Journal of Medicinal Chemistry

Article

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Synthesis and Biological Evaluation of a New Series of Highly Functionalized 7′-*homo*-Anhydrovinblastine Derivatives

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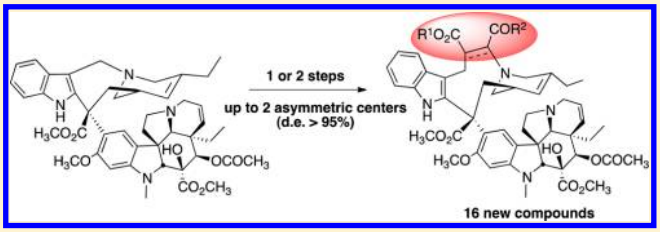
Supporting Information

ABSTRACT: Sixteen new 7′-*homo*-anhydrovinblastine derivatives were prepared in one or two steps from vinorelbine by means of an original and regiospecific rearrangement and subsequent diastereoselective reduction. This strategy has allowed fast access to a family of vinca alkaloid derivatives with an enlarged and functionalized ring C′. Their synthesis and biological evaluation are reported. One compound (compound **35**) is 1.7 times more active than vinorelbine as a tubulin assembly inhibitor. Moreover, some of these compounds are highly cytotoxic, and two of them are more potent than vinorelbine on HCT116 and K562 cell lines. Molecular modeling studies, carried out with two of the new vinca derivatives, provide useful hints about how a given functionalization introduced at positions 7′ and 8′ of the C′ ring results in improved binding interactions between one of the new derivatives and the interdimer interface when compared to the parent compound vinblastine.



INTRODUCTION

Microtubules are major structural components in cells. They play a crucial role in a number of cellular functions, such as cell division, as key constituents of the mitotic spindle. Microtubules are dynamic polymers of α,β-tubulin heterodimers that assemble in a head to tail arrangement.[1] Each monomer binds a GTP molecule[2] which is nonhydrolysable and nonexchangeable at the α subunit but is hydrolyzed to GDP at the β subunit during the polymerization reaction that leads to microtubule formation.

Tubulin-binding molecules interfere with microtubule assembly and functions, thus resulting in mitotic arrest of eukaryotic cells. Among them, Vinca alkaloids[3] (Figure 1) represent one of the most important classes of anticancer agents. Vinca alkaloids are a family of indole−indoline dimeric natural compounds that, from a biogenetic point of view, originate from the oxidative coupling of vindoline **2** to catharanthine **1**.[4] Vinblastine (VLB) **3** and vincristine **4**, isolated at the end of the 1950s,[5] as well as synthetic vindesine **5**,[6] vinorelbine (VLN) **7**,[7] and more recently vinflunine **8**[8] are widely used in cancer chemotherapy.

They inhibit the assembly of tubulin into microtubules by binding in the so-called vinca domain[9] and, consequently, prevent cell division. This binding site is at the longitudinal interface between two α,β-tubulin heterodimers and vinca alkaloids are oriented in such a way that their velbanamine and vindoline moieties interact with both heterodimers, with velbenamine being next to the GTP/GDP nucleotide exchange site of a β-subunit.

Performing selective modifications on these highly complex molecules represents a challenge for organic chemists. For almost 40 years, many derivatives have been elaborated by semi or total synthesis.[10] Nevertheless, most of them were obtained by modifications of the lower vindoline part, as changes in the velbanamine moiety are much less trivial. In addition, it is known that even a slight change in the upper indole part impacts the biological activities (two out of the five vinca alkaloids used in the clinic come from modifications of the velbenamine moiety, either on ring C′ or on rings C′ and D′, see **7** and **8**).

Chemical modifications of the velbanamine part, which do not cause loss or induce an increase of activity, have been reviewed.[3,11,12] For example, small hydrophobic groups can be introduced on C-12′ of ring A′.[13] Ring D′ can also undergo important modifications,[14] but ring-opening results in total loss of pharmacological activities. Thus, very recently, Boger et al. have synthesized original C-20′ urea and thiourea derivatives of vinblastine, some of which are more cytotoxic than vinblastine on selected cancer cell lines.[15] The C′ ring is clearly essential for obtaining antimitotic properties: ring-opening dramatically suppresses any pharmacological activity, whereas ring contraction from 9 to 8 centers, as for vinorelbine **7** and vinflunine **8**, leads to vinca alkaloids exhibiting markedly efficacious antitumor properties. In some cases, however, ring C′ can withstand large changes. Thus, in the course of our work on

Received: March 25, 2013
Published: July 3, 2013

dx.doi.org/10.1021/jm4004347 | J. Med. Chem. 2013, 56, 6088−6100

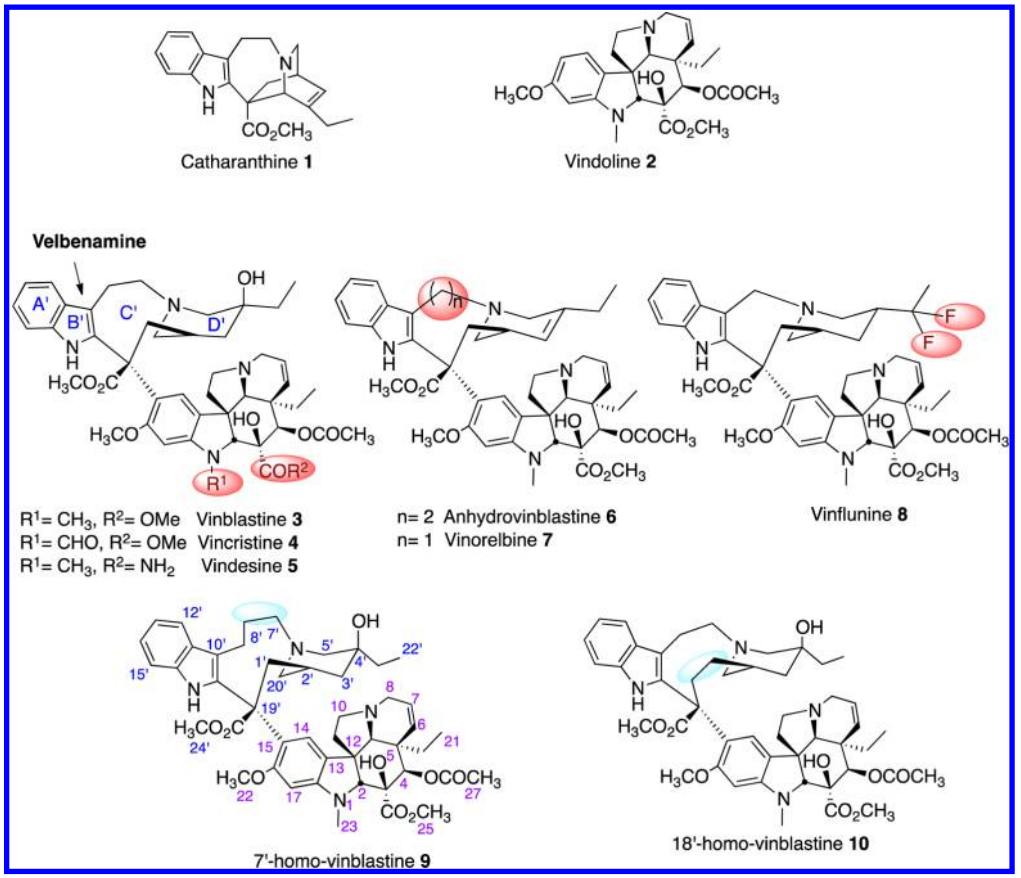
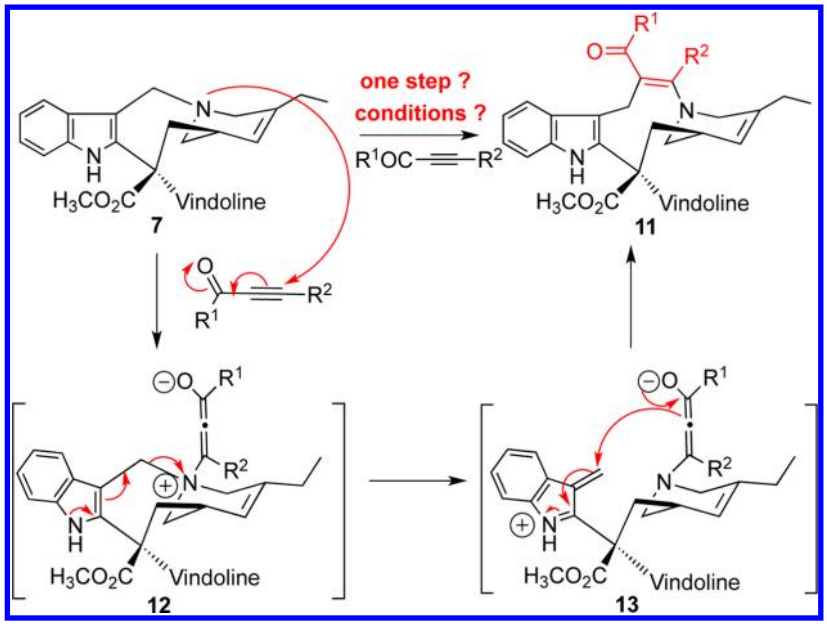


Figure 1. Vinblastine **3** and natural and unnatural congeners.

Scheme 1. Envisaged Synthetic Pathway



vinca−phomopsin hybrids,[16] we linked various peptide chains to the tertiary amine of the velbenamine moiety of anhydrovinblastine **6** and vinorelbine **7** and found that some of the resulting derivatives were very potent inhibitors of tubulin polymerization despite their large size. Molecular modeling showed that they could indeed be accommodated at the interface between α- and β-tubulin. Even more surprisingly, Kuehne et al. have shown that homologation from 9 to 10 carbons on C′ leading to an active compound if it takes place on the "upper" side (7′a-*homo*-vinblastine **9**) but to an inactive one (18′a-*homo*-vinblastine **10**) when this is performed on the "lower" side.[17,18]

All of these results prompted us to design and synthesize a new series of vinca derivatives with an enlarged and functionalized ring C′. We postulated that functionalization of 7′-*homo*-anhydrovinblastine on C-7′ and/or C-8′ might lead to

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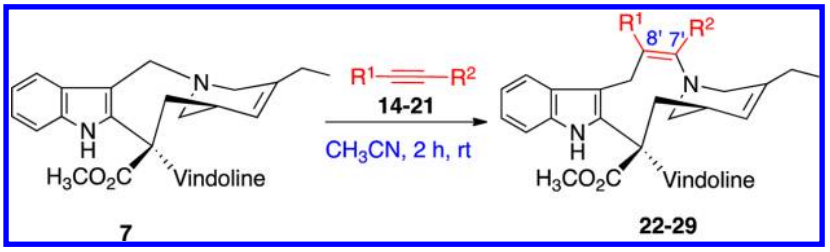
Table 1. Synthesis of a Series of β-Enamino Esters 22−29



entry	alkyne	R^1	R^2	compd	yield (%)[c]
1[a]	**14**	CO_2Me	H	**22**	36
2[a]	**15**	CO_2Et	H	**23**	33
3	**16**	CO_2Bn	H	**24**	32
4	**17**	CO_2Me	CO_2Me	**25**	33
5[b]	**18**	CO_2Me	CONHBn	**26**	50
6[b]	**19**	CO_2Me	CONH*i*Pr	**27**	52
7[b]	**20**	CO_2Me	CONHAll	**28**	50
8[b]	**21**	CO_2Me	CONH*t*Bu	**29**	52

[a]Typical conditions: 1.1 equiv of alkyne, rt for 2 h. [b]Typical conditions: 2 equiv of alkyne, rt for 2 h. [c]Isolated yield.

derivatives with increased potency. In fact, the added groups should be positioned, within the vinca domain, in the vicinity of the exchangeable GTP binding site, as this may reinforce their biological activity.[19]

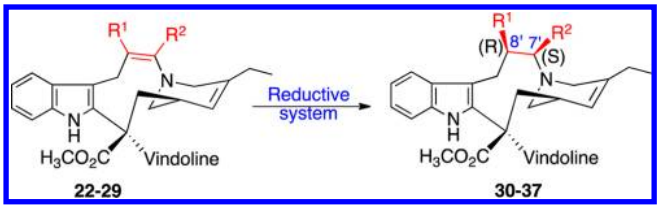
RESULTS AND DISCUSSION

Chemistry. The "naked" 7′-*homo*-vinblastine compound **9**, found to be as active as vinblastine **3**,[17] was prepared by total synthesis by Kuehne et al.. We reasoned that we could elaborate functionalized 7′-*homo*-anhydrovinblastine compound **11** in one step from vinorelbine **7**, by a direct enlargement reaction, using its gramine bridge as a reactive template (Scheme 1). Indeed, in indole chemistry, gramine is known to be a good precursor of alkylideneindolenine that can be trapped by various nucleophiles.[20] A few years ago, a facile coupling of gramine and activated acetylene was shown to generate alkylideneindolenines via elimination of an enamine species.[21] Indeed, we postulated that, in our case, activated acetylenes would play a double role by generating an alkylideneindolenine that could be trapped intramolecularly by the transient enamine intermediate.[22] This reaction may occur via a conjugate Michael addition of the tertiary nitrogen of vinorelbine **7** to acetylenes, generating a zwitterion **12**. Spontaneous fragmentation of the gramine bridge would lead to the reactive alkylideneindolenium ion **13**. This ion could then be trapped intramolecularly to give inserted compounds **11**.

This insertion on vinorelbine **7** was studied using methyl propiolate as the Michael acceptor (Table 1, entry 1). Various conditions were screened in order to perform this reaction in a selective and mild way by preventing any over reactions of the β-enaminone thus formed.[23] The nature of the solvent proved to be crucial for this rearrangement, which was complete in polar aprotic solvents like DMF or CH_3CN in only 2 h at room temperature. Under the conditions of 1.1 equiv of methyl propiolate, the expected compound **22** was isolated in 36% yield. The scope of this insertion was then investigated using various reactive acetylenes (entries 2−8). Overall, the expected compounds were obtained in yields that were deemed to be satisfactory in light of framework complexity. It should be noted, however, that loss of material was observed during the purification by flash chromatography. First, activated acetylenes with one electron withdrawing carboxylate group (entries 2−3) generated the desired products in moderate yields. When the acetylenes were disubstituted with carboxylates, insertions were successful (entries 4−8). The best results were obtained with unsymmetrical ester amide alkynes (entries 5−8). In these cases, using 2 equiv of alkyne increased significantly the yield of this insertion. Remarkably, the regioselectivity was complete and provided, for compounds **26−29**, a perfect control of the groups thus inserted at positions 7′ and 8′.

Afterward, the reduction of these various β-enamino esters **22−29** was considered. Two different reductive systems were tested in anhydrous CH_2Cl_2 (Table 2). All of them were reduced by using either $NaBH_4/ZnI_2$[24] or $NaBH(OAc)_3$,[25] the latter providing the best yields. Moreover, this reduction was highly diastereoselective and up to two asymmetric centers were controlled, leading to enantiopure 8′*R* or 7′*S*,8′*R* β-amino esters **30−37**.

Table 2. Synthesis of β-Amino Esters 30−37



				yield (%)[a]	
entry	compd	R^1	R^2	$NaBH_4/ZnI_2$[b]	$NaBH(OAc)_3$[c]
1	**30**	CO_2Me	H	52	69
2	**31**	CO_2Et	H	51	95
3	**32**	CO_2Bn	H	86	90
4	**33**	CO_2Me	CO_2Me	33	
5	**34**	CO_2Me	CONHBn		95
6	**35**	CO_2Me	CONH*i*Pr	34	81
7	**36**	CO_2Me	CONHAll		78
8	**37**	CO_2Me	CONH*t*Bu		72

[a]Isolated yield. [b]Typical conditions: anhydrous CH_2Cl_2, 3 equiv of ZnI_2 at 0 °C for 1 h, then 5 equiv of $NaBH_4$ overnight at rt. [c]Typical conditions: anhydrous CH_2Cl_2, 3 equiv of $NaBH(OAc)_3$ for 4 h at 0 °C.

dx.doi.org/10.1021/jm4004347 | J. Med. Chem. 2013, 56, 6088−6100

The (8′*R*,7′*S*) stereochemistry in compounds **30**−**37** was determined by NOE experiments (Figure 2). For example, for

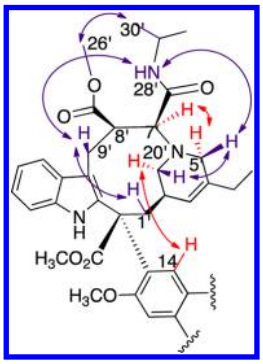


Figure 2. Selected NOE for compound **35**.

compound **35**, correlations could be observed, on the one hand, between H-20′β and H-5′β, H-5′β and NH-28′, NH-28′ and H-9′β, H-9′β and H1′β, CH_3-26′ and CH_3-30′ and, on the other hand, between H-20′α and H-14 and H-5′α and H-7′. This selectivity could be explained by the addition of the hydride on the lower face of velbenamine through the formation of an imino-boron-enol ester complex, as postulated by Palmieri et al.[25]

Overall, this two-step procedure enabled us to elaborate an original series of 16 new vinca alkaloids in a very efficient way.

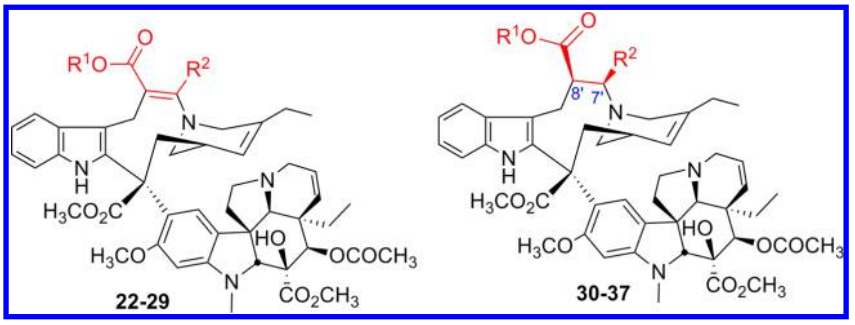
Biological Activity. Compounds **22**−**37** were evaluated for inhibition of tubulin polymerization. The results are summarized in Table 3. All tested compounds except **23**, **24**, **31**, and **32** displayed significant inhibitory activity on microtubule assembly. Compound **35** was even more potent than vinorelbine ($IC_{50\text{-}35}/IC_{50\text{-}VLN}$ = 0.6), and compounds **27** and **37** were almost as active ($IC_{50\text{-}27}/IC_{50\text{-}VLN}$ = 2.0 and $IC_{50\text{-}37}/IC_{50\text{-}VLN}$ = 1.7). Position 8′ did not appear to tolerate large substituents because compounds **23** (R = Et) and **24** (R = Bn) were totally inactive. On the contrary, C-7′ tolerates bulky groups as exemplified by compound **27** that was very active, inhibiting tubulin polymerization. Moreover, most of the reduced compounds were much more active than their parent β-enamino esters, except for compounds **33** and **36**, whose activities were comparable to those of **25** and **28**.

The activities of compounds **22**, **25**−**29**, **30**, and **33**−**37** were evaluated on human HCT-116 colorectal carcinoma, K562 leukemia, U87-MG glioblastoma, and HUVEC endothelial cells (Table 4). Their activities were, in many cases, in a nanomolar range. Once again, the β-amino esters were 10−500 times more potent than their β-enaminone analogues (see, for example, compounds **33** and **25**) except for compounds **26** and **34**, which showed similar cytotoxicity, and compounds **29** and **37**, because in this case the reduced compound was less active.

Notably, compounds **30** and **33** were more active than vinorelbine **7** on HCT-116 and K562 cells, and compound **33** was as active as **7** on U87 (1.5 nM) and HUVEC (2.5 nM) cell lines. It should be pointed out that there was no clear correlation between cytotoxicity and tubulin inhibition assembly as the more potent compounds in the tubulin polymerization assay (i.e., **27**, **35**, and **37**) were not the most cytotoxic.

The effects of the most cytotoxic compounds **30** and **33** were then investigated on cell cycle distribution of K562 and U87-MG cells by flow cytometry at various concentrations (Figure 3). As these compounds were highly cytotoxic, albeit 3−10 times less potent than vinorelbine **7** for inhibition of tubulin polymerization, we intended to ascertain that they lead to cell ring arrest in G_2/M, thus confirming their biological target. After 24 h treatment of K562 cells with 50 nM of **30** or 10 nM of **33**, a net increase in the number of cells arrested at the G_2/M growth phase was observed. A similar cell cycle profile was obtained with U87-MG cells after 24 h treatment with 50 nM of **30** or 5 nM of **33**, and increasing the concentration of **30** or

Table 3. Inhibition of Tubulin Polymerization by Compounds 22−37

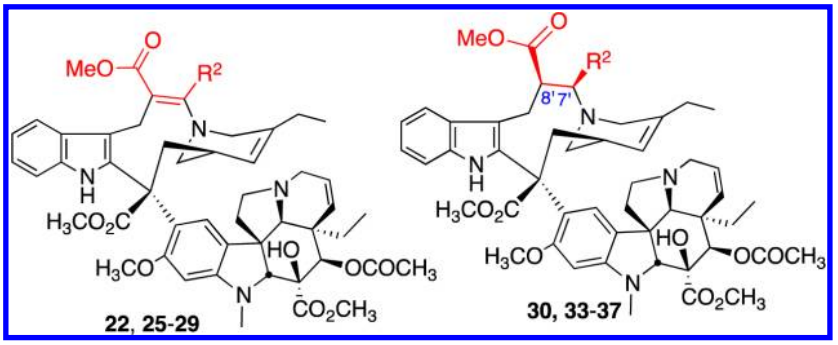


entry	R^1	R^2	compd	microtubule assembly inhibitory activity IC_{50} (μM)[a]	compd	microtubule assembly inhibitory activity IC_{50} (μM)[a]
1	Me	H	**22**	18.0	**30**	2.5
2	Et	H	**23**	>100	**31**	>100
3	Bn	H	**24**	>100	**32**	>100
4	Me	CO_2Me	**25**	6.0	**33**	8.6
5	Me	CONHBn	**26**	10.4	**34**	2.7
6	Me	CONH*i*Pr	**27**	1.6	**35**	0.5
7	Me	CONHAll	**28**	3.7	**36**	4.9
8	Me	CONH*t*Bu	**29**	10.4	**37**	1.1
9			7 (VLN)	0.7		
10			3 (VLB)	2.1		

[a]IC_{50} is the concentration of a compound that inhibits 50% of the rate of microtubule assembly (concentration in tubulin = 3 mg/mL).

dx.doi.org/10.1021/jm4004347 | *J. Med. Chem.* 2013, 56, 6088−6100

Table 4. Cytotoxicity of Compounds 22, 25−29, 30, and 33−37



	IC$_{50}$ (nM)[a]													
			R^2 H		R^2 CO$_2$Me		R^2 CONHBn		R^2 CONH*i*Pr		R^2 CONHAll		R^2 CONH*t*Bu	
	7 (VLN)	3 (VLB)	22	30	25	33	26	34	27	35	28	36	29	37
HCT116	35	3.5	500	18	500	6	250	200	2500	100	1800	45	60	300
K562	20	7	500	15	1000	5	80	75	1500	60	1000	50	50	250
U87	2	1	700	7	700	1.5	10	70	350	20	3000	10	25	80
HUVEC	2	0.8	250	8	100	2.5	40	60	700	150	2000	25	25	70

[a]IC$_{50}$ measures the drug concentration required for the inhibition of 50% cell proliferation after 72 h of incubation.

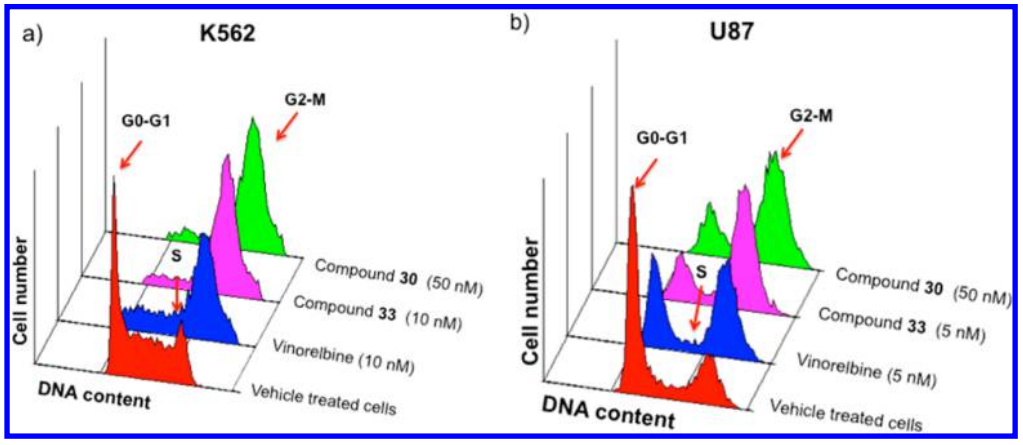


Figure 3. Effect of compounds **30**, **33**, and vinorelbine on cell-cycle distribution in chronic myelogenous leukemia K562 and glioblastoma U87-MG cell lines determined by flow cytometry analysis. DNA content was assessed via propidium iodide staining.

33 led to essentially the same results in K562 and U87-MG cells (data not shown).

Structural Interpretation. To provide a structural rationale to the observed differences in the inhibition of tubulin polymerization between β-enaminones **22**, **25−29**, and their β-amino esters equivalents **30** and **33−37**, we model-built the complexes of **35** and **27** with a $\beta_2\alpha_1$-tubulin interface and carried out molecular dynamics (MD) simulations. Both ligands were located between the GDP-bound β-tubulin subunit of the "bottom" heterodimer (β_1) and the GTP-Mg^{2+}-bound α-tubulin subunit of the "top" heterodimer (α_2).[3] Mutual adaptation between the ligands and the side chains of the amino acids making up the binding site during the MD trajectories improved the intermolecular interactions and provided distinct details for each complex. The van der Waals and hydrogen bonding interactions observed for **35** were overall similar to those already reported for vinblastine,[26] but the modification of ring C′ favors the formation of additional hydrogen bonds that contribute to tightening the interaction with both tubulin subunits (Figure 4). Thus, the hydrogen bond established between the NH-28′ of the CONH*i*Pr moiety at position 7′ and the side chain of Asp$_{\beta1}$179 favors the formation of a direct hydrogen bond between the charged amino group N-6′ in the velbenamine moiety and the backbone carbonyl of Val$_{\beta1}$177 instead of the water-bridged interaction that is established in the vinblastine complex. This improved interaction, along with the repuckering of the C′ ring brought about by the increase in the number of carbons and the position of the substituents at 7′ and 8′, also favors the formation of two hydrogen bonds involving the side chain of Asn$_{\alpha2}$329, one between the carboxamide oxygen and the indolic NH-17′ (on ring B′) and another between the carboxamide nitrogen and the ester carbonyl C-23′ on ring C′. Additionally, the carbonyl ester at 8′ establishes a hydrogen bonding interaction with the backbone NH of Tyr$_{\beta1}$224. Besides, the binding of **35** is further stabilized by a van der Waals interaction between the iPr moiety in the CONHiPr group at 7′ and the side chain of Leu$_{\alpha2}$248 in the T7 loop of α_2-tubulin.

On the other hand, the unsaturation between positions 7′ and 8′ in **27** does not favor a good orientation of the newly introduced moieties (Figure 5) and the two substituents facing the α_2 subunit force a readjustment of the T7 loop of α_2-tubulin. The iPr moiety of the CONHiPr group still establishes van der Waals interactions with Leu$_{\alpha2}$248 and Ala247, but its

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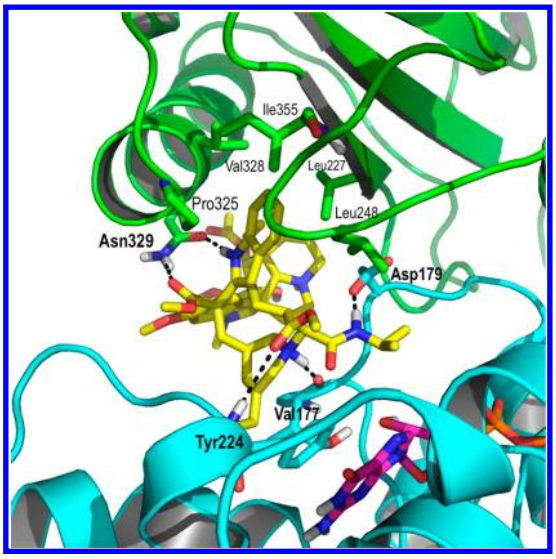

Figure 4. PyMOL cartoon and stick representation of **35** (yellow sticks) bound at the $\beta_1-\alpha_2$ interdimer interface near the GDP molecule (cyan, green, and magenta, respectively). Only polar hydrogens are shown for clarity. The residues lining the binding site are shown as sticks, and those establishing a hydrogen bond (dashed line) are labeled in bold.

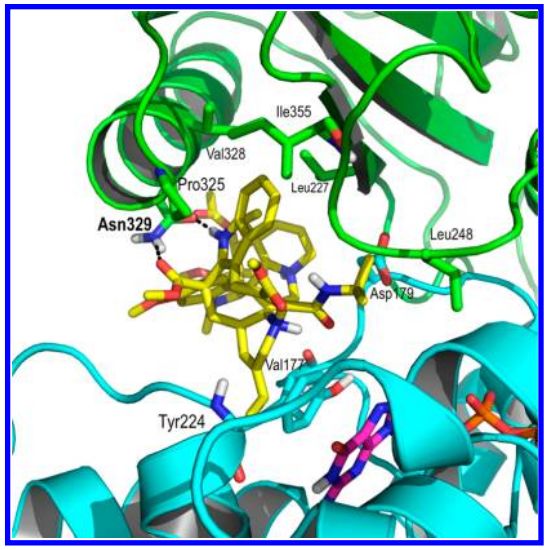

Figure 5. PyMOL cartoon and stick representation of **27** (yellow sticks) bound at the $\beta_1-\alpha_2$ interdimer interface near the GDP molecule (cyan, green, and magenta, respectively). Only polar hydrogens are shown for clarity. The residues lining the binding site are shown as sticks, and those establishing a hydrogen bond (dashed line) are labeled in bold.

orientation prevents formation of a hydrogen bond with either the carboxamide of $Asp_{\beta1}179$ or the backbone of $Tyr_{\beta1}224$. This arrangement therefore results in a different orientation of the vindoline group within the binding site that makes the interaction between the charged amino group in the velbenamine domain and the backbone carbonyl of $Val_{\beta1}177$ possible only through a bridging water molecule. The only hydrogen bonds that are maintained are those established with $Asn_{\alpha2}329$, and these are favored by the puckering of the C′ ring.

CONCLUSION

A series of 16 novel vinca derivatives enlarged and functionalized on ring C′ was designed and synthesized from vinorelbine **7** by means of a new and efficient two-step procedure. First, eight β-enamino esters **22**−**29** were synthesized by an insertion reaction that occurs under mild conditions, with good regioselectivity using several mono or disubstituted, asymmetric or nonasymmetric activated acetylenes. Subsequently, β-amino esters **30** and **33**−**37** were obtained by reduction of these β-enamino esters with $NaBH(OAc)_3$ with a total diastereoselectivity in good to excellent yields. Their ability to inhibit tubulin polymerization was evaluated, and one disubstituted compound, **35**, is shown to be more active than vinorelbine **7**. Interestingly, we demonstrate that β-amino esters **30** and **33**−**37** are globally more active than β-enamino esters **22** and **25**−**29** due to the fact that their greater flexibility allows a better fit in the vinca domain. In addition, position C-7′ is shown to tolerate large substituents in contrast with position C-8′ because the substituents located in the latter position are oriented toward the α_2-tubulin binding pocket, whereas the C-7′ substituents are oriented at the interdimer interface space created by the wedge that this type of antitumor drugs introduce in tubulin. Notably, two β-amino esters, **30** and **33**, proved to be highly cytotoxic, with **33** being much more active against HCT116 and K562 cell lines than vinorelbine **7**. Moreover, **30** and **33** appear to elicit their cytotoxicities in a fashion similar to vinorelbine via inhibition of tubulin polymerization, which then leads to cell cycle arrest in G_2/M.

Taken together, the present results demonstrate that introduction of functionalities on the "upper side" of velbenamine can lead to potent compounds, provided these groups are oriented at the $\beta_1-\alpha_2$ interdimer interface near the GDP molecule, as it is known that this space is also occupied by other tubulin binding molecules like phomopsin A and soblidotin.[27]

EXPERIMENTAL SECTION

General Information. Anhydrous solvents and starting materials were obtained from commercial suppliers and used as received, except for vinorelbine, which was a gift from Institut de Recherche Pierre Fabre, and alkynes **18**−**21**, which were synthesized using a published procedure.[28] All reactions were performed in oven-dried glassware under an argon atmosphere. Flash column chromatographies were performed using normal phase silica gel (60 Å, 40−63 μm). Chemical shifts (δ) for ^{1}H NMR and ^{13}C NMR spectra are reported in ppm relative to acetonitrile or chloroform resonances. Data for ^{1}H NMR are reported as follows: chemical shift (δ in ppm), multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet), coupling constant (Hz), integration. Data for ^{13}C NMR are reported in terms of chemical shift (δ in ppm). $[\alpha]_D$ are expressed in deg cm^3 g^{-1} dm^{-1} for a concentration of compound in mg cm^{-3}. High-resolution mass spectra (HMRS) were recorded by electrospray ionization (ESI). All the tested compounds **22**−**37** possess a purity ≥95% that was determined by UPLC using a C18 column (2.1 mm × 50 mm) and a gradient of water/acetonitrile from 95:5 to 0:100.

General Procedure A for the Synthesis of Alkynes. A solution of methyl propiolate (0.45 mL, 5 mmol) in anhydrous THF (25 mL) was cooled to −78 °C, under argon, before a solution of *n*-BuLi (1.6 M in hexanes, 3.3 mL, 5.25 mmol) was added dropwise. The mixture was stirred at that temperature for 30 min before the isocyanate (5 mmol) was added dropwise. The resulting mixture was stirred at −78 °C for 30 min. A saturated aqueous solution of ammonium chloride (20 mL) was added, and the mixture was allowed to warm to room temperature. The two phases were separated, and the aqueous layer was extracted

dx.doi.org/10.1021/jm4004347 | J. Med. Chem. 2013, 56, 6088−6100

with ethyl acetate. The combined organic extracts were washed with a saturated solution of sodium hydrogenocarbonate (20 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give a brown oil that was purified by column chromatography on silica gel using heptane/ethyl acetate 4:1.

*Methyl 4-(Allylamino)-4-oxobut-2-ynoate **20**.* Following general procedure A, methyl 4-(allylamino)-4-oxobut-2-ynoate was obtained as a yellow oil (261 mg, 31%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.58 (br s, 1H), 5.81−5.73 (ddd, J = 17.0 and 10.2 and 5.4 Hz, 1H), 5.19 (d, J = 17.0 Hz, 1H), 5.14 (d, J = 10.2 Hz, 1H), 3.89 (t, J = 5.4 Hz, 1H), 3.78 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.9, 150.8, 132.6, 117.7, 77.6, 73.8, 53.5, 42.5.

*Methyl 4-(tert-Butylamino)-4-oxobut-2-ynoate **21**.* Following general procedure A, methyl 4-(*tert*-butylamino)-4-oxobut-2-ynoate was obtained as a yellow oil (302 mg, 33%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.81 (s, 3H), 1.37(s, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.1, 149.9, 78.7, 71.9, 53.4, 53.3, 28.6.

General Procedure B for the Synthesis of the Enamino-Esters 22−29. *Method 1.* Alkyne (0.071 mmol, 1.1 equiv) was added to a solution of vinorelbine (50 mg, 0.064 mmol, 1 equiv) in CH_3CN (0.5 mL). The reaction mixture was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure. The resulting residue was purified by column chromatography on silica gel with ethyl acetate/acetone (1:0 to 4:1). The second fraction provided the corresponding vinca derivatives **22**, **23**, or **24**.

Method 2. Alkyne (0.128 mmol, 2 equiv) was added to a solution of vinorelbine (50 mg, 0.064 mmol, 1 equiv) in CH_3CN (0.5 mL). The reaction mixture was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure. The resulting residue was purified by column chromatography on alumina with heptane/ethyl acetate (4:1 to 0:1). The second fraction provided the corresponding vinca derivatives **25**−**29**.

*Compound **22**.* Following the general procedure B (method 1) with methyl propiolate **14**, compound **22** was obtained as a white powder (20 mg, 36%); $[\alpha]_D^{25}$ −55 (c 1.00, $CHCl_3$). ^{1}H NMR (CD_3CN, 300 MHz) δ 8.89 (s, 1H, OH), 8.15 (s, 1H, H-17′), 7.60 (d, J = 7.9 Hz, 1H, H-12′), 7.52 (s, 1H, H-7′), 7.13 (d, J = 7.9 Hz, 1H, H-15′), 6.99 (s, 1H, H-14), 6.98 (t, J = 7.9 Hz, 1H, H-14′), 6.89 (t, J = 7.9 Hz, 1H, H-13′), 6.29 (s, 1H, H-17), 5.76 (dd, J = 10.7 and 5.0 Hz, 1H, H-7), 5.38−5.36 (m, 1H, H-3′), 5.28 (s, 1H, H-4), 5.22 (d, J = 10.7 Hz, 1H, H-6), 4.11 (d, J = 15.1 Hz, 1H, H-9′α), 3.90 (d, J = 15.1 Hz, 1H, H-9′β), 3.72 (s, 3H, H-26′), 3.69 (s, 3H, H-25), 3.66 (s, 3H, H-22), 3.62 (s, 1H, H-2), 3.58 (d, J = 16.1 Hz, 1H, H-5′α), 3.52 (s, 3H, H-24′), 3.35 (d, J = 16.1 Hz, 1H, H-5′β), 3.34 (d, J = 16.1 Hz, 1H, H-8α), 3.32 (dd, J = 12.0 and 5.7 Hz, 1H, H-20′β), 3.27 (td, J = 9.5 and 6.0 Hz, 1H, H-10β), 2.74−2.70 (m, 1H, H-8b), 2.71 (s, 1H, H-19), 2.68 (s, 3H, H-23), 2.52 (dd, J = 12.0 and 3.5 Hz, 1H, H-20′α), 2.46 (td, J = 9.5 and 5.0 Hz, 1H, H-10α), 2.16 (td, J = 13.8 and 6.0 Hz, 1H, H-11β), 2.12−2.10 (m, 2H, H-1′), 2.10 (s, 3H, H-27), 2.09−2.06 (m, 1H, H-11α), 1.94−1.93 (m, 1H, H-2′), 1.90 (dd, J = 15.2 and 7.6 Hz, 2H, H-21′), 1.63 (dd, J = 14.7 and 7.0 Hz, 1H, H-20β), 1.28 (dd, J = 14.7 and 7.0 Hz, 1H, H-20α), 0.95 (t, J = 7.6 Hz, 3H, H-22′), 0.56 (t, J = 7.0 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 75 MHz) δ 175.0 (C-23′), 173.1 (C-24), 171.9 (C-26), 171.5 (C-25′), 159.6 (C-16), 154.4 (C-18), 154.4 (C-7′), 139.8 (C-4′), 136.2 (C-16′), 132.2 (C-18′), 131.2 (C-6), 130.0 (C-11′), 125.5 (C-7), 125.4 (C-13), 125.0 (C-14), 124.7 (C-3′), 122.5 (C-14′), 121.4 (C-15), 121.0 (C-12′), 119.8 (C-13′), 116.5 (C-10′), 111.9 (C-15′), 104.0 (C-8′), 95.4 (C-17), 84.5 (C-2), 80.5 (C-3), 77.4 (C-4), 67.1 (C-19), 56.6 (C-19′), 54.3 (C-12), 54.2 (C-5′), 52.9 (C-25), 52.7 (C-22), 52.1 (C-10), 51.8 (C-20′), 51.6 (C-26′), 51.5 (C-8), 45.3 (C-11), 43.8 (C-5), 38.9 (C-23), 35.2 (C-2′), 35.2 (C-1′), 32.0 (C-20), 28.2 (C-21′), 24.2 (C-9′), 21.0 (C-27), 12.7 (C-22′), 8.7 (C-21). HRMS-ESI calcd for $C_{49}H_{59}N_4O_{10}$ 863.4231, found 863.4257.

*Compound **23**.* Following the general procedure B (method 1) and using ethyl propiolate **15**, compound **23** was obtained as a white powder (18.4 mg, 33%); $[\alpha]^{25}{}_D$ −66.0 (c 1.00, $CHCl_3$). ^{1}H NMR (CD_3CN, 500 MHz) δ 8.16 (s, 1H, H-17′), 7.63 (d, J = 7.7 Hz, 1H, H-12′), 7.52 (s, 1H, H-7′), 7.13 (d, J = 7.7 Hz, 1H, H-15′), 6.98 (s, 1H, H-14), 6.97 (t, J = 7.7 Hz, 1H, H-14′), 6.89 (t, J = 7.7 Hz, 1H, H-13′), 6.29 (s, 1H, H-17), 5.78 (dd, J = 10.2 and 4.8 Hz, 1H, H-7), 5.37 (s, 1H, H-3′), 5.28 (s, 1H, H-4), 5.22 (d, J = 10.2 Hz, 1H, H-6), 4.20 (q, J = 6.9 Hz, 2H, H-26′), 4.11 (d, J = 15.5 Hz, 1H, H-9′α), 3.90 (d, J = 15.5 Hz, 1H, H-9′β), 3.72−3.70 (m, 1H, H-20′β), 3.68 (s, 3H, H-25), 3.66 (s, 3H, H-22), 3.62 (s, 1H, H-2), 3.58 (d, J = 16.3 Hz, 1H, H-5′α), 3.52 (s, 3H, H-24′), 3.37 (d, J = 16.2 Hz, 1H, H-1′β), 3.34 (d, J = 16.3 Hz, 1H, H-5′β), 3.33 (d, J = 15.5, 1H, H-8α), 3.26 (td, J = 9.9 and 4.6 Hz, 1H, H-11a), 2.72 (d, J = 15.5 Hz, 1H, H-8β), 2.71 (s, 1H, H-19), 2.68 (s, 3H, H-23), 2.52 (d, J = 13.1 Hz, 1H, H-20′α), 2.46 (td, J = 19.9 and 6.9 Hz, 1H, H-11β), 2.18−2.08 (m, 1H, H-10β and H-10α), 2.07 (d, J = 16.2 Hz, 1H, H-1′α), 1.94 (s,3H, H-27), 1.94 (s, 1H, H-2′), 1.92−1.89 (m, 2H, H-21′), 1.63 (td, J = 14.4 and 7.0 Hz, 1H, H-20β), 1.30−1.24 (m, 1H, H-20α), 1.27 (t, J = 6.9 Hz, 1H, H-27′), 0.94 (t, J = 7.4 Hz, 3H, H-22′), 0.56 (t, J = 7.0 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 500 MHz) δ 175.3 (C-23′), 173.4 (C-24), 171.9 (C-26), 171.5 (C-25′), 159.9 (C-16), 154.8 (C-18), 154.6 (C-7′), 140.2 (C-4′), 136.5 (C-16′), 132.6 (C-18′), 131.6 (C-6), 130.3 (C-11′), 125.8 (C-7), 125.7 (C-13), 125.3 (C-14), 125.0 (C-3′), 122.8 (C-14′), 121.8 (C-15), 121.3 (C-12′), 120.0 (C-13′), 118.7 (C-10′), 112.2 (C-15′), 105.3 (C-8′), 95.8 (C-17), 84.8 (C-2), 80.9 (C-3), 77.8 (C-4), 67.5 (C-19), 60.9 (C-26′), 57.0 (C-22), 55.7 (C-12), 54.5 (C-19′), 54.5 (C-5′), 53.2 (C-24′), 53.0 (C-25), 52.5 (C-20′), 52.0 (C-10), 51.8 (C-8), 45.7 (C-11), 44.2 (C-5), 39.2 (C-23), 35.6 (C-2′), 35.6 (C-1′), 32.3 (C-20), 28.5 (C-21′), 24.5 (C-9′), 21.6 (C-27), 15.4 (C-27′), 13.0 (C-22′), 9.0 (C-21). HRMS-ESI calcd for $C_{50}H_{61}N_4O_{10}$ 877.4387, found 877.4388.

*Compound **24**.* Following the general procedure B (method 1) and using benzyl propiolate **16**, compound **24** was obtained as a white powder by column chromatography on silica gel (19 mg, 32%); $[\alpha]_D^{25}$ −66 (c 1.00, $CHCl_3$). ^{1}H NMR (CD_3CN, 500 MHz) δ 8.90 (s, 1H, OH), 8.15 (s, 1H, H-17′), 7.60 (s, 1H, H-7′), 7.58 (d, J = 7.3 Hz, 1H, H-12′), 7.41 (d, J = 7.3 Hz, 2H, H-28′), 7.34 (t, J = 7.3 Hz, 2H, H-29′), 7.29 (dd, J = 14.6 and 7.3 Hz, 1H, H-30′), 7.12 (d, J = 7.3 Hz, 1H, H-15′), 6.98 (s, 1H, H-14), 6.96 (t, J = 7.3 Hz, 1H, H-14′), 6.82 (t, J = 7.3 Hz, 1H, H-13′), 6.29 (s, 1H, H-17), 5.79 (dd, J = 10.5 and 5.4 Hz, 1H, H-7), 5.35 (s, 1H, H-3′), 5.28 (s, 1H, H-4), 5.26 (d, J = 13.0 Hz, 1H, H-26′), 5.22 (d, J = 10.5 Hz, 1H, H-6), 5.19 (d, J = 13.0 Hz, 1H, H-26′), 4.13 (d, J = 15.4 Hz, 1H, H-9′α), 3.92 (d, J = 15.4 Hz, 1H, H-9′β), 3.74 (d, J = 13.0 Hz, 1H, H-20′ β), 3.68 (s, 3H, H-25), 3.65 (s, 3H, H-22), 3.61 (s, 1H, H-2), 3.59 (d, J = 4.0 Hz, 1H, H-5′α), 3.50 (s, 3H, H-24′), 3.35−3.33 (m, 2H, H-5′β and H-1′β), 3.31 (d, J = 5.4 Hz, 1H, H-8α), 3.25 (td, J = 9.3 and 4.8 Hz, 1H, H-10β), 2.72−2.70 (m, 1H, H-8β), 2.70 (s, 1H, H-19), 2.67 (s, 3H, H-23), 2.54 (dd, J = 13.0 and 4.3 Hz, 1H, H-20′α), 2.45−2.43 (m, 1H, H-10α), 2.16−2.13 (m, 1H, H-11β), 2.11−2.09 (m, 1H, H-1′b), 2.09−2.07 (m, 1H, H-11α), 1.94 (s, 3H, H-27), 1.93−1.91 (m, 2H, H-21′), 1.90 (s, 1H, H-2′), 1.63 (dd, J = 14.1 and 7.4 Hz, 1H, H-20β), 1.28 (dd, J = 14.1 and 7.4 Hz, 1H, H-20α), 0.93 (t, J = 7.4 Hz, 3H, H-22′), 0.56 (t, J = 7.4 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 125 MHz) δ 174.9 (C-23′), 173.1 (C-24), 171.5 (C-26), 170.9 (C-25′), 159.6 (C-16), 154.7 (C-7′), 154.4 (C-18), 139.8 (C-4′), 138.9 (C-27′), 136.2 (C-16′), 132.2 (C-18′), 131.2 (C-6), 129.9 (C-11′), 129.5 (C-29′), 129.0 (C-28′), 128.8 (C-30′), 125.5 (C-7), 125.4 (C-13), 125.0 (C-14), 124.6 (C-3′), 122.5 (C-14′), 121.4 (C-15), 121.0 (C-12′), 119.7 (C-13′), 116.6 (C-10′), 111.9 (C-15′), 104.0 (C-8′), 95.4 (C-17), 84.5 (C-2), 80.6 (C-3), 77.5 (C-4), 67.1 (C-19), 66.3 (C-26′), 56.7 (C-22), 56.0 (C-19′), 54.3 (C-5′), 54.2 (C-12), 52.9 (C-24′), 52.7 (C-25), 52.1 (C-20′), 51.6 (C-10), 51.5 (C-8), 45.4 (C-11), 43.8 (C-5), 38.9 (C-23), 35.2 (C-1′), 35.2 (C-2′), 32.0 (C-20), 28.2 (C-21′), 24.3 (C-9′), 21.3 (C-27), 12.7 (C-22′), 8.7 (C-21). HRMS-ESI calcd for $C_{55}H_{63}N_4O_{10}$ 939.4544, found 939.4561.

*Compound **25**.* Following the general procedure B (method 2) and using dimethyl acetylenedicarboxylate **17**, compound **25** was obtained as a white powder by column chromatography on silica gel (21.9 mg, 37%); $[\alpha]_D^{25}$ −23 (c 1.00, $CHCl_3$). ^{1}H NMR (CD_3CN, 500 MHz) δ 8.41 (s, 1H, H-17′), 7.34 (d, J = 7.9 Hz, 1H, H-12′), 7.14 (d, J = 7.9 Hz, 1H, H-15′), 6.99 (t, J = 7.9 Hz, 1H, H-14′), 6.93 (s, 1H, H-14), 6.90 (t, J = 7.9 Hz, 1H, H-13′), 6.27 (s, 1H, H-17), 5.78 (dd, J = 10.1 and 5.4 Hz, 1H, H-7), 5.27 (d, J = 10.1 Hz, 1H, H-6), 5.25 (s, 1H, H-3′), 5.23 (s, 1H, H-4), 4.45 (d, J = 15.0 Hz, 1H, H-9′α), 4.00 (d, J =

dx.doi.org/10.1021/jm4004347 | J. Med. Chem. 2013, 56, 6088−6100

15.0 Hz, 1H, H-9′β), 3.72 (s, 3H, H-22), 3.71 (s, 3H, H-26′), 3.71−3.67 (m, 1H, H-5′α), 3.69 (s, 3H, H-28′), 3.67 (s, 3H, H-25), 3.58 (s, 1H, H-2), 3.49 (s, 3H, H-24′), 3.48−3.44 (m, 1H, H-1′β), 3.34 (d, *J* = 15.0 Hz, 1H, H-20′β), 3.29 (dd, *J* = 16.0 and 5.4 Hz, 1H, H-8α), 3.24−3.17 (m, 1H, H-10β), 2.95 (d, *J* = 15.0 Hz, 1H, H-20′α), 2.80 (s, 1H, H-19), 2.73 (d, *J* = 16.0 Hz, 1H, H-8β), 2.70−2.67 (m, 1H, H-5′β), 2.67 (s, 3H, H-23), 2.53−2.50 (m, 1H, H-10α), 2.15−2.11 (m, 1H, H-1′α), 2.10−2.04 (m, 1H, H-11β), 1.94 (s, 3H, H-27), 1.92−1.87 (m, 1H, H-11α), 1.91−1.86 (m, 2H, H-21′), 1.61 (q, *J* = 7.2 Hz, 1H, H-20β), 1.61−1.57 (m, 1H, H-2′), 1.33 (q, *J* = 7.2 Hz, 1H, H-20α), 0.92 (t, *J* = 7.5 Hz, 3H, H-22′), 0.63 (t, *J* = 7.2 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 125 MHz) δ 175.4 (C-23′), 173.3 (C-24), 172.0 (C-25′), 171.9 (C-26), 167.0 (C-27′), 159.7 (C-16), 154.7 (C-18), 152.5 (C-7′), 139.3 (C-4′), 136.7 (C-16′), 133.0 (C-18′), 131.7 (C-6), 130.4 (C-11′), 126.0 (C-14), 125.8 (C-7), 125.3 (C-13), 124.3 (C-3′), 123.2 (C-14′), 122.1 (C-15), 120.7 (C-12′), 120.3 (C-13′), 119.9 (C-8′), 114.9 (C-10′), 112.5 (C-15′), 95.8 (C-17), 84.6 (C-2), 81.1 (C-3), 77.8 (C-4), 66.5 (C-19), 57.3 (C-19′), 57.1 (C-22), 55.2 (C-5′), 54.7 (C-12), 53.2 (C-26′), 53.1 (C-25), 53.0 (C-28′), 51.6 (C-8), 51.5 (C-24′), 51.2 (C-10), 51.1 (C-20′), 46.1 (C-11), 44.1 (C-5), 39.1 (C-23), 36.0 (C-2′), 35.1 (C-1′), 32.3 (C-20), 28.4 (C-21′), 26.4 (C-9′), 21.6 (C-27), 13.0 (C-22′), 9.1 (C-21). HRMS-ESI calcd for $C_{51}H_{61}N_4O_{12}$ 921.4286, found 921.4285.

Compound ***26***. Following the general procedure B (method 2) using alkyne **18**, compound **26** was obtained as a yellow powder (32.0 mg, 50%); $[\alpha]_D^{25}$ −75 (*c* 1.00, $CHCl_3$). ^{1}H NMR (CD_3CN, 700 MHz) δ 8.82 (s, 1H, OH), 8.34 (s, 1H, H-17′), 7.36 (d, *J* = 7.9 Hz, 1H, H-12′), 7.29−7.28 (m, 2H, H-32′ and H-34′), 7.28−7.26 (m, 1H, H-33′), 7.22−7.18 (m, 2H, H-31′ and H-35′), 7.11 (d, *J* = 7.9 Hz, 1H, H-15′), 6.97 (t, *J* = 7.9 Hz, 1H, H-14′), 6.90 (t, *J* = 6.4 Hz, 1H, H-28′), 6.88 (t, *J* = 7.9 Hz, 1H, H-13′), 6.87 (s, 1H, H-14), 6.25 (s, 1H, H-17), 5.75 (dd, *J* = 9.8 and 4.4 Hz, 1H, H-7), 5.24 (d, *J* = 9.8 Hz, 1H, H-6), 5.22 (s, 1H, H-4), 5.22−5.19 (m, 1H, H-3′), 4.40 (dd, *J* = 14.8 and 6.4 Hz, H-29′α), 4.35 (d, *J* = 14.8 Hz, 1H, H-9′β), 4.24 (dd, *J* = 14.8 and 6.4 Hz, H-29′β), 4.01 (d, *J* = 14.8 Hz, 1H, H-9′α), 3.77 (d, *J* = 13.3 Hz, 1H, H-20′β), 3.71 (s, 3H, H-22), 3.65 (s, 3H, H-25), 3.55−3.54 (s, 1H, H-2), 3.53 (s, 3H, H-26′), 3.48 (s, 3H, H-24′), 3.46−3.44 (m, 1H, H-1′β), 3.32 (d, *J* = 14.5 Hz, 1H, H-5′α), 3.26 (d, *J J* = 14.4 Hz, 1H, H-8α), 3.19−3.16 (m, 1H, H-10β), 2.98 (d, *J* = 14.5 Hz, 1H, H-5′β), 2.72 (s, 1H, H-19), 2.70−2.67 (m, 1H, H-20′α), 2.67 (d, *J* = 14.5 Hz, 1H, H-8β), 2.64 (s, 3H, H-23), 2.42−2.38 (m, 1H, H-10α), 2.10 (d, *J* = 16.1 Hz, 1H, H-1′α), 2.08−2.02 (m, 1H, H-11β), 1.92 (s, 3H, H-27), 1.90−1.86 (m, 1H, H-11α), 1.83 (q, *J* = 7.5 Hz, 2H, H-21′), 1.62−1.57 (m, 1H, H-20β), 1.56−1.54 (m, 1H, H-2′), 1.34−1.28 (m, 1H, H-20α), 0.87 (t, *J* = 7.5 Hz, 3H, H-22′), 0.61 (t, *J* = 6.7 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 175 MHz) δ 175.5 (C-23′), 173.4 (C-24), 172.6 (C-25′), 171.9 (C-26), 166.3 (C-27′), 159.7 (C-16), 157.6 (C-7′), 154.8 (C-18), 140.5 (C-30′), 139.6 (C-4′), 136.8 (C-16′), 132.6 (C-18′), 131.7 (C-6), 130.0 (C-11′), 129.9 (C-33′), 129.5 (C-32′ and C-34′), 128.6 (C-31′ and C-35′), 126.0 (C-14), 125.9 (C-7 and C-13), 124.5 (C-3′), 123.2 (C-14′), 122.3 (C-15), 121.0 (C-12′), 120.3 (C-13′), 116.0 (C-10′ and C-8′), 112.4 (C-15′), 95.5 (C-17), 84.7 (C-2), 81.1 (C-3), 77.9 (C-4), 66.8 (C-19), 57.4 (C-19′), 57.2 (C-22), 55.4 (C-20′), 54.7 (C-12), 53.2 (C-24′), 53.0 (C-25), 52.7 (C-26′), 51.7 (C-8), 51.4 (C-10), 51.1 (C-5′), 46.1 (C-11), 44.3 (C-29′), 44.2 (C-5), 39.2 (C-23), 36.0 (C-2′), 35.2 (C-1′), 32.3 (C-20), 28.7 (C-21′), 26.8 (C-9′), 21.7 (C-27), 13.1 (C-22′), 9.2 (C-21). HRMS-ESI calcd for $C_{53}H_{63}N_5O_{11}$ 996.4759, found 996.4789.

Compound ***27***. Following the general procedure B (method 2) and using alkyne **19**, compound **27** was obtained as a yellow powder (31.8 mg, 52%); $[\alpha]_D^{25}$ −28 (*c* 1.00, $CHCl_3$). ^{1}H NMR (CD_3CN, 500 MHz) δ 8.88 (s, 1H, OH), 8.38 (s, 1H, H-17′), 7.39 (d, *J* = 7.9 Hz, 1H, H-12′), 7.13 (d, *J* = 7.9 Hz, 1H, H-15′), 6.99 (t, *J* = 7.9 Hz, 1H, H-14′), 6.90 (t, *J* = 7.9 Hz, 1H, H-13′), 6.89 (s, 1H, H-14), 6.37 (d, *J* = 7.1 Hz, 1H, H-28′), 6.27 (s, 1H, H-17), 5.78 (dd, *J* = 8.9 and 4.5 Hz, 1H, H-7), 5.26 (d, *J* = 8.9 Hz, 1H, H-6), 5.25−5.24 (m, 1H, H-3′), 5.23 (s, 1H, H-4), 4.34 (d, *J* = 14.6 Hz, 1H, H-9′β), 4.03 (d, *J* = 14.0 Hz, 1H, H-9′α), 3.92 (sext, *J* = 7.1 Hz, H-29′), 3.79 (d, *J* = 13.6 Hz, 1H, H-20′β), 3.73 (s, 3H, H-22), 3.68 (s, 3H, H-26′), 3.67 (s, 3H, H-25), 3.57 (s, 1H, H-2), 3.50 (s, 3H, H-24′), 3.46 (t, *J* = 15.3 Hz, 1H, H-1′β), 3.40 (d, *J* = 15.5 Hz, 1H, H-5′α), 3.29 (dd, *J* = 12.3 and 4.5 Hz, 1H, H-8α), 3.20 (td, *J* = 10.5 and 4.9 Hz, 1H, H-10β), 3.05 (d, *J* = 15.5 Hz, 1H, H-5′β), 2.76 (s, 1H, H-19), 2.72 (d, *J* = 13.6 Hz, 1H, H-20′α), 2.71 (dd, *J* = 12.3 Hz, 1H, H-8β), 2.67 (s, 3H, H-23), 2.45 (td, *J* = 10.5 and 6.0 Hz, 1H, H-10α), 2.12 (d, *J* = 15.3 Hz, 1H, H-1′α), 2.09−2.06 (m, 1H, H-11β), 1.94 (s, 3H, H-27), 1.92−1.88 (m, 1H, H-11α), 1.91−1.87 (m, 2H, H-21′), 1.65−1.57 (m, 1H, H-20β), 1.58−1.56 (m, 1H, H-2′), 1.37−1.30 (m, 1H, H-20α), 1.08 (t, *J* = 7.1 Hz, 6H, H-30′), 0.92 (t, *J* = 7.5 Hz, 3H, H-22′), 0.63 (t, *J* = 7.3 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 125 MHz) δ 175.5 (C-23′), 173.3 (C-24), 172.6 (C-25′), 171.9 (C-26), 165.9 (C-27′), 159.6 (C-16), 157.4 (C-7′), 154.7 (C-18), 139.6 (C-4′), 136.8 (C-16′), 132.7 (C-18′), 131.7 (C-6), 130.4 (C-11′), 126.0 (C-14), 125.9 (C-7), 125.3 (C-13), 124.3 (C-3′), 123.1 (C-14′), 122.2 (C-15), 121.0 (C-12′), 120.2 (C-13′), 116.1 (C-10′), 115.1 (C-8′), 112.4 (C-15′), 95.4 (C-17), 84.6 (C-2), 81.1 (C-3), 77.8 (C-4), 66.6 (C-19), 57.2 (C-22), 56.8 (C-19′), 55.5 (C-20′), 54.6 (C-12), 53.1 (C-24′), 53.0 (C-25), 52.8 (C-26′), 51.6 (C-8), 51.3 (C-10), 50.9 (C-5′), 46.0 (C-11), 44.2 (C-5), 42.7 (C-29′), 39.1 (C-23), 35.9 (C-2′), 35.1 (C-1′), 32.3 (C-20), 28.6 (C-21′), 26.7 (C-9′), 22.9 (C-30′), 21.7 (C-27), 13.0 (C-22′), 9.1 (C-21). HRMS-ESI calcd for $C_{53}H_{65}N_5O_{11}$ 948.4759, found 948.4767.

Compound ***28***. Following the general procedure B (method 2) using alkyne **20**, compound **28** was obtained as a yellow powder (30.2 mg, 50%); $[\alpha]_D^{25}$ −32 (*c* 1.00, $CHCl_3$). ^{1}H NMR (CD_3CN, 500 MHz) δ 8.88 (s, 1H, OH), 8.39 (s, 1H, H-17′), 7.39 (d, *J* = 7.8 Hz, 1H, H-12′), 7.13 (d, *J* = 7.8 Hz, 1H, H-15′), 6.99 (t, *J* = 7.8 Hz, 1H, H-14′), 6.90 (t, *J* = 7.8 Hz, 1H, H-13′), 6.89 (s, 1H, H-14), 6.62 (t, *J* = 6.0 Hz, 1H, H-28′), 6.28 (s, 1H, H-17), 5.86−5.80 (m, 1H, H-30′), 5.77 (dd, *J* = 10.3 and 5.0 Hz, 1H, H-7), 5.26 (d, *J* = 10.3 Hz, 1H, H-6), 5.25−5.24 (m, 1H, H-3′), 5.23 (s, 1H, H-4), 5.17 (d, *J* = 17.3 Hz, 1H, H-31′), 5.07 (d, *J* = 10.2 Hz, 1H, H-31′), 4.36 (d, *J* = 14.6 Hz, 1H, H-9′β), 4.03 (d, *J* = 14.6 Hz, 1H, H-9′α), 3.83 (dt, *J* = 15.5 and 6.0 Hz, 1H, H-29′), 3.74 (dt, *J* = 15.5 and 6.0 Hz, 1H, H-29′), 3.79 (d, *J* = 11.7 Hz, 1H, H-20′β), 3.73 (s, 3H, H-22), 3.68 (s, 3H, H-26′), 3.67 (s, 3H, H-25), 3.57 (s, 1H, H-2), 3.50 (s, 3H, H-24′), 3.46 (d, *J* = 14.8 Hz, 1H, H-1′β), 3.39 (d, *J* = 15.5 Hz, 1H, H-5′α), 3.28 (dd, *J* = 16.1 and 5.0 Hz, 1H, H-8α), 3.20 (td, *J* = 10.0 and 5.0 Hz, 1H, H-10β), 3.04 (d, *J* = 15.5 Hz, 1H, H-5′β), 2.76 (s, 1H, H-19), 2.73 (d, *J* = 11.7 Hz, 1H, H-20′α), 2.70 (d, *J* = 16.1 Hz, 1H, H-8β), 2.67 (s, 3H, H-23), 2.44 (td, *J* = 10.0 and 6.3 Hz, 1H, H-10α), 2.12 (d, *J* = 14.8 Hz, 1H, H-1′α), 2.08−2.04 (m, 1H, H-11β), 1.94 (s, 3H, H-27), 1.93−1.90 (m, 1H, H-11α), 1.89 (q, *J* = 7.5 Hz, 2H, H-21′), 1.61 (td, *J* = 14.2 and 7.2 Hz, 1H, H-20β), 1.58−1.56 (m, 1H, H-2′), 1.33 (td, *J* = 14.2 and 7.2 Hz, 1H, H-20α), 0.92 (t, *J* = 7.5 Hz, 3H, H-22′), 0.63 (t, *J* = 7.2 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 125 MHz) δ 175.5 (C-23′), 173.3 (C-24), 172.6 (C-25′), 171.9 (C-26), 166.7 (C-27′), 159.6 (C-16), 157.0 (C-7′), 154.7 (C-18), 139.5 (C-4′), 136.8 (C-16′), 136.1 (C-30′), 132.7 (C-18′), 131.7 (C-6), 130.4 (C-11′), 126.0 (C-14), 125.9 (C-7), 125.3 (C-13), 124.4 (C-3′), 123.1 (C-14′), 122.2 (C-15), 121.0 (C-12′), 120.2 (C-13′), 117.0 (C-31′), 115.9 (C-8′), 115.8 (C-10′), 112.4 (C-15′), 95.4 (C-17), 84.6 (C-2), 81.0 (C-3), 77.8 (C-4), 66.6 (C-19), 57.2 (C-22), 56.7 (C-19′), 55.4 (C-20′), 54.7 (C-12), 53.1 (C-24′), 53.0 (C-25), 52.9 (C-26′), 51.6 (C-8), 51.3 (C-10), 51.0 (C-5′), 46.0 (C-11), 44.1 (C-5), 42.8 (C-29′), 39.1 (C-23), 35.9 (C-2′), 35.1 (C-1′), 32.3 (C-20), 28.5 (C-21′), 26.7 (C-9′), 21.7 (C-27), 13.0 (C-22′), 9.1 (C-21). HRMS-ESI calcd for $C_{53}H_{63}N_5O_{11}$ 946.4602, found 946.4646.

Compound ***29***. Following the general procedure B (method 2) using alkyne **21**, compound **29** was obtained as a yellow powder (32.1 mg, 52%). ^{1}H NMR (CD_3CN, 500 MHz) δ 8.91 (s, 1H, OH), 8.45 (s, 1H, H-17′), 7.44 (d, *J* = 7.6 Hz, 1H, H-12′), 7.17 (d, *J* = 7.6 Hz, 1H, H-15′), 7.02 (t, *J* = 7.6 Hz, 1H, H-14′), 6.94 (t, *J* = 7.6 Hz, 1H, H-13′), 6.92 (s, 1H, H-14), 6.31 (s, 1H, H-28′), 6.28 (s, 1H, H-17), 5.81 (dd, *J* = 10.1 and 4.9 Hz, 1H, H-7), 5.30 (d, *J* = 10.1 Hz, 1H, H-6), 5.27 (s, 1H, H-4), 5.27−5.26 (m, 1H, H-3′), 4.37 (d, *J* = 14.8 Hz, 1H, H-9′β), 4.05 (d, *J* = 14.8 Hz, 1H, H-9′α), 3.81 (d, *J* = 12.7 Hz, 1H, H-20′β), 3.77 (s, 3H, H-22), 3.73 (s, 3H, H-26′), 3.71 (s, 3H, H-25), 3.61 (s, 1H, H-2), 3.53 (s, 3H, H-24′), 3.51−3.46 (m, 1H, H-1′β), 3.46 (d, *J* = 13.5 Hz, 1H, H-5′α), 3.32 (d, *J* = 14.9 Hz, 1H, H-8α), 3.26−3.19 (m, 1H, H-10β), 3.14 (d, *J* = 13.5 Hz, 1H, H-5′β), 2.78 (s, 1H, H-19), 2.73

dx.doi.org/10.1021/jm4004347 | *J. Med. Chem.* 2013, 56, 6088−6100

(d, J = 12.7 Hz, 1H, H-20′α), 2.72−2.68 (m, 1H, H-8β), 2.71 (s, 3H, H-23), 2.47 (dd, J = 16.9 and 8.4 Hz, 1H, H-10α), 2.16 (d, J = 16.3 Hz, 1H, H-1′α), 2.14−2.08 (m, 1H, H-11β), 1.98 (s, 3H, H-27), 1.95−1.92 (m, 2H, H-11α and H-21′), 1.65 (td, J = 14.6 and 6.7 Hz, 1H, H-20β), 1.60−1.58 (m, 1H, H-2′), 1.40−1.36 (m, 1H, H-20α), 0.96 (t, J = 7.2 Hz, 3H, H-22′), 0.66 (t, J = 6.7 Hz, 3H, H-21). ^{13}C NMR (CD3CN, 125 MHz) δ 175.5 (C-23′), 173.3 (C-24), 172.7 (C-25′), 171.9 (C-26), 166.3 (C-27′), 159.6 (C-16), 157.6 (C-7′), 154.7 (C-18), 139.6 (C-4′), 136.7 (C-16′), 132.6 (C-18′), 131.7 (C-6), 130.5 (C-11′), 126.0 (C-14), 125.9 (C-7), 125.3 (C-13), 124.3 (C-3′), 123.1 (C-14′), 122.2 (C-15), 121.1 (C-12′), 120.2 (C-13′), 116.1 (C-10′), 114.8 (C-8′), 112.4 (C-15′), 95.4 (C-17), 84.6 (C-2), 81.1 (C-3), 77.8 (C-4), 66.6 (C-19), 57.3 (C-19′), 57.2 (C-22), 55.5 (C-20′), 54.7 (C-12), 53.1 (C-24′), 53.0 (C-25), 52.9 (C-26′), 52.7 (C-29′), 51.6 (C-8), 51.2 (C-10), 50.9 (C-5′), 46.0 (C-11), 44.1 (C-5), 39.1 (C-23), 36.0 (C-2′), 35.1 (C-1′), 32.2 (C-20), 29.1 (C-30′), 28.6 (C-21′), 26.7 (C-9′), 21.7 (C-27), 13.0 (C-22′), 9.1 (C-21). HRMS-ESI calcd for $C_{54}H_{67}N_5O_{11}$ 962.4915, found 962.4957.

General Synthetic Procedure C for the Synthesis of Amino-Esters 27−32. *Method 1.* To a solution of anhydrous zinc iodide (3 equiv) in anhydrous CH_2Cl_2 (0.2 mL) at 0 °C were added the corresponding enamino-esters **22−29** (20 mg, 1 equiv). The resulting mixture was stirred at the same temperature for 1 h, after which $NaBH_4$ (5 equiv) was added. The solution was allowed to reach room temperature and stirred at room temperature overnight. The reaction was then quenched with a saturated ammonium chloride solution, and the reaction mixture was extracted with CH_2Cl_2. After usual treatments, purification of the crude compound was carried out by column chromatography on silica gel using ethyl acetate/acetone (1:0 to 4:1).

Method 2. $NaBH(OAc)_3$ (3 equiv) was added to a solution of the corresponding enamino-ester **22−29** (20 mg, 1 equiv) in CH_2Cl_2 (0.2 mL) at 0 °C. The reaction mixture was stirred for 4 h at 0 °C. The resulting mixture was diluted with CH_2Cl_2 and washed with a saturated sodium carbonate solution. Evaporation of the solvent provided pure amino-esters **30−37**.

Compound 30. Following the general procedure C (method 2) compound **30** was obtained as a white powder (14 mg, 69%); $[\alpha]_D^{25}$ +30 (c 1.00, $CHCl_3$). ^{1}H NMR (CD_3CN, 600 MHz) δ 8.51 (s, 1H, H-17′), 7.33 (d, J = 7.6 Hz, 1H, H-12′), 7.16 (d, J = 7.6 Hz, 1H, H-15′), 7.01 (t, J = 7.6 Hz, 1H, H-14′), 6.92 (t, J = 7.6 Hz, 1H, H-13′), 6.80 (s, 1H, H-14), 6.25 (s, 1H, H-17), 5.77 (dd, J = 10.7 and 5.1 Hz, 1H, H-7), 5.26 (d, J = 10.7 Hz, 1H, H-6), 5.18 (s, 1H, H-4), 5.13 (d, J = 4.6 Hz, 1H, H-3′), 4.14 (d, J = 14.5 Hz, 1H, H-9′β), 3.75 (s, 3H, H-22), 3.71 (s, 3H, H-26′), 3.66 (s, 3H, H-25), 3.52 (s, 1H, H-2), 3.49 (s, 3H, H-24′), 3.26 (dd, J = 14.9 and 5.1 Hz, 1H, H-8β), 3.21 (dd, J = 16.0 and 11.4 Hz, 1H, H-1′β), 3.16 (td, J = 9.9 and 5.6 Hz, 1H, H-10β), 3.10 (d, J = 14.5 Hz, 1H, H-9′α), 3.02−3.00 (m, 1H, H-8′), 2.99 (d, J = 15.6 Hz, 1H, H-5′β), 2.84 (d, J = 11.3 Hz, 1H, H-20′β), 2.82 (d, J = 15.6 Hz, 1H, H-5′α), 2.80 (s, 1H, H-19), 2.70 (d, J = 12.2 Hz, 1H, H-7′β), 2.68 (d, J = 14.9 Hz, 1H, H-8α), 2.65 (s, 3H, H-23), 2.60−2.56 (m, 1H, H-7′α), 2.44 (td, J = 9.9 and 5.6 Hz, 1H, H-10α), 2.20 (d, J = 16.0 Hz, 1H, H-1′α), 2.01−1.97 (m, 1H, H-11β), 1.94 (s, 3H, H-27), 1.87 (q, J = 7.6 Hz, 2H, H-21′), 1.80 (td, J = 12.2 and 5.6 Hz, 1H, H-11α), 1.68 (d, J = 11.3 Hz, 1H, H-20′α), 1.57 (q, J = 7.6 Hz, 1H, H-20β), 1.30 (q, J = 7.6 Hz, 1H, H-20α), 1.08 (s, 1H, H-2′), 0.92 (t, J = 7.6 Hz, 3H, H-22′), 0.64 (t, J = 6.9 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 150 MHz) δ 178.3 (C-25′), 176.3 (C-23′), 173.1 (C-24), 171.8 (C-26), 159.6 (C-16), 154.2 (C-18), 140.8 (C-4′), 136.7 (C-16′), 132.4 (C-18′), 131.7 (C-6), 130.9 (C-11′), 125.9 (C-14), 125.8 (C-7), 124.9 (C-13), 124.4 (C-3′), 123.3 (C-14′), 122.7 (C-15), 120.2 (C-12′), 120.0 (C-13′), 118.0 (C-10′), 112.3 (C-15′), 94.9 (C-17), 84.4 (C-2), 81.1 (C-3), 77.7 (C-4), 65.8 (C-19), 57.7 (C-19′), 57.1 (C-22), 57.0 (C-5′), 56.3 (C-7′), 54.6 (C-12), 53.0 (C-24′), 53.0 (C-26′), 52.9 (C-25), 51.4 (C-8), 50.7 (C-10), 50.3 (C-20′), 47.8 (C-8′), 45.9 (C-11), 44.0 (C-5), 39.1 (C-23), 35.9 (C-2′), 35.2 (C-1′), 32.1 (C-20), 28.4 (C-21′), 22.1 (C-9′), 21.6 (C-27), 13.1 (C-22′), 9.0 (C-21). HRMS-ESI calcd for $C_{49}H_{61}N_4O_{10}$ 865.4387, found 865.4398.

Compound 31. Following the general procedure C (method 2) compound **31** was obtained as a white powder (18.9 mg, 95%); $[\alpha]^{25}_D$ +28.0 (c 1.00, $CHCl_3$). ^{1}H NMR (CD_3CN, 600 MHz) δ 8.89 (s, 1H, OH), 8.47 (s, 1H, H-17′), 7.36 (d, J = 7.9 Hz, 1H, H-12′), 7.15 (d, J = 7.9 Hz, 1H, H-15′), 7.01 (t, J = 7.9 Hz, 1H, H-14′), 6.92 (t, J = 7.9 Hz, 1H, H-13′), 6.79 (s, 1H, H-14), 6.26 (s, 1H, H-17), 5.77 (dd, J = 10.3 and 5.3 Hz, 1H, H-7), 5.27 (d, J = 10.3 Hz, 1H, H-6), 5.19 (s, 1H, H-4), 5.13 (d, J = 5.3 Hz, 1H, H-3′), 4.19 (dd, J = 10.3 and 7.2 Hz, 2H, H-26′), 4.13 (ddd, J = 15.3 and 10.0 and 5.0 Hz, 1H, H-9′β), 3.76 (s, 3H, H-22), 3.66 (s, 3H, H-25), 3.52 (s, 1H, H-2), 3.49 (s, 3H, H-24′), 3.27 (dd, J = 15.5 and 5.3 Hz, 1H, H-8β), 3.23 (dd, J = 15.0 and 11.6 Hz, 1H, H-1′β), 3.16 (td, J = 10.0 and 5.8 Hz, 1H, H-10β), 3.10 (dd, J = 15.3 and 5.0 Hz, 1H, H-9′α), 3.01 (d, J = 15.5 Hz, 1H, H-5′β), 2.97 (dd, J = 11.0 and 6.8 Hz, 1H, H-8′), 2.85 (d, J = 12.0 Hz, 1H, H-20′β), 2.82 (d, J = 15.5 Hz, 1H, H-5′α), 2.77 (s, 1H, H-19), 2.70 (dd, J = 11.0 and 6.8 Hz, 1H, H-7′β), 2.67 (d, J = 15.5 Hz, 1H, H-8α), 2.65 (s, 3H, H-23), 2.59 (dd, J = 11.0 and 6.8 Hz, 1H, H-7′α), 2.43 (td, J = 10.0 and 5.8 1H, H-10α), 2.21 (d, J = 15.0 Hz, 1H, H-1′α), 1.99 (td, J = 13.5 and 5.8 Hz, 1H, H-11β), 1.94 (s, 3H, H-27), 1.88 (q, J = 7.4 Hz, 2H, H-21′), 1.82 (td, J = 12.6 and 5.8 Hz, 1H, H-11α), 1.68 (d, J = 12.0 Hz, 1H, H-20′α), 1.58 (q, J = 7.1 Hz, 1H, H-20β), 1.30 (q, J = 7.1 Hz, 1H, H-20α), 1.13−1.09 (m, 1H, H-2′), 0.93 (t, J = 7.4 Hz, 3H, H-22′), 0.65 (t, J = 7.1 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 600 MHz) δ 177.9 (C-25′), 176.3 (C-23′), 173.2 (C-24), 171.9 (C-26), 159.7 (C-16), 154.3 (C-18), 140.8 (C-4′), 136.8 (C-16′), 132.5 (C-18′), 131.8 (C-6), 130.0 (C-11′), 126.0 (C-14), 125.9 (C-7), 125.0 (C-13), 124.6 (C-3′), 123.4 (C-14′), 122.9 (C-15), 120.3 (C-12′), 120.0 (C-13′), 118.3 (C-10′), 112.3 (C-15′), 95.1 (C-17), 84.6 (C-2), 81.1 (C-3), 77.9 (C-4), 66.1 (C-19), 62.0 (C-26′), 57.8 (C-19′), 57.2 (C-22), 57.1 (C-5′), 56.5 (C-7′), 54.7 (C-12), 53.1 (C-24′), 52.9 (C-25), 51.5 (C-8), 50.9 (C-10), 50.3 (C-20′), 48.1 (C-8′), 46.0 (C-11), 44.1 (C-5), 39.2 (C-23), 36.0 (C-2′), 35.3 (C-1′), 32.2 (C-20), 28.5 (C-21′), 22.0 (C-9′), 21.7 (C-27), 15.1 (C-27′), 13.2 (C-22′), 9.1 (C-21). HRMS-ESI calcd for $C_{50}H_{63}N_4O_{10}$ 879.4544, found 879.4547.

Compound 32. Following the general procedure C (method 2) compound **32** was obtained as a white powder (19 mg, 90%); $[\alpha]_D^{25}$ +31 (c 1.00, $CHCl_3$). ^{1}H NMR (CD_3CN, 500 MHz) δ 8.47 (s, 1H, H-17′), 7.40 (d, J = 7.5 Hz, 2H, H-28′), 7.36 (t, J = 7.5 Hz, 2H, H-29′), 7.34−7.31 (m, 1H, H-30′), 7.28 (d, J = 7.8 Hz, 1H, H-12′), 7.14 (d, J = 7.8 Hz, 1H, H-15′), 6.99 (t, J = 7.8 Hz, 1H, H-14′), 6.84 (t, J = 7.8 Hz, 1H, H-13′), 6.78 (s, 1H, H-14), 6.24 (s, 1H, H-17), 5.76 (dd, J = 10.2 and 4.7 Hz, 1H, H-7), 5.27 (d, J = 10.2 Hz, 1H, H-6), 5.25 (d, J = 12.2 Hz, 1H, H-26′), 5.19 (s, 1H, H-4), 5.15 (d, J = 12.2 Hz, 1H, H-26′), 5.12 (d, J = 5.0 Hz, 1H, H-3′), 4.15 (dd, J = 13.3 and 4.6 Hz, 1H, H-9′β), 3.75 (s, 3H, H-25), 3.66 (s, 3H, H-22), 3.52 (s, 1H, H-2), 3.50 (s, 3H, H-24′), 3.25 (dd, J = 16.2 and 4.7 Hz, 1H, H-8β), 3.23 (t, J = 14.7 Hz, 1H, H-1′β), 3.16−3.13 (m, 1H, H-10β), 3.11 (dd, J = 13.3 and 4.6 Hz, 1H, H-9′α), 3.05 (td, J = 6.7 and 4.6 Hz, 1H, H-8′), 2.95 (d, J = 15.5 Hz, 1H, H-5′β), 2.83 (d, J = 11.4 Hz, 1H, H-20′β), 2.79 (d, J = 15.5 Hz, 1H, H-5′α), 2.76 (s, 1H, H-19), 2.72 (t, J = 11.5 Hz, 1H, H-7′β), 2.65 (s, 3H, H-23), 2.63 (d, J = 16.2 Hz, 1H, H-8α), 2.62 (dd, J = 11.5 and 6.7 Hz, 1H, H-7′α), 2.40 (td, J = 10.2 and 5.6 Hz, 1H, H-10α), 2.20 (d, J = 14.7 Hz, 1H, H-1′α), 2.01−1.96 (m, 1H, H-11β), 1.94 (s, 3H, H-27), 1.93−1.90 (m, 1H, H-11α), 1.84 (q, J = 7.5 Hz, 2H, H-21′), 1.67 (d, J = 11.4 Hz, 1H, H-20′α), 1.58 (dd, J = 14.1 and 7.4 Hz, 1H, H-20β), 1.29 (dd, J = 14.1 and 7.4 Hz, 1H, H-20α), 1.12−1.08 (m, 1H, H-2′), 0.91 (t, J = 7.5 Hz, 3H, H-22′), 0.63 (t, J = 7.4 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 125 MHz) δ 177.7 (C-25′), 176.3 (C-23′), 173.2 (C-24), 171.9 (C-26), 159.7 (C-16), 154.3 (C-18), 140.8 (C-4′), 137.9 (C-27′), 136.7 (C-16′), 132.6 (C-18′), 131.8 (C-6), 130.9 (C-11′), 130,0 (C-29′), 130.0 (C-28′), 129.6 (C-30′), 126.0 (C-7), 125.8 (C-14), 125.0 (C-13), 124.6 (C-3′), 123.3 (C-15), 122.9 (C-14′), 120.3 (C-12′), 120.1 (C-13′), 118.1 (C-10′), 112.3 (C-15′), 95.1 (C-17), 84.5 (C-2), 81.1 (C-3), 77.8 (C-4), 67.8 (C-19), 66.1 (C-26′), 57.8 (C-19′), 57.2 (C-22), 57.1 (C-5′), 56.9 (C-7′), 54.7 (C-12), 53.1 (C-24′), 52.9 (C-25), 51.5 (C-8), 50.8 (C-10), 50.4 (C-20′), 48.1 (C-8′), 46.0 (C-11), 44.1 (C-5), 39.3 (C-23), 36.0 (C-2′), 35.3 (C-1′), 32.2 (C-20), 28.5 (C-21′), 22.1 (C-9′), 21.7 (C-27), 13.3 (C-22′), 9.1 (C-21). HRMS-ESI calcd for $C_{55}H_{65}N_4O_{10}$ 941.4700, found 941.4730.

Compound 33. Following the general procedure B (method 1) compound **33** was obtained as a white powder (7 mg, 33%); $[\alpha]_D^{25}$ +4

dx.doi.org/10.1021/jm4004347 | J. Med. Chem. 2013, 56, 6088−6100

(*c* 1.00, $CHCl_3$). ^1H NMR (CD_3CN, 600 MHz) δ 8.96 (s, 1H, OH), 8.54 (s, 1H, H-17′), 7.38 (d, *J* = 7.7 Hz, 1H, H-12′), 7.17 (d, *J* = 7.7 Hz, 1H, H-15′), 7.02 (t, *J* = 7.7 Hz, 1H, H-14′), 6.94 (s, 1H, H-14), 6.93 (t, *J* = 7.7 Hz, 1H, H-13′), 6.25 (s, 1H, H-17), 5.78 (dd, *J* = 10.2 and 5.1 Hz, 1H, H-7), 5.29 (d, *J* = 10.2 Hz, 1H, H-6), 5.18 (s, 1H, H-4), 5.15 (d, *J* = 5.5 Hz, 1H, H-3′), 4.10 (dd, *J* = 15.1 and 5.7 Hz, 1H, H-9′β), 3.75 (s, 3H, H-22), 3.69 (s, 3H, H-26′), 3.66 (s, 3H, H-25), 3.59 (s, 3H, H-28′), 3.52 (s, 1H, H-2), 3.48 (s, 3H, H-24′), 3.47 (m, 1H, H-8′), 3.41 (d, *J* = 5.7 Hz, 1H, H-7′), 3.27 (dd, *J* = 16.3 and 5.1 Hz, 1H, H-8β), 3.23 (dd, *J* = 15.4 and 11.7 Hz, 1H, H-1′β), 3.16 (td, *J* = 9.9 and 5.5 Hz, 1H, H-10β), 3.13 (dd, *J* = 15.1 and 5.7 Hz, 1H, H-9′α), 3.05 (d, *J* = 16.0 Hz, 1H, H-5′β), 2.98 (d, *J* = 16.0 Hz, 1H, H-5′α), 2.95 (d, *J* = 12.2 Hz, 1H, H-20′β), 2.85 (s, 1H, H-19), 2.68 (d, *J* = 16.3 Hz, 1H, H-8α), 2.65 (s, 3H, H-23), 2.44 (td, *J* = 9.9 and 5.5 Hz, 1H, H-10α), 2.12 (d, *J* = 15.4 Hz, 1H, H-1′α), 2.02 (d, *J* = 12.2 Hz, 1H, H-20′α), 1.98 (td, *J* = 10.6 and 5.5 Hz, 1H, H-11β), 1.94 (s, 3H, H-27), 1.85 (q, *J* = 7.5 Hz, 2H, H-21′), 1.78 (td, *J* = 10.6 and 5.5 Hz, 1H, H-11α), 1.57 (td, *J* = 14.4 and 7.2 Hz, 1H, H-20β), 1.32 (td, *J* = 14.4 and 7.2 Hz, 1H, H-20α), 1.10−1.07 (m, 1H, H-2′), 0.91 (t, *J* = 7.5 Hz, 3H, H-22′), 0.69 (t, *J* = 7.2 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 150 MHz) δ 176.2 (C-25′), 176.1 (C-23′), 173.4 (C-27′), 173.1 (C-24), 171.8 (C-26), 159.5 (C-16), 154.2 (C-18), 140.4 (C-4′), 136.8 (C-16′), 133.0 (C-18′), 131.7 (C-6), 130.7 (C-11′), 126.0 (C-14), 125.8 (C-7), 124.9 (C-13), 124.1 (C-3′), 123.3 (C-14′), 122.2 (C-15), 120.2 (C-13′), 120.1 (C-12′), 116.1 (C-10′), 112.5 (C-15′), 94.9 (C-17), 84.3 (C-2), 81.0 (C-3), 77.7 (C-4), 67.5 (C-7′), 65.7 (C-19), 57.6 (C-19′), 57.1 (C-22), 54.6 (C-12), 53.1 (C-26′), 53.0 (C-24′), 52.8 (C-25), 52.7 (C-5′), 52.5 (C-28′), 51.5 (C-8), 51.4 (C-20′), 50.6 (C-10), 50.0 (C-8′), 45.9 (C-11), 44.0 (C-5), 39.1 (C-23), 35.9 (C-2′), 35.2 (C-1′), 32.1 (C-20), 28.4 (C-21′), 22.5 (C-9′), 21.6 (C-27), 13.1 (C-22′), 9.1 (C-21). HRMS-ESI calcd for $C_{51}H_{63}N_4O_{12}$ 923.4442, found 923.4360.

Compound ***34***. Following the general procedure C (method 2) compound **34** was obtained as a yellow powder (19.1 mg, 95%); $[\alpha]_D^{25}$ −4 (*c* 1.00, $CHCl_3$). ^1H NMR (CD_3CN, 500 MHz) δ 8.57 (s, 1H, H-17′), 7.43 (d, *J* = 8.1 Hz, 1H, H-12′), 7.37−7.33 (m, 2H, H-32′ and H-34′), 7.33−7.31 (m, 1H, H-33′), 7.26−7.23 (m, 2H, H-31′ and H-35′), 7.18 (d, *J* = 8.1 Hz, 1H, H-15′), 7.03 (t, *J* = 8.1 Hz, 1H, H-14′), 7.01 (t, *J* = 7.7 Hz, 1H, H-28′), 6.94 (t, *J* = 8.1 Hz, 1H, H-13′), 6.77 (s, 1H, H-14), 6.28 (s, 1H, H-17), 5.79 (td, *J* = 10.3 and 5.1 Hz, 1H, H-7), 5.30 (d, *J* = 10.3 Hz, 1H, H-6), 5.21 (s, 1H, H-4), 5.10 (d, *J* = 4.5 Hz, 1H, H-3′), 4.38 (d, *J* = 6.3 Hz, 1H, H-29′), 4.08−4.04 (m, 1H, H-9′β), 3.79 (s, 3H, H-22), 3.69 (s, 6H, H-26′ and H-25), 3.55 (s, 1H, H-2), 3.54 (s, 3H, H-24′), 3.35 (d, *J* = 15.1 Hz, 1H, H-1′β), 3.34−3.29 (m, 1H, H-8β), 3.31 (d, 1H, *J* = 17.5 Hz, H-9′α), 3.32−3.27 (m, 1H, H-8′), 3.22−3.19 (m, 1H, H-10β), 3.15 (d, *J* = 12.0 Hz, 1H, H-20′β), 3.29−3.24 (m, 1H, H-7′), 3.00 (d, *J* = 15.2 Hz, 1H, H-5′β), 2.78 (s, 1H, H-19), 2.71 (d, *J* = 15.2 Hz, 1H, H-5′α), 2.69 (dd, *J* = 13.2 and 5.1 Hz, 1H, H-8α), 2.68 (s, 3H, H-23), 2.46 (td, *J* = 10.6 and 7.0 Hz, 1H, H-10α), 2.23 (d, *J* = 15.1 Hz, 1H, H-1′α), 2.13 (s, 3H, H-27), 2.04−1.99 (m, 1H, H-11β), 1.87−1.83 (m, 1H, H-11α), 1.81 (q, *J* = 7.8 Hz, 2H, H-21′), 1.78 (d, *J* = 12.0 Hz, 1H, H-20′α), 1.59 (dd, *J* = 14.2 and 7.6 Hz, 1H, H-20β), 1.35−1.30 (m, 1H, H-20α), 1.21 (t, *J* = 7.6 Hz, 1H, H-2′), 0.87 (t, *J* = 7.8 Hz, 3H, H-22′), 0.64 (t, *J* = 7.6 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 125 MHz) δ 176.3 (C-25′), 176.2 (C-23′), 173.2 (C-24), 171.9 (C-26), 171.5 (C-27′), 159.6 (C-16), 154.4 (C-18), 141.0 (C-30′), 140.4 (C-4′), 136.8 (C-16′), 132.5 (C-18′), 131.8 (C-6), 130.8 (C-11′), 129.8 (C-33′), 129.3 (C-32′ and C-34′), 128.4 (C-31′ and C-35′), 126.4 (C-14), 125.8 (C-7), 125.1 (C-13), 124.4 (C-3′), 123.4 (C-14′), 123.2 (C-15), 120.5 (C-12′), 120.2 (C-13′), 118.3 (C-10′), 112.4 (C-15′), 95.0 (C-17), 84.5 (C-2), 81.2 (C-3), 77.9 (C-4), 70.1 (C-7′), 66.0 (C-19), 58.2 (C-19′), 57.2 (C-22), 54.7 (C-12), 53.2 (C-5′), 53.1 (C-24′), 53.0 (C-25), 52.9 (C-26′), 51.6 (C-8), 50.8 (C-10), 50.3 (C-8′), 49.6 (C-20′), 46.0 (C-11), 44.1 (C-5), 43.8 (C-29′), 39.2 (C-23), 35.8 (C-2′), 35.0 (C-1′), 32.2 (C-20), 28.6 (C-21′), 21.7 (C-27), 20.0 (C-9′), 13.1 (C-22′), 9.1 (C-21). HRMS-ESI calcd for $C_{53}H_{65}N_5O_{11}$ 998.4915, found 998.4922.

Compound ***35***. Following the general procedure C (method 2) compound **35** was obtained as a yellow powder (16 mg, 81%); $[\alpha]_D^{25}$ +12 (*c* 1.00, $CHCl_3$). ^1H NMR (CD_3CN, 600 MHz) δ 8.54 (s, 1H, H-17′), 7.39 (d, *J* = 7.9 Hz, 1H, H-12′), 7.14 (d, *J* = 7.9 Hz, 1H, H-15′), 6.99 (t, *J* = 7.9 Hz, 1H, H-14′), 6.90 (t, *J* = 7.9 Hz, 1H, H-13′), 6.73 (s, 1H, H-14), 6.45 (d, *J* = 6.9 Hz, 1H, H-28′), 6.25 (s, 1H, H-17), 5.76 (dd, *J* = 10.4 and 4.2 Hz, 1H, H-7), 5.27 (d, *J* = 10.4 Hz, 1H, H-6), 5.19 (s, 1H, H-4), 5.10 (d, *J* = 5.0 Hz, 1H, H-3′), 4.04 (d, *J* = 15.2 Hz, 1H, H-9′β), 3.97 (q, *J* = 6.9 Hz, 1H, H-29′), 3.77 (s, 3H, H-22), 3.69 (s, 3H, H-26′), 3.66 (s, 3H, H-25), 3.53 (s, 1H, H-2), 3.53 (s, 3H, H-24′), 3.29 (t, *J* = 14.8 Hz, 1H, H-1′β), 3.28 (dd, *J* = 13.9 and 4.2 Hz, 1H, H-8β), 3.25 (dd, *J* = 15.2 and 6.5 Hz, 1H, H-9′α), 3.20 (m, 1H, H-8′), 3.17 (td, *J* = 10.1 and 5.3 Hz, 1H, H-10β), 3.12 (d, *J* = 6.5 Hz, 1H, H-7′), 3.09 (d, *J* = 12.3 Hz, 1H, H-20′β), 3.05 (d, *J* = 15.5 Hz, 1H, H-5′β), 2.77 (s, 1H, H-19), 2.67 (d, *J* = 15.5 Hz, 1H, H-5′α), 2.66 (d, *J* = 13.9 Hz, 1H, H-8α), 2.66 (s, 3H, H-23), 2.46 (td, *J* = 10.1 and 5.3 Hz, 1H, H-10α), 2.23 (d, *J* = 14.8 Hz, 1H, H-1′α), 2.00 (dq, *J* = 12.9 and 5.3 Hz, 1H, H-11β), 1.94 (s, 3H, H-27), 1.87 (q, *J* = 7.7 Hz, 2H, H-21′), 1.81 (td, *J* = 12.9 and 5.3 Hz, 1H, H-11α), 1.74 (dd, *J* = 12.3 and 2.6 Hz, 1H, H-20′α), 1.57 (dd, *J* = 14.2 and 7.3 Hz, 1H, H-20β), 1.29 (dd, *J* = 14.2 and 7.3 Hz, 1H, H-20α), 1.20−1.16 (m, 1H, H-2′), 1.12 (d, *J* = 6.9 Hz, 3H, H-30′), 1.09 (d, *J* = 6.9 Hz, 3H, H-31′), 0.91 (t, *J* = 7.7 Hz, 3H, H-22′), 0.61 (t, *J* = 7.4 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 150 MHz) δ 176.3 (C-23′), 176.2 (C-25′), 173.2 (C-24), 171.9 (C-26), 170.3 (C-27′), 159.6 (C-16), 154.3 (C-18), 140.4 (C-4′), 136.8 (C-16′), 132.4 (C-18′), 131.8 (C-6), 130.8 (C-11′), 126.3 (C-14), 125.8 (C-7), 125.0 (C-13), 124.6 (C-3′), 123.4 (C-14′), 123.2 (C-15), 120.5 (C-12′), 120.1 (C-13′), 118.5 (C-10′), 112.3 (C-15′), 95.0 (C-17), 84.5 (C-2), 81.1 (C-3), 77.8 (C-4), 69.7 (C-7′), 66.0 (C-19), 58.1 (C-19′), 57.2 (C-22), 54.7 (C-12), 54.4 (C-5′), 53.1 (C-24′), 53.1 (C-25), 52.9 (C-26′), 51.6 (C-8), 50.8 (C-10), 50.3 (C-8′), 49.5 (C-20′), 46.0 (C-11), 44.1 (C-5), 42.1 (C-29′), 39.1 (C-23), 35.7 (C-2′), 35.1 (C-1′), 32.2 (C-20), 28.7 (C-21′), 23.3 (C-30′), 23.0 (C-31′), 21.7 (C-27), 19.8 (C-9′), 13.3 (C-22′), 9.0 (C-21). HRMS-ESI calcd for $C_{53}H_{68}N_5O_{11}$ 950.4915, found 950.4939.

Compound ***36***. Following the general procedure C (method 2) compound **36** was obtained as a yellow powder (16 mg, 78%); $[\alpha]_D^{25}$ +11 (*c* 1.00, $CHCl_3$). ^1H NMR (CD_3CN, 500 MHz) δ 8.54 (s, 1H, H-17′), 7.38 (d, *J* = 7.7 Hz, 1H, H-12′), 7.14 (d, *J* = 7.7 Hz, 1H, H-15′), 7.00 (t, *J* = 7.7 Hz, 1H, H-14′), 6.90 (t, *J* = 7.7 Hz, 1H, H-13′), 6.81 (t, *J* = 5.7 Hz, 1H, H-28′), 6.75 (s, 1H, H-14), 6.25 (s, 1H, H-17), 5.83 (m, 1H, H-30′), 5.76 (dd, *J* = 10.3 and 4.8 Hz, 1H, H-7), 5.28 (d, *J* = 14.0 Hz, 1H, H-6), 5.19 (s, 1H, H-4), 5.18 (dd, *J* = 14.0 and 1.6 Hz, 1H, H-31′), 5.09 (d, *J* = 5.1 Hz, 1H, H-3′), 5.06 (dd, *J* = 10.6 and 1.6 Hz, 1H, H-31′), 4.02 (d, *J* = 12.7 Hz, 1H, H-9′β), 3.79−3.75 (m, 2H, H-29′), 3.77 (s, 3H, H-22), 3.68 (s, 3H, H-26′), 3.66 (s, 3H, H-25), 3.53 (s, 1H, H-2), 3.52 (s, 3H, H-24′), 3.30 (dd, *J* = 10.9 and 4.8 Hz, 1H, H-8β), 3.29 (t, *J* = 14.7 Hz, 1H, H-1′β), 3.25 (d, *J* = 12.7 Hz, 1H, H-9′α), 3.24−3.22 (m, 1H, H-8′), 3.20−3.19 (m, 1H, H-7′), 3.14 (dd, *J* = 9.9 and 5.4 Hz, 1H, H-10β), 3.11 (d, *J* = 12.1 Hz, 1H, H-20′β), 3.05 (d, *J* = 15.4 Hz, 1H, H-5′β), 2.79 (s, 1H, H-19), 2.69 (d, *J* = 15.4 Hz, 1H, H-5′α), 2.68 (d, *J* = 16.0 Hz, 1H, H-8α), 2.66 (s, 3H, H-23), 2.46 (td, *J* = 9.9 and 5.4 Hz, 1H, H-10α), 2.22 (d, *J* = 14.7 Hz, 1H, H-1′α), 2.00 (td, *J* = 9.9 and 5.4 Hz, 1H, H-11β), 1.94 (s, 3H, H-27), 1.86 (q, *J* = 7.4 Hz, 2H, H-21′), 1.82 (td, *J* = 10.9 and 5.4 Hz, 1H, H-11α), 1.77 (dd, *J* = 12.1 and 2.8 Hz, 1H, H-20′α), 1.57 (td, *J* = 14.5 and 7.2 Hz, 1H, H-20β), 1.30 (td, *J* = 14.5 and 7.2 Hz, 1H, H-20α), 1.20−1.16 (m, 1H, H-2′), 0.91 (t, *J* = 7.4 Hz, 3H, H-22′), 0.61 (t, *J* = 7.2 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 125 MHz) δ 176.3 (C-25′), 176.2 (C-23′), 173.2 (C-24), 171.9 (C-26), 171.3 (C-27′), 159.6 (C-16), 154.3 (C-18), 140.4 (C-4′), 136.8 (C-16′), 136.6 (C-30′), 132.5 (C-18′), 131.8 (C-6), 130.8 (C-11′), 126.4 (C-14), 125.7 (C-7), 125.0 (C-13), 124.5 (C-3′), 123.4 (C-14′), 123.2 (C-15), 120.5 (C-12′), 120.2 (C-13′), 118.3 (C-10′), 116.6 (C-31′), 112.4 (C-15′), 95.0 (C-17), 84.4 (C-2), 81.2 (C-3), 77.8 (C-4), 70.0 (C-7′), 66.1 (C-19), 58.2 (C-19′), 57.2 (C-22), 54.7 (C-5′), 54.7 (C-12), 53.2 (C-24′), 53.1 (C-25), 53.0 (C-26′), 51.6 (C-8), 50.8 (C-10), 50.3 (C-8′), 49.6 (C-20′), 46.0 (C-11), 44.1 (C-5), 42.4 (C-29′), 39.2 (C-23), 35.8 (C-2′), 35.1 (C-1′), 32.2 (C-20), 28.7 (C-21′), 21.7 (C-27), 19.9 (C-9′), 13.2 (C-22′), 9.0 (C-21). HRMS-ESI calcd for $C_{53}H_{65}N_5O_{11}$ 948.4759, found 948.4766.

Compound ***37***. Following the general procedure C (method 2) compound **37** was obtained as a yellow powder (14.5 mg, 72%);

dx.doi.org/10.1021/jm4004347 | *J. Med. Chem.* 2013, 56, 6088−6100

$[\alpha]_D^{25}$ +1 (*c* 1.00, $CHCl_3$). ^1H NMR (CD_3CN, 500 MHz) δ 8.55 (s, 1H, H-17′), 7.44 (d, *J* = 7.8 Hz, 1H, H-12′), 7.17 (d, *J* = 7.8 Hz, 1H, H-15′), 7.03 (t, *J* = 7.8 Hz, 1H, H-14′), 6.94 (t, *J* = 7.8 Hz, 1H, H-13′), 6.78 (s, 1H, H-14), 6.34 (s, 1H, H-28′), 6.28 (s, 1H, H-17), 5.80 (td, *J* = 9.9 and 5.2 Hz, 1H, H-7), 5.30 (d, *J* = 9.9 Hz, 1H, H-6), 5.22 (s, 1H, H-4), 5.25−5.14 (d, *J* = 5.2 Hz, 1H, H-3′), 3.81 (t, *J* = 14.3 Hz, 1H, H-9′β), 3.80 (s, 3H, H-22), 3.74 (s, 3H, H-26′), 3.70 (s, 3H, H-25), 3.61 (s, 1H, H-2), 3.56 (s, 3H, H-24′), 3.39 (d, *J* = 14.3 Hz, 1H, H-9′α), 3.35 (d, *J* = 13.2 Hz, 1H, H-8β), 3.22 (t, *J* = 13.4 Hz, 1H, H-1′β), 3.22−3.21 (m, 1H, H-8′), 3.21−3.18 (m, 1H, H-10β), 3.14 (d, *J* = 11.6 Hz, 1H, H-20′β), 3.12 (d, *J* = 6.5 Hz, 1H, H-7′), 3.10 (d, *J* = 14.5 Hz, 1H, H-5′β), 2.79 (s, 1H, H-19), 2.73 (d, *J* = 14.5 Hz, 1H, H-5′α), 2.69 (s, 3H, H-23), 2.68 (dd, *J* = 13.2 and 5.2 Hz, 1H, H-8α), 2.46 (td, *J* = 10.4 and 5.2 Hz, 1H, H-10α), 2.26 (d, *J* = 13.4 Hz, 1H, H-1′α), 2.06−1.99 (m, 1H, H-11β), 1.97 (s, 3H, H-27), 1.95−1.93 (m, 2H, H-21′), 1.88−1.84 (m, 1H, H-11α), 1.81 (dd, *J* = 11.6 and 4.1 Hz, 1H, H-20′α), 1.60 (dd, *J* = 13.6 and 7.0 Hz, 1H, H-20β), 1.36−1.33 (m, 1H, H-20α), 1.32 (s, 9H, H-30′), 1.24−1.22 (m, 1H, H-2′), 0.96 (t, *J* = 7.7 Hz, 3H, H-22′), 0.64 (t, *J* = 7.0 Hz, 3H, H-21). ^{13}C NMR (CD_3CN, 125 MHz) δ 176.2 (C-25′), 176.0 (C-23′), 173.2 (C-24), 171.9 (C-26), 170.6 (C-27′), 159.6 (C-16), 154.4 (C-18), 140.6 (C-4′), 136.8 (C-16′), 132.5 (C-18′), 131.8 (C-6), 130.8 (C-11′), 126.4 (C-14), 125.8 (C-7), 125.1 (C-13), 124.8 (C-3′), 123.4 (C-14′), 123.1 (C-15), 120.5 (C-12′), 120.2 (C-13′), 118.4 (C-10′), 112.3 (C-15′), 95.0 (C-17), 84.5 (C-2), 81.2 (C-3), 77.9 (C-4), 69.9 (C-7′), 66.1 (C-19), 58.1 (C-19′), 57.2 (C-22), 54.7 (C-12), 54.5 (C-5′), 53.1 (C-24′), 53.1 (C-25), 52.9 (C-26′), 52.0 (C-29′), 51.6 (C-8), 50.8 (C-10), 50.1 (C-8′), 49.6 (C-20′), 46.0 (C-11), 44.1 (C-5), 39.2 (C-23), 35.8 (C-2′), 35.3 (C-1′), 32.2 (C-20), 29.3 (C-30′), 28.7 (C-21′), 21.7 (C-27), 19.9 (C-9′), 13.5 (C-22′), 9.1 (C-21). HRMS-ESI calcd for $C_{54}H_{69}N_5O_{11}$ 964.5072, found 964.5109.

Inhibition of Tubulin Assembly. The drug, dissolved in DMSO at different concentrations, was added to a solution of free tubulin (obtained from sheep brain and prepared according to a published procedure[29]) 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 (the maximum was reached in about 1 min). The maximum rate of assembly was recorded and compared to a drug-free sample. The IC_{50} of the compound was calculated from the effect of several concentrations and compared to the IC_{50} of vinorelbine obtained within the same day with the same tubulin preparation.

Cell Culture and Proliferation Assay. Cancer cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA) and were cultured according to the supplier's instructions. Human K562 leukemia cells and HCT116 colorectal carcinoma cells were grown in RPMI 1640 supplemented with 10% fetal calf serum (FCS) and 1% glutamine. U87-MG human glioblastoma cells were grown in Dulbecco's Minimal Essential Medium (DMEM) containing 10% FCS and L-glutamine. Human umbilical vein endothelial cells (HUVECs) were obtained from Lonza (Walkersville, MD, USA) and cultured according to the supplier's instructions in endothelial cell growth medium (EGM2) containing growth factors and 2% FCS. Cell lines were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cell viability was assessed using Promega CellTiter-Blue reagent (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, the cells were seeded in 96-well plates (2.5 × 103 cells/well) containing 50 μL of growth medium. After 24 h of culture, the cells were supplemented with 50 μL of medium containing different concentrations of the tested compound dissolved in DMSO (less than 0.1% in each preparation). After 72 h of incubation, 20 μL of resazurin was added for 1.5 h before recording fluorescence (λ_{ex} = 560 nm, λ_{em} = 590 nm) using a microtiter plate fluorimeter. The IC_{50} corresponds to the concentration of compound that induced a 50% decrease in fluorescence of drug-treated cells compared with untreated cells. Experiments were performed in triplicate.

Cell Cycle Analysis. Exponentially growing cancer cells were incubated with compound **30**, **33**, or vinorelbine at two concentrations or DMSO. For the K562 cell line, compound **30** was incubated at 10 and 50 nM, compound **33** at 1 and 10 nM, and vinorelbine at 5 and 10 nM. For the U87 cell line, compound **30** was incubated at 10 and 50 nM, compound **33** at 1 and 5 nM, and vinorelbine at 1 and 5 nM. After 24 h, all the cells were harvested, dissociated, and washed with 2 mL of phosphate buffer saline (PBS). The cells were then fixed at +4 °C with cold 70% ethanol for 30 min and stored at −20 °C overnight. Ethanol was removed by centrifugation, and 2 mL of PBS were added to wash the pellets. The cellular DNA was stained with propidium iodide (PI, 50 mg/mL, Sigma) and RNase (100 mg/mL, Sigma) for 30 min. The cell-cycle profiles were determined by flow cytometry on a FC500 flow cytometer (Beckman-Coulter, France).

Molecular Modeling. Compounds **27** and **35** were model-built using vinblastine as a template, essentially as reported earlier for other vinca derivatives.[26] Point charges for the energy-minimized geometries were assigned by fitting the quantum mechanically calculated (RHF/6-31G*//RHF/3-21G*) molecular electrostatic potential (MEP) using Gaussian 03 (Gaussian, Inc., Wallingford, CT). Consistent bonded and nonbonded AMBER parameters for these ligands were assigned by analogy or through interpolation from those already present in the AMBER database for protein atoms (ff03). **27** and **35** were manually docked at the longitudinal interface between two tubulin heterodimers by superimposing them onto the vinblastine structure in the tubulin−vinblastine complex.[3] The resulting complexes were first energy-minimized in vacuo to remove any steric clashes within the binding site and then immersed in a truncated octahedron containing ~32300 TIP3P water molecules and 23 Na^+ ions. The *sander* and *pmemd* modules from the AMBER12 suite of programs (http://ambermd.org/) were used for the restrained and unrestrained MD simulations, respectively. Periodic boundary conditions were applied, and electrostatic interactions were treated using the smooth particle mesh Ewald method with a grid spacing of 1 Å. The cutoff distance for the nonbonded interactions was 9 Å, the SHAKE algorithm was applied to all bonds, and an integration step of 2.0 fs was used throughout. After an initial energy minimization of the water molecules and counterions, the system was heated to 300 K in 25 ps, after which the solvent was allowed to redistribute around the positionally restrained solute for 220 ps. After this time, the restraints were removed and the system was further simulated for 20 ns. Snapshots from each 10 ns MD trajectory were collected every 20 ps for structural and energetic analyses. The *ptraj* module was used to assess the stability of the complexes by calculating the root-mean-square deviations (RMSD) from the initial geometries and for calculating a representative average structure for each complex from the unrestrained MD simulations after removal of the water molecules for visualization purposes. The molecular graphics program PyMOL version 0.99 (DeLano Scientific, LLC, Palo Alto, CA) was employed for visualization and model building.

■ ASSOCIATED CONTENT

Supporting Information

^1H and ^{13}C NMR spectra for compounds **20**−**37**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Jacques Fahy (Pierre Fabre Laboratories) is gratefully acknowledged for a generous gift of vinorelbine. Marie Elise Tran Huu Dau is also acknowledged for fruitful discussions. O.G. gratefully acknowledges the ED425 "Innovation Thérapeutique, du Fondamental à l'Appliqué" for a grant and L'Oréal Foundation, UNESCO, and the French Science Academy for her "Pour les Femmes et la Science" prize. F.G. thankfully acknowledges financial support from Comunidad Autónoma de

Madrid (S-BIO/0214/2006) and Comisión Interministerial de Ciencia y Tecnología (SAF2009-13914-C02-02).

■ ABRREVIATIONS USED

VLB, vinblastine; VLN, vinorelbine; AVLB, anhydrovinblastine

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