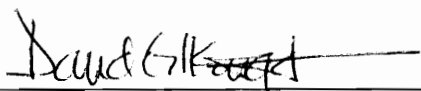


CHEMICAL STUDIES OF THE C-4 POSITION OF BACCATIN III AND TAXOL

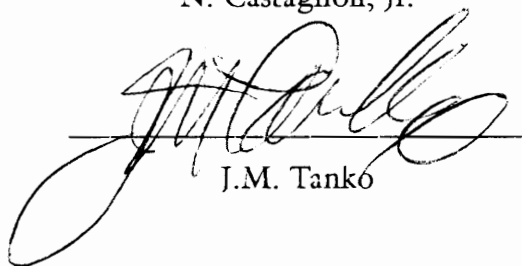
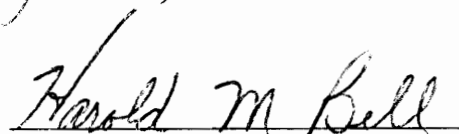
by
Kurt Alan Neidigh

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



David G.I. Kingston, *Chair*


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Key words: Taxol, baccatin III, cancer, chemotherapeutics, SARs.

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Department of Chemistry

ABSTRACT

Because of the efficacy of taxol against a wide variety of cancers, the demand for this drug has vastly increased during the last decade. Due to its limited natural supply, a number of alternative sources of taxol continue to be investigated. One approach toward alleviating the taxol supply problem is by the systematic investigation of the structure-activity relationships of the molecule, in order to establish the structural features and functionalities necessary for biological activity. Research efforts during the last decade have led to the establishment of the molecular domains and functionalities which are crucial for biological activity, however at the inception of this work, the structure-activity relationships of the C-4 position of taxol were unknown. It was thus the major goal of this work to prepare 4-deacetyltaxol, in order to assess the importance of the C-4 acetate for overall activity, as well as to have a template molecule with which to begin studies aimed at determining the effect on activity rendered by replacement of the C-4 acetate with other acyl groups.

Preliminary studies of the deacylation and reacylation of baccatin III were carried out in order to find conditions necessary for the preparation of 4-deacetylbaccatin III, and hence 4-deacetyltaxol. 4-Deacetyltaxol has now been prepared from baccatin III via two synthetic approaches and from taxol via one synthetic approach, and has been shown to be significantly less potent than taxol, suggesting that the C-4 acetate is necessary for biological activity.

From the investigation of several potential synthetic approaches toward the formation of 4-acyltaxol analogs, one methodology has been developed which has allowed the preparation of 4-acyltaxol derivatives from baccatin III or the more readily available 10-deacetylbaccatin III. This particular methodology can be extended to the preparation of other 4-acyltaxol or 4-acyltaxotere derivatives.

Two C-5 α halogenated oxetane ring-opened compounds have been prepared from a 4-deacetyltaxol derivative, offering the opportunity to investigate the potential effects on biological activity generated by modifications to the oxetane ring.

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To Laurel

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*For most of us this is the aim
Never here to be realized;
Who are only undefeated
Because we have gone on trying
We, content at last
If our temporal reversion nourish
(Not too far from the yew tree)
The life of significant soil.*

– T.S. Eliot

I. INTRODUCTION

1.1 Historical

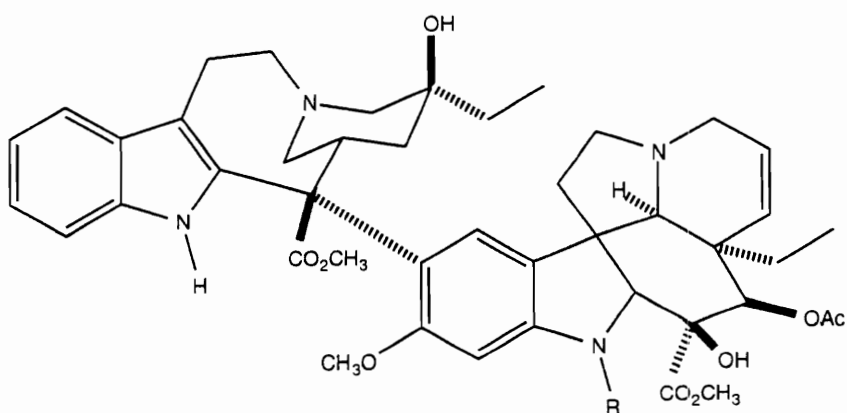
From the nomadic hunter-gatherer tribes of prehistory, through the establishment of agriculture-based societies, to the present time, plants and plant-derived materials have been of paramount importance to humankind. Not only have humans derived sustenance from the plant kingdom through the ages, but we have also sought out plants and plant products for a vast supply of non-nutritive, yet valuable commodities which have been used variously for shelter, clothing, transportation, weapons, poisons, intoxicants, and more importantly in the context of the work to be reported in the following pages, in the treatment of or cure for a myriad of ailments and diseases. In addition to the archeological evidence we have accumulated, giving us a deeper understanding of the diverse lives and activities of primitive peoples, we also have written evidence of the importance of plants in ancient times, especially regarding their medicinal value. Some of the oldest written evidence of the use of plant materials as medicinal agents can be found in the *Rig Vedas* of India. Compiled between 4500 and 1600 BC, it is one of the oldest collections of human learning and many of its 1,028 entries detail the efficacy and therapeutic value of various plants.¹⁻³ Another ancient source of botanical medicine is a Chinese text, the *Pen Ts'ao*, completed in 1596 AD, but representing the culmination of a much older compilation of pharmacological knowledge, traditionally dating to the Emperor Shen Nung about 2700 BC. Included in the *Pen Ts'ao* are hundreds of plant-derived preparations for use in the treatment of various health problems.^{2,3} The *Ebers Papyrus*, another ancient treatise on medical knowledge, was written in Egypt about 1550 BC and contains, in addition to over 800 references to plant-derived medicinal products, some of the earliest references to the use of natural products in the treatment of cancer.²⁻⁴

While it is obvious that the use of plants or plant extracts in the treatment of disease has a long history, it was only relatively recently that these remedies were understood from a scientific point of view. The birth of pharmacognosy (the branch of pharmacy dealing with

the derivation of biologically active compounds from plant sources) in the early 1800s and the advances of organic chemistry between the mid-1700s and the turn of this century led to the realization that the efficacy of traditional medicinally useful plants could most often be attributed to the action of a single compound or a series of related compounds.³ This understanding prompted a relatively rapid decline in the use of crude plant preparations (folk remedies as they are commonly known) and a proportionate increase in the use of the purified active compound(s), such that purified compounds are now nearly exclusively used in the treatment of illnesses.

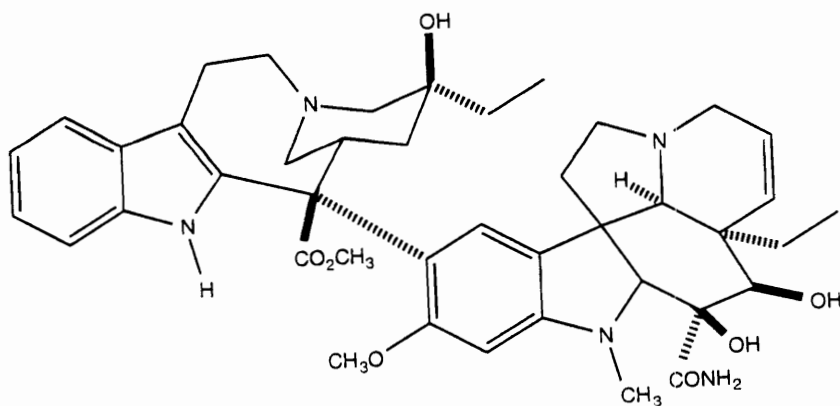
One of the most dynamic areas of chemical research during the past 50 years has been that of cancer chemotherapy, involving either the isolation of biologically active components from natural sources, the synthesis of potential chemotherapeutic agents, or a combination of both (i.e., using plant-derived molecules as starting materials in the semi-synthesis of potentially useful anticancer agents). However, because prospective anticancer drugs may exhibit undesirable side effects, they must pass through a rigorous testing period. Out of literally thousands of compounds which may enter this testing period only one may be found which satisfies the strict testing criteria and be allowed to be marketed as a clinically useful chemotherapeutic agent. Thus, of the large number of potential compounds derived from plant sources and initially showing promising activities in tissue and animal trials, only a relative few have proven to be clinically useful enough to become established cancer chemotherapeutic agents. Among the most successful antineoplastic agents discovered to date are the Vinca alkaloids obtained from *Catharanthus roseus*, commonly referred to as the periwinkle. Vincristine (1.1) is particularly effective against childhood leukemia and in allowing short-term remission of reticulum-cell sarcoma and Hodgkin's disease.^{5,6} Vinblastine (1.2) is useful in the treatment of generalized Hodgkin's disease, lymphocytic lymphoma, advanced testicular carcinoma and Kaposi's sarcoma.⁶ Vindesine (1.3), a semi-synthetic derivative of vinblastine, has also shown promise as a chemotherapeutic agent, especially for use in patients who show resistance to vincristine and vinblastine. The lignans colchicine (1.4), isolated from *Colchicum autumnale*, and podophylltoxin (1.5) and its

derivatives, isolated from several species of *Podophyllum*, have been found to exhibit profound cytotoxic properties. Two semi-synthetic derivatives of podophyllotoxin, teniposide (1.6) and etoposide (1.7), are currently the preferred chemotherapeutic agents in the treatment of small cell lung carcinoma and have shown good activity against Hodgkin's disease and other malignant lymphomas, pediatric leukemias and a variety of other types of cancer.⁷

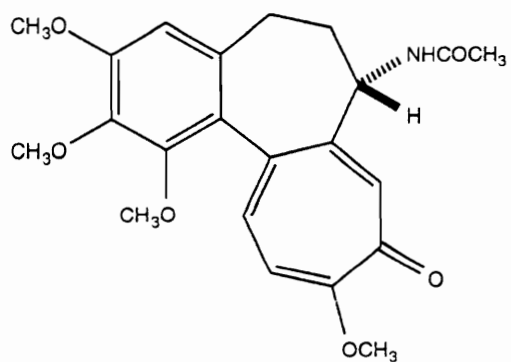


Vincristine (1.1): R = CHO

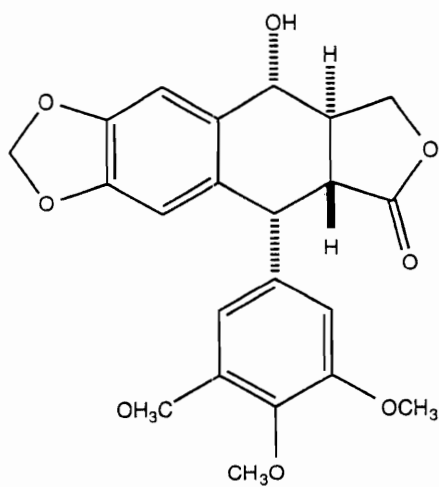
Vinblastine (1.2): R = CH₃



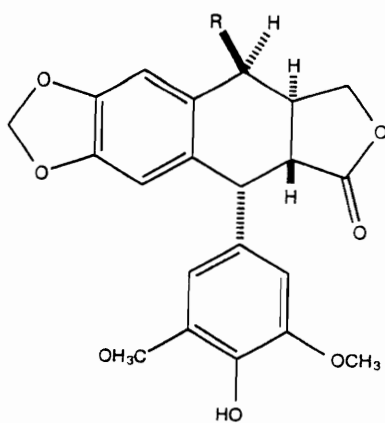
Vindesine (1.3)



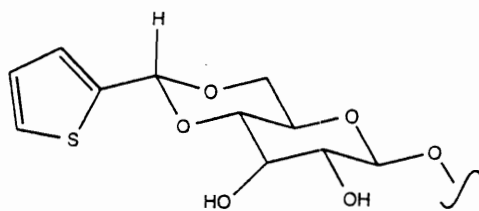
Colchicine (1.4)



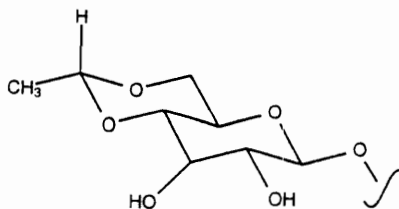
Podophyllotoxin (1.5)



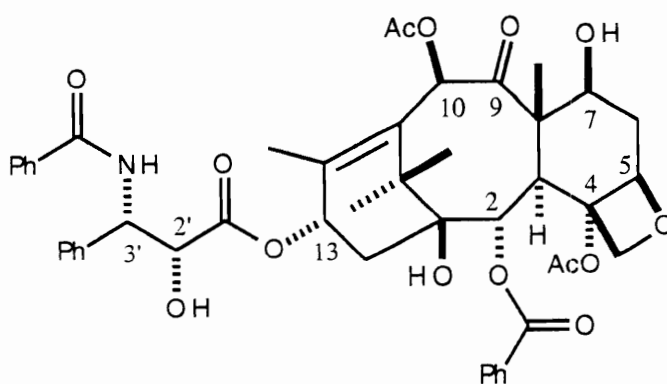
Teniposide (1.6): R=



Etoposide (1.7): R=



One of the most recent additions to this select group of clinically important anticancer agents derived from plants is taxol (**1.8**), a member of the taxane diterpenoid family of natural products originating in various *Taxus* species. While early clinical testing of taxol was hampered by its extremely low water solubility and very limited supply, it has now emerged as one of the most important chemotherapeutic agents isolated to date. Taxol has been shown to have excellent activity against advanced and drug-refractory ovarian cancer⁸ and against breast cancer,^{9,10} as well as very promising activity against lung cancer^{11,12} and melanoma.^{13,14} Based on its behavior in ongoing clinical testing, it is likely that taxol will exhibit activity against other cancers as well, further enhancing its reputation as one of the most intriguing cancer chemotherapeutic agents ever isolated from a plant source.



Taxol (**1.8**)

1.2 Cancer: An Overview

Cancer is the general name used to define a large class of diseases. These diseases are all characterized by two things: uncontrolled growth and division of abnormal cells, and the ability of these cells to spread to other parts of the body and invade other organs and tissues, a process known as metastasis. If the spread of these abnormal cells is not controlled, the activities of normally functioning cells and tissues may be disrupted and death can result. Cancer is caused by a number of both internal and external factors. These include hormonal changes, immune problems and inherited mutations, as well as exposure to chemicals, radiation and viruses. These causal factors may operate singly, in combination or in sequence to initiate cancer formation and/or to promote its spread to other parts of the body.

Cancer is not a new phenomenon. As evidenced by archeological findings which have produced skeletons and mummies bearing the distinctive scarring of cancer or showing signs of attempted surgical intervention, and ancient medical tracts which contain descriptions of diseases we now recognize as cancer and the known remedies of the time, cancer has afflicted humankind through the ages. While having always been greatly feared, cancer was not frequently encountered until the last century. In 1850, approximately one in every 190 deaths in the United States was reported to be related to cancer,¹⁵ whereas it has been estimated that cancer now accounts for one of every five deaths.¹⁶ Most of the increase in cancer mortality has occurred during this century despite the impressive advances made in scientific knowledge and medical practice, as well as the significant improvements made socially and economically. The increase in cancer mortality can be attributed to a number of factors, as discussed below.

During the first half of this century, increasing acceptance of the germ theory of disease led to a series of medical and public health advances which resulted in improvements in water and food supplies, in sewage treatment and waste removal, in antiseptic practices in homes and hospitals and in the administration of vaccines, antitoxins and antibiotics. These innovations resulted in the reduction of both the frequency and the severity of infectious diseases, such that the mortality rate from infectious diseases has fallen from more than half

of all deaths in 1900 to well below one in 30 currently in the United States.¹⁵ As a result of eliminating infectious diseases as a major cause of death, the average life span has increased – in 1900 only 4.1% of the population was 65 years or older, but by 1988, 12.3% of the population was 65 or older; and while the total population tripled in this time, the elderly group increased nearly tenfold.¹⁷ However, as the life expectancy of the population has increased, the death rate from cancer has also increased. Considering that cancer mortality is relatively low among children and teenagers (about seven deaths in 100,000 at ages younger than 15 years), but from age 15 on the death rate from cancer increases very rapidly, to over 170 per 100,000 by ages 45 to 54 and over 1,200 per 100,000 by ages 75 to 84,¹⁵ it is apparent that the reduction of the death rate by infectious diseases has resulted in a greater frequency of cancer-related deaths. That the cancer mortality rate increases with advancing age can be attributed to two factors: 1) the greater likelihood of exposure to environmental carcinogens (it has been estimated that probably more than 90% of cancer is caused by cancer-causing agents found in foods, water, air and clothing, as well as in the workplace or in various substances to which the body is voluntarily or involuntarily exposed, such as tobacco smoke, ethyl alcohol, viruses, ultraviolet radiation, ionizing radiation, vinyl chloride, and asbestos); and 2) for nearly all carcinogens there is a typical delay of five to 30 years or more between initial exposure and the manifestation of cancer. Thus, years of chronic exposure to carcinogens can precede detection of disease, a situation which shifts the prevalence of cancer to the older age groups.¹⁵

While cancer is often a deadly disease, it is by no means predictable in its effects. Different types of cancer do not behave alike and the same kind of tumor in several people may behave in radically different ways, allowing one person to be cured, while another may die, and yet another may be treated and appear disease-free for years before suffering a relapse. Additionally, cancer can vary in its site frequency, depending on environmental factors and behavioral patterns. Thus, while a number of cancers, including those of the stomach, rectum and colon, have declined in their occurrence in the last 60 years, mainly due to improved eating habits, a number of cancers have increased in frequency during the

same period, including lung cancer, breast cancer and non-Hodgkin's lymphoma. Of particular note is that the incidence of death from lung cancer has steadily increased since 1930 from a mortality rate of three per 100,000 to over 70 per 100,000 in 1990, and it is now the leading cause of cancer deaths among men and woman.^{15,16} Mortality from this cancer continues to increase more rapidly than for *any* other cause of death, and the incidence of this cancer has become so prevalent that if lung cancer deaths were excluded from consideration, general cancer mortality would have declined 14% between 1950 and 1990.¹⁶ The rise in lung cancer deaths can be attributed almost exclusively to exposure to tobacco smoke, which is estimated to have at least 43 known carcinogens.¹⁶

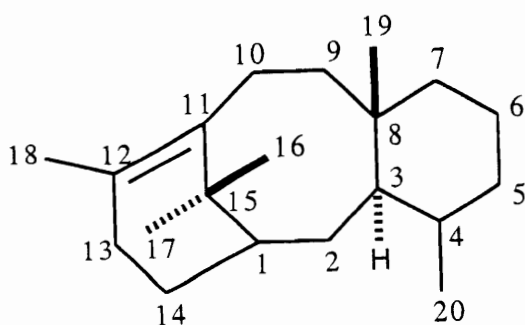
Despite the advances made this century in scientific understanding and medical practices, the treatment of many major types of cancer has proven to be a difficult task. Earlier detection of symptoms has greatly improved the prognosis for survival, especially regarding the treatment options available, which include surgical removal, radiation therapy, chemotherapy, hormone manipulation therapy and immunologic agent therapy; however, depending on the cancer and the treatment option(s) selected, the five-year survival rate is quite variable. For example, the five-year survival rate for lung cancer in all patients is only 13%; for leukemia, the rate is 38%; for ovarian cancer, 41%; for prostate cancer, 78%; and for breast cancer, 93%.¹⁶ In light of these statistics, which show hope for some cancer treatments yet indicate the extent to which effective cancer treatments have eluded discovery, it is not surprising that the search for more active treatments has continued to intensify, especially in the quest for more potent chemotherapeutic agents.

1.3 Taxanes

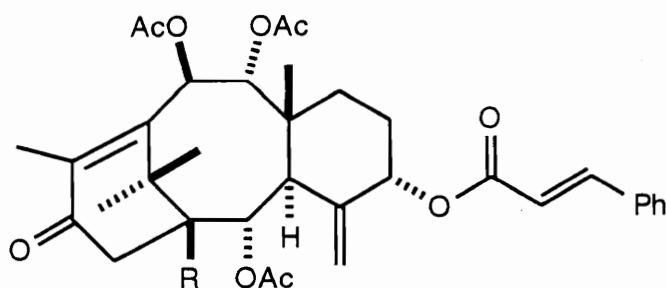
In the search for potential chemotherapeutic agents derived from plants, often a particular plant or plant part is selected for study based on its known toxicity, since it has frequently been shown that the particular component(s) responsible for the toxic nature of the plant or plant part can show significant activity against various diseases or ailments, including cancer. This has been evidenced by the discovery of the Vinca alkaloids and podophyllotoxin and its biological derivatives (as mentioned in Section 1.1), as well as by a class of compounds known as the taxane diterpenoids.

The taxane diterpenoids comprise a unique class of natural products characterized by an unusual multiring carbon skeleton (1.9) or by closely related skeletons (the numbering system for the taxane skeleton is that adopted by IUPAC).¹⁸ This class of compounds originates in the various species of the genus *Taxus* (Taxaceae), commonly referred to as yew plants, which have been known since ancient times to be extremely toxic. In fact, early interest in the chemical composition of *Taxus* species was prompted by the known toxic and often deadly effects elicited by ingestion of various parts of the English yew (*Taxus baccata*). Chemical investigation was initiated in 1856 by Lucas,¹⁹ who reported the isolation of a crude alkaloidal material from *T. baccata*, which he named “taxine.” While this material was held to be largely responsible for the toxicity of the yew, it was later found to be a mixture of related alkaloids. Early investigations²⁰ of the structure of “taxine” were carried out by a number of researchers during the period from 1856 to 1943, but it was the work of Winterstein and his colleagues which produced the first structural elucidation of a significant portion of the mixture. They obtained, upon acid hydrolysis of the mixture, a nitrogen-containing carboxylic acid and determined it to be 3-(dimethylamino)-3-phenylpropanoic acid, later named “Winterstein’s acid.”^{21,22} Work on the structural elucidation of the components of “taxine” continued during the 1940s and the 1950s, mainly centered around the basic Winterstein esters, in the belief that these were the major

active compounds responsible for the toxicity of the yew. Two independent groups converted the mixture of Winterstein esters to the more stable cinnamate ester by β -elimination, each obtaining a different compound, but marking the first successful isolation of homogeneous materials from "taxine." The structures of these two compounds were determined to be O-cinnamoyltaxicin-I triacetate (**1.10**) derived from *T. baccata*,²³ and O-cinnamoyltaxicin-II triacetate, or taxinine (**1.11**) derived from *T. cuspidata*, the Japanese yew.²⁴⁻²⁶



1.9

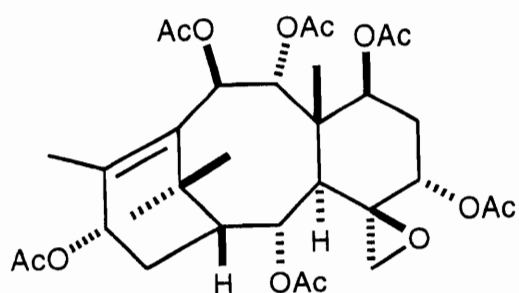


1.10: R = OH

1.11: R = H

While early investigations of *Taxus* constituents had focused on the isolation of basic components through the use of rather harsh acidic conditions, which undoubtedly resulted in substantial decomposition, when milder extraction methods were employed a large

number of neutral compounds could be isolated directly. Thus, in 1963 Taylor reported the isolation of a compound from the heartwood of *T. baccata*, which he named baccatin.²⁷ Taylor's compound was later renamed baccatin I (**1.12**) by Halsall, who had isolated other baccatins from the same species.²⁸⁻³²



1.12

The development of nuclear magnetic resonance spectroscopy during the 1960s and subsequent refinement of NMR techniques, coupled with the application of X-ray crystallography, optical rotary dispersion and circular dichroism,^{25,28,31,33-35} have since allowed the investigation and structural elucidation of taxane diterpenoids to be carried out at a continually increasing pace, leading to the structural characterization of well over 100 taxanes to date.³⁶ These compounds can be included in three main structural classifications, as shown in Figure 1.

Representative examples of taxanes corresponding to the type A structural class are summarized in Table 1. The common structural features of this class of compounds are an exocyclic C-4(20) double bond, a C-5 α -oxygenated substituent and a β -oxygenated substituent at C-10. Variable oxygenated substituent patterns are seen in this class at the C-2, C-7 and C-13 positions.

Table 2 lists a number of representative taxanes of the relatively small structural class B, from Figure 1. This class of taxanes has as its main structural feature a C-4(20) epoxide. In addition, this class is characterized by an acetate group at the C-2 position and varying

oxygenated substituents at C-1, C-5, C-7, C-9, C-10 and C-13. From a biological point of view, it can be supposed that the epoxide functionality common to this class arises from the oxidation of the C-4(20) olefin of the group A class of taxanes.

The third structural class of taxanes, type C, are typified by a C-4(5) oxetane ring, an unusual structural feature for a naturally occurring compound; and, in the majority of the compounds of this class, a bridgehead C-1 hydroxyl group. Representative examples of this structural class are given in Table 3, and as can be seen from the table, this class of taxanes includes taxol (1.8), baccatin III (1.13), the C-13 deacyl analog of taxol, and cephalomannine (1.16).

As is evident from Table 3, cephalomannine, which itself displays some antitumor activity,³⁷ differs from taxol only by the substituent on the side chain nitrogen atom:

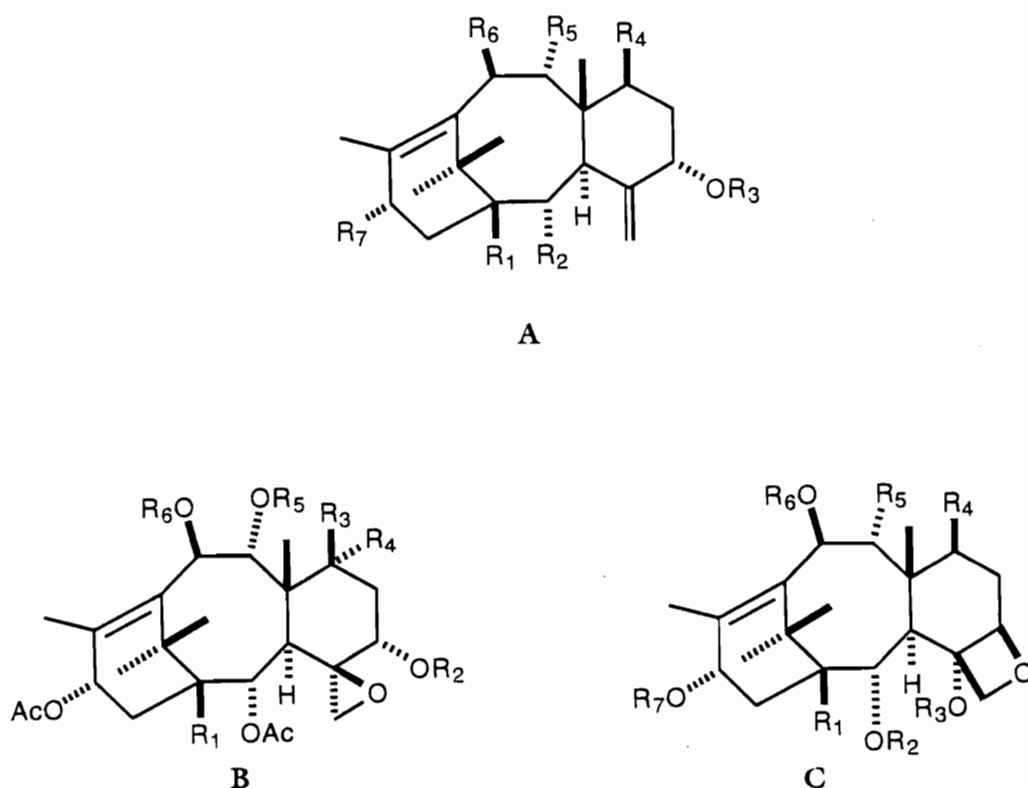
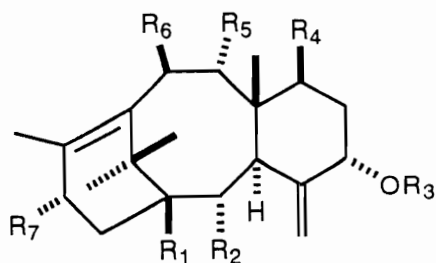


Figure 1. Major structural classes of the taxane diterpenoids.

Table 1. Representative examples of taxanes of the structural class type A from Figure 1.*

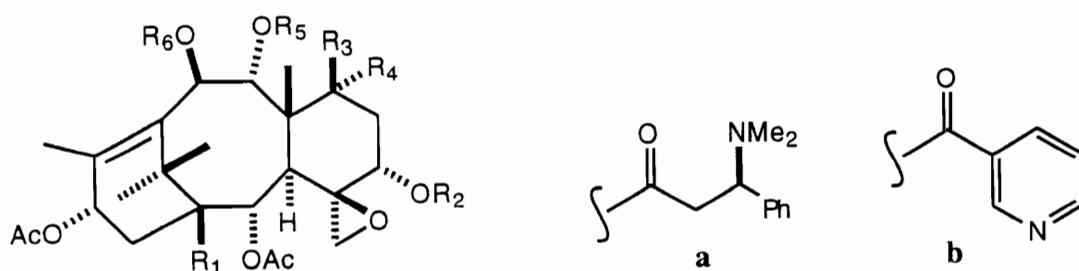


Name	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇
5 α ,9 α ,10 β ,13 α -Tetraacetoxy-4(20),11-taxadiene (Taxusin)	H	H	Ac	H	OAc	OAc	OAc
5 α ,9 α ,10 β ,13 α -Tetrahydroxy-4(20),11-taxadiene	H	H	H	H	OH	OH	OH
9 α ,10 β -Diacetoxy-5 α ,13 α -dihydroxy-4(20),11-taxadiene	H	H	H	H	OAc	OAc	OH
5 α ,7 β ,9 α ,10 β ,13 α -Pentaacetoxy-4(20),11-taxadiene	H	H	Ac	OAc	OAc	OAc	OAc
7 β ,9 α ,10 β -Triacetoxy-2 α ,5 α ,13 α -trihydroxy-4(20),11-taxadiene	H	OH	H	OAc	OAc	OAc	OH
5 α -Hydroxy-2 α ,7 β ,9 α ,10 β ,13 α -tetraacetoxy-4(20),11-taxadiene (Decinnamoyltaxinine J)	H	OAc	H	OAc	OAc	OAc	OAc
1 β ,2 α ,5 α ,9 α ,10 β ,13 α -Hexahydro-4(20),11-taxadiene	OH	OH	H	H	OH	OH	OH
2 α -Benzoyloxy-9 α ,10 β -diacetoxy-1 β ,5 α ,13 α -trihydroxy-4(20),11-taxadiene	OH	OCOPh	H	H	OAc	OAc	OH
2 α -Benzoyloxy-10 β ,13 α -diacetoxy-1 β ,5 α ,9 α -trihydroxy-4(20),11-taxadiene	OH	OCOPh	H	H	OH	OAc	OAc
Brevifoliol	OH	H	H	OCOPh	OAc	OAc	OH
Taxinine A	H	OAc	H	H	OAc	OAc	=O
Taxinine H	H	OAc	Ac	H	OAc	OAc	=O

*Adapted from Reference 36.

whereas taxol has an *N*-benzoyl group, cephalomannine has an *N*-tigloyl group. Baccatin III, on the other hand, displays no biologically relevant effects, suggesting that the variously nitrogen-substituted side chain is partially responsible for the observed biological activity. This hypothesis is further supported by the fact that the semi-synthetically prepared compound taxotere (1.17), characterized by an *N*-*tert*-butoxycarbonyl group and a C-10

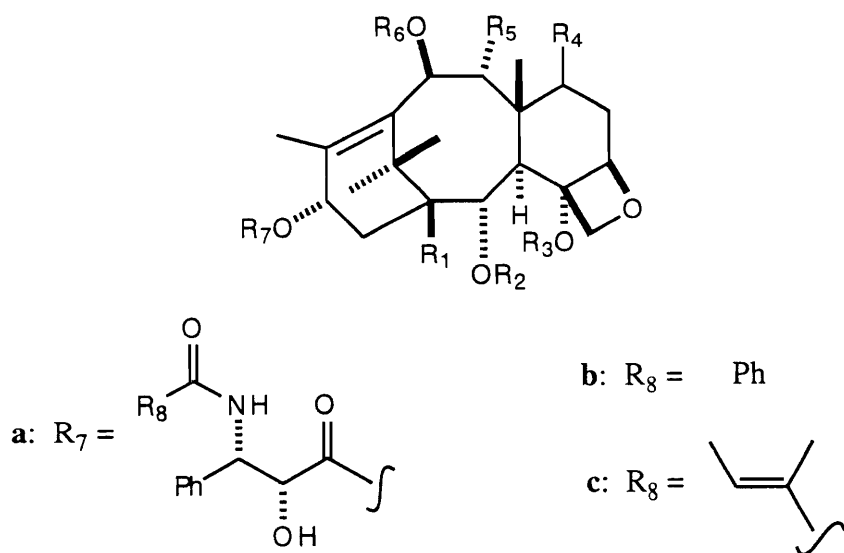
Table 2. Representative examples of taxanes of the structural class type B from Figure 1.*



Name	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
5 α -Deacetylbaccatin I	H	H	OAc	H	Ac	Ac
1 β -Hydroxybaccatin I	OH	Ac	OAc	H	Ac	Ac
1 β -Hydroxy-5 α -deacetylbaccatin I	OH	H	OAc	H	Ac	Ac
1 β -Acetoxy-5 α -deacetylbaccatin I	OAc	H	OAc	H	Ac	Ac
1 β -Hydroxy-7 β -deacetoxy-7 α -hydroxybaccatin I	OH	Ac	H	OH	Ac	Ac
Spicataxine	H	a	H	H	H	Ac
9 α -Acetyl-10 β -deacetylspicataxine	H	a	H	H	Ac	H
Nicaustrine	H	a	H	H	b	Ac

* Adapted from reference 36.

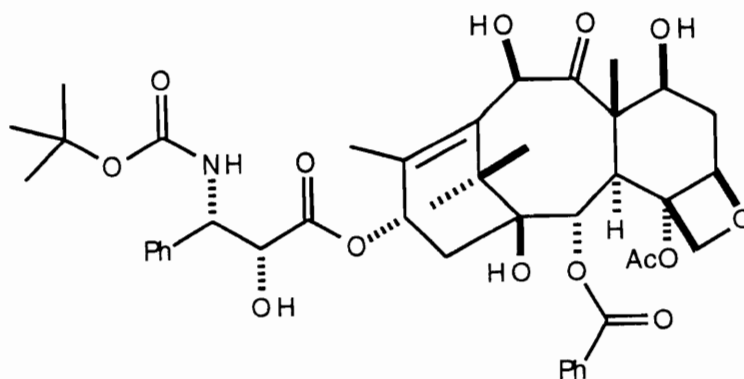
Table 3. Representative examples of taxanes of the structural class type C from Figure 1.*



Name	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈
Baccatin IV	OH	Ac	Ac	β-OAc	OAc	Ac	Ac	–
1β-Acetylbaccatin IV	OAc	Ac	Ac	β-OAc	OAc	Ac	Ac	–
Baccatin VI	OH	COPh	Ac	β-OAc	OAc	Ac	Ac	–
1β-Dehydroxybaccatin VI	H	COPh	Ac	β-OAc	OAc	Ac	Ac	–
Baccatin VII	OH	COC ₅ H ₁₁	Ac	β-OAc	OAc	Ac	Ac	–
Baccatin III (1.13)	OH	COPh	Ac	β-OH	=O	Ac	H	–
10-Deacetylbaccatin III (1.14)	OH	COPh	Ac	β-OH	=O	H	H	–
1-Dehydroxybaccatin III	H	COPh	Ac	β-OH	=O	Ac	H	–
Baccatin V	OH	COPh	Ac	α-OH	=O	Ac	H	–
Taxol (1.8)	OH	COPh	Ac	β-OH	=O	Ac	a	b
7- <i>epi</i> -Taxol	OH	COPh	Ac	α-OH	=O	Ac	a	b
10-Deacetyltaxol (1.15)	OH	COPh	Ac	β-OH	=O	H	a	b
Cephalomannine (1.16)	OH	COPh	Ac	β-OH	=O	Ac	a	c
10-Deacetylcephalomannine	OH	COPh	Ac	β-OH	=O	H	a	c

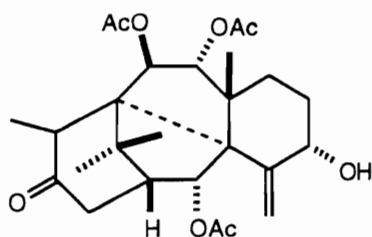
* Adapted from reference 33.

hydroxyl group, has shown improved activity over taxol in some *in vitro*³⁸ and *in vivo*³⁹ assays.

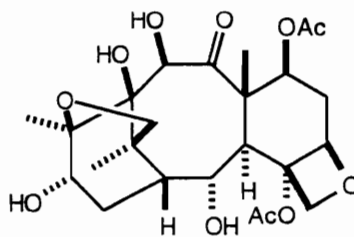


1.17

While the structural classes A, B and C shown in Figure 1 represent the majority of the known taxanes, there are also a number of miscellaneous taxanes displaying a diversity of structural modifications to the taxane carbocyclic skeleton. Some examples of these include taxinine K (1.18),⁴⁰ the C-3(11) transannular analog of taxinine A,⁴¹ taxagifine III (1.19),⁴² taxine A (1.20)^{20,43} and taxchinin A (1.21).⁴⁴

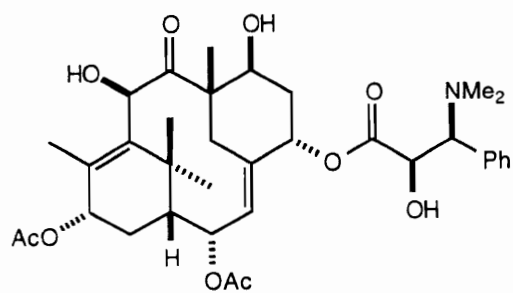


1.18

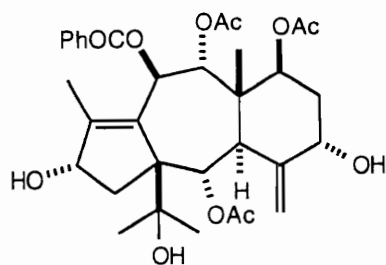


1.19

The latter two compounds obviously do not have the normal taxane carbon framework, taxine A (1.20) having a B-ring expanded skeleton and a differing B(C)-ring linkage and taxchinin A (1.21) having an A(B)-ring contracted skeleton. The biosynthetic processes



1.20



1.21

1.4 The Emergence of Taxol

Despite the unusually complex molecular structures and toxic nature of the taxanes, their isolation and structural elucidation may have remained merely an interesting chemical curiosity were it not for a particularly fortunate sequence of events leading up to what has become a momentous discovery in the fields of cancer research and natural products research.

The knowledge that a wide variety of plants produce compounds which display anticancer activity, coupled with the possibility that many plants may have been overlooked as potential sources of such compounds, led the National Cancer Institute to initiate a cancer chemotherapy program in the late 1950s, based on the assay-directed screening of plant materials, using *in vitro* tumor cell lines and *in vivo* tumor system models to evaluate their potential anticancer activity.⁴⁵ In 1964, it was found that an extract of the stem bark of the Pacific yew (*Taxus brevifolia*) exhibited cytotoxicity in the KB cancer cell line and activity against carcinosarcoma in rats and leukemia in mice.⁴⁵ As part of their ongoing research to assess the biological activity of plant-derived compounds, researchers Wani and Wall of the Research Triangle Institute continued the assay-directed investigation of this extract to determine the compound(s) responsible for the observed activity. The low yield of the active principle of this extract from its natural source made their research efforts difficult, but the structure of the biologically active component of the extract was finally elucidated in 1968, through a combination of chemical degradation, X-ray crystallographic and nuclear magnetic resonance studies. Wani and Wall subsequently reported their findings in 1971, naming the compound “taxol” (1.8).⁴⁶

Although taxol had shown promising chemotherapeutic value due to its potent antileukemic and tumor-inhibitory properties, its initial development into a clinically useful drug was hindered by its extremely low aqueous solubility and its limited natural supply. Its pharmacological activity was gradually forthcoming, however, due to assiduous research efforts conducted throughout the 1970s. The pharmacological data that began to appear showed taxol to have excellent activity against murine B16 melanoma and the MX-1

mammary tumor xenograft in mice.^{47,48} In 1977 the National Cancer Institute decided to begin a preclinical assessment of taxol. Development of taxol as a clinical chemotherapeutic agent was further enhanced by two discoveries made in the next two years. In 1978, Fuchs and Johnson reported their finding that taxol is a mitotic spindle poison (i.e., it inhibits cell division).⁴⁹ In 1979, Horwitz showed that taxol exhibits a unique mode of action.⁵⁰ While the various known spindle poisons, including vinblastine (1.2) and colchicine (1.4), exert their effects by preventing microtubule formation during cell division, taxol actually promotes the assembly of microtubules and inhibits the disassembly of microtubules to tubulin.⁵⁰⁻⁵⁵

Taxol was entered into Phase I clinical trials in 1983 and showed promising results against the drug-refractory tumors common in this testing regimen. A significant number of patients with either non-small cell lung cancer, melanoma, head and neck carcinoma, gastric sarcoma or ovarian cancer showed some response to treatment with taxol, with the most promising results seen against ovarian cancer.⁵⁶ Phase II testing of taxol commenced in 1985 and showed the compound to have excellent activity against advanced chemoresistant ovarian cancer, displaying a 40% overall response rate.^{8,57} Promising activity was also observed against breast cancer⁹ and in non-small cell lung cancer, although the sample size in the latter study was not large enough to be statistically reliable.³⁶ Taxol was approved by the Food and Drug Administration for the treatment of ovarian cancer in 1992 and has since entered Phase III clinical studies in order to more fully establish its efficacy in the treatment of ovarian and various other types of cancers.

The excitement generated by taxol's promising activity in clinical testing, combined with the opportunities: 1) for the biochemist to use taxol as a molecular probe for exploring the biomechanics of microtubule formation and stabilization; 2) for the organic chemist to explore the synthetic challenges presented by the uniquely compelling taxol structure; and 3) for the medicinal chemist to assess the structure-activity relationships of the various functionalities of the taxol molecule, have served to thrust taxol into the forefront of research in a number of scientific disciplines. An extensive review of the clinical applications

and activity of taxol and taxol analogs has appeared,⁵⁸ as have reviews on the chemistry,^{36,45,59} attempted syntheses⁶⁰⁻⁶² and structure-activity relationships,⁶³ and will not be discussed at great length, except as appropriate within the scope of this project.

1.5 Taxol's Mode of Action

1.5.1 The Tubulin-Microtubule System

During the course of normal cell division a cell undergoes a series of distinct transitions, which are characterized by disorganization of the nuclear membrane, formation of the mitotic spindle apparatus during prophase, rearrangement of nuclear material into duplicate pairs of chromatids, and segregation of the chromatids by the mitotic spindle to opposite poles to become the nuclei of the two daughter cells. The mitotic spindle is made up of proteinaceous structures called microtubules which are responsible not only for allowing the separation of the chromatids during cell division, but also serve to maintain cellular shape and other cellular functions, including organelle and surface receptor movement and cell motility.⁶⁴ Microtubules are present in nearly all eukaryotic cells and are formed by the polymerization of two similar but distinct proteins, α - and β -tubulin, each with an approximate molecular weight of 50,000 Daltons, as determined by amino acid sequencing.⁶⁵ Tubulin generally exists in dimeric form, consisting of an α -tubulin unit bound to a β -tubulin unit. Tubulin protein pairs initially align in head-to-tail fashion to form long chains, which in turn further organize into helices, forming tube-like structures – microtubules – which are between 240 and 300 Angstroms in diameter.⁶⁶ Microtubules and tubulin exist in a dynamic equilibrium with each other, with the tubulin dimers preferentially assembling at one end of the microtubule and preferentially disassembling at the opposite end, a process known as “treadmilling.”⁶⁷ A schematic representation of this process is depicted in Figure 2. The tubulin-microtubule equilibrium is apparently determined by the generation of signals by intracellular regulators according to the needs of the cell during the specific phase of its cell cycle.

1.5.2 Tubulin-Microtubule Assembly

Microtubules are believed to assemble from tubulin via a sequential process involving: 1) nucleation; 2) propagation of microtubule growth; and 3) termination.^{67b} Under normal

cellular conditions this process is dependent on certain conditions in the cell, including a critical concentration of tubulin, required for microtubule assembly; the presence of microtubule associated proteins (MAPs) and guanosine triphosphate (GTP) to assist the assembly process; and magnesium and calcium ions, as well as other regulatory factors, which moderate the particular assembly conditions required by the cell.⁶⁸⁻⁷¹ During assembly tubulin dimers are assembled onto one end of the microtubule at a rate greater than the disassembly rate from the other end,^{67a} effectively allowing the microtubule to elongate until its formation stabilizes due to the lowering of the free tubulin concentration. The microtubule then continues to carry out its particular function until cellular signals cause a reversal in the equilibrium and the microtubule disassembles back to free tubulin dimers.

1.5.3 Taxol's Mechanism of Action

Unlike the classic spindle poisons colchicine, podophyllotoxin, vincristine or vinblastine, which all bind to the tubulin dimer and inhibit the assembly of microtubules, taxol displays the unique ability of promoting and stabilizing microtubule assembly through shifting the tubulin-microtubule equilibrium in favor of microtubule assembly. Through work conducted largely by Horwitz and co-workers in the late 1970s and early 1980s,⁵⁰⁻⁵⁵ it was demonstrated that taxol preferentially binds to microtubules rather than tubulin, with saturation occurring at a near stoichiometric ratio of one mole of taxol per mole of tubulin

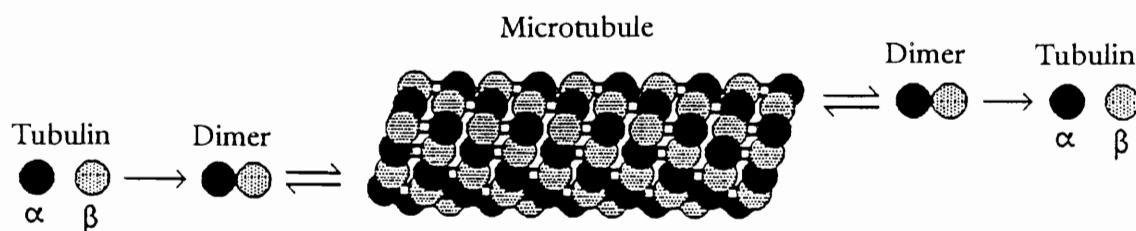


Figure 2. Schematic representation of the tubulin-microtubule equilibrium.

dimer within the assembled microtubule.⁵⁵ This binding is reversible, with an apparent binding constant of approximately $1.0 \times 10^{-6} \text{M}$.⁵⁵ Concentrations of taxol as low as 0.05mM have been observed to promote the *in vitro* assembly of microtubules, while reducing the lag time required for assembly and decreasing the critical concentration of tubulin required for assembly into microtubules.⁷² Additionally, taxol has been found to induce microtubule formation even in the absence of MAPs and GTP,^{53,54} and in binding experiments, no competition was observed between taxol and MAPs or other classic tubulin-binding agents such as colchicine, podophyllotoxin or vinblastine,⁵³ suggesting that taxol binds to a different site on the tubulin dimer than other drugs. Taxol has also been found to stabilize microtubules even after treatment with calcium or lowered temperatures, conditions which normally favor microtubule disassembly.⁷³

Due to its ability to induce microtubule assembly, taxol apparently promotes the formation of abnormal microtubule bodies in the cytoplasm during mitosis.⁵² Typically microtubules are attached to microtubule organizing centers (MTOC), however, in taxol-treated cells it has been observed that microtubule arrays exist independently of any MTOC.⁷⁴ Furthermore, it has been noted that centrosomes and kinetochores essentially lose their capacity to organize microtubule assembly, based on the disappearance of the cytoplasmic microtubule complex as well as the mitotic spindles.^{52,75,76} It is in the disruption of mitotic spindle formation, leading to cellular dysfunction and eventual cell death, that taxol manifests its cytotoxic effects, thus explaining the observed efficacy of taxol against tumor cells.

1.6 Approaches to the Supply of Taxol

1.6.1 Introduction

The efficacy of taxol in the treatment of ovarian cancer along with its promising activity in the treatment of breast, lung and other cancers has placed increased demand on the supply of the drug. Currently, the only approved source of taxol for drug use is in the bark of the slow-growing Pacific yew (*Taxus brevifolia*). The procedure for extracting taxol from the bark is, however, difficult, expensive and results in the destruction of a large number of trees. To exacerbate the problem of maintaining a steady supply of taxol, it has been estimated that it takes the bark of six to ten yew trees to produce the two grams of taxol necessary for the treatment of one patient.⁵⁶ The combined yearly number of ovarian, breast and lung cancers would require 150,000 treatment regimens, or the bark of up to 15 million trees.⁵⁶ Unfortunately, it has been estimated that fewer than 20 million harvestable yew trees remain.⁵⁶ In order to protect this endangered tree from extinction and yet meet the increasing demand for taxol, several alternative sources of taxol are being investigated. These include the isolation of taxol from renewable sources, such as *Taxus* needles and twigs, and the production of taxol by other methods, including tissue or fungal cultures, total synthesis, or semi-synthesis from more plentiful taxanes isolated from renewable sources.

1.6.2 Taxol From Natural Sources

1.6.2.1 *Taxus* Needles as a Renewable Source of Taxol

The extraction of taxol from the needles of various *Taxus* species is an appealing solution to the taxol supply problem. Since various *Taxus* species are used worldwide as ornamental shrubbery and cultivars and their needles (or leaves) are frequently pruned for decorative purposes, the cuttings represent a vast, renewable source of taxol and/or other taxanes. The use of yew needle cuttings as a source of taxol could be extended to the

implementation of large *Taxus* farms, from which large quantities of needles could be continuously harvested. This idea has apparently found favor with several companies, particularly the Weyerhaeuser Company, which has initiated a program to cultivate fast-growing, high-yielding *Taxus* species.⁵⁶ Fortunately, most varieties of yew sold as ornamentals in the United States have been developed from species known to produce taxol in reasonable amounts. It has been estimated that taxol can be isolated from the cuttings of ornamentals in yields comparable to the yield from bark (i.e., approximately 0.01%),⁷⁷ thus avoiding the needless destruction of *T. brevifolia* trees.

1.6.2.2 Taxol Production by Plant Tissue-Culture

Another attractive method of solving the taxol supply problem is the use of plant tissue-culture production. The earliest report of tissue-culture production of taxol from *T. brevifolia* appeared in 1989,⁷⁸ in which taxol was afforded in quantities up to 3 mg per liter of culture supernatant. While the predicted commercial production of taxol using this approach has not yet been made practical, this approach offers the advantage of being able to select superior varieties of plants and variable growth mediums to maximize the yield of taxol. Furthermore, such an approach allows the production of relatively pure taxol, thus simplifying the extraction and purification process.

It has been reported that callus cultures of *T. cuspidata* and *T. canadensis* have produced taxol in yields up to 0.05% dry weight.⁷⁹⁻⁸⁵ Yields have been shown to increase in callus cultures supplemented with various biosynthetic precursors, such as phenylalanine, mevalonate, and acetate.^{80,81,84,85} Such studies are prone, however, to variability due to the difficulty in establishing and maintaining stable cell lines which produce taxol in high yield, and problems inherent in production scale-up, as well as the fact that slow-growing callus cultures are susceptible to fungal and bacterial contamination.

Thus, while taxol production by plant tissue-culture methods has shown some promise as a potential alternative source, this approach is yet in the developmental stages and is probably some years away from being a commercially viable process.

1.6.2.3 Taxol Production by Fungal Culture

It was reported in 1993 that taxol is produced by *Taxomyces andreanae*, a fungus found growing in association with *Taxus brevifolia*.⁸⁶ The fungus was isolated from the inner bark of a particular tree in an old-growth cedar forest in northern Montana, and was found to yield taxol in nanogram quantities. While the yield of taxol from this source is extremely low, variation of growth conditions or genetic engineering could conceivably produce an improved strain of fungus which could show vastly increased production capability. Large-scale fermentation methods could then be implemented at a much lower cost than current plant tissue-culture methods require, thus offering another promising alternative source of taxol.^{87,88}

1.6.3 Taxol From Synthetic Methodologies

1.6.3.1 Taxol Production by Total Synthesis

Another potential source of taxol is from total synthesis. This has been a very active area of investigation for the synthetic organic chemists of more than 30 research groups worldwide ever since the discovery of taxol and its subsequent rise to clinical importance. From a synthetic point of view, taxol is a very formidable molecular target, containing 11 asymmetric centers (i.e., 2,048 possible diastereomers) and a variety of oxygenation patterns. Considering the level of complexity, it is not surprising that a large number of synthetic approaches to taxol have been reported,^{36,59,60} but until only recently no total synthesis of taxol had been reported. In 1994, both Holton⁶¹ and Nicolaou⁶² and their respective co-workers, independently reported the first total syntheses of taxol, representing one of the most impressive accomplishments of organic chemistry to date. However, despite the attainment of one of the most coveted synthetic targets ever envisioned, it remains to be seen whether either of these achievements will ever contribute significantly to the taxol

supply problem. In light of the number of synthetic manipulations necessary to realize taxol, as evidenced by Holton's and Nicolaou's approaches and likely by any other conceivable approaches which may be reported in the foreseeable future, it is doubtful that total synthesis of taxol will ever be a commercially acceptable process, especially since the alternative methods described thus far are much more economical.

1.6.3.2 Taxol Production by Partial Synthesis

Another potentially economical alternative source of taxol is from partial synthesis.^{36,59b} In contrast to total synthesis, the semi-synthesis of taxol from readily available precursors shows enormous potential for large scale production. Such an approach consists of the preparation of an suitably protected taxol side chain acid derivative, and the isolation of an appropriate precursor from *Taxus* species to which the side chain can be attached. A number of side chain preparations have appeared in the literature,^{36,45,60,63,89} several of which will be discussed in the context of the research goals of this project in Section 2.1.3. The most practical naturally occurring substrates to which the side chain acid derivatives can be attached are baccatin III (1.13), the C-13 deacyl analog of taxol, and the related and more readily available 10-deacetylbaccatin III (1.14). 10-Deacetylbaccatin III can be obtained in yields as high as one gram per kilogram from the fresh leaves of *T. baccata*. Considering that various side chain derivatives can be realized in a relatively low number of synthetic steps from readily available and inexpensive starting materials, and that 10-deacetylbaccatin III and/or baccatin III can be readily obtained in relatively good yields from renewable sources, the semi-synthesis of taxol currently seems to be the most practical approach toward alleviating the taxol supply problem. Additionally, the use of baccatin III or 10-deacetylbaccatin III as the substrate in the partial synthesis further allows the investigation of the possible effects on overall activity elicited by subtle modifications to the ring system or side chain functionalities. Such modifications provide the basis for evaluating the structure-activity relationships of the molecule and could potentially lead to the discovery

and preparation of more potent taxol analogs. Obviously, taxol derivatives displaying a higher clinical efficacy could also serve to mitigate the taxol supply problem, since more potent analogs would decrease the per-patient demand for treatment.

1.7 Structure-Activity Relationships of Taxol

1.7.1 Introduction

In general, drugs exert their effects by very specific interactions with a particular receptor. The functional groups and structural features of a given drug may be necessary at various times for: 1) solubilizing the compound upon administration into the body; 2) for assisting in transport through the cell membrane and into the cytoplasm; or 3) for optimizing the interaction(s) with the site of action. Conversely, certain functionalities or structural aspects may be superfluous. In determining what role(s) a particular functional group plays, especially regarding active site interaction, the traditional approach has been to study the structural-activity relationships of the functionality in question. This is typically accomplished by first removing the functional group and assessing the effect this has on activity relative to the parent compound. Substitution of various other functional groups and subsequent evaluation of the relative effect on activity can lead to a better understanding of the functional group requirements at this particular position.

Evaluation of the structure-activity relationships of a drug such as taxol can be complicated due to the many different assay systems which have been developed and implemented by various investigators. Precautions must be taken to ensure that the assay results are reliable, and this is most often accomplished by assaying the parent compound in the same system under the same conditions as the derivative(s).

For the assessment of the activity of taxol derivatives there are three main types of assays which have been employed and are known to give fairly reliable results:

1) *Microtubule assembly assays*:^{59b,73a,74,90,91}

Since taxol has been shown to promote microtubule assembly and to stabilize microtubules against calcium- or low temperature-induced disassembly,^{50,55} assays designed to monitor microtubule assembly can be used to ascertain the relative effectiveness of a taxol analog. Taxol analogs are evaluated according to their ability to enhance microtubule assembly and to prevent microtubule depolymerization, and a direct comparison to taxol's ability to do the same under identical conditions allows the investigator to establish the relative effectiveness and activity of the

particular analog being investigated. There are a number of variations on the microtubule assembly assay, but in general, this family of assays permits the rapid acquisition of reliable test data, and only requires sub-milligram quantities of an analog for evaluation.

2) *Mammalian cell culture cytotoxicity:*

The cytotoxicity of various taxol analogs has been determined predominantly using the KB cell line (human carcinoma of the nasopharynx), the J774-2 cell line (mouse macrophage-like cells), the P-388 mouse lymphocytic leukemia cell line or the HL-60 leukemia cell line.^{59b,74} Measurements of the activity of taxol and taxol derivatives in a particular cell line relative to another have shown these assays to be fairly comparable to each other and to correlate well with results obtained from the microtubule assays.

3) *Mouse assays:*

In vivo evaluation of taxol and its derivatives has been carried out against a number of cancers using the mouse as the animal model. These systems include P-388 leukemia, B16 melanoma, and other mouse-based systems, such as the use of human tumors as xenografts in athymic mice.^{59b,74}

Using various combinations of the assays outlined above as a guide, taxol has been the subject of an extensive number of chemical characterization and bioactivity studies.^{36,59b} From the accumulation of knowledge acquired through these investigations has emerged a considerable body of information on the structure-activity relationships of taxol. This information is summarized in Figure 3 and will be discussed in greater detail in the Sections below.

1.7.2 The Side Chain

Early work on the structural elucidation of taxol led to the observation by Wall and co-workers that the C-13 *N*-benzoyl- β -phenylisoserine side chain plays a critical role in the biological activity of taxol.⁴⁶ Low temperature methanolysis of taxol led to the isolation of baccatin III (1.13) and the corresponding side chain methyl ester. Both of these

compounds were separately tested in the KB cell line and were respectively found to be 10^3 and 10^5 times less active than the parent compound taxol itself.⁴⁶ This preliminary activity data and activity data acquired for other taxanes lacking the C-13 side chain, which show such compounds to be significantly less active than taxol in both tubulin-assembly and cytotoxicity assays,⁹⁰ emphasize the importance of the side chain for activity. The activity data for the side chain methyl ester also indicates that it is not solely the side chain which is responsible for taxol's biological activity, as was once believed.

While baccatin III and its derivatives do not display significant activity relative to taxol, they can serve as potential precursors for the partial synthesis of taxol as well as C-13

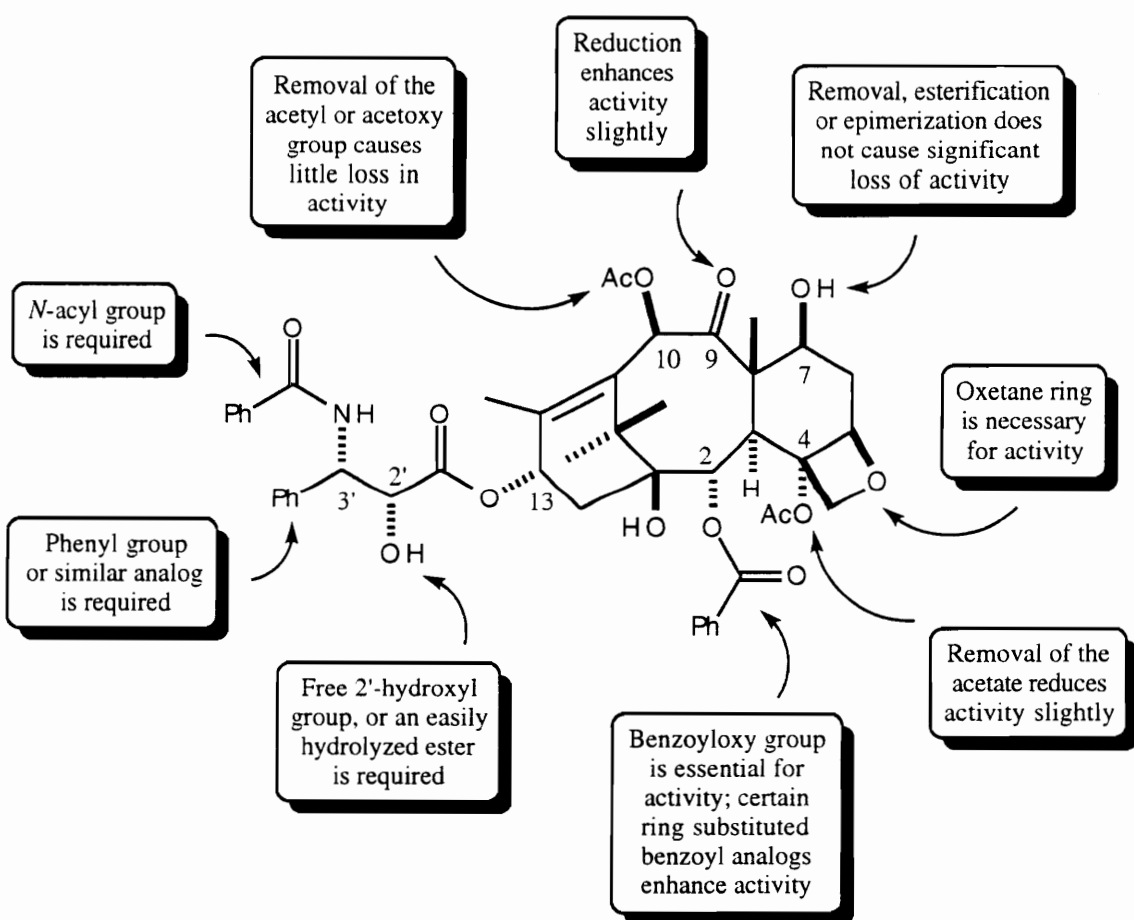


Figure 3. Structure-activity relationships of taxol.

modified taxol analogs. Several methodologies exist for coupling baccatin or its derivatives to an appropriately protected side chain acid derivative, including the direct coupling of a protected baccatin substrate to a C-2'-ethoxyethylether-protected side chain acid⁹² or an oxazolidine side chain derivative,⁹³ or coupling with a β -lactam.⁹⁴⁻⁹⁶ The ability to prepare modified side chains and couple them to protected baccatins has led to an increased understanding of the structure-activity relationships of the pendant side chain.

Various taxol analogs have been prepared semi-synthetically from side chains having modified C-3' functionalities and biological testing of these compounds has shown that the C-3' phenyl group is necessary for activity.^{59b,91} Analogs of substituted C-3' phenyl groups have been prepared and generally display lower activity than taxol.⁹⁷⁻⁹⁹

Taxol analogs have been prepared from side chains which, instead of having the C-3' *N*-benzoyl group, have other C-3' *N*-acyl groups. These derivatives have shown mixed activity results. Most *N*-acyltaxol analogs are less active than taxol,⁹⁷⁻⁹⁹ however, one analog, taxotere (1.17), which has an *N-tert*-butoxycarbonyl group and lacks the C-10 acetate, has shown great therapeutic value.^{63c} It has shown a two-fold enhancement of activity over taxol in several assay systems and is currently in clinical trials in the United States and in France.¹⁰⁰ Taxol derivatives in which the position, functional group attachment and stereochemistry of the side chain C-2' hydroxyl and C-3' benzoylamino groups are altered have been prepared,^{63c,90,91,101} but all such derivatives show less activity than taxol. A taxol derivative possessing a one carbon homologated C-13 side chain has been prepared.¹⁰² This compound was shown to be at least 25 times less active than taxol, indicating that the conformational changes caused by extension of the side chain are substantial enough to reduce the molecule's binding affinity for microtubules.

The structure-activity relationships mentioned above can be used to draw several inferences regarding the side chain:

- 1) Acylation of the C-2' hydroxyl group results in lower activity in the tubulin-assembly assay, but occasionally the activities are unchanged in cytotoxicity assays. This has been attributed to *in vivo* hydrolysis of the 2'-acyl compounds, since in

cases where a 2'-acyl group is hydrolytically stable, the activities have been markedly reduced.

- 2) Deoxygenation of the C-2' hydroxyl group produces an analog displaying a major loss of activity.^{63c}
- 3) Considerable modification of the C-3' carboxamide moiety can be made without significant loss of potency. Examples include replacement of the *N*-benzoyl group with an *N*-tigloyl (cephalomannine) or an *N*-tosyl group, both of which preserve activity; or with an *N-tert*-butoxycarbonyl group, which enhances the activity. The C-3' free amine itself, however, displays lower activity than its *N*-acyl relatives.
- 4) Substitution of a hydrogen for the C-3' phenyl group results in a marked reduction in activity, whereas ring-substituted phenyl groups at this position exhibit only marginal reductions in activity.
- 5) Retention of the normal taxane C-2',C-3' configuration (2'*R*,3'*S*) elicits the highest potency; however, analogs with the 2'*S*,3'*S* configuration and an *N*-acyl moiety tend to show only modest reductions in activity.
- 6) Extension of the C-13 side chain tends to disrupt the molecular conformation necessary for optimal microtubule binding, resulting in a significant reduction in biological activity.

Of the more than 50 side chain-modified taxol derivatives prepared to date, the most potent of these compounds are those with the naturally occurring side chain or the *N-tert*-butoxycarbonyl side chain, indicating that the various functionalities and the particular configuration of the side chain are crucial in the attainment of the particular conformation necessary for optimal interaction with the biological site of action.

1.7.3 The Northern Hemisphere

The northern hemisphere of the taxol molecule is comprised of carbons 6 through 12, and includes the C-7 hydroxyl group, the C-9 carbonyl, the C-10 acetoxy group, and the

C-11(12) double bond (see Figure 3). The structure-activity research that has been carried out on this portion of the taxol molecule has led to the conclusion that, for the most part, modifications to the functional groups of the northern hemisphere do not cause substantial changes in biological activity, as will be discussed below.

1.7.3.1 Effects of Changes at the C-7 Position

The β -hydroxyl group at C-7 of taxol apparently does not play a significant role in the overall activity of taxol. It has been found that neither acylation of, removal of, nor epimerization of the C-7 hydroxyl group results in significant reduction of activity.¹⁰³⁻¹⁰⁶ Oxidation of the C-7 hydroxyl group has been observed to lead to a significant loss of activity, but the lower activity in this case is likely attributable to the known proclivity of 7-oxotaxol to lead to the opening of the oxetane ring under mildly acidic or basic conditions (cf. Section 1.7.4.4).¹⁰¹

1.7.3.2 Effects of Changes at the C-9 Position

Only limited structure-activity relationship data is available for derivatives of taxol modified at C-9, mainly because of the low reactivity of this rather hindered ketone. To date the only modification to C-9 has been the reduction of the ketone to an α -hydroxyl group. This product did however exhibit a slightly higher activity in the tubulin-assembly assay, although no cytotoxicity data exists.^{107,108}

1.7.3.3 Effects of Changes at the C-10 Position

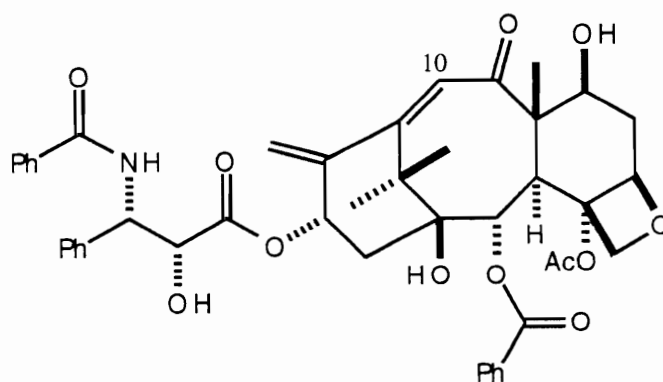
For the most part, modifications to the C-10 position of taxol result in only slight changes in the activity, although some C-10 modifications can induce substantial activity changes depending on the nature of other functional groups in the molecule. Hydrolysis of the C-10 acetate affords 10-deacetyltaxol (**1.15**), which is only slightly less active than taxol.^{73a,109} Taxotere (**1.17**), which has been mentioned previously, has no acetate at the C-

10 position, but accompanied by the change in the C-3' *N*-carboxamide moiety, this compound displays a two-fold increase in activity relative to taxol.^{63c} Oxidation of the C-10 hydroxyl group of 10-deacetyltaxol gives the 10-oxo-taxol derivative, which displays significantly lower activity.^{59c} The removal of the C-10 acetoxy functionality affords the 10-deacetoxy analog,¹¹⁰⁻¹¹³ which shows activity comparable to that of taxol. Preparation of 10-deoxytaxotere and biological evaluation showed this compound to be on the order of 100 times more active than taxol in the P-388 cell culture assay.¹¹⁰ Replacement of the C-10 acetate by various acyl groups has afforded a number of taxol analogs displaying variable biological activities.¹¹⁴ While most of the 10-acyltaxol analogs prepared have shown activities comparable to taxol, three analogs, namely the 10-*N,N*-dimethyl carbamate, the 10-methoxy, and the 10-methyl carbonate, have shown better activity than taxol.¹¹⁴

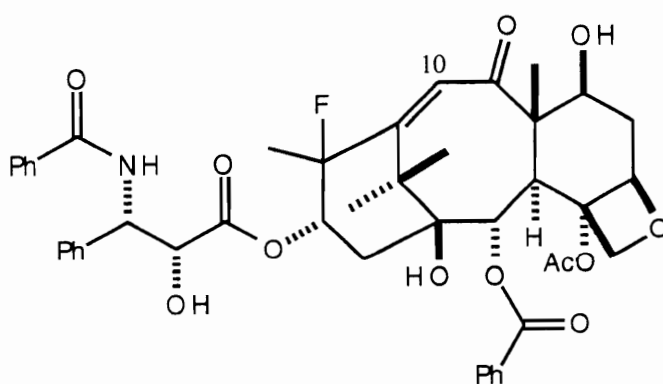
1.7.3.4 Effects of Changes to the C-11(12) Olefin

It has been shown that the C-11(12) tetrasubstituted double bond is completely resistant to hydrogenation¹¹⁵ as well as oxidation,¹¹⁶ thus hindering investigations in this portion of the molecule.¹¹⁷ Recently however, the allylic transposed olefin has been prepared in an attempt to modify the C-10 position.¹¹¹ Treatment of 2,7'-bistrichloroethylchloroformyl-10-deacetyltaxol with Yaravenko's reagent ($\text{ClFHCCF}_2\text{NEt}_2$, a fluorinating agent) did not produce the desired 10-fluoro derivative, but rather, it afforded the dieneone **1.22** through a simultaneous elimination and double bond transposition. The dieneone was hydrogenated to afford 10-deacetoxytaxol to confirm the structure of the starting material. A minor product of the reaction was identified as the fluorinated derivative **1.23**. Both **1.22** and **1.23** were found to be about eight times less cytotoxic than taxol, as determined in the HCT116 human colon carcinoma assay, but both retained their activity in the tubulin-assembly system. These preliminary results seem to indicate that modifications of the C-11(12) double bond may result in decreased biological activity.

With a few exceptions, as noted, the observations summarized above indicate that structural modifications to the northern hemisphere of taxol do not have a significant effect on the biological activity of the molecule. This suggests that this portion of the molecule does not play a major role in the taxol-microtubule binding process.



1.22



1.23

1.7.4 The Southern Hemisphere

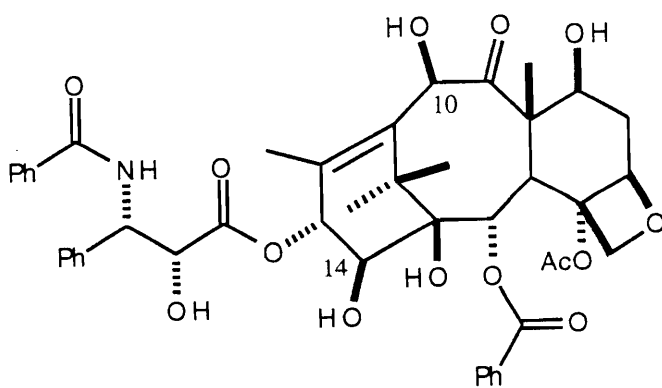
The southern hemisphere of the taxol molecule includes the C-14 and C-1 through C-5 positions, and contains oxygen functionalities at C-1, C-2, C-4, and the unusual C-4(C-20),C-5 oxetane ring (see Figure 3). Until relatively recently very little was known

about the structure-activity relationships of the southern hemisphere (with the exception of the oxetane ring), but based on the accumulating information gathered from extensive chemical modifications to this region and the effect such changes have on biological activity, the southern hemisphere appears to be of vital importance, apparently interacting with a hydrophobic region of the microtubule binding site.⁴⁵

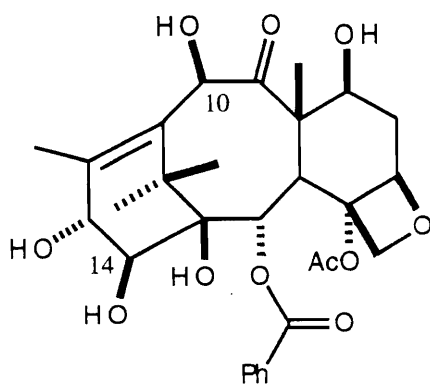
1.7.4.1 Effects of Changes at the C-14 Position

Until very recently, no information on the structure-activity relationships of the C-14 position of taxol was available. However, Kant and co-workers¹¹⁸ have reported the preparation of 14- β -hydroxy-10-deacetyltaxol (**1.24**) from the recently isolated 14- β -hydroxy-10-deacetylbaccatin III (**1.25**).¹¹⁹ 14- β -Hydroxy-10-deacetyltaxol (**1.24**) was found to be slightly less potent than taxol under *in vitro* and *in vivo* assays and in promoting microtubule assembly.¹¹⁸ Kant and co-workers¹¹⁸ have also reported the preparation of 14- β -hydroxy-10-acetyltaxotere (**1.26**) and 14- β -hydroxytaxotere (**1.27**), the latter of which has also reportedly been prepared by Ojima and co-workers¹²⁰ from 14- β -hydroxy-10-deacetylbaccatin III (**1.25**).¹¹⁹ Compounds **1.26** and **1.27** were found to exhibit biological activities comparable to taxotere (**1.17**).^{118,120}

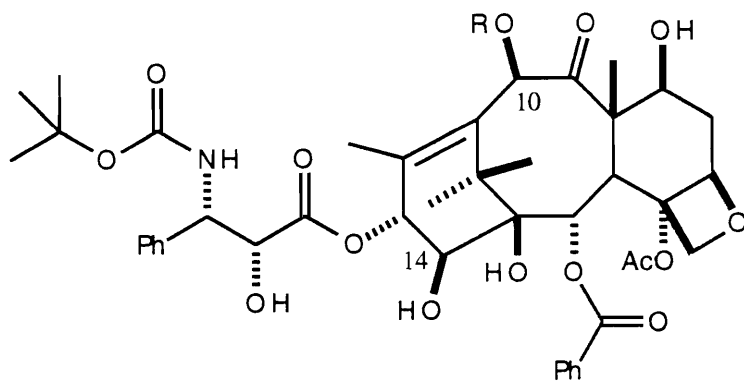
These preliminary results seem to indicate that placement of a β -hydroxyl group at the C-14 position lowers the activity only marginally in taxol and does not appreciably affect activity in the taxotere series. The potential effects on activity rendered by acylation of the C-14- β -hydroxyl group are unknown at the present time, as are the possible changes in activity effected by placement of an α -hydroxyl group at C-14 and subsequent acylation thereof. With the minimal information available for the structure-activity relationships at the C-14 position, modifications of this position to better elucidate its role on activity are likely forthcoming. Other modifications to the C-14 position, having arisen during the course of this research project, and the potential for further studies of the structure-activity relationships of this position will be discussed in the Results and Discussion section.



1.24



1.25

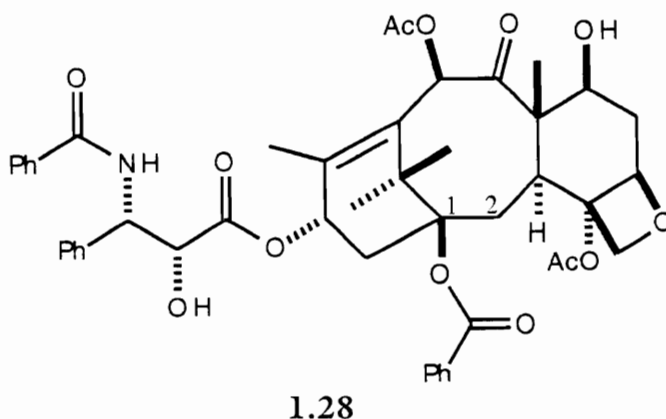


126: R = Ac

127: R = H

1.7.4.2 Effects of Changes at the C-1 Position

Virtually all attempts to modify the C-1 bridgehead hydroxyl group of taxol have been unfruitful, leading to the assessment that this position is essentially inert to even the most vigorous acylation conditions. The contribution that the C-1 hydroxyl group makes to the overall activity of taxol is still unclear, but Kingston^{63a} has reported the preparation of 1-benzoyl-2-debenzoyloxotaxol (**1.28**), which is significantly less active than taxol. The reduction in activity may be partly due to the presence of the benzoyl group at the C-1 position, but is more likely attributable to the absence of the C-2 benzoyloxy functionality, since it is known that the C-2 benzoyloxy is essential for activity, as will be discussed below.



1.7.4.3 Effects of Changes at the C-2 Position

Recent structural modifications to the C-2 position of taxol have indicated that the benzoate group at this position plays a crucial role in the overall activity. It has been found that modifications of the C-2 position by removal of either the benzoate or the benzoyloxy moieties afford derivatives which are significantly lower in biological activity.¹²¹⁻¹²³ The 2-debenzoyloxy analog in particular is approximately 120 times less active than taxol in the HCT116 human colon cancer cell line assay, and shows negligible ability to assemble

tubulin.¹²¹ Migration of the benzoyl group to the C-1 position and concomitant deoxygenation of the C-2 position affords 1-benzoyl-2-debenzoyloxytaxol, which, as mentioned above, shows significantly less activity than taxol. The apparent importance of the C-2 benzoate suggested further modification of this position by replacement with various ring-substituted benzoyl groups, and the preparation of a vast array of these 2-aroyletaxol derivatives has been reported by Kingston and co-workers.^{74,87,124} It was found that the biological activities of these C-2 aroyletaxol analogs is highly dependent on the nature of the particular aroyl group. As a rule, aroyletaxol congeners with a *para*-substituted C-2 benzoate are consistently less active than taxol, with the exception of 2-*para*-fluorobenzoyltaxol which shows comparable activity to taxol, presumably due to its similarity in size to the benzoate. Aroyletaxol analogs containing *meta*-substituted C-2 aryl groups, however, were frequently found to be more cytotoxic than taxol,¹²⁴ particularly the *meta*-chloro, *meta*-azido and *meta*-methoxy derivatives. These three analogs have been found to promote microtubule assembly more effectively than taxol. Furthermore, the *meta*-azido congener and, to a lesser extent, the *meta*-methoxy congener, promote the assembly of microtubules under conditions that taxol does not.^{63a,74,95,124}

It has been proposed that the C-2 benzoate of taxol provides stabilization of the C-13 side chain by π -stacking hydrophobic interactions with the pendant side chain aromatic moieties as well as hydrogen bonding interactions;^{125,126} however, the variable effects on biological activity seen in the C-2 *meta*- and *para*-substituted benzoyl derivatives indicate that steric factors may contribute significantly to the taxol-microtubule binding interaction as well.⁸⁷

1.7.4.4 Effects of Changes at the C-4 Position

Perhaps because of its tertiary disposition and the apparent difficulty in selectively removing the C-4 acetate in the presence of the three secondary acyl groups at C-2, C-10 and C-13, the C-4 position of taxol had not been modified at the inception of this project

and no structure-activity relationships were known. It was therefore a major goal of this research to initiate studies of modifications to the C-4 position. From preliminary results, which will be described at length in the Results and Discussion portion of this work, it has been found that removal of the C-4 acetate causes a slight reduction in activity in the P-388 and CA46 Burkitt cytotoxicity assays and the microtubule assembly assay, apparently indicating that the C-4 acetate is only slightly necessary for activity. These findings have been further substantiated by the investigations of other researchers during the course of this project.^{127,128} During the course of this project it was found in our labs that removal of the C-4 acetoxy group results in a substantial reduction in biological activity relative to taxol.¹²⁹ Substitution of various acyl analogs at C-4 by us and others^{127,128} has allowed the establishment of a preliminary base of information. This data shows that biological activity varies significantly depending on the substituent in the C-4 modified series and is generally observed to be much lower than taxol.^{127,128} These results will also be discussed in the Results and Discussion section.

1.7.4.5 The C-4(C-20),C-5 Oxetane Ring

The earliest evidence of the general importance of the southern hemisphere functionalities to the overall activity of taxol was provided by work carried out by Kingston and co-workers on the C-4(C-20),C-5 oxetane ring.^{63a,101,117,130} It was found that opening of the oxetane by oxidation of the C-7 hydroxyl group, as mentioned previously (cf. Section 1.7.3.1), or through the use of electrophilic reagents,^{101,117,130} causes substantial loss in activity in both cytotoxicity and microtubule assembly assays.^{63a,130,131} Other modifications to the oxetane ring arising during the course of this project have afforded taxol congeners with significantly lower activity than taxol but offer lead compounds for investigating the effect of replacement of the oxygen with various heteroatoms. These modifications will be discussed in greater detail in the Results and Discussion portion of this work.

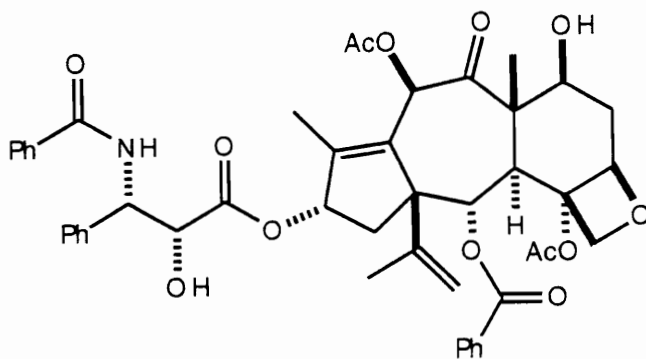
It is clear that the intact oxetane ring plays a vital role in ensuring effective binding on the microtubule,^{132,133} and although the exact function of the oxetane during the taxol-microtubule binding process is not well understood at the present time, it has been

suggested that the relatively inert oxetane ring imparts its effect simply by acting to maintain the conformation of the ring system necessary for optimal interaction.^{74,87,130} These observations, along with activity-guided purification of *Taxus* extracts in which all compounds displaying activity contain an oxetane ring (or a C-2 benzoate),⁴⁵ have led to the conclusion that the oxetane is a crucial feature of the taxol molecule.

From the observations summarized in this Section it is apparent that the functionalities of the southern hemisphere impart their effects on activity differently, either by direct interaction with the microtubule itself or by indirect influence of the conformational and/or electrostatic properties of other functionalities within the molecule. Unlike the northern hemisphere of taxol, the southern hemisphere plays a tremendously important role in microtubule binding, and hence, is largely responsible for the biological activity of the drug.

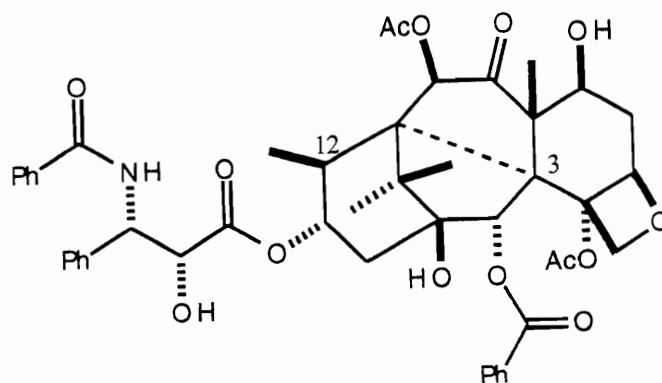
1.7.5 Modifications to the Taxol Ring System

A number of taxol derivatives have been prepared in which the taxane ring system has been modified. The first AB ring-contracted compound was reported by Kingston and was named A-nortaxol (**1.29**).¹³⁰ Compound **1.29** was shown to be nearly as active as taxol in the microtubule assembly assay but was also observed to be much less potent in the P-388 cytotoxicity assay.¹³⁰ Low-level molecular modeling of A-nortaxol shows this compound to be similar in its three-dimensional spatial characteristics to taxol, which may account for its comparable biological activity.⁷⁴



1.29

A transannular B-ring contracted taxol derivative has been reportedly prepared by irradiation of taxol in CCl_4 .¹³⁴ Intramolecular hydrogen transfer from the C-3 to the C-12 position, followed by the formation of a transannular bond affords compound **1.30**, which displays approximately 100 times less activity than taxol. The lowered activity was attributed to the presumed changes in molecular topology arising from ring contraction and the accompanying reduction in microtubule interaction capability. A number of BC ring modified taxol analogs have been reported¹³⁵ and these compounds typically exhibited *in vitro* activities comparable to taxol in several cell line assays. Their similarity in activity to taxol was ascribed to the retention of important structural features even after the ring system modifications had been carried out.

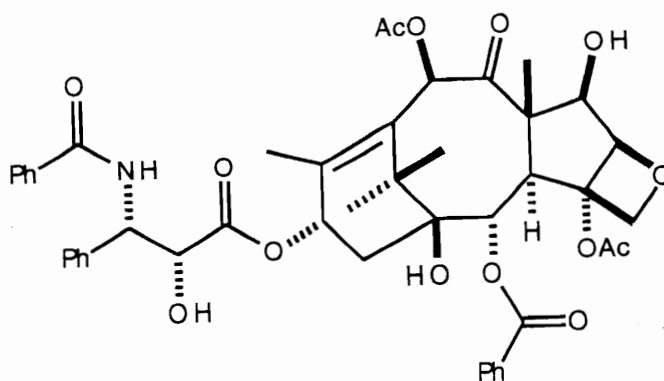


1.30

Very recently, a C ring contracted taxol analog has been produced, having the structure **1.31**.¹³⁶ This compound was found to be less effective in the promotion of microtubule assembly and less cytotoxic towards HCT116 cells than taxol, indicating that changes in the size and conformation of the C ring and the attached oxetane ring cause significant changes in the activity of taxol.

From these reports as well as others which have appeared,^{87,135,137} it is apparent that the effect on activity caused by ring modifications to the taxol skeleton is quite variable, and is

dependent on the extent of ring alteration and whether the resultant molecular topology resembles that of taxol closely enough to maintain effective binding properties.



1.31

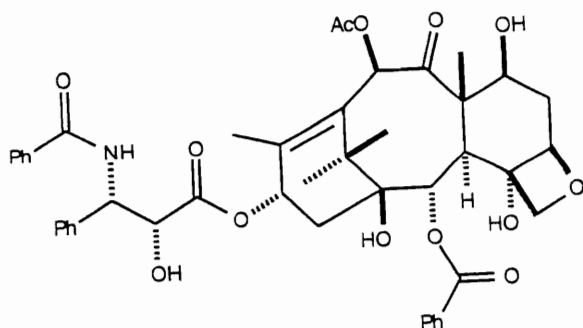
II. RESULTS AND DISCUSSION

2.1 Preparation of 4-Deacetyltaxol

2.1.1 Introduction

At the outset of this project relatively little was known about the structure-activity relationships of the so-called Southern Hemisphere of taxol (**1.8**). In particular, the effect of the acetate at the C-4 position on the overall activity of taxol was unknown. It was thus the major goal of this research to establish the necessity of the C-4 acetate group relative to overall activity and what effect replacement of the C-4 acetate with other acyl groups might display on biological activity.

In order to ascertain the importance of the C-4 acetate for biological activity, it was therefore necessary to prepare 4-deacetyltaxol (**2.1**) to permit direct comparison of its activity to that of taxol (**1.8**), as well as to serve as a lead compound for the investigation of the possible effects on activity of various C-4 acyl taxol analogs.



2.1

In theory, the most direct route to 4-deacetyltaxol would be from taxol itself. However, from the chemical behavior of taxol reported previous to the inception of this project,^{59b,c;131} it was well known that the side chain at C-13 of taxol is particularly prone to hydrolysis. It was thus surmised that any base-catalyzed hydrolysis conditions employed to effect the removal of the C-4 acetate would result in cleavage of the side chain, as well as probable

hydrolysis of the esters at C-2 and C-10 and epimerization of the C-7 hydroxyl group through a retro-aldol reaction. Considering these potential problems, it was envisioned that the cheaper and more readily available baccatin III (**1.13**) would serve as a reasonable starting material for the preparation of 4-deacetyltaxol, through appropriate chemical manipulation followed by attachment of a suitably protected side chain at the C-13 position and any necessary further chemical modifications.

2.1.2 Deacylation Studies of Baccatin III

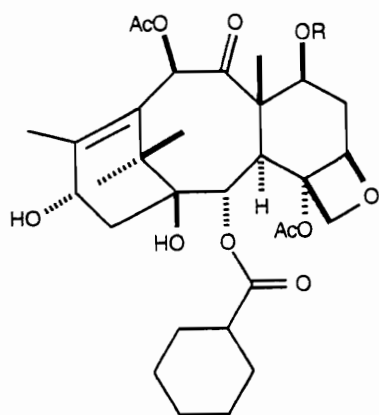
Prior to this study, the deacylation of baccatin III (**1.13**) and the subsequent reacylation of the resultant deacylation products had been investigated by Samaranayake.^{115,138} From this study, the following summary can be drawn:

- 1) Hydrolysis of an aliphatic ester was anticipated to occur more readily than that of a benzoate ester, so baccatin III was first subjected to hydrogenation over platinum to yield the hexahydro derivative **2.2**. The C-7 hydroxyl group of compound **2.2** was then protected as its triethylsilyl derivative **2.3** to prevent the known epimerization of this position under the basic conditions proposed for the hydrolysis of the C-4 acetate.
- 2) 7-(Triethylsilyl)hexahydrobaccatin III (**2.3**) was subjected to treatment with methanolic NaOMe under various conditions and it was found that the order of deacylation of this compound was C-10 > C-4 > C-2.
- 3) The resultant tris-deacyl compound **2.4** was then subjected to reacylation under a variety of conditions with different acylating reagents, and the order of reacylation was found to be C-13 > C-2 > C-10 >> C-4.

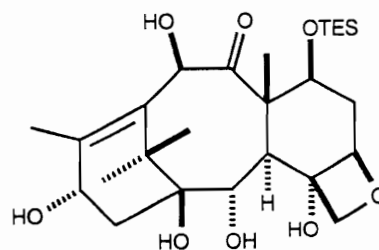
With this information, it was possible to devise a reasonable strategy for the conversion of baccatin III (**1.13**) to 4-deacetyltaxol (**2.1**), as depicted in Scheme 1.

Since the C-2 hexahydrocarbonyl of compound **2.3** apparently has the lowest reactivity of the ester functionalities within the molecule, based on the findings of the aforementioned investigation, it was reasoned that hydrogenation of baccatin III (**1.13**) was superfluous. A

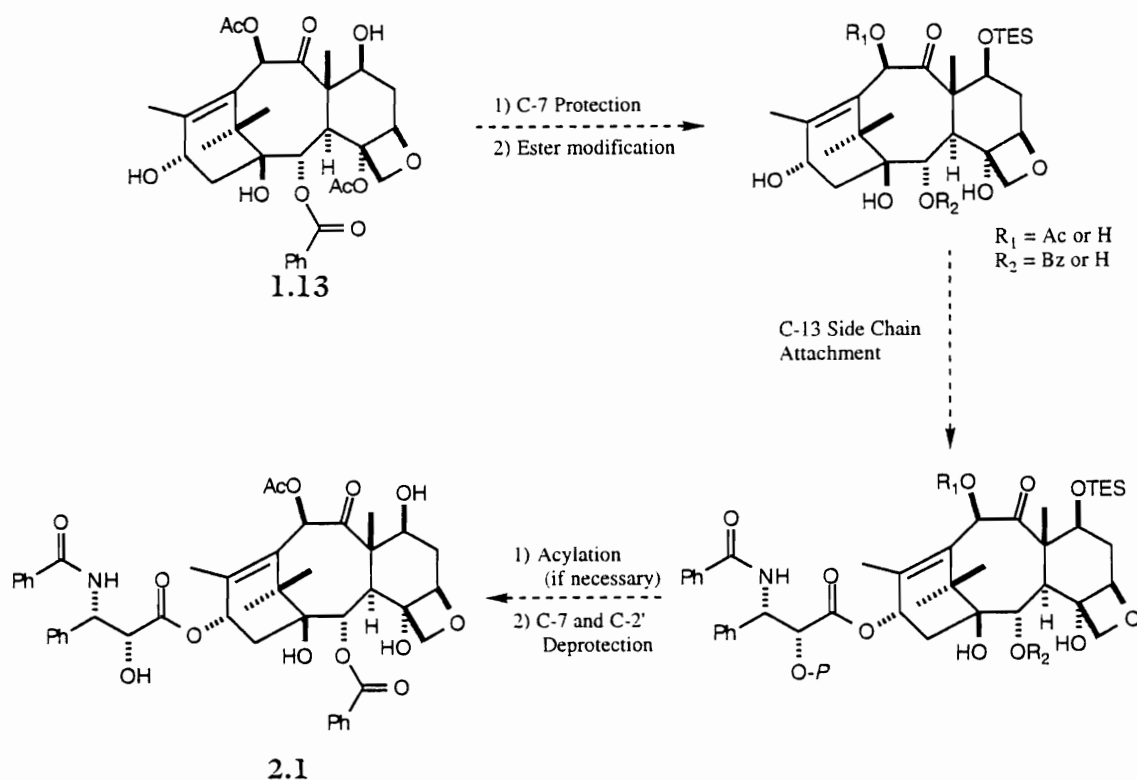
study of the reactivity of baccatin III itself was therefore initiated. Accordingly, baccatin III was first protected to afford 7-(triethylsilyl)baccatin III (**2.5**).



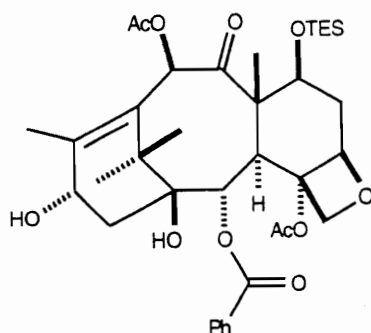
2.2: R = H
2.3: R = TES



2.4



Scheme 1. Proposed general synthetic approach toward 4-deacetyltaxol (**2.1**).



2.5

This compound was then subjected to methanolysis conditions, in order to establish whether it would exhibit the same relative order of deacylation as its hexahydro derivative 2.3. During the course of this reaction, a number of products were noted by TLC analysis, and subsequent purification and characterization of the products from the mixture showed these products to have the structures 2.4, 2.6, 2.7 and 2.8.

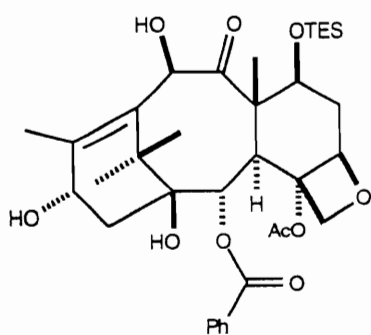
Compound 2.6 was identified as 10-deacetyl-7-(triethylsilyl)baccatin III based on its ^1H NMR spectroscopic and mass spectrometric data. In particular, the chemical shift of the C-10 methine proton moved upfield from 6.46 ppm in 2.5 to 5.16 ppm in 2.6. The presence of a peak for the C-2 proton at 5.59 ppm and five aromatic proton signals between 7.44 and 8.10 ppm led to the assessment that the C-2 benzoate remained intact in compound 2.6. The presence of a signal for the C-7 proton at 4.41 ppm and an acetate methyl signal at 2.27 ppm indicated that the C-4 acetate remained intact. This NMR information, combined with information acquired by FABMS analysis, namely, that the $[\text{MH}]^+$ peak at m/z 659 in the mass spectrum of 2.6 was consistent with a composition $\text{C}_{35}\text{H}_{50}\text{O}_{10}\text{Si}$ for this compound, led to the structure assignment for 2.6.

Compound 2.7 was identified as 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III. The ^1H NMR spectrum for this compound showed the C-10 methine proton to be at 5.21 ppm. The C-2 proton signal at 5.58 ppm appeared to be relatively unchanged from the starting material 2.6, while the signal for the C-7 proton appeared to be shifted upfield relative to compound 2.6, from 4.41 to 3.99 ppm, and the acetate signal at 2.27 ppm in the spectrum

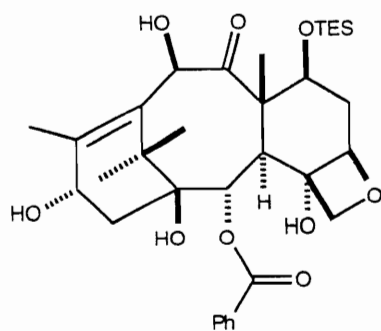
of **2.6** was lacking in the spectrum of **2.7**. The AB quartet for the diastereotopic C-20 protons showed a smaller chemical shift difference than that observed for compounds with an intact acetoxy group at C-4. Baccatin III derivatives typically show a chemical shift difference of 0.25 ppm for these protons, but in compound **2.7** the chemical shift difference was observed to be less than 0.15 ppm. The FABMS $[MH]^+$ peak at m/z 617, combined with the NMR spectral data, is consistent with a composition of $C_{33}H_{48}O_9Si$ and the structure assigned to compound **2.7**.

Compound **2.4** was identified as 4,10-bis(deacetyl)-2-debenzoyl-7-(triethylsilyl)baccatin III on the basis of its NMR and mass spectral data and by comparison of these data to those reported for the same compound prepared from the C-2 hexahydrobaccatin derivative **2.3**.^{115, 138} Specifically, in its ^1H NMR spectrum, the C-2 and C-10 methine protons showed appropriate upfield shifts relative to the starting material **2.5**, from 5.59 to 3.77 ppm for C-2 and from 6.46 to 5.10 ppm for C-10. The C-7 proton shift from 4.41 to 3.94 ppm and the lack of acetate peaks at 2.25 and 2.18 ppm were consistent with the structure assigned to compound **2.4**. Furthermore, the CIMS $[MH]^+$ peak at m/z 513 was consistent with the expected molecular mass of 512 for **2.4**.

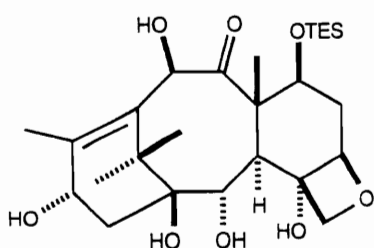
Compound **2.8**, the final compound isolated, was obtained as the most polar of the four products obtained under base hydrolysis conditions. By mass spectrometric analysis, this compound exhibited a molecular ion having the same mass units as compound **2.4**, namely, a CIMS $[MH]^+$ peak at m/z 513. However, by ^1H NMR analysis compound **2.8** displayed significant spectral differences from compound **2.4**, especially regarding the chemical shifts of the C-2, C-5 and C-20 protons. In particular, the signal for the C-2 methine proton shifted downfield from 3.77 ppm in **2.4** to 4.14 ppm in **2.8**; the signal for the C-5 proton, a doublet of doublets, shifted upfield from 4.76 ppm in **2.4**, with J values of 3 and 8 Hz, to 4.22 ppm in **2.8**, with J values of 8 and 10 Hz. The chemical shift of the AB quartet for the diastereotopic C-20 protons of **2.8** was observed to move upfield relative to compound **2.4**, from 4.55 to 3.76 ppm. Compound **2.8** was therefore identified as the rearrangement product of compound **2.4** based on this characterization data, as well as further characterization of the products from the acetylation experiments described below.



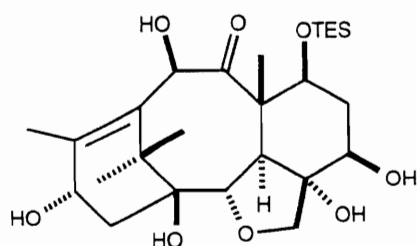
2.6



2.7



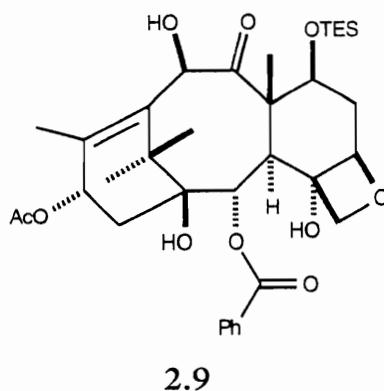
2.4



2.8

Investigations of the methanolysis of 7-(triethylsilyl)baccatin III (2.5) were carried out to monitor the time course of product formation and to explore the relative lability of the various ester functionalities under controlled hydrolysis conditions. These studies led to the observation, as evidenced initially by TLC, and subsequently by ^1H NMR and mass spectrometric analyses, that under base-catalyzed hydrolysis conditions, the 10-deacetyl product 2.6 is formed initially, followed sequentially by the formation of the 4,10-dideacetyl product 2.7, the 4,10-dideacetyl-2-debenzoyl product 2.4, and the isomeric 4,10-dideacetyl-2-debenzoyl product 2.8, thus indicating the order of deacylation to be C-10 > C-4 > C-2, analogous to the order seen in the hexahydrobaccatin series.^{115,138} That the tertiary C-4 acetate is hydrolyzed more readily than the secondary C-2 benzoate ester is somewhat surprising, albeit similar to the observations reported for methanolysis of the C-2 hexahydrobaccatin derivative 2.3.^{115,138} The most reasonable explanation for this

observation is that an intramolecular acetyl transfer from the C-4 position to the C-13 position occurs in compound **2.6** to give the intermediate ester **2.9**, following which a facile hydrolysis of the resultant C-13 acetate group occurs, leading to compound **2.4**.^{115,138}



Indeed, a three-dimensional representation (CSC Chem3D Plus, v.3.1) of compound **2.6** (Figure 4) or a suitable molecular model shows that, given the close proximity of the C-13 hydroxyl group to the C-4 acetate, such an intramolecular acetate transfer is possible.

Furthermore, investigations of the reaction of 7,13-bis(triethylsilyl)hexahydrobaccatin III (**2.10**) under methanolysis conditions by Samaranayake^{115,138} have shown that no C-4 acetate hydrolysis occurs. Rather, the C-10 acetate is readily hydrolyzed, followed by a more sluggish hydrolysis of the C-2 cyclohexanecarbonyl, with no observable hydrolysis of the C-4 acetate. This seems to indicate that the C-13 hydroxyl group plays a vital role during the hydrolysis of the C-4 acetate.

A very recent publication by Georg and co-workers¹³⁹ further substantiates the proposed intramolecular acetate transfer mechanism. It was reported that treatment of 7,13-bis(triethylsilyl)baccatin III (**2.11**) with potassium *tert*-butoxide exhibited a different reactivity than that observed by Samaranayake for compound **2.10**; specifically, only the 2-debenzoylated product was observed. That no C-10 acetate hydrolysis was reported may be attributed to: 1) the reaction conditions employed by Georg; 2) the conformational changes within the baccatin ring system generated by the triethylsilyl protecting group at the C-13 position, thereby resulting in a lower degree of accessibility to the C-10 acetate by the

inherently more bulky base; or 3) to a combination of these factors. The fact that no C-4 acetate hydrolysis was observed for 7,13-bis(triethylsilyl)baccatin III (2.11) led Georg¹³⁹ to independently conclude that an intramolecular acetate transfer mechanism apparently accounts for the lability of the C-4 acetate under hydrolysis conditions, in agreement with the conclusions drawn by Samaranayake.^{115,138}

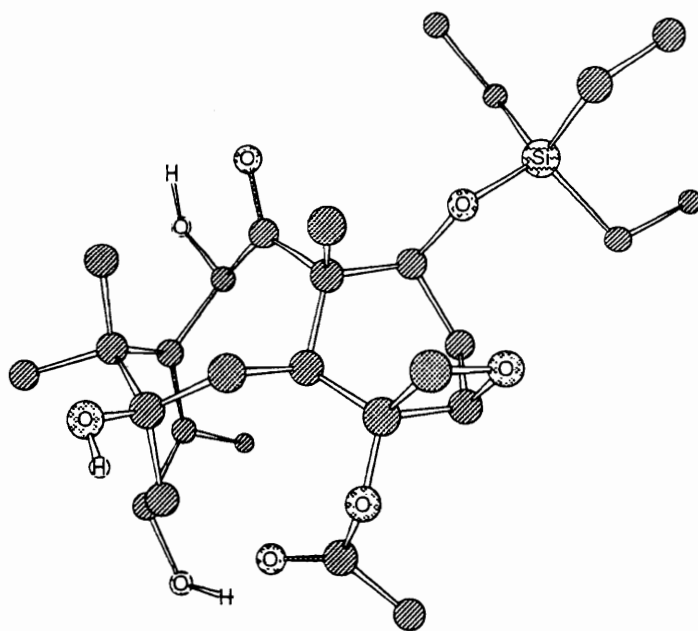
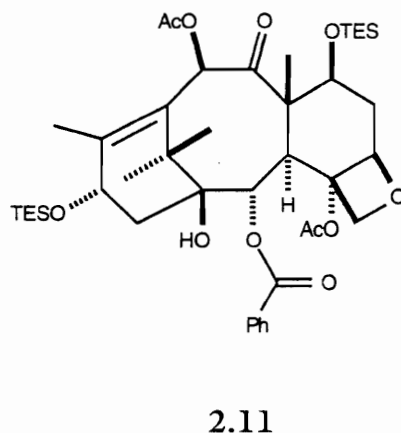
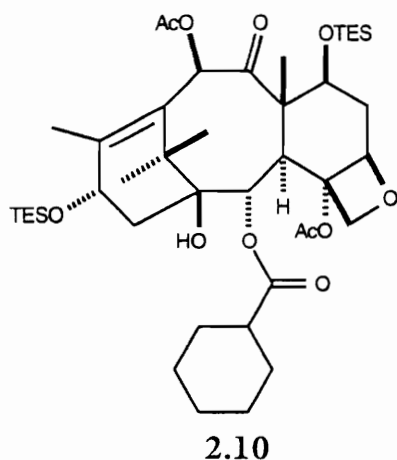
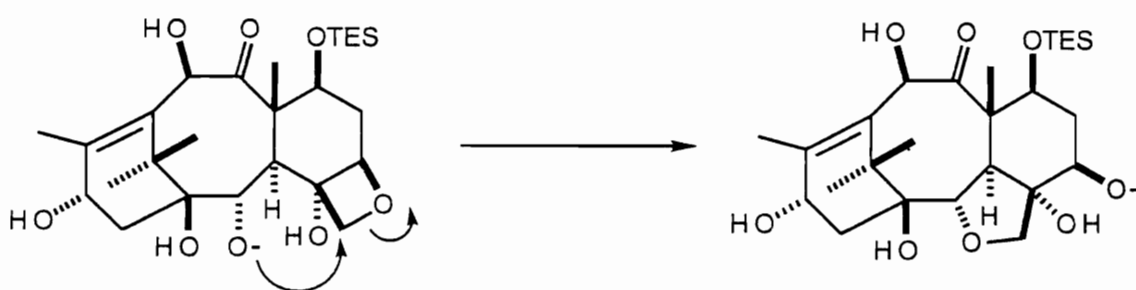


Figure 4. Three-dimensional representation depicting proximity of C-4 and C-13 positions. [For clarity the C-2 benzoate and protons on carbons are not shown.]

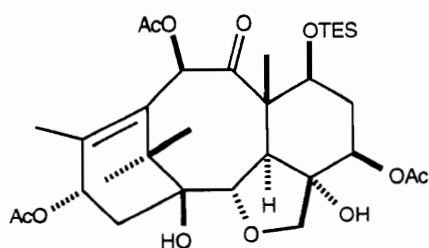
The formation of the rearranged baccatin III derivative **2.8**, which was given the name 4,10-bis(deacetyl)-2-debenzoyl-7-(triethylsilyl)isobaccatin III, was also an unexpected outcome of the methanolysis of 7-(triethylsilyl)baccatin III (**2.5**), and was found to be particularly prevalent with prolonged methanolysis reactions. It was rationalized that the formation of the isomeric product from compound **2.4** can be attributed to an intramolecular S_N2 -type reaction of the C-2 oxyanion with the C-20 carbon of the nearby oxetane (see Scheme 2), since it is well known that oxetanes can readily undergo such ring openings via treatment with nucleophilic reagents.¹⁴⁰

In order to provide more conclusive evidence for the structure of **2.8**, the compound was subjected to acetylation under standard conditions. From this reaction two products were obtained and these compounds were identified as the triacetate **2.12** and the tetraacetate **2.13**. Compound **2.12**, which was the major product and was the more polar of the pair, displayed a ^1H NMR spectrum in which the chemical shift of the C-2 proton was essentially unchanged from the starting material **2.8**. The signal for the C-5 proton of **2.12**, however, was shifted downfield relative to the starting material **2.8**, from 3.79 to 5.19 ppm, indicating that acetylation had occurred at the C-5 position. The chemical shift values for the C-10 and C-13 protons were observed to be shifted downfield, consistent with the expected facile acetylation at these positions. Mass spectral analysis of compound **2.12** indicated an increase in molecular weight of 126 mass units relative to the starting

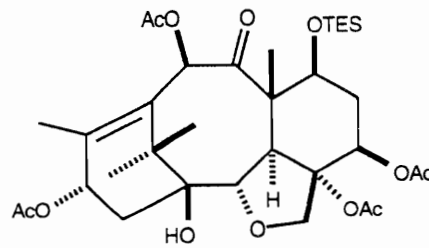


Scheme 2. Intramolecular S_N2 -type displacement leading to isomerization.

material **2.8**, corresponding to the addition of three acetates. Correlation of the above data thus led to the assignment of compound **2.12** as the triacetate.



2.12



2.13

Compound **2.13**, obtained only in low yield from the acetylation of **2.8**, also displayed downfield ^1H NMR chemical shifts in the signals for the C-5, C-10, and C-13 protons, indicative of acetylation at these positions. Additionally, the ^1H NMR spectrum for **2.13** also reflected a downfield shift for the C-7 proton relative to the starting compound **2.8**, from 3.95 to 4.54 ppm, apparently signifying the presence of an additional acetate at C-4. Indeed, mass spectral analysis of compound **2.13** showed an increase in molecular weight of 168 mass units relative to compound **2.8**, leading to the assignment of **2.13** as the tetraacetate.

The stereochemistry of the C-5 hydroxyl or acetoxyl group in compounds **2.8**, **2.12** and **2.13** was initially assigned based on the postulated intramolecular $\text{S}_{\text{N}}2$ -type displacement by the C-2 oxygen on the C-20 position of the oxetane ring, as discussed above and outlined in Scheme 2. Further evidence to support this assumption comes from the observed coupling constants for the C-5 proton in each of these compounds. Thus the C ring of compounds **2.8**, **2.12** and **2.13** is predicted to be in a boat conformation, as shown both by low-level molecular mechanics calculations (using CSC Chem3D Plus, v.3.1) and by the coupling constants of the C-6 and C-7 protons. Accordingly, for compound **2.8** the two $J_{6,7}$ values are calculated to be 8 and 4 Hz for the boat conformation and 6 and 1 Hz for the chair conformation; the observed values of 10 and 8 Hz are clearly in better agreement with the boat conformation. The observed $J_{5,6}$ values of 4 and 9 Hz for this

conformation with a 5β -OH group configuration thus fit well with the calculated values of 3 and 8 Hz; conversely, the 5α configuration would require coupling constants of about 8 and 0.5 Hz. Similar arguments hold for the configuration of the acetates **2.12** and **2.13**. Furthermore, comparison of the characterization data for compounds **2.8**, **2.12** and **2.13** to literature data reported independently soon after this investigation was carried out^{141,142} confirm the structural assignments made for these compounds. Specifically, our data correspond well with that reported for compound **2.8**, formed similarly but independently by the base-catalyzed hydrolysis of 10-deacetyl-7-(triethylsilyl)baccatin III,¹⁴¹ and an analogous isomerization product, reportedly obtained after treatment of 7,13-diacetylbaccatin III with tributyltin methoxide in 1-methyl-2-pyrrolidinone.¹⁴²

While the formation of the isobaccatin III **2.8** was initially observed under basic conditions, it was suspected that the rearrangement of compound **2.4** to the isomeric **2.8** could prove to be more facile under acidic conditions, since the yield of **2.8** obtained from a reaction varied depending upon workup conditions. The lowest yields of **2.8** were observed when the workup involved careful neutralization of the base, while the highest yields were noted when an excess of acid was used to quench the reaction. A study was thus conducted to investigate the formation of **2.8** from **2.4** under various conditions. Treatment of a small quantity of the tris(deacetyl)baccatin III derivative **2.4** with either acidic (AlCl_3 , 1M HCl or *para*-toluenesulfonic acid [TsOH]) or basic (4-dimethylaminopyridine [DMAP] or NaOMe) reagents showed the starting material to be converted over varying times to a more polar product which, upon TLC comparison to a known standard, confirmed the suspected isomerization of **2.4** to **2.8** (see Table 4). In the acid series the relative conversion rate was $\text{AlCl}_3 > 1\text{M HCl} > \text{TsOH}$, with each giving complete conversion within 60 minutes. Relative conversion was much slower in the basic series, with NaOMe affording complete conversion only after two hours, and DMAP affording only partial conversion even after two hours.

2.1.3 Taxol Side Chain Preparation

Having established that 7-(triethylsilyl)baccatin III (**2.5**) could serve as a practical starting material for preparation of the synthetically important tris(deacetyl)baccatin III derivative **2.4**, and that isomerization of **2.4** to **2.8** could be minimized by control of the reaction conditions and/or work-up procedure, the focus of the project shifted to the preparation of a suitable taxol side chain, with which to continue the synthesis of 4-deacetyltaxol (**2.1**) as previously outlined in Scheme 1. At this point in time a number of side chain syntheses had been reported in the literature,^{89,91-96,143-145} including the β -lactam of Holton,⁹⁶ compound **2.14**, and the ethoxyethyl ether-protected taxol side chain acid **2.15**, via the synthetic methodologies of DeAmicis,⁸⁹ Greene^{145a} and Ojima.^{145c}

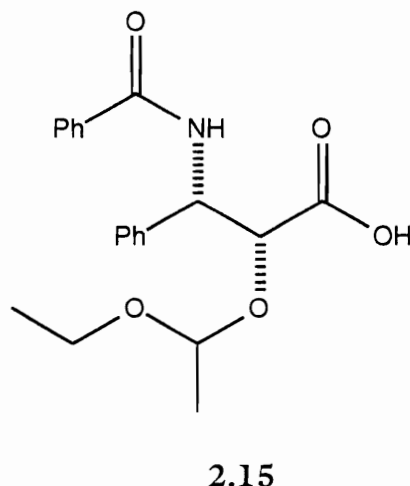
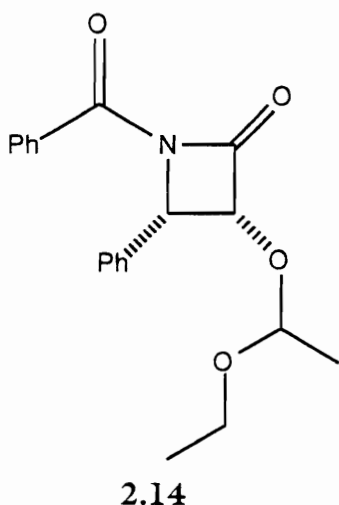


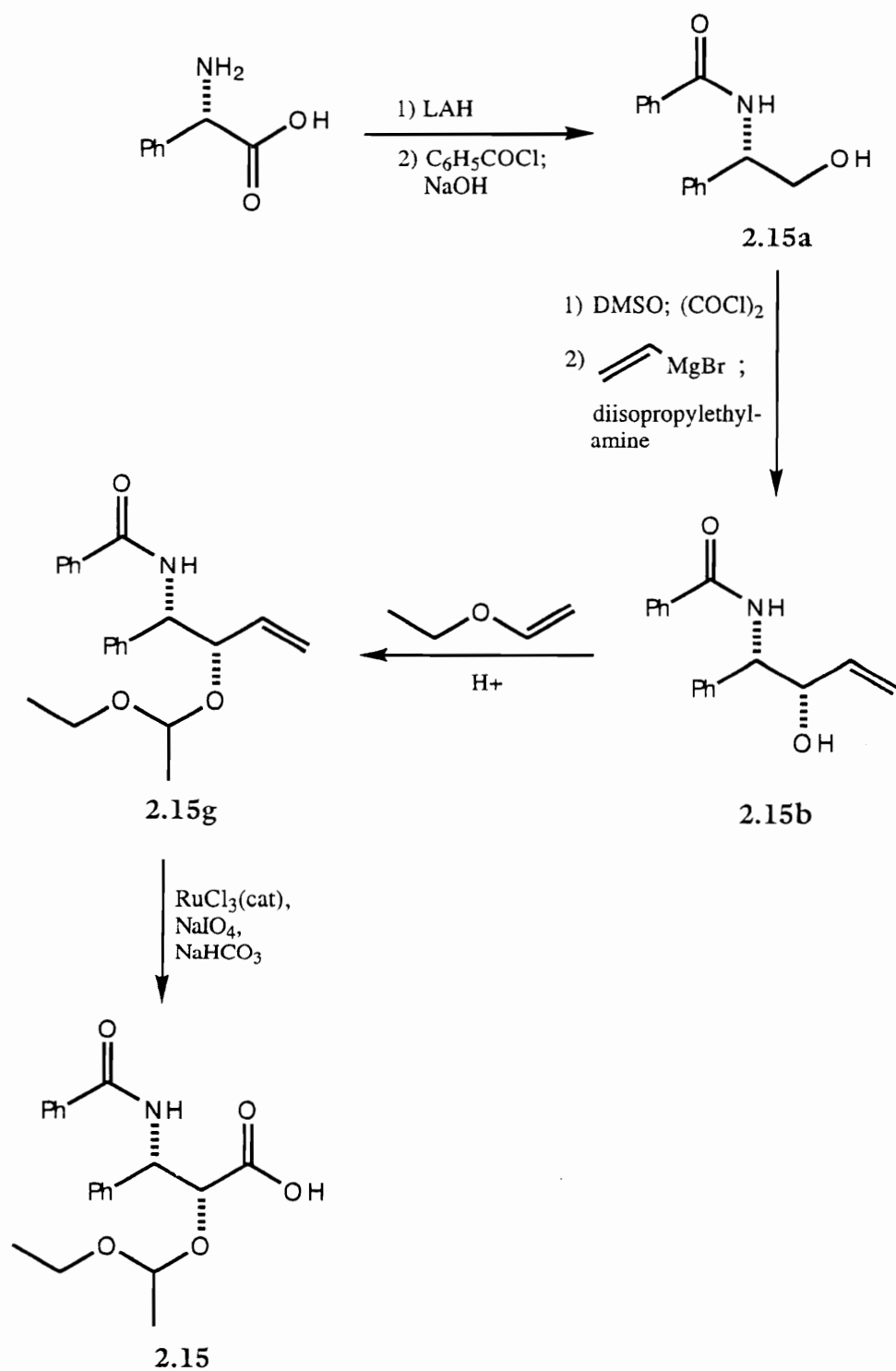
Table 4. Conditions for the conversion of 2.4 to 2.8.

Experiment*	Solvent	Acid/Base	Conversion time
1	CH ₂ Cl ₂	TsOH	1 hour
2	CH ₂ Cl ₂	AlCl ₃	0.5 hours
3	THF	dilute HCl	1 hour
4	CH ₂ Cl ₂	DMAP	> 2 hours
5	MeOH	NaOMe	2 hours

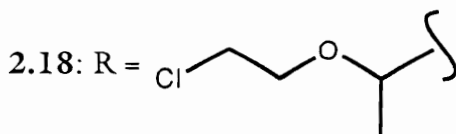
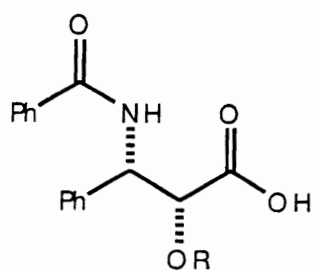
* Each experiment was conducted at room temperature using 640 μ g of **2.4**, 0.5mL solvent and a catalytic amount of either acid or base.

It was decided that the methodology of Greene and coworkers^{145a} would be the most practical route, and thus the side chain **2.15** was prepared according to Scheme 3. While the synthesis of compound **2.15** by Greene's synthesis was straightforward and gave reasonably good yields as determined by initial NMR and TLC analyses, it was found that the compound was inherently unstable, decomposing completely within a time range of 15 minutes to 24 hours at room temperature. Attempts to store the compound at lower temperatures (-20 to -78 °C), as suggested by the authors, increased the lifetime of the compound only marginally, up to a maximum of 36 hours. This instability was presumed to be due to the acid-sensitive nature of the ethoxyethyl ether protecting group and its close proximity to the carboxylic acid functionality of the molecule. Furthermore, model coupling reactions of the side chain acid (**2.15**) with 7-(triethylsilyl)baccatin III (**2.5**) gave disappointingly low yields, on the order of 20 to 30%. Initially, the low yields were attributed variously to the instability of the side chain, which was presumed to have undergone self-condensation under the coupling conditions employed, or to a lack of reactivity of the C-13 hydroxyl of the baccatin III substrate, or to the steric demands of the side chain acid itself. To establish whether the low yields were in fact due to an inherent instability of compound **2.15**, Greene's methodology^{145a} was adapted to substitute several different protecting groups for the ethoxyethyl ether, affording compounds **2.16**, **2.17** and **2.18**. These protecting groups were selected on the basis of their comparatively lower sensitivity to acidic conditions than the ethoxyethyl ether initially employed. Of particular note is that the protecting group selected for use in compound **2.18**, the chlorinated analog of the protecting group used in the original side chain **2.15**, is on the order of 20 to 300 times more stable toward acid-catalyzed hydrolysis than its non-chlorinated relative, depending on the nature of the acid used.¹⁴⁶

While the side chain acids **2.16**, **2.17** and **2.18** proved to be exceptionally stable based on ¹H NMR analyses over the course of several weeks, each gave only modest yields when coupled with 7-(triethylsilyl)baccatin III (**2.5**), again on the order of 20 to 30%.



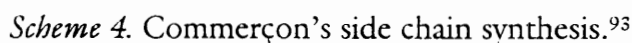
Scheme 3. Preparation of the taxol side chain acid **2.15** via Greene's methodology.^{145a}

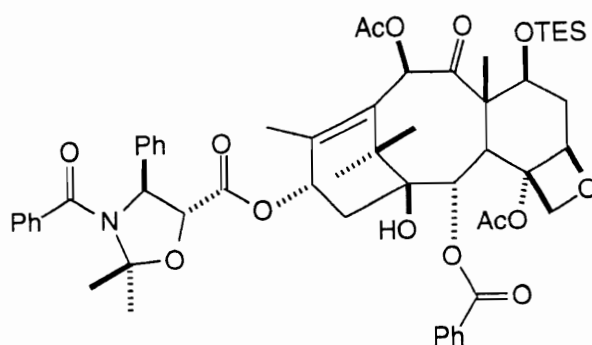


In a model study to determine the relative reactivity of the C-13 hydroxyl group of the baccatin substrate **2.5**, phenylacetic acid was added to **2.5** under standard coupling conditions (DCC/DMAP). It was observed that coupling readily occurred within 34 hours and in moderate yield (~ 50%). This information, combined with the information obtained from the coupling studies described above for the side chains **2.16**, **2.17** and **2.18**, led to the presumption that the low coupling yields observed with each of the side chains could mainly be attributed to the steric demands of the side chains, and in particular, to the close proximity of the rather bulky protecting groups employed to the carboxylic acid functionality. With this in mind, it was thought that a sterically less demanding protecting group on the side chain hydroxyl group might allow a greater yield of coupled product to be realized. At this point in the project a new taxol side chain analog **2.19** had been recently reported by Commerçon,⁹³ the preparation of which is shown in Scheme 4.

It was reasoned that although side chain **2.19** would require subsequent modification of the amido group after the proposed coupling to baccatin or its derivatives in order to realize analogs having the taxol side chain, the acetonide protecting group would effectively serve as a sterically less demanding appendage relative to the protecting groups employed previously, with the additionally attractive feature of being conveniently tethered back and away from the operative portion of the molecule, thus reducing steric hindrance to the coupling process. With this target compound in mind, it was further rationalized that rather than employing Commerçon's approach⁹³ to **2.19**, the methodology of Greene^{145a} could

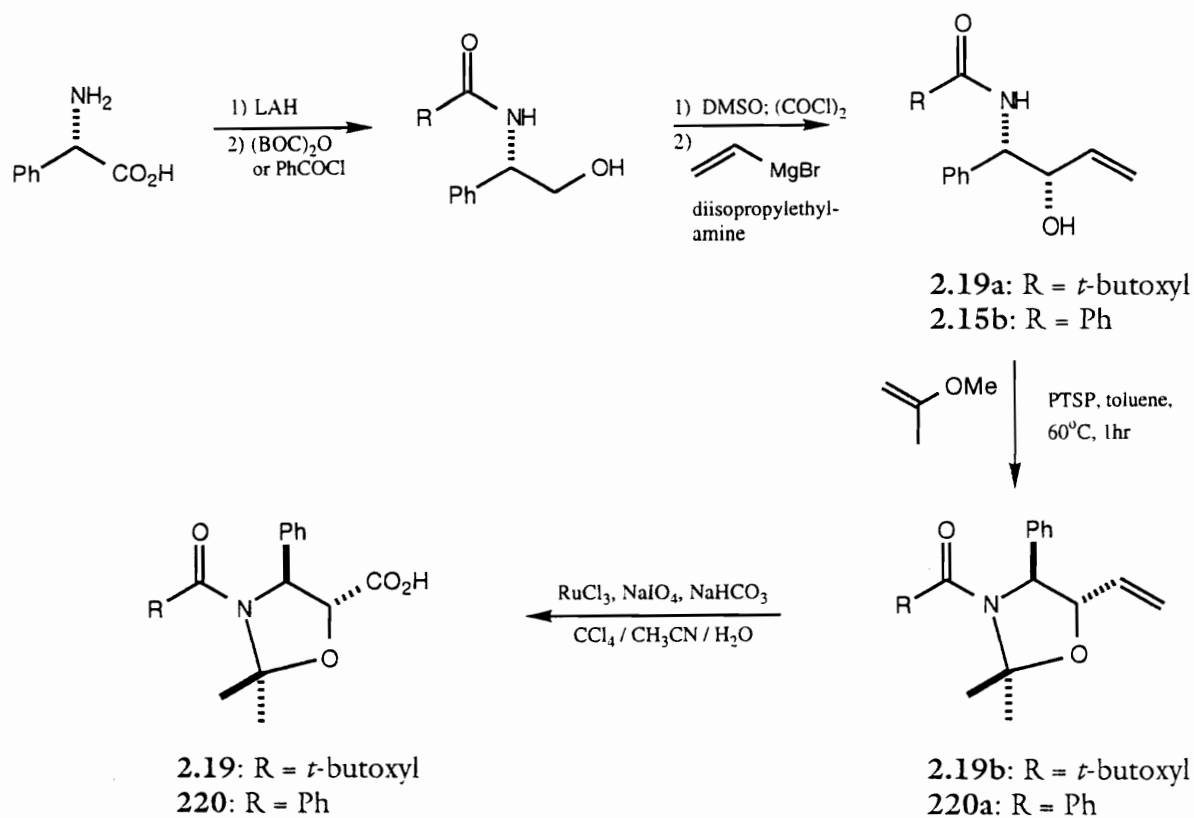
Since coupling of compound **2.20** to a given baccatin substrate would theoretically entail only one further step, namely deprotection of the 7-(triethylsilyl) group and removal of the side chain *N,O*-acetonide, it was thought that the preparation of side chain **2.20** would be the more reasonable approach. Thus, according to the methodology outlined in Scheme 5, compound **2.20** was prepared in good yield, following which it was subjected to model coupling studies with 7-(triethylsilyl)baccatin III (**2.5**). The coupling proved to be quite facile, occurring within 45 minutes to 2 hours and giving product **2.21** in moderate yields from 40 to 70%, apparently indicating that the steric and/or spatial disposition of the protecting group on the side chain directly influences the extent of coupling to a baccatin substrate.





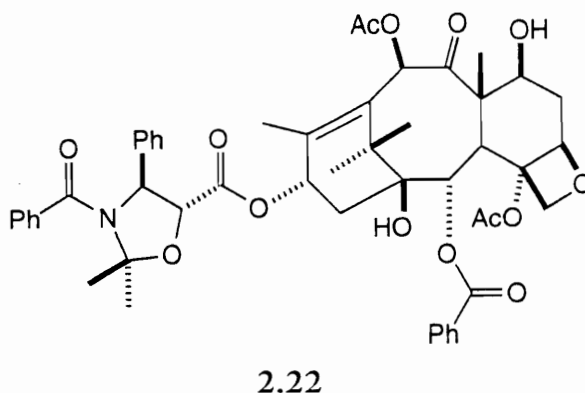
2.21

The protected taxol **2.21** from the coupling reaction was next subjected to deprotection using 99% formic acid, following the deprotection conditions employed by Commerçon.⁹³ After 2 hours, a more polar product was observed by TLC analysis. After isolation, the



Scheme 5. Combination of methodologies for improved side chain.

product was characterized by ^1H NMR spectroscopy and mass spectrometry. The spectral data indicated that removal of the 7-(triethylsilyl) group had occurred, but that the side chain *N,O*-acetonide remained intact. Prolonged treatment of the partially deprotected compound **2.22** with 99% formic acid (4 to 16 hours) showed no change in the compound both by TLC and ^1H NMR analyses.



A series of experiments was then carried out on compound **2.21** to determine whether both the 7-(triethylsilyl) group and the *N,O*-acetonide could be removed under various deprotection conditions. The results of these experiments are summarized in Table 5. From this series of experiments it was observed that under all the conditions employed no *N,O*-acetonide deprotection was effected.

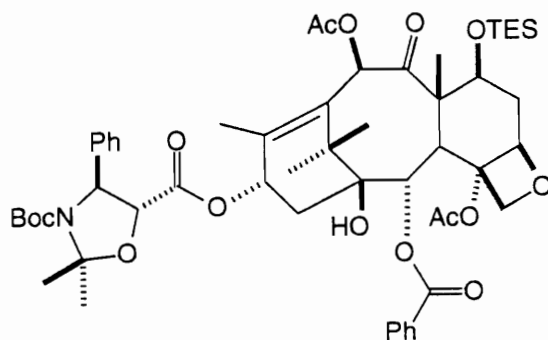
Table 5. Conditions employed to attempt full deprotection of 2.21.

Experiment*	Conditions	Temperature and time	Result
1	99% HCO_2H	r.t.; 12 hours	only TES group removed
2	5% HCl/MeOH	r.t.; 8 hours	only TES group removed
3	1 <i>N</i> HCl/THF	r.t.; 12 hours	only TES group removed
4	6 <i>N</i> HCl/THF	r.t.; 3 hours	product decomposed
5	6 <i>N</i> HCl/THF	reflux; 1 hour	product decomposed
6	4 <i>N</i> $\text{H}_2\text{SO}_4/\text{H}_2\text{O}$	r.t.; 2 hours	product decomposed
7	4 <i>N</i> $\text{H}_2\text{SO}_4/\text{MeOH}$	r.t.; 8 hours	only TES group removed
8	99% $\text{HCO}_2\text{H}/\text{CH}_2\text{Cl}_2$ (2:3)	r.t.; 12 hours	only TES group removed

* Each experiment was conducted using 1.0 mg of **2.21** and 0.5 mL of the solution indicated.

Thus, having been unsuccessful at fully deprotecting compound **2.21** derived from coupling of 7-(triethylsilyl)baccatin III (**2.5**) with side chain **2.20**, it was decided that the use of side chain **2.19**, while entailing an additional *N*-benzoylation step after deprotection, might be a more fruitful approach since deprotection of the *N,O*-acetonide of this side chain was not reported to be problematic by Commerçon.⁹³ Presumably, deprotection of the protected taxol derivative obtained from side chain **2.19** operates through a different mechanism than the attempted deprotection of the protected taxol obtained from side chain **2.20**. Again implementing the methodology shown in Scheme 5, compound **2.19** was realized in good yield (~20% overall yield from phenylglycine). Compound **2.19** was subjected to a model coupling with 7-(triethylsilyl)baccatin III (**2.5**), affording the protected taxol derivative **2.23** in greater than 90% yield.

To ensure that the coupled compound **2.23** could be deprotected fully, it was treated with 99% formic acid for 3 hours, yielding a very polar product (baseline region by TLC analysis) which was then, without purification or characterization, subjected to *N*-benzoylation via Schotten-Baumann conditions.¹⁴⁷ The major product of the *N*-benzoylation reaction was determined to be taxol (**1.8**) by comparison of its ¹H NMR spectrum to known spectral information, and was prepared in 40% yield from compound **2.23**.

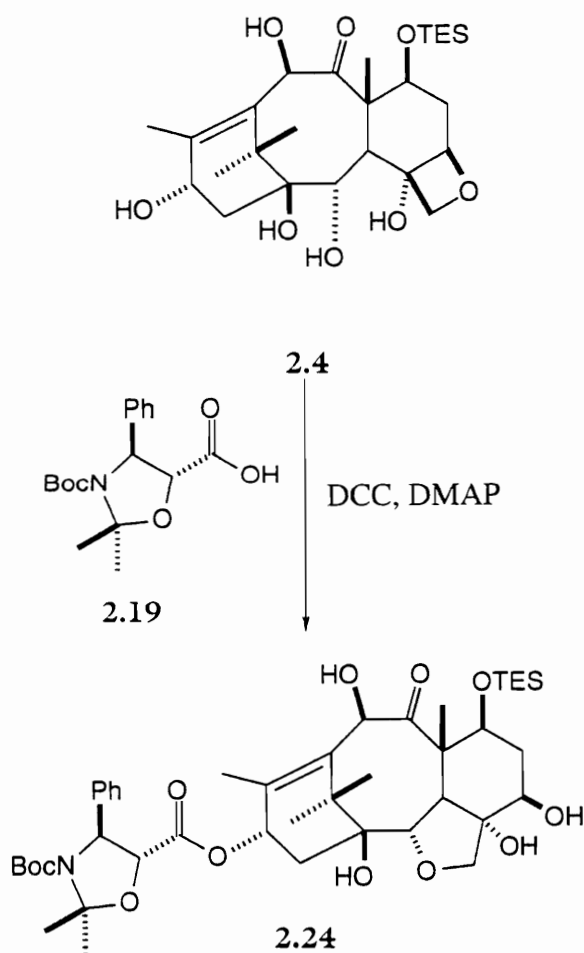


2.23

2.1.4 Coupling of 2,4,10-Tris(deacyl)-7-(triethylsilyl)baccatin III (2.4) with Side Chain 2.19

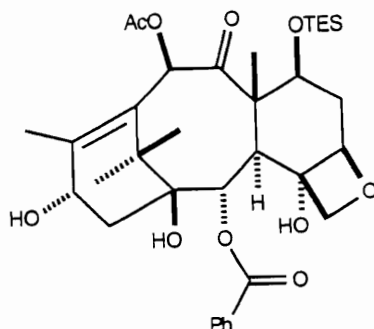
With a suitable side chain having been prepared and having shown promising ability for coupling, the focus of the research shifted once again back to the proposed coupling of 2.19 to 2,4,10-tris(deacyl)-7-(triethylsilyl)baccatin III (2.4). Upon the actual implementation of the coupling reaction, it was found that the side chain 2.19 did indeed couple to the C-13 hydroxyl group of the tris(deacyl)baccatin substrate 2.4 in good yield within 3 hours; however, presumably under the coupling conditions employed, the baccatin portion of the molecule isomerized to the tetrahydrofuranyl derivative 2.24 (see Scheme 6), in a manner analogous to the isomerization of tris(deacyl)baccatin III 2.4 to the isobaccatin analog 2.8, mentioned in the preceding discussion. Previous findings from our group^{63a} had indicated that the corresponding tetrahydrofuranyl derivative of taxol is significantly less active than taxol, and as a result it was deemed necessary to modify the original synthetic strategy toward 4-deacetyltaxol (2.1). In order to preclude the possibility of isomerization, it was obvious that either the C-2 benzoate would have to be retained or the the C-2 hydroxyl would require sufficient protection. Regarding the number of additional steps the latter course indicated, and in light of what was known about the relative deacylation order for 7-(triethylsilyl)baccatin III (2.5), it was decided that the first course would be more practical. From the deacylation studies carried out on 7-(triethylsilyl)hexahydrobaccatin III^{115,138} and on 7-(triethylsilyl)baccatin III (2.5), as mentioned above, it was known that 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7) could be isolated in varying quantities from the treatment of 7-(triethylsilyl)baccatin III with methanolic NaOMe. Considering the possibility that treatment of 7-(triethylsilyl)baccatin III with a different base might result in greater selectivity (i.e., retention of the C-2 benzoate), a series of reactions was conducted to study the effect of the nature of the base on ester hydrolysis, the results of which are summarized in Table 6.

Thus it was found from this study that potassium *tert*-butoxide afforded the desired 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7) in the highest yield, 72%. After this initial study it was found through another series of experiments, using quantities of potassium *tert*-butoxide ranging from 1.1 to 10 equivalents, that the yield of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7) could be optimized to afford greater than 90% yield by using at least 5 equivalents of potassium *tert*-butoxide. It was also observed by TLC, ^1H NMR and mass spectrometric analyses that in every case the C-10 acetate is hydrolyzed more readily than the C-4 acetate. Interestingly, subsequent to the completion



Scheme 6. Isomerization observed upon coupling of 2.4 and 2.19.

of these studies, and as reported recently by Georg and co-workers,¹³⁹ it was found that treatment of 7-(triethylsilyl)baccatin III (2.5) with 1.1 equivalents of potassium *tert*-butoxide, from -20 to 0 °C, over 30 to 45 minutes, yielded 4-deacetyl-7-(triethylsilyl)baccatin III (2.25) as the major product (58% yield), while no corresponding 10-deacetyl compound was reported.



2.25

In light of these different observations, a study was undertaken by us utilizing the conditions employed by Georg,¹³⁹ and it was found from a series of reactions that even after reaction times as long as 4 hours at 0 °C, 4-deacetyl-7-(triethylsilyl)baccatin III (2.25) was formed only to a small extent (less than 20% based on recovered starting material). This would seem to indicate that in our hands the selectivity and yield of product reported by Georg was not reproducible, even after extended reaction times. It was reasoned that the overall differences in selectivity observed can be mainly attributed to the more vigorous conditions employed by us versus the milder conditions employed by Georg.¹³⁹

Table 6. Base-catalyzed formation of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7) from 7-(triethylsilyl)baccatin III (2.5).

Experiment	Conditions	Temperature and time	% Yield of 2.7
1	NaOMe (10 equivalents), MeOH	r.t., 30 minutes	66.8
2	NaOEt (10 equivalents), EtOH	r.t., 30 minutes	33.3
3	LiO <i>i</i> -Pr (10 equivalents), <i>i</i> -PrOH	r.t., 2.5 hours	64.9
4	KO <i>t</i> -Bu (10 equivalents), THF	r.t., 15 minutes	72.2

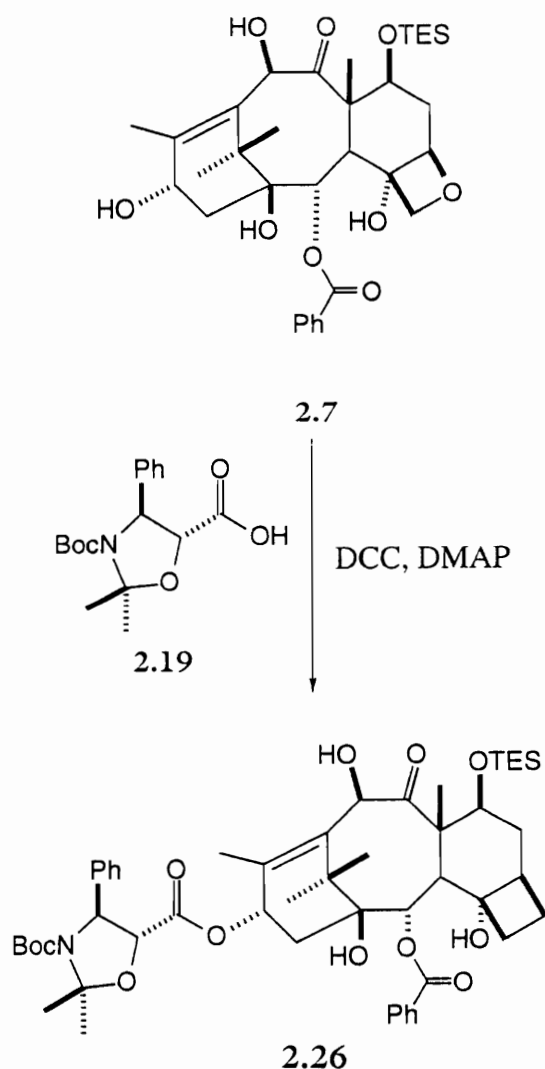
During the time that the base studies were being conducted, as discussed above and summarized in Table 6, and prior to Georg's publication¹³⁹ of the formation of 4-deacetyl-7-(triethylsilyl)baccatin III (**2.25**) from 7-(triethylsilyl)baccatin III (**2.5**) using potassium *tert*-butoxide, an important discovery was made in our labs. It was found, through a series of experiments directed at ascertaining the reactivity and chemistry of 7-(triethylsilyl)baccatin III (**2.5**) upon reaction with various reagents, that treatment of 7-(triethylsilyl)baccatin III (**2.5**) with lithium aluminum hydride yielded 4-deacetyl-7-(triethylsilyl)baccatin III (**2.25**). The formation of this product, while giving a disappointingly low yield of 10%,¹⁴⁸ afforded the opportunity to investigate its potential use for coupling with the side chain **2.19**. It was envisioned that such a coupling would entail one less step than coupling of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (**2.7**) with the same side chain (i.e., subsequent acetylation of the C-10 hydroxyl group).

2.1.5 Coupling of 4,10-Bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7**) with Side Chain **2.19****

From the reacylation studies^{115,138} of 2,4,10-tris(deacetyl)-7-(triethylsilyl)baccatin III (**2.4**), it was well established that the order of reacylation is C-13 > C-2 > C-10 >> C-4. Thus, it was presumed that reaction of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (**2.7**) with the side chain acid **2.19** would afford the desired C-13 acylated product **2.26** preferentially over the corresponding C-10 acylation product, as depicted in Scheme 7. Indeed, when the proposed coupling reaction was carried out using the side chain acid **2.19** and the baccatin III derivative **2.7**, the major product was purified and identified as having the structure **2.26**, based on its ¹H NMR and mass spectral data. Specifically, the ¹H NMR spectrum of compound **2.26** showed the C-13 methine proton to be shifted downfield relative to the starting material **2.7**, from 4.89 to 5.89 ppm, as expected for acylation of this position. The C-10 methine proton of **2.26** appeared at 5.20 ppm, relatively unchanged from the starting material **2.7**, indicating that no acylation had occurred at this position. Other signals in the ¹H NMR spectrum were consistent with the structure assigned and this assignment was further substantiated by the CIMS [MH]⁺ peak at *m/z* 920.

2.1.6 Acetylation of Compound 2.26

Again, with the knowledge of the relative order of reacetylation,^{115,138} it was assumed that compound **2.26** would give preferential reacetylation at the C-10 hydroxyl group, affording the 4-deacetyltaxol derivative **2.27**. On this assumption, compound **2.26** was subjected to standard acetylation conditions (Ac_2O /DCC/DMAP) and produced in a reasonable yield of 65% the 4-deacetyltaxol derivative **2.27**, as depicted in Scheme 8. The ^1H NMR

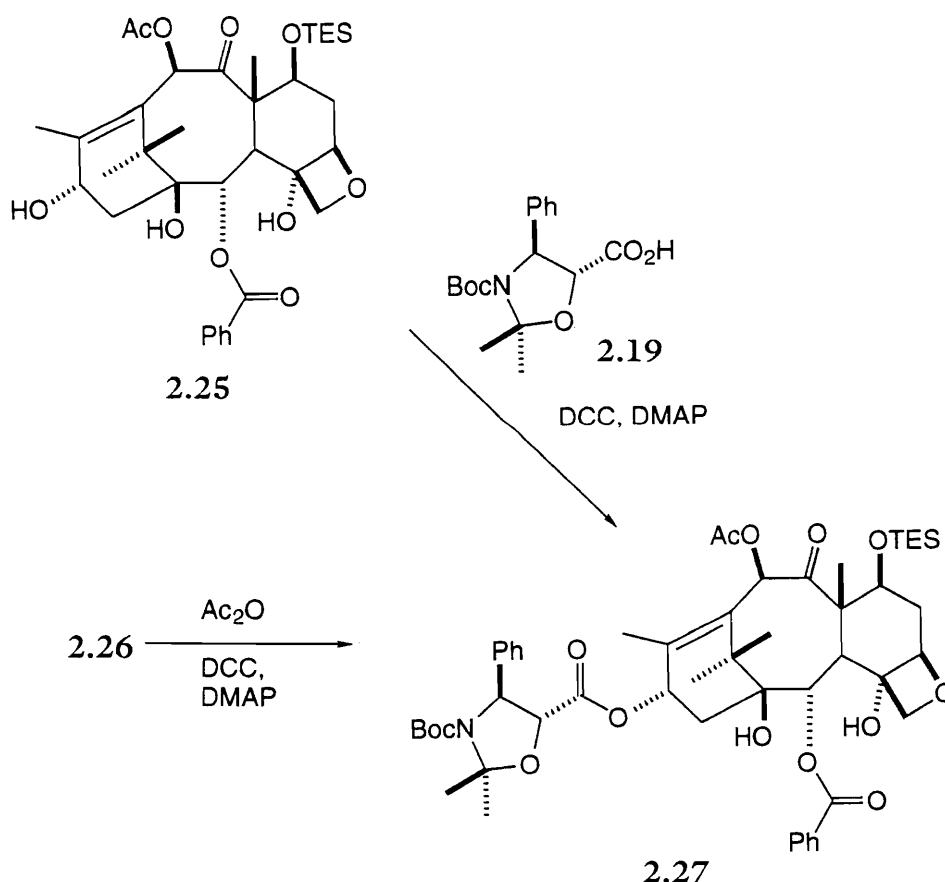


Scheme 7. Coupling of the bis(deacetyl)baccatin **2.7** with the side chain **2.19**.

spectrum of compound **2.27** showed the C-10 proton signal to have shifted downfield relative to the starting material **2.26**, from 5.20 to 6.49 ppm, indicating that acetylation had indeed occurred at the C-10 position. The other signals in the spectrum were consistent with the structure assigned. The FABMS $[MH]^+$ peak at m/z 962 provided further evidence of compound **2.27** having the structure assigned.

2.1.7 Coupling of 4-Deacetyl-7-(triethylsilyl)baccatin III (**2.25**) with Side Chain **2.19**

Based on the successful coupling of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (**2.7**) with the side chain acid **2.19**, it was reasoned that a similar coupling reaction of the side



Scheme 8. Approaches toward the preparation of the 4-deacetyltaxol derivative **2.27**.

chain acid **2.19** with 4-deacetyl-7-(triethylsilyl)baccatin III (**2.25**), as depicted in Scheme 8, would proceed smoothly. Indeed, when such a coupling was carried out, the product isolated was found by TLC, ^1H NMR and mass spectrometric analyses to be identical to compound **2.27**, described in Section 2.1.6, produced from the baccatin III derivative **2.7**.

2.1.8 Deprotection of Compound **2.27**, followed by 3'-N-Benzoylation

With the 4-deacetylated compound **2.27** having been obtained from the two individual substrates **2.7** and **2.25**, as described in the above Sections, the final two steps of the proposed synthesis of 4-deacetyltaxol (**2.1**), namely deprotection and C-3'-N-benzoylation, could be carried out. Thus, using Commerçon's deprotection conditions,⁹³ compound **2.27** was treated with 99% formic acid, yielding 4-deacetyl-3'-N-debenzoyltaxol (**2.28**) in good yield by TLC analysis.

The free amine **2.28** was not purified or characterized, but was immediately subjected to Schotten-Baumann conditions¹⁴⁷ to effect 3'-N-benzoylation. The major product of this reaction was thus realized in 46.2% yield over two steps from compound **2.27**. Full characterization of this product was carried out, allowing definitive identification of this compound as the target molecule 4-deacetyltaxol (**2.1**). The ^1H NMR spectrum of 4-deacetyltaxol (**2.1**) appears in Figure 5, and as can be seen by comparison with a spectrum for taxol (**1.8**), shown in Figure 6, the spectrum for 4-deacetyltaxol (**2.1**) shows the following major features, indicating the lack of an acetate at the C-4 position:

- 1) no acetate methyl singlet at 2.38 ppm;
- 2) the C-7 proton doublet of doublets is shifted upfield, from 4.40 ppm to 4.10 ppm; and
- 3) the C-14 α proton doublet of doublets is shifted downfield, from 2.28 ppm to 2.97 ppm.

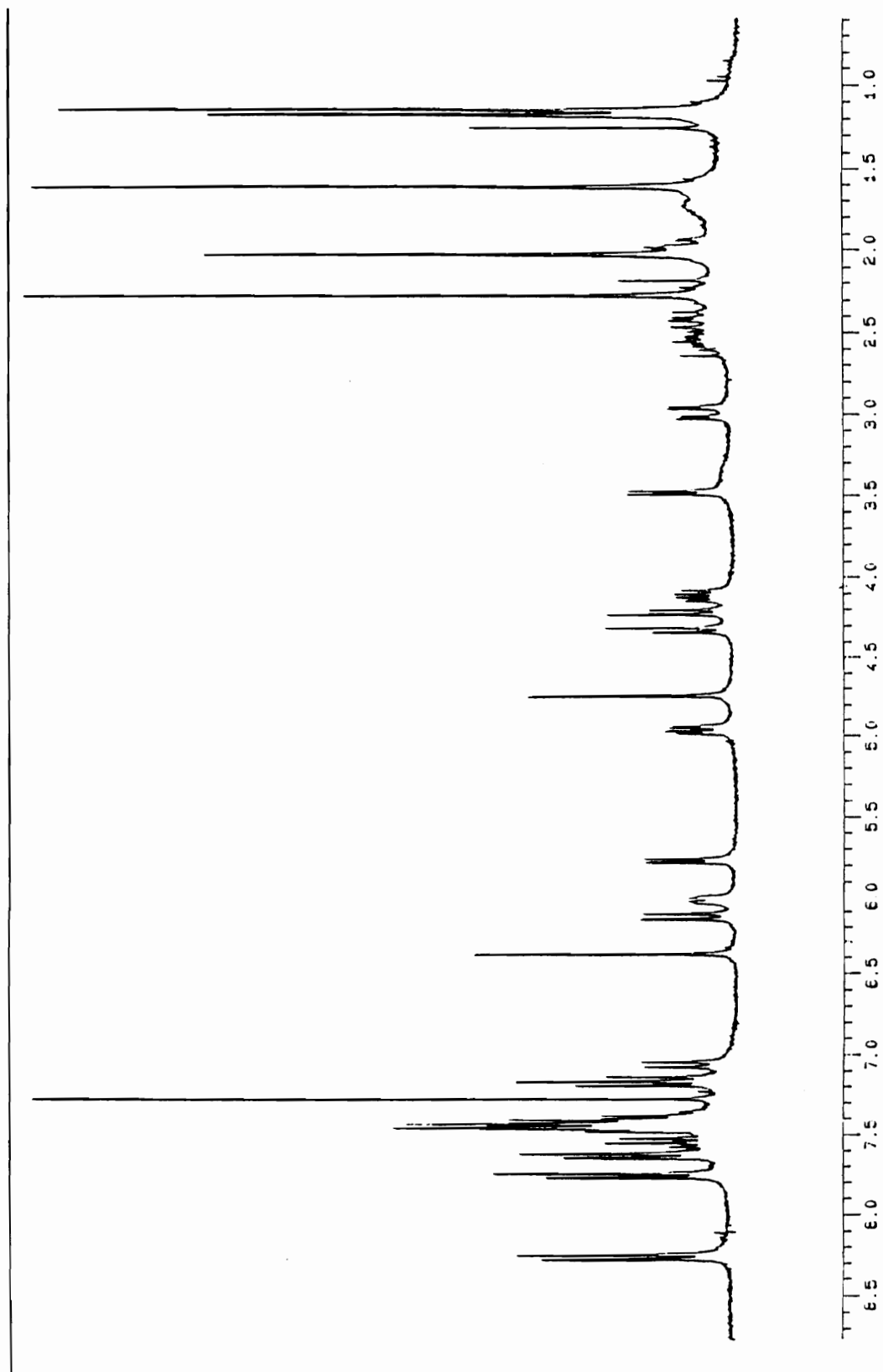
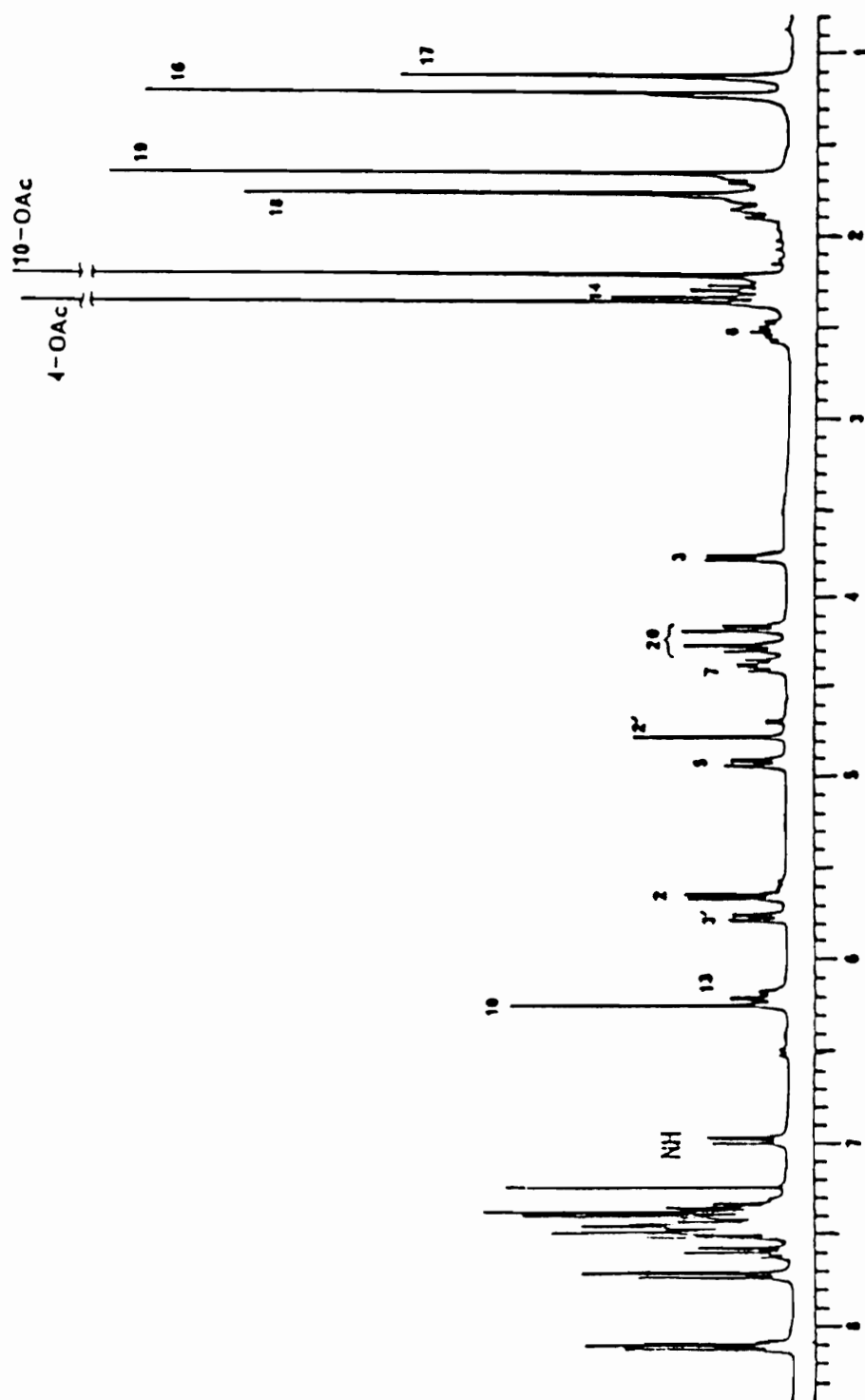
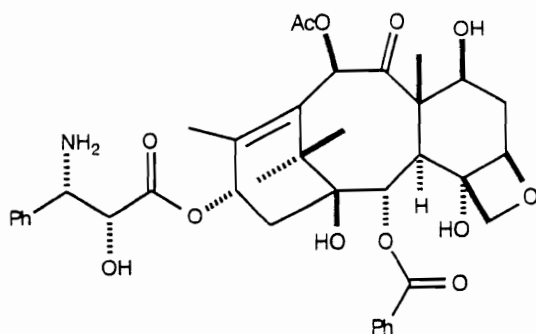


Figure 5. ^1H NMR spectrum of 4-deacetyltaxol (2.1).





2.28

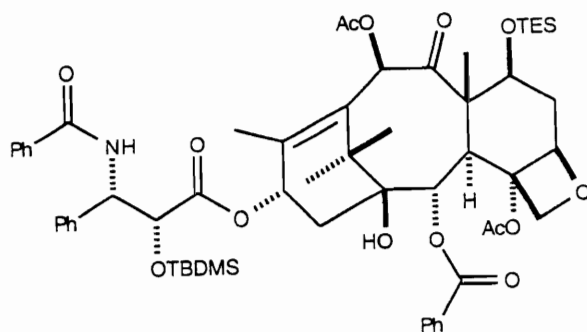
Comparison of the ^{13}C NMR spectra of 4-deacetyltaxol (**2.1**) and taxol (**1.8**) shows the major differences between the compounds to be a lack of the C-4 acetate methyl and C-4 acetate carbonyl peaks at 22.6 ppm and 170.4 ppm, respectively, in the spectrum for 4-deacetyltaxol (**2.1**), with only minor chemical shift differences for the other carbons of the molecule. Furthermore, the FABMS $[\text{MH}]^+$ peak at m/z 812 is in agreement with the expected molecular weight of 811 for 4-deacetyltaxol (**2.1**).

2.1.9 Preparation of 4-Deacetyltaxol (**2.1**) from Taxol (**1.8**)

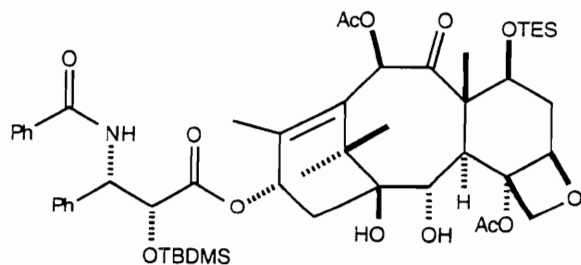
Subsequent to the successful formation of 4-deacetyltaxol (**2.1**) via the two independent procedures leading from 7-(triethylsilyl)baccatin III (**2.5**), as described above, it was found^{74,148} that treatment of 2'-(*tert*-butyldimethylsilyl)-7-(triethylsilyl)taxol (**2.29**): with benzyltrimethylammonium hydroxide ("Triton B") led to the formation of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-7-(triethylsilyl)taxol (**2.30**), which, upon continued treatment with Triton B, was further hydrolyzed to 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-2-debenzoyl-7-(triethylsilyl)taxol (**2.31**), in yields of 55 to 65%.

It was found soon thereafter that treatment of **2.29** with benzyltrimethylammonium methoxide¹⁴⁸ afforded **2.31** in somewhat better yields, on the order of 65 to 85%. That the normally labile taxol side chain does not undergo ready hydrolysis under these conditions

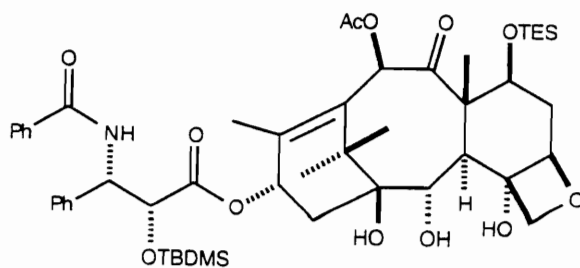
can be attributed mainly to the extremely bulky protecting group at the C-2' position, essentially shielding the ester linkage from hydrolytic cleavage. Indeed, when the 2',7-bis(triethylsilyl)taxol analog (**2.32**) was subjected to the same conditions,⁷⁴ the major products of the reaction were identified as baccatin III derivatives, indicating the crucial role of the more bulky protecting group on the C-2' hydroxyl in keeping the side chain intact.



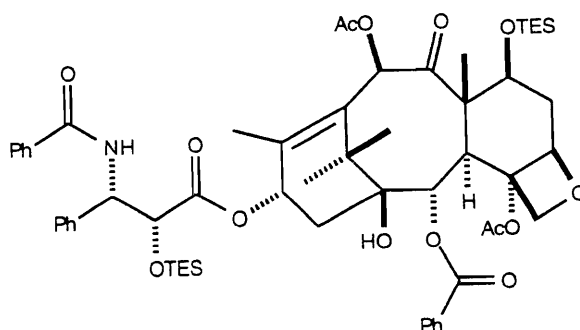
2.29



2.30



2.31

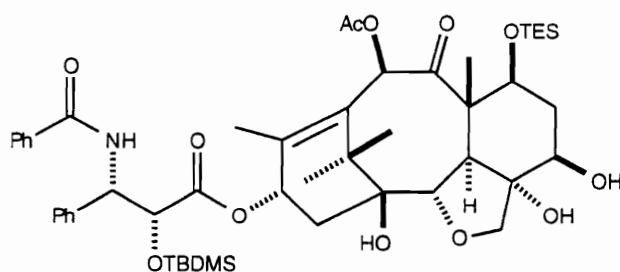


2.32

The selectivity observed under these deacylation conditions, namely C-2 > C-4 > C-13 (no C-10 ester hydrolysis was observed), may be due to the rather bulky nature of the base itself, since a similar order of deacylation was observed in a series of studies conducted to determine the effect on 2'-(*tert*-butyldimethylsilyl)-7-(triethylsilyl)taxol (**2.29**) by treatment with potassium *tert*-butoxide. The yields of product obtained using potassium *tert*-butoxide were, however, substantially lower, on the order of 20 to 35%. This order of deacylation was also independently observed by Georg and coworkers, as described in a recent publication.¹²⁸ That the C-2 benzoate of 2'-(*tert*-butyldimethylsilyl)-7-(triethylsilyl)taxol (**2.29**) is hydrolyzed first seems to indicate that the nearby C-1 hydroxyl group may provide assistance during such a hydrolysis. The observation that the C-4 acetate is hydrolyzed only after the C-2 benzoate has been cleaved implies that, in an analogous fashion to the C-2 benzoate cleavage just described, hydrolysis of the C-4 acetate may be assisted by the neighboring C-2 hydroxyl group. This neighboring hydroxyl group participation explains the observed lack of reactivity of the C-10 acetate, and has also been independently proposed by Georg.¹²⁸

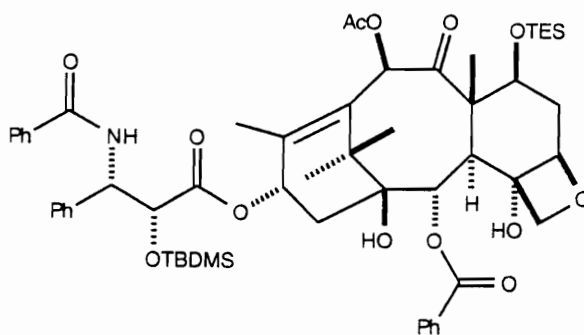
During the initial few experiments directed at converting compound **2.29** to the 2,4-di-deacylated analog **2.31**, it was found that, depending on the work-up conditions employed, a major product isolated from the reaction mixture had the isomeric structure **2.33**. The formation of this particular compound was reasoned to occur in an analogous manner to that described for the conversion of compound **2.4** to the tetrahydrofuranyl isomer **2.8**,

namely by intramolecular attack of the intermediary C-2 oxyanion on the C-20 position in compound **2.31**, thus affording compound **2.33** (cf. Scheme 2).¹¹⁵ As with the baccatin III series, knowledge of this particular process and the conditions under which the tetrahydrofuranyl isomer is less likely to form (i.e., lowered reaction temperatures, limited reaction times, and careful neutralization of the reaction mixture) allowed the conversion of compound **2.29** to **2.31** to be rapidly optimized, such that in subsequent experiments the isomeric product **2.33** was observed only in minute quantities.



2.33

Thus, with 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-2-debenzoyl-7-(triethylsilyl)taxol (**2.31**) available in reasonably good yield in two steps from taxol (**1.8**), compound **2.31** was next subjected to rebenzoylation of the C-2 hydroxyl group using benzoic anhydride under standard coupling conditions to afford 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**) in 65 to 80% yield.



2.34

The diprotected 4-deacetyltaxol **2.34** was subjected to deprotection using 5% methanolic HCl, affording 4-deacetyltaxol (**2.1**) in nearly quantitative yield. Spectroscopic analysis of this compound showed it to be identical in every way to the product obtained from the synthetic approaches discussed in previous Sections, in which baccatin III was used as the starting material.

2.1.10 Biological Test Results of 4-Deacetyltaxol (**2.1**)

2.1.10.1 Biological Test Systems

In order to provide a preliminary assessment of the activity of 4-deacetyltaxol, three different assay systems were used, namely the P-388 lymphocytic leukemia cell line, the CA46 Burkitt cell line, and the *in vitro* tubulin assembly/disassembly assay. Both the P-388 and the CA46 Burkitt cell cultures measure the ability of a potential drug to inhibit the proliferation of cancer cells. In these assays, varying concentrations of the compound of interest are incubated with the cancer cells in order to establish the concentration of compound necessary to inhibit cell growth by 50% of the control (no compound) group. Typically when a taxol derivative is evaluated for activity, taxol is tested under the same conditions to be used as a standard for activity comparison – relative differences in concentrations necessary to inhibit growth by 50% (the inhibition concentration, IC_{50} ; or the effective dose, ED_{50} , sometimes used to express the activity relative to taxol) allow an immediate comparison to be made between taxol and the analog. The disadvantages of using cell line assays are possible variations in cellular uptake; possible enzymatic modifications to the analog of interest; the possibility that an otherwise inactive derivative is toxic to the cell culture, giving a false-positive result; and the fact that several milligrams of the analog are necessary for each assay trial.

On the other hand, the tubulin assembly/disassembly assay is more convenient in rapidly obtaining reliable test data. This assay typically requires less than one milligram of a taxol analog, and when run simultaneously with a taxol standard, allows direct comparison

of a compound's relative activity and potential for use as a chemotherapeutic agent. Because of taxol's unique mode of action, taxol analogs displaying activity in the tubulin assay can be assumed to behave more or less in the same manner, and as such the assay serves as a good diagnostic tool in determining whether taxol analogs warrant further investigation.

2.1.10.2 Biological Test Results

4-Deacetyltaxol (**2.1**) was initially submitted to Dr. W. Lichter of the University of Miami School of Medicine for biological testing in the P-388 murine leukemia cell line. It was found that 4-deacetyltaxol (**2.1**) exhibited an ED_{50} of 0.2 $\mu\text{g/mL}$ relative to taxol (**1.8**), which had an ED_{50} of 0.03 $\mu\text{g/mL}$. These preliminary results indicated that 4-deacetyltaxol was an order of magnitude less active than taxol, however, biological activity data on other compounds submitted by our research group at the time indicated that the activity levels may have been subject to experimental uncertainty. Although these preliminary results indicated that 4-deacetyltaxol was somewhat less active than taxol itself, another sample of 4-deacetyltaxol was submitted to Dr. Ernest Hamel at the National Cancer Institute, National Institutes of Health, for biological testing in the human CA46 Burkitt lymphoma cell line, as well as in a tubulin assembly assay. In the CA46 Burkitt cells 4-deacetyltaxol had no inhibitory effect on cell growth at concentrations as high as 10 μM , as compared with an IC_{50} value of 30nM for taxol.¹⁴⁸ The effects of 4-deacetyltaxol on tubulin polymerization relative to taxol are summarized in Table 7.

As can be seen from Table 7, under conditions in which tubulin assembly occurs without any drug at 37 °C but not at 20 °C, taxol (**1.8**) displayed a slight effect even at 0 °C and a profound effect on assembly at 20 °C. In the absence of drug, depolymerization occurred following assembly when the temperature was lowered to 0 °C, however, as can be seen from the Table, taxol substantially reduced the disassembly rate. On the other hand, 4-deacetyltaxol (**2.1**) showed no activity at 0 °C at concentrations as high as 40 μM , and only very slight activity at 20 °C. At 37 °C and at a concentration of 40 μM 4-deacetyltaxol

displayed tubulin assembly activity only marginally higher than in the control. Furthermore, as shown in Table 7, the disassembly rate for 4-deacetyltaxol was only moderately reduced even at concentrations of 40 μ M. Thus, the data indicate that 4-deacetyltaxol is significantly less active than taxol in promoting tubulin polymerization and in stabilizing tubulin polymer. This suggests that the acetate at the C-4 position of taxol is necessary for biological activity.¹⁴⁸

Table 7. Effects of taxol (1.8) and 4-deacetyltaxol (2.1) on tubulin polymerization.

Compound	Max. assembly rate, unit/min*			Max. depolymerization rate, unit/min*
	0 °C	20 °C	37 °C	
None	0	0	0.46	0.56
1.8 (10 μ M)	0.010	0.98	n.m.	0.17
1.8 (40 μ M)	0.029	0.95	n.m.	0.14
2.1 (10 μ M)	0	0	0.46	0.46
2.1 (40 μ M)	0	0.015	0.60	0.34

* Rates are expressed as change in absorbance units at 350 nm (i.e., ΔA_{350}). Baselines were established with all components (1.0 mg/mL tubulin, 0.6 M monosodium glutamate, 1.0 mM MgCl₂, 0.4 mM GTP) in the reaction mixture except drug or dimethyl sulfoxide (the drug solvent). Drug was added, and the reaction followed for 10 minutes at 0 °C, 15 minutes at 20 °C and 15 minutes at 37 °C. Reaction temperature was then reduced to 0 °C for the depolymerization phase. Averages of duplicate values are presented (n.m. = not meaningful, since the extent of reaction at the lower preceding temperature was sufficiently extensive that the maximum rate at the indicated temperature was relatively low).

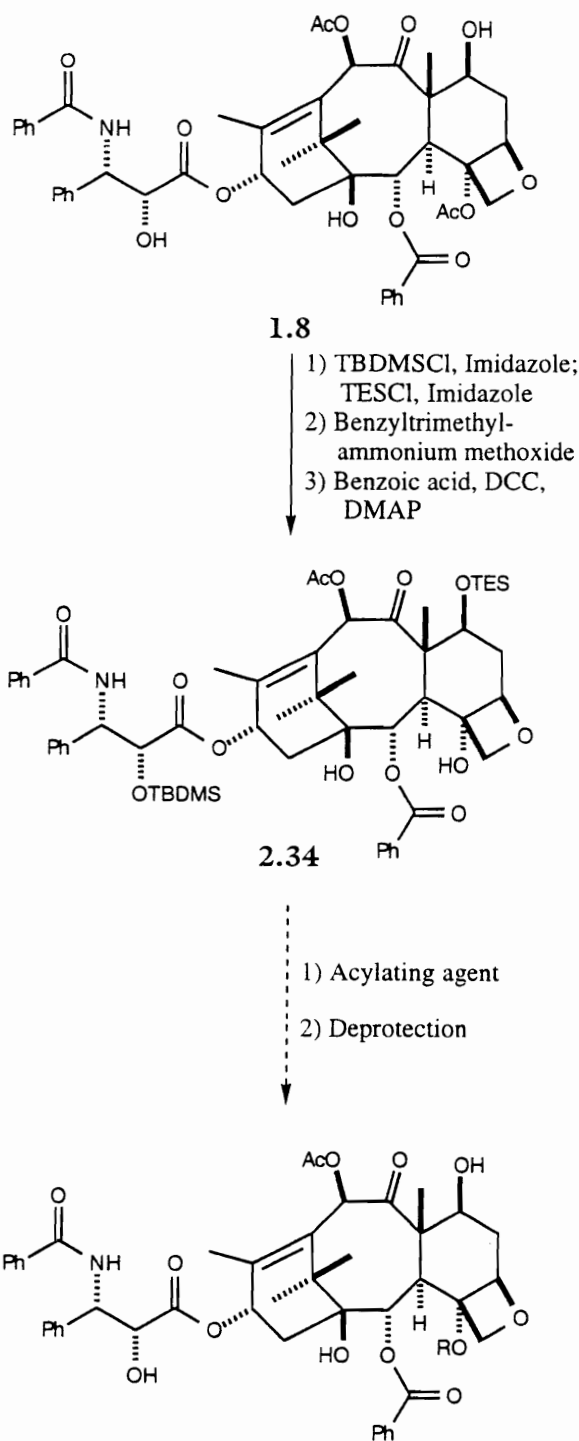
3.1 Preparation of 4-Acyltaxol Analogs

3.1.1 Introduction

Having successfully prepared 4-deacetyltaxol (**2.1**) via three independent synthetic approaches, and thus realizing the first major objective of this project, it was now possible to investigate the preparation of various 4-acyltaxol analogs in order to assess the effect on biological activity that modifications to the C-4 position might display relative to both taxol and the newly prepared 4-deacetyltaxol. It was anticipated, in addition to the possible enhancement of activity that modifications to the C-4 position might render, that any changes in the biological activity observed for the various 4-acyltaxol analogs could be useful in more fully establishing the structural features of the C-4 position vital for biological activity (i.e., whether the pendant group at C-4 is functioning as a “lock”, holding the C ring in a particular conformation and only indirectly playing a role in the tubulin binding process, or whether the C-4 position plays a direct part in binding with tubulin, in either a steric or an electronic sense).

As originally envisioned, synthetic efforts toward preparing 4-acyltaxol derivatives were to focus on 4-deacetyltaxol formed from baccatin III (**1.13**) or other baccatin III derivatives, since at the inception of this work it was reasoned that, while the use of baccatin III or its derivatives would ultimately entail more synthetic steps, the lability of the taxol side chain precluded the use of taxol as the starting material. However, in light of the chemistry of suitably protected taxol involving either benzyltrimethylammonium hydroxide (“Triton-B”) or its methoxide analog,^{74,148} as described in Section 2.1.9, it was thought that this chemically more direct approach might prove to be more fruitful in realizing various 4-acyltaxol derivatives, according to the synthetic methodology outlined in Scheme 9.

In addition to being a more direct synthetic preparation of 4-deacetyltaxol relative to the baccatin III approaches, this approach offers the advantage of producing the 2',7-diprotected 4-deacetyltaxol **2.34** in fairly good yield *prior* to realizing 4-deacetyltaxol. This is in contrast to the 4-deacetyltaxol preparations from baccatin III in which deprotection



R = varying acyl group

Scheme 9. General synthetic approach to 4-acyltaxol derivatives from taxol (1.8).

necessarily occurs before the realization of the target molecule (cf. Section 2.1.8) – subsequent reacylation attempts using 4-deacetyltaxol from the baccatin III approaches would require prior reprotection and subsequent deprotection of the C-7 and C-2' hydroxyl groups, further increasing the number of synthetic steps required to obtain 4-deacetyltaxol by this approach. Accordingly, and as described previously in Section 2.1.9, taxol was first protected to give compound **2.29**, following which treatment with benzyltrimethylammonium methoxide and subsequent rebenzoylation afforded 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**), to be used as the substrate for the reacylation studies described below.

3.1.2 Reacylation Attempts Utilizing 2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**)

The initial attempts at reacylation of the C-4 position of 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**) were performed in four experiments, using different acylating agents under standard coupling conditions (DCC/DMAP) at elevated temperature, as summarized in Table 8. The first of the experiments, employing acetic anhydride as the acylating agent, was carried out primarily to provide direct comparison of the product to the known compound **2.29**, giving firm evidence that acylation would occur as desired at the C-4 position.

*Table 8. Conditions for the initial acylation attempts on 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**).*

Experiment	Acylating agent (equivalents)	Conditions (equivalents)	Temperature and time	Result*
1	(CH ₃ CO) ₂ O (10)	DCC (10), DMAP (cat), tol.	65 °C; 12 hours	n.r.
2	(ClCH ₂ CO) ₂ O (10)	DCC (10), DMAP (cat), tol.	65 °C; 16 hours	n.r.
3	HCO ₂ H (10)	DCC (10), DMAP (cat), tol.	65 °C; 18 hours	n.r.
4	PhCO ₂ H (20)	DCC (20), DMAP (cat), tol.	65 °C; 12 hours	n.r.

* TLC analysis showed only unreacted starting material (n.r. = no reaction).

As evident from Table 8, in each of the four experiments, using different acylating agents under somewhat vigorous conditions and for prolonged times, insignificant product formation was evident by TLC analysis. Subsequent purification and ^1H NMR analysis of the reaction materials confirmed starting material **2.34** to be unreacted. Using the same experimental conditions as shown in Table 8, with the exception that reaction times were extended to 36 hours, *experiments 1* and *2* were repeated to determine whether in fact these conditions were suitable for acylation to occur. Again, however, only unreacted starting material was observed by TLC and ^1H NMR analysis.

Having had no success in the above series of reactions using standard coupling conditions, it was reasoned that the DCC-acyl adduct might be too sterically demanding to allow successful coupling with the C-4 hydroxyl group of compound **2.34**, which, in addition to being inherently hindered by its tertiary disposition, is “shielded” both by its position within the cup-like shape of the taxol ring system and by the partial blocking effect of the side chain, with its bulky C-2' *tert*-butyldimethylsilyl protecting group. To overcome the apparent inaccessibility of the C-4 position under the standard coupling conditions employed above, it was thought that base-catalyzed coupling using an acyl anhydride might prove to be more fruitful. Toward this end, the taxol substrate **2.34** was treated with chloroacetic anhydride in pyridine, under rather vigorous conditions (65 °C, 30 hours). However, TLC and ^1H NMR analyses after purification showed unreacted starting material and a fully deprotected decomposition product in the reaction mixture.

A study was conducted using chloroacetyl chloride and pyridine under a variety of reaction conditions ranging from mild (0 °C, 4 hours) to rather vigorous (65 °C, 30 hours). Again, however, TLC and ^1H NMR analyses after purification showed unreacted starting material and a deprotected, decomposed material to be the only constituents of the reaction mixtures.

Reasoning that the use of a stronger base might lend itself to more desirable results, another series of experiments was carried out using various acid chlorides and triethylamine as the base, the conditions and results of which are summarized in Table 9. As can be seen

from Table 9, in all but four experiments negligible reaction was evident by TLC analysis. Subsequent ^1H NMR analysis showed essentially unreacted starting material to be present in the reaction mixtures of these experiments. On the other hand, the TLC analyses of the reaction mixtures from *experiments 9, 10, 14, and 15*, listed in Table 9, displayed a large number of products (on the order of 12 to 15 UV-active compounds for each, 6 to 10 of which gave colors typical for taxanes upon spraying with a vanillin spray indicator). Purification of the four reaction mixtures was carried out and ^1H NMR analyses were attempted, however, presumably due to the small scale of the reactions (circa 5 mg of compound **2.34**) and the low quantities of individual products isolated (typically less than 200 μg), the ^1H NMR data was inconclusive in determining whether acylation had occurred in any of these experiments.

Experimental conditions for *reactions 9, 10, 14, and 15* from Table 9 were repeated on a larger scale in an effort to better identify the products formed, however on this scale it was noted that the mixtures proved to be even more complex than originally supposed.

Table 9. Conditions for the attempted acylation of 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**) using acid chloride and triethylamine (10 equivalents) as base.

Experiment	Acid chloride (equivalents)	Solvent	Temperature and time	Result*
1	CH_3COCl (10)	CH_2Cl_2	0 °C; 6 hours	n.r.
2	ClCH_2COCl (10)	CH_2Cl_2	0 °C; 6 hours	n.r.
3	PhCOCl (10)	CH_2Cl_2	0 °C; 6 hours	n.r.
4	PhCH_2COCl (10)	CH_2Cl_2	0 °C; 6 hours	n.r.
5	$\text{PhOCH}_2\text{COCl}$ (10)	CH_2Cl_2	0 °C; 6 hours	n.r.
6	CH_3COCl (10)	CH_2Cl_2	r.t.; 8 hours	n.r.
7	ClCH_2COCl (10)	CH_2Cl_2	r.t.; 8 hours	n.r.
8	PhCOCl (10)	CH_2Cl_2	r.t.; 8 hours	n.r.
9	PhCH_2COCl (10)	CH_2Cl_2	r.t.; 8 hours	complex mixture
10	$\text{PhOCH}_2\text{COCl}$ (10)	CH_2Cl_2	r.t.; 8 hours	complex mixture
11	CH_3COCl (10)	CHCl_3	60 °C; 6 hours	n.r.
12	ClCH_2COCl (10)	CHCl_3	60 °C; 6 hours	n.r.
13	PhCOCl (10)	CHCl_3	60 °C; 6 hours	n.r.
14	PhCH_2COCl (10)	CHCl_3	60 °C; 6 hours	complex mixture
15	$\text{PhOCH}_2\text{COCl}$ (10)	CHCl_3	60 °C; 6 hours	complex mixture

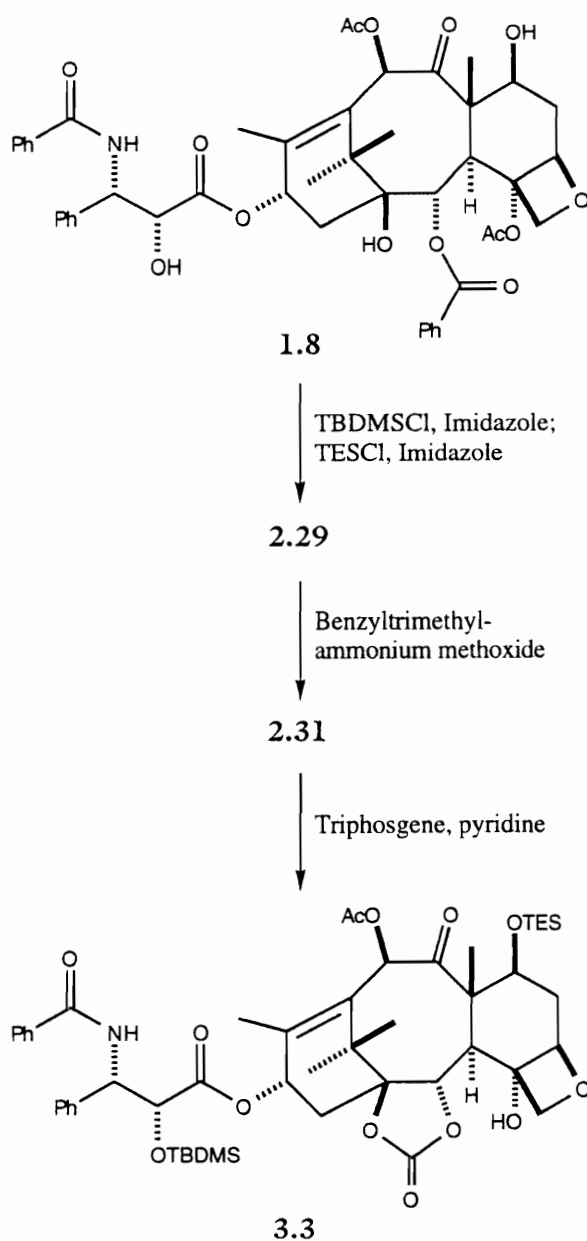
* Except where noted, TLC analysis showed only unreacted starting material (n.r. = no reaction).

Attempted purification of the products by PTLC and subsequent ^1H NMR analysis showed that what were believed to be individual components in the previous series of experiments had actually consisted of mixtures of two to four different compounds. Further attempts to purify the individual components of the mixtures through various PTLC solvent systems proved to be unsuccessful. In light of the complex mixtures obtained from these reactions and the difficulties inherent in their purification, as well as the low potential yield (if any) of the desired C-4 acylated products, it was decided that synthetic approaches using 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**) as the substrate would not be practical for our goal of obtaining C-4 acylated taxol analogs efficiently and in reasonable yield.

3.1.3 Reacylation Attempts Utilizing 2'-(*tert*-Butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**)

Because of the lack of success in the above experiments, it was speculated that, in addition to the inherently low accessibility of the C-4 hydroxyl group of 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**) toward acylation, the C-2 benzoate may further interfere with the approach of any acylating agent to the C-4 position under the various conditions attempted. In the interest of maintaining a relatively short synthetic route directly from taxol, it was reasoned that if the C-2 hydroxyl group was not initially benzoylated and could be protected in a manner such that it would not become acylated itself or interfere with the acylation process occurring in relative close proximity, and that if it could be readily deprotected and rebenzoylated after acylation had occurred at the C-4 position, then such an approach might prove to be chemically viable. While these considerations were being made, Holton⁶¹ and Nicolaou⁶² had published the first total syntheses of taxol and had reported the use of the baccatin III derivatives **3.1** and **3.2**, respectively, as substrates for the acylation of the C-4 position, both with reasonable success. It was thought that incorporation of the 1,2-cyclic carbonate functionality, common to both

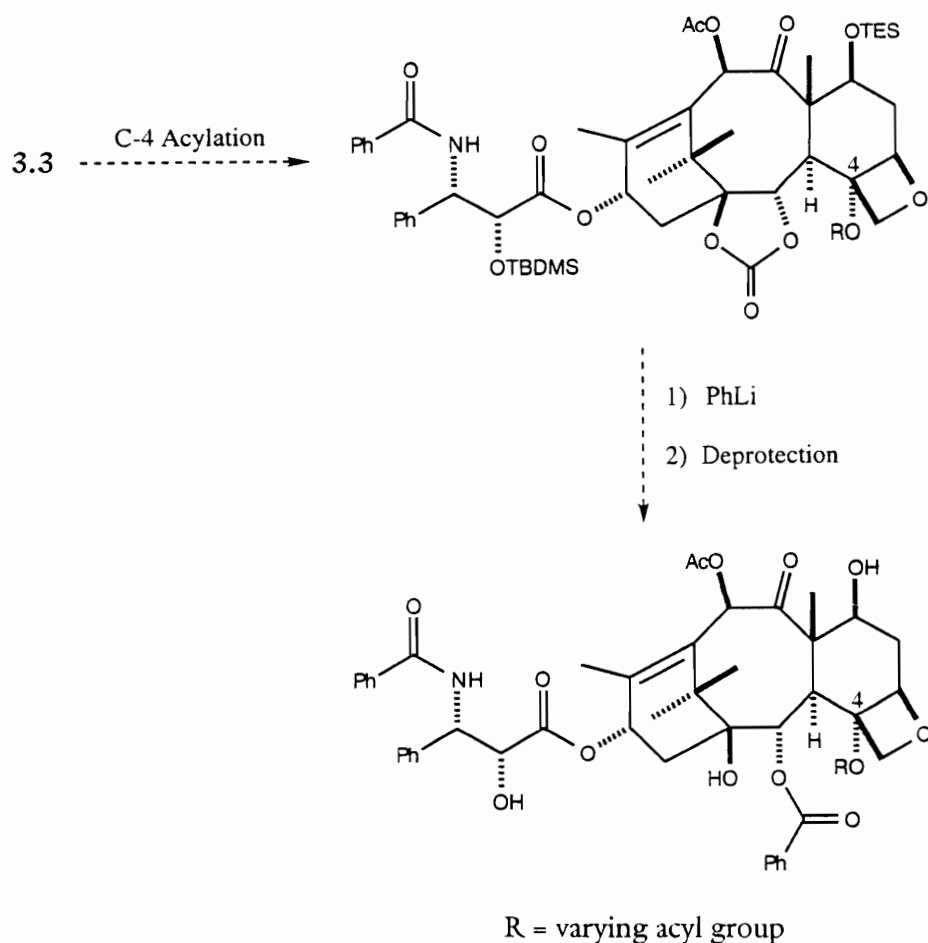
3.1 and 3.2, into the taxol derivative 2.31 to give the 1,2-carbonato-taxol derivative 3.3, as depicted in Scheme 10, might afford a sterically less congested substrate which could allow successful acylation of the C-4 position. Additionally, as Holton and Nicolaou each



Scheme 10. Proposed formation of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3) from taxol (1.8).

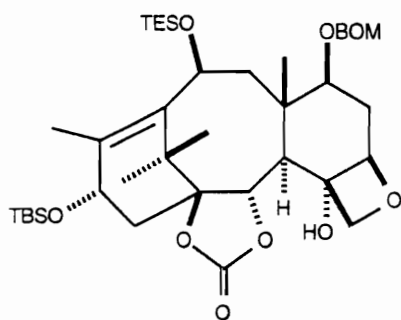
reported,^{61,62} the cyclic carbonate can be regioselectively transformed in one step to the C-1 hydroxy C-2 benzoate configuration of taxol by reaction with phenyl lithium. Therefore, pending successful acylation at the C-4 position, it was conceived that the acylated carbonate could be transformed in two steps to the corresponding 4-acyltaxol congener, as depicted in Scheme 11.

Thus, according to Scheme 10, 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**) was prepared from taxol (**1.8**) in three synthetic steps, in 45% overall yield. In similar fashion to the experiments previously discussed, involving 2'-

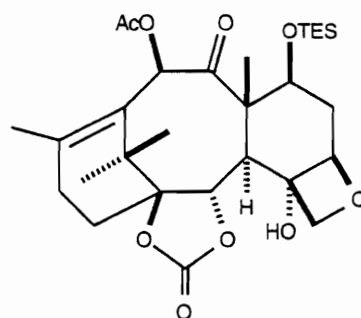


Scheme 11. Proposed general synthetic approach to 4-acyltaxol derivatives from 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**).

(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**), 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**) was next subjected to a systematic study of its reactivity toward acylation at the C-4 position, as discussed in the following pages.



3.1



3.2.

Six reactions were performed using 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**) and either formic acid, acetic acid, propionic acid, acetic anhydride, propionic anhydride or chloroacetic anhydride, under standard coupling conditions (DCC/DMAP), as summarized in Table 10. Thin layer chromatographic analysis of *reactions 1* through 5, from Table 10, indicated that no reaction had occurred after 12 hours, and the unreacted starting material from each was recovered by PTLC and determined by ^1H NMR analysis to be homogeneous. Thin layer

Table 10. Conditions for the initial acylation attempts on 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**).

Experiment	Acyating agent (equivalents)	Conditions (equivalents)	Temperature and time	Result*
1	HCO ₂ H (10)	DCC (10), DMAP (cat), tol.	65 °C; 8 hours	n.r.
2	CH ₃ CO ₂ H (10)	DCC (10), DMAP (cat), tol.	65 °C; 8 hours	n.r.
3	CH ₃ CH ₂ CO ₂ H (10)	DCC (10), DMAP (cat), tol.	65 °C; 12 hours	n.r.
4	(CH ₃ CO) ₂ O (10)	DCC (10), DMAP (cat), tol.	65 °C; 12 hours	n.r.
5	(CH ₃ CH ₂ CO) ₂ O (10)	DCC (10), DMAP (cat), tol.	65 °C; 12 hours	n.r.
6	(ClCH ₂ CO) ₂ O (10)	DCC (10), DMAP (cat), tol.	65 °C; 8 hours	product

* Except where noted, TLC analysis showed only unreacted starting material (n.r. = no reaction).

chromatographic analysis of *reaction 6*, on the other hand, indicated the formation of a small amount of a polar product.

Because of the small scale of this reaction (less than 4 mg of the carbonate **3.3**), no attempt was made to isolate the polar product for characterization. It was speculated that this product might be the C-4 chloroacetyl carbonate derivative, since it was known from comparison of the TLC behavior of 2'-(*tert*-butyldimethylsilyl)-7-(triethylsilyl)taxol (**2.29**) and 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**) in EtOAc/hexanes (1:3) that the C-4 acetylated compound (**2.29**, R_f 0.21) is actually *more* polar than its C-4 deacetylated counterpart (**2.34**, R_f 0.32). To determine whether this same trend would be observed in the carbonate series, a sample of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**) was compared by TLC analysis (EtOAc/hexanes, 1:3) to a sample of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-7-(triethylsilyl)taxol (**3.4**), previously prepared in our labs from reaction of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-7-(triethylsilyl)taxol (**2.31**) with triphosgene. It was

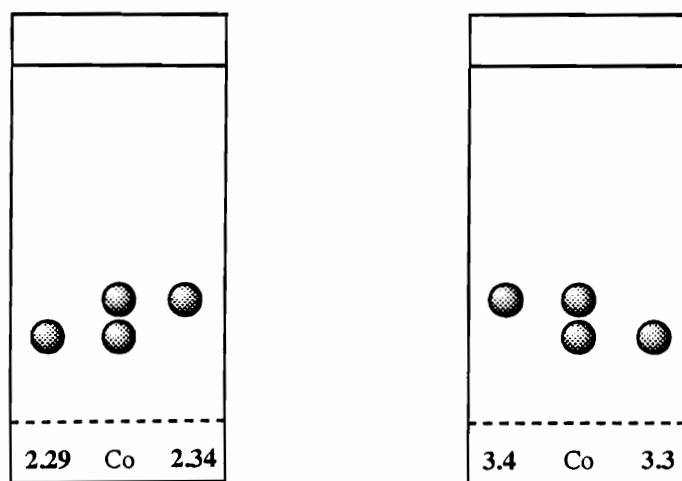
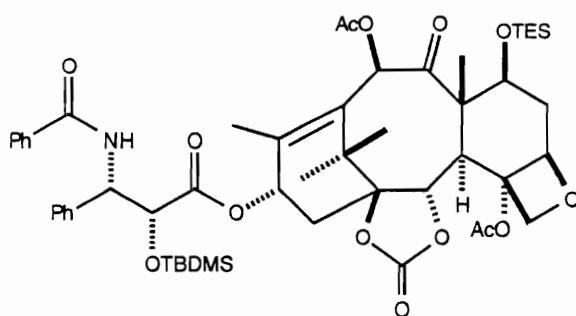


Figure 7. Comparison of the chromatographic behavior of the taxol derivatives **2.29**, **2.34**, **3.3** and **3.4** in the ethylacetate/hexanes (1:3) solvent system.

found that the C-4 acetylated carbonate (3.4, R_f 0.34) is *less* polar than the corresponding C-4 deacetylated compound (3.3, R_f 0.23), as depicted in Figure 7.

Although it was considered that the C-4 chloroacetate might influence chromatographic behavior to a certain extent, the foregoing observation led to the tentative conclusion that the polar material formed from the reaction of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3) with chloroacetyl anhydride was not the corresponding C-4 acylated product, but was, rather, deprotected or decomposed material. In the interest of acquiring the desired C-4 acylated taxol analogs, further investigation of this product was deemed unprofitable at this time.



3.4

Another series of experiments was conducted in which four small-scale reactions were run using 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3) and either acetyl chloride, acetic anhydride, chloroacetyl chloride or

Table 11. Conditions for the attempted acylation of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3) using either acid chloride or acid anhydride and pyridine (as solvent and base).

Experiment	Acid chloride (equivalents)	Temperature and time	Result*
1	CH ₃ COCl (10)	65 °C; 16 hours	n.r.
2	(CH ₃ CO) ₂ O (30)	65 °C; 12 hours	n.r.
3	ClCH ₂ COCl (30)	r.t. to 65 °C; 30 hours	product
4	(ClCH ₂ CO) ₂ O (30)	65 °C; 6 hours	product

* Except where noted, TLC analysis showed only unreacted starting material (n.r. = no reaction).

chloroacetic anhydride in pyridine, as summarized in Table 11. Each reaction was performed at the temperature and for the time period indicated in the Table. Subsequent TLC analysis showed *reactions 1* and *2*, from Table 11, to contain only unreacted starting material, which was recovered by PTLC. *Reactions 3* and *4*, however, were seen to contain a moderate amount of a more polar product, but no products of lower polarity were evident. Based on the small scale of the reactions and the conclusions drawn previously as to the chromatographic behavior of potential C-4 acylated carbonate analogs, isolation and characterization of this product were not pursued.

In another experiment, 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**) was treated with sodium hydride followed by acetyl chloride at 0 °C. No reaction was evident by TLC analysis after 1.5 hours so the reaction vial was allowed to warm to room temperature and stirring was continued for an additional 16 hours. A TLC plate of the reaction mixture taken at this time showed no reaction to have occurred, and the starting material was recovered by PTLC.

Yet another experiment was conducted in which four small-scale reactions were carried out using 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**), triethylamine, and either acetyl chloride, chloroacetyl chloride, phenylacetyl chloride or phenoxyacetyl chloride, as summarized in Table 12. The reactions were each started at 0 °C and, after 1.5 hours, each was warmed to room temperature and allowed to continue. Total reaction times varied between 14 and 18 hours, but TLC analysis

Table 12. Conditions for the attempted acylation of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**) using acid chloride and triethylamine (10 equivalents) as base.

Experiment	Acid chloride (equivalents)	Solvent	Temperature and time	Result*
1	CH ₃ COCl (10)	CH ₂ Cl ₂	0 °C to r.t., 18 hours	n.r.
2	ClCH ₂ COCl (10)	CH ₂ Cl ₂	0 °C to r.t., 14 hours	product
3	PhCH ₂ COCl (10)	CH ₂ Cl ₂	0 °C to r.t., 16 hours	n.r.
4	PhOCH ₂ COCl (10)	CH ₂ Cl ₂	0 °C to r.t., 16 hours	n.r.

* Except where noted, TLC analysis showed only unreacted starting material (n.r. = no reaction).

of the reactions showed only unreacted starting material in *reactions 1, 3 and 4*, from Table 12. Thin layer chromatographic analysis of *reaction 2*, on the other hand, showed the starting material to have been converted to a single product, similar in polarity to the product noted in previous experiments.

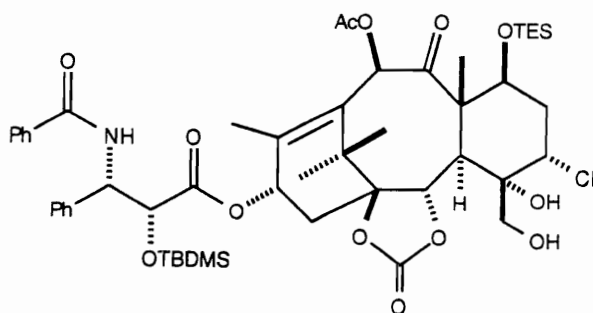
Taking into consideration the virtual lack of product formation from the reaction of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**) with a number of acylating agents under a wide variety of reaction conditions, it was decided that this approach would not be suitable for realizing our goal of efficiently preparing a variety of 4-acyltaxol analogs in reasonable yield.

3.1.4 An Unexpected Oxetane Ring-Opened Product

Before completely abandoning the methodology toward gaining C-4 acylated taxol derivatives described in the previous Section, it was thought that one further study was warranted. The only notable event during the entire course of the investigation of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**) as a possible substrate for attaining 4-acyltaxol derivatives was the formation of a polar product when the carbonate **3.3** was treated with either chloroacetyl chloride or chloroacetic anhydride under various conditions. Because the product did not exhibit the chromatographic properties expected for a C-4 acylated carbonatotaxol analog, and because of the small scale of the reactions in which it was observed, no attempt toward full characterization of this material had been made thus far. Accordingly, it was of interest to prepare this material in order to firmly establish its identity and whether the same compound is formed in each case, as well as to better understand why its formation is exclusive to the reaction of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**) with chloroacetyl chloride or with chloroacetic anhydride. Thus, those reactions producing the polar material were repeated on a larger

scale as summarized in Table 13. Also included in Table 13 is the summary of a reaction conducted to assess the effect of DMAP alone on the formation of the polar product.

As anticipated, each of the reactions yielded a more polar product and after work-up and purification, these products were individually analyzed by ^1H NMR spectroscopy, which showed them to be identical. While this observation was not completely unexpected, given the similarity of the two acylating agents used, what was not expected was that the preliminary NMR data seemed to indicate that the product had undergone opening of the oxetane ring to give the product 3.5.



3.5

The first indication that this product was not a C-4 acylated taxol analog was the lack of a significant downfield chemical shift in the C-7 proton, relative to the starting material 3.3. In general, the observed chemical shift differences between C-4 acetylated compounds and their deacetylated counterparts has been used extensively as a diagnostic tool, and are typically on the order of 0.5 ppm or more. Specifically, the data showed the C-7 proton to

Table 13. Conditions utilized for the formation of the polar product from 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3).

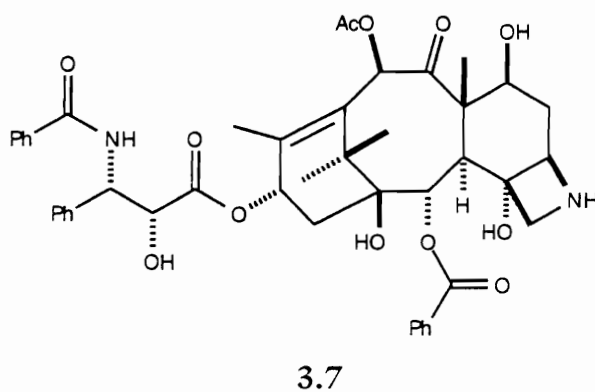
Experiment	Reagent (10 equivalents)	Reaction Conditions (equivalents)	Temperature and time	% Yield
1	(ClCH_2CO) $_2\text{O}$	DCC (10), DMAP (cat), tol.	65 °C; 12 hours	38.9
2	ClCH_2COCl	pyridine (excess)	65 °C; 16 hours	42.7
3	(ClCH_2CO) $_2\text{O}$	pyridine (excess)	65 °C; 6 hours	33.7
4	ClCH_2COCl	triethylamine (10), CH_2Cl_2	r.t.; 14 hours	39.7
5	(ClCH_2CO) $_2\text{O}$	DMAP (5), CH_2Cl_2	r.t.; 24 hours	27.0

be at 4.55 ppm, slightly downfield shifted relative to the starting material value of 4.22 ppm. In addition, the chemical shift of the C-5 proton which, in most taxol or baccatin III derivatives normally appears around 5.0 ppm, was observed to be shifted upfield relative to the starting material, from 4.98 to 4.70 ppm, indicating a major modification had occurred in its vicinity. Furthermore, the broad doublet splitting pattern normally seen for the C-5 proton had changed to a narrow, overlapping doublet of doublets (or a pseudo triplet) with coupling constants of less than 0.5 and 4.5 Hz, leading to the speculation that the C-5 proton of the product was in the β orientation rather than the usual α orientation. These small coupling constants are in agreement with the values of 0.5 Hz and 5.5 Hz for the C-5 β proton predicted by low-level molecular mechanics calculations (CSC Chem3D Plus, v.3.1). The drastic upfield chemical shift changes observed for the two C-20 protons, from 4.60 and 4.40 ppm in the starting material to 4.08 and 3.57 ppm of the product, also indicated that a major change had taken place in this region of the molecule. Significant changes in the C-3 and C-14 α protons were also observed, showing downfield shifts from 3.01 to 3.77 ppm for the C-3 proton and from 2.78 to 3.12 ppm for the C-14 α proton. Subsequent mass spectral analysis of this product showed it to give a FABMS $[MH]^+$ peak at 1000 mass units with a relative intensity of 100, and a secondary peak at 1,002 mass units with a relative intensity of 42. Given that the molecular weight of the starting material is 962 mass units, the addition of 37 mass units with the characteristic chlorine isotope pattern is consistent with the addition of HCl to the starting material. Based on this information, as well as on comparison of these results to the ^1H NMR data reported by Rimoldi,⁷⁴ which showed similar trends in the chemical shift changes upon opening of the oxetane in a related compound, it was concluded that 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**) had undergone oxetane ring-opening upon reaction with either chloroacetyl chloride or chloroacetic anhydride, as discussed above.

The mechanism by which compound **3.5** is formed is uncertain, but it is known that its formation is not derived from the chloride ion of the acid chloride portion of the acylating agent, since the use of chloroacetic anhydride leads to formation of compound **3.5** and the

use of acetyl chloride does not. This indicates that it is the chloride α to the carbonyl which is incorporated into the product. How this chloride is incorporated is not known, but it was initially speculated that the bases employed during the experiments in which compound **3.5** is formed may be nucleophilic enough to displace chloride ion from the acylating agent, such that the chloride is delivered to the C-5 position, causing the observed oxetane ring opening. This is questionable, however, based on the fact that no analogous oxetane ring-opened product was observed when 2'-(*tert*-butyltrimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**) was reacted under the same acylating conditions. It may be speculated that the chloride is delivered to the C-5 position from a pre-coupled C-4 chloroacetate through base-assisted displacement but mechanistically this hypothesis is unlikely due to the fact that there is no other precedent for C-4 acylation of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**).

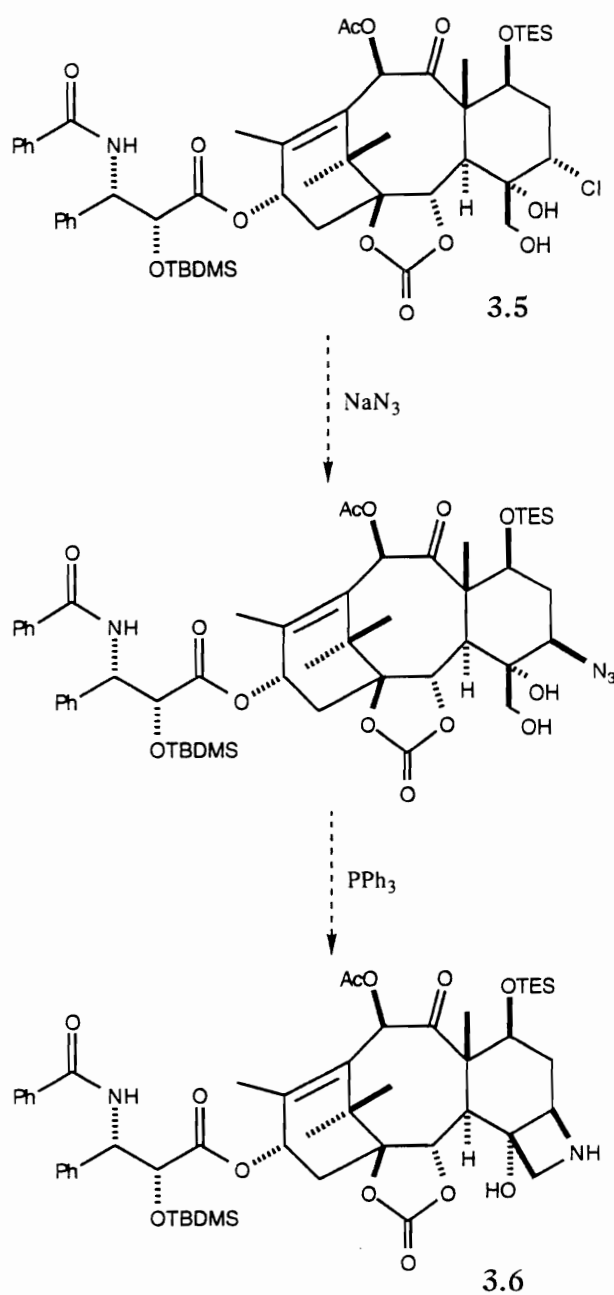
It was realized that the successful formation of the azetidine analog **3.6** would afford the taxol derivative **3.7** which would bear a modified oxetane ring but lack the biologically relevant C-4 acetate group. It was reasoned, however, that compound **3.7** could initially be directly compared to 4-deacetyltaxol (**2.1**) in order to assess the effect of the azetidine on biological activity.



Toward this end, compound **3.5** was treated at room temperature with 12 equivalents of sodium azide with 18-crown-6 ether added to assist the reaction. After 24 hours no

reaction was evident by TLC, so the temperature was increased to 60 °C. Even after an additional 18 hours at elevated temperature, no reaction was observed.

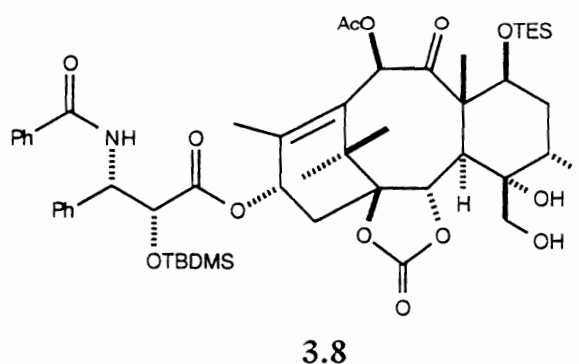
Reasoning that the chlorinated oxetane ring-opened compound **3.5** might not be reactive enough toward nucleophilic reagents, particularly the azide, it was thought that the



Scheme 12. Generalized approach to the azetidine taxol analog **3.6**.

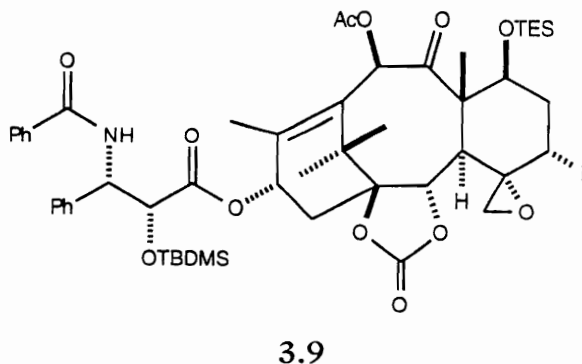
C-5 brominated or iodinated analogs might prove to be better substrates for obtaining oxetane-modified analogs. In order to obtain the C-5 brominated analog it was speculated that reaction of the carbonate 3.3 with zinc bromide might give the desired compound, as it had been reported that the oxetane can be opened by reaction with Lewis acids.¹⁴⁹ It was reasoned that after formation of the oxetane-Lewis acid complex, bromide could be delivered to the C-5 position, affording the desired C-5 α bromo analog. However, when this reaction was attempted, there was no observed change in the starting material even after 8 hours at room temperature.

From the work of Rimoldi,⁷⁴ it was known that treatment of 2'-(*tert*-butyltrimethylsilyl)-7-(triethylsilyl)-taxol (2.29) with iodotrimethylsilane can result in the opening of the oxetane, and it was thought that treatment of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3) with this reagent might afford the C-5 iodinated analog of 3.5. Indeed, when the carbonate 3.3 was treated with iodotrimethylsilane at -20 °C, it was found after 10 minutes that the starting material had been converted to a more polar product which, after work-up, purification and subsequent ¹H NMR and mass spectral analyses, was identified as the C-5 iodinated oxetane ring-opened product 3.8.



This compound has since been converted by reaction with *para*-nitrotoluenesulfonyl chloride to the key intermediate 3.9, which is currently being used for the preparation and eventual biological evaluation of heteroatom-containing oxetane analogs.¹⁵⁰ The

heteroatom derivatives currently being prepared include the nitrogen-, sulfur- and selenium-containing oxetane congeners.



3.1.5 Model Reacylation Studies Performed on 4,10-Bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7)

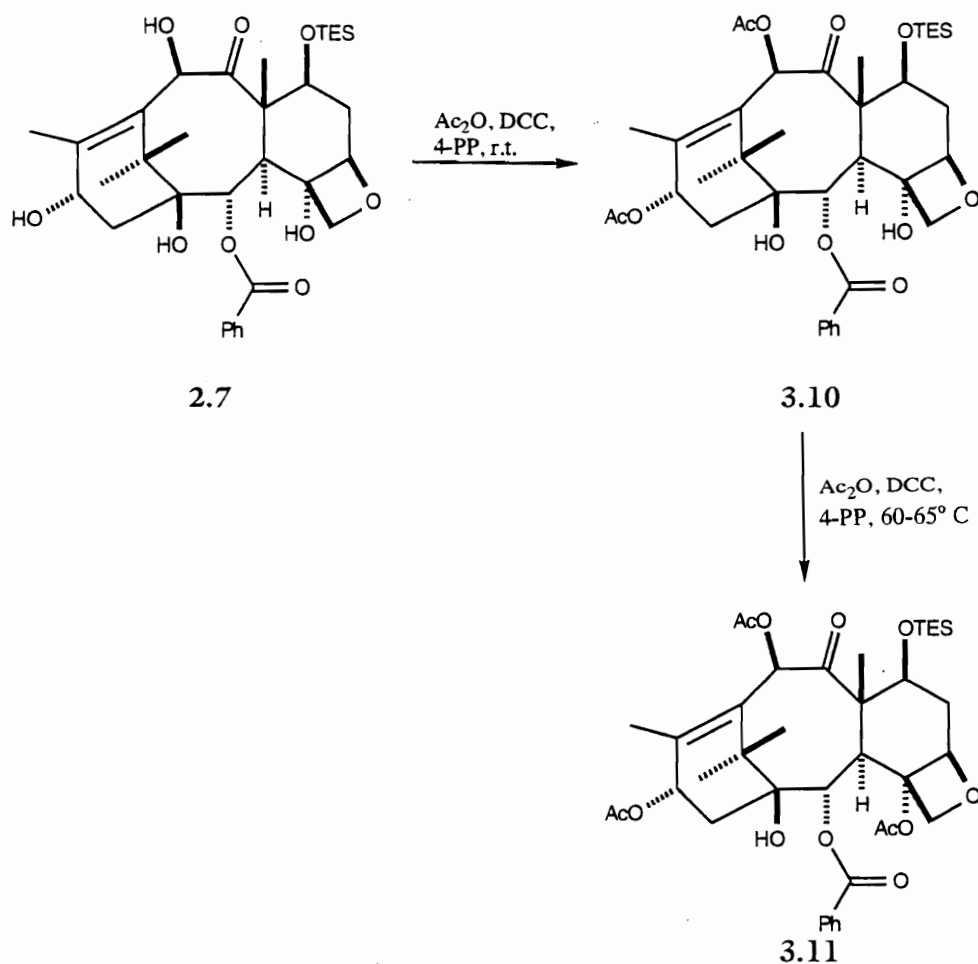
Having had no success at acylating the C-4 position in either of the taxol substrates 2.33 or 3.3, it was postulated that the side chain in each substrate, with its very bulky *tert*-butyltrimethylsilyl protecting group at the C-2' position, may have been responsible for the observed lack of reactivity of the C-4 hydroxyl group, since replacement of the C-2 benzoate with the C-1, C-2 cyclic carbonate apparently failed to increase the accessibility of the C-4 position. With this in mind, the focus of the project again shifted to the baccatin III series as potentially more viable substrates for the proposed C-4 acylation attempts. In order to determine whether C-4 acylation would be possible, the baccatin substrate, 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7) was selected for use in an initial model study. Accordingly, 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7) was subjected to mild acetylation conditions (5.0 equivalents Ac_2O /DCC/4-PP/r.t.), as shown in Scheme 13, and the major product of the reaction was isolated in 71.0% yield and determined by ^1H NMR and mass spectrometric analysis to be 13-acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (3.10). 13-Acetyl-4-deacetyl-7-(triethylsilyl)baccatin III was then subjected to more vigorous acetylation conditions (30 equivalents Ac_2O /DCC/4-PP/60 to 65 $^\circ\text{C}$), and upon purification of the reaction mixture and ^1H NMR analysis, it was found that acetylation did

in fact occur at the C-4 position, albeit in low yield (23.6%), affording 13-acetyl-7-(triethylsilyl)baccatin III (3.11).

3.1.6 Approaches Toward C-4 Reacylation Using Modified Baccatin Substrates

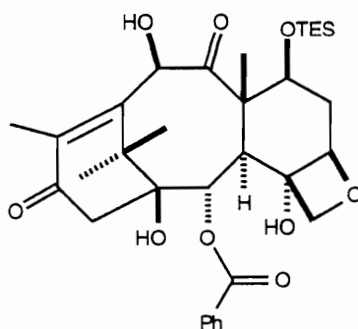
3.1.6.1 Introduction

With the successful acetylation of the C-4 position of 13-acetyl-4-deacetyl-7-(triethylsilyl)baccatin III, it was now believed that this approach, using an appropriate



Scheme 13. Acetylation of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7).

baccatin III substrate, would allow the successful formation of a series of 4-acylbaccatin III derivatives, which could be converted to the corresponding 4-acyltaxol analogs. It was realized, however, that the successful approach employed above would need to be modified somewhat since selective hydrolysis of the C-13 acetate, necessary for introduction of the side chain **2.19**, would likely prove to be difficult in the presence of the three other ester groups in the molecule. Taking this into consideration, it was reasoned that a protecting group at the C-13 position would be more practical for the formation of 4-acylbaccatin III analogs, which in turn could be readily converted to the accordant 4-acyltaxol analogs. Such a protecting group would have to fulfill two major prerequisites, namely, that it be: 1) relatively small, so as not to interfere with the C-4 acylation process; and 2) that it be easily and selectively deprotectable in the presence of the protected C-7 hydroxyl group, to subsequently allow easy coupling of the taxol side chain, concomitant with obtaining 4-acyltaxol analogs. A number of potential protecting groups were considered, but it was envisioned that the most appealing plan for protection would involve no formal protecting group at all, but rather, the oxidation of the C-13 hydroxyl¹⁵⁰ of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (**2.7**) to the corresponding C-13 ketone derivative **3.12**.



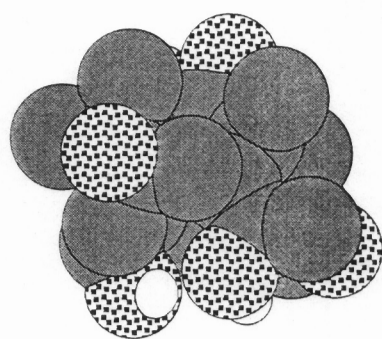
3.12

In addition to being inherently small and apparently inert under the coupling conditions to be employed, the C-13 carbonyl would presumably allow easier access to the C-4 position by slightly opening the mouth of the cup-shaped molecule relative to its C-13

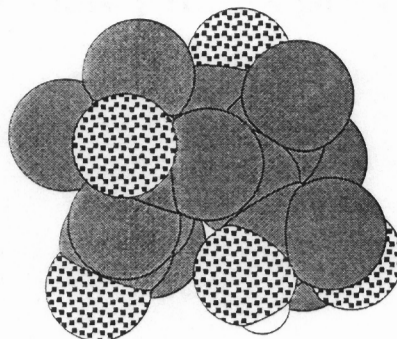
hydroxy counterpart, due to the geometry change of the C-13 rehybridization, as depicted in Figure 8.

It was envisioned that the preparation of the desired substrate, 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (**3.13**), to be used for the proposed C-4 acylation studies, could be achieved in four steps from baccatin III (**1.13**) or 10-deacetylbaccatin III (**1.14**), according to Scheme 14.

On the assumption that acylation of the C-4 position of 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (**3.13**) would proceed as expected, subsequent conversion to the analogous 4-acyltaxol derivatives would involve reduction of the 4-acyl-13-oxobaccatin back to the corresponding C-13 hydroxyl compound. Whether the reduction would occur to give the desired α configuration for the C-13 hydroxyl group preferentially over the β configuration was not known, however it was thought that the cup-like shape of the molecule would tend to direct the reduction to occur from the top face of the C-13 carbonyl, thus affording the desired α isomer. In order to determine whether the proposed reduction would indeed occur in a stereoselective fashion, a model study was undertaken in which 7-(triethylsilyl)baccatin III (**2.5**) was first oxidized to 7-(triethylsilyl)-13-oxobaccatin III (**3.14**), using manganese dioxide.¹⁵⁰ The ketone **3.14** was then subjected to reducing



2.7

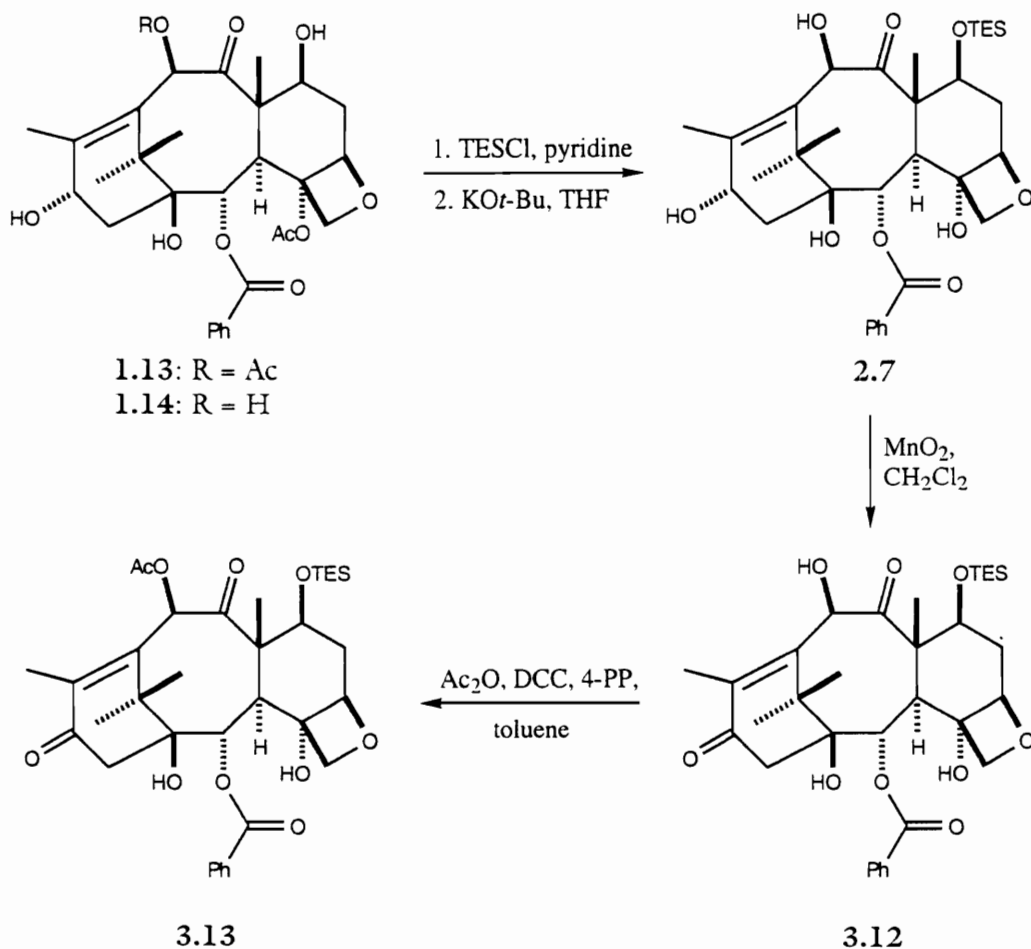


3.21

Figure 8. Spatial accommodation caused by oxidation of the C-13 hydroxyl group of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (**2.7**) to the corresponding ketone (**3.12**). [For clarity the C-2 benzoate, the C-7 triethylsiloxy group and irrelevant protons are not shown.]

conditions, using sodium borohydride in THF-methanol (9:1). As noted by TLC analysis, the starting material was rapidly consumed, forming a pair of more polar products, in roughly a 9:1 ratio. Upon purification of these two products and analysis by ^1H NMR, it was determined by comparison to known spectral data that the major product was 7-(triethylsilyl)baccatin III (**2.5**); the minor component was determined, based on ^1H NMR chemical shift values, to be the isomeric 7-(triethylsilyl)-13-*epi*-baccatin III (**3.15**).

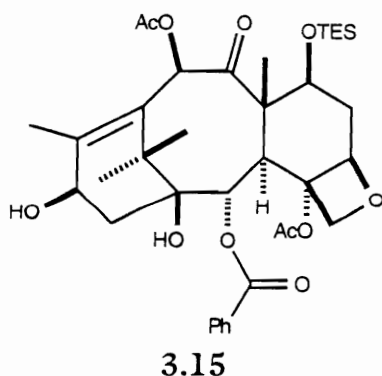
Having established that the C-13 ketone could be selectively reduced to give predominantly the desired C-13 α -hydroxy isomer, it seemed that the synthetic approach



Scheme 14. Proposed formation of 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (**3.13**), for use in acylation studies.

toward obtaining 4-acyltaxol analogs via 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (**3.13**) would prove feasible. Accordingly, the synthetic methodology outlined in Scheme 14 was implemented. Each step proceeded as expected in reasonable yield, and the desired 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (**3.13**) was obtained in 46.0% yield over four steps from baccatin III (**1.13**) or in 42.3% yield over the same number of steps from 10-deacetylbaccatin III (**1.14**).

With the compound **3.13** in hand, it was now possible to determine whether in fact the C-4 hydroxyl group of this particular substrate could be acylated. As a preliminary to such an investigation, it was decided that a model study would be carried out, using acetic anhydride as the acylating agent. Such a model study, if successful, would afford a C-4 acylated product identical to 7-(triethylsilyl)-13-oxobaccatin III (**3.14**), previously prepared from 7-(triethylsilyl)baccatin III (**2.5**), and would allow an immediate direct comparison of spectroscopic information in providing conclusive evidence that acylation can occur at the C-4 position of the substrate **3.13**.



Thus, substrate **3.13** was treated with acetic anhydride under mild coupling conditions (DCC/4-PP/CH₃CN/r.t.) and within two hours the starting material had been converted to a pair of slightly less polar products in an approximate ratio of 5:1, as determined by TLC analysis. The predominant product was purified by PTLC and then analyzed by ¹H NMR. Upon comparison to the spectrum for the known compound 7-(triethylsilyl)-13-oxobaccatin III (**3.14**), the predominant product was identified as the same material, thus

offering conclusive evidence that C-4 acylation had occurred in this substrate. While no definitive assignment was made for the minor product isolated, a crude ^1H NMR spectrum revealed that this material possessed structural modifications in the vicinity of positions C-13 and C-14. No evidence of C-4 acetylation was noted, based on the absence of a downfield shift for the C-7 proton signal, a characteristic of C-4 acetylated compounds.

With the successful conclusion of this model study to establish the utility of the substrate **3.13**, the prospect of preparing various 4-acyltaxol derivatives seemed more likely than in the previously unsuccessful attempts (cf. Sections 3.1.2 and 3.1.3). Of the possible acyl groups which could conceivably be attached at the C-4 position, four were selected on the basis of their practicality and potential effect(s) on biological activity. The four acyl group candidates were: the propionate, the chloroacetate, the trichloroacetate and the benzoate functionalities. Naturally, it was assumed, depending on the successful outcome of the acylation attempts planned for these particular groups as well as the nature of the effect(s) on biological activity of the related taxol analogs, that the synthetic methodology could be extended to the placement of other acyl groups at the C-4 position. This would then provide a broader base of analogs with which to better establish the structure-activity relationships for the C-4 position.

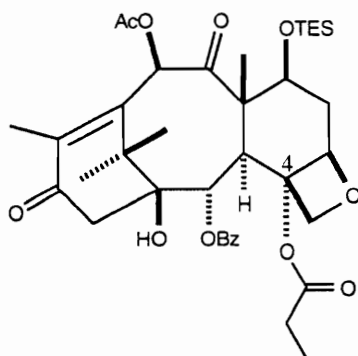
The most practical consideration in selecting the four acyl groups above was that of cost and availability, and each of the candidates have the advantage of being relatively inexpensive and readily available. Practical considerations aside, it was thought that the four acyl groups would serve as a representative lead group in the establishment of the structure-activity relationships of the C-4 position. The propionate group was selected mainly to assess the effect on activity elicited by an acetate homolog. The chloroacetate and trichloroacetate groups were chosen based on their similarity in size to the acetate at this position in taxol, as well as on the potential effects on activity rendered by their varying electronic natures. The benzoate was selected on the basis of the potential effect on biological activity due to its steric and electronic properties and the resultant hydrostatic interactions these properties might provoke. It is believed from solution studies of taxol¹²⁰,

that the *N*-benzoate, 3'-phenyl, and the 2-benzoate groups may be involved in a sterically large π -stacked cluster which directly influences the interaction of taxol with tubulin through assorted hydrophobic and hydrophilic electrostatic interactions. If this is indeed the case, the replacement of the C-4 acetate with other acyl groups would likely result in either a positive or a negative effect on biological activity through enhancement or disruption of the π -stacking array or other modifications to the molecular topology which could result in different tubulin binding affinity.

Thus having selected four 4-acyltaxol analogs as reasonable target compounds with which to continue our study of the structure-activity relationships of the C-4 position, experiments toward obtaining these compounds were initiated. Four reactions were carried out using the different acyl anhydrides and 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13) as the substrate, the results of which are discussed below.

3.1.6.2 Reaction of 4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13) with Propionic Anhydride

4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13), dissolved in acetonitrile, was treated with propionic anhydride (30 equivalents) under standard coupling conditions (DCC/4-PP/r.t. then 65 °C), affording two less polar products in an approximate ratio of 3:1. After purification, ¹H NMR analysis showed the major product to be a C-4 acylated compound, based on the downfield shift of the C-7 proton (a typical indication of the presence of a C-4 acyl group), and additional methylene and methyl signals at about 3.0 ppm and 1.2 ppm, respectively, displaying the characteristic ethyl group splitting pattern. This data, combined with mass spectral data which gave a FABMS [MH]⁺ peak at 713, indicated that the desired acylation had occurred at C-4, thus affording 4-deacetyl-7-(triethylsilyl)-13-oxo-4-propionylbaccatin III (3.16) in 59.5% yield from 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13).



3.16

Mass spectrometric analysis of the minor product showed this compound to be isomeric with the major compound, with a FABMS $[MH]^+$ of 713, however, ^1H NMR analysis of this compound showed this compound to be structurally different from the major compound 3.16. In particular, the C-7 proton signal lacked the downfield shift characteristic of C-4 acylated compounds, remaining essentially unchanged from the starting material. On the other hand, the signals for the two C-14 protons evident in the starting material were completely lacking in the spectrum for the minor product and were replaced with a sharp singlet appearing at 5.30 ppm, characteristic of a vinylic proton signal. Similar to the spectrum for the major product, methylene and methyl protons with the characteristic ethyl splitting pattern, were present at about 2.4 ppm and 1.2 ppm, respectively, indicating the addition of propionyl group to the baccatin substrate 3.13. The spectroscopic information for this compound is consistent with the structure assigned, namely 4-deacetyl-13,14-dehydro-13-propionylbaccatin III (3.17).

It is conceivable that this product may have arisen under the conditions of the coupling reaction through the action of the base catalyst, 4-pyrrolidinopyridine, particularly if minute traces of water were introduced to the small scale reaction. While precautions were taken to ensure that the reaction was run under anhydrous conditions, it is quite possible that traces of water were present in the solvent used, the acylating or the coupling reagents used, or were contained within the amorphous, glassy solid starting material. Since the C-14 protons

are activated toward base abstraction by the C-13 ketone, tautomerism is likely and with the excess of propionic anhydride present in the reaction mixture, it is quite feasible that the intermediate tautomer could react with an anhydride molecule, thus producing the observed enol propionate. Under the conditions of the reaction, the enol propionate would be favored by equilibrium, however, its formation would effectively render the C-4 position inaccessible to acylation due to the shielding effect of the enol propionate over the open end of the molecular cup, as depicted in Figure 9.

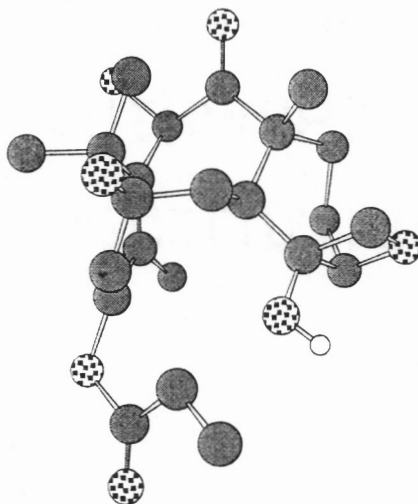
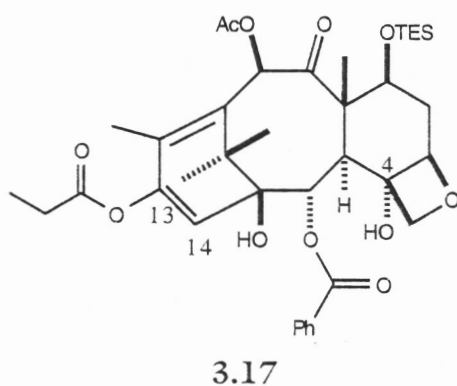
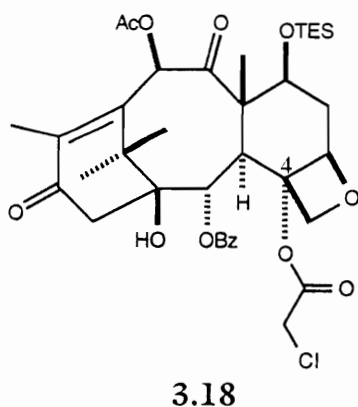


Figure 9. Three-dimensional representation of 4-deacetyl-13,14-dehydro-13-propionylbaccatin III (**3.17**), depicting the reduced accessibility of the C-4 position.

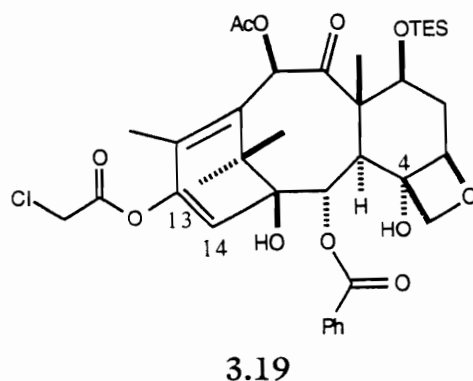
3.1.6.3 Reaction of 4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13) with Chloroacetic Anhydride

4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13), dissolved in acetonitrile, was treated with chloroacetic anhydride (30 equivalents) under standard coupling conditions (DCC/4-PP/r.t. then 65 °C), affording two less polar products in an approximate ratio of 7:1. After purification, ^1H NMR analysis showed the major product to be a C-4 acylated compound, based on the downfield shift of the C-7 proton, as is typical upon the introduction of a C-4 acyl group; and an additional methylene peak at about 3.7 ppm, displaying the characteristic chloroacetate methylene chemical shift. This information, combined with mass spectral data which gave a FABMS $[\text{MH}]^+$ peak at 733, indicated that the desired acylation had occurred at C-4, thus affording 4-deacetyl-4-chloroacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.18) in 56.3% yield from 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13).



Mass spectrometric analysis of the minor product showed this compound to be isomeric with the major compound, with a FABMS $[\text{MH}]^+$ of 733, however, ^1H NMR analysis of this compound showed this compound to be structurally different. The C-7 proton signal lacked the downfield shift characteristic of C-4 acylated compounds and was essentially

unchanged from the starting material. The signals for the two C-14 protons evident in the starting material were completely lacking in the spectrum for the minor product and had been replaced with a sharp singlet appearing at about 5.3 ppm, characteristic of a vinylic proton signal. Similar to the spectrum for the major product, the chloroacetate methylene signal was present at about 3.7 ppm, indicating the addition of chloroacetyl group to the baccatin substrate **3.13**. The spectroscopic information for this compound is consistent with the structure assigned, namely 13-chloroacetyl-4-deacetyl-13,14-dehydrobaccatin III (**3.19**).

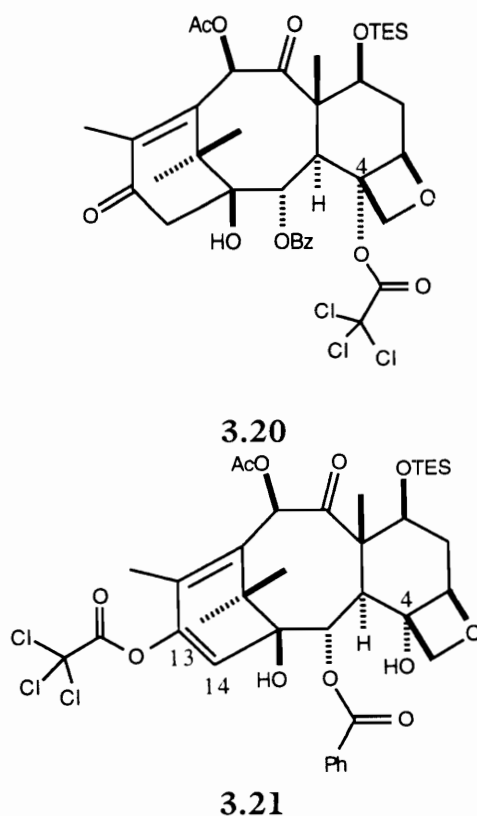


3.1.6.4 Reaction of 4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (**3.13**) with Trichloroacetic Anhydride

4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (**3.13**) was treated in acetonitrile with trichloroacetic anhydride (30 equivalents) under standard coupling conditions (DCC/4-PP/r.t. then 65 °C), affording two less polar products in an approximate ratio of 4:1, along with unreacted starting material. After purification, ^1H NMR analysis showed the major product to be a C-4 acylated compound, based on the downfield shift of the C-7 proton, typical of the introduction of a C-4 acyl group. Subtle changes in the spectrum involving the C-3 and C-5 protons indicated changes in the local magnetic environment, as expected by the addition of a trichloroacetate at the C-4 position. This information, combined with mass spectral data which gave a FABMS $[\text{MH}]^+$ peak at 803, indicated that the desired acylation had occurred at C-4, thus affording 4-deacetyl-4-trichloroacetyl-7-(triethylsilyl)-

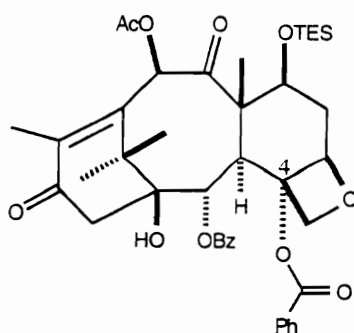
13-oxobaccatin III (**3.20**) in 39.4% yield from 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (**3.13**).

Mass spectrometric analysis of the minor product showed this compound to be isomeric with the major compound, with a FABMS $[MH]^+$ of 803, however, ^1H NMR analysis of this compound showed this compound to be structurally different. The C-7 proton signal lacked the downfield shift characteristic of C-4 acylated compounds and was essentially unchanged from the starting material. The signals for the two C-14 protons evident in the starting material were not present in the spectrum for the minor product and had been replaced with a sharp singlet appearing at about 5.3 ppm, characteristic of a vinylic proton signal. The spectroscopic information for this compound is consistent with the structure assigned, namely 13-trichloroacetyl-4-deacetyl-13,14-dehydrobaccatin III (**3.21**), likely having arisen in similar fashion to the two enol esters discussed above, from the existence of trace amounts of water in the reaction mixture.



3.1.6.5 Reaction of 4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13) with Benzoic Anhydride

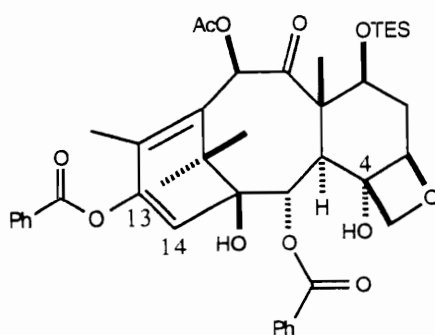
4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13) was treated in acetonitrile with benzoic anhydride (30 equivalents) under standard coupling conditions (DCC/4-PP/r.t. then 65 °C), affording two less polar products in an approximate ratio of 2:1. After purification, ^1H NMR analysis showed the major product to be a C-4 acylated compound, based on the downfield shift of the C-7 proton, typical of the introduction of a C-4 acyl group. The presence of additional peaks in the aromatic region of the NMR spectrum, particularly a doublet of doublets characteristic of *ortho*-benzoate protons, and subtle chemical shift changes for the C-3, C-5 and C-20 protons; combined with mass spectral data which gave a FABMS $[\text{MH}]^+$ peak at 761, indicated that the desired benzoylation had occurred at C-4, thus affording 4-deacetyl-4-benzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.22) in 52.8% yield from 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13).



3.22

Mass spectrometric analysis of the minor product showed this compound to be isomeric with the major compound, with a FABMS $[\text{MH}]^+$ of 761, however, similar to the three cases discussed above, ^1H NMR analysis of this minor compound showed it to be structurally different from the major compound 3.22. The C-7 proton signal lacked the downfield shift characteristic of C-4 acylated compounds and was essentially unchanged

from the starting material. The signals for the two C-14 protons evident in the starting material were not present in the spectrum for the minor product, having been replaced with a sharp vinylic singlet appearing at about 5.5 ppm. Minor chemical shift changes were evident for the C-3, C-5 and C-20 protons, indicating the presence of a modified magnetic environment. The spectroscopic information for this compound is consistent with the structure assigned, namely 13-benzoyl-4-deacetyl-13,14-dehydrobaccatin III (**3.23**), presumably having arisen in similar fashion to the three enol esters previously discussed.



3.23

3.1.6.6 Optimization of C-4 Acylating Conditions

Due to the unexpected formation of the enol esters described above, a series of reactions was carried out in order to evaluate the conditions necessary for optimal formation of the desired C-4 acyl compounds. Because of the suspected involvement of water in the formation of the enol esters, the starting material, 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (**3.13**), was dried using high vacuum; solvents were freshly dried and distilled; and other reagents were either vacuum-dried or were newly obtained. As summarized in Table 14, the reactions were run in various solvents at different temperatures, and the ratio of enol ester formation to C-4 acylation ranged from about 1:2 up to 8:1. In the cases where the starting material was added to previously prepared

mixtures of the acylating agents and DCC as well as in some cases at lower temperature, the ratio between the products was seen to be consistently in favor of the C-4 acylated compounds.

Thus, it was found that the 4-acyl-13-oxobaccatins could be produced in moderate to good yields from the starting material 3.13, without extensive formation of the corresponding enol esters, and it was decided that another set of reactions would be conducted on a larger scale (50 mg of the starting material 3.13) using the four acylating agents employed above to afford the 4-acyl-13-oxobaccatins in reasonably good yields. Accordingly, the acylation reactions were carried out to furnish the 4-acyl-13-oxobaccatins 3.16 (84.4% yield), 3.18 (73.1% yield), 3.20 (61.7% yield) and 3.22 (72.5% yield).

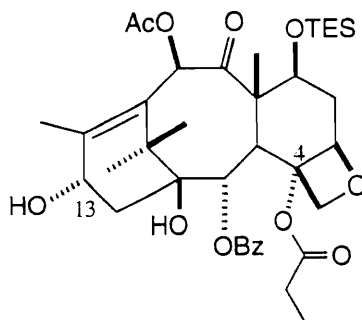
Table 14. Summary of reactions carried out to optimize the formation of 4-acyl-13-oxobaccatins.

Experiment	Acylating agent	Solvent	Temperature and time	Ratio (enolate: 4-acylate)
1	(CH ₃ CO) ₂ O	CH ₂ Cl ₂	r.t., 2 hours	1:2.3
2	(CH ₃ CH ₂ CO) ₂ O	CH ₂ Cl ₂	r.t., 12 hours	1:2.3
3	(ClCH ₂ CO) ₂ O	CH ₂ Cl ₂	r.t., 24 hours	1:1.2
4	(Cl ₃ CCO) ₂ O	CH ₂ Cl ₂	r.t., 24 hours	1.3:1
5	(PhCO) ₂ O	CH ₂ Cl ₂	r.t., 24 hours	n.r.
6	(CH ₃ CO) ₂ O	CH ₃ CN	r.t., 24 hours	1:2.2
7	(CH ₃ CH ₂ CO) ₂ O	CH ₃ CN	r.t., 24 hours	1:2.5
8	(CH ₃ CO) ₂ O	CH ₃ CN	60 °C, 3.5 hours	2.4:1
9	(CH ₃ CH ₂ CO) ₂ O	CH ₃ CN	60 °C, 3.5 hours	2.6:1
10	(ClCH ₂ CO) ₂ O	CH ₃ CN	60 °C, 3.5 hours	3.2:1
11	(Cl ₃ CCO) ₂ O	CH ₃ CN	60 °C, 3.5 hours	4.2:1
12	(PhCO) ₂ O	CH ₃ CN	60 °C, 3.5 hours	8.3:1
13	(CH ₃ CO) ₂ O	toluene	65 °C, 5.5 hours	2.3:1
14	(CH ₃ CH ₂ CO) ₂ O	toluene	65 °C, 5.5 hours	2.4:1
15	(ClCH ₂ CO) ₂ O	toluene	65 °C, 5.5 hours	2.9:1
16	(Cl ₃ CCO) ₂ O	toluene	65 °C, 5.5 hours	3.4:1
17	(PhCO) ₂ O	toluene	65 °C, 5.5 hours	7.6:1
18*	(CH ₃ CH ₂ CO) ₂ O	CH ₂ Cl ₂	r.t., 6 hours	less than 1:20
19*	(ClCH ₂ CO) ₂ O	CH ₃ CN	60 °C, 6 hours	1:3.1
20*	(Cl ₃ CCO) ₂ O	toluene	60 °C, 6 hours	1:2.7
21*	(PhCO) ₂ O	CH ₃ CN	60 °C, 8 hours	1:2.3

* These experiments were carried out by adding 7-(triethylsilyl)-13-oxo-4-deacetylbaecatin III (3.13) to a previously prepared mixture of acylating agent, DCC and 4-PP.

3.1.6.7 Reduction of 4-Deacetyl-7-(triethylsilyl)-13-oxo-4-propionylbaccatin III (3.16) with Sodium Borohydride

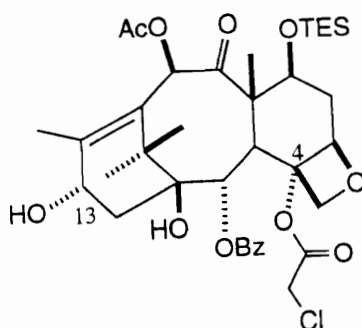
4-Deacetyl-7-(triethylsilyl)-13-oxo-4-propionylbaccatin III (3.16) was treated with NaBH_4 in THF-methanol (9:1), affording a more polar product. Upon purification of this product and analysis of ^1H NMR and mass spectral data, it was determined that the product was the desired C-13 α -hydroxy compound 4-deacetyl-7-(triethylsilyl)-4-propionylbaccatin III (3.24).



3.24

3.1.6.8 Reduction of 4-Deacetyl-4-chloroacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.18) with Sodium Borohydride

4-Deacetyl-4-chloroacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.18) was treated with NaBH_4 in THF-methanol (9:1), affording a more polar product. Upon purification of this product and analysis of its ^1H NMR and mass spectral data, it was determined that the product was the desired C-13 α -hydroxy compound 4-deacetyl-4-chloroacetyl-7-(triethylsilyl)baccatin III (3.25).



3.25

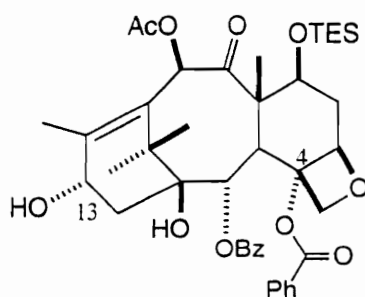
3.1.6.9 Attempted Reduction of 4-Deacetyl-4-trichloroacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.20) with Sodium Borohydride

4-Deacetyl-4-trichloroacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.20) was treated with NaBH_4 in THF-methanol (9:1), affording a major polar product. Upon purification of this product and analysis of its ^1H NMR and mass spectral data, it was found that the compound was identical with 4-deacetyl-7-(triethylsilyl)baccatin III (2.25). Hydrolysis of the C-4 trichloroacetate group of this compound was unexpected, however it was presumed that under the mildly basic conditions of the reduction the labile trichloroacetate group could be easily hydrolyzed, either directly or by intramolecular acyl transfer to the newly formed C-13 hydroxyl group and subsequent cleavage. The presence of unreacted starting material, noted in Section 3.1.6.4, may be indicative of the tendency of the C-4 trichloroacetate to undergo facile hydrolysis under basic conditions. That no corresponding C-10 deacetylated product was observed, in accordance with the hitherto established order of deacylation for baccatin III, may be explained by considering the more reactive nature of the trichloroacetate group relative to the acetate group, and the observation that no C-10 deacetylated products were observed in the NaBH_4 reduction of 7-(triethylsilyl)-13-oxobaccatin III (3.13). In light of the apparent lability of the trichloroacetate group under the above conditions, it was further reasoned that even if it were possible to prepare the C-4 trichloroacetyl taxol analog for *in vitro* and/or *in vivo* biological testing its efficacy would

probably be indeterminate, as potential enzyme-mediated hydrolysis of the inherently activated C-4 acyl group could lead to the formation of 4-deacetyltaxol (**2.1**), which has been previously shown to be less active than taxol (**1.8**). With these considerations in mind, it was decided to abandon any further attempts toward gaining the C-4 trichloroacetate analog.

3.1.6.10 Reduction of 4-Deacetyl-4-benzoyl-7-(triethylsilyl)-13-oxobaccatin III (**3.22**) with Sodium Borohydride

4-Deacetyl-4-benzoyl-7-(triethylsilyl)-13-oxobaccatin III (**3.22**) was treated with NaBH_4 in THF-methanol (9:1), affording a more polar product. Upon purification of this product and analysis of ^1H NMR and mass spectral data, it was determined that the compound was the desired C-13 α -hydroxy compound 4-deacetyl-4-benzoyl-7-(triethylsilyl)baccatin III (**3.26**).

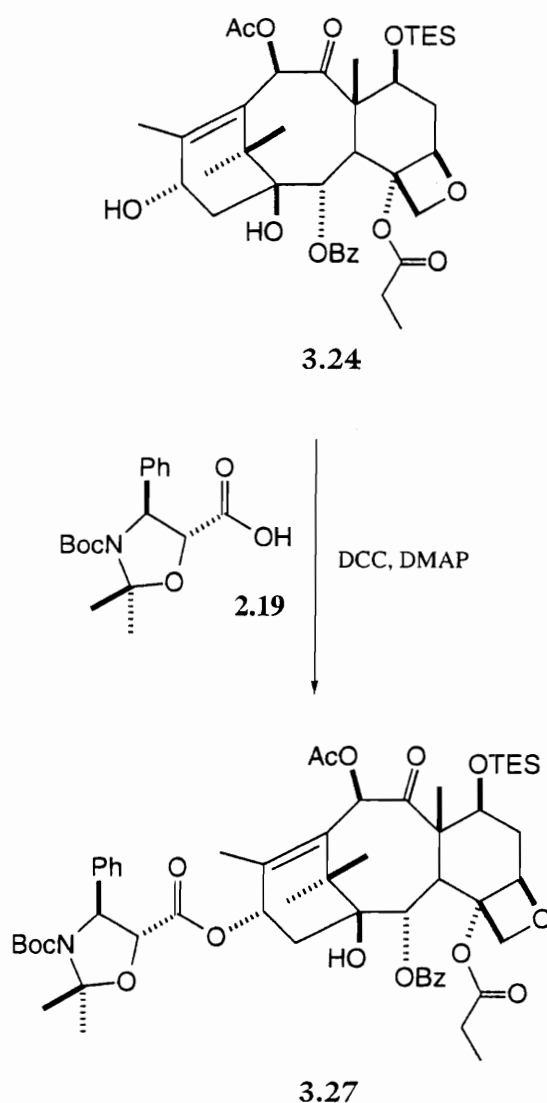


3.26

3.1.6.11 Coupling of 4-Deacetyl-7-(triethylsilyl)-4-propionylbaccatin III (**3.24**) with Side Chain **2.19**

As depicted in Scheme 15, 4-deacetyl-7-(triethylsilyl)-4-propionylbaccatin III (**3.24**) was treated with the side chain **2.19** under standard coupling conditions (DCC/DMAP) in toluene at 62 °C for 8 hours, affording 11.6 mg (62.4%) of a single less polar product. After

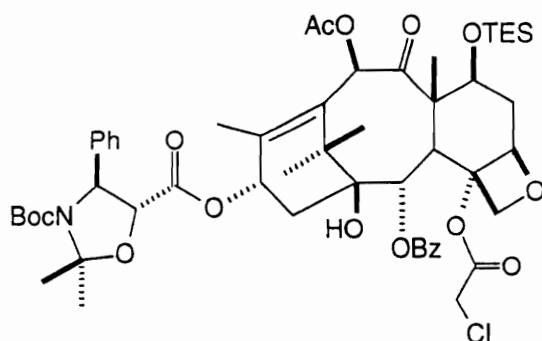
purification, the product was characterized by ^1H NMR, which showed coupling to have occurred as desired. Specifically, the ^1H NMR data showed a downfield shift for the C-13 proton, relative to the starting material, from about 4.8 ppm to 6.23 ppm, and additional signals for the C-2' and C-3' protons at 5.03 and 4.41 ppm, indicative of the addition of the side chain 2.19 to the baccatin substrate 3.24.



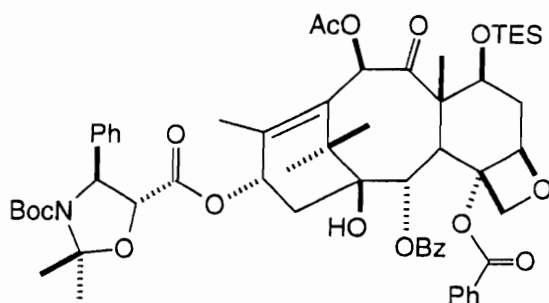
Scheme 15. Coupling of 4-deacetyl-7-(triethylsilyl)-4-propionylbaccatin III (3.24) with side chain 2.19 to afford the coupled 4-deacetyl-4-propionyltaxol derivative 3.27.

3.1.6.12 Coupling of 4-Deacetyl-4-chloroacetyl-7-(triethylsilyl)baccatin III (3.25) with Side Chain 2.19

4-Deacetyl-4-chloroacetyl-7-(triethylsilyl)baccatin III (3.25) was treated with the side chain 2.19 under standard coupling conditions (DCC/DMAP) in toluene at 62 °C for 8 hours, affording 8.3 mg (73.1%) of a single less polar product. After purification, the product was characterized by ^1H NMR, which showed coupling to have occurred as desired. Specifically, the ^1H NMR data showed a downfield shift for the C-13 proton, relative to the starting material, and additional signals for the C-2' and C-3' protons, indicative of the addition of the side chain 2.19 to the baccatin substrate 3.25.



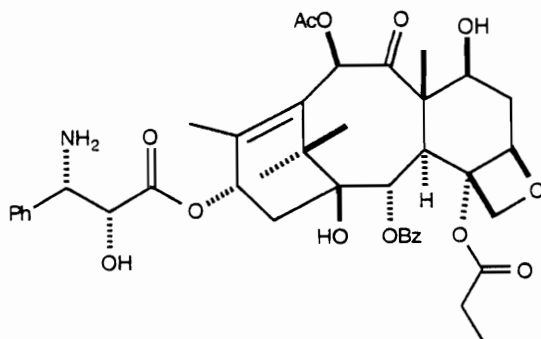
starting material, and additional signals for the C-2' and C-3' protons, indicative of the addition of the side chain 2.19 to the baccatin substrate 3.26.



3.29

3.1.6.14 Deprotection of the Coupled Compound 3.27, followed by 3'-N-Benzoylation

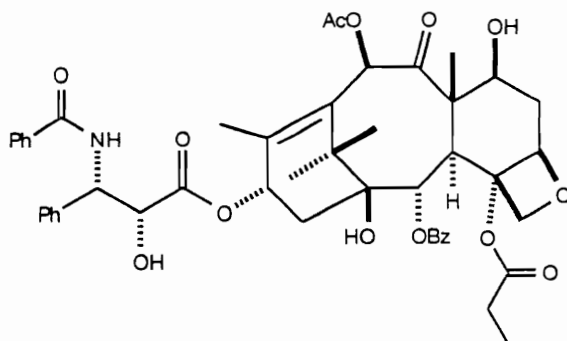
Using Commerçon's deprotection conditions,⁹³ compound 3.27 was treated with 99% formic acid, yielding 4-deacetyl-3'-N-debenzoyl-4-propionyltaxol (3.30) in good yield by TLC analysis.



3.30

The free amine 3.30 was not purified or characterized, but was immediately subjected to Schotten-Baumann conditions¹⁴³ to effect 3'-N-benzoylation. The major product of this reaction was thus realized in 57.2% yield over two steps from compound 3.27.

Characterization of this product allowed definitive identification of this compound as the target molecule 4-deacetyl-4-propionyltaxol (**3.31**).



3.31

The ^1H NMR spectrum of 4-deacetyl-4-propionyltaxol (**3.31**) appears in Figure 10, and as can be seen by comparison with a spectrum for taxol (**1.8**) (cf. Figure 6), the spectrum shows the following major features, indicating reacylation of the C-4 position with a propionate group:

- 1) no C-4 acetate methyl singlet at 2.38 ppm;
- 2) C-7 proton multiplet appears at 4.44 ppm; and
- 3) characteristic propionyl peaks at about 2.6 and 1.19 ppm.

Comparison of the ^{13}C NMR spectra of 4-deacetyl-4-propionyltaxol (**3.31**) and taxol (**1.8**) showed the major differences between the compounds to be the addition of a methylene signal at 28.7 ppm and a methyl signal at 9.2 ppm for compound **3.31**, indicating the presence of a propionate group in the molecule.

3.1.6.15 Deprotection of the Coupled Compound 3.28, followed by 3'-N-Benzoylation

Using Commerçon's deprotection conditions,⁹³ compound **3.28** was treated with 99% formic acid, yielding 4-deacetyl-4-chloroacetyl-3'-N-debenzoyltaxol (**3.32**) in good yield by TLC analysis.

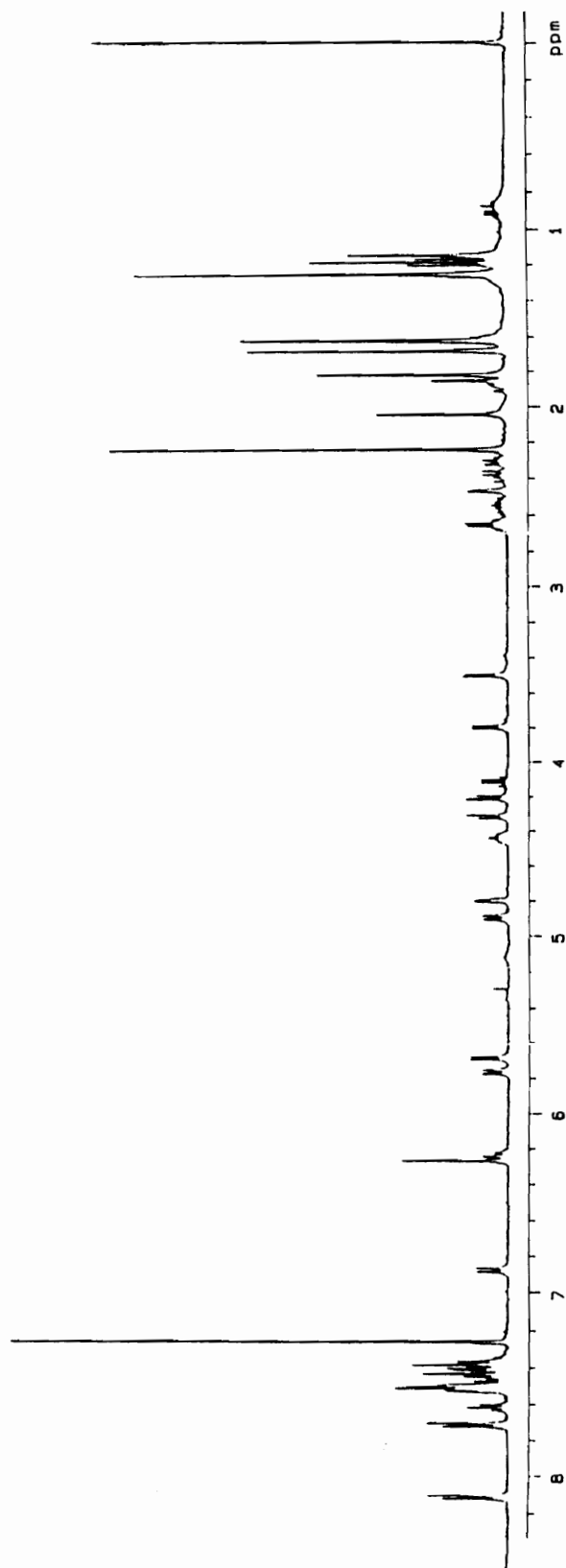
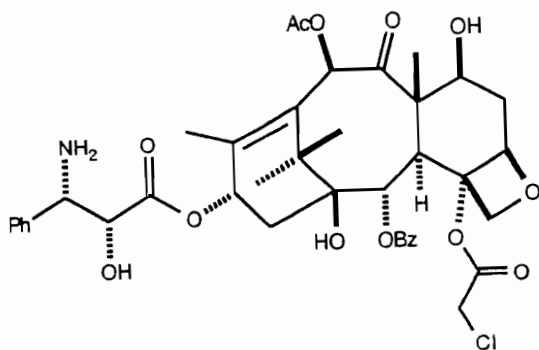


Figure 10. ^1H NMR spectrum of 4-deacetyl-4-propionyl-taxol (3.31).



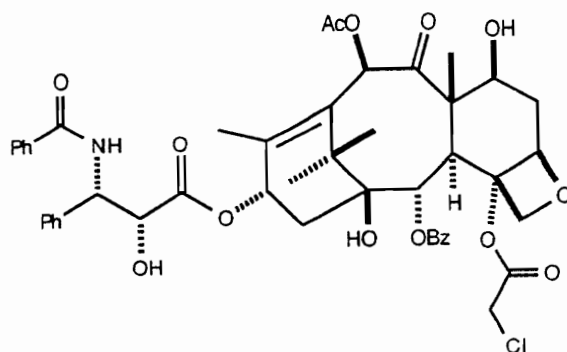
3.32

The free amine **3.32** was not purified or characterized, but was immediately subjected to Schotten-Baumann conditions¹⁴³ to effect 3'-*N*-benzoylation. The resultant material was purified by PTLC and was then analyzed by ¹H NMR. The spectrum obtained had a high level of background signals from impurities eluted from the preparative plate, but clearly indicated the following:

- 1) characteristic chloroacetate protons at 3.94 ppm;
- 2) C-7 proton multiplet at about 4.46 ppm;
- 3) no acetate methyl signal at 2.38 ppm; and
- 4) C-2' and C-3' proton signals at about 4.8 and 5.7 ppm, respectively.

A mass spectrum for this material showed a FAB [MH]⁺ peak at 889, consistent with the expected mass of the product. The typical chlorine isotope pattern was not observed however, presumably due to the high level of background noise in the spectrum obtained.

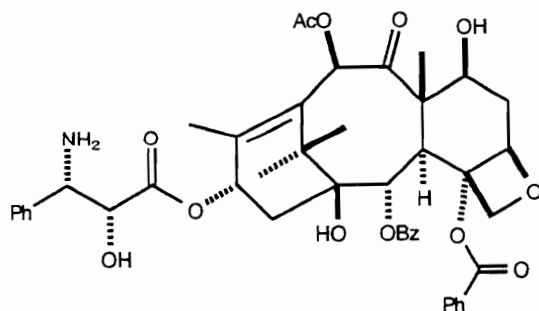
Based on this evidence, and on precedent from reactions described above, it is reasonable to propose that the chloroacetate group had indeed been introduced at the C-4 position. The compound was thus tentatively identified as 4-deacetyl-4-chloroacetyltaxol (**3.33**). Unfortunately, due to the small sample size, further efforts to purify and more rigorously characterize this material were unsuccessful.



3.33

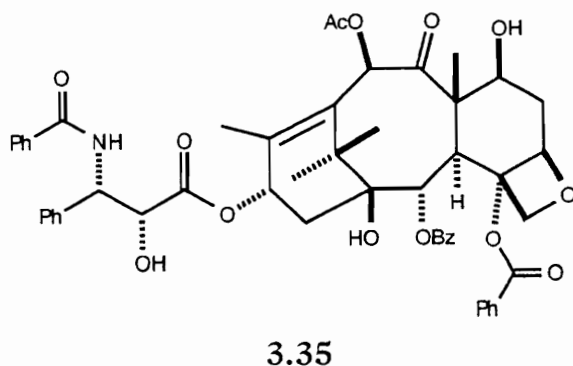
3.1.6.16 Deprotection of the Coupled Compound 3.29, followed by 3'-*N*-Benzoylation

Using Commerçon's deprotection conditions,⁹³ compound 3.29 was treated with 99% formic acid, yielding 4-deacetyl-4-benzoyl-3'-*N*-debenzoyltaxol (3.34) in good yield by TLC analysis.



3.34

The free amine 3.34 was not purified or characterized, but was immediately subjected to Schotten-Baumann conditions¹⁴³ to effect 3'-*N*-benzoylation. The major product of this reaction was thus realized in 45.7% yield over two steps from compound 3.29. Characterization of this product allowed this compound to be identified as 4-deacetyl-4-benzoyltaxol (3.35).



The ^1H NMR spectrum of 4-deacetyl-4-benzoyltaxol (3.35) appears in Figure 11, and as can be seen by comparison with a spectrum for taxol (1.8) (cf. Figure 6), the spectrum shows the following major features, indicating reacylation of the C-4 position with a benzoate group:

- 1) no acetate methyl singlet at 2.38 ppm;
- 2) C-7 proton multiplet appears at about 4.6 ppm; and
- 3) addition of characteristic benzoyl *ortho*-proton peaks at 8.24 and *meta*- and *para*-proton multiplets from 7.2 to 7.7 ppm.

Comparison of the ^{13}C NMR spectra of 4-deacetyl-4-benzoyltaxol (3.35) and taxol (1.8) showed the major differences between the compounds to be additional aromatic carbons between 127 and 131 ppm for compound 3.35, indicating the presence of an additional benzoate group in this molecule.

3.1.6.17 Recent Literature Reports on the Synthesis and Biological Evaluation of C-4 Modified Taxols

Just prior to the successful formation of the three C-4 acyltaxols 3.31, 3.33 and 3.35 in our labs, two research groups independently published the first syntheses of C-4 modified taxols. Chen and co-workers¹²⁷ of the Bristol-Myers Squibb Research Institute reported the

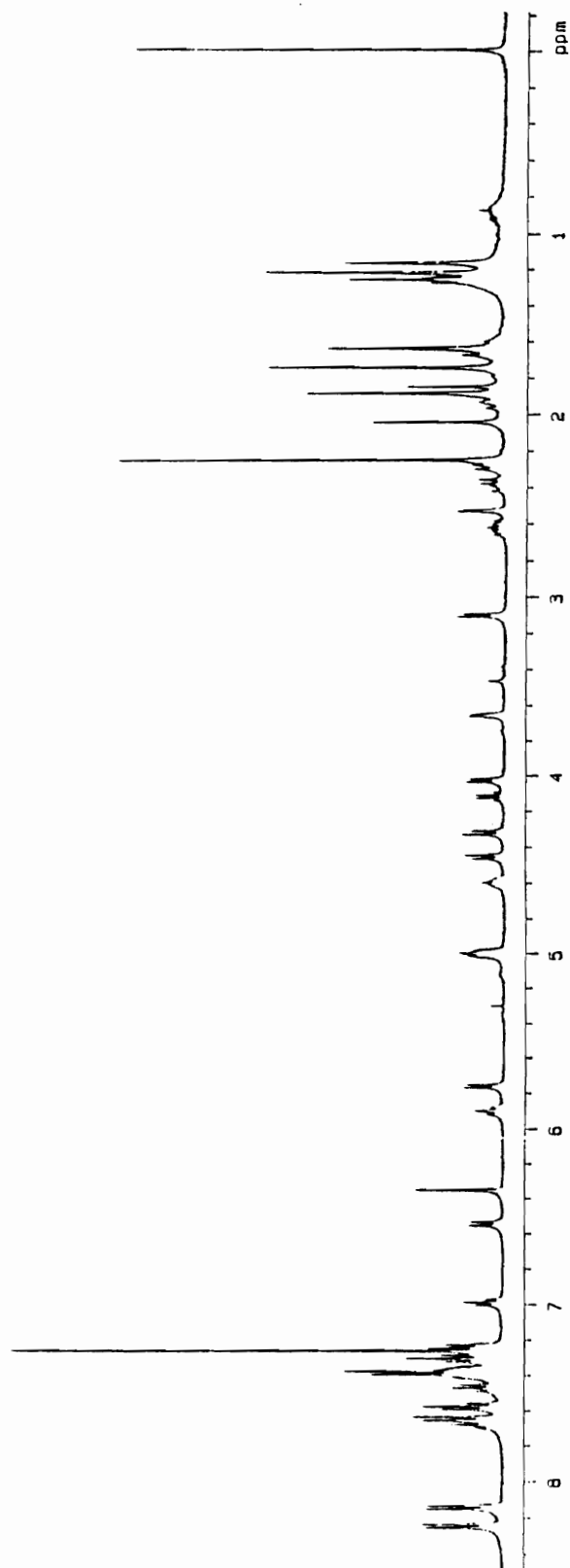
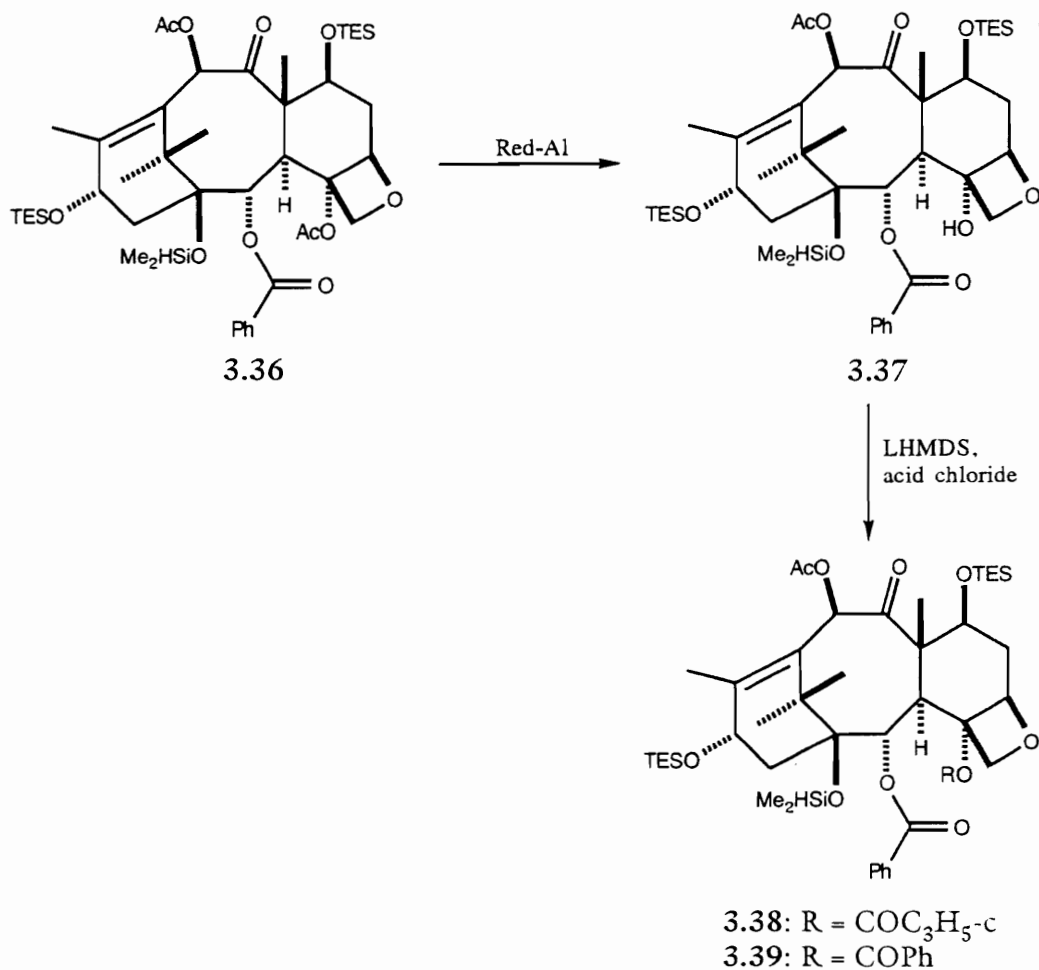


Figure 11. ^1H NMR spectrum of 4-deacetyl-4-benzoyl-taxol (3.35).

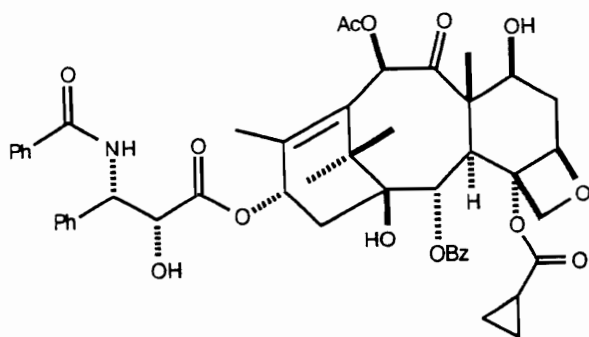
preparation of the C-4 cyclopropyl and the C-4 benzoyl esters of taxol from baccatin III (1.13). As depicted in Scheme 16, the key steps in this reaction sequence involved the selective removal of the C-4 acetate of the appropriately protected baccatin substrate 3.36 using a Red-Al mediated reduction, and the reacylation of the C-4 hydroxyl group using lithium bis(trimethylsilyl)amide and either cyclopropanecarbonyl chloride or benzoyl chloride.

The two corresponding C-4 acylated analogs thus produced, 4-deacetyl-4-cyclopropylcarbonyltaxol (3.40) and 4-deacetyl-4-benzoyltaxol (3.35), were tested for *in*



Scheme 16. Key steps in the reported preparation of two C-4 acylated taxol derivatives by Chen and co-workers.¹²⁷

vitro cytotoxicity in the HCT-116 human lung carcinoma cell line. It was determined from this assay that the C-4 cyclopropylcarbonyltaxol (**3.40**), displaying an IC_{50} value of 1.0nM, is more potent than taxol (1.8, IC_{50} = 2.4nM). The C-4 benzoyltaxol (**3.35**), on the other hand, was found to be much less active than taxol, with an IC_{50} value of 411nM.¹²⁷



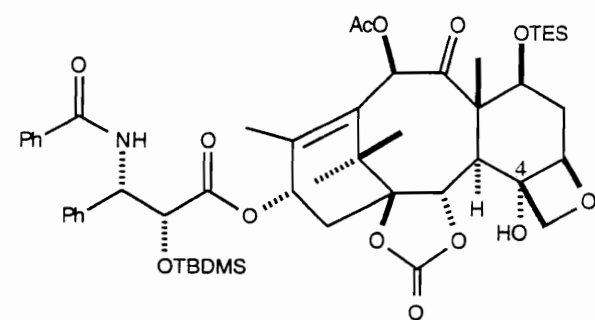
3.40

Georg and co-workers¹²⁸ reported the preparation of 4-deacetyl-4-isobutanoyltaxol (**3.41**) which, ironically, was formed using 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**), as shown in Scheme 17. The key step in this reaction sequence was the reacylation of the C-4 hydroxyl group using isobutyric anhydride (20 equivalents) and *N,N*-dimethylaminopyridine (20 equivalents).

Compound **3.41** was investigated for its ability to promote microtubule assembly and for its cytotoxicity against B16 melanoma cells, and was found to be less active than taxol in both the microtubule and the *in vitro* assays, exhibiting relative ED_{50} values of 2.6 and 4.5, respectively.¹²⁸

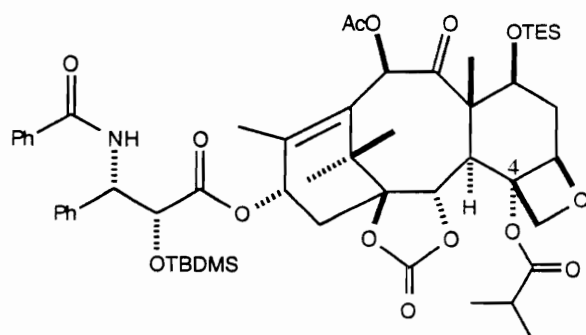
Very recently, the formation of the C-4 aziridine taxol derivative **3.42** was reported by Chen and co-workers as part of their ongoing research on the structure-activity relationships of the C-4 position.¹⁵²

The lead compound in the preparation of the aziridine-bearing taxol **3.42**, the baccatin substrate **3.43**, was formed by adaptation of the synthetic methodology shown earlier in Scheme 16, and the key steps in achieving the C-4 aziridine were the conversion of the C-4

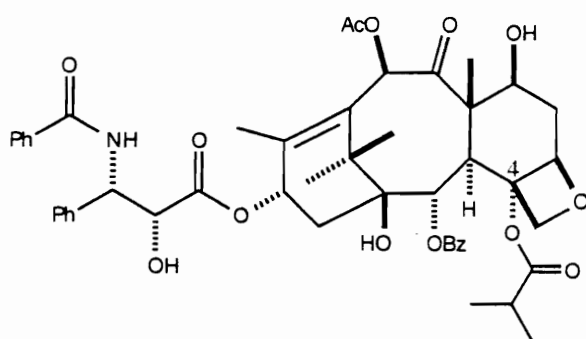


3.3

Isobutyric anhydride,
DMAP



1) PhLi
2) Deprotection

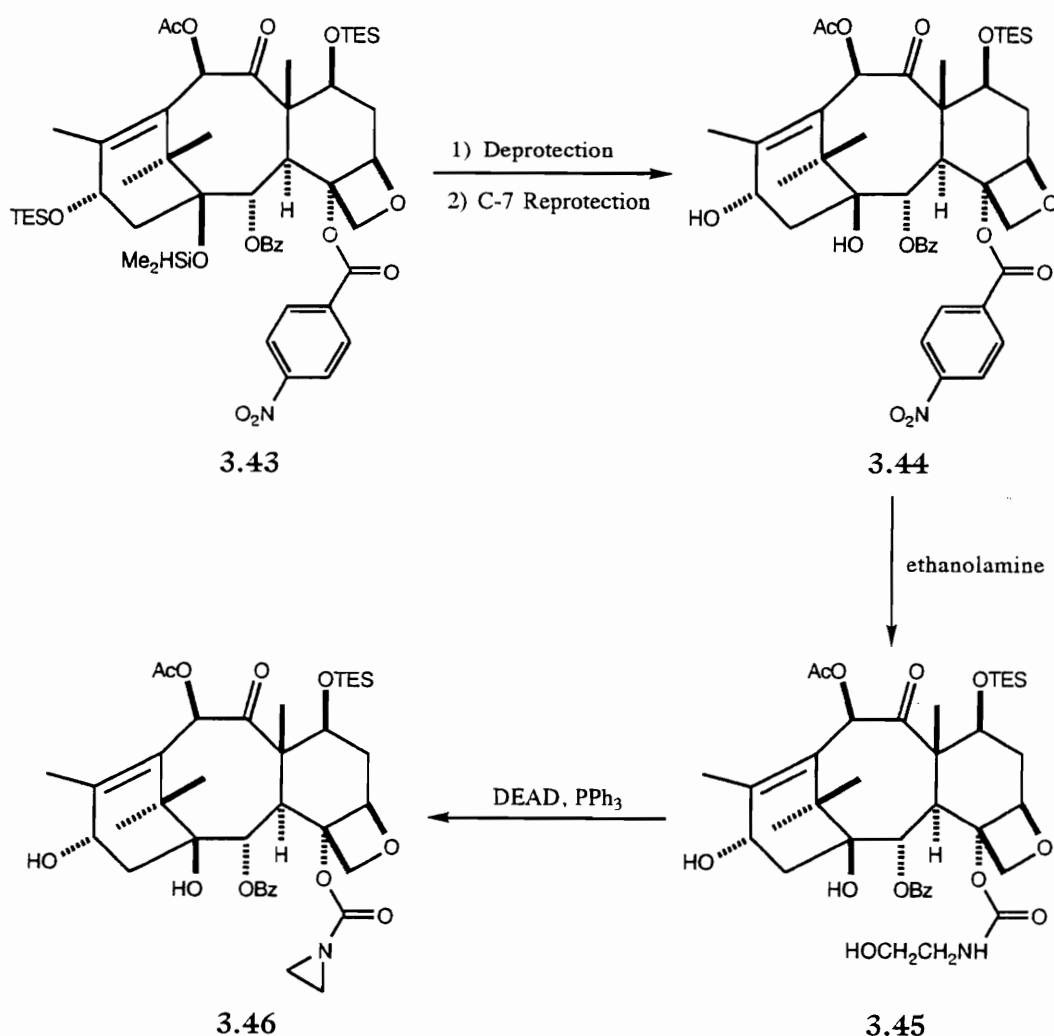


3.41

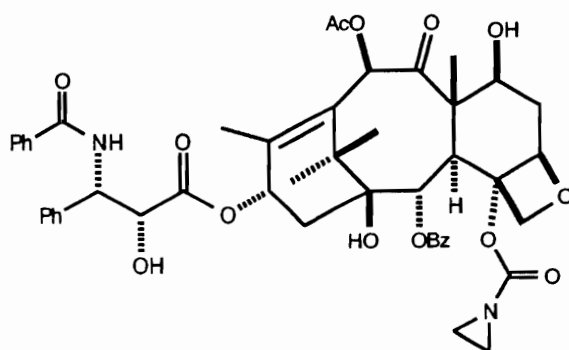
Scheme 17. Key steps in the reported preparation of the C-4 acylated taxol derivative **3.41** by Georg and co-workers.¹²⁸

para-nitrobenzoyl group of compound **3.44** to the corresponding carbamate **3.45**, followed by an intramolecular Mitsunobu reaction to afford the aziridine-bearing baccatin derivative **3.46**, as outlined in Scheme 18.

The resultant C-4 aziridine-bearing taxol derivative (**3.42**) was evaluated for biological activity, and it was found that this compound has lower activity than taxol in both the microtubule assembly assay (relative ED₅₀ of 2.8) and the HCT-116 *in vitro* cytotoxicity assay (IC₅₀ value of 15.6nM).¹⁵¹



Scheme 18. Key steps in the reported preparation of the C-4 aziridine-bearing taxol derivative **3.42** by Chen and co-workers.¹⁵⁰



3.42

Still more recently Chen and fellow researchers at Bristol-Myers Squibb have reported the preparation and biological evaluation of more than 30 taxol derivatives modified at the C-4 position.¹⁵³ With few exceptions, these compounds generally were found to be less active than taxol, indicating that modifications to the C-4 position generally lead to a disruption of the normal taxol-microtubule binding process.

3.1.7 Preliminary Chemical Investigations of the 13-Acyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III Derivatives

While the formation of the 13-acyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III derivatives was an unexpected occurrence during the initial attempts to acylate the C-4 position of 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin (3.13), it was believed that these derivatives could be employed in further investigations of the structure-activity relationships of taxol at the C-14 position. The only reported modifications to this position thus far have been taxol and taxotere derivatives bearing a 14 β -hydroxyl group.^{118,119} Analogs possessing the 14 α -hydroxyl group or acyl groups at either the α or the β position have not been reported to date, and it was thus conceived that the enol ester baccatins could be modified in such a manner that a series of C-14 substituted taxol derivatives could be generated. As a

preliminary to such a study, a number of reactions were conducted in order to assess the reactivity of the enol esters and their potential as precursors of C-14 modified taxols.

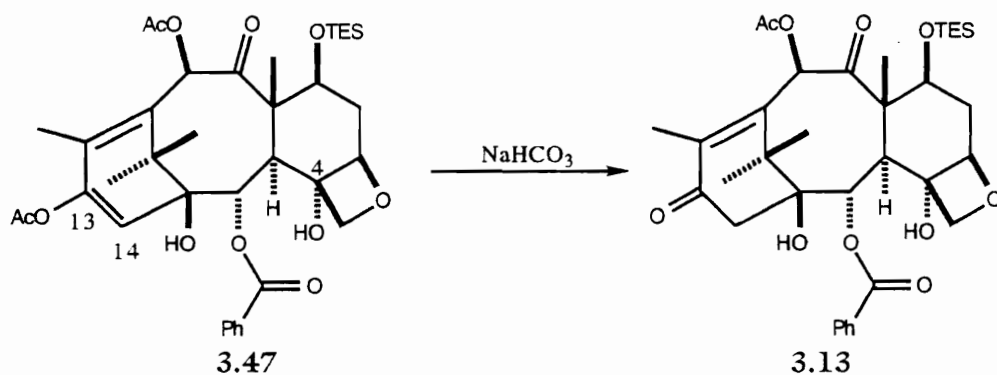
3.1.7.1 Hydrolysis of 13-Acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47)

13-Acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47) was stirred with sodium bicarbonate in methanol, affording one slightly more polar product by TLC analysis, as shown in Scheme 19.

Purification of the product by PTLC and subsequent analysis by ^1H NMR showed this compound to be 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13), the starting material from the acylation reactions previously discussed. In addition to providing an efficient way of regenerating the synthetically important compound 3.13, this reaction also confirmed the assumption that the enol esters would generally prove to be particularly labile under basic hydrolysis conditions.

3.1.7.2 Attempted C-4 Acylation of 13-Acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47)

In order to evaluate the feasibility of C-4 acylation of the enol ester baccatins, it was thought that the substrate with the smallest C-13 pendant acyl group, namely 13-acetyl-



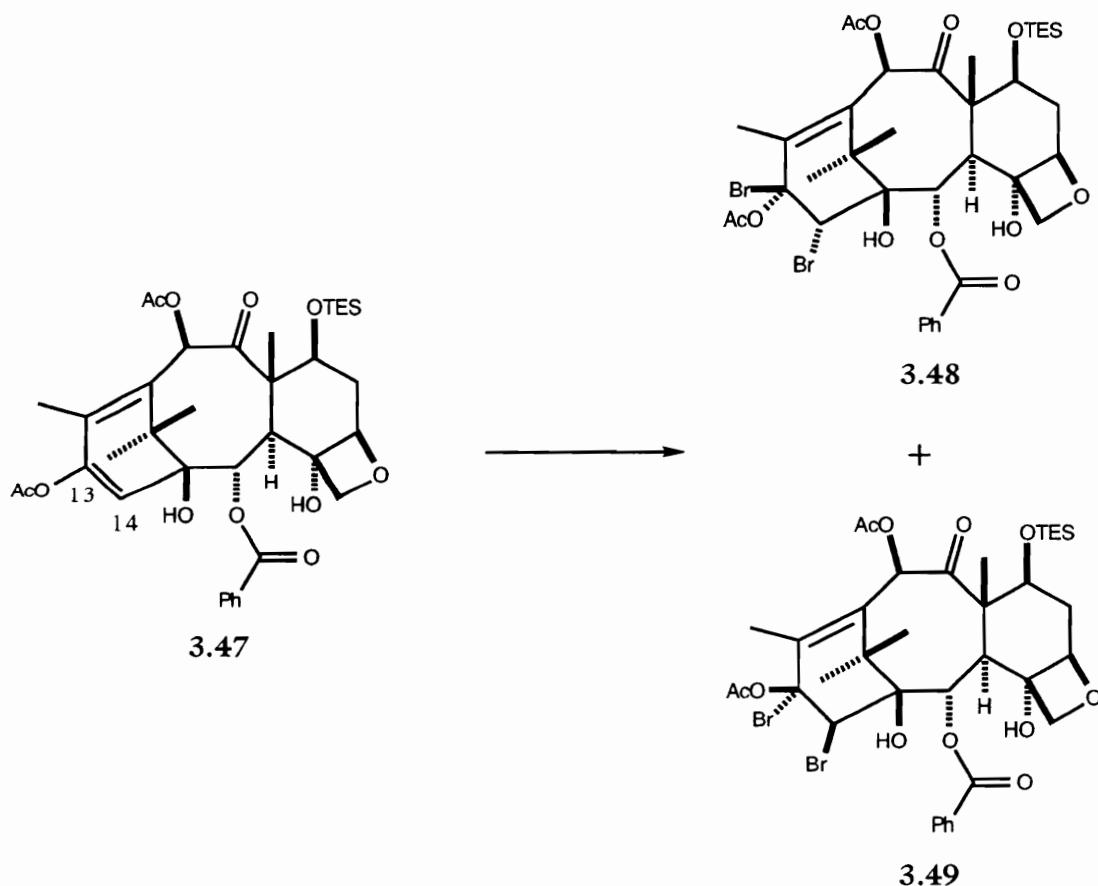
Scheme 19. Hydrolysis of 13-acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47) with sodium bicarbonate.

4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47), would be the best candidate for attempting to reacylate the C-4 position. Since acetylation of the C-4 position, followed by bicarbonate-catalyzed hydrolysis of the enol acetate would afford the known compound 7-(triethylsilyl)-13-oxobaccatin III (3.14), it was reasoned that subjecting 13-acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47) to extremely vigorous acetylation conditions would provide the best evidence of the ability of this compound to be reacylated. Accordingly, 13-acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47) was treated with acetic anhydride under extreme coupling conditions (DCC, DMAP, toluene) at 90 °C for 16 hours, at which time it was determined by TLC analysis that the only change in the starting material was the formation of baseline decomposition material which moved negligibly even in very polar TLC solvent systems. The starting material was isolated and was shown by ¹H NMR to be homogeneous, thus indicating that no C-4 acetylation had occurred. This finding led to the conclusion that if, even under extremely energetic coupling conditions, the C-4 position of 13-acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47) is not accessible to relatively small acylating agents such as the acetic anhydride used in this experiment, any attempts to repeat this experiment using different acylating agents and/or other enol ester baccatins would be in vain. The lack of reactivity of the C-4 position seen under reacetylation conditions indicates the degree to which the mouth of the molecular cup is closed by a relatively small ester such as an acetate at the C-13 position. From this, it can be inferred that larger pendant esters at the C-13 position would be even greater impediments to acylation at the C-4 position (cf. Figure 9). This inference has been substantiated by the observation, as reported herein, that the ratio of 13-acyl-4-deacetyl-13,14-dehydrobaccatin to the corresponding C-4 acylated C-13 keto baccatin increases proportionately with the size of the enol ester and/or the size of the particular acylating agent employed.

3.1.7.3 Bromination of 13-Acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47)

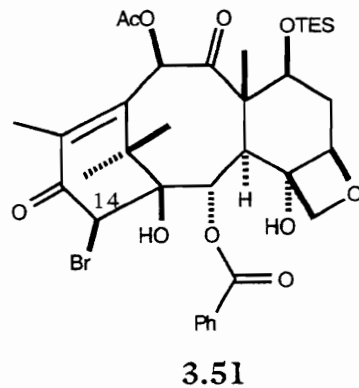
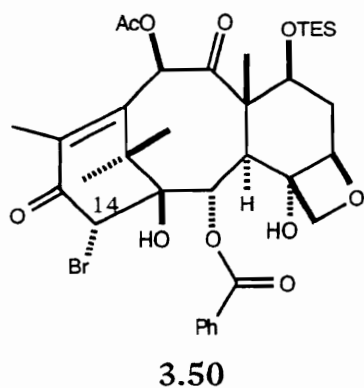
As depicted in Scheme 20, 13-acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47) was treated with bromine, which afforded two slightly more polar products by TLC analysis. Purification of these products by PTLC and subsequent ^1H NMR analysis indicated these compounds to be the diastereotopic bromo compounds 3.48 and 3.49

Conceivably, compounds 3.48 and 3.49 could be converted by treatment with sodium bicarbonate to the corresponding 13-keto-14 α -bromo- and 13-keto-14 β -bromo-baccatins,



Scheme 20. Bromination of 13-acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47).

3.50 and 3.51, affording two substrates for further investigation of the structure-activity relationships of the C-14 position.



3.1.8 Effects of Various Reagents on 7-(Triethylsilyl)-13-oxobaccatin III (3.14)

With the formation of 7-(triethylsilyl)-13-oxobaccatin III (3.14) it now became possible to study the chemical behavior of this molecule upon treatment with various reagents. It had been established that the C-13 ketone could be reduced with sodium borohydride to a 9:1 mixture of 7-(triethylsilyl)baccatin III (2.5) and 7-(triethylsilyl)-13-*epi*-baccatin III (3.15). In an effort to assess the yield of the desired C-13 α -hydroxyl baccatin 2.5, several reactions were run using somewhat bulky reducing agents to gain preferential reduction from the top face of the molecule. The results of these reactions are summarized below. Additionally, three hydrolysis reactions were carried out in order to evaluate the order of deacylation for 7-(triethylsilyl)-13-oxobaccatin III (3.14), and are also discussed below.

3.1.8.1 Treatment of 7-(Triethylsilyl)-13-oxobaccatin III (3.14) with Sodium Cyanoborohydride

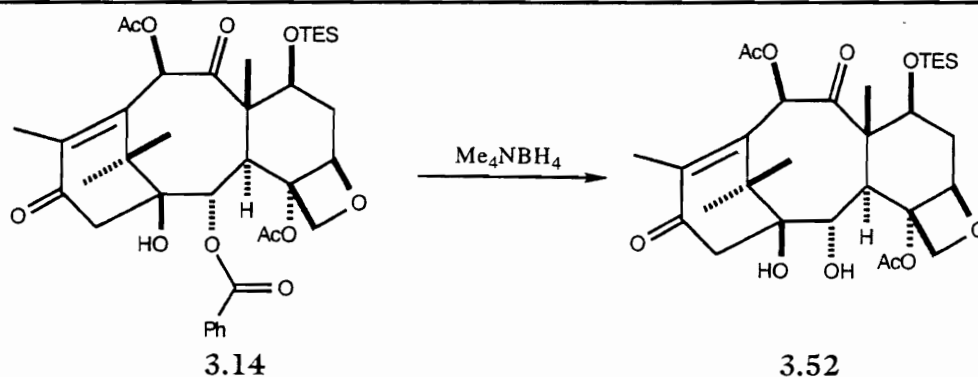
7-(Triethylsilyl)-13-oxobaccatin III (3.14) was treated with 10.0 equivalents of NaCNBH₃ in methanol, however, even after 24 hours no change in the reaction was

evident by TLC analysis. The unreacted starting material was recovered and observed to be homogeneous by ^1H NMR analysis, thus eliminating sodium cyanoborohydride from further consideration as a potentially selective reducing agent.

3.1.8.2 Treatment of 7-(Triethylsilyl)-13-oxobaccatin III (3.14) with Tetramethylammonium Borohydride

As depicted in Scheme 21, 7-(triethylsilyl)-13-oxobaccatin III (3.14) was treated with 20.0 equivalents of tetramethylammonium borohydride at room temperature. After 14 hours, the starting material appeared by TLC analysis to have been converted to a more polar product, which, after purification was identified by ^1H NMR analysis as 2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.52).

Under the conditions of the reaction, instead of the selective reduction of the C-13 ketone as desired, the reagent promoted the cleavage of the C-2 benzoate. It is not certain whether this was due to reduction of the C-2 benzoate via a C-1 hydroxyl group-assisted mechanism (similar to the C-2' hydroxyl group-assisted cleavage of the taxol side chain upon treatment with tetrabutylammonium borohydride¹¹⁷) or whether the C-2 benzoate was simply hydrolyzed under the reaction conditions employed. The lack of C-13 ketone reduction during this experiment led to the decision to eliminate tetramethylammonium borohydride from further consideration.



Scheme 21. Treatment of 7-(triethylsilyl)-13-oxobaccatin III (3.14) with tetramethylammonium borohydride.

3.1.8.3 Treatment of 7-(Triethylsilyl)-13-oxobaccatin III (3.14) with Tetrabutylammonium Borohydride

7-(Triethylsilyl)-13-oxobaccatin III (3.14) was treated with 16.0 equivalents of tetrabutylammonium borohydride at room temperature. Even after 90 hours, no reaction was evident by TLC analysis, leading to the decision to eliminate this ineffectual reducing agent from further consideration as a potentially selective reducing agent.

3.1.8.4 Treatment of 7-(Triethylsilyl)-13-oxobaccatin III (3.14) with Tetramethylammonium Hydroxide

Based on the observed hydrolysis of 7-(triethylsilyl)-13-oxobaccatin III (3.14) when treated with tetramethylammonium borohydride in methanol, the decision was made to investigate the hydrolysis of compound 3.14 using tetramethylammonium hydroxide. Accordingly, 7-(triethylsilyl)-13-oxobaccatin III (3.14) was treated with tetramethylammonium hydroxide, resulting in the starting material being converted to a more polar product. After purification, the product was analyzed by ^1H NMR and determined to be 2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.52), identical to the same compound described above.

3.1.8.5 Treatment of 7-(Triethylsilyl)-13-oxobaccatin III (3.14) with Benzyltrimethylammonium Methoxide

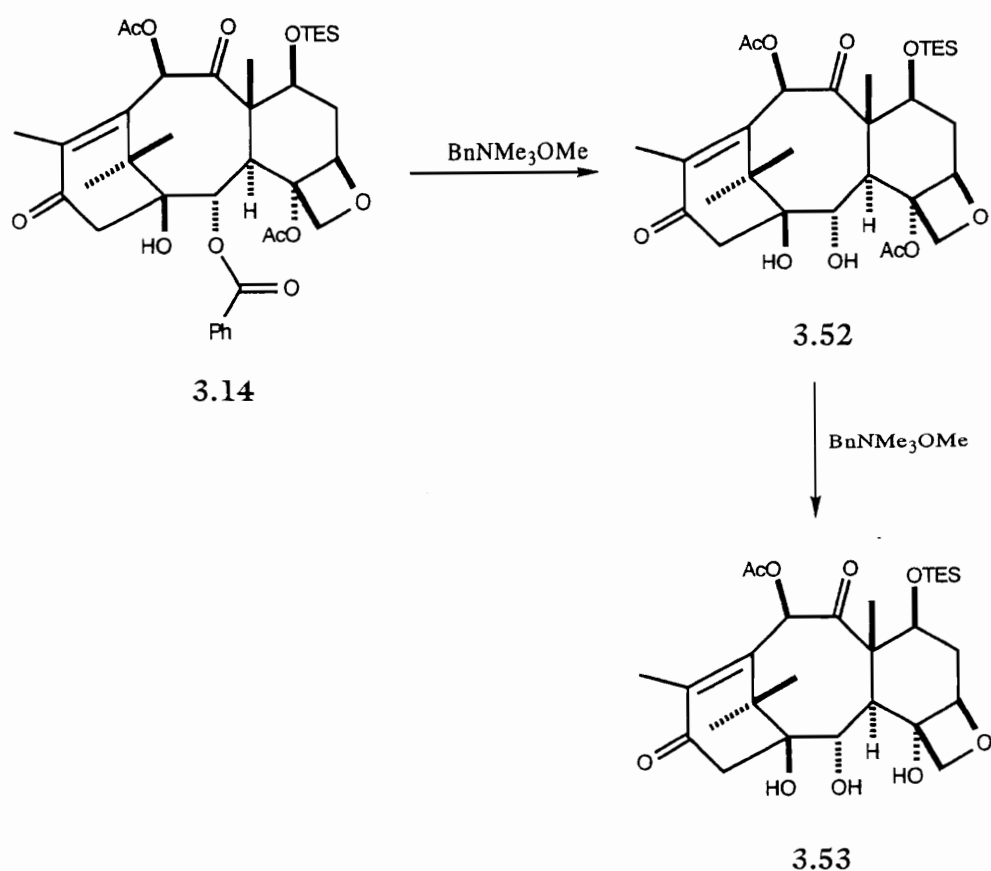
It was found from a preliminary reaction of 7-(triethylsilyl)-13-oxobaccatin III (3.14) with benzyltrimethylammonium methoxide at $-78\text{ }^\circ\text{C}$, as depicted in Scheme 22, that the starting material is initially hydrolyzed to 2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.52), identical to the same compound described above.

Continued exposure to the reagent as the reaction flask gradually warmed to ambient temperature resulted in the formation of 4-deacetyl-2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.53). No deacetylation of the C-10 position was observed by TLC or

^1H NMR analyses, leading to the establishment of the following deacylation order for 7-(triethylsilyl)-13-oxobaccatin III (3.14) treated with benzyltrimethylammonium methoxide: C-2 > C-4, no C-10.

3.1.8.6 Treatment of 7-(Triethylsilyl)-13-oxobaccatin III (3.14) with Sodium Methoxide

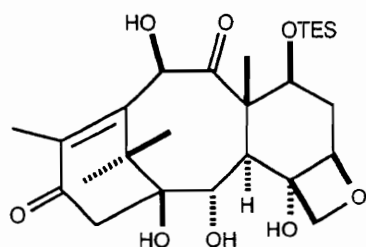
It was of particular interest in this study of the reactivity of 7-(triethylsilyl)-13-oxobaccatin III (3.14) to determine the effect on the reactivity of the C-4 acetate



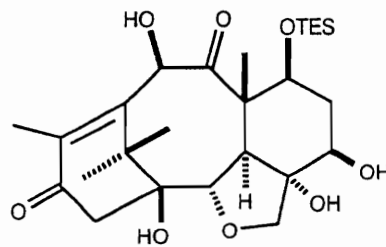
Scheme 22. Treatment of 7-(triethylsilyl)-13-oxobaccatin III (3.14) with benzyltrimethylammonium methoxide.

caused by the conversion of the C-13 α -hydroxy group to the corresponding ketone. As mentioned previously, the apparent lability of the tertiary C-4 acetate in 7-(triethylsilyl)baccatin III (2.5) has been attributed to an intramolecular acetate transfer to the proximate C-13 hydroxyl group, followed by facile hydrolysis.^{115,138} Indeed, Samaranayake reported that after protection of the C-13 hydroxyl group with the triethylsilyl group, C-4 deacetylation is not observed under methanolysis conditions.^{115,138} This seems to indicate that the C-13 hydroxyl group plays an important role in assisting the hydrolysis of the C-4 acetate. Whether this intramolecular acetate transfer is the only mechanism by which the C-4 acetate can be removed was, however, thrown into question upon: 1) the successful acylation of 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13); and 2) the reaction of 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13) with benzyltrimethylammonium methoxide, which showed that the C-4 acetate can apparently be hydrolyzed without the assistance of the C-13 hydroxyl group. It was reasoned that, since acylation of the C-4 position implies a direct attack of the acylating agent, the same position in 7-(triethylsilyl)-13-oxobaccatin III (3.14) should be directly accessible to the smaller methoxide ion or other reagents, as was shown to be the case with benzyltrimethylammonium methoxide. In order to further test this hypothesis, a study of the methanolysis of 7-(triethylsilyl)-13-oxobaccatin III (3.14) was undertaken, as discussed below.

7-(Triethylsilyl)-13-oxobaccatin III (3.14) was initially treated with sodium methoxide under rather vigorous conditions (10 equivalents of sodium methoxide at room temperature), leading to the formation of two more polar products, which were identified by ¹H NMR as 4,10-bis(deacetyl)-2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.54) and 4,10-bis(deacetyl)-2-debenzoyl-7-(triethylsilyl)-13-oxoisobaccatin III (3.55).



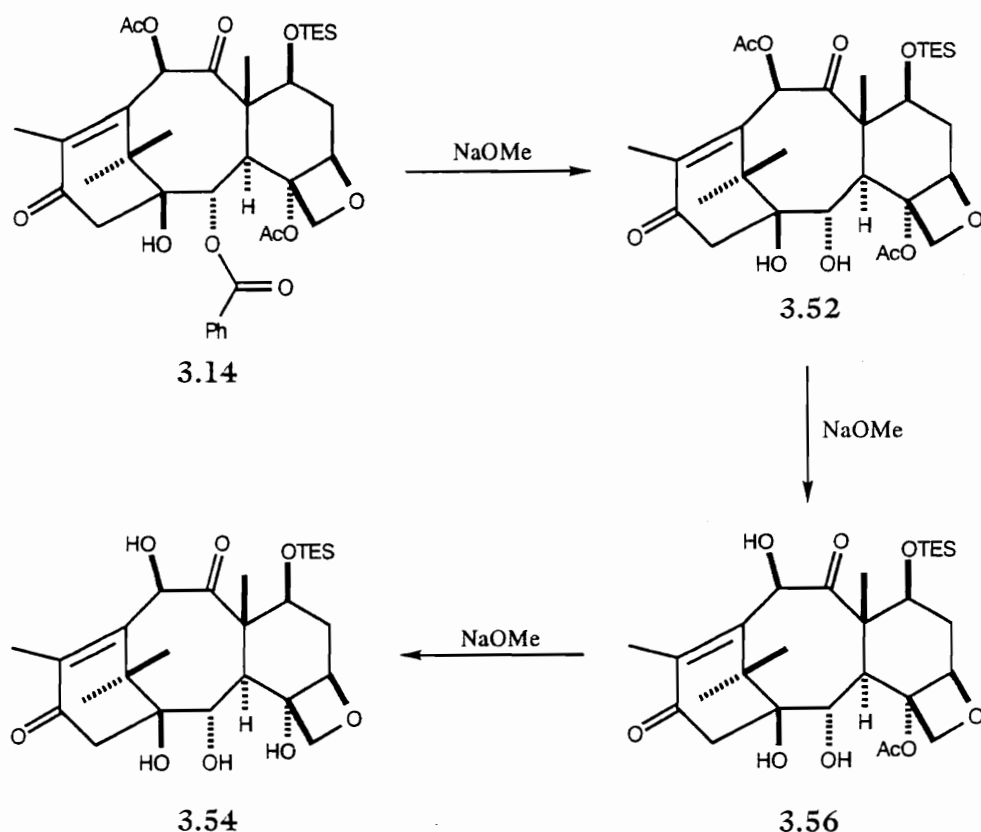
3.54



3.55

Although the formation of these products was anticipated based on the results of the previous experiment with benzyltrimethylammonium methoxide, the order of deacylation obviously could not be established from this single reaction. Therefore, another experiment was initiated in which three simultaneous reactions were run using varying concentrations of methoxide. In the first reaction, 7-(triethylsilyl)-13-oxobaccatin III (3.14) was treated for 15 minutes with 1.0 equivalent of sodium methoxide at room temperature, affording an approximately 1:1 mixture of 2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.52) and 10-deacetyl-2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.56), respectively. In the second reaction, 7-(triethylsilyl)-13-oxobaccatin III (3.14) was treated for 15 minutes with 2.1 equivalents of sodium methoxide at room temperature, affording an approximately 2:5:1 mixture of three products, which were identified as 2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.52), 10-deacetyl-2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.56) and 4,10-bis(deacetyl)-2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.54), respectively. In the third reaction, 7-(triethylsilyl)-13-oxobaccatin III (3.14) was treated for 15 minutes with 4.0 equivalents of sodium methoxide at room temperature, affording an approximately 1:1 mixture of 10-deacetyl-2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.56) and 4,10-bis(deacetyl)-2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.54), respectively. These findings, as represented in Scheme 23, led to the establishment of the following deacylation order for 7-(triethylsilyl)-13-oxobaccatin III (3.14) treated under methanolysis conditions: C-2 > C-10 > C-4.

It is of interest that the orders of deacylation of 7-(triethylsilyl)-13-oxobaccatin III (3.14) are different depending on the base employed. That no C-10 deacetylation is observed in the case when benzyltrimethylammonium methoxide is used seems to indicate that the sterically large base cannot approach this position closely enough to effect deacetylation. Whether the bulky base attacks the C-2 benzoate and then the C-4 acetate by direct approach, is unknown and seems unlikely, but as mentioned previously (cf. Section 2.1.9) it has been postulated that removal of these esters may be facilitated by neighboring group participation, specifically by the assistance of the C-1 hydroxy in the case of removal of the C-2 benzoate, and the assistance of the incipient C-2 hydroxy in the case of removal of the C-4 acetate.¹²⁸



Scheme 23. Order of deacylation observed in the methanolysis of 7-(triethylsilyl)-13-oxobaccatin III (3.14).

On the other hand, when sodium methoxide is employed, the order of deacylation indicates that the C-2 benzoate is still the most labile of the three esters, followed by the C-10 acetate and then the C-4 acetate. This order of reactivity seems to imply that, while neighboring hydroxyl group participation may be responsible to a small extent for the removal of the C-2 and C-4 esters (but not the C-10 acetate), direct attack of the methoxide on the three esters of 7-(triethylsilyl)-13-oxobaccatin III (3.14) is the predominant mechanism of cleavage. The heightened lability of the C-2 benzoate of 7-(triethylsilyl)-13-oxobaccatin III (3.14) relative to its counterpart in 7-(triethylsilyl)baccatin III (2.5) may be due to changes in the overall molecular conformation caused by the oxidation of the C-13 hydroxyl group. Conformational changes and the concomitant exposure of the underside of the molecule upon oxidation of the C-13 hydroxyl group may also account for the observed reactivity of the C-4 acetate of 7-(triethylsilyl)-13-oxobaccatin III (3.14). This further suggests that the lack of C-4 hydrolysis of 7,13-bis(triethylsilyl)hexahydrobaccatin III (2.11) reported by Samaranayake¹³⁸ may to a great extent be attributed to the "umbrella effect" of the C-13 triethylsilyl ether, and is more a reflection of the interference of the protecting group to the approaching base than a complete lack of reactivity when the C-13 hydroxyl has been protected. The importance of the C-13 hydroxyl group of 7-(triethylsilyl)baccatin III (2.5) in promoting the hydrolysis of the C-4 acetate cannot be ignored however, since in cases where the C-13 hydroxyl group is not protected (or oxidized to the ketone) the C-4 acetate is cleaved much more readily than the C-2 benzoate. This indicates that while the C-4 acetate can be hydrolyzed independently of the C-13 hydroxyl group, its removal is further enhanced by C-13 hydroxyl group-assisted intramolecular acetate transfer, as previously discussed.

From the above observations of the effect of base on various baccatin III substrates, the following conclusions can be drawn regarding deacylation order:

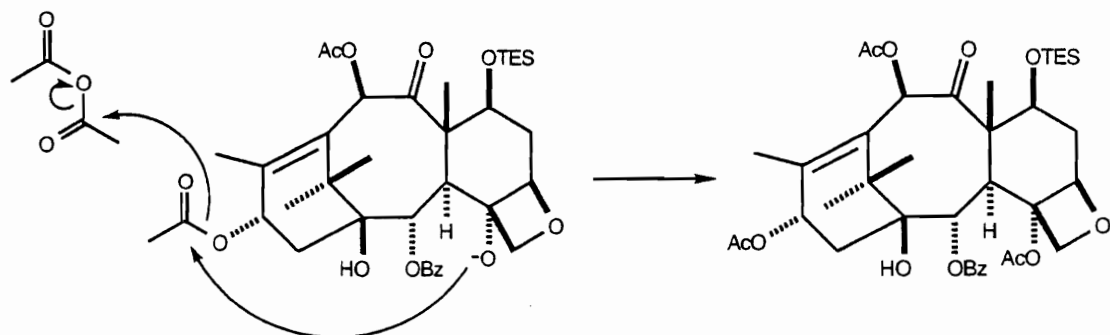
- 1) treatment of 7-(triethylsilyl)baccatin (2.5) with sodium methoxide gives the deacylation order: C-10 > C-4 > C-2;

- 2) treatment of 7-(triethylsilyl)baccatin (2.5) with potassium *tert*-butoxide gives the deacylation order: C-10 > C-4, no C-2;
- 3) treatment of 7-(triethylsilyl)-13-oxobaccatin (3.14) with tetramethylammonium hydroxide results in hydrolysis of the C-2 benzoate but no hydrolysis of the C-4 or C-10 acetates;
- 4) treatment of 7-(triethylsilyl)-13-oxobaccatin (3.14) with benzyltrimethylammonium methoxide gives the deacylation order: C-2 > C-4, no C-10; and
- 5) treatment of 7-(triethylsilyl)-13-oxobaccatin (3.14) with sodium methoxide gives the deacylation order: C-2 > C-10 > C-4.

3.1.9 Preliminary Chemical Investigations of a Possible “Uphill” Intramolecular Acetate Transfer

Based on the accumulating evidence in support of the “downhill” intramolecular acetate transfer from the tertiary C-4 position to the secondary C-13 position during the methanolysis of 7-(triethylsilyl)baccatin III (2.5), speculation arose as to the possibility of an “uphill” intramolecular acetate transfer, in which an acetate at the C-13 position might be transferred to the C-4 position, as depicted in Scheme 24.

By subjecting 13-acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (3.10) to acetylation under forcing conditions (cf. Section 3.1.5), it had been established that the C-4 position



Scheme 24. Proposed “uphill” intramolecular acetate transfer.

could indeed be reacetylated. Whether this occurs by direct attack of the acylating group on the C-4 hydroxyl group or through an intramolecular acetate transfer from the C-13 position under the rather vigorous reaction conditions employed was indeterminate. In order to investigate the proposed “uphill” acetate transfer, it was envisioned that 13-acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (3.10) could be subjected to the same forcing conditions previously employed, but using a deuterium-labeled acetylating agent. Formation of product and subsequent analysis by ^1H NMR would allow the determination of whether the labeled acetate is found at the C-4 position, indicating direct acetate attack; or at the C-13 position, indicating that an intramolecular acetate transfer occurs. These two possibilities are depicted in Scheme 25.

Conversely, 10- d_3 -acetyl-13- d_3 -acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (3.59) could be reacted with unlabeled acetic anhydride under forcing conditions, leading to the two possible products indicated in Scheme 26.

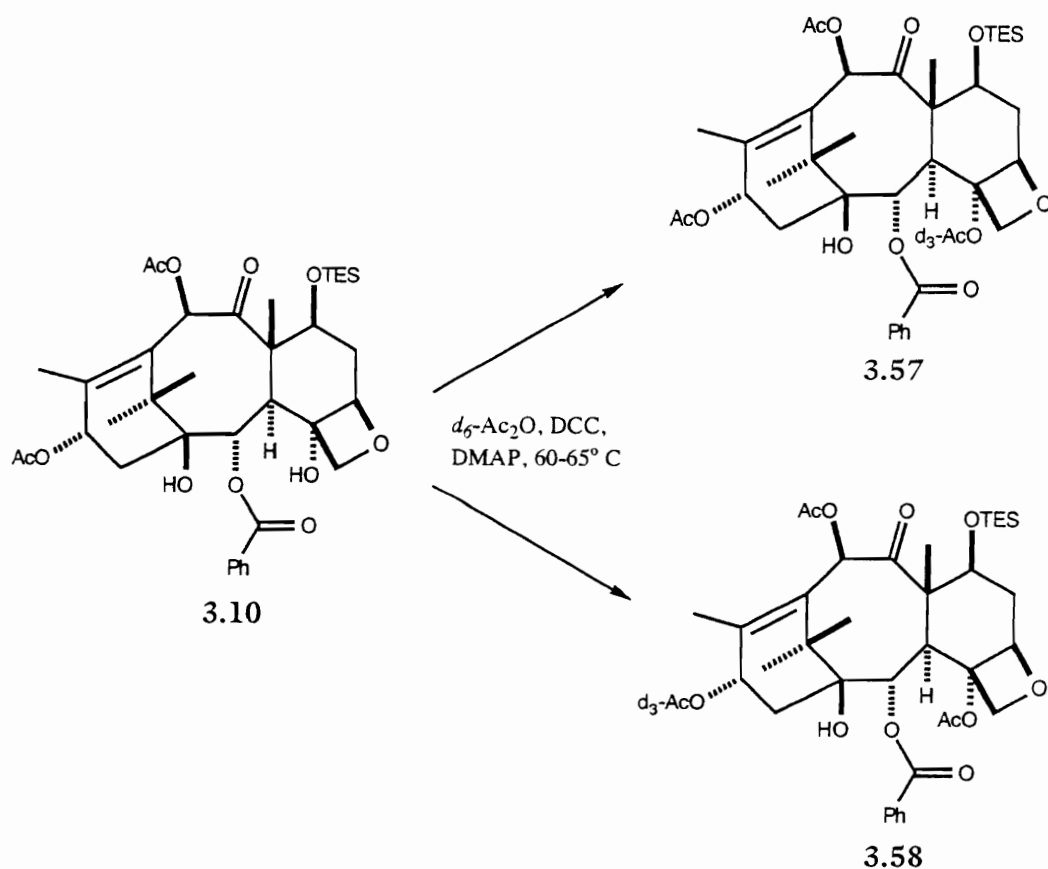
In order to carry out this investigation in a thorough fashion, it was decided that both baccatin substrates, 3.10 and 3.59, would be used. Accordingly, 13-acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (3.10) was prepared from 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7), as described in Section 3.1.5. 10- d_3 -Acetyl-13- d_3 -acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (3.59) was prepared in the same manner, except that d_6 -acetic anhydride was employed as the acetylating agent, as depicted in Scheme 27.

13-Acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (3.10) was treated with d_6 -acetic anhydride under forcing conditions (DCC/4-PP/65 °C), yielding a slightly less polar product which, after purification and subsequent analysis by ^1H NMR, was found to be a mixture of the two compounds 3.57 and 3.58. Evidence for this comes from the observation that the C-4 and C-13 acetate methyl peaks, normally appearing at 2.34 and 2.20 ppm, respectively, integrate for less than three protons each, as would be expected for the incorporation of labeled acetate at both positions.

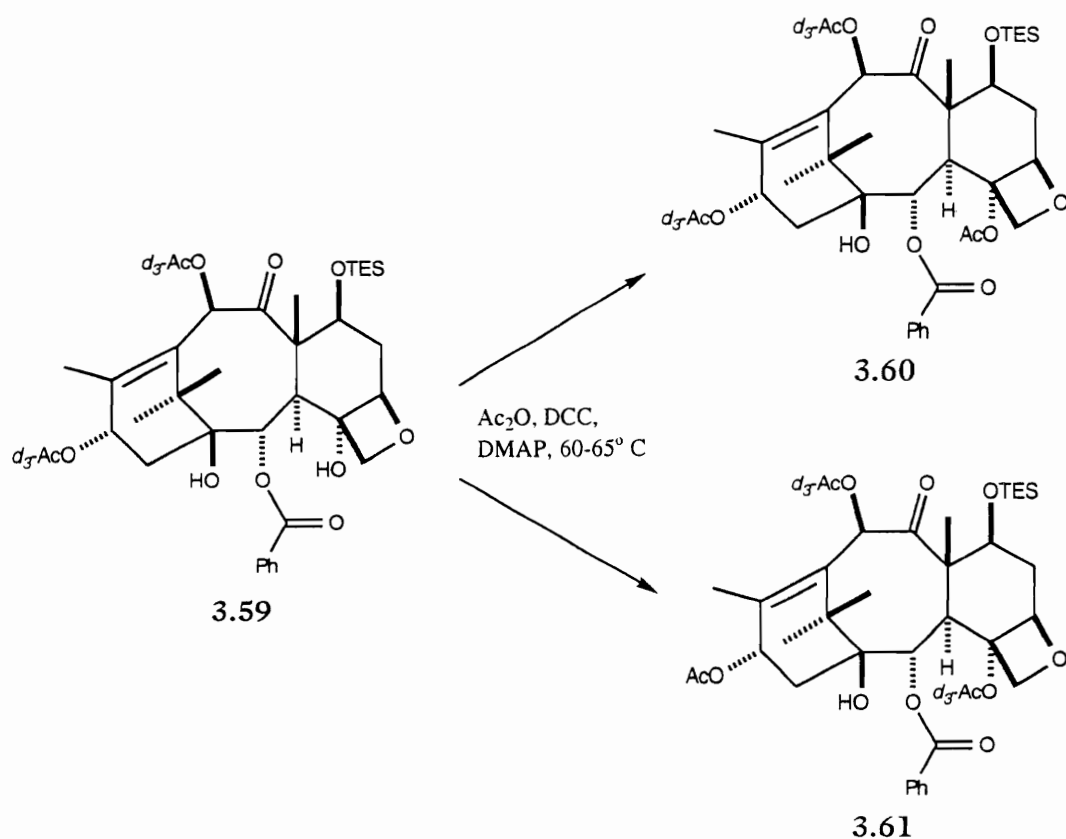
10- d_3 -Acetyl-13- d_3 -acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (3.59) was treated with unlabeled acetic anhydride under forcing conditions (DCC/4-PP/65 °C), to yield a slightly

less polar product which, after purification and subsequent analysis by ^1H NMR, was found to be a mixture of the two compounds **3.60** and **3.61**. Similar to the experiment described above, the NMR data shows that the C-4 and C-13 acetate methyl peaks integrate for less than three protons each, thus indicating the incorporation of labeled acetate at both positions.

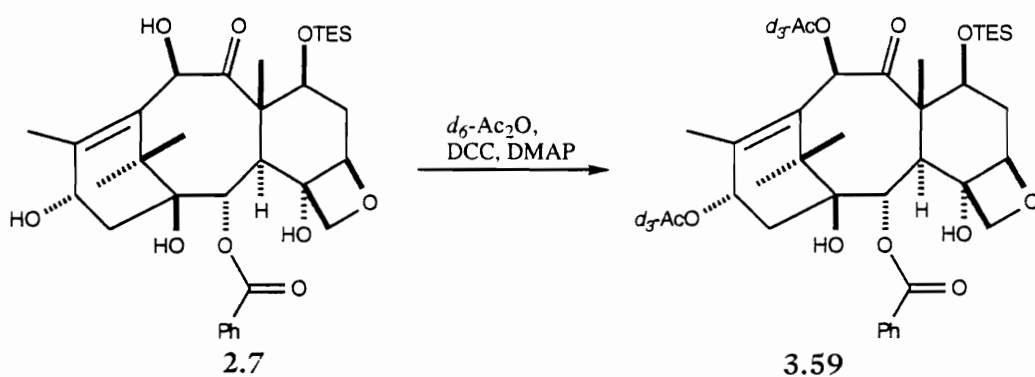
From these preliminary results on the substrates **3.10** and **3.54**, it may be proposed that two pathways can operate during C-4 acetylation, namely, acetylation by direct approach of the acyl group to the C-4 position, and acetylation by intramolecular acetate transfer from the C-13 position. Thus, there is evidence that the proposed “uphill”



Scheme 25. Possible outcomes of the reaction of 13-acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (**3.10**) with d_6 -acetic anhydride under forcing conditions.



Scheme 26. Possible outcomes of the reaction of 10- d_3 -acetyl-13- d_3 -acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (**3.59**) with acetic anhydride under forcing conditions.



Scheme 27. Preparation of 10- d_3 -acetyl-13- d_3 -acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (**3.59**) from 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (**2.7**).

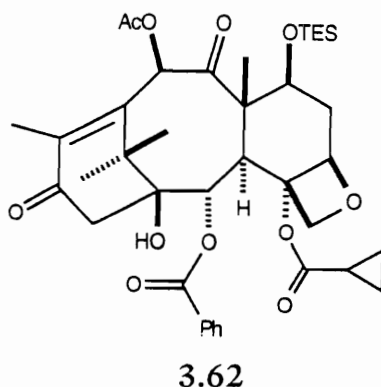
intramolecular acetate transfer can in fact occur, but whether reaction conditions can be varied in such a way as to make this the predominant pathway toward C-4 acetylation, with the potential for extending this methodology to the placement of other acyl groups at the C-4 position, is not known at this time and warrants further investigation.

III. CONCLUSIONS

Because of the absence of information on the structure-activity relationships of the C-4 position of taxol at the inception of this project, it was the goal of this work to prepare 4-deacetyltaxol and 4-acyltaxol congeners in order to evaluate the effect(s) on biological activity these modified compounds might elicit. As is evident from the foregoing discussion, the preparation of these compounds is not a trivial endeavor; however, 4-deacetyltaxol (**2.1**) and the 4-acyltaxol analogs **3.31** and **3.35** have now been prepared, with a tentative identification having been made for a third 4-acyltaxol analog **3.33**. 4-Deacetyltaxol was subjected to biological testing and was found to be significantly less active than taxol. While the 4-acyltaxol analogs have not yet been evaluated for biological activity, it has been shown from recent literature reports that 4-acyltaxol analogs, with the exception of 4-deacetyl-4-cyclopropanecarbonyltaxol (**3.40**), generally exhibit lower activities than taxol.^{127,128,151} In particular, Chen and co-workers¹²⁷ have shown that 4-deacetyl-4-benzoyltaxol (**3.35**) is nearly 200 times less active than taxol. These researchers have also reported the formation of 4-deacetyl-4-aziridinotaxol (**3.42**), which is 2.8 times less active against microtubule assembly and is 15.6 times less active than taxol in the HCT-116 cytotoxicity assay.¹⁵¹ Unpublished results obtained by Chen and co-workers¹⁵² also indicate that other C-4 modified taxol derivatives generally display lower activity than taxol. Georg and co-workers¹²⁸ have prepared 4-deacetyl-4-isobutanoyltaxol (**3.41**), which is 2.6 times less active than taxol. These results seem to indicate that the acetate group is necessary at the C-4 position, possibly serving as a conformational "lock" for the C ring or participating directly at the taxol-microtubule interface. Only slight adjustments can be made at the C-4 position, as evidenced by the fact that only one C-4 modified analog is known to have a higher biological activity than taxol, suggesting that other acyl groups at this position reduce the binding affinity of taxol with its microtubule receptor site.

While the particular synthetic methodology developed during this project for the preparation of 4-acyltaxol derivatives from baccatin III derivatives is somewhat laborious,

this approach does offer a significant degree of versatility in the preparation of potentially more active taxol analogs. For example, since 4-deacetyl-4-cyclopropanecarbonyltaxol (**3.40**) shows enhanced biological activity, it is conceivable that this same acyl group could be attached at the C-4 position of 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (**3.13**), giving 4-deacetyl-4-cyclopropylcarbonyl-7-(triethylsilyl)-13-oxobaccatin III (**3.62**).



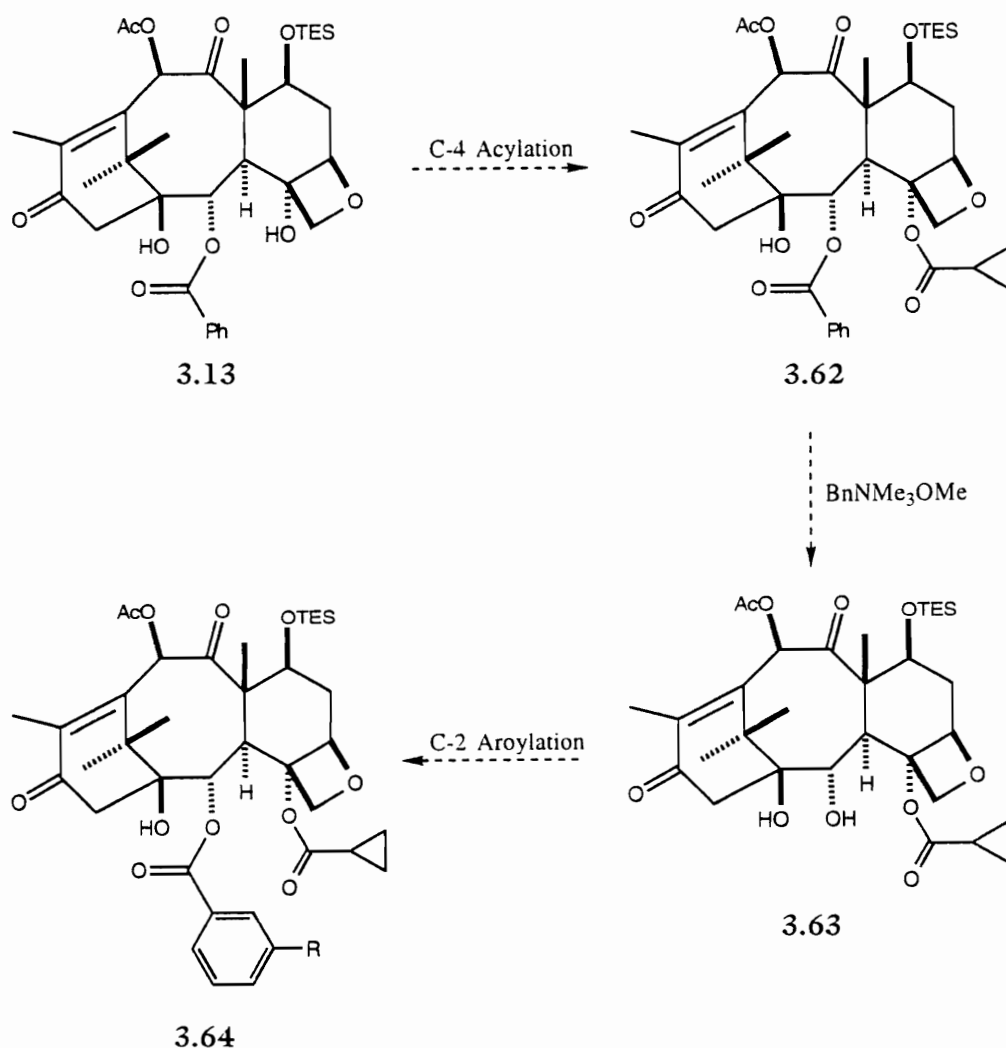
Compound **3.62** could then be modified in a number of ways, leading to potentially more active taxol analogs. One such modification would involve the controlled reaction of compound **3.62** with benzyltrimethylammonium methoxide (cf. Section 3.1.8.5) to effect selective debenzoylation at the C-2 position. The C-2 position of the resultant compound **3.63** could then be reacylated using a *meta*-substituted benzoyl group (cf. Section 1.7.4.3), giving rise to the C-2, C-4 modified compound **3.64**, as shown in Scheme 28.

Compound **3.64** could also be prepared by more liberal reaction of 7-(triethylsilyl)-13-oxobaccatin III (**3.14**) with benzyltrimethylammonium methoxide (cf. Section 3.1.8.5), followed by reacylation of the incipient C-2 hydroxyl group using a *meta*-substituted benzoyl group (cf. Section 1.7.4.3) to give **3.65**, then C-4 reacylation using cyclopropanecarbonyl chloride to afford **3.64**, as depicted in Scheme 29.

Reduction of the C-13 ketone of **3.64**, followed by attachment and final modifications of the side chain **2.19** would afford the corresponding taxol derivatives, bearing two modifications known to enhance biological activity.^{74,87,124,127}

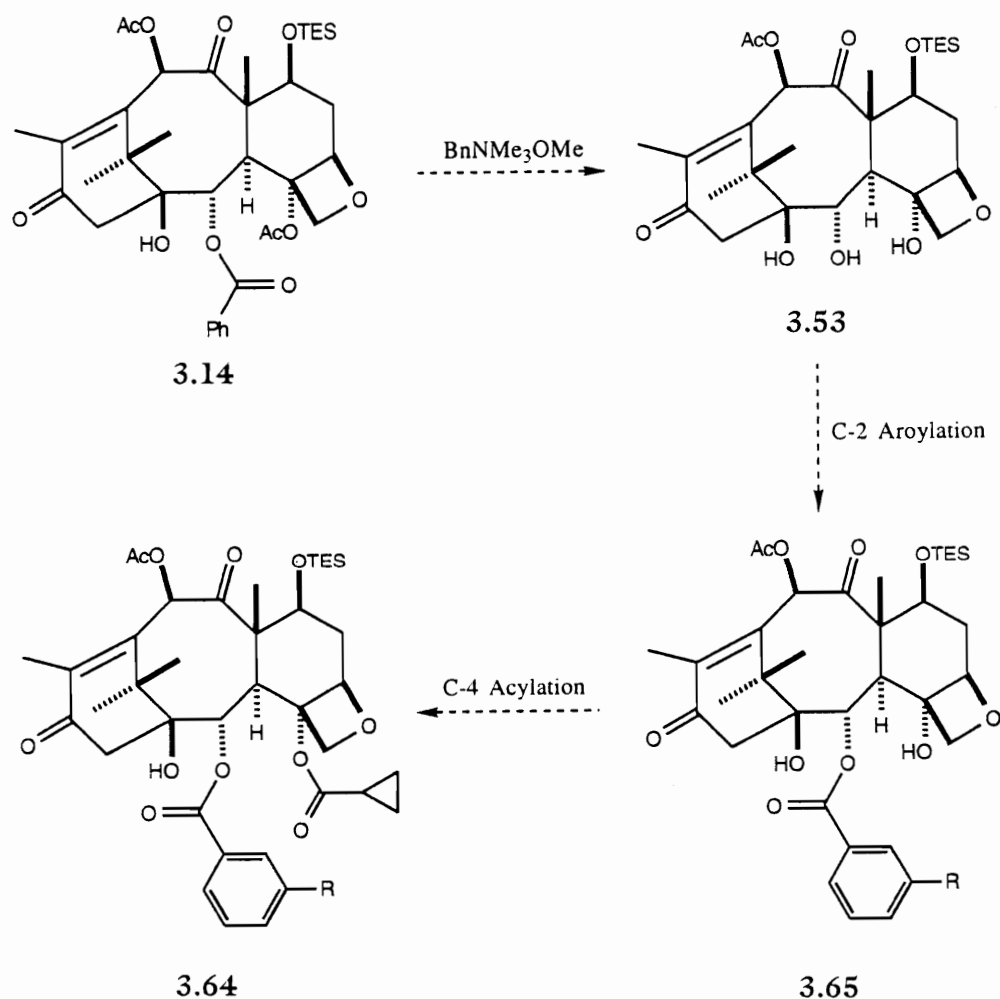
Another possible modification to compound **3.62** would involve the treatment of this compound with sodium methoxide under controlled conditions (cf. Section 3.1.8.6) to afford the 2,10-bis(deacyl)baccatin **3.66**, as shown in Scheme 30.

Reacylation of the C-2 position with a *meta*-substituted benzoyl group (cf. Section 1.7.4.3) to give **3.67**, followed by reduction of the C-13 ketone, attachment of the side chain **2.19**, and appropriate modifications, could conceivably lead to the corresponding C-2, C-4 modified taxotere analogs.



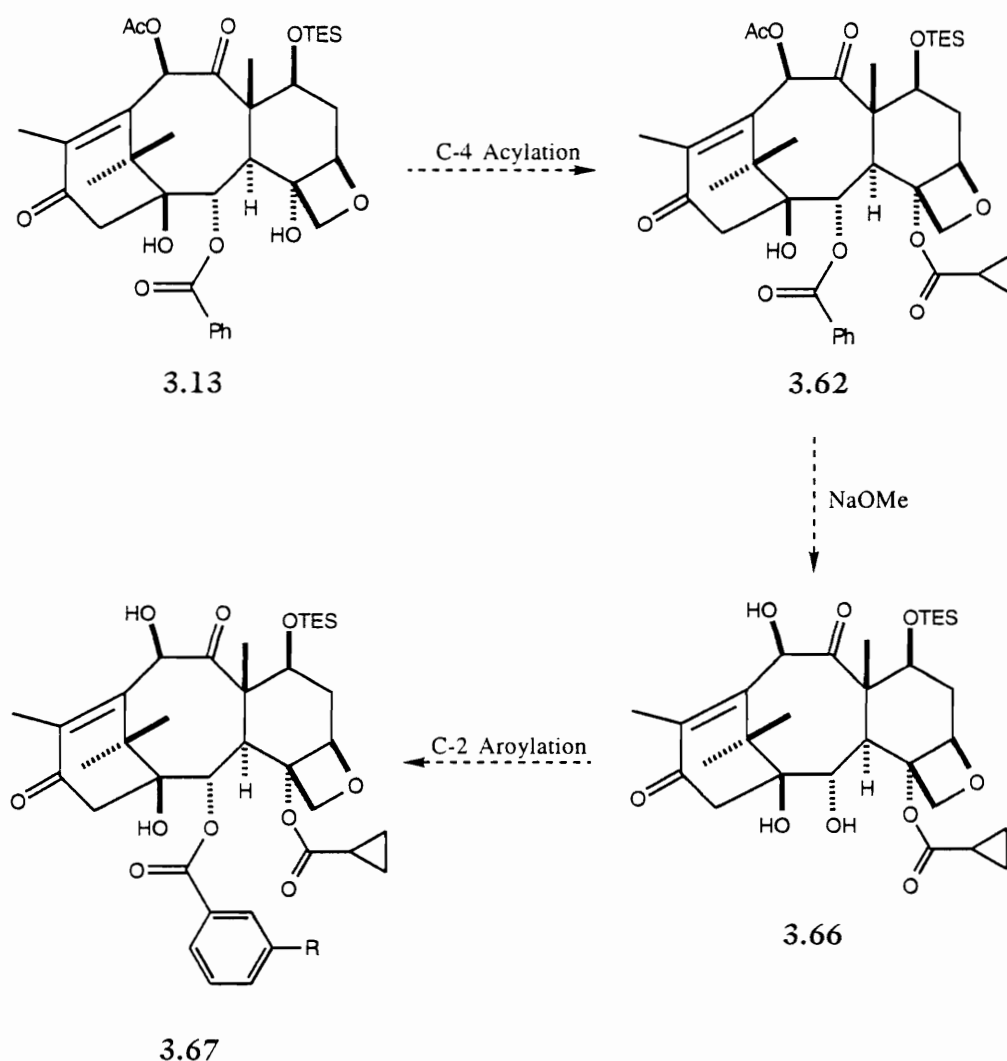
Scheme 28. Proposed formation of C-2, C-4 modified baccatins, leading to the corresponding taxol derivatives.

In addition to the development of synthetic methodologies leading to the successful preparation of 4-deacetyltaxol (2.1) and the three 4-acyltaxol derivatives, 3.31, 3.33 and 3.35, during the course of this investigation several compounds have been formed which may prove to be beneficial in future evaluations of the structure-activity relationships of taxol. The discovery of the C-5 iodinated oxetane ring-opened compound 3.8 and its conversion to the key intermediate compound 3.9 offers the opportunity to investigate modifications of the oxetane ring by substitution of the oxygen with various heteroatoms, such as nitrogen, sulfur or selenium, as shown in Scheme 31.



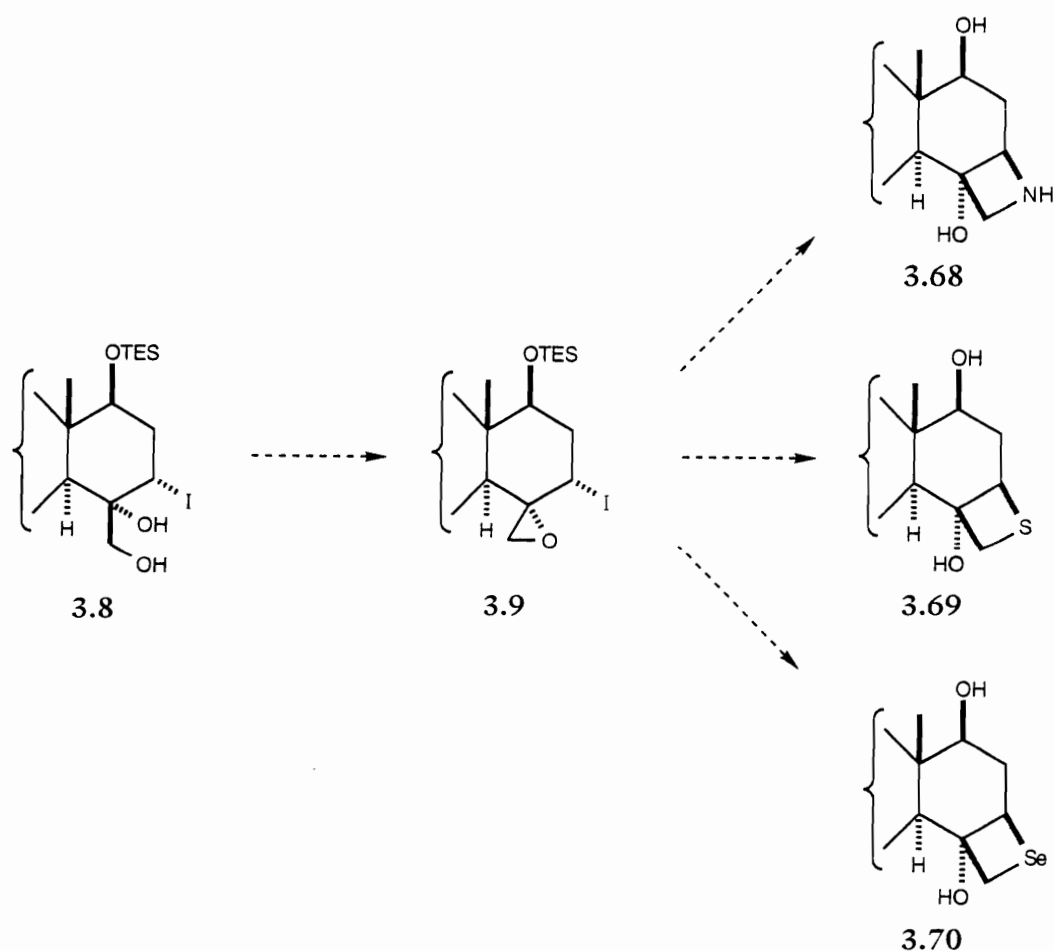
Scheme 29. Proposed formation of C-2, C-4 modified baccatins, leading to the corresponding taxol derivatives.

In fact, the discovery of the oxetane ring-opened product 3.8 has prompted such an investigation in our labs, and at the current time efforts are being made toward the preparation of the taxol analogs 3.68, 3.69 and 3.70, and a number of their respective congeners, for eventual biological testing and evaluation. The nitrogen-containing analog, or azetidine, offers the opportunity for an in-depth investigation of: 1) the effect on activity rendered by possible enhancement of hydrogen bonding in this domain of the molecule; 2) the possible effects rendered on activity by a nucleophilic functionality which may covalently



Scheme 30. Proposed formation of C-2, C-4 modified 10-deacetyl-baccatins, leading to the corresponding taxol derivatives.

bond to amino acid residues at the active site; 3) the possible changes in activity caused by the placement of various acyl groups on the azetidine nitrogen; and 4) the possibility of improved water-solubility due to the presence of the nitrogen atom or of acyl substituents on the nitrogen atom. The sulfur-containing analog, or thietane, offers the opportunity for evaluation of the effects caused by a Group VI-A atom other than oxygen, as well as the potential changes in activity brought on by oxidation of the sulfur atom. Similarly, the selenium-containing analog presents the opportunity to examine the effect on activity caused by replacement of the oxygen with a rather large Group VI-A atom.



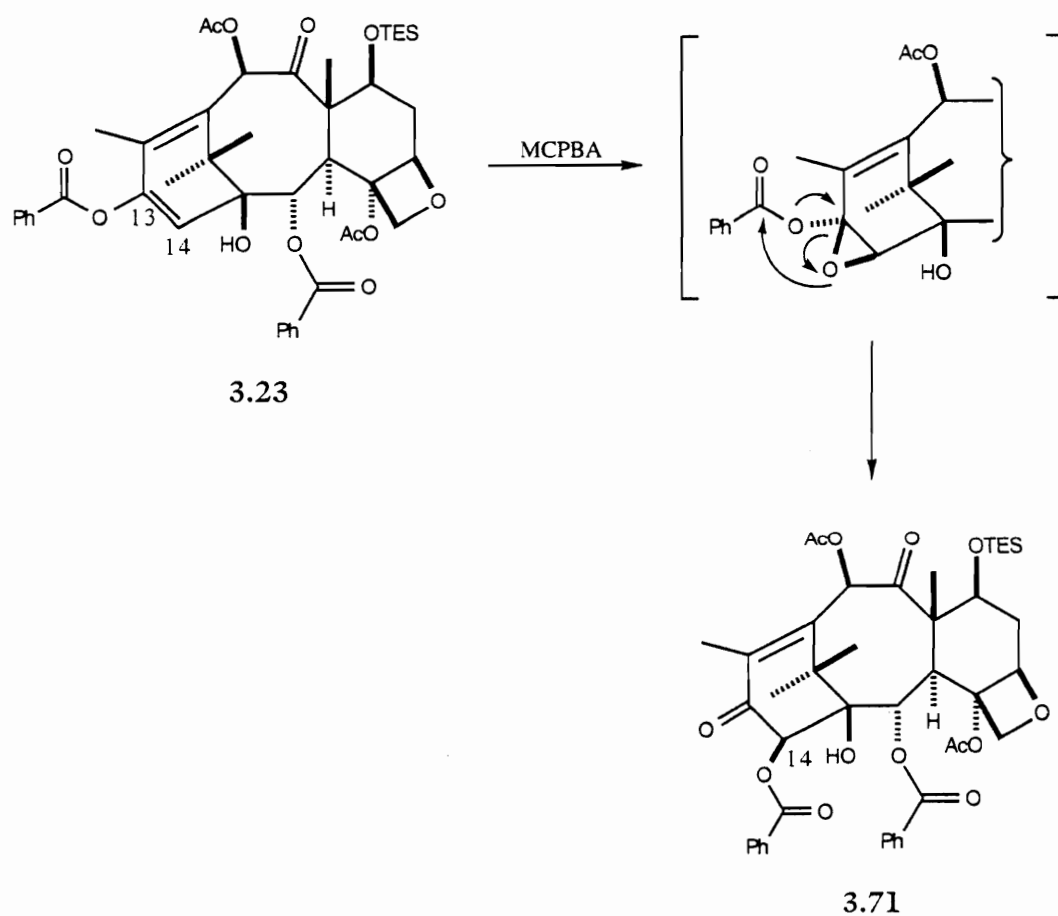
Scheme 31. Proposed formation of the nitrogen, sulfur and selenium analogs of the taxol oxetane ring.

Another group of compounds generated from this work, the enol esters 3.17, 3.19, 3.23 and 3.47, also offers the opportunity to investigate the effects on biological activity rendered by changes to the C-14 position. Preliminary investigations and ongoing work in our labs have shown that the enol esters 3.17, 3.19, 3.23 and 3.47 can be modified by treatment with various reagents, leading to a number of substrates which have potential for investigating the structure-activity relationships of the C-14 position. It is conceivable that reaction of 13-acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47) with bromine followed by sodium bicarbonate-induced hydrolysis could afford the diastereotopic C-14 brominated compounds 3.50 and 3.51. Potentially these compounds could be used as substrates for future investigation of C-14 modified taxol derivatives. Recently in our labs, it has been observed that the reaction of 13-benzoyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.23) (formed from the reaction of 7-(triethylsilyl)-13-oxobaccatin III (3.14) with benzoic anhydride and base) with *meta*-chloroperoxybenzoic acid affords the unexpected C-14 β substituted compound 3.71, presumably through an intermediate benzoyl migration,¹⁵⁴ as depicted in Scheme 32.

In theory, various C-14 β -acylbaccatin derivatives could be similarly generated by the use of other 14,13-enol esters, which, upon treatment with *meta*-chloroperoxybenzoic acid would presumably undergo acyl transfer from the C-13 to the C-14 position, thus leading to a series of 14 β -acyltaxol congeners. In addition to the formation of various C-14 acyl taxol derivatives, it would be of particular interest to prepare taxol analogs in which the side chain was attached at either the C-14 α or the C-14 β configuration, in order to establish what effects these changes might have on activity.

Thus, in addition to having prepared 4-deacetyltaxol and a number of 4-acyltaxol derivatives in an effort toward gaining a better understanding of the structure-activity relationships of the C-4 position of taxol, a number of synthetically useful compounds have been discovered. As discussed above, these compounds have already shown their potential for use in studies of the structure-activity relationships of taxol, especially pertaining to the C-4(C-20), C-5 oxetane and the C-14 position, and will undoubtedly be key synthetic

intermediates in future investigations of this clinically important and chemically intriguing compound.



Scheme 32. Formation of 14β-benzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.71) from the reaction of 13-benzoyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.23) with *meta*-chloroperoxybenzoic acid.

IV. OUTLOOK

Due to the potent chemotherapeutic properties which taxol displays against a wide variety of cancers, the demand for this drug has vastly increased during the last decade. Because of taxol's limited supply from its natural source, the bark of the Pacific yew (*Taxus brevifolia*), a number of alternative sources of this compound continue to be investigated, including isolation from renewable portions of *Taxus* species, production by plant tissue and fungal cultures, and preparation by partial or total synthesis. While the production of taxol via alternative sources shows high potential toward alleviating the supply problem, large-scale production of the drug is not likely to occur for several years.

Another approach toward alleviating the taxol supply problem is by the systematic investigation of the structure-activity relationships of the molecule, in order to establish the structural features and functionalities necessary for biological activity. In the past decade, an enormous research effort to elucidate the structure-activity relationships of taxol has produced a vast amount of information, which has led to the establishment of three primary functional domains which are crucial for biological activity: the C-13 side chain, the C-2 benzoate and the C-4(C-20),C-5 oxetane ring. It has been speculated that these functionalities elicit their effect on activity by direct interaction with hydrophobic binding sites on the microtubule. Investigation of modifications to these functionalities, as well as modifications of the multiring taxane skeleton and positions on the skeleton which have thus far received relatively little attention (e.g., the C-1, C-6, and C-14 positions), is likely to continue well into the future, in an effort to enhance the activity of the drug and to simplify the synthetic preparation of this complex molecule.

Despite the wealth of information which has been generated by studies of the structure-activity relationships of taxol, our understanding of how taxol exerts its activity at the molecular level is far from complete. Complicating this understanding is the fact that neither the three-dimensional structure of the microtubule nor its spatial interaction(s) with taxol have been firmly established. With the current advances in multi-dimensional NMR

solution studies and high-speed computer-assisted molecular modeling, as well as the application of spin-labeling and photoaffinity-labeling techniques, the elucidation of the taxol-microtubule binding site will likely be forthcoming. This information, coupled with that continuing to accumulate on the chemistry and structure-activity relationships of taxol, will afford the opportunity to more completely investigate site-specific modifications to the taxol structure, thereby leading to potentially more active taxol analogs and also providing greater insight into the chemical and mechanistic processes underlying taxol-microtubule interaction as well as normal microtubule assembly.

V. EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES

All non-aqueous reactions were performed in oven- or flame-dried glassware under a positive pressure of argon. Tetrahydrofuran and diethyl ether were distilled from sodium/benzophenone ketyl. Methylene chloride was distilled from calcium hydride. Anhydrous toluene and acetonitrile were obtained from Aldrich Chemical Company or were distilled from calcium hydride. Anhydrous pyridine was obtained from Aldrich Chemical Company. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotation values were obtained on a Perkin-Elmer 241 polarimeter, using sodium D line illumination, at ambient temperature. ^1H and ^{13}C NMR spectra were obtained on Bruker WP 200, WP 270, or Varian Unity 400 MHz spectrometers. NMR chemical shift values are given in parts per million (ppm) from the resonance of the internal standard, tetramethylsilane. Splitting patterns are designated as "s" (singlet), "d" (doublet), "t" (triplet), "q" (quartet) and "m" (multiplet), and coupling constants are given in Hertz (Hz). Except where indicated, all NMR spectra were obtained in CDCl_3 at ambient temperature. Infrared spectral data was obtained on a Perkin-Elmer 283B spectrophotometer using NaCl plates. Low resolution electron ionization (EI), chemical ionization (CI) and fast atom bombardment (FAB) mass spectra were obtained on a VG Analytical 7070E-HF mass spectrometer. Analytical thin layer chromatography was performed on E. Merck aluminum-supported silica gel 60 F_{254} (200 μm) plates. Preparative thin layer chromatography was performed on 20 cm $\times$ 20 cm GF_{254} (500 μm or 1000 μm) glass-supported plates (Analtech). Taxol and baccatin III derivatives were extracted from silica gel with acetone or ethyl acetate, and after removal of the solvent, were dissolved in methylene chloride or chloroform, and hexane was added to precipitate the compound. Taxol and baccatin III derivatives were visualized on TLC plates by UV illumination and by spraying with a vanillin/sulfuric acid solution followed by heating. Silica gel for flash chromatography was E. Merck (230-400 mesh). Low-level molecular mechanics

calculations were performed using the Chem3D Plus (v.3.1) software (Cambridge Scientific Computing, Inc.).

Unless otherwise noted, the phrase “worked up by standard procedure” refers to dilution of the reaction with an excess of organic solvent (e.g., CH₂Cl₂, EtOAc, diethyl ether), neutralization with acid or base as indicated, extraction of the component(s) of interest by liquid-liquid partitioning, successive washing with water and brine, drying over MgSO₄, and *in vacuo* evaporation of the solvent.

Baccatin III was obtained by reductive cleavage of a crude mixture of taxol and cephalomannine;¹¹⁷ the taxol/cephalomannine mixture was obtained from the National Cancer Institute. Purified taxol and 10-deacetylbaccatin III were supplied by Bristol-Myers Squibb.

7-(TRIETHYLSILYL)BACCATIN III (2.5)

Baccatin III (1.13, 630 mg, 1.075 mmol) was dissolved in 27 ml anhydrous pyridine with stirring. After 5 min, chlorotriethylsilane (1.62 g, 10.75 mmol, 10.0 equiv.) was added and the reaction was allowed to proceed, at room temperature under argon, for 36 hr. At this time the reaction was stopped by the addition of 30 ml distilled water, followed by 30 ml ethyl acetate. The ethyl acetate layer was separated and the aqueous layer washed with additional ethyl acetate (2 × 10 ml). The combined organic layers were then washed with aqueous CuSO₄ solution (5 × 50 ml) to remove the pyridine, followed by brine wash (3 × 10 ml). After drying with MgSO₄, the solution was filtered and evaporated to a pale yellow residue, which was subjected to column chromatography (3% isopropanol in CH₂Cl₂), affording 552.3 mg (73.4%) of pure 7-(triethylsilyl)baccatin III (2.5), as a colorless, amorphous solid. FABMS *m/z* (rel int.) [MH]⁺ 701 (100), 679 (16), 669 (11), 661 (9), 655 (15), 641 (63), 623 (29), 611 (21), 593 (32), 587 (87), 581 (52), 563 (34); ¹H NMR (CDCl₃) δ 7.44 - 8.13 (m, 5H), 6.46 (s, 1H), 5.63 (d, *J* = 7Hz, 1H), 4.96 (d, *J* = 8Hz, 1H), 4.83 (br. m, 1H), 4.49 (dd, *J* = 7, 10Hz, 1H), 4.23 (ABq, *J* = 8Hz, Δ*v*_{AB} = 42, 2H), 3.88 (d, *J* = 7Hz, 1H), 2.6 (m, 1H), 2.53 (m, 1H), 2.25 (s, 3H), 2.19 (s, 3H), 2.18 (s,

3H), 1.9 (m, 1H), 1.89 (m, 1H), 1.65 (s, 3H), 1.20 (s, 3H), 1.04 (s, 3H), 0.93 (t, 9H), 0.58 (q, 6H).

METHANOLYSIS OF 7-(TRIETHYLSILYL)BACCATIN III (2.5)

7-(Triethylsilyl)baccatin III (2.5, 59.7 mg, 0.0853 mmol) was dissolved in 0.5M sodium methoxide in methanol (4.0 ml, 2.0 mmol, 23.4 equiv.), and the reaction was allowed to proceed at room temperature under argon for 30 min. At this time the reaction was quenched by treatment with 10% methanolic HCl, following which the solvent was removed by rotary evaporation. The crude reaction mixture was worked up by standard procedure and subjected to purification by PTLC (CH₂Cl₂/isopropanol, 9:1), affording three products. The first product, *R_f* 0.43, a colorless amorphous solid, was identified as 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7) (1.3 mg, 2.5%): FABMS *m/z* (rel int.) [MNa]⁺ 639 (100), 621 (16), 609 (14), 594 (18), 566 (19); ¹H NMR (CDCl₃) δ 7.45 - 8.10 (m, 5H), 5.60 (d, *J* = 7Hz, 1H), 5.17 (s, 1H), 4.96 (d, *J* = 8Hz, 1H), 4.89 (br. m, 1H), 4.43 (dd, *J* = 6, 10Hz, 1H), 4.24 (ABq, *J* = 8Hz, Δ*v*_{AB} = 40, 2H), 3.96 (d, *J* = 7Hz, 1H), 2.52 (m, 1H), 2.45 (m, 1H), 2.09 (s, 3H), 1.91 (m, 1H), 1.88 (m, 1H), 1.73 (s, 3H), 1.24 (s, 3H), 1.09 (s, 3H), 0.94 (t, 9H), 0.56 (q, 6H). The second product, *R_f* 0.13, a colorless amorphous solid, was 4,10-bis(deacetyl)-2-debenzoyl-7-(triethylsilyl)baccatin III (2.4) (22.0 mg, 50.4%) and was shown by ¹H NMR and mass spectral data to be identical to the same compound prepared by Samaranayake^{115,138} from 7-(triethylsilyl)hexahydrobaccatin III (2.3): CIMS *m/z* (rel. int.) [MH]⁺ 513 (1.2), 495 (1.2), 477 (9.3), 459 (11), 441 (6.9), 429 (7.8), 413 (5.9), 401 (5.6), 387 (4.7), 345 (13), 327 (23), 309 (14), 299 (16); ¹H NMR (CDCl₃) δ 5.10 (s, 1H), 4.76 (dd, *J* = 3, 8Hz, 1H), 4.55 (br. d, *J* = 8Hz, 1H), 4.55 (ABq, *J* = 8Hz, Δ*v*_{AB} = 89, 2H), 3.94 (dd, *J* = 5, 11Hz, 1H), 3.77 (d, *J* = 7Hz, 1H), 3.25 (d, *J* = 7Hz, 1H), 2.40 (m, 1H), 2.40 (m, 1H), 2.12 (s, 3H), 2.08 (m, 1H), 1.97 (m, 1H), 1.59 (s, 3H) 1.05 (s, 3H), 0.97 (s, 3H), 0.91 (t, 9H), 0.51 (q, 6H). The third product, *R_f* 0.03, also a colorless amorphous solid, was identified as the rearrangement isomer of compound 2.4, 4,10-bis(deacetyl)-2-debenzoyl-7-

(triethylsilyl)isobaccatin III (2.8) (8.4 mg, 19.2%): FABMS m/z (rel. int.) $[MH]^+$ 513 (9), 495 (7), 477 (20), 459 (12), 419 (11), 399 (9), 381 (14), 363 (39), 345 (38), 327 (35), 177 (64), 149 (100), 133 (97), 121 (97); ^1H NMR ($CDCl_3$) δ 4.96 (s, 1H), 4.49 (br. d, J = 6.5Hz, 1H), 4.22 (dd, J = 8, 10Hz, 1H), 4.14 (d, J = 6Hz, 1H), 3.79 (dd, J = 4, 9Hz, 1H), 3.76 (ABq, J = 10Hz, $\Delta\nu_{AB}$ = 32, 2H), 3.51 (d, J = 6Hz, 1H), 2.51 (m, 1H), 2.38 (m, 1H), 2.11 (m, 1H), 1.99 (s, 3H), 1.81 (m, 1H), 1.32 (s, 3H) 1.06 (s, 3H), 0.94 (t, 9H), 0.92 (s, 3H), 0.54 (q, 6H).

CONTROLLED METHANOLYSIS OF 7-(TRIETHYLSILYL)BACCATIN III (2.5)

7-(Triethylsilyl)baccatin III (2.5, 59.7 mg, 0.0853 mmol) was dissolved in 4.0 ml methanol. After cooling the solution to $-15\text{ }^\circ\text{C}$, 42.9 μl (0.188 mmol, 2.2 equiv.) of a sodium methoxide solution (25wt% in methanol) was added dropwise via syringe over 15 min. The reaction mixture was then allowed to warm to room temperature while stirring was continued. After 30 min an aliquot was taken and, after neutralization and removal of the solvent, was analyzed by ^1H NMR. Upon comparison to known spectral data, the spectrum obtained for the crude reaction mixture showed it to consist of unreacted starting material 7-(triethylsilyl)baccatin III (2.5), 10-deacetyl-7-(triethylsilyl)baccatin III (2.6) and 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7), in a ratio of 9:1:1, respectively. After 90 min, ^1H NMR analysis of another aliquot indicated the presence of unreacted starting material 7-(triethylsilyl)baccatin III (2.5), 10-deacetyl-7-(triethylsilyl)baccatin III (2.6), 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7) and 4,10-bis(deacetyl)-2-debenzoyl-7-(triethylsilyl)baccatin III (2.4), in a ratio of 5:1:2:1, respectively. After 13 hr, the reaction was carefully neutralized to pH 7 with 10% HCl in methanol. After removal of the solvent by rotary evaporator, the crude reaction mixture was purified by PTLC (CH_2Cl_2 /isopropanol, 9:1), and the individual components were analyzed by ^1H NMR. Obtained from the reaction were 1.3 mg (2.5%) of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7); 22.0 mg (50.4%) of 4,10-bis(deacetyl)-2-debenzoyl-7-

(triethylsilyl)baccatin III (2.4); and 8.4 mg (19.2%) of 4,10-bis(deacetyl)-2-debenzoyl-7-(triethylsilyl)isobaccatin III (2.8). All compounds were identical with the same products described above.

**PROLONGED METHANOLYSIS OF 7-(TRIETHYLSILYL)BACCATIN III (2.5):
FORMATION OF 4,10-BIS(DEACETYL)-2-DEBENZOYL-7-
(TRIETHYLSILYL)ISOBACCATIN III (2.8)**

7-(Triethylsilyl)baccatin III (2.5, 20.0 mg, 0.0286 mmol) was dissolved in 0.5 M sodium methoxide in methanol (2.2 ml, 1.0 mmol, 35.0 equiv.), and the reaction was allowed to proceed at room temperature under argon for 12 hr. At this time the reaction was quenched with 10% methanolic HCl, following which the solvent was removed by rotary evaporation. The crude residue was worked up by standard procedure and subjected to purification by PTLC (CH_2Cl_2 /isopropanol, 92:8), affording a colorless, amorphous product at R_f 0.03 which was analyzed by ^1H NMR. This product was found to be 7-(triethylsilyl)-2-debenzoyl-4,10-bis(deacetyl)isobaccatin III (2.8, 13.5 mg, 91.6%), identical to the tetrahydrofuran product described above.

**CONVERSION OF 4,10-BIS(DEACETYL)-2-DEBENZOYL-7-(TRIETHYLSILYL)
BACCATIN III (2.4) TO 4,10-BIS(DEACETYL)-2-DEBENZOYL-7-
(TRIETHYLSILYL)ISOBACCATIN III (2.8)**

The conversion reactions were each carried out at room temperature using 620 μg (1.21 μmol) of 2.4 in 0.5 ml solvent with a catalytic amount of either acid or base, as summarized in Table 4. Each reaction was monitored by TLC analysis (10% isopropanol in CH_2Cl_2) using pure 2.8 as a reference standard, and in each case the conversion of 2.4 to 2.8 proceeded cleanly and, with the exception of the experiment in which compound 2.4 was treated with DMAP, nearly quantitatively.

ACETYLATION OF 4,10-BIS(DEACETYL)-2-DEBENZOYL-7-(TRIETHYLSILYL)
ISOBACCATIN III (2.8)

A solution of 2.8 (7.7 mg, 0.015 mmol) in anhydrous CH_2Cl_2 (1 ml) was treated with DCC (11.4 mg, 0.055 mmol, 3.7 equiv.), Ac_2O (8.0 μl , 0.085 mmol, 5.7 equiv.), and a catalytic amount of 4-PP, and the reaction was allowed to proceed for 20 hr at room temperature. After quenching with aqueous acetic acid, the reaction mixture was worked up by the standard procedure, then purified by PTLC (3% isopropanol in CH_2Cl_2), affording two major products. The first product, R_f 0.42, a colorless, amorphous solid, was identified as the triacetate 2.12 (1.9 mg, 19.8%), based on its NMR and mass spectral data: CIMS m/z (rel. int.) $[\text{MH}]^+$ 639 (1.5), 621 (2.0), 609 (1.4), 579 (9.0), 561 (29), 543 (14), 519 (89), 501 (62), 489 (19), 473 (12), 459 (72), 441 (47), 429 (21), 413 (16), 401 (14), 387 (43), 369 (21), 359 (12), 343 (40), 327 (44), 309 (49); ^1H NMR (CDCl_3) δ 6.33 (s, 1H), 5.91 (ddd, $J = 1, 6, 10\text{Hz}$, 1H), 5.19 (t, $J = 9\text{Hz}$, 1H), 4.06 (d, $J = 7\text{Hz}$, 1H), 3.95 (dd, $J = 5, 10\text{Hz}$, 1H), 3.57 (s, 2H), 3.18 (d, $J = 7\text{Hz}$, 1H), 2.6 (m, 1H), 2.26 (m, 1H), 2.18 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 1.93 (s, 3H), 1.82 (m, 1H), 1.77 (m, 1H), 1.26 (s, 3H), 1.17 (s, 3H), 1.07 (s, 3H), 0.93 (t, 9H), 0.57 (q, 6H). The second product, R_f 0.45, a colorless, amorphous solid, was identified as the tetraacetate 2.13 (1.3 mg, 12.7%) based on its spectral data: CIMS m/z (rel. int.) $[\text{MH}]^+$ 681 (0.5), 663 (0.8), 651 (0.8), 621 (1.9), 603 (1.8), 561 (90), 543 (12), 519 (11), 501 (29), 485 (8.0), 473 (10), 459 (43), 441 (21), 411 (16), 397 (39), 383 (16), 369 (18), 355 (10), 341 (19), 327 (24), 309 (26); ^1H NMR (CDCl_3) δ 6.34 (s, 1H), 6.15 (br. t, $J = 9\text{Hz}$, 1H), 5.43 (dd, $J = 5, 10\text{Hz}$, 1H), 4.54 (dd, $J = 6, 10\text{Hz}$, 1H), 4.07 (d, $J = 7\text{Hz}$, 1H), 3.84 (ABq, $J = 10\text{Hz}$, $\Delta\nu_{\text{AB}} = 43$, 2H), 3.56 (d, $J = 7\text{Hz}$, 1H), 2.68 (m, 1H), 2.28 (m, 1H), 2.22 (s, 3H), 2.17 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 1.89 (s, 3H), 1.89 (m, 1H), 1.79 (m, 1H), 1.25 (s, 3H), 1.23 (s, 3H), 1.09 (s, 3H), 0.93 (t, 9H), 0.57 (q, 6H).

FORMATION OF THE TAXOL SIDE CHAIN (2.15) BY
GREENE'S METHODOLOGY^{145a}

To a stirring mixture of 2.52 g (66.3 mmol, 2.0 equiv.) of lithium aluminum hydride in 120 ml of refluxing THF under argon was carefully added, portionwise through a reflux condenser, (S)-(+)-phenylglycine (5.0 g 33.1 mmol). Upon completion of the addition, the condenser was rinsed with an additional 7 ml of THF, and the mixture was allowed to reflux for 6 hr. At this time, the mixture was cooled to room temperature and was slowly treated with 4.0 ml of 10% aqueous NaOH followed by 5.0 ml of distilled water. Stirring was continued for 15 min while the reaction mixture was cooled to 0 °C, then an additional 53 ml of 10% NaOH solution and 3.3 ml (4.0 g, 28 mmol, 0.85 equiv.) of benzoyl chloride were introduced. The resultant mixture was allowed to stir for 30 min while warming to 20 °C, then CH₂Cl₂ and aqueous potassium sodium tartrate were added. After stirring for 15 min, the crude product was extracted with CH₂Cl₂ according to standard procedure, and then recrystallized from methanol-CH₂Cl₂. Column chromatography (20% EtOAc in CH₂Cl₂) of the mother liquor was carried out, giving a total yield of 5.175 g (64.9%) of 2.15a, according to Scheme 3. Melting point: 177.5-180 °C (lit.^{145a} 179-180 °C); ¹H NMR (CDCl₃) δ 7.79 - 7.83 (m, 2H), 7.31 - 7.52 (m, 8H), 6.86 (br. s, NH), 5.28 (m, 1H), 4.01 (t, 2H), 2.61 (t, OH).

Dimethyl sulfoxide (DMSO, 1.82 ml, 25.6 mmol, 1.64 equiv.) was added to a stirred solution of oxalyl chloride (2.14 ml, 24.0 mmol, 1.54 equiv.) in 32 ml of CH₂Cl₂ at -78 °C under argon, and the mixture was allowed to stir for 5 min at this temperature. The reaction flask was then warmed to -60 °C over 20 min, whereupon the alcohol 2.15a (3.76 g, 15.58 mmol), suspended in 50 ml CH₂Cl₂-DMSO (24:1) was added over 15 min. Residual material from the addition flask was washed into the reaction flask with an additional 10 ml CH₂Cl₂. The reaction mixture was allowed to warm to -35 °C over 20 min, stirred at this temperature for 5 min, and then treated over the next 5 min with 16.72 ml (96.0 mmol, 6.16 equiv.) of diisopropylethylamine. After removing the cooling bath for 5 min, the reaction flask was re-cooled to -78 °C and the mixture was added with a double-tipped

needle to a room temperature solution (104 ml. 1.0M, 104 mmol, 6.68 equiv.) of vinylmagnesium bromide in 1:1 THF-CH₂Cl₂. After stirring for 60 min, the mixture was treated with 16 ml ethanol and 24 ml saturated aqueous ammonium chloride. To the mixture was added CH₂Cl₂ and dilute HCl and the crude reaction product was isolated and worked up by standard methodology. The product was purified by silica gel column chromatography (5% EtOAc in CH₂Cl₂) giving 2.09 g (50.2%) of compound **2.15b**. Melting point: 134.5-136 °C (lit.^{145a} 135-136 °C); ¹H NMR (CDCl₃) δ 7.80 - 7.83 (m, 2H), 7.30 - 7.52 (m, 8H), 6.94 (br. s, NH), 5.96 (ddd, *J* = 5.0, 10.6, 17.3Hz, 1H), 5.43 (d, *J* = 17.2Hz, 1H), 5.28 (m, 1H), 5.25 (m, 1H), 4.57 (m, 1H), 2.21 (d, *J* = 3.9Hz, OH).

A solution of compound **2.15b** (460.2 mg, 1.72 mmol), *para*-toluenesulfonic acid (43.2 mg, 0.169 mmol, 0.10 equiv.) and ethyl vinyl ether (1.64 ml, 17.23 mmol, 10.0 equiv.) in 9 ml CH₂Cl₂ was stirred under argon at room temperature for 4.5 hr. After neutralization with pyridine, the reaction mixture was diluted with CH₂Cl₂ and worked up by the standard procedure. The crude product was purified by column chromatography (Et₂O/hexane, 2:3) to provide 478.5 mg (81.9%) of **2.15c**. Melting point: 85-86.5 °C (lit.^{145a} 85.5-87 °C); ¹H NMR (CDCl₃) δ 7.74 - 7.90 (m, 2H), 7.21 - 7.53 (m, 8H), 7.02 (d, *J* = 8Hz, NH), 5.98 and 5.82 (2ddd, *J* = 7, 10, 17Hz, 1H), 5.08 - 5.48 (br. m, 3H), 4.36 - 4.64 (2q, *J* = 5Hz, 1H), 4.28 - 4.42 (m, 1H), 2.97 - 3.51 (m, 2H), 1.28 and 1.08 (2d, *J* = 5Hz, 3H), 1.06 and 0.99 (2t, *J* = 7Hz, 3H).

To a stirred mixture of 129 mg (0.381 mmol) of compound **2.15c** in 0.75 ml CH₃CN, 0.75 ml CCl₄ and 1.2 ml H₂O at room temperature under argon were added 205 mg (2.44 mmol, 6.4 equiv.) of NaHCO₃ and, in small portions, 441 mg (2.06 mmol, 5.4 equiv.) of NaIO₄. After completion of addition, the reaction mixture was stirred for 5 min, then 12.7 mg (0.06 mmol, 0.16 equiv.) of RuCl₃ was added and the reaction was allowed to proceed for 48 hr. At this time, the reaction mixture was treated with aqueous 1N HCl to pH 4, then was extracted with CH₂Cl₂ and worked up by the standard procedure, giving, without further purification, 96.0 mg (70.7%) of pure taxol side chain **2.15**: Melting point: 91-93.5

°C (lit.^{145a} 93-94 °C); $[\alpha]_D -20.2^\circ$ ($c = 1.0$, CH₃OH) (lit.^{145a} $[\alpha]_D -21^\circ$ ($c = 0.7$, CH₃OH)); ¹H NMR (CDCl₃) δ 8.2 - 8.6 (br. s, 1H), 7.76 - 7.84 (m, 2H), 7.28 - 7.52 (m, 9H), 5.72 - 5.82 (m, 1H), 4.80 and 4.62 (2q, $J = 5$ Hz, 1H), 4.64 and 4.52 (2d, $J = 2$ Hz, 1H), 3.22 - 3.44 and 2.87 - 2.97 (2m, 2H), 1.26 (d, $J = 5$ Hz, 3H), 1.08 and 0.93 (2t, $J = 7$ Hz, 3H).

MODEL COUPLING OF 7-(TRIETHYLSILYL)BACCATIN III (2.5) WITH THE TAXOL SIDE CHAIN 2.15

7-(Triethylsilyl)baccatin III (2.5, 25.0 mg, 0.0357 mmol) was dissolved with stirring in 1.0 ml anhydrous toluene. To the stirred solution was added the taxol side chain 2.15 (63.8 mg, 0.179 mmol, 5.0 equiv.), followed by DCC (36.8 mg, 0.179 mmol, 5.0 equiv.) and a catalytic amount of DMAP. The reaction mixture was heated to 55 °C and allowed to stir at this temperature under argon for 36 hr. At this time TLC analysis (EtOAc/ hexanes, 2:1) showed the formation of a less polar product in addition to unreacted starting material. After allowing the reaction mixture to cool to room temperature, 0.1 N HCl was added to quench, and the mixture was worked up by standard procedure. The crude reaction mixture was purified by PTLC (EtOAc/ hexanes, 2:1), giving 17.4 mg of unreacted starting material and 3.2 mg (22.1% based on recovered starting material) of the model coupling product. The ¹H NMR spectrum obtained for this compound showed the C-13 proton to be shifted downfield relative to the starting material, from 4.83 to 6.17 ppm, indicative of acylation at this position. Further ¹H NMR evidence of side chain coupling was found in the additional proton signals in the spectrum of the product, especially the aromatic proton peaks from 7.1 to 7.6 ppm.

GREENE'S APPROACH USING THE TRICHLOROETHOXYMETHYL ETHER PROTECTING GROUP TO GIVE THE TAXOL SIDE CHAIN 2.16

The taxol side chain hydroxy olefin (2.15b, 50.0 mg, 0.187 mmol) was slowly added to a stirring suspension of NaH (6.7 mg, 0.279 mmol, 1.5 equiv.) in 1.0 ml dry, distilled THF. The mixture was allowed to stir for 5 min at room temperature, during which time the

reaction turned to an opaque yellow color. At this time, trichloroethoxymethyl chloride (55.5 mg, 0.281 mmol, 1.5 equiv.) was added dropwise via syringe over 5 min. The reaction was allowed to proceed at room temperature under argon while monitoring by TLC (5% isopropanol in CH_2Cl_2). After 2.5 hr the reaction appeared to be complete based on consumption of starting material and formation of a less polar product. After quenching the reaction with several mls of water, the product was extracted with CH_2Cl_2 and worked up in the usual manner, giving a clear, slightly yellow oil. The oil was subjected to PTLC (2% isopropanol in CH_2Cl_2) for purification, and 43.7 mg (54.5%) of a clear, colorless oily residue was obtained: CIMS m/z (rel. int.) $[\text{MH}]^+$ 430 (88), 429 (86), 394 (29), 280 (52), 250 (19), 210 (100), 122 (50); ^1H NMR (CDCl_3) δ 7.26 - 7.87 (m, 10H), 7.01 (d, J = 8.7Hz, 1H), 5.88 (ddd, J = 6.9, 10.4, 17.2Hz, 1H), 5.44 (d, J = 2.5Hz, 1H), 5.40 (m, 2H), 4.84 (d, J = 7.0Hz, 1H), 4.75 (d, J = 7.0Hz, 1H), 4.56 (dd, J = 2.5, 6.9Hz, 1H), 3.62 (d, J = 11.8Hz, 1H), 3.22 (d, J = 11.8Hz, 1H).

The protected hydroxy olefin from above (26.0 mg, 0.061 mmol) was dissolved in a mixture of 150 μl CH_3CN , 150 μl CCl_4 and 225 μl H_2O . While stirring was maintained, NaHCO_3 (33.3 mg, 0.396 mmol, 6.5 equiv.), and NaIO_4 (71.9 mg, 0.336 mmol, 5.5 equiv.) were added. After 5 min, RuCl_3 (3.2 mg, 0.0154 mmol, 0.25 equiv.) was added to the mixture and the reaction was allowed to proceed for 32 hr, at which time TLC analysis (5% isopropanol in CH_2Cl_2) showed the reaction to be complete. After acidifying the mixture to pH 4 with 1N HCl and diluting with CH_2Cl_2 , the reaction was worked by the usual procedure, yielding a colorless, glassy residue. The residue was purified by PTLC (10% isopropanol in CH_2Cl_2), affording 16.8 mg (61.7%) of the taxol side chain 2.16: ^1H NMR (CDCl_3) δ 8.8 - 9.0 (br. s, 1H), 7.25 - 7.82 (m, 10H), 7.18 (d, J = 7Hz, 1H), 5.30 (d, J = 2Hz, 1H), 4.91 (d, J = 8Hz, 1H), 4.79 (d, J = 8Hz, 1H), 4.69 (dd, J = 2, 7Hz, 1H), 3.67 (d, J = 13Hz, 1H), 3.19 (d, J = 13Hz, 1H).

GREENE'S APPROACH USING THE TRIMETHYLSILYLETHOXYMETHYL ETHER PROTECTING GROUP TO GIVE THE TAXOL SIDE CHAIN 2.17

The taxol side chain hydroxy olefin (**2.15b**, 32.6 mg, 0.122 mmol), in 1.0 ml anhydrous CH_2Cl_2 , was slowly added to a stirring suspension of NaH (3.7 mg, 0.154 mmol, 1.26 equiv.) in 1.0 ml anhydrous CH_2Cl_2 . The mixture was allowed to stir for 15 min at room temperature, during which time the reaction mixture turned from a clear, colorless appearance to an opaque yellow. At this time, 2-(trimethylsilyl)ethoxymethyl chloride (65 μl , 0.366 mmol, 3.0 equiv.) was added dropwise via syringe over 5 min. The reaction was allowed to proceed at room temperature under argon while monitoring by TLC (5% isopropanol in CH_2Cl_2). After 2.5 hrs, an additional quantity of 2-(trimethylsilyl)ethoxymethyl chloride (65 μl , 0.366 mmol, 3.0 equiv.) was added to the reaction mixture. After 22 hr the reaction appeared to be complete based on consumption of starting material and formation of a less polar product. After quenching the reaction with several mls of water, the product was extracted with CH_2Cl_2 and worked up in the usual manner. The oil was subjected to PTLC (5% isopropanol in CH_2Cl_2) for purification, and 15.4 mg (31.7%) of a clear, colorless viscous oil was obtained: ^1H NMR (CDCl_3) δ 7.30 - 7.92 (m, 10H), 7.05 (d, 1H), 5.96 (m, 1H), 5.48 (d, 1H), 5.39 (m, 2H), 4.43 (dd, 1H), 3.34 (m, 2H), 1.05 (t, 2H), 0.84 (t, 2H), 0.18 (s, 6H), 0.09 (s, 3H).

The protected hydroxy olefin from above (15.4 mg, 0.0387 mmol) was dissolved in a mixture of 100 μl CH_3CN , 100 μl CCl_4 and 150 μl H_2O . While stirring was maintained, NaHCO_3 (21.1 mg, 0.251 mmol, 6.5 equiv.), and NaIO_4 (45.5 mg, 0.213 mmol, 5.5 equiv.) were added. After 5 min, RuCl_3 (1.3 mg, 0.0063 mmol, 0.16 equiv.) was added to the mixture and the reaction was allowed to proceed for 48 hr, at which time TLC analysis (5% isopropanol in CH_2Cl_2) showed the reaction to be complete. After acidifying the mixture to pH 4 with 1 N HCl and diluting with CH_2Cl_2 , the reaction was worked up by the usual procedure, yielding a colorless, glassy residue. The residue was purified by PTLC (10% isopropanol in CH_2Cl_2), affording 8.9 mg (55.3%) of the taxol side chain **2.17**: ^1H

NMR (CDCl₃) δ 7.22 - 7.84 (m, 10H), 7.14 (d, NH), 5.34 (d, 1H), 4.64 (m, 1H), 3.26 (m, 2H), 1.03 (t, 2H), 0.87 (t, 2H), 0.20 (s, 6H), 0.08 (s, 3H).

GREENE'S APPROACH USING THE CHLOROETHOXYETHYL ETHER PROTECTING GROUP TO GIVE THE TAXOL SIDE CHAIN 2.18

A solution of compound 2.15b (101.5 mg, 0.380 mmol), chloroethyl vinyl ether (386 μ l, 3.80 mmol, 10.0 equiv.) and a catalytic amount of *para*-toluenesulfonic acid in 2.5 ml CH₂Cl₂ was stirred under argon at room temperature for 1.5 hr, at which time TLC analysis (3% isopropanol in CH₂Cl₂) indicated the reaction was complete. After neutralization with 5 drops of pyridine, the reaction mixture was diluted with CH₂Cl₂ and worked up by the standard procedure. The crude product was purified by column chromatography (3% isopropanol in CH₂Cl₂) to provide 102.2 mg (71.9%) of a colorless, amorphous solid: ¹H NMR (CDCl₃) δ 7.15 - 7.52 (m, 11H), 5.71 - 6.02 (m, 1H), 5.05 - 5.38 (m, 3H), 4.73 (m, 1H), 4.46 (m, 1H), 3.45 (m, 2H), 3.14 (m, 2H), 1.23 and 1.08 (2d, 3H).

To a stirred mixture of 51.1 mg (0.137 mmol) of the above protected hydroxy olefin in 300 μ l CH₃CN, 300 μ l CCl₄ and 450 μ l H₂O at room temperature under argon were added 74.4 mg (0.885 mmol, 6.5 equiv.) of NaHCO₃ and, in small portions, 160.1 mg (0.749 mmol, 5.5 equiv.) of NaIO₄. After completion of addition, the reaction mixture was stirred for 5 min, then 4.5 mg (0.022 mmol, 0.16 equiv.) of RuCl₃ was added and the reaction was allowed to proceed for 48 hr. At this time, the reaction mixture was treated with aqueous 1N HCl to pH 4, then was extracted with CH₂Cl₂ and worked up by the standard procedure, giving without further purification, 34.3 mg (63.9%) of pure taxol side chain 2.18, as a colorless, amorphous solid: ¹H NMR (CDCl₃) δ 7.18 - 7.78 (m, 11H), 5.66 (m, 1H), 4.67 (m, 1H), 4.52 (m, 1H), 3.38 (m, 2H), 3.17 (m, 2H), 1.20 and 1.11 (2d, 3H).

**MODEL COUPLING OF 7-(TRIETHYLSILYL)BACCATIN III (2.5)
WITH GREENE'S MODIFIED SIDE CHAIN 2.16**

7-(Triethylsilyl)baccatin III (2.5, 4.7 mg, 0.0067 mmol) was dissolved with stirring in 1.0 ml anhydrous toluene. To the stirred solution was added the taxol side chain 2.16 (15.0 mg, 0.0336 mmol, 5.0 equiv.), followed by DCC (6.9 mg, 0.0336 mmol, 5.0 equiv.) and a catalytic amount of DMAP. The reaction mixture was heated to 55 °C and allowed to stir at this temperature under argon for 16 hr. At this time TLC analysis (EtOAc/ hexanes, 2:1) showed the formation of a less polar product in addition to unreacted starting material. After allowing the reaction mixture to cool to room temperature, 0.1 N HCl was added to quench, and the mixture was worked up by standard procedure. The crude reaction mixture was purified by PTLC (EtOAc/ hexanes, 2:1), giving 0.7 mg of unreacted starting material and 1.5 mg (23.3% based on recovered starting material) of the model coupling product. The ¹H NMR spectrum obtained for this compound showed the C-13 proton to be shifted downfield relative to the starting material, from 4.83 to 6.15 ppm, indicating that acylation had occurred at this position. Further ¹H NMR evidence of side chain coupling was found in the additional proton signals in the spectrum of the product, especially the aromatic proton peaks from 7.15 to 7.70 ppm.

**MODEL COUPLING OF 7-(TRIETHYLSILYL)BACCATIN III (2.5)
WITH GREENE'S MODIFIED SIDE CHAIN 2.17**

7-(Triethylsilyl)baccatin III (2.5, 4.7 mg, 0.0067 mmol) was dissolved with stirring in 1.0 ml anhydrous toluene. To the stirred solution was added the taxol side chain 2.17 (8.0 mg, 0.0201 mmol, 3.0 equiv.), followed by DCC (4.2 mg, 0.0201 mmol, 3.0 equiv.) and a catalytic amount of DMAP. The reaction mixture was heated to 55 °C and allowed to stir at this temperature under argon for 24 hours. At this time TLC analysis (EtOAc/ hexanes, 2:1) showed the formation of a less polar product in addition to unreacted starting material. After allowing the reaction mixture to cool to room temperature, 0.1 N HCl was added to quench, and the mixture was worked up by standard procedure. The crude reaction mixture

was purified by PTLC (EtOAc/ hexanes, 2:1), giving 1.3 mg of unreacted starting material and 1.4 mg (26.7% based on recovered starting material) of the model coupling product. The ^1H NMR spectrum obtained for this compound showed the C-13 proton to be shifted downfield relative to the starting material, from 4.83 to 6.18 ppm, indicating that acylation had occurred at this position. Further ^1H NMR evidence of side chain coupling was found in the additional proton signals in the spectrum of the product, especially the aromatic proton peaks from 7.12 to 7.65 ppm.

MODEL COUPLING OF 7-(TRIETHYLSILYL)BACCATIN III (2.5) WITH GREENE'S MODIFIED SIDE CHAIN 2.18

7-(Triethylsilyl)baccatin III (2.5, 10.7 mg, 0.0153 mmol) was dissolved with stirring in 1 ml anhydrous toluene. To the stirred solution was added the taxol side chain 2.18 (30.0 mg, 0.0766 mmol, 5.0 equiv.), followed by DCC (15.8 mg, 0.0766 mmol, 5.0 equiv.) and a catalytic amount of DMAP. The reaction mixture was heated to 55 °C and allowed to stir at this temperature under argon for 18 hours. At this time TLC analysis (EtOAc/hexanes, 2:1) showed the formation of a less polar product in addition to unreacted starting material. After allowing the reaction mixture to cool to room temperature, 0.1 N HCl was added to quench, and the mixture was worked up by standard procedure. The crude reaction mixture was purified by PTLC (EtOAc/ hexanes, 2:1), giving 3.3 mg of unreacted starting material and 2.8 mg (24.7% based on recovered starting material) of the model coupling product. The ^1H NMR spectrum obtained for this compound showed the C-13 proton to be shifted downfield relative to the starting material, from 4.83 to 6.14 ppm, indicating that acylation had occurred at this position. Further ^1H NMR evidence of side chain coupling was found in the additional proton signals in the spectrum of the product, especially the aromatic proton peaks from 7.10 to 7.70 ppm.

MODEL COUPLING STUDY OF 7-(TRIETHYLSILYL)BACCATIN III (2.5) WITH PHENYLACETIC ACID

7-(Triethylsilyl)baccatin III (2.5, 14.9 mg, 0.0213 mmol) was dissolved with stirring in 1.0 ml anhydrous toluene. To the stirred solution was added phenylacetic acid (17.5 mg, 0.129 mmol, 6.1 equiv.), followed by DCC (26.6 mg, 0.129 mmol, 6.1 equiv.) and a catalytic amount of DMAP. The reaction mixture was allowed to stir at room temperature under argon for 34 hr. At this time TLC analysis (5% isopropanol in CH₂Cl₂) showed the formation of a less polar product in addition to unreacted starting material, and 0.1 N HCl was added to quench. The mixture was worked up by standard procedure, and the crude reaction mixture was purified by PTLC (3% isopropanol in CH₂Cl₂), giving 3.3 mg of unreacted starting material and 6.9 mg (50.9% based on recovered starting material) of the model coupling product: FABMS *m/z* (rel. int.) [MH]⁺ 819 (9.0), 801 (7.0), 789 (5.0), 759 (24), 741 (18), 729 (10), 697 (11), 683 (18), 667 (16), 637 (15), 623 (32), 607 (22), 593 (24), 549 (23), 501 (27), 489 (50), 471 (30), 459 (29), 429 (49), 411 (49), 371 (79); ¹H NMR (CDCl₃) δ 8.09, (d, *J* = 8.1 Hz, 2H), 7.31 - 7.68 (m, 8H), 6.45 (s, 1H), 6.18 (m, 1H), 5.69 (d, *J* = 10.8, 1H), 4.98 (d, *J* = 16.2 Hz, 1H), 4.48 (dd, *J* = 8.1, 13.5 Hz, 1H), 4.25 (ABq, *J* = 11 Hz, Δ*v*_{AB} = 39, 2H), 3.86 (d, *J* = 10.8 Hz, 1H), 3.74 (d, *J* = 3.3 Hz, 2H), 2.58 (m, 1H), 2.53 (m, 1H), 2.34 (s, 3H), 2.26 (m, 1H), 2.16 (s, 3H), 1.95 (s, 3H), 1.87 (m, 1H), 1.69 (s, 3H), 1.26 (s, 3H), 1.16 (s, 3H), 0.93 (t, 9H), 0.58 (q, 6H).

SYNTHESIS OF (4*S*,5*R*)-*N*-BENZOYL-2,2-DIMETHYL-4-PHENYL- 5-OXAZOLIDINE CARBOXYLIC ACID (2.20)

The side chain intermediate (-)-*N*-((1*S*,2*S*)-2-hydroxy-1-phenyl-3-butenyl)benzamide (2.15b) was prepared according to the methodology of Greene.^{145a} As depicted in Scheme 7, this intermediate (210 mg, 0.786 mmol) was dissolved in 10 ml anhydrous toluene, and to the stirred solution was added via syringe 2-methoxypropene (453.4 mg, 6.29 mmol, 8.0 equivalents), followed by pyridinium *para*-toluenesulfonate (10 mg, 0.0398 mmol, 0.05

equivalents). The reaction was allowed to proceed at room temperature under argon while being monitored by TLC (5% ethyl acetate in CH_2Cl_2). After 35 min the starting material, R_f 0.16, had been converted to a less polar material, R_f 0.51. The solvent was removed by rotary evaporator, and the faintly yellow-colored crude residue was purified by a short column of silica gel (4% ethyl acetate in CH_2Cl_2), affording 220.2 mg (91.2%) of the cyclic acetal-protected olefin **2.20a**: CIMS m/z (rel int.) $[\text{MH}]^+$ 308 (4), 268 (100), 250 (49), 210 (44), 147 (8), 129 (7), 122 (41), 105 (62), 91 (14), 85 (25), 79 (26); ^1H NMR (CDCl_3) δ 6.84 - 7.15 (m, 10H), 5.85 (ddd, $J = 7, 11, 17\text{Hz}$, 1H), 5.22 (d, $J = 11\text{Hz}$), 5.17 (d, $J = 17\text{Hz}$), 4.54 (br. d, $J = 9\text{Hz}$, 1H), 4.38 (dd, $J = 7, 9\text{Hz}$), 1.96 (br. s, 3H), 1.89 (br. s, 3H).

The above cyclic acetal-protected olefin (**2.20a**, 220.2 mg, 0.717 mmol) was dissolved in 2.8 ml 1:1 $\text{CH}_3\text{CN}/\text{CCl}_4$, and to the stirred solution was added 2.2 ml distilled water. To the biphasic mixture were added sodium bicarbonate (391.5 mg, 4.67 mmol, 6.5 equiv.) and, in small portions, sodium periodate (844.0 mg, 3.94 mmol, 5.5 equiv.). After stirring the mixture for 10 min, following completion of the addition, RuCl_3 (24.3 mg, 0.115 mmol, 0.16 equiv.) was added, and the reaction was allowed to proceed under argon at room temperature. After 48 hr the reaction mixture was diluted with distilled water, followed by diethyl ether. The ether layer was separated and the aqueous layer was acidified with 1 N HCl to pH 4 when turbidity persisted. After extraction of the aqueous layer with CH_2Cl_2 (3 $\times$ 50 ml), the combined CH_2Cl_2 and ether layers were washed with brine (2 $\times$ 50 ml), dried with MgSO_4 , filtered, and the solvents evaporated, yielding 205.6 mg (88.2%) of the colorless, amorphous solid **2.20**: CIMS m/z (rel int.) $[\text{MH}]^+$ 326 (16), 310 (2.7), 282 (3.2), 280 (2.8), 268 (1.8), 252 (2.1), 240 (1.9), 224 (8.0), 176 (9.9), 162 (100), 149 (18), 123 (27), 121 (30), 105 (46), 91 (18), 85 (14); ^1H NMR (CDCl_3) δ 9.79 (s, 1H), 7.07 - 7.21 (m, 8H), 6.87 (s, 2H), 5.19 (d, $J = 5.6\text{Hz}$, 1H), 4.52 (d, $J = 5.6\text{Hz}$, 1H), 1.92 (br. s, 3H), 1.82 (br. s, 3H); ^{13}C NMR (CDCl_3) δ 171.8, 170.0, 138.8, 136.9, 129.5, 128.5, 128.0, 127.8, 126.7, 126.2, 98.2, 80.9, 65.3, 26.1, 25.7.

**COUPLING OF 7-(TRIETHYLSILYL)BACCATIN III (2.5) WITH (4*S*,5*R*)-
N-BENZOYL-2,2-DIMETHYL-4-PHENYL-5-OXAZOLIDINE
CARBOXYLIC ACID (2.20)**

7-(Triethylsilyl) baccatin III (2.5, 30.4 mg, 0.043 mmol) was dissolved in 1.5 ml anhydrous toluene. To the stirred solution was added the taxol side chain carboxylic acid 2.20 (28.3 mg, 0.087 mmol, 2.0 equiv.), followed by dicyclohexylcarbodiimide (18.0 mg, 0.087 mmol, 2.0 equiv.), and finally, a catalytic amount of DMAP. The reaction was allowed to proceed under argon at room temperature while being monitored by TLC (5% isopropanol in CH₂Cl₂). After 12 hr the reaction appeared to be complete based on consumption of the starting material and formation of a less polar product. The solvent was removed by rotary evaporation, and the crude residue was worked up by standard procedure, then subjected to PTLC (3.5% isopropanol in CH₂Cl₂), affording 29.9 mg (69.0%) of a less polar, colorless, amorphous product, which was shown by NMR and mass spectral data to be the desired coupling compound 2.21. CIMS *m/z* (rel. int.) [MH]⁺ 1009 (100), 948 (25), 930 (24), 855 (7.0), 765 (11) 747 (8.0), 704 (13), 682 (39), 666 (9.0), 622 (52); ¹H NMR (CDCl₃) δ 8.00 (m, 2H), 7.11 - 7.63 (m, 13H), 6.93 (d, *J* = 7.0Hz, NH), 6.45 (s, 1H), 6.24 (t, *J* = 7.4Hz, 1H), 5.64 (d, *J* = 7.1Hz, 1H), 5.27 (d, *J* = 6.6Hz, 1H), 4.88 (d, *J* = 7.9Hz, 1H), 4.56 (d, *J* = 6.7Hz, 1H), 4.46 (dd, *J* = 6.6, 10.5Hz, 1H), 4.16 (ABq, *J* = 8.6Hz, Δ*v*_{AB} = 40, 2H), 3.77 (d, *J* = 7.1Hz, 1H), 2.48 (m, 2H), 2.19 (s, 6H), 2.04 (s, 3H), 2.00 (s, 3H), 1.8 - 2.0 (m, 2H), 1.87 (s, 3H), 1.65 (s, 3H), 1.22 (s, 3H), 1.19 (s, 3H), 0.95 (t, 9H), 0.59 (q, 6H).

PARTIAL DEPROTECTION OF COMPOUND 2.21

Compound 2.21 (0.8 mg, 0.00079 mmol) was dissolved with stirring in 0.5 ml of a 5% solution of HCl in methanol. The reaction was allowed to stir at room temperature under argon and after 2 hr TLC analysis (5% isopropanol in CH₂Cl₂) showed the starting material to be converted to a more polar product. This product, however, had an *R_f* of about 0.15 greater than that for the taxol standard used for comparison. Based on this observation, it

was speculated that the triethylsilyl protecting group had been removed but the *N,O*-cyclic acetal protecting group on the side chain remained intact. The reaction was allowed to continue for an additional 6 hr, but no apparent change was noted by TLC analysis. The crude product mixture was purified by PTLC (3% isopropanol in CH₂Cl₂), affording 0.6 mg (85%) of a compound which was identified by ¹H NMR and mass spectral data as the partially deprotected taxol derivative **2.22**. FABMS *m/z* (rel. int.) [MH]⁺ 894 (100), 834 (20), 816 (11), 791 (7.0), 733 (8.0), 714 (7.0), 685 (7.0), 673 (8.0), 657 (9.0), 643 (32), 625 (9.0); ¹H NMR (CDCl₃) δ 8.01 (m, 2H), 7.11 - 7.65 (m, 13H), 6.96 (br. d, NH), 6.29 (s, 1H), 6.28 (m, 1H), 5.63 (d, *J* = 6.8Hz, 1H), 5.27 (d, *J* = 6.9Hz, 1H), 4.92 (dd, *J* = 7.4Hz, 1H), 4.56 (d, *J* = 6.9Hz, 1H), 4.43 (m, 1H), 4.17 (AB q, *J* = 7.4Hz, Δ*v*_{AB} = 33, 2H), 3.76 (d, *J* = 6.8Hz, 1H), 2.53 (m, 1H), 2.46 (m, 1H), 2.26 (s, 6H), 2.18 (m, 1H), 1.90 (m, 1H), 1.88 (s, 1H), 1.64 (s, 3H), 1.55 (s, 3H), 1.26 (s, 3H), 1.13 (s, 3H), 0.95 (s, 3H).

ATTEMPTS AT COMPLETE DEPROTECTION OF COMPOUND **2.21**

Eight separate attempts at full deprotection were carried out, each using 1.0 mg (0.001 mmol) of compound **2.21** in 0.5 ml of acidic solution, as summarized in Table 5. Each reaction was monitored by TLC analysis (3% isopropanol in CH₂Cl₂) using pure taxol as a reference standard. As is evident in Table 5, reaction conditions ranged from mild to fairly vigorous and led to two possible outcomes: 1) the C-7 triethylsilyl protecting group was removed without concomitant deprotection of the side chain *N,O*-cyclic acetal; or 2) the compound decomposed. The reaction mixtures from *experiments 1, 2, 3, 7 and 8*, as listed in Table 5, were each subjected to PTLC (3% isopropanol in CH₂Cl₂) for purification. The major product isolated from each experiment was analyzed by ¹H NMR and all were shown to be the partially deprotected taxol derivative **2.22**, based on comparison to the data for the identical compound described above. *Experiments 4, 5 and 6*, from Table 5, led to decomposition of the reaction components, based on the prevalence of TLC plate baseline

materials, and no attempt was made to isolate any of the decomposition products for identification.

SYNTHESIS OF (4*S*,5*R*)-*N*-BOC-2,2-DIMETHYL-4-PHENYL-5-OXAZOLIDINE CARBOXYLIC ACID (2.19)

The side chain intermediate (+)-1,1-dimethylethyl (*N*-((1*S*,2*S*)-2-hydroxy-1-phenyl-3-butenyl)amino)methanoate (2.19a) was prepared according to the methodology of Greene.^{145a} According to Scheme 7, this intermediate (545.0 mg, 2.039 mmol) was dissolved in 20 ml anhydrous toluene, and to the stirring solution was added 2-methoxypropene (1.18 g, 16.36 mmol, 8.0 equiv.), followed by pyridinium *para*-toluenesulfonate (20 mg, 0.0797 mmol, 0.04 equiv.). The reaction was allowed to proceed at 62 °C under argon for 4 hr. After cooling to room temperature and adding three drops pyridine to neutralize, the solvent was removed by rotary evaporator. The crude product mixture was purified by a short column of silica gel (4% ethyl acetate in CH₂Cl₂), affording 468.7 mg (74.8%) of the cyclic acetal-protected olefin 2.19b. EIMS and CIMS did not yield an identifiable molecular ion. ¹H NMR (CDCl₃) δ 7.20 - 7.34 (m, 5H), 5.87 (ddd, *J* = 6.7, 10.4, 17.1 Hz, 1H), 5.28 (m, 1H), 5.11 (m, 1H), 4.36 (br. m, 1H), 4.25 (t, 1H), 1.74 and 1.73 (2s, 6H), 1.04 (br. s, 9H).

The above cyclic acetal-protected olefin (2.19b, 468.7 mg, 1.525 mmol) was dissolved in 6.0 ml 1:1 CH₃CN/CCl₄, and to the stirred solution was added 4.6 ml distilled water. To the biphasic mixture was added sodium bicarbonate (832.7 mg, 9.91 mmol, 6.5 equiv.) and, in small portions, sodium periodate (1.794 g, 8.39 mmol, 5.5 equiv.). After stirring the mixture for 10 min, following completion of the addition, RuCl₃ (50.6 mg, 0.244 mmol, 0.16 equiv.) was added, and the reaction was allowed to proceed for 28 hr. At this time the reaction mixture was diluted with distilled water, followed by diethyl ether. The ether layer was separated and the aqueous layer was extracted twice with ether (20 ml). The aqueous layer was then treated with 1 N HCl to pH 4, followed by extraction with CH₂Cl₂ (3 × 50

ml). The combined CH_2Cl_2 layers were washed with brine (2×50 ml), dried with MgSO_4 , filtered, and the solvent evaporated, yielding 387.8 mg (78.1%) of the colorless, amorphous solid **2.19**, identical to the same compound reported in the literature,⁹³ but prepared by a different methodology. Melting point: 135-138 °C (lit.⁹³ 137 °C); $[\alpha]_{\text{D}} + 5.2^\circ$ ($c = 1.0$, CHCl_3) (lit.⁹³ $[\alpha]_{\text{D}} + 5.3^\circ$ ($c = 1.1$, CHCl_3)); CIMS m/z (rel int.) $[\text{MH}]^+$ 322 (4), 306 (9), 278 (7), 266 (29), 250 (17), 222 (100), 206 (16), 146 (9), 121 (8), 91 (12); ^1H NMR (CDCl_3) δ 10.3 (s, 1H), 7.25 - 7.34 (m, 5H), 5.09 (br. d, $J = 5\text{Hz}$, 1H), 4.51 (d, $J = 5\text{Hz}$, 1H), 1.78 (s, 3H), 1.72 (s, 3H), 1.20 (br. s, 9H).

**COUPLING OF 7-(TRIETHYLSILYL)BACCATIN III (2.5) WITH (4*S*,5*R*)-
N-Boc-2,2-DIMETHYL-4-PHENYL-5-OXAZOLIDINE
CARBOXYLIC ACID (2.19)**

7-(Triethylsilyl)baccatin III (**2.5**, 24.0 mg, 0.034 mmol) was dissolved in 1.5 ml anhydrous toluene. To the stirred solution was added the taxol side chain carboxylic acid **2.19** (23.4 mg, 0.072 mmol, 2.1 equiv.), followed by dicyclohexylcarbodiimide (14.9 mg, 0.072 mmol, 2.1 equiv.), and finally, a catalytic amount of DMAP. The reaction was allowed to proceed under argon at room temperature for 12 hr, at which time it was determined by TLC analysis (5% isopropanol in CH_2Cl_2) that the reaction was complete based on disappearance of starting material. The solvent was removed by evaporation, and the crude product mixture was subjected to PTLC (3% isopropanol in CH_2Cl_2) for purification, affording 33.1 mg (96.6%) of the coupled compound **2.23**, as a colorless, amorphous solid. FABMS m/z (rel. int.) $[\text{MH}]^+$ 1005 (100), 947 (30), 905 (52), 889 (35), 871 (20); ^1H NMR (CDCl_3) δ 8.04 (m, 2H), 7.05 - 7.62 (m, 13H), 6.45 (s, 1H), 6.24 (br. t, 1H), 5.65 (d, $J = 7.1\text{Hz}$, 1H), 5.05 (m, 1H), 4.87 (br. d, 1H), 4.45 (m, 1H), 4.45 (d, $J = 6.7\text{Hz}$, 1H), 4.17 (ABq, $J = 8.3\text{Hz}$, $\Delta\nu_{\text{AB}} = 41$, 2H), 3.77 (d, $J = 7.1\text{Hz}$, 1H), 2.5 (m, 1H), 2.22 (m, 1H), 2.18 (s, 6H), 1.90 (m, 2H), 1.84 (s, 3H), 1.81 (s, 3H), 1.76 (s, 3H), 1.66 (s, 3H), 1.26 (s, 3H), 1.20 (s, 3H), 1.13 (br. s, 9H), 0.91 (t, $J = 8.3\text{Hz}$, 9H), 0.55 (q, $J = 8.3\text{Hz}$, 6H).

COUPLING OF 4,10-BIS(DEACETYL)-2-DEBENZOYL-7-(TRIETHYLSILYL)BACCATIN III (2.4) WITH (4S,5R)-N-BOC-2,2-DIMETHYL-4-PHENYL-5-OXAZOLIDINE CARBOXYLIC ACID (2.19): FORMATION OF THE TETRAHYDROFURANYL TAXOL ISOMER 2.24

4,10-Bis(deacetyl)-2-debenzoyl-7-(triethylsilyl)baccatin III (2.4, 6.0 mg, 0.0117 mmol) was dissolved in 1.0 ml anhydrous toluene. To the stirred solution was added the taxol side chain carboxylic acid 2.19 (3.2 mg, 0.0098 mmol, 0.84 equiv.), followed by dicyclohexylcarbodiimide (2.4 mg, 0.0117 mmol, 1.0 equiv.), and finally, a catalytic amount of DMAP. The reaction was allowed to proceed under argon at room temperature while being monitored by TLC (5% isopropanol in CH₂Cl₂). After 3 hr, TLC analysis showed the reaction to be complete relative to consumed starting material and formation of a less polar product. The solvent was removed *in vacuo*, and the crude product mixture was subjected to PTLC (3% isopropanol in CH₂Cl₂), affording 5.7 mg (71.0%) of the coupled isomerized compound 2.24: ¹H NMR (CDCl₃) δ 7.24 - 7.40 (m, 5H), 5.93 (br. m, 1H), 4.99 (s, 1H), 4.48 (d, *J* = 6Hz, 1H), 4.38 (br. m, 1H), 4.10 (d, *J* = 6Hz, 1H), 4.08 (d, *J* = 6Hz, 1H), 3.89 (ABq, *J* = 10Hz, Δ*v*_{AB} = 84, 2H), 3.81 (dd, *J* = 4, 15Hz, 1H), 3.24 (d, *J* = 6Hz, 1H), 2.5 (m, 1H), 2.4 (m, 1H), 2.2 (m, 1H), 2.04 (s, 3H), 1.9 (m, 1H), 1.75 (s, 3H), 1.67 (s, 3H), 1.55 (s, 3H), 1.24 (s, 3H), 1.20 (br. s, 9H), 1.02 (s, 3H), 0.90 (t, 9H), 0.54 (q, 6H).

BASE-CATALYZED FORMATION OF 4,10-BIS(DEACETYL)-7-(TRIETHYLSILYL)BACCATIN III (2.7): A COMPARATIVE STUDY OF BASE HYDROLYSIS:

SODIUM METHOXIDE

7-(Triethylsilyl)baccatin III (2.5, 25.0 mg, 0.0357 mmol) was dissolved in 1.5 ml methanol. To the stirred solution was added 81.8 μl (0.357 mmol, 10.0 equiv.) of a 25wt% solution of NaOMe in methanol, and the reaction was allowed to proceed at room

temperature under argon while being monitored by TLC (5% isopropanol in CH_2Cl_2). After 30 min, a TLC plate showed the starting material to be converted to several products, one of which was 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7) by comparison to a known sample. The reaction was stopped by the addition of methanolic 1 N HCl to neutral pH, and after removing the solvent by rotary evaporation, the crude product mixture was worked up according to the standard procedure. The crude material was subjected to purification by PTLC (3.5% isopropanol in CH_2Cl_2) to afford 14.7 mg (66.8%) of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7), which displayed identical spectral characteristics to those previously described for the same compound.

SODIUM ETHOXIDE

7-(Triethylsilyl)baccatin III (2.5, 25.0 mg, 0.0357 mmol) was dissolved in 1.5 ml absolute ethanol. To the stirred solution was added 133.3 μl (0.357 mmol, 10.0 equiv.) of a 21wt% solution of NaOEt in ethanol, and the reaction was allowed to proceed at room temperature under argon while being monitored by TLC (5% isopropanol in CH_2Cl_2). After 30 min, a TLC plate showed the starting material to be converted to several products, one of which was 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7) by comparison to a known sample. The reaction was stopped by the addition of ethanolic 1 N HCl to neutral pH, and after removing the solvent by rotary evaporation, the crude product mixture was worked up according to the standard procedure. The crude material was subjected to purification by PTLC (3% isopropanol in CH_2Cl_2) to afford 3.11 mg (33.3% based on recovered 2.5) of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7), which displayed identical spectral characteristics to those previously described for the same compound.

LITHIUM ISOPROPOXIDE

7-(Triethylsilyl)baccatin III (2.5, 25.0 mg, 0.0357 mmol) was dissolved in 1.5 ml isopropanol. To the stirred solution was added 23.6 mg (0.357 mmol, 10.0 equiv.) of powdered $\text{LiO}i\text{-Pr}$, and the reaction was allowed to proceed at room temperature under argon while being monitored by TLC (5% isopropanol in CH_2Cl_2). After 2.5 hr, a TLC

plate showed the starting material to be converted to several products, one of which was 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7) by comparison to a known sample. The reaction was stopped by the addition of 1N HCl in isopropanol to neutral pH, and after removing the solvent by rotary evaporation, the crude product mixture was worked up according to the standard procedure. The crude material was subjected to purification by PTLC (3% isopropanol in CH₂Cl₂) to afford 7.6 mg (64.9% based on recovered 2.5) of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7), which displayed identical spectral characteristics to those previously described for the same compound.

POTASSIUM *TERT*-BUTOXIDE

7-(Triethylsilyl)baccatin III (2.5, 25.0 mg, 0.0357 mmol) was dissolved in 1.15 ml dry, distilled THF. To the stirred solution was added 357 μ l (0.357 mmol, 10.0 equiv.) of a 1.0 M solution of KO*t*-Bu in THF, and the reaction was allowed to proceed at room temperature under argon while being monitored by TLC (5% isopropanol in CH₂Cl₂). After 25 min, a TLC plate showed the starting material to be converted to one major product, 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7), by comparison to a known sample. The reaction was stopped by the addition of 1N HCl in THF to neutral pH, and after removing the solvent by rotary evaporation, the crude product mixture was worked up according to the standard procedure. The crude material was subjected to purification by PTLC (3% isopropanol in CH₂Cl₂) to afford 15.88 mg (72.2%) of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7), which displayed identical spectral characteristics to those previously described for the same compound.

EFFECT OF POTASSIUM *TERT*-BUTOXIDE CONCENTRATION ON 4,10-BIS (DEACETYL)-7-(TRIETHYLSILYL)BACCATIN III (2.7) FORMATION

A series of experiments was conducted to determine the effect of varying quantities of KO*t*-Bu on the formation of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7). Each experiment was carried out at room temperature using 5.0 mg (0.00714 mmol) of 7-

(triethylsilyl)baccatin III (2.5) and the appropriate amount of KO*t*-Bu solution (1.0M in THF) in 1.0 ml dry, distilled THF. Each reaction was monitored by TLC (5% isopropanol in CH₂Cl₂) for the disappearance of starting material and the formation of product, using pure 2.7 as a reference standard. The results of these experiments are summarized below in Table 15.

4,10-BIS(DEACETYL)-7-(TRIETHYLSILYL)BACCATIN III (2.7)
FROM 7-(TRIETHYLSILYL)BACCATIN III (2.5)

7-(Triethylsilyl)baccatin III (2.5, 27 mg, 0.039 mmol) was dissolved in 1.0 ml dry THF with stirring. After 5 min, 390 μ l (0.39 mmol, 10.0 equiv.) of a 1.0M solution of potassium *tert*-butoxide in THF was added, and the reaction was allowed to proceed for 15 min at room temperature under argon. The reaction was then neutralized with 1N HCl in THF. After evaporation of the solvents, the crude reaction mixture was subjected to PTLC (3% isopropanol in CH₂Cl₂), affording 22.8 mg (94.9%) of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7), which was shown by ¹H NMR spectral data to be identical with the same compound described previously.

Table 15. Comparative reactions to determine the effect of varying potassium-*tert*-butoxide concentration on the formation of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7).

Experiment	KO <i>t</i> -Bu (equivalents)	Reaction time	Yield of 2.7
1	1.1	30 min	1.42 mg; 32.3%
2	2.2	30 min	2.20 mg; 50.1%
3	3.0	25 min	3.27 mg; 74.4%
4	4.0	20 min	3.92 mg; 89.0%
5	5.0	15 min	4.21 mg; 95.6%
6	7.0	10 min	4.03 mg; 91.7%
7	10.0	10 min	4.06 mg; 92.3%

**COUPLING OF 4,10-BIS(DEACETYL)-7-(TRIETHYLSILYL)BACCATIN III (2.7)
WITH (4*S*,5*R*)-*N*-BOC-2,2-DIMETHYL-4-PHENYL-5-OXAZOLIDINE
CARBOXYLIC ACID (2.19)**

4,10-Bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7, 2.9 mg, 0.0047 mmol) was dissolved in 0.5 ml anhydrous toluene. To the stirred solution was added the taxol side chain carboxylic acid 2.19 (1.53 mg, 0.0042 mmol, 1.0 equiv.), followed by dicyclohexylcarbodiimide (0.97 mg, 0.0047 mmol, 1.0 equiv.), and finally, a catalytic amount of *N,N*-dimethylaminopyridine. The reaction was allowed to proceed under argon at room temperature while monitoring by TLC (5% isopropanol in CH₂Cl₂). After 12 hr, the solvent was removed by evaporation, and the crude product mixture was subjected to PTLC (3% isopropanol in CH₂Cl₂), affording 3.8 mg (88.0%) of the coupling compound 2.26. CIMS *m/z* (rel int.) [MH]⁺ 920 (1.2), 846 (0.9), 804 (0.6), 799 (0.5), 757 (0.4), 742 (0.4), 705 (0.4), 685 (0.5), 643 (0.7), 599 (1.6), 433 (4.1), 325 (33), 307 (19), 217 (100); ¹H NMR (CDCl₃) δ 7.99 (dd, *J* = 1.4, 8.5Hz, 2H), 7.31 - 7.58 (m, 8H), 5.89 (br. m, 1H), 5.56 (d, *J* = 5.8Hz, 1H), 5.20 (br. s, 1H), 4.76 (br. d, *J* = 8.1Hz, 1H), 4.62 (d, *J* = 4.1Hz, 1H), 4.30 and 4.07 (2d, *J* = 7.7Hz, 2H), 4.05 (br. m, 2H), 3.42 (br. m, 1H), 2.41 (br. m, 3H), 2.02 (s, 3H), 1.96 (m, 1H), 1.83 (s, 3H), 1.74 (s, 3H), 1.64 (s, 3H), 1.53 (s, 9H), 1.23 (s, 3H), 1.11 (s, 3H), 0.92 (t, 9H), 0.55 (q, 6H).

ACETYLATION OF COMPOUND 2.26

Compound 2.26 (1.0 mg, 0.0011 mmol) was dissolved in 0.5 ml dry THF and to the stirred solution was added acetic anhydride (0.21 μl, 0.0022 mmol, 2.0 equiv.), dicyclohexylcarbodiimide (0.46 mg, 0.0022 mmol, 2.0 equiv.), and finally, a catalytic amount of 4-pyrrolidinopyridine. The reaction was allowed to proceed under argon at room temperature while monitoring by TLC (3% isopropanol in CH₂Cl₂). After 12 hours, the starting material had been converted to a slightly less polar material, and the solvent was removed by evaporation, and the crude product mixture was subjected to PTLC (5%

isopropanol in CH_2Cl_2), affording 0.87 mg (83.2%) of the acetylated compound **2.27**. FABMS m/z (rel int.) $[\text{MH}]^+$ 962 (1.9), 944 (2.0), 932 (1.1), 902 (2.6), 888 (3.2), 860 (3.8), 846 (4.8), 828 (3.9), 816 (2.0), 802 (5.9), 784 (4.0), 641 (8.9), 623 (6.9), 581 (21), 519 (14), 459 (26), 345 (22), 327 (52), 309 (31), 276 (61), 264(36), 251 (44), 222 (100); ^1H NMR (CDCl_3) δ 8.02 (d, $J = 8\text{Hz}$, 2H), 7.3 - 7.6 (m, 8H), 6.49 (s, 1H), 5.87 (br. m, 1H), 5.62 (d, $J = 6\text{Hz}$, 1H), 4.78 (br. d, $J = 8\text{Hz}$, 1H), 4.64 (d, $J = 4\text{Hz}$, 1H), 4.31 and 4.09 (2d, $J = 8\text{Hz}$, 2H), 4.13 (br. m, 2H), 3.38 (br. d, 1H), 2.46 (m, 2H), 2.20 (s, 3H), 2.15 (s, 3H), 1.86 (s, 3H), 1.75 (s, 3H), 1.65 (s, 3H), 1.60 (s, 9H), 1.25 (s, 3H), 1.09 (s, 3H), 0.93 (t, 9H), 0.60 (q, 6H).

4-DEACETYL-7-(TRIETHYLSILYL)BACCATIN III (**2.25**)

7-(Triethylsilyl)baccatin III (**2.5**, 111.8 mg, 0.16 mmol), dissolved in 2.0 ml dry THF, was cooled to $-23\text{ }^\circ\text{C}$. To the cooled, stirred solution was added lithium aluminum hydride (60.6 mg, 1.60 mmol, 10.0 equiv.) in a single portion. After stirring for 6 min, the reaction mixture was poured into 50 ml of a cooled 1:1 mixture of diethyl ether and distilled water to quench. The ether layer was separated and the aqueous layer was extracted with additional ether ($2 \times 10\text{ ml}$). The combined ether layers were washed with brine ($3 \times 15\text{ ml}$) and dried with MgSO_4 . After filtering the drying agent, the solvents were removed by rotary evaporator. The crude product mixture was subjected to PTLC (3% isopropanol in CH_2Cl_2), affording 10.6 mg (10.1%) of 4-deacetyl-7-(triethylsilyl)baccatin III (**2.25**). FABMS m/z (rel int.) $[\text{MH}]^+$ 659 (3.1), 641 (2.7), 599 (2.5), 581 (5.2); ^1H NMR (CDCl_3) δ 8.06 (dd, $J = 1, 7\text{Hz}$, 2H), 7.43 - 7.62 (m, 3H), 6.46 (s, 1H), 5.62 (d, $J = 6\text{Hz}$, 1H), 4.79 (dd, $J = 4, 10\text{Hz}$, 1H), 4.61 (br. d, $J = 9\text{Hz}$, 1H), 4.26 (ABq, $J = 8\text{Hz}$, $\Delta\nu_{\text{AB}} = 57$, 2H), 4.11 (dd, $J = 6, 12\text{Hz}$, 2H), 3.62 (d, $J = 6\text{Hz}$, 1H), 2.61 (dd, $J = 3, 13\text{Hz}$, 1H), 2.45 (m, 2H), 2.21 (s, 3H), 2.20 (s, 3H), 2.0 (m, 1H), 1.57 (s, 3H), 1.21 (s, 3H), 1.01 (s, 3H), 0.91 (t, 9H), 0.57 (q, 6H).

**COUPLING OF 4-DEACETYL-7-(TRIETHYLSILYL)BACCATIN III (2.25)
WITH (4*S*,5*R*)-*N*-BOC-2,2-DIMETHYL-4-PHENYL-5-
OXAZOLIDINE CARBOXYLIC ACID (2.19)**

4-Deacetyl-7-(triethylsilyl)baccatin III (2.25, 10.0 mg, 0.0152 mmol) was dissolved in 0.5 ml anhydrous toluene. To the stirred solution was added a solution of the taxol side chain carboxylic acid 2.19 (5.4 mg, 0.0167 mmol, 1.1 equiv.) and dicyclohexylcarbodiimide (3.4 mg, 0.0167 mmol, 1.1 equiv.) in 0.5 ml anhydrous toluene. A catalytic amount of *N,N*-dimethylaminopyridine was added and the reaction was allowed to proceed under argon at room temperature for 20 min, at which time TLC analysis (5% isopropanol in CH₂Cl₂) showed the starting material to have been converted to a less polar product. The solvent was removed by evaporation, and the crude product mixture was subjected to PTLC (3% isopropanol in CH₂Cl₂), affording 12.7 mg (87.7%) of the coupling compound 2.27, identical with that described earlier.

FORMATION OF 4-DEACETYLTAXOL (2.1) VIA THE BACCATIN III APPROACH

Compound 2.27 (6.3 mg, 0.0065 mmol) was allowed to stir in 1.0 ml 99% formic acid for 4.5 hr at room temperature. The reaction mixture was then diluted with 10 ml ethyl acetate and treated with saturated aqueous sodium bicarbonate solution (3 × 10 ml) to remove the formic acid. The ethyl acetate layer was washed with brine (2 × 10 ml), and the solvent was removed by rotary evaporation. The polar product was assumed to be the free amine and was not subjected to purification or characterization, but was directly carried to the next step. The free amine (4.0 mg, 0.0056 mmol) was dissolved in 2.0 ml ethyl acetate, and to the stirred solution was added 1.0 ml distilled water, followed by sodium bicarbonate (0.52 mg, 1.1 equivalents). To this mixture was added benzoyl chloride (0.78 mg, 0.98 equivalents) and the reaction was allowed to proceed at room temperature for 3 hr. After separating the ethyl acetate layer, the aqueous layer was neutralized with 1% HCl and extracted with ethyl acetate (2 × 5 ml). The combined ethyl acetate layers were washed with

brine (2×10 ml) and dried with MgSO_4 . After removing the drying agent, the solvent was evaporated off, and the crude product mixture was subjected to PTLC (3% isopropanol in CH_2Cl_2), affording 2.1 mg (46.2%) of 4-deacetyltaxol (2.1). FABMS m/z (rel int.) $[\text{MH}]^+$ 812 (1.0), 785 (0.4), 755 (0.5), 738 (0.4), 718 (0.5), 686 (0.7), 645 (0.7), 612 (0.7), 596 (0.4), 580 (0.4), 541 (1.1), 522 (1.0), 504 (1.2), 471 (2.1), 429 (6.6), 413 (6.0), 397 (4.1), 325 (40), 307 (22), 291 (11), 257 (21), 234 (29), 217 (100); ^1H NMR (CDCl_3) δ 8.25 (dd, $J = 1, 7\text{Hz}$, 2H), 7.74 (dd, $J = 1.5, 7\text{Hz}$, 2H), 7.12 - 7.77 (m, 11H), 7.02 (d, $J = 10\text{Hz}$, NH), 6.37 (s, 1H), 6.16 (d, $J = 10\text{Hz}$, 1H), 6.02 (m, 1H), 5.76 (d, $J = 6\text{Hz}$, 1H), 4.96 (dd, $J = 4, 10\text{Hz}$, 1H), 4.73 (br. s, 1H), 4.26 (ABq, $J = 8\text{Hz}$, $\Delta\nu_{\text{AB}} = 29$, 2H), 4.11 (m, 1H), 3.47 (d, $J = 6\text{Hz}$, 1H), 2.99 (m, 1H), 2.53 (m, 1H), 2.40 (m, 1H), 2.27 (s, 3H), 2.02 (s, 3H), 1.9 (m, 1H), 1.61 (s, 3H), 1.25 (s, 3H), 1.15 (s, 3H); ^{13}C NMR (CDCl_3) δ 204.2, 172.2, 171.1, 167.6, 167.5, 141.0, 140.2, 139.6, 135.8, 134.4, 134.2, 133.5, 132.0, 130.4, 129.0, 128.8, 128.6, 128.2, 127.23, 127.17, 88.7, 87.4, 81.9, 74.9, 74.4, 73.3, 72.7, 60.7, 59.0, 53.9, 51.3, 42.9, 35.9, 34.8, 27.8, 20.9, 19.4, 16.6, 9.5.

TREATMENT OF 7-(TRIETHYLSILYL)BACCATIN III (2.5) WITH LITHIUM HYDROXIDE

7-(Triethylsilyl)baccatin III (2.5, 30.9 mg, 0.044 mmol) was dissolved in 3.0 ml THF and to the stirred solution was added 49 μl (0.049 mmol, 1.1 equiv.) of an aqueous 1.0M LiOH solution. The reaction was allowed to proceed at room temperature while being monitored by TLC (10% methanol in CH_2Cl_2). After 72 hr, the starting material had been completely converted to two more polar products, and after dilution with CH_2Cl_2 , and work-up by the standard procedure, the crude reaction mixture was subjected to purification by PTLC (5% methanol in CH_2Cl_2). The first product, R_f 0.40, a colorless glassy solid, was identified by ^1H NMR as 2-debenzoyl-7-(triethylsilyl)baccatin III (3.72, 7.1 mg, 27.1%): ^1H NMR (CDCl_3) δ 6.39 (s, 1H), 4.98 (dd, $J = 2.7, 8.4\text{Hz}$, 1H), 4.85 (br. s, 1H), 4.63 (ABq, $J = 8\text{Hz}$, $\Delta\nu_{\text{AB}} = 21$, 2H), 4.45 (dd, $J = 5.4, 8.1\text{Hz}$, 1H), 3.89 (t, $J = 8.1\text{Hz}$, 1H), 3.51 (d, $J = 8.1\text{Hz}$, 1H), 2.54 (m, 1H), 2.33 (m, 1H), 2.16 (s, 6H), 2.04

(m, 1H), 1.96 (m, 1H), 1.66 (s, 3H), 1.25 (s, 3H), 1.09 (s, 3H), 1.07 (s, 3H), 0.92 (t, 9H), 0.57 (q, 6H). The second product, R_f 0.17, also a colorless glassy solid, was identified by ^1H NMR as 4-deacetyl-2-debenzoyl-7-(triethylsilyl)baccatin III (3.73, 6.0 mg, 24.6%): ^1H NMR (CDCl_3) δ 6.39 (s, 1H), 4.79 (dd, $J = 3.7, 9.7\text{Hz}$, 1H), 4.57 (ABq, $J = 8.6\text{Hz}$, $\Delta\nu_{\text{AB}} = 92$, 2H), 4.55 (t, $J = 8.5\text{Hz}$, 1H), 4.08 (dd, $J = 6.4, 11.3\text{Hz}$, 1H), 4.03 (s, OH), 3.87 (t, $J = 5.9\text{Hz}$, 1H), 3.24 (t, $J = 6.2\text{Hz}$, OH), 3.02 (d, $J = 8.2\text{Hz}$, 1H), 2.36 - 2.50 (m, 2H), 2.17 (s, 3H), 1.98 - 2.05 (m, 2H), 1.62 (s, 3H), 1.56 (s, 3H), 1.09 (s, 3H), 1.03 (s, 3H), 0.90 (t, 9H), 0.58 (q, 6H).

TREATMENT OF 7-(TRIETHYLSILYL)BACCATIN III (2.5) WITH CALCIUM BOROHYDRIDE

7-(Triethylsilyl)baccatin III (2.5, 67.8 mg, 0.097 mmol) was dissolved in 0.5 ml 95% ethanol to which 1 drop of an ethanolic solution of phenolphthalein was added. The solution was titrated to neutral pH by the dropwise addition of aqueous 2M KOH. To the neutral solution was added an additional 2.5 ml 95% ethanol, following which the solution was cooled to 0 °C. To the cooled solution was added CaCl_2 (145.2 mg, 1.308 mmol, 13.5 equiv.), and the resultant slurry was allowed to stir at 0 °C for 20 min. At this time, a solution of NaBH_4 (110.0 mg, 2.910 mmol, 30.0 equiv.) in 1.0 ml 95% ethanol was added dropwise over 30 min to the stirring slurry. The reaction was then allowed to proceed for 2 hr at 0 °C, then at room temperature for another 90 hr, at which time TLC analysis (5% methanol in CH_2Cl_2) showed no discernible change in the starting material. After quenching the reaction by the addition of cold 1N HCl to pH 4, the solvents were concentrated by rotary evaporation and the crude starting material was extracted into EtOAc. Following the standard workup procedure, the crude reaction material was subjected to PTLC (3% methanol in CH_2Cl_2), allowing the recovery of 55.7 mg (82.2%) of pure starting material 7-(triethylsilyl)baccatin III (2.5).

2'-(*tert*-BUTYLDIMETHYLSILYL)-7-(TRIETHYLSILYL)TAXOL (2.29)

Imidazole (170.1 mg, 2.5 mmol, 10.0 equiv.) and *tert*-butyldimethylsilyl chloride (376.8 mg, 2.5 mmol, 10.0 equiv.) were added to a solution of taxol (213.3 mg, 0.25 mmol) in 3.0 ml DMF. The stirred solution was heated at 65-67 °C for 1 hr, then allowed to cool to room temperature, then an additional 170.1 mg (2.5 mmol, 10.0 equiv.) of imidazole and 420 μ l (2.5 mmol, 10.0 equiv.) of triethylsilyl chloride was added. After stirring for an additional 30 min, the solution was treated with 1N HCl (8.0 ml), then extracted with CH₂Cl₂ (2 $\times$ 50 ml) and washed successively with water and brine. After drying over Na₂SO₄ and concentration *in vacuo* the crude material was purified by PTLC (EtOAc/hexanes, 3:7) to yield 236.5 mg (87.4%) of 2'-(*tert*-butyldimethylsilyl)-7-(triethylsilyl)taxol (2.29) as an amorphous white solid. Melting point: 128-130 °C; FABMS *m/z* (rel int.) [M+Na-H]⁺ 1104 (5), 705 (3), 422 (40), 354 (12), 105 (100); HRFABMS *m/z* (rel int.) [M+Na-H]⁺ 1104.4936 (C₅₉H₇₉NO₁₄Si₂Na requires 1104.4937); ¹H NMR (CDCl₃) δ 8.12 (d, *J* = 7Hz, 2H), 7.74 (d, *J* = 7Hz, 2H), 7.28 - 7.60 (m, 11H), 7.08 (d, *J* = 9Hz, NH), 6.45 (s, 1H), 6.26 (m, 1H), 5.74 (dd, *J* = 2, 9Hz, 1H), 5.70 (d, *J* = 7Hz, 1H), 4.95 (br. d, *J* = 10Hz, 1H), 4.68 (d, *J* = 2Hz, 1H), 4.48 (dd, *J* = 7, 10Hz, 1H), 4.25 (ABq, *J* = 8Hz, $\Delta\nu_{AB}$ = 29, 2H), 3.84 (d, *J* = 7Hz, 1H), 2.58 (s, 3H), 2.5 (m, 1H), 2.4 (m, 1H), 2.16 (s, 3H), 2.02 (s, 3H), 1.9 (m, 2H), 1.70 (s, 3H), 1.21 (s, 3H), 1.17 (s, 3H), 0.93 (t, 9H), 0.80 (s, 9H), 0.58 (q, 6H), -0.02 (s, 3H), -0.29 (s, 3H).

2'-(*tert*-BUTYLDIMETHYLSILYL)-4-DEACETYL-2-DEBENZOYL-7-(TRIETHYLSILYL) TAXOL (2.31) VIA BENZYLTRIMETHYL-AMMONIUM HYDROXIDE (TRITON B)

To a solution of 40 mg (0.0370 mmol) of 2.29 dissolved in 2.0 ml of anhydrous CH₂Cl₂ was added 2.0 equivalents (34 ml; 0.0740 mmol) of benzyltrimethyl ammonium hydroxide (40% w/w solution in MeOH) at -78 °C. After 1 min, the cooling bath was removed and the reaction was allowed to warm to room temperature over 15 min, while monitoring the progress of the reaction by TLC (EtOAc/hexanes, 2:3). After the reaction

was complete, the solution was diluted with CH_2Cl_2 and quenched with 2.0 ml of 0.1 *N* HCl. The organic layer was washed successively with water and brine, dried over MgSO_4 , and concentrated to yield the crude residue, which was purified by PTLC (EtOAc/hexanes, 2:3) to yield 20.7 mg (59.8%) of **2.31**. Melting point: 128.5-131 °C; FABMS *m/z* (rel int.) $[\text{M}+\text{Na}]^+$ 958 (5), 400 (7), 354 (30), 177 (20), 105 (100); HRFABMS *m/z* (rel int.) $[\text{M}+\text{Na}]^+$ 958.4551 ($\text{C}_{50}\text{H}_{73}\text{NO}_{12}\text{Si}_2\text{Na}$ requires 958.4569); ^1H NMR (CDCl_3) δ 7.75 (dd, *J* = 1, 8 Hz, 2H), 7.23 - 7.54 (m, 8H), 7.25 (d, *J* = 9 Hz, NH), 6.40 (s, 1H), 5.85 (m, 1H), 5.83 (dd, *J* = 2, 9 Hz, 1H), 4.90 (dd, *J* = 3, 10 Hz, 1H), 4.57 (ABq, *J* = 8 Hz, $\Delta\nu_{\text{AB}}$ = 60, 2H), 4.52 (d, *J* = 2 Hz, 1H), 4.11 (dd, *J* = 6, 11 Hz, 1H), 3.79 (dd, *J* = 7, 7 Hz, 1H), 2.97 (d, *J* = 7 Hz, 1H), 2.55 (m, 1H), 2.5 (m, 1H), 2.16 (s, 3H), 2.05 (m, 1H), 2.04 (s, 3H), 2.0 (m, 1H), 1.52 (s, 3H), 1.09 (s, 3H), 1.04 (s, 3H), 0.94 (t, 9H), 0.84 (s, 9H), 0.58 (q, 6H), -0.07 (s, 3H), -0.26 (s, 3H).

2'-(*tert*-BUTYLDIMETHYLSILYL)-4-DEACETYL-2-DEBENZOYL-7-(TRIETHYLSILYL) TAXOL (2.31) VIA BENZYLTRIMETHYL-AMMONIUM METHOXIDE

To a solution of 150 mg (0.139 mmol) of **2.29** dissolved in 3.0 ml of anhydrous CH_2Cl_2 was added 2.1 equivalents (140 μl ; 0.291 mmol) of benzyltrimethyl ammonium methoxide (40% w/w solution in MeOH) at -78 °C. After 1 min, the cooling bath was removed and the reaction was allowed to warm to room temperature over 15 min, while monitoring the progress of the reaction by TLC (EtOAc/hexanes, 2:3). After the reaction was complete, the solution was diluted with CH_2Cl_2 and quenched with 1.0 ml of 0.1 *N* HCl. The organic layer was washed successively with water and brine, dried over MgSO_4 , and concentrated to yield the crude residue, which was purified by PTLC (EtOAc/hexanes, 2:3) to yield 89.9 mg (69.3%) of **2.31** as a colorless, glassy solid. Analysis by ^1H NMR showed compound **2.31** to be identical to that previously described.

2',7-Bis(TRIETHYLSILYL)TAXOL (2.32)

To 140 mg (0.164 mmol) of taxol in 5.0 ml of dry CH_2Cl_2 was added 115 mg (1.69 mmol, 10.3 equiv.) imidazole and 250 μl (1.49 mmol, 9.1 equiv.) triethylsilyl chloride. After stirring at room temperature for 1 hr, the reaction was diluted with CH_2Cl_2 and washed several times with water and brine, respectively. The CH_2Cl_2 layer was dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by PTLC (EtOAc/hexanes, 3:7) to afford 162 mg (91.3%) of 2.32 as a white powder: Melting point: 122-124 °C; FABMS m/z (rel int.) $[\text{MH}]^+$ 1104 (100), 982 (10); ^1H NMR (CDCl_3) δ 8.13 (dd, $J = 1.4, 8.5\text{Hz}$, 2H), 7.74 (dd, $J = 1.1, 8.5\text{Hz}$, 2H), 7.3 - 7.6 (m, 13H), 7.10 (d, $J = 8.9\text{Hz}$, NH), 6.45 (s, 1H), 6.24 (br. t, 1H), 5.70 (d, $J = 7.0\text{Hz}$, 1H), 5.70 (m, 1H), 4.94 (br. d, $J = 8.8\text{Hz}$, 1H), 4.70 (d, $J = 2.1\text{Hz}$, 1H), 4.48 (dd, $J = 6.6, 10.6\text{Hz}$, 1H), 4.27 (ABq, $J = 8\text{Hz}$, $\Delta\nu_{\text{AB}} = 28$, 2H), 3.83 (d, $J = 7\text{Hz}$, 1H), 2.54 (s, 3H), 2.50 (m, 1H), 2.32 (m, 1H), 2.25 (m, 1H), 2.16 (s, 3H), 2.05 (br. s, 3H), 1.86 (m, 1H), 1.70 (s, 3H), 1.25 (s, 3H), 1.20 (s, 3H), 0.90 (t, 9H), 0.81 (t, 9H), 0.58 (q, 6H), 0.45 (q, 6H).

REACTION OF 2',7-BIS(TRIETHYLSILYL)TAXOL (2.32) WITH BENZYLTRIMETHYLAMMONIUM METHOXIDE

To a solution of 20 mg (0.0185 mmol) of 2.32 dissolved in 1.0 ml of anhydrous CH_2Cl_2 was added 2.0 equivalents (17 μl ; 0.0370 mmol) of benzyltrimethyl ammonium methoxide (40% w/w solution in MeOH) at -78 °C. After 1 min, the cooling bath was removed and the reaction was allowed to warm to room temperature over 15 min, while monitoring the progress of the reaction by TLC (EtOAc/hexanes, 2:3). After the reaction was complete, the solution was diluted with CH_2Cl_2 and quenched with 1.0 ml of 0.1 N HCl. The organic layer was washed successively with water and brine, dried over MgSO_4 , and concentrated to yield the crude residue, which was purified by PTLC (EtOAc/hexanes, 2:3) to yield three major products. One product was identified as 7-(triethylsilyl)baccatin III (2.5, 3.2 mg, 24.7%), identical to the same material described previously. A second product

was identified on the basis of its ^1H NMR spectrum as 2-debenzoyl-7-(triethylsilyl)baccatin III (3.72, 4.6 mg, 41.7%), identical with the same compound previously described. A third product was identified as 4-deacetyl-2-debenzoyl-7-(triethylsilyl)baccatin III (3.73, 1.8 mg, 17.6 %) based on its ^1H NMR spectrum, and was identical with the same compound described previously.

REACTION OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-7-(TRIETHYLSILYL) TAXOL (2.29) WITH POTASSIUM *tert*-BUTOXIDE

2'-(*tert*-Butyldimethylsilyl)-7-(triethylsilyl)taxol (2.29, 23.5 mg, 0.0217 mmol) was dissolved in 250 μl dry THF with stirring. After 5 min, 87 μl (0.0869 mmol, 4.0 equivs.) of a 1.0M solution of potassium *tert*-butoxide in THF was added, and the reaction was allowed to proceed for 15 min at room temperature under argon. The reaction was then neutralized with 1N HCl in THF. After evaporation of the solvents, the crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1). Purification afforded 3.7 mg (17.4%) of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-7-(triethylsilyl)taxol (2.30) as a white powder: Melting point: 132-135 $^{\circ}\text{C}$; FABMS m/z (rel int.) [MNa-H] $^{+}$ 1000 (3), 400 (22), 354 (84), 177 (25), 105 (100); ^1H NMR (CDCl_3) δ 7.74 (dd, J = 1.6, 8.3Hz, 2H), 7.26 - 7.52 (m, 8H), 7.05 (d, J = 9.2Hz, NH), 6.37 (s, 1H), 6.24 (br. t, 1H), 5.67 (dd, J = 1.6, 9.2Hz, 1H), 4.95 (br. d, 1H), 4.63 (br. s, 2H), 4.60 (d, J = 1.6Hz, 1H), 4.41 (dd, J = 6.6, 10.3Hz, 1H), 3.92 (t, J = 6.3Hz, 1H), 3.46 (d, J = 6.9Hz, 1H), 2.79 (d, J = 5.9Hz, OH), 2.51 (m, 1H), 2.42 (s, 3H), 2.4 - 2.5 (m, 1H), 2.14 (s, 3H), 1.96 (s, 3H), 1.9 - 2.0 (m, 2H), 1.62 (s, 3H), 1.15 (s, 3H), 1.07 (s, 3H), 0.92 (t, J = 7.8Hz, 9H), 0.80 (s, 9H), 0.57 (q, J = 7.8Hz, 6H), -0.04 (s, 3H), -0.28 (s, 3H); 8.3 mg (40.9%) of 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-2-debenzoyl-7-(triethylsilyl)taxol (2.31), which was identical to the same material described previously; and 5.6 mg (27.6%) of 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-2-debenzoyl-7-(triethylsilyl)isotaxol (2.33): ^1H NMR (CDCl_3) δ 7.79 (dd, J = 1.5, 8.5Hz, 2H), 7.35 - 7.53 (m, 9H), 6.35 (s, 1H), 5.90 (m, 1H), 5.60 (dd, J = 1.4, 9.1Hz, 1H), 4.51 (d, J = 1.4Hz, 1H), 4.23 (m, 1H), 4.06 (d, J =

6.0Hz, 1H), 3.95 (m, 1H), 3.80 (ABq, $J = 9.9\text{Hz}$, $\Delta\nu_{AB} = 55$, 2H), 3.18 (d, $J = 6.0\text{Hz}$, 1H), 2.5 (m, 2H), 2.30 (s, 3H), 2.03 (s, 3H), 2.0 (m, 2H), 1.40 (s, 3H), 1.30 (s, 3H), 1.08 (s, 3H), 0.95 (t, $J = 7.8\text{Hz}$, 9H), 0.80 (s, 9H), 0.59 (q, $J = 7.8\text{Hz}$, 6H), -0.08 (s, 3H), -0.32 (s, 3H).

FORMATION OF 4-DEACETYLTAXOL (2.1) VIA THE TAXOL APPROACH

Compound **2.31** (10.3 mg, 0.011 mmol), dissolved in 125 ml anhydrous toluene, was added to a slurry of DCC (45.3 mg, 0.22 mmol, 20.0 equiv.) and benzoic acid (26.8 mg, 0.22 mmol, 20.0 equiv.) in 200 μl anhydrous toluene. After adding a catalytic amount of DMAP, the mixture was heated at 65 °C for 10 hr, at which time the reaction appeared to be complete by TLC analysis (EtOAc/hexanes, 1:2). The reaction mixture was cooled then diluted with EtOAc, then was filtered through a pipette of silica gel with EtOAc. After removal of the solvent, the crude residue was subjected to purification via PTLC (EtOAc/hexanes, 1:2) to yield 7.7 mg (67.3%) of 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**): FABMS m/z (rel int.) $[M]^+$ 1040 (70), 982 (60), 964 (100); ^1H NMR (CDCl_3) δ 8.19 (d, $J = 7.3\text{Hz}$, 2H), 7.78 (d, $J = 7.0\text{Hz}$, 2H), 7.19 - 7.54 (m, 12H), 6.49 (s, 1H), 6.11 (d, $J = 10.8\text{Hz}$, 1H), 5.93 (m, 1H), 5.71 (d, $J = 5.9\text{Hz}$, 1H), 4.91 (dd, $J = 2.5$, 8.1Hz, 1H), 4.75 (s, OH), 4.56 (br. s, 1H), 4.24 (ABq, $J = 8.3\text{Hz}$, $\Delta\nu_{AB} = 36$, 2H), 4.08 (m, 1H), 3.44 (d, $J = 5.9\text{Hz}$, 1H), 2.80 (dd, $J = 2.7$, 10.8Hz, 1H), 2.54 (m, 2H), 2.33 (m, 1H), 2.19 (s, 3H), 1.71 (s, 3H), 1.60 (s, 3H), 1.24 (s, 3H), 1.07 (s, 3H), 0.92 (t, 9H), 0.84 (s, 9H), 0.60 (q, 6H), -0.07 (s, 3H), -0.24 (s, 3H).

The above compound (**2.34**, 6.5 mg, 0.0063 mmol) was added to 1.0 ml of 5% HCl in MeOH and stirred at room temperature for 2 hr. The solution was then diluted with EtOAc, washed with water then brine, dried over MgSO_4 , concentrated, and applied to PTLC (EtOAc/hexanes, 3:2) to yield 5.0 mg (98.6%) of 4-deacetyltaxol (**2.1**), which was shown by ^1H NMR and mass spectrometry to be identical to the same compound prepared from baccatin III above.

COMPARATIVE C-4 REACYLATION OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-
4-DEACETYL-7-(TRIETHYLSILYL)TAXOL (2.34) USING
VARIOUS ACYLATING AGENTS:

ACETIC ANHYDRIDE

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34, 7.1 mg, 0.0068 mmol) was dissolved in 100 ml anhydrous toluene. This solution was then added to a stirred mixture of Ac₂O (6.4 μ l, 0.068 mmol, 10.0 equiv.) and DCC (14.1 mg, 0.068 mmol, 10.0 equiv.) in 100 ml anhydrous toluene. Upon the addition of a catalytic amount of DMAP, the reaction was heated to 65 °C and allowed to proceed while being monitored by TLC (EtOAc/hexanes, 1:3). After 12 hr, no apparent change was evident, and after evaporation of the solvent, the starting material was recovered by PTLC (EtOAc/hexanes, 1:3), which afforded 5.4 mg of 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34, 76.1% recovery). Analysis of this material by ¹H NMR confirmed its identity.

CHLOROACETIC ANHYDRIDE

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34, 6.8 mg, 0.0065 mmol) was dissolved in 100 μ l anhydrous toluene. This solution was then added to a stirred mixture of chloroacetic anhydride (11.2 mg, 0.065 mmol, 10.0 equiv.) and DCC (13.5 mg, 0.065 mmol, 10.0 equiv.) in 100 μ l anhydrous toluene. Upon the addition of a catalytic amount of DMAP, the reaction was heated to 65 °C and allowed to proceed while being monitored by TLC (EtOAc/hexanes, 1:3). After 16 hr, no apparent change was evident, and after evaporation of the solvent, the starting material was recovered by PTLC (EtOAc/hexanes, 1:3), which afforded 5.7 mg of 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34, 83.8% recovery). Analysis of this material by ¹H NMR confirmed its identity.

FORMIC ACID

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34, 6.3 mg, 0.0061 mmol) was dissolved in 100 μ l anhydrous toluene. This solution was then added to a stirred

mixture of formic acid (2.3 μ l, 0.061 mmol, 10.0 equiv.) and DCC (12.4 mg, 0.061 mmol, 10.0 equiv.) in 100 μ l anhydrous toluene. Upon the addition of a catalytic amount of DMAP, the reaction was heated to 65 °C and allowed to proceed while being monitored by TLC (EtOAc/hexanes, 1:3). After 18 hr, no apparent change was evident, and after evaporation of the solvent, the starting material was recovered by PTLC (EtOAc/hexanes, 1:3), which afforded 5.3 mg of 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**, 84.1% recovery), as determined by ^1H NMR analysis.

BENZOIC ACID

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**, 7.3 mg, 0.0070 mmol) was dissolved in 100 μ l anhydrous toluene. This solution was then added to a stirred mixture of benzoic acid (17.1 mg, 0.140 mmol, 20.0 equiv.) and DCC (28.9 mg, 0.140 mmol, 20.0 equiv.) in 100 μ l anhydrous toluene. Upon the addition of a catalytic amount of DMAP, the reaction was heated to 65 °C and allowed to proceed while being monitored by TLC (EtOAc/hexanes, 1:3). After 24 hr, no apparent change was evident, and after evaporation of the solvent, the starting material was recovered by PTLC (EtOAc/hexanes, 1:3), which afforded 6.2 mg of 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**, 84.9% recovery). Analysis of this material by ^1H NMR confirmed its identity.

ATTEMPTED LARGE-SCALE C-4 ACETYLATION OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-4-DEACETYL-7-(TRIETHYLSILYL)TAXOL (**2.34**)

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**, 25.3 mg, 0.0243 mmol) was dissolved in 200 μ l anhydrous toluene. This solution was then added to a stirred mixture of Ac_2O (23.0 μ l, 0.243 mmol, 10.0 equiv.) and DCC (50.2 mg, 0.243 mmol, 10.0 equiv.) in 200 μ l anhydrous toluene. Upon the addition of a catalytic amount of DMAP, the reaction was heated to 65 °C and allowed to proceed while being monitored by TLC (EtOAc/hexanes, 1:3). After 36 hr, no apparent change was evident, and after evaporation of the solvent, the starting material was recovered by PTLC (EtOAc/hexanes,

1:3), which afforded 19.6 mg of 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**, 77.5% recovery), as determined by ¹H NMR analysis.

ATTEMPTED LARGE-SCALE C-4 ACYLATION OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-4-DEACETYL-7-(TRIETHYLSILYL)TAXOL (**2.34**) USING CHLOROACETIC ANHYDRIDE UNDER STANDARD COUPLING CONDITIONS

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**, 24.7 mg, 0.0238 mmol) was dissolved in 200 μ l anhydrous toluene. This solution was then added to a stirred mixture of chloroacetic anhydride (40.6 mg, 0.238 mmol, 10.0 equiv.) and DCC (49.0 mg, 0.238 mmol, 10.0 equiv.) in 200 μ l anhydrous toluene. Upon the addition of a catalytic amount of DMAP, the reaction was heated to 65 °C and allowed to proceed while being monitored by TLC (EtOAc/hexanes, 1:3). After 36 hr, no apparent change was evident, and after evaporation of the solvent, the starting material was recovered by PTLC (EtOAc/hexanes, 1:3), which afforded 18.8 mg of 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**, 76.1% recovery), as determined by ¹H NMR analysis.

ATTEMPTED C-4 ACYLATION OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-4-DEACETYL-7-(TRIETHYLSILYL)TAXOL (**2.34**) USING CHLOROACETIC ANHYDRIDE AND PYRIDINE

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**, 6.4 mg, 0.0062 mmol) was dissolved with stirring in pyridine. To the stirred solution was added chloroacetic anhydride (31.6 mg, 0.185 mmol, 29.8 equiv.), and the reaction vial was immersed in an oil bath at 65 °C. The reaction was allowed to proceed at this temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:2). Within 2 hr, the reaction turned from clear and colorless to an opaque, dark red-brown; however, TLC analysis showed very little reaction to have occurred. After 30 hr, a TLC showed the formation of a more polar product, and after allowing the reaction to cool to room

temperature and dilution with CH_2Cl_2 , the solution was treated with aqueous CuSO_4 solution to remove the pyridine, then was treated with brine and dried with MgSO_4 . The solvents were removed *in vacuo* and the crude residue was subjected to PTLC (EtOAc/hexanes, 1:2) for purification. The bands corresponding to unreacted starting material and the product were isolated and analyzed by ^1H NMR, which confirmed the starting material to be unchanged. The spectrum for the product of the reaction, on the other hand, showed major changes to have occurred in the molecule, including deprotection of both the C-2' and C-7 positions and epimerization of the resultant unprotected C-7 hydroxyl group. No C-4 chloroacetyl incorporation was evident and therefore no further attempt was made at fully characterizing this compound.

ATTEMPTED C-4 ACYLATION OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-4-DEACETYL-7-(TRIETHYLSILYL)TAXOL (2.34) USING CHLOROACETYL CHLORIDE AND PYRIDINE: A COMPARATIVE STUDY OF REACTION CONDITIONS:

EXPERIMENT 1

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**, 5.0 mg, 0.0048 mmol) was dissolved with stirring in 500 μl pyridine. To the stirred solution was added chloroacetic chloride (3.8 μl , 0.048 mmol, 10.0 equiv.), and the reaction vial was immersed in an cooling bath at 0 $^\circ\text{C}$. The reaction was allowed to proceed at this temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:2). After 4 hr, TLC analysis showed no reaction to have occurred. After dilution with CH_2Cl_2 , the solution was treated with aqueous CuSO_4 solution to remove the pyridine, then was treated with brine and dried with MgSO_4 . The solvents were removed *in vacuo* and the crude starting material was subjected to PTLC (EtOAc/hexanes, 1:2) for purification, yielding 4.1 mg (82.0%) of recovered starting material. Analysis of this material by ^1H NMR confirmed its identity as 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**).

EXPERIMENT 2

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**, 5.0 mg, 0.0048 mmol) was dissolved with stirring in 500 μ l pyridine. To the stirred solution was added chloroacetic chloride (3.8 μ l, 0.048 mmol, 10.0 equiv.), and the reaction was allowed to proceed at room temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:2). After 12 hr, TLC analysis showed no reaction to have occurred. After dilution with CH_2Cl_2 , the solution was treated with aqueous CuSO_4 solution to remove the pyridine, then was treated with brine and dried with MgSO_4 . The solvents were removed *in vacuo* and the crude starting material was subjected to PTLC (EtOAc/hexanes, 1:2) for purification, yielding 4.3 mg (86.0%) of recovered starting material. Analysis of this material by ^1H NMR confirmed its identity as 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**).

EXPERIMENT 3

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**, 5.0 mg, 0.0048 mmol) was dissolved with stirring in 500 μ l pyridine. To the stirred solution was added chloroacetic chloride (3.8 μ l, 0.048 mmol, 10.0 equiv.), and the reaction vial was immersed in an oil bath at 65 $^\circ\text{C}$. The reaction was allowed to proceed at this temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:2). Even after 30 hr, TLC analysis showed no reaction to have occurred, and after dilution with CH_2Cl_2 , the solution was treated with aqueous CuSO_4 solution to remove the pyridine, then was treated with brine and dried with MgSO_4 . The solvents were removed *in vacuo* and the crude starting material was subjected to PTLC (EtOAc/hexanes, 1:2) for purification, affording 3.8 mg (76.0%) of recovered starting material. Analysis of this material by ^1H NMR confirmed its identity as 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**).

EXPERIMENT 4

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (**2.34**, 5.0 mg, 0.0048 mmol) was dissolved with stirring in 500 μ l pyridine. To the stirred solution was added chloroacetic chloride (11.5 μ l, 0.144 mmol, 30.0 equiv.), and the reaction vial was

immersed in an cooling bath at 0 °C. The reaction was allowed to proceed at this temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:2). After 4 hr, TLC analysis showed no reaction to have occurred. After dilution with CH₂Cl₂, the solution was treated with aqueous CuSO₄ solution to remove the pyridine, then was treated with brine and dried with MgSO₄. The solvents were removed *in vacuo* and the crude starting material was subjected to PTLC (EtOAc/hexanes, 1:2) for purification, affording 4.6 mg (92.0%) of recovered starting material. Analysis of this material by ¹H NMR confirmed its identity as 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34).

EXPERIMENT 5

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34, 5.0 mg, 0.0048 mmol) was dissolved with stirring in 500 µl pyridine. To the stirred solution was added chloroacetic chloride (11.5 µl, 0.144 mmol, 30.0 equiv.), and the reaction was allowed to proceed at room temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:2). After 12 hr, TLC analysis showed no reaction to have occurred. After dilution with CH₂Cl₂, the solution was treated with aqueous CuSO₄ solution to remove the pyridine, then was treated with brine and dried with MgSO₄. The solvents were removed *in vacuo* and the crude starting material was subjected to PTLC (EtOAc/hexanes, 1:2) for purification, affording 4.4 mg (88.0%) of recovered starting material. Analysis of this material by ¹H NMR confirmed its identity as 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34).

EXPERIMENT 6

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34, 5.0 mg, 0.0048 mmol) was dissolved with stirring in 500 µl pyridine. To the stirred solution was added chloroacetic chloride (11.5 µl, 0.144 mmol, 30.0 equiv.), and the reaction vial was immersed in an oil bath at 65 °C. The reaction was allowed to proceed at this temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:2). Even after 30 hr, TLC analysis showed no reaction to have occurred, and after dilution with CH₂Cl₂, the solution

was treated with aqueous CuSO_4 solution to remove the pyridine, then was treated with brine and dried with MgSO_4 . The solvents were removed *in vacuo* and the crude starting material was subjected to PTLC (EtOAc/hexanes, 1:2) for purification, yielding 3.3 mg (66.0%) of recovered starting material. Analysis of this material by ^1H NMR confirmed its identity as 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34).

ATTEMPTED C-4 ACYLATION OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-4-DEACETYL-7-(TRIETHYLSILYL)TAXOL (2.34) USING VARIOUS ACYL CHLORIDES AND TRIETHYLAMINE: A COMPARATIVE STUDY OF REACTION CONDITIONS

A series of experiments was conducted to determine the outcome of treating 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34) with various acylating agents at several temperatures at a set concentration of triethylamine. Each experiment was carried out using 5.0 mg (0.0048 mmol) of 2'-(*tert*-butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34), 10.0 equivalents (0.048 mmol) of either CH_3COCl , ClCH_2COCl , PhCOCl , PhCH_2COCl or $\text{PhOCH}_2\text{COCl}$, and 10.0 equivalents (0.048 mmol) of dry, distilled triethylamine, according to Table 9. The first set of reactions was performed in 150 μl of anhydrous CH_2Cl_2 at 0 °C for 6 hr each, the second set of reactions was performed in 150 μl of anhydrous CH_2Cl_2 at room temperature for 8 hr each, and the third set of reactions was performed in 150 μl of anhydrous CHCl_3 at 60 °C for 6 hr each, according to Table 9. All experiments were monitored by TLC (EtOAc/hexanes, 1:3), and with the exception of four experiments, as shown in Table 9 and as described in the text, no products from the reactions were evident. Purified starting material was recovered from each reaction by PTLC (EtOAc/hexanes, 1:3). The four experiments in which the starting material was consumed, however, were shown by TLC and ^1H NMR analyses to be very complex mixtures, as shown in Table 9 and as discussed in the text. Attempts to further purify these product mixtures using different PTLC solvent systems were unsuccessful.

ATTEMPTS AT C-4 ACYLATION OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-4-
DEACETYL-7-(TRIETHYLSILYL)TAXOL (2.34), USING TWO
ACID CHLORIDES WITH TRIETHYLAMINE AS BASE:

PHENYLACETYL CHLORIDE

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34, 6.3 mg, 0.006 mmol) was dissolved with stirring in 100 μ l anhydrous CH_2Cl_2 . To the stirred solution was added triethylamine (8.5 μ l, 0.06 mmol, 10.0 equiv.) and phenylacetyl chloride (8.0 μ l, 0.06 mmol, 10.0 equiv.), and the reaction was allowed to proceed at room temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:3). After 20 hr, a TLC showed the formation of a series of more polar products, and after addition of H_2O and dilution with CH_2Cl_2 , the reaction mixture was worked up by the standard procedure. The crude residue was subjected to PTLC (EtOAc/hexanes, 1:3) for purification, affording several products which, upon ^1H NMR analysis, were shown to be complex mixtures. Attempts to further purify these mixtures by PTLC using various solvent systems proved to be fruitless.

PHENOXYACETYL CHLORIDE

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34, 6.3 mg, 0.006 mmol) was dissolved with stirring in 100 μ l anhydrous CH_2Cl_2 . To the stirred solution was added triethylamine (8.5 μ l, 0.06 mmol, 10.0 equiv.) and phenoxyacetyl chloride (8.4 μ l, 0.06 mmol, 10.0 equiv.), and the reaction was allowed to proceed at room temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:3). After 20 hr, a TLC showed the formation of a series of more polar products, and after addition of H_2O and dilution with CH_2Cl_2 , the reaction mixture was worked up by the standard procedure. The crude residue was subjected to PTLC (EtOAc/hexanes, 1:3) for purification, affording several products which, upon ^1H NMR analysis, were shown to be complex mixtures. Further attempts to purify these mixtures by PTLC using various solvent systems were unfruitful.

FURTHER ATTEMPTS AT C-4 ACYLATION OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-4-DEACETYL-7-(TRIETHYLSILYL)TAXOL (2.34),

USING ELEVATED TEMPERATURE:

PHENYLACETYL CHLORIDE

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34, 25.0 mg, 0.024 mmol) was dissolved with stirring in 200 μ l anhydrous CHCl_3 . To the stirred solution was added triethylamine (33.4 μ l, 0.24 mmol, 10.0 equiv.) and phenylacetyl chloride (31.7 μ l, 0.24 mmol, 10.0 equiv.), and the reaction was heated to 60 $^{\circ}\text{C}$ and allowed to proceed at this temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:3). After 18 hr, a TLC showed the formation of a series of more polar products, and after addition of H_2O and dilution with CH_2Cl_2 , the reaction mixture was worked up by the standard procedure. The crude residue was subjected to PTLC (EtOAc/hexanes, 1:3) for purification, affording several products which, upon ^1H NMR analysis, were shown to be complex mixtures. Attempts to further purify these mixtures by PTLC using various solvent systems proved to be fruitless.

PHENOXYACETYL CHLORIDE

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-7-(triethylsilyl)taxol (2.34, 25.0 mg, 0.024 mmol) was dissolved with stirring in 200 μ l anhydrous CHCl_3 . To the stirred solution was added triethylamine (33.4 μ l, 0.24 mmol, 10.0 equiv.) and phenoxyacetyl chloride (33.2 μ l, 0.24 mmol, 10.0 equiv.), and the reaction was heated to 60 $^{\circ}\text{C}$ and allowed to proceed at this temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:3). After 18 hr, a TLC showed the formation of a series of more polar products, and after addition of H_2O and dilution with CH_2Cl_2 , the reaction mixture was worked up by the standard procedure. The crude residue was subjected to PTLC (EtOAc/hexanes, 1:3) for purification, affording several products which, upon ^1H NMR analysis, were shown to be complex mixtures. Further attempts to purify these mixtures by PTLC using various solvent systems were unfruitful.

PREPARATION OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-2-DEBENZOYL-1,2-CARBONATO-4-DEACETYL-7-(TRIETHYLSILYL)TAXOL (3.3) FROM 2'-(*tert*-BUTYLDIMETHYLSILYL)-4-DEACETYL-2-DEBENZOYL-7-(TRIETHYLSILYL)TAXOL (2.31)

2'-(*tert*-Butyldimethylsilyl)-4-deacetyl-2-debenzoyl-7-(triethylsilyl)taxol (2.31, 115.9 mg, 0.124 mmol) was dissolved in 3.7 ml CH₂Cl₂, and the solution was cooled to 0 °C. To the stirred solution was added 300 µl anhydrous pyridine, following which triphosgene (36.7 mg, 0.124 mmol, 1.0 equiv.) was added. The flask was warmed to room temperature over 20 min, and the reaction was allowed to proceed at this temperature under argon for an additional 45 min, at which time a TLC (EtOAc/hexanes, 1:2) showed the reaction to be complete based on consumption of starting material and formation of a less polar product. The solution was diluted with CH₂Cl₂, then was worked up by standard procedure. After evaporation of the solvent, the crude residue was subjected to purification by PTLC (EtOAc/hexanes, 2:5), affording 84.4 mg (70.8%) of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3): FABMS *m/z* (rel int.) [MH]⁺ 963 (100), 943 (27), 921 (23), 905 (31), 873 (22), 820 (18), 804 (12), 790 (17), 756 (20), 726 (13), 662 (22), 647 (17), 627 (22); ¹H NMR (CDCl₃) δ 7.77 (dd, *J* = 1.6, 8.3Hz, 2H), 7.30 - 7.54 (m, 9H), 6.45 (s, 1H), 5.90 (m, 1H), 5.86 (d, *J* = 8.8Hz, 1H), 5.21 (s, OH), 4.96 (d, *J* = 7.3Hz, 1H), 4.58 (ABq, *J* = 8.7Hz, Δ*v*_{AB} = 19, 2H), 4.54 (d, *J* = 1.6Hz, 1H), 4.41 (d, *J* = 5.4Hz, 1H), 4.22 (dd, *J* = 6.8, 10.5Hz, 1H), 3.01 (d, *J* = 5.4Hz, 1H), 2.78 (dd, *J* = 4.6, 15.6Hz, 1H), 2.44 - 2.65 (m, 2H), 2.18 (s, 3H), 1.96 - 2.10 (m, 1H), 1.67 (s, 3H), 1.59 (s, 3H), 1.25 (s, 3H), 1.19 (d, 3H), 0.94 (t, 9H), 0.84 (s, 9H), 0.61 (q, 6H), -0.10 (s, 3H), -0.26 (s, 3H).

COMPARATIVE C-4 REACYLATION STUDIES OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-
2-DEBENZOYL-1,2-CARBONATO-4-DEACETYL-7-(TRIETHYLSILYL)
TAXOL (3.3) UNDER STANDARD COUPLING CONDITIONS

A series of six reactions was conducted to determine the outcome of treating 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3) with various acylating agents under standard coupling conditions. Each experiment was carried out using around 5 mg (0.0052 mmol) of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3), 10.0 equivalents (0.052 mmol) of either HCO_2H , $\text{CH}_3\text{CO}_2\text{H}$, $\text{CH}_3\text{CH}_2\text{CO}_2\text{H}$, $(\text{CH}_3\text{CO})_2\text{O}$, $(\text{CH}_3\text{CH}_2\text{CO})_2\text{O}$ or $(\text{ClCH}_2\text{CO})_2\text{O}$, 10.0 equivalents (0.052 mmol) of DCC and a catalytic amount of DMAP, according to Table 10. Upon combination of the reagents, the reactions were each heated to 65 °C, and allowed to proceed for up to 12 hr. The reactions were checked by TLC (EtOAc/hexanes, 1:3), and with the exception of the sixth reaction, as shown in Table 10 and as described in the text, essentially no reaction products were evident. Purified starting material was recovered from *reactions 1* through 5, from Table 10, by PTLC (EtOAc/hexanes, 1:3). Because of the scale of the reactions in this experiment and the expected behavior of the C-4 acylated carbonate, the product formed in *reaction 6* was not further characterized.

COMPARATIVE C-4 REACYLATION STUDIES OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-
2-DEBENZOYL-1,2-CARBONATO-4-DEACETYL-7-(TRIETHYLSILYL)TAXOL
(3.3) IN PYRIDINE USING ACYL ANHYDRIDE OR ACYL CHLORIDE

A series of four reactions was conducted to determine the outcome of treating 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3) with various acylating agents in pyridine. Each reaction was carried out using 5 to 10 mg (0.0052 to 0.0104 mmol) of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3) and 30.0 equivalents (0.156 to 0.312 mmol) of either CH_3COCl , $(\text{CH}_3\text{CO})_2\text{O}$, ClCH_2COCl or $(\text{ClCH}_2\text{CO})_2\text{O}$ in anhydrous pyridine, according to Table 11. After combining the reagents at r.t., the reaction vials were

immersed in an oil bath at 65 °C, and the reactions were allowed to proceed at this temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:3). Thin layer chromatographic analyses (EtOAc/hexanes, 1:3) after the reaction times indicated in Table 11, showed no reaction to have occurred in *reactions 1* and 2, and the purified starting material from these reactions was recovered by PTLC (EtOAc/hexanes, 1:3). *Reactions 3* and 4, from Table 11, were seen to have produced a more polar product in an approximate ratio of 1-to-5 to starting material, but because of the scale of the reactions in these experiments and the expected behavior of the C-4 acylated carbonate, the products formed in the latter two reactions were not fully characterized.

**ATTEMPTED C-4 REACYLATION OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-2-DEBENZOYL-1,2-CARBONATO-4-DEACETYL-7-(TRIETHYLSILYL)TAXOL
(3.3) USING ACETYL CHLORIDE AND SODIUM HYDRIDE**

2'-(*tert*-Butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3, 5.0 mg, 0.0052 mmol), dissolved in 100 µl dry, distilled DMF, was treated with sodium hydride (1.2 mg, 0.052 mmol, 10.0 equivs.) at 0 °C. Following the addition of acetyl chloride (4.1 mg, 0.052 mmol, 10.0 equivs.), the reaction was allowed to warm to r.t. and allowed to proceed under argon while monitoring by TLC (EtOAc/hexane, 1:3). After 16 hr, a TLC plate of the reaction mixture showed no reaction to have occurred, and 3.6 mg (72.0%) of the starting material 3.3 was recovered by PTLC (EtOAc/hexanes, 1:3).

**COMPARATIVE C-4 REACYLATION STUDIES OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-2-DEBENZOYL-1,2-CARBONATO-4-DEACETYL-7-(TRIETHYLSILYL)TAXOL
(3.3) USING ACID CHLORIDE AND TRIETHYLAMINE**

A series of four reactions was conducted to determine the outcome of treating 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3) with various acyl chlorides in triethylamine. Each experiment was carried out using 3.5 mg (0.0036 mmol) of 2'-(*tert*-butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-

(triethylsilyl)taxol (3.3), 10.0 equivalents (0.036 mmol) of either CH_3COCl , ClCH_2COCl , PhCH_2COCl or $\text{PhOCH}_2\text{COCl}$ and 10.0 equivalents (0.036 mmol) dry, distilled triethylamine in 100 μl of CH_2Cl_2 , according to Table 12. After adding the acylating agent to the solution of substrate 3.3 and triethylamine in CH_2Cl_2 at 0 $^\circ\text{C}$, the reaction vials were allowed to warm gradually to r.t., and then allowed to proceed at this temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:3). Thin layer chromatographic analyses (EtOAc/hexanes, 1:3) after the reaction times indicated in Table 12, showed no reaction to have occurred in *reactions 1, 3 and 4*, and the purified starting material from each was recovered by PTLC (EtOAc/hexanes, 1:3). *Reaction 2*, from Table 12, however, was seen to have produced a more polar product in an approximate ratio of 1:6 to starting material, but again, because of the scale of the reactions in these experiments and the expected behavior of the C-4 acylated carbonate, the product formed in this reaction was not isolated or characterized.

FORMATION OF THE OXETANE RING-OPENED DERIVATIVE OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-2-DEBENZOYL-1,2-CARBONATO-4-DEACETYL-7-(TRIETHYLSILYL)TAXOL (3.3):

EXPERIMENT I

2'-(*tert*-Butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3, 25.0 mg, 0.026 mmol) was added to a mixture of 44.4 mg (0.26 mmol, 10.0 equiv.) of chloroacetic anhydride and 53.6 mg (0.26 mmol, 10.0 equiv.) of DCC in 500 μl anhydrous toluene. After the addition of a catalytic amount of DMAP, the reaction vial was immersed in an oil bath at 65 $^\circ\text{C}$, and the reaction was allowed to proceed under argon for 12 hr. At this time, the reaction was checked by TLC (EtOAc/hexanes, 1:3), which showed the formation of a more polar product. The solvent was removed by rotary evaporator, and the crude residue was subjected to PTLC (EtOAc/hexanes, 1:3) for purification, affording 10.1 mg (38.9%) of purified product which, after analysis by ^1H NMR and mass

spectrometry, was determined to have the structure **3.5**: FABMS m/z (rel. int.) $[MH]^+$ 1000 (76), 986 (18), 965 (17), 942 (18), 927 (11), 897 (9.0), 866 (11), 780 (14), 754 (16), 730 (18), 702 (13), 689 (18), 667 (22), 617 (21); ^1H NMR ($CDCl_3$) δ 7.73 (dd, J = 1.7, 6.7Hz, 2H), 7.28 - 7.54 (m, 8H), 7.20 (d, J = 8.1Hz, NH), 6.60 (s, 1H), 5.98 (br. d, J = 9.1Hz, 1H), 5.97 (m, 1H), 4.70 (t, 1H), 4.55 (m, 1H), 4.53 (d, J = 1.6Hz, 1H), 4.39 (s, OH), 4.25 (d, J = 4.6Hz, 1H), 3.83 (ABq, J = 11.4Hz, $\Delta\nu_{AB}$ = 139, 2H), 3.77 (d, J = 4.6Hz, 1H), 3.12 (dd, J = 5.0, 15.5Hz, 1H), 2.54 (dd, J = 9.9, 15.7Hz, 1H), 2.34 (s, 3H), 2.15 - 2.39 (m, 2H), 2.18 (s, 3H), 1.65 (s, 3H), 1.21 (s, 3H), 1.15 (s, 3H), 0.94 (t, 9H), 0.79 (s, 9H), 0.59 (q, 6H), -0.14 (s, 3H), -0.35 (s, 3H).

EXPERIMENT 2

2'-(*tert*-Butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**, 25.0 mg, 0.026 mmol) was dissolved in 500 μ l anhydrous pyridine. To the stirred solution was added 20.7 μ l (0.26 mmol, 10.0 equiv.) of chloroacetyl chloride and the reaction vial was then immersed in an oil bath at 65 $^{\circ}C$. The reaction was allowed to proceed at this temperature under argon for 16 hr, at which time the reaction was checked by TLC (EtOAc/hexanes, 1:3), which showed the formation of a more polar product. After dilution of the reaction with CH_2Cl_2 , the mixture was treated with aqueous $CuSO_4$ solution, then was worked up by the standard procedure. After removal of the solvent by rotary evaporator, the crude residue was subjected to PTLC (EtOAc/hexanes, 1:3) for purification, affording 11.1 mg (42.7%) of purified product **3.5**, which displayed the same ^1H NMR and mass spectral data as the identical compound from above.

EXPERIMENT 3

2'-(*tert*-Butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (**3.3**, 10.0 mg, 0.0104 mmol) was dissolved in 300 μ l anhydrous pyridine. To the stirred solution was added 53.3 mg (0.312 mmol, 30.0 equiv.) of chloroacetic anhydride and the reaction vial was then immersed in an oil bath at 65 $^{\circ}C$. The reaction was allowed to proceed at this temperature under argon for 30 hr, at which time the reaction was checked

by TLC (EtOAc/hexanes, 1:3), which showed the formation of a more polar product. After dilution of the reaction with CH₂Cl₂, the mixture was treated with aqueous CuSO₄ solution, then was worked up by the standard procedure. After removal of the solvent by rotary evaporator, the crude residue was subjected to PTLC (EtOAc/hexanes, 1:3) for purification, affording 3.5 mg (33.7%) of purified product 3.5, which displayed the same ¹H NMR and mass spectral data as the identical compound from above.

EXPERIMENT 4

2'-(*tert*-Butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3, 25.0 mg, 0.026 mmol) was dissolved in 500 µl anhydrous CH₂Cl₂ at room temperature. To the stirred solution was added 36.2 µl (0.26 mmol, 10.0 equiv.) anhydrous triethylamine, followed by 20.7 µl (0.26 mmol, 10.0 equiv.) of chloroacetyl chloride. The reaction was allowed to proceed at room temperature under argon for 14 hr, at which time the reaction was checked by TLC (EtOAc/hexanes, 1:3), which showed the formation of a more polar product. After dilution of the reaction with CH₂Cl₂, the mixture was treated with 1 N HCl, then was worked up by the standard procedure. After removal of the solvent by rotary evaporator, the crude residue was subjected to PTLC (EtOAc/hexanes, 1:3) for purification, affording 10.3 mg (39.7%) of purified product 3.5, which displayed the same ¹H NMR and mass spectral data as the identical compound from above.

EXPERIMENT 5

2'-(*tert*-Butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3, 25.0 mg, 0.026 mmol) was dissolved in 500 µl anhydrous CH₂Cl₂ at room temperature. To the stirred solution was added 31.8 mg (0.26 mmol, 10.0 equiv.) of DMAP, followed by 44.4 mg (0.26 mmol, 10.0 equiv.) of chloroacetic anhydride. The reaction was allowed to proceed at room temperature under argon for 24 hr, at which time the reaction was checked by TLC (EtOAc/hexanes, 1:3), which showed the formation of a more polar product. After dilution of the reaction with CH₂Cl₂, the mixture was treated with 1 N HCl, then was worked up by the standard procedure. After removal of the solvent

by rotary evaporator, the crude residue was subjected to PTLC (EtOAc/hexanes, 1:3) for purification, affording 7.0 mg (27.0%) of the purified product 3.5, which displayed the same ^1H NMR and mass spectral data as the identical compound from above.

TREATMENT OF COMPOUND 3.5 WITH SODIUM AZIDE

Compound 3.5 (2.5 mg, 0.0025 mmol) was dissolved with stirring in 1.0 ml dry, distilled THF. To the stirred solution was added NaN_3 (2.0 mg, 0.0308 mmol, 12.3 equivs.), followed by a catalytic amount of 18-crown-6 ether. The reaction was allowed to proceed at room temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:3). After 6 hr, no reaction was apparent so the reaction was warmed to 40 °C. After 4 hr at this temperature, no reaction was apparent and the reaction was heated further to 60 °C and allowed to continue stirring for another 12 hr. Thin layer chromatographic analysis at this time showed no appreciable change in the starting material. The crude reaction mixture was then worked up by standard procedure and the unreacted starting material was recovered by PTLC (EtOAc/hexanes, 1:3). Afforded was 2.1 mg (84% recovery) of material which, upon analysis by ^1H NMR, was confirmed to be compound 3.5.

TREATMENT OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-2-DEBENZOYL-1,2-CARBONATO-4-DEACETYL-7-(TRIETHYLSILYL)TAXOL (3.3) WITH ALUMINUM TRICHLORIDE

2'-(*tert*-Butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3, 15.0 mg, 0.0156 mmol) was dissolved in 1.0 ml anhydrous CH_2Cl_2 at room temperature. To the stirred solution was added 41.6 mg (0.468 mmol, 30.0 equiv.) of AlCl_3 , and the reaction was allowed to proceed at this temperature under argon for 30 min, at which time TLC analysis (EtOAc/hexanes, 1:2) showed the starting material to be completely converted to baseline material ($R_f < 0.05$). This material, lacking the

characteristic taxol/derivative color upon spraying with vanillin/sulfuric acid solution, was presumed to consist of decomposition products and therefore no further attempt was made at isolation.

TREATMENT OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-1,2-CARBONATO-4-DEACETYL-2-DEBENZOYL-7-(TRIETHYLSILYL)TAXOL (3.3) WITH ZINC BROMIDE

2'-(*tert*-Butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3, 20.0 mg, 0.0208 mmol) was dissolved in 100 μ l anhydrous CH_2Cl_2 at room temperature. To the stirred solution was added 46.8 mg (0.208 mmol, 10.0 equiv.) of ZnBr_2 , and the reaction was allowed to proceed at this temperature under argon while being monitored by TLC analysis (EtOAc/hexanes, 1:2). Even after 8 hr, no change in the starting material was evident, and the reaction mixture was worked up by the standard procedure then purified by PTLC (EtOAc/hexanes, 1:2) to recover 18.2 mg (91.0%) of unreacted starting material 3.3, as confirmed by ^1H NMR.

TREATMENT OF 2'-(*tert*-BUTYLDIMETHYLSILYL)-2-DEBENZOYL-1,2-CARBONATO-4-DEACETYL-7-(TRIETHYLSILYL)TAXOL (3.3) WITH IODOTRIMETHYLSILANE

2'-(*tert*-Butyldimethylsilyl)-2-debenzoyl-1,2-carbonato-4-deacetyl-7-(triethylsilyl)taxol (3.3, 15.6 mg, 0.0162 mmol) was dissolved in 100 μ l anhydrous CH_2Cl_2 at $-20\text{ }^\circ\text{C}$. To the stirred solution was added 4.6 μ l (0.0324 mmol, 2.0 equiv.) of TMSI, and the reaction was allowed to proceed at this temperature under argon while being monitored by TLC analysis (EtOAc/hexanes, 1:2). After 10 min, the reaction appeared to be complete based on disappearance of starting material (R_f 0.57) and formation of a predominant, more polar product (R_f 0.28). The reaction was diluted with CH_2Cl_2 and treated with distilled water, then worked up by the standard procedure. The solvent was removed *in vacuo* and the product was purified by PTLC (EtOAc/hexanes, 1:2), affording 10.1 mg (57.2%) of a

material which was identified as the iodinated oxetane ring-opened product **3.8**: FABMS m/z (rel. int.) $[M+Na-H]^+$ 1112 (21), $[MH]^+$ 1091 (47), $[M]^+$ 1090 (63), 1048 (16), 998 (18), 984 (23), 972 (18), 964 (33), 962 (38), 944 (40), 932 (33), 629 (41), 587 (83), 514 (100), 400 (13), 354 (11), 197 (7), 177 (12), 149 (13), 133 (32), 105 (100), 91 (45), 73 (95); ^1H NMR ($CDCl_3$) δ 7.72 (d, $J = 6.9$ Hz, 2H), 7.26 - 7.53 (m, 8H), 7.17 (d, $J = 8.4$ Hz, NH), 6.57 (s, 1H), 6.10 (m, 1H), 5.93 (d, $J = 8.7$ Hz, 1H), 5.17 (t, 1H), 4.63 (br. s, 1H), 4.54 (dd, $J = 2.0, 10.8$ Hz, 1H), 4.43 (s, OH), 4.26 (d, $J = 5.1$ Hz, 1H), 3.78 (ABq, $J = 13.5$ Hz, $\Delta\nu_{AB} = 77$, 2H), 3.65 (d, $J = 5.1$ Hz, 1H), 3.07 (dd, $J = 6.1, 17.2$ Hz, 1H), 2.1 - 2.6 (m, 3H), 2.49 (s, 3H), 2.18 (s, 3H), 1.25 (s, 3H), 1.20 (s, 3H), 1.13 (d, 3H), 0.94 (t, 9H), 0.79 (s, 3H), 0.78 (s, 6H), 0.59 (q, 6H), -0.14 (s, 3H), -0.34 (s, 3H).

ACETYLATION OF 4,10-BIS(DEACETYL)-7-(TRIETHYLSILYL)

BACCATIN III (2.7)

4,10-Bis(deacetyl)-7-(triethylsilyl)baccatin III (**2.7**, 20.6 mg, 0.0334 mmol) was dissolved with stirring in 1.2 ml anhydrous CH_2Cl_2 . To the solution were added DCC (34.5 mg, 0.167 mmol, 5.0 equiv.) and acetic anhydride (15.8 μ l, 0.167 mmol, 5.0 equiv.), followed by a catalytic amount of 4-pyrrolidinopyridine. The reaction was allowed to proceed at room temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:1). After 2.5 hr, the reaction appeared to be complete based on conversion of the starting material to a less polar product. The reaction was quenched by the addition of 0.5N HCl. After dilution with CH_2Cl_2 , the reaction mixture was worked up by standard procedure. The crude material was subjected to PTLC (EtOAc/hexanes, 45:55), affording 16.6 mg (71.0%) of 13-acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (**3.10**): FABMS m/z (rel. int.) $[MH-H_2O]^+$ 683 (10), 665 (5), $[MH-HOAc]^+$ 641 (25), 623 (25), 581 (30), 501 (40), 371 (60), 235 (100); ^1H NMR ($CDCl_3$) δ 8.01 (dd, $J = 1.3, 8.3$ Hz, 2H), 7.44 - 7.63 (m, 3H), 6.45 (s, 1H), 5.93 (m, 1H), 5.66 (d, $J = 5.9$ Hz, 1H), 4.81 (dd, $J = 3.5, 9.7$ Hz, 1H), 4.23 (ABq, $J = 8.2$ Hz, $\Delta\nu_{AB} = 53$, 2H), 4.07 (dd, $J = 6.2, 11.5$ Hz, 1H), 3.36 (d, $J = 6.3$ Hz, 1H), 2.41 - 2.63 (m, 2H), 2.22 (s, 3H), 2.20 (s, 3H),

2.07 (s, 3H), 1.92 - 2.18 (m, 2H), 1.61 (s, 3H), 1.23 (s, 3H), 1.10 (s, 3H), 0.91 (t, 9H), 0.58 (q, 6H).

ACETYLATION OF 13-ACETYL-4-DEACETYL-7-(TRIETHYLSILYL) BACCATIN III (3.10)

13-Acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (3.10, 8.4 mg, 0.0120 mmol) was dissolved with stirring in 1.0 ml anhydrous CH_3CN . To the solution were added DCC (74.3 mg, 0.360 mmol, 30.0 equiv.) and acetic anhydride (34 μl , 0.360 mmol, 30.0 equiv.), followed by a catalytic amount of 4-pyrrolidinopyridine. The reaction was heated to 65 $^\circ\text{C}$ and allowed to proceed under argon while being monitored by TLC (EtOAc/hexanes, 3:2). After 30 min, the starting material appeared to be converted to a slightly less polar product. The reaction was quenched by the addition of 0.5N HCl and after dilution with CH_2Cl_2 , the reaction mixture was worked up by standard procedure. The crude material was subjected to PTLC (EtOAc/hexanes, 1:1), affording 2.1 mg (23.6%) of 13-acetyl-7-(triethylsilyl)baccatin III (3.11) as a colorless, amorphous solid identical to the same material reported by Samaranayake^{115,138}: ^1H NMR (CDCl_3) δ 8.08 (br. d, J = 6.1Hz, 2H), 7.45 - 7.64 (m, 3H), 6.46 (s, 1H), 6.15 (t, J = 8.2Hz, 1H), 5.68 (d, J = 7.0Hz, 1H), 4.96 (br. d, J = 7.7Hz, 1H), 4.48 (dd, J = 6.7, 10.5Hz, 1H), 4.23 (ABq, J = 8.4Hz, $\Delta\nu_{\text{AB}}$ = 41, 2H), 3.84 (d, J = 7.0Hz, 1H), 2.41 - 2.55 (m, 2H), 2.34 (s, 3H), 2.21 (s, 3H), 2.18 (s, 3H), 2.04 (d, J = 1.4Hz, 3H), 1.84 - 1.99 (m, 2H), 1.69 (s, 3H), 1.23 (s, 3H), 1.18 (s, 3H), 0.93 (t, 9H), 0.60 (q, 6H).

MODEL OXIDATION OF 7-(TRIETHYLSILYL)BACCATIN III (2.5)¹⁵⁰

7-(Triethylsilyl)baccatin III (2.5, 88.6 mg, 0.127 mmol) was dissolved in 3.0 ml anhydrous CH_2Cl_2 . To the stirring solution was added 550 mg (6.33 mmol, 50.0 equiv.) of MnO_2 , and the reaction was allowed to proceed at room temperature under argon for 10 min. At this time TLC analysis (EtOAc/hexanes, 1:2) showed the complete conversion of

the starting material (R_f 0.06) to a less polar product at R_f 0.26. The reaction mixture was filtered through Celite and the solvent was removed by rotary evaporation. The crude material was then purified by PTLC (EtOAc/hexanes, 1:2), giving 88.1 mg (91.5%) of 7-(triethylsilyl)-13-oxobaccatin III (3.14) as a white, amorphous solid: ^1H NMR (CDCl_3) δ 8.08 (dd, J = 1.1, 8.4 Hz, 2H), 7.46 - 7.66 (m, 3H), 6.59 (s, 1H), 5.70 (d, J = 6.8 Hz, 1H), 4.93 (br. d, J = 9.3 Hz, 1H), 4.49 (dd, J = 6.8, 10.4 Hz, 1H), 4.23 (ABq, J = 8.4 Hz, $\Delta\nu_{\text{AB}}$ = 56, 2H), 3.92 (d, J = 6.7 Hz, 1H), 2.81 (ABq, J = 18.7 Hz, $\Delta\nu_{\text{AB}}$ = 82, 2H), 2.54 (m, 1H), 2.23 (s, 3H), 2.19 (s, 3H), 1.88 (m, 1H), 1.67 (s, 3H), 1.27 (s, 3H), 1.25 (s, 3H), 1.19 (s, 3H), 0.92 (t, 9H), 0.59 (q, 6H).

MODEL REDUCTION OF 7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.14)

7-(Triethylsilyl)-13-oxobaccatin III (3.14, 256.9 mg, 0.368 mmol) was dissolved in 10.0 ml THF-methanol (9:1), and to the stirred solution was added NaBH_4 (69.6 mg, 1.84 mmol, 5.0 equiv.). The reaction was allowed to proceed at room temperature while being monitored by TLC (EtOAc/hexanes, 1:1), and after 1 hr the reaction appeared to be complete based on disappearance of starting material and appearance of two products in an approximate ratio of 9:1. After workup by standard procedure, the product mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification. The major product was isolated to give 200.7 mg (77.9%) of a white, amorphous powder, which was identified as 7-(triethylsilyl)baccatin III (2.5) by comparison to known ^1H NMR and mass spectral data. The minor product was isolated to give 17.3 mg (6.7%) of a white, amorphous powder, which was identified as 7-(triethylsilyl)-13-*epi*-baccatin III (3.15) by comparison to known ^1H NMR and mass spectral data.

10-DEACETYL-7-(TRIETHYLSILYL)BACCATIN III (2.6)

10-Deacetylbaccatin III (1.14, 1.02 g, 1.882 mmol) was dissolved in 35 ml anhydrous pyridine with stirring. After 5 min, chlorotriethylsilane (3.16 ml, 18.82 mmol, 10.0 equiv.) was added and the reaction was allowed to proceed, at room temperature under argon, for

24 hr. At this time the reaction was stopped by the addition of 50 ml distilled water, followed by 35 ml ethyl acetate. The ethyl acetate layer was separated and the aqueous layer washed with additional ethyl acetate (2 × 10 ml). The combined organic layers were then washed with aqueous CuSO₄ solution (5 × 50 ml) to remove the pyridine, followed by brine wash (3 × 10 ml). After drying with MgSO₄, the solution was filtered and evaporated to a pale yellow residue, which was subjected to column chromatography (EtOAc/hexanes, 1:1), affording 1.02 g (82.1%) of pure 10-deacetyl-7-(triethylsilyl)baccatin III (2.6), indistinguishable from the same compound prepared previously by a different methodology: CIMS *m/z* (rel. int.) [MH]⁺ 659 (18), 641 (17), 623 (12), 581 (10), 563 (15), 551 (1.8), 537 (5.9), 519 (10), 501 (4.1), 459 (36), 441 (17), 429 (7.0), 413 (6.0), 401 (8.0), 387 (10), 369 (6.0), 345 (10), 327 (27), 309 (17), 299 (17); ¹H NMR (CDCl₃) δ 8.11 (dd, *J* = 1.4, 8.3Hz, 2H), 7.45 - 7.63 (m, 3H), 5.60 (d, *J* = 7.0Hz, 1H), 5.17 (d, *J* = 1.9Hz, 1H), 4.96 (br. d, *J* = 8.2Hz, 1H), 4.88 (m, 1H), 4.41 (dd, *J* = 6.6, 10.6Hz, 1H), 4.25 (d, *J* = 2.0Hz, OH), 4.24 (ABq, *J* = 8.3Hz, Δ*v*_{AB} = 41, 2H), 3.95 (d, *J* = 7.0Hz, 1H), 2.42 - 2.56 (m, 1H), 2.29 (s, 3H), 2.09 (s, 3H), 1.84 - 2.07 (m, 3H), 1.74 (s, 3H), 1.25 (s, 3H), 1.09 (s, 3H), 0.94 (t, 9H), 0.56 (q, 6H).

4,10-Bis(DEACETYL)-7-(TRIETHYLSILYL)BACCATIN III (2.7) FROM 10-DEACETYL-7-(TRIETHYLSILYL)BACCATIN III (2.6)

10-Deacetyl-7-(triethylsilyl)baccatin III (2.6, 192 mg, 0.292 mmol) was dissolved in 3.5 ml dry THF with stirring. After 5 min, 1.46 ml (1.46 mmol, 5.0 equiv.) of a 1.0*M* solution of potassium *tert*-butoxide in THF was added, and the reaction was allowed to proceed for 15 minutes at room temperature under argon. The reaction was then neutralized with 1*N* HCl in THF. After evaporation of the solvents, the crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1), affording 147.1 mg (81.8%) of 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7), identical with the same compound described previously.

OXIDATION OF 4,10-BIS(DEACETYL)-7-(TRIETHYLSILYL)

BACCATIN III (2.7)

4,10-Bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7, 107.6 mg, 0.175 mmol) was dissolved in 6.0 ml CH_2Cl_2 , and to the stirring solution was added MnO_2 (304 mg, 3.49 mmol, 19.9 equiv.). The reaction was allowed to proceed at room temperature under argon for 10 min, at which time a TLC (EtOAc/hexanes, 1:1) showed the reaction to be complete. After dilution with CH_2Cl_2 , the reaction mixture was filtered through Celite, and the solvent was removed *in vacuo*. The crude residue was purified by PTLC (EtOAc/hexanes, 2:3), affording 91.4 mg (85.1%) of pure 4,10-bis(deacetyl)-7-(triethylsilyl)-13-oxobaccatin III (3.12) as a colorless, glassy solid: ^1H NMR (CDCl_3) δ 8.00 (dd, $J = 1.3, 8.4\text{Hz}$, 2H), 7.44 - 7.64 (m, 3H), 5.67 (d, $J = 6.2\text{Hz}$, 1H), 5.29 (d, $J = 2.5\text{Hz}$, 1H), 4.73 (dd, $J = 3.8, 9.6\text{Hz}$, 1H), 4.33 (d, $J = 2.5\text{Hz}$, 1H), 4.24 (ABq, $J = 8.5\text{Hz}$, $\Delta\nu_{\text{AB}} = 82$, 2H), 3.93 (dd, $J = 6.1, 11.6\text{Hz}$, 1H), 3.34 (d, $J = 6.2\text{Hz}$, 1H), 3.07 (ABq, $J = 19.4\text{Hz}$, $\Delta\nu_{\text{AB}} = 233$, 2H), 2.75 (s, OH), 2.33 - 2.44 (m, 1H), 2.11 (s, 3H), 1.94 - 2.05 (m, 1H), 1.66 (s, 3H), 1.25 (s, 3H), 1.17 (s, 3H), 0.92 (t, 9H), 0.55 (q, 6H).

MILD ACETYLATION OF 4,10-BIS(DEACETYL)-7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.12)

4,10-Bis(deacetyl)-7-(triethylsilyl)-13-oxobaccatin III (3.12, 145 mg, 0.236 mmol) was dissolved in 2.5 ml anhydrous CH_2Cl_2 . The solution was then added by syringe to a stirred mixture of acetic anhydride (51.2 μl , 0.543 mmol, 2.3 equiv.) and DCC (112.1 mg, 0.543 mmol, 2.3 equiv.) in 2.5 ml anhydrous CH_2Cl_2 at room temperature. To the mixture was added a catalytic amount of 4-pyrrolidinopyridine, and the reaction was allowed to proceed under argon for 8 hr, at which time a TLC (EtOAc/hexanes, 1:1) showed the starting material to be converted to a less polar product. After work up by standard procedure, the solvents were removed *in vacuo*. The crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification, and 125.4 mg (81.0%) of the product, 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13) was thus obtained: FABMS m/z (rel int.)

[M+Na+H]⁺ 680 (3.4), [MH]⁺ 657 (4.2), 639 (3.9), 623 (1.8), 597 (8.1), 579 (6.6), 517 (18), 475 (14), 445 (18), 429 (10), 403 (15), 385 (14), 361 (12), 193 (20), 165 (19), 145 (46), 115 (100); ¹H NMR (CDCl₃) δ 8.00 (dd, *J* = 1.3, 8.5 Hz, 2H), 7.37 - 7.56 (m, 3H), 6.52 (s, 1H), 5.66 (d, *J* = 6.1 Hz, 1H), 4.70 (dd, *J* = 3.7, 9.6 Hz, 1H), 4.21 (ABq, *J* = 8.4 Hz, Δ*v*_{AB} = 64, 2H), 4.03 (dd, *J* = 6.1, 11.6 Hz, 1H), 3.28 (d, *J* = 6.1 Hz, 1H), 3.10 (ABq, *J* = 19.5 Hz, Δ*v*_{AB} = 269, 2H), 2.33 - 2.48 (m, 1H), 2.20 (s, 3H), 2.14 (s, 3H), 1.86 - 2.02 (m, 1H), 1.57 (s, 3H), 1.25 (s, 3H), 1.18 (s, 3H), 0.90 (t, 9H), 0.54 (q, 6H).

ACETYLATION OF 4-DEACETYL-7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.13)

4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13, 10.0 mg, 0.0152 mmol) was dissolved in 500 μl anhydrous acetonitrile. The solution was then added by syringe to a stirred mixture of acetic anhydride (43.1 μl, 0.457 mmol, 30.1 equiv.) and DCC (94.4 mg, 0.457 mmol, 30.1 equiv.) in 500 μl anhydrous acetonitrile at room temperature. To the mixture was added a catalytic amount of 4-pyrrolidinopyridine, and the reaction was heated to 60 °C, and allowed to proceed under argon for 4 hr, at which time a TLC (EtOAc/hexanes, 1:1) showed the starting material to be converted to a slightly less polar mixture of products. After cooling to room temperature and dilution with CH₂Cl₂, the reaction mixture was worked up by standard procedure and the solvents were removed *in vacuo*. The crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification, affording two products. The first product, *R_f* 0.69, the more prevalent of the two, was found by its ¹H NMR and mass spectrometric data to be 7-(triethylsilyl)-13-oxobaccatin III (3.14, 7.6 mg, 71.6%), identical with the same product previously described. The second product, *R_f* 0.60, was later identified by ¹H NMR spectroscopy as 13-acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47, 2.1 mg, 19.8%): ¹H NMR (CDCl₃) δ 8.01 (br. d, *J* = 7.1 Hz, 2H), 7.37 - 7.59 (m, 3H), 6.34 (s, 1H), 5.82 (d, *J* = 6.9 Hz, 1H), 5.42 (s, 1H), 4.83 (dd, *J* = 2.2, 6.5 Hz, 1H), 4.23 (s, 2H), 4.01 (dd, *J* = 6.0, 12.2 Hz, 1H), 3.75 (s, OH), 3.18 (d, *J* = 6.9 Hz, 1H), 2.35 - 2.50 (m, 1H), 2.32 (s, 3H),

2.21 (s, 3H), 1.97 (s, 3H), 1.80 - 2.02 (m, 1H), 1.68 (s, 3H), 1.37 (s, 3H), 1.17 (s, 3H), 0.91 (t, 9H), 0.53 (q, 6H).

REACTION OF 4-DEACETYL-7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.13) WITH PROPIONIC ANHYDRIDE

4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13, 15.0 mg, 0.0229 mmol) was dissolved in 250 μ l anhydrous acetonitrile. The solution was then added by syringe to a stirred mixture of propionic anhydride (88.0 μ l, 0.686 mmol, 30.0 equiv.) and DCC (141.5 mg, 0.686 mmol, 30.0 equiv.) in 250 μ l anhydrous acetonitrile at room temperature. To the mixture was added a catalytic amount of 4-pyrrolidinopyridine, and the reaction was heated to 65 °C, and allowed to proceed under argon for 25 min, at which time a TLC (EtOAc/hexanes, 1:1) showed the starting material to be converted to two less polar products, in an approximate ratio of 3:1. After cooling to room temperature, diluting with CH₂Cl₂ and adding 1.0 ml 1N HCl, the reaction mixture was worked up by standard procedure and the solvents were removed *in vacuo*. The crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification, affording two products. The first product, *R_f* 0.73, the more prevalent of the two, was identified by ¹H NMR and mass spectrometry as 4-deacetyl-7-(triethylsilyl)-13-oxo-4-propionylbaccatin III (3.16, 9.7 mg, 59.5%): FABMS *m/z* (rel int.) [M+Na]⁺ 735 (18), [MH]⁺ 713 (7.5), 670 (7.0), 655 (12), 457 (1.4), 427 (1.7); ¹H NMR (CDCl₃) δ 8.08 (d, *J* = 7.0Hz, 2H), 7.43 - 7.61 (m, 3H), 6.60 (s, 1H), 5.73 (d, *J* = 6.6Hz, 1H), 4.92 (br. d, *J* = 7.9Hz, 1H), 4.58 (dd, *J* = 6.7, 10.4Hz, 1H), 4.28 (ABq, *J* = 8.4Hz, $\Delta\nu_{AB}$ = 48, 2H), 4.02 (d, *J* = 6.5Hz, 1H), 2.72 (ABq, *J* = 19.8Hz, $\Delta\nu_{AB}$ = 95, 2H), 2.89 - 3.08 (m, 1H), 2.41 - 2.78 (m, 2H), 2.22 (d, 3H), 1.94 (s, 3H), 1.9 - 2.1 (m, 1H), 1.79 (s, 3H), 1.71 (s, 3H), 1.57 (s, 3H), 1.12 - 1.32 (br. m, 3H), 0.93 (t, 9H), 0.59 (q, 6H). The second product, *R_f* 0.62, was identified by ¹H NMR and mass spectrometry as 4-deacetyl-13,14-dehydro-7-(triethylsilyl)-13-propionylbaccatin III (3.17, 2.8 mg, 17.2%): FABMS *m/z* (rel. int.) [M+Na]⁺ 735 (8.5),

[MH]⁺ 713 (6.0), 669 (8.2), 655 (14); ¹H NMR (CDCl₃) δ 8.03 (dd, *J* = 1.5, 8.5Hz, 2H), 7.42 - 7.61 (m, 3H), 6.55 (s, 1H), 5.70 (d, *J* = 5.8Hz, 1H), 5.30 (s, 1H), 4.75 (dd, *J* = 3.7, 9.6Hz, 1H), 4.24 (ABq, *J* = 8.4Hz, Δ*v*_{AB} = 62, 2H), 4.06 (dd, *J* = 6.1, 11.5Hz, 1H), 3.31 (d, *J* = 6.1Hz, 1H), 2.37 - 2.52 (m, 3H), 2.23 (s, 3H), 2.17 (s, 3H), 1.9 - 2.1 (m, 1H), 1.60 (s, 3H), 1.28 (s, 3H), 1.25 (s, 3H), 1.08 - 1.21 (m, 3H), 0.91 (t, 9H), 0.56 (q, 6H).

REACTION OF 4-DEACETYL-7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.13) WITH CHLOROACETIC ANHYDRIDE

4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13, 7.5 mg, 0.0114 mmol) was dissolved in 250 μl anhydrous acetonitrile. The solution was then added by syringe to a stirred mixture of chloroacetic anhydride (58.6 mg, 0.343 mmol, 30.0 equiv.) and DCC (70.8 mg, 0.343 mmol, 30.0 equiv.) in 250 μl anhydrous acetonitrile at room temperature. To the mixture was added a catalytic amount of 4-pyrrolidinopyridine, and the reaction was heated to 65 °C, and allowed to proceed under argon for 22 hr, at which time a TLC (EtOAc/hexanes, 1:1) showed the starting material to be converted to two less polar products, in an approximate ratio of 7:1. After cooling to room temperature, diluting with CH₂Cl₂ and adding 1.0 ml 1N HCl, the reaction mixture was worked up by standard procedure and the solvents were removed *in vacuo*. The crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification, affording two products. The first product, *R_f* 0.70, the more prevalent of the two, was identified by ¹H NMR and mass spectrometry as 4-deacetyl-4-chloroacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.18, 4.7 mg, 56.3%): FABMS *m/z* (rel int.) [M+Na]⁺ 755 (12), [MH]⁺ 733 (9.3), 697 (5.4), 690 (6.1), 654 (11); ¹H NMR (CDCl₃) δ 8.06 (d, *J* = 7.8Hz, 2H), 7.45 - 7.62 (m, 3H), 6.75 (s, 1H), 5.66 (d, *J* = 5.2Hz, 1H), 4.51 (dd, *J* = 4.4, 10.7Hz, 1H), 4.41 (t, *J* = 3.0Hz, 1H), 4.24 (ABq, *J* = 12.0Hz, Δ*v*_{AB} = 41, 2H), 3.99 (d, *J* = 5.2Hz, 1H), 3.71 (ABq, *J* = 14.9Hz, Δ*v*_{AB} = 27, 2H), 3.24 (s, OH), 3.22 (ABq, *J* = 19.6Hz, Δ*v*_{AB} = 300, 2H), 2.28 (s, 3H), 2.24 (s, 3H), 1.8 - 2.2 (m, 2H), 2.16 (s, 3H), 1.30 (s, 3H), 1.23 (d, 3H), 0.92 (t, 9H),

0.58 (q, 6H). The second product, R_f 0.61, was identified by ^1H NMR and mass spectrometry as 13-chloroacetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.19, 0.7 mg, 8.4%): FABMS m/z (rel int.) $[\text{M}+\text{Na}]^+$ 755 (3.4), $[\text{MH}]^+$ 733 (4.6), 697 (2.9), 689 (3.1), 683 (6.2), 653 (9.0); ^1H NMR (CDCl_3) δ 8.05 (br. d, $J = 7.2\text{Hz}$, 2H), 7.44 - 7.64 (m, 3H), 6.68 (s, 1H), 5.65 (d, $J = 5.4\text{Hz}$, 1H), 5.29 (s, 1H), 4.52 (br. d, $J = 8\text{Hz}$, 1H), 4.22 (ABq, $J = 10\text{Hz}$, $\Delta\nu_{\text{AB}} = 44$, 2H), 4.08 (m, 1H), 3.63 (m, 2H), 3.28 (d, $J = 5.4\text{Hz}$, 1H), 2.3 - 2.4 (m, 1H), 2.26 (s, 3H), 2.20 (s, 3H), 1.8 - 2.1 (m, 1H), 1.77 (s, 3H), 1.27 (s, 3H), 1.21 (s, 3H), 0.93 (t, 9H), 0.59 (q, 6H).

REACTION OF 4-DEACETYL-7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.13) WITH TRICHLOROACETIC ANHYDRIDE

4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13, 7.5 mg, 0.0114 mmol) was dissolved in 250 μl anhydrous acetonitrile. The solution was then added by syringe to a stirred mixture of trichloroacetic anhydride (62.7 μl , 0.343 mmol, 30.0 equiv.) and DCC (70.8 mg, 0.343 mmol, 30.0 equiv.) in 250 μl anhydrous acetonitrile at room temperature. To the mixture was added a catalytic amount of 4-pyrrolidinopyridine, and the reaction was heated to 65 $^\circ\text{C}$, and allowed to proceed under argon for 16 hr, at which time a TLC (EtOAc/hexanes, 1:1) showed some unreacted starting material as well as two less polar products, in an approximate ratio of 4:1. After cooling to room temperature, diluting with CH_2Cl_2 and adding 1.0 ml 1N HCl, the reaction mixture was worked up by standard procedure and the solvents were removed *in vacuo*. The crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification, affording two products. The first product, R_f 0.61, the more prevalent of the two, was identified by ^1H NMR and mass spectrometry as 4-deacetyl-4-trichloroacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.20, 3.6 mg, 39.4%): FABMS m/z (rel int.) $[\text{MH}]^+$ 803 (7.4), 766 (3.1), 759 (3.7), 731 (4.6), 724 (8.8); ^1H NMR (CDCl_3) δ 8.02 (d, $J = 8.1\text{Hz}$, 2H), 7.43 - 7.68 (m, 3H), 6.74 (s, 1H), 5.76 (d, $J = 5.4\text{Hz}$, 1H), 4.56 (dd, $J = 5.1, 12.0\text{Hz}$, 1H), 4.27 (d, $J = 5.4\text{Hz}$, 1H), 4.21 (t,

$J = 2.7\text{Hz}$, 1H), 4.13 (ABq, $J = 10.5\text{Hz}$, $\Delta\nu_{\text{AB}} = 103$, 2H), 3.20 (ABq, $J = 21.6\text{Hz}$, $\Delta\nu_{\text{AB}} = 238$, 2H), 2.63 (s, OH), 2.29 (d, 3H), 2.19 (s, 3H), 1.97 - 2.35 (m, 2H), 1.73 (s, 3H), 1.27 (s, 3H), 1.23 (d, 3H), 0.92 (t, 9H), 0.60 (q, 6H). The second product, R_f 0.55, was identified by ^1H NMR and mass spectrometry as 13-trichloroacetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.21, 0.8 mg, 8.8%): FABMS m/z (rel int.) $[\text{M}+\text{Na}]^+$ 825 (3.1), $[\text{MH}]^+$ 803 (2.3), 767 (4.4), 759 (6.1), 731 (1.2), 724 (8.1); ^1H NMR (CDCl_3) δ 8.04 (br. d, $J = 7.9\text{Hz}$, 2H), 7.44 - 7.66 (m, 3H), 6.73 (s, 1H), 5.72 (d, $J = 5.3\text{Hz}$, 1H), 5.31 (s, 1H), 4.61 (br. d, $J = 9\text{Hz}$, 1H), 4.16 (s, 2H), 3.42 (d, $J = 5.4\text{Hz}$, 1H), 2.3 - 2.5 (m, 1H), 2.27 (s, 3H), 2.18 (s, 3H), 1.9 - 2.2 (m, 1H), 1.68 (s, 3H), 1.28 (s, 3H), 1.22 (s, 3H), 0.91 (t, 9H), 0.58 (q, 6H).

REACTION OF 4-DEACETYL-7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.13) WITH BENZOIC ANHYDRIDE

4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13, 10.0 mg, 0.0152 mmol) was dissolved in 250 μl anhydrous acetonitrile. The solution was then added by syringe to a stirred mixture of benzoic anhydride (103.5 mg, 0.457 mmol, 30.0 equiv.) and DCC (94.3 mg, 0.457 mmol, 30.0 equiv.) in 250 ml anhydrous acetonitrile at room temperature. To the mixture was added a catalytic amount of 4-pyrrolidinopyridine, and the reaction was heated to 65 $^\circ\text{C}$, and allowed to proceed under argon for 12 hr, at which time a TLC (EtOAc/hexanes, 1:1) showed the starting material to be converted to two less polar products, in an approximate ratio of 2:1. After cooling to room temperature, diluting with CH_2Cl_2 and adding 1.0 ml 1N HCl, the reaction mixture was worked up by standard procedure and the solvents were removed *in vacuo*. The crude reaction mixture was subjected to PTL (EtOAc/hexanes, 1:1) for purification, affording two products. The first product, R_f 0.62, the more prevalent of the two, was identified by ^1H NMR and mass spectrometry as 4-deacetyl-4-benzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.22, 6.1 mg, 52.8%): FABMS m/z (rel int.) $[\text{M}+\text{Na}]^+$ 783 (1.2), $[\text{MH}]^+$ 761 (1.7), 746 (3.2), 718 (4.0),

703 (3.6), 656 (6.7); ^1H NMR (CDCl_3) δ 8.22 (m, 4H), 7.30 - 7.79 (m, 6H), 6.53 (s, 1H), 5.99 (d, $J = 5.4\text{Hz}$, 1H), 5.10 (br. d, $J = 6.4\text{Hz}$, 1H), 4.68 (dd, $J = 3.9, 8.7\text{Hz}$, 1H), 4.43 (ABq, $J = 8.0\text{Hz}$, $\Delta\nu_{\text{AB}} = 25$, 2H), 4.07 (d, $J = 5.4\text{Hz}$, 1H), 3.14 (ABq, $J = 19.5\text{Hz}$, $\Delta\nu_{\text{AB}} = 273$, 2H), 2.58 - 2.73 (m, 1H), 2.21 (s, 3H), 2.08 (s, 3H), 1.9 - 2.1 (m, 1H), 1.81 (s, 3H), 1.28 (s, 3H), 1.24 (s, 3H), 0.94 (t, 9H), 0.62 (q, 6H). The second product, R_f 0.54, was identified by ^1H NMR and mass spectrometry as 13-benzoyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (**3.23**, 2.3 mg, 19.9%): FABMS m/z (rel int.) $[\text{MH}]^+$ 761 (3.4), 717 (5.3), 703 (4.8), 655 (7.9); ^1H NMR (CDCl_3) δ 8.20 (dd, $J = 1.5, 8.4\text{Hz}$, 2H), 8.08 (dd, $J = 1.5, 8.7\text{Hz}$, 2H), 7.43 - 7.73 (m, 6H), 6.39 (s, 1H), 5.89 (d, $J = 6.9\text{Hz}$, 1H), 5.55 (s, 1H), 4.92 (dd, $J = 3.2, 9.8\text{Hz}$, 1H), 4.27 (s, 2H), 4.05 (dd, $J = 6.3, 11.4\text{Hz}$, 1H), 4.02 (s, 1H), 3.30 (d, $J = 6.8\text{Hz}$, 1H), 2.41 - 2.54 (m, 1H), 2.21 (s, 3H), 2.02 (s, 3H), 1.88 - 2.01 (m, 1H), 1.70 (s, 3H), 1.45 (s, 3H), 1.22 (s, 3H), 0.89 (t, 9H), 0.55 (q, 6H).

OPTIMIZATION OF C-4 ACYLATION CONDITIONS

A series of reactions was conducted in order to systematically eliminate the formation of the enol ester baccatins **3.51**, **3.17**, **3.19**, **3.21** and **3.23** while maximizing yields of the corresponding 4-acyl-13-oxobaccatins **3.14**, **3.16**, **3.18**, **3.20** and **3.22**, as summarized in Table 14. All reactions were carried out using vacuum-dried 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (**3.13**) and 20.0 to 30.0 equivalents of acylating agent, 20.0 to 30.0 equivalents of DCC and a catalytic amount of 4-pyrrolidinopyridine, all of which had been either vacuum-dried or newly opened. The solvents used were all anhydrous, and the glassware and stir bars employed were oven- or flame-dried. As shown in Table 14, variation of reaction conditions (i.e., solvent, temperature, duration) resulted in a significant fluctuation of product profile. As noted in Table 14 it was found that the preferential formation of the desired 4-acyl-13-oxobaccatins could be optimized at lower temperatures in some cases or simply by the addition of 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III

(3.13) to a previously prepared anhydrous mixture of the acylating agent, DCC and the catalyst, 4-pyrrolidinopyridine.

FORMATION OF 4-DEACETYL-7-(TRIETHYLSILYL)-13-OXO-4-PROPIONYLBACCATIN III (3.16)

4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13, 50.0 mg, 0.0762 mmol) was dissolved in 500 μ l anhydrous CH_2Cl_2 . The solution was then added by syringe to a stirred mixture of propionic anhydride (293 μ l, 2.29 mmol, 30.0 equiv.) and DCC (472 mg, 2.29 mmol, 30.0 equiv.) in 500 μ l anhydrous CH_2Cl_2 at room temperature. To the mixture was added a catalytic amount of 4-pyrrolidinopyridine, and the reaction was allowed to proceed at room temperature under argon for 12 hr, at which time a TLC (EtOAc/hexanes, 1:1) showed the starting material to be converted to a less polar product. After dilution with CH_2Cl_2 and addition of 1.0 ml 1 N HCl, the reaction mixture was worked up by standard procedure and the solvents were removed *in vacuo*. The crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification, affording 45.8 mg (84.4%) of 4-deacetyl-7-(triethylsilyl)-13-oxo-4-propionylbaccatin III (3.16), which displayed ^1H NMR and mass spectral characteristics identical with the same compound described above.

FORMATION OF 4-DEACETYL-4-CHLOROACETYL-7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.18)

4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13, 50.0 mg, 0.0762 mmol) was dissolved in 500 μ l anhydrous acetonitrile. The solution was then added by syringe to a stirred mixture of chloroacetic anhydride (391 mg, 2.29 mmol, 30.0 equiv.) and DCC (472 mg, 2.29 mmol, 30.0 equiv.) in 500 μ l anhydrous acetonitrile at room temperature. To the mixture was added a catalytic amount of 4-pyrrolidinopyridine, and the reaction was heated to 60 $^\circ\text{C}$, and allowed to proceed under argon for 16 hr, at which time a TLC (EtOAc/hexanes, 1:1) showed the starting material to be converted to a less polar product. After dilution with CH_2Cl_2 and addition of 1.0 ml 1 N HCl, the reaction mixture was

worked up by standard procedure and the solvents were removed *in vacuo*. The crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification, affording 40.8 mg (73.1%) of 4-deacetyl-4-chloroacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.18), which displayed ¹H NMR and mass spectral characteristics identical with the same compound described above.

FORMATION OF 4-DEACETYL-4-TRICHLOROACETYL-7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.20)

4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13, 50.0 mg, 0.0762 mmol) was dissolved in 500 µl anhydrous acetonitrile. The solution was then added by syringe to a stirred mixture of trichloroacetic anhydride (418 µl, 2.29 mmol, 30.0 equiv.) and DCC (472 mg, 2.29 mmol, 30.0 equiv.) in 500 µl anhydrous acetonitrile at room temperature. To the mixture was added a catalytic amount of 4-pyrrolidinopyridine, and the reaction was heated to 60 °C, and allowed to proceed under argon for 16 hr, at which time a TLC (EtOAc/hexanes, 1:1) showed the starting material to be converted to a less polar product. After dilution with CH₂Cl₂ and addition of 1.0 ml 1N HCl, the reaction mixture was worked up by standard procedure and the solvents were removed *in vacuo*. The crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification, affording 37.7 mg (61.7%) of 4-deacetyl-4-trichloroacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.20), which displayed ¹H NMR and mass spectral characteristics identical with the same compound described above.

FORMATION OF 4-DEACETYL-4-BENZOYL-7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.22)

4-Deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13, 50.0 mg, 0.0762 mmol) was dissolved in 500 µl anhydrous acetonitrile. The solution was then added by syringe to a stirred mixture of benzoic anhydride (517 mg, 2.29 mmol, 30.0 equiv.) and DCC (472 mg,

2.29 mmol, 30.0 equiv.) in 500 μ l anhydrous acetonitrile at room temperature. To the mixture was added a catalytic amount of 4-pyrrolidinopyridine, and the reaction was heated to 60 °C, and allowed to proceed under argon for 18 hr, at which time a TLC (EtOAc/hexanes, 1:1) showed the starting material to be converted to a less polar product. After dilution with CH_2Cl_2 and addition of 1.0 ml 1N HCl, the reaction mixture was worked up by standard procedure and the solvents were removed *in vacuo*. The crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification, affording 42.0 mg (72.5%) of 4-deacetyl-4-benzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.22), which displayed ^1H NMR and mass spectral characteristics identical with the same compound described above.

SODIUM BOROHYDRIDE REDUCTION OF 4-DEACETYL-7-(TRIETHYLSILYL)-13-OXO-4-PROPIONYLBACCATIN III (3.16)

4-Deacetyl-7-(triethylsilyl)-13-oxo-4-propionylbaccatin III (3.16, 29.5 mg, 0.0414 mmol) was dissolved in 3.0 ml THF-methanol (9:1), and to the stirred solution was added NaBH_4 (7.8 mg, 0.206 mmol, 5.0 equiv.). The reaction was allowed to proceed at room temperature while being monitored by TLC (EtOAc/hexanes, 1:1), and after 30 min the reaction appeared to be complete, based on disappearance of the starting material and appearance of a predominant, more polar product. After workup by standard procedure, the crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification. The product was isolated to give 24.6 mg (83.2%) of a white, amorphous powder, which was identified by ^1H NMR and mass spectral data as 4-deacetyl-7-(triethylsilyl)-4-propionylbaccatin III (3.24): FABMS m/z (rel int.) $[\text{MH}]^+$ 715 (6.7), 700 (11), 672 (9.4), 657 (14); ^1H NMR (CDCl_3) δ 8.12 (dd, $J = 1.5, 8.5\text{Hz}$, 2H), 7.44 - 7.63 (m, 3H), 6.46 (s, 1H), 5.63 (d, $J = 7.0\text{Hz}$, 1H), 4.91 (br. d, $J = 9.6\text{Hz}$, 1H), 4.82 (m, 1H), 4.51 (dd, $J = 6.7, 10.4\text{Hz}$, 1H), 4.23 (ABq, $J = 8.3\text{Hz}$, $\Delta\nu_{\text{AB}} = 43$, 2H), 3.88 (d, $J = 7.1\text{Hz}$, 1H), 2.49 - 2.63 (m, 4H), 2.20 - 2.28 (m, 1H), 2.18 (s, 3H), 1.82 - 1.96 (m, 1H), 1.69 (s, 3H), 1.27 (s, 3H), 1.25 (m, 3H), 1.20 (s, 3H), 1.04 (s, 3H), 0.93 (t, 9H), 0.59 (q, 6H).

SODIUM BOROHYDRIDE REDUCTION OF 4-DEACETYL-4-CHLOROACETYL-7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.18)

4-Deacetyl-4-chloroacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.18, 15.9 mg, 0.0217 mmol) was dissolved in 3.0 ml THF-methanol (9:1), and to the stirred solution was added NaBH_4 (1.6 mg, 0.0423 mmol, 1.9 equiv.). The reaction was allowed to proceed at room temperature while being monitored by TLC (EtOAc/hexanes, 1:1), and after 30 min the reaction appeared to be complete, based on disappearance of the starting material and appearance of a single, more polar product. After workup by standard procedure, the crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification. The product was isolated to give 13.1 mg (82.2%) of a white, amorphous powder, which was identified by ^1H NMR and mass spectral data as 4-deacetyl-4-chloroacetyl-7-(triethylsilyl)baccatin III (3.25): FABMS m/z (rel int.) $[\text{MH}]^+$ 736 (1.7), 718 (2.2), 714 (2.3), 699 (5.6), 694 (4.3), 658 (5.2); ^1H NMR (CDCl_3) δ 8.04 (d, $J = 5.4\text{Hz}$, 2H), 7.42 - 7.63 (m, 3H), 6.59 (s, 1H), 5.46 (d, $J = 8.4\text{Hz}$, 1H), 4.67 (t, $J = 1.8\text{Hz}$, 1H), 4.62 (t, $J = 7.8\text{Hz}$, 1H), 4.48 (dd, $J = 5.4, 5.6\text{Hz}$, 1H), 4.40 (s, 2H), 3.94 (br. d, $J = 4.8\text{Hz}$, 1H), 3.93 (d, $J = 8.2\text{Hz}$, 1H), 2.82 (s, OH), 2.28 - 2.52 (m, 3H), 2.21 (s, 3H), 2.09 - 2.25 (m, 1H), 1.57 (s, 3H), 1.35 (s, 3H), 1.26 (s, 3H), 1.07 (s, 3H), 0.93 (t, 9H), 0.58 (q, 6H).

SODIUM BOROHYDRIDE REDUCTION OF 4-DEACETYL-4-TRICHLOROACETYL-7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.20)

4-Deacetyl-4-trichloroacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.20, 14.2 mg, 0.0177 mmol) was dissolved in 3.0 ml THF-methanol (9:1), and to the stirred solution was added NaBH_4 (1.4 mg, 0.0370 mmol, 2.1 equiv.). The reaction was allowed to proceed at room temperature while being monitored by TLC (EtOAc/hexanes, 1:1), and after 30 min the reaction appeared to be complete, based on disappearance of the starting material and appearance of a single, more polar product. After workup by standard procedure, the crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification. The

product was isolated to give 7.8 mg (67.0%) of a white, amorphous powder, which was determined by ^1H NMR and mass spectral data to be the hydrolysis product, 4-deacetyl-7-(triethylsilyl)baccatin III (**2.25**).

SODIUM BOROHYDRIDE REDUCTION OF 4-DEACETYL-4-BENZOYL-7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.22**)**

4-Deacetyl-4-benzoyl-7-(triethylsilyl)-13-oxobaccatin III (**3.22**, 42.0 mg, 0.0553 mmol) was dissolved in 3.0 ml THF-methanol (9:1), and to the stirred solution was added NaBH_4 (10.5 mg, 0.276 mmol, 5.0 equiv.). The reaction was allowed to proceed at room temperature while being monitored by TLC (EtOAc/hexanes, 1:1), and after 30 min the reaction appeared to be complete, based on disappearance of the starting material and appearance of a predominant, more polar product. After workup by standard procedure, the crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification. The product was isolated to give 31.4 mg (74.5%) of a white, amorphous powder, which was identified by ^1H NMR and mass spectral data as 4-deacetyl-4-benzoyl-7-(triethylsilyl)baccatin III (**3.26**): FABMS m/z (rel int.) $[\text{M}+\text{Na}]^+$ 785 (2.1), $[\text{MH}]^+$ 763 (2.7), 745 (3.4), 727 (2.6), 718 (3.5); ^1H NMR (CDCl_3) δ 8.18 (m, 4H), 7.32 - 7.77 (m, 6H), 6.48 (s, 1H), 5.94 (d, $J = 5.8\text{Hz}$, 1H), 5.05 (br. d, $J = 6.6\text{Hz}$, 1H), 4.78 (m, 1H), 4.63 (dd, $J = 3.4, 8.8\text{Hz}$, 1H), 4.42 (ABq, $J = 8.0\text{Hz}$, $\Delta\nu_{\text{AB}} = 24$, 2H), 3.90 (d, $J = 5.7\text{Hz}$, 1H), 2.54 - 2.72 (m, 2H), 2.19 (s, 3H), 2.06 (s, 3H), 1.9 - 2.1 (m, 2H), 1.78 (s, 3H), 1.27 (s, 3H), 1.20 (s, 3H), 0.92 (t, 9H), 0.60 (q, 6H).

COUPLING OF 4-DEACETYL-7-(TRIETHYLSILYL)-4-PROPIONYLBACCATIN III (3.24**) WITH (4S,5R)-N-Boc-2,2-DIMETHYL-4-PHENYL-5-OXAZOLIDINE CARBOXYLIC ACID (**2.19**)**

4-Deacetyl-7-(triethylsilyl)-4-propionylbaccatin III (**3.24**, 13.0 mg, 0.0182 mmol) was dissolved in 0.5 ml anhydrous toluene. To the stirred solution was added the taxol side

chain carboxylic acid **2.19** (12.4 mg, 0.0381 mmol, 2.1 equiv.) and DCC (8.0 mg, 0.0388 mmol, 2.1 equiv.) in 1.0 ml anhydrous toluene. After the addition of a catalytic amount of DMAP, the reaction was warmed to 62 °C and allowed to proceed under argon at this temperature while being monitored by TLC (5% isopropanol in CH₂Cl₂). After 12 hours, the solvent was removed by evaporation, and the crude product mixture was subjected to PTLC (3% isopropanol in CH₂Cl₂), affording 11.6 mg (62.4%) of the coupling compound **3.27**: ¹H NMR (CDCl₃) δ 8.06 (d, *J* = 7.2Hz, 2H), 7.34 - 7.64 (m, 8H), 6.44 (s, 1H), 6.23 (t, *J* = 10.8Hz, 1H), 5.65 (d, *J* = 7.1Hz, 1H), 5.03 (br. m, 1H), 4.81 (br. d, *J* = 8.1Hz, 1H), 4.48 (dd, *J* = 6.1, 12.2Hz, 1H), 4.41 (d, *J* = 6.6Hz, 1H), 4.17 (ABq, *J* = 8.3Hz, Δ*v*_{AB} = 40, 2H), 3.75 (d, *J* = 7.0Hz, 1H), 2.42 - 2.58 (m, 2H), 2.17 (s, 3H), 2.02 (s, 3H), 1.85 - 2.20 (br. m, 4H), 1.80 (s, 3H), 1.76 (s, 3H), 1.66 (s, 3H), 1.25 (s, 3H), 1.22 (s, 9H), 1.02 - 1.20 (m, 6H), 0.91 (t, 9H), 0.57 (q, 6H).

COUPLING OF 4-DEACETYL-4-CHLOROACETYL-7-(TRIETHYLSILYL)BACCATIN III (3.25) WITH WITH (4*S*,5*R*)-*N*-BOC-2,2-DIMETHYL-4-PHENYL-5-OXAZOLIDINE CARBOXYLIC ACID (2.19)

4-Deacetyl-4-chloroacetyl-7-(triethylsilyl)baccatin III (**3.25**, 8.0 mg, 0.0109 mmol) was dissolved in 0.5 ml anhydrous toluene. To the stirred solution was added the taxol side chain carboxylic acid **2.19** (7.1 mg, 0.0218 mmol, 2.0 equiv.) and DCC (4.5 mg, 0.0218 mmol, 2.0 equiv.) in 1.0 ml anhydrous toluene. After the addition of a catalytic amount of DMAP, the reaction was warmed to 62 °C and allowed to proceed under argon at this temperature while being monitored by TLC (5% isopropanol in CH₂Cl₂). After 12 hr, the solvent was removed by evaporation, and the crude product mixture was subjected to PTLC (3% isopropanol in CH₂Cl₂), affording 8.3 mg (73.1%) of the coupling compound **3.28**: ¹H NMR (CDCl₃) δ 8.14 (d, *J* = 7.4Hz, 2H), 7.38 - 7.62 (m, 8H), 6.56 (s, 1H), 6.16 (t, *J* = 10.4Hz, 1H), 5.72 (br. d, *J* = 7.2Hz, 1H), 5.04 (d, *J* = 7.4Hz, 1H), 4.96 (d, *J* = 7.8Hz, 1H), 4.48 (m, 1H), 4.43 (d, *J* = 7.4Hz, 1H), 4.31 (ABq, *J* = 9Hz, Δ*v*_{AB} = 44, 2H), 3.84 (d, *J* = 9.8Hz, 1H), 3.71 (d, *J* = 7.1Hz, 1H), 2.4 - 2.6 (m, 2H), 2.19 (s, 3H), 2.04 (s,

3H), 1.9 - 2.1 (m, 2H), 1.81 (s, 3H), 1.67 (s, 3H), 1.25 (s, 3H), 1.19 (s, 9H), 1.0 - 1.2 (m, 6H), 0.92 (t, 9H), 0.57 (q, 6H).

COUPLING OF 4-DEACETYL-4-BENZOYL-7-(TRIETHYLSILYL)BACCATIN III (3.26) WITH WITH (4*S*,5*R*)-*N*-Boc-2,2-DIMETHYL-4-PHENYL-5-OXAZOLIDINE CARBOXYLIC ACID (2.19)

4-Deacetyl-4-benzoyl-7-(triethylsilyl)baccatin III (3.26, 11.3 mg, 0.0148 mmol) was dissolved in 0.5 ml anhydrous toluene. To the stirred solution was added the taxol side chain carboxylic acid 2.19 (9.6 mg, 0.0297 mmol, 2.0 equiv.) and DCC (6.1 mg, 0.0297 mmol, 2.0 equiv.) in 1.0 ml anhydrous toluene. After the addition of a catalytic amount of DMAP, the reaction was heated to 62 °C and allowed to proceed under argon at this temperature while being monitored by TLC (5% isopropanol in CH₂Cl₂). After 16 hours, the solvent was removed by evaporation, and the crude product mixture was subjected to PTLC (3% isopropanol in CH₂Cl₂), affording 10.7 mg (67.6%) of the coupling compound 3.29: ¹H NMR (CDCl₃) δ 8.20 (d, *J* = 7.4Hz, 2H), 8.14 (d, *J* = 7.6Hz, 2H), 7.10 - 7.64 (m, 11H), 6.40 (s, 1H), 5.97 (t, *J* = 10.6Hz, 1H), 5.74 (d, *J* = 6.8Hz, 1H), 5.08 (m, 1H), 5.01 (d, *J* = 6.7Hz, 1H), 4.59 (m, 1H), 4.54 (m, 1H), 4.38 (ABq, *J* = 8Hz, Δ*v*_{AB} = 43, 2H), 3.84 (d, *J* = 6.8Hz, 1H), 2.4 - 2.7 (m, 2H), 2.18 (s, 3H), 2.04 (s, 3H), 1.9 - 2.1 (m, 2H), 1.84 (s, 3H), 1.76 (s, 3H), 1.26 (s, 3H), 1.22 (s, 9H), 1.05 - 1.20 (m, 6H), 0.92 (t, 9H), 0.58 (q, 6H).

FORMATION OF 4-DEACETYL-4-PROPIONYL TAXOL (3.31)

Compound 3.27 (7.0 mg, 0.00685 mmol) was allowed to stir in 1.0 ml 99% formic acid for 4 hr at room temperature. The reaction mixture was then diluted with 10 ml ethyl acetate and treated with saturated aqueous sodium bicarbonate solution (3 × 10 ml) to remove the formic acid. The ethyl acetate layer was washed with brine (2 × 5 ml), and the solvent was removed by rotary evaporator. The polar product (4.3 mg, 0.00564 mmol) was not subjected to purification or characterization, but was directly carried to the next step by first dissolving it in 1.0 ml ethyl acetate. To the stirred solution was added 1.0 ml distilled

water, followed by sodium bicarbonate (6.2 μ l of an aqueous 1.0 mM solution, 0.0062 mmol, 1.1 equivalents). To this mixture was added benzoyl chloride (5.5 μ l of a 1.0 mM solution in EtOAc, 0.0055 mmol, 0.98 equivalents) and the reaction was allowed to proceed at room temperature for 3 hr, at which time TLC analysis (5% isopropanol in CH_2Cl_2) showed the formation of a single, less polar product. After separating the ethyl acetate layer, the aqueous layer was neutralized with aqueous 1% HCl and extracted with ethyl acetate (2 $\times$ 5 ml). The combined ethyl acetate layers were washed with brine (2 $\times$ 10 ml) and dried with MgSO_4 . After removing the drying agent, the solvent was evaporated off, and the crude product mixture was subjected to PTLC (3% isopropanol in CH_2Cl_2), affording 3.4 mg (57.2%) of 4-deacetyl-4-propionyltaxol (**3.31**). FABMS m/z (rel int.) $[\text{M}+\text{Na}]^+$ 890 (7.1), $[\text{MH}]^+$ 868 (6.4), 852 (5.5), 824 (6.9), 809 (9.0), 762 (12); ^1H NMR (CDCl_3) δ 8.16 (dd, J = 1.5, 8.6 Hz, 2H), 7.72 (dd, J = 1.4, 8.4 Hz, 2H), 7.37 - 7.68 (m, 11H), 6.88 (d, J = 8.7 Hz, NH), 6.27 (s, 1H), 6.24 (t, J = 9.2 Hz, 1H), 5.76 (dd, J = 2.3, 9.2 Hz, 1H), 5.69 (d, J = 7.2 Hz, 1H), 4.89 (br. d, J = 7.5 Hz, 1H), 4.80 (m, 1H), 4.44 (br. m, 1H), 4.26 (ABq, J = 8.5 Hz, $\Delta\nu_{\text{AB}}$ = 45, 2H), 3.79 (d, J = 7.3 Hz, 1H), 3.49 (d, J = 5.0 Hz, 1H), 2.50 - 2.70 (br. m, 3H), 2.25 - 2.50 (br. m, 2H), 2.25 (s, 3H), 2.05 (s, 3H), 1.83 - 1.92 (m, 1H), 1.83 (s, 3H), 1.69 (s, 3H), 1.26 (s, 3H), 1.19 (t, J = 7.6 Hz, 3H); ^{13}C NMR (CDCl_3) δ 203.7, 173.5, 173.1, 171.3, 167.1, 167.0, 142.0, 138.0, 133.7, 133.6, 133.1, 132.0, 130.2, 129.2, 129.0, 128.7, 128.4, 127.1, 127.0, 84.6, 81.0, 79.1, 77.2, 76.4, 75.5, 75.1, 72.7, 72.6, 72.1, 58.5, 54.9, 45.6, 43.2, 35.8, 35.5, 28.7, 26.9, 22.1, 20.8, 14.7, 9.6, 9.2.

FORMATION OF 4-DEACETYL-4-CHLOROACETYLTAXOL (**3.33**)

Compound **3.28** (5.3 mg, 0.00509 mmol) was allowed to stir in 1.0 ml 99% formic acid for 4 hr at room temperature. The reaction mixture was then diluted with 10 ml ethyl acetate and treated with saturated aqueous sodium bicarbonate solution (3 $\times$ 10 ml) to remove the formic acid. The ethyl acetate layer was washed with brine (2 $\times$ 5 ml), and the solvent was removed by rotary evaporator. The polar product (2.6 mg, 0.00332 mmol) was

not subjected to purification or characterization, but was directly carried to the next step by initially dissolving it in 1.0 ml ethyl acetate. To the stirred solution was added 1.0 ml distilled water, followed by sodium bicarbonate (3.7 μ l of an aqueous 1.0 mM solution, 0.0037 mmol, 1.1 equivalents). To this mixture was added benzoyl chloride (3.3 μ l of a 1.0 mM solution in EtOAc, 0.0033 mmol, 0.98 equivalents) and the reaction was allowed to proceed at room temperature for 3 hr, at which time TLC analysis (5% isopropanol in CH_2Cl_2) showed the formation of a single, less polar product. After separating the ethyl acetate layer, the aqueous layer was neutralized with aqueous 1% HCl and extracted with ethyl acetate (2 $\times$ 5 ml). The combined ethyl acetate layers were washed with brine (2 $\times$ 10 ml) and dried with MgSO_4 . After removing the drying agent, the solvent was evaporated off, and the crude product mixture was subjected to PTLC (3% isopropanol in CH_2Cl_2), affording 1.9 mg (42.1%) of 4-deacetyl-4-chloroacetyltaxol (**3.33**). FABMS m/z (rel int.) $[\text{M}+\text{Na}]^+$ 911 (7.7), $[\text{MH}]^+$ 889 (11), 853 (8.3), 845 (8.1), 810 (7.4), 783 (8.2); ^1H NMR (CDCl_3) δ 8.18 (dd, J = 1.5, 8.4Hz, 2H), 7.74 (dd, J = 1.4, 8.6Hz, 2H), 7.28 - 7.64 (m, 11H), 6.80 (d, J = 8.0Hz, NH), 6.52 (s, 1H), 6.28 (t, J = 9.0Hz, 1H), 5.74 (d, J = 7.5Hz, 1H), 5.64 (d, J = 7.0Hz, 1H), 4.97 (m, 1H), 4.76 (m, 1H), 4.46 (m, 1H), 4.28 (ABq, J = 9.1Hz, $\Delta\nu_{\text{AB}}$ = 44, 2H), 3.82 (d, J = 14.4Hz, 1H), 3.68 (d, J = 6.9Hz, 1H), 2.25 - 2.57 (m, 3H), 2.20 (s, 3H), 1.8 - 2.1 (m, 1H), 1.81 (s, 3H), 1.65 (s, 3H), 1.23 (s, 3H), 1.17 (s, 3H).

FORMATION OF 4-DEACETYL-4-BENZOYLTAXOL (**3.35**)

Compound **3.29** (6.9 mg, 0.00645 mmol) was allowed to stir in 1.0 ml 99% formic acid for 4 hr at room temperature. The reaction mixture was then diluted with 10 ml ethyl acetate and treated with saturated aqueous sodium bicarbonate solution (3 $\times$ 10 ml) to remove the formic acid. The ethyl acetate layer was washed with brine (2 $\times$ 5 ml), and the solvent was removed by rotary evaporator. The polar product (3.7 mg, 0.00456 mmol) was not subjected to purification or characterization, but was directly carried to the next step by initially dissolving it in 1.0 ml ethyl acetate. To the stirred solution was added 1.0 ml

distilled water, followed by sodium bicarbonate (5.0 μ l of an aqueous 1.0 mM solution, 0.0050 mmol, 1.1 equivalents). To this mixture was added benzoyl chloride (4.5 μ l of a 1.0 mM solution in EtOAc, 0.0045 mmol, 0.98 equivalents) and the reaction was allowed to proceed at room temperature for 3 hr, at which time TLC analysis (5% isopropanol in CH_2Cl_2) showed the formation of a single, less polar product. After separating the ethyl acetate layer, the aqueous layer was neutralized with aqueous 1% HCl and extracted with ethyl acetate (2 $\times$ 5 ml). The combined ethyl acetate layers were washed with brine (2 $\times$ 10 ml) and dried with MgSO_4 . After removing the drying agent, the solvent was evaporated off, and the crude product mixture was subjected to PTLC (3% isopropanol in CH_2Cl_2), affording 2.7 mg (45.7%) of 4-deacetyl-4-benzoyltaxol (**3.35**). FABMS m/z (rel int.) $[\text{MH}]^+$ 916 (7.3), 898 (4.4), 880 (4.7), 872 (3.8), 810 (5.4), 767 (7.8), 705 (13); ^1H NMR (CDCl_3) δ 8.24 (dd, J = 1.5, 8.2 Hz, 2H), 8.14 (dd, J = 1.2, 8.4 Hz, 2H), 7.22 - 7.70 (m, 15H), 6.99 (t, J = 7.4 Hz, 1H), 6.54 (d, J = 8.9 Hz, NH), 6.35 (s, 1H), 5.90 (t, J = 9.0 Hz, 1H), 5.76 (d, J = 6.9 Hz, 1H), 5.00 (br. m, 1H), 4.60 (m, 1H), 4.39 (ABq, J = 8.5 Hz, $\Delta\nu_{\text{AB}}$ = 56, 2H), 4.03 (d, J = 7.3 Hz, 1H), 3.65 (dd, J = 2.5, 5.4 Hz, 1H), 3.10 (d, J = 5.3 Hz, 1H), 2.52 - 2.66 (m, 2H), 2.25 - 2.42 (m, 1H), 2.25 (s, 3H), 1.85 - 1.97 (m, 1H), 1.88 (s, 3H), 1.74 (s, 3H), 1.64 (s, 3H), 1.19 (d, 3H); ^{13}C NMR (CDCl_3) δ 203.7, 173.1, 171.3, 167.0, 166.8, 165.7, 142.5, 138.2, 133.9, 133.8, 132.9, 131.8, 130.1, 129.5, 129.3, 128.71, 128.67, 128.62, 128.54, 128.1, 127.8, 126.9, 84.5, 81.9, 79.1, 77.2, 76.5, 75.6, 75.2, 73.0, 72.2, 71.8, 58.7, 54.6, 45.9, 43.2, 35.7, 35.6, 29.7, 26.8, 22.0, 20.9, 14.9, 9.6.

HYDROLYSIS OF 13-ACETYL-4-DEACETYL-13,14-DEHYDRO-7-(TRIETHYLSILYL)BACCATIN III (**3.47**)

13-Acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (**3.47**, 16.2 mg, 0.0232 mmol) was dissolved in 100 μ l methanol. To the stirred solution was added sodium bicarbonate (4.9 mg, 0.0580 mmol, 2.5 equiv.), and the reaction was allowed to continue stirring at room temperature for 2 hr. At this time a TLC (EtOAc/hexane, 1:1) showed the

starting material to have been converted to a slightly more polar product. After workup by the usual procedure, the solvent was evaporated, and the crude reaction mixture was purified by PTLC (EtOAc/hexane, 1:1). The product was analyzed by ^1H NMR and mass spectrometry and was found to be 4-deacetyl-7-(triethylsilyl)-13-oxobaccatin III (3.13), identical with the same compound described previously.

ATTEMPTED C-4 ACETYLATION OF 13-ACETYL-4-DEACETYL-13,14-DEHYDRO-7-(TRIETHYLSILYL)BACCATIN III (3.47)

13-Acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47, 12.7 mg, 0.0182 mmol) was dissolved in 100 μl anhydrous toluene. The solution was added to a mixture of Ac_2O (34.3 μl , 0.364 mmol, 20.0 equiv.) and DCC (75.1 mg, 0.364 mmol, 20.0 equiv.) in 100 μl anhydrous toluene. After adding a catalytic amount of 4-pyrrolidinopyridine, the reaction was heated to 90 $^\circ\text{C}$ and allowed to stir at this temperature under argon for 16 hr. At this time a TLC (EtOAc/hexane, 1:1) showed no change to have occurred in the starting material, with the exception of the formation of baseline material, which moved negligibly even upon attempted elution with 20% methanol in CH_2Cl_2 . After workup by the usual procedure, the solvent was evaporated, and the crude starting material was purified by PTLC (EtOAc/hexane, 1:1), affording 8.1 mg (63.8% recovery) of the enol acetate 3.47.

BROMINATION OF 13-ACETYL-4-DEACETYL-13,14-DEHYDRO-7-(TRIETHYLSILYL)BACCATIN III (3.47)

13-Acetyl-4-deacetyl-13,14-dehydro-7-(triethylsilyl)baccatin III (3.47, 21.2 mg, 0.032 mmol) was dissolved in 2.0 ml anhydrous CH_2Cl_2 , then cooled to 0 $^\circ\text{C}$. To the stirring solution was added bromine (33.3 μl , 0.646 mmol, 20.2 equiv.), and the reaction was allowed to warm to room temperature over 15 min. At this time a TLC (EtOAc/hexane, 1:1) showed the starting material to have been converted to two slightly more polar products in an approximate ratio of 1:1. After workup by the usual procedure, the solvent

was evaporated, and the crude reaction mixture was purified by PTLC (EtOAc/hexane, 1:1), affording two compounds which were subsequently analyzed by ^1H NMR spectroscopy. The more polar of the two products (6.6 mg, 25.3%) was tentatively assigned as the C-14 α brominated compound **3.48**, based on its spectral information: ^1H NMR (CDCl_3) δ 8.04 (dd, $J = 1.5, 8.5\text{Hz}$, 2H), 7.45 - 7.61 (m, 3H), 6.51 (s, 1H), 6.13 (s, 1H), 5.88 (d, $J = 6.0\text{Hz}$, 1H), 4.70 (dd, $J = 5, 9\text{Hz}$, 1H), 4.26 (ABq, $J = 9.1\text{Hz}$, $\Delta\nu_{\text{AB}} = 136$, 2H), 4.02 (m, 1H), 3.32 (d, $J = 6.0\text{Hz}$, 1H), 2.78 (s, OH), 2.35 - 2.52 (m, 1H), 2.23 (d, 3H), 2.17 (s, 3H), 1.95 - 2.20 (m, 1H), 1.65 (s, 3H), 1.32 (s, 3H), 1.25 (s, 3H), 1.14 (s, 3H), 0.91 (t, 9H), 0.57 (q, 6H). The less polar of the two products (7.1 mg, 27.2%) was given tentative assignment as the C-14 β brominated compound **3.49**, based on its spectral information: ^1H NMR (CDCl_3) δ 8.07 (br. d, $J = 7.1\text{Hz}$, 2H), 7.46 - 7.62 (m, 3H), 6.73 (s, 1H), 6.30 (s, 1H), 5.85 (d, $J = 5.5\text{Hz}$, 1H), 4.57 (dd, $J = 4, 10\text{Hz}$, 1H), 4.48 (t, $J = 3\text{Hz}$, 1H), 4.12 (ABq, $J = 12.1\text{Hz}$, $\Delta\nu_{\text{AB}} = 79$, 2H), 4.03 (d, $J = 5.4\text{Hz}$, 1H), 3.44 (s, OH), 2.88 (s, OH), 2.42 (s, 3H), 2.23 (s, 3H), 2.17 (s, 3H), 2.0 - 2.4 (m, 2H), 1.38 (s, 3H), 1.26 (d, 3H), 1.13 (s, 3H), 0.93 (t, 9H), 0.58 (q, 6H).

TREATMENT OF 7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.14) WITH SODIUM CYANOBOROHYDRIDE

7-(Triethylsilyl)-13-oxobaccatin III (**3.14**, 10.0 mg, 0.0143 mmol) was dissolved in 1.0 ml methanol, and to the stirred solution was added NaCNBH_3 (9.0 mg, 0.143 mmol, 10.0 equiv.). The reaction was allowed to proceed at room temperature while being monitored by TLC (5% isopropanol in CH_2Cl_2). Even after 24 hr no change in the reaction was evident, and after workup by standard procedure, the reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification. The unreacted starting material was recovered to give 7.2 mg (72.0%) of a white, amorphous powder, which was confirmed by ^1H NMR to be 7-(triethylsilyl)-13-oxobaccatin III (**3.14**).

**TREATMENT OF 7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.14)
WITH TETRAMETHYLAMMONIUM BOROHYDRIDE**

7-(Triethylsilyl)-13-oxobaccatin III (3.14, 10.0 mg, 0.0143 mmol) was dissolved in 500 μL CH_2Cl_2 , and to the stirred solution was added tetramethylammonium borohydride (25.5 mg, 0.287 mmol, 20.0 equiv.). The reaction was allowed to proceed at room temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:1). After 14 hr, the starting material appeared to have been converted to a more polar product, and after evaporation of the solvent the reaction was worked up by standard procedure. The crude material was subjected to PTLC (EtOAc/hexanes, 1:1) for purification, affording 4.0 mg (47.1%) of a colorless, glassy material, R_f 0.17, which upon analysis by ^1H NMR was identified as 2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.52): ^1H NMR (CDCl_3) δ 6.52 (s, 1H), 4.92 (br. d, $J = 7.7\text{Hz}$, 1H), 4.61 (ABq, $J = 8.6\text{Hz}$, $\Delta\nu_{\text{AB}} = 23$, 2H), 4.44 (dd, $J = 6.7, 10.4\text{Hz}$, 1H), 3.96 (t, $J = 6.3\text{Hz}$, 1H), 3.55 (d, $J = 6.3\text{Hz}$, 1H), 2.68 (ABq, $J = 19.7\text{Hz}$, $\Delta\nu_{\text{AB}} = 69$, 2H), 2.45 - 2.62 (m, 1H), 2.21 (s, 3H), 2.13 (s, 3H), 2.05 (s, 3H), 1.82 - 1.97 (m, 1H), 1.66 (s, 3H), 1.18 (d, 3H), 0.92 (t, 9H), 0.58 (q, 6H).

**TREATMENT OF 7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.14)
WITH TETRABUTYLAMMONIUM BOROHYDRIDE**

7-(Triethylsilyl)-13-oxobaccatin III (3.14, 9.5 mg, 0.0136 mmol) was dissolved in 500 μL CH_2Cl_2 , and to the stirred solution was added tetrabutylammonium borohydride (14.1 mg, 0.0548 mmol, 4.0 equiv.). The reaction was allowed to proceed at room temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:1). Even after 90 hr, no reaction was evident and the solution was treated with 1N HCl then diluted with CH_2Cl_2 . After workup by standard procedure, the crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification. The unreacted starting material was recovered to give 7.1 mg (74.7%) of a white, amorphous powder, which was confirmed by ^1H NMR to be 7-(triethylsilyl)-13-oxobaccatin III (3.14).

TREATMENT OF 7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.14)

WITH TETRAMETHYLAMMONIUM HYDROXIDE

7-(Triethylsilyl)-13-oxobaccatin III (3.14, 22.6 mg, 0.0324 mmol) was dissolved in 1.0 ml CH_2Cl_2 , and to the stirred solution was added 28.6 μl (0.0680 mmol, 2.1 equiv.) of tetramethylammonium hydroxide (25wt% solution in methanol). The reaction was allowed to proceed at room temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:1). Within 10 min, the starting material had been converted to a more polar product, and the solution was treated with 1 N HCl, then diluted with CH_2Cl_2 . After workup by standard procedure, the crude reaction mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification, affording 9.7 mg (50.4%) of a colorless, glassy solid, R_f 0.17, which was identified by ^1H NMR as 2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.52), identical to the same compound described above.

CONTROLLED REACTION OF 7-(TRIETHYLSILYL)-13-OXOBACCATIN III

(3.14) WITH BENZYLTRIMETHYLAMMONIUM METHOXIDE

7-(Triethylsilyl)-13-oxobaccatin III (3.14, 7.2 mg, 0.0103 mmol) was dissolved in 120 μl CH_2Cl_2 and cooled to -78°C . To the stirred solution was slowly added by syringe 10.6 μl (0.0217 mmol, 2.1 equiv.) of benzyltrimethylammonium methoxide (40wt% solution in methanol). After stirring at this temperature for 2 min, the reaction was quenched by the addition of 1.0 ml 1 N HCl. After dilution with CH_2Cl_2 , the reaction was worked up by standard procedure and the crude mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification, affording 6.1 mg (99.5%) of a colorless, glassy solid, R_f 0.17, which was identified by ^1H NMR as 2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.52), identical to the same compound described above.

**TREATMENT OF 7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.14)
WITH BENZYLTRIMETHYLAMMONIUM METHOXIDE**

7-(Triethylsilyl)-13-oxobaccatin III (3.14, 5.2 mg, 0.00745 mmol) was dissolved in 120 μl CH_2Cl_2 and cooled to $-78\text{ }^\circ\text{C}$. To the stirred solution was slowly added by syringe 7.7 μl (0.0156 mmol, 2.1 equiv.) of benzyltrimethylammonium methoxide (40wt% solution in methanol). After stirring at this temperature for 2 min, the reaction was allowed to warm to room temperature. After 15 min, a TLC (EtOAc/hexane, 1:1) showed the starting material to have been converted to a mixture of two more polar products. The reaction was quenched by the addition of 1.0 ml 1N HCl, and after dilution with CH_2Cl_2 the reaction was worked up by standard procedure. The crude mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification, affording 2.6 mg (58.8%) of a colorless, glassy solid, R_f 0.17, which was identified by ^1H NMR as 2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.52), identical to the same compound described above. The second product isolated, a colorless, glassy solid, R_f 0.26, was identified by ^1H NMR as 4-deacetyl-2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.53, 1.2 mg, 29.1% yield): ^1H NMR (CDCl_3) δ 6.47 (s, 1H), 4.76 (dd, $J = 3.1, 9.8\text{Hz}$, 1H), 4.55 (ABq, $J = 8.7\text{Hz}$, $\Delta\nu_{\text{AB}} = 92$, 2H), 4.26 (s, OH), 4.03 (dd, $J = 6.4, 11.0\text{Hz}$, 1H), 3.96 (t, $J = 6.2\text{Hz}$, 1H), 3.54 (d, $J = 5.8\text{Hz}$, 1H), 3.37 (s, OH), 2.83 (ABq, $J = 19.6\text{Hz}$, $\Delta\nu_{\text{AB}} = 128$, 2H), 2.81 (d, $J = 6.6\text{Hz}$, 1H), 2.42 - 2.55 (m, 1H), 2.21 (s, 3H), 2.12 (s, 3H), 1.93 - 2.05 (m, 1H), 1.61 (s, 3H), 1.21 (s, 3H), 1.17 (s, 3H), 0.90 (t, 9H), 0.57 (q, 6H).

METHANOLYSIS OF 7-(TRIETHYLSILYL)-13-OXOBACCATIN III (3.14)

7-(Triethylsilyl)-13-oxobaccatin III (3.14, 15.0 mg, 0.0215 mmol) was dissolved in 500 μl CH_3OH . To the stirred solution was slowly added by syringe 49.1 μl (0.215 mmol, 10 equiv.) of sodium methoxide (25wt% solution in methanol), and the reaction was allowed to proceed at room temperature under argon while being monitored by TLC (EtOAc/hexanes, 3:1). After 25 min, the starting material was seen to be consumed and

the reaction was neutralized by the addition of 10% methanolic HCl to pH 6. After dilution with CH₂Cl₂, the reaction was worked up by standard procedure and the crude mixture was subjected to PTLC (EtOAc/hexanes, 1:1) for purification, affording two polar products. The first product, *R_f* 0.14, a colorless, glassy solid, was identified as 4,10-bis(deacetyl)-2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (**3.54**) (6.8 mg, 62.0%): FABMS *m/z* (rel int.) [MH]⁺ 511 (3.8), 493 (7.1), 475 (5.1), 463 (2.4), 447 (2.0), 429 (1.7), 327 (10), 315 (5.6), 299 (6.6), 281 (16), 225 (7.8), 193 (26), 163 (42), 149 (50), 137 (47), 115 (100); ¹H NMR (CDCl₃) δ 5.25 (d, *J* = 2.3Hz, 1H), 4.78 (dd, *J* = 3.1, 9.5Hz, 1H), 4.55 (ABq, *J* = 8.7Hz, Δ*v*_{AB} = 87, 2H), 4.29 (d, *J* = 2.4Hz, OH), 3.92 (t, *J* = 6.9Hz, 1H), 3.91 (dd, *J* = 6.4, 11.3Hz, 1H), 2.85 (d, *J* = 6.9Hz, 1H), 2.79 (ABq, *J* = 19.4Hz, Δ*v*_{AB} = 121, 2H), 2.35 - 2.51 (m, 1H), 2.06 (s, 3H), 1.93 - 2.05 (m, 1H), 1.68 (s, 3H), 1.25 (s, 3H), 1.06 (s, 3H), 0.91 (t, 9H), 0.55 (q, 6H). The second product, *R_f* 0.04, also a colorless, glassy solid, was identified as the rearrangement isomer of compound **3.54**, 4,10-bis(deacetyl)-2-debenzoyl-7-(triethylsilyl)-13-oxo-isobaccatin III (**3.55**) (1.2 mg, 10.9%): FABMS *m/z* (rel int.) [MH]⁺ 511 (5.0), 493 (2.8), 475 (3.3), 431 (4.7), 327 (5.4), 307 (2.5), 279 (7.4), 257 (4.3), 217 (79), 197 (20), 181 (31), 165 (18), 149 (34), 126 (32), 109 (100); ¹H NMR (CDCl₃) δ 5.03 (d, *J* = 1.9Hz, 1H), 4.36 (d, *J* = 1.9Hz, OH), 4.20 (m, 2H), 3.79 (s, 2H), 3.77 (m, 1H), 3.25 (d, *J* = 3.0Hz, 1H), 2.97 (ABq, *J* = 19.6Hz, Δ*v*_{AB} = 154, 2H), 2.04 - 2.18 (m, 1H), 2.01 (s, 3H), 1.77 - 1.93 (m, 1H), 1.38 (s, 3H), 1.27 (s, 3H), 1.03 (s, 3H), 0.94 (t, 9H), 0.53 (q, 6H).

CONTROLLED METHANOLYSIS OF 7-(TRIETHYLSILYL)-13-OXOBACCATIN III (**3.14**): A COMPARATIVE STUDY OF SODIUM METHOXIDE CONCENTRATION:

EXPERIMENT I (1.0 Molar Equivalent)

7-(Triethylsilyl)-13-oxobaccatin III (**3.14**, 10.0 mg, 0.0143 mmol) was dissolved in 500 μl CH₃OH. To the stirred solution was slowly added by syringe 3.3 μl (0.0143 mmol)

of sodium methoxide (25wt% solution in methanol), and the reaction was allowed to proceed at room temperature under argon while being monitored by TLC (EtOAc/hexanes, 3:1). After 15 min, the starting material was observed to be converted to a more material. The reaction was neutralized by the addition of 5% methanolic HCl to pH 6, and, after dilution with CH₂Cl₂, the reaction was worked up by the standard procedure. The crude material was subjected to PTLC (EtOAc/hexanes, 3:1) for purification, affording 3.0 mg of a colorless, glassy solid, which was identified by ¹H NMR as an approximately 1:1 mixture of 2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.52) and 10-deacetyl-2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.56). The ¹H NMR data for 3.52 matched spectral data for the identical compound previously described above; for compound 3.56: ¹H NMR (CDCl₃) δ 5.26 (d, *J* = 2.5Hz, 1H), 4.92 (br. d, *J* = 8.2Hz, 1H), 4.62 (ABq, *J* = 9.1Hz, Δ*v*_{AB} = 26, 2H), 4.35 (dd, *J* = 6.7, 10.5Hz, 1H), 4.30 (d, *J* = 2.5Hz, OH), 3.92 (t, *J* = 6.3Hz, 1H), 3.60 (d, *J* = 6.3Hz, 1H), 2.70 (ABq, *J* = 19.7Hz, Δ*v*_{AB} = 59, 2H), 2.43 - 2.62 (m, 1H), 2.21 (s, 3H), 2.05 (s, 3H), 1.82 - 1.98 (m, 1H), 1.71 (s, 3H), 1.57 (s, 3H), 1.20 (s, 3H), 0.92 (t, 9H), 0.58 (q, 6H).

EXPERIMENT 2 (2.1 Molar Equivalents)

7-(Triethylsilyl)-13-oxobaccatin III (3.14, 10.0 mg, 0.0143 mmol) was dissolved in 500 μl CH₃OH. To the stirred solution was slowly added by syringe 6.9 μl (0.0300 mmol, 2.1 equiv.) of sodium methoxide (25wt% solution in methanol), and the reaction was allowed to proceed at room temperature under argon while being monitored by TLC (EtOAc/hexanes, 3:1). After 15 min, the starting material was observed to be converted to a more material. The reaction was neutralized by the addition of 5% methanolic HCl to pH 6, and, after dilution with CH₂Cl₂, the reaction was worked up by the standard procedure. The crude material was subjected to PTLC (EtOAc/hexanes, 3:1) for purification, affording 9.3 mg of a colorless, glassy solid, which was identified by ¹H NMR as a mixture of three components in an approximate ratio of 2:5:1. These products were identified as 2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.52), 10-deacetyl-2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.56) and 4,10-bis(deacetyl)-2-debenzoyl-7-

(triethylsilyl)-13-oxobaccatin III (3.54), each displaying ^1H NMR spectral data identical with its respective compound described previously.

EXPERIMENT 3 (4.0 Molar Equivalents)

7-(Triethylsilyl)-13-oxobaccatin III (3.14, 10.0 mg, 0.0143 mmol) was dissolved in 500 μl CH_3OH . To the stirred solution was slowly added by syringe 13.0 μl (0.0573 mmol) of sodium methoxide (25wt% solution in methanol), and the reaction was allowed to proceed at room temperature under argon while being monitored by TLC (EtOAc/hexanes, 3:1). After 15 min, the starting material was observed to have been converted to two more polar products. The reaction was neutralized by the addition of 5% methanolic HCl to pH 6, and, after dilution with CH_2Cl_2 , the reaction was worked up by the standard procedure. The crude mixture was subjected to PTLC (EtOAc/hexanes, 3:1) for purification, affording 4.2 mg of a colorless, glassy solid, R_f 0.28, which was identified by ^1H NMR as 10-deacetyl-2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.56), identical with the same compound previously described. The second product, R_f 0.10, a colorless, glassy solid, was isolated in 3.2 mg yield and identified by ^1H NMR as 4,10-bis(deacetyl)-2-debenzoyl-7-(triethylsilyl)-13-oxobaccatin III (3.54), identical with the same compound described previously.

REACTION OF 13-ACETYL-4-DEACETYL-7-(TRIETHYLSILYL)BACCATIN III (3.10) WITH d_6 -ACETIC ANHYDRIDE

13-Acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (3.10, 10.0 mg, 0.0143 mmol) was dissolved with stirring in 250 μl anhydrous CH_3CN . The solution was added by syringe to a mixture of d_6 -acetic anhydride (40.4 μl , 0.429 mmol, 30.0 equiv.) and DCC (88.4 mg, 0.429 mmol, 30.0 equiv.) in 250 μl CH_3CN . After the addition of a catalytic amount of 4-pyrrolidinopyridine, the reaction was heated to 65 $^\circ\text{C}$ and allowed to proceed under argon while being monitored by TLC (EtOAc/hexanes, 1:1). After 3.5 hr, the reaction appeared to be complete based on conversion of the starting material to a slightly less polar product. The reaction was quenched by the addition of 0.5N HCl and after dilution with CH_2Cl_2 , the reaction mixture was worked up by standard procedure. The crude material was

subjected to PTLC (EtOAc/hexanes, 1:1), affording 6.3 mg (59.1%) of a colorless, amorphous solid which was shown by ^1H NMR integration data to have a mixture of deuterium labeled acetates at the C-4 and C-13 positions: ^1H NMR (CDCl_3) δ 8.08 (br. d, $J = 6.1\text{Hz}$, 2H), 7.45 - 7.64 (m, 3H), 6.46 (s, 1H), 6.15 (t, $J = 8.2\text{Hz}$, 1H), 5.68 (d, $J = 7.0\text{Hz}$, 1H), 4.96 (br. d, $J = 7.7\text{Hz}$, 1H), 4.48 (dd, $J = 6.7$, 10.5Hz , 1H), 4.23 (ABq, $J = 8.4\text{Hz}$, $\Delta\nu_{\text{AB}} = 41$, 2H), 3.84 (d, $J = 7.0\text{Hz}$, 1H), 2.41 - 2.55 (m, 2H), 2.34 (s, 0.4H), 2.21 (s, 3H), 2.18 (s, 2.6H), 2.04 (d, $J = 1.4\text{Hz}$, 3H), 1.84 - 1.99 (m, 2H), 1.69 (s, 3H), 1.23 (s, 3H), 1.18 (s, 3H), 0.93 (t, 9H), 0.60 (q, 6H).

REACTION OF 4,10-BIS(DEACETYL)-7-(TRIETHYLSILYL)BACCATIN III (2.7) WITH d_6 -ACETIC ANHYDRIDE

4,10-Bis(deacetyl)-7-(triethylsilyl)baccatin III (2.7, 47.3 mg, 0.0768 mmol) was dissolved in 750 μl CH_2Cl_2 . The solution was added by syringe to a mixture of d_6 -acetic anhydride (21.7 μl , 0.2304 mmol, 3.0 equiv.) and DCC (47.5 mg, 0.2304 mmol, 3.0 equiv.) in 750 μl CH_2Cl_2 . After the addition of a catalytic amount of 4-pyrrolidinopyridine, the reaction was allowed to proceed at room temperature under argon while being monitored by TLC (EtOAc/hexanes, 1:1). After 4 hr, the reaction appeared to be complete based on consumption of starting material and formation of a less polar product. The reaction mixture was quenched with 1 ml 1N HCl then diluted with CH_2Cl_2 , following which the standard workup procedure was implemented. After removal of the solvent, the crude material was subjected to PTLC (EtOAc/hexanes, 1:1), affording 19.2 mg (35.4%) of a colorless, amorphous solid which was identified by ^1H NMR and mass spectral data as 10- d_6 -acetic-13- d_3 -acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (3.59): FABMS m/z (rel int.) $[\text{M}+\text{Na}+\text{H}]^+$ 730 (0.3), $[\text{MH}]^+$ 707 (0.5), 690 (0.5), 644 (2.0), 626 (2.4), 581 (1.2), 459 (2.1), 429 (1.5), 413 (1.1), 389 (1.2), 371 (1.0); ^1H NMR (CDCl_3) δ 8.02 (d, $J = 7.8\text{Hz}$, 2H), 7.44 - 7.63 (m, 3H), 6.45 (s, 1H), 5.93 (m, 1H), 5.66 (d, $J = 6.3\text{Hz}$, 1H), 4.81 (dd, $J = 3.3$, 9.6Hz , 1H), 4.23 (ABq, $J = 8.2\text{Hz}$, $\Delta\nu_{\text{AB}} = 53$, 2H), 4.07 (dd, $J = 6.1$,

11.5Hz, 1H), 3.36 (d, $J = 6.3\text{Hz}$, 1H), 2.38 - 2.72 (m, 3H), 2.08 (s, 3H), 1.91 - 2.07 (m, 1H), 1.60 (s, 3H), 1.23 (s, 3H), 1.10 (s, 3H), 0.91 (t, 9H), 0.57 (q, 6H).

REACTION OF 10- d_3 -ACETYL-13- d_3 -ACETYL-4-DEACETYL-7-(TRIETHYLSILYL) BACCATIN III (3.59) WITH ACETIC ANHYDRIDE

10- d_3 -Acetyl-13- d_3 -acetyl-4-deacetyl-7-(triethylsilyl)baccatin III (3.59, 5.9 mg, 0.00836 mmol) was dissolved in 125 ml CH_3CN . The solution was added by syringe to a mixture of acetic anhydride (15.8 μl , 0.1671 mmol, 20.0 equiv.) and DCC (34.5 mg, 0.1671 mmol, 20.0 equiv.) in 125 μl CH_3CN . After the addition of a catalytic amount of 4-pyrrolidinopyridine, the reaction vial was immersed in an oil bath at 65 °C and the reaction was allowed to proceed at this temperature, under argon, while being monitored by TLC (EtOAc/hexanes, 1:1). After 4 hr, the reaction appeared to be complete based on consumption of starting material and formation of a less polar product. The reaction mixture was quenched with 1 ml 1N HCl then diluted with CH_2Cl_2 , following which the standard workup procedure was implemented. After removal of the solvent, the crude material was subjected to PTLC (EtOAc/hexanes, 1:1), affording 5.6 mg (89.6%) of a colorless, amorphous solid which was shown by ^1H NMR integration data to have a mixture of deuterium labeled acetates at the C-4 and C-13 positions: ^1H NMR (CDCl_3) δ 8.08 (d, $J = 8.2\text{Hz}$, 2H), 7.45 - 7.64 (m, 3H), 6.46 (s, 1H), 6.15 (t, $J = 8.9\text{Hz}$, 1H), 5.67 (d, $J = 7.0\text{Hz}$, 1H), 4.96 (br. d, $J = 8.5\text{Hz}$, 1H), 4.48 (dd, $J = 6.6, 10.5\text{Hz}$, 1H), 4.23 (ABq, $J = 8.4\text{Hz}$, $\Delta\nu_{\text{AB}} = 42$, 2H), 3.84 (d, $J = 7.0\text{Hz}$, 1H), 2.43 - 2.62 (m, 2H), 2.33 (s, 2.7H), 2.17 (s, 0.3H), 2.04 (s, 3H), 1.82 - 1.97 (m, 2H), 1.69 (s, 3H), 1.22 (s, 3H), 1.18 (s, 3H), 0.93 (t, 9H), 0.58 (q, 6H); FABMS m/z (rel int.) $[\text{M}+\text{Na}]^+$ 771 (0.3), $[\text{MH}]^+$ 749 (1.3), 686 (0.9), 668 (0.9), 624 (0.8), 504 (2.2), 459 (3.7), 441 (4.3), 411 (3.9), 371 (14), 327 (9.0), 309 (10), 235 (19), 199 (31), 185 (22), 148 (79), 115 (100).

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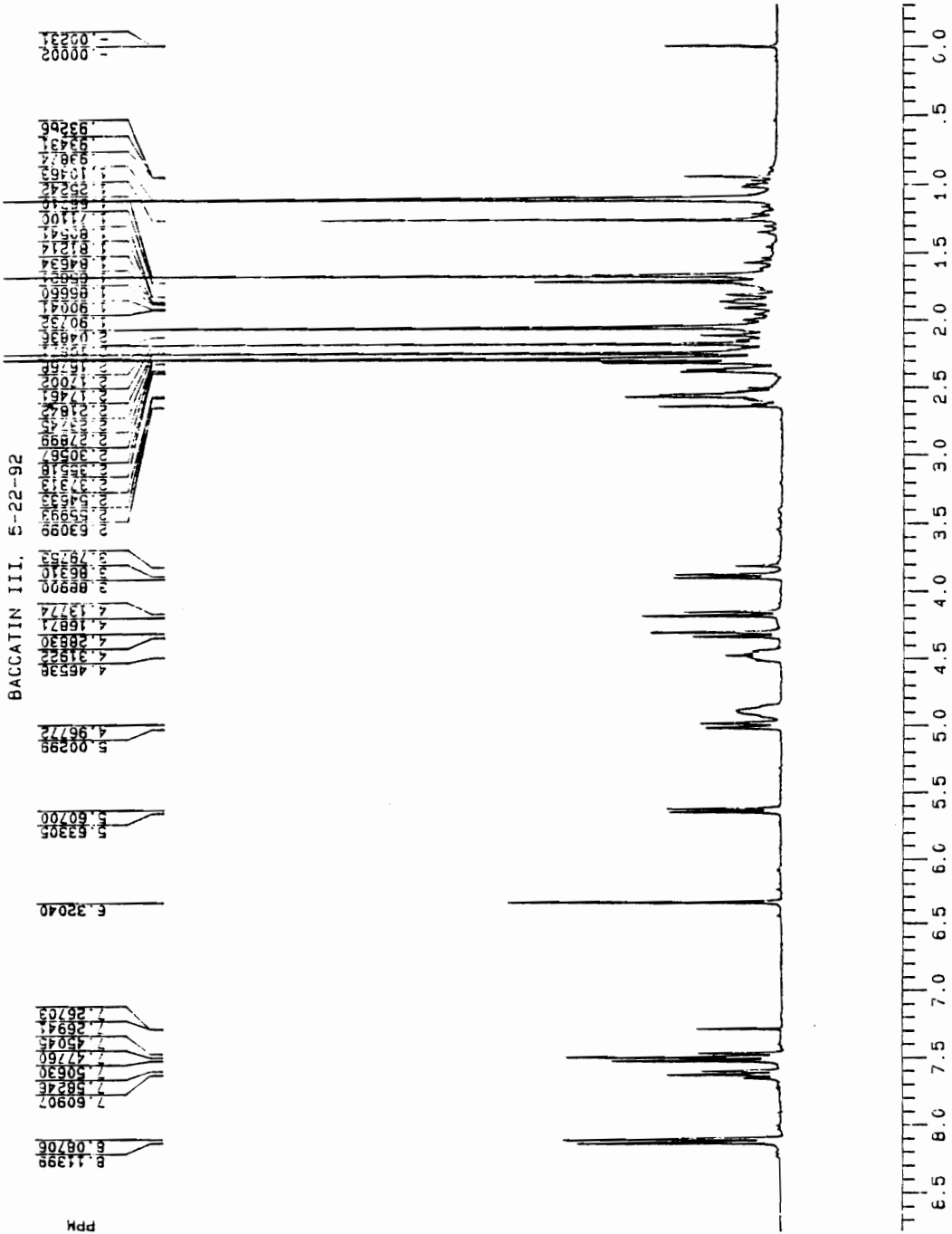
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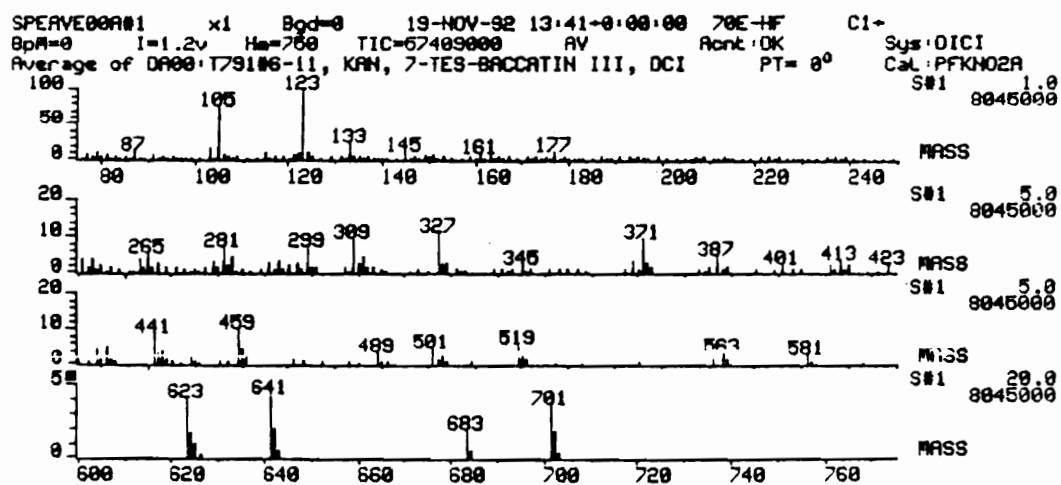
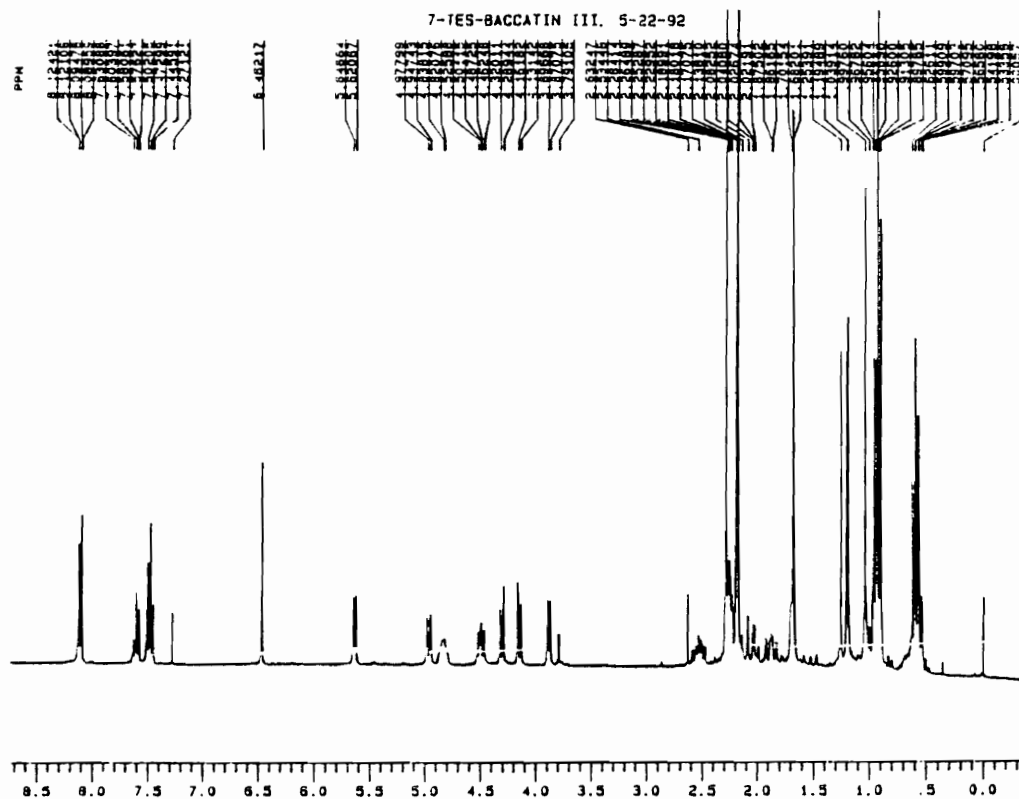
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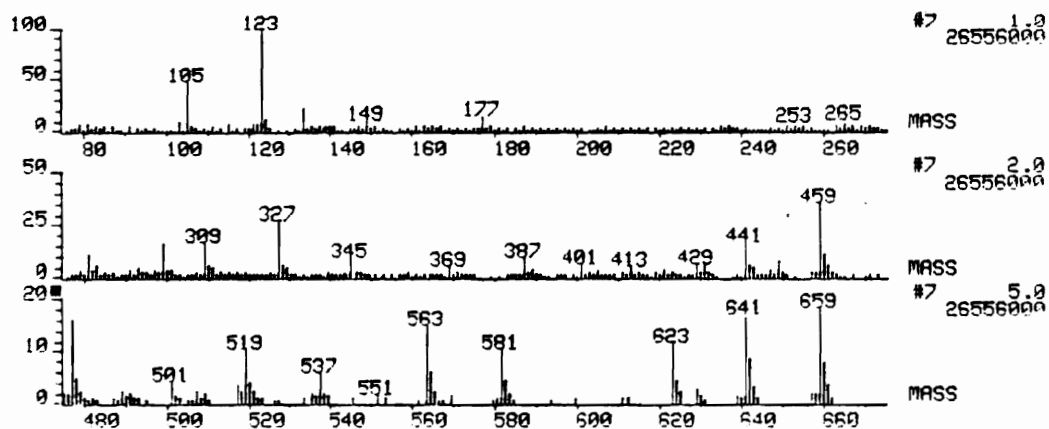
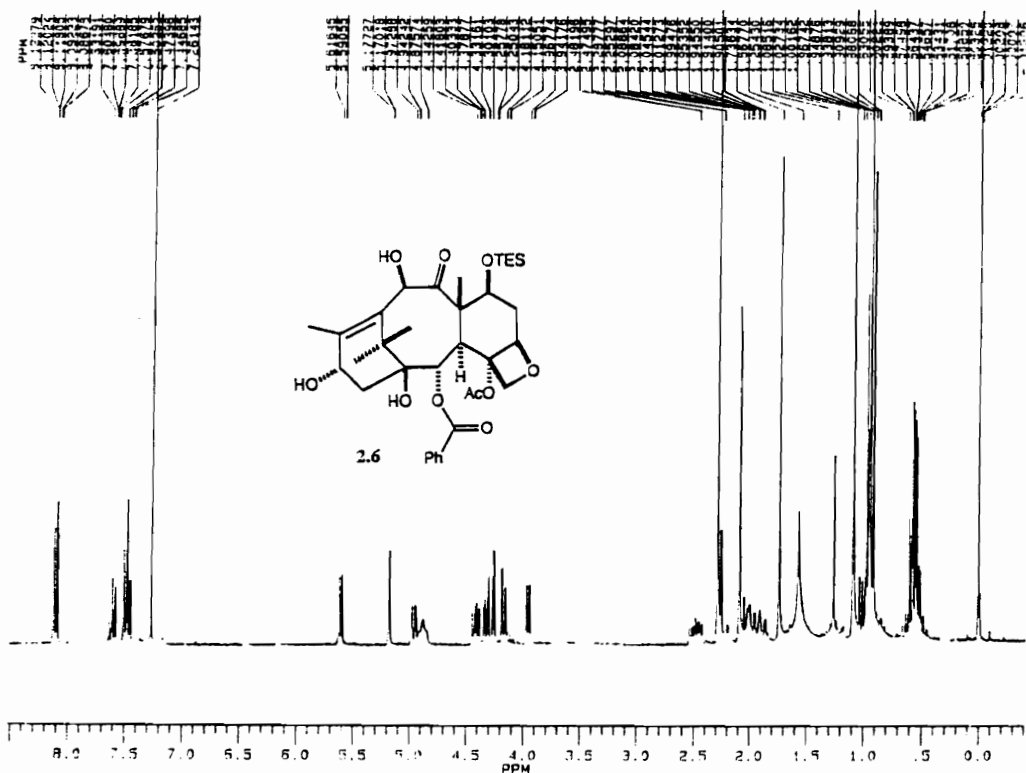
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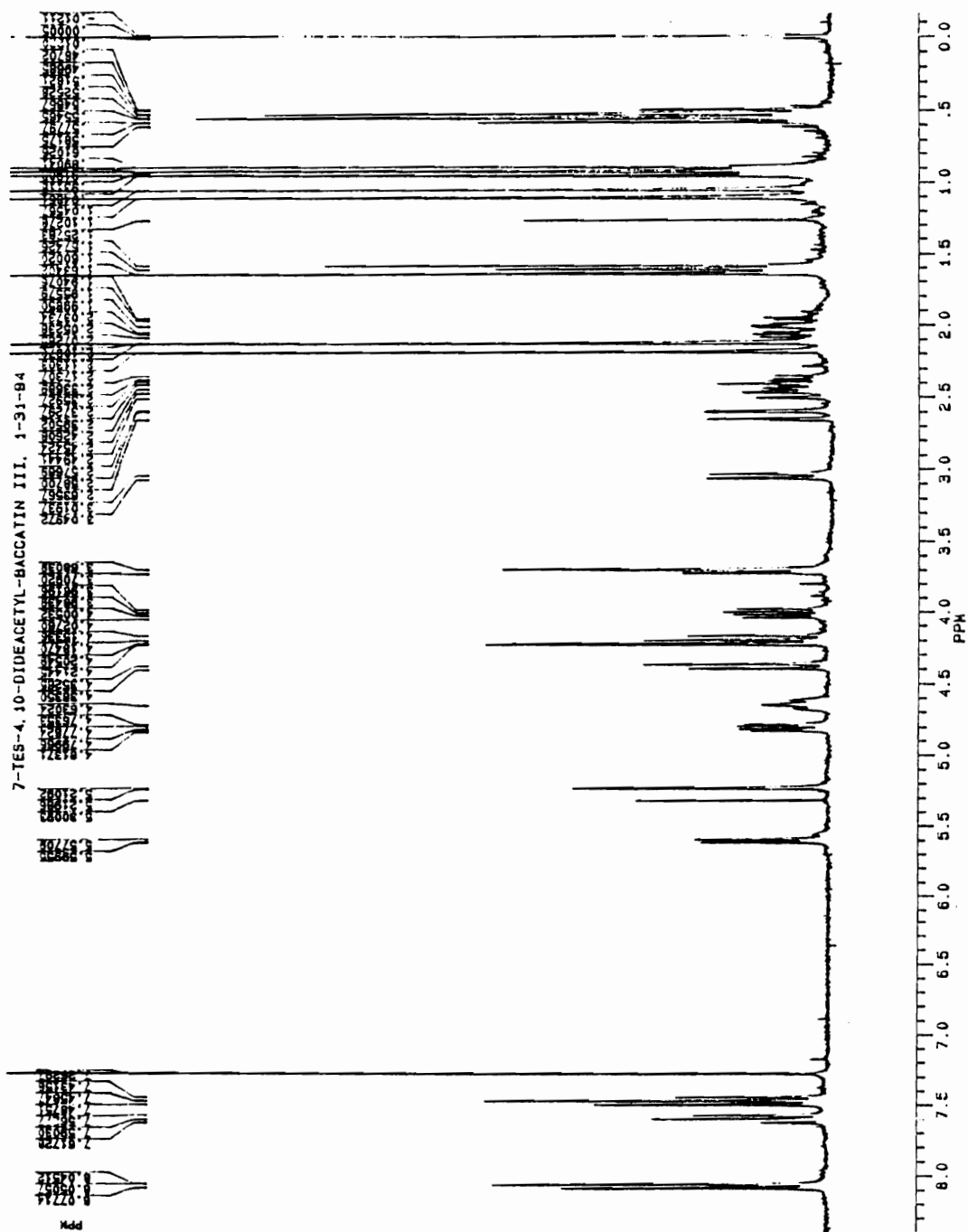
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(b) Baumann, E. *Chem. Ber.* **1886**, *19*, 3218.
(c) For a review see: Sonntag, N.O.V. *Chem. Rev.* **1953**, *52*, 272.
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VII. APPENDIX

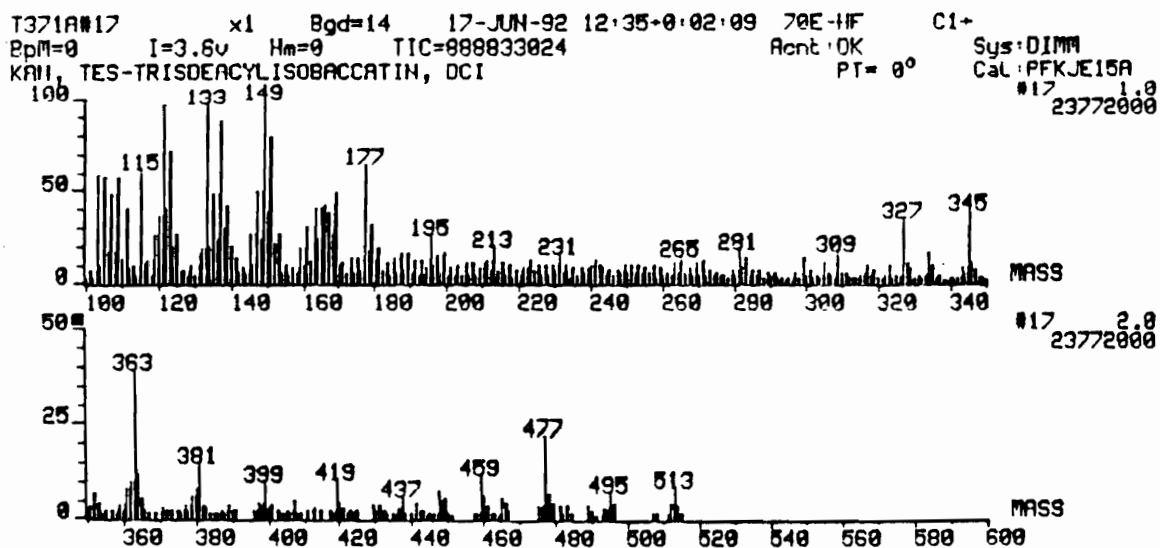
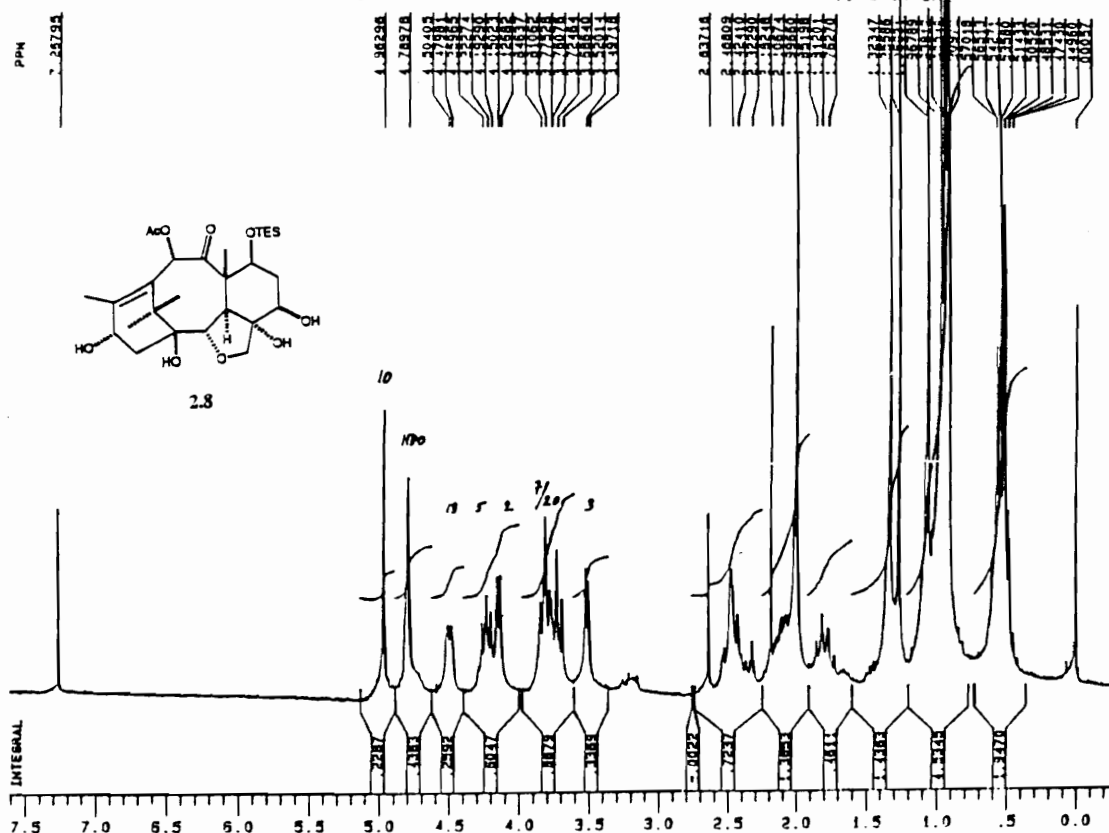


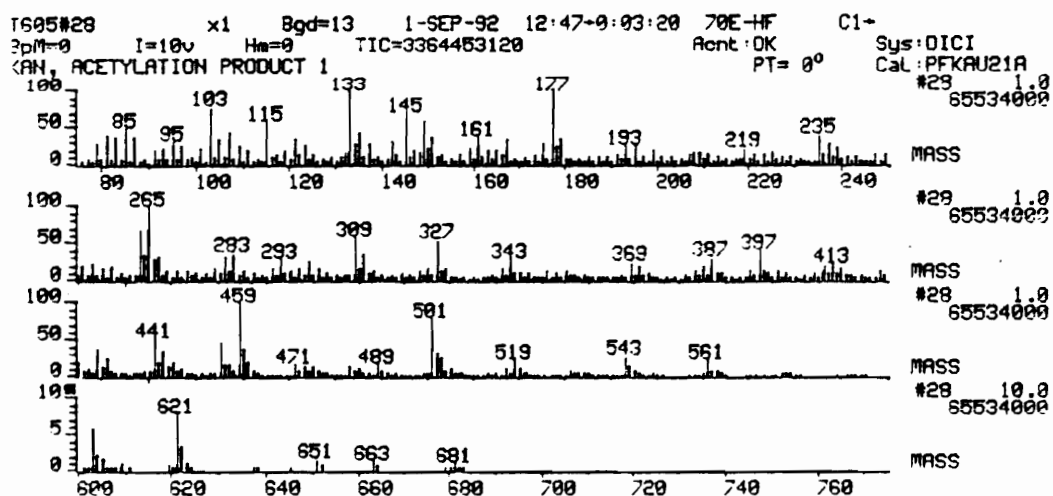
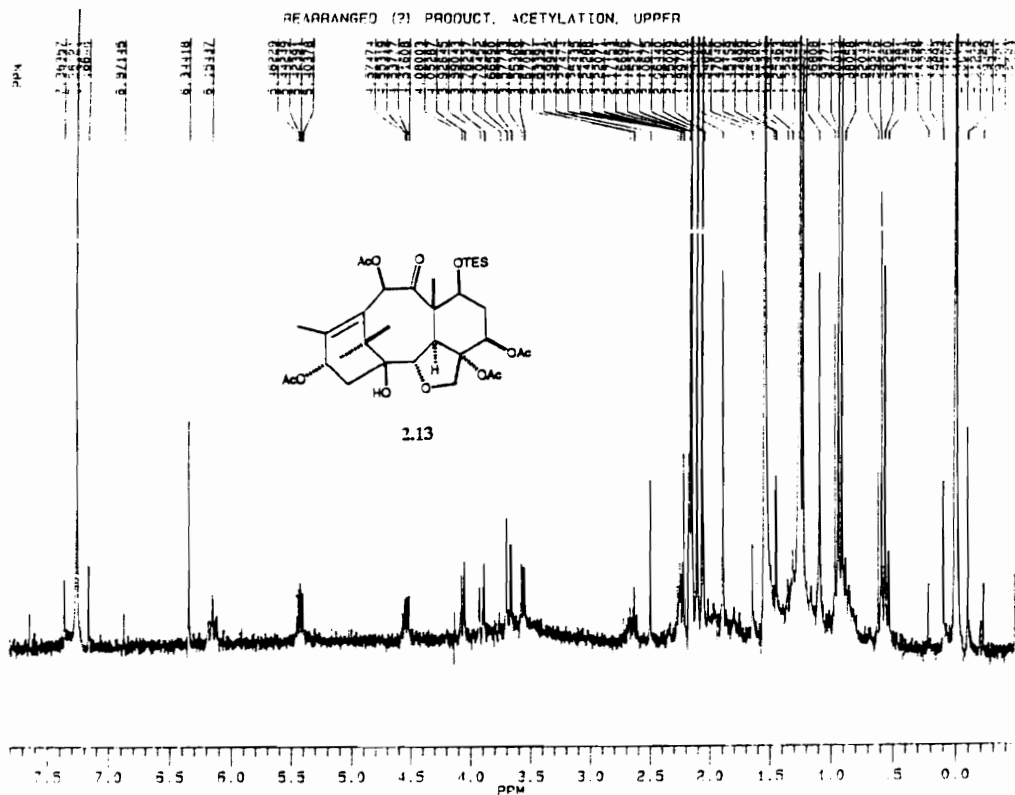


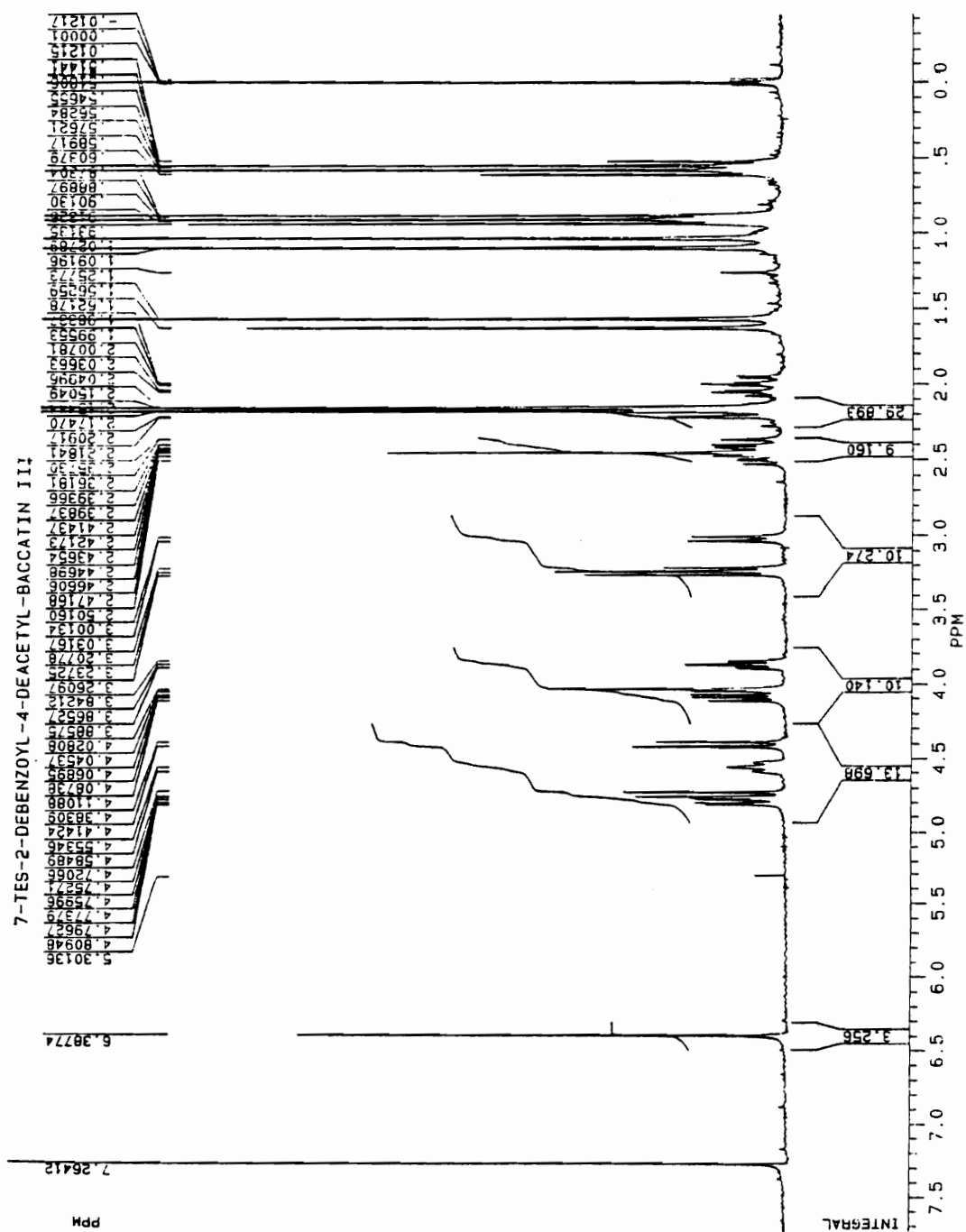




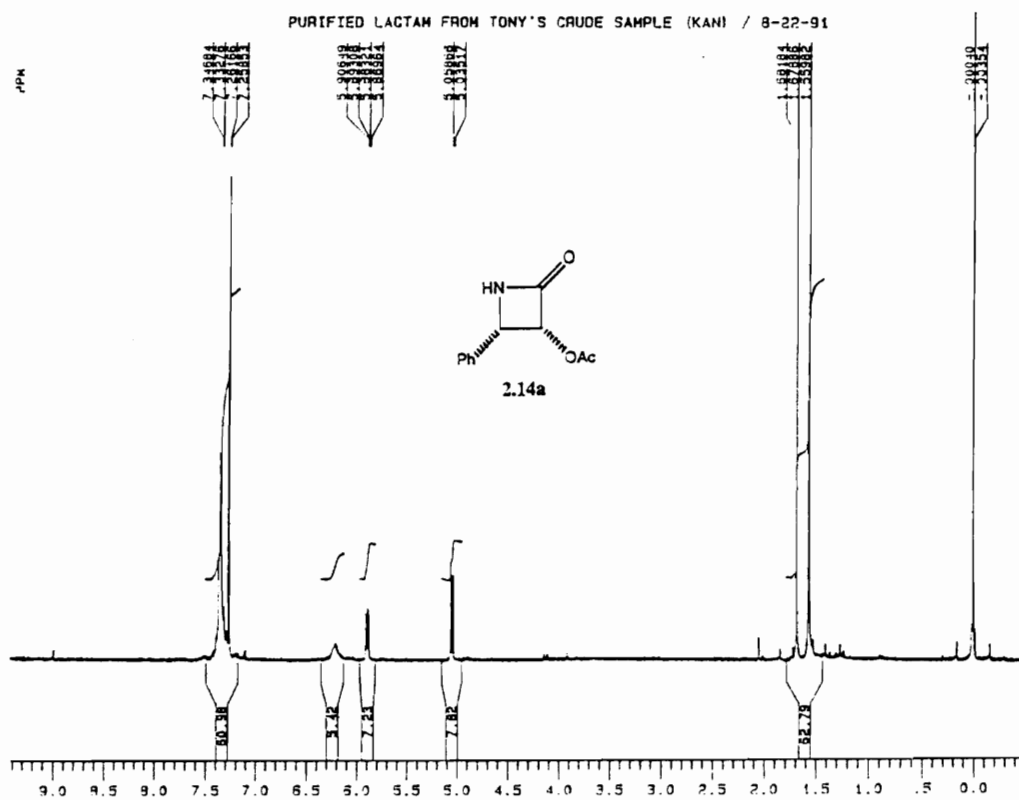
METHANOLYSIS 'BASELINE MATERIAL' (REARRANGED PRODUCT?), 5-14-92



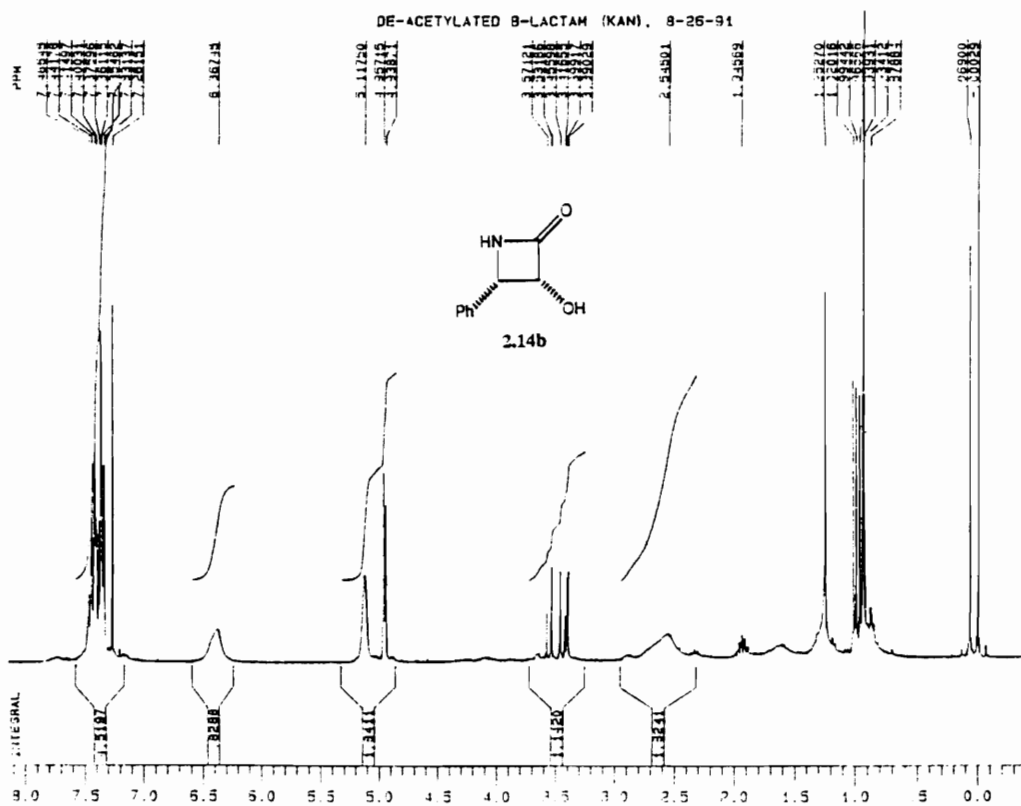


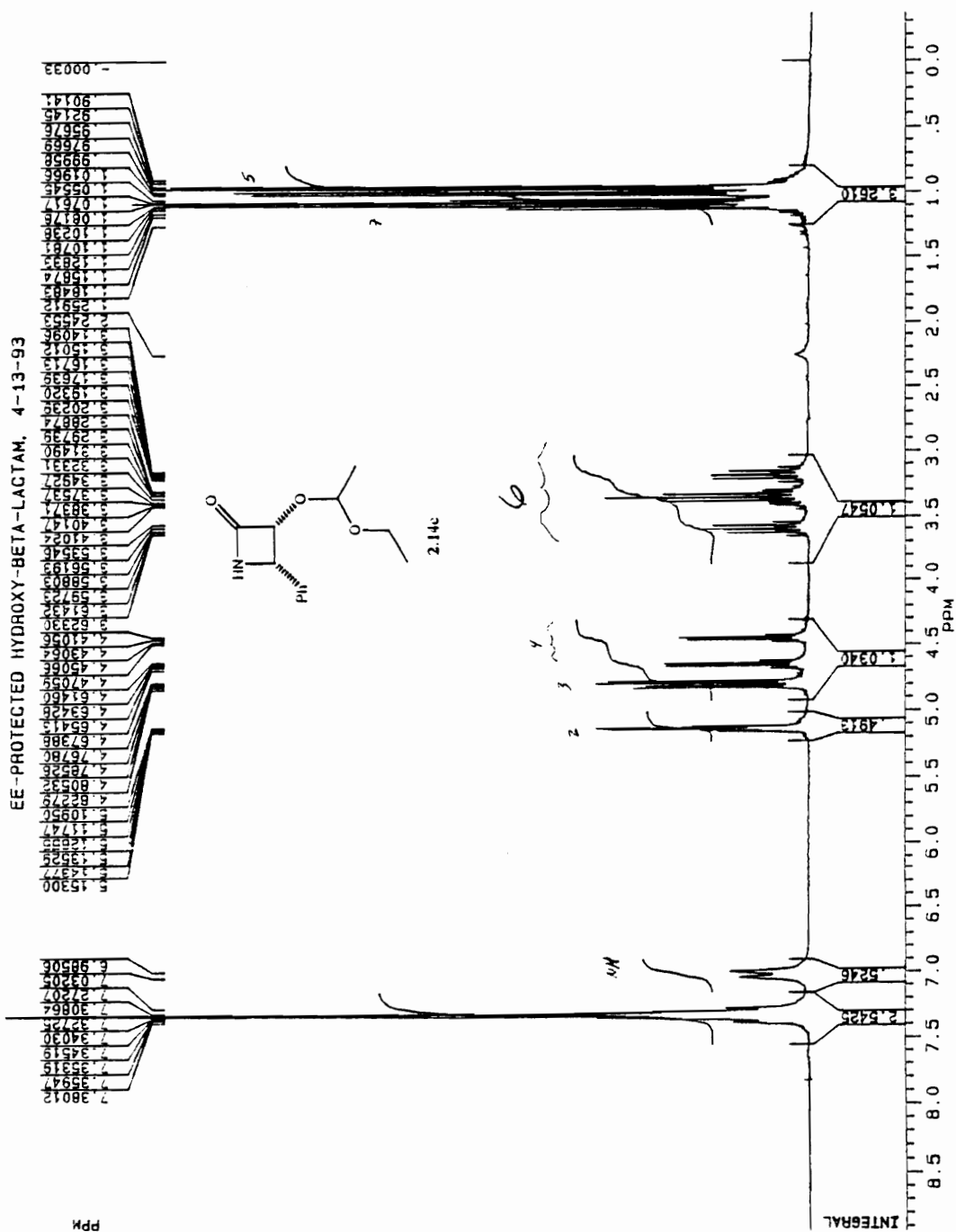


PURIFIED LACTAM FROM TONY'S CRUDE SAMPLE (KAN) / 8-22-91

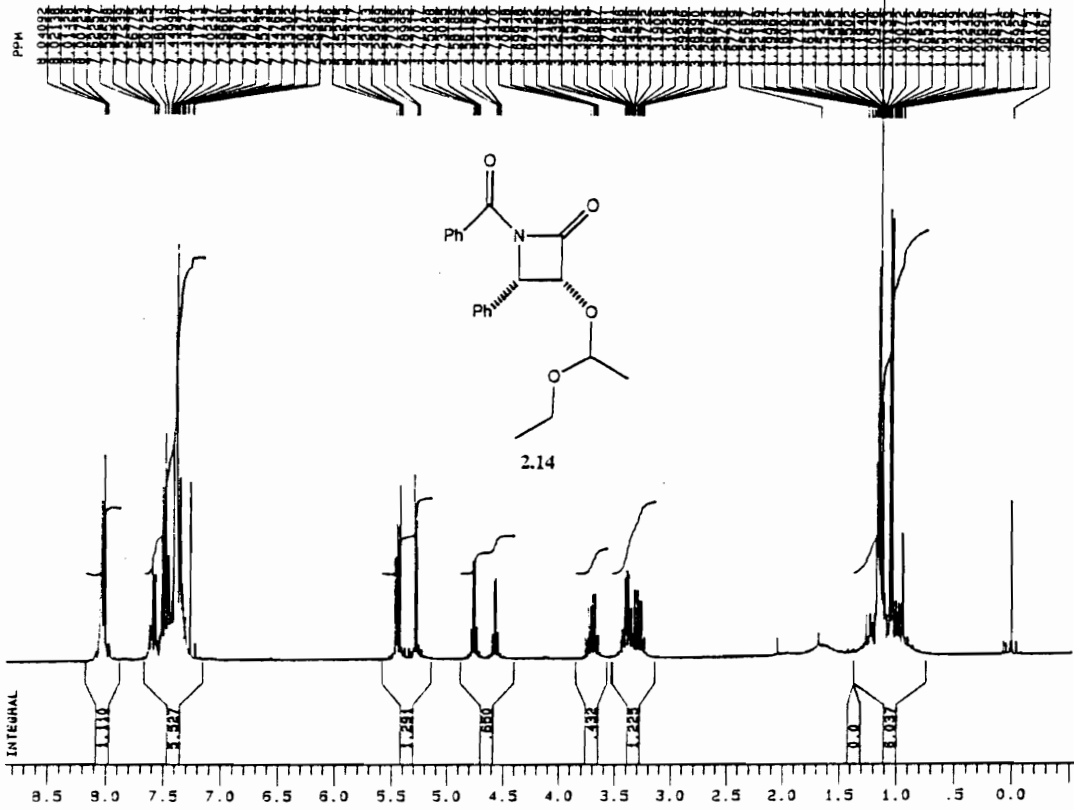


DE-ACETYLATED 8-LACTAM (KAN), 8-26-91

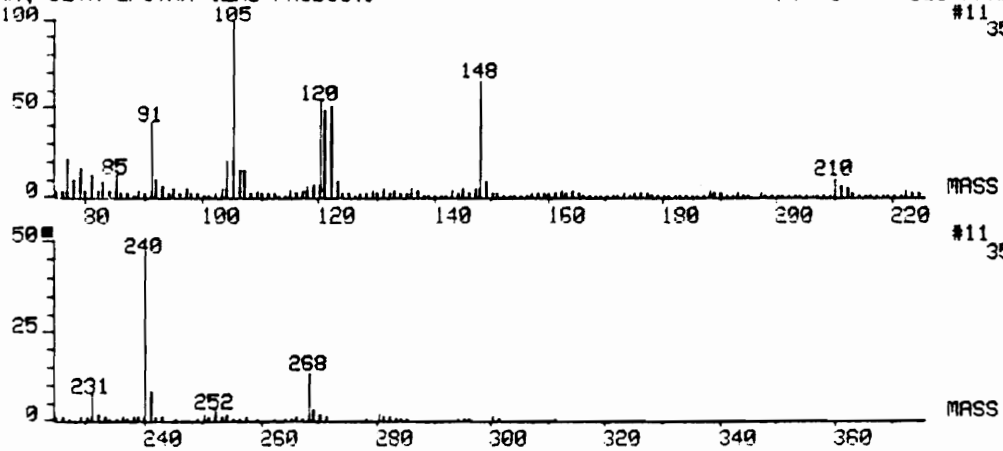




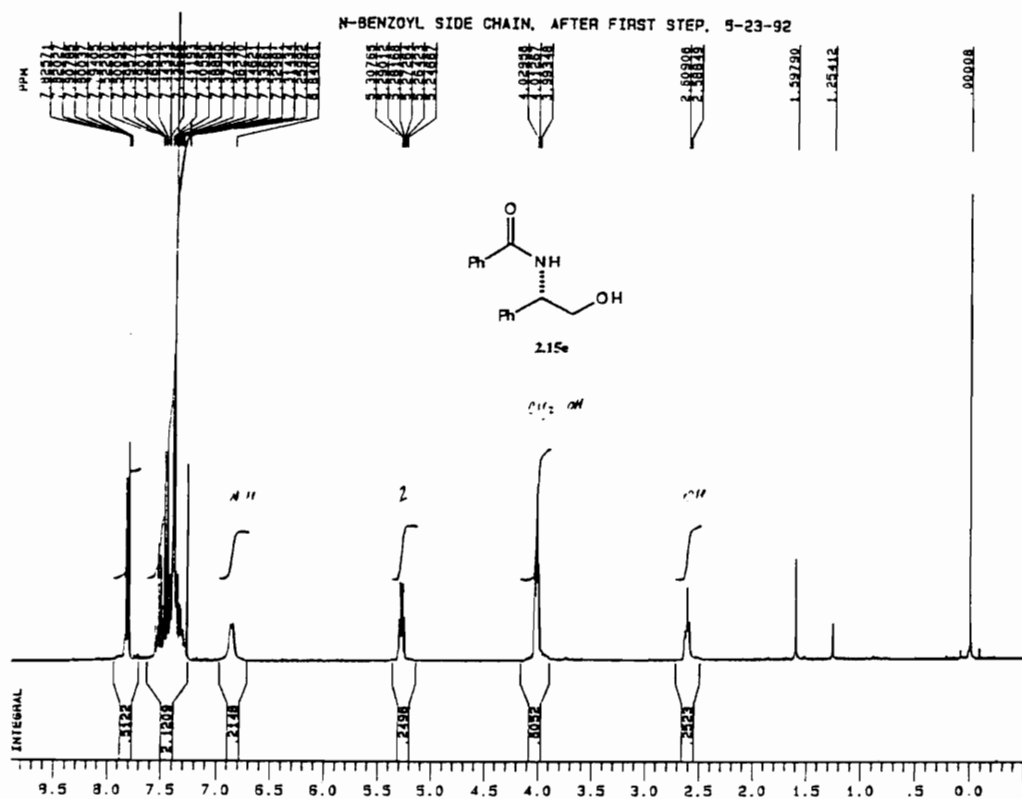
BETA-LACTAM (END PRODUCT). PURIFIED VIA PTLC. 12-9-91



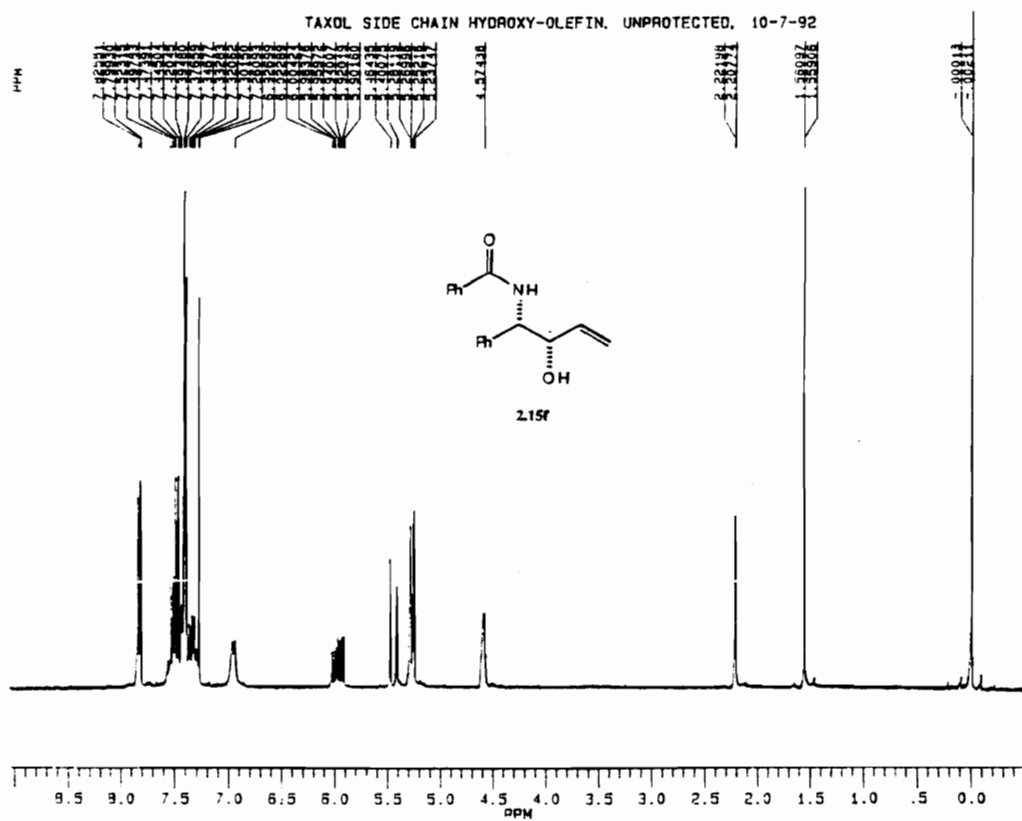
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 SpM=0 I=5.30 Hm=0 TIC=271174016 Acnt:OK Sys:OICI
 KAN, BETA-LACTAM (END PRODUCT) PT= 0° Cal: PFKMR24C
 #11 1.0
 35014000

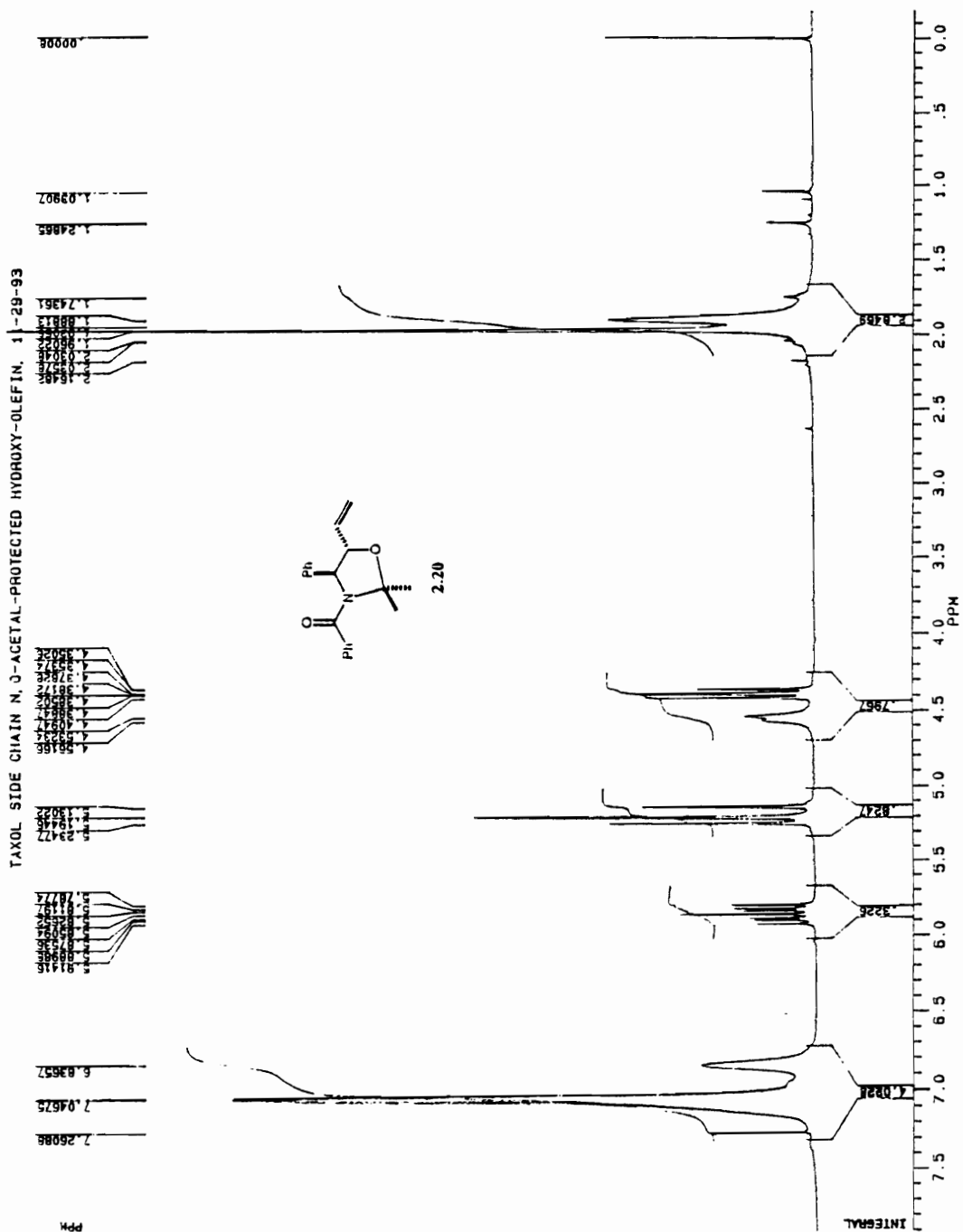


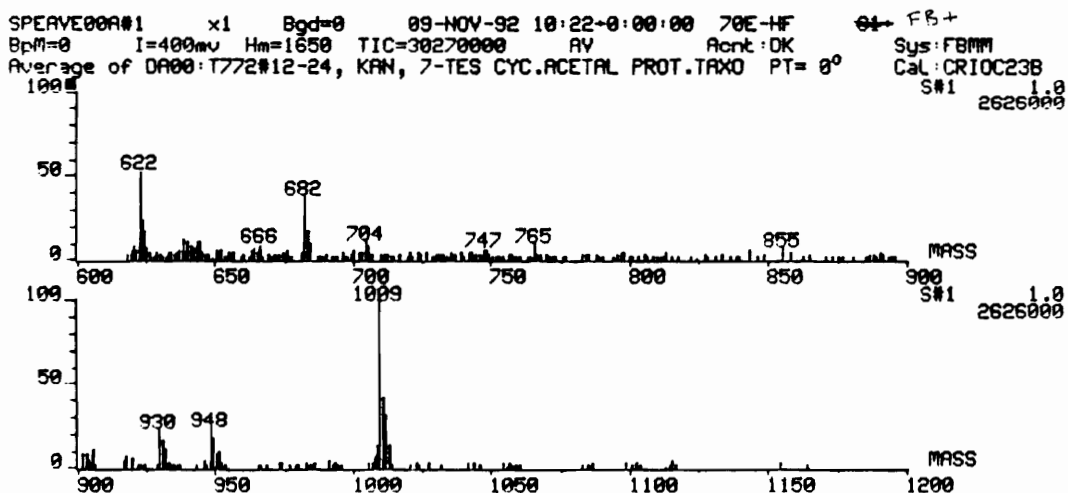
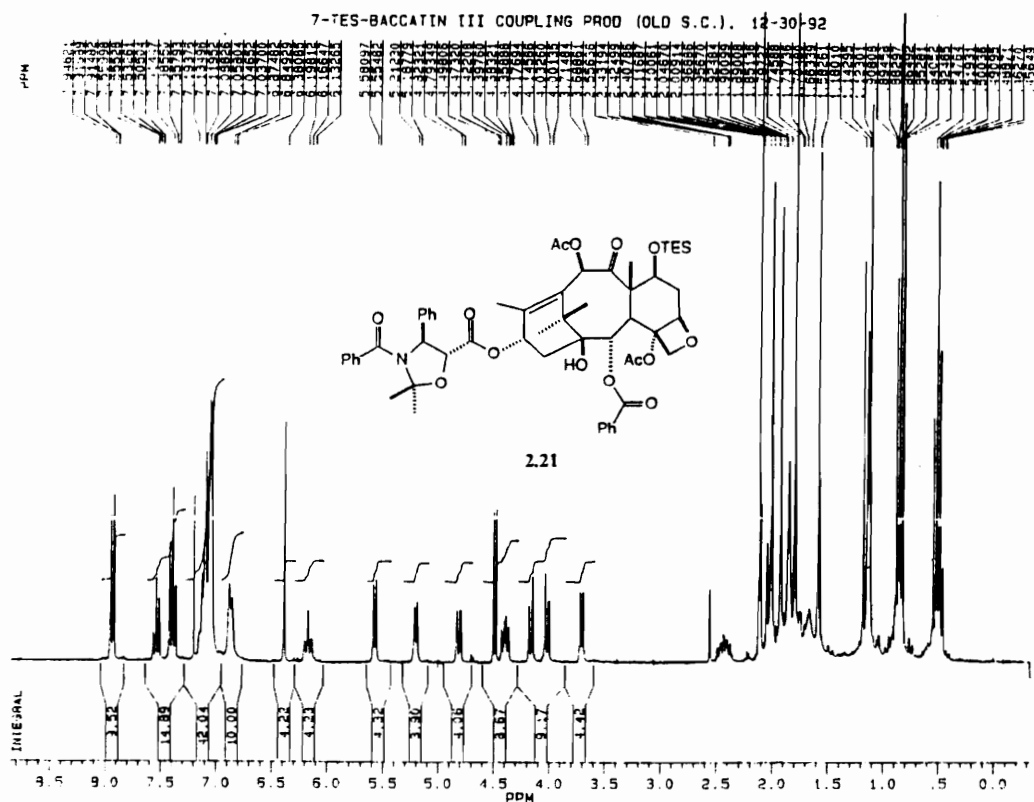
N-BENZOYL SIDE CHAIN, AFTER FIRST STEP, 5-23-92

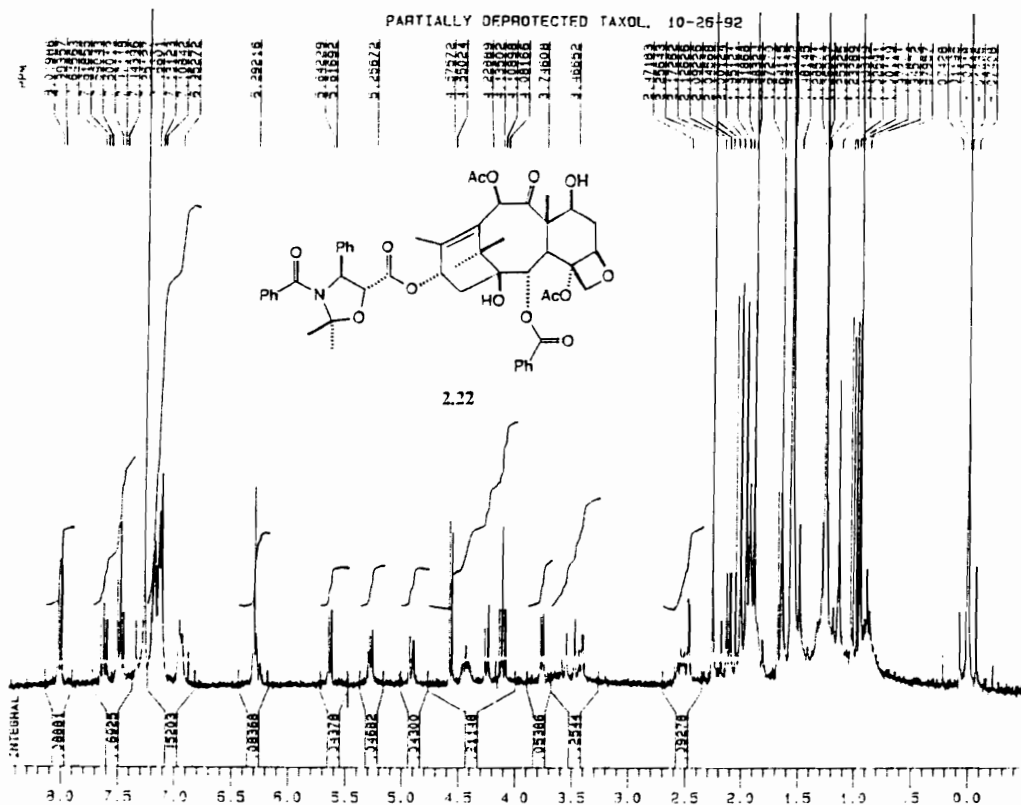


TAXOL SIDE CHAIN HYDROXY-OLEFIN. UNPROTECTED, 10-7-92



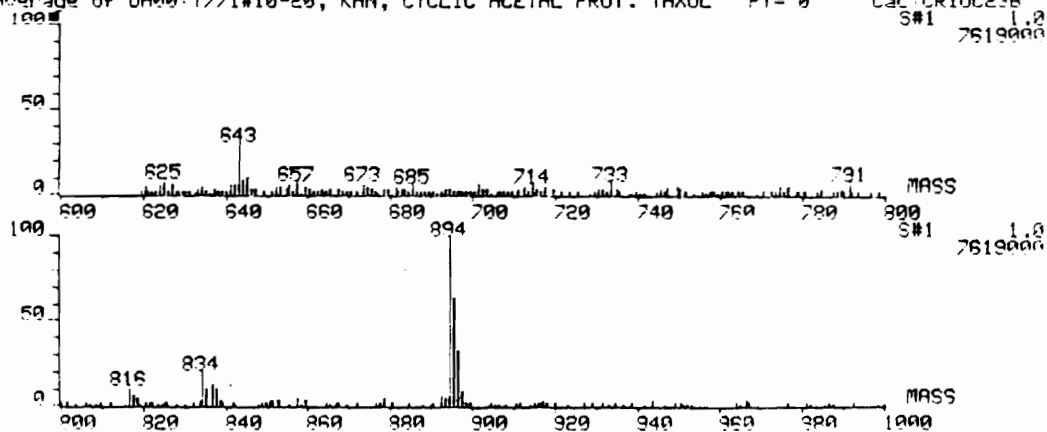




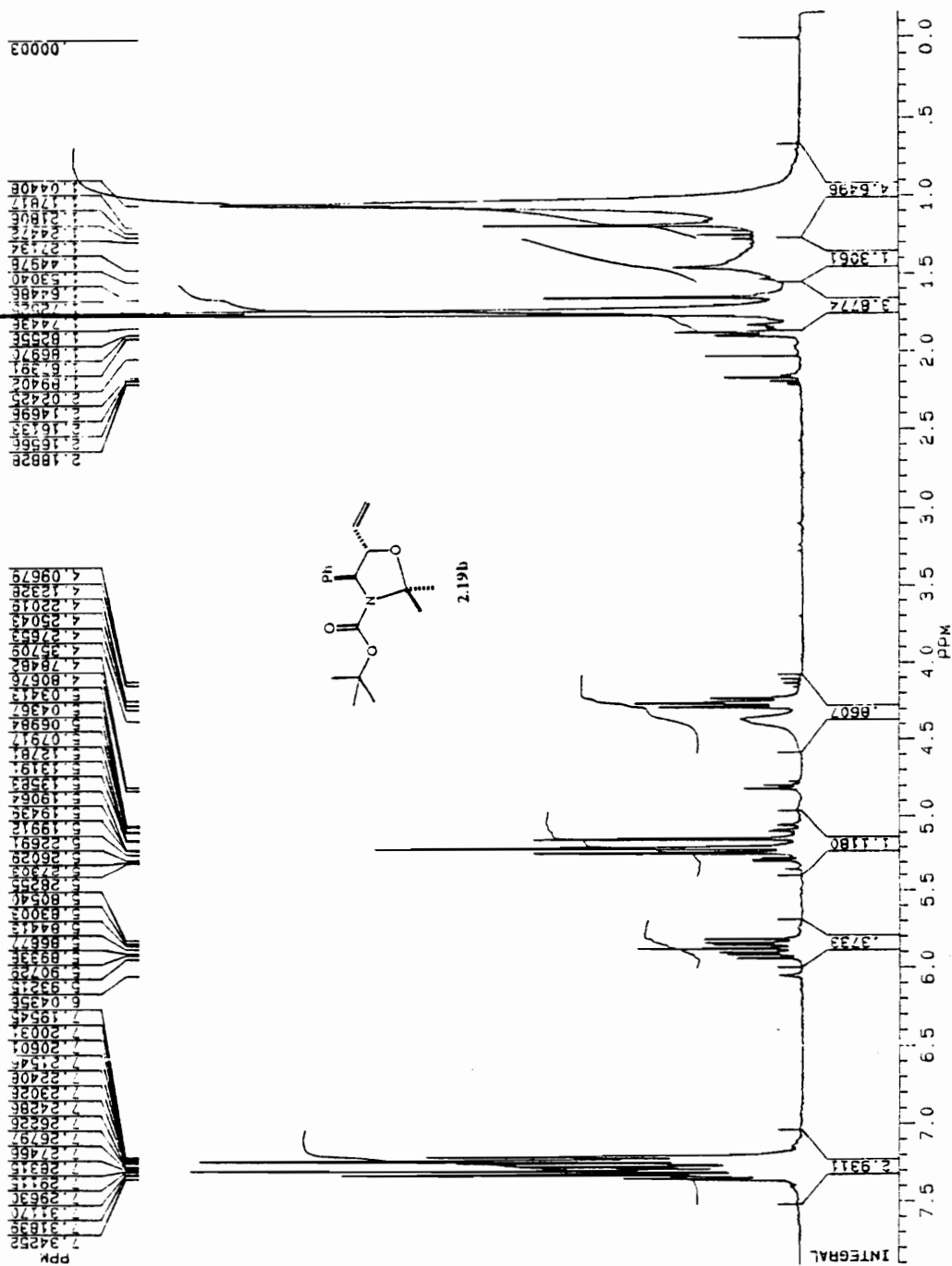


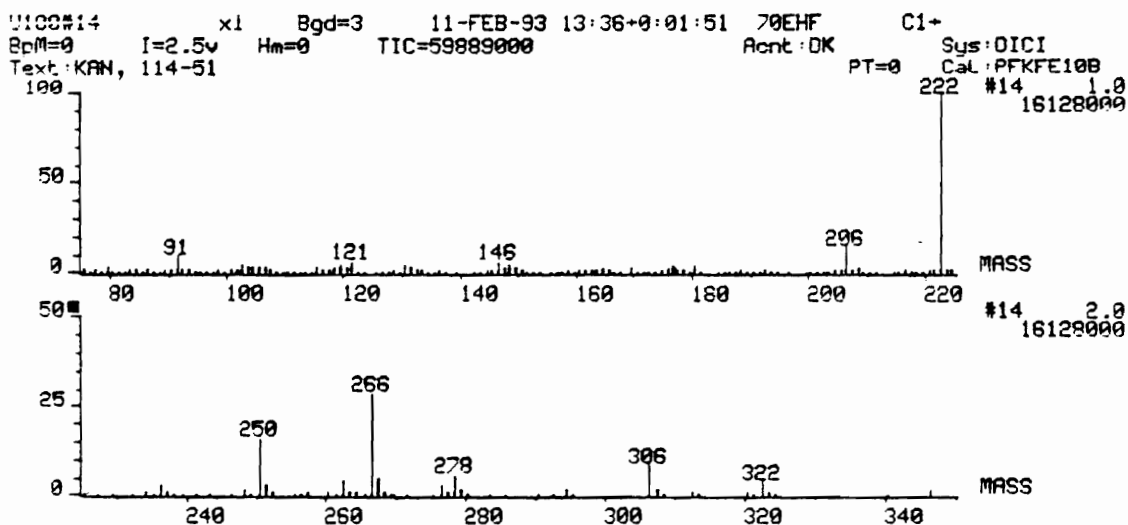
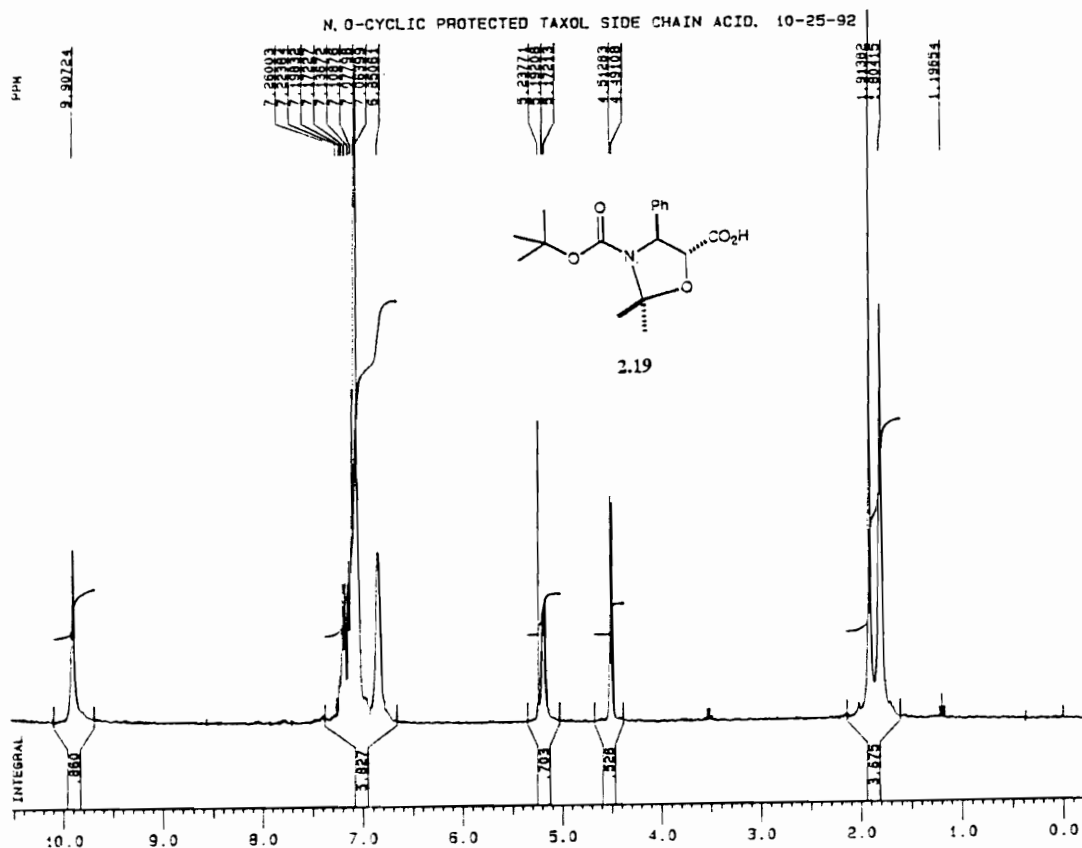
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 RPM=0 I=1.2v Hm=1650 TIC=58531000 AV Acnt:OK
 Average of 0A00:T771#10-20, KAN, CYCLIC ACETAL PROT. TAXOL PT= 0°

FS+
 Sys:FBMM
 Cal:CR10C23B
 S#1 1.2
 7619000

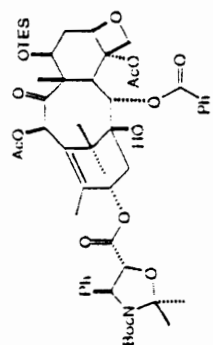


N-TBUC N, O-CYCLIC ACETAL-PROT'D HYDROXY-OLEFIN, 11-9-92

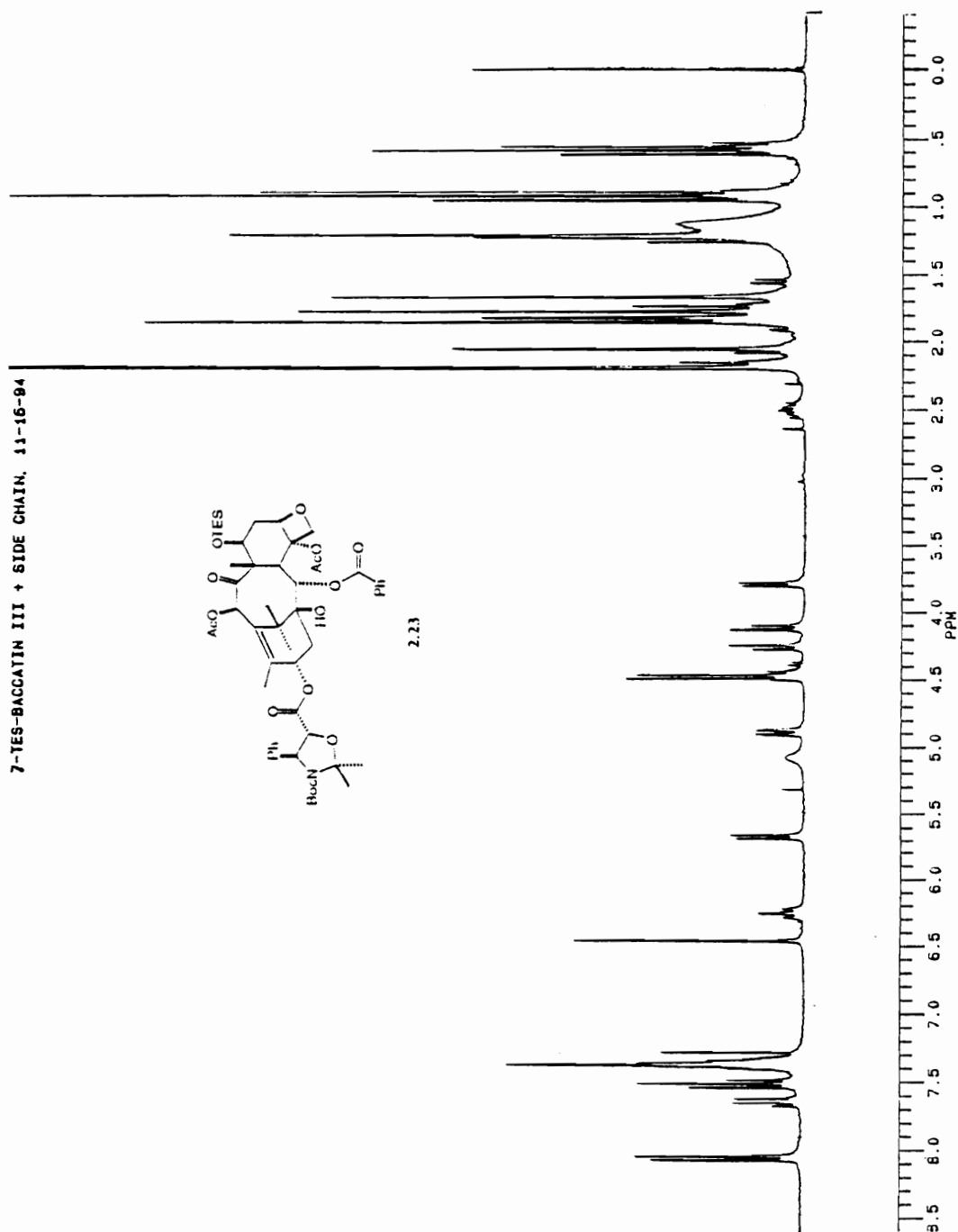


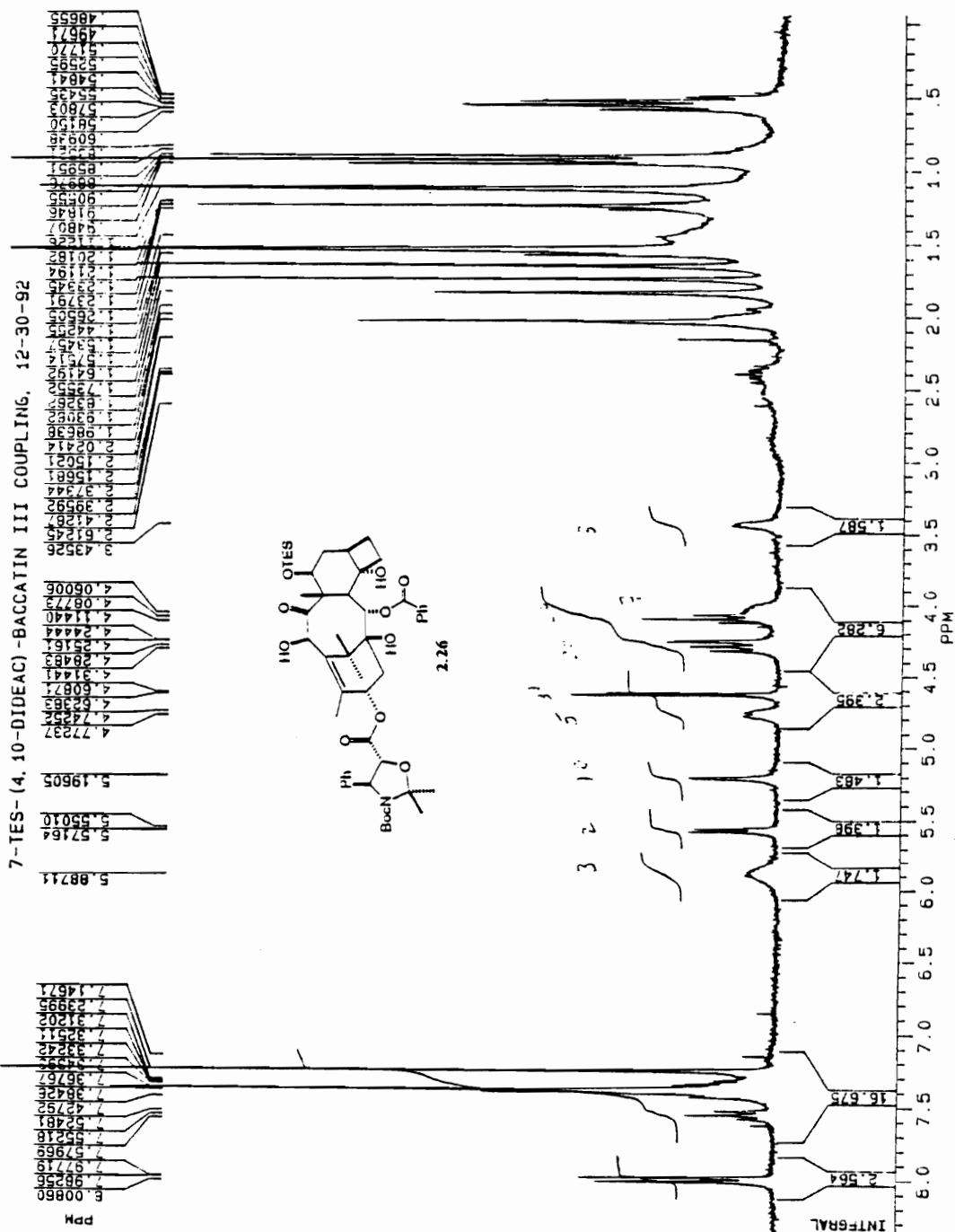


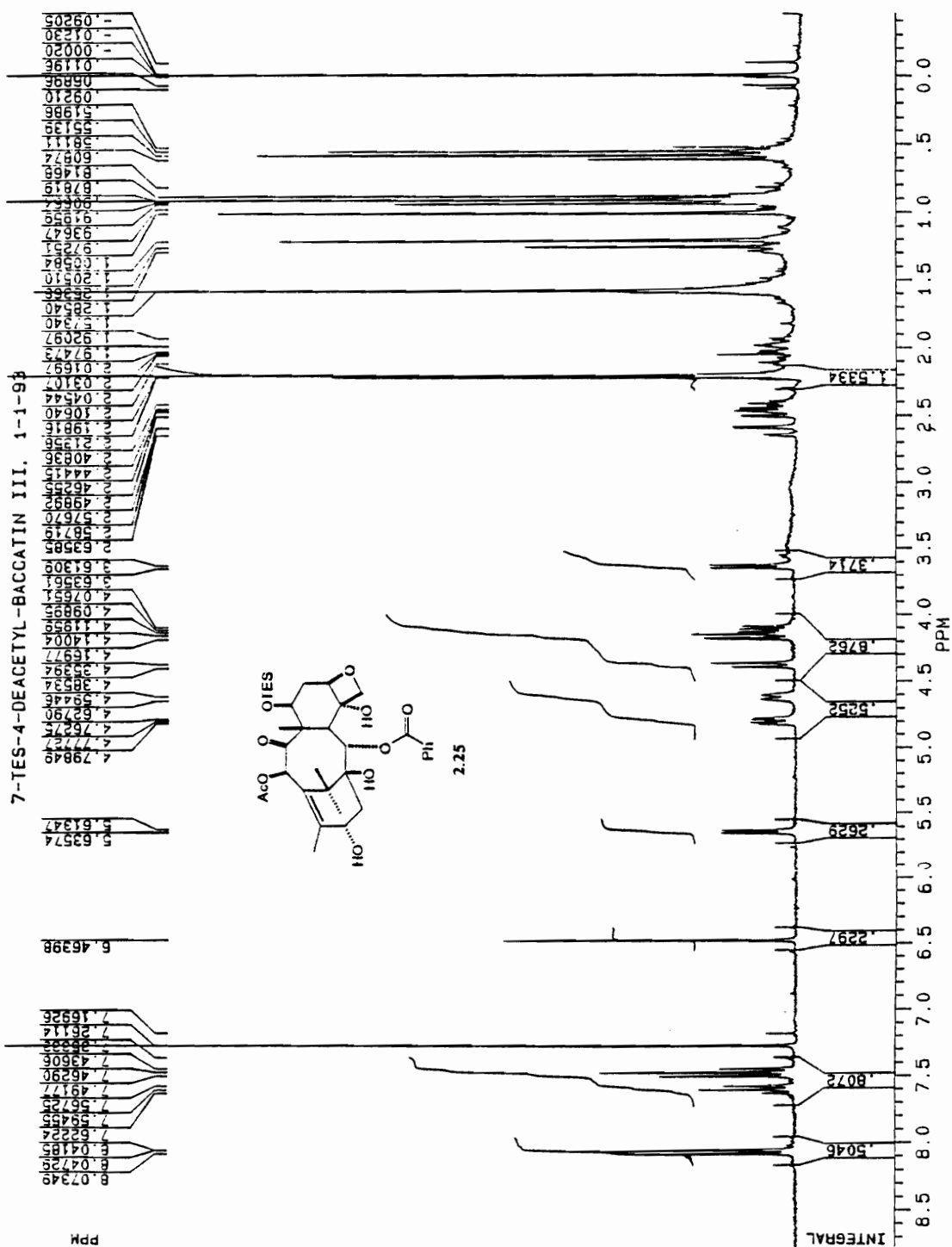
7-TES-BACCATIN III + SIDE CHAIN, 11-16-94

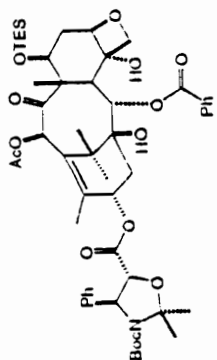


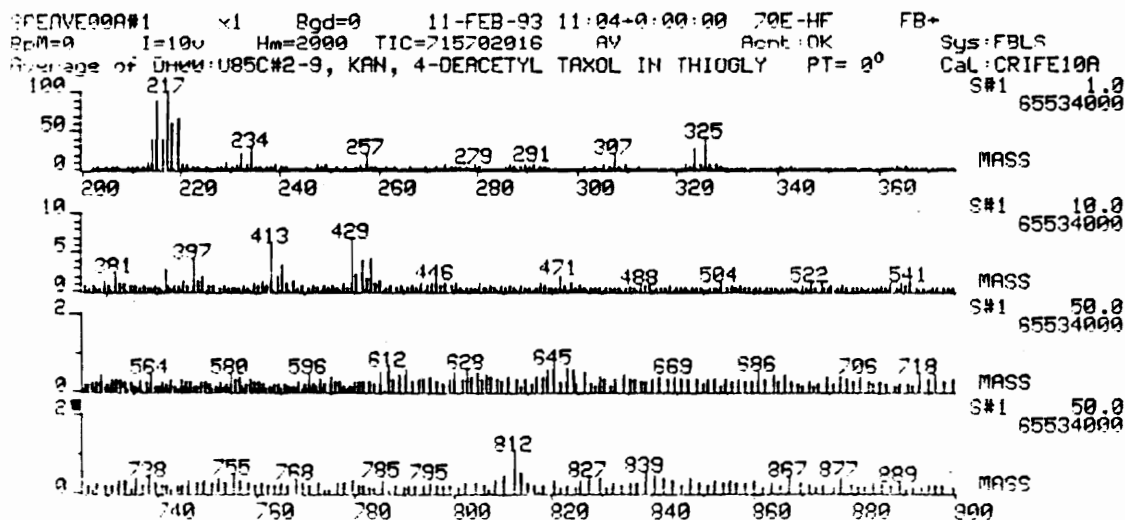
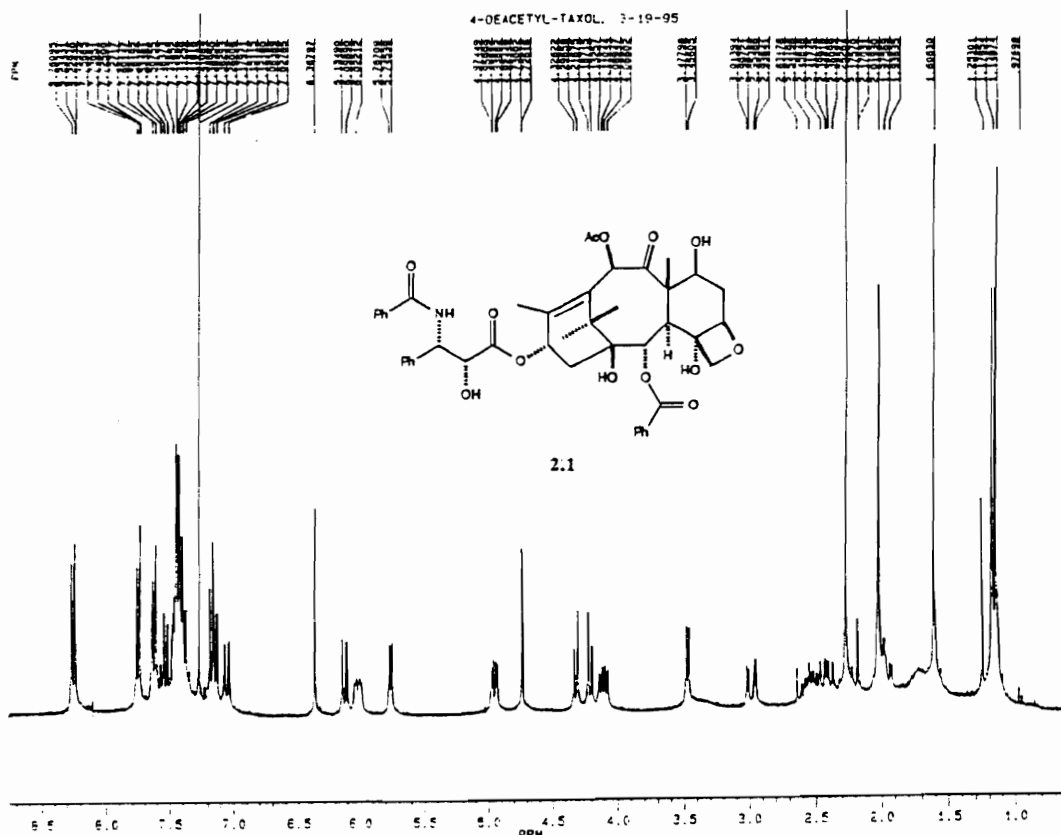
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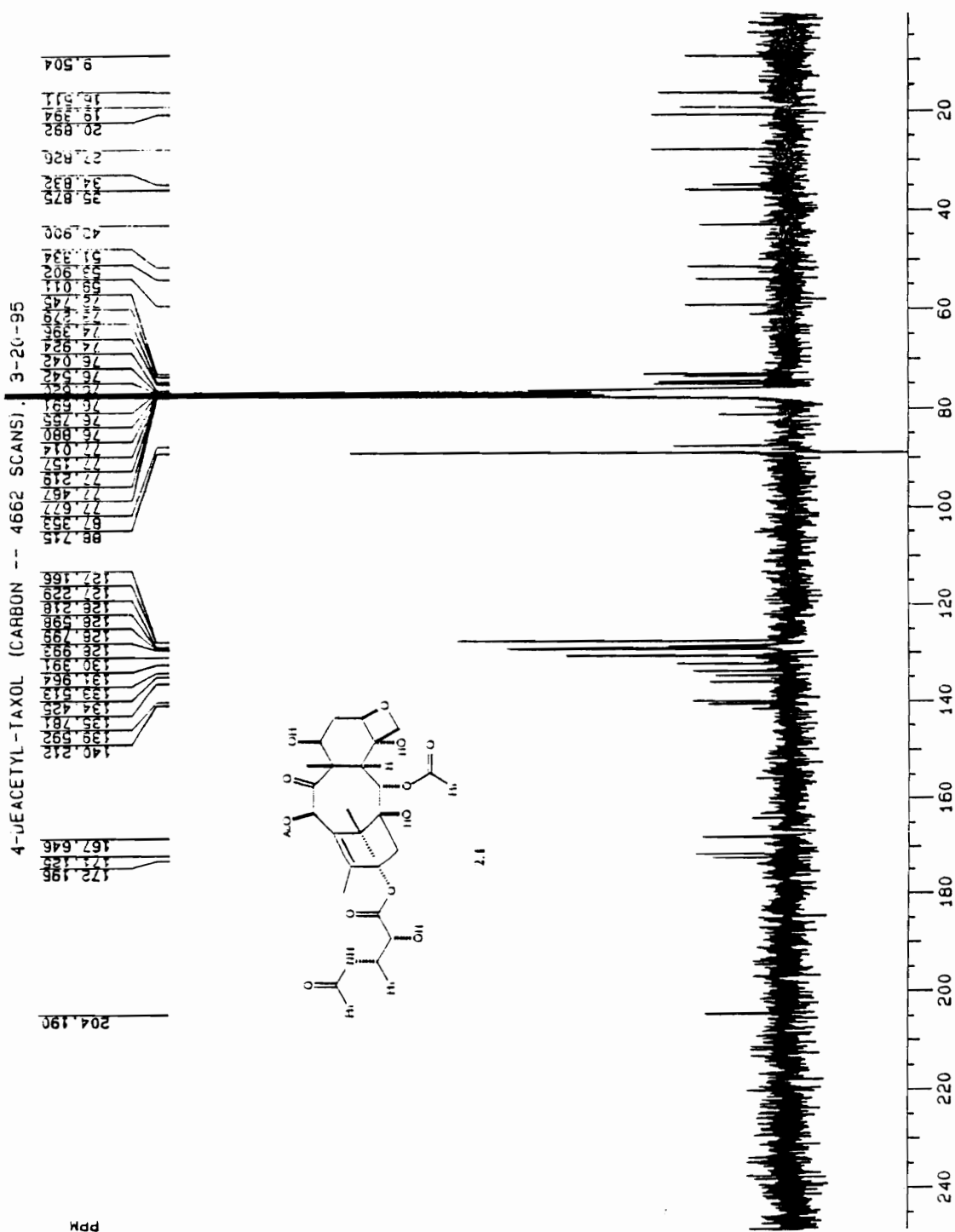


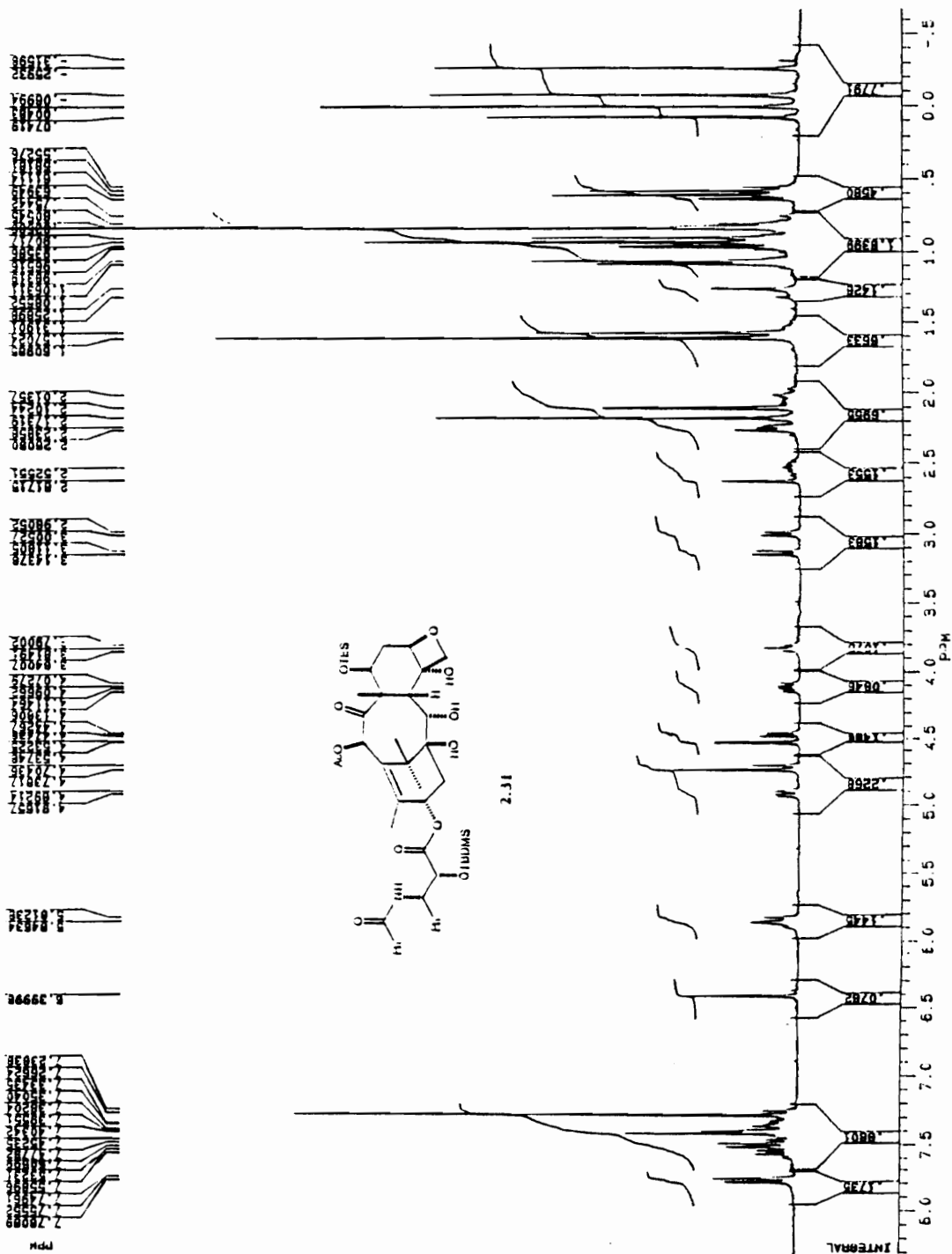


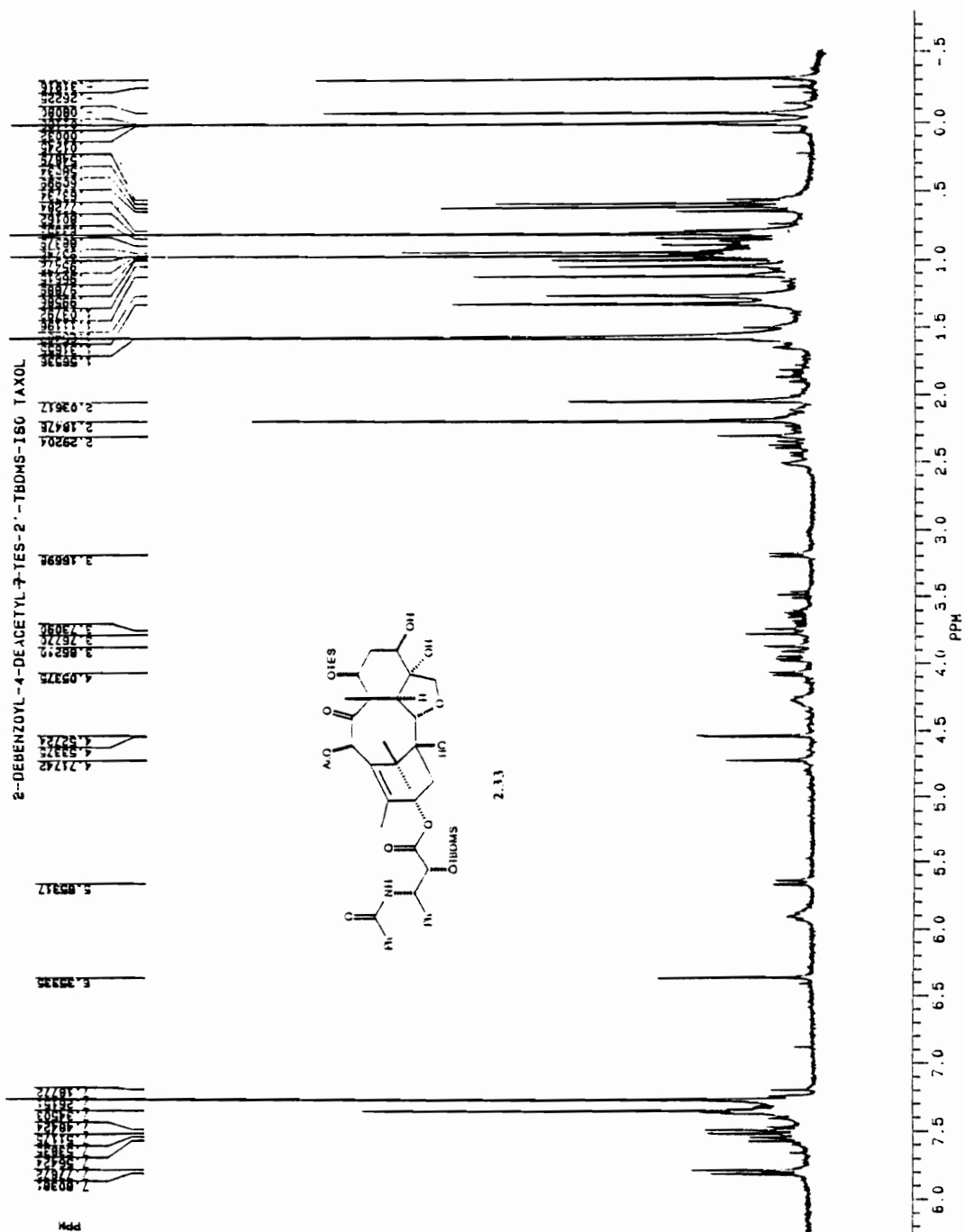


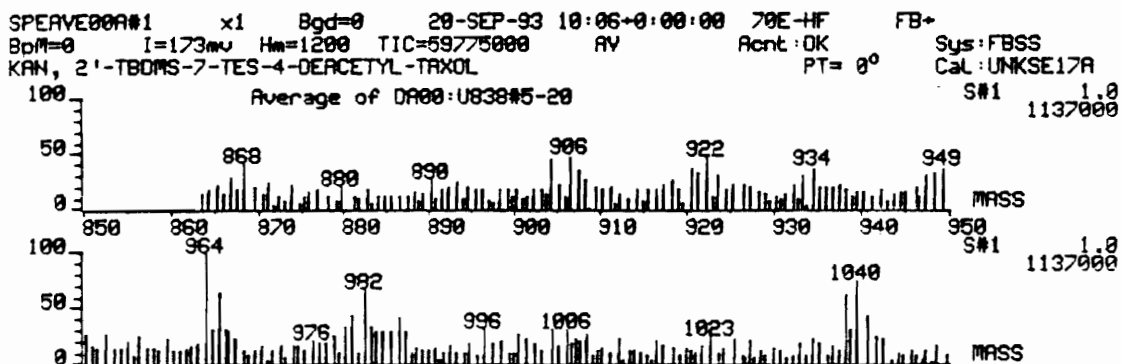
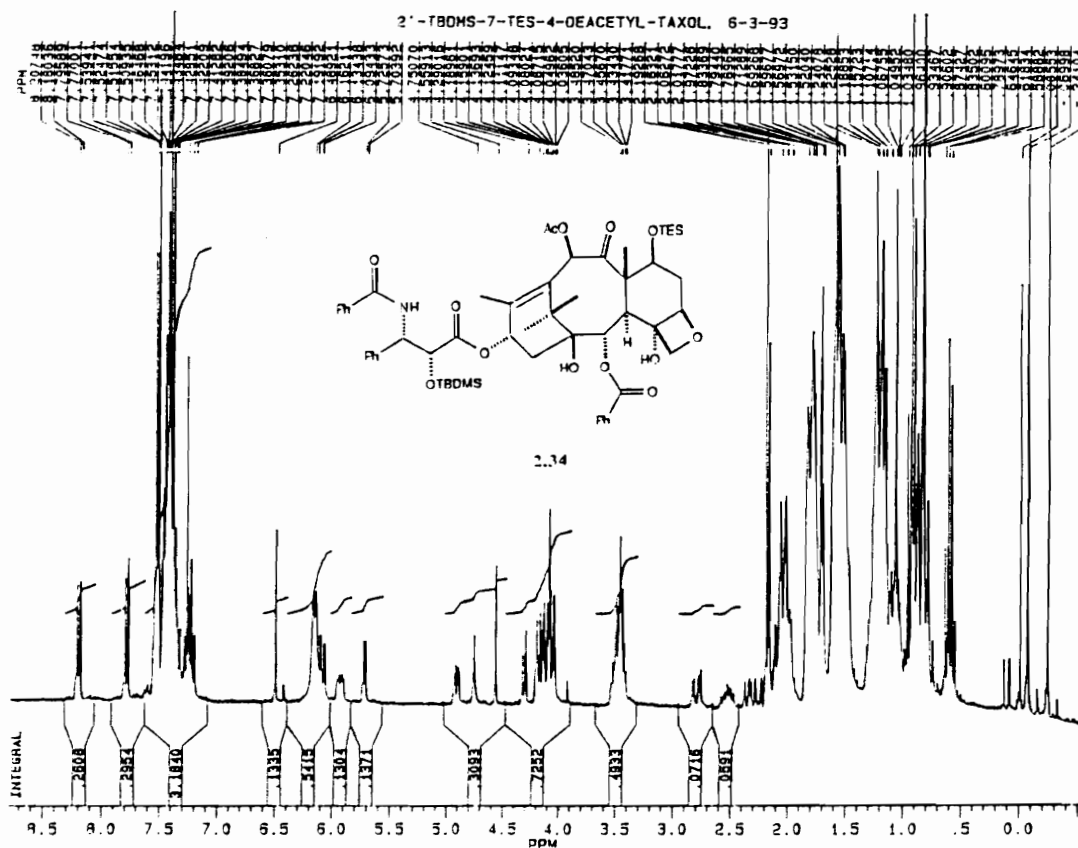
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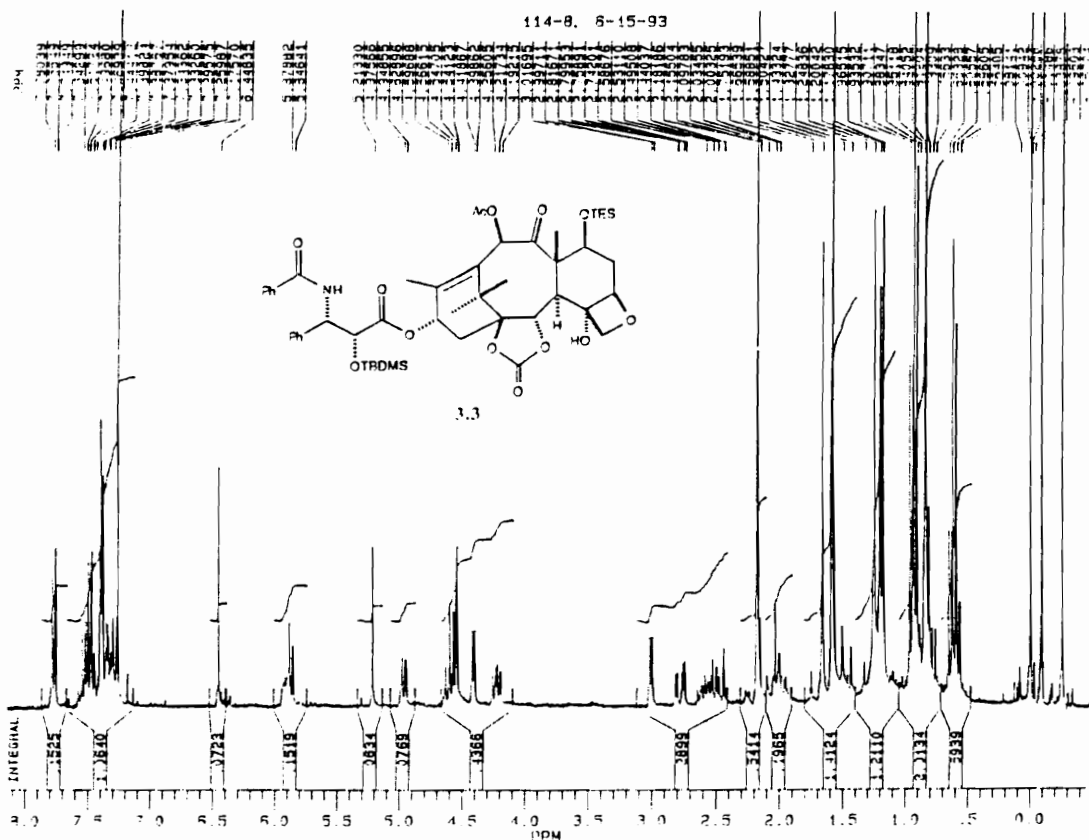




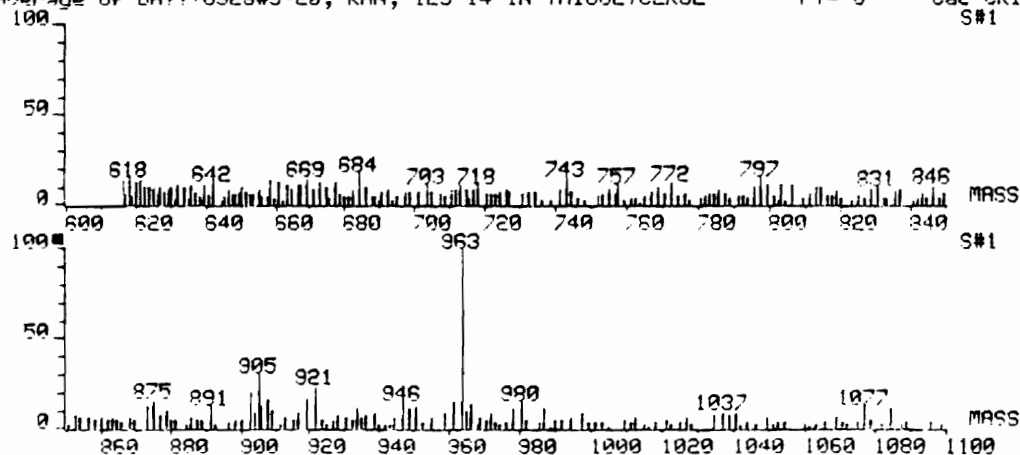


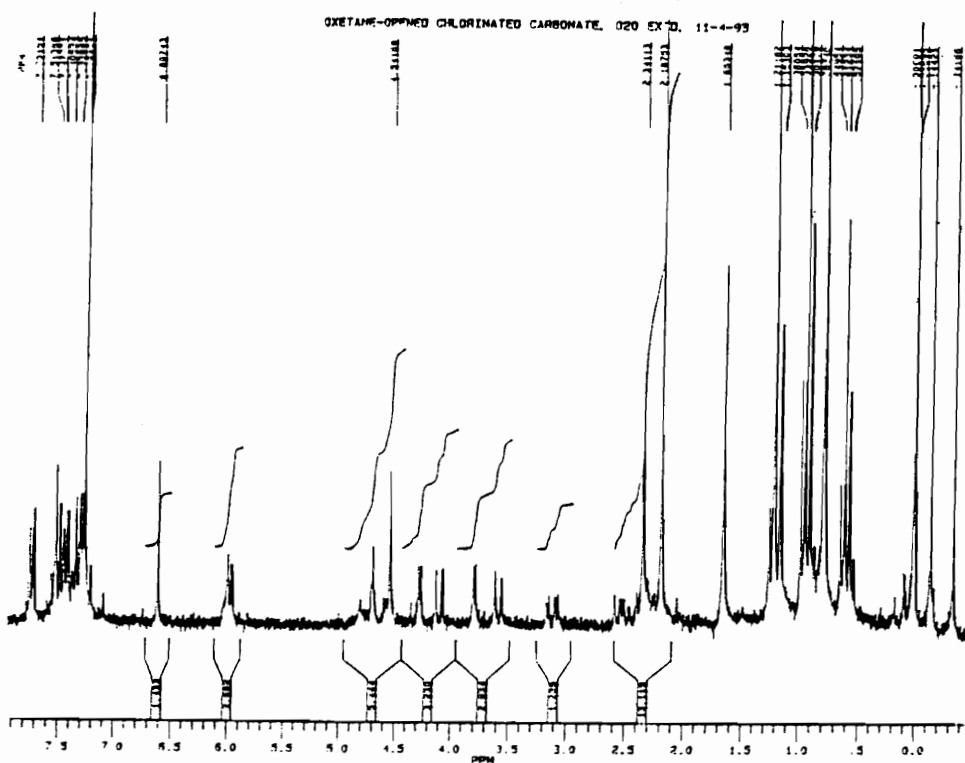


114-8, 8-15-93

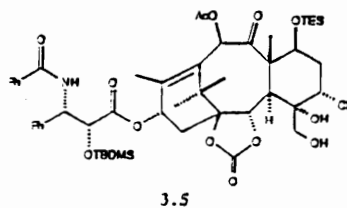
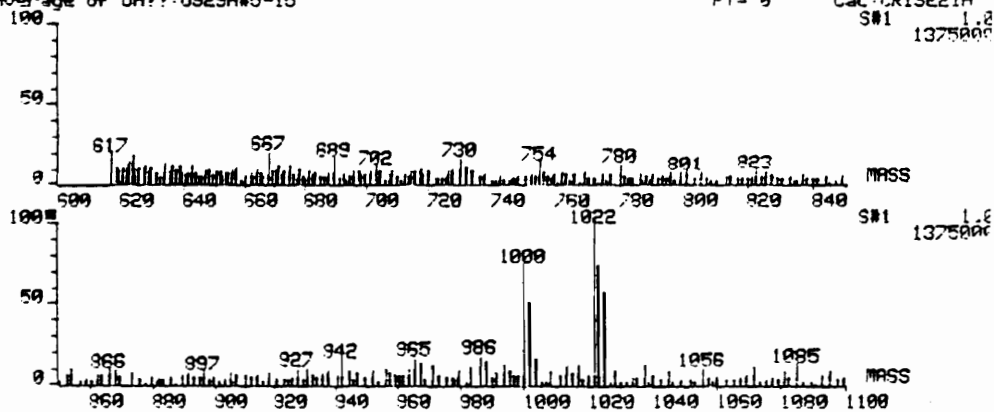


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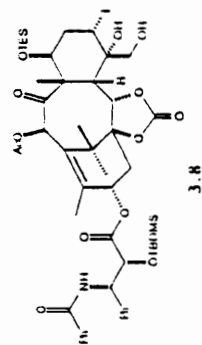
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 S#1 1375000 1.2



LG-131-157-2
 OBSERVED
 FREQUENCY 399.951 MHz
 SPECTRAL WIDTH 5000.0 Hz
 ACQUISITION TIME 3.14 sec
 RELAXATION DELAY 1.100 sec
 PULSE WIDTH 3.6 usec
 AMBIENT TEMPERATURE
 NO. REPLICATIONS 240
 DOUBLE PRECISION ACQUISITION
 DATA PROCESSING
 FT SIZE 65536
 TOTAL ACQUISITION TIME 18 minutes
 Oct 29 95
 Virginia Tech STC NMR Facility

8 7 6 5 4 3 2 1 0 ppm
 8.51 90.00 4.71 4.52 3.70 3.96 3.61 7.90 3.40 3.36 19.95 22.95 41.02 39.74 10.62 11.09
 10.62 11.09 39.74 39.1924.71

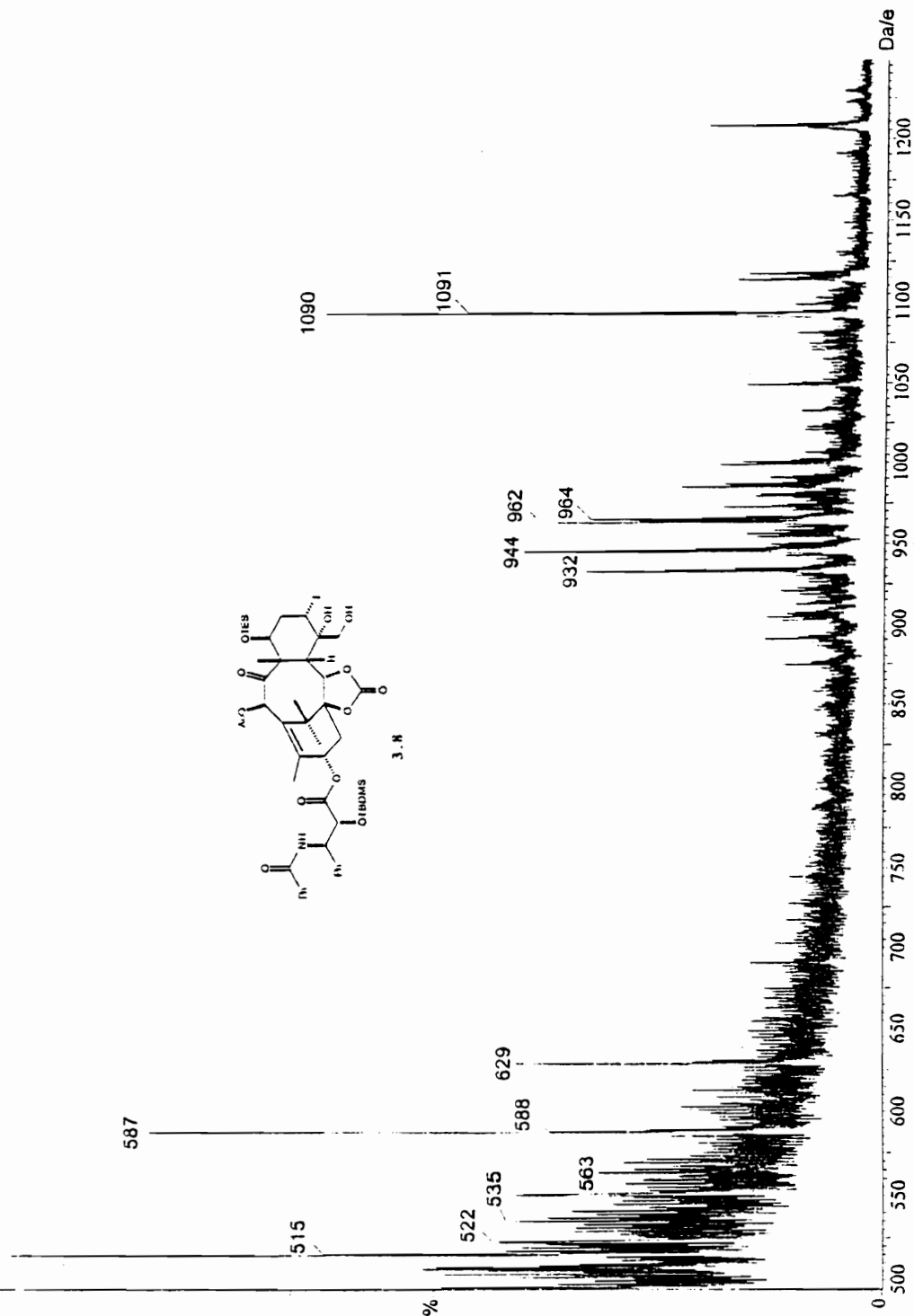
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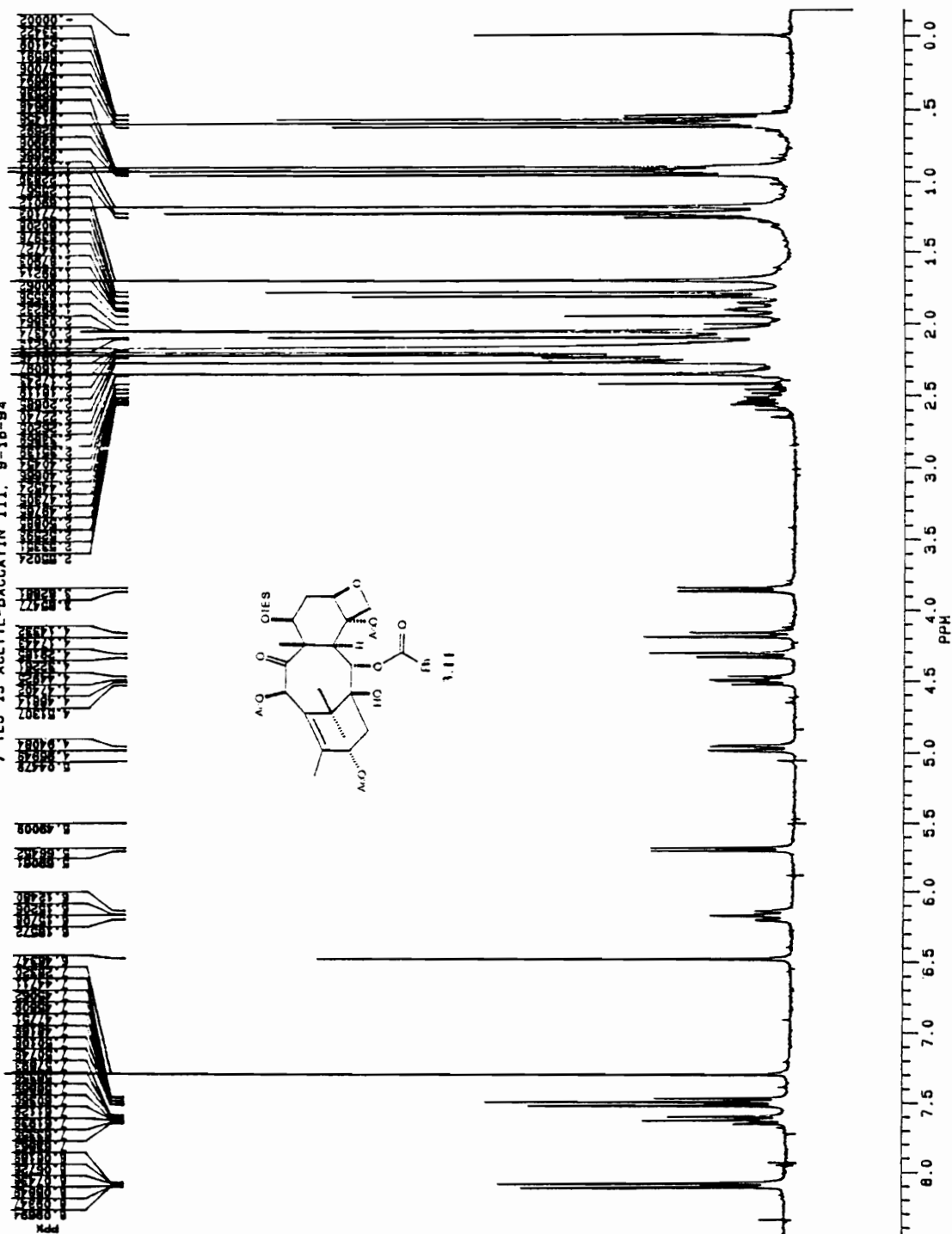
KAN-130-3 IN THIOGLYCEROL
Analytical Services
K11173.1 (2.617)

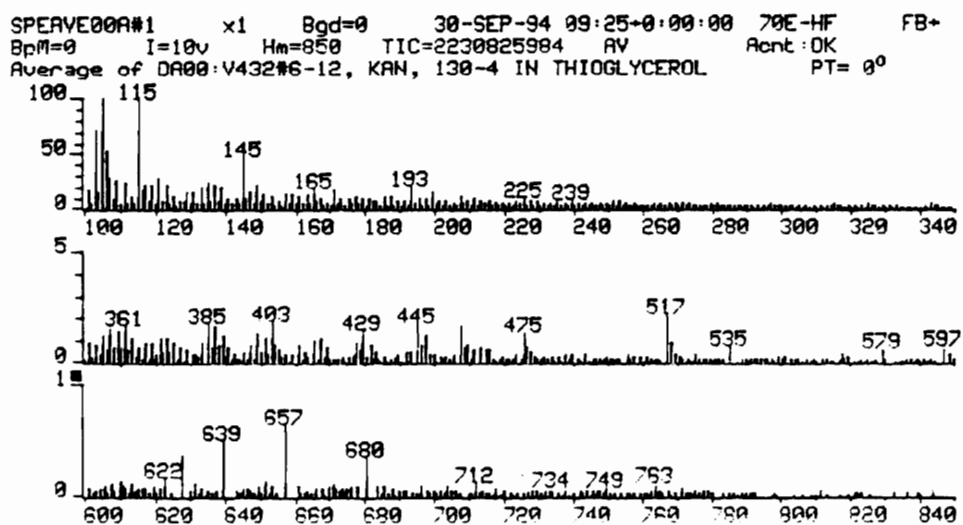
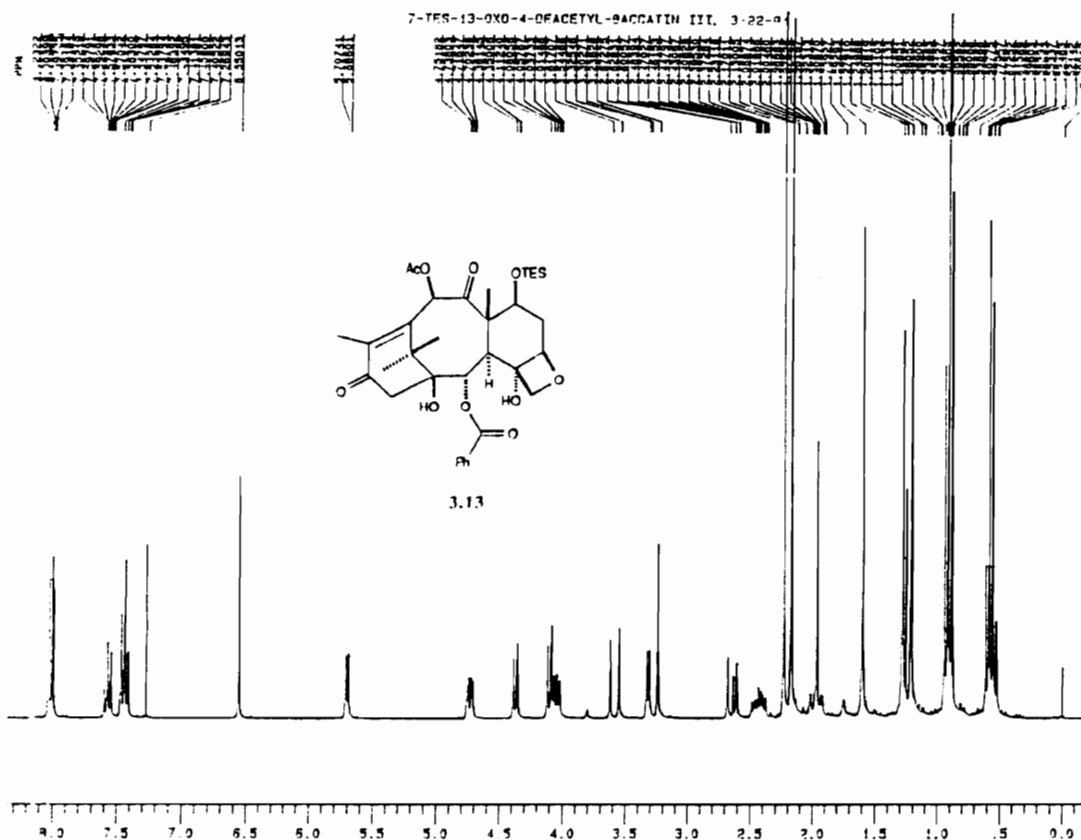
VG Quattro

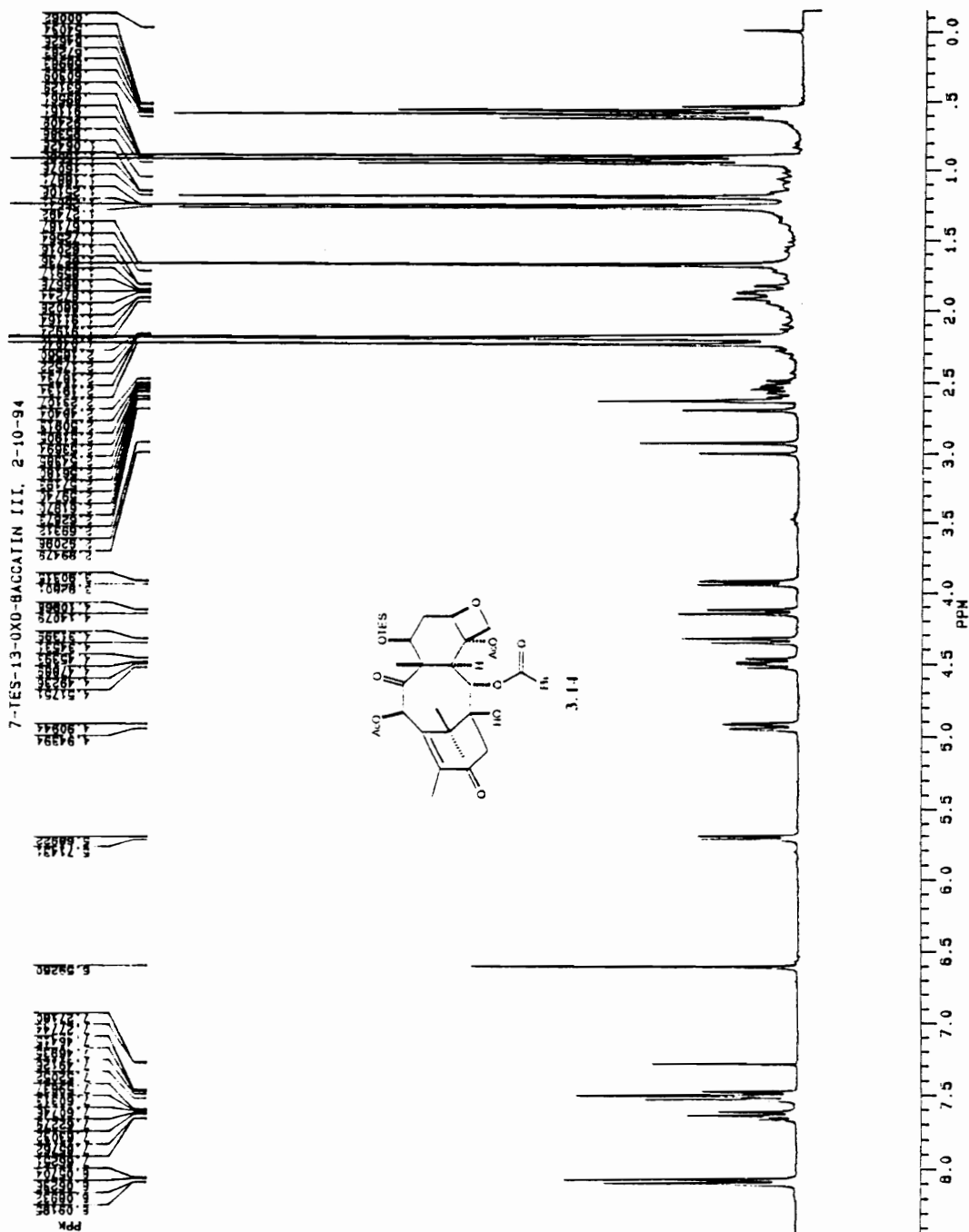
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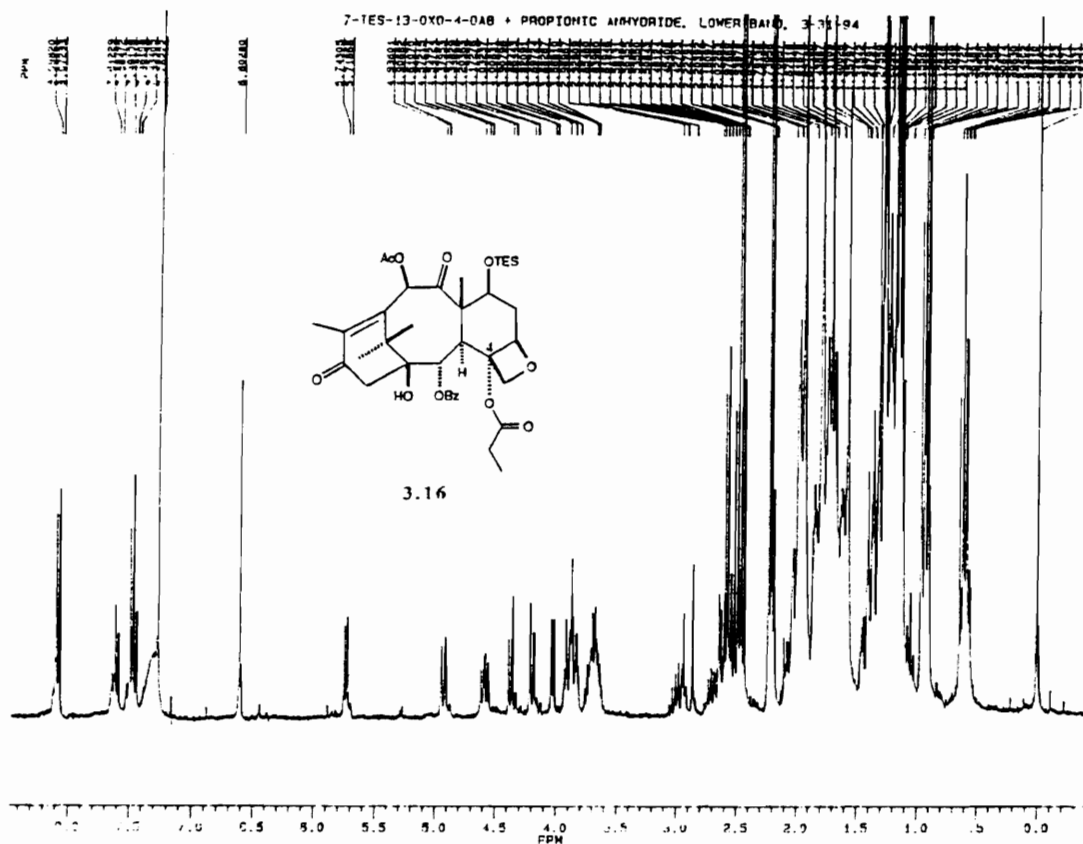


7-TES-13-ACETYL-BACCATIN III, 9-18-94

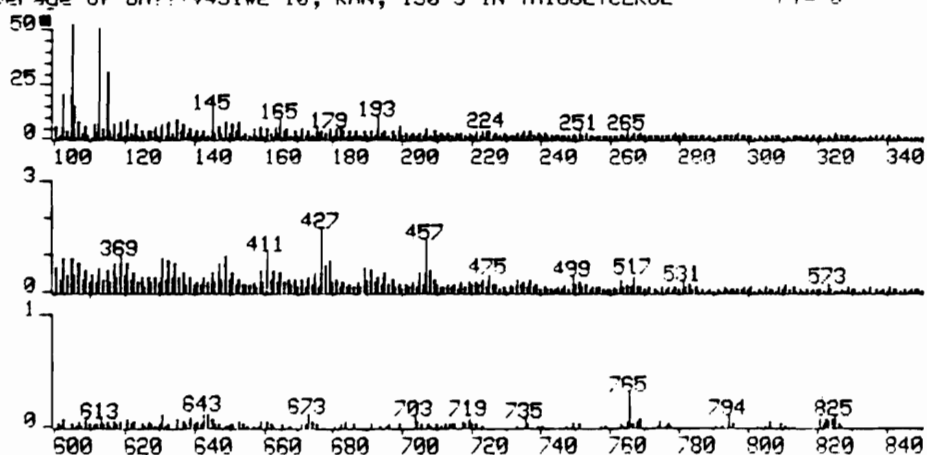




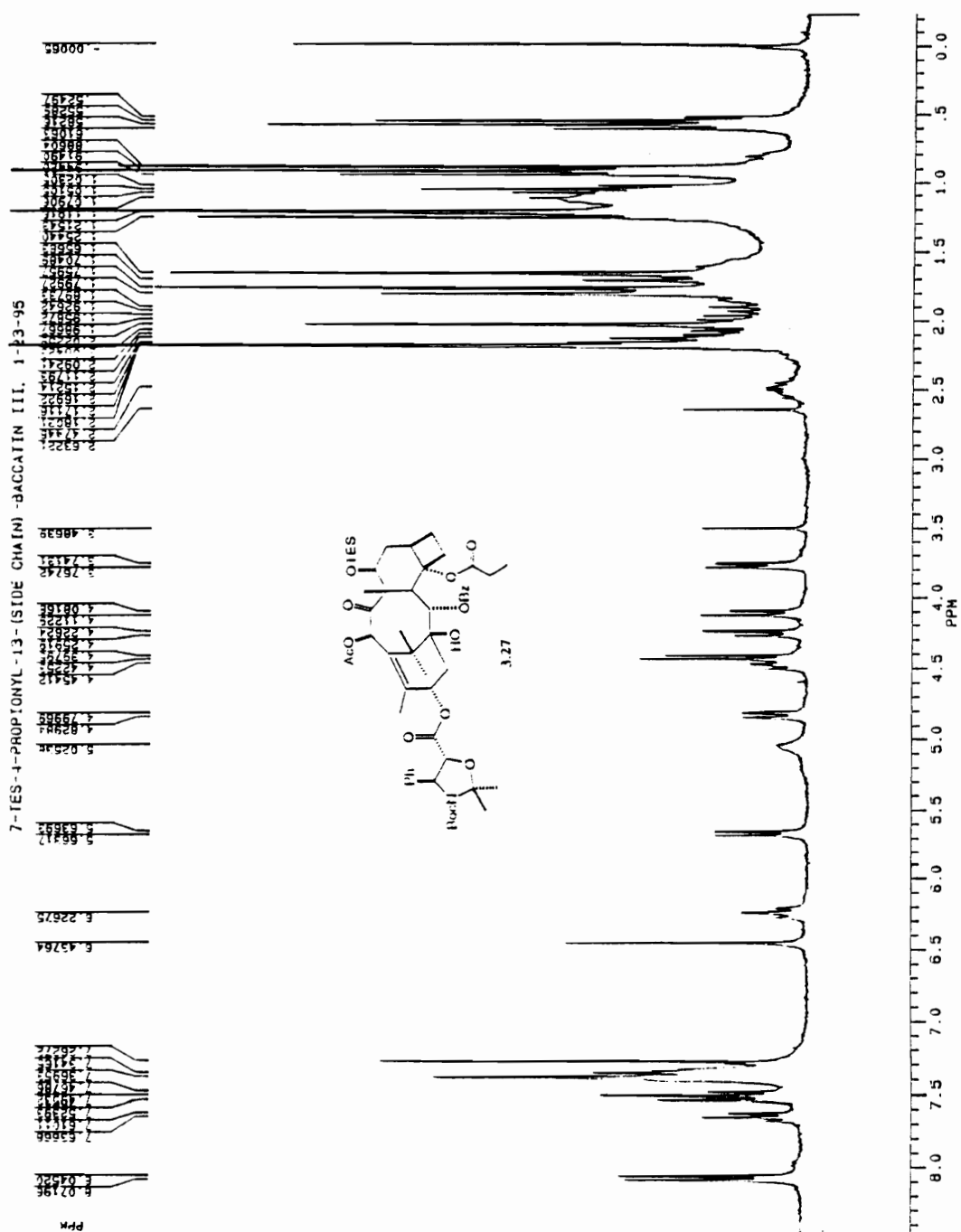




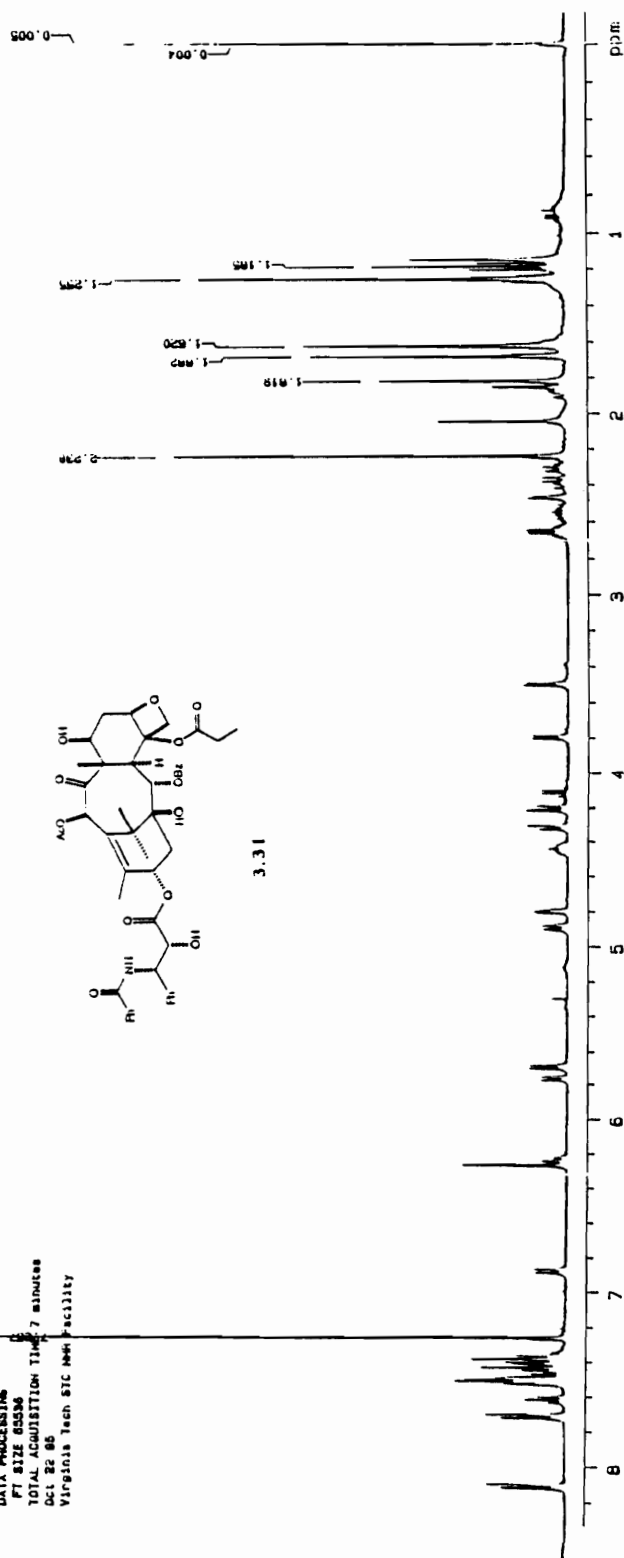
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 Average of DA???:V431#2-10, KAN, 130-9 IN THIOGLYCEROL PT= 0°



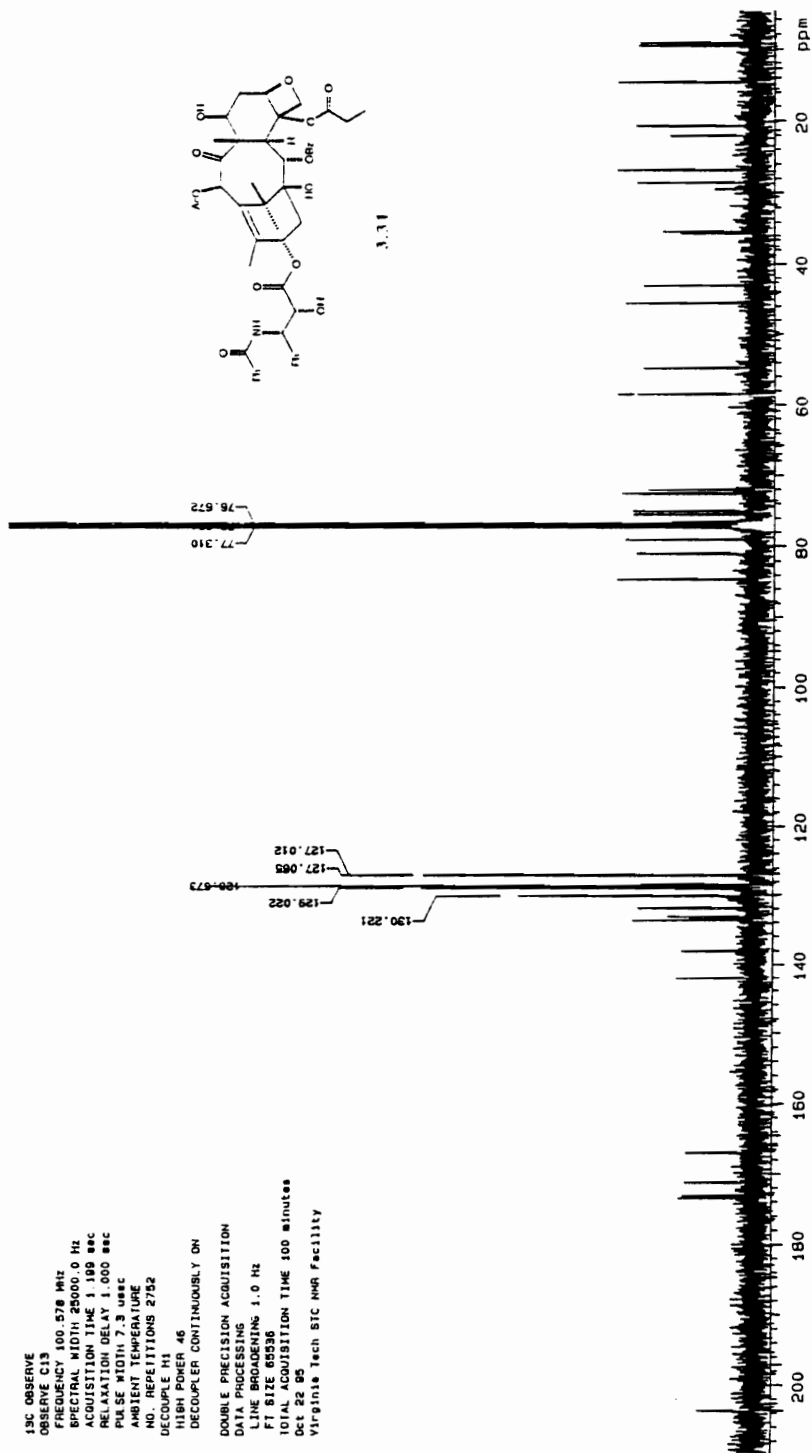




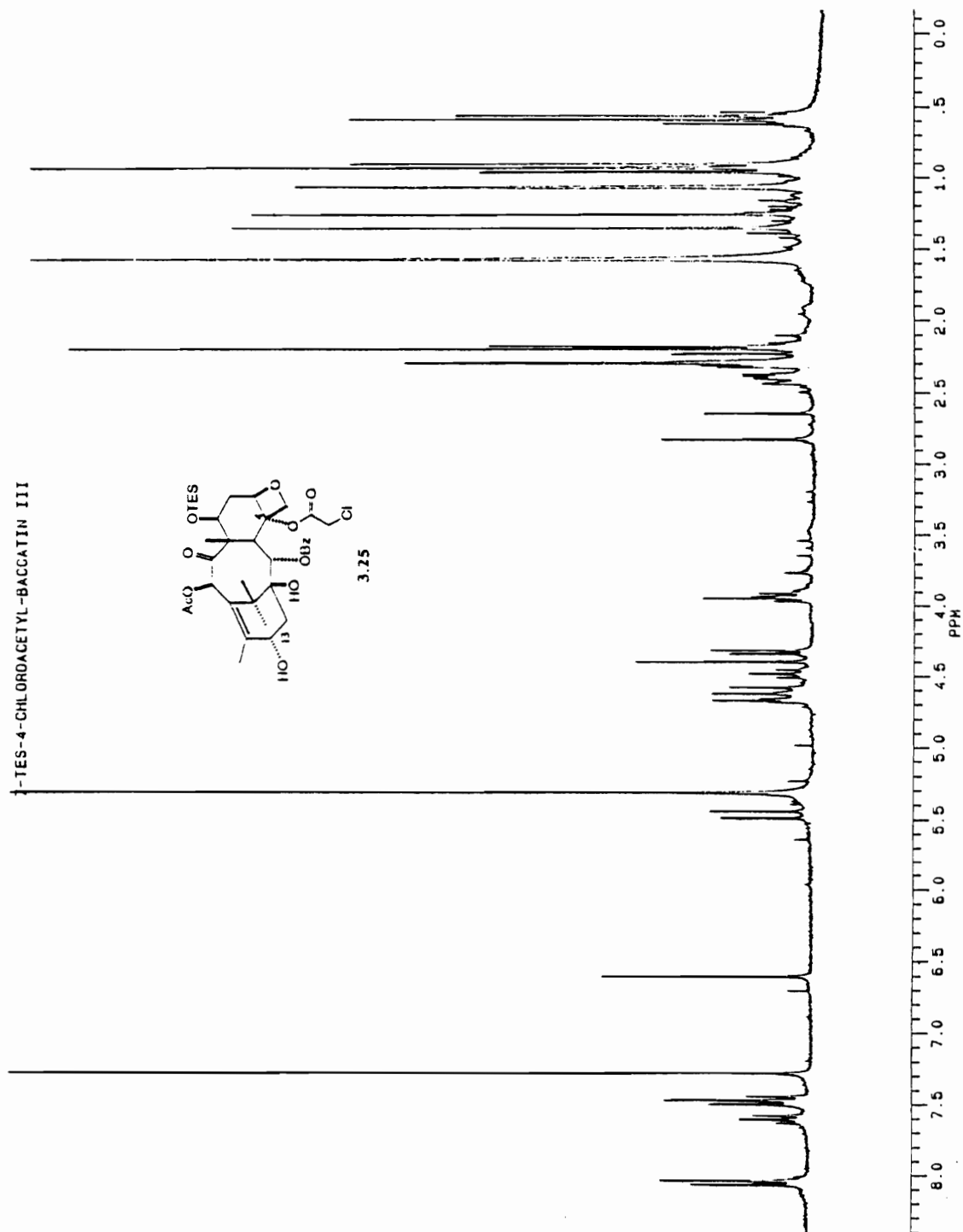
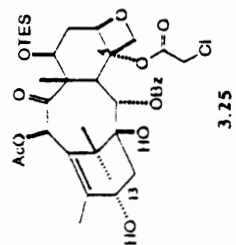
The chemical structure of compound 1 is a complex polycyclic molecule. It features a central ring system with several fused and fused-to rings. Key functional groups include a hydroxyl group (OH) on the left, a ketone group (C=O) at the top, an ester group (COOCH₃) on the right, and a carboxylic acid group (COOH) at the bottom. The structure is highly substituted with various side chains and functional groups, including a methyl group (CH₃) and a hydroxyl group (OH) on the right side.



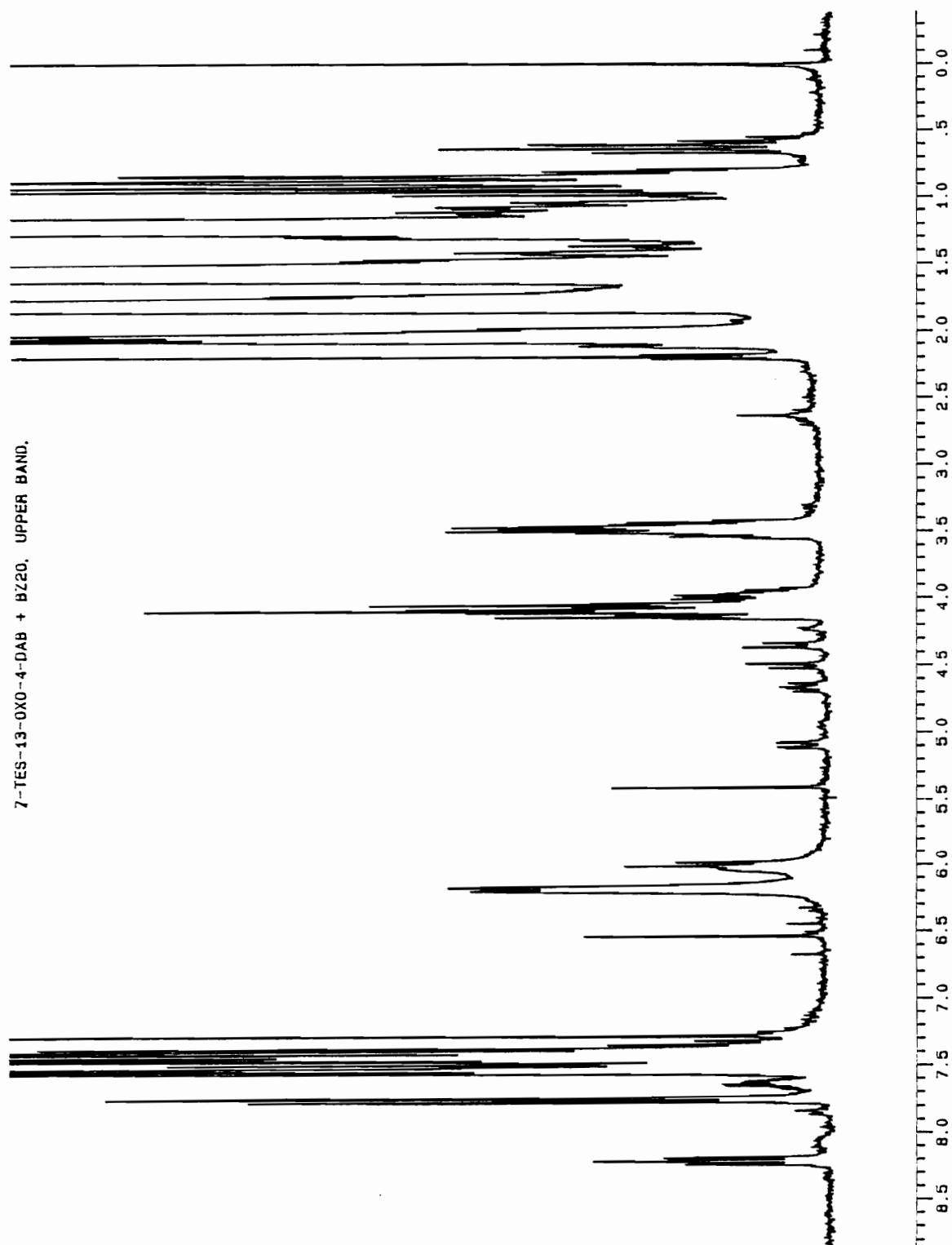
The chemical structure of compound 1 is a complex polycyclic molecule. It features a central fused ring system. Key functional groups include a carboxylic acid group (COOH) at the top left, an amide group (NH-CO-CH₂-CH₃) at the bottom left, and a ketone group (C=O) at the bottom right. There are also several other functional groups and substituents, including a hydroxyl group (OH), a methoxy group (OMe), and a carboxylic acid group (COOH) on the right side. The structure is highly detailed with stereochemistry indicated by wedges and dashes.

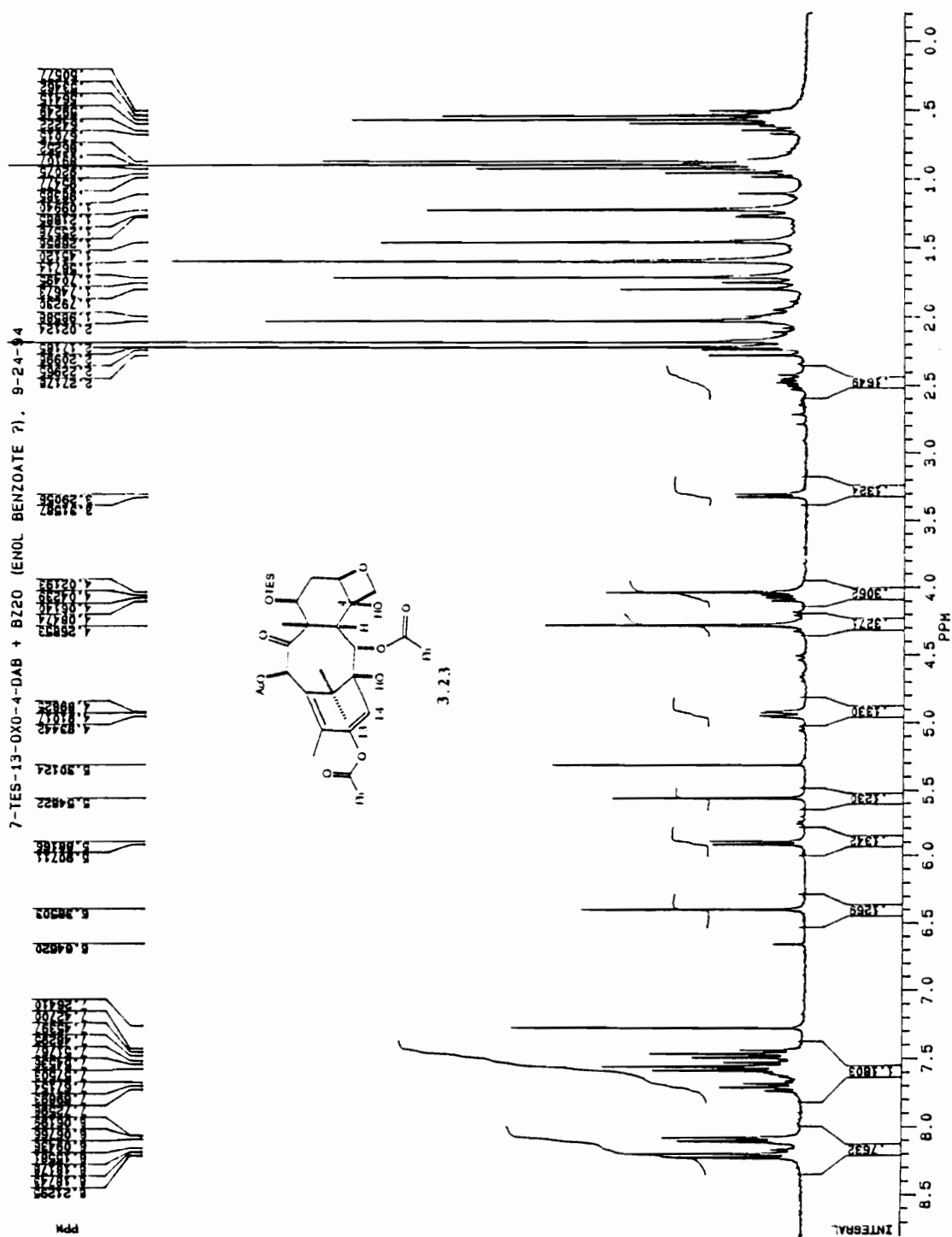


1-TES-4-CHLOROACETYL-BACCATIN III

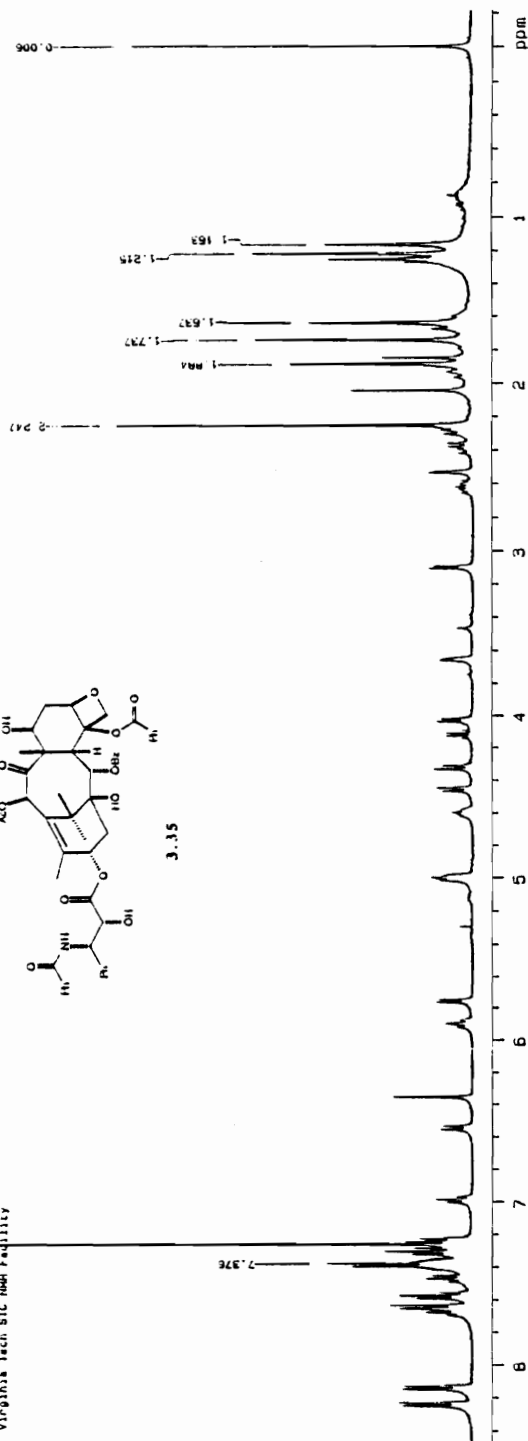
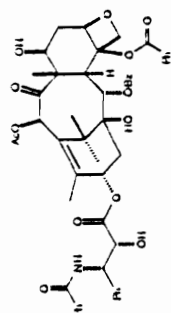


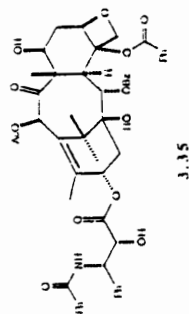
7-TES-13-0X0-4-DAB + BZ20, UPPER BAND.





1H OBSERVE
 OBSERVE M1
 FREQUENCY 300 M51 M12
 SPECTRAL WIDTH 5000 0 Hz
 ACQUISITION TIME 3.74 sec
 RELAXATION DELAY 1.000 sec
 PULSF WIDTH 9.6 usec
 AMBIENT TEMPERATURE
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 DATA PROCESSING
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 TOTAL ACQUISITION TIME 7.376 minutes
 OCT 22 95
 Virginia Tech SIC NMR Facility

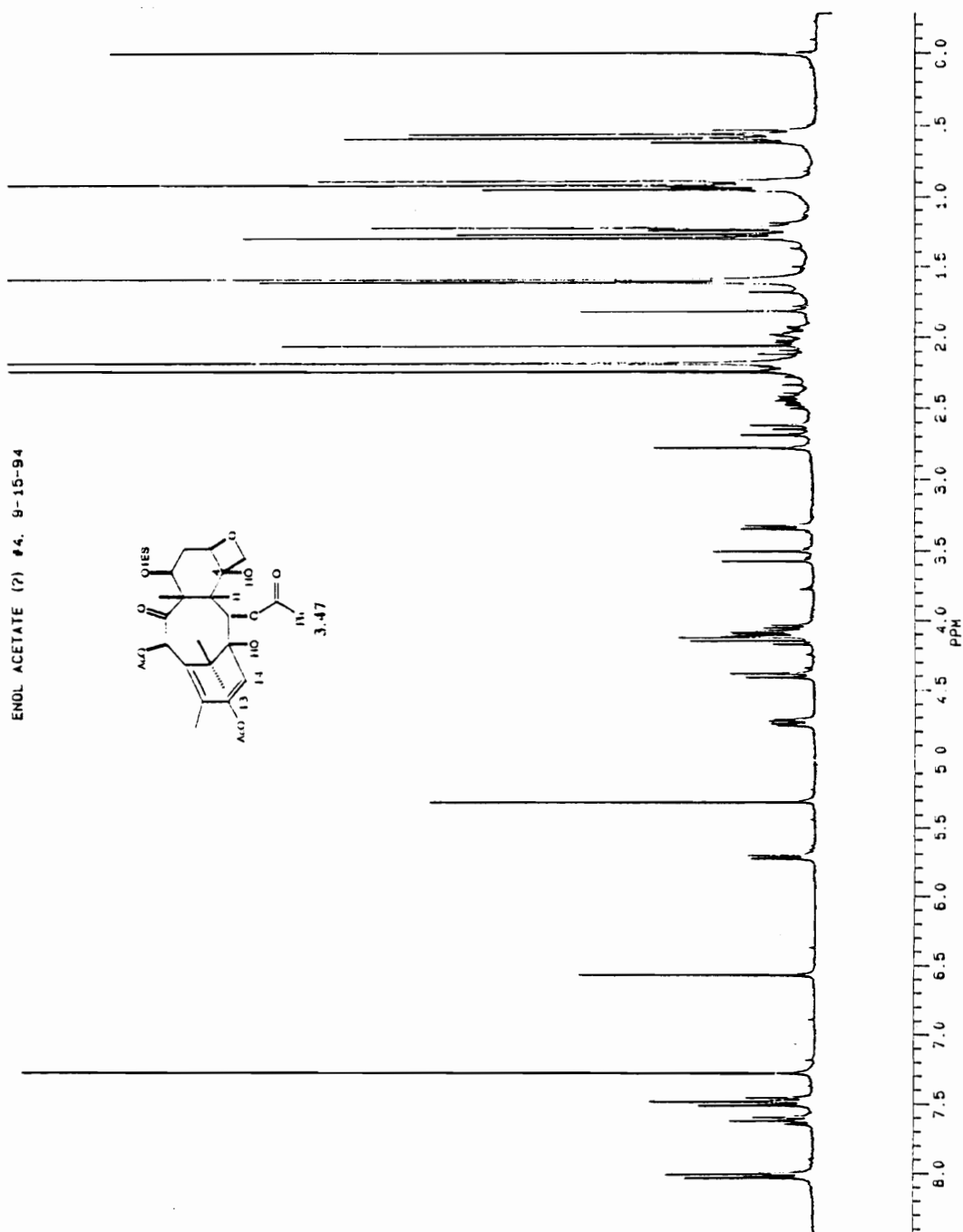
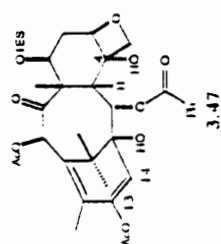


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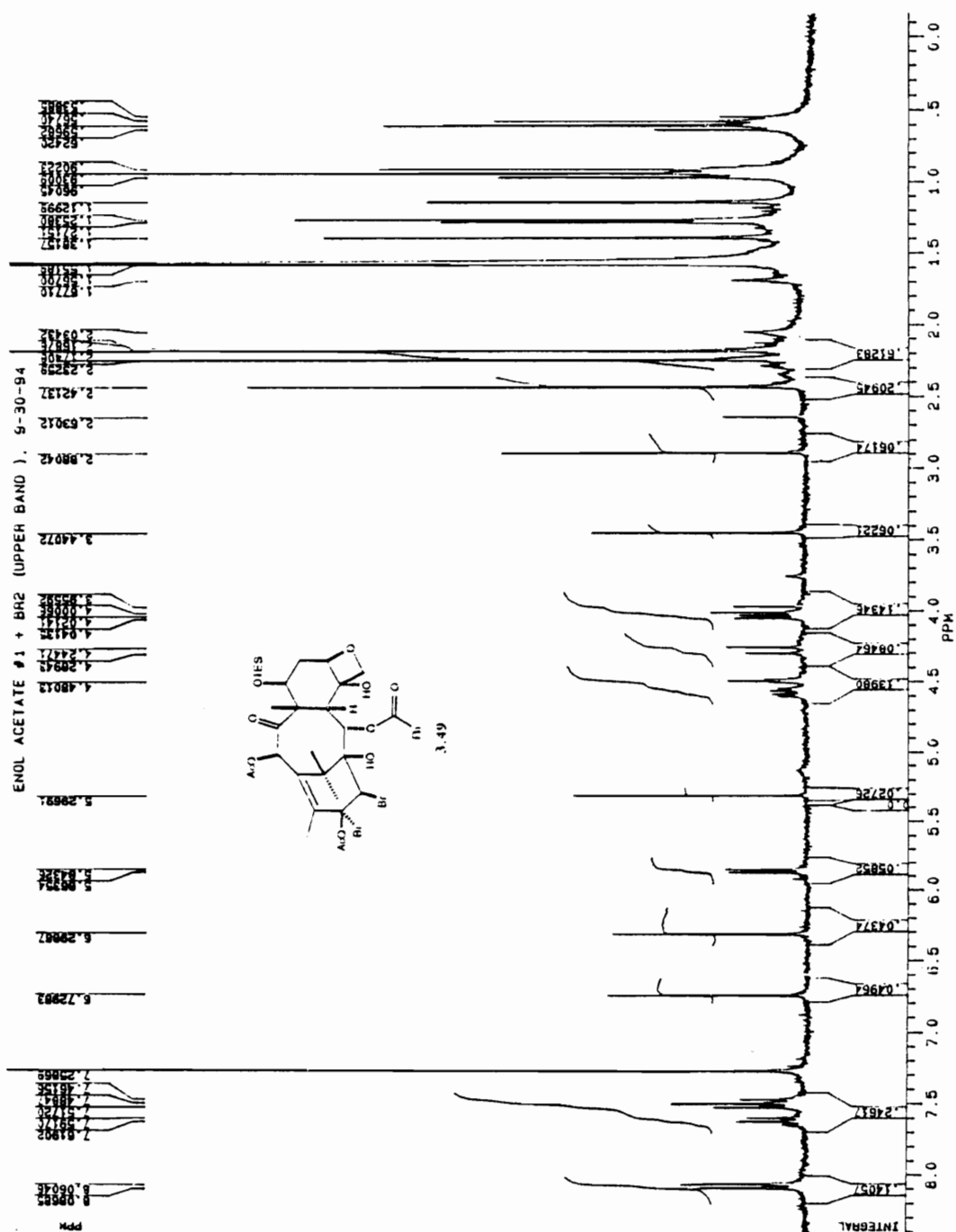
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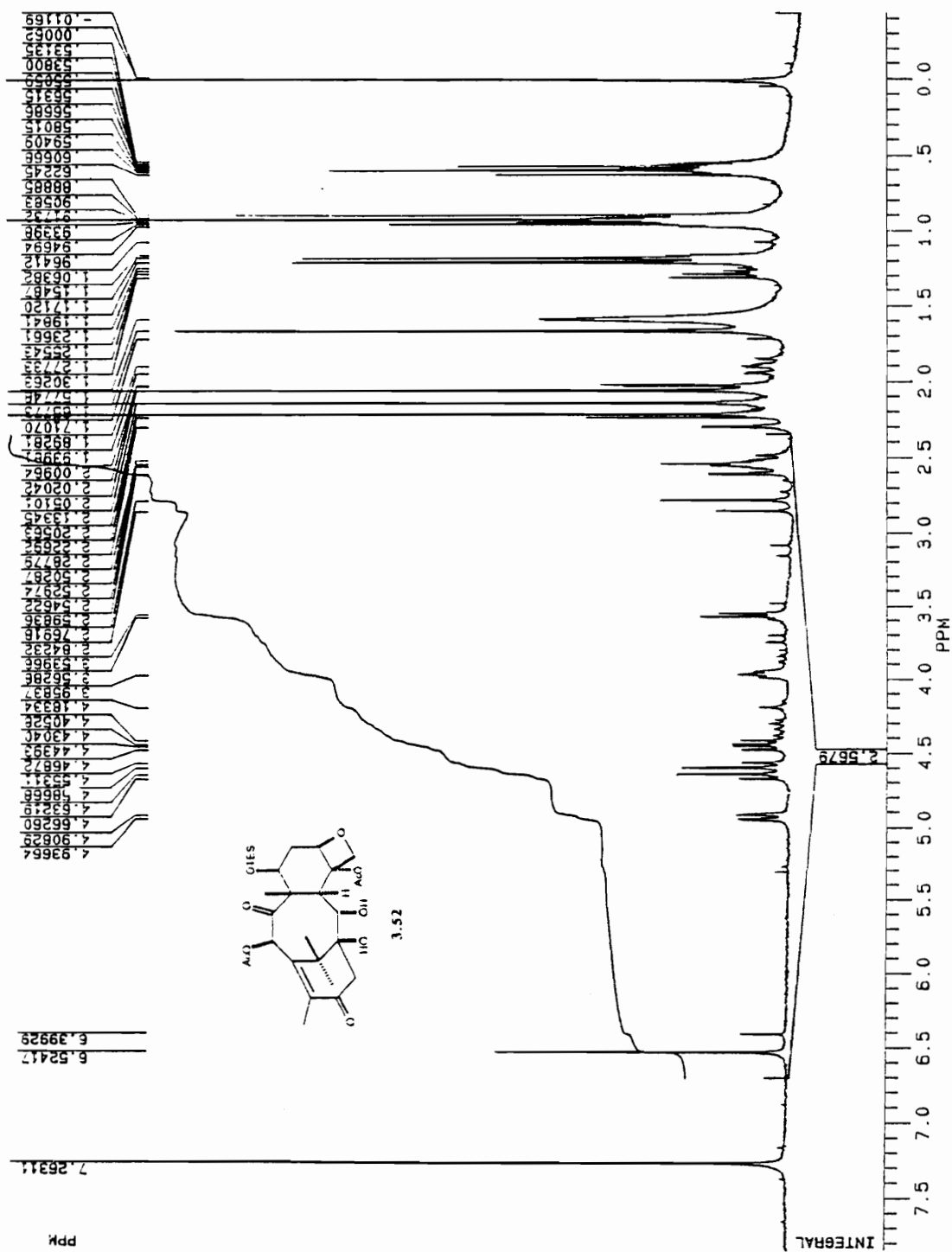
300

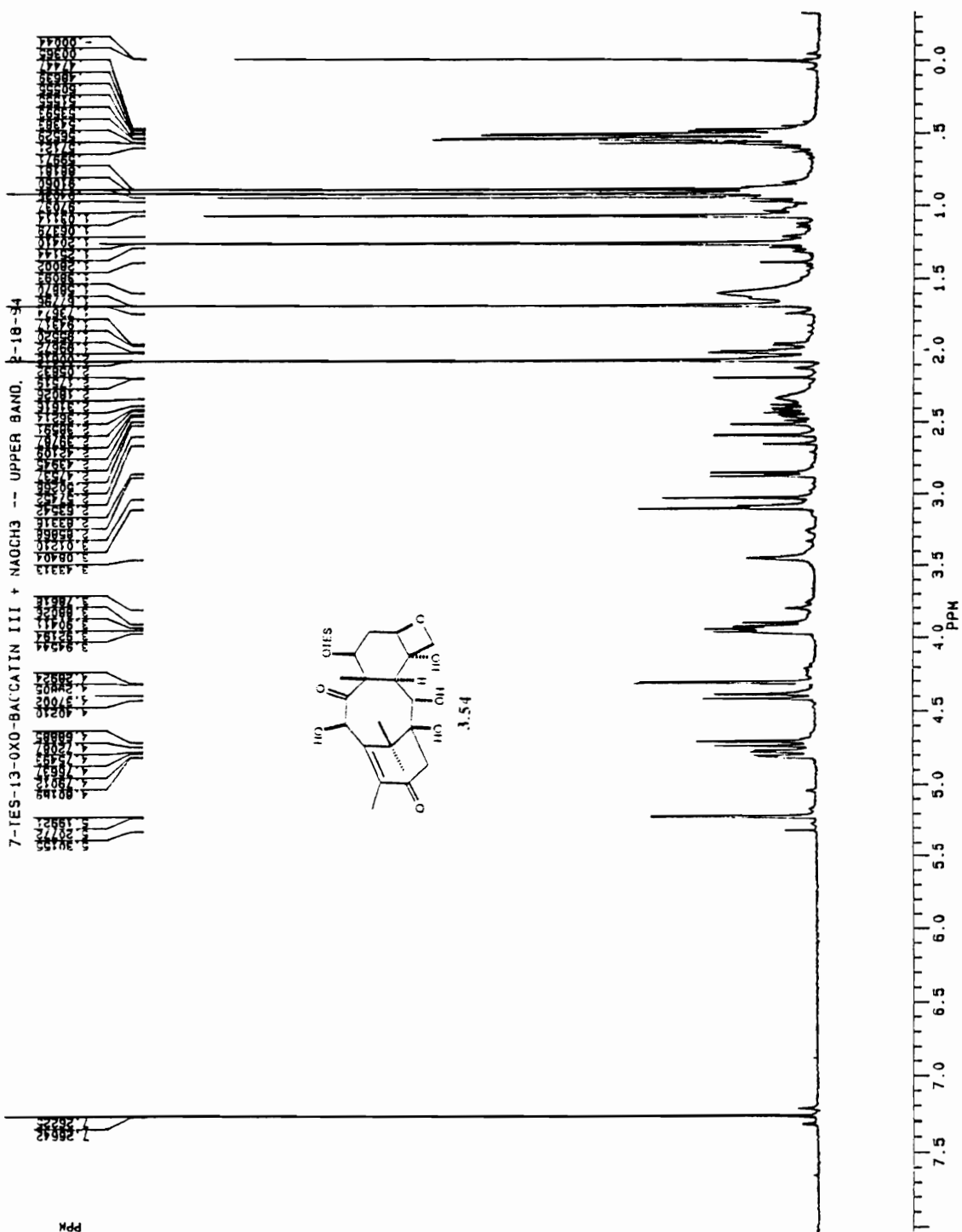
ENDL ACETATE (?) #4. 9-15-94

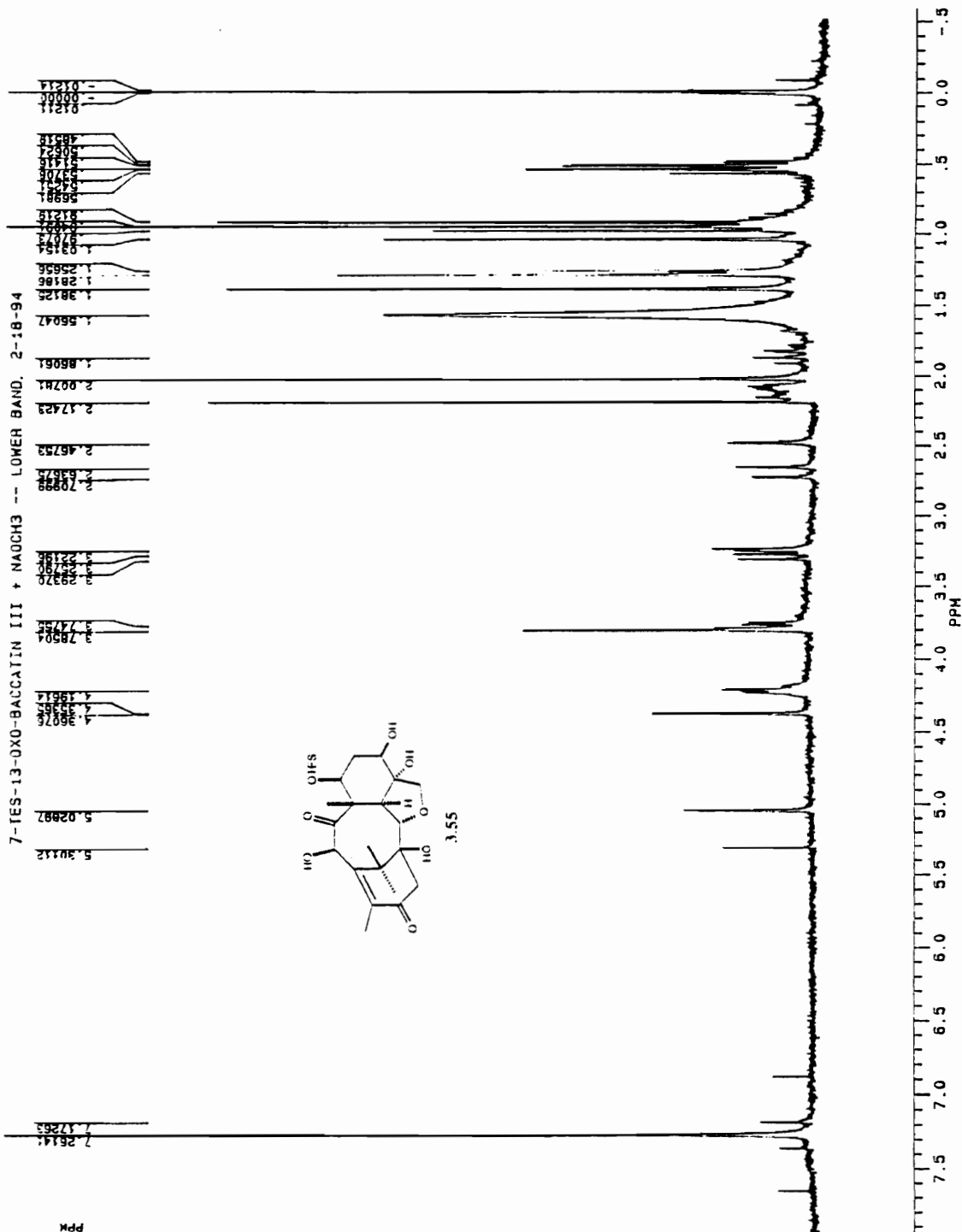


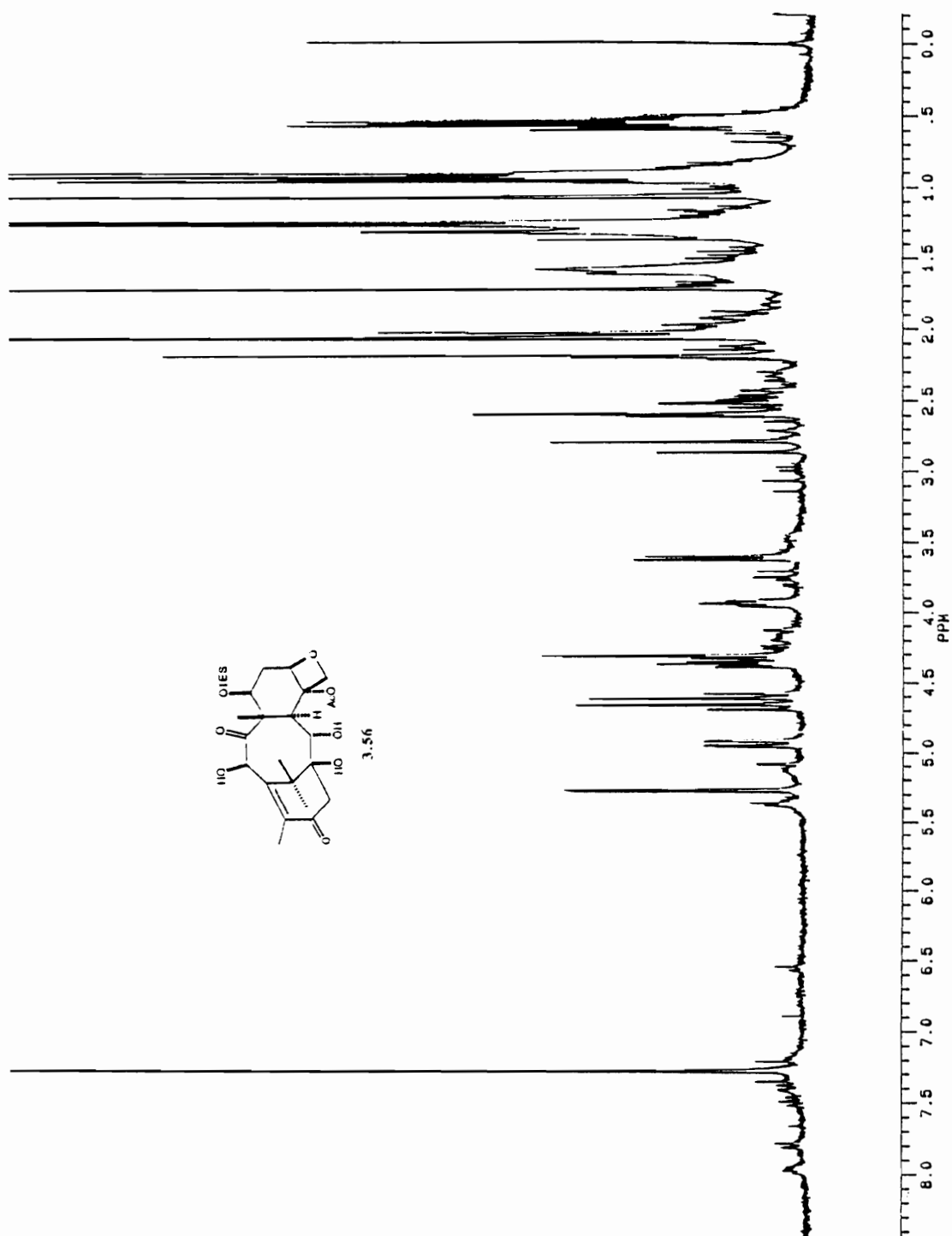


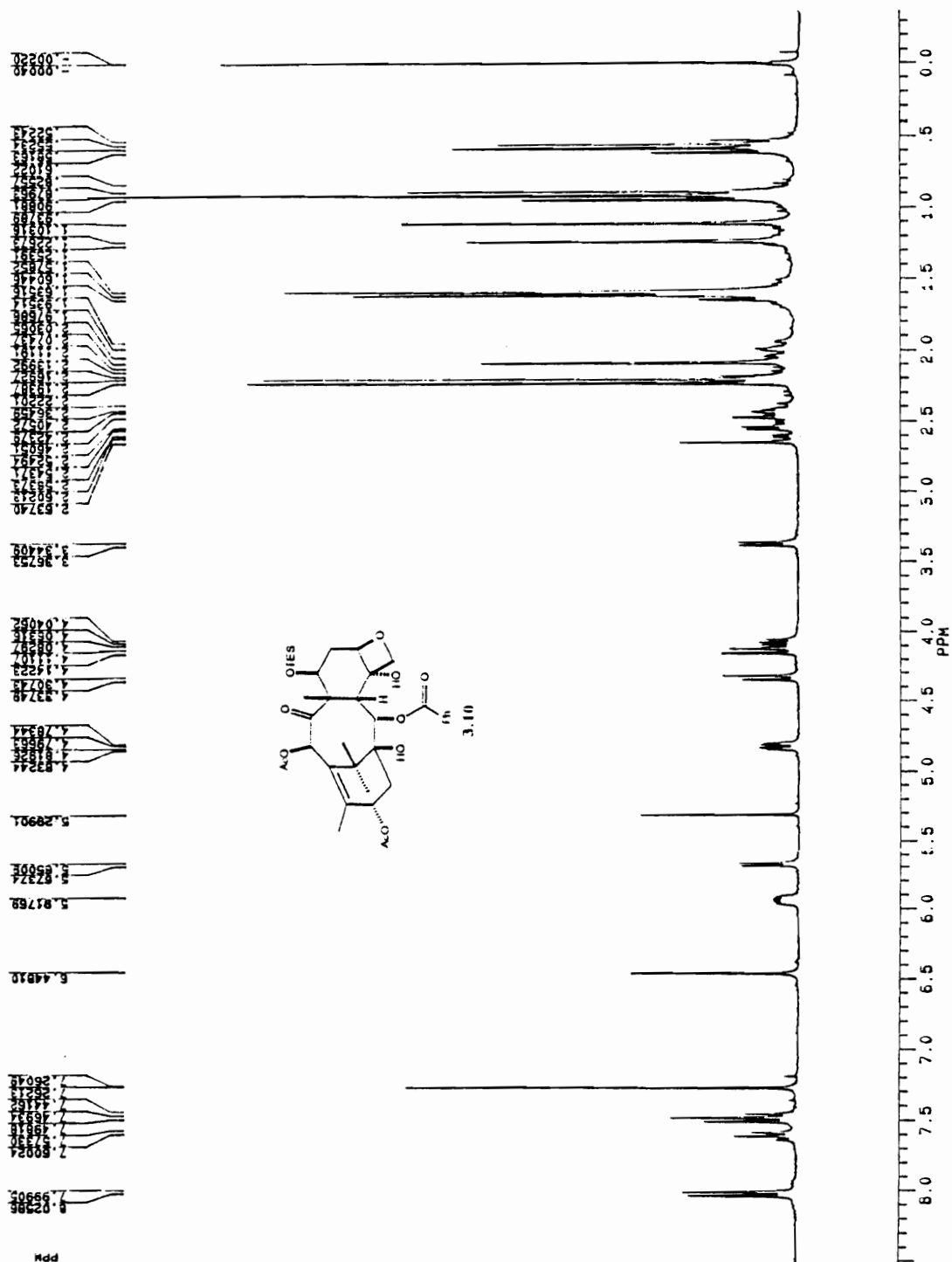


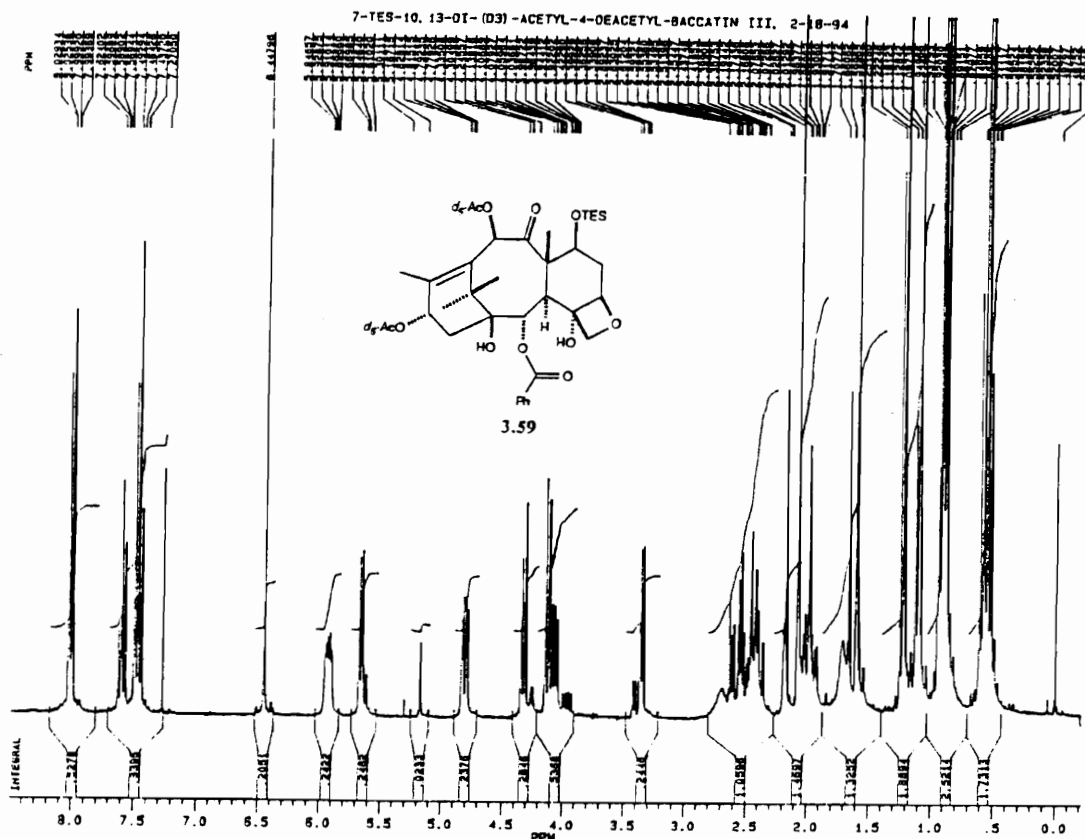




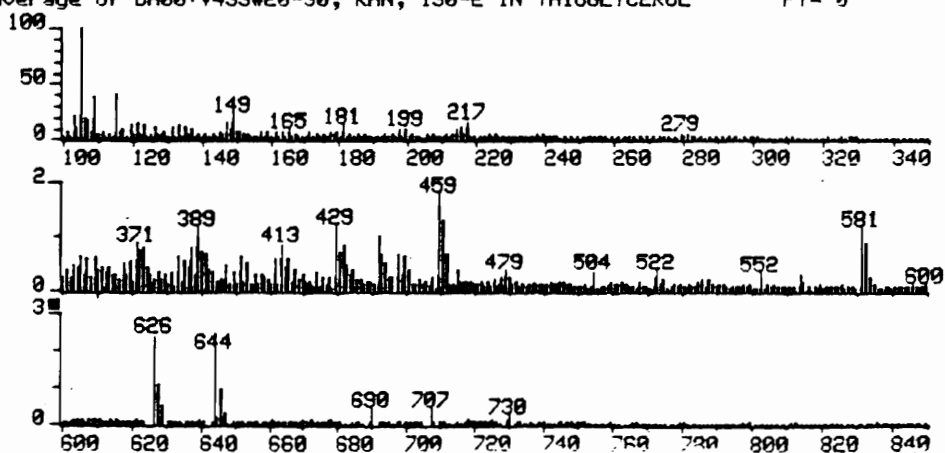


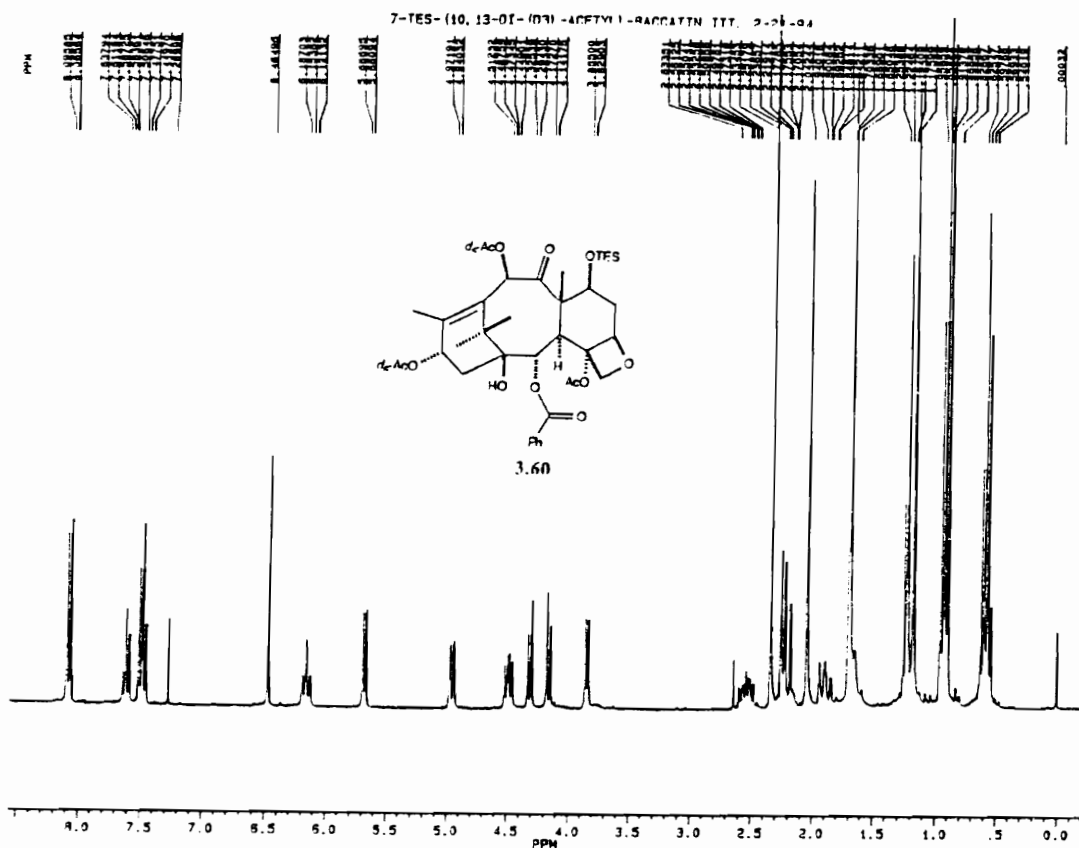




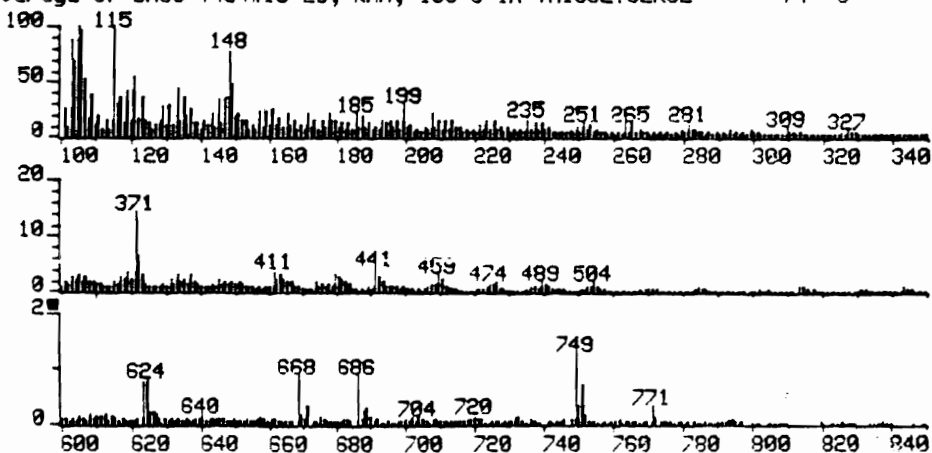


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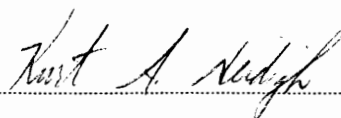


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 Average of DA00:V434#13-23, KAN, 130-5 IN THIOLYCEOL PT= 0°



VIII. VITA

Kurt Alan Neidigh was born on July 18, 1962, in Washington DC. After graduating from Spotsylvania High School (Spotsylvania, Virginia) in June of 1980, he attended Germana Community College (Locust Grove, Virginia) where he graduated Magna Cum Laude with Associate of Science degrees in Science, Education and Business Administration in June of 1982. In June of 1986, he received his Bachelor of Science degree in Chemistry with a Mathematics minor from the University of Utah (Salt Lake City, Utah). After working as a Laboratory Assistant in the Department of Bioengineering at the University of Utah and as a Laboratory Specialist in the Department of Biomedical Sciences at the Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University (Blacksburg, Virginia), he enrolled in the Chemistry graduate program at Virginia Polytechnic Institute and State University where he received his Master of Science degree under the direction of Dr. David G.I. Kingston in May of 1992. Continuing under the tutelage of Professor Kingston, he completed the requirements for the Doctor of Philosophy degree in December of 1995. He is currently a Public Health Service post-doctoral fellow, working under the direction of Dr. Richard A. Glennon in the Departments of Medicinal Chemistry and Pharmacology and Toxicology at the Medical College of Virginia, Virginia Commonwealth University (Richmond, Virginia), where he is preparing nicotine analogs in order to more fully investigate the nicotine pharmacophore and to determine the pharmacological properties and abuse potential of these nicotinoids.

A handwritten signature in cursive script, reading "Kurt A. Neidigh", is positioned above a horizontal dotted line.

Kurt A. Neidigh, Ph.D.