Antitumor Agents. II[1]: Regio- and Stereospecific Syntheses of 1-β-Alkyl-1-desoxypodophyllotoxin Derivatives and Biological Activity

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1-β-Alkyl derivatives of 1-desoxypodophyllotoxin were synthesized, and their cytotoxicity and inhibitory effects on DNA topoisomerase II (Topo-II) and tubulin polymerization were examined.

The reaction of epipodophyllotoxin derivatives (1a—c) with trimethylallylsilane in the presence of boron trifluoride etherate gave 1-β-allylated compounds (2a—c). The regiochemistry and the β-stereochemistry of the 1-allyl group were confirmed by comparison of the ^{13}C-NMR spectra and NOE's (%) of 2c, podophyllotoxin (POD) and epipodophyllotoxin (1b). 1-β-Alkyl-1-desoxypodophyllotoxin derivatives (3—8) were prepared from 2b.

None of the tested compounds (3—8) showed any inhibitory effect on Topo-II. 1-β-Propyl compound (3) and its 4′-demethyl compound (4) inhibited tubulin polymerization and the cytotoxicities of these compounds were equal to that of VP-16. 1-β-(2,3-Dihydroxypropyl) compounds (5 and 8) and 1-β-(2,3-diacetoxypropyl) compounds (6 and 7) showed no inhibitory effect on tubulin polymerization. Although 5 did not inhibit either Topo-II activity or tubulin polymerization, it showed a high cytotoxicity against sarcoma 180.

Keywords antitumor agent; 1-β-alkyl-1-desoxypodophyllotoxin; stereospecific synthesis; biological activity; *in vitro*

Podophyllotoxin (POD), a natural product isolated from *Podophyllum peltatum* L. and *P. emodi* WALL., has been reported to be a potent inhibitor of mitosis.[2] Several related compounds have been evaluated as antitumor agents in clinical trials (Fig. 1).[3] Among such compounds, 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 not been used as antitumor agents because of severe side effects.[5] In contrast, the analogues etoposide (VP-16) and teniposide (VM-26) (Fig. 1), both widely used in clinical cancer chemotherapy, are potent inhibitors of DNA topoisomerase II (Topo-II) but do not inhibit microtubule polymerization.[6] These results suggest that inhibition of DNA Topo-II, but not microtubule polymerization, is an important biochemical determinant of the antitumor activity of podophyllotoxin derivatives.

Recently, we have reported syntheses of 1-desoxypodophyllotoxin derivatives and the relationships between structure and biological activities *in vitro* and *in vivo*.[1] The inhibitory effect on DNA Topo-II contributed to the antitumor activity *in vivo*. However, strong cytotoxicity alone was not a good indicator of antitumor activity *in vivo*, in so far as it was due to the inhibition of tubulin polymerization.

On the other hand, though VP-16 shows a good efficacy in the treatment of small cell lung cancer (SCLC), its effectiveness against non-small cell lung cancer (NSCLC), which is found in about 80% of patients with lung cancer, is low.[7] The following factors can be assumed to contribute to the low activity of VP-16 against NSCLC: 1) a short half-life time of VP-16,[8] 2) a rapid biotransformation *in vivo*,[9] 3) different cell kinetics (a long doubling time of NSCLC),[10] 4) lower DNA Topo-II activity in NSCLC in comparison to SCLC.[11]

Therefore, we were interested in finding 1-desoxypodophyllotoxin derivatives showing specific inhibition of DNA Topo-II, resistance to metabolic cleavage at the 1-position and long retention time in the target tissue, in an attempt to improve the antitumor activity *in vivo* against NSCLC. We hypothesized that 1-β-alkylated derivatives of 1-desoxypodophyllotoxin would be promising candidates.

This paper describes the regio- and stereospecific synthesis of 1-β-alkyl derivatives of 1-desoxypodophyllotoxin, and their biological activity *in vitro* (cytotoxicity against sarcoma 180, and inhibitory effects on mammalian DNA Topo-II and tubulin polymerization).

Chemistry

Numerous C-1-substituted analogues of VP-16 have been synthesized.[12] But, there is no report of regio- and stereoselective C–C bond introduction at the C-1 position of the podophyllotoxin skeleton. Numerous methods have been reported for C–C bond formation. However, most of these reactions cannot be applied to podophyllotoxin and related compounds. It is well known that under basic conditions or strongly acidic conditions, ring opening or epimerization easily occurs in the lactone moiety of podophyllotoxin derivatives.[13] Therefore, we selected 4′-demethylepipodophyllotoxin (**1a**)[14] as a starting material and examined the reactions with trimethylallylsilane[15] or alkoxytrimethylsilanes[16] in the presence of various Lewis acids or other catalysts. In most cases under neutral or acidic conditions, reactions either did not occur or exclusively gave dehydrogenated compounds, or gave coupling products with epimerization. The reaction of **1a** with trimethylallylsilane in the presence of boron trifluoride etherate gave the 1-β-allylated compound (**2a**) in moderate yield (Chart 1). To prevent the 4′-hydroxy group from affecting the reaction, epipodophyllotoxin

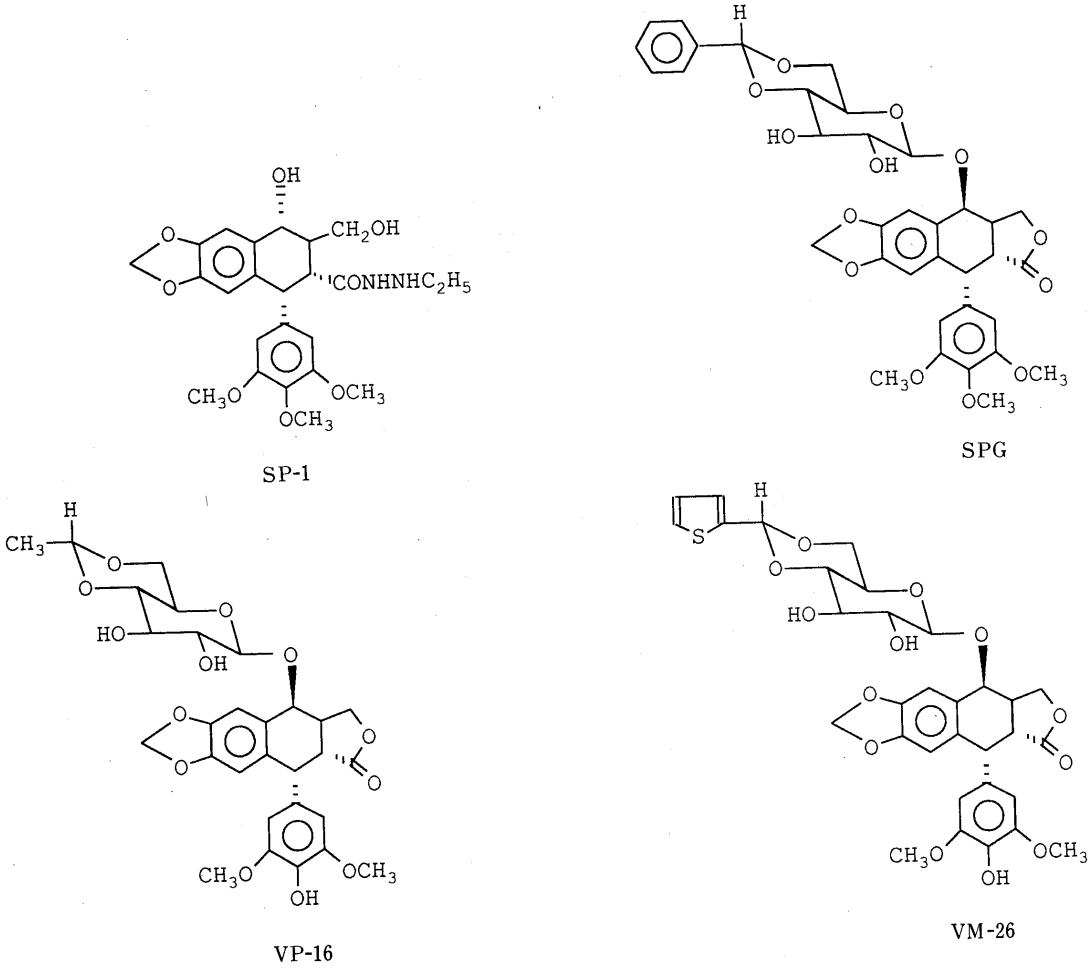
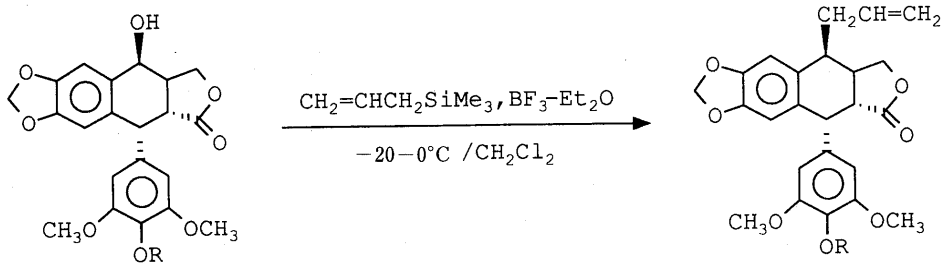


Fig. 1



1a : R = H

1b : R = Me

1c : R = Z (benzyloxycarbonyl)

2a : R = H (49.1%)

2b : R = Me (91%)

2c : R = Z (95.7%)

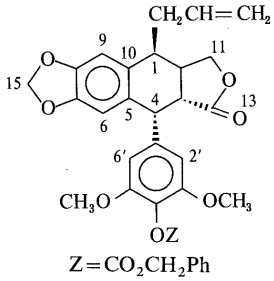
Chart 1

(**1b**)[17] and 4′-demethyl-4′-*O*-benzyloxycarbonylepipodophyllotoxin (**1c**)[18] were used, and the expected compounds (**2b** and **2c**) were obtained in high yield.

The regiochemistry of the allyl group in **2c** was confirmed by comparison of the ^{13}C-NMR spectra of **2c**, POD and **1b** (Table I). In $CDCl_3$, the C-1 carbon of **2c** resonated upfield from the corresponding carbon of POD and **1b** by 28.37 and 32.97 ppm, respectively, because of the allyl group at the C-1 position. The vicinal coupling constant for compound **2c** (6 Hz) at the $H_{1\text{-}2}$ position was between the vicinal coupling constant (9 Hz) of POD and that (3.5 Hz) of compound **1b**. It could not be used to determine the stereochemistry of the allyl group. The β-stereochemistry of the allyl group in **2c** was determined by comparison of NOE's at the C-1 position with those of POD and **1b** (Table II). The NOE's (%) of **2c** at $H_{1\text{-}2}$, $H_{1\text{-}9}$ and $H_{1\text{-}11}$ were nearly equal to those of **1b**, and not equal to those of POD. These results confirmed the regio- and β-stereochemistry of the allyl group in **2c**.

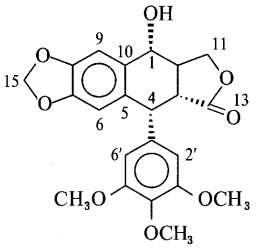
The syntheses of **3**—**8** are shown in Chart 2. Hydrogenation of **2b** with palladium on carbon in a mixture of methanol and ethyl acetate gave the 1-β-propyl compound

TABLE I. Carbon NMR Chemical Shift Assignments for (**2c**), Podophyllotoxin (POD) and Epipodophyllotoxin (**1b**)

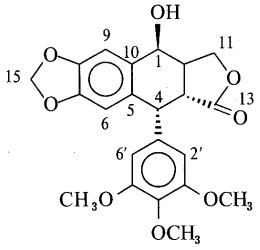


$Z = CO_2CH_2Ph$

2c



POD



1b

Carbon		Chemical shift in $CDCl_3$		
		2c	POD	**1b**
C(1)	d	38.4	71.4	67.8
C(2)	d	36.0	40.6	38.4
C(3)	d	42.2	45.2	40.5
C(4)	d	44.1	44.1	44.0
C(5)	s	130.45	130.9	131.9
C(6)	d	110.1	109.6	110.4
C(7)	s	146.84	147.6	147.5
C(8)	s	147.1	147.6	148.5
C(9)	d	108.64	106.4	109.1
C(10)	s	133.09	133.4	131.9
C(11)	t	68.86	71.4	66.7
C(13)	s	174.8	174.8	175.3
C(15)	t	101.2	101.4	101.6
C(1′)	s	139.04	135.6	135.2
C(2′,6′)	d	107.88	108.4	108.3
C(3′,5′)	s	151.32	152.5	152.6
C(4′)	s	128.26	137.0	137.2
C(3′,5′-OCH_3)	q	56.11	56.2	56.2
C(4′-OCH_3)	q	—	60.8	60.8
C(Z-CO)	s	153.01	—	—
C(Ph(Z)-1)	s	135.06	—	—
C(Ph(Z)-2,6)	d	128.39	—	—
C(Ph(Z)-3,5)	d	128.33	—	—
C(Ph(Z)-4)	d	128.11	—	—
C($CH_2CH=CH_2$)	d	136.55	—	—
C($CH_2CH=CH_2$)	t	116.80	—	—
C($CH_2CH=CH_2$)	t	37.58	—	—

(**3**). The 4′-methyl group of **3** was easily cleaved with a mixture of hydrogen bromide and acetic acid, affording the 4′-demethyl-1-β-propyl compound (**4**). Oxidation of **2b** with osmic acid and *N*-methyl morpholine *N*-oxide (NMO) in acetone gave the diol compound (**5**). Acetylation of **5** with acetic anhydride in tetrahydrofuran (THF) gave **6**. The 4′-demethylation of **6**, under the same conditions used to prepare **4**, gave **7**. Compound **8** was obtained by deacetylation with $Zn(OAc)_2$ in methanol. Physical constants of **2a**—**8** are summarized in Table III.

Biological Results and Discussion

We have reported that measurements of both cytotoxicity and inhibitory effects on DNA Topo-II and tubulin polymerization *in vitro* were necessary to predict *in vivo* antitumor activity of podophyllotoxin derivatives.[1] The biological results for compounds **3**—**8** are summarized in Table IV. Compounds **3** and **4**, possessing a propyl group, exhibited strong inhibitory effects on tubulin polymerization; the IC_{50} values were of the order of 10 μM. Compounds **5**, **6**, **7** and **8**, containing a 2,3-dihydroxypropyl group or 2,3-diacetoxypropyl group, did not inhibit tubulin polymerization even at 60 μM concentration. The inhibitory effect on tubulin polymerization, which does not contribute to antitumor activity *in vivo*, is affected by the kind of substituent group on the 1-position of 1-β-alkyl-1-desoxypodophyllotoxin derivatives. All the tested compounds (**3**—**8**) showed no inhibitory effect on DNA Topo-II at higher concentrations than the IC_{50} value (59.2 μM) of VP-16. Therefore, although the cytotoxicities of **3**, **4** and **1a** toward sarcoma 180 were equal to that of VP-16, their mechanism of action was the inhibition of tubulin polymerization, which would not contribute to antitumor activity *in vivo*.[1] On the other hand, the result with compound **5** was different. This compound did

TABLE II. Vicinal Coupling Constants and NOE at the C-1 Position of **2c**, Podophyllotoxin (POD) and Epipodophyllotoxin (**1b**)

		2c	POD	**1b**
J (Hz)	1-2	6.0	9.0	3.5
NOE (%)	1-2	11.0	4.0	10.0
	1-3	0	8.0	0
	1-4	0	0	0
	1-6	0	0	0
	1-9	13.0	6.0	13.0
	1-11α	0	0	0
	1-11β	0	5.0	0

TABLE III. Physical Constants of 1-β-Alkyl-1-desoxypodophyllotoxin Derivatives

Compd.	Yield (%)	Recryst.[a] solvent	mp (°C)	$[\alpha]_D^{20}$ (°) (*c*, solvent)	Formula	IR (KBr) cm^{-1}	Mass (FAB) *m/z*
2a	49.0	Et	181—185	−90.94 (0.552, $CHCl_3$)	$C_{24}H_{24}O_7$	3420, 1768,	425 ($M^+ + H$)
2b	90.9	Et–Hex	93—95	−85.20 (0.500, $CHCl_3$)	$C_{25}H_{26}O_7$	1770	439 ($M^+ + H$)
2c	95.8	Et	135—137	−70.03 (1.300, DMSO)	$C_{32}H_{30}O_9$	3440, 1768	559 ($M^+ + H$)
3	79.7	Et–Hex	114—115	−74.28 (0.520, $CHCl_3$)	$C_{25}H_{28}O_7$	1770	441 ($M^+ + H$)
4	71.7	Et–Hex	189—191	−78.03 (0.346, $CHCl_3$)	$C_{24}H_{26}O_7$	3610, 1770	427 ($M^+ + H$)
5	89.3	Et–Hex	144—145	−73.42 (0.552, $CHCl_3$)	$C_{25}H_{28}O_9$	3680, 3610, 1770	473 ($M^+ + H$)
6	87.7	Et–Hex	92—93	−52.52 (0.495, $CHCl_3$)	$C_{29}H_{32}O_{11}$	1770, 1740	557 ($M^+ + H$)
7	30.7	Et	169—170	−106.25 (0.448, $CHCl_3$)	$C_{28}H_{30}O_{11}$	3610, 1770, 1740	543 ($M^+ + H$)
8	72.8	Et	131—133	−74.53 (0.530, $CHCl_3$)	$C_{24}H_{26}O_9$	3610, 1770	459 ($M^+ + H$)

a) Et, Et_2O; Hex, *n*-hexane.

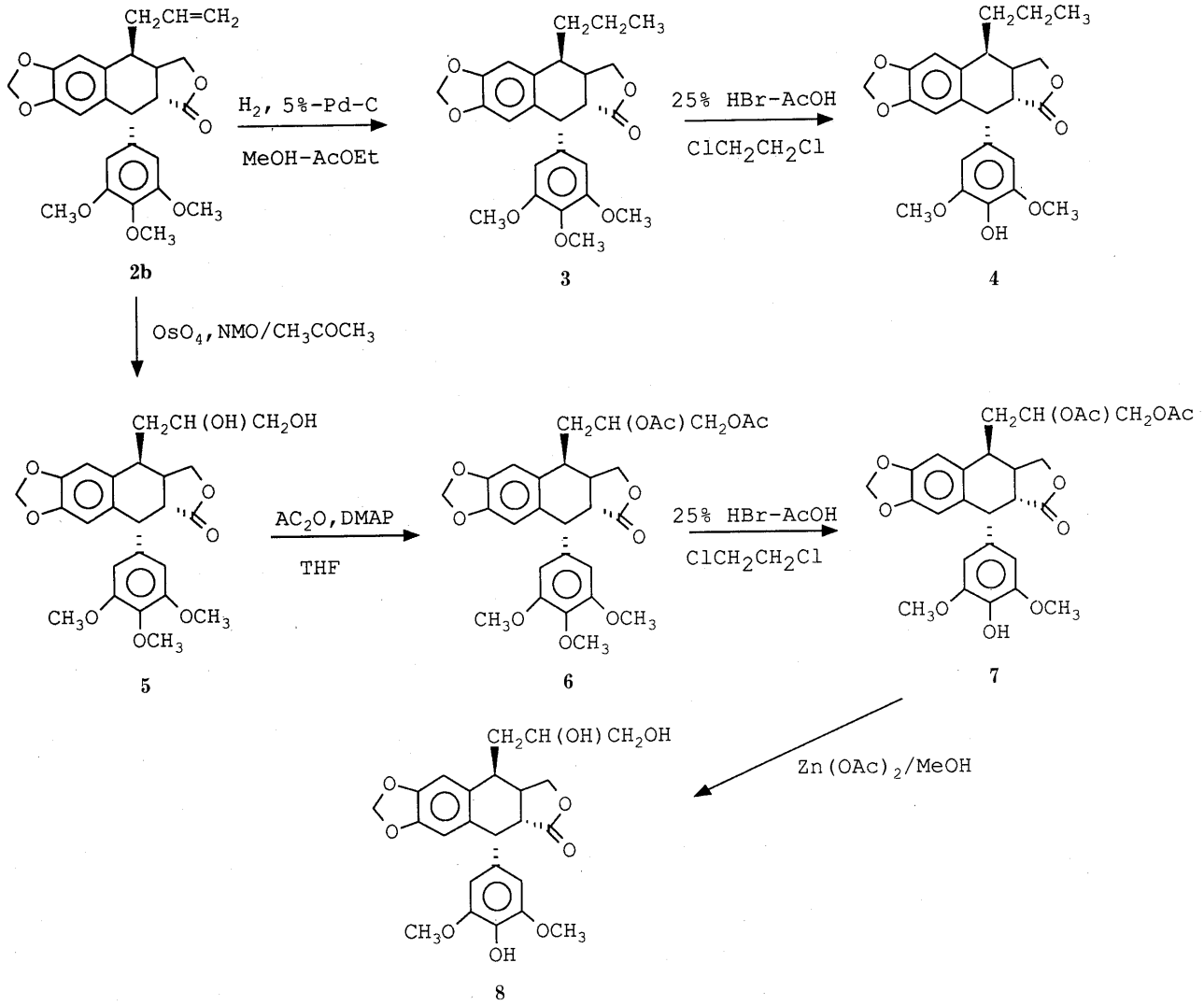


Chart 2

TABLE IV. Biological Evaluation of Compounds Shown in Chart 2

Compd. No.	Cytotoxicity[a] S 180 (IC_{50}; M)	Microtubulin[a] polymerization inhibition (IC_{50}; μM)	Topo-II[a] inhibition (IC_{50}; μM)
3	3×10^{-7}	10	>125
4	1×10^{-7}	10	>125
5	4×10^{-7}	>60	>125
6	1×10^{-6}	>60	>125
7	2×10^{-6}	>60	>125
8	2×10^{-6}	>60	>500
1a	2×10^{-7}	4.5	144.1
VP-16	1×10^{-7}	>60	59.2

a) See Experimental.

not inhibit either DNA Topo-II or tubulin polymerization. Nevertheless, it exhibited cytotoxicity against S-180 ($IC_{50}=4\times10^{-7}$ M) almost equivalent to that of VP-16 ($IC_{50}=1\times10^{-7}$ M). These results raise the possibility that the introduction of a C–C bond at the 1β-position of 1-desoxypodophyllotoxin destroys the ability to inhibit tubulin polymerization, without affecting the cytotoxicity against S-180.

Experimental

All melting points were determined on a Yanagimoto MP-3 micro melting point apparatus and are uncorrected. The instruments used in this study were as follows; elemental analyses, Yanagimoto MT-3, IR spectra, Hitachi I-3000 IR spectrometer; specific rotation, Horiba SEPA-200 spectropolarimeter; FAB-MS, JMS-DX303; ^{1}H-NMR spectra, FT NMR JNM-FX90Q spectrometer; ^{13}C-NMR spectra and nuclear Overhauser effect (NOE), JEOL JNM-GSX400 spectrometer. Chemical shifts were reported in ppm (δ) downfield from tetramethylsilane as an internal standard; coupling constants are given in hertz (Hz). Signals are expressed as s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad). 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).

4′-Demethyl-1-β-allyl-1-desoxypodophyllotoxin (2a) BF_3–Et_2O (74 μl) was added to a mixture of 4′-demethylepipodophyllotoxin (**1a**)[14] (100 mg, 0.25 mmol) and trimethylallylsilane (60 mg, 0.53 mmol) in CH_2Cl_2 (2.5 ml) at −20—0 °C with stirring. After 3 h, the reaction was quenched with pyridine (74 μl) and the mixture was extracted with AcOEt (100 ml). The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with AcOEt–*n*-hexane (3 : 2). Recrystallization from Et_2O gave **2a** (52 mg, 49.0%). mp 181—185 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.72 (1H, s, 9-H), 6.47 (1H, s, 6-H), 6.29 (2H, s, 2′-, 6′-H), 5.94 (2H, s, OCH_2O), 5.60—5.87 (1H, m, $-CH_2\underline{C}H=CH_2$), 5.41 (1H, s, 4′-OH), 5.03—5.20 (2H, m, $-CH=C\underline{H}_2$), 4.55 (1H, d, J=4.8, 4-H), 4.29, 4.20 (each 1H, m, 11α,β-H), 2.87—3.44 (3H, m, 1-, 2-, 3-H), 2.34—2.77 (2H, m, $-C\underline{H}_2CH=CH_2$).

Anal. Calcd for $C_{24}H_{24}O_7$: C, 67.91; H, 5.69. Found: C, 67.72; H, 5.72.

1-β-Allyl-1-desoxypodophyllotoxin (2b) BF_3–Et_2O (0.35 ml) was added to a mixture of epipodophyllotoxin (**1b**)[17] (500 mg, 1.18 mmol) and trimethylallylsilane (274 mg, 2.4 mmol) in CH_2Cl_2 (10 ml) at −20—0 °C with stirring. After 4 h, the reaction was quenched with pyridine (0.35 ml) and the mixture was extracted with AcOEt (100 ml). The extract was washed consecutively with cold dilute HCl and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with AcOEt–*n*-hexane (1 : 2). Recrystallization from Et_2O–*n*-hexane gave **2b** (470 mg, 90.9%). mp 93—95 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.73 (1H, s, 9-H), 6.46 (1H, s, 6-H), 6.29 (2H, s, 2′-, 6′-H), 5.93 (2H, s, OCH_2O), 5.48—5.80 (1H, m, $-CH_2CH=CH_2$), 4.88—5.20 (2H, m, $-CH_2CH=CH_2$), 4.52 (1H, d, J=3.6, 4-H), 4.24 (2H, m, 11α,β-H), 3.78 (3H, s, 4′-OCH_3), 3.74 (6H, s, 3′-, 5′-OCH_3), 2.80—3.40 (3H, m, 1-, 2-, 3-H), 2.24—2.60 (2H, m, $-CH_2CH=CH_2$). *Anal.* Calcd for $C_{25}H_{26}O_7$: C, 68.48; H, 5.97. Found: C, 68.27; H, 6.01.

4′-Demethyl-4′-*O*-benzyloxycarbonyl-1-β-allyl-1-desoxypodophyllotoxin (2c) BF_3–Et_2O (0.6 ml) was added to a mixture of 4′-demethyl-4′-*O*-benzyloxycarbonylepipodophyllotoxin (**1c**)[18] (1 g, 1.87 mmol) and trimethylallylsilane (426 mg, 3.73 mmol) in CH_2Cl_2 (15 ml) at −20—0 °C with stirring. After 1 h, the reaction mixture was quenched with pyridine (0.6 ml) and extracted with AcOEt (100 ml). The extract was washed consecutively with cold dilute HCl and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with $CHCl_3$. Recrystallization from Et_2O gave **2c** (1 g, 95.8%). mp 135—137 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.30—7.43 (5H, m, $PhCH_2OCO$–), 6.72 (1H, s, 9-H), 6.46 (1H, s, 6-H), 6.31 (2H, s, 2′-, 6′-H), 5.94 (1H, d, J=1.5, OCH_2O), 5.93 (1H, d, J=1.5, OCH_2O), 5.80 (1H, ddt, J=17, 10.5, 6.5, $-CH_2CH=CH_2$), 5.25 (2H, s, $PhCH_2OCO$–), 5.12 (1H, ddt, J=17, 2, 1.5, $-CH_2CH=CH_2$), 5.11 (1H, ddt, J=10.5, 1.5, 1.5, $-CH_2CH=CH_2$), 4.58 (1H, d, J=5, 4-H), 4.25 (2H, m, 11-H), 3.68 (6H, s, 3′-, 5′-OCH_3), 3.27 (1H, m, 1-H), 3.07 (1H, dd, J=14.5, 5, 3-H), 2.93 (1H, m, 2-H), 2.57 (1H, m, $-CH_2CH=CH_2$), 2.42 (1H, m, $-CH_2CH=CH_2$). *Anal.* Calcd for $C_{32}H_{30}O_9$: C, 68.80; H, 5.41. Found: C, 68.57; H, 5.52.

1-β-Propyl-1-desoxypodophyllotoxin (3) **2b** (100 mg, 0.23 mmol) was reduced for 2 h on 5%-Pd/C (10 mg) with H_2 (1 atm) in MeOH–AcOEt (1 : 1, 1 ml). The reaction mixture was filtered through Celite, then washed with AcOEt, and the filtrate was concentrated *in vacuo*. Recrystallization from Et_2O–*n*-hexane gave **3** (80 mg, 79.7%). mp 114—115 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.69 (1H, s, 9-H), 6.45 (1H, s, 6-H), 6.28 (2H, s, 2′-, 6′-H), 5.93 (2H, s, OCH_2O), 4.57 (1H, d, J=5, 4-H), 4.20—4.40 (2H, m, 11-H), 3.79 (3H, s, 4′-OCH_3), 3.74 (6H, s, 3′-, 5′-OCH_3), 2.80—3.20 (3H, m, 2-, 3-, 4-H), 1.20—1.80 (4H, m, $-CH_2CH_2CH_3$), 0.98 (3H, t, J=6.1, $-CH_3$). *Anal.* Calcd for $C_{25}H_{28}O_7$: C, 68.16; H, 6.40. Found: C, 68.13; H, 6.39.

4′-Demethyl-1-β-propyl-1-desoxypodophyllotoxin (4) A solution of **3** (80 mg, 0.18 mmol) and 25% HBr–AcOH (1 ml) in $ClCH_2CH_2Cl$ (5 ml) was stirred for 15 h at room temperature. The reaction mixture was added to a saturated solution of $NaHCO_3$ (30 ml) mixed with ice (50 g), and extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* at 30 °C. The residue was purified by silica gel column chromatography with AcOEt–*n*-hexane (30 : 1). Recrystallization from Et_2O–*n*-hexane gave **4** (55 mg, 71.7%). mp 189—191 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.69 (1H, s, 9-H), 6.45 (1H, s, 6-H), 6.29 (2H, s, 2′-, 6′-H), 5.94 (2H, s, OCH_2O), 5.41 (1H, s, –OH), 4.57 (1H, d, J=5, 4-H), 4.00—4.40 (2H, m, 11-H), 3.77 (6H, s, 3′-, 5′-OCH_3), 2.80—3.20 (3H, m, 1-, 2-, 3-H), 1.20—1.80 (4H, m, $-CH_2CH_2CH_3$), 0.98 (3H, t, J=5.9, $-CH_3$). *Anal.* Calcd for $C_{24}H_{26}O_7$: C, 67.59; H, 6.14. Found: C, 67.65; H, 5.97.

1-β-(2,3-Dihydroxy-1-propyl)-1-desoxypodophyllotoxin (5) A mixture of **2b** (100 mg, 0.23 mmol), *N*-methylmorpholine-*N*-oxide (NMO) (28 mg, 0.24 mmol) and osmic acid (OsO_4) (5.8 mg, 0.023 mmol) in acetone (2 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 successively washed with dilute 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). Recrystallization from Et_2O–*n*-hexane gave **5** (97 mg, 89.3%). mp 144—145 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.87, 6.80 (1H, s, 9-H), 6.44, 6.42 (1H, s, 6-H), 6.28 (1H, s, 2′- or 6′-H), 6.26 (1H, s, 2′- or 6′-H), 5.91 (2H, s, OCH_2O), 4.53 (1H, m, 4-H), 4.00—4.40 (2H, m, 11-H), 3.79 (3H, s, 4′-OCH_3), 3.73 (6H, s, 3′-, 5′-OCH_3), 3.58—3.68 (1H, m, $-CH(OH)CH_2OH$), 3.24—3.58 (2H, m, $-CH(OH)CH_2OH$), 2.60—3.24 (5H, m, 1-, 2-, 3-H, $-CH(OH)CH_2OH$), 1.28—2.00 (2H, m, $-CH_2CH(OH)CH_2OH$). *Anal.* Calcd for $C_{25}H_{28}O_9$: C, 63.55; H, 5.97. Found: C, 63.65; H, 5.86.

1-β-(2,3-Diacetoxy-1-propyl)-1-desoxypodophyllotoxin (6) A solution of **5** (900 mg, 1.90 mmol) and acetic anhydride (428 mg, 4.19 mmol) in THF (5 ml) was stirred in the presence of 4-dimethylaminopyridine (10 mg) for 2 d at room temperature. The reaction mixture was added to dilute HCl, and extracted with AcOEt. The extract was washed successively with brine 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 (50 : 1). Recrystallization from Et_2O–*n*-hexane gave **6** (927 mg, 87.7%). mp 92—93 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.65 (1H, s, 9-H), 6.45 (1H, s, 6-H), 6.26 (2H, s, 2′-, 6′-H), 5.95 (2H, s, OCH_2O), 4.80—5.40 (1H, br, $-CH(OAc)CH_2OAc$), 4.54 (1H, m, 4-H), 3.88—4.40 (4H, m, $-CH(OAc)CH_2OAc$, 11-H), 3.79 (3H, s, 4′-OCH_3), 3.74 (6H, s, 3′-, 5′-OCH_3), 2.80—3.20 (3H, m, 1-, 2-, 3-H), 2.20, 2.12, 2.11, 2.08 (6H, S, $-OCOCH_3$), 1.68—2.00 (2H, m, $-CH_2OH(OAc)CH_2OAc$). *Anal.* Calcd for $C_{29}H_{32}O_{11}$: C, 62.58; H, 5.79. Found: C, 62.65; H, 5.67.

4′-Demethyl-1-β-(2,3-diacetoxy-1-propyl)-1-desoxypodophyllotoxin (7) A solution of **6** (500 mg, 0.90 mmol) and 25% HBr–AcOH (1 ml) in $ClCH_2CH_2Cl$ (12 ml)–Et_2O (1 ml) was stirred for 20 h at room temperature. The reaction mixture was added to a mixture of 1% aqueous $NaHCO_3$ (50 ml) and AcOEt (100 ml). The mixture was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* at below 30 °C. The residue was purified by silica gel column chromatography with AcOEt–*n*-hexane (30 : 1). The fractions containing the compound with an *Rf* of 0.6 were collected and concentrated *in vacuo*. Recrystallization from Et_2O gave **7** (150 mg, 30.7%). mp 169—170 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.65 (1H, s, 9-H), 6.45 (1H, s, 6-H), 6.28 (2H, s, 2′-, 6′-H), 5.95 (2H, s, OCH_2O), 5.44 (1H, s, –OH), 4.72—5.40 (1H, br, $-CH(OAc)CH_2OAc$), 4.53 (1H, m, 4-H), 4.40—4.84 (4H, m, $-CH(OAc)CH_2OAc$, 11-H), 3.77 (6H, s, 3′-, 5′-OCH_3), 2.60—3.20 (3H, m, 1-, 2-, 3-H), 2.20, 2.12, 2.11, 2.08 (6H, s, $-OCOCH_3$), 1.48—2.00 (2H, m, $-CH_2CH(OAc)CH_2OAc$). *Anal.* Calcd for $C_{28}H_{30}O_{11}$: C, 61.98; H, 5.57. Found: C, 61.76; H, 5.27.

4′-Demethyl-1-β-(2,3-dihydroxy-1-propyl)-1-desoxypodophyllotoxin (8) A solution of **7** (100 mg, 0.18 mmol) and $Zn(OAc)_2$ (30 mg, 0.16 mmol) in MeOH (5 ml) was refluxed with stirring for 6 h. The reaction mixture was concentrated *in vacuo* at 30 °C. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (15 : 1). Recrystallization from Et_2O gave **8** (60 mg, 72.8%). mp 131—133 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.87, 6.80 (1H, s, 9-H), 6.43, 6.42 (1H, s, 6-H), 6.27, 6.26 (2H, s, 2′-, 6′-H), 5.91 (2H, s, OCH_2O), 5.41 (1H, s, 4′-OH), 4.53 (1H, m, 4-H), 4.00—4.40 (2H, m, 11-H), 3.73 (6H, s, 3′-, 5′-OCH_3), 3.58—3.68 (1H, m, $-CH(OH)CH_2OH$), 3.24—3.58 (2H, m, $-CH(OH)CH_2OH$), 2.60—3.24 (5H, m, 1-, 2-, 3-H, $-CH(OH)CH_2OH$), 1.28—2.00 (2H, m, $-CH_2CH(OH)CH_2OH$). *Anal.* Calcd for $C_{24}H_{26}O_9$: C, 62.87; H, 5.71. Found: C, 62.65; H, 5.77.

Biological Screening. Cell Lines and Cytotoxicity Test S-180 (a murine sarcoma cell line) was kept in continuous culture in RPMI 1640 medium supplemented with 10% fetal calf serum. Cell lines were seeded in 24-well culture plates (Corning, type 25820), and incubated for 96 h with individual test compounds. On day 4, the cells 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.[19] Exponentially growing cells were collected by centrifugation and washed with ice-cold NB buffer (NB consists of 2 mM K_2HPO_4, 5 mM $MgCl_2$, 150 mM NaCl, 1 mM EGTA, and 0.1 mM dithiothreitol, pH adjusted to 6.5). The washed cells were resuspended in NB, and a solution of 9 ml of NB supplemented with 0.35% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride was added slowly down the side of the tube. The cell suspension was mixed by rotation for 5 min at 4 °C, then centrifuged at 1000 × *g* for 10 min, and the nuclear pellets were washed with Triton-free NB. 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 17000 × *g* for 10 min and the supernatant was decanted. Protein concentration in the supernatant was determined by the method of Bradford.[20]

Topo-II Catalytic Activity Assay Topo-II catalytic activity was measured by using the decatenation assay.[21] The standard reaction mixture for this assay 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. Decatenation of kinetoplast DNA was carried out by incubating 4 μl of nuclear extract (0.05 μg protein) with 1 μg of

kinetoplast DNA in standard reaction mixture for 30 min at 30 °C. Reactions were terminated by the addition of 5 μl of 5% sodium dodecyl sulfate, 0.13% bromophenol blue, and 50% glycerol. Samples were then electrophoresed 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 transillumination with UV and photographed on Polaroid type 665 positive/negative film. Inhibitory activity was calculated from the densitometric scanning of gel negatives. The IC_{50} value was defined as the drug concentration needed to produce a 50% reduction in the amount of mini-circle DNA relative to the control.

Tubulin Preparation and Anti-microtubular Activity Test Bovine brain tubulin was prepared as described previously.[22] Purification was carried out in a buffer composed of 100 mM Mes (2-(*N*-morpholino)ethanesulfonic acid), 1 mM ethyleneglycol-bis-*N*,*N*-tetraacetic acid (EGTA), 1 mM $MgSO_4$, 5 mM NaH_2PO_4 and 0.02% NaN_3, pH 6.75 (MES buffer). After one cycle of polymerization–depolymerization, the final pellets were stored at −80 °C. Tubulin polymerization was carried out by incubating 50 μl of tubulin preparation (200 μg protein) with 250 μl of MEM buffer containing 1 mM GTP for 15 min at 37 °C. Tubulin polymerization was monitored spectrophotometrically 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.

References

1) T. Terada, K. Fujimoto, M. Nomura, J. Yamashita, T. Kobunai, S. Takeda, K. Wierzba, Y. Yamada, and H. Yamaguchi, *Chem. Pharm. Bull.*, **40**, 2720 (1992).
2) L. S. King and M. S. Sullivan, *Science*, **104**, 244 (1946).
3) W. J. Gensler, C. A. Murthy, and M. H. Trannell, *J. Med. Chem.*, **20**, 635 (1977); D. Loike, C. F. Brewer, H. Strenlicht, W. J. Gensler, H. Stänhelin, and A. von Wartburg, *Progress Drug Res.*, **33**, 169 (1989); I. Jardine, "Podophyllotoxin in Anticancer Agents Based on Natural Product Models," Academic Press, Inc., New York, 1980, p. 319; B. H. Long, S. F. Musial, and M. G. Brattain, *Biochemistry*, **23**, 1183 (1984).
4) H. Stähelin and A. Cerletti, *Schweiz. Med. Wschr.*, **94**, 1490 (1964).
5) G. Falkson, A. G. Sandison, and J. Vanzyl, *Afr. J. Radiol.*, **2**, 1 (1964); V. K. Vaitkevicius and M. I. Reed, *Cancer Chemother. Rep.*, **50**, 565 (1966).
6) J. D. Loike and S. B. Horwitz, *Biochemistry*, **15**, 5435 (1976); M. H. Cohen, L. L. Broder, and B. H. Fossieck, *Cancer Treat. Rep.*, **61**, 489 (1977); B. F. Issell, *Cancer Chemother. Pharmacol.*, **7**, 73 (1982); B. H. Long and A. Minocha, *Proc. Am. Assoc. Cancer Res.*, **24**, 321 (1983); B. S. Glisson, S. E. Smallwood, and W. E. Ross, *Biochim. Biophys. Acta*, **783**, 74 (1984).
7) R. Chapman, L. Itri, R. Gralla, D. Kelsen, E. Casper, and R. Golby, *Cancer Chemother. Pharmacol.*, **7**, 205 (1982); B. F. Issell, *ibid.*, **7**, 73 (1982).
8) W. E. Evans, J. A. Sinkule, W. R. Crom, L. Dow, and A. T. Look, *Cancer Chemother. Pharmacol.*, **7**, 147 (1982); J. van Hoogenhuijze, J. Lankelma, J. Stam, and H. M. Pinedo, *Eur. J. Cancer Clin. Oncol.*, **23**, 807 (1987).
9) A. Gouyette, A. Deniel, and J-L. Pico, *Eur. J. Cancer Clin. Oncol.*, **23**, 1627 (1987); W. E. Ewans, J. A. Sinkule, and W. R. Crom, *Cancer Chemother. Pharmacol.*, **7**, 147 (1982).
10) M. J. Straus and R. E. Moran, *Seminars in Respiratory Medicine*, **3**, 3, 194 (1982).
11) K. Kasahara, Y. Fujiwara, Y. Sugimoto, K. Nishio, T. Tamura, T. Matsuda, and N. Saijo, *J. Nat. Cancer Inst.*, **84**, 2, 113 (1992).
12) R. K. Levy, I. H. Hall, and K. H. Lee, *J. Pharm. Sci.*, **72**, 1158 (1983); H. Saito, H. Yoshikawa, Y. Nishimura, S. Kondo, T. Takeuchi, and H. Umezawa, *Chem. Pharm. Bull.*, **34**, 3733 (1986); H. Saito, Y. Nishimura, S. Kondo, and T. Takeuchi, *Bull. Chem. Soc. Jpn.*, **61**, 2493 (1988); L. S. Thurston, H. Irie, S. Tani, F. S. Han, Z. C. Liu, Y. C. Cheng, and H. Lee, *J. Med. Chem.*, **29**, 1547 (1986); S. A. Beers, Y. Imakura, H. J. Dai, Y. C. Cheng, and K. H. Lee, *J. Nat. Prod.*, **51**, 901 (1988); L. S. Thurston, Y. Imakura, M. Haruna, D. H. Li, Z. C. Liu, S. Y. Liu, Y. C. Cheng, and K. H. Lee, *J. Med. Chem.*, **32**, 604 (1989); K. H. Lee, Y. Imakura, M. Haruna, S. A. Beers, L. S. Thurston, H. Dai, C. H. Chen, S. Y. Liu, and Y. C. Cheng, *J. Nat. Prod.*, **52**, 606 (1989); S. Y. Liu, B. D. Hwang, M. Haruna, Y. Imakura, K. H. Lee, and Y. C. Cheng, *Mol. Pharmacol.*, **36**, 78 (1989); K. H. Lee, S. A. Beers, M. Mori, Z. Q. Wang, Y. H. Kuo, L. Li, S. Y. Liu, and Y. C. Cheng, *J. Med. Chem.*, **33**, 1364 (1990); Z. Q. Wang, Y. H. Kuo, D. Schur, J. P. Bowen, S. Y. Liu, F. S. Han, J. Y. Chang, Y. C. Cheng, and K. H. Lee, *ibid.*, **33**, 2660 (1990); R. S. Ward, *Synthesis*, **1992**, 719.
13) M. Kuhn and A. von Wartburg, *Helv. Chim. Acta*, **52**, 948 (1969); W. J. Gensler and C. D. Gatsonis, *J. Org. Chem.*, **31**, 3224 (1966); J. H. Beijnen, J. J. Holthuis, H. G. Kerkdijk, O. A. G. J. van der Houwen, A. C. A. Paalman, A. Bult, and W. J. M. Underberg, *Int. J. Pharmaceut.*, **41**, 169 (1988); Y. Aso, Y. Hayashi, S. Yoshioka, Y. Takeda, Y. Kita, Y. Nishimura, and Y. Arata, *Chem. Pharm. Bull.*, **37**, 422 (1989).
14) M. Kuhn, C. Keller-Julsen, and A von Wartburg, *Helv. Chim. Acta*, **52**, 944 (1969).
15) A. Hosomi and H. Sakurai, *Tetrahedron Lett.*, **16**, 1295 (1976); A. Hosomi, M. Endo, and H. Sakurai, *Chem. Lett.*, **1976**, 941; T. Tsunoda, M. Suzuki, and R. Noyori, *Tetrahedron Lett.*, **21**, 71 (1980); G. Deleris, J. Dunogues, and R. Calas, *ibid.*, **28**, 2449 (1976); T. Mukaiyama, T. Harada, and S. Shoda, *Chem. Lett.*, **1980**, 1507; H. Nishiyama and K. Itoh, *J. Org. Chem.*, **47**, 2496 (1982); H. Nishiyama, T. Naritomi, K. Sakuta, and K. Itoh, *ibid.*, **48**, 1557 (1983).
16) A. K. Kraus and M. Shimagaki, *Tetrahedron Lett.*, **22**, 1171 (1981); S. Murata, M. Suzuki, and R. Noyori, *J. Am. Chem. Soc.*, **102**, 3248 (1980); S. Murata, M. Suzuki, and R. Noyori, *Tetrahedron Lett.*, **21**, 2527 (1980); H. Sakurai, K. Sasaki, and A. Hosomi, *Bull. Chem. Soc. Jpn.*, **56**, 3195 (1983); A. Hosomi, Y. Sakata, and H. Sakurai, *Chem. Lett.*, **1983**, 405; M. T. Reetz, *Angew. Chem. Int. Ed.*, **23**, 556 (1984).
17) H. Yamaguchi, M. Arimoto, S. Nakajima, M. Tanoguchi, and Y. Fukada, *Chem. Pharm. Bull.*, **34**, 2056 (1986).
18) M. Kuhn and A. von Wartburg, *Helv. Chim. Acta*, **52**, 948 (1969).
19) A. M. Deffie, J. K. Batra, and G. J. Goldenberg, *Cancer Res.*, **49**, 58 (1989).
20) M. M. Bradford, *Anal. Biochem.*, **72**, 248 (1976).
21) M. Duguet, C. Lavenot, F. Harper, G. Mirambeau, and A. M. Derecondo, *Nucleic Acids Res.*, **11**, 1059 (1983); J. C. Marini, K. G. Miller, and P. T. Englund, *J. Biol. Chem.*, **255**, 4976 (1980).
22) D. G. Vogel, R. L. Margolis, and N. K. Mott, *Toxicol. Appl. Pharmacol.*, **80**, 473 (1985).