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New Compounds Related to Podophyllotoxin and Congeners: Synthesis, Structure Elucidation and Biological Testing

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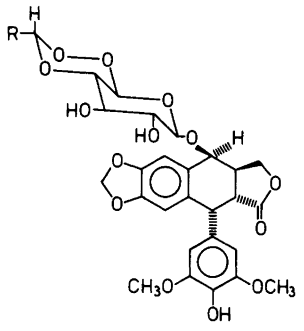
4-Azido, 4-amino, 4-amido and 4-alkoxy compounds related to the lignans podophyllotoxin and 4′-demethylepipodophyllotoxin have been synthesized, and their structures elucidated. The Ritter reaction was shown to be useful in the preparation of the 4-amido compounds with the required stereochemistry. A preparative method for 4-chloro-4-deoxypicrophyllotoxin, for which all earlier synthetic attempts resulted in the two dehydrated compounds, α- and β-apopicropodophyllotoxin, was developed. Supplementary preliminary studies of the biological activities of some of the compounds were performed. All compounds had pronounced inhibitory effect on the *in vitro* growth of human cervical cancer cells and TC-mouse cells with 4-amino-4-deoxypodophyllotoxin and 4-azido-4-deoxypodophyllotoxin showing the highest activity. Alkaline elution studies indicate that the toxicity of the 4′-demethoxy derivatives is due to protein-mediated DNA nicking. None of the compounds were found to have antiviral effect against herpes simplex type 2 (HSV-2), human immunodeficiency (HIV), and cytomegalovirus (CMV) in doses not toxic to the cells.

Podophyllotoxin (PT, **1a**, Table 1) has proved to be the most efficient chemotherapeutic agent for the treatment of venereal warts (*Condyloma acuminata*)[1] and it is a potent inhibitor of microtubule assembly. PT itself has too many side effects due to general toxicity, to be of clinical value in cancer chemotherapy,[2,3] but there has been considerable effort to synthesize derivatives with preferential cytotoxicity against tumor cells. This has led to two drugs, etoposid (VM 26) and vepesid (VP 16), Scheme 1 which are glucopyranosyl derivatives of 4′-demethylepipodophyllotoxin (*epi*DPT, **2c**).[4,5] The latter is an antitumor agent used for the treatment of testicular and small-cell lung cancer.[6] The cytostatic effect of compounds derived from **2c** that have a hydroxy group at the 4′-position and a bulky group at position 4β does not stem from inhibition of microtubule assembly but rather from protein-mediated DNA-breaking.[7–9] The structures of the PT congeners and derivatives have a pronounced influence on their toxicities, i.e., epimerization of PT at position 2 leads to the nearly inactive picropodophyllotoxin (PPT, **1b**).[10–13]

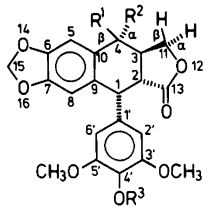


Vepeside (VP-16, R = CH_3)
Etopside (VP-26, R = S)

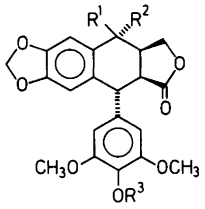
Scheme 1.

As a part of our attempts to develop new biologically active semisynthetic compounds from lignans, especially from PT and congeners, the 4-OH groups in PT, PPT and DPT were exchanged with nitrogen functional groups such as azido, amino and amido, Table 1, Scheme 2A, B, C. The epimeric compounds were synthesized by more

Table 1. The endpoint dose for inhibition of human cervix cancer cells MS 751 and the LD100 on TC mouse cells, and ^{1}H NMR data for derivatives of podophyllotoxin.

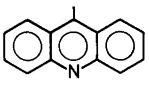
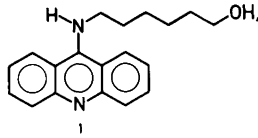


Podophyllotoxin configuration
1a–12a, 1c–12c[a]

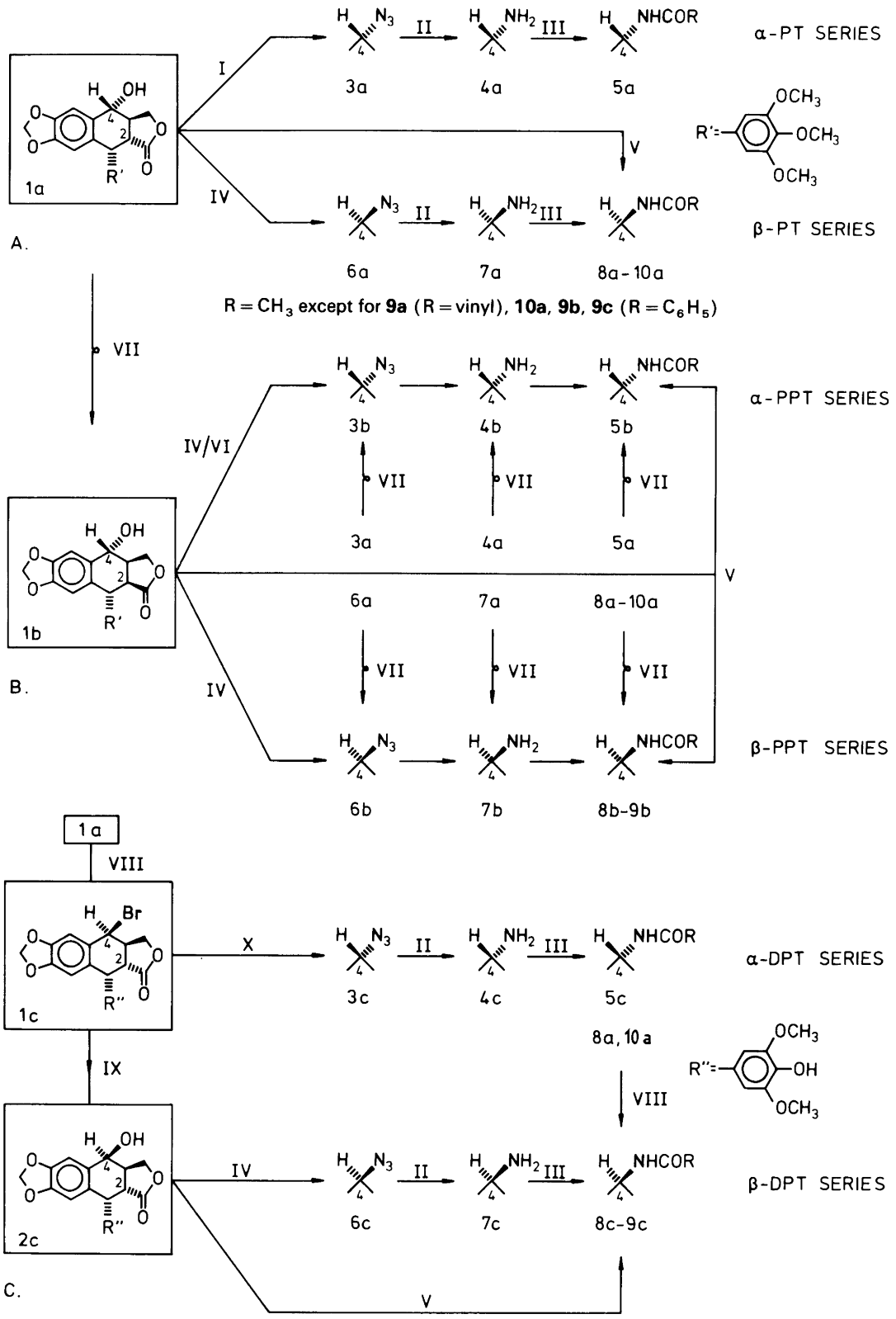


Picropodophyllotoxin configuration
1b–12b[a]

Compound[e]	R1(β)	R2(α)	R3	TC cell LD100 ng/ml	Endpoint dose for inhibition on MS 751 cells mmol	^{1}H NMR data $J_{3,4}$ Hz	δ (2-H, 5-H, 3-H)
1a[b]	H	OH	CH_3	1	0.03	9.0	2.84, 7.22
1b[b]	H	OH	CH_3	10	3	8.0	2.74
2a[c]	Br	H	CH_3			3.6	
2b[c]	Cl	H	CH_3				
3a	H	N_3	CH_3	0.001	1	9.7	2.83, 7.04
3b	H	N_3	CH_3	100	3	9.5	2.84
4a	H	NH_2	CH_3	1	0.003	8.3	2.83, 7.11
4b	H	NH_2	CH_3	100	3	4.7	2.45
5a	H	$NHCOCH_3$	CH_3			9.5	2.88, 6.82
5b	H	$NHCOCH_3$	CH_3	100	5	5.0	3.00
6a	N_3	H	CH_3			3.7	3.16, 6.78
6b	N_3	H	CH_3			4.6	3.15
7a	NH_2	H	CH_3			3.7	3.27, 6.81
7b	NH_2	H	CH_3			4.2	3.16
8a	$NHCOCH_3$	H	CH_3	100	2	ud	2.83, 6.77
8b	$NHCOCH_3$	H	CH_3			5.4	3.29
9a	$NHCOCH=CH_2$	H	CH_3	100	40	4.4	2.90, 6.78
9b	NHCOPh	H	CH_3			3.8	3.22
10a	NHCOPh	H	CH_3	30	2	4.3	2.92, 6.82 3.05
10b[b]	OH	H	CH_3			5.0	3.10
11a[b]	OH	H	CH_3			3.5	3.27, 6.88
11b[b]	H	OCH_2CH_3	CH_3			8.0	2.84
12a[b]	OCH_2CH_3	H	CH_3			3.5	3.45, 6.83
12b[b]	OCH_2CH_3	H	CH_3			4.5	3.12
1c	Br	H	H			3.4	3.36, 6.89
2c	OH	H	H	10	0.2	ud	3.27, 6.87
3c	H	N_3	H	100	0.04	9.8	3.39, 6.81
4c	H	NH_2	H			ud	2.78, 7.08
5c	H	$NHCOCH_3$	H			ud	2.85, 6.82
6c	N_3	H	H			3.0	3.15, 6.79
7c	NH_2	H	H			3.9	3.28, 6.80
8c	$NHCOCH_3$	H	H	10	2	4.2	2.90, 6.77
9c	NHCOPh	H	H	100	0.03	4.5	2.96, 6.82
10c[b]	H	OH	H			9.0	3.22, 7.17
11c[d]	$O(CH_2)NHAcr$	H	H	10	0.07	ud	3.35, 6.79
12c	H	OCH_2CH_3	H	100	40	8.0	3.30, 6.81
1					0.2		
2[e]					7		
3[e]							

[a] **1a–12a** refer to derivatives related to PT, **1b–12b** to PPT and **1c–12c** to DPT. [b] NMR data for PT (**1a**), PPT (**1b**), *epi*PT (**11a**), 4β-ethoxy-4-deoxyPT (**12a**), *epi*PPT (**10b**), 4α-ethoxy-4-deoxyPPT (**11b**), 4β-ethoxy-4-deoxyPPT (**12b**), DP (**10c**), Ref. 11. [c] Refers to the predominant species in a mixture of the α- and β-epimers. [d] Acr =  and **1** = 

. [e] 4′-Demethyl-β-*apo*PPT(2), α-*apo*PPT(3), Scheme 3. ud: undetermined.



Scheme 2A,B,C. Structure elucidation of 4-deoxy derivatives of podophyllotoxin (PT, **1a**), picropodophyllotoxin (PPT, **1b**) and 4′-demethylpodophyllotoxin (DPT, **1c**), Table 1.

than one method and from different starting materials. Together with H NMR spectral analysis, this served to elucidate the structures of all the compounds prepared. Determination of the structures is a necessary condition for understanding the relationship between the structure and biological activity. A one-step method based on the Ritter reaction for the preparation of the until now unknown amido compounds was developed. In addition some alkoxy derivatives of PT (**1a**) and 4′-demethylpodophyllotoxin (DPT, **10c**) were also synthesized.

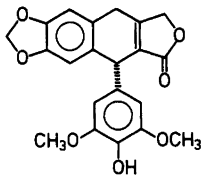
The inhibitory effect on the growth of colonies of human cervical cancer cells, herpes simplex type 2 virus (HSV-2), cytomegalovirus (CMV) and human immunodeficiency virus (HIV) and a murine cell line were evaluated for selected substances.

Results and discussion

Chemistry and Structure (Table 1, Scheme 2A, B, C). PT (**1a**) and PPT (**1b**) served as the starting material for the preparations of all the derivatives. The incorporation of the bromo, chloro, azido, amino and amido groups at the 4-position of PT and PPT followed standard procedures (Table 1, Scheme 2A, B, C). Phosphorus tribromide or thionyl chloride were successfully used as halogenating agents, and sodium azide as a nucleophile. In contrast with the findings of Hartwell *et al.*[14] who recorded that the action of phosphorus trichloride, thionyl chloride or acetyl chloride on PPT gave the dehydrated product α-apopicropodophyllotoxin (α-*apo*PPT, **3**, Scheme 3), we were able to obtain 4-chloro-4-deoxyPPT (**2b**) by brief treatment of **1b** with thionyl chloride at room temperature. The reaction of 4-bromo-4-deoxyPT (**2a**) led to a 4:1 mixture of 4α- and 4β-azido-4-deoxyPT (**3a**, **6a**) and for 4-chloro-4-deoxyPPT (**2b**) to a 3:1 mixture of 4α- and 4β-azido-4-deoxyPPT (**3b**, **6b**). In both cases the pure α-forms were obtained by recrystallization. Pure 4β-azido-4-deoxyPT (**6a**) was produced from the carbocation reaction of **1a** with NaN_3 and trifluoroacetic acid (TFA).[15] The 4β-azido-4-deoxyPPT (**6b**) was obtained by piperidine-catalyzed rearrangement of **6a**. In contrast with PT (**1a**) the NaN_3–TFA method used on PPT (**1b**) was of little use because the reaction led to a 2:5 mixture of 4α- and 4β-azido-4-deoxyPPT (**3b**, **6b**). The reduction of the azides to amines took place in ethanol at a slightly elevated temperature using Pd as a catalyst. In the formation of the amides of PT (**1a**), PPT (**1b**) and *epi*DPT (**2c**), we employed the Ritter reaction. Like the NaN_3–TFA method, the Ritter reaction with **1a** and **2c** led to the stereochemically pure 4β-amido compounds (**8a–10a**, **8c–9c**). With PPT (**1b**) the reaction was less stereospecific with acetonitrile than with benzonitrile, and resulted in a 2:1 mixture of 4α- and 4β-acetamido-4-deoxyPPT (**5b**, **8b**), and nearly pure 4β-benzamido-4-deoxyPPT (**9b**), respectively.

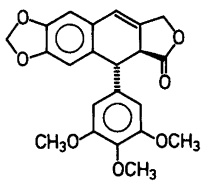
The preparation of the *epi*DPT (**2c**) was made by a modified version of Kuhn's method[16] (Scheme 2C). The reaction of **1a** with hydrogen bromide simultaneously *O*-demethylated at the 4′ position and generated the reactive 4β-bromo-4-deoxyDPT (**1c**) as the major product. The latter was easily converted into *epi*DPT (**2c**) or into the corresponding ethers 4β-[6-(9-acridinylamino)hexyloxy]-4-deoxy-DPT (**11c**) and 4α-ethoxy-4-deoxy-DPT (**12c**) by treatment with water, 9-(6-hydroxyhexylamino)-acridine (**1**, Table 1) or ethanol, respectively. The linking of 6-amino-1-hexanol to 9-aminoacridine to form 9-(6-hydroxyhexylamino)acridine (**1**) was carried out in phenol in accordance with Nielsen *et al.*[17] The 4α-azido-4-deoxyDPT (**3c**) was formed from **1c** by treatment with NaN_3 in DMF and the 4β-azido compound (**6c**) was also prepared from the *epi*DPT (**2c**) using the NaN_3–TFA method.[17] Successful *O*-demethylation of the acetamide or benzamide of **1a**, without disturbing the amido function, was also accomplished by the HBr method. Alternatively, all of the amides could be prepared by appropriate acylation of amino derivatives, which were formed as mentioned above by catalytic hydrogenation of the 4-azido compounds. The formation of 4′-demethyl-β-*apo*PPT (**2**, Scheme 3) could be accomplished either by dehydration of **2c**, or by prolonged heating of solutions of the ethyl ether of the DPTs (**12c**) in analogy with the reaction of the PT.[11]

Scheme 2A, B, C shows that all nitrogen compounds reported in this paper, are, by their chemistry, structurally interconnected. If only the configuration at C-4 is known or determined for one of these compounds, we know the configurations of them all since the absolute configuration of PT (**1a**), *epi*PT (**11a**), PPT (**1b**), and *epi*PPT (**10b**) are known.[18] It was possible, based on ^1H NMR data, to correlate ^1H NMR data for other derivatives of PT and their epimers with configuration (Table 1). The configuration at C-4 of the PT compounds was assigned on the basis of the chemical shift for the protons at C-2, C-5 and the coupling constants $J_{3,4}$. However, the latter in some cases were impossible to establish. The proton at C-5, showing a singlet around δ 6.8, was always less shielded



4′-Demethyl-β-apopicropodophyllotoxin

2



α-Apopicropodophyllotoxin

3

Scheme 3. 4′-Demethyl-β-*apo*PPT (**2**) and α-*apo*PPT (**3**).

in the 4β substituted than in the 4α-substituted compounds.[11] This difference is due to the anisotropic effect of the aromatic ring attached to the α- or β-substituted C-4.[12,13] It was also a constant feature that the C-2 proton was less shielded with a substituent in the 4α- than in the 4β-position. The coupling constants $J_{3,4} < 4.5$ Hz for the C-4β substituted compounds are in good agreement with the *cis* relationship between H-3 and H-4, whereas C-4α compounds, which have a *trans* relationship, have $J_{3,4} \geqslant 8.5$ Hz,[12,13] as found for PT (4α, **1a**) and *epi*PT (4β, **11a**).[11] The magnitude of $J_{3,4}$ was systematically larger in the PT system for the 4α- than for the 4β-epimer. This enabled us to elucidate the structures in this series, but could not be used to solve structures in the PPT system. The only general ^{1}H NMR feature found in the PPT system was that the proton at C-3 is less shielded in compounds with a substituent at the 4α than at the 4β position. Owing to a rather large variety of chemical shifts for the C-3 proton in different PPT compounds, it was necessary to synthesize both the 4α- and 4β-epimer of the 4-substituted PPT compound, in order to observe the significant difference in the H-3 chemical shifts of the two epimers to establish the structure. As shown in Scheme 2B, it is also possible to establish the structure of only one of the two PPT epimers from the corresponding PT epimer by transforming PT derivatives, through base-catalyzed C-2-epimerization, into their PPT analogues. We have consequently, on the basis of the chemistry shown in Scheme 2A, B, C and the analysis of the ^{1}H NMR data given above (Table 1), assigned all the structures shown in Scheme 2. Similar assignments have been undertaken for 4β-acrylamido(benzamido)-4-deoxyPT (**9a**, **10a**), 4β-benzamido-4-deoxyPPT (**9b**), 4β-benzamido-4-deoxy-DPT (**9c**) and 4β-[6-(9-acridinylamino)hexyloxy]-4-deoxy-DPT (**11c**), which all are derivatives not chemically connected with their corresponding amines (azides).

The Ritter reaction both on PT (**1a**) and *epi*DPT (**2c**) resulted only in the 4β-epimer. The epimer formed at C-4 seems to be directed by steric hindrance of the bulky pseudoaxial trimethoxyphenyl *E*-ring acting on the α-side of the C-4 carbocation of PT. In PPT (**1b**) the Ritter reaction resulted in a 2:1 formation of 4α- and 4β-acetamido-4-deoxyPPT (**5b**, **8b**). This agrees with the fact that the *E*-ring in the picro-system is pseudoequatorial and thereby gives rise to a lesser difference in steric hindrance on either side in the molecule. We believe that the preference for the 4α-epimer is caused by the steric hindrance by the lactone *D*-ring which bends toward the β-side of PPT (**1b**), which is especially pronounced in the carbocation conformation. As mentioned above, the α/β product ratio will be radically changed from 2:1 to 0:1 by an exchange of the sterically small methyl group (**8b**) in RCONH– with the sterically larger phenyl group (**9b**). Because of the similarity between the Ritter reaction and the NaN_3–TFA method, both of the carbocation type, the latter reaction likewise resulted in pure 4β-azido-4-deoxyPT (**6a**) and a mixture of 4α- and 4β-azido-4-deoxyPPT (**3b** and **6b**).

Biology. All the derivatives of PT showed a pronounced *in vitro* inhibitory effect on the growth of human cervical cancer cells MS 751 in a test designed for screening potentially antineoplastic compounds (Table 1). These compounds were likewise able to inhibit the growth of TC mouse cells in suspension culture (Table 1).

With the exception of the 4α-amino-4-deoxyPT (**4a**), which exhibited a tenfold higher activity than podophyllotoxin (**1a**), all other compounds were found less active on the human cell line. In the murine cell line the 4α-azido derivative of PT (**3a**) showed an extraordinarily high toxicity. Such unexpectedly high toxicity has been observed before for the 4β-phenylacetyl ester of DPT by Thurston *et al.*[19] In a toxicity assay with a monolayer murine 3T3 cell line, compound **3a** had no remarkable cytotoxicity (data not shown), indicating that suspension cells may be more sensitive than monolayer cells towards compound **3a**.

It has been demonstrated that the toxicity of the *epi*DPTs, especially compounds with a bulky group at C-4, is due to their interaction with topoisomerase-II, which results in protein-mediated DNA nicking, rather than the tubulin inhibition activity of PT.[8,12,13] In the present study it seems that the human cervix cancer cell line is more susceptible to the topoisomerase II mode of action than is the murine cell line, as demonstrated by the relatively higher activity of demethylated compounds **3c**, **9c** and **11c** in the MS 75I cell assay. These compounds were shown to produce protein associated DNA strand breaks by the alkaline elution technique [Fig. 1(a), (b)].

The relatively high toxicity of the DPT–acridine conjugate, **11c**, is interesting since the action mechanism of this compound may parallel that of *m*-AMSA Scheme 4. It is believed that *m*-AMSA, upon interfering with the function of topoisomerase II, interacts *both* with the topoisomerase (via the *m*-methoxysulfonamido moiety) and the DNA (via the acridine moiety) in a ternary complex.[20] Thus compound **11c** could be the first example of a new generation of DPT-derived topoisomerase inhibitors having the same dual mechanism of action as *m*-AMSA. Titration of **11c** with calf-thymus DNA gives rise to a 6 nm bathochromic shift and 27% hypochromicity of the 408 nm acridine absorption band which is indicative of DNA binding by intercalation, and alkaline elution results indicate that **11c** does indeed, as does *m*-AMSA, interfere with topoisomerase II. This aspect deserves further attention. Owing to the pronounced difference in sensitivity of the two cell lines used in the present study towards PT derivatives—most dramatically illustrated by the toxicity of compound **3a**—it is not sensible to engage in detailed structure–activity relationship discussions. On the other hand, compounds that exhibit such differentiated toxicity towards different cell-lines (types) deserve special attention since differential toxicity (towards malignant versus normal cells) is what is sought for in anticancer drugs. This aspect is now being pursued using a large variety of cell lines (types).

None of the compounds inhibited HSV-2, HIV or

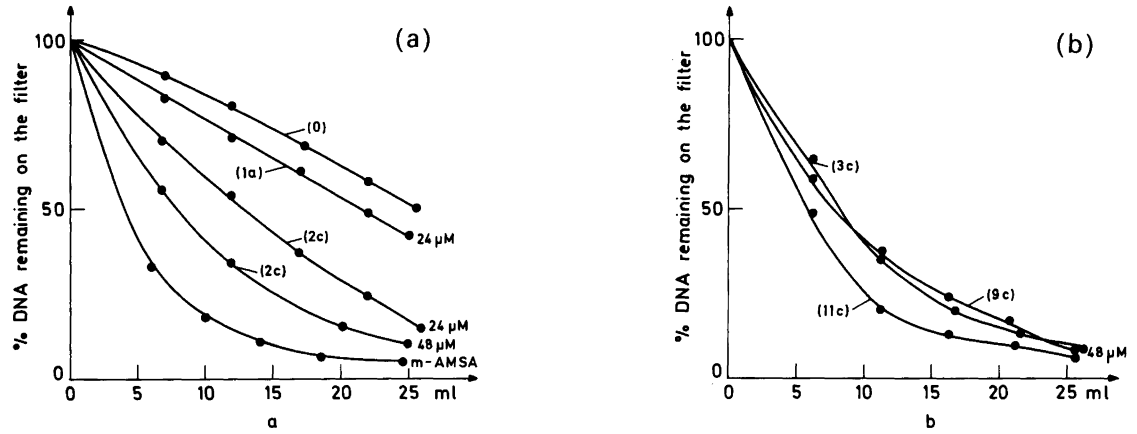
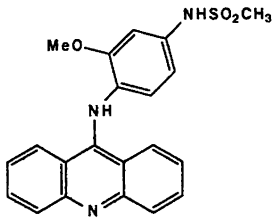


Fig. 1. Alkaline elution analysis of SEWA cells. The cells (10^6 in 1 ml of medium) were incubated for 60 min at 37°C in the presence of: (a) PT (**1a**) (24 μM); *m*-AMSA, [4-(9-acridinylamino)-*N*-(methanesulfonyl)-*m*-anisidine] (0.1 μg ml^{-1}); *epi*DPT (**2c**) (24,48 μM); without drug (0); (*b*) 4β-[6-(9-acridinylamino)hexyloxy]-4-deoxyDPT (**11c**); 4β-benzamido-4-deoxyDPT (**9c**) (48 μM); 4α-azido-4-deoxyDPT (**3c**) (48 μM). The cells were applied on the filters, lysed, heated with protease (0.1 mg ml^{-1}, 60 min), and the DNA was eluted at pH 12.1 as previously described.[24,25]



m-AMSA.

Scheme 4.

CMV infections in the highest dose not toxic to the target cells used in *in vitro* virus testing and thus they do not show any immediate promise as antiviral drugs.

In conclusion, methods for the preparation of 4α- and 4β-azido, amino- or amido- 4-deoxyPT (4-deoxyDPT) and their corresponding PPT-compounds have been developed of which the azido derivatives of the former exhibited high biological activity against various tumor cells. In the wider sense, the Ritter reaction opens up an easy way of preparing PTs substituted in the 4β-position with groups as –COOH, $-NH_2$, –NHCOR, –NHRCONHR′ and –NHCOSR. In addition it is worth mentioning that the well known DPT drugs, etoposide and vepesid, Scheme 1, used as mentioned above in cancer therapy are both substituted in the 4β-position.

Materials and methods

Podophyllotoxin (**1a**) {>98%, HPLC, m.p. 181–182°C, $[\alpha]_D^{20} - 131°$ (*c* 1, $CHCl_3$)} and picropodophyllotoxin (**1b**) {>99%, HPLC, m.p. 229–231°C, $[\alpha]_D^{20} + 4.5°$ (*c* 1, $CHCl_3$)} were donated by Nycomed-DAK A/S. All other chemicals for synthesis and analysis were commercially available. The ^1H NMR spectra were recorded on a Bruker 250 MHz spectrometer. IR spectra were recorded in KBr on a Perkin-Elmer 500 spectrometer and mass spectra on a Masslab V G, 12-250 instrument. HPLC chromatography was performed with a system consisting of a Kontron Analytical LC pump 414 and a Kontron Analytical UV Detector Model 750 with a Sperisorb S50DS2 column. The eluent was methanol–1% aq. KH_2PO_4 (6:4 v/v), the flow rate was 1 ml min^{-1} and the effluent was monitored at 252 nm.

Inhibition of Colony Formation in Soft Agar. A cell suspension of 3×10^5 MS 751 cervical cancer cells in 2 ml 0.4% agar in minimum essential medium (MEM) supplemented with 10% fetal calf serum was seeded on top of 5 ml 0.5% agar in the same medium (feeder layer). A 6 mm diameter filter-paper dish was placed on top and treated with 10 ml of the test compound in 99% ethanol. The dishes were sealed in a small box and incubated at 37°C to allow the individual cells to grow into colonies. After 12 days, the width of the colony-free zone around the filter paper—if any—was measured. Using triplicates of tenfold dilutions the lowest dose giving evidence of colony inhibition was determined. Positive and negative controls were included with each test.

Inhibition of Virus Infection. Herpes simplex virus (HSV-2) type 2 strain MS CATCC was plaque titered on MS 751 cells in 1 cm^2 tissue culture wells.[21] Human immunodeficiency virus (HIV) strain RF from Dr. Peter Nara, NCl, Frderick, MD, USA, was tested for syncytia plaque formation on CEM cells in 96-well microtiter dishes.[22] Multiplication of cytomegalovirus (CMV) strain AD 169 was determined by infection of subconfluent monolayers of MRC-5 (human embryo fibroblasts) in 1 cm^2 tissue culture wells and ELISA-testing for cell-associated CMV antigen on the 5th day after infection using antibodies to CMV harvested from hyperimmunized rhesus monkeys. All test compounds were first titered for *in vitro* cytotoxicity against target cells and the

highest non-cell-toxic doses were duplicated for effect on virus infection. The compounds were added to some cultures 24 h prior to infection in order to detect an interferon-like effect and added to other cultures 24 h after infection where virus multiplication was established. In both test situations the cells were exposed to the compounds for 24 h.

Toxicity assay with TC-SEWA cells. Suspension cultures of the SEWA cell line (a dedifferentiated osteosarcoma)[23] were grown in McCoy medium containing 10% foetal calf serum supplemented with glutamine, penicillin and streptomycin in a CO_2 incubator at 37°C.

Toxicity assays were done by adding the desired amount of drug to 200 ml cell culture (5×10^4 cells/ml) in the wells of microtitre plates. The drugs were tested in concentrations ranging from 10^{-4} to 10^{-10} M using ten-fold dilution steps and the concentration which caused complete inhibition of growth was scored after six–seven days, taking advantage of the fact that extensive cell growth results in acidification of the medium and thus gives a color shift of the pH indicator from red to yellow.

Synthesis

4α-Azido-4-deoxypodophyllotoxin (**3a**). *Method I (M I), Scheme 2A, B, C.* A solution of anhydrous **1a** (10 g, 24.1 mmol) in benzene (100 ml) was refluxed with PBr_3 (2.3 g, 8.5 mmol) for 1 h. The supernatant was decanted and the residue was washed with warm benzene (2 × 25 ml). The benzene was removed under reduced pressure. The resulting glassy material was taken up in warm benzene (50 ml), which, again, was removed under reduced pressure at 30°C. The last traces of benzene were removed by further heating to 70°C for 1.5 h under reduced pressure to leave *4-bromo-4-deoxypodophyllotoxin* (**2a**), Table 1 (11.1 g, 23.3 mmol, 96%) as a white foam which was used without further purification. To **2a** (11.1 g, 23.3 mmol, 96%) in *N,N*-dimethylformamide (DMF, 50 ml) was added NaN_3 (5 g, 76.9 mmol). The solution was stirred overnight. The reaction was followed by TLC (silica gel 60 Merck, 1:1 ethyl acetate–hexane, 1, R_f 0.1; 2, R_f 0.4). The solution was filtered to remove undissolved NaN_3 and the solvent removed under reduced pressure to leave an oil. The oil was washed with water (20 ml), and the remainder of the solvents was removed under reduced pressure at 70°C to give **3a** (4.2 g, 9.6 mmol, 91%) as a white foamy material; m.p. 185–186°C (decomp.) IR (KBr): 2100, 1785, 1580, 1490 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.83 (dd, 1 H, 2-H), 2.93 (m, 1 H, 3-H), 3.74 (s, 6 H, 3′,5′-OCH_3), 3.81 (s, 3 H, 4′-OCH_3), 4.05 (dd, 1 H, 11α-H), 4.30 (d, 1 H, 11β-H), 4.63 (d, 1 H, 1-H), 4.65 (d, 1 H, 4-H), 6.02 (d, 2 H, 15-H), 6.36 (s, 2 H, 2′,6′-H), 6.55 (s, 1 H, 8-H), 7.04 (s, 1 H, 5-H). Anal. $C_{22}H_{21}N_3O_7$: C, H, N. MS: *m/z* 439 (M^+).

4α-Amino-4-deoxypodophyllotoxin (**4a**). *M II.* To a solution of **3a** (2.5 g, 5.7 mmol) in ethanol (100 ml) was added Pd–C (0.5 g, 5%). The solution was stirred for 4 h under an atmosphere of hydrogen at 40°C. The solution was filtered and evaporated under reduced pressure. Recrystallization of the residue from ethanol gave **4a** (1.8 g, 4.4 mmol, 76%). M.p. 184–185°C. IR (KBr): 1770, 1590, 1485 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.61 (s, 2 H, NH_2), 2.55 (m, 1 H, 3-H), 2.83 (dd, 1 H, 2-H), 3.74 (s, 6 H, 3′,5′-OCH_3), 3.79 (s, 3 H, 4′-OCH_3), 4.20 (d, 2 H, 11α, 11β-H), 4.29 (d, 1 H, 4-H), 4.55 (d, 1 H, 1-H), 5.94 (s, 2 H, 15-H), 6.30 (s, 2 H, 2′,6′-H), 6.48 (s, 1 H, 8-H), (s, 1 H, 5-H). Anal. $C_{22}H_{23}NO_7 . 0.5\ H_2O$: C, H, N. MS: *m/z* 414 (M^+).

4α-Acetamido-4-deoxypodophyllotoxin (**5a**). *M III.* **4a** (1.03 g, 2.5 mmol) was dissolved in pyridine (15 ml) and acetic anhydride (2.7 mmol, 0.25 ml) was added at 0°C. The reaction mixture was stirred for 1 h at 0°C. The solvents were removed under reduced pressure at 60°C. The solid residue was stirred with water (100 ml) at room temperature for 24 h, then filtered and washed with water. Recrystallization from $CHCl_3$ gave **5a** (1.02 g, 2.2 mmol, 90%). M.p. 255–256°C. IR (KBr): 1777, 1674, 1589, 1466 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.09 (s, 3 H, CH_3), 2.63 (m, 1 H, 3-H), 2.88 (dd, 1 H, 2-H), 3.75 (s, 6 H, 3′,5′-OCH_3), 3.79 (s, 3 H, 4′-OCH_3), 4.18 (t, 1 H, 11α-H), 4.37 (dd, 1 H, 11β-H), 4.59 (d, 1 H, 1-H), 5.11 (t, 1 H, 4-H), 5.82 (d, 1 H, 4-NH), 5.96 (d, 2 H, 15-H), 6.36 (s, 2 H, 2′,6′-H), 6.52 (s, 1 H, 8-H), 6.82 (s, 1 H, 5-H). Anal. Found: C 62.64; H 5.50; N 3.03. Calc. for $C_{24}H_{25}NO_8$: C 63.31; H 5.50; N 3.08. MS: *m/z* 455 (M^+).

4β-Azido-4-deoxypodophyllotoxin (**6a**).[16] *M IV.* To **1a** (10.3 g, 25 mmol) and NaN_3 (8.3 g, 125 mmol) in $CHCl_3$ (100 ml) was added trifluoroacetic acid (TFA, 25 ml, 330 mmol) dropwise. The reaction mixture was stirred for 1 h, but in order to avoid gel formation during the reaction it was necessary to add further TFA (100 ml, 1.35 mol). The solution was neutralized with aqueous saturated $NaHCO_3$. The phases were separated. The aqueous phase was extracted twice with $CHCl_3$ (50 ml). The combined organic phases were washed with water and dried over Na_2SO_4. Evaporation of $CHCl_3$ and recrystallization from methanol (80%) gave **6a** (9.6 g, 22 mmol, 87%). M.p. 195–196°C. IR (KBr): 2104, 1770, 1588, 1506, 1487 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.89 (m, 1 H, 3-H), 3.16 (dd, 1 H, 2-H), 3.71 (s, 6 H, 3′,5′-OCH_3), 3.77 (s, 3 H, 4′-OCH_3), 4.27 (dd, 2 H, 11α, 11β-H), 4.60 (d, 1 H, 1-H), 4.75 (d, 1 H, 4-H), 5.98 (dd, 2 H, 15-H), 6.23 (s, 2 H, 2′,6′-H), 6.56 (s, 1 H, 8-H), 6.78 (s, 1 H, 5-H). Anal. $C_{22}H_{21}N_3O_7$: C, H, N. MS: *m/z* 439 (M^+).

4β-Amino-4-deoxypodophyllotoxin (**7a**). A solution of **6a** (4.4 g, 10 mmol) in ethanol (110 ml, abs.) and Pd–C (3 g, 10%) was hydrogenated as described for **4a**, *M II*, at 25°C for 8 h. After filtration, washing and evaporation, the remaining solid was recrystallized from ethanol to give **7a** (3.2 g, 7.8 mmol, 78%). M.p. 142–143°C. IR (KBr): 1772, 1589, 1505, 1482 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.01 (s, 2 H, NH_2), 2.82 (m, 1 H, 3-H), 3.27 (dd, 1 H,

2-H), 3.71 (s, 6 H, 3',5'-OCH_3), 3.77 (s, 3 H, 4'-OCH_3), 4.29 (m, 3 H, 1, 11α, 11β-H), 4.53 (d, 1 H, 4-H), 5.93 (d, 2 H, 15-H), 6.27 (s, 2 H, 2',6'-H), 6.47 (s, 1 H, 8-H), 6.81 (s, 1 H, 5-H). Anal. $C_{22}H_{23}NO_7 . H_2O$: C, H, N. MS: *m/z* 413 (M^+).

4β-Acetamido-4-deoxypodophyllotoxin (**8a**). *M V.* A solution of **1a** (5 g, 12.1 mmol) in acetonitrile (50 ml) was stirred and kept cool while conc. H_2SO_4 (3 ml, 54 mmol) acid was added. The mixture was heated to room temperature and after 1 h water (50 ml) containing crushed ice was added. After 1 h of stirring the solution was neutralized with solid $NaHCO_3$ and the solvents were removed under reduced pressure. The white foamy residue was dissolved in $CHCl_3$, filtered and kept in a refrigerator overnight whereupon **8a** (4.7 g, 10.3 mmol, 85%) also precipitated. **8a** was also prepared from **7a** (1.03 g, 25 mmol) as described for **5a**, *M III.* Recrystallization from $CHCl_3$ gave **8a** (1.05 g, 23 mmol, 92%). M.p. 216–217°C. IR (KBr): 1780, 1680, 1595, 1490 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.99 (s, 3 H, CH_3), 2.83 (dd, 1 H, 2-H), 2.91 (m, 1 H, 3-H), 3.70 (s, 6 H, 3',5'-OCH_3), 3.73 (s, 3 H, 4'-OCH_3), 3.78 (d, 1 H, 1-H), 4.45 (m, 2 H, 11α, 11β-H), 5.23 (d, 1 H, 4-H), 5.93 (d, 2 H, 15-H), 6.25 (s, 2 H, 2',6'-H), 6.46 (s, 1 H, 8-H), 6.77 (s, 1 H, 5-H). Anal. $C_{24}H_{25}NO_8$: C, H, N. MS: *m/z* 455 (M^+).

4β-Acrylamido-4-deoxypodophyllotoxin (**9a**) was prepared as above from **1a** (5.0 g, 12.3 mmol) and acrylonitrile according to **8a**, *M V.* The target compound **9a** remained dissolved in the excess of acrylonitrile. The solution was neutralized with $NaHCO_3$. The organic and aqueous phases were separated and washed with water and acrylonitrile, respectively. The organic phases were combined and evaporated to dryness. Recrystallization of the residue from ether gave **9a** (4.6 g, 9.8 mmol, 81%). M.p. 213°C. IR (KBr): 1775, 1670, 1585, 1500 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.87 (s, 1 H, NH), 2.90 (m, 2 H, 2,3-H), 3.73 (s, 6 H, 3',5'-OCH_3), 3.78 (s, 3 H, 4'-OCH_3), 4.45 (m, 3 H, 1, 11α, 11β-H), 4.71 (s, 2 H, $CH_2=C$), 5.30 (dd, 1 H, 4-H), 5.76 (dd, 1 H, C=CH), 5.95 (d, 2 H, 15-H), 6.27 (s, 2 H, 2',6'-H), 6.5 (s, 1 H, 8-H), 6.78 (s, 1 H, 5-H). Anal. $C_{25}H_{25}NO_8$: C, H, N. MS: *m/z* 467 (M^+).

4β-Benzamido-4-deoxypodophyllotoxin (**10a**) was prepared from **1a** (5.0 g, 12.1 mmol) and benzonitrile in analogy with **8a**, *M V.* Recrystallization from $CHCl_3$–ether gave **10a** (4.8 g, 9.3 mmol, 77%). M.p. 164–165°C. IR (KBr): 1770, 1640, 1580, 1480 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.92 (dd, 1 H, 2-H), 3.05 (m, 1 H, 3-H), 3.75 (s, 6 H, 3',5'-OCH_3), 3.79 (s, 3 H, 4'-OCH_3), 3.93 (m, 1 H, 1-H), 4.50 (m, 2 H, 11α, 11β-H), 5.35 (dd, 1 H, 4-H), 5.97 (d, 2 H, 15-H), 6.30 (s, 2 H, 2',6'-H), 6.46 (d, 1 H, NH), 6.53 (s, 1 H, 8-H), 6.82 (s, 1 H, 5-H), 7.7–8.3 (aromatic H). Anal. $C_{29}H_{27}NO_8$: C, H, N. MS: *m/z* 517 (M^+).

4α-Azido-4-deoxypicropodophyllotoxin (**3b**). *M VI.* To a solution of **1b** (4.0 g, 9.7 mmol) in chloroform (100 ml) was added thionyl chloride (4.0 ml, 55.6 mmol). The solution was stirred for 10 min at room temperature after which the solvents were removed under reduced pressure to leave *4-chloro-4-deoxypicropodophyllotoxin* (**2b**) (3.6, 9.1 mmol) as a white foam. **2b** (3.6 g, 9.1 mmol) was dissolved without further purification in DMF (40 ml). On addition of NaN_3 (4 g, 61.1 mmol) **3b** began to precipitate. The suspension was stirred at room temperature overnight after which ice–water (100 ml) was added. The suspension was filtered and washed with water and cold diethyl ether to leave **3b** (3.6 g, 8.2 mmol, 84%) on the filter.

M VII. A solution of **3a** (11.0 g, 25 mmol) and piperidine (0.10 ml) in ethanol (100 ml) was refluxed for 10 h. The solvent was removed under reduced pressure to give a white foamy material which after recrystallization from ethanol gave **3b** (9.7 g, 22.1 mmol, 88%). M.p. 185–186°C. IR (KBr): 2075, 1765, 1590, 1480 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.84 (m, 1 H, 3-H), 3.20 (dd, 1 H, 2-H), 3.79 (s, 6 H, 3',5'-OCH_3), 3.82 (s, 3 H, 4'-OCH_3), 4.31 (m, 3 H, 1, 4, 11α-H), 4.41 (dd, 1 H, 11β-H), 5.96 (dd, 2 H, 15-H), 6.36 (s, 2 H, 2',6'-H), 6.49 (s, 1 H, 8-H), 6.96 (s, 1 H, 5-H). Anal. $C_{22}H_{21}NO_3$: C, H, N. FAB MS: *m/z* 439 (M^+).

4α-Amino-4-deoxypicropodophyllotoxin (**4b**). **3b** (3.0 g, 6.8 mmol) was hydrogenated as described for **4a**, *M II*, to give **4b** (2.5 g, 5.9 mmol, 61%). M.p. 182–184°C. IR (KBr): 1770, 1585, 1480 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.58 (s, 2 H, NH_2), 2.45 (m, 1 H, 3-H), 3.20 (dd, 1 H, 2-H), 3.62 (d, 1 H, 1-H), 3.82 (s, 6 H, 3',5'-OCH_3), 3.86 (s, 3 H, 4'-OCH_3), 4.07 (d, 1 H, 11β-H), 4.45 (d, 1 H, 11α-H), 4.55 (d, 1 H, 4-H), 5.92 (q, 2 H, 15-H), 6.33 (s, 1 H, 8-H), 6.46 (s, 2 H, 2',6'-H), 7.04 (s, 1 H, 5-H). Anal. $C_{22}H_{23}NO_7 . 0.5\ H_2O$: C, H, N. MS: *m/z* 414 (M^+).

4α-Acetamido-4-deoxypicropodophyllotoxin (**5b**). **5b** was prepared in analogy with the preparation of **8a**, *M V*, using **1b** (5.2 g, 12.1 mmol) and acetonitrile. Recrystallization from acetone gave **5b** (4.8 g, 10.5 mmol, 87%). **5b** was also prepared from **4b** (1.03 g, 2.5 mmol) as described for **8a**, *M III.* Recrystallization from acetone gave **5b** (1.00 g, 2.3 mmol, 88%). **5a** (0.41 g, 10 mmol) was rearranged using piperidine as the catalyst as described for **3b**, *M VII*, to give **5b** (0.36 g, 7.9 mmol, 79%). M.p. 243–245°C. IR (KBr): 1770, 1655, 1591, 1481 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.74 (s, 3 H, $COCH_3$), 3.00 (m, 1 H, 3-H), 3.44 (dd, 1 H, 2-H), 3.81 (s, 6 H, 3',5'-OCH_3), 3.82 (s, 3 H, 4'-OCH_3), 4.19 (dd, 1 H, 11α-H), 4.42 (d, 1 H, 1-H), 4.46 (dd, 1 H, 11β-H), 4.86 (dd, 1 H, 4-H), 5.35 (d, 1 H, NH), 5.98 (dd, 2 H, 15-H), 6.41 (s, 2 H, 2',6'-H), 6.63 (s, 1 H, 8-H), 6.79 (s, 1 H, 5-H). Anal. $C_{24}H_{25}NO_8$: C, H, N. MS: *m/z* 455 (M^+).

4β-Azido-4-deoxypicropodophyllotoxin (**6b**). **6b** was prepared from **1b** (1.1 g, 2.5 mmol), TFA and NaN_3 as described for **6a**, *M IV*, which gave a mixture of **6b** and **3b** (1.07 g, 2.42 mmol) in the ratio 2:1, as shown by HPLC.

6b was also prepared from **6a** (11 g, 25 mmol) as described for **2b**, *M VI*. Recrystallization from ethanol gave **6b** (10 g, 23 mmol, 92%). M.p. 165–166°C. IR (KBr): 2106, 1771, 1505, 1484 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.15 (m, 1 H, 3-H), 3.37 (dd, 1 H, 2-H), 3.79 (s, 6 H, 3′,5′-OCH_3), 3.83 (s, 3 H, 4′-OCH_3), 4.23 (dd, 1 H, 11α-H), 4.32 (d, 1 H, 1-H), 4.40 (dd, 1 H, 11β-H), 4.68 (d, 1 H, 4-H), 5.96 (dd, 2 H, 15-H), 6.39 (s, 2 H, 2′,6′-H), 6.51 (s, 1 H, 8-H), 6.87 (s, 1 H, 5-H). Anal. $C_{22}H_{21}N_3O_7$: C, H, N. MS: *m/z* 439 (M^+).

4β-Amino-4-deoxypicropodophyllotoxin (**7b**). **6b** (4.4 g, 10 mmol) was hydrogenated as described for **4a**, *M II*, to give after recrystallization from ethanol, **7b** (2.6 g, 6.3 mmol, 63%). M.p. 172–173°C. IR (KBr): 1766, 1589, 1505, 1483 cm^{-1}. ^1H NMR : δ 3.16 (m, 1 H, 3-H), 3.46 (dd, 1 H, 2-H), 3.74 (s, 6 H, 3′,5′-OCH_3), 3.78 (s, 3 H, 4′-OCH_3), 4.15 (m, 2 H, 11α-H), 4.31 (t, 1 H, 11β-H), 4.45 (d, 1 H, 4-H), 5.92 (d, 2 H, 15-H), 6.32 (s, 2 H, 2′,6′-H), 6.61 (s, 1 H, 8-H), 6.91 (s, 1 H, 5-H). Anal. $C_{22}H_{23}NO_7$. 0.5 H_2O: C, H, N. FAB MS: *m/z* 413 (M^+).

4β-Acetamido-4-deoxypicropodophyllotoxin (**8b**). **8b** was prepared from **7b** (1.03 g, 25 mmol) as described for **5a**, *M III*. Recrystallization from acetone gave **8b** (1.01 g, 22 mmol, 88%). **8b** was also prepared from **8a** (0.41 g, 10 mmol) and piperidine (40 µl, 10.4 mmol) as described for **3b**, *M VII*. Recrystallization from acetone gave **8b** (0.38 g, 8.3 mmol, 83%). M.p. 174°C. IR (KBr): 1766, 1676, 1590, 1505, 1484 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.01 (s, 3 H, CH_3), 3.29 (m, 1 H, 3-H), 3.43 (dd, 1 H, 2-H), 3.78 (s, 6 H, 3′,5′-OCH_3), 3.80 (s, 3 H, 4′-OCH_3), 4.12 (dd, 1 H, 11α-H), 4.26 (t, 1 H, 11β-H), 4.44 (d, 1 H, 1-H), 5.37 (t, 1 H, 4-H), 5.91 (dd, 2 H, 15-H), 6.35 (s, 2 H, 2′,6′-H), 6.61 (s, 1 H, 8-H), 6.68 (d, 1 H, NH), 6.77 (s, 1 H, 5-H). Anal. $C_{24}H_{25}NO_8$. 0.5 H_2O: C, H, N. MS: *m/z* 455 (M^+).

4β-Benzamido-4-deoxypicropodophyllotoxin (**9b**). **9b** was prepared from **1b** (3.0 g, 7.2 mmol) and benzonitrile in analogy to **8a**, *M V*. Recrystallization from EtOH gave **9b** (3.2 g, 6.2 mmol, 86%). M.p. 301–302°C. IR (KBr): 1769, 1676, 1590, 1505, 1484 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.22 (m, 1 H, 3-H), 3.59 (dd, 1 H, 2-H), 3.71 (s, 6 H, 3′,5′-OCH_3), 3.81 (s, 3 H, 4′-OCH_3), 4.19 (dd, 1 H, 11α-H), 4.54 (d, 1 H, 1-H), 4.58 (dd, 1 H, 11β-H), 5.13 (dd, 1 H, 4-H), 5.98 (dd, 2 H, 15-H), 6.10 (d, 1 H, NH), 6.39 (s, 2 H, 2′,6′-H), 6.72 (s, 1 H, 8-H), 6.88 (s, 1 H, 5-H), 7.25–7.44 (aromatic H). Anal. $C_{29}H_{27}NO_8$: C, H, N. MS: *m/z* 517 (M^+).

4β-Bromo-4-deoxy-4′-demethylpodophyllotoxin (**1c**). *M VIII*. A solution of **1a** (10 g, 24.2 mmol) in 1,2-dichloroethane (200 ml) and diethyl ether (20 ml) was cooled to 0°C and saturated with hydrogen bromide. After 6 h the solvents were removed under reduced pressure to leave a pink foam. Recrystallization of this from acetone gave **1c** (7.2 g, 15.5 mmol, 64%). M.p. 156°C (decomp.) IR (KBr): 1760, 1610, 1480 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.90 (m, 1 H, 3-H), 3.36 (dd, 1 H, 2-H), 3.77 (s, 6 H, 3′,5′-OCH_3), 4.36 (d, 2 H, 11α, 11β-H), 4.66 (d, 1 H, 1-H), 5.61 (d, 1 H, 4-H), 5.98 (q, 2 H, 15-H), 6.28 (s, 2 H, 2′,6′-H), 6.46 (s, 1 H, 8-H), 6.89 (s, 1 H, 5-H). Anal. $C_{21}H_{19}BrO_7$: C, H, N. Br. MS: *m/z* 463 (M^+).

4′-Demethylepipodophyllotoxin (**2c**). *M IX*. To a solution of **1c** (5 g, 10.8 mmol) in acetone (40 ml) was added water (40 ml) and $BaCO_3$ (1 g, 5.1 mmol). The solution was stirred for 4 h at 40°C. The solvents were removed under reduced pressure. Recrystallization from acetone gave **2c** (3.1 g, 7.7 mmol, 71%). M.p. 143–145°C. IR (KBr): 1775, 1615, 1485 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.87 (m, 1 H, 3-H), 3.27 (dd, 1 H, 2-H), 3.77 (s, 6 H, 3′,5′-OCH_3), 4.30 (s, 1 H, 11α-H), 4.41 (d, 1 H, 11β-H), 4.60 (d, 1 H, 1-H), 4.85 (d, 1 H, 4-H), 5.41 (s, 1 H, OH), 5.97 (q, 2 H, 15-H), 6.29 (s, 2 H, 2′,6′-H), 6.54 (s, 1 H, 8-H), 6.87 (s, 1 H, 5-H). Anal. $C_{21}H_{20}O_8$: C, H. MS: *m/z* 400 (M^+).

4α-Azido-4-deoxy-4′-demethylpodophyllotoxin (**3c**). *M X*. To a solution of **1c** (5 g, 10.8 mmol) in DMF (40 ml) was added NaN_3 (5 g, 77 mmol). The solution was stirred overnight after which the solvents were removed under reduced pressure and the residue was washed with water. Recrystallization from acetone gave **3c** (4.1 g, 9.6 mmol, 89%). M.p. 172–174°C (decomp.) IR (KBr): 2240, 1785, 1610, 1490 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.90 (m, 1 H, 3-H), 3.39 (dd, 1 H, 2-H), 3.76 (s, 6 H, 3′,5′-OCH_3), 4.27 (s, 1 H, 11α-H), 4.37 (s, 1 H, 11β-H), 4.48 (d, 1 H, 1-H), 4.59 (d, 1 H, 4-H), 5.97 (d, 2 H, 15-H), 6.27 (s, 2 H, 2′,6′-H), 6.53 (s, 1 H, 8-H), 6.81 (s, 1 H, 5-H). Anal. $C_{21}H_{19}N_3O_7$: C, H, N. MS: *m/z* 425 (M^+).

4α-Amino-4-deoxy-4′-demethylpodophyllotoxin (**4c**). **3c** (4.4 g, 10 mmol) was hydrogenated as described for **4a**, *M II*. Recrystallization from ethanol (96%) gave **4c** (2.5 g, 6.3 mmol, 63%). M.p. 214°C. IR (KBr): 1774, 1608, 1483 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.50 (m, 1 H, 3-H), 2.78 (dd, 1 H, 2-H), 3.76 (s, 6 H, 3′,5′-OCH_3), 3.85 (dd, 1 H, 11α-H), 4.00 (dd, 1 H, 11β-H), 4.56 (m, 2 H, 1,4-H), 5.94 (dd, 2 H, 15-H), 6.35 (s, 2 H, 2′,6′-H), 6.48 (s, 1 H, 8-H), 7.08 (s, 1 H, 5-H). Anal. $C_{21}H_{21}NO_7$: C, H, N. MS: *m/z* 399 (M^+).

4α-Acetamido-4-deoxy-4′-demethylpodophyllotoxin (**5c**). **4c** (1.03 g, 2.5 mmol) was prepared according to **5a**, *M III*. Recrystallization from $CHCl_3$ gave **5c** (1.02 g, 2.0 mmol, 90%). M.p. 230°C. IR (KBr): 1775, 1672, 1484 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.09 (s, 3 H, CH_3), 2.64 (m, 1 H, 3-H), 2.85 (dd, 1 H, 2-H), 3.78 (s, 6 H, 3′,5′-OCH_3), 4.17 (dd, 1 H, 11α-H), 4.36 (dd, 1 H, 11β-H), 4.58 (d, 1 H, 1-H), 5.11 (dd, 1 H, 4-H), 5.80 (d, 1 H, NH), 5.95 (dd, 2 H, 15-H), 6.38 (s, 2 H, 2′,6′-H), 6.52 (s, 1 H, 8-H), 6.82 (s, 1 H, 5-H). Anal. Found: C 59.37, H 4.90, N 2.80. Calc. for $C_{23}H_{23}NO_8$, 1.5 H_2O: C 58.97, H 5.59, N 2.99. MS: *m/z* 441 (M^+).

4β-Azido-4-deoxy-4'-demethylpodophyllotoxin (**6c**). **6c** was prepared from **2c** (10 g, 25 mmol) in analogy with **6a**, *M IV*. Recrystallization from a mixture of ethyl acetate and $CHCl_3$ (10:1) gave **6c** (9.4 g, 22 mmol, 88%). M.p. 207–209°C. IR (KBr): 2096, 1763, 1612, 1505, 1485 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.94 (m, 1 H, 3-H), 3.15 (dd, 1 H, 2-H), 3.76 (s, 6 H, 3',5'-OCH_3), 4.29 (d, 2 H, 11α, 11β-H), 4.61 (d, 1 H, 1-H), 4.75 (d, 1 H, 4-H), 5.41 (s, 1 H, OH), 6.00 (dd, 2 H, 15-H), 6.26 (s, 2 H, 2',6'-H), 6.58 (s, 1 H, 8-H), 6.79 (s, 1 H, 5-H). Anal. $C_{21}H_{19}N_3O_7$: C, H, N. MS: *m/z* 425 (M^+).

4β-Amino-4-deoxy-4'-demethylpodophyllotoxin (**7c**). **6c** (4.4 g, 10 mmol) was hydrogenated as described for **4a**, *M II*. Recrystallization from ethanol (96%) gave **7c** (2.3 g, 5.8 mmol, 58%). M.p. 210–211°C. IR (KBr): 1772, 1611, 1484 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.02 (d, 2 H, NH), 2.85 (m, 1 H, 3-H), 3.28 (dd, 1 H, 2-H), 3.76 (s, 6 H, 3',5'-OCH_3), 4.26 (m, 3 H, 4, 11α, 11β-H), 4.53 (d, 1 H, 1-H), 5.96 (dd, 2 H, 15-H), 6.29 (s, 2 H, 2',6'-H), 6.49 (s, 1 H, 8-H), 6.80 (s, 1 H, 5-H). Anal. $C_{21}H_{21}NO_7 \cdot 0.5\ H_2O$: C, H, N. MS: *m/z* 399 (M^+).

4β-Acetamido-4-deoxy-4'-demethylpodophyllotoxin (**8c**). **8a** (5 g, 11.0 mmol) was prepared according **1c**, *M VIII*. Recrystallization from acetone gave **8c** (4.5 g, 10.2 mmol, 93%). **8c** was also prepared from **2c** in acetonitrile according to **8a**, *M V* and led to crystals identical with **8c**. **8c** was also prepared from **7c** (1.00 g, 2.5 mmol) as described for **5c**, *M III*. Recrystallization from acetone gave **8c** (0.86 g, 1.95 mmol, 78%). M.p. 156–157°C. IR (KBr): 1780, 1680, 1595, 1490 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.05 (s, 3 H, CH_3), 2.90 (m, 2 H, 2,3-H), 3.77 (s, 6 H, 3',5'-OCH_3), 3.86 (m, 1 H, 1-H), 4.36 (d, 1 H, 11α-H), 4.55 (d, 1 H, 11β-H), 5.23 (dd, 1 H, 4-H), 5.71 (d, 1 H, NH), 5.97 (q, 2 H, 15-H), 6.28 (s, 2 H, 2',6'-H), 6.52 (s, 1 H, 8-H), 6.77 (s, 1 H, 5-H). Anal. $C_{23}H_{23}NO_8$: C, H, N. MS: *m/z* 441 (M^+).

4β-Benzamido-4-deoxy-4'-demethylpodophyllotoxin (**9c**). **9c** was prepared from **2c** (5.0 g, 12.5 mmol) in analogy with **10a**, *M V*. Recrystallization from $CHCl_3$ gave **9c** (4.6 g, 9.1 mmol, 73%). Preparation according to **8c**, *M VIII*, also leads to crystals identical with **9c**. M.p. 212–217°C. IR (KBr): 1770, 1650, 1580, 1460 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.96 (m, 2 H, 2,3-H), 3.78 (s, 6 H, 3',5'-OCH_3), 3.85 (d, 1 H, 1-H), 4.42 (d, 1 H, 11α-H), 4.58 (d, 1 H, 11β-H), 5.41 (dd, 1 H, 4-H), 5.97 (d, 2 H, 15-H), 6.31 (s, 2 H, 2',6'-H), 6.54 (s, 1 H, 8-H), 6.82 (s, 1 H, 5-H), 7.40–7.82 (aromatic H). Anal. $C_{28}H_{25}NO_8$: C, H, N. MS: *m/z* 503 (M^+).

4β-[6-(9-Acridinylamino)hexyloxy]-4-deoxy-4'-demethylpodophyllotoxin (**11c**). 9-(6-Hydroxyhexylamino)acridine. HCl (**1**), prepared from 6-amino-1-hexanol and 9-chloroacridine as described by Nielsen *et al.*[11] (m.p. 108–109°C) was dissolved in acetone. To the solution was added **1c** (5 g, 10.8 mmol) and $BaCO_3$ (1 g, 5.1 mmol). The solution was refluxed for 1 h and stirred overnight at room temperature. The solvents were removed under reduced pressure and the residue washed with water. Recrystallization from ethanol–acetic acid (100:1) gave **11c** (4.1 g, 6.1 mmol, 56%). M.p. 173–174°C. IR (KBr): 1750, 1610, 1480 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.55 (s, 8 H, CH_2), 2.24 (s, 2 H, CH_2N), 2.88 (m, 1 H, 3-H), 3.35 (dd, 1 H, 2-H), 3.52 (m, 2 H, CH_2O), 3.74 (s, 6 H, 3',5'-OCH_3), 4.27 (d, 2 H, 11α, 11β-H), 4.47 (d, 1 H, 4-H), 4.55 (d, 1 H, 1-H), 5.93 (d, 2 H, 15-H), 6.25 (s, 2 H, 2',6'-H), 6.50 (s, 1 H, 8-H), 6.79 (s, 1 H, 5-H), 7.15–7.46 and 7.93–8.33 (acridine H) 9.39 (d, 1 H, NH). Anal. $C_{40}H_{40}N_2O_8$: C, H, N. FAB MS: *m/z* 677.77 (M^+).

4α-Ethoxy-4-deoxy-4'-demethylpodophyllotoxin (**12c**). To a solution of **1c** (5 g, 10.8 mmol) in ethanol (40 ml, abs.) was added $BaCO_3$ (1 g, 5.1 mmol) and the mixture was stirred for 4 h. The solvents were removed under reduced pressure. Recrystallization of the residue from ethanol (96%) gave **12c** (4.6 g, 10.8 mmol, 100%). M.p. 225–226°C. IR (KBr): 1770, 1610, 1480 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.22 (t, 3 H, CH_3), 2.93 (m, 1 H, 3-H), 3.30 (dd, 1 H, 2-H), 3.75 (s, 6 H, 3',5'-OCH_3), 4.30 (d, 2 H, 11α, 11β-H), 4.43 (d, 1 H, 4-H), 4.58 (d, 1 H, 1-H), 5.96 (q, 2 H, 15-H), 6.27 (s, 2 H, 2',6'-H), 6.53 (s, 1 H, 8-H), 6.81 (s, 1 H, 5-H). Anal. $C_{23}H_{24}O_8$: C, H. MS: *m/z* 428 (M^+).

4'-Demethyl-β-apopicropodophyllin (**2**). To a solution of **2c** (5 g, 12.5 mmol) in acetic anhydride (20 ml) was added conc. sulfuric acid (1 ml, 18 mmol). The solution was refluxed for 1 h and neutralized with an saturated solution of $NaHCO_3$ in water. The solvents were removed under reduced pressure and the residue was washed with water. Recrystallization from chloroform gave **2** (4.3 g, 11.2 mmol, 90%). M.p. 247–249°C. IR (KBr): 1750, 1690, 1610, 1485 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.79 (d, 2 H, 4-H), 3.80 (s, 6 H, 3',5'-OCH_3), 4.83 (m, 3 H, 11α, 11β-H), 5.94 (s, 2 H, 15-H), 6.38 (s, 2 H, 2',6'-H), 6.62 (s, 1 H, 8-H), 6.71 (s, 1 H, 5-H). Anal. $C_{21}H_{18}O_7$: C, H. MS, FAB MS: *m/z* 383 (M^+).

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