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Synthesis and bioactivity of a side chain bridged paclitaxel: A test of the T-Taxol conformation

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ABSTRACT

A knowledge of the bioactive tubulin-binding conformation of paclitaxel (Taxol™) is crucial to a full understanding of the bioactivity of this important anticancer drug, and potentially also to the design of simplified analogs. The bioactive conformation has been shown to be best approximated by the T-Taxol conformation. As a further test of this conclusion, the paclitaxel analog **4** was designed as a compound which has all the chemical functionality necessary for activity, but which cannot adopt the T-Taxol conformation. The synthesis and bioassay of **4** confirmed its lack of activity, and thus provided further support for the T-Taxol conformation as the bioactive tubulin-binding conformation.

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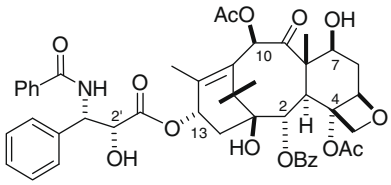
The anticancer drug paclitaxel (**1**, Taxol™, PTX) is one of the most important anticancer natural products ever discovered, and has contributed significantly to human health. First reported from the Western yew, *Taxus brevifolia*, in 1971,[1] it went through a long period of development, and was finally approved by the FDA for clinical use in 1992.



1

A turning point in its development came with the discovery of its then unique mechanism of action as a promoter of the assembly of tubulin into microtubules.[2] The microtubule has been called 'the single best cancer target identified to date',[3] and several other microtubule-stabilizing natural products have been identified as promising cancer therapeutics.[4] The first of the non-taxane microtubule-stabilizing drugs, the modified epothilone ixabepilone, has recently been approved for clinical use.[5]

The normal functioning of tubulin assembly and disassembly is crucial to cell division, and any interference with this process disrupts cell division and leads to cell death by apoptosis. PTX owes its activity to its ability to bind to tubulin and suppress microtubule assembly dynamics.[3] Thus the nature of the binding of paclitaxel to tubulin, and in particular the tubulin-binding conformation of PTX, is an important question with potential applications in the design of improved taxoid drugs.

Since PTX has a large flexible side chain at C13 and smaller flexible side chains at C2, C4, and C10, many different conformations are possible. Various proposals for the binding conformation have been made based on studies of the solution NMR spectra of PTX. NMR studies in nonpolar solvents suggested a 'nonpolar' conformation,[6–8] while a 'polar' conformation featuring hydrophobic interactions between the C2 benzoate, the C3′ phenyl group and the C4 acetate was proposed on the basis of NMR studies in polar solvents.[9–12] A third approach involved NMR studies using the NAMFIS deconvolution method, and this showed that PTX adopts 9–10 conformations in $CDCl_3$.[13] An analysis of the electron crystallographic data in combination with the NAMFIS results suggested that the actual binding conformation has a T-shaped structure, designated T-Taxol.[14]

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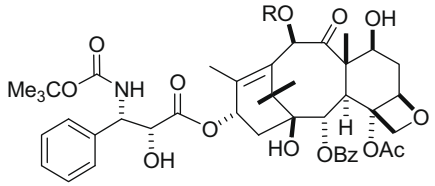
The validity of the T-Taxol model was demonstrated in several ways. Direct evidence for the conformation was obtained by REDOR NMR studies of labeled PTXs directly bound to tubulin. The derived internuclear distances were compatible with the T-Taxol tubulin-binding conformation, ruling out most competitors.[15,16] The predictive value of this conformation was then tested by the synthesis of bridged PTXs constrained by the bridging to the T-Taxol conformation. The bridges were constructed between the C3′-phenyl group and the C4 acetate, since these two positions are adjacent in the T-Taxol conformation. Two of the resulting bridged compounds with two carbon chains connecting the *ortho*-position on the C3′-phenyl group to the acetate methyl group showed excellent bioactivity, with activities 2–30-fold greater than those of PTX, depending on the assay used.[17,18]

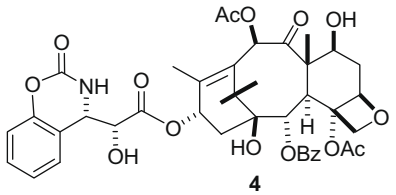
These results provided strong positive support for the T-Taxol conformation as the bioactive conformation of PTX. It was also desirable, however, to devise a negative test, to determine if the T-Taxol conformation could also be used as a criterion for inactive taxanes. Such a test is possible because of the extensive knowledge of the structure–activity relationships of PTX.[19–21]

The PTX analog docetaxel (**2**) and its 10-acetyl derivative **3** were both prepared by Potier and his group from 10-deacetylbaccatin III, and docetaxel has been developed into the second taxane for clinical use.[22] The 10-acetyl analog **3** is almost as active as docetaxel, so the presence of the 10-acetyl group does not have a major effect on activity.[23]



2 R = H
3 R = Ac

A simple modification of structure of **3** yields the target cyclic carbamate **4**, a compound with all the atom connectivity and structural features of PTX or docetaxel needed for bioactivity: an intact tetracyclic taxane ring system, a C13 side chain with a C3′-phenyl ring and an *N*-acyl group, and a free C2′-hydroxyl group. The methyl carbamate **5a** was selected as the best 'open chain' version of **4** for comparison purposes, since it differs only by one carbon atom from **4**. Although the bioactivity of this compound has been reported,[24] we elected to synthesize it so as to allow it to be evaluated in the same assays used for the constrained compound **4**. The tolyl derivative **5b** has been prepared previously,[25] and was used as a second comparison structure.



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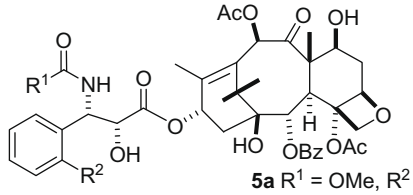


5a R^1 = OMe, R^2 = H
5b R^1 = Ph, R^2 = Me

What the planar depiction does not show, however, is that compound **4** cannot adopt the 'T' shape of T-Taxol represented by the spatial arrangement of the three phenyl rings in **1**.[26] Thus, the two well-separated aromatic rings at the termini of C13 have been collapsed into a single bicycle occupying space between them as shown in the manual docking of Figure 1a. This space is likewise occupied by Val23 of the β-tubulin protein. If the T-Taxol geometry or anything similar to it[16,27] is required for bioactivity, then it is predicted that **4** should be inactive, and such lack of activity would be an important 'negative test' of these conformations.

The synthesis of **4** was accomplished by the Holton–Ojima β-lactam synthon method[28,29] from 10-deacetylbaccatin III, as shown in Scheme 1. The key β-lactam **7** was prepared by a modification of the standard conditions for β-lactam formation.[30] In brief, 2-benzyloxybenzaldehyde was converted to the β-lactam **6** by reaction of its *p*-anisidine imine with acetoxyacetyl chloride in the presence of Hünig's base. Attempted resolution of **6** using *Pseudomonas cepacia* lipase[31] was unsuccessful, presumably because of the steric hindrance from the ortho-benzyloxy group, and so the racemic mixture was carried forward in the expectation that a kinetic resolution could be effected.[32]

Lactam **6** was hydrolyzed to the alcohol and reprotected as its triisopropyl silyl derivative **7**, which was then converted to the *t*-butyloxycarbonyl derivative **8** by deprotection of the *p*-methoxyphenyl group with ceric ammonium nitrate and acylation with di-*tert*-butyldicarbonate. β-Lactam **8** was then coupled with 7-(triethylsilyl)baccatin III[33] under standard conditions to give the coupled product **9**. Hydrogenolysis of **9** followed by treatment with formic acid gave the aminodiol **10**, which was reacted with triphosgene to give the cyclic carbamate **11**. Deprotection of the 2′-TIPS group then gave the final product **4** (Scheme 1). The ^{1}H NMR spectrum of **4** and of its precursors **9**–**11** showed only one set of signals for each of the protons, with no doubling of signals due to the presence of two diastereomers.[34] This indicated that coupling occurred stereoselectively to give the desired product, in agreement with the literature.[32]

The methyl carbamate **5a** was prepared by standard methods. The known non-racemic β-lactam **12**[35] was acylated with methyl chloroformate to give the carbamate **13** (Scheme 2), and acylation of 7-(triethylsilyl)baccatin III with **13** followed by deprotection gave the analog **5a**.

Constrained cyclic carbamate **4**, open chain carbamate **5a**, and PTX were evaluated for tubulin-affinity, tubulin-assembly activity,[36] and antiproliferative activity against the A2780 and PC3 cell lines (Table 1). Association constants for PTX and **4** binding to GTP-microtubules were assessed by a fluorescent competition assay as described in the Supporting Information. Owing to limited solubil-

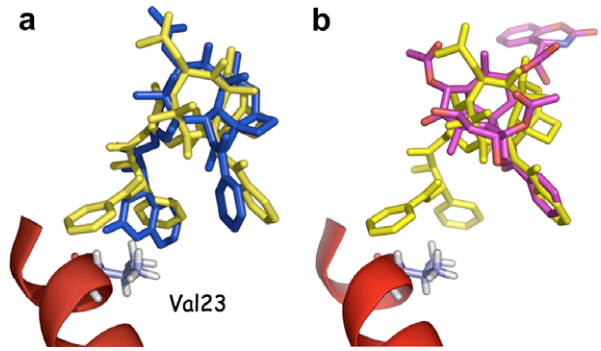
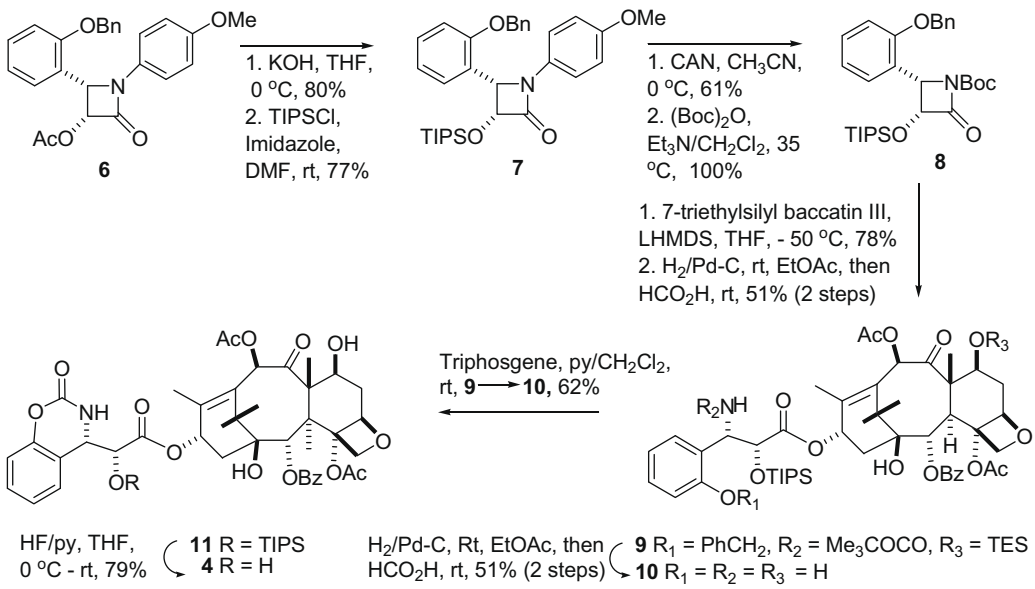
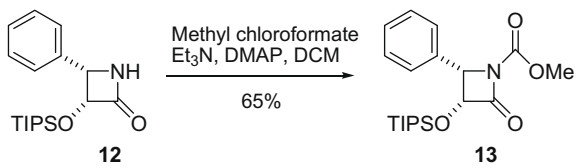


Figure 1. T-Taxol in β-tubulin (yellow): (a) best ROCS 3-D fit of T-Taxol and the most similar T-conformer of compound **4** (blue) manually docked into β-tubulin. Val23 on Helix 1 is in steric conflict with **4**; (b) the best Glide docking of **4** (magenta) flips the ligand and directs the carbamate out into solvent.



Scheme 1. Synthesis of cyclic carbamate **4**.



Scheme 2. Synthesis of β-lactam **13**.

Table 1
Bioactivity of PTX and carbamates **4** and **5**

Compd	Tubulin assembly	Tubulin affinity	Antiproliferative activity (IC_{50}, nM)	
	ED_{50} (μM)	K_d ($\times 10^7$ M^{-1})[a]	A2780[b]	PC3[b]
PTX	0.48 ± 0.09[c]	4.76 ± 0.28	14.9 ± 4.0	1.40 ± 0.32
4	>25	~0.03	>20,000	>5,000
5a	0.43 ± 0.19	1.11 ± 0.04	9 ± 1.9	2.00 ± 0.08

[a] Disassociation constants (K_d) determined as described in Supplementary data.
[b] Cytotoxicity determined as described in Supplementary data.
[c] Data from Ref. 34.

ity and low activity of compound **4**, only a partial inhibition curve could be obtained (Fig. 1 in Supplementary data). The association constant measured for the binding of **4** to microtubules is therefore an estimate based on limited data. However, the affinity of **4** for the PTX site on microtubules is at least 150-fold less than that of PTX (Table 1). The ability of **4** to promote in vitro tubulin assembly was also evaluated. The efficacy of **4** in this assay is at least 50-fold less than that of PTX. In addition, the cyclic carbamate **4** was unable to stabilize microtubules against cold-induced disassembly (Figs. 2 and 3 in Supplementary data). Model compound **5a**, on the other hand, strongly promotes tubulin polymerization, with an activity comparable to that of PTX. Model compound **5b** is reported to have an ED_{50} for tubulin assembly 5.1-fold greater than PTX, so the introduction of an *ortho* substituent onto the 3′-phenyl group causes only a modest reduction in tubulin assembly activity.[25]

Cyclic carbamate **4** also exhibits 1900- to about 6000-fold less antiproliferative activity than PTX in the two cell lines tested (Table 1). Model compound **5a** has approximately the same antiproliferative activity as PTX in two different cell lines, being slightly more active against the A2780 cell line and slightly less active against the PC3 cell line. This result contrasts significantly with the reported cytotoxicity data for compound **5a** to P388 cells, where it was 150-fold less active than docetaxel.[24] The lack of activity of **4** can thus be attributed, at least in large measure, to the geometric constraint caused by the cyclic carbamate.

To place these results in a biostructural context, **4** was docked flexibly into β-tubulin with Glide XP (See Supplementary data for details).[37] While the molecule can assume an overall shape similar to the T-Taxol conformation, the cyclic carbamate moiety is repelled by Val23-ligand congestion, rotates **4** ca. 180° relative to Figure 1a and lifts it out of the binding pocket (Fig. 1b). Thus, the best tolerated docking pose at the taxane site places **4** in a conformation directing the carbamate ring out toward solvent. The conformation incorporates 9–10 kcal/mol internal strain energy relative to the corresponding global minima using both MMFF and OPLS2005 force fields.[35] This estimate complements the observations by Buey et al.[38] that taxane binding is enthalpy driven and that increasing binding enthalpy predicts cytotoxicity. The ΔH contribution to ΔG incorporates both intermolecular ligand–protein interactions as well as intramolecular conformational strain energy. The calculation of substantial ligand torsional strain for **4** clearly counterbalances the otherwise favorable intermolecular enthalpy factor and aids in the interpretation of its low tubulin assembly and antiproliferative activities.

Although these results do not prove that the T-Taxol shape is the correct binding geometry, they do support the notion that a taxane incapable of adopting a close mimic of the T-conformation will not show significant tubulin-assembly activity, even though it may possess all the correct chemical functionality for such activity. Assessment of the alternative PTX-NY ('REDOR-taxol')[26] conformation cannot be made, since this high energy conformer appears to significantly reorganize the tubulin-taxane binding site, precluding a simple comparison.[39] Independent support for the T-Taxol conformation has been provided by a detailed analysis of REDOR NMR data of labeled PTX analogs bound to microtubules.[16]

Acknowledgements

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Supplementary data

Supplementary data (experimental procedures for the synthesis of all compounds, for the molecular modeling, and for the tubulin bioassays) provided as supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.063.

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34. *Characterization data for* **4**: mp 192–193°; $[\alpha]_D^{25}$ −214.0 (c = 1.0, $CHCl_3$); ^1H NMR (500 MHz, CD_3OD) δ 7.98 (2H, d, J = 7.6), 7.58 (1H, t, J = 7.6), 7.46 (2H, t, J = 7.8), 7.21 (2H, m), 6.84 (1H, t, J = 7.6), 6.79 (1H, t, J = 8.0), 6.39 (1H, s), 6.20 (1H, t, J = 8.7), 5.57 (1H, d, J = 7.3), 5.17 (1H, d, J = 5.7), 4.93 (1H, d, J = 5.7), 4.53 (2H, s), 4.23 (1H, dd, J = 11.0, 6.6), 4.06 (2H, s), 3.75 (1H, d, J = 7.1), 2.35 (1H, m), 2.23 (3H, m), 2.09 (3H, s), 2.06 (3H, s), 1.91 (3H, s), 1.82 (3H, s), 1.69 (1H, m), 1.56 (3H, s), 1.19 (2H, m), 1.06 (3H, s); ^{13}C NMR: δ 205.3, 171.9, 171.3, 170.8, 167.7, 156.9, 141.9, 135.4, 134.7, 131.3 (×2), 129.9, 128.9, 126.1, 121.0, 116.8, 101.2, 86.1, 82.3, 80.8, 79.3, 77.5, 76.9, 76.3, 73.3, 72.5, 59.4, 57.4, 48.2, 44.8, 37.7, 37.0, 30.9, 27.1, 22.3, 20.9, 14.9, 10.6; HRFABMS m/z 792.2876 (+1.1 ppm/+0.9 mmu) $[M+H^+]$ (calcd for $C_{41}H_{46}NO_{15}$, 792.2867).
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