

Isolation and characterization of two new drimanes from *Zygogynum baillonii* and synthesis of analogues

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Abstract

Two new drimanes were isolated and characterized from the bark of *Zygogynum baillonii*, an endemic New-Caledonian tree of the Winteraceae family. One of it shows a significant cytotoxic activity. In order to define structure–activity relationships, analogues were synthesized and their cytotoxicity was evaluated.

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1. Introduction

In our search to find new cytotoxic compounds, we screened on KB cell line, a series of 1200 plant extracts prepared from species belonging to the New-Caledonian biodiversity. This screening revealed the cytotoxicity of the EtOAc extract of the bark of *Zygogynum baillonii*, an endemic New-Caledonian tree of the Winteraceae family. Bioassay-guided chemical investigation on this extract led to the isolation of two new drimane sesquiterpenoids.

Drimane sesquiterpenoids have been discovered sixty years ago with the isolation¹ and the characterization² of drimenol (**1**) from the bark of *Drimys winterii* Forst. Since then, a large number of drimanes have been isolated from different families of plants (for example, Cannellaceae, Winteraceae or Polygonaceae) and from several fungi.³

Drimane sesquiterpenes possess attractive biological properties including antibacterial, antifungal, antifeedant, and cytotoxic effects.⁴ For example, insulicolide A (**2**) displays significant

cytotoxicity against HCT-116 human colon carcinoma cells⁵ and polygodial (**3**) exhibits antifungal activity (Fig. 1).^{6,7}

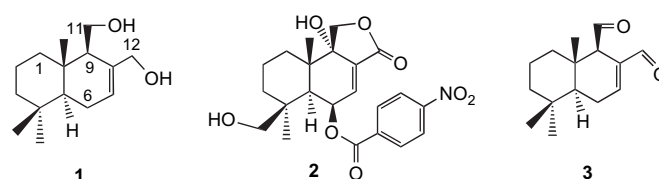


Figure 1. Some examples of drimane sesquiterpenoids.

In this report, we describe the isolation and characterization of two new drimanes **4** and **5** from the bark of *Z. baillonii*, together with the synthesis and cytotoxic activity of the analogues (Fig. 2).

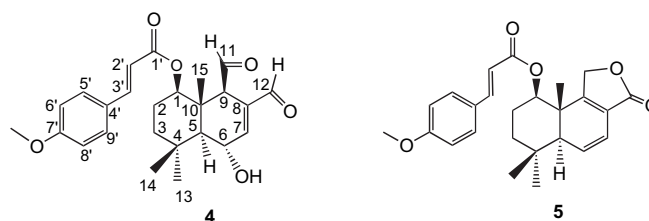


Figure 2. New drimanes from the bark of *Zygogynum baillonii*.

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2. Results

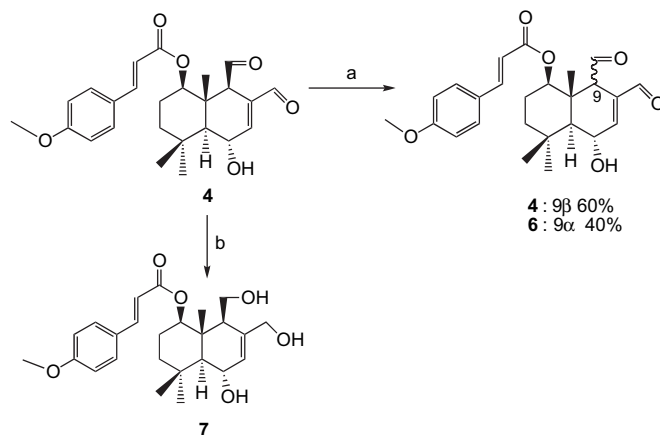
The genus *Zygogynum* consists of some 41 species distributed in the west pacific region including Australia, Papua-New-Guinea, and New-Caledonia. The chemical constituents of the genus *Zygogynum* are scarcely known.⁸ Compound **4** was obtained as a yellowish amorphous powder. Its molecular formula $C_{25}H_{30}O_6$ was supported by HREIMS of the $[M+Na]^+$ ion peak at m/z 449.1967 (calcd 449.1940). The 1H and ^{13}C NMR spectra of **4** were closely related to those of L- β -(*p*-methoxy-(*E*)-cinnamoyl)-polygodial.⁹ The only structural difference was the presence of an additional hydroxyl group at δ 1.97 (OH-6). This was confirmed by the presence of an oxygen-bearing methine carbon at δ 68.1 (C-6) and a corresponding proton signal at δ 4.66 (m, 1H, H-6). Correlations between H-6 (δ 4.66) and H-5 (δ 1.41) and H-7 (δ 6.83) in the COSY experiment allowed to place the hydroxyl group in position C-6. The relative configuration of the asymmetric carbons, that is, β positions of the aldehyde group at C-9 and the *trans*-cinnamic ester at C-1, as well as the α position of the hydroxyl group at C-6 could be easily assigned by the NOESY experiment in which correlations between H-6 (δ 4.66) with H₃-14 (δ 1.15) and H₃-15 (δ 1.09), and H-5 (δ 1.41) with H-1 (δ 4.83), H-9 (δ 3.32), and H₃-13 (δ 1.20) were observed (Fig. 3). On the basis of these evidences, the structure of compound **4** was determined as 1 β -*O*-(*p*-methoxy-(*E*)-cinnamoyl)-6 α -hydroxypolygodial and was named isodrimanial.

Compound **5** was obtained as a white amorphous powder. Its molecular $[M+Na]^+$ ion peak at m/z 431.1858 (calcd 431.1834) by HREIMS, revealed the molecular formula $C_{25}H_{28}O_5$. Compound **5** was also a *p*-methoxycinnamoyl derivative as evidenced by the typical signals of H-2', H-3', H-5', and H-6' in its 1H NMR and the signals of C-1'–C-7' and OMe in its ^{13}C NMR. The presence of an α,β -unsaturated lactone was readily recognized by signals of quaternary carbons at δ 171.6, 166.8, and 123.9 in the ^{13}C NMR. This was supported by a strong absorption band at 1754 cm^{-1} in the IR spectrum. Moreover, examination of the 1H NMR spectra revealed the presence of signals at δ 4.70 (d, $J=18\text{ Hz}$, 1H, H-11a) and 4.79 (d, $J=18\text{ Hz}$, 1H, H-11b) for an oxymethylene group and the presence of signals at δ 6.00 (dd, $J=2.3$, 9.4 Hz, 1H, H-6) and 6.36 (dd, $J=2.9$, 9.4 Hz, 1H, H-7) for an additional double bond when compared with **4**. These structural assignments with the combination of 1H COSY, HMQC, HMBC, and NOESY experiments allowed us to determine the structure of **5**. Long range correlations of H-5, H-6, H-7, CH₂-11, Me-13, Me-14, and Me-15 with neighboring carbons were particularly helpful in determining the structure of the A and B rings. Assignment of the lactone carbonyl to C-12 was established on the basis of the chemical shifts of C-8 and C-9 at

δ 123.9 and 165.8, respectively, and confirmed by observation of long range correlations from H-6 to C-8 and from H-5 to C-9 in the HMBC spectrum. Finally, correlations of H-5 with H-1 and H₃-13 in the NOESY spectrum, allowed us to determine the relative configuration of the asymmetric centers, that is, the *trans* orientation of the AB ring junction and the β position of the *trans*-cinnamic ester at C-1 (Fig. 3). Thus, the structure of compound **5** was determined as 1 β -*O*-(*p*-methoxy-(*E*)-cinnamoyl)-bemadienolide.

Compound **4** revealed a cytotoxicity on KB cell line with an IC_{50} of 0.30 μM whereas **5** was devoid of activity. As compound **4** was available on a gram scale, some structural modifications were undertaken in order to underline the structure–activity relationships.

Compound **4** was first easily epimerized at C-9 in basic conditions at room temperature to give a 40:60 mixture of separable **6** 9 α and **4** 9 β compounds. The aldehyde functions were then selectively reduced to give the triol **7**¹⁰ as a single diastereomer (Scheme 1).



Scheme 1. Reagents and conditions: (a) DMAP, toluene, rt, 50%; (b) NaBH_4 (4.6 equiv), MeOH, 0 °C, 33%.

In order to oxidize the aldehyde functions, sodium chlorite and different HOCl scavengers were used. A dioxidation could be performed on compound **4** with sodium chlorite/ H_2O_2 to give compound **8** in a nearly quantitative yield (Scheme 2).

A selective mono oxidation could also be performed at C-11 with sodium chlorite/sulfamic acid (Scheme 2). This oxidation was followed by an intramolecular lactonization on C-12 to give compound **9** as a single diastereomer. The relative configuration of the new asymmetric center could be determined, thanks to NOE effects between H-12 and H₃-15. Compound **9** was then esterified either on C-6 as an acetate (compound **10**) or a bromobenzoate (compound **12**) or on C-6 and C-12 as a diacetate (compound **11**).

Compound **4** was quite tricky to handle compared with other polygodial derivatives and was sensitive to acid or basic conditions. For example, in an attempt to mono protect the C-11 aldehyde (APTS, ethylene glycol), we noticed that compound **4** rearranged spontaneously in acidic conditions to give the lactone **5** (NMR and mass spectra identical to those of the natural compound). This reaction was quantitative with a catalytic

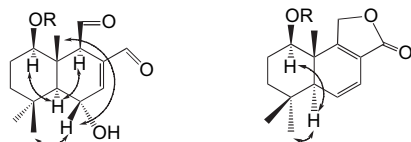
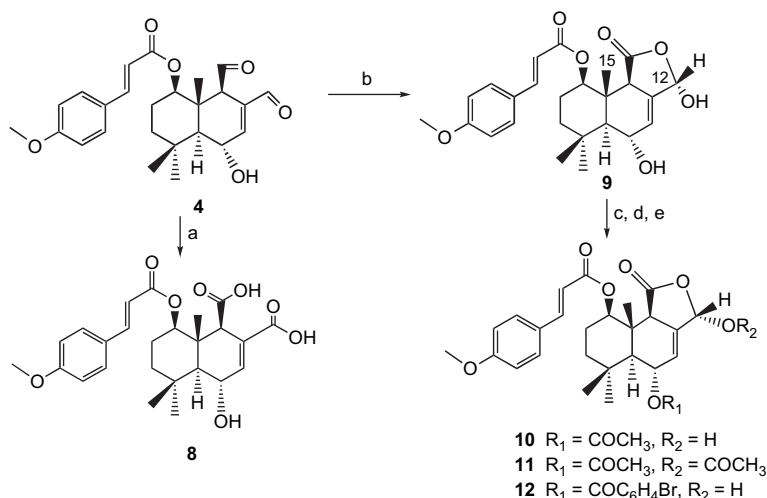
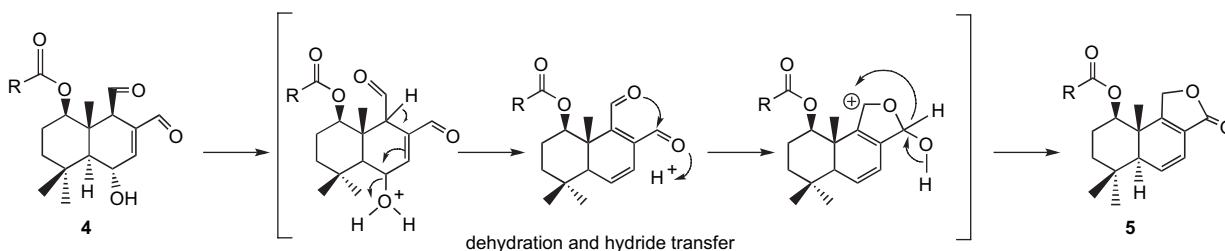


Figure 3. Observed NOE for compounds **4** and **5**.



Scheme 2. Reagents and conditions: (a) NaClO_2 (5 equiv), H_2O_2 (4 equiv), acetonitrile, rt, 96%; (b) NaClO_2 (2 equiv), $\text{NH}_2\text{SO}_3\text{H}$ (2 equiv), 1,4-dioxane/water (4:1), 0°C , 94%; (c) CH_3COCl (1.1 equiv), pyridine (1.1 equiv), CH_2Cl_2 , rt, 25%; (d) CH_3COCl (3.3 equiv), pyridine (3.3 equiv), CH_2Cl_2 , rt, 31%; (e) DCC (1.1 equiv), DMAP (0.2 equiv), $\text{BrC}_6\text{H}_4\text{CO}_2\text{H}$ (1.1 equiv), toluene, rt, 50%.



Scheme 3. Reagents and conditions: (a) APTS (0.2 equiv), benzene, reflux, 99%.

amount of APTS in refluxing benzene and was not observed with polygodial **3**¹¹ (Scheme 3). The presence of the $6\alpha\text{-OH}$ plays a crucial role in this rearrangement. Lactone **5** may be formed by a dehydration process followed by a hydride transfer in a Tishchenko-type reaction.¹² We may emphasize that this disproportionation reaction may also occur in the plant.

The biological activity of all the new synthesized compounds **6**–**12** was evaluated on KB cell line. Compound **6** was the only one to be cytotoxic on KB cells with an IC_{50} of $4.5\ \mu\text{M}$. Thus the presence of the dialdehyde functions was shown essential for the cytotoxic activity.

3. Experimental section

3.1. General remarks

All solvents and reagents were purified by standard techniques or used as supplied from commercial sources as appropriate. Flash chromatography was carried out on 230–400 mesh gel or 70–230 mesh aluminum oxide.

3.2. Plant material

Bark of *Z. baillonii* were collected in December 2000 in the high altitude scrubland of ‘Monts Dzumac’, South Province, New Caledonia, by one of us (M.L.). A voucher specimen

(LIT-1275) is deposited in the Herbarium of the Botanical and Tropical Ecology Department of the IRD Center, Noumea, New Caledonia.

3.3. Extraction and isolation

The powdered air-dried bark of *Z. baillonii* (600 g) were extracted sequentially with heptane ($3 \times 1.5\ \text{L}$), EtOAc ($3 \times 2\ \text{L}$), and MeOH ($3 \times 1.5\ \text{L}$) at room temperature to afford 4.1 g, 50.6 g, and 25.0 g of heptane, EtOAc, and MeOH extracts, respectively. EtOAc extract displayed a significant inhibitory activity for the growth of KB cells (85% inhibition at a concentration of $10\ \text{Hg/mL}$). The EtOAc extract ($2 \times 10\ \text{g}$) was then subjected to a flash column chromatography on silica (Versa-Pak™ $40 \times 150\ \text{mm}$) eluting with heptane/AcOEt/MeOH (80:20:0 to 0:0:100) to give 14 fractions according to their TLC profile (fractions 1–14). Fractions 5+6 (9.57 g) contained compound **4** with a purity over 90% and fractions 7+8 (0.65 g) contained compound **4** with a purity of 80%. Fraction 6 (310 mg) was submitted to a silica gel column chromatography using a gradient of heptane/AcOEt (60:40 to 0:100) to afford compound **4** (156 mg). Fraction 2 (3.49 g) was submitted to a silica gel column chromatography using a gradient of heptane/AcOEt (60:40 to 0:100) to give 10 fractions (fractions 2-1 to 2-10). Fractions 2–4 (1.35 g) were submitted to a flash chromatography on reversed-phase (VersaPack™, C18 Cartridge $40 \times 75\ \text{mm}$) using

a gradient mobile phase consisting of H₂O/MeCN 50:50 to 0:100 at 10 mL/min to give 8 fractions (fractions 2-4-1 to 2-4-8). Fraction 2-4-3 (138 mg) was finally submitted to a preparative HPLC (Kromasil C-18 column 250×21.2 mm, I.D. 5 μm) using an isocratic mobile phase MeCN/H₂O 70:30 at a flow rate of 21.2 mL min⁻¹ to give 44 mg of compound **5** (*t*_R: 19.0 min).

3.3.1. Isodrimanial **4**

Yellowish amorphous powder; ¹H NMR (CDCl₃, 300 MHz) δ: 1.09 (s, 3H, H-15); 1.15 (s, 3H, H-14); 1.20 (s, 3H, H-13); 1.41 (m, 1H, H-5); 1.53 (m, 1H, H-3); 1.79 (m, 2H, H-2); 1.97 (s, 1H, OH); 3.32 (br s, 1H, H-9); 3.82 (s, 3H, OMe); 4.66 (m, 1H, H-6); 4.83 (dd, *J*=11.2 Hz, 3.6 Hz, 1H, H-1); 6.22 (d, *J*=15.9 Hz, 1H, H-2'); 6.83 (br s, 1H, H-7); 6.88 (d, *J*=8.6 Hz, 2H, H-6', H-8'); 7.47 (d, *J*=8.6 Hz, 2H, H-5', H-9'); 7.59 (d, *J*=15.9 Hz, 1H, H-3'); 9.35 (s, 1H, H-12); 9.75 (d, *J*=2.4 Hz, 1H, H-11). ¹³C NMR (CDCl₃, 75 MHz) δ: 11.4 (C-15); 22.8 (C-14); 24.0 (C-2); 33.2 (C-4); 35.9 (C-13); 40.5 (C-3); 44.5 (C-10); 55.4 (OMe); 55.8 (C-5); 58.4 (C-9); 68.1 (C-6); 80.8 (C-1); 114.3 (C-6'+C-8'); 115.0 (C-2'); 126.8 (C-4'); 130.0 (C-5'+C-9'); 139.7 (C-8); 145.7 (C-3'); 153.5 (C-7); 161.6 (C-7'); 166.3 (C-1'); 192.4 (C-12); 199.9 (C-11). HRESIMS *m/z* 449.1967 [M+Na]⁺ (calcd for C₂₅H₃₀O₆Na 449.1940). IR (cm⁻¹): 3443; 2773; 1705; 1662; 1627; 1603; 1158. [α]_D²⁵ +237 (c 0.3, CH₂Cl₂).

3.3.2. 1β-*O*-(*p*-Methoxy-(*E*)-cinnamoyl)-bemadienolide **5**

White amorphous powder; ¹H NMR (CDCl₃, 300 MHz) δ: 1.01 (s, 3H, H-13); 1.07 (s, 3H, H-14); 1.16 (s, 3H, H-15); 1.49 (m, 1H, H-3a); 1.57 (m, 1H, H-3b); 1.78 (m, 1H, H-2a); 1.94 (m, 1H, H-2b); 2.32 (s, 1H, H-5); 3.83 (s, 3H, OMe); 4.70 (d, *J*=18.0 Hz, 1H, H-11a); 4.79 (d, *J*=18.0 Hz, 1H, H-11b); 5.03 (dd, *J*=4.1, 11.2 Hz, 1H, H-1); 6.00 (dd, *J*=2.3, 9.4 Hz, 1H, H-6); 6.28 (d, *J*=15.8 Hz, 1H, H-2'); 6.36 (dd, *J*=2.9, 9.4 Hz, 1H, H-7); 6.91 (d, *J*=8.7 Hz, 2H, H-6', H-8'); 7.48 (d, *J*=8.7 Hz, 2H, H-5', H-9'); 7.65 (d, *J*=15.9 Hz, 1H, H-3'). ¹³C NMR (CDCl₃, 75 MHz) δ: 11.3 (C-15); 23.0 (C-14); 24.4 (C-2); 32.0 (C-13); 32.8 (C-4); 38.9 (C-3); 42.2 (C-10); 52.2 (C-5); 55.6 (OMe); 69.1 (C-11); 76.6 (C-1); 114.6 (C-6'+C-8'); 118.3 (C-7); 114.9 (C-2'); 123.9 (C-8); 126.9 (C-4'); 130.1 (C-5'+C-9'); 130.9 (C-6); 145.9 (C-3'); 161.9 (C-7'); 166.6 (C-1'); 166.8 (C-9); 171.6 (C-12). MS (ESI⁺, CH₂Cl₂+MeOH): *m/z* 431.2 [M+Na]⁺. HRESIMS 431.1834 (calcd for C₂₅H₂₈O₈Na 431.1871). IR (cm⁻¹): 2922; 1754; 1703; 1601. [α]_D²⁵ -60 (c 0.5, CHCl₃). Compound **4** (20 mg; 0.05 mmol) and APTS (2 mg; 0.01 mmol) were refluxed in 1 mL of benzene for 3 h. Solvent was removed in vacuo. The crude mixture was diluted with CH₂Cl₂ (3 mL). The organic layer was washed with a saturated solution of NaHCO₃ and brine, dried over MgSO₄, filtered, and evaporated under reduced pressure. The crude mixture was purified by column chromatography on aluminum oxide (EtOAc/heptane 15:85) to afford compound **5** (19 mg, 99%) as a white amorphous solid. It provided spectral and analytical data identical to those of the natural product.

3.3.3. 9α-Dialdehyde **6**

Compound **4** (50 mg; 0.12 mmol) and DMAP (15 mg; 0.12 mmol) were dissolved in 1 mL of toluene at room temperature. The reaction mixture was stirred for 24 h. Dichloromethane was added and the organic layer was washed with an aqueous 1 N HCl solution and brine, dried over MgSO₄, filtered, and concentrated. The crude product was purified on preparative TLC of silica gel (EtOAc/heptane 1:1) to give pure compounds **4** (12 mg) and **6** (9 mg). ¹H NMR (CDCl₃, 300 MHz) δ: 1.08 (s, 3H, H-14); 1.12 (s, 3H, H-15); 1.15 (s, 3H, H-13); 1.46 (m, 2H, H-3); 1.57 (d, *J*=9.6 Hz, 1H, H-5); 1.77 (m, 2H, H-2); 3.63 (ls, 1H, H-9); 3.83 (s, 3H, OMe); 4.48 (m, 1H, H-6); 4.84 (dd, *J*=5.7, 10.5 Hz, 1H, H-1); 6.30 (d, *J*=15.9 Hz, 1H, H-2'); 6.88 (ls, 1H, H-7); 6.90 (d, *J*=8.7 Hz, 2H, H-6, H'-8'); 7.48 (d, *J*=8.7 Hz, 2H, H-5', H-9'); 7.66 (d, *J*=15.9 Hz, 1H, H-3'); 9.44 (s, 1H, H-12); 9.82 (d, *J*=1.5 Hz, 1H, H-11). ¹³C NMR (CDCl₃, 75 MHz) δ: 17.3 (C-15); 22.6 (C-14); 24.1 (C-2); 33.1 (C-4); 35.5 (C-13); 40.1 (C-3); 44.4 (C-10); 51.3 (C-5); 54.1 (C-9); 55.4 (OMe); 68.0 (C-6); 77.0 (C-1); 114.4 (C-6'+C-8'); 115.1 (C-2'); 126.8 (C-4'); 130.0 (C-5'+C-9'); 139.7 (C-8); 145.4 (C-3'); 152.3 (C-7); 161.6 (C-7'); 166.3 (C-1'); 192.4 (C-12); 201.6 (C-11). MS (ESI⁺, CH₂Cl₂+MeOH): *m/z* 449.2 [M+Na]⁺. HRESIMS 449.1913 (calcd for C₂₅H₃₀O₆Na 449.1940). IR (cm⁻¹): 3440; 2931; 1682; 978. [α]_D²⁵ -32 (c 0.5, CHCl₃).

3.3.4. Triol **7**

Compound **4** (150 mg; 0.35 mmol) was dissolved in 3 mL of anhydrous methanol under argon and the resulting solution was cooled to 0 °C. Sodium borohydride (48 mg; 1.26 mmol) was added and the reaction mixture was stirred at 0 °C for 3 h. Additional NaBH₄ (13 mg; 0.35 mmol) was then added. After one more hour at 0 °C, 1 mL of water was added. Solvent was evaporated under reduced pressure and the residue was diluted with ethyl acetate (20 mL) and water (10 mL). The organic layer was decanted, washed with an aqueous 1 N HCl solution and brine, dried over MgSO₄, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (CH₂Cl₂/MeOH 95:5) to afford the desired compound (49 mg, 33%). ¹H NMR (DMSO-*d*₆, 300 MHz) δ: 1.05 (s, 3H, H-15); 1.08 (s, 3H, H-14); 1.13 (s, 3H, H-13); 1.23 (ls, 1H, H-5); 1.36 (ls, 2H, H-3); 1.53 (m, 2H, H-2); 1.90 (s, 1H, OH); 2.07 (ls, 1H, H-9); 3.53 (d, *J*=4.8 Hz, 1H, H-11); 3.80 (s, 3H, OMe); 3.92 (m, 4H, H-11, H-12, OH-11, OH-12); 4.18 (m, 1H, H-6); 4.44 (d, *J*=6.9 Hz, 1H, H-12); 4.69 (ls, 1H, H-1); 5.63 (ls, 1H, H-7); 6.48 (d, *J*=15.9 Hz, 1H, H-2'); 6.98 (d, *J*=8.4 Hz, 2H, H-6, H'-8'); 7.59 (d, *J*=8.4 Hz, 2H, H-5, H'-9'); 7.67 (d, *J*=15.9 Hz, 1H, H-3'). ¹³C NMR (DMSO-*d*₆, 75 MHz) δ: 11.4 (C-15); 22.6 (C-14); 24.9 (C-2); 32.9 (C-4); 35.9 (C-13); 40.0 (C-3); 42.2 (C-10); 53.0 (C-9); 55.2 (OMe); 55.3 (C-5); 59.6 (C-11); 63.1 (C-12); 66.4 (C-6); 81.3 (C-1); 114.3 (C-6'+C-8'); 116.2 (C-2'); 126.7 (C-4'); 128.9 (C-7); 130.1 (C-5'+C-9'); 137.4 (C-8); 143.9 (C-3'); 161.1 (C-7'); 165.8 (C-1'). MS (ESI⁺, MeOH): *m/z* 453.2 [M+Na]⁺. HRESIMS 453.2256 (calcd for C₂₅H₃₄O₆Na 453.2253). IR (cm⁻¹): 3283; 2920; 1697; 1146; 978. [α]_D²⁵ -6 (c 0.3, DMSO).

3.3.5. Diacid **8**

Sodium phosphate (10 mg; 0.08 mmol) in 0.2 mL of water and H₂O₂ (56 μ L; 0.64 mmol) were added to a solution of compound **4** (70 mg; 0.16 mmol) and dissolved in 2 mL of acetonitrile. Sodium chlorite (93 mg; 0.8 mmol) in 0.5 mL of water was added dropwise over a period of 15 min. The reaction mixture was stirred for 24 h at room temperature. Solid Na₂SO₃ (5 mg) was added and the mixture was stirred for 5 min then acidified by an aqueous solution of 10% HCl. Aqueous layer was washed three times with CH₂Cl₂. Combined organic layers were washed with brine, dried over MgSO₄, filtered, and concentrated in vacuo. Pure compound **8** was isolated as a white powder (73 mg; 96%). ¹H NMR (CDCl₃, 300 MHz) δ : 1.11 (s, 3H, H-15); 1.19 (s, 3H, H-14); 1.30 (s, 3H, H-13); 1.40 (d, J =11.0 Hz, 1H, H-5); 1.60 (m, 2H, H-3); 1.81 (m, 2H, H-2); 3.34 (ls, 1H, H-9); 3.74 (s, 3H, OMe); 4.55 (1H, H-6); 4.86 (1H, H-1); 5.45 (ls, 3H, OH); 6.04 (d, J =15.9 Hz, 1H, H-2'); 6.66 (d, J =8.7 Hz, 2H, H-6', H-8'); 6.90 (ls, 1H, H-7); 7.11 (d, J =8.7 Hz, 2H, H-5', H-9'); 7.43 (d, J =15.6 Hz, 1H, H-3'). MS (ESI[−], CH₂Cl₂+MeOH): m/z 457.2 [M−H[−]]. HRESIMS 457.1835 (calcd for C₂₅H₂₉O₈ 457.1862). IR (cm^{−1}): 2931; 1697; 1168. [α]_D²⁵+34 (c 0.7, CHCl₃).

3.3.6. Monooxidized compound **9**

Compound **4** (150 mg; 0.35 mmol) was dissolved in a 4:1 mixture of dioxane and water (3 mL) and cooled to 0 °C. Sulfamic acid (NH₂SO₃H: 75 mg; 0.77 mmol) and sodium chlorite (NaClO₂: 70 mg; 0.77 mmol) were successively added. The reaction mixture was stirred for 1 h and diluted with ethyl acetate (10 mL). A 95:5 mixture of saturated solutions of NaHCO₃ and Na₂SO₃ (2 mL) was added and the mixture was stirred for 10 min. The organic layer was decanted, washed with brine, dried over MgSO₄, filtered, and concentrated to give a pure yellow compound as a single diastereomer (150 mg; 94%), which was used without further purification. ¹H NMR (CDCl₃, 300 MHz) δ : 0.89 (s, 3H, H-15); 0.98 (s, 3H, H-14); 1.10 (s, 3H, H-13); 1.41 (ls, 1H, H-5); 1.60 (m, 2H, H-3); 1.70 (m, 2H, H-2); 2.50 (ls, 1H, H-9); 2.78 (s, 1H, OH); 3.74 (s, 3H, OMe); 4.30 (m, 1H, H-6); 4.65 (dd, J =3.9, 10.8 Hz, 1H, H-1); 5.70 (ls, 1H, H-12); 5.80 (ls, 1H, −OH); 6.17 (d, J =15.9 Hz, 1H, H-2'); 6.52 (1H, H-7); 6.79 (d, J =8.7 Hz, 2H, H-6', H-8'); 7.38 (d, J =8.7 Hz, 2H, H-5', H-9'); 7.43 (d, J =15.9 Hz, 1H, H-3'). ¹³C NMR (CDCl₃, 75 MHz) δ : 11.1 (C-15); 22.7 (C-14); 24.4 (C-2); 33.1 (C-4); 35.3 (C-13); 40.4 (C-3); 43.7 (C-10); 55.4 (OMe); 56.5 (C-5); 57.0 (C-9); 67.7 (C-6); 79.9 (C-1); 100.4 (C-12); 114.3 (C-6'+C-8'); 115.7 (C-2'); 127.1 (C-4'); 130.1 (C-5'+C-9'); 135.8 (C-7); 144.5 (C-8); 144.6 (C-3'); 161.4 (C-7'); 167.1 (C-1'); 168.3 (C-11). MS (ESI⁺, CH₂Cl₂+MeOH): m/z 465.1 [M+Na⁺]. HRESIMS 465.1886 (calcd for C₂₅H₃₀O₇Na 465.1889). IR (cm^{−1}): 3404; 2931; 1697; 978. [α]_D²⁵+13 (c 0.7, CHCl₃).

3.3.7. Compound **10**

Compound **9** (50 mg; 0.11 mmol), acetyl chloride (9 μ L; 0, 12 mmol), and dry pyridine (10 μ L; 0.12 mmol) were dissolved in 1.6 mL of anhydrous CH₂Cl₂. The reaction mixture was stirred at room temperature under argon for 24 h then

washed with brine, dried over MgSO₄, and concentrated. The crude product was purified on preparative TLC of silica gel (EtOAc/heptane 1:1) to afford pure compound **10** (14 mg, 25%). ¹H NMR (CDCl₃, 300 MHz) δ : 0.89 (s, 3H, H-13); 0.99 (s, 3H, H-14); 1.02 (s, 3H, H-15); 1.44 (m, 2H, H-3); 1.63 (m, 1H, H-2); 1.72 (d, J =9.3 Hz, 1H, H-5); 1.78 (m, 1H, H-2); 2.00 (s, 3H, CH₃−OAc); 2.62 (d, 1H, H-9); 3.77 (s, 3H, OMe); 4.77 (dd, J =4.2, 11.1 Hz, 1H, H-1); 5.62 (m, 1H, H-6); 5.76 (sl, 1H, H-12); 6.20 (d, J =15.9 Hz, 1H, H-2'); 6.59 (t, J =3.6 Hz, 1H, H-7); 6.83 (d, J =8.7 Hz, 2H, H-6', H-8'); 7.40 (d, J =8.7 Hz, 2H, H-5', H-9'); 7.52 (d, J =15.9 Hz, 1H, H-3'). ¹³C NMR (CDCl₃, 75 MHz) δ : 11.5 (C-15); 21.4 (CH₃−OAc); 22.3 (C-14); 24.3 (C-2); 33.1 (C-13); 33.9 (C-4); 39.9 (C-3); 43.7 (C-10); 53.0 (C-5); 55.4 (OMe); 57.2 (C-9); 69.8 (C-6); 79.3 (C-1); 99.7 (C-12); 114.4 (C-6'+C-8'); 115.4 (C-2'); 128.6 (C-4'); 129.9 (C-5'+C-9'); 131.6 (C-7); 144.5 (C-8); 144.6 (C-3'); 161.6 (C-7'); 166.4 (C-11); 166.7 (C-1'); 170.4 (CO−OAc). MS (ESI⁺, CH₂Cl₂+MeOH): m/z 507.1 [M+Na⁺]. HRESIMS 507.2002 (calcd for C₂₇H₃₂O₈Na 507.1995). IR (cm^{−1}): 2968; 1603. [α]_D²⁵+58 (c 0.7, CHCl₃).

3.3.8. Compound **11**

Compound **9** (50 mg; 0.11 mmol), acetyl chloride (27 μ L; 0.36 mmol), and dry pyridine (30 μ L; 0.36 mmol) were dissolved in 1.6 mL of anhydrous CH₂Cl₂. The reaction mixture was stirred at room temperature under argon for 24 h then washed with brine, dried over MgSO₄, and concentrated. The crude product was purified on preparative TLC of silica gel (EtOAc/heptane 1:1) to afford compounds **11** (18 mg, 31%) and **10** (5 mg). ¹H NMR (CDCl₃, 300 MHz) δ : 0.89 (s, 3H, H-15); 0.99 (s, 3H, H-14); 1.06 (s, 3H, H-13); 1.46 (m, 2H, H-3); 1.63 (m, 1H, H-2); 1.74 (d, J =11.6 Hz, 1H, H-5); 1.80 (s, 3H, OAc); 1.81 (m, 1H, H-2); 2.00 (s, 3H, OAc); 2.90 (dd, J =4.6, 9.2 Hz, 1H, H-9); 3.77 (s, 3H, OMe); 4.69 (dd, J =3.8, 10.8 Hz, 1H, H-1); 5.64 (m, 1H, H-6); 6.09 (d, J =16.7 Hz, 1H, H-2'); 6.51 (d, J =7.2 Hz, 1H, H-12); 6.69 (t, J =5.1 Hz, 1H, H-7); 6.84 (d, J =8.2 Hz, 2H, H-6', H-8'); 7.41 (d, J =8.2 Hz, 2H, H-5', H-9'); 7.56 (d, J =16.7 Hz, 1H, H-3'). ¹³C NMR (CDCl₃, 75 MHz) δ : 11.0 (C-13); 20.5 (CH₃−OAc); 20.8 (CH₃−OAc); 22.1 (C-14); 24.1 (C-2); 33.2 (C-4); 33.6 (C-15); 39.9 (C-3); 43.8 (C-10); 52.7 (C-5); 53.9 (C-9); 54.9 (OMe); 69.5 (C-6); 79.8 (C-1); 94.6 (C-12); 114.2 (C-6'+C-8'); 114.7 (C-2'); 126.7 (C-4'); 129.8 (C-5'+C-9'); 131.3 (C-7); 136.2 (C-8); 145.8 (C-3'); 161.8 (C-7'); 166.3 (C-11); 169.1 (C-1'); 170.3 (2 CO−OAc). MS (ESI, CH₂Cl₂+MeOH): m/z 549.2 [M+Na⁺]. HRESIMS 549.2101 (calcd for C₂₉H₃₄O₉Na 549.2099). IR (cm^{−1}): 2937; 1778; 1735; 1706. [α]_D²⁵+12 (c 0.8, CHCl₃).

3.3.9. Compound **12**

A solution of compound **9** (50 mg; 0.11 mmol), 4-bromobenzoic acid (24 mg; 0.12 mmol), DMAP (2.4 mg; 0.02 mmol), and DCC (25 mg; 0.12 mmol) in 1 mL of toluene was stirred at room temperature under argon for 48 h. The reaction mixture was filtered and the solvent was removed under reduced pressure. The crude mixture was diluted with CH₂Cl₂ (10 mL). The organic layer was washed with aqueous 1 M HCl, a saturated solution of NaHCO₃, and brine. The organic layer was dried over MgSO₄, filtered, and evaporated

under reduced pressure. The crude mixture was purified by column chromatography on silica gel (EtOAc/heptane 3:7) to afford compound **12** (34 mg, 50%) as a white amorphous solid. ^1H NMR (CDCl_3 , 300 MHz) δ : 1.07 (s, 3H, H-15); 1.09 (s, 3H, H-14); 1.15 (s, 3H, H-13); 1.51 (d, $J=9$ Hz, 1H, H-5); 1.62 (m, 2H, H-2); 1.83 (2H, H-3); 3.02 (d, $J=5.4$ Hz, 1H, H-9); 3.40 (s, 1H, OH); 3.80 (s, 3H, OMe); 4.52 (m, 1H, H-6); 4.72 (dd, $J=3.6, 10.0$ Hz, 1H, H-1); 5.82 (d, $J=15.9$ Hz, 1H, H-2'); 6.66 (d, $J=5.4$ Hz, 1H, H-12); 6.78 (1H, H-7); 6.81 (d, $J=8.7$ Hz, 2H, H-6', H-8'); 6.84 (d, $J=15.9$ Hz, 1H, H-3'); 7.13 (d, $J=8.7$ Hz, 2H, H-5', H-9'); 7.26 (d, $J=8.7$ Hz, 2H, H-4'', H-6''); 7.46 (d, $J=8.7$ Hz, 2H, H-3'', H-7''). ^{13}C NMR (CDCl_3 , 75 MHz) δ : 10.9 (C-15); 22.3 (C-14); 24.8 (C-2); 33.7 (C-4); 35.3 (C-13); 40.3 (C-3); 40.3 (C-10); 55.4 (OMe); 56.8 (C-5); 54.1 (C-9); 67.8 (C-6); 80.0 (C-1); 95.2 (C-12); 114.1 (C-6'+C-8'); 114.2 (C-2'); 127.4 (C-2''); 127.6 (C-8); 129.0 (C-4'); 129.9 (C-5'+C-9'); 131.2 (C-4''+C-6''); 131.5 (C-3''+C-7''); 136.8 (C-7); 145.4 (C-3'); 161.6 (C-7'); 164.1 (C-5''); 166.1 (C-11); 166.2 (C-1'). MS (ESI^+ , $\text{CH}_2\text{Cl}_2+\text{MeOH}$): m/z 647.2 $[\text{M}+\text{Na}^+]$. HRESIMS 647.1318 (calcd for $\text{C}_{32}\text{H}_{33}\text{O}_8\text{NaBr}$ 647.1256). IR (cm^{-1}): 3325; 2925; 981. $[\alpha]_{\text{D}}^{25} -223$ (c 0.8, CHCl_3).

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