# Isolation, Biomimetic Synthesis, and Cytotoxic Activity of Bis(pseudopterane) Amines

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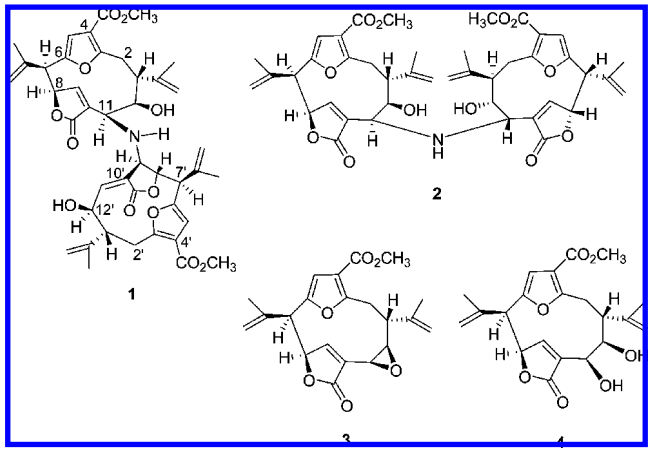
LC-MS/MS-based screening of the dichloromethane extract of the gorgonian coral *Pseudopterogorgia acerosa* led to the isolation of a novel bis(pseudopterane) amine (**1**). The structural assignment of **1** was achieved by 1D and 2D NMR and mass spectrometry analysis. A biomimetic synthesis of **1** and the known symmetrical diterpene **2** from pseudopterolide (**3**) is described in this report. Bis(pseudopterane) amine showed selective growth inhibition activity against cancer cell lines with $IC_{50}$ values of 4.2 $\mu$M (HCT116) and 42 $\mu$M (HeLa).

The gorgonian coral *Pseudopterogorgia acerosa* has received considerable attention from the natural products community due to a diversity of terpene chemistry.[1−6] Fenical and Clardy reported[2] the isolation of pseudopterolide, a structurally intriguing diterpene with an apparently ring-contracted cembranoid skeleton from *P. acerosa* collected in the Florida Keys. Later studies of this gorgonian collected in Puerto Rico, Tobago, and Martinique indicated the presence of an extensive array of diterpenes with the pseudopterane skeleton.[1−6] We recently reported[7] the isolation of a series of lipidyl pseudopteranes from a Bahamian collection of *P. acerosa*, which exhibit selective inhibitory activity against protein tyrosine phosphatase 1B. From a subsequent expedition to the Bahamas, a collection of *P. acerosa* has led to the isolation of a novel amine bridging bis(pseudopterane) amine (**1**) along with several known diterpenes including pseudopterolide (**3**), gorgiacerodiol (**4**), and bis(gorgiacerol) amine (**2**).

The mass spectrometric fragmentation pattern of the pseudopterane diterpenoids were useful for the development of a data-dependent screening method called the doubleplay method.[8,9] The advantage of this method is that minor components can be identified in the presence of major metabolites of extract fractions. After development of the doubleplay method with an ion-trap mass spectrometer, a range of fractions from a $C_{18}$ flash column of the dichloromethane extract of *P. acerosa* was screened. The resulting data indicated the presence of several peaks within the *m/z* range 740 to 790 with fragmentation similar to that of gorgiacerodiol (**4**), and we subsequently isolated two of the more abundant of these compounds. We report here the isolation, characterization, biomimetic synthesis, and cytotoxic activity of one new and one previously reported[4] bis-diterpenoid (**1** and **2**).




The sample of *P. acerosa* investigated in this study was collected at Sweetings Cay, Bahamas, and the dried coral was exhaustively extracted with $CH_2Cl_2/CH_3OH$ (1:1). The crude extract was partitioned between $CH_3OH/H_2O$ and hexanes and then with $CH_2Cl_2$. The latter organic extract was subjected to $C_{18}$ flash chromatography to afford six fractions. UPLC-MS/MS analysis of the $CH_3CN$ fraction from the $C_{18}$ column showed the presence of two peaks (**1** and **2**) both with pseudomolecular ions of *m/z* 758. The $MS^2$ fragmentation pattern for those two peaks indicated that these metabolites were related. Both compounds were purified by preparative TLC followed by semipreparative HPLC using a phenylhexyl column. The $^1H$ NMR spectrum of both **1** and **2** confirmed that these are two closely related compounds, and $^1H$ and $^{13}C$ NMR, MS, and database searches confirmed the identification of **2** as the previously isolated bis(gorgiacerol) amine (**2**).[4]

HRMS analysis of **1** indicated a pseudomolecular ion $[M + H]^+$ of *m/z* 758.3127, which corresponds to a molecular formula of $C_{42}H_{47}NO_{12}$ and indicates 20 degrees of unsaturation. The IR spectrum showed absorptions characteristic for an unsaturated $\gamma$-lactone (1739 $cm^{-1}$), conjugated ester (1721 $cm^{-1}$), hydroxyl group (3464 $cm^{-1}$), and alkene (3078 $cm^{-1}$). The $^{13}C$ NMR spectrum of **1** indicated the presence of 42 carbons including six methyl, six methylene, 14 methine, and 16 quaternary carbons on the basis of HMQC and DEPTQ experiments (Table 1). Proton resonances at $\delta$ 6.67 (1H) and 5.42 (1H) with corresponding carbon signals at $\delta$ 149.2 and 80.6, and quaternary carbons at $\delta$ 132.61 and 173.6 with long-range HMBC correlations to the signal at $\delta$ 6.67, suggested the presence of an $\alpha,\gamma$-disubstituted $\alpha,\beta$-unsaturated $\gamma$-lactone, illustrated in Figure 1 (partial structure A).

Quaternary carbon signals at $\delta$ 131.9 and 170.7 showed HMBC correlations with a proton signal at $\delta$ 4.87, which was attached to a carbon at $\delta$ 86.0 (HMQC). The proton at $\delta$ 4.87 also exhibited an HMBC correlation with a signal at $\delta$ 62.9 (CH), while a proton at $\delta$ 5.70 showed HMBC correlations with signals at $\delta$ 170.7 and 62.9. This, together with a broad IR absorption at 1739 $cm^{-1}$, suggests the presence of partial structure B. Proton signals at $\delta_H$ 6.34 (1H), 6.31 (1H), 3.82 (3H), and 3.85 (3H) along with carbon signals at $\delta_C$ 163.8 (C), 163.7 (C), 160.3 (C), 160.4 (C), 150.4 (C), 150.6 (C), 116.2 (C), 116.1 (C), 112.0 (CH), 110.1 (CH), 51.56 ($CH_3$), and 51.64 ($CH_3$) suggested the presence of two $\alpha,\alpha'$-disubstituted $\beta$-furan moieties, as shown in substructure C. This was supported by IR and long-range HMBC correlations. A methyl singlet at $\delta$ 1.82 showed HMBC correlations with carbons at $\delta$ 114.0, 145.1, and 49.9, suggesting an isopropylene group. A similar pattern was observed with three other methyl signals at $\delta$ 1.95, 1.94, and 1.91, indicating the presence of four isopropylene groups (substructure D).

These substructures were connected using $^1H-^1H$ COSY and HMBC correlations (Figure 2). The presence of three exchangeable protons was confirmed by a $D_2O$ exchange experiment. The amine

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**Table 1.** NMR Data for Bis(pseudopterane) Amine (**1**) in $CDCl_3$ (300 MHz for $^1H$ and 75 MHz for $^{13}C$)

| position | $^{13}C^a$ | $^1H$ ($J$ in Hz) | COSY | NOESY | HMBC $^{13}C$ |
|---|---|---|---|---|---|
| 1 | 43.9, CH | 3.13, dd (13.1, 3.6) | 2a, 2b | 2b | |
| 2 | 31.7, $CH_2$ | 3.65, dd (15.5, 13.1) | 2b, 1 | 2b | |
| | | 2.67, dd (15.5, 3.6) | 2a, 1 | 2a, 1 | |
| 3 | 160.4, qC | | | | |
| 4 | 116.2, qC | | | | |
| 5 | 110.1, CH | 6.34, s | | 7 | 4, 6, 3 |
| 6 | 150.4, qC | | | | |
| 7 | 48.1, CH | 3.78, d (4.3) | 8, 18a, 18b, 19 | 5, 8, 9 | |
| 8 | 80.6, CH | 5.42, dd (4.38, <1.5) | 7, 9 | 7, 19, 9 | 9, 6 |
| 9 | 149.2, CH | 6.67, d (<1.5) | 8 | 8, 7, 11 | 8, 10, 20 |
| 10 | 132.6, qC | | | | |
| 11 | 61.3, CH | 3.48, dd (11.5, 3.7) | NH, 12 | 9′ | |
| 12 | 75.8,CH | 3.04, dd (11.8, 3.5) | 11 | | |
| 13 | 144.3, qC | | | | |
| 14 | 116.8, $CH_2$ | 5.08, s | 15 | 15 | 15, 1 |
| | | 5.07, s | 15 | 15 | 15 |
| 15 | 22.3, $CH_3$ | 1.91, s | 14a, 14b | 14a, 14b | 14, 1, 13 |
| 16 | 163.8, qC | | | | |
| 17 | 139.2, qC | | | | |
| 18 | 115.3, $CH_2$ | 5.01, s | 18b, 7, 19 | 18b, 19 | 19 |
| | | 4.76, s | 18a, 19, 7 | 18a | 7, 19 |
| 19 | 21.5, $CH_3$ | 1.94, s | 18a, 18b, 7 | 18a, 8 | 18, 17, 7 |
| 20 | 173.6, qC | | | | |
| 21 | 51.56, $CH_3$ | 3.81, s | | | 16 |
| NH | | 3.29, dd (11.4, 7.6) | 11, 9′ | 12(OH), 12′(OH) | |
| 12(OH) | | 2.96, d (11.8) | | NH, 12′(OH) | |
| 1′ | 49.9, CH | 3.82, nr[b] | 2′a, 2′b (w) | | |
| 2′ | 29.2, $CH_2$ | 3.56, dd (12.2, nr) | 2′b, 1′ | 2′b | |
| | | 2.60, dd (12.2, <0.5) | 2′a, 1′ | 2′a | |
| 3′ | 160.3, qC | | | | |
| 4′ | 116.1, qC | | | | |
| 5′ | 112.0, CH | 6.31, s | | 7′, 9′ | 4′, 6′, 3′ |
| 6′ | 150.6, qC | | | | |
| 7′ | 48.8, CH | 3.53, d (4.1) | 8′, 19′, 18′a, 18′b | 5′, 8′, 18′b | |
| 8′ | 86.0, CH | 4.87, d (4.1) | 7′ | 7′, 19′ | 9′, 10′, 20′, 6′ |
| 9′ | 62.9, CH | 3.17, d (7.1) | NH | 11, 11′ | |
| 10′ | 131.9, qC | | | | |
| 11′ | 140.2, CH | 5.70, d (7.3) | 12′ | 12′, 9′ | 20′, 12′, 9′ |
| 12′ | 69.7, CH | 3.85, nr | 11′, 12′ (OH) | 11′, 12′OH | |
| 13′ | 145.1, qC | | | | |
| 14′ | 114.0, $CH_2$ | 4.98, s | 15′ | 15′ | 15′ |
| | | 4.98, s | 15′ | 15′ | 15′, 1′ |
| 15′ | 18.9, $CH_3$ | 1.82, s | 14′a, 14′b | 14′a, 14′b | 14′, 1′, 13′ |
| 16′ | 163.7, qC | | | | |
| 17′ | 140.8, qC | | | | |
| 18′ | 116.0, $CH_2$ | 5.08, s | 7′, 19′, 18′b | 19′, 18′b | 19′ |
| | | 4.91, s | 7′, 19′, 18′a | 18′a, 7′ | 19′ |
| 19′ | 22.1, $CH_3$ | 1.94, s | 18′a, 18′b, 7′ | 18′a | 18′, 17′, 7′ |
| 20′ | 170.7, qC | | | | |
| 21′ | 51.64, $CH_3$ | 3.85, s | | | 16′ |
| 12′(OH) | | 4.15, d (12.3) | 12′ | 12, 12(OH), NH | |

[a] $^{13}C$ multiplicity was assigned on the basis of DEPTQ and HMQC experiments. [b] nr = not resolved.

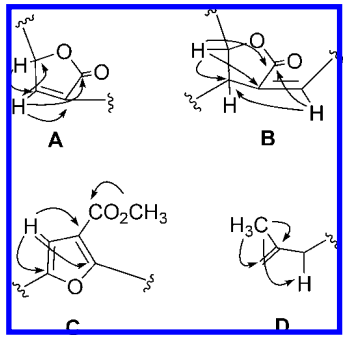



**Figure 1.** Partial structures A−D of **1** based on HMBC correlations.

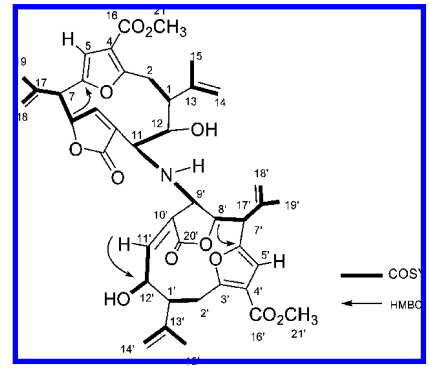



**Figure 2.** Key $^1H-^1H$ COSY and HMBC correlations of **1**.

proton at $\delta$ 3.29 appeared as a doublet of doublets ($J$ = 11.4 and 7.6 Hz), and the COSY experiment indicated correlations with C-11 and C-9′ protons. Thus, **1** appears to be related to **2** but is linked in an asymmetrical fashion through C11 and C9′.

The relative configuration of **1** was deduced from analysis of $^1H-^1H$ coupling constants along with NOESY data (Table 1) and molecular modeling simulations. In the NOESY spectrum, the

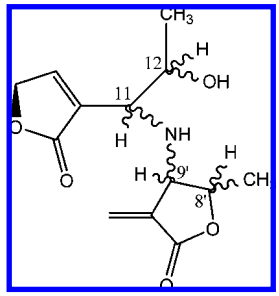


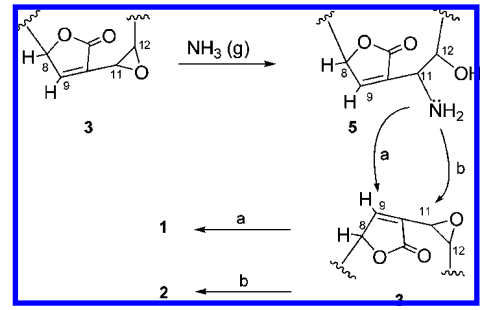
**Figure 3.** Model of **1** used to perform quantum chemical determination of coupling constants between H-8′ and H-9′ as well as H-11 and H-12.

proton at $\delta$ 5.43 (H-8) showed a cross-peak with the proton at $\delta$ 3.78 (H-7), suggesting a *cis* orientation of these protons. This was supported by a coupling constant value ($J = 4.4$ Hz) between the proton at $\delta$ 3.78 (H-7) and $\delta$ 5.42 (H-8). A *trans* relative configuration between H-12 and H-1 was deduced due to the absence of a NOESY correlation. H-11 showed a large coupling constant ($J = 11.5$ Hz) with the amine proton, which then further showed coupling with H-9′ ($J = 7.1$ Hz). The configurations were assigned due to a NOESY cross-peak between H-9′ and H-11.

To aid in the assignment of the absolute stereochemical configuration of positions 8′, 9′, 11, and 12, it was necessary to employ molecular modeling techniques. Allowing the absolute configuration at four centers to vary between *R* and *S* generates 16 possible stereoisomers of **1**, and each of these structures was optimized using the MMFF force field as implemented in the Spartan ’08 molecular mechanics package.[10] A systematic search was then performed over all possible conformations of each stereoisomer to locate the equilibrium conformer of each according to the MMFF method. The resulting 16 structures were then truncated as illustrated in Figure 3 to make high-level calculations more tractable, and the vicinal $^1H-^1H$ coupling constants between H-8′ and H-9′, as well as H-11 and H-12, were calculated[11] using density functional theory (DFT) for the purpose of comparison with experiment. In particular, Becke’s three-parameter exchange functional[12] (B3) was employed in conjunction with the correlation functional proposed by Lee, Yang, and Parr[13] (LYP) and the 6-31G* doubly split-valence Pople basis set. The effect of an aqueous solvent was incorporated with the polarizable continuum model (PCM)[14] and under these conditions DFT (B3LYP) has been shown to produce reliable coupling constant data for small molecules[15] and larger natural products;[16] therefore it is a suitable choice for the current work.

The measured coupling constant between H-8′ and H-9′ was minimal (i.e., <1 Hz), and that between H-11 and H-12 was 3.5 Hz. Of the 16 stereoisomers that were computationally explored, two yielded satisfactory agreement with the experimental NMR parameters. The 8′*S*, 9′*R*, 11*R*, 12*S* and the 8′*S*, 9′*R*, 11*S*, 12*R* configurations both indicated the same *trans* relationship of H-8′ and H-9′ with the two *cis* orientations of H-11 and H-12. The 8′*S*, 9′*R*, 11*R*, 12*S* configuration was found to have calculated *J*(H-8′, H-9′) and *J*(H-11, H-12) coupling constants of 0.1 and 2.7 Hz, and the 8′*S*, 9′*R*, 11*S*, 12*R* configuration was found to have values of −0.1 and 1.4 Hz. These are both in good agreement with the experimental values given the observation that DFT generally underestimates such properties.[14] More accuracy is expected for the *J*(H-8′, H-9′) coupling constant given the greater conformational mobility of the 11-membered ring. Since compound **1** is biosynthetically related to pseudopterolide (vide infra), for which the configuration of the epoxide is known, we suggest that the structure of **1** has the C-12 hydroxy substituent with the same configuration as in pseudopterolide with a *cis* relationship to the C-11 amino functionality, as shown in Figure 3. All 14 remaining structures yielded coupling constant data that were in marked contrast to experimental values. The proposed configuration of compound **1** is in agreement with the *cis* diol and *cis* amine/alcohol relationships indicated for gorgiacerodiol and bis(gorgiacerol) amine, respectively.[4]

**Scheme 1.** Proposed Biosynthetic Route to Bis-diterpenoids (**1** and **2**)




Tinto and co-workers suggested[4] that bis(gorgiacerol) amine (**2**) can be viewed as the product of the addition of ammonia to 2 equiv of a pseudopteranoid. The co-occurrence of compounds **1** and **2** suggested to us that such bis-diterpenoids most likely arise from a reaction of ammonia with 2 equiv of pseudopterolide (**3**) as depicted in Scheme 1. Specifically, ammonia could react with pseudopterolide (**3**) to give intermediate **5**, which could then react with a second equivalent of **3** at the epoxide to give **2** or in a Michael addition to afford **1**. Interestingly, both bis-diterpenoids were produced by bubbling $NH_3$ through a solution of pseudopterolide at room temperature for 19 h, supporting this biosynthetic proposal. The production of **1** (1.4%) and **2** (0.8%) was confirmed by LCMS and comparison with authentic samples of **1** and **2**. No attempt to optimize the yields of **1** and **2** was made. The remainder of the material was unreacted pseudopterolide and a compound with a pseudomolecular ion corresponding to a molecular formula of $C_{21}H_{25}NO_6$ and thus consistent with proposed intermediate **5** (1.0%). Given this biosynthetic relationship of pseudopterolide (**3**) with **1** and **2** (Scheme 1), the absolute configurations of unreacted stereocenters of **3** are assumed to be the same in **1**. Hence based on the discussion of relative configurations of **1** and the absolute configuration of pseudopterolide,[2] the assignments are as depicted in structure **1** (1*R*, 7*R*, 8*R*, 11*S*, 12*R*, 1′*R*, 7′*R*, 8′*S*, 9′*R*, 12′*R*).

Bis(pseudopterane) amine (**1**) and bis(gorgiacerol) amine (**2**) were evaluated in cytotoxicity assays using SYBR Green dye with HeLa and HCT116 cell lines. The known amine **2** was found to be inactive (>100 $\mu$M) against HeLa cells and has modest activity against HCT116 ($IC_{50}$ 28 $\mu$M); however **1** was more active against both cell lines and was found to exhibit selective activity against HeLa and HCT116 (42 and 4 $\mu$M, respectively) cell lines.

## Experimental Section

**General Experimental Procedures.** NMR data were recorded in $CDCl_3$ solution and referenced with residual solvent signal with resonances at $\delta_{1H/13C}$ at 7.27/77.00 ($CDCl_3$). UPLC-MS/MS analysis was performed on a Thermo Accela LC system with a LXQ linear ion trap mass spectrometer. HRESIMS spectral determination was performed by the Maritime Mass Spectrometry Laboratories at Dalhousie University, Halifax, using a Bruker MicroTOF. Flash column chromatography was performed using Bakerbond octadecyl ($C_{18}$) 40 $\mu$m preparative LC packing. Silica gel 60 $F_{254}$ precoated plates with thickness 250 $\mu$m were used for preparative TLC. HPLC was carried out using a Perkin-Elmer system consisting of a Series 250 pump and LC-235 diode array detector for semipreparative purposes. A Luna 5$\mu$ Phenyl-Hexyl 250 × 10 mm Phenomenex column was used for purification of all compounds discussed.

**Collection and Extraction Procedure.** Bis(pseudopterane) amine (**1**), bis(gorgiacerol) amine (**2**), pseudopterolide (**3**), and gorgiacerodiol (**4**) were isolated from a 2006 collection of the gorgonian octocoral *Pseudopterogorgia acerosa* from Sweetings Cay, Bahamas. A voucher (#230506-01-024) is maintained in the Department of Biomedical Sciences at UPEI. The lyophilized coral (1.2 kg) was cut into small

pieces and soaked in 2.5 L of $CH_3OH/CH_2Cl_2$ (1:1) for 72 h. The extraction was repeated twice, and after filtration, the solvent was evaporated under vacuum to yield 103.9 g of crude extract (brownish-green). After partitioning the crude extract between hexane and $CH_3OH/H_2O$ (90:10), the $CH_3OH/H_2O$ composition was made up to 1:1 and further partitioned with $CH_2Cl_2$. The dichloromethane fraction was dried under vacuum to afford 20.3 g of a viscous brown oil, which was chromatographed over $C_{18}$ (232 g) eluting with $CH_3OH/H_2O$ (10:90), $CH_3OH/H_2O$ (50:50), $CH_3OH:/H_2O$ (80:20), $CH_3CN$, acetone, and $CH_2Cl_2$. All six fractions were analyzed by UPLC-MS/MS, which indicated the presence of compounds **1** and **2** in the $CH_3CN$ fraction, which was further purified by preparative TLC (silica) using EtOAc/hexane/acetone (73:18:9), followed by semipreparative HPLC (using acetonitrile/water in gradient) to give **1** (7.8 mg) and **2** (7.3 mg). Compounds **3** and **4** were isolated from the $CH_3OH/H_2O$ (80:20) fraction by HPLC.

**Bis(pseudopterane) amine (1):** white solid; $[\alpha]_D$ −14.8 (*c* 0.80, $CHCl_3$); UV ($CH_3CN$) $\lambda_{max}$, 244 nm; IR (neat) $\nu_{max}$ 3464, 3078, 2957, 1739, 1720, 1649 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR ($CDCl_3$) data, see Table 1; LRMS *m/z* 758.22 $[M + H]^+$ $MS^2$ fragments (CID = 35) 740.17 (BP), 726.12, 708.15, 694.24, 676.27, 658.17, 464.25, 388.04, 353.01, 321.08, 247.10; HRESIMS *m/z* 758.3127 $[M + H]^+$ (calcd for $C_{42}H_{48}NO_{12}$, 758.3171).

**Synthesis of Bis(pseudopterane) Amine (1) and Bis(gorgiacerol) Amine (2) from Pseudopterolide.** Ammonia was bubbled through a 1 mg/mL solution of pseudopterolide in $CH_3CN$ for 19 h at ambient temperature. Analysis of the reaction mixture by LCMS/MS indicated the presence of **1** (1.4%) and **2** (0.8%). Yields were determined by use of a calibration curve generated from authentic standards.

**Cytotoxicity Assay.** Bis(pseudopterane) amine (**1**) and bis(gorgiacerol) amine (**2**) were tested for cytotoxicity against HeLa and HCT116 cell lines using the CyQUANT NF cell proliferation assay kit (Invitrogen) as per published protocol.[17]

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**Supporting Information Available:** Copies of the HRESIMS, $^1H$ NMR (300 MHz), $^{13}C$ NMR (75 MHz), DEPTQ, COSY, NOESY, HMQC, and HMBC for compound **1** are available free of charge via the Internet at http://pubs.acs.org.

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