The crude material was used in the next step without further purification.

The sample was dissolved in 1 mL of dry CH_2Cl_2 and cooled to 0°C using an ice bath. To this solution 7 mg of crotonic acid, 16 mg of DCC, and a catalytic amount of DMAP were added. The resulting mixture was warmed to room temperature and then stirred overnight. The crude mixture was streaked directly on a preparative TLC plate (3:2 ethyl acetate/hexane) for purification. The result was 17.3 mg (26%) of (**3.76**): mp 168-171°C; $[\alpha]^{23}_D$ = -69.2°(*c*=2.60, $CHCl_3$); ^{1}H NMR see Table 3.7; FABMS *m/z* (rel. int.) $[MH]^+$ 818 (2), 509 (10), 318 (5), 250 (35), 105 (100); HRFABMS *m/z* $[MH]^+$ 818.3375 ($C_{44}H_{52}NO_{14}$ requires 818.3388).

REACTION OF 2", 3"-DIBROMOCEPHALOMANNINE (**3.65**) WITH SODIUM AZIDE- A 33 mg sample of (**3.65**) was dissolved in 0.5 mL of dry DMF under argon. A 9 mg quantity of NaN_3 was added to the solution which was then warmed to 60°C and left to stir overnight. The resulting mixture was diluted with 15 mL of ethyl acetate and washed with water (2 x 10 mL)and brine (1 x 10 mL). After drying ($MgSO_4$), the solvent was removed *in vacuo*. Purification by preparative TLC afforded 29 mg (95%) of 3"-bromocephalomannine (**3.66**): mp 171-174°C; $[\alpha]^{23}_D$ = -110.8° (*c*=2.69, $CHCl_3$); ^1H NMR δ 7.30-8.12 (m, 10H, 2-OCOPh, 3'-Ph), 6.51 (d, J=8.9, 1H, NH), 6.44 (qd, J=7.0, 1.2, C-3"H), 6.27 (s, 1H, C-10H), 6.21 (br t, J=8.4, 1H, C-13H), 5.67 (d, J=7.0, 1H, C-2H), 5.61 (dd, J=8.8, 2.7, 1H, C-3'H), 4.93 (br d, J=7.7, 1H, C-5H), 4.41 (dd, J=5.3, 2.7, 1H, C-2'H), 4.40 (m, 1H, C-7H), 4.29 and 4.18 (ABq, J=8.4, 2H, C-20H_2), 3.78 (d, J=7.0, 1H, C-3H), 2.54 (m, 1H, C-6H), 2.36 (s, 3H, 4-OCOCH_3), 2.24 (s, 3H, 10-OCOCH_3), 1.80 (s, 3H, C-5"H_3), 1.79 (s, 3H, C-18H_3), 1.72 (d, J=7.0, C-4"H_3), 1.68 (s, 3H, C-19H_3), 1.26 (s, 3H, C-17H_3), 1.15 (s, 3H, C-16H_3).; FABMS *m/z* (rel. int.) $[MH]^+$ 910 (30), 852 (5), 509 (100); HRFABMS *m/z* $[MH]^+$ 910.2690 ($C_{45}H_{53}{}^{79}BrNO_{14}$ requires 910.2649).

4. Mechanistic Studies

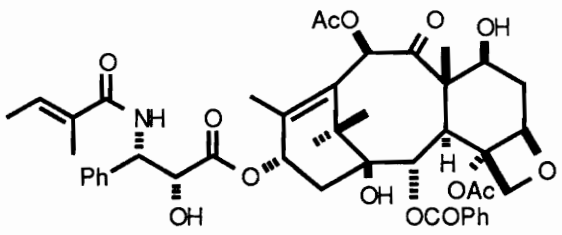
Taxol (**4.1**) has drawn widespread attention not only because of its clinically demonstrated anticancer activity, but also because of its novel mechanism of action. Early work revealed that Taxol is an antimitotic agent and potent inhibitor of cell replication.[40] However, among antimitotic drugs Taxol is unique in that it promotes assembly of stable microtubules from tubulin under normally unfavorable conditions. Moreover, it binds to microtubules stabilizing them against depolymerization. A presumed consequence of these effects is the significant anticancer activity Taxol exhibits.[7]



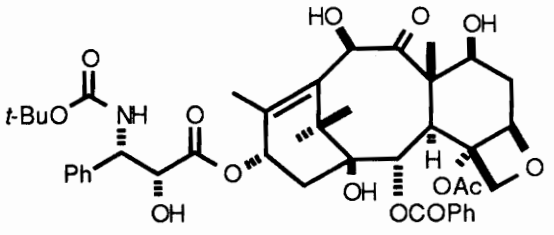
4.1

As might be expected, subsequent studies have shown that, in addition to Taxol, other biologically active taxanes such as cephalomannine (**4.2**)[68] and the semisynthetic analog Taxotere (**4.3**)[73] also operate in a similar fashion. However, a recent collaborative effort between Dr. D. G. I. Kingston's group at Virginia Polytechnic Institute

& State University and Dr. E. Hamel's group at the National Cancer Institute suggest there might be some important differences.[187]



4.2



4.3

The collaborative studies being conducted were designed to evaluate the biological activity and binding characteristics of several Taxol analogs which possessed various photoreactive substituents. These evaluations were part of a much larger effort to identify the binding site of Taxol on polymerized tubulin using photoaffinity labels. An unanticipated finding of these evaluations was that the Taxol analogs had some, but not all of the properties of Taxol.[187] The results seemed to indicate that these photolabeled analogs differed little from Taxol in their ability to stabilize tubulin polymers to cold-induced depolymerization, but, unlike Taxol, they failed to promote assembly of microtubule at low temperature, in the absence of microtubule associated proteins, or in the absence of GTP. This failure to promote assembly while retaining the ability to stabilize intact microtubules suggests that the hypernucleation of microtubule assembly and the stabilization of microtubules observed with Taxol represent two distinct properties of the drug. These findings prompted a detailed investigation of cephalomannine (**4.2**) and Taxotere (**4.3**) as well as

several other selected analogs to determine whether they differ from Taxol in this respect, too. The results of this investigation are described in this section.

4.1. Background

The discovery of Taxol's mode of action by Horwitz and coworkers[40] in 1979 gave a significant boost to the development of Taxol as an anticancer agent. Although Taxol had been reported eight years earlier[3] and its anticancer activity firmly established[38], problems with water solubility as well as difficulties in obtaining requisite amounts prevented its development. Interest in Taxol suddenly increased when Horwitz *et. al.* reported that it functioned as an antimitotic agent, blocking cell replication in HeLa and fibroblast cells.[101] Their studies indicated that replication was blocked in late G_2 and/or M and appeared to be the result of Taxol's ability to promote the assembly of microtubules from tubulin. Earlier *in vitro* studies had shown Taxol decreased the lag time for microtubule assembly and shifted the equilibrium in favor of the microtubule by reducing the critical concentration of tubulin normally required for assembly.[40] Furthermore, the microtubules formed in the presence of Taxol were found to be resistant to depolymerization by calcium ions, cold, dilution, or microtubule disassembling drugs such as steganacin.[101] These observations were in stark contrast with other antimicrotubule agents (e.g. colchicine, podophyllotoxin, and the vinca alkaloids)

which inhibit assembly. Although it is possible that Taxol and its analogs exert their anticancer activity by some other as yet undiscovered mechanism, current evidence seems to indicate that they are effective anticancer agents precisely because they interfere so effectively with mitosis by stabilizing abnormal microtubules through specific interactions with polymerized tubulin.[102]

The process by which Taxol promotes the assembly of microtubules and consequently makes them resistant to depolymerization is only partially understood. It has been established that the binding is noncovalent and that the binding site is on the intact microtubule rather than the tubulins or tubulin dimers.[40] The maximum effects of the drug are observed at Taxol concentrations stoichiometric with the tubulin dimer.[103]

While a wealth of information about the interactions of Taxol with microtubules on the cellular level has accumulated, less is known about the mechanism of action on the molecular level. Since the binding of Taxol to microtubules appears to play a fundamental role in its activity, a detailed understanding of the binding site would be of particular interest. Theoretically, if this could be determined in sufficient detail to allow the development of a three-dimensional map of the site, then it might prove possible to design synthetic analogs that would fit the binding site and thus show greatly enhanced biological activity.[187]

Present knowledge of the binding site on microtubules is severely limited by the fact that it has thus far proved impossible to

obtain an X-ray structure of either tubulin or microtubules. Although the structure of hydrated cytoplasmic microtubules has been determined at 18 Å by X-ray fiber diffraction,[188] the resolution of this study was not sufficient for a detailed analysis of the binding site. Furthermore, little headway has been made in preparing crystalline Taxol-microtubule complexes suitable for X-ray analysis.

In the absence of direct physical methods such as X-ray diffraction, photoaffinity labeling was chosen as a suitable technique for identification of the binding site of Taxol on microtubules. This technique has emerged as a powerful technique for investigating drug receptor interactions and has previously been applied to the analysis of vinblastine,[189] colchicine,[190-194] GTP,[195-198] and ATP and GTP derivatives[199-201] with the tubulin-microtubule system. A recent direct photoaffinity labeling study indicated that Taxol selectively binds to β-tubulin,[202] but the actual location still remains a mystery.

A typical experiment requires the synthesis of an appropriate photoaffinity reagent which will serve as the ligand which will bind to the receptor (Figure 4.1). Photoaffinity reagents are modified ligands which contain a photosensitive group which masks a highly reactive intermediate, often a carbene or nitrene. Once synthesized, the photoaffinity reagent is exposed to the receptor where binding can occur. Upon photolysis, the photoaffinity reagent is converted to a highly reactive species which then reacts with regions of the receptor it is in contact with and covalently links the ligand. The covalently bound ligand-receptor complex is degraded to identify the regions of

the receptor labeled by the reagent. Thus, the domains of the receptor which are in contact with the ligand can be identified.

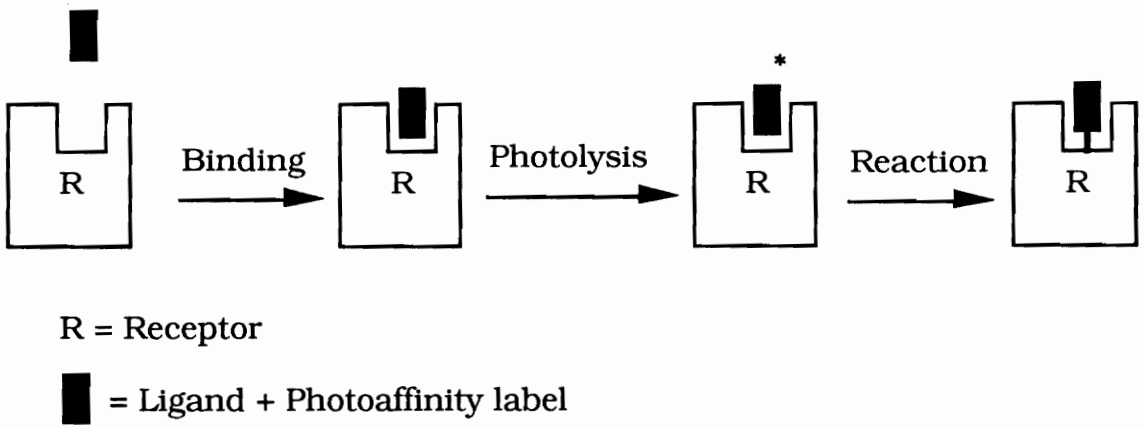
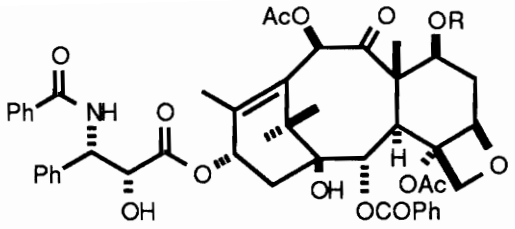
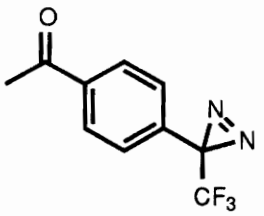


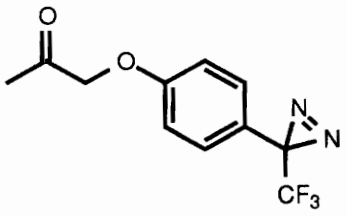
Figure 4.1. Basic photoaffinity labeling experiment.

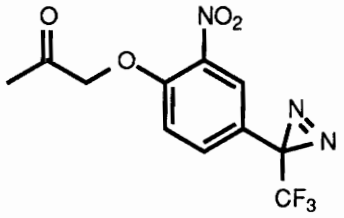
In the early 90's a collaborative project designed to develop an appropriate photoaffinity reagent to be used to identify the binding site of Taxol on microtubules began between Dr. D. G. I Kingston at Virginia Polytechnic Institute & State University and Dr. E. Hamel at the National Cancer Institute. John Rimoldi and Ashok Chaudhary of Dr. Kingston's group synthesized several modified Taxols (**4.4**-**4.7**) to be evaluated as possible photoaffinity reagents and the compounds were sent to Dr. E. Hamel for testing.

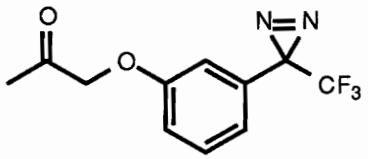
Dr. Hamel and coworkers chose to use purified tubulin as opposed to the microtubule protein (microtubules obtained from brain tissue and containing microtubule associated proteins in addition to tubulin) to examine the interactions of the photolabeled Taxol



4.4 R = 

4.6 R = 

4.5 R = 

4.7 R = 

analogs.[187] The intent in choosing this system was to eliminate the possibilities of nonspecific interactions with nontubulin components. Microtubule assembly was monitored by the increase in turbidity. In the system used by Hamel and his colleagues, 1.0 M glutamate replaced microtubule-associated proteins (MAPs) as an inducer of polymerization. Typical assembly-disassembly curves are illustrated in Figure 4.2 with 10 μM tubulin ± 10 μM Taxol. In the absence of Taxol, little reaction occurred at 15° C, while extensive formation of microtubules occurred at 37° C. With Taxol present, extensive polymerization was observed at the lower temperature. Moreover, in the absence of Taxol, microtubules are cold labile, with about 80% of

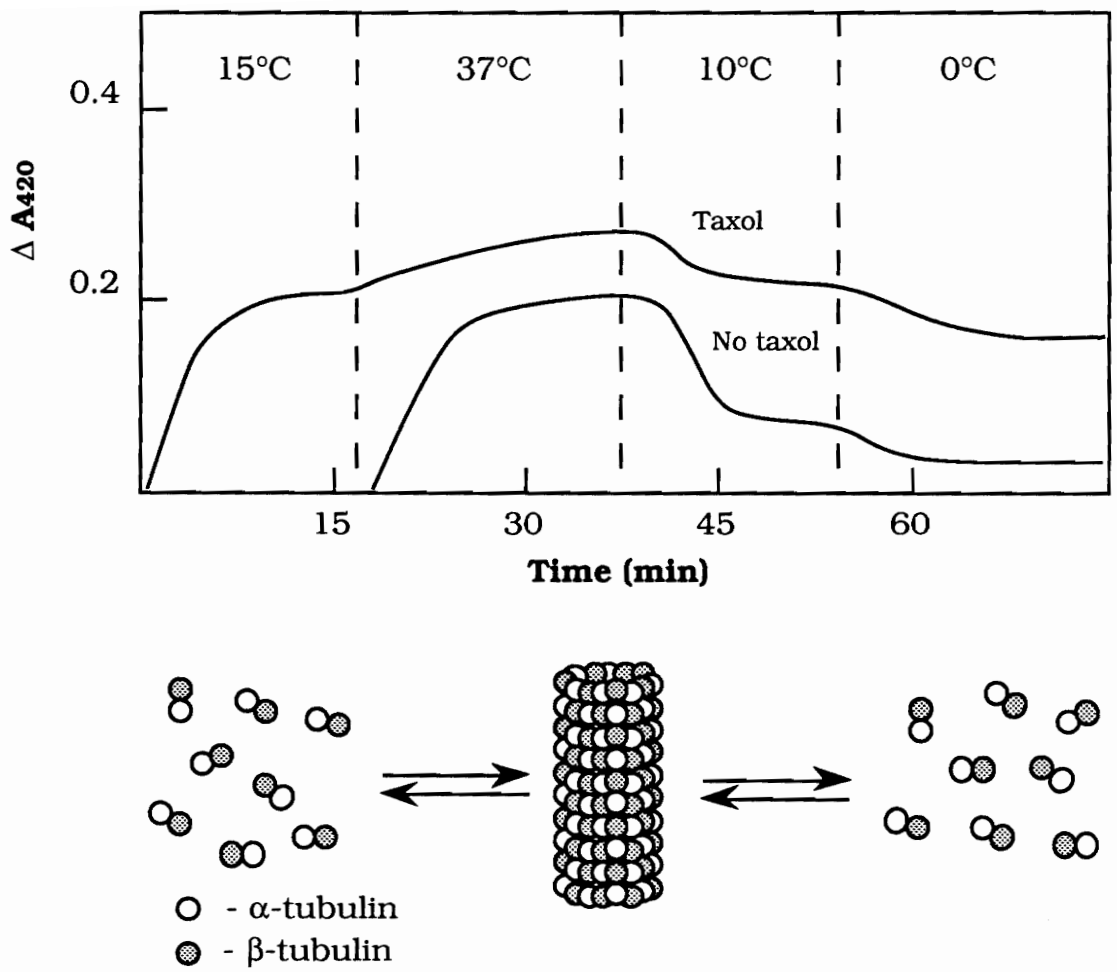


Figure 4.2. Taxol induced polymerization of purified tubulin.[187]

the depolymerization occurring at 10° C. With Taxol, less that 50% depolymerization occurred and the majority of that was observed at 0° C.

Hamel *et. al.* examined the photolabeled analogs (**4.4-4.7**) using the same system.[187] Initially, IC_{50} values, defined as the concentration of drug which reduced the rate of depolymerization by 50%, were

obtained. While all potential photolabeled compounds were found to be slightly less active than Taxol, they were deemed suitable for possible photoaffinity reagents.

Using a radiolabeled version of (**4.8**), Hamel and coworkers began to investigate its incorporation into the tubulin polymer. Surprisingly, unlike Taxol, this analog failed to induce polymerization, even at superstoichiometric concentrations. This prompted a reexamination of photolabeled analogs (**4.4**-**4.7**).

The results of this reinvestigation were completely unanticipated.[187] Hamel and his colleagues found that these analogs differed little from Taxol in there ability to stabilize tubulin polymers to cold-induced depolymerization, even at substoichiometric concentrations (Figure 4.3). In contrast, even superstoichiometric concentrations of these photolabeled analogs failed to promote the assembly of tubulin polymers at low temperature, in the absence of microtubule-associated proteins, or in the absence of GTP. These findings suggest that the hypernucleation of microtubule assembly and the ability to stabilize the resulting microtubules observed with Taxol represent two distinct properties.

If the ability to promote the assembly of microtubules and the ability to stabilize the resulting tubulin polymers actually are two distinct properties, this represents a unique problem. Both assembly and disassembly assays have been used to evaluate the biological activity of Taxol analogs,[4] but these results suggest that disassembly assays may not correlate well.

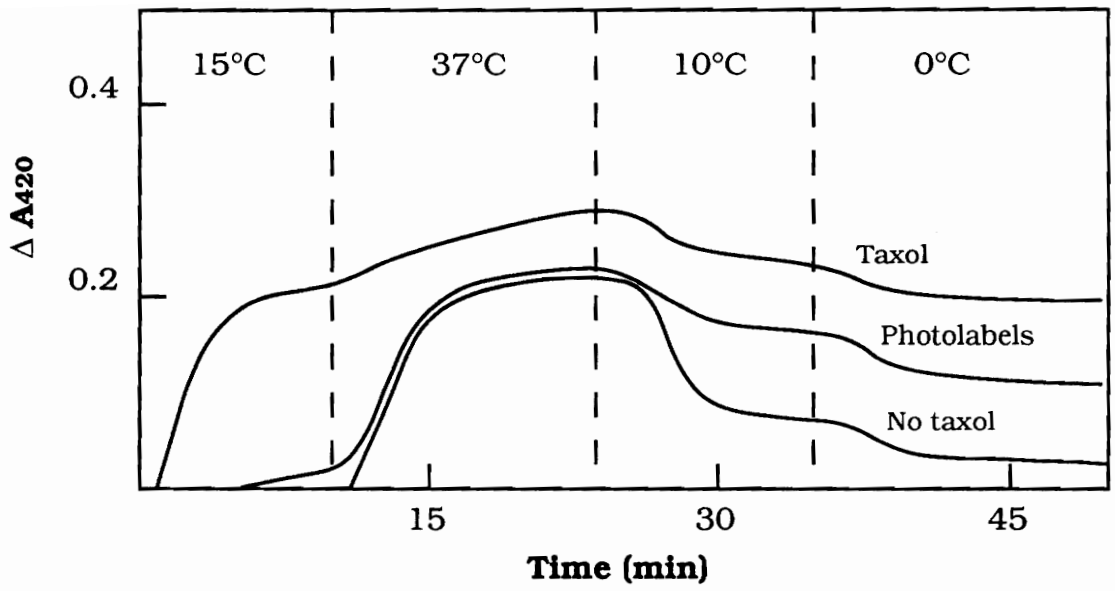


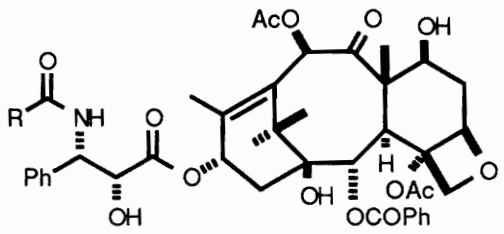
Figure 4.3. Effects of photolabeled analogs on tubulin polymerization.

In order to further investigate these differences, Hamel was interested in examining a variety of 3'-N-acyl analogs including cephalomannine, Taxotere, and 10-acetyltaxotere.

4.2. Results and Discussion

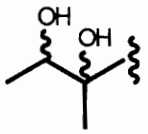
The 3'-N position was one of the earliest to be examined for its effect on the biological activity of Taxol, and a considerable number of analogs have been reported. Hamel's interest primarily focused on cephalomannine (**4.2**) and Taxotere (**4.3**). Cephalomannine (**4.2**), a naturally occurring 3'-N-acyl analog, is slightly less cytotoxic than

Taxol and thus, would be an excellent compound to test in Hamel's tubulin assembly-disassembly system. Interest in Taxotere (**4.3**) stemmed not only from the fact that it was the first semisynthetic analog found to be more active than Taxol, but also because it is currently of clinical interest. Furthermore, it would allow Hamel to determine whether his reaction systems are in accord with those of others.[203] Since Taxotere (**4.3**) differs from Taxol (**4.1**) at the C-10 as well as the C-3', 10-acetyltaxotere (**4.9**) was also of interest. N-debenzoyl-N-(phenoxyacetyl)taxol (**4.10**) was chosen because the N-(phenoxyacetyl) moiety was similar to the photoreactive substituents used in the earlier experiments. The final compound investigated was the cephalomannine diol (**4.11**). While not of any special interest, it was readily prepared and provided another compound similar to cephalomannine that was known to be less active than Taxol.



4.9 R = *t*-BuO

4.10 R = $PhOCH_2$

4.11 R = 

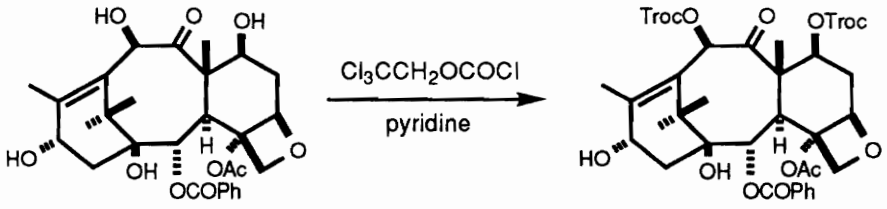
Taxotere (**4.3**), or RP56976, is considered a potentially important chemotherapeutic agent for the treatment of cancer.[73] Like taxol, it is a potent inhibitor of cell replication and is known to promote the *in vitro* assembly of microtubules in the absence of guanosine triphosphate (GTP).[73] Taxotere (**4.3**) was initially prepared at the Institut de Chemie des Substances Naturelles of the Centre National de la Recherche Scientifique, France by Potier and his colleagues.[135] Since that time, a variety of methods have been reported for its preparation.[130, 137, 168-171] In spite of the fact that Taxotere (**4.3**) is currently of clinical interest and well known, it was unavailable and thus needed to be synthetically prepared. After considering various routes, the decision was made to prepare it using the methodology describe in section 3.2 by which several other 3'-N-acyl analogs were prepared.

Because Taxotere (**4.3**) lacks the C-10 acetyl group the synthetic route previously used to prepare 3'-N-acyl analogs required minor modification. Selective cleavage of the C-10 acetate has proven difficult[89] and consequently any synthetic scheme chosen must take this into account. Potier and coworkers originally prepared Taxotere (**4.3**) from 10-deacetylbaccatin III (**4.12**), a naturally occurring taxane isolated from the leaves of the English Yew, *Taxus baccata*.[73] Structurally, 10-deacetylbaccatin III (**4.12**) is Taxotere (**4.3**) minus the side chain and thus circumvents the necessity of acetate cleavage. Although the use of 10-deacetylbaccatin III (**4.12**) requires selective

protection of the C-7 and C-10 hydroxyls, it was readily available and thus the obvious choice.

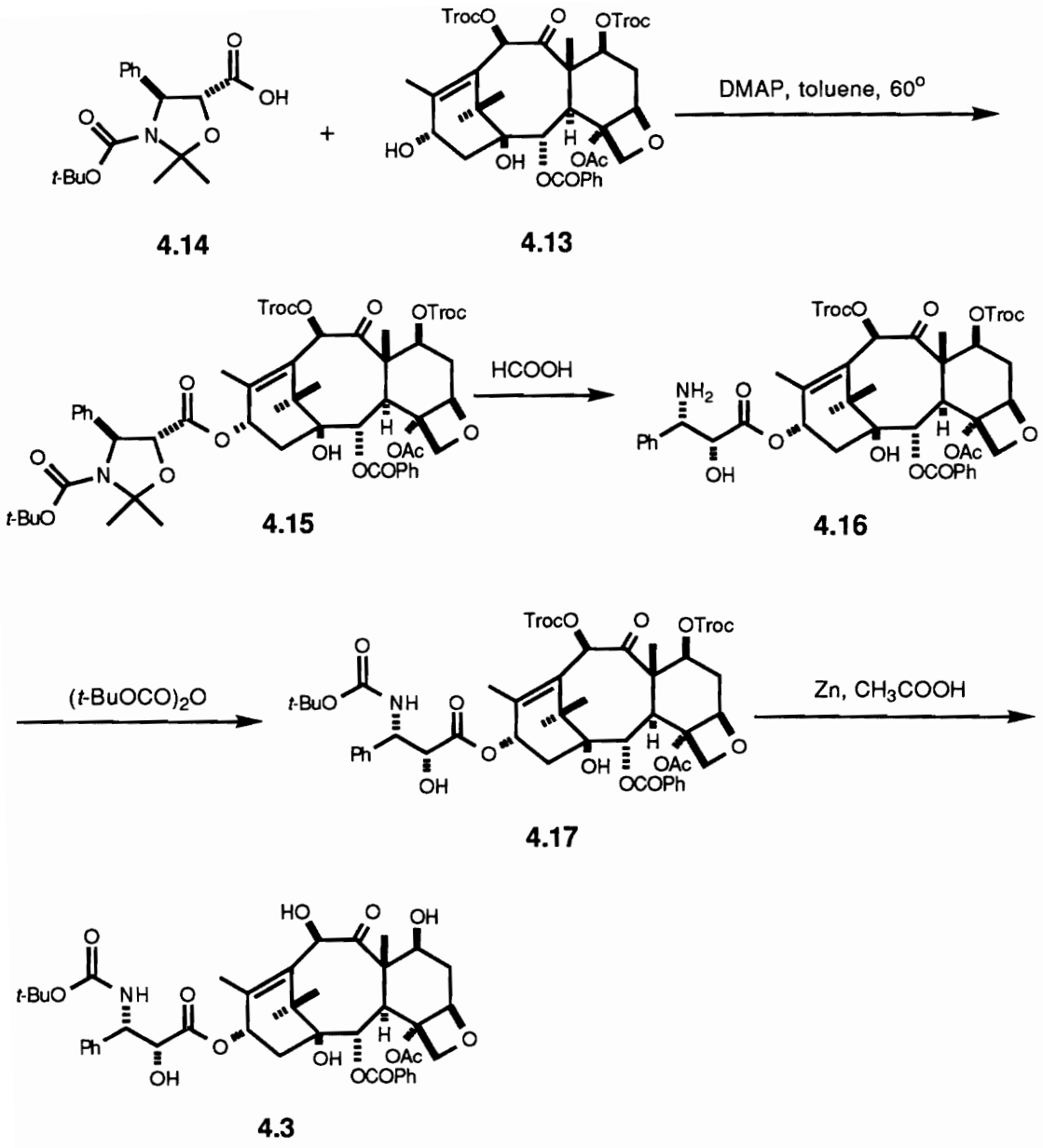
The synthetic sequence began with the selective protection of the 7- and 10-hydroxyls leaving the 13-hydroxyl free for the side chain coupling. While the TES group had been successfully used in earlier side chain coupling reactions to baccatin III, it was not suitable here. Prior work in this area had revealed that the TES group could not be introduced selectively.[204] Several attempts had shown that the 7-hydroxyl was the first to be protected, but reaction at the 10- and 13 hydroxyls was competitive, leading to mixtures.

The alternative was the 2, 2, 2-trichloroethoxycarbonyl, or Troc group. This protecting group had previously been used by Potier and coworkers[135] and while it was known to be selective, its removal would require an extra step in the synthetic sequence. Fortunately, both protection and deprotection occur in reasonably high yield. Thus, 10-deacetylbaccatin III (**4.12**) was treated with 2, 2, 2-trichloroethyl chloroformate in the presence of pyridine to yield 7, 10-diTrocbaccatin III (**4.13**) in 84% yield (*Scheme* 4.1).



4.12 **4.13**

Scheme 4.1. Preparation of 7, 10-diTrocbaccatin III (**4.13**).



Scheme 4.2. Preparation of Taxotere (**4.3**).

After protection, the side chain (**4.14**), prepared as described in section 3.2, was coupled to the diprotected derivative (**4.13**) using DCC and DMAP in toluene at 60°C (*Scheme* 4.2). As was observed with 7-TES baccatin III, side chain coupling was rapid and clean. Next, exposure of the diprotected side chain analog (**4.15**) to 99% formic acid led to the free amine (**4.16**) which was used in the next step without purification. Acylation of the free amine (**4.16**) was accomplished using di-*tert*-butyl dicarbonate and a catalytic amount of DMAP to give 7, 10-diTroctaxotere (**4.17**). The final step of the sequence was deprotection of (**4.17**) which was achieved using zinc in acetic acid. This led to Taxotere (**4.3**) in 74% yield.

The ^{1}H NMR data is shown in Table 4.1 and was in accord with the literature values.[135]

With Taxotere (**4.3**) in hand, attention was turned to the preparation of 10-acetyltaxotere (**4.9**). Like Taxotere, 10-acetyl-taxotere is slightly more active than Taxol. Potier and coworkers were, likewise, responsible for its synthesis and biological evaluation.[135] It has received less attention than Taxotere simply because it was found to be less water soluble. Structurally, it is identical to Taxotere except that it possesses the C-10 acetate. Synthetically, it can be readily prepared using the side chain route described in section 3.2.

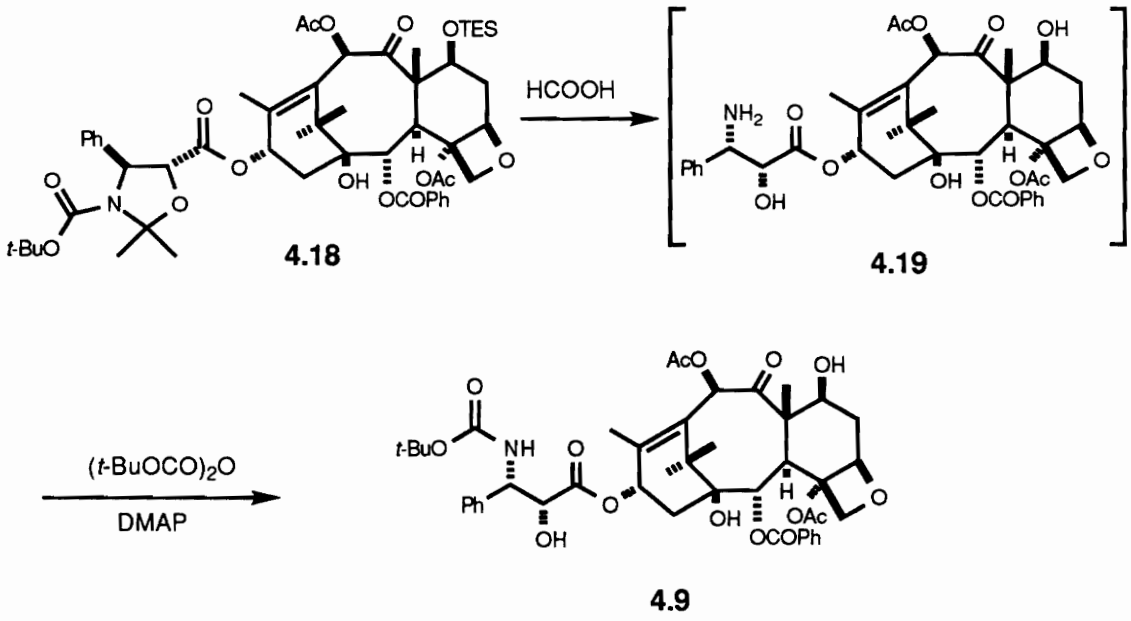
Side chain coupled product (**4.18**), prepared as described in section 3.2, was treated with 99% formic acid for 3 hours to give the

Table 4.1. ^{1}H NMR Spectrum of Taxotere (**4.3**).[a, b]

Protons on	Taxotere (Prepared)	Taxotere (Literature)[c]
C-2	5.70 (d, 7.0)	5.68 (d, 7)
C-3	3.80 (d, 7.0)	3.91 (d, 7)
C-5	4.95 (d, 7.6)	4.94 (d, 9)
C-6	2.58 (m)	2.58 (m)
	1.88 (m)	
C-7	4.28 (m)	4.26 (m)
C-10	5.21 (s)	5.22 (s)
C-13	6.23 (t, 8)	6.22 (t, 9)
C-14	2.28 (m)	2.28 (m)
C-16-CH_3	1.25 (s)	1.24 (s)
C-17-CH_3	1.14 (s)	1.12 (s)
C-18-CH_3	1.87 (s)	1.87 (s)
C-19-CH_3	1.77 (s)	1.77 (s)
C-20	4.33 (d, 8.4)	4.32 (d, 9)
	4.19 (d, 8.3)	4.19 (d, 9)
C-2'	4.63 (br s)	4.62 (br s)
C-3'	5.27 (dd, 8.8, 2.6)	5.26 (br d, 9)
3'-NH	5.46 (d, 8.8)	5.46 (d, 9)
4-OAc	2.39 (s)	2.37 (s)
2-OBz, 3'-Ph	7.32-8.15	7.38-8.12
3'-NHCOC*t*-Bu	1.35 (s)	1.35 (s)

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. [c]From reference 135.

free amine (**4.19**) (*Scheme* 4.3). The free amine (**4.19**) was then acylated using di-*tert*-butyl dicarbonate and a catalytic amount of DMAP to give 10-acetyltaxotere (**4.9**) in modest yield.



Scheme 4.3. Synthesis of 10-acetyltaxotere (**4.9**).

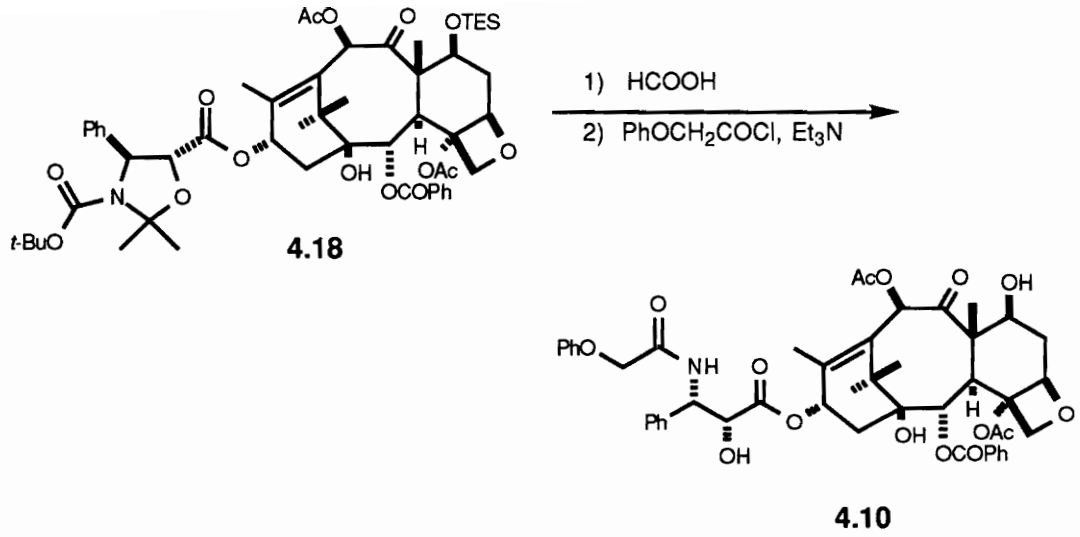
The ^{1}H NMR data is shown in Table 4.2 and was found to be consistent with the literature values.[135]

Like 10-acetyltaxotere (**4.9**), N-debenzoyl-N-(phenoxyacetyl)-taxol (**4.10**) could be prepared using the side chain methodology previously described (Figure 4.4). Thus, protected side chain adduct (**4.18**) was exposed to 99% formic acid for 3 hours to generate the free amine which was then acylated using phenoxyacetyl chloride and

Table 4.2. ^{1}H NMR Spectrum of 10-Acetyltaxotere (**4.9**).[a, b]

Protons on	10-Acetyltaxotere (Prepared)	10- Acetyltaxotere (Literature)[c]
C-2	5.67 (d, 7)	5.65 (d, 7)
C-3	3.81 (d, 7)	3.8 (d, 7)
C-5	4.96 (d, 9)	4.95 (d, 9)
C-6	2.56 (m)	2.55 (m)
C-7	4.41 (m)	4.4 (dd, 12, 7)
C-10	6.23 (s)	6.28 (s)
C-13	6.26 (t, 8)	6.2 (t, 8)
C-14	2.28 (m)	2.28 (m)
C-16-CH_3	1.25 (s)	1.25 (s)
C-17-CH_3	1.15 (s)	1.15 (s)
C-18-CH_3	1.85 (s)	1.85 (s)
C-19-CH_3	1.68 (s)	1.67 (s)
C-20	4.30 (d, 9)	4.3 (d, 9)
	4.19 (d, 9)	4.17 (d, 9)
C-2'	4.64 (br s)	4.6 (br s)
C-3'	5.26 (d, 9)	5.25 (d, 9)
3'-NH	5.38 (d, 9)	5.4 (d, 9)
4-OAc	2.39 (s)	2.38 (s)
10-OAc	2.26 (s)	2.25 (s)
2-OBz, 3'-Ph	7.32-8.13	7.35-8.07
3'-NHCOC*t*-Bu	1.33 (s)	1.3 (s)

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. [c]From reference 135.



Scheme 4.4. Preparation of N-(phenoxyacetyl) analog (**4.10**).

triethylamine. This produced the desired analog (**4.10**) in 33% yield. The ^{1}H NMR data is shown in Table 4.3.

The final derivative to be prepared was the cephalomannine diol (**4.11**). With cephalomannine readily available, this was easily prepared using the procedure reported by Kingston *et. al*[54] and as described in section 3.2.

The four analogs prepared were forwarded to Dr. Ernest Hamel at the National Cancer Institute for the testing in various tubulin assembly-disassembly systems. Dr. Hamel and Dr. Grover were responsible for the all the biochemical studies and what follows is only a brief description of their work. Their results are included here to give the reader a complete picture of the collaborative project. For a more in depth discussion, the reader is referred to reference 203.

Table 4.3. ^{1}H NMR Spectrum of N-(Phenoxyacetyl) Analog (**4.10**).[a, b]

Protons on	N-debenzoyl-N-(phenoxyacetyl)taxol
C-2	5.67 (d, 7.2)
C-3	3.79 (d, 6.9)
C-5	4.94 (d, 8.0)
C-6	2.54 (m)
	1.88 (m)
C-7	4.40 (m)
C-10	6.26 (s)
C-13	6.19 (t, 8.8)
C-14	*c*
C-16-CH_3	1.15 (s)
C-17-CH_3	1.27 (s)
C-18-CH_3	1.78 (s)
C-19-CH_3	1.68 (s)
C-20	4.30 (d, 8.4)
	4.19 (d, 8.4)
C-2'	3.43 (dd, 5.4, 2.4)
C-3'	5.63 (dd, 9.5, 2.4)
3'-NH	*d*
4-OAc	2.33 (s)
10-OAc	2.25 (s)
2-OBz, 3'-Ph, 3'-$NHCOCH_2O\underline{Ph}$	6.90-8.12
3'-$NHCOC\underline{H_2}Ph$	4.51 (s)

[a]Measured in $CDCl_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. [b]Multiplicity: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. [c]Hidden under the methyl peaks. [d]Hidden in the aromatic region.

Hamel and Grover initially examined the ability of cephalomannine (**4.2**), Taxotere (**4.3**), 10-acetyltaxotere (**4.9**), and C-3'-modified analogs (**4.10**) and (**4.11**) to stabilize tubulin polymers in a glutamate-GTP polymerization system. In this system, glutamate and GTP induce assembly and consequently microtubule associated proteins (MAPs) are not required. This is the same tubulin-microtubule system in which the C-7-modified photolabeled analogs had been examined and found to stabilize microtubules to the same extent as Taxol, but did not induce assembly.

Depolymerization IC_{50} values, defined as the concentration of drug required to reduce the depolymerization rate by 50% (measured spectrophotometrically), were obtained from reaction mixtures containing 1.0 M monosodium glutamate, 1.0 mM $MgCl_2$, 1.0 mg/mL tubulin, 0.1 mM GTP, and varying drug concentrations (4% v/v dimethylsulfoxide as the vehicle).[205] Tubulin polymerization was initiated by a 0°C to 37°C temperature jump and then after 20 minutes reset to 0°C. The maximum depolymerization rates, defined as the greatest interval drop in turbidity following the reduction in temperature, were determined. The results are summarized in Table 4.4.[205]

Taxotere (**4.3**) and 10-acetyltaxotere (**4.9**) were the most active (IC_{50} values, 0.63 μM and 0.64 μM respectively). Cephalomannine (**4.2**) and N-debenzoyl-N-(phenoxyacetyl)taxol (**4.10**) were found to be slightly less active (IC_{50} values, 1.3 μM and 1.26 μM respectively) while the cephalomannine diol (**4.11**) was the least active with an IC_{50}

value of 3.1 μM. During the course of these studies, Hamel and Grover noted that under these conditions all C-3'-modified analogs were able to stabilize tubulin polymer, but invariably had reduced activity in enhancing assembly.[203]

Table 4.4. IC_{50} Values of Taxol and Taxol Analogs.

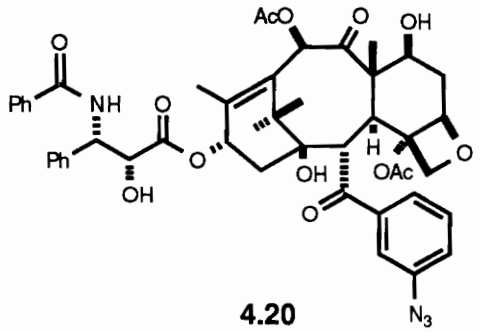
Compound	IC_{50} (μM)	IC_{50}^{rel}
Taxol (**4.1**)	0.86	1
Cephalomannine (**4.2**)	1.3	1.5
Taxotere (**4.3**)	0.63	0.73
10-Acetyltaxotere (**4.9**)	0.64	0.74
N-debenzoyl-N-(phenoxyacetyl)taxol (**4.10**)	2.6	3.0
Cephalomannine diol (**4.11**)	3.1	3.6

As part of a more detailed study, the depolymerization IC_{50} values of taxol (**4.1**), Taxotere (**4.3**), and N-debenzoyl-N-(phenoxyacetyl)taxol (**4.10**) were redetermined in the same system except the concentration of GTP was increased from 0.1 mM to 0.4 mM.[203] The results are shown in Table 4.5. Under these conditions, the IC_{50} values for Taxol (**4.1**) and Taxotere (**4.3**) were approximately one-half

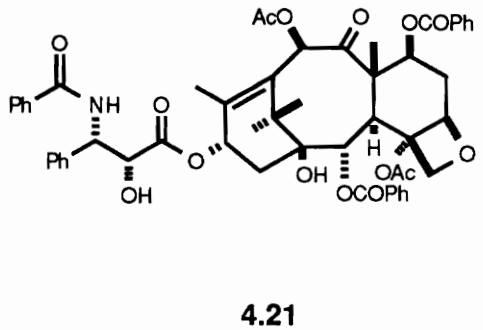
Table 4.5. IC_{50} Values of Taxol and Selected Taxol Analogs.[203]

Compd	IC_{50} (μM)	IC_{50rel}
Taxol (**4.1**)	0.42	1
Taxotere (**4.3**)	0.28	0.67
2-debenzoyl-2-(*m*-azidobenzoyl) taxol (**4.20**)	0.31	0.74
7-benzoyltaxol (**4.21**)	0.65	1.5
N-debenzoyl-N-(phenoxyacetyl)taxol (**4.10**)	0.78	1.9

the value of those under the earlier conditions (0.42 μM and 0.28 μM compared to 0.86 μM and 0.63 μM). In contrast, N-debenzoyl-N-(phenoxyacetyl)taxol (**4.10**) was slightly more active than in the previous system (0.78 μM compared to 2.6 μM). Hamel and Grover also chose to include C-2-modified analog (**4.20**) and C-7-modified derivative (**4.21**) in this investigation. The C-2 *m*-azido analog (**4.20**)



4.20



4.21

proved to be more active than taxol (IC_{50} value, 0.31) while the C-7 benzoyl derivative (**4.21**) was slightly less active (IC_{50} value, 0.65).

After IC_{50} values were determined, Hamel and Grover examined the analogs in a variety of polymerization systems including polymerization in the presence of MAPs and GTP, polymerization with MAPs but not GTP, polymerization with GTP but not MAPs, and polymerization with neither MAPs nor GTP.[203] Only in the system containing both MAPs and GTP was polymerization observed without Taxol or Taxol analogs present. Polymerization was measured by adding the compounds to otherwise complete reaction mixtures at 0°C, and then monitoring the turbidity change successively at 0°, 10°, and 37°C. Polymerization stability was then evaluated by monitoring the turbidity changes as the temperature was reduced first to 10° then 0°C.

In the polymerization system containing both MAPs and GTP, considered the "complete system", Hamel and Grover found that tubulin polymer assembled at 37°C and disassembled at 10°C when Taxol or its analogs were not present (Figure 4.4).[203] In the presence of Taxol, some assembly occurred at 0°C, but the majority occurred at 10°C. The Taxol containing polymers were stable at 10°C, but a significant decrease in turbidity (40%) was noted at 0°C.

The analogs were examined at both substoichiometric concentrations (10 μM) (Figure 4.4) and superstoichiometric

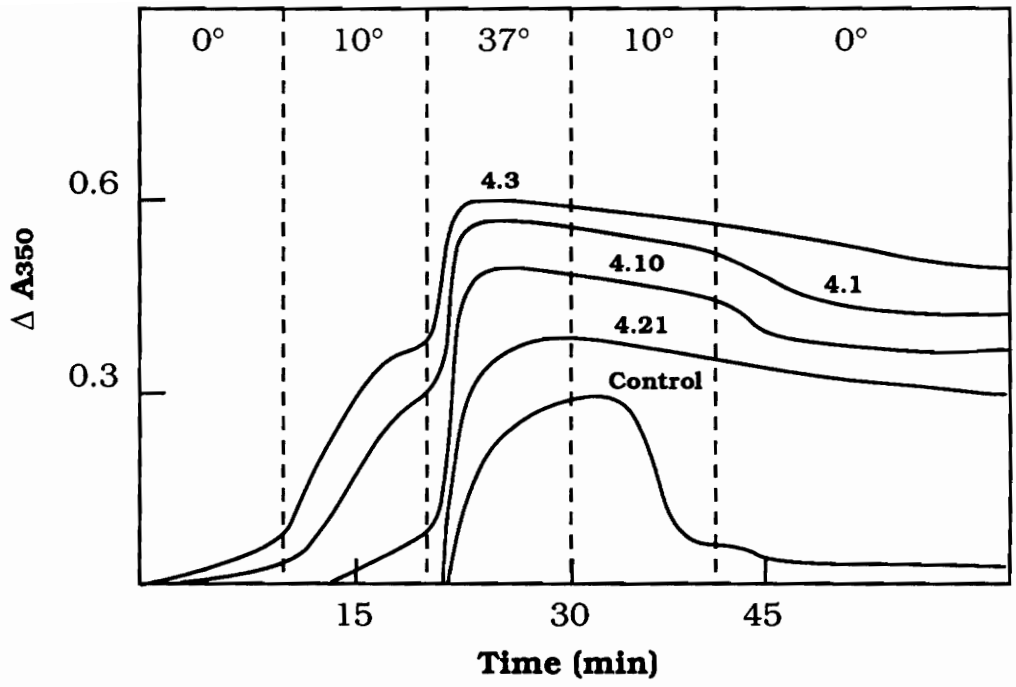


Figure 4.4. Polymerization with MAPs and GTP at 10 μM drug concentration. Reaction mixtures contained 0.1 M Mes (pH 6.9), 1.0 mg/mL (10 μM) tubulin, 0.5 mg/mL heat treated MAPs, 100 μM GTP, and 4% (v/v) dimethylsulfoxide. Data was reproduced from reference 203.

concentrations (40 μM) (Figure 4.5). The results in both systems were similar with the primary difference being an enhanced reaction of Taxotere (**4.3**) at 0°C at 40 μM. At both concentrations, the C-7 benzoyl analog (**4.21**) showed little ability to assemble tubulin polymers. The N-debenzoyl-N-(phenoxyacetyl) analog (**4.10**) displayed only slight activity at 10 μM, but moderate assembly occurred at 40 μM.

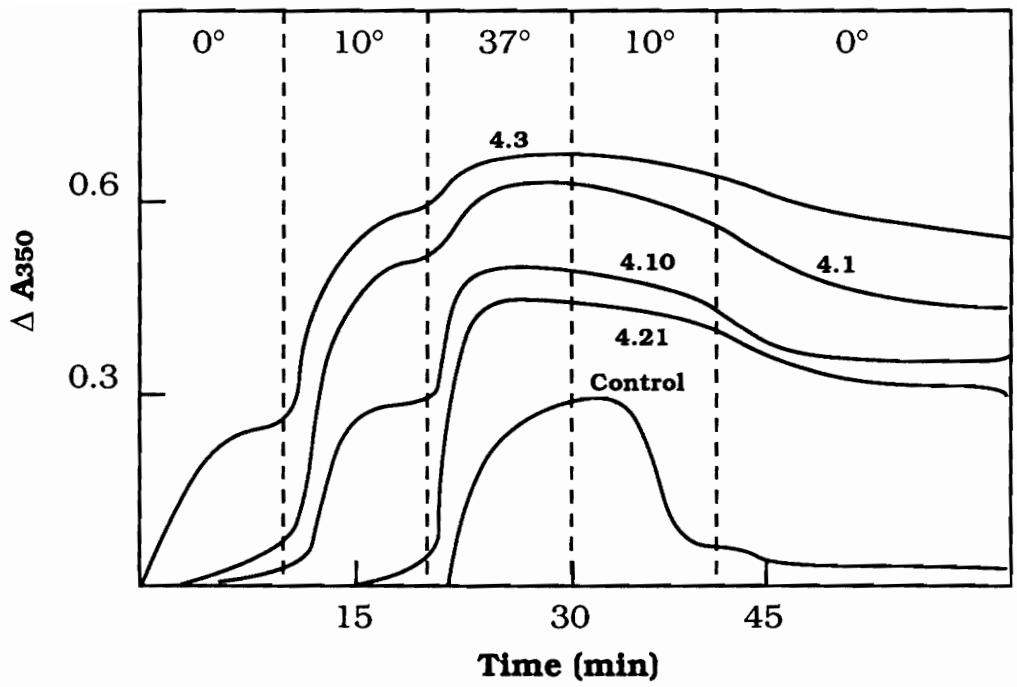


Figure 4.5. Polymerization with MAPs and GTP at 40 μM drug concentration. Reaction mixtures contained 0.1 M Mes (pH 6.9), 1.0 mg/mL (10 μM) tubulin, 0.5 mg/mL heat treated MAPs, 100 μM GTP, and 4% (v/v) dimethylsulfoxide. Data was reproduced from reference 203.

When Hamel and Grover examined the analogs under more "restrictive conditions" (no MAPs; no GTP) where tubulin normally does not polymerize, the differences became more obvious (Figure 4.6).[203, 205] As was previously done, the analogs were examined at 10 μM and 40 μM concentrations. At substoichiometric concentrations (10 μM) assembly failed to occur with any of the analogs except the C-2 *m*-azido derivative (**4.20**). At superstoichiometric concentrations (40 μM), Taxol (**4.1**), Taxotere (**4.3**), 10-acetyltaxotere (**4.9**), and the

C-2 *m*-azido derivative all promoted assembly, but none of the C-3' analogs, including cephalomannine (**4.2**) gave any observable reaction.

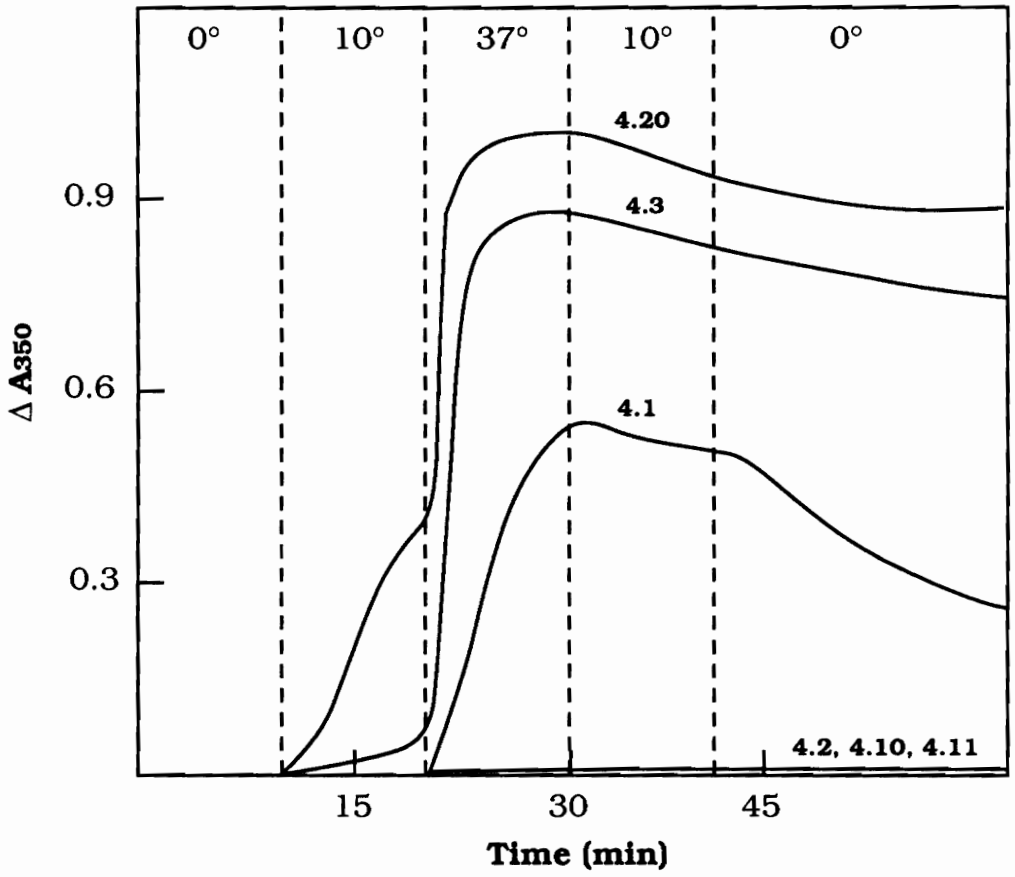


Figure 4.6. Polymerization without MAPs or GTP at 40 μM drug concentration. Reaction mixtures contained 0.1 M Mes, 2 mg/mL tubulin, and 4% (v/v) dimethyl sulfoxide. Data was reproduced from references 203 and 205.

4.3. Conclusions

The results of the polymerization studies clearly support the belief that the hypernucleation of tubulin assembly and polymer

stabilization observed with Taxol represent two distinct properties of the compound.[203] Hamel and Grover's analysis of a wide variety of derivatives modified at the C-2, C-3', and C-7 showed that cold stabilization of tubulin polymer frequently occurred and the differences from the Taxol control were minimal. However, when "restrictive conditions" were used (no MAPs; no GTP) the majority of the analogs failed to induce polymerization. Moreover, polymer stabilization was frequently observed with substoichiometric concentrations, while even superstoichiometric concentrations of most compounds failed to promote extensive polymerization at low temperatures or in the absence of MAPs or GTP. Thus, with a variety of Taxol analogs, stabilization of tubulin polymers to cold does not correlate well with the ability to promote assembly under "restrictive reaction conditions".

Of the compounds that were examined in detail, Hamel and Grover found that the most striking example of the separation of the stabilization and hypernucleation properties was with 7-benzoyltaxol (**4.21**), although N-debenzoyl-N-(phenoxyacetyl)taxol (**4.10**) also displayed this dissociation.[203] When the ability to stabilize polymer to cold was evaluated as a function of analog concentration, minimal differences between Taxol and these two analogs were observed (IC_{50} values of 0.42, 0.65, and 0.78 for Taxol, 7-benzoyltaxol, and N-debenzoyl-N-(phenoxyacetyl)taxol). In contrast, when examined in the absence of MAPs and GTP, Taxol promoted assembly while both analogs failed to induce any observable polymerization.

Conversely, Hamel and Grover noted that the ability of Taxotere (**4.3**) and the *m*-azido derivative (**4.20**) to enhance hypernucleation could not be predicted from the cold stability of the polymer formed in their presence.[203] The IC_{50} values for these two analogs were very close to Taxol, but when examined under restrictive conditions, their enhanced ability to promote assembly was detected.

One area these results may play a significant role in is the biological testing of analogs. Disassembly assays have frequently been used in the past,[4] but these results suggest that this may not be the best approach. Instead, assembly assays may be more valuable. However, whether assembly assays correlate well with *in vivo* anticancer activity is a question that remains to be answered.

5. Experimental

GENERAL EXPERIMENTAL PROCEDURES.- The general experimental procedures used were as described in section 2.4 and section 3.4.

As previously noted, Taxol derivatives typically form glassy solids when the solvent is removed under vacuum. Consequently, after pure compounds were isolated they were dissolved in a small quantity of CH_2Cl_2 and hexane was added to precipitate them. This procedure typically led to white amorphous solids.

7, 10-BIS(2, 2, 2-TRICHLOROETHYLOXYCARBONYL)BACCATIN III (**4.13**) - A 53 mg (98 μmoles) sample of 10-deacetylbaccatin III (**4.12**)

was dissolved in 1 mL of pyridine and then heated to 80°C. To the hot solution 43 μL (312 mmoles, 3.2 equivalents) of 2, 2, 2-trichloroethyl chloroformate was added. After stirring 8 minutes a second 43 μL (312 mmoles, 3.2 equivalents) aliquot of 2, 2, 2-trichloroethyl chloroformate was added. Approximately 4 minutes after the second addition, the reaction was cooled and diluted with ethyl acetate (15 mL). The diluted solution was washed with 1 N HCl (2 x 15 mL), water (1 x 15 mL, and brine 1 x 15 mL). After drying ($MgSO_4$), the solvent was removed *in vacuo*. Purification by preparative TLC (1:3 ethyl acetate/hexane) led to 73.6 mg (84%) of (**4.13**). All spectral data was in accord with the literature values[151]: ^1H NMR δ 7.50-8.07 (m, 15H, 2-OCOPh), 6.27 (s, 1H, C-10H), 5.65 (d, J=6.7, 1H, C-2H), 5.60 (m, 1H, C-7H), 4.99 (d, J=8.2, 1H, C-5H), 4.92 and 4.61 (d, J=11.8, 2H, 7-OCOC$\underline{H_2}CCl_3$), 4.78 (d, J=4.4, 2H, 10-OCOC$\underline{H_2}CCl_3$), 4.89 (m, 1H, C-13H), 4.34 and 4.16 (ABq, J=8.4, C-20H_2), 3.98 (d, J=6.9, 1H, C-3H), 2.65 and 2.05 (m, 2H, C-6H), 2.30 (s, 3H, 4-OCOCH_3), 2.16 (s, 3H, C-18H_3), 1.85 (s, 3H, C-19H_3), 1.12 (s, 3H, C-17H_3), 1.16 (s, 3H, C-16H_3).

SYNTHESIS OF THE (4S,5R)-N-BOC-2, 2-DIMETHYL-4-PHENYL-5-OXAZOLIDINE CARBOXYLIC ACID (**4.14**)- Protected side chain (**4.14**) was prepared as described by J. Rimoldi.[172] This procedure was a modification of the methodology of Greene *et. al.*[167] and A. Commerçon *et. al.*[137]: mp 135-138°C (lit. 137°C), ^1H NMR δ 7.34 (m,

5H, 3'-Ph), 5.11 (br d, 1H, C-3'H), 4.52 (d, J=5.7, 1H, C-2'H), 1.79 (s, 3H, acetal CH_3), 1.74 (s, 3H, acetal CH_3), 1.19 (br s, 9H, $C(CH_3)_3$).

7, 10- BIS(2, 2, 2-TRICHLOROETHYLOXYCARBONYL) PROTECTED SIDE CHAIN COUPLED PRODUCT (**4.15**) - A 26 mg (29 μmoles) sample of 7,10-protected-10-deacetylbaccatin III (**4.13**) was dissolved in 0.5 mL of toluene and then 14 mg (43 μmoles, 1.5 equivalents) of side chain (**4.14**), 9 mg (43 μmoles, 1.5 equivalents) of DCC, and a catalytic amount of DMAP were added. The mixture was heated to 60°C and stirred for 30 minutes. Purification by preparative TLC (3:7 ethyl acetate/hexane) give 35 mg (99%) of (**4.15**). The ^1H NMR spectral data was in agreement with the literature values[137]: ^1H NMR δ 7.31-8.06 (m, 10H, 2-OCOPh, 3'-Ph), 6.28 (br t, 1H, C-13H), 6.24 (s, 1H, C10H), 5.67 (d, J=7.0, 1H, C-2H), 5.58 (m, 1H, C-7H), 5.08 (br s, 1H, C-3'H), 4.91 (d, 1H, C-5H), 4.91 and 4.40 (d, J=11.8, 2H, 7-$OCOOC\underline{H_2}CCl_3$),4.78 (s, 2H, 10-$OCOOC\underline{H_2}CCl_3$), 4.47 (d, J=6.6, 1H, C-2'H), 4.28 and 4.12 (ABq, J=8.4, C-$20H_2$), 3.89 (d, J=7.0, 1H, C-3H), 2.54 (m, 1H, C-6H), 2.05 (s, 3H, 4-$OCOCH_3$), 1.94 (s, 3H, C-$18H_3$), 1.84 (s, 3H, acetonide CH_3), 1.81 (s, 3H, acetonide CH_3), 1.76 (s, 3H, C-$19H_3$), 1.26 (s, 3H, C-$17H_3$), 1.16 (s, 3H, C-$16H_3$), 1.12 (br s, 9H, $OC(CH_3)_3$).

7, 10- BIS(2, 2, 2-TRICHLOROETHYLOXYCARBONYL)TAXOTERE (**4.17**) - A 34.7 mg (29 μmoles) of (**4.15**) was dissolved in 0.5 mL of 99% formic acid and stirred at room temperature. After 3 hours the

mixture was diluted with ethyl acetate (15 mL) and washed with saturated sodium bicarbonate (2 x 10 mL), water, (1 x 10 mL), and brine (1 x 10 mL). After drying over $MgSO_4$, the solvent was removed under vacuum to give crude (**4.16**).

The crude amine (**4.16**) was dissolved in 0.5 mL of freshly distilled THF and then 11 mg of di-*tert*-butyl dicarbonate and 10 mg of $NaHCO_3$ were added. After stirring at room temperature 2 hours the solution was filtered to remove the $NaHCO_3$ and evaporated to dryness. The crude product was purified by preparative TLC (3:7 ethyl acetate/ hexane) to give 18 mg (54%) of (**4.17**). The ^1H NMR spectral data was consistent with the literature values[135]: ^1H NMR δ 7.33-8.11 (m, 10H, 2-OCOPh, 3'-Ph), 6.24 (s, 1H, C-10H), 6.21 (br t, J=8.6, 1H, C-13H), 5.70 (d, J=6.9, 1H, C-2H), 5.54 (m, 1H, C-7H), 5.39 (d, J=9.4, 1H, C-3'NH), 5.26 (br d, J=9.6, 1H, C3'H), 4.96 (d, J=8.1, 1H, C-5H), 4.91 and 4.60 (d, J=11.8, 2H, 7-$OCOOCH_2CCl_3$), 4.78 (s, 2H, 10-$OCOOCH_2CCl_3$), 4.65 (m, 1H, C-2'H), 4.33 and 4.18 (ABq, J=8.5, C-$20H_2$), 3.91 (d, J=6.8, 1H, C-3H), 2.61 and 2.06 (m, 2H, C-6H), 2.39 (s, 3H, 4-$OCOCH_3$), 1.96 (s, 3H, C-$18H_3$), 1.85 (s, 3H, C-$19H_3$), 1.35 (br s, 9H, $OC(CH_3)_3$), 1.27 (s, 3H, C-$16H_3$), 1.20 (s, 3H, C-$17H_3$).

TAXOTERE (**4.3**) - An 18 mg sample of (**4.17**) was dissolved in 0.5 mL of methanol and then 0.5 mL of glacial acetic acid was added along with 26 mg of zinc dust. The resulting heterogeneous mixture was heated to 60°C and stirred for 90 minutes. After cooling, the solution was filtered to remove the zinc dust and then diluted with ethyl

acetate (15 mL). The diluted solution was washed with saturated sodium bicarbonate (2 x 10 mL), water (1 x 10 mL), and brine (1 x 10 mL). After drying ($MgSO_4$) the solvent was removed under vacuum. Purification by preparative TLC (3:2 ethyl acetate/hexane) led to 9 mg (74%) of Taxotere (**4.3**) as a white amorphous solid. The ^1H NMR spectral data was in accord with the literature values[135]: ^1H NMR see Table 4.1.

ACETONIDE PROTECTED SIDE CHAIN PRODUCT (**4.18**) - Acetonide (**4.13**) was prepared as described in section 3.4: mp 148-150°C; ^1H NMR δ 7.31-8.05 (m, 10H, 2-OCOPh, 3'-Ph), 6.45 (s, 1H, C-10H), 6.24 (br t, J=8.4, 1H, C13H), 5.65 (d, J=7.1, 1H, C-2H), 5.06 (br s, 1H, C-3'H), 4.87 (br d, J=8.0, 1H, C-5H), 4.47 (d, J=6.7, 1H, C-2'H), 4.45 (m, 1H, C-7H), 4.24 and 4.09 (ABq, J=8.3, C-20H_2), 3.77 (d, J=7.0, 1H, C-3H), 2.54 (m, 1H, C-6H), 2.18 (s, 3H, 4-OCOCH_3), 1.84 (s, 3H, acetonide CH_3), 1.81 (s, 3H, acetonide CH_3), 1.76 (s, 3H, C-18H_3), 1.66 (s, 3H, C-19H_3), 1.26 (s, 3H, C-17H_3), 1.20 (s, 3H, C-16H_3), 1.13 (br s, 9H, OC(CH_3)$_3$), 0.91 (t, J=8.3, 6H, Si($CH_2C\underline{H}_3$)$_3$), 0.55 (q, J=8.3, 6H, Si($C\underline{H}_2CH_3$)$_3$); FABMS *m/z* (rel. int.) [MH^+] 1004 (3), 902 (1), 844 (1), 276 (5), 176 (95), 105 (100).

10-ACETYLTAXOTERE (**4.9**)- A 30 mg (34 μmoles) quantity of (**4.18**) was dissolved in 1 mL of 99% formic acid and stirred at room temperature for 2 hours. The solution was then diluted with ethyl acetate (10 mL) and washed with saturated sodium bicarbonate (3 x 10

mL), water (1 x 10 mL), and brine (1 x 10 mL). After drying over $MgSO_4$, the solvent was removed under vacuum to yield crude (**4.19**).

The crude product was dissolved in 1 mL of dry THF and 6.4 mg of di-*tert*-butyldicarbonate and a small quantity of $NaHCO_3$ was added. The resulting heterogeneous solution was stirred at room temperature and monitored by TLC until the reaction was complete. The reaction was worked-up by diluting with ethyl acetate (10 mL) and washing with water (2 x 10 mL) and brine (1 x 10 mL). Ater drying (MgSO4), the solvent was removed under vacuum. The crude product was purified by preparative TLC (3:2 ethyl acetate/hexane) to afford 10.8 mg (37%) of 10-acetyltaxotere (**4.9**). All data was consistent with the literature values[135]: mp 162-165°C; ^{1}H NMR see Table 4.2; FABMS *m/z* (rel. int.) $[MH]^+$ 850 (15), 569 (28), 509 (60), 327 (55), 206 (90); HRFABMS *m/z* $[MH]^+$ 850.3627 ($C_{45}H_{56}NO_{15}$ requires 850.3650).

N-DEBENZOYL-N-(PHENOXYACETYL)TAXOL (**4.10**)- A 35 mg (35 μmoles) quantity of side chain coupled product (**4.18**) was dissolved in 0.50 mL of 99% formic acid and stirred at room temperature for 3 hours. The solution was then diluted with ethyl acetate (10 mL) and washed with saturated sodium bicarbonate (1 x 15 mL), water (1 x 15 mL), and brine (1 x 15 mL). After drying over $MgSO_4$, the solvent was removed *in vacuo* to yield (**4.19**) as a glassy solid.

The crude solid was dissolved in 0.5 mL of dry methylene chloride and cooled to 0°C using an ice bath. To this solution, 6 μL (41 μmoles) of phenoxyacetyl chloride and 8.5 μL (50 μmoles) of

DIPEA were added. The resulting mixture was stirred at 0°C for 10 minutes. The product was purified by preparative TLC (3:2 ethyl acetate/hexane) to afford 10.3 mg (33%) of (**4.10**) as a white solid: $[\alpha]^{23}_{D}$ = -123.5° (*c*=7.2, $CHCl_3$); ^1H NMR see Table 4.3; FABMS *m/z* (rel. int.) $[MH]^+$ 884.2 (1), 569.2 (1), 460.1 (2), 307.0 (20), 154.0 (100); HRFABMS *m/z* $[MH]^+$ 884.3498 ($C_{48}H_{54}NO_{15}$ requires 884.3493).

CEPHALOMANNINE DIOLS (**4.11**)- The cephalomannine diols (**4.11**) were prepared according to the catalytic procedure described by Kingston *et. al.*[54]

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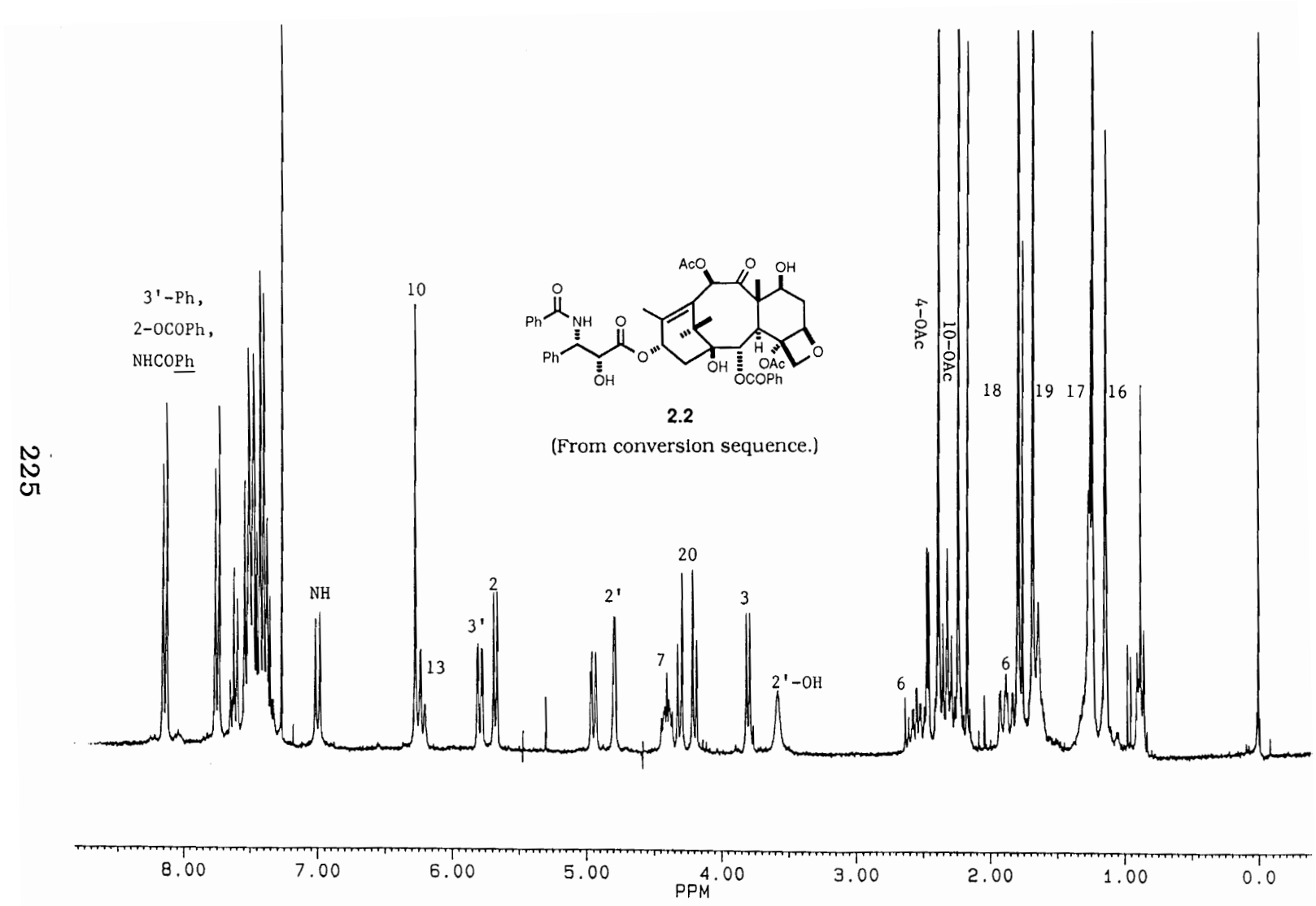
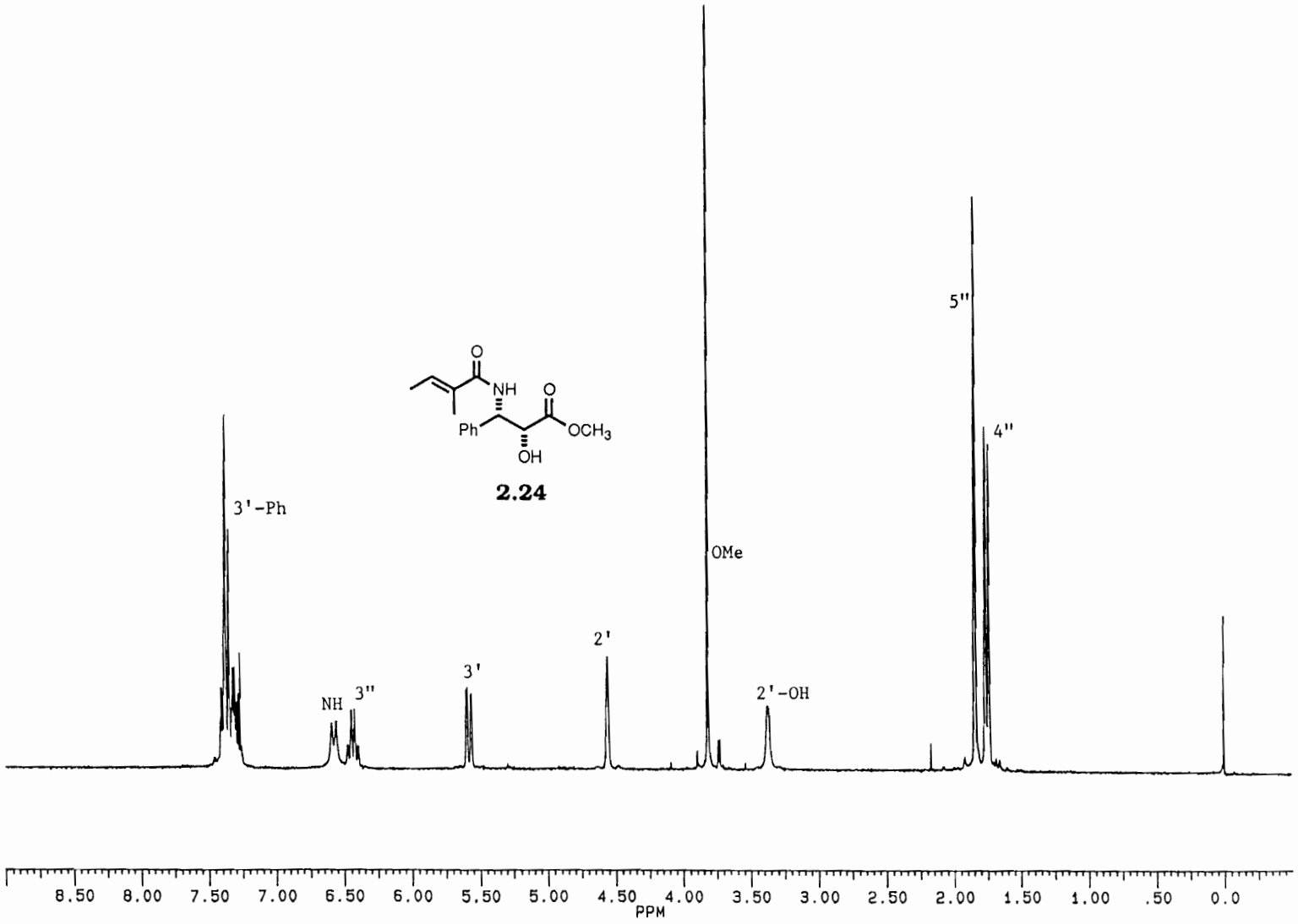
Appendix

^1H NMR spectra (270 mHz or 400 mHz, $CDCl_3$) of selected compounds. Due to the fact that many of the compounds were glassy solids or viscous oils, it was difficlut to remove all traces of solvent. Where solvent peaks are known they are noted. The spectra are arranged in numerical order according to compound number.

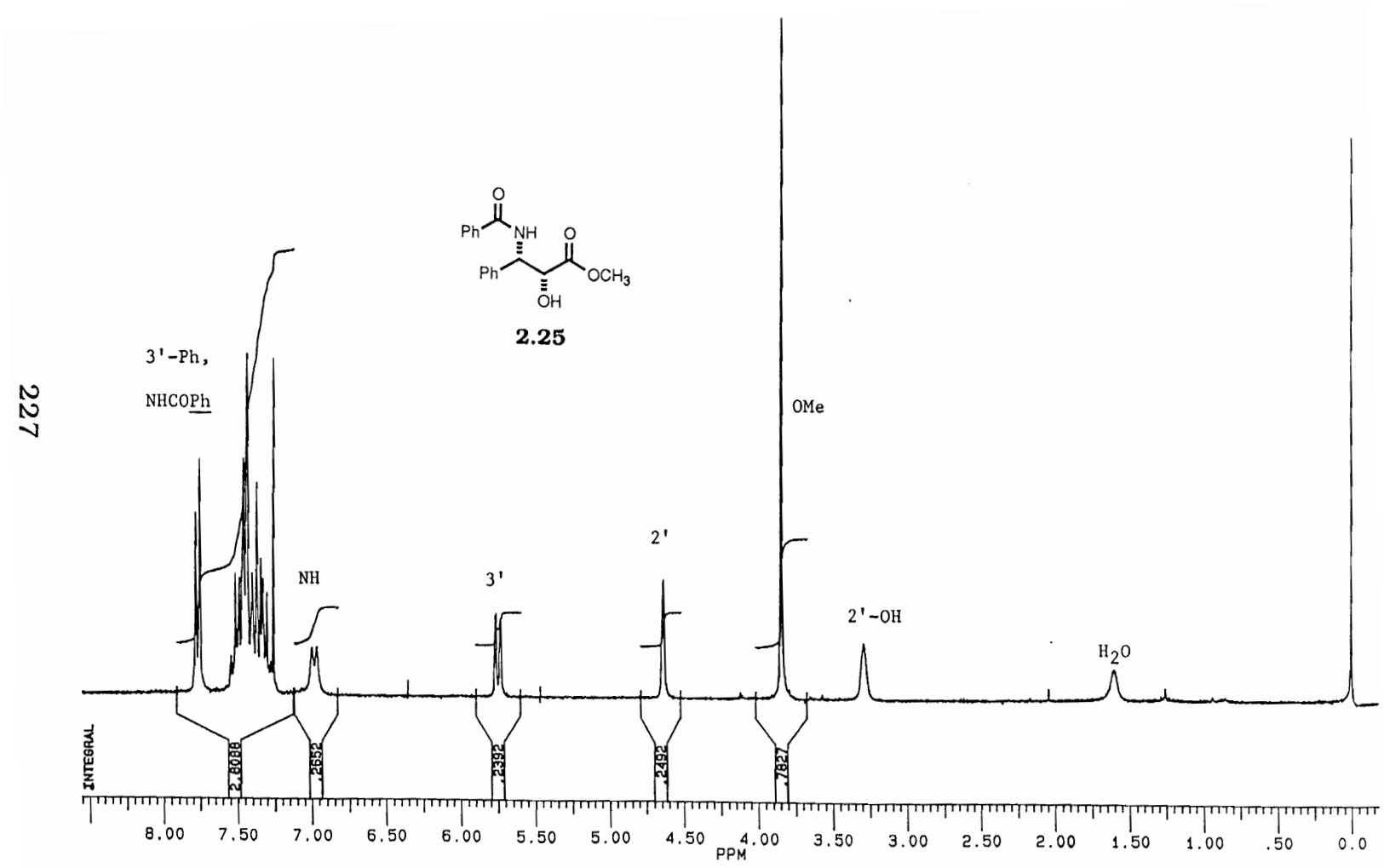


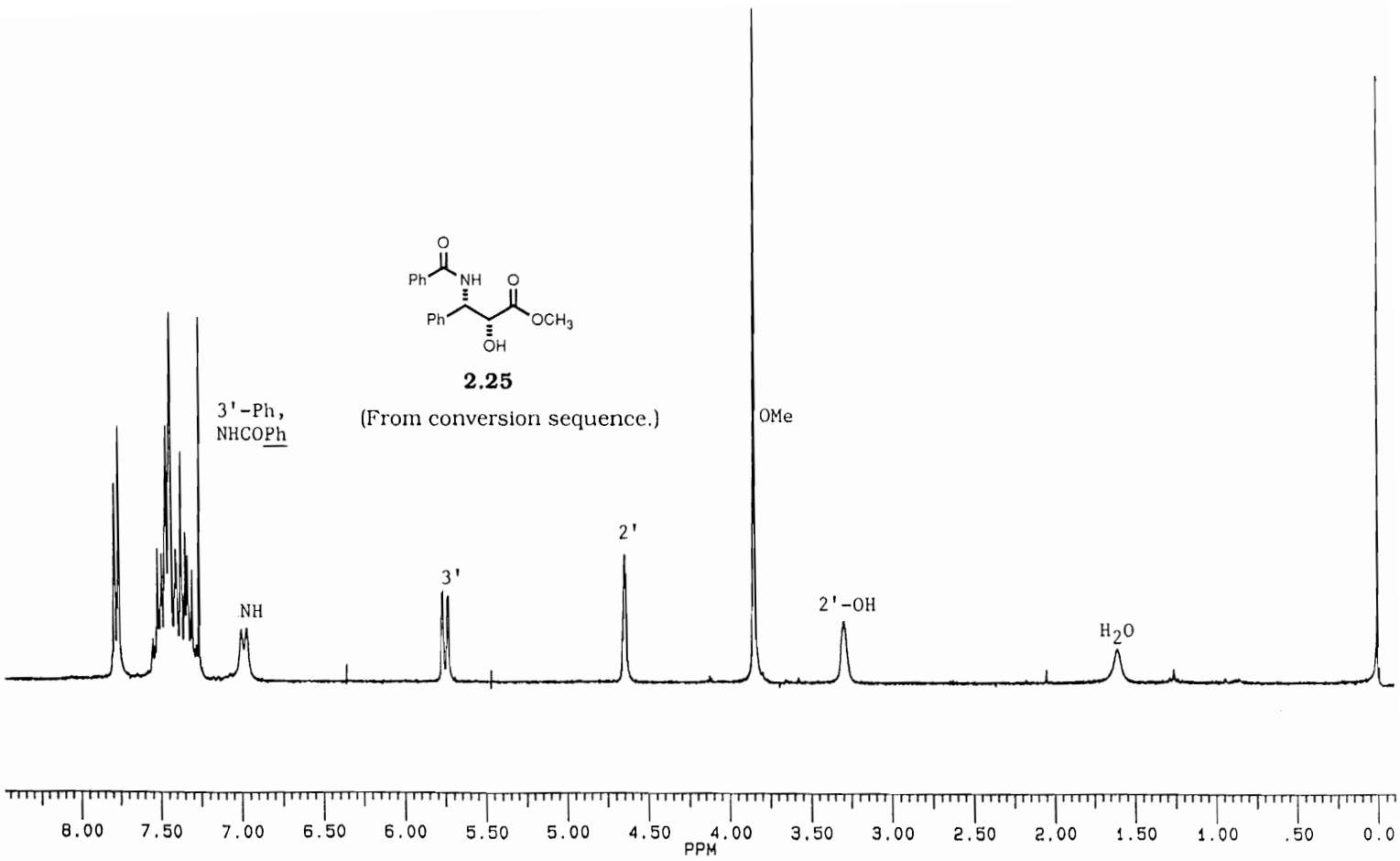
2.2

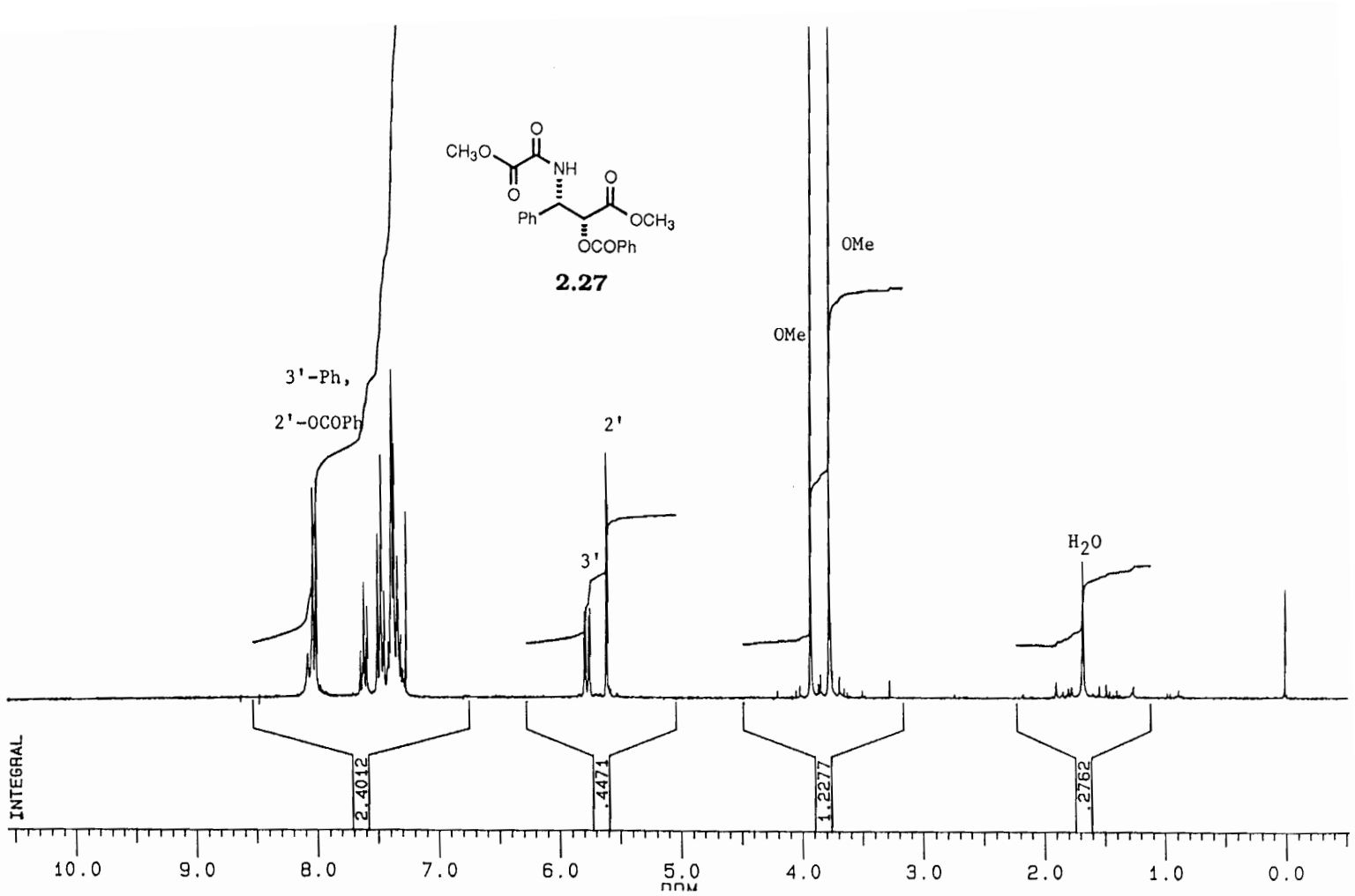
(From conversion sequence.)

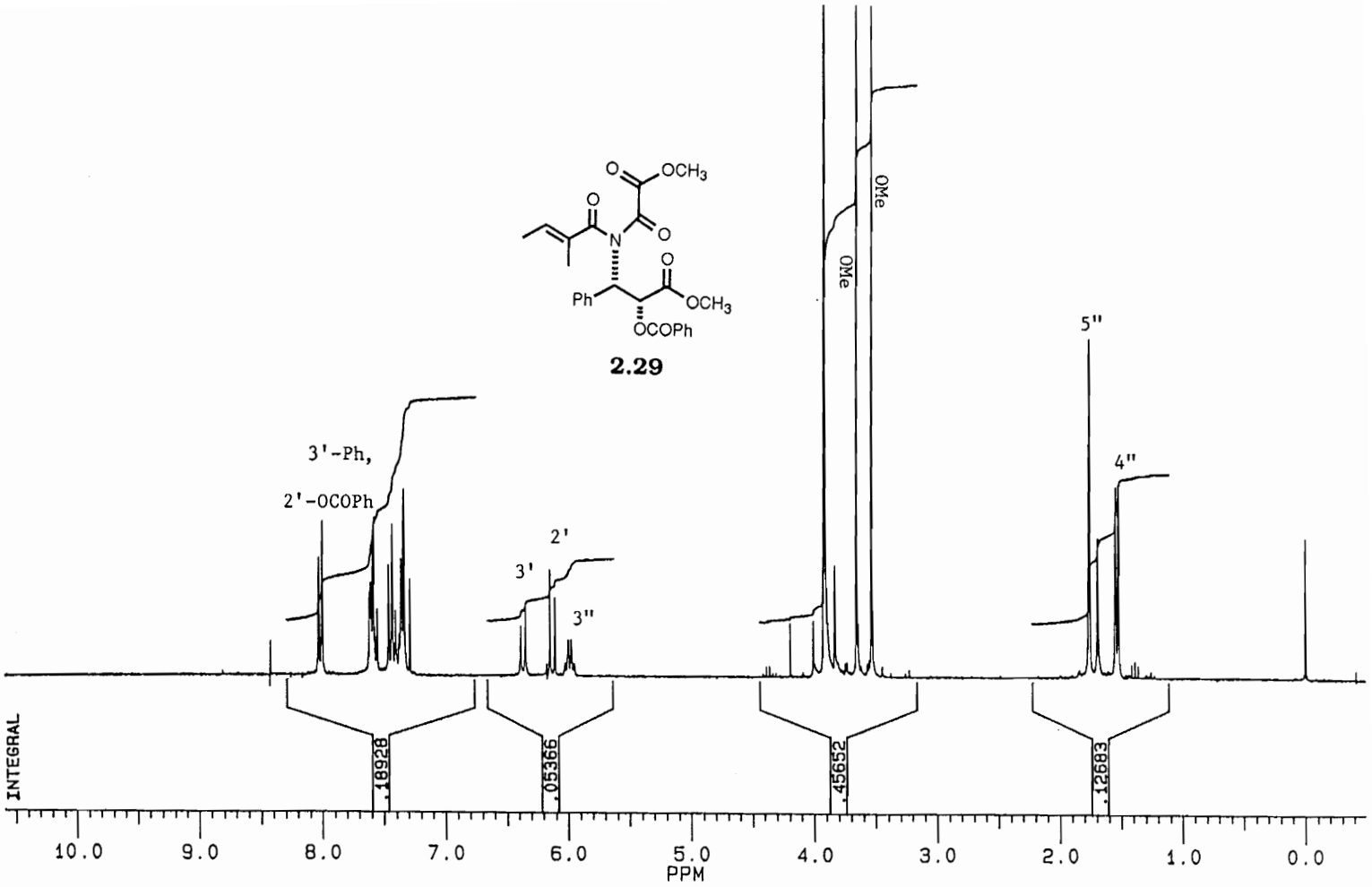

3'-Ph
NH
3"
3'
2'
OMe
2'-OH
5"
4"
8.50 8.00 7.50 7.00 6.50 6.00 5.50 5.00 4.50 4.00 3.50 3.00 2.50 2.00 1.50 1.00 .50 0.0
PPM

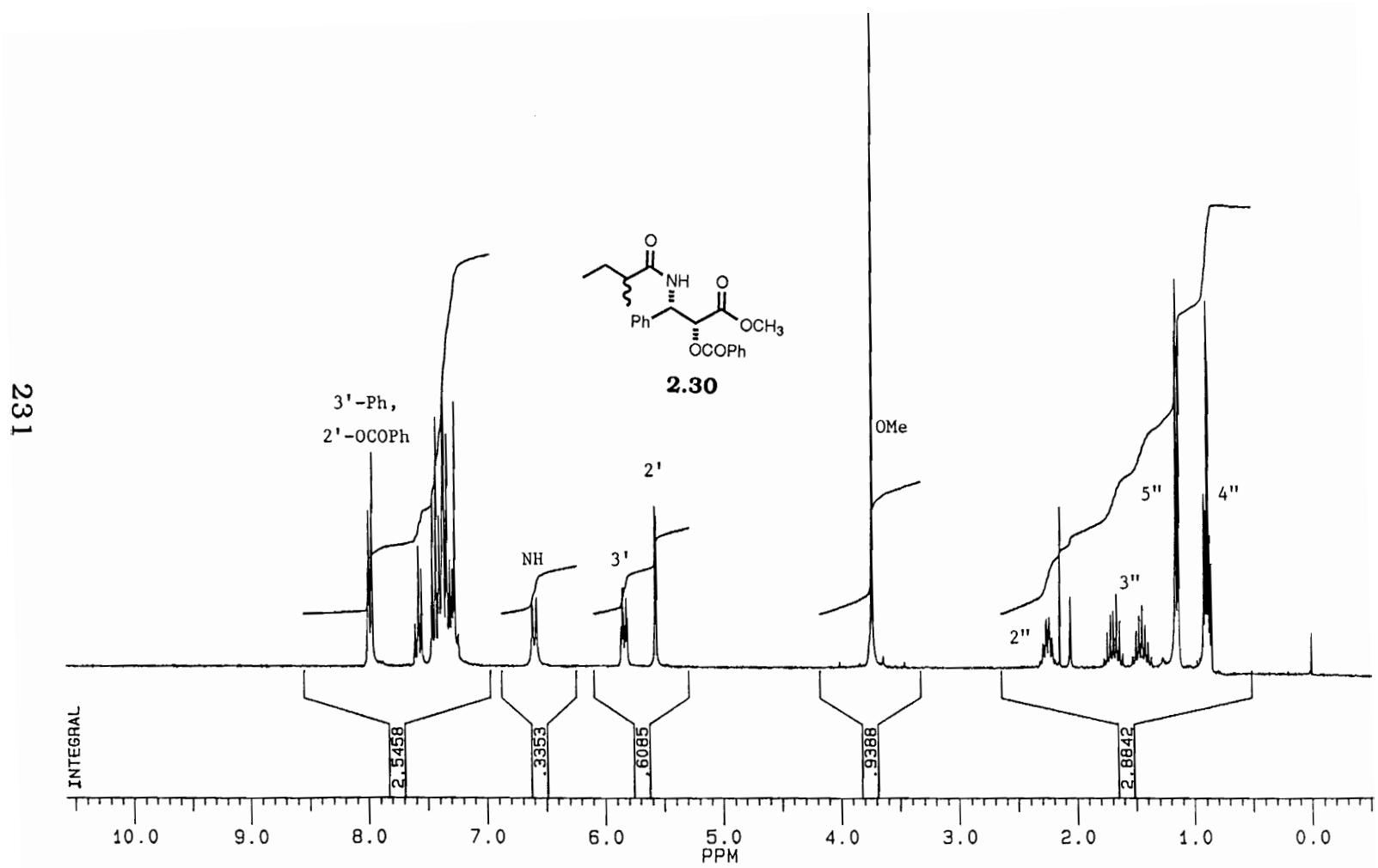
2.24

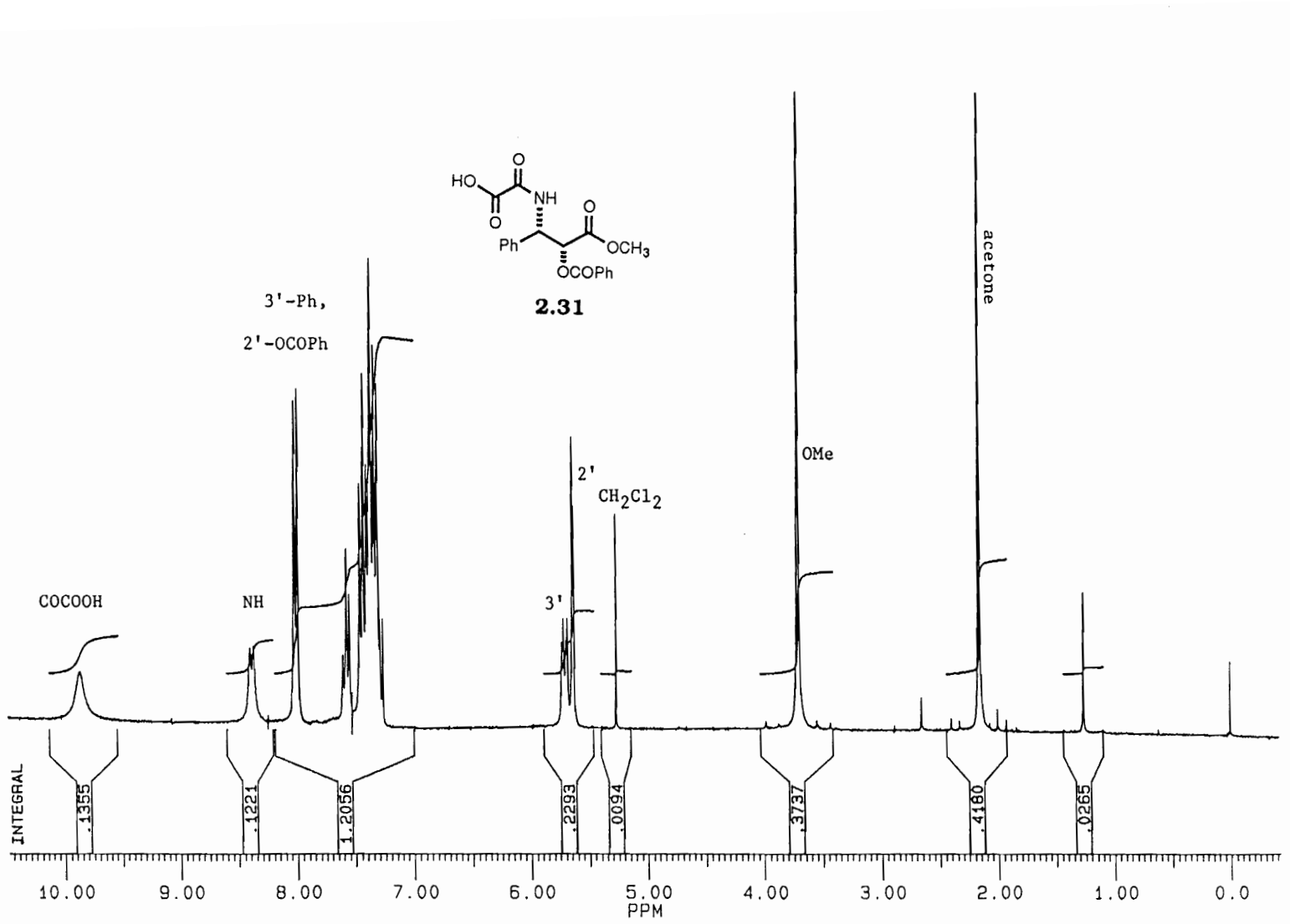

Ph
NH
O
O
Ph
OCH3
OH
2.25
3'-Ph,
NHCOPh
NH
3'
2'
OMe
2'-OH
H2O
INTEGRAL
2.8088
.2652
.2392
.2492
.7827
8.00
7.50
7.00
6.50
6.00
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
.50
0.0
PPM

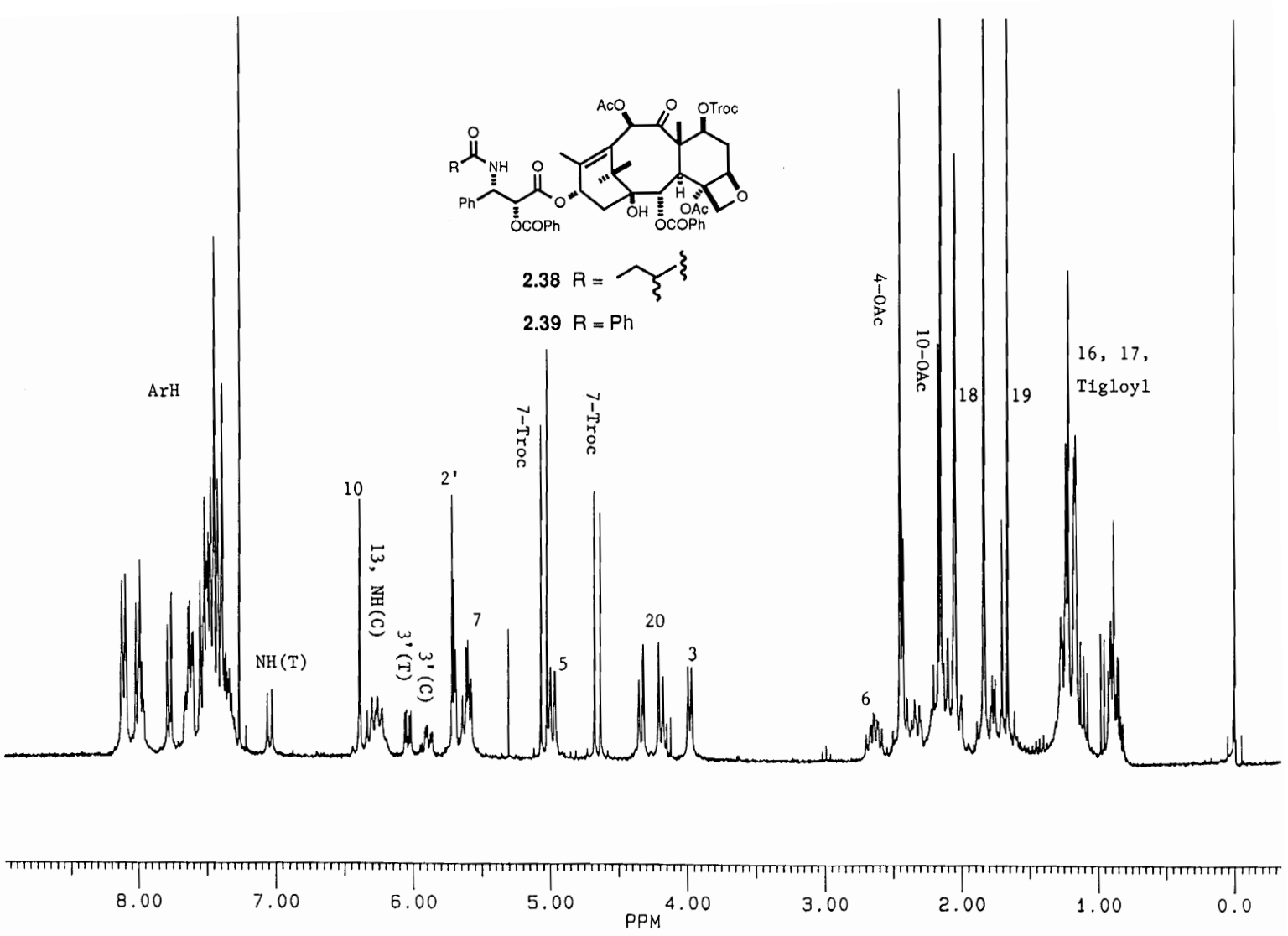

O
Ph
NH
O
Ph
OCH3
OH
2.25
(From conversion sequence.)
3'-Ph,
NHCOPh
NH
3'
2'
OMe
2'-OH
H2O
8.00
7.50
7.00
6.50
6.00
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
.50
0.0
PPM

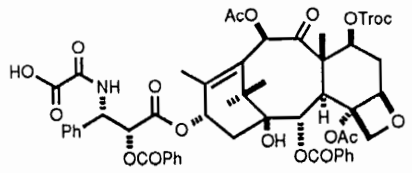

CH3O
O
NH
O
O
Ph
OCH3
OCOPh
2.27
3'-Ph,
2'-OCOPh
2'
3'
OMe
OMe
H2O
INTEGRAL
2.4012
.4471
1.2277
.2762
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
PPM


OCH3
O
O
N
O
O
Ph
OCH3
OCOPh
2.29
OMe
OMe
5"
4"
3'-Ph,
2'-OCOPh
2'
3'
3"
INTEGRAL
.18928
.05366
.45652
.12683
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
PPM


O
NH
O
Ph
OCH3
OCOPh
2.30
3'-Ph,
2'-OCOPh
NH
3'
2'
OMe
5"
4"
3"
2"
INTEGRAL
2.5458
.3353
.6085
.9388
2.8842
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
PPM

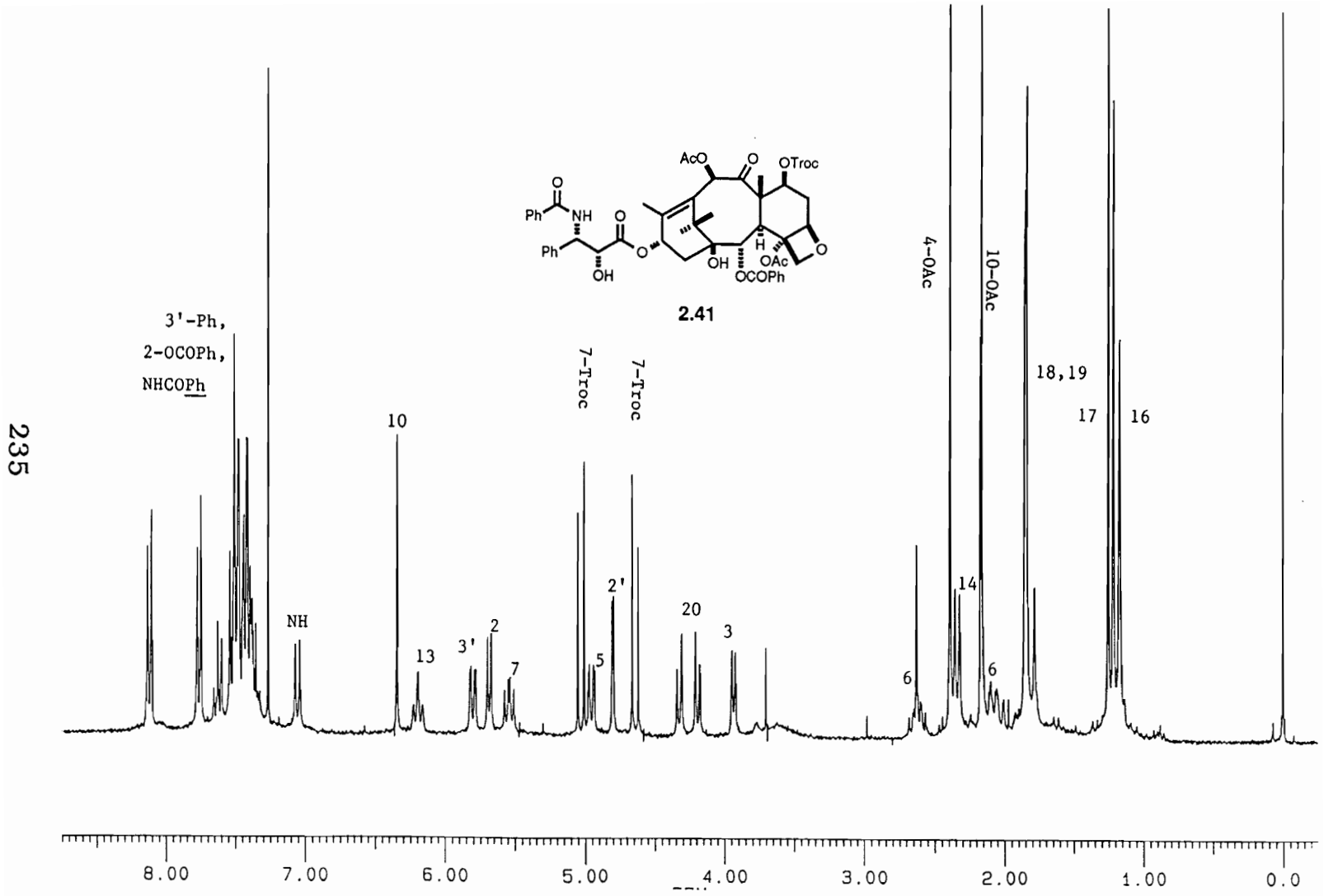

HO
O
O
NH
O
Ph
OCH3
OCOPh
2.31
3'-Ph,
2'-OCOPh
COCOOH
NH
3'
2'
CH2Cl2
OMe
acetone
INTEGRAL
.1355
.1221
1.2056
.2293
.0094
.3737
.4180
.0265
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

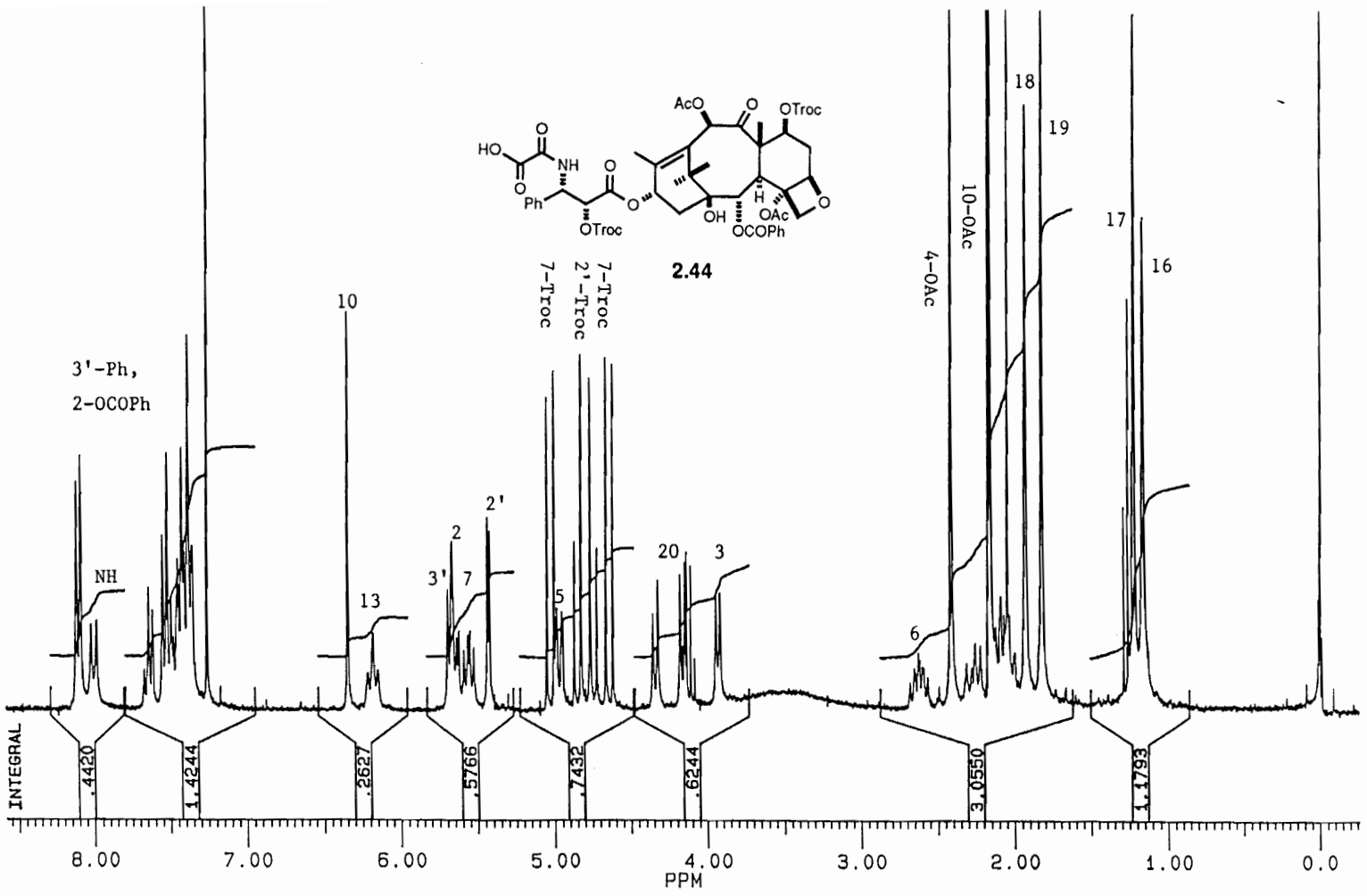

AcO
O
OTroc
R
NH
O
Ph
OCOPh
O
OH
H
OAc
OCOPh
2.38 R =
2.39 R = Ph
ArH
NH(T)
10
13, NH(C)
3'(T)
3'(C)
2'
7
7-Troc
5
7-Troc
20
3
6
4-OAc
10-OAc
18
19
16, 17,
Tigloyl
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

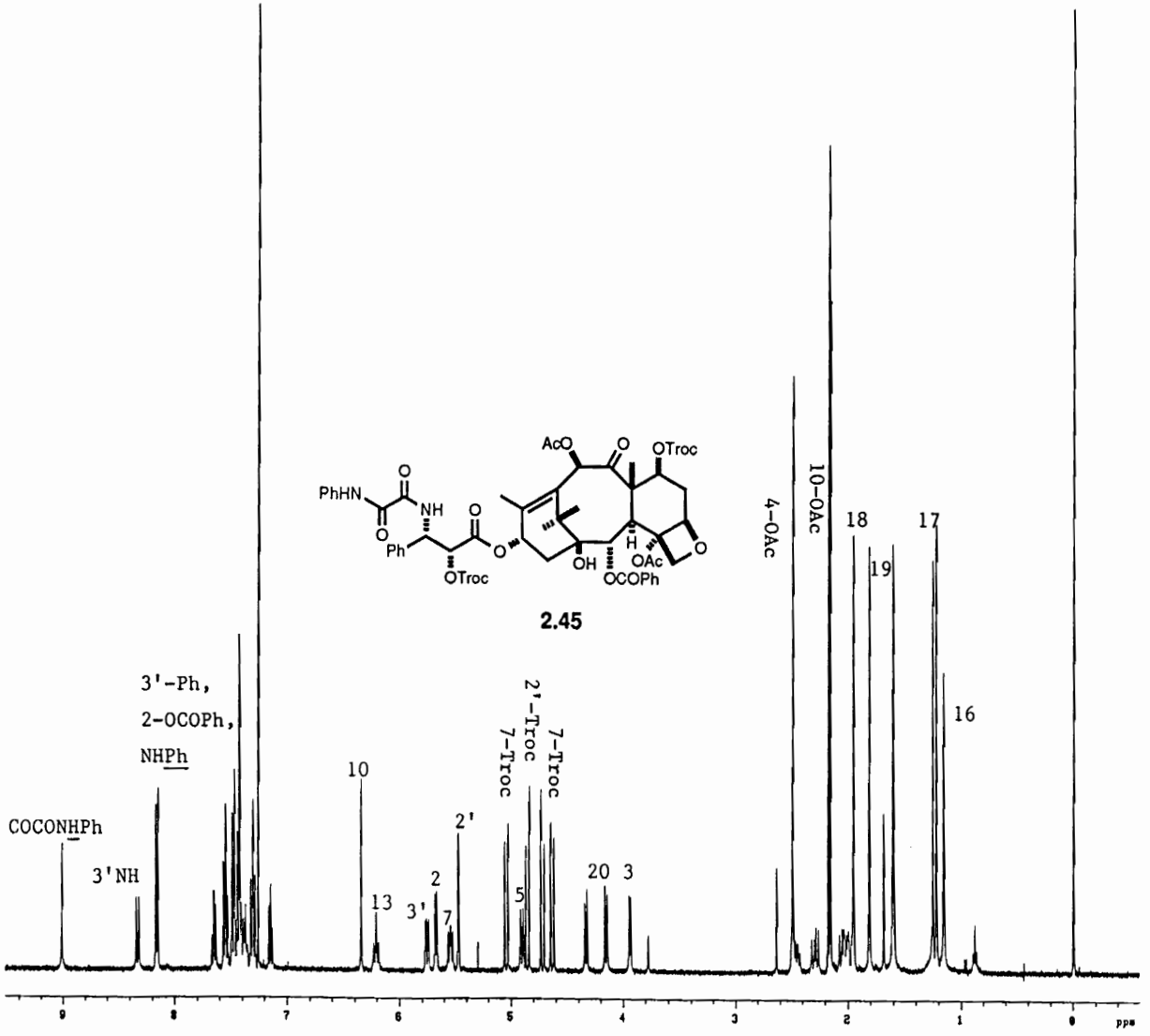


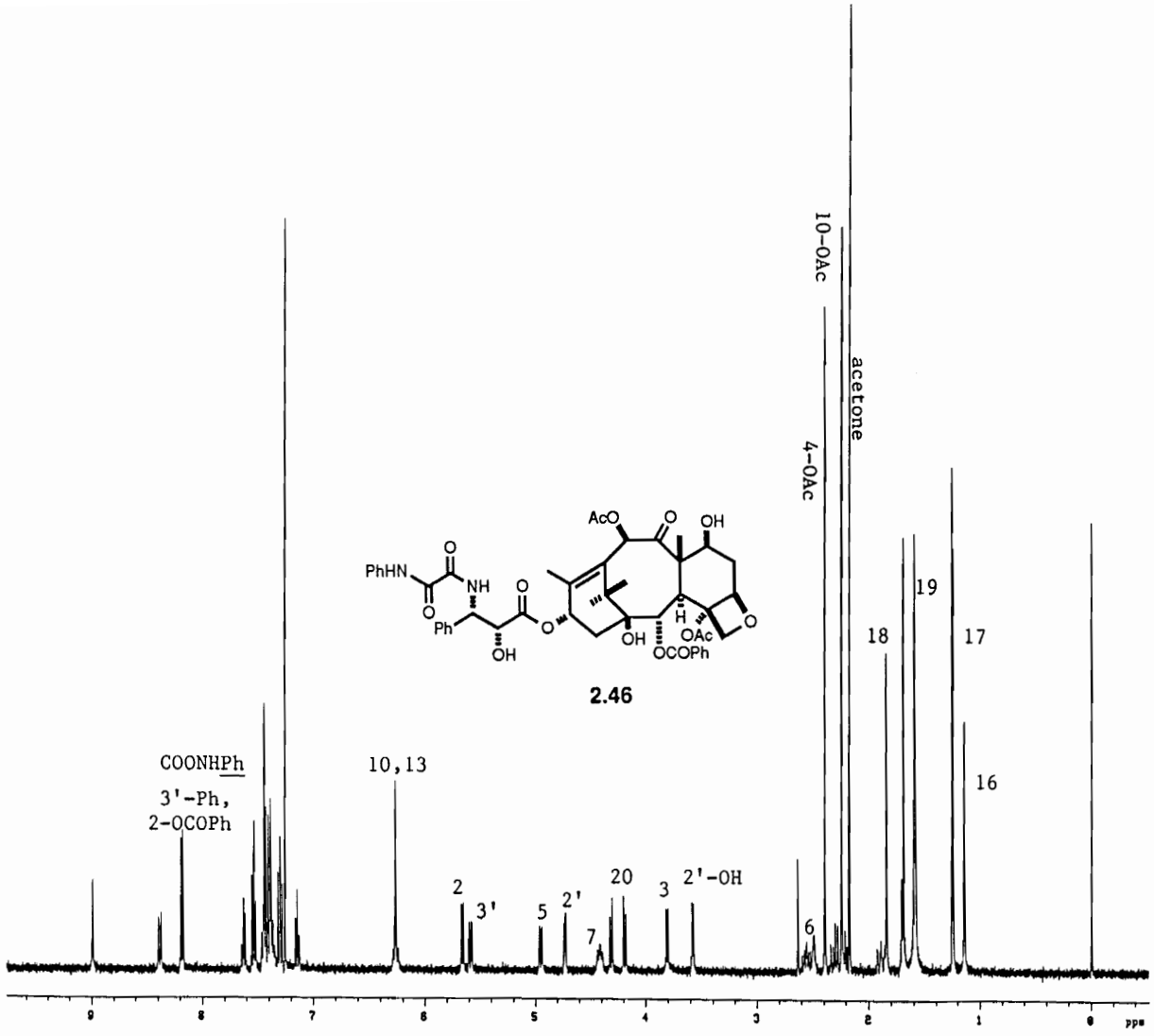
2.40

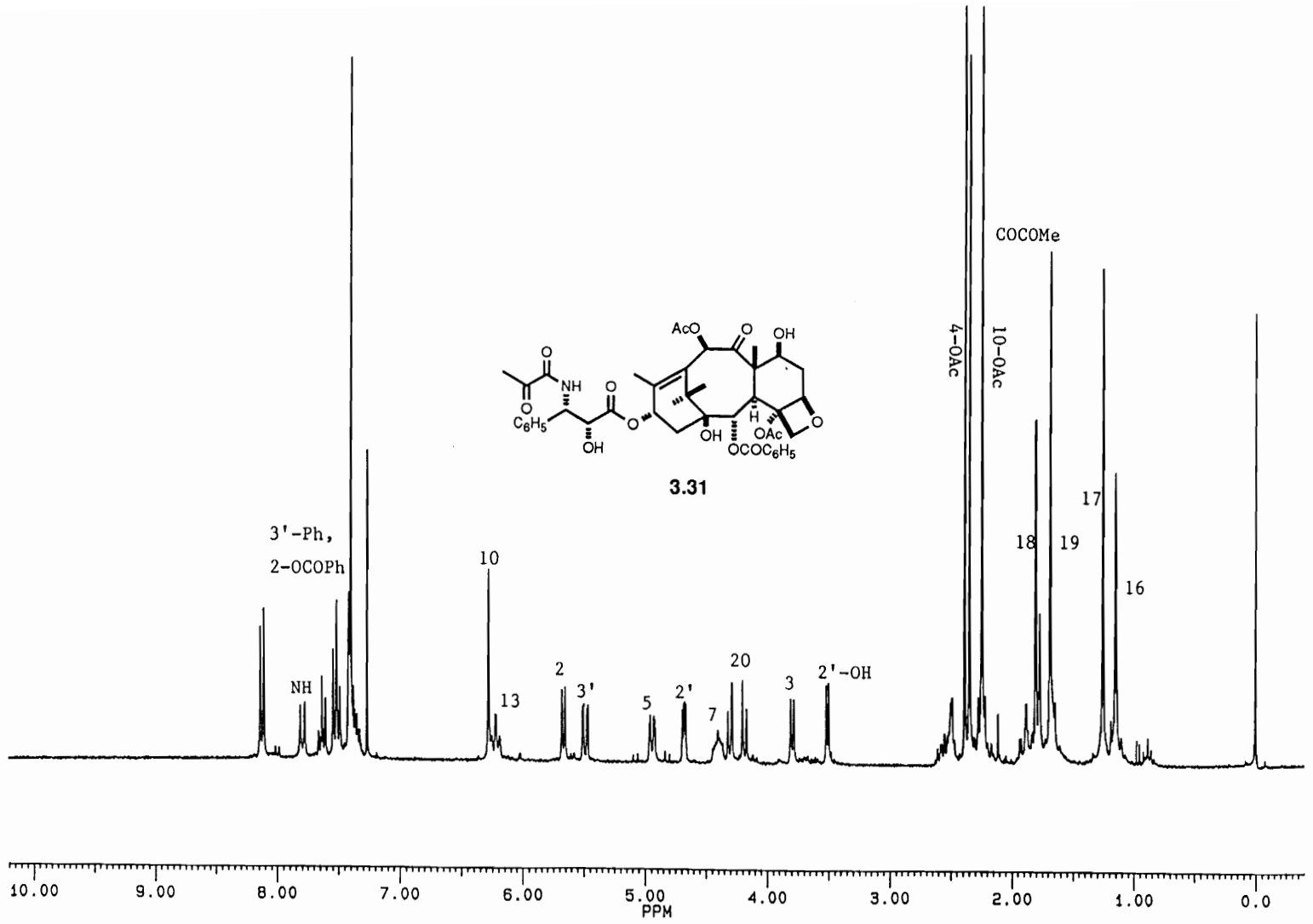
(Crude oxamic acid)


AcO
OTroc
Ph
NH
Ph
OH
OAc
OCOPh
2.41
3'-Ph,
2-OCOPh,
NHCOPh
10
NH
13
3'
2
7
7-Troc
5
2'
7-Troc
20
3
6
4-OAc
14
10-OAc
6
18,19
17
16
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0

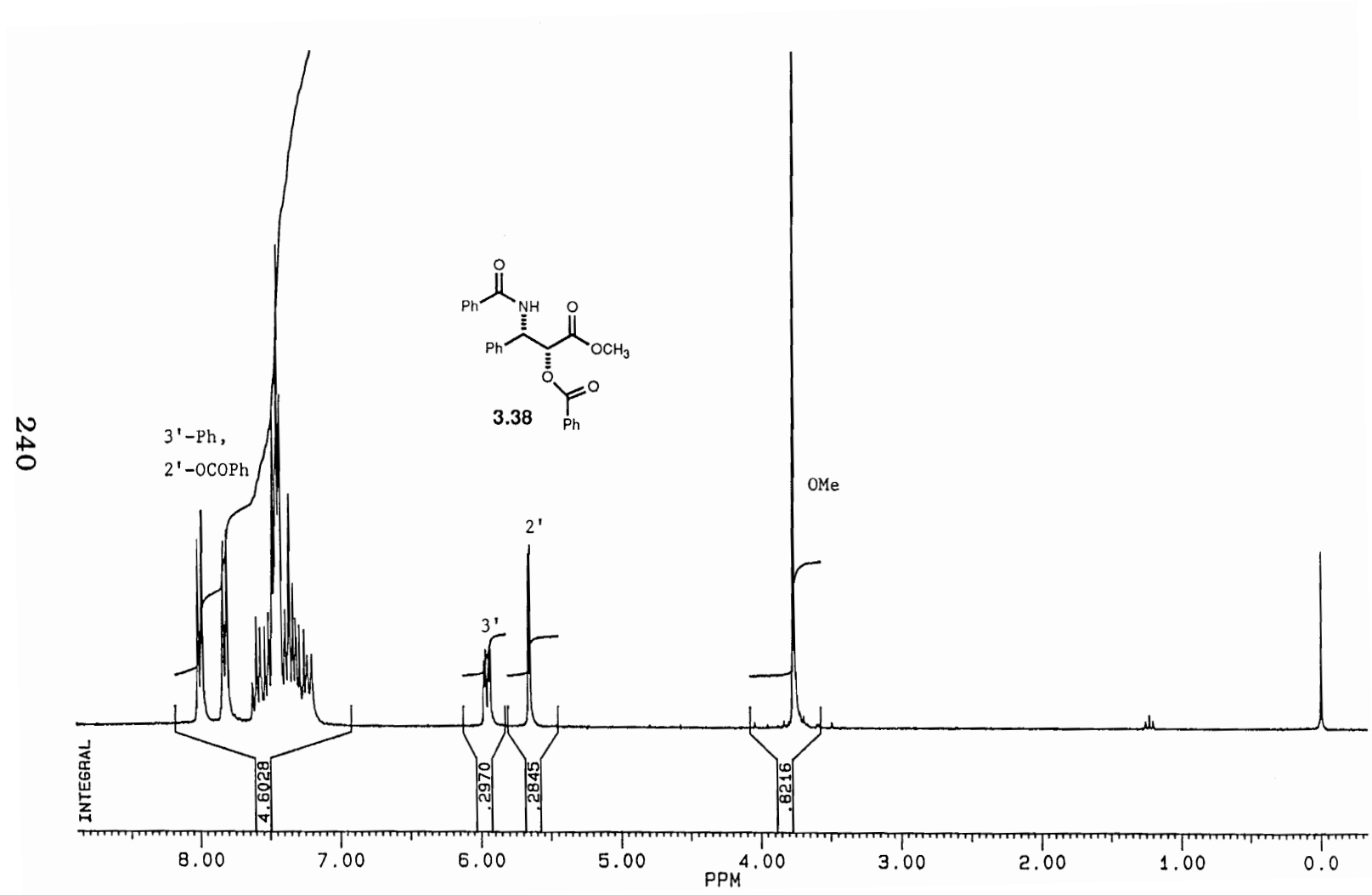

2.44
3'-Ph,
2-OCOPh
NH
10
13
3'
2
2'
7
5
7-Troc
2'-Troc
7-Troc
20
3
6
4-OAc
10-OAc
18
19
17
16
INTEGRAL
.4420
1.4244
.2627
.5766
.7432
.6244
3.0550
1.1793
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM


PhHN
NH
Ph
OTroc
AcO
OTroc
OH
OAc
OCOPh
2.45
4-OAc
10-OAc
18
19
17
16
3'-Ph,
2-OCOPh,
NHPh
COCONHPh
3'NH
10
13
3'
2
7
2'
7-Troc
2'-Troc
7-Troc
5
20
3
9
8
7
6
5
4
3
2
1
0
ppm

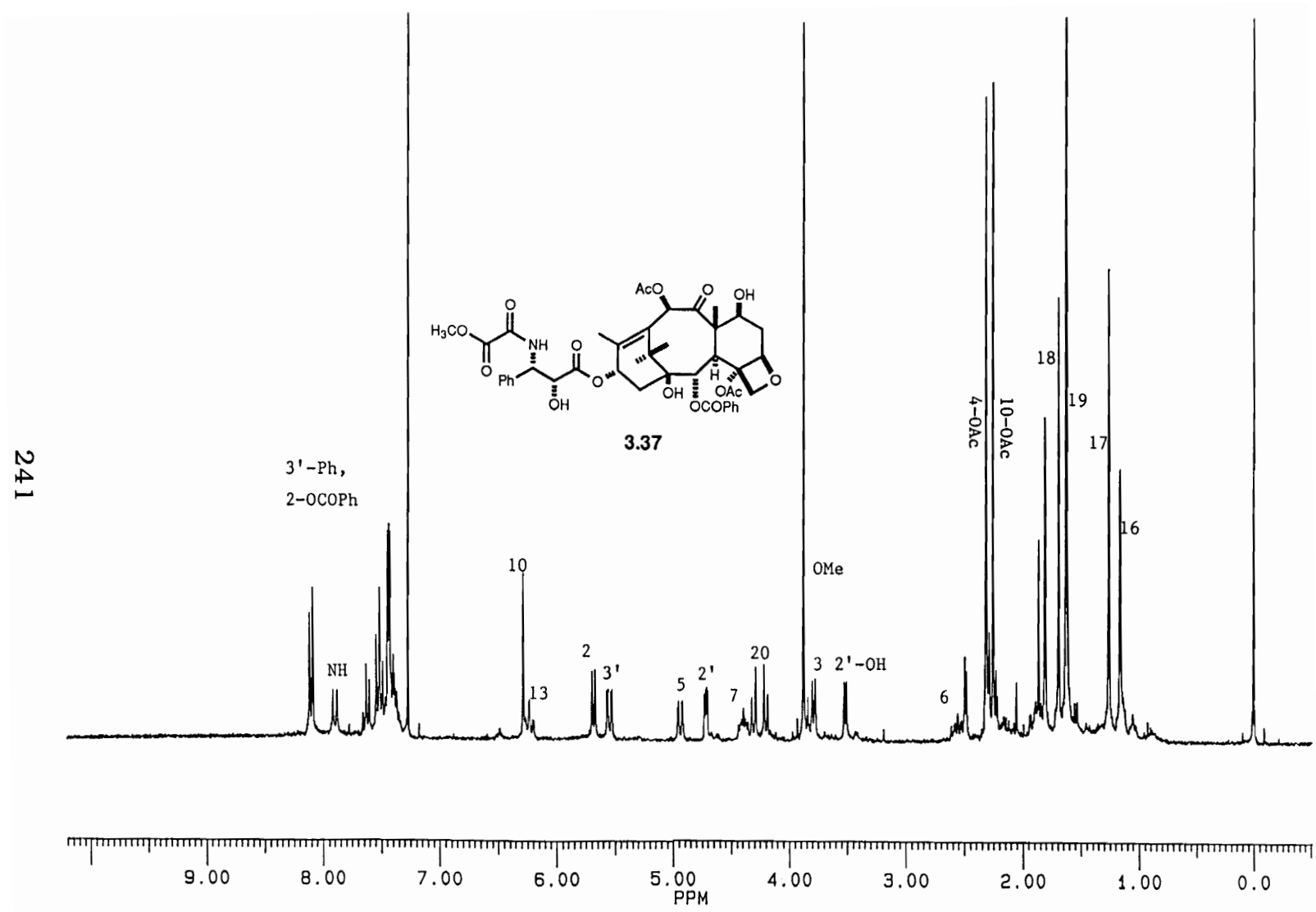

10-OAc
4-OAc
acetone
18
19
17
16
COONHPh
3'-Ph,
2-OCOPh
10,13
2
3'
5
2'
7
20
3
2'-OH
6
PhHN
NH
Ph
OH
AcO
OH
OAc
OCOPh
H
2.46
9
8
7
6
5
4
3
2
1
0
ppm


3'-Ph,
2-OCOPh
NH
10
13
2
3'
5
2'
7
20
3
2'-OH
4-OAc
10-OAc
COCOMe
18
19
17
16
10.00
9.00
8.00
7.00
6.00
5.00
PPM
4.00
3.00
2.00
1.00
0.0

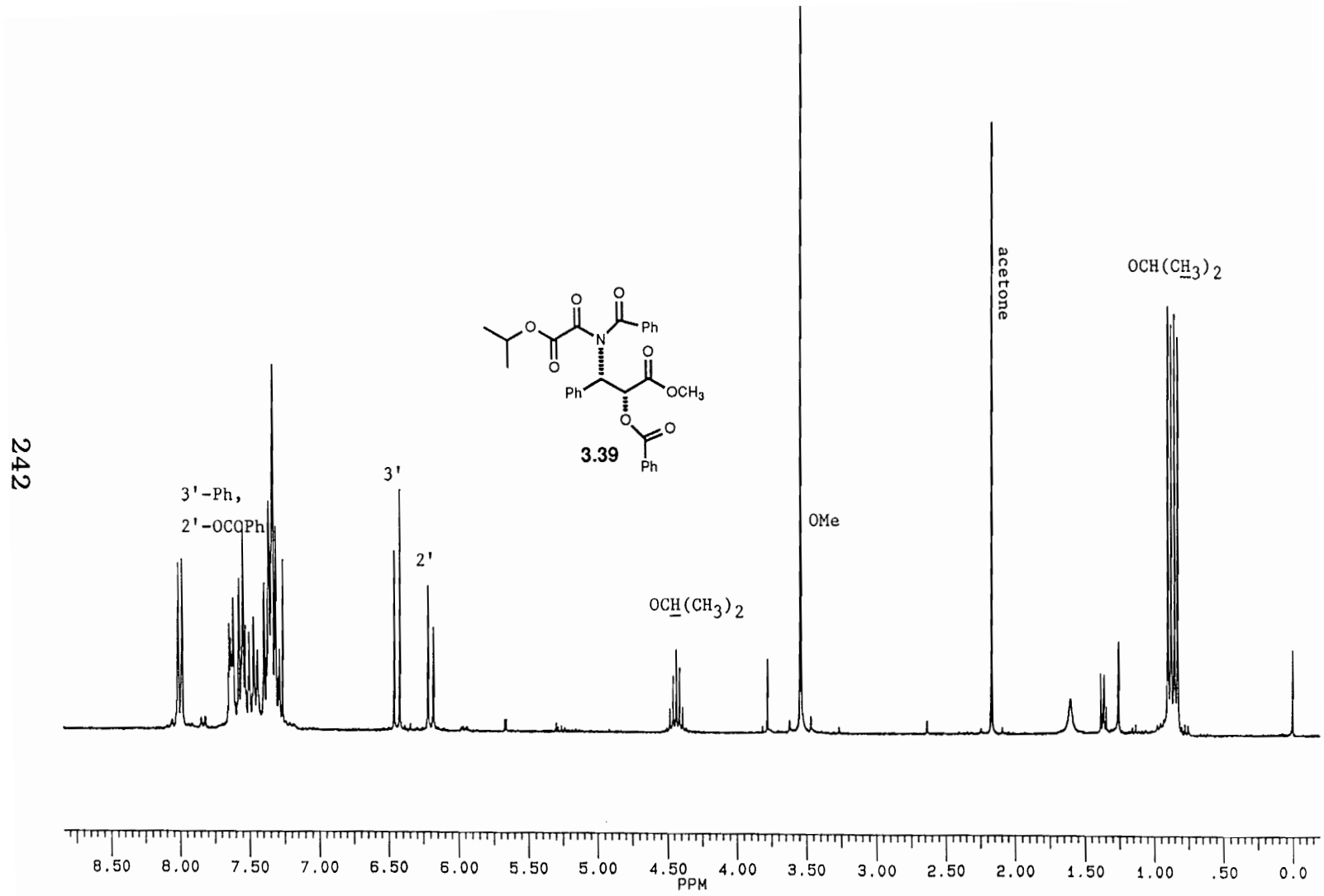
3.31

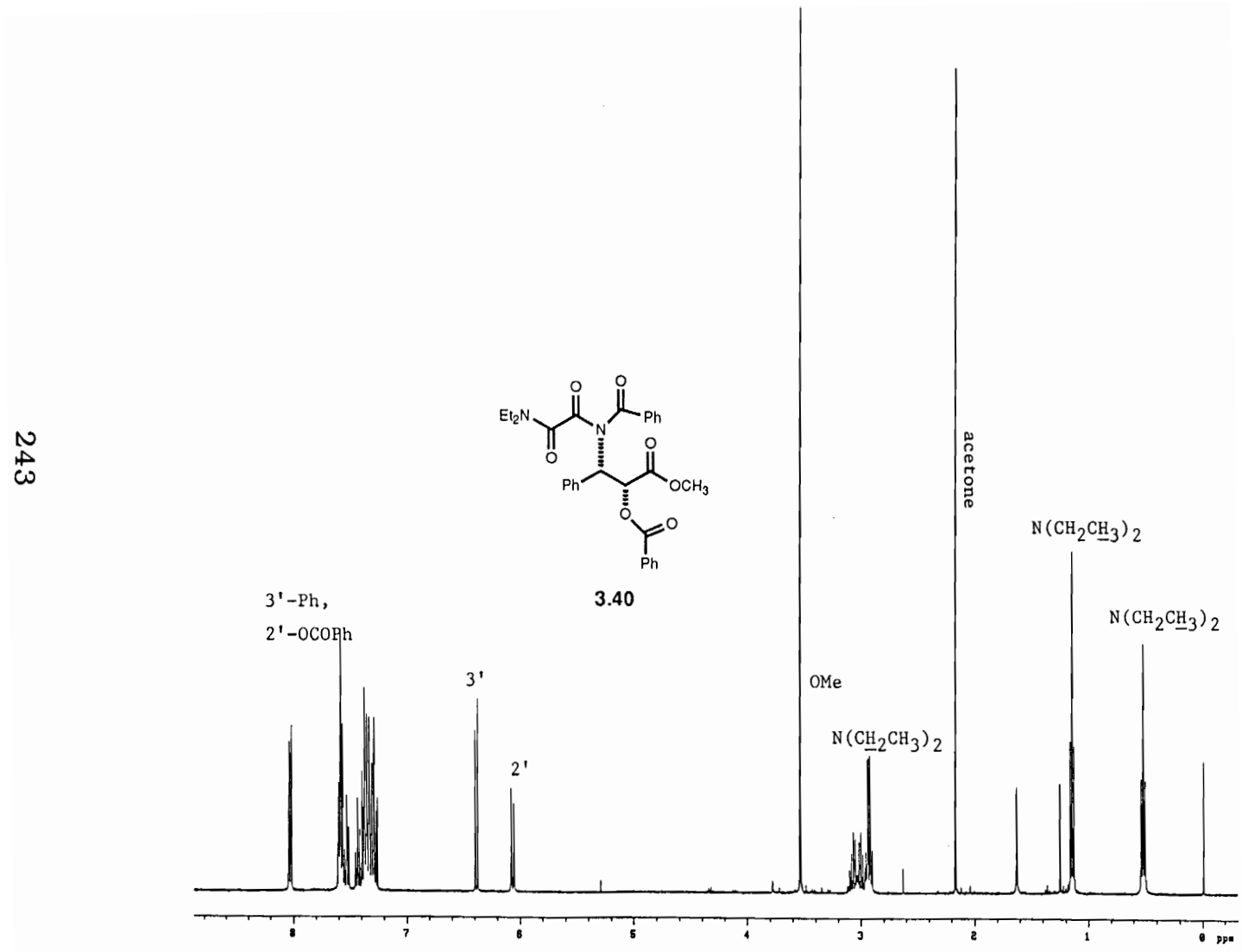

3'-Ph,
2'-OCOPh
3'
2'
OMe
INTEGRAL
4.6028
.2970
.2845
.8216
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

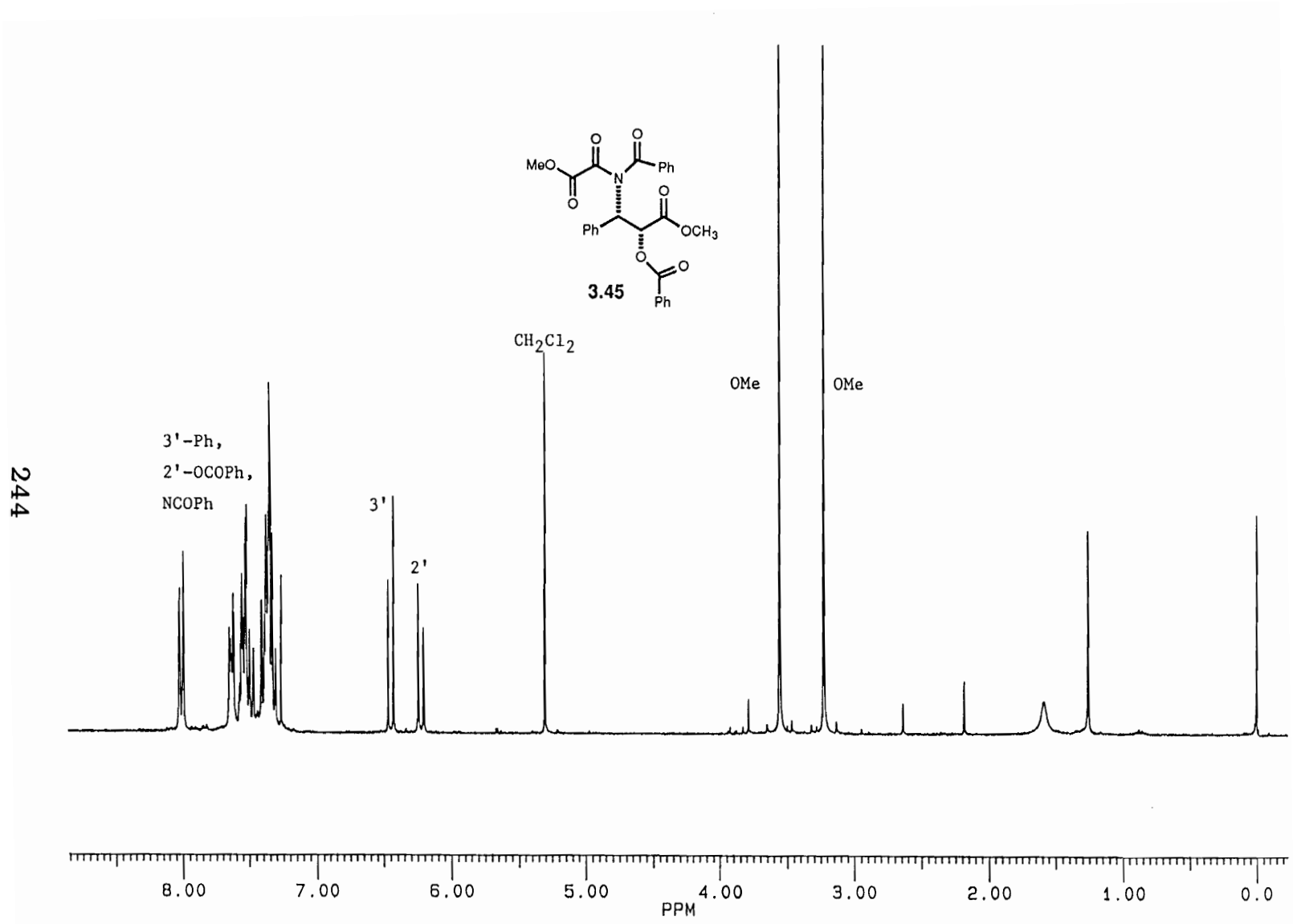
3.38

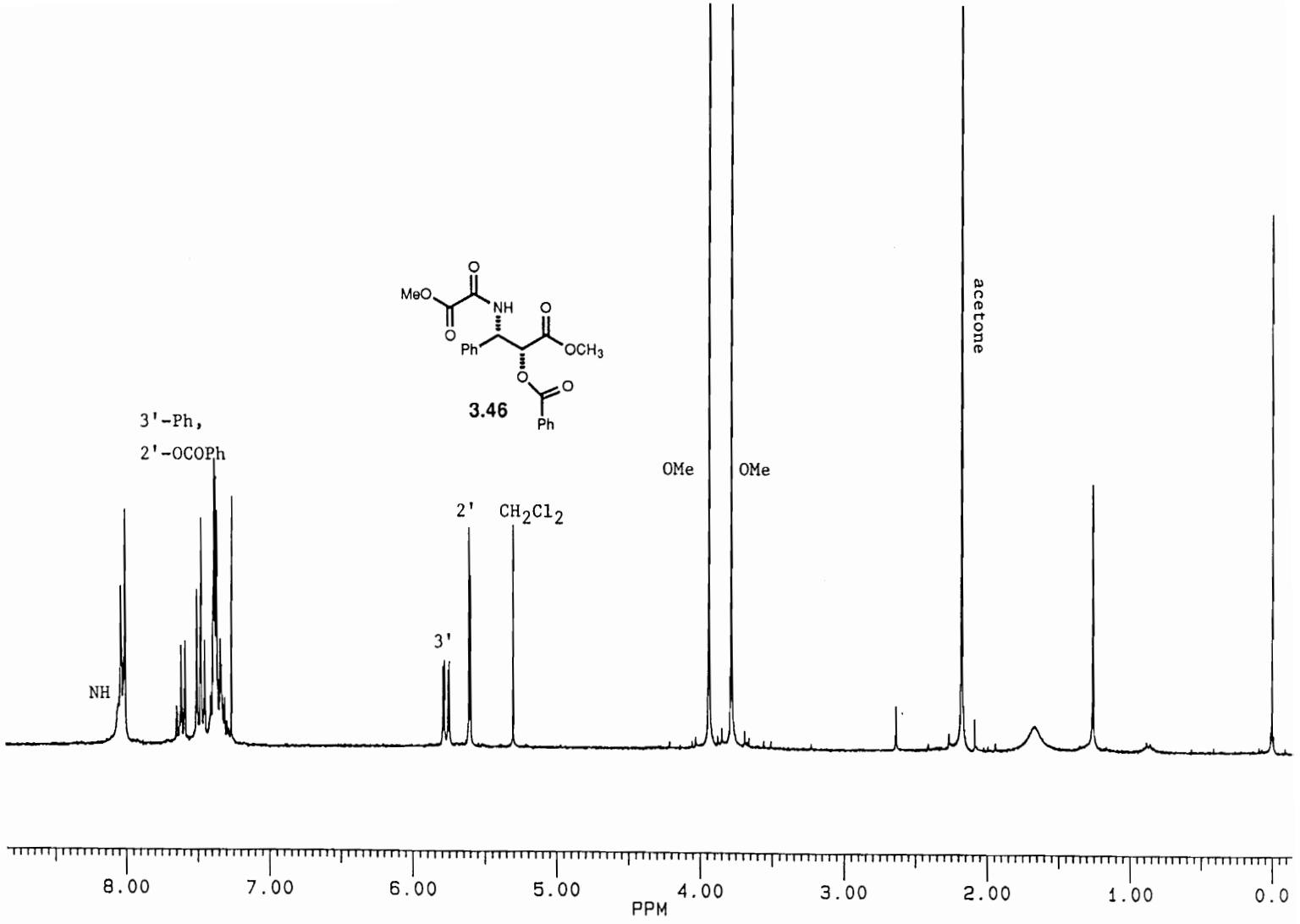

3'-Ph,
2-OCOPh
NH
10
13
2
3'
5
2'
7
20
OMe
3
2'-OH
6
4-OAc
10-OAc
18
19
17
16
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

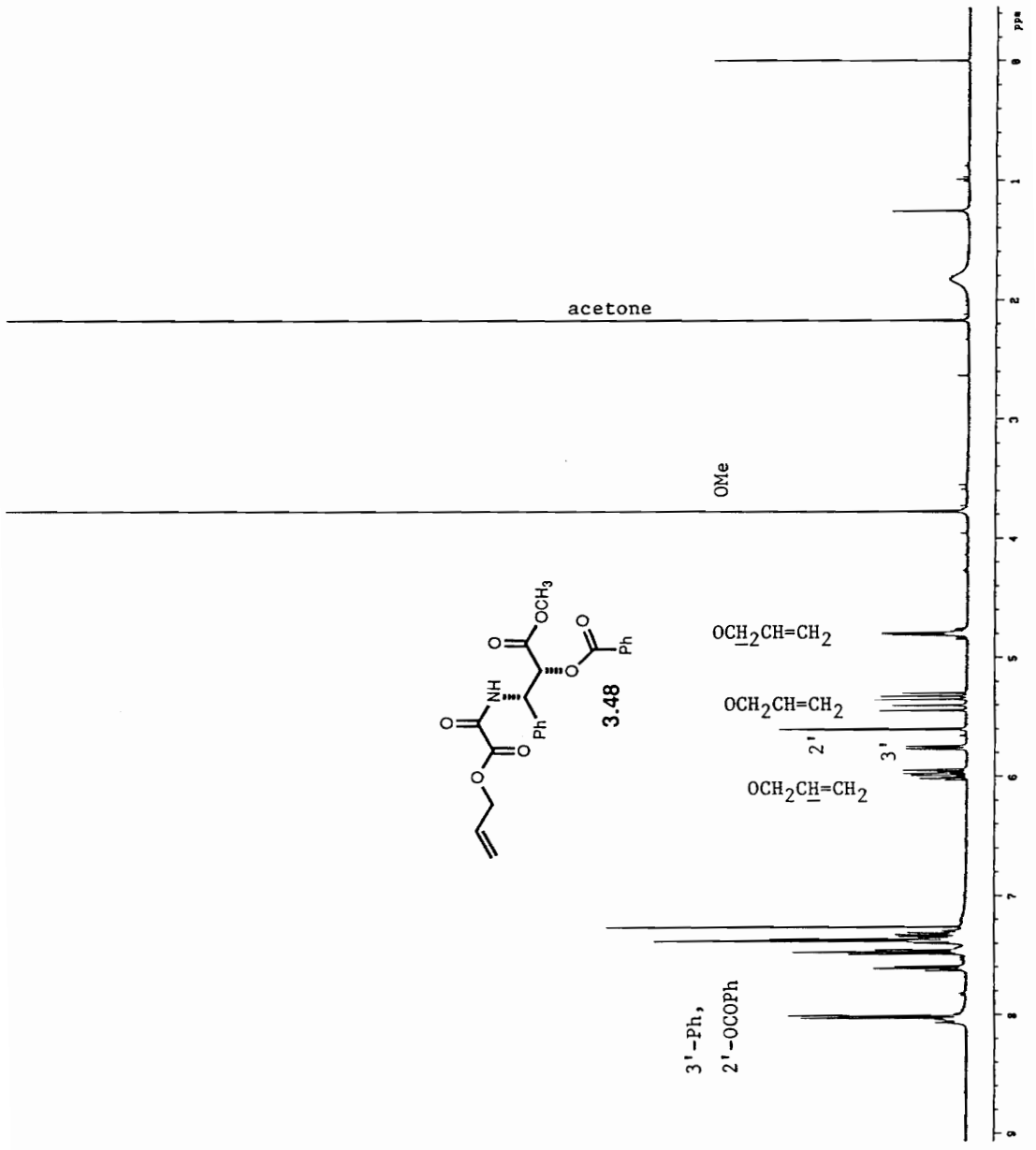
3.37

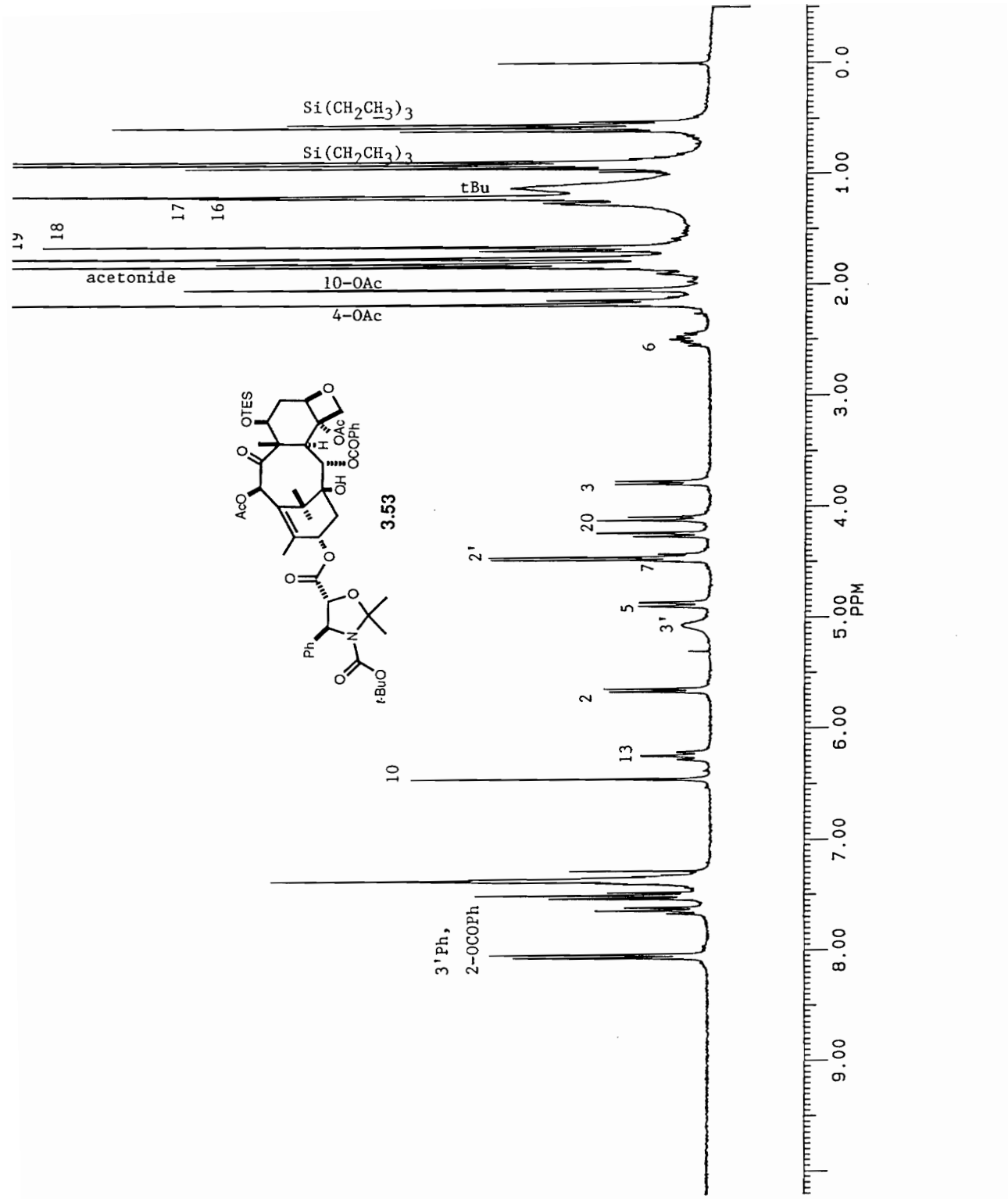

3'-Ph,
2'-OCOPh
3'
2'
OCH(CH3)2
OMe
acetone
OCH(CH3)2
3.39
8.50
8.00
7.50
7.00
6.50
6.00
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
.50
0.0
PPM

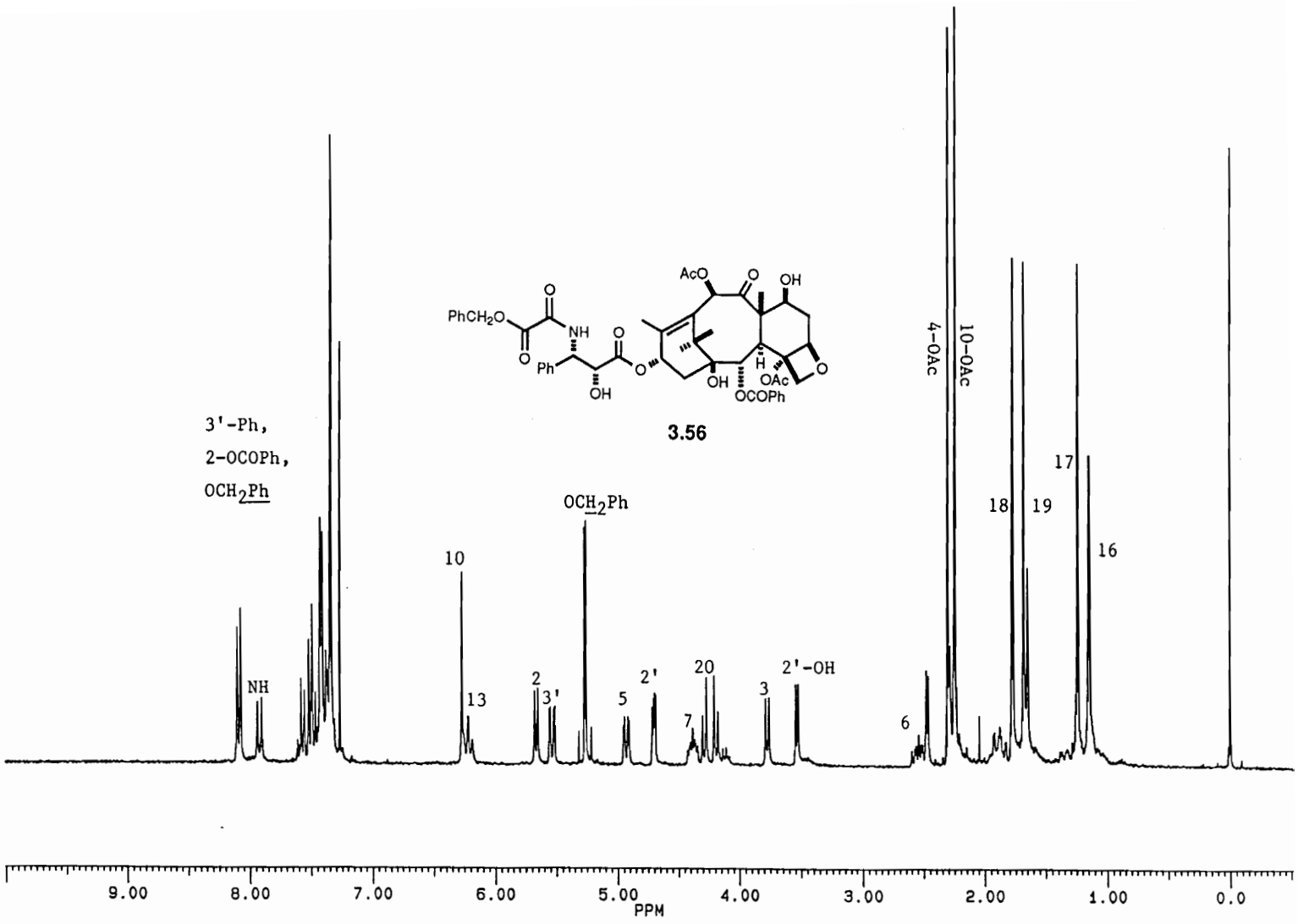

Et2N
Ph
OCH3
Ph
Ph
3.40
3'-Ph,
2'-OCOPh
3'
2'
OMe
N(CH2CH3)2
acetone
N(CH2CH3)2
N(CH2CH3)2
8
7
6
5
4
3
2
1
0 ppm


MeO
O
O
N
O
Ph
Ph
O
OCH3
O
O
Ph
3.45
CH2Cl2
OMe
OMe
3'-Ph,
2'-OCOPh,
NCOPh
3'
2'
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

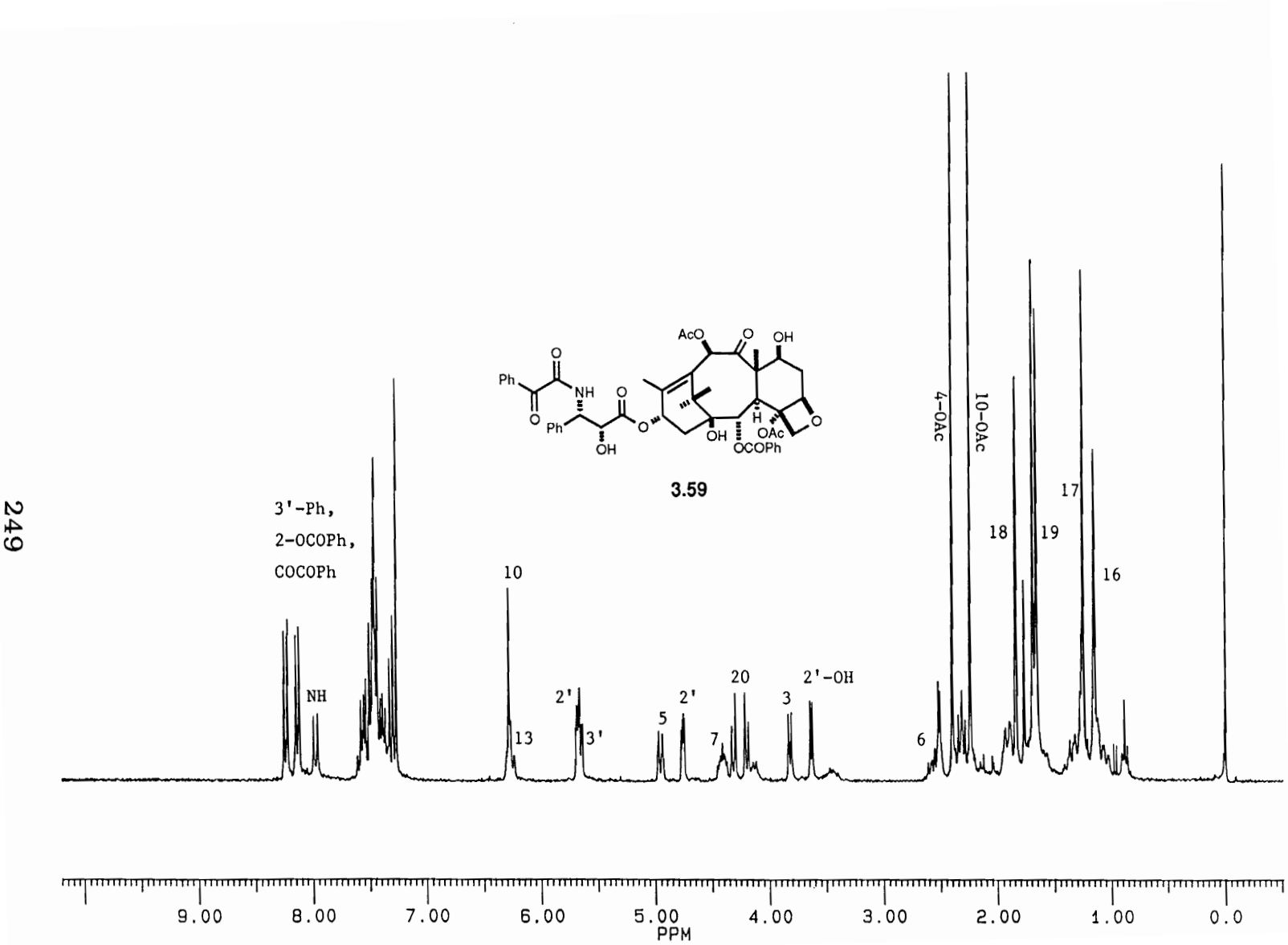

MeO
NH
Ph
OCH3
Ph
3.46
3'-Ph,
2'-OCOPh
NH
3'
2'
CH2Cl2
OMe
OMe
acetone
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM


acetone
OMe
OCH2CH=CH2
OCH2CH=CH2
2'
3'
OCH2CH=CH2
3'-Ph,
2'-OCOPh
3.48

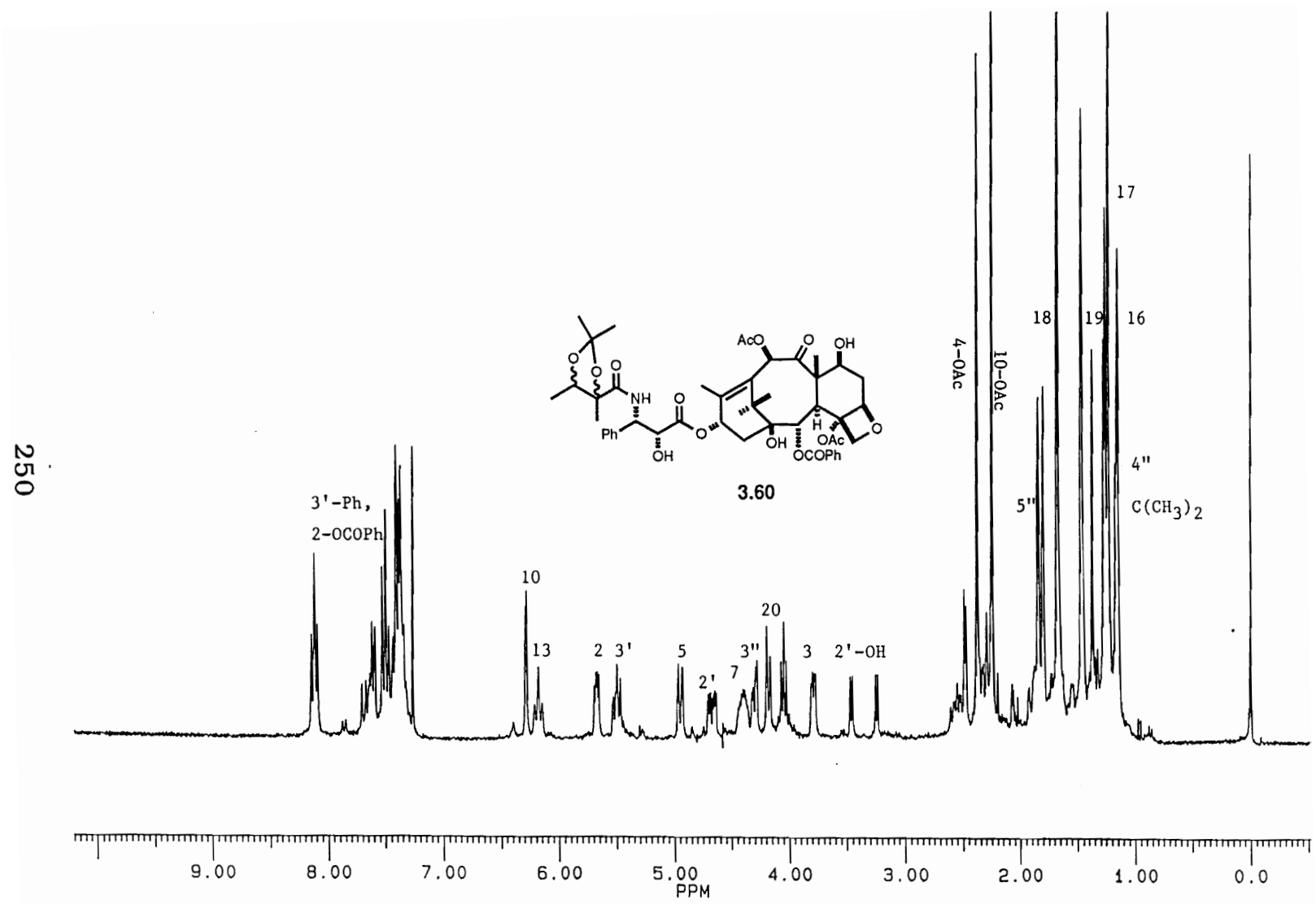

Si(CH2CH3)3
Si(CH2CH3)3
tBu
17
16
18
acetonide
10-OAc
4-OAc
6
20
3
2'
7
5
3'
2
13
10
3'Ph,
2-OCOPh
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM
OTES
OAc
OCOPh
AcO
OH
Ph
tBuO
3.53


3'-Ph,
2-OCOPh,
OCH2Ph
NH
10
13
2
3'
OCH2Ph
5
2'
7
20
3
2'-OH
6
4-OAc
10-OAc
18
19
17
16
9.00
8.00
7.00
6.00
5.00
PPM
4.00
3.00
2.00
1.00
0.0

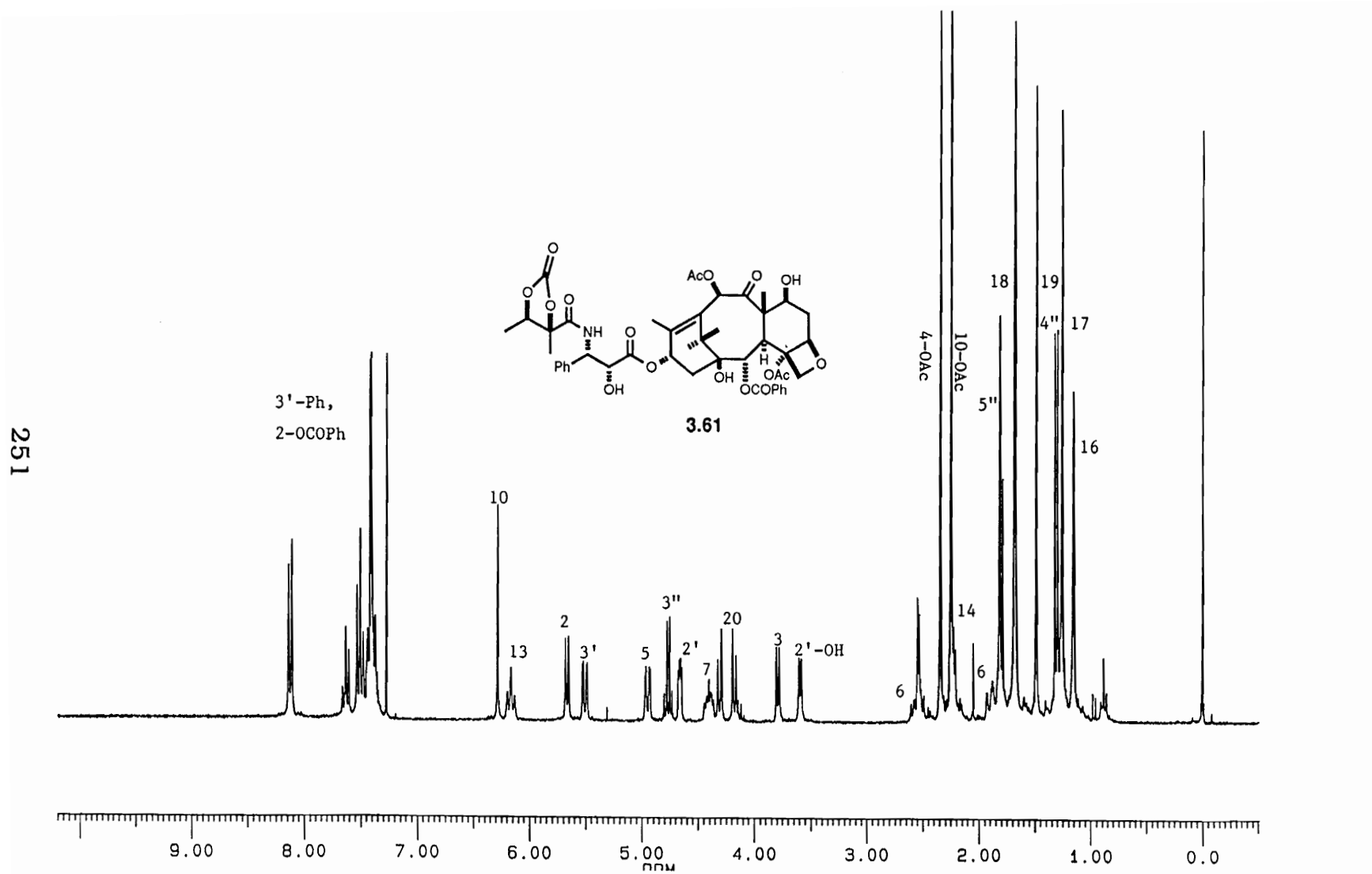
3.56


3'-Ph,
2-OCOPh,
COCOPh
NH
10
13
2'
3'
5
2'
7
20
3
2'-OH
6
4-OAc
10-OAc
18
19
17
16
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

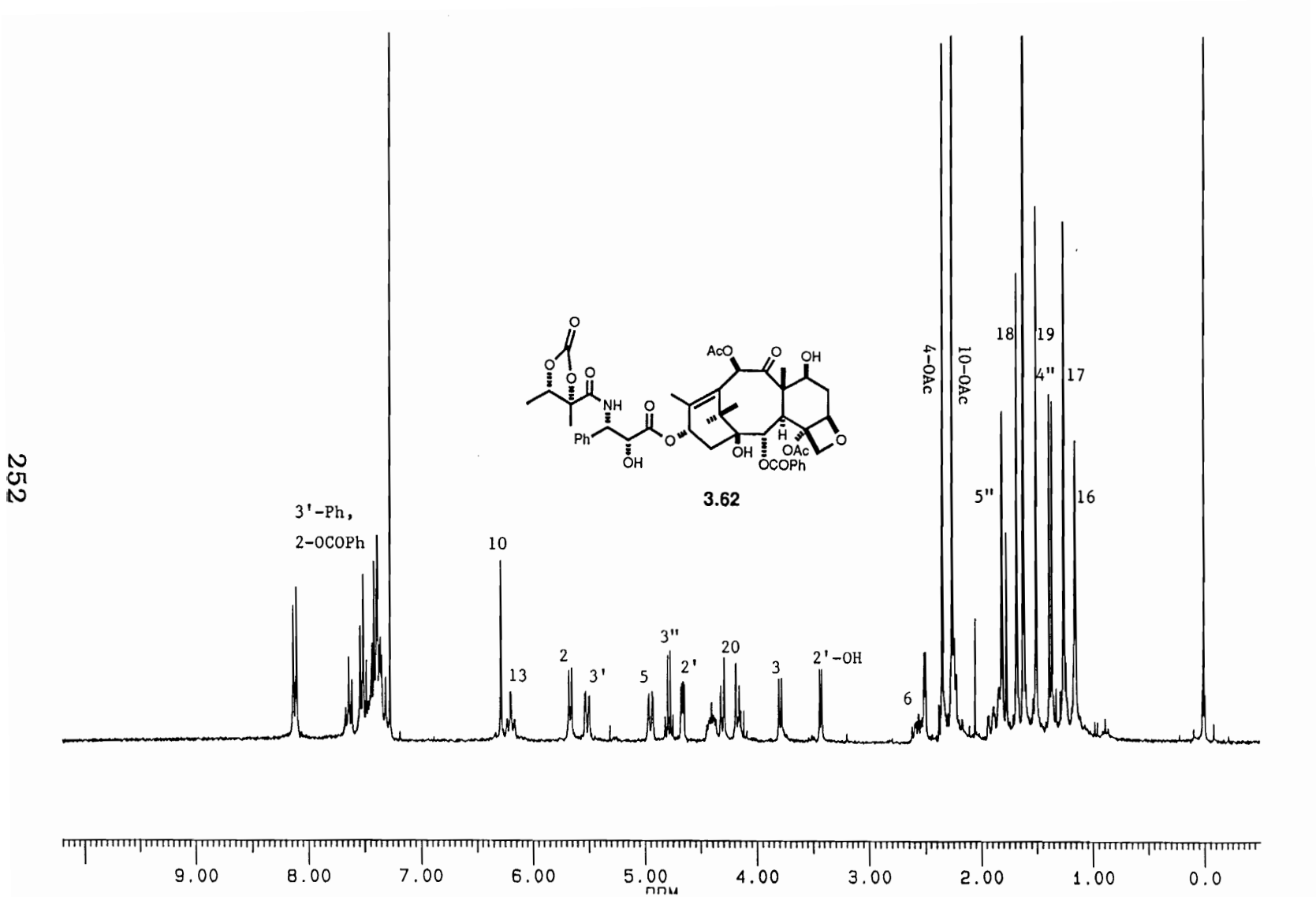
3.59


3'-Ph,
2-OCOPh
10
13
2
3'
5
2'
7
3"
20
3
2'-OH
4-OAc
10-OAc
18
19
17
16
5"
4"
C(CH3)2
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

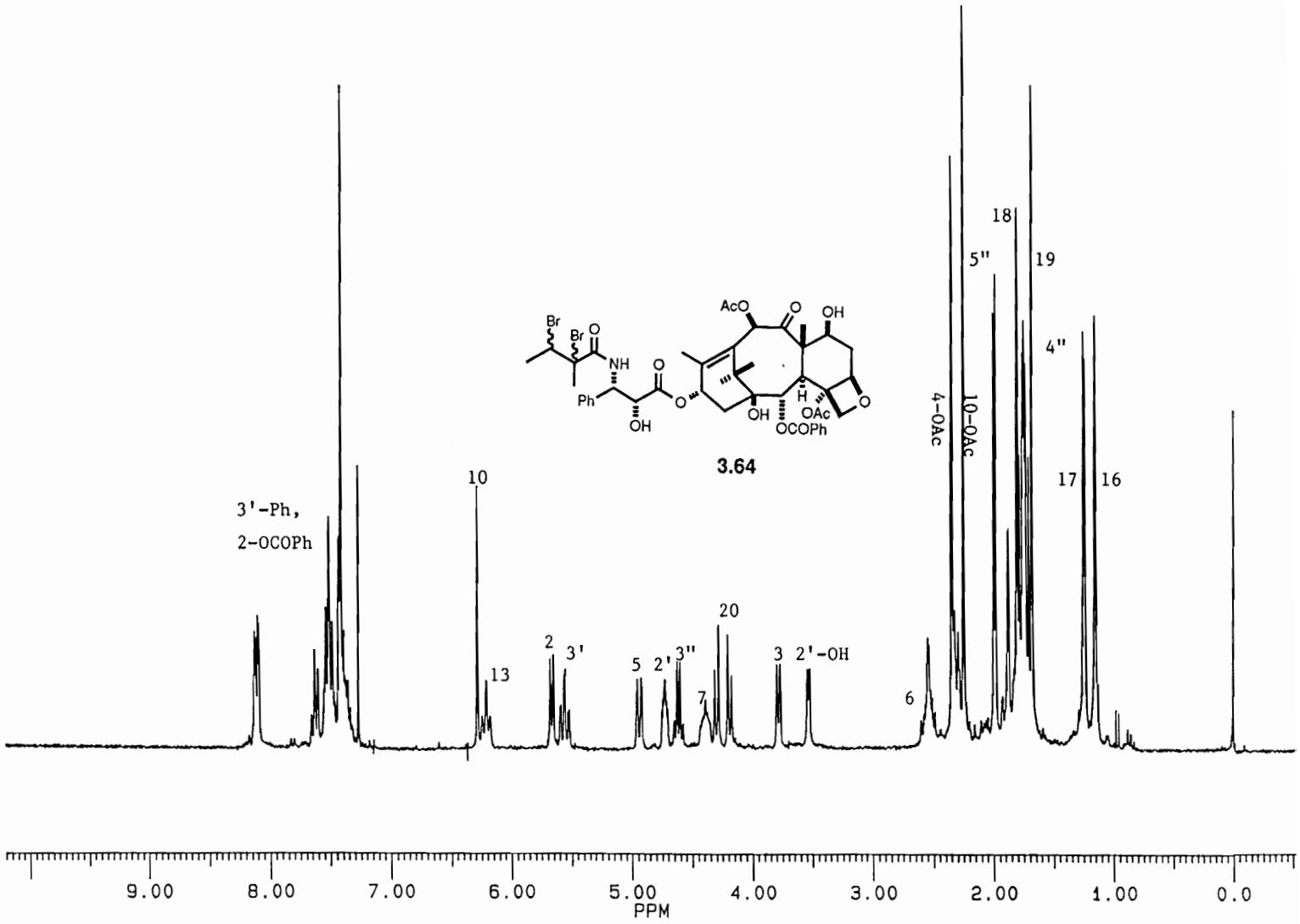
3.60


3'-Ph,
2-OCOPh
10
13
2
3'
5
3"
2'
7
20
3
2'-OH
6
4-OAc
10-OAc
14
6
18
5"
19
4"
17
16
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

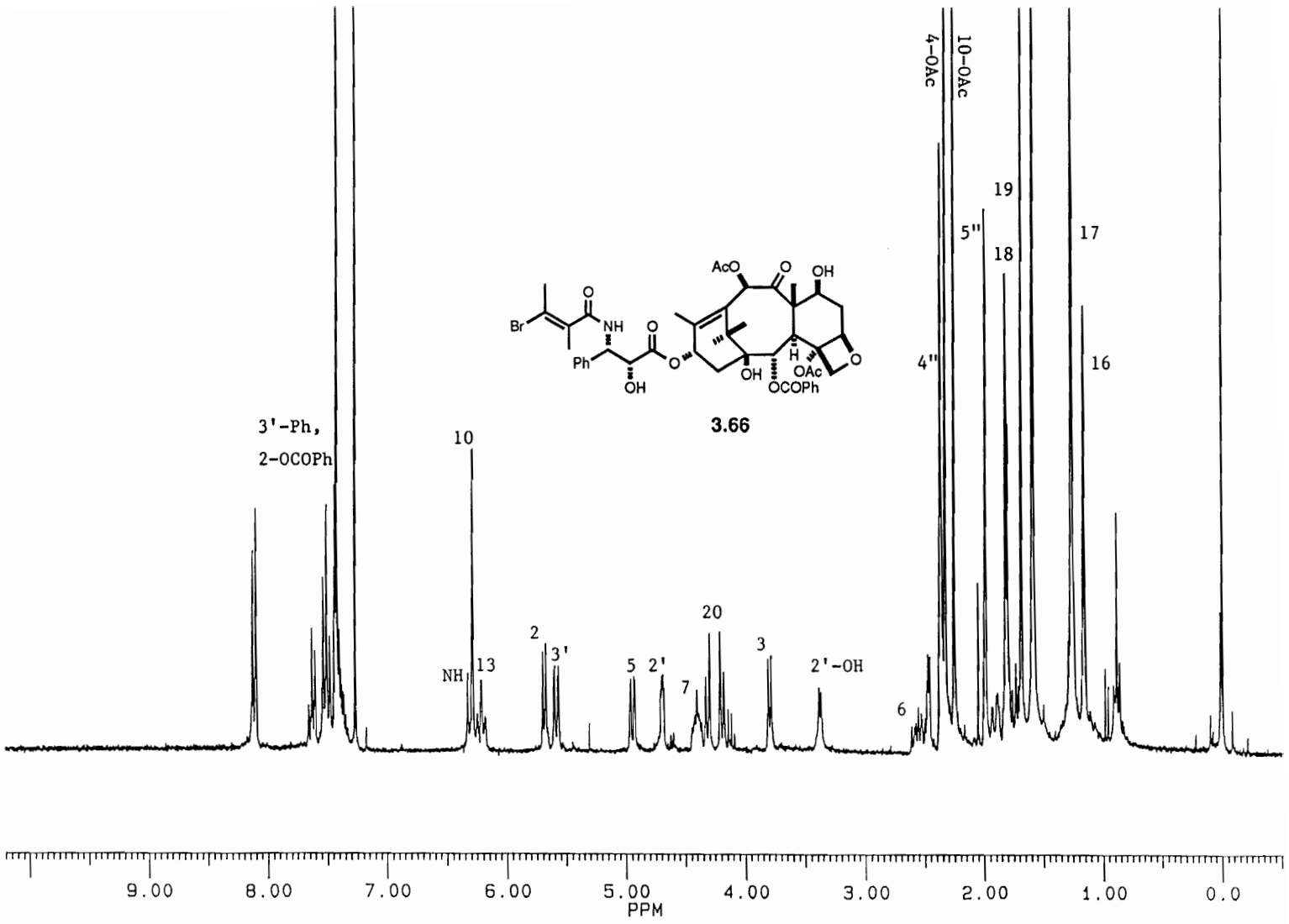
3.61


3'-Ph,
2-OCOPh
10
13
2
3'
5
3"
2'
20
3
2'-OH
6
4-OAc
10-OAc
18
19
4"
17
5"
16
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

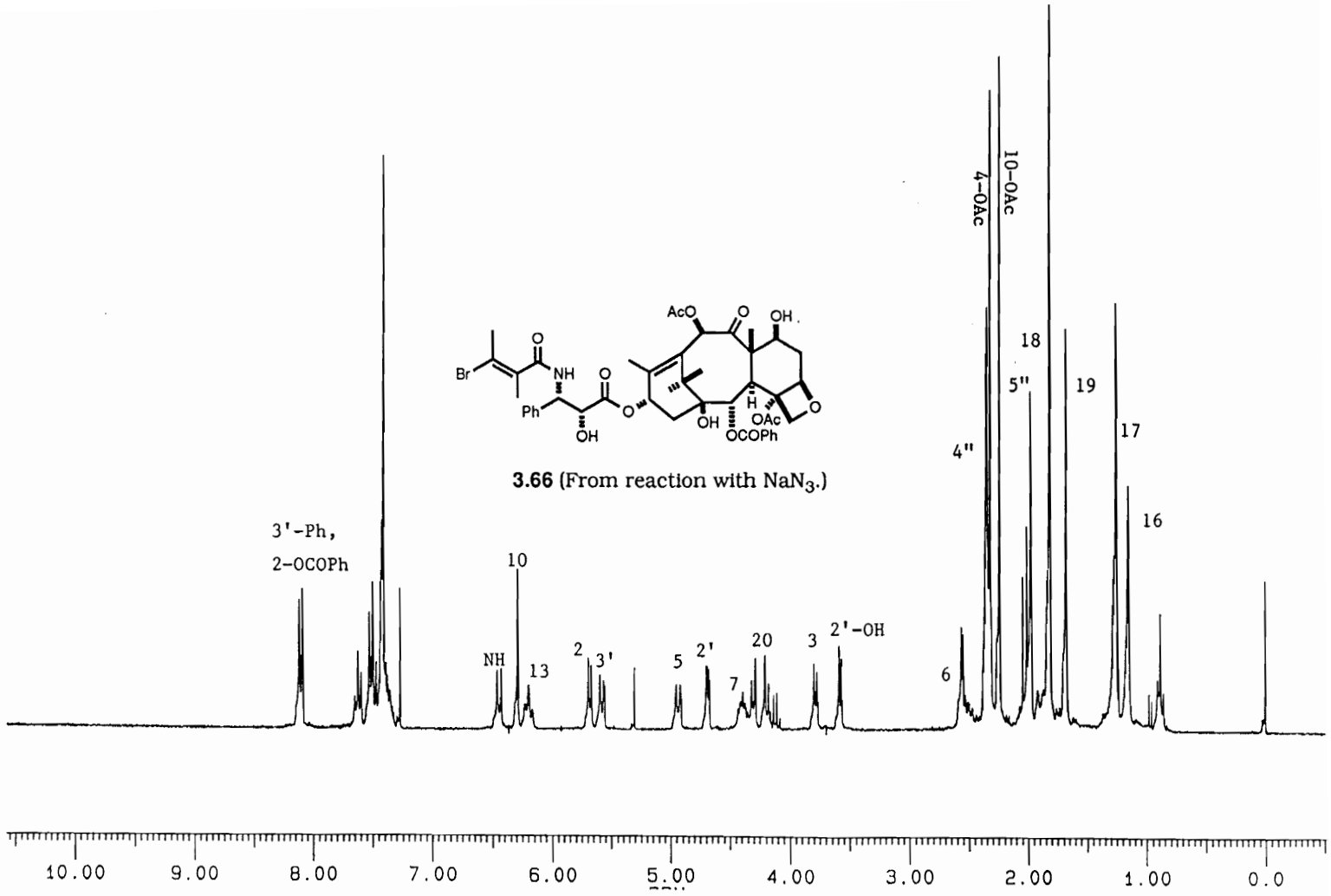
3.62


3'-Ph,
2-OCOPh
10
13
2
3'
5
2'
3"
7
20
3
2'-OH
6
4-OAc
10-OAc
5"
18
19
4"
17
16
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

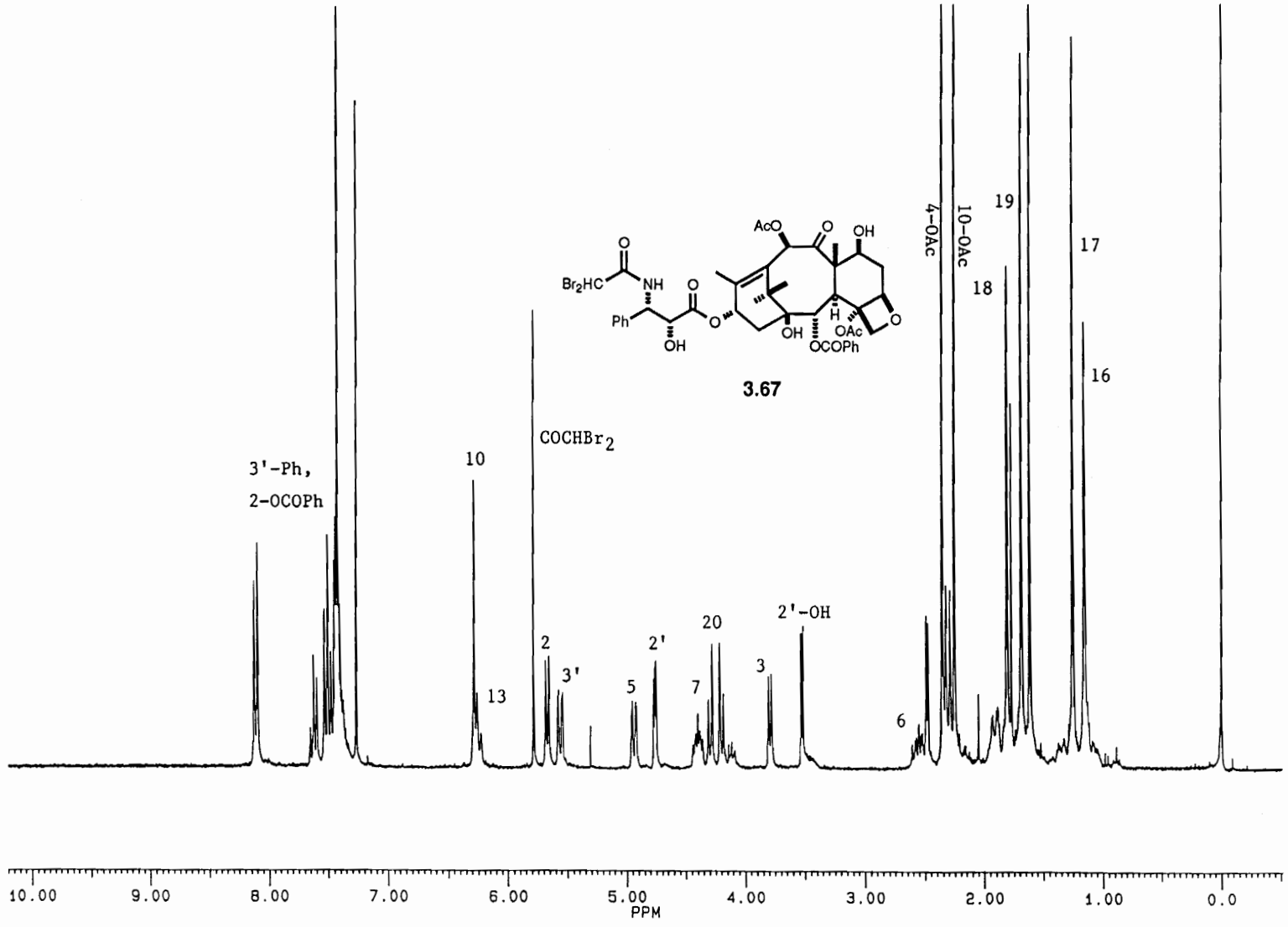
3.64


4-OAc
10-OAc
19
5"
18
17
4"
16
3'-Ph,
2-OCOPh
10
NH
13
2
3'
5
2'
7
20
3
2'-OH
6
9.00
8.00
7.00
6.00
5.00
PPM
4.00
3.00
2.00
1.00
0.0

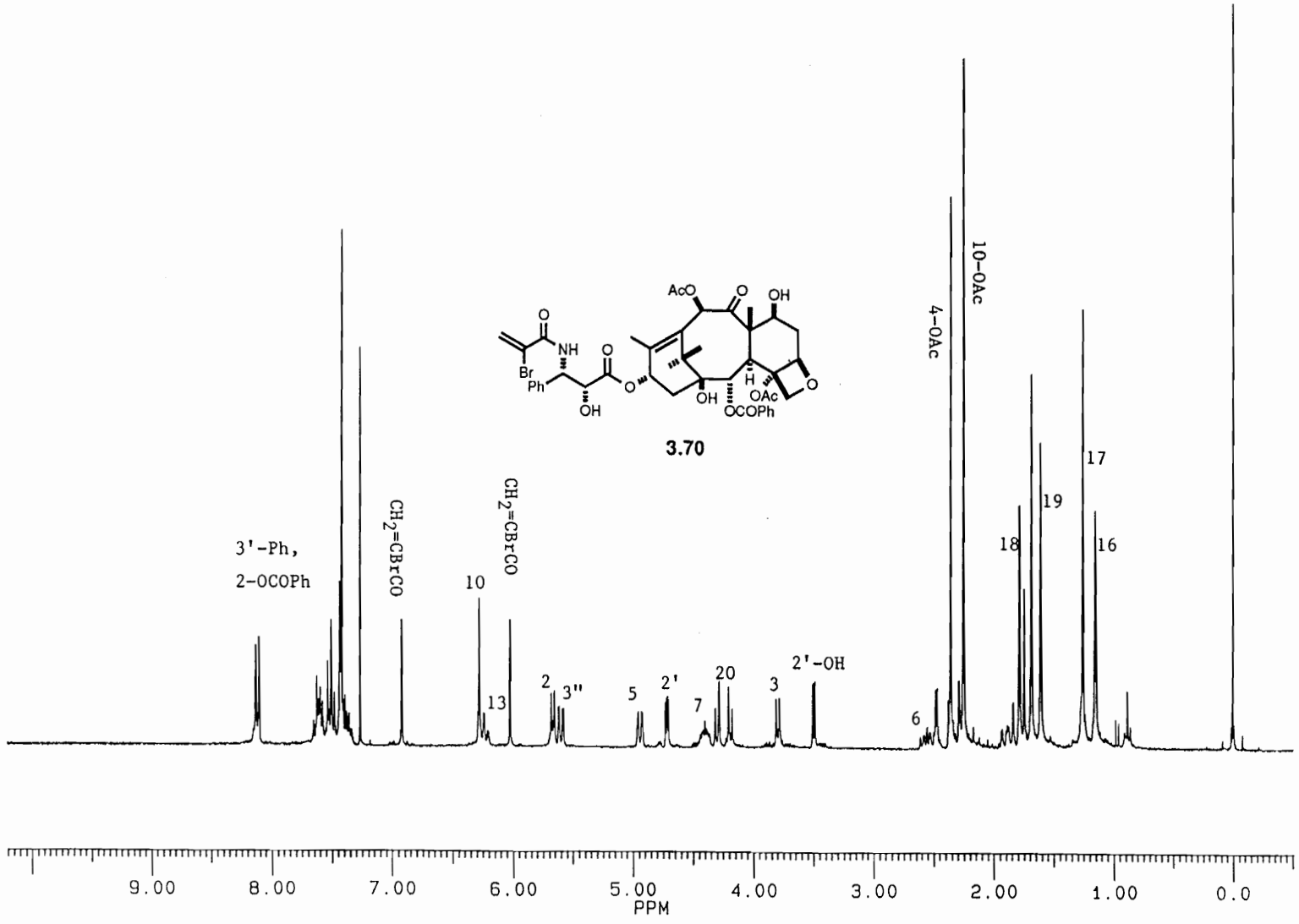
3.66



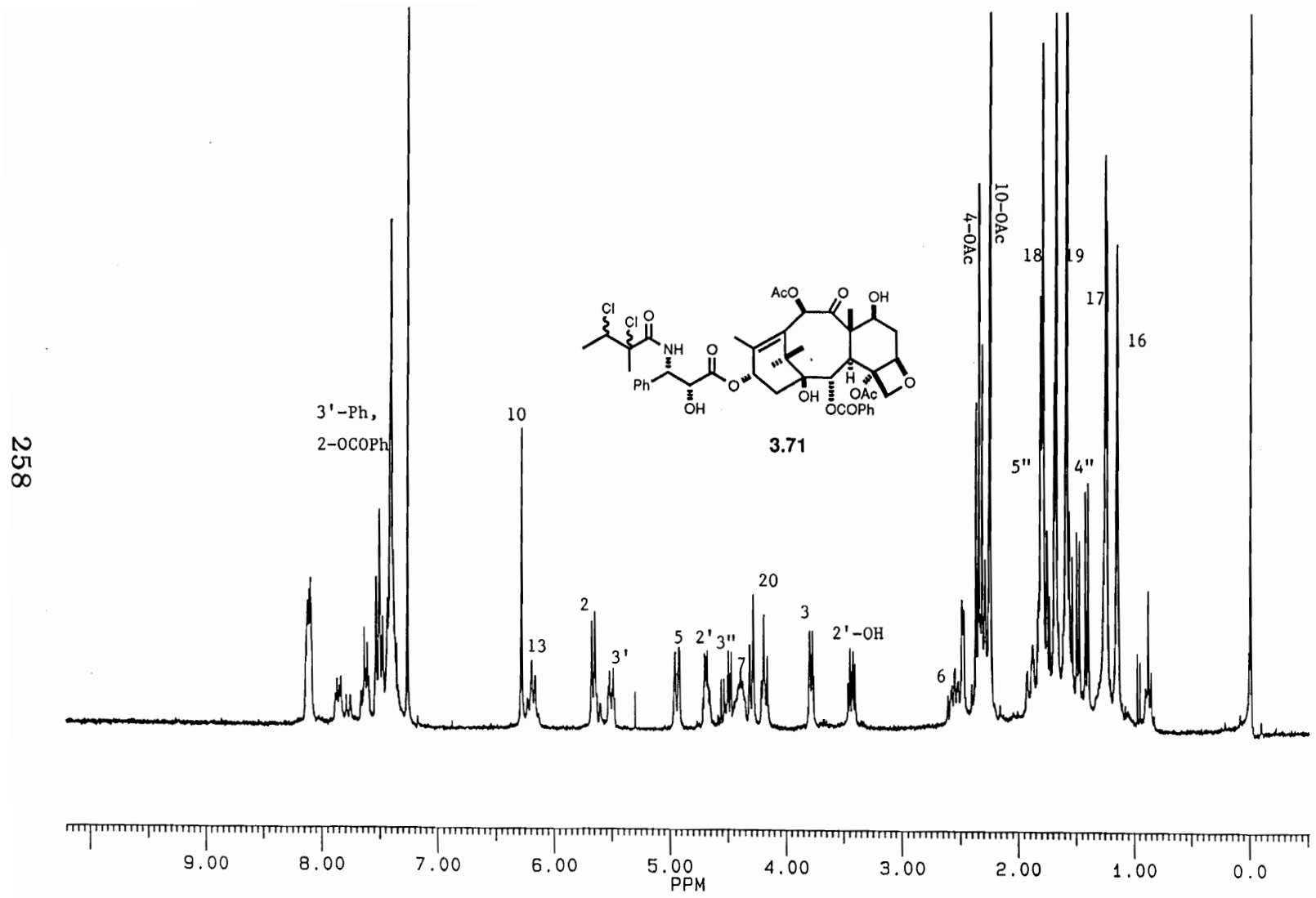
3.66 (From reaction with NaN_3.)


3'-Ph,
2-OCOPh
10
13
COCHBr2
2
3'
5
2'
7
20
3
2'-OH
6
4-OAc
10-OAc
19
18
17
16
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

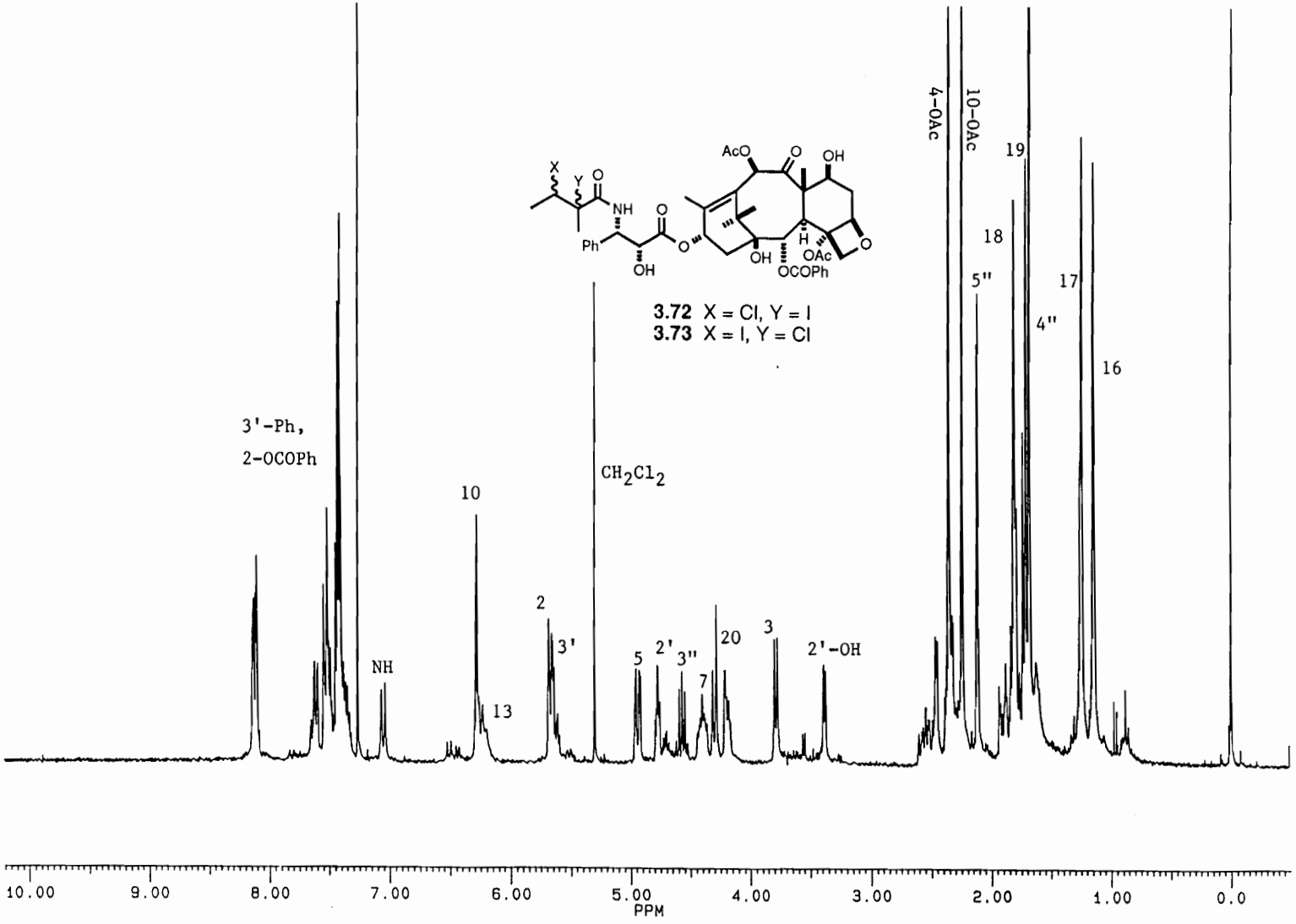
3.67


3'-Ph,
2-OCOPh
CH2=CBrCO
CH2=CBrCO
10
13
2
3"
5
2'
7
20
3
2'-OH
6
4-OAc
10-OAc
18
19
17
16
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

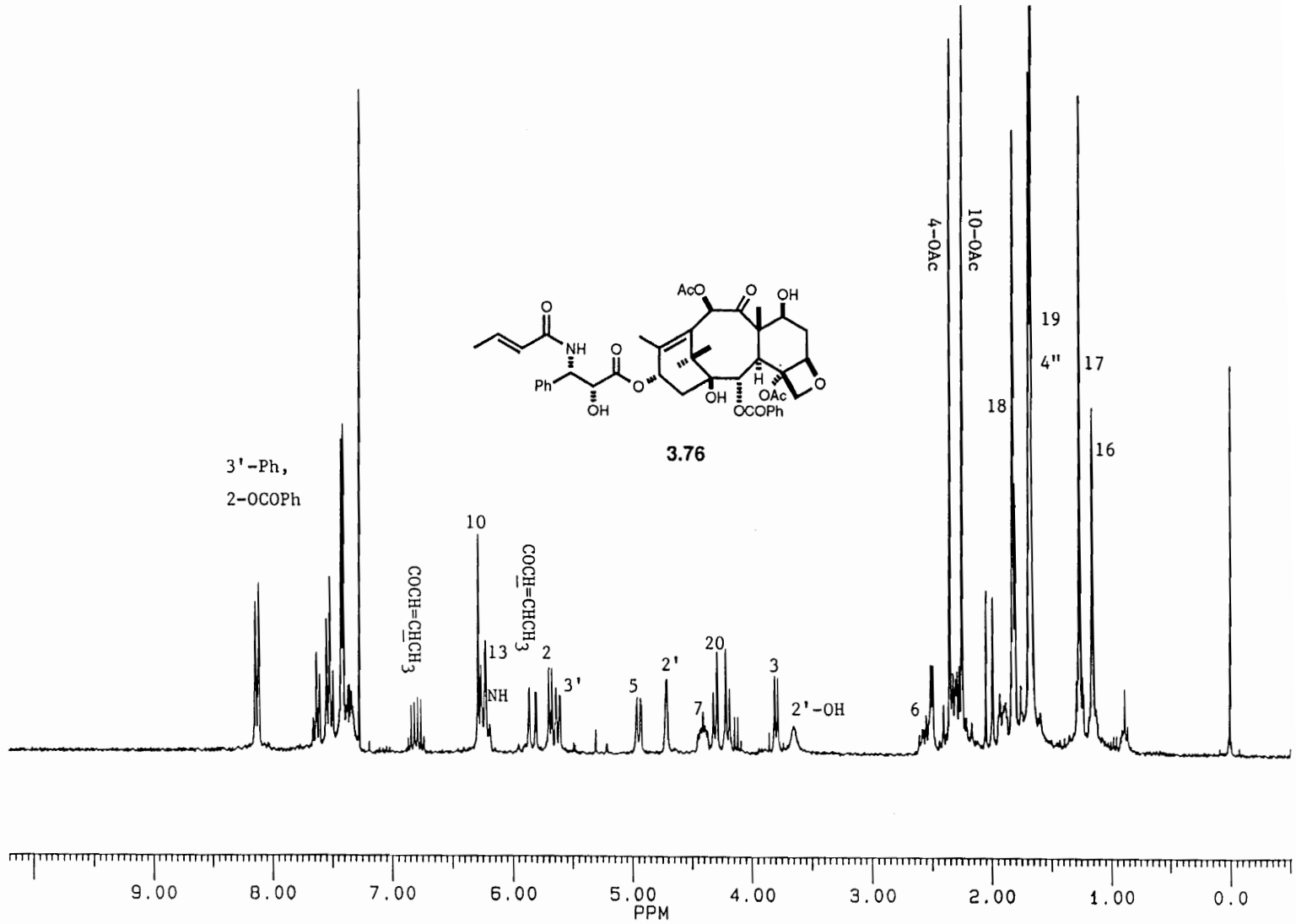
3.70


3'-Ph,
2-OCOPh
10
13
2
3'
5
2'
3"
7
20
3
2'-OH
6
4-OAc
10-OAc
18
19
17
16
5"
4"
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

3.71

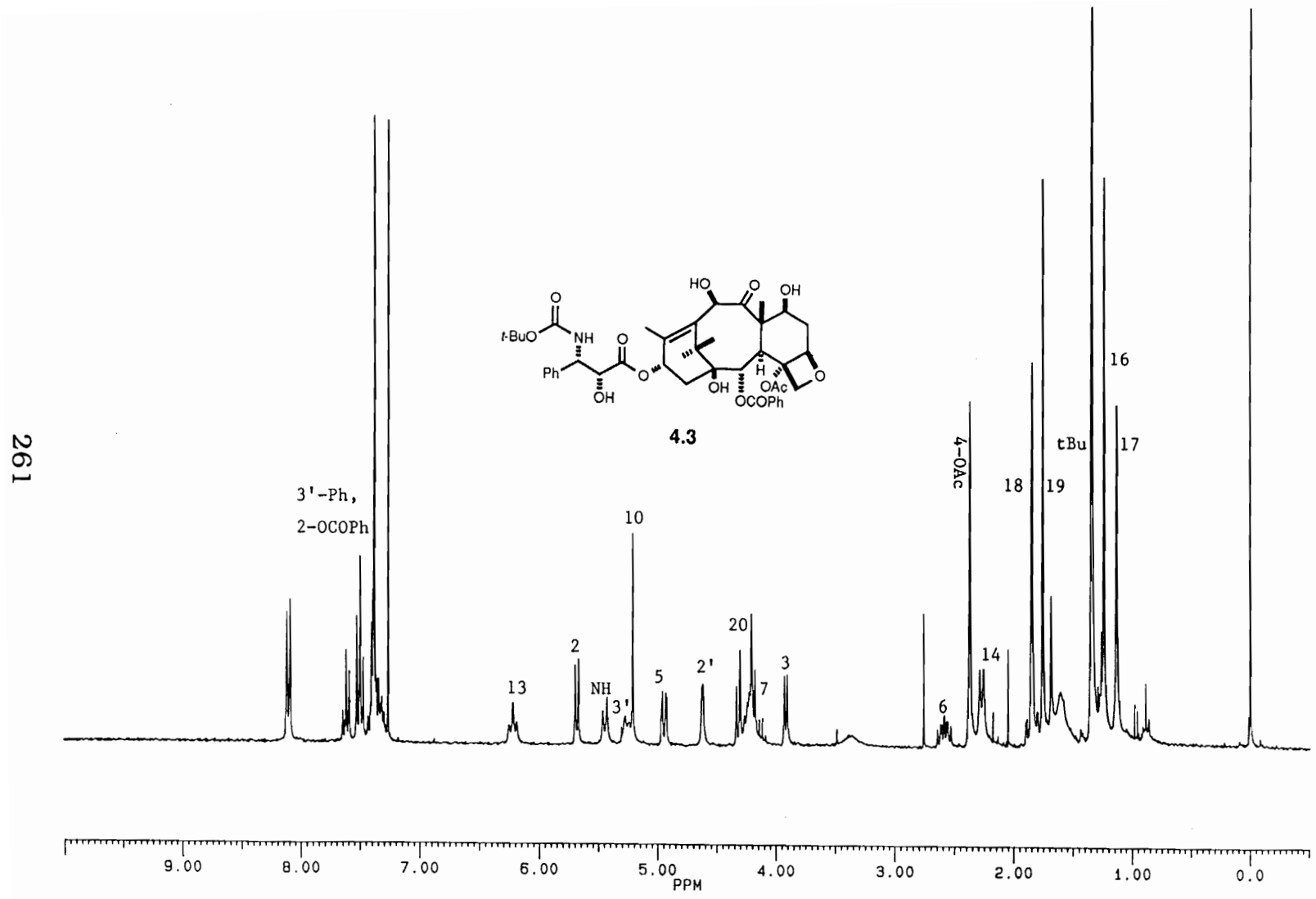


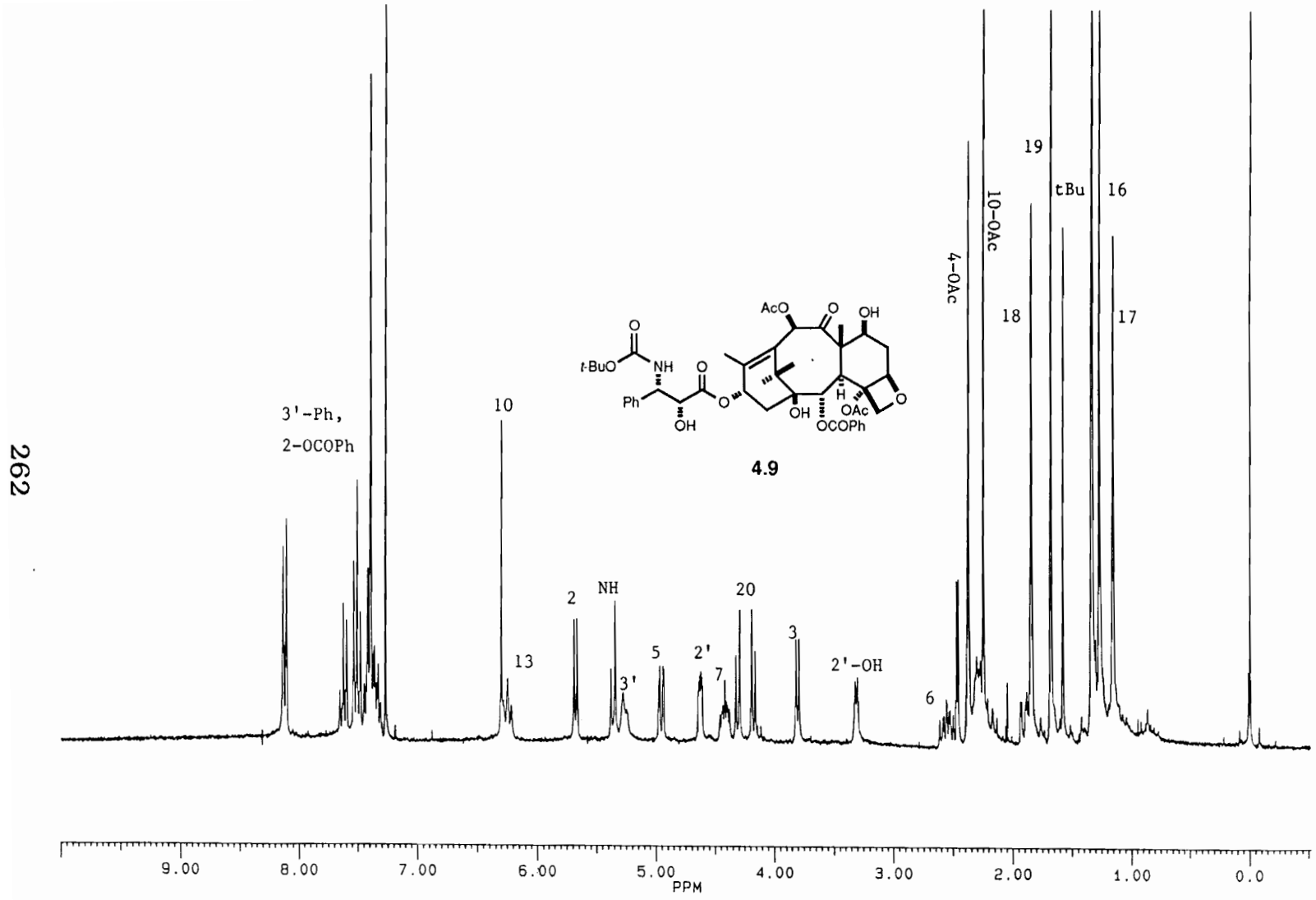
3.72 X = Cl, Y = I
3.73 X = I, Y = Cl


3'-Ph,
2-OCOPh
COCH=CHCH3
10
13
NH
COCH=CHCH3
2
3'
5
2'
7
20
3
2'-OH
6
4-OAc
10-OAc
19
4"
17
18
16
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

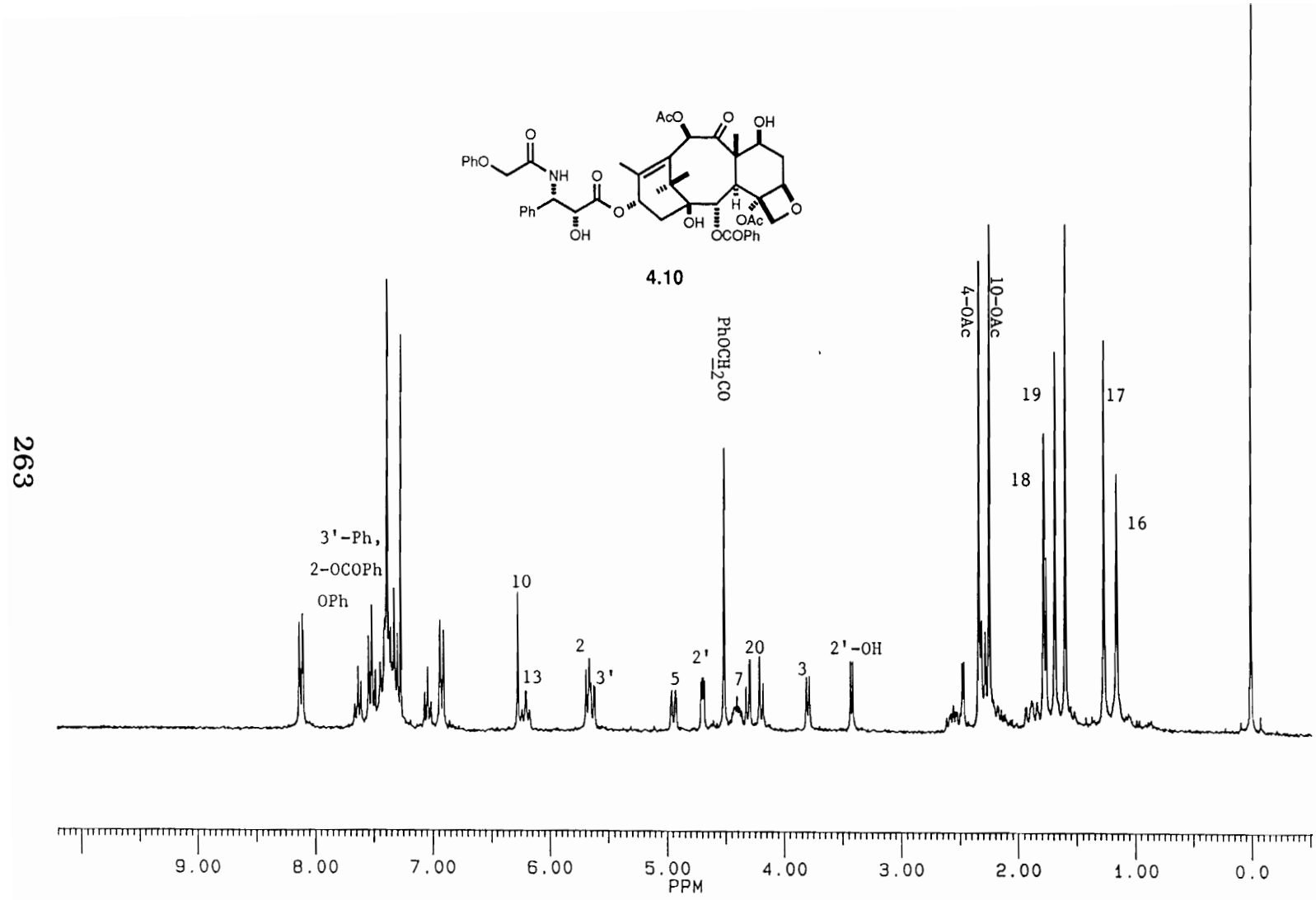
AcO
O
OH
NH
O
Ph
OH
O
H
OH
OAc
OCOPh

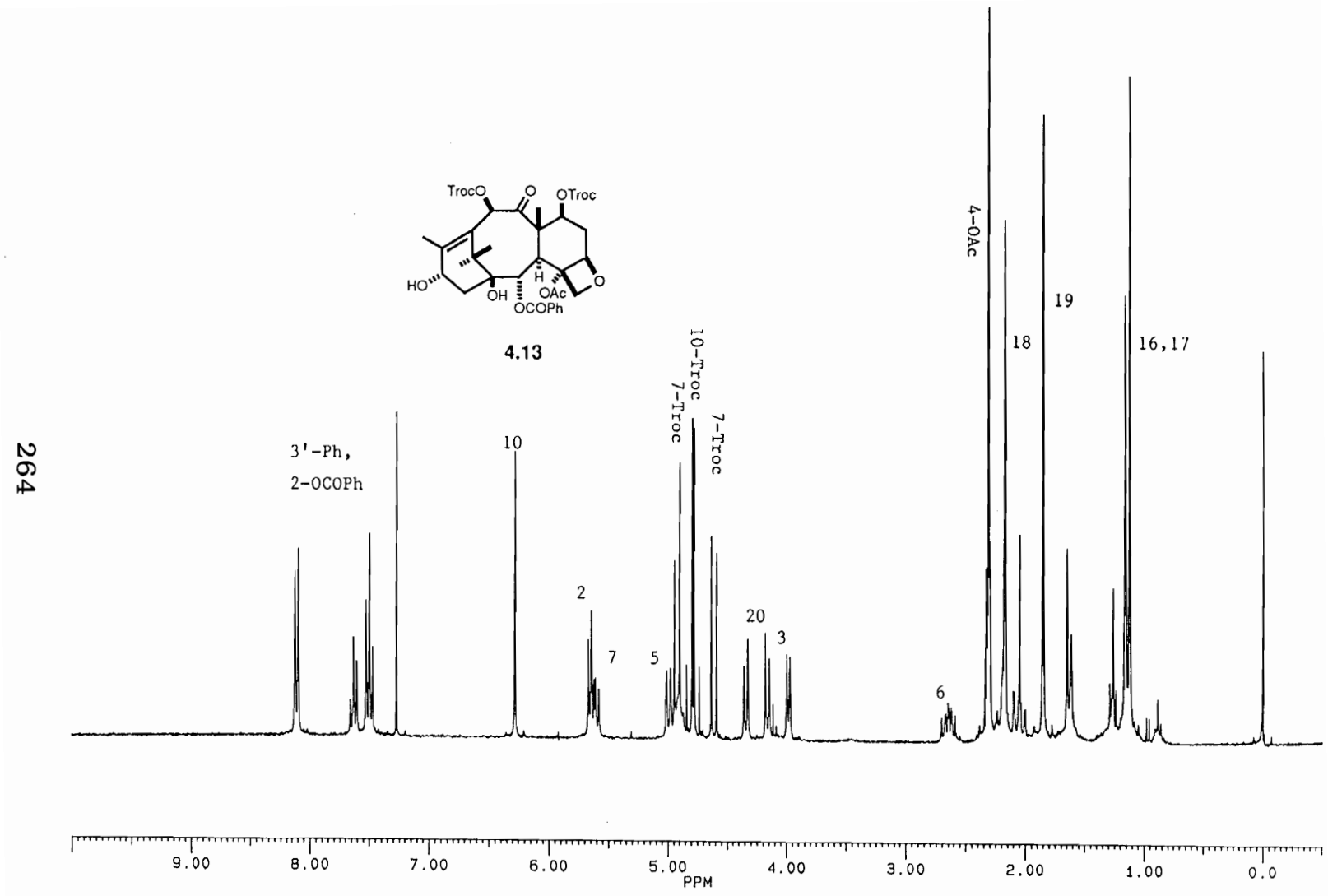
3.76

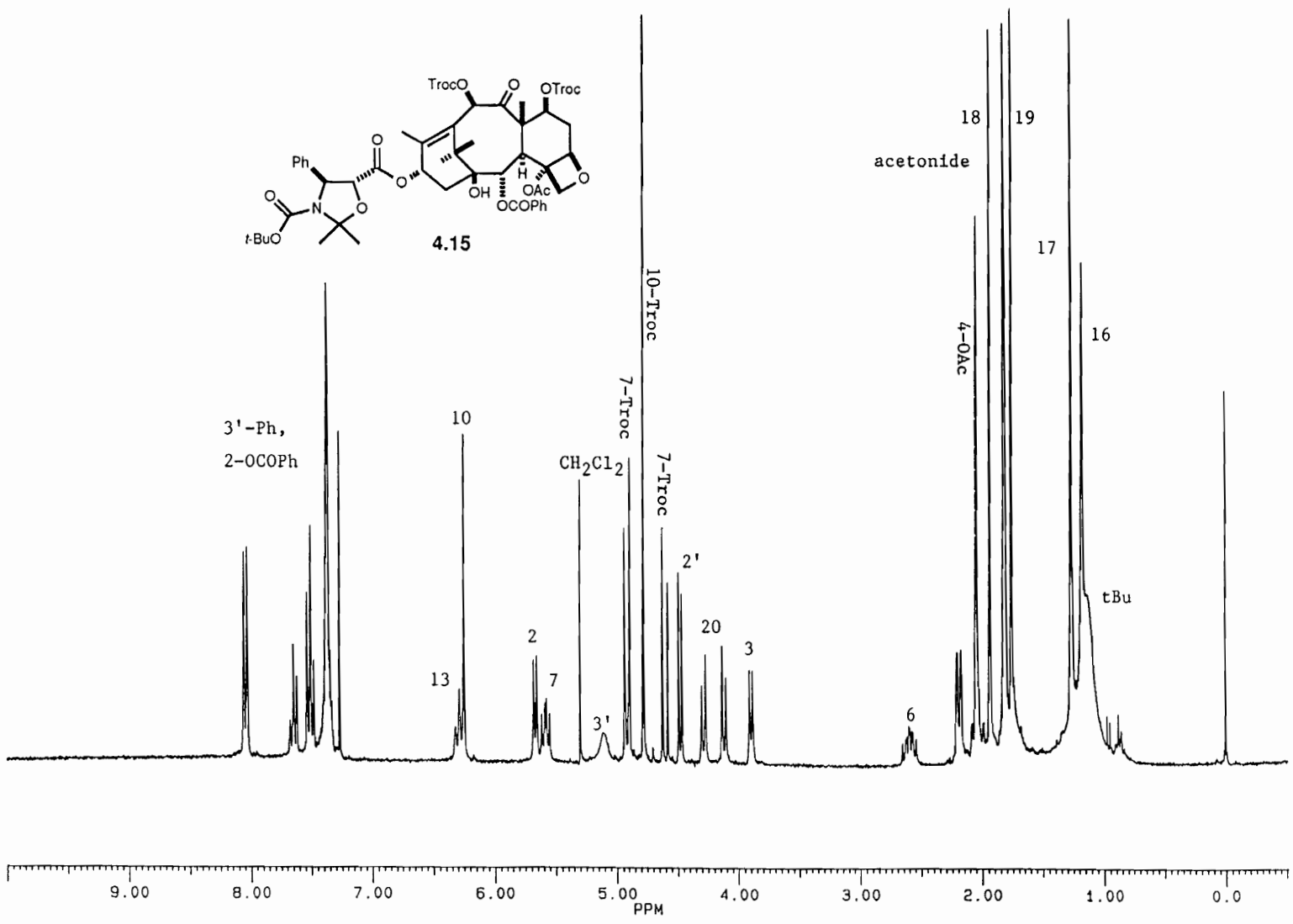

t-BuO
NH
Ph
OH
HO
OH
OAc
OCOPh
4.3
3'-Ph,
2-OCOPh
10
2
13
NH
3'
5
2'
20
7
3
6
4-OAc
14
18
19
tBu
16
17
9.00
8.00
7.00
6.00
5.00
PPM
4.00
3.00
2.00
1.00
0.0

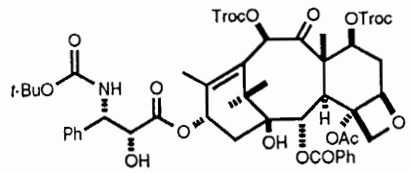

3'-Ph,
2-OCOPh
10
13
2
NH
3'
5
2'
7
20
3
2'-OH
6
4-OAc
10-OAc
18
19
tBu
16
17
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

4.9


PhO
NH
Ph
OH
AcO
OH
OAc
OCOPh
4.10
PhOCH2CO
4-OAc
10-OAc
19
17
18
16
3'-Ph,
2-OCOPh
OPh
10
13
2
3'
5
2'
7
20
3
2'-OH
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM


TrocO
O
OTroc
HO
OH
H
OAc
OCOPh
O
4.13
3'-Ph,
2-OCOPh
10
2
7
5
7-Troc
10-Troc
7-Troc
20
3
6
4-OAc
18
19
16,17
9.00
8.00
7.00
6.00
5.00
PPM
4.00
3.00
2.00
1.00
0.0


TrocO
OTroc
Ph
OH
OAc
OCOPh
t-BuO
4.15
3'-Ph,
2-OCOPh
10
13
2
7
CH_2Cl_2
7-Troc
3'
10-Troc
7-Troc
2'
20
3
6
acetonide
18
19
4-OAc
17
16
tBu
9.00
8.00
7.00
6.00
5.00
PPM
4.00
3.00
2.00
1.00
0.0


TrocO
O
OTroc
t-BuO
NH
O
Ph
OH
O
H
OH
OAc
OCOPh
O

4.17

3'-Ph,
2-OCOPh
10
13
2
NH
7
3'
5
7-Troc
10-Troc
7-Troc
20
3
2'-OH
6
4-OAc
14
6
18
18
tBu
17
16
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.0
PPM

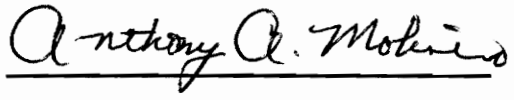
Vita

Anthony Aaron Molinero was born on April 14, 1956 in Tacoma Washington to Dr. and Mrs. Donald P. Molinero. His early years were spent on the Olympic Peninsula. One of the highlights during this time was the 1980 eruption of Mt. St. Helens. During the eruption, he was visiting his fiancée who lived only 17 miles from the mountain.

He received a B.S. in chemistry from Seattle Pacific University in 1978 and a M.S. in chemistry from Virginia Polytechnic Institute and State University in 1982. After receiving his M.S. he returned to Washington where he taught high school for five years at Snohomish High School. In 1989, he returned to Virginia to pursue a Ph.D. in organic chemistry.

Since 1993, he has been an Assistant Professor of Chemistry at Belmont University. His regular teaching duties include general chemistry, organic chemistry, advanced organic chemistry, and biochemistry.

He currently resides in Ashland City, Tennessee with his wife, Rhonda, and children, Amy, Christine, David, and Peter. During his spare time, he frequently can be found fishing, hiking, or gardening with his family.



Anthony Aaron Molinero