Tetrahedron Vol. 49, No. 9, pp. 1891-1900, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Design, Synthesis, and Antitumor Activity-Absolute Configuration Relationships of Podophyllotoxin Aza-Analogues[†]

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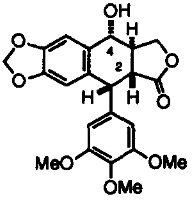
(*Received in Japan* 4 *November* 1992)

*Abstract: Optically active and racemic podophyllotoxin aza-analogues **3**~**7** were designed and synthesized by a highly stereoselective condensation reaction of a cyclic urethane **10** or **16** with 3,4,5-trimethoxybenzaldehyde **11** and were found to show a promising in vitro and in vivo antitumor activity.*

A long and fascinating history of podophyllotoxin **1** as medicinals has recently culminated in the semi-synthetic epi-analogue of clinically useful anticancer drug **2** (etoposide).[1] Since isomerization of cytotoxic **1** to inactive picropodophyllin is suggested to occur *via* epimerization at the C2 center under physiological conditions,[2] it is quite interesting to explore new podophyllotoxin analogues which are incapable to lose configurational integrity at the C2 center.[3] As our continuing studies towards synthesis and evaluation of antitumor activity of lignanes and analogues,[4,5] we designed and synthesized azapodophyllotoxins of which sp^3 C2 carbon was replaced with sp^2 nitrogen expecting configurational integrity. Azapodophyllotoxins were prepared in both racemic and optically pure forms, and evaluated their antitumor activity.[6,7]



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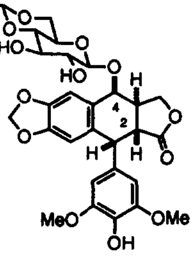

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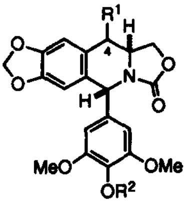


3 R^1 = H, R^2 = Me
4 R^1 = R^2 = H
5 R^1 = α-OH, R^2 = Me
6 R^1 = β-OH, R^2 = Me
7 R^1 = β-OH, R^2 = H

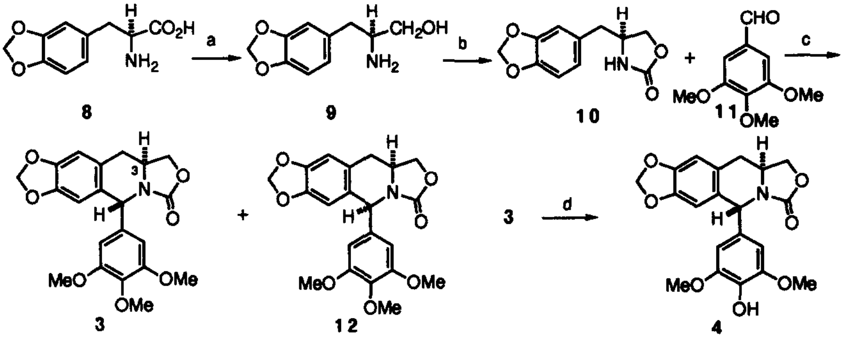
Design of Azapodophyllotoxin

The guide lines we set for the creation of candidate were categorized as follows: (1) the stereochemical structure has the most similarity to podophyllotoxin; (2) carbonyl oxygen should have enough electron density to form hydrogen-bond; (3) the compounds have minimum stereoisomers; (4) the synthetic route is as short as possible; (5) optically pure compounds are readily available.

On the basis of the guidelines we designed aza-analogues **3**~**7**. Aza-analogues have an sp^2 nitrogen at the corresponding C2 center of **1** and therefore epimerization at this center can be avoided.[4,5] From synthetic view point, the compounds **3**~**7**, both in racemic and optically pure forms, would be available in a quite short step starting from the known amino acid **8** *via* condensation of a cyclic urethane **9** and 3,4,5-trimethoxybenzaldehyde **11**.

Synthesis of Azadeoxypodophyllotoxin

The synthesis began with the preparation of racemic amino acid **8**. According to the reported procedure[8] acetylaminomalonate was alkylated with piperonyl chloride to afford, after hydrolysis and decarboxylation, the corresponding known amino acid **8**. Reduction of **8** with lithium aluminum hydride and treatment of the corresponding amino alcohol **9** (mp 80-82 °C) with diethyl carbonate provided the urethane **10**. A cyclic urethane **10** was prepared from a racemic **8** in 62% two-step yield. Optically pure (*S*)-(-)- and (*R*)-(+)-**10** were also prepared starting from the corresponding optically active L- and D-amino acid **8**, respectively.[8]



a) $LiAlH_4$/THF, reflux 2 h, 70%; b) $OC(OEt)_2$-NaOEt/EtOH, reflux 4 h, 89%; c) 11-H_2SO_4/CH_2Cl_2, rt 4 h, 93% for 3 and 3% for 12; d) HBr/$Cl(CH_2)_2Cl$, 0 °C 14 h, 80%.

Condensation of racemic **10** with 3,4,5-trimethoxybenzaldehyde **11** in the presence of H_2SO_4 (2 equiv) in CH_2Cl_2 at room temperature for 4 h provided a mixture of separable two diastereomers **3** and **12** in 93 and 3% yields, respectively. The stereostructure was determined by observing NOE (in $CDCl_3$). An enhancement of 8% was observed between a proton of trimethoxyphenyl ring (δ 6.47) and a methine proton at the C3 center (δ 4.03) of the major product and none of enhancement was observed between the corresponding protons (δ 6.50 and 4.12) of the minor product, indicating **3** and **12** as major and minor products. It is quite important to note that *trans*-**3** was formed predominantly, in sharp contrast to the podorhizol cyclization[9] and Pictet-Spengler reaction[10] providing a *cis*-product.

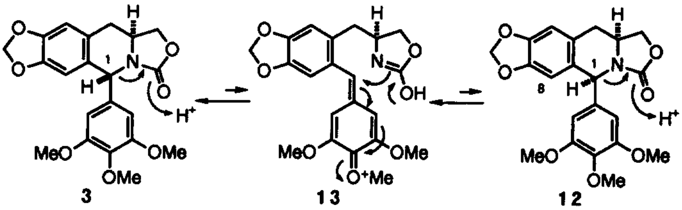
Stereoselective formation of the *trans* product **3** in the condensation is general regardless to the reagents used in the reaction as summarized in Table I. Table I also clearly demonstrates that stronger acids provide higher yields of the product.

The constant *trans*/*cis* (**3**/**12**) ratio regardless to the conditions suggests the thermodynamic equilibrium between the *trans*- and *cis*-products. Treatment of **12** with acid (H_2SO_4/CH_2Cl_2, HBr/CH_2Cl_2, CF_3CO_2H/benzene, etc.) established a constant equilibrium to afford a mixture of **3** and **12** in a ratio of 28:1 (determined by HPLC analysis). In turn, acid treatment of **3** also provided a mixture in the same ratio. These equilibrium between **3** and **12** is considered to occur *via* an intermediate **13** formed by C1-N bond cleavage. A trimethoxyphenyl ring of **12** is oriented pseudo-equatorial and sterically unfavorably interacted with C=O and C8-H bonds on a plane, being epimerized to a pseudo-

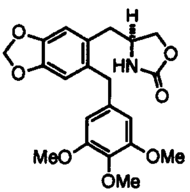
Table I. Stereoselective Formation of **3** from **10**

entry	reagent	solvent	temp / °C	time / h	**3** / %	**12** / %
1	HCO_2H	HCO_2H	100	24	43	3
2	$BF_3{\cdot}OET_2$	CH_2Cl_2	40	45	59	4
3	CF_3CO_2H	CH_2Cl_2	40	40	71	1
4	CF_3CO_2H	CF_3CO_2H	72	11	51	1
5	CF_3CO_2H	benzene	80	93	86	4
6	CF_3SO_3H	CH_2Cl_2	25	5	84	1
7	H_2SO_4	CH_2Cl_2	25	4	93	3

axial position in **3**. Predominant formation of **3** by equilibration rationalizes the highly stereoselective condensation reaction of **10** with **11**.



3 **13** **12**



14

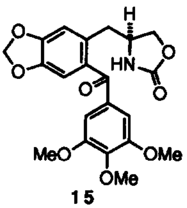
The existence of the intermediary of **13** was experimentally supported. Thus treatment of **3** with triethylsilane in trifluoroacetic acid at 72 °C for 44 h gave the reduction product **14** in 68% yield.

4'-Demethoxy derivative **4** was prepared in 80% yield from **3** or **12** by treating with HBr in $Cl(CH_2)_2Cl$ at 0 °C for 14 h.

Optically pure (-)- **3**, (-)-**12** and (+)- **3**, (+)-**12** were prepared starting from (-)-(*S*)- and (+)-(*R*)-**10**, respectively, without any event.

Synthesis of Azapodophyllotoxin

A direct introduction of oxygen functionality at the requisite benzylic position of azadeoxypodophyllotoxin **3** seems to be the straightforward way to azapodophyllotoxins **5**, **6**. Attempted oxidation of azadeoxypodophyllotoxin **3**, however, was not successful. For example, **15** was obtained in 86% yield by treating **3** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).



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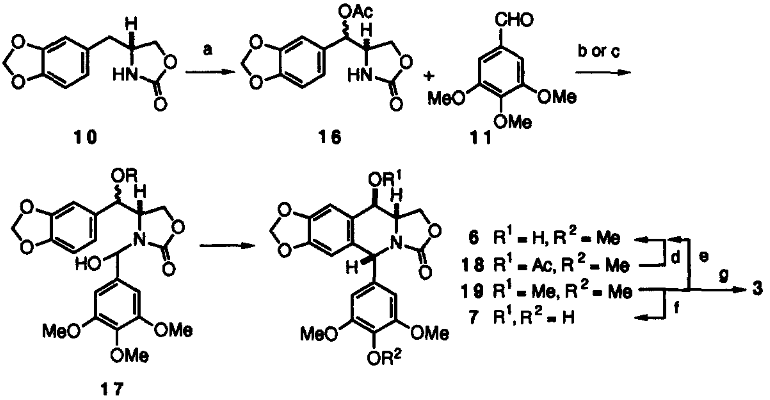
After an exhaustive and fruitless trial, we then turned our focus to cyclization of **16** with **11**. Treatment of **10** with DDQ[11] in acetic acid at 60 °C for 48 h provided **16** as a mixture of two diastereomers (6:4) in 70% yield.

Treatment of a mixture of **16** and **11** with H_2SO_4 under the best conditions for **3** did not give any condensation products, resulting in decomposition of **16**. Table II summarizes some of the results in condensation. A mixture of **16** and **11** was treated with triflic acid (2 equiv) in CH_2Cl_2 at 0 °C to provide intractable mixture. However dilution with acetic acid was found to effective in producing desired **18**. Thus a reaction of **16** with **11** in a mixture of CH_2Cl_2 and acetic acid (10:1) in the presence of triflic acid (2 equiv) at 4 °C for 24 h afforded **18** as a single isomer in 34% yield. Dilution with methanol (CH_2Cl_2-MeOH 10:1) was much more interesting to afford **19** as a single isomer in 94% yield. The structure of **19** was determined by NMR analysis and by chemical conversion to **3**. Nuclear Overhauser effect was observed by irradiation of aromatic protons of trimethoxyphenyl ring resulting in a 6% increase of integration at the H-3 methine proton and this indicates that **19** has 1,3-*trans* relation. Furthermore coupling constant between H-4 and H-3 is 1.8 Hz, indicating β-OMe at the C4 position. Ionic reduction of **19** with Et_3SiH in CF_3CO_2H afforded **3** in 93% yield, also supporting the structural assignment of **19**.

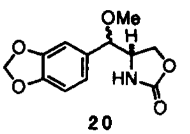


a) DDQ/AcOH, 60 °C 48 h, 70%; b) CF_3SO_3H/AcOH-CH_2Cl_2, 4 °C 24 h, 34% for **18**; c) CF_3SO_3H/MeOH-CH_2Cl_2, 4 °C 37 h, 94% for **19**; d) K_2CO_3/MeOH, rt 10 h, 95%; e) 10% aq. HCl/dioxane, 50 °C 5 h, 82% for **6** and 12% for **5**; f) HBr/Cl$(CH_2)_2$Cl, 4 °C 22 h, and then $BaCO_3$/aq. THF, rt 16 h, 60% for **7**; g) Et_3SiH/CF_3CO_2H, rt 1 h, 93%.

Table II. Stereoselective Formation of **18** and **19** from **16**

entry	reagent	solvent	temp / °C	time / h	**18** / %	**19** / %
1	CF_3CO_2H	benzene	80	90	0	-
2	H_2SO_4	CH_2Cl_2	0	1	0	-
3	CF_3SO_3H	CH_2Cl_2	0	3	27	-
4	CF_3SO_3H	CH_2Cl_2-AcOH	4	24	34	-
5	CF_3SO_3H	CH_2Cl_2-MeOH	4	37	-	94

Since the intermediate **20** (3:2 mixture of two diastereomers) was isolated in 78% yield along with **19** (19%) when reaction was conducted in the presence of sulfuric acid in CH_2Cl_2-MeOH (10:1), **19** would be produced *via* **20** formed by methanol attack to the corresponding benzylic cation species of **16**. Cyclization is considered to proceed regioselectively *via* benzylic cation species derived from a possible intermediate **17** to **18** and **19** without formation of **21** which would arise from alternative benzylic cation species. Stereoselective formation of 1,3-*trans*-cyclization product is reasonable in light of stability of 1,3-*trans* compound much more preferable than 1,3-*cis* isomer. Stereoselective formation of C4 center would be the result of preferential attack of oxy-functionality (MeOH and acetic acid) to the C4 benzylic cation of **18** and **19** by avoiding steric interference of pseudo-axial trimethoxyphenyl ring.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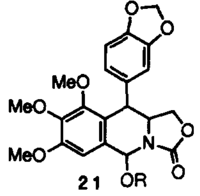
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Ester exchange reaction of the acetate **18** in the presence of K_2CO_3 in methanol provided the hydroxy compound **6** in 95% yield. The methoxy compound **19** was also converted to **6** and **5** in 82 and 12% yields, respectively, by treating with 10% aq. HCl in dioxane.

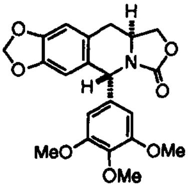
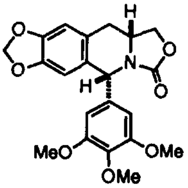
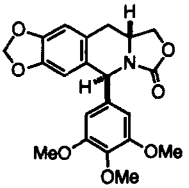
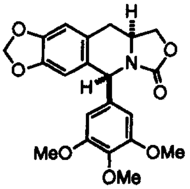
4'-Demethylepipodophyllotoxin analogue **7** was synthesized in 60% yield by treating **19** with hydrogen bromide in 1,2-dichloroethane and then with $BaCO_3$ in aq. THF. C_2 symmetry of 3',5'-dimethoxy-4'-hydroxyphenyl group of **7** was reasonably characterized by 400 MHz NMR in $CDCl_3$ (δ: 3.81 (6H, s, OCH_3 x 2), 5.53 (1H, s, ArOH), and 6.43 (2H, s, ArH)).

Optically pure (-)-**7** and (-)- **19** were prepared starting from (-)-(*S*)-**10** without any event.

Antitumor Activity-Absolute Configuration Relationships

To our delightful, the racemic compounds **3**, **4**, **5**, **6**, **7**, **12**, **18**, and **19** exhibited promising growth inhibition of KB cell (ED_{50} (μg/mL) **3**: <0.3, **4**: <0.3, **5**: <0.3, **6**: <0.3, **7**: 4.55, **18**: 0.62, **19**: 2.75) and *in vivo* activity against P-388 mouse (T/C 145 (**3**) and 170 (**12**)).[12]

It is also quite important to note that cytotoxicity of azadeoxypodophyllotoxin relies mostly on the absolute configuration at the C1 position, not that at the C3 position as shown.

	(-)-3	(+)-12	(+)-3	(-)-12
ED_{50} (μg/ml)	< 0.3	< 0.3	10.3	38.5

We have also succeeded in the preparation of aza-etoposide starting from (-)-**19** which will be the subject of further publication.[12]

Experimental[13]

(-)-(*S*)-3,4-Methylenedioxyphenylalaninol (9): A mixture of L-3,4-methylenedioxyphenylalanine((*S*)-**8**)[8] (1.37 g, 6.55 m mol) and $LiAlH_4$ (0.75 g, 19.7 m mol) in THF (35 ml) was stirred under reflux for 6 h. Successive addition of water (0.75 ml), 15 % NaOH (0.75 ml), and water (2.25 ml) and following filtration provided a colorless solution. Concentration provided colorless solids (1.27 g, mp

87-97°C). Recrystallization from benzene (5 ml) afforded (-)-(*S*)-**9**: (1.08 g, 85 %) as colorless needles of mp 91-92 °C. $[\alpha]_D^{25}$ -21.5 °(c=1.108, $CHCl_3$). IR (KBr): 3355 cm^{-1}. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.69 (3H, brs), 2.45 (1H, dd, J=8.4, 13.6 Hz), 2.71 (1H, dd, J=5.1, 13.6 Hz), 3.06 (1H, dddd, J=4.0, 5.1, 7.3, 8.4 Hz), 3.37 (1H, dd, J=7.3, 10.6 Hz), 3.62 (1H, dd, J=4.0, 10.6 Hz), 5.94 (2H, s), 6.64 (1H, dd, J=1.7, 7.7 Hz), 6.69 (1H, d, J=1.7 Hz), 6.75 (1H, d, J=7.7 Hz). MS m/z: 195. Anal. ($C_{10}H_{13}O_3N$).

(+)-(*R*)-**9**: Colorless needles of mp 88-89 °C. $[\alpha]_D^{20}$ +21.3 °(c=1.29, $CHCl_3$).

(±)-**9**: Colorless needles of mp 80-82 °C (benzene).

(-)-(*S*)-4-(3,4-Methylenedioxybenzyl)-1,3-oxazolidin-2-one (10): A solution of (-)-(*S*)-**9** (0.98 g, 5.0 m mol), diethyl carbonate (5.93 g, 50 m mol), and sodium methoxide (0.5 m mol) in ethanol (4.5 ml) was stirred under reflux for 3 h. After concentration the residue was diluted with 10 % aq. HCl (20 ml) and extracted with ethyl acetate (30 ml x 3). The extracts were washed with water (30 ml), satd. aq. $NaHCO_3$ (30 ml), and brine (30 ml), and then dried over Na_2SO_4. Concentration gave pale yellow solids (1.13 g). Recrystallization from ethyl acetate (1.3 ml)-ether (1 ml) gave (-)-(*S*)-**10** (0.99 g, 90 %) as colorless pillars of mp 98-99.5 °C. $[\alpha]_D^{20}$ -59.9 °(c=1.362, $CHCl_3$). IR ($CHCl_3$): 3450, 1755 cm^{-1}. MS m/z: 221. ^{1}H-NMR ($CDCl_3$, TMS) δ: 2.78 (1H, dd, J=6.4, 13.7 Hz), 2.80 (1H, dd, J=7.1, 13.7 Hz), 4.03 (1H, dddd, J=5.5, 6.4, 7.1, 8.6 Hz), 4.13 (1H, dd, J=5.5, 8.6 Hz), 4.43 (1H, dd, J=8.6, 8.6 Hz), 5.89 (1H, brs), 5.95 (2H, s), 6.63 (1H, dd, J=1.8, 7.7 Hz), 6.66 (1H, d, J=1.8 Hz), 6.77 (1H, d, J=7.7 Hz). Anal. ($C_{11}H_{11}NO_4$).

(+)-(*R*)-**10**: Colorless pillars of .mp 97-98 °C. $[\alpha]_D^{20}$ +60.8 °(c=1.174, $CHCl_3$).

(±)-**10**: Colorless prisms of mp 82-83.5 °C (ethyl acetate-ether (1/6)).

(-)-2-Azadeoxypodophyllotoxin (3) and **(-)-2-Azaisodeoxypodophyllotoxin (12)**: A solution of (-)-**10** (75.1 mg, 0.339 m mol), 3,4,5-trimethoxybenzaldehyde (83.9 mg, 0.441 m mol), and conc. H_2SO_4 (0.036 ml, 0.679 m mol) in CH_2Cl_2 (3 ml) was stirred at rt for 5 h. After dilution with CH_2Cl_2 (50 ml), the whole was washed with satd. $NaHCO_3$ (20 ml) and brine (40 ml), and then dried over Na_2SO_4. Concentration gave a yellow caramel (161 mg). Purification by SiO_2 column chromatography (CH_2Cl_2-acetone (2/1)) gave (-)-**3** (125.4 mg, 93 %) and (-)-**12** (4.3 mg, 3 %).

(-)-**3**: Colorless needles of mp 200-201 °C ($CHCl_3$-benzene). $[\alpha]_D^{24}$ -171.8 °(c=1.134, $CHCl_3$). IR (KBr): 1725, 1592 cm^{-1}. ^{1}H-NMR ($CDCl_3$, TMS) δ: 2.89 (1H, dd, J=10.2, 15.4 Hz), 2.93 (1H, dd, J=5.1, 15.4 Hz), 3.80 (6H, s), 3.84 (3H, s), 4.03 (1H, m), 4.11 (1H, dd, J=4.2, 8.4 Hz), 4.48 (1H, dd, J=8.4, 8.4 Hz), 5.85 (1H, s), 5.94 and 5.97 (each 1H, d, J=1.5 Hz), 6.46 (1H, s), 6.47 (2H, s), 6.64 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 34.3 (t), 48.1 (d), 56.1 (q), 56.4 (d), 60.7 (q), 68.3 (t) 101.0 (t), 105.7 9d), 108.0 (d), 108.2 (d), 125.4 (s), 126.5 (s), 137.3 (s), 137.6 (s), 146.5 (s), 146.9 (s), 153.0 (s), 156.2(s). MS m/z: 399. Anal. ($C_{21}H_{21}NO_7$).

(-)-**12**: Colorless needles of mp 241-242 °C ($CHCl_3$-benzene). $[\alpha]_D^{24}$ -56.2 °(c=1.00, $CHCl_3$). IR (KBr): 1740, 1592 cm^{-1}. ^{1}H-NMR ($CDCl_3$, TMS) δ: 2.96 (1H, dd, J=3.7, 14.5 Hz), 3.00 (1H, dd, J=9.3, 14.5 Hz), 3.80 (3H, s), 3.82 (6H, s), 4.05 (1H, dd, J=7.0, 11.0 Hz), 4.12 (1H, m), 4.55 (1H, dd, J=7.0, 7.1 Hz), 5.49 (1H, s), 5.90 and 5.93 (each 1H, d, J=0.7 Hz), 6.50 (2H, s), 6.53 and 6.62 (each 1H, s). ^{13}C-NMR ($CDCl_3$) δ: 34.1 (t), 54.5 (d), 56.0 (q), 59.6 (d), 60.6 (q), 68.2 (t), 101.1 (t), 104.7 (d), 107.8 (d), 108.4 (d), 124.1 (s), 129.6 (s), 137.3 (s), 137.8 (s), 146.6 (s), 147.0 (s), 152.9 (s), 156.5 (s). MS m/z: 399. Anal. ($C_{21}H_{21}NO_7 \cdot 1/3\ H_2O$).

(+)-**3**: Colorless needles of mp 200-201 °C ($CHCl_3$-benzene). $[\alpha]_D^{23}$ +166.5 °(c=1.176, $CHCl_3$).

(±)-**3**: Colorless needles of mp 185-186 °C (benzene).

(+)-**12**: Colorless needles of mp 236-238 °C ($CHCl_3$-benzene). $[\alpha]_D^{23}$ +54.3 °(c=0.99, $CHCl_3$).

(±)-12: Colorless needles of mp 237.5-238.5 °C ($CHCl_3$-ether).

Isomerization of (±)-3 and (±)-12: A solution of (±)-**3** (36 mg, 0.09 m mol) and conc. H_2SO_4 (17 mg, 0.18 m mol) in CH_2Cl_2 (0.9 ml) was stirred at rt for 1 h. After dilution with CH_2Cl_2 (30 ml), the whole was washed with satd. $NaHCO_3$ (10 ml) and brine (40 ml), and then dried over $MgSO_4$. Concentration gave pale yellow solids (36 mg) as a mixture of (±)-**3** and (±)-**12** in a ratio of 95:5 (determined by HPLC analysis (Waters μ Polasil, AcOEt-hexane (2/1), 2.0 ml/min, 254 nm, 4.7 min for **3**, 7.2 min for **12**).

By the same way, (±)-**12** was converted to a mixture of (±)-**3** and (±)-**12** in a ratio of 95:5.

(±)-4-(4,5-Methylenedioxy-2-(3,4,5-trimethoxybenzyl)benzyl)-1,3-oxazolidin-2-one (14): A mixture of (±)-**3** (32.0 mg, 0.08 m mol) and Et_3SiH (57 mg, 0.49 m mol) in CF_3CO_2H (0.4 ml) was stirred under reflux for 44 h. After dilution with $CHCl_3$ (30 ml), the whole was washed with satd. $NaHCO_3$ (20 ml) and brine (20 ml), and then dried over Na_2SO_4. Concentration gave a yellow oil which was purified by SiO_2 column chromatography (CH_2Cl_2-acetone (20/1)) to give (±)-**3** (9.2 mg, 29 %) and (±)-**14** (15.4 mg, 48 %) as colorless needles of mp 159-159.5 °(benzene). IR ($CHCl_3$): 3450, 1760 cm^{-1}. ^1H-NMR ($CDCl_3$, TMS) δ: 2.79 (2H, m), 3.79 (6H, s), 3.82 (3H, s), 3.86 (1H, d, J=2.9 Hz), 3.88-3.95 (1H, m), 4.06 (1H, dd, J=5.5, 8.4 Hz), 4.41 (1H, dd, J=8.4, 8.4 Hz), 5.37 (1H, brs), 5.95 (2H, s), 6.27 (2H, s), 6.63 and 6.65 (each 1H, s). MS m/z: 401. Anal. ($C_{21}H_{23}NO_7$).

(±)-4-(4,5-Methylenedioxy-2-(3,4,5-trimethoxybenzoyl)benzyl)-1,3-oxazolidin-2-one (15): A mixture of (±)-**3** (24.1 mg, 0.06 m mol) and DDQ (28.6 mg, 0.126 m mol) in CH_3CO_2H (0.12 ml) was stirred at 65 °C for 2.5 h. The mixture was diluted with CH_2Cl_2 (50 ml) and washed with satd. $NaHCO_3$ (20 ml), 10 % NaOH (20 ml x 2), and brine (20 ml x 2), and then dried over $MgSO_4$. Concentration gave white solid (23.5 mg) which was purified by SiO_2 column chromatography (benzene-acetone (3/1)) to give (±)-**15** (21.5 mg, 86 %) as white solids of mp 159-161 °C. IR ($CHCl_3$): 1754, 1649 cm^{-1}. ^1H-NMR ($CDCl_3$, TMS) δ: 2.78 (1H, dd, J=6.9, 13.6 Hz), 3.01 (1H, dd, J=4.4, 13.6 Hz), 3.87 (6H, s), 3.94 (3H, s), 4.09-4.67 (3H, m), 5.91 (1H, s), 6.05 (1H, d, J=1.5 Hz), 6.06 (1H, d, J=1.5 Hz), 6.82 (1H, s), 6.89 (1H, s), 7.00 (2H, s). MS m/z: 415. Anal. ($C_{21}H_{21}NO_8$).

Synthesis of 4-(Acetyloxy-(3,4-methylenedioxyphenyl)methyl)-1,3-oxazolidin-2-one (16) from (-)-10: A suspension of (-)-**10** (1.26g, 5.7 m mol), DDQ (2.73 g, 11.6 m mol) in AcOH (12 ml) was stirred at 60 °C for 50 h. After concentration and following dilution with CH_2Cl_2 (300 ml), the whole was washed with 10 % NaOH (200 ml x 2) and brine (100 ml), and then dried over $MgSO_4$. Concentration provided a mixture of (-)-**10** and **16** (1.29 g). Purification by SiO_2 column chromatography (CH_2Cl_2-acetone) afforded (-)-**10** (26% recovery) and **16** (826 mg, 52%, 70% based on the consumed **10**) as a mixture of 6:4 two diastereomers. IR($CHCl_3$): 3450, 1764, 1750 cm^{-1}. ^1H-NMR ($CDCl_3$, TMS) δ: 2.12 (3H, s), 4.02-4.54 (3H, m), 5.05 (0.4H, brs), 5.76 (0.6H, brs), 5.56 (0.6H, d, J=7.5 Hz), 5.66 (0.4H, d, J=6.3 Hz), 5.98 (2H, s), 6.81 (3H, brs). Ms m/z: 279. Anal. ($C_{13}H_{13}NO_6$).

(±)-Acetyl-2-azaepipodophyllotoxin (18): To a mixture of (±)-**16** (6:4 diastereomers mixture, 16.3 mg, 0.058 m mol) and 3,4,5-trimethoxybenzaldehyde (16.4 mg, 0.084 m mol) in CH_2Cl_2-AcOH (10:1, 0.5 ml) was added at -20°C triflic acid (19.3 mg, 0.129 m mol). The mixture was stirred at 4 °C for 15 h. After addition of satd. $NaHCO_3$ (2 ml) at -20 °C, the mixture was stirred for 10 min and then extracted with CH_2Cl_2 (50 ml). The extracts were washed with satd. $NaHCO_3$ (20 ml) and brine (20 ml x 2) and then dried over $MgSO_4$. Concentration gave a yellow oil (32 mg) which was purified by SiO_2

column chromatography (CH_2Cl_2-acetone, 20/1) to give (±)-**18** (9.0 mg, 34 %) as colorless prisms of mp 236.5-237.5 °C (CH_2Cl_2-benzene). IR (KBr): 1745, 1735 cm^{-1}. ^1H-NMR ($CDCl_3$, TMS) δ: 2.10 (3H, s), 3.80 (6H, s), 3.84 (3H, s), 4.22-4.26 (2H, m), 4.42 (1H, dd, J=9.5, 9.5 Hz), 5.94 (1H, s), 5.95 (1H, d, J=2.2 Hz), 5.97 and 6.01 (each 1H, d, J=1.5 Hz), 6.44 (2H, s), 6.49 (1H, s), 6.93 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 21.07 (q), 51.98 (d), 56.30 (q), 56.42 (q), 60.83 (q), 64.19 (t), 68.10 (d), 101.67 (t), 105.69 (d), 108.26 (d), 109.93 (d), 124.67 (s), 129.05 (s), 136.90 (s), 138.07 (s), 147.38 (s), 148.84 (s), 153.48 (s), 156.89 (s), 170.88 (s). MS m/z: 457. Anal. ($C_{22}H_{23}NO_3$). Diastereomerically pure **16**, obtained by columun chromatography of the mixture, was also converted to **18** in 40 % yield.

(-)-4-Methoxy-2-azaepideoxypodophyllotoxin (19): To a mixture of optically active **16** derived from (-)-**10** (10:7 diastereomers mixture, 19 mg, 0.068 m mol) and 3,4,5-trimethoxybenzaldehyde (19.2 mg, 0.098 m mol) in CH_2Cl_2-MeOH (10:1, 0.5 ml) was added triflic acid (22.6 mg, 0.15 m mol). The mixture was stirred at rt for 14 h. Satd. $NaHCO_3$ (2 ml) was added at 0°C. The whole was extracted with CH_2Cl_2 (20 ml). The extracts were washed with brine (30 ml) and then dried over Na_2SO_4. Concentration gave yellow solid (39.5 mg) which was purified by SiO_2 column chromatography (CH_2Cl_2-acetone, 20/1) gave (-)-**19** (27.4 mg, 94 %) as colorless prisms of mp 245-246 °C (CH_2Cl_2-benzene). $[\alpha]^{25}_{D}$ -138.8 °(c=1.13, $CHCl_3$). IR (KBr): 1741 cm^{-1}. ^1H-NMR ($CDCl_3$, TMS) δ: 3.31 (3H, s), 3.80 (6H, s), 3.84 (3H, s), 4.07 (1H, d, J=1.8 Hz), 4.14 (1H, ddd, J=1.8, 3.7, 8.4 Hz), 4.41 (1H, dd, J=8.4, 8.4 Hz), 4.58 (1H, dd, J=3.7, 8.4 Hz), 5.80 (1H, s), 5.98 and 6.00 (each 1H, d, J=1.5 Hz), 6.45 (2H, s), 6.49 (1H, s), 6.76 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 53.70 (d), 56.01 (q), 56.21 (q), 56.79 (d), 60.73 (q), 64.35 (t), 75.61 (d), 101.37 (t), 105.55 (d), 106.86 (d), 109.61 (d), 125.06 (s), 129.00 (s), 137.53 (s), 137.63 (s), 146.34 (s), 148.16 (s), 153.12 (s), 157.43 (s). MS m/z: 429. Anal. ($C_{22}H_{23}NO_3$).

(±)-**19**: white powder of mp 222.5-226.5 °C (benzene).

Reduction of (±)-19 to (±)-3: A mixture of (±)-**19** (3.0 mg, 0.007 m mol) and Et_3SiH (1.0 mg, 0.08 m mol) in CF_3CO_2H (0.04 ml) was stirred at rt for 1 h. After addition of satd. $NaHCO_3$ (3 ml), the mixture was extracted with $CHCl_3$ (10 ml). The extract was washed with brine (20 ml) and then dried over Na_2SO_4. Concentration gave a yellow oil (3.1 mg) which was purified by SiO_2 column chromatography (benzene-AcOEt, 2/1) to give (±)-**3** (2.6 mg, 93 %).

(±)-2-Azapodophyllotoxin (5) and 2-Azaepipodophyllotoxin (6): A mixture of (±)-**19** (16.2 mg, 0.038 m mol) and 10 % HCl (0.4 ml) in dioxane (0.6 ml) was stirred at 40 °C for 16 h and then at 50 °C for 5.5 h. The mixture was diluted with $CHCl_3$ (30 ml). After neutralization with satd. $NaHCO_3$ (20 ml), the mixture was extracted with $CHCl_3$ (20 ml x 2). The combined extracts were washed with brine (30 ml) and then dried over Na_2SO_4. Concentration gave a colorless oil (17 mg) which was purified by SiO_2 column chromatography (CH_2Cl_2-acetone, 9/1) to give (±)-**5** (1.8 mg, 12 %) and (±)-**6** (12.9 mg, 82 %).

(±)-**5**: Colorless prisms of mp 225-225.5 °C ($CHCl_3$-benzene). IR ($CHCl_3$): 3440, 1740 cm^{-1}. ^1H-NMR ($CDCl_3$, TMS) δ: 2.33 (1H, brs), 3.76 (1H, ddd, J=3.7, 8.1, 9.5 Hz), 3.80 (6H, s), 3.84 (3H, s), 4.46 (1H, dd, J=3.7, 9.2 Hz), 4.52 (1H, dd, J=8.1, 9.2 Hz), 4.63 (1H, brd, J=9.5 Hz), 5.82 (1H, s), 5.97 and 6.00 (each 1H, d, J=1.5 Hz), 6.45 (1H, s), 6.48 (2H, s), 7.14 (1H, s). Ms m/z: 415. Anal. ($C_{21}H_{21}NO_8$).

(±)-**6**: Colorless prisms of mp 223-225 °C (dec) ($CHCl_3$-benzene). IR (KBr): 3420, 1738 cm^{-1}. ^1H-NMR ($CDCl_3$, TMS) δ: 2.44 (1H, d, J=8.0 Hz, D_2O exchangeable), 3.78 (6H, s), 3.83 (3H, s), 4.09 (1H, ddd, J=2.4, 4.4, 8.8 Hz), 4.40 (1H, dd, J=8.4, 8.8 Hz), 4.50 (1H, dd, J=2.4, 8.0 Hz), 4.69 (1H, dd, J=4.4, 8.4 Hz), 5.88 (1H, s), 5.97 and 6.00 (each 1H, d, J=1.5 Hz), 6.42 (2H, s), 6.48 (1H, s), 6.88 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 52.95 (d), 56.19 (d), 56.28 (q), 60.80 (q), 64.10 (t), 66.99 (d), 101.58 (t), 105.81 (d), 108.23 (d), 109.52 (d), 127.76 (s), 128.46 (s), 136.64 (s), 138.04 (s), 147.55 (s), 148.52 (s), 153.36 (s), 157.16 (s). MS m/z: 415. Anal. ($C_{21}H_{21}NO_8$).

(±)-**6** was also prepared by transesterification of (±)-**18**: A mixture of (±)-**18** and K_2CO_3 in MeOH was stirred at rt for 10 h. After filtration and concentration, the residue was purified by SiO_2 column chromatography to give (±)-**6** in 80 % yield.

(±)-2-Aza-4'-demethyldeoxypodophyllotoxin (4): A solution of (±)-**3** (3.0 g, 7.5 m mol) in CH_2Cl_2 (24 ml) was treated with HBr gas at 0 °C for 30 min. The mixture was stirred at 0 °C for 14 h under sealing. Concentration gave a yellow caramel (3.2 g). Purification by SiO_2 column chromatography ($CHCl_3$-acetone 10/1) gave (±)-**4** (2.3 g, 80 %) and (±)-catechol (0.29 g, 11 %).

(±)-**4**: White powder of mp 210-212 °C (acetone). IR ($CHCl_3$): 3530, 1743, 1613 cm^{-1}. ^{1}H-NMR ($CDCl_3$, TMS) δ: 2.86 (1H, d, J=15.4 Hz), 2.93 (1H, dd, J=5.1, 15.4 Hz), 3.83 (6H, s), 4.0-4.07 (1H, m), 4.10 (1H, dd, J=4.4, 8.4 Hz), 4.46 (1H, dd, J=8.4, 8.4 Hz), 5.53 (1H, brs), 5.85 (1H, s), 5.93 and 5.96 (each 1H, d, J=1.3 Hz), 6.46 (1H, s), 6.48 (2H, s), 6.63 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 34.4 (t), 48.0 (d), 56.5 (q), 56.5 (d), 68.4 (t), 101.2 (t), 105.6 (d), 108.3 (d), 108.4 (d), 125.6 (s), 126.9 (s), 133.2 (s), 134.7 (s), 146.7 (s), 147.0 (s), 147.1 (s), 156.5 (s). MS m/z: 385. Anal. ($C_{20}H_{19}NO_7$).

(±)-Catechol: Colorless prisms of mp 226.5-228 °C (acetone). ^{1}H-NMR ($CDCl_3$, TMS) δ: 2.8-2.9 (2H, m), 3.88 (3H, s), 4.0-4.16 (1H, m), 4.08 (1H, dd, J=4.0, 9.6 Hz), 4.46 (1H, dd, J=9.6, 9.6 Hz), 5.38 and 5.43 (each 1H, brs), 5.82 (1H, s), 5.93 (2H, s), 6.23 (1H, d, J=2.0 Hz), 6.44 and 6.61 (each 1H, s), 6.66 (1H, d, J=1.7 Hz). MS m/z: 371. Anal. ($C_{19}H_{17}NO_7$).

(-)-2-Aza-4'-demethylepipodophyllotoxin (7): Hydrogen bromide gas was bubbled through a solution of (-)-**19** (3.0 g, 7.0 m mol) in $(CH_2)_2Cl_2$ (0.5 ml) at 0 °C for 30 min. The mixture was stirred at 0 °C for 26 h under sealing. Concentration gave a yellow solid (3.72 g) which was treated with $BaCO_3$ (3 g) in THF-water (9:1, 150 ml) at rt for 24 h and then diluted with EtOH. The mixture was filtered and then concentrated to give a brown oil. Purification by SiO_2 column chromatography (CH_2Cl_2-acetone 4/1) gave (-)-**7** (1.6 g, 71%) as colorless prisms of mp 203-206 °C (AcOEt-hexane). $[\alpha]^{23}_{D}$ -131.4 °(c=1.012, $CHCl_3$). IR ($CHCl_3$): 3530, 3360, 1741 cm^{-1}. ^{1}H-NMR ($CDCl_3$, TMS) δ: 2.28 (1H, brs, D_2O exchangeable), 3.81 (6H, s), 4.08 (1H, ddd, J=2.4, 4.4, 8.8 Hz), 4.50 (1H, brs, (d, J=2.4 Hz by D_2O exchange)), 4.69 (1H, dd, J=4.4, 8.4 Hz), 5.53 (1H, s, D_2O exchangeable), 5.89 (1H, s), 5.97 and 6.00 (each 1H, d, J=1.5 Hz), 6.43 (2H, s), 6.48 (1H, s), 6.88 (1H, s). MS m/z: 401. Anal. ($C_{20}H_{19}NO_8$·1/2 H_2O). HRMS Calcd for $C_{20}H_{19}NO_8$ 401.1108. Found 401.sa1052.

(±)-7: Colorless prisms of mp 246-248 °C ($CHCl_3$-MeOH).

[†] Dedicated to Emeritus Professor Shun-ichi Yamada on the occasion of his 77th birthday.

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References and Notes

1 Stähelin, H.; von Wartburg, A. in *Progress in Drug Research*, ed. by Jucker, E. **1989**, *33*, 169; Jardin, I. in "Anticancer Agents Based on Natural Product Models," ed. by Cassady, J. M.; Douros, J. D. 1980, Academic Press, p 319.

2 Emmenegger, H.; Stähelin, H.; Rutschmann, J.; Renz, J.; von Wartburg, A. *Arzneim.-Forsch* **1961**, *11*, 327, 459.

3 Synthesis of podophyllotoxin is still challenging problem: Gensler, W. J.; Gatsonis, C. D. *J. Org. Chem.* **1966**, *31*, 4004; Kende, A. S.; King, M. L.; Curran, D. P. *Ibid.* **1981**, *46*, 2826; Rajapaksa D.; Rodorigo, R. *J. Am. Chem. Soc.* **1981**,*103*, 6208; Murphy, W. S.; Wattanasin, S. *J. Chem. Soc. Parkin I* **1982**, 271; Van der Eycken, J.; De Clercq, P.; Vandewalle, M. *Tetrahedron* **1986**, *42*, 4297; Vyas, D. M.; Skonezny, P. M.; Jenks, T. A.; Doyle, T. W. *Tetrahedron Lett.* **1986**, *27*, 3099; Jung, M. E., Lowen, G. T. *Ibid.* **1986**, *27*, 5319; Kaneko, T.; Wong, H. *Ibid.* **1987**, *28*, 517; Jones, D. W.; Thompson, A. M. *J. Chem. Soc., Chem. Commun.* **1987**, 1797; MacDonald, D. I.; Durst, T. *J. Org. Chem.* **1988**, *53*, 3663; Andrews, R. C.; Teague, S. J. Meyers, A. I. *J. Am. Chem. Soc.* **1988**, *110*, 7854.

4 Tomioka, K.; Ishiguro, T.; Mizuguchi, H.; Komeshima, N.; Koga, K.; Tsukagoshi, S.; Tsuruo, T.; Tashiro, T.; Tanida, S. and Kishi, T. *J. Med. Chem.* **1991**, *34*, 54.

5 Tomioka, K.; Kubota, Y.; Kawasaki, H.; Koga, K. *Tetrahedron Lett.* **1989**, *30*, 2949.

6 Preliminary communication: Tomioka, K.; Kubota, Y.; Koga, K. *Tetrahedron Lett.* **1989**, *30*, 2953; *Idem*, *J. Chem. Soc., Chem. Commun.* **1989**, 1622.

7 Similar approach has been described. Pearce, H. L.; Bach, N. J.; Cramer, T. L. *Tetrahedron Lett.* **1989**, *30*, 907; Bosmans, J.-P.; Van der Eycken, J.; Vandewalle, M.; Hulkenberg, A.; Van Hes, R.; Veerman, W. *ibid.* **1989**, *30*, 3877.

8 Both racemic and optically active amino acids are available. Yamada, S.; Fujii, T.; Shioiri, T. *Chem. Pharm. Bull.* **1962**, *10*, 680.

9 Tomioka, K.; Ishiguro, T.; Koga, K. *Chem. Pharm. Bull.* **1985**, *33*, 4333.

10 Konda, M.; Shioiri, T.; Yamada, S. *Chem. Pharm. Bull.* **1975**, *23*, 1025.

11 Tomioka, K.; Mizuguchi, H.; Ishiguro, T.; Koga, K. *Chem. Pharm. Bull.* **1985**, *33*, 121.

12 We are grateful to Drs. S. Tsukagoshi, T. Tsuruo, T. Tashiro, Cancer Institute, for activity evaluation. A part of the work was financially supported by grants from The Research Foundation for Optically Active Compounds, the Hoansha Foundation and by Grant-in-Aid for Scientific Research, the Ministry of Education, Science and Culture, Japan.

13 Satisfactory analytical data (±0.3% for C,H,N) were obtained for new comppounds described in the experimental section.