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Taxol, a molecule for all seasons

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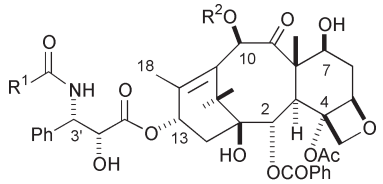
The diterpenoid natural product taxol, first discovered in the 1960's has proved to be the most important new anticancer drug introduced in the last ten years. A brief history of taxol's development from laboratory curiosity to blockbuster drug is followed by a summary of its chemistry, including an overview of the six completed syntheses of taxol. The review concludes with a discussion of its bioactivity as a promoter of tubulin polymerization and as a drug.

Introduction

With the possible exception of Viagra, probably no new drug in the last 20 years has generated as much public interest and excitement as has taxol (paclitaxel or Taxol™, **1**).[1] This interest



1 R^1 = Ph, R^2 = Ac
2 R^1 = Me_3CO, R^2 = H

was fueled primarily by taxol's excellent clinical activity against ovarian and breast cancers, but it was intensified by the severe supply problems that were in effect during the period leading up to its general clinical use. These problems were successfully solved, as will be described below, and taxol is now widely available and is the largest-selling anticancer drug of all time, with sales of over $1.5 billion in 1999.[2] This review will give a brief history of the development of taxol from a laboratory curiosity to a blockbuster drug, and will then describe some recent advances in the chemistry and biology of this fascinating compound.

History of taxol's development as an anticancer drug

Discovery of the bioactivity now known to be due to taxol was made in 1962, when Arthur Barclay, a botanist working for the US Department of Agriculture under contract to the US National Cancer Institute (NCI), made a collection of the stem and bark of *Taxus brevifolia* Nutt. in Washington State. These plant samples, along with many others, were duly extracted and tested for bioactivity, and in 1964 the extract from *T. brevifolia* was found to be cytotoxic to KB cells. The extract was assigned to Dr Monroe Wall at Research Triangle Institute, and taxol was isolated in 1967.[3] The structure was elucidated by a combination of X-ray studies of two degradation products and ^{1}H-NMR analysis of the intact molecule, and was published in 1971.[4]

Initial reaction to taxol as a potential anticancer drug was less than enthusiastic. Although it was clearly an active compound, with activity both in cell culture and also *in vivo* against various leukemias and the Walker 256 carcinosarcoma, its activity was only modest in these assays. To add to its problems, it was highly insoluble in water, and would thus clearly present formidable formulation problems, and it was isolated in only very modest yield from the bark of a relatively uncommon and slow-growing tree. Not a good outlook for a potential drug candidate!

Fortunately testing was carried out in some new *in vivo* bioassays that were introduced by NCI in the early 1970's, and it proved to be strongly active in a B16 mouse melanoma model. On the basis of this activity, and with enthusiastic support from Dr Matthew Suffness at NCI and Dr Monroe Wall, taxol was selected as a development candidate in 1977. Development of taxol as a drug was a challenging task because of the problems with solubility and supply noted earlier, and also because of its relatively low potency. The solubility problem was successfully overcome with a formulation in ethanol and Cremophor EL, and this turned out to be important in both negative and positive ways. On the negative side, the high levels of Cremophor required led to hypersensitivity reactions and almost led to the withdrawal of taxol from clinical trials. On the positive side, there is some evidence that Cremophor has a pharmaceutical effect over and above its surfactant properties, and may act to reverse multidrug resistance.[3]

Interest in taxol as a drug candidate was increased significantly when Susan Horwitz reported in 1979 that it had what was then a completely new mechanism of action, in that it promoted the assembly of the proteins α- and β-tubulin into microtubules.[5] A schematic representation of taxol's effect on the tubulin polymerization process is shown in Fig. 1.

Microtubules are required for chromosome segregation and for other operations such as intracellular transport and positioning of internal cellular organelles, and all of these activities require that the microtubules be in dynamic equilibrium with monomeric tubulins. Several compounds, including the clinically used drugs vinblastine (Velban™) and vincristine (Oncovin™), were known to operate as spindle poisons by *preventing* the assembly of tubulin into microtubules, but taxol was the first compound in which the activity was linked to *promotion* of microtubule assembly. This discovery proved to be important in maintaining interest in the development of taxol at a time when its initial clinical results were discouraging. Taxol's mechanism

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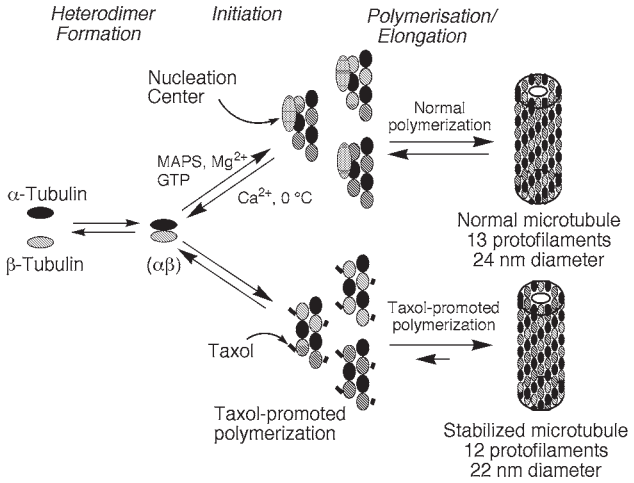


Fig. 1 Schematic representation of normal microtubule assembly (upper) and taxol-promoted microtubule assembly (lower).

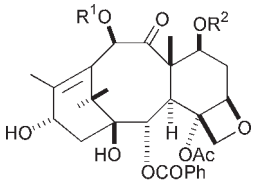
of action, and specifically its binding to microtubules, will be discussed in more detail in a later section of this review.

Taxol went into Phase I clinical trials in 1984, and into Phase II trials in 1985. These trials were limited by the supply of the drug, but gave the first clear evidence of activity with clinically relevant responses in ovarian cancer and in breast cancer reported in 1989[6] and 1991[7] respectively. Taxol and its semisynthetic analog docetaxel (Taxotere™, **2**) are now used (either as single agents or in combination with other drugs such as cisplatin) for the treatment of ovarian cancer, breast cancer, and non-small-cell lung cancer.[8]

The taxol supply crisis and its solution

The recognition of taxol's clinical activity created a crisis in the supply of the drug, since at the time it was only available in low yield from the bark of *T. brevifolia*. Since this tree is relatively uncommon, occurring most abundantly in the old-growth forests of the Pacific Northwest of the USA, the prospects of the large-scale logging needed to supply taxol to the clinical market raised serious environmental concerns.[9]

The responsibility for solving this problem fell to the Bristol-Myers Squibb Company, which had obtained the rights to develop taxol from the NCI under a Cooperative Research and Development Agreement. The various options for increasing the supply included increased harvesting of *T. brevifolia* bark, the isolation of taxol from a renewable resource such as yew needles, the semisynthesis of taxol from other taxoids in yew, and bioproduction through plant tissue culture. Initial supplies of taxol for clinical use were obtained by increased harvesting of *T. brevifolia* bark. The real breakthrough came from a combination of Potier's discovery that needles of the English yew, *T. baccata*, contained substantial amounts of 10-deacetylbaccatin III (**3**), in addition to smaller amounts of baccatin III (**4**),[10] with Holton's discovery of an efficient semisynthesis of taxol from the protected baccatin III (**5**) through a β-lactam



3 $R^1 = R^2 = H$
4 $R^1 = Ac$, $R^2 = H$
5 $R^1 = Ac$, $R^2 = SiEt_3$

intermediate; the details of this synthesis will be described later.[11] This invention was licensed to Bristol-Myers Squibb, and enabled the company to produce enough taxol to meet an escalating demand, with sales rising from about $400 million in 1994 to an estimated $1600 million in 2000.[2]

In addition to this and other semisynthetic routes, several workers have investigated the production of taxol by plant tissue culture methods, and at least two companies (Phyton Inc. and ESCA Genetics) have developed production methods.[12] Reported yields range from 0.012–0.05%, with specific production rates of 0.3 mg g^{-1} dry cell weight per day for up to 40 days.[13] These yields are close to those needed for commercially viable production, and it is thus quite probable that full-scale bioproduction of taxol will begin within the next few years, especially since Bristol-Myers Squibb signed agreements in 1998 with Phyton Inc. to commercialize Phyton's plant cell fermentation technology. The production of taxol by the fungus *Taxomyces andreanae* was reported by Stierle *et al*. in 1993, but the yield was extremely low (25–50 ng L^{-1}) and a large increase in this yield through strain improvement will be needed before a fungal culture method would be commercially viable. More recently Strobel and his coworkers have found that the fungus *Periconia* sp. isolated from *Torreya grandiflora* produces taxol at the 800 ng L^{-1} level when stimulated with benzoic acid.[14] Other sources of taxol have also been discovered, with one of the more interesting ones being hazelnut cultivars and their associated fungal endophytes.[15] Since taxol appears to be produced by the hazelnut cultivars as well as by their fungal endophytes, this is the first example of taxol being found in a plant outside the Taxaceae family.

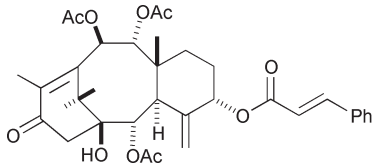
The quest to understand taxol's structure–activity relationships

The chemistry of taxol has been thoroughly investigated by a large number of academic and industrial researchers, and this review will thus not attempt to provide a complete coverage. Readers interested in a more comprehensive review can consult any of the several available books[16–19] and reviews,[20–23] including a comprehensive forthcoming review.[24] This section will thus describe some of the early work from the author's laboratory, and will then summarize the major findings for each section of taxol's structure.

Early studies

Our work on taxol began in 1979 with a conversation between the author and Bob Holton, who at that time was a member of the Virginia Tech faculty. He had developed the outline of a synthetic approach to taxol, and we agreed that we would collaborate on this synthesis. He would start at the beginning in a 'bottom up' approach, while I would start with taxol and study its chemistry in a 'top down' approach. Since we did not have a supply of taxol, I decided to begin by looking at the possible conversion of *O*-cinnamoyltaxicin I triacetate (**6**), available by Lythgoe's procedure from taxine B (**7**)[25] into taxol or taxol-like compounds. I isolated about 100 mg of crude taxine B from yew bushes growing on the Virginia Tech campus, converted it into **6**, and did some preliminary work on forming the oxetane ring. At this point I also contacted Dr Matthew Suffness at the US National Cancer Institute, and was able to obtain a small amount of pure taxol, together with larger amounts of various side cuts from the large-scale purification of taxol for the pending clinical trials. With these extracts in hand the need for the conversion of **6** into taxol-like compounds was removed, and this aspect of the project was dropped. It was thus very gratifying to find that Scheeren,[26] Potier,[27] and Saicic[28] have all published conversions of taxine to taxol-like compounds within the last few years.

Bob Holton and I submitted a proposal to the National Institutes of Health in 1980 for funding of our joint approach to taxol, but there was little interest in the compound at that time



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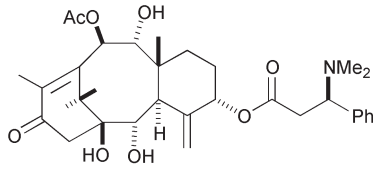

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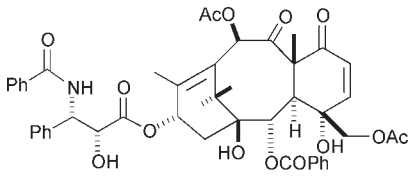
and the proposal was not funded. At this point we decided to try our luck at separate proposals, and so while Bob went back to NIH (where he eventually did get funded for his work) I chose to apply to the American Cancer Society. To their credit the reviewers at the ACS saw the value of studying the chemistry of taxol, and we received a modest grant in 1982.

Our basic approach in these early days, when little was known about the chemistry of taxol, was to try to modify its functional groups one at a time, to see whether they were individually necessary for the activity of the drug. We began by showing that the side-chain hydroxy group at the 2′-position was much more reactive to acetylation than that at the 7-position, and Susan Horwitz, with whom we had begun to collaborate, showed that the resulting taxol acetates still retained much of taxol's activity.[29] The reverse proved true on oxidation, however, and the 7-hydroxy group could be oxidized selectively to the corresponding 7-oxo derivative. This product proved to be unstable, and readily underwent β-elimination to give the ring-opened product **8**. Compound **8** turned out to be essentially non-cytotoxic, thus providing the first hint of the importance of the oxetane ring to taxol's activity.[30]



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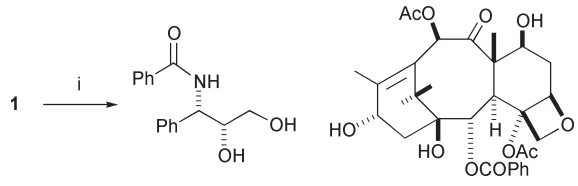
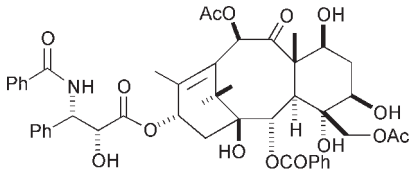
With a molecule as complex as taxol, not every experiment gave the expected result. Sometimes the unexpected results were useful, although more often they were not. One of the useful results came from our desire to reduce the C-9 keto group to an alcohol. Treatment of taxol with sodium borohydride left the keto group essentially untouched, but removed the side chain in good yield; the yield was later improved by the use of tetrabutylammonium borohydride in dichloromethane (Scheme 1).[31]



Scheme 1 *Reagents and conditions*: i, Bu_4NBH_4 (excess), CH_2Cl_2, rt, 24 h 98%.

This unexpected reduction of an ester in the presence of a keto group was due to the extremely hindered location of the 9-keto group and to the presence of an α-hydroxy group on the side chain. The reduction products were the diol **9** and baccatin III (**4**), and this formation of baccatin III from taxol or (equally well) from crude taxol–cephalomannine mixtures available from the National Cancer Institute gave us and other workers ready access to baccatin III.

Another surprising reaction was that of taxol with Meerwein's reagent, $Et_3O^+BF_4^-$. The reaction was originally carried out in an attempt to cleave the side-chain amide group, since it was known that amides react selectively with Meerwein's reagent in the presence of esters to yield imino ethers.[32] In the event, the amide group was unreactive but the oxetane ring underwent an assisted ring-opening to give compound **10**.[33] Compound **10**, like compound **8**, was essentially inactive in tubulin-assembly and cytotoxicity assays, thus emphasizing the importance of the oxetane ring to taxol's activity.



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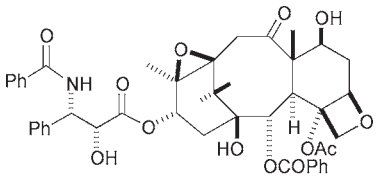
In the following sections the chemistry and structure–activity relationships of taxol will be summarized, with an emphasis on work carried out at Virginia Tech but including key results from other laboratories. The discussion will follow a clockwise route, starting with the 'northern hemisphere' and proceeding around the oxetane ring to the 'southern hemisphere' and thence to the side chain.

The northern hemisphere

The C-11–C-12 double bond in the A-ring of taxol is relatively unreactive, as indicated by the fact that hydrogenation of baccatin III gives a hexahydro product in which the C-2 benzoyl group is reduced to a cyclohexylcarbonyl group while the C-11–C-12 double bond is untouched.[34] Epoxidation of taxol with *m*-chloroperbenzoic acid also fails, but the epoxide **11** could be prepared by oxidation of 10-deacetoxytaxol; compound **11** turned out to be more active than taxol in a tubulin-assembly assay but less cytotoxic to B16 melanoma cells.[35]



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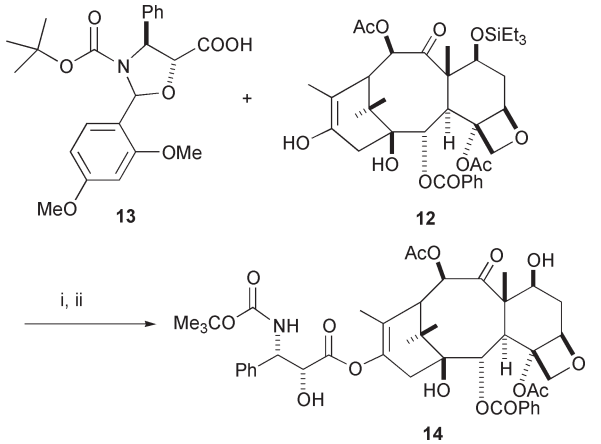
Interestingly the enol **12**, obtained by reduction of 7-(triethylsilyl)-13-oxobaccatin III with Zn–AcOH, is stable and can be acylated. Acylation with the oxazolidine **13** (Scheme 2) gave the isotaxol derivative **14**; compound **14** is slightly more cytotoxic than taxol.[36]

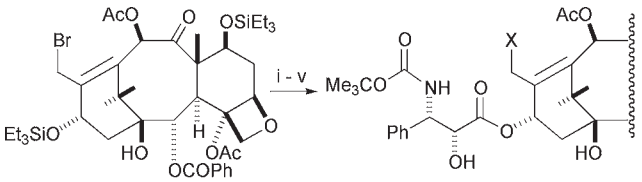
Allylic bromination of 7,13-di(triethylsilyl)baccatin III gave the bromide **15**, which gave the corresponding C-18 analogs on treatment with nucleophiles such as Me_2CuLi, NaN_3, Bu_4NOAc and KCN. Attachment of the taxol side chain and deprotection gave the C-18 analogs **16** (X = Me, N_3, OAc or CN), all of which were less cytotoxic than taxol, with the methyl derivative being the most active (Scheme 3).[37]

Taxol can be selectively deacetylated at C-10 to give 10-deacetyltaxol by treatment with hydrazine[38] or with sodium bicarbonate and hydrogen peroxide.[39] Deoxygenation of the 10-hydroxy group was originally achieved using Barton's xanthate chemistry,[40] but a simpler method was developed by treating taxol with samarium diiodide.[41,42] The resulting 10-deacetoxytaxol **17** had a similar cytotoxicity to taxol,[40] and



13 **12** **14**

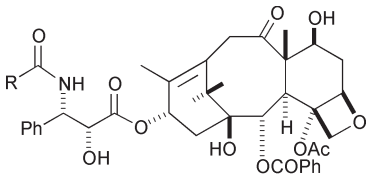
Scheme 2 *Reagents and conditions*: i, DCC, DMAP; ii, AcOH–H_2O, rt, 4 days, 40% overall.



15 **16**

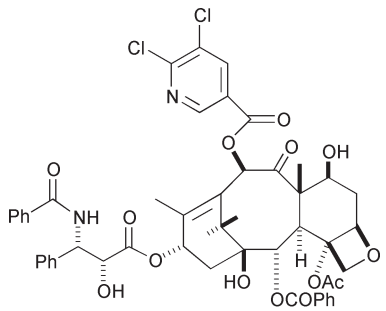
Scheme 3 *Reagents and conditions*: i, Me_2CuLi, THF, −78 °C; ii, HF–pyridine, rt; iii, TES-Cl, imidazole, DMF, 0 °C, 40%; iv, NaHMDS, THF, −78 °C, then β-lactam synthon; v, HF–pyridine, rt, 53%.

the same was true for the corresponding docetaxel analog **18**.[42]



17 R = Ph
18 R = Bu^tO

Acylation of 10-deacetyltaxol at the C-10 position has yielded a number of useful analogs, including the dichloronicotinyl derivative **19** which is more cytotoxic than taxol



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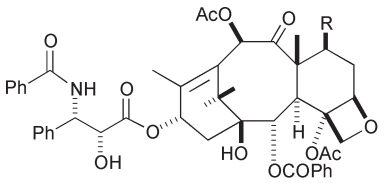
against both normal and drug-resistant MCF-7 cells.[43] Taxol analogs with isobutyl or isobutenyl substituents replacing the 3′-phenyl group and various C-10 acyl groups have been prepared by Ojima, and several of these have shown improved cytotoxicity against the resistant cell line MCF7-R.[44]

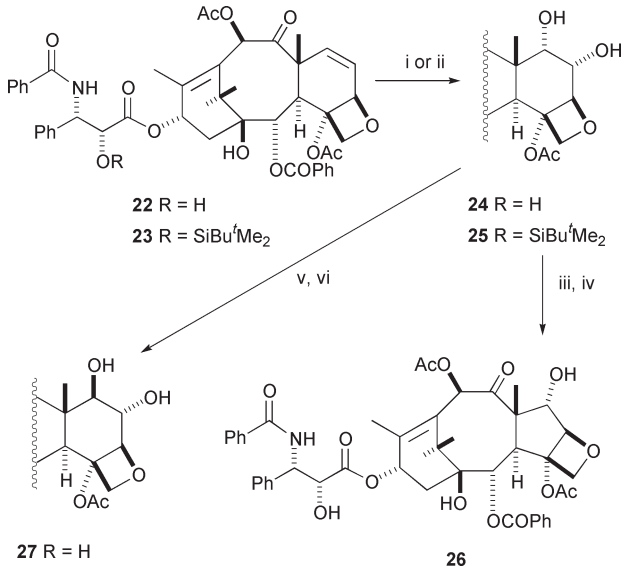
The C-7 hydroxy group can be removed selectively by the Barton xanthate deoxygenation route to give 7-deoxytaxol **20**, and the same intermediate can be used to convert 7-*epi*taxol to taxol.[45] 7-Deoxytaxol has the same cytotoxicity as taxol in the HCT116 cell line, suggesting that the 7-hydroxy group is not essential for taxol's activity.[45,46]

Although the 7-hydroxy group may not be necessary for taxol's activity, modifications at C-7 have yielded analogs with improved properties, and the thiomethyl derivative **21** has been selected by Bristol-Myers Squibb for development as a 'second-generation' taxol analog: it is currently in clinical trials, with preliminary reports that it is performing well.[47]



20 R = H
21 R = OCH_2SCH_3

Dehydration at C-7 can be accomplished by treatment of the 7-triflate derivative with a non-nucleophilic base such as DBU to give 6,7-dehydrotaxol (**22**),[38,48] which was slightly less cytotoxic than taxol to CA46 cells.[48] Osmylation of **22** gave the 6α,7α-diol **24**. Protection of **22** as its TBDMS ether gave **23**, which could be converted to the 2′-protected diol **25**; this was converted to the C-nortaxol analog **26** by reaction with lead tetraacetate and deprotection.[49] Compound **26** was significantly less cytotoxic than taxol, which is surprising given the very similar shapes of the two compounds. Diol **25** could also be converted to 6α-hydroxytaxol (**27**), which is the major human metabolite of taxol.[50] Although the yield was low, the starting material was recovered unchanged and could be recycled to effect a good overall conversion (Scheme 4).



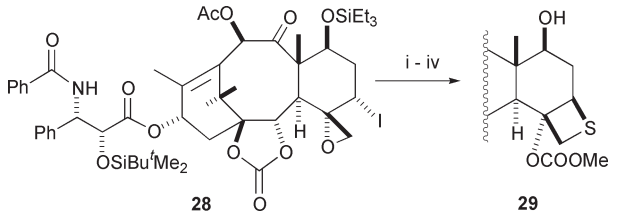
22 R = H
23 R = $SiBu^tMe_2$
24 R = H
25 R = $SiBu^tMe_2$
27 R = H
26

Scheme 4 *Reagents and conditions*: i, **22**, OsO_4, NMO, 25 °C, 9 h, 71% of **24**; ii, **23**, OsO_4, NMO, 25 °C, 9 h, 78% of **25**; iii **25**, $Pb(OCc)_4$, $NaHCO_3$, 2 h, 0 °C, 67%; iv, THF, HF–pyridine, 1.5 h, rt, 86% of **26**; v, DBU, PhMe, 80 °C, 12% (95% based on unrecovered starting material); vi, THF, HF–pyridine, 75%.

The oxetane ring

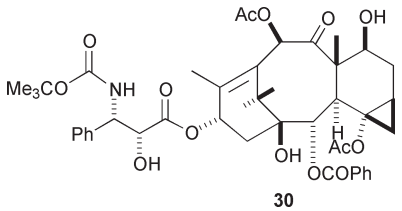
As noted earlier, opening of the oxetane ring to give compounds **8** or **10** essentially eliminated taxol's cytotoxicity and tubulin-assembly activity. The question thus arose as to the reason for this effect; putting the question another way, why is the oxetane ring necessary for taxol's activity? It is clearly not involved in any covalent binding to tubulin, since taxol can be exchanged with labeled taxol on tubulin, and we thus elected to test whether changing the size and electronegativity of the hetero-

atom had any effect. The sulfetane analog **29** was selected for study, and was prepared by reaction of the key intermediate **28** with Li_2S, followed by acylation at C-4, reaction with phenyllithium to open the cyclic carbonate, and deprotection (Scheme 5). Sulfetane **29** was significantly less active than taxol in both tubulin-assembly and cytotoxicity assays.[51]



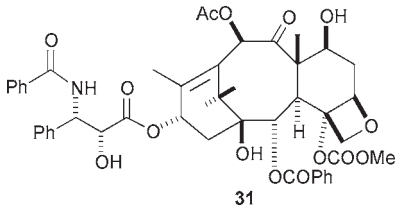
Scheme 5 *Reagents and conditions*: i, Li_2S, THF, rt, 28 h, then Im_2CO, imidazole, rt, 12 h, 56%; ii, LHMDS, THF, −78 °C (7 min), rt (1 min), −78 °C (2 min) then $ClCO_2Me$, 39%; iii, PhLi, THF, −78 °C, 3 min, 61%; iv, HF–pridine, rt, 9 h, 76%.

The reason for the lack of activity of **29** as compared with taxol, and the reason for the lack of activity of oxetane ring-opened analogs, has been discussed by Snyder on the basis of a minireceptor model of the binding site for taxol on tubulin.[52] These authors note that oxetane ring-opened analogs such as **8** and **11** also lack the C-4 acetate function, which has been shown to be necessary for activity, and conclude that hydrogen bond acceptor properties and the rigidification of the taxol ring system by the oxetane ring also play a role in stabilizing the taxol–tubulin complex. The lack of activity of the thietane analog **29** is explicable because the larger sulfur atom does not fit well in the binding site. The predictions of the Snyder model were supported by the observation that the cyclopropyl derivative **30** is almost as active as taxol in a tubulin assembly assay.[53]

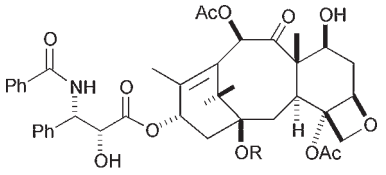


The southern hemisphere

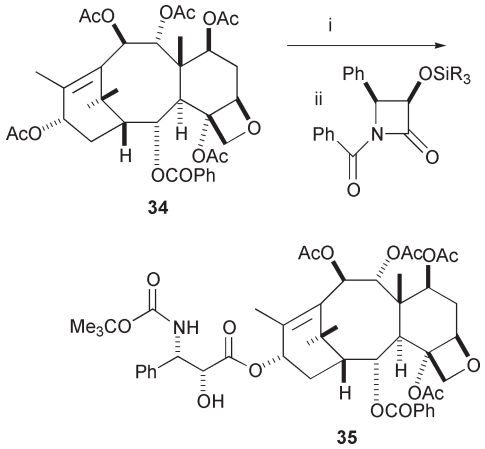
The C-4 acetate function, or at least a C-4 acyl function, is necessary for activity, as mentioned above. Thus C-4 deacetyltaxol is significantly less active than taxol,[54] and 4-deacetoxytaxol is also much less active than taxol.[55] Some C-4 derivatives are more active than taxol, however, and the carbonate **31** is in clinical trials.[56,47]



Modifications of the C-2 benzoate have yielded a number of interesting derivatives. The benzoyloxy group or a similar group is necessary for activity, since 2-debenzoyloxytaxol (**32**)[57] and 1-benzoyl-2-debenzoyloxytaxol (**33**)[58] are both inactive. Replacement of the C-2 benzoyl group with other acyl groups results in taxol analogs which can be significantly less active or more active than taxol.[59,60] Interestingly the difference in activity is modulated by the position of substituents on the benzene ring of substituted benzoyl groups; 2-*p*-azidobenzoyltaxol is essentially inactive, while 2-*m*-azidobenzoyltaxol is almost an order of magnitude more active than taxol.[59]

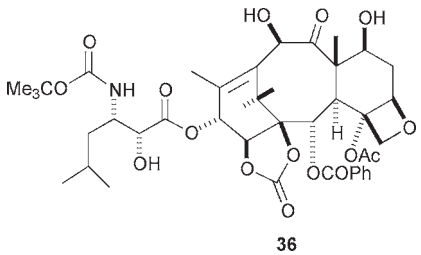


It has not proved possible to remove the C-1 hydroxy group by Barton deoxygenation or other deoxygenation methods; an attempt to do so led to formation of the C-2 deoxy product **33**.[58] Some C-1 deoxytaxol derivatives were, however, prepared from the natural product baccatin VI (**34**) by selective deacetylation and side-chain attachment (Scheme 6). The 1-deoxy-9-dihydrodocetaxel analog **35** was about one third as active as taxol in tubulin assembly and cytotoxicity assays.[61]



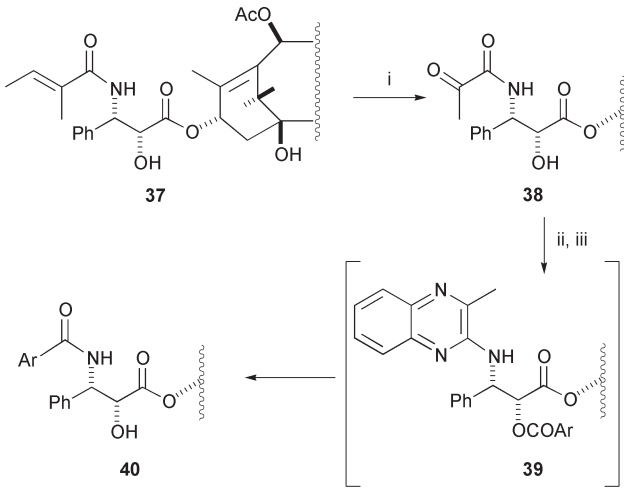
Scheme 6 *Reagents and conditions*: i, Red Al, THF, 77%; ii, NaH, THF, β-lactam, 0 °C–rt, 86%; HF–pyridine, −20 °C, 87%.

Analogs at the C-14 position have also been prepared by Ojima and Appendino from the naturally-occurring 14β-hydroxy-10-deacetylbaccatin III. The most interesting analogs have come from a series of compounds with a carbonate group linking the C-14 and C-1 hydroxy groups;[62] the derivative **36** is under development by Bayer as an orally active anticancer drug.[63]



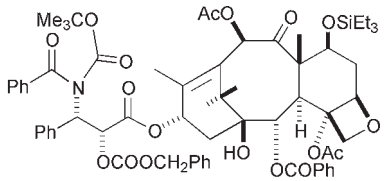
The side chain

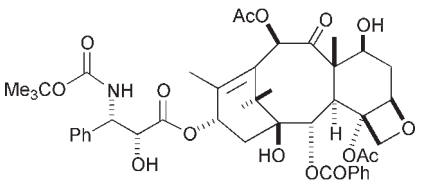
Our work on the side chain has been concerned largely with finding direct methods for the conversion of taxol or its congener cephalomannine (**37**) into other N-acyl analogues. The initial success came with the finding that cephalomannine could be ozonized selectively in high yield to give the ketoamide **38** (Scheme 7). Acylation of **38** with a desired substituted benzoic acid gave the corresponding 2′-acyl derivative selectively, and this could be reacted with *o*-phenylenediamine under anhydrous acidic conditions to give an *N*-acyl analog of taxol. Reaction proceeds *via* the 2-aminoquinoxaline derivative **39**, which is cleaved under the acidic conditions to the free 3′-amine. This amine then undergoes a spontaneous *O* → *N* intramolecular acyl transfer reaction to give the final product **40**.[64]



Scheme 7 *Reagents and conditions*: i, O_3, CH_2Cl_2, −78 °C, 30 min, 97%; ii, $ArCO_2OH$, DCC, 4-pyrrolidonopyridine, EtOAc, 2 h, 90%; iii, 4 Å sieves, PhH, *o*-phenylenediamine, *p*-TsOH (cat.), reflux, 12 h, 80%.

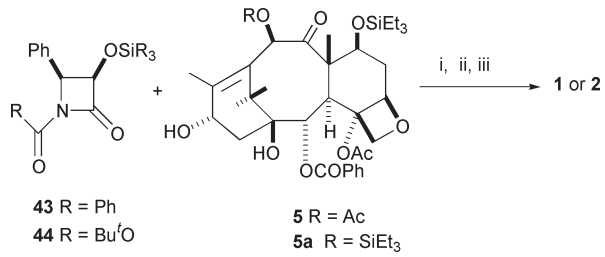
A second pathway was developed to convert taxol into docetaxel.[65] Protection of taxol as its 2′-(benzyloxycarbonyl)-7-(triethylsilyl) derivative was followed by reaction with di-*tert*-butyl dicarbonate and DMAP to give the imide **41**. Compound **41** was then converted into 10-acetyldocetaxel **42** by reaction with magnesium methoxide followed by HF–pyridine to deprotect the 7-position.





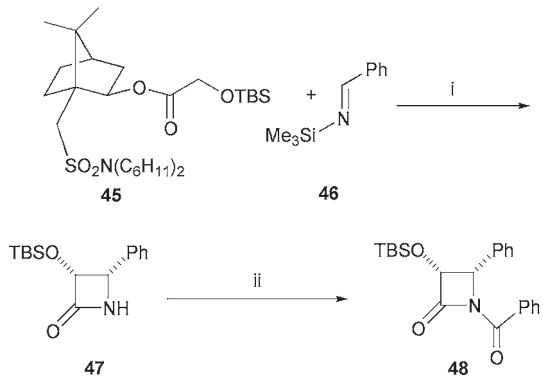
Many routes have been developed to prepare the taxol side chain and to attach it to a protected baccatin III derivative to prepare taxol or an analog thereof, and this chemistry has been reviewed.[11,66] Because of the congested position of the 13-hydroxy group of baccatin III, esterification with a simple protected taxol side chain is difficult and proceeds in only modest yield.[67] The most important synthetic methods are thus those which use a cyclically protected form of the taxol side chain, and these are the methods used commercially in the synthesis of taxol and docetaxel.

As mentioned earlier, taxol is prepared commercially by acylation of a 7-protected baccatin III derivative (**5**) with a β-lactam such as **43** (Scheme 8); the use of other β-lactams such as **44** yields docetaxel analogs.[11] The β-lactams needed for the synthesis of Scheme 8 are prepared by condensation of an enolate with an imine, but there have been many different approaches to the details of this synthesis. In Holton's original approach the β-lactam was prepared by a Staudinger reaction of acetyl glycolyl chloride with the imine from benzaldehyde and *p*-anisidine; this gave the desired *syn* lactam stereoselectively, but of course in racemic form.[11] Several enantioselective syntheses of suitably protected β-lactams have been developed, with Georg's route from Oppolzer's chiral auxiliary[68]

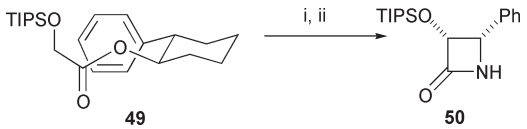


Scheme 8 *Reagents and conditions*: i, **5** (0.1 M in THF), *n*-BuLi, −45 °C; ii, **43** or **44** (0.2 M in THF), warm to 0 °C, 2 h, 95%; iii, HF–pyridine, MeCN, 98%.

(Scheme 9) and Ojima's synthesis from (1*R*,2*S*)-2-phenylcyclohexan-1-ol[69] (Scheme 10) being early and effective approaches.



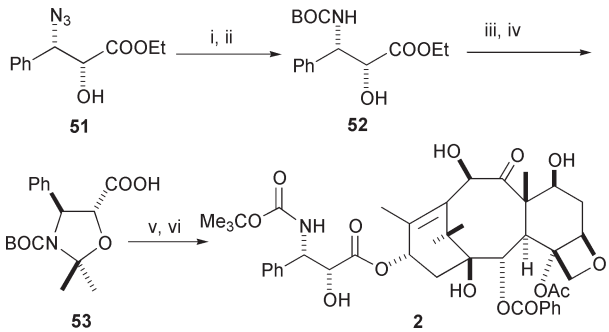
Scheme 9 *Reagents and conditions*: i, LDA, THF, 94%; ii, PhCOCl, CH_2Cl_2, Et_3N, DMAP, 96%, 93–97% ee.



Scheme 10 *Reagents and conditions*: i, LDA; ii, PhCH=NTMS, 85%, 96% ee.

Other chiral syntheses of the β-lactam have been developed by Farina,[70] Commerçon,[71] and Fujisawa[72] from chiral imine precursors, by Holton[73] and by Palomo[74] from oxazolidinone auxiliaries, and by Lee by means of a Sharpless oxidation,[75] to name just a few. A convenient enzyme-catalyzed resolution of racemic β-lactam has also been published by Sih.[76]

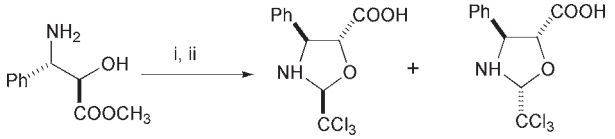
The second major synthetic route is through an oxazolidine carboxylic acid intermediate. This pathway was originally developed by Commerçon and his co-workers, and is illustrated in Scheme 11 below.[77] The azido ester **51** can be prepared by



Scheme 11 *Reagents and conditions*: i, H_2, Pd/C; ii, $(BOC)_2O$, 65%; iii, $CH_2=C(OCH_3)CH_3$, PPTS; iv, LiOH, EtOH–H_2O, 99%; v, 7,10-diTroc-10-deactylbaccatin, III, DCC, DMAP, 99%; vi, HCO_2H, then $(BOC)_2O$, then Zn, AcOH, H_2O, 62%.

ring-opening of an epoxide derived from a Sharpless epoxidation,[78] by the Staudinger reaction previously mentioned,[71] or in

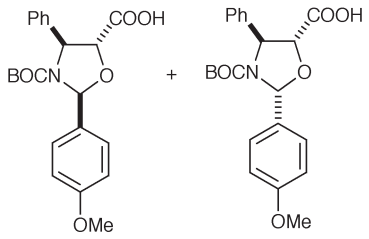
several other ways. Reduction and protection of **51** gave the carbamate **52**, which was converted to the oxazolidine **53** and thence to docetaxel (**2**). The weakness of the original approach lay in the deprotection and reprotection steps necessary to open the oxazolidine ring, but this weakness has been resolved in several ways. In one approach (Scheme 12) the trichloromethyl



54 55 56

Scheme 12 *Reagents and conditions*: i, CCl_3CHO, PPTS; ii, LiOH, MeOH, then HCl.

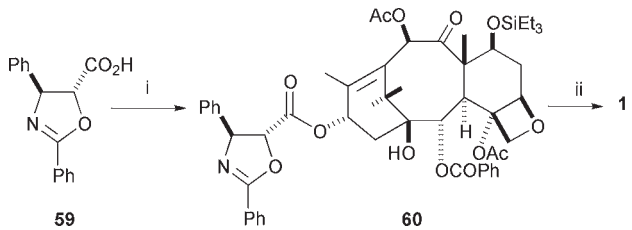
oxazolidines **55** and **56** could be coupled directly with 7,10-ditrocbaccatin III without the necessity of protecting the nitrogen; the resulting coupled product was converted to docetaxel by treatment with zinc and acetic acid followed by N-acylation.[79] In a second approach from the same group it was found that the *p*-methoxybenzylidene oxazolidines **57** and **58**



57 58

could be coupled with protected baccatin III derivatives in almost quantitative yield, and that the coupled products could then be treated with toluenesulfonic acid to remove the *p*-methoxybenzylidene group selectively;[80] this approach thus provides a very efficient route to docetaxel.

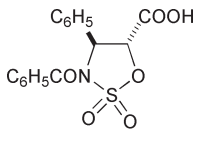
A third general approach is through an oxazoline intermediate. Coupling of the oxazoline **59** with 7-(triethylsilyl)baccatin III proceeded in excellent yield to give the coupled product **60**, which could be hydrolyzed to taxol in one step (Scheme 13).[81] The simplicity of this route has attracted several



59 60

Scheme 13 *Reagents and conditions*: i, dry PhMe, 4-pyrrolidonopyridine (cat.), DCC, rt, 30 min, 95%; ii, 0.1 M HCl, 95 °C, 2 h, 75%.

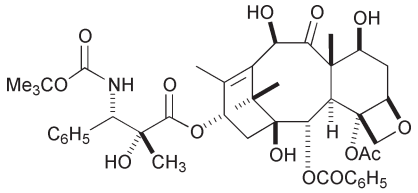
syntheses of suitable oxazolines, including syntheses of racemic material from cinnamyl alcohol through addition of phenylselenyl triflate and azide ion[82,83] and a synthesis of chiral oxazolines from (L)-phenylglycine.[84] One unexpected synthesis of the oxazoline derivative **60** was derived from the dioxooxathiazolidine **61**, which gave **60** on coupling with 7-(triethylsilyl)baccatin III.[85]



61

Many taxol analogs with modified side chains have been prepared in studies to find improved analogs of taxol. The best-known such compound is docetaxel (**2**), which was prepared early on by Potier and his colleagues and has entered clinical use.[86] The other noteworthy compound with a modified side chain is the 14β-hydroxytaxol derivative **36**, which as noted earlier is in clinical trials. The side chain of this compound differs from that of taxol by having an isobutyl group replacing the 3′-phenyl group and a *tert*-butoxycarbonyl group replacing the 3′-*N*-benzoyl group. Other analogs with modified side chains have been reviewed.[21,66]

Some taxol analogs with highly modified side chains are of interest because of the light they throw on the conformation of the side chain. Thus the methylated analog **62** is more cytotoxic than taxol to HCT116 colon carcinoma cells and has an increased binding affinity to tubulin.[87–89] Its enhanced potency may be due to a reduction in the degree of freedom of rotation of the C-2′–C-3′ bond, or possibly to an additional hydrophobic binding interaction of the methyl group with the microtubule binding site. The conformationally restricted analog **63** has



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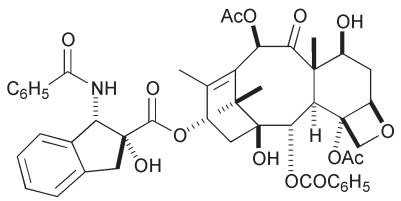


63

comparable cytotoxicity to taxol in several cell lines,[90] suggesting that the side chain may exist in an 'open' conformation rather than the hydrophobically collapsed conformation that has been proposed.[91]

The synthesis of taxol

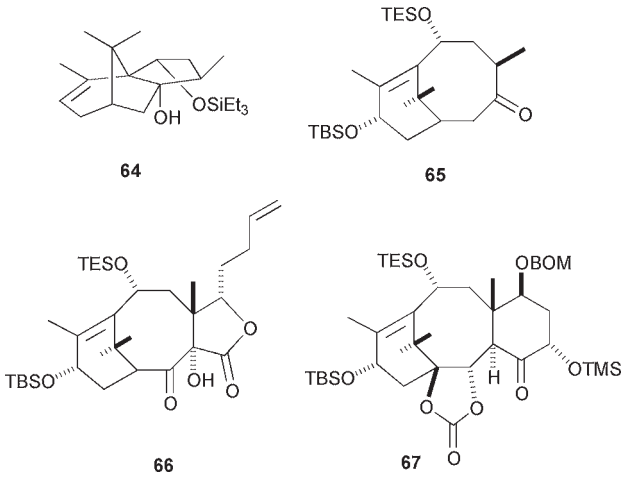
The synthesis of taxol presented one of the more difficult challenges to synthetic chemists, both because of its complex ring system and because of its many chiral centers. It is a tribute to the current development of synthetic methodology that six independent syntheses have been achieved to date, using a variety of approaches.

Since baccatin III has been converted into taxol by many different routes, as noted earlier, a synthesis of baccatin III constitutes a synthesis of taxol. The six routes will not be discussed in detail because of space limitations, but a brief summary of each will be provided.

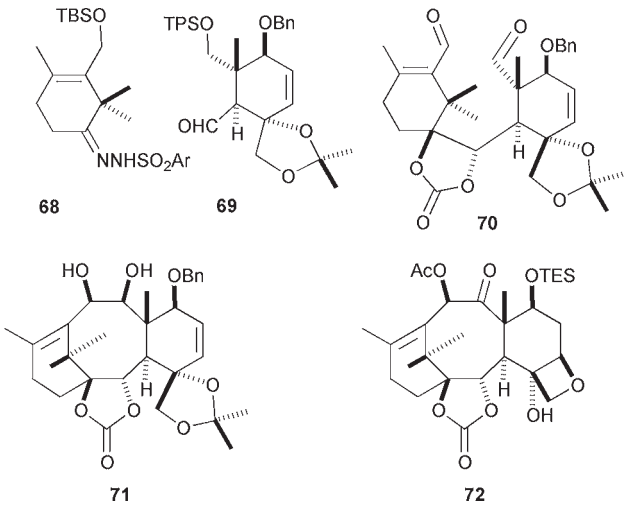
The first two syntheses were published essentially simultaneously in 1994 by Holton and Nicolaou. In the Holton synthesis (Scheme 14), the natural product β-patchoulene served as the starting material to generate the protected diol **64**. A clever ring-opening of the epoxide of **64** then gave the AB ring system **65**, and this was elaborated to the ABC system **67** through intermediate **66**. Final elaboration of ring D and functional group manipulations then gave baccatin III.[92]

In the Nicolaou synthesis (Scheme 15), the A and C ring precursors **68** and **69** were both made by Diels–Alder chemistry, and were then coupled by a Shapiro reaction and elaborated to the AC system **70**. A McMurry coupling of **70** generated the ABC system **71**, which was then converted to the ABCD system **72** and thence to baccatin III.[93]

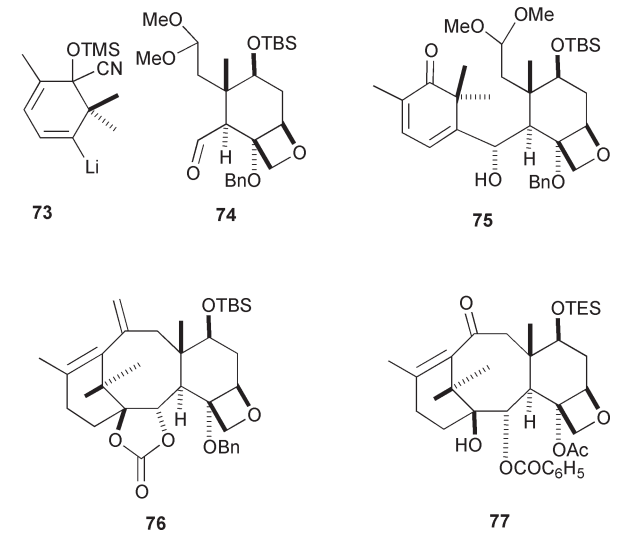
The Danishefsky synthesis (Scheme 16) is the only one to date to start with a preformed D-ring. The key to the success of



Scheme 14 The Holton synthesis.



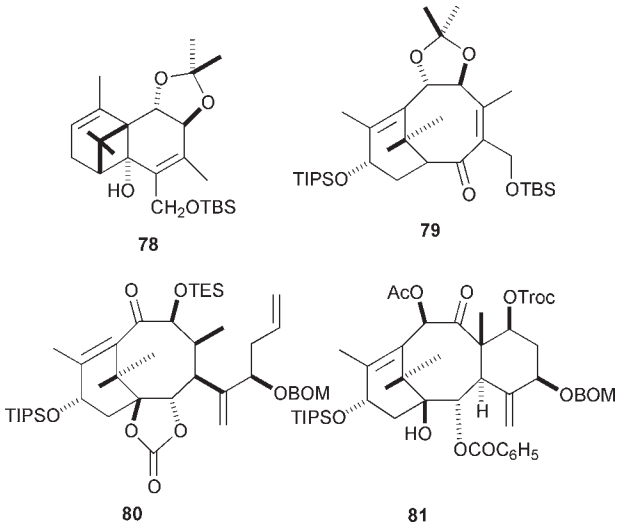
Scheme 15 The Nicolaou synthesis.



Scheme 16 The Danishevsky synthesis.

this approach was the protection of the C-4 hydroxy group as a benzyl ether rather than as an acetate, thus avoiding complications from neighbouring group participation by this group. The CD ring system **74** was prepared from the Wieland–Miescher ketone, and this was then coupled with the A-ring synthon **73** to give the A–CD unit **75**. Cyclization to the ABCD system **76** was achieved by the Heck reaction, and oxidation and functional group manipulations gave **77**, which was converted to baccatin III by appropriate oxidation chemistry.[94]

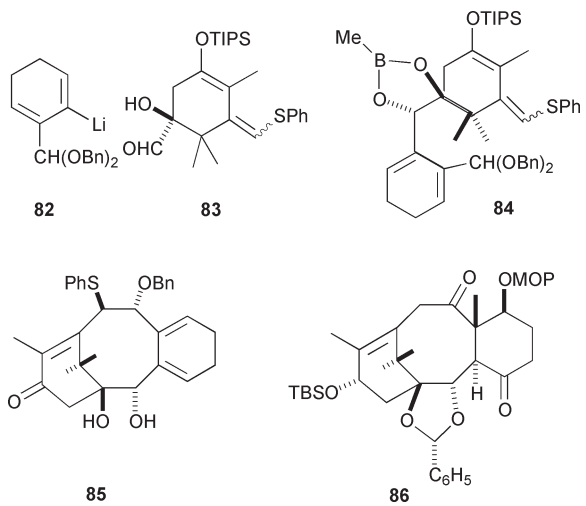
The Wender synthesis (Scheme 17), like the Holton synthesis, is of the form A → AB → ABC → ABCD, but it started



Scheme 17 The Wender synthesis.

from verbenone, which provided 10 of the 20 carbons of the baccatin III ring system. Some ingenious chemistry converted verbenone to intermediate **78**, which then underwent oxidative cleavage in a manner reminiscent of the conversion of **64** to **65** in the Holton synthesis. Intermediate **79** was then converted into **80** through elaboration of the C-3 position and aldol condensation, and the synthesis was completed by formation of the oxetane ring. The overall synthesis, at 37 steps from verbenone, is claimed to be the shortest recorded synthesis of taxol.[95]

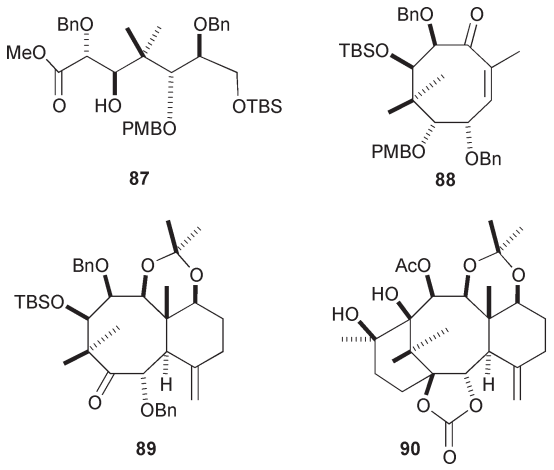
The Kuwajima synthesis, first reported in 1998 with the full report appearing in 2000, uses the A + C → A–C → ABC → ABCD approach (Scheme 18).[96] The A-ring synthon **83** was



Scheme 18 The Kuwajima synthesis.

prepared in 16 steps from propargyl alcohol, and this was coupled with the C-ring synthon **82** to give the A–C adduct **84**. Adduct **84** was cyclized by a novel reaction to give **85**, and this was elaborated to **86**; the C-18 methyl group was introduced *via* a cyclopropane intermediate. Final incorporation of the oxetane ring gave baccatin III.

The final synthesis by Mukaiyama, reported in 1999 (Scheme 19), is unique in starting with the acyclic precursor **87** and cyclizing it to the stereochemically defined B-ring synthon **88**. Rings C and A (in that order) were then built onto ring B to



Scheme 19 The Mukaiyama synthesis.

give intermediate **89** and then **90**, and addition of ring D completed the synthesis.[97]

The bioactivity of taxol

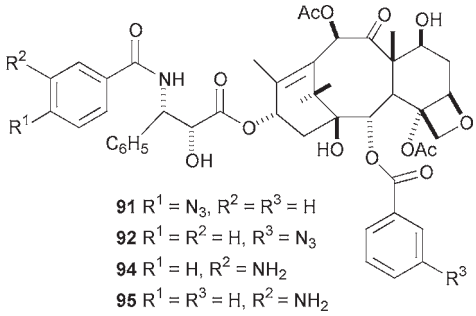
As noted in the introduction to this review, taxol was discovered on the basis of the antileukemic and cytotoxic activities of *T. brevifolia* extracts, but its mechanism of action was found to be its ability to promote the assembly of tubulin into microtubules. In brief, taxol binds to the assembled microtubule with a stoichiometry of approximately 1 mole of taxol to 1 mole of tubulin dimer and stabilizes it to dissociation (Fig. 1). This binding occurs in the absence of any cofactors, and the resulting disruption of the equilibrium between tubulin and microtubules also disrupts cell division and ultimately leads to cell death by apoptosis.[98,99]

The binding of taxol to tubulin polymers and the associated interruption of the cell cycle was thought for a long time to be its only significant mechanism of action, but in recent years it has been increasingly clear that taxol can bring about apoptotic cell death by a second mechanism which is independent of mitotic arrest.[100,101] The protein Bcl-2 has been identified as a second taxol-binding protein[102] which undergoes dose-dependent hyperphosphorylation in the presence of taxol.[103] The situation is complex, however, since it has also been shown that Bcl-2 phosphorylation in the presence of taxol is linked to the latter's tubulin-assembly activity, and it has thus been proposed that taxol-promoted assembly of microtubules leads to Raf-1 activation and Bcl-2 phosphorylation, and thence to apoptosis.[104] The binding of taxol to tubulin is thus clearly biologically significant, and has been studied extensively by several methods. A detailed understanding of this binding has become much more achievable in recent years thanks to the work of Downing and his collaborators, who have reported the structure of tubulin at a resolution of 3.7 Å using electron crystallography on crystalline sheets formed in the presence of zinc.[105,106]

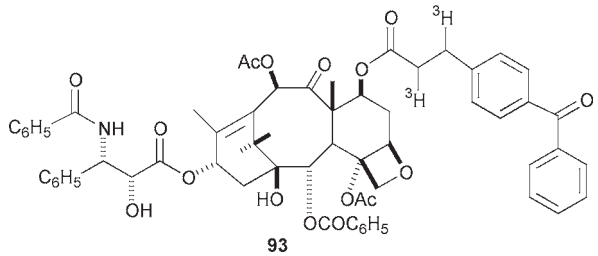
The interaction of taxol with tubulin

Several different methods have been used to study the interaction of taxol with tubulin. In studies by the photoaffinity labeling technique, various labeled taxol analogs have been used to study the location of the binding site of taxol on tubulin. Thus 3′-(*p*-azidobenzamido)taxol (**91**) photolabeled the N-terminal 31 amino acid unit of β-tubulin preferentially,[107] while 2-(*m*-azidobenzoyl)taxol (**92**) labeled a peptide containing amino acids 217–231 of β-tubulin,[108] and the photoaffinity probe **93** was shown to bind to Arg^{282} in β-tubulin.[109]

A second useful technique has been that of fluorescence spectroscopy. This technique has the significant advantage that



91 $R^1 = N_3$, $R^2 = R^3 = H$
92 $R^1 = R^2 = H$, $R^3 = N_3$
94 $R^1 = H$, $R^2 = NH_2$
95 $R^1 = R^3 = H$, $R^2 = NH_2$

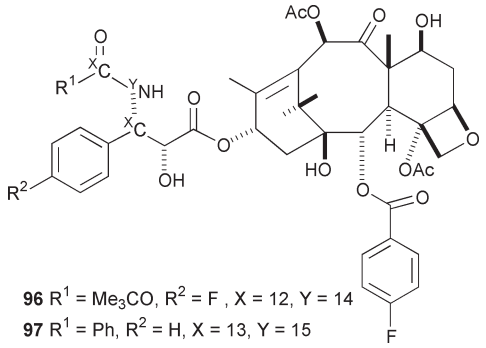


93

it is performed on systems in solution, thus avoiding potential problems with changes in shape on preparing solid samples for analysis. As one example, 2-(*m*-aminobenzoyl)taxol (**94**) gave solvent-dependent absorption and emission spectra. Using this information it was possible to show that the fluorophore binding site on the microtubule is in an environment of intermediate polarity, and also that tubulin has two binding sites for taxol, one high affinity site and one low affinity site.[110] Studies using fluorescence resonance energy transfer measurements showed that the distance between the taxol and colchicine binding sites is approximately 17 Å.[111] A recent paper describes results with the 3′-*N*-(*m*-aminobenzoyl)taxol **95**.[112] It was found that **95** bound to two types of site on the microtubules, with binding affinities of 61 nM and 3.3 μM. It bound to a single site on microtubules assembled from GDP-tubulin with a dissociation constant of 2.5 μM, and it bound to a single site on microtubules assembled from the GTP analog GMPCPP with a dissociation constant of 15 nM. It was thus proposed that although all the subunits of the microtubule at the steady state are the same 'GTP–tubulin–taxol', they are formed through two different pathways: either from taxol binding to a tubulin subunit before GTP hydrolysis (a high affinity binding) or taxol binding to a tubulin subunit after GTP hydrolysis (a low affinity binding). Studies with fluorescent taxol derivatives have also been carried out by others.[113,114]

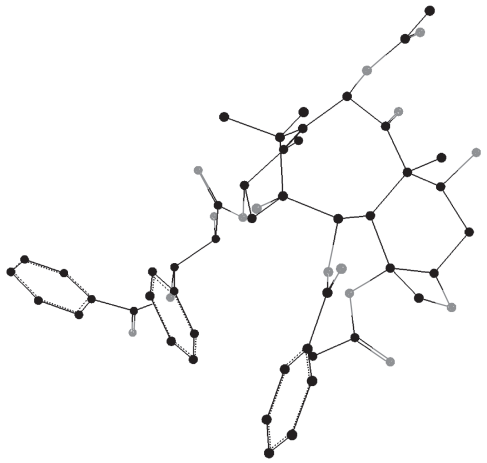
A number of investigators have studied the NMR spectra of taxol in various solvents in an attempt to determine the solution conformation. In non-polar solvents such as chloroform taxol seems to exist primarily in a set of 'open' conformations in which the side chain is oriented away from the 2-benzoyl group,[115,116] but in polar aqueous solvents it adopts a set of 'hydrophobically collapsed' conformations in which the 3′-phenyl group is oriented towards the 2-benzoyl group.[91] Over-interpretation of these results in terms of a 'binding conformation' of taxol to tubulin is, however, dangerous, since taxol exists in chloroform (and presumably also in polar solvents) as a population of different conformations.[117] It is possible that taxol's relatively weak association with tubulin may be due in part to the presence of a large number of nonproductive conformers.

NMR studies of taxol bound to microtubules can be made using the technique of solid state magic angle spinning, and two such studies have been reported. In one study fluorine-containing taxol analogs were used to obtain an F–F distance of 6.5 Å in the difluoro analog **96**.[118,119] In the second study, internuclear distances between ^{13}C and F were obtained by the REDOR technique on the quadruply labeled analog **97**, and these results were coupled with fluorescence data to lead to the



96 $R^1 = Me_3CO$, $R^2 = F$, X = 12, Y = 14
97 $R^1 = Ph$, $R^2 = H$, X = 13, Y = 15

proposal of structure **98** as the most probable conformation of taxol on tubulin.[120]



98

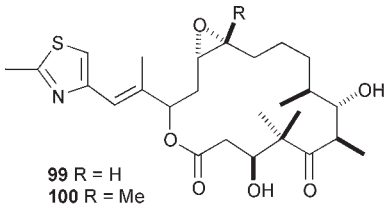
Taxol mimics

For many years taxol was the only compound known to promote the assembly of tubulin into microtubules, but over the past few years several other natural products have been discovered with the same or similar activity. The most important compounds of this class are the epothilones A (**99**) and B (**100**),[121,122] discodermolide (**101**),[123] and eleutherobin (**102**),[124,] but other compounds with this activity have also been discovered. These include rhazinilam,[125] which inhibits the disassembly of microtubules but has a different mechanism of action than taxol, laulimalide and isolaulimalide,[126] WS9885B,[127] and poly-isoprenylated benzophenones such as guttiferone E.[128] The naturally occurring 3(2*H*)-furanone derivative geiparvin has been found to counteract the microtubule-assembly effects of taxol, suggesting that it is a competitive inhibitor at the taxol-binding site of tubulin.[129]

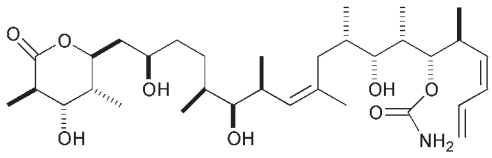
The taxol pharmacophore

Much of the chemical work reported in the previous sections was carried out with a view to determining structure–activity relationships for taxol and its analogs and thus to defining the pharmacophore in chemical terms. Some of this work can be summarized as in Fig. 2 opposite, where the key structure–activity relationships of taxol are shown.

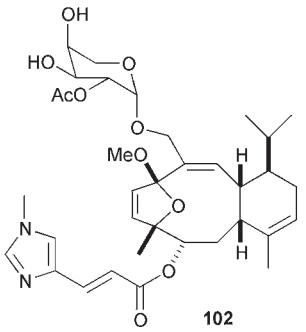
The discovery of the tubulin-assembly properties of the natural products referred to in the previous section opened up a second way of delineating the taxol pharmacophore, since comparisons could be made between the structures of taxol and of these taxol mimics. Various approaches to this important question have been made, with most studies concentrating on a



99 R = H
100 R = Me



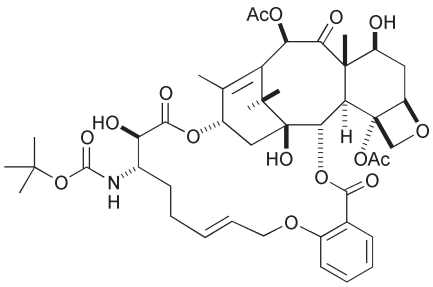
101



102

comparison of the structures and activities of taxol and its analogs and the epothilones.

In one approach various bridged analogs of taxol such as **103** were prepared by olefin methathesis.[130] Three related analogs



103

were found to be cytotoxic to the human breast cancer cell line MDA-435/LCC6-WT with IC_{50} values of less than 1 μM. These activities are significantly less than that of taxol in the same cell line (0.0031 μM), but the compounds also showed tubulin-assembly activity that was only only slightly less than that of taxol, so they are presumably binding to the same binding site as taxol. These data were used to support a model of the pharmacophore in which the aryl sector of epothilone overlaps the C-13 acyl side chain of taxol and in which the baccatin portion of the taxol molecule is relatively non-essential.

A second approach, developed by Giannakakou and his collaborators, was based on a comparison of the effects of taxol and various epothilone analogs on the polymerization of native tubulin and of modified tubulins carrying β-tubulin mutations near the taxol-binding site.[131] Two possible common overlaps of the epothilones and taxol were found. In the first the C-2 benzoyl group of taxol overlapped with much of the 1-methyl-2-thiazolyl side chain of the epothilones, while in the second the thiazole portion of the epothilones overlapped with the side chain of taxol.

A third approach deduced a different binding based on the finding that the side chain of taxol is not as essential for activity as was previously thought, since 2-(*m*-azidobenzoyl)baccatin III is significantly active as a promoter of tubulin polymeriza-

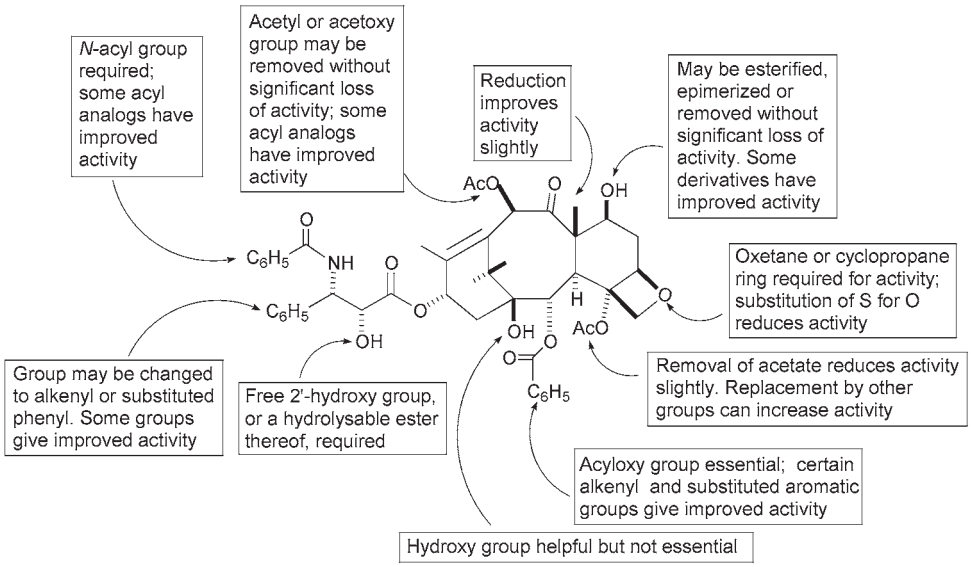


Fig. 2 Structure–activity relationships of taxol.

tion and is a competitive inhibitor of the binding of taxol to microtubules.[132] These studies led to the proposal of a pharmacophore model similar to the first binding mode of Giannakakou *et al*. A perceptive evaluation of the various models has been published.[133]

A different approach was taken by Snyder, who developed a minireceptor model for the binding of taxol and epothilone to the microtubule based on an analysis of tubulin-assembly data.[134] This model places the thiazole ring of the epothilones in the same region of the receptor as the side chain of taxol, consistent with the second binding mode of Giannakakou *et al*.[131] It also predicts much of the SAR data for taxol and the epothilones and appears to be an interesting first step towards the development of a full binding site model.

In summary, the taxol pharmacophore is still under development, with at least two rather different competing hypotheses, and further work will be needed to clarify the situation. It is reasonable however to expect that a final model of the taxol pharmacophore will eventually be developed and will be used in a predictive way to create new and improved taxol analogs.

Conclusion

The preceding review has just scratched the surface of the enormous amount of work that has been done on taxol. In addition to the chemical work summarized above, an enormous amount of work has been done on optimizing the clinical use of taxol in cancer treatment, and several recent reviews of this work have appeared.[8,135–137] The big question, of course, is what sort of difference have the taxoid drugs (taxol and docetaxel) made to cancer treatment? Have they improved cancer survival significantly? The literature on this subject is surprisingly sparse, in part because optimum combination therapies of the taxoids with other drugs are still being worked out; it is expected that such combinations will significantly enhance their use.[137] At present the taxoids are routinely used for the treatment of breast, lung, and ovarian carcinomas, and for AIDS-related Kaposi's sarcoma, and they have been called 'the most powerful compounds' (among the chemotherapeutic drugs introduced in the last decade).[135] In spite of the wide use of the taxoid drugs over the last decade, it appears that improvement in patient survival is modest at best. The greatest number of studies has been done with breast cancer, and for this disease Miller and Sledge state 'Combination therapy (of taxol or docetaxel with other drugs) has increased response rates but as yet has not improved the overall survival of patients with metastatic disease. Improved survival with the addition of paclitaxel to standard adjuvant therapy reported in a recent trial suggests the true impact of the taxanes has not yet been realized.'[137] A slightly more cautious view is expressed by Nabholtz and his colleagues 'However, the impact of taxanes on the natural history of breast cancer is yet to be defined, despite the trend of results suggesting that these agents have the potential for significant improvements in advanced and, most importantly, adjuvant therapy of breast cancer.'[136] A third evaluation was made by a panel of experts convened by the US National Institutes of Health in November 2000. The NIH Consensus Statement approved by this panel reads in part 'Currently available data are inconclusive and do not permit definitive recommendations regarding the impact of taxanes on either relapse-free or overall survival.'[138]

Although the impact of the taxoid drugs on patient survival is thus still a matter of research and debate, the outlook remains bright. The new analogs referred to earlier in this review will almost certainly improve patient survival, while increased understanding of the way taxol binds to tubulin and advances in such areas as drug targeting[139] will most probably lead to even better agents in the future. These factors thus suggest that taxol and its analogs will continue to be important cancer chemotherapeutic drugs well into the new millennium.

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Cancer Institute from grants CA48974, CA55731, and CA69571, and I am most grateful for this crucial component of our work.

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