

Published in final edited form as:

J Nat Prod. 2007 April; 70(4): 686-688.

# Guttiferones K and L, Antiproliferative Compounds of *Rheedia* calcicola from the Madagascar Rain Forest

Shugeng Cao<sup>†</sup>, Peggy J. Brodie<sup>†</sup>, James S. Miller<sup>‡</sup>, Fidy Ratovoson<sup>§</sup>, Chris Birkinshaw<sup>§</sup>, Sennen Randrianasolo<sup>±</sup>, Etienne Rakotobe<sup>±</sup>, Vincent E. Rasamison<sup>±</sup>, and David G. I. Kingston\*,†

Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061. Missouri Botanical Garden. P. O. Box 299. St. Louis, Missouri 63166-0299, and Centre National d'Application et Recherches Pharmaceutiques, B. P. 702, Antananarivo 101, Madagascar

#### **Abstract**

Bioassay-guided fractionation of the ethanol extract obtained from the fruits of *Rheedia calcicola* led to the isolation of two new guttiferone analogues, guttiferones K (1) and L (16-hydroxyguttiferone K) (2). The structures of 1 and 2 were established on the basis of extensive interpretation of one and two dimensional NMR spectroscopic data. Both compounds were tested for their antiproliferative activity against the A2780 human ovarian cancer cell line.

> As part of our continuing investigation of Madagascar plants for cytotoxic principles, we found that an ethanol extract (MG 2796) of the fruits of Rheedia calcicola Jum. & H. Perrier (Clusiaceae) showed cytotoxicity in the A2780 assay with an IC<sub>50</sub> value of 15 µg/mL. This extract was selected for bioassay-guided fractionation based on its cytotoxicity against the A2780 human ovarian cancer cell line, and also on the absence of any previous chemical investigation of the species. Our bioassay-guided fractionation of Rheedia calcicola resulted in the isolation of two new cytotoxic guttiferone analogues, guttiferones K (1) and L (16hydroxyguttiferone K) (2)

> The genus *Rheedia* has been found to be a rich sources of xanthones, <sup>2–4</sup> biflavonoids, <sup>5–7</sup> polyisoprenylated benzophenones (7- and 15-epiclusianone and xanthochymol), <sup>7-9</sup> and triterpenoids. <sup>10</sup> Their biological properties including brine shrimp lethality, <sup>11</sup> as well as antibacterial <sup>10,11</sup> and analgesic activity, <sup>6</sup> have been reported. 7-Epiclusianone and xanthochymol showed anti-HIV activity, <sup>12</sup> and xanthochymol also displayed antimicrobial activity <sup>13</sup> and cytotoxicity. <sup>14</sup> There is no information on traditional uses of the plant, and only lemurs eat its fruit. 15

> Extract MG 2796 was partitioned between hexane, CH<sub>2</sub>Cl<sub>2</sub>, and MeOH, and the CH<sub>2</sub>Cl<sub>2</sub> extract was found to be the most active with an IC<sub>50</sub> value of 10 µg/mL. The CH<sub>2</sub>Cl<sub>2</sub> extract was purified by filtration through a C18 cartridge followed by HPLC on a C18 column to yield compound 1 from the second fraction. Further HPLC separation of the first fraction using a C8 column yielded compound 2.

<sup>\*</sup>To whom correspondence should be addressed. Tel: (540) 231-6570. Fax: (540) 231-7702. E-mail: dkingston@vt.edu. †Virginia Polytechnic Institute and State University.

<sup>&</sup>lt;sup>‡</sup>Missouri Botanical Garden, P. O. Box 299, St. Louis, Missouri 63166-0299

Missouri Botanical Garden, B. P. 3391, Antananarivo 101, Madagaascar

Centre National d'Application des Recherches Pharmaceutiques, B. P. 702, Antananarivo 101, Madagascar

Compound 1 was obtained as a yellow oil. Its positive ion HRFABMS revealed a pseudo-molecular ion  $[(M+H)^+]$  consistent with the molecular formula  $C_{38}H_{50}O_6$ , requiring fourteen double-bond equivalents. The IR spectrum for 1 displayed bands for hydroxyl (3348 cm<sup>-1</sup>) and carbonyl groups (1728, 1670, 1640 cm<sup>-1</sup>), while the UV absorptions at  $\lambda_{max}$  241, 255, and 325 revealed aromatic and conjugated carbonyl chromophores. NMR data were collected in  $CD_3OD/0.1\%$  TFA for comparisons with the literature data, and in pyridine- $d_5$  to reduce signal overlap for the purpose of structure elucidation. The  $^1H$  NMR spectrum (Table 1) exhibited the presence of a 1,2,4-trisubstituted benzene ring. Four olefinic protons, one tertiary methyl and eight vinyl methyl groups, six methylenes, and one methine were also observed in the  $^1H$  NMR and HSQC spectra of 1, indicating the presence of four 3-methylbut-2-enyl groups and a fifth C5 unit.

The <sup>13</sup>C NMR spectrum of 1 (Table 1, CD<sub>3</sub>OD/0.1%TFA) showed resonances for six aromatic carbons, a conjugated carbonyl group at  $\delta_C$  196.7, an enolized 1,3-diketone ( $\delta_C$  191.0, 119.9, and 196.7), a non-conjugated carbonyl at  $\delta C$  209.2, two quaternary carbons [ $\delta_C$  69.4 (C-4) and  $\delta$ C 64.1 (C-8)], and 25 signals assignable to four isoprene units and a fifth C5 unit.  $^2J$  and  $^3J$ HMBC correlations (Figure 1) indicated the presence of two fragments, I (a 3,4dihydroxybenzoyl group) and II (a 2,2-dimethylbicyclo[3.3.1]nonane ring system substituted with four 3-methylbut-2-enyl groups). The <sup>1</sup>H and <sup>13</sup>C NMR spectra of **1** were very similar to those of guttiferone A  $(3)^{12b}$  suggesting that 1 was a stereoisomer of guttiferone A. The correlations between CH<sub>3</sub>-22 and CH<sub>2</sub>-17/H-7α in the ROESY spectrum of 1 indicated that CH<sub>3</sub>-22 ( $\delta_H$  0.81/ $\delta_C$  16.4, in CD<sub>3</sub>OD/0.1%TFA) must be in the  $\alpha$ -orientation like CH<sub>2</sub>-17. The  $^{13}$ C NMR chemical shift of C-6 at  $\delta_{\rm C}$  42.0 in CD<sub>3</sub>OD/0.1% TFA suggested that the C-6 substituent was in the equatorial position, since the signal of C-6 with an axial substituent is located at lower field ( $\delta_C$  46–48). <sup>12a</sup> The coupling patterns of H-7 $\alpha$  ( $\delta_H$  1.44, dd,  $J_{7\alpha,7\beta}$  = 13.3,  $J_{7a,6} = 12.9$  Hz, in CD<sub>3</sub>OD/0.1%TFA) further revealed axial orientations for CH<sub>3</sub>-22, H-6, and H-7 $\alpha$ , and equatorial orientations for H-7 $\beta$ , the 3-methylbut-2-enyl group at the 6-position, and the 4-methylpent-3-enyl group at the 5-position. These data supported a chair conformation of the bicyclo[3.3.1]nonane ring system, and enabled the structure of 1 to be assigned as 5-epi, 6-epi-guttiferone A. The orientations of CH<sub>3</sub>-22 and H-6 of 1 were the same as those of guttiferone G, 16a isolated from Garcinia macrophylla from the Suriname rainforest, and of its enantiomer (+) oblongifolin C. 16b The optical rotation of 1 was small but negative, suggesting but not demanding that it belongs to the guttiferone G series rather than the oblongifolin C series.

Compound **2** was also obtained as a yellow oil, and its molecular formula was determined as  $C_{38}H_{50}O_7$  by HRFABMS and  $^{13}C$  NMR spectroscopy. The  $^1H$  NMR signals of fragment II were very similar to those of **1**, but fragment I was identified as a 1,2,4,5-tetrasubstituted benzoyl group by the presence of two singlets ( $\delta_H$  6.82 and 7.44, s, in CD<sub>3</sub>OD/0.1%TFA) in the aromatic region. The  $^{13}C$  NMR chemical shifts of fragment I of **2** were similar to those of orirubenones **A** and **B**,  $^{17}$  indicating that it was a 2,4,5-trihydroxybenzoyl group. The stereochemistries of the 4-, 5-, 6- and 8-positions were determined to be the same as those of **1** on the basis of observed ROESY correlations between CH<sub>3</sub>-22 and CH<sub>2</sub>-17/H-7 $\alpha$ . The structure of **2** was thus assigned as guttiferone L (16-hydroxyguttiferone K).

It is reported that guttiferones I and J from *Garcinia virgata* were weakly cytotoxic against the KB cancer cell line with IC $_{50}$  values of 4.7 and 5.0 µg/mL, respectively. <sup>16c</sup> Guttiferone G isolated from *Garcinia macrophylla* also displayed weak antiproliferative activity against the A2780 ovarian cancer cell line, with an IC $_{50}$  value of 8.00 µg/mL. <sup>16a</sup> Compounds 1 and 2 were evaluated for their antiproliferative activity against the A2780 human ovarian cancer cell line, and had IC $_{50}$  values of 3.6 and 3 µg/mL, respectively, which is in good agreement with previous observations that guttiferone analogs are weakly active against certain cancer cell lines.

## **Experimental Section**

## **General Experimental Procedures**

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR and UV spectra were measured on a Spectrum One FT-IR Spectrometer (PerkinElmer Instruments) and a Shimadzu UV-1201 spectrophotometer, respectively. NMR spectra were obtained at room temperature on a JEOL Eclipse 500 spectrometer (5 mm BB probe for  $^1H$  and  $^{13}C$  NMR, and PFG probe for 2D NMR) and a Varian INOVA 400 spectrometer (5 mm AUTOSW PFG probe) in CD<sub>3</sub>OD/0.1%TFA ( $\delta_H$  3.31 and  $\delta_C$  49.5) or  $C_5D_5N$  ( $\delta_H$  8.71 and  $\delta_C$  149.9). The chemical shifts are given in  $\delta$  (ppm), and coupling constants are reported in Hz. Mass spectra were obtained on a JEOL JMS-HX-110 instrument, in the positive ion mode. HPLC was performed on a Shimadzu LC-10AT instrument with a semi-preparative C8 Varian Dynamax column (5  $\mu$ m, 250  $\times$  10 mm) and a preparative C18 Varian Dynamax column (8  $\mu$ m, 250  $\times$  21.4 mm). Finnigan LTQ LC/MS with a C18 Hypersil column (5  $\mu$ m, 100  $\times$  2.1 mm) was also used for crude sample analysis.

# **Antiproliferative Bioassays**

Antiproliferative activity measurements were performed at Virginia Polytechnic Institute and State University against the human A2780 ovarian cancer cell line as previously described. The A2780 cell line is a drug - sensitive ovarian cancer cell line. 18

#### **Plant Material**

Fruits of *Rheedia calcicola* Jum. & H. Perrier (Clusiaceae) were collected from a tree 8 m high and trunk diameter at breast height of 10 cm, growing on sand near a stream on November 12th, 2004 in the forest of Sahafary/Saharenana, in the Province of Antsiranana, Madagascar (12.34.36 S / 49.27.15 E, elevation 268 m). The herbarium voucher specimen for the sample is Sennen Randrianasolo et al. 503. Duplicate voucher specimens were deposited at herbaria of: the Centre National d'Application des Recherches Pharmaceutiques, Madagascar (CNARP); the Parc Botanique et Zoologique de Tsimbazaza, Madagascar (TAN); at the Missouri Botanical Garden, St. Louis, Missouri (MO); and at the Muséum National d'Histoires Naturelles, Paris, France (P).

## **Extraction and Isolation**

Dried fruit of *R. calcicola* 130 g) were ground in a hammer mill, then extracted with EtOH by percolation for 24 h at rt to give the crude extract MG 2796 (7.0 g), of which 1.3 g was shipped to Virginia Polytechnic Institute and State University (VPISU) for fractionation. MG 2796 (IC<sub>50</sub>: 15 µg/mL, 106.1 mg) was suspended in aqueous MeOH (MeOH-H<sub>2</sub>O, 9:1, 10 mL) and extracted with hexane (3 × 10 mL portions). The aqueous layer was then diluted to 70% MeOH (v/v) with H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 10 mL portions). Both the hexane and the CH<sub>2</sub>Cl<sub>2</sub> extracts were evaporated *in vacuo* to leave 31.3 and 44.8 mg of residues (IC<sub>50</sub>: 11 and 10 µg/mL, respectively). The aqueous MeOH extract (30 mg) was inactive. The CH<sub>2</sub>Cl<sub>2</sub> extract was selected due to its relatively greater quantity and potency as compared with the hexane extract. The CH<sub>2</sub>Cl<sub>2</sub> extract was dissolved in MeOH, and the MeOH solution was filtrated through an SPE cartridge over C18 before being injected into the HPLC (85% MeOH-H<sub>2</sub>O). Four fractions were collected (I, II, III, and IV). Fraction II yielded compound 1 (20 mg,  $t_R$  33 min). Further purification of fraction I was carried out by C8 HPLC with 85% MeOH as the eluent to yield compound 2 (0.6 mg,  $t_R$  24 min).

### **Guttiferone K (1)**

yellow oil; [ $\alpha$ ] $_D^{23}$   $-2^{\circ}$  (c 0.35, CHCl $_3$ ); IR (film)  $V_{max}$  3348, 2968, 2916, 2857, 1728, 1670, 1640, 1602, 1542, 1519, 1440, 1376, 1289, 1191, 1116 cm $^{-1}$ ; UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 241

(sh), 255 (4.54), 325 (4.14) nm;  $^{1}$ H NMR (500 MHz, CD<sub>3</sub>OD and pyridine- $d_5$ ) and  $^{13}$ C NMR (125 MHz, CD<sub>3</sub>OD and pyridine- $d_5$ ) see Table 1; HRFABMS m/z 603.3712 (calcd for C<sub>38</sub>H<sub>51</sub>O<sub>6</sub>, 603.3686).

## 16-Hydroxyguttiferone K (2)

yellow oil;  $[\alpha]_D^{23}$  –8° (c 0.06, CHCl<sub>3</sub>); IR (film) V<sub>max</sub> 3450, 2966, 2922, 2856, 1732, 1672, 1604, 1494, 1461, 1384, 1292, 1267, 1191 cm<sup>-1</sup>; UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 251 (4.0), 285 (sh), 364 (3.6) nm; <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD and pyridine- $d_5$ ) and <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD) see Table 1; HRFABMS m/z 601.3562 [M-OH]. (calcd for C<sub>38</sub>H<sub>49</sub>O<sub>6</sub>, 601.3529).

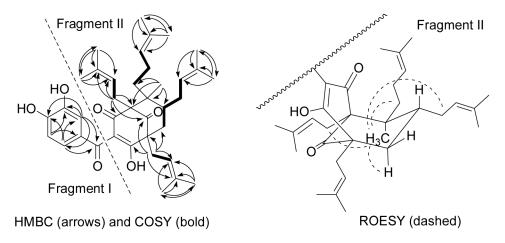
#### Acknowledgments

This International Cooperative Biodiversity Group project was supported by the Fogarty International Center, the National Cancer Institute, the National Science Foundation, the National Heart, Lung and Blood Institute, the National Institute of Mental Health, the Office of Dietary Supplements, and the Office of the Director of NIH, under Cooperative Agreement U01 TW000313 from the National Institutes of Health, and this support is gratefully acknowledged. We thank Mr. B. Bebout for obtaining the mass spectra and Mr. T. Glass for assistance with the NMR spectra. Field work essential for this project was conducted under a collaborative agreement between the Missouri Botanical Garden and the Parc Botanique et Zoologique de Tsimbazaza and a multilateral agreement between the ICBG partners, including the Centre National d'Applications des Recherches Pharmaceutiques. We gratefully acknowledge courtesies extended by the Government of Madagascar.

#### **References and Notes**

- Biodiversity Conservation and Drug Discovery in Madagascar, Part 27. For Part 26, see Cao S, Brodie PJ, Miller JS, Randrianaivo R, Ratovoson F, Callmander M, Andriantsiferana R, Rasamison VE, Kingston DGI. J. Nat. Prod. in press (np060627g)
- Delle Monache G, Delle Monache F, Waterman PG, Crichton EG, Alves de Lima R. Phytochemistry 1984;23:1757–1759.
- 3. Delle Monache G, Botta B, De Mello JF, Coelho JS, de B, Menichini F. J. Nat. Prod 1984;47:620–625.
- Delle Monache G, Delle Monache F, Marini Bettolo GB, Alves de Lima R. J. Nat. Prod 1983;46:655–659.
- Li XC, Joshi AS, Tan B, ElSohly HN, Walker LA, Zjawiony JK, Ferreira D. Tetrahedron 2002;58:8709–8717.
- Luzzi R, Guimaraes CL, Verdi LG, Simionatto EL, Delle Monache F, Yunes RA, Floriani AEO, Cechinel-Filho V. Phytomedicine 1997;4:141–144.
- Botta B, Marquina McQuhae M, Delle Monache G, Delle Monache F, De Mello JF. J. Nat. Prod 1984;47:1053.
- 8. Dos Santos MH, Nagem TJ, Braz-Filho R, Lula IS, Speziali NL. Mag. Res. Chem 2001;39:155-159.
- Alves TM, de A, Alves R, de O, Romanha AJ, Dos Santos MH, Nagem TJ, Zani CL. J. Nat. Prod 1999;62:369–371. [PubMed: 10075791]
- 10. Torrico F, Velasco P, Gimenez A, Almanza GR. Rev. Boliv. Quim 2001;18:38–42.
- 11. Verdi LG, Pizzolatti MG, Montanher ABP, Brighente IMC, Smania Junior A, Smania E, de FA, Simionatto EL, Delle Monache F. Fitoterapia 2004;75:360–363. [PubMed: 15158995]
- 12. (a) Piccinelli AL, Cuesta-Rubio O, Chica MB, Mahmood N, Pagano B, Pavone M, Barone V, Rastrelli L. Tetrahedron 2005;61:8206–8211. (b) Gustafson KR, Blunt JW, Munro MHG, Fuller RW, McKee TC, Cardellina JH II, McMahon JB, Cragg GM, Boyd MR. Tetrahedron 1992;48:10093–10102. (c) Roux D, Hadi HA, Thoret S, Guénard D, Thoison O, Paies M, Sévenet T. J. Nat. Prod 2000;63:1070–1076. [PubMed: 10978200]
- 13. Iinuma M, Tosa H, Tanaka T, Kanamaru S, Asai F, Kobayashi Y, Miyauchi K, Shimano R. Biol. Pharm. Bull 1996;19:311–314. [PubMed: 8850331]
- 14. (a) Baggett S, Protiva P, Mazzola EP, Yang H, Ressler E, Basile MJ, Weinstein I, Bernard K, Edward J. J. Nat. Prod 2005;68:354–360. [PubMed: 15787435] (b) Matsumoto K, Akao Y, Kobayashi E, Ito T, Ohguchi K, Tanaka T, Iinuma M, Nozawa Y. Biol. Pharm. Bull 2003;26:569–571. [PubMed:

- 12673047] (c) Ito C, Itoigawa M, Miyamoto Y, Onoda S, Rao KS, Mukainaka T, Tokuda H, Nishino H, Furukawa H. J. Nat. Prod 2003;66:206–209. [PubMed: 12608850]
- 15. Personal communication from a Malagasy forest guide to Fidy Ratovoson.
- 16. (a) Williams RB, Hoch J, Glass TE, Evans R, Miller JS, Wisse JH, Kingston DGI. Planta Med 2003;69:864–866. [PubMed: 14598218] (b) Hamed W, Brajeul S, Mahuteau-Betzeer F, Thoison O, Mons S, Delpech B, Van Hung N, Sévenet T, Marazano C. J. Nat. Prod 2006;69:774–777. [PubMed: 16724839] (c) Merza J, Mallet S, Litaudon M, Dumontet V, Seraphin D, Richomme P. Planta Med 2006;72:87–89. [PubMed: 16450306]
- 17. Kawagishi H, Tonomura Y, Yoshida H, Sakai S, Inoue S. Tetrahedron 2004;60:7049–7052.
- 18. Louie KG, Behrens BC, Kinsella TJ, Hamilton TC, Grotzinger KR, McKoy WM, Winker MA, Ozols RF. Cancer Res 1985;45:2110–2115. [PubMed: 3986765]



**Figure 1.** Key HMBC, COSY, and ROESY correlations for **1**.

Table 1 Table 1 Table 1 Table 1 and  $^{13}\mathrm{C}$  NMR Spectral Data for Compounds 1 and  $2^a$ 

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

no		1				2	
	MeOH-d4	$^{1}$ H $_{\mathrm{SD_{S}N}}$	MeOH-d <sub>4</sub>	$^{13}$ C $_{5}D_{5}N$	$MeOH-d_4$	$^{1}$ H $_{c_{5}D_{5}N}$	<sup>13</sup> C MeOH-d <sub>4</sub>
			196.7	190.9			190.4
2			119.9	121.9			120.4
3			191.0	187.1			182.5
4,			69.4	69.4			70.3
o,	1		51.6	49.4	!	,	51.7
9	1.75 m 2.03 dd 13.3, 3.3 1.44 dd	2.62 dd 13.5, 10.1 2.47 dd 14.0, 3.9 1.73 dd	42.0 43.2	41.0 41.8	1.75 m 2.04 dd 13.1, 3.5	2.11 m 2.43 dd 13.3, 3.2	39.4 43.8
۰	13.3, 12.9	14.0, 10.1	1 72	0.03	1.52 m	1.75 m	3 33
0 0			209.2	02.9 210 6			207.5
10			196.7	196.9			196.1
			130.3	131.3	ţ		117.9
12	7.20 d 2.1	7.92 br s	117.4	117.4	7.44 s	8.06 s	109.8
51 41			152.6	152.7			151.2
15	6.69 d 8.4	7.17 d 8.3	115.3	115.7	6.82 s	7.36 s	104.2
9 1	6.95 dd 8.4, 2.1	7.66 dd 8.3, 2.5	125.1	124.0		0	155.0
1.)	2.73 dd 13.0, 7.8 2.65 dd 13.0 4 3	3.10 dd 13.8, 7.1 3.03 dd 13 8 4 2	7.97	26.7	3.00 dd 14.2, 9.0 2 87 dd 14.2 4.0	3.18 m 3.18 m	7.97
18	4.88 m	5.52 br t 7.0	121.5	123.4	4.70 m	5.21 br t 7.0	120.8
19			135.2	$132.1^{b}$			135.3
20	1.69 s	1.78 s	18.5	18.7	1.83 s	1.68 s	18.8
21	1.62 s	1.57 s	26.4	26.3	1.50 s	1.47 s	26.2
22	0.81 s	0.99 s	16.4	16.6	0.90 s	0.99 s	17.6
23	1.68 m	1.98 br t 8.1	37.6	37.0	1.95 m; 1.58 m	1.67 m	37.5
24 24	2.0/ m; 1.// m	2.25 m; 1.91 m	30.2	29.9	2.00 m; 1.28 m	2.09 m; 1.85 m	30.3
25 26	5.00 br t 7.0	2.22 m	123.7	$\frac{124.0}{133.2}$	4.95 m	3.00 m	123.2
27	1.67 s	1.60 s	18.3	18.3	1.59 s	1.58 s	18.3
28	1.57 s	1.56 s	26.0	26.0	1.55 s	1.57 s	25.8
29	2.51 dd 14.5, 8.8 2.44 dd	2.91 d 6.4	31.8	31.6	2.52 m	2.94 m; 2.85 m	31.4
30	5.10 br t 7.1	5.73 br t 7.0	121.1	122.5	4.95 m	5.42 br t 7.0	120.9
31	,		135.7	133.3	•	i t	135.4
32	1.6/ s	1.73 s	18.4	18.5	1.65 s	1.75 s	18.3
33	1./I s	1.65 s	26.4	26.3	1.59 s	1.52 s	26.2
35 35	5.04 br t 6.9	5.24 m	23.3 125.6	23.0 126.1	1.78 m 4.95 m	5.12 m	24.3 124.7
36			132.7	132.0			133.3
37 38	1.66 s 1.59 s	1.63 s 1.63 s	26.1 18.0	26.1 18.1	1.69 s 1.59 s	1.63 s 1.37 s	26.0 17.7

 $<sup>^</sup>a\delta$  (ppm), 500 MHz for  $^1\mathrm{H}$  NMR and 125 MHz for  $^{13}\mathrm{C}$  NMR; multiplicities; J values (Hz).

 $\frac{b}{b}$  Interchangeable.