

Synthesis and Cytotoxicity of Novel Lignans

Oskar Middel,^{†,‡} Herman J. Woerdenbag,[†] Wim van Uden,[†] Arjan van Oeveren,[‡] Johan F. G. A. Jansen,[‡] Ben L. Feringa,[‡] Antonius W. T. Konings,[§] Niesko Pras,[†] and Richard M. Kellogg^{*,‡}

Department of Pharmaceutical Biology, University Centre for Pharmacy, Groningen Institute for Drug Studies, University of Groningen, Antonius Deusinglaan 2, NL-9713 AW Groningen, The Netherlands, Department of Organic and Molecular Inorganic Chemistry, Groningen Center for Catalysis and Synthesis, University of Groningen, Nijenborgh 4, NL-9747 AG Groningen, The Netherlands, and Department of Radiobiology, Faculty of Medicine, Groningen Institute for Drug Studies, University of Groningen, Bloemensingel 1, NL-9713 BZ Groningen, The Netherlands

Received January 10, 1995[®]

In this study the syntheses of 11 novel lignans are described. Their cytotoxicities are studied in GLC₄, a human small cell lung carcinoma cell line, using the microculture tetrazolium (MTT) assay. Ten of these compounds were substituted with a menthyloxy group on the 5-position of the lactone. These compounds can easily be prepared in (novel) 'one-pot', three- or four-step syntheses. In addition, methods for controlling the stereogenic centers are described. Furthermore, five naturally occurring podophyllotoxin-related compounds were tested. The cytotoxicities of all lignan compounds, and of three non-lignan intermediates originating from the syntheses, were compared with the clinically applied anticancer agents etoposide, teniposide, and cisplatin. Most compounds showed moderate to high activities against GLC₄, and two of the compounds containing a menthyloxy group showed activities comparable to the reference cytotoxic agents.

Introduction

Lignans form an important class of compounds that are being applied in cancer chemotherapy. There is a demonstrated need of novel anticancer agents, and these can be found among lignan-related structures. These compounds can be investigated either by isolating lignans from plant material, which can be followed by (semisynthetic) derivatization, or by total synthesis. In this study the structure-activity relationships (SAR) among a range of naturally occurring lignans,¹ synthetically prepared lignans,² intermediates from these syntheses, and semisynthetic derivatives obtained from naturally occurring lignans have been investigated.³ The compounds for SAR-investigation (Table 1) were divided into four groups in which structural elements were altered, (a) removal or addition of the benzyl-, ethoxyethyl-, 1,3-propylidithiane-, thiophenyl-, and methylenedioxy-protective groups, (b) removal or alteration of the menthyloxy group, (c) podophyllotoxin-like compounds, and (d) non-lignan compounds synthesized for assignment of the stereogenic centers of the lignan compounds. The cytotoxicity of these compounds was evaluated in GLC₄,⁴ a human small cell lung carcinoma cell line, using the microculture tetrazolium (MTT) assay.⁵ The GLC₄ cell line was used because etoposide (VP-16-213) and teniposide (VM-26), two clinically applied anticancer agents that are semisynthetic derivatives of podophyllotoxin,⁶ are known to be particularly active against small cell lung tumors. These two compounds induce double-stranded DNA breaks by inhibiting the topoisomerase II repair system. In contrast, podophyllotoxin and deoxypodophyllotoxin are known to act as antimitotic spindle poisons. By binding to tubulin, these compounds inhibit microtubule formation.

The total syntheses of 5-methoxypodophyllotoxin (1), (-)-pluviatolide² (2), and (-)-hinokinin² (3) (Figure 1)

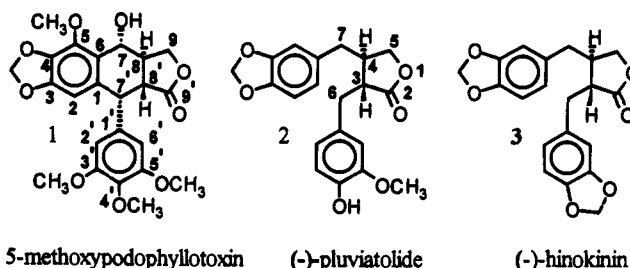


Figure 1. Three naturally occurring lignans obtained via total syntheses.

yielded several lignans and lignan intermediates which are based structurally on l-menthol. These compounds are biologically interesting because a large menthyloxy group might block the binding to tubulin. Our first aim was to study whether the compounds containing a menthyloxy group at the lactone segment might exhibit promising cytotoxic activity. In addition, to enlarge the scope of this study, other related podophyllotoxin-like compounds were isolated from *Podophyllum hexandrum* Royle and *Linum flavum* L.¹ or synthesized from podophyllotoxin.³ As reference compounds the clinically applied anticancer agents etoposide, teniposide, and cisplatin were used.

Chemistry

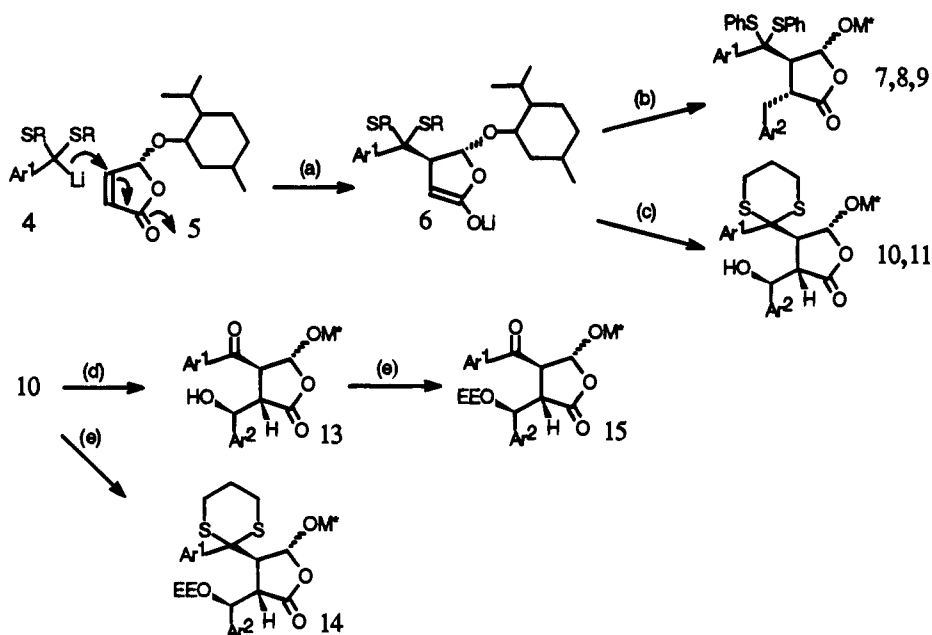
A Michael addition in dry tetrahydrofuran (THF), using (5*R*)-(1-menthyloxy)-2(5*H*)-furanone (5) as a Michael acceptor and freshly prepared lithiated dithianes 4 as Michael donors, yielded lithium enolate 6 (Scheme 1). Lignans could then be obtained in two different ways. Quenching this enolate with the appropriate benzylic bromides yielded the lignan compounds 7-9² under conditions providing total control of all stereogenic centers. Reaction of the enolate formed after a Michael addition with the appropriate benzaldehydes yielded the lignan compounds 10 and 11. The desired stereochemistry at the hydroxyl functionality formed during the

[†] Department of Pharmaceutical Biology.

[‡] Department of Organic and Molecular Inorganic Chemistry.

[§] Department of Radiobiology.

[®] Abstract published in *Advance ACS Abstracts*, May 15, 1995.

Scheme 1^a

^a (a) $-78\text{ }^{\circ}\text{C}$, THF; (b) ArCH_2Br ; (c) (1) ethylene chloroboronate, $-50\text{ }^{\circ}\text{C}$ or chlorotitanium tris(diethylamide), $-45\text{ }^{\circ}\text{C}$, (2) ArCHO , -110 to $-100\text{ }^{\circ}\text{C}$, (3) NH_4F ; (d) $\text{HgCl}_2/\text{CaCO}_3$, $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, $20\text{ }^{\circ}\text{C}$; (e) vinyl ethyl ether/ H^+ , THF, $-12\text{ }^{\circ}\text{C}$.

aldol condensation was achieved by transmetalation of the lithium enolate **6** to the boron⁷ or titanium⁸ enolates (Scheme 1). Using this method, one of the π -faces is effectively shielded so that only the syn-product can be formed in the condensation reaction with an aldehyde. In the synthesis of compound **11**, compound **12** (Table 1) was isolated as a side product (11%).

Hydrolysis of the thioketal functionality in compound **10** was accomplished using mercury(II) chloride in aqueous acetonitrile;⁹ compound **13** was obtained. Protection of the C6-hydroxyl functionality of compounds **10** and **13** with an ethoxyethyl group provided compounds **14** and **15**, respectively. Protection was accomplished by reaction with vinyl ethyl ether and a catalytic amount of toluene sulfonic acid in dry toluene.

Reduction of the thioketal in compound **9** was performed with Raney nickel^{2a} in THF, to provide compound **16**. Reduction of the thioketal in compound **8** was performed in methanol with ' Ni_2B ' generated in situ from NiCl_2 and NaBH_4 . Removal of the benzyl-protective group provided compound **2**. The reduction of the ketal moiety in compound **8**, with removal of the chiral auxiliary, was accomplished by adding aqueous potassium hydroxide and sodium borohydride to this reaction mixture. After acidic workup and subsequent deprotection of the 4'-hydroxyl functionality using palladium-black under a hydrogen atmosphere, (-)-pluviatolide (**2**) was obtained.^{2b}

(5*R*)-(1-Menthyloxy)-2(5*H*)-furanone (**5**) was also used for the synthesis of (-)-hinokinin (**3**). An analogous procedure using 5-methoxy-2(5*H*)-furanone yielded compound **18**.^{2b}

The total syntheses yielded several other lactones (**24**–**26**; Table 1). Using the same procedures as those used for the Michael additions in the previously described lignan syntheses, the enolate anion **6** was quenched with an aqueous ammonium chloride solution to give the monobenzylated furanone. These compounds enabled us to prove the antiselectivity of the Michael additions by two-dimensional ^1H NMR, the trans-

relationship between the substituents at C4 and C5 of the lactone could easily be determined by the small coupling constant ($J < 2\text{ Hz}$). The trans-stereochemistry at the C3 stereocenter at the lactone ring was easily deduced from ^1H NMR and, in one case, confirmed by X-ray analysis as described previously.^{2c} Assignment of the stereochemistry of the fourth exocyclic stereogenic center, which was formed during the aldol condensation, was more difficult because no NOE effect was observed in two-dimensional NMR for this proton; this is consistent with the stereochemistry indicated, but it is not absolute proof. For crystalline compounds, the stereochemistry of this stereogenic center can be assigned unambiguously, using X-ray crystallography, as described by Jansen.^{2c}

Deoxypodophyllotoxin (**23**) was synthesized from podophyllotoxin (**20**) by using palladium-black in acetic acid³ under a hydrogen atmosphere. 5-Methoxypodophyllotoxin (**1**) was isolated from cell cultures of *L. flavum* L.¹ (Linaceae), and 4'-demethylpodophyllotoxin (**21**) was isolated from cell cultures of *P. hexandrum* Royle³ (Berberidaceae). Podophyllotoxin glucoside (**19**) was synthesized following procedures that have been described for the syntheses of etoposide and teniposide.⁶ The compounds described above are summarized in Table 1.

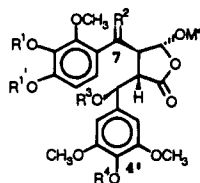
Biological Results

Biological results are listed in Table 2. For the structural formulas, see Table 1. Comparing the cytotoxic effects of compounds within the structural groups a–d (see Introduction), based on continuous as well as 2 h incubation, some general effects were observed with respect to the chemical structures and their substituents. These are summarized below.

R¹. Removal of the methylenedioxy group of compound **11** to give **12**, resulting in two free hydroxyl groups, significantly enhanced the activity following continuous incubation. If methoxy groups were intro-

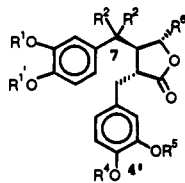
Table 1. Compounds Used for Cytotoxicity Tests^a

(a) Removal and Addition of Protective Groups



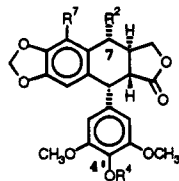
	R ¹	R ^{1'}	R ²	R ³	R ⁴
10	-CH ₂ -		S(CH ₂) ₃ S	H	CH ₃
11	-CH ₂ -		S(CH ₂) ₃ S	H	Bn
12	H	H	S(CH ₂) ₃ S	H	Bn
13	-CH ₂ -		O	H	CH ₃
14	-CH ₂ -		S(CH ₂) ₃ S	EE	CH ₃
15	-CH ₂ -		O	EE	CH ₃

(b) Addition and Removal of Protective Groups; Alteration or Removal of the Menthyl group



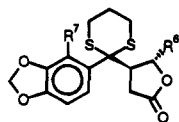
	R ¹	R ^{1'}	R ²	R ⁴	R ⁵	R ⁶
2	-CH ₂ -		H	H	CH ₃	H
7	CH ₃	CH ₃	H	CH ₃	CH ₃	OM*
8	-CH ₂ -		SPh	Bn	CH ₃	OM*
9	-CH ₂ -		SPh		-CH ₂ -	OM*
16	-CH ₂ -		H		-CH ₂ -	OM*
18	-CH ₂ -		SPh		-CH ₂ -	OCH ₃

(c) Podophyllotoxin-like Compounds



	R ²	R ⁴	R ⁷
1	OH	CH ₃	OCH ₃
19	OGlu	CH ₃	H
20	OH	CH ₃	H
21	OH	H	H
23	H	CH ₃	H

(d) Removal of the Menthyl group



	R ⁶	R ⁷
24	OM*	OCH ₃
25	H	OCH ₃
26	H	H

^a EE = ethoxyethyl, Br = benzyl, M* = 1-menthyl, Glu = glucoside.

duced instead of a methylenedioxy group (7 versus 16), no significant difference in cytotoxicity was observed.

R². Compounds with a carbonyl functionality at C7 showed the highest cytotoxicity (13, 15). Replacing a carbonyl by a dithiane reduced the activity against GLC₄ ($p < 0.05$, 13 versus 10 and 15 versus 14).**Table 2.** Cytotoxic Effects of the Test Compounds against GLC₄; IC₅₀ Mean Values \pm SD ($n = 3$) (μ M)

compd	continuous incubation	2 h incubation ^a
7	35 \pm 4	not tested
8	>100	not tested
9	40 \pm 3	not tested
10	8.8 \pm 1.5	75 \pm 9
11	48 \pm 3	not tested
12	10 \pm 2	>100
13	1.7 \pm 0.3	7.2 \pm 0.7
14	10 \pm 2	33 \pm 4
15	0.83 \pm 0.07	2.0 \pm 0.2
16	44 \pm 5	not tested
2	6.5 \pm 0.8	>100
18	>100	not tested
19	10 \pm 2	58 \pm 6
20	0.06 \pm 0.01	0.15 \pm 0.04
21	0.03 \pm 0.01	3.1 \pm 0.2
1 (= 22)	0.06 \pm 0.01	0.25 \pm 0.04
23	0.008 \pm 0.001	0.12 \pm 0.02
24	29 \pm 4	not tested
25	54 \pm 7	not tested
26	36 \pm 4	not tested
etoposide	0.82 \pm 0.04	4.0 \pm 0.4
teniposide	0.48 \pm 0.02	2.8 \pm 0.2
cisplatin	1.1 \pm 0.2	4.6 \pm 0.2

^a Compounds showing an IC₅₀ value exceeding 10 μ M in continuous incubations were not tested in 2 h incubations.

Further enlargement of the dithiane moiety reduced activity (compare Table 1, parts a and b). Cytotoxic effects comparable to etoposide, teniposide, and cisplatin were observed for compounds 13 and 15. Comparing these results with Table 1c of the podophyllotoxin-like compounds, a free hydroxyl group at C7 and ring closure further enhanced the activity (20–22). Converting podophyllotoxin (20) to deoxypodophyllotoxin (23) even provided a stronger cytotoxicity. Glycosilation of the hydroxyl group (20) with a glucose moiety (19) strongly reduced the activity.

R³. How a free hydroxyl group at the benzylic position (10–13) influences cytotoxicity cannot be concluded from our experiments. On the other hand, protecting the hydroxyl functionality with an ethoxyethyl group significantly enhanced the activity found in 2 h incubations (compare 10 to 14 and 13 to 15). Following continuous incubation, however, this effect was less pronounced or even absent. Ring-closed podophyllotoxin-like structures (20–23) showed the highest cytotoxic effects (Table 1c).**R⁴.** Compared to a methoxy group, a benzyl group at R⁴ reduced the activity ($p < 0.05$, 10 compared to 11). Within the range of podophyllotoxin-like compounds (20 and 21), a free hydroxyl compared to a methoxy substituent at C4' (Figure 1), significantly reduced the activity after a 2 h incubation period. In continuous incubation it slightly enhanced the activity.**R⁵.** Introduction of a methylenedioxy functionality instead of a methoxy substituent did not lead to significant differences in cytotoxicity. Compounds 7 and 16 showed similar activities.**R⁶.** A compound containing a methoxy group showed a much higher IC₅₀ value than one with a menthyl group ($p < 0.05$, 18 versus 9), indicating that a menthyl group at this position could enhance the activity. From our experiments the possible effects of removing the menthyl group cannot be deduced.**R⁷.** A methoxy substituent at R⁷ did not significantly influence the activity (25 versus 26). Podophyllotoxin

Table 3. Evaluation of the General Structure–Activity Relationships of the Tested Compounds

structural group	structural elements
a (Table 1a,b)	R ¹ = CH ₃ << CH ₂ < H R ² = SPh < S(CH ₂) ₃ S < H < O R ³ = H < OH < OEE < ring-closed structure R ⁴ = Bn < CH ₃ < H
b (Table 1c,d)	R ⁶ = OCH ₃ < OM*
c (Table 1c)	R ² = OGluc << OH ~ H R ⁴ = H < CH ₃ R ⁷ = H ~ OCH ₃

(20) and 5-methoxypodophyllotoxin (1) showed comparable cytotoxicities.

The results of this study are summarized in the Table 3 in which the structural elements are arranged in order of their influence on the activities.

Discussion

Compounds 10 and 11 were synthesized in a novel,² 'one-pot' four-step synthesis. These kinds of compounds would normally be prepared step by step. The large advantage of this approach is that these compounds can now readily be synthesized within 1 day, with total control of all stereogenic centers. Purification steps take less time because all starting materials can now easily be removed by flash chromatography, and purification of the products can be carried out by column chromatography. Synthesizing these compounds step by step can imply a number of purification steps, which in some cases can be a real problem as described in this paper for compound 25.

From Table 2 it can be seen that for several compounds (2, 10, 12, 21) a very large difference in cytotoxicity was found between 2 h and continuous incubation. These differences may be explained in terms of the structure of these compounds. Probably the substituents are too bulky, or possess a low-lipophilic character, resulting in low intracellular concentration after 2 h exposure. Increasing cytotoxicity has been found with increasing lipophilicity.¹⁰ The difference in cytotoxicity (continuous incubation) between a compound with a methoxy group and compounds with a menthyloxy group (9 versus 18) could also be explained in terms of different lipophilicities. The benzyl substituent in compounds 8 and 11 seems to be too bulky; although these compounds possess a more lipophilic character, their cytotoxic effect is reduced compared to compounds with a methoxy substituent at this position (10, 13–15). The more bulky phenyldithianes (8, 9, 18) showed lower cytotoxic effects compared to the less bulky propyldithianes (10–12, 14).

From our study it may be concluded that some structural elements influence the cytotoxicity. An ethoxyethyl group (14, 15 compared to 10, 13), a carbonyl (13, 15 compared to 10, 14), a free hydroxyl (12, 16), and ring closure (20–23) enhanced cytotoxicity within our range of lignan compounds. A benzyl-protective group (11, 15) and dithiane-protective groups (10, 14, 18 versus 13, 15, 2) seemed to reduce the cytotoxic effects.

On the basis of IC₅₀ values, deoxypodophyllotoxin (23) was about 8 times more toxic than podophyllotoxin (20) in continuous incubations, in contrast to the difference of a factor of only 2 times found in 2 h incubations. The difference in biological activity as found in our study is

not in agreement with earlier SAR studies carried out with podophyllotoxin,¹¹ in which it was concluded that the free hydroxyl at C7 is partially responsible for the binding to tubulin. Both compounds are known to act as antimitotic spindle poisons.

Compounds 13 and 15 showed activities to GLC₄ in the same range as etoposide, teniposide, and cisplatin. Whether their cytotoxicity is a result of binding to the enzyme topoisomerase II, which is essential in the DNA breakage–reunion process, or whether they act as spindle poisons by binding to tubulin is not clear from our experiments. Further studies should reveal whether one of the substituents (for instance a menthyloxy group) is able to block the binding to tubulin. Compounds 13 and 15 are the most interesting of the synthesized compounds based upon their IC₅₀ values.

Experimental Section

Cell Line. GLC₄, a human small cell lung carcinoma cell line, was derived from a pleural effusion at the Department of Internal Medicine, University Hospital Groningen, The Netherlands.⁴ This cell line grows in suspension culture, partly floating and partly attached, in RPMI 1640 medium (Gibco, Paisly, U.K.), supplemented with 10% heat-inactivated (30 min, 56 °C) fetal calf serum (Gibco) plus 50 µg/mL streptomycin and 50 IU/mL penicillin. It was maintained at 37 °C in a humidified atmosphere with 5% CO₂. The doubling time was 18–21 h. Cells were in the exponential phase of growth at the moment of testing the compounds. The viability of the cells used in the experiments exceeded 90% as determined with trypan blue.

Cytotoxicity Assay. Cytotoxicity after treatment of the tumor cells with the test compounds was determined using the microculture tetrazolium (MTT) assay.⁵ This assay is based on the reduction of a soluble tetrazolium salt by mitochondrial dehydrogenase activity of viable tumor cells to an insoluble, colored formazan product.

The amount of this formazan product can be measured spectrophotometrically after dissolution¹⁰ in dimethyl sulfoxide (Merck, Darmstadt, FRG). Under the experimental conditions used in the present study, the enzyme activity and the amount of formazan formed were proportional to the amount of cells. Reduction in the amount of cells by a particular agent can generally be explained by killing of cells and/or inhibition of cell proliferation.

Concentrated stock solutions (200×) were made in dimethyl sulfoxide and stored at –20 °C. Exponentially growing cells were harvested. The cell suspension was diluted appropriately with RPMI 1640 medium plus 10% fetal calf serum. Of this cell suspension, 50 µL containing 3750 cells was pipetted into 96-well microtiter plates (Nunc, Roskilde, DK).

Subsequently, 50 µL of a solution of the test compounds, obtained by diluting the stocks with RPMI 1640 medium plus 10% fetal calf serum, was added to each well. The small amount of dimethyl sulfoxide present in the wells (maximum 0.25%) proved not to affect the experiments. The highest concentration of the compounds tested was 100 µM.

The cells were exposed to the test compounds either continuously (4 days) or for 2 h. Only compounds with an IC₅₀ ≤ 10 µM following continuous exposure were used for the 2 h incubations. After the 2 h exposure period at 37 °C in a humidified atmosphere with 5% CO₂, the cells were washed three times with 200 µL of culture medium (10 min, 20 °C, 210g). After the washing steps, the plates were incubated in the same atmosphere for a culture period of 4 days.

A 5 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma, St. Louis, MO) was prepared in phosphate-buffered saline (PBS; 1.5 mM KH₂PO₄, 6.5 mM Na₂HPO₄, 137 mM NaCl, 2.7 mM KCl; pH 7.4). Of this solution, 20 µL was pipetted into each well. Following an incubation time of 3 h 45 min with MTT at 37 °C in a

humidified atmosphere with 5% CO₂ and centrifugation of the plates (15 min, 20 °C, 210g), the medium was carefully aspirated.

The formazan product formed was then dissolved in 200 μL of dimethyl sulfoxide. After thorough mixing, the absorbance was measured at 520 nm using a scanning microtiterwell spectrophotometer (Titertek Multiscan; Flow Laboratories, Irvine, U.K.).

Cell growth inhibition (or killing) was calculated using the formula: growth inhibition (%) = [1 - [(absorbance of treated cells - absorbance of culture medium) / (absorbance of untreated cells - absorbance of culture medium)]] × 100. The IC₅₀ value (the concentration of test compound causing 50% growth inhibition of the tumor cells) was used as a parameter for cytotoxicity.

Statistics. For comparison of the results of the experiments, the Student's *t*-test was used. A *p*-value < 0.05 was considered significant. All experiments were carried out in triplicate (separate experiments).

Chemistry. ¹H-NMR spectra were recorded on a Varian XL-200 (200 MHz), Varian XR-300S (300 MHz), or Bruker AMX 400 nb instrument. ¹³C-NMR spectra were recorded on Nicolet NT 200 (50.32 MHz) and Varian 300 VXR 300S (75.48 MHz) spectrometers. The chemical shifts are given in δ units (ppm) relative to TMS (δ = 0 ppm), and signal multiplicity was designated according to the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; br, broad singlet; m, multiplet. HRMS spectra were recorded on an AEI MS-902 spectrometer (EI). Melting points were determined on a Mettler FP-2 (uncorrected). Optical rotations were measured at the Na D-line on a Perkin Elmer 241 MC instrument at room temperature. Chloroform was used as a solvent. Chromatography was performed using silica gel 60 F₂₅₄ (Merck, Art. No. 7734 or 9385). *R_f* values were determined on TLC plates precoated with 0.25 mm thick silica gel 60 F₂₅₄ (Merck Art. No. 5714). Unless otherwise noted, *R_f* = 0.3 for compounds purified by chromatography. Solvents were purified using standard procedures. (5*R*)-(1-Menthyloxy)-2(5*H*)-furanone (**5**) was synthesized according to a procedure previously described by de Jong and Feringa.¹² 2-Methoxy-3,4-(methylenedioxy)-benzaldehyde was synthesized according to a procedure described by Baker et al.¹³ Chlorotitanium tris(diethylamide) was freshly prepared as described by Reetz and Urz.⁸ Ethylene chloroboronate was synthesized following a procedure described Hoffmann and Dittrich.¹⁴ 3,4,5-Trimethoxybenzaldehyde, podophyllotoxin, and syringaldehyde were purchased from Janssen Chimica (Beerse, Belgium). Compound **20** was synthesized according to a procedure described in the literature.¹⁵ Syringaldehyde was benzylated using a procedure described by Loirot et al.¹⁶ The syntheses of compounds **7–9** and **16–18** have been described elsewhere (protocols and analyses).² Isolation procedures for compounds **20–22** also have been described elsewhere.¹ Reactions that were sensitive to air and moisture were carried out under nitrogen atmosphere using flame-dried glassware.

1,2-(Methylenedioxy)-3-methoxy-4-[(1,3-propylenedithio)methyl]benzene (4). To a solution of 2-methoxy-3,4-(methylenedioxy)benzaldehyde (12 g, 80 mmol) in toluene (80 mL) were added 1,3-propanedithiol (6.1 g, 80 mmol) and toluenesulfonic acid (0.1 g, catalytic amount). The reaction was carried out overnight with azeotropic removal of water. The solvent was removed in vacuo and the residue redissolved in chloroform (50 mL). The organic layer was washed with water (100 mL) subsequently and twice with 1 N NaOH and dried over magnesium sulfate. Removal of the solvent under reduced pressure yielded a yellowish colored oil, which was recrystallized from THF-hexane to provide colorless cubic crystals of the pure 1,3-propyldithiane of 2-methoxy-3,4-(methylenedioxy)benzaldehyde (**4**) (17.5 g, 81%); mp 155 °C; ¹H NMR (CDCl₃) δ 6.82 (dd, 2 H, *J*_{AB} = 18.2 Hz, Ar-H), 5.92 (s, 2 H, OCH₂O), 5.56 (s, 1 H, (SR)₂C-H), 4.05 (s, 3 H, OCH₃), 2.83–3.19 (m, 4 H, SCH₂), 1.76–2.14 (m, 2 H, CH₂); ¹³C NMR (CDCl₃) δ 149.1 (s), 140.1 (s), 136.2 (s), 124.6 (d), 122.2 (d), 103.3 (t), 101.1 (d), 60.2 (q), 43.7 (d), 32.5 (t), 25.3 (t); HRMS 270.038, calcd for C₁₂H₁₄O₅S₂ 270.038; MS (rel intensity) 272

(M⁺, 10), 271 (15), 270 (100), 197 (12), 196 (69), 195 (14), 167 (14), 165 (19), 163 (17).

General Procedure for the Formation of the Enolate; Michael Addition of Lithiated 4 to (5*R*)-(1-Menthyloxy)-2(5*H*)-furanone (5**) (Table 1a).** To a solution of **4** (1.12 g, 4.16 mmol) in dry THF (40 mL) at -78 °C was added *n*-BuLi (2.00 mL, 2.5 M, in hexane, 5 mmol) dropwise within a period of 30 min. Stirring was continued for 30 min, and then a solution of (5*R*)-(1-menthyloxy)-2(5*H*)-furanone (**5**) (1.00 g, 4.58 mmol) in THF (5 mL) was added dropwise over a period of 20 min at -90 °C. The orange-brown lithium enolate solution of **6** was used as such in further syntheses.

General Procedure for Transmetalation to the Titanium and Boron Enolates (Table 1a). At -90 °C chlorotitanium tris(diethylamide)⁸ (4.0 mL, 12.6 mmol) was added to the lithium enolate solution. The temperature was allowed to rise to -45 °C over a period of 40 min, resulting in the formation of the dark red titanium enolate solution. This solution was used as such in further syntheses.

At -50 °C ethylene chloroboronate (1.0 g, 9.4 mmol) was added to the lithium enolate solution. The temperature was allowed to rise to -15 °C over a period of 1 h; this resulted in the formation of the brightly yellow boron enolate solution.

((3*R*,4*R*,5*R*)7*αR*)-3-[(3,4,5-Trimethoxyphenyl)hydroxymethyl]-4-[[2-methoxy-3,4-(methylenedioxy)phenyl]-1,3-propylenedithio)methyl]-5-(1-menthyloxy)dihydro-2(3*H*)-furanone (10**).** To the titanium (at -110 °C) or boron enolate solution (at -100 °C) was added a solution of 3,4,5-trimethoxybenzaldehyde (0.88 g, 4.56 mmol) in THF (5 mL) dropwise over a period of 2 h. The reaction mixture was poured into a concentrated aqueous ammonium fluoride solution (ca. 200 mL) and extracted with diethyl ether (2 × 100 mL). The organic layer was washed with water (50 mL) and brine (50 mL) and dried over anhydrous sodium sulfate. Removal of the solvent yielded a brown-red oil that was submitted to flash chromatography with dichloromethane as eluent (*R_f* 10 = 0), to remove some starting material. Then diethyl ether was used as eluent to give the crude product mixture. Column chromatography (diethyl ether/hexane, 3:2) yielded compound **10**, 1.55 g (2.2 mmol, 53%), as a white foam that became a slightly yellow oil upon standing: [α]_D -10.2° (*c* = 1); ¹H NMR (CDCl₃) δ 0.65–1.5 (m, 16 H, menthyl), 1.7–2.3 (m, 4 H, CH-menthyl and CH₂-dithiane), 2.4–2.8 (m, 5 H, SCH₂ and C8-H), 2.9–3.1 (m, 1 H, OCH-menthyl), 3.3–3.35 (br, 1 H, C8'-H or OH), 3.64 (s, 6 H, OCH₃), 3.75 (s, 3 H, OCH₃), 3.8 (br, 1 H, Ar-CHOR), 4.05 (s, 3 H, OCH₃), 4.65–4.8 (br, 1 H, CH-Omenthyl), 5.99 (d, 2 H, ²*J* = 7 Hz, OCH₂O), 6.25 (s, 2 H, Ar-H), 6.41 (d, 1 H, ³*J*_{AB} = 8.5 Hz, Ar-H), 7.08 (d, 1 H, ³*J*_{AB} = 8.5 Hz); ¹³C NMR (CDCl₃) δ 14.1 (d), 15.5 (q), 21.0 (q), 22.1 (d), 22.8 (t), 24.0 (t), 25.7 (q), 26.9 (t), 27.6 (t), 31.2 (d), 31.5 (t), 34.2 (t), 39.5 (t), 47.6 (q), 52.5 (d), 55.7 (d), 59.0 (q), 60.6 (s), 60.7 (q), 75.2 (d), 78.2 (d), 101.1 (d), 101.5 (t), 102.1 (d), 124.7 (d), 135.0 (s), 137.4 (s), 139.4 (s), 142.1 (s), 143.0 (s), 149.6 (s), 152.7 (s), 178.4 (s); HRMS 704.763, calcd for C₃₆H₄₇O₁₀S₂ 704.763; MS (rel intensity) 704 (M⁺, 0.36), 508 (6), 360 (4), 324 (4), 296 (6), 269 (75), 250 (19), 196 (39), 195 (27), 181 (15), 165 (12), 83 (10), 55 (12), 41 (19), 39 (11), 32 (21), 28 (100).

((3*R*,4*R*,5*R*)7*αR*)-3-[[4-(Benzoyloxy)-3,5-dimethoxyphenyl]hydroxymethyl]-4-[[2-methoxy-3,4-(methylenedioxy)phenyl]-1,3-propylenedithio)methyl]-5-(1-menthyloxy)dihydro-2(3*H*)-furanone (11**).** The same procedure was used as described above for **10** except that 4-(benzyloxy)-3,5-dimethoxybenzaldehyde was used instead of the 3,4,5-trimethoxybenzaldehyde. In the final column chromatography, diethyl ether/hexane (2:1) was used as the eluent. Compound **11**, 1.71 g (2.2 mmol, 53%), was obtained as a white solid, which became an oil upon standing: [α]_D -12.5° (*c* = 1); ¹H NMR (CDCl₃) δ 0.6–1.4 (m, 16 H, menthyl), 1.4–2.0 (m, 4 H, CH-menthyl and CH₂-dithiane), 2.0–2.2 (br, 1 H, C8-H), 2.2–3.0 (m, 4 H, SCH₂), 3.25–3.3 (br, 1 H, OCH-menthyl), 3.3–3.6 (m, 1 H, C8'-H), 3.62 (s, 3 H, OCH₃), 3.82 (s, 6 H, OCH₃), 3.88 (s, 3 H, OCH₃), 3.9–4.0 (br, 1 H, ArCHOHR), 4.65–4.85 (br, 1 H, OCH-Omenthyl), 5.00 (s, 2 H, OCH₂Ph), 5.96 (d, 2 H, ²*J* = 10.3 Hz, OCH₂O), 6.52 (d, 1 H, ³*J*_{AB} = 8.3 Hz, Ar-H), 6.53 (s, 1 H, Ar-H), 6.53 (s, 1 H, Ar-H), 7.36 (d, 1 H, ³*J*_{AB} = 8.3 Hz, Ar-H), 7.3–7.4 (m, 3 H, Ar-H), 7.50 (d, 2 H,

$^3J = 6.8$ Hz, Ar-H); ^{13}C NMR (CDCl_3) δ 15.4 (d), 15.5 (q), 20.9 (q), 22.0 (d), 22.8 (t), 24.0 (t), 25.7 (q), 26.9 (t), 28.8 (t), 30.9 (d), 31.0 (t), 34.0 (t), 39.3 (t), 47.5 (q), 52.3 (d), 55.6 (d), 55.8 (d), 58.7 (q), 59.8 (q), 60.4 (s), 74.7 (t), 75.1 (d), 78.0 (d), 101.4 (t), 101.8 (d), 102.0 (d), 122.1 (d), 124.6 (d), 127.5 (d), 127.9 (d), 128.2 (d), 134.8 (s), 136.4 (s), 137.8 (s), 142.2 (s), 142.3 (s), 149.4 (s), 152.8 (s), 178.3 (s); HRMS calcd for $\text{C}_{42}\text{H}_{52}\text{O}_{10}\text{S}_2$ 780.968; the parent peak was not observed. Immediate fragmentation occurs between C3 and C6 (Figure 1). This results in the masses of 508.41 and 272.11: MS (rel intensity) 569 (0.14), 508 (2), 272 (6), 269 (14), 250 (8), 196(6), 179 (4), 165 (9), 163 (5), 106 (7), 91 (100). As a side product (11%) in this synthesis, compound **12** was isolated as a yellowish colored oil.

(3R,4R,5R)7aR-3-[(3,4,5-Trimethoxyphenyl)hydroxymethyl]-4-[[2-methoxy-3,4-(methylenedioxy)phenyl]oxomethyl]-5-(l-menthyloxy)dihydro-2(3H)-furanone (13). To a stirred solution of **10** (190 mg, 0.273 mmol) in acetonitrile/acetone/water, volume ratio 6:3:2.2 at room temperature, HgCl_2 (289 mg, 0.612 mmol) and CaCO_3 (114 mg, 1.139 mmol) were added. Reaction was followed on TLC (diethyl ether/hexane, 2:1) and was complete after about 30 min. Subsequently, the reaction mixture was filtered and extracted with dichloromethane (50 mL). The organic layer was washed with water (2×30 mL) and brine (30 mL). Column chromatography with diethyl ether/hexane (3:2) yielded compound **13**, 89 mg (0.147 mmol, 67%), as a colorless oil, which colorizes upon standing. This compound had to be repurified (column chromatography; diethyl ether/hexane, 3:2) before it could be used in cytotoxicity experiments: ^1H NMR (CDCl_3) δ 0.65–1.50 (m, 16 H, menthyl), 1.70–2.3 (m, 2 H, CH-menthyl), 2.4–2.8 (m, 1 H, C8-H), 2.9–3.1 (m, 1 H, OCH-menthyl), 3.64 (s, 6 H, OCH_3), 3.75 (s, 3 H, OCH_3), 3.8–3.9 (br, 1 H, ArCHOR), 3.8 (br, 1 H, C8'-H), 4.05 (s, 3 H, OCH_3), 4.65–4.80 (br, 1 H, CH-Omenthyl), 5.99 (d, 2 H, $^2J = 7$ Hz, OCH_2O), 6.25 (s, 2 H, Ar-H), 6.41 (d, 1 H, $^3J_{AB} = 8.5$ Hz, Ar-H), 7.08 (d, 2 H, $^3J_{AB} = 8.5$ Hz, Ar-H); ^{13}C NMR (CDCl_3) δ 14.1 (d), 15.5 (q), 21.0 (q), 22.1 (d), 22.8 (t), 24.0 (t), 25.7 (q), 26.9 (t), 27.6 (t), 31.2 (d), 34.2 (t), 47.6 (q), 52.5 (d), 55.7 (d), 58.8 (q), 60.7 (q), 75.2 (d), 78.2 (d), 101.1 (d), 101.5 (t), 102.1 (d), 124.7 (d), 135.0 (s), 137.4 (s), 139.4 (s), 142.1 (s), 143.0 (s), 149.6 (s), 152.7 (s), 178.4 (s), 198.1 (s); HRMS 614.273, calcd for $\text{C}_{33}\text{H}_{42}\text{O}_{11}$ 614.273; MS (rel intensity) 614 (M^+ , 9), 278 (14), 250 (8), 234 (5), 207 (5), 197 (11), 196 (44), 195 (6), 181 (20), 179 (11), 178 (100).

(3R,4R,5R)7aR-3-[(3,4,5-Trimethoxyphenyl)(ethoxyethyl)oxymethyl]-4-[[2-methoxy-3,4-(methylenedioxy)phenyl](1,3-propylenedithio)methyl]-5-(l-menthyloxy)dihydro-2(3H)-furanone (14). At -12°C , vinyl ethyl ether (0.50 mL, 0.377 g, 5.92 mmol) was added to a stirred solution of **10** (276 mg, 0.393 mmol) and toluenesulfonic acid (0.1 g, catalytic amount) in dry THF (8.5 mL). After 1 h the reaction mixture was neutralized to pH 7.0 with 0.05 N KOH in absolute EtOH. The reaction mixture was diluted with diethyl ether (50 mL) and washed with an aqueous KOH solution of pH 8 (30 mL). The solvent was removed under a vacuum which was followed by flash chromatography with dichloromethane/hexane/triethylamine (16:1:0.01) to remove some impurities (R_f **14** = 0). Flash chromatography with diethyl ether/triethylamine (1:0.001) yielded **14**, 274 mg (0.354 mmol, 90%), as a white foam that became an oil upon standing: ^1H NMR (CDCl_3) δ 0.6–1.95 (m, 21 H, menthyl and CH_3 -ethoxyethyl), 1.46 (d, 3 H, $^3J = 5.9$ Hz, CH_3 -ethoxyethyl), 1.99 (t, 2 H, $^3J = 6.7$ and 7.3 Hz, CH_2 -dithiane), 2.72 (t, 2 H, $^3J = 7.8$ Hz, CH_2 -dithiane), 2.8–2.9 (m, 1 H, C8-H), 3.02 (t, 2 H, $^3J = 7.1$ Hz, CH_2 -dithiane), 3.40 (q, 2 H, $^3J = 7.1$ Hz, CH_2 -ethoxyethyl), 3.5–3.6 (m, 1 H, OCH-menthyl), 3.6–3.8 (m, 1 H, C8'-H), 3.9–4.0 (br, 1 H, ArCHOR), 3.87 (s, 6 H, OCH_3), 3.88 (s, 3 H, OCH_3), 3.99 (q, 1 H, $^3J = 26$ Hz, CH-ethoxyethyl), 3.99 (s, 3 H, OCH_3), 4.1–4.3 (m, 1 H, OCH-Omenthyl), 5.95 (s, 2 H, OCH_2O), 6.50 (d, 1 H, $^3J = 8.3$ Hz, Ar-H), 7.19 (s, 2 H, Ar-H), 7.34 (d, 1 H, $^3J = 8.6$ Hz, Ar-H); ^{13}C NMR (CDCl_3) δ 14.8 (q), 15.3 (q), 15.4 (q), 19.8 (q), 21.1 (d), 22.1 (t), 22.6 (t), 24.1 (q), 25.2 (t), 25.5 (t), 31.2 (d), 34.2 (t), 39.7 (t), 47.7 (d), 49.0 (q), 52.9 (d), 55.8 (q), 58.8 (t), 59.2 (d), 60.7 (q), 61.1 (s), 76.5 (d), 77.7 (d), 80.0 (d), 97.0 (d), 101.2 (t), 101.8 (d), 124.8 (d), 125.0 (d), 134.5 (s), 137.1 (s), 139.5 (s), 142.4 (s), 149.4 (s), 152.7 (s), 175.0 (s); HRMS 776.326, calcd for $\text{C}_{40}\text{H}_{56}\text{O}_{11}\text{S}_2$

776.326; MS (rel intensity) 776 (M^+ , 0.7), 704 (4), 508 (8), 418 (2), 352 (2), 269 (100).

(3R,4R,5R)7aR-3-[(3,4,5-Trimethoxyphenyl)(ethoxyethyl)oxymethyl]-4-[[2-methoxy-3,4-(methylenedioxy)phenyl]oxomethyl]-5-(l-menthyloxy)dihydro-2(3H)-furanone (15). The same procedure on the same scale was used as that for compound **13**; compound **14** was used as starting material, and 1% triethylamine was added to the eluent for chromatography. Compound **15**, 150 mg (0.219 mmol, 80%), was obtained as a colorless oil: ^1H NMR (CDCl_3) δ 0.6–2.3 (m, 21 H, menthyl and CH_3 -ethoxyethyl), 2.4–2.8 (m, 1 H, C8-H), 3.2–4.0 (m, 4 H, OCH-menthyl, C8'-H, CH_2 -ethoxyethyl), 3.76 (s, 6 H, OCH_3), 3.85–3.95 (br, 1 H, ArCHOR), 3.89 (s, 3 H, OCH_3), 3.40 (s, 3 H, OCH_3), 4.0–4.2 (br, 1 H, CH-Omenthyl), 6.00 (d, 2 H, $^2J = 6.7$ Hz, OCH_2O), 6.25 (s, 2 H, Ar-H), 6.41 (d, 1 H, $^3J = 8.7$ Hz, Ar-H), 7.08 (d, 1 H, $^3J = 7.9$ Hz, Ar-H); ^{13}C NMR (CDCl_3) δ 15.0 (q), 15.4 (q), 15.5 (q), 19.7 (q), 21.1 (d), 22.2 (t), 22.6 (t), 24.1 (q), 25.2 (t), 25.5 (t), 31.2 (d), 47.7 (d), 49.0 (q), 52.8 (d), 55.8 (q), 58.8 (t), 59.2 (d), 60.7 (q), 76.5 (d), 77.7 (d), 80.0 (d), 97.1 (d), 101.1 (t), 101.8 (d), 124.9 (d), 125.0 (d), 134.5 (s), 137.1 (s), 139.5 (s), 142.5 (s), 149.4 (s), 152.7 (s), 175.1 (s), 197.1 (s); HRMS 686.377, calcd for $\text{C}_{37}\text{H}_{50}\text{O}_{12}$ 686.377; MS (rel intensity) 686 (M^+ , 0.62), 278 (14), 250 (8), 234 (5), 207 (5), 197 (11), 196 (44), 195 (6), 181 (20), 179 (11), 178 (100).

(4R,5R)4-[[3-Methoxy-1,2-(methylenedioxy)phenyl](1,3-propylenedithio)methyl]-5-(l-menthyloxy)dihydro-2(4H)-furanone (24). The lithium enolate solution was poured into 100 mL of concentrated ammonium chloride solution and extracted with diethyl ether (3×150 mL). Subsequently, the organic layer was washed with 1 N HCl (2×50 mL), water (100 mL), and brine (100 mL) and dried over magnesium sulfate. The solvent was removed under reduced pressure. Column chromatography with diethyl ether/hexane (1:2) (R_f **24** = 0) was followed by column chromatography with toluene/ether (20:1) and yielded **24**, 1.09 g (2.20 mmol, 53%), as a colorless oil: $[\alpha]_D^{25} +16.95^\circ$ ($c = 1$); ^1H NMR (CDCl_3) δ 0.5–2.05 (m, 19 H, menthyl and lactone), 2.6–3.3 (m, 6 H, dithiane), 3.55–3.65 (m, 1 H, lactone), 3.94 (s, 3 H, OCH_3), 5.4 (br, 1 H, OCHO), 6.07 (s, 2 H, OCH_2O), 6.58 (d, 1 H, $^3J = 3.14$ Hz, Ar-H), 7.47 (d, 1 H, $^3J = 8.45$ Hz, Ar-H); ^{13}C NMR (CDCl_3) δ 15.7 (q), 20.9 (q), 22.0 (q), 22.8 (t), 24.0 (t), 25.4 (d), 27.6 (t), 27.9 (t), 30.4 (t), 30.4 (d), 34.0 (t), 39.2 (t), 47.3 (d), 49.2 (d), 59.0 (s), 59.2 (d), 77.9 (d), 101.8 (t), 102.2 (d), 125.1 (s), 125.3 (d), 139.7 (s), 142.4 (s), 149.5 (s), 176.0 (s).

(4R)4-[[3-Methoxy-1,2-(methylenedioxy)phenyl](1,3-propylenedithio)methyl]dihydro-2(5H)-furanone (25). To a stirred solution of **24** (3.53 g, 7.11 mmol) in EtOH (absolute) was added sodium borohydride (2.02 g, 53.5 mmol). Subsequently, KOH (0.81 g, 14.4 mmol) was added at once, and stirring was continued for 1 h. The reaction mixture was acidified to pH 4.0 with 1 N HCl and extracted with diethyl ether. Compound **4** was the side product of this reaction, and we were not able to find a successful purification method. Only a small amount of **25** was isolated (<1%): ^1H NMR (CDCl_3) δ 1.75–2.0 (m, 2 H, lactone), 2.42–2.53 (q, 1 H, $^3J = 3.5$ Hz, lactone), 2.68–3.0 (m, 4 H, dithiane), 3.90 (s, 3 H, OCH_3), 4.1–4.5 (m, 2 H, lactone), 5.96 (s, 2 H, OCH_2O), 6.52 (d, 1 H, $^3J_{AB} = 8$ Hz, Ar-H), 7.45 (d, 1 H, $^3J_{AB} = 8$ Hz, Ar-H); ^{13}C (CDCl_3) δ 24.7 (t), 27.2 (t), 30.22 (t), 32.1 (s), 48.1 (d), 59.4 (q), 69.0 (t), 101.8 (t), 102.0 (d), 125.1 (d), 125.4 (s), 139.3 (s), 141.9 (s), 149.2 (s), 176.5 (s); HRMS 354.60, calcd for $\text{C}_{16}\text{H}_{18}\text{O}_5\text{S}_2$ 354.60; MS (rel intensity) 354 (M^+ , 8), 271 (10), 270 (16), 269 (100), 251 (17), 233 (11), 221 (12), 195 (16).

Deoxypodophyllotoxin (23). Podophyllotoxin (1.3 g, 3.05 mmol) was dissolved in 70 mL of acetic acid, and 1.0 g of palladium (10% on carbon) was added. Podophyllotoxin was reduced under an atmosphere of hydrogen at 95°C during 48 h with stirring. Subsequently the reaction mixture was filtered, and ca. 100 mL of diethyl ether was added to the clear solution. The acetic acid was removed by washing the ether layer with a large amount of water (1.5 L). The ether layer was concentrated to ca. 10 mL and submitted to flash chromatography with diethyl ether as eluent. Removal of the solvent yielded a slightly yellow residue (1.0 g, 81%) consisting of pure deoxypodophyllotoxin: $[\alpha]_D -116.4^\circ$ ($c = 0.80$); ^1H NMR

(CDCl₃) δ 6.67 (s, 1 H, C5-H), 6.52 (s, 1 H, C2-H), 6.35 (s, 2 H, H-2' and H-6'), 5.95 (d, 1 H, $J = 1.3$ Hz, C10-H), 5.93 (d, 1 H, $J = 1.3$ Hz, C10-H), 4.6 (m, 1 H, C7-H), 4.5 (m, 1 H, C9-H), 3.9 (m, 1 H, C9-H), 3.81 (s, 3 H, 4'OCH₃), 3.75 (s, 6 H, 3' and 5'OCH₃), 3.08 (m, 1 H, C7'-H), 2.7–2.8 (m, 3 H, C8-H, C8'-H, and C7'-H); ¹³C NMR (CDCl₃) δ 175.0 (s), 152.4 (s), 146.9 (s), 146.6 (d), 136.8 (s), 136.3 (s), 130.5 (s), 128.3 (s), 110.3 (s), 108.4 (d), 108.2 (s), 101.1 (t), 72.0 (t), 60.6 (d), 56.1 (q), 47.3 (d), 43.7 (d), 32.9 (d), 32.7 (d); MS (rel intensity) 398 (M⁺, 100), 230 (23), 199 (16), 185 (67), 181 (100), 173 (84), 168 (43), 153 (40); MS (PCI, NH₃) m/e 399 (M + 1), 416 (M + NH₄⁺).

References

- (1) (a) Van Uden, W.; Homan, B.; Woerdenbag, H. J.; Pras, N.; Malingré, Th. M.; Wichers, H. J.; Harkes, M. Isolation, Purification, and Cytotoxicity of 5-Methoxypodophyllotoxin, a Lignan from a Root Culture of *Linum flavum*. *J. Nat. Prod.* **1992**, *55*, 102–110. (b) Van Uden, W.; Pras, N.; Malingré, Th. M. The Accumulation of Podophyllotoxin- β -D-glucoside by Cell Suspension Cultures Derived from the Conifer *Callitris drummondii*. *Plant Cell Rep.* **1990**, *9*, 257–260.
- (2) (a) Van Oeveren, A.; Jansen, J. F. G. A.; Feringa, B. L. Enantioselective Synthesis of Natural Dibenzylbutyrolactone Lignans (-)-Enterolactone, (-)-Hinokinin, (-)-Pluviatolide, (-)-Enterodiol, and Furofuran Lignan (-)-Eudesmin via Tandem Conjugate Addition to γ -Alkoxybutenolides. *J. Org. Chem.* **1994**, *59*, 5999–6007. (b) Jansen, J. F. G. A.; Feringa, B. L. Asymmetric 1,4-Additions to γ -Menthylxybutenolides (Part III). Enantioselective Synthesis of (-)-Eudesmin. *Tetrahedron Lett.* **1991**, *32*, 3239–3242. (c) Jansen, J. F. G. A. Stereoselective 1,4-Additions. Thesis, University of Groningen, 1991; 31.
- (3) Van Uden, W.; Bouma, A. S.; Bracht Waker, A. F.; Middel, O.; Wichers, H. J.; de Waard, P.; Woerdenbag, H. J.; Kellogg, R. M.; Pras, N. The Production of Podophyllotoxin and its 5-Methoxy Derivative through Bioconversion of Cyclodextrin-complexed

- Desoxypodophyllotoxin by Plant Cell Cultures. *Plant Cell, Tissue Organ Cult.* **1995**, in press.
- (4) Meijer, C.; Mulder, N. H.; Timmer-Bosscha, H.; Zijlstra, J. G.; de Vries, E. G. E. Role of Free Radicals in an Adriamycin Resistant Human Small Cell Lung Cancer Cell Line. *Cancer Res.* **1987**, *47*, 4613–4617.
 - (5) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. Evaluation of a Tetrazolium-based Colorimetric Assay: Assessment of Chemosensitivity Testing. *Cancer Res.* **1987**, *47*, 936–942.
 - (6) Kuhn, M.; von Wartburg, A. Über ein neues Glycosidierungsverfahren. Synthese von Epipodophyllotoxin- β -D-glucopyranosid. (Synthesis of Epipodophyllotoxin- β -D-glucopyranoside.) *Helv. Chim. Acta* **1968**, *51*, 1631–1636.
 - (7) Pelter, A.; Smith, K.; Brown, H. C. *Borane Reagents*, 1st ed.; Academic Press: New York, 1988; Chapter 4, pp 328–329.
 - (8) Reetz, M. T.; Urz, R.; Schuster, T. An Economical Large-Scale Synthesis of Titanium Tetrakis[diethylamide] and Chlorotitanium Tris[diethylamide]. *Synthesis* **1983**, 540.
 - (9) Corey, E. J.; Erickson, B. W. Oxidative Hydrolysis of 1,3-Dithiane Derivatives to Carbonyl Compounds Using N-Halosuccinimide Reagents. *J. Org. Chem.* **1971**, *36*, 3553–3560.
 - (10) Woerdenbag, H. J.; Moskal, T. A.; Pras, N.; Malingré, Th. M.; El-Ferally, F. S.; Kampinga, H. H.; Konings, A. W. T. Cytotoxicity of Artemisinin-related Endoperoxides to Ehrlich Ascites Tumor Cells. *J. Nat. Prod.* **1993**, *56*, 849–856.
 - (11) Ayres, D. C.; Loike, J. D. *Lignans: Chemical, Biological and Clinical Properties*, 1st ed.; Cambridge University Press: Cambridge, U.K., 1990; Chapter 3, pp 85–106.
 - (12) De Jong, J. C.; van Bolhuis, F.; Feringa, B. L. Asymmetric Diels-Alder Reactions with 5-Menthylxy-2(5H)-furanone. *Tetrahedron: Asymmetry* **1991**, *2*, 1247–1253.
 - (13) Baker, W.; Montgomery, L. V.; Smith, H. A. Synthesis of Derivatives of Myristicin. *J. Chem. Soc.* **1932**, 1281–1283.
 - (14) Hoffmann, R. W.; Ditrich, K. Oxidation of Arylmagnesium Bromides or Aryllithium to Phenols. *Synthesis* **1983**, 107–109.
 - (15) Van Speybroeck, R.; Guo, H.; van der Eyken, J.; Vandewalle, M. Enantioselective Total Synthesis of (-)-Epipodophyllotoxin and (-)-Podophyllotoxin. *Tetrahedron* **1991**, *47*, 4675–4686.
 - (16) Loiro, M.; Brown, E.; Robin, J. P. Synthèse Totale du (\pm)-Atenuol. (Total Synthesis of (\pm)-Atenuol.) *Tetrahedron* **1983**, *39*, 2795–2799.

JM9500131