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ARTICLE *in* JOURNAL OF MEDICINAL CHEMISTRY · FEBRUARY 2005

Impact Factor: 5.45 · DOI: 10.1021/jm0495733 · Source: PubMed

Synthesis and Biological Study of a New Series of 4′-Demethylepipodophyllotoxin Derivatives

Maria Duca,[†,‡] Dominique Guianvarc'h,[†,§] Philippe Meresse,[‡] Emmanuel Bertounesque,[‡] Daniel Dauzonne,[‡] Laurence Kraus-Berthier,[⊥] Sylvie Thirot,[‡] Stéphane Léonce,[⊥] Alain Pierré,[⊥] Bruno Pfeiffer,[⊥] Pierre Renard,[⊥] Paola B. Arimondo,[†] and Claude Monneret*[,‡]

Laboratoire de Biophysique, UMR 5153 CNRS, Muséum National d'Histoire Naturelle, USM 0503, INSERM UR565, 43 rue Cuvier, 75231 Paris Cedex 05, France, Section de Recherche, Institut Curie, UMR 176 CNRS, 26 rue d'Ulm, 75248 Paris Cedex 05, France, and Division Recherche Cancérologie, Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy sur Seine, France

Received June 4, 2004

Etoposide (VP-16) is a potent human DNA topoisomerase II poison, derived from 4′-demethylepipodophyllotoxin, widely used in cancer chemotherapy. Continuous efforts have driven to synthesize new related compounds, presenting decreased toxic side effects, metabolic inactivation, drug resistance, and increased water solubility. Identified structure−activity relationships have pointed out the importance of the 4β-substitution and of the configuration of the D ring. Here we report the synthesis of two novel series of derivatives of 4′-demethylepipodophyllotoxin. The first bears a carbamate chain in the 4 position (**13a**−**f**), whereas, in the second series, in addition to this chain, the lactone ring has been modified by shifting the carbonyl from position 13 to position 11 (**27a**−**f**). Moreover, an analogue of TOP-53 having this lactone modification has also been prepared (**32**). From this study, structure−activity relationships were established. Compounds **13a** and **27a** displayed potent cytotoxic activity against the L1210 cell line (10 to 20-fold higher than VP-16) and proved to be strong topoisomerase II poisons more potent than VP-16. From preliminary in vivo investigation of both compounds against P388 leukemia and orthotopically grafted human A549 lung carcinoma, it appeared that **13a** and **27a** constitute promising leads for a new class of antitumor agents.

Introduction

Etoposide (VP-16, Figure 1) is a semisynthetic derivative of podophyllotoxin (**1**, Figure 1) and one of the most commonly used drugs in cancer chemotherapy, particularly in the treatment of small cell lung cancer, testicular carcinoma, lymphoma, and Kaposi's sarcoma.[1−3] However, while **1** inhibits the assembly and disassembly of microtubules, etoposide is an inhibitor of DNA topoisomerase II (topo II).[4,5] This is a ubiquitous and essential enzyme that modulates DNA topology by passing an intact DNA double helix through a transient double-stranded break. This enzyme plays important roles in many aspects of the DNA metabolism.[6,7]

The DNA/topoisomerase II covalent complex, called the cleavage complex, is the key intermediate of the topoisomerase reaction. In this complex each monomer of the enzyme is covalently linked, through a phosphotyrosine bond, to one strand of the duplex, to the 5′-phosphate of the four base pair staggered DNA double-stranded break. This intermediate, which normally has a short lifetime, is stabilized by different molecules, such as etoposide or its derivatives, called topoisomerase II poisons. These poisons form a ternary complex, drug/topoisomeraseII/DNA, and thus prevent DNA religation.[4,8,9] Thereby, these molecules induce breaks in the DNA strands and cause cell death. However, the mechanism by which etoposide forms the ternary drug/topoisomerase II/DNA complex remains unclear.

Although VP-16 is widely used in therapy, it presents several limitations, such as moderate potency, poor water solubility, development of drug resistance, metabolic inactivation, and toxic effects.[10] Therefore, the structure of VP-16 has been extensively modified, thus increasing the information about its structure−activity relationships. The most important modification is that of the substituent in the 4β-position leading to potent inhibitors of topoisomerase II, such as TOP-53 (Figure 1)[11] or GL331,[12] which proved to be more active than VP-16. The amino derivative TOP-53 displays twice the inhibitory activity of VP-16 against topoisomerase II and exhibits in vivo superior antitumor activity than VP-16 against several types of cancer.[13,14] Accordingly, TOP-53 has arrived in phase II clinical assays, but the study was interrupted in 2002. It is therefore important to synthesize less toxic derivatives of TOP-53 and to find new VP-16 analogues to improve its clinical efficacy, overcome the problems cited above, and also clarify the molecular mechanism of this class of topoisomerase II inhibitors.

In the course of our general program aimed at the discovery of new antitumor drugs and, especially, of new 4′-demethylepipodophyllotoxin derivatives as topoisomerase II inhibitors,[15,16] we report here the synthesis of two novel series of such derivatives and their biological activities. In analogy with TOP-53, we decided to introduce an aminoalkyl chain, containing, moreover, a carbamate group in the 4-β-position, thus leading to the first series of derivatives: the 4′-demethylepipodo-

* Corresponding author. Phone: (33) 1 42 34 66 55. Fax: (33) 1 42 34 66 31. E-mail: claude.monneret@curie.fr.
† Muséum National d'Histoire Naturelle.
‡ Institut Curie.
§ Present address: Laboratoire de Chimie Organique Biologique, Université Paris 6, UMR 7613, 4, place Jussieu, 75005 Paris, France.
⊥ Institut de Recherches Servier.

10.1021/jm0495733 CCC: $30.25
Published on Web 12/31/2004

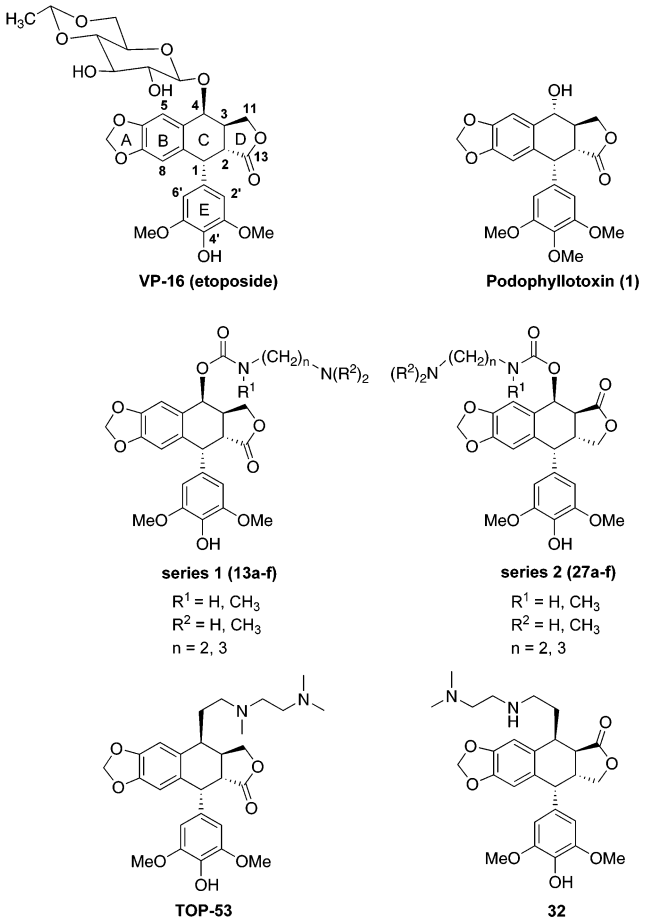


Figure 1. Structure of etoposide, podophyllotoxin, TOP-53, and the new series of compounds substituted in 4-position.

phyllotoxin 4-aminoalkylcarbamates **13a**−**f** (Figure 1). In this context, it is worth mentioning that another class of 4-carbamate analogues of etoposide has been previously prepared and showed good features of topoisomerase II inhibition.[17] Furthermore, because the main metabolites of etoposide are its lactone ring modified analogues,[18] to overcome metabolic inactivation and hopefully maintain topoisomerase II inhibition activity, we decided to synthesize analogues with a shift in the carbonyl group position on the lactone ring from carbon 13 to carbon 11 (called retrolactone), as previously made for etoposide (retroetoposide).[19] Here we developed a new synthetic method, giving improved yields. This generated the second series of compounds, **27a**−**f**, depicted in Figure 1. In addition, we synthesized a 4-β-aminoalkyl analogue of TOP-53 with the retrolactone modification (**32**, Figure 1) to study the influence of the latter on the activity.

The main chemical differences introduced in these new series of compounds are (a) the substitution of the nitrogen of the carbamate group (methylated or free), (b) the length of the side chain (ethyl or propyl), and (c) the substitution of the terminal nitrogen (methylated or not). All compounds were evaluated for inhibition of topoisomerase II and cytotoxicity.

Chemistry

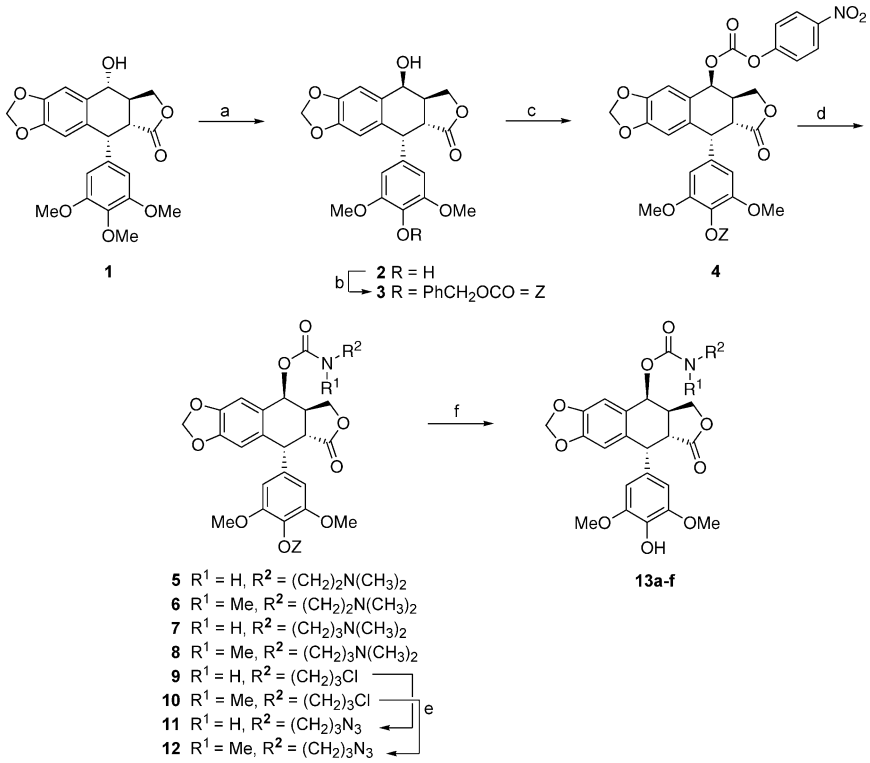
Synthesis of 4′-Demethylepipodophyllotoxin 4-Aminoalkylcarbamate Derivatives 13a−f. The synthetic route to the new derivatives **13a**−**f** is depicted in Scheme 1. The podophyllotoxin **1** was first regioselectively demethylated by using a previously described procedure[16] by treatment with trimethylsilyl iodide (TMSI) and barium carbonate to afford **2**. This latter was subsequently protected at the 4′-hydroxy position as its benzyloxycarbonyl derivative, **3**.[20] Compound **3** was then converted into its 4β-activated derivative **4** by simple treatment with *p*-nitrophenylchloroformate in pyridine. Introduction of the carbamate chains was performed by reacting **4** with the appropriate amines in the presence of triethylamine, affording compounds **5**−**8** in good yields. To introduce a primary amino group at the end of the side chain, the chloro intermediates **9** and **10** were prepared to be subsequently and quantitatively converted, by using NaN_3 in DMF, into their azido derivatives **11** and **12**, respectively. This two-step procedure was necessary, as we observed dimerization as a side reaction when **4** was reacted with diamines containing a terminal primary amino group. Finally the 4′-demethylepipodophyllotoxin 4-aminoalkylcarbamate derivatives **13a**−**f** were obtained by treatment of **5**−**8**, **11**, and **12** with 10% Pd/C in ethyl acetate. This allowed both the cleavage of the benzyloxycarbonyl protecting group and the reduction of the azido group in the case of compounds **11** and **12**.

Synthesis of 11-Oxo-13-deoxo-4′-demethylepipodophyllotoxin 18 (Scheme 2). Compound **15** was obtained in two steps after protection of the 4 and 4′-hydroxyl groups of **2** with *tert*-butyldimethylsilyl triflate and subsequent reduction of the lactone ring in the presence of lithium aluminum hydride ($LiAlH_4$) following a previously reported procedure.[19,20] The diol **15** was further treated with a catalytic amount of tetrapropylammonium perruthenate in the presence of NMO, in dichloromethane. This reaction provided a mixture of two products, **14** and **16**, inseparable by column chromatography or by crystallization.

When this mixture was submitted to the prolonged action of tetrabutylammonium fluoride (TBAF) in THF, the complete deprotection of **14** and **16** provided the 4′-DMEP **2** and the wanted 11-oxo-13-deoxo derivative **18**, respectively, but these compounds remained inseparable. However, during this deprotection step, we observed that **14** was completely deprotected after 20 min to give **2**, while **16** was only partially deprotected to afford **17**. So, the reaction was stopped at this step and the two products, **2** and **17**, were then successfully separated by flash chromatography. Compound **2** was recycled and **17** was further completely deprotected in the presence of TBAF for 23 h to provide **18** in good yield. This new synthesis of retrolactone proved to be faster and more efficient than the previously reported procedure.[19]

Synthesis of 11-Oxo-13-deoxo-4′-demethylepipodophyllotoxin 4-Aminoalkylcarbamate Derivatives 27a−f. The syntheses of derivatives **27a**−**f** bearing various carbamate chains are depicted in Scheme 3. Compounds **27a**−**f** were obtained from **18** in four steps similar to those reported for **13a**−**f**: (a) phenol protection (**18**→**19**), (b) activation of the 4-hydroxy position (**19**→**20**), (c) amine coupling (**20**→**21**−**26**), and, finally, (d) cleavage of the protecting group (**21**−**26**→**27a**−**f**). In the case of these retrolactone

Scheme 1[a]



[a] Reagents: (a) TMSI then $BaCO_3$; (b) $PhCH_2OCOCl$, Et_3N, CH_2Cl_2; (c) *p*-$NO_2PhOCOCl$, pyridine; (d) HNR^1R^2, Et_3N, CH_2Cl_2; (e) NaN_3, DMF; (f) H_2, Pd/C 10%, EtOAc.

derivatives having a primary amino group at the end of the carbamate side chain (**27c** and **27f**), we could perform the direct substitution, without the preparation of chloro and azido intermediates as in the first series (**13e** and **13f**). This afforded the wanted compounds quickly (in 30 min) and in high yields without formation of dimeric products. It was hypothesized that the presence of the carbonyl group in the 11-position changes the steric hindrance and consequently the kinetic of the dimerization reaction.

Synthesis of the 11-Oxo-13-deoxo-4′-demethyl-4-β-[2-[[2-(*N,N*-dimethylamino)ethyl]amino]ethyl]-4-desoxypodophyllotoxin 32 (Scheme 4). To prepare this analogue, the starting material was the 11-oxo-13-deoxo-4′-benzyloxycarbonyl-4′-demethylepipodophyllotoxin (**19**). Following the synthesis previously reported for TOP-53,[11] an allyl group was first introduced in the 4-β-position of **19** upon reaction with trimethylallylsilane in the presence of boron trifluoride etherate to provide compound **28**. Oxidation of **28** with osmium tetroxide and NMO in acetone, followed by oxidation with lead(IV) tetraacetate ($Pb(OAc)_4$) in benzene, gave 11-oxo-13-deoxo-4′-demethyl-4′-benzyloxycarbonyl-4β-(formylmethyl)-4-desoxypodophyllotoxin (**30**). Reductive amination of **30** with sodium cyanoborohydride and *N,N*-dimethylethylamine in a mixture AcOH−CH_3OH gave the 4-β-aminoalkyl derivative **31**. Finally, the TOP-53 analogue **32** was obtained in 65% yield after deprotection of the hydroxy group by catalytic hydrogenation.

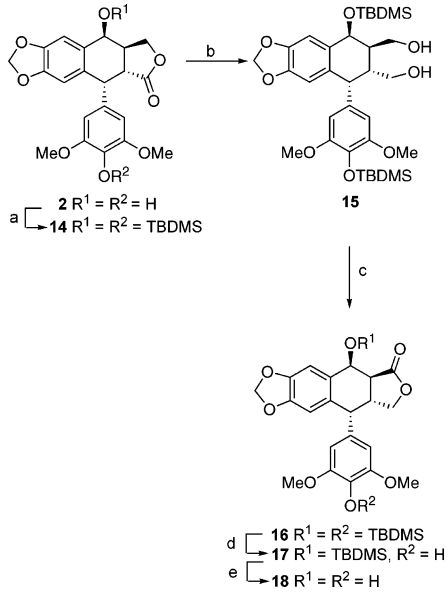
Biological Results and Discussion

The stability of this new series of compounds in DMSO solution was followed by HPLC measurements over a 1-month period. As revealed by HPLC analysis, all of the compounds **13a**−**f** are stable, and the formation of secondary products was only observed in the case of the retrolactone derivatives **27a** (14% byproducts) and **27c** (16% byproducts), when stored at room temperature as a 5 mM DMSO solution over 1 week. In contrast, the powder is completely stable. The related compounds **27d** and **27f**, bearing a longer side chain, are completly stable in solution. The TOP-53 analogue **32**, which bears the retrolactone modification and a secondary amino group instead of a tertiary one, was also stable under these conditions. To avoid the formation of secondary products, all compounds were dissolved in DMSO immediately before use.

In Table 1 are summarized the activities of all compounds for inhibition of DNA topoisomerase II and cytotoxicity toward murine L1210 leukemia cell line. For topoisomerase II inhibition, the percentage of linear DNA over a range of concentrations for each compound has been tested. The profiles being the same at the different concentrations, we have reported in Table 1 only the percentage of linear DNA measured at a drug concentration of 20 μM. The first compounds studied were the series of 4′-demethylepipodophyllotoxin 4-aminoalkylcarbamates (**13a**−**f**). The biological activities are discussed on the basis of the following chemical properties: (a) the nitrogen of the carbamate group that can be methylated or not (R^1), (b) the length of the side chain (*n*), and (c) the terminal nitrogen that can be disubstituted or not (R^2). The second series of compounds synthesized shares these chemical differences and, in addition, encompasses the retrolactone modification, where the carbonyl group is shifted from C-13 to C-11.

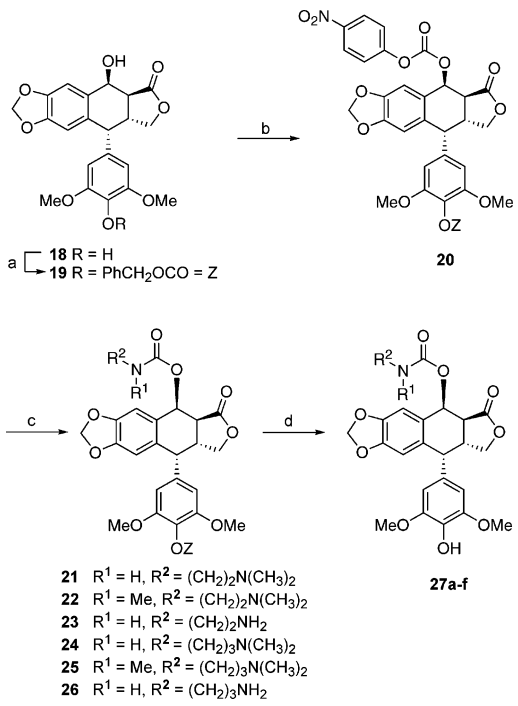
Concerning topoisomerase II poisoning, the comparison of the compounds of this first series suggests that

Scheme 2[a]



[a] Reagents: (a) TBDMSTf, 2,6-lutidine, CH_2Cl_2; (b) $LiAlH_4$, THF; (c) NMO, tetrapropylammonium perruthenate, CH_2Cl_2; (d) tetrabutylammonium fluoride, THF, 20 min; (e) tetrabutylammonium fluoride, THF, 23 h.

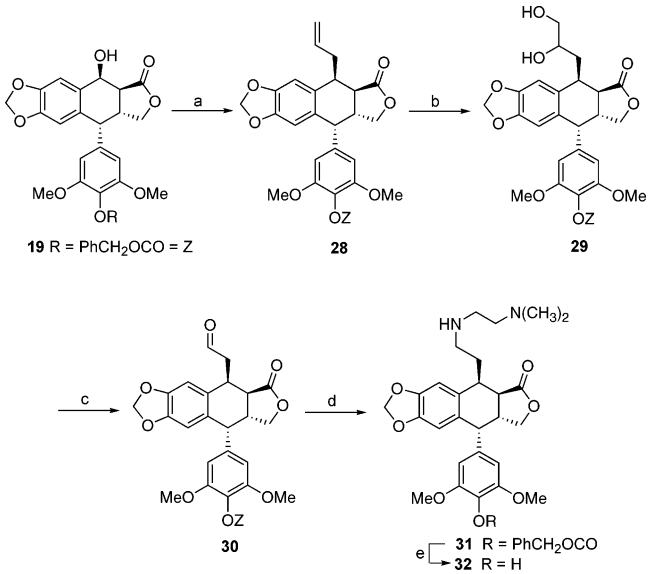
Scheme 3[a]



[a] Reagents: (a) $PhCH_2OCOCl$, Et_3N, CH_2Cl_2; (b) *p*-$NO_2PhOCOCl$, pyridine; (c) HNR_1R_2, Et_3N, CH_2Cl_2; (d) H_2, Pd/C 10%, EtOAc.

an unsubstituted carbamate nitrogen is more favorable than a methylated one (R^1 = H > CH_3). In fact, compounds **13a**, **13c**, and **13e** are considerably more active than their methylated analogues **13b**, **13d**, and **13f**, respectively. Moreover, as the length of the side chain increases (from ethyl to propyl), topoisomerase II inhibition activity is comparable for the primary carbamates derivatives (compare **13a** to **13c**) and decreases slightly for the more hindered *N*-methylated compounds (compare **13b** to **13d**). Finally, a dimethyl-substituted terminal nitrogen is more favorable than the corresponding primary amino group (**13c** > **13e** and **13d** > **13f**).

Scheme 4[a]

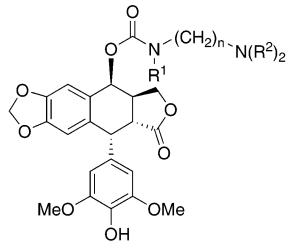


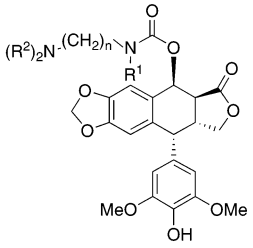
[a] Reagents: (a) CH_2=$CHCH_2SiMe_3$, BF_3·Et_2O, CH_2Cl_2; (b) OsO_4, NMO, acetone; (c) $Pb(OAc)_4$, benzene; (d) *N*,*N*-dimethylethylamine, $NaCNBH_3$, AcOH, MeOH; (e) H_2, Pd/C 10%, EtOAc.

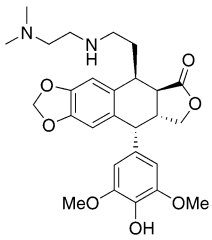
For this series of compounds cytotoxicity quite well follows the topoisomerase II inhibition activity. Compound **13a,** a very potent topoisomerase II poison, shows the higher cytotoxicity (0.038 μM compared to 0.83 μM for VP-16). The only exception is compound **13f**, which bears a terminal primary amino group and is methylated on the carbamate. This compound, which is cytotoxic (IC_{50} = 0.24 μM for L1210 cell line), is poorly active against topoisomerase II. This could suggest another cellular target. Because it is known that derivatives of podophyllotoxin (Figure 1) can be either topoisomerase II poison or tubulin polymerization/depolymerization inhibitors, we tested this compound for tubulin polymerization inhibition (TPI). Compound **13f** is indeed a significant tubulin inhibitor with an IC_{50} of 3.9 μM (IC_{50} = 3 μM for podophyllotoxin), while the most active topoisomerase II poisons, **13a** and **13c**, show only very poor inhibition of tubulin polymerization (49% and 30% at 19 mM, respectively).

Next, we studied the relationships between structure and biological activity for the second series of compounds, **27a**−**f** (Table 1). Here again, a carbamate with a secondary nitrogen atom (R^1 = H) and an aminoethyl chain (n = 2) proved to be favorable parameters for topoisomerase II inhibition. In fact, freshly prepared solutions of **27a** and **27c** were the only derivatives to inhibit topoisomerase II and proved to be very efficient. Replacement of aminoethyl chains by the longer aminopropyl chains induced a complete loss of topoisomerase II inhibition activity. As observed in the first series, a dimethyl-substituted terminal nitrogen (as in **27a**) is more favorable than the corresponding primary amino group (as in **27c**). Compound **27a**, which bears a dimethylamino group and is the more potent topoisomerase II inhibitor (better than VP-16), is 10-fold more cytotoxic than VP-16 (IC_{50}= 0.088 μM) and has the most potent effects on the cell cycle. Its ability to inhibit tubulin polymerization/depolymerization has

Table 1. Biological Evaluation







compound	R^1		R_2	inhibition of topoisomerase IIα (% linear DNA)[a]	cytotoxicity IC_{50} (μM)[b]	cell cycle effect[c]
VP-16				50	0.83	76% (2.5 μM)
TOP-53				57	0.6	75% (2.5 μM)
13a	H	2	CH_3	56	0.038	67% (0.1 μM)
13b	CH_3	2	CH_3	27	1.7	77% (2.5 μM)
13c	H	3	CH_3	58	0.34	72% (1 μM)
13d	CH_3	3	CH_3	17	0.96	nt[d]
13e	H	3	H	38	1.3	nt
13f	CH_3	3	H	8	0.24	nt
27a	H	2	CH_3	64	0.088	83% (1 μM)
27b	CH_3	2	CH_3	0	5.2	77% (25 μM)
27c	H	2	H	48	3.2	nt
27d	H	3	CH_3	0	1.4	65% (10 μM)
27e	CH_3	3	CH_3	0	21	nt
27f	H	3	H	0	4.3	nt
32				0	27.9	nt

[a] Each value reported here is a medium value of at least three independent experiments and at 20 μM of drug. [b] IC_{50}: concentration of drug required to reduce to 50% L1210 cell growth. [c] % of L1210 cells in the G2M phase at the specified drug concentration. [d] nt = not tested.

Table 2. Antitumor Activity of Compounds **13a**, **13b**, and **27a**

	P388 leukemia (treatment on day 1)			A549 non-small-cell lung carcinoma (treatment on days 14, 21, and 28)		
compd	dose range (mg/kg)	MTD (mg/kg)[a]	T/C[b]	dose range (mg/kg)	MTD (mg/kg)[a]	T/C[b]
VP-16	6.25−100	100	233	70−100	70	131
13a	1.56−12.5	6.25	192	1.56−6.25	6.25	151
13b	12.5−200	50	182	12.5−100	25	102
27a	1.56−100	12.5	214	1.56−6.25	6.25	152

[a] MTD = maximum tolerated dose, i.e., dose which does not induce toxic death and/or weight loss higher than 20%. [b] T/C = % median survival time of treated animals/median survival time of control animals × 100.

also been studied. It results that this compound weakly inhibits tubulin polymerization (IC_{50}= 37 μM) and even less the depolymerization (40% at 67 μM). As for its primary amino analogue **27c**, it is poorly cytotoxic. Replacement of aminoethyl chains by longer aminopropyl chains strongly reduces cytotoxicity, with the exception of compound **27d**, which exhibits an average cytotoxicity, as observed for the topoisomerase II inhibition activity. Therefore, compound **27d** was evaluated for its effect on tubulin polymerization/depolymerization and proved to be completely inactive (0% at 20 mM).

To understand why the aminopropyl chain gave such an important loss of activity in the case of the retrolactone series, we studied the position of the carbamate side chain by computational simulation[21] (data not shown). We observed that, in the case of compound **13c** and its analogue **27d**, the spatial position of the carbamate chain in the minimized structure is considerably different, showing that, due to a steric clash between the *N*-methylated carbamate and the carbonyl group of the retrolactone, the side chain rotates away from the latter. Such a change in the orientation of the side chain could explain the decrease in topoisomerase II inhibition activity, probably because of an unfavorable interaction of the compound in the DNA/topoisomerase II cleavage complex.

To investigate the role of the 4-carbamate chain in the retrolactone compounds, we synthesized compound **32**, bearing a 2-[2-(*N*,*N*-dimethylamino)ethyl]aminoethyl side chain, instead of the 2-(*N*,*N*-dimethylamino)-ethyl carbamate side chain of compound **27a**. Compound **32** can be considered as an analogue of TOP-53 with the retrolactone modification and a secondary amine instead of a tertiary amine in the side chain. This compound is weakly cytotoxic (IC_{50}= 27.9 μM) and has no effect on topoisomerase II (Table 1). Thus, the 4-carbamate substituents seem essential for the activity in this retrolactone series.

The most cytotoxic and active compounds **13a** and **27a**, as well as the average compound **13b** (used as control), have been further investigated in vivo in P388 leukemia tumor model and A549 orthotopic model of human lung cancer.

These three compounds, **13a**, **13b**, and **27a**, are active against P388 with T/C values of 192%, 182%, and 214%, respectively (versus 233% for VP-16). Compounds **13a** and **27a**, but not **13b**, are also very active against A549 with T/C values of 151%, 152%, and 102%, respectively (versus 131% for VP16). The most active compound, **27a**, belongs to the second series and is also the most potent topoisomerase II inhibitor.

Our study underlines some new relationships between structure and activity that allowed us to design **13a** and **27a** as new promising leading drugs. Noteworthy, the spatial organization of 4′-demethylepipodophyllotoxin

derivatives is very important for the interactions in the ternary drug/topoisomerase II/DNA complex. It has been shown that etoposide binds to topoisomerase II, but not to DNA.[22,23] It has also been suggested that the interaction of the poison with the enzyme could precede the binding to DNA.[4,22] However, the molecular features of the ternary complex have not yet been elucidated and other paths are not yet excluded, as, for example, the direct interaction of the drug with the already formed binary DNA/topoisomerase II complex. More recent results suggested that there are two interaction sites for etoposide in the ternary complex.[4] Many efforts have been made to improve biological features and to identify structure−activity relationships for 4-substituted derivatives of 4′-demethylepipodophyllotoxin. It has been shown that the 4-glucosidation, present in etoposide, is not essential for anti-topoisomerase activity and that other types of substituents can be introduced in the 4-position while topoisomerase II inhibition activity is maintained.[24] The pharmacophore model proposed by MacDonald,[25,26] common for all classes of topoisomerase II inhibitors, is supported by 3D-QSAR studies,[27,28] and it shows that three domains are necessary for topoisomerase II inhibition activity: a DNA pseudo-intercalating moiety, a minor groove binding site, and a molecular region that can accommodate a number of structurally different substituents (4-substituents for 4′-demethylepipodophyllotoxin derivatives). Moreover, the pharmacophore model suggests that the 4-substituent could also interact with the DNA minor groove. In addition, Q-SAR electrostatic contour plots showed that active compounds should have positive charged functional groups near the minor groove of DNA. Accordingly, the carbamate chains we have examined in the present study proved to be good 4-substituents for 4′-demethylepipodophyllotoxin, and we have found new derivatives with an activity comparable to (and even better than) that of etoposide, as well as inactive compounds. This has allowed us to outline structure−activity relationships for this class of compounds and to predict the features that are favorable to topoisomerase II inhibition. In conclusion, the highest cytoxicity and topoisomerase II inhibition properties have been obtained with compounds containing an unsubstituted carbamate nitrogen with an *N*,*N*-dimethylamino alkyl side chain. For the retrolactone series, the presence of the aminopropyl chain induced a complete loss of topoisomerase II inhibition activity due to a different positioning of the side chain. The two 2-dimethylaminoethylcarbamate compounds **13a** and **27a** are promising for further in-depth evaluation as anticancer agents.

Experimental Section

Chemistry. Solvents and most of the starting materials were purchased from Acros, Aldrich, or Avocado. The commercially unavailable *N*-methyl-3-chloropropylamine was prepared according to a previously reported procedure.[29] Reference etoposide was obtained from Sigma Chemicals Co. Melting points were measured on a Kofler hot stage apparatus and were uncorrected. Mass spectra were obtained with a Nermag-Ribermag R10-10C spectrometer applying either the desorption chemical ionization (CI) method (operating in the positive ion mode using ammonia as the reagent gas) or the fast atom bombardment method (FAB). Infrared spectra were obtained with a Perkin-Elmer 1710 spectrophotometer for chloroform solutions or KBr disks. Specific rotations were measured with a Perkin-Elmer 241 polarimeter. The ^{1}H NMR (300 MHz) spectra were recorded on a Bruker AC 300 spectrometer. Chemical shifts are expressed as parts per million from tetramethylsilane. Splitting patterns have been designated as follows: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), m (multiplet), and br (broad signal). Coupling constants (J values) are listed in hertz (Hz). Reactions were monitored by analytical thin-layer chromatography and products were visualized by exposure to UV light. Merck silica gel (230−400 mesh ASTM) was used for column chromatography. Acetone, methanol, and dichloromethane employed as eluents for column chromatography were distilled on a rotary evaporator prior to use. Anhydrous DMF was obtained by prolonged contact with activated Linde-type 4 Å molecular sieves. Dry THF was prepared by distillation from benzophenone/sodium. All yields reported are unoptimized. Elemental analysis for most of the new substances was performed by CNRS Laboratories (Vernaison, France), and unless noted otherwise, the results obtained are within 0.4% of the theoretical values.

HPLC analysis was carried out on a reverse phase column (X-Terra MS C_{18} 5 μM 4.6 × 250 mm) using a gradient of H_2O/acetonitrile 0−40% and H_2O/methanol 0−30% (both containing 0.01% of trifluoroacetic acid) with UV detection at 260 nm.

4′-Benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-(*p*-Nitrophenyl)carbonate (4). To a solution of *p*-nitrophenylchloroformate (2.6 g, 12.7 mmol) in anhydrous dichloromethane (10 mL) was added dry pyridine (1.2 mL). Instantaneously a white precipitate was formed. A solution of **3** (2 g, 3.74 mmol) in dry dichloromethane (10 mL) was added dropwise under an argon atmosphere. The mixture was stirred for 45 min at room temperature and then washed twice with water (2 × 40 mL) and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography (CH_2Cl_2/Acetone 97:3) led to **4.** The following recrystallization in hexane gave analytically pure **4** (2.3 g, 88%) as yellow crystals. R_f = 0.27 (cyclohexane/ethyl acetate 2:1). Mp = 125−130 °C. $[\alpha]^{20}_D$ = −101.5 (*c* = 0.988, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 8.30 (d, 2H, *J* = 8.3 Hz, *Ph*NO_2), 7.43−7.30 (m, 7H, CH_2*Ph*, *Ph*NO_2), 7.04 (s, 1H, 5-H), 6.58 (s, 1H, 8-H), 6.30 (s, 2H, 2′,6′-H), 6.08 (d, 1H, *J* = 3.0 Hz, 4-H), 6.02 (d, 2H, *J* = 6.3 Hz, CH_2O_2), 5.26 (s, 2H, *CH*$_2$Ph), 4.74 (d, 1H, *J* = 5.0 Hz, 1-H), 4.46 (t, 1H, *J* = 8.2 Hz, 11a-H), 4.16 (t, 1H, *J* = 7.1 Hz, 11b-H), 3.69 (s, 6H, 3′,5′-OCH_3), 3.36 (dd, 1H, *J* = 5.1, 14.2 Hz, 2-H), 3.15−3.00 (m, 1H, 3-H). MS (CI) *m/z*: 717 $[M + NH_4]^+$. Anal. ($C_{36}H_{29}NO_{14}$) C, H, N.

General Synthetic Method for Compounds 5−8. To a stirred solution of **4** (0.36 mmol, 248.3 mg) in CH_2Cl_2 (10 mL) was added the appropriate amine (0.48 mmol, 1.1 equiv) and triethylamine (0.48 mmol) at room temperature. After stirring for the reported times, the reaction mixture was washed with cold saturated $NaHCO_3$ and then water until pH 6−7. The extract was dried over $MgSO_4$ and concentrated in vacuo at 30 °C. The residue was purified by silica gel column chromatography using a mixture of cyclohexane/ethyl acetate 1:1 as the eluent to afford compounds **5**−**8** in the reported yields. In the cases of compounds **6**−**8**, the chromatographed compounds were directly employed in the subsequent deprotection step without further purification.

4′-Benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-[*N*-[2-(*N*′,*N*′-Dimethylaminoethyl)]]carbamate (5). Reaction time: 3 h. White crystals from hexane. Yield: 85%. R_f = 0.42 (CH_2Cl_2/MeOH 90:10). Mp = 104−106 °C. $[\alpha]^{20}_D$ = −74.5 (*c* 0.395, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.47−7.30 (m, 5H, CH_2*Ph*), 6.94 (s, 1H, 5-H), 6.53 (s, 1H, 8-H), 6.30 (s, 2H, 2′,6′-H), 6.03−5.94 (m, 3H, CH_2O_2, 4-H), 5.50 (br s, 1H, NH), 5.25 (s, 2H, *CH*$_2$Ph), 4.66 (d, 1H, *J* = 5.1 Hz, 1-H), 4.38 (t, 1H, *J* = 8.4 Hz, 11a-H), 4.03 (t, 1H, *J* = 9.8 Hz, 11b-H), 3.68 (s, 6H, 3′,5′-OCH_3), 3.38−3.18 (m, 3H, $CH_2\alpha$, 2-H), 3.00−2.85 (m, 1H, 3-H), 2.47 (t, 2H, *J* = 5.8 Hz, $CH_2\beta$), 2.26 (s, 6H, $N(CH_3)_2$). MS (CI) *m/z*: 649 $[M + H]^+$, 666 $[M + NH_4]^+$. Anal. ($C_{34}H_{36}N_2O_{11}$) C, H, N.

4′-Benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-[*N*-[2-(*N*′,*N*′-Dimethylaminoethyl)]-*N*-methyl]carbamate (6). Reaction time: 1 h. Yellow amorphous solid.

Yield: 85%. R_f = 0.14 (CH_2Cl_2/MeOH 95:5). Mp = 102−104 °C. $[\alpha]^{20}_D$ = −59.0 (*c* 0.998, $CHCl_3$). ^{1}H NMR ($CDCl_3$) *δ*: 7.44−7.33 (m, 5H, CH_2*Ph*), 6.94 (s, 0.5H, 5-H (rot.)), 6.91 (s, 0.5H, 5-H (rot.)), 6.54 (s, 1H, 8-H), 6.31 (s, 2H, 2′,6′-H), 6.04 (d, 1H, *J* = 3.5 Hz, 4-H), 6.01 (s, 2H, CH_2O_2), 5.27 (s, 2H, *CH*$_2$Ph), 4.67 (d, 1H, *J* = 5.0 Hz, 1-H), 4.40 (br t, 1H, *J* = 7.1 Hz, 11a-H), 4.05−3.99 (m, 1H, 11b-H), 3.71 (s, 6H, 3′,5′-OCH_3), 3.60−3.54 (m, 0.5H, CH_2α (rot.)), 3.39−3.30 (m, 1H, CH_2α (rot.)), 3.25−3.17 (m, 1.5H, CH_2α (rot.), 2-H), 3.00 (s, 1.5H, NCH_3 (rot. 1.5H)), 2.98−2.92 (m, 1H, 3-H), 2.89 (s, 1.5H, NCH_3 (rot. 1.5H)), 2.52−2.42 (m, 1H, $CH_2\beta$), 2.40−2.35 (m, 1H, $CH_2\beta$), 2.29 (s, 3H, $N(CH_3)_2$), 2.14 (s, 3H, $N(CH_3)_2$). MS (CI) *m/z*: 663 $[M + H]^+$.

4′-Benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-[*N*-[3-(*N*′,*N*′-Dimethylaminopropyl)]]carbamate (7). Reaction time: 1.5 h. Yield: 85%. R_f = 0.20 (CH_2Cl_2/MeOH 90:10). Mp = 62−64°C (white solid). $[\alpha]^{20}_D$ = −77.0 (*c* 0.225, $CHCl_3$). ^{1}H NMR ($CDCl_3$) *δ*: 7.45−7.31 (m, 5H, CH_2*Ph*), 6.94 (s, 1H, 5-H), 6.55 (s, 1H, 8-H), 6.30 (s, 2H, 2′,6′-H), 6.00−5.97 (m, 3H, 4-H, CH_2O_2), 5.89 (br s, 1H, NH), 5.27 (s, 2H, *CH*$_2$Ph), 4.66 (d, 1H, *J* = 4.9 Hz, 1-H), 4.42−4.37 (m, 1H, 11a-H), 4.04−3.98 (m, 1H, 11b-H), 3.69 (s, 6H, 3′,5′-OCH_3), 3.35−3.29 (m, 2H, CH_2α), 3.25−3.16 (m, 1H, 2-H), 3.04−2.93 (m, 1H, 3-H), 2.44−2.40 (t, 2H, *J* = 6.8 Hz, $CH_2\gamma$), 2.29−2.18 (m, 6H, $N(CH_3)_2$), 1.73−1.69 (m, 2H, $CH_2\beta$). MS (CI) *m/z*: 663 $[M + H]^+$.

4′-Benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-[*N*-[3-(*N*′,*N*′-Dimethylaminopropyl)]-*N*-methyl]carbamate (8). Reaction time: 2 h. White crystals from hexane. Yield 75%. R_f = 0.10 (cyclohexane/ethyl acetate 1:1). Mp 83−85 °C. $[\alpha]^{20}_D$ = −61.0 (*c* 0.589, $CHCl_3$). ^{1}H NMR ($CDCl_3$) *δ*: 7.42−7.30 (m, 5H, CH_2*Ph*), 6.92 (s, 0.5H, 5-H (rot.)), 6.89 (s, 0.5H, 5-H (rot.)), 6.50 (s, 1H, 8-H), 6.29 (s, 2H, 2′,6′-H), 6.04−6.00 (m, 1H, 4-H), 5.93 (d, 2H, *J* = 6.5 Hz, CH_2O_2), 5.22 (s, 2H, *CH*$_2$Ph), 4.65 (d, 1H, *J* = 4.9 Hz, 1-H), 4.35 (t, 1H, *J* = 8.2 Hz, 11a-H), 4.02−3.90 (m, 1H, 11b-H), 3.65 (s, 6H, 3′,5′-OCH_3), 3.40−3.22 (m, 1H, CH_2α (rot.)), 3.21−3.09 (m, 2H, CH_2α (rot.), 2-H), 3.00−2.86 (m, 3.5H, 3-H, N*CH*$_3$ (rot.)), 2.73 (s, 1.5H, N*CH*$_3$ (rot.)), 2.31−2.28 (m, 2H, $CH_2\gamma$), 2.07 (s, 6H, $N(CH_3)_2$), 1.78−1.66 (m, 1H, $CH_2\beta$), 1.64−1.49 (m, 1H, CH_2b). MS (CI) *m/z*: 677 $[M + H]^+$.

General Procedure for the Synthesis of 9 and 10. To a solution of **4** (800 mg, 1.14 mmol) in anhydrous CH_2Cl_2 (33 mL) were added methyl-3-chloropropylamine (prepared as previously described[29]) or chloropropylamine (2.2 equiv, 2.28 mmol) and then dry triethylamine (4.56 mmol, 0.64 mL). After the solution was stirred at room temperature for the reported times, it was diluted with CH_2Cl_2 (70 mL) and washed with a saturated solution of $NaHCO_3$ and then with water until pH 7. The organic layer was separated and dried over anhydrous $MgSO_4$. Flash chromatography (cyclohexane/ethyl acetate 1:1) afforded **9** and **10** as pure compounds in the reported yields. The chromatographed compounds were directly employed in the subsequent deprotection step without further purification.

4′-Benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-[*N*-(3-Chloropropyl)]carbamate (9). Reaction time: 3 h. Yellow amorphous solid. Yield: 96%. R_f = 0.34 (cyclohexane/ethyl acetate 1:1). Mp = 125−127 °C. $[\alpha]^{20}_D$ = −75.5 (*c* 0.520, $CHCl_3$). ^{1}H NMR ($CDCl_3$) *δ*: 7.46−7.30 (m, 5H, CH_2*Ph*), 6.92 (s, 1H, 5-H), 6.52 (s, 1H, 8-H), 6.28 (s, 2H, 2′,6′-H), 6.04−5.91 (m, 3H, 4-H, CH_2O_2), 5.24 (s, 2H, *CH*$_2$Ph), 5.08 (br s, 1H, NH), 4.64 (d, 1H, *J* = 4.7 Hz, 1-H), 4.37 (t, 1H, *J* = 8.1, 11a-H), 3.98 (t, 1H, *J* = 9.8 Hz, 11b-H), 3.66 (s, 6H, 3′,5′-OCH_3), 3.63−3.50 (m, 2H, CH_2α), 3.40−3.27 (m, 1H, $CH_2\gamma$), 3.19 (dd, 1H, *J* = 4.8, 14.0 Hz, 2-H), 3.00−2.97 (m, 2H, 3-H, $CH_2\gamma$), 2.06−1.80 (m, 2H, $CH_2\beta$). MS (CI) *m/z*: 671 $[M + NH_4]^+$.

4′-Benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-[*N*-(3-Chloropropyl)-*N*-methyl]carbamate (10). Reaction time: 6 h. Yellow amorphous solid. Yield 75%. R_f = 0.51 (cyclohexane/ethyl acetate 1:1). Mp = 116−118 °C. $[\alpha]^{20}_D$ = −59.0 (*c* 0.275, $CHCl_3$). ^{1}H NMR ($CDCl_3$) *δ*: 7.56−7.30 (m, 5H, CH_2*Ph*), 6.93 (s, 0.5H, 5-H), 6.92 (s, 0.5H, 5-H (rot.), 6.53 (s, 1H, 8-H), 6.30 (s, 2H, 2′,6′-H), 6.05−5.93 (m, 3H, 4-H, CH_2O_2), 5.25 (s, 2H, *CH*$_2$Ph), 4.67 (d, 1H, *J* = 5.0 Hz, 1-H), 4.39 (t, 1H, *J* = 8.1 Hz, 11a-H), 3.57 (dd, 1H, *J* = 7.1, 14.2 Hz, 11b-H), 3.68 (s, 6H, 3′,5′-OCH_3), 3.62−3.55 (m, 1H, CH_2α (rot.)), 3.50−3.36 (m, 2H, 2-H, CH_2α (rot.)), 3.27−3.11 (m, 1H, 3-H), 2.97 (s, 1.5H, NCH_3 (rot.)), 2.88 (s, 1.5H, NCH_3 (rot.)), 1.95−1.87 (m, 1H, $CH_2\gamma$), 1.77−1.61 (m, 3H, CH_2g, $CH_2\beta$). MS (CI) *m/z*: 685 $[M + NH_4]^+$.

General Procedure for the Synthesis of 11 and 12. To a solution of **9** or **10** (0.8 mmol) in dry DMF (40 mL) was added NaN_3 (4.0 mmol, 260.0 mg). The solution was stirred for 60 h at 50 °C. After this time, the reaction mixture was diluted with ethyl acetate and washed with a saturated solution of NH_4Cl and then with water, until pH 7. The organic layer was dried over anhydrous $MgSO_4$ and purified by silica gel column chromatography, using a mixture CH_2Cl_2/acetone 90:10 as the eluent, to give **11** and **12** in the reported yields. The chromatographed compound was directly employed in the subsequent deprotection step without further purification.

4′-Benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-[*N*-(3-Azidopropyl)]carbamate (11). Yellow amorphous solid. Yield 85%. R_f 0.10 (cyclohexane/ethyl acetate 1:1). Mp 80−82 °C. $[\alpha]^{20}_D$ = −83.0 (*c* 0.360, $CHCl_3$). ^{1}H NMR ($CDCl_3$) *δ*: 7.47−7.30 (m, 5H, CH_2*Ph*), 6.92 (s, 1H, 5-H), 6.52 (s, 1H, 8-H), 6.26 (s, 2H, 2′,6′-H), 6.05−5.89 (m, 3H, 4-H, CH_2O_2), 5.55 (br s, 1H, NH), 5.23 (s, 2H, *CH*$_2$Ph), 4.60 (d, 1H, *J* = 4.9 Hz, 1-H), 4.34 (br t, 1H, *J* = 7.2 Hz, 11a-H), 3.97 (dd, 1H, *J* = 9.0, 10.5 Hz, 11b-H), 3.66 (s, 6H, 3′,5′-OCH_3), 3.40−3.20 (m, 5H, 2-H, $CH_2\alpha,\gamma$), 3.19−3.08 (m, 1H, 3-H), 1.86−1.70 (m, 2H, $CH_2\beta$). IR ($CHCl_3$) *ν*: 3452 (NH), 2934 (aliphatic C-H), 2102 (N_3), 1777 (lactone), 1719 (carbamate), 1623, 1507, 1485 (aromatic C=C). MS (CI) *m/z*: 678 $[M + NH_4]^+$.

4′-Benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-[*N*-(3-Azidopropyl)-*N*-methyl]carbamate (12). Yellow amorphous solid. Yield 75%. R_f = 0.48 (CH_2Cl_2/acetone 90:10). Mp = 92−95 °C. $[\alpha]^{20}_D$ = −64.0 (*c* 0.470, $CHCl_3$). ^{1}H NMR ($CDCl_3$) *δ*: 7.50−7.34 (m, 5H, CH_2*Ph*), 6.97 (s, 1H, 5-H), 6.55 (s, 1H, 8-H), 6.27 (s, 2H, 2′,6′-H), 6.10−5.85 (m, 3H, 4-H, CH_2O_2), 5.41 (s, 2H, *CH*$_2$Ph), 4.65 (d, 1H, *J* = 5.0 Hz, 1-H), 4.38 (t, 1H, *J* = 13.1 Hz, 11a-H), 3.97 (dd, 1H, *J* = 8.8, 10.7 Hz, 11b-H), 3.84 (s, 3H, NCH_3), 3.77 (s, 6H, 3′,5′-OCH_3), 3.47−3.30 (m, 4H, $CH_2\alpha,\gamma$), 3.25 (dd, 1H, *J* = 5.1, 14.2 Hz, 2-H), 3.05−2.90 (m, 3H, 3-H, $CH_2\beta$). IR ($CHCl_3$) *ν*: 2941 (aliphatic C-H), 2102 (N_3), 1776 (lactone), 1697 (carbamate), 1621, 1506, 1486 (aromatic C=C). MS *m/z*: 692 $[M + NH_4]^+$.

General Procedure for the Synthesis of 13a−f, 27a−f, and 32. A solution of **5**−**8**, **11**, **12**, **21**−**26**, or **31** (0.250 mmol) was stirred under hydrogen atmosphere in the presence of palladium on activated carbon (10%). The mixture was stirred for 3 h. After removal of the catalyst by filtration through a pad of Celite, the filtrate was concentrated under reduced pressure and the product purified by flash chromatography, using a mixture CH_2Cl_2/MeOH 90:10 as the eluent, to give pure compounds in the reported yields.

4′-Demethylepipodophyllotoxin 4-[*N*-[2-(*N*′,*N*′-Dimethylaminoethyl)]]carbamate (13a). White crystals from hexane. Yield: 70%. R_f = 0.16 (CH_2Cl_2/MeOH 90:10). Mp = 110−112 °C. $[\alpha]^{20}_D$ = −104.5 (*c* 0.520, $CHCl_3$). ^{1}H NMR ($CDCl_3$) *δ*: 6.94 (s, 1H, 5-H), 6.54 (s, 1H, 8-H), 6.29 (s, 2H, 2′,6′-H), 6.05−5.92 (m, 3H, CH_2O_2, 4-H), 5.48−5.38 (m, 2H, OH, NH), 4.62 (d, 1H, *J* = 4.9 Hz, 1-H), 4.35 (t, 1H, *J* = 8.2 Hz, 11a-H), 4.02 (t, 1H, *J* = 9.8 Hz, 11b-H), 3.76 (s, 6H, 3′,5′-OCH_3), 3.36−3.25 (m, 2H, CH_2α), 3.17 (dd, 1H, *J* = 5.0, 14.1 Hz, 2-H), 3.02−2.88 (m, 1H, 3-H), 2.42 (t, 2H, *J* = 5.7 Hz, $CH_2\beta$), 2.21 (s, 6H, $N(CH_3)_2$). MS (CI) *m/z*: 515 $[M + H]^+$. Anal. ($C_{26}H_{30}N_2O_9$): C, H, N.

4′-Demethylepipodophyllotoxin 4-[*N*-[2-(*N*′,*N*′-Dimethylaminoethyl)]-*N*-methyl]carbamate (13b). White amorphous solid. Yield: 60%. R_f = 0.39 (CH_2Cl_2/MeOH 95:5). Mp = 184−186°C. $[\alpha]^{20}_D$ = −81.5 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) *δ*: 6.93 (s, 0.5H, 5-H (rot.)), 6.90 (s, 0.5H, 5-H (rot.)), 6.54 (s, 1H, 8-H), 6.29 (s, 2H, 2′,6′-H), 6.03 (d, 1H, *J* = 3.5 Hz, 4-H), 5.98 (d, 2H, *J* = 6.0 Hz, CH_2O_2), 5.41 (s, 1H, 4′-OH), 4.64 (d, 1H, *J* = 4.9 Hz, 1-H), 4.37 (br t, 1H, *J* = 9.9 Hz, 11a-H), 4.06−3.92 (m, 1H, 11b-H), 3.78 (s, 6H, 3′,5′-OCH_3), 3.58−3.42 (m, 1H, CH_2α (rot.)), 3.39−3.25 (m, 1H, CH_2α (rot.)), 3.38−3.22−

3.10 (m, 1H, 2-H), 3.05−2.95 (m, 2.5H, 3-H, NCH_3 (rot.)), 2.86 (s, 1.5H, NCH_3 (rot.)), 2.50−2.40 (m, 1H, $CH_2\beta$), 2.38−2.30 (m, 1H, $CH_2\beta$), 2.27 (m, 3H, $(NCH_3)_2$), 2.14 (s, 3H, $N(CH_3)_2$). MS (CI) *m/z*: 529 $[M + H]^+$, 551 $[M + Na]^+$. Anal. ($C_{27}H_{32}N_2O_9$): C, H, N.

4′-Demethylepipodophyllotoxin 4-[*N*-[3-(*N*′,*N*′-Dimethylaminopropyl)]]carbamate (13c). Yield: 76%. White amorphous solid. $R_f = 0.21$ (CH_2Cl_2/MeOH 90:10). Mp 150−152 °C. $[\alpha]^{20}_D = -23.0$ (*c* 0.035, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 6.91 (s, 1H, 5-H), 6.55 (s, 1H, 8-H), 6.29 (s, 2H, 2′,6′-H), 6.07 (br s, 1H, NH), 5.99−5.97 (m, 3H, 4-H, CH_2O_2), 5.41 (s, 1H, 4′-OH), 4.64 (d, 1H, $J = 5.1$ Hz, 1-H), 4.33 (t, 1H, $J = 8.1$ Hz, 11a-H), 3.99 (t, 1H, $J = 10.6$ Hz, 11b-H), 3.78 (s, 6H, 3′,5′-OCH_3), 3.40−3.35 (m, 2H, $CH_2\alpha$), 3.27 (dd, 1H, $J = 5.1$, 14.1 Hz, 2-H), 3.24−3.18 (m, 2H, $CH_2\gamma$), 3.15−3.06 (m, 1H, 3-H), 2.81 (s, 6H, $N(CH_3)_2$), 1.44−1.40 (m, 2H, $CH_2\beta$). MS (CI) *m/z*: 529 $[M + H]^+$.

4′-Demethylepipodophyllotoxin 4-[*N*-[3-(*N*′,*N*′-Dimethylaminopropyl)]-*N*-methyl]carbamate (13d). White crystals from hexane. Yield 70%. $R_f = 0.28$ (CH_2Cl_2/MeOH 90:10). Mp = 94−96 °C. $[\alpha]^{20}_D = -74.0$ (*c* 0.455, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 6.92 (s, 0.5H, 5-H (rot.)), 6.84 (s, 0.5H, 5-H (rot.)), 6.52 (s, 1H, 8-H), 6.26 (s, 2H, 2′,6′-H), 6.04−5.92 (m, 3H, 4-H, CH_2O_2), 5.43 (br s, 1H, 4′-OH), 4.63 (br d, 1H, $J = 4.5$ Hz, 1-H), 4.33 (t, 1H, $J = 8.1$ Hz, 11a-H), 4.00−3.83 (m, 1H, 11b-H), 3.74 (s, 6H, 3′,5′-OCH_3), 3.51−3.30 (m, 0.5H, $CH_2\alpha$ (rot.)), 3.28−3.15 (m, 1.5H, $CH_2\alpha$ (rot.)), 3.14 (dd, 1H, $J = 4.9$, 14.1 Hz, 2-H), 3.04−2.91 (m, 4H, 3-H, CH_3), 2.81 (s, 6H, $N(CH_3)_2$), 2.73−2.60 (m, 2H, $CH_2\gamma$), 2.21−1.94 (m, 2H, $CH_2\beta$). MS (CI) *m/z*: 543 $[M + H]^+$.

4′-Demethylepipodophyllotoxin4-[*N*-(3-Aminopropyl)]-carbamate (13e). White amorphous solid. Yield 45%. $R_f = 0.15$ (CH_2Cl_2/MeOH 90:10). Mp = 195−197 °C. $[\alpha]^{20}_D = -68.0$ (*c* 0.470, DMSO). ^1H NMR (DMSO-d_6) δ: 6.90 (s, 1H, 5-H), 6.53 (s, 1H, 8-H), 6.19 (s, 2H, 2′,6′-H), 5.99 (d, 2H, $J = 11.1$ Hz, CH_2O_2), 5.87 (d, 1H, $J = 2.7$ Hz, 4-H), 4.55 (d, 1H, $J = 4.7$ Hz, 1-H), 4.34 (t, 1H, $J = 7.7$ Hz, 11a-H), (t, 1H, $J = 9.4$ Hz, 11b-H), 3.60 (s, 6H, 3′,5′-OCH_3), 3.12−2.90 (m, 4H, 2,3-H, $CH_2\alpha$), 2.79−2.65 (m, 2H, $CH_2\gamma$), 1.69−1.57 (m, 2H, $CH_2\beta$). MS (CI) *m/z*: 501 $[M + H]^+$.

4′-Demethylepipodophyllotoxin 4-[*N*-(3-Aminopropyl)-*N*-methyl]carbamate (13f). White amorphous solid. Yield 40%. $R_f = 0.36$ (CH_2Cl_2/MeOH 90:10). Mp 122−124 °C. $[\alpha]^{20}_D = -74.5$ (*c* 0.495, DMSO). ^1H NMR (DMSO-d_6) δ: 6.98 (s, 1H, 5-H), 6.54 (s, 1H, 8-H), 6.18 (s, 2H, 2′,6′-H), 5.99 (d, 2H, $J = 10.0$ Hz, CH_2O_2), 5.90 (d, 1H, $J = 2.9$ Hz, 4-H), 4.54 (d, 1H, $J = 5.0$ Hz, 1-H), 4.34 (t, 1H, $J = 7.9$ Hz, 11a-H), 3.91 (t, 1H, $J = 9.4$ Hz, 11b-H), 3.72 (s, 3H, NCH_3), 3.70−3.64 (m, 6H, $3CH_2$), 3.60 (s, 6H, 3′,5′-OCH_3), 3.16 (dd, 1H, $J = 5.1$, 14.4 Hz, 2-H), 3.12−2.99 (m, 1H, 3-H). MS (CI) *m/z*: 515 $[M + H]^+$. Anal. ($C_{26}H_{30}N_2O_9$) C, H, N.

4,4′-bis-*O*-(*tert*-Butyldimethylsilyl)-11-oxo-13-deoxo-4′-demethylepipodophyllotoxin (16). To a stirred solution of **15** (9.49 mmol, 6 g), prepared as previously described,[17] in CH_2Cl_2 were added *N*-methylmorpholine *N*-oxide (NMO) (28.3 mmol, 3.3 g), tetrapropylammonium perruthenate (0.96 mmol, 334 mg), and molecular sieves (4Å). After 2 h, the reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The product was then purified by flash chromatography, using a mixture cyclohexane/ethyl acetate 3:1 as eluent, to give an inseparable mixture of **14** and **16**. Overall yield: 83%. $R_f = 0.65$ (cyclohexane/ethyl acetate 3:1). MS (CI) *m/z*: 646 $[M + NH_4]^+$.

4-*O*-(*tert*-Butyldimethylsilyl)-11-oxo-13-deoxo-4′-demethylepipodophylloto xin (17). To a solution of **14** and **16** (7.08 mmol, 4.45 g) in anhydrous THF (150 mL) was added dropwise a solution of 1 M tetrabutylammonium fluoride in THF (10.62 mmol). After stirring for 20 min, the reaction mixture was washed with a saturated NH_4Cl solution and then with water until pH 6−7. The extract was dried over $MgSO_4$ and concentrated under reduced pressure at 30 °C. The residue was further purified by silica gel column chromatography using a mixture cyclohexane/ethyl acetate 1:1 as the eluent to afford pure compound **17**. Compound **2** was obtained as the second product and can be recycled. White crystals from hexane. Yield: 48%. $R_f = 0.45$ (cyclohexane/ethyl acetate 1:1). Mp = 195−197 °C. $[\alpha]^{20}_D = -18.1$ (*c* 0.210, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 6.76 (s, 1H, 5-H), 6.47 (s, 1H, 8-H), 5.96 (br s, 4H, 2′,6′-H, CH_2O_2), 5.46 (s, 1H, 4′-OH), 5.12 (d, 1H, $J = 2.6$ Hz, 4-H), 4.41 (t, 1H, $J = 6.2$ Hz, 13a-H), 4.34 (d, 1H, $J = 5.4$ Hz, 1-H), 3.78 (s, 6H, 3′,5′-OCH_3), 3.58−3.47 (m, 2H, 2,13b-H), 2.72 (dd, 1H, $J = 2.6$, 13.8 Hz, 3-H), 0.81 (s, 9H, tBu), 0.25 (s, 3H, Me), 0.11 (s, 3H, Me). MS (CI) *m/z*: 532 $[M + NH_4]^+$. Anal. ($C_{27}H_{34}O_8Si$) C, H.

11-Oxo-13-deoxo-4′-demethylepipodophyllotoxin (18). Compound **17** (988 mg, 1.92 mmol) was treated under conditions similar to those described in the previous step, but maintaining stirring for 23 h, to provide **18** in 80% yield after flash chromatography using cyclohexane/ethyl acetate 1:2 as the eluent. Subsequent recrystallization from hexane gives pure **17** as white crystals. Yield: 80%. $R_f = 0.34$ (cyclohexane/ethyl acetate 1:2). Mp = 129−131 °C. $[\alpha]^{20}_D = -16.0$ (*c* 0.100, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 6.94 (s, 1H, 5-H), 5.50 (s, 1H, 8-H), 6.03 (s, 2H, 2′,6′-H), 5.96 (d, 2H, $J = 10.4$ Hz, CH_2O_2), 5.48 (s, 1H, 4′-OH), 5.15−5.10 (m, 1H, 4-H), 4.45 (t, 1H, $J = 7.6$ Hz, 13a-H), 4.33 (d, 1H, $J = 5.9$ Hz, 1-H), 3.78 (s, 6H, 3′,5′-OCH_3), 3.61 (dd, 1H, $J = 8.4$, 11.3 Hz, 13b-H), 3.42−3.29 (m, 1H, 2-H), 2.77 (dd, 1H, $J = 3.3$, 14.4 Hz, 3-H), 2.64 (br s, 1H, 4-OH). MS (CI) *m/z*: 418 $[M + NH_4]^+$. Anal. ($C_{21}H_{20}O_8$) C, H.

11-Oxo-13-deoxo-4′-benzyloxycarbonyl-4′-demethylepipodophyllotoxin (19). Compound **18** (614 mg, 1.53 mmol) was treated under conditions similar to those described for the preparation of **3**, giving **19** in 82% yield (after flash chromatography using cyclohexane/ethyl acetate 1:2 as the eluent) as a white amorphous solid. $R_f = 0.44$ (cyclohexane/ethyl acetate 1:2). Mp = 128−130 °C. $[\alpha]^{20}_D = -7.5$ (*c* 0.1, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.45−7.35 (m, 5H, Ph), 6.93 (s, 1H, 5-H), 6.49 (s, 1H, 8-H), 6.03 (s, 2H, 2′,6′-H), 5.97 (d, 2H, $J = 10.3$ Hz, CH_2O_2), 5.27 (s, 2H, CH_2Ph), 5.16 (t, 1H, $J = 3.3$ Hz, 4-H), 4.48 (t, 1H, $J = 8.0$ Hz, 13a-H), 4.39 (d, 1H, $J = 5.9$ Hz, 1-H), 3.71 (s, 6H, 3′,5′-OCH_3), 3.66 (dd, 1H, $J = 8$, 11.5 Hz, 13b-H), 3.37 (m, 1H, 2-H), 2.77 (dd, 1H, $J = 3.3$, 14.4 Hz, 3-H), 2.62 (d, 1H, $J = 3.3$ Hz, 4-OH). MS (CI) *m/z*: 552 $[M + NH_4]^+$. HRMS (DIC/NH_3) *m/z* 552.1870 $[M + NH_4]^+$ $C_{29}H_{26}O_{10}$ requires 552.1866 $[M + NH_4]^+$. Anal. ($C_{29}H_{26}O_{10}$) C, H.

11-Oxo-13-deoxo-4′-benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-(*p*-nitrophenyl)carbonate (20). Compound **19** (523.6 mg, 0.980 mmol) was treated under conditions similar to those described for the preparation of **4**, to afford **20** (0.95 mmol) in 97% yield (after flash chromatography using a mixture cyclohexane/ethyl acetate 2:1 as the eluent) as a white amorphous solid. $R_f = 0.56$ (cyclohexane/ethyl acetate 2:1). Mp = 125−130 °C. $[\alpha]^{20}_D = -67.0$ (*c* 0.510, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 8.27 (d, 2H, $J = 7.1$ Hz, *Ph*NO_2), 7.46−7.35 (m, 7H, CH_2*Ph* (5H), *Ph*NO_2 (2H)), 7.10 (s, 1H, 5-H), 6.55 (s, 1H, 8-H), 6.40 (d, 1H, $J = 3.4$ Hz, 4-H), 6.03 (m, 3H, 2′,6′-H, CH_2O_2), 6.01 (d, 1H, $J = 1.1$ Hz, CH_2O_2), 5.27 (s, 2H, CH_2Ph), 4.54−4.47 (m, 2H, 1-H, 13a-H), 3.72 (s, 6H, 3′,5′-OCH_3), 3.66 (dd, 1H, $J = 8.4$, 11.2 Hz, 13b-H), 3.36 (m, 1H, 2-H), 2.98 (dd, 1H, $J = 3.4$, 14.4 Hz, 3-H). MS *m/z*: 717 $[M + NH_4]^+$. Anal. ($C_{36}H_{29}NO_{14}$) C,H,N.

General Procedure for the Synthesis of Compounds 21−26. To a stirred solution of **20** (0.286 mmol, 200 mg) in CH_2Cl_2 (10 mL) was added the appropriate amine (0.429 mmol, 1.5 equiv) and triethylamine (0.429 mmol) at room temperature. After the reported times, the reaction mixture was washed with a cold saturated $NaHCO_3$ solution and then with water until pH 6−7. The extract was dried over $MgSO_4$ and concentrated in vacuo at 30 °C. The residue was purified by silica gel column chromatography, using the mixture CH_2Cl_2/MeOH 90:10 as the eluent, to afford pure compounds **17**−**22**. The chromatographed compounds were directly employed in the subsequent deprotection step without further purification.

11-Oxo-13-deoxo-4′-benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-[*N*-[2-(*N*′,*N*′-Dimethylaminoethyl)]]carbamate (21). Yellow amorphous solid. Reaction time: 1.30 h. Yield 78%. $R_f = 0.20$ (CH_2Cl_2/MeOH 90:10). Mp = 113−

116 °C. $[\alpha]^{20}_D = -45.5$ (*c* 0.910, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.45−7.36 (m, 5H, CH_2Ph), 7.21 (s, 1H, 5-H), 6.49 (s, 1H, 8-H), 6.38 (d, 1H, J = 3.5 Hz, 4-H), 6.03 (br s, 2H, 2′,6′-H), 5.98 (d, 2H, J = 7.6 Hz, CH_2O_2), 5.28 (s, 2H, CH_2Ph), 5.18 (br t, 1H, J = 5.3 Hz, NH), 4.44 (br t, 1H, J = 8.3 Hz, 13a-H), 4.41 (d, 1H, J = 6.1 Hz, 1-H), 3.71 (s, 6H, 3′,5′-OCH_3), 3.59 (dd, 1H, J = 8.3, 11.2 Hz, 13b-H), 3.37−3.24 (m, 3H, 2-H, $CH_2\alpha$), 2.86 (dd, 1H, J = 3.5, 14.4 Hz, 3-H), 2.41 (m, 2H, $CH_2\beta$), 2.21 (s, 6H, $N(CH_3)_2$). MS (CI) *m/z*: 649 $[M + H]^+$. HRMS (FAB^+) *m/z*: 649.2388 $[M + H^+]$ $C_{34}H_{36}N_2O_{11}$ requires 649.2397 $[M + H^+]$.

11-Oxo-13-deoxo-4′-benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-[*N*-[2-(*N*′,*N*′-Dimethylaminoethyl)]-*N*-methyl]carbamate (22). Reaction time: 2 h. White amorphous solid. Yield: 78%. R_f = 0.37 (CH_2Cl_2/MeOH 90:10). Mp = 102−104 °C. $[\alpha]^{20}_D = -42.5$ (*c* 1.04, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.49−7.34 (m, 5H, CH_2Ph), 7.25 (s, 1H, 5-H), 6.49 (s, 1H, 8-H), 6.36−6.45 (m, 1H, 4-H), 6.03 (br s, 2H, 2′,6′-H), 5.98 (d, 2H, J = 6.9 Hz, CH_2O_2), 5.28 (s, 2H, CH_2Ph), 4.51−4.40 (m, 2H, 1,13a-H), 3.71 (s, 6H, 3′,5′-OCH_3), 3.59 (dd, 1H, J = 8.1, 11.3 Hz, 13b-H), 3.65−3.55 (m, 2H, $CH_2\alpha$), 3.50−3.40 (m, 1 H, 2-H), 2.98 (s, 1.5H, NCH_3 (rot.)), 2.92−2.87 (m, 1H, 3-H), 2.82 (s, 1.5H, NCH_3 (rot.)), 2.56−2.47 (m, 2H, $CH_2\beta$), 2.31 (br s, 3H, $N(CH_3)_2$), 2.23 (br s, 3H, $N(CH_3)_2$). MS (CI) *m/z*: 663 $[M + H]^+$.

11-Oxo-13-deoxo-4′-benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-[*N*-(2-Aminoethyl)]carbamate (23). Reaction time: 30 min. White amorphous solid. Yield 76%. R_f = 0.12 (CH_2Cl_2/MeOH 90:10). Mp = 114−116 °C. $[\alpha]^{20}_D = -59.0$ (*c* 0.350, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.46−7.29 (m, 5H, CH_2Ph), 7.16 (s, 1H, 5-H), 6.47 (s, 1H, 8-H), 6.41−6.30 (m, 1H, 4-H), 6.02 (s, 2H, 2′,6′-H), 5.94 (d, 2H, J = 7.8 Hz, CH_2O_2), 5.25 (s, 2H, CH_2Ph), 4.49−4.32 (m, 2H, 1,13a-H), 3.69 (s, 6H, 3′,5′-OCH_3), 3.57 (br t, 1H, J = 8.7 Hz, 13b-H), 3.40−3.17 (m, 3H, 2-H, $CH_2\beta$), 2.93−2.76 (m, 3H, 3-H, $CH_2\alpha$), 2.13 (br s, 3H, NH, NH_2). MS (CI) *m/z*: 621 $[M + H]^+$.

11-Oxo-13-deoxo-4′-benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-[*N*-[2-(*N*′,*N*′-Dimethylaminopropyl)]]carbamate (24). Reaction time: 1.30 h. White amorphous solid. Yield 79%. R_f = 0.14 (CH_2Cl_2/MeOH 90:10). Mp = 102−104 °C. $[\alpha]^{20}_D = -51.0$ (*c* 0.500, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.48−7.30 (m, 5H, CH_2Ph), 7.19 (s, 1H, 5-H), 6.48 (s, 1H, 8-H), 6.38 (d, 1H, J = 3.4 Hz, 4-H), 6.02 (s, 2H, 2′,6′-H), 5.95 (d, 2H, J = 6.4 Hz, CH_2O_2), 5.45 (br s, 1H, NH), 5.27 (s, 2H, CH_2Ph), 4.49−4.33 (m, 2H, 1-H, 13a-H), 3.70 (s, 6H, 3′,5′-OCH_3), 3.58 (t, 1H, J = 9.8 Hz, 13b-H), 3.41−3.19 (m, 3H, 2-H, $CH_2\alpha$), 2.85 (dd, 1H, J = 4.0, 14.0 Hz, 3-H), 2.38−2.30 (m, 2H, $CH_2\gamma$), 2.23 (s, 3H, $N(CH_3)_2$), 2.21 (s, 3H, $N(CH_3)_2$), 1.80−1.65 (m, 2H, $CH_2\beta$). MS *m/z*: 663 $[M + H]^+$. HRMS (FAB^+) *m/z*: 663.2539 $[M + H^+]$ $C_{35}H_{38}N_2O_{11}$ requires 663.2554 $[M + H^+]$.

11-Oxo-13-deoxo-4′-benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-[*N*-[3-(*N*′,*N*′-Dimethylaminopropyl)]-*N*-methyl]carbamate (25). Reaction time: 1 h. White amorphous solid. Yield: 65%. R_f = 0.30 (CH_2Cl_2/MeOH 90:10). Mp = 124−126 °C. $[\alpha]^{20}_D = -46.0$ (*c* 0.410, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.48−7.31 (m, 5H, CH_2Ph), 7.24 (s, 0.5H, 5-H (rot.)), 7.05 (s, 0.5H, 5-H (rot.)), 6.50 (s, 1H, 8-H), 6.41 (d, 1H, J = 3.8 Hz, 4-H), 6.03 (s, 2H, 2′,6′-H), 5.97 (d, 2H, J = 6.0 Hz, CH_2O_2), 5.27 (s, 2H, CH_2Ph), 4.62−4.50 (m, 1H, 13a-H), 4.45 (d, 1H, J = 7.6 Hz, 1-H), 3.70 (s, 6H, 3′,5′-OCH_3), 3.68−3.54 (m, 1H, 13b-H), 3.48−3.44 (m, 0.5H, $CH_2\alpha$ (rot.)), 3.40−3.20 (m, 1.5H, $CH_2\alpha$ (rot.)), 3.15−3.00 (m, 2H, 2,3-H), 2.95 (s, 1.5H, NCH_3 (rot.)), 2.80 (s, 1.5H, NCH_3 (rot.)), 2.68 (s, 6H, $N(CH_3)_2$), 2.30−2.10 (m, 2H, $CH_2\gamma$), 1.87−1.60 (m, 2H, $CH_2\beta$). MS (CI) *m/z*: 677 $[M + H]^+$. HRMS (FAB^+) *m/z*: 677.2725 $[M + H^+]$ $C_{36}H_{40}N_2O_{10}$ requires 677.2710 $[M + H^+]$.

11-Oxo-13-deoxo-4′-benzyloxycarbonyl-4′-demethylepipodophyllotoxin 4-[*N*-(3-Aminopropyl)]carbamate (26). Reaction time: 30 min. White amorphous solid. Yield 60%. R_f = 0.10 (CH_2Cl_2/MeOH 90:10). Mp = 92−94 °C. $[\alpha]^{20}_D = -33.5$ (*c* 0.475, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.46−7.30 (m, 5H, CH_2Ph), 7.13 (s, 1H, 5-H), 6.47 (s, 1H, 8-H), 6.40−6.30 (m, 1H, 4-H), 6.01 (br s, 2H, 2′,6′-H), 5.95 (d, 2H, J = 7.2 Hz, CH_2O_2), 5.26 (s, 2H, CH_2Ph), 4.47−4.30 (m, 2H, 1-H, 13a-H), 3.78 (s, 6H, 3′,5′-OCH_3), 3.55 (t, 1H, J = 11.3 Hz, 13b-H), 3.32−3.18 (m, 4H, $CH_2\alpha,\gamma$), 3.00−2.73 (m, 5H, 2,3-H, NH, NH_2), 1.80−1.60 (m, 2H, $CH_2\beta$). MS *m/z*: 635 $[M + H]^+$.

11-Oxo-13-deoxo-4′-demethylepipodophyllotoxin 4-[*N*-[2-(*N*′,*N*′-Dimethylaminoethyl)]]carbamate (27a). White amorphous solid. Yield 90%. R_f = 0.30 (CH_2Cl_2/MeOH 85:15). Mp = 140−142 °C. $[\alpha]^{20}_D = -64.0$ (*c* 0.520, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.19 (s, 1H, 5-H), 6.48 (s, 1H, 8-H), 6.37 (d, 1H, J = 3.5 Hz, 4-H), 6.01 (br s, 2H, 2′,6′-H), 5.96 (d, 2H, J = 8.3 Hz, CH_2O_2), 5.19 (br s, 2H, 4′-OH, NH), 4.42 (br t, 1H, J = 8.2 Hz, 13a-H), 4.35 (d, 1H, J = 5.9 Hz, 1-H), 3.79 (s, 6H, 3′,5′-OCH_3), 3.55 (dd, 1H, J = 8.2, 11.3 Hz, 13b-H), 3.32−3.21 (m, 3H, 2-H, $CH_2\alpha$), 2.87 (dd, 1H, J = 3.5, 14.1 Hz, 3-H), 2.41 (m, 2H, $CH_2\beta$), 2.20 (s, 6H, $N(CH_3)_2$). MS (CI) *m/z*: 515 $[M + H]^+$. HRMS (FAB^+) *m/z*: 515.2043 $[M + H^+]$ $C_{26}H_{30}N_2O_9$ requires 515.2030 $[M + H^+]$.

11-Oxo-13-deoxo-4′-demethylepipodophyllotoxin 4-[*N*-[2-(*N*′,*N*′-Dimethylaminoethyl)]-*N*-methyl]carbamate (27b). White amorphous solid. Yield 94%. R_f = 0.32 (CH_2Cl_2/MeOH 90:10). Mp = 133−135 °C. $[\alpha]^{20}_D = -50.0$ (*c* 0.750, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.25 (s, 1H, 5-H), 6.50 (s, 1H, 8-H), 6.42−6.38 (m, 1H, 4-H), 6.02 (s, 2H, 2′,6′-H), 5.97 (d, 2H, J = 6.0 Hz, CH_2O_2), 4.43 (br t, 1H, J = 8.2 Hz, 13a-H), 4.37 (d, 1H, J = 5.9 Hz, 1-H), 3.80 (s, 6H, 3′,5′-OCH_3), 3.56 (dd, 1H, J = 8.2, 11.2 Hz, 13b-H), 3.44−3.38 (m, 1H, CH_2a), 3.40−3.20 (m, 2H, 2-H, $CH_2\alpha$), 2.98 (s, 1.5H, NCH_3 (rot.)), 2.95−2.86 (m, 1H, 3-H), 2.81 (s, 1.5H, NCH_3 (rot.)), 2.52−2.43 (m, 2H, $CH_2\beta$), 2.28 (s 3H, $N(CH_3)_2$), 2.19 (s 3H, $N(CH_3)_2$). MS (CI) *m/z*: 529 $[M + H]^+$. HRMS (FAB^+) *m/z*: 529.2180 $[M + H^+]$ $C_{27}H_{32}N_2O_9$ requires 529.2186 $[M + H^+]$.

11-Oxo-13-deoxo-4′-demethylepipodophyllotoxin 4-[*N*-(2-Aminoethyl)]carbamate (27c). White amorphous solid. Yield: 65%. R_f = 0.16 (CH_2Cl_2/MeOH 85:15). Mp = 161−163 °C. $[\alpha]^{20}_D = -76.5$ (*c* 0.350, $CHCl_3$). ^1H NMR (CD_3OD) δ: 7.02 (s, 1H, 5-H), 6.49 (s, 1H, 8-H), 6.35−6.26 (m, 1H, 4-H), 6.14 (s, 2H, 2′,6′-H), 5.93 (d, 2H, J = 3.9 Hz, CH_2O_2), 4.60−4.37 (m, 2H, 1,13a-H), 3.73 (s, 6H, 3′,5′-OCH_3), 3.60−3.43 (m, 1H, 13b-H), 3.40−3.20 (m, 4H, 2,3, $CH_2\alpha$), 3.15−2.94 (m, 2H, $CH_2\beta$). MS *m/z*: 487 $[M + H]^+$. Anal. ($C_{24}H_{26}N_2O_9$) C, H, N.

11-Oxo-13-deoxo-4′-demethylepipodophyllotoxin 4-[*N*-[3-(*N*′,*N*′-Dimethylaminopropyl)]]carbamate (27d). White amorphous solid. Yield: 81%. R_f = 0.16 (CH_2Cl_2/MeOH 85:15). Mp = 103−105 °C. $[\alpha]^{20}_D = -68.0$ (*c* 0.285, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.03 (s, 1H, 5-H), 6.49 (s, 1H, 8-H), 6.37 (d, 1H, J = 3.5 Hz, 4-H), 6.01 (s, 2H, 2′,6′-H), 5.96 (d, 2H, J = 7.5 Hz, CH_2O_2), 5.80 (br s, 1H, 4′-OH), 4.45 (t, 1H, J = 7.7 Hz, 13a-H), 4.36 (d, 1H, J = 5.6 Hz, 1-H), 3.79 (s, 6H, 3′,5′-OCH_3), 3.57 (dd, 1H, J = 8.5, 11.1 Hz, 13b-H), 3.39−3.21 (m, 3H, 2-H, $CH_2\alpha$), 3.20−3.08 (m, 1H, 2-H), 2.85 (dd, 1H, J = 3.5, 14.6 Hz, 3-H), 2.72 (s, 6H, $N(CH_3)_2$), 2.26−2.10 (m, 2H, $CH_2\gamma$), 2.00−1.85 (m, 2H, $CH_2\beta$). MS *m/z*: 529 $[M + H]^+$. HRMS (FAB^+) *m/z*: 529.2180 $[M + H^+]$ $C_{27}H_{32}N_2O_9$ requires 529.2186 $[M + H^+]$. Anal. ($C_{27}H_{32}N_2O_9$) C, H, N.

11-Oxo-13-deoxo-4′-demethylepipodophyllotoxin 4-[*N*-[3-(*N*′,*N*′-Dimethylaminopropyl)]-*N*-methyl]carbamate (27e). White amorphous solid. Yield: 73%. R_f = 0.10 (CH_2Cl_2/MeOH 85:15). Mp = 135−137°C. $[\alpha]^{20}_D = -67.0$ (*c* 0.468, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.23 (s, 0.5H, 5-H (rot.)), 7.02 (s, 1H, 5-H (rot.)), 6.51 (s, 1H, 8-H), 6.41 (d, 1H, J = 3.6 Hz, 4-H), 6.01 (s, 2H, 2′,6′-H), 6.97 (d, 2H, J = 7.0 Hz, CH_2O_2), 5.47 (br s, 1H, 4′-OH), 4.46 (t, 1H, J = 5.8 Hz, 13a-H), 4.38 (d, 1H, J = 5.3 Hz, 1-H), 3.79 (s, 6H, 3′,5′-OCH_3), 3.59 (br t, 1H, J = 9.9 Hz, 13b-H), 3.30−3.04 (m, 3H, 2-H, $CH_2\alpha$), 2.95 (s, 1.5H, NCH_3 (rot.)), 2.81 (s, 6H, $N(CH_3)_2$), 2.78 (s, 1.5H, NCH_3 (rot.)), 2.77−2.70 (m, 3H, 3-H, $CH_2\gamma$), 2.23−2.07 (m, 2H, $CH_2\beta$). MS (CI) *m/z*: 543 $[M + H]^+$. HRMS (FAB^+) *m/z*: 543.2346 $[M + H^+]$ $C_{28}H_{34}N_2O_{10}$ requires 543.2342 $[M + H^+]$. Anal. ($C_{28}H_{34}N_2O_9$): C, H, N.

11-Oxo-13-deoxo-4′-demethylepipodophyllotoxin 4-[*N*-(3-Aminopropyl)]carbamate (27f). White amorphous solid. Yield: 75%. R_f = 0.10 (CH_2Cl_2/MeOH 85:15). Mp = 165−167 °C. $[\alpha]^{20}_D = -24.5$ (*c* 0.430, DMSO). ^1H NMR (CD_3OD) δ: 6.97 (s, 1H, 5-H), 6.50 (s, 1H, 8-H), 6.30 (d, 1H, J = 3.5 Hz, 4-H), 6.15 (s, 2H, 2′,6′-H), 5.94 (d, 2H, J = 3.0 Hz, CH_2O_2), 4.60−

4.40 (m, 2H, 1,13a-H), 3.73 (s, 6H, 3′,5′-OCH_3), 3.52 (dd, 1H, J = 8.5, 11.1 Hz, 13b-H), 3.30−3.22 (m, 2H, 2,3-H), 3.10−2.94 (m, 4H, $CH_2\alpha,\gamma$), 1.96−1.72 (m, 2H, $CH_2\beta$). MS (CI) *m/z*: 501 $[M + H]^+$. Anal. $C_{25}H_{28}N_2O_9$ (C, H, N).

11-Oxo-13-deoxo-4′-demethyl-4′-benzyloxycarbonyl-4-β-allyl-4-desoxypodophyllotoxin (28). To a cooled (0 °C) solution of **19** (0.842 mmol, 450 mg) and trimethylallylsilane (1.68 mmol, 267 μL) in CH_2Cl_2 was added $BF_3{\cdot}Et_2O$ (0.27 mL). After 4 h, the reaction mixture was quenched with pyridine (0.27 mL). The mixture was successively washed with cold 1 N HCl and a saturated NaCl solution and then dried over $MgSO_4$. The obtained solution was concentrated in vacuo at 30 °C. The chromatographed compound was directly involved in the subsequent deprotection step without further purification. White amorphous solid. R_f = 0.72 (CH_2Cl_2/acetone 95:5). Mp = 88−90 °C. $[\alpha]^{20}_D$ = −8.0 (*c* 0.540, DMSO). ^{1}H NMR ($CDCl_3$) δ: 7.42−7.28 (m, 5H, Ph), 6.68 (s, 1H, 5-H), 6.38 (s, 1H, 8-H), 6.05 (s, 2H, 2′,6′-H), 5.90 (s, 2H, CH_2O_2), 5.84−5.70 (m, 1H, $CH_2CH{=}CH_2$), 5.23 (s, 2H, CH_2Ph), 5.04−4.96 (m, 2H, $CH_2CH{=}CH_2$), 4.40−4.30 (m, 2H, 1,13a-H), 3.66 (s, 6H, 3′,5′-OCH_3), 3.39 (dd, 1H, J = 8.7, 11.0 Hz, 13b-H), 3.32−3.26 (m, 1H, 4-H), 3.20−3.06 (m, 1H, 2-H), 2.79 (dd, 1H, J = 5.3, 14.4 Hz, 3-H), 2.61−2.50 (m, 1H, $CH_2CH{=}CH_2$), 2.40−2.25 (m, 1H, $CH_2CH{=}CH_2$). MS (CI) *m/z*: 576 $[M + NH_4]^+$.

11-Oxo-13-deoxo-4′-demethyl-4′-benzyloxycarbonyl-4β-(formylmethyl)-4-desoxypodophyllotoxin (30). A solution of **28** (0.758 mmol, 423 mg), *N*-methylmorpholine *N*-oxide (NMO) (0.834 mmol, 97.7 mg), and osmium tetraoxide 2.5 wt % solution in 2-methyl-2-propanol (0.0758 mmol) in acetone (5 mL) was stirred for 3 h at room temperature. A saturated solution of $NaHSO_3$ (2 mL) was then added and the reaction mixture was extracted with ethyl acetate to afford the diol **29**. This crude compound (0.405 mmol, 240 mg) and $Pb(OAc)_4$ (0.405 mmol, 1 equiv) in dry benzene (18 mL) were stirred for 3 h at room temperature. The reaction mixture was filtered off. The solid residue was washed with ethyl acetate. The filtrate was concentrated in vacuo at 30°C and the residue was purified by silica gel column chromatography using a mixture CH_2Cl_2/MeOH 90:10 as the eluent to give **30** as a white amorphous solid in 65% yield. R_f 0.16 (CH_2Cl_2/MeOH 90:10). Mp = 116−118 °C. $[\alpha]^{20}_D$ = −23.5 (*c* 0.335, $CDCl_3$). ^{1}H NMR ($CDCl_3$) δ: 9.88 (s, 1H, CHO), 7.48−7.30 (m, 5H, CH_2Ph), 6.75 (s, 1H, 5-H), 6.42 (s, 1H, 8-H), 6.05 (s, 2H, 2′,6′-H), 5.92 (d, 2H, J = 5.9 Hz, CH_2O_2), 5.27 (s, 2H, CH_2Ph), 4.50−4.40 (m, 1H, 13a-H), 4.34 (d, 1H, J = 5.2 Hz, 1-H), 3.70 (s, 6H, 3′,5′-OCH_3), 3.52 (t, 1H, J = 11.0 Hz, 13b-H), 3.00−2.79 (m, 4H, 2,4-H, CH_2), 2.67−2.50 (m, 1H, 3-H). MS (CI) *m/z*: 578 $[M + NH_4]^+$.

11-Oxo-13-deoxo-4′-demethyl-4′-benzyloxycarbonyl-4-β-[2-[[2-(*N*′,*N*′-dimethylamino)ethyl]amino]ethyl]-4-desoxypodophyllotoxin (31). Compound **30** (0.338 mmol, 190 mg) was added to a stirred mixture of *N*,*N*-dimethylethylenediamine (0.372 mmol, 40.8 μL), AcOH (0.2 mL), and $NaCNBH_3$ (0.406 mmol, 25.5 mg) in MeOH (10 mL) at 0 °C. The mixture was allowed to warm to room temperature and then stirred for an additional 3 h. Ethyl acetate (50 mL) was added. The mixture was washed with a cold saturated $NaHCO_3$ solution, followed by washing to pH 6−7 with a saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated in vacuo below 30 °C. The residue was purified by silica gel chromatography using CH_2Cl_2/MeOH 5:1 as the eluent to provide pure **31** as a white amorphous solid in 60% yield. R_f = 0.40 (CH_2Cl_2/MeOH 85:15). Mp = 95−97 °C. $[\alpha]^{20}_D$ = −37.0 (*c* 0.320, $CDCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.42−7.30 (m, 5H, Ph), 6.62 (s, 1H, 5-H), 6.33 (s, 1H, 8-H), 6.16 (s, 2H, 2′,6′-H), 5.88 (s, 2H, CH_2O_2), 5.25 (s, 2H, CH_2Ph), 4.31−4.20 (m, 1H, 13a-H), 4.18−4.10 (m, 1H, 1-H), 3.67 (s, 6H, 3′,5′-OCH_3), 3.50−3.38 (m, 4H, 2,4,13b−H, NH), 3.30−2.19 (m, 1H, 3-H), 3.05−2.90 (m, 4H, $2CH_2$ α,β), 2.82 (s, 6H, $N(CH_3)_2$), 2.63−2.50 (m, 2H, CH_2NH), 2.10−1.98 (m, 2H, CH_2). MS (CI) *m/z*: 633 $[M + H]^+$. Anal. ($C_{35}H_{40}N_2O_9$) C, H, N.

11-Oxo-13-deoxo-4′-demethyl-4-β-[2-[[2-(*N*,*N*-dimethylamino)ethyl]amino]ethyl]-4-desoxypodophyllotoxin (32). White amorphous solid. Yield: 65%. R_f = 0.11 (CH_2Cl_2/MeOH 5:1). Mp = 115−117 °C. $[\alpha]^{20}_D$ = −10.0 (*c* 0.285, DMSO). ^{1}H NMR ($CDCl_3$) δ: 6.63 (s, 1H, 5-H), 6.35 (s, 1H, 8-H), 6.14 (s, 2H, 2′,6′-H), 5.88 (s, 2H, CH_2O_2), 4.31−4.20 (m, 1H, 13a-H), 4.09 (d, 1H, J = 4.6 Hz, 1-H), 3.92−3.82 (m, 1H, CH_2a), 3.76 (s, 6H, 3′,5′-OCH_3), 3.70−3.60 (m, 1H, $CH_2\alpha$), 3.50−3.38 (m, 1H, 13b-H), 3.30−3.19 (m, 2H, 4-H, NH), 3.10−3.00 (m, 2H, 2,3-H), 2.81−2.62 (m, 8H, $CH_2\beta$, $N(CH_3)_2$), 2.52−2.48 (m, 2H, CH_2), 2.10−1.98 (m, 2H, CH_2). MS (CI) *m/z*: 499 $[M + H]^+$. Anal. ($C_{27}H_{34}N_2O_7$) C, H, N.

DNA and Biochemicals. The plasmid pBS was from Stratagene. Human topoisomerase IIα was purchased from TopoGEN Inc. and etoposide from Sigma Chemicals.

Compounds were dissolved immediately before use in dimethyl sulfoxide at 5 mM and then diluted to working concentrations in distilled water.

Topoisomerase II-mediated DNA Cleavage Assay. Supercoiled pBS DNA (0.1 μg) was incubated for 15 min at 30 °C, in a 50 mM Tris-HCl buffer, pH 7.5, containing 1 mM ATP, 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 0.1 mM EDTA, and 30 μg BSA, in the presence of increasing concentrations of drug (from 0.05 to 50 μM) (total reaction volume 10 μL). Two units of human DNA topoisomerase IIα was added to the sample, preincubated as described, and incubated for 20 min at 30°C. The DNA−topoisomerase II cleavage complexes were dissociated by addition of sarcosyl to a 0.5% final concentration. Loading dye was added and DNA samples were electrophoresed (35 V/cm) in a 1% agarose gel in TBE × 1, containing ethidium bromide (1 μg/mL), at room temperature for 2 h. Gels were washed in water and photographed under UV light. The amount of linearized DNA in the presence of the drugs was measured and considered as the parameter to evaluate the ability of these compounds to stabilize the cleavage complex.

Cell Culture and Cytotoxicity Assays. Cells were cultivated in RPMI 1640 medium (Invitrogen Inc.) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH 7.4). Cytotoxicity was measured by the microculture tetrazolium assay (MTA) as described.

The nonadherent L1210 cells in exponential phase of growth were incubated for 48 h with serial dilutions of the compounds. At the end of this period, 15 μL of 5 mg/mL of 3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) were added to each well and the plates were incubated for 4 h at 37 °C. The medium was aspirated and the formazan solubilized by 100 μL of DMSO. The IC_{50}, the concentration reducing by 50% the optical density at 540 nm, was calculated by a linear regression performed on the linear zone of the dose−response curve. All the measurements were performed in triplicate.

Cell Cycle Analysis.[30,31] L1210 cells (2.5×10^5 cells/mL) were incubated for 21 h with various concentrations of the compounds. Cells were then fixed in 70% ethanol (v/v) and washed and incubated in Dulbecco's phosphate-buffered saline (D-PBS) containing 100 μg/mL RNase A and 25 μg/mL propidium iodide for 30 min at 20 °C. For each sample, 10^4 cells were analyzed on a Epics XL/MCL flow cytometer (Beckman Coulter).

Antitumor Activity in Vivo. Murine P388 leukemia tumor model was used as previously described.[32] B6D2F1 mice were inoculated ip with 10^6 tumor cells (day 0) and drugs were iv administered on day 1.

A549 human lung carcinoma tumor cells were cultured and grafted into immunodeficient mice as previously described.[33] Briefly, 10^6 cells in a volume of 100 μL were implanted through the chest wall into the left pleural space of anesthetized BALB/C nude mice. Mice were treated iv when the tumors were established, on days 14, 21, and 28 after the injection of tumor cells.

The antitumor activity was assessed by the increase in the life span of mice. The median survival time (MST) of the treated group (T) was compared with that of the control group (C), and the results were expressed as T/C (%) = (MST of treated group/MST of control group) × 100. All experiments were approved by an ethical committee and in accordance with

the guidelines approved by the UKCCCR for the welfare of animals in experimental neoplasia.[34]

Tubulin Test. Tubulin polymerization inhibition was determined as reported previously.[35]

Acknowledgment. Thanks are due to S. Thoret (ICSN-CNRS-Gif) for monitoring the tubulin assays and Dr. Brahim Kerkar (Gif-sur-Yvette) for the SYBYL calculations. This work was supported by grants from Fondation de France (to D.G.), from Ligue Nationale contre le Cancer and Ministère de la Recherche et de l′Industrie (ACI "Molécules et Cibles Thérapeutiques") (to P.B.A.), and from Association pour la Recherche contre le Cancer (ARC) (to M.D.).

Supporting Information Available: Infrared spectra data for compounds **4**−**10** and **13**−**32** and elemental analysis or HPLC analysis data for compounds **4**, **5**, **13, 17**−**20**, **27**, **31**, **32**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Belani, C. P.; Doyle, L. A.; Aisner, J. Etoposide: Current status and future perspectives in the management of malignant neoplasms. *Cancer Chemother. Pharmacol* **1994**, *34 Suppl.* S118−126.
(2) Hande, K. R. Etoposide: Four decades of development of a topoisomerase II inhibitor. *Eur. J. Cancer* **1998**, *34*, 1514−1521.
(3) Pommier, Y.; Fesen, M. R.; Goldwasser, F. *Cancer Chemotherapy and Biotherapy: Principles and Practice;* Chabner, B. A., Longo, D. L., Eds.; Lippincott-Raven Publishers: Philadelphia, PA, 1996; pp 435−461.
(4) Bromberg, K. D.; Burgin, A. B.; Osheroff, N. A two-drug model for etoposide action against human topoisomerase IIα. *J. Biol. Chem.* **2003**, *278*, 7406−7412.
(5) Fortune, J. M.; Osheroff, N. Topoisomerase II as a target for anticancer drugs: When enzymes stop being nice. *Prog. Nucleic Acid Res. Mol. Biol.* **2000**, *64*, 221−253.
(6) Osheroff, N. DNA topoisomerases. *Biochim. Biophys. Acta* **1998**, *1400*, 1−2.
(7) Champoux, J. J. DNA topoisomerases: Structure, function, and mechanism. *Annu. Rev. Biochem.* **2001**, *70*, 369−413.
(8) Capranico, G.; Binaschi, M. DNA sequence selectivity of topoisomerases and topoisomerase poisons. *Biochim. Biophys. Acta* **1998**, *1400*, 185−194.
(9) Burden, D. A.; Osheroff, N. Mechanism of action of eukaryotic topoisomerase II and drugs targeted to the enzyme. *Biochim. Biophys. Acta* **1998**, *1400*, 139−154.
(10) Kobayashi, K.; Ratain, M. J. Pharmacodynamics and long-term toxicity of etoposide. *Cancer Chemother. Pharmacol.* **1994**, *34 Suppl*. S64−8.
(11) Terada, T.; Fujimoto, K.; Nomura, M.; Yamashita, J.; Wierzba, K.; Yamazaki, R.; Shibata, J.; Sugimoto, Y.; Yamada, Y.; Kobunai, T.; et al. Synthesis and biological activity of 4-beta-alkyl derivatives containing hydroxy, amino, and amido groups of (4′-*O*-demethyl-4-desoxypodophyllotoxin as antitumor agents. *J. Med. Chem*. **1993**, *36*, 1689−1699.
(12) Huang, T. S.; Lee, C. C.; Chao, Y.; Shu, C. H.; Chen, L. T.; Chen, L. L.; Chen, M. H.; Yuan, C. C.; Whang-Peng, J. A novel podophyllotoxin-derived compound GL-331 is more potent than its congener VP-16 in killing refractory cancer cells. *Pharm Res.* **1999**, *16*, 997−1002.
(13) Utsugi, T.; Shibata, J.; Sugimoto, Y.; Aoyagi, K.; Wierzba, K.; Kobunai, T.; Terada, T.; Oh-hara, T.; Tsuruo, T.; Yamada, Y. Antitumor activity of a novel podophyllotoxin derivative (TOP-53) against lung cancer and lung metastatic cancer. *Cancer Res.* **1996**, *56*, 2809−2814.
(14) Byl, J. A.; Cline, S. D.; Utsugi, T.; Kobunai, T.; Yamada, Y.; Osheroff, N. DNA topoisomerase II as the target for the anticancer drug TOP-53: Mechanistic basis for drug action. *Biochemistry* **2001**, *40*, 712−718.
(15) Guianvarc'h, D.; Duca, M.; Boukarim, C.; Kraus-Berthier, L.; Leonce, S.; Pierré, A.; Pfeiffer, B.; Renard, P.; Arimondo, P.; Monneret, C.; Dauzonne, D. Synthesis and biological activity of sulfonamide derivatives of epipodophyllotoxin. *J. Med. Chem*. **2004**, *47*, 2365−2374.
(16) Daley, L.; Guminski, Y.; Demerseman, P.; Kruczynski, A.; Etievant, C.; Imbert, T.; Hill, B. T.; Monneret, C. Synthesis and antitumor activity of new glycosides of epipodophyllotoxin, analogues of etoposide and NK611. *J. Med. Chem.* **1998**, *41*, 4475−4485.
(17) Zhang, Y.; Lee, K. H. Recent progress in the development of novel antitumor etoposide analogs. *Chin. Pharm. J.* **1994**, *46*, 319−369.
(18) Dow, L.; Sinkule, J. A.; Look, A. T.; Horvath, A.; Evans W. E. Comparative cytotoxic and cytokinetic effects of the epipodophyllotoxins 4′-demethylepipodophyllotoxin-9-(4,6-*O*-2-ethylidene-β-D-glucopyranoside) and 4′-demethylepipodophyllotoxin-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) and their metabolites on human leukemic lymphoblasts. *Cancer Res.* **1983**, *43*, 5699−5706.
(19) Meresse, P.; Magiatis, P., Bertounesque, E.; Monneret, C. Synthesis and antiproliferative activity of retroetoposide. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4107−4109.
(20) Zhou, X. M.; Lee, K. J.; Cheng, J.; Wu, S. S.; Chen, H. X.; Guo, X.; Cheng, Y. C.; Lee, K. H. New γ-lactone ring-modified arylamino etoposide analogs as inhibitors of human DNA topoisomerase II. *J. Med. Chem.* **1994**, *37*, 287−292.
(21) The Sybyl program (version 6.0) is available from Tripos Associates, 1699 South Hanley Road, St. Louis, MO 63144. CS ChemBats3D Pro software is available from CambridgeSoft Corp., Cambridge, MA).
(22) Kingma, P. S.; Burden, D. A.; Osheroff, N. Binding of etoposide to topoisomerase II in the absence of DNA: Decreased affinity as a mechanism of drug resistance. *Biochemistry* **1999**, *38*, 3457−3461.
(23) Leroy, D.; Kajava, A. V.; Frei, C.; Gasser, S. M. Analysis of etoposide binding to subdomains of human DNA topoisomerase II alpha in the absence of DNA. *Biochemistry* **2001**, *40*, 1624−1634.
(24) Damayanthi, Y.; Lown J. W. Podophyllotoxins: Current status and recent developments. *Curr. Med. Chem.* **1998**, *5*, 205−252.
(25) Macdonald, T. L.; Lehnert, E. K.; Loper, J. T.; Chow, K.-C.; Ross, W. E. *On the mechanism of interaction of DNA topoisomerase II with chemotherapeutic agents in DNA Topoisomerases in Cancer;* Potmesil, M., Kohn, K. W., Eds.; Oxford University Press: New York, 1991; pp 199−214.
(26) Tepe, J. J.; Madalengoitia, J. DS.; Slunt, K. M.; Werbovetz, K. W.; Spoors, P. G.; Macdonald, T. L. Inhibition of DNA topoisomerase II by azaelliptitoxins functionnalized in the variable substituent domain. *J. Med. Chem*. **1996**, *39*, 2188−2196.
(27) Xiao, Z.; Xiao, Y.-D.; Feng, J.; Golbraikh, A.; Tropsha, A.; Lee, K. H. Antitumor Agents. 213. Modeling of epipodophyllotoxin derivatives using variable selection *k* nearest neighbor QSAR Method. *J Med. Chem.* **2002**, *45*, 2294−2309.
(28) Cho, S. J.; Tropsha, A.; Suffness, M.; Cheng, Y. C.; Lee, K. H. Three-dimensional quantitative structure−activity relationship study of 4′-demethylepipodophyllotoxin analogs using the modified CoMFA/q^2-GRS approach. *J. Med. Chem.* **1996**, *39*, 1383−1395.
(29) Avison, A. W. D.; Morrison, A. L. Synthetic Analgesics. Part VIII. Further attempts to prepare 3-Phenylpiperidine derivatives. *J. Chem. Soc.* **1950**, 1474−1477.
(30) Leonce, S.; Anstett, M.; Combe-Perez, V.; Pierre, A. Flow cytometric evaluation of the cell cycle perturbations induced by S12363, a new vinca alkaloid derivative. *Anti Cancer Drugs* **1990**, *1*, 179−183.
(31) Pierré, A.; Perez, V.; Leonce, S.; Boutin, J. A.; Saint-Dizier, D.; Hautefaye, P.; Lavielle, G.; Atassi, G. Relationship between the cellular accumulation and the cytotoxicity of S12363, a new Vinca alkaloid derivative. *Cancer Chemother. Pharmacol*. **1992**, *29*, 367−374.
(32) Guilbaud, N.; Kraus-Berthier, L.; Saint-Dizier, D.; Rouillon, M. H.; Jan, M.; Burbridge, M.; Visalli, M.; Bisagni, E.; Pierré, A.; Atassi, G. In vivo antitumor activity of S 16020−2, a new olivacine derivative. *Cancer Chemother. Pharmacol*. **1996**, *38*, 513−521.
(33) Kraus-Berthier, L.; Jan, M.; Guilbaud, N.; Naze, M.; Pierré, A.; Atassi, G. Histology and sensitivity to anticancer drugs of two human non-small cell lung carcinomas implanted in the pleural cavity of nude mice. *Clin. Cancer Res.* **2000**, *6*, 297−304.
(34) Workman, P.; Balmain, A.; Hickman, J. A.; McNally, N. J.; Rohas, A. M.; Mitchison, N. A.; Pierrepoint, C. G.; Raymond, R.; Rowlatt, C.; Stephens, T. C.; Wallace, J.; Straughan, D. W. UKCCCR guidelines for the welfare of animals in experimental neoplasia. *Lab. Anim.* **1988**, *22*, 195−201.
(35) Zavala, F.; Guenard, D.; Robin, J.-P.; Brown, E. Structure-Antitubulin activity relationships in steganacin congeners and analogues. Inhibition of tubulin polymerization in vitro by (±)-isodoxypodophyllotoxin. *J. Med. Chem*. **1980**, *23*, 546−549.

JM0495733