JOURNAL OF NATURAL PRODUCTS

NOTE

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Chisomicines A−C, Limonoids from *Chisocheton ceramicus*

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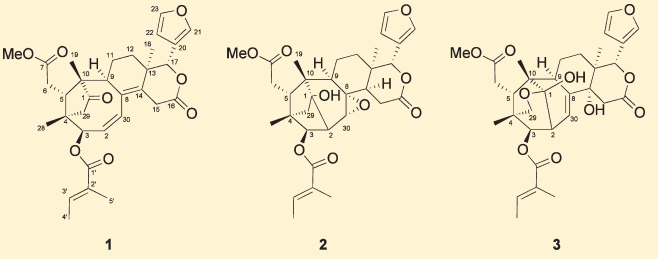
Supporting Information

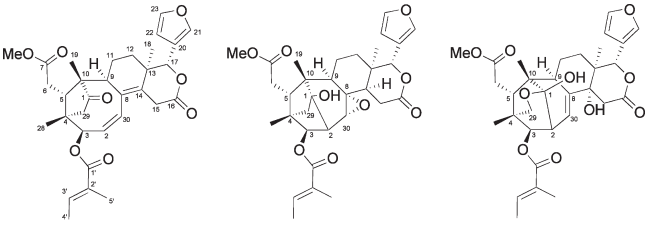
ABSTRACT: Three new limonoids, chisomicines A−C (**1**−**3**), have been isolated from the bark of *Chisocheton ceramicus*. Their structures were determined by 2D NMR, CD spectroscopic methods, and X-ray analysis. Chisomicine A (**1**) exhibited NO production inhibitory activity in J774.1 cells stimulated by LPS dose-dependently at high cell viability.



1 **2** **3**

Limonoids from Meliaceae plants have been the subject of various studies due to their antifeedant, insecticide, antitumor, and antimalarial activities.[1,2] In addition, their diverse structures, with the oxidized backbone and the side chain moiety bonded to ring D in the intact tetranortriterpenoid nucleus, have attracted great interest.[3]

We have found that an alcoholic extract of *Chisocheton ceramicus* (Miq.) C. Dc. (Meliaceae) bark is a rich source of interesting limonoids, named as a series of ceramicines.[4] In continuation of our research,[5] we have isolated three new limonoids: an A2,B,D-*seco* type (**1**) named chisomicine A, a phragmalin type (**2**) named chisomicine B, and an A1,A2-*seco*-phragmalin type (**3**) named chisomicine C. Two known mexicanolide-type limonoids, 14-deoxyxyloccensin K[6] and proceranolide,[7] were also isolated. We now wish to report the isolation and structure elucidation of compounds **1**−**3** and the NO production inhibitory activity of **1**.



1 **2** **3**

Chisomicine A (**1**) was obtained as white, amorphous powder, and the HRESIMS displayed a pseudomolecular ion peak at 573.2468 $(M + Na)^+$ corresponding to the molecular formula $C_{32}H_{38}O_8$. IR absorption at 1734 cm^{-1} indicated the presence of a carbonyl group. The ^{13}C NMR and DEPT spectra revealed 32 carbon resonances due to four carbonyls, four sp^2 quaternary carbons, three sp^3 quaternary carbons, six sp^2 methines, four sp^3 methines, five sp^3 methylenes, and six methyls. Among them, two sp^3 methines (δ_C 76.8 and 80.2), one sp^3 methyl (δ_C 52.0), and two sp^2 methines (δ_C 141.7 and 142.8) were ascribed to those bearing an oxygen atom.

Five partial structures, **a** (C-2, C-3, and C-30), **b** (from C-5 to C-6), **c** (from C-9 to C-12), **d** (from C-22 to C-23), and **e** (from C-3′ to C-4′), were deduced from $^1H-^1H$ COSY analysis of **1** in $CDCl_3$ (see Supporting Information). The presence of a bicyclo-[5.2.1]dec-3-en-8-one unit containing the partial structure **a** was supported by HMBC correlations. HMBC correlations for H-3, H-5, H_3-28, and H_2-29 of C-4 (δ_C 43.3) provided the connectivity of partial structures **a** and **b** through the C-4 atom. The presence of a cyclopentanone ring connected with the partial structure **b** was assigned by the HMBC correlations for H_2-29 of C-1 (δ_C 220.6), C-5 (δ_C 40.5), and C-10 (δ_C 54.2) and for H-5 of C-1 and C-10. Connection of partial structures **a**, **b**, and **c** was assigned based on HMBC correlations for H_3-19 of C-5, C-9 (δ_C 44.4), and C-10 and for H-9 of C-8 (δ_C 131.0) and C-10. The presence of a methoxy carbonyl group connected to the partial structure **b** was supported by the HMBC correlations for H_2-6 and H_3-OMe of C-7 (δ_C 174.1). Partial structure **e** constructing

Received: January 6, 2011
Published: March 23, 2011

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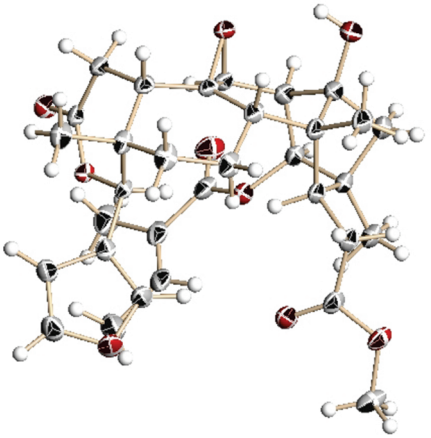

Figure 1. X-ray crystallographic structure of **2**.

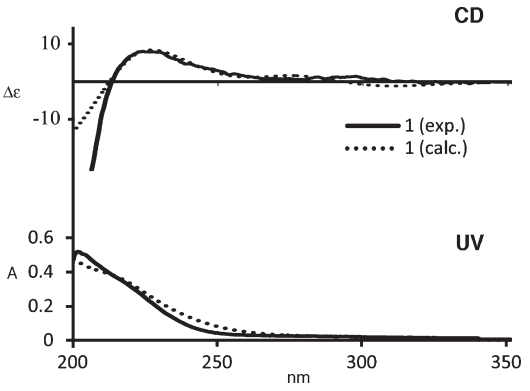

Figure 2. Experimental and calculated CD and UV spectra of **1**.

(*E*)-2-methylbut-2-enoic acid was attached at C-3 by the HMBC correlations for H-3 and H-3′ of C-1′ (δ_C 167.1). The presence of a β-furyl ring at C-17 was also assigned from the HMBC correlations. In addition, the HMBC correlations for H_3-18 of C-12 (δ_C 28.5), C-13 (δ_C 37.8), C-14 (δ_C 131.6), and C-17 (δ_C 80.2) and for H-15 of C-8, C-13, C-14, and C-16 (δ_C 169.2) indicated the presence of an isochromenone containing the partial structure **c** and a tetrahydropyran-2-one ring. Thus, chisomicine A (**1**) was concluded to be a unique limonoid possessing a bicyclo[5.2.1]dec-3-en-8-one ring system, an isochromenone, and a β-furyl ring at C-17.

NOESY correlations among H-2, H-3, and H-29b indicated that the ester at C-3 and Me-28 at C-4 took a β-orientation. Furthermore, the relative configurations at C-5, C-13, and C-17 were deduced from NOESY correlations among H-5, H-12a, and H-17. The relative configurations at C-9 and C-10 were assigned by NOESY correlations of H-9/H-30 and H_3-19 and of H-30/H_2-15.

Chisomicine B (**2**) was obtained as colorless needles (mp 176−178 °C), and the HRESIMS displayed a pseudomolecular ion peak at 569.2706 $(M + H)^+$, corresponding to the molecular formula $C_{32}H_{40}O_9$. IR absorptions implied the presence of carbonyl (1735 and 1703 cm^{-1}) functionalities. The ^{13}C NMR spectrum revealed 32 carbon resonances due to three carbonyls, two for an ester at δ_C 168.4 (C-1′) and δ_C 173.8 (C-7) and one for a lactone at δ_C 169.4 (C-16), two sp^2 quaternary carbons, five sp^3 quaternary carbons, four sp^2 methines, seven sp^3 methines, five sp^3 methylenes, and six methyls. Among them, two sp^3 quaternary carbons (δ_C 80.0 and 61.3), three sp^3 methines (δ_C 78.6, 77.6, and 59.6), one sp^3 methyl (δ_C 52.0), and two sp^2 methines (δ_C 141.4 and 143.1) were ascribed to those bearing an oxygen atom.

Analysis of the 2D NMR data revealed a similar skeleton to that of **1** except for the connection between C-1 and C-2. The connection between C-1 and C-2 was suggested by the HMBC correlation of H-30 to C-1 (δ_C 80.0), and on the basis of the HRESIMS data and the ^{13}C chemical shift of C-8 and C-30 (δ_C 61.3 and 59.6, respectively) **2** was assumed to have a C-8−C-30 epoxy ring. Thus the gross structure of **2** was suggested to possess a phragmalin-type skeleton with β-furan, δ-lactone, epoxy rings, and α-methyl crotonate moieties. The relative structure and the presence of the C-8−C-30 epoxy ring of **2** were confirmed by X-ray crystallography, as shown in Figure 1.

Chisomicine C (**3**) was isolated as a white, amorphous powder. The HRESIMS displayed a pseudomolecular ion peak at 607.2512 $(M + Na)^+$ corresponding to the molecular formula $C_{32}H_{40}O_{10}$. IR absorptions implied the presence of carbonyl functionalities (1732, 1718, and 1706 cm^{-1}).

On the basis of analysis of both 1D and 2D NMR data of **3**, the structure was deduced to be an oxidized-phragmalin-type limonoid with a rare C-1−C-29 oxygen bridge similar to granaxylocarpin C.[8] The C-8−C-30 double bond was confirmed by the $^1H-^1H$ COSY correlation of H-2/H30 and by the HMBC correlation of H-2/C-8. The relative structure of **3** was assumed to be similar to **2** and granaxylocarpin C and was confirmed by the observed NOESY correlations. The α-orientation of an OH at C-14 was deduced by the NOESY correlations of H-5/H-17 and H-17/H-3′.

The absolute configuration of **1** was assigned by comparing the experimental CD spectrum with the calculated CD spectrum (CD calculations were performed by Turbomole 6.1[9] using the RI-TD-DFT-BP86/aug-cc-pVDZ[10] level of theory on RI-DFT-BP86/SVP[10]-optimized geometries). The calculated CD spectrum of the 3*R*, 4*R*, 5*S*, 9*S*, 10*R*, 13*R*, 17*R* isomer of **1** showed a CD pattern similar to that of the experimental spectrum (Figure 2). Therefore, the absolute configuration of **1** was proposed as shown in the structures.

A plausible biogenetic pathway for chisomicines A−C (**1**−**3**) would be that they could be derived from a mexicanolide skeleton (see Supporting Information). Chisomicine B (**2**), with a phragmalin skeleton, could be biosynthesized from the coupling of C-1−C-29 of a mexicanolide skeleton, and an oxidative cleavage of the C-1−C-2 bond in chisomicine B (**2**) followed by dehydroxylation would yield chisomicine A (**1**), whereas chisomicine C (**3**) could be biosynthesized from a mexicanolide skeleton after oxidation at C-29 followed by formation of a hemiketal linkage.

Chisomicine A (**1**) inhibited NO production in J774.1 cells dose dependently stimulated by LPS and also showed little effect on cell viability (Figure 3, IC_{50} 20.2 μM).[11] However, chisomicines B (**2**) and C (**3**), 14-deoxyxyloccensin K, and proceranolide did not show NO production inhibitory activity.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 automatic digital polarimeter. UV spectra were obtained on an Ultrospec 2100 Pro spectrophotometer, and IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. High-resolution ESIMS were obtained on a LTQ Orbitrap XL (Thermo Scientific). ^1H and 2D NMR spectra were recorded on Bruker AV700 and JEOL ECA400 spectrometers, and chemical shifts were referenced to the residual solvent peaks (δ_H 7.26 and δ_C 77.0 for chloroform-*d*). Standard pulse sequences were employed for the 2D NMR experiments. $^1H-^1H$ COSY, HOHAHA,

and NOESY spectra were measured with spectral widths of both dimensions of 4800 Hz, and 32 scans with two dummy scans were accumulated into 1K data points for each of 256 t_1 increments. NOESY spectra in the phase-sensitive mode were measured with a mixing time of 800 ms. For HMQC spectra in the phase-sensitive mode and HMBC spectra, a total of 256 increments of 1K data points were collected. For HMBC spectra with *Z*-axis PFG, a 50 ms delay time was used for long-range C−H coupling. Zero-filling to 1K for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation.

Material. The bark of *C. ceramicus* was collected in 2000 from Hutan Simpan Bukit Enggang, Malaysia. The plant species was identified by Mr. Teo Leong Eng, with a voucher specimen (No. KL 4973), and the herbarium specimen was deposited in the herbarium of the Chemistry Department, University of Malaya.

Extraction and Isolation. The dried and powdered bark of *C. ceramicus* (900 g) was extracted successively with MeOH, and the MeOH extract (200 g) was partitioned with 10% MeOH(aq) and EtOAc. EtOAc-soluble materials (10 g) were subjected to a silica gel column (hexane/EtOAc, 1:0 → 0:1), after which a fraction eluted by hexane/EtOAc (3:7) was further purified on a silica gel column with CH_2Cl_2/hexane/EtOAc (5:3:2) to afford **1** (250 mg; 2.5% yield). The second fraction eluted by hexane/EtOAc (1:4) was further separated on a silica gel column with EtOAc/acetone/hexane (65:10:25). The first subfraction has been subjected to an ODS HPLC (80% MeOH(aq) with 0.1% formic acid, 3.0 mL/min, 254 nm) to give pure proceranolide (15 mg, 0.15%), and the second subfraction was subjected to an ODS HPLC (75% MeOH(aq) with 0.1% formic acid, 2.5 mL/min, 254 nm) to give **2** (25 mg, 0.25%) and 14-deoxyxyloccensin K (19 mg, 0.19%). The third subfraction was subjected to the preparative TLC with EtOAc/acetone/hexane (65:10:25) to give **3** (16 mg, 0.16%).

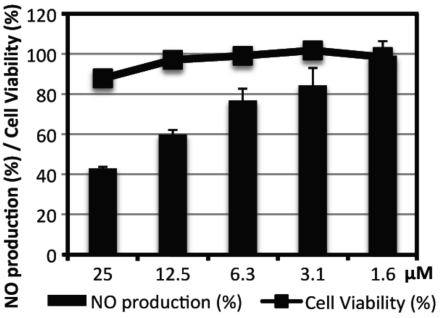

Figure 3. NO production ratio in J774.1 cells stimulated by LPS of chisomicine A (**1**). The assay was performed with $n = 3$.

Chisomicine A (1): white, amorphous powder; $[\alpha]^{27}_D$ −125 (*c* 0.7, MeOH); IR (KBr) ν_{max} 2938, 1734, and 1266 cm^{-1}; UV (MeOH) λ_{max} (log ε) 202 (4.15), and 214 (sh, 4.02); CD (MeOH) λ_{max} 201 ($\Delta\varepsilon$ −36.2), 213 (0), 227 (7.73), 290 (1.24) nm; ^{1}H NMR data (Table 1) and ^{13}C NMR data (Table 2); ESIMS *m/z* 573 $(M + Na)^+$; HRESIMS *m/z* 573.2464 $(M + Na)^+$; calcd for $C_{32}H_{38}O_8Na$, 573.2464.

Chisomicine B (2): colorless needles; mp 176−178 °C; $[\alpha]^{27}_D$ −66 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3391, 2972, 1735, 1703, and 1268 cm^{-1}; UV (MeOH) λ_{max} (log ε) 216 (3.92); CD (MeOH) λ_{max} 205 ($\Delta\varepsilon$ 0), 209 (0.71), 213 (0), 223 (−3.42), 236 (0), 245 (0.92) nm; ^{1}H NMR data (Table 1) and ^{13}C NMR data (Table 2); ESIMS *m/z* 591 $(M + Na)^+$; HRESIMS *m/z* 569.2706 $(M + H)^+$; calcd for $C_{32}H_{41}O_9$, 569.2751.

Chisomicine C (3): white, amorphous powder; $[\alpha]^{27}_D$ −86 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3441, 2980, 1732, 1718, 1706, and 1269 cm^{-1}; UV (MeOH) λ_{max} (log ε) 206 (4.16); CD (MeOH) λ_{max} 201 ($\Delta\varepsilon$ −4.86), 208 (0), 211 (0.44), 217 (0), 221 (−0.3), 227 (0), 235 (0.48), 263 (0.93) nm; ^{1}H NMR data (Table 1) and ^{13}C NMR data (Table 2); ESIMS *m/z* 607 $(M + Na)^+$; HRESIMS *m/z* 607.2512 $(M + Na)^+$; calcd for $C_{32}H_{40}O_{10}Na$, 607.2519.

X-ray Crystallography. Measurement was made on a Bruker SMART APEX CCD diffractometer with graphite-monochromated Mo Kα radiation at −173 °C. Crystal data of chisomicine B: colorless platelet crystal, orthorhombic, $C_{32}H_{40}O_9$, M = 568.64, space group $P2_12_12_1$ (#9), a = 12.4910(2) Å, b = 13.4647(2) Å, c = 34.3327(5) Å, V = 5774.33 Å^3, Z = 8, D_{calc} = 1.308 g/cm^3. Of the 55 933 reflections that

Table 1. ^{1}H NMR Spectroscopic Data (*J*, Hz) of Chisomicines A−C (1−3) in $CDCl_3$[a]

H	1	2	3
2	5.85 (1H, dd, 11.6, 6.4)	2.94 (1H, dd, 10.9, 3.4)	2.96 (1H, m)
3	4.79 (1H, dd, 6.4, 1.6)	4.80 (1H, d, 10.9)	4.80 (1H, d, 10.0)
5	3.82 (1H, brd, 12.0)	3.03 (1H, dd, 11, 2. 3)	2.90 (1H, dd, 12.0, 10.0)
6a	2.52 (1H, dd, 16.0, 12.8)	2.33 (1H, dd, 17.2, 2.3)	2.37 (1H, d, 11.3)
6b	2.35 (1H, m)	2.27 (1H, m)	2.30 (1H, d, 11.3)
9	2.66 (1H, brd, 6.0)	1.80 (1H, m)	2.44 (1H, dd, 5.5, 4.6)
11a	1.87 (1H, brd, 14.4)	1.90 (1H, brd, 11.1)	1.66 (1H, bd, 14.0)
11b	1.63 (1H, m)	1.81 (1H, m)	1.51 (1H, m)
12a	1.30 (1H, m)	1.62 (1H, m)	1.25 (1H, m)
12b	1.04 (1H, m)	1.34 (1H, d, 11.1)	1.17 (1H, m)
14		2.04(1H, dd, 6, 1.2)	
15a	3.07 (2H, brs)	2.50 (1H, dd, 18.4, 7.3)	2.94 (1H, d, 18.8)
15b		2.30 (1H, dd, 18.4, 1.8)	2.79(1H, d, 18.8)
17	5.44 (1H, s)	5.40 (1H, s)	5.38 (1H, s)
18	1.09 (3H, s)	1.00 (3H, s)	1.01 (3H, s)
19	0.97 (3H, s)	1.00 (3H, s)	0.63 (3H, s)
21	7.54 (1H, brs)	7.70 (1H, brs)	7.78 (1H, brs)
22	6.46 (1H, brs)	6.40 (1H, brs)	6.46 (1H, brs)
23	7.39 (1H, brs)	7.40 (1H, brs)	7.39 (1H, brs)
28	1.13 (3H, s)	0.86 (3H, s)	1.01 (3H, s)
29a	2.40 (1H, d, 17.6)	2.00 (1H, d, 11)	3.93 (1H, d, 9.6)
29b	2.05 (1H, d, 17.6)	1.33 (1H, dd, 11, 1.5)	3.48 (1H, d,9.6)
30	5.83 (1H, brd, 11.6)	3.25 (1H, d, 3.2)	5.48 (1H, d, 6.2)
OMe	3.72 (3H, s)	3.69 (3H, s)	3.69(3H, s)
3′	7.29 (1H, qd, 7.0, 1.6)	6.97 (1H, qd, 9.0, 1.6)	6.92 (1H, d, 7.0)
4′	1.70 (3H, d, 7.0)	1.30 (3H, d, 9.0)	1.60 (3H, d, 7.0)
5′	1.76 (3H, brs)	1.80 (3H, brs)	1.77 (3H,brs)

[a] δ in ppm.

dx.doi.org/10.1021/np200013g |*J. Nat. Prod.* 2011, 74, 1313–1317

Table 2. ^{13}C NMR Spectrscopic Data (δ) of Chisomicines A−C (1−3) in $CDCl_3$

C	1	2	3
1	220.6	80.0	97.3
2	135.5	43.9	45.2
3	76.8	78.6	75.3
4	43.3	44.8	43.3
5	40.5	39.4	34.8
6	33.8	34.2	31.8
7	174.1	173.8	173.9
8	131.0	61.3	140.9
9	44.4	41.6	43.8
10	54.2	45.1	41.4
11	19.1	21.5	19.1
12	28.5	33.7	28.6
13	37.8	36.0	41.2
14	131.6	44.8	72.9
15	33.0	27.3	39.3
16	169.2	169.4	169.3
17	80.2	77.6	76.9
18	16.4	22.0	14.9
19	22.9	18.8	14.7
20	120.7	120.8	120.0
21	141.7	141.4	142.1
22	109.9	109.7	109.9
23	142.8	143.1	143.1
28	22.6	15.0	15.5
29	46.4	43.3	67.9
30	129.1	59.6	121.8
OMe	52.0	52.0	52.2
1′	167.1	168.4	167.7
2′	127.9	128.0	127.4
3′	139.5	139.3	140.0
4′	14.3	14.2	14.7
5′	12.0	12.1	11.8

were obtained, 7315 were unique (R_{int} = 0.0556). The structure was solved by direct methods. R_1 = 0.0479 ($I > 2.00\sigma(I)$). All refinements were performed using SHELXL-97. The refined fractional atomic coordinates, bond lengths, bond angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC). CCDC 805753 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/deposit, or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

NO Production Assay by J774.1 Cells. The J774.1 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and penicillin/streptomycin. Cells were seeded onto a 96-well microtiter plate at 1×10^5 cells in 100 μL of solution per well and were preincubated for 12 h at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were cultured in the medium containing LPS (5 μg/mL) with or without the test sample at different concentrations for 24 h. NO production was then determined by the Griess assay. Supernatant of the cultured medium (100 μL) was transferred to a 96-well microtiter plate, and then 100 μL of Griess reagent (1% sulfanilamide, 0.1% *N*-l-naphthylethylenediamine dihydrochloride in 2.5% H_3PO_4) was added. After incubation at room temperature for 15 min, the absorbance at 540 and 620 nm was measured with a microplate reader (Benchmark Plus microplate spectrometer, Bio-Rad). L-NMMA, an NO synthase inhibitor, was used as a positive control (IC_{50} 13.8 μg/mL).

Cell Viability Assay. The cell viability was determined by MTT assay. MTT (15 μL of a 5 mg/mL solution) was added into each well of the cultured medium. After a 2 h incubation period, the medium was removed, and then 50 μL of DMSO was added to resolve the formazan crystals. Optical density measurements were made using a microplate reader equipped with a two-wavelength system at 550 and 700 nm. In each experiment, three replicates were prepared for each sample. The ratio of living cells was determined on the basis of the difference of the absorbance between those of samples and controls.

■ ASSOCIATED CONTENT

Supporting Information. Selected 2D NMR correlations, plausible biogenetic path, ^1H and ^{13}C NMR spectra, 2D NMR correlations of **1**−**3**, and atom coordinate of **1** are available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

This work was partly supported by University Malaya Research grants PS378/2009B, a CNRS (France) grant, a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and a grant from the Open Research Center Project.

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