Antitumor Agents. 3.[1] Synthesis and Biological Activity of 4β-Alkyl Derivatives Containing Hydroxy, Amino, and Amido Groups of 4'-*O*-Demethyl-4-desoxypodophyllotoxin as Antitumor Agents

Tadafumi Terada,* Katsuhiko Fujimoto, Makoto Nomura, Jun-ichi Yamashita, Konstanty Wierzba, Ryoko Yamazaki, Jiro Shibata, Yoshikazu Sugimoto, and Yuji Yamada

Hanno Research Center, Taiho Pharmaceutical Co. Ltd., 216-1 Nakayashita Yaoroshi, Hanno-shi, Saitama 357, Japan

Takashi Kobunai, Setsuo Takeda, Yoshinori Minami, and Ken-ichirou Yoshida

Tokushima Institute, Taiho Pharmaceutical Co., Ltd., Hiraishi, Ebisuno, Kawauchi-cho, Tokushima-shi, Tokushima 771-01, Japan

Hideo Yamaguchi

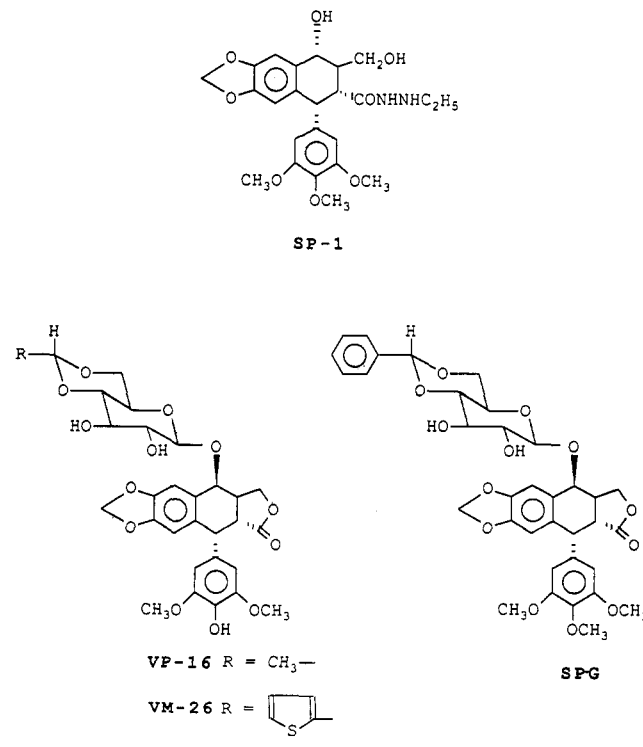
Osaka University of Pharmaceutical Sciences, Kawai 2-10-65, Matsubara, Osaka 580, Japan

Received October 16, 1992

A series of 4β-alkyl (**7–10**), 4β-aminoalkyl (**12a–y**), and 4β-amidoalkyl derivatives (**14a–g**) of 4'-*O*-demethyl-4-desoxypodophyllotoxin have been synthesized, and their cytotoxicity, inhibition of DNA topoisomerase II (Topo II), and tubulin polymerization were evaluated. All derivatives of **12a–y** and **14a–g** did not inhibit tubulin polymerization. Many compounds exhibited cytotoxicity and inhibition of Topo II. In particular, **12o**, **12s**, **12t**, and **12u** strongly inhibited Topo II (IC_{50} (μM) 32.5, 60.9, 58.8, and 33.6, respectively) and were strong cytotoxicity against P388 cells (IC_{50} (M) 1.0, 4.1, 3.3, and 3.0×10^{-9}, respectively), compared with VP-16 (IC_{50} (μM) 59.2, IC_{50} (M) 1×10^{-8}, respectively). These compounds were nearly equal to or superior to VP-16 in antitumor activity in vivo (L1210, P388, and Lewis lung) and were more cytotoxic against various human cell lines in vitro than VP-16.

Many derivatives of podophyllotoxin (POD) that are potent inhibitors of mitosis[2] have been synthesized and examined as antitumor agents.[3] Among these, podophyllinic acid ethyl hydrazide (SP-1) and podophyllotoxin benzylidene-β-D-glucopyranoside (SP-G), whose main mechanism of action is inhibition of microtubule polymerization,[4] have been examined as clinical antitumor agents (Chart I). However, these agents have been little used as clinical antitumor agents because of severe side effects.[5] In contrast, the analogous 4'-demethylepipodophyllotoxin 4-(4,6-*O*-ethylidene)-β-D-glucopyranoside (etoposide; VP-16) and 4'-demethylepipodophyllotoxin 4-(4,6-*O*-thienylidene)-β-D-glucopyranoside (teniposide; VM-26) (Chart I) are widely used in clinical cancer chemotherapy.[6] VP-16 does not inhibit tubulin polymerization and induces dose-dependent DNA strand breakage which is associated with its ability to inhibit DNA topoisomerase II (Topo II).[7] Therefore, much attention has been given to the modification of podophyllotoxin glucoside as a potent Topo II inhibitor.[8] Recently, we reported that some nonglucoside podophyllotoxin derivatives with an aminoalkoxy residue instead of glucose at the 4β-position of 4-desoxypodophyllotoxin, inhibited Topo II without inhibiting microtubulin polymerization, in a manner similar to that of VP-16, and showed antitumor activity in vitro and in vivo.[9]

Chart I

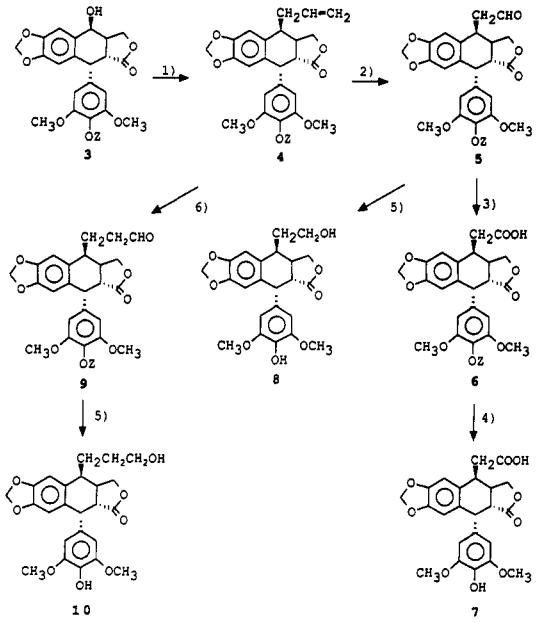


Though VP-16 has shown high response rates (40–60%) against small cell lung cancer (SCLC),[10] those against nonsmall cell lung cancer (NSCLC) have been very low (8%).[11] NSCLC has the following biological and biochemical characters which differ from SCLC: (1) a 2–3-fold longer doubling time;[12] (2) a lower labeling index;[12] and (3) a lower amount and activity of Topo II.[13] The low sensitivity of VP-16 to NSCLC could be due to insufficient inhibition of Topo II and/or poor distribution in the lung tissue.[14]

A compound of POD that overcame the following factors should be a more effective agent against NSCLC: (1) more potent inhibition of Topo II and (2) a higher concentration and longer distribution of the active form of the compound

0022-2623/93/1836-1689$04.00/0

Scheme I[a]



[a] (1) CH_2=$CHCH_2SiMe_3$, BF_3·Et_2O/CH_2Cl_2–20 to 0 °C; (2) (i) cat. OsO_4–NMO/CH_3COCH_3, room temperature; (ii) $Pb(OAc)_4$/benzene; (3) CrO_3–H_2SO_4/H_2O–CH_3COCH_3, 0 °C; (4) 5% Pd–C, H_2 (1 atm)/CH_2Cl_2, room temperature; (5) (i) $Zn(BH_4)_2$/THF, 0 °C to room temperature; (ii) 5% Pd–C, H_2 (1 atm)/CH_2Cl_2, to room temperature; (6) (i) 2M BH_3·Me_2S/THF, 0 °C to room temperature; (ii) pyridinium chlorochromate/CH_2Cl_2, 0 °C to room temperature.

to the lung tissue. Therefore, we attempted to synthesize compounds which are more stable metabolically and have more potent inhibition of Topo II and more potent cytotoxicity against NSCLC than VP-16.

In this paper, we describe the synthesis of 4β-alkyl derivatives containing hydroxy, amino, and amido groups of 4′-demethyl-4-desoxypodophyllotoxin. We studied the relationships between structure and biological activities, especially cytotoxicity against human NSCLC and inhibitory activity against Topo II. In addition, we examined the in vivo antitumor activity of the selected compounds.

Chemistry

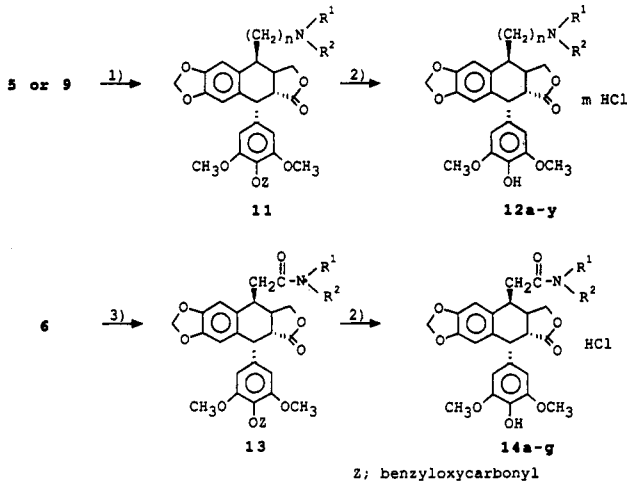
The synthesis of compounds **4–10** is shown in Scheme I. Regio- and stereospecific introduction of allyl group at the 4β-position of 4′-demethyl-4′-*O*-(benzyloxycarbonyl)-epipodophyllotoxin (**3**) was obtained using trimethylallylsilane in the presence of boron trifluoride etherate, at a high yield according to the previously reported method.[1] Oxidation of **4** with osmic acid and *N*-methylmorpholine *N*-oxide (NMO) in acetone, followed by oxidation with lead tetraacetic acid ($Pb(OAc)_4$) in benzene gave 4β-(formylmethyl)-4-desoxypodophyllotoxin (**5**). Jones oxidation of **5** gave 4β-(2-oxo-2-hydroxyethyl)-4-desoxypodophyllotoxin (**6**) in moderate yield (59.4%). Deprotection of the 4′-benzyloxycarbonyl group of **6** was carried out with H_2 (1 atm) on 5% palladium–carbon in dichloromethane to give **7**. Reduction of the allyl group of **4** with a borane dimethyl sulfide complex in THF, followed by oxidation with pyridinium chlorochromate in dichloromethane gave 4β-(2-formylethyl)-4-desoxypodophyllotoxin (**9**). Reduction of the aldehyde group of **5** or **9** with zinc borohydride ($Zn(BH_4)_2$) in THF, followed by deprotection of 4′-benzyloxycarbonyl group with H_2 (1 atm) on 5% palladium–carbon in dichloromethane, gave the 2-hydroxyethyl compound (**8**) or 3-hydroxypropyl compound (**10**), respectively. The physical properties of **4–10** are listed in Table I.

Table I. Physical Properties of the 4β-Alkyl-4-desoxypodophyllotoxin Derivatives Shown in Scheme I

compd	yield, %	mp, °C[a]	$[\alpha]^{20}_D$, deg (*c*., solvent[b])	formula[c]
4	95.8	135–137	−70.03 (1.300)	$C_{32}H_{30}O_9$
5	92.9	180–182	−71.04 (1.320)	$C_{31}H_{28}O_{10}$
6	59.4	138–140	−68.59 (0.554)	$C_{31}H_{28}O_{11}$
7	66.3	222–225	−93.95 (0.430)	$C_{23}H_{22}O_9$
8	74.5	120–122	−78.72 (0.194)	$C_{23}H_{24}O_8$
9	44.9	153–155	−69.09 (0.380)	$C_{32}H_{30}O_{10}$
10	48.5	174–175	−81.30 (0.214)	$C_{24}H_{26}O_8$

[a] All compounds were recrystallized from Et_2O. [b] All compounds were dissolved in DMSO. [c] Analyses for C and H were within ±0.4% of the calculated values.

Scheme II[a]



Z; benzyloxycarbonyl

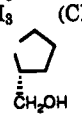
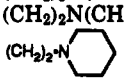
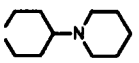
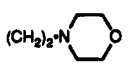
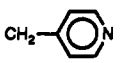
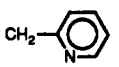
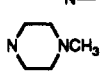
[a] (1) HNR^1R^2, $NaCNBH_3$/AcOH–CH_3OH, 0 °C to room temperature; (2) (i) 10% Pd–C, H_2 (1 atm)/CH_2Cl_2; (ii) 4 N HCl–AcOEt/AcOEt, 0 °C; (3) $(CH_3)_3CCOCl$, HNR^1R^2, DMAP/AcOEt, 0 °C (*n*, 2 or 3; *m*, 1 or 2).

The syntheses of 4β-alkyl-4-desoxypodophyllotoxin derivatives containing various amino and amido groups are shown in Scheme II. Reductive amination of **5** or **9** with sodium cyanoborohydride ($NaCNBH_3$) and various appropriate amines in AcOH–CH_3OH gave the 4β-aminoalkyl derivatives **11**, followed by deprotection of the 4′-benzyloxycarbonyl group with H_2 (1 atm) on 10% palladium–carbon in dichloromethane and by treatment with 4 N HCl/AcOEt, gave 4β-(aminoalkyl)-4-desoxypodophyllotoxin derivatives (**12a–y**). Physical properties of **12a–y** are listed in Table II. The formation of mixed anhydrides with **6** and pivaloyl chloride in the presence of (dimethylamino)pyridine (DMAP), followed by reaction with various appropriate amines gave 4β-amidoalkyl derivatives **13**. The use of DCC (dicyclohexylcarbodiimide) as a condensing agent with **6** and various appropriate amines was not successful. Deprotection of the 4′-benzyloxycarbonyl group of **13** with H_2 (1 atm) on 10% palladium–carbon in dichloromethane, followed by treatment with 4 N HCl/AcOEt, gave 4β-(amidoalkyl)-4-desoxypodophyllotoxin derivatives **14a–g**. The physical properties of **14a–g** are listed in Table III.

Biological Results and Discussion

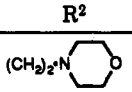
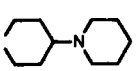
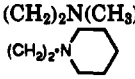
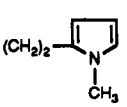
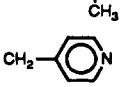
Recently, we reported that cytotoxicity and Topo II inhibitory activity alone were not good indicators of antitumor activity in vivo for POD derivatives, insofar as their derivatives inhibit tubulin polymerization.[9] Therefore, we examined the inhibition of tubulin polymerization and Topo II, as well as cytotoxicity. The biological effects

Table II. Physical Properties of 12 Shown in Scheme II

compd	R^1	R^2	n	m	yield,[a] %	mp, °C[b]	$[\alpha]^{20}_D$, deg (c, solvent[c])	formula[d]
12a	CH_3	CH_3	2	1	81.8	226–228	−66.77 (0.910)	$C_{25}H_{29}NO_7 \cdot HCl \cdot H_2O$
12b	CH_3	CH_3	3	1	61.5	240–243	−79.91 (0.510)	$C_{26}H_{31}NO_7 \cdot HCl \cdot H_2O$
12c	CH_3	CH_2CH_2OH	2	1	72.0	234–237	−60.89 (0.335)	$C_{26}H_{31}NO_8 \cdot HCl \cdot 2H_2O$
12d	CH_3	$CH(CH_2OH)_2$	2	1	67.0	222–225	−69.31 (0.550)	$C_{27}H_{33}NO_9 \cdot HCl \cdot 0.5H_2O$
12e	CH_3	$CH_2CH_2OCH_3$	2	1	92.0	193–195	−71.72 (0.488)	$C_{27}H_{33}NO_8 \cdot HCl \cdot H_2O$
12f	CH_3	$(CH_2)_5CH_3$	2	1	66.1	210–214	−65.65 (0.385)	$C_{30}H_{39}NO_7 \cdot HCl \cdot H_2O$
12g			2	1	59.3	239–244	−58.38 (0.590)	$C_{28}H_{33}NO_8 \cdot HCl \cdot 0.5H_2O$
12h	CH_3		2	1	81.0	240–242	−66.77 (0.910)	$C_{30}H_{37}NO_7 \cdot HCl \cdot H_2O$
12i			2	1	61.0	250–252	−70.25 (0.550)	$C_{28}H_{33}NO_7 \cdot HCl \cdot H_2O$
12j	CH_3	CH_2Ph	2	1	69.8	204–205	−64.94 (0.610)	$C_{31}H_{33}NO_7 \cdot HCl \cdot 2.5H_2O$
12k			2	1	70.0	251–253	−63.38 (0.183)	$C_{27}H_{31}NO_8 \cdot HCl \cdot H_2O$
12l	CH_3	$N(CH_3)_2$	2	1	89.2	224–226	−71.42 (0.580)	$C_{26}H_{32}N_2O_7 \cdot HCl \cdot 0.5H_2O$
12m	CH_3	$N(CH_3)Ph$	2	1	67.4	170–172	−87.93 (0.600)	$C_{31}H_{34}N_2O_7 \cdot HCl \cdot H_2O$
12n	H	$(CH_2)_2N(CH_3)_2$	2	2	55.0	213 dec[e]	−64.09 (0.493)	$C_{27}H_{34}N_2O_7 \cdot 2HCl \cdot 2.5H_2O$
12o	CH_3	$(CH_2)_2N(CH_3)_2$	2	2	71.7	203–205	−67.44 (0.495)	$C_{28}H_{36}N_2O_7 \cdot 2HCl \cdot H_2O$
12p	CH_3	$(CH_2)_3N(CH_3)_2$	2	2	92.0	238 dec	−56.76 (0.303)	$C_{29}H_{38}N_2O_7 \cdot 2HCl \cdot 3H_2O$
12q	CH_3	$(CH_2)_6N(CH_3)_2$	2	2	75.0	198–199	−57.14 (0.119)	$C_{32}H_{44}N_2O_7 \cdot 2HCl \cdot 1.5H_2O$
12r	CH_3	$(CH_2)_2N(CH_2CH_3)_2$	2	2	61.5	195–197	−59.73 (0.298)	$C_{30}H_{40}N_2O_7 \cdot 2HCl \cdot 2H_2O$
12s	CH_3		2	2	69.0	210–213	−63.89 (1.155)	$C_{31}H_{40}N_2O_7 \cdot 2HCl \cdot 2H_2O$
12t			2	2	65.4	280 dec	−48.95 (0.527)	$C_{33}H_{42}N_2O_7 \cdot 2HCl \cdot H_2O$
12u			2	2	66.7	232–236	−59.35 (0.556)	$C_{28}H_{34}N_2O_7 \cdot 2HCl \cdot 2H_2O$
12v	CH_3		2	2	66.0	210–216	−57.07 (0.820)	$C_{30}H_{38}N_2O_8 \cdot 2HCl \cdot 2H_2O$
12w	CH_3		2	2	31.0	181–183	−64.56 (0.285)	$C_{30}H_{32}N_2O_7 \cdot 2HCl \cdot 2H_2O$
12x	CH_3		2	2	57.0	185–186	−58.21 (0.119)	$C_{30}H_{32}N_2O_7 \cdot 2HCl \cdot 1.5H_2O$
12Y	CH_3		2	2	67.0	188–190	−64.58 (0.384)	$C_{29}H_{37}N_3O_7 \cdot 2HCl \cdot 3H_2O$

[a] Yield from **5** or **9**. [b] All compounds were recrystallized from Et_2O. [c] All compounds dissolved in DMSO, except for **12c**, **12t** (in H_2O), and **12m** (in DMF). [d] Analyses for C, H, and N were within ±0.4% of the calculated values. [e] Dec: decomposed.

Table III. Physical Properties of 14 Shown in Scheme II

compd	R^1	R^2	yield,[a] %	mp, °C[b]	$[\alpha]^{20}_D$, deg (c, solvent[c])	formula[d]
14a	H		84.3	250 dec[e]	−64.71 (0.479)	$C_{29}H_{34}N_2O_9 \cdot HCl \cdot H_2O$
14b			76.1	209 dec	−42.83 (0.831)	$C_{33}H_{40}N_2O_8 \cdot HCl \cdot H_2O$
14c	CH_3	$(CH_2)_2N(CH_3)_2$	78.4	195–197 dec	−44.06 (0.463)	$C_{28}H_{34}N_2O_8 \cdot HCl \cdot H_2O$
14d	H		88.3	225–228 dec	−69.81 (0.424)	$C_{30}H_{36}N_2O_8 \cdot HCl \cdot H_2O$
14e			78.6	205–210 dec	−44.08 (0.549)	$C_{28}H_{32}N_2O_8 \cdot HCl \cdot H_2O$
14f	H		50.5	156–158	−84.49 (0.258)	$C_{30}H_{32}N_2O_8 \cdot HCl \cdot H_2O$
14g	H		34.0	179–180	−82.41 (0.381)	$C_{29}H_{28}N_2O_8 \cdot HCl \cdot H_2O$

[a] Yield from **6**. [b] All compounds were recrystallized from Et_2O. [c] All compounds were carried out in DMSO. [d] Analyses for C, H, and N were within ±0.4% of the calculated values. [e] Dec: decomposed.

of compounds **7–10**, **12a–y** and **14a–g** are summarized in Table IV. Compounds **8** and **10**, possessing a hydroxyalkyl group, exhibited a stronger inhibitory effect on Topo II than that of VP-16, with similar cytotoxicity. However, these compounds also inhibited tubulin polymerization. On the other hand, all compounds of **12a–y** with various amino groups and **14a–g** with various amido groups showed no inhibitory effect against tubulin polymerization. The

Table IV. Biological Evaluation of 4β-Alkyl-4-desoxypodophyllotoxin Derivatives and VP-16

compd	cytotoxicity[a] P388 leukemia (IC_{50}, M)	tubulin[a] polymerization (IC_{50}, μM)	Topo II[a] (IC_{50}, μM)
VP-16	1.0×10^{-8}	>60	59.2 (1.0)[b]
7	1.5×10^{-5}	NT[c]	NT
8	5.0×10^{-8}	2	13.8 (0.23)
10	5.0×10^{-8}	26	42.1 (0.71)
12a	6.0×10^{-8}	>100	36.7 (0.62)
12b	1.2×10^{-8}	>167	73.5 (1.24)
12c	7.0×10^{-8}	>100	17.2 (0.29)
12d	6.6×10^{-7}	>167	25.1 (0.42)
12e	1.6×10^{-7}	>100	75.8 (1.28)
12f	1.9×10^{-8}	>167	61.4 (1.03)
12g	6.3×10^{-8}	>62	112.1 (1.89)
12h	3.3×10^{-8}	>167	60.9 (1.02)
12i	1.2×10^{-8}	>167	67.7 (1.14)
12j	1.5×10^{-7}	>167	97.3 (1.64)
12k	2.6×10^{-8}	>167	58.3 (0.98)
12l	2.0×10^{-8}	>100	58.3 (0.98)
12m	$>1.0 \times 10^{-6}$	NT	NT
12n	4.0×10^{-8}	>100	13.3 (0.22)
12o	1.0×10^{-9}	>99	32.5 (0.54)
12p	5.5×10^{-8}	>100	26.9 (0.45)
12q	3.7×10^{-8}	>133	30.0 (0.50)
12r	3.7×10^{-8}	>100	53.8 (0.90)
12s	4.1×10^{-9}	>167	60.9 (1.02)
12t	3.3×10^{-9}	>167	29.8 (0.53)
12u	3.0×10^{-9}	>132	33.6 (0.56)
12v	2.6×10^{-7}	>167	115.7 (1.95)
12w	1.0×10^{-7}	>100	31.4 (0.52)
12x	1.4×10^{-7}	>100	31.4 (0.52)
12y	4.3×10^{-9}	>100	32.3 (0.54)
14a	2.2×10^{-7}	>100	NT
14b	8.4×10^{-9}	>100	266.4 (4.50)
14c	3.2×10^{-8}	>100	251.6 (4.25)
14d	3.0×10^{-8}	>100	60.6 (1.02)
14e	1.2×10^{-9}	>100	296 (5.00)
14f	1.5×10^{-6}	>100	NT
14g	2.2×10^{-6}	>100	NT

[a] See the Experimental section. [b] Value in parentheses is the ratio of IC_{50} of individual compound/IC_{50} of VP-16. [c] NT: not tested.

compounds with various amino or amido groups are classified as follows: (1) those with a linear alkyl chain containing one N atom (**12a–f**), (2) those with a cyclic alkyl chain or benzene ring containing one N atom (**12g–k**), (3) those with a linear alkyl chain or a benzene ring containing two N atoms (**12l–r**), (4) those with a cyclic alkyl chain containing two or three N atoms, or with a pyridine ring (**12s–y**), and (5) those with an amido group (**14a–g**).

In the first group (**12a–f**), a comparison of compounds containing an aminoethyl group (**12a**) and an aminopropyl group (**12b**) against Topo II suggests that the introduction of the N atom at the β-position of the 4β-alkyl group of 4-desoxypodophyllotoxin was better for the inhibition of Topo II than that at the *r*-position. However, their cytotoxicities against P388 were similar. Therefore, we synthesized compounds with a N atom at the β-position of the 4β-alkyl group of 4-desoxypodophyllotoxin. By a comparison of **12c–f**, the introduction of hydroxy groups (but not ether groups) at the end of the linear alkyl chain containing one N atom enhanced the inhibitory effect against Topo II. However, the degree of Topo II inhibition did not correlate with cytotoxicity.

In the second group (**12g–k**), the inhibitory effects against Topo II and cytotoxicity were nearly equal to those of **12f**. These linear aminoalkyl chain groups were not necessary for the inhibition of Topo II or cytotoxicity. No significant improvement of the inhibition of Topo II and cytotoxicity was found in the above two groups containing one N atom.

Table V. Antitumor Activity against L1210 in Vivo of 4β-Alkyl-4-desoxypodophyllotoxin Derivatives and VP-16

compd	dose (mg/kg/day)	ILS[a] max.(%)	survivors (30 days)
VP-16	10	102	3/6
12a	2.5	67	0/6
12f	20	106	0/6
12j	10	53	0/6
12k	10	81	0/6
12l	40	74	0/6
12o	2.5	103	3/6
12s	5		6/6
12t	2.5	102	5/6
12u	1.3	140	1/6

[a] Male CDF_1 mice (7 weeks old) were inoculated ip with 1×10^5 L1210 cells on day 0, and each compound was administered ip on days 1–5. Compounds were dissolved in saline containing 3.5% DMSO and 6.5% Tween 80. Each group except the control consisted of six mice. The control group consists of 10 mice. The percentage increase in the life span (ILS) was maximal, which was calculated from the mean survival period of the treated group compared with that of the control group. The numbers of mice that survived for 30 days are not included in calculations of the ILS value.

In the third group (**12l–r**) containing two N atoms, the inhibitory effect against Topo II and cytotoxicity against P388 were only slightly affected by the distance between the N atoms. Compound **12o** was more potent in both cytotoxicity against P388 (1.0×10^{-9} M) and inhibition of Topo II, compared with VP-16.

In the fourth group (**12s–y**), the introduction of a pyridine ring (**12w,x**) induced more inhibition of Topo II than that of VP-16, but decreased the cytotoxicity dramatically. On the other hand, **12s–u,y** exhibited similar or more Topo II inhibition than VP-16, and each compound was also highly cytotoxic (IC_{50}: 4.1, 3.3, 3.0, and 4.3×10^{-9} M, respectively).

The fifth group with various amido groups (**14a–g**) tended toward less inhibition of Topo II, but the cytotoxicity remained.

We were unable to determine a good correlation between the potency of Topo II inhibition and the cytotoxicity of the compounds tested. We therefore selected representative compounds which possessed the same or stronger inhibition of Topo II and cytotoxicity than VP-16 and examined the antitumor activity in vivo. In antitumor activity in vivo against L1210 leukemia (ip–ip) (Table V), compounds **12o,s–u** were nearly equal or superior to VP-16. Antitumor activity against P388 leukemia (sc–iv) of **12s** and **12t** were nearly equal or superior to that of VP-16 (Table VI). On the other hand, **12o,s–u** exhibited more tumor growth inhibition against Lewis lung (sc–iv) (Table VI) than VP-16. In addition, **12o,s,u** had better antitumor activity against Lewis lung carcinoma (iv–iv) than VP-16 (Table VII).

Furthermore, we examined the cell growth inhibitory effects of **12o,s–u** and VP-16 upon various human non-small cell lung cancer cell lines in vitro to predict the effect against NSCLC (Table VIII). All of the tested compounds exhibited growth inhibition at lower concentrations than those of VP-16 in vitro (Table VIII). We also observed that these compounds were distributed throughout the lung tissue at higher concentrations and for longer than VP-16.[15] These results suggest that **12o,s–u** could be appropriate compounds against NSCLC.

The fact that these C–C bond compounds with various amino groups exhibited stronger antitumor activity and a different distribution from VP-16 suggests that both the β-D-glucose and acetal group, which was reported as an active structural requirements of VP-16,[16] are not

Table VI. Antitumor Activity against P-388 and Lewis Lung in Vivo of **12o**, **12s**, **12t**, **12u**, and VP-16

compd	P388[a] dose (mg/kg/day)	ILS (%)	Lewis lung[c] dose (mg/kg/day)	tumor growth inhibition (%)
VP-16	7	226 (2/7)[b]	12	60**[d]
	4	196	7	39*
	2.3	151		
12o	2.3	236 (1/7)	4	95***
	1.3	196	2.3	51**
	0.8	116		
12s	4	259 (3/7)	7	97***
	2.3	165	4	72*
	1.3	131		
12t	2.3	231 (2/7)	4	91***
	1.3	202	2.3	50**
	0.8	143	1.3	40*
12u	0.8	182	2.3	96***
	0.4	112	1.3	81***
	0.3	67	0.3	37*

[a] Male CDF_1 mice (5 weeks old) were inoculated sc with 1×10^6 P388 cells on day 0, and each compound was administered iv on days 1–5. Compounds were dissolved in saline containing 3.5% DMSO and 6.5% Tween 80. Each group except control consisted of seven mice. The control group consisted of 10 mice. The percentage increase of life span (ILS) was maximal, which was calculated from the mean survival period of the treated group compared with that of the control group. The numbers of mice that survived for 30 days are not included in calculating the ILS value. [b] Number of mice that survived for 30 days: survived mice/number of control mice. [c] Lewis lung carcinoma (2 mm^3) were inoculated sc into male BDF_1 mice (5 weeks old) on day 0, and each compound was administered iv for 5 days on days 4–8. Compounds were dissolved in saline, and VP-16 was dissolved in saline containing 3.5% DMSO and 6.5% Tween 80. Each group, except the control, consisted of seven mice. The control group consisted of 10 mice. The percentage inhibition of tumor growth was calculated from the mean tumor weight of the treated group compared with that of the control group on day 17. [d] *, **, ***: Significantly different from the control at $p < 0.05$, 0.01, and 0.001, respectively.

Table VII. Antitumor Activity against Lewis Lung[a] in Vivo of **12o**, **12s**, **12t**, **12u**, and VP-16

compd	dose (mg/kg/day)	bwc[b] (g, day 6-0)	Lewis lung ILS[c] (%)
control		+2.3	
VP-16	21	−3.8	91**(1/7)[d]
	12	−2.6	81***
	7	−0.8	21***
	4	−0.4	24***
	2.3	+0.0	17**
12o	4	−2.9	209** (6/7)
	2.3	−0.7	90*** (2/7)
	1.3	+0.2	72***
	0.77	+0.44	38***
12s	7	−3.5	55 (5/7)
	4	−1.0	108***
	2.3	−0.6	75***
	1.3	+0.1	14**
	0.77	+0.7	20**
12t	7	−4.1	−44***
	4	−1.6	−13 (1/7)
	2.3	−0.5	133***
	1.3	−0.2	63***
	0.77	+0.3	43***
12u	1.3	−3.3	127*** (3/7)
	0.77	−0.9	91***
	0.44	+0.3	67***
	0.25	+0.9	24***

[a] Lewis lung carcinoma, 3.2×10^5 cells were inoculated into male BDF_1 mice (5 weeks old) on day 0 intraveneously, and compounds were administered iv from days 1–5. Each group consists of seven mice, except the control group which included 14 mice. [b] bwc: mean body weight change. [c] Number of 60-day survivors is not included to calculations to ILS (increased life span) value. [d] Number of mice that survived for 30 days/number of control mice. **, ***: significantly different from the control at $p < 0.005$, $p < 0.001$.

Table VIII. Growth Inhibition of **12o**, **12s**, **12t**, **12u**, and VP-16 against Various Human Cell Lines

cell line	ED_{50} (μg/mL)[a] VP-16	12o	12s	12t	12u
lung small cell ca.					
RERF-LC-MA	>59	14	27	35	5.8
SBC-3	1.6	0.41	0.54	0.16	0.28
lung non-small cell ca.					
A-549	2.9	0.82	1.8	1.0	0.76
PC-7	35	3.5	4.4	4.0	2.9
hepatoma					
HLF	19	2.8	4.0	2.7	1.6
HLE	1.5	0.23	0.36	0.31	0.13
renal cancer					
ACHN	13	2.0	2.7	1.1	2.0
G-402	4.8	0.33	0.35	0.18	0.25
colon carcinoma					
COLO201	59	13	29	13	9.3
COLO320DM	14	2.0	3.2	2.1	0.99

[a] See the Experimental Section. ED_{50} was the concentration of compound which afforded a 50% reduction in cell number after 4 h.

necessary for inhibition of Topo II or antitumor activity in vitro and in vivo. Further detailed biological effects of the selected compounds are being evaluated.

Experimental Section

All melting points were determined on a Yanagimoto MP-3 micro melting point apparatus and are uncorrected. The instruments used were as follows: elemental analyses, Yanagimoto MT-3; IR spectra, Hitachi I-3000 IR spectrometer; specific rotation, Horiba SEPA-200 spectropolarimeter; FAB-MS spectra, JMS-DX303; ^1H NMR spectra, FT NMR JNM-FX90Q spectrometer, JEOL JNM-GSX400 spectrometer. Chemical shifts were reported in ppm (δ) downfield from tetramethylsilane as the internal standard, and coupling constants are given in hertz. Column chromatography was carried out on Merck silica gel (Kieselgel 60; 70–230 mesh). Preparative thin-layer chromatography (PTLC) was carried out on precoated silica gel plates (Merck Kieselgel $60F_{254}$, 0.5-mm thickness). All new compounds were characterized by melting point, optical rotation, ^1H NMR, FAB-MS, and IR spectral analyses as well as elemental analyses.

4′-Demethyl-4′-*O*-(benzyloxycarbonyl)-4β-allyl-4-desoxypodophyllotoxin (4). BF_3·Et_2O (0.6 mL) was added to a mixture of 4′-demethyl-4′-*O*-(benzyloxycarbonyl)epipodophyllotoxin (**3**) (1 g, 1.87 mmol)[17] and trimethylallylsilane (426 mg, 3.73 mmol) in CH_2Cl_2 (15 mL) at 0 °C with stirring. After 1 h, the reaction mixture was quenched with pyridine (0.6 mL), and the mixture was extracted with AcOEt (100 mL). The extract was washed consecutively with cold 1 N HCl and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo at 30 °C. The residue was purified by silica gel column chromatography with $CHCl_3$. Recrystallization from Et_2O gave **3c** (1 g, 95.8%): ^1H NMR ($CDCl_3$) δ 7.30–7.43 (5H, m, $PhCH_2OCO$), 6.72 (1H, s, 5-H), 6.46 (1H, s, 8-H), 6.31 (2H, s, 2′,6′-H), 5.94 (1H, d, J = 1.5 Hz, OCH_2O), 5.93 (1H, d, J = 1.5 Hz, OCH_2O), 5.80 (1H, ddt, J = 17, 10.5, 6.5 Hz, $CH_2CH{=}CH_2$), 5.25 (2H, s, $PhCH_2OCO$), 5.12 (1H, dq, J = 17, 2, 1.5 Hz, $CH_2CH{=}CH_2$), 5.11 (1H, dq, J = 10.5, 1.5 Hz, $CH_2CH{=}CH_2$), 4.58 (1H, d, J = 5 Hz, 1-H), 4.25 (2H, m, 11-H), 3.68 (6H, s, 3′,5′-OCH_3), 3.27 (1H, m, 4-H), 3.07 (1H, dd, J = 14.5, 5 Hz, 2-H), 2.93 (1H, m, 3-H), 2.57 (1H, m, $CH_2CH{=}CH_2$), 2.42 (1H, m, $CH_2CH{=}CH_2$). Anal. ($C_{32}H_{30}O_9$) C, H.

4′-Demethyl-4′-*O*-(benzyloxycarbonyl)-4β-(formylmethyl)-4-desoxypodophyllotoxin (5). A mixture of 4

(1 g, 1.79 mmol), *N*-methylmorphorine *N*-oxide (NMO) (225 mg, 1.92 mmol) and osmic acid (OsO_4) (43 mg, 0.17 mmol) in acetone (13 mL) was stirred for 1 h at room temperature. A saturated solution of $NaHSO_3$ (5 mL) mixed with ice (50 g) was added, and the reaction mixture was extracted with AcOEt. The extract was washed consecutively with 1 N HCl and H_2O, dried over $MgSO_4$, and concentrated in vacuo at 30 °C. The residue was purified by silica gel column chromatography with $CHCl_3$/MeOH (20/1). The oxidation product (1 g) and $Pb(OAc)_4$ (795 mg, 1.79 mmol) in benzene (80 mL) was stirred for 0.5 h at room temperature. The reaction mixture was filtered off and then washed with AcOEt. The filtrate was concentrated in vacuo at 30 °C. The residue was purified by silica gel column chromatography with $CHCl_3$/MeOH (20/1). Recrystallization from Et_2O gave **5** (934 mg, 92.9%): ^{1}H NMR (DMSO-d_6) δ 9.73 (1H, s, C*H*O), 7.39 (5H, s, CO_2CH_2*Ph*), 6.88 (1H, s, 5-H), 6.46 (1H, s, 8-H), 6.35 (2H, s, 2′,6′-H), 5.96 (2H, s, OCH_2O), 5.23 (2H, s, CO_2CH_2Ph), 4.54 (1H, d, J = 3.6 Hz, 1-H), 3.96–4.32 (1H, m, 11α-H), 3.63 (6H, s, 3′,5′-OCH_3), 3.48–3.92 (1H, m, 11β-H), 2.56–3.40 (5H, m, 2,3,4-H, CH_2CHO). Anal. ($C_{31}H_{28}O_{10}$) C, H.

4′-Demethyl-4′-*O*-(benzyloxycarbonyl)-4β-(2-oxo-2-hydroxyethyl)-4-desoxypodophyllotoxin (6). A solution of CrO_3 (178 mg, 1.78 mmol), concentrated H_2SO_4 (0.2 mL), and H_2O (0.9 mL) was added dropwise to acetone solution (20 mL) of **5** (1 g, 1.78 mmol) at 0 °C. After stirring for 3 h, i-PrOH (1 mL) was added. The mixture was extracted with AcOEt. The extract was washed with saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo at 30 °C. The residue was purified by silica gel column chromatography with $CHCl_3$/MeOH (10/1). Recrystallization from Et_2O gave **6** (609 mg, 59.4%): ^{1}H NMR (DMSO-d_6) δ 12.5–13.0 (1H, b, CO_2H), 7.40 (5H, s, CO_2CH_2*Ph*), 6.94 (1H, s, 5-H), 6.46 (1H, s, 8-H), 6.34 (2H, s, 2′,6′-H), 5.96 (2H, s, OCH_2O), 5.23 (2H, s, CO_2CH_2Ph), 4.52 (1H, d, J = 5.6 Hz, 1-H), 4.27 (1H, dd, J = 7.6, 3.8 Hz, 11α-H), 3.86 (1H, m, 11β-H), 3.63 (6H, s, 3′,5′-OCH_3), 3.20–3.40 (3H, m, 2,3,4-H), 2.80–3.00 (2H, m, CH_2CO_2H). Anal. ($C_{31}H_{28}O_{11}$) C, H.

4′-Demethyl-4β-(2-oxo-2-hydroxyethyl)-4-desoxypodophyllotoxin (7). **6** (200 mg, 0.347 mmol) was reduced for 3 h on 5% Pd/C (40 mg) with H_2 (2 atm) in CH_2Cl_2 (10 mL). The reaction mixture was filtered off and washed with AcOEt, and the filtrate was concentrated in vacuo at 30 °C. Recrystallization from CH_3CN gave **7** (98 mg, 66.3%): ^{1}H NMR (DMSO-d_6) δ 12.3–12.4 (1H, b, COO*H*), 8.22 (1H, s, 4′-OH), 6.91 (1H, s, 5-H), 6.43 (1H, s, 8-H), 6.21 (2H, s, 2′,6′-H), 5.96 (1H, s, OCH_2O), 5.95 (1H, s, OCH_2O), 4.40 (1H, d, J = 5.3 Hz, 1-H), 4.24 (1H, dd, J = 8.1, 4.1 Hz, 11α-H), 3.84 (1H, dd, J = 1.98, 8.91 Hz, 11β-H), 3.62 (6H, s, 3′,5′-OCH_3), 3.10–3.57 (3H, m, 2,3,4-H), 2.80–3.00 (2H, m, CH_2COOH). Anal. ($C_{23}H_{22}O_9$) C, H.

4′-Demethyl-4β-(2-hydroxyethyl)-4-desoxypodophyllotoxin (8). $Zn(BH_4)_2/Et_2O$ (27 mL, 4.14 mmol) was added dropwise to a solution of **5** (543 mg, 0.97 mmol) in THF (60 mL) with stirring at −5 to 0 °C. After being stirred for 30 min, the reaction mixture was added to cold 1 N HCl. The mixture was extracted with AcOEt, washed with saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo at 30 °C. The residue was purified by silica gel column chromatography with AcOEt/*n*-hexane (1:1). The product (435 mg) was reduced for 2 h on 5% Pd/C (43 mg) with H_2 (1 atm) in CH_3OH (10 mL). The reaction mixture was filtered off and washed with AcOEt, and the filtrate was concentrated in vacuo at 30 °C. The residue was purified by silica gel column chromatography with $CHCl_3$/CH_3OH (20/1). Recrystallization from Et_2O gave **8** (309 mg, 74.5%): ^{1}H NMR (DMSO-d_6) δ 6.77 (1H, s, 5-H), 6.46 (1H, s, 8-H), 6.29 (2H, s, 2′,6′-H), 5.94 (1H, d, J = 1.0 Hz, OCH_2O), 5.93 (1H, d, J = 1.0 Hz, OCH_2O), 5.38 (1H, s, 4′-OH), 4.55 (1H, d, J = 4.5 Hz, 1-H), 4.33 (1H, m, 11α-H), 4.16 (1H, m, 11β-H), 3.8–3.7 (2H, overlapped, C*H*$_2$OH), 3.77 (6H, s, 3′,5′-OCH_3), 3.32 (1H, m, 4-*H*), 2.99 (2H, m, 2,3-H), 2.03 (1H, m, C*H*$_2$CH$_2$OH), 1.74 (1H, m, C*H*$_2$CH$_2$OH), 1.45 (1H, t, J = 4.0 Hz, CH$_2$O*H*). Anal. ($C_{23}H_{24}O_8$) C, H.

4′-Demethyl-4′-*O*-(benzyloxycarbonyl)-4β-(2-formylethyl)-4-desoxypodophyllotoxin (9). 2 M BH_3·Me_2S/THF (4.3 mL, 8.6 mmol) was added dropwise to a solution of **4** (4 g, 7.17 mmol) in THF (40 mL) at 0 °C and then stirred for 1 h at room temperature. The reaction mixture was concentrated in vacuo at 30 °C. The residue was oxidized by pyridinium chlorochromate (3.0 g, 13.9 mmol) in CH_2Cl_2 (10 mL) for 10 h at room temperature. To the reaction mixture were added AcOEt (100 mL) and florisil (10 g). The reaction mixture was then filtered and washed with AcoEt, and the filtrate was concentrated in vacuo at 30 °C. The residue was purified by silica gel column chromatography with *n*-hexane/AcOEt (1/1). Recrystallization from Et_2O gave **9** (1.85 g, 44.9%): ^{1}H NMR ($CDCl_3$) δ 9.83 (1H, s, C*H*O), 7.40 (5H, m, CO_2CH_2*Ph*), 6.83 (1H, s, 5-H), 6.45 (1H, s, 8-H), 6.28 (2H, s, 2′,6′-H), 5.94 (2H, s, OCH_2O), 5.25 (2H, s, CO_2CH_2Ph), 4.58 (1H, d, J = 4.6 Hz, 1-H), 4.37 (1H, m, 11α-H), 4.11 (1H, m, 11β-H), 3.67 (6H, s, 3′,5′-OCH_3), 3.04–3.10 (1H, m, 4-H), 2.86–3.00 (2H, m, 2,3-H), 2.49–2.67 (2H, m, C*H*$_2$CHO), 1.83–2.21 (2H, m, C*H*$_2$CH$_2$CHO). Anal. ($C_{32}H_{30}O_{10}$) C, H.

4′-Demethyl-4β-(3-hydroxypropyl)-4-desoxypodophyllotoxin (10). Compound **10** (214 mg, 48.5%, recrystallized from Et_2O) was synthesized from **9** (574 mg, 1.0 mmol) and $Zn(BH_4)_2/Et_2O$ (27 mL, 4.14 mmol) by the method described for the synthesis of **8**: ^{1}H NMR (DMSO-d_6) δ 8.19 (1H, s, 4′-OH), 6.83 (1H, s, 5-H), 6.42 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.94 (1H, d, J = 1.0 Hz, OCH_2O), 5.93 (1H, d, J = 1.0 Hz, OCH_2O), 4.4 (1H, d, J = 5.5 Hz, 1-H), 4.41 (1H, t, J = 5.0 Hz, CH$_2$O*H*), 4.36 (1H, t, J = 8.0 Hz, 11α-H), 4.10 (1H, dd, J = 11.0, 8.0 Hz, 11β-H), 3.61 (6H, s, 3′,5′-OCH_3), 3.44 (2H, q, J = 5.0 Hz, C*H*$_2$OH), 2.86–3.11 (3H, m, 2,3,4-H), 1.82 (1H, m, C*H*$_2$CH$_2$CH$_2$OH), 1.53 (1H, m, CH$_2$C*H*$_2$CH$_2$OH), 1.45 (2H, m, C*H*$_2$C*H*$_2$CH$_2$OH). Anal. ($C_{24}H_{26}O_8$) C, H.

General Synthetic Method for Type 12 Compounds (12a–y). Compound **5** (91 mg, 0.162 mmol) or **9** (93 mg, 0.162 mmol) was added to a mixture of the appropriate amine (0.167 mmol), AcOH (0.1 mL), and $NaCNBH_3$ (10 mg, 0.19 mmol) in CH_3OH (5 mL) at 0 °C with stirring. After the mixture was stirred at room temperature for 1 h, AcOEt (100 mL) was added, washed with cold saturated $NaHCO_3$, followed by washing to pH 6–7 with H_2O. The extract was dried over $MgSO_4$ and concentrated in vacuo below 30 °C. The residue was purified by silica gel column chromatography with $CHCl_3$/CH_3OH (5/1). The main spot was collected, concentrated in vacuo below 30 °C, and dried in vacuo at room temperature. The residue was reduced for 10 h at room temperature on 5% Pd–C (20 mg) with H_2 (1 atm) in MeOH (10 mL). The reaction mixture was filtered off and washed with AcOEt, and then the filtrate was concentrated below 30 °C. The residue was purified by preparative TLC with $CHCl_3$/MeOH (10/1). The eluate with $CHCl_3$–MeOH (10/1) was concentrated

below 30 °C. To the residue in $ClCH_2CH_2Cl$ (2 mL) was added 4 N HCl–AcOEt (0.1 mL). The reaction mixture was concentrated in vacuo at below 30 °C. Recrystallization from Et_2O gave compounds **12a–y**, respectively.

4′-Demethyl-4β-[2-(*N*,*N*-dimethylamino)ethyl]-4-desoxypodophyllotoxin hydrochloride (12a): yield 81.8%; ^1H NMR (CD_3OD) δ 6.81 (1H, s, 5-H), 6.47 (1H, s, 8-H), 6.29 (2H, s, 2′,6′-H), 5.93 (1H, d, J = 1.0 Hz, OCH_2O), 5.92 (1H, d, J = 1.0 Hz, OCH_2O), 4.56 (1H, d, J = 5.5 Hz, 1-H), 4.42 (1H, dd, J = 8.5, 7.5 Hz, 11α-H), 4.17 (1H, dd, J = 11, 8.5 Hz, 11β-H), 3.71 (6H, s, 3′,5′-OCH_3), 3.30 (2H, overlapped, CH_2N), 3.24 (1H, m, 4-H), 3.17 (1H, dd, J = 14.5, 5.5 Hz, 2-H), 3.05 (1H, m, 3-H), 2.87 (6H, s, $N(CH_3)_2$), 2.19 (1H, m, CH_2CH_2N), 1.91 (1H, m, CH_2CH_2N). Anal. ($C_{25}H_{29}NO_7$·HCl·H_2O) C, H, N.

4′-Demethyl-4β-[3-(*N*,*N*-dimethylamino)propyl]-4-desoxypodophyllotoxin hydrochloride (12b) was prepared by using **9** as starting material: yield 61.5%; ^1H NMR (DMSO-d_6) δ 9.74 (1H, b, N^+H), 8.22 (1H, s, 4′-OH), 6.89 (1H, s, 5-H), 6.45 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.98 (1H, d, J = 1.0 Hz, OCH_2O), 5.96 (1H, d, J = 1.0 Hz, OCH_2O), 4.43 (1H, d, J = 5.5 Hz, 1-H), 4.38 (1H, t, J = 8.0 Hz, 11α-H), 4.12 (1H, dd, J = 11.0, 8.0 Hz, 11β-H), 3.62 (6H, s, 3′,5′-OCH_3), 3.12 (1H, dd, J = 14.5, 5.5 Hz, 2-H), 3.08 (2H, m, CH_2N), 3.05 (1H, m, 4-H), 2.86 (1H, m, 3-H), 2.75 (6H, s, $N(CH_3)_2$), 1.84 (1H, m, $CH_2CH_2CH_2N$), 1.76 (1H, m, $CH_2CH_2CH_2N$), 1.61 (1H, m, $CH_2CH_2CH_2N$), 1.41 (1H, m, $CH_2CH_2CH_2N$). Anal. ($C_{26}H_{31}NO_7$·HCl·H_2O) C, H, N.

4′-Demethyl-4β-[2-[*N*-methyl-*N*-(2-hydroxyethyl)amino]ethyl]-4-desoxypodophyllotoxin hydrochloride (12c): yield 72.0%; ^1H NMR (CD_3OD) δ 6.83 (1H, s, 5-H), 6.47 (1H, s, 8-H), 6.29 (2H, s, 2′,6′-H), 5.93 (1H, d, J = 1.0 Hz, OCH_2O), 5.92 (1H, d, J = 1.0 Hz, OCH_2O), 4.56 (1H, d, J = 5.5 Hz, 1-H), 4.42 (1H, dd, J = 8.5, 7.5 Hz, 11α-H), 4.14 (1H, dd, J = 11.0, 8.5 Hz, 11β-H), 3.86 (2H, t, J = 5.5 Hz, NCH_2CH_2OH), 3.71 (6H, s, 3′,5′-OCH_3), 3.30 (2H, m, $CH_2CH_2NCH_2CH_2OH$), 3.2–3.3 (3H, m, 4-H, CH_2CH_2OH), 3.17 (1H, dd, J = 14.5, 5.5 Hz, 2-H), 3.05 (1H, m, 3-H), 2.91 (3H, s, NCH_3), 2.23 (1H, m, $CH_2CH_2NCH_2CH_2OH$), 1.91 (1H, m, $CH_2CH_2NCH_2CH_2OH$). Anal. ($C_{26}H_{31}NO_8$·HCl·$2H_2O$) C, H, N.

4′-Demethyl-4β-[2-[*N*-methyl-*N*-(1,3-dihydroxy-2-propyl]amino]ethyl]-4-desoxypodophyllotoxin hydrochloride (12d): yield 67.0%; ^1H NMR (DMSO-d_6) δ 9.29 (1H, b, N^+H), 7.96 (1H, s, 4′-OH), 6.95 (1H, s, 5-H), 6.45 (1H, s, 8-H), 6.23 (2H, s, 2′,6′-H), 5.97 (1H, d, J = 1.0 Hz, OCH_2O), 5.96 (1H, d, J = 1.0 Hz, OCH_2O), 5.24 (2H, b, $CH(CH_2OH)_2$), 4.45 (1H, d, J = 5.5 Hz, 1-H), 4.36 (1H, t, J = 8.0 Hz, 11α-H), 4.17 (1H, dd, J = 11.0, 8.0 Hz, 11β-H), 3.73 (4H, b, $CH(CH_2OH)_2$), 3.64 (6H, s, 3′,5′-OCH_3), 3.2–3.5 (2H, m, CH_2N), 3.39 (1H, b, $CH(CH_2OH)$, 3.15 (1H, m, 4-H), 3.09 (1H, overlapped, 2-H), 2.89 (1H, m, 3-H), 2.81 (3H, b, NCH_3), 2.28 (1H, m, CH_2CH_2N), 1.91 (1H, m, CH_2CH_2N). Anal. ($C_{27}H_{33}NO_9$·HCl·$0.5H_2O$) C, H, N.

4′-Demethyl-4β-[2-[*N*-methyl-*N*-(2-methoxyethyl)amino]ethyl]-4-desoxypodophyllotoxin hydrochloride (12e): yield 92.0%; ^1H NMR (DMSO-d_6) δ 9.29 (1H, b, N^+H), 8.26 (1H, s, 4′-OH), 6.97 (1H, d, J = 7.9 Hz, 5-H), 6.46 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.99 (1H, s, OCH_2O), 5.97 (1H, s, OCH_2O), 4.45 (1H, d, J = 5.6 Hz, 1-H), 4.36 (1H, t, J = 7.2 Hz, 11α-H), 4.17 (1H, m, 11β-H), 3.69 (2H, m, CH_2OCH_3), 3.62 (6H, s, 3′,5′-OCH_3), 3.30 (3H, s, CH_2OCH_3), 2.82–3.39 (7H, m, 2,3,4-H), CH_2NCH_2), 2.76 (3H, s, NCH_3), 2.29 (1H, m, CH_2CH_2N), 1.86 (1H, m, CH_2CH_2N). Anal. ($C_{27}H_{33}NO_8$·HCl·H_2O) C, H, N.

4′-Demethyl-4β-[2-(*N*-methyl-*N*-hexylamino)ethyl]-4-desoxypodophyllotoxin hydrochloride (12f): yield 66.1%; ^1H NMR (DMSO-d_6) δ 9.73, 9.69 (1H, b, N^+H), 8.23 (1H, s, 4′-OH), 7.00, 6.96 (1H, d, J = 7.9 Hz, 5-H), 6.46 (1H, s, 8-H), 6.19 (2H, s, 2′,6′-H), 5.99 (1H, s, OCH_2O), 5.97 (1H, s, OCH_2O), 4.45 (1H, d, J = 5.5 Hz, 1-H), 4.36 (1H, t, J = 8.0 Hz, 11α-H), 4.14, 4.11 (1H, dd, J = 14.5, 5.5 Hz, 11β-H), 3.62 (6H, s, 3′,5′-OCH_3), 3.20 (1H, m, 4-H), 3.14 (1H, dd, J = 14.5, 5.5 Hz, 2-H), 3.09 (1H, b, $NCH_2(CH_2)_4$), 2.96 (1H, b, $NCH_2(CH_2)_4$), 2.88 (1H, b, 3-H), 2.73 (3H, s, NCH_3), 2.23 (1H, m, $CH_2CH_2NCH_3$), 1.82 (1H, m, $CH_2CH_2NCH_3$), 1.63 (2H, b, $NCH_2CH_2(CH_2)_3$), 1.29 (6H, b, $NCH_2CH_2(CH_2)_3CH_3$), 0.88 (3H, t, J = 7.0 Hz, $N(CH_2)_5CH_3$). Anal. ($C_{30}H_{39}NO_7$·HCl·H_2O) C, H, N.

4′-Demethyl-4β-[2-[α-(hydroxymethyl)pyrrolidino]ethyl]-4-desoxypodophyllotoxin hydrochloride (12g): yield 59.3%; ^1H NMR (DMSO-d_6) δ 9.63 (1H, b, N^+H), 8.22 (1H, s, 4′-OH), 7.02 (1H, d, s, 5-H), 6.45 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.99 (1H, s, OCH_2O), 5.96 (1H, s, OCH_2O), 5.44 (1H, t, J = 5 Hz, CH_2OH), 4.44 (1H, d, J = 5.5 Hz, 1-H), 4.35 (1H, t, J = 8.0 Hz, 11α-H), 4.13 (1H, dd, J = 11.0, 8.0 Hz, 11β-H), 3.77 (1H, m, CH_2OH), 3.62 (6H, s, 3′,5′-OCH_3), 3.54 (1H, m, $NCHCH_2OH$ in pyrrolidine ring), 3.54 (1H, m, NCH_β in pyrrolidine ring), 3.54 (1H, m, CH_2CH_2N), 3.13 (1H, dd, J = 15.0, 5.0 Hz, 2-H), 3.13 (1H, m, $NCH\alpha$ in pyrrolidine ring), 3.13 (1H, m, 4-H), 3.00 (1H, m, CH_2N), 2.86 (1H, m, 3-H), 2.27 (1H, m, CH_2CH_2N), 2.08 (1H, m, $NCH(CH_2OH)CH_\beta CH_2$ in pyrrolidine ring), 1.95 (1H, m, $NCH_2CH_\beta CH_2$ in pyrrolidine ring), 1.90 (1H, m, CH_2CH_2N), 1.86 (1H, m, $NCH_2CH_\alpha CH_2$ in pyrrolidine ring), 1.76 (1H, m, $NCH(CH_2OH)CH_\alpha CH_2$ in pyrrolidine ring). Anal. ($C_{28}H_{33}NO_8$·HCl·$0.5H_2O$) C, H, N.

4′-Demethyl-4β-[2-(*N*-methyl-*N*-cyclohexylamino)ethyl]-4-desoxypodophyllotoxin hydrochloride (12h): yield 81.0%; ^1H NMR (DMSO-d_6) δ 9.94 (1H, b, N^+H), 8.23 (1H, s, 4′-OH), 7.05, 7.02 (1H, d, 5-H), 6.46 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.99 (1H, d, J = 1.0 Hz, OCH_2O), 5.97 (1H, d, J = 1.0 Hz, OCH_2O), 4.45 (1H, d, J = 5.5 Hz, 1-H), 4.38 (1H, m, 11α-H), 4.15 (1H, m, 11β-H), 3.62 (6H, s, 3′,5′-OCH_3), 3.20 (1H, m, $NCHCH_2$ in cyclohexane ring), 3.12 (2H, m, 2,4-H), 2.87 (1H, m, 3-H), 2.80 (1H, m, CH_2N), 2.65 (3H, s, NCH_3), 2.27 (1H, m, CH_2CH_2N), 1.97 (2H, m, $NCHCH_\beta CH_2$ in cyclohexane ring), 1.86 (1H, m, CH_2CH_2N), 1.84 (2H, m, $NCHCH_2CH_B$ in cyclohexane ring), 1.62 (1H, m, $NCHCH_2CH_2CH_B$ in cyclohexane ring), 1.42 (1H, m, $NCHCH_\alpha CH_2$ in cyclohexane ring), 1.30 (2H, m, $NCHCH_2CH_\alpha$ in cyclohexane ring), 1.13 (1H, m, $NCHCH_2CH_2CH_\alpha$ in cyclohexane ring). Anal. ($C_{30}H_{37}NO_7$·HCl·H_2O) C, H, N.

4′-Demethyl-4β-[2-(1-piperidino)ethyl]-4-desoxypodophyllotoxin hydrochloride (12i): yield 61.0%; ^1H NMR (DMSO-d_6) δ 9.48 (1H, b, N^+H), 8.23 (1H, s, 4′-OH), 6.96 (1H, s, 5-H), 6.46 (1H, s, 8-H), 6.19 (2H, s, 2′,6′-H), 5.99 (1H, d, J = 0.5 Hz, OCH_2O), 5.96 (1H, d, J = 0.5 Hz, OCH_2O), 4.45 (1H, d, J = 5.5 Hz, 1-H), 4.37 (1H, t, J = 8.0 Hz, 11α-H), 4.12 (1H, dd, J = 11.0, 8.0 Hz, 11β-H), 3.62 (6H, s, 3′,5′-OCH_3), 3.44 (2H, m, $NCH_\beta CH_2$ in piperidine ring), 3.26 (1H, m, CH_2CH_2N), 3.13 (1H, dd, J = 14.5, 5.5 Hz, 2-H), 3.13 (1H, m, 4-H), 2.89 (1H, m, 3-H), 2.86 (2H, m, $NCH_\alpha CH_2$ in piperidine ring), 2.24 (1H, m, CH_2CH_2N), 1.85 (1H, m, CH_2CH_2N), 1.79 (2H, m, $NCH_2CH_\beta CH_2$ in piperidine ring), 1.68 (2H, m, $NCH_2CH_\beta CH_2$ in piperidine ring), 1.68 (1H, m, $NCH_2CH_2CH_\beta$ in piperidine ring), 1.38

(1H, m, $NCH_2CH_2CH_\alpha$ in piperidine ring). Anal. ($C_{28}H_{33}NO_7$·HCl·H_2O) C, H, N.

4′-Demethyl-4β-[2-(*N*-methyl-*N*-benzylamino)ethyl]-4-desoxypodophyllotoxin hydrochloride (12j): yield 69.8%; ^{1}H NMR (DMSO-d_6) δ 10.76 (1H, b, N^+H), 7.96 (1H, b, 4′-OH), 7.55 (2H, b, *Ph*), 7.46 (3H, b, *Ph*), 6.90, 6.86 (1H, s, 5-H), 6.45 (1H, s, 8-H), 6.22 (2H, s, 2′,6′-H), 5.98 (1H, d, J = 1.0 Hz, OCH_2O), 5.96 (1H, d, J = 1.0 Hz, OCH_2O), 4.45 (1H, d, J = 5.5 Hz, 1-H), 4.39 (1H, b, CH_2-Ph), 4.31 (1H, m, 11α-H), 4.22 (1H, b, CH_2Ph), 4.11 (1H, t, J = 8.5 Hz, 11β-H), 3.64 (6H, s, 3′,5′-OCH_3), 3.10 (1H, overlapped, 2-H), 2.89 (1H, m, 3-H), 2.67 (3H, b, NCH_3), 2.33 (1H, m, CH_2CH_2N), 1.96 (1H, m, CH_2CH_2N), proton signals of 4-H and CH_2CH_2N were not clearly observed since their signals were extremely broadened. Anal. ($C_{31}H_{33}NO_7$·HCl·2.5H_2O) C, H, N.

4′-Demethyl-4β-(2-morpholinoethyl)-4-desoxypodophyllotoxin hydrochloride (12k): yield 70.0%; ^{1}H NMR (DMSO-d_6) δ 10.48 (1H, b, N^+H), 8.22 (1H, b, 4′-OH), 6.96 (1H, s, 5-H), 6.46 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.99 (1H, s, OCH_2O), 5.97 (1H, s, OCH_2O), 4.45 (1H, d, J = 5.5 Hz, 1-H), 4.37 (1H, t, J = 8.0 Hz, 11α-H), 4.14 (1H, dd, J = 11.0, 8.0 Hz, 11β-H), 3.97 (2H, b, NCH_2CH_2O in morpholine ring), 3.73 (2H, t, J = 12.0 Hz, NCH_2CH_2O in morpholine ring), 3.62 (6H, s, 3′,5′-OCH_3), 3.45 (1H, d, J = 12.0 Hz, $NCH_\beta CH_2O$ in morpholine ring), 3.40 (1H, d, J = 12.0 Hz, $NCH_\alpha CH_2O$ in morpholine ring), 3.30 (1H, m, CH_2N), 3.15 (1H, m, 4-H), 3.11 (1H, dd, J = 14.0, 5.5 Hz, 2-H), 3.06 (3H, m, CH_2CHN, NCH_2CH_2O in morpholine ring), 2.88 (1H, m, 3-H), 2.28 (1H, m, CH_2-CH_2N), 1.88 (1H, m, CH_2CH_2N). Anal. ($C_{27}H_{31}NO_8$·HCl·H_2O) C, H, N.

4′-Demethyl-4β-[2-(*N*,*N*,*N*′-trimethylhydrazino)ethyl]-4-desoxypodophyllotoxin hydrochloride (12l): yield 89.2%; ^{1}H NMR (DMSO-d_6) δ 10.76 (1H, b, N^+H), 8.10 (1H, b, 4′-OH), 6.81 (1H, s, 5-H), 6.45 (1H, s, 8-H), 6.30 (2H, s, 2′,6′-H), 5.93 (1H, d, J = 1.0 Hz, OCH_2O), 5.92 (1H, d, J = 1.0 Hz, OCH_2O), 4.55 (1H, d, J = 5.5 Hz, 1-H), 4.43 (1H, dd, J = 8.5, 7.5 Hz, 11α-H), 4.16 (1H, dd, J = 11.0, 8.5 Hz, 11β-H), 3.71 (6H, s, 3′,5′-OCH_3), 3.30 (2H, m, CH_2N), 3.24 (1H, m, 4-H), 3.15 (1H, dd, J = 14.5, 5.5 Hz, 2-H), 3.02 (1H, m, 3-H), 2.80 (6H, s, $N(CH_3)_2$), 2.72 (3H, s, $N(CH_3)N$), 2.13 (1H, m, CH_2CH_2N), 1.80 (1H, m, CH_2-CH_2N). Anal. ($C_{26}H_{32}N_2O_7$·HCl·0.5H_2O) C, H, N.

4′-Demethyl-4β-[2-(*N*,*N*′-dimethyl-*N*′-phenylhydrazino)ethyl]-4-desoxypodophyllotoxin hydroxhloride (12m): yield 67.4%; ^{1}H NMR (DMSO-d_6) δ 7.17 (2H, dd, J = 8.5, 7.0 Hz, N*Ph*), 7.01 (2H, d, J = 8.5 Hz, N*Ph*), 6.67 (1H, t, J = 7.0 Hz, N*Ph*), 6.66 (1H, s, 5-H), 6.39 (1H, s, 8-H), 6.18 (2H, s, 2′,6′-H), 5.92 (1H, s, OCH_2O), 5.90 (1H, s, OCH_2O), 4.38 (1H, d, J = 5.5 Hz, 1-H), 4.30 (1H, t, J = 8.0 Hz, 11α-H), 4.02 (1H, dd, J = 12.0, 8.0 Hz, 11β-H), 3.61 (6H, s, 3′,5′-OCH_3), 3.17 (1H, m, 4-H), 3.09 (1H, dd, J = 14.5, 5.5 Hz, 2-H), 2.84 (1H, m, 3-H), 2.75 (3H, s, $N(CH_3)$Ph), 2.69 (2H, m, $CH_2N(CH_3)$), 2.38 (3H, s, $N(CH_3)$Ph), 1.93 (1H, m, CH_2CH_2N), 1.53 (1H, m, CH_2-CH_2N). Proton signals of N^+H and 4′-OH were not clear. Anal. ($C_{31}H_{34}N_2O_7$·HCl·H_2O) C, H, N.

4′-Demethyl-4β-[2-[[2-(*N*,*N*-dimethylamino)ethyl]amino]ethyl]-4-desoxypodophyllotoxin dihydrochloride (12n): yield 55.0%; ^{H} NMR (CD_3OD) δ 6.80 (1H, s, 5-H), 6.47 (1H, s, 8-H), 6.27 (2H, s, 2′,6′-H), 5.95 (1H, d, J = 1.0 Hz, OCH_2O), 5.94 (1H, d, J = 1.0 Hz, OCH_2O), 4.55 (1H, d, J = 4.5 Hz, 1-H), 4.41 (1H, m, 11α-H), 4.26 (1H, m, 11β-H), 3.76 (6H, s, 3′,5′-OCH_3), 3.61 (2H, m, $NHCH_2CH_2N$), 3.47 (2H, br, $NHCH_2CH_2N$), 3.27 (1H, m, 4-H), 3.15 (2H, m, $CH_2NHCH_2CH_2N$), 3.05 (2H, overlapped, 2,3-H), 2.96 (6H, s, $N(CH_3)_2$), 2.27 (1H, m, CH_2-$CH_2NHCH_2CH_2N$), 2.04 (1H, m, $CH_2CH_2NHCH_2CH_2N$). Anal. ($C_{27}H_{34}N_2O_7$·2HCl·2.5H_2O) C, H, N.

4′-Demethyl-4β-[2-[*N*-[2-(*N*′,*N*′-dimethylamino)ethyl]-*N*-methylamino]ethyl]-4-desoxypodophyllotoxin dihydrochloride (12o): yield 71.7%; ^{1}H NMR (DMSO-d_6) δ 6.95 (1H, s, 5-H), 6.44 (1H, s, 8-H), 6.23 (2H, s, 2′,6′-H), 5.95 (1H, d, J = 1.0 Hz, OCH_2O), 5.94 (1H, d, J = 1.0 Hz, OCH_2O), 4.44 (1H, d, J = 4.5 Hz, 1-H), 4.36 (1H, t, J = 8.0 Hz, 11α-H), 4.16 (1H, dd, J = 11.0, 8.0 Hz, 11β-H), 3.64 (6H, s, 3′,5′-OCH_3), 3.42 (6H, b, $CH_2NCH_2CH_2N$), 3.17 (1H, m, 4-H), 3.11 (1H, dd, J = 14.0, 5.5 Hz, 2-H), 2.90 (1H, m, 3-H), 2.80 (6H, s, $N(CH_3)_2$), 2.74 (3H, s, NCH_3), 2.26 (1H, m, $CH_2CH_2NCH_2CH_2N$), 1.89 (1H, m, CH_2CH_2-NCH_2CH_2N). Proton signals of N^+H and 4′-OH were not clear. Anal. ($C_{28}H_{36}N_2O_7$·2HCl·H_2O) C, H, N.

4′-Demethyl-4β-[2-[*N*-[3-(*N*′,*N*′-dimethylamino)propyl]-*N*-methylamino]ethyl]-4-desoxypodophyllotoxin dihydrochloride (12p): yield 92.0%; ^{1}H NMR (DMSO-d_6) δ 8.26 (1H, s, 4′-OH), 7.03 (1H, s, 5-H), 6.45 (1H, s, 8-H), 6.19 (2H, s, 2′,6′-H), 5.99 (1H, s, OCH_2O), 5.97 (1H, s, OCH_2O), 4.44 (1H, d, J = 5.2 Hz, 1-H), 4.37 (1H, m, 11α-H), 4.34 (1H, dd, m, 11β-H), 3.62 (6H, s, 3′,5′-OCH_3), 2.90–3.40 (9H, m, $CH_2NCH_2CH_2CH_2N$, 2,3,4-H), 2.75 (9H, m, $N(CH_3)(CH_2)_3N(CH_3)_2$), 1.91–2.34 (4H, m, $CH_2CH_2NCH_2CH_2CH_2N$). Proton signals of N^+H were not clear. Anal. ($C_{29}H_{38}N_2O_7$·2HCl·3H_2O) C, H, N.

4′-Demethyl-4β-[2-[*N*-[6-(*N*′,*N*′-dimethylamino)hexyl]-*N*-methylamino]ethyl]-4-desoxypodophyllotoxin dihydrochloride (12q): yield 75.0%; ^{1}H NMR (DMSO-d_6) δ 11.0 (2H, b, N^+H), 8.26 (1H, s, 4′-OH), 7.05 (1H, s, 5-H), 6.44 (1H, s, 8-H), 6.19 (2H, s, 2′,6′-H), 5.99 (2H, s, OCH_2O), 4.18–4.43 (3H, m, 1, 11α,11β-H), 3.62 (6H, s, 3′,5′-OCH_3), 2.51–3.62 (9H, m, 2,3,4-H, $CH_2NCH_2(CH_2)_4$-CH_2N), 2.75 (6H, s, $N(CH_3)_2$), 2.74 (3H, s, NCH_3), 2.27 (1H, m, $CH_2CH_2NCH_2CH_2N$), 1.85 (1H, m, $CH_2CH_2NCH_2$-CH_2N), 1.33–1.65 (8H, m, $NCH_2(CH_2)_4CH_2N$). Anal. ($C_{32}H_{44}N_2O_7$·2HCl·1.5H_2O) C, H, N.

4′-Demethyl-4β-[2-[*N*-methyl-*N*-[2-(*N*′,*N*′-diethylamino)ethyl]amino]ethyl]-4-desoxypodophyllotoxin dihydrochloride (12r): yield 61.5%; ^{1}H NMR (DMSO-d_6) δ 11.17 (1H, b, N^+H), 8.20 (1H, b, 4′-OH), 7.05, 7.00 (1H, s, 5-H), 6.45 (1H, s, 8-H), 6.19 (2H, s, 2′,6′-H), 5.99 (1H, s, OCH_2O), 5.96 (1H, s, OCH_2O), 4.45 (1H, d, J = 5.6 Hz, 1-H), 4.37 (1H, t, J = 7.2 Hz, 11α-H), 4.10–4.30 (1H, m, 11β-H), 3.61 (6H, s, 3′,5′-OCH_3), 3.00–3.60 (12H, m, 4, 2-H, $CH_2NCH_2CH_2N(CH_2CH_3)_2$)), 2.90 (1H, m, 3-H), 2.81 (3H, s, NCH_3), 2.32 (1H, m, CH_2CH_2N), 1.91 (1H, m, CH_2-CH_2N), 1.26 (6H, t, $N(CH_2CH_3)_2$). Anal. ($C_{30}H_{40}N_2O_7$·2HCl·2H_2O) C, H, N.

4′-Demethyl-4β-[2-[*N*-methyl-*N*-[2-(1-piperidino)ethyl]amino]ethyl]-4-desoxypodophyllotoxin dihydrochloride (12s): yield 69.0%; ^{1}H NMR (DMSO-d_6) δ 11.41 (1H, b, N^+H), 10.72, 10.66 (1H, b, N^+H), 8.24 (1H, s, 4′-OH), 7.03, 6.97 (1H, s, 5-H), 6.47 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.99 (1H, s, OCH_2O), 5.97 (1H, s, OCH_2O), 4.45 (1H, d, J = 5.5 Hz, 1-H), 4.37 (1H, t, J = 7.5 Hz, 11α-H), 4.13–4.17 (1H, m, 11β-H), 3.63 (2H, m, NCH_2CH_2N, NCH_2CH_2N), 3.62 (6H, s, 3′,5′-OCH_3), 3.57 (2H, m, NCH_β-CH_2 in piperidine ring), 3.50 (2H, m, NCH_2CH_2N, NCH_2CH_2N), 3.45 (1H, m, $CH_2CH_2NCH_2CH_2N$), 3.16 (1H, m, 4-H), 3.14 (1H, dd, J = 14.5, 5.5 Hz, 2-H), 3.08 (1H, m, $CH_2CH_2NCH_2CH_2N$), 2.92 (2H, m, $NCH_\alpha CH_2$ in piperidine ring), 2.90 (1H, m, 3-H), 2.84 (3H, b, NCH_3), 2.30 (1H, m, CH_2CH_2N), 1.90 (1H, m, CH_2CH_2N), 1.7–1.9 (5H,

m, $NCH_2CH_2CH_\beta$ in piperidine ring), 1.42 (1H, m, $NCH_2CH_2CH_\alpha$ in piperidine ring). Anal. ($C_{31}H_{40}N_2O_7 \cdot 2HCl \cdot 2H_2O$) C, H, N.

4′-Demethyl-4β-[2-(4-piperidinopiperidin-1-yl)ethyl]-4-desoxypodophyllotoxin dihydrochloride (12t): yield 65.4%; ^1H NMR (DMSO-d_6 + D_2O (2:1)) δ 6.87 (1H, s, 5-H), 6.49 (1H, s, 8-H), 6.25 (2H, s, 2′,6′-H), 5.95 (2H, s, OCH_2O), 4.51 (1H, d, J = 5.5 Hz, 1-H), 4.44 (1H, t, J = 8.0 Hz, 11α-H), 4.13 (1H, dd, J = 11.0, 8.0 Hz, 11β-H), 3.68 (2H, m, $CHNCH_\beta CH_2$ in piperidinopiperidine ring), 3.65 (6H, s, 3′,5′-OCH_3), 3.43 (3H, m, $NCH_\beta CH_2CHN$ in piperidinopiperidine ring), 3.10–3.30 (1H, m, 4-H, CH_2CH_2N), 3.18 (1H, dd, J = 15.0, 5.5 Hz, 2-H), 2.40–3.10 (5H, overlapped, 3-H, $NCH_\alpha CH_2CHN$ in piperidinopiperidine ring, $CHNCH_\alpha CH_2$ in piperidinopiperidine ring), 2.30 (2H, m, $NCH_2CH_\beta CHN$ in piperidinopiperidine ring), 2.17 (1H, m, CH_2CH_2N), 1.80–2.00 (5H, m, CH_2CH_2N, $NCH_2CH_\alpha CHN$ in piperidinopiperidine ring, $CHNCH_2CH_\beta CH_2$ in piperidine ring), 1.60–1.80 (3H, m, $NCH_2CH_\alpha CH_\beta CH_2$ in piperidine ring), 1.43 (1H, m, $NCH_2CH_2CH_\alpha CH_2$ in piperidine ring). Anal. ($C_{33}H_{42}N_2O_7 \cdot 2HCl \cdot H_2O$) C, H, N.

4′-Demethyl-4β-[2-(4-methylpiperazin-1-yl)ethyl]-4-desoxypodophyllotoxin dihydrochloride (12u): yield 66.7%; ^1H NMR (DMSO-d_6) δ 6.91 (1H, s, 5-H), 6.43 (1H, s, 8-H), 6.23 (2H, s, 2′,6′-H), 5.94 (1H, d, J = 1.0 Hz, OCH_2O), 5.93 (1H, d, J = 1.0 Hz, OCH_2O), 4.43 (1H, d, J = 5.5 Hz, 1-H), 4.35 (1H, t, J = 8.0 Hz, 11α-H), 4.15 (1H, dd, J = 11.0, 8.0 Hz, 11β-H), 3.64 (6H, s, 3′,5′-OCH_3), 3.20–3.50 (10H, m, CH_2CH_2N, NCH_2CH_2N in piperazine ring), 3.16 (1H, m, 4-H), 3.08 (1H, dd, J = 14.5, 5.5 Hz, 2-H), 2.88 (1H, m, 3-H), 2.72 (3H, s, NCH_3), 2.17 (1H, m, CH_2CH_2N), 1.80 (1H, m, CH_2CH_2N). Proton signals of N^+H and 4′-OH were not clear. Anal. ($C_{28}H_{34}N_2O_7 \cdot 2HCl \cdot 2H_2O$) C, H, N.

4′-Demethyl-4β-[2-[*N*-methyl-*N*-(2-morpholinoethyl)amino]ethyl]-4-desoxypodophyllotoxin dihydrochloride (12v): yield 66.0%; ^1H NMR (DMSO-d_6) δ 11.30 (1H, b, N^+H), 10.78 (1H, b, N^+H), 8.23 (1H, s, 4′-OH), 7.00 (1H, b, 5-H), 6.46 (1H, s, 8-H), 6.20 (2H, s, 2′,6′-H), 5.99 (1H, s, OCH_2O), 5.97 (1H, s, OCH_2O), 4.45 (1H, d, J = 5.5 Hz, 1-H), 4.37 (1H, t, J = 8.0 Hz, 11α-H), 4.15 (1H, m, 11β-H), 3.62 (6H, s, 3′,5′-OCH_3), 3.16 (1H, m, 4-H), 3.14 (1H, dd, J = 14.5, 5.5 Hz, 2-H), 3.00–4.10 (14H, m, $CH_2CH_2NCH_2CH_2N$, NCH_2CH_2O in morpholine ring), 2.89 (1H, m, 3-H), 2.83 (3H, brs, NCH_3), 2.30 (1H, m, CH_2CH_2N), 1.91 (1H, m, CH_2CH_2N). Anal. ($C_{30}H_{38}N_2O_8 \cdot 2HCl \cdot 2H_2O$) C, H, N.

4′-Demethyl-4β-[2-[*N*-methyl-*N*-(4-pyridinylmethyl)amino]ethyl]-4-desoxypodophyllotoxin dihydrochloride (12w): yield 31.0%; ^1H NMR (DMSO-d_6) δ 8.85 (2H, m, pyridine ring), 8.02 (2H, m, pyridine ring), 6.90, 7.00 (1H, b, 5-H), 6.45 (1H, s, 8-H), 6.18 (2H, s, 2′,6′-H), 5.99 (1H, s, OCH_2O), 5.97 (1H, s, OCH_2O), 4.17–4.70 (5H, m, 1-H, NCH_2Py, 11-H), 3.61 (6H, s, 3′,5′-OCH_3), 3.00–4.00 (4H, m, $CH_2CH_2N(CH_3)CH_2$, 2,4-H), 2.90 (1H, m, 3-H), 2.67 (3H, s, NCH_3), 2.40 (1H, m, CH_2CH_2N), 2.00 (1H, m, CH_2CH_2N). Proton signals of N^+H and 4′-OH were not clear. Anal. ($C_{30}H_{32}N_2O_7 \cdot 2HCl \cdot 2H_2O$) C, H, N.

4′-Demethyl-4β-[2-[*N*-methyl-*N*-(2-pyridinylmethyl)amino]ethyl]-4-desoxypodophyllotoxin dihydrochloride (12x): yield 57.0%; ^1H NMR (DMSO-d_6) δ 8.68 (1H, d, J = 5.0 Hz, pyridine ring), 7.99 (1H, m, pyridine ring), 7.76 (1H, d, J = 7.6 Hz, pyridine ring), 7.53 (1H, m, pyridine ring), 6.95 (1H, s, 5-H), 6.44 (1H, s, 8-H), 6.19 (2H, s, 2′,6′-H), 5.99 (1H, s, OCH_2O), 5.97 (1H, s, OCH_2O), 4.50 (2H, s, NCH_2Py), 4.44 (1H, d, J = 5.3 Hz, 1-H), 4.32 (1H, t, J = 7.9 Hz, 11α-H), 4.17 (1H, m, 11β-H), 3.62 (6H, s, 3′,5′-OCH_3), 3.11–3.40 (4H, m, $CH_2CH_2N(CH_3)CH_2$, 2,4-H), 2.89 (1H, m, 3-H), 2.76 (3H, s, NCH_3), 2.40 (1H, m, CH_2CH_2N), 1.99 (1H, m, CH_2CH_2N). Proton signals of N^+H and 4′-OH were not clear. Anal. ($C_{30}H_{32}N_2O_7 \cdot 2HCl \cdot 1.5H_2O$) C, H, N.

4′-Demethyl-4β-[2-[*N*-methyl-*N*-(4-methylpiperazin-1-yl)amino]ethyl]-4-desoxypodophyllotoxin dihydrochloride (12y): yield 67.0%; ^1H NMR (DMSO-d_6) δ 13.00 (1H, b, N^+H), 10.94 (1H, b, N^+H), 7.05 (1H, s, 5-H), 6.44 (1H, s, 8-H), 6.19 (2H, s, 2′,6′-H), 5.98 (2H, s, OCH_2O), 4.45 (2H, m, 4, 11α-H), 4.12 (1H, m, 11β-H), 3.61 (6H, s, 3′,5′-OCH_3), 2.20–3.60 (13H, m, 2,3,4-H, CH_2CH_2NN, $NNCH_2CH_2N$ in piperazine ring), 2.92 (6H, s, $N(CH_3)N$, NCH_3), 2.28 (1H, m, CH_2CH_2N), 2.04 (1H, m, CH_2CH_2N). Proton signals of 4′-OH were not clear. Anal. ($C_{29}H_{37}N_3O_7 \cdot 2HCl \cdot 3H_2O$) C, H, N.

General Synthetic Method for Type 14 Compounds (14a–g). Pivaloyl chloride (60 mg, 0.5 mmol) in AcOEt (2 mL) was added dropwise to a mixture solution of **6** (288 mg, 0.5 mmol) and (dimethylamino)pyridine (74 mg, 0.6 mmol) in AcOEt (5 mL) at 0 °C and stirred. After 0.5 h, the appropriate amine (1.0 mmol) in AcOEt (2 mL) was added dropwise to the reaction mixture. After the mixture was stirred for 0.5 h, AcOEt (100 mL) was added to the reaction mixture, which was then washed with cold saturated $NaHCO_3$, followed by washing to pH 6–7 with saturated NaCl. The extract was dried over $MgSO_4$ and concentrated in vacuo below 30 °C. The residue was purified by silica gel column chromatography with $CHCl_3$/CH_3OH (10/1). The main spot was collected, concentrated in vacuo below 30 °C, and dried in vacuo at room temperature. The residue was reduced for 10 h at room temperature on 10% Pd–C (50 mg) with H_2 (2 atm) in CH_2Cl_2 (15 mL). The reaction mixture was filtered off and washed with AcOEt, and then the filtrate was concentrated below 30 °C. The residue was purified by PTLC with $CHCl_3$/MeOH (10/1). The eluate with $CHCl_3$/MeOH (10/1) was concentrated below 30 °C. To the residue in AcOEt (2 mL) was added 4 N HCl–AcOEt (0.3 mL). The reaction mixture was concentrated in vacuo below 30 °C. Recrystallization from Et_2O gave compounds **14a–g**, respectively.

4′-Demethyl-4β-[2-oxo-2-[(2-morpholinoethyl)amino]ethyl]-4-desoxypodophyllotoxin hydrochloride (14a): yield 84.3%; ^1H NMR (DMSO-d_6) δ 10.62–10.69 (1H, b, N^+H), 8.19–8.29 (2H, b, 4′-OH, CONH), 6.87 (1H, s, 5-H), 6.44 (1H, s, 8-H), 6.22 (2H, s, 2′,6′-H), 5.97 (1H, s, OCH_2O), 5.96 (1H, s, OCH_2O), 4.44 (1H, d, J = 5.6 Hz, 1-H), 4.22 (1H, t, J = 8.2 Hz, 11α-H), 3.94 (1H, m, 11β-H), 3.50–3.90 (4H, m, NCH_2CH_2O in morpholine ring), 3.62 (6H, s, 3′,5′-OCH_3), 3.43 (2H, m, $NCH_\beta CH_2O$ in morpholine ring), 3.14 (5H, m, 4-H, $CONHCH_2CH_2N$, $NCH_\alpha CH_2O$ in morpholine ring), 3.05 (1H, m, 2-H), 2.92 (1H, m, 3-H), 2.72 (1H, m, CH_2CONH), 2.38–2.51 (2H, m, $CONHCH_2CH_2N$), 2.33 (1H, m, CH_2CONH). Anal. ($C_{29}H_{34}N_2O_9 \cdot HCl \cdot H_2O$) C, H, N.

4′-Demethyl-4β-[2-oxo-2-(4-piperidinopiperidin-1-yl)ethyl]-4-desoxypodophyllotoxin hydrochloride (14b): yield 76.1%; ^1H NMR (DMSO-d_6) δ 8.24 (1H, s, 4′-OH), 6.85–6.83 (1H, b, 5-H), 6.45 (1H, s, 8-H), 6.22 (2H, s, 2′,6′-H), 5.96 (1H, s, OCH_2O), 5.94 (1H, s, OCH_2O), 4.58 (1H, d, J = 4.95 Hz, 1-H), 4.10 (1H, m, 11α-H), 4.00 (1H, m, 11β-H), 3.63 (6H, s, 3′,5′-OCH_3), 3.55–3.80 (2H, m, $CHNCH_\beta CH_2$ in piperidinopiperidine ring), 3.33–3.55 (3H,

m, NCH_βCH_2CHN in piperidinopiperidine ring), 3.10–3.30 (1H, m, 4-H), 2.80–3.10 (6H, m, 2,3-H, NCH_αCH_2-CHN in piperidinopiperidine ring, CHNCH_αCH_2 in piperidinopiperidine ring), 2.40–2.70 (2H, m, CH_2CONH), 1.92–2.15 (2H, m, NCH_2CH_βCHN in piperidinopiperidine ring), 1.80–2.00 (4H, m, NCH_2CH_αCHN in piperidinopiperidine ring, CHNCH_2CH_βCH_2 in piperidinopiperidine ring), 1.60–1.80 (3H, m, CHNCH_2CH_αCH_β in piperidinopiperidine ring), 1.30–1.60 (1H, m, CHNCH_2CH_2CH_α in piperidinopiperidine ring). Proton signals of N$^+$H were not clear. Anal. ($C_{33}H_{40}N_2O_8$·HCl·H_2O) C, H, N.

4′-Demethyl-4β-[2-oxo-2-[*N*-[2-(*N*′,*N*′-dimethylamino)ethyl]-*N*-methylamino]ethyl]-4-desoxypodophyllotoxin hydrochloride (14c): yield 78.4%; ^{1}H NMR (DMSO-d_6) δ 10.15 (1H, b, N$^+$H), 8.25 (1H, s, 4′-OH), 6.96 (1H, s, 5-H), 6.45 (1H, s, 8-H), 6.23 (2H, s, 2′,6′-H), 5.98 (1H, d, J = 1.0 Hz, OCH_2O), 5.95 (1H, d, J = 1.0 Hz, OCH_2O), 4.42 (1H, d, J = 4.9 Hz, 1-H), 4.15 (1H, t, J = 7.9 Hz, 11α-H), 3.88 (1H, m, 11β-H), 3.62 (6H, s, 3′,5′-OCH_3), 3.00–3.53 (4H, m, 2,4-H, CONCH_2CH_2N), 2.59–3.00 (3H, m, 3-H, CONCH_2CH_2N), 2.93 (3H, s, CON(CH_3)CH_2), 2.85 (6H, s, N(CH_3)$_2$), 2.40–2.60 (2H, m, CH_2CON). Anal. ($C_{28}H_{34}N_2O_8$·HCl·H_2O) C, H, N.

4′-Demethyl-4β-[2-oxo-2-[[2-(1-piperidino)ethyl]amino]ethyl]-4-desoxypodophyllotoxin hydrochloride (14d): yield 88.3%; ^{1}H NMR (DMSO-d_6) δ 10.02 (1H, b, N$^+$H), 8.24 (1H, s, 4′-OH), 8.20–8.30 (1H, b, CONHCH$_2$), 6.86 (1H, s, 5-H), 6.44 (1H, s, 8-H), 6.21 (2H, s, 2′,6′-H), 5.97 (1H, s, OCH_2O), 5.95 (1H, s, OCH_2O), 4.43 (1H, d, J = 5.6 Hz, 1-H), 4.22 (1H, t, J = 7.9 Hz, 11α-H), 3.93 (1H, m, 11β-H), 3.62 (6H, s, 3′,5′-OCH_3), 3.40–3.50 (4H, m, CONHCH_2CH_2N, NCH_βCH_2 in piperidine ring), 3.07–3.14 (2H, m, 2,4-H), 2.80–3.00 (5H, m, 3-H, CONHCH_2CH_2, NCH_αCH_2 in piperidine ring), 2.75 (1H, d, J = 6.6 Hz, CH_2CON), 2.37 (1H, d, J = 6.6 Hz, CH_2CON), 1.67–1.80 (5H, m, NCH_2CH_2CH_β in piperidine ring), 1.39 (1H, b, NCH_2CH_2CH_α in piperidine ring). Anal. ($C_{30}H_{36}N_2O_8$·HCl·H_2O) C, H, N.

4′-Demethyl-4β-[2-oxo-2-(4-methylpiperazin-1-yl)ethyl]-4-desoxypodophyllotoxin hydrochloride (14e): yield 78.6%; ^{1}H NMR (DMSO-d_6) δ 8.32 (1H, b, 4′-OH), 6.82 (1H, s, 5-H), 6.45 (1H, s, 8-H), 6.23 (2H, s, 2′,6′-H), 5.97 (1H, s, OCH_2O), 5.95 (1H, s, OCH_2O), 4.46 (1H, d, J = 5.3 Hz, 1-H), 4.21 (1H, t, J = 8.6 Hz, 11α-H), 3.92 (1H, m, 11β-H), 3.64 (6H, s, 3′,5′-OCH_3), 2.80–3.40 (11H, m, 2,3,4-H, NCH_2CH_2N in piperazine ring), 2.75 (3H, s, NCH_3), 2.50–2.60 (2H, m, CH_2CON). Proton signals of N$^+$H were not clear. Anal. ($C_{28}H_{32}N_2O_8$·HCl·H_2O) C, H, N.

4′-Demethyl-4β-[2-oxo-2-[[2-(*N*-methylpyrrol-2-yl)ethyl]amino]ethyl]-4-desoxypodophyllotoxin hydrochloride (14f): yield 50.5%; ^{1}H NMR (DMSO-d_6) δ 7.95 (1H, b, 4′-OH), 7.90–8.00 (1H, b, CH$_2$CONH), 6.82 (1H, s, 5-H), 6.59 (1H, m, pyrrole ring), 6.43 (1H, s, 8-H), 6.21 (2H, s, 2′,6′-H), 5.96 (1H, s, OCH_2O), 5.94 (1H, s, OCH_2O), 5.86 (1H, dd, J = 3.3, 2.9 Hz, pyrrole), 5.78 (1H, m, pyrrole), 4.43 (1H, d, J = 5.6 Hz, 1-H), 4.20 (1H, m, 11α-H), 3.92 (1H, m, 11β-H), 3.62 (6H, s, 3′,5′-OCH_3), 3.60 (1H, m, 4-H), 3.51 (3H, s, NCH_3), 3.26 (2H, t, J = 6.9 Hz, CONHCH_2), 3.07 (1H, dd, J = 9.9, 5.2 Hz, 2-H), 2.80–3.00 (1H, m, 3-H), 2.62 (2H, t, J = 8.5 Hz, CONHCH$_2$CH_2), 2.20–2.60 (2H, m, CH_2CON). Proton signals of N$^+$H were not clear. Anal. ($C_{30}H_{32}N_2O_8$·HCl·H_2O) C, H, N.

4′-Demethyl-4β-[2-oxo-2-[(4-pyridinylmethyl)amino]ethyl]-4-desoxypodophyllotoxin hydrochloride (14g): yield 34.0%; ^{1}H NMR (DMSO-d_6) δ 8.78 (2H, d, J = 6.3 Hz, pyridine ring), 8.65 (1H, s, 4′-OH), 8.60–8.70 (1H, m, CH$_2$CONH), 7.77 (2H, d, J = 6.2 Hz, pyridine ring), 6.84 (1H, s, 5-H), 6.46 (1H, s, 8-H), 6.22 (2H, s, 2′,6′-H), 5.97 (2H, s, OCH_2O), 4.52 (1H, d, J = 5.6 Hz, 1-H), 4.45 (2H, d, J = 5.3 Hz, CONHCH_2), 4.20 (1H, m, 11α-H), 3.95 (1H, m, 11β-H), 3.62 (6H, s, 3′,5′-OCH_3), 3.60 (1H, m, 4-H), 3.16 (1H, dd, J = 14.3, 4.6 Hz, 2-H), 2.80–3.00 (1H, m, 3-H), 2.70–2.80 (1H, m, CH_2CON), 2.40–2.60 (1H, m, CH_2-CON). Proton signals of N$^+$H were not clear. Anal. ($C_{29}H_{28}N_2O_8$·HCl·H_2O) C, H, N.

Biological Screening. Cell Lines and Cytotechnology. Cells were continuously cultured in RPMI 1640 medium (P388) or minimal essential medium (human cell lines) supplemented with 10% FCS. Cells were plated in 24-well flat-bottomed plates (Corning, type 25820) and cultured for 24 h in a CO_2 incubator. Thereafter, test compounds were added and cultured for 96 or 4 h. Cell numbers were counted using a hemocytometer. The IC_{50} value was defined as the drug concentration needed to produce a 50% reduction in cell number relative to the control.

Preparation of Crude Nuclear Extracts. Crude nuclear extracts were prepared by a modification of a published procedure.[18] Exponentially growing cells were collected by centrifugation and washed in ice-cold NB (NB consists of 2 mM K_2HPO_4, 5 mM $MgCl_2$, 150 mM NaCl, 1 mM EGTA, and 0.1 mM dithiothreitol, pH 6.5). The washed cells were resuspended in NB, and 9 mM NB supplemented with 0.35% Triton X-100 and 1 mM phenylmethyl sulfonyl fluoride was added slowly down the side of the tube. The cell suspension was mixed by rotation for 5 min at 4 °C and then centrifuged at 1000g for 10 min, and then the nuclear pellet was washed in Triton-free NB. The nuclear protein was extracted from the nuclei for 30 min at 4 °C with ice-cold NB containing 0.35 M NaCl. DNA and nuclear debris were pelleted by centrifugation at 17000g for 10 min, and the supernatant was decanted. The protein concentration in the supernatant was determined by the method of Bradford.[19]

Topo II Catalytic Activity Assay. Topo II catalytic activity was measured using the decatenation assay.[20] The standard reaction mixture was 50 mM Tris-HCl (pH 7.5), 8.5 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM EDTA, bovine serum albumin (0.03 mg/mL), and 1 mM ATP. Kinetoplast DNA was decatenated by incubating 4 μL of nuclear extract (0.05 μg of protein) with 1 μg of kinetoplast DNA in the standard reaction mixture for 30 min at 30 °C. Reactions were terminated with 5 μL of 5% SDS containing 0.13% bromophenol blue and 50% glycerol. Samples were then electrophored in 1% agarose with 40 mM Tris, 2 mM EDTA, 19 mM acetic acid, pH 8.1 at 50 V for 1 h. Gels were stained with ethidium bromide (1.0 μg/mL) for 30 min and destained for 1 h in H_2O. DNA bands were visualized by UV transillumination and photographed using Polaroid type 665 positive/negative film. Inhibitory activity was calculated from densitometrically scanning gel negatives. The IC_{50} value was defined as the drug concentration needed to produce a 50% reduction in the amount of minicircle DNA relative to the control.

Tubulin Preparation and Antimicrotubular Activity Test. Bovine brain tubulin was prepared as described previously.[21] Purification was proceeded in a buffer composed of 100 mg of Mes (2-(*N*-morpholino)-ethanesulfonic acid), 1 mM ethylene glycol-bis-*N*,*N*-tetraacetic acid (EGTA), 1 mM $MgSO_4$, 5 mM NaH_2PO_4,

and 0.02% NaN_3, pH 6.75 (MEM buffer). After one cycle of polymerization–depolymerization, the pellets were stored at −80 °C. Tubulin was polymerized by incubating 50 μL of tubulin (200 μg protein) with 250 μL of MEM buffer containing 1 mM GTP for 15 min at 37 °C. For assembly measurements, turbidity was monitored at 350 nm with a temperature-controlled Hitachi U3210 spectrophotometer. The IC_{50} value was defined as the drug concentration needed to produce a 50% reduction of polymerization relative to the control.

Acknowledgment. We are grateful to Dr. T. Asao for helpful discussions and to T. Yamazaki and Dr. K. Kobayashi for mass spectra recording.

Supplementary Material Available: Characterization data (^1H NMR spectral, FAB-MS spectral, IR spectral, melting points, optical rotation, and microanalytical) for compounds **4–10**, **12a–12x**, and **14a–14g** (12 pages). Ordering information is given on any current masthead page.

References

(1) Terada, T.; Fujimoto, K.; Nomura, M.; Yamashita, J.; Kobunai, T.; Takeda, S.; Wierzba, K.; Yamada, Y.; Minami, Y.; Yoshida, K.; Yamaguchi, H. Antitumor Agents II: Regio- and Stereospecific Syntheses of 1-β-Alkyl-1-Desoxypodophyllotoxin Derivatives and Biological Activity. *Chem. Pharm. Bull.*, in press.

(2) King, L. S.; Sullivan, M. S. The Similarty of the Effect of Podophyllin and Colchicine and Their Use in the Treatment of Condylomata Acuninata. *Science* **1946**, *104*, 244–245.

(3) (a) Gensler, W. J.; Murthy, C. D.; Trammell, M. H. Nonenolizable Podophyllotoxin Derivatives. *J. Med. Chem.* **1977**, *20*, 635–644. (b) Loike, J. D.; Brewer, C. F.; Sternlicht, H.; Gensler, W. J.; Horwitz, S. B. Structure-Activity Study of the Inhibition of Microtubule Assembly in Vitro by Podophyllotoxin and Its Congeners. *Cancer Res.* **1978**, *38*, 2688–2693. (c) Stahelin, H.; Wartburg, A. V. From Podophyllotoxin Glucoside to Etoposide. *Prog. Drug. Res.* **1989**, *33*, 169–266.

(4) Stahelin, H.; Cerletti, A. Experimental Results with Podophyllum-Cylostatic SP-I and SP-G. *Schweiz. Med. Wschr.* **1964**, *94*, 1490–1502.

(5) (a) Falkson, G.; Sandison, A. G.; Vanzyl, J. Podophyllin Derivatives SPG 827 and SPI 77 in the Treatment of Advanced Cancer. *S. Afr. J. Radiol.* **1964**, *2*, 1–7. (b) Vaitkevicius, V. K.; Reed, M. I. Clinical Studies with Podophyllum Compounds SPI-77 (NSC-72274) and SPG-827 (NSC-42076). *Cancer Chemother. Rep.* **1966**, *50*, 565–571.

(6) (a) Cohen, M. H.; Broder, L. E. Fossieck, B. E.; Ihde, D. C.; Minna, J. D. Phase II Clinical Trial of Weekly Administration of VP-16-213 in Small Cell Bronchogenic Carcinoma. *Cancer Treat. Rep.* **1977**, *61*, 489–490. (b) Issell, B. F. The Podophyllotoxin Derivatives VP16-213 and VM-26. *Cancer Chemother Pharmacol.* **1982**, *7*, 73–80. (c) Issell, B. F.; Muggia, F. M.; Carter, S. K. Etoposide [VP-16] Current Status and New Developments; Academic Press: Orlando, 1984; pp 1–353.

(7) (a) Grieder, A.; Maurer, R.; Stahelin, H. Comparative Study of Early Effects of Epipodophyllotoxin Derivatives and Other Cytostatic Agents on Mastocytoma Cultures. *Cancer Res.* **1977**, *37*, 2998–3005. (b) Loike, J. D.; Horwitz, S. B. Effects of VP16-213 on the Intracellular Degradation of DNA in HeLa Cells. *Biochemistry* **1976**, *15*, 5443–5448. (c) Loike, J. D.; Horwitz, S. B. Effects of Podophyllotoxin and VP-16-213 on Microtubule Assembly in Vitro and Nucleoside Transport in HeLa Cells. *Biochemistry* **1976**, *15*, 5435–5443. (d) Long, B. H.; Minocha, A. Inhibition of Topoisomerase II by VP-16-213 (etoposide) VM-26 (teniposide), and Structural Congeners as an Explanation for in Vivo DNA Breakage and Cytotoxicity. *Proc. Am. Assoc. Cancer Res.* **1983**, *24*, 321 (Abstr). (e) Glisson, B. S.; Smallwood, S. E.; Ross, W. E. Characterization of VP-16-induced DNA Damage in Isolated from L1210 Cells. *Biochim. Biophys. Acta* **1984**, *783*, 74–79. (f) Minocha, A.; Long, B. H. *Biochim. Biophys. Res. Commun.* **1984**, *122*, 165–170.

(8) (a) Saitoh, H.; Yoshikawa, H.; Nishimura, Y.; Kondo, S.; Takeuchi, T.; Umezawa, H. Studies on Lignan Lactone Antitumor Agents. I. Synthesis of Aminoglycosidic Lignan Variants Related to Podophyllotoxin. *Chem. Pharm. Bull.* **1986**, *34*, 3733–3740. (b) Saitoh, H.; Yoshikawa, H.; Nishimura, Y.; Kondo, S.; Takeuchi, T.; Umezawa, H. Studies on Lignan Lactone Antitumor Agents. II. Synthesis of N-Alkylamino- and 2,6-Dideoxy-2-aminoglycosidic Lignan Variants Related to Podophyllotoxin. *Chem. Pharm. Bull.* **1986**, *34*, 3741–3746. (c) Saitoh, H.; Nishimura, Y.; Kondo, S.; Umezawa, H. Syntheses of All Four Possible Diastereomers of Etoposide and Its Aminoglycosidic Analogues via Optical Resolution of (±)-Podophyllotoxin by Glycosidation with D- and L-Sugars. *Chem. Lett.* **1987**, 799–802. (d) Showalter, H. D.; Winter, R. H.; Sercel, A. D.; Michel, A. Facile Synthesis of Thioglucose Analogs of the Anticancer Agent Etoposide. *Tetrahedron Lett.* **1991**, *25*, 2849–2852.

(9) Terada, T.; Fujimoto, K.; Nomura, M.; Yamashita, J.; Kobunai, T.; Takeda, S.; Wierzba, K.; Yamada, Y.; Yamaguchi, H. Antitumor Agents. I. DNA Topoisomerase II Inhibitory Activity and the Structural Relationship of Podophyllotoxin Derivatives as Antitumor Agents. *Chem. Pharm. Bull.* **1992**, *40*, 2720–2727.

(10) Slevin, M. L.; Clark, P. I.; Joel, S. P.; Malik, S.; Osborne, R. J.; Gregory, W. M.; Lowe, D. G.; Reznek, R. H.; Wrigley, P. F. A Randomized Trial to Evaluate the Effect of Schedule on the Activity of Etoposide in Small-Cell Lung Cancer. *J. Clin. Oncol.* **1989**, *7*, 1333–1340.

(11) (a) Chapman, R.; Itri, L.; Gralla, R.; Kelsen, D.; Capser, E.; Golby, R. Phase II Trial of VP16-213 in Non-small Cell Lung Cancer (NSCLC). *Cancer Chemother. Pharmacol.* **1982**, *7*, 205–207. (b) Eagan, R.; Carr, D.; Frytak, S. Vp16-213 versus Polychemotherapy in Patients with Advanced Lung Cancer. *Cancer Treat. Rep.* **1976**, *60*, 949–951. (c) Falkson, G.; Dyk, J.; Med, M.; Eden, E. B.; Merwe, A. M.; Bergh, J. A.; Falkson, H. C. A Clinical Trial of the Oral Form of 4′-Demethylepipodophyllotoxin-β-D-ethylidene Glucoside (NSC 141540) VP16-213. *Cancer* **1975**, *35*, 1141–1144. (d) Nissen, N. I.; Pajak, T. F.; Leone, L. A.; Bloomfield, C. D.; Kennedy, B. J.; Ellison, R. R.; Silver, R. T.; Weiss, R. B.; Cuttner, J.; Falkson, G.; Kung, F.; Bergevin, P. R.; Holland, J. F. Clinical Trial of VP16-213 (NSC 141540) I.V. Twice Weekly in Advanced Neoplastic Disease. *Cancer* **1980**, *45*, 232–235.

(12) Straus, M. J.; Moran, R. E. Cell Cycle Kinetics of Human Lung Cancer. *Semin. Resp. Med.* **1982**, *3*, 194–199.

(13) Kasahara, K.; Fujiwara, Y.; Sugimoto, Y.; Nishio, K.; Tamura, T.; Matsuda, T.; Saijo, N. Determination of Response to the DNA Topoisomerase II Inhibitors Doxorubicin and Etoposide in Human Lung Cancer Cell Lines. *J. Natl. Cancer Inst.* **1992**, *84*, 2, 113–118.

(14) (a) Colombo, T.; Broggini, M.; Torti, L.; Erba, E.; D'Incalci, M. Pharmacokinetics of VP16-213 in Lewis Lung Carcinoma Bearing Mice. *Cancer Chemother. Pharmacol.* **1982**, *7*, 127–131. (b) Evans, W. E.; Sinkule, J. A.; Crom, W. R.; Dow, L.; Look, A. T.; Rivera, G. Pharmacokinetics of Teniposide (VM-26) and Etoposide (VP16-213) in Children with Cancer. *Cancer Chemother. Pharmacol.* **1982**, *7*, 147–150. (c) Broggini, M.; Colombo, T.; D'Incalci, M. Activity and Pharmocokinetics of Teniposide in Lewis Lung Carcinoma-Bearing Mice. *Cancer Treat. Rep.* **1983**, *67*, 555–559. (d) Arbuck, S. G.; Douglass, H. O.; Crom, W. R.; Goodwin, P.; Silk, Y. Etoposide Pharmocokinetics in Patients with Normal and Abnormal Organ Function. *J. Clin. Oncol.* **1986**, *4*, 1690–1695. (e) Hoogenhuijze, J. V.; Lankelma, J.; Stam, J.; Pinedo, H. M. Unchanged Pharmocokinetics of VP-16-213 (etoposide, NSC141540) during Concominant Administration of Doxorubicin and Cyclophosphamide. *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 807–812. (f) Gouyette, A.; Deniel, A.; Pico, J.-L.; Baume, D.; Ostronoff, M.; Bail, L. N.; Hayat, M. Clinical Pharmacology of High-dose Etoposide Associated with Cisplatin. Pharmacokinetic and Metabolic Studies. *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 1627–1632.

(15) VP-16 (10 mg/kg) and selected compounds (10 mg/kg) were administered iv to S-180 bearing mice (sc). The AUC_{0-4} (mg/mL per h) value of all selected compounds in lung tissue was higher than that of VP-16. For example, the AUC_{0-4} (mg/mL per h) value of **12s** was 81.8 (mg/mL per h), compared with 4.7 (mg/mL per h) of VP-16. These results will be reported elsewhere (unpublished).

(16) (a) Jardine, I. Podophyllotoxin. In *Anticancer Agents Based on Natural Product Models*; Cassady, J., Douros, J., Es.; Academic Press, Inc.: Orlando, 1980; pp 319–351. (b) Long, B. H.; Musial, S. T.; Brattain, M. G. Comparison of Cytotoxicity and DNA Breakage Activity of Congeners of Podophyllotoxin Including VP16-213 and VM-26: A Quantitative Structure-Activity Relationship. *Biochemistry* **1984**, *23*, 1183–1188.

(17) Kuhn, M.; Wartburg, A. V. New Glycosidation Process. II. Glycosides of 4′-Demethylepipodophyllotoxin. *Helv. Chim. Acta* **1969**, *52*, 948–955.

(18) Deffie, A. M.; Batra, J. K.; Goldenberg, G. J. Direct Correlation between DNA Topoisomerase II Activity and Cytotoxicity in Adriamycin-sensitive and -resistant P388 Leukemia Cell Lines. *Cancer Res.* **1989**, *49*, 58–62.

(19) Bradford, M. M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254.

(20) (a) Duguet, M.; Lavenot, C.; Harper, F.; Mirambeau, G.; Derecondo, A. M. DNA Topoisomerase from Rat Liver: Physiological Variations. *Nucleic Acids Res.* **1983**, *11*, 1059–1075. (b) Marini, J. C.; Miller, K. G.; Englund, P. T. Decatenation of Kinetoplast DNA by Topoisomerases. *J. Biol. Chem.* **1980**, *255*, 4976–4979.

(21) Vogel, D. G.; Margolis, R. L.; Mottet, N. K. The Effects of Methyl Mercury Binding to Microtubules. *Toxicol. Appl. Pharmacol.* **1985**, *80*, 473–486.