# Synthesis of C2−C3′N-Linked Macrocyclic Taxoids. Novel Docetaxel Analogues with High Tubulin Activity

Olivier Querolle, Joëlle Dubois,* Sylviane Thoret, Fanny Roussi, Françoise Guéritte, and Daniel Guénard

*Institut de Chimie des Substances Naturelles, CNRS, 91190 Gif sur Yvette, France*

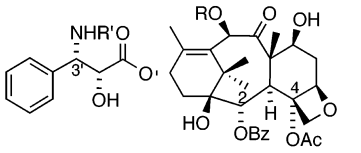
*Received March 11, 2004*

Novel C2−C3′N-linked macrocyclic taxoids **4** bearing an aromatic ring at position C2 were synthesized. These compounds, tethered between N3′ and the C2-aromatic ring at the ortho, meta, or para position, were constructed by ring-closing metathesis. The para-substituted derivatives were unable to stabilize microtubules, whereas the ortho- and meta-substituted compounds show significant activity in cold-induced microtubule disassembly assay. The meta derivative **4c** is the first C2−C3′-linked cyclic analogue to be equipotent to paclitaxel in this assay and to show significant cytotoxicity. Computational studies of the conformational behavior of these compounds indicate that they can adopt several conformations including mainly the "T-shaped" forms. Docking experiments have shown that the "T-shaped" form is preferred for a good interaction of these compounds with the $\beta$-tubulin binding pocket.

## Introduction

The anticancer drugs paclitaxel **1a** (Taxol)[1] and docetaxel **1b** (Taxotere),[2] and their congeners,[3] stabilize microtubular proteins[4] by binding to a specific site on $\beta$-tubulin.[5,6] During the past 10 years, extensive studies have been devoted to the elucidation of the bioactive conformation of the taxoids, i.e. the conformation they adopt when they are bound to microtubules.

Conformational analyses, based on X-ray structures of paclitaxel and docetaxel, NMR, and molecular modeling studies, have led to the proposal of two major conformations for taxoids in which there is a hydrophobic collapse between the C2-benzoyl group and the C3′ substituents. The "polar" form, exhibiting hydrophobic interactions between the C3′-phenyl group and the C2 benzoate, has been identified in the crystal structure of paclitaxel[7] and by NMR in polar solvents.[8] On the other hand, the "nonpolar" conformation where the N3′ substituent is close to the C2 benzoate, has been observed in the docetaxel solid state[9] and by NMR in apolar solvents.[10] More recently, a third conformation has been calculated using the electron crystallographic density of tubulin $\beta$-sheets[11] in conjunction with an analysis of paclitaxel conformations.[12] Contrary to the former ones, this so-called "T-shaped" structure is devoid of intramolecular hydrophobic interactions. A few taxoids with built-in conformational restrictions have been designed to mimic the presumed bioactive conformations. Macrocyclic taxoids covalently linked between the C2-benzoyl and the C3′-phenyl groups were designed to mimic the "polar" form but few of them retain tubulin assembly activity, and those that do have a lower activity than that of paclitaxel.[13] On the other hand, the first compounds designed to mimic the T-shaped form bearing either a constrained C-13 side chain[14] or a tether between the C3′-phenyl and C4-acetyl groups[15] have shown cytotoxicity and tubulin interaction. However, these compounds were not sufficiently constrained




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

**Figure 1.**

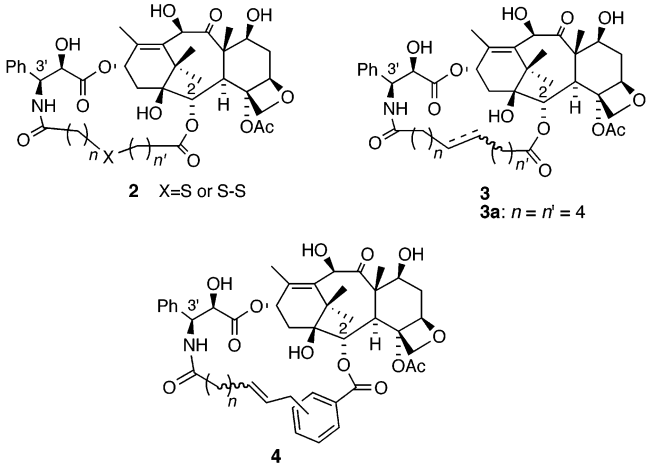



**Figure 2.**

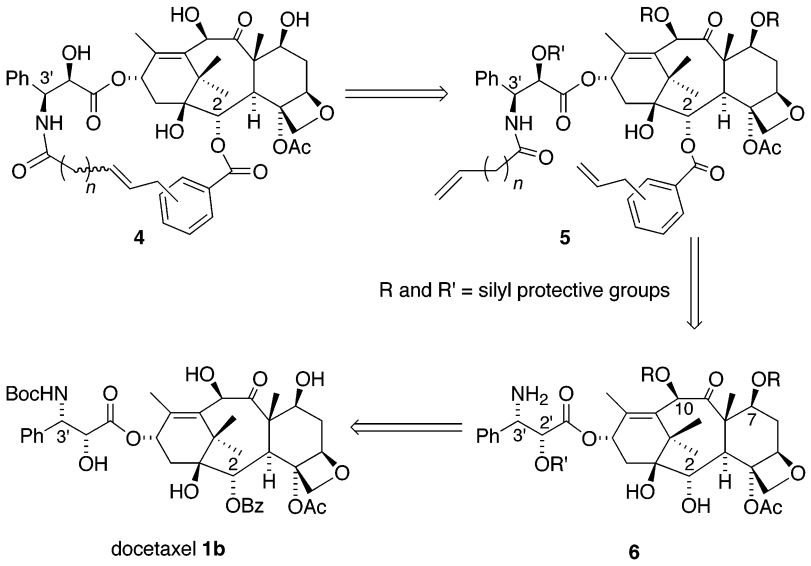
to allow a definite conclusion. Finally, to mimic the "nonpolar" form, C2−C3′N-tethered macrocyclic taxoids have been designed by our team[16] and by Ojima's group.[17] This group has synthesized compounds with significant cytotoxicity but has reported neither their tubulin activity nor their conformational behavior. Therefore, no conclusion on the bioactive conformation could be drawn from these results.

During the submission of this manuscript, more constrained C3′Ph−C4-linked macrocyclic taxoids that actually mimic the T-shaped form have been described to possess high cytotoxicity and tubulin interaction.[18] The authors conclude that the T-shaped form is therefore the bioactive conformation of taxoids. The results

* To whom correspondence should be addressed. Phone: 33 (0)1 69 82 30 58. Fax: 33 (0)1 69 07 72 47. E-mail: joelle.dubois@icsn.cnrs-gif.fr.

10.1021/jm0497996 CCC: $27.50 
Published on Web 10/22/2004

**Scheme 1.** Retrosynthesis of Compounds **4**




presented in this paper on C2−C3′N-linked macrocyclic taxoids corroborate this conclusion.

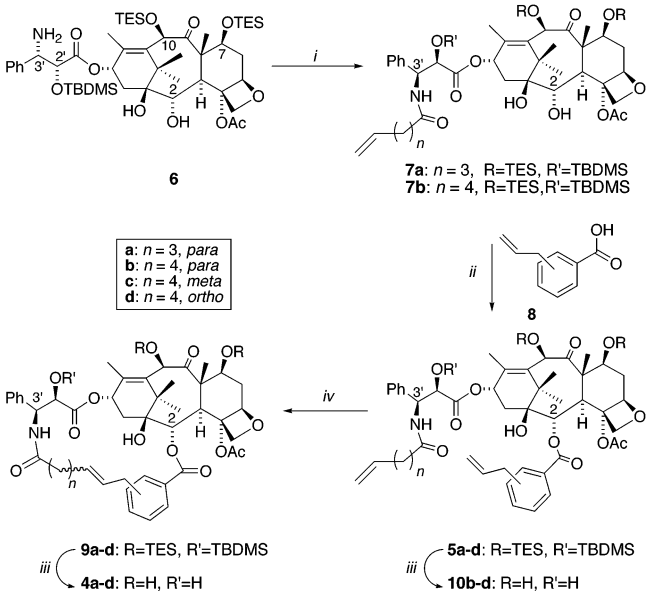
In the field of "nonpolar" form mimics, we have synthesized two series of macrocyclic taxoids **2**,[16a] **3**[16b] with C2−C3′N tether (Figure 2). We have found that the macrocycle ring size is crucial for tubulin activity as well as the functionalities on the linker. The 22-membered ring taxoid **3a**, bearing a C−C double bond on the tether, was found to be only 7-fold less active than paclitaxel on microtubule disassembly assay and moderately cytotoxic. Molecular modeling studies have shown that this compound adopts a conformation situated between the "nonpolar" and T-shaped forms. To get more insight into the bioactive conformation, it was important to increase tubulin interaction in our series of macrocyclic taxoids. We have therefore designed new compounds that resemble docetaxel more closely by adding an aromatic ring at the C2 position.

We present, in this paper, the synthesis and biological evaluation of macrocyclic taxoids **4** bearing a 21−23 membered ring in which the N3′ position is linked to the ortho-, meta-, or para-position of the C2-aromatic ring (Figure 2).

**Chemistry.** The synthesis of compounds **4** has been realized by using the same strategy as for compounds **3** (Scheme 1). The macrocyclic ring was formed by ring-closing metathesis on the open chain precursor **5** bearing a C2-allyl benzoate and a C3′N $\omega$−alkenyl chain which can be obtained from the C2-OH and C3′-$NH_2$ taxoid **6**. This derivative is easily obtained from docetaxel as previously described.[16a]

For this series of compounds **4**, acylation of the C-3′ amine of **6** was first carried out with 2 equiv of 5-hexenoic or 6-heptenoic acid in the presence of EDCI and DMAP leading to **7a** or **7b**[16b] in good yields (Scheme 2). The subsequent step of the synthesis required the *o*-, *m*-, and *p*-allylbenzoic acids **8** (***o,m,p***) that are not commercially available. A few methods have been described in the literature to synthesize allylbenzoates from aryl halides and generally involve a metal in the reaction such as Ni,[19] Zn,[20] Pd,[21] Co,[22] Mg,[23] and Mg with copper catalysis.[24] The three allybenzoic acids were synthesized in 42−59% overall yield according to the

**Scheme 2.** Synthesis of Compounds **4**[a]




[a] (*i*) EDCI, DMAP, $CH_2{=}CH(CH_2)_nCOOH$ (2 equiv), $CH_2Cl_2$, rt, 3 h (**7a**, 79%; **7b**, 77%). (*ii*) **8** (30 equiv), DCC (30 equiv), DMAP (1 equiv), toluene, 60 °C, 24 h (**5a**, 54%; **5b**, 32%, **5c**, 62%; **5d**, 72%). (*iii*) HF/pyridine, pyridine, $CH_3CN$, 0 °C, 1 h then room temp, 6 h (**4a**, 46%; **4b**, 50%, **4c**, 75%; **4d**, 43%; **10b**, 89%, **10c**, 72%; **10d**, 65%). (*iv*) $(Cy_3P)_2Ru(=CHPh)Cl_2$ (6% mol), $CH_2Cl_2$, reflux, 3 h (**9a**, 60%; **9b**, 77%, **9c**, 60%; **9d**, 64%).

Knochel procedure[24] starting from the commercially available iodobenzoic acids.

Contrary to our previous results obtained in the esterification of the C2 hydroxyl group with alkyl acids,[16] the same reaction with allylbenzoic acids **8** proved to be very difficult and a large excess of acid and DCC was needed to achieve acylation. Such conditions have already been used by Kingston et al. to realize C2-OH esterification.[25]

The major drawback of the reaction with DCC is a significant side reaction that yields, after formation of the intermediate *O*-acylisourea, the *N*-acylurea via O to N acyl migration.[26] Modifications of the experimental conditions (coupling reagent, solvent, activation of the acid) did not improve the yield in the desired compound,

**Table 1.** Ring-Closure Metathesis Results on Compounds **9a**−**d**

| compd | n | ring size | yield, % | *E*/*Z* |
|---|---|---|---|---|
| **9a** | 3 | 22 | 60 | 80/20 |
| **9b** | 4 | 23 | 77 | 90/10 |
| **9c** | 4 | 22 | 60 | 65/35 |
| **9d** | 4 | 21 | 64 | 85/15 |

and the only way to achieve acceptable yields was to increase the amount of reagents.

Compounds **5a**−**d** were then subjected to ring-closing metathesis[27] using Grubb's catalyst as previously described (Scheme 2).[16b] Macrocyclic taxoids **9a**−**d** were obtained in 60−77% yield as an *E*/*Z* mixture with a 1.8 to 9 ratio, determined by $^1$H NMR (Table 1).

The *E*-isomer was predominantly formed as previously observed in the synthesis of 21- and 22-membered macrocycles.[16b] The *E*- and *Z*-isomers were inseparable, and their configurations were determined by $^{13}$C NMR after examination of the benzylic carbon chemical shift, that of the *E*-isomer being about 7 ppm downfield of the *Z*-isomer's. Full deprotection of **9a**−**d** by HF/pyridine afforded compounds **4a**−**d** in moderate yields. Only the *Z*- and *E*-isomers of the meta derivative **4c** were separated by chromatography. To compare the biological activities of the macrocyclic taxoids with their open-chain precursors, compounds **5b**−**d** were also subjected to HF/pyridine deprotection to afford **10b**−**d** in good yields (Scheme 2).

## Results and Discussion

**Biological Evaluation.** Macrocyclic taxoids **4a**−**d** and their acyclic analogues **10b**−**d** were evaluated for their inhibition of cold-induced microtubule disassembly[28] and for their cytotoxicity against the KB cell line[29] (Table 2). Though less cytotoxic than docetaxel, these novel macrocyclic taxoids **4a**−**d** show a significant activity against KB cell line with a marked increased cytotoxicity compared to our former aliphatic series **3** (from 10 to 100-fold). Thus, addition of an aromatic ring at the C2-position is beneficial to activity. This effect is much more pronounced in the acyclic series where compounds **10b**−**d** are at least 10-fold more cytotoxic than their macrocyclic analogues. It is to be noted that the ortho-substituted compound **10d** is only one log less potent than docetaxel on KB cell line and more active than the meta derivative **10c**, contrary to what have been previously reported for other ortho-substituted benzoyl analogues.[30]

The cytotoxicity of compounds **4c(*E*)**, **4c(*Z*)**, **4d**, and **10d** was also evaluated on two other cancer cell lines (HCT 116 and MCF7) as well as that of compound **3a-(*E*)** (Figure 2), the most active derivative of our previous series[16b] (Table 2). The results clearly show the importance of the C2-benzoyl group for cytotoxicity since compound **3a(*E*)** is poorly active, whereas compounds **4** and **10** exhibit significant $IC_{50}$ values. Compound **10d** is again the most active derivative of this series showing an $IC_{50}$ value in the nanomolar range, and the *E*-isomer of compound **4c** is about 30-fold more cytotoxic than the *Z*-isomer. Like docetaxel, these compounds were found poorly active on a resistant MCF 7 cell line expressing the P-glycoprotein (data not shown). This result suggests that they are also recognized by this protein.

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The most meaningful result for the elucidation of the bioactive conformation of taxoids is the activity on cold-induced microtubule disassembly. As we have hypothesized, the addition of an aromatic ring at position 2 greatly improves tubulin interaction, except for the para-substituted compounds **4a**,**b** and **10b** which do not interact at all with tubulin. The lack of tubulin activity of taxoids bearing a para-substituted C2-benzoate has already been reported for azido, methoxy, or halo derivatives.[25,30,31] The discrepancy between cytotoxicity and microtubule binding for these para-substituted compounds deserves additional comments. Though the taxoid biological activity has been reported to be closely related to their interaction with microtubules, some derivatives have already been reported to be cytotoxic

**Table 2.** Biological Activities of Compounds **4a**−**d** and **10b**−**d** Compared with Docetaxel **1b** and Compound **3b(*E*)**

| compd | *n* | subst | ring size | microtubule disassembly inhibitory activity $IC_{50}$/$IC_{50}$(paclitaxel)[a] | cytotoxicity against cancer cell lines, $IC_{50}$[b] ($\mu$M) | | |
|---|---|---|---|---|---|---|---|
| | | | | | KB | HCT 116 | MCF 7 |
| **1b** | - | - | - | 0.5 | 0.0003 | 0.0022 | 0.001 |
| **4a** | 3 | para | 22 | inactive | 1.6 | - | - |
| **4b** | 4 | para | 23 | inactive | 0.28 | - | - |
| **4c(*E*)** | 4 | meta | 22 | 1 | 0.07 | 0.7 | 0.35 |
| **4c(*Z*)** | 4 | meta | 22 | 1.4 | 0.2 | 8 | >10 |
| **4d** | 4 | ortho | 21 | 6 | 0.7 | 2.4 | 2.1 |
| **10b** | 4 | para | - | inactive | 0.04 | - | - |
| **10c** | 4 | meta | - | 1 | 0.014 | - | - |
| **10d** | 4 | ortho | - | 1.2 | 0.0032 | 0.012 | 0.009 |
| **3a(*E*)** | 4 | - | 22 | 7.3 | 8 | >10 | >10 |

[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 $\mu$M. [b] $IC_{50}$ measures the drug concentration required for the inhibition of 50% cell proliferation after 72 h of incubation.

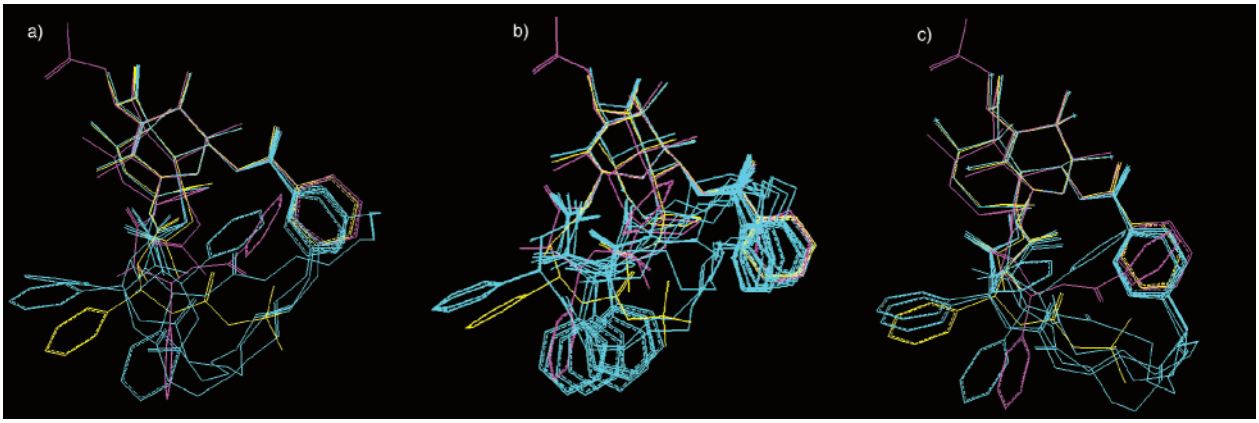


**Figure 3.** (a) Superimposition of the nonpolar form of docetaxel (yellow), T-shaped paclitaxel (magenta), and some representative conformers (cyan) of a) **4c(*E*)**, (b) **4d(*E*)** and (c) **4b(*E*)**.

but deprived of any interaction with tubulin.[16a,32] The compounds bearing such biological behavior may be transformed in the cell to more active derivatives either by hydrolysis of esters in the case of 7-, 9-, or 10-substituted derivatives[32b,c] or by biotransformation into active metabolites. However, these explanations are not appropriate for the para derivatives **4a**,**b** and **10b** and another mechanism of action must be hypothesized for these compounds. This would deserve further investigation and **10b** is a good candidate for this study due to its high cytotoxicity.

Compound **4c(*E*)** is the most active derivative on cold-induced microtubule disassembly and the first C2−C3′-linked macrocyclic taxoid equipotent to paclitaxel in this assay. It is to be noted that this macrocyclic compound bears the same activity as its acyclic precursor **10c**, proving that the presence of the N3′−C2 tether is not detrimental to activity and suggesting that the conformational lock induced by the presence of the macrocycle mimics the bioactive conformation. But, if it is so, compound **4c(*E*)** should have been more potent than docetaxel because of the favorable entropic factor achieved by cyclization. However, the entropic gain may not be so important because of the presence of many other easily rotated single bonds in that compound, and the absence of an increased activity may be due to unfavorable interactions with the protein. To get further information, we have studied more extensively the conformational behavior in solution and in the tubulin binding site of these derivatives.

**Conformational Studies.** Compounds **4b**, **4c(*E*)**, and **4d** were subjected to conformational analysis by NMR and molecular modeling. As previously reported,[16b] the H2′/H3′coupling constant gives a good indication of the major form present in solution, a low value (<3 Hz) revealing a major "nonpolar" conformation, whereas a higher value is the sign of a major "polar" form (>6 Hz). Unconstrained taxoids show a small $J_{H2'/H3'}$ (<2 Hz) in $CDCl_3$ and a larger value (6−8 Hz) in DMSO-$d_6$.[33] For compound **3a**, the H2′/H3′ coupling constant was small in both polar and apolar solvent (<1.5 and 2.5 Hz, respectively), suggesting a real restricted conformation with a major "nonpolar" form. For compounds **4b**, **4c(*E*)**, and **4d** the coupling constant is still <1.5 Hz in $CDCl_3$ and it slightly increases to 2, 3.5, and 3 Hz, respectively, in DMSO-$d_6$. Thus, the conformational constraint on the C13 side chain has been really achieved but it is slightly weaker for compounds **4c(*E*)** and **4d** than for **3a** and **4b**. NMR conformational analysis (NOESY and ROESY) in polar (DMSO-$d_6$) and apolar ($CDCl_3$) solvents showed an intense nuclear Overhauser effect (NOE) between 2′-H and 3′-H, characteristic of a gauche interaction between these two protons. Other NOE connectivity patterns were very similar for the three compounds with a slight difference for **4b** that showed strong NOE between 3′-Ph and 4-OAc that was not detected for the two other derivatives and inversely did not exhibit 2′-H/4-OAc NOE whereas they are present in the spectra of the two other compounds. Contrary to **4b**, **4c(*E*)**, and **4d**, both connectivities (2′-H/4-OAc and 3′-Ph/4-OAc) were observed for docetaxel in apolar solvent, suggesting that the major solution conformation of **4b**, **4c(*E*)**, and **4d** is somewhat different from the "nonpolar" form.

Molecular dynamic modeling studies were performed on compounds **4b**−**d(*E*)** in two different media (vacuum or $H_2O$) using Sybyl 6.8 software and MMFF94 force field. For each compound, conformational searching produced a great number of conformers, and only the conformers with a trans lactam function have been considered. No significant differences were observed between the two media. For the ortho derivative **4d**, all the conformers adopt closely related forms situated between the "nonpolar" and the "T-shaped" conformations, the latter being the most frequent (Figure 3b). For the para compound **4b**, two sets of conformers have been observed, the major one is very similar to that of the ortho derivative, and the other group bears the 3′-phenyl group on the other side of the median plane of the molecule (Figure 3c).

Finally the conformers obtained for the meta derivative **4c** can be classified in three families, two of which are similar to the para population, and a third group where the 3′-phenyl group lies in the median plane of the molecule. It is to be noted that when the phenyl group lies on the upper side of the macrocycle, it is located near the benzamide moiety of the T-shaped paclitaxel (Figure 3a).

Docking experiments of the most representative conformers of each compound in the refined model of α$\beta$-tubulin[11] have been realized. None of the **4b** conformers were able to fit the $\beta$-tubulin binding pocket. For the ortho derivative **4d**, only "T-shaped" conformers have led to a good interaction with the binding site, and

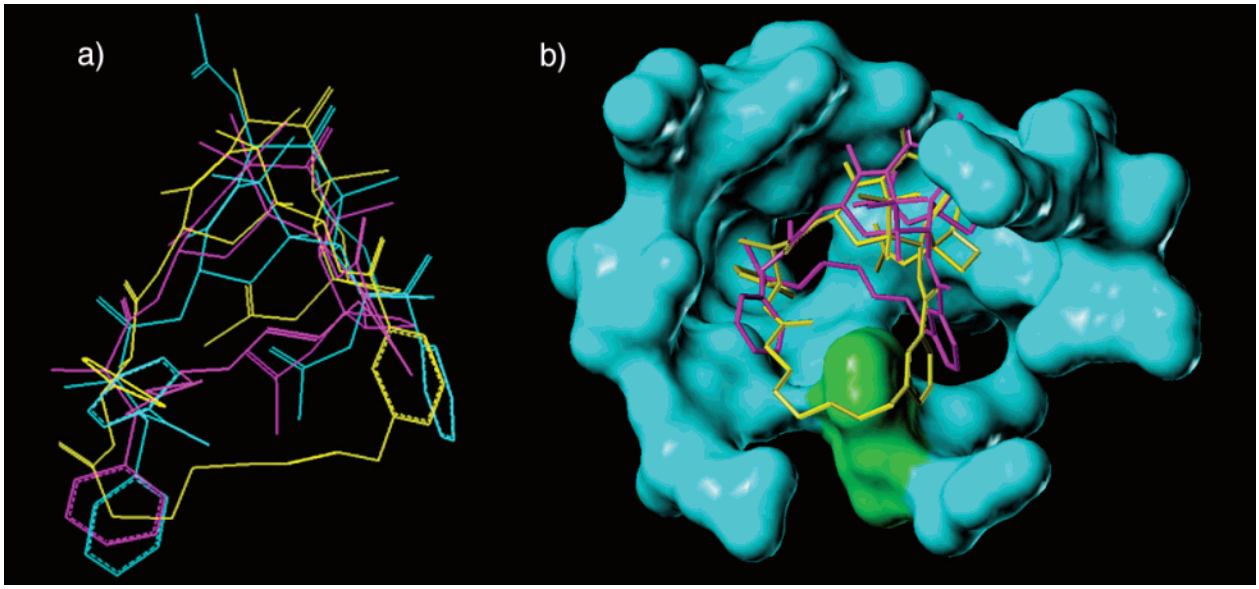


**Figure 4.** (a) Superimposition of two conformers of compound **4c(*E*)** (yellow and magenta) with "T-shaped" paclitaxel (cyan). (b) The two conformers of compound **4c(*E*)** (yellow and magenta) bound to $\beta$-tubulin in globular form (cyan) with $\pi$-stacking with His 229 (green).

finally for the meta compound **4c**, two sets of conformers gave satisfactory docking with the $\beta$-tubulin binding site (Figure 4b). For these conformers, the 3′-phenyl group occupies either the binding area of the 3′-phenyl or of the 3′-benzamide, allowing both conformers to be considered as "T-shaped" forms (Figure 4a).

On these docking models, the binding on $\beta$-tubulin is favored by a $\pi$-stacking interaction between the imidazolyl moiety of His 229 and the phenyl ring of the benzoate. This interaction has been depicted in the model of paclitaxel bound to $\beta$-tubulin.[11] It should be mentioned that none of the conformers that resemble the "nonpolar" form were able to dock in the $\beta$-tubulin binding site.

## Summary and Conclusions

Seven novel taxoids bearing a C2-aroyl group have been synthesized including four macrocyclic derivatives. In agreement with previously reported data, para-substituted compounds **4a**,**b** and **10b** were deprived of any tubulin interaction. On the other hand, the four other compounds **4c**,**d** and **10c**,**d** show increased activity on cold-induced microtubule disassembly compared to the aliphatic series **2** and **3**. Thus, as expected, introduction of an aromatic ring in the C2-position as in docetaxel greatly increased tubulin interaction. The most active derivative, the meta-substituted macrocyclic taxoid **4c**, is equipotent to paclitaxel and to its acyclic analogue **10c** on microtubule stabilization. Though **4c** is less cytotoxic than docetaxel, it is one of the most potent C2−C3′-linked macrocyclic taxoids so far described in the literature with submicromolar $IC_{50}$ values on three different cell lines. Examination of the conformational behavior of the macrocyclic taxoids **4b**−**d** has shown that these compounds, though relatively constrained, can adopt several conformations, most of them being situated between the "nonpolar" and the "T-shaped" conformations.

The results obtained from docking experiments are in good agreement with the biological results, i.e. inactivity of the para-substituted derivatives and a good interaction with the $\beta$-tubulin binding pocket for the ortho- and meta-substituted compounds. The observation of a possible $\pi$-stacking interaction between the His229-imidazolyl and C2-benzoyl rings as seen with paclitaxel may explain the increased activity of this series compared to the former aliphatic ones **2** and **3**. The better docking of "T-shaped" conformers of **4c** or **4d** with tubulin supports the hypothesis of a "T-shaped" bioactive conformation of taxoids as suggested by Snyder et al.[11]

Thus two different series of macrocyclic taxoids which mainly adopt the "T-shaped" form, bearing a tether either between the C4-acetate and the C3′-phenyl moiety[18] or between the C2-benzoate and the C3′-amine, have led to the same conclusion on the bioactive conformation of taxoids. Though these experiments are based on a 3.5 Å refined structure of tubulin, both results present evidence of a "T-shaped" conformation of taxoids when they are bound to microtubules.

It would have been expected that our new macrocyclic derivatives would be more active than docetaxel if they mimic the bioactive conformation. However, since our tether was essentially designed to well-orientate the different substituents of the C13 side chain, these compounds keep a relative flexibility that may counterbalance the favorable entropic factor achieved by cyclization. It should also be noted that the difficulty with C2,C3′-linked taxoids was to find the right tether that still allows $\pi$-stacking between the benzoate and the His229 imidazole ring. This also may account for the lower biological activity of our compounds compared to the C4−C3′-linked taxoids.[18] Even though our 22-membered-ring macrocycle **4c(*E*)** is at present the most active compound in this series, the biological activity may be most likely improved by making some modifications on the tether, what is under current investigation.

## Experimental Section

**General.** $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker AC 300 or AV 300 spectrometer. NOESY and ROESY spectra have been performed on a Bruker AMX 400 spectro-

meter. Chemical shifts are given as $\delta$ 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 COSY, HMQC, and HMBC on a Bruker AV 300. Mass spectra were obtained on an AQA Navigator ThermoQuest. High-resolution mass spectra were performed on 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 Dr. Alain Commerçon (Aventis-Pharma), and compound **6** has been prepared according to the previously described procedure.[16a] Microtubular proteins were purified from mammalian brain as previously described.[34] Cytotoxicity and microtubule disassembly inhibition were carried out according to established literature protocols.[28,29]

**Synthesis of 2-Debenzoyl-2′-*O*-*tert*-butyldimethylsilyl-3′-de-*tert*-butoxycarbonyl-3′-hex-5-enoyl-7,10-ditriethylsilyldocetaxel (7a).** Hex-5-enoic acid (6.3 $\mu$L, 0.08 mmol, 1.5 equiv) was added to a solution of **6** (50 mg, 0.053 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (15.2 mg, 0.11 mmol, 1.5 equiv) and DMAP (13 mg, 0.106 mmol, 2 equiv) in $CH_2Cl_2$ (0.5 mL). The reaction mixture was stirred at room temperature for 3 h and then 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′-hex-5-enoyl-7,10-ditriethylsilyldocetaxel **7a** (43.5 mg, 79%) as a white amorphous solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ = −0.28 (s, 3H), −0.07 (s, 3H), 0.49−0.70 (m, 12H), 0.77 (s, 9H), 0.93−1.01 (m, 18H), 1.08 (s, 3H), 1.20 (s, 3H), 1.55 (s, 3H), 1.67−1.78 (m, 2H), 1.78 (s, 3H), 1.83−2.01 (m, 1H), 2.02−2.18 (m, 4H), 2.21−2.33 (m, 2H), 2.37 (s, 3H), 2.42−2.59 (m, 1H), 3.49 (d, $^3J_{H,H}$ = 6.5 Hz, 1H), 3.94 (br d, $^3J_{H,H}$ = 6.5 Hz, 1H), 4.34 (dd, $^3J_{H,H}$ = 10.5 Hz and $^3J_{H,H}$ = 6.5 Hz, 1H), 4.49 (br s, 1H), 4.63 and 4.69 (qAB, $^3J_{H,H}$ = 9.5 Hz, 2H), 4.92 (d, $^3J_{H,H}$ = 8.5 Hz, 1H), 4.99−5.12 (m, 3H), 5.55 (br d, $^3J_{H,H}$ = 9.5 Hz, 1H), 5.67−5.85 (m, 1H), 6.18 (t, $^3J_{H,H}$ = 9.0 Hz, 1 H), 6.38 (d, $^3J_{H,H}$ = 9.5 Hz, 1H), 7.12−7.40 (m, 5H).$^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ = −5.8, −5.2, 5.4, 6.1, 7.0, 10.8, 13.7, 18.3, 21.0, 23.4, 25.0, 25.6, 26.5, 33.0, 35,8, 36.0, 37.6, 43.2, 47.0, 54.8, 58.4, 72.4, 72.9, 74.2, 75.1, 75.6, 78.2, 78.6, 82.6, 84.0, 115.8, 126.5, 128.0, 128.7, 133.7, 137.7, 138.0, 138.5, 170.0, 171.6, 172.9, 206.3. MS (ESI$^+$): *m/z* 1064 [M + $Na^+$].

Synthesis of compound **7b** is described in ref 16b.

**General Procedure for the Synthesis of Allylbenzoic Acids (8).** A typical procedure is described for the synthesis of 3-allylbenzoic acid.

**(a) Ethyl 3-Iodobenzoate**. To a solution of 3-iodobenzoic acid (5 g, 20 mmol) in ethanol (50 mL) was added dropwise thionyl chloride (2.93 mL, 40 mmol, 2 equiv). The solution was heated at 60 °C for 4 h, and the reaction mixture was concentrated in vacuo. After standard workup, the residue was purified by flash chromatography (heptane/$CH_2Cl_2$, 70/30) to afford pure ethyl 3-iodobenzoate (5.2 g, 93%) as a yellow oil. Proton NMR and mass spectrometry data are in agreement with the literature.[34] $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ =14.4, 61.4, 93.9, 129.8, 130.1, 132.4, 138.5, 141.6, 165.1.

**(b) Ethyl 3-Allylbenzoate.** To a solution of ethyl 3-iodobenzoate (3 g, 10.8 mmol) in dry THF (114 mL) cooled to −40 °C was added dropwise isopropylmagnesium chloride (2 M in THF, 8.1 mL, 1.5 equiv). The reaction mixture was stirred at −40 °C for 1 h before adding a daily prepared CuCN, 2LiCl solution in THF (0.34 M, 32 mL, 10.8 mmol, 1 equiv) followed, after a further 15 min, by allyl bromide (3.8 mL, 43.2 mmol, 4 equiv). After being stirred for 1 h, the reaction mixture was diluted with EtOAc and filtered over Celite. After standard workup, the residue was purified by flash chromatography (heptane/$CH_2Cl_2$, 70/30) to afford pure ethyl 3-allylbenzoate (1.6 g, 77%) as a yellow-brown oil. Analytical data are in agreement with the literature.[21b]

**(c) 3-Allylbenzoic acid (8m).** To an aqueous solution of sodium hydroxide (2 M, 75 mL) was added an ethanolic solution of ethyl 3-allylbenzoate (55 mM, 150 mL, 8.2 mmol). The solution was stirred at room temperature for 3 h, and the reaction was stopped by addition of an excess of 1 N HCl. After removal of ethanol, the aqueous layer was extracted three times with EtOAc. The organic layer was concentrated in vacuo, and the residue was purified by flash chromatography ($CH_2Cl_2$/MeOH, 97/3) to afford 3-allylbenzoic acid (1.1 g, 83%) as a white amorphous solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ = 3.44 (d, $^3J_{H,H}$ = 6.5 Hz, 2 H), 5.03−5.17 (m, 2H), 5.85−6.07 (m, 1H), 7.35−7.50 (m, 2H), 7.87−8.02 (m, 2H).$^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ = 39.9, 116.6, 128.2, 128.6, 129.5, 130.3, 134.2, 136.7, 140.6, 172.8. MS (EI): *m/z* 162 [M•].

In the same manner, 2- and 4-allylbenzoic acids (**8o** and **8p**, respectively) were synthesized (see Supporting Information).

**General Procedure for the Synthesis of 2-Allylbenzoyl-2′-*O*-*tert*-butyldimethylsilyl-3′-de-*tert*-butoxycarbonyl-3′-alkenoyl-7,10-ditriethylsilyldocetaxel (5a−d).** A typical procedure is described for the synthesis of 2-*m*-allylbenzoyl-2′−O-*tert*-butyldimethylsilyl-3′-de-*tert*-butoxycarbonyl-3′-heptenoyl-7,10-ditriethylsilyldocetaxel (**5c**). A solution of taxoid **7b** (100 mg, 0.095 mmol), DCC (577 mg, 2.8 mmol, 30 equiv), and DMAP (11.6 mg, 0.095 mmol, 1 equiv) in anhydrous toluene (0.4 mL) was stirred at 60 °C for 15 min before adding 3-allylbenzoic acid **8m** (460 mg, 2.8 mmol, 30 equiv). The solution was stirred at 60 °C for 24 h and then filtered through a Celite/silica gel (1/1) column in EtOAc/heptane (3/7) and concentrated in vacuo. After standard workup, the residue was purified by flash chromatography (heptane/acetone, 92/8 then heptane/acetone, 90/10) to afford pure **5c** (70.8 mg, 62%) as a white amorphous solid: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ = −0.27 (s, 3H), −0.10 (s, 3H), 0.50−0.74 (m, 12H), 0.79 (s, 9H), 0.89−1.09 (m, 18H), 1.24 (s, 6H), 1.29−1.47 (m, 2H), 1.51−1.65 (m, 2H), 1.69 (s, 3H), 1.86 (s, 3H), 1.86−2.06 (m, 3H), 2.08−2.22 (m, 1H), 2.22−2.30 (m, 2H), 2.32−2.46 (m, 1H), 2.50−2.62 (m, 1H), 2.53 (s, 3H), 3.46 (d, $^3J_{H,H}$ = 6.5 Hz, 2H), 3.86 (d, $^3J_{H,H}$ = 7.0 Hz, 1H), 4.20 and 4.30 (qAB, $^2J_{H,H}$ = 8.5 Hz, 2H), 4.41 (dd, $^3J_{H,H}$ = 6.5 Hz and $^3J'_{H,H}$ = 11.0 Hz, 1H), 4.56 (br s, 1H), 4.84−5.01 (m, 3H), 5.03−5.22 (m, 2H), 5.30 (s, 1H), 5.68 (br d, $^3J_{H,H}$ = 9.0 Hz, 1H), 5.64−5.82 (m, 2H), 5.88−6.04 (m, 1H), 6.22 (t, $^3J_{H,H}$ = 9.0 Hz, 1 H), 6.34 (d, $^3J_{H,H}$ = 9.0 Hz, 1H), 7.20−7.48 (m, 7H), 7.96 (br s, 2H). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ = −5.6, −5.3, 5.4, 6.1, 7.0, 10.6, 13.9, 18.3, 20.9, 23.3, 25.2, 25.6, 26.7, 28.4, 33.4, 35.6, 36.4, 37.5, 40.0, 43.4, 46.7, 55.2, 58.5, 71.9, 72.8, 75.2, 75.4, 76.9, 78.8, 81.4, 84.1, 114.7, 116.6, 126.6, 127.9, 128.1, 128.7, 128.8, 129.5, 130.5, 133.4, 134.2, 136.8, 138.0, 138.5, 138.7, 140.6, 167.1, 170.2, 171.7, 172.2, 205.4. MS (ESI$^+$): *m/z* 1222 [M + $Na^+$].

Compounds **5a**,**b**,**d** were prepared according to this method. For experimental details and spectral data, see the Supporting Information.

**General Procedure for the Synthesis of Macrocyclic Taxoids 9 by Ring-Closure Metathesis.** A typical procedure is described for the synthesis of macrocyclic taxoid **9c**. To a solution of taxoid **5c** (69.7 mg, 0.058 mmol) in $CH_2Cl_2$ (4 mL) was added dropwise a solution of bis(tricyclohexylphosphine)-benzylideneruthenium(IV) dichloride (2.9 mg, 0.0035 mmol) in $CH_2Cl_2$ (1.6 mL). The solution was refluxed for 3 h and concentrated in vacuo. After standard workup, the residue was purified by silica gel chromatography (EtOAc/heptane, 75/35) to afford an inseparable *E*/*Z* mixture (65/35) of pure **9c** (40.5 mg, 60%) as a white amorphous solid.

**9c**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ = −0.32 (s, 3H), −0.07 (s, 3H), 0.53−0.79 (m, 21H), 0.90−1.09 (m, 18H), 1.25 (s, 3H), 1.28 (s, 3H), 1.28−1.36 (m, 2H), 1.56−1.76 (m, 2H), 1.70 (s, 3H), 1.81−1.91 (m, 1H), 1.92 (s, 3H), 1.93−2.12 (m, 2H), 2.12−2.42

(m, 4H), 2.48−2.60 (m, 1H), 2.60 (s, 3H), 3.23−3.50 (m, 2H), 3.78−3.91 (m, 1H), 4.14−4.35 (m, 2H), 4.35−4.48 (m, 1H), 4.53−4.64 (br s, 1H), 4.85−4.95 (m, 1H), 5.10−5.55 (m, 2H), 5.56−5.85 (m, 3H), 6.24 (d, $^3J_{H,H}$ = 9.0 Hz, 1H), 6.40 (t, $^3J_{H,H}$ = 9.0 Hz, 1H), 7.22−7.49 (m, 7H), 7.82−8.10 (m, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ (*E*-isomer) = −5.9−5.2, 5.4, 6.1, 7.0, 7.1, 10.7, 14.1, 18.3, 21.2, 22.8, 23.3, 25.6, 26.8, 27.1, 30.1, 34.8, 35.7, 37.6, 39.4, 43.5, 47.8, 54.7, 58.7, 70.9, 73.0, 75.2, 75.4, 75.6, 77.0, 79.2, 81.3, 84.3, 126.6, 127.7, 128.7, 129.7, 130.1, 130.5, 133.7, 134.4, 137.7, 138.8, 141.7, 167.6, 171.1, 171.4, 172.2, 205.5. Modifications for the $^{13}$C NMR chemical shifts for the *Z*-isomer: $\delta$ ($\delta$ *E*-isomer) = 32.3 (39.4), 132.4, 132.6 (130.1, 130.5), 142.8 (141.7). MS (ESI$^+$): *m/z* 1194 [M + Na$^+$].

In the same manner, **9a**,**b**,**d** were synthesized, see the Supporting Information

**General Procedure for Removal of the Silyl Protecting Groups.** A typical procedure is described for the synthesis of macrocyclic taxoid **4c**. To a cooled solution of **9c** (25 mg, 0.021 mmol) in pyridine−acetonitrile (7/93, 0.825 mL) was added dropwise HF/pyridine (70%, 120 $\mu$L, 0.85 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 additional 6 h at room temperature. The reaction was quenched by addition of saturated aqueous sodium hydrogenocarbonate (50 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 **4c**-*Z* (4.3 mg, 24%) and **4c**-*E* (8.9 mg, 50%) as white amorphous solids.

**4c**-*E*: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ = 1.14 (s, 3H), 1.27 (s, 3H), 1.27−1.43 (m, 2H), 1.51−1.65 (m, 2H), 1.78 (s, 3H), 1.88 (s, 3H), 1.88−2.10 (m, 3H), 2.13−2.38 (m, 4H), 2.48 (s, 3H), 2.53−2.69 (m, 1H), 3.13 (br s, 1H), 3.38 (d, $^3J_{H,H}$ = 5.5 Hz, 2H), 3.91 (d, $^3J_{H,H}$ = 7.0 Hz, 1H), 4.15−4.30 (m, 2H), 4.34 (d, $^3J_{H,H}$ = 8.5 Hz, 1H), 4.67 (br.s, 1H), 4.94 (d, $^3J_{H,H}$ = 9.0 Hz, 1H), 5.18 (s, 1H), 5.24−5.37 (m, 2H), 5.62−5.83 (m, 3H), 6.24 (t, $^3J_{H,H}$ = 9.0 Hz, 1H), 6.36 (d, $^3J_{H,H}$ = 9.5 Hz, 1H), 7.30−7.47 (m, 7H), 7.85 (br s, 1H), 8.02 (s, 1H). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ = 10.1, 14.6, 20.9, 22.6, 23.5, 26.8, 27.4, 30.8, 35.5, 36.2, 37.2, 39.8, 43.2, 46.5, 53.5, 57.7, 72.2, 72.4, 73.0, 74.5, 74.9, 76.6, 79.2, 81.1, 84.3, 127.1, 128.2, 128.7, 129.0, 129.7, 130.0, 130.8, 134.5, 136.1, 138.3, 141.5, 167.3, 170.7, 172.3, 172.8, 211.5. HRMS (MALDI-TOF): *m/z* calcd for $C_{46}H_{55}NO_{13}$.Na$^+$ 852.3571, found 852.3587 ($\Delta$ = −1.9 ppm).

**4c**-*Z*: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ = 1.14 (s, 3H), 1.29 (s, 3H), 1.29−1.49 (m, 2H), 1.50−1.72 (m, 2H), 1.73−1.96 (m, 3H), 1.80 (s, 3H), 1.95 (s, 3H), 2.14−2.38 (m, 3H), 2.40−2.53 (m, 1H), 2.46 (s, 3H), 2.53−2.68 (m, 1H), 3.22 (br s, 1H), 3.35−3.50 (m, 1H), 3.77−3.90 (m, 1H), 3.94 (d, $^3J_{H,H}$ = 6.5 Hz, 1H), 4.17−4.40 (m, 3H), 4.74 (br.s, 1H), 4.92 (d, $^3J_{H,H}$ = 10.0 Hz, 1H), 5.19 (s, 1H), 5.31 (s, 1H), 5.57−5.75 (m, 2H), 5.75−5.88 (m, 2H), 6.16 (d, $^3J_{H,H}$ = 9.5 Hz, 1H), 6.43 (t, $^3J_{H,H}$ = 9.0 Hz, 1H), 7.30−7.53 (m, 7H), 7.89 (d, $^3J_{H,H}$ = 6.5 Hz, 1H), 8.07 (s, 1H). $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta$ = 10.2, 14.4, 21.4, 22.9, 26.2, 26.7, 27.7, 29.7, 32.4, 35.5, 36.4, 37.0, 43.1, 46.4, 53.5, 57.6, 72.0, 72.4, 72.7, 74.4, 75.3, 77.3, 79.4, 81.0, 84.3, 126.8, 127.3, 128.1, 128.8, 129.4, 130.1, 132.4, 132.8, 135.9, 138.4, 142.1, 167.3, 170.8, 172.1, 173.2, 211.8. HRMS (MALDI-TOF): *m/z* calcd for $C_{46}H_{55}NO_{13}$.Na$^+$ 852.3571, found 852.3556 ($\Delta$ = 1.8 ppm).

In the same manner macrocylic taxoids **4a**,**b**,**d** were synthesized as the acyclic taxoids **10b**−**d**. For experimental details and spectral data see the Supporting Information.

**Computational Procedures.** All calculations were performed on a SiliconGraphics Indigo2 Extreme workstation. All modelizations were done using Sybyl 6.8 software. The MMFF94 force field was used for minimization and partial charge calculations, a dielectric constant of 1.0 or 78 being employed. In all cases A, B, C, and D rings of the taxoids were considered as an aggregate. Compounds **4b**−**d** were subjected to an unrestrained molecular dynamics simulation at 1600 K for 20 000 fs. Conformations were sampled every 100 fs during the simulation. Each of these conformers was minimized and compared with others with a RMS of 0.3 Å. The structures were analyzed using Sybyl 6.8 software, and superimposition of conformers was based on the backbone atoms of the taxane core.

Docking experiments on compounds **4b**−**d**-*E* were realized using the DOCK software of Sybyl 6.8 with Tripos force field for minimization.

**Acknowledgment.** The authors thank Dr Alain Commerçon (Aventis-Pharma) for a gift of docetaxel. Christiane Gaspard and Jérôme Bignon are acknowledged for cytotoxicity evaluations, Jean-François Gallard and Marie-Thérèse Martin for performing NMR spectra, and Vincent Guérineau for high-resolution mass spectra.

**Supporting Information Available:** Experimental details and characterization data for new compounds **4a**,**b**,**d**, **5a**,**b**,**d**, **8p**,**o**, **10b**−**d**, and **11a**,**b**,**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0497996