Antitumor Agents. 144.§ New γ-Lactone Ring-Modified Arylamino Etoposide Analogs as Inhibitors of Human DNA Topoisomerase II

Xiao-Ming Zhou,[†] Kenneth Jiann-Hung Lee,[†,∥] Ji Cheng,[†] Shihn-Sheng Wu,[†] Hong-Xing Chen,[‡] Xin Guo,[‡] Yung-Chi Cheng,[‡] and Kuo-Hsiung Lee[*,†]

Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, and Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510

The *trans*-fused γ-lactone ring of etoposide is readily epimerized to its *cis* epimer, which is biologically inactive, or is metabolized to the inactive ring-opened hydroxy acids. Modification of this γ-lactone ring of 4β-(arylamino)-4′-O-demethyl-4-desoxypodophyllotoxin resulted in several compounds (15–16, 21–22, and 24) that should block this epimerization and the resulting biological deactivation. In a topoisomerase II inhibition assay, compounds 21, 22, and 24 showed comparable activity to etoposide. In a protein-linked DNA complex formation assay, compounds 21 and 22 were more active than etoposide.

Introduction

Etoposide (VP-16, 1) is an important drug used in the treatment of small-cell lung cancer, testicular carcinoma, leukemia, lymphoma, and Kaposi's sarcoma.[2,3] Although 1 is widely used in the clinic, several problems hinder its clinical efficacy.[4,5] Metabolic deactivation is one of these problems. The major metabolites of 1 are the corresponding *trans*- and *cis*-hydroxy acids (2 and 3, respectively), formed by hydrolysis of the lactone ring, and the *cis*-picro lactone isomer (4), formed by epimerization at the lactone ring (Figure 1). Picroetoposide (4) is found in plasma, serum, urine, cerebrospinal fluid, and liver.[6] The hydroxy acids (2 and 3) are found in urine, bile, and tissue following administration of etoposide.[7] None of these metabolites exhibit biological activity *in vitro*.[8] Furthermore, compound 1 is susceptible to nonenzymatic epimerization by even weak bases. Conversion of the *trans*-lactone ring to the thermodynamically stable *cis*-lactone probably occurs via the formation of an enol at C-2 by proton abstraction, followed by an inversion of the configuration at C-2.[6,9] Synthesis of a derivative of 1 that cannot be so readily inactivated could provide a better therapeutic agent.

The 4β-arylamino derivatives NPF (5) and W-68 (6) (Figure 2) are 40-fold more cytotoxic than 1 against P-388/adriamycin resistant cells (P-388/adr), which have a decreased content of topoisomerase II, whereas 5 exhibits almost equivalent activity to 1 against nonresistant P-388 cells. In addition, compound 5 is at least 7 times less toxic *in vivo* compared with 1.[10] Therefore, we have synthesized and evaluated the biological activity of 4β-(4″-arylamino)-4′-demethylpodophyllotoxin γ-cyclic acetal, γ-cyclic ether, and γ-lactol 15, 16, 21, 22, and 24. The structures of these compounds were determined from ¹H NMR (1D, 2D) and IR spectral data, as well as elemental analyses.
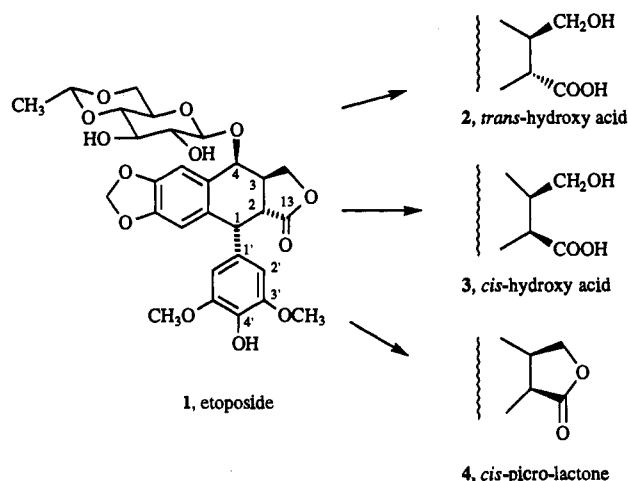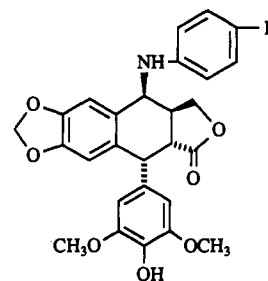

Figure 1. Inactive metabolites of etoposide.

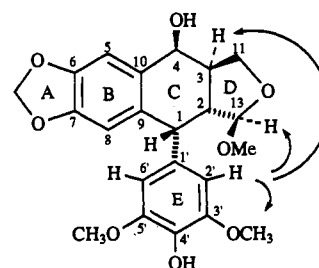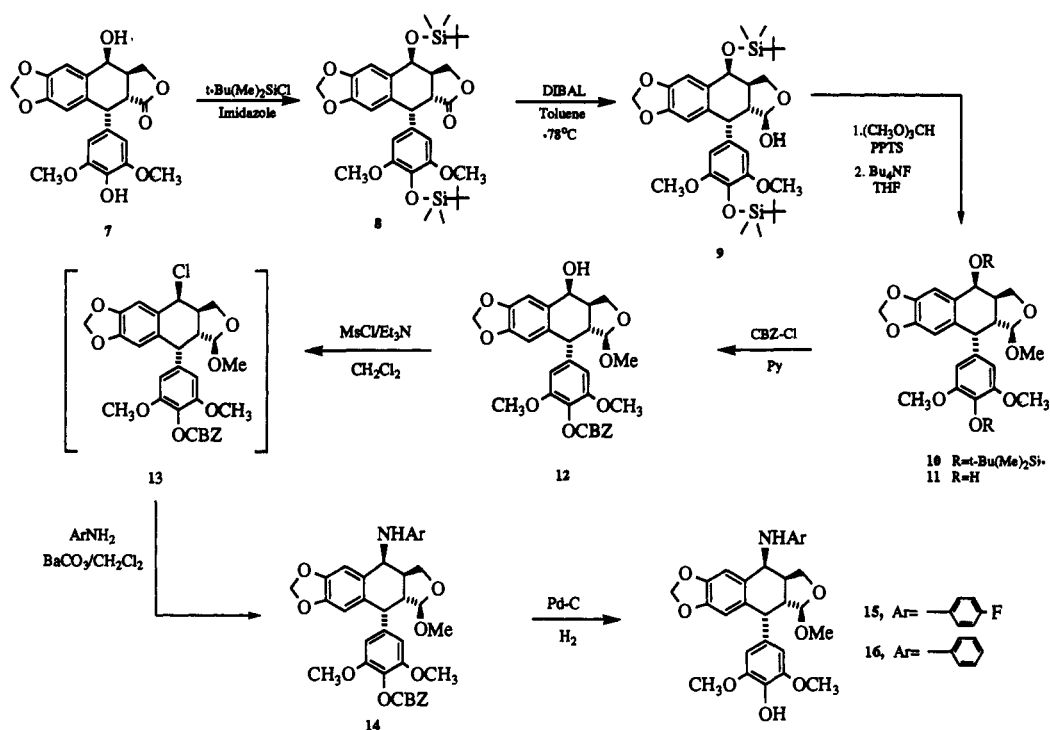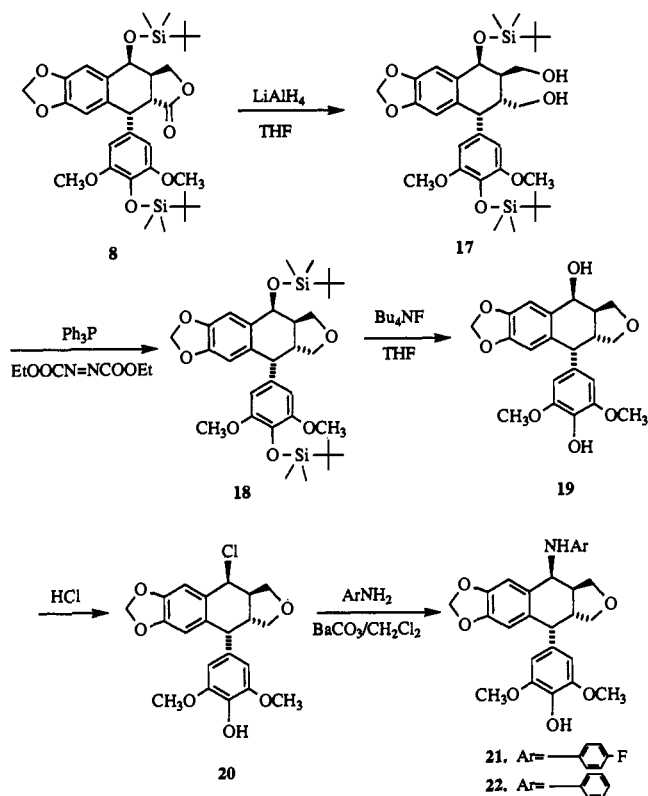Figure 2. NPF (5), R = F; W-68 (6), R = NO₂.

Figure 3. NOE correlation of 11.

Chemistry

a. Synthesis of γ-Cyclic Acetal Etoposide Analogs 15 and 16. As shown in Scheme 1, 4β-(4″-arylamino)-

Scheme 1

Scheme 2

4'-demethylpodophyllotoxin γ-cyclic acetals 15 and 16 were synthesized from 7. Treatment of 7 with dimethyl-*tert*-butylsilyl chloride (DMTBSiCl) and imidazole afforded 8, in which the 4,4'-hydroxyl groups are blocked.[11] Reduction of 8 with diisobutylaluminum hydride (DIBAL) produced lactol 9, which under mildly acidic conditions with trimethyl orthoformate formed methyl acetal 10. The silyl protecting group was removed using tetra-*n*-butyl-ammonium fluoride (Bu4NF) to give 11.[11] The phenol

was selectively protected by treatment of 11 with benzyl chloroformate (CBZ-Cl) to produce 12. Reaction of 12 with methanesulfonyl chloride gave intermediate 13, which was immediately treated with the appropriate arylamine and BaCO3 to produce 14. Removal of the phenolic protecting group by catalytic hydrogenation furnished 15 or 16.[12]

The configuration at C-13 was determined by a NOESY spectrum of 11. A crosspeak between H-13 and H-2',6' was observed. In order to confirm that this peak is due to an NOE effect, a difference NOE experiment was performed. With irradiation at δ 6.14 (H-2',6'), NOE peaks appear at δ 4.40 (d, H-13 NOE peak) and 2.45 (m, H-3, NOE peak). The observation of NOE between H-13 and H-2',6' suggests that the methoxy group at C-13 is β. The observation of NOE between H-3 and H-2',6' suggests that the E ring is freely rotating. The NOE correlation of H-2',6' in 11 is shown in Figure 3.

The *trans*-lactone of 4'-demethylpodophyllotoxin can be epimerized to an inactive *cis*-lactone under even mildly basic conditions. To assure that epimerization of 9 at C-2 did not occur prior to formation of the methyl acetal, the *trans* relationship of the protons at C-2 and C-3 of ring D had to be established. The coupling patterns of H-2 and H-3 in 11 were complex. However, after the H-13 proton was decoupled from H-2, the coupling pattern of H-2 was simplified to a double doublet (Figure 4). $J_{2,3}$ in 11 was calculated to be 14 Hz, thus indicating the *trans* relationship of H-2 and H-3.

b. Synthesis of the Cyclic Ether Etoposide Analogs 21 and 22. As shown in Scheme 2, 18 was synthesized by reduction of 8 with lithium aluminum hydride (LiAlH4), followed by closure of the resulting diol 17 to a cyclic ether by a mixture of triphenylphosphine (TPP) and diethyl azodicarboxylate (DEAD).[13] Compound 18 was deprotected with Bu4NF to produce the cyclic ether 19. Compound 20 was obtained via chlorination of 19 and, due to its high reactivity, was directly treated with the appropriate arylamine to give 21 or 22.
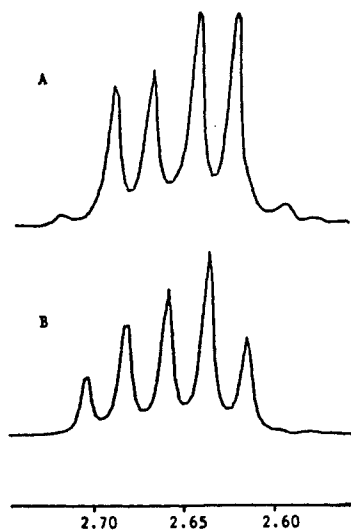
Figure 4. Sections of the 300-MHz ^{1}H NMR spectra of compound 11. A: H-2 has been decoupled from H-13 by irradiation at δ 4.42. B: The resonance for H-2.
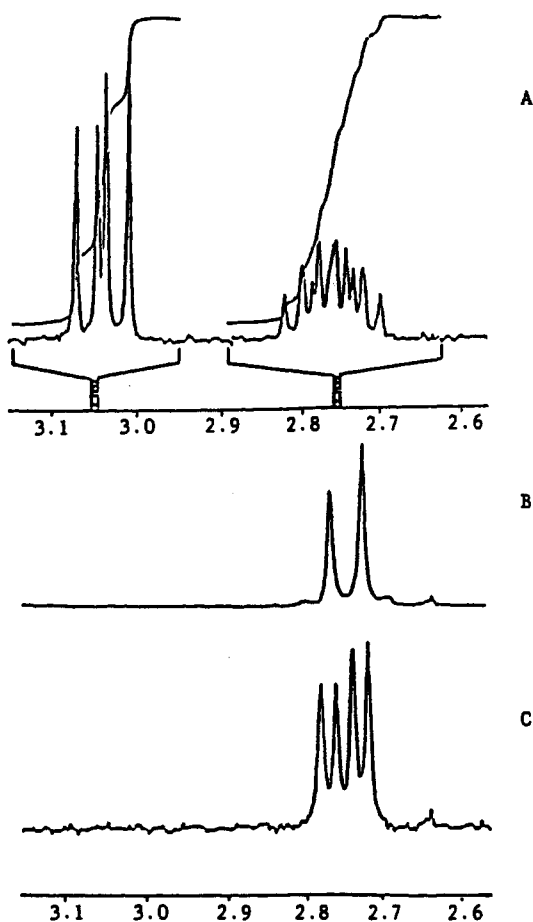

Figure 5. Sections of the 300-MHz ^{1}H NMR spectra of compound 19. A: Nondeuterated 19. B: In deuterated 19, the H-1 proton has been decoupled from H-2. C: Deuterated 19.

In order to determine the *trans* fusion of ring D and to assign resonances for the H-11 and H-13 protons, a parallel synthesis was carried out using LiAlD$_4$. In the ^{1}H NMR spectrum of deuterated 19, the H-13 proton resonances disappeared, and the coupling pattern of H-2 was simplified to a double doublet. $J_{2,3}$ in deuterated 19 was observed directly after irradiation of H-1 (Figure 5). $J_{2,3}$ in 19 is 14 Hz, which is consistent with a *trans* fusion of ring D.

Scheme 3
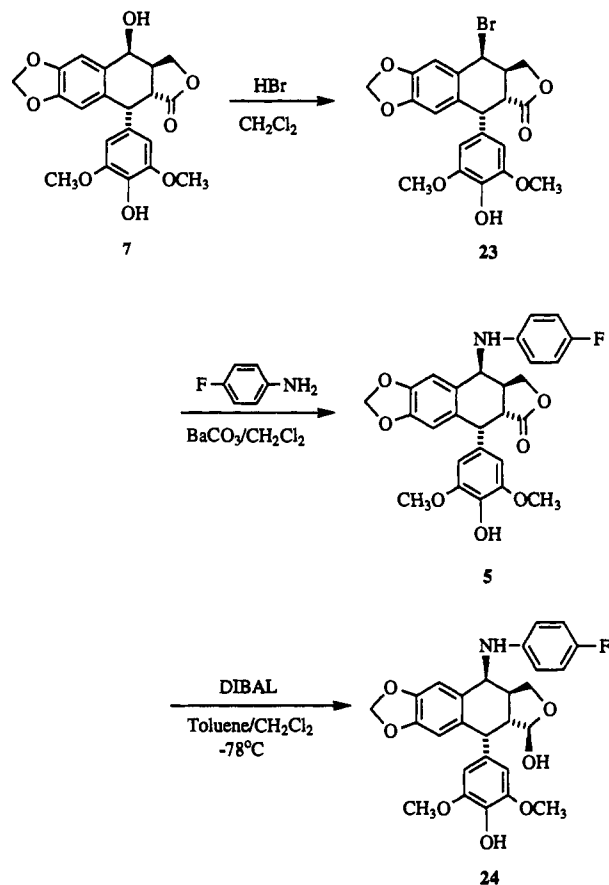
c. Synthesis of the Lactol Etoposide Analog 24. As shown in Scheme 3, 24 was synthesized by reduction of 5 with DIBAL at -78 °C. As described previously, 5 was prepared by nucleophilic substitution of 23 with the appropriate arylamine; 23 was obtained by bromination of 7.[14] The configuration of C-13 in 24 was determined by a difference NOE experiment. With irradiation at δ 6.14 (H-2',6'), an NOE peak appears at δ 4.85 (d, H-13). This NOE peak suggests that the hydroxy group at C-13 is β.

Biological Results and Discussion

Table 1 shows the activities of Topo II inhibition, protein-linked DNA complex formation, and cytotoxicity toward the KB-ATCC cell line for the γ-lactone ring-modified arylamino etoposide analogs. In the Topo II inhibition assay, compounds 21, 22, and 24 show comparable activity to etoposide (1), while compounds 15 and 16 are less active. In the protein-linked DNA complex formation assay, only compounds 21 and 22 are more active than 1; 15, 16, and 24 are less active. In the KB-ATCC cell toxicity assay, all compounds have higher ID$_{50}$ values compared with 1. Thus, there is a lack of correlation between the ability of the compounds to cause protein-linked DNA breaks and their cytotoxicity. This suggests that, in addition to etoposide's inhibition of the catalytic activity of Topo II, other mechanisms of action may also be involved in the cytotoxicity observed with this class of compound. Within this series of compounds, the γ-cyclic etoposide analogs (21 and 22) are the most active; however, they are less active than their corresponding lactone congener (5). Therefore, while the lactone moiety is not an absolute requirement for antitumor activity, its alteration reduces the activity of the parent drug. Evaluation

Table 1. Biological Evaluation of D-Ring-Modified Arylamino Etoposide Analogs

compound	R or R'	cytotoxicity[a] ID$_{50}$ KB (μM)			inhibition of DNA topoisomerase II activity[b] ID$_{50}$ (μM)			cellular protein–DNA complex formation[c] (%), 20 μM		
		A	B	C	A	B	C	A	B	C
1	(sugar)	0.20			50			100		
5	p-HN-C$_6$H$_4$-F	0.24			5			213		
15 and 16	OCH$_3$		5.5	5.5		>100	100		66	96
21 and 22	H		4.1	2.6		50	50		125	139
24	OH		2.2			50			87	

[a] ID$_{50}$ is the concentration of drug that affords 50% reduction in cell number after a 3-day incubation.[15] [b] Each compound was examined in triplicate at concentrations of 5, 10, 25, 50, and 100 μM. The ID$_{50}$ value was established based on the degree of inhibition at these concentrations.[16] [c] The method is described in ref 17.

of the γ-cyclic ethers (21 and 22) in an *in vivo* assay will be important to determine a rationale.

Experimental Section

General Experimental Procedures. All melting points were taken on a Fischer-Johns melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1320 spectrophotometer, and ¹H NMR spectra were obtained using a Bruker AC-300 NMR spectrometer. All chemical shifts are reported in ppm from TMS. Elemental analyses were performed by Atlantic MicroLab, Inc., Norcross, GA. Optical rotations were measured with a Rudolph Research Autopol III polarimeter. Analytical thin-layer chromatography (TLC) was carried out on Merck precoated silica gel F-254 plates. EM Kieselgel 60 (230–400-mesh ASTM) was used for column chromatography. All new target compounds were characterized by melting point, optical rotation, ¹H NMR and IR spectral analyses, and elemental analyses.

4,4′-Bis-*O*-(dimethyl-*tert*-butylsilyl)-4′-*O*-demethylepipodophyllotoxin (8). To 1.0 g (2.mmol) of 4′-*O*-demethylepipodophyllotoxin (7) and 1.8 g (26 mmol) of imidazole in 4 mL of DMF was added 2.0 g (13.3 mmol) of DMTBSiCl. The solution was stirred for 20 h and then poured into 200 mL water. The solution was filtered, and the solid was dissolved in CH$_2$Cl$_2$ and dried over MgSO$_4$. After the solvent was removed *in vacuo*, the crude product was purified by column chromatography (CH$_2$-Cl$_2$/hexanes, 50:4) to give 1.5 g of 16 (98%): mp 166–168 °C (crystals from CH$_2$Cl$_2$); ¹H NMR (CDCl$_3$) δ 6.76 (s, 1H, 5-H), 6.52 (s, 1H, 8-H), 6.22 (s, 2H, 2′,6′-H), 5.98 (s, 2H, OCH$_2$O), 4.87 (d, 1H, J = 3.1, 4-H), 4.58 (d, 1H, J = 5.3, 1-H), 4.25 (d, 2H, J = 9.1, 11-H$_2$), 3.68 (s, 6H, 3′,5′-OCH$_3$), 3.39 (dd, 1H, J = 5.3, J = 13.8, 2-H), 2.85 (m, 1H, 3-H), 0.99 (s, 9H, (CH$_3$)$_3$CSi), 0.89 (s, 9H, (CH$_3$)$_3$CSi), 0.16 (s, 3H, CH$_3$Si), 0.11 (s, 9H, CH$_3$Si, (CH$_3$)$_2$Si). Anal. (C$_{33}$H$_{48}$O$_8$Si$_2$) C, H, N.

4,4′-Bis(*tert*-butyldimethylsilyl)-4′-*O*-demethylepipodophyllotoxin 13-Alcohol (9). To 300 mg (0.48 mmol) of 8 in 7 mL toluene was added 1.2 mL of DIBAL (1.0 M in hexanes) at −78 °C. After 20 min, the reaction mixture was quenched with 1 mL MeOH at −78 °C. Then water and CH$_2$Cl$_2$ were added, and the mixture was stirred for 0.5 h. The organic layer was washed with water, and the solvent was removed *in vacuo*. The crude product was purified by column chromatography (CH$_2$Cl$_2$/acetone/EtOAc, 100:2:2) to give 200 mg of lactol (9) (67%) and 39 mg of the diol: mp 200–201 °C (crystals from CH$_2$Cl$_2$); ¹H NMR (CDCl$_3$) δ 6.72 (s, 1H, 5-H), 6.47 (s, 1H, 8-H), 6.10 (s, 2H, 2′,6′-H), 5.95 (s, 2H, OCH$_2$O), 4.81 (d, 1H, J = 6.9, 4-H), 4.73 (d, 1H, J = 6.9, 13-H), 4.33 (d, 1H, J = 6.8, 1-H), 4.08 (m, 1H, 11-H), 3.86 (t, 1H, 11′-H), 3.68 (s, 6H, 3′,5′-OCH$_3$), 2.74 (m, 1H, 2-H),

2.48 (m, 1H, 3-H), 1.00 (s, 9H, (CH$_3$)$_3$CSi), 0.88 (s, 9H, (CH$_3$)$_3$-CSi), 0.13 (s, 3H, CH$_3$Si), 0.11 (s, 6H, (CH$_3$)$_2$Si), 0.07 (s, 3H, CH$_3$Si); IR (KBr) 3340 (OH) cm⁻¹. Anal. (C$_{33}$H$_{50}$O$_8$Si$_2$) C, H, N.

4′-*O*-Demethylepipodophyllotoxin β-Methyl Ether (11). To 2.0 g (3.2 mmol) of 9 in 5 mL trimethyl orthoformate was added 60 mg of PPTS at room temperature. The reaction mixture was stirred for 30 min. CH$_2$Cl$_2$ was added, the solution was washed with water, and the solvent was evaporated. The crude product was dried by oil pump and was used in the next reaction without further purification.

To 2.0 g of the crude product (10) in 20 mL of THF was added 3.3 g (12.6 mmol) of Bu$_4$NF. The reaction mixture was stirred overnight at room temperature. THF was removed *in vacuo*, and CH$_2$Cl$_2$ was added. The solution was then washed with water. After the solvent was evaporated, the crude product was purified by flash column chromatography (CH$_2$Cl$_2$/EtOAC/acetone, 100:20:20) to give 1.12 g of 11 (85%, calculated from 9): mp 239–240 °C (powder from EtOAc and hexanes); ¹H NMR (CDCl$_3$) δ 6.86 (s, 1H, 5-H), 6.48 (s, 1H, 8-H), 6.14 (s, 2H, 2′,6′-H), 5.96 (s, 1H, OCHO), 5.94 (s, 1H, OCHO), 5.47 (s, 1H, OH), 4.78 (d, 1H, J = 3.3, 4-H), 4.41 (d, 1H, J = 6.8, 13-H), 4.32 (d, 1H, J = 6.4, 1-H), 4.10 (m, 1H, 11-H), 3.97 (m, 1H, 11′-H), 3.79 (s, 6H, 3′,5′-OCH$_3$), 3.51 (s, 3H, 13-OCH$_3$), 2.64 (m, 1H, 2-H), 2.47 (m, 1H, 3-H). Anal. (C$_{22}$H$_{24}$O$_8$) C, H, O.

4′-*O*-Carbobenzoxy-4′-*O*-demethylepipodophyllotoxin 13-Methyl Ether (12). To 400 mg (0.96 mmol) of 11 in 6 mL of acetone was added 0.36 mL of pyridine (4.4 mmol) and 0.4 mL (2.8 mmol) of CBZ-Cl at 0 °C. The reaction mixture was stirred at room temperature for 3 h, and then ice was added. After the organic layer was washed with water, the solvent was removed *in vacuo*. The crude product was purified by column chromatography (CH$_2$Cl$_2$/EtOAc/acetone, 100:10:10) to give 440 mg of 7 (83%). Starting material (30 mg) was also recovered: mp 209–210 °C (powder from EtOAc and hexanes); ¹H NMR (CDCl$_3$) δ 7.41 (m, 5H, CBZ aromatic), 6.86 (s, 1H, 5-H), 6.47 (s, 1H, 8-H), 6.15 (s, 2H, 2′,6′-H), 5.95 (AB q, 2H, J = 1.3, OCH$_2$O), 5.30 (s, 1H, OH), 4.79 (t, 1H, 4-H), 4.40 (d, 1H, J = 6.7, 13-H), 4.36 (d, 1H, J = 6.3, 1-H), 4.10 (m, 1H, 11-H), 3.97 (t, 1H, 11′-H), 3.70 (s, 6H, 3′,5′-OCH$_3$), 3.42 (s, 3H, 13-OCH$_3$), 2.65 (m, 1H, 2-H), 2.46 (m, 1H, 3-H).

General Procedure for the Synthesis of 15 and 16. To 50 mg (0.09 mmol) of 7 in 1.5 mL of anhydrous CH$_2$Cl$_2$ was added 38 mL of Et$_3$N (0.27 mmol) and 21 mL of methanesulfonyl chloride (0.27 mmol) at 0 °C. The reaction mixture was stirred for 3 h. Then 1 mL of anhydrous CH$_2$Cl$_2$, 50 mg of BaCO$_3$, and 0.36 mmol of the appropriate arylamine were added. The reaction mixture was stirred overnight. The solvent was removed *in vacuo*, and the crude product was purified by preparative TLC (CH$_2$-

Cl_2) to give **14** (α and β mixture). The product was used in the next reaction without further purification.

To the isomeric compound from the last reaction in 2 mL of EtOAc was added 8 mg of 10% palladium on activated carbon. The reaction mixture was stirred under hydrogen for 3 h. The mixture was filtered, and the filtrate was evaporated. The crude product was purified by preparative TLC (toluene/EtOAc/acetone, 100:5:5, developing four times) to give the β isomer.

4β-(4″-Fluoroanilino)-4′-O-demethylepipodophyllo-toxin 13-Methyl Ether (15): yield 40% (calculated from **12**); mp 154–156 °C (powder from acetone and hexanes); $[\alpha]^{25}_D$ –129 (c = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.91 (t, 2H, J = 8.7, 3″,5″-H), 6.72 (s, 1H, 5-H), 6.47 (m, 3H, 8, 2″,6″-H), 6.18 (s, 2H, 2′,6′-H), 5.94 (s, 1H, OCHO), 5.93 (s, 1H, OCHO), 5.45 (s, 1H, OH), 4.56 (d, 1H, J = 2.3, 4-H), 4.45 (d, 1H, J = 6.1, 13-H), 4.32 (d, 1H, J = 5.5, 1-H), 3.97 (m, 1H, 11-H), 3.82 (s, 6H, 3′,5′-OCH₃), 3.76 (m, 1H, 11′-H), 3.40 (s, 3H, 13-OCH₃), 2.59 (m, 2H, 2, 3-H). Anal. ($C_{28}H_{28}O_7NF$) C, H, N.

4β-Anilino-4′-O-demethylpodophyllotoxin 13-Methyl Ether (16): yield 32% (calculated from **12**); mp 137–139 °C (powder from acetone and hexanes); $[\alpha]^{25}_D$ –146 (c = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.19 (t, 2H, J = 78, 3″,5″-H), 6.72 (m, 2H, 4″,5-H), 6.55 (d, 2H, J = 8.0, 2′,6′-H), 6.47 (s, 1H, 8-H), 6.19 (s, 2H, 2′,6′-H), 5.94 (s, 1H, OCHO), 5.92 (s, 1H, OCHO), 5.45 (s, 1H, OH), 4.65 (s, br, 1H, 4-H), 4.45 (d, 1H, J = 5.6, 13-H), 4.33 (d, 1H, J = 4.8, 1-H), 3.98 (m, 2H, 11-H, NH), 3.83 (s, 6H, 3′,5′-OCH₃), 3.76 (m, 1H, 11′-H), 2.62 (m, 2H, 2, 3-H). Anal. ($C_{28}H_{29}O_7N$·^{1}/₂H₂O) C, H, N.

4′,4-Bis(*tert*-butyldimethylsilyl)-4′-O-demethylepipodo-phyllol (17). To 2.0 g (3.2 mmol) of **8** in 40 mL of THF was added 182 mg (4.8 mmol) of $LiAlH_4$. The reaction mixture was stirred at room temperature for 3 h. Ice was added to quench the reaction. THF was removed *in vacuo*. CH_2Cl_2 was added, and the organic layer was washed with water. After evaporation of solvent, the crude product was purified by column chromatography (CH_2Cl_2/EtOAc/acetone = 100:20:20) to give 1.46 g of **17** (73%): mp 160–162 °C (powder from acetone and hexanes); ^{1}H NMR ($CDCl_3$) δ 6.78 (s, 1H, 5-H), 6.41 (s, 1H, 8-H), 6.22 (s, 2H, 2′,6′-H), 5.92 (s, 2H, OCH₂O), 5.02 (d, 1H, J = 3.2, 4-H), 4.17 (d, 1H, J = 6.3, 1-H), 3.85 (m, 2H, 11-H₂), 3.71 (s, 6H, 3′,5′-OCH₃), 3.68 (m, 2H, 13-H₂), 2.63 (m, 1H, 2-H), 2.33 (m, 1H, 3-H), 1.01 (s, 9H, (CH₃)₃CSi), 0.89 (s, 9H, (CH₃)₃CSi), 0.20 (s, 3H, CH₃-Si), 0.11 (s, 6H, (CH₃)₂Si), 0.02 (s, 3H, CH₃Si). Anal. ($C_{33}H_{52}O_8$-Si_2) C, O, N.

4,4′-Bis(*tert*-butyldimethylsilyl)-4′-O-demethylanhy-droepipodophyllol (18). To 1.3 g (2.1 mmol) of **17** in 25 mL of CH_2Cl_2 were added 853 mg (3.2 mmol) of TPP and 0.52 mL (3.2 mmol) of DEAD under nitrogen. The reaction mixture was stirred for 3 h. After the solvent was removed, the crude product was purified by column chromatography (CH_2Cl_2/hexanes, 10:1) to give 1.2 g of **18** (92%): mp 166–168 °C (crystals from CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 6.75 (s, 1H, 5-H), 6.46 (s, 1H, 8-H), 5.99 (s, 2H, 2′,6′-H), 5.95 (s, 2H, OCH₂O), 4.85 (d, 1H, J = 2.7, 4-H), 4.25 (d, 1H, J = 5.6, 1-H), 3.88 (m, 3H, 11-H₂, 13-H), 3.67 (s, 6H, 3′,5′-OCH₃), 2.93 (m, 2H, 2-H, 13′-H), 2.39 (m, 1H, 3-H), 1.00 (s, 9H, (CH₃)₃CSi), 0.86 (s, 9H, (CH₃)₃CSi), 0.13 (s, 3H, CH₃Si), 0.11 (s, 6H, (CH₃)₂CSi), 0.07 (s, 3H, CH₃Si). Anal. ($C_{33}H_{50}O_7Si_2$) C, H.

4′-O-Dmethylanhydroepipodophyllol (19). Compound **19** was prepared from **18** in an analogous manner to **11** from **10**: mp 231–232 °C (powder from acetone and hexanes); ^{1}H NMR ($CDCl_3$) δ 6.90 (s, 1H, 5-H), 6.50 (s, 1H, 8-H), 6.05 (s, 2H, 2′,6′-H), 5.97 (s, 1H, OCHO), 5.95 (s, 1H, OCHO), 5.44 (s, 1H, OH), 4.84 (s, br, 1H, 4-H), 4.29 (d, 1H, J = 6.2, 1-H), 3.95 (m, 3H, 11-H₂, 13-H), 3.79 (s, 6H, 3′,5′-OCH₃), 3.03 (m, 1H, 13′-H), 2.74 (m, 1H, 2-H), 2.44 (m, 1H, 3-H). Anal. ($C_{21}H_{22}O_7$) C, H.

General Procedure for the Synthesis of Compounds 21 and 22. Compound **19** (0.19 mmol) was dissolved in CH_2Cl_2 (4 mL). Dry HCl gas was bubbled through the solution for 60 min at 0 °C. The solvent was removed, and the product was dried using an oil pump. The chloride was displaced by the appropriate arylamine (0.23 mmol) as in the preparation of **15** and **16**.

4β-(4″-Fluoroanilino)-4′-demethylanhydropodophyllol (21). Yield: 47% (calculated from **19**); mp: 163–164 °C (powder from EtOAc and hexanes); $[\alpha]^{25}_D$ –97 (c = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.91 (t, 2H, J = 8.6, 3″,5″-H), 6.74 (s, 1H, 5-H), 6.48 (m, 3H,

2″,6″, 8-H), 6.09 (s, 2H, 2′,6′-H), 5.95 (s, 1H, OCHO), 5.93 (s, 1H, OCHO), 5.44 (s, 1H, OH), 4.59 (d, 1H, J = 3.8, 4-H), 4.27 (d, 1H, J = 6.0, 1-H), 4.01 (t, 2H, 11-H₂), 3.81 (s, 6H, 3′,5′-OCH₃), 3.58 (q, 1H, 13-H), 3.07 (q, 1H, 13′-H), 2.68 m, 1H 2-H), 2.55 (m, 1H, 3-H). Anal. ($C_{27}H_{26}O_6NF$·½H₂O) C, H, N.

4β-Anilino-4′-O-demethylanhydropodophyllol (22): yield 61% (calculated from **19**); mp 232–234 °C (powder from acetone and hexanes); $[\alpha]^{25}_D$ –116 (c = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.20 (t, 2H, J = 7.6, 3″,5″-H), 6.74 (m, 2H, 4″,5-H), 6.57 (d, 2H, J = 8.0, 2″,6″-H), 6.47 (s, 1H, 8-H), 6.10 (s, 2H, 2′,6′-H), 5.94 (s, 1H, OCHO), 5.92 (s, 1H, OCHO), 5.44 (s, 1H, OH), 4.59 (d, 1H, J = 4.0, 4-H), 4.27 (d, 1H, J = 5.9, 1-H), 4.01 (m, 2H, 11-H₂), 3.81 (s, 6H, 3′,5′-OCH₃), 3.59 (q, 1H, 13-H), 3.07 (q, 1H, 13′-H), 2.69 (m, 1H, 2-H), 2.54 (m, 1H, 3-H). Anal. ($C_{27}H_{27}O_6N$·½H₂O) C, H, N.

4β-(4″-Fluoroanilino)-4′-O-demethylpodophyllotoxin 13-Alcohol (24). Compound **5** (prepared as in ref 14) was reduced with DIBAL using the same procedure as in the synthesis of **9**: mp 203–205 °C (powder from acetone and hexanes); $[\alpha]^{25}_D$ –131 (c = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.91 (t, 2H, 8.7, 3″,5″-H), 6.72 (s, 1H, 5-H), 6.48 (m, 3H, 2″,6″, 8-H), 6.20 (s, 2H, 2′,6′-H), 5.95 (s, 1H, OCHO), 5.93 (s, 1H, OCHO), 5.46 (s, 1H, OH), 4.86 (d, 1H, J = 6.0, 13-H), 4.55 (d, 1H, J = 3.7, 4-H), 4.34 (d, 1H, J = 6.1, 1-H), 3.96 (t, 1H, 11-H), 3.88–3.82 (m, 7H, 11′,3′,5′-OCH₃), 2.87 (m, 1H, 2-H), 2.52 (m, 1H, 3-H). Anal. ($C_{27}H_{26}O_7$-NF·½H₂O) C, H, N.

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