Studies on Water-Soluble Taxol Derivatives

by

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Chemistry

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Blacksburg, Virginia

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(ABSTRACT)

The importance of taxol as an anticancer drug lies not only in its activity in antitumor assays but also in its unique mechanism of action. Unfortunately, taxol is not water-soluble and therefore must be given in conjunction with emulsifying agents. Modifications of taxol were carried out in order to prepare water-soluble taxol derivatives. The C-2′ hydroxyl group of taxol was substituted with various groups to increase water solubility. The synthesized taxol derivatives, 2′-((3-sulfo-1-oxopropyl)oxy)taxol sodium salt, 2′- ((4-((2- sulfoethyl)amino)- 1,4- dioxobutyl)oxy)taxol sodium salt, and 2′-((4-((3-sulfopropyl)amino)- 1,4-dioxobutyl)oxy)taxol sodium salt were more water-soluble than taxol. The synthetic pathways to these compounds are compared and discussed.

Acknowledgements

The author wishes to express his gratitude and appreciation to Professor David G. I. Kingston for his supervision and encouragement during this research project.

Grateful acknowledgements are also made to Professors Harold M. Bell, Harry C. Dorn, and James F. Wolfe for their valuable advice and discussions.

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Table of Contents

List of Illustrations

List of Tables

INTRODUCTION

Natural Products in Cancer Chemotherapy

Drugs from plants and animals have played important roles in the treatment of various diseases for several hundred years. Approximately one fourth of the drugs currently used in Western medicine are derived from plants. The National Cancer Institute Program for discovery of new and clinically useful anticancer drugs from plants has demonstrated that 3-4% of plant species produced a great variety of anticancer agents of very diverse structural types (1-4).

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

Taxol and Taxane Derivatives

The constituents in the Taxaceae family have been studied for over a hundred years. In 1856, Lucas(5) reported the isolation of an alkaloid called taxin from the needles and other parts of the English yew, Taxus baccata L. It was later found to be a mixture of many alkaloids (6-8). In 1963, Taylor reported the isolation of a compound called baccatin from the heartwood of T. baccata (9). Taylor's compound was later named baccatin I by Halsall who himself isolated baccatin II, baccatin III and baccatin IV (10).

Between 1967 and 1970, many taxane derivatives were isolated from various plants (11). Among these, only the baccatin-type compounds have the unique oxetane ring at C-4 and 5; other derivatives either have an exocyclic double bond at C-4 or no substituent at this position.

In 1971, Wani and co-workers (12), guided by a biological activity test, isolated taxol [1] from the stem bark of the Pacific yew, T. brevifolia. Taxol is also found in other Taxus species, such as T. baccata and T. cuspidata (12). It possesses important antileukemic and other anticancer activities and is currently in Phase II clinical trial at the National Institutes of Health, where it shows encouraging activity against ovarian cancer. The structure of taxol, numbered according to the IUPAC system, is shown in Figure 1.

In 1979, a new antitumor alkaloid called cephalomannine [**2**] was isolated from T. wallichiana by Powell et al. (13,14) and was found to be cytotoxic in KB cell culture and also showed potent inhibition of PS leukemia in mice. The structure of cephalomannine is also shown in Figure 1. Two new taxane derivatives, 10-deacetyltaxol and

10-deacetylcephalomannine, were also isolated from T. wallichiana by McLaughlin et al. (15).

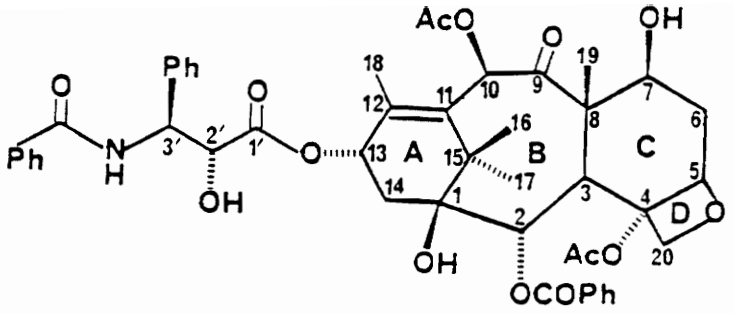
Other taxane derivatives were reported in 1982 and 1986 by Kingston and co-workers(16, 17) who isolated various taxane derivatives from T. brevifolia. In addition, Potier and co-workers obtained other new taxanes from T. baccata (18).

Due to the important antileukemic and cytotoxic activity and the unique structure of taxol, several research groups have been engaged in synthetic approaches to this compound. Semisynthesis of some taxane derivatives has recently been reported(19).

Structure of the Taxane Skeleton

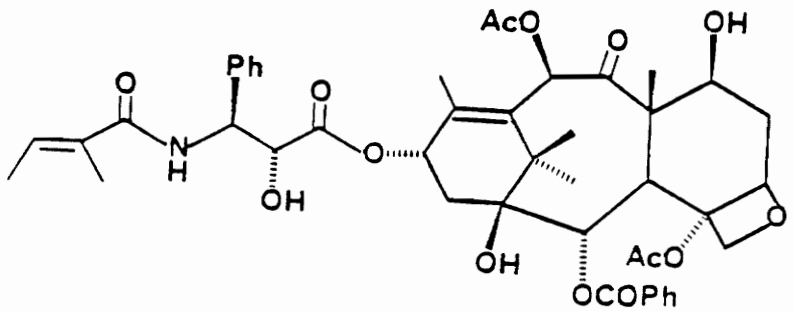
Taxol and other taxane derivatives have a relatively complex structure. The structure of taxol was established by a combination of chemical and spectroscopic studies, and X-ray crystallographic techniques(12, 20).

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



Taxol

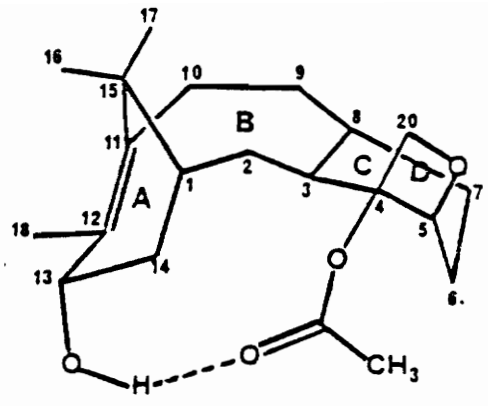
1



Cephalomannine

2

Figure 1.



Three-Dimensional Structure of the Taxane Skeleton

Figure 2.

Nuclear Magnetic Resonance Spectra of Taxol

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

^{1}H -NMR peak shapes and chemical shifts are always good criteria for the structure of taxane derivatives. In taxol, the acetate groups at the C-4 and C-10 give sharp intense singlet methyls. Signals of other methyl groups are generally less intense. Long-range coupling of the C-18 methyl protons to the C-13 proton causes the C-18 singlet to be broader than the C-19 signal (20). Similarly, the C-17 signal is also broader than the C-16 methyl singlet. The effects may be also due to different relaxation times caused by restricted rotation. The other singlet belongs to the C-10 proton which appears at 6.25 ppm. The C-2 and C-3 proton signals are seen as doublets at 5.56 and 3.75 ppm respectively. The other doublet at 4.71 ppm is assigned to the C-2′ proton of the C-13 side chain. The fourth doublet represents the C-3′ NH signal and is seen at 7.0 ppm.

The signals for the C-3′ and C-7 protons consist of a set of doublets at 5.8 and 4.4 ppm respectively. The only AB quartet belongs to the C-20 of the oxetane ring with a

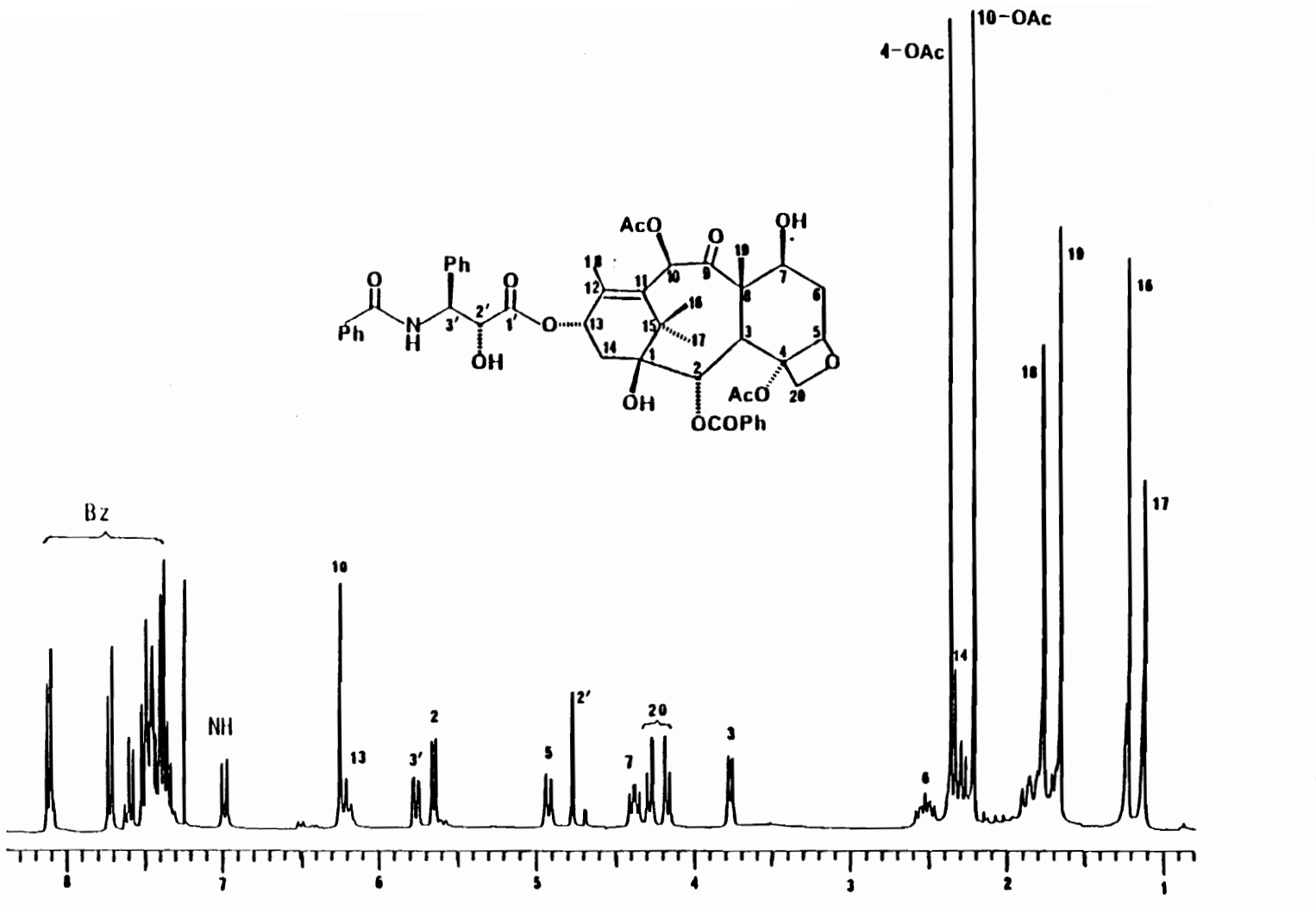


Figure 3. ^{1}H NMR Spectrum of Taxol

geminal coupling constant of 8-9 Hz. When this oxetane ring is cleaved the coupling constant generally increases to about 12 Hz while the peak shape is unchanged. The β proton of C-13 is seen as a broad triplet at 6.15 ppm, and its long-range coupling with the C-18 methyl protons can be observed by the 2 Hz coupling constant of the latter signal. The chemical shift of the 6.15 ppm triplet is a good criterion for the presence of an ester group at the C-13 hydroxyl group of baccatin III. Similarly the chemical shift of the doublet at 4.7 ppm is diagnostic for esterification of C-2′ hydroxyl group.

Mass Spectra and Other Physical Data

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

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

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

Mechanism of Action of Taxol

The importance of taxol as an anticancer drug lies not only in its activity in the anticancer assays but also in its unique mechanism of action. In order to understand taxol's mechanism of action knowledge of what takes place during cell division is necessary as this is where taxol exerts its effect.

Cell division involves the synthesis of nucleic acids and proteins, replication of the DNA, division of the nuclear material and formation of two new cells. The mitotic spindle is responsible for pulling the pairs of chromosomes apart during mitosis, and is composed of threads of protein which are polymers of the protein tubulin, made up of alpha and beta subunits. After the chromosomes have been separated and before the cell has finished dividing the microtubule polymers must depolymerize so that the mitotic spindle is destroyed (Figure 4).

Investigation of the mechanism of the biological activity of taxol showed that it acted as a mitotic spindle poison (21). Further investigation showed that taxol acted by increasing the extent to which microtubule polymers form and by preventing the microtubule polymer from disassembling once formed (22, 23). In <u>in vitro</u> tests it was found that tubulin will polymerize in the presence of taxol alone, without the presence of exogenous guanine 5'-triphosphate or microtubule associated proteins, materials which had previously been necessary for the polymerization of tubulin <u>in vitro</u> (24, 25). Taxol was found not only to promote microtubule polymer formation but also to bind to the polymers formed in a ratio of one mole of taxol to one mole of tubulin dimer (one mole each of alpha and beta tubulin) (26, 27).

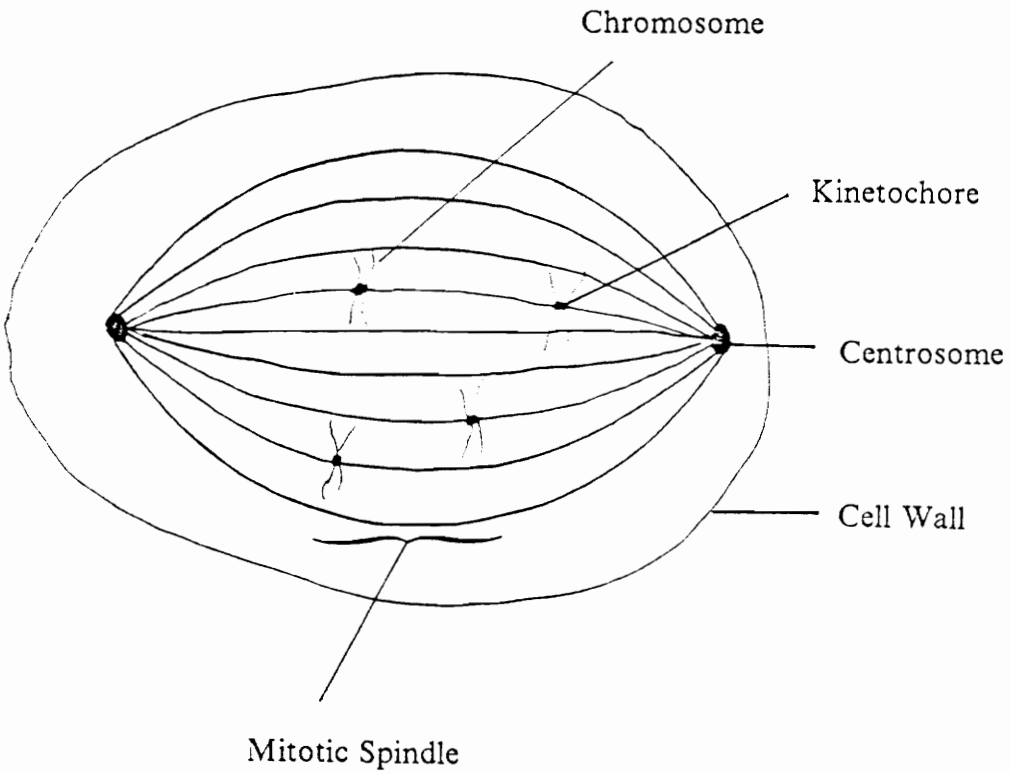

Chromosome
Kinetochore
Centrosome
Cell Wall
Mitotic Spindle

Figure 4.

It is not yet completely understood how taxol promotes the formation of a structure which is necessary for cell division and causes cell division to stop. Research has been conducted which suggests that the structures in a cell normally responsible for production and organization of the microtubule polymers that is -the centrosomes and kinetochores- no longer have the power to effect the synthesis of the organized spindle required for cell division (28-30). It has also been shown that the growth of spindles in the presence of taxol during anaphase when the chromosomes are separating, and when spindles normally have stopped forming, can push the nuclear material back together thereby temporarily reversing cell division (31).

Taxol's mechanism of action is unique. While there are other anticancer drugs such as vinblastine and colchicine which exert their activity by interacting with microtubule polymer formation, these other drugs work by preventing formation of the microtubule polymers (32). Taxol is the only drug that works by promoting the formation of these polymers. It is this unique feature of taxol's mechanism of action that makes it such a promising candidate for clinical use.

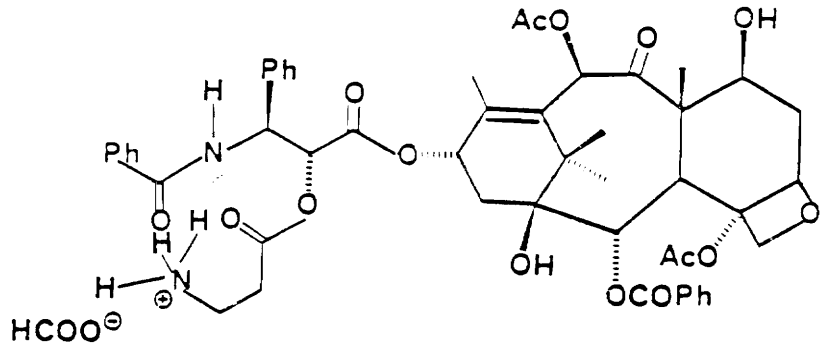
Problems with Taxol as an Anticancer Drug

While the final administration of cancer chemotherapy lies with the physician it must begin with the chemist. Isolation and characterization or synthesis of new chemotherapy agents is the first step that is taken by the chemist in producing a new cancer drug. Once a lead compound has been obtained, there are several ways in which the chemist can contribute further. One of these ways is to structurally modify the drug to produce a more active or less toxic drug.

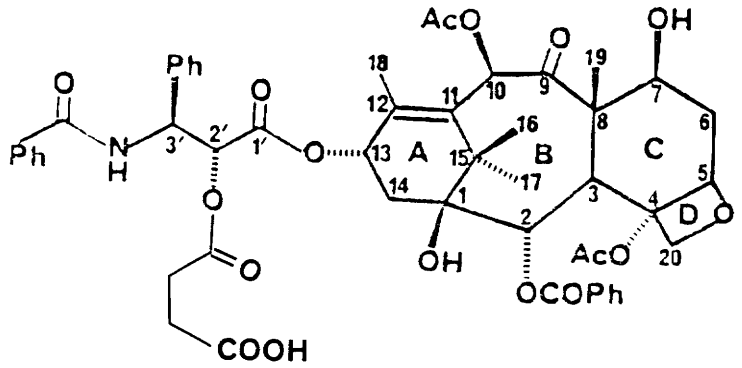
One of the major obstacles for routine clinical usage of taxol is its lack of water solubility. that is, it must be given in conjunction with emulsifying agents. In its Phase I clinical trials taxol itself did not show excessive toxic effects, but severe allergic reactions were caused by the emulsifiers given in conjunction with taxol. In one case the shock caused by allergic reaction caused the death of a patient (32). If taxol did not possess the unique mechanism of action it might not have been approved for Phase II clinical trials because of this problem of formulating it.

PREVIOUS WORK

In the design of water-soluble congeners of taxol, Kingston and co-workers (33) took advantage of the fact that acyl substituents at the C-2′ position are readily hydrolyzed under in vivo condition and thus a suitable 2′-acyltaxol would serve as a prodrug form of taxol itself. Two taxol derivatives with increased water-soluble were prepared, the 2′-(β-alanyl) derivative [3] and the 2′-succinyl derivative [4] The structures of 2′-(β-alanyl)taxol and 2′-succinyltaxol are showed in Figure 5. Although both these derivative were active in vivo and in vitro, the former was too unstable and latter not active enough to make suitable prodrugs of taxol.


AcO
OH
Ph
H
O
Ph
N
O
O
O
H
H
O
O
H
N
⊕
HCOO⊖
OH
AcO
OCOPh

3


AcO
OH
O
Ph
18
19
O
Ph
N
H
3′
2′
1′
O
12
11
10
9
7
8
6
13
A
15
16
B
C
5
14
17
1
3
4
O
O
2
D
O
OH
AcO
20
OCOPh
O
COOH

4

Figure 5.

RESULTS AND DISCUSSION

INTRODUCTION

The work to be presented in this research project addresses the synthesis of biologically active water-soluble taxols. One of the major obstacles that taxol faces in becoming a routinely clinically used drug is its lack of water solubility. The production of an active water-soluble taxol derivative would generate two major benefits. The first of these is that taxol could be given in a water-based solution. Taxol itself must be given in conjuction with emusifying agents and this is not the vehicle of choice for clinical administration of a drug. The second benefit is that once the drug was present in vivo it could be more easily transported throughout the body.

A basic prerequisite for the prodrug approach to be useful in solving drug delivery problems is the ready availability of chemical derivative types satisfying the prodrug requirements, the most prominent of these being reconversion of prodrug to the parent drug in vivo. This prodrug--drug conversion may take place before absorption, during absorption, after absorption or at the specific site of drug action in the body, all de-

pendent upon the specific goal for which the prodrug is designed. Ideally, the prodrug should be converted to the drug as soon as the goal is achieved. The prodrug is an inactive species, and therefore, once its job is completed, intact prodrug represents unavailable drug. For example, prodrug designed to overcome solubility problems in formulating intravenous injection solution should preferably be converted immediately to drug following injection so that the concentration of circulating prodrug would rapidly become insignificant in relation to that of active drug.

The necessary conversion or activation of prodrugs to parent drug molecules in the body can take place by a variety of reactions. The most common prodrugs are those requiring a hydrolytic cleavage mediated by enzymic catalysis. Active drug species containing hydroxyl or carboxyl groups can often be converted to prodrug esters from which the active forms are regenerated by esterases within the body, e.g., in the blood (34).

Based on the prodrug idea, the methods which were used to prepare water soluble taxols involved the addition of polar groups to the C-2′ position. These polar groups included an amino acid, γ-amino-butyric acid, and three different sulfonate derivatives.

SYNTHESIS

1. 2′-((3-Sulfo-1-oxopropyl)oxy) Taxol Sodium Salt

Preparation of 2′-(3-sulfo-1-oxopropyl)oxytaxol sodium salt was achieved via coupling of taxol with acrylic acid followed by Michael addition of bisulfite ion (Figure 6). Reaction of taxol with acrylic acid using isobutyl chloroformate (iBuOCOCl) as the cou-

pling agent produced 2′-acryloyltaxol [**5**] in 94% yield after purification via flash chromatography (silica gel, 1/1 dichloromethane/ethyl acetate). The reaction, monitored by TLC, was 90% complete in 15 hours at 60°*C*, but extended reaction times were also employed to allow the reaction to proceed close to completion. Extended reaction times did not cause any C-2′, C-7 disubstituted product to form.

Characterization data for 2′-acryloyltaxol are shown in Table 1. The only major change for the taxol-derived protons in the ^{1}H -NMR spectrum when contrasted with the ^{1}H -NMR spectrum of taxol was that the signal for the C-2′ proton at 4.73 ppm (d, J = 3) in taxol, was shifted downfield to 5.46 ppm (d, J = 3). This downfield shift is consistent with acylation of the C-2′ hydroxyl group. The signal for the C-7 proton of **5** at 4.43 ppm (dd, J = 6,10) was essentially unchanged when compared with the analogous taxol signal at 4.38 ppm (dd, J = 4,10). The C-7 proton signal for **5** showed that no reaction had taken place at that position. The double bond protons were observed at 6.45 ppm and 5.95 ppm. The other signals for the 2′-acryloyltaxol could be assigned without difficulty. The mass spectrum for **5** indicated a molecular weight of 907 by the presence of peaks at m/z 930 (MNa^+) and 908 (MH^+) .

Michael addition of 2′-acryloyltaxol to yield [**6**] was effected by using sodium bisulfite. Sodium bisulfite was used for two reasons; it is a good nucleophile, and also provides a suitable pH condition for the reaction.

The characterization data for **6** are shown in Table 2. The differences in the ^{1}H -NMR spectrum of **6** when contrasted with the 2′-acryloyltaxol **5** are due to the absence of the double bond protons. Two triplets at 3.14 ppm and 2.93 ppm, respectively, indicated the presence of two new methylene groups. The mass spectrum for **6** indicated a molecular weight of 1011 by the presence of peaks at m/z 1034 (MNa^+) and 1012 (MH^+) .

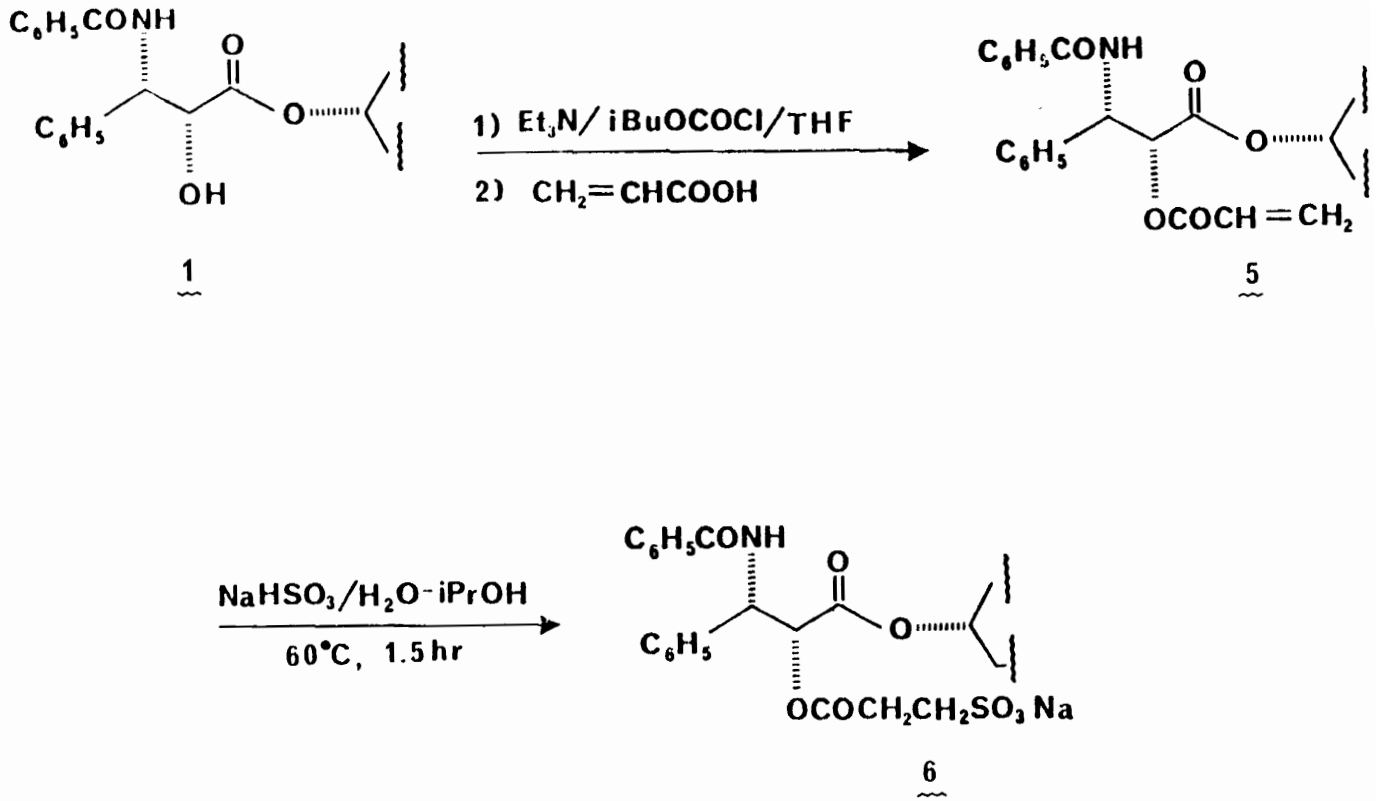

C6H5CONH
C6H5
OH
O
O
1
1) Et3N/ iBuOCOCl/THF
2) CH2=CHCOOH
C6H5CONH
C6H5
OCOCH=CH2
O
O
5
NaHSO3/H2O-iPrOH
60°C, 1.5hr
C6H5CONH
C6H5
OCOCH2CH2SO3Na
O
O
6

Figure 6.

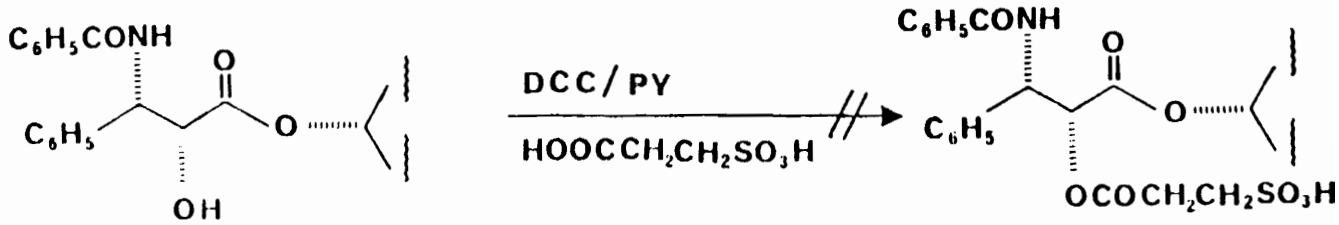

C6H5CONH
O
C6H5
O
OH
DCC/PY
HOOCCH2CH2SO3H
C6H5CONH
O
C6H5
O
OCOCH2CH2SO3H

Figure 7.

Synthesis of the product **6** was attempted by the one-step reaction as shown in Figure 7, but no product was obtained. The possible reason is that the reaction intermediate was attacked intramolecularly by sulfonyl group.

2. 2′-((4-((2-Sulfoethyl)amino)-1,4-dioxobutyl)oxy) Taxol Sodium Salt

Preparation of 2′-((4-((-sulfoethyl)amino)-1,4-dioxobutyl)oxy)taxol sodium salt [**8**] was achieved in 88% overall yield via coupling of taxol with succinic anhydride followed by coupling of taurine tetrabutylammonium salt and ion exchange (35, 36) (Figure 8).

Reaction of taxol with succinic anhydride yielded 2′-succinyltaxol **4** in 96% yield after a reaction time of 2 hours at room temperature. After simple-work up, the product was shown to be homogeneous on TLC and ^{1}H -NMR showed no other products present. No trace of taxol or a disubstituted taxol was present. The characterization data for **4** were the same as the known compound (33). Contrasted with taxol the major change seen in the ^{1}H -NMR spectrum of 2′-succinyltaxol is the downfield shift of the C-2′ proton signal to 5.51 ppm (d, J = 3). The succinyl protons are seen as multiplets centered at 2.6 ppm.

Reaction of succinyltaxol with taurine tetrabutylammonium salt using isobutylchloroformate as the coupling agent produced 2′-((4-((2-sulfoethyl)amino) -1,4-dioxobutyl)oxy) taxol tetrabutyl ammonium salt [**7**] in 100% yield after isolation via flash chromatography (silica gel, 7/1 dichloromethane/methanol). The reaction, monitored by TLC, was 80% complete in 2 hours, so extended reaction times were employed to allow the reaction to proceed to completion. Characterization data for **7** are shown in Table 3. The taxol derived protons in the ^{1}H -NMR spectrum when contrasted with

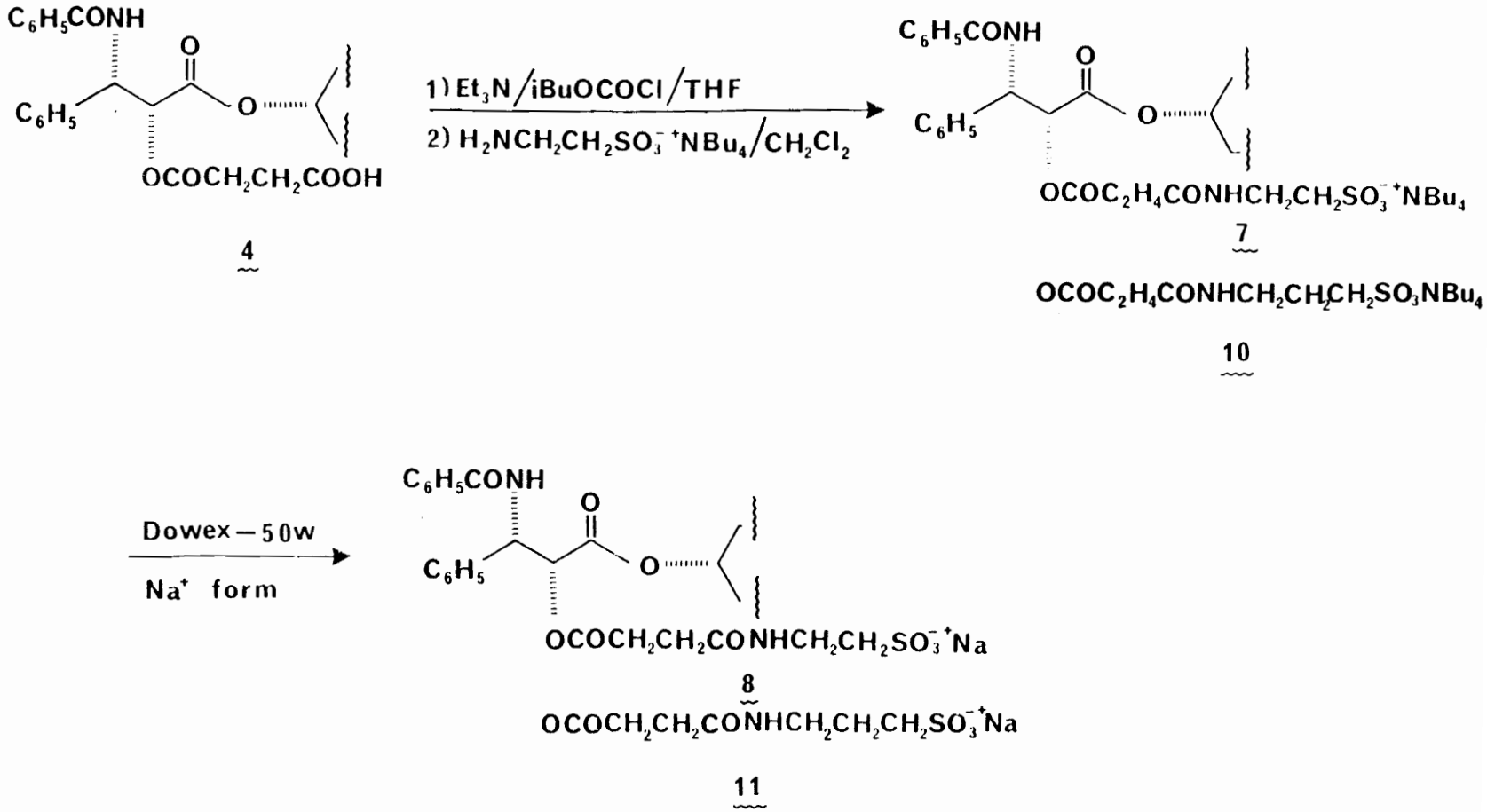

C6H5CONH
C6H5
O
OCOCH2CH2COOH
4
1) Et3N/iBuOCOCl/THF
2) H2NCH2CH2SO3⁻ +NBu4/CH2Cl2
OCOC2H4CONHCH2CH2SO3⁻ +NBu4
7
OCOC2H4CONHCH2CH2CH2SO3NBu4
10
Dowex—50w
Na+ form
OCOCH2CH2CONHCH2CH2SO3⁻ +Na
8
OCOCH2CH2CONHCH2CH2CH2SO3⁻ +Na
11

Figure 8.

those of the ^{1}H -NMR spectrum of succinyltaxol were essentially unchanged, except for new peaks at 3.6 ppm and 2.94 ppm, respectively, for two methylene groups. The vicinal nature of these methylene protons was confirmed by decoupling. Signals for the tetrabutyl groups were easily distinguished from the other peaks by integration.

The preparation of 2′-((4((2-sulfoethyl)amino)-1,4-dioxobutyl)oxy) taxol sodium salt **8** was achieved by passing **7** through a Dowex 50 ion-exchange column (Na^+ form). The characterization data for **8** are shown in Table 4. Contrasted with **7** the major change seen in the ^{1}H -NMR spectrum of **8** is the absence of signals for the tetrabutyl groups. The mass spectrum for **8** indicated a molecular weight of 1082 by the presence of peaks at m/z 1105 (MNa^+) and 1083 (MH^+) .

Preparation of 2′-((4-((2-sulfoethyl)amino)-1,4-dioxobutyl)oxy)taxol **8** from taxol-2′-succinate was also tried by a one-step reaction as shown in Figure 9. No desired product was obtained, however, due to the presence of water, which was needed to make the taurine soluble; the water hydrolyses the intermediate back to starting material, as shown in Figure 10. Since this reaction did not work, non aqueous conditions were also tried. The reaction still did not succeed because the taurine did not dissolve in any organic solvents; and only the conditions described above were successful.

Preparation of **8** via 2′-((4-((2-ethanethiol)amino)-1,4-dioxobutyl)oxy) taxol [**9**] was also tried. But this approach (Figure 11) was also not successful because of the poor yield, although we did succeed in obtaining the intermediate, 2′-((4-((2-ethanethiol)amino)-1,4-dioxobutyl)oxy)taxol **9** , in low yield. However, oxidation of this thiol to the desired sulfonic acid did not yield the desired product **8** in acceptable amounts.

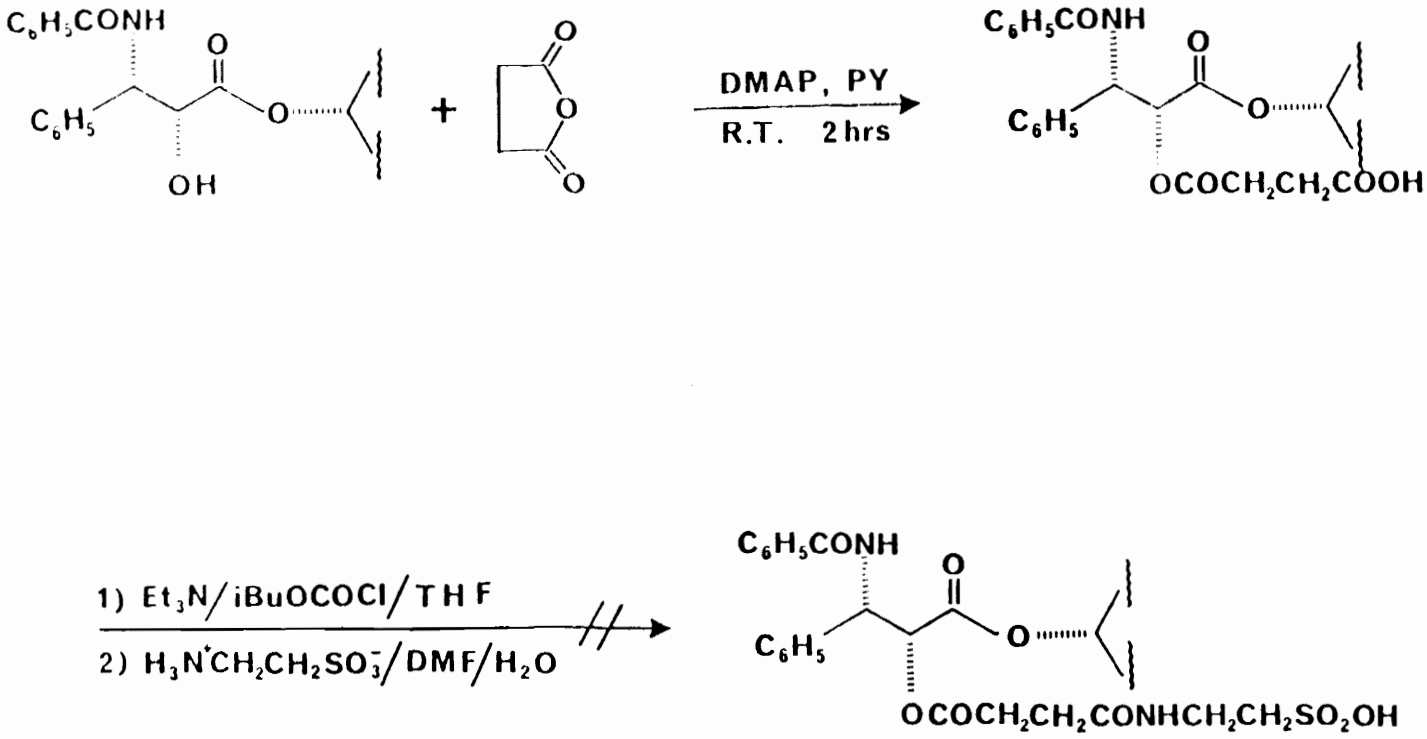

C6H5CONH
C6H5
OH
O
O
O
O
+
DMAP, PY
R.T. 2hrs
C6H5CONH
C6H5
OCOCH2CH2COOH
1) Et3N/iBuOCOCl/THF
2) H3N+CH2CH2SO3-/DMF/H2O
C6H5CONH
C6H5
OCOCH2CH2CONHCH2CH2SO2OH

Figure 9.

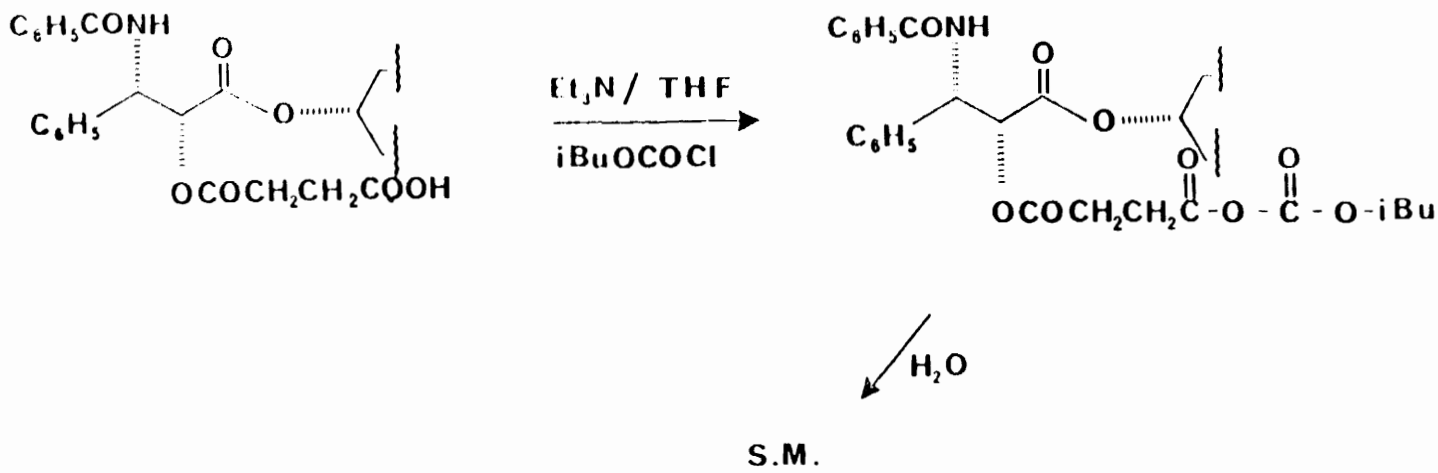

C6H5CONH
C6H5
O
O
OCOCH2CH2COOH
Et3N / THF
iBuOCOCl
C6H5CONH
C6H5
O
O
O
O
OCOCH2CH2C-O-C-O-iBu
H2O
S.M.

Figure 10.

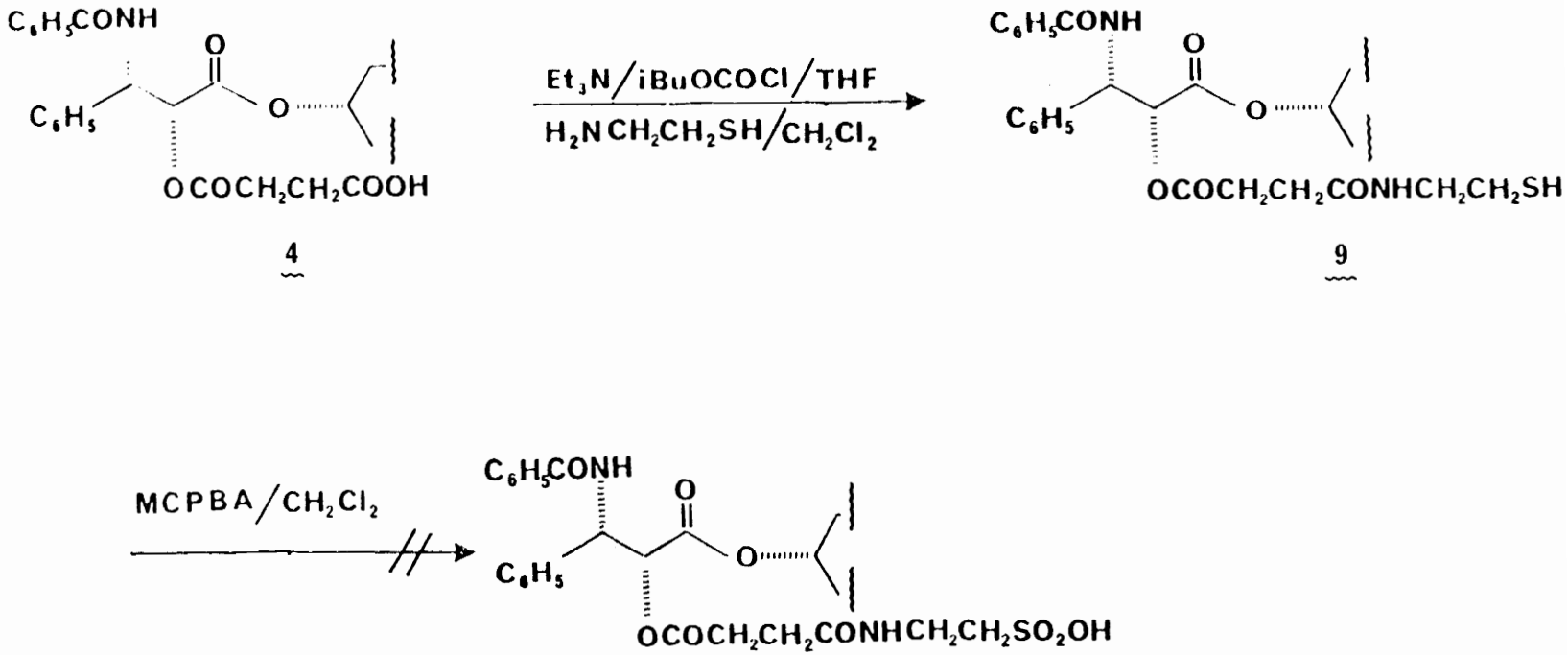

C6H5CONH
C6H5
O
OCOCH2CH2COOH
4
Et3N/iBuOCOCl/THF
H2NCH2CH2SH/CH2Cl2
C6H5CONH
C6H5
O
OCOCH2CH2CONHCH2CH2SH
9
MCPBA/CH2Cl2
C6H5CONH
C6H5
O
OCOCH2CH2CONHCH2CH2SO2OH

Figure 11.

3. 2′-((4-((3-Sulfopropyl)amino)-1,4-dioxobutyl)oxy)Taxol Sodium Salt

Preparation of 2′-((4-((3-sulfopropyl)amino)-1,4-dioxobutyl)oxy)taxol sodium salt [**11**] was achieved by same method as for **8** via coupling of succinyltaxol with 3-amino-1-sulfopropionic acid tetrabutylammonium salt. (Figure 8). Reaction of 2′-succinyltaxol with isobutylchloroformate followed by 3-amino-1-sulfopropyl tetrabutylammonium salt yielded 2′-((4-((3-sulfopropyl)amino)-1,4-dioxobutyl)oxy)taxol tetrabutylammonium salt [**10**] in 71.2% yield after purification via flash chromatography(silica gel, 10/1 dichloromethane/methanol). The reaction, monitored by TLC, was 90% completed in 1 hour but extended reaction times were employed to allow it to proceed to completion. Characterization data for **10** are shown in Table 5. The only major difference compared with the ^{1}H -NMR spectrum of succinyltaxol is the presence of new peaks at 3.28, 1.98 and 2.87 ppm, for three additional methylene groups.

The preparation of **11** was achieved by passing the **10** through a Dowex 50 ion-exchange column (Na^+ form). The characterization data for **11** are shown in Table 6. Contrasted with **10** the major change seen in the ^{1}H -NMR spectrum of **11** is the absence of signals for the tetrabutyl groups. The mass spectrum for **11** indicated a molecular weight of 1096 by the presence of peaks at m/z 1119 (MNa^+) and 1097 (MH^+) .

4. 2′-((4-((2-Hydroxylethyl)oxy)-1,4-dioxobutyl)oxy) Taxol

Preparation of 2′-((4-((hydroxylethyl)oxy)-1,4-dioxobutyl)oxy)taxol [**12**] was achieved via coupling of succinyltaxol with ethylene glycol in 83% yield after a reaction time of 20 hours at room temperature(Figure 12). The purpose of making **12** was to convert the secondary hydroxyl group in taxol at the 2′-position to a primary hydroxyl group, so that the hydroxyl group in the product would be more reactive than that of taxol, therefore will be possible to make other taxol derivatives under mild conditions.

The characterization data for **12** are shown in Table 7. Contrasted with succinyltaxol the major change seen in the ^{1}H -NMR spectrum of **12** is the presence of new peaks at 3.7 ppm and 4.1 ppm, respectively, assigned to the two new methylene groups. The mass spectrum for **12** indicated a molecular weight of 997 by the presence of peaks at m/z 1020 (MNa^+) and 998 (MH^+) .

5. 2′-γ-AminobutyrylTaxol Formate

Preparation of 2′-γ-aminobutyryltaxol [**14**] was achieved via coupling of taxol with the N-carbobenzyloxy(CBZ)-γ-aminobutyric acid followed by deprotection of the amine(Figure 13). Reaction of taxol with N-CBZ-γ-aminobutyric acid using dicyclohexylcarbodiimide (DCC) as the coupling agent produced 2′-N-CBZ-γ-aminobutyryltaxol [**13**] in 75% yield after purification via preparative TLC (silica gel, 3/2 hexanes/ethyl acetate). Excess reagents were used to drive the reaction to completion. The excess reagents present at the end of the reaction did not present a problem, since the excess DCC was decomposed to dicyclohexylurea by the addition of

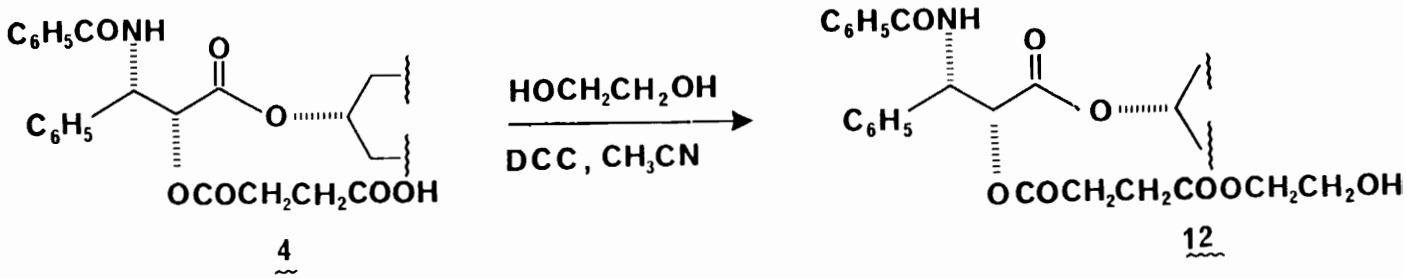

C6H5CONH
O
C6H5
O
OCOCH2CH2COOH
4
HOCH2CH2OH
DCC, CH3CN
C6H5CONH
O
C6H5
O
OCOCH2CH2COOCH2CH2OH
12

Figure 12.

water, and most of the dicyclohexylurea and N-CBZ-γ-aminobutyric acid were removed by filtration.

Characterization data for **13** are shown in Table 8. The only major change for the taxol derived protons in the ^{1}H -NMR spectrum of **13** when contrasted with the ^{1}H -NMR spectrum of taxol was that the signal for C-2′, at 4.73 ppm in taxol, was shifted downfield to 5.47 ppm. This downfied shift is consistent with acylation of the C-2′ hydroxyl group. The signal for the C-7 proton of 13, at 4.47 ppm was essentially unchanged when compared with the analogous taxol signals 4.38 ppm, showing that no reaction had taken place at that position. Integration of the aromatic region of the ^{1}H -NMR spectrum clearly showed the presence of 20 aromatic protons. The other signals could be assigned without difficulty.

Deprotection of **13** to yield 2′-γ-aminobutyryltaxol formate **14** was effected using 5% Pd/C as catalyst and formic acid as hydrogen source. Formic acid was used as hydrogen source for two reasons; it is a much more active form of hydrogen for removal of CBZ protecting groups, and the reaction yields the γ -aminobutyryltaxol derivative as a formate salt, which is more water soluble than the neutral form. The proton NMR spectrum showed that the C-2′ proton was still at 5.46 ppm (J = 3 Hz) and only 15 aromatic protons were left. The proton signal of OCH_2Ph (4.95 ppm s) had disappeared, but the three new methylene groups were still there.

Unfortunately, the product **14** was unstable in methanol solution. The product decomposed back to taxol in methanol solution after a few hours. The evidence is that the C-2′ proton signal was shifted upfield to 4.75 ppm, the same chemical shift as for the C-2′ proton in taxol, on standing in methanol. The result of TLC showed that it had same R_f value as taxol by co-chromatography (CH_2CH_2 / EtOAc/AcOH, 2/1/0.02). This

reactivity of **14** parallels that observed for 2'-(β -alanyl)taxol (33), and precludes further consideration of this derivative as a prodrug form of taxol.

WATER SOLUBILITY

The partition coefficients between 1-octanol and water have been determined for the sodium salt derivatives, **6** , **8** , and **11** . For the partitioning, octanol saturated with distilled water and distilled water saturated with octanol were used. Usually 10 ml portions of octanol were used with 10 ml portions of water. The volume ratio of the two phases and the amount of sample were chosen so that, in most cases, the absorbance of a sample from the water layer after partitioning had a value between 0.2 and 0.9 using a 1-cm cell. The concentration of the sample in both the water layer and the octanol layer were determined. Previous studies showed that the very small amount of octanol dissolved in the water had no effect on the absorption curve at wave lengths higher than 220 nm (37). The absorption peak of the E-band at 228 nm was used. It is known that, when dilute solution are used and solvents are quite insoluble in each other, that the partition coefficient is not very sensitive to variations in temperature (38), so measurement were obtained at ambient temperature.

The partition coefficient was calculated as following:

$$P = \frac{C_{octanol}}{C_{water}}$$

$$P' = \frac{C_{water}}{C_{octanol}}$$

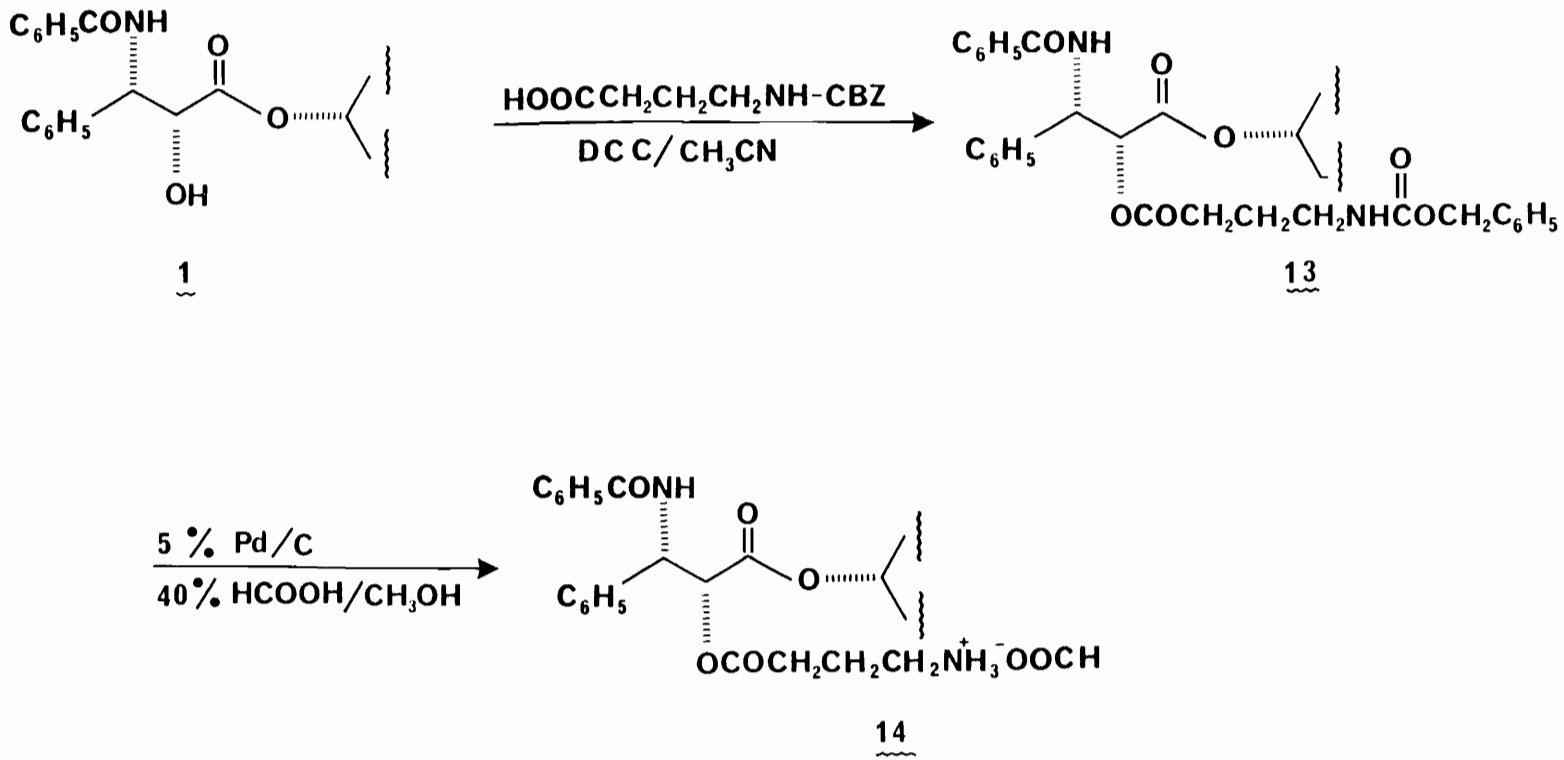

C6H5CONH
C6H5
OH
O
O
1
HOOCCH2CH2CH2NH-CBZ
DCC/CH3CN
C6H5CONH
C6H5
O
O
O
OCOCH2CH2CH2NHCOCH2C6H5
13
5 % Pd/C
40% HCOOH/CH3OH
C6H5CONH
C6H5
O
O
OCOCH2CH2CH2NH3OOCH
14

Figure 13.

$$K' = \frac{P'_x}{P'_{taxol}}$$

The results showed that the product **6** was 210 times more water soluble than that of taxol and the products **8**, **11** were 191 and 118 times more water soluble than that of taxol, respectively.

EXPERIMENTAL PROCEDURES

General Experimental Techniques

A. General

All melting points were determined on a hot stage apparatus and were uncorrected. Solvents for anhydrous reactions were dried according to procedures in Loewenthal (39). Reactions requiring anhydrous conditions were carried out under an argon atmosphere.

B. Spectra

^{1}H -NMR and ^{13}C -NMR spectra were taken on a Bruker WP 270SY 270 MHz spectrometer. Two-dimensional NMR spectra were obtained on a Bruker WP200 200 MHz spectrometer.

Chemical shifts were recorded in parts per million (ppm) downfield from TMS in the case of proton NMR, while ^{13}C -NMR chemical shifts were based on the chloroform chemical shift at 77.0 ppm or on the TMS chemical shift at 0 ppm. Spectra were generally recorded in $CDCl_3$ or CD_3OD solution at ambient temperature.

Infrared (IR) spectra were recorded on a Perkin Elmer 710 B infrared spectrophotometer. Ultraviolet (UV) spectra were taken on Perkin-Elmer 330 UV-visible spectrophotometer. Mass spectra (MS) were obtained by the fast atom bombardment (FAB) method on a VG 7070 HF or MAT-112S mass spectrometer.

C. Chromatography

Analytical thin-layer chromatography (TLC) wes carried out on silica gel 60 F_{254} (0.2 mm thickness) (E. Merck). Preparative TLC was performed on silica gel GF plates, $20 \times 20cm \times 1000\mu m$ thick (Analtech). Flash chromatography was performed by using silica gel 60, 0.040-0.063 mm (230-400 mesh) particle size. Column sizes were varied and are specified in the pertinent experimental sections.

1. Preparation of 2′-((3-Sulfo-1-oxopropyl)oxy)Taxol Sodium Salt

Preparation of C-2′-acryloyltaxol 5

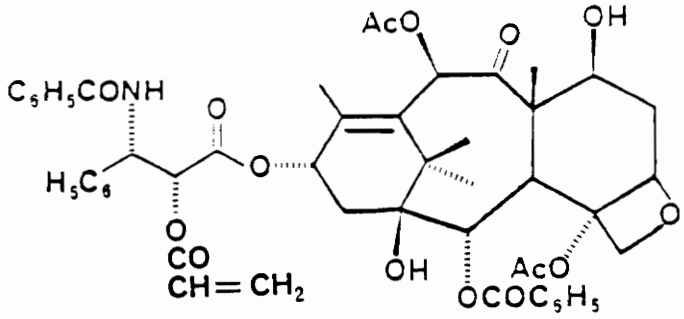
Triethylamine (Et_3N, 50 μl) and acrylic acid (30 μl) were dissolved in 5 ml of dry THF in a 25 ml round-bottomed flask along with argon gas. The solution was cooled down to 0^0C by an ice bath. To the solution, 50 μl of isobutylchloroformate were added and

the reaction mixture was warmed to room temperature in 15 minutes. Then 100 mg of taxol were added and the reaction was stirred at 60°C for 15 hours, monitored by TLC with dichloromethylene/ethyl acetate (2/1). At the end of reaction , the triethylamine hydrochloride which had precipitated was removed by filtration, the solvent removed in vacuo and the product purified by flash chromatography, silica gel, 1/1 dichloromethane/ethyl acetate, to yield 100 mg (94%) of pure **5** . The characterization data for **5** are shown in Table 1.

Preparation of 2'-((3-sulfo-1-oxopropyl)oxy)taxol sodium salt 6

2'-acryloyltaxol **5** (85 mg) was dissolved in about 3 ml of distilled iPrOH and 85 mg of sodium meta-bisulfite were dissolved in about 1 ml of distilled water. These two solutions were mixed together and the reaction mixture was stirred at 60°C for about 15 hours. The reaction was checked by TLC with 10/1 dichloromethane/methanol. At the end of the reaction, the solvents were removed under vacuum; the water was removed by azeotroping with acetonitrile. The product was purified by flash chromatography with 2/1 CH_2Cl_2 /iPrOH, to yield 83.5 mg of **6** (83.5%). Characterization data for **6** are shown in Table 2.

Characterization Data for 5.



5

m.p.	160-161°C
$[\alpha]_D^{20}$	-32° (0.002 g/ml, MeOH)
IR (KBr):	3500, 2970, 2370, 1740, 1660, 1385, 1260, 1190, 990 cm^{-1}
UV λ_{max}^{MeOH}	280 nm (ε 968), 275 nm (ε 1272)
MS (FAB):	930 (MNa^+), 908 (MH^+)

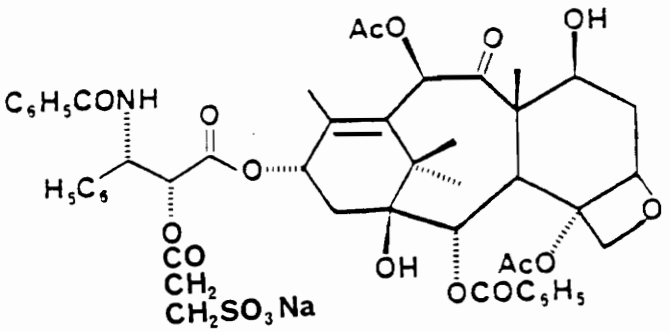
Table 1.

Position	^1H Shift (ppm from TMS) Coupling (hertz)	^{13}C Shift (ppm from TMS)
C-1		78.8
C-2	5.67 (d, 7)	75
C-3	3.78 (d, 7)	45.8
C-4		80.7
C-5	4.96 (d, 9)	84.2
C-6	2.55 m	35.2
C-7	4.48 m	75.8

Table 1 continued.

C-8		58.1
C-9		203.5
C-10	6.27 s	70.8
C-11		132
C-12		142.2
C-13	6.25 (t, 8)	75
C-14	2.3 m	35.2
C-15		43
C-16	1.2 s	26.8
C-17	1.11 s	20.8
C-18	1.92 s	14.8
C-19	1.67 s	9.6
C-20	4.17 (d, 8) 4.30 (d, 8)	71
C-1′		174.3
C-2′	5.5 (d, 3)	74
C-3′	5.97 (dd, 3,9)	53.2
N-H	6.94 (d,9)	
CH_3(OAc)	2.21 s	21.2
CH_3(OAc)	2.43 s	21.9
Bz	7.4-8.1 m	126.8-138.1
CO(OAc)		170
CO(OAc)		171
CO(OBz)		167.2
CO(NBz)		167.9
C-1″		172.5
C-2″	6.20 (dd 12,10)	133
C-3″	5.95 (dd 3,10) 6.45 (dd 3,12)	132.6

Characterization Data for 6.



6

m.p.	175-176°C
$[\alpha]_D^{20}$	-30° (0.0012, MeOH)
IR (KBr):	3500, 2950, 1760, 1730, 1660, 1380, 1250, 1190, 1100, 800 cm^{-1}
UV λ_{max}^{MeOH}:	279 nm (ε 579), 270 nm (ε 869), 228 nm (ε 15072)
MS (FAB):	1034 (MNa^+), 1012 (MH^+)

Table 2.

Position	^1H Shift (ppm from TMS) Coupling (hertz)	^{13}C Shift (ppm from TMS)
C-1		*
C-2	6.2 (d, 7)	75
C-3	3.82 (d, 7)	45.8
C-4		80.5
C-5	5.0 (d, 9)	84
C-6	2.48 m	35.2
C-7	4.35 m	76

Table 2 continued.

C-8		57.9
C-9		203.8
C-10	6.45 s	70.8
C-11		131
C-12		141
C-13	6.09 (t, 8)	75.4
C-14	2.48 m	35.8
C-15		43
C-16	1.15 s	25.9
C-17	1.15 s	19.8
C-18	1.95 s	13.8
C-19	1.67 s	9.4
C-20	4.21 s	70.8
C-1′		171
C-2′	5.45 (d, 3)	74
C-3′	5.84 (d, 7)	53.1
N-H	7.26 (t, 9)	
CH_3(OAc)	2.2 s	21
CH_3(OAc)	2.4 s	21.9
Bz	7.4-8.1 m	126.8-138.1
CO(OAc)		168.4
CO(OAc)		169.9
CO(OBz)		166.2
CO(NBz)		168.2
C-1″		170.2
C-2″	2.93 (t 8)	29.2
C-3″	3.14 (t 8)	63.2

* under $CHCl_3$ signal

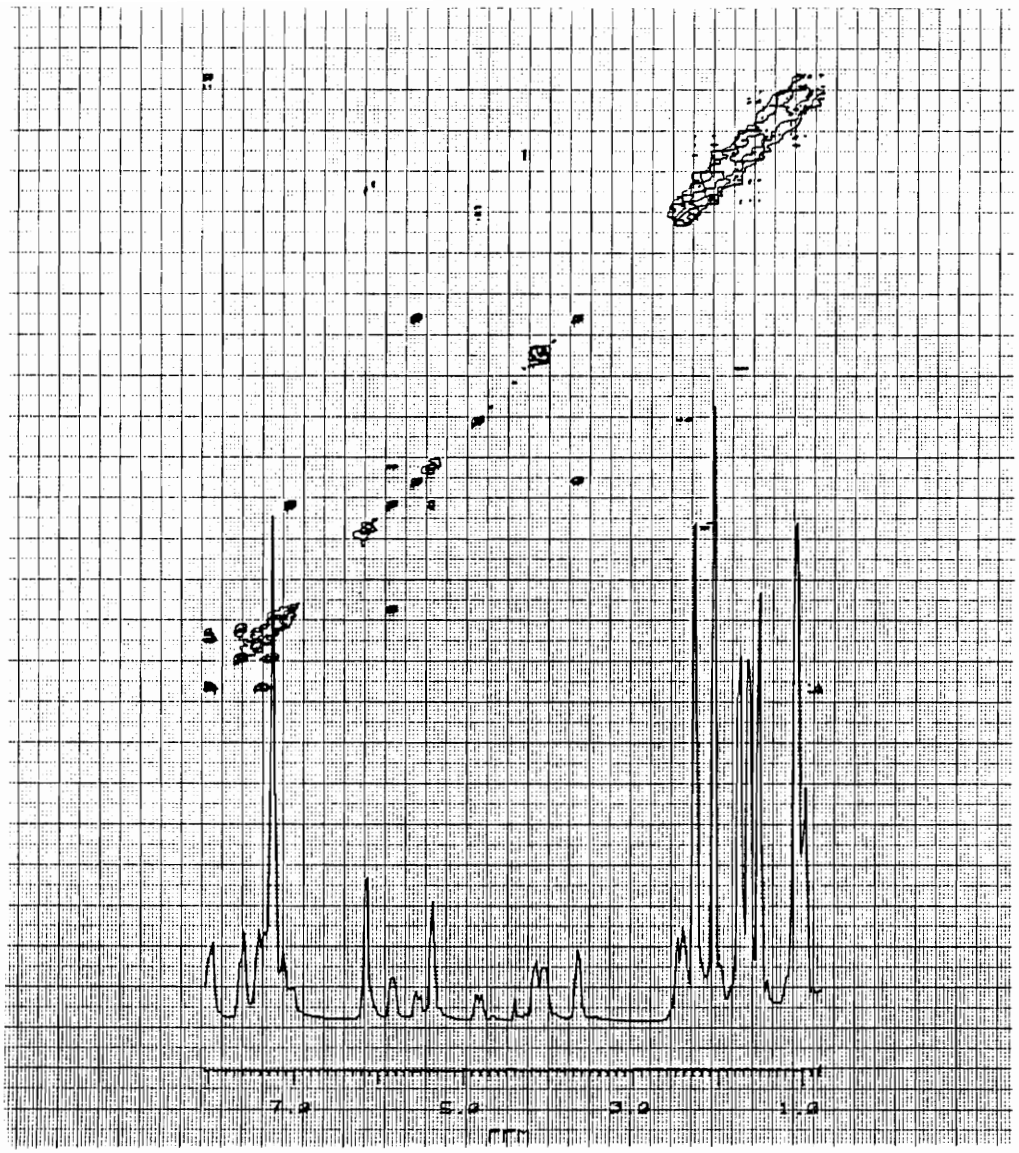
2. Preparation of 2′-((4-((2-Sulfoethyl)amino)-1,4-dioxobutyl)oxy)Taxol Sodium Salt

Preparation of succinyltaxol 4

Taxol (206 mg), 4-dimethylaminopyridine (DMAP) (2.9 mg) and succinic anhydride (49 mg) were added to a 25 ml flask equipped with a magnetic stirrer. Dry pyridine (2.0 ml) was added and the solution was stirred at room temperature for 2.5 hours. At the end of the reaction, several ml of water were added which produced a white precipitate and opaque suspension. Several ml of CH_2Cl_2 were then added to extract the products. The white aqueous suspension did not disappear until 1 ml of concentrated HCl was added. The dichloromethane layer was dried with Na_2SO_4 , filtered and evaporated. Analysis of the sample at this point by TLC with 7/1 CH_2Cl_2 /MeOH showed only a trace of pyridine left. Several ml of heptanes were added and evaporated for several times to remove the pyridine residue to yield succinyltaxol **4** (218 mg, 96.6%) homogeneous by ^{1}H -NMR and TLC (CH_2Cl_2 /MeOH, 7/1). The ^{1}H-NMR of the product matched the values given in the literature (33). The 2D-NMR HOMO COSY spectrum for **4** is shown in Figures 14 and 15.

Preparation of taurine tetrabutylammonium salt

Taurine (250 mg) was dissolved in a minimum volume of distilled water and 1 ml of aqueous tetrabutylammonium hydroxide was added. The solution was stirred at room temperature for 1 hour, and the solution was evaporated to dryness. The products were



2D-NMR of 2′-Succinyltaxo (HOMO COSY)

Figure 14.

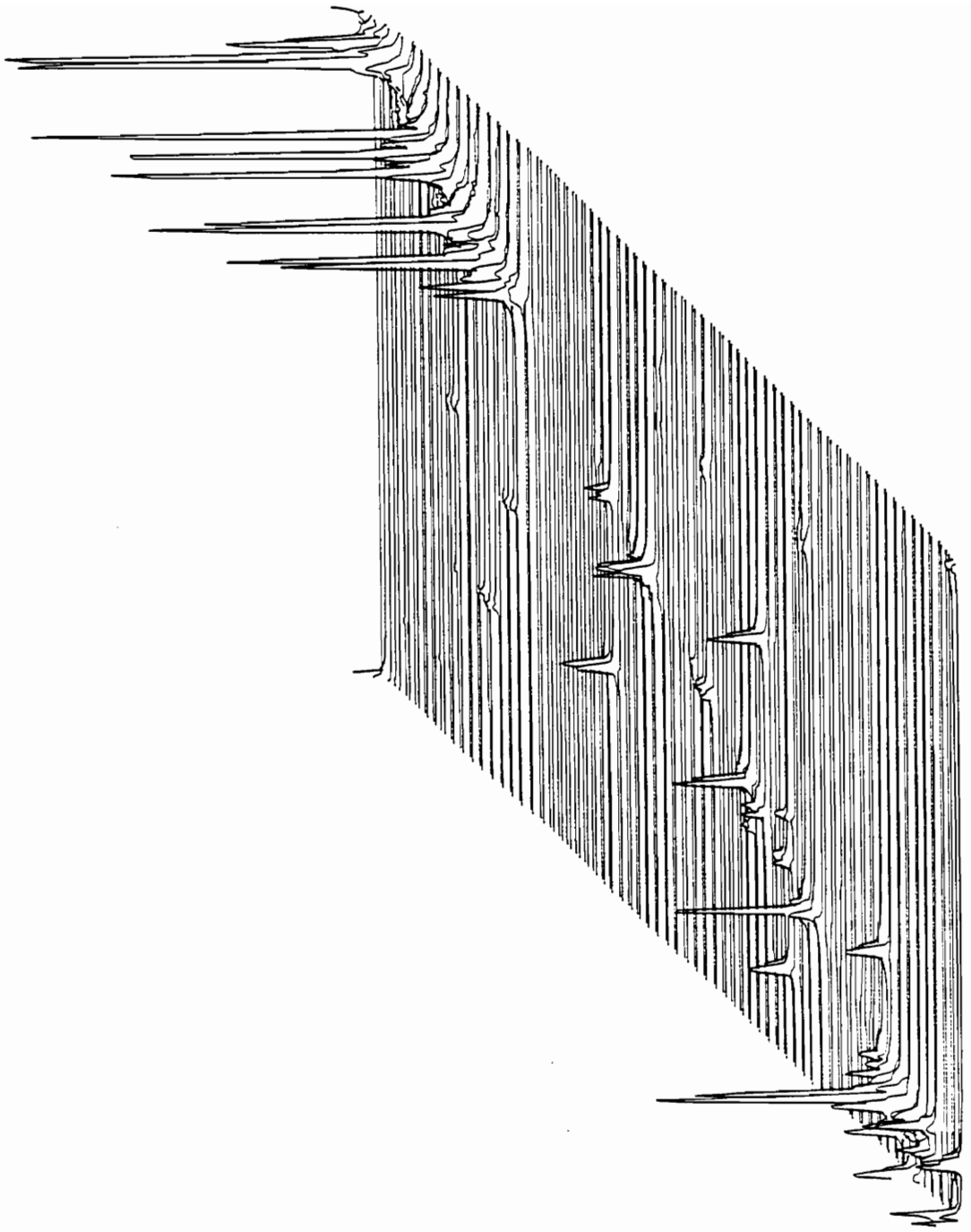


Figure 15. 2D-NMR of 2′-Succinyltaxo (HOMO COSY)

dissolved in dry THF (about 15 ml), filtered, and the filtrate was evaporated to dryness. The product was dissolved in 2 ml of dry THF for the subsequent reaction.

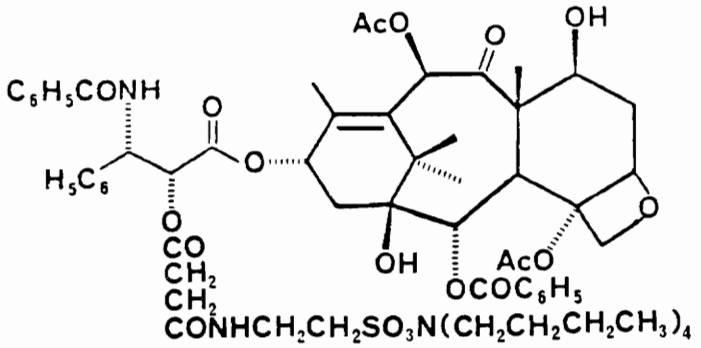
Preparation of 2'-((4-((2-sulfoethyl)amino)-1,4-dioxobutyl)oxy)taxol tetrabutylammonium salt 7

Taxol succinate (122 mg) was dissolved in about 4 ml dry THF, 50 μl of Et_3N were added, and the solution was cooled down to 0^oC. To the solution, 50 μl of isobutylchloroformate (iBuOCOCl) were added and the reaction mixture was warmed to room temperature in 15 minutes, and 0.5 ml of taurine tetrabutylammonium salt in THF solution (equivalent to 91 mg of taurine tetrabutylammonium salt) were added. The reaction mixture was stirred at room temperature for 5 hours, monitored by TLC with 2/1 EtOAc/MeOH. The solution was evaporated after filtration. The product was purified by flash chromatography (silica gel 300 X 15 mm, 7/1 CH_2Cl_2 /MeOH), to yield 168 mg (100%) of **7** . Characterization data for **7** are shown in Table 3.

Preparation of 2'-((4-((2-sulfoethyl)amino)-1,4-dioxobutyl)oxy)taxol sodium salt 8

The tetrabutylammonium salt **7** (160 mg) was placed in a beaker with Dowex 50 ion exchange resin in the Na^+ form (about 3 ml of resin in 3 ml of deionized water). The mixture was stirred at room temperature for 1.5 hours, then the mixture was passed though a small resin column which contained 2 ml of resin in the Na^+ form, using deionized water as solvent. The solution was azeotroped with CH_3CN to yield 122 mg (91.7%) of sodium salt **8** . Characterization data for **8** are shown in Table 4.

Characterization Data for 7.



7

m.p.	168-170°C
$[\alpha]_D^{20}$	−24.06° (0.0086, MeOH)
IR (KBr)	3460, 3000, 1760, 1740, 1670, 1560, 1400, 1260, 1180, 1060 cm^{-1}
UV λ_{max}^{MeOH}	278 nm (ε 587), 271 nm (ε 844)

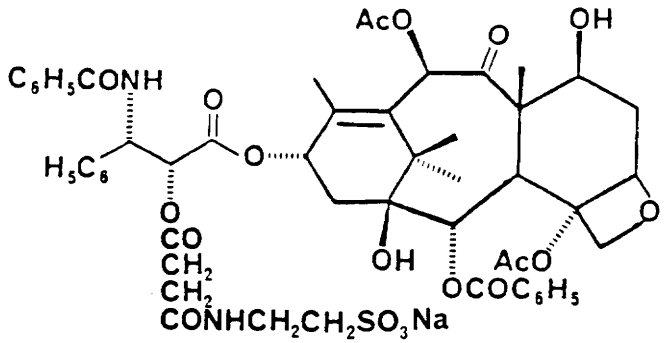
Table 3.

Position	^1H Shift (ppm from TMS) Coupling (hertz)	^{13}C Shift (ppm from TMS)
C-1		78.8
C-2	5.62 (d, 7)	75.8
C-3	3.78 (d, 7)	45.8
C-4		80.6
C-5	4.95 (d, 9)	84.2
C-6	2.52 m	35.2
C-7	4.45 m	76

Table 3 continued.

C-8		58
C-9		203.8
C-10	6.28 s	71.8
C-11		132.2
C-12		142.6
C-13	6.08 (t, 8)	75
C-14	2.18 m	35.8
C-15		42.8
C-16	1.22 s	26.7
C-17	1.12 s	20.2
C-18	1.90 s	14.8
C-19	1.65 s	9.8
C-20	4.17 (d, 8) 4.29 (d, 8)	71.6
C-1′		171.8
C-2′	5.5 (d, 7)	74.2
C-3′	5.82 (dd 7,7)	54
N-H	7.2 (t, 7)	
CH_3(OAc)	2.22 s	21.8
CH_3(OAc)	2.45 s	22.6
Bz	7.4-8.1 m	126.8-138.1
CO(OAc)		167
CO(OAc)		168.3
CO(OBz)		166.8
CO(NBz)		170
C-1″		170.2
C-2″	2.82 (t, 8)	30.6
C-3″	2.74 (t, 8)	30.6
C-4″		170.4
C-1‴	3.6 m	47.2
C-2‴	2.94 m	50
N-H	3.77 (t, 7)	
N-CH_2	3.28 (t, 9)(8H)	59.8
$NCH_2\underline{CH_2}$	1.68 m (8H)	24.2
$\underline{CH_2}CH_3$	1.45 m (8H)	20.2
$CH_2\underline{CH_3}$	1.05 (t, 7)(12H)	14

Characterization Data for 8.



8

m.p.	174-175°C
$[\alpha]_D^{20}$	−29.8° (0.0055, MeOH)
IR (KBr)	3450, 3000, 1760, 1730, 1660, 1560, 1400, 1260, 1190, 1050 cm^{-1}
UV λ_{max}^{MeOH}	279 nm (ε 649), 271 nm (ε 8920), 228 nm (ε 12824)
MS (FAB)	1105 (MNa^+), 1083 (MH^+)

Table 4.

Position	^1H Shift (ppm from TMS) Coupling (hertz)	^{13}C Shift (ppm from TMS)
C-1		79
C-2	5.66 (d, 7)	76.6
C-3	3.8 (d, 7)	47.2
C-4		81.6
C-5	5.02 (d, 9)	85.4
C-6	2.52 m	36
C-7	4.35 m	77.3

Table 4 continued.

C-8		58.8
C-9		204.8
C-10	6.43 s	72.8
C-11		132.6
C-12		142.2
C-13	6.05 (t, 8)	75.9
C-14	2.14 m	36.2
C-15		44.1
C-16	1.18 s	26.8
C-17	1.18 s	21
C-18	1.94 s	14.9
C-19	1.67 s	10.2
C-20	4.23 s	72
C-1′		173.4
C-2′	5.46 (d, 7)	75.8
C-3′	5.8 (dd 7,7)	55
N-H	7.27 (t, 7)	
CH_3(OAc)	2.2 s	22.2
CH_3(OAc)	2.4 s	23.3
Bz	7.4-8.1 m	126.8-138.1
CO(OAc)		170.2
CO(OAc)		170.2
CO(OBz)		167.2
CO(NBz)		171.2
C-1″		173.1
C-2″	2.72 m	30
C-3″	2.52 m	30
C-4″		173.1
C-1‴	3.58 m	47
C-2‴	2.96 m	51
N-H	3.58 (t, 7)	

3. Preparation of 2′-((4-((3-Sulfopropyl)amino)-1,4-dioxobutyl)oxy)Taxol Sodium Salt

Preparation of 3-amino-1-sulfo-propionic acid tetrabutylammonium salt

3-Amino-1-sulfo-propionic acid (280 mg) were dissolved in a little distilled water and 1 ml of tetrabutylammonium hydroxide was added. The solution was stirred at 60°*C* for 1 hour. The solution was then evaporated to dryness and the products were dissolved in THF (about 15 ml). Excess 3-amino-1-sulfo-propionic acid was removed by filtration and the filtrate was evaporated and redissolved in 2 ml dry THF for the subsequent reaction.

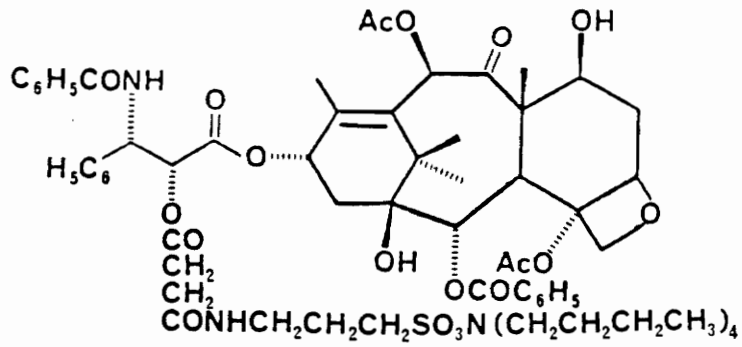
Preparation of 2′-((4-((3-sulfopropyl)amino)-1,4-dioxobutyl)oxy)taxol tetrabutylammonium salt 10

Succinyltaxol (130 mg) was dissolved in 4 ml of dry THF and 50 μl of Et_3N were added, and the solution was cooled down to 0°*C*. To the solution, 50 μl of iBuOCOCl were added and reaction mixture was warmed to room temperature in 15 minutes. Then 0.6 ml of 3-amino-1-sulfo-propionic acid tetrabutylammonium salt in THF solution, equivalent to 108 mg of 3-amino-1-sulfo-propionic acid tetrabutylammonium salt, were added. The reaction mixture was stirred at room temperature for 3 hours, monitored by TLC with 4/1 EtOAc/MeOH. At the end of the reaction, the solution was filtered and evaporated, and the product was purified by flash chromatography (silica gel 300 mm high, 15 mm diameter, 10/1 CH_2Cl_2/MeOH). The yield of homogeneous tetrabutylammonium salt **10** was 128 mg (71.2%). Characterization data for **10** are shown in Table 5.

Preparation of 2'-((4-((3-sulfopropyl)amino)-1,4-dioxobutyl)oxy)taxol sodium salt 11

The tetrabutylammonium salt **10** (120 mg) was placed in a beaker with Dowex 50 ion exchange resin in the Na^+ form (about 3 ml resin in 3 ml of deionized water). The mixture was stirred at room temperature for 1.5 hours, and then passed through a resin column which contained 2 ml of resin in the Na^+ form, using deionized water as a solvent. The solution was azeotroped with CH_3CN , to yield 84 mg of **11** , 79.3%. Characterization data for **11** are showed in Table 6.

Characterization Data for 10.



10

m.p.	165-166°C
$[\alpha]_D^{20}$	−17.9° (0.0033 MeOH)
IR (KBr)	3460, 3000, 1760, 1740, 1670, 1560, 1400, 1260, 1180, 1060, cm^{-1}
UV λ_{max}^{MeOH}	278 nm (ε 880), 272 nm (ε 1180)

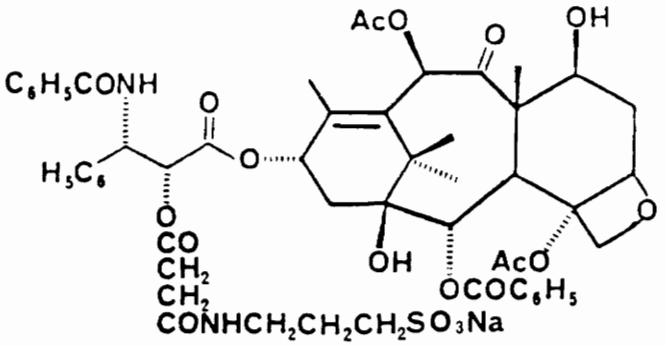
Table 5.

Position	^{1}H Shift (ppm from TMS) Coupling (hertz)	^{13}C Shift (ppm from TMS)
C-1		79.2
C-2	5.64 (d, 7)	75.8
C-3	3.8 (d, 7)	46.1
C-4		81.2
C-5	4.97 (d, 9)	84.2
C-6	2.49 m	35.5
C-7	4.35 m	76

Table 5 continued.

C-8		58.5
C-9		203.9
C-10	6.44 s	71
C-11		131.8
C-12		142.8
C-13	6.05 (t, 8)	75.8
C-14	2.14 m	35.7
C-15		43.6
C-16	1.18 s	27
C-17	1.18 s	20.8
C-18	1.93 s	14.8
C-19	1.67 s	10
C-20	4.21 s	71.8
C-1′		172.8
C-2′	5.45 (d, 7)	75.2
C-3′	5.8 (dd 7,7)	54.2
N-H	7.24 (t, 7)	
CH_3(OAc)	2.0 s	21.2
CH_3(OAc)	2.41 s	22
Bz	7.4-8.1 m	126.8-138.1
CO(OAc)		167.8
CO(OAc)		167.8
CO(OBz)		167
CO(NBz)		169.2
C-1″		171
C-2″	2.79 (t, 7)	30
C-3″	2.55 (t,7)	30.8
C-4″		170.5
C-1‴	3.28 m	38.5
C-2‴	1.98 m	28.2
C-3‴	2.87 (t, 7)	50.6
N-CH_2	3.25 (t, 9)(8H)	60
$NCH_2\underline{CH_2}$	1.69 m (8H)	25
$\underline{CH_2}CH_3$	1.43 m (8H)	20.6
$CH_2\underline{CH_3}$	1.09(t, 9)(12H)	14.3

Characterization Data for 11.



11

m.p.	168-169°C
$[\alpha]_D^{20}$	−29° (0.001 MeOH)
IR (KBr)	3480, 3000, 1760, 1740, 1660, 1550, 1400, 1260, 1050 cm^{-1}
UV λ_{max}^{MeOH}	279 nm (ε 974), 271 nm (ε 1240), 228 nm (ε 12719)
MS (FAB)	1119 (MNa^+), 1097 (MH^+)

Table 6.

Position	^1H shift (ppm from TMS) Coupling (hertz)	^{13}C Shift (ppm from TMS)
C-1		*
C-2	5.63 (d, 7)	74.8
C-3	3.8 (d, 7)	46
C-4		80.8
C-5	4.99 (d, 9)	84.2
C-6	2.5 m	34.7
C-7	4.34 m	75.9

Table 6 continued.

C-8		57.5
C-9		204.2
C-10	6.44 s	71.3
C-11		131.6
C-12		141.2
C-13	6.05 (t, 8)	75.2
C-14	2.14 m	35.7
C-15		42.8
C-16	1.16 s	25.6
C-17	1.16 s	19.4
C-18	1.93 s	13.6
C-19	1.67 s	8.9
C-20	4.21 s	70.8
C-1′		172
C-2′	5.44 (d, 7)	74.2
C-3′	5.79 (dd 7,7)	53.6
N-H	7.25 (t, 7)	
CH_3(OAc)	2.2 s	20.9
CH_3(OAc)	2.4 s	21.6
Bz	7.4-8.1 m	126.8-138.1
CO(OAc)		169
CO(OAc)		170.2
CO(OBz)		166.4
CO(NBz)		170.2
C-1″		171.9
C-2″	2.75 (t, 7)	29
C-3″	2.54 (t,7)	29.8
C-4″		171.9
C-1‴	3.25 m	37.9
C-2‴	1.98 m	28.3
C-3‴	2.85 (t, 7)	**

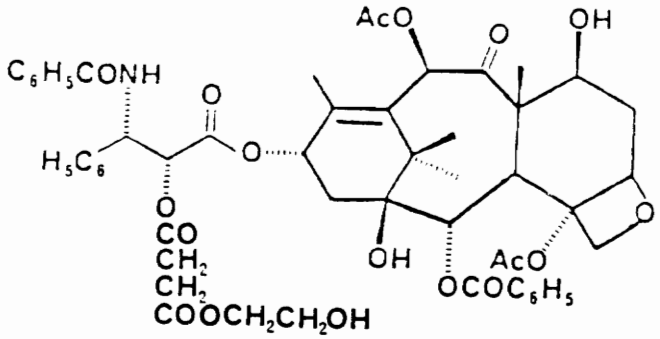
* under $CHCl_3$ signal ** under MeOH signal

4. Preparation of 2′-((4-((2-Hydroxyethyl)oxy)-1,4-dioxobutyl)oxy)Taxol

Preparation of 2′-((4-((2-hydroxyethyl)oxy)-1,4-dioxobutyl)oxy)taxol 12

Taxol succinate (26 mg) was dissolved in 2 ml of dry THF under argon gas and then 20 μl of Et_3N were added. The solution was cooled down to -1°C. To the solution, 10 μl of iBuOCOCl were added and the reaction mixture was warmed to room temperature in 15 minutes. Then 5 μl of ethylene glycol were added and the reaction mixture was stirred at room temperature for 15 hours, monitored by TLC with CH_2Cl_2 /EtOAc (1:1). The reaction was stopped by filtering the precipitate and evaporating the solvent. The crude products were purified by PTLC with CH_2Cl_2/EtOAc (1:3), to yield 25 mg of **12** , 83.3%. Characterization data for **12** are showed in Table 7.

Characterization Data for 12.



12

m.p.	164-165°C
$[\alpha]_D^{20}$	−32.5° (0.002, MeOH)
IR (KBr)	3500, 2950, 1760, 1740, 1660, 1390, 1260, 1160, 1080, 1040 cm^{-1}
UV λ_{max}^{MeOH}	279 nm (ε 609), 272 nm (ε 831), 228 nm (ε 14404)
MS (FAB):	1020 (MNa^+), 998 (MH^+)

Table 7.

Position	^1H Shift (ppm from TMS) Coupling (hertz)	^{13}C Shift (ppm from TMS)
C-1		79.1
C-2	5.7 (d, 7)	75.8
C-3	3.8 (d, 7)	45.8
C-4		81
C-5	4.95 (d, 9)	84.3
C-6	2.56 m	35.6
C-7	4.43 m	75.8

Table 7 continued.

C-8		58.2
C-9		204
C-10	6.29 s	72.1
C-11		132
C-12		142.3
C-13	6.23 (t, 8)	75.8
C-14	2.42 m	35.6
C-15		43.2
C-16	1.23 s	26.8
C-17	1.15 s	20.5
C-18	1.94 s	14.3
C-19	1.70 s	9.8
C-20	4.19 (d, 8) 4.32 (d, 8)	72.1
C-1′		172.2
C-2′	5.48 (d, 3)	74.3
C-3′	5.97 (dd 3,9)	52.9
N-H	7.14 (d, 9)	
CH_3(OAc)	2.25 s	22.1
CH_3(OAc)	2.45 s	22.8
Bz	7.4-8.1 m	126.8-138.1
CO(OAc)		168
CO(OAc)		169.9
CO(OBz)		167
CO(NBz)		167.3
C-1″		171
C-2″	2.65 m	29
C-3″	2.78 m	29
C-4″		171
C-1‴	3.7 (t,7)	66.2
C-2‴	4.1 m	61

5. Preparation of 2′-γ-Aminobutyryltaxol Formate

Preparation of 2′-N-CBZ-γ-aminobutyryltaxol 13

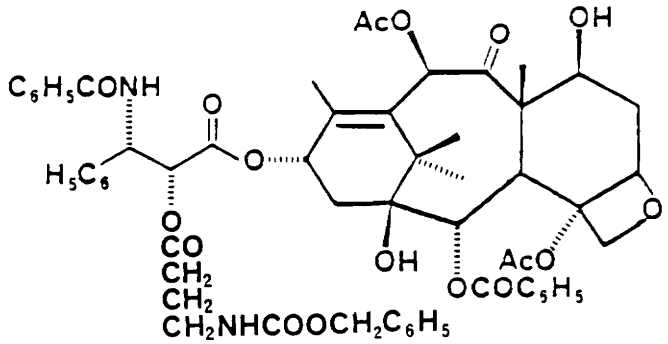
Taxol (20 mg) was added to a 10 ml flask along with 40 mg of DCC and 20 mg of N-CBZ- γ- aminobutyric acid. The reactants were dissolved in 4 ml of dry acetonitrile (dry acetonitrile was obtained by passing acetonitrile though activated alumina). The reaction was stirred at room temperature for 30 hours. At the end of the reaction, the dicyclohexylurea which had precipitated was removed by filtration. The solvent was removed under vacuum. The crude products were seperated by PTLC with hexanes/ethyl acetate (45:55),to yield 19.1 mg of pure product **13** (75.9%). Characterization data for **13** are showed in Table 8.

Preparation of 2′-γ-aminobutyryltaxol formate 14

2′-N-CBZ-γ-aminobutyryltaxol **13** (6 mg) was dissolved in 1.5 ml of methanol and 1 ml of formic acid was added to form a 40% formic acid/MeOH solution. To the solution, 5 mg of 5% Pd/C was added. The mixture was stirred at room temperature for 26 hours. The reaction was stopped by filtering the Pd/C and the filtration was dried under vacuum. The product was checked by proton NMR without further purification. The proton NMR spectrum showed that it was the expected product. The proton NMR spectrum for the key protons were showed below: C-2′ proton, 5.46 ppm (d, J = 3 Hz), C-2″ protons 3.14 ppm m, C-3″ protons, 2.42 ppm m, C-4″ protons, 3.40 ppm m, and signal for OCH_2Ph was disappeared as well as only 15 arometic protons were left according to the integration. After a few hours, it was checked by proton NMR and TLC

with CH_2Cl_2 /EtOAc/AcOH (2:1:0.02) again, which showed that the product **14** had decomposed back to taxol.

Characterization Data for 13.



13

m.p.	168-170°C
$[\alpha]_D^{20}$	−22.5° (0.002, MeOH)
IR (KBr):	3450, 2950, 1740, 1720, 1660, 1530, 1375, 1240, 1070, 1020, cm^{-1}
UV λ_{max}^{MeOH}	272 nm (ε 913), 268 nm (ε 1263), 264 nm (ε 1570)

Table 8.

Position	^{1}H Shift (ppm from TMS) Coupling (hertz)	^{13}C Shift (ppm from TMS)
C-1		79
C-2	5.7 (d, 7)	75.5
C-3	3.82 (d, 7)	45.8
C-4		81.3
C-5	5.0 (d, 9)	84.2
C-6	2.58 m	34
C-7	4.47 m	75.5

Table 8 continued.

C-8		58.2
C-9		203.8
C-10	6.29 s	72.4
C-11		132.4
C-12		142.8
C-13	6.26 (t, 8)	75.5
C-14	2.45 m	35.4
C-15		43.4
C-16	1.23 s	26.8
C-17	1.14 s	20.7
C-18	1.95 s	14.8
C-19	1.67 s	9.9
C-20	4.21 (d, 8) 4.33 (d, 8)	72.1
C-1′		172
C-2′	5.47 (d, 3)	74.5
C-3′	6.07 (dd 3,9)	52.8
N-H	7.4 (d, 9)	
CH_3(OAc)	2.22 s	22
CH_3(OAc)	2.50 s	22.6
Bz	7.4-8.1 m	126.8-138.1
CO(OAc)		168.2
CO(OAc)		170
CO(OBz)		167
CO(NBz)		167.6
C-1″		171.8
C-2″	3.14 m	29.8
C-3″	2.42 m	30.2
C-4″	3.4 m	30.8
OCH_2Ph	4.95 s	66.4
N-H	4.87 (t, 7)	
C-1‴		156.4

6. Measurement of Water Solubilities

Measurement of water solubility for taxol

Taxol (1.6 mg) was disolved in 10 ml of distilled water saturated with 1-octanol in a 60 ml separatory funnel. Then 10 ml of 1-octanol saturated with distilled water were added to the separatory funnel. The funnel was shaken for a few times and stood for about 30 minutes until these two layers were completely separated. Samples of both the water layer and octanol layer were taken for UV absorption measurement at 228 nm. The octanol layer was diluted 5 times (1 ml diluted to 5 ml) before measurement.

The concentration of the taxol in water layer C_{water} was 1.6×10^{-6} M and $C_{octanol}$ was 8.3×10^{-5} M.

$$P = \frac{C_{octanol}}{C_{water}} = \frac{8.3 \times 10^{-5} M}{1.6 \times 10^{-6} M} = 51.87$$

$$P' = \frac{C_{water}}{C_{octanol}} = \frac{1.6 \times 10^{-6} M}{8.3 \times 10^{-5} M} = 0.019$$

$$K' = \frac{0.019}{0.019} = 1$$

Measurement of water solubility for 6

Product **6** (0.8 mg) was disolved in 10 ml of distilled water saturated with octanol in a 60 ml separatory funnel. Then 10 ml of octanol saturated with distilled water were added

to the separatory funnel. The funnel was shaken for a few times and stood for about 30 minutes until these two layers were completely separated. Sample of both the water layer and octanol layer were taken for UV absorption measurement at 228 nm.

The concentration of the **6** in the water layer C_{water} was 5.31×10^{-5} M and $C_{octanol}$ was 1.33×10^{-5} M.

$$P = \frac{C_{octanol}}{C_{water}} = \frac{1.33 \times 10^{-5} M}{5.31 \times 10^{-5} M} = 0.25$$

$$P' = \frac{C_{water}}{C_{octanol}} = \frac{5.31 \times 10^{-5} M}{1.33 \times 10^{-5} M} = 3.99$$

$$K' = \frac{P'_x}{P'_{taxol}} = \frac{3.99}{0.019} = 210$$

Measurement of water solubility for 8

The same manner was used as for product **6** and 0.8 mg of **8** was taken.

The concentration of **8** in the water layer C_{water} was 4.83×10^{-5} M and $C_{octanol}$ was 1.33×10^{-5} M.

$$P = \frac{1.33 \times 10^{-5}}{4.83 \times 10^{-5}} = 0.28 \quad P' = \frac{4.83 \times 10^{-5}}{1.33 \times 10^{-5}} = 3.63 \quad K' = \frac{3.63}{0.019} = 191$$

Measurement of water solubility for **11**

The same manner was used as for product **6** and 0.7 mg of **11** was taken.

The concentration of the **11** in the water layer, C_{water}, was 4.87×10^{-5} M and $C_{octanol}$ was 2.16×10^{-5} M.

$$P = \frac{2.16 \times 10^{-5}}{4.87 \times 10^{-5}} = 0.44 \quad P' = \frac{4.87 \times 10^{-5}}{2.16 \times 10^{-5}} = 2.26 \quad K' = \frac{2.26}{0.019} = 118$$

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VITA

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