

STUDIES ON THE CHEMISTRY OF PACLITAXEL

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Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in the partial fulfillment of the requirement for the degree of

Doctor of Philosophy
in
Chemistry

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August 11, 1998

Blacksburg, Virginia

Keywords: Paclitaxel, Taxol[®], synthesis, analog, SAR

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HAIQING YUAN

(ABSTRACT)

Paclitaxel is a natural occurring diterpene alkaloid originally isolated from the bark of *Taxus brevifolia*. It is now one of the most important chemotherapeutic agents for clinical treatment of ovarian and breast cancers. Recent clinical trials have also shown paclitaxel's potential for the treatment of non-small-cell lung cancer, head and neck cancer, and other types of cancers. While tremendous chemical research efforts have been made in the past years, which established the fundamental structure-activity relationships of the paclitaxel molecule, and provided analogs for biochemical studies to elucidate the precise mechanism of action and for the development of second-generation agents, many areas remain to be explored.

In continuation of our efforts in the structure-activity relationships study of *A-nor*paclitaxel, five new analogs modified at the C-1 substituent and analogs with expanded B-ring or contracted C-ring have now been prepared. Preliminary biological studies indicated that the volume rather than functionality at the C-1 position plays a role in determining the anticancer activity by controlling the relative position of the tetracyclic ring system, which in turn controls the positions of the most critical functionalities such as the C-2 benzoyl, the C-4 acetate, and the C-13 side chain. The optimum conformation could possibly be modulated by ring contraction or expansion, as suggested by the improved activity of a B-lactone-*A-nor*paclitaxel analog.

Chemical investigations were also carried out in the C-6 and C-7 positions and led to the synthesis of five new analogs. Of particular importance, 6 α -hydroxy-paclitaxel, the major human metabolite of paclitaxel, was synthesized for the first time through a C-7 epimerization reaction. The availability of the major human metabolite through synthesis makes it possible to perform *in vivo* biological investigations on the metabolite, and it also offers an important opportunity for the production of standard HPLC samples of the

metabolites which could be useful in the clinical monitoring of paclitaxel's disposition in human patients.

Previous modifications at the C-4 position suggested that analogs with an acyl group other than an acetate at C-4 may exert similar activity to paclitaxel. Little was known, however, on the conformation-activity relationships of the C-4 position. In order to further explore the C-4 chemistry, a mild C-4 acylation method using acid as the acyl source was successfully developed. The new method was exemplified by the synthesis of water-soluble paclitaxel analogs with hydrophilic functional groups at the terminal of the C-4 acyl moiety. This method should be applicable to a variety of similar carboxylic acids and offer an alternative or even better approach for the preparation of C-4 modified paclitaxel analogs.

Lastly, in addition to the extension of paclitaxel analog library, specially designed analogs have been sought to probe the active conformation of paclitaxel. An analog that has a bridge to tie up the C-4 acyl group with an inert position would be useful for this purpose. With successful demonstration of the above C-4 acylation method, combined with the well established C-6 chemistry, the synthesis of such a novel C-4 and C-6 bridged paclitaxel analog was completed.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude and appreciation to my research advisor, Professor David G. I. Kingston, for the guidance, support, encouragement, and patience he has given throughout the course of this work.

I am also grateful to my committee members, Professors M. Calter, N. Castagnoli, Jr., R. Gandour, L. Taylor, and J. Tanko for their guidance and encouragement.

Thanks are extended to all the past and present members of the Kingston research group, especially Doctors Xian Liang, Prakash Jagtap, Lakshman Samala, Bing-nan Zhou, Leslie Gunatilaka, Mahendra Chordia, Maria Sarragiotto, Maged Abdel-Kader, and Mr. Chongming Wu and Erkan Baloglu, for many helpful discussions and friendship.

Financial support and supplies of paclitaxel provided by Bristol-Myers Squibb is gratefully acknowledged.

Finally, I give my deepest gratitude and love to my wife Lucy, my daughter Amy, my parents Yunting and Jin, and my brother Yiqing for their continuing love and support.

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1. Introduction

1.1 *Cancer: The Basics*

Cancer is the most common name given by the ancient Romans for a large group of diseases that are characterized by three basic features: (1) Uncontrolled cell proliferation; (2) Loss of cellular differentiation; and (3) The ability to invade surrounding tissues and to establish new growth thereafter. There are a number of other terms to describe cancer, which are either more descriptive or more specific, e.g., “tumor”, “neoplasm”, and the root “onco-” all relate to a swelling of tissue, while “carcinoma” refers to the type of cancer that develops in the lining and covering tissue of organs (such as skin, the lining of the mouth and intestine), “sarcoma” refers to cancer that develops in the connective and supportive tissue of the body (such as bone, muscle, fat, etc.). It is noteworthy that, of these terms, tumor is often used in a narrow sense to refer to malignant tumor, which is synonymous to cancer, and both are used in the following text.

Just as there are different variations of names for cancers, in terms of the original site of occurrence, cancer has been found in almost every part of the body, including the head and neck, brain, eyes, salivary gland, thyroid, lung, breast, ovary, cervix, uterus, esophagus, stomach, bowel, liver, adrenal, pancreas, kidney, bladder, prostate, bone, blood, skin, lymphomas, etc. In fact, beside human beings and other mammalian species, all multicellular living organisms are susceptible to cancer, due to its disordered cell proliferation nature.

Cancer causes various types of harmful consequences to the patient, such as obstruction of airways, blood vessels, bowel and other drainage channels, causing severe pains, bleeding, alteration or malfunction of organs, and ultimately death. It is reported that the percentage of deaths caused by cancer has risen quickly from about 0.5% in

1850,¹ to about 11% in 1946, 16% in 1970,² and nearly 20% in 1994.³ It is now second only to cardiovascular diseases in mortality rate. Based on the history of cancer's occurrence rate and the current status of cancer prevention and therapy, it was even estimated that cancer will surpass cardiovascular disease to be the number one killer in about the year of 2000. Statistical results also astonishingly revealed that about 1 in 3 of the population will finally develop cancer at some time during their lives.⁴

This rapid increase in the mortality rate of cancer could be attributed to several reasons. Firstly, although cancer has been known to human society as early as 3600 years ago, it was not frequently encountered in previous generations because most cancers develop late in life, and life expectancy was much shorter in previous centuries. The great improvements that have been made in public health and medical care in the past century have eliminated or at least reduced many of the major causes of death such as infectious diseases. This in turn has increased life expectancy, which caused an increasing number of people to survive into the "cancer age". Secondly, worldwide industrialization and economic development have also inevitably deteriorated the entire living environment of mankind. People are now subjected to increasing pollution from industrial processes and automobile exhausts, and are also exposed to unprecedented cosmic radiation due to the disappearance of the ozone layer above the polar regions. Finally, the increase in smoking over the last 50 years can be directly correlated with an increased incidence of lung cancer. All of these factors, along with others, combined to make cancer one of the most dreadful, and often deadly diseases for the past century and for the near future until successful preventative strategies or cures can be developed.

From an epidemiological point of view, there are many different factors involved in the development of cancer. Chemical carcinogens are no doubt one of the causes of cancer, though many times it is overstated because man himself is composed of chemicals, as is food and everything that provides the comforts of modern life, and that

¹ Prescott, D. M.; Flexer, A. S. *Cancer: The Misguided Cell*, 2nd Ed.; Sinauer Associates, Inc.: Sunderland, MA, 1986.

² Lafond, R. E., Ed. *Cancer: The Outlaw Cell*, 2nd Ed.; American Chemical Society: Washington, DC, 1988.

³ *Cancer Facts and Figures*; American Cancer Society: Washington, DC, 1994.

⁴ Whitehouse, M.; Slevin, M. *Cancer: The Facts*, 2nd Ed.; Oxford University Press: New York, 1996.

provides prevention and treatment of diseases. It is estimated that only about 4% of human cancer is due to occupational exposure to chemicals. Nevertheless, all chemicals should be treated with respect, particularly those known carcinogens and new compounds that have not been tested.

Physical carcinogens, primarily radiation or ultraviolet light (a radiation of particular frequency), have been known to be associated with an increased risk of cancer. Carcinogenesis is thought to involve interaction of the radiation with DNA, through radiation induced genetic mutation or activation of oncogenes. Extensive exposure to X-rays, sunlight, contact with radioactive materials, including certain rocks used as building materials, and even painted watch dials containing radium and thorium, not to say the massive radiation released from atom bomb or nuclear reactor disasters, will cause various cancers such as leukemia and bone cancer. Interestingly, cancer risk from radiation exposure depends largely on the type of radiation and the rate of exposure. When radiation is used in a controlled way, it becomes a method for cancer treatment, and the chance of causing other tumor is extremely low.

Viruses are also found to induce cancer. Almost every DNA virus family and one in the RNA virus family (retroviruses) have been shown to produce tumors in animals or to cause cell transformation in culture under appropriate circumstances. These viruses contain a subset of genes that can alter the growth of the host cell, by encoding proteins, altering cellular gene expression and prohibiting cell division.

Of particular importance, tobacco smoking has been widely recognized to be associated with cancers, especially lung cancer, although there is considerable individual dependence. Cancer of the larynx, pancreas, kidney, and bladder have also been attributed partly to tobacco smoking. Indeed, tobacco smoking has been assessed to contribute to about 35% of all cancer deaths. The risks have also been realized to extend to non-smokers who share the air space with smokers. Although cigarette smoke is not a new factor causing cancers, it does need particular consideration by the population, since lung cancer deaths comprise almost one third of all cancer mortality.

Diet also influences the risk of cancer in many ways, mainly via consumption of carcinogens such as aflatoxin (causing liver cancer), bracken fern (causing esophageal

cancer), or via consumption of chemicals that could influence the formation of carcinogens in the body (such as nitrites and nitrates).

Besides all these external factors, internal genetic factors play a critical role in cancer initiation. This is because cancer ultimately begins as a genetic defect. There is an overwhelming amount of evidence obtained in the past two decades that has determined effects of genes on cancer formation. Basically, three classes of genes are pertinent to cancer formation or inhibition: (1) genes that affect DNA synthesis and repair, in which mutations lead to production of defective DNA; (2) genes whose mutation promotes tumor formation; (3) genes that suppress or inhibit tumor formation.

1.2 Natural Products in Cancer Chemotherapy

There are many ways to treat cancer medicinally. Surgery, radiotherapy, chemotherapy and immunotherapy are the most common treatments to choose. Among these cancer treatment methods, chemotherapy is a relatively new one. Surgical removal of the tumor has been in existence for over a century, and radiotherapy has been used for more than eighty years. Systematic chemotherapy only made its appearance in the middle of World War II, when Farber prescribed methotrexate to treat childhood leukemia in 1940. Since then enormous progress has been made, especially in recent decades, in the drugs themselves, in methods of delivering the drugs, and in the ways to alleviate side effects. In fact, chemotherapy gives the first promise of eradicating cancer that has disseminated. This is because chemotherapeutic agents work by interfering with the process by which cancer cells divide to produce new cells. The drugs are introduced into the bloodstream and circulate around the body, killing cancer cells that reside at the original site of occurrence as well as those migrated to other tissues (metastasis). This has a great advantage over other methods in which treatments can only be applied locally to a particular part of the body.

It is very important, though, to realize that chemotherapeutic agents damage both normal cells and cancer cells, because cancer cells are only subtly different from normal cells in that they have lost the mechanism which controls their growth and reproduction. In most other respects their biological behavior and chemical processes are almost the same. Usually, a therapeutic drug is given followed by a break of a period of time, during which time normal cells recover fully and more rapidly than the cancer cells. It is on this narrow difference that practical clinical chemotherapy relies.

Like other cancer therapeutic methods, however, cancer chemotherapy has its own limitations. These include its inability to destroy all cancer cells of a large tumor, the occurrence of drug resistance, and its side effects, such as nausea, vomiting, diarrhea, hair loss, and increased susceptibility to infections. Most of these side effects are the consequences of the destruction of normal cells.

It is now very clear that chemotherapy's most effective role in cancer treatment is its combination with surgery, radiotherapy and immunotherapy, as well as with a combination of anticancer drugs themselves. It is possible, however, that some types of cancer can be cured by chemotherapy alone, particularly when selective chemotherapeutic agents are available.

In 1970, because of increasing awareness of the threat of cancer to a rapidly increasing population, a panel of consultants was called together by the United States Senate through the efforts of many prominent scientists and Federal Government agencies to report on a national program for the conquest of cancer. The most direct result of this event was the passage of the National Cancer Act in 1971 and the financial backing associated with it, which led to the establishment of several national cancer research centers and to considerable progress made in the understanding and treatment of cancer.

In the course of searching for new, more effective anticancer agents, natural products become an extremely important, productive and readily available domain. In fact, terrestrial plants have been used for the treatment of human diseases for thousands of years, although the actual rationale of the plant's effect may have been largely semi-empirical or even unknown. As summarized recently,⁵ approximately 120 drugs in the market are obtained from plants. They represent about 25% of the prescription drugs sold in the United States,⁶ and represented a retail value of about \$16 billion in 1990.⁷ The situation in the realm of anticancer agents is similar. Currently, there are about 87 anticancer drugs available, among which 62% are natural products or are modeled on natural products. Out of about 300 pre-NDA (new drug application) anticancer candidates that were in preclinical or clinical development during 1989-1995, about 61% have a natural origin.⁸ It has long been recognized that natural sources, being a virtually

⁵ Pezzuto, J. M. *Biochem. Pharm.* **1997**, 53, 121.

⁶ Farnsworth, N. R.; Soejarto, D. D. *Econ. Bot.* **1985**, 39, 231.

⁷ Principe, P. P. Monetizing the Pharmacological Benefits of Plants. In: *Medicinal Resources of the Tropical Forests*, Balic M. J.; Elisabetsky, E.; Laird S. A., Eds.; Columbia University Press: New York, 1996.

⁸ Cragg, G. M.; Newman, D. J.; Snader K. M. *J. Nat. Prod.* **1997**, 60, 52.

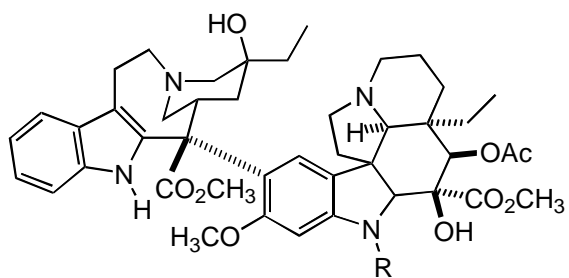
untapped library of complex compounds, represents a “reservoir of novel drugs awaiting imaginative and progressive organizations”.⁹

Current cancer chemotherapeutic agents can be classified into five categories by their mechanism of action, they are:

- (1) Antimetabolites, which have similar structures to those natural metabolites and are able to disrupt nuclei acid synthesis either by falsely substituting for biosynthetic precursors of DNA/RNA or by inhibition of normal precursor biosynthesis.
- (2) DNA interactive agents, which alter DNA structure so as to interfere with its template functions for replication. This is the broadest class including the alkylating agents, the DNA strand-breaking agents, intercalating topoisomerase II inhibitors, non-intercalating topoisomerase II inhibitors, and topoisomerase I inhibitors.
- (3) Tubulin interactive agents, which are essentially mitotic inhibitors and act by interfering with the cellular mechanism of mitosis. This class will be discussed in more detail in the following text.
- (4) Hormonal agents, which are often natural or synthetic hormonal substances such as steroids, steroid analogs or hormone-like compounds, interact with hormone receptors to reduce tumors whose growth are sensitive to hormonal controls. A few drugs that inhibit hormone synthesis are also categorized in this class.
- (5) Other drugs, which do not fit any of the above classes.

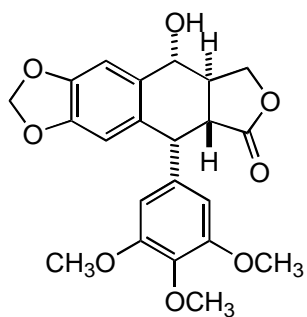
There are currently over 80 anticancer drugs available, many of which are either derived directly from natural sources (usually plants), or having natural products as a prototype. For example, two well known tubulin interactive vinca alkaloids Vinblastine[®] and Vincristine[®] were discovered in the 1960's from the periwinkle plant (*Vinca rosea*) and are particularly useful against leukemia and lymphomas. Other mitotic inhibitors such as the lignans podophyllotoxin and colchicine are also isolated from *Podophyllum* and *Colchicum autumnale*, respectively (Figure 1.1).

⁹ Farnsworth, N. R. *Pharm. Technol.* **1995**, August, 14.

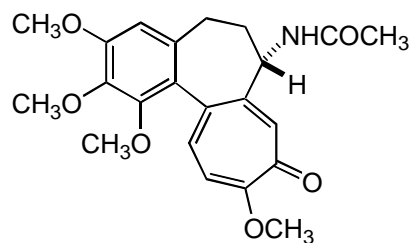


Vinblastine R = CH₃

Vincristine R = CHO



Podophyllotoxin



Colchicine

Figure 1.1 Some selected anticancer agents originated from natural products

1.3 Natural Taxanes

Taxanes are a class of structurally complex yet homogenous diterpene alkaloids that occur in the genus *Taxus*, commonly known as the yew. This family of diterpenoids has long been known for its toxicity as well as for other biological activities. The first chemical study of the metabolites of the yew dates back to the mid-nineteenth century, when a mixture of taxanes was obtained by the German pharmacist Lucas in 1856.¹⁰ The structure characterization of these compounds which were named as taxine by Lucas, however, was extremely slow, due to the complexity of the structure and the lack of modern spectroscopic techniques. Moreover, the taxine obtained earlier had inconsistent physical properties (m.p.: 82–124 °C, $[\alpha]_D^{25}$: +35–90°), and was later shown by Graf¹¹ to be a mixture of at least seven compounds. Graf was able to isolate three of the pure components and named them as taxines A, B and C. Their structure was identified only very recently as **1.1**, **1.2**, and **1.3**,¹² as shown in Figure 1.2. In 1963, the constitution of the taxane nucleus was established for the first time by independent work of Lythgoe's group,¹³ Nakanishi's group¹⁴ and Uyeo's group¹⁵ as tricyclic polyalcohols esterified with acids, such as taxinine (**1.4**), whose stereochemistry was established three years later.¹⁶

¹⁰ Lucas, H. *Arch. Pharm.* **1856**, 85, 145.

¹¹ Graf, E. *Angew. Chem.* **1956**, 68, 249.

¹² (a) Graf, E.; Kirfel, A.; Wolff, G. J.; Breitmaier, E. *Liebigs Ann. Chem.* **1989**, 376. (b) Ettouati, L.; Ahond, A.; Poupat, C.; Potier, P. *J. Nat. Prod.* **1991**, 54, 1455.

¹³ Eyre, D. H.; Harrison, J. W.; Scrowston, R. M.; Lythgoe, B. *Proc. Chem. Soc.* **1963**, 271.

¹⁴ Kurono, M.; Nakadaira, Y.; Onuma, S.; Sasaki, K.; Nakanishi, K. *Tetrahedron Lett.* **1963**, 2153.

¹⁵ Ueda, K.; Uyeo, S.; Yamamoyo, Y.; Maki, Y. *Tetrahedron Lett.* **1963**, 2167.

¹⁶ Eyre, D. H.; Harrison, J. W.; Lythgoe, B. *J. Chem. Soc., Chem. Commun.* **1967**, 452.

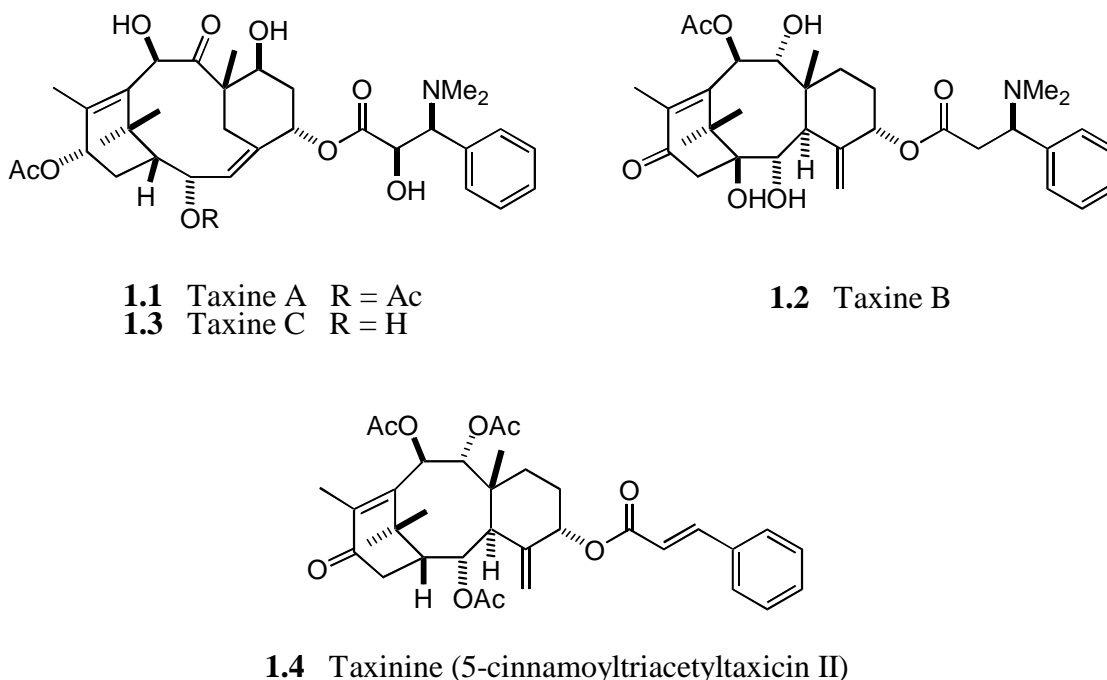


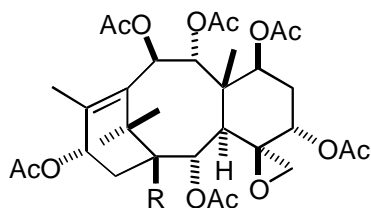
Figure 1.2 Natural taxanes

In the later 1960s, systematic studies on the non-alkaloidal components of the yew tree were conducted and several new members of the taxane family were discovered. For example, Halsall *et al.*¹⁷ isolated baccatin (**1.5**, see Figure 1.3.) and gave the numbering system for the taxane skeleton which is still used today, as shown in Figure 1.4. More importantly, in 1971, Wani and Wall discovered the highly potent anti-cancer agent taxol, whose isolation and characterization will be discussed in full detail afterwards.¹⁸ This remarkable accomplishment not only shifted the attention of the scientific community to paclitaxel itself, but also attracted extensive studies on various species of yew tree that led to the isolation of many new taxane family members. To date, over 100 taxanes have

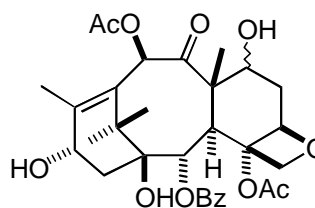
¹⁷ (a) Chan, W. R.; Hassal, T. G.; Hornby, G. M.; Oxford, A. W.; Sabel, W.; Bjamer, K.; Ferguson, G.; Monteath, R. J. *J. Chem. Soc., Chem. Commun.* **1966**, 923. (b) Della Casa de Marcano, D. P.; Hasall, T. G. *J. Chem. Soc., Chem. Commun.* **1969**, 1282. (c) Della Casa de Marcano, D. P.; Hasall, T. G. *J. Chem. Soc., Chem. Commun.* **1970**, 216.

¹⁸ Taxol was isolated in the late 1960's and its structure was published in 1971. The name taxol was assigned by Wall *et al.*, and this name continued in general use until 1992. At this time Bristol-Myers Squibb claimed the name Taxol as a trademark, based on the earlier use of this trademark for a laxative drug. The generic name paclitaxel was offered as a substitute, and this name will thus be used in this dissertation.

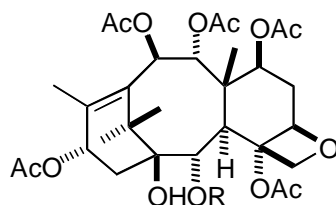
been isolated and structurally elucidated.¹⁹ They can be divided into several structural sub-classes:



1.5a Baccatin I R = H
1.5b Baccatin II R = OH



1.5c Baccatin III R = 7 β -OH
1.5b Baccatin V R = 7 α -OH



1.5e Baccatin IV R = Ac
1.5f Baccatin VI R = Bz
1.5g Baccatin VII R = n-hexanoyl

Figure 1.3 Baccatins

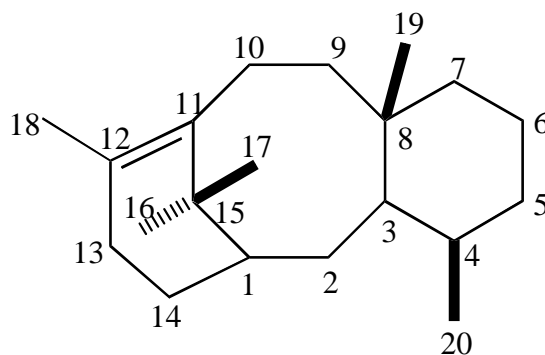


Figure 1.4 Taxane skeleton numbering system

¹⁹ For a review, see Kingston, D. G. I. *Natural Taxoids: Structure and Chemistry*. In *Taxol: Science and Applications*, Suffness, M. Ed.; CRC Press: Boca Raton, FL, 1994, pp. 287.

1.3.1 Taxanes with an Exocyclic Double Bond between C-4 and C-20

This is the most abundant subclass of taxanes, including compounds with or without side chains at C-5, different oxidation states at C-1, C-13, C-7 and ester functionalities at C-2, C-9, C-10. Some examples are shown in Figure 1.5.

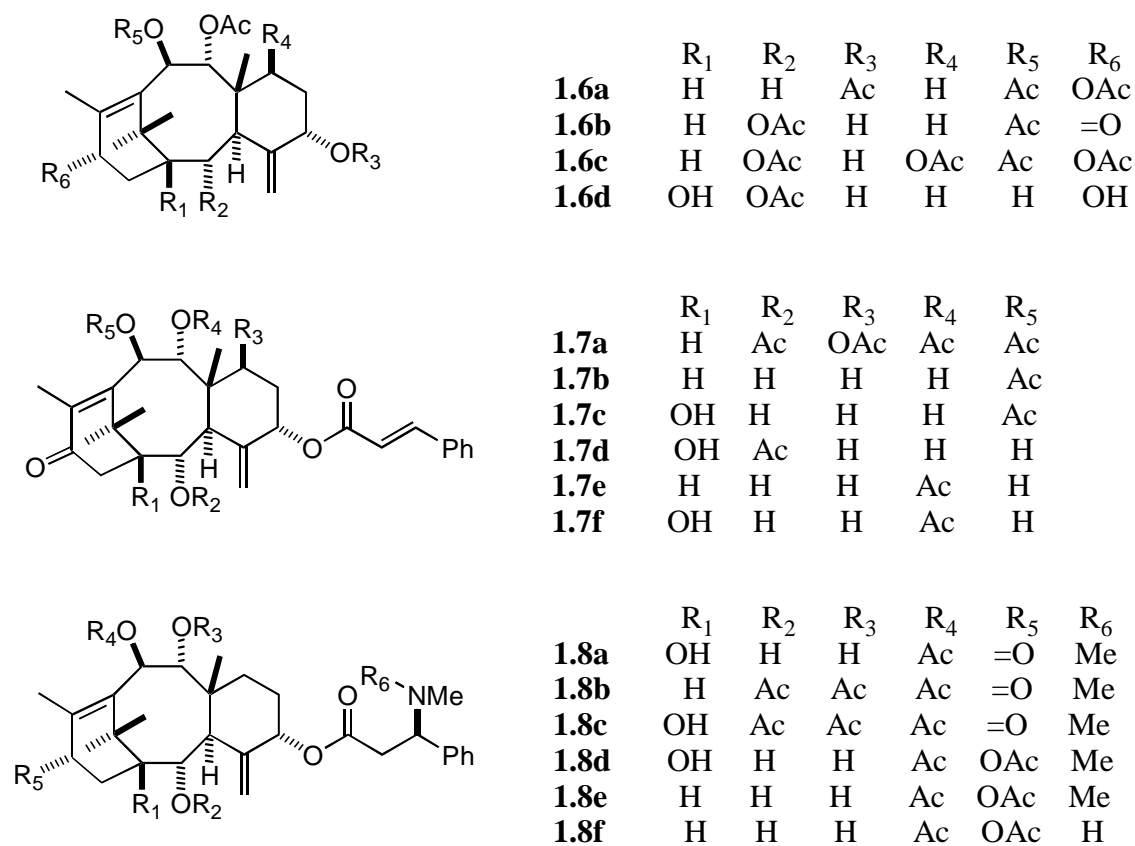
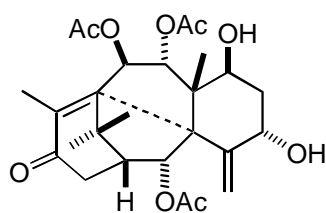


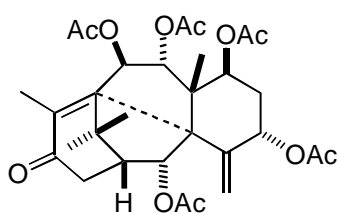
Figure 1.5 Some taxanes with an exocyclic double bond between C-4 and C-20

1.3.2 Taxanes with an Extra Bond Connecting Transannular Atoms

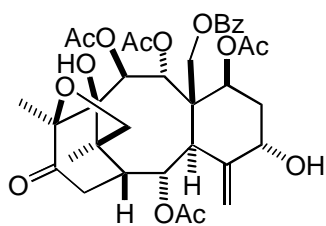
This class shares the common feature of a transannular bond, although the structure may look quite different at a glance. It is exemplified by taxinine K (**1.9**), taxinine L (**1.10**), and taxinine M (**1.11**). Variations of functional groups at C-1, C-2, C-5, C-7, C-9, C-10, and C-13 are also common, e.g., taxagifine (**1.12**) (Figure 1.6).



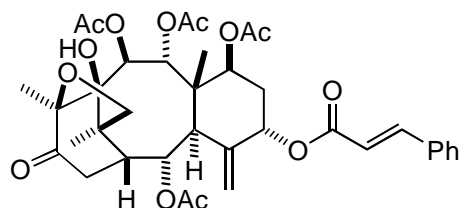
1.9 Taxinine K



1.10 Taxinine L



1.11 Taxinine M



1.12 Taxagifine

Figure 1.6 Some taxanes with a extra bond connecting transannular atoms

1.3.3 Taxanes with a C-4 (20) Epoxide

This class contains relatively fewer compounds. Representative compounds are listed in Figure 1.7, including baccatin I (**1.5a**), 1- β -hydroxy-baccatin I (**1.13**), C-5 isophenylalanine side chain derivatives (**1.14**) and some novel derivatives having nicotinate substituent at C-9 (**1.15**).

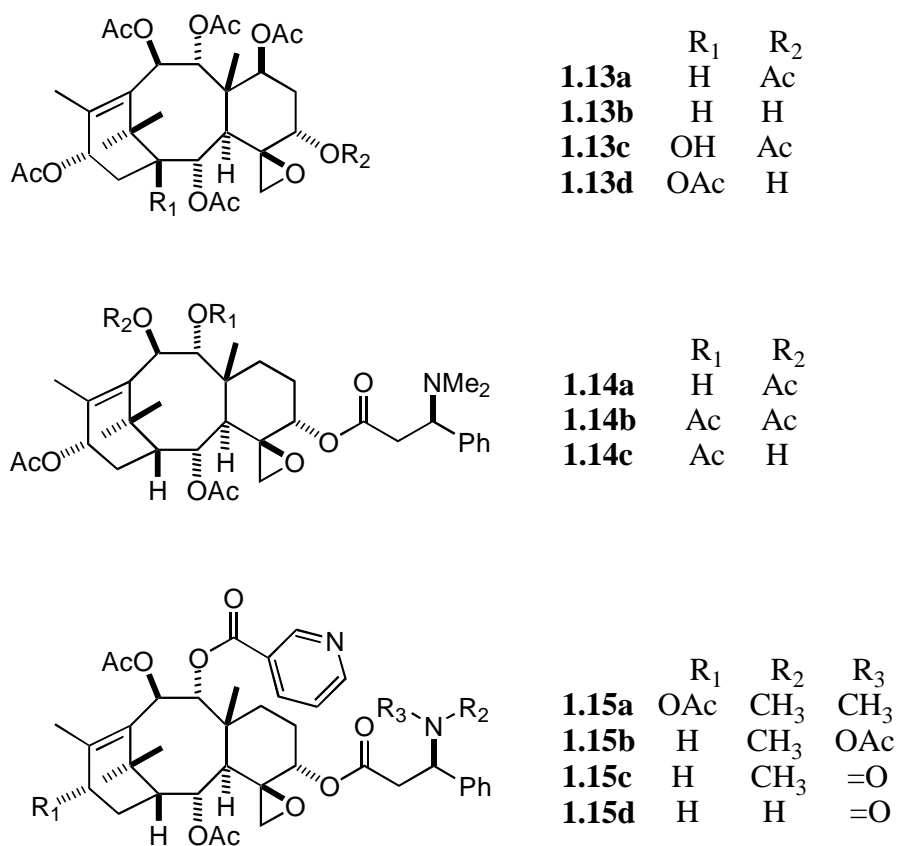


Figure 1.7 Some taxanes with a C-4 (20) epoxide

1.3.4 Taxanes Having an Oxetane Ring at C-4 and C-5

This class of compounds, including paclitaxel (**1.16**, Figure 1.8) as the key character, are usually present in fairly small quantities in various parts of the yew tree, yet they represent the most intriguing class of taxanes owing to their promising anti-cancer potential. They are usually characterized by a ketone group at C-9 and a complex side chain at the C-13 position rather than at the C-5 position that is often seen in other classes of taxanes.²⁰

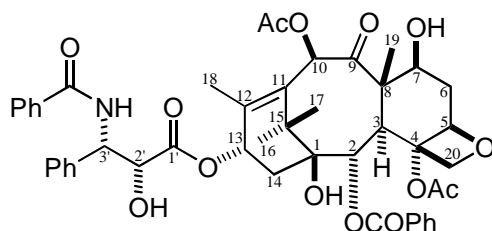


Figure 1.8 Paclitaxel (Taxol[®]) **1.16**

The first compound of this class was isolated as baccatin III from the heartwood of *Taxus baccata* in 1966 by Chan *et al.*²¹ and its structure was eventually shown to be **1.5c**. Many substances have been extracted from the bark of *Taxus baccata*, *Taxus wallichiana*, or *Taxus brevifolia* that differ only in the nature of the substituents at C-1, C-2 and C-4 positions, as shown in compounds **1.17a-e** (Figure 1.9).

²⁰ Suffness, M.; Cordell, G. A. In *The Alkaloids*, Brossi, A. Ed.; Vol. 25, Academic Press: New York, 1985, pp. 10.

²¹ Chan, W. R.; Hassal, T. G.; Hornby, G. M.; Oxford, A. W.; Sabel, W.; Bjammer, K.; Ferguson, G.; Monteath, R. J. *J. Chem. Soc., Chem. Commun.* **1966**, 923.

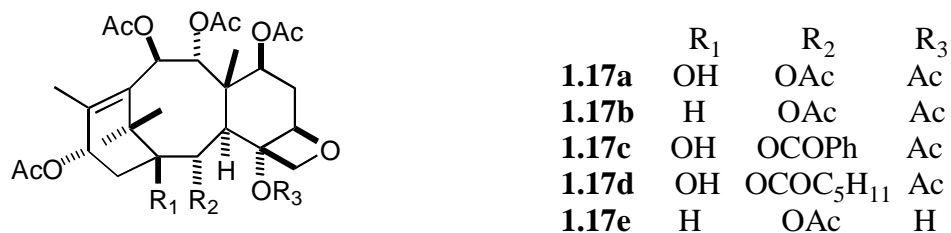
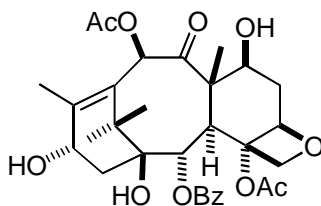


Figure 1.9 Other taxanes having an oxetane ring at C-4 and C-5

An important biosynthetic precursor of paclitaxel, 10-deacetylbaccatin III (**1.18**) was also isolated in reasonably good yield from the leaves of *Taxus baccata* in 1981²² as well as from the bark of *Taxus brevifolia* in 1982.²³ It serves as the starting material for the semi-synthesis of paclitaxel through a coupling reaction with an appropriately protected side chain that can be prepared synthetically.



1.18 10-Deacetylbaccatin III

²² Chauviere, G.; Guenard, D.; Picot, F.; Senilh, V.; Potier, P. *Acad. Sci. Ser.* **1981**, 2, 293, 501.

²³ Kingston, D. G. I; Hawkins, D. R.; Ovington, L. *J. Nat. Prod.* **1982**, 45, 466.

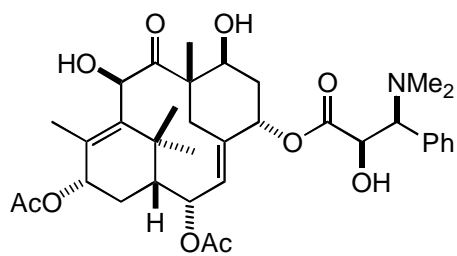
1.3.5 Miscellaneous Taxanes

In addition to the structural classes of taxanes discussed above, there are also a number of miscellaneous taxanes displaying a diversity of structural modifications to the tricyclic skeleton. Some examples include taxine A (**1.19**),^{17(a)} which has a distinctive rearranged skeleton; taxchinine A (**1.20a**)²⁴ and brevifoliol (**1.20b**),²⁵ which have a five-membered A ring in place of the normal six-member ring; hydroxylated analogs (**1.21a-b**)²⁶ of the taxanes with an exocyclic double bond between C-4 and C-20 (class A), which can also be viewed as the oxetane ring-opened compounds of the taxane class D (Figure 1.10).

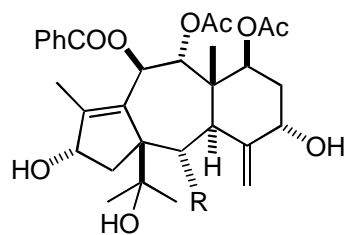
²⁴ Fuji, K.; Tanaka, K.; Li, B.; Singu, T.; Sun, H.; Taga, T. *Tetrahedron Lett.* **1992**, 33, 7915.

²⁵ (a) Georg, G. I.; Gollapudi, S. R.; Grunewald, G. L.; Gunn, C. W.; Himes, R. H.; Rao, B. K.; Liang, X. Z.; Mirhom, Y. W.; Mitscher, L. A.; Vander Velde, D. G.; Ye, Q. M. *Bioorg. Med. Chem. Lett.* **1993**, 3, 1345. (b) Balza, F.; Tachibana, S.; Barrios, H.; Towers, G. H. N. *Phytochemistry* **1991**, 30, 1613.

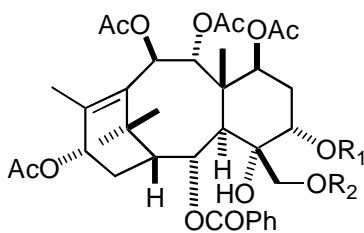
²⁶ Liang, J.; Kingston, D. G. I. *J. Nat. Prod.* **1993**, 56, 594.



1.19 Taxine A



1.20a taxchinin R = OAc
1.20b brevifoliol R = H



1.21a R₁ = H, R₂ = Ac
1.21b R₁ = Ac, R₂ = H

Figure 1.10 Miscellaneous taxanes

1.4 The Advent of Paclitaxel

As part of a screening and discovery program for cancer chemotherapeutics established at the National Cancer Institute (NCI) in 1960, thousands of plant samples were collected by the U. S. Department of Agriculture (USDA) and extracts of these samples were supplied to NCI for evaluation for anticancer activities. In August 1962, USDA botanist Arthur S. Barclay and his student assistants collected 650 plant samples in California, Washington and Oregon, including bark, twigs, leaves and fruit of *Taxus brevifolia* (pacific yew or western yew) in Washington State.²⁷ Initial samples of *Taxus brevifolia* were assigned to Dr. Monroe Wall's group at the Research Triangle Institute (RTI) under contract to the NCI. Its cytotoxicity to KB cells derived from a nasopharyngeal tumor was confirmed in 1964, and *in vivo* anti-cancer activity against the Walker 256 carcinosarcoma, P1534 leukemia, and L1210 leukemia models was also observed later.²⁸ Bioassay-directed fractionation was finally completed by June 1967 and yielded approximately 0.5 g of pure paclitaxel from 12 kg of air-dried stem and bark of *Taxus brevifolia*, corresponding to a yield of about 0.004%. Structure determination of paclitaxel was achieved after about a year in 1968 through a combination of spectroscopic techniques, chemical degradation, and X-ray crystallography of derivatives of degraded moieties. It was evident that paclitaxel had the empiric formula of C₄₇H₅₁NO₁₄ with a molecular weight of 853.9, and it was comprised of a taxane nucleus to which a rare four-membered oxetane ring was linked to C-4 and C-5, and an ester was attached at the C-13 position, as shown in **1.16**.²⁹ The complete IUPAC name for paclitaxel is tax-11-en-9-one-5 β ,20-epoxyl-1,2 α ,4,7 β ,13 α -hexahydroxy-4,10-diacetate-2-benzoate 13- β -benzoyl-amino- α -hydroxybenzenepropionate.

Development efforts for paclitaxel ceased for nearly a decade, however, due to its lack of significantly superior activity in bioassay systems available at that time, the

²⁷ *Washington Insight*, Persinos, E., Ed.; Sept. 15, 1990.

²⁸ Suffness, M. Taxol: From Discovery to Therapeutic Use. In *Annual Reports in Med. Chem.* Vol. 28, Bristol, J. A., Ed.; Academic Press: San Diego, CA, 1993, pp. 305.

formulation problems arising from its poor aqueous solubility (less than 0.01 mg/mL),³⁰ and the anticipated difficulties in collecting, extracting and isolating this scarce natural product on a large scale.³¹ Fortunately, one of the new models, the B16 melanoma, was introduced in the early 1970's with an emphasis on predicting activity against solid tumors. This was because it had become apparent that those intensively studied models such as the L1210 leukemia were less useful to this end, although they were valuable in discovering clinical agents for rapid growing tumors such as leukemia, lymphomas and childhood cancers. It was on this B16 melanoma animal model that paclitaxel showed good activity and thus by 1977, paclitaxel was selected by NCI as a development candidate.

NCI's decision spurred studies on paclitaxel in many areas, including chemistry, biology, formulation, pharmacology, toxicology, etc., which in turn, promoted the development of paclitaxel. In 1978, Fuchs and Johnson³² indicated that paclitaxel inhibited cell proliferation at the G₂-M phase of the cell cycle and blocked mitosis. A more detailed investigation by Horwitz's group³³ in 1979 established that paclitaxel inhibited mitosis by a unique mechanism in that it stabilized microtubules and inhibited depolymerization back to tubulin. At that time paclitaxel was the only one in a series of anti-mitotic agents that act by this mechanism, while all others bind to tubulin and inhibited polymerization of tubulin to microtubules.³⁴ Both the uniqueness of its structure and its mechanism of action made paclitaxel an even more worthy candidate for development.

Paclitaxel was found to be highly active in numerous preclinical tumor models,³⁵ and it entered Phase I clinical trials in 1981, which established toxicity profiles and dose schedules for further trials. Partial or minor responses were observed in patients with melanoma, ovarian, head and neck, and non-small cell lung cancer, as well as neutropenia

²⁹ Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.

³⁰ Adams, J. D.; Flora, K. F.; Goldspiel, B. R.; Wilson, J. W.; Arbuck, S. G.; Finley, R. *Monogr. Natl. Cancer Inst.* **1993**, *15*, 141.

³¹ Borman, S. *Chem. Eng. News* **1991**, *69*, 11.

³² Fuchs, D. A.; Johnson, R. K. *Cancer Treat. Repts.* **1978**, *62*, 1219.

³³ Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *22*, 665.

³⁴ Horwitz, S. B. *Trends Pharmacol. Sci.* **1992**, *13*, 134.

³⁵ Rose, W. C.; Crosswell, A. R.; Cassaza, A. M. *Proc. Am. Assoc. Cancer Res.* **1992**, *33*, 518.

and hypersensitivity reactions. Phase II trials began in 1985, and during those trials paclitaxel showed quite remarkable activity against refractory and advanced ovarian cancer with 30% response rate³⁶ which was confirmed by other investigators.³⁷ Activities had also been reported in metastatic breast cancer with 56%³⁸ and 62%³⁹ response rates, as well as in non-small cell lung cancer⁴⁰ and head and neck cancers.³⁸

In order to obtain an adequate supply of paclitaxel, the National Cancer Institute issued a Cooperative Research and Development Award in 1991 to Bristol-Myers Squibb after an open competition. The company then moved rapidly to market the drug. A new drug application (NDA) for paclitaxel was approved by the FDA in 1992 for treatment of ovarian cancer and in 1994 for breast cancer. In 1997, the U. S. sales of Taxol has reached \$970,000,000.

Paclitaxel has also received extensive chemical studies during its development. Various chemical transformations have been carried out, which provided structure-activity relationships (SAR) as well as analogs with greater anti-cancer potency. This area will be discussed in full detail later. Significant progress has also been made on the synthesis of the side chain of paclitaxel,⁴¹ which permits the facile semisynthesis of paclitaxel starting from baccatin III (**1.5c**) or 10-deacetylbaccatin III (**1.18**) that occur in other *Taxus* species in much greater quantity. These new natural sources of paclitaxel's semisynthetic precursor, e.g., the needles of *Taxus baccata*,⁴² a European yew, as renewable source, along with potential plant cell culture pathways for taxane

³⁶ McGuire, W. P.; Rowinsky, E. K.; Rosenheim, N. B.; Grumbine, F. C.; Ettinger, D. S.; Armstrong, D. K.; Donehower, R. C. *Ann. Intern. Med.* **1989**, *111*, 273.

³⁷ (a) Thigpen, T.; Blessing, J.; Ball, H.; Hummel, S.; Barret, R. *Proc. Am. Soc. Clin. Oncol.* **1990**, *9*, 156. (b) Einzig, A. I.; Wiernik, P. H.; Sasloff, J.; Runowicz, C. D.; Goldberg, G. *J. Clin. Oncol.* **1992**, *10*, 1748.

³⁸ Holmes, F. A.; Walters, R. S.; Thierault, R. L.; Forman, A. D.; Newton, L. K.; Raber, M. N.; Buzdar, A. U.; Frye, D. K.; Hortobagyi, G. N. *J. Natl. Cancer, Inst.* **1991**, *83*, 1797.

³⁹ (a) Rowinsky, E. K.; Onetto, N.; Canetta, R. M.; Arbuck, S. G. *Semin. Oncol.* **1992**, *19*, 646. (b) Seidman, A.; Reichman, B.; Crown, J.; Begg, C.; Heelan, R.; Hakes, T.; Surbon, A.; Gilewski, T.; Lebwohl, D.; Currie, V.; Huddis, C.; Klecker, R.; Collins, J.; Toomasi, F.; Berkery, R.; Quinlivan, S.; Kelsen, D.; Norton, L. *Proc. Am. Soc. Clin. Oncol.* **1992**, 1164.

⁴⁰ Murphy, W. K.; Fossella, F. V.; Winn, R. J.; *J. Natl. Cancer Inst.* **1993**, *85*, 384.

⁴¹ A number of side chain preparation have appeared in the literature. For excellent reviews, see Holton, R. A.; Biegiger, R. J.; Boatman, P. D. Semisynthesis of Taxol and Taxotere. In *Taxol: Science and Application*, Suffness, M. Ed.; CRC Press: Boca Raton, FL, 1994.

⁴² Suffness, M. In *Ann. Repts. Med. Chem.* Bristol, J. A. Ed.; Academic Press: San Diego, CA, **1993**, 305.

production,⁴³ had essentially assured paclitaxel's continuing supply. The total synthesis⁴⁴ of paclitaxel has also been a challenging target of a number of research groups. In particular, as one of the most exciting achievements in modern organic synthesis, both the Holton⁴⁵ group and the Nicolaou⁴⁶ group synthesized paclitaxel almost simultaneously in 1994, albeit that both syntheses are too long to have commercial value.

⁴³ (a) Christen, A. A.; Gibson, D. M.; Bland, J. *Proc. Am. Assoc. Cancer Res.* **1989**, *30*, 566. (b) Christen, A. A.; Gibson, D. M.; Bland, J. 1991, U.S. Patent 5,019,504. (c) Ma, W.; Park, G. L.; Gomez, G. A.; Nieder M. H.; Adams, T. L.; Aynsley, J. S.; Sahai, O. P.; Smith, R. J.; Stahlhut, R. W.; Hylands, P. J. *J. Nat. Prod.* **1994**, *57*, 116.

⁴⁴ For an excellent review, see Wender, P. A.; Natchus, M. G.; Shuker, A. J. Toward the Total Synthesis of Taxol and its Analogs. In *Taxol: Science and Application*, Suffness, M. Ed.; CRC Press: Boca Raton, FL, 1995, pp. 123.

⁴⁵ (a) Holton, R. A.; Somoza, C.; Kim, H.-B.; Liang, F.; Biegiger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K. K.; Gentile, L. N.; Liu, J. H. *J. Am. Chem. Soc.* **1994**, *116*, 1597. (b) Holton, R. A.; Somoza, C.; Kim, H.-B.; Liang, F.; Biegiger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K. K.; Gentile, L. N.; Liu, J. H. *J. Am. Chem. Soc.* **1994**, *116*, 1599.

⁴⁶ Nicolaou, K. C.; Yang, Z.; Liu, J. J.; Ueno, H.; Nantermet, P. G.; Guy, R. K.; Claiborne, C. F.; Renaud, J.; Couladouros, E. A.; Paulvanan, K. *Nature* **1994**, *367*, 630.

1.5 Paclitaxel's Mechanism of Action

Both normal cells and cancer cells have a characteristic life cycle during which a number of fundamental processes take place and important materials for cell division are synthesized. The cell cycle is divided into several transition periods that are called phases, as shown in Figure 1.11.

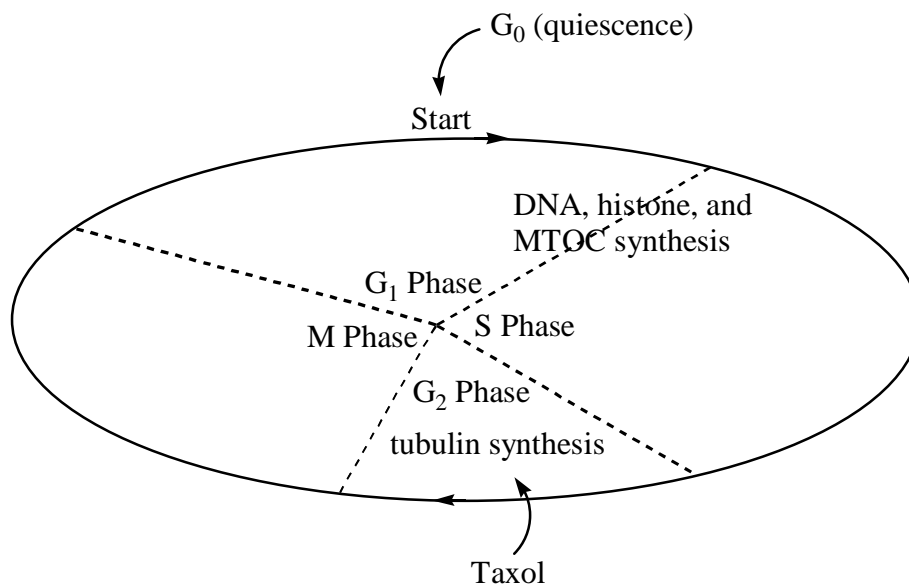


Figure 1.11 The cell cycle

Some of the cells in the human body, such as neurons, are generally nondividing once they are differentiated (a state called terminal differentiation). Other cells replicate at different rates, e.g., skeletal muscle cells and liver cells about once a year; stem cells about twice a day. The proliferation rate of a certain type of cell, however, is not determined only by the time needed to traverse the cell cycle, but primarily by the fraction of cells in the cell cycle. In normal circumstances, out of approximately 10^{13} cells in the human body, there are about 25 million cell divisions occurring at any time.⁴⁷ Therefore, the majority of cells spend most of their time in a nonproliferation state called quiescence

(G₀).⁴⁸ These quiescent cells can reenter the cell cycle at G₁ through the influence of tissue-specific growth factors or internal controls, etc., such as in the case of healing of a wound. In contrast, cancer cells often lack these control mechanisms and continue to replicate in conditions that normally induce quiescence. The cell cycle time of cancerous cells is therefore not necessarily shorter than that of normal cells, but the uncontrolled growth of tumors is usually caused by the greater fraction of the cells in the cell cycle.⁴⁹

In the cell cycle, cells pass through the G₁ phase during which DNA, histone, and microtubule organizing centers (MTOC) are synthesized. The cells then enter the G₂ phase, a period for preparation for chromosome condensation and mitosis, including the synthesis of tubulin and histone phosphorylation; the former is a major component of the mitotic spindle and the latter is a prerequisite for the interaction of chromosomal fibers into high order structures. Next, in mitosis, cells pass through five sequential periods: prophase, prometaphase, metaphase, anaphase, and telophase. Important events during these periods include chromosome condensation, spindle formation, attachment of the chromosomes to the spindle and their movement toward the center of spindle, separation of chromatids and their movement to opposite poles, and formation of two new nuclei of the daughter cells.

As a major component of the mitotic spindle, microtubules are essential in cell mitosis for all eukaryotic cells. Moreover, they are required for the maintenance of cell structure, motility, and cytoplasmic motion within the cell. Microtubules are structurally hollow tubes of about 30 nm in diameter, while their lengths vary between cell types and species. The walls of microtubules are usually made up of 13 subunits that are called protofilaments or tubulin (Figure 1.12). Tubulin, in turn, exists as a dimer of two alternating protein monomers: tubulin α and tubulin β . Each has a molecular weight of 55,000, and the two tubulins are structurally very similar to each other.

⁴⁷ Sadava, D. E. Chapter 12: Cell Cycle. In *Cell Biology*, Johns and Bartlett Publishers: Boston, MA, 1993.

⁴⁸ Pardee, A. B. *Science* **1989**, 249, 603.

⁴⁹ Baserga, R. *Biology of Cell Reproduction*; Harvard University Press: Cambridge, MA, 1985.

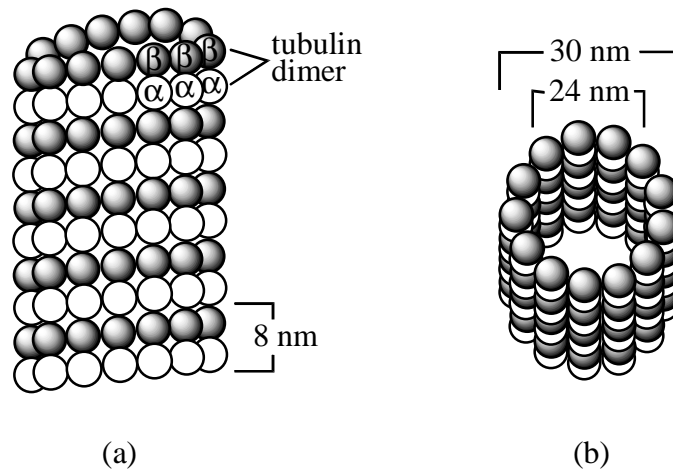


Figure 1.12 Microtubule (a) longitudinal view. (b) cross section view.

Microtubules are in a state of dynamic equilibrium with their subunit tubulins, with preferential growth at one end and dissociation at the other end, a process termed “threadmilling”. Under steady-state conditions, the net tubulin assembly rate equals the net disassembly rate and thus the length of the microtubule remains constant. In terms of the cell cycle, the synthesis of tubulin and the assembly of microtubules occurs during the G₂ phase and the prophase of mitosis, the period that paclitaxel was found to alter the course of cancer cell division (normal cell division as well) and finally lead to cell death. See Figure 1.13 for a schematic picture of the process.

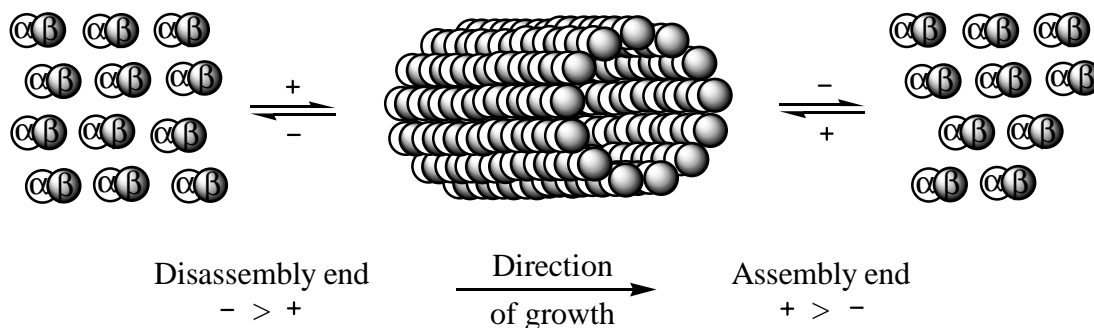


Figure 1.13 Microtubule “threadmilling”

Before the discovery of paclitaxel, there were several clinical and pre-clinical anti-cancer drugs that belong to the class of spindle poisons/tubulin interactive agents.

Colchicine, vinblastine, vincristine and podophyllotoxin all bind to tubulin, stabilize it and prevent it from assembling into microtubules.

In contrast, Horwitz *et al.* found in 1979 that paclitaxel displayed a unique ability in promoting microtubule assembly by binding and stabilizing microtubules. It was demonstrated that in the presence of paclitaxel, tubulin can be assembled into microtubules under appropriate conditions (even at a lower than usual temperature of 4°C)⁵⁰ in the absence of microtubule-associated proteins (MAPs), guanosine triphosphate (GTP) and ethylene glycol-bis-β-aminoethyl ether N,N'-tetraacetic acid (EGTA), which are the normal conditions for *in vitro* tubulin assembly.⁵¹

Paclitaxel has been found to bind to microtubules without competing with other microtubule interactive agents such as colchicine, podophyllotoxin or vinblastine, suggesting that paclitaxel binds to a different site on the tubulin.⁵² Paclitaxel stabilized microtubules containing MAPs were also found to be stable to both low temperature and to Ca²⁺ in the cell, which will usually cause depolymerization, while under the same conditions, paclitaxel treated microtubules in the absence of MAPs will depolymerize slowly.⁵³ This result supports the conclusion that paclitaxel does not compete with MAPs for binding to the microtubule surface and that the effects of MAPs and paclitaxel on microtubule assembly are additive.

Displacement studies with ³H-labelled paclitaxel and 7-acetyl paclitaxel established a binding site on preformed microtubules.⁵⁴ Photolysis of bound ³H-labelled paclitaxel followed by depolymerization specifically located the binding site at the β-tubulin subunit.⁵⁵ The maximum effects of paclitaxel were found when the paclitaxel:tubulin ratio approached a stoichiometric ratio (1:1), while tubulin polymerization was promoted with a concentration of paclitaxel of as low as 0.05 mM⁵⁶

⁵⁰ (a) see ref. 32. (b) Schiff, P. B.; Horwitz, S. B. *Biochemistry* **1981**, *20*, 3247.

⁵¹ Gaskin, F.; Cantor, C. R.; Shelanski, M. L.; *J. Mol. Biol.* **1974**, *89*, 737.

⁵² (a) Caplow, M.; Zeeberg, B. *J. Biochem.* **1982**, *127*, 319. (b) Choudhury, G. G.; Bhattacharyya, B.; Biswas, B. B. *Biochem. Cell Biol.* **1987**, *65*, 558.

⁵³ Collins, C. A. Vallee, R. B. *J. Cell. Biol.* **1987**, *105*, 2847.

⁵⁴ (a) Parness, J.; Horwitz, S. B. *J. Cell Biol.* **1981**, *91*, 479. (b) Takoudji, M.; Wright, M.; Chenu, J.; Gueritte-Voegelein, F.; Guenard, D. *FEBS Lett.* **1988**, *227*, 96.

⁵⁵ Rao, S.; Horwitz, S. B.; Ringel, I. *J. Natl. Cancer Inst.* **1992**, *84*, 785.

⁵⁶ (a) Schiff, P. B.; Horwitz, S. B. *Biochemistry* **1981**, *20*, 3247. (b) Horwitz, S. B.; Parness, J.; Schiff, P. B.; Manfredi, J. J. *Cold Spring Harbor Symp. Quant. Biol.* **1982**, *46*, 219.

and 0.01 mM.⁵⁷ The binding of paclitaxel to microtubules was also found to be reversible, with a binding constant of about 0.9 $\mu\text{mol/L}$.⁵⁸ Further studies using photoaffinity paclitaxel analogs suggest more specifically that the 3'-(*p*-azidobenzamido) group of the corresponding paclitaxel analog binds to the N-terminal 31 amino acids in the β -tubulin protein sequence,⁵⁹ and that the 2-benzoyl group of paclitaxel binds to 217-231 amino acids in the β -tubulin protein sequence.⁶⁰

Recently, other natural products such as epothilone⁶¹ and discodermolide⁶² have also been reported to share paclitaxel's mechanism of action in promoting microtubule assembly and have shown potential anti-cancer activity.

⁵⁷ Jordan, M. A.; Wilson, L. Microtubule Polymerization Dynamics, Mitotic Block, and Cell Death by Paclitaxel at Low Concentrations. In *Taxane Anticancer Agents: Basic Science and Current Status*, Goerg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M., Eds.; ACS Symposium Series 583, American Chemical Society: Washington, DC, 1995, pp. 138.

⁵⁸ see ref 52(a).

⁵⁹ Rao, S.; Krauss, N. E.; Heerding, J. M.; Swindell, C. S.; Ringel, I.; Orr, G. A.; Horwitz, S. B. *J. Biol. Chem.* **1994**, *269*, 3132.

⁶⁰ Rao, S.; Orr, G. A.; Chaudhary, A. G.; Kingston, D. G. I.; Horwitz, S. B. *J. Biol. Chem.* **1995**, *270*, 20235.

⁶¹ Hofle, G.; Bedorf, N.; Reichenbach, H. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2801.

⁶² Ter Haar, E.; Kowalski, R. J.; Day, B. W.; *Biochemistry* **1996**, *35*, 243.

1.6 Structure-Activity Relationships of Paclitaxel

One of the major areas that organic chemists can contribute to the development of new therapeutic agents is the study of the structure-activity relationships (SAR) of lead compounds. Analog synthesis of potential natural products will not only extend the resources from natural metabolites to an almost unlimited degree, but also help identify functional groups or structure features that are responsible for specific interactions of the drug molecule with receptors in the body, thus allowing the design of analogs with improved activity. In addition, the availability of synthetic drug analogs is often critical in cell biology studies, especially with appropriate techniques such as radioactive, fluorescent, and photoaffinity labeling.

Extensive studies on the synthesis of paclitaxel analogs and the establishment of structure-activity relationships (SARs) for paclitaxel have been carried out over the last decade. While new analogs are continually being made and SARs are constantly being refined, a considerable body of information on the SARs of paclitaxel has essentially been established, as summarized in Figure 1.14.⁶³ Instead of giving detailed biological data on a vast number of analogs modified at each functional groups and the ring skeleton, which can be found in a number of comprehensive reviews,⁶⁴ only general trends will be discussed here. More detailed discussions will be incorporated into the Results and Discussion section as pertinent and necessary.

⁶³ Adapted from Kingston, D. G. I. *Trends Biotechnol.* **1994**, *12*, 222.

⁶⁴ For excellent reviews, see (a) ref. 53. (b) Kingston, D. G. I. Recent Advances in the Chemistry and Structure-Activity Relationships of Paclitaxel. In *Taxane Anticancer Agents: Basic Science and Current Status*, Goerg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M., Eds.; ACS Symposium Series 583, American Chemical Society: Washington, DC, 1995, pp. 203. (c) George G. I.; Harriman, G. C. B.; Vander Velder, D. G.; Boge, T. C.; Cheruvallath, Z. S.; Datta, A.; Hepperle, M.; Park, H.; Himes, R.; Jayasinghe, L. Medicinal Chemistry of Paclitaxel: Chemistry, Structure-Activity Relationships, and Conformational analysis. In *Taxane Anticancer Agents: Basic Science and Current Status*, Goerg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M., Eds.; ACS Symposium Series 583, American Chemical Society: Washington, DC, 1995, pp. 217. (d) George G. I.; Boge, T. C.; Cheruvallath, Z. S.; Clowers, J. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. The Medicinal Chemistry of Taxol. In *Taxol: Science and Application*, Suffness, M. Ed.; CRC Press: Boca Raton, FL, 1995, pp. 317.

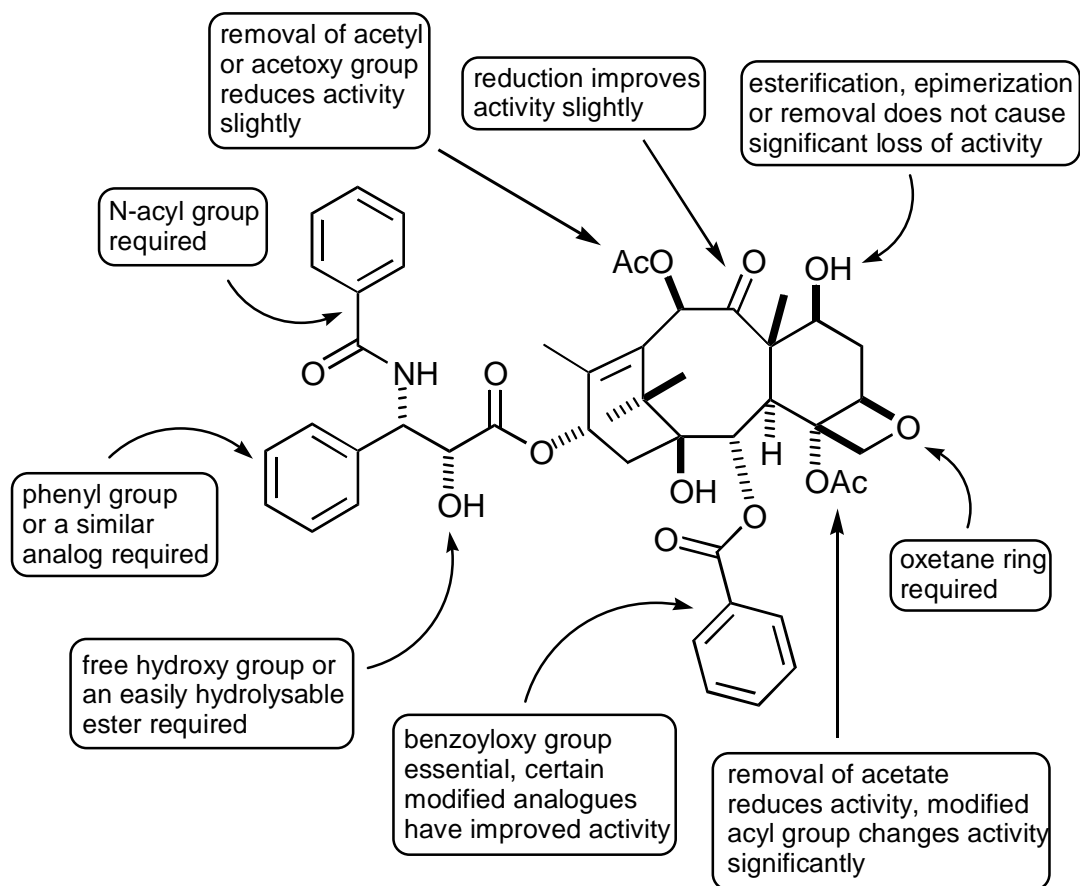


Figure 1.14 Structure-activity relationships of paclitaxel

Due to the poor crystalline property of paclitaxel and its analogs, the first X-ray crystal structure of a bioactive taxoid (docetaxol) appeared only in 1990,⁶⁵ and that of paclitaxel was not available until 1995.⁶⁶ NMR studies⁶⁷ and molecular modeling⁶⁸ were also used in determining the three-dimensional structure of paclitaxel and correlating the

⁶⁵ Gueritte-Voegelein, F.; Mangatal, L.; Guenard, D.; Potier, P.; Guilhem, J.; Cesario, M.; Pascard, C. *Acta Cryst.* **1990**, C46, 781.

⁶⁶ Mastropaolo, D.; Camerman, A.; Luo, Y.; Brayer, G. D.; Camerman, N. *Proc. Natl. Acad. Sci. USA* **1995**, 92, 6920.

⁶⁷ (a) Dubios, J.; Guenard, D.; Gueritte-Voegelein, F.; Guedira, N.; Potier, P.; Gillet, B.; Beloeil, J.-C. *Tetrahedron*, **1993**, 49, 6533. (b) Vander Velde, D. G.; Georg, G. I.; Grunewald, C. G.; Gunn, W.; Mitscher, L. A. *J. Am. Chem. Soc.* **1993**, 115, 11650. (c) Baker, J. K. *Spectrosc. Lett.* **1992**, 25, 31. (d) Hilton, B. D.; Chmurny, G. N.; Muschik, G. M. *J. Nat. Prod.* **1992**, 55, 1157. (e) Chmurny, G. N.; Hilton, B. D.; Brobst, S. Look, S. A.; Witherup, K. M.; Beutler, J. A. *J. Nat. Prod.* **1992**, 55, 414.

⁶⁸ Williams, H. J.; Scott, A. I.; Dieden, R. A.; Swindell, C. S.; Chirlian, L. E.; Francl, M. M.; Heering, J. M.; Krauss, N.-E. *Tetrahedron*, **1993**, 49, 6545.

conformation of paclitaxel with its microtubule-binding ability. Figure 1.15 gives a three-dimensional structure of paclitaxel modeled from the docetaxel crystal structure.⁶⁹

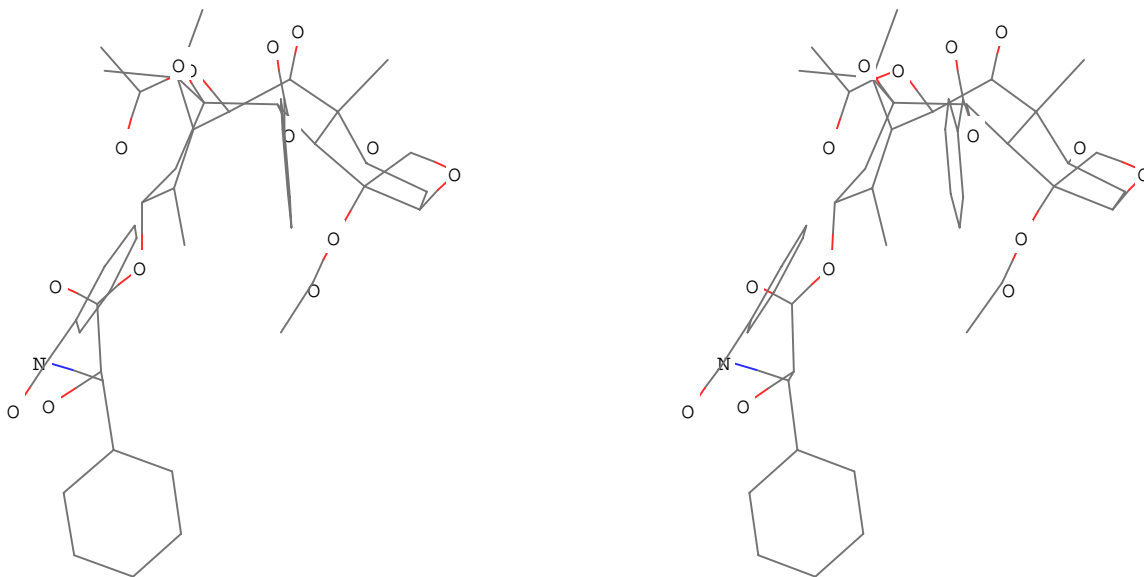


Figure 1.15 Stereoview of three-dimensional structure of paclitaxel

⁶⁹ Downloaded from The Three-Dimensional Drug Structure Databank (http://cmm.info.nih.gov/modeling/drugbank/compounds/cancer_compounds.html) and transformed using Chem3D Version 3.5.1.

1.7 Biological Evaluation of Paclitaxel Analogs

A number of methods have been adopted to evaluate microtubule assembly activity and cytotoxicity of paclitaxel analogs.

1.7.1 *In Vitro* Mammalian Microtubule Assembly Assays

These assays are designed based on paclitaxel's ability to promote microtubule assembly and to stabilize microtubules against disassembly at low temperature. There are three major variations of this method. (1) Initial slope of tubulin polymerization method was introduced by the Horwitz group,⁷⁰ in which a paclitaxel analog was incubated with tubulin and microtubule associated proteins (MAPs) in the absence of guanosine triphosphate (GTP). Turbidity was measured and plotted against time to give an initial slope value, and this was compared with that of paclitaxel control. (2) Extent of microtubule assembly method was developed by Himes⁷¹ and involved MAPs-free incubation conditions that do not cause microtubule assembly in the absence of paclitaxel. After incubation, the solution was centrifuged and the unassembled tubulin remaining in solution was determined by turbidity or colorimetric measurement. (3) Microtubule disassembly method was developed by the Potier⁷² group based on the unusual stability of paclitaxel bound microtubules. In the test, a solution of microtubule protein was assembled in the presence of the paclitaxel analog at 38 °C and was then cooled to 4 °C, with observation of the turbidity as a function of time to determine the initial rate of disassembly of microtubules in comparison with that of a paclitaxel control.

⁷⁰ (a) Swindell, C. S.; Krauss, N. E.; Horwitz, S. B.; Ringel, I. *J. Med. Chem.* **1991**, *34*, 1176. (b) Ringel, I.; Horwitz, S. B. *J. Natl. Cancer Inst.* **1991**, *83*, 288.

⁷¹ (a) Georg, G. I.; Cheruvallath, Z. S.; Himes, R. H.; Mejillano, M. R.; Burkner, C. T. *J. Med. Chem.* **1992**, *35*, 4230. (b) Mathew, A. E.; Mejillano, M. R.; Nath, J. P.; Himes, R. H.; Stella, V. *J. Med. Chem.* **1992**, *35*, 145.

⁷² Lataste, H.; Senih, V.; Wright, M.; Guenard, D.; Potier, P. *Proc. Natl. Acad. Sci., U. S. A.* **1984**, *81*, 4090.

All three methods use microtubule proteins isolated from mammalian organisms and the tests are conducted *in vitro*. The final results are often expressed as the ratio of the value of the investigated analog to that of paclitaxel (such as $ID_{50}/ID_{50(\text{paclitaxel})}$).

1.7.2 *In Vitro* Cytotoxicity Assays⁷³

The cytotoxicity of paclitaxel analogs could be determined using various cancer cell lines. The most frequently used are: KB cell lines, derived from human carcinoma of the nasopharynx; HCT116 cell lines derived from human colon carcinoma; P388 mouse lymphocytic leukemia cell lines; J774.2 mouse macrophages; L1210 leukemia, and B16 melanoma cell lines, etc. Relative cytotoxicity results are usually in good agreement with relative tubulin assembly activities, unless the analog possesses different metabolism or uptake pathways.⁷⁴

1.7.3 *In Vivo* Assays

In vivo assays can be conducted using either mouse tumors or human tumor xenografts in mice. Coverage of this topic has been reviewed⁷⁵ and can be found elsewhere.⁷⁶

⁷³ (a) Kinston, D. G. I. *Pharmacol. Ther.* **1991**, *52*, 1. (b) Rowinsky, E. K.; Donehower, R. C. *Pharmacol. Ther.* **1991**, *52*, 35. (c) Suffness, M.; Cordell, G. A. The Alkaloids: Chemistry and Pharmacology. In *The Alkaloids*, Vol. 25, Brossi, A., Ed.; Academic Press: New York, 1985, pp. 3.

⁷⁴ Gueritte-Voegelien, F.; Guenard, D.; Lavelle, F.; Le Goff, M. T.; Mangatal, L.; Potier, P. *J. Med. Chem.* **1991**, *34*, 992.

⁷⁵ (a) Suffness, M. *Annu. Rep. Med. Chem.* **1993**, *28*, 305. (b) Zee-Chang, R. K. Y. Cheng, C. C. *Drugs Future* **1986**, *11*, 45. (c) Rose, W. C. *Anti-Cancer Drugs* **1992**, *3*, 311.

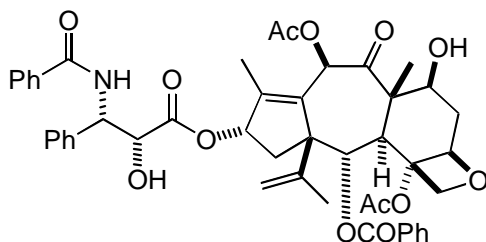
⁷⁶ Rose, W. C. Preclinical Antitumor Activity of Taxanes. In *Taxol: Science and Application*, Suffness, M. Ed.; CRC Press: Boca Raton, FL, 1995, pp. 209.

2. Results and Discussion

2.1 Synthesis of A-norpaclitaxel Analogs

2.1.1 Introduction

Paclitaxel has been found to undergo a rearrangement reaction under a number of acidic conditions to give the A-ring contracted analog A-norpaclitaxel (**2.1**) that contains a five-member A-ring fused with a seven-member B-ring instead of the bridged six-eight A-B ring system.



2.1 A-norpaclitaxel

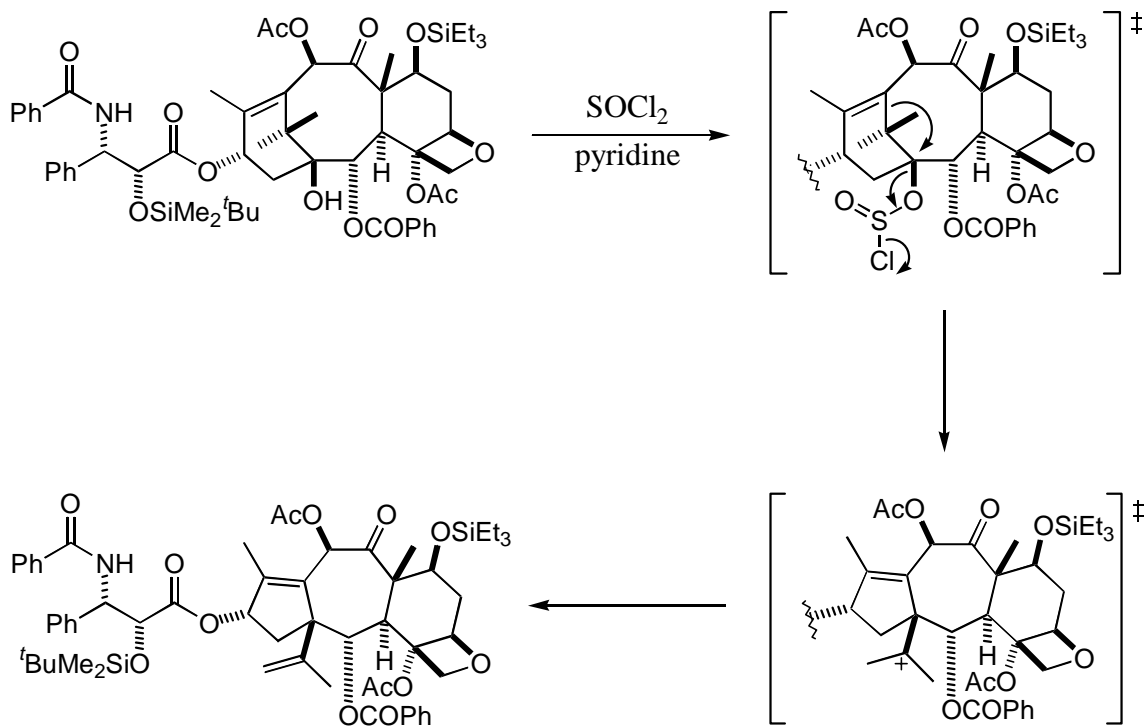
A-ring contraction was first observed by the Kingston group when paclitaxel was treated with acetyl chloride under vigorous conditions.⁷⁷ Many other Brønsted or Lewis acids, such as mesylating reagents, trifluoroacetic acid,⁷⁸ the fluorinating reagent diethylaminosulfur trifluoride (DAST),⁷⁹ etc. were also found to induce this rearrangement. While in many cases this type of rearrangement was an undesired side reaction, it has been optimized to nearly quantitative yield using thionyl chloride and

⁷⁷ Samaranayake, G.; Magri, N. F.; Jitrangri, C.; Kingston, D. G. I. *J. Org. Chem.* **1991**, *56*, 5114.

⁷⁸ Wahl, A.; Gueritte-Voegelein, G.; Guenard, D. *Tetrahedron* **1992**, *48*, 6965.

⁷⁹ Chen, S. H.; Huang, S.; Wei, J.; Farina, V. *J. Org. Chem.* **1993**, *58*, 4520.

excess pyridine in dry methylene chloride, and is thus a useful synthetic approach to a novel ring system. The proposed mechanism of the reaction is depicted in Scheme 2.1.



Scheme 2.1 Paclitaxel's A-ring contraction pathway

Preliminary biological studies indicated that *A-norpaclitaxel* was nearly one third as active as paclitaxel in the tubulin assembly assay but was much less cytotoxic against Burkitt lymphoma CA 46 cells than paclitaxel,⁸⁰ which suggests a different physiological pathway. Due to this incongruent tubulin assembly activity and cytotoxicity and the similarity of its conformation to that of paclitaxel, derivatization of *A-norpaclitaxel* was desired and conducted in order to find analogs with improved activity and simpler structure, as well as to extend SAR knowledge of this region.

At the outset of this project, analogs of *A-norpaclitaxel* modified at the C-2 phenyl ring and the double bond of the C-1 isopropenyl moiety had been made and their

⁸⁰ Chordia, M. D.; Kingston, D. G. I.; Hamel, E.; Lin, C. M.; Long, B. H.; Fairchild, C. A.; Johnston, K. A.; Rose, W. C. *Bioorg. Med. Chem.* **1997**, *5*, 941.

biological activities evaluated. Figure 2.1 and Table 2.1 give the structure and biological data of these compounds, respectively.

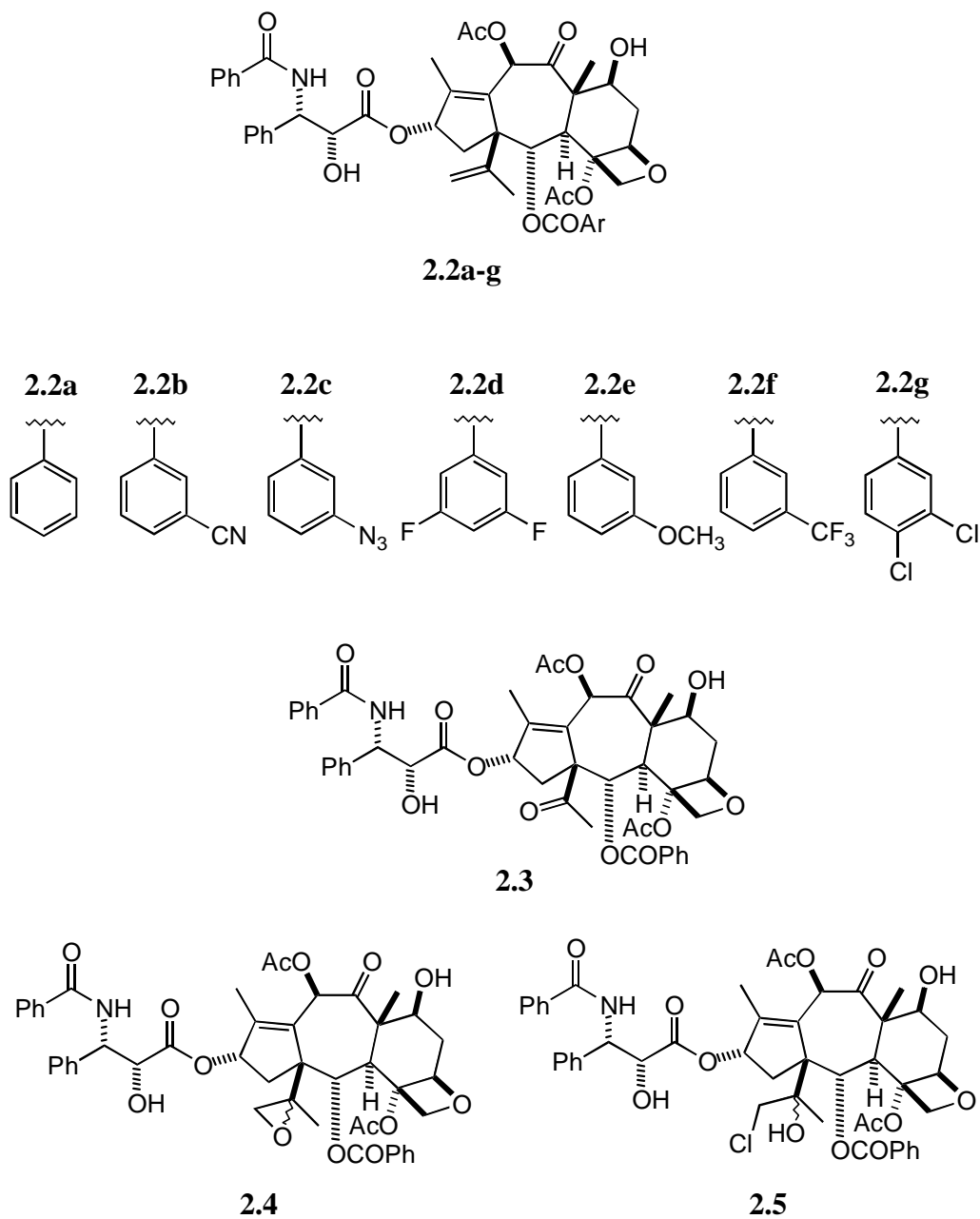


Figure 2.1 Some reported *A-norpaclitaxels*

Table 2.1 Biological activity of some reported *A-norpaclitaxel* analogs

Compound	EC ₅₀ (μM) ^a	IC ₅₀ (nM) ^b
Paclitaxel	4.1	9
2.2a	>40	>100
2.2b	>40	>100
2.2c	>40	>100
2.2d	>40	>100
2.2e	>40	>100
2.2f	>40	>100
2.2g	>40	>100
2.3	>40	>100
2.4	3.8	20
2.5	17	50

^a EC₅₀ (μM) for polymerization in 1.0M glutamate at RT in the absence of GTP. Data from reference 70.

^b IC₅₀ (nM) for cytotoxicity to Burkitt lymphoma CA 46 cells.

Interestingly, unlike paclitaxel where certain selected modifications at the C-2 phenyl group usually favorably alter the tubulin assembly activity, substitution at the same position of *A-norpaclitaxel* (**2.2a-g**) uniformly decreased tubulin assembly activity slightly. On the other hand, modifications at the C-1 isopropenyl moiety (**2.4**, **2.5**) increased the tubulin assembly activity, even to the level of paclitaxel.

It seems that the spatial volume of the substituent at C-1 may play a role in determining tubulin assembly activity by forcing the ring skeleton to adopt the best conformation and thus place the C-2 benzoyl moiety and possibly the side chain as well at the right position for interacting with microtubules, which in turn, is believed to be critical to the tubulin assembly activity.⁸¹ It was also noticed that *A-norpaclitaxel* analogs that have retained or even better anti-cancer activity could be potential second-generation

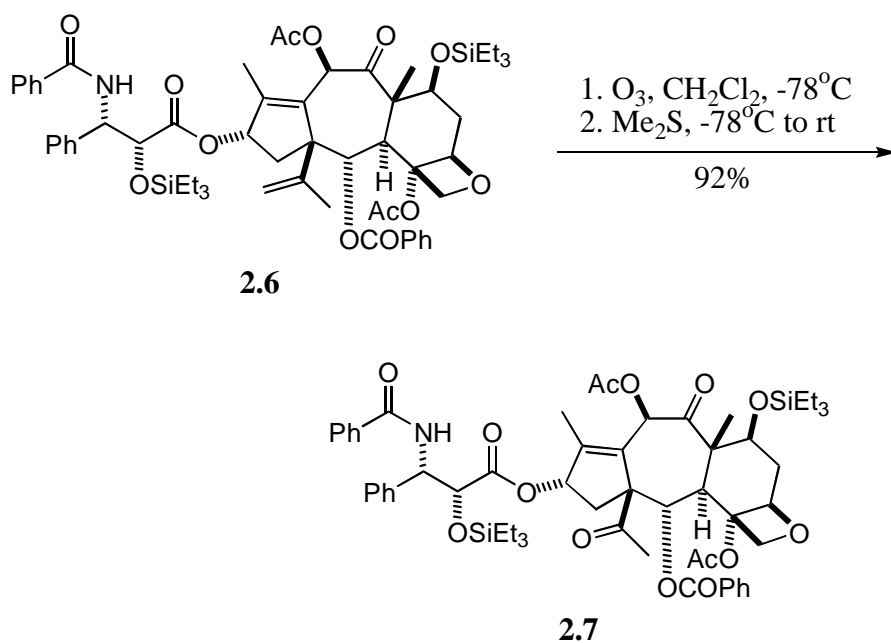
⁸¹ Kingston, D. G. I. *Taxane Journal* **1996**, *II*, 20.

drugs due to their simplified ring structure which could conceivably be synthesized more easily in the laboratory than isolated from the Nature.

In order to test this assumption, and to obtain more structure-activity relationship (SAR) information for this region, with an aim of preparing analogs with enhanced anti-cancer activity, we decided to modify the C-1 substituent, with a focus on the double bond functionality.

2.1.2 Synthesis of 1-deisopropenyl-1-acetoxy-A-norpaclitaxel

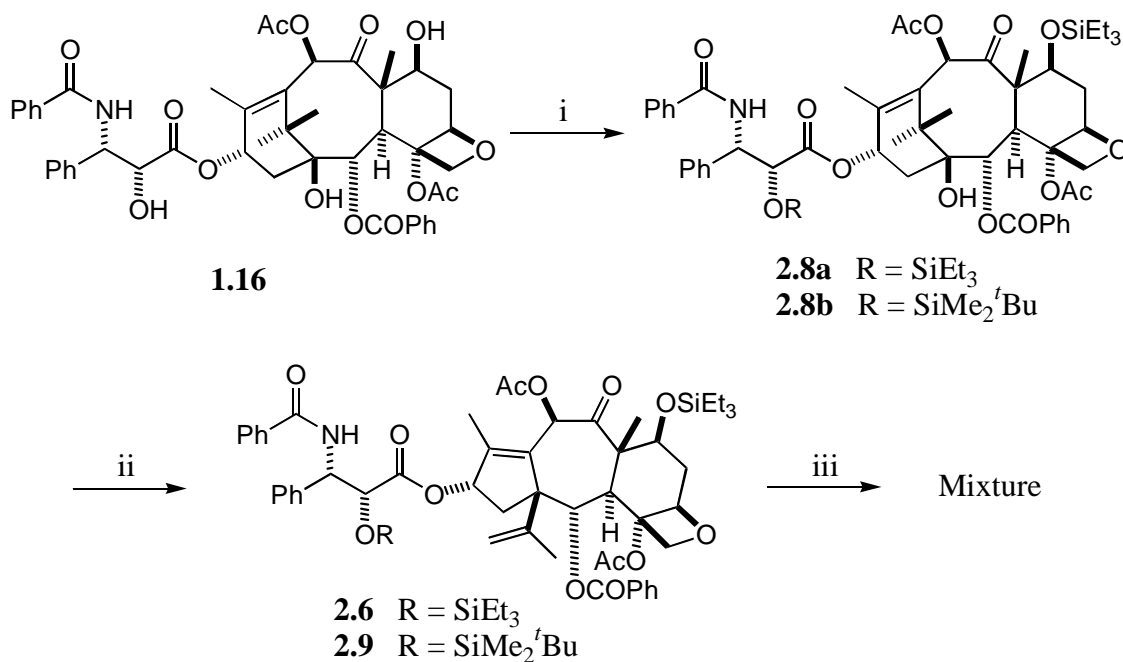
2',7-Bistriethylsilyl-1-deisopropenyl-1-acetyl-A-norpaclitaxel (**2.7**) had been prepared before in Kingston's group by ozonolysis of 2',7-bistriethylsilyl-A-norpaclitaxel (**2.6**) in methylene chloride followed by reduction with dimethyl sulfide (Me₂S) and desilylation. The cleavage of the olefin is shown in Scheme 2.2.



Scheme 2.2 Reported ozonolysis of the olefin **2.6**

It was envisioned that the ketone **2.7** could serve as an intermediate for the preparation of other desired A-norpaclitaxel analogs, for example, further oxidation of the ketone to an ester through Baeyer-Villiger reaction would afford a new acetoxy substituent at the C-1 position. To this end, paclitaxel (**1.16**) was converted to 2',7-bis-triethylsilyl-A-norpaclitaxel (**2.6**) or 2'-tert-butyltrimethylsilyl-7-triethylsilyl-A-norpaclitaxel (**2.9**) in three steps (Scheme 2.3). However, ozonolysis of **2.6** or **2.9** under the same conditions as reported previously reproducibly gave a mixture of products among which the major product was found to be the desired keto-compound **2.7** or **2.10**

in a low yield of 50-70%. It was also observed that some of the minor products were unstable and were converted to at least two other products, whose identification will be discussed later.

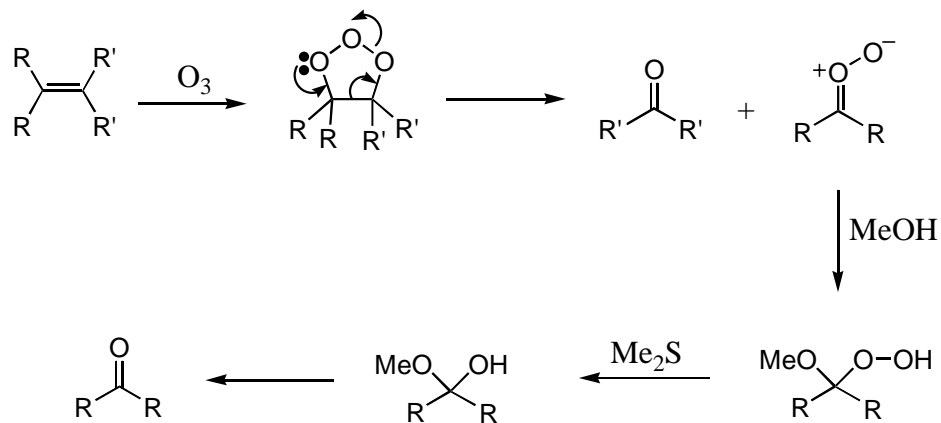


- i. for **2.8a**, Et₃SiCl, imidazole, DMF; 97%;
for **2.8b**, (a) ^tBuMe₂SiCl, imidazole, DMF;
(b) Et₃SiCl, imidazole, DMF; 95%, (2steps).
ii. SOCl₂, pyridine, CH₂Cl₂, 98%.
iii. (a) O₃, CH₂Cl₂, -78°C, (b) Me₂S, -78°C to rt.

Scheme 2.3 Preparation of 2',7-disilylated A-norpaclitaxel and its ozonolysis using reported procedures

Since literature evidence⁸² clearly indicated that the reductive workup of an ozonization reaction using dimethyl sulfide (Me₂S) relies on the formation of a peroxy hemiacetal, which is formed in the presence of alcohol, as illustrated in Scheme 2.4, a revised condition using methanol as co-solvent was chosen for the ozonization reaction.

⁸² (a) Pappas, J. J.; Keaveney, W. P.; Gancher, E.; Berger, M. *Tetrahedron Lett.* **1966**, 4273. (b) Stotter, P. L.; Eppner, J. B. *Tetrahedron Lett.* **1973**, 26, 2417.

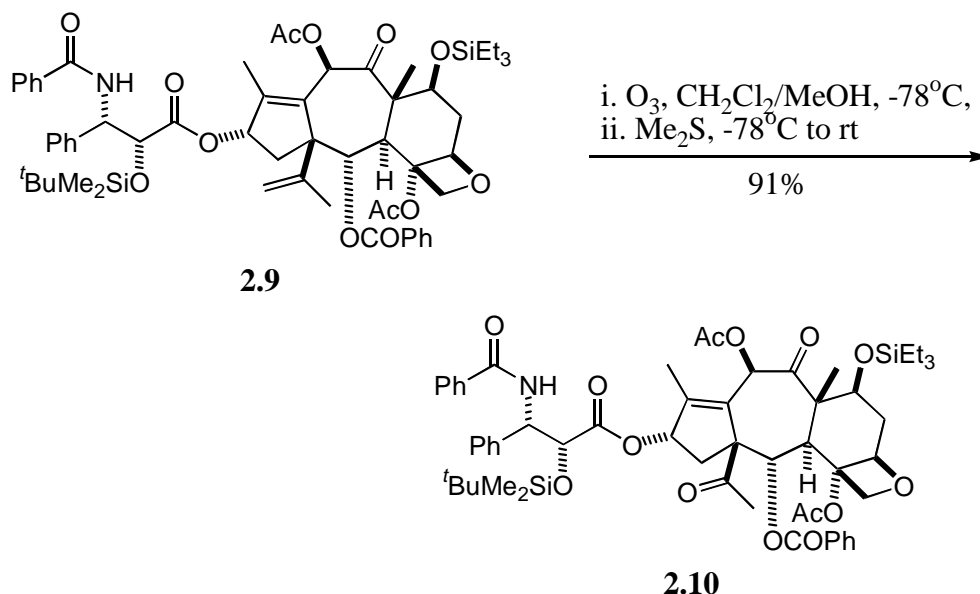


Scheme 2.4 Reductive workup of ozonization

The amount of methanol was varied from 3 equivalents⁸³ of methanol in CH₂Cl₂ to 1:1 ratio of MeOH and CH₂Cl₂, and finally to methanol only. In all cases the reaction proceeded very cleanly, differing only in the time needed to complete the reaction; larger quantities of methanol tended to slow down the reaction. When methanol was used as the only solvent, the ozonization was finished in a matter of hours, while in 3 equivalents of methanol in methylene chloride the reaction was usually finished in 10-15 minutes⁸⁴ (Scheme 2.5).

⁸³ According to reference 82(b), 1 equivalent of MeOH is needed. Due to the small scale of the reaction, approximately 3 equivalents was used in this experiment.

⁸⁴ The time needed for the completion of the ozonization also depended on the amount of olefin and the flow rate of the O₂ that carries O₃. These data were obtained under comparable conditions.



Scheme 2.5 Revised ozonolysis conditions

Thin layer chromatography (TLC) analysis was used to monitor the progress of ozonization and the subsequent reduction. It was apparent that ozonolysis in the presence of methanol proceeded in a different way from that in pure methylene chloride. Figure 2.2 shows a comparison of the TLC behavior of the reaction under both conditions.

In the presence of methanol, the starting olefin was converted to two compounds, one of which was the desired keto-analog (spot x). The other compound (spot y), which was the major product and was presumably the peroxyhemiacetal, was reduced afterward with dimethyl sulfide to give the keto-analog (spot x) in greater than 91% isolated yield. Ozonization in the absence of methanol, however, gave a mixture of products with similar polarities. The mixture contained the keto-analog and other intermediates that were not stable and decomposed in the course of reaction, and were not affected by the addition of dimethyl sulfide.⁸⁵

⁸⁵ Ozonization reactions in the absence of methanol were carried out both with/without dimethyl sulfide reduction. TLC analysis displayed identical pattern.

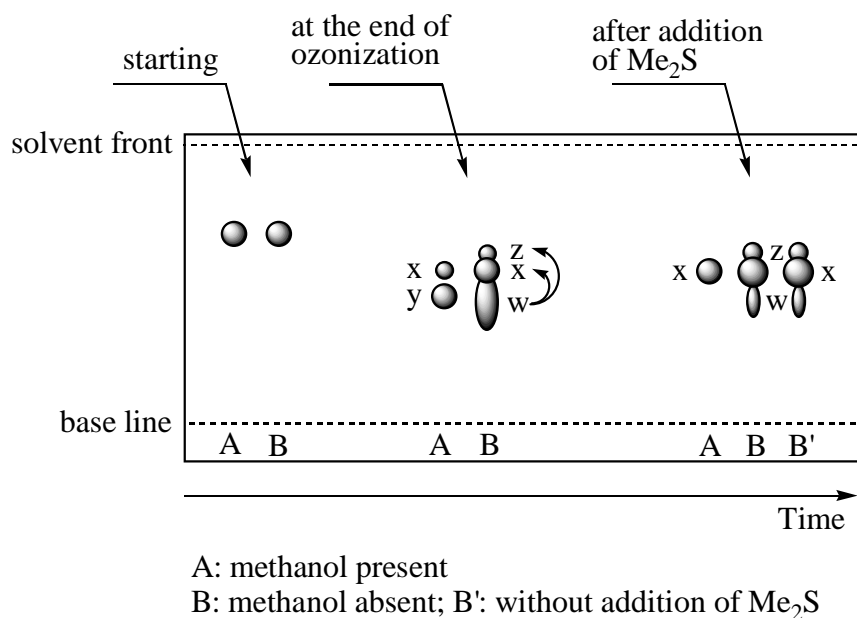


Figure 2.2 A comparison of the thin layer chromatogram of the ozonolysis under methanol and methanol-free conditions

Having established the right procedures for the preparation of 2'-*tert*-butyldimethylsilyl-7-triethylsilyl-1-deisopropenyl-1-acetyl-*A-nor*paclitaxel (**2.10**) in excellent yield, the focus of the study proceeded to Baeyer-Villiger oxidation of compound **2.10**. Initial reactions using *meta*-chloroperoxybenzoic acid (mCPBA) or trifluoroacetic anhydride and the urea-hydrogen peroxide complex⁸⁶ were extremely slow, and prolonged treatment led to decomposition of the starting material (Table 2.2). While investigating the conditions of the reaction, it was noted that one of the parallel tests using mCPBA lost all the solvent (methylene chloride) after overnight reaction due to a loose cap of the reaction vial, and the yield was found to be much higher than the other reactions (TLC). Keeping the solid mixture in the vial for a longer time did not change the reaction mixture composition any more. It was thus realized that the key to this reaction was to keep a very high concentration of the reactants.

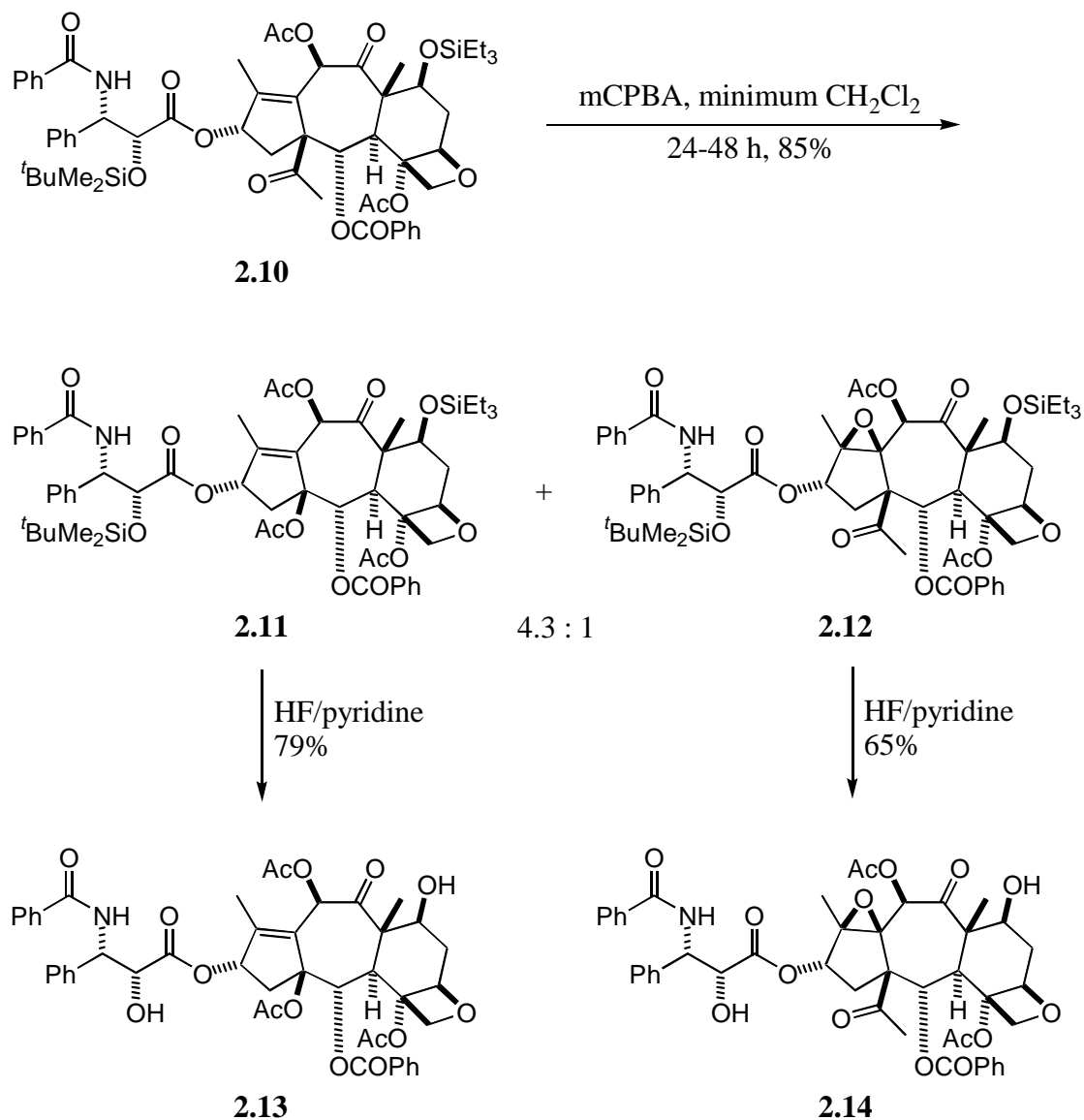
⁸⁶ (a) Cooper, M. S.; Heaney, H.; Newbold, A. J.; Sanderson, W. R. *Synlett* **1990**, 533. (b) Jackson, W. P. *Synlett* **1990**, 536.

Table 2.2 Conditions for the Baeyer-Villiger oxidation

Experiment	Solvent	Reagent	Conversion ^a
1	CH ₂ Cl ₂	mCPBA	< 10%
2	CH ₂ Cl ₂	mCPBA/NaHCO ₃	< 10%
3	EtOAc	mCPBA	< 10%
4	CH ₂ Cl ₂	(CF ₃ CO) ₂ O/H ₂ O ₂ -urea	< 5%

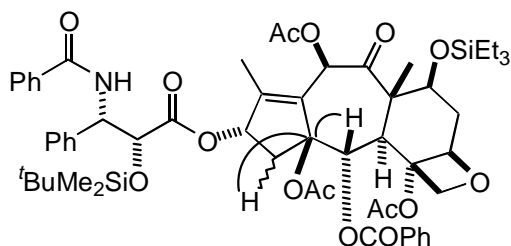
^a estimated yield after 48 hours based on TLC absorption.

Therefore, **2.10** was reacted with mCPBA in a minimum amount of methylene chloride for 24-48 hours to afford the desired compound 2'-*tert*-butyldimethylsilyl-7-triethylsilyl-1-deisopropenyl-1-acetoxy-*A-nor*paclitaxel (**2.11**) in 69 % yield, along with the 11,12-epoxy analog (**2.12**) of the keto- compound **2.10** in 16% yield. Subsequent deprotection of both products gave 1-deisopropenyl-1-acetoxy-*A-nor*paclitaxel (**2.13**) in 79% yield and 1-deisopropenyl-1-acetyl-11,12-epoxy-*A-nor*paclitaxel (**2.14**) in 65% yield (Scheme 2.6).



Scheme 2.6 Synthesis of 1-deisopropenyl-1-acetoxy-A-norpaclitaxel and 1-deisopropenyl-1-acetyl-11,12-epoxy-A-norpaclitaxel

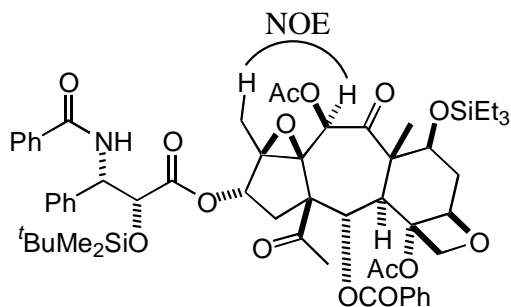
The structure of compound **2.11** was determined by ^1H , ^{13}C , TOCSY, HMQC, HMBC, and NOESY spectroscopy. The key information was the ^{13}C chemical shift of C-15 from 204.5 ppm to 170.1 ppm and C-1 from 69.8 ppm to 93.6 ppm. HMQC and HMBC established the proton-carbon and carbon-carbon connectivity (Figure 2.3). The composition was confirmed by high resolution fast atom bombardment mass spectroscopy (HRFABMS).



2.11

Figure 2.3 Key HMBC correlation of **2.11**

The structure of compound **2.12** was determined by ¹H, APT, and TOCSY spectroscopy and its composition was confirmed by HRFABMS. The chemical shift of both protons at C-10 and C-13, which were shifted upfield from 6.36 ppm to 5.76 ppm (C-10) and from 5.99 ppm to 5.30 ppm (C-13) were consistent with an oxidation reaction at the C-11,12 double bond. The ¹³C chemical shifts of both C-11 and C-12, which shifted from the olefinic region (133.3 ppm and 148.0 ppm) to the oxygenated region (two extra carbons observed between 70-84 ppm), assured this assignment. The stereochemistry of the epoxide was determined by a NOESY experiment on the protected compound **2.12**, in which a correlation of the C-18 methyl group and the C-10 proton was observed (Figure 2.4).

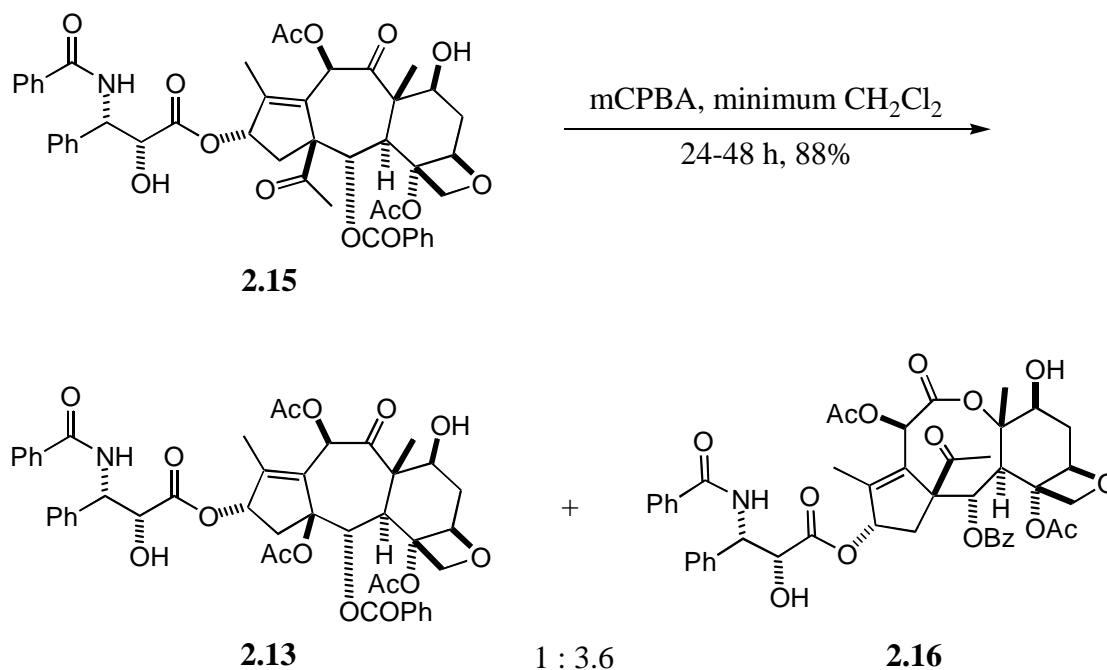


2.12

Figure 2.4 Key NOESY correlation of the epoxide **2.12**

2.1.3 Synthesis of 1-deisopropenyl-1-acetyl-8,9-oxido-A-norpaclitaxel

In the course of the preparation of compound **2.13** on a larger scale for *in vivo* biological study, a batch of recovered 1-deisopropenyl-1-acetyl-A-nor-paclitaxel **2.15** obtained from deprotection of **2.10** was used to react with mCPBA. Surprisingly, a different compound was obtained and characterized as 1-deisopropenyl-1-acetyl-8,9-oxido-A-nor-paclitaxel (**2.16**) along with the desired compound **2.13** in a ratio of about 3.6:1 (**2.16:2.13**) (Scheme 2.7).



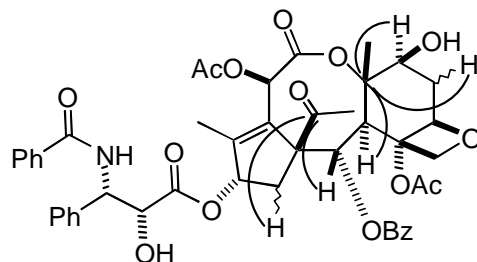
Scheme 2.7 Synthesis of 1-deisopropenyl-1-acetyl-8,9-oxido-A-nor-paclitaxel

The ¹³C spectrum of **2.16** was very similar to that of **2.13** in that both compounds have the same number of carbonyl, ester carbonyl, quaternary carbon, and oxygenated quaternary carbon signals. See Table 2.3 for a comparison of the ¹³C signals of the compounds **2.15**, **2.13**, and **2.16**.

Table 2.3 ^{13}C signals of compounds **2.13**, **2.15**, and **2.16**

2.15	2.13	2.16
204.4(C-15)	202.1(C-9)	204.8(C-15)
202.5(C-9)	172.9	171.9
172.4	171.7	171.2
171.1	170.1	169.5
170.4	168.8	169.3
166.8	166.8	166.6
165.1	164.6	165.1
147.9	146.4	149.2
137.9	137.6	138.5
133.7	133.8	133.72
133.5	133.7	133.67
131.9	131.8	132.0
131.7	129.9	131.8
130.0	129.5	129.7
128.8	129.3	129.1
128.7	128.61	128.8
128.6	128.60	128.71
128.5	128.5	128.68
128.1	128.0	128.2
126.9	127.2	127.1
126.9	126.9	127.0
84.7	93.4(C-1)	92.5(C-8)
79.6	84.7	84.3
79.0	81.3	82.1
74.6	79.7	80.5
73.2	74.4	74.5
71.8	72.7	74.3
71.7	71.7	72.9
71.1	71.4	71.3
69.4(C-1)	70.5	69.2
56.0(C-3')	55.15(C-3')	67.6(C1)
55.1(C-8)	55.12(C-8)	55.0(C-3')
44.0	44.5	48.6
36.4	37.1	35.5
35.4	34.7	34.8
25.5	22.1	25.5
21.6	21.0	22.3
20.3	20.5	20.2
12.4	13.4	14.2
8.4	9.6	12.4

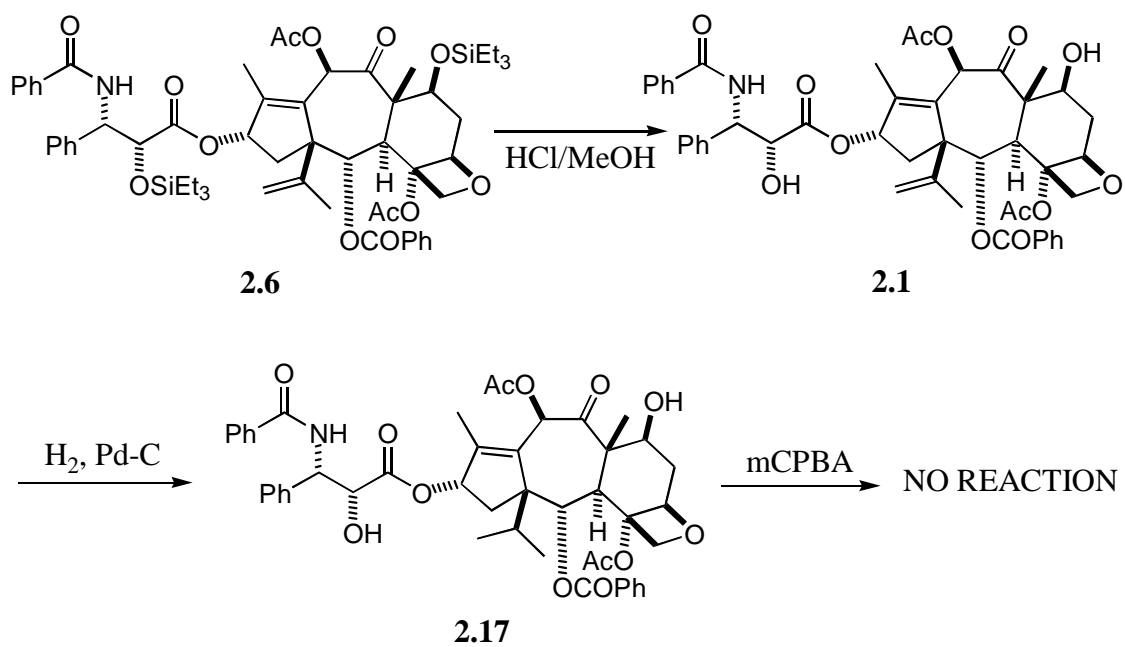
The structure of **2.16** was assigned unambiguously using a combination of ^1H , ^{13}C , DEPT, TOCSY, HMQC, and HMBC spectroscopic techniques, and its composition was confirmed by HRFABMS. Figure 2.5 shows the key HMBC (2-3 bonds) correlation used to determine the position of the inserted oxygen atom.



2.16

Figure 2.5 Key HMBC correlation of compound **2.16**

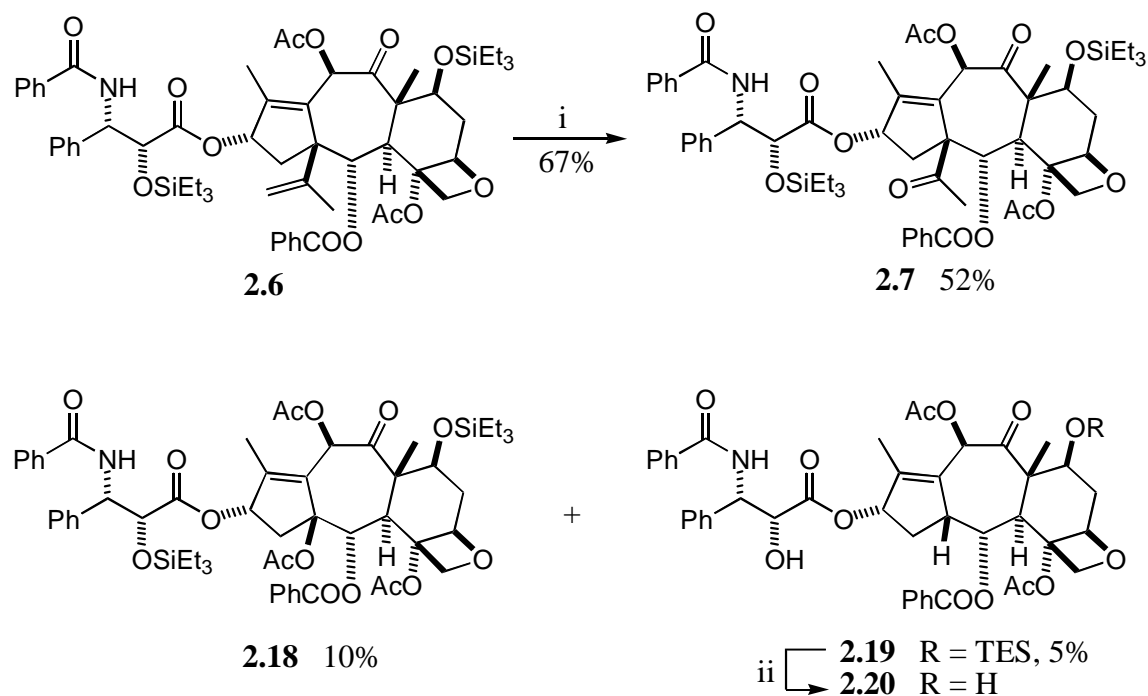
The formation of compound **2.16** was first assumed to be due to the anchimeric effect of the C-7 free hydroxyl group. In order to test this assumption, and to prepare analogs of the B-ring expanded (B-lactone) paclitaxel (**2.16**), *A-norpaclitaxel* (**2.1**) was prepared and hydrogenated to afford 15,16-dihydro-*A-norpaclitaxel* (**2.17**). When **2.17** was treated with mCPBA under the same conditions, no reaction was observed (See Scheme 2.8). It thus appeared that the delivery of mCPBA must be assisted by the C-15 keto group rather than the C-7 hydroxyl group, as assumed earlier.



Scheme 2.8 Preparation of 15,16-dihydro-A-norpaclitaxel and its reaction with mCPBA

2.1.4 Synthesis of 1-deisopropenyl-A-norpaclitaxel

As described in section 2.1.2, ozonolysis of **2.6** in methylene chloride gave a mixture of products, among which the desired ketone (**2.7**) was the major component. Other products included unstable intermediate(s) that decomposed upon standing. Two of the final stable components were isolated and identified as 2,7-bis-triethylsilyl-1-deisopropenyl-1-acetoxy-A-norpaclitaxel (**2.18**) in 10% yield and 7-triethylsilyl-1-deisopropenyl-A-norpaclitaxel (**2.19**) in 5% yield (Scheme 2.9).



i. (a) O₃, CH₂Cl₂, -78°C, (b) Me₂S, -78°C to rt; ii. HF/pyridine, 86%

Scheme 2.9 Ozonolysis products of the olefin **2.6** in methylene chloride

¹H NMR showed that the new compound (**2.19**) lacked a methyl peak corresponding to the C-17 methyl group and that it had an extra signal for one proton at 2.95 ppm. A TOCSY experiment using 0.012 ms mixing time (for detection of coupling

through 2-3 bonds) showed that the extra proton was coupled to the C-14 α proton at 1.67 ppm and the C-14 β proton at 2.42 ppm, as well as the C-2 proton at 5.60 ppm (Figure 2.6).

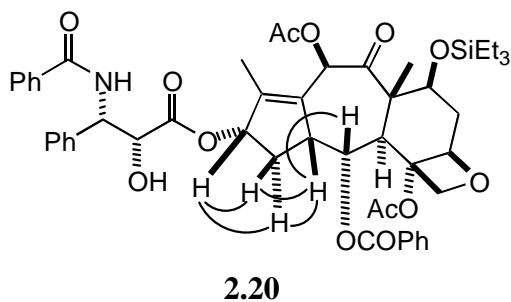


Figure 2.6 Key TOCSY correlation of the 1-deacetyl *A-norpaclitaxel* **2.20**

A ^{13}C NMR spectrum indicated the same loss of the C-17 methyl carbon signal at 25.5 ppm, as well as the C-15 carbonyl carbon signal at 204.4 ppm. The C-1 carbon was shifted from 69.4 to 47.4, suggested a transformation of the quaternary carbon bearing an acyl group to a bridgehead methine carbon. Other signals were very similar to the corresponding signals in the spectrum of **2.7**. Based on this information, the structure of the new compound was assigned as 7-triethylsilyl-1-deisopropenyl-*A-norpaclitaxel* (**2.19**).

The composition of this new compound was confirmed by HRFABMS. A NOESY experiment using 0.05 second mixing time and 5 second delay time established the stereochemistry of the C-1 stereogenic center, as shown in Figure 2.7, based on the observed correlation between the C-1 proton and the C-13 and C-19 protons.

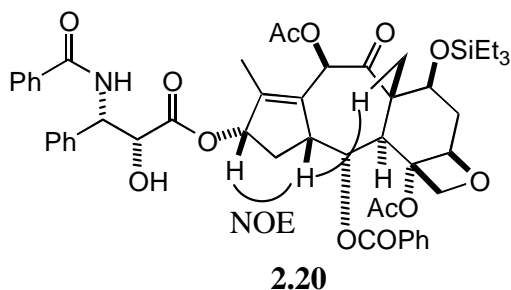
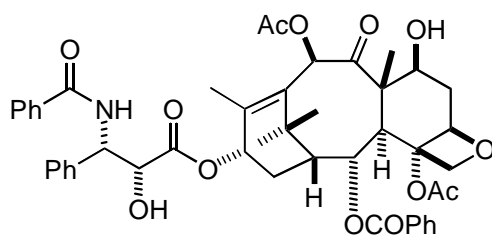


Figure 2.7 Key NOESY correlation for determining the stereochemistry of **2.20**

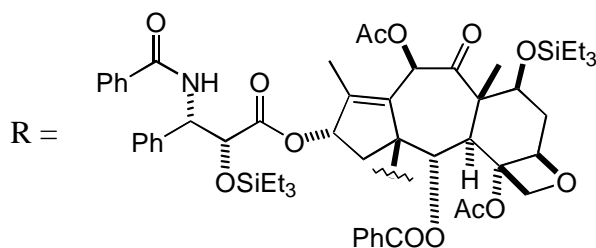
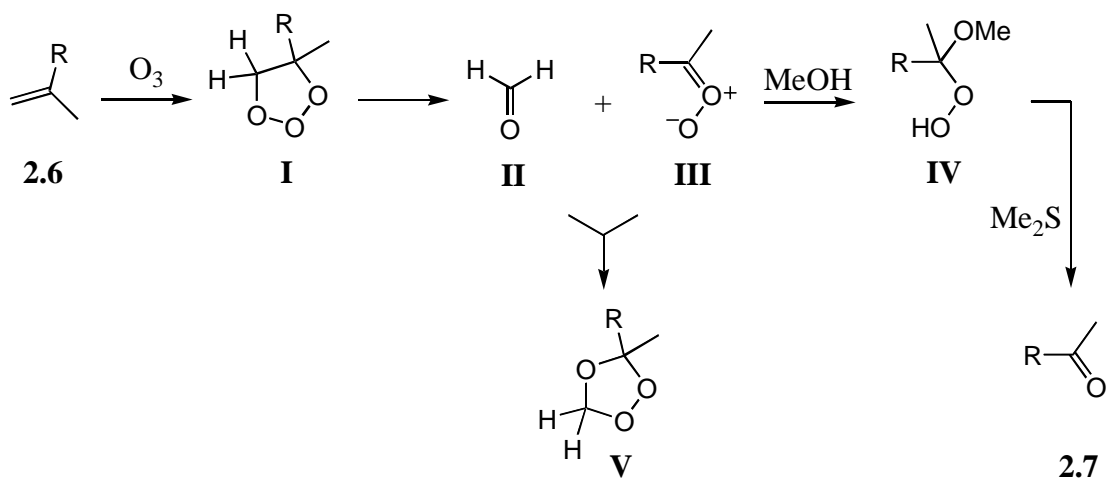
The isolation and identification of compound **2.20** was considered very important in three respects. Firstly, this compound represents one of the extreme cases of *A-nor*paclitaxel derivatives where the substituent at C-1 is the smallest possible, a hydrogen atom. This analog could thus possibly provide us a sense about the effects of the spatial volume of C-1 substituents on their anticancer activities. Secondly, compound **2.20** would have been very difficult to prepare by standard chemical reactions. Lastly, this compound is the *A-nor* analog of the semi-synthetic compound 1-deoxypaclitaxel (**2.21**) which shows promising biological properties.⁸⁷



2.21 1-deoxypaclitaxel

According to the thin layer chromatographic behavior of the reaction mixture of the ozonization of **2.6** in methylene chloride, both compounds **2.18** and **2.19** must be formed via the intermediate secondary ozonide (spot w in Figure 2.2). As described in Scheme 2.10, the primary ozonide **I** falls apart quickly and gives the ketone **II** and the carbonyl oxide **III**, which is trapped by methanol if present to form the hemiacetal **IV**. The hemiacetal is then reduced with dimethyl sulfide to give **2.7**. In the absence of methanol, however, the ketone **II** and the carbonyl oxide **III** recombine and form secondary ozonide **V**.

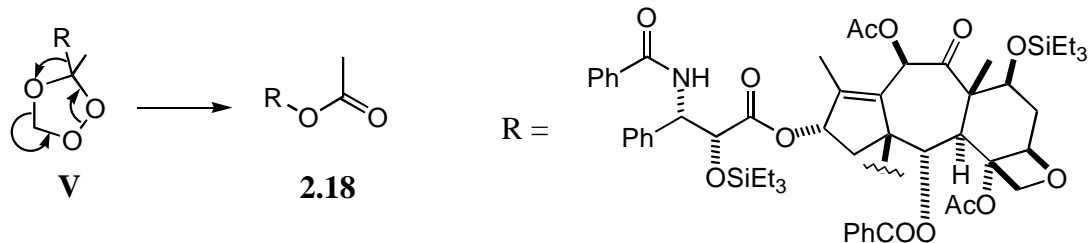
⁸⁷ Kingston, D. G. I.; Chordia, M. D.; Jagtap, P. G. In US Patent 5,739,359, Apr. 14, 1997.



Scheme 2.10 Mechanistic analysis of the ozonolysis of **2.6**⁸⁸

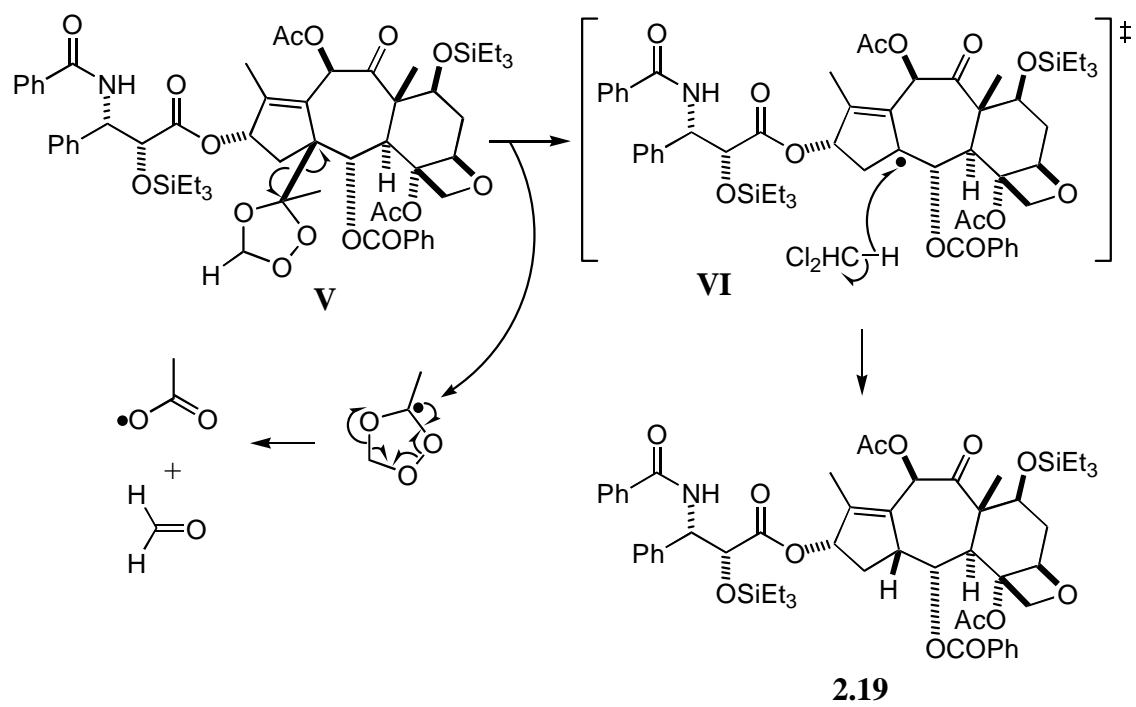
It should be pointed out that many other secondary ozonides could also be formed for an unsymmetrical substrate such as **2.6**, including tetraoxolanes, stereoisomers, and structural isomers arising from cross combinations of **II** and **III**. These secondary ozonides tend to fragment again to release the energy of the O-O bond and the conformational strains present in the ring to give a variety of stable products. Compound **2.18** is believed to be formed from the secondary ozonide **V** through the fragmentation shown in Scheme 2.11.

⁸⁸ Another fragmentation pathway of **I** that gives directly **2.7** and formaldehyde oxide is similar to this one and is not shown in the Scheme.



Scheme 2.11 Proposed mechanism for the formation of **2.18**

Without literature precedence, the mechanism for the formation of **2.19** is unknown. However, it could possibly arise from an allylic radical **VI** which could be generated in the way shown in Scheme 2.12. Abstraction of a hydrogen atom from solvent (CH_2Cl_2) by **VI** could then take place to give **2.19**. This process is regioselective, apparently because **2.19** has a lower strain energy than the other regioisomer. The stereoselectivity must be controlled by kinetic factors in that the approach of the hydrogen donor from the less sterically hindered top face is greatly favored. Another pathway involving heterolytic cleavage of the $\text{C}_1\text{-C}_{15}$ bond to give a tertiary allylic anion at C-1, however, can not be excluded, although it would be less energetically favorable.



Scheme 2.12 Proposed mechanism for the formation of **2.19**

2.1.5 Synthesis of A-nor-C-norpaclitaxel

C-ring contraction has also been observed in a number of structure modification studies.⁸⁹ In particular, C-norpaclitaxel has been synthesized when the carbon-carbon bond between C-6 and C-7 of 2'-*tert*-butyldimethylsilyl-6 α -hydroxy-7-*epi*paclitaxel (**2.22**) was cleaved oxidatively.⁹⁰ Aiming at examining the effects of modification of the ring skeleton on the anticancer activity of A-norpaclitaxel, we planned a seven-step synthesis of A-nor-C-norpaclitaxel (Scheme 2.13).

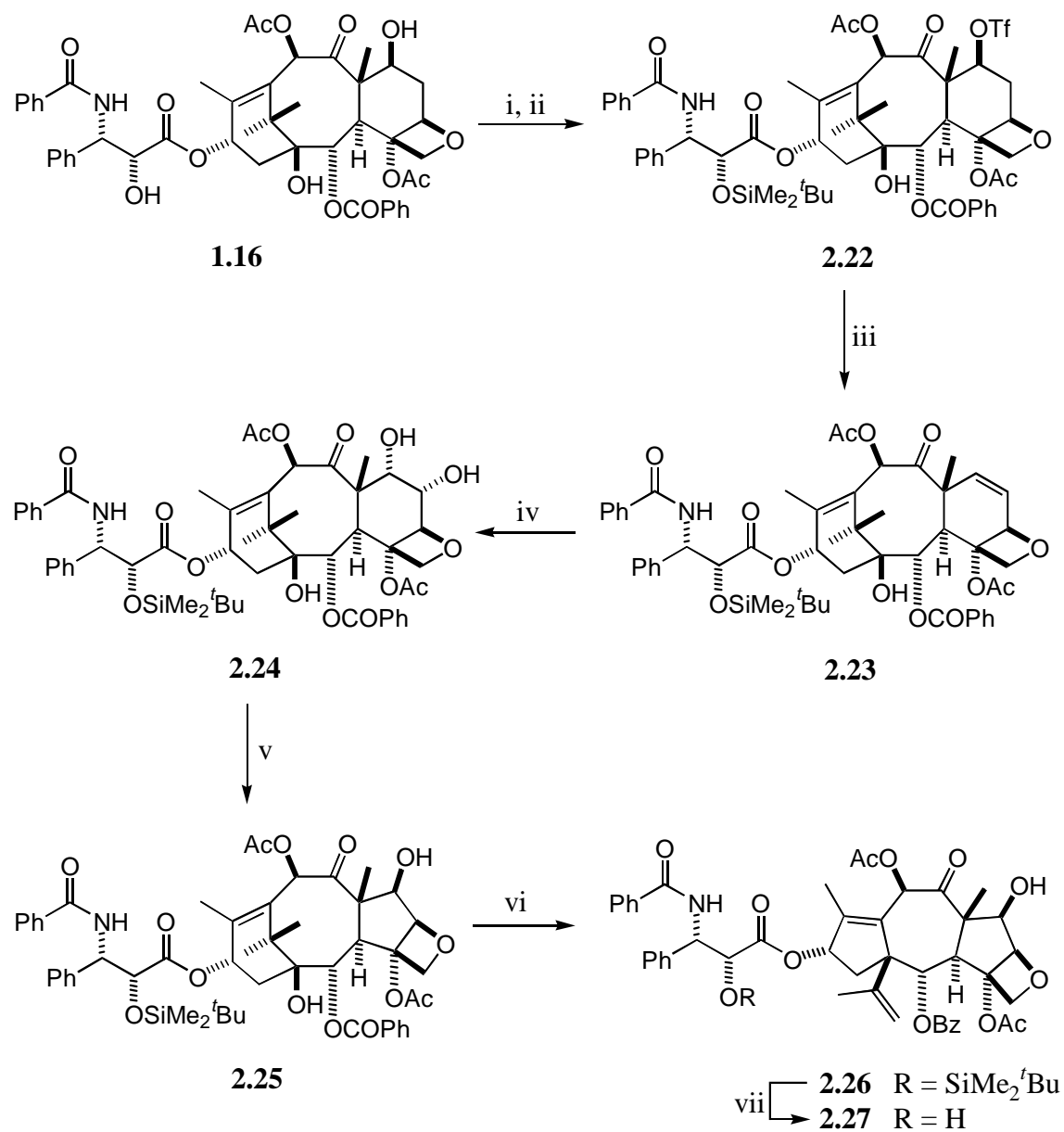
Compound **2.24** has been reported⁹¹ previously to be prepared in four steps from paclitaxel in about 55% yield. It was an important intermediate in the preparation of A-nor-C-norpaclitaxel as well as a number of other paclitaxel analogs that will be described later. In the synthesis of A-nor-C-norpaclitaxel, 2'-*O-tert*-butyldimethylsilyl-6 α -hydroxy-7-*epi*paclitaxel (**2.24**) was prepared through the same sequence in 75% yield. Compound **2.24** was then treated with lead tetraacetate in the presence of sodium bicarbonate as a buffer to convert it to 2'-*O-tert*-butyldimethylsilyl-C-norpaclitaxel (**2.25**) in 50% yield, along with oxetane ring opened products, as reported previously.⁹² Compound **2.25** was then subjected to the A-ring contraction conditions previously described followed by desilylation to give the desired A-nor-C-norpaclitaxel (**2.27**).

⁸⁹ Chen, S. H.; Huang, S.; Roth, G. P. *Tetrahedron Lett.* **1995**, 36, 8933.

⁹⁰ Liang, X.; Kingston, D. G. I. *Tetrahedron* **1997**, 53, 3441.

⁹¹ Liang, X.; Kingston, D. G. I. *Tetrahedron Lett.* **1995**, 36, 2901.

⁹² Also see ref. 90 and 91.

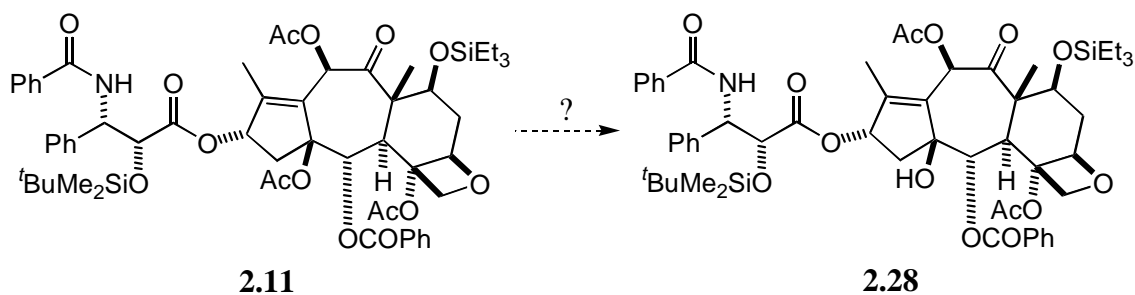


- i. ^tBuMe₂SiCl, imidazole, DMF, 98%; ii. CF₃SO₂Cl, DMAP, CH₂Cl₂; 99%;
 iii. DBU, CH₂Cl₂, 40 °C, 92%; iv. OsO₄, NMO, acetone/H₂O, 84%;
 v. Pb(OAc)₄, NaHCO₃, CH₂Cl₂, 0°C, 50%; vi. SOCl₂, pyridine, CH₂Cl₂, 57%;
 vii. HF/pyridine, THF, 76%.

Scheme 2.13 Preparation of A-nor-C-norpaclitaxel **2.27**

2.1.6 Attempts at the Synthesis of 1-deisopropenyl-1-hydroxy-*A-nor*paclitaxel

In continuation of efforts aimed at preparing *A-nor*paclitaxel analogs with different substituents at C-1 position, we turned our attention to the synthesis of 1-deisopropenyl-1-hydroxy-*A-nor*paclitaxel **2.28**. This compound was desired since it is the closest *A-nor*paclitaxel analog to paclitaxel, and should thus provide the clearest information on the effects of the *A-nor* structural modification on the activity of paclitaxel. It was thought that selective hydrolysis/cleavage of the substrate **2.11** might be the best approach, as proposed in Scheme 2.14.



Scheme 2.14 Proposed approach to 1-deisopropenyl-1-hydroxy-*A-nor*paclitaxel

Selective hydrolysis/cleavage of C-2, C-4, C-10, and C-13 esters of paclitaxel has been achieved using different reaction conditions. These methods provide access to a variety of structurally modified paclitaxel analogs. For instance, selective C-2 benzoyl hydrolysis and the following C-4 acetate hydrolysis was effected using anhydrous hydroxide under phase transfer conditions⁹³ or by a nucleophilic deesterification reaction.⁹⁴ Selective C-10 deacetylation⁹⁵ was achieved using H₂O₂/NaHCO₃⁹⁶ or

⁹³ Ashok, G. C.; Gharpure, M. M.; Rimoldi, J. M.; Chordia, M. D.; Gunatilaka, A. A. L.; Kingston, D. G. I. *J. Am. Chem. Soc.* **1994**, *116*, 4097.

⁹⁴ Datta, A.; Jayasinghe, L. R.; Georg, G. I. *J. Org. Chem.* **1994**, *59*, 4689.

⁹⁵ See ref. 77.

⁹⁶ Zheng, Q. Y.; Darbie, L. G.; Cheng, X.; Murray, C. K. *Tetrahedron Lett.* **1995**, *36*, 2001.

hydrazine monohydrate.⁹⁷ The C-13 side chain could also be cleaved reductively with tetrabutylammonium borohydride in very good yield.⁹⁸

A number of experiments were conducted using various conditions that had given success in the selective hydrolysis/cleavage of an ester functionality either in paclitaxel or in other molecules, as summarized in Table 2.4.

Table 2.4 Attempted selective hydrolysis/cleavage of C-1 acetate of **2.11**

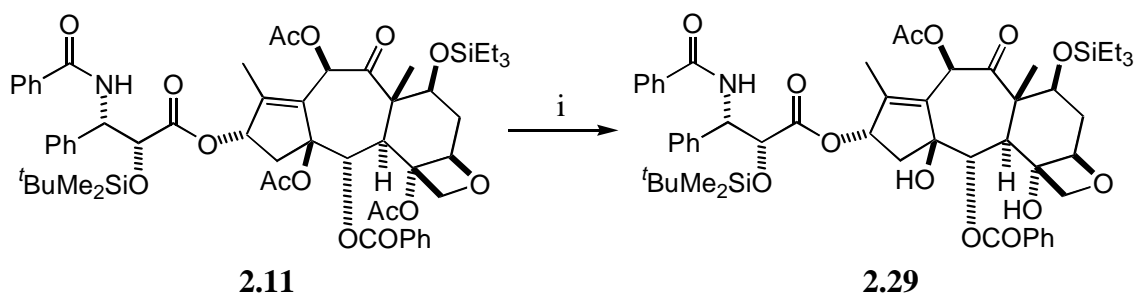
Experiments	Conditions	Results
1	7 eq. MeLi, THF, -25 °C, 1 h	mixture, side chain cleaved and multi-deacylated
2	1.2 eq. MeLi, THF, -40 °C, 30 min.	slow reaction, similar mixture as in experiment 1
3	6 eq. MeLi, THF, -40 °C, 30 min.	slow reaction, similar mixture as in experiment 1
4	4 eq. BuLi, THF, -78 °C to -40 °C, 1.5 h	no reaction
5	4 eq. BuLi, THF, rt, 1 h	2.29 and side chain cleaved product and other mixture
6	2 eq. BuLi, THF, rt, 1 h	complex mixture containing 2.29
7	1.2 eq. Red-Al, THF, -78 °C to 0 °C, 2.5 h	complex mixture
8	1.2-7 eq. DBU, MeOH, 10-30 min.	complex mixture
9	trifluoroacetic acid (excess), CH ₂ Cl ₂ , 45 min.	complex mixture containing oxetane opened product
10	2-4 eq. Triton-B, CH ₂ Cl ₂ , 0 °C, 1 h	complex mixture
11	formic acid (neat), 30 min.	complex mixture
12	0.7 eq. K ₂ CO ₃ , MeOH, 2 h	complex mixture

⁹⁷ Datta, A.; Hepperle, M.; Georg, G. I. *J. Org. Chem.* **1995**, *60*, 761.

⁹⁸ Magri, N. F.; Kingston, D. G. I.; Jitrangsi, C.; Piccariello, T. *J. Org. Chem.* **1986**, *51*, 3239.

All of the above reactions were performed with careful monitoring of the reaction mixture using TLC, and it was found that most of the reactions proceeded non-selectively, as evidenced by the formation of complex products simultaneously rather than sequentially. This could be due to two reasons: (1) the C-1 acetate is sterically hindered because of its tertiary nature. (2) the substrate **2.11** lacks an intramolecular handle (such as nearby hydroxyl group) to assist selective reaction at the desired C-1 position, and this type of assistance has been postulated to be critical to selectivity.

Among those many products isolated from the above experiments, compound **2.29** obtained from the reaction of **2.11** with BuLi (Scheme 2.15) was found to be closest to the target. ¹H NMR indicated the compound had two fewer acetyl methyl groups compared with the starting material **2.11**, and the acetyl group at C-10 must have been retained since the chemical shift of the proton at C-10 was unchanged. The rest of the molecule was assumed to be the same, based on its NMR spectrum, and the composition was confirmed by HRFABMS.



i. BuLi (2 equiv.), THF, rt, 1 h, 12%

Scheme 2.15 Nucleophilic cleavage of C-1 and C-4 acetate of **2.11**

Because compound **2.29** lacks a C-4 acetate, which is believed to be necessary for activity, and because it has two structural changes at a time, the biological activity of this compound was not evaluated. Selective re-acetylation of **2.29** was not investigated due to foreseen difficulties that will be discussed in more depth in section 2.4.

2.1.7 Biological Evaluation of Selected *A-norpaclitaxel* Analogs

Biological studies of the new *A-norpaclitaxel* analogs are still underway. Preliminary results are summarized in Table 2.5.

Table 2.5 Biological evaluation of *A-norpaclitaxels*

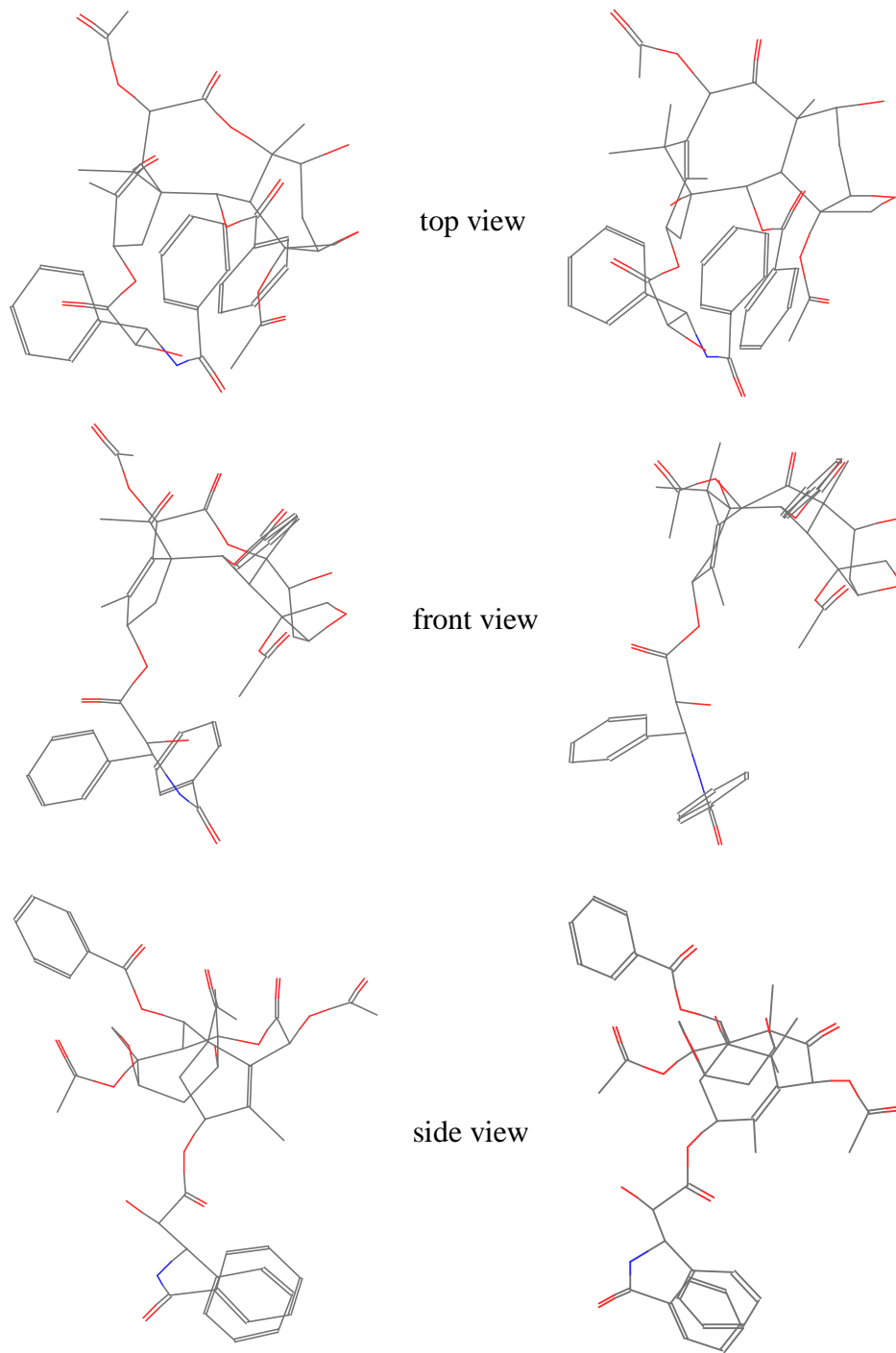
Compounds	Tubulin Assembly Activity (μM) ^a	Cytotoxicity (nM) ^b
paclitaxel	5.8 \pm 0.6	1.50
2.13	>1000	-
2.16	5.3 \pm 0.8	-
2.20	-	-
2.27	9.2 \pm 2.0	121.7

^a EC₅₀ for polymerization in 1.0M glutamate at room temperature in the absence of GTP.

^b IC₅₀ for cytotoxicity to HCT 116 cell line.

It seems that alteration in functionality at the C-1 position does not give a consistent trend in activity. It is probably the spatial volume of the substituent at C-1 that plays a role in determining the activity by forcing the molecule to adopt such a conformation that brings all the functionalities necessary for binding to microtubules to the right position.

The ring-B lactone analog **2.16** displayed a slightly stronger activity in promoting microtubule assembly, although its structure is quite different from paclitaxel. Its geometry and energy optimized conformation using Chem3D MM2 minimization, however, indicated that **2.16** is very similar to paclitaxel in space, especially in the so called “southern hemisphere” region (Figure 2.8).



1-deisopropenyl-8,9-oxido-A-nor-paclitaxel

paclitaxel

Figure 2.8 Similar conformation of compound **2.16** to paclitaxel

2.2 Synthesis of the Major Human Metabolite of Paclitaxel

2.2.1 Introduction

During the commercialization of paclitaxel as a new anticancer agent, extensive pharmacological investigations concerning its metabolism were performed, especially after it entered Phase II clinical trials in the late 1980's.⁹⁹ Both *in vitro* and *in vivo* studies on the metabolism of paclitaxel in animals and humans have been reported in recent years¹⁰⁰ (Figure 2.9). In particular, among other hydroxylated metabolites, 6 α -hydroxy-paclitaxel (**P5**) was reported to be the principal metabolite of paclitaxel in human hepatic microsomes, human liver slices, and patient biliary excretions, while in rats it is apparently not a biotransformation product. Table 2.6 listed the distribution of some of the paclitaxel metabolites found in the human and in the rat. Due to the small quantity of purified materials (usually sub nanomolar) that could be isolated through biological pathways, a synthetic method for the preparation of 6 α -hydroxy-paclitaxel from the parent compound paclitaxel in a relatively larger amount was desirable for studies of biological activity as well as for providing an HPLC standard for monitoring drug disposition in patients who take paclitaxel.

⁹⁹ (a) Rowinsky, E. K.; Wright, M.; Monsarrat, B.; Lesser, G.; Donehower, R. C.; *Cancer Survey Volume 17: Pharmacokinetics and Cancer Chemotherapy*; Imperial Cancer Research Fund: London, 1993, pp. 283. (b) Wright, M.; Monsarrat, B.; Royer, I.; Rowinsky, E. K.; Donehower, R. C.; Cresteil, T.; Guenard, D. In *The Chemistry and Pharmacology of Taxol and its Derivatives*; Farina, V., Ed.; 1995, Vol. 22; pp. 131. (c) Vuilhorgne, M.; Gailard, C.; Sanderink, G. J.; Royer, I.; Monsarrat, B.; Dubios, J.; Wright, M. In *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M.; Eds., ACS Symposium Series 583, American Chemical Society: Washington, DC, 1995, pp. 98.

¹⁰⁰ (a) Arrat, B.; Mariel, E.; Cros, S.; Gares, M.; Guenard, D.; Gueritte-Voegelein, F.; Wright, M. *Drug Metab. Dispos.* **1990**, *18*, 895. (b) Walle, T.; Kumar, G. N.; McMillan, J. M.; Thornburg, K. R.; Walle, U. K. *Biochem. Pharm.* **1993**, *46*, 1661. (c) Harris, J. W.; Katki, A.; Anderson, L. W.; Chmurny, G. N.; Paukstelis, J. V.; Collins, J. M. *J. Med. Chem.* **1994**, *37*, 706. (d) Kumar, G. N.; Oatis, J. E.; Thornburg, K. R.; Heldrich, F. J.; Hazard, E. S.; Walle, T. *Drug Metab. Dispos.* **1994**, *22*, 177. (e) Chaudhary, A. G.; Gharpure, M.; Rimoldi, J. M.; Kingston, D. G. I. *J. Am. Chem. Soc.* **1994**, *116*, 4097.

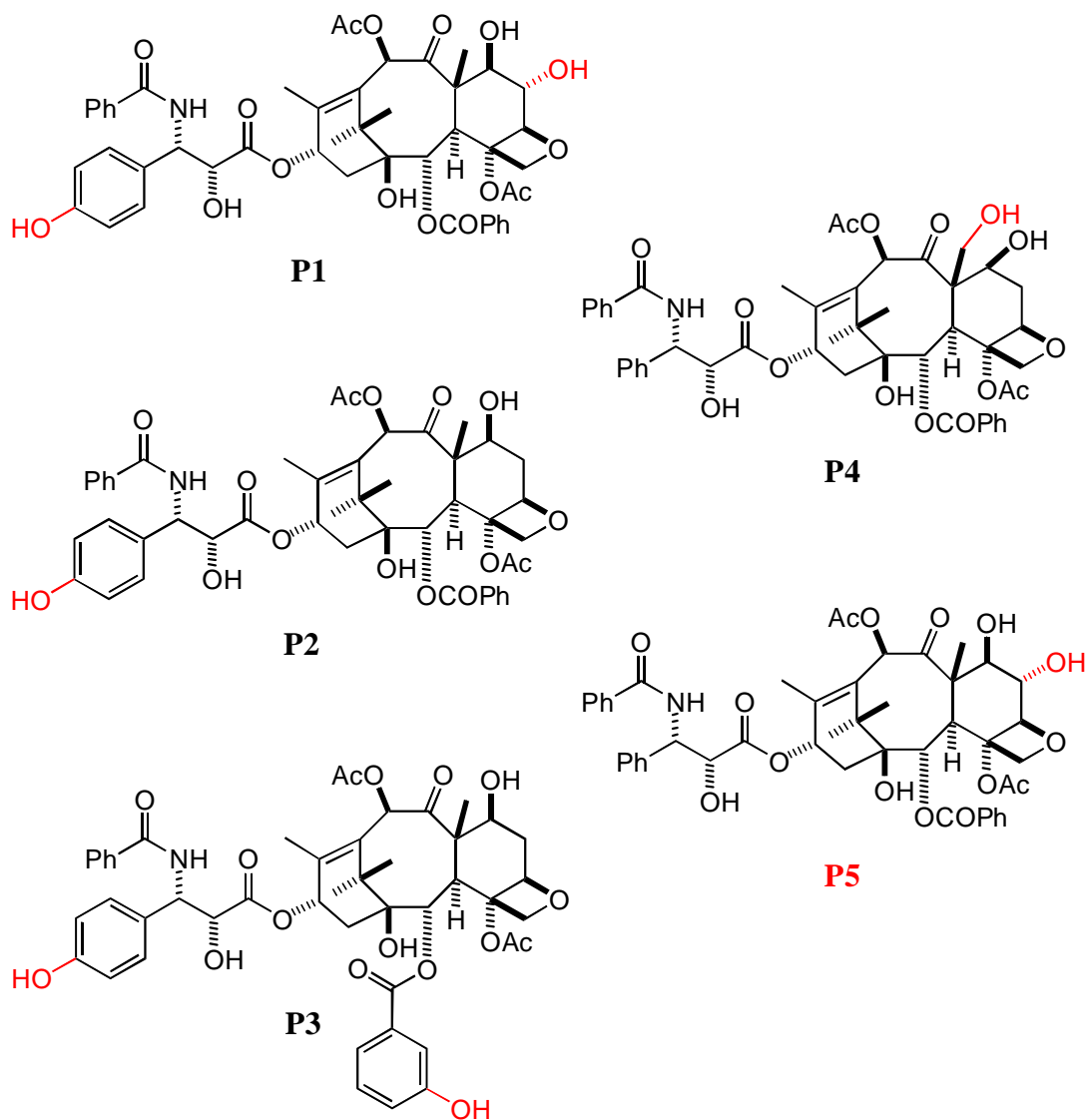


Figure 2.9 Selected paclitaxel metabolites

Table 2.6 Paclitaxel distribution in the human and rat

	P1	P2	P3	P4	P5	Paclitaxel	baccatin III
Human	minor	2%	/	/	12%	3%	/
Rat	/	13%	5%	minor	/	12%	minor

Unlike other metabolites where hydroxylation occurs on the aromatic rings of paclitaxel, and whose synthesis could thus be easily effected¹⁰¹ using known chemistry such as 2-debenzoylation followed by 2-arylation,¹⁰² or through the synthesis and attachment of appropriately modified side chains,¹⁰³ the preparation of 6 α -hydroxy-paclitaxel required modification of the taxane ring system itself, and proved to be a difficult one.

¹⁰¹ Park, H.; Hepperle, M.; Boge, T.C.; Himes, R. H.; Georg, G. I. *J. Med. Chem.* **1996**, *39*, 2705.

¹⁰² (a) See ref. 100(e). (b) Nicolaou, K. C.; Renaud, J.; Nantermet, P. G. *J. Am. Chem. Soc.* **1995**, *117*, 2409.

¹⁰³ (a) Holton, R. A.; Biediger, R. J.; Boatman, P. D. In *Taxol: Science and Applications*, Suffness, M., Ed.; CRC Press, Inc.: Boca Raton, FL, 1995, pp. 97. (b) Boge, T. C.; Georg, G. I. In *Enantioselective Synthesis of β -Amino Acids*, Juaristi, E., Ed.; Wiley-VCH, Inc.: New York, 1997, pp. 1.

2.2.2 Synthesis of 6 α -hydroxy-paclitaxel: the Major Human Metabolite

Our initial approach was to open the epoxide ring of 2'-*O*-*tert*-butyl-dimethyl-6 β ,7 β -epoxypaclitaxel (**2.30**), which was prepared from paclitaxel as previously described.¹⁰⁴ It was envisioned that this transformation would occur under acidic conditions, and this strategy was expected to be both straightforward and stereoselective. In the event, however, the oxirane ring proved to be much more stable under acidic conditions than the oxetane ring, and various attempts to carry out a selective oxirane ring-opening only yielded products with opened oxetane rings. This result thus confirms the great susceptibility of the oxetane ring of paclitaxel to electrophilic reagents, as observed previously for paclitaxel itself.¹⁰⁵

In the course of our investigations on the ring opening of **2.30** we became aware that its stereochemistry might have been assigned incorrectly. We thus carried out further studies of this question, and found that a NOESY experiment, far from indicating the β -configuration of the epoxide as previously supposed,⁸⁵ in fact gave unambiguous support for the alternative α -configuration, and showed that paclitaxel 6,7-epoxide has the structure and stereochemistry **2.31**. The most convincing correlation observed was between H-7 β at 3.0 ppm and CH₃-19 at 1.86 ppm (Figure 2.10). No correlation were observed between H-6 or H-7 and H-3, and we thus assume that the NOE correlation previously reported⁸⁵ must have been due to some spectroscopic artifact. The stereochemistry of paclitaxel 6,7-epoxide has been confirmed independently by X-ray crystallography.¹⁰⁶

¹⁰⁴ Liang, X.; Kingston, D. G. I.; Lin, C. M.; Hamel, E. *Tetrahedron Lett.* **1995**, 36, 2901.

¹⁰⁵ Chen, S. H.; Huang, S.; Wei, J.; Farina, V. *Tetrahedron* **1993**, 49, 2805.

¹⁰⁶ Alstadt, T. J.; Gao, Q.; Wittman, M. D.; Kadow, J. F.; Vyas, D. M. *Tetrahedron Lett.* **1998**, 39, 4965.

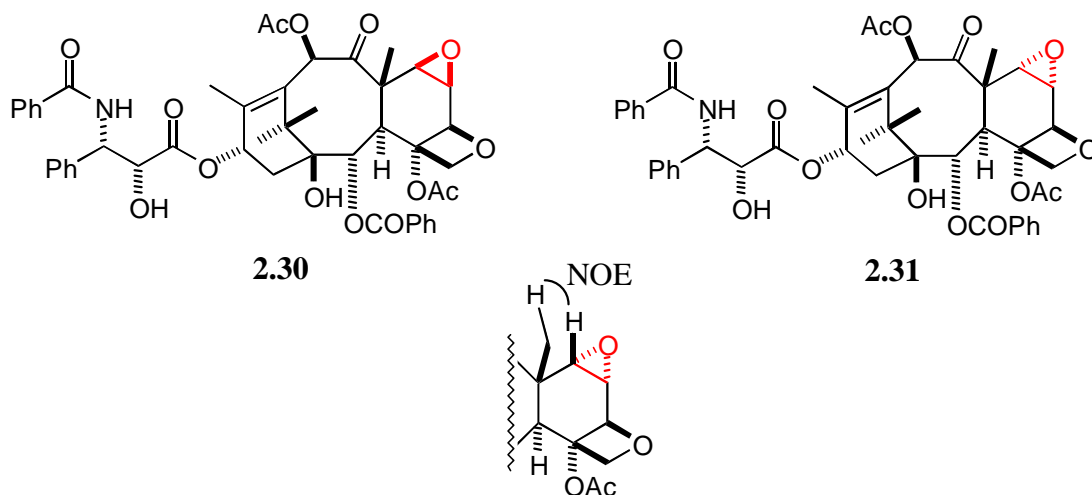
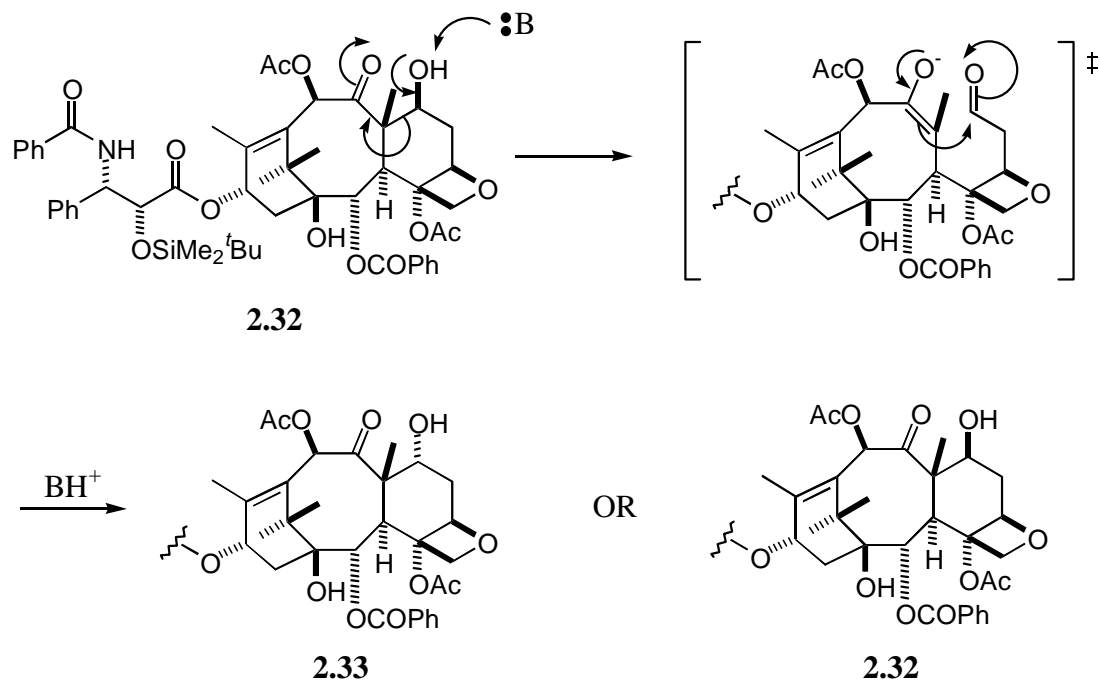


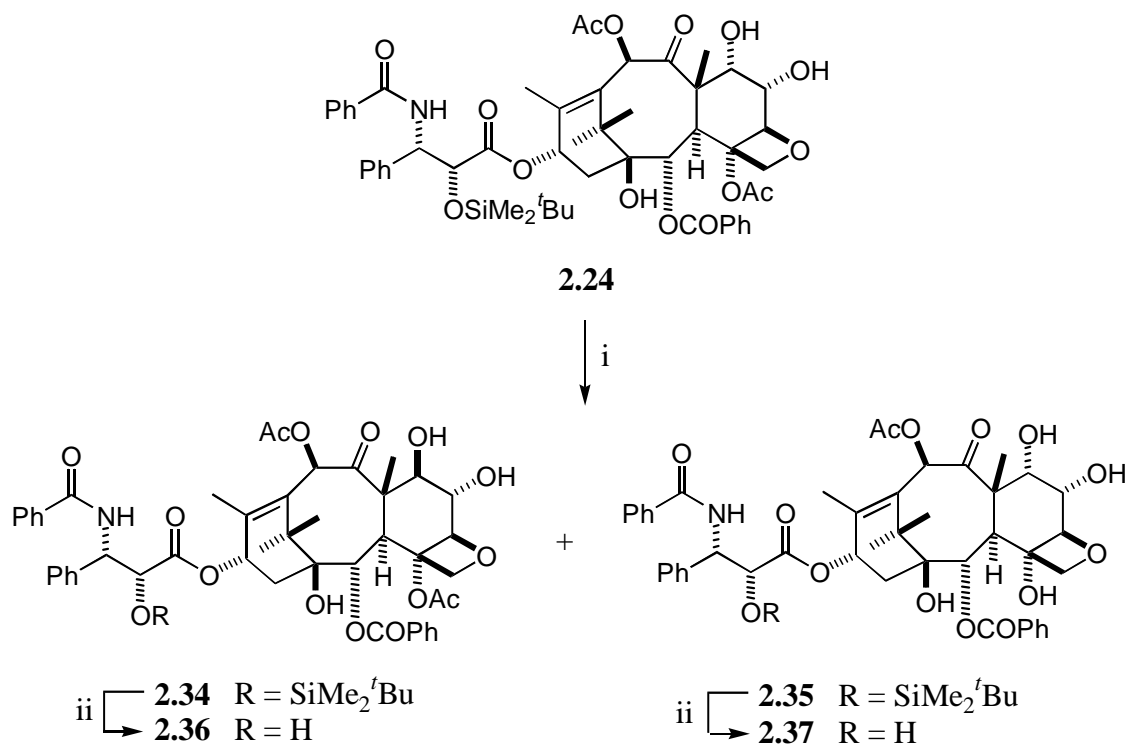
Figure 2.10 Stereochemistry of the two possible epoxides

Since *2'-O-tert-butyl*dimethylsilyl-6 α -hydroxy-7-*epi*paclitaxel (**2.24**) can be obtained in high yield in four steps from paclitaxel via the intermediate alkene **2.23**, epimerization of the 7 α -hydroxyl group in **2.24** to the normal position was also investigated. The desired epimerization is actually the reverse process of the epimerization observed before for paclitaxel itself (in the protected form **2.32**) that leads to 7-*epi*paclitaxel. The process involves a retro-aldol reaction that breaks the bond between C-7 and C-8, and forms an enolate transition state. When the enolate re-attacks the C-7 aldehyde carbonyl from one face it forms 7-*epi*paclitaxel (**2.33**), while from the other face it re-forms the starting material (paclitaxel). See Scheme 2.16 for a proposed mechanism for the epimerization.



Scheme 2.16 Mechanism of the C-7 epimerization of paclitaxel

Thus, treatment of **2.24** in anhydrous toluene with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) at 80 °C afforded a single product which was isolated and fully characterized as 2'-*O*-*tert*-butyldimethylsilyl-6 α -hydroxy-paclitaxel (**2.34**) in 12% yield along with 84% unreacted starting material (Scheme 2.17).



i. DBU, toluene, 80°C, or entry 2-7 in Table 2-7; ii. HF/py, THF, 72-82%.

Scheme 2.17 Synthesis of 6 α -hydroxy-paclitaxel

This result parallels a recent study on the epimerization of the 7-hydroxyl group of paclitaxel itself and of other related derivatives.¹⁰⁷ Although the yield obtained was low in absolute terms, the yield based on unrecovered starting material was an acceptable 75%, suggesting that this would be a viable route for the preparation of the desired metabolite. Since the epimerization was essentially an equilibrium reaction, we decided to optimize reaction conditions to achieve the best turnover and yield. The variants investigated included the use of similar hindered bases to DBU such as 1,5-Diazabicyclo[4,3,0]non-5-ene (DBN) and 1,4-Diazabicyclo[2,2,2]-octane (Dabco™), the use of different temperatures, and the presence of activated 4 Å molecular sieves. In addition to the desired product **2.34**, small amounts of the 4-deacetyl product **2.35** were formed under most conditions; the results of these studies are summarized in Table 2.7.

¹⁰⁷ Fang, W. S.; Fang, Q. C.; Liang, X. T. *Syn. Comm.* **1997**, 27, 2305.

Table 2.7 Isolated yields of the epimerization reaction under different conditions

Entry	Reaction Conditions	2.24	2.34	2.35
1	DBU, toluene, 80 °C, 1.5 h	84%	12%	<2%
2	DBU, toluene, 90 °C, 2.5 h	43%	9-11%	11-12%
3	DBU, xylenes, 80 °C, 1.5 h	83%	15%	<2%
4	DBU, xylenes, mol. sieves, 80 °C, 1.5 h	86%	12%	<2%
5	DBN, xylenes, 80 °C, 1.5 h	92%	7%	<2%
6	Dabco TM , xylenes, 80 °C, 1.5 h	99%	0	0
7	DBU, xylenes, 70 °C, 1 h	92%	7%	1%

From the studies it is clear that DBU in xylenes at 80 °C (entry 3) gives the best results with 15% absolute yield and 88% yield based on unrecovered starting material. Lower temperatures (entry 7) give lower yields for both products **2.34** and **2.35**, while higher temperatures and prolonged treatment (entry 2) give larger amounts of 4-deacetyl derivative **2.35** together with other side products. DBN was worse than DBU in terms of turnover and DabcoTM was almost unreactive, a fact which could be attributed to its steric bulk and weaker basicity or both. Anhydrous conditions were believed to be critical but molecular sieves were unnecessary as long as the solvents and bases were appropriately dried. Attempts were made to trap the 7 β -hydroxyl group with chlorotriethylsilane so as to drive the equilibrium to completion, but the use of different bases (DBU or NaH) and different substrates failed to give any of the desired 7 β -O-silylated derivatives.

Interestingly, the 4-deacetylated product **2.35** still retained the 7 α hydroxyl group, which is in contrast to the assumption that the strong intramolecular hydrogen bonding observed between 7 α hydroxyl proton and the acyl oxygen of the C-4 acetate might be the main reason for the facile and favorable epimerization of paclitaxel to 7-epipaclitaxel in base. A possible explanation for the preferential formation of **2.24** is that a hydrogen bond between the 6 α -hydroxyl group and the oxygen of the presumed 7-aldehyde

intermediate in the transition state of the retro-aldol reaction may lock the conformation in such a way that the following aldol addition generates starting material (Figure 2.11).

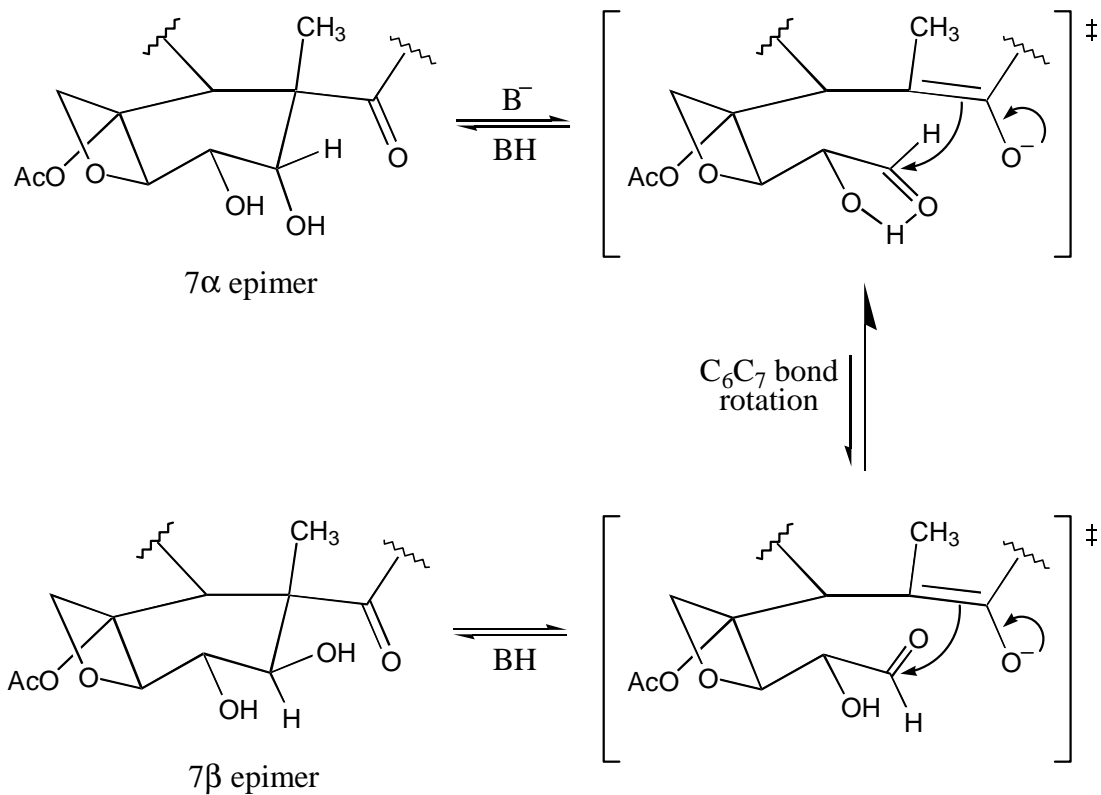


Figure 2.11 Hydrogen bonding in the transition state of the epimerization favors the 7 α epimer kinetically

2'-*O*-*tert*-Butyldimethylsilyl-6 α -hydroxy-paclitaxel (**2.34**) was deprotected with HF/pyridine to give the major human metabolite **2.36** in good yield. The ^1H NMR spectrum of **2.36** was identical to that reported in the literature¹⁰⁸ and other spectra including ^{13}C NMR, TOCSY, HMQC, and NOESY were consistent with the assigned structure. The composition was confirmed by HRFABMS.

¹⁰⁸ (a) Harris, J. W.; Katki, A.; Anderson, L. W.; Chmurny, G. N.; Paukstelis, J. V.; Collins, J. M. *J. Med. Chem.* **1994**, 37, 706. (b) Kumar, G. N.; Oatis, J. E.; Thornburg, K. R.; Heldrich, F. J.; Hazard, E. S.; Walle, T. *Drug Metab. Dispos.* **1994**, 22, 177.

2.3 Synthesis of Paclitaxel Analogs Modified at the C-6 and C-7 Positions

2.3.1 Introduction

Structure modifications of paclitaxel at the C-6 and C-7 positions have been achieved by our group and others, mainly via the intermediates 6,7-olefin or 6,7-diol. A number of analogs have been synthesized, such as 6 α -hydroxy-7-deoxy-paclitaxel, C-6 monoesters of 6 α -hydroxy-7-epipaclitaxel, and C-6 and C-7 cyclic ester/thioester of 6 α -hydroxy-7-epipaclitaxel (Figure 2.12).

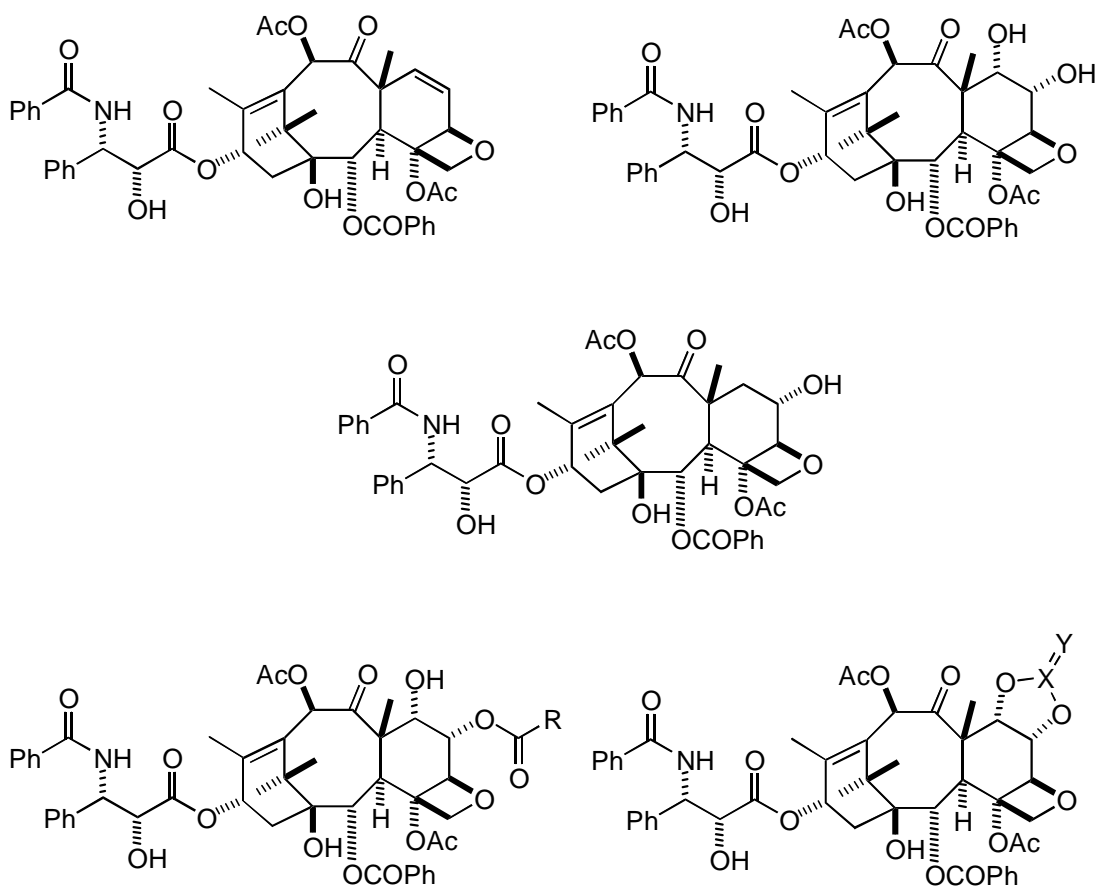


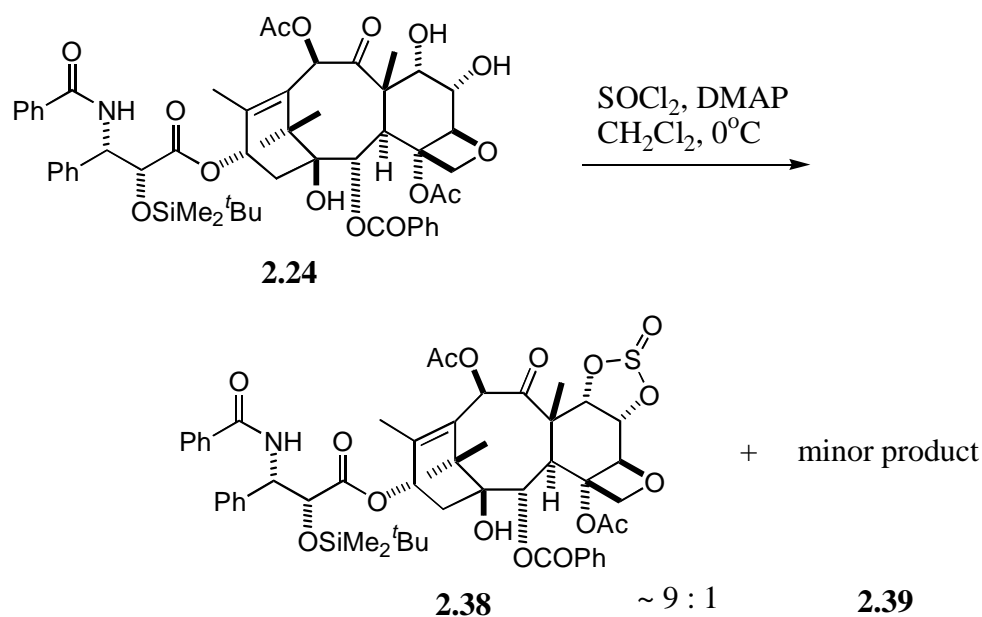
Figure 2.12 Known paclitaxel analogs modified at the C-6 and/or C-7 positions

Tubulin assembly/microtubule disassembly activity of most of these C-6/C-7 modified paclitaxel analogs were found to fluctuate around that of paclitaxel's. It thus seemed that both positions are not critical to the interaction between microtubule and the molecule. In pursuing new analogs with improved biological activity and desired properties (such as water solubility), we further investigated the C-6, C-7 chemistry.

2.3.2 Synthesis of the C-6,C-7 Cyclic Sulfite and Cyclic Sulfate of 6 α -hydroxy-7-*epi*paclitaxel

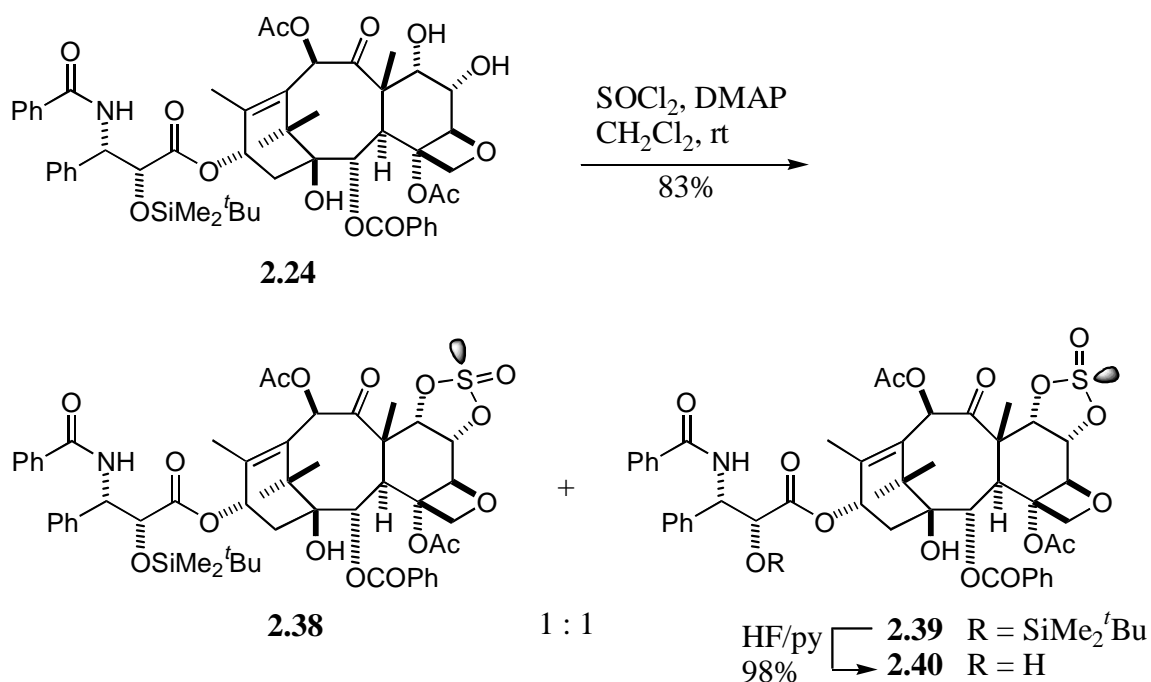
6 α ,7 α -*O*-Cyclosulfonylpaclitaxel (**2.38**) has been synthesized previously in our group by treating 2'-*O*-(*tert*-butyldimethylsilyl)-6 α -hydroxy-7-*epi*paclitaxel (**2.24**) with thionyl chloride and 4-dimethylamino pyridine at 0 °C. Preliminary biological evaluation of this compound indicated that it was about as active as paclitaxel in stabilizing microtubules.¹⁰⁹ The stereochemistry of compound **2.38** with respect to the orientation of the lone pair electrons of the sulfur atom, however, was not clear. Isomerism due to the asymmetric nature of a cyclic sulfite is a common phenomenon, and with appropriate asymmetric induction, diastereomeric or enantiomeric products may result. Careful examination of the previously obtained ¹H NMR spectrum of compound **2.38** clearly indicated the presence of a paclitaxel-like impurity which could presumably be the other isomer of the sulfite. The reaction was thus repeated and the same mixture was obtained (Scheme 2.18, **2.38:2.39** ~ 9:1 based on ¹H NMR integration).

¹⁰⁹ Liang, X. *Studies on Ring C Modified Paclitaxel Analogs*, Virginia Polytechnic Institute and State University, 1996.



Scheme 2.18 Reported synthesis of C-6 C-7 cyclic sulfite of 6 α -hydroxy-7-epipaclitaxel

In order to obtain the minor product (**2.39**) in greater proportion in the mixture, the reaction was rerun at a higher temperature (room temperature). Under these conditions both products were obtained in about a 50:50 ratio (Scheme 2.19). The new compound had a very similar polarity to **2.38** and could be isolated only by careful multi-elution on preparative TLC.



Scheme 2.19 Reinvestigation of the reaction between 2'-*O*-(*tert*-butyldimethylsilyl)-6 α -hydroxy-7-*epi*paclitaxel (**2.24**) with thionyl chloride

Not only did the two compounds have similar polarities, but they also shared many common resonances in both the ¹H and ¹³C NMR spectra. The significant differences were the protons at the C-5, C-7, C-14 α , and C-20 β positions. These are listed in Table 2.8 for comparison (corresponding data for the cyclic sulfate **2.41** are also included).

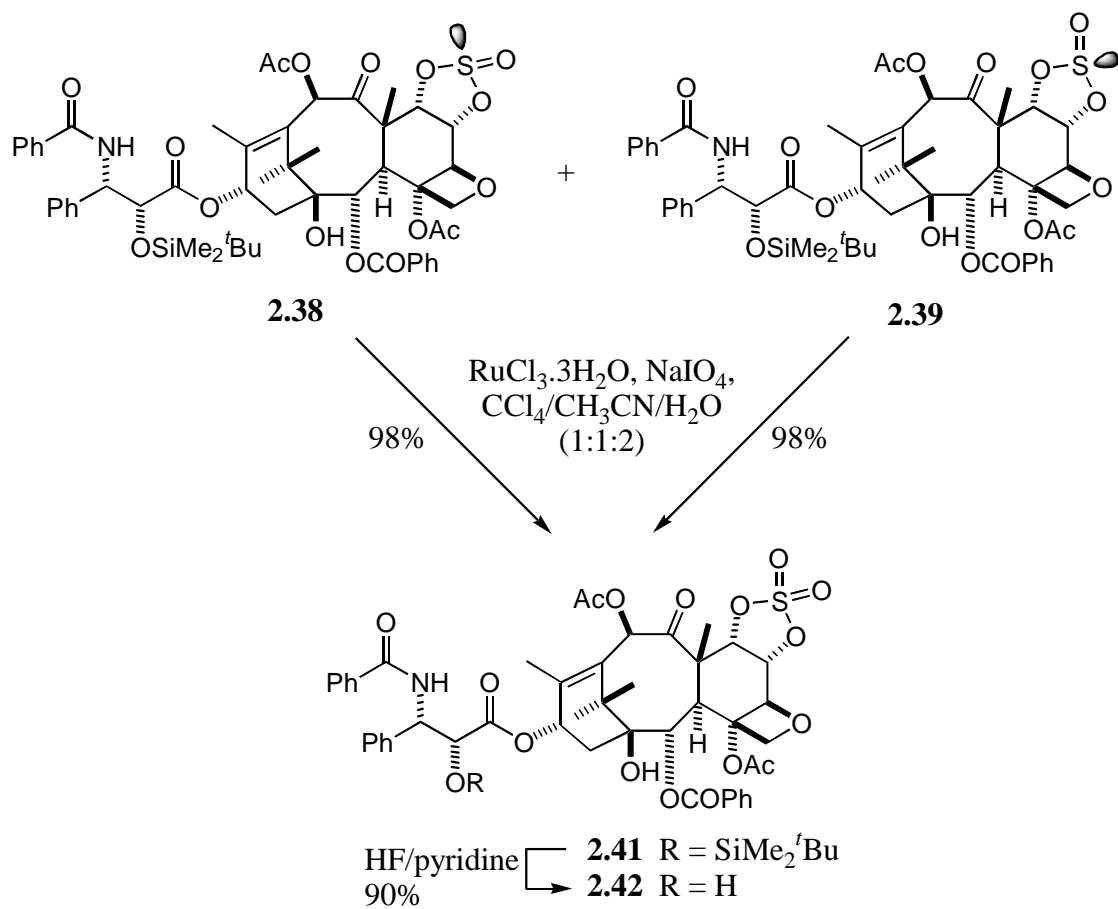
Table 2.8 Comparison of selected proton chemical shifts of compounds **2.38** and **2.39**

protons	2.38	2.39	2.41
H-5 (ppm)	4.76	5.86	5.03
H-7 (ppm)	4.89	4.39	4.86
H-14 α (ppm)	2.40	3.08	2.47
H-20 β (ppm)	4.35	5.04	4.46

HRFABMS revealed that the new compound has a molecule weight of 1030.3701 (M+H)⁺, corresponding to the molecular formula of C₅₃H₆₃NO₁₆SSi, which was identical to that of the known compound 6 α ,7 α -O-cyclosulfonylpaclitaxel. It was thus determined that the two compounds formed in the reaction were indeed a pair of diastereomers that differ only in the orientation of the lone pair electrons of the sulfur atom.

In order to verify and identify each of the two diastereomers, as well as to obtain a versatile substrate for subsequent transformations, e.g., nucleophilic substitution, both of the sulfites were oxidized to the sulfate with ruthenium trichloride and sodium periodate. As expected, both **2.38** and **2.39** were converted to the same compound 6 α ,7 α -O-cyclosulfonyl-paclitaxel (**2.41**, Scheme 2.20), whose HRFABMS (after desilylation) gave the molecular formula of C₄₇H₄₉NO₁₇S, indicating an extra oxygen atom in the molecule.

Selected ¹H NMR chemical shifts of **2.41** are also listed in Table 2-8 for comparison. Compounds **2.38** and **2.41** have very similar proton and carbon signals, and both are considered normal as compared with paclitaxel. The unusual downfield chemical shift of the H-5 and H-14 α protons in compound **2.39** suggested that the lone pair electrons of the sulfur atom in this compound should be in close proximity to these protons, which in turn established the orientation of the lone pair electrons of this compound as being on the α face. The opposite orientation was thus assigned to sulfite **2.38**.



Scheme 2.20 Oxidation of the two isomers of the cyclic sulfite

2.3.3 Attempts to Open the Cyclic Sulfate with Nucleophiles

The 1,2 cyclic sulfate has been known as a useful synthon similar to an epoxide,¹¹⁰ and in many cases it is even better than an epoxide for nucleophilic reactions.¹¹¹ It was thus expected that nucleophilic substitution at either the C-6 or the C-7 position of 6 α ,7 α -O-cyclosulfurylpaclitaxel (**2.41**) would give trans-disubstituted paclitaxel analogs (Figure 2.13).

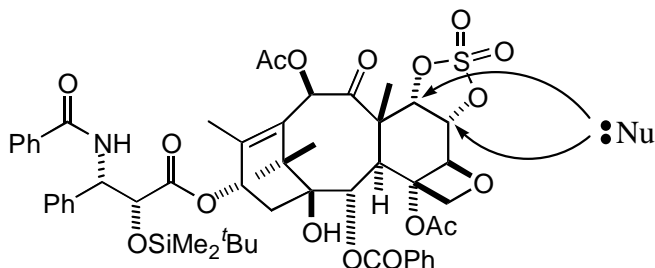


Figure 2.13 Proposed nucleophilic substitution of the cyclic sulfate **2.41**

Compound **2.41** was therefore treated with a variety of nucleophiles including the good nucleophiles sodium azide and tetrabutylammonium azide.¹¹² Unfortunately, no detectable reaction was observed. It was reasoned that the stability of the cyclic sulfate **2.41** toward nucleophilic reaction was due to the steric hindrance of the β face of the molecule caused by the C-19 angular methyl group and probably the oxetane ring as well (Figure 2.14). This same steric hindrance initially determined the stereochemistry of the dihydroxylation of the 6,7-olefin, even in the presence of asymmetric induction that favors the other (β) isomer, and uniformly gave 6 α ,7 α -diol (Figure 2.15).¹¹³

¹¹⁰ For a review, see Lohray, B. B. *Synthesis* **1992**, 1035.

¹¹¹ (a) Gao, Y.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, 7538. (b) Kim, B. M.; Sharpless, K. B. *Tetrahedron Lett.* **1989**, 30, 655.

¹¹² Prepared from the reaction of tetrabutylammonium hydroxide and sodium azide, see Brandstrom, A.; Lamm, B.; Palmertz, I. *Acta. Chem. Scand.* **1974**, B28, 699.

¹¹³ Liang, X.; Kingston, D. G. I. unpublished results.

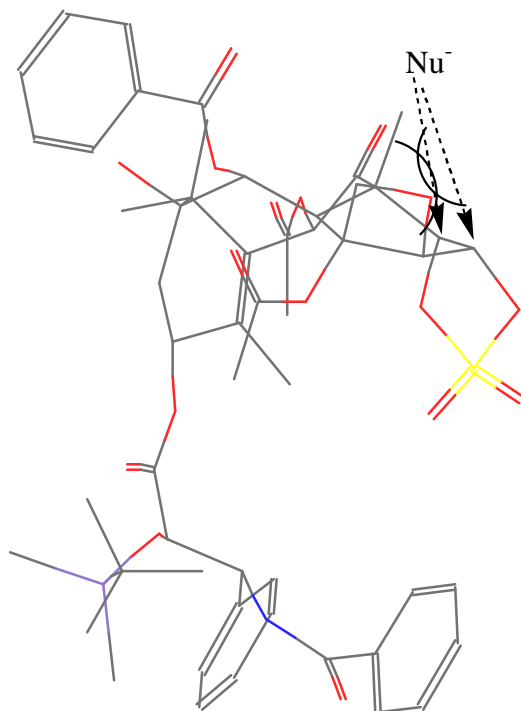


Figure 2.14 Steric hindrance of nucleophilic attack at the 6 α ,7 α -O-cyclosulfurylpaclitaxel (**2.41**)

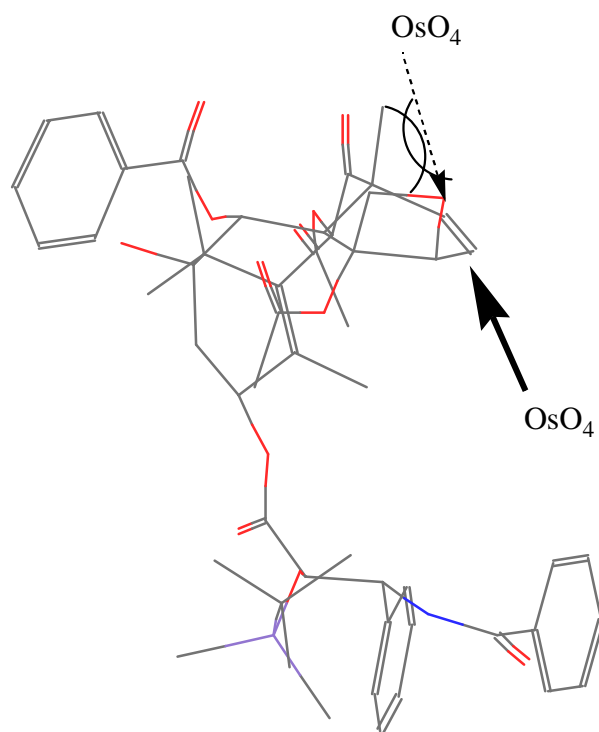


Figure 2.15 Stereochemistry of the dihydroxylation of the 6,7 olefin **2.23**

This phenomenon is actually in accordance with the observed stability of paclitaxel 6,7-epoxide, which has been discussed earlier in section 2.2.2. When the epoxide was treated with a variety of reagents with the intention of opening the epoxide ring, only starting material was recovered, and under harsh conditions the epoxide ring remained intact while the oxetane ring was usually opened. Once the stereochemistry of the epoxide was revised from β to α , its stability was explainable on the basis of hindered nucleophilic attack as shown in Figure 2.16.

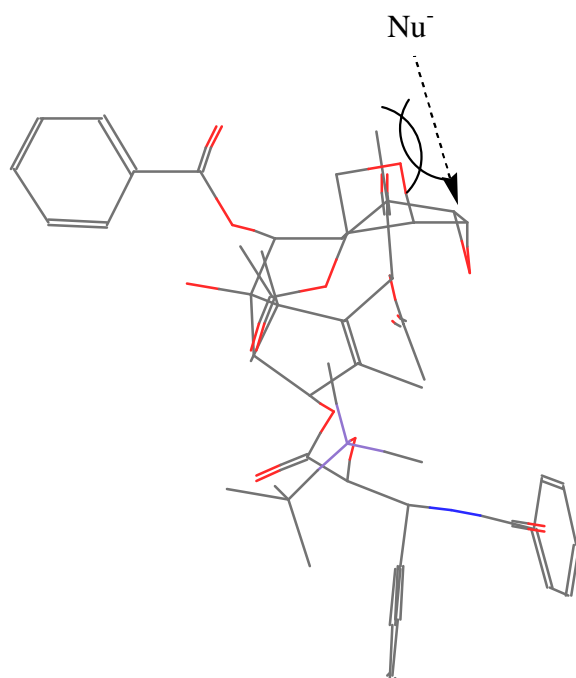
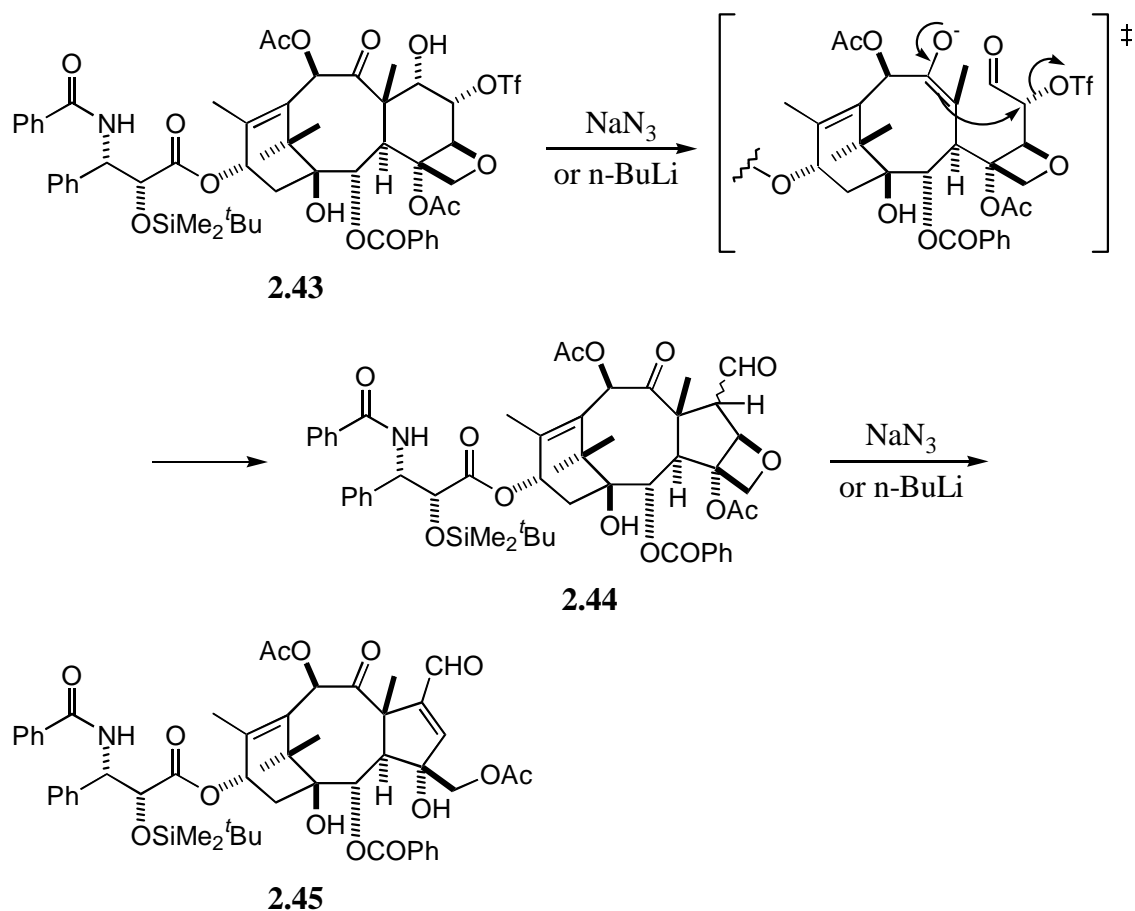


Figure 2.16 Steric hindrance of nucleophilic attack at the 6,7- α -epoxide

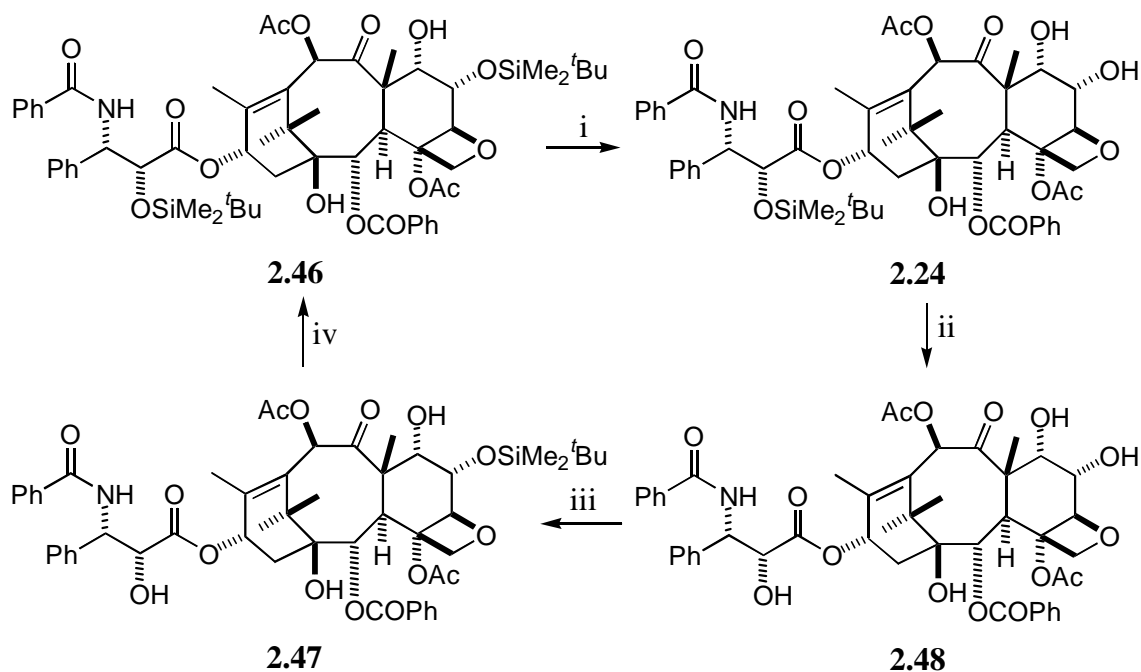
2.3.4 Synthesis of 6 β -azido-7-*epi*paclitaxel

Another approach was to convert the 6 α hydroxyl group into a good leaving group which could be substituted by nucleophiles directly. Treatment of 2'-*O-tert*-butyldimethylsilyl-6 α -hydroxy-7-*epi*paclitaxel (**2.24**) with trifluoromethanesulfonyl chloride (5 equivalents) and 4-dimethylamino pyridine (7 equivalents) in anhydrous dichloromethane at room temperature furnished the desired 2'-*O-tert*-butyldimethylsilyl-6 α -*O*-trifluoromethanesulfonyl-7-*epi*paclitaxel (**2.43**) in quantitative yield. The following reaction with a nucleophile such as sodium azide was however troublesome. Instead of giving the desired substitution product, a retro-aldol reaction promoted by the weak base - NaN₃ took place and led to a product with a rearranged C-ring (**2.44** and **2.45**, see Scheme 2.21).



Scheme 2.21 Previous efforts in the synthesis of C-6 azido paclitaxel analogs

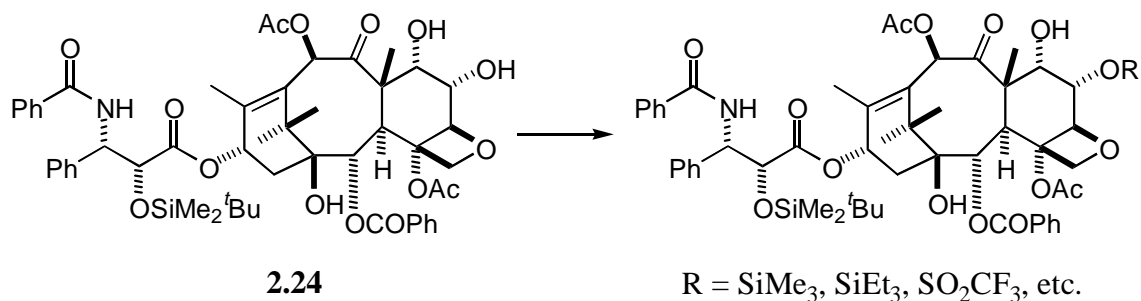
A simple way to avoid this undesired reaction was to eliminate the retro-aldol pathway by protection of the 7-hydroxy group. According to our knowledge about the reactivity of the three hydroxyl groups present in the compound **2.24**, the C-6 α hydroxyl group is the most reactive one, and it could be even more reactive (either chemically or sterically or both) than the C-2' hydroxyl group, which was evidenced by selective silylation and desilylation under controlled reaction conditions (Scheme 2.22).



- i. 1% HCl/MeOH, 1 h, 85%. ii. 5% HCl/MeOH, 1-2 h, 90%.
 iii. ^tBuMe₂SiCl, imidazole, 40°C, 1-2 h, 80%.
 iv. ^tBuMe₂SiCl, imidazole, 60°C, 2 h, 95%

Scheme 2.22 Reactivity of C-6 α hydroxyl and C-2' hydroxyl groups

On the other hand, C-7 α hydroxyl group is very hindered, especially when the neighboring C-6 α hydroxyl group is functionalized, which makes the steric problem even more serious. Before the launch of this synthetic investigation, the only derivative of this group was a methyl xanthate when 2',6 α -bis-*O-tert*-butyldimethylsilyl-7-*epi*paclitaxel was treated with sodium hydride (NaH) in THF at room temperature and quenched with carbon disulfide (CS₂) and methyl iodide (MeI). All other esterification conditions, including the least sterically demanding acetylation, yielded C-6 mono esters. Silylation using *tert*-butyldimethylsilyl chloride, triethylsilyl chloride and trimethylsilyl chloride also gave C-6 mono silyl ethers only (Scheme 2.23).

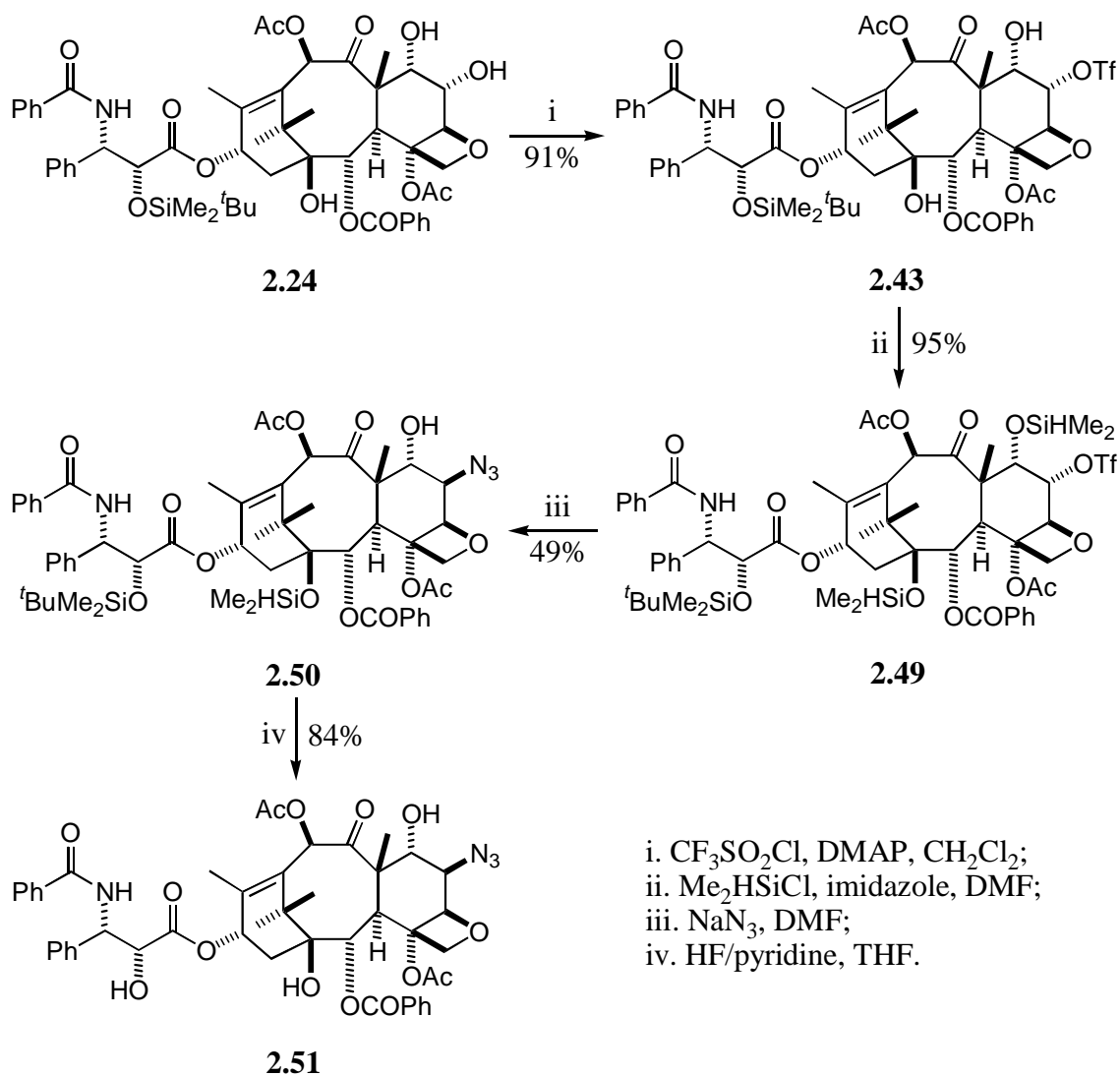


Scheme 2.23 Reactivity of C-7 α hydroxyl group

It was based on this known chemistry and on unsuccessful trials with bulkier reagents (not shown) that the even smaller silylation reagent dimethylsilyl chloride was chosen to mask the C-7 *epi* hydroxyl group. Dimethylsilyl chloride has previously been used for the protection of the C-1 hydroxyl group,¹¹⁴ which is a tertiary hydroxyl group and is presumably more sterically hindered. In the event, 2'-*O-tert*-butyldimethylsilyl-6 α -*O*-trifluoromethanesulfonyl-7-*epi*paclitaxel (**2.43**) was treated with chlorodimethylsilane and imidazole in DMF at room temperature. A less polar compound was formed shortly and the starting material was completely consumed in 30 minutes (Scheme 2.24). The proton NMR spectrum of the purified product clearly indicated that it was the expected 2'-*O-tert*-butyldimethylsilyl-1,7 α -bis-*O*-dimethylsilyl-6 α -*O*-trifluoromethanesulfonyl-7-*epi*paclitaxel (**2.49**), with the characteristic presence of two dimethylsilyl groups (two multiplets at 4.84 ppm and 4.32 ppm for two protons and four doublets at 0.36, 0.33, -0.17, and -0.46 ppm for four CH₃ groups) and most of the other proton chemical shifts remained unchanged. Having protected the C-7 hydroxyl group, the azide substitution reaction was effected by treating compound **2.49** with sodium azide in anhydrous DMF at room temperature for 18 hours. A new more polar compound was formed and isolated in 60% yield. The upfield shift of the C-6 proton peak from 5.33 ppm to 4.13 ppm indicated the change of functional group at C-6 and this change was consistent with that expected for azide substitution. ¹³C and APT NMR also showed similar consistency, in which the number of oxygenated methine carbons decreased by one and there was an extra methine carbon peak at 62.1 ppm that is normal for a carbon attached to an azide function. A FT-

¹¹⁴ Chen, S. H.; Kadow, J. F.; Farina, V. *J. Org. Chem.* **1994**, *59*, 6156.

IR spectrum further confirmed the introduction of the azide by showing a characteristic strong absorption at 2105 cm^{-1} . It is noteworthy that one dimethylsilyl protecting group was removed according to the ^1H NMR spectrum. After careful examination of the proton spectrum and comparison with paclitaxel C-1 dimethylsilyl ether, it was determined that the remaining dimethylsilyl group was on C-1. Desilylation of **2.50** afforded **2.51** in 84% yield. Its structure was verified by ^1H and ^{13}C NMR and the composition was confirmed by HRFABMS.



Scheme 2.24 Synthesis of 6 β -azido-7-epipaclitaxel

A NOESY experiment was performed in order to determine the stereochemistry of the azide **2.51**. The only through-space NOE correlation observed for H-6 was with 7-*epi* OH, which in turn had correlation with H-3. This suggests an α proton at the C-6 position.

To ensure this stereochemistry assignment, the coupling constants of the H-5, H-6, and H-7 protons of **2.51** were compared with that of C-6 and C-7 modified analogs with known stereochemistry (Table 2.9). The observed coupling constants of compound **2.51** (H₅-H₆ 8.0-8.4, H₆-H₇ 1.6-2.0) also supported an α proton at the C-6 position. The structure of **2.51** was thus determined to be 6 β -azido-7-*epi*paclitaxel. This stereochemistry is apparently the results of an S_N2 reaction.

Table 2.9 Selected coupling constants of paclitaxel and its known C-6 and C-7 analogs

Compound	H ₅ -H _{6α}	H ₅ -H _{6β}	H _{6α} -H _{7β}	H _{6β} -H _{7β}
7- <i>epi</i> -paclitaxel	9.0-9.2	3.5-3.7	2.1	5.0
2.24	-	2.0-2.4	-	4.4-4.8
2.43	-	2.4	-	4.8
6 α - <i>O</i> -esters of 2.24 ^a	-	2.5-2.6	-	4.7-4.8
paclitaxel	9.5-9.6	2.0-2.3	-	-
6 α -hydroxy-paclitaxel	-	0	-	-

^a data reported by X. Liang for 2'-*O*-*tert*-butyldimethylsilyl-6 α -*O*-acetyl-7-*epi*paclitaxel, 2'-*O*-*tert*-butyldimethylsilyl-6 α -*O*-benzoyl-7-*epi*paclitaxel, 2'-*O*-*tert*-butyldimethylsilyl-6 α -*O*-(*o*-methyl-benzoyl)-7-*epi*paclitaxel, and 2'-*O*-*tert*-butyldimethylsilyl-6 α -*O*-cyclopropylcarbonyl-7-*epi*paclitaxel.¹¹⁵

Along with the new compound **2.50**, other more polar products were also observed by TLC analysis but were not isolated and characterized. Since the required product **2.50** bore a free C-7 α hydroxyl group, it is very likely that the side products arose from the retro-aldol reaction followed by the enolate attack at the C-6 carbon atom bearing a leaving group, a process similar to the one described in Scheme 2-21. The

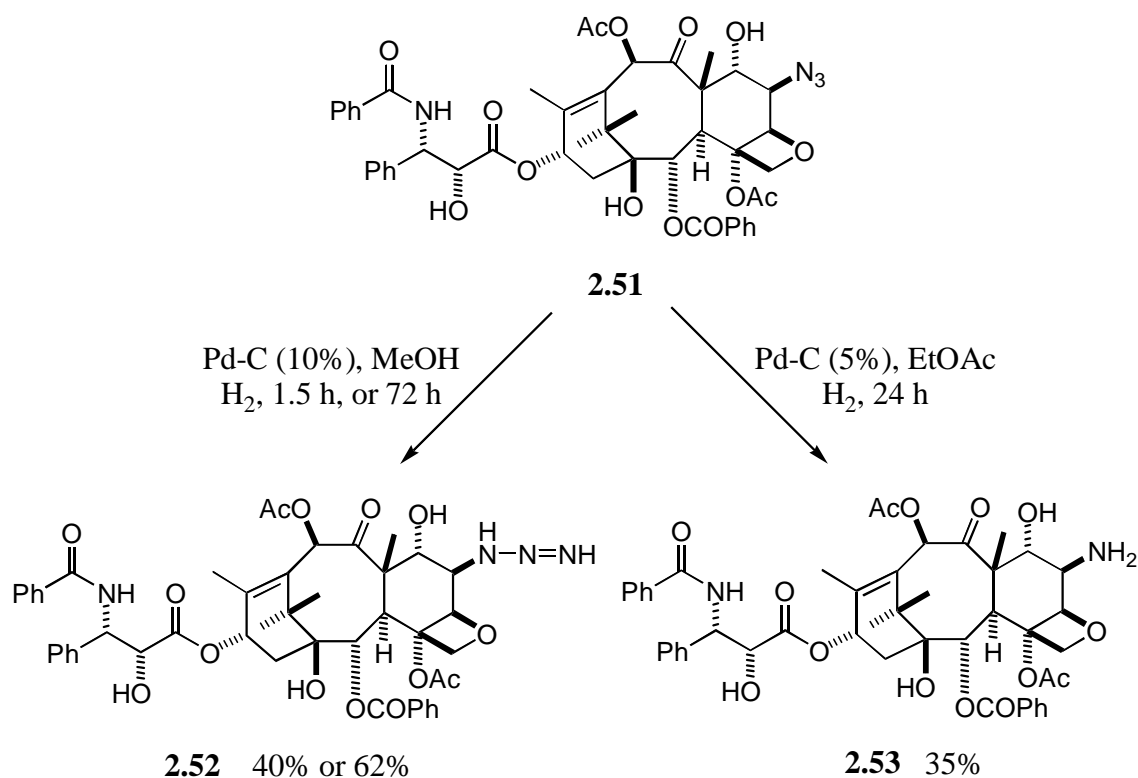
¹¹⁵ See ref. 109.

competition of C-7 desilylation process with the C-6 azide substitution is thus the cause of the undesired side reactions. Since this is the only protecting group that can be easily put on and taken off, however, it is the best compromise for this synthetic approach.

2.3.5 Hydrogenolysis of 6 β -azido-7-*epi*paclitaxel

Having successfully prepared 6 β -azido-7-*epi*paclitaxel (**2.51**), its reduction to the amino analog was the next immediate goal in order to evaluate the effect of the amino group on the anticancer activity as well as to afford an analog with improved water solubility in an acidic environment.

Compound **2.51** was hydrogenated in methanol catalyzed by 5% palladium on activated carbon under atmospheric pressure. TLC analysis after 4 hours using 7:3 EtOAc:hexanes indicated complete conversion of the starting material ($R_f \sim 0.6$) to a very polar product ($R_f \sim 0$). After filtration of the solid and removal of the solvent *in vacuo*, ^1H NMR was taken and showed that the product was a mixture of three components. Separation of the mixture was then achieved with MeOH:CH₂Cl₂ (8:92) to afford the major component **2.52** at 40% yield (Scheme 2.25).



Scheme 2.25 Hydrogenation of the azide **2.51**

The ¹H NMR spectrum of **2.52** features an upfield shift of the C-6 proton from 4.12 ppm to 3.0 ppm, in accordance with the reduction of the azide functionality. The HRFABMS revealed, however, that compound **2.52** had a molecular formula of C₄₇H₅₂O₁₄N₄, corresponding to the partially hydrogenated dihydroazido analog, as in Scheme 2.25.

A stoichiometric amount of Pd-C was then used along with a much longer reaction time, in an effort to completely reduce the azide. However, a complex reaction mixture again resulted after three days. The major product, isolated in greater yield (65%), was identical with **2.52**. It suggested that, due to certain reasons, the 6β azide or its partially reduced form is relatively inert toward hydrogenation. This had in fact been observed earlier in our group when the 7α azide was hydrogenated under similar conditions or with borohydride reductants.¹¹⁶ The only reported hydrogenation of paclitaxel azide in the literature was effected on the 7α azide under 48 psi hydrogen for

72 hours and gave only 50% yield.¹¹⁷ A hydrogenation experiment under 50 psi hydrogen pressure was thus conducted and yielded the desired 6 β -amino-paclitaxel (**2.53**) in 35% yield (Scheme 2.25).

The structure of compound **2.53** was assigned by ¹H, ¹³C, TOCSY, and HMQC spectra. 6 α proton was found to shift from 4.12 ppm to 3.35 ppm, while C-6 carbon shifted from 6.23 ppm to 54.6 ppm. Both changes are in consistent with the expected reduction of azide to amine. The molecular formula was confirmed by HRFABMS.

¹¹⁶ See ref. 109.

¹¹⁷ Menichincheri, M.; Botta, M.; Ceccarelli, W.; Ciomei, M.; Correli, F.; D'Attello, M.; Fusar-Bassini, D.; Mongelli, N.; Pesenti, E.; Pinciroli, V.; Tafi, A.; Vanotti, E. *Med. Chem. Res.* **1995**, 534.

2.4 A New Method for C-4 Acylation and Synthesis of Water Soluble Paclitaxel Analogs with Hydrophilic Groups at C-4

2.4.1 Introduction

In spite of paclitaxel's great promise in the treatment of refractory and untreatable human cancers, it is afflicted with formulation and administration problems, which are associated with its extremely low aqueous solubility. As a complex diterpenoid having extended fused ring systems and a number of hydrophobic substituents, together with its lack of ionizable functional groups, paclitaxel was reported to have water solubility of as low as 0.25 µg/mL.¹¹⁸ Higher values have also appeared in the literature, such as 0.7 µg/mL,¹¹⁹ 6 µg/mL,¹²⁰ and 30 µg/mL,¹²¹ but the differences are mainly due to non-comparable measurement methods adopted to determine the solubility. For example, the measurement of 30 µg/mL was made immediately after preparation of a saturated solution of paclitaxel, and was verified to be time-dependent. It was hypothesized that paclitaxel exists as a polymorphic solid which will form an energetically more stable crystalline solid over time. The equilibrium solubility of paclitaxel was believed to be around 5-6 µg/mL, dropping to less than 1 µg/mL after 24-36 hours.¹²²

This water solubility problem blocked paclitaxel's development as a cancer chemotherapeutic agent for almost 10 years. Many attempts at formulation were made using mixed solvents, emulsions, and liposomes.¹²³ The current formulation selected for clinical application consists of paclitaxel solubilized at a concentration of 6 mg/mL in

¹¹⁸ Vyas, D. M.; Wong, H.; Crosswell, A. R.; Casazza, A. M.; Knipe, J. O.; Mamber, S. W.; Doyle, T. W. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1357.

¹¹⁹ Mathew, A. E.; Mejillano, M. R.; Nath, J. P.; Himes, R. H.; Stella, V. J. *J. Med. Chem.* **1992**, *35*, 145.

¹²⁰ Tarr, B. D.; Yalkowsky, S. H. *J. Parenteral Sci. Technol.* **1987**, *41*, 31.

¹²¹ (a) Swindell, C. S.; Krauss, N. E. *J. Med. Chem.* **1991**, *34*, 1176. (b) Ringel, I.; Horwitz, S. B. *J. Natl. Cancer Inst.* **1991**, *83*, 288.

¹²² Sharma, U.; Balasubramanian, S. V.; Straubinger, R. M. *J. Pharm. Sci.* **1995**, *84*, 1223.

¹²³ Adams, J. D.; Flora, K. F.; Goldspiel, B. R.; Wilson, J. W.; Arbuck, S. G.; Finley, R. *Monogr. Natl. Cancer Inst.* **1993**, *15*, 141.

Cremophor EL[®] (polyoxyethylated castor oil) containing 50% ethanol. It is diluted 5 to 20 fold in 0.9% saline or 5% dextrose prior to administration. The amount of Cremophor EL[®] required for paclitaxel formulation represents the highest amount that has ever been used for any drug. As a consequence, patients often experience hypersensitivity reactions that are caused by histamine release. Antihistamine medications are therefore recommended prior to paclitaxel therapy. In an effort to overcome these problems caused by paclitaxel's poor water solubility, the design and synthesis of alternative forms of paclitaxel with improved water solubility and retained pharmacological activity has received much attention.

There are two approaches to achieve these alternative forms: paclitaxel derivatives and prodrugs. A paclitaxel derivative bearing a functional group that can significantly increase its water solubility and at the same time retain or even increase its anticancer activity would be a potent new generation agent. Several paclitaxel analogs were prepared possessing ionizable groups at the C-7 and/or C-10 position, mainly because modifications at these two positions have less impact on the tubulin assembly activity. Analogs of this category include glutaric acid derivatives,¹²⁴ amino acid derivatives,¹²⁵ and phosphate derivatives.¹²⁶ Some C-7 modified water-soluble derivatives are shown in Figure 2.17.

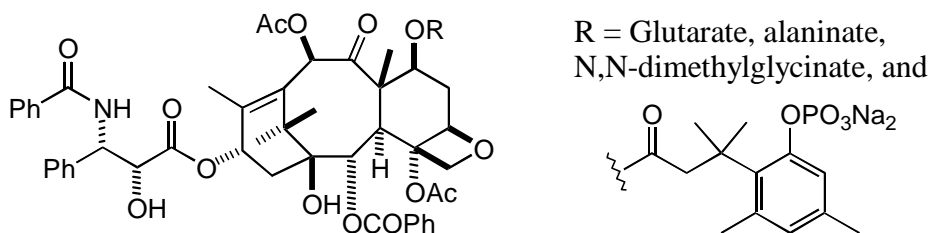


Figure 2.17 Some C-7 modified paclitaxel derivatives with improved water solubility

¹²⁴ Gueritte-Voegelein, F.; Guenard, D.; Lavelle, F.; LeGoff, M.-T.; Mangatal, L.; Potier, P. *J. Med. Chem.* **1991**, *34*, 992.

¹²⁵ Mathew, A. E.; Mejillano, M. R.; Nath, J. P.; Himes, R. H.; Stella, V. *J. Med. Chem.* **1992**, *35*, 145.

¹²⁶ Ueda, Y.; Mikkilineni, A. B.; Knipe, J. O.; Rose, W. C.; Casazza, A. M.; Vyas, D. M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1761.

A prodrug is a chemically modified form of a drug that possesses favorable physical or biological properties but is inactive by itself. It undergoes *in vivo* transformation to resume its active form. Common approaches to paclitaxel prodrugs¹²⁷ are esterifications at the C-2', C-7, and possibly C-10 hydroxyl groups with acyl groups that incorporate amines,¹²⁸ carboxylic acids,¹²⁹ sulfonic acids,¹³⁰ amino acids,¹³¹ phosphates,¹³² and esters and carbonates.¹³³ See Figure 2.18 for some representative C-2' modified prodrugs.¹³⁴

¹²⁷ DeBont, D. B. A.; Leenders, R. G. G.; Haisma, H. J.; van der Meulen-Muileman, I.; Scheeren, H. W. *Bioorg. Med. Chem.* **1997**, *5*, 405.

¹²⁸ (a) Magri, N. F.; Kingston, D. G. I. *J. Nat. Prod.* **1988**, *51*, 298. (b) Zhao, Z.; Kingston, D. G. I.; Crosswell, A. R. *J. Nat. Prod.* **1991**, *54*, 1067. (c) See ref. 126.

¹²⁹ (a) Deutsch, H. M.; Glinski, J. A.; Hernandez, M.; Haugwitz, R. D.; Narayanan, V. L.; Suffness, M.; Zalkow, L. H. *J. Med. Chem.* **1989**, *32*, 788. (b) see ref. 128(a).

¹³⁰ see ref. 128(b).

¹³¹ see ref. 128(a), (b), and 126.

¹³² (a) Vyas, D. M.; Wong, H.; Crosswell, A. R.; Casazza, A. M.; Knipe, J. O.; Mamber, S. W.; Doyle, T. W. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1357. (b) Ueda, Y.; Mikkilineni, A. B.; Knipe, J. O.; Rose, W. C.; Casazza, A. M.; Vyas, D. M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1761.

¹³³ Nicolaou, K. C.; Riemer, C.; Kerr, M. A.; Rideout, D.; Wrasidlo, W. *Nature* **1993**, *364*, 464.

¹³⁴ For a review, see Georg, G. I.; Boge, T. C.; Cheruvallath, Z. S.; Clowers, J. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. *The Medicinal Chemistry of Taxol*. In *Taxol: Science and Applications*, Suffness, M., Ed.; CRC Press: Boca Raton, FL, 1995, pp. 317, and references therein.

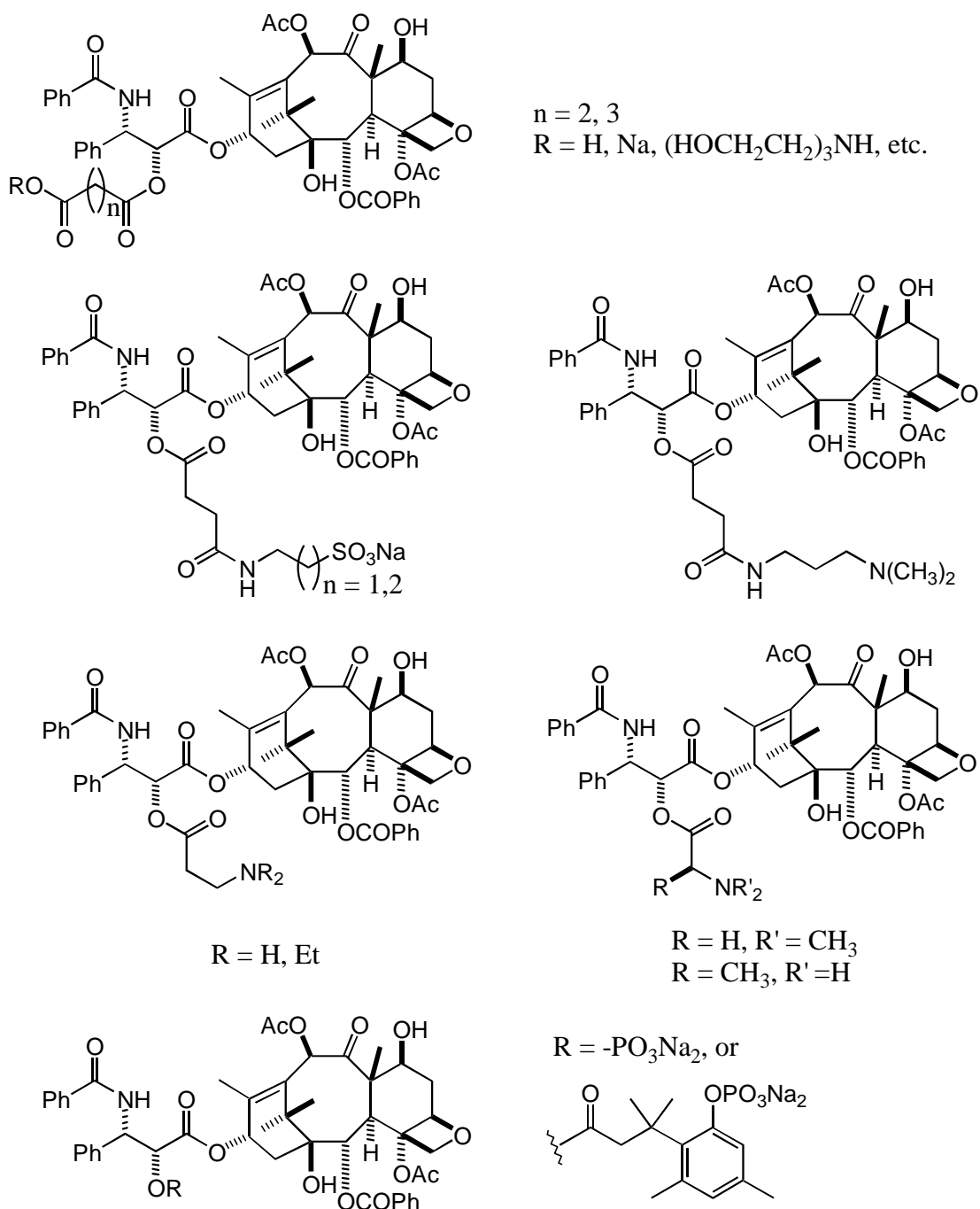


Figure 2.18 Some C-2' modified prodrugs of paclitaxel

In spite of the fact that a few of these synthetic prodrugs or more water-soluble derivatives showed interesting properties, none of them to date have progressed to clinical evaluation. Most of them are either less active in promoting tubulin assembly, or have

only marginal water solubility, or are unstable for clinical administration, or are too stable to be unmasked at a rate appropriate for therapy.

In a continuation of synthetic studies toward the preparation of paclitaxel analogs with improved water solubility, we turned our focus onto the C-4 position. Selective hydrolysis of the C-2 benzoate and the C-4 acetate with anhydrous base in phase transfer conditions has been achieved before, and allowed manipulations at both the C-2 and the C-4 positions. In particular, a C-4 deacetyl analog was found to be essentially inactive, while C-4 acyl analogs usually displayed similar activity to the parent compound paclitaxel (with C-4 acetyl), and some of them were more active than paclitaxel. It seemed that a C-4 acyl group may be crucial to the biological activity. It was thus the purpose of the study to prepare C-4 acyl analogs bearing hydrophilic functional groups at the acyl moiety.

2.4.2 C-4 Acylation Methods

Being a tertiary hydroxyl group, C-4 hydroxyl is in a very hindered position and is difficult to acylate. This is also partly due to the fact that it sits beneath the inverted cup-shaped part of the molecule, which is best depicted by three orthogonal views of the energy minimized conformation (See Figure 2.19).

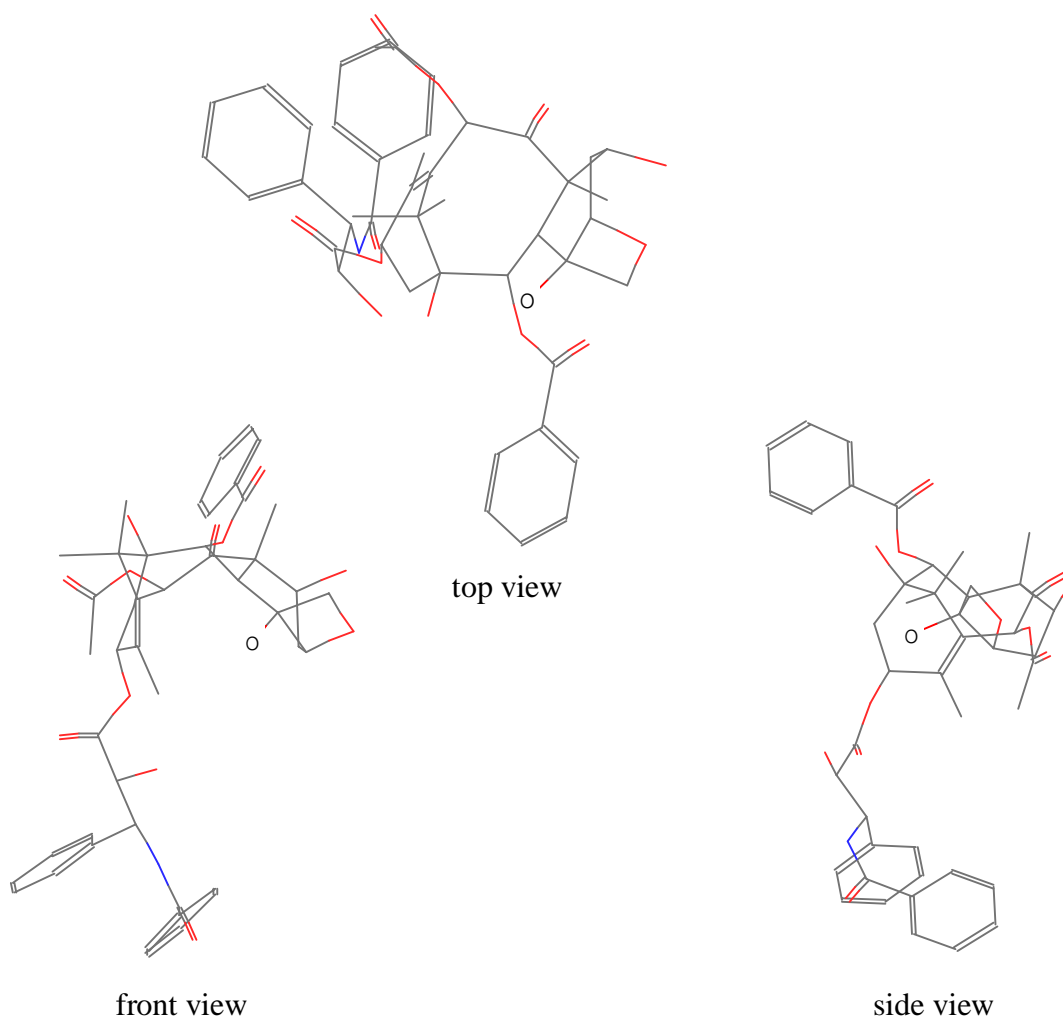
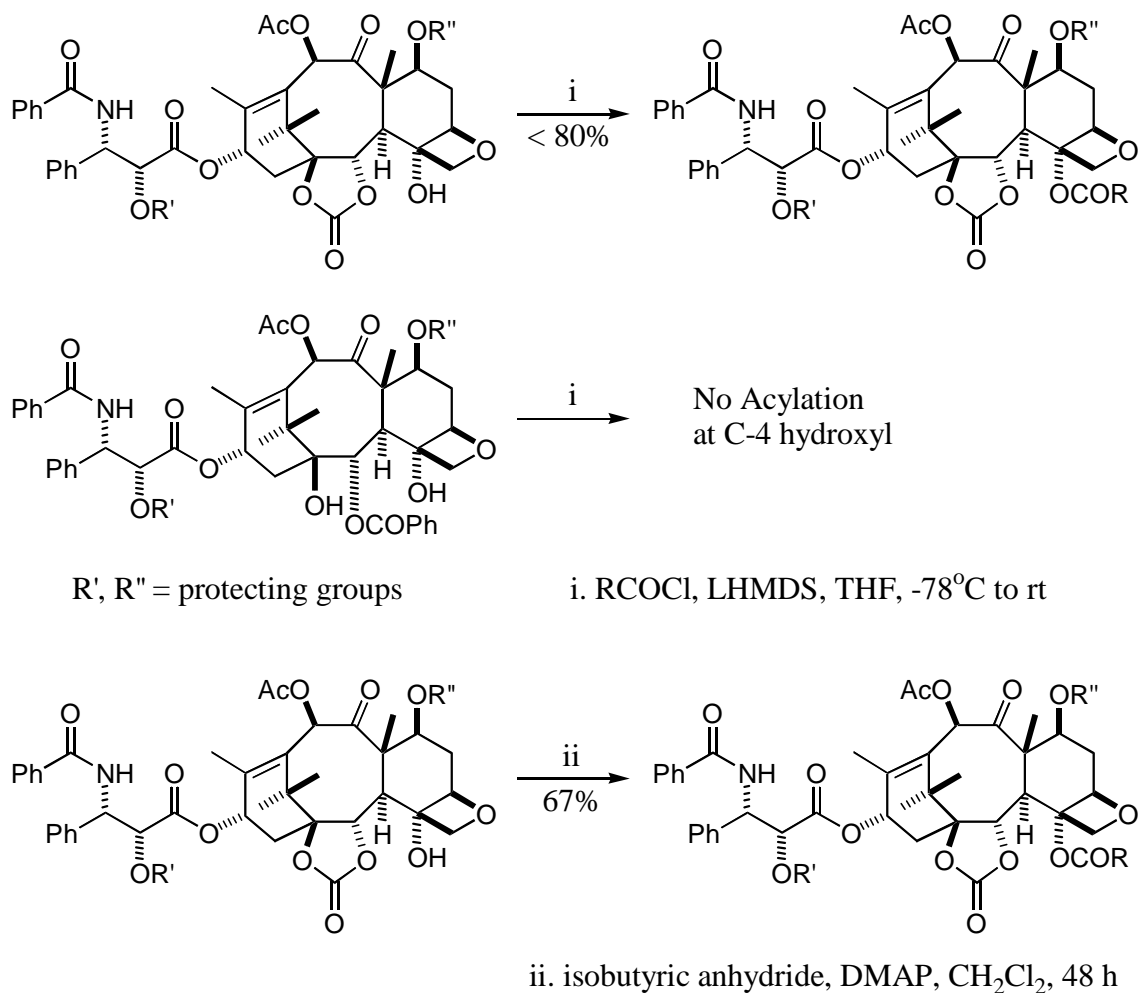


Figure 2.19 Steric hindrance of the C-4 hydroxyl group

Esterification at this position has been reported for both paclitaxel and baccatin III. In the case of paclitaxel, C-4 acylation requires the protection of the C-2 hydroxyl group in the form of a 1,2-carbonate. With a C-2 benzoyl group installed, the C-4 hydroxyl group of paclitaxel does not undergo acylation under any conditions (See Scheme 2.26). It is believed that the C-2 benzoate blocks the acylating reagent from approaching the C-4 hydroxyl group, and the cyclic carbonate not only masks the more reactive C-2 hydroxyl group, but is also small enough not to exert any significant steric hindrance to acylation at C-4. The reaction conditions reported involve the use of lithium hexamethyldisilylamide (LHMDS) and acyl chloride, or acid anhydride and DMAP. LHMDS conditions are hard to control, especially for small scale reactions, due to the reagent's moisture and temperature sensitivity as well as the very short reaction time.¹³⁵ The best yield obtained was about 75-80%. The later conditions using anhydride and DMAP are much milder, but there is only one precedence in the literature using isobutyric anhydride and this gave a 67% yield of the C-4 acylated product.¹³⁶ Efforts in our laboratory using other anhydrides under this condition failed to give any detectable C-4 acylated product.

¹³⁵ The reaction usually requires the addition of LHMDS to the solution of the substrate in THF at -78 °C and then raise the temperature to certain point between -45 °C to 0 °C by removal of the cool bath, followed by the addition of acyl chloride at appropriate speed. The uncertainty arise from the difficulties in controlling temperature and the addition speed of the acyl chloride.

¹³⁶ Georg, G. I.; Ali, S. M.; Boge, T. C.; Datta, A.; Falborg, L. *Tetrahedron Lett.* **1994**, 35, 8931.



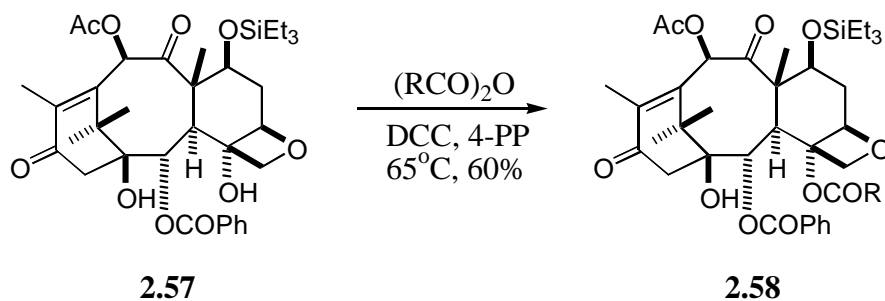
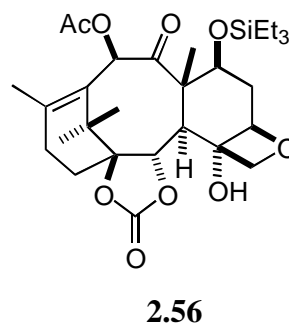
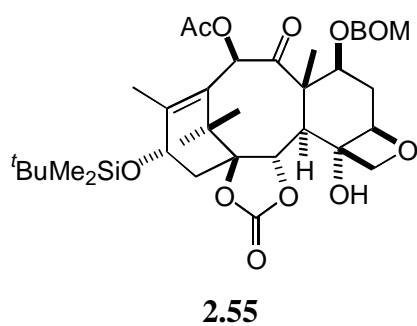
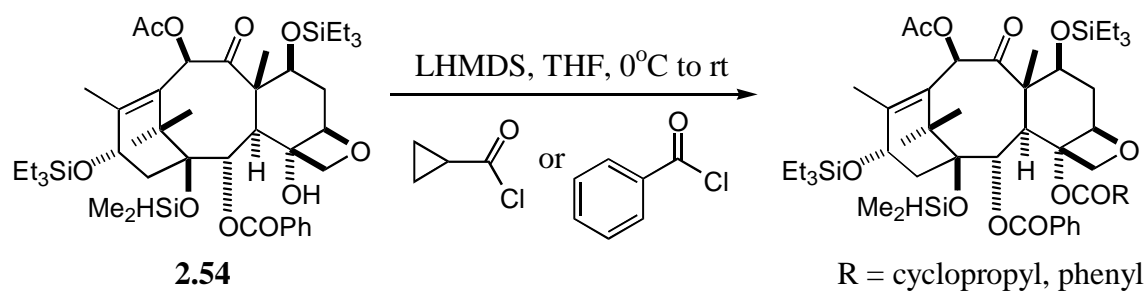
Scheme 2.26 Reported C-4 acylation of paclitaxel derivatives

On the other hand, C-4 acylation of baccatin III derivatives has been achieved both in the presence and absence of the C-2 benzoyl group (See Scheme 2.27). For example, Chen *et al.*¹³⁷ reported acylation of 1,7,13-triprotected baccatin III (**2.54**) using LHMDS and acid chloride. Neidigh¹³⁸ also successfully acylated the C-4 hydroxyl group using a 13-oxo baccatin III derivative (**2.57**). In both cases, C-4 acylation occurred in the presence of a C-2 benzoate. Holton¹³⁹ and Nicolaou¹⁴⁰ used 1,2-carbonato-baccatin III derivatives (**2.55** and **2.56**) to acylate at C-4 in their total synthesis of paclitaxel.

¹³⁷ Chen, S.-H.; Kadow, J. F.; Farina, V. *J. Org. Chem.* **1994**, *59*, 6156.

¹³⁸ Neidigh, K. A. *Chemical Studies of the C-4 Position of Baccatin III and Taxol*, Virginia Polytechnic Institute and State University, 1995.

¹³⁹ See ref. 45.



Scheme 2.27 C-4 acylation of baccatin III derivative

¹⁴⁰ See ref. 46.

2.4.3 A New Method for C-4 Acylation

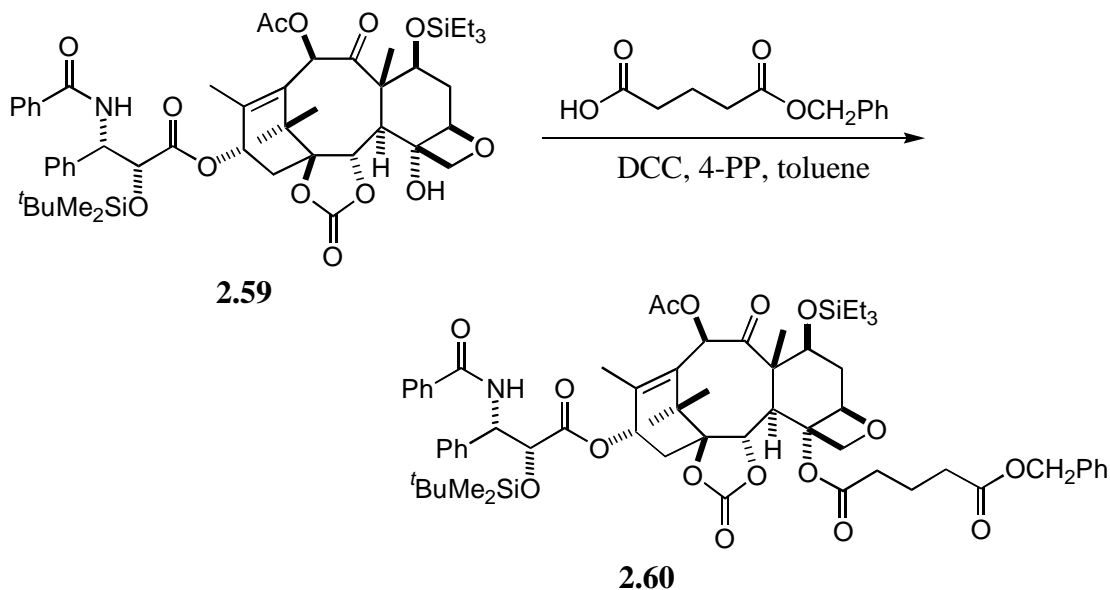
The C-4 acylation of paclitaxel through baccatin III derivatives would apparently entail more synthetic steps and was therefore inferior to the more direct 1,2-carbonato paclitaxel approach. With the aim of preparing paclitaxel derivatives bearing functional groups at the C-4 position that would assist in improving its water solubility, initial attempts were performed using both acid chloride/LHMDS and acid anhydride/DCC/DMAP conditions, as summarized in Table 2.10. Glutaric acid was chosen to investigate the effectiveness of different C-4 acylation methods. As sometimes observed earlier in the same laboratory when preparing other C-4 modified paclitaxel derivatives, acid chloride/LHMDS conditions often gave a complex reaction mixture that contained the desired product in variable but low yield (<20% based on TLC and/or ¹H NMR). Acid anhydride/DCC/DMAP conditions were also studied by treating 2'-*O*-*tert*-butyldimethylsilyl-7-triethylsilyl-2-debenzoyl-1,2-carbonato-4-deacetyl-paclitaxel (**2.59**) with glutaric anhydride and DCC/DMAP for 48 hours; TLC analysis indicated that a product was formed in about 20% yield along with unreacted starting material. The product was not isolated but was believed to be the desired product because of the acid like polarity and TLC behavior as well as the indicative greenish color when sprayed with vanillin.

Table 2.10 Conditions for initial acylation attempts on 2'-*O*-*tert*-butyldimethylsilyl-7-triethylsilyl-2-debenzoyl-1,2-carbonato-4-deacetyl-paclitaxel (**2.59**)

Experiment	Acylating agent	Conditions	Rxn. Time	Result
1	CICO(CH ₂) ₃ CO ₂ CH ₂ Ph	LHMDS, THF, -78 °C	24 h	mixture
2	glutaric anhydride	DMAP, CH ₂ Cl ₂	48 h	product

Reasoning that the low yield of the anhydride approach might be due to the steric demands of the cyclic anhydride, another experiment was carried out using mono-

protected glutaric acid and DCC/4-PP. When 2'-*O*-*tert*-butyldimethylsilyl-7-triethylsilyl-2-debenzoyl-1,2-carbonato-4-deacetyl-paclitaxel (**2.59**) was treated with 10 equivalents of monobenzyl glutaric acid, 12 equivalents of DCC and a catalytic amount of 4-PP for 24 hours at room temperature, careful analysis of the TLC of the reaction mixture by eluting three times with 30% ethyl acetate in hexanes indicated the complete conversion of the starting material to a slightly more polar product (Scheme 2.28). More clearly, the product gave a distinctive greenish color upon spraying with vanillin after TLC, while the starting material gave brownish color. The difference in color, however, was very subtle and required quick and short heating. A number of other development systems were examined later but none of them gave better separation. The required critical judgement for the completion of similar reactions thus relied solely on multiple elution and careful visualization of the TLC.

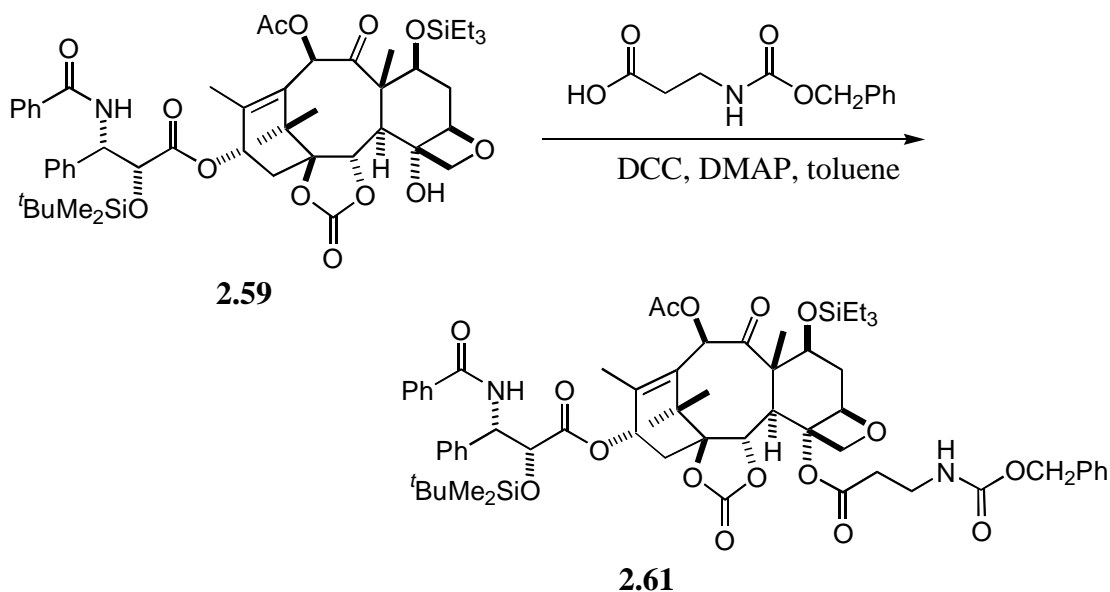


Scheme 2.28 C-4 acylation using glutaric acid monobenzyl ester

The reaction mixture was purified and afforded a pure product in 87% yield. The ^1H NMR spectrum of the product was typical for a C-4 acylated compound. A characteristic upfield shift of the 3'-NH proton from about 7.3 ppm (hidden in the aromatic region) to 7.01 ppm confirmed the occurrence of C-4 acylation. The appearance

of benzylic protons at 5.08 ppm as an AB quartet and a series of multiplets for six extra protons from 2.75 to 2.05 ppm were also consistent with the installation of a glutaryl group onto paclitaxel. The ^{13}C NMR spectrum was also consistent with this product.

Having successfully reacylated 2'-*O*-*tert*-butyldimethylsilyl-7-triethylsilyl-2-debenzoyl-1,2-carbonato-4-deacetyl-paclitaxel in good yield under very mild conditions, it was desirable to expand the scope of this reaction to other acids. With the intention of preparing water-soluble paclitaxel analogs modified at C-4, we chose N-carbobenzyoxy- β -alanine as the other acylating reagent. Under the same conditions, 2'-*O*-*tert*-butyldimethylsilyl-7-triethylsilyl-2-debenzoyl-1,2-carbonato-4-deacetyl-paclitaxel (**2.59**) was reacted with N-carbobenzyoxy- β -alanine to give 2'-*O*-*tert*-butyldimethylsilyl-7-triethylsilyl-2-debenzoyl-1,2-carbonato-4-deacetyl-4-(N-carbobenzyoxy- β -alanyl)-paclitaxel (**2.61**) in 72% yield (Scheme 2.29).



Scheme 2.29 C-4 acylation using N-Cbz- β -alanine

The ^1H NMR spectrum of the compound **2.61** also showed a typical upfield shift of the 3'-NH proton from about 7.3 ppm to 7.07 ppm, along with the oxygenated benzylic protons at 4.96 ppm as a broad singlet and an extra proton for NH (β -alanyl) at 5.71 ppm

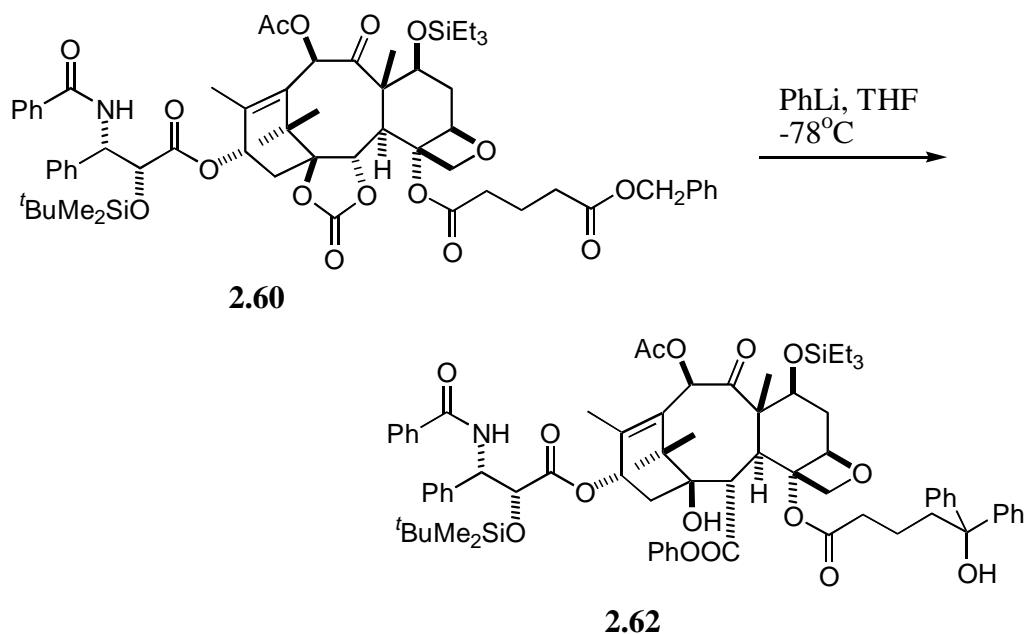
as well as the methylene protons of the alanine unit. The ^{13}C NMR spectrum was also consistent with the assigned C-4 acylated structure.

2.4.4 Preparation of Water-Soluble Paclitaxel Analogs with a Hydrophilic Group at the C-4 Position

To complete the preparation of water-soluble paclitaxel analogs with a hydrophilic group at the C-4 position, it was necessary to reinstall the C-2 benzoyl group of compounds **2.60** and **2.61**. Holton¹⁴¹ and Nicolaou¹⁴² had reported independently that the 1,2-cyclic carbonate can be regioselectively transformed into the C-1 hydroxyl C-2 benzoyl configuration of paclitaxel by reaction with phenyl lithium. Generally speaking, the carbonate carbonyl group is less reactive toward nucleophilic addition of organometallic reagents than ester carbonyl groups; the success of the above mentioned phenyl lithium opening of the cyclic carbonate is therefore the consequence of steric factors. Considering the presence of a benzyl ester at C-4 in compound **2.60**, and that this ester would very likely protrude out of the molecule and would thus be easily accessible, it was expected that the phenyl lithium reaction with this compound would be troublesome. Indeed, when **2.60** was reacted with PhLi in THF at -78 °C for 15-20 minutes, a single product was formed and was found after purification to be the product that had reacted both at the carbonate and at the benzyl ester (Scheme 2.30). Compound **2.62** was characterized by comparison of its ¹H NMR spectrum with starting material and other relevant authentic compounds. The downfield shift of the C-2 proton from 4.50 ppm to 5.69 ppm was indicative of the opening of the 1,2 carbonate, so was the appearance of the two ortho-protons of the C-2 phenyl ring at 8.10 ppm. However, the disappearance of the two benzyl ester protons at 5.08 ppm suggested that reaction also occurred at the C-4 benzyl ester end. The integration of aromatic protons suggested there were two phenyl rings at the C-4 end, and the polarity of compound **2.62** was in agreement with the proposed tertiary alcohol structure.

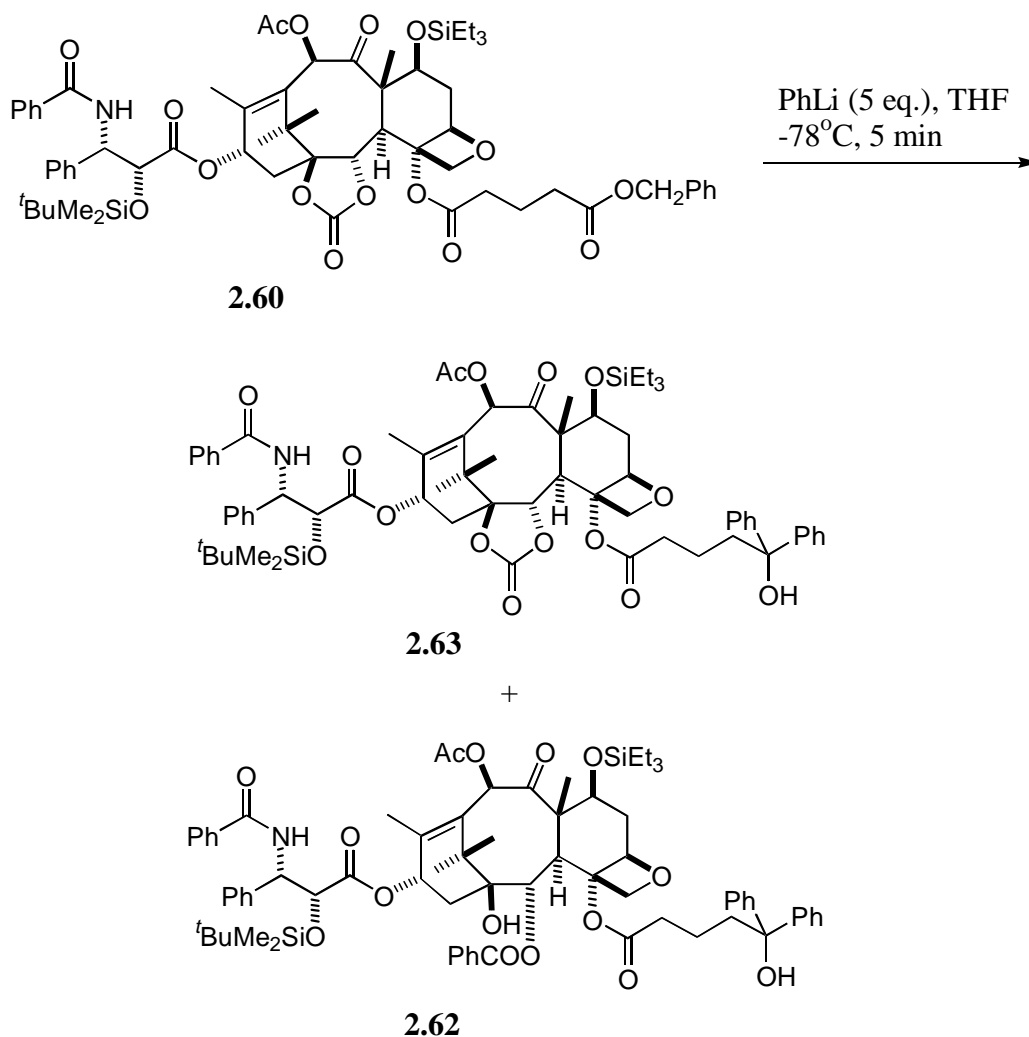
¹⁴¹ See ref. 45.

¹⁴² See ref. 46.



Scheme 2.30 Attempt at selective opening of 1,2-carbonate in the presence of an reactive ester

Hoping that a shorter reaction time might preferentially give the carbonate addition product without formation of product formed by attack at the benzyl ester carbonyl group, the experiment was repeated with a smaller amount of phenyl lithium (5 equivalents) and was stopped after 5 minutes. Unfortunately, the ^1H NMR spectrum of the crude reaction mixture indicated that it was a mixture of starting material, compound **2.62** and **2.63** in approximately equal amounts (Scheme 2.31).

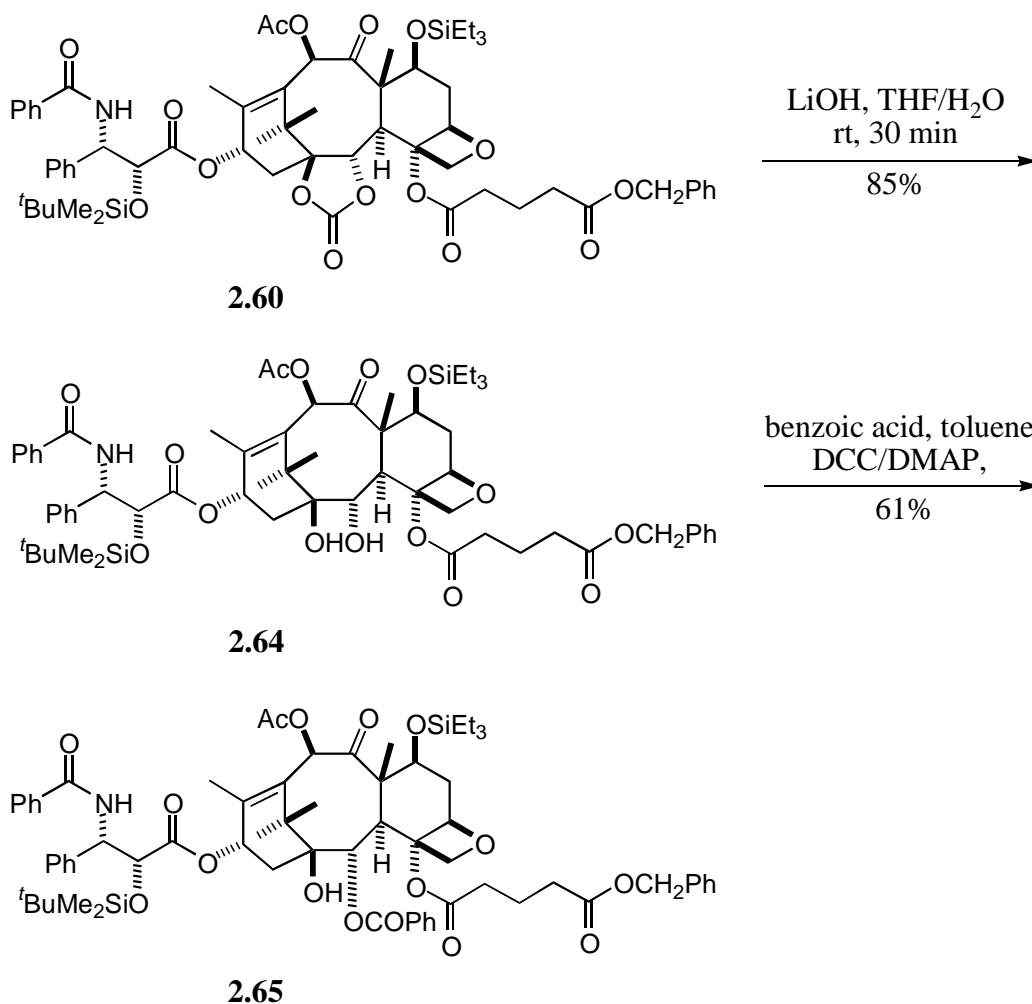


Scheme 2.31 More attempt at selective opening of 1,2-carbonate in the presence of an reactive ester

Having had no success in the direct benzylation at C-2 position, an indirect approach was adopted to get around the reactivity problem. Hydrolysis of the 1,2 carbonate with lithium hydroxide (LiOH) has been used previously in the preparation of C-2 benzoyl modified paclitaxel analogs from 1,2 carbonate derivatives.¹⁴³ It was found that the remaining labile groups, i.e. the C-4, C-10, and C-13 esters, are stable enough to allow selective hydrolysis of the 1,2-cyclic carbonate. Although the C-4 benzyl ester of compound **2.60** could also be hydrolyzed, this should not affect subsequent esterification

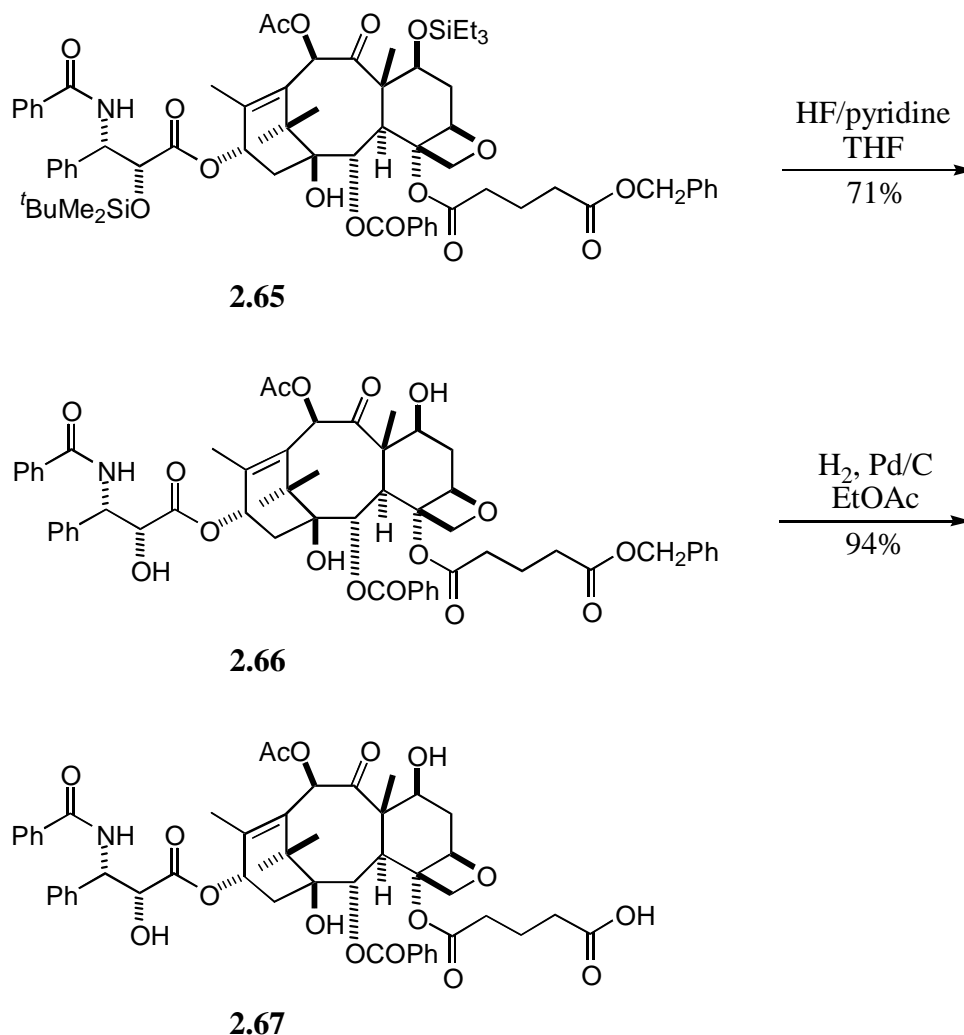
¹⁴³ Chordia, M. D.; Choudhary, A. G.; Kingston, D. G. I. *Abstr. Paper Am. Chem. Soc.* 208: 54-Med., Part 1. Aug 21, 1994.

using excess benzoic acid and DCC/DMAP. Thus, compound **2.60** was treated with lithium hydroxide in THF/H₂O at room temperature, affording a more polar product in 85% yield. After purification, ¹H NMR analysis showed it to be the desired 1,2-diol with the C-4 benzyl ester intact. With the diol **2.64** in hand, C-2 benzylation was effected as usual by reacting **2.64** with 10 equivalents of benzoic acid, 12 equivalents of DCC, and a catalytic amount of DMAP. The reaction gave a less polar product in 61% yield after purification (Scheme 2.32). ¹H NMR analysis showed it to be the desired product 2'-*O*-*tert*-butyldimethylsilyl-7-triethylsilyl-4-deacetyl-4-(*O*-benzylglutaryl)-paclitaxel (**2.65**) based on the typical downfield shift of the C-2 proton from 4.50 ppm to 5.66 ppm and observation of the appearance of two ortho-benzoate protons at 8.12 ppm.



Scheme 2.32 An alternative method for the re-installation of the C-2 benzoyl group

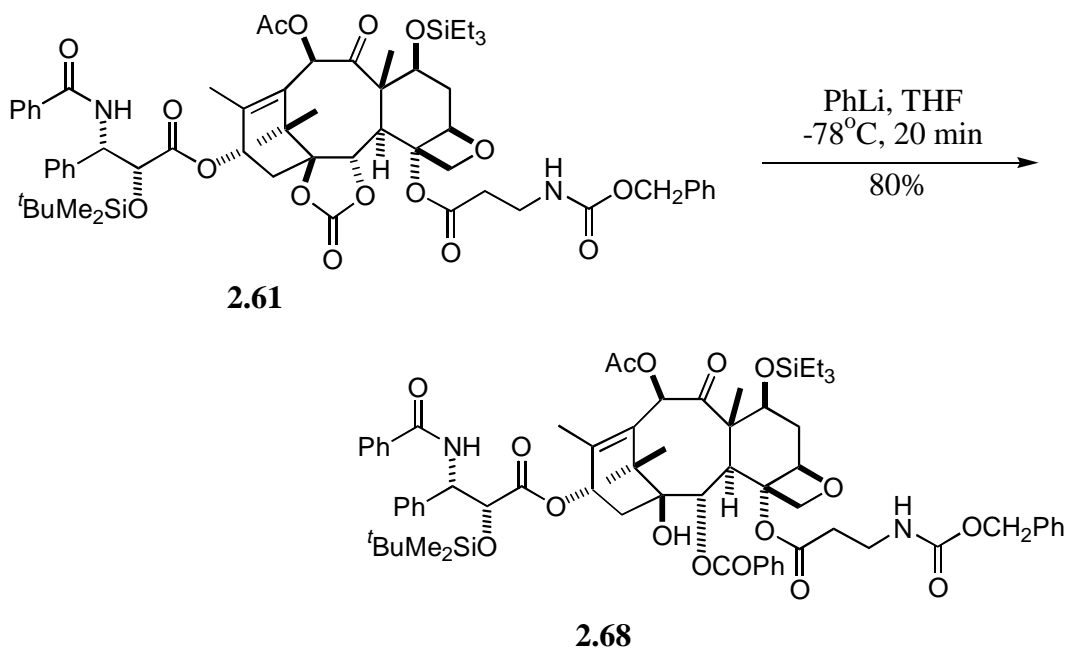
Compound **2.65** was then subjected to desilylation and hydrogenation reactions to remove the protecting groups, affording a highly polar product in 67% yield in two steps (Scheme 2.33). ^1H NMR analysis of the purified product **2.67** showed it was the desired compound 4-deacetyl-4-glutaryl-paclitaxel. Compared with paclitaxel, compound **2.67** had all the proton signals at the normal position except that the methyl group of the C-4 acetate at about 2.38 ppm was replaced with a multiply coupled spin system at 2.75-2.06 ppm corresponding to the $\text{CH}_2\text{CH}_2\text{CH}_2$ of the C-4 glutaryl group. ^{13}C NMR data were also consistent with the structure assignment. Specifically, compound **2.67** had one extra carboxylic carbon at about 176 ppm and three extra secondary carbons at 22.1 ppm, 30.0 ppm, and 32.9 ppm. Mass spectral data further confirmed the molecular formula of the compound to be $\text{C}_{50}\text{H}_{55}\text{NO}_{16}$, agreeing with the structure.



Scheme 2.33 Preparation of a water-soluble paclitaxel analog bearing a carboxylic acid at the C-4 position

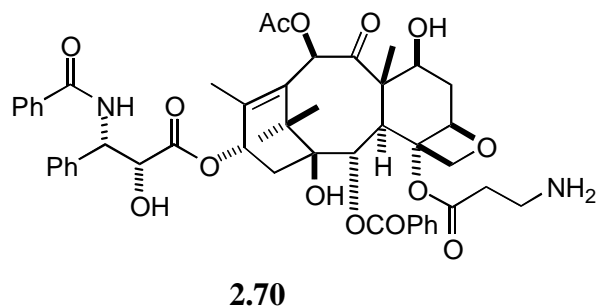
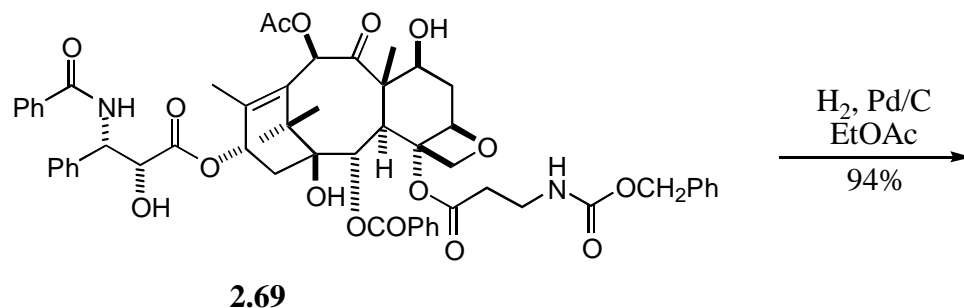
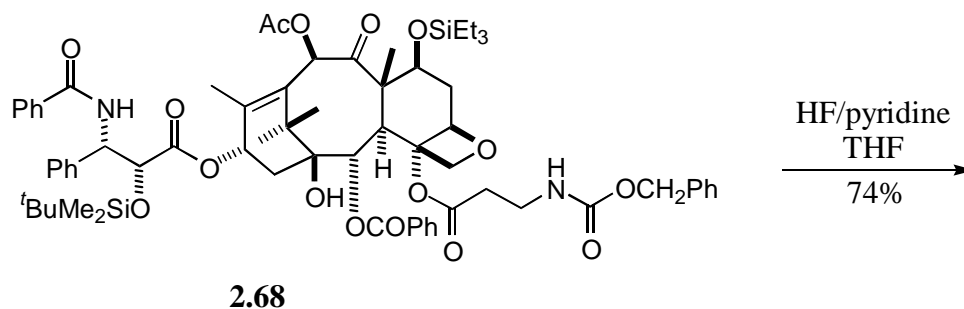
Unlike **2.60**, 2'-*O*-*tert*-butyldimethylsilyl-7-triethylsilyl-4-deacetyl-4-(*N*-benzyloxycarbonyl- β -amino-propionyl)-1,2-carbonato-paclitaxel (**2.61**) has a carbamate group at the terminal of the C-4 acyl moiety instead of an ester, and can therefore undergo selective reaction with phenyllithium. Compound **2.61** was treated with 10 equivalents of PhLi in THF at -78 °C for 20 minutes, affording a product with very similar polarity on TLC to that of starting material. Changing eluting solvent systems or conducting multiple elution did not improve separation significantly, but monitoring of the reaction could still be achieved by differentiating the distinctive colors of the starting material and the product upon vanillin spray. The 1,2-carbonato analogs usually give a brownish color

while compounds with a C-2 benzoate often give a green-blue color. After purification, the product **2.68** was obtained in 80% yield. ^1H NMR analysis of **2.68** indicated that the regioselective 1,2 carbonate opening had occurred and that the rest of the molecule remained intact. The key NMR evidence for this assignment was the downfield shift of the C-2 proton from 4.50 ppm to 5.65 ppm and the appearance of two ortho-protons of the C-2 benzoate at 8.07 ppm (Scheme 2.34).



Scheme 2.34 Selective opening of the 1,2-carbonate in the presence of a carbamate

Deprotection of compound **2.68** proceeded smoothly as expected with HF/pyridine in THF, followed by hydrogenation catalyzed by 5% palladium on activated carbon to remove the Cbz protecting group. This afforded the desired 4-deactyl-4- β -alanyl-paclitaxel (**2.70**) in 70% yield (2 steps) (Scheme 2.35). The desilylated product was purified by preparative TLC, eluting with 7:3 EtOAc:hexanes. The final product amine was obtained by filtering the hydrogenation mixture through a pad of Celite on the top of a short silica gel column and collected with acetone elution.



Scheme 2.35 Preparation of a water-soluble paclitaxel analog bearing a amino group at the C-4 position

^1H NMR spectra of both compounds **2.69** and **2.70** showed normal trends for the reactions taken place. For **2.69**, signals corresponding to the *tert*-butyldimethylsilyl and triethylsilyl groups were absent while chemical shifts for other protons were essentially unchanged. For compound **2.70**, the β -alanyl NH proton at 5.43 ppm shifted upfield (overlapped with others), and the two benzyl protons at 4.96 ppm were absent. The β -protons of the C-4 β -alanyl group at 3.56 ppm also shifted upfield, in agreement with the removal of the electron withdrawing benzyloxy carbonyl group. ^{13}C NMR data were in agreement with the structure as well. HRFABMS confirmed the molecular formula of $\text{C}_{48}\text{H}_{54}\text{N}_2\text{O}_{14}$ for the proposed structure.

2.5 Synthesis of a C-4, C-6 Tethered Paclitaxel Analog

2.5.1 Introduction

It is the ultimate goal for structure-activity relationships study to understand thoroughly the direct interaction between a drug molecule and the receptor that is responsible for the drug's curing action. This includes identifying and mapping the active site at the molecular level in three dimensional space, as well as understanding the biological processes associated with the interaction. In this aspect, X-ray diffraction is the most powerful tool in that it can possibly give a direct picture of the drug and the receptor when the two are interacting. This is extremely difficult for paclitaxel and microtubules because of two reasons: (1) the resolution (18Å) of the X-ray structure of the microtubules is insufficient to recognize the binding site;¹⁴⁴ (2) X-ray analysis of paclitaxel and its analogs is very difficult due to their poor crystalline properties. Many efforts have thus been made towards the synthesis of paclitaxel analogs bearing functional groups such as radioactive isotopes, photoaffinity labels, and fluorescent chromophores. With the help of these specific analogs and appropriate techniques, an indirect visualization of the binding between microtubules and paclitaxel or its analogs has been realized for some area of the molecule.¹⁴⁵ It is noteworthy that the structure of tubulin has been obtained in a much improved resolution (6.5 Å) in 1995 by electron crystallography of zinc-induced two-dimensional crystals of the tubulin.¹⁴⁶ In the study, each paclitaxel molecule was visualized to bind to one tubulin dimer, which is in agreement with the known stoichiometry of paclitaxel in microtubules.

On the other hand, conformational analysis of paclitaxel using high level molecular modeling and 2D-NMR techniques such as NOESY has also provided

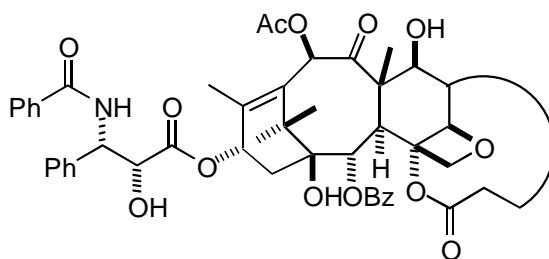
¹⁴⁴ Beese, L.; Stubbs, G.; Cohen, C. *J. Mol. Biol.* **1987**, *194*, 257.

¹⁴⁵ (a) Rao, S.; Horwitz, S. B.; Ringel, I. *J. Natl. Cancer Inst.* **1992**, *84*, 785. (b) Rao, S.; Krauss, N. E.; Heerding, J. M.; Swindell, C. S.; Ringel, I.; Orr, G. A.; Horwitz, S. B. *J. Biol. Chem.* **1994**, *269*, 3132.

¹⁴⁶ Nogales, E.; Wolf, S. G.; Khan, I. A.; Luduena, R. F.; Downing, K. H. *Nature* **1995**, *375*, 424.

information about the active conformation of the molecule. To ensure the effectiveness and reliability of this study, and to further explore the steric constraints of the paclitaxel binding site on tubulin, the rational design and synthesis of a paclitaxel analog that helps reveal the active conformation becomes necessary.

The C-4 acetate of paclitaxel has been believed to be important to its activity, as discussed in Section 2.4.1. While many investigations have been conducted to study the active conformation of other important pharmacophores of paclitaxel such as the C-2 benzoyl and the C-13 side chain, due to relatively easy synthetic access to those areas, little is known about the active conformation of the C-4 acetate or about the effect of groups constrained to lie underneath ring C. Biological evaluation of paclitaxel analogs modified at C-4 indicated that an acyl substitution at the C-4 acetyl group can have a significant effect on bioactivity. It was thus our plan to link the C-4 acetyl group to another part of the molecule so that the conformation of the C-4 acyl group would be constrained. The conformation of this constrained compound could then be studied through NOESY experiments and modeling. (Figure 2.20). The correlation of its conformation and activity data would be very useful in revealing the interaction of the C-4 acetate and the lower face of the C-ring and microtubules.



2.71

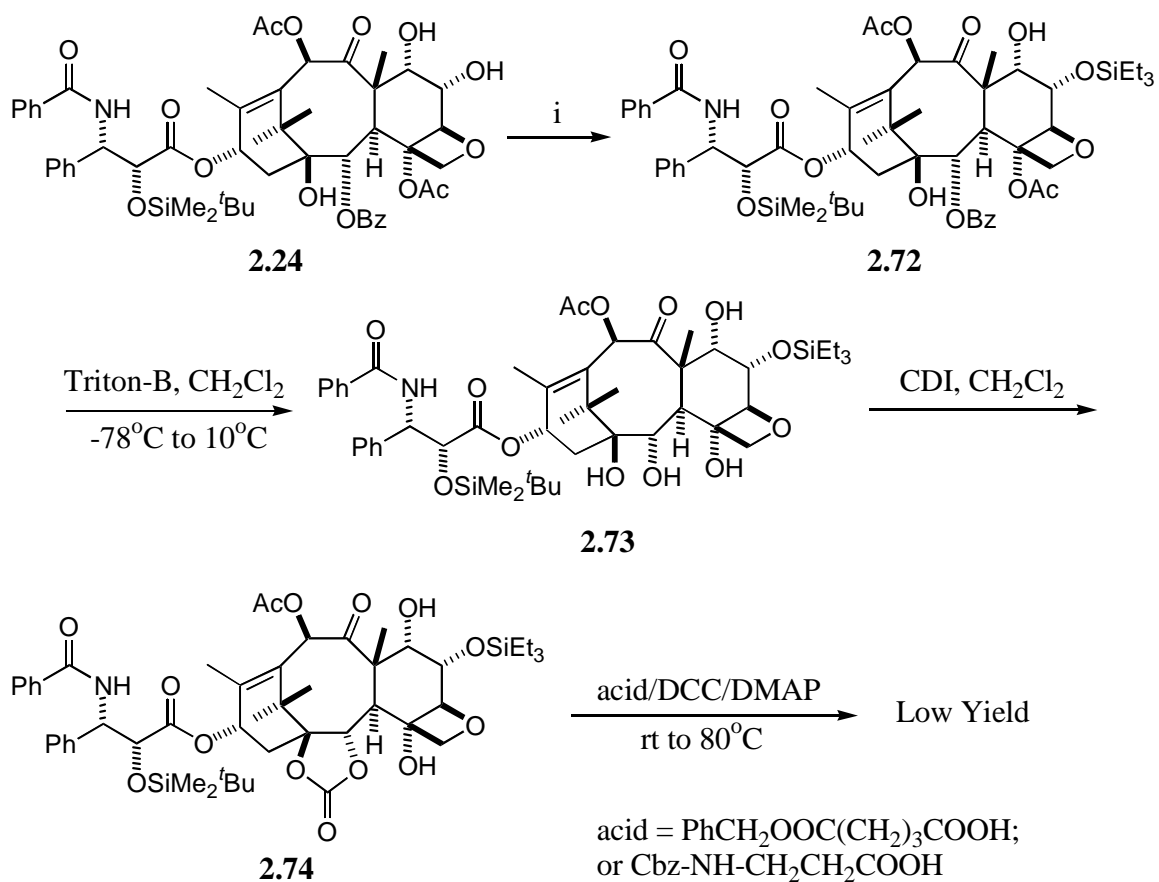
Figure 2.20 A schematic paclitaxel analog with frozen conformation at C-4

Having successfully developed a mild C-4 acylation method using acid/DCC/DMAP conditions (Section 2.4.3), synthetic efforts were made to tether the C-4 acyl group to the C-6 position, which should be the best anchor to lock the C-4 acyl group. The C-6 α hydroxyl group (such as in **2.24**) generated in the dihydroxylation of

compound **2.23** provided a useful handle for the installation of a linker between C-6 and C-4, and modifications at the C-6 α hydroxyl group of paclitaxel only slightly changed the activity, according to previous studies. Thus the change in activity of the proposed tethered analog could be contributed primarily to any conformation change at the C-4 acyl group and to the effect of a substituent on the α face of ring C. A detailed discussion concerning this synthetic approach is presented hereafter.

2.5.2 Attempts at the C-4 Acylation of Paclitaxel Analog Having a C-6 α Hydroxyl Group

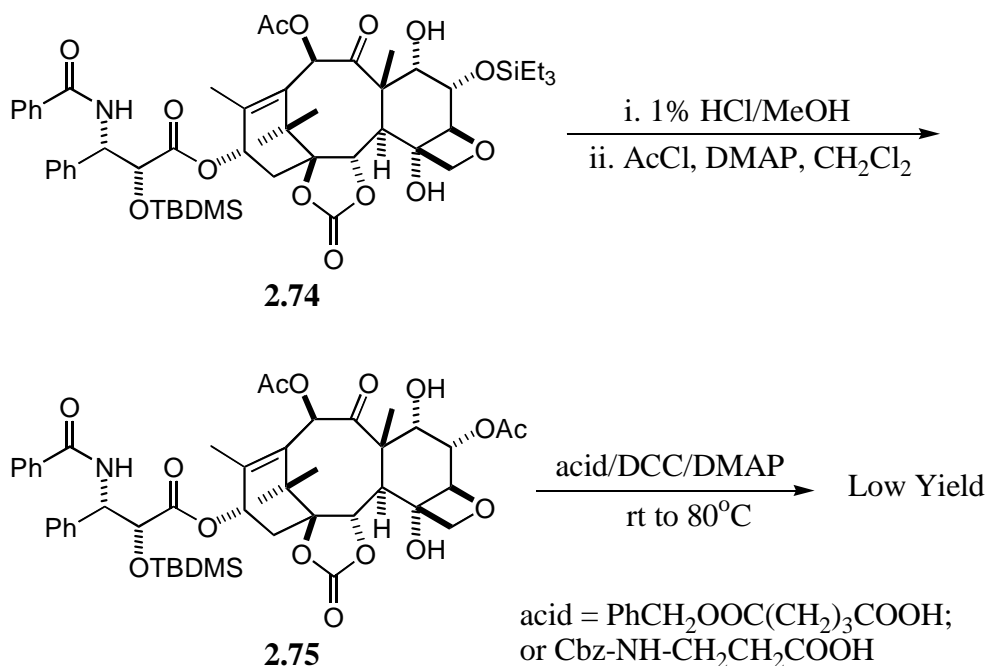
The 6 α hydroxyl group of 2'-*O-tert*-butyldimethylsilyl-6 α -hydroxy-7-*epi*paclitaxel (**2.24**) was protected to form 2'-*O-tert*-butyldimethylsilyl-6 α -*O*-triethylsilyl-7-*epi*paclitaxel (**2.72**) and was subjected to the selective hydrolysis conditions using Triton-B. The reaction proceeded in the same way as with 2'-*O-tert*-butyldimethylsilyl-7-*O*-triethylsilyl-paclitaxel (**2.8b**) and yielded the tetra-ol **2.73**. The 1,2-dihydroxyl groups in compound **2.73** were then protected as previously described by reaction with N,N'-carbonyl diimidazole (CDI) in methylene chloride to afford compound **2.74** (Scheme 2.36).



i. Et₃SiCl, imidazole, DMF.

Scheme 2.36 Attempts at the C-4 acylation of 2.74

The reacylation reactions under conditions similar to that described in Section 2.43, however, failed to give the desired product in an adequate yield for further investigation. In most cases, unreacted starting material was recovered and the desired product was obtained in less than 10-20% yield. It was reasoned that the low yield might be due to the steric bulkiness of the C-6 α silyl ether group. In order to test the extent of the effect of steric hindrance at this position, the model compound 2'-*O*-*tert*-butyldimethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-6 α -*O*-acetyl-*epi*paclitaxel (2.75) was prepared and subjected to the same reacylation conditions as in Scheme 2.37. Similar low yields resulted from these model reactions.

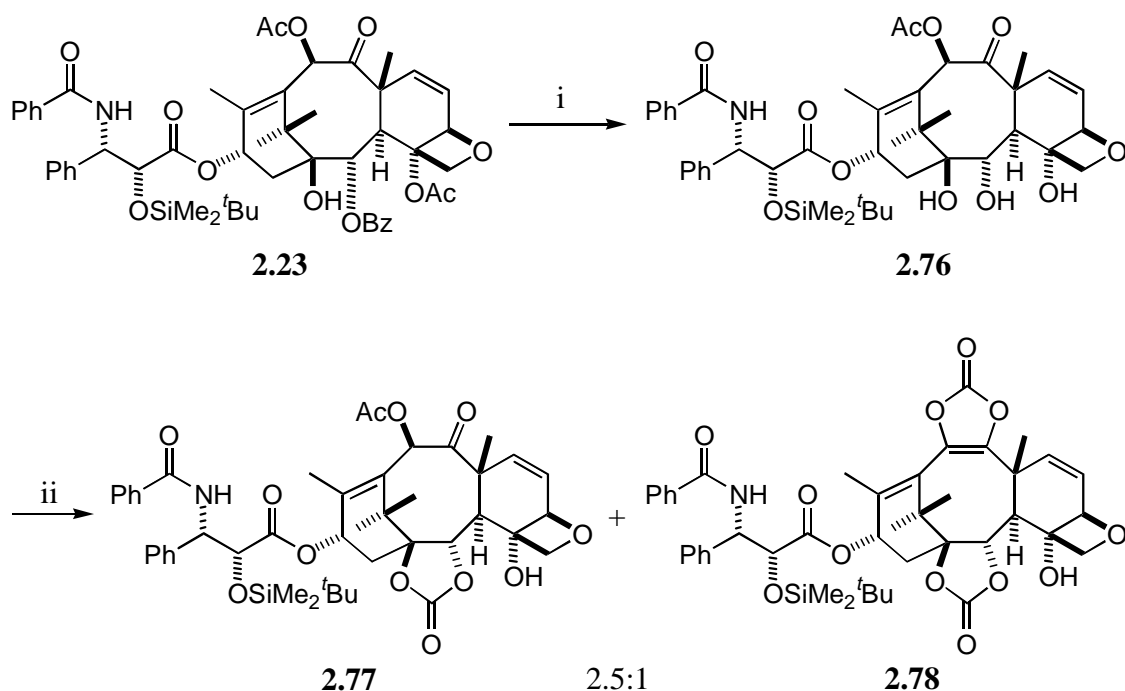


Scheme 2.37 A model acylation reaction of C-6 α protected substrate **2.75**

This results excluded the possibility of reacylating the C-4 position in the presence of a pre-installed C-6 α hydroxyl group due to the necessity for protection of this group and the resulting steric hindrance to acylation at C-4. Since even a protecting group as small as an acetate will block reacylation at the C-4 position, an alternative method had to be developed.

2.5.3 Acylation at the C-4 Position Prior to the Installation of the C-6 α Hydroxyl Group

Realizing that any substituent on the α face of C-6 of compound **2.75** will interfere with the C-4 acylation reaction, a 6,7-dehydro version of **2.75** was believed to be ideal choice for the reaction. Compound **2.23** was thus treated with Triton-B in methylene chloride, the standard reaction conditions for the selective hydrolysis of the C-2 benzoyl and the C-4 acetate. TLC analysis showed sequential removal of the C-2 benzoyl and the C-4 acetate groups and the formation of a very polar product, in a cleaner way than that was observed with 2'-*O*-*tert*-butyldimethylsilyl-7-triethylsilyl-paclitaxel (**2.8b**). The product was subjected directly to the following reaction after workup to protect the 1,2-diol as its cyclic carbonate (Scheme 2.38).

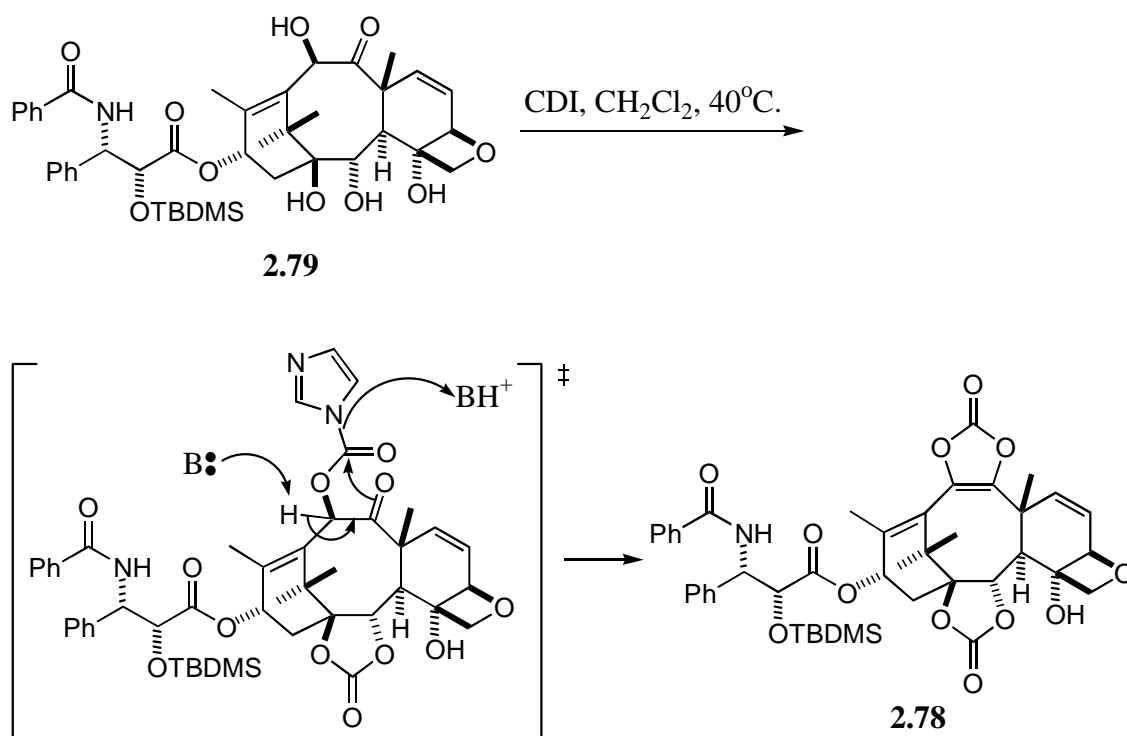


- i. Triton-B, CH₂Cl₂, -78°C to 10°C, %;
 ii. CDI, CH₂Cl₂, 40°C, 40% for **2.77** (2 steps).

Scheme 2.38 Preparation of the key intermediate **2.77**

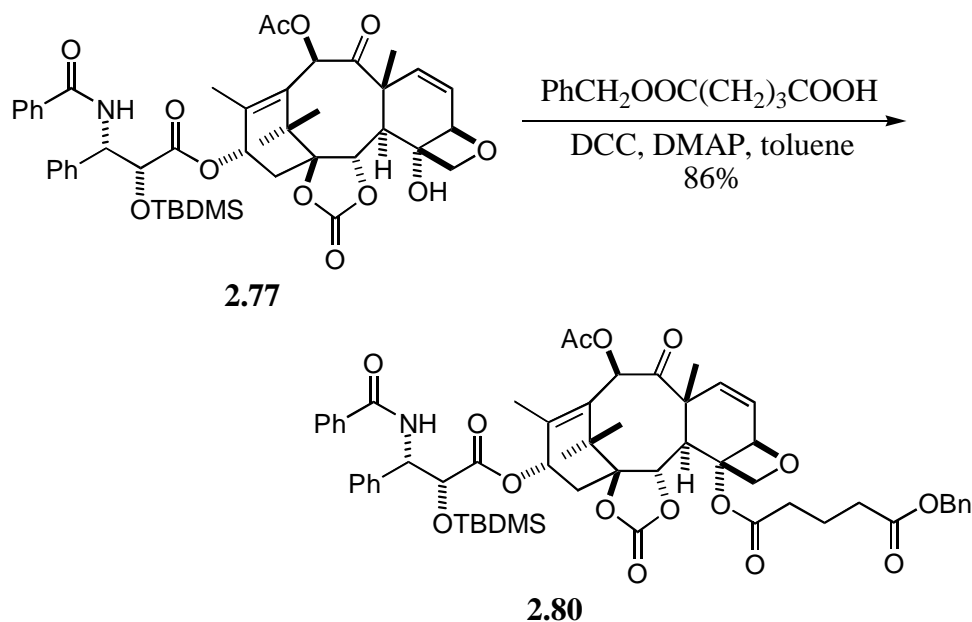
In the reaction of the crude product **2.76** with *N,N'*-carbonyl diimidazole (CDI), the desired product **2.77** and another product **2.78** were isolated in a ratio of about 2.5:1. Compound **2.78** must be formed via a 10-deacetyl intermediate such as **2.79** (Scheme 2.39),¹⁴⁷ whose formation could presumably take place during the Triton-B reaction. The 10-deacetylation has not been observed before in this reaction with 2'-*O*-*tert*-butyldimethylsilyl-7-triethylsilyl-paclitaxel (**2.8b**), but the conformation changes due to the 6,7 olefin in **2.23** may increase the reactivity of the C-10 acetate by decreasing the steric hindrance at this position.

¹⁴⁷ For a similar example, see Datta, A.; Aube, J.; Georg, G. I.; Mitscher, L. A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1831.



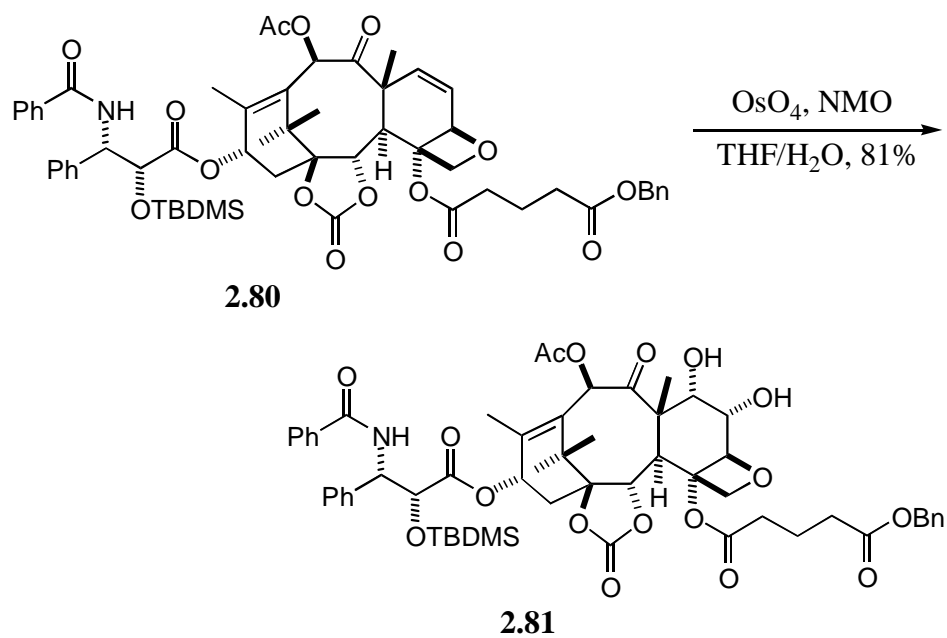
Scheme 2.39 A proposed mechanism for the formation of **2.78**

Having converted **2.23** to **2.77** in two steps in a reasonably good yield (40%), the C-4 acylation reaction was effected using the acid/DCC/DMAP conditions. As expected, compound **2.77** was successfully acylated with monobenzyl glutaric acid in 24 hours, affording the new compound **2.80** in 86% yield. Again, the polarity of **2.80** was almost the same as that of the starting material. The ^1H NMR spectrum of **2.80** showed the presence of a broad singlet for the benzyl protons at 5.08 ppm, along with methylenic protons of the glutaryl moiety at about 2.74-1.78 ppm (Scheme 2.40). It is noteworthy that purification of compound **2.80** was difficult due to the excess DCC used for the coupling. However, a rough column chromatography on silica gel provided a mixture containing DCC/DCU that was suitable for the next dihydroxylation reaction, in which a much more polar product was expected to form and was easier to purify.



Scheme 2.40 C-4 acylation of the 6,7 olefinic substrate **2.77**

The C-4 acylated olefin **2.80** was then treated with osmium tetroxide (OsO_4) and N-methylmorpholine-N-oxide (NMO) in THF/ H_2O for 24 hours, affording a more polar product in 81% yield (Scheme 2.41). ^1H NMR data of the product **2.81** was typical for the desired transformation in that the signal of the C-6 olefinic proton at 6.15 ppm shifted to 4.22 ppm, and the signal of the C-7 olefinic proton at 6.02 ppm shifted to 3.78 ppm; both of these changes in chemical shift were similar to those observed in the known dihydroxylation of 2'-*O*-*tert*-butyl-dimethylsilyl-6,7-dehydro-paclitaxel (**2.23**). The chemical shift of the C-10 proton also changed from 6.30 ppm in compound **2.80** to 6.73 ppm in compound **2.81**, which is characteristic for α dihydroxylation of the 6,7-olefinic paclitaxel derivatives.



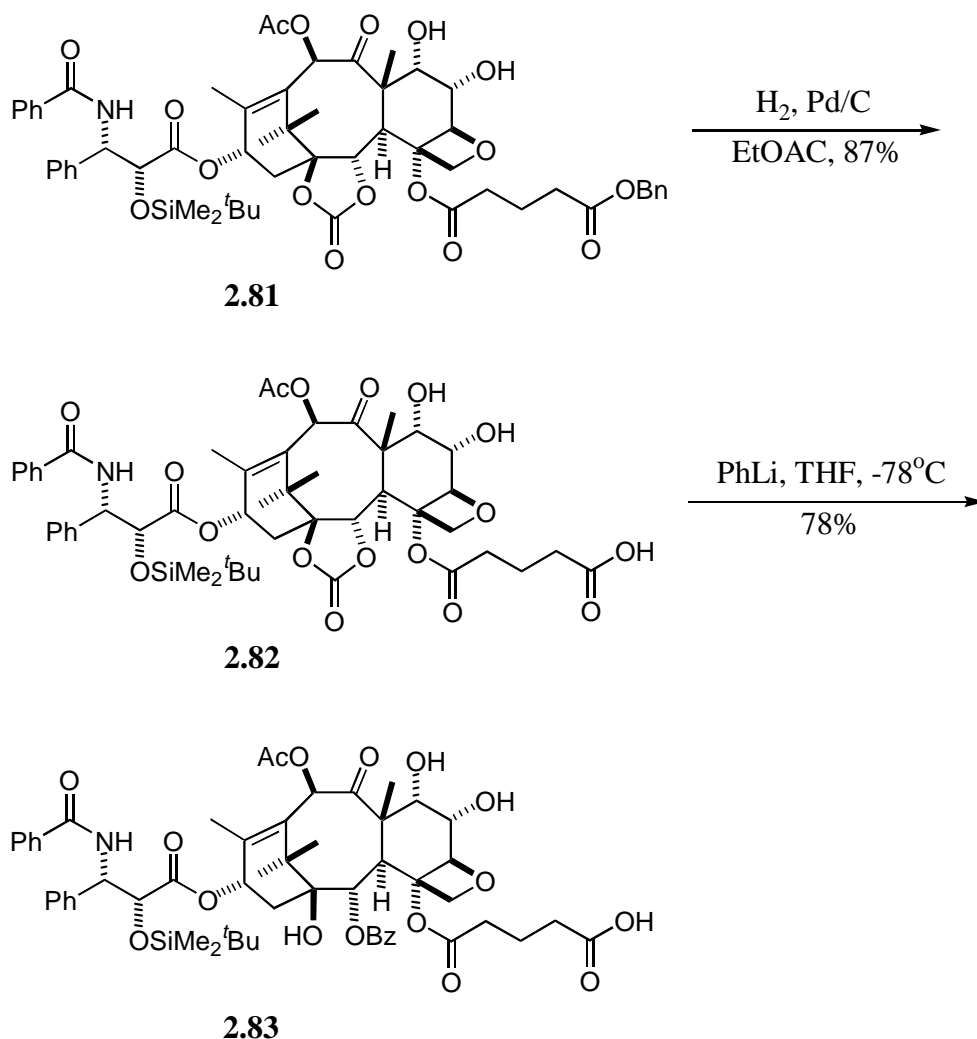
Scheme 2.41 α dihydroxylation of the C-4 acylated olefin **2.80**

2.5.4 Synthesis of a Hydroxyl Acid: the Precursor for a C-4 and C-6 Bridged Paclitaxel Analog

It was envisioned that the reaction involving the bridge formation between C-4 and C-6 would be the most critical and difficult step, and it would thus be beneficial to leave that step to the very last of the synthesis. The C-2 benzylation was therefore the next transformation of choice. As discussed in Section 2.4.4, direct C-2 benzylation using phenyl lithium in the presence of a reactive benzyl ester at C-4 failed due to the selectivity problem. The indirect approach using lithium hydroxide to hydrolyze the 1,2 carbonate followed by benzoic acid coupling reaction should work but is cumbersome for the substrate **2.81**, because that approach necessitates the protection of the more reactive C-6 α hydroxyl group and deprotection afterwards.

Because carbonyl groups in esters and acids have different reactivity toward nucleophilic reactions, regioselective reactions have been achieved for many substrates containing both types of carbonyl group using organo-lithium or Grignard reagents. It was postulated that a terminal acid at the C-4 position of compound **2.81** instead of the benzyl ester might change the reactivity sequence of the carbonyl groups in the molecule so that selective C-2 benzylation could be achieved in a direct manner.

Compound **2.81** was thus hydrogenated to afford the acid **2.82** in 87% yield. Reaction of **2.82** in THF at -78 °C with 10 equivalents of phenyl lithium gave a new product. The starting material and the product of the reaction were very hard to resolve on TLC plates, but the formation of the product was easily indicated with vanillin visualization after development. The product displayed a green color which was distinctive for a paclitaxel analog bearing a C-2 benzoyl or aryl group, while the 1,2 carbonate displayed a gray-brown color (Scheme 2.42).



Scheme 2.42 Direct re-benzoylation at the C-2 position

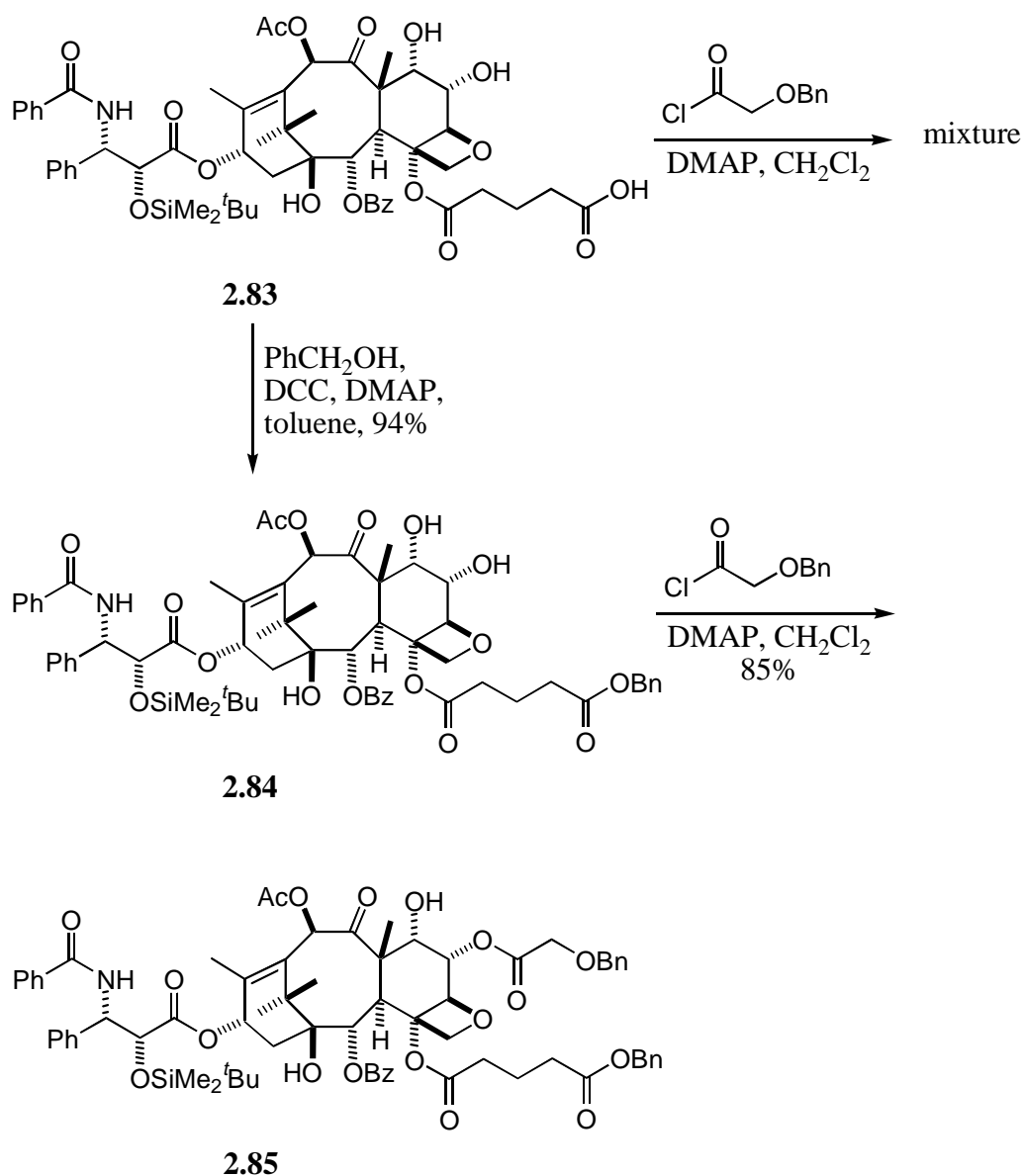
The ¹H NMR spectrum of compound **2.83** showed the characteristic shift of the C-2 proton from 4.63 ppm to 5.74 ppm, similar to that described in Section 2.4.4. The appearance of the two *ortho*-protons at 8.13 ppm also assured the presence of the C-2 benzoyl group. This direct C-2 benzoylation reaction sequences should also be applicable to the compound **2.59** and other analogs of **2.81** with different lengths of the acid chain at C-4.

A preliminary molecular modeling study using MacSpartan's Sybyl level geometry optimization and energy minimization suggested that the best linker between

the C-6 α hydroxyl group and the C-4 glutarate would be a 2-3 carbon chain.¹⁴⁸ During the modeling studies, it was found that certain chains such as unsaturated di-acids or phenylene dialkyl di-acids at the C-4 position might be better than the glutaric acid in both terms of entropy (less degree of freedom) and product stability (less trans-annular interactions). Because of the unavailability of the appropriate reagents and the problems encountered in the C-4 acylation using these reagents, it was decided to simplify the question by choosing glutaric acid as the acyl source at C-4.

2-Benzyloxy acetyl chloride was thus selected as the linker, and it was attached to the C-6 α hydroxyl group of the compound **2.83** in methylene chloride and DMAP. The C-4 acid also reacted with the acid chloride to give an intermediate mixed anhydride which was then hydrolyzed during aqueous workup. Other side products were also formed in the reaction. Furthermore, due to the presence of excess 2-benzyloxy acetyl chloride used in the reaction, the desired product was difficult to isolate from the reaction mixture. To overcome this problem, the C-4 acid in compound **2.83** was masked again as its benzyl ester (**2.84**) using benzyl alcohol under DCC/DMAP conditions to give **2.84** in 94% yield. The formation of compound **2.84** was evidenced by the appearance of the two benzylic protons as a broad singlet at about 5.08 ppm, as well as the great decrease in polarity of compound **2.84**. The reaction of the protected acid **2.84** with 2-benzyloxy acetyl chloride proceeded smoothly and cleanly in a yield of 85% (Scheme 2.43).

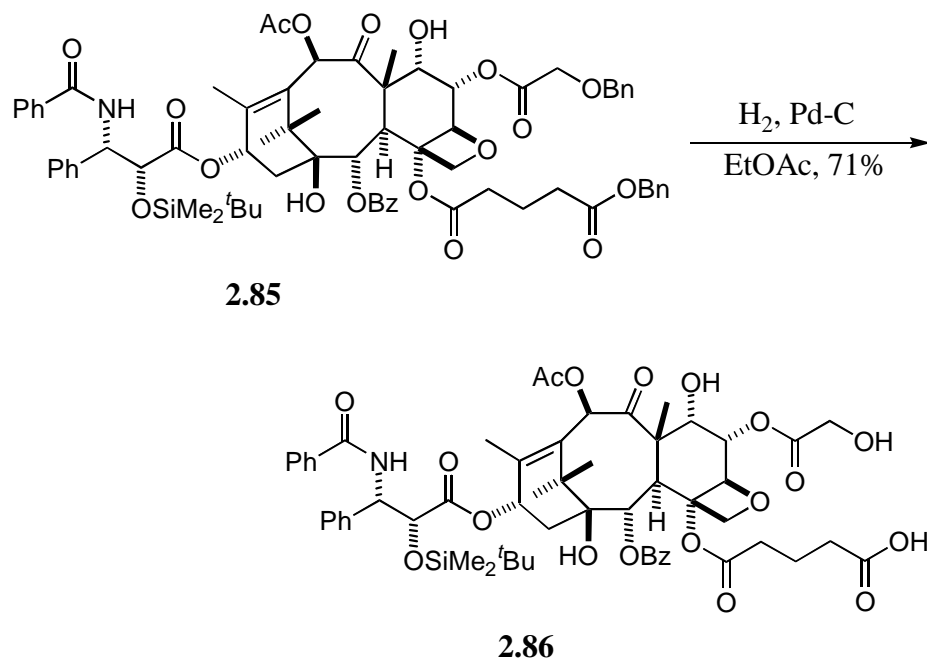
¹⁴⁸ Various hypothetical bridged analogs were examined by changing the linker between C-4 and C-6 as well as the C-4 acyl moiety, the minimized energy was compared to determine the relative stability of the analogs.



Scheme 2.43 Attachment of the linker at the C-6 α hydroxyl group

In the ^1H NMR spectrum of compound **2.85**, the resonance of the C-6 proton shifted from 4.17 ppm in **2.84** to 5.39 ppm, as expected for C-6 esterification. The presence of a broad singlet for two protons at 4.62 ppm corresponding to the benzylic protons of the benzyl ether, and a broad singlet at 4.16 ppm for two protons corresponding to the β -protons of the benzyloxy acetic ester, confirmed the attachment of the linker at the C-6 position. Hydrogenation of compound **2.85** unmasked both of the

benzyl protection groups and afforded the key intermediate, the ω -hydroxy acid **2.86** in 71% yield (Scheme 2.44).



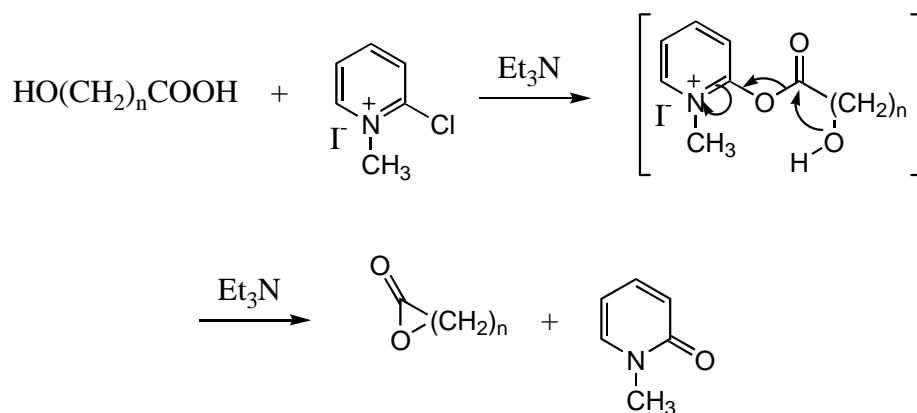
Scheme 2.44 The preparation of the ω -hydroxy acid **2.86**

2.5.5 The preparation of the C-4 C-6 Bridged Paclitaxel via Macrolactonization

There are a large number of natural products that possess a macrocyclic lactone structure. Many of them have displayed interesting biological properties, which has accelerated the development of numerous synthetic methods for the construction of macrocyclic lactones.

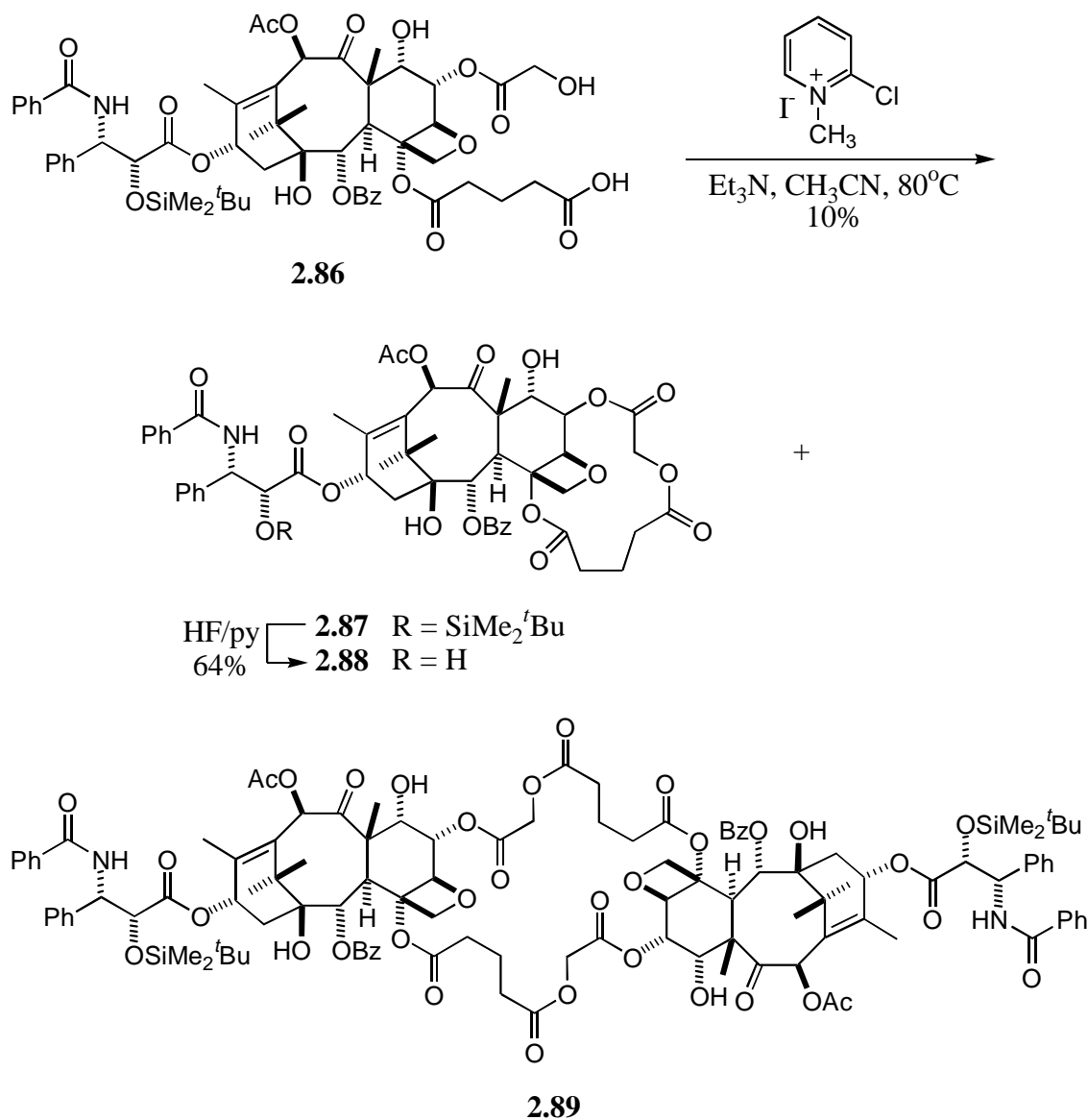
Beside general strategies such as high dilution method, more advanced synthetic approaches require activation of the carboxyl group or the hydroxyl group or both. Among well studied agents, 2-chloro-1-methylpyridinium iodide has been used in the synthesis of various natural products to bring about ring closure reactions of ω -hydroxy acids. The method is based on having a good leaving group with stabilization of the final step transition state (Scheme 2.45).¹⁴⁹ The reaction also forms dilactones, and the yield of lactone increases with ring size whereas that of dilactone decreases. According to a systematic study on a straight chain hydroxy acid, the ratio of the yield of a 13 member ring lactone to that of a 26 member ring dilactone was about 69:14. Considering the complexity of the hydroxy acid **2.86**, and the mildness of the Mukaiyama conditions, 2-chloro-1-methylpyridinium iodide was selected as the activating agent to promote the key macrocyclization.

¹⁴⁹ Mukaiyama, T.; Usui, M.; Saigo, K. *Chem. Lett.* **1976**, 49.



Scheme 2.45 Cyclization of ω -hydroxy acids by 2-chloro-1-methylpyridinium iodide in the presence of triethylamine

Thus, a solution of the hydroxy acid **2.86** and triethylamine in acetonitrile was added slowly via a syringe pump into a solution of acetonitrile containing 2-chloro-1-methylpyridinium iodide refluxing at 80 °C over 8 hours. After completion of the addition, the solution was kept refluxing for another hour. TLC analysis indicated the formation of two less polar products, with $R_f = 0.50$ and 0.70 (EtOAc:hexanes 1:1), along with starting material and other minor products that gave a series of spots at various R_f values. The product with R_f value 0.5 was isolated in about 10% yield, and was identified by ^1H and ^{13}C NMR as the desired lactone (Scheme 2.46). The other product with R_f value 0.7 was also isolated in a yield of 12%, and it was tentatively identified as the dilactone **2.89** because its ^1H NMR displayed a pair of peaks for each of the proton resonances.



Scheme 2.46 Macrolactonization of the hydroxy acid **2.86**

The ¹H NMR spectrum of compound **2.87** was essentially the same as that of **2.86**, except that the two β protons of the hydroxyacetyl group at the C-6 position shifted downfield and were split into a pair of doublets. This downfield shift is in accordance with the expected lactonization reaction, as is the observed splitting of the two protons (Figure 2.21). In compound **2.86**, the two β protons of the hydroxyacetyl group at the C-6 position were almost equivalent due to the free rotation of the chain, whereas in lactone **2.87** they experienced different electron-magnetic environments because of the rigidity of

3. Conclusions

Chemical studies on paclitaxel have been performed in several areas of the molecule during this work. New paclitaxel analogs modified on the A-ring, the C-ring, and the C-4 position have been synthesized in order to establish or extend the structure-activity relationship (SAR) information for these regions. Analogs possessing desired physical or chemical properties have also been prepared through newly developed chemistry, and they serve as important materials for various biological studies.

Previous studies indicated that *A-nor*paclitaxel displayed similar anti-cancer activity to that of paclitaxel, and modifications at the C-1 substituent had a greater influence on the activity than those at the C-2 position, which is believed to have direct interaction with microtubules. New paclitaxel analogs modified at the C-1 substituent were thus synthesized, as well as a novel B-ring expanded *A-nor*paclitaxel and a C-ring contracted *A-nor*paclitaxel analog. Biological evaluation of these new *A-nor*paclitaxel analogs revealed that most of the C-1 modified *A-nor*paclitaxel analogs were about 10 fold less active than paclitaxel. This suggests that the volume of the C-1 substituent rather than its functionality plays a role in determining the anticancer activity. A direct interaction between the C-1 substituent and microtubules is likely to be trivial due to the fact that the C-1 substituent is located on the top face of the molecule, which is opposite to the side chain, the C-2, and the C-4 groups that have been well established as the key functionalities (southern functional groups). The ideal disposition of these microtubule-interacting groups requires the right rigid conformation of the taxane ring system. Interestingly, the novel B-ring expanded *A-nor*paclitaxel analog, namely, 1-deisopropenyl-1-acetyl-8,9-oxido-*A-nor*paclitaxel (**2.16**) was found to be slightly more active than paclitaxel, while the corresponding *A-nor* counterpart 1-deisopropenyl-1-acetyl-*A-nor*paclitaxel (**2.15**) was about 10 fold less active than paclitaxel. It seems that the smaller 1-acetyl group causes divergence of the southern functional groups from the optimum position, and the insertion of a oxygen between C-8 and C-9 rectifies this

deviation. Indeed, a molecular modeling study clearly showed that the southern functional groups of 1-deisopropenyl-1-acetyl-8,9-oxido-A-norpaclitaxel overlapped nicely with that of paclitaxel (Figure 2.8).

Chemistry at the C-6 and C-7 positions has been elaborated, in an effort to prepare paclitaxel analogs with enhanced activity and water solubility. Both diastereomers of paclitaxel 6 α ,7 α -cyclicsulfite were synthesized, and it was found that the ratio of the two sulfites can be controlled by changing the reaction temperature. Paclitaxel 6 α ,7 α -cyclicsulfate was also synthesized through oxidation of the corresponding sulfite, but it was found to be a poor substrate for nucleophilic substitution reactions due to steric hindrance. This finding helped in the revision of the stereochemistry of 6,7-epoxy-paclitaxel, which had been considered as a versatile intermediate for the synthesis of other paclitaxel analogs but proved to be remarkably unreactive. The desired 6-azido paclitaxel analog was synthesized by an alternative approach in good yield. Hydrogenation of the azido analog was investigated. Agreeing with a literature precedent, hydrogenation of the azido group was difficult and resulted in only partially reduced product. The corresponding amino analog was finally obtained under medium pressure hydrogenation conditions.

Of particular importance, 6 α -hydroxy-paclitaxel (**2.36**), the major human metabolite of paclitaxel, was synthesized for the first time through a C-7 epimerization reaction. *In vivo* biological investigations on the metabolite can now be performed, because they require large quantities of the material that can only be provided by organic synthesis. The availability of the major human metabolite through synthesis also offers an opportunity for the production of standard HPLC samples of paclitaxel metabolites which could be useful in the clinical monitoring of the drug's disposition in human patients.

The C-4 acetate group has been shown to be necessary for paclitaxel's anticancer activity, perhaps by participating directly in binding to microtubules. Previous modifications at the C-4 position have seemed to indicate that analogs with an acyl group other than acetate at C-4 may exert similar or even increased activity to paclitaxel. Little was known, however, on the conformation-activity relationships of the C-4 position. In order to further explore the C-4 chemistry, a mild C-4 acylation method using acid as the

acyl source was successfully developed. The new method was exemplified by the synthesis of water-soluble paclitaxel analogs with hydrophilic functional groups at the terminal of the C-4 acyl moiety. This method should be applicable to a variety of similar carboxylic acids and offer an alternative or even better approach for the preparation of C-4 modified paclitaxel analogs.

In addition to extension of the paclitaxel analog library, specially designed analogs have been sought to probe the active conformation of paclitaxel. An analog that has a bridge to link the C-4 acyl group with an inert position has been developed for this purpose. With successful demonstration of the above C-4 acylation method, combined with well established C-6 chemistry, the synthesis of such a novel C-4 and C-6 bridged paclitaxel analog was completed. It was found to be inactive in tubule assembly assay, however, presumably due to the crowding beneath the C-ring caused by the newly formed macrolactone.

4. Experimental

General Experimental Methods. Unless otherwise noted, all materials were used as received from a commercial supplier without further purification. All anhydrous reactions were performed in oven-dried glassware under argon. THF and diethyl ether were distilled from sodium/benzophenone. Anhydrous toluene was distilled from sodium. Dichloromethane was distilled from calcium hydride. All reactions were monitored by E. Merck analytical TLC plates (silica gel 60 GF, aluminum back) and analyzed with 254 nm UV light and/or vanillin/sulfuric acid spray. Silica gel for column chromatography was purchased from E. Merck (230-400 mesh). Preparative thin layer chromatography (PTLC) plates (silica gel 60 GF) were purchased from Analtech. ^1H and ^{13}C NMR spectra were obtained in CDCl_3 on a Varian Unity 400 spectrometer (operating at 399.951 MHz for ^1H and 100.578 MHz for ^{13}C) or a Bruker WP 360 spectrometer (operating at 360.140 MHz for ^1H and 90.562 MHz for ^{13}C), and were assigned by comparison of chemical shifts and coupling constants with those of related compounds and by appropriate 2D-NMR techniques. All 2D-NMR spectra were obtained on the Varian Unity 400 spectrometer. Chemical shifts were reported as δ -values relative to tetramethylsilane (TMS) as internal reference, and coupling constants were reported in Hertz. Mass spectra (LRFABMS/HRFABMS) were obtained at the Nebraska Center for Mass Spectrometry, University of Nebraska. All samples were purified until homogeneous on TLC in two or three solvent systems before being submitted for biological assays and MS.

2'-O-(tert-Butyldimethylsilyl)-7-O-triethylsilyl-1-deisopropenyl-1-acetyl-A-norpaclitaxel (2.10) - Ozone generated from a micro-ozonizer¹⁵⁰ was carried by oxygen and passed through a solution of 2'-O-(tert-butyldimethylsilyl)-7-O-triethylsilyl)-A-norpaclitaxel (**2.9**, 22 mg, 0.021 mmol) in anhydrous dichloromethane (1 mL) and methanol (1 mL) pre-cooled to -78 °C for 18 minutes. TLC analysis indicated complete conversion of the starting material to a more polar compound. The solution was purged with oxygen gas for 30 minutes. Dimethyl sulfide (Me₂S, 250 μL, excess) was then added at -78 °C and the mixture was warmed up to room temperature and stirred for 30 minutes during which the intermediate was transformed to the final product. The solvent was evaporated under reduced pressure and the residue was purified by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 3:7) to afford 2'-O-(tert-butyldimethylsilyl)-7-O-triethylsilyl-1-deisopropenyl-1-acetyl-A-nor-paclitaxel (**2.10**) (20 mg, 91%) as an amorphous white solid. ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.14 (dd, 2H), 7.67 (dd, 2H), 7.56-7.20 (m, 9H), 6.97 (d, J=9.1, 1H), 6.84 (m, 2H), 6.48 (s, 1H), 5.95 (m, 1H), 5.80 (d, J=9.3, 1H), 5.17 (dd, J=9.1,2.2, 1H), 4.86 (d, J=8.4, 1H), 4.52 (d, J=8.0, 1H), 4.43(d, J=8.0, 1H), 4.43(dd, 1H), 4.25 (d, J=2.2, 1H), 3.21 (d, J=9.3, 1H), 2.57 (m, 1H), 2.55(m, 1H), 2.15 (s, 3H), 2.13 (m, 1H), 1.95 (s, 3H), 1.76 (s, 9H), 0.94 (t, 9H), 0.77 (s, 9H), 0.58 (q, 6H), -0.14 (s, 3H), -0.31 (s, 3H). ¹³C NMR (CDCl₃, TMS, 100.578 MHz) δ 199.1, 171.1, 170.1, 169.5, 169.0, 166.7, 164.6, 147.5, 138.3, 134.3, 133.7, 131.6, 131.6, 130.3, 129.5, 128.6, 128.3, 127.6, 127.0, 126.5, 93.6, 84.4, 79.5, 79.4, 77.2, 75.0, 74.6, 71.9, 71.1, 69.9, 55.7, 54.9, 44.6, 37.6, 37.6, 25.5, 22.0, 21.3, 20.3, 18.2, 12.8, 10.0, 6.9, 5.2, -5.4, -5.8.

2'-O-(tert-Butyldimethylsilyl)-7-O-triethylsilyl-1-deisopropenyl-1-acetoxy-A-norpaclitaxel (2.11) and 2'-O-(tert-butyldimethylsilyl)-7-O-triethylsilyl-1-deisopropenyl-1-acetyl-11,12-epoxy-A-norpaclitaxel (2.12) - A solution of 2'-O-(tert-butyldimethylsilyl)-7-O-triethylsilyl-1-deisopropenyl-1-acetyl-A-nor-paclitaxel (**2.10**, 250 mg, 0.235 mmol), *meta*-chloroperoxybenzoic acid (57-86%, 175 mg, ~0.7 mmol), and sodium bicarbonate (44 mg, 0.52 mmol) in dichloromethane was stirred at room

¹⁵⁰ Beroza, M.; Bierl, B. A. *Mikrochimica Acta* [Wien] **1969**, 720.

temperature for 18 hours. The reaction mixture was diluted with EtOAc and washed with saturated sodium sulfite (Na_2SO_3), water, and brine. The organic layer was combined and dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000 μ , EtOAc:hexanes 3:7) to afford 2'-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-1-deisopropenyl-1-acetoxy-*A*-norpaclitaxel (**2.11**, 151 mg, 69%) and 2'-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-1-deisopropenyl-1-acetyl-11,12-epoxy-*A*-norpaclitaxel (**2.12**, 35 mg, 16%).

2'-*O*-(*tert*-Butyldimethylsilyl)-7-*O*-triethylsilyl-1-deisopropenyl-1-acetoxy-*A*-norpaclitaxel (2.11**)** ^1H NMR (CDCl_3 , TMS, 399.951 MHz) δ 8.14 (d, $J=7.2$, 2H), 7.67 (d, $J=7.2$, 2H), 7.56-7.20 (m, 9H), 6.97 (d, $J=8.8$, 1H), 6.84 (m, 2H), 6.48 (s, 1H), 5.99 (m, 1H), 5.80 (d, $J=9.6$, 1H), 5.17 (d, $J=8.4$, 1H), 4.86 (d, $J=9.2$, 1H), 4.52 (d, $J=8.8$, 1H), 4.43(d, $J=8.4$, 1H), 4.43(d, 1H), 4.25 (d, $J=2.0$, 1H), 3.21 (d, $J=10.0$, 1H), 2.57-2.55 (m, 2H), 2.15 (s, 3H), 2.11 (m, 1H), 1.95 (s, 3H), 1.76 (s, 3H), 1.75 (s, 2CH₃), 0.94 (t, 9H), 0.78 (s, 9H), 0.58 (q, 6H), -0.14 (s, 3H), -0.31 (s, 3H). ^{13}C NMR (CDCl_3 , TMS, 100.578 MHz) δ 199.1, 171.1, 170.1, 169.5, 169.0, 166.7, 164.6, 147.5, 138.3, 134.3, 133.7, 131.6, 131.6, 130.3, 129.5, 128.6, 128.3, 127.6, 127.0, 126.5, 93.6, 84.4, 79.5, 79.4, 77.2, 75.0, 74.6, 71.9, 71.1, 69.9, 55.7, 54.9, 44.6, 37.6, 37.6, 25.5, 22.0, 21.3, 20.3, 18.2, 12.8, 10.0, 6.9, 5.2, -5.4, -5.8.

2'-*O*-(*tert*-Butyldimethylsilyl)-7-*O*-triethylsilyl-1-deisopropenyl-1-acetyl-11,12-epoxy-*A*-norpaclitaxel (2.12**)** ^1H NMR (CDCl_3 , TMS, 399.951 MHz) δ 8.09 (d, $J=7.2$, 2H), 7.69 (d, $J=7.2$, 2H), 7.56-7.24 (m, 11H), 7.16 (d, $J=8.8$, 1H, NH), 6.16 (d, $J=10.4$, 1H), 5.76 (s, 1H), 5.51 (dd, $J=8.4, 2.1$, 1H), 5.30 (t, $J=8.0$, 1H), 5.07 (d, $J=8.8$, 1H), 4.60 (dd, $J=10.0, 6.8$, 1H), 4.44 (d, $J=2.0$, 1H), 4.39 (d, $J=8.4$, 1H), 4.26(d, $J=8.4$, 1H), 3.95 (d, $J=10.4$, 1H), 2.59 (m, 1H), 2.34 (s, 3H), 2.32 (s, 3H), 2.17 (m, 1H), 2.10(s, 3H), 1.89 (m, 1H), 1.74 (m, 1H), 1.64 (s, 3H), 1.19 (s, 3H), 0.91 (t, 9H), 0.73 (s, 9H), 0.55 (q, 6H), -0.30 (s, 3H), -0.45 (s, 3H). ^{13}C NMR (CDCl_3 , TMS, 100.578 MHz) δ 199.1, 171.1, 170.1, 169.5, 169.0, 166.7, 164.6, 147.5, 138.3, 134.3, 133.7, 131.6, 131.6, 130.3, 129.5, 128.6, 128.3, 127.6, 127.0, 126.5, 93.6, 84.4, 79.5, 79.4, 77.2, 75.0, 74.6, 71.9, 71.1, 69.9, 55.7, 54.9, 44.6, 37.6, 37.6, 25.5, 22.0, 21.3, 20.3, 18.2, 12.8, 10.0, 6.9, 5.2, -5.4, -5.8.

1-Deisopropenyl-1-acetoxyl-A-norpaclitaxel (2.13) - A solution of 2',7-*O*-bis-triethylsilyl-1-deisopropenyl-1-acetoxyl-A-norpaclitaxel (**2.11**, 20 mg, 0.019 mmol) in 3% HCl/MeOH (1.0 mL) was stirred at room temperature for 2 hours. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate, the organic layer was combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000 μ , i-PrOH:CH₂Cl₂:hexanes 6:47:47) to afford 1-deisopropenyl-1-acetoxyl-A-norpaclitaxel (**2.13**, 12.5 mg, 79%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.04 (dd, J=8.0,2.0, 2H), 7.64 (d, J=7.2, 2H), 7.48-7.25 (m, 9H), 7.02 (dd, J=8.0,2.0, 2H), 6.62 (d, J=8.8, 1H), 6.24 (s, 1H), 5.87 (br d, J=8.4, 1H), 5.76 (d, J=10.0, 1H), 5.08 (dd, J=8.8,2.8, 1H), 4.84 (d, J=9.2, 1H), 4.68 (d, J=8.8, 1H), 4.46 (d, J=8.8, 1H), 4.42 (m, 1H), 4.35 (dd, J=6.0,2.8, 1H), 3.29 (d, J=6.0, 1H), 3.14 (d, J=10.4, 1H), 2.60 (m, 1H), 2.57 (m, 1H), 2.39 (d, J=3.6, 1H), 2.21 (m, 1H), 2.20 (s, 3H), 1.95 (s, 3H), 1.91 (m, 1H), 1.79 (s, 3H), 1.78 (s, 3H), 1.39 (s, 3H). ¹³C NMR (CDCl₃, TMS, 100.578 MHz) δ 202.1, 172.9, 171.7, 170.1, 168.8, 166.8, 164.6, 46.4, 137.6, 133.8, 133.7, 131.8, 129.9, 129.5, 129.3, 128.61, 128.60, 128.5, 128.0, 127.2, 126.9, 93.4, 84.7, 81.3, 79.7, 74.4, 72.7, 71.7, 71.4, 70.5, 55.15, 55.12, 44.5, 37.1, 34.7, 22.1, 21.0, 20.5, 13.4, 9.6. HRFABMS calculated for C₄₆H₄₇NO₁₅ (M+H)⁺ 854.3024, found 854.3024, error 0.0 ppm.

1-Deisopropenyl-1-acetyl-11,12-epoxy-A-norpaclitaxel (2.14) - To a solution of 2'-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-1-deisopropenyl-1-acetyl-11,12-epoxy-A-norpaclitaxel (**2.12**, 6.8 mg, 0.0063 mmol) in THF (0.3 mL) was added HF-pyridine (70%, 100 μ L) and the solution was stirred at room temperature for 3 hours. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate, the organic layer was combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 500 μ , EtOAc:hexanes 6:4) to afford 1-deisopropenyl-1-acetyl-11,12-epoxy-A-norpaclitaxel (**2.14**, 3.5 mg, 65%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.12 (d, J=7.2, 2H), 7.67-7.24 (m, 13H), 6.67 (d, J=8.8, 1H), 6.23 (d, J=11.2, 1H), 5.84 (s,

1H), 5.44 (dd, J=8.0,2.4, 1H), 5.22 (t, J=8.0, 1H), 5.05 (d, J=9.6, 1H), 4.69 (m, 1H), 4.62 (br s, 1H), 4.35 (br s, 2H), 3.83 (d, J=11.2, 1H), 2.62 (m, 1H), 2.35 (s, 3H), 2.21 (s, 3H), 2.20 (m, 1H), 2.19 (s, 3H), 1.93 (m, 1H), 1.79 (m, 1H), 1.59 (s, 3H), 1.53 (s, 3H). HRFABMS calculated for C₄₆H₄₇NO₁₅ (M+H)⁺ 854.3024, found 854.3001, error 2.7 ppm.

1-Deisopropenyl-1-acetyl-8,9-oxido-A-norpaclitaxel (2.16) - A solution of 1-deisopropenyl-1-acetyl-A-norpaclitaxel (**2.15**, 32 mg, 0.038 mmol) and *meta*-chloroperoxybenzoic acid (mCPBA, 70%, 83 mg) in CH₂Cl₂ (0.3 mL) was stirred at room temperature for 48 hours. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate, the organic layer was combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 7:3) to afford a mixture of two products, which was further separated by preparative TLC (silica gel, 1000μ, MeOH:CH₂Cl₂ 5:95) to afford 1-deisopropenyl-1-acetyl-8,9-oxido-A-norpaclitaxel (**2.16**, 22 mg, 69%) and 1-deisopropenyl-1-acetoxyl-A-norpaclitaxel (**2.13**, 6.0 mg, 19%).

1-deisopropenyl-1-acetyl-8,9-oxido-A-norpaclitaxel (2.16) ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 7.91 (d, J=7.2, 2H), 7.84 (d, J=7.2, 2H), 7.60-7.32 (m, 11H), 7.21 (d, J=8.8, 1H, NH), 6.65 (d, J=6.4, 1H, H-2), 6.00 (m, 1H, H-13), 5.81 (s, 1H, H-10), 5.80 (m, 1H, H-3'), 4.90 (d, J=8.8, 1H, H-5), 4.75 (dd, J=2.4,2.4, 1H, H-2'), 4.43 (d, J=8.4, 1H, H-20), 4.20-4.12 (m, 3H, H-7, 2'-OH, and H-20), 2.95 (m, 2H, H-3 and H-14), 2.63 (m, 1H, H-6), 2.47 (br s, 1H, 7-OH), 2.26 (s, 6H, 2CH₃), 2.07 (s, 3H), 2.02 (s, 3H), 1.94-1.84 (m, 2H, H-14 and H-6), 1.53 (s, 3H, CH₃). ¹³C NMR (CDCl₃, TMS, 100.578 MHz) δ 204.8, 171.9, 171.2, 169.5, 169.3, 166.6, 165.1, 149.2, 138.5, 133.72, 133.67, 132.0, 131.8, 129.7, 129.1, 128.8, 128.71, 128.68, 128.2, 127.1, 127.0, 92.5, 84.3, 82.1, 80.5, 74.5, 74.3, 72.9, 71.3, 69.2, 67.6, 55.0, 48.6, 35.5, 34.8, 25.5, 22.3, 20.2, 14.2, 12.4. HRFABMS calculated for C₄₆H₄₇NO₁₅ (M+Na)⁺ 876.2843, found 876.2819, error 2.8 ppm.

Ozonolysis of 2',7-O-bis-triethylsilyl-A-norpaclitaxel (2.6) in methylene chloride - Ozone generated from micro-ozonizer was carried by oxygen and passed through a solution of 2'-O-(*tert*-butyldimethylsilyl)-7-O-triethylsilyl-A-norpaclitaxel (**2.6**, 237 mg, 0.22 mmol) in anhydrous dichloromethane (10 mL) pre-cooled to -78 °C for 12 minutes. The solution was purged with oxygen gas for 30 minutes. Dimethyl sulfide (Me₂S, 1 mL, excess) was then added at -78 °C and the mixture was warmed up to room temperature and stirred for 30 minutes. The solvent was evaporated under reduced pressure and the residue was purified by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 3:7) to afford 2',7-O-bis-triethylsilyl-1-deisopropenyl-1-acetyl-A-nor-paclitaxel (**2.7**, 124 mg, 52%), 2',7-O-bis-triethylsilyl-1-deisopropenyl-1-acetoxy-A-nor-paclitaxel (**2.18**, 25 mg, 11%), and 7-O-triethylsilyl-1-deisopropenyl-A-nor-paclitaxel (**2.19**, 11 mg, 5%).

7-O-Triethylsilyl-1-deisopropenyl-A-nor-paclitaxel (2.19). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 7.99 (dd, J=8.0,1.6, 2H), 7.65 (dd, J=8.8,1.6, 2H), 7.47-7.21 (m, 9H), 6.90 (dd, J=8.0, 1.6, 2H), 6.59 (br s, 1H), 6.51 (d, J=8.8, 1H), 5.61 (m, 2H), 4.98 (dd, J=8.8,2.8, 1H), 4.79 (d, J=8.4, 1H), 4.70 (d, J=8.4, 1H), 4.48 (m, 2H), 3.98 (s, 1H), 3.16 (br d, J=3.2, 1H), 3.02 (d, J=9.6, 1H), 2.95 (m, 1H), 2.54 (m, 1H), 2.41 (m, 1H), 2.17 (s, 3H), 1.84 (m, 1H), 1.73 (s, 3H), 1.66 (m, 1H), 1.59 (s, 3H), 1.33 (s, 3H), 0.92 (t, 9H), 0.56 (q, 6H).

1-Deisopropenyl-A-nor-paclitaxel (2.20) - To a solution of 7-O-triethylsilyl-1-deisopropenyl-A-nor-paclitaxel (**2.19**, 10 mg, 0.011 mmol).in dry THF (0.7 mL) was added HF-pyridine (70%, 200 μL) and the solution was stirred at room temperature for 1 hour. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate, the organic layer was combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 6:4) to afford 1-deisopropenyl-A-nor-paclitaxel (**2.20**, 7.5 mg, 86%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.01 (dd, J=8.0,1.6, 2H), 7.65 (dd, J=8.8,1.6, 2H), 7.47-7.19 (m, 9H), 6.90 (dd, J=8.0, 1.6, 2H), 6.49 (d, J=8.8, 1H), 6.27 (s, 1H), 5.64 (m, 2H), 5.00 (dd, J=8.8,2.8, 1H), 4.85 (d, J=8.8, 1H), 4.70 (d, J=8.8, 1H), 4.52 (d, J=8.8, 1H), 4.52 (m, 1H), 4.01 (s, 1H),

3.20 (br s, 1H), 3.08 (d, J=9.6, 1H), 2.92 (m, 1H), 2.56 (m, 1H), 2.41 (m, 1H), 2.21 (s, 3H), 1.88 (m, 1H), 1.84 (s, 3H), 1.70 (m, 1H), 1.61 (s, 3H), 1.29 (s, 3H). ¹³C NMR (CDCl₃, TMS, 100.578 MHz) δ 205.3, 173.1, 171.6, 170.1, 166.8, 165.2, 141.2, 137.8, 133.9, 133.4, 131.7, 130.0, 129.8, 129.6, 128.6, 129.5, 128.4, 127.8, 127.2, 126.9, 84.3, 82.8, 80.0, 74.6, 73.1, 72.5, 70.2, 69.3, 55.2, 54.9, 47.6, 45.5, 34.8, 29.6, 20.8, 20.5, 13.2, 9.4. HRFABMS calculated for C₄₄H₄₅NO₁₃ (M+Na)⁺ 818.2789, found 818.2791, error - 0.4 ppm.

2'-O-(tert-Butyldimethylsilyl)-A-nor-C-norpaclitaxel (2.26) - To a solution of 2'-O-(tert-butyldimethylsilyl)-C-norpaclitaxel (**2.25**, 36 mg, 0.038 mmol) in dry CH₂Cl₂ (1.5 mL,) was added anhydrous pyridine (72 μL, 0.89 mmol) followed by thionyl chloride (SOCl₂) at room temperature. The solution was stirred for 20 minutes. The reaction mixture was then diluted with EtOAc and washed with dilute HCl (1N). The organic layer was combined and washed with dilute sodium bicarbonate, water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 5:5) to afford 2'-O-(tert-butyldimethylsilyl)-A-nor-C-norpaclitaxel (**2.26**, 20 mg, 57%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.15 (d, J=7.6, 2H, ArH), 7.82 (d, J=7.2, 2H, ArH), 7.56-7.29 (m, 11H, ArH), 7.09 (d, J=9.2, 1H, 3'-NH), 6.74 (d, J=9.6, 1H, H-2), 6.04 (t, J=8.8, 1H, H-13), 5.98 (s, 1H, H-10), 5.73 (d, J=9.2, 1H, H-3'), 5.19 (d, J=7.2, 1H, H-5), 5.02 (s, 1H, H-16), 4.84 (d, J=10.4, 1H, H-20), 4.78 (s, 1H, H-16), 4.68 (d, J=7.2, 1H, H-6), 4.56 (d, J=2.4, 1H, H-2'), 4.41 (d, J=10.4, 1H, H-20), 3.38 (d, J=9.6, 1H, H-3), 2.30 (m, 1H, H-14), 2.19 (m, 1H, H-14), 2.13 (s, 3H, 4-OAc), 2.07 (s, 3H, 10-OAc), 1.98 (s, 3H, 19-CH₃), 1.80 (s, 3H, 18-CH₃), 1.57 (s, 3H, 16-CH₃), 0.77 (s, 9H, SiMe₂^tBu), -0.11 (s, 3H, SiMe₂^tBu), -0.33 (s, 3H, SiMe₂^tBu). ¹³C NMR (CDCl₃, TMS, 100.578 MHz) δ 201.8, 170.8, 170.3, 168.8, 166.5, 165.5, 146.3, 143.1, 138.7, 134.6, 134.1, 133.5, 131.6, 130.2, 129.3, 128.7, 128.6, 128.5, 127.7, 127.0, 126.6, 111.2, 87.8, 81.1, 79.7, 78.4, 77.2, 75.6, 73.0, 69.9, 62.8, 58.1, 55.6, 44.9, 39.2, 25.5, 21.5, 20.48, 20.44, 18.2, 11.9, 11.7, -5.6, -6.1.

A-nor-C-norpaclitaxel (2.27) - To a solution of 2'-*O*-(*tert*-butyldimethylsilyl)-*A-nor-C-norpaclitaxel* (**2.26**, 18 mg, 0.019 mmol) in dry THF (1 mL) was added HF-pyridine (70%, 240 μ L) and the solution was stirred at room temperature for 1.5 hours. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate, the organic layer was combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000 μ , EtOAc:hexanes 6:4) to afford *A-nor-C-norpaclitaxel* (**2.27**, 12 mg, 76%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.10 (d, J=8.4, 2H), 7.81 (dd J=8.0,1.6, 2H), 7.57-7.27 (m, 11H), 7.06 (d, J=9.6, 1H, NH), 6.99 (s, 1H, H-10), 6.74 (d, J=9.6, 1H, H-2), 5.96 (m, 1H, H-13), 5.94 (s, 1H, H-10), 5.80 (br d, J=9.2, 1H, H-3'), 5.14 (d, J=4.8, 1H, H-5), 5.01 (s, 1H, H-16), 4.80 (m, 2H, H-16 and H-20), 4.68 (dd, J=3.6,2.0, 1H, H-2'), 4.57 (m, 1H, H-6), 4.40 (d, J=8.4, 1H, H-20), 3.65 (d, J=3.6, 1H, 2'-OH), 3.35 (d, J=9.6, 1H, H-3), 2.50 (d, J=6.8, 1H, 6-OH), 2.43 (dd, J=15.2,8.0, 1H, H-14), 2.19 (dd, J=15.2,6.0, 1H, H-14), 2.07 (br s, 6H, 4-OAc and 10-OAc), 1.95 (s, 3H, CH₃), 1.65 (s, 3H, CH₃), 1.58 (s, 3H, CH₃). ¹³C NMR (CDCl₃, TMS, 100.578 MHz) δ 201.7, 172.1, 170.5, 168.8, 166.4, 165.6, 146.1, 142.5, 138.5, 134.9, 134.0, 133.6, 131.8, 130.0, 129.3, 128.8, 128.7, 128.6, 128.0, 127.0, 126.8, 111.3, 87.8, 81.5, 81.3, 78.3, 76.9, 73.9, 73.0, 69.8, 63.3, 58.2, 54.6, 44.9, 39.4, 21.5, 20.45, 20.43, 11.9, 11.7. HRFABMS calculated for C₄₇H₅₁NO₁₅ (M+H)⁺ 822.3126, found 822.3129, error -0.4 ppm.

2'-*O*-(*tert*-Butyldimethylsilyl)-6 α -hydroxy-paclitaxel (2.34) - To a solution of 2'-*O*-(*tert*-butyldimethylsilyl)-6 α -hydroxy-7-*epi*paclitaxel (**2.24**, 20.3 mg, 0.021 mmol) in xylenes (0.8 mL, distilled from calcium hydride) was added 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU, 105 μ L, 0.70 mmol) and the mixture was stirred at 80 °C for 2 hours. The reaction mixture was then diluted with EtOAc and washed with dilute HCl (1N). The organic layer was combined and washed with dilute sodium bicarbonate, water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000 μ , EtOAc:hexanes 6:4) to afford 2'-*O*-(*tert*-butyldimethylsilyl)-6 α -hydroxy-paclitaxel (**2.34**,

3.0 mg, 15%) and 2'-*O*-(*tert*-butyldimethylsilyl)-6 α -hydroxy-7-epipaclitaxel (**2.24**, 16.9 mg, 83%).

2'-*O*-(*tert*-butyldimethylsilyl)-6 α -hydroxy-paclitaxel (2.34**)** ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.13 (d, J=8.0, 2H), 7.73 (d, J=8.0, 2H), 7.62-7.32 (m, 11H), 7.07 (d, J=10.0, 1H, NH), 6.28 (s, 1H, H-10), 6.28 (m, 1H, H-13), 5.74 (br d, J=9.6, 1H, H-3'), 5.68 (d, J=8.0, 1H, H-2), 4.86 (s, 1H, H-5), 4.65 (d, J=2.4, 1H, H-2'), 4.34 (d, J=9.2, 1H, H-20), 4.26 (m, 1H, H-7), 4.23 (d, J=9.8, 1H, H-20), 3.98-3.95 (m, 2H, H-6 and H-3), 2.89 (d, J=4.0, 1H, OH), 2.59 (s, 3H, 4-OAc), 2.55 (d, J=4.0, 1H, OH), 2.42 (m, 1H, H-14), 2.24 (s, 3H, 10-OAc), 2.16 (m, 1H, H-14), 1.92 (s, 3H, CH₃), 1.66 (s, 3H, CH₃), 1.24 (s, 3H, CH₃), 1.11 (s, 3H, CH₃), 0.80 (s, 9H), -0.05 (s, 3H), -0.31 (s, 3H).

6 α -Hydroxy-paclitaxel (2.36**)** - A solution of 2'-*O*-(*tert*-butyldimethylsilyl)- 6 α -hydroxy-paclitaxel (**2.34**, 15 mg, 0.015 mmol) in 5% HCl/MeOH (0.5 mL) was stirred at room temperature for 2 hours. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate, the organic layer was combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 500 μ , EtOAc:hexanes 8:2) to afford 6 α -hydroxy-paclitaxel (**2.36**, 9.5 mg, 72%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.12 (d, J=8.0, 2H), 7.73 (d, J=7.2, 2H), 7.62-7.35 (m, 11H), 7.01 (d, J=8.8, 1H, NH), 6.26 (s, 1H, H-10), 6.23 (t, J=8.4, 1H, H-13), 5.77 (dd, J=8.4,2.0, 1H, H-3'), 5.66 (d, J=6.8, 1H, H-2), 4.82 (s, 1H, H-5), 4.78 (dd, J=9.2, 2.4, 1H, H-2'), 4.30 (d, J=8.4, 1H, H-20), 4.20 (d, J=8.4, 1H, H-20), 4.20 (d, J=8.4, 1H, H-7), 3.93 (m, 2H, H-6 and H-3), 3.66 (d, J=5.2, 1H, 2'-OH), 3.02 (d, J=3.6, 1H, 7-OH), 2.75 (br s, 1H, 6-OH), 2.39 (s, 3H, 4-OAc), 2.32 (m, 2H, H-14), 2.23 (s, 3H, 10-OAc), 1.90 (br s, 1H, OH), 1.82 (s, 3H, 18-CH₃), 1.64 (s, 3H, 19-CH₃), 1.23 (s, 3H, 17-CH₃), 1.12 (s, 3H, 16-CH₃). ¹³C NMR (CDCl₃, TMS, 100.578 MHz) δ 202.4, 172.8, 171.3, 170.6, 167.1, 166.9, 142.3, 137.9, 133.8, 133.6, 133.1, 130.2, 129.0, 128.8, 128.7, 128.4, 127.1, 127.0, 91.5, 82.8, 79.0, 77.2, 76.5, 76.2, 75.4, 74.6, 73.1, 72.3, 57.8, 55.0, 45.4, 43.1, 35.8, 26.9, 22.6, 21.9, 20.9, 14.9, 10.5. HRFABMS calculated for C₄₇H₅₁NO₁₅ (M+H)⁺ 870.3337, found 870.3332, error 0.6 ppm.

2'-O-(tert-Butyldimethylsilyl)-6 α ,7 α -O-cyclosulfonyl(α)-paclitaxel (2.38) and 2'-O-(tert-butyldimethylsilyl)-6 α ,7 α -O-cyclosulfonyl(β)-paclitaxel (2.39) - To a solution of 2'-O-(tert-butyldimethylsilyl)-6 α -hydroxy-7-epipaclitaxel (**2.24**, 40 mg, 0.041 mmol) in dry CH₂Cl₂ (2mL) was added DMAP (20 mg, 0.16 mmol) and SOCl₂ (9 μ L, 0.12 mmol) and the mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then diluted with EtOAc and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000 μ , EtOAc:hexanes 4:6) to afford 27.8 mg of a mixture of 2'-O-(tert-butyldimethylsilyl)-6 α ,7 α -O-cyclosulfonyl(α)-paclitaxel (**2.39**) and 2'-O-(tert-butyldimethylsilyl)-6 α ,7 α -O-cyclosulfonyl(β)-paclitaxel (**2.38**) along with starting material (**2.24**, 10 mg). The mixture was further separated by preparative TLC (silica gel, 1000 μ , EtOAc:hexanes 2.5:7.5, 6 times elution) to afford 2'-O-(tert-butyldimethylsilyl)-6 α ,7 α -O-cyclosulfonyl(α)-paclitaxel (**2.39**, 9 mg, 37% based on unrecovered starting material) and 6 α ,7 α -O-cyclosulfonyl(β)-paclitaxel (**2.38**, 11 mg, 46% based on unrecovered starting material) along with starting material (**2.24**, 6 mg, decomposed on PTLC plate).

2'-O-(tert-butyldimethylsilyl)-6 α ,7 α -O-cyclosulfonyl(α)-paclitaxel (2.39) ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.02 (d, J=7.2, 2H), 7.75 (d, J=7.2, 2H), 7.58-7.28 (m, 11H), 7.11 (d, J=9.6, 1H), 6.79 (s, 1H), 6.33 (t, J=8.8, 1H), 5.85 (s, 1H), 5.80 (m, 2H), 5.04 (d, J=8.4, 1H), 4.94 (d, J=6.8, 1H), 4.65 (d, J=1.6, 1H), 4.39 (d, J=6.4, 1H), 4.21 (d, J=8.4, 1H), 3.80 (d, J=7.2, 1H), 3.07 (m, 1H), 2.52 (s, 3H), 2.22 (s, 3H), 2.10 (m, 1H), 1.98 (s, 3H), 1.91 (s, 3H), 1.21 (s, 3H), 1.17 (s, 3H), 0.78 (s, 9H), -0.06 (s, 3H), -0.33 (s, 3H). ¹³C NMR (CDCl₃, TMS, 90.562 MHz) δ 204.4, 171.2, 169.7, 169.6, 167.2, 166.9, 141.5, 138.3, 134.2, 133.8, 131.9, 131.8, 129.9, 129.1, 128.9, 128.8, 128.7, 127.9, 127.0, 126.3, 88.0, 84.2, 81.7, 80.1, 78.9, 77.2, 75.6, 75.2, 74.6, 70.8, 55.5, 54.2, 42.6, 42.0, 35.8, 25.8, 25.5, 22.9, 21.4, 20.8, 18.1, 15.4, 15.0, -5.3, -5.9. HRFABMS calculated for C₅₃H₆₃NO₁₆SSi (M+H)⁺ 1030.3716, found 1030.3701, error 1.4 ppm.

6 α ,7 α -O-Cyclosulfonyl(α)-paclitaxel (2.40) - To a solution of 2'-O-(*tert*-butyldimethylsilyl)-6 α ,7 α -O-cyclosulfonyl(α)-paclitaxel (**2.39**, 3.2 mg, 0.0031 mmol) in dry THF (100 μ L) was added HF-pyridine (70%, 20 μ L) and the solution was stirred at room temperature for 4 hours. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate and dilute HCl (1N), the organic layer was combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by preparative TLC (silica gel, 500 μ , EtOAc:hexanes 6:4) to afford 6 α ,7 α -O-cyclosulfonyl(α)-paclitaxel (**2.40**, 2.8 mg, 98%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 7.98 (d, J=8.4, 2H), 7.78 (dd, J=8.0,1.2, 2H), 7.60-7.32 (m, 11H), 7.13 (d, J=9.6, 1H), 6.72 (s, 1H), 6.17 (t, J=8.0, 1H), 5.81 (m, 2H), 5.80 (s, 1H), 5.00 (d, J=8.4, 1H), 4.94 (d, J=6.4, 1H), 4.78 (m, 1H), 4.36 (d, J=6.8, 1H), 4.16 (d, J=8.0, 1H), 3.77 (d, J=6.8, 1H), 3.43 (d, J=5.2, 1H), 3.02 (m, 1H), 2.36 (s, 3H), 2.33 (m, 1H), 2.23 (s, 3H), 1.89 (s, 3H), 1.72 (s, 3H), 1.18 (s, 3H), 1.17 (s, 3H). ¹³C NMR (CDCl₃, TMS, 90.562 MHz) δ 203.6, 172.3, 169.6(2C), 167.1, 166.7, 140.7, 138.1, 133.9, 133.8, 132.8, 131.9, 129.8, 129.1, 128.9(2C), 128.7, 128.3, 127.0(2C), 87.6, 84.2, 81.7, 80.3, 78.4, 77.2, 75.4, 74.1, 73.4, 72.3, 54.8, 54.4, 42.4, 42.0, 35.6, 26.5, 22.6, 20.8, 20.5, 15.7, 14.8. HRFABMS calculated for C₄₇H₄₉NO₁₆S (M+H)⁺ 916.2851, found 916.2844, error 0.7 ppm.

2'-O-(*tert*-Butyldimethylsilyl)-6 α ,7 α -O-cyclosulfuryl-paclitaxel (2.41) - A. To a solution of 2'-O-(*tert*-butyldimethylsilyl)-6 α ,7 α -O-cyclosulfonyl(α)-paclitaxel (**2.39**, 4.6 mg, 0.0045 mmol) in CCl₄/H₂O/CH₃CN (120 μ L/240 μ L/120 μ L) was added sodium periodate (NaIO₄, 4.8 mg, 0.022 mmol) and ruthenium trichloride (RuCl₃H₂O, 1.2 mg, 0.0046 mmol) and the mixture was stirred at room temperature for 2 hours. The reaction mixture was then filtered through a pad of silica gel and rinsed with EtOAc (1 mL x 2). The filtrate was concentrated under reduced pressure to afford 2'-O-(*tert*-butyldimethylsilyl)-6 α ,7 α -O-cyclosulfuryl-paclitaxel (**2.41**, 4.6 mg, 98%).

B. To a solution of 2'-O-(*tert*-butyldimethylsilyl)-6 α ,7 α -O-cyclosulfonyl(β)-paclitaxel (**2.38**, 23 mg, 0.022 mmol) in CCl₄/H₂O/CH₃CN (0.6mL/1.2mL/0.6mL) was added sodium periodate (NaIO₄, 24 mg, 0.11 mmol) and ruthenium trichloride (RuCl₃H₂O, 6

mg, 0.023 mmol) and the mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then filtered through a pad of silica gel and rinsed with EtOAc (2 mL x 2). The filtrate was concentrated under reduced pressure to afford 2'-*O*-(*tert*-butyldimethylsilyl)-6 α ,7 α -*O*-cyclosulfuryl-paclitaxel (**2.41**, 23 mg, 98%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.15 (dd, J=9.2,1.6, 2H), 7.73 (dd, J=9.2,1.6, 2H), 7.62-7.32 (m, 11H), 7.08 (d, J=10.0, 1H), 6.69 (s, 1H), 6.30 (t, J=9.6, 1H), 5.80 (2d, 2H), 5.03 (s, 1H), 5.01 (d, J=7.6, 1H), 4.86 (d, J=7.6, 1H), 4.69 (d, J=2.4, 1H), 4.46 (d, J=9.6, 1H), 4.24 (d, J=9.6, 1H), 4.03 (d, J=7.6, 1H), 2.64 (s, 3H), 2.48 (m, 1H), 2.21 (s, 3H), 2.13 (m, 1H), 1.99 (br s, 3H), 1.90 (s, 3H), 1.21 (s, 3H), 1.17 (s, 3H), 0.78 (s, 9H), -0.04 (s, 3H), -0.32 (s, 3H).

6 α ,7 α -*O*-Cyclosulfuryl-paclitaxel (2.42) - To a solution of 2'-*O*-(*tert*-butyldimethylsilyl)-6 α ,7 α -*O*-cyclosulfuryl-paclitaxel (**2.41**, 4.6 mg, 0.0044 mmol) in dry THF (250 μ L) was added HF-pyridine (70%, 40 μ L) and the solution was stirred at room temperature for 4 hours. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate and dilute HCl (1N), the organic layer was combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by preparative TLC (silica gel, 500 μ , EtOAc:hexanes 6:4) to afford 6 α ,7 α -*O*-cyclosulfuryl-paclitaxel (**2.42**, 3.7 mg, 90%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.13 (d, J=7.2, 2H), 7.74 (d, J=7.6, 2H), 7.64-7.35 (m, 11H), 7.01 (d, J=9.2, 1H), 6.65 (s, 1H), 6.22 (t, J=8.8, 1H), 5.82 (2d, 2H), 4.99 (d, J=7.2, 1H), 4.98 (s, 1H), 4.84 (br s, 1H), 4.83 (d, J=7.2, 1H), 4.43 (d, J=8.0, 1H), 4.21 (d, J=8.0, 1H), 4.03 (d, J=7.2, 1H), 3.59 (br d, J=4.4, 1H), 2.45 (s, 3H), 2.44 (m, 1H), 2.33 (m, 1H), 2.21 (s, 3H), 1.88 (s, 3H), 1.86 (s, 3H), 1.21 (s, 3H), 1.18 (s, 3H). ¹³C NMR (CDCl₃, TMS, 90.562 MHz) δ 202.1, 172.7, 169.5, 169.1, 167.1, 166.9, 141.5, 138.0, 134.0, 132.0, 131.9, 130.2, 129.0, 128.9, 128.7, 128.3, 127.0, 84.8, 82.5, 80.3, 80.0, 78.3, 77.3, 74.1, 73.1, 71.8, 55.2, 54.7, 42.5, 39.2, 35.9, 25.8, 22.3, 21.1, 20.6, 15.1, 13.5. HRFABMS calculated for C₄₇H₄₉NO₁₇S (M+H)⁺ 932.2800, found 932.2813, error -1.4 ppm.

1-*O*-Dimethylsilyl-2'-*O*-(*tert*-butyldimethylsilyl)-6 α -*O*-trifluoromethanesulfonyl-7 α -*O*-dimethylsilyl-paclitaxel (2.49) - To a solution of 2'-*O*-(*tert*-butyldimethylsilyl)-6 α -*O*-trifluoromethanesulfonyl-7-*epi*paclitaxel (**2.43**, 68 mg, 0.060 mmol) in dry CH₂Cl₂ (3 mL) was added imidazole (80 mg, 1.2 mmol) and chlorodimethylsilane (DMSCl, 35 μ L, 0.31 mmol) and the solution was stirred at room temperature for 1 hour and 45 minutes. The reaction mixture was then directly subjected to column chromatography (silica gel, EtOAc:hexanes 3:7) to afford 1-*O*-dimethylsilyl-2'-*O*-(*tert*-butyldimethylsilyl)-6 α -*O*-trifluoromethanesulfonyl-7 α -*O*-dimethylsilyl-paclitaxel (**2.49**, 71 mg, 95%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.16 (d, J=8.4, 2H), 7.75 (m, 2H), 7.61-7.32 (m, 11H), 7.05 (d, J=9.2, 1H), 6.39 (s, 1H), 6.36 (t, J=8.4, 1H), 5.91 (dd, J=9.2,2.0, 1H), 5.64 (d, J=7.2, 1H), 5.33 (dd, J=6.4,2.4, 1H), 5.02 (d, J=6.4, 1H), 4.84 (m, 1H), 4.71 (d, J=2.4, 1H), 4.65 (d, J=8.4, 1H), 4.32 (m, 1H), 4.19 (2d, 2H), 4.04 (d, J=2.0, 1H), 2.69 (s, 3H), 2.50 (m,1H), 2.35 (m, 1H), 2.19 (s, 3H), 1.91 (br s, 3H), 1.69 (s, 3H), 1.15 (s, 3H), 1.07 (s, 3H), 0.78 (s, 9H), 0.36 (d, J=2.8, 3H), 0.33 (d, J=2.8, 3H), -0.04 (s, 3H), -0.18 (d, J=2.8, 1H), -0.30 (s, 3H), -0.47 (d, J=2.8, 3H).

1-*O*-Dimethylsilyl-2'-*O*-(*tert*-butyldimethylsilyl)-6 β -azido-7-*epi*paclitaxel (2.50) - To a solution of 1-*O*-dimethylsilyl-2'-*O*-(*tert*-butyldimethylsilyl)-6 α -*O*-trifluoromethanesulfonyl-7 α -*O*-dimethylsilyl-paclitaxel (**2.49**, 71 mg, 0.057 mmol) in DMF (1.5 mL) was added sodium azide (NaN₃, 100 mg, 1.5 mmol) and the solution was stirred at room temperature for 18 hours. The reaction mixture was then diluted with EtOAc and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by preparative TLC (silica gel, 1000 μ , EtOAc:hexanes 4:6) to afford 1-*O*-dimethylsilyl-2'-*O*-(*tert*-butyldimethylsilyl)-6 β -azido-7-*epi*paclitaxel (**2.50**, 25 mg, 49%, based on unrecovered starting material), the starting material (**2.49**, 11 mg) along with other products that were not characterized. ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.18 (d, J=8.0, 2H), 7.72 (m, 2H), 7.60-7.31 (m, 11H), 7.03 (d, J=9.6, 1H), 6.79 (s, 1H), 6.32 (t, J=8.8, 1H), 5.87 (dd, J=9.6,2.4, 1H), 5.78 (d, J=7.6, 1H), 5.05 (d, J=8.0, 1H), 4.68 (d, J=2.4, 1H), 4.57 (d, J=11.2, 1H), 4.36 (s, 2H), 4.26 (m, 1H), 4.13 (d, J=9.2, 1H), 3.86 (d, J=7.2, 1H), 3.68 (dd, J=10.8,1.2, 1H), 2.72 (s,

3H), 2.31 (m,2H), 2.19 (s, 3H), 2.04 (s, 3H), 1.88 (s, 3H), 1.79 (s, 3H), 1.13 (s, 3H), 1.11 (s, 3H), 0.76 (s, 9H), -0.05 (s, 3H), -0.15 (d, J=2.4, 1H), -0.31 (s, 3H), -0.49 (d, J=2.8, 3H). ¹³C NMR (CDCl₃, TMS, 90.562 MHz) δ 206.0, 172.6, 170.9, 169.5, 166.8, 165.6, 139.5, 138.0, 133.9, 133.4, 132.8, 131.7, 130.4, 129.9, 128.75, 128.69, 128.6, 127.9, 127.1, 126.4, 82.7, 81.9, 80.7, 78.6, 77.8, 75.7, 75.4, 70.5, 62.1, 57.5, 55.4, 43.5, 39.9, 34.8, 26.8, 25.5, 22.7, 22.1, 20.9, 18.2, 14.8, 14.7, 0.3, -0.4, -5.3, -6.0. FT-IR 2104.5 cm⁻¹ (strong).

6β-Azido-7-epipaclitaxel (2.51) - To a solution of 1-*O*-dimethylsilyl-2'-*O*-(*tert*-butyldimethylsilyl)-6β-azido-7-epipaclitaxel (**2.50**, 25 mg, 0.023 mmol) in dry THF (0.5 mL) was added HF-pyridine (70%, 100 μL) and the solution was stirred at room temperature for 3 hours. The reaction mixture was then diluted with EtOAc and washed with dilute sodium bicarbonate and dilute HCl (1N), the organic layer was combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 6:4) to afford 6β-azido-7-epipaclitaxel (**2.51**, 17.6 mg, 84%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.16 (d, J=7.6, 2H), 7.71 (m, 2H), 7.62-7.35 (m, 11H), 7.05 (d, J=8.8, 1H), 6.77 (s, 1H), 6.20 (t, J=8.8, 1H), 5.80 (dd, J=8.8,2.0, 1H), 5.76 (d, J=7.6, 1H), 5.03 (d, J=8.0, 1H), 4.79 (m, 1H), 4.49 (d, J=10.8, 1H), 4.42 (d, J=8.8, 1H), 4.27 (d, J=8.8 1H), 4.12 (m, 1H), 3.86 (d, J=7.2, 1H), 3.68 (br s, 1H), 3.66 (m, 1H), 2.50 (s, 3H), 2.35 (m,1H), 2.26 (m, 1H), 2.18 (s, 3H), 1.77 (s, 3H), 1.76 (s, 3H), 1.17 (s, 3H), 1.13 (s, 3H). ¹³C NMR (CDCl₃, TMS, 90.562 MHz) δ 205.6, 172.6, 172.4, 169.5, 167.1, 167.07, 139.6, 137.9, 133.8, 133.6, 133.3, 132.0, 130.2, 129.1, 129.0, 128.8, 128.7, 128.3, 127.0, 126.9, 82.6, 80.5, 79.0, 78.4, 78.1, 77.2, 75.0, 73.2, 72.0, 62.3, 57.7, 54.9, 42.6, 40.1, 36.0, 25.8, 22.3, 21.1, 20.8, 14.76, 14.73. HRFABMS calculated for C₄₇H₅₀N₄O₁₄ (M+H)⁺ 895.3402, found 895.3401, error 0.1 ppm.

Hydrogenation of 6β-azido-7-epipaclitaxel (2.51) - To a solution of 6β-azido-7-epipaclitaxel (**2.51**, 10 mg, 0.011 mmol) in MeOH (1mL) was added a catalytic amount of palladium on activated carbon (10%). Hydrogen gas was bubbled through a needle into

the suspension for 1.5 hours. TLC analysis showed complete disappearance of the starting material and formation of a very polar spot. The suspension was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 500 μ , MeOH:CH₂Cl₂ 8:92) gave a complex reaction mixture. The major component (4.0 mg, 40%) was isolated and identified as 6 α -dihydroazido-7-epipaclitaxel (**2.52**, ¹H and ¹³C NMR data see below).

6 β -Dihydroazido-7-epipaclitaxel (2.52) - To a solution of 6 β -azido-7-epipaclitaxel (**2.51**, 7.8 mg, 0.0088 mmol) in MeOH (2mL) was added excess amount of palladium on activated carbon (10%). This suspension was stirred under hydrogen atmosphere for 72 hours. The suspension was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 500 μ , MeOH:EtOAc 5:95) to furnish 6 β -dihydroazido-7-epipaclitaxel (**2.52**, 4.8 mg, 62%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.12 (dd, J=8.0,1.2, 2H), 7.77 (dd, J=8.0,1.2, 2H), 7.62-7.34 (m, 11H), 7.17 (d, J=9.6, 1H), 6.80 (s, 1H), 6.15 (t, J=8.4, 1H), 5.83 (dd, J=8.8,2.4, 1H), 5.80 (d, J=6.0, 1H), 5.15 (d, J=3.2, 1H), 4.80 (d, J=2.4, 1H), 4.39 (d, J=8.0, 1H), 4.19 (d, J=7.6, 1H), 4.03 (d, J=6.0, 1H), 3.82 (d, J=6.4, 1H), 3.80 (m, 1H), 3.61 (br s, 1H), 3.01 (m, 1H), 2.41 (s, 3H), 2.33 (m,1H), 2.23 (m, 1H), 2.21 (s, 3H), 1.91 (s, 3H), 1.75 (s, 3H), 1.20 (s, 3H), 1.18 (s, 3H). ¹³C NMR (CDCl₃, TMS, 90.562 MHz) δ 203.8, 171.9, 170.9, 169.4, 167.0, 166.8, 139.3, 138.1, 133.9, 133.8, 133.7, 131.9, 130.1, 129.1, 128.9, 128.7, 128.2, 127.02, 127.05, 82.5(C-5), 80.8(C-4), 79.3(C-10), 78.3(C-1), 77.2(C-20), 75.3(C-2), 73.8(C-7), 73.4(C-2'), 71.8(C-13), 69.1(C-6), 57.8(C-8), 54.7(C-3'), 42.8(C-15), 42.6(C-6), 40.5(C-3), 35.7(C-14), 25.8(C-17), 22.5(4-OAc), 20.9(10-OAc), 20.8(C-16), 17.5(C-19), 14.7(C-18). LRFABMS calculated for C₄₇H₅₂N₄O₁₄ (M+H)⁺ 897.8, found 897.4; (M+Na)⁺ 919.3, found 919.4; (M+Li)⁺ 903.4, found 903.4.

6 β -Amino-7-epipaclitaxel (2.53) - To a solution of 6 β -azido-7-epipaclitaxel (**2.51**, 34 mg, 0.038 mmol) in EtOAc (10 mL) was added catalytic amount of palladium on activated carbon (5%). This suspension was stirred under 50 psi hydrogen atmosphere for 24 hours. The suspension was filtered through Celite and the filtrate was concentrated

under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000 μ , MeOH:CH₂Cl₂ 8:92) to furnish 6 β -amino-7-epipaclitaxel (**2.53**, 12 mg, 35%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.17 (dd, J=7.2,1.6, 2H), 7.71 (dd, J=6.8,1.6, 2H), 7.62-7.34 (m, 11H), 7.11 (d, J=9.2, 1H, 3'-NH), 6.81 (s, 1H, H-10), 6.19 (t, J=8.8, 1H, H-13), 5.79 (dd, J=8.8,2.4, 1H, H-3'), 5.75 (d, J=7.6, 1H, H-2), 4.90 (d, J=8.4, 1H, H-5), 4.78 (d, J=2.4, 1H, H-2'), 4.39 (d, J=8.0, 1H, H-20), 4.17 (d, J=8.4, 1H, H-20), 3.85 (d, J=7.6, 1H, H-3), 3.52 (br s, 1H, H-7), 3.35 (d, J=8.0, 1H, H-6), 2.47 (s, 3H, 4-OAc), 2.36 (m, 1H, H-14), 2.23 (m, 1H, H-14), 2.1-2.2 (br, NH₂ and OH), 2.17 (s, 3H, 10-OAc), 1.75 (s, 3H, 18-CH₃), 1.73 (s, 3H, 19-CH₃), 1.16 (s, 3H, 17-CH₃), 1.13 (s, 3H, 16-CH₃). ¹³C NMR (CDCl₃, TMS, 90.562 MHz) δ 206.5(C-9), 172.6, 172.1, 169.5, 167.2, 167.1, 139.4, 138.0, 133.7, 133.6, 133.2, 131.9, 130.2, 129.3, 129.0, 128.8, 128.6, 128.3, 127.1, 126.9, 83.5(C-5), 83.0(C-7), 80.7, 79.1, 78.0(C-10), 76.2(C-20), 75.2(C-2), 73.2(C-2'), 72.0(C-13), 58.0(C-8), 54.9(C-3'), 54.6(C-6), 42.5(C-15), 40.2(C-3), 36.0(C-14), 25.8(C-17), 22.3(4-OAc), 21.1(C-16), 20.9(10-OAc), 14.8(C-18), 14.5(C-19). HRFABMS calculated for C₄₇H₅₂N₂O₁₄ (M+H)⁺ 869.3497, found 869.3504, error -0.8 ppm.

Glutaric acid monobenzyl ester - To a solution of glutaric anhydride (4.43 g, 38.9 mmol) in dry CH₂Cl₂ (40 mL) was added DMAP (100 mg, catalytic amount) and benzyl alcohol (4.5 mL, 43.8 mmol) followed by triethylamine (EtN₃, 7 mL, 50 mmol). The resulting solution was stirred at room temperature for 12 hours. The reaction mixture was diluted with diethyl ether and extracted with dilute sodium bicarbonate. The combined aqueous layer was acidified with dilute HCl (1N) and was then extracted with diethyl ether. The organic layer was separated and combined, dried over anhydrous sodium sulfate, and was concentrated *in vacuo* to give glutaric acid monobenzyl ester as a clear oily liquid. (5.5g, 60%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 7.34 (m, 5H, ArH), 5.11 (s, 2H, COOCH₂Ph), 2.43 (m, 4H, OCOCH₂CH₂CH₂COO), 1.96 (m, 2H, OCOCH₂CH₂CH₂COO). ¹H NMR was identical with the literature data.¹⁵¹

¹⁵¹ English, A. R.; Girard, D.; Jasys, V. J.; Martingano, R. J.; Kellogg, M. S. *J. Med. Chem.* **1990**, *33*, 344.

2'-O-(tert-Butyldimethylsilyl)-7-O-triethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(O-benzylglutaryl)-paclitaxel (2.60) - To a solution of 2'-O-(tert-butylidimethylsilyl)-7-O-triethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-paclitaxel (**2.59**, 57 mg, 0.059 mmol) in dry toluene (3.0 mL) was added DCC (310 mg, 1.50 mmol), 4-PP (7 mg, catalytic amount), and glutaric acid monobenzyl ester (133 μ L, 0.72 mmol). The resulting suspension was stirred at room temperature for 24 hours and was then filtered through a pad of silica gel (rinsed with EtOAc. The filtrate was concentrated and applied directly on preparative TLC (silica gel, 1000 μ , EtOAc:hexanes 4:6) to afford 2'-O-(tert-butylidimethylsilyl)-7-O-triethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(O-benzylglutaryl)-paclitaxel (**2.60**, 60 mg, 87%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 7.77 (m, 2H), 7.55-7.27 (m, 13H), 7.01 (d, J=10.4, 1H), 6.42 (s, 1H), 6.12 (t, J=9.6, 1H), 5.62 (dd, J=10.4,2.4, 1H), 5.08 (AB q, 2H), 4.91 (d, J=9.6, 1H), 4.64 (d, J=9.6, 1H), 4.63 (d, J=2.4, 1H), 4.50 (d, J=6.0, 1H), 4.49 (d, J=9.6, 1H), 4.41 (dd, J=10.8,8.0, 1H), 3.45 (d, J=6.4, 1H), 2.76 (m, 1H), 2.61-1.92 (m, 9H), 2.15 (s, 3H), 2.00 (s, 3H), 1.75 (s, 3H), 1.32 (s, 3H), 1.22 (s, 3H), 0.91 (t, J=8.8, 9H), 0.83 (s, 9H), 0.57 (q, J=8.8, 6H), -0.02 (s, 3H), -0.20 (s, 3H). ¹³C NMR (CDCl₃, TMS, 90.562 MHz) δ 201.9, 172.31, 172.27, 171.8, 169.0, 167.0, 152.6, 143.7, 137.9, 131.8, 131.2, 128.8, 128.7, 128.6, 128.4, 128.24, 128.18, 127.0, 126.8, 89.7, 84.2, 81.3, 80.1, 77.2, 76.3, 75.6, 74.6, 71.5, 70.6, 66.5, 60.0, 55.7, 43.4, 41.5, 37.9, 35.1, 32.9, 32.3, 25.5, 25.4, 21.0, 20.7, 18.2, 14.8, 10.1, 6.7, 5.2, -5.1, -5.5.

Reaction of 2'-O-(tert-butylidimethylsilyl)-7-O-triethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(O-benzylglutaryl)-paclitaxel (2.60) with phenyl lithium - To a solution of 2'-O-(tert-butylidimethylsilyl)-7-O-triethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(O-benzylglutaryl)-paclitaxel (**2.60**, 38mg, 0.033 mmol) in dry THF (2.5 mL) at -78 °C was added PhLi (1.8 M in hexanes, 94 μ L, 1.7 mmol). The solution was stirred at -78 °C for 12 minutes, and was then diluted with EtOAc, quenched with dilute HCl (1N), the organic layer was separated. The aqueous layer was extracted three times with EtOAc. The combined organic layer was washed with dilute sodium bicarbonate, water, and brine, dried over anhydrous sodium sulfate, concentrated under reduced pressure. The

residue was purified by preparative TLC (silica gel, 1000 μ , EtOAc:hexanes 4:6) to give 2'-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-4-deacetyl-4-(5''-hydroxy-5'',5''-diphenylpentanoyl)-paclitaxel (**2.62**, mg, %).¹⁵² ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.10 (dd, J=8.4,1.2, 2H), 7.69 (dd, J=8.4,1.2, 2H), 7.60-7.13 (m, 21H), 7.04 (d, J=8.8, 1H), 6.45 (s, 1H), 6.32 (t, J=8.8, 1H), 5.79 (dd, J=9.2,2.0, 1H), 5.69 (d, J=6.8, 1H), 4.85 (d, J=8.0, 1H), 4.69 (d, J=2.0, 1H), 4.66 (d, J=6.0, 1H), 4.49 (dd, J=10.0,6.4, 1H), 4.25 (d, J=8.4, 1H), 4.17 (d, J=8.4, 1H), 3.81 (d, J=6.8, 1H), 3.33 (s, 1H), 3.08 (m, 1H), 2.69 (m, 1H), 2.53 (m, 1H), 2.37-1.75 (m, 7H), 2.17 (s, 3H), 2.01 (s, 3H), 1.70 (s, 3H), 1.21 (s, 3H), 1.20 (s, 3H), 0.94 (t, 9H), 0.76 (s, 9H), 0.59 (q, 6H), -0.02 (s, 3H), -0.32 (s, 3H). ¹³C NMR (CDCl₃, TMS, 100.578 MHz) δ 201.7, 172.6, 171.2, 169.3, 167.2, 167.0, 147.7, 146.3, 140.2, 138.0, 133.62, 133.59, 133.5, 131.9, 130.1, 129.0, 128.9, 128.7-125.8 (11C), 84.4, 81.1, 79.0, 77.4, 76.4, 74.94, 74.89, 74.85, 72.2, 71.0, 58.4, 55.7, 46.6, 43.3, 40.8, 37.2, 36.7, 35.6, 26.7, 25.4, 21.7, 20.9, 19.8, 18.1, 14.1, 10.1, 6.7, 5.3, -5.1, -5.9.

2'-*O*-(*tert*-Butyldimethylsilyl)-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(N-carbobenzyloxy- β -alanyl)-paclitaxel (2.61**)** - To a solution of 2'-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-paclitaxel (**2.59**, 31 mg, 0.032 mmol) in dry toluene (2.0 mL) was added DCC (80 mg, 0.39 mmol), 4-PP (2 mg, catalytic amount), and N-carbobenzyloxy-alanine (Cbz-NH-CH₂CH₂COOH, 72 mg, 0.32 mmol). The resulting suspension was stirred at room temperature for 12 hours and was then filtered through a pad of Celite. The filtrate was concentrated and applied directly on preparative TLC (silica gel, 1000 μ , EtOAc:hexanes 5:5) to afford 2'-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(N-carbobenzyloxy- β -alanyl)-paclitaxel (**2.61**, 27 mg, 72%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 7.77 (m, 2H), 7.54-7.28 (m, 13H), 7.06 (d, J=9.2, 1H, 3'-NH), 6.39 (s, 1H, 10-H), 6.21 (t, J=8.8, 1H, 13-H), 5.71 (t, J=5.2, 1H, 3''-NH), 5.65 (dd, J=8.8,2.8, 1H, 3'-H), 4.99 (m, 3H, 5-H, -COOCH₂Ph), 4.70 (d, J=2.8, 1H, 2'-H), 4.66 (d, J=8.8, 1H, 20-H), 4.50 (d, J=6.8, 1H, 2-H), 4.48 (d, J=8.8, 1H, 20-H), 4.38 (dd, J=9.6,7.2,

¹⁵² Reaction with a smaller excess of PhLi gave a mixture containing the starting material, **2.62**, and another product (**2.63**); see page 107 for discussion.

1H, 7-H), 3.63 (m, 1H, 3''-H), 3.51 (m, 1H, 3''-H), 3.41 (d, J=6.0, 3-H), 2.94 (t, J=6.8, 2H, 2''-H), 2.59 (m, 1H, 6-H), 2.32 (m, 1H, 14-H), 2.16 (m, 1H, 14-H), 2.15 (s, 3H, 10-OAc), 1.94 (br s, 3H, 18-CH₃), 1.92 (m, 1H, 6-H), 1.75 (s, 3H, 19-CH₃), 1.32 (s, 3H, 17-CH₃), 1.21 (s, 3H, 16-CH₃), 0.92 (t, J=8.8, 9H, -SiEt₃), 0.80 (s, 9H, -SiMe₂^tBu), 2.58 (q, J=8.8, 6H, -SiEt₃), -0.02 (s, 3H, -SiMe₂^tBu), -0.24 (s, 3H, -SiMe₂^tBu). ¹³C NMR (CDCl₃, TMS, 100.578 MHz) δ 201.8, 171.5, 170.9, 169.0, 167.1, 156.4, 152.4, 143.5, 137.6, 136.3, 133.7, 132.0, 131.2, 128.80, 128.76, 128.4, 128.2, 128.0, 127.0, 126.7, 89.6, 83.8, 81.3, 80.3, 76.2, 75.5, 74.9, 71.6, 70.0, 66.7, 60.0, 55.9, 43.4, 41.5, 37.8, 37.0, 36.6, 33.9, 32.3, 25.6, 25.5, 24.9, 21.1, 20.7, 18.1, 14.7, 14.2, 10.1, 6.7, 5.2, -5.1, -5.6.

2'-O-(tert-Butyldimethylsilyl)-7-O-triethylsilyl-2-debenzoyl-4-deacetyl-4-(O-benzylglutaryl)-paclitaxel (2.64) - To a solution of 2'-O-(tert-butyldimethylsilyl)-7-O-triethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(O-benzylglutaryl)-paclitaxel (**2.60**, 30 mg, 0.025 mmol) in THF (1.5 mL) was added 7 drops of water and lithium hydroxide monohydrate (LiOH·H₂O, 26 mg, excess). The mixture was stirred at room temperature for 2 hours and was then diluted with EtOAc, washed with dilute HCl (1N), dilute sodium bicarbonate, water, and brine. The combined organic layer was dried over anhydrous NaSO₄, concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 6:4) to afford 2'-O-(tert-butyldimethylsilyl)-7-O-triethylsilyl-2-debenzoyl-4-deacetyl-4-(O-benzylglutaryl)-paclitaxel (**2.64**, 25 mg, 85%) which was directly subjected to the next reaction.

2'-O-(tert-Butyldimethylsilyl)-7-O-triethylsilyl-4-deacetyl-4-(O-benzylglutaryl)-paclitaxel (2.65) - To a solution of 2'-O-(tert-butyldimethylsilyl)-7-O-triethylsilyl-2-debenzoyl-4-deacetyl-4-(O-benzylglutaryl)-paclitaxel (**2.64**, 30 mg, 0.026 mmol) in dry toluene (3.0 mL) was added DCC (64 mg, 0.31 mmol), DMAP (2 mg, catalytic amount), and benzoic acid (30 mg, 0.25 mmol). The resulting suspension was stirred at 80 °C for 48 hours and was then applied directly on preparative TLC (silica gel, 1000μ, EtOAc:hexanes 4:6) to afford 2'-O-(tert-butyldimethylsilyl)-7-O-triethylsilyl-4-deacetyl-4-(O-benzylglutaryl)-paclitaxel (**2.65**, 20 mg, 61%) along with other products that were

not characterized. ^1H NMR (CDCl_3 , TMS, 399.951 MHz) δ 8.12 (m, 2H), 7.72 (m, 2H), 7.56-7.27 (m, 16H), 7.07 (d, $J=9.2$, 1H), 6.44 (s, 1H), 6.21 (t, $J=8.8$, 1H), 6.15 (br d, $J=7.6$, 1H), 5.68 (d, $J=8.0$, 1H), 5.09 (s, 2H), 4.88 (br d, $J=8.8$, 1H), 4.67 (d, $J=2.4$, 1H), 4.47 (dd, $J=10.4, 6.8$, 1H), 4.29 (d, $J=8.4$, 1H), 4.20 (d, $J=8.4$, 1H), 3.81 (d, $J=6.8$, 1H), 3.01 (m, 1H), 2.74 (m, 1H), 2.52-1.70 (m, 8H), 2.16 (s, 3H), 2.03 (s, 3H), 1.72 (s, 3H), 1.21 (s, 3H), 1.17 (s, 3H), 0.92 (t, 9H), 0.79 (s, 9H), 0.58 (q, 6H), -0.02 (s, 3H), -0.27 (s, 3H).

4-Deacetyl-4-(*O*-benzylglutaryl)-paclitaxel (2.66) - To a solution of 2'-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-4-deacetyl-4-(*O*-benzylglutaryl)-paclitaxel (**2.65**, 6.0 mg, 0.0048 mmol) in dry THF (1 mL) was added HF-pyridine (70%, 150 μL , excess) and the solution was stirred at room temperature for 4 hours. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate and dilute HCl (1N), the organic layer was combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by preparative TLC (silica gel, 500 μ , EtOAc:hexanes 7:3) to afford 4-deacetyl-4-(*O*-benzylglutaryl)-paclitaxel (**2.66**, 3.5 mg, 71%). ^1H NMR (CDCl_3 , TMS, 399.951 MHz) δ 8.11 (m, 2H), 7.75 (m, 2H), 7.60-7.27 (m, 16H), 7.09 (d, $J=8.4$, 1H), 6.24 (s, 1H), 6.19 (t, $J=8.8$, 1H), 5.73 (dd, $J=8.8, 3.2$, 1H), 5.66 (d, $J=7.2$, 1H), 5.07 (s, 2H), 4.87 (br d, $J=8.0$, 1H), 4.77 (dd, $J=6.0, 3.2$, 1H), 4.41 (m, 1H), 4.28 (d, $J=8.4$, 1H), 4.18 (d, $J=8.4$, 1H), 3.77 (d, $J=6.8$, 1H), 3.65 (d, $J=6.0$, 1H), 2.75-2.50 (m, 3H), 2.46 (d, $J=4.0$, 1H), 2.44-1.84 (m, 7H), 2.24 (s, 3H), 1.76 (br s, 3H), 1.68 (s, 3H), 1.25 (s, 3H), 1.13 (s, 3H).

4-Deacetyl-4-glutaryl-paclitaxel (2.67) - A catalytic amount of palladium on activated carbon (5%) was added to a solution of 4-deacetyl-4-(*O*-benzylglutaryl)-paclitaxel (**2.66**, 3.5 mg, 0.0035 mmol) in EtOAc (1 mL). This suspension was stirred in an atmosphere of hydrogen gas for 1 hour. TLC analysis indicated complete conversion of the starting material to a very polar compound. The solid was removed by filtration and the filtrate was concentrated under reduced pressure to afford 4-deacetyl-4-glutaryl-paclitaxel (**2.67**, 3.0 mg, 94%). ^1H NMR (CDCl_3 , TMS, 399.951 MHz) δ 8.10 (d, $J=8.0$, 2H), 7.79 (d,

J=7.6, 2H), 7.62-7.28 (m, 12H), 6.24 (s, 1H), 6.15 (t, J=8.4, 1H), 5.70 (dd, J=8.8,4.8, 1H), 5.66 (d, J=7.2, 1H), 4.88 (br d, J=8.0, 1H), 4.77 (d, J=4.0, 1H), 4.41 (m, 1H), 4.28 (d, J=8.8, 1H), 4.19 (d, J=8.8, 1H), 3.75 (d, J=6.8, 1H), 3.42 (m, 1H), 2.75-2.40 (m, 5H), 2.23 (s, 3H), 2.20-1.89 (m, 5H), 1.75 (br s, 3H), 1.67 (s, 3H), 1.23 (s, 3H), 1.12 (s, 3H). ¹³C NMR (CDCl₃, TMS, 100.578 MHz) δ 203.8, 175.5, 173.8, 172.8, 172.3, 171.3, 167.2, 142.5, 137.4, 133.7, 132.9, 132.0, 130.1, 129.3, 128.8, 128.65, 128.61, 128.3, 127.3, 127.1, 84.6, 81.2, 77.8, 76.4, 75.5, 75.0, 73.2, 71.8, 71.7, 58.4, 55.9, 45.5, 43.8, 35.4, 34.6, 32.9, 29.9, 26.8, 22.1, 20.8, 20.7, 14.6, 9.6. HRFABMS calculated for C₅₀H₅₅NO₁₆ (M+Li)⁺ 932.2931, found 932.2955, error 2.6 ppm.

2'-O-(tert-Butyldimethylsilyl)-7-O-triethylsilyl-4-deacetyl-4-(N-carbobenzyloxy-β-alanyl)-paclitaxel (2.68) - To a solution of 2'-O-(tert-butyldimethylsilyl)-7-O-triethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(N-carbobenzyloxy-β-alanyl)-paclitaxel (**2.61**, 7.0 mg, 0.0060 mmol) in dry THF (1.0 mL) at -78 °C was added PhLi (1.8 M in hexanes, 20 μL, 0.036 mmol). The solution was stirred at -78 °C for 15 minutes, and was then diluted with EtOAc, quenched with dilute HCl (1N), the organic layer was separated. The aqueous layer was extracted three times with EtOAc. The combined organic layer was washed with dilute sodium bicarbonate, water, and brine, dried over NaSO₄, concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 500μ, EtOAc:hexanes 4:6) to give 2'-O-(tert-butyldimethylsilyl)-7-O-triethylsilyl-4-deacetyl-4-(N-carbobenzyloxy-β-alanyl)-paclitaxel (**2.68**, 6.0 mg, 80%) which was directly subjected to the next reaction.

4-Deacetyl-4-(N-carbobenzyloxy-β-alanyl)-paclitaxel (2.69) - A solution of 2'-O-(tert-butyldimethylsilyl)-7-O-triethylsilyl-4-deacetyl-4-(N-carbobenzyloxy-β-alanyl)-paclitaxel (**2.68**, 15 mg, 0.012 mmol) in freshly prepared 5% HCl/MeOH (1 mL) was stirred at room temperature for 2 hours. The reaction mixture was diluted with EtOAc, washed with dilute sodium bicarbonate, water, and brine, dried over NaSO₄, concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 500μ, EtOAc:hexanes 7:3) to afford 4-deacetyl-4-(N-carbobenzyloxy-β-alanyl)-paclitaxel (**2.69**,

9.0 mg, 74%). ^1H NMR (CDCl_3 , TMS, 399.951 MHz) δ 8.06 (d, $J=7.2$, 2H, 2-*o*-COPh), 7.81 (d, $J=7.6$, 2H), 7.62-7.26 (m, 17H), 6.21 (s, 1H, 10-H), 6.20 (t, $J=8.8$, 1H, 13-H), 5.69 (dd, $J=8.4, 4.0$, 1H, 3'-H), 5.65 (d, $J=7.2$, 1H, 2-H), 5.43 (m, 1H, 3''-NH), 4.99 (br s, 2H, -COOCH₂Ph), 4.89 (d, $J=8.0$, 1H, 5-H), 4.72 (dd, $J=6.8, 4.4$, 1H, 2'-H), 4.38 (m, 1H, 7-H), 4.27 (d, $J=8.8$, 1H, 20-H), 4.17 (d, $J=8.8$, 1H, 20-H), 4.15 (m, 1H, 3''-H), 3.70 (d, $J=7.2$, 3-H), 3.57 (d, $J=6.8$, 1H, 2'-OH), 3.56 (m, 1H, 3''-H), 2.86 (t, $J=6.8$, 2H, 2''-H), 2.52 (m, 1H, 6-H), 2.48 (d, $J=4.0$, 7-OH), 2.23 (s, 3H, 10-OAc), 2.12 (m, 1H, 14-H), 2.05 (m, 1H, 14-H), 1.87 (m, 1H, 6-H), 1.75 (br s, 3H, 18-CH₃), 1.66 (s, 3H, 19-CH₃), 1.24 (s, 3H, 17-CH₃), 1.12 (s, 3H, 16-CH₃).

4-Deacetyl-4- β -alanyl-paclitaxel (2.70) - A catalytic amount of palladium on activated carbon (5%) was added to a solution of 4-deacetyl-4-(N-carbobenzyloxy- β -alanyl)-paclitaxel (**2.69**, 9.0 mg, 0.0089 mmol) in EtOAc (2 mL). This suspension was stirred in an atmosphere of hydrogen gas for 2 hours. TLC analysis indicated complete conversion of the starting material to a very polar compound. The solid was removed by filtration and the filtrate was concentrated under reduced pressure to afford 4-deacetyl-4- β -alanyl-paclitaxel (**2.70**, 7.5 mg, 94%). ^1H NMR (CDCl_3 , TMS, 399.951 MHz) δ 8.04 (m, 2H, 2-*o*-COPh), 7.82 (m, 2H, ArH), 7.62-7.28 (m, 12H, ArH and 3'-NH), 6.22 (s, 1H, 10-H), 6.21 (t, $J=8.8$, 1H, 13-H), 5.73 (dd, $J=8.8, 4.0$, 1H, 3'-H), 5.67 (d, $J=7.2$, 1H, 2-H), 4.93 (d, $J=8.0$, 1H, 5-H), 4.73 (d, $J=4.0$, 1H, 2'-H), 4.41 (m, 1H, 7-H), 4.29 (d, $J=8.4$, 1H, 20-H), 4.16 (d, $J=8.8$, 1H, 20-H), 3.70 (d, $J=7.6$, 3-H), 3.19 (m, 1H), 2.92-2.77 (m, 3H), 2.53 (m, 2H), 2.35-2.15 (m, 6H), 2.24 (s, 3H, 10-OAc), 1.87 (m, 1H, 6-H), 1.78 (br s, 3H, 18-CH₃), 1.66 (s, 3H, 19-CH₃), 1.26 (s, 3H, 17-CH₃), 1.12 (s, 3H, 16-CH₃). ^{13}C NMR (CDCl_3 , TMS, 90.562 MHz) δ 203.5, 172.8, 172.7, 171.4, 167.0, 166.4, 142.6, 139.0, 134.0, 133.8, 132.9, 131.7, 130.0, 129.1, 128.7, 128.64, 128.58, 128.0, 127.6, 127.0, 84.4, 81.5, 79.7, 77.2, 76.2, 75.4, 75.1, 73.5, 72.0, 71.1, 58.3, 55.6, 45.7, 43.2, 36.8, 36.3, 35.3, 34.8, 26.7, 22.4, 20.9, 14.7, 9.7. HRFABMS calculated for $\text{C}_{48}\text{H}_{54}\text{N}_2\text{O}_{14}$ ($\text{M}+\text{H}$)⁺ 883.3653, found 883.3647, error 0.7 ppm.

2'-O-(tert-Butyldimethylsilyl)-2-debenzoyl-4-deacetyl-6,7-dehydro-paclitaxel (2.76) - 2'-O-(tert-butyldimethylsilyl)-6,7-dehydro-paclitaxel (**2.23**, 723 mg, 0.76 mmol) was dissolved in dry CH₂Cl₂ (18 mL) and was cooled to -78 °C for 5 minutes. To the solution was added benzyldimethylammonium hydroxide (Triton-B, 30% in MeOH, 0.90 mL, 1.82 mmol). The solution was stirred at -78 °C (acetone-dry ice bath) for 5 minutes and was allowed to warm up to 15 °C by replacing the acetone-dry ice bath with ethanol. The reaction proceeded at 15 °C for 10 minutes and was monitored by TLC every 3 minutes. TLC analysis (EtOAc:hexanes 6:4) indicated that the starting material (Rf~0.7) was converted to a more polar compound (Rf~0.3). The reaction mixture was diluted with ethyl acetate (dry ice cooled) and quenched with dilute HCl (1N). The organic layer was separated. The aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, concentrated under reduced pressure. The residue was filtered through a short column (silica gel, EtOAc:hexanes 5:5) to give crude product 2'-O-(tert-butyldimethylsilyl)-2-debenzoyl-4-deacetyl-6,7-dehydro-paclitaxel (**2.76**, 650 mg) which was subjected to the next reaction without further purification.

2'-O-(tert-Butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-6,7-dehydro-paclitaxel (2.77) and **2'-O-(tert-butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-6,7-dehydro-10-deacetyl-9,10-carbonato-paclitaxel (2.78)** - To a solution of 2'-O-(tert-butyldimethylsilyl)-2-debenzoyl-4-deacetyl-6,7-dehydro-paclitaxel(**2.76**, 650 mg, crude) in dry CH₂Cl₂ (10 mL) was added N,N'-carbonyldiimidazole (CDI, 2.0 g, 12.3 mmol) and imidazole (catalytic amount) at room temperature. The solution was then stirred at 40 °C for 4 hours. The reaction mixture was dilute with EtOAc, washed with water and brine, dried over anhydrous sodium sulfate, concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, EtOAc:hexanes 4:6) to afford 2'-O-(tert-butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-6,7-dehydro-paclitaxel (**2.77**, 253 mg, 40% 2 steps) and 2'-O-(tert-butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-6,7-dehydro-10-deacetyl-9,10-carbonato-paclitaxel (**2.78**, 101 mg).

2'-O-(tert-Butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-6,7-dehydro-paclitaxel (2.77) ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 7.79 (d, J=7.6, 2H, ArH), 7.56-7.30 (m, 9H, ArH and 3'-NH), 6.34 (s, 1H, H-10), 6.19-6.12 (m, 2H, H-6 and H-7), 5.90 (d, J=8.8, 1H, H-3'), 5.87 (m, 1H, H-13), 5.20 (s, 1H, 4-OH), 5.13 (d, J=7.2, H-5), 4.73 (d, J=8.4, 1H, H-20), 4.67 (d, J=8.4, 1H, H-20), 4.48 (m, 2H, H-2 and H-2'), 3.16 (d, J=5.2, 1H, H-3), 2.90 (m, 1H, H-14), 2.56 (m, 1H, H-14), 2.24 (s, 3H, 10-OAc), 1.98 (s, 3H, 18-CH₃), 1.78 (s, 3H, 19-CH₃), 1.23 (s, 3H, 17-CH₃), 1.17 (s, 3H, 16-CH₃), 0.84 (s, 9H, 2'-OSiMe₂^tBu), -0.10 (s, 3H, 2'-OSiMe₂^tBu), -0.29 (s, 3H, 2'-OSiMe₂^tBu).

2'-O-(tert-butylidimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-6,7-dehydro-10-deacetyl-9,10-carbonato-paclitaxel (2.78) ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 7.80 (d, J=7.6, 2H, ArH), 7.56-7.28 (m, 9H, ArH and 3'-NH), 6.38 (d, J=10.0, 1H, H-7), 6.13 (dd, J=10.0,6.0, 1H, H-6), 5.92 (m, 1H, H-13), 5.79 (d, J=8.4, 1H, H-3'), 5.08 (d, J=5.6, 1H, H-5), 5.06 (s, 1H, 4-OH), 4.75 (d, J=4.8, H-2), 4.70 (AB quartet, J=9.6, 2H, H-20), 4.45 (d, J=1.6, 1H, H-2'), 3.00 (m, 1H, H-14), 2.59 (m, 1H, H-14), 2.35 (d, J=4.8, 1H, H-3), 1.82 (d, J=1.2, 3H, 18-CH₃), 1.75 (s, 3H, 19-CH₃), 1.31 (s, 3H, 17-CH₃), 1.20 (s, 3H, 16-CH₃), 0.83 (s, 9H, 2'-OSiMe₂^tBu), -0.11 (s, 3H, 2'-OSiMe₂^tBu), -0.32 (s, 3H, 2'-OSiMe₂^tBu). ¹³C NMR (CDCl₃, TMS, 100.578 MHz) δ 169.9, 167.4, 152.4, 151.7, 151.3, 141.7, 139.2, 138.3, 134.8, 133.4, 132.2, 128.8, 128.6, 128.0, 127.0, 126.2, 125.5, 124.5, 89.6, 84.5, 80.6, 80.3, 75.1, 73.8, 70.1, 55.5, 43.8, 42.5, 40.6, 32.7, 25.5, 23.3, 20.3, 19.9, 18.2, 18.1, -5.6, -6.0.

2'-O-(tert-Butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(O-benzylglutaryl)-6,7-dehydro-paclitaxel (2.80) - To a solution of 2'-O-(tert-butylidimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-6,7-dehydro-paclitaxel (2.77, 82 mg, 0.099mmol) in dry toluene (7.0 mL) was added DCC (600 mg, 2.9 mmol), DMAP (10 mg, catalytic amount), and glutaric acid monobenzyl ester (250 μL, 1.35 mmol). The resulting suspension was stirred at room temperature for 4 days and was then filtered through a pad of Celite and was rinsed with EtOAc. The filtrate was concentrated and applied directly on preparative TLC (silica gel, 1000μ, EtOAc:hexanes 4:6) to afford 2'-O-(tert-butylidimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(O-benzylglutaryl)-

6,7-dehydro-paclitaxel (**2.80**, 88 mg, 86%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 7.79 (d, J=7.6, 2H, ArH), 7.52-7.29 (m, 13H, ArH), 7.04 (d, J=10.4, 1H, 3'-NH), 6.30 (s, 1H, H-10), 6.15-6.09 (m, 2H, H-6 and H-13), 6.02 (d, J=10.8, 1H, H-7), 5.65 (dd, J=10.0,2.4, 1H, H-3'), 5.21 (d, J=6.4, 1H, H-5), 5.08 (br s, 2H, 4-COOCH₂Ph), 4.82 (d, J=10.0, 1H, H-20), 4.64 (m, 2H, H-2 and H-2'), 4.60 (d, J=9.6, 1H, H-20), 3.57 (d, J=6.0, 1H, H-3), 2.74-1.78 (m, 8H, H-14 and 4-OCOCH₂CH₂CH₂COO), 2.20 (s, 3H, 10-OAc), 1.88 (s, 3H, 19-CH₃), 1.84 (s, 3H, 18-CH₃), 1.37 (s, 3H, 17-CH₃), 1.18 (s, 3H, 16-CH₃), 0.81 (s, 9H, 2'-OSiMe₂^tBu), -0.05 (s, 3H, 2'-OSiMe₂^tBu), -0.23 (s, 3H, 2'-OSiMe₂^tBu).

2'-O-(tert-Butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(O-benzylglutaryl)-6 α -hydroxy-7-epipaclitaxel (2.81) - To a solution of 2'-O-(tert-butyl dimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(O-benzylglutaryl)-6,7-dehydro-paclitaxel (**2.80**, 60 mg, 0.058 mmol) in acetone/H₂O (4mL/0.8mL) was added N-methylmorpholine-N-oxide (NMO, 60 mg, 0.51 mmol) and osmium tetroxide (OsO₄, catalytic amount). The solution was stirred at room temperature for 40 hours and was then diluted with EtOAc and washed with saturated sodium sulfite (Na₂SO₃) until the aqueous layer was clear. The combined aqueous layer was extracted three times with EtOAc. The organic layer was combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, EtOAc:hexanes 6:4) to 2'-O-(tert-butyl dimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(O-benzylglutaryl)-6 α -hydroxy-7-epipaclitaxel (**2.81**, 50 mg, 81%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 7.78 (d, J=8.4, 2H, ArH), 7.53-7.28 (m, 13H, ArH), 7.04 (d, J=9.2, 1H, 3'-NH), 6.73 (s, 1H, H-10), 6.18 (t, J=8.8, 1H, H-13), 5.63 (dd, J=9.2,2.4, 1H, H-3'), 5.07 (AB quartet, 2H, 4-COOCH₂Ph), 4.77 (s, 1H, H-5), 4.75 (d, J=9.6, 1H, H-20), 4.64 (d, J=6.0, H-2), 4.62 (d, J=2.4, 1H, H-2'), 4.56 (d, J=9.2, 1H, H-20), 4.22, (m, 1H, H-6), 4.17 (d, J=9.2, 1H, 7-OH), 3.78 (dd, J=9.2,5.6, 1H, H-7), 3.63 (d, J=6.4, 1H, H-3), 2.84 (m, 2H, OCOCH₂CH₂CH₂COO and OH), 2.70 (m, 1H, OCOCH₂CH₂CH₂COO), 2.51-1.99 (m, 6H, OCOCH₂CH₂CH₂COO and H-14), 2.18 (s, 3H, 10-OAc), 1.89 (s, 3H, 18-CH₃), 1.63 (s, 3H, 19-CH₃), 1.35 (s, 3H, 17-CH₃), 1.16 (s, 3H, 16-CH₃), 0.81 (s, 9H, 2'-OSiMe₂^tBu), -0.04 (s, 3H, 2'-OSiMe₂^tBu), -0.23 (s,

3H, 2'-OSiMe₂^tBu). ¹³C NMR (CDCl₃, TMS, 90.562 MHz) δ 205.2, 173.8, 172.2, 171.6, 169.4, 167.0, 152.4, 144.6, 137.9, 135.6, 134.0, 131.8, 130.5, 128.7, 128.6, 128.3, 128.24, 128.16, 127.0, 126.6, 90.6, 89.8, 82.0, 81.0, 78.4, 75.0, 74.7, 71.7, 70.2, 66.5, 58.8, 55.7, 40.7, 36.3, 34.7, 32.7, 25.5, 24.9, 21.6, 20.7, 20.4, 18.1, 15.9, 14.4, -5.1, -5.6.

2'-O-(tert-Butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-4-glutaryl-6α-hydroxy-7-epipaclitaxel (2.82) - A catalytic amount of palladium on activated carbon (5%) was added to a solution of 2'-O-(tert-butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(O-benzylglutaryl)-6α-hydroxy-7-epipaclitaxel (**2.81**, 50 mg, 0.047 mmol) in EtOAc (2 mL). This suspension was stirred in an atmosphere of hydrogen gas for 2 hours. TLC analysis indicated complete conversion of the starting material to a very polar compound. The solid was removed by filtration and the filtrate was concentrated under reduced pressure to afford 2'-O-(tert-butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-4-glutaryl-6α-hydroxy-7-epipaclitaxel (**2.82**, 41 mg, 87%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 7.80 (d, J=8.8, 2H, ArH), 7.54-7.21 (m, 8H, ArH and 3'-NH), 6.70 (s, 1H, H-10), 6.26 (t, J=10.0, 1H, H-13), 5.64 (d, J=9.2, 1H, H-3'), 4.95 (br s, 1H, H-5), 4.83 (d, J=10.8, 1H, H-20), 4.63 (d, J=6.8, 1H, H-2), 4.60 (d, J=2.0, 1H, H-2'), 4.56 (d, J=10.4, 1H, H-20), 4.20 (d, J=6.4, 1H, H-6), 3.75 (d, J=6.0, 1H, H-7), 3.62 (d, J=6.8, 1H, H-3), 2.90 (m, 1H, OCOCH₂CH₂CH₂COO), 2.66-2.54 (m, 3H, OCOCH₂CH₂CH₂COO and OH), 2.44-1.96 (m, 5H, OCOCH₂CH₂CH₂COO and H-14), 2.16 (s, 3H, 10-OAc), 1.87 (s, 3H, 18-CH₃), 1.62 (s, 3H, 19-CH₃), 1.34 (s, 3H, 17-CH₃), 1.13 (s, 3H, 16-CH₃), 0.79 (s, 9H, 2'-OSiMe₂^tBu), -0.08 (s, 3H, 2'-OSiMe₂^tBu), -0.36 (s, 3H, 2'-OSiMe₂^tBu).

2'-O-(tert-Butyldimethylsilyl)-4-deacetyl--4-glutaryl-6α-hydroxy-7-epipaclitaxel (2.83) - To a solution of 2'-O-(tert-butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-4-glutaryl-6α-hydroxy-7-epipaclitaxel (**2.82**, 285 mg, 0.29 mmol) in dry THF (10 mL) at -78 °C was added PhLi (1.8 M in hexanes, 1.95 mL, 3.48 mmol). The solution was stirred at -78 °C for 20 minutes, and was then diluted with EtOAc, quenched with dilute HCl (1N). The organic layer was separated, and the aqueous layer was extracted

twice with EtOAc. The combined organic layer was washed with dilute sodium bicarbonate, water, and brine, dried over sodium sulfate, concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000 μ , EtOAc:hexanes 7:3) to give 2'-*O*-(*tert*-butyldimethylsilyl)-4-deacetyl-4-glutaryl-6 α -hydroxy-7-*epi*paclitaxel (**2.83**, 240 mg, 78%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.13 (d, J=8.4, 2H, ArH), 7.75 (d, J=7.2, 2H, ArH), 7.57-7.30 (m, 11H, ArH), 7.21 (d, J=8.8, 1H, 3'-NH), 6.81 (s, 1H, H-10), 6.32 (t, J=8.8, 1H, H-13), 5.74 (d, J=7.6, 1H, H-2), 5.67 (d, J=8.8, 1H, H-3'), 4.69-4.65 (m, 3H, H-5, H-2' and OH), 4.39 (d, J=8.8, 1H, H-20), 4.33 (d, J=8.8, 1H, H-20), 4.17 (m, 1H, H-6), 3.84 (d, J=7.6, 1H, H-3), 3.69 (m, 1H, H-7), 3.15 (m, 1H, OCOCH₂CH₂CH₂COO), 2.79 (m, 1H, OCOCH₂CH₂CH₂COO), 2.63 (s, 1H, OH), 2.39-1.87 (m, 6H, OCOCH₂CH₂CH₂COO and H-14), 2.20 (s, 3H, 10-OAc), 1.89 (s, 3H, 18-CH₃), 1.64 (s, 3H, 19-CH₃), 1.22 (s, 3H, 17-CH₃), 1.12 (s, 3H, 16-CH₃), 0.77 (s, 9H, 2'-OSiMe₂^tBu), -0.03 (s, 3H, 2'-OSiMe₂^tBu), -0.35 (s, 3H, 2'-OSiMe₂^tBu).

2'-*O*-(*tert*-Butyldimethylsilyl)-4-deacetyl-4-(*O*-benzylglutaryl)-6 α -hydroxy-7-*epi*paclitaxel (2.84**)** - To a solution of 2'-*O*-(*tert*-butyldimethylsilyl)-4-deacetyl-4-glutaryl-6 α -hydroxy-7-*epi*paclitaxel (**2.83**, 140 mg, 0.13 mmol) in dry toluene (1.5 mL) was added DCC (50 mg, 0.24 mmol), DMAP (catalytic amount), and benzyl alcohol (120 μ L, 1.2 mmol). The solution was stirred at room temperature for 24 hours. TLC analysis indicated complete conversion of the starting material to a less polar product. The solution was then concentrated under reduced pressure. The resulting syrup was purified on preparative TLC (silica gel, 1000 μ , EtOAc:hexanes 6:4) to afford 2'-*O*-(*tert*-butyldimethylsilyl)-4-deacetyl-4-(*O*-benzylglutaryl)-6 α -hydroxy-7-*epi*paclitaxel (**2.84**, 143 mg, 94%) which was directly subjected to the next reaction.

2'-*O*-(*tert*-Butyldimethylsilyl)-4-deacetyl-4-(*O*-benzylglutaryl)-6 α -(2-benzyloxy-acetoxy)-7-*epi*paclitaxel (2.85**)** - To a solution of 2'-*O*-(*tert*-butyldimethylsilyl)-4-deacetyl-4-(*O*-benzylglutaryl)-6 α -hydroxy-7-*epi*paclitaxel (**2.84**, 86 mg, 0.075 mmol) in CH₂Cl₂ (3.0 mL) was added DMAP (64 mg, 0.75 mmol) and 2-benzyloxyacetyl chloride (60 μ L, 0.38 mmol) at 0 °C. The solution was stirred at room temperature for 1 hour. The

reaction mixture was diluted with EtOAc, washed with dilute sodium bicarbonate, water, and brine, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000 μ , EtOAc:hexanes 4:6) to afford 2'-*O*-(*tert*-butyldimethylsilyl)-4-deacetyl-4-(*O*-benzylglutaryl)-6 α -(2-benzyloxy-acetoxy)-7-*epi*paclitaxel (**2.85**, 82 mg, 85%) which was directly subjected to the next reaction.

2'-*O*-(*tert*-Butyldimethylsilyl)-4-deacetyl--4-glutaryl-6 α -(2-hydroxy-acetoxy)-7-

***epi*paclitaxel (2.86)** - A catalytic amount of palladium on activated carbon (5%) was added to a solution of 2'-*O*-(*tert*-butyldimethylsilyl)-4-deacetyl-4-(*O*-benzylglutaryl)-6 α -(2-benzyloxy-acetoxy)-7-*epi*paclitaxel (**2.85**, 74 mg, 0.057 mmol) in EtOAc (3 mL). This suspension was stirred in an atmosphere of hydrogen gas for 2 hours. The solid was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000 μ , EtOAc:hexanes 7:3) to afford 2'-*O*-(*tert*-butyldimethylsilyl)-4-deacetyl-4-glutaryl-6 α -(2-hydroxy-acetoxy)-7-*epi*paclitaxel (**2.86**, 45 mg, 71%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.13 (d, J=7.2, 2H, ArH), 7.74 (d, J=7.6, 2H, ArH), 7.58-7.30 (m, 11H, ArH), 7.19 (d, J=8.8, 1H, 3'-NH), 6.77 (s, 1H, H-10), 6.30 (t, J=8.8, 1H, H-13), 5.75 (d, J=7.2, 1H, H-2), 5.69 (d, J=8.8, 1H, H-3'), 5.29 (dd, J=5.2,2.4, 1H, H-6), 4.82 (d, J=2.0, 1H, H-5), 4.81 (d, J=6.4, 1H, OH), 4.66 (d, J=1.6, 1H, H-2'), 4.45 (d, J=8.4, 1H, H-20), 4.37 (d, J=8.8, 1H, H-20), 4.17 (br s, 2H, 6-OCOCH₂O), 3.92-3.89 (m, 2H, H-7 and H-3), 3.14 (m, 1H, 4-COCCH₂CH₂CH₂COO), 2.80 (m, 1H, 4-COCCH₂CH₂CH₂COO), 2.62 (s, 1H, OH), 2.42-2.02 (m, 6H, 4-COCCH₂CH₂CH₂COO and H-14), 2.18 (s, 3H, 10-OAc), 1.87 (s, 3H, 18-CH₃), 1.71 (s, 3H, 19-CH₃), 1.20 (s, 3H, 17-CH₃), 1.12 (s, 3H, 16-CH₃), 0.77 (s, 9H, 2'-OSiMe₂^tBu), -0.04 (s, 3H, 2'-OSiMe₂^tBu), -0.32 (s, 3H, 2'-OSiMe₂^tBu). ¹³C NMR (CDCl₃, TMS, 100.578 MHz) δ 205.6, 175.0, 174.1, 171.7, 171.1, 169.5, 167.6, 166.9, 140.5, 137.8, 133.9, 133.6, 132.8, 132.1, 130.2, 129.0, 128.9, 128.8, 128.77, 128.1, 127.1, 126.2, 87.4, 84.0, 79.1, 77.8, 75.3, 75.1, 74.9, 74.8, 70.9, 60.6, 57.7, 55.8, 53.7, 42.7, 39.9, 35.0, 32.6, 29.2, 26.0, 25.5, 21.7, 20.8, 20.3, 18.1, 15.5, 14.8, -5.2, -5.9.

2'-O-(tert-Butyldimethylsilyl)-4-deacetyl-6 α -(2''-hydroxy-acetoxy)-7-epipaclitaxel

4,2''-O-cycloglutarate (2.87) - To a solution of 2-chloro-1-methyl-pyridinium iodide (49 mg, 0.19mmol) in acetonitrile (2.5 mL) at 80 °C was added by syringe pump a solution of 2'-O-(tert-butyldimethylsilyl)-4-deacetyl-4-glutaryl-6 α -(2-hydroxy-acetoxy)-7-epipaclitaxel (**2.86**, 29 mg, 0.026 mmol) and triethylamine (37 μ L, 0.27 mmol) in acetonitrile (5.0 mL) over a period of 8 hours. The solution was kept stirring for an additional 1 hour at 80 °C and was then concentrated under reduced pressure. The mixture was purified by preparative TLC (silica gel, 1000 μ , EtOAc:hexanes 5:5) to afford 2'-O-(tert-butyldimethylsilyl)-4-deacetyl-6 α -(2''-hydroxy-acetoxy)-7-epipaclitaxel 4,2''-O-cycloglutarate (**2.87**, 2.5 mg, 9%). ¹H NMR (CDCl₃, TMS, 399.951 MHz) δ 8.13 (d, J=7.2, 2H, ArH), 7.70 (d, J=7.2, 2H, ArH), 7.60-7.35 (m, 11H, ArH), 7.05 (d, J=9.2, 1H, 3'-NH), 6.77 (s, 1H, H-10), 6.27 (t, J=8.8, 1H, H-13), 5.74-5.71(m, 2H, H-3' and H-2), 5.33 (dd, J=5.2,2.4, 1H, H-6), 4.95 (d, J=2.0, 1H, H-5), 4.91 (d, J=16.0, 1H, 6-OCOCH₂O), 4.76 (d, J=11.6, 1H, 7-OH), 4.65 (d, J=2.0, 1H, H-2'), 4.40 (d, J=15.2, 1H, 6-OCOCH₂O), 4.38 (br s, 2H, H-20), 3.91 (dd, J=11.6,5.2, 1H, H-7), 3.82 (d, J=7.6, 1H, H-3), 3.18 (m, 1H, 4-COCCH₂CH₂CH₂COO), 2.86 (m, 2H, 4-COCCH₂CH₂CH₂COO), 2.51 (m, 2H, 4-COCCH₂CH₂CH₂COO), 2.31 (m, 1H, H-14), 2.19 (s, 3H, 10-OAc), 2.14 (m, 2H, 4-COCCH₂CH₂CH₂COO), 1.89 (s, 3H, 18-CH₃), 1.69 (s, 3H, 19-CH₃), 1.19 (s, 3H, 17-CH₃), 1.12 (s, 3H, 16-CH₃), 0.79 (s, 9H, 2'-OSiMe₂^tBu), -0.02 (s, 3H, 2'-OSiMe₂^tBu), -0.26 (s, 3H, 2'-OSiMe₂^tBu).

4-Deacetyl-4-glutaryl-6 α -(2''-hydroxy-acetoxy)-7-epipaclitaxel

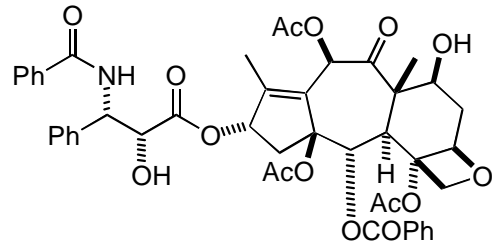
4,2''-O-

cycloglutarate (2.88) - To a solution of 2'-O-(tert-butyldimethylsilyl)-4-deacetyl-6 α -(2''-hydroxy-acetoxy)-7-epipaclitaxel 4,2''-O-cycloglutarate (**2.87**, 2.5 mg, 0.0023 mmol) in dry THF (0.5 mL) was added HF-pyridine (70%, 200 μ L, excess) and the solution was stirred at room temperature for 4 hours. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate and dilute HCl (1N). The organic layers was combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 500 μ , EtOAc:hexanes 7:3) to **2.88**, (1.4 mg, 64%). ¹H NMR (CDCl₃, TMS, 399.951

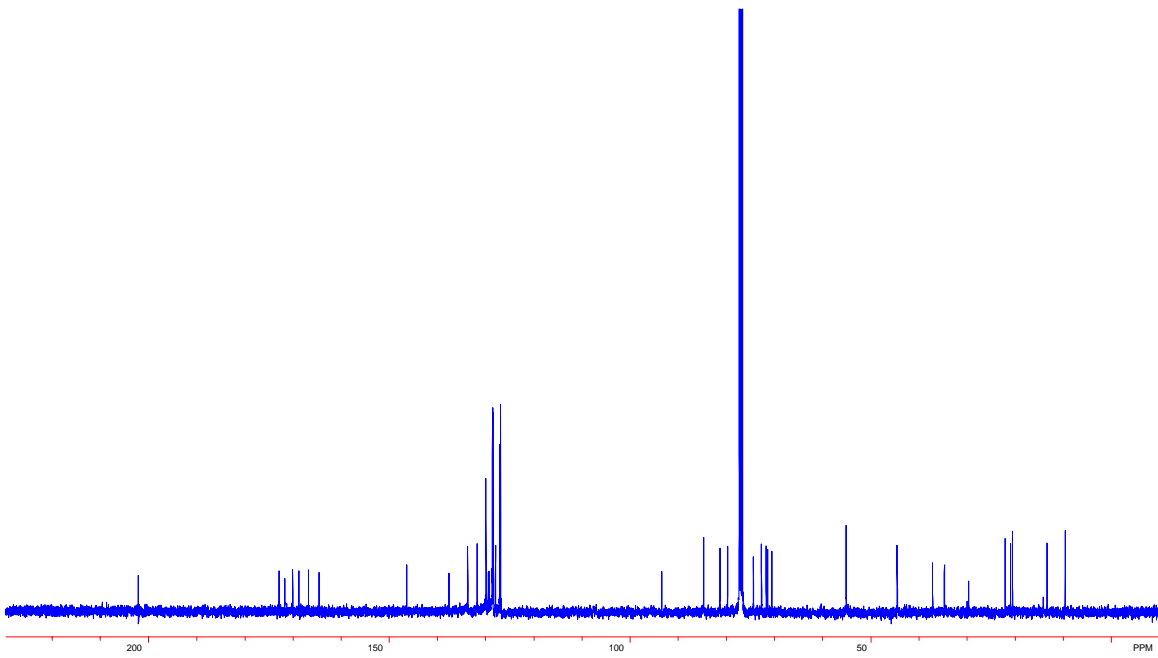
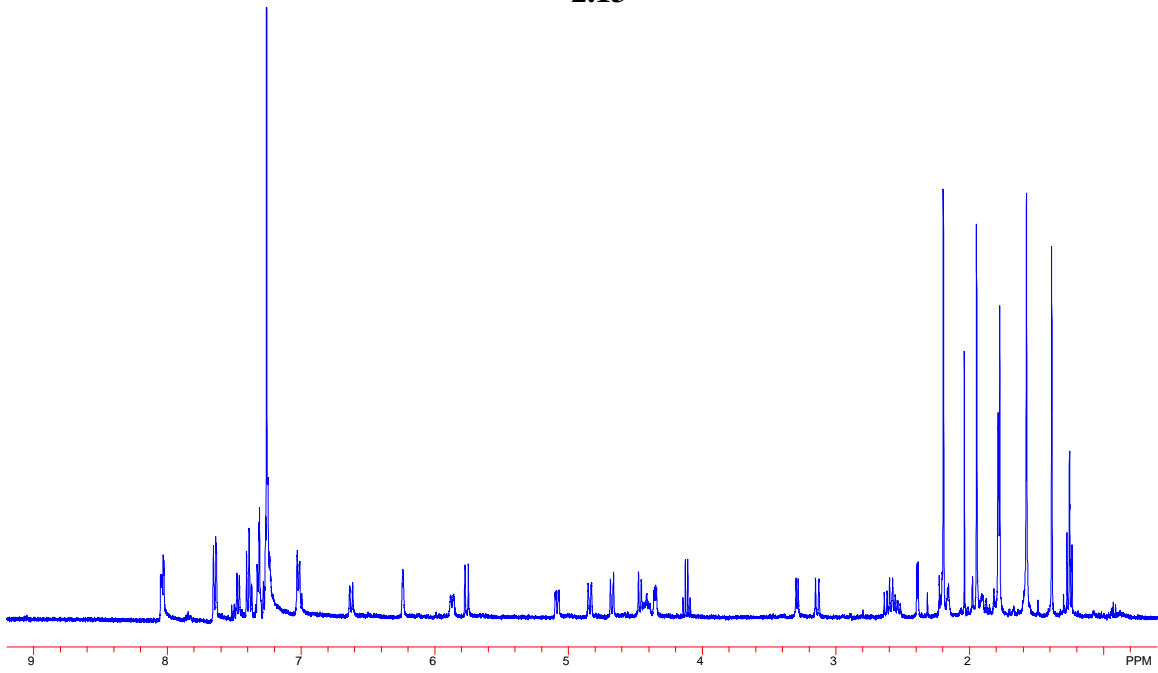
MHz) δ 8.12 (d, $J=7.6$, 2H, ArH) 7.77 (d, $J=7.2$, 2H, ArH), 7.64-7.30 (m, 12H, ArH and 3'-NH), 6.78 (s, 1H, H-10), 6.15 (t, $J=8.8$, 1H, H-13), 5.81 (dd, $J=8.8, 2.8$, 1H, H-3'), 5.73 (d, $J=7.2$, 1H, H-2), 5.32 (dd, $J=4.0, 4.0$, 1H, H-6), 5.12 (d, $J=11.6$, 1H, 7-OH), 5.02 (m, 1H, H-5), 4.66 (d, $J=2.8$, 1H, H-2'), 4.47 (d, $J=8.8$, 1H, H-20), 4.46 (d, $J=16.0$, 1H, 6-OCOCH₂O), 4.39 (d, $J=16.0$, 1H, 6-OCOCH₂O), 4.34 (d, $J=8.8$, H-20), 4.29 (d, $J=4.8$, 1H, OH), 3.86 (d, $J=7.2$, 1H, H-3), 3.74 (dd, $J=12.0, 4.0$, 1H, H-7), 2.84 (t, $J=6.8$, 2H, 4-COCCH₂CH₂CH₂COO), 2.47-1.86 (m, 6H, H-14, 4-COCCH₂CH₂CH₂COO), 2.09, 1.91, 1.69, 1.19, 1.12. ¹³C NMR (CDCl₃, TMS, 90.562 MHz) δ 205.8, 174.3, 172.1, 171.8, 170.3, 167.2, 166.9, 166.6, 141.1, 138.5, 134.05, 133.95, 132.6, 131.7, 130.2, 128.9, 128.8, 128.4, 127.9, 127.4, 127.2, 86.8, 84.4, 79.2, 78.0, 77.2, 76.2, 75.5, 74.9, 72.7, 71.3, 60.7, 57.8, 55.1, 42.5, 40.2, 36.6, 34.6, 32.8, 25.8, 21.5, 20.8, 19.2, 16.7, 14.8. FABMS calculated for C₅₂H₅₅NO₁₈ (M+Li)⁺ 988, found 988.

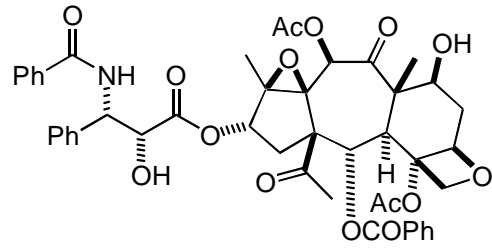
5. Appendix

^1H and ^{13}C NMR spectra of new paclitaxel analogs are attached. 2D-NMR spectra are available, but not included.

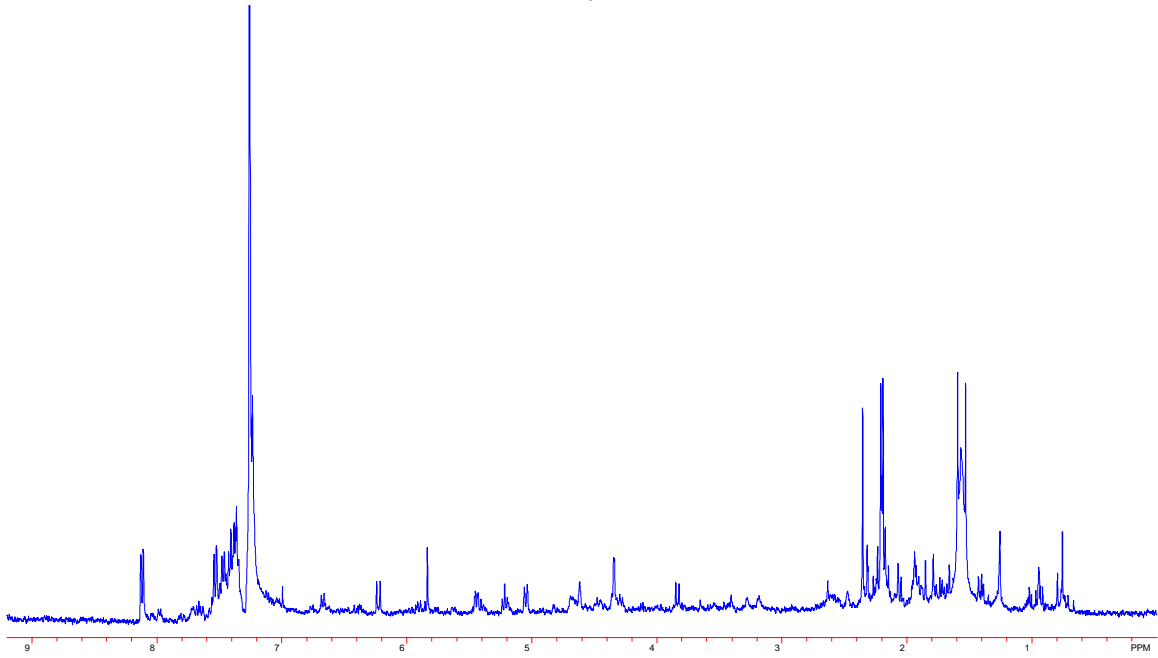


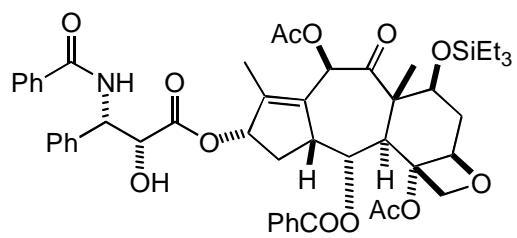
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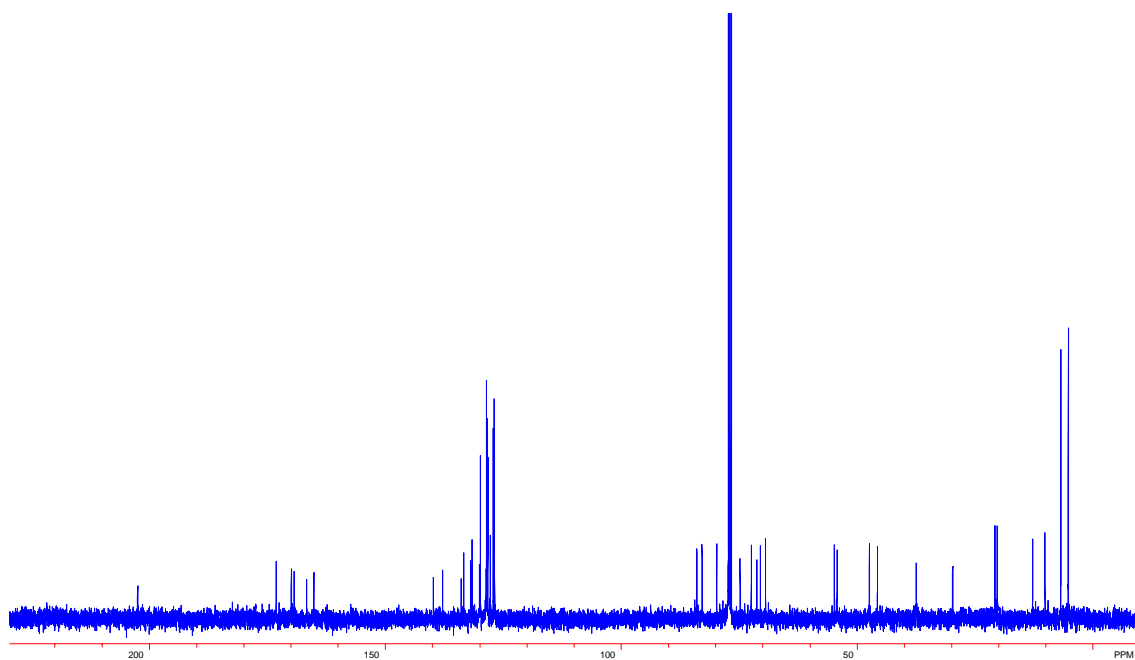
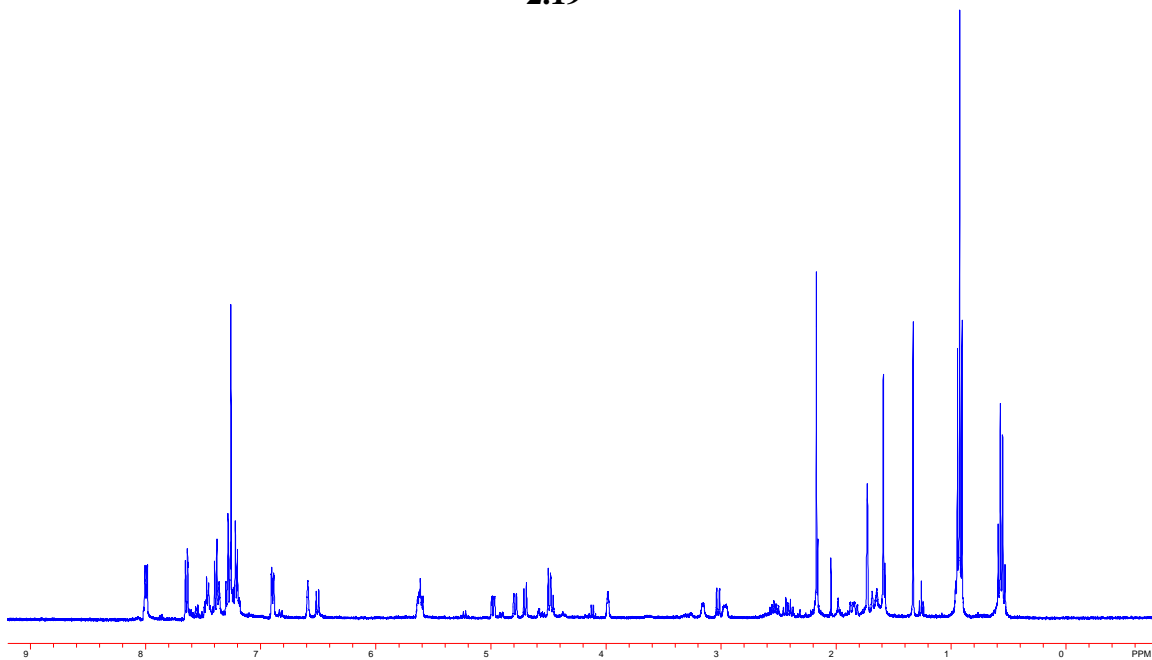


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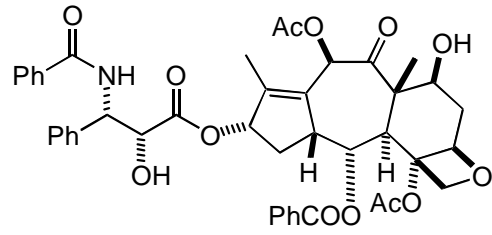




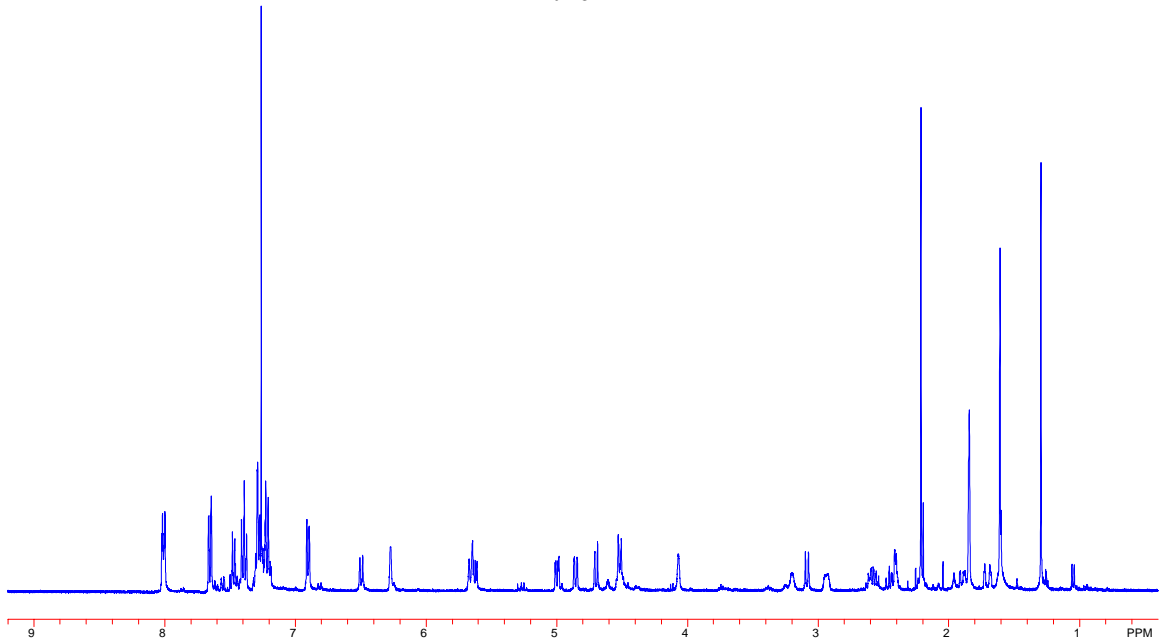
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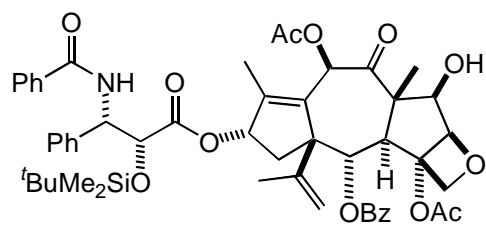
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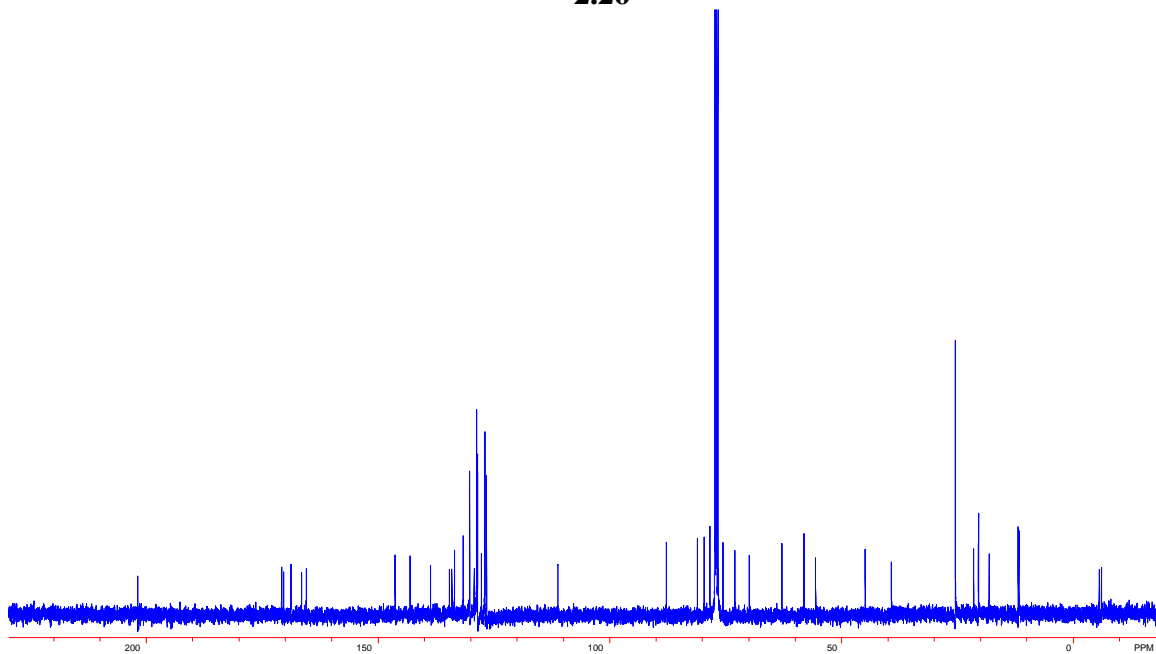
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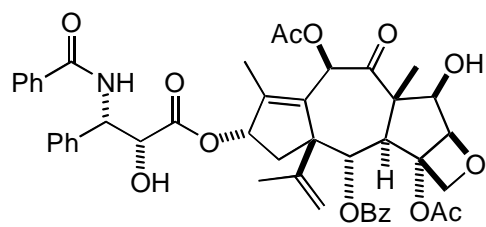


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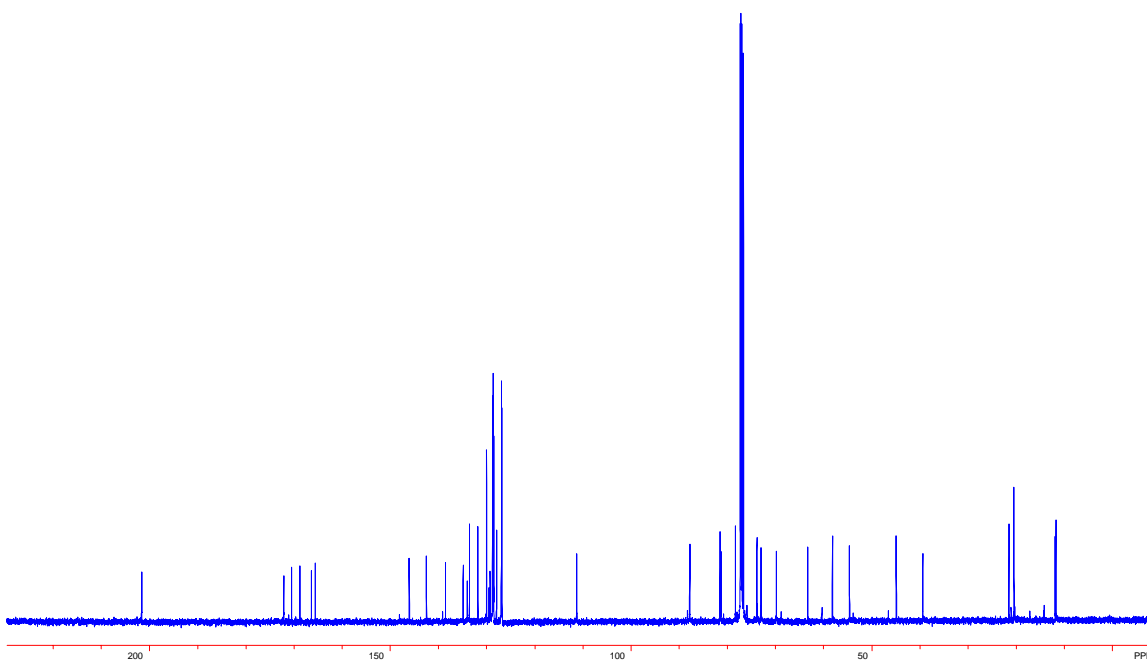
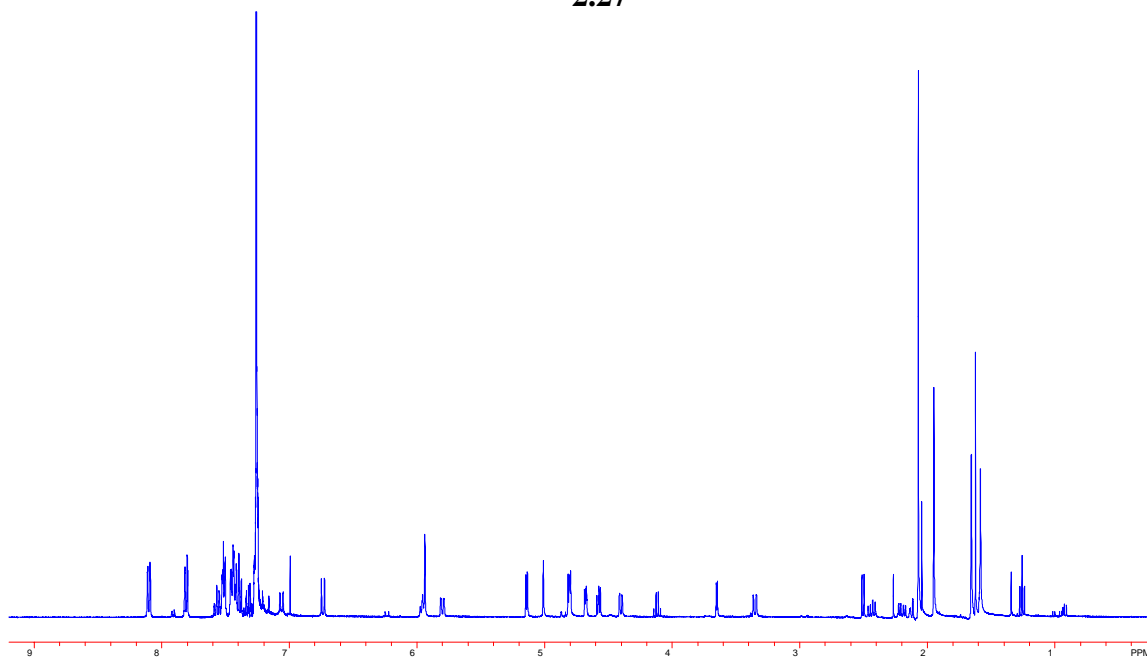


2.26

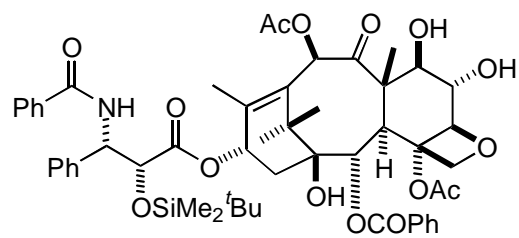




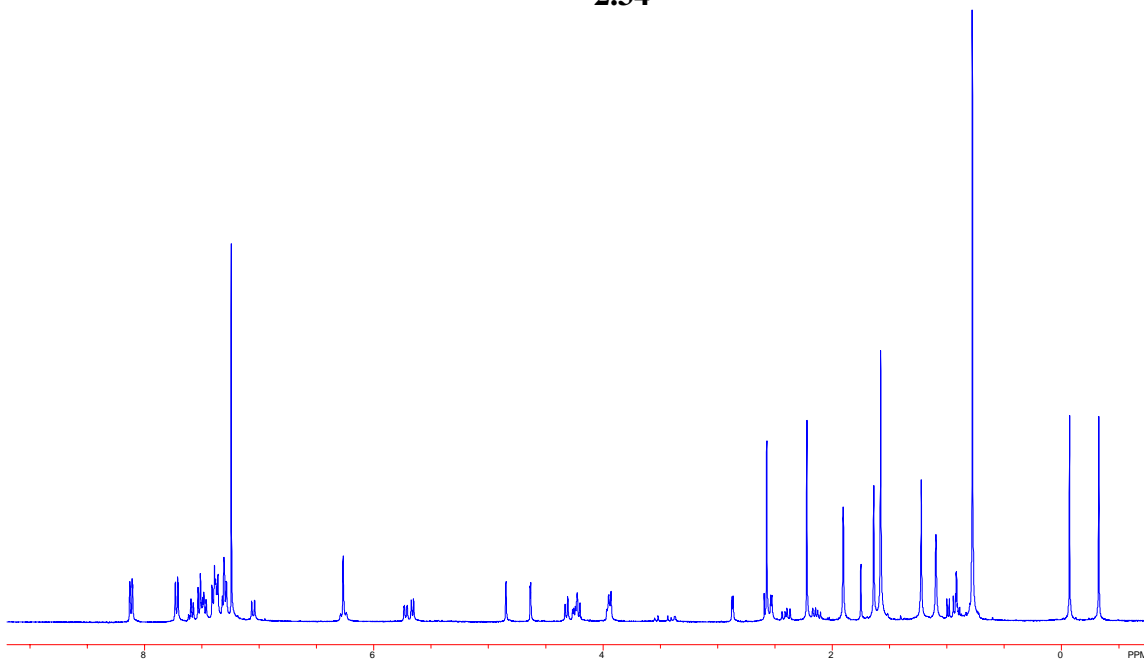
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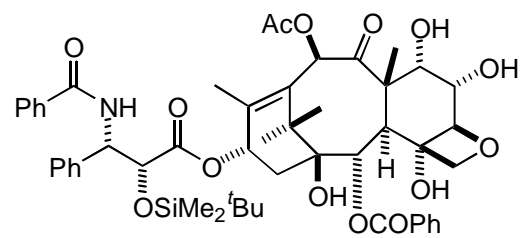


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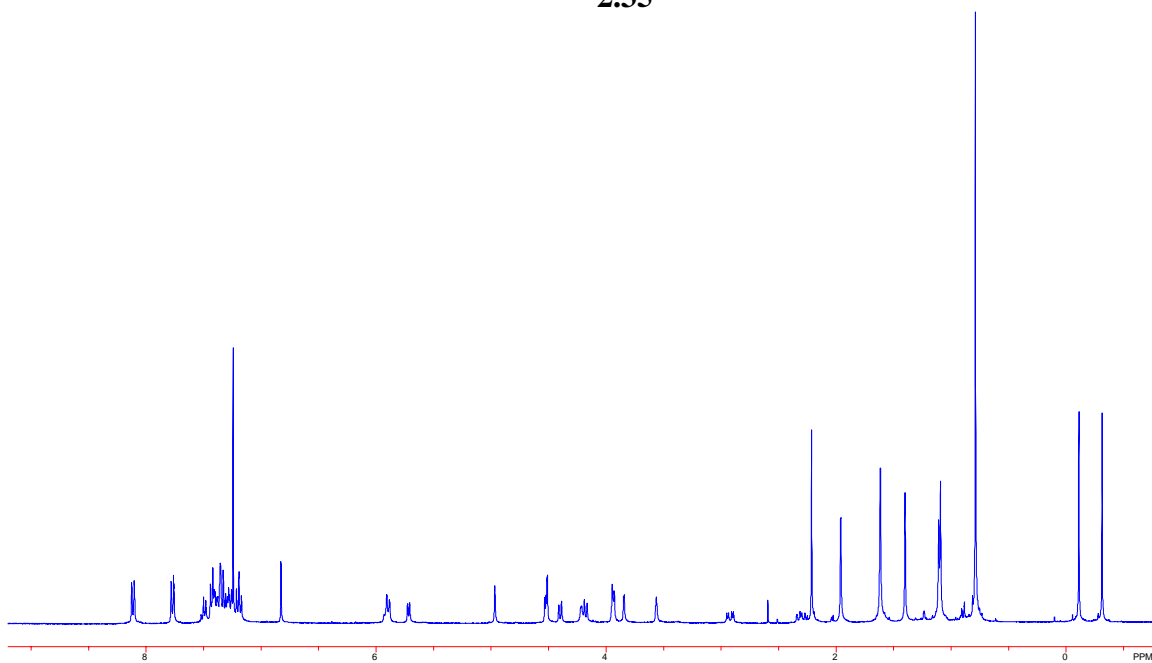


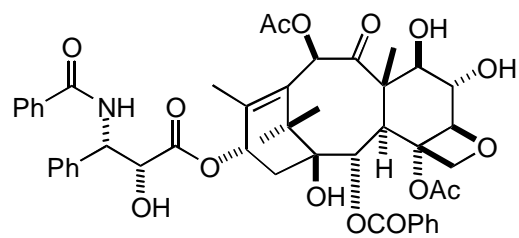
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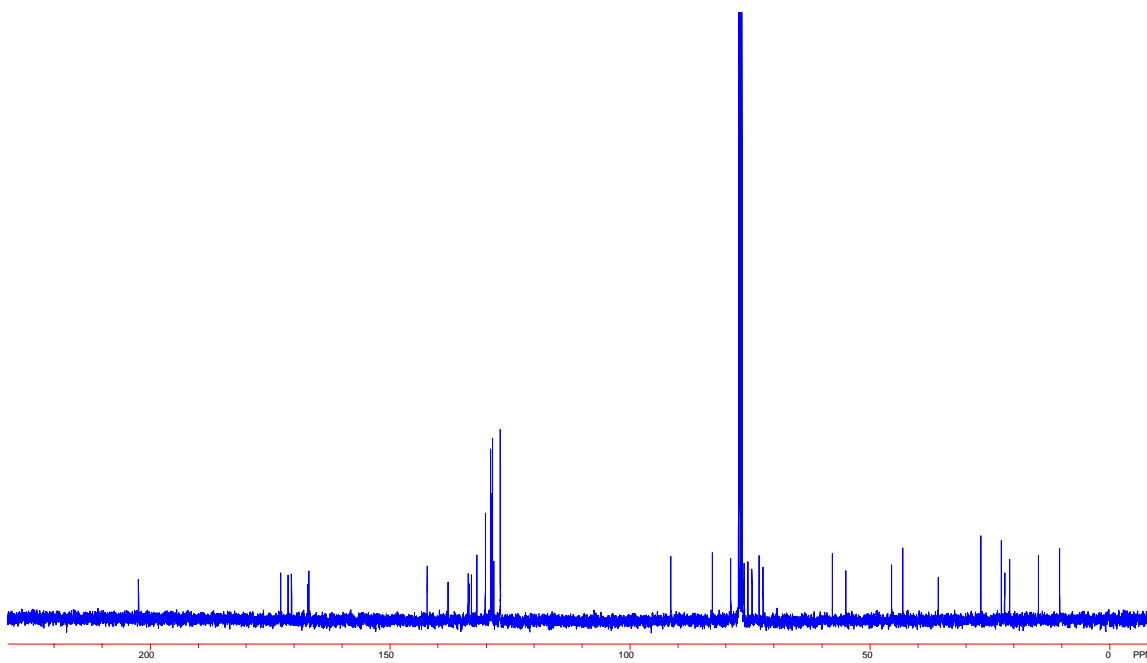
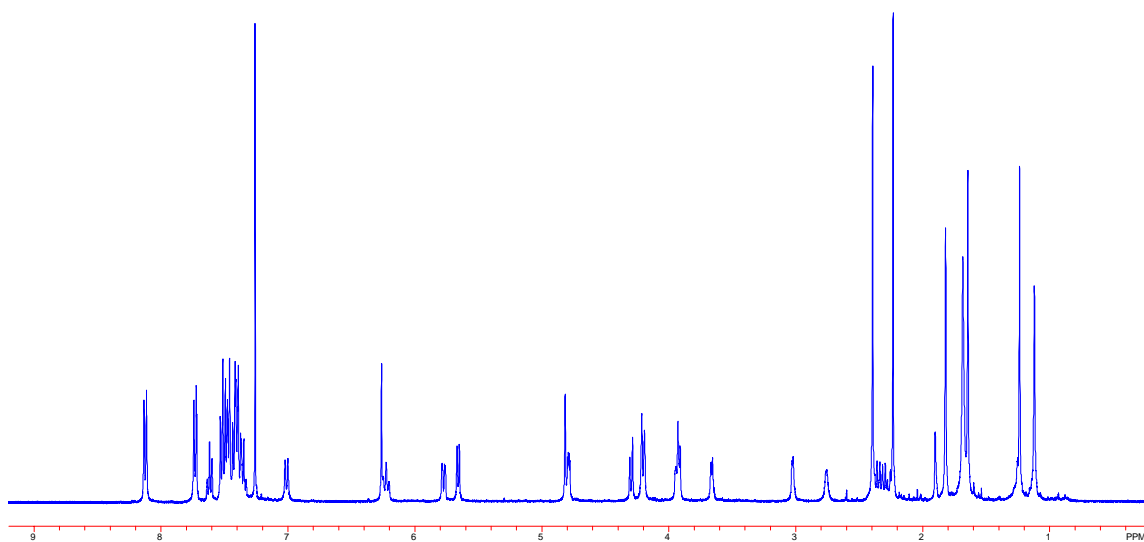


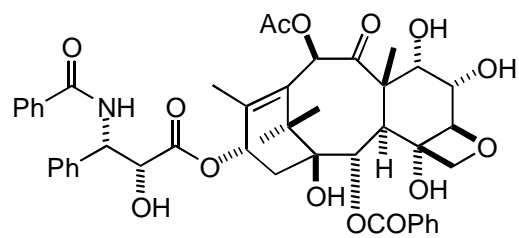
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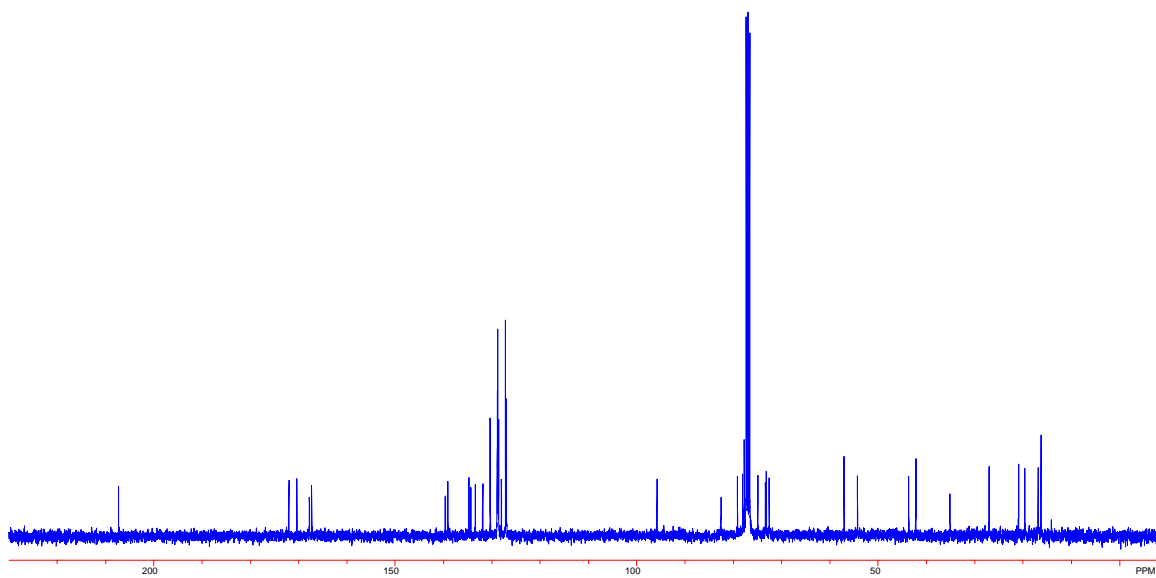
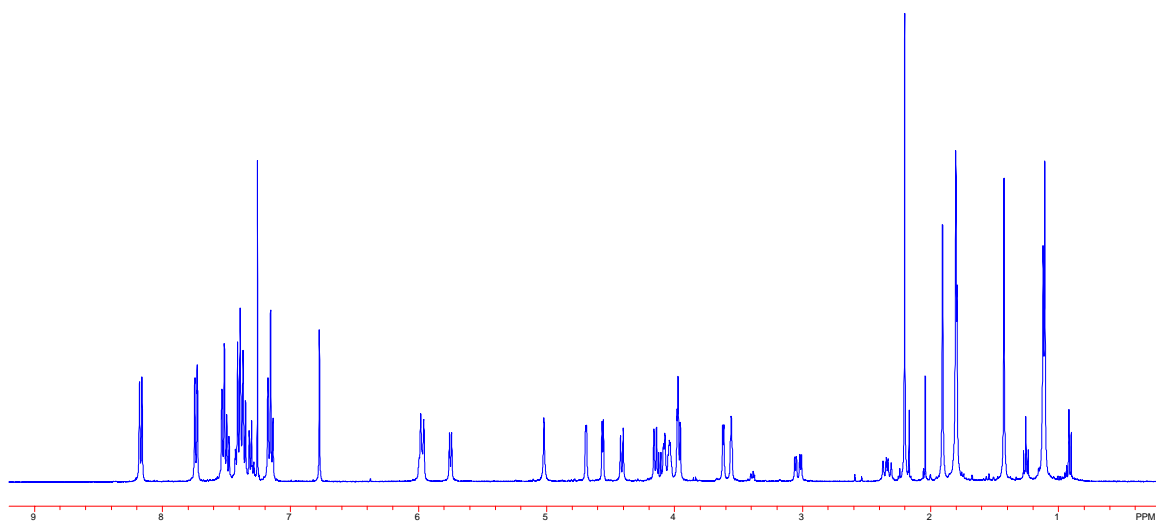


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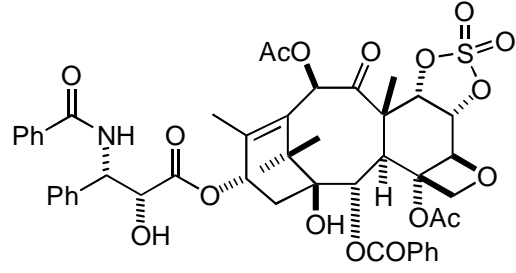




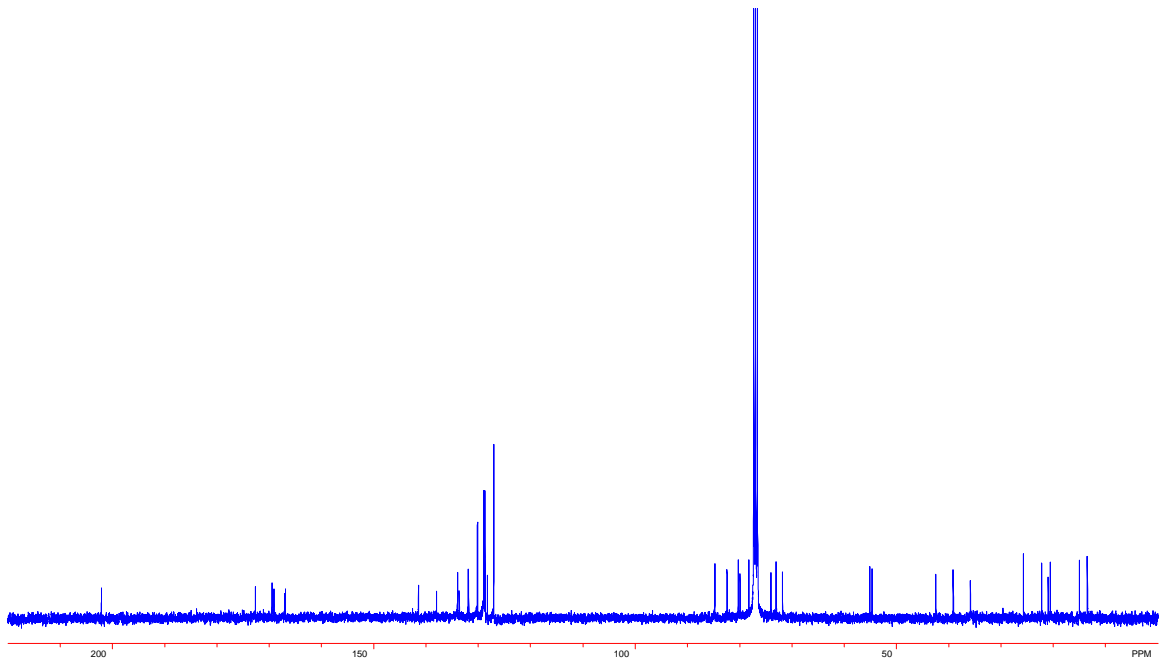
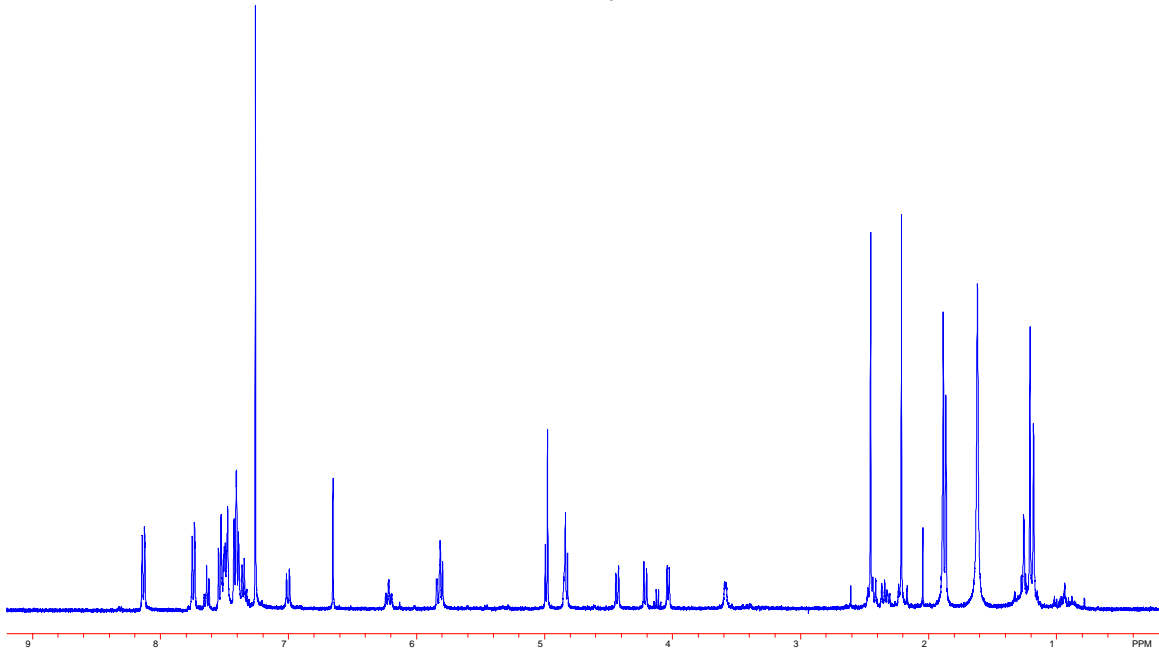
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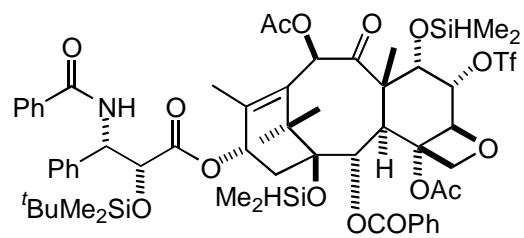


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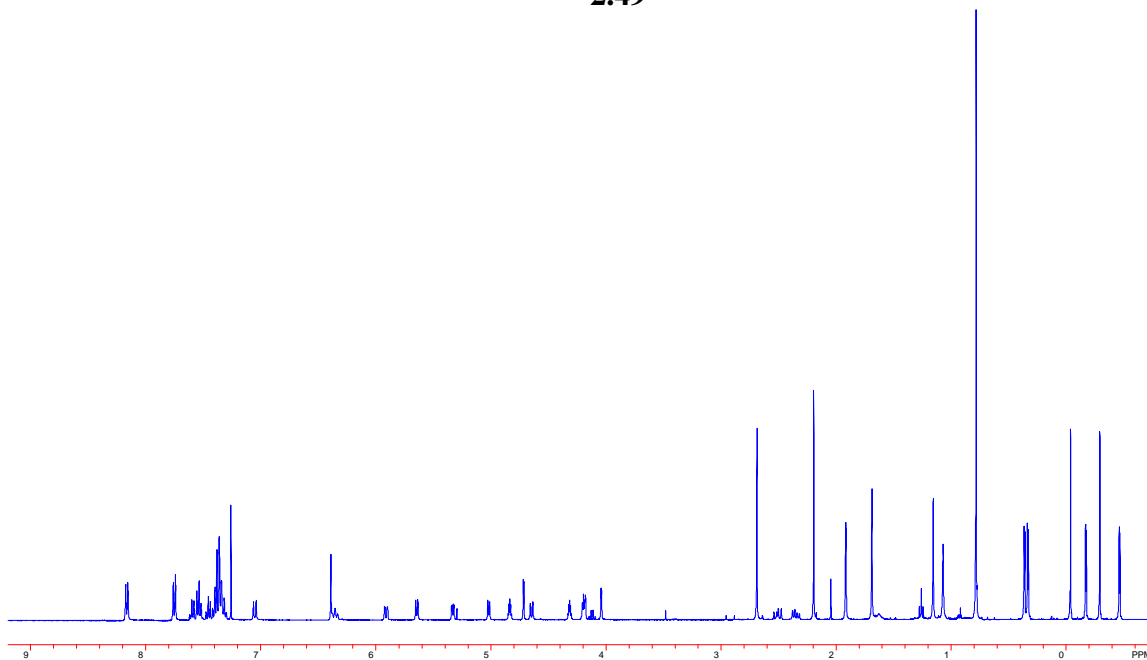


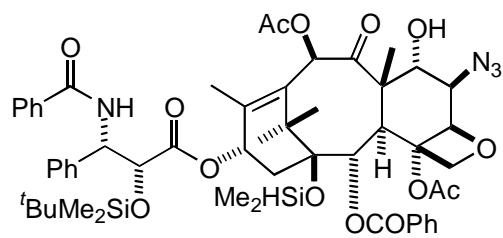
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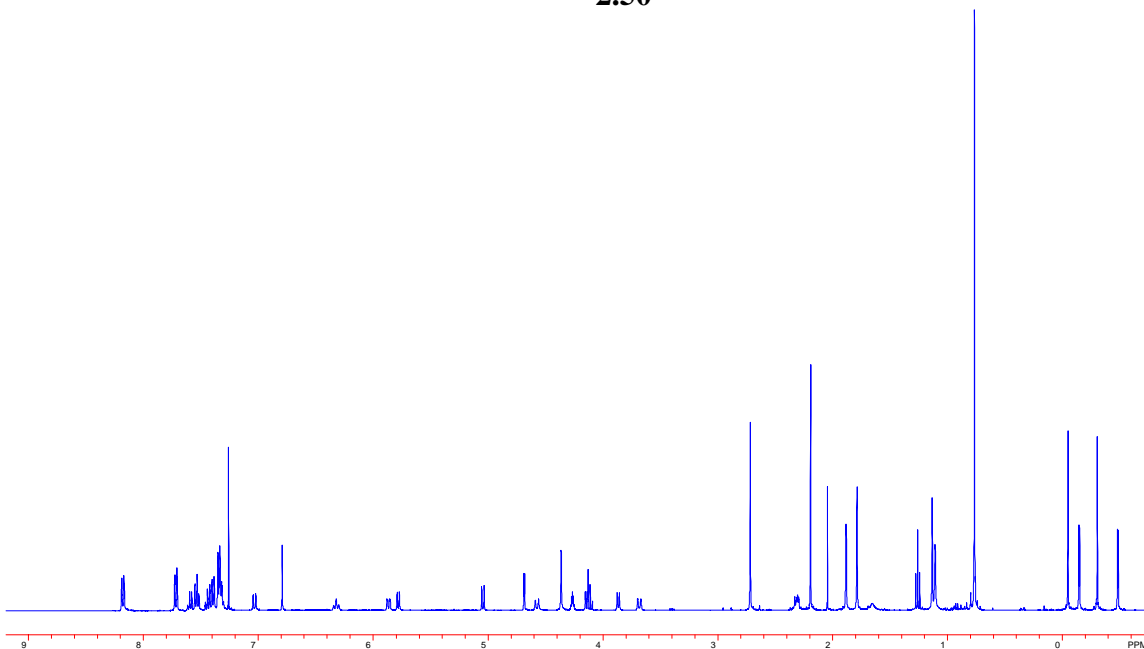


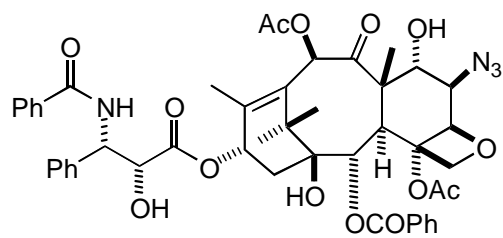
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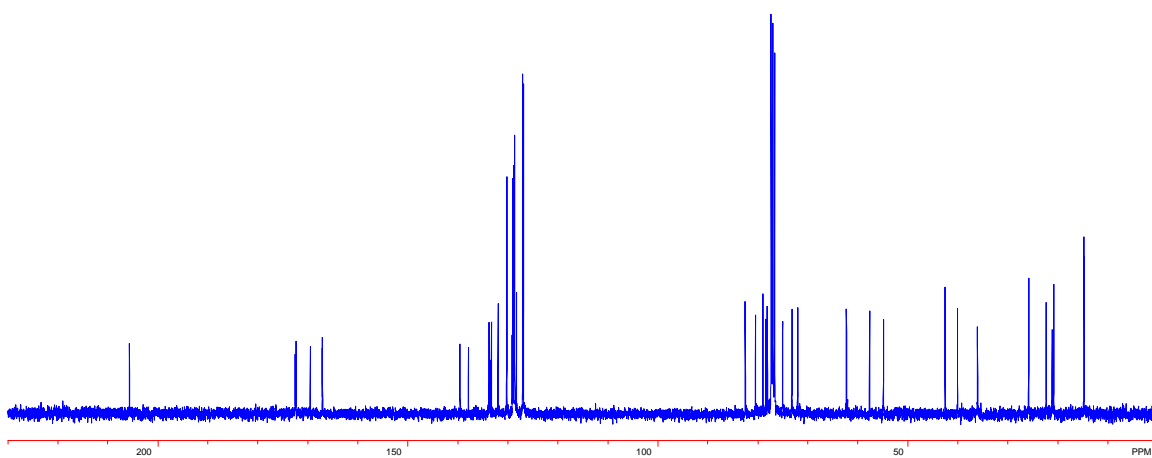
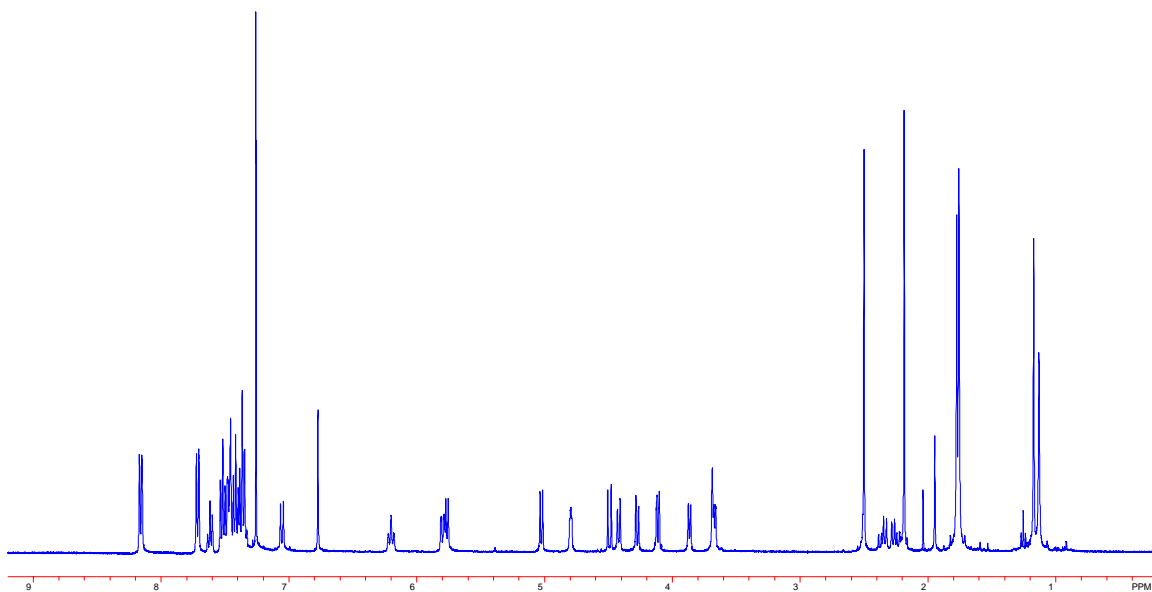


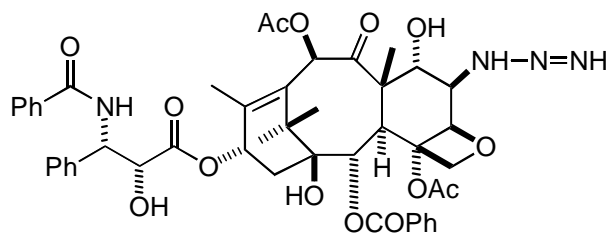
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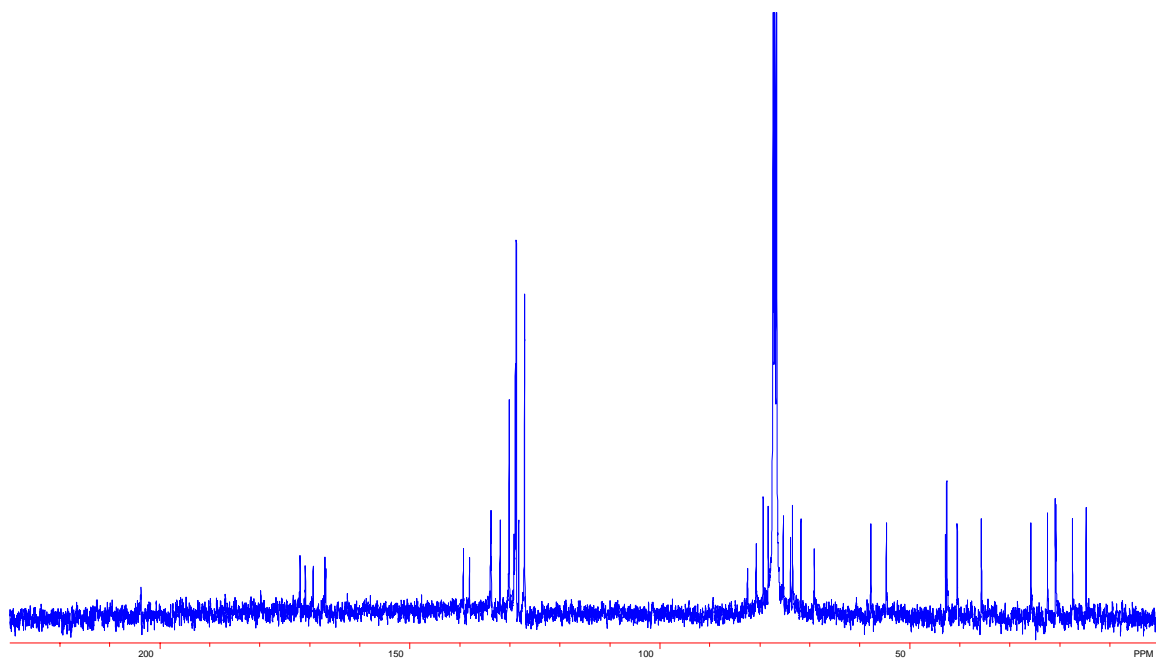
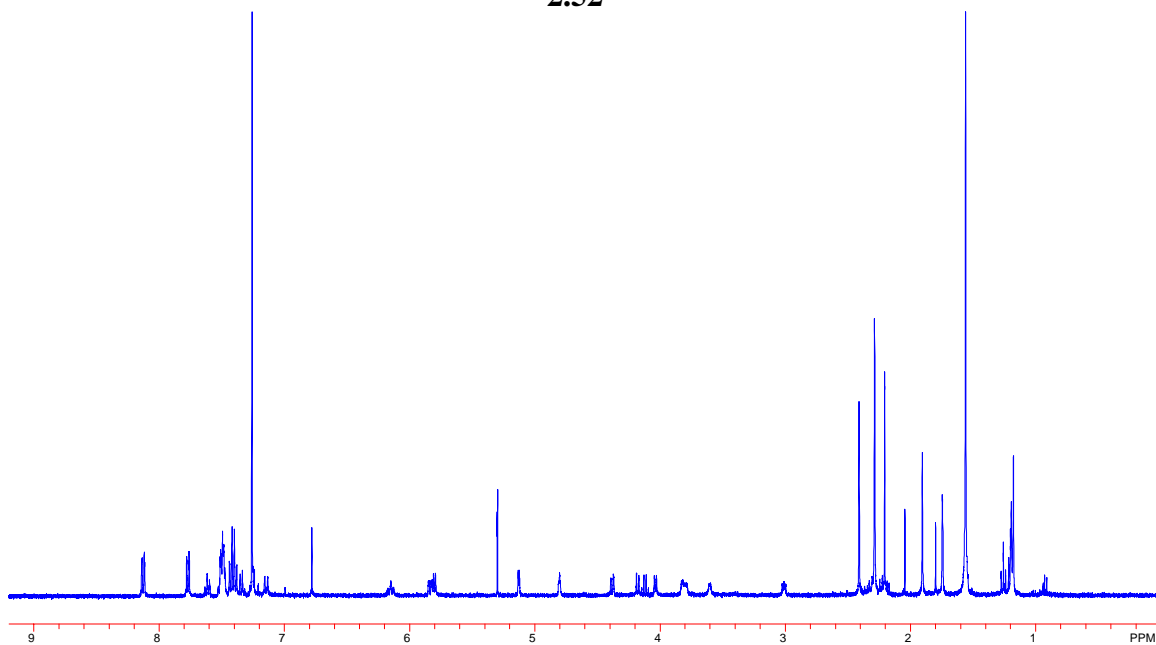


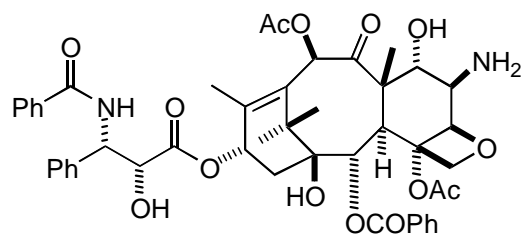
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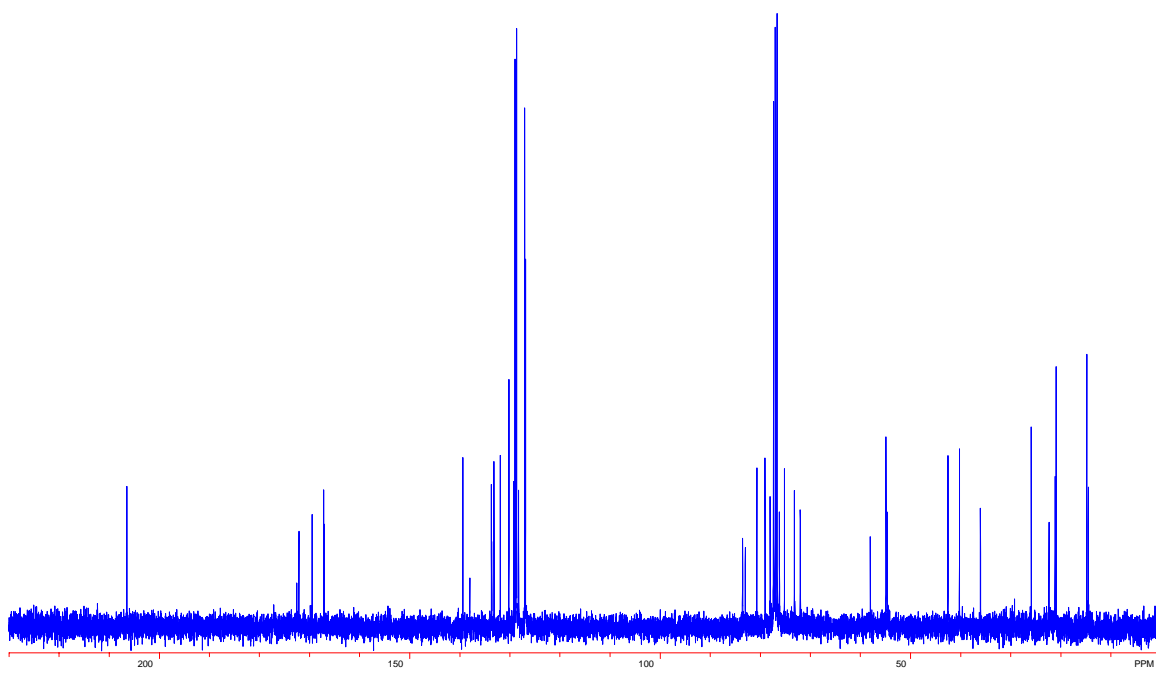
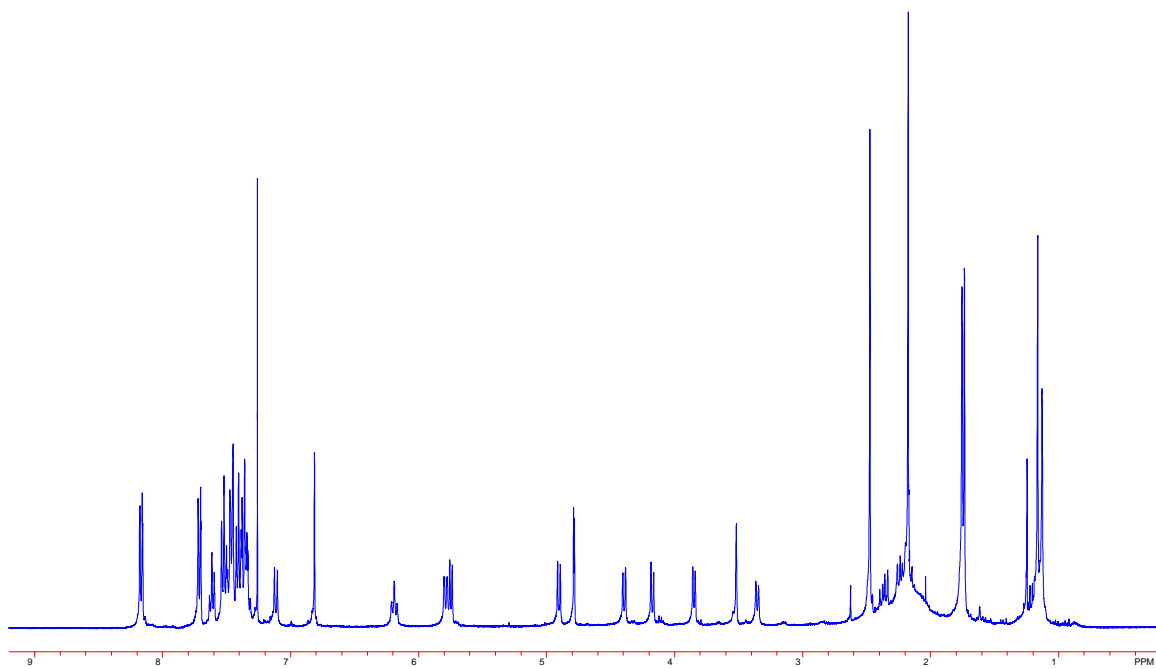


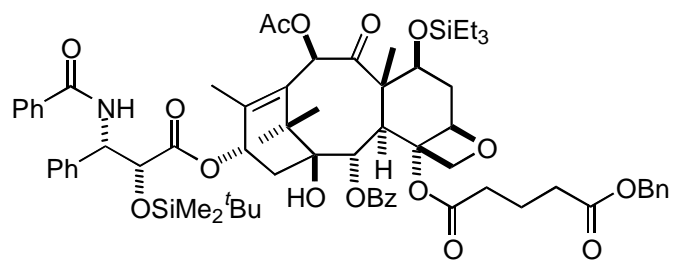
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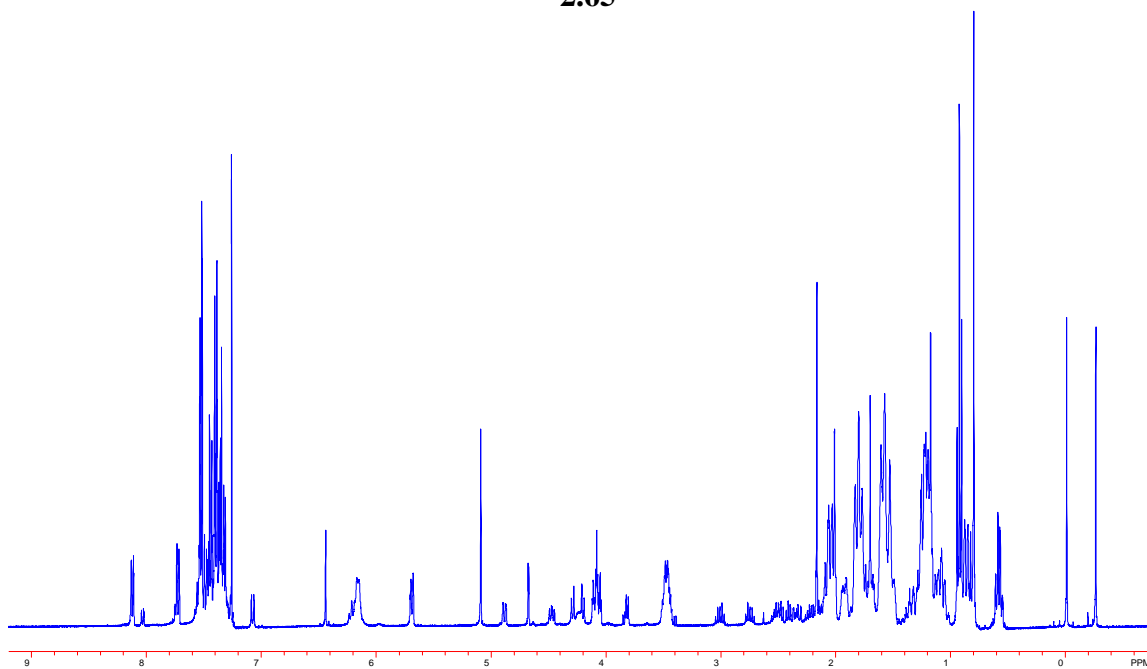


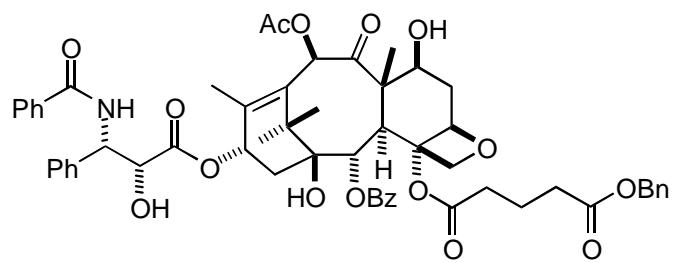
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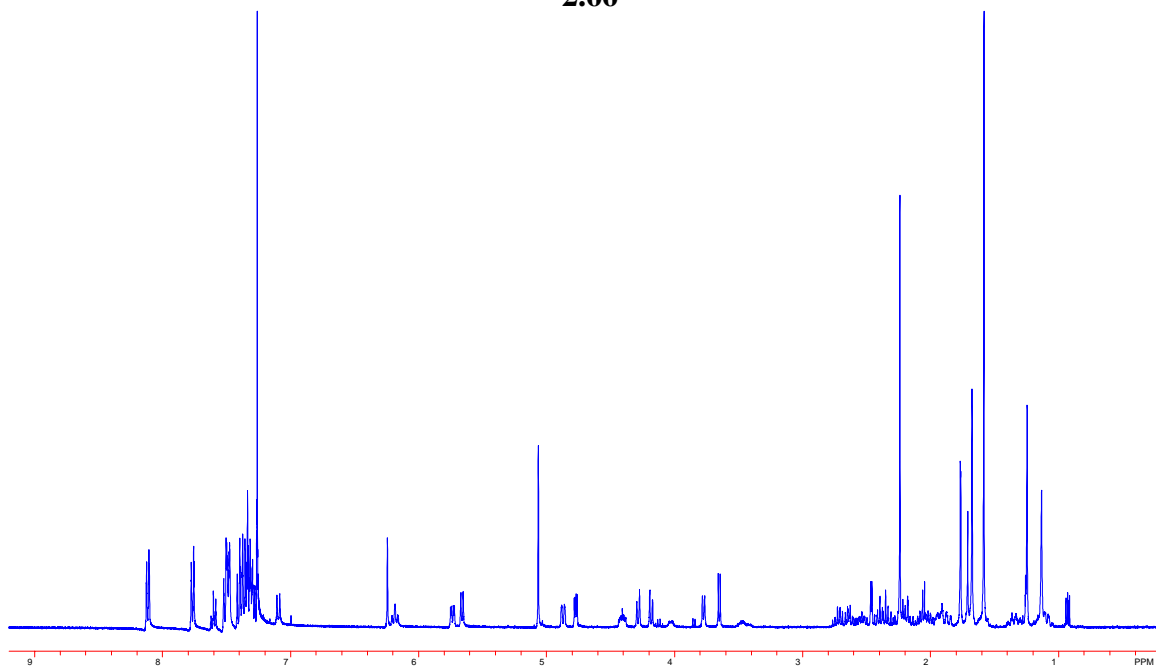


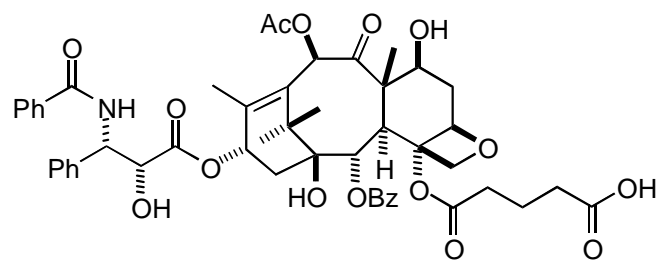
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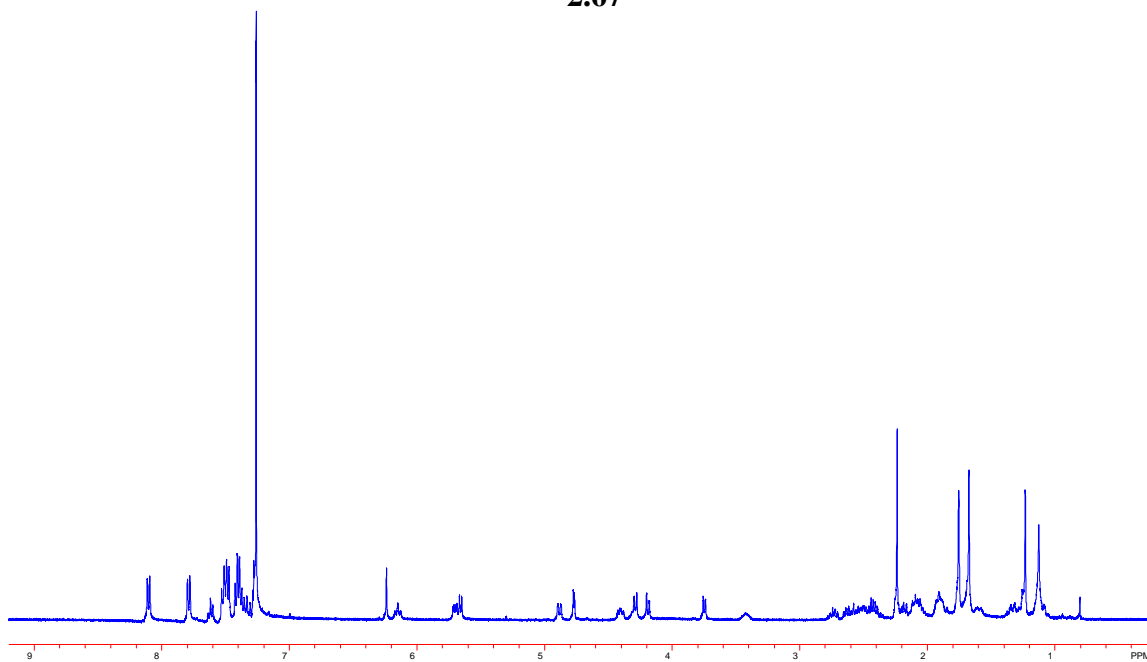


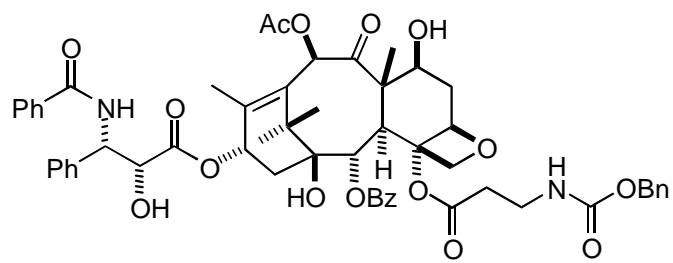
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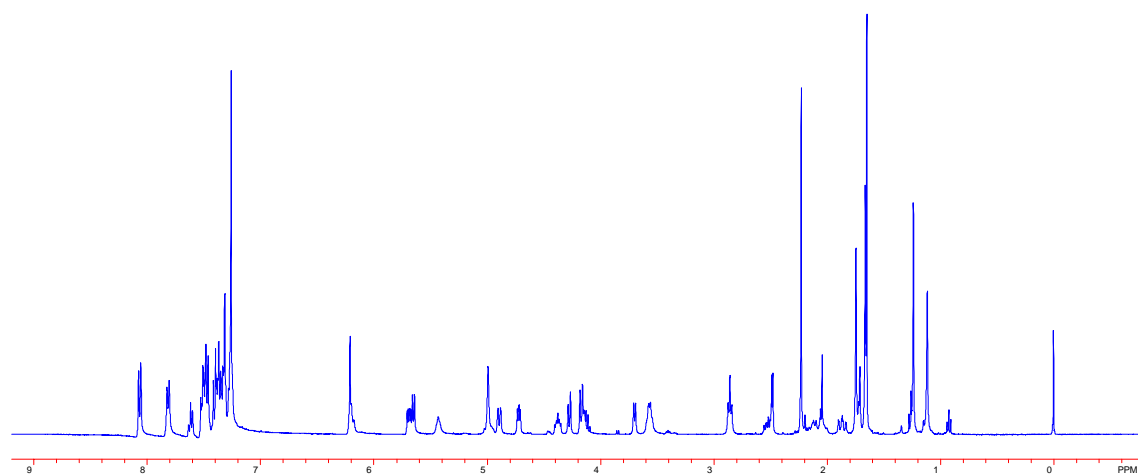


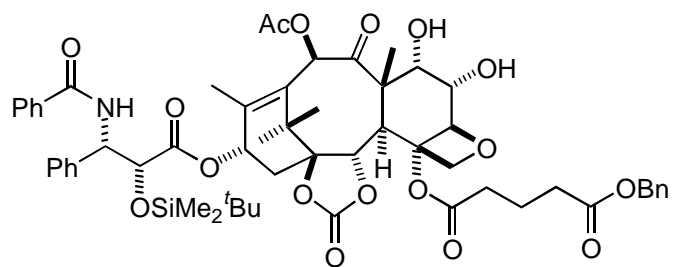
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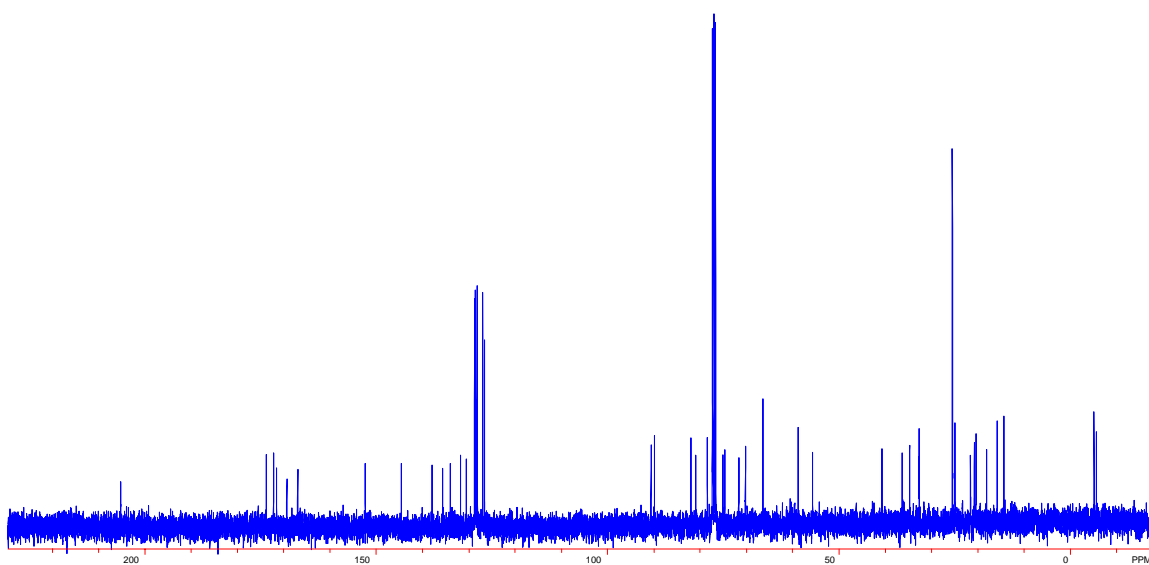
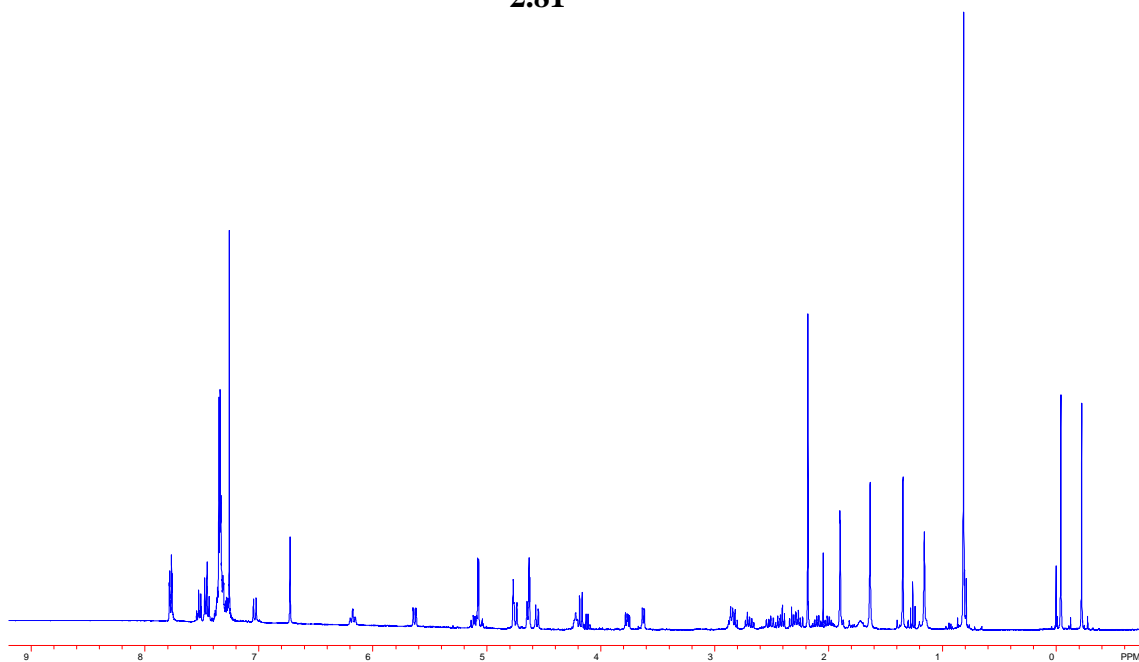


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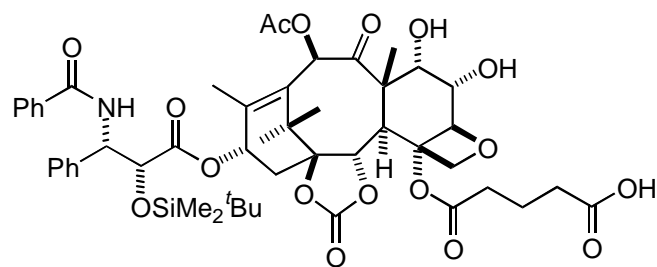




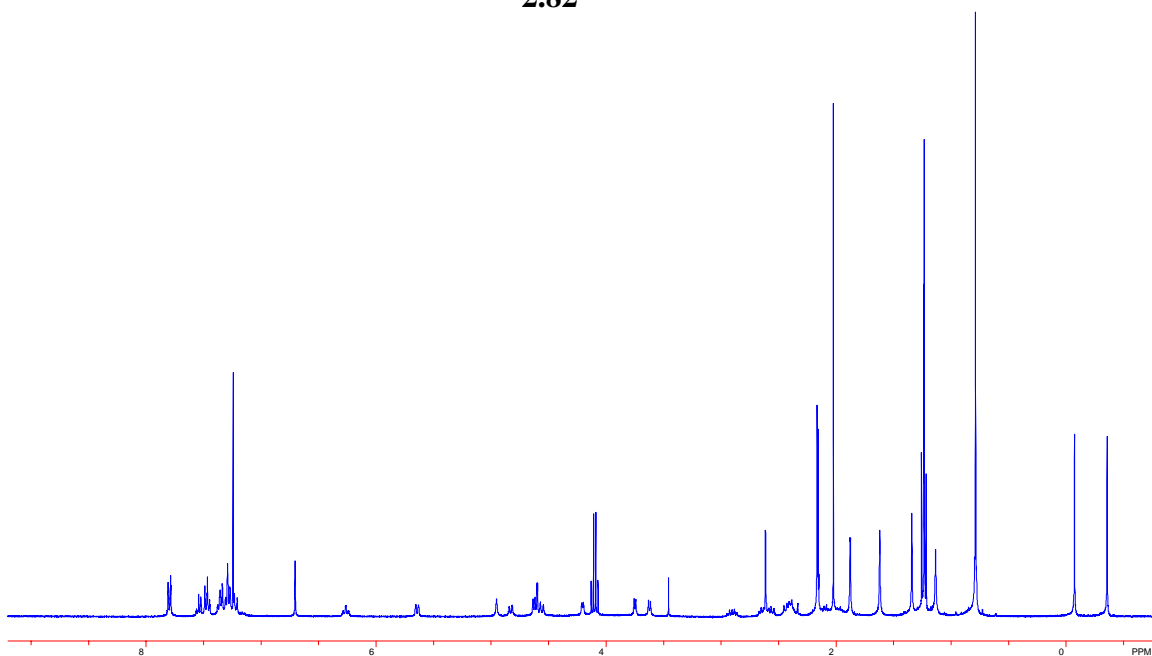
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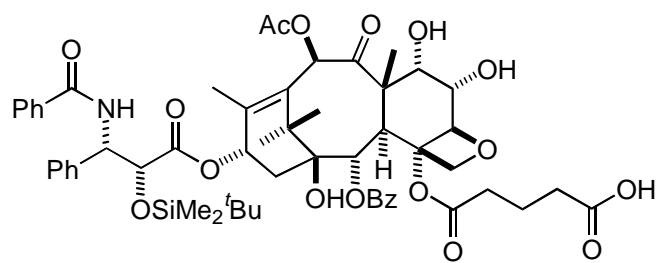
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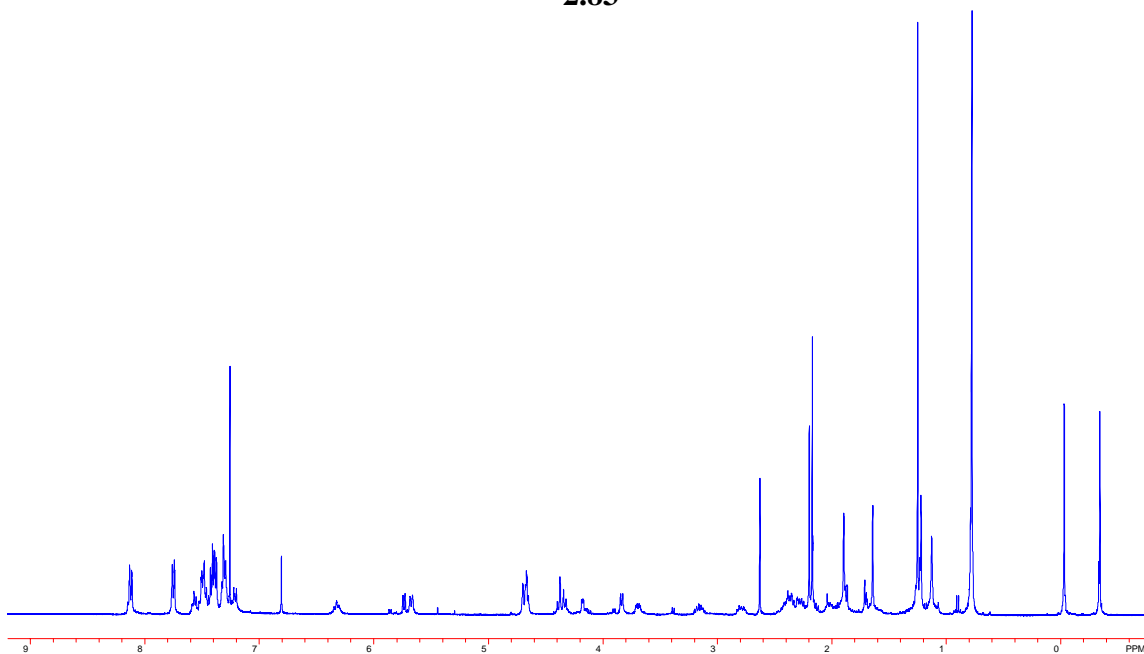
2.82



HH



2.83



6. Vita

HAIQING YUAN

Born on May 21, 1966 in Zhengjiang, Jiangsu, China, he attended the First High School of Zhengjiang and graduated in 1984. He received his Bachelor of Science degree *summa cum laude* in Chemistry from Fudan University in July, 1988. He then worked as an analytical chemist in Shenzhen Science & Technology Industry Park for two years. In 1990, he moved back to Nanjing, Jiangsu and was employed by the Research Institute of Yangzi Petrochemical Corporation. In 1994, he decided to pursue a Ph.D. degree in Chemistry and moved abroad to Blacksburg, Virginia to join Dr. David G. I. Kingston's group in Virginia Polytechnic Institute and State University.