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

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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 sp3 C2 carbon was replaced with sp2 nitrogen expecting configurational integrity. Azapodophyllotoxins were prepared in both racemic and optically pure forms, and evaluated their antitumor activity.6,7

Design of Azapodophyllotoxin

The guidelines 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 sp2 nitrogen at the corresponding C2 center of 1 and therefore epimerization at this center can be avoided.4,5 From
synthetic viewpoint, 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.\(^8\)

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\text{O} \quad \text{O} \\
\begin{array}{c}
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\text{MeO} \\
\text{MeO}
\end{array}
\text{H}_{\text{COOH}} \quad \text{NH}_{2} \\
\text{O} \quad \text{O} \\
\begin{array}{c}
\text{MeO} \\
\text{MeO} \\
\text{MeO}
\end{array}
\]

\(a) \text{LiAlH}_4/\text{THF}, \text{reflux} \ 2 \text{ h}, 70\%; b) \text{OC(OEt)}_2-\text{NaOEt}/\text{EtOH}, \text{reflux} \ 4 \text{ h}, 89\%; c) \text{H}_{2}\text{SO}_4/\text{CH}_2\text{Cl}_2, \text{rt} \ 4 \text{ h}, 93\% \text{ for 3 and 3}\% \text{ for 12; d) HBr/Cl(CH}_2)_2\text{Cl, 0 }^\circ \text{C} \text{14 h, 80}\%.
\]

Condensation of racemic 10 with 3,4,5-trimethoxybenzaldehyde 11 in the presence of H\(_2\text{SO}_4\) (2 equiv) in CH\(_2\text{Cl}_2\) 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\(_2\text{SO}_4/\text{CH}_2\text{Cl}_2\), HBr/CH\(_2\text{Cl}_2\), CF\(_3\text{CO}_2\text{H/benzene}, \text{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 Cl-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-
axial position in 3. Predominant formation of 3 by equilibration rationalizes the highly stereoselective condensation reaction of 10 with 11.

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(CH2)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).

After an exhaustive and fruitless trial, we then turned our focus to cyclization of 16 with 11. Treatment of 10 with DDQ11 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 H2SO4 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 CH2Cl2 at 0 °C to

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

<table>
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<th>entry</th>
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<th>temp / °C</th>
<th>time / h</th>
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<th>12 / %</th>
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*Podophyllotoxin aza-analogues*
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 CH₂Cl₂ 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 (CH₂Cl₂-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 Et₃SiH in CF₃CO₂H afforded 3 in 93% yield, also supporting the structural assignment of 19.

![Chemical structures](image)

a) DDQ/AcOH, 60 °C 48 h, 70%; b) CF₃SO₃H/MeOH·AcOH·CH₂Cl₂, 4 °C 24 h, 34% for 18; c) CF₃SO₃H/MeOH·CH₂Cl₂, 4 °C 37 h, 94% for 19; d) K₂CO₃/MeOH, rt 10 h, 95%; e) 10% aq. HCl/dioxane, 50 °C 5 h, 82% for 6 and 12% for 5; f) HBr/Cl(CH₂)₂Cl, 4 °C 22 h, and then BaCO₃/aq. THF, rt 16 h, 60% for 7; g) Et₃SiH/CF₃CO₂H, rt 1 h, 93%.

Table II. Stereoselective Formation of 18 and 19 from 16

<table>
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<tr>
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<td>34</td>
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<tr>
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<td>CH₂Cl₂·MeOH</td>
<td>4</td>
<td>37</td>
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<td>94</td>
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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 CH₂Cl₂-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.

Ester exchange reaction of the acetate 18 in the presence of K₂CO₃ 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 BaCO₃ in aq. THF. C₂ symmetry of 3',5'-dimethoxy-4'-hydroxyphenyl group of 7 was reasonably characterized by 400 MHz NMR in CDCl₃ (δ: 3.81 (6H, s, OCH₃ 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 delight, the racemic compounds 3, 4, 5, 6, 7, 12, 18, and 19 exhibited promising growth inhibition of KB cell (ED₅₀ (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)).

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.

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

**Experimental**

(-)-(S)-3,4-Methylenedioxyphenylalaninol (9): A mixture of L-3,4-methylenedioxyphenylalanine(S)-8 (1.37 g, 6.55 m mol) and LiAlH₄ (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. [α]D25 -21.5° (c=1.108, CHCl3). IR (KBr): 3355 cm⁻¹. ¹H-NMR (CDCl3, 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. (ClcH1303N).

(−)-(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. NaHCO₃ (30 ml), and brine (30 ml), and then dried over Na₂SO₄. Concentration gave pale yellow solids (1.13 g). Recrystallization from ethyl acetate (1.3 ml)−ether (1 ml) gave (−)-O-10 (0.99 g, 90 %) as colorless pillars of mp 98-99.5°C. [α]D20 -59.9° (c=1.362, CHCl3). IR (CHCl3): 3450, 1755 cm⁻¹. MS m/z: 221. ¹H-NMR (CDCl3, TMS) δ: 2.78 (1H, dd, J=6.4, 13.7 Hz), 2.80 (1H, dd, J=7.1, 13.7 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. (C₁₁H₁₁NO₄).

(+)-(R)-10: Colorless pillars of mp 97-98°C. [α]D20 +60.8° (c=1.174, CHCl3).

(-)-2-Azadeoxypodophyllotoxin (3) and (-)-2-Azaisodeoxypodophyllotoxin (12): A solution of (-)-10 (7.51 mg, 0.339 m mol), 3,4,5-trimethoxybenzaldehyde (83.9 mg, 0.441 m mol), and conc. H₂SO₄ (0.036 ml, 0.679 m mol) in CH₂Cl₂ (3 ml) was stirred at rt for 5 h. After dilution with CH₂Cl₂ (50 ml), the whole was washed with satd. NaHCO₃ (20 ml) and brine (40 ml), and then dried over Na₂SO₄. Concentration gave a yellow caramel (161 mg). Purification by SiO₂ column chromatography (CH₂Cl₂-acetone (2/1)) gave (−)-3 (125.4 mg, 93 %) and (−)-12 (4.3 mg, 3 %).

(-)-3: Colorless needles of mp 200-201°C (CHCl₃-benzene). [α]D20 -171.8° (c=1.134, CHCl₃). IR (KBr): 1725, 1592 cm⁻¹. ¹H-NMR (CDCl₃, 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). ¹³C-NMR (CDCl₃) δ: 34.3 (t), 48.1 (d), 56.1 (q), 56.4 (d), 60.7 (q), 68.3 (t), 191.1 (t), 104.7 (d), 107.8 (d), 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), 156.5 (s). MS m/z: 399. Anal. (C₂₁H₂₁N₀₇·1/13 H₂O).

(+)-3: Colorless needles of mp 185-186°C (benzene). [α]D20 +166.5° (c=1.176, CHCl₃).

(−)-12: Colorless needles of mp 236-238°C (CHCl₃-benzene). [α]D20 +54.3° (c=0.99, CHCl₃).
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(±)-12: Colorless needles of mp 237.5-238.5 °C (CHCl3-ether).

**Isomerization of (±)-3 and (±)-12:** A solution of (±)-3 (36 mg, 0.09 m mol) and conc. H2SO4 (17 mg, 0.18 m mol) in CH2Cl2 (0.9 ml) was stirred at rt for 1 h. After dilution with CH2Cl2 (30 ml), the whole was washed with satd. NaHCO3 (10 ml) and brine (40 ml), and then dried over MgSO4. 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 Et3SiH (57 mg, 0.49 m mol) in CF3CO2H (0.4 ml) was stirred under reflux for 44 h. After dilution with CHC13 (30 ml), the whole was washed with satd. NaHCO3 (20 ml) and brine (20 ml x 2), and then dried over Na2SO4. Concentration gave a yellow oil which was purified by SiO2 column chromatography (CH2Cl3-acetone (20/1)) to give (±)-3 (9.2 mg, 29 %) and (±)-14 (15.4 mg, 48 %) as colorless needles of mp 159-159.5 °C (benzene). IR (CHC13): 3450, 1760 cm⁻¹. 1H-NMR (CDCl3, 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=8.4, 8.4 Hz), 4.41 (1H, dd, J=5.5, 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. (C21H18N2O6).

(±)-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 CH3C02H (0.12 ml) was stirred at 65 OC for 2.5 h. The mixture was diluted with CH2Cl2 (50 ml) and washed with satd. NaHCO3 (20 ml) and brine (20 ml x 2), and then dried over MgSO4. Concentration gave white solid (23.5 mg) which was purified by SiO2 column chromatography (benzene-acetone (3/1)) to give (±)-15 (21.5 mg, 86 %) as white solids of mp 159-161 °C. IR (CHCl3): 1754, 1649 cm⁻¹. 1H-NMR (CDCl3, 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. (C21H21NO7).

**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 CH2Cl2 (300 ml), the whole was washed with 10 % NaOH (200 ml x 2) and brine (100 ml), and then dried over MgSO4. Concentration provided a mixture of (-)-10 and 16 (1.29 g). Purification by SiO2 column chromatography (CH2Cl2-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(CHCl3): 3450, 1764, 1750 cm⁻¹. 1H-NMR (CDCl3, 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. (C19H21NO6).

(±)-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 CH2Cl2-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. NaHCO3 (2 ml) at -20 °C, the mixture was stirred for 10 min and then extracted with CH2Cl2 (50 ml). The extracts were washed with satd. NaHCO3 (20 ml) and brine (20 ml x 2) and then dried over MgSO4. Concentration gave a yellow oil (32 mg) which was purified by SiO2
column chromatography (CH$_2$Cl$_2$-acetone, 20/1) to give (±)-18 (9.0 mg, 34 %) as colorless prisms of mp 236.5-237.5 °C (CH$_2$Cl$_2$-benzene). IR (KBr): 1745, 1735 cm$^{-1}$. $^1$H-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$_{23}$N$_2$O$_3$). Diastereomerically pure 16, obtained by column 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$_2$Cl$_2$-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. Saturated NaHCO$_3$ (2 ml) was added at $0^\circ$C. The whole was extracted with CH$_2$Cl$_2$ (20 ml). The extracts were washed with brine (30 ml) and then dried over Na$_2$SO$_4$. Concentration gave yellow solid (39.5 mg) which was purified by SiO$_2$ column chromatography (CH$_2$Cl$_2$-acetone, 20/1) gave (-)-19 (27.4 mg, 94 %) as colorless prisms of mp 245-246 °C (CH$_2$Cl$_2$-benzene). [α]$_D$ = -138.8 (c=1.13, CHCl$_3$). IR (KBr): 1741 cm$^{-1}$. $^1$H-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$_{23}$H$_{23}$N$_2$O$_3$).

Reduction of W-19 to (±)-3: A mixture of W-19 (3.0 mg, 0.007 m mol) and Et$_3$SiH (1.0 mg, 0.08 m mol) in CF$_3$CO$_2$H (0.04 ml) was stirred at rt for 1 h. After addition of saturated NaHCO$_3$ (3 ml), the mixture was extracted with CH$_2$Cl$_2$ (10 ml). The extract was washed with brine (20 ml) and then dried over Na$_2$SO$_4$. 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 CH$_2$Cl$_2$ (30 ml). After neutralization with saturated NaHCO$_3$ (3 ml), the mixture was extracted with CH$_2$Cl$_2$ (30 ml x 2). The combined extracts were washed with brine (30 ml) and then dried over Na$_2$SO$_4$. Concentration gave a colorless oil (17 mg) which was purified by SiO$_2$ column chromatography (CH$_2$Cl$_2$-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$^{-1}$. $^1$H-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), 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$_{21}$N$_2$O$_4$).

(±)-6: Colorless prisms of mp 223-223 °C (dec) (CHCl$_3$-benzene). IR (KBr): 3420, 1738 cm$^{-1}$. $^1$H-NMR (CDCl$_3$, TMS) δ: 2.44 (1H, d, J=8.0 Hz, D$_2$O 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,
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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). 13C-NMR (CDCl3) 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. (C21H21NO6).

(±)-6 was also prepared by transesterification of (±)-18: A mixture of (±)-18 and K2CO3 in MeOH was stirred at rt for 10 h. After filtration and concentration, the residue was purified by SiO2 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 CH2Cl2 (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 SiO2 column chromatography (CHCl3-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 (CHCl3): 3530, 1743, 1613 cm⁻¹. 1H-NMR (CDCl3, 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). 13C-NMR (CDCl3) 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), 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. (C20H19NO7).

(±)-Catechol: Colorless prisms of mp 226.5-228 °C (acetone). 1H-NMR (CDCl3, 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. (C19H17N2O7).

(-)-2-Aza-4'-demethylepipodophyllotoxin (7): Hydrogen bromide gas was bubbled through a solution of (-)-19 (3.0 g, 7.0 m mol) in (CH2)2Cl2 (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 BaCO3 (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 SiO2 column chromatography (CH2Cl2-acetone 4/1) gave (-)-7 (1.6 g, 71%) as colorless prisms of mp 203-206 °C (AcOEt-hexane). [α]D20 -131.4 °c=1.012, CHCl3). IR (CHCl3): 3530, 3360, 1741 cm⁻¹. 1H-NMR (CDCl3, TMS) δ: 2.28 (1H, brs, D2O 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 D2O exchange)), 4.69 (1H, dd, J=4.4, 8.4 Hz), 5.53 (1H, s, D2O 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. (C20H19NO7H2O). HRMS Calcd for C20H19NO7 401.1108. Found 401.1052.

(-)-7: Colorless prisms of mp 246-248 °C (CHCl3-MeOH).

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

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References and Notes

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