Synthesis and Biological Evaluation of Vinca Alkaloids and Phomopsin Hybrids

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Ten hybrids of vinca alkaloids and phomopsin A have been synthesized by linking the octahydrophomopsin lateral chain to the tertiary amine of the cleavamine moiety of anhydrovinblastine (AVLB) and vinorelbine. These compounds have been elaborated in order to obtain original products that may interfere with both binding sites of vinblastine (VLB) and phomopsin in tubulin. Although NMR and molecular modeling studies have shown that the orientation of the added peptide chains of these hybrids is not the same as those of phomopsin A, most of them are very potent inhibitors of microtubules assembly and they present good cytotoxicity against KB cell line. These interesting biological activities may eventually be explained by the fact that their lateral chain resides in a pocket distinct from that of the phomopsin A peptide, at the interface of tubulins β and α.

Introduction

Microtubules, dynamic polymers of $\alpha\beta$ tubulin heterodimers,[1] are major structural components in cells. They play a crucial role in a number of cellular functions such as cell division, as they are key constituents of the mitotic spindle. GTP[a] hydrolysis into GDP is responsible for the dynamic instability of these microtubules. Tubulin binding molecules are one of the most important classes of anticancer agents with major drugs already on the market and many promising compounds in clinical trials.[2] Most of these are derived from complex natural products. They interfere with microtubule assembly and functions, thus resulting in mitotic arrest of eukaryotic cells.

Vinca alkaloids are successful antimitotic drugs inhibiting tubulin polymerization into microtubules. Vinblastine (**1**) and vincristine (**2**) isolated at the end of the 1950s,[3] as well as synthetic vinorelbine or nor-anhydrovinblastine (**4**),[4] are widely used in cancer chemotherapy (Figure 1). Many derivatives have also been elaborated so far,[5] by modification of either the cleavamine[6] or the vindoline moieties.[7] Recently, vinflunine[8] and anhydrovinblastine (**3**) reached advanced clinical phases.[9] Other inhibitors of tubulin polymerization have been shown to bind in the vinca domain.[10] This is the case for the antimitotic cyclopeptides[11,12] phomopsins A and B[13,14] and ustiloxins A−F.[15,16] These novel hexapeptides contain a 13-membered cyclic core that includes a rare tertiary aryl alkyl ether linkage and a lateral chain that, for the phomopsins, consists of three dehydroamino acids. Though potent antimitotic compounds, ustiloxins and phomopsin A are relatively weak cytotoxins. The binding site of vinblastine remained largely unknown until Knossow and co-workers[17] published the X-ray structure of vinblastine bound to the tubulin-colchicine:RB3-SLD complex ($(Tc)_2R$) with a resolution of 4.1 Å. This binding site is at the interface between two tubulin heterodimers in a head-to-tail arrangement, and vinblastine is oriented so that its cleavamine and vindoline moieties each interact with both heterodimers. In addition, these authors showed that the vinblastine site in this complex is very similar to the vinblastine site in tubulin. Very recently, this group also published the 4.1 and 3.8 Å X-ray structures of phomopsin A and soblidotin[18] (a dolastatin 10 analogue) bound to the same tubulin complex.[19] Most importantly, superimposition of both binding sites revealed that they significantly overlap: the cleavamine moiety of vinblastine, the cyclic core of phomopsin A, and the first two amino acids of soblidotin occupy the same area and define the core of the vinca domain (Figure 2). However, the vindoline moiety of vinblastine and the lateral chain of phomopsin A and the main part of soblidotin are oriented in opposite directions. In particular, phomopsin A and soblidotin possess extensive contacts with Tyr β224, one of the residues sandwiching the GDP−GTP nucleotide exchangeable site.

These very important results prompted us to elaborate hybrids of vinblastine and phomopsin A in order to obtain original compounds that may interfere with both binding sites leading to an increased cytotoxicity (Figure 2). Moreover, such hybrids could also be valuable in order to better understand the interactions of these antimitotic drugs with tubulin. We chose to link vinca type compounds to the lateral chain of phomospin A by formation of quaternary ammonium salts on the tertiary amine of the cleavamine moiety (Scheme 1). Anhydrovinblastine and vinorelbine hybrids were synthesized in order to compare their biological activities and, if possible, to see if these are correlated with the configuration at N6′ and thus with the orientation of the added peptide chain.

Chemistry

Even if unsaturated amino acids generally introduce elements of conformational rigidity as well as changes in reactivity, Lacey et al.[14] have shown that *rac*-octahydrophomopsin A (**7**) is as potent as phomopsin A (**5**) on tubulin (IC_{50} of 0.40 μM versus 0.56 μM). Consequently, as the synthesis of the phomopsin tripeptide side chain is far from obvious,[20] we have elaborated lateral chains of various lengths starting from commercially available L-proline, L-isoleucine, and L-aspartic acid (Scheme 1). Pro-Ile-Obzl **12** and Pro-Ile-Asp-$(Obzl)_2$ **17** were synthesized according to classical

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[a] Abbreviations: AVLB, anhydrovinblastine; VLB, vinblastine; nor-AVLB, nor-anhydrovinblastine; Pro, proline; Ile, isoleucine; Asp, aspartic acid; GDP, guanosine diphosphate; GTP, guanosine triphosphate; Tyr, tyrosine.

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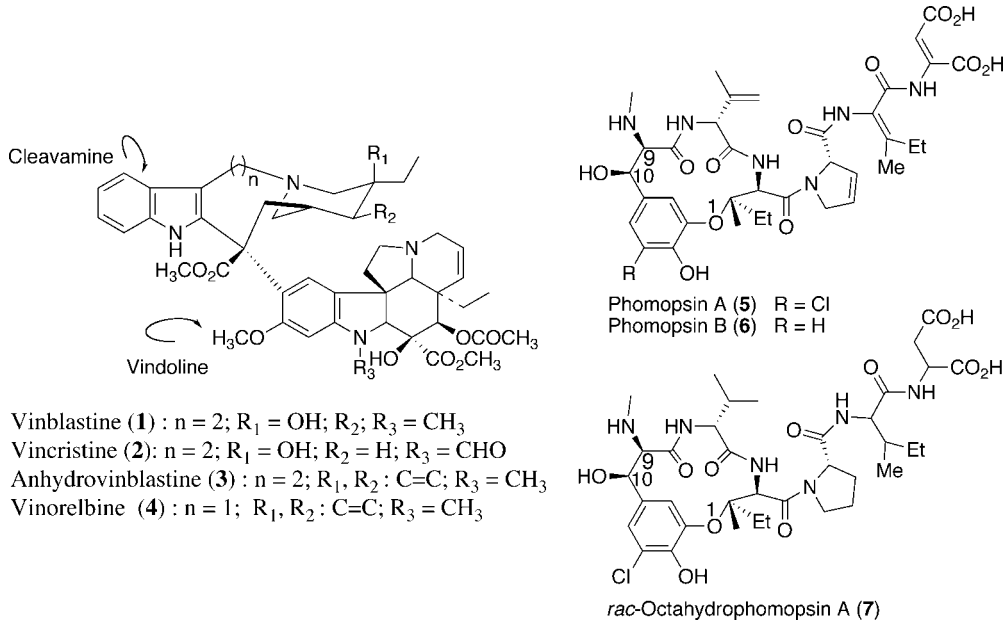


Vinblastine (**1**) : n = 2; R_1 = OH; R_2; R_3 = CH_3
Vincristine (**2**): n = 2; R_1 = OH; R_2 = H; R_3 = CHO
Anhydrovinblastine (**3**) : n = 2; R_1, R_2 : C=C; R_3 = CH_3
Vinorelbine (**4**) : n = 1; R_1, R_2 : C=C; R_3 = CH_3

Figure 1. Vinca alkaloids and phomopsin series.

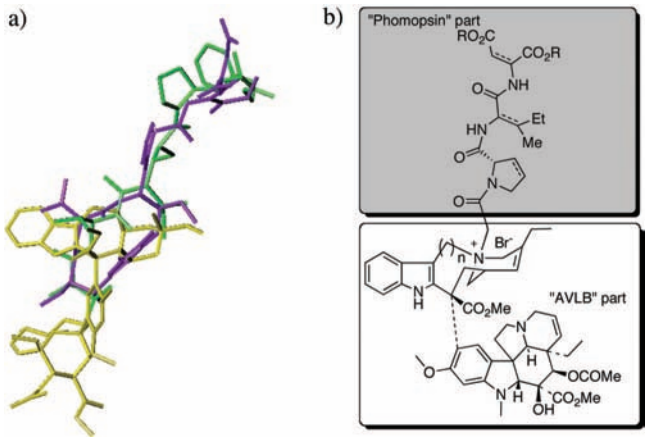


Figure 2. (a) Superimposition of vinblastine (yellow), phomopsin A (violet), and soblidotin (green) in their binding site. (b) Conceived hybrids of vinblastine and phomopsin.

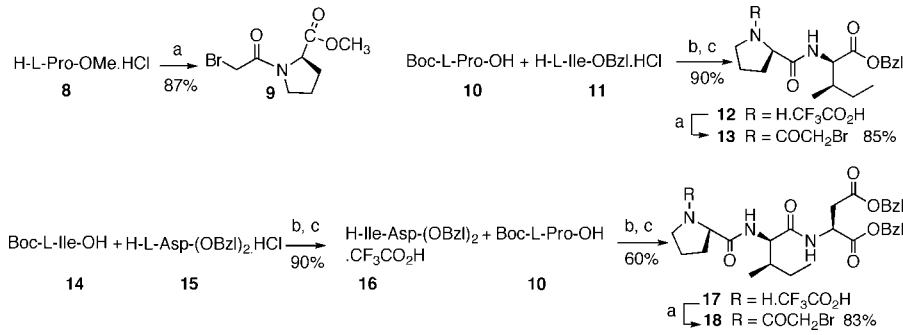
procedures. They, as well as commercial Pro-OMe, were coupled with bromoacetic acid to give three analogues **9**, **13**, and **18** of the phomopsin side chain. These peptide chains were then condensed with anhydrovinblastine synthesized through ferric-ion-mediated coupling of catharanthine with vindoline[21] to give three quaternary ammonium salts[22] **19**, **20**, **22** (Scheme 2). Compounds **20** and **22** were deprotected under mild conditions to give the free acids **21** and **23**. In the same manner, the peptide side chains were condensed with nor-anhydrovinblastine **4** synthesized from **3** in two steps.[23] Five new quaternary ammonium salts **24**−**28** were thus obtained. All these complex compounds were fully characterized, and their NMR spectra were assigned using correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC). Compared with starting compounds **3** and **4**, important modifications of their NMR spectra were observed around N6′, particularly for positions 5′, 7′, 19′, and 24′ that are very deshielded.

Results and Discussion

Biological Evaluation. Compounds **19**−**23** and **24**−**28** were evaluated for inhibition of tubulin polymerization as well as for cytotoxicity on KB cell lines.The results are summarized in Table 1. It was found that all the tested compounds except **28** showed significant microtubule assembly inhibitory activity. Compound **19** is even more potent than vinblastine (IC_{50}/IC_{50VLB} = 0.9), and it is noteworthy that compounds **22** and **27** with a large protected tripeptide side chain (ProIleAsp$(OBzl)_2$) are almost as active as vinblastine ($IC_{50\text{-}19}/IC_{50VLB}$ = 1.3 and $IC_{50\text{-}24}/IC_{50VLB}$ = 1.8) on tubulin. In addition, except compound **27**, all these products showed notable cytotoxicity (IC_{50} < 1 μM) on the KB cell line, especially compounds **19**, **25**, and **26** (0.08, 0.05, and 0.09 μM).

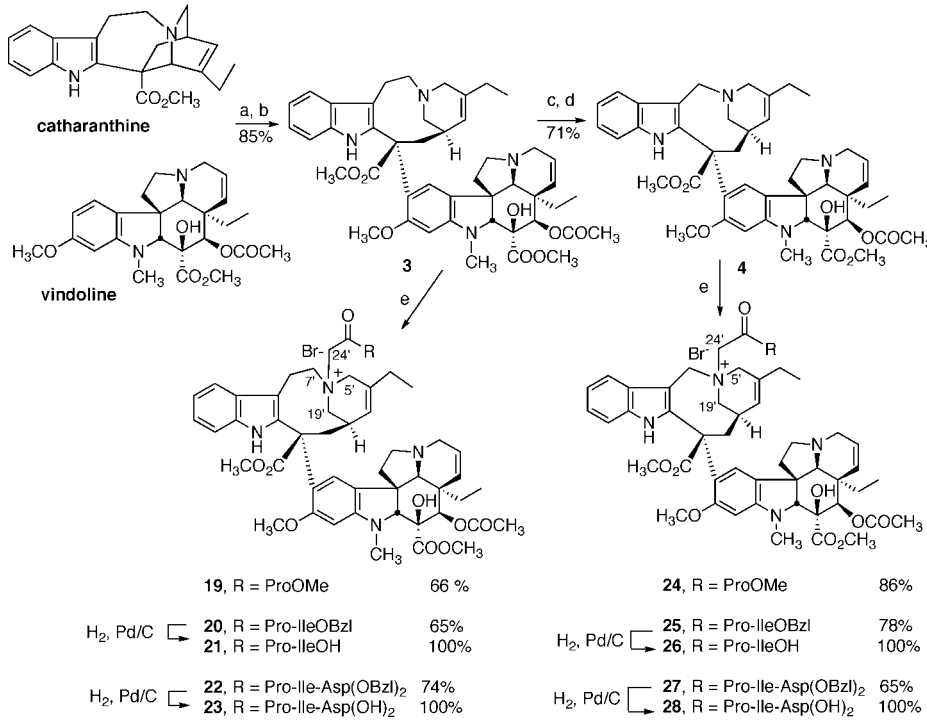
Conformational Studies. The configuration at the N6′ quaternary center is the determining factor for the orientation of the peptide chain, which itself is fundamental for the interaction of these compounds with tubulin. Ideally, it should be *R* to allow an orientation of the peptide chain similar to that of phomopsin. According to the X-ray structure of vinblastine,[24] the nitrogen lone pair is oriented such that the absolute configuration of the amino group is *S*. Both *R* and *S* configurations of the amino group could be expected for the vinorelbine derivatives **24**−**28**; because of the gramine bridge, these ammonium ions could be in equilibrium with an open enaminium intermediate (Figure 3). All these compounds **24**−**28** are obtained as one diastereomer, and their absolute configuration at N6′ was determined by a NOESY experiment that was performed on a simplified compound **29** obtained by condensation of vinorelbine **4** with bromomethyl acetate. Two nuclear Overhauser effects (NOE) between H1′ and H8′ and between H19′ and H14 were observed (Figure 4). Such correlations are only consistent with an *S* absolute configuration at N6′ for compounds **24**−**29**. In order to rationalize the interesting biological results and to explain the good activity on tubulin of these very large quaternary ammonium salts, molecular dynamic modeling studies and docking experiments were performed on bulky and potent compounds **22** (AVLB- ProIleAsp$(OBzl)_2$) and **27** (nor-AVLB-ProIleAsp$(OBzl)_2$) using SYBYL 7.3 software and MMFF94 force field (Figure 5). For each compound, conformational searching produced a great number of conformers that adopt closely related geometries; in a 10 kcal interval, all these conformers present a similar orientation of the peptide side chain. The orientation of the lateral chain is slightly different

Scheme 1. Synthesis of Simplified Phomopsin A Side Chains[a]



[a] Reagents: (a) NMM, bromoacetic acid, EDC, HOBt, CH_2Cl_2; (b) NMM, EDC, HOBt, CH_2Cl_2; (c) TFA, CH_2Cl_2.

Scheme 2. Synthesis of Vinca Alkaloids and Phomopsin A Hybrids[a]

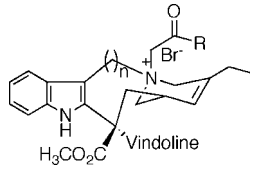


[a] Reagents: (a) $FeCl_3$, glycine buffer, 0.1 N HCl, 35 °C; (b) $NaBH_4$, NH_4OH; (c)TFA,NBS, CH_2Cl_2, −78 °C; (d) $AgBF_4$, THF/H_2O, 50 °C; (e) $BrCH_2COR$, CH_3CN, room temp.

between **22** and **27** even if they are both in the same plane. However, in both cases, their orientation is different from that of the phomopsin A peptide chain. Docking experiments were realized on the lowest energy conformers of each compound in the binding site of vinblastine. These experiments also suggest that the peptide chains of hybrids **22** and **27** do not superimpose with that of phomopsin but extend in another direction at the interface between tubulin α and tubulin β. In particular, these peptide chains do not interact with Tyr β224 near the GDP−GTP nucleotide site (Figure 6). The binding mode of vinca domain ligands has been found to correlate with inhibition of nucleotide exchange, with the strongest inhibitors contacting residue Tyr β224.[19] We therefore examined the effect of compound **22** on GDP−GTP exchange and compared it with those of vinblastine and phomopsin A (Figure 7). The exchange of GDP for GTP at the β-tubulin nucleotide site is almost totally inhibited when the experiment is run with 20 μM phomospsin A (tubulin concentration: 10 μM). In contrast, 20 μM vinblastine has only a small impact on this exchange (which decreases by only 25%). Concentrations of 20 and 40 μM compound **22** have no impact, but a 100 μM concentration partially inhibits this exchange (∼55% of exchange still takes place). Therefore, compound **22** inhibits the GDP−GTP exchange slightly less than vinblastine (since it is less active on tubulin) but much less than phomopsin A, which is consistent with the orientation of the peptide moiety as suggested by docking experiments.

In summary, a set of 10 complex and original hybrids of anhydrovinblastine or vinorelbine and phomopsine A has been elaborated. Their biological activities have been evaluated. Some of these are potent inhibitors of tubulin polymerization in spite of their large size. These results are very promising, since among the many SAR studies that have been done on vinblastine derivatives, most have shown that even a slight modification, particularly on the cleavamine moiety, dramatically decreases the activity on tubulin. NMR experiments proved that the absolute configuration of the vinorelbine quaternary ammonium salts is *S*, as for the anhydrovinblastine compounds. Molecular modeling experiments showed that even if the peptide side chains of all these compounds are not oriented in the same direction as that of phomopsin, they may still be accommodated

Table 1. Biological Evaluation of Compounds **19**−**28**



compd	*n*	R	microtubule assembly, inhibitory activity IC_{50}/IC_{50VLB}[a]	cytotoxicity against KB cell line, IC_{50}[b] (μM)
19	2	ProOMe	0.9	0.08
20	2	ProlleOBzl	1.4	0.3
21	2	ProlleOH	1.7	0.7
22	2	$ProlleAsp(OBzl)_2$	1.3	0.7
23	2	$ProlleAsp(OH)_2$	2.0	0.1
24	1	ProOMe	2.0	0.1
25	1	ProlleOBzl	9.4	0.05
26	1	ProlleOH	9.4	0.09
27	1	$ProlleAsp(OBzl)_2$	1.8	1.3
28	1	$ProlleAsp(OH)_2$	19.4	0.4
VLB			1	0.0010
AVLB (**3**)				0.04
nor-AVLB (**4**)				0.0010

[a] IC_{50} is the concentration of a compound that inhibits 50% of the rate of microtubule assembly. IC_{50VLB} is the concentration of vinblastine that inhibits 50% of the rate of microtubule assembly within the same day with the same tubuline preparation. IC_{50VLB} found in our assays was 2.1 μM. [b] IC_{50} measures the drug concentration required for the inhibition of 50% cell proliferation after 72 h of incubation. Values are reported as the mean values of two independant determinations.

at the interface of tubulin α and β. The poor effects of compound **22** on tubulin nucleotide exchange is in agreement with the hypothesis that the added peptide chains of all the synthesized hybrids are far from the GDP−GTP binding site. Two assumptions can then be made: either the added peptide chains have no impact on the biological activity of these ammonium salts, in which case a simplification of the vinblastine moiety should decrease it, or the peptide chains have a real influence on activity. In the latter case, a new family of antimitotic compounds has been elaborated. A simplification of the vinca part (particularly on the vindoline moiety) and/or a reorientation of the peptide chains should maintain or increase the activity. Work is currently underway in order to address these questions.

Experimental Section

General Remarks. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 300 or ARX 500 spectrometer. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane as an internal standard. The spectra were fully assigned using correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC). High resolution mass spectra were performed on a AQA Navigator ThermoQuest. IR spectra were recorded on a Perkin-Elmer Spectrum BX spectrometer. The $[\alpha]_D$ values were measured using a JASCO P-1010 polarimeter. Catharanthine was a gift from Jacques Fahy and Philippe Maillas (Pierre Fabre Laboratories). Microtubular proteins were purified from mammalian brain as previously described.[25] Measurement of the inhibition of tubulin assembly was carried out on Shimidazu UV 2401 PC spectrophotometer equipped with a temperature controlled cell. UPLC analyses were performed on a Waters system (Acquity) equipped with a photodiode array detector (monitoring at 200−400 nm), a UV detector, and a mass detector (TQD Waters), using an Acquity UPLC HSS C18 1.8 μm column (2.1 mm × 50 mm).

General Procedure for the Elaboration of the Quaternary Ammonium Salts 19, 20, 22, and 24, 25, 27. Anhydrovinblastine or nor-anhydrovinblastine and compound **9**, **13**, **18** or methyl bromoacetate (1 eq) were dissolved in acetonitrile (∼1.5 mL). The reaction mixture was stirred at room temperature for 3 h, and the solvent was removed in vacuo. The crude product was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 97:3 and then a gradient of MeOH).

General Procedure for the Deprotection of the Benzyl Groups To Give Compounds 21, 23, 26, and 28. A catalytic amount of 10% Pd−C (0.1 equiv) was added to a solution of **20**, **22**, **25**, or **27** in EtOH (0.01−0.02 mol/L). The resulting suspension was stirred for 3 h under an atmospheric pressure of hydrogen. The catalyst was removed by filtration on a pad of Celite and the filtrate was evaporated in vacuo to give the pure expected **21**, **23**, **26**, or **28**.

Compound 19. Reaction was performed with anhydrovinblastine **3** (32 mg, 0.04 mmol) and compound **9** (10 mg, 0.04 mmol) to give, after purification, compound **19** (26 mg, 66%) as a translucent oil. ^1H NMR (500 MHz, $CDCl_3$) δ 0.77 (t, J = 7.3 Hz, 3H, H-21), 0.98 (t, J = 7.0 Hz, 3H, H-21′), 1.28 (m, 1H, H-20), 1.68 (m, 1H, H-20), 1.95 (m, 3H, H-2′, H-20′), 1.97 (m, 4H, H-11, H-28′, H-29′), 2.03 (s, 3H, H-27), 2.15 (m, 1H, H-11), 2.20 (m, 1H, H-28′), 2.47 (d, J = 14.5 Hz, 1H, H-1′), 2.66 (m, 1H, H-10), 2.67 (s, 3H, H-23), 2.70 (s, 1H, H-19), 2.94 (m, 1H, H-8), 3.08 (d, J = 14.5 Hz, 1H, H-1′), 3.16 (m, 1H, H-8′), 3.25 (m, 1H, H-10), 3.29 (m, 1H, H-8), 3.30 (m, 1H, H-7′), 3.60 (s, 3H, H-23′), 3.66 (m, 2H, H-30′), 3.71 (s, 1H, H-2), 3.73 (s, 3H, H-32′), 3.76 (m, 1H, H-8′), 3.77 (s, 3H, H-25), 3.80 (m, 1H, H-7′), 3.82 (s, 3H, H-22), 3.97 (m, 1H, H-19′), 4.36 (m, 1H, H-24′), 4.40 (s, 2H, H-5′), 4.46 (m, 1H, H-19′), 4.47 (m, 1H, H-27′), 4.51 (m, 1H, H-24′), 5.26 (d, J = 11.5 Hz, 1H, H-6), 5.32 (s, 1H, H-4), 5.50 (s, 1H, H-3′), 5.82 (dd, J = 11.5 and 4.5 Hz, 1H, H-7), 6.04 (s, 1H, H-17), 6.35 (s, 1H, H-14), 7.08−7.20 (m, 3H, H-12′, H-13′, H-14′), 7.76 (d, J = 8.0 Hz, 1H, H-11′), 8.26 (s, 1H, H-16′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.5 (C-21), 11.7 (C-21′), 19.8 (C-8′), 20.9 (C-27), 25.4 (C-29′), 27.6 (C-20′), 28.9 (C-28′), 30.7 (C-2′), 30.9 (C-20), 34.4 (C-1′), 38.0 (C-23), 42.3 (C-5), 45.9 (C-11), 47.7 (C-30′), 47.8 (C-7′), 50.2 (C-8); 50.3 (C-10), 52.0 (C-25), 52.8 (C-32′), 53.1 (C-23′), 53.3 (C-12), 54.1 (C-18′), 55.4 (C-22), 58.0 (C-5′), 59.7 (C-27′), 63.0 (C-24′), 63.9 (C-19′), 65.0 (C-19), 76.4 (C-4), 79.8 (C-3), 83.0 (C-2), 94.5 (C-17), 108.0 (C-9′), 111.6 (C-14′), 117.8 (C-11′), 118.3 (C-15), 120.6 (C-13′), 122.0 (C-3′), 122.4 (C-14), 123.7 (C-12′), 124.4 (C-13), 125.3 (C-7), 125.7 (C-10′), 129.8 (C-6), 131.7 (C-15′), 132.7 (C-17′), 133.9 (C-4′), 154.1 (C-18), 158.3 (C-16), 169.1 (C-31′), 170.0 (C-25′), 170.7 (C-24), 171.0 (C-25), 172.0 (C-22′); IR (cm^{-1}) 3452, 2950, 1740, 1235; $[\alpha]_D$ +35 (c 0.9, $CHCl_3$); HR-EI-MS m/z 962.4894 (M^+, calcd for $C_{54}H_{68}N_5O_{11}$ 962.4915).

Compound 20. Reaction was performed with anhydrovinblastine **3** (32 mg, 0.04 mmol) and compound **13** (18 mg, 0.04 mmol) to give, after purification, compound **20** (30 mg, 65%) as a translucent oil. ^1H NMR (500 MHz, $CDCl_3$) δ (OH is missing) 0.70−1.00 (m, 12H, H-21′, H-21, H-36′, H-37′), 1.16 (m, 1H, H-35′), 1.28 (m, 1H, H-20), 1.36 (m, 1H, H-35′), 1.68 (m, 1H, H-20), 1.83−2.13 (m, 10H, H-2′, H-20′, H-28′, H-29′, H-34′, H-11), 2.03 (s, 3H, H-27), 2.46 (m, 1H, H-1′), 2.56 (m, 1H, H-10), 2.67 (s, 3H, H-23), 2.70 (s, 1H, H-19), 2.82 (m, 1H, H-8), 2.96 (m, 1H, H-10), 3.08 (m, 1H, H-1′), 3.12−3.79 (m, 8H, H-7′, H-8′, H-30′, H-2, H-8), 3.58 (s, 3H, H-23′), 3.72 (s, 3H, H-25), 3.77 (s, 3H, H-22), 3.98 (m, 1H, H-19′), 4.28−4.50 (m, 6H, H-5′, H-19′, H-24′, H-27′), 4.66 (m, 1H, H-33′), 5.07 (d, J = 11.8 Hz, 1H, H-39′), 5.16 (d, J = 11.8 Hz, 1H, H-39′), 5.25 (d, J = 11.0 Hz, 1H, H-6), 5.35 (s, 1H, H-4), 5.44 (s, 1H, H-3′), 5.81 (m, 1H, H-7), 6.05 (s, 1H, H-17), 6.57 (m, 2H, H-32′, H-14), 7.04−7.22 (m, 4H, H-11′, H-12′, H-13′, H-14′), 7.22−7.30 (m, 5H, H_{ar}), 8.25 (s, 1H, 16′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.5 (C-21), 11.7 (C-21′, C-36′), 15.7 (C-37′), 19.8 (C-8′), 21.3 (C-27), 24.9 (C-29′), 25.4 (C-35′), 27.4 (C-20′), 28.7 (C-28′), 30.7 (C-2′), 30.9 (C-20), 34.0 (C-1′), 37.4 (C-34′), 38.3 (C-23), 42.3 (C-5), 45.0 (C-11), 47.7 (C-7′, C-30′), 50.2 (C-8), 50.3 (C-10), 52.3 (C-25), 53.0 (C-23′), 53.2 (C-12), 54.1 (C-18′), 55.8 (C-22), 56.1 (C-33′), 57.4 (C-5′), 61.2 (C-27′), 63.4 (C-24′), 63.9 (C-19′), 65.2 (C-19), 67.3 (C-39′), 76.6 (C-4), 80.3 (C-3), 83.2 (C-2), 94.2 (C-17), 108.0 (C-9′), 110.9 (C-14′), 117.6 (C-11′), 118.7 (C-15), 120.3 (C-12′), 121.6 (C-3′), 122.5 (C-14), 123.6 (C-13′), 124.4 (C-13), 125.0 (C-7), 125.7 (C-10′), 128.3 (6 C_{ar}), 129.6 (C-

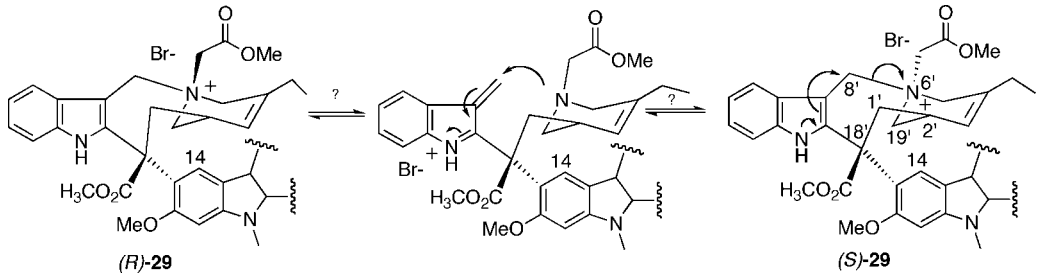


Figure 3. Potential equilibration from (*S*)-**29** to (*R*)-**29**.

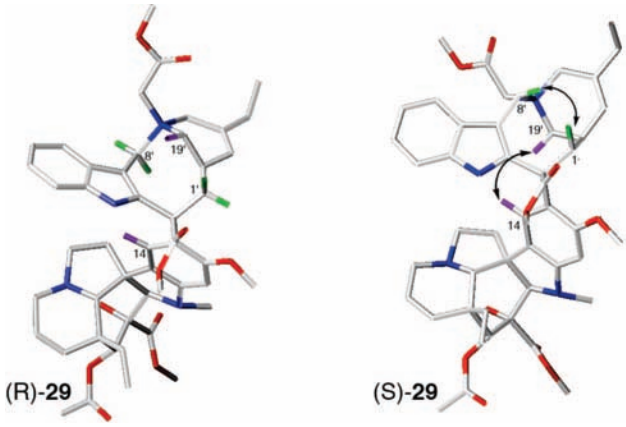


Figure 4. Observed nuclear Overhauser effects for compound **29**.

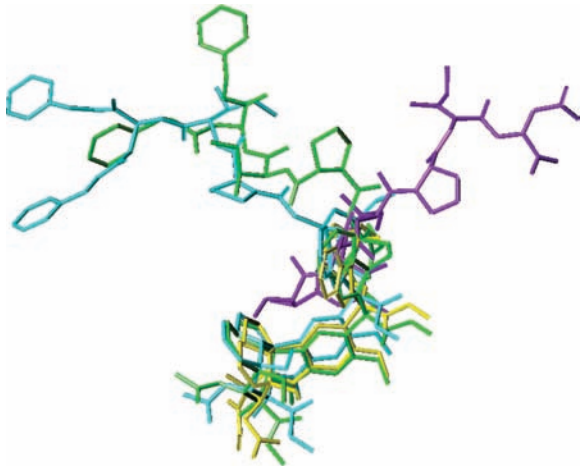


Figure 5. Superimposition of the lowest energy conformers of compounds **22** (green) and **27** (cyan) with phomopsin (violet) and vinblastine (yellow) in their active conformation.

6), 131.7 (C-15′), 132.5 (C-17′), 134.0 (C-4′), 154.6 (C-18), 158.9 (C-16), 169.3 (C-31′), 170.0 (C-25′), 171.6 (C-38′), 172.2 (C-26), 172.8 (C-24), 174.6 (C-22′); IR (cm^{-1}) 2959, 1738, 1234; $[\alpha]_D$ +20 (*c* 0.8, $CHCl_3$); HR-EI-MS *m*/*z* 1151.6025 (M^+, calcd. for $C_{66}H_{83}N_6O_{12}$ 1151.6069).

Compound 21. Reaction was performed with compound **20** (30 mg, 0.02 mmol) to give quantitatively compound **21** (27 mg). ^1H NMR (500 MHz, MeOD) δ 0.70−1.00 (m, 12H, H-21′, H-21, H-36′, H-37′), 1.13 (m, 1H, H-35′), 1.46 (m, 1H, H-35′), 1.70 (m, 2H, H-20), 1.81 (m, 1H, H-34′), 1.83−2.13 (m, 9H, H-2′, H-20′, H-28′, H-29′, H-11), 2.03 (s, 3H, H-27), 2.46 (m, 1H, H-1′), 2.56 (m, 1H, H-10), 2.64 (s, 3H, H-23), 2.70 (s, 1H, H-19), 2.77 (m, 1H, H-8), 2.96 (m, 1H, H-10), 3.18 (m, 2H, H-1′, H-8′), 3.26 (m, 1H, H-7′), 3.31 (m, 1H, H-8), 3.58 (s, 3H, H-23′), 3.62 (m, 2H, H-30′), 3.67 (s, 3H, H-25), 3.77 (m, 5H, H-2, H-22, H-7′), 3.81 (m, 1H, H-8′), 3.86 (m, 1H, H-19′), 4.02 (m, 1H, H-5′), 4.19 (m, 1H, H-24′), 4.37 (m, 1H, H-5′), 4.39 (m, 1H, H-19′), 4.40 (m, 1H, H-27′), 4.53 (m, 1H, H-24′), 4.66 (m, 1H, H-33′), 5.25 (s, 1H, H-6), 5.38 (s, 1H, H-4), 5.60 (s, 1H, H-3′), 5.78 (m, 1H, H-7), 6.29 (s, 1H, H-17), 6.55 (s, 1H, H-14), 6.95−7.20 (m, 3H, H-12′, H-13′, H-14′), 7.73 (m, 1H, H-11′); ^{13}C NMR (75 MHz, MeOD) δ 7.7 (C-21), 11.0 (C-21′,36′), 15.2 (C-37′), 19.4 (C-8′), 21.3 (C-27), 24.2 (C-29′), 24.7 (C-35′), 27.0 (C-20′), 29.3 (C-28′), 30.5 (C-20), 31.0 (C-2′), 33.5 (C-1′), 37.4 (C-34′), 37.7 (C-23), 42.9 (C-5), 44.6 (C-11), 47.1 (C-7′, C-30′), 49.2 (C-8), 50.0 (C-10), 51.7 (C-25), 53.0 (C-23′), 55.2 (C-22), 57.6 (C-5′), 58.6 (C-33′), 60.6 (C-27′), 62.6 (C-24′), 62.9 (C-19′), 65.2 (C-19), 76.4 (C-4), 82.5 (C-2), 94.2 (C-17), 108.0 (C-9′), 111.3 (C-14′), 117.6 (C-11′), 119.5 (C-15), 120.1 (C-12′), 122.6 (C-13′), 123.9 (C-14), 124.6 (C-3′, C-13), 125.0 (C-7), 129.5 (C-10′), 131.3 (C-6), 134.6 (C-4′), 136.6 (C-15′), 154.7 (C-18), 159.0 (C-16), 169.3 (C-31′), 170.0 (C-25′), 171.7 (C-26), 173.0 (C-24), 174.6 (C-22′); IR (cm^{-1}) 2966, 2359, 1733, 1653, 1238; $[\alpha]_D$ −9 (*c* 0.85, MeOH); HR-EI-MS *m*/*z* 1061.5555 (M^+, calcd for $C_{59}H_{77}N_6O_{12}$ 1061.5599).

Compound 22. Reaction was performed with anhydrovinblastine **3** (32 mg, 0.04 mmol) and compound **18** (26 mg, 0.04 mmol) to give, after purification, compound **22** (40 mg, 74%) as a translucent oil. ^1H NMR (500 MHz, $CDCl_3$) δ (OH is missing) 0.70−1.00 (m, 12H, H-21′, H-21, H-36′, H-37′), 1.16 (m, 1H, H-35′), 1.30 (m, 1H, H-20), 1.46 (m, 1H, H-35′), 1.69 (m, 1H, H-20), 1.83−2.13 (m, 10H, H-2′, H-20′, H-28′, H-29′, H-34′, H-11), 2.03 (s, 3H, H-27), 2.46 (d, *J* = 15.1 Hz, 1H, H-1′), 2.67 (s, 3H, H-23), 2.70 (s, 1H, H-19), 2.80 (m, 1H, H-41′), 2.86 (m, 1H, H-10), 2.87 (m, 1H, H-8), 2.98 (m, 1H, H-10), 3.02 (m, 1H−H41′), 3.06 (dd, *J* = 15.1 and 4.4 Hz, 1H, H-1′), 3.13 (m, 1H, H-8′), 3.25 (m, 1H, H-8), 3.38 (m, 1H, H-7′), 3.50 (m, 2H, H-30′), 3.58 (s, 3H, H-23′), 3.70 (s, 1H, H-2), 3.72 (s, 3H, H-25), 3.77 (s, 3H, H-22), 3.85 (m, 1H, H-8′), 4.00 (m, 1H, H-19′), 4.30−4.51 (m, 8H, H-5′, H-7′, H-19′, H-24′, H-27′, H-33′), 4.84 (m, 1H, H-40′), 5.00 (d, *J* = 9.1 Hz, 2H, H-43′), 5.04 (d, *J* = 5.3 Hz, 2H, H-45′), 5.25 (m, 1H, H-6), 5.35 (s, 1H, H-4), 5.44 (s, 1H, H-3′), 5.81 (m, 1H, H-7), 6.05 (s, 1H, H-17), 6.57 (m, 2H, H-14), 6.75 (m, 2H, H-32′, H-39′), 7.06−7.30 (m, 13H, H-12′, H-13′, H-14′ and $10H_{ar}$), 7.79 (d, *J* = 8.2 Hz, 1H, H-11′), 8.25 (s, 1H, H-16′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.6 (C-21), 11.5 (C-21′, C-36′), 15.2 (C-37′), 19.8 (C-8′), 21.3 (C-27), 24.8 (C-20′, C-35′), 27.4 (C-29′), 29.0 (C-28′), 30.6 (C-2′), 30.9 (C-20), 34.1 (C-1′), 36.2 (C-41′), 38.2 (C-23), 42.2 (C-5), 45.2 (C-11), 47.6 (C-7′, C-30′), 48.5 (C-40′), 50.0 (C-10), 50.2 (C-8), 52.2 (C-25), 52.7 (C-23′), 53.2 (C-12), 54.1 (C-18′), 56.0 (C-22), 57.0 (C-19′), 57.5 (C-33′), 60.2 (C-5′), 61.4 (C-27′), 63.8 (C-24′), 64.7 (C-19), 67.0 (C-43′), 67.7 (C-45′), 76.6 (C-4), 79.6 (C-3), 83.0 (C-2), 94.2 (C-17), 108.0 (C-9′), 111.1 (C-14′), 117.7 (C-11′), 118.7 (C-15), 120.4 (C-12′), 121.4 (C-3′), 122.7 (C-14), 123.4 (C-13′), 124.4 (C-13), 125.2 (C-7), 128.2−128.7 (10 C_{ar} and C-10′), 129.8 (C-6), 133.2 (C-4′), 134.5 (C-15′), 135.0 (2 $C_{q.ar}$), 153.5 (C-18), 157.8 (C-16), 170.0 (C-42′, C-44′), 170.5 (C-25′, C-31′, C-38′), 170.7 (C-26), 171.2 (C-24), 172.8 (C-24), 173.1 (C-22′); IR (cm^{-1}) 2962, 1737, 1660, 1229; $[\alpha]_D$ +40 (*c* 1.2, $CHCl_3$); HR-EI-MS *m*/*z* 1356.6860 (M^+, calcd for $C_{77}H_{94}N_7O_{15}$ 1356.6808).

Compound 23. Reaction was performed with compound **22** (40 mg, 0.03 mmol) to give quantitatively compound **23** (35 mg). ^1H NMR (500 MHz, MeOD) δ 0.70−1.00 (m, 12H, H-21′, H-21, H-36′, H-37′), 1.03−1.86 (m, 5H, H-34′, H-35′, H-20), 1.88−2.20 (m, 9H, H-2′, H-20′, H-28′, H-29′, H-11), 2.03 (s, 3H, H-27), 2.38 (m, 1H, H-1′), 2.56−2.80 (m, 5H, H-41′, H-8, H-10, H-19), 2.64

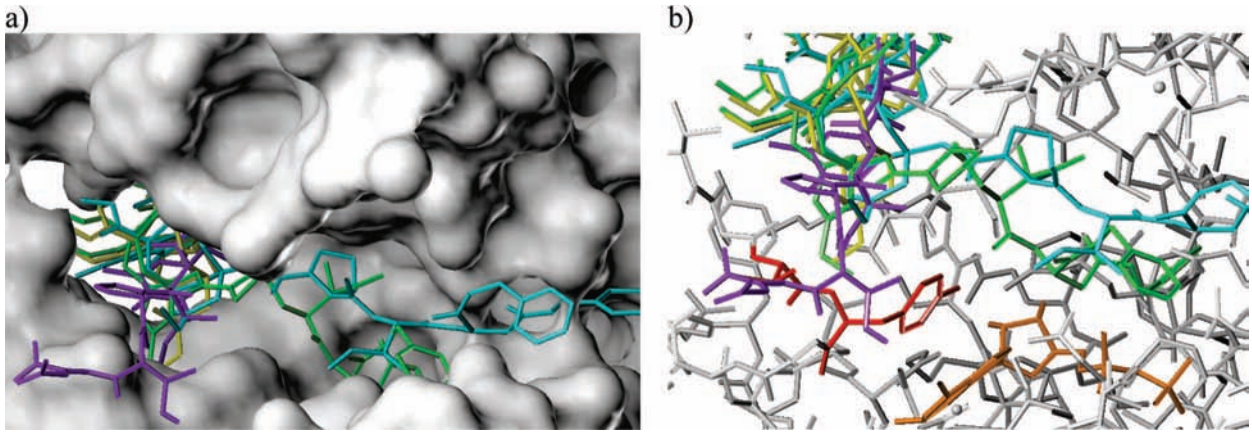


Figure 6. (a) Superimposition of the lowest energy conformers of compounds **22** (green) and **27** (cyan) after docking with phomopsin (violet) and vinblastine (yellow) in tubulin. (b) Superimposition of the lowest energy conformers of compounds **22** (green) after docking with phomopsin (violet) and vinblastine (yellow) in tubulin: interaction of phomopsin with Thr β223 and Tyr β224 (red) close to GDP (orange).

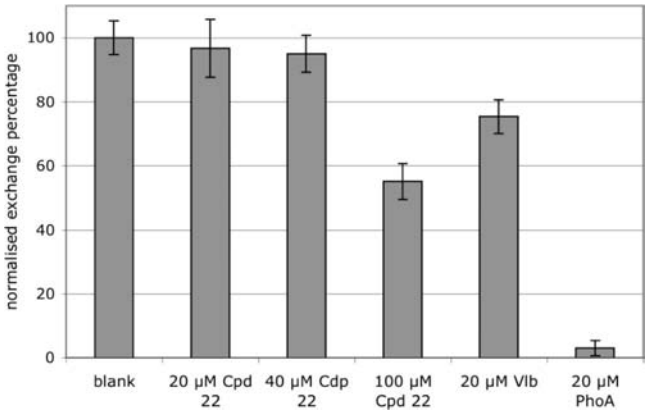


Figure 7. Compound **22** effects on tubulin nucleotide exchange.

(s, 3H, H-23), 2.96 (m, 1H, H-10), 2.98 (m, 1H, H-8′), 3.05−3.81 (m, 6H, H-1′, H-7′, H-8′, H-30′, H-8), 3.58 (s, 3H, H-23′), 3.69 (s, 3H, H-25), 3.78 (s, 4H, H-2, H-22), 3.86 (m, 1H, H-19′), 4.00 (m, 1H, H-5′), 4.18 (m, 1H, H-33′), 4.22 (m, 1H, H-24′), 4.40 (m, 3H, H-7′, H-19′, H-27′), 4.44 (m, 1H, H-5′), 4.48 (m, 1H, H-24′), 4.58 (m, 1H, H-40′), 5.28 (s, 1H, H-4), 5.39 (s, 1H, H-6), 5.57 (s, 1H, H-3′), 5.81 (m, 1H, H-7), 6.32 (s, 1H, H-17), 6.64 (s, 1H, H-14), 6.93−7.21 (m, 3H, H-12′, H-13′, H-14′), 7.80 (s, 1H, H-11′); ^{13}C NMR (75 MHz, MeOD) δ 7.6 (C-21), 10.4 (C-36′), 11.0 (C-21′), 14.5 (C-37′), 18.0 (C-8′), 20.9 (C-27), 24.2 (C-29′, C-35′), 27.0 (C-20′), 29.3 (C-28′), 30.7 (C-20), 31.1 (C-2′), 33.5 (C-1′), 36.2 (C-41′), 37.3 (C-23), 42.3 (C-5), 44.6 (C-11), 47.2 (C-30′), 48.2 (C-7′), 49.2 (C-18′), 49.8 (C-8), 50.0 (C-10), 51.7 (C-25), 53.0 (C-23′), 55.2 (C-22), 57.2 (C-5′), 58.0 (C-33′), 59.8 (C-27′), 60.4 (C-40′), 62.6 (C-24′), 63.2 (C-19′), 65.2 (C-19), 75.6 (C-4), 81.7 (C-2), 94.2 (C-17), 108.0 (C-9′), 111.1 (C-14′), 117.4 (C-11′), 120.4 (C-15), 119.4 (C-12′), 122.4 (C-3′), 122.6 (C-13′), 122.9 (C-6), 123.7 (C-14), 128.6 (C-10′), 130.6 (C-7), 133.2 (C-4′), 135.7 (C-15′), 153.3 (C-18), 158.0 (C-16), 163.4 (C-31′), 167.2 (C-38′), 172.2 (C-26), 172.4 (C-25′), 173.7 (C-24), 174.6 (C-22′); IR (cm^{-1}) 3365, 2966, 2360, 1732, 1650, 1230; $[\alpha]_D$ −39 (*c* 1.0, MeOH); HR-EI-MS *m*/*z* 1176.5892 (M^+, calcd for $C_{63}H_{82}N_7O_{15}$ 1176.5869).

Compound 24. Reaction was performed with nor-anhydrovinblastine **4** (31 mg, 0.04 mmol) and compound **9** (10 mg, 0.04 mmol) to give, after purification, compound **24** (34 mg, 86%) as a translucent oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.69 (t, J = 7.2 Hz, 3H, H-21), 1.04 (t, J = 7.2 Hz, 3H, H-21′), 1.14 (m, 1H, H-20), 1.67 (m, 1H, H-20), 1.73 (m, 1H, H-11), 1.80−2.30 (m, 8H, H-2′, H-11, H-20′, H-28′, H-29′), 2.02 (s, 3H, H-27), 2.38 (m, 2H, H-1′, H-10), 2.55 (s, 1H, H-19), 2.68 (s, 3H, H-23), 2.78 (m, 1H, H-8), 3.18 (m, 2H, H-1′, H-10), 3.20 (m, 2H, H-30′), 3.30 (m, 1H, H-8), 3.32 (m, 1H, H-19′), 3.66 (s, 3H, H-23′), 3.70 (s, 1H, H-2), 3.71 (s, 3H, H-32′), 3.72 (s, 3H, H-23′), 3.73 (s, 3H, H-25), 3.80 (s, 3H, H-22), 4.03 (m, 1H, H-19′), 4.52 (m, 2H, H-5′, H-27′), 4.78 (m, 1H, H-8′), 4.66 (m, 1H, H-24′), 4.89 (d, J = 17.5 Hz, 1H, H-5′), 5.21 (m, 1H, H-6), 5.31 (s, 1H, H-4), 5.69 (m, 1H, H-8′), 5.76 (s, 1H, H-3′), 5.81 (m, 1H, H-7), 6.02 (s, 1H, H-17), 6.05 (m, 1H, H-24′), 6.06 (s, 1H, H-14), 7.12−7.30 (m, 3H, H-12′, H-13′, H-14′), 7.65 (s, 1H, H-11′), 8.79 (s, 1H, H-16′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.0 (C-21), 11.8 (C-21′), 21.1 (C-27), 24.9 (C-29′), 27.0 (C-20′), 27.9 (C-2′), 28.8 (C-28′), 30.5 (C-20), 31.8 (C-1′), 38.1 (C-23), 42.5 (C-5), 44.6 (C-11), 47.4 (C-30′), 50.1 (C-8), 50.3 (C-10), 51.0 (C-8′), 52.2 (C-25), 52.4 (C-32′), 53.0 (C-12), 53.4 (C-23′), 55.3 (C-19′), 55.8 (C-22), 59.2 (C-24′), 59.6 (C-27′), 60.4 (C-5′), 65.2 (C-19), 76.3 (C-4), 79.2 (C-18′), 79.6 (C-3), 82.9 (C-2), 94.1 (C-17), 100.5 (C-9′), 111.5 (C-14′), 117.5 (C-15), 119.1 (C-11′), 121.9 (C-14), 122.0 (C-3′), 122.8 (C-12′), 123.6 (C-13), 124.6 (C-13′), 124.8 (C-7), 129.8 (C-6), 130.5 (C-10′), 132.4 (C-4′), 134.6 (C-15′), 135.9 (C-17′), 153.6 (C-18), 158.4 (C-16), 163.1 (C-25′), 170.9 (C-24), 171.5 (C-26), 172.8 (C-31′), 175.0 (C-22′); IR (cm^{-1}) 3422, 2956, 2359, 1736, 1241; $[\alpha]_D$ +27 (*c* 1.4, CH_2Cl_2); HR-EI-MS *m*/*z* 948.4734 (M^+, calcd for $C_{53}H_{66}N_5O_{11}$ 948.4759).

Compound 25. Reaction was performed with nor-anhydrovinblastine **4** (31 mg, 0.04 mmol) and compound **13** (18 mg, 0.04 mmol) to give, after purification, compound **25** (36 mg, 78%) as a translucent oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (OH is missing) 0.64−1.08 (m, 12H, H-21′, H-21, H-36′, H-37′), 1.12 (m, 1H, H-20), 1.23 (m, 1H, H-35′), 1.46 (m, 1H, H-35′), 1.65 (m, 1H, H-20), 1.67 (m, 1H, H-11), 1.80−2.13 (m, 7H, H-20′, H-28′, H-29′, H-34′), 2.03 (s, 3H, H-27), 2.19 (m, 1H, H-2′), 2.22 (m, 1H, H-11), 2.36 (m, 2H, H-1′, H-10), 2.67 (s, 3H, H-23), 2.70 (s, 1H, H-19), 2.83 (d, J = 16.7 Hz, 1H, H-8), 3.20 (m, 2H, H-1′, H-10), 3.26 (m, 1H, H-19′), 3.31 (m, 1H, H-8), 3.54 (m, 2H, H-30′), 3.66 (s, 3H, H-23′), 3.70 (s, 1H, H-2), 3.72 (s, 3H, H-25), 3.79 (s, 3H, H-22), 4.10 (m, 1H, H-19′), 4.49 (m, 2H, H-5′, H-27′), 4.60 (m, 1H, H-33′), 4.64 (m, 1H, H-24′), 4.75 (m, 1H, H-8′), 5.06 (m, 1H, H-5′), 5.07 (d, J = 12.3 Hz, 1H, H-39′), 5.16 (d, J = 12.3 Hz, 1H, H-39′), 5.19 (m, 1H, H-6), 5.30 (s, 1H, H-4), 5.70 (s, 1H, H-3′), 5.80 (d, J = 3.8 Hz, 1H, H-7), 5.97 (m, 1H, H-8′), 6.02 (s, 1H, H-17), 6.04 (s, 1H, H-14), 6.10 (d, J = 14.2 Hz, 1H, H-24′), 6.76 (m, 1H, H-32′), 7.11−7.33 (m, 9H, H_{ar}), 8.76 (s, 1H, H-16′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.2 (C-21), 12.1 (C-21′, C-36′), 15.6 (C-37′), 21.4 (C-27), 24.9 (C-29′), 25.0 (C-35′), 27.0 (C-20′), 27.4 (C-28′), 30.7 (C-2′), 30.0 (C-20), 34.0 (C-1′), 37.5 (C-34′), 38.0 (C-23), 42.3 (C-5), 45.0 (C-11), 47.6 (C-30′), 50.2 (C-8), 50.3 (C-10), 52.3 (C-25), 53.0 (C-23′), 53.2 (C-12), 54.1 (C-18′), 55.3 (C-19′), 55.8 (C-22), 56.9 (C-33′), 57.0 (C-8′), 59.2 (C-24′), 60.2 (C-27′), 60.4 (C-5′), 65.2 (C-19), 67.3 (C-39′), 76.6 (C-4), 80.3 (C-3), 83.2 (C-2), 93.9 (C-17), 100.5 (C-9′), 111.5 (C-14′), 118.7 (C-15), 119.1 (C-11′), 121.1 (C-14), 122.8 (C-3′), 122.8 (C-12′), 124.4 (C-7), 124.6 (C-13′), 125.0 (C-13), 128.2−128.7 (5 C_{ar}), 129.8 (C-6), 130.5 (C-10′), 133.0 (C-4′), 134.6 (C-15′), 135.9 (C-17′), 154.6

(C-18), 158.9 (C-16), 170.0 (C-25′), 171.6 (C-38′), 172.2 (C-26), 172.8 (C-24), 174.6 (C-22′); IR (cm^{-1}) 2962, 1737, 1651, 1236; $[\alpha]_D$ +38.5 (*c* 1.2, CH_2Cl_2); HR-EI-MS *m/z* 1137.5935 (M^+, calcd for $C_{65}H_{81}N_6O_{12}$ 1137.5912).

Compound 26. Reaction was performed with compound **25** (36 mg, 0.03 mmol) to give quantitatively compound **26** (34 mg). ^1H NMR (500 MHz, MeOD) δ 0.64 (m, 3H, H-21), 0.77−0.87 (m, 6H, H-36′, H-37′), 1.06 (m, 3H, H-21′), 1.21 (m, 1H, H-35′), 1.28 (m, 1H, H-20), 1.49 (m, 1H, H-35′), 1.51 (m, 1H, H-11), 1.57 (m, 1H, H-20), 1.80−2.15 (m, 7H, H-20′, H-28′, H-29′, H-34′), 1.93 (s, 3H, H-27), 2.03 (m, 1H, H-2′), 2.16 (m, 1H, H-11), 2.57 (m, 2H, H-1′, H-10), 2.65 (s, 3H, H-23), 2.70 (s, 1H, H-19), 2.85 (m, 1H, H-8), 2.96 (m, 1H, H-10), 3.10 (m, 1H, H-1′), 3.40 (m, 1H, H-8), 3.54 (m, 2H, H-30′), 3.66 (s, 3H, H-23′), 3.67 (s, 3H, H-25), 3.70 (s, 1H, H-2), 3.79 (s, 3H, H-22), 4.00 (d, J = 15.9 Hz, 1H, H-19′), 4.13 (d, J = 15.9 Hz, 1H, H-19′), 4.24 (d, J = 15.8 Hz, 1H, H-5′), 4.37 (m, 2H, H-5′, H-27′), 4.60 (m, 1H, H-33′), 4.95 (d, J = 14.6 Hz, 1H, H-8′), 5.28 (m, 1H, H-6), 5.38 (s, 1H, H-4), 5.39 (m, 1H, H-24′), 5.56 (d, J = 14.6 Hz, 1H, H-8′), 5.79 (m, 3H, H-7, H-3′, H-24′), 6.28 (s, 1H, H-17), 6.55 (s, 1H, H-14), 7.17 (m, 2H, H_{ar}), 7.35 (d, J = 7.9 Hz, 1H, H_{ar}), 7.61 (d, J = 7.5 Hz, 1H, H_{ar}), 7.79 (s, 1H, H-16′); ^{13}C NMR (75 MHz, MeOD) δ (main carbons) 7.7 (C-21), 11.0 (C-21′, C-36′), 15.2 (C-37′), 21.3 (C-27), 24.2 (C-29′), 24.7 (C-35′), 27.0 (C-20′), 29.3 (C-28′), 30.5 (C-20), 31.0 (C-2′), 33.5 (C-1′), 37.4 (C-34′), 37.7 (C-23), 42.9 (C-5), 44.6 (C-11), 47.1 (C-30′), 49.2 (C-8), 50.0 (C-10), 51.7 (C-25), 53.0 (C-23′), 54.6 (C-8′), 55.2 (C-22), 57.6 (C-5′), 58.6 (C-33′), 60.6 (C-27′), 62.6 (C-24′), 62.9 (C-19′), 65.2 (C-19), 76.4 (C-4), 82.5 (C-2), 94.2 (C-17), 108.0 (C-9′), 111.3 (C-14′), 117.6 (C-11′), 119.5 (C-15), 120.1 (C-12′), 122.6 (C-13′), 123.9 (C-14), 124.6 (C-3′, C-13), 125.0 (C-7), 129.5 (C-10′), 131.3 (C-6), 134.6 (C-4′), 136.6 (C-15′), 154.7 (C-18), 159.0 (C-16), 169.3 (C-31′), 170.0 (C-25′), 171.7 (C-26), 173.0 (C-24), 174.6 (C-22′); IR (cm^{-1}) 3396, 1650, 1246; $[\alpha]_D$ −4 (*c* 1.1, MeOH); HR-EI-MS *m/z* 1047.5416 (M^+, calcd for $C_{58}H_{75}N_6O_{12}$ 1047.5443).

Compound 27. Reaction was performed with nor-anhydrovinblastine **4** (31 mg, 0.04 mmol) and compound **18** (26 mg, 0.04 mmol) to give, after purification, compound **27** (35 mg, 65%) as a translucent oil. ^1H NMR (500 MHz, $CDCl_3$) δ 0.67 (m, 3H, H-21), 0.83 (t, J = 7.4 Hz, 3H, H-36′), 0.97 (t, J = 6.6 Hz, 3H, H-37′), 1.06 (t, J = 7.0 Hz, 3H, H-21′), 1.11 (m, 1H, H-20), 1.20 (m, 1H, H-35′), 1.58 (m, 1H, H-35′), 1.66 (m, 2H, H-11, H-20), 1.85−2.24 (m, 9H, H-2′, H-20′, H-28′, H-29′, H-34′, H-11), 2.03 (s, 3H, H-27), 2.36 (m, 1H, H-1′, H-10), 2.67 (s, 3H, H-23), 2.70 (s, 1H, H-19), 2.81 (m, 1H, H-8), 2.83 (m, 1H, H-41′), 3.05 (m, 1H, H-41′), 3.20 (m, 2H, H-1′, H-10), 3.27 (m, 1H, H-19′), 3.30 (m, 1H, H-8), 3.54 (m, 2H, H-30′), 3.66 (s, 3H, H-23′), 3.70 (s, 1H, H-2), 3.72 (s, 3H, H-25), 3.80 (s, 3H, H-22), 4.08 (m, 1H, H-19′), 4.44 (m, 1H, H-27′), 4.51 (d, J = 16.6 Hz, 1H, H-5′), 4.60 (m, 1H, H-33′), 4.65 (d, J = 14.0 Hz, 1H, H-24′), 4.77 (m, 1H, H-8′), 4.84 (m, 1H, H-40′), 4.97 (m, 1H, H-5′), 4.99 (s, 2H, H-43′), 5.07 (s, 2H, H-45′), 5.2 (s, 1H, H-6), 5.30 (s, 1H, H-4), 5.71 (s, 1H, H-3′), 5.75 (m, 1H, H-8′), 5.80 (m, 1H, H-7), 6.02 (s, 1H, H-17), 6.04 (s, 1H, H-14), 6.06 (d, J = 14.0 Hz, 1H, H-24′), 6.83 (d, J = 7.9 Hz, 1H, H-39′), 7.14−7.33 (m, 14H, H_{ar}), 8.78 (s, 1H, H-16′); ^{13}C NMR (75 MHz, $CDCl_3$) δ (main carbons) 8.2 (C-21), 11.6 (C-21′, C-36′), 15.4 (C-37′), 21.2 (C-27), 24.5 (C-35′), 24.9 (C-29′), 27.0 (C-20′), 27.9 (C-28′), 29.0 (C-2′), 29.5 (C-20), 34.0 (C-1′), 36.3 (C-41′), 37.1 (C-34′), 38.0 (C-23), 42.3 (C-5), 45.0 (C-11), 47.6 (C-30′), 48.5 (C-40′), 50.2 (C-8), 50.3 (C-10), 52.3 (C-25), 53.0 (C-23′), 53.2 (C-12), 54.1 (C-18′), 55.8 (C-22), 56.9 (C-33′), 57.0 (C-8′), 58.3 (C-19′), 59.2 (C-24′), 60.5 (C-27′), 60.4 (C-5′), 65.2 (C-19), 66.9 (C-43′), 67.6 (C-45′), 76.6 (C-4), 80.3 (C-3), 83.2 (C-2), 93.9 (C-17), 100.5 (C-9′), 111.5 (C-14′), 118.7 (C-15), 119.1 (C-11′), 121.1 (C-14), 122.8 (C-3′), 122.8 (C-12′), 124.4 (C-7), 124.6 (C-13′), 125.0 (C-13), 128.2−128.7 (10 C_{ar}), 129.8 (C-6), 130.5 (C-10′), 133.0 (C-4′), 134.6 (C-15′), 135.6 (2 $C_{q.ar}$), 135.9 (C-17′), 154.6 (C-18), 158.9 (C-16), 170.0 (C-25′), 170.8 (C-38′), 171.0 (C-42′, C-44′), 172.2 (C-26), 172.8 (C-24), 174.6 (C-22′); IR (cm^{-1}) 2962, 2358, 1739, 1656, 1243; $[\alpha]_D$ +18 (*c* 1.2, CH_2Cl_2); HR-EI-MS *m/z* 1342.6667 (M^+, calcd for $C_{76}H_{92}N_7O_{15}$ 1342.6651).

Compound 28. Reaction was performed with compound **27** (35 mg, 0.03 mmol) to give quantitatively compound **28** (29 mg). ^1H NMR (500 MHz, MeOD) δ 0.64 (m, 3H, H-21), 0.77−0.87 (m, 6H, H-36′, H-37′), 1.06 (m, 3H, H-21′), 1.21 (m, 1H, H-35′), 1.28 (m, 1H, H-20), 1.49 (m, 1H, H-35′), 1.51 (m, 1H, H-11), 1.57 (m, 1H, H-20), 1.80−2.15 (m, 7H, H-20′, H-28′, H-29′, H-34′), 1.93 (s, 3H, H-27), 2.03 (m, 1H, H-2′), 2.16 (m, 1H, H-11), 2.57 (m, 2H, H-1′, H-10), 2.59 (s, 3H, H-23), 2.70 (m, 3H, H-19, H-41′), 2.85 (m, 1H, H-8), 2.96 (m, 1H, H-10), 3.10 (m, 1H, H-1′), 3.28 (m, 2H, H-30′), 3.40 (m, 1H, H-8), 3.66 (s, 3H, H-23′), 3.67 (s, 3H, H-25), 3.70 (s, 1H, H-2), 3.76 (s, 3H, H-22), 3.79−4.50 (m, 7H, H-5′, H-19′, H-27′, H-33′, H-40′), 4.97 (m, 1H, H-8′), 5.28 (m, 1H, H-6), 5.38 (s, 1H, H-4), 5.35 (m, 1H, H-24′), 5.56 (m, 1H, H-8′), 5.77 (m, 3H, H-7, H-3′, H-24′), 6.27 (s, 1H, H-17), 6.30 (s, 1H, H-14), 6.90−7.47 (m, 4H, H_{ar}); ^{13}C NMR (75 MHz, MeOD) δ (main carbons) 7.7 (C-21), 11.0 (C-21′, C-36′), 15.2 (C-37′), 21.3 (C-27), 24.2 (C-29′), 24.7 (C-35′), 27.0 (C-20′), 29.3 (C-28′), 30.5 (C-20), 31.0 (C-2′), 33.5 (C-1′), 36.2 (C-41′), 37.4 (C-34′), 37.7 (C-23), 42.9 (C-5), 44.6 (C-11), 47.1 (C-30′), 49.2 (C-8), 50.0 (C-10), 51.7 (C-25), 53.0 (C-23′), 54.6 (C-8′), 55.2 (C-22), 57.6 (C-5′), 58.6 (C-33′), 60.4 (C-40′), 60.6 (C-27′), 62.6 (C-24′), 62.9 (C-19′), 65.2 (C-19), 76.4 (C-4), 82.5 (C-2), 94.2 (C-17), 108.0 (C-9′), 111.3 (C-14′), 117.6 (C-11′), 119.5 (C-15), 120.1 (C-12′), 122.6 (C-13′), 123.9 (C-14), 124.6 (C-3′, C-13), 125.0 (C-7), 129.5 (C-10′), 131.3 (C-6), 134.6 (C-4′), 136.6 (C-15′), 154.7 (C-18), 159.0 (C-16), 169.3 (C-31′), 170.0 (C-25′), 171.7 (C-26), 173.0 (C-24), 174.6 (C-22′); IR (cm^{-1}) 3384, 2360, 1650, 1244; $[\alpha]_D$ −2 (*c* 1.32, MeOH); HR-EI-MS *m/z* 1162.5688 (M^+, calcd for $C_{62}H_{80}N_7O_{15}$ 1162.5712).

Compound 29. Reaction was performed with nor-anhydrovinblastine **4** (31 mg, 0.04 mmol) and methyl bromoacetate (6 mg, 0.04 mmol) to give, after purification, compound **29** (27 mg, 77%). ^1H NMR (500 MHz, $CDCl_3$) δ 0.68 (t, J = 7.8 Hz, 3H, H-21), 1.09 (t, J = 7.4 Hz, 3H, H-21′), 1.13 (m, 1H, H-20), 1.69 (m, 1H, H-20), 1.76 (m, 1H, H-11), 2.02 (s, 3H, H-27), 2.08 (m, 2H, H-20′), 2.15 (m, 1H, H-2′), 2.16 (m, 1H, H-11), 2.38 (m, 2H, H-1′, H-10), 2.51 (s, 1H, H-19), 2.67 (s, 3H, H-23), 2.78 (d, J = 16.0 Hz, 1H, H-8), 3.22 (m, 2H, H-1′, h10), 3.30 (dd, J = 16.0 and 3.8 Hz, 1H, H-8), 3.39 (m, 1H, H-19′), 3.66 (s, 3H, H-26′), 3.71 (s, 1H, H-2), 3.72 (s, 3H, H-23′), 3.73 (s, 3H, H-25), 3.80 (m, 4H, H-19′, H-22), 4.80 (m, 1H, H-5′), 4.84 (m, 1H, H-8′), 4.82 (s, 2H, H-24′), 4.99 (d, J = 17.5 Hz, 1H, H-5′), 5.21 (d, J = 10.5 Hz, 1H, H-6), 5.31 (s, 1H, H-4), 5.69 (m, 1H, H-8′), 5.76 (s, 1H, H-3′), 5.80 (dd, J = 10.5 Hz, 4.7 Hz, 1H, H-7), 6.05 (s, 2H, H-14, H-17), 7.18−7.32 (m, 3H, H-12′, H-13′, H-14′), 7.99 (d, J = 7.9 Hz, 1H, H-11′), 8.83 (s, 1H, H-16′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.0 (C-21), 11.8 (C-21′), 21.1 (C-27), 26.9 (C-20′), 28.8 (C-2′), 30.5 (C-20), 33.5 (C-1′), 38.1 (C-23), 42.5 (C-5), 44.6 (C-11), 50.1 (C-8), 50.3 (C-10), 52.2 (C-25), 52.6 (C-26′), 53.0 (C-12), 53.3 (C-23′), 53.6 (C-19′), 54.3 (C-18′), 54.8 (C-8′), 55.8 (C-22), 59.6 (C-24′), 60.3 (C-5′), 65.2 (C-19), 76.3 (C-4), 79.6 (C-3), 82.9 (C-2), 94.1 (C-17), 101.7 (C-9′), 111.2 (C-14′), 117.5 (C-15), 118.9 (C-11′), 121.4 (C-3′), 121.9 (C-14), 122.3 (C-12′), 123.6 (C-13), 124.5 (C-13′), 124.8 (C-7), 129.2 (C-10′), 129.8 (C-6), 132.2 (C-4′), 134.3 (C-15′), 136.6 (C-17′), 153.6 (C-18), 158.4 (C-16), 166.2 (C-25′), 170.9 (C-24), 171.5 (C-26), 173.7 (C-22′); IR (cm^{-1}) 1741, 1233; $[\alpha]_D$ +48 (*c* 0.78, $CHCl_3$); HR-EI-MS *m/z* 851.4232 (M^+, calcd for $C_{48}H_{59}N_4O_{10}$ 851.4231).

Computational Procedures. All calculations were performed on a PC workstation. All modeling studies were performed using Sybyl 7.3 software. The MMFF94 force field was used for minimization and partial charge calculations, a dielectric constant of 1.0 being employed. Compounds **22** and **27** 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 ranked according to energy. They were analyzed using Sybyl 7.3 software, and superimposition of conformers was based on the backbone atoms of vinblastine. Docking experiments on compounds **22** and **27**

were realized using the DOCK software of Sybyl 7.3 with Tripos force field for minimization.

Inhibition of Tubulin Assembly. The drug, dissolved in ethanol at different concentrations, was added to a solution of free tubulin at 0 °C. Then the solution was placed in a temperature controlled cell at 37 °C (microtubule assembly) and the increase of the optical density was monitored in a UV spectrophotometer at 350 nm for 1 min. The maximum rate of assembly was recorded and compared to a sample without drug. The IC_{50} of the compound was calculated from the effect of several concentrations and compared to the IC_{50} of vinblastine obtained within the same day with the same tubulin preparation.

Cytotoxicity Assays. The effect of the drugs on the growth of KB human cell lines was monitored at the Laboratoire de Cultures Cellulaires, ICSN, Gif sur Yvette, France. The IC_{50} refers to the concentration of drug corresponding to 50% growth inhibition after 72 h of incubation.[26]

GDP−GTP Exchange. The exchange of GDP for GTP at the β-tubulin nucleotide site is estimated by measuring the displacement of "cold" GDP by [α-^{32}P]GTP following a procedure adapted from Bai et al.[27] 10 μM GDP-tubulin is incubated for 5 min on ice with the candidate inhibitor in 80 mM Pipes-KOH, pH 6.9, 0,5 mM $MgCl_2$. 50 μM [α-^{32}P]GTP is added, and the reaction mixture is incubated for an additional 15 min. Tubulin is separated from unbound nucleotide by rapid gel filtration on Micro Bio-Spin P6 column (BioRad) previously equilibrated with the same buffer.[28] There is no release of tubulin-bound nucleotide during centrifugation.[29] We also quantified the free nucleotide that is eluted from the column and corrected the values accordingly. The radioactivity of the tubulin-containing eluted fraction is counted and compared to the radioactivity of a known concentration of [α-^{32}P]GTP. The nucleotide exchange with tubulin alone is found to be ~1 nmol of GTP bound per nmol of tubulin.

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Supporting Information Available: Experimental details and characterization data for new compounds **9**, **12**, **13**, **16**−**18** and chromatographic tracings of compounds **19**−**28**. This material is available free of charge via the Internet at http://pubs.acs.org.

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