Dapholdhamines A−D, Alkaloids from *Daphniphyllum oldhami*

Yu Zhang,[†,‡] Ying-Tong Di,[†] Shu-Zhen Mu,[§] Chun-Shun Li,[†] Qiang Zhang,[†] Cheng-Jian Tan,[†] Zhen Zhang,[†] Xin Fang,[†] and Xiao-Jiang Hao*[,†]

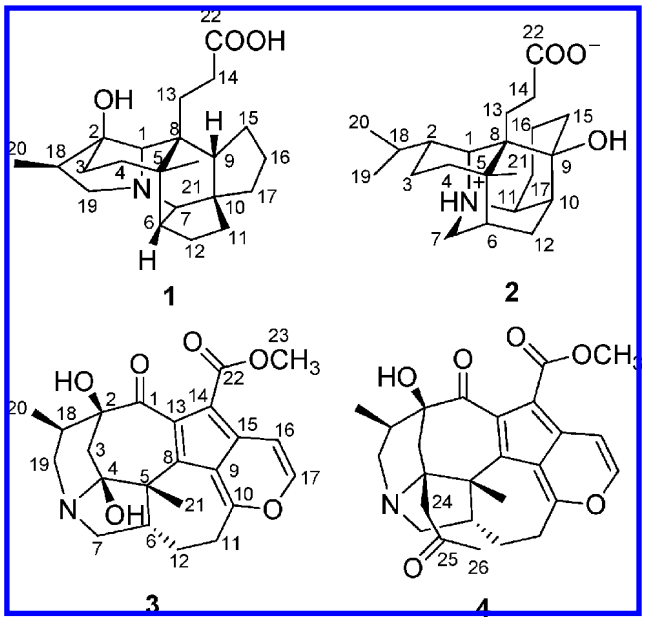
State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China, Graduate School of Chinese Academy of Sciences, Beijing 100039, People's Republic of China, and The Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, Guiyang 550002, People's Republic of China

Received February 19, 2009

Four new *Daphniphyllum* alkaloids, dapholdhamines A−D (**1**−**4**), were isolated from the leaves of *Daphniphyllum oldhami*. The structures and relative configurations of **1**−**4** were elucidated on the basis of spectroscopic data.

Daphniphyllum alkaloids are a family of structurally diverse natural products with complex polycyclic systems elaborated by plants of the genus *Daphniphyllum*.[1] Their unique structural features have attracted much attention regarding their biosynthesis and total synthesis.[2]

Recently, we reported some new *Daphniphyllum* alkaloids from *Daphniphyllum oldhami* (Hemsl.) Rosenth. and other species of the family Daphniphyllaceae, some of which possessed unprecedented ring systems.[3] In our continuing investigations of structurally unique and biogenetically interesting alkaloids from the leaves of *D. oldhami*, four new alkaloids, dapholdhamines A−D (**1**−**4**), together with eight known ones, have been isolated. This paper describes the isolation and structural elucidation of these new compounds.



Dapholdhamine A (**1**) was obtained as a white solid, and its molecular formula, $C_{22}H_{33}NO_3$, was established by HRESIMS (*m*/*z* 360.2546 [M + H]$^+$, calcd 360.2538), corresponding to seven degrees of unsaturation. IR absorptions at 3392 and 1722 cm^{-1} suggested the presence of hydroxy and carbonyl functionalities,

Table 1. ^{1}H NMR Data of Dapholdhamines A−D (**1**−**4**)

	1[a]	**2**[b]	**3**[b]	**4**[a]
1	3.15 (s)	3.35 (d, 3.2)		
2		1.60 (m)		
3a	1.69 (m)	1.80 (m)	2.14 (d, 14.2)	2.10 (d, 15.6)
3b	1.84 (m)	2.28 (m)	2.54 (d, 14.2)	2.74 (d, 15.6)
4a	1.67 (m)	1.48 (m)		
4b	2.16 (m)	2.04 (m)		
6	2.40 (m)	1.70 (m)	2.89 (m)	2.66 (m)
7a	3.58 (m)	3.45 (br d, 12.8)	2.24 (dd, 11.6, 8.0)	2.32 (dd, 8.8, 11.2)
7b		3.91 (d, 12.8)	3.12 (m)	3.08 (m)
9	2.06 (m)			
10		2.32 (m)		
11a	1.88 (m)	3.89 (d, 6.4)	2.94 (dd, 2.8, 5.2)	2.92 (m)
11b	2.01 (m)		3.61 (ddd, 2.8, 13.6, 16.8)	3.55 (ddd, 2.8, 14.0, 16.4)
12a	1.55 (m)	1.41 (m)	1.68 (m)	1.66 (m)
12b	1.37 (m)	1.92 (m)	2.55 (m)	2.58 (m)
13a	1.54 (m)	1.33 (m)		
13b	1.79 (m)	2.06 (m)		
14a	2.35 (m)	2.16 (m)		
14b	2.44 (m)	2.29 (m)		
15a	1.56 (m)	1.57 (m)		
15b	1.80 (m)	1.92 (m)		
16a	1.77 (m)	1.38 (m)	7.68 (br s)	7.69 (d, 4.8)
16b	1.93 (m)	2.63 (m)		
17a	1.69 (m)	1.39 (m)	7.68 (br s)	7.67 (d, 4.8)
17b	1.85 (m)	1.80 (m)		
18	2.37 (m)	1.58 (m)	2.55 (m)	2.66 (m)
19a	3.53 (m)	0.93 (d, 5.0)	2.68 (dd, 2.4, 15.6)	2.57 (m)
19b	3.60 (dd, 11.2, 8.0)		3.24 (dd, 10.4, 15.6)	3.08 (m)
20	1.01 (d, 6.0)[c]	1.00 (d, 5.1)	0.90 (d, 6.8)	0.86 (d, 6.8)
21	1.02 (s)[c]	0.96 (s)	1.53 (s)	1.38 (s)
23			3.82 (s)	3.81 (s)
24a				2.15 (d, 12.8)
24b				2.93 (m)
26				2.18 (s)

[a] Measured in $CDCl_3$. [b] Measured in $CDCl_3/CD_3OD$ (2:1). [c] Overlapped.

respectively. ^{13}C NMR and DEPT spectra data (Table 2) revealed 22 carbon signals due to one carbonyl, four sp^3 quaternary, five sp^3 methine, 10 sp^3 methylene, and two methyl carbon atoms. One methylene (δ_C 57.4, δ_H 3.57) and two methines (δ_C 68.8, δ_H 3.15; δ_C 62.9, δ_H 3.58) were typical of nitrogenated groups. The NMR data showed high similarity to those of bukittinggine,[4] except that the C-2 methine in bukittinggine was replaced by an oxygenated quaternary carbon at δ_C 82.1 and the absence of the six-membered lactone ring in **1**. This assignment was confirmed by ^{1}H−^{1}H COSY and HMBC spectra, especially by the HMBC correlations from H-1 and H$_3$-20 to C-2. Thus, the gross structure of dapholdhamine A

* To whom correspondence should be addressed. Tel: +86-871-5223263. Fax: +86-871-5223070. E-mail: haoxj@mail.kib.ac.cn.
† Kunming Institute of Botany.
‡ Graduate School of the Chinese Academy of Sciences, Beijing.
§ The Key Laboratory of Chemistry for Natural Product of Guizhou Province.

10.1021/np900112d CCC: $40.75
Published on Web 05/14/2009

Table 2. ^{13}C NMR Data of Dapholdhamines A−D (**1**−**4**)

	1[a]	**2**[b]	**3**[b]	**4**[a]
1	68.8	64.4	210.0	210.8
2	82.1	37.3	76.7	76.0
3	31.8	31.3	37.1	35.1
4	34.9	35.0	93.8	68.6
5	42.6	36.3	51.2	49.3
6	37.5	38.3	40.8	41.6
7	62.9	45.0	57.7	58.6
8	38.1	47.1	126.2	125.9
9	53.1	80.5	122.0	122.0
10	50.0	48.2	165.1	164.9
11	37.0[c]	66.8	29.9	29.8
12	19.2	28.4	26.5	27.4
13	26.2	26.8	141.4	141.9
14	30.9	34.1	107.9	108.3
15	29.7	25.6	132.6	132.4
16	29.0	34.5	110.8	110.6
17	37.1	24.6	143.4	143.2
18	37.0[c]	29.8	37.3	36.2
19	57.4	20.5	50.7	52.1
20	10.4	21.1	13.2	13.3
21	22.0	24.5	25.4	25.4
22	177.1	179.1	166.5	166.3
23			51.2	51.1
24				51.9
25				208.9
26				33.3

[a] Measured in $CDCl_3$. [b] Measured in $CDCl_3/CD_3OD$ (2:1). [c] Overlapped.

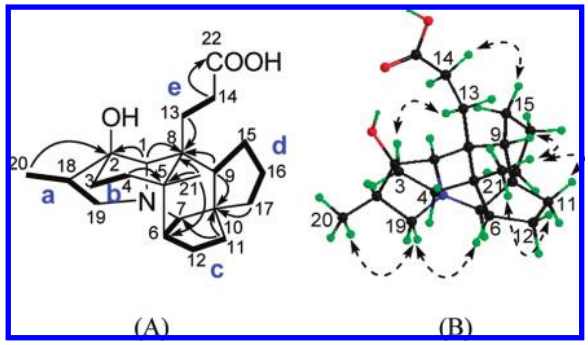


Figure 1. (A) $^1H-^1H$ COSY (bold) and key HMBC (arrow, H→C) correlations of **1**; (B) key ROESY (dashed) correlations of **1**.

was assigned as **1**, as shown in Figure 1A. The relative configuration of **1** was elucidated by ROESY data, in which correlations of H_3-21/H-9, H_3-21/H-11β, H_3-20/H-19β, and H-19β/H-6 suggested that CH_3-21, H-9, and H-6 were β-oriented, as shown in a computer-generated 3D drawing (Figure 1B).

Dapholdhamine B (**2**) showed a molecular formula of $C_{22}H_{35}NO_3$, as determined by HRESIMS at *m*/*z* 362.2695 (calcd 362.2695), with six degrees of unsaturation. The IR absorptions indicated the presence of hydroxy (3418 cm^{-1}) and carboxylate (1569 and 1391 cm^{-1}) functionalities. Its ^{13}C NMR spectrum (Table 2) showed 22 carbon signals including one carbonyl, three quaternary, six sp^3 methine, nine methylene, and three methyl carbon atoms. Since the carbonyl group accounted for one out of the six degrees of unsaturation, the remaining five degrees of unsaturation were ascribed to the presence of a pentacyclic system, similar to that of daphnezomine A.[5] The planar structure of **2** was further established by 2D NMR (HSQC, $^1H-^1H$ COSY, and HMBC). The HMBC correlations of H-11, H-12b, H-13a, and H-16a with C-9 (δ_C 80.5) indicated that the hydroxy group was located at C-9. Therefore, the planar structure of **2** was established as shown in Figure 2A. The relative configuration of **2** was the same as that of daphnezomine A, which was verified by the ROESY spectrum, as shown in the computer-generated 3D drawing (Figure 2B). Structurally, compounds **1** and **2** are very similar. However, compound **2** exists as a zwitterion while **1** does not, as supported by the solubility of

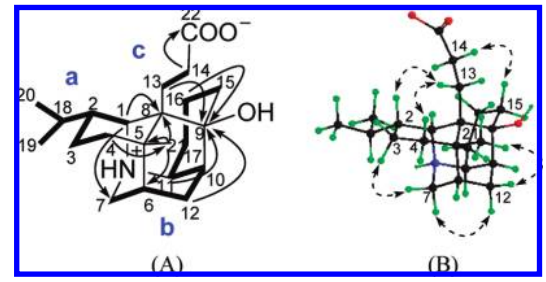


Figure 2. (A) $^1H-^1H$ COSY (bold) and key HMBC (arrow, H→C) correlations of **2**; (B) key ROESY (dashed) correlations of **2**.

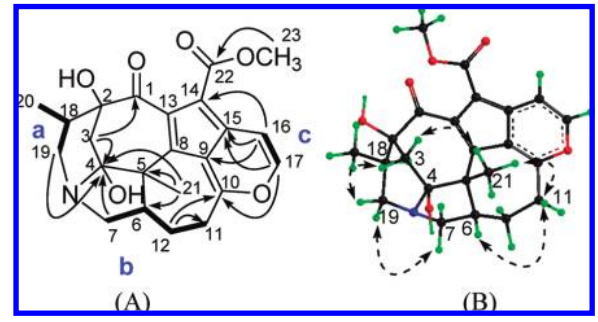


Figure 3. (A) $^1H-^1H$ COSY (bold) and key HMBC (arrow, H→C) correlations of **3**; (B) key ROESY (dashed) correlations of **3**.

1 in $CDCl_3$. This could be explained by the presence of an additional hydroxy group at C-2 in **1**, which reduces the nitrogen basicity through an inductive effect.

Dapholdhamine C (**3**) was obtained as a light yellow solid, and its positive HRESIMS signal at *m*/*z* 434.1573 ($[M + Na]^+$, calcd 434.1579) established the molecular formula $C_{23}H_{25}NO_6$ with 12 degrees of unsaturation. The IR spectrum showed the presence of hydroxy (3433 cm^{-1}) and two conjugated carbonyl groups (1711 and 1630 cm^{-1}, respectively). The ^{13}C NMR and DEPT spectral data (Table 2) of **3** showed 23 carbon signals including four double bonds, two carbonyls, three sp^3 quaternary carbons, two sp^3 methines, five sp^3 methylenes, two methyls, and one methoxy group. The NMR data of **3** are strikingly similar to those of paxiphylline B,[3f] except for the presence of signals corresponding to a quaternary carbon (δ_C 93.8), which was assigned as an amino ketal carbon[6] instead of a nitrogenated methylene group (δ_C 66.3). The hydroxy group of the amino ketal moiety was located at C-4 on the basis of the HMBC correlations from H_2-3, H-7β, H_2-19, and H_3-21 to C-4. The planar structure of dapholdhamine C was eventually established by the 2D NMR experiments as **3** (Figure 3A). ROESY correlations as shown in Figure 3B verified that the relative configuration of dapholdhamine C (**3**) was the same as that of paxiphylline B, in which H-4, H-6, CH_3-20, and CH_3-21 were β-oriented. On biosynthesis considerations, the OH group at C-4 must be β-oriented, and the assignment could also be supported by the γ-steric compression effect from oxygen atoms of 4-OH to H_3-21 (δ_H 1.53, $\Delta\delta_H$ +0.08), which also resulted in the upfield shift of Me-21(δ_C 25.4, $\Delta\delta_C$ −2.8).

Dapholdhamine D (**4**) had a molecular formula of $C_{26}H_{29}NO_6$ as established by HRESIMS at *m*/*z* 474.1901 ($[M + Na]^+$, calcd for $C_{26}H_{29}NO_6Na^+$, 474.1892). Comparison of the NMR data of **4** (Tables 1 and 2) with those of **3** suggested that both alkaloids likely shared the same basic skeleton, but the molecular weight of **4** is larger than that of **3** by 40 units, namely, the hydroxy group of the amino ketal moiety in **3** was replaced by a 2-oxopropyl unit [δ_C 51.9, δ_H 2.15, 2.93 (each 1H); δ_C 208.9; δ_C 33.3, δ_H 2.18 (3H)]. The planar structure of **4** was further established by 2D NMR (HSQC, $^1H-^1H$ COSY, and HMBC), as shown in Figure 4A. HMBC correlations of H_2-24 to C-4, C-5, C-25, and C-26 indicated

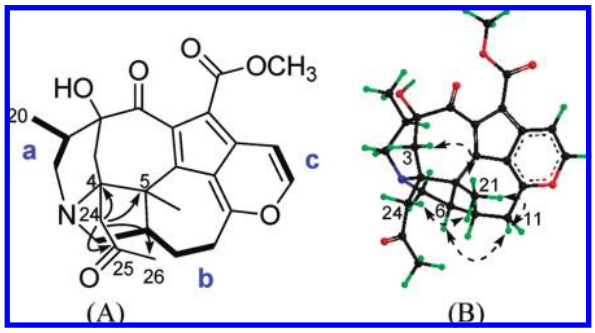


Figure 4. (A) ^{1}H−^{1}H COSY (bold) and key HMBC (arrow, H→C) correlations of **4**; (B) key ROESY (dashed) correlations of **4**.

that C-24 was attached to C-5 via C-4. The ROESY spectrum showed that dapholdhamine D (**4**) had the same relative configuration as dapholdhamine C (**3**). Especially, the β-orientation of H-24a was recognized by the ROESY correlation of H-24a with H_3-21. Considering that acetone had not been used in any of purification processes, compound **4** might be biogenetically derived from daphnicyclidin B, and the "extra" unit (C-24 to C-26) might be provided by acetyl coenzyme A (Supporting Information).[7]

In addition to the above four new alkaloids, the other eight *Daphniphyllum* alkaloids, deoxyisocalyciphylline B,[8] deoxycalyciphylline B,[8] daphnicyclidins A and D,[9] daphnilactone A,[10] methyl homodaphniphyllate,[11] zwitterionic alkaloid,[12] and calyciphylline E,[13] were all identified by comparison of experimental and reported physical data.

Dapholdhamines A−D (**1**−**4**) were evaluated in a bioassay for cytotoxicity against six cell lines, HCT116, HL-60, HT-29, SW480, KB, and K562, respectively. The results indicated that all of them were inactive.

Experimental Section

General Experimental Procedures. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. Optical rotations were obtained on a Perkin-Elmer model 241 polarimeter. ESIMS and HRESIMS were measured on a Finnigan MAT 90 instrument and VG Auto Spec-3000 spectrometer, respectively. 1D and 2D NMR spectra were measured on a Bruker DRX-500 or AM-400 spectrometer. Column chromatography was performed on Si gel H (10−40 μm; Qingdao Marine Chemical Factory) and Sephadex LH-20 (40−70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden).

Plant Material. The leaves of *D. oldhami* were collected from Hunan Province, People's Republic of China, in September 2007, and the plant sample was identified by Prof. Xun Gong of Kunming Institute of Botany, Chinese Academy of Sciences (CAS). A voucher specimen (KIB 07090411) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Science (CAS).

Extraction and Isolation. The air-dried and powdered leaves (8.0 kg) of *D. oldhami* were extracted three times with 95% EtOH. The extract was concentrated under reduced pressure, followed by partitioning between EtOAc and 3% tartaric acid. The aqueous phase was adjusted to pH 10 with saturated Na_2CO_3 and extracted with $CHCl_3$ to give crude alkaloids (9.0 g). The crude alkaloids were subjected to a Si gel column ($CHCl_3$/MeOH, 1:0 → 0:1) to obtain four major fractions (F_1−F_4). Fraction 3 (F_3) was further chromatographed over a Si gel (300−400 mesh) column ($CHCl_3$/MeOH, 20:1 → 10:1) followed by Sephadex LH-20 CC eluted with MeOH to afford dapholdhamine C (**3**, 7 mg) and dapholdhamine D (**4**, 5 mg). Fraction 4 (F_4) was subjected to a RP-18 Si gel column (MeOH/H_2O) to give four subfractions (P_1−P_4). Subraction 2 (P_2) was subjected to Si gel ($CHCl_3$/MeOH, 8:1) followed by Sephadex LH-20 CC eluted with MeOH to afford dapholdhamine A (**1**, 10 mg) and dapholdhamine B (**2**, 13 mg).

Dapholdhamine A (1): white solid; $[\alpha]^{26}_D$ −38.8 (*c* 0.40, MeOH); IR (KBr) ν_{max} 3392, 2950, 1722, 1594, 1383, and 1188 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS 360 [M + H]$^+$; HRESIMS *m/z* 360.2546 (calcd for $C_{22}H_{34}NO_3^+$, 360.2538).

Dapholdhamine B (2): colorless solid; $[\alpha]^{26}_D$ −34.9 (*c* 0.215, MeOH); IR (KBr) ν_{max} 3418, 2964, 1568, and 1391 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS 362 [M + H]$^+$; HRESIMS *m/z* 362.2695 (calcd for $C_{22}H_{36}NO_3^+$, 362.2695).

Dapholdhamine C (3): light yellow solid; $[\alpha]^{26}_D$ −263.8 (*c* 0.115, MeOH); UV (MeOH) λ_{max} (log ε) 222 (4.19), 241 (4.08), 274 (4.27); IR (KBr) ν_{max} 3433, 2933, 1711, 1630, 1441, and 1233 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS 434 [M + Na]$^+$; HRESIMS *m/z* 434.1573 (calcd for $C_{23}H_{25}NO_6Na^+$, 434.1579).

Dapholdhamine D (4): light yellow solid; $[\alpha]^{27}_D$ −260.6 (*c* 0.165, MeOH); UV (MeOH) λ_{max} (log ε) 221 (4.25), 240 (4.11), 274 (4.26); IR (KBr) ν_{max} 3449, 2959, 2930, 1712, 1675, 1628, 1442, and 1235 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS 474 [M + Na]$^+$; HRESIMS *m/z* 474.1901 (calcd for $C_{26}H_{29}NO_6Na^+$, 474.1892).

Acknowledgment. This work was financially supported by the Ministry of Science and Technology (2009CB940900 and 2009CB522303) and by the National Natural Science Foundation (20672120) of PRC. The authors thank Prof. Xun Gong of Kunming Institute of Botany, Chinese Academy of Sciences (CAS), for the identification of the plant material.

Supporting Information Available: 1D and 2D NMR spectra of dapholdhamines A−D (**1**−**4**) are supplied, and this material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) For a review of *Daphniphyllum* alkaloids: Kobayashi, J.; Morita, H. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: New York, 2003; Vol, 60, pp 165−205, and references therein.
(2) (a) Wallence, G. A.; Heathcock, C. H. *J. Org. Chem.* **2001**, *66*, 450–454. (b) Heathcock, C. H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14323–14327. (c) Heathcock, C. H.; Joe, D. *J. Org. Chem.* **1995**, *60*, 1131–1142. (d) Heathcock, C. H. *Angew. Chem., Int. Ed.* **1992**, *31*, 665–681.
(3) (a) Kong, N. C.; Zhang, Y.; Gao, S.; Lu, Y.; Zheng, Q. T.; Sun, Q. Y.; Yang, F. M.; Di, Y. T.; Hao, X. J. *Tetrahedron Lett.* **2009**, *50*, 957–959. (b) Li, C. S.; Di, Y. T.; Mu, S. Z.; He, H. P.; Zhang, Q.; Fang, X.; Zhang, Y.; Li, S. L.; Lu, Y.; Gong, Y. Q.; Hao, X. J. *J. Nat. Prod.* **2008**, *71*, 1202–1206. (c) Mu, S. Z.; Wang, J. S.; Yang, X. S.; He, H. P.; Li, C. S.; Di, Y. T.; Wang, Y.; Zhang, Y.; Fang, X.; Huang, L. J.; Hao, X. J. *J. Nat. Prod.* **2008**, *71*, 564–569. (d) Di, Y. T.; Liu, L. L.; Li, C. S.; Zhang, Y.; Zhang, Q.; Mu, S. Z.; Sun, Q. Y.; Yang, F. M.; Liu, H. Y.; Hao, X. J. *Helv. Chim. Acta* **2008**, *91*, 838–843. (e) Tan, C. J.; Di, Y. T.; Wang, Y. H.; Wang, Y.; Mu, S. Z.; Gao, S.; Zhang, Y.; Kong, N. C.; He, H. P.; Zhang, J. X.; Fang, X.; Li, C. S.; Lu, Y.; Hao, X. J. *Tetrahedron Lett.* **2008**, *49*, 3376–3379. (f) Zhang, Y.; He, H. P.; Di, Y. T.; Mu, S. Z.; Wang, Y. H.; Wang, J. S.; Li, C. S.; Kong, N. C.; Gao, S.; Hao, X. J. *Tetrahedron Lett.* **2007**, *48*, 9104–9107. (g) Li, C. S.; Di, Y. T.; He, H. P.; Gao, S.; Wang, Y. H.; Lu, Y.; Zhong, J. L.; Hao, X. J. *Org. Lett.* **2007**, *9*, 2509–2512. (h) Di, Y. T.; He, H. P.; Wang, Y. S.; Li, L. B.; Lu, Y.; Gong, J. B.; Fang, X.; Kong, N. C.; Li, S. L.; Zhu, H. J.; Hao, X. J. *Org. Lett.* **2007**, *9*, 1355–1358. (i) Mu, S. Z.; Wang, Y.; He, H. P.; Yang, X. W.; Wang, Y. H.; Di, Y. T.; Lu, Y.; Chang, Y.; Hao, X. J. *J. Nat. Prod.* **2006**, *69*, 1065–1069.
(4) (a) Arbain, D.; Byrne, L. T.; Cannon, J. R.; Patric, V. A.; White, A. H. *Aust. J. Chem.* **1990**, *43*, 185–190. (b) Heathcock, C. H.; Stafford, J. A.; Clark, D. L. *J. Org. Chem.* **1992**, *57*, 2575–2585. (c) Yahata, H.; Kutoba, T.; Kobayashi, J. *J. Nat. Prod.* **2009**, *72*, 148–151.
(5) Morita, H.; Yoshida, N.; Kobayashi, J. *J. Org. Chem.* **1999**, *64*, 7208–7212.
(6) Lindquist, N.; Fenical, W. *Tetrahedron Lett.* **1990**, *31*, 2521–2524.
(7) Hoye, T. R.; Bjorklund, J. A.; Koltun, D. O.; Renner, M. K. *Org. Lett.* **2000**, *2*, 3–5.
(8) Yang, S. P.; Yue, J. M. *J. Org. Chem.* **2003**, *68*, 7961–7966.
(9) Kobayashi, J.; Inaba, Y.; Shiro, M.; Yoshida, N.; Morita, H. *J. Am. Chem. Soc.* **2001**, *123*, 11402–11408.
(10) Sasaki, K.; Hirata, Y. *Tetrahedron Lett.* **1972**, *13*, 1275–1278.
(11) Toda, M.; Yamamura, S.; Hirata, Y. *Tetrahedron Lett.* **1969**, 2585–2586.
(12) Yamamura, S.; Toda, M.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 830–844.
(13) (a) Zhan, Z. J.; Zhang, C. R.; Yue, J. M. *Tetrahedron* **2005**, *61*, 11038–11045. (b) Saito, S.; Kubota, T.; Fukushi, E.; Kawabata, J.; Zhang, H.; Kobayashi, J. *Org. Lett.* **2007**, *9*, 1207–1209.

NP900112D