Synthesis of Novel Macrocyclic Docetaxel Analogues. Influence of Their Macrocyclic Ring Size on Tubulin Activity

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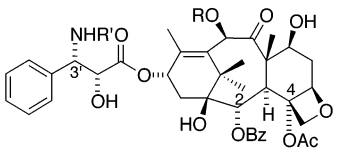
This work describes the synthesis of a series of novel macrocyclic taxoids **3** and **3(H)** designed to mimic the docetaxel solid-state ("nonpolar") conformation. These compounds, bearing 18-, 20-, 21-, and 22-membered rings connecting the C-2 OH and C-3′ NH moieties, were constructed by ring-closing olefin metathesis of the taxoid-ω,ω'-dienes **4**. Biological evaluation of these new taxoids showed that activity is dependent on the ring size, and only the 22-membered ring taxoid **3d** exhibits significant tubulin binding. Synthesis of the open-chain analogues **7** and **7(H)** and comparison of their biological activities with macrocyclic taxoids show that the carbon tether between C-2 OH and C-3′ NH does not hamper tubulin binding. Computational studies of the conformational behavior of the macrocyclic taxoids **3** indicate that the 18-, 20-, and 21-membered-ring **3a**−**c** adopt mainly conformations that are not recognized by tubulin. The most active taxoid **3d** appears to adopt a conformation that is between the "nonpolar" and T-shaped forms.

Introduction

Taxoids are a series of anticancer drugs[1] that inhibit cell growth by interacting with microtubules.[2] Paclitaxel (Taxol) **1a**[3] and docetaxel (Taxotere) **1b**[4] (Figure 1), the leading compounds of this series, are effective clinical agents for the treatment of various cancers. Because of their original activity and their efficiency in cancer treatment, extensive studies have been made on structure−activity relationships.[5] The recently determined structure of tubulin[6] clearly shows the location of the taxoid binding site, but the 3.5 Å resolution does not allow complete determination of the ligand conformation. The first information on the 3D structure of taxoids was obtained by X-ray diffraction on the crystal of docetaxel.[7] Since the past 10 years, several conformational studies (X-rays, NMR, and molecular modeling) have led to the proposal of three major "active" conformations for antitumor taxoids. Whereas the taxane core is conformationally rigid, the C-13 β-phenylisoserine side chain possesses a high degree of freedom and the three conformers refer to its relative orientation. Two of these conformers show hydrophobic collapse between the C-2 benzoyl group and the C-3′ substituents. The "polar" form, observed by NMR in polar solvents[8] and in the crystal structure of paclitaxel,[9] displays a hydrophobic interaction between the C-2 benzoyl moiety and the phenyl group at position C-3′. Otherwise, this interaction involves the C-3 NH substituent in the "nonpolar" conformer observed in the docetaxel solid state[7] and by NMR in apolar solvents.[10] The third conformer is devoid of intramolecular interactions, and



Paclitaxel (Taxol®) **1a** R=Ac, R'=Bz
Docetaxel (Taxotere®) **1b** R=H, R'=Boc

Figure 1.

the centroid of the C-2 benzoyl phenyl ring is almost equidistant from the centroids of both hydrophobic groups at the C-3′ position (T-shaped structure). This conformation has been recognized in the deconvolution of the average spectra of paclitaxel in chloroform[11] and proposed to be the bound conformation on β-tubulin.[12]

To delineate the bound conformation of taxoids on tubulin, the synthesis of analogues with built-in conformational restrictions has been designed.[13] Several compounds with bridges linking the C-2 and C-3′ phenyl groups have been synthesized to mimic the "polar" conformation.[13a−c] Only a few of them retain an interaction with microtubules and to a lesser extent than paclitaxel (19−36%).[13c] Analogues of the T-shaped conformation have been recently designed bearing either a conformationally constrained side chain[13e] or a tether between the C-4 acetyl group and the C-3′ phenyl ring.[13d] These compounds show significant cytotoxicity and tubulin binding. Conformational analysis of these derivatives is compatible with the hypothesis of a bioactive T-shaped conformation but does not rule out the existence of another one. Finally, the first derivatives designed to mimic the "nonpolar" conformation have been described with a tether between the C-2 and N-3′ groups.[13f] Though these new compounds were reported to be cytotoxic, their binding to microtubules was not reported.

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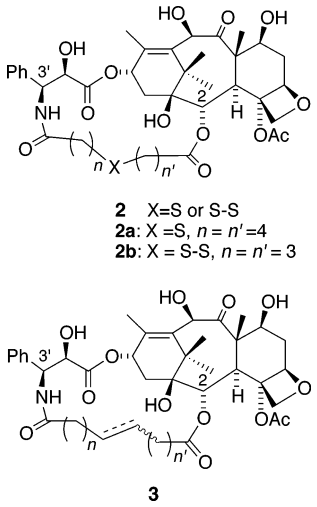


Figure 2.

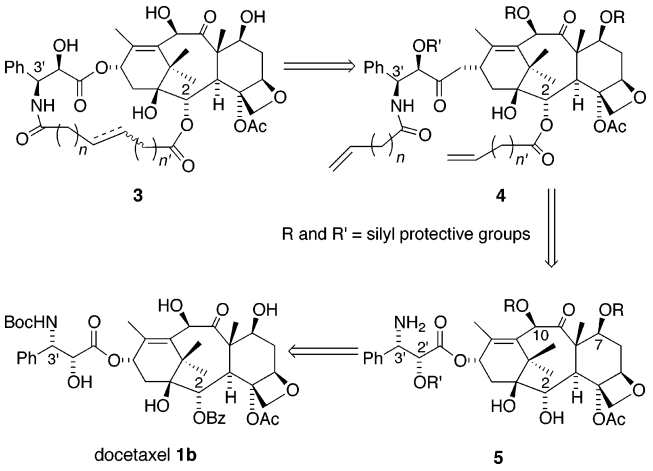
In an attempt to model the "nonpolar" conformation, we have designed macrocyclic taxoids with bridges linking the C-2 benzoyl phenyl group and the N-3′ acyl moiety. We have shown with 19- to 25-membered-ring thio- or dithiomacrocyclic taxoids **2** that the macrocyclic ring size had an influence on tubulin binding[14] (Figure 2). The 21-membered-ring sulfide **2a** and 20-membered-ring disulfide **2b** were the most active compounds though much less than docetaxel on microtubule disassembly and cytotoxicity. Suspecting the presence of sulfur to be deleterious to microtubule binding, we designed new C-2,C-3′ N-linked macrocyclic taxoids **3** with carbon tethers (Figure 2). With the 20- and 21-membered-ring taxoids **2a** and **2b** being the most active compounds in the sulfur-containing series, we decided to vary the macrocyclic ring size below and above these values in order to investigate its influence on biological activities. Therefore, we present, in this paper, the syntheses of compounds **3** bearing 18-, 20-, 21- and 22-membered rings together with the examination of their conformational behavior related to their biological activities.

Chemistry

In the design of the synthesis of compounds **3**, ring-closing metathesis (RCM)[15] methodology was chosen for the crucial macrocyclization step. This strategy has proven its efficacy for the synthesis of macrocyclic taxoids. First employed by Ojima and his collaborators for the synthesis of a large number of C-2,C-3′ linked macrocyclic taxoids,[13b,c] this methodology has been also used in preparing analogues with C-4,C-3′[13d] and C-2,C-3′N[13f] linkers. By use of the same strategy as for compounds **2**, macrocyclic taxoids **3** can be obtained by RCM on the open-chain ω,ω'-diene precursor **4**, which can be synthesized from the C-2 OH and C-3′ NH_2 taxoid **5**. This deacylated derivative **5** is easily obtained from docetaxel as previously described[14] (Scheme 1).

The introduction of the alkenyl chains on compound **5** was performed by acylation of C-2 OH and C-3′ NH_2 with commercially available alkenoic acids in the presence of DCC and DMAP (Scheme 2). For the 21-membered macrocyclic taxoid, acylation of the amine has been first realized with 2 equiv of 6-heptenoic acid.

Scheme 1. Retrosynthesis of Compounds **3**



Then, after intermediate purification leading to the N-acylated derivative (88% yield), esterification at C-2 has been achieved by addition of 5 equiv of 5-hexenoic acid together with DCC and DMAP. Compounds **4a**−**d** have been thus synthesized in good yields (60−79%) (Scheme 2). These diene precursors were subjected to RCM using Grubbs' catalyst in refluxing CH_2Cl_2 to afford the macrocyclic taxoids **6a**−**d** in 85−90% yield (Table 1).

All compounds were obtained as an *E*/*Z* mixture with a 0.5 to 4 ratio, determined by NMR, depending on the macrocycle formed. Because of the superposition of ethylenic ^1H signals with other protons, the configuration of the isolated compounds was determined either by nuclear Overhauser effect spectrometry (NOESY) or by comparison of the ^{13}C chemical shifts of the allylic carbons. The *Z*-isomer prevailed only for the 18-membered-ring compound **6a**, and the amount of the *E*-isomer increased with the ring size. Both isomers were found in similar amounts for **6b**, whereas the *E*-isomer was preferentially formed for 21- and 22-membered macrocyclic taxoids **6c** and **6d**. *Z* and *E* isomers were easily separated by silica gel chromatography for **6a** and **6b** to give **6a**-*Z*, **6a**-*E*, **6b**-*Z*, and **6b**-*E*. *E* and *Z* isomers were inseparable in **6c**, and only the *E*-isomer of **6d** has been obtained in pure form. Hydrogenation of macrocyclic taxoids **6a**−**d** on Pd/C afforded the corresponding macrocyclic taxoids **6(H)a**−**d** bearing a saturated linker in satisfactory yield (72−84%) (Scheme 2).

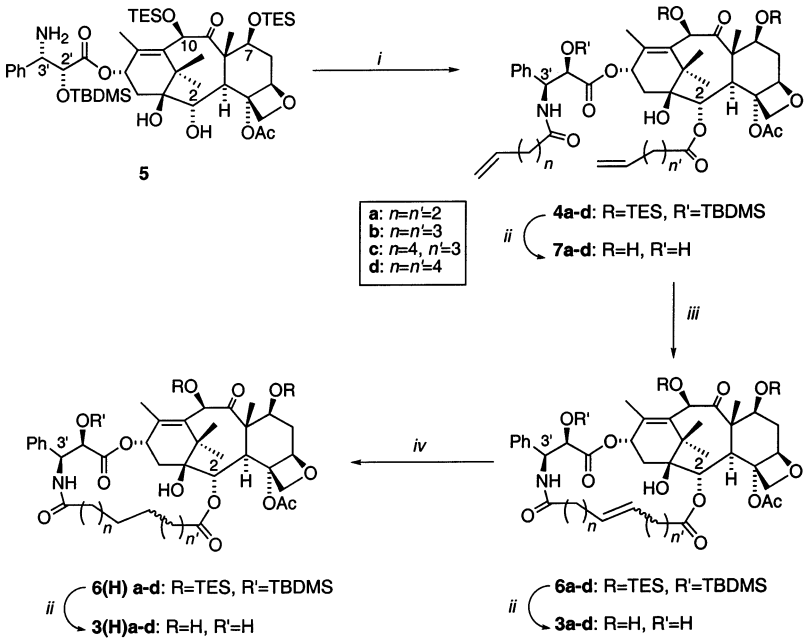
To compare the biological activities of the macrocyclic taxoids with their open-chain precursors, compounds **4a**−**d** were also subjected to hydrogenation on Pd/C to afford the corresponding saturated taxoids **4(H)a**−**d** in 70−80% yield (Scheme 3). All these compounds were fully deprotected by HF/pyridine with moderate to good yields, affording 19 compounds **3a**-*E* and -*Z*, **3b**-*E* and -*Z*, **3c**, **3d** and **3d**-*E*, **3(H)a**−**d**, **7a**−**d** (Scheme 2), and **7(H)a**−**d** (Scheme 3).

Results and Discussion

Biological Evaluation. The 19 new compounds were evaluated for their inhibition of cold-induced microtubule disassembly[16] and for their cytotoxicity against the KB cell line[17] (Table 2).

All compounds are less cytotoxic than docetaxel with cytotoxicity in agreement with tubulin activity, in

Scheme 2[a]



[a] (i) For **a**, **b**, **d**: DCC, DMAP, $CH_2{=}CH(CH_2)_nCOOH$ (5 equiv), toluene, 60 °C, 3 h (**5a**, 62%; **5b**, 60%; **5d**, 77%). For **c**: DCC, DMAP, $CH_2{=}CH(CH_2)_4COOH$ (2 equiv), toluene, room temp, 3 h, then DCC, DMAP, $CH_2{=}CH(CH_2)_3COOH$ (5 equiv), toluene, 60 °C, 3.5 h (**5c**, 79%). (ii) HF/pyridine, pyridine, CH_3CN, 0 °C, 1 h. then room temp, 6.5−8 h (**3a**-*Z*, 57%; **3a**-*E*, 51%; **3b**-*Z*, 69%; **3b**-*E*, 68%; **3c**, 70%; **3d**, 50%; **3d**-*E*, 61%; **3(H)a**, 45%; **3(H)b**, 55%; **3(H)c**, 64%; **3(H)d**, 56%; **7a**, 46%; **7b**, 71%; **7c**, 75%; **7d**, 75%). (iii) $(Cy_3P)_2Ru(=CHPh)Cl_2$ (6% mol), CH_2Cl_2, reflux, 4 h (**6a**, 89%; **6b**, 90%; **6c**, 90%; **6d**, 74%); (iv) H_2, Pd/C, EtOAc, 3.5 h (**6(H)a**, 84%; **6(H)b**, 75%; **6(H)c**, 76%; **6(H)d**, 72%).

Table 1. Ring-Closure Metathesis Results on Compounds **4a**−**d**

compd	*n*	*n*′	ring size	yield, %	*E*/*Z*
6a	2	2	18	89	35/65
6b	3	3	20	90	55/45
6c	4	3	21	90	80/20
6d	4	4	22	85	75/25

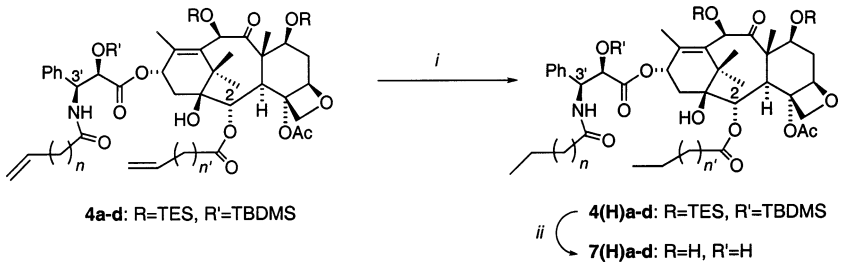
contrast to what has been observed with the sulfur-containing macrocyclic taxoids **2**.[14] The only compounds that are inactive on microtubule disassembly but still cytotoxic are the *E*-isomer of compound **3b** and its reduced analogue **3(H)b**. It is noted that the acyclic taxoids are in the same range of activity except for **7b**, which is the most cytotoxic (only 10 times less than docetaxel), and for **7(H)a**, which is the less active compound on KB cells. Compounds **7a**−**d** and **7(H)a**−**d** are the first docetaxel derivatives substituted both at C-2 and N-3′ by alkyl chains. They show similar activities on microtubule disassembly compared to taxoids bearing a hydrophobic group either at C-2 or at N-3′.[18,19]

The activities of macrocyclic compounds **3a**−**d** and **3(H)a**−**d** on microtubule disassembly are very interesting. Their interaction with microtubules seems to be dependent on the ring size because compounds bearing less than 21 atoms in the macrocycle are devoid of any activity. Only the 22-membered-ring compound **3d** is well recognized by tubulin in the unsaturated series with an activity 3 and 6 times higher than, respectively, the 20-membered-ring disulfide **2b** and the 21-membered-ring sulfide **2a**.[14] These results confirm that the presence of the sulfur atom is deleterious to microtubule binding. Inhibition of microtubule disassembly has been observed with the 21-membered-ring derivative **3c** at high concentration, but the activity was too low to enable any IC_{50} measurement. In the reduced series, only a weak microtubule disassembly inhibition was observed with **3(H)c** and **3(H)d**. The difference between **3d** and **3(H)c**,**d** may be due to the presence of the double bond in **3d** that either induces a more favorable conformation by additional constraint or promotes hydrophobic interaction in the tubulin binding site. It is noted that inhibition of microtubule disassembly seems to be independent of the stereochemistry of the double bond. Finally these results show that the presence of an alkenyl tether between C-2 and N-3′ does not prevent tubulin binding as shown by the close values obtained for **3d** and its acyclic analogue **7d**.

Conformational Studies. To investigate how these macrocyclic taxoids were conformationally restricted, we first studied their conformational behavior in solution. As mentioned in the Introduction, taxoids may exist in solution under different conformers. To clarify our discussion, we have taken into account the highest population conformations. Thus, the "nonpolar" form is the major conformation in chloroform characterized by a small H2′/H3′ coupling constant ($J_{H2'/H3'} < 2$ Hz), whereas taxoids adopt preferentially the "polar" conformation in DMSO indicated by a larger coupling constant ($J_{H2'/H3'} = 6-8$ Hz).[20]

In contrast to paclitaxel and docetaxel, the H2′/H3′ coupling constant was very similar in both polar and apolar solvents for sulfur-containing macrocyclic taxoids **2** bearing at the most a 23-membered ring ($J_{H2'/H3'} < 2$ Hz in $CDCl_3$ and $J_{H2'/H3'} = 2.5$ Hz in DMSO-d_6). The same coupling values were obtained for the 22-membered-ring taxoid **3d** in both solvents. NMR conformational analysis (NOESY and rotating-frame Overhauser enhancement spectroscopy (ROESY)) in polar and apolar solvents showed an intense nuclear Overhauser

Scheme 3[a]



[a] (i) H_2, Pd/C, EtOAc, 4 h (**4(H)a**, 80%; **4(H)b**, 78%; **4(H)c**, 70%; **4(H)d**, 74%). (ii) HF/pyridine, pyridine, CH_3CN, 0 °C, 1 h, then room temp, 6.5−8 h (**7(H)a**, 68%; **7(H)b**, 71%; **7(H)c**, 65%; **7(H)d**, 64%).

Table 2. Biological Activities of Macrocyclic Taxoids and of Their Acyclic Analogues

compd	*n*	*n′*	ring size	microtubule disassembly inhibitory activity IC_{50}/IC_{50}(paclitaxel) [a]	cytotoxicity against KB cell line IC_{50} [b] (μM)
3a-*Z*	2	2	18	inactive	>100
3a-*E*	2	2	18	inactive	>100
3b-*Z*	3	3	20	inactive	>100
3b-*E*	3	3	20	inactive	45
3c	4	3	21	>100[c]	14
3d-*E*	4	4	22	7.3	8
3d	4	4	22	7	14
3(H)a	2	2	18	inactive	>100
3(H)b	3	3	20	inactive	55
3(H)c	4	3	21	30	13
3(H)d	4	4	22	45	23
7a	2	2		5.1	1.6
7b	3	3		4.6	0.01
7c	4	3		2.4	0.65
7d	4	4		1.1	0.5
7(H)a	2	2		2.9	10
7(H)b	3	3		2.3	1
7(H)c	4	3		6.7	0.45
7(H)d	4	4		2.7	0.55

[a] IC_{50} is the concentration that inhibits 50% of the rate of microtubule disassembly. The ratio IC_{50}/IC_{50}(paclitaxel) gives the activity with respect to paclitaxel. IC_{50}(paclitaxel) = 1 μM. [b] IC_{50} measures the drug concentration required for the inhibition of 50% cell proliferation after 72 h of incubation. IC_{50}(docetaxel) = 0.001 μM. [c] **3c** showed a dose−response curve, but the 50% inhibition of microtubule disassembly was only achieved at high concentration (>100 μM).

effect (NOE) between 2′-H and 3′-H, characteristic of a gauche interaction between these two protons, and other NOE connectivity patterns were very similar to those observed with docetaxel in apolar solvents. NMR spectra of **3d** obtained at low temperature were identical to those obtained at room temperature. These NMR studies suggest that **3d** adopts mainly the "nonpolar" conformation, whatever the solvent polarity. Considering that **3d** possesses the larger ring size of the series, it is likely that the other macrocyclic derivatives **3a**−**c** are also sufficiently restricted to mimic a docetaxel-like solid-state conformation, especially for the C2′ and C3′ substituents.

To better characterize the conformational behavior of these macrocyclic taxoids, molecular dynamic modeling studies were performed on compounds **3a**−**d**. Though it has been described for paclitaxel that such calculations could lead to different energy ranking of side chain conformations according to the force field used,[21] we have always observed results in good agreement with experimental data for docetaxel (ref 10 and unpublished data). To minimize misinterpretation, two force fields (MMFF94 and Tripos) have been used for this study,

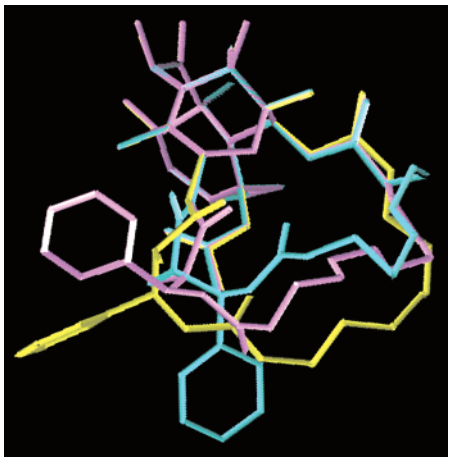


Figure 3. Superimposition of conformer A (cyan) found for **3c** and conformers B (yellow) and C (magenta) found for **3d**.

Table 3. Mole Fraction (MF) of the A, B, and C Conformations for Compounds **3a**−**d**

compd	ring size (atoms)	MF (%) A	B	C
3a	18	93	0	7
3b	20	87.5	0	12.5
3c	21	72.5	27.5	0
3d	22	0	37	63

leading to similar results. Furthermore, though the carbon chain of compounds **3a**−**d** between N3′ and O2′ possesses a relatively high degree of freedom, it generates sufficient constraints to restrict the orientation of the C2′ and C3′ substituents, which was the scope of our study. For each compound, conformational searching produced a great number of conformers and only the conformers within 3.5 kcal/mol of the lowest energy structure have been considered. These lower energy conformers were clustered into three families (A, B, and C) based on the orientation of the C-13 side chain functional groups (mainly the C-2′ OH and C-3′ Ph) (Figure 3).

The first two families A and B are very similar, the main difference being the orientation of the phenyl group. The C conformation has already been reported in the literature[8b] but does not resemble any of the proposed "polar", "nonpolar", or T-shaped forms, whereas the B conformer is very similar to the "nonpolar" conformation. The mole fraction (MF) of each conformer was calculated according to the Boltzmann distribution equation (Table 3).

This study shows that the major conformation of compounds **3a**−**d** changes from one derivative to an-

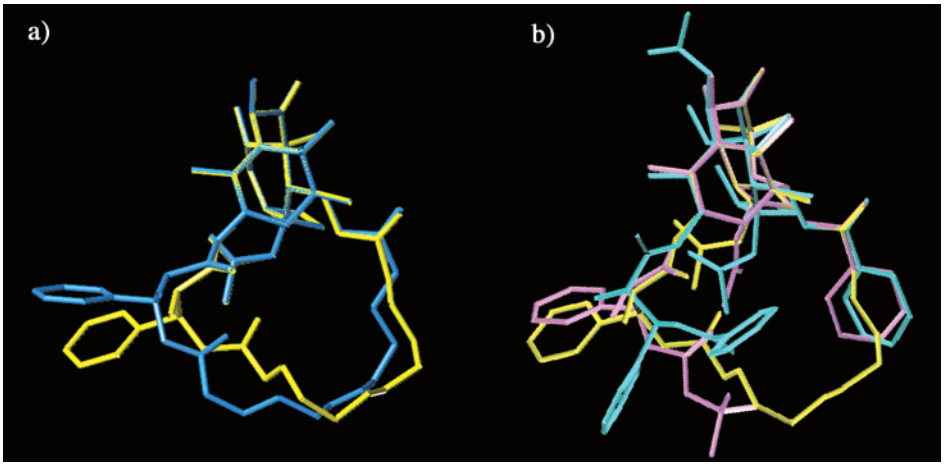


Figure 4. Superimposition the lowest energy conformer of compound **3d** obtained by simulated annealing (yellow, torsion angles T1 (O13−C1′−C2′−C3′) = 63.9°; T2 (H2′−C2′−C3′−H3′) = 78.2°) (a) with conformer B (blue, T1 = 63.3°, T2 = 85.2°); (b) with docetaxel in "nonpolar form" (magenta, T1 = 59.4°, T2 = 89.2°) and T-shaped (cyan, T1 = 55.7°, T2 = 55.4°).

other depending on the ring size and consequently on the ring rigidity. Since **3d** is the only compound showing significant tubulin activity, we can assume that the bioactive conformation of these taxoids would be like the B or C forms.

To have more information on the solution conformation of **3d**, this compound was then subjected to constraint simulated annealing using NMR data. After minimization, the lowest energy conformer was very similar to the B conformation (Figure 4a) and situated between the "nonpolar" and T-shaped docetaxel forms (Figure 4b).

Summary and Conclusions

Nineteen new taxoids have been synthesized including 11 18-membered- to 22-membered-ring macrocyclic compounds. As expected, replacement of the sulfur atom on the tether by a double bond greatly increased microtubule interaction. Though the reduced macrocyclic taxoids **3(H)c** and **3(H)d** (21- and 22-membered ring, respectively) exhibit weak activity on microtubule disassembly, the unsaturated 22-membered-ring derivative **3d** is the only compound showing a real inhibition. The small difference between the IC_{50} of **3d** and its acyclic analogue **7d** proves that the carbon tether between C-2 and N-3′ does not hamper tubulin binding. Examination of the conformation of the unsaturated derivatives reveals that the macrocyclic taxoids **3a**−**c** adopt mainly a conformation that is not recognized by tubulin. Molecular modeling studies have shown that compound **3d** can adopt two conformations B and C, the former being very similar to the "nonpolar" conformation. In solution, this macrocyclic taxoid seems to adopt a conformation situated between the "nonpolar" and T-shaped forms. It is noted that these two conformations are not so different, the main discrepancy being the position of the C-3′ phenyl group with respect to the median plane of the taxane core. All these results suggest that the bioactive conformation of taxoids interacting with microtubules is either the "nonpolar" or the T-shaped form or another conformation between these two forms. Other analogues bearing a more restricted conformation must be designed to definitively discriminate between these two forms.

Experimental Section

General. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 300 or AV 300 spectrometer. NOESY and ROESY data were obtained from a Bruker AMX 400 spectrometer. Chemical shifts are given as δ values and are referenced to the residual solvent proton or carbon peak, i.e., chloroform (C^1HCl_3 = 7.27 ppm and $^{13}CHCl_3$ = 77.14 ppm). The spectra were fully assigned using correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) on a Bruker AV 300. For compounds **3a**−**d**, **3(H)a**−**d**, **4a**−**d**, **6a**−**d**, **7a**−**d**, and **7(H)a**−**d**, all NMR spectra were very similar; the only modifications were the added acyl chains. So, only the first compound of each series will be fully described and the characterization of the following ones can be found in the Supporting Information. Mass spectra were obtained on an AQA Navigator ThermoQuest. High-resolution mass spectra were obtained from a Voyager-DE STR (PerSeptive Biosystems) with gentisic acid as matrix and paclitaxel and docetaxel as internal standards. Merck silica gel 60 (230−400 mesh) was used for the flash chromatography purification of some compounds. All chemicals were purchased from Fluka, Aldrich, or Acros and were used without further purification unless indicated otherwise. Solvents were purchased from SDS. Toluene and THF were dried and distilled before use. Standard workup means extraction with a suitable solvent (EtOAc unless otherwise specified), washing the extract with H_2O or brine, drying over Na_2SO_4, and evaporation under reduced pressure. Docetaxel **1b** was a gift from Alain Commerçon (Aventis-Pharma), and compound **5** has been prepared according to the previously described procedure.[14] Microtubular proteins were purified from mammalian brain as previously described.[22] Cytotoxicity and microtubule disassembly inhibition were carried out according to established literature protocols.[16,17]

Synthesis of 2-Debenzoyl-2-alkenoyl-2′-*O*-*tert*-butyldimethylsilyl-3′-de-*tert*-butoxycarbonyl-3′-alkenoyl-7,10-ditriethylsilyldocetaxel (4a−d). General Procedure for compounds 4a,b,d. A typical procedure is described for the synthesis of 2-debenzoyl-2-pent-4-enoyl-2′-*O*-*tert*-butyldimethylsilyl-3′-de-*tert*-butoxycarbonyl-3′-pentenoyl-7,10-ditriethylsilyldocetaxel (**4a**). Pent-4-enoic acid (81 μL, 0.8 mmol, 5 equiv) was added dropwise to a toluene (1 mL) solution of **5** (150 mg, 0.16 mmol) and DCC (163 mg, 0.8 mmol, 5 equiv) and 4-DMAP (97 mg, 0.8 mmol, 5 equiv). The reaction mixture was stirred at 60 °C for 3 h and then filtered through a Celite/silica gel (1/1) column in EtOAc/heptane (1/1) and concentrated in vacuo. After standard workup, the residue was purified by flash chromatography (CH_2Cl_2/acetone, 98/2) to afford pure **4a** (110 mg, 62%) as a white amorphous solid. ^1H NMR (300 MHz, $CDCl_3$): δ −0.30 (s, 3H), −0.11 (s, 3H), 0.52−0.73 (m, 12H),

0.78 (s, 9H), 0.93−1.0 (m, 18H), 1.16 (s, 3H), 1.20 (s, 3H), 1.52 (s, 3H), 1.81 (s, 3H), 1.88 (m, 1H), 2.05−2.23 (m, 2H), 2.31−2.69 (m, 12H), 3.7 (d, $^3J_{H,H}$ = 7.0 Hz), 4.20 and 4.46 (qAB, $^2J_{H,H}$ = 8.0 Hz, 2H), 4.35 (dd, $^3J_{H,H}$ = 6.5 Hz and $^3J'_{H,H}$ = 10.5 Hz), 4.49 (br s, 1H), 4.93 (d, $^3J_{H,H}$ = 9.0 Hz, 1H, C-5*H*), 4.99−5.08 (m, 4H), 5.12 (s, 1H), 5.44 (d, $^3J_{H,H}$ = 7.0 Hz, 1H), 5.47 (br d, $^3J_{H,H}$ = 9.0 Hz, 1H), 5.75−5.92 (m, 2H), 6.15 (t, $^3J_{H,H}$ = 9.0 Hz, 1 H), 6.44 (d, $^3J_{H,H}$ = 9.0 Hz, 1H), 7.17−7.41 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.7, −5.3, 5.4, 6.1, 6.9, 7.0, 10.5, 13.8, 18.3, 20.7, 23.3, 25.6, 26.6, 28.5, 29.4, 34.1, 35.4, 35.6, 37.4, 43.4, 46.6, 55.3, 58.5, 72.0, 72.7, 74.8, 75.2, 75.5, 76.9, 78.7, 81.1, 84.3, 115.8, 116.0, 126.6, 127.9, 128.6, 134.3, 136.7, 137.0, 138.0, 138.6, 170.1, 171.7, 171.9, 174.0, 205.4. MS (ESI$^+$), *m*/*z*: 1132 [M + Na$^+$].

For **4b** and **4d**, see the Supporting Information.

Synthesis of 2-Debenzoyl-2-hex-5-enoyl-2′-*O*-*tert*-butyldimethylsilyl-3′-de-*tert*-butoxycarbonyl-3′-hept-6-enoyl-7,10-ditriethylsilyldocetaxel (4c). Hept-6-enoic acid (111 μL, 0.82 mmol, 2 equiv) was added to a solution of **5** (390 mg, 0.41 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (158.2 mg, 0.82 mmol, 2 equiv), and DMAP (152.3 mg, 1.25 mmol, 3 equiv) in CH_2Cl_2 (3.9 mL). The reaction mixture was stirred at room temperature for 3 h, and the reaction mixture was concentrated in vacuo. After standard workup, the residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95/5) to afford pure 2-debenzoyl-2′-*O*-*tert*-butyldimethylsilyl-3′-de-*tert*-butoxycarbonyl-3′-hept-6-enoyl-7,10-ditriethylsilyldocetaxel (335.5 mg, 77%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.28 (s, 3H), −0.07 (s, 3H), 0.48−0.70 (m, 12H), 0.77 (s, 9H), 0.93−1.01 (m, 18H), 1.07 (s, 3H), 1.20 (s, 3H), 1.41 (m, 2H), 1.53 (s, 3H), 1.60 (m, 2H), 1.78 (s, 3H), 1.93 (m, 1H), 1.99−2.16 (m, 4H), 2.26 (m, 2H), 2.36 (s, 3H), 2.49 (m, 1H), 3.49 (d, $^3J_{H,H}$ = 6.0 Hz, 1H), 3.69 (d, $^3J_{H,H}$ = 11.3 Hz, 1H), 3.95 (dd, $^3J_{H,H}$ = 11.3 Hz and $^3J_{H,H}$ = 6.0 Hz, 1H), 4.34 (dd, $^3J_{H,H}$ = 10.4 Hz and $^3J_{H,H}$ = 6.5 Hz, 1H), 4.49 (br s, 1H), 4.66 and 4.73 (qAB, $^3J_{H,H}$ = 9.0 Hz, 2H), 4.91−5.07 (m, 4H), 5.56 (br d, $^3J_{H,H}$ = 9.6 Hz, 1H), 5.66−5.92 (m, 1H), 6.18 (t, $^3J_{H,H}$ = 8.8 Hz, 1 H), 6.40 (d, $^3J_{H,H}$ = 9.6 Hz, 1H), 7.06−7.37 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.8, −5.2, 5.4, 6.1, 7.0, 7.1, 10.8, 13.7, 21.0, 23.4, 25.4, 25.6, 26.6, 28.4, 33.4, 35.7, 36.7, 37.6, 43.2, 47.1, 54.7, 58.3, 72.4, 72.9, 74.1, 75.1, 75.1, 78.1, 78.7, 82.6, 84.0, 115.0, 126.5, 128.0, 128.7, 133.6, 138.1, 138.4, 138.5, 170.1, 171.6, 173.0, 206.3. MS (ESI$^+$), *m*/*z*: 1078 [M + Na$^+$].

Hex-5-enoic acid (140 μL, 1.17 mmol, 5 equiv) was added to a toluene (2.25 mL) solution of 2-debenzoyl-2′-*O*-*tert*-butyldimethylsilyl-3′-de-*tert*-butoxycarbonyl-3′-hept-6-enoyl-7,10-ditriethylsilyldocetaxel (247 mg, 0.23 mmol), DCC (241 mg, 1,17 mmol, 5 equiv), and DMAP (142 mg, 1.16 mmol, 5 equiv). The reaction mixture was stirred at 60 °C for 3.5 h and then filtered through a Celite/silica gel (1/1) column in EtOAc/heptane (1/1) and concentrated in vacuo. After standard workup, the residue was purified by flash chromatography (CH_2Cl_2/acetone, 98/2) to afford pure **4c** (209 mg, 79%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.29 (s, 3H), −0.11 (s, 3H), 0.50−0.71 (m, 12H), 0.79 (s, 9H), 0.88−1.06 (m, 18H), 1.16 (s, 3H), 1.19 (s, 3H), 1.43 (m, 2H), 1.62 (s, 3H), 1.67 (m, 2H), 1.76 (m, 2H), 1.80 (s, 3H), 1.88 (m, 1H), 1.96−2.21 (m, 6H), 2.24−2.35 (m, 3H), 2.40 (m, 1H), 2.41 (s, 3H), 2.52 (m, 1H), 3.72 (d, $^3J_{H,H}$ = 7.0 Hz, 1H), 4.19 and 4.45 (qAB, $^3J_{H,H}$ = 8.0 Hz, 2H), 4.34 (dd, $^3J_{H,H}$ = 10.5 Hz and $^3J_{H,H}$ = 6.5 Hz, 1H), 4.49 (bs, 1H), 4.92−5.09 (m, 5H), 5.12 (s, 1H), 5.42 (d, $^3J_{H,H}$ = 7.0 Hz, 5.45 (br d, $^3J_{H,H}$ = 9.0 Hz, 1H), 5.70−5.87 (m, 2H), 6.14 (t, $^3J_{H,H}$ = 8.5 Hz, 1 H), 6.37 (d, $^3J_{H,H}$ = 9.0 Hz, 1H), 7.16−7.41 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.7, −5.3, 5.4, 6.1, 7.0, 7.1, 10.5, 13.8, 18.3, 20.7, 23.3, 23.8, 25.2, 25.6, 26.6, 28.4, 33.0, 33.5, 34.8, 35.3, 36.5, 37.4, 43.4, 46.6, 55.3, 58.5, 72.1, 72.7, 74.6, 75.2, 75.5, 76.9, 78.7, 81.1, 84.3, 114.9, 115.6, 126.6, 127.9, 128.7, 134.3, 137.8, 138.0, 138.5, 138.7, 170.1, 171.7, 172.6, 174.7, 205.4. MS (ESI$^+$), *m*/*z*: 1174 [M + Na$^+$].

General Procedure for the Synthesis of Macrocyclic Taxoids 6 by Ring-Closure Metathesis. A typical procedure is described for the synthesis of macrocyclic taxoid **6a**. To a solution of taxoid **4a** (52.7 mg, 0.047 mmol) in CH_2Cl_2 (4 mL) was added dropwise a solution of bis(tricyclohexylphosphine)-benzylideneruthenium(IV) dichloride (2.3 mg, 0.0028 mmol) in CH_2Cl_2 (1.3 mL). The solution was refluxed for 4 h and concentrated in vacuo. After standard workup, the residue was purified by silica gel chromatography (EtOAc/heptane, 50/50) to afford pure **6a**-*Z* (30 mg, 58%) and **6a**-*E* (16.3 mg, 31%) as white amorphous solids.

6a-*Z*. ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.33 (s, 3H), −0.11 (s, 3H), 0.54−0.78 (m, 12H), 0.71 (s, 9H), 0.93−1.07 (m, 18H), 1.13 (s, 3H), 1.25 (s, 3H), 1.64 (s, 3H), 1.89 (s, 3H), 1.89−2.14 (m, 5H), 2.26−2.59 (m, 4H), 2.50 (s, 3H), 2.59−2.74 (m, 3H), 3.76 (d, $^3J_{H,H}$ = 7.8 Hz, 1H), 4.16 and 4.39 (qAB, $^3J_{H,H}$ = 8.0 Hz, 2H), 4.44 (dd, $^3J_{H,H}$ = 10.0 Hz and $^3J_{H,H}$ = 6.3 Hz, 1H), 4.52 (br s, 1H), 4.86 (d, $^3J_{H,H}$ = 8.0 Hz, 1H), 5.11 (s, 1H), 5.24−5.42 (m, 2H), 5.44−5.53 (m, 2H), 6.21−6.36 (m, 2H), 7.16−7.40 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ −6.0, −5.3, 5.5, 6.1, 7.1, 10.0, 13.7, 21.6, 22.6, 22.7, 23.2, 25.5, 26.7, 34.7, 35.5, 35.8, 37.1, 43.3, 47.0, 55.3, 58.1, 70.9, 72.5, 74.9, 75.4, 77.6, 80.7, 84.6, 126.5, 127.6, 128.5, 129.1, 130.1, 133.7, 137.5, 138.4, 170.9, 171.4, 173.9, 205.1. MS (ESI$^+$), *m*/*z*: 1104 [M + Na$^+$].

In the same manner, **6b**−**d** were synthesized (see the Supporting Information).

General Procedure for the Reduction of the Double Bond(s). A typical procedure is described for the synthesis of macrocyclic taxoid **6(H)a**. A solution of **6a**-*Z* (32.5 mg, 0.03 mmol) in EtOAc (3.1 mL) was added to 10% palladium on carbon (32.5 mg) under a hydrogen atmosphere, and the reaction mixture was stirred for 3.5 h at room temperature. The reaction mixture was then filtered through Celite and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/heptane, 40/60) to afford pure **6(H)a** (27.2 mg, 84%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.33 (s, 3H), −0.14 (s, 3H), 0.54−0.81 (m, 12H), 0.72 (s, 9H), 0.93−1.08 (m, 18H), 1.15 (s, 3H), 1.27 (s, 3H), 1.22−1.43 (m, 4H), 1.47−1.69 (m, 2H), 1.60 (s, 3H), 1.76 (m, 1H), 1.89 (m, 1H), 1.92 (s, 3H), 2.00 (m, 1H), 2.16 (m, 1H), 2.22−2.37 (m, 3H), 2.43−2.58 (m, 2H), 2.47 (s, 3H), 3.77 (d, $^3J_{H,H}$ = 7.5 Hz, 1H), 4.13 (qAB, $^3J_{H,H}$ = 8.0 Hz, 2H), 4.39−4.50 (m, 2H), 4.51 (d, $^3J_{H,H}$ = 2.5 Hz, 1H), 4.88 (d, $^3J_{H,H}$ = 8.0 Hz, 1H), 5.12 (s, 1H), 5.32−5.40 (m, 2H), 6.27 (t, $^3J_{H,H}$ = 8.5 Hz, 1H), 6.36 (d, $^3J_{H,H}$ = 8.0 Hz, 1H), 7.18−7.40 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ −6.0, −5.5, 5.5, 6.0, 7.0, 7.1, 10.9, 13.7, 18.2, 21.6, 23.5, 24.1, 25.3, 25.5, 26.8, 27.0, 28.1, 34.1, 36.1, 36.3, 37.2, 43.5, 47.0, 53,5, 58.1, 71.4, 72.5, 74.8, 75.0, 76.7, 80.3, 80.8, 84.5, 126.8, 127.7, 128.5, 133.7, 137.6, 138.3, 171.2, 172.7, 175.0, 205.1. MS (ESI$^+$), *m*/*z*: 1106 [M + Na$^+$].

Compounds **6(H)b**−**d** and **4(H)a**−**d** were prepared according to this method. For experimental details and spectral data, see the Supporting Information.

General Procedure for Removal of the Silyl Protecting Groups. A typical procedure is described for the synthesis of macrocyclic taxoid **3a**-*Z*. To a cooled solution of **6a**-*Z* (38.5 mg, 0.035 mmol) in pyridine/acetonitrile (0.08/1, 1 mL) was added dropwise HF/pyridine (70%, 170 μL, 1.4 mmol, 40 equiv) at 0 °C. The solution was stirred for 1 h at 0 °C and then warmed to room temperature and stirred for 6.5 h more at room temperature. The reaction was quenched by addition of saturated aqueous sodium hydrogenocarbonate (25 mL), and the aqueous layer was extracted three times with EtOAc (50 mL). After standard workup, the crude residue was purified by silica gel chromatography (CH_2Cl_2/MeOH, 93/7) to afford pure **3a**-*Z* (14.7 mg, 57%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05 (s, 3H), 1.29 (s, 3H), 1.71 (s, 3H), 1.86 (m, 1H), 1.94 (s, 3H), 2.11 (m, 2H), 2.17−2.88 (m, 8H), 2.51 (s, 3H), 3.82 (d, $^3J_{H,H}$ = 7.7 Hz, 1H), 4.19 and 4.46 (qAB, $^3J_{H,H}$ = 8.1 Hz, 2H), 4.29 (dd, $^3J_{H,H}$ = 11.3 Hz and $^3J_{H,H}$ = 6.5 Hz, 1H), 4.71 (br s, 1H), 4.92 (d, $^3J_{H,H}$ = 9.5 Hz, 1H), 5.14 (s, 1H), 5.31 (m, 1H), 5.40 (d, $^3J_{H,H}$ = 7.7 Hz, 1H), 5.48 (m, 1H), 5.58 (br d, $^3J_{H,H}$ = 9.4 Hz, 1H), 6.23 (d, $^3J_{H,H}$ = 9.4 Hz, 1H), 6.44 (t, $^3J_{H,H}$ = 9.3 Hz, 1H), 7.27−7.44 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.6, 14.0, 22.0, 22.5, 22.6, 22.8, 26.7, 34.6, 35.5, 36.1, 36.6, 43.1, 46.4, 55.8, 57.4, 71.6, 72.0, 72.8, 74.9, 75.2, 76.5, 80.5, 80.6, 84.7, 126.6, 128.0, 128.8, 129.4, 129.8,

135.5, 138.2, 138.4, 171.3, 171.7, 172.9, 174.0, 211.6. HRMS (MALDI-TOF), *m/z*: calcd for $C_{39}H_{49}NO_{13}{\cdot}Na^+$, 762.3102; found, 762.3087 (Δ = 2.0 ppm).

In the same manner macrocylic taxoids **3b**−**d** and **3(H)a**−**d** were synthesized as the acyclic taxoids **7a**−**d** and **7(H)a**−**d**. For experimental details and spectral data, see the Supporting Information.

Computational Procedures. All calculations were performed on a SiliconGraphics Indigo2 Extreme workstation. All modeling was done using Sybyl 6.8 software. The MMFF94 and Tripos force fields were used for minimization and partial charge calculations, a dielectric constant of 1.0 being employed.[22] In all cases, the A, B, C, and D rings of the taxoids were considered as an aggregate. Compounds **3a**−**d**-*E* were subjected to an unrestrained molecular dynamics simulation at 1600 K for 20 000 fs. Conformations were sampled every 100 fs during the simulation, resulting in 200 randomized structures. Each of these conformers was minimized and compared with others with a rms of 0.3 Å. The obtained structures were then ranked according to energy, and the conformers within 3.5 kcal/mol of the lowest energy structure were retained for further analysis. The structures were analyzed using Sybyl 6.8 software, and superimposition of conformers was based on the backbone atoms of the taxane core.

Compound **3d**-*E* was subjected to 10 cycles of constraint simulated annealing. The taxane skeleton was considered as an aggregate, and the constraints were derived from NOE data between H2′ and H3′ and between these protons and the taxane core. The annealing protocol consisted of an initial equilibration step during which the temperature was gradually increased to 1000 K, followed by a production step of 1000 fs and by gradual cooling to 100 K over a 1000 fs period. After minimization using the previously described protocol, the 10 obtained conformer families were ranked by energy.

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Supporting Information Available: Experimental details and characterization data for new compounds **4b**, **4d**, **6b**−**d**, **6(H)b**−**d**, **4(H)a**−**d**, **3b**−**d**, **3(H)a**−**d**, **7a**−**d**, and **7(H)a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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