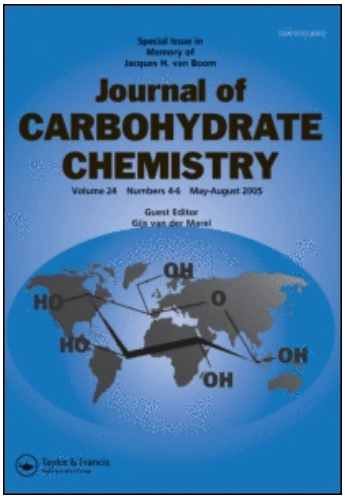

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A SIMPLE SYNTHESIS OF *C*-GLUCOSIDES RELATED TO THE ANTITUMOR AGENT ETOPOSIDE

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ABSTRACT

The synthesis of four new lignan derivatives **2a, 2b, 3a** and **3b** related to the anticancer agent etoposide has been accomplished. In these compounds a methylene or ethylene group of a *C*-glucoside separates the glucosidic moiety as present in etoposide (**1**) from its lignan aglycone bonded by an ethereal function. The key step in the synthesis involves the reaction of a benzylated alditol **4** or **5** and the unprotected 4'-demethylepipodophyllotoxin (**6**).

INTRODUCTION

Etoposide (**1**) is a clinically useful antitumor agent derived from the naturally occurring lignan podophyllotoxin. Currently, etoposide is widely used alone or in combination with other drugs for the treatment of small cell lung cancer and testicular cancer.[1-4] The principal mechanism of action of **1** is the inhibition of the catalytic activity of DNA topoisomerase II and of the concurrent enzyme-mediated production of lethal DNA strand breaks.[5]

The favourable pharmacological properties of etoposide have stimulated much activity in the search for analogs with enhanced activity or activity against etoposide-resistant tumor cells.[6] Thus it was found that the replacement of the 4β-*O*-glucosidic moiety of **1** with a 4β-*N*-, 4β-*S*- or 4β-*O*-aliphatic or aromatic substituents

affords a number of compounds which, in some cases, are as active or more active than etoposide (**1**) against human DNA topoisomerase II.[7,8]

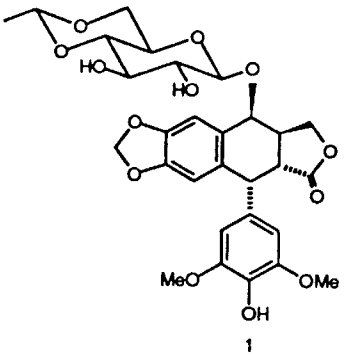
RESULTS AND DISCUSSION

Pursuing our interest[9] in analogues of etoposide (**1**), we planned the synthesis of the *C*-glucosides **2a, 2b, 3a** and **3b** which contain an ethereal function bonding the lignan moiety of **1** to a *C*-glucoside derived from the ethylidene glucose present in **1**. The ethereal function present in **2a, 2b, 3a** and **3b** and the *C*-glycosidic bond of the pyranose ring should assure significant stability of these compounds to possible biological hydrolysis while the different distances of the glucosidic portions from the aglycone would afford derivatives able to satisfy different stereochemical requirements for the biological action.

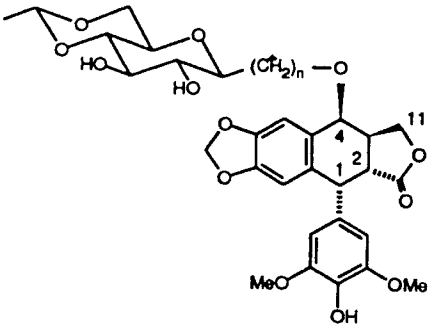
The key step in the synthesis of compounds **2a, 2b, 3a** and **3b** is the direct formation, under boron trifluoride-diethyl ether complex ($BF_3.Et_2O$) catalysis, of the ethereal bond between the 2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-D-*glycero*-D-*gulo*-heptitol (**4**) or the 3,7-anhydro-2-deoxy-4,5,6,8-tetra-*O*-benzyl-D-*glycero*-D-*gulo*-octitol (**5**) and 4'-demethylepipodophyllotoxin (**6**), to afford benzylated *C*-glucosides **7a, 7b, 8a** and **8b** (Scheme).

Interestingly, we have found that on performing this reaction, it is not necessary to protect the phenolic group of **6**. This result is noteworthy since in all previously reported processes for the preparation of etoposide,[10-15] or analogues having an ethereal function in place of the glycosidic bond,[8] the selective protection of the 4'-phenolic group of 4'-demethylepipodophyllotoxin (**6**) was found necessary.

Since it has been reported that the stereochemistry of C-4 substituents on the aglycone is important in determining the biological activity of the etoposide and its analogues, the β-isomers being generally much more potent than α-isomers,[7] we first designed experiments to obtain stereoselectively the β-isomers **7a** and **7b**, reasoning that they are the favoured products in a possible SN_1 process which should involve preferential attack of the nucleophilic reagent at the C-4 benzylic carbocation of **6** from the side opposite to the bulky aromatic ring pendant at C-1. Thus we found that using 0.5 and 1 molar equivalents of Lewis acid and relatively long reaction time (7 and 21 h) it was possible to obtain, in good yields and with favourable stereoselectivity, respectively the β-compounds **7a** (in 58% yield, accompanied by **8a**, formed in 3% yield) and **7b** (in 52% yield, accompanied by **8b**, formed in 3% yield).



1



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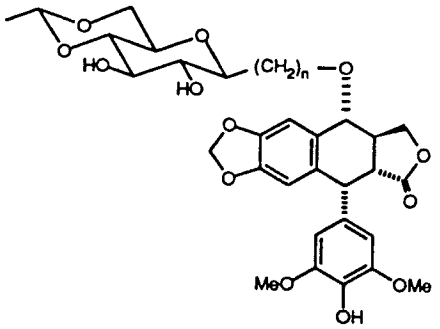


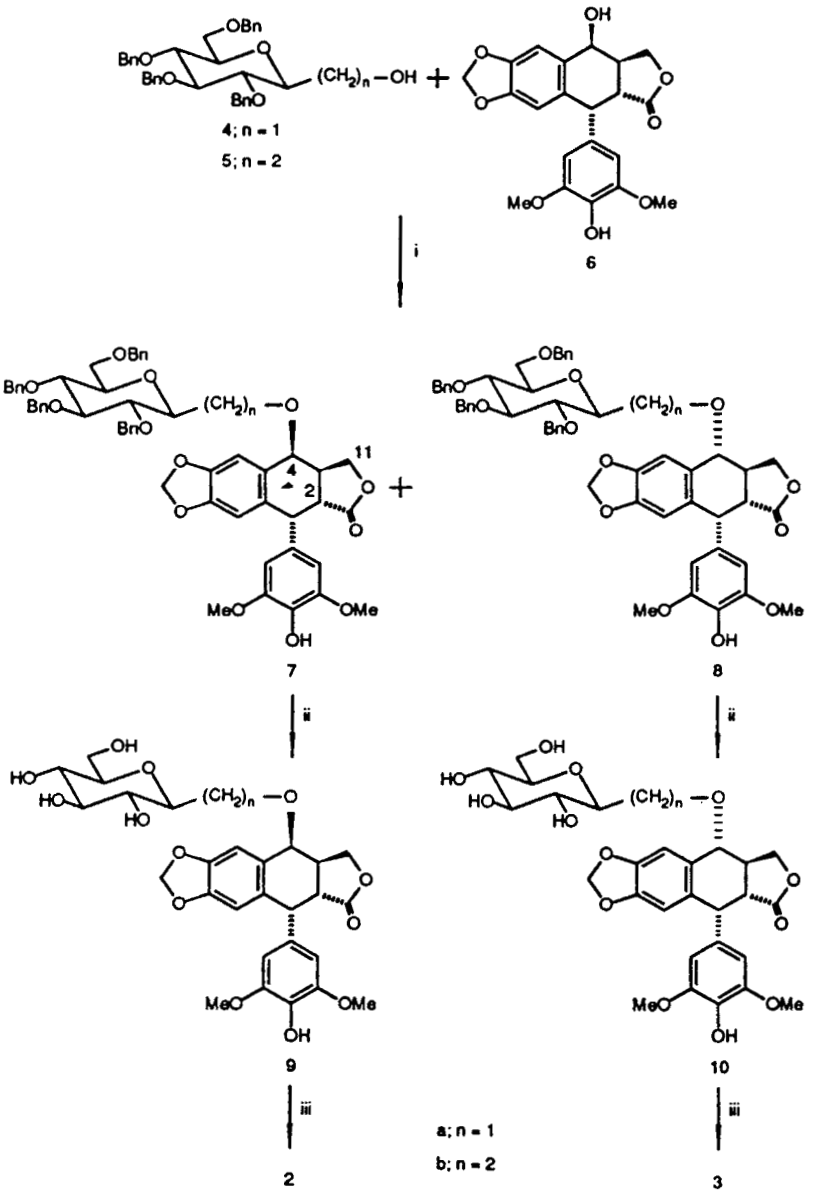
3

a; n = 1
b; n = 2
c; n = 1, epimer at C-2

In other experiments we established also the best conditions for obtaining the α-isomers **8a** and **8b** with comparable yields and stereoselectivity. In fact, it was not possible to exclude *a priori* that the derived compounds **3a** and **3b** also could possess favourable biological activity. The synthesis of these compounds was possible since simple variations of the reaction conditions, e.g. using excess amounts of Lewis acid (5 and 3 molar equivalents) and relatively short reaction times (2 h), allowed us to obtain also **8a** (in 60% yield, accompanied by **7a**, formed in 14% yield) and **8b** (in 45% yield, accompanied by **7b**, formed in 7% yield) with good stereoselectivity.

Compounds **7a, 7b, 8a** and **8b** were separately transformed, by debenzylation, into the corresponding polyhydroxy compounds **9a, 9b, 10a** and **10b** which then were treated with 1,1-dimethoxyethane/H^+ to afford the desired compounds **2a, 2b, 3a** and **3b** (Scheme).

SCHEME



Scheme Reagents: i, $BF_3.Et_2O$; ii, H_2, Pd-C; iii, $MeCH(OMe)_2$, p-TSA

The stereochemistry of all compounds obtained was deduced from the examination of their ^{1}H NMR spectra (500 MHz) (Table 1 and 2). In particular, in all these compounds the observed coupling constants between the proton at C-3 and the adjacent protons at C-2 and C-11 ($J_{2,3}$ = 14.0 Hz, $J_{3,11}$ = 10.0-10.5 and 7.0-8.0 Hz) show on that in no case had epimerisation of the lactonic function occurred, to afford picropodophyllotoxin analogues. In fact, the observed values are typical[16] for the podophyllotoxin series and quite different from those observed for picropodophyllotoxin ($J_{2,3}$ = 9.0 Hz, $J_{3,11}$ = 6.0 and 1.5 Hz) and for the derivatives **2c** and **3c** ($J_{2,3}$ = 10.5 Hz, $J_{3,11}$ = 7.0 and 3.0 Hz) obtained by mild basic epimerization at C-2 of **2a** and **3a**.

The configuration at C-4 of each compound was derived from the value of the coupling constant between the H-3 and H-4 protons. In fact because of the rigidity conferred on the system by the 2,3-*trans* fusion,[17] these protons in the 4-β isomers are in an axial-equatorial relationship (J = 3.5 Hz), while in the α isomers they are in an axial-axial geometry (J = 9.5-10.0 Hz).[7]

Additional support to the assigned stereochemistry at C-4 derived from the differences observed between the chemical shifts of the C-11 methylenic protons which were always smaller in the β series (Δδ 0.03-0.18 ppm) than in the α series (Δδ 0.45-0.53 ppm). These differences are attributable to a different influence of the α and β oxygen substituents present at C-4 of the isomeric compounds.[17]

The mass spectra of all synthesized 4β-derivatives of podophyllotoxin (**2a, 2b, 7a, 7b, 9a, 9b**) in respect to those of the 4α-epimers (**3a, 3b, 8a, 8b, 10a, 10b**) show a significant difference in the ratio of the abundance of the ion at m/z 382 (corresponding to the loss of the *C*-glucosidic moiety and OH) to the ion at m/z 298 (attributable to the further loss of an α,β unsaturated γ-butyrolactone molecule). The ratio varies between 3 and 18.2 for the 4α-series and between 0.74 and 1.25 for 4β-series.

In previous work[17,18] the observation has been made that the abundance of the [M^+ - H_2O or M^+ - ROH]-ion, corresponding in our products (**2a, 2b, 3a, 3b, 7a, 7b, 8a, 8b, 9a, 9b, 10a** and **10b**) to the ion at m/z 382, may bear a relationship to the stereochemistry of the C-4 oxygen substituent and of the stereochemistry of the lactone ring; easier elimination being possible in the more flexible *cis* lactone than in the *trans*. Examination of the mass spectra of our diastereomeric lactones shows that stereochemical differences in the lactone ring junction did not cause any major differences in the relative abundance of the ion at m/z 382 which is the parent peak in podophyllotoxin and picropodophyllotoxin derivatives (**2a, 3a** and **2c, 3c**) and is always high in all compounds. However, the observation may be made that the peak at m/z 298, corresponding to the further loss of the lactone ring, is always higher in 4α-series than in the 4β-series compounds, independently from the stereochemistry at C-2.

TABLE 1. ^{1}H NMR[a] chemical shifts δ_H in ppm and coupling constants J in Hz for compounds 2a, 3a, 7a, 8a, 9a, 10a

Proton (J)	2a	3a	7a	8a	9a	10a
2	3.32dd	2.77dd	3.36dd	2.72dd	3.41dd	3.02dd
(1,2)	(5.0)	(4.5)	(5.5)	(5.0)	(4.8)	(4.5)
(2,3)	(14.0)	(14.0)	(14.0)	(14.0)	(14.0)	(14.0)
3	2.82dddd	2.90dddd	2.80dddd	2.86dddd	2.92dddd	2.88dddd
(3,4)	(3.5)	(10.0)	(3.5)	(9.5)	(3.5)	(9.5)
(3,11a)	(10.5)	(7.0)	(10.5)	(7.0)	(10.4)	(7.5)
(3,11b)	(8.0)	(10.5)	(8.0)	(10.5)	(8.0)	(10.5)
4	4.48d	4.69d	4.51d	4.64d	4.70d	4.76d
11a	4.33dd	4.54dd	4.40dd	4.50dd	d	4.68dd
(11a,11b)	(8.0)	(9.0)	(8.0)	(8.5)	-	(8.5)
11b	4.29dd	4.06dd	4.22dd	3.98dd	d	4.23dd
1"a	3.83bd	3.74dd	3.72dd	c	3.88dd	3.89dd
(1"a,1"b)	-	(10.5)	(10.5)	-	(12.0)	(12.0)
(1"a,2")	(3.5)	(3.0)	(1.5)	(1.5)	(1.5)	(1.5)
1"b	3.83bd	3.70dd	3.64dd	c	3.65dd	3.67dd
(1"b,2")	(3.5)	(5.0)	(4.5)	(4.0)	(5.6)	(5.5)
2"	3.47dd	3.51ddd	3.46ddd	3.43ddd	-	f
(2",3")	(9.5)	(9.0)	(9.0)	(9.0)	-	-
3"	3.51dd	3.55dd	3.55dd	c	e	f
(3",4")	(9.5)	(9.0)	(9.0)	-	-	-
4"	3.65dd	3.68dd	3.66dd	c	e	f
(4",5")	(9.5)	(9.0)	(9.0)	-	-	-
5"	3.21dd	3.25dd	3.52dd	c	e	f
(5",6")	(9.5)	(9.0)	(9.0)	(9.0)	(9.0)	(9.0)
6"	3.29ddd	3.32ddd	3.40ddd	3.39ddd	3.40ddd	3.45ddd
(6",7"a)	(5.0)	(5.0)	(3.5)	(4.0)	(1.5)	(2.5)
(6",7"b)	(9.5)	(9.0)	(2.0)	(2.0)	(5.6)	(5.5)
7"a	4.16dd	4.15dd	3.88dd	c	3.97dd	3.93dd
(7"a,7"b)	(10.5)	(10.5)	(11.0)	-	(11.0)	(11.0)
7"b	3.47dd	3.48dd	3.75dd	c	3.83dd	3.79dd

a. For solutions $CDCl_3$, except **9a** and **10a** which are in CD_3OD.
b. These protons are magnetically equivalents.
c. Overlapping 3.58-3.77 ppm.
d. Overlapping 4.38-4.44 ppm.
e. Overlapping 3.22-3.33 ppm.
f. Overlapping 3.26-3.40 ppm.

In order to obtain insight on the mechanisms of formation of the 4α and 4β isomers **7a, 7b, 8a** and **8b**, the 4-β isolated pure compounds were submitted to the action of $BF_3.Et_2O$ or of the same Lewis acid in the presence of the appropriate benzylated alditol **4** or **5**; however in all cases untreatable mixtures of decomposition compounds were

TABLE 2. ^{1}H NMR[a] chemical shifts δ_H in ppm and coupling constants J in Hz for compounds 2b, 3b, 7b, 8b, 9b, 10b

Proton (J)	2b	3b	7b	8b	9b	10b
2	3.31dd	2.77dd	3.32dd	2.72dd	3.39dd	3.01dd
(1,2)	(5.0)	(5.0)	(5.5)	(4.5)	(5.5)	(4.5)
(2,3)	(14.0)	(14.0)	(14.0)	(14.0)	(14.0)	(14.0)
3	2.83dddd	2.88dddd	2.79dddd	2.84dddd	2.92dddd	2.89dddd
(3,4)	(3.5)	(10.0)	(3.5)	(10.0)	(3.5)	(9.5)
(3,11a)	(8.0)	(7.0)	(10.5)	(7.0)	(8.0)	(7.0)
(3,11b)	(10.5)	(10.0)	(8.0)	(10.5)	(10.5)	(10.5)
4	4.40d	4.65d	4.34d	4.54d	4.55d	4.70d
11a	4.30dd	4.53dd	4.27dd	4.52dd	4.40dd	4.67dd
(11a,11b)	(8.0)	(9.0)	(8.0)	(9.0)	(8.0)	(8.5)
11b	4.27dd	4.06dd	4.24dd	3.99dd	4.32dd	4.19dd
1"a	3.81ddd	3.62ddd	3.00ddd	c	3.91ddd	d
(1"a,1"b)	(9.0)	(9.0)	(8.5)	-	(9.0)	-
(1"a,2"a)	(9.0)	(5.5)	(8.5)	(8.5)	(9.0)	(9.0)
(1"a,2"b)	(5.5)	(4.5)	(6.5)	(5.0)	(5.5)	(5.0)
1"b	3.64ddd	3.58ddd	3.61ddd	c	3.74ddd	d
(1"b,2"a)	(6.5)	(9.0)	(7.5)	(7.5)	(6.5)	(6.5)
(1"b,2"b)	(5.5)	(4.5)	(5.0)	(5.0)	(4.5)	(5.0)
2"a	2.12dddd	2.19dddd	2.10dddd	2.18dddd	2.15dddd	2.28dddd
(2"a,2"b)	(14.0)	(14.0)	(13.5)	(14.0)	(14.0)	(14.0)
(2"a,3")	(3.5)	(3.5)	(2.5)	(2.5)	(2.5)	(2.5)
2"b	1.71dddd	1.75dddd	1.64dddd	1.70dddd	1.64dddd	1.70dddd
(2"b,3")	(8.0)	(8.0)	(9.0)	(9.0)	(9.5)	(9.0)
3"	3.32ddd	3.45ddd	3.29ddd	3.39ddd	3.21ddd	3.35ddd
(3",4")	(9.0)	(9.5)	(9.0)	(9.0)	(9.5)	(9.0)
4"	3.28dd	3.31dd	3.25dd	3.29dd	3.05dd	3.09dd
(4",5")	(9.0)	(9.5)	(9.0)	(9.0)	(9.5)	(9.0)
5"	3.58dd	3.64dd	b	3.68dd	3.31dd	3.33dd
(5",6")	(9.0)	(9.5)	-	(9.0)	(9.5)	(9.0)
6"	3.20dd	3.22dd	b	c	3.27dd	3.29dd
(6",7")	(9.0)	(9.5)	(9.0)	(9.0)	(9.5)	(9.0)
7"	3.17ddd	3.25ddd	3.32ddd	3.35ddd	3.12ddd	3.18ddd
(7",8"a)	(4.5)	(4.5)	(3.0)	(2.5)	(2.5)	(2.5)
(7",8"b)	(10.0)	(9.5)	(3.0)	(3.5)	(5.5)	(5.5)
8"a	4.11dd	4.06dd	b	c	3.85dd	3.78dd
(8"a,8"b)	(10.5)	(9.5)	-	-	(12.0)	(12.0)
8"b	3.44dd	3.51dd	b	c	3.65dd	3.63dd

a. For solutions $CDCl_3$, except **9b** and **10b** which are in CD_3OD.
b. Overlapping 3.59-3.68 ppm.
c. Overlapping 3.57-3.65 ppm.
d. Overlapping 3.70-3.78 ppm.

obtained. Thus complex mechanisms appear responsible for the formation of the 4-α and 4-β isomers in the reaction.

EXPERIMENTAL

General Procedures. All melting points (mps) are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM-500 instrument for solutions in $CDCl_3$ or CD_3OD and are reported in δ units relative to $CHCl_3$ fixed at 7.24 ppm or to CH_3OD fixed at 3.31 ppm. Mass spectra were determined on a Varian 112 S mass spectrometer by direct inlet. Routine optical rotations were recorded with a Perkin-Elmer 141 spectropolarimeter; $[\alpha]^t_D$ values are given in degrees. TLC was performed on precoated silica gel G plates (E. Merck, HF_{254}), visualized by spraying with 70% sulphuric acid, followed by heating. Column chromatography was performed by Still's method (flash chromatography).[19]

HPLC analyses were performed on a Jasco twinkle pump system and on a Uvidec 100 II. The analyses were carried out on a reverse-phase Lichrosorb C-18 column (3 μm; 4 x 250 mm; Merck), using as solvent system the reported mixtures; the flow rate was 1 mL min^{-1} and detection was performed at 254 nm. For compounds **7a-b** and **8a-b** the eluting system was MeOH-H_2O 90:10 v/v and retention times (R_t), were related to 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose. For compounds **2a-c, 3a-c, 9a-b** and **10a-b** the eluting system was CH_3CN-AcOH-H_2O 35:1:70 v/v/v and the R_t was related to etoposide (**1**). The progress of all reactions and column chromatography was monitored by TLC and by HPLC.

The usual work-up refers to washing the organic layer with water, drying over Na_2SO_4, and removing the solvent under reduced pressure.

Synthesis of 2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-D-*glycero*-D-*gulo*-heptitol (4). 2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose (10 g, 18.5 mmol) in dichloromethane (50 mL) was reacted with trifluoroacetic anhydride (3.76 mL, 27.8 mmol) for 30 min at room temperature. The solvent, the excess anhydride and the formed trifluoroacetic acid were evaporated under reduced pressure and the residue in dichloromethane (50 mL) was reacted with trimethylsilyl cyanide (8.88 mL, 64.8 mmol) in the presence of $BF_3.Et_2O$ (1.16 mL, 9.4 mmol) for 30 min at 0 °C under argon. Usual work-up followed and column chromatography (dichloromethane-hexane 70:30) afforded first 2,3,4,6-tetra-*O*-benzyl-1-deoxy-1-cyano-α-D-glucopyranose (4.56 g, 45%), as an oil: $[\alpha]_D^{23}$ + 28.7 ($CHCl_3$, *c* 1) [lit.,[20] $[\alpha]_D$ + 37].

Further elution yielded 2,3,4,6-tetra-*O*-benzyl-1-deoxy-1-cyano-β-D-glucopyranose (3.86 g, 38%), mp 85.5-86 °C (from MeOH); $[\alpha]^{23}_D$ + 24.5 ($CHCl_3$, *c* 1) [lit.,[20]

mp 76-78 °C, $[\alpha]_D$ + 29]; ^{1}H NMR ($CDCl_3$) δ 3.41 (1H, ddd, $J_{5,6a}$ = 2.3, $J_{5,6b}$ = 4.0 and $J_{5,4}$ = 9.0 Hz, H-5), 3.59 (1H, dd, $J_{3,2}$ = 9.5 and $J_{3,4}$ = 9.5 Hz, H-3), 3.65 (1H, dd, $J_{4,3}$ = 9.5 and $J_{4,5}$ = 9.5 Hz, H-4), 3.67 (1H, dd, $J_{6b,5}$ = 4.0 and $J_{6b,6a}$ = 11.0 Hz, H-6b), 3.71 (1H, dd, $J_{6a,5}$ = 2.3, and $J_{6a,6b}$ = 11.0 Hz, H-6a), 3.77 (1H, dd, $J_{2,1}$ = 9.8 and $J_{2,3}$ = 9.5 Hz, H-2), 4.05 (1H, d, $J_{1,2}$ = 9.8 Hz, H-1), 4.51-4.93 (8H, 8 x d, J = 10-12 Hz, 8 x OC*H*Ph) and 7.10-7.36 (20H, m, aromatics).

2,3,4,6-Tetra-*O*-benzyl-1-deoxy-1-cyano-ß-D-glucopyranose (3.5 g) was treated with sodium methoxide in methanol (200 mL, 3.10^{-2}M) at room temperature for 3 h. The solution was acidified with aqueous HCl (2 M), the solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate. Work-up followed by crystallization afforded methyl 2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-D-*glycero*-D-*gulo*-heptanoate (3.2 g, 85%), mp 75-76 °C (from MeOH); $[\alpha]^{23}_D$ + 11.2 ($CHCl_3$, *c* 1); ^{1}H NMR ($CDCl_3$) δ 3.50 (1H, ddd, $J_{5,6a}$ = 2.0, $J_{5,6b}$ = 5.0 and $J_{5,4}$ = 10.0 Hz, H-5), 3.61 (1H, dd, $J_{4,3}$ = 10.0 and $J_{4,5}$ = 10.0 Hz, H-4), 3.67 (1H, dd, $J_{6b,5}$ = 5.0 and $J_{6b,6a}$ = 12.0 Hz, H-6b), 3.71 (1H, dd, $J_{3,2}$ = 10.0 and $J_{3,4}$ = 10.0 Hz, H-3), 3.71 (3H, s, OCH_3), 3.71 (1H, dd, $J_{6a,5}$ = 2.0 and $J_{6a,6b}$ = 12.0 Hz, H-6a), 3.80 (1H, dd, $J_{2,1}$ = 10.0 and $J_{2,3}$ = 10.0 Hz, H-2), 3.89 (1H, d, $J_{1,2}$ = 10.0 Hz, H-1), 4.52, 4.54, 4.58, 4.59, 4.76, 4.80, 4.86, 4.89 (8H, 8 x d, J = 11-12 Hz, 8 x OC*H*Ph) and 7.05-7.35 (20H, m, aromatics).

Methyl 2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-D-*glycero*-D-*gulo*-heptanoate (3 g, 5.15 mmol) dissolved in anhydrous diethyl ether (100 mL) was added to a $LiAlH_4$ (200 mg, 5.3 mmol) solution in anhydrous diethyl ether (50 mL) at 0 °C. After 30 min at room temperature the excess hydride was destroyed by addition of ethyl acetate and water. Usual work-up and crystallisation afforded 2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-D-*glycero*-D-*gulo*-heptitol (**4**) (2.58 g, 90%), mp 92-93 °C (from diisopropyl ether); $[\alpha]^{23}_D$ + 13 ($CHCl_3$, *c* 1); [lit.,[21] mp 93-95 °C; $[\alpha]_D$ + 8.6 ($CHCl_3$, *c* 1.1)]; ^{1}H NMR ($CDCl_3$) δ 2.09 (1H, dd, J = 6.5 and J = 6.5 Hz, OH), 3.35 (1H, ddd, $J_{2,1a}$ = 2.5, $J_{2,1b}$ = 5.0 and $J_{2,3}$ = 9.0 Hz, H-2), 3.47 (1H, ddd, $J_{6,7b}$ = 2.0, $J_{6,7a}$ = 4.3 and $J_{6,5}$ = 10.0 Hz, H-6), 3.55 (1H, dd, $J_{3,2}$ = 9.0 and $J_{3,4}$ = 9.0 Hz, H-3), 3.58 (1H, dd, $J_{5,4}$ = 9.0 and $J_{5,6}$ = 9.0 Hz, H-5), 3.67 (1H, dd, $J_{7a,6}$ = 4.3 and $J_{7a,7b}$ = 11.0 Hz, H-7a), 3.68 (1H, ddd, $J_{1b,OH}$ = 5.0, $J_{1b,2}$ = 5.0 and $J_{1b,1a}$ = 12.0 Hz, H-1b), 3.70 (1H, dd, $J_{7b,6}$ = 2.0 and $J_{7b,7a}$ = 11.0 Hz, H-7b), 3.71 (1H, dd, $J_{4,3}$ = 9.0 and $J_{4,5}$ = 9.0 Hz, H-4), 3.86 (1H, ddd, $J_{1a,2}$ = 2.5, $J_{1a,OH}$ = 5.0 and $J_{1a,1b}$ = 12.0 Hz, H-1a), 4.51, 4.54, 4.56, 4.65, 4.81, 4.85, 4.88, 4.91 (8H, 8 x d, J = 11-12 Hz, 8 x OC*H*Ph) and 7.13-7.33 (20H, m, aromatics).

General procedure for the synthesis of compounds 7a-b and 8a-b. The appropriate benzylated anhydro alditol (1.5 mmol) and 4'-demethylepipodophyllotoxin (**6**, 1 mmol) dissolved in dichloromethane (100 mL) were treated with $BF_3.Et_2O$ at the temperature and for the time required under the best conditions for obtaining each

compound. The reaction mixture was poured into a saturated $NaHCO_3$ aqueous solution and worked-up to afford, after a flash chromatography (eluting with dichloromethane-ethyl acetate 100:10) a mixture of the 4α and 4β epimers in each series (**7a, 8a** or **7b, 8b**). Additional chromatography (eluting with hexane-ethyl acetate 60:40) afforded each pure epimer. In the following paragraphs we report the synthesis of each compound under the best conditions.

i) 4'-*O*-Demethyl-4-(2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-D-*glycero*-D-*gulo*-heptitolyloxy)-4-desoxyepipodophyllotoxin (7a). A lditol **4** (830 mg, 1.5 mmol) in the presence of $BF_3.Et_2O$ (62 µL, 0.5 mmol), at 0 °C, for 7 h, afforded after the first chromatography a mixture (586 mg) of **7a** and **8a** in 95:5 ratio. Subsequent chromatographic separation afforded first the α-epimer **8a** (28 mg, 3%), R_t 1.54; a glass; $[\alpha]^{23}_D$ - 59 ($CHCl_3$, *c* 1); m/z 382 (48%), 298 (65), 181 (100).

Anal. Calcd for $C_{56}H_{56}O_{13}$: C, 71.78; H, 6.02. Found: C, 71.43; H, 6.21.

Further elution afforded the 4β-epimer **7a** (540 mg, 58%), R_t 1.67; mp 161-163 °C (from methanol); $[\alpha]^{23}_D$ - 44.5 ($CHCl_3$, *c* 1); m/z 382 (92%), 298 (18), 181 (100).

Anal. Calcd for $C_{56}H_{56}O_{13}$: C, 71.78; H, 6.02. Found: C, 71.57; H, 6.15.

ii) 4'-*O*-Demethyl-4-(2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-D-*glycero*-D-*gulo*-heptitolyloxy)-4-desoxypodophyllotoxin (8a). Alditol **4** (830 mg, 1.5 mmol) in the presence of $BF_3.Et_2O$ (0.62 mL, 5 mmol), at 0 °C, for 2 h, afforded first a mixture (702 mg) of **7a** and **8a** in 19:81 ratio, and, after separation, the 4α-epimer **8a** (560 mg, 60%) and the 4β-epimer **7a** (130 mg, 14%), both identical with those described above.

iii) 4'-*O*-Demethyl-4-(3,7-anhydro-2-deoxy-4,5,6,8-tetra-*O*-benzyl-D-*glycero*-D-*gulo*-octitolyloxy)-4-desoxyepipodophyllotoxin (7b). Alditol **5**[22] (850 mg, 1.5 mmol) in the presence of $BF_3.Et_2O$ (0.124 mL, 1 mmol), at - 20 °C, for 21 h, afforded after the first chromatography a mixture (524 mg) of **7b** and **8b** in 95:5 ratio. Subsequent chromatographic separation afforded first the α-epimer **8b** (27 mg, 3%), R_t 1.67; a glass; $[\alpha]^{23}_D$ - 53 ($CHCl_3$, *c* 1); m/z 382 (59%), 298 (75), 181 (100).

Anal. Calcd for $C_{57}H_{58}O_{13}$: C, 71.98; H, 6.15. Found: C, 72.12; H, 6.23.

Further elution afforded the 4β-epimer **7b** (490 mg, 52%), R_t 1.87; mp 108-110 °C (from dichloromethane-diisopropyl ether); $[\alpha]^{23}_D$ - 49 ($CHCl_3$, *c* 1); m/z 382 (100%), 298 (31), 181 (98).

Anal. Calcd for $C_{57}H_{58}O_{13}$: C, 71.98; H, 6.15. Found: C, 71.83; H, 6.33.

iv) 4'-*O*-Demethyl-4-(3,7-anhydro-2-deoxy-4,5,6,8-tetra-*O*-benzyl-D-*glycero*-D-*gulo*-octitolyloxy)-4-desoxypodophyllotoxin (8b). Alditol **5**[22] (850 mg, 1.5 mmol) in the presence of $BF_3.Et_2O$ (0.372 mL, 3 mmol), at 0 °C, for 2 h, afforded first a mixture (510 mg) of **7b** and **8b** in 13:87 ratio, and after separation the 4α-epimer **8b** (428 mg, 45%) and the 4β-epimer **7b** (63 mg, 7%), both identical with those described above.

Reductive debenzylation - General procedure. The benzylated compounds **7a-b** or **8a-b** (1 mmol) in methanol-acetone (300 mL, 1:1) were hydrogenated over 10% palladium on carbon (300 mg). After 10 h the hydrogen uptake ceased, and the mixture was filtered and evaporated. Concentration under reduced pressure afforded the debenzylated compound in nearly quantitative yields.

i) 4'-*O*-Demethyl-4-(2,6-anhydro-D-*glycero*-D-*gulo*-heptitol-1-yloxy)-4-desoxy-epipodophyllotoxin (9a), R_t 0.47; mp 212-214 °C (from methanol-ethyl acetate); $[\alpha]^{23}_D$ - 66 ($MeOH:H_2O$, 9:1, *c* 0.5); m/z 576 (M^+, 1%), 382 (100), 298 (5).

Anal. Calcd for $C_{28}H_{32}O_{13}$: C, 58.33; H, 5.59. Found: C, 58.05; H, 5.40.

ii) 4'-*O*-Demethyl-4-(2,6-anhydro-D-*glycero*-D-*gulo*-heptitol-1-yloxy)-4-desoxy-podophyllotoxin (10a), R_t 0.52; mp 95-96 °C (decomp; from methanol); $[\alpha]^{23}_D$ - 80 (MeOH, *c* 1); m/z 576 (M^+, 5%), 382 (96), 298 (100).

Anal. Calcd for $C_{28}H_{32}O_{13}$: C, 58.33; H, 5.59. Found: C, 58.20; H, 5.62.

iii) 4'-*O*-Demethyl-4-(3,7-anhydro-2-deoxy-benzyl-D-*glycero*-D-*gluco*-octitol-1-yloxy)-4-desoxyepipodophyllotoxin (9b), R_t 0.49; mp 227-230 °C (from ethyl acetate-methanol); $[\alpha]^{23}_D$ - 63 (MeOH, *c* 1); m/z 590 (M^+, 0.1%), 382 (100), 298 (11).

Anal. Calcd for $C_{29}H_{34}O_{13}$: C, 58.98; H, 5.80. Found: C, 58.65; H, 5.63.

iv) 4'-*O*-Demethyl-4-(3,7-anhydro-2-deoxy-D-*glycero*-D-*gluco*-octitol-1-yloxy)-4-desoxypodophyllotoxin (10b), R_t 0.57; mp 117 °C (decomp; from ethyl acetate); $[\alpha]^{23}_D$ - 93 (MeOH, *c* 1); m/z 590 (M^+, 2%), 382 (100), 298 (80).

Anal. Calcd for $C_{29}H_{34}O_{13}$: C, 58.98; H, 5.80. Found: C, 58.59; H, 5.94.

Acetalisation - General procedure. To a mixture of the appropriate hydroxy compound **9a-b** or **10a-b** (1 mmol) and 1,1-dimethoxyethane (4 mL) in nitromethane (20 mL) was added *p*-toluenesulphonic acid monohydrate (20 mg) and the mixture was stirred at room temperature for 2 h. Usual work-up and rapid chromatography afforded the corresponding ethylidene derivatives **2a-b** or **3a-b**.

i) 4'-*O*-Demethyl-4-(2,6-anhydro-5,7-*O*-ethylidene-D-*glycero*-D-*gulo*-heptitol-1-yloxy)-4-desoxyepipodophyllotoxin (2a) was obtained in 63% yield, R_t 1.02; mp 213-217 °C (from diisopropyl ether); $[\alpha]^{23}_D$ - 76 ($CHCl_3$, *c* 1); m/z 602 (M^+, 1%), 382 (100), 298 (6).

Anal. Calcd for $C_{30}H_{34}O_{13}$: C, 59.80; H, 5.69. Found: C, 59.62; H, 5.63.

ii) 4'-*O*-Demethyl-4-(2,6-anhydro-5,7-*O*-ethylidene-D-*glycero*-D-*gulo*-heptitol-1-yloxy)-4-desoxypodophyllotoxin (3a) was obtained in 64% yield, R_t 1.22; mp 154-156 °C (145 °C softens; from diisopropyl ether); $[\alpha]^{23}_D$ - 101 ($CHCl_3$, *c* 1); m/z 602 (M^+, 8%), 382 (100), 298 (98).

Anal. Calcd for $C_{30}H_{34}O_{13}$: C, 59.80; H, 5.69. Found: C, 59.57; H, 5.82.

iii) 4'-*O*-Demethyl-4-(3,7-anhydro-2-deoxy-6,8-*O*-ethylidene-D-*glycero*-D-*gulo*-octitol-1-yloxy)-4-desoxyepipodophyllotoxin (2b) was obtained in 65% yield, R_t 1.22; mp 187-189 °C (from dichloromethane-diisopropyl ether); $[\alpha]^{23}_D$ - 88 ($CHCl_3$, *c* 1); m/z 616 (M^+, 1%), 382 (100), 298 (9).

Anal. Calcd for $C_{31}H_{36}O_{13}$: C, 60.38; H, 5.88. Found: C, 60.05; H, 5.63.

iv) 4'-*O*-Demethyl-4-(3,7-anhydro-2-deoxy-6,8-*O*-ethylidene-D-*glycero*-D-*gulo*-octitol-1-yloxy)-4-desoxypodophyllotoxin (3b) was obtained in 68% yield, R_t 1.34; mp 142-144 °C (from dichloromethane-diisopropyl ether); $[\alpha]^{23}_D$ - 116 ($CHCl_3$, *c* 1); m/z 616 (M^+, 3%), 382 (100), 298 (89).

Anal. Calcd for $C_{31}H_{36}O_{13}$: C, 60.38; H, 5.88. Found: C, 60.86; H, 5.78.

Epimerization of 2a and 3a - General procedure. The podophyllotoxin derivative **2a** or **3a** (0.5 mmol) was dissolved in piperidine (5 mL). After stirring at room temperature (24 h for **2a** and 7 h for **3a**) the mixture was poured in aqueous HCl (14 mL, 4 N) and extracted with a mixture of ethyl acetate-butanol (3:1). Usual work-up and rapid chromatography afforded the picropodophyllotoxin derivative **2c** or **3c**, respectively.

i) 4'-*O*-Demethyl-4-(2,6-anhydro-5,7-*O*-ethylidene-D-*glycero*-D-*gulo*-heptitol-1-yloxy)-4-desoxyepipicropodophyllotoxin (2c) was obtained in 92% yield, R_t 1.14; mp 132-136 °C (softens), 155-158 °C (melts) (from diisopropyl ether); ^1H NMR ($CDCl_3$, signals diagnostic for the structure) δ 3.01 (1H, dddd, $J_{3,11a}$ = 3.0, $J_{3,4}$ = 4.0, $J_{3,11b}$ = 7.0 and $J_{3,2}$ = 10.5 Hz, H-3), 3.25 (1H, dd, $J_{2,1}$ = 5.0 and $J_{2,3}$ = 10.5 Hz, H-2), 4.37 (1H, dd, $J_{11b,3}$ = 7.0 and $J_{11b,11a}$ = 9.5 Hz, H-11b), 4.41 (1H, dd, $J_{11a,3}$ = 3.0 and $J_{11a,11b}$ = 9.5 Hz, H-11a), 4.45 (1H, d, $J_{4,3}$ = 4.0 Hz, H-4), 6.40 (1H, s, H-8), 6.41 (2H, s, H-2' and H-6') and 6.83 (1H, s, H-5); m/z 602 (M^+, 1%), 382 (100), 298 (4).

Anal. Calcd for $C_{30}H_{34}O_{13}$: C, 59.80; H, 5.69. Found: C, 59.49; H, 5.58.

ii) 4'-*O*-Demethyl-4-(2,6-anhydro-5,7-*O*-ethylidene-D-*glycero*-D-*gulo*-heptitol-1-yl-oxy)-4-desoxypicropodophyllotoxin (3c) was obtained in 80% yield, R_t 1.03; mp 240-242 °C (decomp) (from diisopropyl ether); ^1H NMR ($CDCl_3$, signals diagnostic for the structure) δ 2.88 (1H, dddd, $J_{3,11b}$ = 3.0, $J_{3,4}$ = 6.5, $J_{3,11a}$ = 7.0 and $J_{3,2}$ = 10.5 Hz, H-3), 3.22 (1H, dd, $J_{2,1}$ = 5.0 and $J_{2,3}$ = 10.5 Hz, H-2), 4.18 (1H, d, $J_{4,3}$ = 6.5 Hz, H-4), 4.31 (1H, dd, $J_{11b,3}$ = 3.0 and $J_{11b,11a}$ = 9.5 Hz, H-11b), 4.38 (1H, dd, $J_{11a,3}$ = 7.0 and $J_{11a,11b}$ = 9.5 Hz, H-11b), 6.37 (1H, s, H-8), 6.40 (2H, s, H-2' and H-6') and 6.94 (1H, s, H-5); m/z 602 (M^+, 6%), 382 (100), 298 (68).

Anal. Calcd for $C_{30}H_{34}O_{13}$: C, 59.80; H, 5.69. Found: C, 59.92; H, 5.47.

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