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Stereoselective Synthesis and Anticancer Activities of New Podophyllotoxin Derivatives: 4-β-Cyano-4-Deoxy-4′-Demethylepipodophyllotoxin and 4-β-Carboxyl-4-Deoxy-4′-Demethylepipodophyllotoxin

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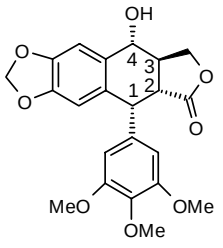
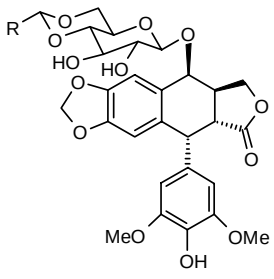
Abstract: 4-β-Cyano-4-deoxy-4'-demethylepipodophyllotoxin **4** was synthesized from 4'-de -methylepipodophyllotoxin and Me_3SiCN in the presence of $BF_3 \cdot Et_2O$. 4-β-Carboxyl-4-deoxy-4'-demethylepipodophyllotoxin **5** was obtained by hydrolyzing **4** in HOAc. Both of them show very high anticancer activities against L_{1210} and KB cell lines *in vitro*.

Keywords: Podophyllotoxin, derivative, anticancer activities.

Podophyllotoxin **1**, isolated from *Podophyllum pelatum L.* or *Podophyllum emodi L.*, shows high antitumor activity. But, it cannot be used as antitumor agent clinically because of its serious side-effect. Its semisynthetic derivatives, Etoposide **2** and Teniposide **3**, are wide-used as important anticancer drugs[1]. However, they have several limitations such as poor water solubility, metabolic inactivation and development of drug resistance. To overcome these limitations, many derivatives of podophyllotoxin have been synthesized in many laboratories[2~5]. It has been recognized that β substitution at C-4 position is necessary to the compounds with high activity. Our group has established a methodology to synthesize 4-β-substituted derivative of podophyllotoxin stereoselectively[3]. This communication reports the synthesis and anticancer activities of 4-β-cyano-4-deoxy-4′-demethylepipodophyllotoxin and 4 - β - carboxy- 4 -deoxy-4′-de-methylepipodophyllotoxin, which are important intermediates for synthesizing 4-β-carbon atom substituted derivatives.



Podophyllotoxin **1**

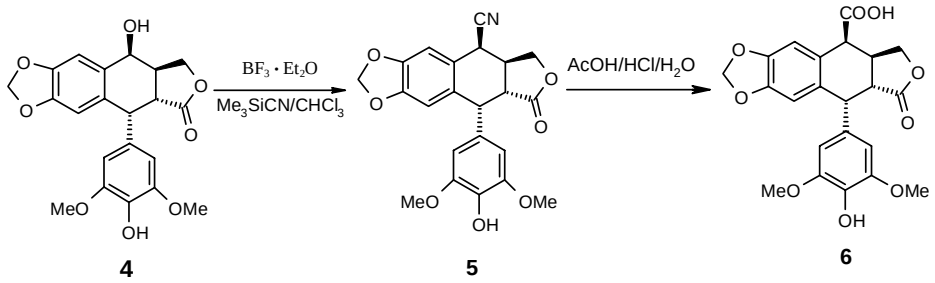


Etoposide:R=CH_3 **2**

Teniposide:R=  **3**

The synthetic approach started from 4'-demethylepipodophyllotoxin **4**. On the basis of our established methodology[3], 4-β-substituted derivative of **1** or **4** can be obtained from **1** or **4** with corresponding HX (X = nucleophilic anion), in the presence of $BF_3 \cdot Et_2O$ at –15 ℃. We, at first, used potassium cyanide as the nucleophilic agent. The yield was rather low, because of low solubility of KCN in organic solvent and no sufficient amount of CN^- being formed. HCN is very toxic. However, in silicon agents, trimethylsilyl group often reacts as if it were a proton. So we used trimethylsilyl cyanide, a versatile silicon agent, to replace HCN. The result is very satisfying with high yield and 100% stereospecificity ($J_{3,4}$ = 7.34Hz). In a mixed solvent of acetic acid and hydro -chloric acid compound **5** can be easily hydrolyzed to the carboxylic acid **6**.

Scheme 1



4 **5** **6**

In vitro anticancer potencies compounds **5** and **6**, and of the control compound Etoposide, against L_{1210} and KB cell lines are reported in **Table 1**. The further synthesis of derivatives will be reported in due course.

Table 1

Compound	Inhibition of L_{1210} IC_{50} (μ g/ml)	Inhibition of KB IC_{50} (μ g/ml)
5	0.00348	0.0531
6	0.164	10
Etoposide	0.155	0.308

Experimental

All melting points were taken on a Fischer-Johns melting point apparatus and uncorrected. EI-MS and HR-MS spectral analyses were determined on a Varian MAT212 instrument and computer SS-MAT. ^{1}H-NMR specta wre obtained using Brucker AM-400 spectrometers with TMS as the internal standard. All chemical shifts are reported in ppm. Optical rotations were measured with a Perkin-Elmer 214MC polarimeter, using DMF as solvent.

4-β-Cyano-4-deoxy-4'-demethylepipodophyllotxin 5

4'-Demethylepipodophyllotoxin (10 g, 10 mmol) was dissolved in 350 mL of $CHCl_3$, Me_3SiCN (4 mL, 30 mmol) was added to the solution. $BF_3 \cdot Et_2O$ (4.5 mL) was added to the mixture dropwise at –15℃ (about 1h). After completion of addition of $BF_3 \cdot Et_2O$, stirring was continued for 1~2 h. The reaction was quenched with 4.5 mL of pyridine. The solution was washed with 5% $NaHCO_3$ solution, 5% HCl, water and brine, dried over Na_2SO_4, concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with AcOEt/cyclohexane (1/1). Recrystallization from acetone gave **5** (7.8 g, 76.3%), mp: 246~248℃. $[\alpha]_D^{25}$ 66.4 (c 0.5, DMF). EI-MS: 409 (M^+), 382 (M^+-CN). HR-MS: $C_{22}H_{19}NO_7$ calcd 409.116140, obsd 409.11572. ^{1}H-NMR (400MHz, DMSO-d_6): δ_{ppm} 3.00 (m, 1H, H-3), 3.13 (m, 1H, H-2), 3.63 (s, 6H, 2xOCH_3), 4.08 (t, 1H, *J* = 8.14, H-11$_\beta$), 4.55 (t, 1H, *J* = 7.91, H-11$_\alpha$), 4.60 (d, 1H, *J*=5.43, H-1), 4.69 (d, 1H, *J* = 7.34, H-4), 6.05 (d, 2H, *J* = 10.15, OCH_2O), 6.20 (s, 2H, H-2′,6′), 6.59 (s, 1H, H-8), 7.10 (s, 1H, H-5). ^{13}C-NMR (400MHz, DMSO-d_6): δ_{ppm} 32.7 (C-3), 32.9 (C-2), 42.4 (C-1), 42.6 (C-4), 56.0 (OCH_3), 68.9 (C-11), 101.6 (OCH_2O), 105.8 (C-6'), 108.0 (C-2'), 108.5 (C-5), 110.3 (C-8), 118.7 (CN), 123.4 (C-14a), 130.0 (C-8a), 131.6 (C-6), 134.8 (C-7), 146.7 (C-4'), 147.1 (C-5'), 147.7 (C-3'), 173.1 (C=O).

4-β-Carboxy-4-deoxy-4'-demethylepipodophyllotxin (6)

Compound **5** (1 g) was dissolved in acetic acid (60 mL), and added concentrated HCl (10 mL) and water (10 mL). The flask was heated in an oil bath 75 ℃. The solution was stirred until it turned to clear, and concentrated *in vacuo*. The residue was extracted with AcOEt, washed with water several times and brine, dried over $NaSO_4$. The solvent was evaporated *in vacuo*. The solid was purified on silica gel column chromatography with acetone/CH_2Cl_2/AcOH (75/25/0.8). Recrystallization from MeOH gave **6** (0.5 g, 47.8%), mp: 254~256℃. $[\alpha]_D^{25}$-70 (c 0.2, DMF). EI-MS: 428 (M^+),382 (M^+-COOH-H). HR-MS: $C_{22}H_{20}O_9$ calcd 428.110719, obsd 428.11037. H^1-NMR (400MHz, $CDCl_3$): 3.03-3.12 (m, 2H, H-2, 3), 3.65 (s, 6H, 2*OCH_3), 3.88 (d, 1H, *J* = 5.84, H-1), 4.19 (d, 1H, *J* = 6.23, H-4), 4.45 (m, 2H, H-11), 5.92 (d, 2H, *J* = 9.27, OCH_2O), 6.12 (s, 2H, H-2',6'), 6.47 (s, 1H, H-8), 7.10 (s, 1H, H-5). ^{13}C-NMR (400MHz, $CDCl_3$): δ_{ppm} 31.7 (C-3), 41.3 (C-2), 46.1 (C-1), 46.4 (C-4), 56.3 (OCH_3), 70.2 (C-11), 101.2 (OCH_2O), 106.0 (C-2', 6'), 108.8 (C-5), 109.2 (C-8), 121.5 (C-14a), 130.3 (C-8a), 130.8 (C-6), 134.2 (C-7), 146.7 (C-4'), 147.1 (C-5'), 147.6 (C-3'), 175.8 (C=O), 176.5 (COOH).

Acknowledgments

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