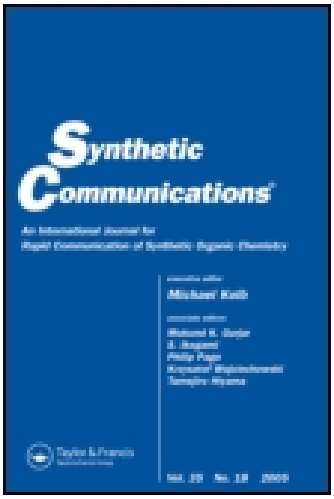

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Yves Guminski [a], Martial Grousseaud [a], Sandrine Cugnasse [a] & Thierry Imbert [a]

[a] Centre de Recherche Pierre Fabre, Division de Chimie Médicinale, Castres, France

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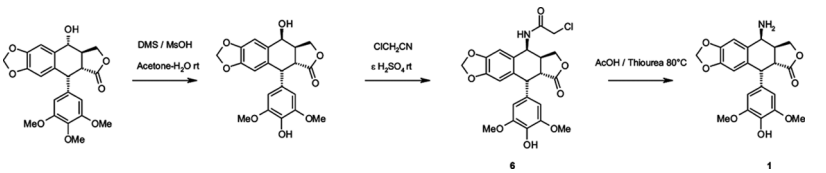
PRACTICAL DEMETHYLATION OF PODOPHYLLOTOXIN AND EFFICIENT PREPARATION OF 4-AMINO-4-DEOXY-4′-DEMETHYLEPIPODOPHYLLOTOXIN

Yves Guminski, Martial Grousseaud, Sandrine Cugnasse, and Thierry Imbert

Centre de Recherche Pierre Fabre, Division de Chimie Médicinale, Castres, France

GRAPHICAL ABSTRACT



Abstract *4′-Demethylepipodophyllotoxin (4′-DMEP) was readily available through demethylation of podophyllotoxin using methionine in methanesulfonic acid in the presence of TFA (or acetone/water). Thus, 4-amino-4-deoxy-4′-demethylepipodophyllotoxin was obtained in three steps in excellent yield by a Ritter reaction on 4′-DMEP, followed by treatment with thiourea in AcOH.*

Keywords 4-Amino-4-deoxy-4′-demethylepipodophyllotoxin; demethylation; 4′-DMEP; methionine; thiourea

INTRODUCTION

As part of our ongoing program to identify new antitumor compounds within the podophyllotoxin series, we needed easy and cheap access to 4-amino-4-deoxy-4′-demethylepipodophyllotoxin **1** as a building block, which is presented here. In addition, we report our investigations about the demethylation of podophyllotoxin **2**.

Demethylation of aromatic methoxy groups is a usual and challenging problem in synthetic organic chemistry, and this reaction is particularly useful in the field of natural products, as reviewed by Bhatt and Kulkarni.[1] We needed a reliable and efficient method of synthetic value to yield a large amount of the key intermediate 4′-demethylepipodophyllotoxin (4′-DMEP) **3**, as starting material, without any tedious chromatographic purification step. This compound **3** was formerly obtained by Kuhn et al.,[2] according to a method involving the treatment with hydrogen bromide

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Address correspondence to Yves Guminski, Centre de Recherche Pierre Fabre, Division de Chimie Médicinale, 17 av. Jean Moulin, 81106 Castres Cedex, France. E-mail: yves.guminski@pierre-fabre.com

Table 1. Demethylation of podophyllotoxin **2** or -4-substituted podophyllotoxin **4a, 4b** to 4′-DMEP **3**, using MsOH (30 eq.), D,L-methionine (5 eq.), and various carbocation trapping reagents

Entry	Starting material	Reagent	Temperature (°C)	Time	Yield (%)
1	**2**	No	RT	5 min	Degradation
2	**4a**	No	−10	1 h	52
3	**4b**	No	−10	2 h	54
4	**2**	TFA	40	45 min	93
5	**2**	H_3PO_4	0 ->RT	6 h	88
6	**2**	HCOOH	40	25 min	48
7	**2**	Acetone/water (5/1)			
		Gram scale	RT	2 h	90
		Large scale	40 ->RT	2 h	80

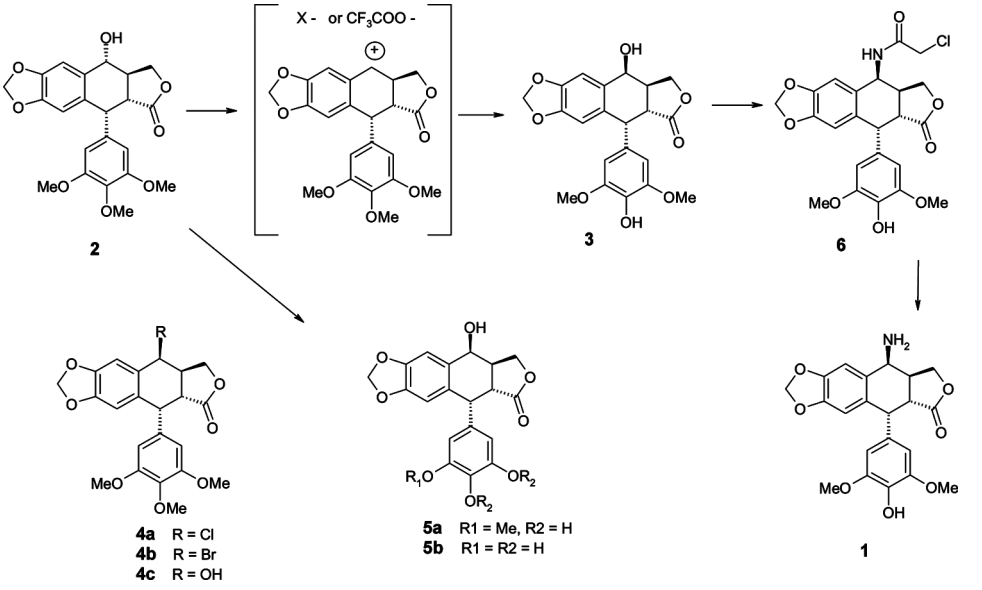
as a gas in methylene chloride/Et_2O at 0 °C, followed by a basic treatment ($BaCO_3$). This procedure was then routinely used by different groups,[3–6] giving an average yield of 40% in our hands and requiring long reaction and purification times. Daley et al.[7] improved this reaction using $ISiMe_3$. However, this procedure was not convenient for scale-up purposes. Kamal et al.[8] used methanesulfonic acid/sodium iodide as a reagent system to elegantly overcome this problem.

Classical reagents[1] were attempted for such a transformation ($AlCl_3$/EtSH; pyridine/HCl; BBr_3; BCl_3; $AlCl_3$; LiI/2,4,6-collidine) but proved unsuccessful because degradation of the starting material was observed.

A method for demethylation involving methanesulfonic acid and methionine as reagent was previously mentioned by Yajima et al.[9–12] Particularly, André et al.[13] pointed out its use for demethylation in the naloxone synthesis, leading with transposition[14–18] to the apo series. This method has been used only rarely up to now.[19,20]

This reagent was used without success when applied to podophyllotoxin **2**. The reaction medium rapidly turned dark red and gave only degradation products, aromatization of the C ring, or tars (Table 1, entry 1). It was rationalized that the known lability of the benzylic hydroxy group of podophyllotoxin in a strongly acidic medium might undergo this degradation during demethylation. In contrast, encouraging results were obtained when demethylation was carried out with derivatives with a chloro, bromo substituent at this 4-position. These starting materials (**4a, 4b**) were conveniently prepared by treatment with HCl and HBr as already described.[21] In these cases, 4′-demethylation with MsOH/methionine gave fair yields of **3** (Table 1, entries 2 and 3). It was necessary to work with an excess of methionine for a nearly complete reaction. However this methodology needed a two-step process.

Interestingly, the HBr-AcOH method proved to be efficient in the case of 4-deoxypodophyllotoxin.[22] In the case of **2**, the first intermediate formed was the benzylic carbocation generated at the 4-position under acidic medium (Lewis acid), in agreement to Kuhn and Von Wartburg.[23] This species is not stable enough during the demethylation process involving methanesulfonic acid and methionine. However, the benzylic cation might be protected against its degradation by trapping this species with an appropriate group, yielding the 4-hydroxy in the β-position after



Scheme 1.

hydrolysis. Thus, trifluoroacetic acid was used both as a cosolvent and as an entity protecting the carbocation immediately after its formation. This putative mechanism, related to that published by Berenyi et al.,[17] is shown in Scheme 1. The best results were obtained by addition of trifluoroacetic acid (93% yield) (Table 1, entry 4). Phosphoric acid gave good yields (Table 1, entry 5) but a less convenient reaction for scaling up because of the difficulty of stirring the reaction medium. Acetic or formic acid (Table 1, entry 6) worked as well, but gave lesser yields.

In a second series of experiments, to reduce this difficulty on a large scale, we used acetone as a cosolvent. However, an excess of acetone lowered the yield. The optimum ratio for the mixture of methanesulfonic acid/acetone was 10/1. Next, it was thought that H_2O might trap the transient carbocation. It was observed that the reaction proceeded without degradation. So, the presence of water was not detrimental for such a reaction. Thus, trifluroacetic acid (TFA) could be replaced by a small amount of water in acetone (mixture 5/1), giving good yields (90% or 80% according to the scale of experiment) (Table 1, entry 7).

Badet and Julia[24] prepared trimethylsulfonium salt with dimethylsulfide (DMS) and anisole in MsOH. Based on this demethylation procedure, our objective was to use DMS as a demethylating reagent. Experiments with several sulfides were undertaken. Use of DMS gave results similar to methionine, featuring well-stirred slurry (Table 2, entries 1–4). A longer reaction time or an excess of demethylating reagent led to a small amount of di- and tri-demethylation, yielding **3** and a mixture of 3',4'-dihydroxy **5a**[25] and 3',4',5'-trihydroxy **5b** that was easy to separate (Table 2, entry 3 and 4). Conversely, a shorter reaction time and an excess of DMS led to **3** and a little amount (2%) of the 4-epipodophyllotoxin **4c** (Table 2, entry 2). The best conditions were determined as 7 molar excess of DMS in 30 molar excess of MsOH

Table 2. Demethylation of podophyllotoxin **2** to 4′-DMEP **3**, using MsOH and various sulfides as demethylating reagent in acetone/water (5/1) at room temperature

Entry	Demethylating reagent	Time	Yield (%)
1	DMS (5 eq.)	1 h	60
2	DMS (7 eq.)	4 h	90[a]
3	DMS (10 eq.)	48 h	95[b]
4	DMS (40 eq.)	15 min	95[c]
5	Dithiane (5 eq.)	2 h	50
6	Thiophene, thioethanol, thioanisole	—	Degradation
7	Thiodiacetic acid	—	Degradation
8	Methylthioacetic acid (5 eq.)[d]	3 h	60

[a]Presence of 2% of **4c** (4-epimer of starting material).
[b]Presence of di- and tri-demethylated compounds **5a** (1%) and **5b** (0.5%).
[c]Presence of di-demethylated compound **5a** (0.5%).
[d]In the absence of acetone/water.

in the presence of a mixture of acetone/water (5/1) (Table 2, entry 2). Under the best conditions, the crude crystalline compound 4′-DMEP **3** was obtained in 90% yield, with a purity of 83% (by analytical high-performance liquid chromatography HPLC), the by-products essentially consisted of an equilibrated proportion of 4-epimer of starting material **4c**, and di- and tridemethylated compounds, **5a** and **5b**. It was considered adequate for most synthetic purposes. Nevertheless, a purity enhanced up to 95% could be obtained by a further recrystallization in hot AcOEt, with an overall yield of 69% from podophyllotoxin **2**.

Some other sulfides such as dithiane gave 50% of **3** (Table 2, entry 5). Thiophene, thioethanol, and thioanisole did not react as expected (Table 2, entry 6). Methylthioacetic acid and thiodiacetic acid were tried for their ability to both trap the intermediate and act as demethylating reagent. Only methylthioacetic acid gave 60% of **3** (Table 2, entry 7).

The conventional approach to give 4-amino-4′-*O*-demethylepipodophyllotoxin **1** from 4′-DMEP **3** was the reduction of the 4-azido intermediate, prepared from **3** with the couple NaN_3/TFA. This methodology actually led to the mixture of both α and β amino epimers. Though this problem was partially resolved by Zhou and coworkers,[26] it needed improvement for scale-up purposes. Another method has been reported with $HN_3/Et_2O\text{-}BF_3$ at $-15\,^{\circ}C$ and reduction with SmI_2 in t-BuOK or $FeSO_4/NH_3$.[27,28] Besides the necessary chromatographic purification of both azido epimers,[29] the drawbacks of these methods were (1) the risk of using an azido derivative, potentially explosive, during large-scale synthesis and (2) the cost of a catalytic reduction step. We present here our results to overcome these problems.

Within the podophyllotoxin series, Ritter reaction with chloroacetonitrile was reported,[3] and this reaction was applied to 4′-DMEP **3**. Chloroacetonitrile was used as a solvent (10 eq) and H_2SO_4 at room temperature, this reaction led directly to the already known[30] 4-chloroacetamido-4-deoxy-4′-demethylepipodophyllotoxin **6**, with an excellent yield (Scheme 1). The configuration at C4 was confirmed by large coupling value in the NMR spectrum (*trans* relationship $J_{H3\text{-}H4} = 7$ Hz). The cleavage of chloroacetamido group to amino with thiourea in ethanol in the presence

Table 3. Experimental conditions for the preparation of 4-amino-4-deoxy-4′-demethylepipodophyllotoxin **1** from 4-acetamido-4-deoxy-4′-demethylepipodophyllotoxin **6**

Entry	Conditions	Reaction time (h)	Yield (%)	Purity (%)
1	EtOH/AcOH (5/1)	10	<10	Presence of starting material and degradation products
2	Glacial AcOH at 80 °C	2	93	>95
3	EtOH/HCl1 N	9	90	95
4	EtOH/H_2O/AcOH (5/1/1)	10	60	95
5	Dioxan or DMA/AcOH/H_2O (5/1/1)	5-6	70	95

of AcOH is a method already reported by Jirgensons et al.[31] We used this procedure with some modifications to adapt it to our purpose. In our hands, the reported procedure afforded the target compound **1** with a poor yield (<10%). A longer reaction time did not improve the transformation and promoted degradation. However, when 4′-DMEP **3** was heated at 80 °C in glacial AcOH, followed by addition of thiourea, the reaction was completed in less than 2 h. The initial insoluble slurry became quite limpid and then a precipitate was observed, leading to the 4-amino-4-deoxy-4′-demethylepipodophyllotoxin **1** in 93% yield.

Different conditions were tested (Table 3), demonstrating that (1) the presence of water (i.e., HCl 1 N, entry 3) with ethanol appeared to be more efficient than anhydrous medium and (2) in contrast to the previously described procedure, the presence of ethanol (entry 4) or other solvents such as dioxan or DMA (entry 5) gave less yield than glacial AcOH alone (entry 2).

In conclusion, 4-amino-4-deoxy-4′-demethylepipodophyllotoxin **1** was obtained in three steps without purification from natural podophyllotoxin **2** with a 70% overall yield. Demethylation of podophyllotoxin with methionine or DMS in methanesulfonic acid in the presence of acetone and water (80% yield), followed by a Ritter reaction with chloroacetonitrile (93% and yield), and then, finally, an acidic cleavage with AcOH gave the requested 4-amino-4-deoxy-4′-demethylepipodophyllotoxin **1** (93% yield). This three-step method appeared to be the best way for a large-scale reaction.[32,33]

EXPERIMENTAL

All solvents and reagents used were obtained commercially as "pure for synthesis" grade (min. 98%) and were used without further purification unless indicated otherwise in the experimental section. Melting points were determined using an Electrothermal 9300 capillary apparatus and were uncorrected. Elemental analyses were performed on a Fisons 1108 microanalyzer and were within 0.4% of the theoretical values. Karl-Fisher analyses were performed on a Mettler DL 37 coulometer. The reaction progress was monitored by thin-layer chromatography (TLC) on silica-gel plates (60 F-254, Merck art. 1.05554), examined under ultraviolet (UV) light at 254 nm and sprayed with Salkowski or Draggendoff reagent. Purification was performed by flash-column chromatography using Merck silica gel (35–70 μ) with Intelliflash 280 (Analogix), gradient, and flow of 40 or 80 mL/min for 30- or

45-cm columns respectively. ^{1}H and ^{13}C NMR spectra were recorded in dimethylsulfoxide (DMSO-d_6) solution (unless otherwise specified) on Brucker AC 200 and Advance DPX 400 spectrometers; chemical shifts are given in parts per million (ppm) relative to tetramethylsilane (TMS) as internal standard. Liquid chromatography coupled with mass spectra was performed on ThermoElectron MSQ (simple quadripole) spectrometer, and mass spectra for structural analysis were recorded on TSQ ThermoElectron (triple quadripole).

4′-Demethylepipodophyllotoxin (4′-DMEP) 3

Procedure using trifluoroacetic acid (Table 1, Entry 4). Methanesulfonic acid (84 mL, 140 mol) and D,L-methionine (21.6 g, 140 mmol) were introduced to a stirred solution of podophyllotoxin **2** (12 g, 29 mmol) in 20 mL of trifluoroacetic acid. The temperature rose to 40 °C, and stirring was maintained for 45 min. Reaction mixture was poured into 500 mL of acetone and cold water (1/1). The stirred mixture was cautiously neutralized up to pH 5 with K_2CO_3 and extracted (twice) with AcOEt. Organic layers were separated, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was crystallized with iPr_2O and gave 10.7 g (93%) of 4′-DMEP **3**; mp 252 °C. ^{1}H NMR (200 MHz, d_6-DMSO): δ 8.30(s, 1H), 6.94 (s, 1H), 6.50 (s, 1H), 6.21 (s, 2H), 5.99 (d, 2H, $J = 3.9$ Hz), 4.71 (d, 1H, $J = 3.4$ Hz), 4.49 (d, 1H, $J = 5.2$ Hz), 4.33 (dd, 1H, $J = J' = 8$ Hz), 4.17 (dd, 1H, $J = 8.4$ Hz, $J' = 10.5$ Hz), 3.61 (s, 6H), 3.24 (dd, 1H, $J = 5.25$ Hz, $J' = 14.2$ Hz), 2.77 (m, 1H).

Procedure using 4-chloroepipodophyllotoxin 4a (Table 1, Entry 2). Podophyllotoxin **2** (1 g, 2.4 mmol) was dissolved in 15 mL CH_2Cl_2 and 5 mL Et_2O. Through this solution, cooled at −10 °C, a light stream of hydrochloric acid was introduced for 15 min. The solution was evaporated at rt and then were added to the residue methane sulfonic acid (5 mL, 8.3 mmol) and D,L-methionine (0.54 g, 3.5 mmol). After solubilization and stirring for 1 h at rt, the slurry was poured into a mixture of acetone/water (1/1) and $BaCO_3$ was added up to pH 7. Extraction with AcOEt yielded after evaporation a residue, which was crystallized with Et_2O to afford 0.5 g (52%) of 4′-DMEP **3**, as white crystals.

Procedure using phosphoric acid (Table 1, Entry 5). Podophyllotoxin **2** (5 g, 12 mmol) was added to the viscous mixture of orthophosphoric acid (2.5 mL), methanesulfonic acid (40 mL, 600 mmol) and methionine (11 g, 72 mmol) at 0 °C. The stirring continued for 6 h at rt. The reaction mixture was poured into ice water (500 mL) to yield a white precipitate. This solid was extracted (three times) with AcOEt, and the organic layers were washed with $NaHCO_3$ solution. The organic layers were dried over Na_2SO_4 and filtered. After evaporation, 4.2 g (88%) of 4′-DMEP **3** were obtained (HPLC purity 79%).

Procedure using formic acid (Table 1, Entry 6). A drop of MsOH, D,L-methionine (10.5 g, 68 mmol), and 49 mL of MsOH were introduced to a limpid solution of podophyllotoxin (7 g, 17 mmol) in 3.5 mL of formic acid and under stirring. The temperature rose to 40 °C, and again D,L-methionine (10.5 g, 68 mmol) was added. Stirring was maintained during 15 min, and the reaction mixture was

poured into ice water. The obtained precipitate was extracted with AcOEt and washed with a solution of $NaHCO_3$. The organic layer was decanted, dried over Na_2SO_4, filtered, and evaporated. The residue was crystallized in a mixture of iPr_2O/CH_2Cl_2, to yield 3.2 g (48%) of 4′-DMEP **3**.

Procedure using D,l-Methionine/acetone/water (Table 1, Entry 7). A mixture of D,L-methionine (4 g, 27 mmol) and methanesulfonic acid (20 mL, 310 mmol) with H_2O (0.4 mL) was added to a solution of podophyllotoxin **2** (2 g, 4.8 mmol) in 2 mL of acetone under stirring at rt. After 2 h, the reaction medium was poured into ice water (200 mL) and extracted with AcOEt. The organic layers were washed with a solution of $NaHCO_3$, dried over Na_2SO_4, and evaporated, affording 1.75 g of 4′-DMEP **3** (yield 90%).

Large Scale Procedure

A solution of podophyllotoxin **2** (50 g, 0.12 mol) in acetone (50 mL) was added under stirring at room temperature to a mixture of D,L-methionine (100 g, 0.67 mol), methane sulfonic acid (500 mL, 7.7 mol), and water (10 mL). The temperature rose to 40 °C, and stirring was maintained for 2 h. Then, the mixture was poured onto ice to obtain a precipitate, which was extracted with ethyl acetate (3×100 mL). The organic layers were washed with a $NaHCO_3$ solution, separated, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to afford the crude demethylated compound (45 g, yield 94%) as a white solid. The residue was taken up in isopropyl ether, and the 4′-DMEP **3** (38 g, 80%) was crystallized as a white powder. A sample was recrystallized from acetone–water mixture. Mp 248–250 °C.

Procedure using DMS in acetone/water (Table 2, Entry 2). DMS (5.2 mL) and MsOH (23 mL, 0.36 mol) were successively added to a stirred suspension of podophyllotoxin **2** (5 g, 12 mmol) in 2.5 mL of a mixture of acetone/H_2O (5/1). The temperature rose up to 40 °C, and stirring was maintained for 1.5 h at rt. The reaction mixture was poured into ice water (500 mL) to yield a white precipitate. This solid was filtered and washed with water and Et_2O to give **3** (4 g crude yield 90%). The purity was determined as 83% with HPLC. These obtained crystals could be recrystallized if necessary in AcOEt, affording 3.3 g of pure 4′-DMEP **3** (overall yield 69%). Another experiment with 500 g of podophyllotoxin gave essentially the same yield. A lengthened reaction time up to 5 h gave a purity of 75% (analytical HPLC).

Procedure using dithiane (Table 2, Entry 5). Dithiane (3 mL, 25 mmol) and MsOH (15 mL, 0.23 mol) were added to a suspension of podophyllotoxin (2 g, 5 mmol) in 2 mL of a mixture of acetone/H_2O (5/1). Stirring was maintained for 2 h at rt. The reaction mixture was poured into ice water, and the white precipitate was filter and washed with water and Et_2O to afford 1.2 g (yield 50%) of 4′-DMEP **3**.

Procedure using methylthioacetic acid. (Table 2, Entry 7). Methylthioacetique acid (1.28 g, 12 mmol) was added to a stirred solution of podophyllotoxin (1 g, 2.4 mmol) in MsOH (10 mL). The stirred slurry was kept 3 h at rt and poured into water. The formed precipitate was filtered, washed with water, and dried with Et_2O, affording 580 mg (yield 60%) of 4′-DMEP **3**.

Preparation of 3′,4′-Didemethylated Epipodophyllotoxin 5a and 3′,4′,5′-Tridemethylated Epipodophyllotoxin 5b

Podophyllotoxin **2** (10 g, 24 mmol) was dissolved in trifluoroacetic acid (60 mL). DMS (5.4 mL, 72 mmol), and MsOH (47 mL, 72 mmol) were successively added. Stirring was maintained for 9 h. Then the medium was poured onto ice (600 mL) and extracted with ethyl acetate (3×300 mL). The organic layers were washed with water and then with a $NaHCO_3$ solution to neutral pH. After drying over anhydrous Na_2SO_4, the solution was filtered and concentrated under vacuum. Crude demethylation products were obtained. Flash chromatography on silica gel (elution : CH_2Cl_2/acetone 9/1) successively afforded 550 mg of 4′-DMEP **3**, 1.1 g of 3′,4′-didemethylepipodophyllotoxin **5a**, ($C_{20}H_{18}O_8$, 0.15 H_2O; calc. C%: 61.74; H%: 4.74; found: C%: 61.67, H%: 4.68) and 1.9 g of 3′,4′,5′-tridemethylepipodophyllotoxin **5b** (^{1}H NMR 400 MHz d_6-DMSO) δ 8.65 (m, 2H), 7.95 (m, 1H), 6.71 (s, 1H), 6.47 (s, 1H), 5.98 (d, 2H, $J = 2$ Hz), 5.93 (s, 2H), 4.68 (d, 1H, $J = 3.2$ Hz), 4.34 (t, 1H, $J = 8$ Hz), 4.29 (d, 1H, $J = 5.2$ Hz), 4.16 (dd, 1H, $J = 8$ Hz, $J' = 10$ Hz), 3.17 (dd, 1H, $J = 5.2$ Hz and J' = 14 Hz), 2.76 (m, 1H).

Preparation of 4-Chloroacetamido-4-deoxy-4′-demethylepipodophyllotoxin 6

To a suspension of 30 g of 4′-DMEP **3** (0.075 mol) in 47.5 mL (0.75 mol) of chloroacetonitrile was added 0.5 mL of concentrated H_2SO_4 at rt under stirring. Solubilization was observed and followed by reprecipitation, and 300 mL of iPrOH were added under vigorous stirring. The precipitate was filtered washed with 200 mL of iPrOH and water to pH 7. The obtained white powder was dried under vacuum at 40 °C to provide 32.9 g (yield 93%) of 4-chloroacetamido-4-deoxy-4′-demethylepipodophyllotoxin **6**.[29] TLC Rf 0.44 (CH_2Cl_2/MeOH/NH_4OH 95/4.5/0.5) mp 240 °C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.65 (d, 1H, $J = 7$ Hz), 8.26 (s, 1H), 6.78 (s, 1H), 6.54 (s, 1H), 6.24 (s, 2H), 5.99 (d, 2H, $J = 11.3$ Hz), 5.17 (dd, 1H, $J = 4.56$ and 7 Hz), 4.51 (d, 1H, $J = 5{,}2$ Hz), 4.29 (t, 1H, $J = 8$ Hz), 4.10 (s, 2H), 3.97 (m, 1H), 3.78 (dd, 1H, $J = 8$ Hz and 10 Hz), 3.63 (s, 6H), 3.15 (dd, 1H, $J = 5.2$ and 14 Hz).

Preparation of 4-Amino-4-deoxy4′-demethylepipodophyllotoxin 1

A suspension of 4-chloracetamido-4-deoxy-4′-demethylepipodophyllotoxin **6** (17 g, 0.0358 mol) in glacial acetic acid was heated at 80 °C under stirring. Thiourea (4.2 g, 0.0537 mol) was added and the slurry was stirred at this temperature for 1 h 30 min. Dissolution and precipitation were observed. The reaction mixture was filtrated, washed with glacial acetic acid (75 mL) and diisopropyl ether. The obtained white solid was dried under vacuum at 40 °C to give **1** as its hydrochloride (14.6 g, 93%).[28] Mp > 260 °C.

^{1}H NMR (400 MHz, d_6-DMSO) 8.63 (m, 2H), 8.32 (m, 1H), 7.23 (s, 1H), 6.60 (s, 1H), 6.8 (s, 2H), 6.05 (d, 2H, $J = 2.1$ Hz), 4.73 (d, 1H, $J = 4.5$ Hz), 4.56 (d, 1H, $J = 5.2$ Hz), 4.34 (m, 2H), 3.65 (dd, 1H, $J = 5.2$ Hz), 3.62 (s, 6H), 3.06 (m, 1H).

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