Synthesis and Biological Activity of Sulfonamide Derivatives of Epipodophyllotoxin†

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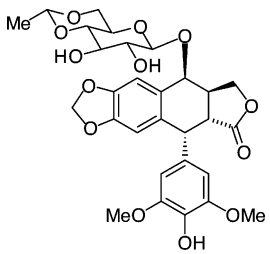
Received December 1, 2003

A series of novel 4β-substituted sulfonamide derivatives of 4′-*O*-demethyl-4-desoxypodophyllotoxin has been synthesized. Their effects on human DNA topoisomerase II and, in some cases, on tubulin polymerization were evaluated. Compounds **8a**, **8c**, **8f**, **8g**, **8n**, **8q**, **8r**, and **8s** and the synthetic precursor **4** are potent topoisomerase II poisons that induce double-stranded breaks in DNA, with either improved or similar activity compared to etoposide. Only the amino precursor, compound **5**, was slightly active in tubulin polymerization inhibition assays. We observed that the derivatives bearing an aromatic ring on the 4β-sulfonamide substituent were either less cytotoxic or equivalent to the parent drug, while the sulfonamides containing an aliphatic side chain and the amino-sulfonamide derivatives, except **8d** and **8g**, exhibited increased cytoxicity compared to etoposide. In vivo, against the P388 leukemia and the A-549 orthotopic model of lung carcinoma, the most promising compounds were the morpholino- and the piperazino-containing sulfonamides derivatives **8r** and **8s**.

Introduction

The podophyllotoxin derivative etoposide (VP16, **1**) is a semisynthetic glycoside derivative of podophyllotoxin first synthesized in 1966, tested clinically as an anticancer agent starting in 1971, and officially approved for clinical use in 1983.[1,2] Eighteen years after its introduction in medical practice, etoposide remains one of the most extensively used antitumor agents in clinical use directed against various types of cancers, including mainly breast cancer, testicular cancer, small-cell lung cancer, lymphoma, and chilhood leukemia.[3,4] In contrast to the parent podophyllotoxin, etoposide neither binds to tubulin nor inhibits microtubule assembly. The mechanism by which etoposide exerts its antineoplastic effects was elucidated in the 1980s after the discovery of the DNA-cleaving enzyme topoisomerase II.[5] In fact, molecules such as etoposide, amsacrine, and mitoxantrone are topoisomerase II inhibitors that induce cell death by enhancing the topoisomerase II-mediated DNA cleavage through the stabilization of the transient DNA/topoisomerase II cleavage complex. In such a complex, DNA is cleaved on both strands and covalently linked to the enzyme; the topoisomerase II poison prevents it from dissociating.[6] Recently, it has been suggested that etoposide−topoisomerase II interactions mediate cleavage complex stabilization, rather than etoposide−DNA, as is the case with amsacrine, another potent topoisomerase II inhibitor.[7]



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Despite its extensive use, etoposide is not devoid of toxic side effects. Bone-marrow depression is a frequent dose-limiting toxicity encountered in patients receiving etoposide, and the efficacy of the drug is calamitously associated with an increased risk of secondary acute myelogenous leukemia.[8,9] For this reason, the development of more active and more potent analogues remains highly valuable. Extensive structure−activity studies with etoposide analogues[10−12] have suggested that the activity of the epipodophyllotoxin derivatives is related to three structurally distinct pharmacophoric domains: the "DNA-intercalating" moiety (central part of the molecule), the binding site (southern part), and a variable substituent region (glycoside moiety).[11,12] Numerous glycoside analogues of etoposide have been synthesized, but only one of them has reached the preclinical level before the trials were stopped, NK611.[13] For our part, we have developed a series of 3-*N*,*N*-dimethylamino-2-deoxy analogues[14] that exhibited relevant antitumor activities in mice. The glycoside moiety of etoposide has thus also been replaced with nonsugar groups such as pyrrolecarboxamidino elements[15] and, above all, by a *p*-nitroanilino substituent. This latter afforded the drug GL-331, which was undergoing a

† Dedicated to the memory of Prof. Claude Hélène, deceased on February 11, 2003.

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10.1021/jm031117b CCC: $27.50
Published on Web 03/30/2004

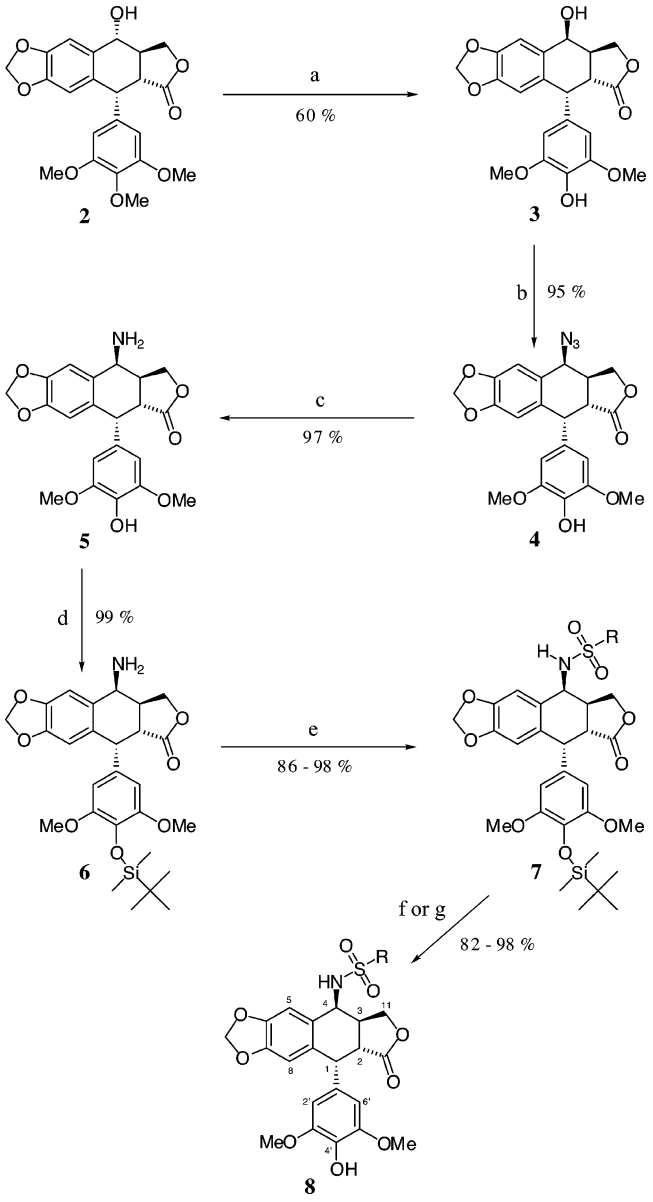
phase II clinical trial for treatment of various cancers,[16] but the trial was stopped in 2001.

In an ongoing effort to develop new and more potent anticancer agents that are water-soluble and less toxic, we synthesized 4β-substituted sulfonamide derivatives containing alkyl (type A), aromatic (type B), or amino groups (type C) on the 4′-*O*-demethyl-4-desoxypodophyllotoxin pharmacophore (Table 1). The choice of the sulfonamide group was dictated by the fact that the methanesulfonyl-*m*-anisidide group plays an important role in the topoisomerase II inhibitory activity of amsacrine,[17−19] and by the fact that the replacement of the glycoside moiety of **1** by a methanesulfonyl-*m*-anisidide group still maintains the topoisomerase II inhibition, depending on the length of the linker between the latter group and the aglycon moiety.[20] The relationship between cytotoxicity and inhibition of topoisomerase II, as well as the DNA binding affinity, was studied.

Chemistry

The synthesis of the novel 4β-(sulfonamido)-4′-*O*-demethyl-4-desoxypodophyllotoxin (**8a**−**s**) studied herein is depicted in Scheme 1. The 4β-amino-4′-*O*-demethyl-4-desoxypodophyllotoxin (**5**) requisite intermediate was prepared on a 20-g scale according to known procedures starting from podophyllotoxin (**2**) via 4′-*O*-demethyl-4-epipodophyllotoxin (**3**)[21] and then 4β-azido-4′-*O*-demethyl-4-desoxypodophyllotoxin (**4**).[22] The preparation of the new silyl-protected derivative **6** was achieved in excellent yield following a classical method involving *tert*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in DMF. The 4′-silylated 4β-alkylsulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin **7a**−**e**, 4β-arylsulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin **7h**−**j**, 4β-(thiophen-2-yl)sulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin **7l**, 4β-(thiazol-5-yl)sulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin **7m**, and 4β-aminosulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (**7n**−**s**) were further obtained by reacting **6** and the appropriate sulfonyl chloride in the presence of 1,4-diazabicyclo-[2.2.2]octane (DABCO) in CH_2Cl_2. The synthesis of the alkylazido derivative **7f** was performed by treatment of the ω-chloro compound **7e** by NaN_3 in dry DMF at 60 °C. It must be noted that during the course of this reaction, an important desilylation occurred and only a little **7f** was isolated aside from a large amount of the deprotected compound **8f**. The arylamino derivative **7k** was easily prepared by catalytic reduction (Pd/C 10%) in dioxane of the azido group of **7j**. The subsequent regenerations of the 4′-hydroxyl group from compounds **7a**−**q** were performed, in most cases (**7a**−**l** and **7n**−**r**), in methanol by using a DOWEX 50 × 2−200 ion-exchange resin to provide the wanted phenolic compounds **8a**−**l** and **8n**−**r** in good to excellent yields. With regard to the protected derivatives **7m** and **7s**, the above desilylation method gave unsatisfactory yields, probably because of an important retention of the product on the resin, and the procedure involving tetrabutylammonium fluoride (Bu_4NF) in THF was successfully employed to obtain **8m** and **8s**. The 4β-(3-aminopropyl)sulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (**8g**) and 4β-(4-aminophenyl)sulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (**8k**) were directly obtained from the corresponding already deprotected azido derivative **8f** and **8j** by catalytic reduction under atmospheric pressure.

Scheme 1[a]

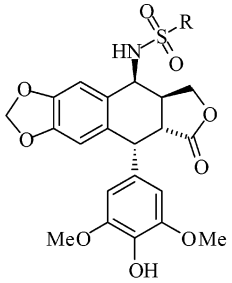


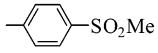
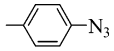
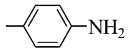
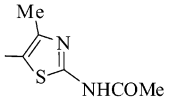
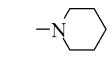
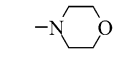
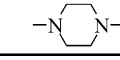
[a] Reagents: (a) $MeSO_3H$, *d*,*l*-methionine, H_2O, acetone; (b) NaN_3, $CHCl_3$, CF_3COOH; (c) H_2, Pd/C (10%) AcOEt; (d) TBDMSCl, imidazole, anhydrous DMF; (e) RSO_2Cl, DABCO, anhydrous CH_2Cl_2; (f) Dowex 50 × 2−200, MeOH for **8a-1** and **8a**−**r** or (g) Bu_4NF, THF for **8m** and **8s**.

Biological Results and Discussion

As illustrated in Table 1, in the sulfonamides of the type A series, the alkyl side chain was found to have an important effect on the activity of inhibiting human DNA topoisomerase II. Indeed, the compound containing the shortest chain, the methylsulfonamide analogue **8a**, showed the strongest inhibition value. As the length of the chain was increased, the inhibition activity decreased strongly for the butylsulfonamide derivative **8b** (5-fold less active), and this activity was completely lost in the case of the compound containing the longest side chain **8d** [R = $(CH_2)_{15}CH_3$]. Surprisingly, the octylsulfonamide **8c** was found to be more active on topoisomerase II inhibition than the shorter analogue **8b**.

Table 1. Biological Evaluation of 4β-Substituted Sulfonamide Derivatives of 4′-*O*-Demethyl-4-desoxypodophyllotoxin



compound	R	Topo II [a] (% linear DNA)	IC_{50} (μM)[b]	Cell cycle effect[c]
1 (VP16)	-	50	0.83	80% (2.5 μM)
4	-	44	0.57	61% (2.5 μM)
5	-	0	0.63	57% (2.5 μM)
	R = aliphatic chain : Sulfonamides type A			
8a	$-CH_3$	50	0.07	77% (0.5 μM)
8b	$-(CH_2)_3CH_3$	10	0.033	75% (0.2 μM)
8c	$-(CH_2)_7CH_3$	35	0.25	76% (1 μM)
8d	$-(CH_2)_{15}CH_3$	0	5.5	73% (25 μM)
8e	$-(CH_2)_3Cl$	14	0.045	66% (0.25 μM)
8f	$-(CH_2)_3N_3$	32	0.035	65% (0.25 μM)
8g	$-(CH_2)_3NH_2$	45	2.5	75% (50 μM)
	R = aromatic or heterocyclic ring : Sulfonamides type B:			
8h		10	0.55	75% (2.5 μM)
8i		0	1	52% (5μM) Toxic at 10 μM
8j		9	0.21	69% (0.5 μM)
8k		0	0.34	75% (0.2 μM)
8l		9	0.17	71% (0.5 μM)
8m		7	3.6	68% (10 μM)
	R = amine : Sulfonamides type C :			
8n	$-N(Me)_2$	44	0.037	66% (0.25 μM)
8o	$-N(Et)_2$	24	0.083	86% (0.25 μM)
8p	$-N(Bu)_2$	0	0.51	60% (2.5 μM)
8q		34	0.12	77% (0.5 μM)
8r		31	0.1	76% (0.25 μM)
8s		45	0.048	84% (0.1 μM)

[a] Each value reported here is a medium value of three independent experiments in the presence of the drug at 50 μM. [b] IC_{50}: concentration of drug required to reduce by 50% L1210 cell growth. [c] Percent of L1210 cells in the G2+M phases at the indicated concentration.

The most potent topoisomerase II inhibitor **8a** was also more cytotoxic than VP16 on L1210 murine leukemia cells (IC_{50} = 0.07 and 0.83 μM, respectively). On the other hand, the presence of the butyl chain in compound **8b**, while it strongly decreased the topoisomerase II inhibitory activity, it increased 2-fold the inhibition of tumor cell growth. In fact, not always the degree of topoisomerase II inhibition correlates with cytotoxicity. To further study the cellular target of compound **8b**, we investigated its ability to inhibit tubulin polymerization, as its precursor (the podophyllotoxin) is a potent anti-microtubule agent. Only a slight inhibition was observed with this compound (18% of inhibition of tubulin polymerization at 67 μM, compared to an IC_{50} = 3 μM for podophyllotoxin), as for **8a** (TPI = 3% at 67 μM), indicating that tubulin is probably not its cellular target. On the contrary, the synthetic precursor **5** (Scheme 1) was more active in inhibiting the polymerization of tubulin (56% at 67 μM), while it was completely inactive against topoisomerase II and showed a modest cytoxicity (Table 1). For the other alkyl derivatives **8c** and **8d**, as the length of the chain increases, the cytotoxicity decreases dramatically in correlation with their activity on topoisomerase II.

We next compared three analogues of compound **8b**. The substitution by a terminal amine on the alkyl chain of the cytotoxic butylsulfonamide **8b** increased by 3-fold the topoisomerase II inhibitory activity with complete loss of cytotoxicity for compound **8g**. The presence of a terminal halogen, as in compound **8e**, did not modify either the inhibition of topoisomerase II, the tubuline polymerization (TPI = 8% at 67 μM), or the cytotoxicity. The presence of an azido group as in compound **8f** increased the topoisomerase II activity and maintained the same cell growth inhibition potency and inactivity against tubulin polymerization (TPI = 19% at 67 μM). The azide precursor **4** inhibited strongly topoisomerase II but was less cytotoxic.

The poor correlation between topoisomerase II inhibition and cytotoxicity, for compounds **4** and **8g**, could be related to the drug uptake or metabolism.

Compounds of type B, bearing an aromatic ring on the 4β-sulfonamide substituent, displayed low topoisomerase II inhibition but a cytotoxic potency comparable to or slightly better than that of VP16, except compounds **8i** and **8m**.

In group C, the substitution of the 4β-sulfonamide group by six different amino substituents preserved the high topoisomerase II inhibition and the cytotoxic activity of the molecule, except for the dibutyl compound **8p**. For this class of derivatives, fairly good correlation between activity against topoisomerase II and inhibition of tumor cell proliferation was observed. The most potent drugs are the *N*,*N*-(dimethylamino)-substituted sulfonamide **8n** and the pyrazine analogue **8s** (IC_{50} = 0.037 and 0.048 μM, respectively, with anti-topoisomerase II activity comparable to that VP16). Lengthening of the alkyl chain on the amine decreased by 2-fold the activity of the *N*,*N*-diethylamino-substituted sulfonamide **8o** compared to its dimethylamino-analogue **8n** and drastically so (up to 12-fold) for the dibutyl analogue **8p**. The piperidine **8q** and morpholine **8r** analogues showed similar topoisomerase II inhibition and cytotoxic activities compared to compound **8o**. Only the most interesting derivative **8n** was tested for tubulin polymerization inhibition and proved inactive (TPI = 13% at 67 μM).

Table 2. Antiproliferative Effects of **1** (VP16) and **8b** against Various Cell Lines

	IC_{50} (nM)			IC_{50} (nM)	
cell lines	**1** (VP16)	**8b**	cell lines	**1** (VP16)	**8b**
L1210	201.4	33.5	IGROV1	984.5	61.4
P388	29.8	16.1	A549	664.0	62.6
K-562	522.2	138.6	NCI-H69	1192.2	316.3
A431	591.0	141.9	KB-3-1	566.5	186.1
DU-145	2373.3	303.7	SK-N-MC	275.0	85.7
MDA-MB-231	600.0	69.2	HT-29	2366.7	237.4

To have some indications on the drug−DNA interaction, we studied perturbations of ethidium bromide−DNA complexes in agarose gel by increasing concentrations (10, 50, 100 μM) of sulfonamide derivatives **8a**−**s**. While, under the same conditions, the known intercalating drug daunorubicin shifts the ethidium bromide in the DNA, the sulfonamide derivatives had no effect. This result argues for a nonintercalative binding mode such as known for VP16.

The perturbation of the cell cycle induced by these compounds was studied on the L1210 cell line. All the compounds induced a marked accumulation (>70%) of cells in the G2+M phases at a concentration between 0.1 and 2.5 μM, except alkylsulfonamides **8d**, **8g**, and **8m**, which were less cytotoxic than the others, requiring a 25, 50, and 10 μM concentration, respectively. Compound **8i** was revealed to be very inactive. Noteworthy, there is no correlation between the arrest in G2+M phase and topoisomerase II inhibition; the former is rather correlated to cell growth inhibition.[23]

Among the most cytotoxic compounds against L1210 cell lines, compound **8b** (IC_{50} = 34 nM) was further evaluated on a panel of 12 cell lines. Cell growth inhibition by **8b** was observed on all the cell lines (Table 2), the most sensitive cell lines being P388 leukemia, L1210 leukemia, and, in a much more moderate way, human tumor cell lines such as breast MDA-MB-231, ovarian IGROV1, lung A-549, and neuroblastoma SK-NMC. A comparison with VP16 shows that **8b** is more cytotoxic for each cell line tested (from 2- to 16-fold).

Compounds **8b**, **8l**, **8n**, **8o**, **8q**, **8r**, and **8s** were subsequently tested in vivo against P388 leukemia and the orthotopic model of human lung cancer A549,[24] with iv administration at J1 in the first case and at J14, J21, and J28 in the second (Table 3). Against P388, the majority of these compounds were active (T/C > 120%), except the thiophene derivative **8l** (T/C = 102%) and the diethylamine derivative **8o** (T/C = 106%). The most active compounds were the morpholine-containing (**8r**) and the piperazine-containing (**8s**) sulfonamides with T/C of 235% and 229%, respectively. However, they did not induce long-term survivors. Comparatively, VP16 exhibits a T/C = 233%, with 6/42 long-term survivors (LTS). Against the A-549 orthotopic model of lung cancer, **8r** and **8s** were more efficient than etoposide with T/C = 155% and 159%, respectively; VP16 presenting a T/C = 122−131%. The cytotoxic and potent topoisomerase II poison **8n** showed next best antitumor properties comparable to VP16 in both tumor models (T/C = 192% in P388 and T/C = 133% in A549), but again no long-term survivors were recorded.

Table 3. Antitumor Activity of Compounds **1** (VP16), **8b**, **8l**, **8n**, **8o**, **8q**, **8r**, and **8s**

	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[a]	median T/C[b]	LTS D60[c]	dose range, mg/kg	MTD[a]	T/C[b]
1 (VP16)	6.25−100	100	233	6/42	70−100	70	122−131
8b	6.25−100	100	161	0/6	25−100	50	105
8l	3.12−50	12.5	102	0/6	−	NT[d]	−
8n	12.5−200	100	192	0/6	6.25−25	6.25	133
8o	1.56−25	25	106	0/6	3.12−12.5	6.25	120
8q	12.5−200	200	121	0/6	−	NT	−
8r	12.5−200	200	235	0/6	25−100	50	155
8s	1.56−25	12.5	229	0/6	1.56−6.25	6.25	126−159

[a] MTD (mg/kg): maximum tolerated dose; i.e., the dose that 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. [c] LTS: long-term survivors, scored at day 60. [d] NT: not tested.

Conclusions

We have synthesized a series of new derivatives of 4-desoxy-4-amino-4′-demethylepipodophyllotoxin in which the glucose acetal moiety has been replaced by various sulfonamide substituents. This led, especially in the case of morpholine- and piperazine-containing sulfonamide side chains (**8r** and **8s**) to highly promising new antitumor compounds. This relevant activity correlated with inhibition activity against topoisomerase II inhibition and with marked accumulation of cells in the G2+M phase.

More complete evaluation of the most active compounds is now in progress against tumor models in vivo to evaluate whether they are worthy of further development as anticancer agents.

Experimental Section

Chemistry. Solvents and most of the starting materials were purchased from Acros, Aldrich, or Avocado. The commercially unavailable diethylsulfamoyl chloride,[25] dibutylsulfamoyl chloride,[25] morpholine-4-sulfonyl chloride,[26] piperidine-1-sulfonyl chloride,[27] and 4-methylpiperazine-1-sulfonyl chloride[28] where prepared according to previously reported procedures. Reference etoposide (**1**) was obtained from Sigma Chemical Co. Melting points were measured on a Köfler hot stage apparatus and are uncorrected. Mass spectra were obtained with a Nermag-Ribermag R10-10C spectrometer applying either desorption chemical ionization (CI) (operating in the positive ion mode using ammonia as the reagent gas) or fast atom bombardment (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), dt (double 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.

4*β*-Amino-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (6). A solution of **5** (14.73 g, 36.9 mmol),[21,22] TBDMSCl (9.8 g, 65 mmol), and imidazole (20.4 g, 0.3 mol) in anhydrous DMF (1115 mL) was stirred in a 2 L dry round-bottomed flask under inert atmosphere for 17 h. The reaction mixture was poured in a 5 L separating funnel, and water (2 L) was added. (**Caution:** The reaction is slightly exothermic.) The mixture was allowed to cool to room temperature and extracted with Et_2O (1 L then 4 × 500 mL). The combined organic extracts were dried ($MgSO_4$) and then filtered. Removal of the solvents in vacuo (increasing gradually the temperature from 30 to 100 °C) gave a crude material (18.75 g, 99%) directly recrystallized in benzene/heptane to afford analytically pure **6** (15.16 g, 80%) as white plates. R_f = 0.23 (CH_2Cl_2/acetone 90:10). Mp = 227−229 °C. $[\alpha]^{20}_D$ = −62.5 (*c* = 0.55, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 6.80 (1H, s, 5-H), 6.50 (1H, s, 8-H), 6.27 (2H, s, 2′,6′-H), 5.95 (2H, d, *J* = 6.0 Hz, CH_2O_2), 4.55 (1H, d, *J* = 5.1 Hz, 1-H), 4.28 (2H, d, *J* = 9.3 Hz, 11-H), 4.19 (1H, d, *J* = 4.1 Hz, 4-H), 3.67 (6H, s, 3′,5′-OCH_3), 3.26 (1H, dd, *J* = 5.1, 14.0 Hz, 2-H), 2.89−2.78 (1H, m, 3-H), 1.95−1.60 (2H, m, NH_2), 0.98 (9H, s, tBu), 0.10 (6H, s, 2Me). IR ($CHCl_3$) ν: 3573 (NH_2), 2931 (aliphatic C−H), 1775 (C═O lactone), 1621, 1505, 1465 (aromatic C═C). MS (CI) *m*/*z*: 531 $[M + NH_4]^+$. Anal. ($C_{27}H_{35}NO_7Si$) C, H, N.

General Procedure for the Synthesis of Compounds 7a−e, 7h−l, and 7n−s. To a solution of **6** (309 mg, 0.6 mmol) and DABCO (45 mg, 0.4 mmol) in anhydrous CH_2Cl_2 (10 mL), under inert atmosphere, was added the appropriate sulfonyl chloride (1 mmol). The reaction mixture was further stirred at room temperature for the reported times (monitored by TLC). The medium was next taken up with CH_2Cl_2 (50 mL) and water (20 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$) and then filtered. Evaporation of the solvents in vacuo afforded a crude material chromatographed on silica gel (100 g; eluent, various mixtures CH_2Cl_2/acetone; see R_f) to provide satisfactorily pure compounds in the reported yields. In the cases of **7a−e**, **7h−l**, **7n**, **7p**, and **7r**, the product was recrystallized from benzene/hexane. In the cases of **7o**, **7q**, and **7s**, the chromatographed silylated compound was directly employed in the subsequent deprotection step without further purification.

4⟨*β*-Methylsulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7a). Reaction time: 16 h. Yield: 91%. R_f = 0.39 (CH_2Cl_2/acetone 96:4). Mp = 206−208 °C (white crystals). $[\alpha]^{20}_D$ = −79.0 (*c* = 0.345, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 6.84 (1H, s, 5-H), 6.53 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.99 (2H, d, *J* = 7.0 Hz, CH_2O_2), 4.84−4.78 (1H, m, 11a-H), 4.56 (1H, d, *J* = 4.1 Hz, 1-H), 4.57−4.32 (3H, m, 11b,4-H, NH), 3.67 (6H, s, 3′,5′-OCH_3), 3.15 (3H, s, CH_3SO_2), 2.92−2.89 (2H, m, 2,3-H), 0.98 (9H, s, tBu), 0.10 (6H, s, 2Me). IR ($CHCl_3$) ν: 3380 (NH), 2934 (aliphatic C−H), 1776 (C═O lactone), 1587, 1507, 1485 (aromatic C═C), 1389, 1132 (SO_2). MS (CI) *m*/*z*: 609 $[M + NH_4]^+$. Anal. ($C_{28}H_{37}NO_9SSi$) C, H, N.

4*β*-Butylsulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7b). Reaction time: 18 h. Yield: 90%. R_f = 0.59 (CH_2Cl_2/acetone 96:4). mp = 127−129 °C (white crystals). $[\alpha]^{20}_D$ = −61.0 (*c* = 0.535,

$CHCl_3$). ^1H NMR ($CDCl_3$) δ: 6.83 (1H, s, 5-H), 6.52 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.99 (2H, d, J = 5.1 Hz, CH_2O_2), 4.82−4.76 (1H, m, 11a-H), 4.56 (1H, d, J = 3.8 Hz, 1-H), 4.42−4.23 (3H, m, 11b, 4-H, NH), 3.67 (6H, s, 3′,5′-OCH_3), 3.22−3.10 (2H, m, CH_2SO_2), 2.96−2.88 (2H, m, 2,3-H), 1.96−1.78 (2H, m, $CH_2\beta$), 1.61−1.42 (2H, m, $CH_2\gamma$), 1.07−0.91 (12H, m, CH_3, tBu), 0.10 (6H, s, 2Me). IR ($CHCl_3$) ν: 3395 (NH), 2934 (aliphatic C−H), 1775 (C=O lactone), 1586, 1507, 1485 (aromatic C=C), 1335, 1132 (SO_2). MS (CI) m/z: 651 $[M + NH_4]^+$. Anal. ($C_{31}H_{43}NO_9SSi$) C, H, N.

4β-Octylsulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7c). Reaction time: 16 h. Yield: 94%. R_f = 0.67 (CH_2Cl_2/acetone 96:4). Mp = 110−112 °C (white crystals). $[\alpha]^{20}_D$ = −57.0 (c = 0.320, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 6.83 (1H, s, 5-H), 6.52 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.99 (2H, d, J = 6.2 Hz, CH_2O_2), 4.82−4.74 (1H, m, 11a-H), 4.56 (1H, br d, J = 3.5 Hz, 1-H), 4.41−4.21 (3H, m, 11b,4-H, NH), 3.67 (6H, s, 3′,5′-OCH_3), 3.22−3.09 (2H, m, CH_2SO_2), 2.99−2.83 (2H, m, 2,3-H), 1.98−1.79 (2H, m, $CH_2\beta$), 1.61−1.41 (2H, m, $CH_2\gamma$), 1.40−1.19 (8H, m, $4CH_2$), 0.98 (9H, s, tBu), 0.93−0.81 (3H, m, CH_3), 0.10 (6H, s, 2Me). IR ($CHCl_3$) ν: 3395 (NH), 2930 (aliphatic C−H), 1776 (C=O lactone), 1587, 1506, 1485 (aromatic C=C), 1335, 1132 (SO_2). MS (FAB) m/z: 712 $[M + Na]^+$. Anal. ($C_{35}H_{51}NO_9SSi$) C, H, N.

4β-Hexadecylsulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7d). Reaction time: 16 h. Yield: 92%. R_f = 0.42 (CH_2Cl_2/acetone 96:4). mp = 126−128 °C (white crystals). $[\alpha]^{20}_D$ = −61.5 (c = 0.275, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 6.84 (1H, s, 5-H), 6.52 (1H, s, 8-H), 6.22 (2H, s, 2′,6′-H), 5.99 (2H, d, J = 6.8 Hz, CH_2O_2), 4.82−4.76 (1H, m, 11a-H), 4.57 (1H, br d, J = 3.3 Hz, 1-H), 4.42−4.28 (3H, m, 11b,4-H, NH), 3.67 (6H, s, 3′,5′-OCH_3), 3.21−3.11 (2H, m, CH_2SO_2), 2.94−2.88 (2H, m, 2,3-H), 1.95−1.80 (2H, m, $CH_2\beta$), 1.54−1.42 (2H, m, $CH_2\gamma$), 1.38−1.20 (24H, m, $12CH_2$), 0.98 (9H, s, tBu), 0.88 (3H, t, J = 6.6 Hz, CH_3), 0.10 (6H, s, 2Me). IR ($CHCl_3$) ν: 3388 (NH), 2929 (aliphatic C−H), 1776 (C=O lactone), 1587, 1506, 1485 (aromatic C=C), 1335, 1132 (SO_2). MS m/z: (FAB) 802 $[M + H]^+$, 824 $[M + Na]^+$. Anal. ($C_{43}H_{67}NO_9SSi$) C, H, N.

4β-(3-Chloropropyl)sulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7e). Reaction time: 22 h. Yield: 88%. R_f = 0.53 (CH_2Cl_2/acetone 96:4). Mp = 133−135 °C (white crystals). $[\alpha]^{20}_D$ = −72.0 (c = 0.555, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 6.87 (1H, s, 5-H), 6.53 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.99 (2H, d, J = 4.5 Hz, CH_2O_2), 4.85−4.76 (1H, m, 11a-H), 4.57 (1H, d, J = 4.3 Hz, 1-H), 4.41−4.25 (3H, m, 11b,4-H, NH), 3.81−3.70 (2H, m, CH_2Cl), 3.67 (6H, s, 3′,5′-OCH_3), 3.38 (2H, t, J = 6.7 Hz, CH_2SO_2), 3.00−2.88 (2H, m, 2,3-H), 2.48−2.25 (2H, m, $CH_2\beta$), 0.98 (9H, s, tBu), 0.10 (6H, s, 2Me). IR ($CHCl_3$) ν: 3385 (NH), 2930 (aliphatic C−H), 1776 (C=O lactone), 1587, 1507, 1485 (aromatic C=C), 1336, 1132 (SO_2). MS (CI) m/z: 671, 673 $[M + NH_4]^+$. Anal. ($C_{30}H_{40}ClNO_9SSi$) H, N; C, calcd 55.07; found 54.36.

4β-(4-Methylsulfonamidophenyl)sulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7h). Reaction time: 5 h. Yield: 98%. R_f = 0.22 (CH_2Cl_2/acetone 94:6). Mp = 167−169 °C (white crystals). $[\alpha]^{20}_D$ = −40.0 (c = 0.25, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 8.21 (4H, dd, J = 8.6 Hz, Ph), 6.45 (1H, s, 8-H), 6.20 (2H, s, 2′6′-H), 5.89 (2H, s, CH_2O_2), 5.44 (1H, s, 5-H), 5.41 (1H, s, NH), 4.62−4.51 (2H, m, 1,4-H), 4.40−4.28 (1H, m, 11-H), 3.75 (6H, s, 3′,5′-OCH_3), 3.16 (3H, s, CH_3), 2.95−2.83 (2H, m, 2,3-H), 0.86 (9H, s, tBu), 0.01 (6H, s, 2Me). IR ($CHCl_3$) ν: 3371 (NH), 2931 (aliphatic C−H), 1776 (C=O lactone), 1587, 1507, 1485 (aromatic C=C), 1333, 1132 (SO_2). MS (FAB) m/z: 754 $[M + Na]^+$. Anal. ($C_{34}H_{41}NO_{11}S_2Si$) C, H, N.

4β-Pentafluorophenylsulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7i). Reaction time: 24 h. Yield: 97%. R_f = 0.60 (CH_2Cl_2/acetone 96:4). ^1H NMR ($CDCl_3$) δ: 6.53 (1H, s, 8-H), 6.17 (2H, s, 2′,6′-H), 6.03 (1H, s, 5-H), 5.96 (2H, d, J = 5.1 Hz, CH_2O_2), 5.41 (1H, d, J = 5.1 Hz, NH), 4.90−4.82 (1H, m, 4-H), 4.58 (1H, d, J = 4.2 Hz, 1-H), 4.40 (1H, t, J = 6.9 Hz, 11a-H), 4.26 (1H, t, J = 9.8 Hz, 11b-H), 3.67 (6H, s, 3′,5′-OCH_3), 3.02−2.90 (2H, m, 2,3-H), 0.98 (9H, s, tBu), 0.10 (6H, s, Me). IR ($CHCl_3$) ν: 3369 (NH), 2931 (aliphatic C−H), 1779 (C=O lactone), 1520, 1500, 1485 (aromatic C=C), 1337, 1132 (SO_2). MS (CI) m/z: 761 $[M + NH_4]^+$

4β-(4-Azidophenyl)sulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7j). Reaction time: 18 h. Yield: 94%. R_f = 0.61 (CH_2Cl_2/acetone 96:4). Mp = 148−150 °C (white crystals). $[\alpha]^{20}_D$ = −71.5 (c = 0.770, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.93 (2H, d, J = 8.7 Hz, Ph), 7.26 (2H, d, J = 8.7 Hz, Ph), 6.46 (1H, s, 8-H), 6.16 (2H, s, 2′,6′-H), 5.91 (2H, s, CH_2O_2), 5.72 (1H, s, 5-H), 4.70−4.59 (3H, m, 1,4-H, NH), 4.27 (2H, d, J = 8.1 Hz, 11-H), 3.65 (6H, s, 3′,5′-OCH_3), 2.92−2.80 (2H, m, 2,3-H), 0.97 (9H, s, tBu), 0.10 (6H, s, 2Me). IR ($CHCl_3$) ν: 3525 (NH), 2930 (aliphatic C−H), 2133 (N_3), 1774 (C=O lactone), 1597, 1505, 1485 (aromatic C=C), 1336 (SO_2). MS (CI) m/z: 712 $[M + NH_4]^+$. Anal. ($C_{33}H_{38}N_4O_9SSi$) C, H, N.

4β-(Thiophen-2-yl)sulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7l). Reaction time: 7 h. Yield: 96%. R_f = 0.63 (CH_2Cl_2/acetone 96:4). Mp = 151−153 °C (white crystals). $[\alpha]^{20}_D$ = −41.5 (c = 0.635, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.80−7.71 (2H, m, Ph), 7.30−7.20 (1H, m, Ph), 6.47 (1H, s, 8-H), 6.18 (2H, s, 2′,6′-H), 5.90 (2H, s, CH_2O_2), 5.62 (1H, s, 5-H), 4.68−4.49 (3H, m, 1,4-H, NH), 4.40−4.30 (2H, m, 11-H), 3.66 (6H, s, 3′,5′-OCH_3), 3.00−2.82 (2H, m, 2,3-H), 0.98 (9H, s, tBu), 0.10 (6H, s, Me). IR ($CHCl_3$) ν: 3365 (NH), 2943 (aliphatic C−H), 1775 (C=O lactone), 1559, 1507, 1485 (aromatic C=C), 1332, 1161 (SO_2). MS (CI) m/z: 677 $[M + NH_4]^+$. Anal. ($C_{31}H_{37}NO_9S_2Si$) C, H, N.

4β-(2-Acetamido-4-methylthiazol-5-yl)sulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7m). Reaction time: 20 h. Yield: 98%. R_f = 0.50 (CH_2Cl_2/acetone 85:15). Mp = 191−193 °C (white crystals). $[\alpha]^{20}_D$ = −100.0 (c = 0.57, MeOH). ^1H NMR ($CDCl_3$) δ: 6.48 (1H, s, 8-H), 6.19 (2H, s, 2′,6′-H), 6.18 (1H, s, NH), 6.03 (1H, s, 5-H), 5.94 (2H, d, J = 3.8 Hz, CH_2O_2), 5.02 (1H, d, J = 6.6 Hz, NH), 4.74−4.68 (1H, m, 4-H), 4.59−4.52 (1H, m, 1-H), 4.37−4.28 (2H, m, 11-H), 3.68 (6H, s, 3′,5′-OCH_3), 2.97−2.89 (2H, m, 2,3-H), 2.54 (3H, s, CH_3), 2.34 (3H, s, CH_3), 0.98 (9H, s, tBu), 0.10 (6H, s, Me). IR ($CHCl_3$) ν: 3336 (NH), 2932 (aliphatic C−H), 1775 (C=O lactone), 1587, 1510, 1485 (aromatic C=C), 1337, 1150 (SO_2). MS (CI) m/z: 732 $[M + H]^+$. Anal. ($C_{33}H_{41}N_3O_{10}S_2Si$) C, H, N.

4β-Dimethylaminosulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7n). Reaction time: 28 h. Yield: 96%. R_f = 0.51 (CH_2Cl_2/acetone 96:4). Mp = 172−174 °C (white crystals). $[\alpha]^{20}_D$ = −61.0 (c = 0.430, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 6.97 (1H, s, 5-H), 6.51 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.98 (2H, d, J = 5.8 Hz, CH_2O_2), 4.80−4.69 (1H, m, 11a-H), 4.60−4.51 (1H, m, 1-H), 4.45−4.30 (2H, m, 4-H, NH), 4.28−4.19 (1H, m, 11b-H), 3.67 (6H, s, 3′,5′-OCH_3), 2.98−2.82 (8H, m, 2,3-H + $2CH_3$), 0.98 (9H, s, tBu), 0.10 (6H, s, 2Me). IR ($CHCl_3$) ν: 3391 (NH), 2930 (aliphatic C−H), 1775 (C=O lactone), 1587, 1506, 1485 (aromatic C=C), 1336, 1132 (SO_2). MS (CI) m/z: 638 $[M + NH_4]^+$. Anal. ($C_{29}H_{40}N_2O_9SSi$) C, H, N.

4β-Diethylaminosulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7o). Reaction time: 28 h. Yield: 89%. R_f = 0.65 (CH_2Cl_2/acetone 92:8). ^1H NMR ($CDCl_3$) δ: 7.04 (1H, s, 5-H), 6.52 (1H, s, 8-H), 6.23 (2H, s, 2′,6′-H), 6.00 (2H, d, J = 4.9 Hz, CH_2O_2), 4.79−4.70 (1H, m, 11a-H), 4.57 (1H, br d, J = 2.5 Hz, 1-H), 4.47−4.31 (2H, m, 4-H, NH), 4.01 (1H, d, J = 6.2 Hz, 11b-H), 3.69 (6H, s, 3′,5′-OCH_3), 3.36 (4H, q, J = 7.2, 14.5 Hz, $2CH_2$), 2.98−2.88 (2H, m, 2,3-H), 1.27 (6H, t, J = 7.1 Hz, $2CH_3$), 0.98 (9H, s, tBu), 0.10 (6H, s, 2Me). MS (CI) m/z: 666 $[M + NH_4]^+$.

4β-Dibutylaminosulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7p). Reaction time: 48 h. Yield: 86%. R_f = 0.55 (CH_2Cl_2/acetone 95:5). Mp = 112−114 °C (white crystals). $[\alpha]^{20}_D$ = −45.0 (c = 0.235, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.03 (1H, s, 5-H), 6.50 (1H, s, 8-H), 6.27 (2H, s, 2′,6′-H), 5.98 (2H, d, J = 6.0 Hz, CH_2O_2),

4.80−4.70 (1H, m, 11a-H), 4.60−4.51 (1H, m, 1-H), 4.55−4.40 (2H, m, 4-H, NH), 3.91 (1H, d, J = 6.0 Hz, 11b-H), 3.77 (6H, s, 3′,5′-OCH_3), 3.30−3.16 (4H, m, $CH_2\alpha$), 2.95−2.84 (1H, m, 2,3-H), 1.71−1.49 (4H, m, $CH_2\beta$), 1.48−1.20 (4H, m, $CH_2\gamma$), 1.02−0.90 (6H, m, CH_3), 0.86 (9H, s, tBu), 0.10 (6H, s, Me). IR ($CHCl_3$) ν: 3338 (NH), 2937 (aliphatic C−H), 1775 (C=O lactone), 1519, 1506, 1485 (aromatic C=C), 1333, 1118 (SO_2). MS (CI) m/z: 722 $[M + NH_4]^+$. Anal. ($C_{35}H_{52}N_2O_9SSi$) C, H, N.

4β-Piperidinosulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7q). Reaction time: 28 h. Yield: 98%. R_f = 0.62 (CH_2Cl_2/acetone 92:8). ^{1}H NMR ($CDCl_3$) δ: 7.02 (1H, s, 5-H), 6.50 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.98 (2H, d, J = 3.4 Hz, CH_2O_2), 4.75−4.68 (1H, m, 11a-H), 4.55 (1H, br d, J = 3.4 Hz, 1-H), 4.43−4.32 (2H, m, 4-H, NH), 4.17 (1H, d, J = 6.7 Hz, 11b-H), 3.67 (6H, s, 3′,5′-OCH_3), 3.40−3.18 (4H, m, 2 CH_2N), 2.97−2.88 (2H, m, 2,3-H), 1.86−1.66 (4H, m, 2 CH_2), 1.65−1.50 (2H, m, CH_2), 0.98 (9H, s, tBu), 0.10 (6H, s, Me). MS (CI) m/z: 661 $[M + H]^+$. 678 $[M + NH_4]^+$.

4β-Morpholinosulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7r). Reaction time: 24 h. Yield: 95%. R_f = 0.43 (CH_2Cl_2/acetone 96:4). Mp = 138−140 °C (white crystals). $[\alpha]^{20}_D$ = −58.0 (c = 0.595, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 6.99 (1H, s, 5-H), 6.51 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.99 (2H, d, J = 5.4 Hz, CH_2O_2), 4.80−4.70 (1H, m, 11a-H), 4.56 (1H, d, J = 4.1 Hz, 1-H), 4.46−4.32 (2H, m, 4-H, NH), 4.31−4.22 (1H, m, 11b-H), 3.89−3.76 (4H, m, CH_2N), 3.67 (6H, s, 3′,5′-OCH_3), 3.46−3.20 (4H, m, CH_2O), 2.97−2.87 (2H, m, 2,3-H), 0.98 (9H, s, tBu), 0.09 (6H, s, 2Me). IR ($CHCl_3$) ν: 3392 (NH), 2930 (aliphatic C−H), 1776 (C=O lactone), 1587, 1506, 1485 (aromatic C=C), 1337, 1132 (SO_2). MS (CI) m/z: 680 $[M + NH_4]^+$. Anal. ($C_{31}H_{42}N_2O_{10}SSi$) C, H, N.

4β-(4-Methylpiperazinyl)sulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7s). Reaction time: 30 h. Yield: 89%. R_f = 0.46 (CH_2Cl_2/acetone 90:10). ^{1}H NMR ($CDCl_3$) δ: 7.01 (1H, s, 5-H), 6.51 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.99 (2H, d, J = 5.4 Hz, CH_2O_2), 4.76−4.72 (1H, m, 11a-H), 4.56 (1H, d, J = 3.5 Hz, 1-H), 4.40−4.24 (3H, m, 11b,4-H, NH), 3.67 (6H, s, 3′,5′-OCH_3), 3.40−3.25 (4H, m, CH_2N), 2.98−2.80 (2H, m, 2,3-H), 2.60−2.50 (4H, m, CH_2N), 2.30 (3H, s, CH_3), 0.98 (9H, s, tBu), 0.10 (6H, s, 2Me). MS (CI) m/z: 676 $[M + H]^+$.

4β-(3-Azidopropyl)sulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7f). To a solution of **7e** (496 mg, 0.758 mmol) in anhydrous DMF (27 mL), under argon atmosphere, was added dry sodium azide (246 mg, 3.79 mmol). The mixture was heated at 60 °C for 60 h. After cooling, water (50 mL) was added and the reaction medium was extracted with ethyl acetate (150 mL, then 5 × 100 mL). Drying of the organic extract ($MgSO_4$), followed by filtration and then evaporation under reduced pressure, gave a crude residue chromatographed on silica gel (90 g, CH_2Cl_2/acetone 90:10). Because of the major formation of the deprotected product **8f**, isolated in 61% yield (253 mg), the title compound **7f** is thus obtained in only 20% yield (100 mg). R_f = 0.56 (CH_2Cl_2/acetone 90:10). Mp = 110−112 °C (white crystals). $[\alpha]^{20}_D$ = −64.0 (c = 0.52, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 6.86 (1H, s, 5-H), 6.53 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 6.00 (2H, d, J = 5.5 Hz, CH_2O_2), 4.85−4.77 (1H, m, 11a-H), 4.57 (1H, d, J = 4.4 Hz, 1-H), 4.44−4.25 (3H, m, 11b,4-H, NH), 3.67 (6H, s, 3′,5′-OCH_3), 3.59 (2H, br t, J = 6.0 Hz, CH_2N_3), 3.36−3.21 (2H, m, CH_2SO_2), 2.98−2.88 (2H, m, 2,3-H), 2.21−2.08 (2H, m, $CH_2\beta$), 0.98 (9H, s, tBu), 0.10 (6H, s, 2Me). IR ($CHCl_3$) ν: 3291 (NH), 2103 (N_3), 2931 (aliphatic C−H), 1775 (C=O lactone), 1587, 1510, 1485 (aromatic C=C), 1335, 1132 (SO_2). MS (CI) m/z: 678 $[M + NH_4]^+$. Anal. ($C_{30}H_{40}N_4O_9SSi$) H, N; C, calcd 54.53; found 54.02.

4β-(4-Aminophenyl)sulfonamido-4′-*tert*-butyldimethylsilyloxy-4′-*O*-demethyl-4-desoxypodophyllotoxin (7k). A solution of **7j** (643 mg, 0.093 mmol) in dioxane (50 mL) was vigorously stirred under hydrogen at atmospheric pressure in the presence of 10% palladium on charcoal (100 mg) for 16 h. Filtration on a pad of Celite and rinsing with MeOH, followed by evaporation of the filtrate, afforded a crude material flash-chromatographed on silica gel (100 g, CH_2Cl_2/acetone 90:10) to provide **7k** in 96% yield. R_f = 0.55 (CH_2Cl_2/acetone 90:10). Mp = 280−282 °C (white powder). $[\alpha]^{20}_D$ = −73.0 (c = 0.550, MeOH). ^{1}H NMR ($CDCl_3$) δ: 7.72 (2H, d, J = 8.7 Hz, Ph), 6.80 (2H, d, J = 8.7 Hz, Ph), 6.44 (1H, s, 8-H), 6.17 (2H, s, 2′,6′-H), 5.90 (2H, s, CH_2O_2), 5.83 (1H, s, 5-H), 4.58−4.50 (1H, m, 1-H), 4.40−4.23 (4H, m, 11,4-H, NH), 3.65 (6H, s, 3′,5′-OCH_3), 3.00−2.80 (2H, m, 2,3-H), 1.58 (2H, br s, NH_2), 0.97 (9H, s, tBu), 0.08 (6H, s, 2Me). IR (KBr) ν: 3482, 3378 (NH, NH_2), 2930 (aliphatic C−H), 1774 (C=O lactone), 1597, 1505, 1485 (aromatic C=C), 1336, 1153 (SO_2). MS (CI) m/z: 686 $[M + NH_4]^+$. Anal. ($C_{33}H_{40}N_2O_9SSi$) H, N; C, calcd 59.26; found 59.70.

General Procedure for the Synthesis of Compounds 8a−f, 8h−j, 8l, and 8n−r. Dowex 50 × 2−200 ion-exchange resin (3 g), previously washed with water and then MeOH, was added to a solution of **7** (0.6 mmol) in MeOH (65 mL). The mixture was vigorously stirred for 16 h. The resin was removed by filtration and thoroughly washed with MeOH. Evaporation of the filtrate in vacuo gave the wanted compound in the reported yields. The compounds were directly recrystallized from acetone/heptane (for **8a−f** and **8h−j**) or benzene/heptane (for **8l** and **8n−r**).

4β-Methylsulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8a). Yield: 94%. R_f = 0.52 (CH_2Cl_2/MeOH 90:10). Mp = 169−171 °C (white powder). $[\alpha]^{20}_D$ = −76.5 (c = 0.225, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 6.95 (1H, s, 5-H), 6.49 (1H, s, 8-H), 6.24 (2H, s, 2′,6′-H), 5.98 (2H, d, J = 9.7 Hz, CH_2O_2), 5.45 (1H, br s, 4′-OH), 4.88−4.72 (2H, m, 11a-H, NH), 4.60−4.50 (1H, m, 1-H), 4.42−4.22 (2H, m, 11b,4-H), 3.75 (6H, s, 3′,5′-OCH_3), 3.13 (3H, s, CH_3SO_2), 2.97−2.84 (2H, m, 2,3-H). IR ($CHCl_3$) ν: 3539 (OH), 3382 (NH), 2935 (aliphatic C−H), 1775 (C=O lactone), 1519, 1506, 1485 (aromatic C=C), 1330, 1155 (SO_2). MS (CI) m/z: 495 $[M + NH_4]^+$. Anal. ($C_{22}H_{23}NO_9S$) C, H, N.

4β-Butylsulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8b). Yield: 92%. R_f = 0.56 (CH_2Cl_2/MeOH 90:10). Mp = 222−224 °C (white powder). $[\alpha]^{20}_D$ = −92.0 (c = 0.485, DMSO). ^{1}H NMR ($CDCl_3$) δ: 6.84 (1H, s, 5-H), 6.52 (1H, s, 8-H), 6.26 (2H, s, 2′,6′-H), 6.00 (2H, d, J = 6.1 Hz, CH_2O_2), 5.41 (1H, br s, 4′-OH), 4.90−4.79 (1H, m, 11a-H), 4.64−4.52 (1H, m, 1-H), 4.46−4.30 (3H, m, 11b,4-H, NH), 3.77 (6H, s, 3′,5′-OCH_3), 3.29−3.10 (2H, s, CH_2SO_2), 2.98−2.87 (2H, m, 2,3-H), 1.98−1.80 (2H, m, $CH_2\beta$), 1.61−1.52 (2H, m, $CH_2\gamma$), 1.00 (3H, t, J = 7.2 Hz, CH_3). IR ($CHCl_3$) ν: 3691 (OH), 3226 (NH), 2965 (aliphatic C−H), 1770 (C=O lactone), 1519, 1506, 1485 (aromatic C=C), 1331, 1116 (SO_2). MS (CI) m/z: 537 $[M + NH_4]^+$. Anal. ($C_{25}H_{29}NO_9S$) C, H, N.

4β-Octylsulfonamido-*O*-demethyl-4-desoxypodophyllotoxin (8c). Yield: 91%. R_f = 0.64 (CH_2Cl_2/MeOH 90:10). Mp = 138−140 °C (white powder). $[\alpha]^{20}_D$ = −59.0 (c = 0.505, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 6.84 (1H, s, 5-H), 6.51 (1H, s, 8-H), 6.25 (2H, s, 2′,6′-H), 5.99 (2H, d, J = 8.6 Hz, CH_2O_2), 5.43 (1H, br s, 4′-OH), 4.84−4.76 (1H, m, 11a-H), 4.72−4.61 (1H, m, 1-H), 4.52−4.37 (3H, m, 11b,4-H, NH), 3.77 (6H, s, 3′,5′-OCH_3), 3.25−3.08 (2H, m, CH_2SO_2), 2.98−2.83 (2H, m, 2,3-H), 1.98−1.80 (2H, m, $CH_2\beta$), 1.65−1.42 (2H, m, $CH_2\gamma$), 1.41−1.20 (8H, m, 4 CH_2), 0.98−0.80 (3H, m, CH_3). IR ($CHCl_3$) ν: 3545 (OH), 3282 (NH), 2929 (aliphatic C−H), 1775 (C=O lactone), 1519, 1507, 1485 (aromatic C=C), 1331, 1117 (SO_2). MS (CI) m/z: 576 $[M + H]^+$. Anal. ($C_{29}H_{37}NO_9S$) C, H, N.

4β-Hexadecylsulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8d). Yield: 96%. R_f = 0.76 (CH_2Cl_2/MeOH 90:10). Mp = 132−133 °C (white powder). $[\alpha]^{20}_D$ = −64.0 (c = 0.560, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 6.84 (1H, s, 5-H), 6.51 (1H, s, 8-H), 6.25 (2H, s, 2′,6′-H), 5.99 (2H, d, J = 9.0 Hz, CH_2O_2), 5.43 (1H, br s, 4′-OH), 4.86−4.80 (1H, m, 11a-H), 4.60−4.55 (1H, m, 1-H), 4.45−4.29 (3H, m, 11b,4-H, NH), 3.77 (6H, s, 3′,5′-OCH_3), 3.22−3.10 (2H, m, CH_2SO_2), 2.96−2.88 (2H, m, 2,3-H), 1.93−1.80 (2H, m, $CH_2\beta$), 1.55−1.42 (2H, m, $CH_2\gamma$), 1.39−1.20 (24H, m, 12 CH_2), 0.88 (3H, t, J = 6.6 Hz, CH_3). IR ($CHCl_3$) ν: 3545 (OH), 3385 (NH), 2927 (aliphatic C−H), 1775

(C=O lactone), 1519, 1507, 1485 (aromatic C=C), 1331, 1117 (SO_2). MS (CI) *m/z*: 688 $[M + H]^+$. Anal. ($C_{37}H_{53}NO_9S$) C, H, N.

4β-(3-Chloropropyl)sulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8e). Yield: 94%. R_f = 1.57 (CH_2Cl_2/MeOH 90:10). Mp = 240−242 °C (white powder). $[\alpha]_D^{20}$ = −87.5 (*c* = 0.490, DMSO). ^{1}H NMR (DMSO-d_6) δ: 8.29 (1H, br s, 4′-OH), 7.87 (1H, d, *J* = 8.6 Hz, NH), 7.03 (1H, s, 5-H), 6.53 (1H, s, 8-H), 6.22 (2H, s, 2′,6′-H), 6.03 (2H, d, *J* = 3.5 Hz, CH_2O_2), 4.85−4.78 (1H, m, 4-H), 4.49 (1H, d, *J* = 5.2 Hz, 1-H), 4.37 (1H, t, *J* = 8.1 Hz, 11a-H), 4.12 (1H, t, *J* = 9.5 Hz, 11b-H), 3.81 (2H, t, *J* = 6.2 Hz, CH_2Cl), 3.64 (6H, s, 3′,5′-OCH_3), 3.49−3.32 (2H, m, CH_2SO_2), 3.23 (1H, dd, *J* = 5.3, 14.3 Hz, 2-H), 3.03−2.89 (1H, m, 3-H), 2.25−2.11 (2H, m, $CH_2\beta$). IR (KBr) ν: 3629 (OH), 3227 (NH), 2907 (aliphatic C−H), 1767 (C=O lactone), 1521, 1507, 1483 (aromatic C=C), 1363, 1116 (SO_2). MS (CI) *m/z*: 557, 559 $[M + NH_4]^+$. Anal. ($C_{24}H_{26}ClNO_9S$) H, N; C, calcd 53.38; found 52.96.

4β-(3-Azidopropyl)sulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8f). Yield: 97%. R_f = 0.61 (CH_2Cl_2/acetone 90:10). Mp = 207−209 °C (pale yellow powder). $[\alpha]^{20}_D$ = −57.0 (*c* = 0.49, MeOH). ^{1}H NMR (DMSO-d_6) δ: 8.29 (1H, br s, 4′-OH), 7.82 (1H, br s, NH), 7.04 (1H, s, 5-H), 6.52 (1H, s, 8-H), 6.22 (2H, s, 2′,6′-H), 6.02 (2H, d, *J* = 7.2 Hz, CH_2O_2), 4.81−4.75 (1H, m, 4-H), 4.49 (1H, d, *J* = 5.3 Hz, 1-H), 4.37 (1H, t, *J* = 7.8 Hz, 11a-H), 4.12 (1H, t, *J* = 9.0 Hz, 11b-H), 3.64 (6H, s, 3′,5′-OCH_3), 3.54 (2H, t, *J* = 6.7 Hz, CH_2N_3), 3.41−3.18 (3H, m, 2-H, CH_2SO_2), 3.02−2.88 (1H, m, 3-H), 2.01−1.90 (2H, m, $CH_2\beta$). IR (KBr) ν: 3585 (OH), 3502 (NH), 3303 (aliphatic C−H), 2105 (N_3), 1768 (C=O lactone), 1522, 1505, 1482 (aromatic C=C), 1330, 1154 (SO_2). MS (CI) *m/z*: 564 [M + $NH_4]^+$. Anal. ($C_{24}H_{26}N_4O_9S$) C, H, N.

4β-(4-Methylsulfonamidophenyl)sulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8h). Yield: 87%. R_f = 0.64 (CH_2Cl_2/MeOH 90:10). Mp = 310−311 °C. $[\alpha]^{20}_D$ = −111.0 (*c* = 0.385, DMSO). ^{1}H NMR (DMSO-d_6) δ: 8.65 (1H, br s, 4′-OH), 8.31 (1H, br s, NH), 8.24 (2H, d, *J* = 8.4 Hz, Ph), 8.16 (2H, d, *J* = 8.4 Hz, Ph), 6.48 (1H, s, 8-H), 6.18 (2H, s, 2′,6′-H), 5.95 (2H, s, CH_2O_2), 5.88 (1H, s, 5-H), 4.77 (1H, d, *J* = 4.4 Hz, 4-H), 4.47 (1H, d, *J* = 5.2 Hz, 1-H), 4.09 (1H, t, *J* = 8.0 Hz, 11a-H), 3.85 (1H, t, *J* = 9.1 Hz, 11b-H), 3.62 (6H, s, 3′,5′-OCH_3), 3.34 (3H, s, CH_3), 3.16 (1H, dd, *J* = 5.2, 14.3 Hz, 2-H), 3.00−2.88 (1H, m, 3-H). IR (KBr) ν: 3474 (OH), 3261 (NH), 2922 (aliphatic C−H), 1775 (C=O lactone), 1519, 1508, 1485 (aromatic C=C), 1329 (SO_2). MS (CI) *m/z*: 635 $[M + NH_4]^+$. Anal. ($C_{28}H_{27}NO_{11}S_2$) C, H, N.

4β-Pentafluorophenylsulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8i). Yield: 93%. R_f = 0.59 (CH_2Cl_2/MeOH 90:10). Mp = 303−305 °C (white powder). $[\alpha]^{20}_D$ = −61.5 (*c* = 0.615, MeOH). ^{1}H NMR ($CDCl_3$) δ: 6.48 (1H, s, 5-H), 6.30 (1H, s, 8-H), 6.23 (2H, s, 2′,6′-H), 5.94 (2H, d, *J* = 1.2 Hz, CH_2O_2), 5.54 (1H, br s, 4′-OH), 4.87 (1H, d, *J* = 4.7 Hz, 4-H), 4.56 (1H, d, *J* = 5.2 Hz, 1-H), 4.29 (1H, t, *J* = 8.6 Hz, 11a-H), 4.14 (1H, t, *J* = 9.0 Hz, 11b-H), 3.75 (6H, s, 3′,5′-OCH_3), 3.33 (1H, dd, *J* = 5.2, 14.3 Hz, 2-H), 3.00−2.85 (1H, m, 3-H). IR (KBr) ν: 3504 (OH), 3227 (NH), 2942 (aliphatic C−H), 1774 (C=O lactone), 1519, 1505, 1434 (aromatic C=C), 1342, 1112 (SO_2). MS (CI) *m/z*: 647 $[M + NH_4]^+$. Anal. ($C_{27}H_{20}F_5NO_9S$) H, N; C, calcd 51.51; found 51.09.

4β-(4-Azidophenyl)sulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8j). Yield: 92%. R_f = 0.69 (CH_2Cl_2/MeOH 90:10). Mp = 173−175 °C (pale yellow powder). $[\alpha]^{20}_D$ = −87.5 (*c* = 0.360, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.94 (2H, d, *J* = 8.7 Hz, Ph), 7.25 (2H, d, *J* = 8.7 Hz, Ph), 6.45 (1H, s, 8-H), 6.21 (2H, s, 2′,6′-H), 5.91 (2H, s, CH_2O_2), 5.73 (1H, s, 5-H), 5.40 (1H, s, 4′-OH), 4.60−4.51 (3H, m, 1,4-H, NH), 4.30 (2H, d, *J* = 8.1, 11-H), 3.75 (6H, s, 3′,5′-OCH_3), 2.92−2.81 (2H, m, 2,3-H). IR ($CHCl_3$) ν: 3538 (OH), 3369 (NH), 2919 (aliphatic C−H), 2133 (N_3), 1774 (C=O lactone), 1519, 1506, 1486 (aromatic C=C), 1332, 1165 (SO_2). MS (CI) *m/z*: 598 [M + $NH_4]^+$. Anal. ($C_{27}H_{24}N_4O_9S$) C, H, N.

4β-(Thiophen-2-yl)sulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8l). Yield: 95%. R_f = 0.65 (CH_2Cl_2/MeOH 90:10). Mp = 276−277 °C (white powder). $[\alpha]^{20}_D$ = −54.5 (*c* = 0.570, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.78−7.76 (2H, m, arom), 7.28−7.25 (1H, m, arom), 6.46 (1H, s, 8-H), 6.23 (2H, s, 2′,6′-H), 5.93 (2H, d, *J* = 2.2 Hz, CH_2O_2), 5.63 (1H, s, 5-H), 5.41 (1H, s, 4′-OH), 4.70−4.59 (2H, m, 4-H, NH), 4.54 (1H, d, *J* = 4.2 Hz, 1-H), 4.36 (2H, d, *J* = 7.8 Hz, 11-H), 3.76 (6H, s, 3′,5′-OCH_3), 3.00−2.85 (2H, m, 2,3-H). IR ($CHCl_3$) ν: 3544 (OH), 3362 (NH), 2928 (aliphatic C−H), 1774 (C=O lactone), 1519, 1507, 1485 (aromatic C=C), 1332, 1160 (SO_2). MS (CI) *m/z*: 563 $[M + NH_4]^+$. Anal. ($C_{25}H_{23}NO_9S_2$) C, H, N.

4β-Dimethylaminosulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8n). Yield: 96%. R_f = 0.64 (CH_2Cl_2/MeOH 90:10). Mp = 228−230 °C (white powder). $[\alpha]^{20}_D$ = −77.5 (*c* = 0.390, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 6.98 (1H, s, 5-H), 6.50 (1H, s, 8-H), 6.26 (2H, s, 2′,6′-H), 5.99 (2H, d, *J* = 7.1 Hz, CH_2O_2), 5.41 (1H, s, 4′-OH), 4.80−4.70 (1H, m, 11a-H), 4.60−4.55 (1H, m, 1-H), 4.45−4.31 (2H, m, 4-H, NH), 4.24 (1H, d, *J* = 7.0 Hz, 11b-H), 3.77 (6H, s, 3′,5′-OCH_3), 2.98−2.77 (8H, m, 2,3-H + $2CH_3$). IR ($CHCl_3$) ν: 3539 (OH), 3391 (NH), 2941 (aliphatic C−H), 1775 (C=O lactone), 1519, 1506, 1482 (aromatic C=C), 1331, 1117 (SO_2). MS (CI) *m/z*: 524 $[M + NH_4]^+$. Anal. ($C_{23}H_{26}N_2O_9S$) C, H, N.

4β-Diethylaminosulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8o). Yield: 95%. R_f = 0.66 (CH_2Cl_2/MeOH 90:10). Mp = 129−131 °C (white powder). $[\alpha]^{20}_D$ = −73.0 (*c* = 0.480, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.02 (1H, s, 5-H), 6.49 (1H, s, 8-H), 6.26 (2H, s, 2′,6′-H), 5.98 (2H, d, *J* = 6.1 Hz, CH_2O_2), 5.41 (1H, s, 4′-OH), 4.77−4.68 (1H, m,11a-H), 4.61−4.52 (1H, m, 1-H), 4.47−4.30 (2H, m, 4-H, NH), 4.00 (1H, d, *J* = 5.7 Hz, 11b-H), 3.77 (6H, s, 3′,5′-OCH_3), 3.35 (4H, q, *J* = 7.0 Hz, 2 CH_2), 2.98−2.82 (2H, m, 2,3-H), 1.24 (6H, t, *J* = 6.9 Hz, CH_3). IR ($CHCl_3$) ν: 3541 (OH), 3392 (NH), 2940 (aliphatic C−H), 1775 (C=O lactone), 1519, 1506, 1485 (aromatic C=C), 1330, 1150 (SO_2). MS (CI) *m/z*: 552 $[M + NH_4]^+$. Anal. ($C_{25}H_{30}N_2O_9S$) C, H, N.

4β-Dibutylaminosulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8p). Yield: 93%. R_f = 0.76 (CH_2Cl_2/MeOH 90:10). Mp = 98−100 °C (white crystals). $[\alpha]^{20}_D$ = −53.5 (*c* = 0.510, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.03 (1H, s, 5-H), 6.50 (1H, s, 8-H), 6.26 (2H, s, 2′,6′-H), 5.98 (2H, d, *J* = 5.4 Hz, CH_2O_2), 5.42 (1H, s, 4′-OH), 4.79−4.70 (1H, m, 11a-H), 4.60−4.51 (1H, m, 1-H), 4.46−4.30 (2H, m, 4-H, NH), 3.91 (1H, d, *J* = 6.0 Hz, 11b-H), 3.77 (6H, s, 3′,5′-OCH_3), 3.30−3.21 (4H, m, 2 $CH_2\beta$), 2.94−2.83 (1H, m, 2,3-H), 1.70−1.50 (4H, m, $CH_2\gamma$), 1.42−1.23 (9H, m, CH_3), 1.02−0.88 (6H, m, $2CH_3$). IR ($CHCl_3$) ν: 3538 (OH), 3339 (NH), 2964 (aliphatic C−H), 1775 (C=O lactone), 1519, 1506, 1485 (aromatic C=C), 1330, 1117 (SO_2). MS (CI) *m/z*: 608 $[M + NH_4]^+$. Anal. ($C_{29}H_{38}N_2O_9S$) C, H, N.

4β-Piperidinosulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8q). Yield: 87%. R_f = 0.61 (CH_2Cl_2/MeOH 90:10). Mp = 249−250 °C (white powder). $[\alpha]^{20}_D$ = −69.5 (*c* = 0.480, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.03 (1H, s, 5-H), 6.49 (1H, s, 8-H), 6.26 (2H, s, 2′,6′-H), 5.98 (2H, d, *J* = 5.2 Hz, CH_2O_2), 5.41 (1H, s, 4′-OH), 4.76−4.70 (1H, m, 11a-H), 4.60−4.52 (1H, m, 1-H), 4.42−4.33 (2H, m, 4-H, NH), 4.21 (1H, d, *J* = 6.8 Hz, 11b-H), 3.77 (6H, s, 3′,5′-OCH_3), 3.31−3.18 (4H, m, 2 CH_2N), 2.93−2.84 (2H, m, 2,3-H), 1.80−1.69 (4H, m, 2 CH_2), 1.60−1.55 (2H, m, CH_2). IR ($CHCl_3$) ν: 3541 (OH), 3392 (NH), 2945 (aliphatic C−H), 1775 (C=O lactone), 1519, 1506, 1485 (aromatic C=C), 1331, 1159 (SO_2). MS (CI) *m/z*: 564 $[M + NH_4]^+$. Anal. ($C_{26}H_{30}N_2O_9S$) C, H, N.

4β-Morpholinosulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8r). Yield: 98%. R_f = 0.59 (CH_2Cl_2/MeOH 90:10). Mp = 248−250 °C (white crystals). $[\alpha]^{20}_D$ = −68.5 (*c* = 0.470, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.00 (1H, s, 5-H), 6.50 (1H, s, 8-H), 6.25 (2H, s, 2′,6′-H), 5.99 (2H, d, *J* = 6.3 Hz, CH_2O_2), 5.42 (1H, s, 4′-OH), 4.80−4.70 (1H, m, 11a-H), 4.57 (1H, d, *J* = 3.7 Hz, 1-H), 4.46−4.29 (3H, m, 11b,4-H, NH), 3.83 (4H, t, *J* = 4.6 Hz, $2CH_2O$), 3.77 (6H, s, 3′,5′-OCH_3), 3.37−3.22 (4H, m, $2CH_2N$), 2.98−2.90 (2H, m, 2,3-H). IR ($CHCl_3$): 3526 (OH), 3386 (NH), 2920 (aliphatic C−H), 1776 (C=O lactone), 1519, 1506, 1485 (aromatic C=C), 1330, 1117 (SO_2). MS (CI) *m/z*: 566 $[M + NH_4]^+$. Anal. ($C_{25}H_{28}N_2O_{10}S$) C, H, N.

4β-(2-Acetamido-4-methylthiazol-5-yl)sulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8m). To a solution

of **7m** (439 mg, 0.6 mmol) in anhydrous THF (10 mL) and under inert atmosphere was added Bu_4NF (1.6 mL of a 1 N solution in THF, 1.6 mmol). The reaction mixture was stirred overnight at room temperature. THF was removed in vacuo and CH_2Cl_2 (25 mL) was added. The solution was then washed with water (10 mL). After drying ($MgSO_4$) and filtration, the solvent was evaporated. The obtained crude material was purified by flash column chromatography on silica gel (100 g, eluent CH_2Cl_2/MeOH 90:10) to give pure **8m** (303 mg, 82%). $R_f = 0.58$ (CH_2Cl_2/MeOH 90:10). Mp = 202−204 °C (pale yellow crystals from acetone/heptane). $[\alpha]^{20}_D = -133.5$ ($c = 0.115$, MeOH). ^{1}H NMR (DMSO-d_6) δ: 8.55 (1H, br s, NH), 8.27 (1H, s, 4′-OH), 6.50 (1H, s, 8-H), 6.22 (2H, s, 2′,6′-H), 5.98 (2H, d, $J = 7.4$ Hz, CH_2O_2), 4.70 (1H, d, $J = 4.1$ Hz, 4-H), 4.47 (1H, d, $J = 5.2$ Hz, 1-H), 4.17 (1H, t, $J = 8.1$ Hz, 11a-H), 3.95 (1H, t, $J = 10.1$ Hz, 11b-H), 3.63 (6H, s, 3′,5′-OCH_3), 3.11 (1H, dd, $J = 5.3$, 14.4 Hz, 2-H), 3.08−2.92 (1H, m, 3-H), 2.47 (3H, s, CH_3), 2.21 (3H, s, CH_3). IR (KBr) ν: 3626 (OH), 3271 (NH), 2990 (aliphatic C−H), 1772 (C═O lactone), 1520, 1505, 1485 (aromatic C═C), 1375, 1154 (SO_2). MS (CI) m/z: 618 $[M + H]^+$. Anal. ($C_{27}H_{27}N_3O_{10}S_2$) C, H, N.

4β-(4-Methylpiperazinyl)sulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8s). Compound **8s** was prepared on a 0.5 mmol scale from **7s** in an analogous manner to **8m** from **7m**. Yield: 84%. $R_f = 0.37$ (CH_2Cl_2/MeOH 90:10). Mp = 223−224 °C (white powder from benzene/heptane). $[\alpha]^{20}_D = -75.5$ ($c = 0.280$, DMSO). ^{1}H NMR (DMSO-d_6) δ: 8.24 (1H, s, 4′-OH), 7.86 (1H, d, $J = 8.6$ Hz, NH), 6.97 (1H, s, 5-H), 6.49 (1H, s, 8-H), 6.19 (2H, s, 2′,6′-H), 6.00 (2H, d, $J = 1.3$ Hz, CH_2O_2), 4.71−4.61 (1H, m, 4-H), 4.44 (1H, d, $J = 5.4$ Hz, 1-H), 4.30 (1H, t, $J = 7.9$ Hz, 11a-H), 4.12 (1H, t, $J = 10.4$ Hz, 11b-H), 3.61 (6H, s, 3′,5′-OCH_3), 3.22 (2H, dd, $J = 5.3$, 14.6 Hz, 2-H), 3.18−2.89 (9H, m, 3-H, 4 CH_2), 2.16 (3H, s, CH_3). IR (KBr) ν: 3567 (OH), 3342 (NH), 2937 (aliphatic C−H), 1769 (C═O lactone), 1520, 1508, 1482 (aromatic C═C), 1337, 1151 (SO_2). MS (CI) m/z: 579 $[M + NH_4]^+$. Anal. ($C_{26}H_{31}N_3O_9S$) C, H, N.

4β-(3-Aminopropyl)sulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8g). Compound **8g** was prepared on a 0.4 mmol scale from **8f** in an analogous manner to **7k** from **7j**: yield 86%. $R_f = 0.73$ (CH_2Cl_2/MeOH 90:10). Mp = 204−206 °C (white powder from benzene/heptane). $[\alpha]^{20}_D = -92.0$ ($c = 0.39$, DMSO). ^{1}H NMR (DMSO-d_6) δ: 9.06 (1H, d, $J = 8.3$ Hz, NH), 6.82 (2H, s, NH_2), 6.79 (1H, s, 5-H), 6.82 (2H, s, NH_2), 6.77 (1H, s, 4′-OH), 6.55 (1H, s, 8-H), 6.26 (2H, s, 2′,6′-H), 6.02 (2H, d, $J = 5.7$ Hz, CH_2O_2), 5.38−5.22 (1H, m, 4-H), 4.51 (1H, d, $J = 4.7$ Hz, 1-H), 4.30 (1H, t, $J = 7.8$ Hz, 11a-H), 4.13 (1H, t, $J = 10.5$ Hz, 11b-H), 3.65 (6H, s, 3′,5′-OCH_3), 3.16 (1H, dd, $J = 5.1$, 14.4 Hz, 2-H), 3.18−2.98 (1H, s, 3-H), (2H, m, CH_2-SO_2) (The signals of $CH_2\alpha$, -β, -γ are masked by the presence of a broad signal of H_2O in the spectra). IR (KBr) ν: 3654 (OH), 3345, 3292 (NH, NH_2), 2935 (aliphatic C−H), 1768 (C═O lactone), 1523, 1505, 1482 (aromatic C═C), 1338, 1117 (SO_2). MS (CI) m/z: 521 $[M + H]^+$, 538 $[M + NH_4]^+$. Anal. ($C_{24}H_{28}N_2O_9S$) C, H, N.

4β-(4-Aminophenyl)sulfonamido-4′-*O*-demethyl-4-desoxypodophyllotoxin (8k). Compound **8k** was prepared from **8j** in an analogous manner to **7k** from **7j**: Yield: 95%. $R_f = 0.49$ (CH_2Cl_2/MeOH 90:10). Mp = 286−288 °C (pale yellow crystals from benzene/heptane). $[\alpha]^{20}_D = -73.5$ ($c = 0.385$, DMSO). ^{1}H NMR (DMSO-d_6) δ: 8.23 (1H, br s, 4′-OH), 7.74 (1H, d, $J = 7.6$ Hz, NH), 7.50 (2H, d, $J = 7.8$ Hz, Ph), 6.66 (2H, d, $J = 8.1$ Hz, Ph), 6.41 (1H, s, 8-H), 6.16 (2H, s, 2′,6′-H), 6.04 (2H, br s, NH_2), 5.98 (1H, s, 5-H), 5.91 (2H, d, $J = 4.2$ Hz, CH_2O_2), 4.59−4.48 (1H, m, 4-H), 4.39 (1H, d, $J = 4.9$ Hz, 1-H), 4.00 (1H, t, $J = 8.0$ Hz, 11a-H), 3.91 (1H, t, $J = 9.1$ Hz, 11b-H), 3.58 (6H, s, 3′,5′-OCH_3), 3.09 (1H, dd, $J = 4.3$, 14.3 Hz, 2-H), 2.96−2.73 (1H, m, 3-H). IR (KBr): 3649 (OH), 3392, 3355 (NH, NH_2), 2940 (aliphatic C−H), 1770 (C═O lactone), 1521, 1507, 1486 (aromatic C═C), 1332, 1151 (SO_2). MS (CI) m/z: 572 $[M + NH_4]^+$. Anal. ($C_{27}H_{26}N_2O_9S$) C, H, N.

DNA and Biochemicals. The plasmid pBS was obtained from Stratagene. Purified human topoisomerase IIα was purchased from TopoGEN Inc. and etoposide from Sigma Chemicals. Compound were dissolved in dimethyl sulfoxide at 5 mM and then diluted to working concentrations in distilled water immediately before use.

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 the drug at 50 μM (total reaction volume 10 μL). Two units of human DNA topoisomerase II were added to the duplex, preincubated as described, and incubated for 30 min at 30 °C. The DNA-topoisomerase II cleavage complexes were dissociated by addition of SDS (final concentration 0.5%) and of proteinase K (Sigma) to 500 μg/mL, followed by incubation for 30 min at 55 °C. DNA samples were then added to the electrophoresis dye mixture (5 μL) and 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 and photographed under UV light.

Cell Culture and Cytotoxicity Assays. Cells were cultivated in RPMI 1640 medium (Gibco) 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.

Briefly, adherent cells were seeded in 96 well-microplates and incubated for 2 days. Tested compounds were then added and plates incubated for four doubling times. The nonadherent L1210 cells were directly incubated for 48 h with the compounds. At the end of this period, 15 μL of 5 mg/mL 3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) was 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.[29,30] 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), 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 an Epics XL/MCL flow cytometer (Beckman Coulter).

Antitumor Activity in Vivo. Murine P388 leukemia tumor model was used as previously described.[31] B6D2F1 mice were inoculated ip with 10^6 tumor cells, and drugs were administered iv the first day of the experiment.

A549 human pulmonary tumor cells were cultured and grafted into immunodeficient mice as previously described.[32] Briefly, 10^6 cells in a volume of 100 L 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.

All experiments were approved by an internal ethical committee and in accordance with the guidelines approved by the UKCCCR for the welfare of animals in experimental neoplasia.[33] The evaluation of the antitumor activity was represented by 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.

Tubuline Test. Tubulin polymerization inhibition was determined as previously reported.[34]

Acknowledgment. This work was supported by grants from Fondation de France (to D.G.) and from Ligue National contre le Cancer and Ministère de la Recherche et de l'Industrie (ACI "Molécules et Cibles Thérapeutiques") (to P.B.A.).

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JM031117B