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Diarylheptanoid Glucosides from *Pyrostria major* and Their Antiprotozoal Activities

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Eight new diarylheptanoid glucosides **1**–**8** have been isolated from the leaves of *Pyrostria major* (A. Rich & DC.) Cavaco [syn. *Canthium major* (A. Rich & DC.)] Cavaco. Their structures were determined by 1D and 2D NMR spectroscopic and HRMS analyses. The absolute configurations of the diarylheptanoids **1**–**4** were determined to be 3*S* and 5*S* by the application of the CD exciton chirality method to the corresponding 3,5-bis(*p*-bromobenzoyl) (**1**–**3**) and 3,5-bis(*p*-dimethylaminobenzoyl) (**4**) derivatives. Their antiparasitic activities against *Plasmodium falciparum*, *Leishmania donovani* (amastigote forms), and *Trypanosoma brucei* (trypomastigote bloodstream forms) as well as their cytotoxic activities against the HL-60, KB, and MRC5 cell lines of naturally occurring diarylheptanoid glucosides and their derivatives are reported.

Introduction

Neglected tropical diseases are a group of chronic, disabling, and disfiguring pathologies caused by parasitic, bacterial, and other infections. Among numerous parasitic diseases, malaria is the most important one in the world. It represents a major problem for public health services in tropical and subtropical regions of the planet. About 300 million people contract malaria each year, with 2–3 million dying annually,[1] mainly due to an increase in parasite resistance to available drugs and the failure to apply existing effective drugs in areas where they would be of greatest benefit. The introduction of new drugs, notably the artemisinin-based combination therapies or ACTs,[2] could replace ineffective treatments (chloroquine and sulfadoxine-pyrimethamine), but remain more expensive than single-agent treatments.[3] In addition, lately, there have been signs that the efficacy of ACTs and artesunate monotherapy have decreased in western Cambodia.[4] There is therefore an urgent need for the discovery of new efficient and cheap antimalarial drugs to cure this disease and to prevent the emergence of resistance. Recently, we screened a series of 40 plant extracts prepared from various species of the Madagascarian biodiversity for their inhibitory activity against blood stages of the *Plasmodium falciparum* strain. The results of this screening led us to investigate chemically the leaves of *Pyrostria major* (Rubiaceae). The ethyl acetate extract yielded, besides β-sitosterol as one of the major components, eight new diarylheptanoid glucosides **1**–**8** (Scheme 1), closely related to the previously reported *Tacca chantrieri* metabolites.[5] If β-sitosterol displayed moderate antiplasmodial activity, the diarylheptanoid glucosides were found to be inactive. However, we discovered by chance that minor structural modifications of these glucosides led to active compounds. Moreover, despite the fact that a large number of Rubiaceae species have been chemically investigated, so far no studies have revealed the presence of diarylheptanoids in this family. Naturally occurring diarylheptanoids are common in Zingiberaceae and Betulaceae, and are encountered in many small botanical families such as Myricaceae and Casuarinaceae.[6] They possess various biological activities, such as anti-inflammatory, antioxidant, and anti-tumoral properties.[7]

In this paper we describe the isolation, structure elucidation, and biological activities of the natural diarylheptanoid glucosides and their derivatives.

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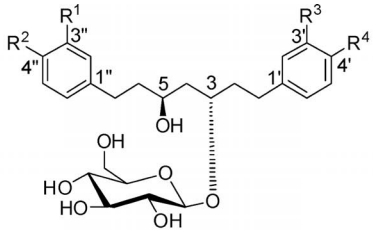
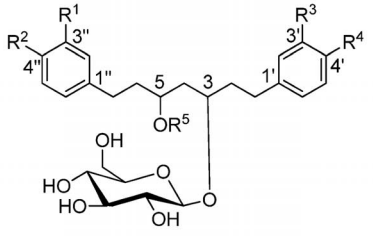
Results and Discussion

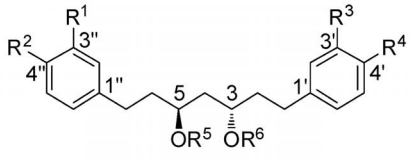
The leaves of *Pyrostria major* (375 g) were extracted with EtOAc to give a dry crude extract (13 g) after removal of the solvent. This extract exhibited a significant antiplasmodial activity (100% at 10 μg mL^{-1}) with no cytotoxicity against the MRC-5 cell line. It was subjected to silica gel chromatography using as eluent a gradient of CH_2Cl_2/



	R^1	R^2	R^3	R^4
1	H	OMe	H	OMe
2	H	OMe	OH	OMe
3	H	OMe	OH	OH
4	OH	OH	OH	OH



	R^1	R^2	R^3	R^4	R^5
5	OH	OMe	OMe	H	H
6	OH	OMe	OH	OH	H
7	H	OMe	OH	OH	CHO
8	OH	OH	OH	OH	CHO



	R^1	R^2	R^3	R^4	R^5	R^6
1a	H	OMe	H	OMe	H	H
1b	H	OMe	H	OMe	*p*-Br-Bz	*p*-Br-Bz
2a	H	OMe	OH	OMe	H	H
2b	H	OMe	OMe	OMe	H	H
2c	H	OMe	OMe	OMe	*p*-Br-Bz	*p*-Br-Bz
3a	H	OMe	OH	OH	H	H
4a	OH	OH	OH	OH	H	H
4b	OMe	OMe	OMe	OMe	H	H
4c	OMe	OMe	OMe	OMe	*p*-$N(CH_3)_2$-Bz	*p*-$N(CH_3)_2$-Bz

Scheme 1. Chemical structures of natural diarylheptanoid glucosides **1**–**8** and their derivatives **1a**, **1b**, **2a**–**2c**, **3a**, **4a**–**4c**.

MeOH (1:0 to 8:2) of increasing polarity, which led to 16 fractions. Only fraction 5, manly containing β-sitosterol, showed a significant antiplasmodial activity (100% at 1 μg mL^{-1}). All the fractions were then subjected to HPLC analysis to assess qualitatively and quantitatively their chemical content. Owing to their highly complex compositions, we decided to investigate chemically fractions 15 and 16, and subsequent HPLC preparative purification afforded compounds **1**–**8** (Figure 1).

Compounds **1**–**8** are closely related to the diarylheptanoids isolated from the rhizomes of *Tacca chantrieri*.[5] Indeed, they share several common spectral characteristics: The UV spectra show absorption bands at 223 and 278 nm, consistent with substituted aromatic rings, and the IR spectra exhibit bands arising from hydroxy groups (3350 cm^{-1}) and aromatic rings (1600 cm^{-1}). The ^{1}H NMR spectra show signals for substituted aromatic rings, an aliphatic moiety, and an anomeric proton belonging to a sugar moiety.

Compound **1** was obtained as an amorphous solid. HRESIMS indicates a $[M + Na]^+$ ion peak at m/z = 529.2406, which suggests a molecular formula of $C_{27}H_{38}O_9$. The ^{1}H NMR spectrum shows signals for two 1,4-disubstituted aromatic ring protons [δ_H = 6.80 (d, J = 8.5 Hz, 2 H), 6.81 (d, J = 8.5 Hz, 2 H), 7.09 (d, J = 8.5 Hz, 2 H), and 7.14 (d, J = 8.5 Hz, 2 H) ppm]. In addition, a signal for two methoxy groups [δ_H = 3.74 (s, 2 × 3 H, 4′-OMe and 4′′-OMe) ppm] and a signal due to an anomeric proton [δ_H = 4.39 (d, J = 7.8 Hz) ppm] are observed, which indicate the β configuration. Hydrolysis of **1** gave D-glucose and **1a**. D-Glucose, including its absolute configuration, was identified by direct supercritical fluid chromatography (SFC) analysis of the hydrolysate lyophilized against D- and L-glucose as references. The ^{13}C and DEPT 135 ^{13}C NMR spectra reveal signals for 12 sp^2 aromatic carbon atoms and 15 sp^3 carbon atoms, among which there are five methylenes and two hydroxymethines for the aliphatic moiety, one hydroxymethylene and five methines for the sugar moiety, and two methoxy groups, which suggests a diarylheptanoid glucoside structure. Each structural fragment constituting the C_7 unit of **1** was connected by analysis of the ^{1}H–^{1}H COSY, HMQC, and HMBC correlations. Finally, HMBC correlations of 2′′-H and 6′′-H to C-7, and of 2′-H and 6′-H to C-1 allowed the two aromatic rings to be located at the terminal methylene groups. Furthermore, the linkage position of D-glucose at C-3 can be determined by a long-range correlation from the anomeric proton at δ_H = 4.29 ppm to the C-3 carbon at δ_C = 78.3 ppm. All these connectivities are similar to those reported by Yokosuka et al.[5]

The absolute configurations of the 3,5-dihydroxy groups were determined by application of the CD exciton chirality method[8] to acyclic 1,3-dibenzoates.[9] Compound **1a** was converted into the corresponding 3,5-bis(*p*-bromobenzoate) derivative **1b**. Its CD spectrum shows positive (252.7 nm, $\Delta\varepsilon$ = +9.3 M^{-1} cm^{-1}) and negative (235.5 nm, $\Delta\varepsilon$ = –9.2 M^{-1} cm^{-1}) Cotton effects, which is consistent with a positive chirality. Thus, the absolute configurations at C-3 and C-5 were assigned as *S*. The structure of **1** was thus shown to be (3*S*,5*S*)-3,5-dihydroxy-1,7-bis(4-methoxyphenyl)heptyl 3-*O*-β-D-glucopyranoside.

Compound **2** was obtained as an amorphous solid. Its molecular formula was determined to be $C_{27}H_{38}O_{10}$ on the basis of the ion peak $[M + Na]^+$ at m/z = 545.2410 in the HRESIMS spectrum. The ^{1}H NMR spectrum shows aromatic signals for 1,3,4-trisubstituted and 1,4-disubstituted benzene rings, and signals for two methoxy groups. HMBC correlations were observed from the methoxy protons at δ_H = 3.72 ppm to C-4′′, and at δ_H = 3.78 ppm to C-4′. Acidic

hydrolysis of **2** gave **2a** and D-glucose. The location of the sugar at C-3 was confirmed by an HMBC correlation of the anomeric proton signal at δ_H = 4.39 ppm to C-3 at δ_C = 78.3 ppm. Compound **2a** was methylated to form the trimethylated derivative **2b**, which was then converted into the 3,5-bis(*p*-bromobenzoate) derivative **2c**. The CD spectrum of **2c** exhibits similar Cotton effects to **1b**. All of these data are consistent with the structure (3*S*,5*S*)-3,5-dihydroxy-1-(3-hydroxy-4-methoxyphenyl)-7-(4-methoxyphenyl)heptyl 3-*O*-β-D-glucopyranoside, which was assigned to compound **2**.

Compound **3** was obtained as an amorphous solid. The HRESIMS spectrum exhibits a $[M + Na]^+$ ion peak at *m*/*z* = 531.2285, which corresponds to the molecular formula $C_{26}H_{36}O_{10}$, lower by CH_2 than that of **2**. The ^1H NMR spectrum of **3** shows signals for a 1,3,4-trisubstituted aromatic ring, a 1,4-disubstituted aromatic ring, and a signal for only one methoxy group. An HMBC correlation between these protons and C-4′′ confirmed the location of the methoxy group. Acidic hydrolysis of **3** gave **3a** and D-glucose, and consecutive methylation of **3a** afforded **2b**. Consequently, **3** was identified as (3*S*,5*S*)-1-(3,4-dihydroxyphenyl)-3,5-dihydroxy-7-(4-methoxyphenyl)heptyl 3-*O*-β-D-glucopyranoside.

Compound **4** was obtained as an amorphous solid. Its molecular formula $C_{25}H_{34}O_{11}$ was deduced from the ion peak $[M + Na]^+$ at *m*/*z* = 533.2015 in the HRESIMS spectrum. The ^1H NMR spectrum of **4** exhibits a signal for an anomeric proton at δ_H = 4.39 (d, *J* = 7.8 Hz, 1 H) ppm and signals for six aromatic protons, but it did not exhibit any signals for methoxy groups. Furthermore, the ^{13}C NMR spectrum shows signals for four oxygenated sp^2 carbon atoms (δ_C = 144.2, 144.3, 146.1, and 146.2 ppm), which suggests the presence of two 3,4-dihydroxyphenyl groups. Acidic hydrolysis of **4** gave **4a** and D-glucose. Thus, the planar structure of **4** was identified as 1,7-bis(3,4-dihydroxyphenyl)-3,5-dihydroxyheptyl 3-*O*-β-D-glucopyranoside by comparison of its NMR spectroscopic data with those reported in the literature.[5] To assign the absolute configuration of compound **4**, **4a** was methylated to give derivative **4b**, which was converted into the 3,5-bis[*p*-(dimethylamino)-benzoate] derivative **4c**. Its CD spectrum exhibits positive (319.8 nm, $\Delta\varepsilon$ = +24.5 $M^{-1}cm^{-1}$) and negative (296 nm, $\Delta\varepsilon$ = –22.1 $M^{-1}cm^{-1}$) Cotton effects, which is consistent with a positive chirality by comparison with reported values.[10] The resulting exciton chirality interaction between the two (dimethylamino)benzoyloxy chromophores, in a redshifted region, provides an unambiguous configurational assignment.[11] Moreover, compounds **4a** and **4b** are enantiomers of the corresponding arylheptanoids described by Mimaki and co-workers,[5] as illustrated by their similar values and opposite signs of specific rotation. Thus, compound **4** is shown to be (3*S*,5*S*)-3,5-dihydroxy-1,7-bis(3,4-dihydroxyphenyl)heptyl 3-*O*-β-D-glucopyranoside.

Compound **5** was shown to have a molecular formula of $C_{27}H_{38}O_{10}$ on the basis of the ion peak $[M + Na]^+$ at *m*/*z* = 545.2404 in the HRESIMS spectrum, which is the same as that of **2**. In the HMBC spectrum, correlation was observed from the anomeric proton signal (δ_H = 4.39 ppm) to C-3 (δ_C = 78.4 ppm) of the heptane moiety. Other HMBC correlations indicate that **5** is an isomer of **2**. Unfortunately, the small amount of **5** did not allow us to determine its absolute configuration. However, compound **5** is supposed to have the same absolute configuration as compounds **1**–**4** on the basis of the same negative sign of their specific rotations. Thus, the structure was tentatively assigned as (3*S*,5*S*)-3,5-dihydroxy-7-(3-hydroxy-4-methoxyphenyl)-1-(4-methyoxyphenyl)heptyl 3-*O*-β-D-glucopyranoside.

The molecular formula $C_{26}H_{36}O_{11}$ of compound **6** was deduced from the $[M + Na]^+$ ion peak at *m*/*z* = 547.2166 in the HRESIMS spectrum. The ^1H NMR spectrum exhibits a signal for an anomeric proton at δ_H = 4.39 (d, *J* = 7.8 Hz, 1 H) ppm, signals for six aromatic protons, and a signal for a methoxy group. In addition, the ^{13}C NMR spectrum shows signals for four oxygenated sp^2 carbon atoms at δ_C = 144.2, 146.1, 147.2, and 147.5 ppm, which suggests the presence of two 1,3,4-trisubstituted aromatic rings. HMBC correlations between the anomeric proton and C-3 confirmed the location of the sugar moiety. The closeness of the carbon resonances of C-3′′ (δ_C = 147.5 ppm) and C-4′′ (δ_C = 147.2 ppm) makes it difficult to determine the attachment of the methoxy group. The NOESY correlation between the aromatic proton 5′′-H at δ_H = 6.80 ppm and the methoxy protons at δ_H = 3.81 ppm confirmed the location of this group at C-4′′. Thus, the planar structure of **6** was elucidated as 1-(3,4-dihydroxyphenyl)-3,5-dihydroxy-7-(3-hydroxy-4-methoxyphenyl)heptyl 3-*O*-β-D-glucopyranoside. The negative sign of the specific rotation of **6**, similar to compounds **1**–**4**, allowed us to tentatively assign the absolute configuration 3*S*,5*S* to compound **6**.

Compound **7** was obtained as an amorphous solid. Its molecular formula $C_{27}H_{36}O_{11}$ was deduced from the quasi-molecular peak $[M + Na]^+$ at *m*/*z* = 559.2241 in the HRESIMS spectrum. Comparison of the ^1H and DEPT 135 ^{13}C NMR spectra of **7** and **3** revealed that **7** is closely related to **3** but the spectra contain additional deshielded signals at δ_H = 5.19 (m, 1 H) and 8.12 (s, 1 H) ppm, and at δ_C = 163.5 ppm. These data, along with the presence of a strong IR absorption at 1710 cm^{-1}, clearly indicate the presence of a formyloxy group. The HMBC correlation between the formyl proton at δ_H = 8.12 ppm and the C-5 carbon at δ_C = 73.1 ppm confirmed that the formyloxy group is attached to carbon C-5. Compound **7** is therefore the formyl ester of **3** and the planar structure of **7** was assigned as 1-(3,4-dihydroxyphenyl)-5-formyl-3-hydroxy-7-(4-methoxyphenyl)heptyl 3-*O*-β-D-glucopyranoside. The negative sign of the specific rotation of **7**, similar to compounds **1**–**4**, allowed us to assign tentatively the absolute configuration 3*S*,5*S* to compound **7**.

Compound **8** ($C_{26}H_{34}O_{12}$) was assigned as a diarylheptanoid glucoside structurally related to **4**. The ^1H and ^{13}C NMR spectroscopic data for **8** are very similar to those of compound **4** but with signals for an additional formyl group, as was the case for **7**. The location of this group at C-5 was deduced from HMBC correlation between the formyl proton at δ_H = 8.12 ppm and the C-5 carbon at δ_C =

73.1 ppm. Other ^1H–^1H COSY and HMBC correlations are similar to those of compound **4** and confirm that compound **8** is 1,7-bis(3,4-dihydroxyphenyl)-5-formyl-3-hydroxyheptyl 3-*O*-β-D-glucopyranoside. The negative sign of the specific rotation of **8**, similar to compounds **1**–**4**, allowed us to assign tentatively the absolute configuration 3*S*,5*S* to compound **8**.

Naturally occurring diarylheptanoid glucosides and their derivatives were evaluated in vitro for their antiparasitic activity against *Plasmodium falciparum* (erythrocytic stage), *Leishmania donovani* (amastigote forms), and *Trypanosoma brucei* (trypomastigote bloodstream forms) as well as for their cytotoxic activity against the HL-60, KB, and MRC-5 cell lines (Table 1). The diarylheptanoid glucosides and their derivatives showed weak or no antiparasitic activity and no cytotoxic activity except for the derivatives **4b** and **4c** against *P. falciparum*, compound **2c** against the intramacrophage amastigotes of *L. donovani*, and compound **6**, which exhibited significant antitrypanosomal activity. Although the antiplasmodial activity is low, because compounds are considered interesting in vitro against *P. falciparum* with activities in the nanomolar range, the antileishmanial activity of compound **2c** and the antitrypanosomal activity of compound **6** against *T. brucei* in the micromolar range, compared with pentamidine, could justify further in vivo evaluation.

Table 1. Antiplasmodial, antileishmanial, and antitrypanosomal activities of compounds **1**–**8** and their derivatives.[a]

Compound	IC_{50} [μM]		
	P. falciparum strain FcB1	*L. donovani* LV9 intramacrophage amastigotes	*T. brucei* CMP strain, MEC
1	>20	39.7 ± 3.2	>100
1a	60.40 ± 0.92	53.0 ± 4.2	>100
1b	27.25 ± 7.25	35.0 ± 2.8	>100
2	>20	31.2 ± 2.5	>100
2a	40.78 ± 1.03	37.4 ± 2.9	50
2b	32.71 ± 3.47	66.8 ± 5.3	50
2c	16.45 ± 0.79	10.5 ± 0.8	25
3	>20	33.2 ± 2.6	>100
3a	25.39 ± 2.44	58.5 ± 4.7	25
4	>20	33.3 ± 2.6	25
4a	45.44 ± 2.31	67.5 ± 5.4	50
4b	4.1 ± 0.8	>100	50
4c	4.5 ± 0.2	26.1 ± 2.0	>100
5	>20	41.4 ± 3.3	>100
6	>20	>100	12.5
7	>20	33.0 ± 2.6	25
8	>20	34.6 ± 2.7	25
Chloroquine	0.05 ± 0.02	n.d.	n.d.
Pentamidine	n.d.	11.2 ± 1.3	3.1
Amphotericin B	n.d.	0.10 ± 0.02	n.d.

[a] Data are IC_{50} values in μm ± standard errors and are the average of three independent experiments unless indicated otherwise. FcB1: Chloroquine-resistant strain of *Plasmodium falciparum*; MEC: minimum effective concentration; n.d.: not done.

Although compounds **4b** and **4c** exhibited a moderate antiplasmodial activity (IC_{50} = 4.1 and 4.5 μM, respectively), they showed no cytotoxic activity against the MRC-5, KB, and HL-60 cell lines (at >100 μM for **4b** and >25 μM for **4c**), which makes **4b** a good candidate for the preparation of analogues for further study. Note that, in contrast to **4b**, its enantiomer was reported to be cytotoxic against the HL-60 cancer cell line[6] (IC_{50} = 12.1 μM). The lack of cytotoxic activity of saturated arylheptanoids was confirmed by Ishida et al.,[12] who showed that the saturation of the olefinic bonds of curcumin analogues eliminated cytotoxic activity. The antiplasmodial activity of the EtOAc leaves extract is probably due to the presence of β-sitosterol (IC_{50} = 2.1 μM) as a major constituent of the extract. This activity could be due to a stomatocytogenic effect, as was reported for lupeol.[13] Further investigation using transmission electron microscopy would be required to confirm this hypothesis.

Conclusions

A chemical investigation of the ethyl acetate extract of leaves of *Pyrostria major* led to the isolation of eight new diarylheptanoid glucosides **1**–**8**, described for the first time in a plant of the Rubiaceae family. Their absolute configurations were successfully determined by the CD exciton chirality[4] method. Surprisingly, this structural study has allowed us to obtain two selective antiplasmodial compounds **4b** and **4c** with no cytotoxicity, as well as a potential antileishmanial-active compound **2c**. Further structure–activity relationship studies, as well as in vivo experimentation, are expected to identify serious drug candidates for use against malaria and leishmaniasis.

Experimental Section

General Procedures: Optical rotations were measured at 25 °C with a JASCP P1010 polarimeter. IR spectra were recorded with a Nicolet FTIR 205 spectrophotometer. The UV spectra were recorded with a Perkin–Elmer Lambda 5 spectrophotometer. CD spectra were recorded at 25 °C with a JASCO J-810 spectropolarimeter using the software SpecDis version 1.51.[14] The NMR spectra were recorded with a Bruker Avance 500 instrument for all compounds except **2**–**4** for which a Bruker 300 MHz instrument was employed. CD_3OD or $CDCl_3$ was used as solvent. HRESIMS were recorded with a Thermoquest TLM LCQ Deca ion-trap spectrometer. Kromasil analytical and preparative C_{18} columns (250 × 4.6 mm, 250 × 21.2 mm i.d. 5 μm Thermo) were used for preparative HPLC separations using a "Waters autopurification system" equipped with a sample manager (Waters 2767), a column fluidics organizer, a binary pump (Waters 2525), a UV/Vis diode-array detector (190–600 nm, Waters 2996), and a PL-ELS 1000 ELSD Polymer Laboratory detector. SFC analyses were performed with a Thar Waters SFC Investigator II System using a photodiode array detector (Waters 2998), an ELS detector (Waters 2424), and a chiralpack analytical IA column (250 × 4.6 mm, 5 μM, Daicel Chemical industries). Silica gel 60 (6–35 μm) and analytical TLC plates (silica gel 60 F254) were purchased from SDS (France). All other chemicals and solvents were purchased from SDS (France).

Plant Material: Leaves of *Pyrostria major* were collected in the Mahanoro region of the Eastern coastal rainforest of Madagascar in September 2005. The plant was identified by Armand Rakotozafy

by comparison with an authentic specimen held in the Department of Botany, Parc Botanique et Zoologique de Tsimbazaza, Antananarivo. Botanical identification was confirmed by Dr. Sylvain Razafimandimbison (The Bergius Foundation, Royal Swedish Academy of Sciences, SE-10504, Stockholm, Sweden). A voucher specimen (MAD-0086) was deposited at the Institut Malgache de Recherches Appliqueés.

Extraction and Isolation: The plant material (dry wt., 375 g) was extracted with EtOAc three times (each 2 L) at 40 °C. The EtOAc extract was concentrated in vacuo at 40 °C to yield 13 g of residue. The EtOAc extract (10 g) was subjected to silica gel column chromatography by using a gradient of CH_2Cl_2/MeOH (1:0 to 8:2) of increasing polarity, which gave 16 fractions on the basis of TLC. Fraction 5 (63.2 mg) was identified as β-sitosterol. Fraction 15 (200 mg) was subjected to preparative C_{18} column chromatography by using the mobile phase MeOH/H_2O (60:40 to 100:0 + 0.1% formic acid) over 20 min at 21 mL min^{-1} to afford **1** (80 mg, 6.2×10^{-6}%), **2** (50 mg, 2.7×10^{-6}%), and **5** (4 mg, 7×10^{-7}%). Fraction 16 (400 mg) was subjected to preparative C_{18} chromatography by using MeOH/H_2O (40:60 to 70:30 + 0.1% formic acid) over 20 min at 21 mL min^{-1} to afford **3** (30 mg, 5.2×10^{-6}%), **4** (20 mg, 3.87×10^{-6}%), **6** (2 mg, 3.4×10^{-7}%), **7** (2 mg, 3.4×10^{-7}%), and **8** (2 mg, 3.4×10^{-7}%).

Compound 1: Amorphous solid. $[\alpha]_D^{25}$ = –39 (c = 0.05, MeOH). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 224 [4.36], 278 nm [3.6]. IR: $\tilde{\nu}_{max}$ = 1634, 1512, 1244, 1078, 710, 2155, 3220 cm^{-1}. ^{1}H NMR (CD_3OD, 500 MHz): δ_H = 1.61–1.75 (m, 4 H, 4-H_2, 6-H_2), 1.79 (m, 1 H, 2a-H), 1.93 (m, 1 H, 2b-H), 2.56 (m, 1 H, 7a-H), 2.64 (t, J = 8.5 Hz, 2 H, 1-H_2), 2.68 (m, 1 H, 7b-H), 3.19 (t, J = 8.5 Hz, 1 H, Glc-2), 3.26 (m, 1 H, Glc-5), 3.33 (m, 1 H, Glc-4), 3.34 (m, 1 H, Glc-3), 3.71 (d, J = 11.8, 5.5 Hz, 1 H, Glc-6a), 3.74 (s, 2 × 3 H, 2 × OMe), 3.87 (dd, J = 11.8, 2.7 Hz, 1 H, Glc-6b), 3.91 (m, 1 H, 5-H), 3.96 (m, 1 H, 3-H), 4.39 (d, J = 7.8 Hz, 1 H, Glc-1), 6.80 (d, J = 8.5 Hz, 2 H, 3′-H, 5′-H), 6.81 (d, J = 8.5 Hz, 2 H, 3′′-H, 5′′-H), 7.09 (d, J = 8.5 Hz, 2 H, 2′′-H, 6′′-H), 7.14 (d, J = 8.5 Hz, 2 H, 2′-H, 6′-H) ppm. ^{13}C NMR: see Table 2. HRMS (ESI): calcd. for $C_{27}H_{38}O_9Na$ [M + Na]$^+$ 529.2414; found 529.2406.

Acid Hydrolysis of 1: Compound **1** (23 mg) in 1 N HCl (10 mL) was heated at 100 °C for 10 h. The resulting hydrolysate was extracted with EtOAc (3 × 10 mL), dried (Na_2SO_4), and evaporated under reduced pressure to afford pure **1a** (14 mg). The aqueous phase was freeze-dried. The residue containing the sugar was analyzed by SFC under the following conditions: Co-solvent (%): MeOH (12%), CO_2 flow rate: 3.52 mL min^{-1}, co-solvent flow rate: 0.48 mL min^{-1}, total flow: 4 mL min^{-1}, detection: ELSD. The D-glucose present in the sugar fraction was identified by comparison of its retention time with those of authentic samples of D- and L-glucose [t_R = 5.20/6.20 (α/β form) and 4.50/5.50 min (α/β form), respectively].

Compound 1a: Amorphous solid. $[\alpha]_D^{25}$ = –3.7 (c = 0.05, $CHCl_3$). UV ($CHCl_3$): λ_{max} [log(ε/M^{-1} cm^{-1})] = 241 [3.2], 278 nm [3.5]. IR: $\tilde{\nu}_{max}$ = 1513, 1246, 3373, 761, 768 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H = 1.64 (t, J = 5.7 Hz, 2 H, 4-H_2), 1.67–1.84 (m, 4 H, 2-H_2, 6-H_2), 2.10 (br. s, 2 H, 3-OH, 5-OH), 2.56–2.72 (m, 4 H, 1-H_2, 7-H_2), 3.76 (s, 2 × 3 H, 2 × OMe), 3.95 (m, 2 H, 3-H, 5-H), 6.81 (d, J = 8.5 Hz, 4 H, 3′-H, 5′-H, 3′′-H, 5′′-H), 7.09 (d, J = 8.5 Hz, 4 H, 2′-H, 6′-H, 2′′-H, 6′′-H) ppm. ^{13}C NMR: see Table 2. HRMS (ESI): calcd. for $C_{21}H_{28}O_4Na$ [M + Na]$^+$ 367.1885; found 367.1884.

Bis(*p*-bromobenzoate) 1b of 1a: NEt_3 (32 μL) was added dropwise to a solution of **1a** (13 mg) in anhydrous CH_2Cl_2 (0.6 mL) containing DMAP (15 mg) as catalyst and *p*-bromobenzoyl chloride (40 mg), and the mixture was stirred at 60 °C for 9 h. The reaction mixture was diluted with H_2O (20 mL) and extracted with EtOAc (3 × 10 mL). After concentration of the organic phase, it was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (9.5:0.5) to yield **1b** (12 mg).

Table 2. ^{13}C NMR data for compounds **1**, **1a**, **2**, **2a**, **2b**, and **3a**.[a]

C atom		δ_C [ppm]						
		1	**1a**	**2**	**2a**	**2b**	**3**	**3a**
	1	31.6	31.4	31.8	31.7	32.0	31.9	32.5
	2	39.1	39.5	38.9	39.2	39.5	39.1	41.4
	3	78.3	69.1	78.3	69.1	69.2	78.4	69.8
	4	43.1	42.7	42.9	42.7	42.8	43.0	45.7
	5	68.4	69.1	68.4	69.1	69.2	68.5	68.9
	6	41.3	39.5	41.2	39.4	39.5	41.3	41.4
	7	32.3	31.4	32.2	31.4	31.4	32.3	32.2
	1′	136.0[b]	134.0	136.9	135.3	134.7	135.6[b]	135.4
	2′	130.5	129.4	116.8	114.8	111.9	116.9	116.7
	3′	114.9	114.1	147.3	145.7	149.1	146.1	146.2
	4′	159.4	158.0	147.1	145.0	147.5	144.2	144.3
	5′	114.9	114.1	113.0	110.9	111.5	116.4	116.4
	6′	130.5	129.4	120.8	119.9	120.3	120.9	120.8
	1′′	135.8[b]	134.0	135.8	134.1	134.0	135.8[b]	135.8
	2′′	130.4	129.4	130.4	129.4	129.4	130.4	130.4
	3′′	114.8	114.1	114.9	114.1	114.1	114.9	114.9
	4′′	159.3	158.0	159.3	158.0	158.1	159.4	159.7
	5′′	114.8	114.1	114.9	114.1	114.1	114.9	114.9
	6′′	130.4	129.4	130.4	129.4	129.4	130.4	130.4
Glc	1	104.4		104.2			104.3	
	2	75.6		75.5			75.6	
	3	78.3		78.1			78.4	
	4	71.8		71.7			71.8	
	5	78.0		78.0			78.0	
	6	62.9		62.9			62.5	
	3′-OMe					56.0		
	4′-OMe	55.7	55.4	56.6	56.2	56.1		
	3′′-OMe							
	4′′-OMe	55.7	55.4	55.7	55.4	55.5	55.8	55.7

[a] Spectra recorded in CD_3OD (**1**–**3**, **3a**) or $CDCl_3$ (**1a**, **2a**, **2b**) at 75 (**2**) and 125 MHz (**1**–**3**, **1a**, **2a**, **2b**, **3a**). [b] May be interchanged.

Compound 1b: Amorphous solid. $[\alpha]_D^{25}$ = +10 (c = 0.1, $CHCl_3$). UV ($CHCl_3$): λ_{max} [log(ε/M^{-1} cm^{-1})] = 318 [3.6], 247 [4.3], 228 nm [4.1]. CD (EtOH): λ_{max} ($\Delta\varepsilon$) = 252.7 (+9.31), 235.6 nm (–9.22 M^{-1} cm^{-1}). IR: $\tilde{\nu}_{max}$ = 2930, 2864, 1716, 1589, 1513, 1032, 1011, 848, 809, 682 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H = 1.86–2.08 (m, 4 H, 2-H_2, 6-H_2), 2.10 (m, 2 H, 4-H_2), 2.59 (m, 4 H, 1-H_2, 7-H_2), 3.73 (s, 2 × 3 H, 2 × OMe), 5.23 (m, 2 H, 3-H, 6-H), 6.75 (d, J = 8.5 Hz, 4 H, 3′-H, 5′-H, 3′′-H, 5′′-H), 7.02 (d, J = 8.5 Hz, 4 H, 2′-H, 6′-H, 2′′-H, 6′′-H), 7.43 (d, J = 8.5 Hz, 4 H), 7.68 (d, J = 8.5 Hz, 4 H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C = 30.9 (t, C-1, C-7), 36.4 (t, C-2, C-6), 38.2 (t, C-4), 55.4 (q, 4′-OMe, 4′′-OMe), 71.5 (d, C-3, C-5), 114.1 (d, C-3′, C-5′, C-3′′, C-5′′), 129.4 (d, C-2′, C-6′, C-2′′, C-6′′), 133.2 (s, C-1′, C-1′′), 158.0 (s, C-4′, C-4′′); *p*-bromobenzoate substituents: 128.1 (2 s), 129.3 (2 s), 131.2 (4 d), 131.7 (4 d), 165.4 (2 s) ppm. HRMS (ESI): calcd. for $C_{35}H_{34}Br_2O_6Na$ [M + Na]$^+$ 731.0620; found 731.0616.

Compound 2: Amorphous solid. $[\alpha]_D^{25}$ = –31 (c = 0.17, MeOH). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 224 [4.36], 278 nm [3.7]. IR: $\tilde{\nu}_{max}$ = 3291, 1512, 1432, 1364, 1271, 1232, 1078, 1033, 815 cm^{-1}. ^{1}H NMR (CD_3OD, 300 MHz): δ_H = 1.59–1.74 (m, 4 H, 4-H_2, 6-H_2), 1.75 (m, 1 H, 2a-H), 1.94 (m, 1 H, 2b-H), 2.51–2.73 (m, 4 H, 7-H_2, 1-H_2), 3.19 (t, J = 8.5 Hz, 1 H, Glc-2), 3.26 (m, 1 H, Glc-5), 3.33 (m, 1 H, Glc-4), 3.35 (m, 1 H, Glc-3), 3.71 (dd, J = 11.8, 5.5 Hz, 1 H, Glc-6a), 3.72 (s, 3 H, 4′′-OMe), 3.78 (s, 3 H, 4′-OMe),

3.87 (dd, J = 11.8, 2.7 Hz, 1 H, Glc-6b), 3.91 (m, 1 H, 5-H), 3.96 (m, 1 H, 3-H), 4.39 (d, J = 7.8 Hz, 1 H, Glc-1), 6.63 (dd, J = 8.5, 2 Hz, 1 H, 6′-H), 6.73 (d, J = 2 Hz, 1 H, 2′-H), 6.78 (d, J = 8.5 Hz, 1 H, 5′-H), 6.79 (d, J = 8.5 Hz, 2 H, 3′′-H, 5′′-H), 7.07 (d, J = 8.5 Hz, 2 H, 2′′-H, 6′′-H) ppm. ^{13}C NMR, see Table 2. HRMS (ESI): calcd. for $C_{27}H_{38}O_{10}Na$ [M + Na]$^+$ 545.2363; found 545.2410.

Acid Hydrolysis of 2: Compound **2** (30 mg) was hydrolyzed by the same procedure as described for **1** to yield **2a** (22 mg). SFC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucose.

Compound 2a: Amorphous solid. $[\alpha]_D^{25}$ = –10 (c = 0.1, $CHCl_3$). UV ($CHCl_3$): λ_{max} [log(ε/M^{-1} cm^{-1})] = 279 nm [3.6]. IR: $\tilde{\nu}_{max}$ = 3407, 1590, 1514, 812, 764 cm^{-1}. ^{1}H NMR (CDCl3, 500 MHz): δ_H = 1.62 (t, J = 5.7 Hz, 2 H, 4-H$_2$), 1.66–1.83 (m, 4 H, 2-H$_2$, 6-H$_2$), 2.51–2.71 (m, 4 H, 1-H$_2$, 7-H$_2$), 3.76 (s, 3 H, 4′′-OMe), 3.83 (s, 3 H, 4′-OMe), 3.94 (m, 2 H, 3-H, 5-H), 6.64 (dd, J = 8.5, 2 Hz, 1 H, 6′-H), 6.74 (d, J = 8.5 Hz, 1 H, 5′-H), 6.75 (d, J = 2 Hz, 1 H, 2′-H), 6.79 (d, J = 8.5 Hz, 2 H, 3′′-H, 5′′-H), 7.08 (d, J = 8.5 Hz, 2 H, 2′′-H, 6′′-H) ppm. ^{13}C NMR, see Table 2. HRMS (ESI): calcd. for $C_{21}H_{28}O_5Na$ [M + Na]$^+$ 383.1834; found 383.1816.

Methylation of 2a: Compound **2a** (20 mg) and Cs_2CO_3 (13 mg) were dissolved in THF (0.5 μL). A large excess of CH_3I was added to the reaction mixture, which was stirred at 50 °C for 9 h. The reaction mixture was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (9.5:0.5) to yield **2b** (12 mg).

Compound 2b: Amorphous solid. $[\alpha]_D^{25}$ = –2.5 (c = 0.07, MeOH). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 225 [4.8], 279 nm [4.3]. IR: $\tilde{\nu}_{max}$ = 3407, 1611, 1590, 1155, 1030, 812, 764 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H = 1.65 (t, J = 5.7 Hz, 2 H, 4-H$_2$), 1.68–1.84 (m, 4 H, 2-H$_2$, 6-H$_2$), 2.56–2.73 (m, 4 H, 1-H$_2$, 7-H$_2$), 3.76 (s, 3 H, 4′′-OMe), 3.83 (s, 3 H, 4′-OMe), 3.84 (s, 3 H, 3′-OMe), 3.96 (m, 2 H, 3-H, 5-H), 6.70 (d, J = 2 Hz, 1 H, 2′-H), 6.71 (dd, J = 8.5, 2.0 Hz, 1 H, 6′-H), 6.77 (d, J = 8.5 Hz, 1 H, 5′-H), 6.81 (d, J = 8.5 Hz, 2 H, 3′′-H, 5′′-H), 7.09 (d, J = 8.5 Hz, 2 H, 2′′-H, 6′′-H) ppm. ^{13}C NMR, see Table 2. HRMS (ESI): calcd. for $C_{21}H_{28}O_5Na$ [M + Na]$^+$ 383.1834; found 383.1816.

Bis(*p*-bromobenzoate) 2c of 2b: Compound **2b** (6 mg) was treated by the same procedure as for **1a** to yield **2c** (6 mg).

Compound 2c: Amorphous solid. $[\alpha]_D^{25}$ = +30 (c = 0.1, $CHCl_3$). UV ($CHCl_3$): λ_{max} [log(ε/M^{-1} cm^{-1})] = 225 [4.3], 246 nm [4.6]. CD (EtOH): λ_{max} ($\Delta\varepsilon$) = 253 nm (+15.5), 237 nm (–11.77 M^{-1} cm^{-1}). IR: $\tilde{\nu}_{max}$ = 2930, 1720, 1513, 1480, 1270, 1140, 1016, 809, 755, 682 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H = 1.86–2.08 (m, 4 H, 2-H$_2$, 6-H$_2$), 2.10 (m, 2 H, 4-H$_2$), 2.59 (m, 4 H, 1-H$_2$, 7-H$_2$), 3.73 (s, 3 H, 4′′-OMe), 3.79 (s, 3 H, 4′-OMe), 3.80 (s, 3 H, 3′-OMe), 5.26 (m, 2 H, 3-H, 6-H), 6.63 (dd, J = 8.5, 2.0 Hz, 1 H, 6′-H), 6.64 (d, J = 2.0 Hz, 1 H, 2′-H), 6.70 (d, J = 8.5 Hz, 1 H, 5′-H), 6.75 (d, J = 8.5 Hz, 2 H, 3′′-H, 5′′-H), 7.02 (d, J = 8.5 Hz, 2 H, 2′′-H, 6′′-H), 7.43 (d, J = 8.5 Hz, 4 H), 7.68 (d, J = 8.5 Hz, 4 H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C = 30.9 (t, C-7), 31.4 (t, C-1), 36.4 (t, C-2, C-6), 38.3 (t, C-4), 55.4 (q, 4′′-OMe), 56.0 (q, 4′-OMe), 56.1 (q, 3′-OMe), 71.4–71.5 (d, C-3, C-5), 111.4 (d, C-5′), 111.9 (d, C-2′), 114.0 (d, C-3′′,C-5′′), 120.3 (d, C-6′), 129.4 (d, C-2′′, C-6′′), 133.2 (s, C-1′′), 133.7 (s, C-1′), 147.5 (s, C-3′), 149.1 (s, C-4′), 158.1 (s, C-4′′); *p*-bromobenzoate substituents: 128.2 (2 s), 129.3 (2 s), 131.2 (4 d), 131.7 (4 d), 165.4 (2 s) ppm. HRMS (ESI): calcd. for $C_{36}H_{36}Br_2O_7Na$ [M + Na]$^+$ 761.0725; found 761.0761.

Compound 3: Amorphous solid. $[\alpha]_D^{25}$ = –10 (c = 0.13, MeOH). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 222 [4.2], 284 nm [3.6]. IR: $\tilde{\nu}_{max}$ = 3291, 1512, 1432, 1364, 1271, 1232, 1078, 1033, 815 cm^{-1}. ^{1}H NMR (CD_3OD, 300 MHz): δ_H = 1.59–1.72 (m, 4 H, 4-H$_2$, 6-H$_2$), 1.75 (m, 1 H, 2a-H), 1.94 (m, 1 H, 2b-H), 2.51–2.61 (m, 3 H, 7a-H, 1-H$_2$), 2.67 (m, 1 H, 7b-H), 3.20 (t, J = 8.5 Hz, 1 H, Glc-2), 3.30 (m, 1 H, Glc-5), 3.33 (m, 1 H, Glc-4), 3.36 (m, 1 H, Glc-3), 3.71 (dd, J = 11.8, 5.5 Hz, 1 H, Glc-6a), 3.74 (s, 3 H, 4′′-OMe), 3.89 (dd, J = 11.8, 2.7 Hz, 1 H, Glc-6b), 3.90 (m, 1 H, 5-H), 3.96 (m, 1 H, 3-H), 4.39 (d, J = 7.8 Hz, 1 H, Glc-1), 6.52 (dd, J = 8, 2 Hz, 1 H, 6′-H), 6.66 (d, J = 8 Hz, 1 H, 5′-H), 6.71 (d, J = 2 Hz, 1 H, 2′-H), 6.81 (d, J = 8.5 Hz, 2 H, 3′′-H, 5′′-H), 7.08 (d, J = 8.5 Hz, 2 H, 2′′-H, 6′′-H) ppm. ^{13}C NMR, see Table 2. HRMS (ESI): calcd. for $C_{26}H_{36}O_{10}Na$ [M + Na]$^+$ 531.2206; found 531.2285.

Acid Hydrolysis of 3: Compound **3** (30 mg) was hydrolyzed by the same procedure as described for **1** to yield **3a** (22 mg). SFC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucose.

Compound 3a: Amorphous solid. $[\alpha]_D^{25}$ = –10 (c = 0.1, MeOH). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 222 [4.2], 284 nm [3.6]. IR: $\tilde{\nu}_{max}$ = 3340, 1513, 1246, 824, 797 cm^{-1}. ^{1}H NMR (CD_3OD, 500 MHz): δ_H = 1.54 (t, J = 6.2 Hz, 2 H, 4-H$_2$), 1.68 (m, 4 H, 2-H$_2$, 6-H$_2$), 2.46–2.71 (m, 4 H, 1-H$_2$, 7-H$_2$), 3.74 (s, 3 H, 4′-OMe), 3.80 (m, 2 H, 3-H, 5-H), 6.50 (dd, J = 8.5, 2 Hz, 1 H, 6′-H), 6.63 (d, J = 2 Hz, 1 H, 2′-H), 6.66 (d, J = 8.5 Hz, 1 H, 5′-H), 6.80 (d, J = 8.5 Hz, 2 H, 3′-H, 5′-H), 7.09 (d, J = 8.5 Hz, 2 H, 2′′-H, 6′′-H) ppm. ^{13}C NMR, see Table 2. HRMS (ESI): calcd. for $C_{20}H_{26}O_5Na$ [M + Na]$^+$ 369.1678; found 369.1678.

Methylation of 3a: Compound **3a** (7 mg) was methylated by the same procedure as described for **2** to yield **2b** (4 mg).

Compound 4: Amorphous solid. $[\alpha]_D^{25}$ = –3.7 (c = 0.1, MeOH). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 227 [3.6], 281 nm [3.5]. IR: $\tilde{\nu}_{max}$ = 1634, 1512, 1244, 1078, 710, 2155, 3220 cm^{-1}. ^{1}H NMR (CD_3OD, 300 MHz): δ_H = 1.57–1.81 (m, 5 H, 4-H$_2$, 6-H$_2$, 2a-H), 1.93 (m, 1 H, 2b-H), 2.46–2.67 (m, 4 H, 7-H$_2$, 1-H$_2$), 3.20 (t, J = 8.5 Hz, 1 H, Glc-2), 3.28 (m, 1 H, Glc-5), 3.33 (m, 1 H, Glc-4), 3.37 (m, 1 H, Glc-3), 3.71 (dd, J = 11.8, 5.5 Hz, 1 H, Glc-6a), 3.89 (dd, J = 11.8, 2.7 Hz, 1 H, Glc-6b), 3.93 (m, 1 H, 5-H), 3.96 (m, 1 H, 3-H), 4.39 (d, J = 7.8 Hz, 1 H, Glc-1), 6.50 (dd, J = 8, 2 Hz, 1 H, 6′′-H), 6.53 (dd, J = 8, 2 Hz, 1 H, 6′-H), 6.63 (d, J = 2 Hz, 1 H, 2′′-H), 6.66 (d, J = 8 Hz, 2 H, 5′-H, 5′′-H), 6.70 (d, J = 2 Hz, 1 H, 2′-H) ppm. ^{13}C NMR, see Table 3. HRMS (ESI): [M + Na]$^+$ calcd. for $C_{25}H_{34}O_{11}Na$ 533.1999; found 533.2015.

Acid Hydrolysis of 4: Compound **4** (27 mg) was hydrolyzed by the same procedure as described for **1** to yield **4a** (15 mg). SFC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucose.

Compound 4a: Amorphous solid. $[\alpha]_D^{25}$ = –10 (c = 0.1, MeOH). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 221 [4.0], 283 nm [3.7]. IR: $\tilde{\nu}_{max}$ = 3440, 1519, 1370, 1058, 815, 782 cm^{-1}. ^{1}H NMR (CD_3OD, 500 MHz): δ_H = 1.53 (t, J = 6.2 Hz, 2 H, 4-H$_2$), 1.67 (m, 4 H, 2-H$_2$, 6-H$_2$), 2.46–2.64 (m, 4 H, 1-H$_2$, 7-H$_2$), 3.80 (m, 2 H, 3-H, 5-H), 6.51 (dd, J = 8.5, 2 Hz, 2 H, 6′-H, 6′′-H), 6.63 (d, J = 2 Hz, 2 H, 2′-H, 2′′-H), 6.66 (d, J = 8.5 Hz, 2 H, 5′-H, 5′′-H) ppm. ^{13}C NMR, see Table 3. HRMS (ESI): calcd. for $C_{19}H_{25}O_6$ [M + H]$^+$ 349.1651; found 349.1653.

Methylation of 4a: Compound **4a** (11 mg) was methylated by the same procedure as described for **2** to yield **4b** (5 mg).

Compound 4b: Amorphous solid. $[\alpha]_D^{25}$ = –20 (c = 0.1, MeOH). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 228 [5.0], 279 nm [4.6]. IR: $\tilde{\nu}_{max}$ = 3280, 1600, 1580, 1420, 1145, 1020, 803, 787, 765 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H = 1.65 (t, J = 5.7 Hz, 2 H, 4-H$_2$), 1.70–1.86

Table 3. ^{13}C NMR data for compounds **4**, **4a**, **4b**, and **5**–**8**.[a]

	C atom	4	4a	4b	5	6	7	8
	1	31.8	32.5	32.0	31.6	31.9	31.6	31.7
	2	39.1	41.4	39.5	39.2	39.2	38.8	38.9
	3	78.4	68.9	69.2	78.4	78.4	77.1	77.2
	4	43.0	45.7	42.8	42.0	43.1	40.6	40.7
	5	68.5	68.9	69.2	68.4	68.5	73.1	73.3
	6	41.3	41.4	39.5	41.2	41.2	38.0	38.1
	7	32.5	32.5	32.0	32.5	32.6	31.7	32.0
	1′	135.6[b]	135.4	134.6	136.0	135.6	135.4	135.4
	2′	116.9	116.7	112.0	130.5	116.9	117.0	116.9
	3′	146.1	146.2	149.1	114.8	146.1	146.2	146.3
	4′	144.3	144.2	147.5	159.3	144.2	144.3	144.3
	5′	116.5	116.4	111.6	114.8	116.4	116.4	116.5
	6′	120.9	120.8	120.4	130.5	120.9	121.0	120.8
	1′′	135.5[b]	135.4	134.6	136.9	136.9	134.9	134.5
	2′′	116.7	116.7	112.0	116.6	116.7	130.4	116.7
	3′′	146.2	146.2	149.1	114.8	147.5	115.0	146.1
	4′′	144.2	144.2	147.5	147.2	147.2	159.6	144.5
	5′′	116.4	116.4	111.6	113.1	113.1	115.0	116.4
	6′′	120.7	120.8	120.4	120.6	120.7	130.4	121.0
Glc	1	104.3			104.4	104.3	104.0	104.1
	2	75.6			75.6	75.7	75.5	75.6
	3	78.3			78.3	78.3	78.3	78.3
	4	71.7			71.8	71.8	71.8	71.8
	5	78.0			78.0	78.1	78.0	78.0
	6	62.9			62.9	63.0	62.9	62.9
	3′-OMe			56.0				
	4′-OMe			56.1	56.6	56.7		
	3′′-OMe			56.0				
	4′′-OMe			56.1	55.7		55.8	
	5-CHO						163.5	163.5

[a] Spectra recorded in CD_3OD (**4**–**8**, **4a**) or $CDCl_3$ (**4b**) at 75 (**4**) and 125 MHz (**5**–**8**, **4a**, **4b**). [b] Assignments may be interchanged.

(m, 4 H, 2-H_2, 6-H_2), 2.54–2.74 (m, 4 H, 1-H_2, 7-H_2), 3.83 (s, 2 × 3 H, 4′-OMe, 4′′-OMe), 3.85 (s, 2 × 3 H, 3′-OMe, 3′′-OMe), 3.98 (m, 2 H, 3-H, 5-H), 6.70 (d, J = 2 Hz, 2 H, 2′-H, 2′′-H), 6.71 (dd, J = 8.5, 2 Hz, 2 H, 6′-H, 6′′-H), 6.77 (d, J = 8.5 Hz, 2 H, 5′-H, 5′′-H) ppm. ^{13}C NMR, see Table 3. HRMS (ESI): calcd. for $C_{23}H_{32}O_6Na$ [M + Na]$^+$ 427.2097; found 427.2084.

Bis[*p*-(dimethylamino)benzoate] (4c) of 4b: NEt_3 (10 μL) was added dropwise to a solution of **4b** (4 mg) in anhydrous CH_2Cl_2 (0.6 mL) containing DMAP (15 mg) as catalyst and *p*-(dimethylamino)benzoyl chloride (10 mg), and the mixture was stirred at 60 °C for 9 h. The reaction mixture was diluted with H_2O (20 mL) and extracted with EtOAc (3 × 10 mL). After concentration of the organic phase, it was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (9.5:0.5) to yield **4c** (2 mg).

Compound 4c: Amorphous solid. $[a]_D^{25}$ = +30 (c = 0.07, EtOH). UV (EtOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 229 [4.4], 311 nm [4.7]. CD (EtOH): λ_{max} ($\Delta\varepsilon$) = 319.8 (+24.5), 296 nm (–22.1 M^{-1} cm^{-1}). IR: $\tilde{\nu}_{max}$ = 2989, 1702, 1435, 1020, 803, 787, 765 cm^{-1}. NMR spectroscopic data: see ref.[10]. HRMS (ESI): calcd. for $C_{41}H_{54}N_3O_8$ [M + NH_4]$^+$ 716.3911; found 716.3916.

Compound 5: Amorphous solid. $[a]_D^{25}$ = –6 (c = 0.05, MeOH). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 223 [4.7], 278 nm [3.25]. IR: $\tilde{\nu}_{max}$ = 3291, 1512, 1432, 1364, 1271, 1232, 1078, 1033, 815 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz): δ_H = 1.60–1.77 (m, 4 H, 4-H_2, 6-H_2), 1.79 (m, 1 H, 2a-H), 1.94 (m, 1 H, 2b-H), 2.51 (m, 1 H, 7a-H), 2.62 (m, 1 H, 7b-H), 2.64 (t, J = 7.7 Hz, 2 H, 1-H_2), 3.19 (t, J = 8.5 Hz, 1 H, Glc-2), 3.26 (m, 1 H, Glc-5), 3.33 (m, 1 H, Glc-4), 3.35 (m, 1 H, Glc-3), 3.71 (dd, J = 11.8, 5.5 Hz, 1 H, Glc-6a), 3.74 (s, 3 H, 4′-OMe), 3.80 (s, 3 H, 4′′-OMe), 3.87 (dd, J = 11.8, 2.7 Hz, 1 H, Glc-6b), 3.90 (m, 1 H, 5-H), 3.95 (m, 1 H, 3-H), 4.39 (d, J = 7.8 Hz, 1 H, Glc-1), 6.62 (dd, J = 8.5, 2 Hz, 1 H, 6′′-H), 6.66 (d, J = 2 Hz, 1 H, 2′′-H), 6.80 (d, J = 8.5 Hz, 1 H, 5′′-H), 6.81 (d, J = 8.5 Hz, 2 H, 3′-H, 5′-H), 7.12 (d, J = 8.5 Hz, 2 H, 2′-H, 6′-H) ppm. ^{13}C NMR, see Table 3. HRMS (ESI): calcd. for $C_{27}H_{38}O_{10}Na$ [M + Na]$^+$ 545.2363; found 545.2404.

Compound 6: Amorphous solid. $[a]_D^{25}$ = –16 (c = 0.016, MeOH). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 221 [4.1], 282 nm [3.7]. IR: $\tilde{\nu}_{max}$ = 3291, 1512, 1432, 1364, 1271, 1232, 1078, 1033, 815 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz): δ_H = 1.60–1.72 (m, 4 H, 4,-H_2, 6-H_2), 1.74 (m, 1 H, 2a-H), 1.93 (m, 1 H, 2b-H), 2.48–2.66 (m, 4 H, 1-H_2, 7-H_2), 3.19 (t, J = 8.5 Hz, 1 H, Glc-2), 3.28 (m, 1 H, Glc-5), 3.33 (m, 1 H, Glc-4), 3.37 (m, 1 H, Glc-3), 3.71 (dd, J = 11.8, 5.5 Hz, 1 H, Glc-6a), 3.81 (s, 3 H, 4′′-OMe), 3.89 (dd, J = 11.8, 2.7 Hz, 1 H, Glc-6b), 3.90 (m, 1 H, 5-H), 3.95 (m, 1 H, 3-H), 4.39 (d, J = 7.8 Hz, 1 H, Glc-1), 6.53 (dd, J = 8.5, 2.0 Hz, 1 H, 6′-H), 6.61 (dd, J = 8.5, 2.0 Hz, 1 H, 6′′-H), 6.65 (d, J = 8.5 Hz, 1 H, 5′-H), 6.66 (d, J = 2.0 Hz, 1 H, 2′′-H), 6.69 (d, J = 2.0 Hz, 1 H, 2′-H), 6.80 (d, J = 8.5 Hz, 1 H, 5′′-H) ppm. ^{13}C NMR, see Table 3. HRMS (ESI): calcd. for $C_{26}H_{36}O_{11}Na$ [M + Na]$^+$ 547.2155; found 547.2166.

Compound 7: Amorphous solid. $[a]_D^{25}$ = –10 (c = 0.032, MeOH). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 223 [4.1], 279 nm [3.6]. IR: $\tilde{\nu}_{max}$ = 3291, 1512, 1432, 1364, 1271, 1232, 1078, 1033, 815 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz): δ_H = 1.75 (m, 1 H, 2a-H), 1.80–1.95 (m, 5 H, 4-H_2, 6-H_2, 2b-H), 2.49–2.60 (m, 4 H, 1-H_2, 7-H_2), 3.20 (t, J = 8.5 Hz, 1 H, Glc-2), 3.23 (m, 1 H, Glc-5), 3.32 (m, 1 H, Glc-4), 3.34 (m, 1 H, Glc-3), 3.71 (dd, J = 11.8, 5.5 Hz, 1 H, Glc-6a), 3.74 (m, 1 H, 3-H), 3.75 (s, 3 H, 4′′-OMe), 3.87 (dd, J = 11.8, 2.7 Hz, 1 H, Glc-6b), 4.39 (d, J = 7.8 Hz, 1 H, Glc-1), 5.19 (m, 1 H, 5-H), 6.52 (dd, J = 8.0, 2.0 Hz, 1 H, 6′-H), 6.66 (d, J = 8.0 Hz, 1 H, 5′-H), 6.70 (d, J = 2.0 Hz, 1 H, 2′-H), 6.82 (d, J = 8.5, 2.0 Hz, 2 H, 3′′-H, 5′′-H), 7.06 (d, J = 8.5, 2.0 Hz, 2 H, 2′′-H, 6′′-H), 8.12 (s, 1 H, CHO-5) ppm. ^{13}C NMR, see Table 3. HRMS (ESI): calcd. for $C_{27}H_{36}O_{11}Na$ [M + Na]$^+$ 559.2255; found 559.2241.

Compound 8: Amorphous solid. $[a]_D^{25}$ = –61 (c = 0.024, MeOH). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 222 [3.9], 281 nm [3.6]. IR: $\tilde{\nu}_{max}$ = 3409, 1660, 1069 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz): δ_H = 1.75 (m, 1 H, 2a-H), 1.80–1.95 (m, 5 H, 4-H_2, 6-H_2, 2b-H), 2.49–2.60 (m, 4 H, 1-H_2, 7-H_2), 3.18 (t, J = 8.5 Hz, 1 H, Glc-2), 3.23 (m, 1 H, Glc-5), 3.32 (m, 1 H, Glc-4), 3.34 (m, 1 H, Glc-3), 3.71 (dd, J = 11.8, 5.5 Hz, 1 H, Glc-6a), 3.72 (m, 1 H, 3-H), 3.87 (dd, J = 11.8, 2.7 Hz, 1 H, Glc-6b), 4.39 (d, J = 7.8 Hz, 1 H, Glc-1), 5.19 (m, 1 H, 5-H), 6.47 (dd, J = 8.0, 2.0 Hz, 1 H, 6′′-H), 6.52 (dd, J = 8.0, 2.0 Hz, 1 H, 6′-H), 6.60 (d, J = 2.0 Hz, 2′′-H), 6.65 (d, J = 8.0 Hz, 1 H, 5′-H), 6.66 (d, J = 8.0 Hz, 1 H, 5′′-H), 6.70 (d, J = 2.0 Hz, 1 H, 2′-H), 8.12 (s, 1 H, CHO-5) ppm. ^{13}C NMR, see Table 3. HRMS (ESI): calcd. for $C_{26}H_{34}O_{12}Na$ [M + Na]$^+$ 561.1948; found 561.2037.

Antiplasmodial Evaluation: The chloroquine-resistant strain FcB1/Colombia of *Plasmodium falciparum* was maintained in vitro on human erythrocytes in RPMI 1640 medium supplemented by 8% (v/v) heat-inactivated human serum at 37 °C under an atmosphere of 3% CO_2, 6% O_2, and 91% N_2. In vitro drug susceptibility was measured by [^{3}H]hypoxanthine incorporation as described previously.[15] Stock solutions of drugs were prepared in DMSO. Compounds were serially diluted two-fold with 100 μL culture medium in 96-well plates. Asynchronous parasite cultures (100 μL, 1% parasitemia and 1% final haematocrit) were then added to each well, which were incubated for 24 h at 37 °C prior to the addition of 0.5 μCi of [^{3}H]hypoxanthine (GE Healthcare, 1–5 Ci mmol mL^{-1})

per well. After further incubation for 24 h, the plates were frozen and thawed. Cell lysates were then collected on glass-fiber filters and counted in a liquid scintillation spectrometer. The growth inhibition at each drug concentration was determined by comparison of the radioactivity incorporated in the treated culture with that in the control culture maintained on the same plate. The concentration causing 50% growth inhibition (IC_{50}) was determined from the drug concentration–response curve and the results are expressed as the mean values ± standard deviations determined from three independent experiments. Chloroquine diphosphate, used as a positive control, was purchased from Sigma (purity >99%).

Antileishmanial Evaluation: Promastigote forms of the *Leishmania donovani* (MHOM/ET/67/HU3) line, called LV9, were grown in M-199 medium (Sigma, Saint-Quentin Fallavier, France) supplemented with 10% inactivated foetal calf serum (Invitrogen, Eragnie, France), 40 mM HEPES (VWR, Paisley, Scotland), 100 μM adenosine (Sigma, Saint-Quentin Fallavier, France), and 0.5 mg L^{-1} Hemin (Sigma, Saint-Quentin Fallavier, France) in the presence of 50 μg mL^{-1} gentamicin at 26 °C in 5% CO_2. Peritoneal macrophages were harvested from female CD1 mice (Charles River, Cléon, France) three days after an intraperitoneal injection of 1.5 mL of sodium thioglycolate (Biomérieux) and were dispensed into eight-well chamber slides (LabTek Ltd.) at a density of 5×10^4 per well (400 μL per well) in RPMI 1640 medium supplemented with 10% hi-FCS, 25 mM HEPES, and 2 mM L-glutamine (Life Technologies, Cergy-Pontoise, France). Four hours after the macrophages were plated, they were washed to eliminate fibroblasts. After a 24 h incubation period, the macrophages were infected with promastigote forms of *L. donovani* LV9 in a stationary phase in a ratio of 10 parasites per macrophage to obtain 87% of infected macrophages and 10 ± 3 amastigotes per macrophage. After 18 h the free promastigotes were eliminated and intramacrophagic amastigotes were treated with various concentrations of the compounds. Pentamidine and amphotericin B were used as reference compounds. The culture medium was renewed 48 h later and a new culture medium containing the drug was added. The experiment was stopped after five days and the percentages of infected macrophages were evaluated microscopically after Giemsa staining. The 50% inhibitory concentrations (IC_{50}) were determined by linear regression analysis and expressed in μM ± standard errors. Each experiment was performed in triplicate.

Antitrypanosomal Evaluation: The method used has been described previously by Loiseau et al.[16] Briefly, the bloodstream forms of *T. brucei brucei* were maintained in vitro for 24 h in the dark at 37 °C in a 5% CO_2 atmosphere in a minimum essential medium (Gibco BRL) that includes 25 mM HEPES and Earle's salts and is supplemented with 2 mM L-glutamine, 1 g L^{-1} of additional glucose, 10 mL L^{-1} of minimum essential medium nonessential amino acids (100×, Gibco BRL), 0.2 mM 2-mercaptoethanol, 2 mM sodium pyruvate, 0.1 mM hypoxanthine, 0.016 mM thymidine, 15% heat-inactivated horse serum (Gibco BRL), and 50 μg mL^{-1} of gentamycin. The drug evaluation was carried out in 96-well tissue culture plates in a final volume of 200 μL containing 2×10^5 trypomastigotes previously purified by centrifugation from the blood of an infected mouse, collected aseptically from the retro-orbital sinus and the compounds to be tested. Pentamidine was used as reference compound. The minimum effective concentration (MEC) was defined as the minimum concentration at which no viable parasite was observed microscopically. This value was confirmed by injecting intraperitoneally the culture from the well corresponding to the MEC into a mouse to confirm that the non-motile parasites were really killed and not able to divide.

Cytotoxicity Evaluation: The human KB tumor cell line (mouth epidermoid carcinoma) was originally obtained from ATCC. The human diploid embryonic lung cells MRC-5 were obtained from ECACC. The human promyelocytic leukemia cells HL-60 were obtained from ICSN. They were seeded into 96-well microplates at 2000 cells per well. The cytotoxicity assays were performed according to a published procedure.[17] Taxotere (in-house product, purity >99%) was used as a reference compound.

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[1] R. W. Snow, C. A. Guerra, A. M. Noor, H. Y. Myint, S. I. Hay, *Nature* **2005**, *434*, 214–217.
[2] *Antimalarial drug combination therapy*, report of the World Health Organization (WHO) technical consultation (Ed.: M. Geyer), WHO, Geneva, **2001**.
[3] N. J. White, *N. Engl. J. Med.* **2006**, *355*, 1956–1957.
[4] A. M. Dondorp, F. Nosten, P. Yi, D. Das, A. P. Phyo, J. Tarning, K. M. Lwin, F. Ariey, W. Hanpithakpong, S. J. Lee, P. Ringwald, K. Silamut, M. Imwong, K. Chotivanich, P. Lim, T. Herdman, S. S. An, S. Yeung, P. Singhasivanon, N. P. J. Day, N. Lindegardh, D. Socheat, N. J. White, *N. Engl. J. Med.* **2009**, *361*, 455–467.
[5] A. Yokosuka, Y. Mimaki, H. Sakagami, Y. Sashida, *J. Nat. Prod.* **2002**, *65*, 283–289.
[6] C. Per, U. P. Claeson, P. Tuchinda, V. Reutrakul, in: *Studies in Natural Products Chemistry*, vol. 26, part 7 (Ed.: Atta ur Rahman), Elsevier, Amsterdam, **2002**, pp. 881–908.
[7] P. Anand, S. Thomas, A. Kunnumakkara, C. Sundaram, K. Harikumar, B. Sung, S. Tharakan, K. Misra, I. Priyadarsini, K. Rajasekharan, *Biochem. Pharmacol.* **2008**, *76*, 1590–1611.
[8] N. Harada, K. Nakanishi, *Acc. Chem. Res.* **1972**, *5*, 257–263.
[9] N. Harada, A. Saito, H. Ono, J. Gawronski, K. Gawronska, T. Sugioka, H. Uda, T. Kuriki, *J. Am. Chem. Soc.* **1991**, *113*, 3842–3850.
[10] S.-I. Uehara, K. Akiyama, H. Morita, K. Takeya, H. Itokawa, *Chem. Pharm. Bull.* **1987**, *35*, 3298.
[11] H. Yagi, H. Akagi, D. R. Thakker, H. D. Mah, M. Koreeda, D. M. Jerina, *J. Am. Chem. Soc.* **1977**, *99*, 2358–2359.
[12] J. Ishida, H. Ohtsu, Y. Tachibana, Y. Nakanishi, K. F. Bastow, M. Nagai, H.-K. Wang, H. Itokawa, K.-H. Lee, *Bioorg. Med. Chem.* **2002**, *10*, 3481–3487.
[13] H. L. Ziegler, D. Staerk, J. Christensen, L. Hviid, H. Hagerstrand, J. W. Jaroszewski, *Antimicrob. Agents Chemother.* **2002**, *46*, 1441–1446.
[14] Y. H. T. Bruhn, A. Schaumlöffel, G. Bringmann, *SpecDis*, version 1.51, University of Würzburg, Germany, **2011**.
[15] J. Guillon, P. Grellier, M. Labaied, P. Sonnet, J.-M. Léger, R. Déprez-Poulain, I. Forfar-Bares, P. Dallemagne, N. Lemaître, F. Péhourcq, J. Rochette, C. Sergheraert, C. Jarry, *J. Med. Chem.* **2004**, *47*, 1997–2009.
[16] P. M. Loiseau, P. Lubert, J.-G. Wolf, *Antimicrob. Agents Chemother.* **2000**, *44*, 2954–2961.
[17] C. Tempête, G. H. Werner, F. Favre, A. Rojas, N. Langlois, *Eur. J. Med. Chem.* **1995**, *30*, 647–650.

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