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Synthesis and Biological Evaluation of Novel Macrocyclic Paclitaxel Analogues

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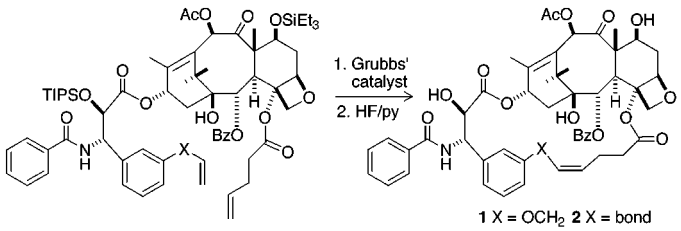
ABSTRACT



This work describes the synthesis of two novel macrocyclic taxoid constructs by ring-closing olefin metathesis (RCM) and their biological evaluation. Computational studies examine conformational profiles of 1 and 2 for their fit to the *β*-tubulin binding site determined by electron crystallography. The results support the hypothesis that paclitaxel binds to microtubules in a "T" conformation.

The important diterpenoid natural product paclitaxel (Taxol) is currently the world's best-selling anticancer drug, with sales of over $1.5 billion in 2000.[1] It operates, in part at least, by binding to microtubules and stabilizing them to disassembly,[2] and several recent studies have attempted to delineate the binding conformation of paclitaxel on assembled microtubules. Three models have been proposed on the basis of the published electron crystallographic coordinates of polymerized α*β*-tubulin.[3] A study based on REDOR NMR and fluorescence spectroscopy by two of our groups led to the proposal of a hydrophobically collapsed conformation,[4] as did the modeling by Rao et al. based on photoaffinity labeling.[5] Giannakakou et al.[6] conceived epothilone binding models from the single-crystal X-ray structure of taxotere[7] docked into *β*-tubulin.[3] A more recent study of the binding of paclitaxel to tubulin using the crystallographic density in conjunction with an analysis of paclitaxel conformations indicates that the T-shaped conformer, deprived of hydrophobic collapse, is the most likely bioactive form.[8]

A fruitful approach to the delineation of the conformation of paclitaxel in its bound conformation on tubulin has been through the synthesis of analogues with built-in conformational restrictions. A number of experiments in this direction have been published, including the synthesis of analogues with conformationally restricted side chains[9] and studies of analogues with bridges linking the C3′ and C2 phenyls with

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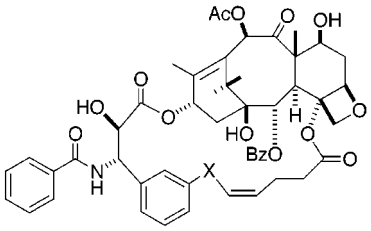
both shorter[10] and longer[11] linkers. Only the latter of the two bridged approaches yielded analogues having tubulin assembly activity, and the activity of the best analogues was less than that of paclitaxel.

In an attempt to model the T-paclitaxel conformation, we have designed and synthesized the two analogues **1** and **2** linked between the C3′-phenyl group and the C4 position. The T-paclitaxel conformation sustains nearly equal 9−10 Å distances between the C13 side chain terminal phenyl rings and the C2-benzoyl phenyl group. Short, conformationally directing linkers between these centers are out of the question. On the other hand, the C4-acetate methyl lies very close to one edge of the C3′ phenyl moiety in this conformer, suggesting a possibility for conformational control. A variety of positional isomers and C3′−C4 linkers were modeled as T-Taxol mimics. In the end, proof of principle and synthetic ease were integrated in structures **1** and **2**.

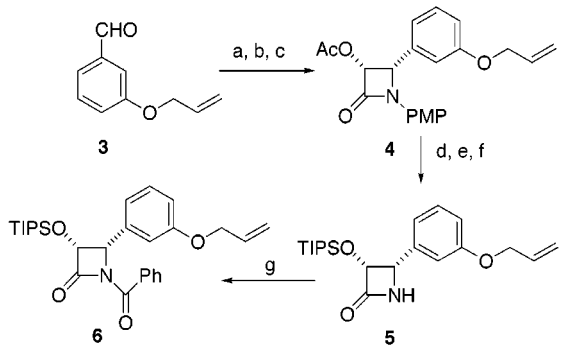


1 X = OCH_2 **2** X = bond

While the latter were not our optimal designs, they nonetheless permitted a straightforward approach to targets by means of the elegant ring-closing metathesis (RCM) methodology in the crucial macrocyclization step. This synthetic strategy was used effectively by Ojima and his collaborators in preparing a large number of paclitaxel analogues with C3′−C2 linkers,[11,12] and it has the advantage of tolerating a wide variety of functional groups.[13] The syntheses, bioactivities, and modeling of these compounds are described below.

The synthesis started with the preparation of the β-lactam **6** as shown in Scheme 1. The starting material **3** was reacted with *p*-anisidine to form *p*-methoxyphenyl (PMP) imine and then converted to a racemic β-lactam by a Staudinger [2 + 2] cyclocondensation between a ketene generated from acetoxyacetyl chloride and the PMP imine.[14] The racemic β-lactam was then subjected to an enzymatic resolution using lipase; the yield was 98%, based on the desired enantiomer **4**.[15] Functional group manipulations then gave the target lactam **6** through intermediate **5**.

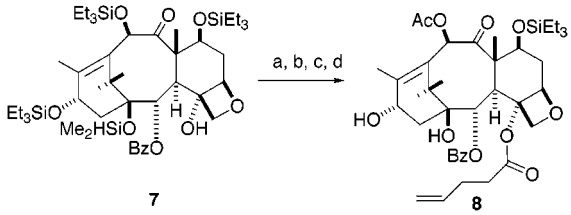
Scheme 1[a]



[a] Reagents and conditions: (a) *p*-$MeOC_6H_4NH_2$, $MgSO_4$, CH_2Cl_2 (100%); (b) CH_3COOCH_2COCl, Et_3N, −78 °C to room temperature, 12 h (85%); (c) lipase PS Amano, phosphate buffer, pH = 7.2, CH_3CN, 24 h (98%); (d) 1 M, KOH, THF, 0 °C (quantitative); (e) TIPSCl, imidazole, DMF (94%); (f) CAN, CH_3CN, −5 °C (92%); (g) PhCOCl, Et_3N, DMAP, CH_2Cl_2 (90%).

The starting material in Scheme 2, the C4 hydroxy baccatin derivative **7**, was prepared from 10-deacetylbaccatin III (10-DAB) using a reported protocol.[16] Initial attempts to acylate the hindered C-4 hydroxy group using the DCC/DMAP method gave low yields, but the use of acid chloride and LiHMDS in THF at 0 °C gave the desired C-4 modified baccatin in 78% yield. A minor product acylated at the C-4 position but with a loss of the 1-dimethylsilyl group was recovered and used for the subsequent step. Global deprotection of the silyl groups using HF/pyridine followed by a selective C-10 acetylation with 0.1 mol % of $CeCl_3$ and acetic anhydride in THF gave a 94% yield of the desired 10-acetyl derivative,[17] and selective reprotection of the C7 hydroxy as the triethylsilyl ether afforded **8** in good yield.

Scheme 2[a]



[a] Reagents and conditions: (a) LiHMDS, THF, 0 °C, $ClCOCH_2$-$CH_2CH{=}CH_2$, (78%); (b) HF/py, THF, (91%); (c) 0.1 mol % of $CeCl_3$, acetic anhydride, THF, (94%); (d) TESCl, imidazole, DMF, 92%

The coupling reaction of C4-modified baccatin derivative **8** and β-lactam **6** using the Holton−Ojima−Georg protocol[18−20] gave the precursor taxoid-ω,ω'-diene **9** (92%), setting

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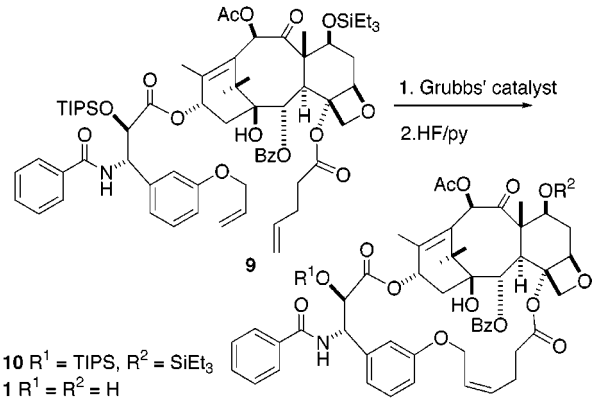
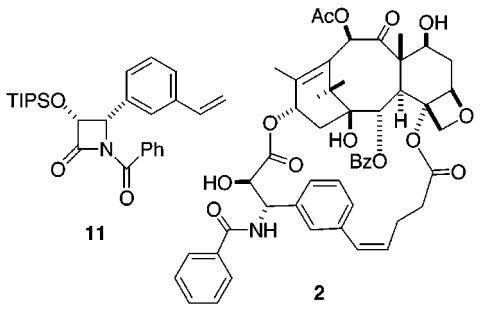
Scheme 3[a]



[a] Reagents and conditions: (a) $((Cy_3)P)_2Cl_2Ru{=}CHPh$, CH_2Cl_2, 20 h, (72%); (b) HF/py THF 12 h, (78%).

the stage for the RCM reaction shown in Scheme 3. The RCM of **9** was catalyzed by Grubbs' catalyst in CH_2Cl_2 at room temperature and gave the desired 21-membered macrocyclic analogue **10** in 72% yield; the *Z* isomer was formed exclusively, as evidenced by NMR coupling constants. Deprotection of **10** gave the final bridged paclitaxel analogue **1** in 78% yield.

A repeat of the above reaction sequence using β-lactam **11** in place of **6** gave the second bridged analogue, **2**.



Bioassays on the cyclic products **1** and **2** were carried out in a microtubule assembly assay and a cytotoxicity assay; the results are shown in Table 1. In the cytotoxicity bioassays, compounds **1** and **2** have comparable bioactivities, and these are only about eight times less than that of paclitaxel. In the tubulin assembly assay, both compounds are also less active than paclitaxel, with compound **1** being about 10-fold less active and compound **2** about 30-fold less active. These results can be compared with those obtained by Ojima et al.; the cytotoxicity values relative to paclitaxel are greater than those observed by him, but the tubulin assembly activities are not as high as those of the best compounds he obtained.[12]

Table 1. Bioactivity Results for Compounds **1** and **2**

compound	tubulin assembly activity (I_{50}, mM)	cytotoxicity to A2780 mammalian cells (IC_{50}, mg/mL)[a]
paclitaxel	0.27 ± 0.05	0.136 ± 0.08
1	2.54 ± 0.53	1.14 ± 0.03
2	8.1 ± 1.0	1.07 ± 0.05

[a] The strain of A2780 used in these experiments consistently gave IC_{50} values in the range 0.06−0.15 μg/mL for paclitaxel. These values are higher than those of other workers and are presumably due to variations in the strain used. The same strain was used for paclitaxel and for compounds **1** and **2**.

In an effort to understand the reduced activities of **1** and **2** relative to that of paclitaxel, we performed Monte Carlo conformational searches on the structures with several force fields tailored to small molecules (MM2*, MM3*, and MMFF (GBSA/H_2O))[21] and a 5 kcal/mol energy window. For **1** and **2**, the combined set of 500−1200 unique and fully optimized conformers was found to contain torsional isomers with excellent superposition of both diterpenoid core and C2, C4, and C13 side chains on the same moieties of T-paclitaxel. If the latter is the bioactive conformation at β-tubulin, and if **1** and **2** can adopt this conformation, why are the hybrid structures less efficacious than paclitaxel? One possibility is that the T-forms are simply too high in energy. To test this idea, we sought to define the conformational profile of compound **1** in solution by means of a NAMFIS conformer deconvolution treatment, a procedure that avoids the capriciousness of raw molecular mechanics energies.[22,23] Careful integration of 2-D ROESY NMR cross-peaks for **1** in $CDCl_3$ led to 27 well-defined intramolecular distances accompanied by $J(H2'-H3') = 1.54$ Hz. NAMFIS analysis, combining the latter NMR quantities and the full 514 conformer data set, yielded eight conformations with predicted populations ranging from 2 to 35% ($\Delta\Delta G = 0-1.7$ kcal/mol). These comprise two nonpolar forms (40% total) and extended conformers (60% total). Among them is an unambiguous 3-D mimic of the T-conformation (5%). The latter fully optimized structure is clearly within reasonable energetic limits for binding to tubulin. Unlike the projected mole fractions of paclitaxel torsional minima in $CDCl_3$,[23] no polar forms appear in the current NAMFIS solution.

Further pursuing the source of reduced activity in **1** and **2**, we docked the T-conformers derived by Monte Carlo searching into the paclitaxel binding pocket of the refined electron crystallographic structure[3] of β-tubulin with the

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DOCK protocol.[24] This approach has been remarkably successful for understanding a range of taxoid−tubulin interactions: analysis of the role of the oxetane ring for tubulin binding,[25] prediction of the activity of a cyclopropyl analogue of oxetane,[25,26] and rationalization of the SAR of C2-benzoyl derivatives.[27,28] The best rigid fit for **1** is pictured in Figure 1. The bridged paclitaxel does not fit nearly as snugly within the hydrophobic binding pocket as does T-Taxol. However, since the present docking involves a rigid body for both the ligand and the protein, it lacks any degree of induced fit, thereby maximizing any differences. A similar result was obtained for **2**. Examination of the floor of the cleft reveals both a kink in the C4−C3′ tether and close contact between Ala233 and Phe272 of the protein and the propene fragment of the linker. In an attempt to introduce a degree of induced fit, we have performed molecular dynamics on the complex of Figure 1 under different conditions at several temperatures for 20 ps. The Phe272−tether interaction is not abated. Thus, while the present results are compatible with the T-Taxol binding motif, they suggest that a flexible and extended bridge can be deleterious to full expression of taxoid activity.

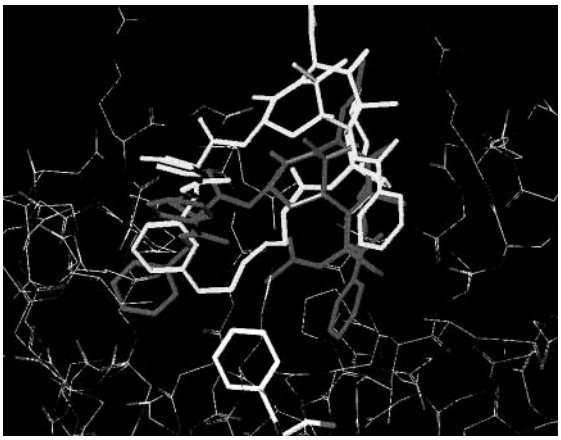

Figure 1. T-Taxol (magenta) bound to β-tubulin as described in ref 7. The best T-form of compound **1** (yellow) is seated higher in the same pocket as a result of close contact between the propene moiety of the tether and Phe272 of the protein (white) at the bottom of the illustration.

In conclusion, we have prepared the C4OAc−C3′Ph bridged analogues **1** and **2**. Both compounds are active but somewhat less so than paclitaxel in tubulin assembly and cytotoxicity assays. While we had hoped that the intramolecular tethers would provide sufficient molecular rigidity to mimic T-Taxol exclusively, NMR conformational analysis suggests that compound **1** can adopt near-nonpolar, T- and side-chain extended conformational types without experiencing undue torsional strain. A recent study of paclitaxel analogues constrained around C3′ within the C13 side chain led to a similar result.[9] These outcomes offer an important caveat to NMR interpretations that lead to single conformational solutions of the averaged ROE and *J* data.[11,22] In the present work, complementary docking studies strongly suggest that although T-shapes of **1** and **2** fill the β-tubulin binding pocket, they unfortunately encounter steric resistance at the bottom of the cleft. While the bioassay and docking studies are compatible with a bioactive T-form, they likewise illustrate that the ideal tether length and constitution has not yet been achieved. Future work will explore this issue in some detail.

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Supporting Information Available: Experimental procedures and full characterization data for compounds **1**, **2**, and **6**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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