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Novel C2–C3′ *N*-peptide linked macrocyclic taxoids. Part 2: Synthesis and biological activities of docetaxel analogues with a peptide side chain at C2 and their macrocyclic derivatives

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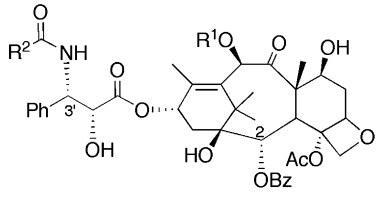
Abstract—The synthesis of a series of novel docetaxel analogues possessing a peptide side chain at the C2 position as well as peptide macrocyclic taxoids is described. These compounds were designed to mimic a region of the α-tubulin loop equivalent to the paclitaxel binding pocket of β-tubulin. Fifteen new peptide taxoids were obtained and evaluated as inhibitors of microtubule disassembly as well as cell proliferation. The relationships between these new taxoids and the tau protein motif interacting with microtubules are discussed.

1. Introduction

The diterpenoid natural product Taxol® (paclitaxel)[1] **1a** and its semi-synthetic analogue Taxotere® (docetaxel)[2] **1b** are two important chemotherapeutic drugs widely used today for the treatment of various types of cancer. They are known to exert their therapeutic effect, at least in part, by promoting the assembly of tubulin into microtubules[3] and stabilizing the resulting microtubules. They thereby inhibit microtubule disassembly[4] and normal dynamic reorganization of the microtubular network required for mitosis.



Paclitaxel (Taxol®) **1a** R^1= Ac, R^2= Ph
Docetaxel (Taxotere®) **1b** R^1= H, R^2= O*t*Bu

In 1998, the tubulin structure was determined by electron crystallography on zinc-induced sheets of tubulin stabilized with paclitaxel **1a**.[5,6] The 3.7 Å resolution obtained not only provided information on the structural and functional composition of the α,β-tubulin dimer, but enabled localization of the paclitaxel **1a** binding site on β-tubulin. Moreover, it was shown that the region of α-tubulin that is equivalent to the paclitaxel binding pocket is occupied by an eight amino acid peptide (Val362-Ala369) belonging to the loop connecting the B9 and B10 strands (Fig. 1).[7,8] It was proposed that this loop could act as an endogenous microtubule-stabilizing factor.[7–10] Snyder et al.[7] have compared the electron density of the paclitaxel-binding pocket in the tubulin map with all the known paclitaxel conformations. The T-shaped or butterfly-like structure with the particular orientation of its C13 side chain and ester groups at C2 and C4 was identified as the most probable one.

Based on these results, we performed molecular modeling to study the superimposition of the α- and β-tubulin structures in this region and we observed conformational similarities between the octapeptide Val362-Val-Pro-Gly-Gly-Asp-Leu-Ala369 and the structure of paclitaxel when it is bound to β-tubulin (Fig. 2).[11] We noted that six amino acids, Val363-Pro-Gly-Gly-Asp-Leu368, of the α-tubulin octapeptide showed good insertion

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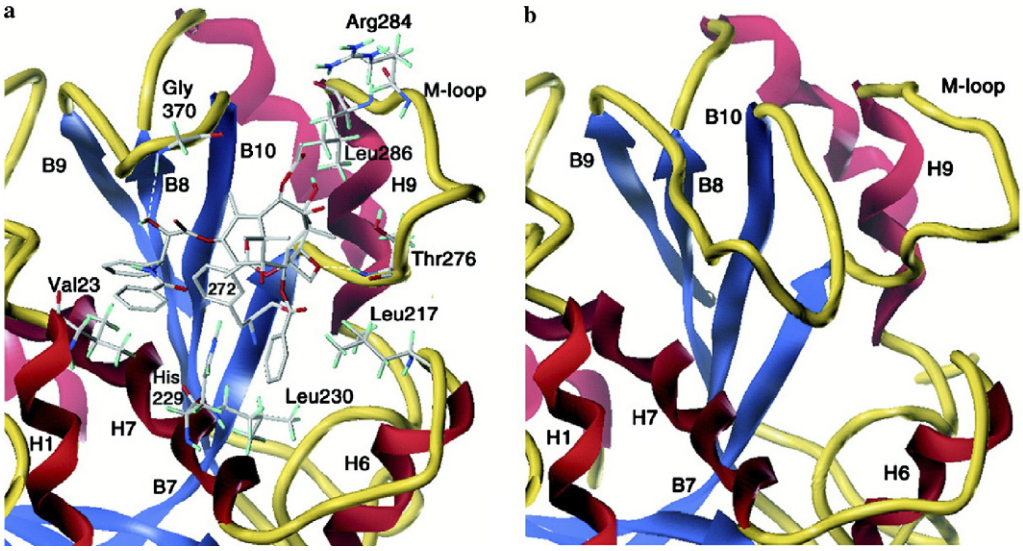


Figure 1. (a) The paclitaxel binding site in β-tubulin and (b) the B9–B10 loop in α-tubulin.[7]

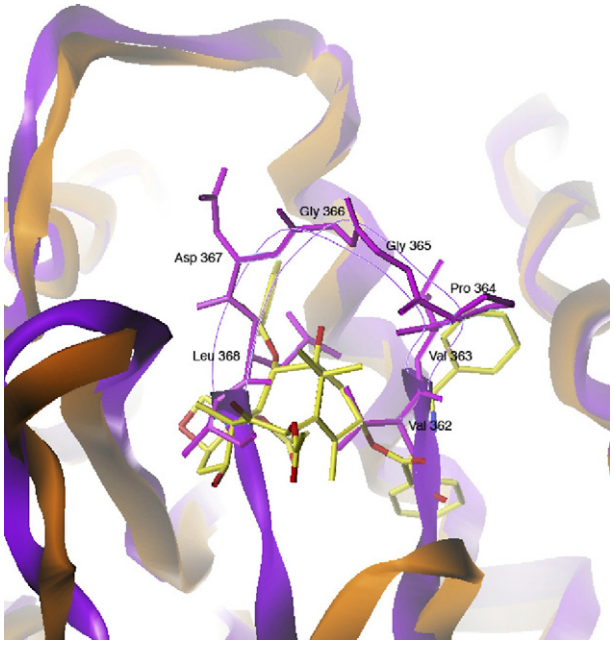


Figure 2. Overlapping of paclitaxel (yellow) and octapeptide V362-A369 (magenta) after manual superimposition of α- and β-tubulin secondary structure (Sybyl software).

between positions 3′ and 2 of paclitaxel **1a**, while the other amino acids mimic a part of the paclitaxel skeleton.

From these observations, we designed new macrocyclic analogues by insertion of specific amino acids of the α-tubulin loop between the 2 and 3′ positions of docetaxel (Fig. 3) and, as reported in a previous communication, we synthesized taxoids **2a**–**2d** and **3a**–**3d** bearing from one to four amino acids (Val-Pro-Gly-Gly) at the C3′ position.[11] The new taxoids exhibited excellent interaction with microtubules, showing IC_{50} values similar to that of docetaxel. To confirm the specificity

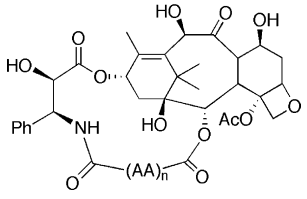
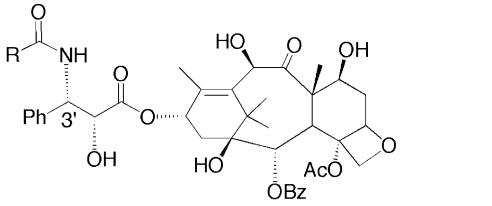


Figure 3. Macrocyclic peptide taxoids (AA, amino acids of the α-tubulin region equivalent to the paclitaxel binding site).

of the interaction between tubulin and the peptide analogues, we also synthesized three other compounds (**4**, **5a**–**5b**) in which the amino acids at the C3′ position are different from those constituting the octapeptide residue. Compounds **4** and **5b** also interacted with microtubules but to a lesser extent than the previous series thereby showing some specificity of the amino acids of the α-tubulin loop toward the interaction with β-tubulin.



2a R= Val
2b R= Val-Pro
2c R= Val-Pro-Gly
2d R= Val-Pro-Gly-Gly

3a R= Val-OBn
3b R= Val-Pro-OBn
3c R= Val-Pro-Gly-OBn
3d R= Val-Pro-Gly-Gly-OBn

4 R= Val-Phe-Met-OMe
5a R= Gly-Ser(OBn)-Tyr(OBn)-OBn
5b R= Gly-Ser-Tyr

These preliminary results encouraged us to continue our investigation on the synthesis of taxoids bearing amino acids at the C2 position (compounds **6a**–**6d**) as well as macrocyclic analogues with a peptide bridge between

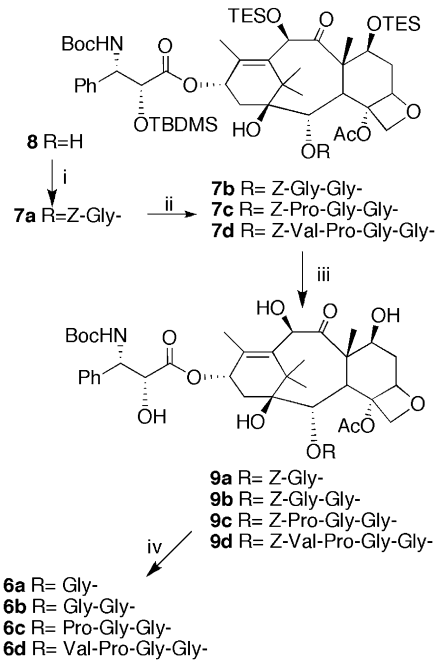
the C3′ and C2 positions (Fig. 3). The synthesis and biological evaluation of these novel taxoids are presented in this paper as well as a discussion of the relationships between these new taxoids and the tau protein motif interacting with microtubules.

2. Chemistry

2.1. Taxoids with a peptide side chain at C2

From a retrosynthetic point of view, compounds **6a**–**6d** may be obtained, after deprotection of the C7, C10, and C2′ hydroxyl groups and of the amine function of the amino acids, from docetaxel **1b** conveniently protected after selective removal of benzoate at C2 and step by step addition of the amino acids.

The protected docetaxel analogue **8** was chosen as starting material.[12] To mimic the desired α-tubulin loop, it was necessary to have the C-terminal part of the peptide linked to the 2 position of **8**. Based on molecular modeling (Fig. 2), leucine should be the first amino acid to be linked. Unfortunately, the linkage of the protected Z-leucine proved to be problematic. The failure of **8** to undergo esterification with Z-protected leucine may be attributed to steric hindrance. For that reason, we decided to esterify compound **8** with the less hindered Z-protected glycine and consequently, only the four amino acids of the α-tubulin loop were used. Compounds **6a**–**6d** could thus be obtained from the protected docetaxel analogue **8** after addition of the protected Z-amino acids added step by step at the C2 position. The coupling with Z-protected glycine was realized by esterification using DCC and DMAP. Contrary to our previous results,[13–15] the esterification of the C2 hydroxyl group with protected amino acids required careful control to avoid rearrangement of the oxetane ring.[16] A large excess of the protected amino acid and DCC (30 equiv) was needed to achieve esterification, compound **7a** being obtained with a yield of 63% (Scheme 1). To lengthen the peptide chain, we used the same strategy as for compounds **3a**–**3d**[11]: deprotection of the benzyloxycarbonyl group by hydrogenolysis and formation of a peptide bond using EDCI and 1-HOBt. Thus, compounds **7b**–**7d** were obtained with good yields ranging from 75% to 95% (Scheme 1). All silyl protecting groups were removed with HF/pyridine affording compounds **9a**–**9d**, while the terminal amino acids were all deprotected by hydrogenolysis, leading to compounds **6a**–**6d** (Scheme 1).



Scheme 1. Reagents and conditions: (i) DCC (30 equiv), DMAP (0.1 equiv), ZNH-Gly-OH (30 equiv), toluene, rt, 15 h (63%); (ii) Pd/C 10%, H_2, MeOH, rt, 5 h and EDCI (5 equiv), HOBt (5 equiv), ZNH-AA-OH (5 equiv), NMM, CH_2Cl_2, rt, 30 min (**7b**, 85%; **7c**, 95%; **7d**, 75%); (iii) HF/pyridine 70% (45 equiv), pyridine, CH_3CN, 0 °C and rt (**9a**, 65%; **9b**, 74%; **9c**, 70%; **9d**, 69%); (iv) Pd/C 10%, H_2, MeOH, rt, 5 h (**6a**, 67%; **6b**, 84%; **6c**, 51%; **6d**, 36%).

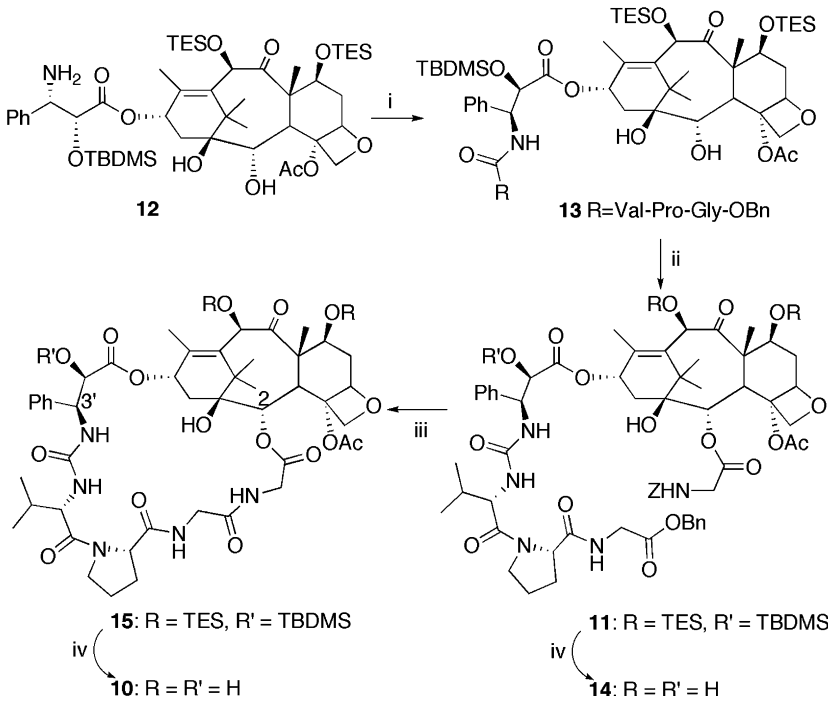
2.2. Macrocyclic taxoids

To synthesize compound **10**, we used previously adapted reactions: introduction of urea function on the amino group at C3′ with CDI[11] followed by the addition of the tripeptide Val-Pro-Gly-OBn using classical coupling reagents (compound **13**) and esterification of the C2 hydroxyl group by Z-Gly as described above (compound **11**). After hydrogenolysis of the terminal amino acid protecting groups, the resulting compound was then subjected to macrocyclization using conditions developed by Boger et al.[17] The hydroxyl groups at C7, C10, and C2′ of compound **15** were finally deprotected affording the expected macrocyclic compound **10** (Scheme 2).

Two other macrocyclic taxoids (compounds **21** and **26**) were also synthesized using the same strategy that led to compound **10**. Taxoid **21** (Scheme 3) bears a peptide chain between the N3′ position and the meta-position of the C2 aromatic ring, while taxoid **26** (Scheme 4) bears a peptide N3′–C2 tether formed with amino acids of the α-loop. The meta-position for the substitution on the benzoate at C2 was chosen because meta-substituted derivatives are generally more active as shown in a previous study on other taxoid macrocycles.[15]

To synthesize **21**, we first added glycine benzyl ester at the C-terminal part of the tripeptide taxoid **13** after hydrogenolysis (compound **16**). Esterification of the C2 hydroxyl group of **16** with 3-nitrobenzoic acid was then realized to afford compound **17** in good yield. After simultaneous hydrogenolysis of the benzyl ester and hydrogenation of the nitro group, macrocyclization was accomplished using Boger's conditions.[17] Compound **21** was finally obtained by full deprotection of the hydroxyl groups (Scheme 3).

The synthesis of taxoid **26** started from **16** (Scheme 4). After deprotection of the terminal amino acid at C3′, coupling with the amino acid H_2N-Asp(O*t*-Bu)-OBn under the usual conditions led to compound **22**. The



Scheme 2. Reagents and conditions: (i) CDI (3 equiv), tripeptide H_2N-Val-Pro-Gly-OBn (2 equiv), NMM (6.5 equiv), CH_2Cl_2/CH_3CN 1:1, 60 °C, 15 h (81%); (ii) DCC (30 equiv), DMAP (0.1 equiv), ZNH-Gly-OH (30 equiv), toluene, rt, 15 h (64%); (iii) Pd/C 10%, H_2, MeOH, rt, 5 h and EDCI (5 equiv), HOBt (5 equiv), NMM, CH_2Cl_2/DMF 5:1, 0 °C, 2 h (58%); (iv) HF/pyridine 70% (45 equiv), pyridine, CH_3CN, 0 °C and rt (**10**, 53%; **14**, 74%).

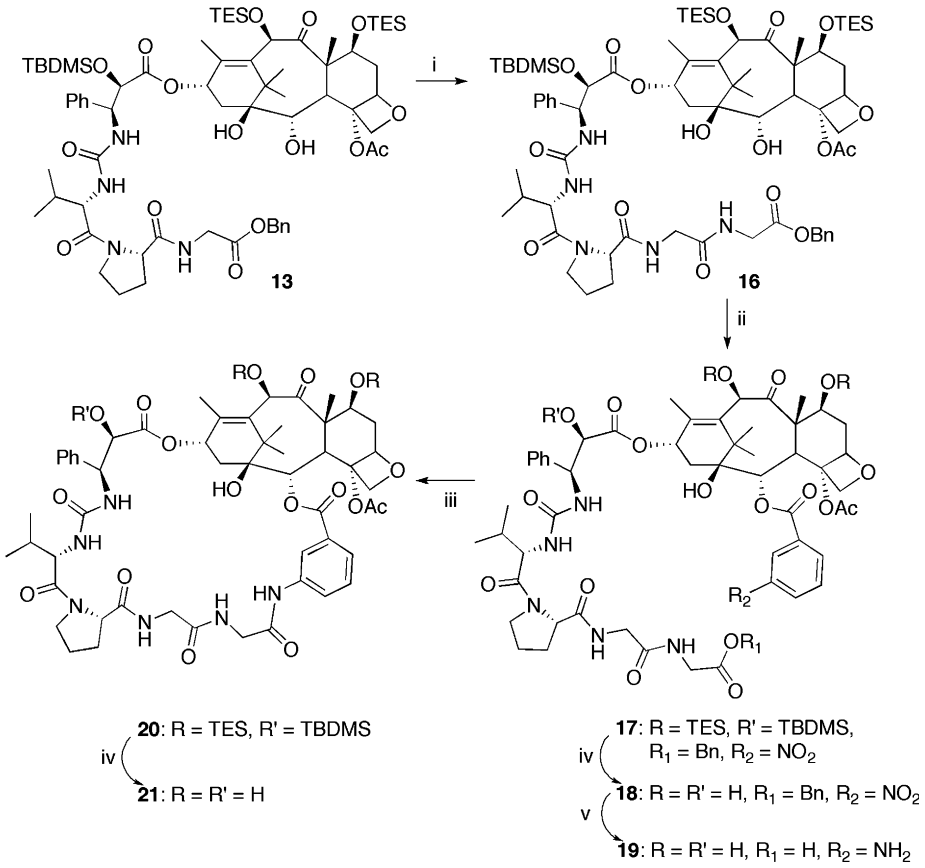
protected aspartic acid was prepared from commercially available Fmoc-Asp(O*t*-Bu)-OH by esterification to its benzyl ester and removal of the Fmoc group. Introduction of the glycine residue at C2 led to compound **23** which after macrocyclization and final deprotection afforded the macrocyclic taxoid **26**. Attempted removal of the t-butyl ester group of the aspartic acid residue of **26** using a variety of methods proved unsuccessful, only degradation products being obtained.[18]

To compare the biological activities of the macrocyclic taxoids with their open-chain precursors, compounds **11**, **17**, and **23** were also subjected to HF/pyridine deprotection to afford **14** (Scheme 2), **18** (Scheme 3), and **24** (Scheme 4), respectively, while taxoid **19** was obtained by hydrogenolysis of compound **18** (Scheme 3).

3. Biological results and discussion

The new compounds **6a**–**6d**, **9a**–**9d**, **10**, **14**, **18**, **19**, **21**, **24**, and **26** were evaluated for their inhibition of cold-induced microtubule disassembly[19] and for their cytotoxicity against the KB cancer cell line.[20] In Table 1, only the active compounds together with analogues **2a**–**2d** and **3a**–**3d** described previously are included. The compounds of the **6a**–**6d** and **9a**–**9d** series are inactive on microtubule disassembly except for analogue **6a** which presents only a weak inhibition. The difference between these series and the first series **2a**–**2d** and **3a**–**3d** is the position of the peptide and the presence of benzoate at the C2 position. These results confirm that a hydrophobic group at C2 plays a major role in the interaction with the amino acids of the binding site. We also noted that this hydrophobic group needs to be directly linked to the hydroxyl group at C2 since compound **9a** bearing a hydrophobic carbobenzoxy group exhibits no activity on microtubule disassembly. This shows that an important steric constraint exists in this area. Compounds **6a**–**6d** and **9b**–**9d** show no activity against the KB cell line while compound **9a**, though inactive on microtubules, exhibits significant cytotoxicity equivalent to that of compound **5a**. In the taxoid series, a few derivatives have already been reported to be inactive toward tubulin but still cytotoxic.[13,21,22] This may be due to a different cellular mode of action which would deserve further investigation. Concerning the three macrocyclic taxoids **10**, **21**, **26** and their open-chain analogues **14**, **18**, **19**, **24**, only compounds **18** and **19** exhibit very good interaction with microtubules, demonstrating again the importance of a hydrophobic group at the C2 position and that macrocyclization induces a loss of activity on microtubule disassembly. All the compounds are non-cytotoxic toward the KB cell line.

To explain the inactivity of the macrocyclic taxoids, molecular modeling studies were performed on compounds **10**, **21**, and **26** which bear a peptide present in the α-loop. At first, we verified whether the peptide bonds, the urea and amide functions, of these macrocyclic analogues could be in a trans conformation as in the natural peptide. NMR conformational analysis (rotating-frame Overhauser enhancement spectroscopy (ROESY)) in DMSO-d_6 was performed and the observation of exchange spots between some protons showed



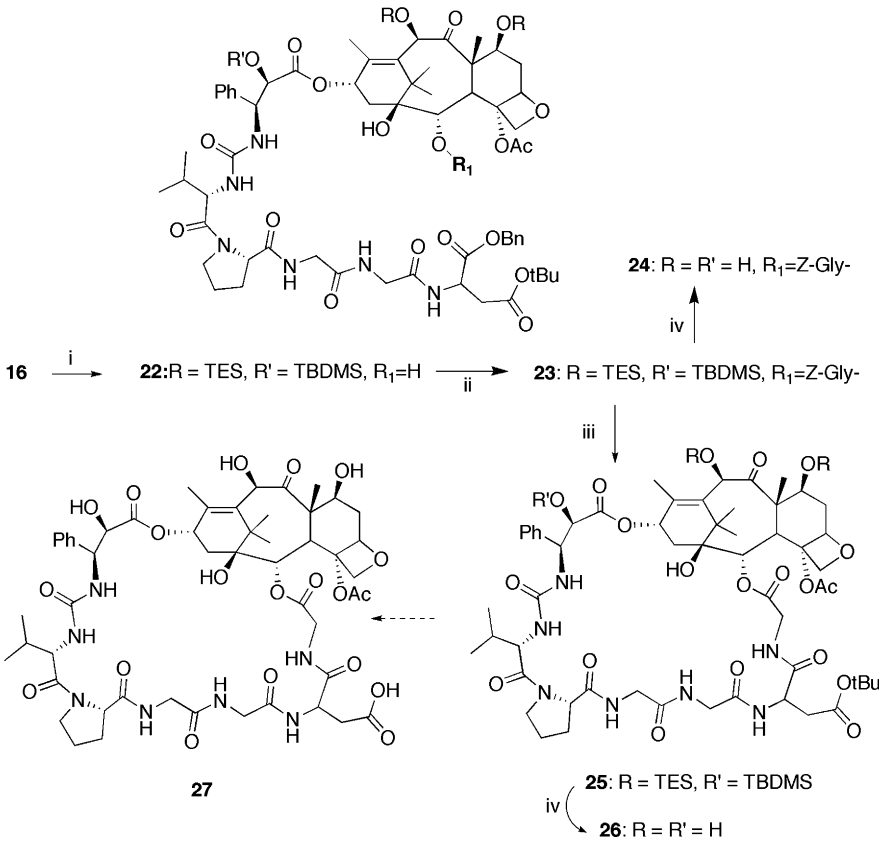
Scheme 3. Reagents and conditions: (i) Pd/C 10%, H_2, MeOH, rt, 5 h and EDCI (5 equiv), HOBt (5 equiv), H_2N-Gly-OBn·HCl (5 equiv), NMM, CH_2Cl_2, rt, 1.25 h (78%); (ii) DCC (30 equiv), DMAP (10 equiv), 3-nitrobenzoic acid (30 equiv), toluene, 60 °C, 5 h (70%); (iii) Pd/C 10%, H_2, MeOH, rt, 5 h and DCC (10 equiv), HOBt (10 equiv), CH_2Cl_2, 0 °C and rt, 5 h (44%); (iv) HF/pyridine 70% (45 equiv), pyridine, CH_3CN, 0 °C and rt (**18**, 56%; **17**, 70%); (v) Pd/C 10%, H_2, MeOH, rt, 5 h (77%).

that the three taxoids **10**, **21**, and **26** were present in solution as two major conformers which were not the result of *cis–trans* isomerization of the urea or amide functions. The correlations observed in the spectra between groups of the molecule were very abundant and did not permit us to conclude on the conformation (T-shaped or 'non-polar' form) adopted by our compounds. Molecular dynamic modeling studies in vacuum media were performed on these macrocyclic taxoids using Sybyl 6.9 software and Tripos force field. For each compound, the lower energy conformers were determined and manually docked in the β-tubulin binding site[7] to observe the possible interactions. For derivative **10**, most of the best conformers obtained were unable to fit the β-tubulin binding pocket (Fig. 4).

We noted that the peptide chain is far from the taxane skeleton such that some amino acids are in close contact with the binding site and there is an important overlap with the His-229 environment thereby explaining the inactivity of this compound. The tridimensional structure of **21** was determined by X-ray crystallography. The good superimposition of this X-ray structure and one of the lower energy conformers of **21** (Fig. 5) allowed us to validate our molecular dynamic modeling study of this compound. The superimposition of the X-ray structure of **21** and paclitaxel **1a** agreed with the T-shaped conformation of **1a**, with a good superimposition of the 3′-phenyl and 2-benzoate but like compound **10**, the peptide chain is far from the taxane skeleton and follows neither the 3′-benzamide orientation nor the orientation of the α-loop.

Thus, this peptide chain conformation induces unfavorable interactions within the β-tubulin binding site particularly at the His-229 level. On the other hand, the peptide chain conformation of lower energy conformer **26** is very different from that of the previous macrocycles and this chain seems to pass around His-229. However the *tert*-butyl ester protecting group of the aspartic acid moiety produces steric bulk and certainly prevents binding to β-tubulin (Fig. 6). The results obtained from docking visualizations and supported by NMR and X-ray experiments are in good agreement with the biological results and explain the inactivity of the three macrocyclic taxoids. It can also be deduced that the conformation of these macrocycles is too rigid in water or in the presence of tubulin, preventing these compounds from adopting a favorable geometry for microtubule interaction.

Concerning the good biological activity of compounds **18** and **19**, some molecular modeling studies have been carried out. Because of the many degrees of freedom,



Scheme 4. Reagents and conditions: (i) Pd/C 10%, H_2, MeOH, rt, 5 h and EDCI (5 equiv), HOBt (5 equiv), H_2N-Asp(OtBu)-OBn (5 equiv), NMM, CH_2Cl_2, rt, 5.75 h (57%); (ii) DCC (30 equiv), DMAP (0.1 equiv), ZNH-Gly-OH (30 equiv), toluene, rt, 15 h (60%); (iii) Pd/C 10%, H_2, MeOH, rt, 5 h and EDCI (5 equiv), HOBt (5 equiv), CH_2Cl_2/DMF 5:1, 0 °C, 7.5 h (53%); (iv) HF/pyridine 70% (45 equiv), pyridine, CH_3CN, 0 °C and rt (**26**, 60%; **24**, 73%).

Table 1. Biological activities of compounds **6a**–**6d**, **9a**–**9d**, **10**, **14**, **17**–**19**, **22**, and **23** compared with docetaxel **1b** and compounds **2a**–**2d**, **3a**–**3d**, **4**, and **5a**–**b**[11]

Compound	Microtubule disassembly IC_{50}[a] (μM)	Cytotoxicity against KB IC_{50}[b] (μM)
Docetaxel **1b**	0.5	0.0001
2a	0.8	0.67
3a	0.8	0.004
2b	1.7	4.50
3b	0.5	0.05
2c	0.6	>10
3c	0.4	0.10
2d	0.5	>10
3d	0.3	4.00
4	2.6	1.10
5a	Inactive	0.09
5b	1.0	1.05
6a	51	>10
9a	Inactive	0.09
18	0.5	>10
19	1.0	>10

[a] IC_{50} is the concentration that inhibits 50% of the rate of microtubule disassembly.

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

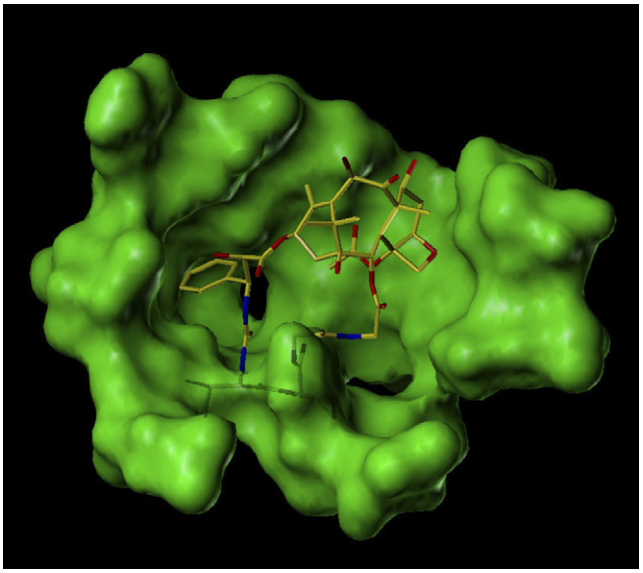


Figure 4. Lower energy conformer of **10** (yellow) bound to β-tubulin in globular form (green).

it was not possible to isolate one or two particular conformers of **18** and **19**. Thus, the taxane moiety of these molecules can easily be docked to the taxol site while the peptide chain is located on the exterior and can adopt multiple conformations whatever the nature of the amino acids of the chain.

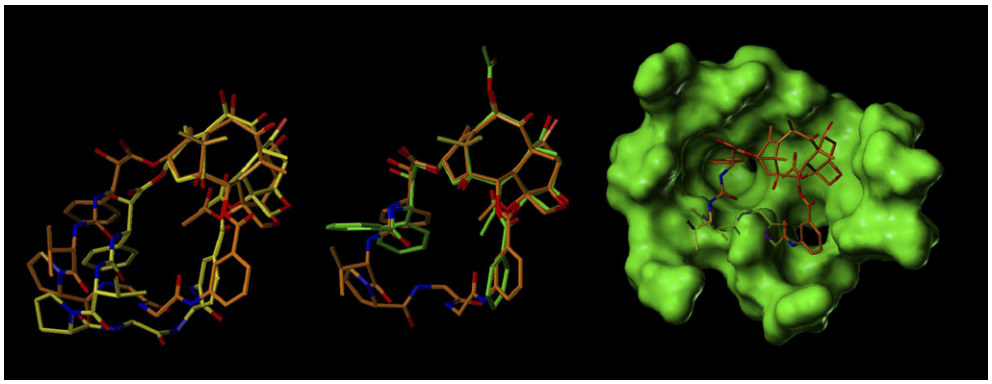


Figure 5. X-ray structure **21** (orange) (a) superimposed with lower energy conformer of compound **21** (yellow), (b) superimposed with T-shaped paclitaxel **1a** (green), and (c) bound to β-tubulin in globular form (green).

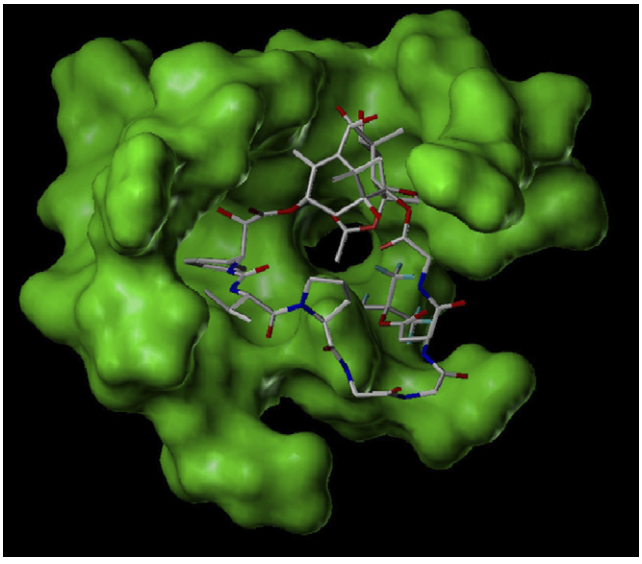


Figure 6. Lower energy conformer of **26** (white) bound to β-tubulin in globular form (green).

4. Conclusion

Twenty-six new taxoids bearing amino acids have been synthesized including three macrocyclic derivatives. Compared to the C3′ analogues **2a**–**2d**, **3a**–**3d**, compounds **6a**–**6d** and **9a**–**9d** having a peptide side chain at C2 were devoid of any tubulin interaction as were macrocyclic compounds **10**, **21**, **26** and the open-chain compounds **14** and **24**. Only acyclic taxoids **18** and **19** exhibit significant activity on microtubule disassembly. These results confirm the importance of the C2-hydrophobic group for a good interaction with tubulin and also the steric constraint in this area due to the presence of histidine 229. The insertion of the α-loop peptide between the 3′-NH and 2-C positions of docetaxel did not improve the interactions with the β-tubulin binding site. However, in this configuration, the peptide is perhaps too constrained and cannot fit in the binding site.

However, taken as a whole, our studies show that specific amino acids of the α-loop as in compounds **2a**–**2d**, **3a**–**3d**, **18**, and **19** can maintain or increase the activity on microtubule disassembly. The good interaction of these derivatives can be compared with the results obtained by Amos et al. on tau protein.[10] This protein has a microtubule binding domain containing several copies of a conserved motif which binds to a site on β-tubulin that overlaps with the paclitaxel binding site.[23,10] Futhermore, part of the tau repeat motif has the sequence Thr-His-Val-Pro-Gly-Gly-Asn, resembling the conserved sequence Thr-Val-Val-Pro-Gly-Gly-Asp-Leu in the extended loop of α-tubulin.[24] It has been proposed[10] that this motif could bind close to the taxol site on β-tubulin and our results strongly support this hypothesis since, for instance, compounds **2d** or **3d**, as or more active than docetaxel, possess the Val-Pro-Gly-Gly motif with a favorable interaction at the taxol binding site. These amino acids could occupy the same area as the one taken by the corresponding peptide chain of tau in its complex with tubulin. The successive addition of the amino acids at N3′ shows that, after a decrease of activity for **2b**, the addition of the following two glycines allows the peptide chain to recover a conformation for a better interaction. Another peptide chain can be easily placed in this area, but the lower activity of **4** and **5b** indicates a preference in favor of the α-loop-specific amino acids, thereby confirming our working hypothesis.

On the contrary, the same peptide chain introduced at the C2 position cannot adopt the geometry observed in **2d** or **3d** and mainly interacts with His-229. Concerning macrocyclic compound **21**, the ortho position would be worth further study in order to place the peptide chain far from the histidine residue.

The results may lead to the design of new taxoid compounds bearing a tau repeat motif at strategic positions on docetaxel or paclitaxel in order to improve tubulin interaction.

5. Experimental

5.1. General

^{1}H and ^{13}C NMR spectra were recorded with a Bruker AM 300 or 500 MHz. ROESY spectra were

performed on a Bruker AM 500 spectrometer. Chemical shifts are given as δ values (in parts per million: ppm) and are referenced to tetramethylsilane or to the residual solvent proton or carbon peak. Mass and high-resolution mass spectra were obtained with an LCT Micromass or performed with a Voyager-DE STR (PerSeptive Biosystems®). SDS silica gel 60 (35–70μm) was used for flash chromatography or preparative chromatography purification of some compounds. LH-20 Sephadex (Pharmacia) was used to purify some products synthesized with DCC. All chemicals were purchased from Fluka, Aldrich or Acros and the protected amino acids used without further purification from Senn Chemicals or Neosystem. Solvents were purchased from SDS and were dried before use. Standard workup means extraction with a suitable solvent (EtOAc unless otherwise specified), washing the organic layer with a solution of saturated aqueous sodium hydrogen carbonate solution and H_2O or brine, drying over Na_2SO_4, and evaporation under reduced pressure. Docetaxel was a gift from Dr. Alain Commerçon (Aventis-Pharma). Microtubular proteins were purified from mammalian brain as previously described.[25] Cytotoxicity and microtubule disassembly inhibition were carried out according to established literature protocols.[19,20]

5.2. General procedure for esterification of C2 hydroxyl

A typical procedure is described for the synthesis of compound **7a**. A solution of the amino acid ZNH-Gly-OH (119.2 mg, 0.57 mmol, 30 equiv), DCC (117.7 mg, 0.57 mmol, 30 equiv), and DMAP (0.23 mg, 0.0019 mmol, 0.1 equiv) in anhydrous toluene (1 mL) was stirred at room temperature for 15 min before adding taxoid **8** (20 mg, 0.019 mmol) in anhydrous toluene (1 mL). The solution was stirred at room temperature for 15 h and then filtered through a Celite gel column using EtOAc and concentrated in vacuo. After standard workup, the crude product was dissolved in petroleum ether with a few drops of MeOH and stored at 4 °C. White crystals of *N*-acylurea were removed by filtration and the filtrate was purified by preparative chromatography (CH_2Cl_2/MeOH 95:5) to afford pure **7a** (15 mg, 63%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: −0.33 (s, 3H), −0.11 (s, 3H), 0.54–0.70 (m, 12H), 0.75 (s, 9H), 0.93–1.03 (m, 18H), 1.16 (s, 3H), 1.19 (s, 3H), 1.41 (s, 9H), 1.64 (s, 3H), 1.70–1.80 (m, 1H), 1.78–1.96 (m, 1H), 1.82 (s, 3H), 2.02–2.20 (m, 1H), 2.32 (s, 3H), 2.46–2.58 (m,1H), 3.74 (d, $J_{H,H}$ = 7 Hz, 1H), 3.97 (d, $J_{H,H}$ = 5 Hz, 2H), 4.21 (dAB, $J_{H,H}$ = 8 Hz, 1H), 4.33–4.38 (q,$J_{H,H}$ = 7 Hz, 1H), 4.46 (br s, 1H), 4.47 (dAB, $J_{H,H}$ = 8 Hz, 1H), 4.91 (d, $J_{H,H}$ = 8 Hz, 1H), 5.07–5.13 (d, $J_{H,H}$ = 12 Hz, 2H), 5.19 (s, 1H), 5.23 (dl hidden, 1H), 5.46 (d, $J_{H,H}$ = 7 Hz, 1H), 5.53 (m, 1H), 6.15 (t, $J_{H,H}$ = 9 Hz, 1H), 7.19–7.38 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: −6.0, −5.4, 5.2, 5.9, 6.8, 6.9, 10.4, 13.7, 18.1, 20.7, 22.9, 25.4, 26.3, 28.2, 35.3, 37.2, 43.0, 43.3, 46.4, 56.7, 58.2, 67.3, 71.9, 72.6, 75.3, 77.2, 78.5, 80.0, 80.8, 84.0, 126.4, 127.6, 128.5, 128.1, 128.4, 134.4, 136.0, 137.5, 138.7, 155.2, 156.6, 169.9, 170.3, 171.6, 205.3. MS (ESI^+): *m/z* 1259.9 [M+Na^+].

The synthesis of compound **8** is described in Ref. 12.

Compounds **11**, **17**, and **23** were prepared according to this method. For experimental details and spectral data, see the Supporting Information.

5.3. General procedure for removal of the benzyloxycarbonyl and the benzyl ester protecting groups by hydrogenolysis

A typical procedure is described for the synthesis of compound **19**. Compound **18** (20 mg, 0.01 mmol) was stirred at room temperature with MeOH (2 mL) and Pd on charcoal (10%) under H_2 for 5 h. After filtration and washing of the catalyst, the organic layer was concentrated and pure compound **19** was obtained (14 mg, 77%). ^{1}H NMR (500 MHz, CD_3OD) δ: 0.90 (d, $J_{H,H}$ = 6.5 Hz, 3H), 0.97 (d, $J_{H,H}$ = 6.5 Hz, 3H), 1.14 (s, 3H), 1.21 (s, 3H), 1.71 (s, 3H), 1.75–1.85 (m, 1H), 1.79–1.90 (m, 1H), 1.93 (s, 3H), 1.94–2.08 (m, 1H), 1.95–2.06 (m, 1H), 2.10–2.20 (m, 2H), 2.15–2.24 (m, 1H), 2.31–2.42 (m, 1H), 2.41–2.52 (m, 1H), 2.43 (s, 3H), 3.51–3.59 (m, 1H), 3.59 (d, $J_{H,H}$ = 17 Hz, 1H), 3.73–3.80 (m, 1H), 3.76 (d, $J_{H,H}$ = 17 Hz, 1H), 3.90 (d, $J_{H,H}$ = 7 Hz, 1H), 3.97 (t, $J_{H,H}$ = 17 Hz, 2H), 4.18–4.27 (m, 3H), 4.29–4.37 (m, 2H), 4.61 (d, $J_{H,H}$ = 3 Hz, 1H), 5.02 (d, $J_{H,H}$ = 9.5 Hz, 1H), 5.29 (s, 1H), 5.38 (d, $J_{H,H}$ = 3 Hz, 1H), 5.63 (d, $J_{H,H}$ = 7 Hz, 1H), 6.23 (t, $J_{H,H}$ = 9 Hz, 1H), 6.95 (d, $J_{H,H}$ = 7 Hz, 1H), 7.23–7.28 (m, 2H), 7.32–7.44 (m, 5H), 7.47 (s, 1H). ^{13}C NMR (125 MHz, CD_3OD) δ: 10.5, 14.6, 18.1, 20.0, 21.7, 23.3, 26.2, 27.2, 30.4, 32.2, 37.0, 37.6, 43.8, 44.4, 44.6, 48.0, 48.9, 57.6, 57.9, 58.9, 62.1, 72.7, 72.9, 75.2, 75.7, 76.4, 77.7, 79.3, 82.4, 86.0, 117.5, 120.4, 121.0, 128.1, 128.5, 129.5, 130.2, 132.2, 138.0, 139.6, 141.3, 149.6, 159.9, 168.4, 171.3, 172.1, 173.7, 174.7, 175.2, 176.5, 211.3. HRMS: *m/z* calcd for $C_{53}H_{68}N_6O_{18}{\cdot}Na^+$ 1099.4488; found 1099.4441 (Δ = −4.3 ppm).

Compounds **6a**–**6d** were prepared according to this method. For experimental details and spectral data, see the Supporting Information.

5.4. General procedure for peptide bond formation on taxoids

A typical procedure is described for the synthesis of compound **7d**. To a solution of the taxoid **7c** obtained after deprotection of the benzyl ester by hydrogenolysis (215 mg, 0.154 mmol) in CH_2Cl_2 (12 mL) were added the amino acid ZHN-Val-OH (193.5 mg, 0.77 mmol, 5 equiv), NMM (85 μL, 0.77 mmol, 5 equiv), EDCI (147.6 mg, 0.77 mmol, 5 equiv), and HOBt (104 mg, 0.77 mmol, 5 equiv). The solution was stirred for 1 h at room temperature with a $CaCl_2$ drying tube. After workup, the residue was purified by silica gel chromatography (Et_2O/MeOH 1:0 to 96:4) to afford pure **7d** (171 mg, 75%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: −0.33 (s, 3H), −0.09 (s, 3H), 0.50–0.68 (m, 12H), 0.75 (s, 9H), 0.85–1.01 (m, 24H), 1.12 (s, 6H), 1.41 (s, 9H), 1.62 (s, 3H), 1.62–1.75 (m, 1H), 1.80–2.05 (m, 6H), 1.80 (s, 3H), 2.05–2.34 (m, 1H), 2.34 (s, 3H), 2.37–2.53 (m,1H), 3.45–3.58

(m, 1H), 3.62–3.74 (m, 1H), 3.72 (d, $J_{H,H}$ = 7 Hz, 1H), 3.97–4.28 (m, 4H), 4.28–4.40 (m, 4H), 4.43 (s, 1H), 4.46 (d, $J_{H,H}$ = 8 Hz, 1H), 4.76 (d, $J_{H,H}$ = 9 Hz, 1H), 5.11 (s, 3H), 5.25 (d, $J_{H,H}$ = 10 Hz, 1H), 5.43 (d, $J_{H,H}$ = 7 Hz, 1H), 5.58 (d, $J_{H,H}$ = 8 Hz, 1H), 6.10 (t, $J_{H,H}$ = 8 Hz, 1H), 7.16 (d, $J_{H,H}$ = 7 Hz, 1H), 7.26–7.42 (m, 10H), 8.04 (d, $J_{H,H}$ = 5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: −6.0, −5.3, 5.3, 6.0, 6.8, 6.9, 10.3, 13.7, 17.5, 18.1, 19.1, 20.6, 23.0, 25.0, 25.4, 26.2, 28.1, 28.5, 31.5, 35.4, 37.2, 43.0, 43.2, 46.4, 47.9, 50.4, 56.9, 57.5, 58.3, 61.1, 67.2, 72.3, 72.6, 75.1, 75.4, 75.5, 77.2, 80.6, 80.8, 84.0, 126.3, 127.9, 128.6, 128.3, 134.1, 136.0, 138.1, 138.5, 155.7, 156.3, 169.5, 169.7, 170.5, 171.5, 171.7, 172.3, 205.1. MS (ESI^+): *m/z* 1513.1 [M+Na^+].

In the same manner, compounds **7b**, **7c**, **16** and **22** were synthesized (see Supporting Information).

5.5. General procedure for insertion of the urea function at NH-3′ position

A typical procedure is described for the synthesis of compound **13**. CDI (129 mg, 0.79 mmol, 3 equiv) in CH_2Cl_2/CH_3CN 1:1 (2 mL) was stirred at room temperature and a solution of tripeptide H_2N-Val-Pro-Gly-OBn (245 mg, 0.53 mmol, 2 equiv) with NMM (189 μL, 1.71 mmol) in CH_2Cl_2/CH_3CN 1/1 (4 mL) was added dropwise and the reaction mixture was stirred at 60 °C. After 5 min compound **12** (250 mg, 0.26 mmol) in CH_2Cl_2/CH_3CN 1/1 (6 mL) was added and the reaction mixture was stirred at 60 °C for 1 h. After workup, the crude product was purified by silica gel chromatography (heptane/EtOAc 2:8) to afford pure compound **13** (white powder, 286 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: −0.34 (s, 3H), −0.14 (s, 3H), 0.54–0.74 (m, 12H), 0.74 (s, 9H), 0.87–1.02 (m, 24H), 1.10 (s, 3H), 1.18 (s, 3H), 1.63 (s, 3H), 1.80 (s, 3H), 1.80–2.30 (m, 8H), 2.43 (s, 3H), 2.43–2.60 (m,1H), 3.51 (d, $J_{H,H}$ = 7 Hz, 1H), 3.52–3.61 (m, 1H), 3.62–3.78 (m, 1H), 3.92 (d, $J_{H,H}$ = 7 Hz, 1H), 4.01 (d, $J_{H,H}$ = 4 Hz, 2H), 4.29–4.44 (m, 2H), 4.44 (d, $J_{H,H}$ = 1.5 Hz, 1H), 4.49–4.53 (m, 1H), 4.64 (q, $J_{H,H}$ = 9 Hz, 2H), 4.96 (d, $J_{H,H}$ = 9 Hz, 1H), 5.09 (s, 1H), 5.14 (s, 2H), 5.36 (br s, 1H), 5.74 (br s, 1H), 6.14 (t, $J_{H,H}$ = 9 Hz, 1H), 7.13–7.36 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: −6.0, −5.5, 5.3, 6.0, 6.9, 7.0, 10.7, 13.7, 17.4, 18.2, 19.4, 20.8, 23.2, 25.0, 25.5, 26.2, 27.6, 31.3, 35.8, 37.4, 41.4, 42.8, 46.6, 47.7, 56.4, 58.3, 59.9, 60.0, 67.2, 72.8, 74.1, 74.8, 75.5, 77.2, 78.2, 82.6, 83.7, 126.5, 127.7, 128.3, 128.4, 128.5, 128.6, 133.9, 134.5, 135.1, 138.0, 156.7, 169.7, 171.4, 171.8, 172.9, 206.0. MS (ESI^+): *m/z* 1356.0 [M+Na^+].

Synthesis of compound **12** is described in Ref 13.

5.6. Synthesis of macrocyclic taxoids 15, 20, and 25

5.6.1. Compound 15. A solution of taxoid **11** (85 mg, 0.056 mmol) previously deprotected by hydrogenolysis, containing HOBt (37.8 mg, 0.28 mmol, 5 equiv) in CH_2Cl_2/DMF 5:1 (26 mL), was stirred at 0 °C for 15 min and a solution of EDCI (53.7 mg, 0.28 mmol, 5 equiv) in CH_2Cl_2/DMF 5:1 (2 mL) was added dropwise. The mixture was stirred at 0 °C, for 2 h water was added (15.8 mL), and the aqueous layer was extracted three times with EtOAc. After standard workup, the crude residue was purified by preparative chromatography (CH_2Cl_2/MeOH 9:1) to afford pure **15** (41.5 mg, 58%) as a white amorphous solid. ^{1}H NMR (300 MHz, CD_3OD) δ (major compound): −0.32 (s, 3H), −0.27 (s, 3H), 0.58–0.73 (m, 12H), 0.76 (s, 9H), 0.99–1.11 (m, 24H), 1.19 (s, 3H), 1.27 (s, 3H), 1.66 (s, 3H), 1.66–1.80 (m, 2H), 1.80–2.03 (m, 2H), 1.88 (s, 3H), 2.03–2.35 (m, 4H), 2.51 (s, 3H), 2.51–2.72 (m, 1H), 3.46–3.62 (m, 2H), 3.73 (d, $J_{H,H}$ = 7 Hz, 1H), 3.74–4.26 (m, 4H), 4.27–4.54 (m, 5H), 4.82 (d, $J_{H,H}$ = 2 Hz, 1H), 5.06 (d, $J_{H,H}$ = 9 Hz, 1H), 5.24 (s, 1H), 5.53 (d, $J_{H,H}$ = 6.5 Hz, 1H), 5.58 (d, $J_{H,H}$ = 2 Hz, 1H), 6.29 (t, $J_{H,H}$ = 9 Hz, 1H), 6.83 (d, $J_{H,H}$ = 7 Hz, 1H), 6.95 (d, $J_{H,H}$ = 10 Hz, 1H), 7.25–7.43 (m, 5H). δ (minor compound): 1.32 (s, 3H), 2.55 (s, 3H), 4.98 (d, $J_{H,H}$ = 9 Hz, 1H), 5.21 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ: −5.8, −4.8, 6.3, 7.0, 7.3, 7.4, 10.9, 14.8, 18.6, 19.1, 19.7, 22.5, 22.9, 26.1, 26.6, 27.4, 29.7, 30.1, 36.7, 37.1, 42.7, 43.2, 44.3, 47.2, 47.4, 54.8, 56.8, 58.7, 61.8, 72.2, 74.3, 75.3, 76.8, 77.2, 78.7, 82.4, 85.4, 127.9, 128.3, 129.5, 136.6, 138.5, 140.8, 160.3, 170.4, 171.3, 172.0, 173.4, 174.4, 177.7, 212.0. MS (ESI^+): *m/z* 1304.8 [M+Na^+].

5.6.2. Compound 25. Compound **25** was synthesized in the same manner. Application of the macrocyclic procedure to **23** (40 mg, 0.023 mmol) and purification by preparative chromatography (CH_2Cl_2/MeOH 85:15) afforded pure compound **25** (18.3 mg, 53%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ (major compound): −0.40 (s, 3H), −0.08 (s, 3H), 0.50–0.69 (m, 12H), 0.78 (s, 9H), 0.83–1.01 (m, 24H), 1.16 (s, 3H), 1.18 (s, 3H), 1.38 (s, 9H), 1.63 (s, 3H), 1.81–2.24 (m, 8H), 1.82 (s, 3H), 2.40 (s, 3H), 2.39–2.67 (m, 1H), 2.65–2.87 (m, 2H), 3.52–3.69 (m, 2H), 3.73 (d, $J_{H,H}$ = 7 Hz, 1H), 3.77–4.22 (m, 6H), 4.23–4.37 (m, 5H), 4.41 (d, $J_{H,H}$ = 1.5 Hz, 1H), 4.48–4.53 (m, 1H), 4.91 (d, $J_{H,H}$ = 8 Hz, 1H), 5.10 (s, 1H), 5.11 (d hidden, 1H), 5.47 (d, $J_{H,H}$ = 7 Hz, 1H), 6.16 (t, $J_{H,H}$ = 9 Hz, 1H), 7.22–7.43 (m, 5H), 7.59 (br s, 1H), 7.73 (d, $J_{H,H}$ = 8 Hz, 1H). δ (minor compound): −0.36 (s, 3H), 0.72 (s, 9H), 1.45 (s, 9H), 2.52 (s, 3H), 5.53 (d, $J_{H,H}$ = 7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: −6.0, −5.3, 5.3, 6.0, 6.9, 10.4, 13.8, 18.1, 19.0, 21.0, 23.3, 25.5, 26.4, 27.9, 28.7, 28.8, 36.3, 37.2, 42.8, 42.9, 43.8, 46.5, 47.8, 58.3, 61.0, 72.2, 72.5, 74.8, 75.3, 81.1, 82.2, 84.2, 126.7, 128.4, 128.7, 133.4, 138.4, 138.6, 157.0, 168.7, 168.8, 168.9, 169.7, 170.3, 171.1, 171.8, 171.9, 172.7, 204.9. MS (ESI^+): *m/z* 1532.6 [M+Na^+].

5.6.3. Compound 20. A solution of taxoid **17** (183 mg, 0.119 mmol) previously deprotected by hydrogenolysis, and HOBt (160.8 mg, 1.19 mmol, 10 equiv) in CH_2Cl_2 (33 mL) was stirred at 0 °C for 15 min and a solution of DCC (245.5 mg, 1.19 mmol, 10 equiv) in CH_2Cl_2 (10 mL) was added dropwise. The mixture was stirred at 0 °C for 1 h and then stirred at room temperature for an additional 4 h. The solution was filtered through a Celite gel column using EtOAc and concentrated in vacuo. After standard workup, the crude residue was

purified on a LH-20 Sephadex column (Ø 2.5 cm × H 72 cm, bed: 353 mL, flux: 2.35 mL/min, CH_2Cl_2/MeOH 1:1) followed by preparative chromatography (EtOAc/MeOH 95:5) to afford pure compound **20** (73.6 mg, 44%) as a white amorphous solid. ^{1}H NMR (500 MHz, CD_3OD) δ (major compound): −0.36 (s, 3 H), −0.04 (s, 3H), 0.60–0.75 (m, 12H), 0.82 (s, 9H), 0.95 (d, $J_{H,H}$ = 7 Hz, 3H), 1.00–1.06 (m, 21H), 1.25 (s, 3H), 1.32 (s, 3H), 1.70 (s, 3H), 1.83–1.93 (m, 3H), 1.94 (s, 3H), 1.94–2.12 (m, 1H), 2.14–2.22 (m, 3H), 2.41–2.50 (m, 1H), 2.56–2.70 (m, 1H), 2.63 (s, 3H), 3.18 (d, $J_{H,H}$ = 16 Hz, 1H), 3.53–3.81 (m, 2H), 3.63–3.70 (m, 2H), 3.85 (d, $J_{H,H}$ = 6.5 Hz, 1H), 4.08 (d, $J_{H,H}$ = 16 Hz, 1H), 4.16 (dAB, $J_{H,H}$ = 8 Hz, 1H), 4.23 (dAB, $J_{H,H}$ = 8 Hz, 1H), 4.25–4.36 (m, 1H), 4.43–4.57 (m, 2H), 4.74 (br s, 1H), 4.99–5.05 (m, 1H), 5.26 (s, 1H), 5.45 (br s, 1H), 5.75 (d, $J_{H,H}$ = 6.5 Hz, 1H), 6.54 (t, $J_{H,H}$ = 9 Hz, 1H), 7.25–7.47 (m, 7H), 7.80 (t, $J_{H,H}$ = 8 Hz, 1H), 8.94 (s, 1H). δ (minor compound): −0.33 (s, 3H), −0.07 (s, 3H), 0.77 (s, 9H), 0.80 (d hidden, 3H), 0.87 (d, $J_{H,H}$ = 7 Hz, 3H), 1.17 (s, 3H), 1.18 (s, 3H), 1.71 (s, 3H), 1.81–1.96 (m, 1H), 1.91 (s, 3H), 2.00–2.06 (m, 2H), 2.13–2.21 (m, 1H), 2.16–2.26 (m, 2H), 2.56–2.70 (m, 1H), 2.61–2.70 (m, 1H), 2.71 (s, 3H), 3.44–3.53 (m, 2H), 3.94 (d, $J_{H,H}$ = 7 Hz, 1H), 4.25–4.36 (m, 2H), 4.43–4.57 (m, 2H), 4.69 (d, $J_{H,H}$ = 2 Hz, 1H), 4.99–5.05 (m, 1H), 5.24 (s, 1H), 5.56 (d, $J_{H,H}$ = 7 Hz, 1H), 5.59 (br s, 1H), 6.17 (t, $J_{H,H}$ = 9 Hz, 1H), 7.23–7.47 (m, 5H), 8.75 (s, 1H). ^{13}C NMR (125 MHz, CD_3OD) δ (major compound): −5.7, −5.1, 6.3, 7.0, 7.3, 7.4, 11.1, 15.1, 19.2, 19.8, 22.0, 23.3, 24.0, 24.1, 26.2, 27.5, 29.7, 33.7, 36.6, 36.9, 43.8, 44.5, 44.8, 47.9, 48.1, 57.2, 57.6, 60.1, 63.5, 72.1, 74.5, 76.0, 76.4, 77.0, 77.8, 78.8, 82.4, 85.3, 125.3, 126.5, 128.0, 129.5, 129.0, 130.0, 131.8, 132.1, 136.3, 139.2, 140.3, 142.3, 160.0, 167.6, 171.7, 173.1, 174.1, 174.4, 175.3, 207.9. δ (minor compound): 6.4, 11.2, 14.9, 19.1, 19.6, 21.7, 26.1, 27.0, 33.0, 43.5, 45.0, 47.7, 57.3, 57.9, 59.6, 62.4, 73.1, 74.2, 76.9, 77.1, 77.9, 79.7, 81.9, 85.4, 122.6, 128.2, 129.7, 136.2, 139.5, 172.6. MS (ESI$^+$): *m/z* 1423.78 [M+Na$^+$].

5.7. General procedure for removal of the silyl protecting groups

A typical procedure is described for the synthesis of compound **9d**. To a cooled solution of **7d** (160 mg, 0.107 mmol) in pyridine–acetonitrile (6:93, 7 mL) was added dropwise HF/pyridine (70%, 625 μL, 4.86 mmol, 45 equiv.) at 0 °C, and the mixture was stirred at room temperature for 4h. HF/pyridine (625 μL, 70%, 4.86 mmol, 45 equiv.) was added dropwise at 0 °C. The solution was stirred at room temperature for additional 3.5 h. The reaction was quenched by addition of saturated aqueous sodium hydrogencarbonate solution and the aqueous layer was extracted three times with EtOAc. After standard workup, the crude residue was purified by preparative chromatography (CH_2Cl_2/MeOH 9:1) to afford pure **9d** (85 mg, 69%) as a white amorphous solid. ^{1}H NMR (500 MHz, CD_3OD) δ: 0.94 (d, $J_{H,H}$ = 6.5 Hz, 3H), 0.99 (d, $J_{H,H}$ = 6.5 Hz, 3H), 1.07 (s, 3H), 1.19 (s, 3H), 1.45 (s, 9H), 1.66 (s, 3H), 1.73–1.95 (m, 1H), 1.83–2.00 (m, 1H), 1.87–2.05 (m, 2H), 1.90 (s, 3H), 1.94–2.15 (m, 1H), 2.05–2.25 (m, 1H), 2.10–2.27 (m, 2H), 2.28 (s, 3H), 2.34–2.56 (m,1H), 3.47–3.55 (m, 1H), 3.65–3.70 (m, 1H), 3.77 (d, $J_{H,H}$ = 6.5 Hz, 1H), 3.78–4.07 (m, 4H), 4.18–4.25 (m, 3H), 4.33–4.37 (m, 1H), 4.52 (br s, 1H), 4.60 (d, $J_{H,H}$ = 8.5 Hz, 1H), 4.98 (d, $J_{H,H}$ = 9.5 Hz, 1H), 5.09 (br s, 2H), 5.15 (s, 1H), 5.26 (s, 1H), 5.41 (d, $J_{H,H}$ = 6.5 Hz, 1H), 6.14 (t, $J_{H,H}$ = 8 Hz, 1H), 7.30–7.42 (m, 10H). ^{13}C NMR (125 MHz, CD_3OD) δ: 10.4, 14.5, 18.7, 19.7, 21.5, 23.2, 26.2, 27.1, 28.8, 30.3, 31.9, 37.0, 37.6, 42.8, 43.7, 44.3, 47.9, 49.7, 58.3, 59.0, 59.7, 62.2, 67.8, 72.7, 72.9, 75.2, 75.7, 77.6, 77.9, 78.8, 80.7, 82.1, 86.0, 128.2, 128.7, 129.6, 128.9, 129.1, 129.5, 137.9, 138.2, 139.5, 140.6, 157.7, 158.7, 171.2, 172.0, 172.6, 173.4, 174.5, 175.0, 211.5. HRMS: *m/z* calcd for $C_{58}H_{77}N_5O_{19}{\cdot}Na^+$ 1170.5110; found 1170.5104 (Δ = −0.6 ppm).

Compounds **9a**–**9c**, **10**, **14**, **21**, **18**, **26**, and **24** were prepared according to this method. For experimental details and spectral data, see the Supporting Information.

5.8. X-ray crystal structure analysis for compound 21

A small prismatic colorless crystal of (0.35 × 0.35 × 0.20) mm, obtained from a mixture (methanol:dioxane:ether), was chosen. To prevent crystal decay during data collection, the crystal was placed in a Linderman capillary with the mother liquor. The X-ray structure analysis showed that the crystal compound formula was $C_{53}H_{66}N_6O_{17} + CH_3OH$ with M_w = 1091.16 (Fig. 7). The compound crystallizes in the monoclinic system, space group $P2_1$, with two identical molecules (Z = 2) in the unit-cell, of parameters: a = 13.888(6), b = 15.741(5), c = 16.009(6) Å, β = 110.97(3)°, V = 3268 Å^3; d_{cal} = 1.109 g cm^{-3}, $F(000)$ = 1160, (λ(Mo-Kα) = 0.71073 Å), μ = 0.084 mm^{-1}. Refinement of 688 parameters converged to R1(F) = 0.0535 for the 5090 observed reflections and wR2(F^2) = 0.2054 for all the 6682 unique data with a goodness-of-fit S factor of 1.003 (Fig. 7). CCDC 607125 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at //www.ccdc.cam.ac.uk/deposit [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB 1EZ, UK].

5.9. General procedure for peptide synthesis

A typical procedure is described for the synthesis of dipeptide BocNH-Val-Pro-OBn. To a solution of the amino acid BocNH-Val-OH (500 mg, 2.3 mmol) in CH_2Cl_2 (15 mL) were added the amino acid H_2N-Pro-OBn·HCl (556 mg, 2.3 mmol, 1 equiv), NMM (253 μL, 2.3 mmol, 1 equiv), EDCI (529 mg, 2.76 mmol, 1.2 equiv), and HOBt (373 mg, 2.76 mmol, 1.2 equiv). The solution was stirred for 3.5 h at room temperature with a $CaCl_2$ drying tube. After workup, the residue was purified by silica gel chromatography (heptane/EtOAc 65:35) to afford pure BocNH-Val-Pro-OBn (913 mg, 99%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90 (d, $^3J_{H,H}$ = 7 Hz, 3H), 1.00 (d, $^3J_{H,H}$ = 7 Hz, 3H), 1.42 (s, 9H), 1.91–2.09 (m, 4H), 2.14–2.29 (m, 1H), 3.60–3.70 (m, 1H), 3.73–3.84 (m,

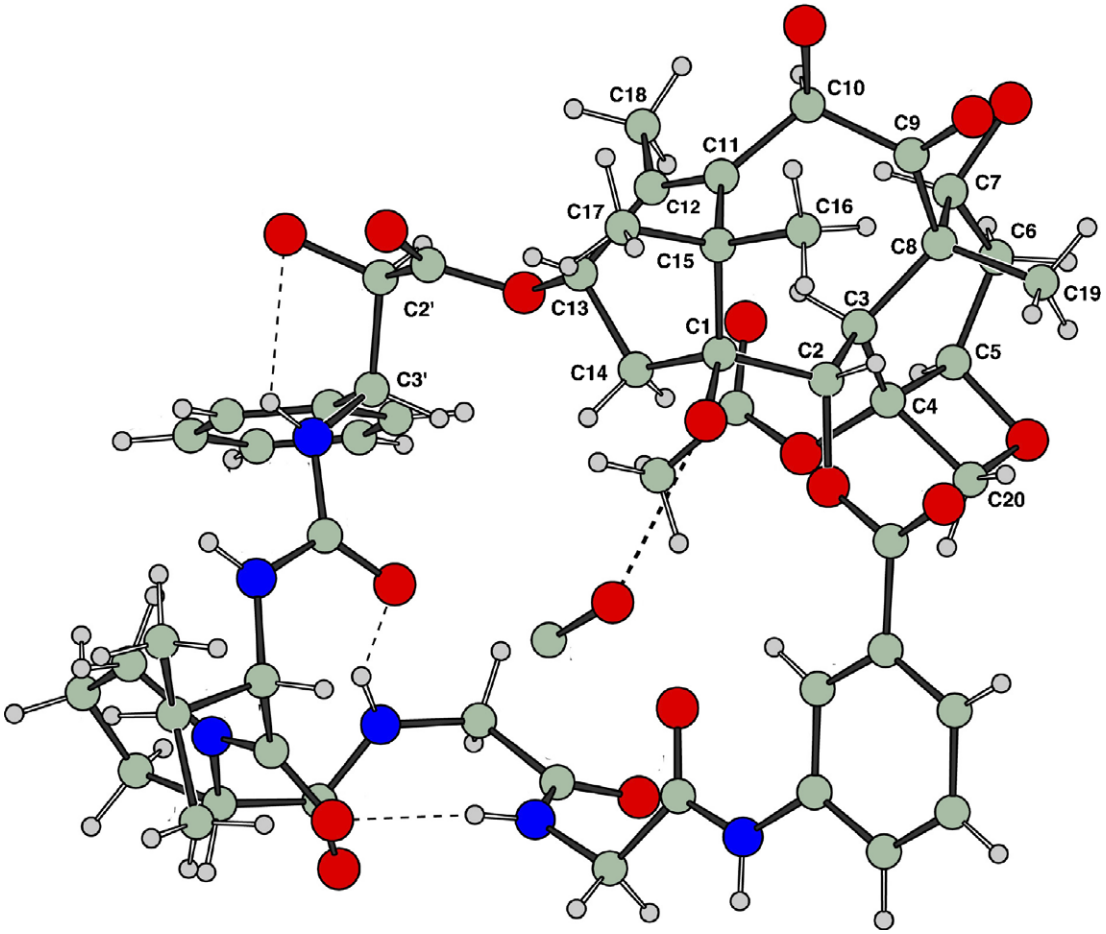


Figure 7. X-ray structure of compound **21**.

1H), 4.24–4.32 (m, 1H), 4.56–4.63 (m, 1H), 5.16 (q, $J_{H,H}$ = 12 Hz, 2H), 5.22 (d, $J_{H,H}$ = 9 Hz, 1H), 7.30–7.40 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.4, 19.3, 25.0, 28.3, 29.0, 31.4, 47.1, 56.8, 58.9, 66.9, 79.5, 128.2, 128.3, 128.5, 135.6, 155.9, 171.2, 171.9. MS (ESI$^+$): *m*/*z* 427.1251 [M+Na$^+$].

In the same manner, the tripeptide BocNH-Val-Pro-Gly-OBn was synthesized: application of the general procedure using dipeptide BocNH-Val-Pro-OBn (600 mg, 1.9 mmol), after hydrogenolysis, with H_2N-Gly-OBn·HCl (396 mg, 1.9 mmol, 1 equiv) in DMF (4 mL) and purification by flash chromatography (CH_2Cl_2/MeOH 95:5) afforded pure tridipeptide BocNH-Val-Pro-Gly-OBn (721.7 mg, 82%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90 (d, $J_{H,H}$ = 7 Hz, 3H), 0.97 (d, $J_{H,H}$ = 7 Hz, 3H), 1.43 (s, 9H), 1.80–2.16 (m, 4H), 2.33–2.43 (m, 1H), 3.52–3.63 (m, 1H), 3.65–3.78 (m, 1H), 4.08 (d, $J_{H,H}$ = 5 Hz, 2H), 4.28 (q,$J_{H,H}$ = 6 Hz, 1H), 4.65 (dd, $J_{H,H}$ = 7.5 Hz,$J'_{H,H}$ = 2 Hz, 1H), 5.15 (q, $J_{H,H}$ = 12 Hz, 2H), 5.22 (d, $J_{H,H}$ = 9 Hz, 1H), 7.30–7.42 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.3, 19.5, 25.1, 28.3, 28.4, 31.3, 41.4, 47.5, 56.8, 59.6, 67.1, 79.6, 128.4, 128.5, 128.6, 135.2, 155.8, 169.4, 171.2, 171.8. HRMS: *m*/*z* calcd for $C_{24}H_{35}N_3O_6$·Na$^+$ 484.2424; found 484.2403 (Δ = −4.2 ppm). $[\alpha]_D^{22}$ −86.7 (*c* 1.0, MeOH).

5.10. Protection of amino acid FmocNH-Asp(OtBu)-OH

To a cooled solution of FmocNH-Asp(OtBu)-OH (500 mg, 1.2 mmol) and NMM (134 μL, 1.2 mmol, 1 equiv) in distilled THF (6 mL) was added dropwise 158.5 μL of isobutylchloroformate (1.2 mmol, 1 equiv) at −15 °C. The mixture was stirred for 5 min and a solution of benzyl alcohol (252 μL, 2.43 mmol, 2 equiv) in THF (1 mL) was added dropwise. The solution was stirred at room temperature for 50 min, filtered through a Celite gel column, and then concentrated in vacuo. The crude residue was purified by flash chromatography (heptane/EtOAc 8:2) and then purified by crystallization in ether with drops of EtOAc, to afford pure compound FmocNH-Asp(OtBu)-OBn (469 mg, 77%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.41 (s, 9H), 2.75–3.00 (m, 2H), 4.23 (t, $J_{H,H}$ = 7 Hz, 1H), 4.30–4.45 (m, 2H), 4.65 (td, $J_{H,H}$ = 4.5 Hz, $J'_{H,H}$ = 9 Hz, 1H), 5.20 (q, $J_{H,H}$ = 12 Hz, 2H), 5.84 (d, $J_{H,H}$ = 9 Hz, 1H), 7.25–7.42 (m, 9H), 7.59 (d, $J_{H,H}$ = 7.5 Hz, 2H), 7.76 (d, $J_{H,H}$ = 7.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.0, 37.7, 47.1, 50.7, 67.3, 67.5, 81.9, 120.0, 125.1, 125.2, 125.2, 125.2, 128.2,

128.4, 128.6, 135.3, 141.3, 143.7, 143.9, 156.0, 170.0, 170.8. HRMS: *m/z* calcd for $C_{30}H_{31}NO_6{\cdot}Na^+$ 524.2049; found 524.2039 ($\Delta = -1.9$ ppm). $[\alpha]_D^{22}$ −15.9 (*c* 1.0, DMF) (in literature[26] $[\alpha]_D^{20}$ −19.4 (*c* 1.0, DMF)). Melting point: 109–110 °C (literature[26] 107–108 °C).

5.11. Computational procedures

All calculations were performed on a PC workstation. All modeling studies were performed using Sybyl 6.9 software. The Tripos force field was used for minimization (Gasteiger-Hückel charges), dielectric constant of 1.0 being employed. In all cases, A, B, C, and D rings of the taxoids were fixed and not submitted to dynamics. Compounds were subjected to molecular dynamics simulation at 800 K for 10,000 fs. Conformations were sampled every 50 fs during the simulation. Each of these conformers was minimized and compared with others with a RMS of 0.3 Å. Superimposition of compounds was based on the backbone atoms of the taxane core.

Docking experiments on macrocyclic compounds were realized first putting manually the molecules in the site and after that using the DOCK software of Sybyl 6.9 with Tripos force field for minimization.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.09.030.

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