Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 14 (2006) 3447–3454
Bioorganic & Medicinal Chemistry

Design, synthesis, and bioactivity of simplified paclitaxel analogs based on the T-Taxol bioactive conformation

Thota Ganesh,[a] Andrew Norris,[a] Shubhada Sharma,[b] Susan Bane,[b] Ana A. Alcaraz,[c] James P. Snyder[c] and David G. I. Kingston[a,*]

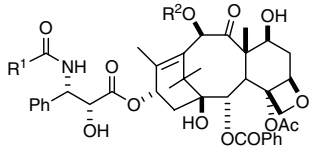
[a]*Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA*
[b]*Department of Chemistry, State University of New York of Binghamton, Binghamton, NY 13902-6016, USA*
[c]*Department of Chemistry, Emory University, Atlanta, GA 30322, USA*

Received 7 November 2005; revised 29 December 2005; accepted 3 January 2006
Available online 24 January 2006

Abstract—A strategy for the design and synthesis of simplified paclitaxel analogs based on the T-Taxol conformation is presented. The resulting compounds have both cytotoxic and tubulin polymerization activities, although less so than those of paclitaxel itself.

1. Introduction

The diterpenoid paclitaxel (Taxol®) (**1**), first reported by Wall and co-workers in 1971,[1] emerged from being a laboratory curiosity in the 1970s and 1980s to a drug of major clinical importance in the 1990s, and it is currently used for the treatment of breast and ovarian cancers, and for AIDS-related Kaposi's sarcoma. It is also used or under investigation for the treatment of a wide variety of other cancers.[2] It is currently one of the largest selling anticancer drugs in history, with combined annual sales of it and its semisynthetic analog docetaxel (Taxotere®)[3] (**2**) of well over $1 billion.



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

Paclitaxel's importance as an anticancer drug has spurred a large amount of work on its chemistry and mechanism of action. In the chemistry area, virtually every position on the ring and on the side chain has been subjected to structural modifications.[4–6] The work described in these reviews has led to the development of several analogs of paclitaxel which are in clinical trial as second-generation taxanes.

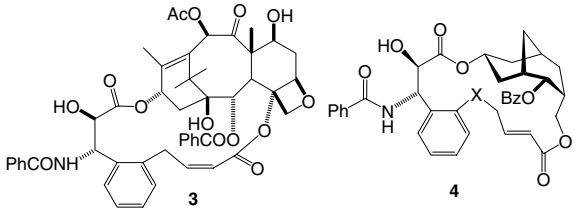
It would be highly desirable if future generations of this class of drugs were structurally much simpler than paclitaxel, while retaining the full activity of the parent compound. Several reports of approaches to this goal have appeared in the literature, and various simplified paclitaxel analogs have been prepared.[7–11] Although some of these analogs showed very weak cytotoxicity, only the bridged paclitaxel-like compounds prepared by Ojima caused any tubulin polymerization;[11] the other compounds were not reported to show this activity.[7–10] Very recently Ojima and co-workers reported the synthesis of simplified macrocyclic PTX analogs[9] based on the 'non-polar' PTX conformation.[12] These analogs likewise do not display tubulin polymerization activity, but they did exhibit micromolar cytotoxicity to two cell lines in spite of having drastically simplified structures.

The difference between our strategy and those previously reported is in the maintenance of the relative orientations of the principal taxane pharmacophore at the β-tubulin-binding site, namely the C-2 benzoate, the C-3′ phenyl, and the C-4 acyl group, by means of a 5-atom bridge. The rational design of such simplified molecules requires a clear understanding of the tubulin-binding conformation of the parent molecule. The T-Taxol conformation was initially discovered on the basis of

Keywords: Taxol; Paclitaxel; Tubulin; Cytotoxicity; Molecular modeling; Synthesis; Olefin metathesis.
* Corresponding author. Tel.: +1 540 231 6570; fax: +1 540 231 3255; e-mail: dkingston@vt.edu

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.01.002

NAMFIS experiments[13] and proposed as the binding conformer in connection with electron crystallographic analysis of tubulin sheets.[14] The binding model has recently been established by the synthesis of macrocyclic PTX analog **3**. This compound adopts the T-Taxol conformation and is significantly more active than PTX in both cytotoxicity and tubulin polymerization assays.[15] The bridged analog **3**, in its 3-D representation, thus defines the required conformation for the effective binding of paclitaxel analogs to tubulin.

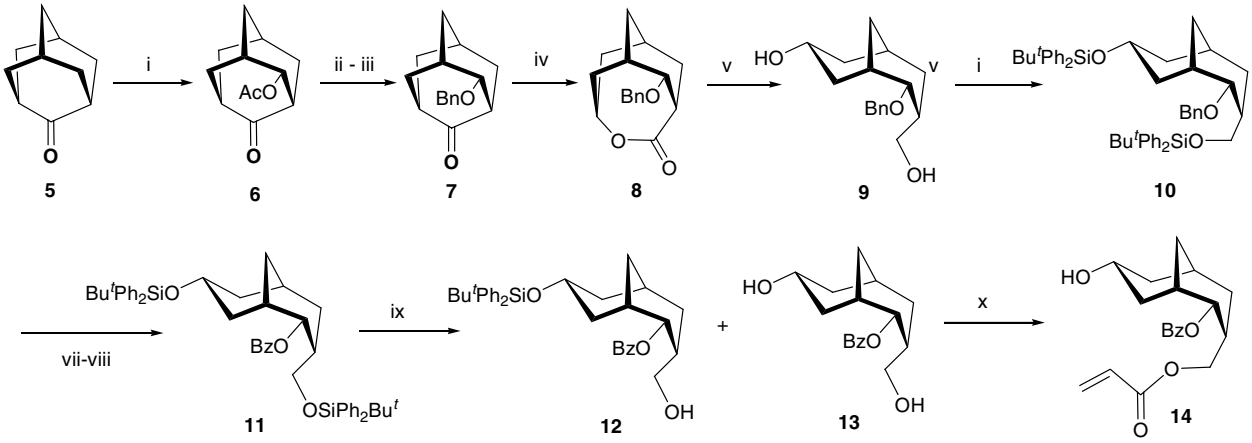


Based on this analysis, and also on the results of SAR studies which indicate that modifications to the northern hemisphere of paclitaxel do not cause significant detriment to its bioactivity,[5] we designed simplified PTX analogs of general structure **4**, by deleting the baccatin core of PTX and replacing it with a modified hydrophobic [3.3.1]-bicyclononane moiety. These compounds do not contain an oxetane ring, which was previously thought to be necessary for the activity of paclitaxel-like compounds. However, it has since been shown that an oxetane ring is not absolutely necessary for activity and can be replaced by other functional groups or ligand–protein-binding features.[16] The design, synthesis, preliminary biological investigation, and biostructural analysis of these model compounds are described in this communication.

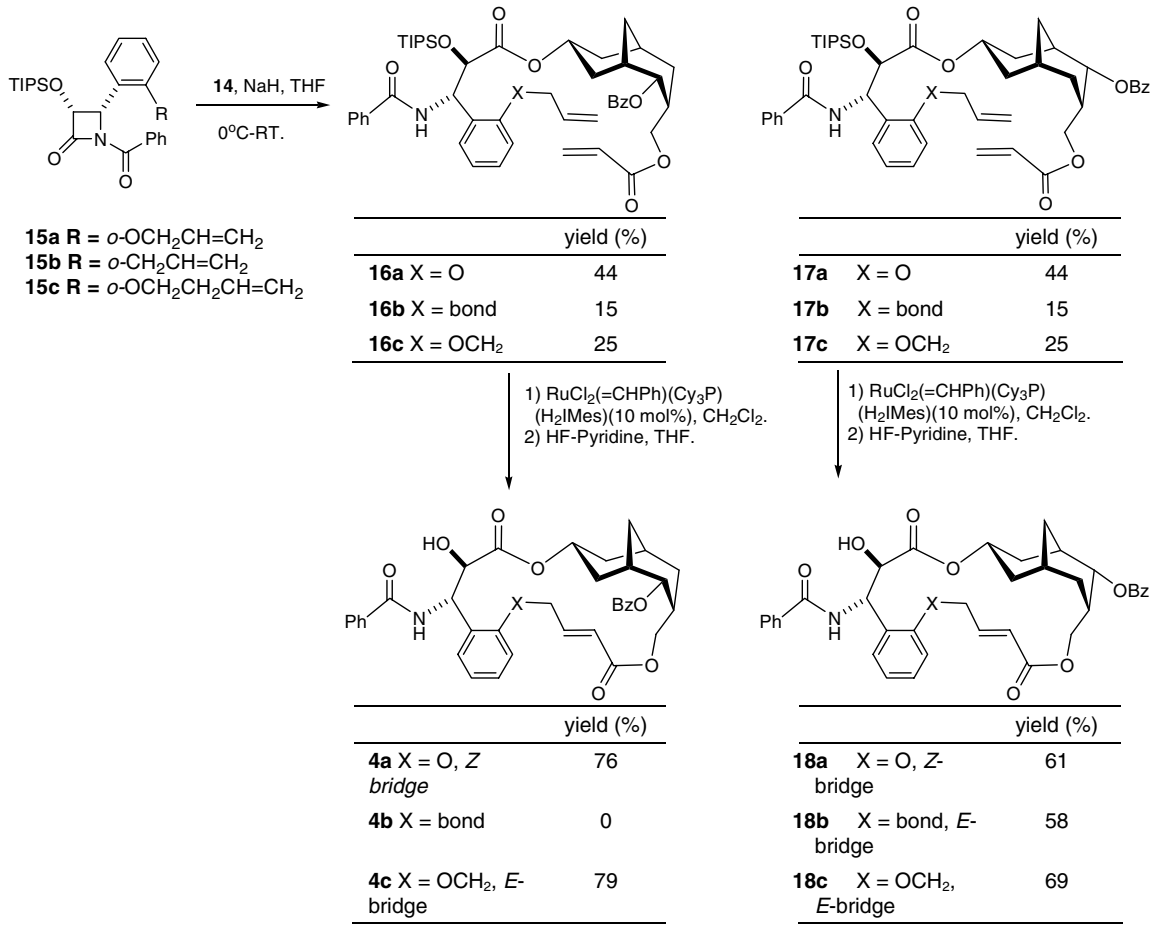
2. Results and discussion

The synthesis of **4** was achieved starting from commercially available 2-adamantanone **5** (Scheme 1). Adamantanone **5** was treated with NaN_3 as previously reported to give acetoxy derivative **6**.[17] The acetyl functional group was then converted to the benzyl-protected compound **7**. Baeyer–Villiger oxidation of **7** produced lactone **8** regioselectively.[18] Lactone **8** was reduced with $LiAlH_4$ to provide diol **9** in satisfactory yield. Attempts to selectively protect one hydroxyl group of **9** failed to give monoprotected alcohol, and only the bis (*tert*-butyldiphenyl)silyl ether **10** was formed even when the more selective silyl protecting group $^tBuPh_2SiCl$ was used. Bis-silyl ether **10** was hydrogenolyzed with H_2–$Pd(OH)_2/C$ at 50 psi to deprotect the benzyl group. The resulting free hydroxyl group was benzoylated with benzoyl chloride and lithium hexamethyldisilazide to give the benzoyl bis-silyl ether **11**. Selective deprotection of the primary OTBDPS ether of **11** was not possible under standard conditions such as HF·Py diluted with pyridine, since these conditions produced a mixture of mono- and bis-deprotected alcohols **12** and **13** in a 1:5 ratio. However, compound **11** was converted to diol **13** in 80% yield by the use of undiluted HF·Py and prolonged reaction conditions. Initial attempts to acylate diol **13** with acryloyl chloride using lithium hexamethyldisilazide or BuLi failed to produce any desired product **14**, instead producing complex mixtures. Analysis of the ^1H NMR spectra of these mixtures indicated that the C2-benzoyl group was migrating among the three hydroxyl groups. This difficulty was circumvented by the use of acrylic acid in the presence of DMAP and EDCI to produce the acryloyl derivative **14** in 80% yield.

The synthesis of β-lactams **15a–c** (Scheme 2) was carried out by standard methods, as previously described.[19] With the building blocks **14** and **15a–c** in hand, the crucial coupling was achieved using sodium hydride, and resulted in the preparation of the three sets of diastereomers **16a–c** and **17a–c** in a nearly 1:1 ratio. The pure individual compounds could be separated by chromatography. Efforts to determine the configuration of the C2-benzoate center were unsuccessful, so both stereoisomeric series were continued to their respective final products.[20] Each diastereomer was subjected to a ring closing metathesis reaction[21] using Grubbs' second-generation catalyst to produce the triisopropylsilyl-protected macrocyclic derivatives. Deprotection of the



Scheme 1. Reagents and conditions: (i) NaN_3, H_2SO_4, Ac_2O (57%); (ii) KOH, THF (85%); (iii) NaH, BnBr, THF (95%); (iv) Ac_2O, AcOH, H_2O_2 (77%); (v) LAH, THF (76%); (vi) TBDPSiCl, imidazole, DMF (92%); (vii) $Pd(OH)_2/C$, 50 psi, 24 h, THF (90%); (viii) BzCl, LHMDS, THF (95%); (ix) HF–pyridine, THF, 0 °C to rt (**13**, 80%); (x) acrylic acid, EDCI, CH_2C1_2, rt (81%).



	yield (%)
16a X = O	44
16b X = bond	15
16c X = OCH_2	25

	yield (%)
17a X = O	44
17b X = bond	15
17c X = OCH_2	25

	yield (%)
4a X = O, *Z* bridge	76
4b X = bond	0
4c X = OCH_2, *E*-bridge	79

	yield (%)
18a X = O, *Z*-bridge	61
18b X = bond, *E*-bridge	58
18c X = OCH_2, *E*-bridge	69

Scheme 2. Synthesis of compounds **4** and **18**.

triisopropylsilyl ethers generated the simplified PTX-like molecules **4a**,**c** and **18a**–**c**; compound **4b** was not formed (Scheme 2). The failure of **16b** to undergo metathesis to the TIPS-protected precursor of **4b** might be attributed to a higher degree of strain relative to diastereomer **18b** from **17b**. However, molecular mechanics conformational searches for isomers **4b** and **18b** suggest they possess similar strain energies.[22] Since the b-series involves the shortest bridge between C4-C=O and *ortho*-C3′-Ph (three carbons), we surmise that the lack of ring closure is kinetic rather than thermodynamic in origin. The intermediate Ru-complex or the corresponding metathesis transition state within the short tether most likely experiences a high energy steric clash with the *syn*-OBz group. Because of the tentative nature of this argument, the regiochemical assignment of the benzoate group in compounds **4a**–**c** and **18a**–**c** should be regarded as provisional.

In our earlier work with bridged PTX analogs such as **3** the double bond in the bridge had the *Z* configuration, but in the present work the compounds were obtained in both *Z* (**4a** and **18a**) and *E* (**4c** and **18b**,**c**) configurations. The configurations were assigned based on NMR coupling constants ($J = 8.4$–8.8 Hz, $J = 15.6$ Hz for protons of the *Z* and *E* bridges, respectively).

3. Biological results and discussion

Cytotoxicity determinations of compounds **4a**,**c** and **18a**–**c** were performed against the A2780 ovarian cell line (Table 1). These measurements were made difficult by the very low solubility of the compounds in DMSO–H_2O mixtures, and it is possible that the reported data underestimate the true cytotoxicities.

All the compounds were cytotoxic, but were significantly less active than PTX. Interestingly, the cytotoxicities of compounds **4a**,**c** and **18a**–**c** were all comparable within a factor of 2, indicating that the relative regiochemistry of the benzoate group and the stereochemistry of the

Table 1. Bioactivity of PTX and analogs **4** and **18**

Compound	IC_{50}[a] (μM) A2780
PTX	0.02 ± 0.01
4a	15.1 ± 2
4b	NA
4c	20 ± 2
18a	11.3 ± 2.5
18b	10.9 ± 1.5
18c	18 ± 1.2

[a] Mean of two determinations.

bridging double bond do not affect the activity significantly. It should also be noted that our simplified analogs have cytotoxicities comparable to those prepared by Ojima and co-workers.[9]

Compounds **4a**,**c** and **18a**,**c** were also tested for microtubule assembly activity in vitro (Fig. 1). The rate and extent of DMSO-induced tubulin assembly was enhanced by these four molecules at concentrations of 30 μM, which was near the limit of solubility for these molecules under the assay conditions.

The substances also appear to stabilize microtubules to cold induced disassembly. In the absence of added ligand, DMSO-induced microtubules are completely depolymerized by dropping the temperature from 37 to 4 °C. In the presence of the compounds, some polymer remains in the samples at 4 °C. Compound **4c** enhanced assembly to the greatest extent, while compound **4a** was most active in stabilizing assembled microtubules.

Modeling of **4c** in the β-tubulin taxoid site demonstrates that the compound is able to adopt the essential elements of the bioactive T-conformation; the C-4 and C-13 side chains matching those of PTX closely. The C-2 benzoyl phenyl overlaps that of PTX, but is forced deeper into the hydrophobic pocket as a result of a somewhat different positioning occasioned by the nonane core by comparison with the PTX baccatin core. This elicits steric congestion via short H–H contacts with the Leu230 and Leu275 tubulin side chains. Apart from insolubility, the reduced activity of **4c** relative to PTX most likely derives from this source. Structure **18c** does not dock into the taxane-binding pocket in the T-form, but can assume a number of alternative conformations and binding modes. Considering the low potency of the compound, we regard none of these as predictive. The diminished activity of our diastereomers as well as that exhibited by the Ojima compounds[9] make it clear that neither class is sufficiently tailored to fully exploit the T-Taxol concept.

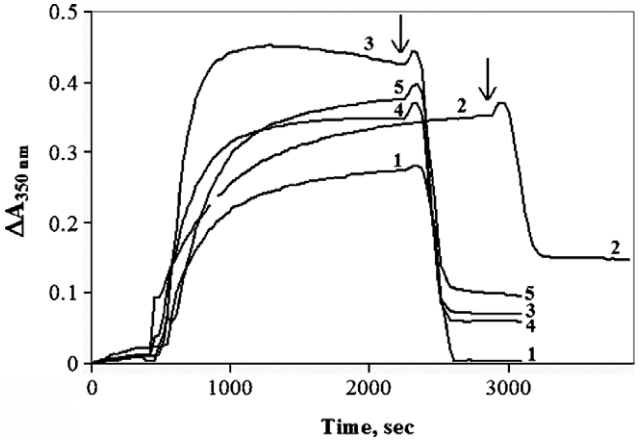


Figure 1. Enhancement of DMSO-induced tubulin assembly (**1**) by **4a** (**2**), **4c** (**3**), **18a** (**4**), and **18c** (**5**). Pure tubulin (15 μM) in buffer (100 mM PIPES, 1 mM $MgSO_4$, 2 mM EGTA, and 1 mM GTP, pH 6.9) was equilibrated to 37 °C in the spectrophotometer. Assembly was initiated by addition of DMSO (curve **1**) or DMSO containing the ligand (curves **2**–**5**) to yield a final concentration of 4% (v/v) DMSO. The final concentration of each ligand was 30 μM. Assembly was monitored by light scattering (apparent absorption at 350 nm). At the time indicated by the arrows, the temperature was dropped to 4 °C.

In summary, we have designed a new class of cytotoxic PTX-like molecules that can approximate, but not precisely achieve, the T-Taxol conformation. Synthesis and biological evaluation of these truncated taxanes provide compounds that exhibit diminished levels of cytotoxicity and tubulin polymerization activity relative to the paclitaxel parent. However, incorporation of polar functional groups in the structures to make them water-soluble may increase their bioactivity. Studies toward this end are in progress and will be reported in due course.

4. Experimental

4.1. General experimental methods

All of the reagents and solvents received from commercial sources were used without further purification. ^1H and ^{13}C NMR spectra were obtained on Varian Unity 400 MHz, Inova 400 MHz, and JEOL Eclipse 500 MHz spectrometers in $CDCl_3$. High resolution FAB mass spectra were obtained on a JEOL HX-110 instrument. Reaction mixtures were worked up by the standard procedure of quenching the reaction, extracting the resulting aqueous mixture with EtOAc or Et_2O, washing the organic solution with water and brine, drying over Na_2SO_4, filtering, and concentration to give crude product.

4.2. Synthesis of 4-benzyloxyadamantan-2-one (7)

A solution of 4-acetoxyadamantanone (**6**)[17] (2.99 g, 14.3 mmol) in THF (35 mL) was added to the stirred solution of 1M KOH (150 mL) in THF (50 mL) at 0 °C. The resulting reaction mixture was stirred for 1 h. After complete hydrolysis, as determined by TLC, two layers were separated. The aqueous layer was extracted with ether (2× 50 mL). Standard workup gave crude 4-hydroxyadamantan-2-one[17] (2.2 g, 95%).

The solution of 4-hydroxyadamantan-2-one (3.24 g, 19.4 mmol) in THF (20 mL) was added to a stirred suspension of NaH (3.88 g, 160 mmol, 8 equiv) in THF (60 mL), followed by benzyl bromide (4.27 mL, 35 mmol, 1.8 equiv) at 0 °C and the resulting reaction mixture was brought to room temperature over night. Saturated brine solution was added to quench the reaction and the product was extracted with Et_2O (3× 100 mL). Standard workup gave crude product, which was subjected to silica gel chromatography with 6–10% EtOAc in hexane to provide 4-benzyloxyadamantan-2-one (**7**) (4.6 g, 95%): ^1H NMR (400 MHz) δ 7.32 (3H, m), 7.28 (2H, m), 4.58 (1H, d, J = 12 Hz), 4.48 (1H, d, J = 12 Hz), 3.90 (1H, m),2.89 (1H, br s), 2.50 (1H, br s), 2.38 (1H, br d, J = 12 Hz), 2.20 (1H, br s), 2.20–1.75 (8H, m); ^{13}C NMR (100 MHz) δ

216.2, 138.4, 128.3, 128.0, 84.2, 69.4, 51.2, 46.5, 39.0, 38.0, 35.3, 33.4, 31.6, 26.7; HRMS (FAB+) Calcd for $C_{17}H_{21}O_2$ (M+H): 257.1542. Found: 257.1539 ($\Delta = -1.0$); Anal. Calcd for $C_{17}H_{20}O_2$: C, 74.97; H, 7.40. Found: C, 74.47; H, 7.47.

4.3. Synthesis of 4-benzyloxyadamantano-lactone (8)

To the solution of 4-benzyloxyadamantan-2-one (**7**) (3.54 g, 13.8 mmol) in acetic acid (30 mL) and Ac_2O (30 mL) H_2O_2[18] (44 mL) was added. The resulting reaction mixture was stirred for four days. Standard workup gave crude product, which was subjected to silica gel chromatography with 10–20% EtOAc in hexane to yield **8** (1.8 g, 60%): ^{1}H NMR (500 MHz) δ 7.32 (3H, m), 7.26 (2H, m), 4.72 (1H, d, $J = 12$ Hz), 4.52 (1H, d, $J = 12$ Hz), 4.42 (1H, t, $J = 2$Hz), 3.54 (2H, m), 2.45 (1H, dt, $J = 15$, 2 Hz), 2.44 (1H, br s), 2.05–1.72 (7H, m), 1.60 (1H, br d, $J = 13$ Hz); ^{13}C NMR (125 MHz) δ 176.0, 138.2, 128.4, 127.66, 127.62, 77.7, 72.7, 69.8, 46.9, 35.8, 33.2, 30.8, 30.3, 29.5, 25.5; HRMS (FAB+) Calcd for $C_{17}H_{21}O_3$ (M+1): 273.1491. Found: 273.1486 ($\Delta = -1.3$).

4.4. Synthesis of 2-benzyloxy-3-hydroxymethyl-[3,3,1]-bicyclononane-7-ol (9)

LAH powder (0.684 g, 18.5 mmol, 3 equiv) was added in portions to a solution of lactone **8** (1.68 g, 6.17 mmol) in THF (40 mL) at 0 °C over 20 min. The resulting reaction mixture was brought to room temperature overnight. A saturated solution of sodium potassium tartrate was added to quench the reaction and the product was obtained by the usual workup. The crude product was subjected to silica gel chromatography with 50% EtOAc in hexane to yield **9** (1.3 g, 76%): ^{1}H NMR (500 MHz) δ 7.36 (5H, m), 4.70 (1H, d, $J = 11.4$ Hz), 4.42 (1H, d, $J = 11.4$ Hz), 4.13 (1H, m), 3.93 (1H, m), 3.66 (1H, dd, $J = 10.7$, 7.5 Hz), 3.46 (1H, dd, $J = 10.7$, 7.5 Hz), 2.59 (1H, br s), 2.13–1.60 (8H, m), 1.30 (1H, dt, $J = 13$, 2.7 Hz); ^{13}C NMR (125 MHz) δ 137.2, 128.8, 128.4, 73.3, 64.7, 64.4, 39.8, 38.0, 34.1, 29.5, 28.6, 25.4, 23.5; HRMS (FAB+) Calcd for $C_{17}H_{25}O_3$ (M+H): 277.1804. Found: 277.1808 ($\Delta = +1.5$).

4.5. Synthesis of 2-benzyloxy-3-(*tert*-butyldiphenylsilyl-oxymethyl)-7-(*tert*-butyldiphenylsilyloxy)-[3,3,1]-bicyclononane (10)

To the solution of diol **9** (1.3 g, 4.74 mmol) in DMF (6 mL) were added imidazole (3.22 g, 47 mmol, 10 equiv) and *tert*-butyldiphenylsilylchloride (5 mL, 19 mmol, 4 equiv) at rt. The resulting mixture was stirred for four days. Saturated methanolic $NaHCO_3$ solution was added to quench the reaction and the mixture was subjected to the usual workup. The crude product was subjected to silica gel chromatography with 1.5–2% Et_2O in hexane to yield **10** (3.5 g, 98%): ^{1}H NMR (400 MHz) δ 7.70 (4H, m), 7.68 (4H, m), 7.42–7.25 (17H, m), 7.15 (2H, m), 4.39 (1H, t, $J = 10.4$ Hz), 4.30 (2H, s), 4.15 (1H, dd, $J = 10$, 4.8 Hz), 4.02 (1H, m), 3.50 (1H, dd, $J = 7.2$, 5.2 Hz), 2.40 (1H, m), 2.20 (2H, br d, $J = 8$ Hz) 2.06 (1H, m), 1.98 (1H, br s), 1.78–1.62 (3H, m), 1.52 (2H, m), 1.14 (9H, s), 1.06 (9H, s), 0.96 (1H, br d, $J = 12$ Hz); ^{13}C NMR (100 MHz) δ 139.2, 136.1, 136.0, 135.87, 135.82, 134.9, 134.8, 134.7, 134.5, 129.7, 129.69, 129.66, 129.62, 128.4, 127.9, 127.8, 127.6, 127.4, 127.3, 80.6, 69.8, 67.1, 63.1, 39.8, 36.8, 31.5, 30.5, 30.3, 27.3, 27.2, 27.1, 25.6, 19.7, 19.3; HRMS (FAB+) Calcd for $C_{49}H_{61}O_3Si_2$ (M+1): 753.4159. Found: 753.4143 ($\Delta = -2.2$).

4.6. Synthesis of 2-benzoyl-3-(*tert*-butyldiphenylsilyl-oxymethyl)-7-(*tert*-butyldiphenylsilyloxy)-[3,3,1]-bicyclononane (11)

To a solution of 2-*O*-benzyl-bis-TBDPS ether (**10**) (1.31 g, 1.74 mmol) in THF (85 mL) was added $Pd(OH)_2/C$ (0.4 g) and the resulting reaction mixture was hydrogenated at 50 psi at room temperature for 24 h. The catalyst was filtered off using a short plug of silica gel and the filtrate was concentrated to yield the crude product. The crude product was then subjected to silica gel chromatography with 1.5–4% Et_2O in hexane to provide 2-hydroxy-bis-OTBDPS ether (0.8 g, 90% based on unrecovered starting material).

To the solution of 2-hydroxy-bis-OTBDPS ether obtained from the above reaction (500 mg, 0.755 mmol) in THF (28 mL) was added lithium hexamethyldisilazide (1.13 mL, 1.13 mmol, 1.5 equiv) at 0 °C and the resulting solution was stirred for 10 min. Benzoyl chloride (0.32 mL, 2.4 mmol, 2 equiv) was added to the above reaction mixture, which was then stirred for 4 h at 0 °C. Saturated NH_4Cl solution was added to quench the reaction and the product was worked up in the usual way. The resulting crude product was subjected to silica gel chromatography using 4% EtOAc in hexane to furnish **11** (560 mg, 97%): ^{1}H NMR (400 MHz) δ 7.90 (2H, dd, $J = 8.2$, 1.2 Hz), 7.72–7.20 (23H, m), 5.26 (1H, t, $J = 7.2$ Hz), 4.26 (1H, t, $J = 9.6$ Hz), 4.05 (2H, m), 2.51 (1H, m), 2.38 (1H, m), 2.18 (1H, br d, $J = 14$ Hz), 2.06 (2H, m), 1.72 (2H, m), 1.52 (1H, d, $J = 12$ Hz), 1.22 (1H, m), 1.04 (18H, 2 singlets), 1.00 (1H, m); ^{13}C NMR (100 MHz) δ 166.0, 136.0, 135.9, 135.7, 135.6, 134.8, 134.6, 134.1, 134.0, 132.8, 130.8, 129.8, 129.7, 129.66, 129.64, 128.4, 127.9, 127.78, 127.7, 127.6, 76.0, 67.0, 63.6, 39.4, 37.4, 31.3, 31.0, 30.2, 27.2, 27.0, 26.5, 25.0, 19.4, 19.3; HRMS (FAB+) Calcd for $C_{49}H_{58}O_4Si_2Na$ (M+Na): 789.3771. Found: 789.3738 ($\Delta = -3.9$).

4.7. Synthesis of 2-benzoyl-3-hydroxymethyl-[3,3,1]-bicyclononane-7-ol (13)

To a solution of **11** (800 mg, 1.04 mmol) in THF (30 mL) was added HF·Py (3 mL, 70% HF) at 0 °C and the resulting solution was brought to room temperature over 36 h. Saturated $NaHCO_3$ solution was added to quench the reaction and the product was subjected to the usual workup. The resulting crude product was subjected to silica gel chromatography with 2% MeOH in CH_2Cl_2 to furnish **13** (244 mg, 80%): ^{1}H NMR (400 MHz) δ 8.04 (2H, dd, $J = 8.2$, 1.2 Hz), 7.57 (1H, t, $J = 7.6$ Hz), 7.45 (2H, t, $J = 7.6$ Hz), 5.66 (1H, dd, $J = 9.3$, 6.2 Hz), 4.00 (1H, t, $J = 4.8$ Hz), 3.73 (1H, dd,

J = 11, 8 Hz), 3.63 (1H, dd, J = 11, 8 Hz), 2.68 (1H, m), 2.39–2.24 (3H, m), 2.20 (1H, m), 2.04 (1H, m), 1.82 (6H, m), 1.09 (1H, dt, J = 13.6, 2.8 Hz); ^{13}C NMR (100 MHz) δ 166.4, 133.6, 129.9, 129.7, 129.0, 74.2, 65.5, 63.7, 39.1, 37.9, 35.5, 29.2, 28.0, 26.8, 23.6. HRMS (FAB+) Calcd for $C_{17}H_{23}O_4$ (M+1): 291.1596. Found: 291.1589 (Δ = −2.5); Anal. calcd for $C_{17}H_{22}O_4$: C, 70.32; H, 7.58. Found: C, 70.18, H, 8.15.

4.8. Synthesis of 2-benzoyl-3-acryloyloxymethyl-[3,3,1]-bicyclononane-7-ol (14)

Acrylic acid (43 mg, 0.608 mmol, 1.05 equiv) followed by DMAP (7. 5 mg, 0.1 mol %) was added to a solution of **13** (168 mg, 0.58 mmol) in CH_2Cl_2 (5 mL), and the resulting reaction mixture was stirred at room temperature for 6 h. EtOAc was added followed by water to quench the reaction, and standard workup gave a crude product, which was purified by preparative TLC on silica gel with 2% MeOH in CH_2Cl_2 to furnish **14** (90 mg, 81% yield, based on unrecovered starting material **13**): ^{1}H NMR (500 MHz) δ 8.00 (2H, dd, J = 8.2, 1.3 Hz), 7.56 (1H, t × t, J = 7.6, 1.1 Hz), 7.44 (2H, t, J = 7.6 Hz), 6.27 (1H, dd, J = 17.2, 1.6 Hz), 5.98 (1H, dd, J = 17.4, 10.5 Hz), 5.72 (1H, dd, J = 10.5, 1.3 Hz), 5.58 (1H, dd, J = 9.1, 6.4 Hz), 4.29 (2H, m), 3.98 (1H, t, J = 4.3 Hz), 2.69 (1H, m), 2.49 (2H, m), 2.17 (3H, m), 1.94–1.73 (6H, m), 1.46 (1H, dt, J = 13.3, 2.5 Hz); ^{13}C NMR (100 MHz) δ 166.0, 165.6, 133.4, 130.8, 129.7, 129.5, 128.8, 128.2, 73.3, 65.27, 65.21, 38.6, 34.2, 33.5, 29.0, 27.7, 26.9, 23.4; HRMS (FAB+) Calcd for $C_{20}H_{24}O_5Na$ (M+Na): 367.1521. Found: 367.15388 (Δ = +3.5).

4.9. Synthesis of open chain ω,ω-diene precursors 16a and 17a

A solution of **14** (7.5 mg, 0.022 mmol) in THF (1 mL) was added to the stirred suspension of NaH (45 mg, excess), THF (2 mL) at 0 °C and the resulting reaction mixture was stirred for 10 min. A solution of **15a**[23] (21 mg, 0.043 mmol, 2 equiv) in THF (0.8 mL) was added to the above reaction mixture at 0 °C and the resulting solution was brought to room temperature over 4 h. Saturated brine solution (5 mL) was added to quench the reaction and the product was worked up in the usual way. The resulting crude mass was subjected to preparative TLC on silica gel with 14% EtOAc in hexane to furnish **16a** (8 mg, 44%) and **17a** (8 mg, 44%). Compound **16a**: $[\alpha]_D^{22}$ −20.5 (c 2.1, $CHCl_3$); ^{1}H NMR (400 MHz) δ 8.02 (2H, dd, J = 8.4, 1.6 Hz), 7.82 (2H, dd, J = 8.8, 1.4 Hz), 7.55 (1H, m), 7.49–7.40 (5H, m), 7.28 (1H, d, J = 8 Hz), 7.19 (2H, m), 6.87 (2H, t, J = 7.6 Hz), 6.27 (1H, dd, J = 17.2, 1.6 Hz), 6.08 (1H, m), 5.95 (1H, dd, J = 17.4, 10.8 Hz), 5.88 (1H, dd, J = 8.8, 2 Hz), 5.70 (1H, dd, J = 10.4, 1.6 Hz), 5.54 (1H, dq, J = 17.2, 1.6 Hz), 5.33 (1H, t, J = 6.4 Hz), 5,24 (1H, dd, J = 10.8, 1.2 Hz), 5.14 (1H, m), 4.93 (1H, d, J = 2 Hz), 4.60 (2H, m), 4.57 (1H, dd, J = 10.8, 8 Hz), 4.41 (1H, dd, J = 10.8, 8 Hz), 2.62 (2H, m), 2.22 (2H, m), 2.02 (1H, m), 1.90–1.80 (3H, m), 1.72 (1H, m), 1.48 (1H, m), 1.39 (1H, m), 0.88 (18H, 2 singlets) 0.82 (3H, m); ^{13}C NMR (100 MHz) δ 172.0, 166.6, 166.0, 155.7, 134.9, 133.2, 131.6, 130.8, 130.4, 129.8, 128.9, 128.8, 128.6, 128.4, 128.0, 127.2, 127.0, 120.8, 117.2, 111.7, 74.2, 73.5, 69.7, 68.9, 65.2, 53.0, 35.2, 33.4, 32.4, 29.9, 26.7, 25.7, 24.3, 17.9, 17.8, 12.4; HRMS (FAB+) Calcd for $C_{48}H_{62}NO_9Si_2$ (M+1): 824.4194. Found: 824.4208 (Δ = +1.7). Compound **17a**: $[\alpha]_D^{22}$ −1.5 (c 2.2, $CHCl_3$); ^{1}H NMR (400 MHz) δ 8.08 (2H, dd, J = 8.4, 1.6 Hz), 7.81(2H, dd, J = 8.8, 1.4 Hz), 7.56 (1H, m), 7.43 (5H, m), 7.28 (1H, dd, J = 8.8 Hz), 7.18 (1H, m), 6.85 (2H, m), 6.25 (1H, dd, J = 17.2, 1.6 Hz), 6.11 (1H, m), 5.91 (1H, dd, J = 17.4, 10.8 Hz), 5.87 (1H, dd, J = 8.8, 2 Hz), 5.68 (1H, dd, J = 10.4, 1.6 Hz), 5.56 (1H, dq, J = 17.2, 1.6 Hz), 5.29 (1H, m), 5.27 (1H, m), 5.15 (1H, m), 4.97 (1H, d, J = 2 Hz), 4.60 (2H, m), 4.59 (1H, dd, J = 10.8, 7.6 Hz), 4.54 (1H, dd, J = 10.8, 7.6 Hz), 2.68 (1H, m), 2.59 (1H, m), 2.40 (1H, m), 2.20 (3H, m), 2.00–1.78 (5H, m), 1.40–1.24 (2H, m), 0.90 (18H, 2 singlets) 0.89 (3H, m); ^{13}C NMR (100 MHz) δ 172.0, 166.4, 165.8, 155.8, 134.8, 133.25, 133.21, 131.6, 130.8, 129.9, 128.9, 128.8, 128.6, 128.4, 128.0, 127.2, 126.9, 120.8, 117.3, 111.6, 74.6, 73.7, 69.6, 68.9, 65.4, 53.3, 35.5, 33.3, 32.9, 30.1, 26.3, 26.1, 24.6, 17.9, 17.8, 12.4; HRMS (FAB+) Calcd for $C_{48}H_{62}NO_9Si_2$ (M+1): 824.4194. Found: 824.41888 (Δ = −0.6).

Compounds **16b** and **17b** were prepared in 30% yield and compounds **16c** and **17c** were prepared in 51% yield by similar procedures; all four derivatives had satisfactory characterization data.

4.10. Simplified paclitaxel analog 18a

To the solution of **17a** (7 mg, 0.01 mmol) in CH_2Cl_2 (2.5 mL) was added Grubbs's second-generation catalyst (3 mg, 30 mol %) in CH_2Cl_2 (2 mL) over 3 h and the resulting solution was stirred for an additional hour. The CH_2Cl_2 was concentrated, and the crude product was subjected to preparative TLC on silica gel using 18% EtOAc in hexane to provide the macrocyclic product (5.7 mg, 85% yield).

To the solution of product obtained from the above reaction (5.5 mg, 0.0043 mmol) in THF (2 mL) was added HF·Py (0.1 mL, 70% HF) at 0 °C and the resulting solution was brought to room temperature overnight. Saturated $NaHCO_3$ solution was added to quench the reaction and the product was extracted with EtOAc (3× 10 mL). Standard workup was followed by purification by preparative TLC with 40% EtOAc in hexane to furnish **18a** (3.2 mg, 72%) (61% overall two steps): ^{1}H NMR (400 MHz) δ 7.98 (2H, dd, J = 8.0, 1.6 Hz), 7.81 (2H, dd, J = 8.0, 1.2 Hz), 7.53 (2H, m), 7.45 (5H, m), 7.26 (1H, m), 7.06 (d, J = 9.2 Hz), 6.99 (2H, m), 6,91 (d, J = 8 Hz), 6.25 (1H, d, J = 8.8 Hz), 5.38 (1H, dd, J = 9.6, 5.2 Hz), 5.32 (1H, m), 4.80 (4H, m), 4.35 (1H, dd, J = 13.6, 3.2 Hz), 4.07 (1H, dd, J = 13.6, 3.2 Hz), 3.20 (1H, br s), 2.82–2.64 (3H, m), 2.54 (1H, m), 2.38 (1H, m), 2.22–2.10 (2H, m), 2.00 (2H, m), 1.80 (1H, m), 1.22 (1H, m); ^{13}C NMR (100 MHz) δ 173.2, 166.8, 166.6, 166.1, 154.4, 143.0, 133.4, 132.0, 129.9, 129.1, 128.9, 128.8, 127.3, 127.0, 126.6, 121.5, 120.5, 111.0, 75.5, 72.7, 71.8, 66.2, 66.0, 50.6, 36.8, 32.7, 30.9, 29.4,

26.7, 24.5, 24.2; HRMS (FAB+) Calcd for $C_{37}H_{38}NO_9$ (M+1): 640.2547. Found: 640.25287 (Δ = −2.7).

Similar two-step procedures were employed to synthesize the other analogs **4a**,**c** and **18b**,**c**. Yields are as follows: **4a** (76% overall yield for two steps), **4c** (79% overall yield for two steps), **18b** (58% overall yield for two steps), and **18c** (51% overall yield for two steps). Compound **4b** was not obtained from **16b**.

4.11. Simplified paclitaxel analog 4a

^{1}H NMR (400 MHz) δ 7.98 (2H, dd, *J* = 8.0, 1.6 Hz), 7.82 (2H, dd, *J* = 8.0, 1.2 Hz), 7.54 (2H, m), 7.45 (4H, m), 7.30 (2H, m), 7.08–7.00 (3H, m), 6.91 (d, *J* = 8.4 Hz), 6.28 (1H, d, *J* = 10 Hz), 5.38–5.30 (2H, m), 4.84 (2H, m), 4.77 (1H, d, *J* = 1.2 Hz), 4.47 (1H, dd, *J* = 12.4, 1.6 Hz), 4.03 (1H, dd, *J* = 12.4, 1.6 Hz), 2.78 (1H, m), 2.58 (1H, m), 2.28–2.02 (6H, m), 1.98 (1H, m), 1.82 (1H, br d, *J* = 14.8 Hz), 1.27 (1H, m); ^{13}C NMR (100 MHz) δ 173.1, 166.7, 166.6, 165.9, 154.4, 142.9, 134.5, 133.4, 132.0, 130.1, 129.8, 129.1, 128.9, 128.7, 127.3, 127.2, 126.7, 121.5, 120.6, 111.1, 75.8, 72.4, 72.0, 66.1, 65.1, 50.3, 35.1, 32.6, 30.7, 29.1, 26.6, 25.0, 24.3; HRMS (FAB+) Calcd for $C_{37}H_{38}NO_9$ (M+1): 640.2547. Found: 640.2529 (Δ = −2.7).

4.11.1. Simplified paclitaxel analog 18b. ^{1}H NMR (400 MHz) δ 8.01 (2H, dd, *J* = 8.4, 1.2 Hz), 7.74 (1H, m), 7.62 (2H, dd, *J* = 8.4 Hz), 7.48 (2H, q, *J* = 7.2 Hz), 7.39 (2H, t, *J* = 8 Hz) 7.28 (4H, m), 6.75 (d, *J* = 9.2 Hz), 6.34 (1H, d, *J* = 15.6 Hz), 5.98 (1H, d, *J* = 9.2 Hz), 5.28 (2H, m), 4.66 (1H, d, *J* = 12.4 Hz), 4.26 (1H, d, *J* = 3.2 Hz), 4.18 (1H, m), 3.45 (1H, dd, *J* = 5.6, 1.6 Hz), 3.25 (1H, d, *J* = 3.6 Hz), 2.65 (1H, m), 2.55 (1H, br s), 2.38–1.90 (6H, m), 1.73 (2H, m), 1.22 (1H, m); ^{13}C NMR (100 MHz) δ 173.1, 168.0, 161.5, 161.1, 141.6, 134.1, 132.7, 127.9, 126.5, 125.7, 124.4, 123.4, 123.3, 122.6, 121.8, 118.4, 71.2, 67.3, 66.2, 57.2, 44.1, 32.3, 23.6; HRMS (FAB+) Calcd for $C_{37}H_{38}NO_8$ (M+): 627.2597. Found: 627.2625 (Δ = +4.4).

4.11.2. Simplified paclitaxel analog 18c. ^{1}H NMR (400 MHz) δ 8.13 (2H, dd, *J* = 8.0, 1.6 Hz), 7.67 (2H, dd, *J* = 8.4, 1.2 Hz), 7.54 (1H, m), 7.44 (3H, m), 7.30 (4H, m), 7.12 (1H, dd, *J* = 15.6, 8.4 Hz), 6.97 (1H, d, *J* = 7.6 Hz), 6.89 (2H, d, *J* = 8.4 Hz) 6.46 (1H, d, *J* = 15.6 Hz), 6.04 (1H, d, *J* = 9.2, 2 Hz), 5.43 (1H, m), 5.32 (1H, dd, *J* = 8.8, 5.6 Hz), 4.92 (1H, d, *J* = 2 Hz), 4.56 (1H, d, *J* = 12.4 Hz), 4.31 (1H, m), 4.14 (2H, m), 2.74 (3H, m), 2.42 (1H, m), 2.28–2.02 (5H, m), 1.90 (2H, m), 1.8 (1H, m), 1.36 (1H, br d, *J* = 14 Hz); ^{13}C NMR (100 MHz) δ 173.2, 166.4, 166.06, 166.03, 155.2, 145.0, 134.3, 133.0, 131.4, 129.98, 129.90, 128.9, 128.5, 127.3, 126.9, 126.1, 124.9, 120.8, 110.7, 75.4, 71.6, 71.5, 65.0, 63.4, 51.1, 32.8, 32.6, 32.4, 31.3, 29.7, 28.9, 26.6, 25.3; HRMS (FAB+) Calcd for $C_{38}H_{40}NO_9$(M+1): 654.2703. Found: 654.26904 (Δ = −2.0).

4.11.3. Simplified paclitaxel analog 4c. ^{1}H NMR (400 MHz) δ 8.00 (2H, dd, *J* = 8.0, 1.2 Hz), 7.76 (2H, dd, *J* = 8.4, 1.2 Hz), 7.52 (2H, m), 7.45 (3H, m), 7.28 (4H, m), 6.94 (1H, t × d, *J* = 7.6, 1.2 Hz), 6.88 (1H, d, *J* = 8.4 Hz), 6.83 (1H, d, *J* = 9.2 Hz) 6.32 (1H, d, *J* = 15.6 Hz), 6.00 (1H, dd, *J* = 9.4, 2 Hz), 5.38 (1H, dd, *J* = 8.8, 5.6 Hz), 5.30 (1H, m), 4.85 (1H, d, *J* = 2.4 Hz), 4.44 (1H, dd, *J* = 12.4, 3.2 Hz), 4.28 (1H, m), 4.18 (2H, m), 2.70 (3H, m), 2.58 (1H, m), 2.40 (1H, m), 2.10 (1H, m), 2.00–1.78 (6H, m), 1.38 (1H, d, *J* = 14 Hz); ^{13}C NMR (100 MHz) δ 173.7, 166.6, 166.4, 166.1, 155.5, 145.5, 134.6, 133.3, 131.9, 130.3, 129.8, 129.2, 128.9, 128.6, 127.4, 127.2, 126.2, 124.7, 121.0, 111.1, 75.5, 72.1, 71.5, 65.7, 65.0, 51.1, 33.8, 33.1, 32.7, 32.4, 29.4, 26.8, 25.8, 24.0; HRMS (FAB+) Calcd for $C_{38}H_{40}NO_9$ (M+1): 654.2703. Found: 654.27545 (Δ = +8.0).

4.12. Computational details

Structures **4c** and **18c** were constructed using T-Taxol as the template in MacroModel 6.5. Each was fully optimized using the MM3* force field including the GBSA/H_2O solvation model.[24] The structures were manually docked into the β-tubulin-binding site by superimposing them on bound paclitaxel.[14] Molecular dynamics was then performed on a 10 Å sphere around the binding site of each model for 250 fs at 20 K using the Tripos force field,[25] followed by full optimization of the complex, while holding the backbone of the protein aggregate fixed. The structure of **4c** retains the essential features of the T-Taxol-binding conformation by locating the bicyclononane core on the baccatin core and by superposing the C-13 phenyl rings on those of PTX. The C-2 phenyl rings overlap, but that of **4c** is offset and buried deeper in the pocket where it encounters a steric clash with the protein. When the T-Taxol model of **18c** is docked into the β-tubulin protein coordinates, the C-13 terminal phenyl rings are positioned similar to those of paclitaxel. However, the diastereomerically relocated C-2 phenyl ring is positioned in the northern part of the binding site near Leu371, Pro274, and Phe272 (ligand/side-chain H–H separations from 2.2 to 2.7 Å). The resulting steric clash pushes the ligand out of the binding site during MD simulations.

Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.01.002.

References and notes

1. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325–2327.
2. Brown, D. T. In *Taxus: The Genus Taxus*; Itokawa, H., Lee, K.-H., Eds.; Taylor and Francis: London, 2003; pp 387–435, Chapter 11.
3. Guénard, D.; Gueritte-Voegelein, F.; Potier, P. *Acc. Chem. Res.* **1993**, *26*, 160–167.
4. Ojima, I.; Kuduk, S.; Chakravarty, S. *Adv. Med. Chem.* **1999**, *4*, 69–124.
5. Kingston, D. G. I.; Jagtap, P. G.; Yuan, H.; Samala, L. *Prog. Chem. Org. Nat. Prod.* **2002**, *84*, 53–225.

6. Wang, X.; Itokawa, H.; Lee, K.-H. Structure–Activity Relationships of Taxoids. In *Taxus: The Genus Taxus*; Itokawa, H., Lee, K.-H., Eds.; Taylor and Francis: London, 2003; pp 298–386.
7. (a) Blechert, S.; Kleine-Klausing, A. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 412–414; (b) Blechert, S.; Jansen, R.; Velder, J. *Tetrahedron* **1994**, *50*, 9649–9656.
8. Klar, U.; Graf, H.; Schenk, O.; Rohr, B.; Schulz, H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1397–1402.
9. Geng, X.; Geney, R.; Pera, P.; Bernacki, R. J.; Ojima, I. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3491–3494.
10. Almqvist, F.; Manner, S.; Thornqvist, V.; Berg, U.; Wallin, M.; Frejd, T. *Org. Biomol. Chem.* **2004**, *2*, 3085–3090.
11. Geney, R.; Sun, L.; Pera, P.; Bernacki, R. J.; Xia, S.; Horwitz, S. B.; Simmerling, C. L.; Ojima, I. *Chem. Biol.* **2005**, *12*, 339–348.
12. For discussion of the non-polar conformation, see (a) Vander Velde, D. G.; Georg, G. I.; Grunewald, G. L.; Gunn, C. W.; Mitscher, L. A. *J. Am. Chem. Soc.* **1993**, *113*, 11650–11651; (b) Paloma, L. G.; Guy, R. K.; Wrasidlo, W.; Nicolaou, K. C. *Chem. Biol.* **1994**, *1*, 107–112; (c) Ojima, I.; Kuduk, S. D.; Chakravarty, S.; Ourevitch, M.; Bégué, J.-P. *J. Am. Chem. Soc.* **1997**, *119*, 5519–5527; (d) Mastropaolo, D.; Camerman, A.; Luo, Y.; Brayer, G. D.; Camerman, N. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 6920–6924; (e) Gao, Q.; Parker, W. L. *Tetrahedron* **1996**, *52*, 2291–2300.
13. Snyder, J. P.; Nevins, N.; Cicero, D. O.; Jansen, J. *J. Am. Chem. Soc.* **2000**, *122*, 724–725.
14. Snyder, J. P.; Nettles, J. H.; Cornett, B.; Downing, K. H.; Nogales, E. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5312–5316.
15. Ganesh, T.; Guza, R. C.; Bane, S.; Ravindra, R.; Shanker, N.; Lakdawala, A. S.; Snyder, J. P.; Kingston, D. G. I. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10006–10011.
16. (a) Wang, M.; Cornett, B.; Nettles, J.; Liotta, D. C.; Snyder, J. P. *J. Org. Chem.* **2000**, *65*, 1059–1068; (b) Barboni, L.; Giarlo, G.; Ricciutelli, M.; Ballini, R.; Georg, G. I.; Vander Velde, D. G.; Himes, R. H.; Wang, M.; Lakdawala, A.; Snyder, J. P. *Org. Lett.* **2004**, *6*, 461–464.
17. Sasaki, T.; Eguch, S.; Toru, T. *J. Org. Chem.* **1970**, *35*, 4109–4114.
18. Duddeck, H.; Kaiser, M. *Z. Naturforsch.* **1982**, *37b*, 1672–1674.
19. Metaferia, B. B.; Hoch, J.; Glass, T. E.; Bane, S. L.; Chatterjee, S. K.; Snyder, J. P.; Lakdawala, A.; Cornett, B.; Kingston, D. G. I. *Org. Lett.* **2001**, *3*, 2461–2464.
20. Compounds **16a** and **17a** were subjected to base hydrolysis (LiOH or KOH) to generate [3,3,1]-bicyclononane diols or triols in an attempt to make Mosher esters at the C2 position. NMR analysis of the hydrolysis products indicated the loss of the acryloyl ester, with concomitant migration of the benzoate and side-chain esters. Lack of material precluded further investigations in this area.
21. (a) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18–29, and references cited therein; (b) Furstner, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 3012–3043; (c) Grubbs, R. H.; Chang, S. *Tetrahedron* **1998**, *54*, 4413–4450, and references cited therein.
22. The steric energies for the global minima of *trans*-**4b** and *trans*-**18b** on the MM3* force field potential energy surface[24] were calculated to be 83 kcal/mol for both isomers. For *cis*-**4b**, the strain energy is estimated to be 5 kcal/mol higher.
23. Compounds **15a**–**c** were prepared as described in Refs. 15,19.
24. (a) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liscamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467; (b) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127–6129; (c) <http://www.schrodinger.com/Products/macromodel.html/>.
25. (a) Clark, M.; Cramer, R. D., III; Van Opdenbosch, N. *J. Comp. Chem.* **1989**, *10*, 982–1012(b) SYBYL: <http://www.tripos.com/sciTech/inSilicoDisc/moleculeModeling/sybyl.html/>; <http://uwmml.pharmacy.wisc.edu/SYBYL_user.html/>.