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Daphmalenines A and B: Two New Alkaloids with Unusual Skeletons from *Daphniphyllum himalense*

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Two new biogenetically related yuzurine-type *Daphniphyllum* alkaloids of the rare (14*R*,15*S*) series, daphmalenines A (**1**) and B (**2**), have been isolated from the leaves of *Daphniphyllum himalense*. Their structures and absolute configurations were elucidated by a combination of spectroscopic data, X-ray crystallographic, and computational methods. A plausible biosynthetic pathway of **1** and **2** was also proposed.

Introduction

Daphniphyllum alkaloids, elaborated by plants of the genus *Daphniphyllum* (Daphniphyllaceae), are a family of structurally diverse natural products with complex polycyclic skeletons.[1] Their unique structural features have attracted great interest as challenging projects for total synthesis[2] and biosynthetic research[3] for several decades. Quite a number of new *Daphniphyllum* alkaloids have been isolated and identified,[1,4] and some of them possessed novel skeletons proposed by the unique biogenetic process involving repeated fissions of C–C and/or C–N bonds followed by rearrangements, recyclization, and so on.[1]

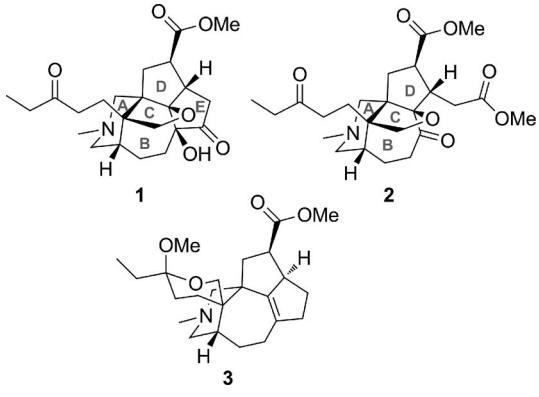

Figure 1. Molecular structures of **1**–**3**.

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In our further search for structurally unique *Daphniphyllum* alkaloids, two new yuzurine-type alkaloids of the rare (14*R*,15*S*) series, daphmalenines A and B (**1** and **2**, respectively; Figure 1), with an unusual penta- or tetracyclic ring system, respectively, were isolated from the leaves of *Daphniphyllum himalense*. In addition, a known yuzurine-type alkaloid, yuzurine (**3**), was found to coexist with **1** and **2**. This paper describes herein the isolation and structural elucidation of the new compounds and a plausible biosynthetic pathway for their generation.

Results and Discussion

Daphmalenine A (**1**), isolated as a colorless block crystal (MeOH), showed a molecular ion at *m*/*z* = 419.2305 in its high-resolution mass spectrum (EI), corresponding to a molecular formula of $C_{23}H_{33}NO_6$ (calcd. 419.2308); this requires eight degrees of unsaturation. The IR spectrum indicated the absorptions of a hydroxy (3468 cm^{-1}) and three carbonyl groups (1754, 1737, and 1707 cm^{-1}). The ^{13}C NMR and DEPT spectra of **1** (Table 1) displayed 23 carbon resonances, including 2 ketone groups (δ_C = 210.4 and 210.8 ppm), 1 ester group (δ_C = 174.9), 4 sp^3 quaternary carbon atoms, 3 sp^3 methine carbon atoms, 10 sp^3 methylene groups, 2 methyl groups, together with 1 methoxy group. Among them, two methylene groups (δ_C = 54.5 and 57.9 ppm) and one methyl group (δ_C = 45.0 ppm) were ascribed as attached to a nitrogen atom, whereas one methylene group (δ_C = 73.1 ppm) and two quaternary carbon atoms (δ_C = 79.8 and 102.3 ppm) were ascribed as bearing a oxygen atom. Because two ketone and one ester groups accounted for three out of eight degrees of unsaturation, the remaining five degrees of unsaturation were assumed for the presence of a pentacyclic system in **1**.

Detailed 2D NMR (HMQC, ^1H–^1H COSY, and HMBC experiments) studies revealed that **1** possesses four spin cou-

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of **1** and **2** (δ in ppm, J in Hz).

Position	**1**[a]		**2**[b]	
	δ_H (multiplicity, J)	δ_C	δ_H (multiplicity, J)	δ_C
1a	2.19 (d, 12.4)	57.9	1.99 (d, 11.5)	56.7
1b	2.52 (d, 12.4)[c]		2.49 (d, 11.5)[c]	
2		210.4		213.4
3a	2.10 (m)	37.7	2.31 (dd, 11.0, 5.5)	38.5
3b	2.34 (m)		2.52 (dd, 11.0, 5.5)	
4a	1.49 (m)	24.7	1.51 (ddd, 16.5, 11.0, 5.5)	23.7
4b	2.04 (m)[c]		1.89 (m)	
5		46.2		46.8
6	1.66 (m)	33.7	1.87 (m)	36.2
7a	2.39 (m)	54.5	2.50 (m)[c]	56.5
7b	2.52 (d, 12.4)[c]			
8		57.9		61.9
9		102.3		98.2
10		79.8		213.9
11a	1.55 (dt, 14.8, 4.0)	29.7	2.41 (dd, 17.0, 9.0)	41.9
11b	3.17 (m)		2.53 (m)	
12a	1.76 (m)	26.2	1.76 (m)	27.5
12b	2.00 (m)		2.36 (m)	
13a	1.74 (dd, 12.8, 7.6)	39.1	1.62 (dd, 12.5, 5.0)	37.2
13b	2.26 (t, 12.8)		2.09 (t, 12.5)	
14	2.72 (m)	50.1	2.72 (m)	50.7
15	3.15 (m)	46.3	2.71 (m)	53.3
16a	1.98 (m)	44.3	2.57 (dd, 17.0, 5.5)	35.3
16b	3.27 (dd, 18.4, 12.4)		3.08 (dd, 17.0, 9.5)	
17		210.8		175.1
18	2.41 (q, 7.2)	36.1	2.47 (q, 7.5)	36.6
19	1.05 (t, 7.2)	7.8	1.00 (t, 7.5)	8.1
21a	3.90 (br. s)	73.1	3.93 (d, 8.9)	74.7
21b	3.90 (br. s)		4.00 (d, 8.9)	
22		174.9		175.5
23	3.70 (s)	52.1	3.67 (s)	52.4
24	2.04 (s)[c]	45.0	1.98 (s)	44.6
25			3.57 (s)	51.8
10-OH	4.03 (s)			

[a] Data were measured in $CDCl_3$ at 400 MHz (^{1}H) and 100 MHz (^{13}C). [b] Data were measured in CD_3OD at 500 MHz (^{1}H) and 125 MHz (^{13}C). [c] Overlapped.

pling systems: *a* (C-18/C-19), *b* (C-3/C-4), *c* (C-7/C-6/C-12/C-11), and *d* (C-13/C-14/C-15/C-16) as outlined with bold bonds (Figure 2a). The linkages of components *a*–*d* with the quaternary carbon atoms and heteroatoms were finally established by HMBC experiments. HMBC correlations of 7-Hb [δ_H = 2.52 (d, J = 12.4 Hz) ppm] and 24-H_3 [δ_H = 2.03 (s) ppm] to C-1 and of 24-H_3 to C-7 indicated that C-1, C-7, and CH_3-24 (δ_C = 45.0 ppm) were connected to each other through the nitrogen atom. The linkages of C-21, C-4, and C-6 to C-5 were fixed by the HMBC correlations from 21-H_2, 4-Ha, and 6-H to C-5. The connectivity of fragments *a* and *b* through a ketone carbonyl (δ_C = 210.4 ppm) assigned to C-2 was elucidated by correlations of 3-Hb, 18-Ha, and 19-H_3 to C-2. In addition, the attachment of C-1, C-5, and C-13 to C-8 was established by HMBC correlations of 21-Ha/C-8, 13-Ha/C-8, 13-Ha/C-1 and 1-Ha/C-8. The formation of an ether linkage between C-21 and C-9 was illustrated by the key HMBC correlations from 21-Ha to C-9. The quaternary carbon signal at δ_C = 210.8 ppm for the other ketone carbonyl was assigned to C-17 by the HMBC correlations from 10-OH, H-15, and 16-H_2 to C-17. HMBC correlations of 10-OH and 11-Ha to C-10 (δ_C = 79.8 ppm) established one of the two oxygenated

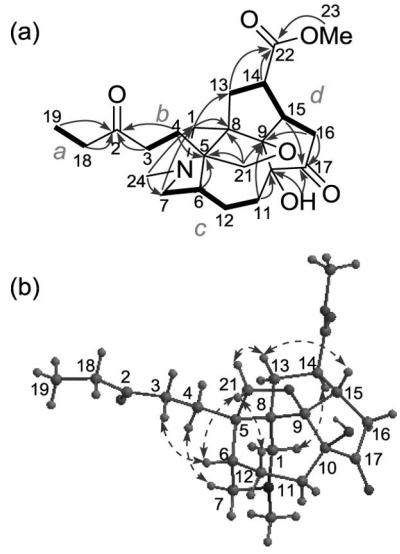


Figure 2. (a) ^{1}H–^{1}H COSY (bold) and selected HMBC (arrow) correlations of **1** and (b) key ROESY correlations of **1**.

quaternary carbon atoms was located at C-10. Moreover, HMBC correlations of 13-Hb, 14-H, and 23-H_3 to C-22

indicated a methoxy group was attached to C-22. Thus, the planar structure of **1**, possessing a yuzurine-type skeleton was assigned as shown in Figure 2a.

The relative stereochemistry of **1** elucidated by a ROESY experiment seems consistent with those of yuzurine-type *Daphniphyllum* alkaloids except for the configuration of 15-H (Figure 2b). The 15-H hydrogen of **1** possesses an opposite configuration to that of 14-H, which is beyond the common expectation for the same configuration of the proton pair 14-H/15-H, as all the *Daphniphyllum* alkaloids normally possess the same configuration of the proton pair 14-H/15-H to satisfy the stereospecific requirement.[1]

The unique stereochemistry of the proton pair H-14/H-15 in *Daphniphyllum* alkaloids raises our interest to assign the absolute configuration of **1**, which was finally accomplished by single-crystal X-ray diffraction using the Flack parameter [0.08(18)],[5] as shown in Figure 3. Thus, the absolute configuration of **1** was unambiguously assigned as (5*R*,6*S*,8*R*,9*R*,10*S*,14*R*,15*S*).

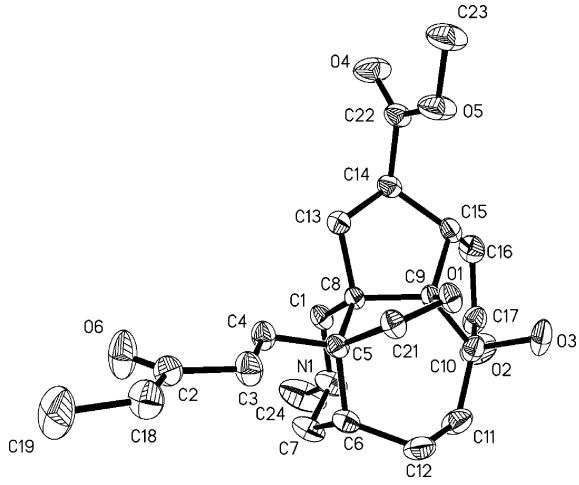


Figure 3. X-ray structure of daphmalenine A (**1**).

Daphmalenine B (**2**), obtained as a colorless solid, possesses a molecular formula of $C_{24}H_{35}NO_7$ determined by HRMS (EI) with *m*/*z* = 449.2419 $[M]^+$ (calcd. 449.2414) and eight degrees of unsaturation. IR absorptions at 1737 and 1704 cm^{-1} implies the presence of ester carbonyl and ketone functionalities. All 24 carbon signals observed in the ^{13}C NMR and DEPT spectra of **2** (Table 1) could be classified into 2 ketone groups (δ_C = 213.4 and 213.9 ppm), 2 ester groups (δ_C = 175.1 and 175.5 ppm), 3 sp^3 quaternary carbon atoms (an oxygenated one at δ_C = 98.2 ppm), 3 sp^3 methine carbon atoms, 10 sp^3 methylene groups (two being linked with the N atom at δ_C = 56.5, 56.7 ppm, and an oxygenated one at δ_C = 74.7 ppm), 2 methyl groups (one being linked with the N-atom at δ_C = 44.6 ppm), together with 2 methoxy groups. The two ketone and two ester groups accounted for four out of eight degrees of unsaturation, and the remaining four degrees of unsaturation required alkaloid **2** to be tetracyclic.

Detailed 2D NMR (^1H–^1H COSY, TOCSY, HMQC, and HMBC experiments) studies revealed that alkaloid **2** had a skeleton partially similar to **1**. The striking difference between them was the presence of two methyl ester groups in **2**. The HMBC correlations suggested that the aliphatic chain with five carbon atoms and the A–D rings in alkaloid **2** were consistent with **1**, as shown in Figure 4a. In the HMBC spectrum of **2**, cross-peaks of 16-H_2 and 25-H_3 to C-17 (δ_C = 175.1 ppm) indicated 25-OMe was attached to C-17, which illustrated the absence of a cyclopentanone E ring with a hydroxy group at C-10 in **2**. HMBC correlations of 11-H_2 and 12-Ha to C-10 (δ_C = 213.9 ppm) implied that one of the two ketone carbonyl groups was assigned to C-10. Thus, the planar structure of **2** was assigned as shown in Figure 4a, containing a ring-open system (C-9, C-15, C-16, C-17, and C-10) due to the unique cleavage of the C-10/C-17 bond, which is the first *seco*-10,17-yuzurine type *Daphniphyllum* alkaloid.

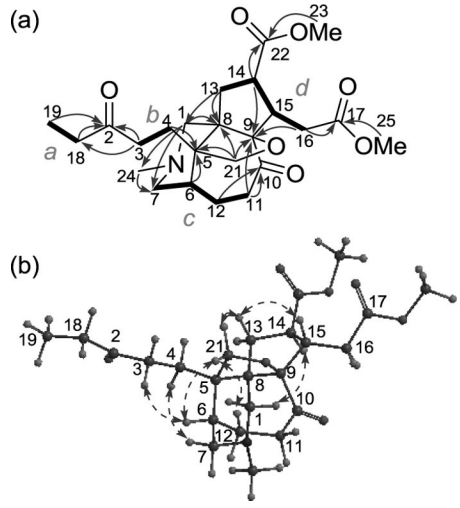
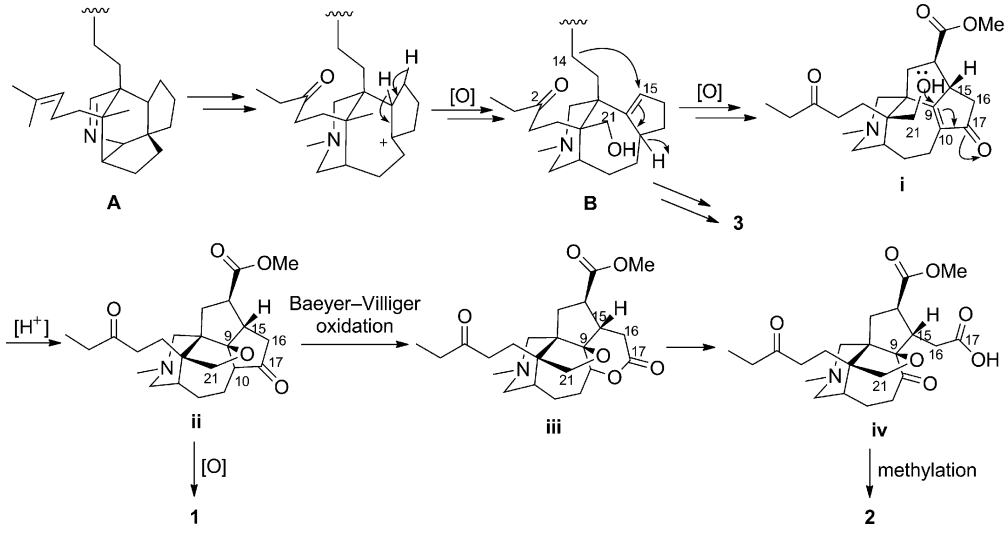


Figure 4. (a) ^1H–^1H COSY (bold) and selected HMBC (arrow) correlations of **2** and (b) key ROESY correlations of **2**.

The relative stereochemistry of **2**, as shown in Figure 4b, was assigned by a ROESY experiment and by comparison of NMR spectroscopic data with that of **1**. Correlations of 21-Hb/6-H, 21-Ha/13-Ha, 13-Ha/15-H, 13-Hb/4-Ha, 4-Hb/7-Ha, 21-Hb/12-Ha, and H-14/H-1b indicated that **2** had the same configuration as **1**.

To allocate the absolute configuration of daphmalenine B (**2**), the optical rotation (OR) value of **2** was calculated by using density functional theory (DFT) methods[4b,6] in the Gaussian 03 program package.[7] The "self-consistent reaction field" method (SCRF) was employed to perform the OR calculation of the most-stable conformer of **2** in MeOH solution at the B3LYP/6-31+G** level. The calculated OR value (–63.3°) for **2** is close to its experimental values (–62.7°), and thus the absolute configuration of **2** was assigned as (5*R*,6*S*,8*R*,9*R*,14*R*,15*S*), which is identical to that of **1**.

The cytotoxic activities of **1** and **2** against the growth of human tumor cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480) by using the MTT method were evaluated.[8] The results indicated that **1** and **2** were inactive against the above cancer cells ($IC_{50} > 40$ μM).



Scheme 1. Hypothetical biosynthetic pathway of **1**–**3**.

Conclusions

Two new yuzurine-type *Daphniphyllum* alkaloids, daphmalenines A (**1**) and B (**2**), of the rare (14*R*,15*S*) series have been isolated from the leaves of *Daphniphyllum himalense*. Their unique structures and relative stereochemistry were elucidated on the basis of spectroscopic data, and the absolute configurations of **1** and **2** were determined by X-ray diffraction by using the Flack parameter and computational methods, respectively. Biogenetically, these secondary metabolites (i.e., **1**–**3**) could be derived from common imine intermediate A,[1] which involves an alternative route during the process of the formation of the C-14 and C-15 bond,[9] as proposed in Scheme 1.

Experimental Section

General: Optical rotations were measured with a Perkin–Elmer model 241 polarimeter. UV spectra were recorded with a Shimadzu UV-250 spectrophotometer using KBr disks. NMR spectra were recorded with a Bruker AM-400 and DRX-500 NMR spectrometer with TMS as internal standard. MS and HRMS (EI) were performed by using a Finnigan MAT 90 instrument and VG Auto Spec-3000 spectrometer, respectively. Column chromatography was performed on silica gel (90–150 μm; Qingdao Marine Chemical Inc.), Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Lichroprep RP-18 gel (20–45 μm, Merck, Darmstadt, Germany). Precoated silica gel GF254 and HF254 plates (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) were used for TLC.

Plant Material: The leaves of *D. himalense* were collected in October 2008 from Gaoligong Mountain, Yunnan Province, People's Republic of China. A voucher specimen (no. KIB 08090418) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Kunming, People's Republic of China.

Extraction and Isolation: The air-dried and powdered leaves of *D. himalense* (16 Kg) were extracted with 95% EtOH, and the crude extract was partitioned between EtOAc and an acidic liquor with pH 3. The aqueous layer was then basified to pH 10 with saturated Na_2CO_3, followed by exhaustive extraction with $CHCl_3$. The $CHCl_3$-soluble material (60 g) was subjected to silica gel column chromatography ($CHCl_3$/MeOH, 1:0 → 0:1) to give four major fractions. Fraction 1 (2.8 g) was purified by chromatography on a silica gel column ($CHCl_3$/MeOH, 30:1) to give a major alkaloid, which was purified on normal H silica gel (petroleum ether/acetone, 8:1 → 2:1) and then by a Sephadex LH-20 gel column (MeOH) to afford daphmalenines A (**1**, 18 mg) and B (**2**, 6 mg), and yuzurine (**3**, 7 mg).

Daphmalenine A (1): Colorless block crystals (MeOH). $[a]_D^{22}$ = –95.4 (c = 0.35, MeOH). M.p. 142–144 °C. IR (KBr): $\tilde{\nu}$ = 3468, 2926, 1754, 1737, 1707, 1453, 1285, 1202, 1167, 1030 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data, see Table 1. MS (EI): m/z (%) = 419 (45) $[M]^+$, 402 (100), 362 (48), 85 (58), 57 (91). HRMS (EI): calcd. for $C_{23}H_{33}NO_6$ $[M]^+$ 419.2308; found 419.2305.

Crystal Data for Daphmalenine A (1): Colorless orthorhombic crystals, $C_{23}H_{33}NO_6$. Space group $P2_12_12_1$, a = 8.145(6) Å, b = 14.890(7) Å, c = 18.096(8) Å, V = 2194.0(2) $Å^3$, Z = 4, d = 1.270 g/cm^3, crystal dimensions 0.98 × 1.46 × 1.82 mm^3 were used for measurements on a Rigaku MicroMax 002+ diffractometer with graphite monochromated (ω and κ scan, 2θ max 144.36°) Cu-K_α radiation. The total number of independent reflections measured was 4235, of which 4223 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: R_1 = 0.0387, wR_2 = 0.1091, S = 1.083. The crystal structure of **1** was solved by the direct method SHELXS-97,[10] expanded using geometrical calculations and difference Fourier techniques, and refined by least-squares calculations. The absolute stereochemistry was determined by the X-ray diffraction of the single crystal using the Flack parameter [χ = 0.08(18)].[5] CCDC-795106 (for **1**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Daphmalenine B (2): Colorless solid. $[a]_D^{22}$ = –62.7 (c = 0.68, MeOH). IR (KBr): $\tilde{\nu}$ = 3440, 2949, 1737, 1704, 1457, 1437, 1290, 1189, 1173 cm^{-1}. For ^1H and ^{13}C spectroscopic data, see Table 1. MS (EI): m/z = 449 (10) $[M]^+$, 418 (12), 376 (12), 319 (12), 278 (30), 187 (39), 155 (100), 149 (64), 57 (68). HRMS (EI): calcd. for $C_{24}H_{35}NO_7$ $[M]^+$ 449.2414; found 449.2419.

Cytotoxicity Bioassays: The following human tumor cell lines were used: HL-60, SMMC-7721, A-549, MCF-7, and SW480. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT method in 96-well microplates.[8] Briefly, adherent cells (100 μL) were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with an initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the tested compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM in triplicate for 48 h with cisplatin (Sigma, USA) as the positive control. After treatment, cell viability was measured and cell growth curve was plotted. IC_{50} values were calculated by the Reed and Muench method.[11]

Supporting Information (see footnote on the first page of this article): IR, MS (EI), and 1D and 2D NMR spectra of daphmalenines A (**1**) and B (**2**); optimized standard orientation of **2** at the B3LYP/6-31+G** level of theory.

Acknowledgments

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[1] a) J. Kobayashi, H. Morita in *The Alkaloids* (Ed.: G. A. Cordell), Academic Press, New York, **2003**, vol. 60, pp. 165–205; b) J. Kobayashi, T. Kubota, *Nat. Prod. Rep.* **2009**, *26*, 936–962 and references cited therein.

[2] a) C. H. Heathcock, *Angew. Chem.* **1992**, *104*, 675; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 665–681; b) C. H. Heathcock, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 14323–14327; c) G. A. Wallence, C. H. Heathcock, *J. Org. Chem.* **2001**, *66*, 450–454.

[3] a) H. Niwa, Y. Hirata, K. T. Suzuki, S. Yamamura, *Tetrahedron Lett.* **1973**, *14*, 2129–2132; b) K. T. Suzuki, S. Okuda, H. Niwa, M. Toda, Y. Hirata, S. Yamamura, *Tetrahedron Lett.* **1973**, *14*, 799–802.

[4] a) C. R. Zhang, H. B. Liu, S. H. Dong, J. Y. Zhu, Y. Wu, J. M. Yue, *Org. Lett.* **2009**, *11*, 4692–4695; b) Y. Zhang, Y. T. Di, Q. Zhang, S. Z. Mu, C. J. Tan, X. Fang, H. P. He, S. L. Li, X. J. Hao, *Org. Lett.* **2009**, *11*, 5414–5417; c) Y. Zhang, Y. T. Di, S. Z. Mu, C. S. Li, Q. Zhang, C. J. Tan, Z. Zhang, X. Fang, X. J. Hao, *J. Nat. Prod.* **2009**, *72*, 1325–1327; d) Q. Zhang, Y. T. Di, C. S. Li, X. Fang, C. J. Tan, Z. Zhang, Y. Zhang, H. P. He, S. L. Li, X. J. Hao, *Org. Lett.* **2009**, *11*, 2357–2359.

[5] H. D. Flack, *Acta Crystallogr., Sect. A* **1983**, *39*, 876–881.

[6] a) X. Fang, Y. T. Di, Z. L. Geng, C. J. Tan, J. Guo, J. Ning, X. J. Hao, *Eur. J. Org. Chem.* **2010**, 1381–1387; b) H. M. Ge, C. H. Zhu, D. H. Shi, D. L. Zhang, Q. D. Xie, J. Yang, W. S. Ng, R. X. Tan, *Chem. Eur. J.* **2008**, *14*, 376–381; c) D. Z. Liu, F. Wang, T. G. Liao, J. G. Tang, W. Steglich, H. J. Zhu, J. K. Liu, *Org. Lett.* **2006**, *8*, 5749–5752; d) P. K. Kondru, W. Peter, D. N. Beratan, *J. Am. Chem. Soc.* **1998**, *120*, 2204–2205; e) S. P. Karna, M. Dupuis, *J. Comput. Chem.* **1991**, *12*, 487–504.

[7] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman Jr., J. A. Montgomery, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, *Gaussian 03*, Revision D.01, Gaussian, Inc., Wallingford, CT, **2005**.

[8] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55–63.

[9] Z. J. Zhan, C. R. Zhang, J. M. Yue, *Tetrahedron* **2005**, *61*, 11038–11045.

[10] G. M. Sheldrick, *SHELXL-97, Program for X-ray Crystal Structure Solution and Refinement*, University of Göttingen, Göttingen, Germany, **1997**.

[11] L. J. Reed, H. Muench, *Am. J. Hyg.* **1938**, *27*, 493–497.

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