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Note

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Tyrosyl-DNA Phosphodiesterase I Inhibitors from the Australian Plant *Macropteranthes leichhardtii*

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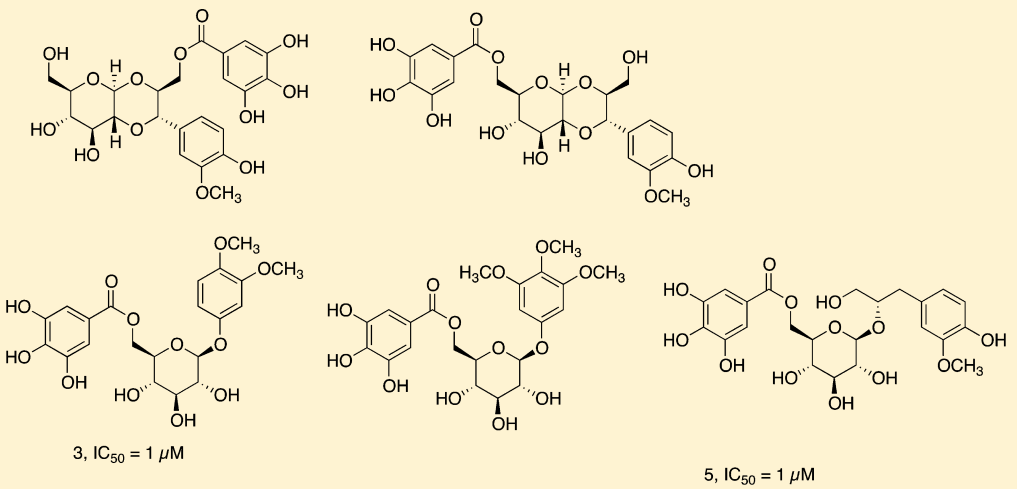
Supporting Information



ABSTRACT: Mass-directed isolation of the CH_2Cl_2/MeOH extract from the bark of an Australian plant, *Macropteranthes leichhardtii*, resulted in the purification of a new phenylpropanoid glucoside, macropteranthol (**1**), together with four known analogues (**2**–**5**). The structure of compound **1** was elucidated by NMR and MS data analyses and quantum chemical calculations. Compounds **3** and **5** showed inhibitory activity against tyrosyl-DNA phosphodiesterase I with IC_{50} values of ∼1.0 μM.

Tyrosyl-DNA phosphodiesterase I (Tdp1), an enzyme involved in the repair of DNA lesions,[1] catalyzes hydrolysis of the topoisomerase I (Top1) tyrosine residue covalently bound to the 3′-phosphate moiety of DNA.[2] Recent studies have shown that a mutation of the human Tdp1 gene is responsible for the neurological disorder spinocerebellar ataxia.[3] Further studies have shown that Tdp1 knockout mice are hypersensitive to camptothecin (CPT), a Top1 inhibitor,[4−6] while cells over-expressing Tdp1 showed resistance to CPT- and etoposide-induced DNA damage.[7] This evidence suggested that Tdp1 inhibitors could act synergistically with Top1 inhibitors in cancer combinational therapy.

Early studies on Tdp1 led to the discovery of Tdp1 inhibitors with millimolar activity, including aminoglycoside antibiotics and the ribosome inhibitors.[8,9] Several new molecules including 5-arylidenethioxothiazolidinones were later identified through high-throughput screening with improved activity.[10] In our continuing research on bioactive lead compounds from natural products, a high-throughput screening assay was established and used to screen a prefractionated natural product library for Tdp1 inhibitors. The library comprises over 200 000 fractions and was constructed by fractionation of over 18 000 terrestrial and marine biota samples. One fraction from the bark of the Australian plant *Macropteranthes leichhardtii* F. Muell. ex Benth. showed activity against Tdp1. LC-MS analysis of the active fraction showed four molecular ions in the (+)-LRESIMS, at *m*/*z* 511, 513, 469, and 499, which were used to guide isolation. Ten grams of air-dried and ground bark was extracted by CH_2Cl_2 and MeOH. Mass-directed fractionation and purification of the combined CH_2Cl_2/MeOH extracts resulted in the isolation of a new phenylpropanoid glucoside, macropteranthol (**1**), together with four known analogues, namely, mallophenol A (**2**),[11] 3,4-dimethoxyphenol-1-β-D-(6′-*O*-galloyl)glucopyranoside (**3**),[12] 3,4,5-trimethoxyphenol-1-β-D-(6′-*O*-galloyl)glucopyranoside (**4**),[13] and 3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol 2-β-D-(6-*O*-galloyl)glucopyranoside (**5**) (Figure 1).[14] In this paper, we

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report the isolation and structure elucidation of macropteranthol (**1**), as well as the Tdp1 inhibitory activity of compounds **1**−**5**.

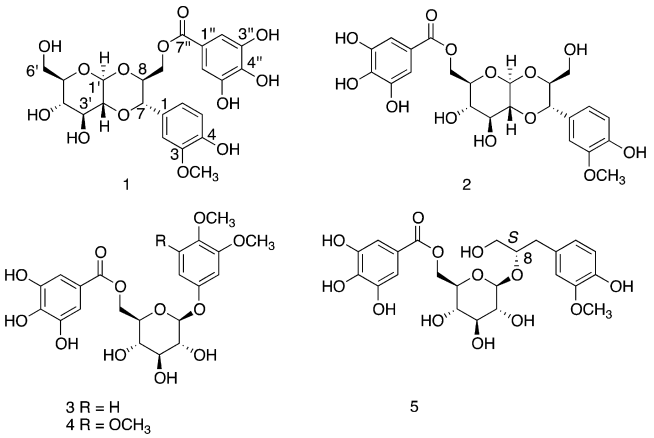

Figure 1. Structures of compounds **1**−**5** isolated from *M. leichhardtii*.

The air-dried and ground bark of *M. leichhardtii* was sequentially extracted with *n*-hexane, CH_2Cl_2, and MeOH. The CH_2Cl_2 and MeOH extracts were combined and chromatographed using reversed-phase C_{18}-bond silica HPLC (MeOH/H_2O/0.1% TFA) to give 60 fractions. Fractions 22−26 contained the ions of interest [LRESIMS (+) at *m*/*z* 513, 511, 499, and 469; LRESIMS (−) at *m*/*z* 511, 509, 497, and 467]. Further purification of fractions 22−26 by C_{18}-bonded silica HPLC (MeOH/H_2O/0.1% TFA) afforded compounds **1**−**5**.

Macropteranthol (**1**) was obtained as a pale, amorphous powder. HRESIMS exhibited a pseudomolecular ion peak at *m*/*z* 533.1266 $[M + Na]^+$ (calcd 533.1255 for $C_{23}H_{26}O_{13}Na$), consistent with a molecular formula of $C_{23}H_{26}O_{13}$, the same as that of mallophenol (**2**). Detailed analysis and comparison of the ^{1}H, gHSQC, and gCOSY NMR spectra of **1** with those of **2** revealed that **1** also has a galloyl group (δ_H 6.96; δ_C 109.0), a 1,3,4-trisubstituted benzene moiety (δ_H 6.90 d, *J* = 1.7 Hz, 6.79 dd, *J* = 8.1, 1.7 Hz, and 6.74 d, *J* = 8.1 Hz), a *β*-glucopyranosyl moiety with the anomeric proton at δ_H 4.54 (d, *J* = 7.7 Hz), and the tetrasubstituted 1,4-dioxane moiety (δ_H 4.46, 4.10; δ_C 78.7, 77.7). The obvious differences are a shielded methylene, H_2-6′, in a glucopyranosyl moiety and a corresponding deshielded methylene, H_2-9, in **1**. Further gHMBC NMR data analysis suggested that, instead of a C-6′ galloyl esterification of the glucopyransyl moiety in **2**, the galloyl group of **1** was connected with C-9 through an ester bond by observation of the gHMBC correlations from H-2″/H-6″ (δ_H 6.96) and methylene H_2-9 (δ_H 4.05, 3.86) to the carbonyl carbon (δ_C 165.9). The substitution position of the methoxy group and the presence of a 1,4-dioxane ring system were similar to those of **2** and were supported by gHMBC correlations (Figure 2). On the basis of the above evidence, the structure of macropteranthol was established as **1**.

The applications of quantum chemical calculations to the prediction of the optical rotations (ORs) of chiral organic molecules have greatly facilitated the reliable determination of their absolute configurations.[15,16] The methods have been utilized successfully to assign the absolute configurations of natural products.[17−20] A conformational search for the NMR-established relative configuration (7*S*,8*S*,1′*S*,2′*R*,3′*S*,4′*S*,5′*R*)-isomer led to the identification of 14 conformers within a relative energy window of 1 kcal/mol. Geometry optimization followed by OR calculations at D-sodium line radiation (wavelength of

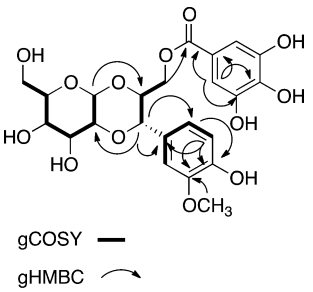

Figure 2. gCOSY and gHMBC correlations of **1**.

589 nm) and subsequently the Boltzmann-weighted average based on the energy of each conformer resulted in the calculated OR. Here two different levels, Hartree−Fock (HF) and density functional theory (DFT), were performed to calculate the ORs. The OR values of the (7*S*,8*S*,1′*S*,2′*R*,3′*S*,4′*S*,5′*R*)-stereoisomer and its enantiomer were predicted to be +22.17 and −22.17, respectively, at the HF level, or +14.21 and −14.21, at the DFT level, respectively. The positive signs of the calculated ORs of the (7*S*,8*S*,1′*S*,2′*R*,3′*S*,4′*S*,5′*R*)-isomer obtained via two calculation levels were in agreement with the experimental OR value ($[\alpha]_D$ +2.75). Therefore, the absolute configuration of (+)-**1** was determined as (7*S*, 8*S*, 1′*S*, 2′*R*, 3′*S*, 4′*S*, 5′*R*).

Four known natural products, mallophenol A (**2**), 3,4-dimethoxyphenol-1-*β*-D-(6′-*O*-galloyl)glucopyranoside (**3**), 3,4,5-trimethoxyphenol-1-*β*-D-(6′-*O*-galloyl)glucopyranoside (**4**), and 3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol 2-*β*-D-(6-*O*-galloyl)glucopyranoside (**5**), were also isolated. Their ^{1}H and ^{13}C NMR data were identical to reported data. Previously, mallophenol A (**2**) was reported only from *Mallotus furetianus*,[11] while 3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol 2-*β*-D-(6-*O*-galloyl)glucopyranoside (**5**) was reported from the berries of *Pimentadioica*, but the C-8 absolute configuration was not assigned.[14] In this study, the absolute configuration of C-8 was assigned via acid hydrolysis and subsequent assessment of the specific rotation of 3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol ($[\alpha]_D$ −6.2, *c* 0.005, MeOH). The absolute configuration of C-8 in **5** was defined as *S* by $[\alpha]_D$ comparison with 3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol {8(*S*)-isomer, $[\alpha]_D$ −23, *c* 0.69, EtOH; 8(*R*)-isomer, $[\alpha]_D$ +18, *c* 0.73, EtOH} literature values.[21]

Tdp1 inhibitory activities of compounds **1**−**5** were evaluated in vitro (Figure 3), using an assay previously described.[22] The assay was performed with the substrate at its K_m for the enzyme used, and the reaction monitored using a kinetic read. Compounds **3** and **5** were the most active, with IC_{50} values of ∼1.0 *μ*M. Compound **1**, its regiomer **2**, and compound **4** showed no inhibitory activity against Tdp1 at concentrations up to 40 *μ*M. Preliminary structure−activity relationship studies suggested that the relative orientations of the aromatic system on the sugar moiety in compounds **1**−**5** may play a part in their interaction with Tdp1 and, therefore, their biological activity. Molecular modeling is currently under way to investigate the binding of compounds **1**−**5** to Tdp1.

In conclusion, five compounds (**1**−**5**) were isolated from the bark of *M. leichhardtii*, including the new compound **1** and compounds **3** and **5**, being active against Tdp1 (IC_{50} 1.0 *μ*M). The 8(*S*)-absolute configuration of **5** was defined for the first time. The current study is the first report of the chemical constituents of a *Macropteranthes* species. The results provide

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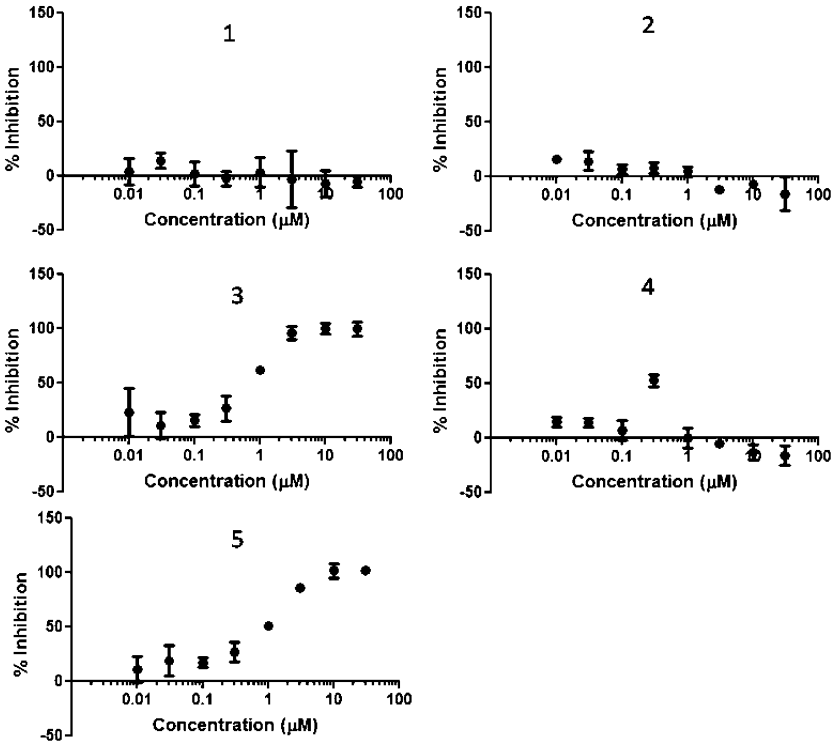

Figure 3. In vitro Tdp1 inhibitory activity curves of compounds **1**−**5**.

more information about the structure−activity relationship of this class of compounds.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a HORIBA SEPA-300 high-sensitive polarimeter. UV spectra were recorded on a Jasco V650 UV/vis spectrophotometer.

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data for Compound 1 (DMSO-d_6)

position	^{13}C	^{1}H (mult., J in Hz, int.)	HMBC correlation
1	128.2		
2	112.3	6.90 d (1.7)	4, 6, 7
3	147.8		
4	147.4		
5	115.5	6.74 d (8.1)	1, 3
6	120.9	6.79 dd (1.7, 8.1)	2, 4, 7
7	78.7	4.46 d (9.4)	1, 2, 6, 8, 2′
8	77.7	4.10 m	7
9	63.6	4.05 dd (1.7, 12.8)	7, 7″
		3.86 dd (5.8, 12.8)	
1′	98.2	4.54 d (7.7)	2′
2′	80.0	3.03 dd (7.7, 8.9)	1′, 3′
3′	73.5	3.38 m	
4′	71.0	3.28 dd (9.0, 9.0)	
5′	76.0	3.38 m	
6′	61.3	3.69 br d (12.0)	5′
		3.48 dd (5.9, 12.0)	
1″	119.4		
2″, 6″	109.0	6.96 s	1″, 2″, 3″, 4″, 5″, 7″
3″, 5″	146.0		
4″	139.1		
7″	165.9		
3-OCH$_3$	55.9	3.71 s	3

NMR spectra were recorded at 30 °C on a Varian 600 MHz Unity INOVA spectrometer equipped with a triple-resonance cryoprobe. The ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent peak for methanol-d_4 at δ_H 3.31 and δ_C 49.5 or DMSO-d_6 at δ_H 2.50 and δ_C 39.5. LRESIMS data were recorded on a Waters ZQ mass spectrometer. HRESIMS data were recorded on a Bruker Daltronics Apex III 4.7e Fourier-transform mass spectrometer. AlltechDavisil 40−60 μmÅ C$_{18}$-bonded silica was used for flash chromatography. A Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler was used for HPLC. A Thermo Scientific C$_{18}$ Betasil 5 μm 143 Å column (21.2 mm × 150 mm) and a Phenomenex Luna C$_{18}$ 5 μm 143 Å column (21.2 mm × 250 mm) were used for semipreparative HPLC separations. All solvents used for chromatography, UV, and MS were Lab-Scan HPLC grade, and the H$_2$O was Millipore Milli-Q PF filtered. A BIOLINE orbital shaker was used for the large-scale extraction of the sponge material.

Plant Materials. Vegetative material of *Macropteranthes leichhardtii* F. Muell. ex Benth. (family: Combretaceae; genus: *Macropteranthes*) was collected at Palmgrove National Park (25°01 S, 149°15 E), south-central Queensland, Australia. It was air-dried prior to freeze-drying and extraction. A voucher sample (Forster PIF26052 (BRI AQ493829)) has been lodged at the Queensland Herbarium, Toowong, Australia.

Extraction and Isolation. The air-dried and ground bark (10 g) was poured into a conical flask (1 L), *n*-hexane (250 mL) was added, and the flask was shaken at 200 rpm for 2 h. The *n*-hexane extract was filtered under gravity, and the liquid phase discarded. CH_2Cl_2 (250 mL) was added to the plant material and shaken at 200 rpm for 2 h. The resulting extract was filtered under gravity. MeOH (250 mL) was added, and the MeOH/plant mixture was shaken for a further 2 h at 200 rpm. Following gravity filtration the plant material was extracted with another volume of MeOH (250 mL), while being shaken at 200 rpm for 16 h. All CH_2Cl_2/MeOH extracts were combined and dried under reduced pressure to yield a dark brown solid (1.35 g). The crude extract was preadsorbed to C$_{18}$-bonded silica (1 g) and packed into a stainless steel cartridge (10 × 30 mm) that was subsequently attached to a C$_{18}$ preparative HPLC column. Isocratic HPLC conditions of 90% H$_2$O (0.1% TFA)/10% MeOH (0.1% TFA) were initially employed for the first 10 min; then a linear gradient to 100% MeOH (0.1% TFA) was run over 40 min, followed by isocratic conditions of 100% MeOH (0.1% TFA) for a

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further 10 min, all at a flow rate of 9.0 mL/min. Sixty fractions (60 × 1 min) were collected every minute from the start of the HPLC run, and fractions 20−30 were analyzed by LC-MS. Fractions 22 (+ESIMS m/z 511), 23 (+ESIMS m/z 511), 24 (+ESIMS m/z 513), 25 (+ESIMS m/z 513, 469), and 26 (+ESIMS m/z 499) showed molecular ions of interests. Fractions 22−26 were further purified using a C_{18} semi-preparative HPLC column eluting with isocratic conditions from H_2O/MeOH (8:2) (0.1% TFA) to MeOH (0.1% TFA) within 40 min to afford **1** (0.4 mg, 0.004% of dry wt), **2** (0.8 mg, 0.008% of dry wt), **3** (0.3 mg, 0.003% of dry wt), **4** (0.2 mg, 0.002% of dry wt), and **5** (0.3 mg, 0.003% of dry wt).

*Macropteranthol (**1**):* pale, amorphous powder; $[\alpha]_D$ +2.75 (c 0.04, MeOH); λ_{max} (log ε) 278 (3.76), 219 (4.12) nm; IR (KBr) ν_{max} 3242, 2926, 2887, 1770, 1562, 1394 cm^{-1}; (+)-LRESIMS m/z 511 $[M + H]^+$, 349 $[M + H - 162]^+$, 179 $[M + H - 162 - 170]^+$; HRESIMS m/z 533.1266 $[M + Na]^+$ (calcd for $C_{23}H_{26}O_{13}Na$, 533.1255).

Computational Details. A conformational search used the MMFFs force field on Macromodel interfaced to the Maestro program.[23] Fourteen conformers having internal relative energies within 1 kcal/mol were subjected to geometry optimization in MeOH at the HF level using the 6-31G(d,p) basis set or at the DFT level using the B3LYP functional and the 6-31G(d,p) basis set. Optimized conformers were subjected to OR calculations in MeOH (SCRF) using the 6-31G(d) basis set for HF and the B3LYP functional and the 6-31G(d) basis set for DFT in Gaussian 09.[24] Final calculated ORs were obtained as the result of the Boltzmann-weighted average.

Acid Hydrolysis of 5. Compound **5** (0.3 mg) was heated in 2 N HCl (1 mL) at 70 °C for 4 h. The resulting hydrolysate was analyzed by LC-MS. The LC-MS result revealed the disappearance of compound **5** and the appearance of an aglycone {+ESIMS m/z 199 $[M + H]^+$}. The hydrolysate was dried under vacuum, preadsorbed to cotton, followed by packing into a stainless steel cartridge (10 × 30 mm) that was subsequently attached to a C_{18} preparative HPLC column. Isocratic HPLC conditions of 90% H_2O (0.1% TFA)/10% MeOH (0.1% TFA) were initially employed for the first 10 min; then a linear gradient to 100% MeOH (0.1% TFA) was run over 40 min, followed by isocratic conditions of 100% MeOH (0.1% TFA) for a further 10 min, all at a flow rate of 9.0 mL/min. Fraction 5 contained the aglycone, 3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol (0.1 mg), which gave an $[\alpha]_D$ −6.2 (c 0.005, MeOH). ^1H NMR (600 MHz, in DMSO-d_6) δ_H 6.74 (d, J = 1.8 Hz, H-2), 6.63 (d, J = 7.9 Hz, H-5), 6.56 (dd, J = 7.9, 1.8 Hz, H-6), 3.72 (m, H-8), 3.56 (m, H-9a), 3.42 (dd, J = 6.8, 13.6 Hz, H-9b), 2.62 (m, H-7a), 2.41 (dd, J = 7.4, 13.6 Hz, H-7b), 3.71 (s, 3-OCH_3).

Tdp1 Inhibitory Assay. The Tdp1 in vitro activity assay was designed as a linear quenched fluorescent substrate that negated interaction with DNA intercalators. A Wellmate microplate dispenser (Matrix) was used to dispense 25 μL/well of a Tdp1 enzyme solution (2.5 nM final concentration in 10 mM Tris-HCl, pH 7.5, 50 mM KCl, 1 mM EDTA, 1 mM DTT, and 0.01% Brij-35) into wells of a black 384-well plate (Costar). A 70 nL amount of each test fraction was pinned into assay plates using an FP3-384 pin tool (VP Scientific) on a PlateMate Plus (Thermo Scientific) and incubated at room temperature for 30 min. The μFill dispenser was used to add 25 μL/well of linear oligonucleotide substrate [50 nM, 5′-/6-TAMN/AGGATCTAAAA-GACTT/3BHQ_2/-3′; Integrated DNA Technologies] in dH_2O. The fluorophore tetramethylrhodamine (TAMN) was coupled to the 5′ terminus, whereas the Black Hole Quencher (BHQ)_2 was coupled to the 3′ terminus. The whole plate was immediately read four times using a kinetic read on the Varioskan Flash multimode reader (Thermo) at Ex_{557}/Em_{582} nm. Tdp1 percentage inhibition was calculated by comparing the rate of increase in fluorescence throughout time for the compound-treated wells to that of DMSO control wells. Activity data were the average of triplicate measurements.

■ ASSOCIATED CONTENT

Supporting Information

^1H, gCOSY, gHSQC, and gHMBC NMR spectra of **1**, computational details, and acid hydrolysis for **5** are included. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00211.

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Notes

The authors declare no competing financial interest.

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