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Hemi-synthesis and Biological Activity of New Analogues of Podophyllotoxin

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Abstract—Various 4-analogues of podophyllotoxin and epipodophyllotoxin were obtained via the formation of the corresponding 4-keto derivatives. Methyloximation of podophyllotoxone, followed by subsequent catalytic hydrogenation, gave stereoselective access to 4-α-amino-4-deoxypodophyllotoxin and from there, to the corresponding acetamido and formamido derivatives. Base-catalyzed isomerisation of 4-α-amino-4-deoxypodophyllotoxin led to the corresponding picropodophyllin isomer while the 4-β-amino afforded a neopodophyllotoxin-like derivative. On the other hand, oxirane and hydroxymethyl-containing analogues were prepared from podophyllotoxin and 4-*epi*-4′-demethyl-podophyllotoxin, using a Takai olefination strategy. In the latter series, carboxaldehyde- and carboxylic acid-containing derivatives were also synthesized.
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Introduction

(−)-Podophyllotoxin **1** is a well-known occurring lignan endowed with potent cytotoxicity which acts as a mitotic spindle poison.[1] In spite of some use as a medicinal drug, human clinical trials were soon abandoned due to its toxicity. An extensive semi-synthetic programme at Sandoz resulted in the development of etoposide **2** and teniposide **3**, two glycoside derivatives of 4-*epi*-4′-demethoxy-podophyllotoxin.[2] Their mechanism of action is different from that of podophyllotoxin and there is evidence[3] that the relevant target of these semi-synthetic derivatives is the interference with DNA topoisomerase II (Fig. 1).

Over the last 10 years, chemical transformations of podophyllotoxin D-ring and/or 4-substituent undertaken by the groups of Gordaliza and Lee led to the discovery of new compounds endowed with cytotoxic[4–7] antiviral[8,9] or immunosuppressive[10,11] activities.

D-ring analogues of podophyllotoxin which are potent cytotoxic compounds were also reported by Subrahmanyam et al.[12,13]

For our part, we have been interested in developing new glycosides[14] and A-ring pyridazine analogues of podophyllotoxin[15] as well as podophyllotoxin δ-lactones.[16] Besides the search for new antitumour drugs, we undertook the design and elaboration of new podophyllotoxin derivatives that can selectively destroy the tumour vasculature at doses below the maximum tolerated dose (MTD), like combretastatin[17] and colchinoids,[18] two drugs which are currently under clinical investigation. Such a general property reported for tubulin binding agents[17] has not been exploited in the podophyllotoxin family although the anti-vascular effects of podophyllotoxin were noted as early as 1954 by Algire et al.[19]

Chemistry

Therefore, we started some structural modifications at the level of podophyllotoxin itself, by preparing new derivatives including a double bond at C-4, like an oxime or in which the 4-OH group had been replaced by an amine or an amide. Thus, podophyllotoxin **1** was oxidized by pyridinium chlorochromate to give podophyllotoxone **4**.[20] Next, podophyllotoxone **4** was almost quantitatively converted into its *Z*-methyloxime **5**[21] by treatment with *O*-methylhydroxylamine hydrochloride in MeOH. Catalytic hydrogenation of **5** stereoselectively

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led to the corresponding 4-amino-4-deoxypodophyllotoxin **6** in 71% yield; this compound was subsequently converted into the *N*-formyl **7** and *N*-acetyl derivative **8**. It must be noted that the NMR spectrum of **6** is different from that previously reported by Hansen et al.[22] for the H-4 and H-11 signals, although mp and IR spectrum are in complete agreement. Concerning the *N*-acetyl derivative **8**, mp, IR and NMR spectra were identical to those noted by these authors (Scheme 1).[22]

As already reported by Hansen et al.,[22] base-catalyzed epimerisation of 4-amino-4-deoxypodophyllotoxin **6** afforded the corresponding *cis*-fused lactone, the picropodophyllin analogue **9**. In contrast, when the 4-amino-4-epipodophyllotoxin **10** was treated under the same conditions (piperidine/ethanol), an unexpected compound was formed, which was not the *cis*-fused lactone as claimed by the same authors. The aza-neopodophyllotoxin structure **11** was unambiguously assigned to this compound by spectroscopic analysis (300 MHz ^{1}H NMR, COSY, HMBC, IR). The HMBC spectrum showed a J_3 correlation between H-4 and the C=O. This was corroborated by the IR absorption at 1703 cm^{-1} indicative of the presence of a lactam functionality. In addition, as suggested by molecular modelling using MM2 force field in Chem 3-D, the H-1 and H-4 protons are nearly orthogonal to the H-2 and H-3 protons, respectively and thus would lead to very small coupling constants $J_{1,2}$ and $J_{4,3}$ in the ^{1}H NMR spectrum. Indeed, both H-1 and H-4 protons appeared as singlets.

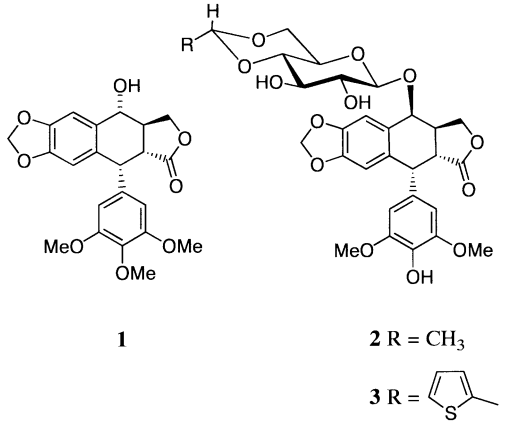
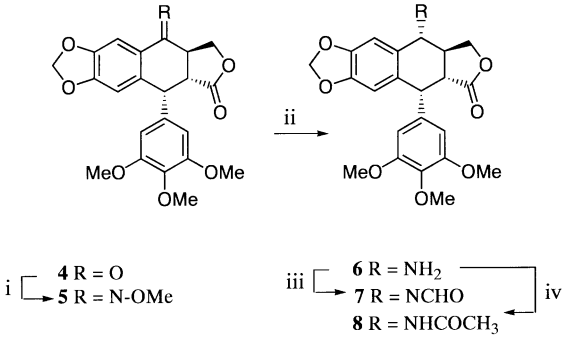


Figure 1.

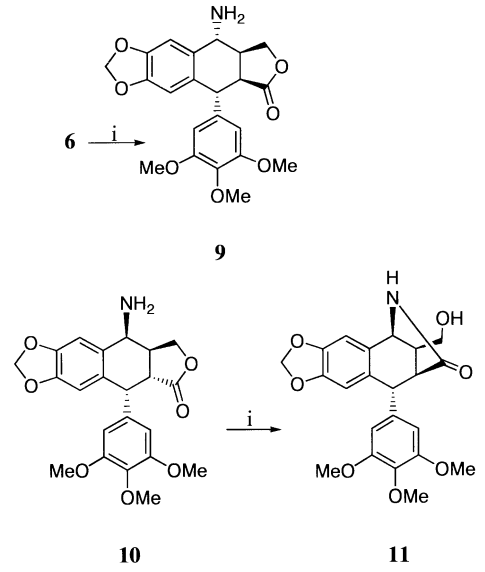


Scheme 1. (i) CH_3NHOH, HCl, NaOAc, MeOH–THF, reflux, (ii) 10% Pd/C, MeOH, H_2; (iii) $HCO_2C_2H_5$, reflux, (iv) Ac_2O, pyridine.

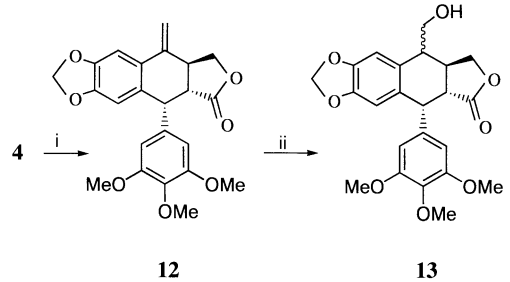
Obviously, such an intramolecular reaction is very similar to that leading to the neopodophyllotoxin-like structure[23] and results from the *cis* relationship between the 4-amino group and the 2-carboxaldehyde anion transitory formed under the basic conditions (Scheme 2).

Our next goal was to create a C–C σ-bond at C-4. As the classical Wittig olefination procedure ($CH_3PPh3Br$, *n*-BuLi, THF, −78 °C) failed with ketone **4** because of the acidic α proton (H-3), we envisioned introducing the methylene moiety at position 4 by mild Takai[24] olefination. This led to the methylene derivative **12** in 50% yield without epimerisation of the *trans*-fused lactone. Hydroboration of **12**, followed by mild hydrolysis of the borane derivative, gave **13** as a mixture of diastereoisomers. Despite the fact that the separation was difficult, a small amount of one of the diastereoisomers could be obtained and fully characterized (Scheme 3).

Introduction of a C–C bond at C-4 was subsequently developed from 4-*epi*-4′-demethylepidodophyllotoxin **14**. However, prior to the oxidation step, the 4′-phenol was selectively protected by using TBDMSCl and imidazole in DMF. This afforded compound **15** (86%) which was then oxidized by Dess–Martin periodinane[25] in dichloromethane, leading to 4′-demethylpodophyllotoxone **16** (88% yield). As above, introduction of the methylene functionality at C-4 was realized using Takai



Scheme 2. (i) EtOH, piperidine, reflux.



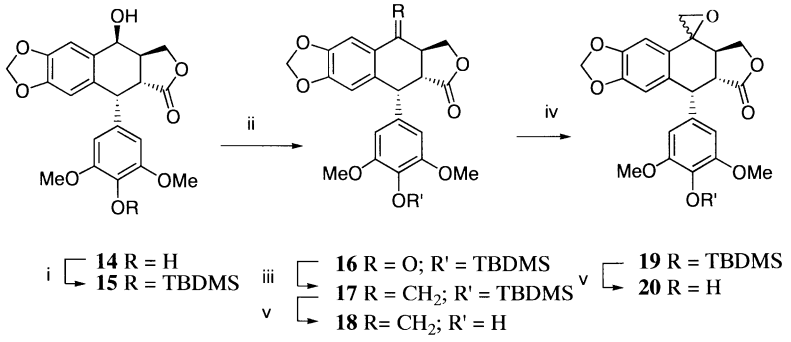
Scheme 3. (i) Zn, CH_2I_2, THF then $TiCl_4$, CH_2Cl_2; (ii) BH_3/SMe_2, THF then phosphate buffer, MeOH, H_2O_2 (30%).

methodology and gave **17** in 73% yield. Further treatment of **17** by iodine and silver II oxide in THF in presence of water led to oxirane **19** (87%) as a single diastereoisomer. Unfortunately, the configuration at C-10 could not be established from the NOESY spectrum together with molecular modeling of both stereoisomers of **19**. Final step required removing of the TBDMS ether. Thus, under mild acidic conditions (PPTS in refluxing ethanol), compounds **17** and **19** led to the corresponding derivatives **18** and **20** in 99 and 63% yields, respectively (Scheme 4).

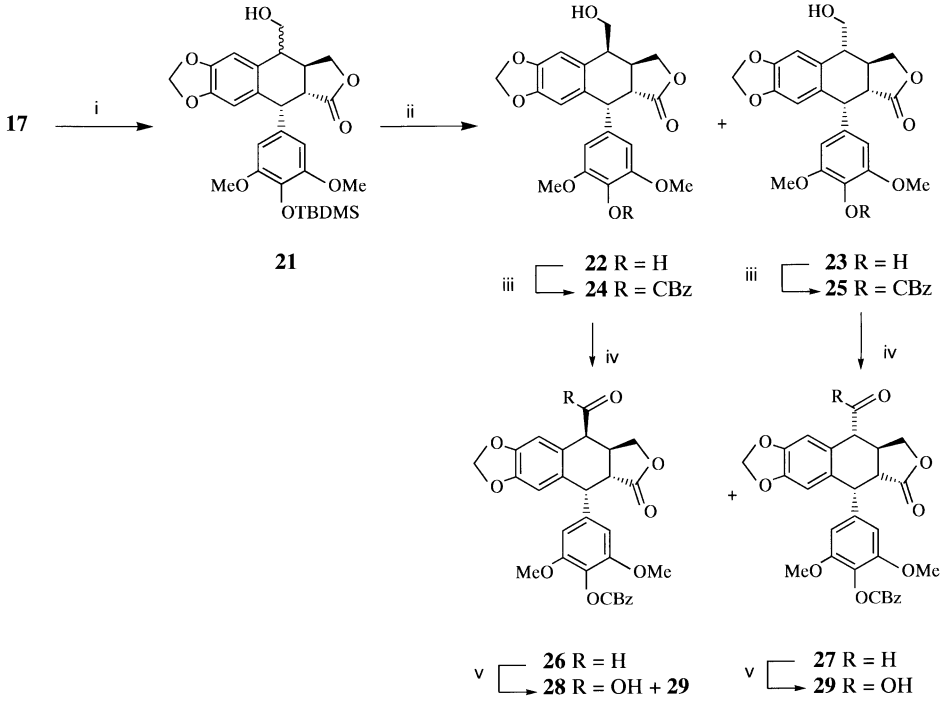
The methylene derivative **17** was also hydroborated (BH_3/SMe_2) and subsequent mild hydrolysis (phosphate buffer pH 7, methanol and H_2O_2) of the intermediate borane furnished the alcohol derivative **21** as an inseparable mixture of isomers (82% overall yield and ratio 3/2 from H-5 or H-8 integrations). Acidic hydrolysis of the TBDMS ether of **21** (PPTS, EtOH, reflux) led to isomers **22** and **23** which were separated by flash chromatography. Their stereochemistry was determined by NMR analysis (22:23 = 3:2, 87% overall yield). Prior to conversion of the C-4 hydroxyl into aldehyde, the hydroxyl at C-4′ needed to be protected. Therefore, diols **22** and **23** were treated with benzyloxycarbonyl chloride. This afforded, in almost quantitative yields, alcohols **24** (92%) and **25** (94%) which were next oxidized with Dess–Martin periodinane to provide aldehydes **26** (94%) and **27** (97%), respectively. Oxidation of aldehyde **27** by sodium chlorite[26] gave the carboxylic acid **29** (42%) whereas, under the same conditions, aldehyde **26** led to a mixture of acids **28** and **29** in 69% overall yield. Obtaining such a mixture could be explained by the isomerisation of the homobenzylic aldehyde **26** to **27** in the acidic medium (acetate buffer pH 4) used for the oxidation reaction (Scheme 5).

Biological Evaluations

All compounds, except the neopodophyllotoxin-like derivative **11**, exhibited from moderate (**10**, **20**, **22**, **23**)



Scheme 4. (i) TBDMSCl, imidazole, DMF; (ii) periodinane, CH_2Cl_2; (iii) Zn, CH_2I_2, THF then $TiCl_4$, CH_2Cl_2; (iv): Ag_2O, I_2, THF, H_2O; (v) PPTS, EtOH, reflux.



Scheme 5. (i) 9-BBN, THF then, phosphate buffer pH 7; (ii) PPTS, MeOH reflux; (iii) ClCOOBn, NEt_3, CH_2Cl_2; (iv) periodinane, CH_2Cl_2; (v) $NaClO_2$, resorcinol, *t*-BuOH/H_2O.

Table 1. Biological evaluation of derivatives **7**–**11**, **18**, **20**, **22** and **23**

Compound	IC_{50} L1210 (μM)	Cells cycle[a]			Inhibition of microtubule assembly[b] IC_{50} (μM)	Topoisomerase inhibition (%)	
		% G2M	% 8 N	Conc (μM)		I	II
7	0.06	17	66	0.1	0.7	0	18
8	0.07	53	21	5	0.7	0	21
9	0.08	35	58	0.25	1.6	0	13
10	0.63	57	34	2.5	nd	nd	nd
11	60.00	nd	nd		Inactive	nd	nd
18	0.035	60	27	0.1	2.3	nd	nd
20	1.30	69	20	5	8.3	nd	nd
22	0.24	42	44	0.25	nd	nd	nd
23	0.10	69	15	0.5	7.3	nd	nd

nd, not determined.
[a]Untreated control cells: 24% G2M, 1% 8 N.
[b]4-Deoxypodophyllotoxin $IC_{50} = 1.1$ μM.

to high (**7**–**9, 18**) antiproliferative activity against L1210 cell line, the most active compound being the 4-methylene 4-deoxypodophyllotoxin derivative **18** with an IC_{50} of 35 nM. The perturbation of the cell cycle induced by these compounds was studied on the same L1210 cell line. Compounds **8**, **10**,**18**, **20**–**23** induced a partial accumulation of cells in the G2 + M phase of the cell cycle (in the range of 42–69% versus 24% for untreated control cells) at relative moderate concentration (e.g. 0.1 μM for **18** (Table 1), for example) (Table 1).

Compounds **7**–**9**, which belong to the series of podophyllotoxin (4β-substitution and 4′-OMe), were almost devoid of topoisomerase II, as well as topoisomerase I, activities. In contrast, they exhibited an inhibition of microtubule assembly of the same magnitude as 4-deoxypodophyllotoxin used as standard[28] with IC_{50} in the range of 0.7–2.3 μM. In agreement with the antimitotic character of such derivatives, they highly induced apparition of 8 N cells with a less marked effect in the case of the acetamido derivative **8**. Although belonging to the 4′-demethyl-4-epipodophyllotoxin series, high accumulation of 8 N cells was also observed in the case of compound **22**.

Antivascular effects as well as apoptotic and necrotic effects are currently under investigation and will be reported later.

Experimental

Chemistry

Methods. Reactions were carried out under an argon atmosphere with dry, freshly distilled solvents under anhydrous conditions unless otherwise noted. All reactions were magnetically stirred and monitored by thin-layer chromatography with Merck 0.25 mm silica gel plates (60F-254). Flash chromatography was performed with silica gel (particle size 40–63 μm). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Etoposide was obtained from Pierre Fabre Médicament. Melting points were determined on an Electrothermal digital melting point apparatus and are not corrected. Infrared spectra were recorded on a Perkin-Elmer 1710 spectrophotometer. Proton NMR spectra were recorded on a Bruker Avance 300 spectrometer. Proton assignments were made according to the usual nomenclature of podophyllotoxin derivatives. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Mass spectra (MS) were obtained with a Nermag R10-10C under chemical ionization (CI) or electrospray (ES/MS) conditions. Microanalyses were performed by the 'Service d'Analyse du CNRS, Vernaison, France'.

Podophyllotoxone, methyloxime (5). To a solution of podophyllotoxone **4**[20] (1 g, 2.4 mmol) in tetrahydrofuran (5 mL), methylhydroxylamine [prepared from its hydrochloride (0.5 g, 6 mmol) and NaOAc (0.5 g, 6.1 mmol) in MeOH (15 mL)] was added. The solution was refluxed overnight and, after cooling to room temperature, was extracted with dichloromethane. Usual work-up afforded 1.15 g of crude residue. Purification by flash chromatography with dichloromethane/acetone (8/2, v/v) as the eluent gave 1 g (91%) of **5** as a crystalline compound. A sample was recrystallized from isopropyl ether: mp 158 °C; $[\alpha]_D^{20}$ −163 (*c* 1, $CHCl_3$); Lit:[21] mp 139–141 °C ($MeOH/CH_2Cl_2$); NMR spectrum was in agreement with that reported in the literature.

4-Amino-4-deoxypodophyllotoxin (6). A solution of methyloxime **5** (700 mg, 1.59 mmol) in MeOH (75 mL) containing Pd/C 10% (150 mg) was stirred under hydrogen atmosphere (10 bar) for 40 h at room temperature. It was then filtered, the solvent was evaporated under reduced pressure and the residue was purified by flash chromatography (CH_2Cl_2/MeOH, 97/3) to give **6** (465 mg, 71%): mp 184 °C; Lit:[22] mp 184–185 °C (MeOH); $[\alpha]_D^{20}$ −127 (*c* 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 1776 (C=O), 1590, 1486; ^1H NMR (300 MHz, $CDCl_3$) δ 1.62 (br s, 2H, NH_2), 2.55 (m, 1H, H-3), 2.81 (dd, 1H, $J = 14.3$ Hz, 4.7 Hz, H-2), 3.75 (s, 6H, OMe-3′ and -5′), 3.80 (s, 3H, OMe-4′), 3.88 (d, 1H, $J = 10.2$ Hz, H-4), 4.03 (dd, 1H, $J = 10.2$, 8.8 Hz, H-11b), 4.59 (dd, 1H, $J = 8.8$ Hz, 7.3 Hz, H-11a), 4.60 (d, 1H, $J = 4.7$ Hz,

H-1), 5.95 (d, 1H, $J=1.1$ Hz, OCH_2O), 5.96 (d, 1H, $J=1.1$ Hz, OCH_2O), 6.36 (s, 2H, H-2′, H-6′), 6.50 (s, 1H, H-8), 7.11 (s, 1H, H-5); MS (DCI/NH_3) m/z 414 $[M + H]^+$.

4-Formamido-4-deoxypodophyllotoxin (7). To a solution of amine **6** (100 mg, 0.24 mmol) in CH_2Cl_2 (2 mL) was added ethyl formate (5 mL) and the reaction mixture was refluxed for 1.5 h. The solvents were then evaporated under reduced pressure, and the residue was purified by flash chromatography (CH_2Cl_2/MeOH, 98/2) to give **7** (85 mg, 80%): mp 258 °C; $[\alpha]_D^{20}$ −122 (c 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3427 (N–H), 1776 (C=O lactone), 1693 (CHO), 1590, 1485; ^1H NMR (300 MHz, $CDCl_3$) δ 2.70 (m, 1H, H-3), 2.90 (dd, 1H, $J=14.2$ Hz, 4.7 Hz, H-2), 3.76 (s, 6H, OMe-3′ and –5′), 3.80 (s, 3H, OMe-4), 4.19 (t, 1H, $J=9.5$ Hz, H-11b), 4.51 (dd, 1H, $J=9.5$ Hz, 7.5 Hz, H-11a), 4.62 (d, 1H, $J=4.7$ Hz, H-1), 5.20 (t, 1H, $J=9.3$ Hz, H-4), 5.96 (d, 1H, $J=1.0$ Hz, OCH_2O), 5.97 (d, 1H, $J=9.3$ Hz, N–H), 5.98 (d, 1H, $J=1.0$ Hz, OCH_2O), 6.37 (s, 2H, H-2′, H-6′), 6.55 (s, 1H, H-8), 6.85 (s, 1H, H-5), 8.38 (s, 1H, CHO); MS (DCI/NH_3) m/z 459 $[M + NH_4]^+$. Anal. calcd for $C_{23}H_{22}O_7$: C, 67.31; H, 5.40. Found: C, 67.60; H, 5.55.

4-Acetamido-4-deoxypodophyllotoxin (8). To a solution of amine **6** (100 mg, 0.24 mmol) in pyridine (2 mL) acetic anhydride (5 mL) was added and the reaction mixture was stirred at room temperature for 12 h. Then the solvents were evaporated under reduced pressure and the residue was purified by flash chromatography ($CHCl_2$/acetone, 85/15) to give **8** (105 mg, 96%): mp 256 °C; Lit:[22] mp 255–256 °C ($CHCl_3$); $[\alpha]_D^{20}$ −125 (c 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3434 (N–H), 1777 (C=O lactone), 1681 (C=O amide), 1590, 1485; ^1H NMR (300 MHz, $CDCl_3$) δ 2.11 (s, 3H, $COCH_3$), 2.62 (m, 1H, H-3), 2.89 (dd, 1H, $J=14.0$ Hz, 4.7 Hz, H-2), 3.77 (s, 6H, OMe-3′ and -5′), 3.81 (s, 3H, OMe-4′), 4.21 (t, 1H, $J=9.5$ Hz, H-11b), 4.40 (dd, 1H, $J=9.5$ Hz, 7.3 Hz, H-11a), 4.61 (d, 1H, $J=4.7$ Hz, H-1), 5.13 (t, 1H, $J=9.0$ Hz, H-4), 5.71 (d, 1H, $J=9.0$ Hz, N–H), 5.97 (d, 1H, $J=1.3$ Hz, OCH_2O), 5.98 (d, 1H, $J=1.3$ Hz, OCH_2O), 6.38 (s, 2H, H-2′, H-6′), 6.54 (s, 1H, H-8), 6.84 (s, 1H, H-5); MS (DCI/NH_3) m/z 473 $[M + NH_4]^+$.

4-Amino-4-deoxypicropodophyllotoxin (9). To a solution of amine **6** (100 mg, 0.24 mmol) in EtOH (10 mL), piperidine (0.05 mL) was added, and the reaction mixture was refluxed for 10 h. The solvent was then removed under reduced pressure and the residue was purified by flash chromatography (CH_2Cl_2/MeOH, 97/3) to give **9** (93 mg, 93%): mp 184 °C; Lit:[22] mp 182–184 °C; $[\alpha]_D^{20}$ −9.4 (c 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 1772 (C=O), 1592, 1505; ^1H NMR (300 MHz, $CDCl_3$) δ 1.66 (br s, 2H, NH_2), 2.45 (m, 1H, H-3), 3.19 (dd, 1H, $J=9.1$ Hz, $J=5.9$ Hz, H-2), 3.62 (d, 1H, $J=9.5$ Hz, H-4), 3.82 (s, 6H, OMe-3′ and -5′), 3.85 (s, 3H, OMe-4′), 4.06 (d, 1H, $J=5.9$ Hz, H-1), 4.41 (dd, 1H, $J=9.3$ Hz, 6.4 Hz, H-11b), 4.59 (dd, 1H, $J=9.3$ Hz, 2.0 Hz, H-11a), 5.90 (d, 1H, $J=1.1$ Hz, OCH_2O), 5.92 (d, 1H, $J=1.1$ Hz, OCH_2O), 6.33 (s, 2H, H-2′, H-6′), 6.45 (s, 1H, H-8), 7.03 (s, 1H, H-5); MS (DCI/NH_3) m/z 414 $[M+H]^+$.

4-Amino-4-deoxy-neo-epipodophyllotoxin (11). Treatment of the 4-amino-4-deoxyepipodophyllotoxin **10**,[22] (100 mg, 0.24 mmol) under the same conditions as that previously used for the conversion of **6** into **9** afforded **11** (91 mg, 91%): mp 95 °C; $[\alpha]_D^{20}$ +80.0 (c 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3429 (N–H), 1703 (C=O), 1591, 1505; ^1H NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 1H, H-2), 2.74 (dd, 1H, $J=9.1$ Hz, 5.5 Hz, H-3), 3.58 (dd, 1H, $J=10.8$ Hz, $J=5.5$ Hz, H-11b), 3.69 (dd, 1H, $J=10.8$ Hz, 9.1 Hz, H-11a), 3.75 (s, 6H, OMe-3′ and -5′), 3.81 (s, 3H, OMe-4′), 4.26 (s, 1H, H-4), 4.31 (s, 1H, H-1), 5.88 (d, 1H, $J=1.3$ Hz, OCH_2O), 5.94 (d, 1H, $J=1.1$ Hz, OCH_2O), 6.29 (s, 2H, H-2′, H-6′), 6.45 (s, 1H, H-8), 6.49 (br s, 1H, N-H), 6.60 (s, 1H, H-5); MS (DCI/NH_3) m/z 414 $[M + H]^+$. Anal. calcd for $C_{22}H_{28}NO_7$: C, 63.91; H, 5.61. Found: C, 64.01; H, 5.70.

4-Deoxy-4-methylene-podophyllotoxin (12). To a suspension of zinc (1.68 g, 25.77 mmol) in anhydrous tetrahydrofuran (30 mL) at 20 °C under argon atmosphere, diiodomethane (1.15 mL, 14.32 mmol) was added and the mixture was stirred at room temperature for 0.5 h before cooling to 0 °C and addition of $TiCl_4$ in dichloromethane (4.72 mL of 0.9 N solution). The reaction mixture was allowed to reach room temperature, after which a solution of podophyllotoxone **4** (1.18 g, 2.86 mmol) in tetrahydrofuran (40 mL) was added. After stirring for one additional hour, the reaction mixture was diluted with dichloromethane (100 mL) and with a 1 N aqueous solution of HCl. The organic layer was separated, washed with water and with brine before drying over $MgSO_4$. Filtration and evaporation in vacuo afforded a crude residue which was purified by flash chromatography (cyclohexane/EtOAc, 2/1) to afford 0.64 g (50%) of **12** as a colourless solid: mp 161 °C; $[\alpha]_D^{20}$ −142 (c 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 2999, 2965, 2942, 2904, 2841 (CH), 1781 (C=O lactone); ^1H NMR (1D, COSY, 300 MHz, $CDCl_3$) δ 2.86 (dd, 1H, $J=4.9$ Hz, 14.7 Hz, H-2), 3.32 (m, 1H, H-3), 3.76 (s, 6H, OMe-3′ and -5′), 3.82 (s, 3H, OMe-4′), 4.24 (dd, 1H, $J=8.7$ Hz, 1.6 Hz, H-11a), 4.59 (dd, 1H, $J=8.4$ Hz, $J=7.4$ Hz, H-11b), 4.66 (d, 1H, $J=4.6$ Hz, H-1), 4.71 (d, 1H, $J=2.2$ Hz, H-12a), 5.55 (d, 1H, $J=2.4$ Hz, H-12b), 5.98 (d, 1H, $J=1.5$ Hz, OCH_2O), 6.02 (d, 1H, $J=1.5$ Hz, OCH_2O), 6.37 (s, 2H, H-2′, H-6′), 6.56 (s, 1H, H-8), 7.23 (s, 1H, H-5); MS (DCI/NH_3) m/z 411 $[M+H]^+$; 428 $[M + NH_4]^+$.

4-Deoxy-4-hydroxymethyl-podophyllotoxin (13). To a cooled (0 °C) solution of **12** (276 mg, 0.67 mmol) in anhydrous tetrahydrofuran (30 mL) was added, under argon atmosphere, 0.67 mL of a solution of BH_3/SMe_2 (2 mol/L in tetrahydrofuran). After stirring for 72 h at room temperature, a solution of phosphate buffer (12 mL, pH 7) was added, followed by addition of methanol (30 mL) and of an aqueous solution of H_2O_2 (30%, 12 mL). Stirring was maintained for 4 h at room temperature prior to extraction with dichloromethane. Usual work-up led to the isolation of a crude compound which was purified by flash chromatography using cyclohexane/ethyl acetate (1/1) as eluent. This afforded mainly a mixture of diastereoisomers (220 mg, 76%) along with a small amount of a pure diastereoisomer: mp 93 °C;

$[\alpha]_D^{20}$ −78 (*c* 0.7, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3617 (OH), 2940, 2840 (aliphatic and aromatic C-H), 1774 (C=O lactone); ^1H NMR (300 MHz, $CDCl_3$) δ 1.80 (t, 1H, OH), 2.93 (m, 1H, H-3), 3.20 (m, 1H, H-4), 3.24 (dd, 1H, *J* = 14 Hz, 5.2 Hz, H-2), 3.74 (s, 6H, OMe-3′ and -5′), 3.79 (s, 3H, OCH_3-4′), 3.96 (m, 2H, H-12), 4.41 (m, 2H, H-11a and H-11b), 4.56 (d, 1H, *J* = 5 Hz, H-1), 5.94 (dd, 1H, *J* = 1 Hz, OCH_2O), 5.96 (d, 1H, *J* = 1 Hz, OCH_2O) 6.51 (s, 1H, H-8), 6.78 (s, 1H, H-5); MS (DCI/NH_3) *m*/*z* 446 $[M + NH_4]^+$; 429 $[M + NH_4 - OH]^+$. Anal. calcd for $C_{23}H_{24}O_8$: C, 64.48; H, 5.65. Found: C, 64.69; H, 5.63.

4′-*tert*-Butyldimethylsilanyloxy-4′-demethyl-4-epipodophyllotoxin (15). 4′-Demethyl-4-epipodophyllotoxin (**14**) (9.0 g, 22.5 mmol) and imidazole (13.14 g, 8 equiv) were dissolved in anhydrous DMF (580 mL) under argon atmosphere before addition of *tert*-butyldimethylsilyl chloride (6.37 g, 8 equiv). After stirring for 4.5 h, the reaction was quenched by addition of 1.2 L of water and the reaction mixture was extracted with ether in the usual manner. The crude material obtained after evaporation of the solvent in vacuo was purified by flash chromatography (cyclohexane/ethyl acetate: 3/1). This afforded compound **15** as a colourless solid (9.96 g, 86%): mp 116 °C. $[\alpha]_D^{20}$ −55 (*c* 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3610, 2800–3000, 1775; ^1H NMR (300 MHz, CDCl3) δ 0.10 (s, 6H, $SiMe_2$), 0.99 (s, 9H, Si-*t*Bu), 1.74 (d, 1H, *J* = 4.3 Hz, OH), 2.85 (m, 1H, H-3), 3.25 (dd, 1H, *J* = 5.0 Hz, 14.1 Hz, H-2), 3.67 (s, 6H, OMe-3′and -5′), 4.35 (m, 2H, H-11a, H-11b), 4.60 (d, 1H, *J* = 5.0 Hz, H-1), 4.85 (t, 1H, H-4), 5.96 (d, 1H, *J* = 1 Hz, OCH_2O), 5.98 (d, 1H, *J* = 1 Hz, OCH_2O), 6.24 (s, 2H, H-2′, H-6′), 6.56 (s, 1H, H-8), 6.87 (s, 1H, H-5); MS (DCI/NH_3): *m*/*z* 532 $[M + NH4]^+$. Anal. calcd for $C_{27}H_{34}O_8Si$: C, 63.01; H, 6.66. Found: C, 62.77; H, 6.76.

4′-*tert*-Butyldimethylsilanyloxy-4′-demethyl-podophyllotoxone (16). Dess–Martin periodinane reagent (2.05 g, 3.99 mmol) was added to a solution of **15** (2.05 g, 3.99 mmol) in 240 mL of anhydrous dichloromethane. The reaction was quenched 20 min later by addition of 200 mL of a 10% aqueous solution of sodium thiosulfate and 200 mL of a saturated solution of sodium hydrogenocarbonate. After 20 min of vigorous stirring, the organic phase was separated, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. Purification by flash chromatography (cyclohexane/ethyl acetate: 3/1) led to **16** as a crystalline compound (1.79 g, 88%): mp 95 °C. $[\alpha]_D^{20}$ −94 (*c* 1, $CHCl_3$); IR ($CHCl_3$) 2931, 2858, 1785, 1688 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.11 (s, 6H, $SiMe_2$), 0.99 (s, 9H, Si-*t*Bu), 3.25 (dd, 1H, *J* = 15 Hz, 5 Hz, H-2), 3.68 (s, 6H, OMe-3′ and -5′), 3.50 (m, 1H, H-3), 4.35 (dd, 1H, *J* = *J*′ = 10 Hz, H-11a), 4.50 (dd, 1H, *J* = *J*′ = 10 Hz, H-11b), 4.82 (d, 1H, *J* = 4.1 Hz, H-1), 6.71 (s, 1H, H-8), 6.09 (d, 1H, *J* = 1.5 Hz, OCH_2O), 6.11 (d, 1H, *J* = 1.5 Hz, OCH_2O), 6.33 (s, 2H), 7.55 (s, 1H, H-5); MS (DCI/NH_3): *m*/*z* 530 $[M + NH_4]^+$. Anal. calcd for $C_{27}H_{32}O_8Si$: C, 63.26; H, 6.29. Found: C, 63.70; H, 6.70.

4′-*tert*-Butyldimethylsilanyloxy-4′-demethyl-4-deoxy-4-methylene-podophyllotoxin (17). To a suspension of activated zinc powder (418 mg, 13.5 equiv) in 5 mL of anhydrous THF under argon atmosphere, diiodomethane (286 μL, 7.5 equiv) was added at 20 °C and, 30 min later, the mixture was cooled to 0 °C. A solution of titane tetrachloride in dichloromethane [(585 μL), *c* 1.82 mol/L, 2.25 equiv] was then added and the reaction mixture was allowed to reach room temperature prior to addition of a solution of the epipodophyllotoxone derivative **16** (243 mg, 0.47 mmol) in 6 mL of anhydrous THF. The reaction mixture was stirred for 20 h, diluted with dichloromethane (20 mL), and a 2% aqueous solution of HCl (20 mL), added. The organic phase was separated, washed with brine, dried over magnesium sulfate and filtered. After concentration under reduced pressure, the residue was purified by flash chromatography (cyclohexane/ethyl acetate: 7/1). Compound **17** was obtained as a crystalline derivative (177 mg, 73%): mp 156 °C. $[\alpha]_D^{20}$ −140 (*c* 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3000–2800, 1780; ^1H NMR (300 MHz, $CDCl_3$) δ 0.11 (s, 3H, $SiMe_2$), 0.99 (s, 9H, Si-*t*Bu), 2.85 (dd, 1H, *J* = 10.2 Hz, 4.5 Hz, H-3), 3.30 (m, 1H, H-3), 3.68 (s, 6H, OMe-3′ and -5′), 4.21 (dd, 1H, *J* = 10.4 Hz, 8 Hz, H-11a), 4.55 (dd, 1H, *J* = 7.5 Hz, 8 Hz, H-11b), 4.64 (d, 1H, *J* = 4.7 Hz, H-1), 4.69 and 5.53 (d, 2H, *J* = 2.2 Hz, H-vinyl), 5.97 (d, 1H, *J* = 1 Hz, OCH_2O), 5.99 (s, 1H, *J* = 1 Hz, OCH_2O), 6.32 (s, 2H, H-2′, H-6′), 6.56 (s, 1H, H-8), 7.21 (s, 1H, H-5); MS (DCI/NH_3): *m*/*z* 528 $[M + NH_4]^+$. Anal. calcd for $C_{28}H_{34}O_7Si$: C, 65.86; H, 6.71. Found: C, 65.91; H, 6.53.

4′-Demethyl-4-deoxy-4-methylene-podophyllotoxin (18). An ethanolic solution of **17** (68.7 mg, 134 μmol in 45 mL) was refluxed in the presence of pyridinium *para*-toluol sulfonate (PPTS) (16.9 mg, 1/2 equiv) until completion of the deprotection as followed by TLC control (22 h). After cooling to room temperature, 10 mL of water and 20 mL of dichloromethane were added. The organic phase was separated, dried over anhydrous magnesium sulfate, filtered and the solvent was removed under reduced pressure. Purification by flash chromatography (cyclohexane/ethyl acetate: 2/1) afforded the derivative **18** as a crystalline residue (53.1 mg, 99%): mp 114 °C. $[\alpha]_D^{20}$ −165 (*c* 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 3538, 2846, 2906, 2967, 1780; ^1H NMR (300 MHz, $CDCl_3$) δ 2.85 (dd, 1H, *J* = 14.5 Hz, 4.6 Hz, H-2), 3.30 (m, 1H, H-3), 3.79 (s, 6H, OMe-3′ and -5′), 4.23 (dd, 1H, *J* = 10.3 Hz, 8.5 Hz, H-11a), 4.57 (dd, 1H, *J* = 7.0 Hz, 8.0 Hz, H-11b), 4.66 (d, 1H, *J* = 4.6 Hz, H-1), 4.70 and 5.55 (2d, 2H, H-vinyl), 5.42 (s, 1H, OH), 6.02 (d, 1H, *J* = 1 Hz, OCH_2O), 6.00 (1H, *J* = 1 Hz, OCH_2O), 6.38 (s, 2H, H-2′, H-6′), 6.56 (s, 1H, H-8), 7.23 (s, 1H, H-5); MS (DCI/NH_3): *m*/*z* 414 $[M + NH_4]^+$. Anal. calcd for $C_{22}H_{20}O_7$: C, 66.66; H, 5.09. Found: C, 66.54; H, 5.03.

4′-*tert*-Butyldimethylsilanyloxy-4′-demethyl-4-deoxy-4-spiroepoxy-podophyllotoxin (19). To the derivative **17** (88.4 mg, 173 μmol) was dissolved in 2 mL of THF were successively added 375 mL of water, iodine (87 mg, 2 equiv) and silver(II) oxide (80 mg, 2 equiv). The mixture was stirred at 20 °C for 20 min and subsequently quenched by addition of 20 mL of water prior to addition of dichloromethane (20 mL) and a few drops of a 10%

sodium hydrogenosulfite solution until discolouration of the medium. The organic phase was separated, dried over anhydrous magnesium sulfate, filtered and the solvent was removed under reduced pressure. Purification by flash chromatography on a silica gel column (cyclohexane/ethyl acetate: 4/1) afforded the oxirane **19** as a colourless solid (79.1 mg, 87%): mp 108 °C. $[\alpha]_D^{20}$ −126 (*c* 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 2931, 2857, 1775; ^1H NMR (300 MHz, $CDCl_3$) δ 0.11 (s, 6H, Me_2Si), 1.01 (s, 9H, Si-*t*Bu), 2.90 (dd, 1H, *J* = 14.0 Hz, 5.0 Hz, H-2), 3.10 (m, 1H, H-3), 3.10 (d, 1H, *J* = 11.0 Hz, H-12a), 3.30 (d, 1H, *J* = 11.0 Hz, H-12b), 3.55 (s, 6H, OMe-3′ and -5′), 3.94–4.07 (m, 2H, H-11a, H-11b), 4.41 (d, 1H, *J* = 5.0 Hz, H-1), 5.56 (d, 1H, *J* = 1, OCH_2O), 5.60 (d, 1H, *J* = 1, OCH_2O), 6.38 (s, 1H, H-8), 6.42 (s, 2H, H-2′, H-6′), 6.55 (s, 1H, H-5); MS (DCI/NH_3): *m*/*z* 544 $(M+NH_4)^+$. Anal. calcd for $C_{28}H_{34}O_8Si$: C, 63.86; H, 6.51. Found: C, 63.51; H, 6.42.

4′-Demethyl-4-deoxy-4-spiroepoxy-podophyllotoxin (20). The oxirane derivative **19** (31 mg, 59 μmol) was treated as previously described for obtaining **18** from **17**. Flash chromatography (cyclohexane/ethyl acetate: 2/1) of the crude product afforded compound **20** as an amorphous solid (15.4 mg, 63%): $[\alpha]_D^{20}$ −145 (*c* 1, $CHCl_3$);. IR ($CHCl_3$, cm^{-1}): 3539, 2928, 1777; ^1H NMR (300 MHz, $CDCl_3$) δ 2.91 (dd, 1H, *J* = 5.0 Hz, 14.1 Hz, H-2), 3.1 (m, 1H, H-3), 3.09 (d, 1H, *J* = 11.0 Hz, H-12a), 3.31 (d, 1H, *J* = 11.0 Hz, H-12b), 3.59 (s, 6H, OMe-3′ and -5′), 3.95–4.09 (m, 2H, H-11a, H-11b), 4.40 (d, 1H, *J* = 5.0 Hz, H-1), 5.28 (s, 1H, OH), 5.59 (d, 1H, *J* = 1.5 Hz, OCH_2O), 5.64 (d, 1H, *J* = 1.5 Hz, OCH_2O), 6.38 (s, 1H, H-8), 6.41 (s, 2H, H-2′, H-6′); 6.54 (s, 1H, H-5); MS (DCI/NH_3): *m*/*z* 430 $[M + NH_4]^+$. Anal. calcd for $C_{22}H_{20}O_8$: C, 64.07; H, 4.89. Found: C, 64.25; H, 5.02.

4′-*tert*-Butyldimethylsilanyloxy-4′-demethyl-4-deoxy-4-hydroxymethyl-podophyllotoxin (21). After the derivative **17** (232.6 mg, 455 μmol) was dissolved in 10 mL of anhydrous THF under argon atmosphere, BH_3/Me_2S (2 M THF solution, 455 μL) was added. After stirring for 2 h, the borane formed was hydrolyzed by addition of 10 mL of pH 7 phosphate buffer, 20 mL of methanol and 10 mL of a 30% H_2O_2 solution in water. The mixture was stirred for 1.5 h, and next 100 mL of water and 100 mL of dichloromethane were added. The organic phase was separated, dried over anhydrous magnesium sulfate, filtered and the solvent was removed under reduced pressure. Purification by flash chromatography (cyclohexane/ethyl acetate: 2/1) afforded a mixture (3/2) of the inseparable alcohols **21** as a colourless solid (197.7 mg, 82%): IR ($CHCl_3$, cm^{-1}): 3619, 2931, 2896, 2858, 1774; ^1H NMR (300 MHz, $CDCl_3$). For the isomer **21β**: δ 0.10 (s, 6H, Me_2Si), 0.98 (s, 9H, Si-*t*.Bu), 2.80–3.00 (m, 1H, H-3), 3.15–3.22 (m, 2H, *J* = 5.0 Hz, 9.1 Hz, H-2, H-4), 3.67 (s, 6H, OMe-3′ and -5′), 3.94 (m, 2H, CH_2OH), 4.37–4.43 (m, 2H, H-11a, H-11b), 4.54 (d, 1H, *J* = 5.0 Hz, H-1), 5.93 (d, 1H, *J* = 1 Hz, OCH_2O), 5.96 (d, 1H, *J* = 1 Hz, OCH_2O), 6.25 (s, 2H, H-2′, H-6′), 6.52 (s, 1H, H-8), 6.76 (s, 1H, H-5). For the isomer **21α**: δ 0.10 (s, 6H, Me_2Si), 0.98 (s, 9H, Si-*t*Bu), 2.80–3.00 (m, 1H, H-3), 2.70 (dd, 1H,), 3.67 (s, 6H, OMe-3′, OMe-5′), 3.80–4.10 (m, 4H), 4.60 (d, 1H, *J* = 5 Hz, H-1), 5.93 (d, 1H, *J* = 1 Hz, O–CH_2–O), 5.95 (d, 1H, *J* = 1 Hz, O–CH_2–O), 6.34 (s, 2H, H-2′, H-6′), 6.58 (s, 1H, H-8), 6.82 (s, 1H, H-5); MS (DCI/NH_3): *m*/*z* 546 $[M + NH_4]^+$, (DCI/CH_4) *m*/*z* 529 $[M + H]^+$; DCI-HRMS *m*/*z* calcd for $C_{28}H_{37}O_8Si$: 529.2258. Found: 529.2224.

4′-Demethyl-4-deoxy-4-hydroxymethyl-epipodo- and podophyllotoxin (22 and 23). A solution of alcohols **21** (752.2 mg, 1.42 mmol) in 60 mL of 95% ethanol was refluxed for 17 h in the presence of PPTS (179 mg, 1/2 equiv). After addition of 10 mL of water and 20 mL of dichloromethane, the organic phase was separated, dried over anhydrous magnesium sulfate and filtered. The solvent was removed under reduced pressure and the residue purified by chromatography on a silica gel column (cyclohexane/ethyl acetate: 1/2, the weight of silica was 100 times equal to that of the crude material). This led successively to **22** and **23** which were both obtained as colourless solids (53.1 mg, 87% overall yield). **22**: mp 130–135 °C; $[\alpha]_D^{20}$ −125.5 (*c* 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 3619, 3539, 2923, 1773; ^1H NMR (300 MHz, $CDCl_3$) δ 2.13 (dd, 1H, *J* = 6.0 Hz, 11.0 Hz, H-4), 2.50 (m, 1H, H-3), 2.53 (dd, 1H, *J* = 13.7 Hz, 4.6 Hz, H-2), 3.25 (t, 2H, H-12a, H-12b), 3.52 (s, 6H, OMe-3′ and -5′), 3.95 (dd, 1H, *J* = 8.5 Hz, 7.0 Hz, H-11a), 4.09 (dd, 1H, *J* = 8.5 Hz, 9.1 Hz, H-11b), 4.52 (d, 1H, *J* = 4.6 Hz, H-1), 5.35 (d, 1H, OCH_2O), 5.39 (d, 1H, OCH_2O), 6.55 and 6.56 (s, 2H, H-5, H-8), 6.62 (s, 1H, H-2′, H-6′); MS (DCI/NH_3): *m*/*z* 432 $[M + NH_4]^+$. Anal. calcd for $C_{22}H_{22}O_6$: C, 63.76; H, 5.35. Found: C, 63.90; H, 5.51. **23**: mp 130–135 °C; $[\alpha]_D^{20}$ −77.8 (*c* 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 3539, 2928, 1775; ^1H NMR (300 MHz, $CDCl_3$) δ 2.13 (dd, 1H, *J* = 4.5 Hz, *J* = 14.0 Hz, H-2), 2.36 (m, 1H, H-4), 2.53 (m, 1H, H-3), 3.28 (m, 2H, H-12a, H-12b), 3.41 (dd, 1H, *J* = 8.5 Hz, *J* = 10.4 Hz, H-11a), 3.52 (s, 6H, OMe-3′ and -5′), 4.16 (t, 1H, *J* = 8.2 Hz, *J* = 7.5 Hz, H-11b), 5.35 (d, 1H, OCH_2O), 5.38 (d, 1H, OCH_2O), 5.41 (s, 1H, OH), 6.58 (s, 1H, H-8), 6.70 (s, 1H, H-5), 6.72 (s, 2H, H-2′, H-6′); MS (DCI/NH_3): *m*/*z* 432 $[M + NH_4]^+$. Anal. calcd for $C_{22}H_{22}O_6$: C, 64.07; H, 4.89. Found: C, 63.93; H, 5.05.

4′-Benzyloxycarbonyl-4′-demethyl-4-deoxy-4-hydroxymethyl-podophyllotoxin (25). To a solution of the diol **23** (101 mg, 244 μmol) in anhydrous dichloromethane (7 mL) kept under an argon atmosphere and cooled to 0 °C, 70 μL of triethylamine (2 equiv) and 50 μL of benzylchloroformate (1.4 equiv) were added. After stirring for 45 min at 0 °C, 20 mL of water were added and the organic phase was separated, dried over anhydrous magnesium sulfate, filtered and the solvent was removed under reduced pressure. Purification by flash chromatography (cyclohexane/ethyl acetate: 1/1) afforded the alcohol **25** as a colourless solid (126 mg, 94%): mp 115–120 °C. $[\alpha]_D^{20}$ −104 (*c* 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 3539, 2828, 1775, 1672; ^1H NMR (300 MHz, $CDCl_3$) δ 1.75 (t, 1H, *J* = 4.3 Hz, OH), 2.80 (m, 1H, H-3), 2.86 (dd, 1H, *J* = 4.3 Hz, 13.9 Hz, H-2), 3.11 (m, 1H, H-4), 3.76 (s, 6H, OMe-3′ and -5′), 3.89 (m, 1H, H-12a), 4.03 (t, 1H, *J* = 9.9 Hz, 8.9 Hz, H-11a), 4.14 (m, 1H, H-12b), 4.65 (m, 1H, *J* = 6.73 Hz, H-11b), 4.67 (d, 1H, *J* = 4.7

Hz, H-1), 5.33 (s, 2H, CH_2), 6.03 (s, 2H, OCH_2O), 6.52 (s, 2H, H-2′, H-6′), 6.63 (s, 1H, H-8), 6.91 (s, 1H, H-5), 7.40–7.51 (m, 5H, Ar); MS (DCI/NH_3): *m/z* 432 [M + NH_4]$^+$. Anal. calcd for $C_{30}H_{28}O_{10}$: C, 65.69; H, 5.15. Found: C, 65.16; H, 5.27.

4′-Benzyloxycarbonyl-4′-demethyl-4-deoxy-4-hydroxymethyl-epipodophyllotoxin (24). To a solution of the diol **23** (339.5 mg, 817 mol) in 22 mL of anhydrous dichloromethane, cooled to 0 °C, under argon atmosphere, were added 227 μL of triethylamine (2 equiv) and 163 μL of benzylchloroformate (1.4 equiv). After stirring for 35 min at the same temperature, water (30 mL) was added and the organic phase was separated, dried over anhydrous magnesium sulfate, filtered and the solvent was removed under reduced pressure. After purification by flash chromatography (cyclohexane/ethyl acetate: 1/2), the alcohol **24** was obtained as a colourless solid (413.5 mg, 92%): mp 105–110 °C. $[\alpha]_D^{20}$ −50 (*c* 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 3650, 2800–3100, 1767; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.90 (m, 1H, H-3), 3.20 (m, 1H, *J* = 6.3 Hz, H-4), 3.22 (dd, 1H, *J* = 5.2 Hz, 14.3 Hz, H-2), 3.75 (s, 6H, OMe-3′ and -5′), 3.95 (m, 2H, H-12a, H-12b), 4.40 (m, 2H, H-11a, H-11b), 4.59 (d, 1H, *J* = 5.1 Hz, H-1), 5.25 (s, 2H, CH_2), 5.93 (d, 1H, OCH_2O), 5.95 (d, 1H, OCH_2O), 6.32 (s, 2H, H-2′, H-6′), 6.51 (s, 1H, H-8), 6.78 (s, 1H, H-5), 7.32–7.44 (m, 5H, Ar); MS (DCI/NH_3): *m/z* 566 [M + NH_4]$^+$, 432 [M + NH_4 −benzyloxycarbonyl]$^+$. Anal. calcd for $C_{30}H_{28}O_{10}$: C, 65.69; H, 5.15. Found: C, 65.63; H, 5.42.

4′-Benzyloxycarbonyl-4′-demethyl-4-deoxy-4-carboxaldehyde-epipodophyllotoxin (27). The alcohol **25** (111 mg, 203 μmol) was dissolved in 30 mL of anhydrous dichloromethane and Dess–Martin periodinane (340 mg, 4 equiv) was added to this solution. The solution, which took a milky aspect, was stirred for 2 h at 20 °C. The reaction was then quenched by addition of 30 mL of a 10% sodium thiosulfate solution and 20 mL of a saturated solution of sodium hydrogenocarbonate. After 20 min of strong stirring, the solution was transferred into a separating funnel, the organic phase was separated and filtered, dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. Purification by flash chromatography (cyclohexane/ethyl acetate: 1/1) afforded the aldehyde **27**, obtained as a colourless solid (104 mg, 94%): mp 110–120 °C. $[\alpha]_D^{20}$ −97 (*c* 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 2906, 1769, 1727; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.78 (dd, 1H, *J* = 14.1 Hz, 4.4 Hz, H-2), 2.40 (m, 1H, H-3), 2.76 (dd, 1H, *J* = 10.7 Hz, H-4), 3.04 (dd, 1H, *J* = 10.3 Hz, 8.8 Hz, H-11a), 3.35 (s, 6H, OMe-3′ and -5′), 3.63 (t, 1H, *J* = 7.5 Hz, 8.1 Hz, H-11b), 4.28 (d, 1H, *J* = 4.4 Hz, H-1), 4.97 (s, 2H, CH_2), 6.23 (d, 1H, *J* = 1.0 Hz, OCH_2O), 6.25 (d, 1H, *J* = 1.0 Hz, OCH_2O), 6.32 (s, 1H, H-8), 6.46 (s, 1H, H-5), 6.48 (s, 2H, H-2′, H-6′), 6.99–7.11 (m, 5H, Ar), 9.08 (d, 1H, *J* = 2.86 Hz, CHO); MS (DCI/NH_3): *m/z* 564 [M + NH_4]$^+$, 430 [M + NH_4 −benzyloxycarbonyl]$^+$. Anal. calcd for $C_{30}H_{26}O_{10}$: C, 65.93; H, 4.80. Found: C, 65.68; H, 4.73.

4′-Benzyloxycarbonyl-4′-demethyl-4-deoxy-4-carboxaldehyde-podophyllotoxin (26). The alcohol **24** (383 mg, 700 μmol) was treated as above. Flash chromatography (cyclohexane/ethyl acetate: 1/1) led to the aldehyde **26** as a colourless solid (371 mg, 97%): mp 115–120 °C; $[\alpha]_D^{20}$ −85 (*c* 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 2843, 2905, 2943, 1768, 1723; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.05 (m, 1H, H-3, H-3), 3.29 (dd, 1H, *J* = 14.6 Hz, 5.1 Hz, H-2), 3.76 (s, 6H, OMe-3′ and -5′), 3.94 (dd, 1H, *J* = 5.7 Hz, 2.5 Hz, H-4), 4.42–4.54 (m, 2H, H-11a, H-11b), 4.75 (d, 1H, *J* = 5.1 Hz, H-1), 5.33 (s, 2H, CH_2), 6.06 (d, 1H, OCH_2O), 6.08 (d, 1H, OCH_2O), 6.40 (s, 2H, H-2′, H-6′), 6.68 (s, 1H, H-8), 6.79 (s, 1H, H-5), 7.40–7.51 (m, 5H, Ar), 9.88 (d, 1H, *J* = 2.6 Hz, CHO); MS (DCI/NH_3): *m/z* 564 [M + NH_4]$^+$, 430 [M + NH_4−benzyloxycarbonyl]$^+$. Anal. calcd for $C_{30}H_{26}O_{10}$: C, 65.93; H, 4.80. Found: C, 65.52; H, 4.96.

4′-Benzyloxycarbonyl-4′-demethyl-4-deoxy-4-carboxylic-epipodophyllotoxin acid (28). The aldehyde **26** (354 mg, 650 μmol) and resorcinol (143 mg, 2 equiv) were dissolved in 35 mL of *tert*-butanol at 25 °C. Then, 10 mL of pH 4 acetate buffer, 9 mL of water and sodium chlorite (70 mg, 1.2 equiv) were added and the mixture was stirred for 40 min. The reaction was then quenched by addition of 100 mL of dichloromethane and 100 mL of water. After extraction, the organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent was removed under reduced pressure. Purification by flash chromatography (cyclohexane/ethyl acetate/acetic acid: 50/50/1) afforded a mixture of acids **28** and **29**, obtained as a colourless solid (250 mg, 69%); IR ($CHCl_3$, cm^{-1}): 2908, 1769, 1709; ^{1}H NMR of **28** (300 MHz, $CDCl_3/C_6D_6$) δ 2.96–3.04 (m, 1H, H-3), 3.68 (dd, 1H, *J* = 5.2 Hz, 12.7 Hz, H-2), 3.72 (s, 6H, OMe-3′ and -5′), 4.07 (d, 1H, *J* = 6.2 Hz, H-4), 4.11 (dd, 1H, H-11a), 4.41 (t, 1H, *J* = 8.2 Hz, H-11b), 4.70 (d, 1H, *J* = 5.1 Hz, H-1), 5.30 (s, 2H, CH_2), 5.99 (d, 1H, *J* = 1 Hz, OCH_2O), 6.02 (d, 1H, *J* = 1 Hz, OCH_2O), 6.37 (s, 2H, H-2′, H-6′), 6.59 (s, 1H, H-8), 6.87 (s, 1H, H-5), 7.37–7.48 (m, 5H, Ar); MS (DCI/NH_3): *m/z* 580 [M + NH_4]$^+$.

4′-Benzyloxycarbonyl-4′-demethyl-4-deoxy-4-carboxylic-podophyllotoxin acid (29). Using the same protocol as above, the aldehyde derivative **27** (42 mg) led to the acid **29** as a colourless solid (18 mg, 42%) after purification by flash chromatography: mp 128–130 °C; $[\alpha]_D^{20}$ −121 (*c* 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 2928, 1768, 1712; ^{1}H NMR (300 MHz, $CDCl_3/C_6D_6$) δ 2.84 (dd, 1H, *J* = 4.4 Hz, 14.3 Hz, H-2), 3.05–3.19 (m, 1H, H-3), 3.74 (s, 6H, OMe-3′ and -5′), 3.91 (d, *J* = 11.1 Hz, H-4), 4.07 (dd, *J* = 10.5 Hz, 8.8 Hz, H-11a), 4.54 (dd, *J* = 7.6 Hz, 8.2 Hz, H-11b), 4.67 (d, 1H, *J* = 4.4 Hz, H-1), 5.31 (s, 2H, CH_2), 6.03 (s, 2H, OCH_2O), 6.52 (s, 2H, H-2′, H-6′), 6.62 (s, 1H, H-8), 6.87 (s, 1H, H-5); MS (DCI/CH_4): *m/z* 563.15 [M + H]$^+$; DCI-HRMS *m/z* calcd for $C_{30}H_{26}O_{12}$: 563.1553. Found: 563.1541.

Growth inhibition assays and cell-cycle analysis

Tumour cells were provided by American Type Culture Collection (Frederik, MD, USA). They were cultivated in RPMI 1640 medium (Life Science Technologies, Cergy-Pontoise, France) supplemented with 10%

fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH = 7.4). Cytotoxicity was measured by the microculture tetrazolium assay (MTA) as described.[27] Cells were continuously exposed to graded concentrations of the compounds for four doubling times, then 15 μL of 5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were added to each well and the plates were incubated for 4 h at 37 °C. The medium was then aspirated and the formazan solubilized by 100 μL of DMSO. Results are expressed as IC_{50} concentrations, which reduced by 50% the optical density of treated cells with respect to untreated controls.

For the cell cycle analysis, L1210 cells (5×10^5 cells/mL) were incubated for 21 h with various concentration of drugs. Cells were then fixed by 70% ethanol (vv), washed, and incubated in PBS containing 100 μg/mL propidium iodide for 30 min at 20 °C. For each sample, 10,000 cells were analyzed on a XLMCL flow cytometer (Beckman Coulter, France).

Microtubule test[28]

Porcine brain tubulin was purified by three cycles of polymerisation–depolymerisation and was dissolved in the assembly buffer containing 0.1 M 2-(*N*-morpholino)-ethanesulfonic acid, 0.5 mM $MgCl_2$, 1 mM EDTA, and 1 mM GTP, pH 6.6.

Protein concentration, estimated with bovine serum albumin as standard, was kept at ca. 1 mg/mL for the experiments. Compounds dissolved in DMSO at 37 °C were added at different concentrations to the solution of microtubules, the temperature was lowered to 0 °C and the optical density recorded at 350 nM. The IC_{50} of microtubule assembly was calculated and 4-deoxypodophyllotoxin was used as standard.[29]

Topoisomerases cleavage assays

They were determined as reported previously.[30]

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