Notes

Calyciphyllines N−P, Alkaloids from *Daphniphyllum calycinum*

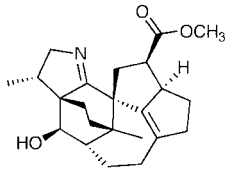
Hiroko Yahata, Takaaki Kubota, and Jun'ichi Kobayashi*

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

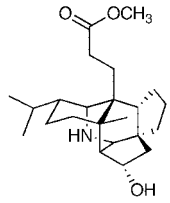
Received August 18, 2008

Three new *Daphniphyllum* alkaloids, calyciphyllines N−P (**1**−**3**), were isolated from the leaves and stems of *Daphniphyllum calycinum*. The structures and relative stereochemistry of **1**−**3** were elucidated on the basis of spectroscopic data.

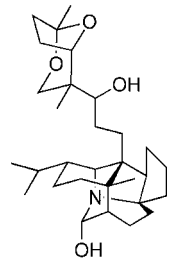
Trees of the genus *Daphniphyllum* (Daphniphyllaceae) are known to elaborate a structurally diverse group of alkaloids with unique polycyclic fused ring systems.[1−6] These *Daphniphyllum* alkaloids have been attractive targets for biogenetic and synthetic studies.[7] Recently, some novel alkaloids with unusual skeletons such as calyciphyllines C−M[2a−e] have been isolated from *Daphniphyllum calycinum* in our laboratory. Further investigation of extracts of this plant resulted in the isolation of three new alkaloids, calyciphyllines N−P (**1**−**3**). In this paper, we describe the isolation and structure elucidation of **1**−**3**.



1



2



3

The leaves and stems of *D. calycinum* were extracted with MeOH, respectively, and each MeOH extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated Na_2CO_3, were extracted with $CHCl_3$ to give crude alkaloidal fractions. The crude alkaloidal materials prepared from the leaves were subjected to passage over an amino silica gel column (hexane/EtOAc and then $CHCl_3$/MeOH), followed by separation over a silica gel column ($CHCl_3$/MeOH), to give calyciphyllines N (**1**, 0.00031% yield) and O (**2**, 0.00010%). The crude alkaloidal materials prepared from the stems were separated by the same procedure as described above to yield calyciphylline P (**3**, 0.00002%).

Calyciphylline N (**1**) showed a pseudomolecular ion peak at *m*/*z* 370 $[M + H]^+$ in the ESIMS, and the molecular formula, $C_{23}H_{31}NO_3$, was established by HRESIMS (*m*/*z* 370.2375, $[M + H]^+$, Δ −0.7 mmu). IR absorptions at 3420 and 1733 cm^{-1} suggested the presence of hydroxyl groups and ester carbonyl functionalities, respectively. ^1H and ^{13}C NMR data (Table 1) and the HMQC spectra of **1** revealed 23 carbon signals due to one ester carbonyl, three sp^2 quaternary carbons, three sp^3 quaternary carbons, five sp^3 methines, eight sp^3 methylenes, one methoxy, and two methyls. These data suggested that the structure of **1** is similar to that of daphmanidin A.[8] The $^1H-^1H$ COSY and TOCSY spectra of **1** revealed four partial structures, **a** (C-3 to C-4), **b** (C-6 to C-7 and C-12, and C-11 to C-12), **c** (C-13 to C-17), and **d** (C-18 to C-19 and C-20), which were connected to each other on the basis of HMBC correlations as shown in Figure 1. The NOESY correlations, as shown in Figure 2, indicated the relative configuration of **1** and the conformation of the bicyclo[2.2.2]octane moiety

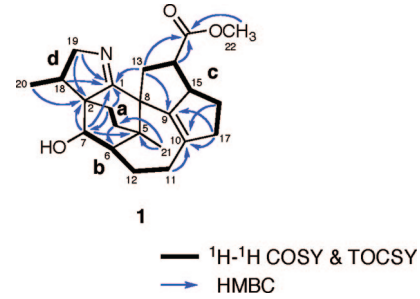


Figure 1. Selected 2D NMR correlations for calyciphylline N (**1**).

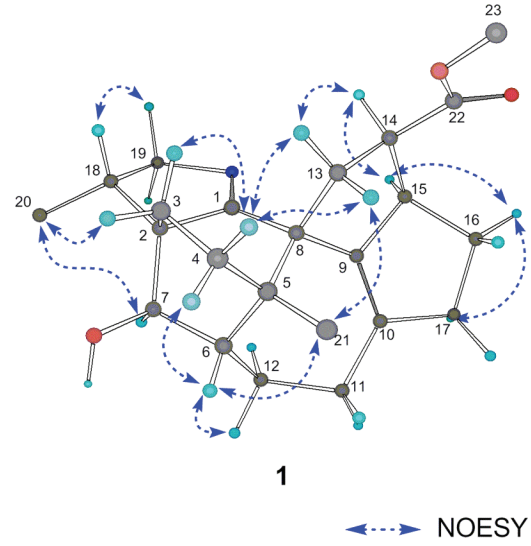


Figure 2. Selected NOESY correlations and relative stereochemistry of calyciphylline N (**1**) (hydrogen atoms of methyl groups are omitted).

* To whom correspondence should be addressed. Tel: +81-11-706-3239. Fax: +81-11-706-4989. E-mail: jkobay@pharm.hokudai.ac.jp.

10.1021/np800515s CCC: $40.75
Published on Web 12/19/2008

Table 1. ^{1}H and ^{13}C NMR Data of Calyciphyllines N (**1**) and O (**2**) in $CDCl_3$

calyciphylline N (**1**)				calyciphylline O (**2**)			
position	δ_H	δ_C		position	δ_H	δ_C	
1		185.7	s	1	2.97 (1H, brs)	48.2	d
2		57.0	s	2	0.91 (1H, m)	42.9	d
3a	2.06 (1H, m)	23.3[a]	t	3	1.49 (2H, m)	20.5	t
3b	1.20 (1H, m)			4a	1.58 (1H, m)	37.8	t
4a	1.41 (1H, m)	34.6	t	4b	1.29 (1H, m)		
4b	1.27 (1H, m)			5		36.0	s
5		39.4	s	6	1.81 (1H, d, 4.6 Hz)	57.2	d
6	1.56 (1H, m)	51.8	d	7	3.03 (1H, d, 4.6 Hz)	56.9	d
7	4.00 (1H, d, 5.9 Hz)	67.8[b]	d	8		35.8	s
8		52.6	s	9	1.67 (1H, m)	52.5	d
9		141.2	s	10		50.4	s
10		132.2	s	11a	2.04 (1H, dd, 14.6, 7.2 Hz)	51.5	t
11	2.12 (2H, m)	25.2	t	11b	1.58 (1H, m)		
12a	2.12 (1H, m)	22.8[a]	t	12	4.20 (1H, d, 6.9 Hz)	71.4	d
12b	1.88 (1H, m)			13a	1.65 (1H, m)	27.8	t
13a	2.53 (1H, dd, 13.6, 5.0 Hz)	38.8	t	13b	1.58 (1H, m)		
13b	2.31 (1H, dd, 13.6, 9.4 Hz)			14a	2.37 (1H, m)	29.5	t
14	3.13 (1H, m)	42.2	d	14b	2.20 (1H, m)		
15	3.54 (1H, m)	54.3	d	15a	1.70 (1H, m)	30.0	t
16a	1.81 (1H, m)	27.3	t	15b	1.26 (1H, m)		
16b	1.16 (1H, m)			16a	1.75 (1H, m)	26.6	t
17a	2.48 (1H, m)	42.7	t	16b	1.40 (1H, m)		
17b	2.27 (1H, dd, 14.6, 8.6 Hz)			17a	1.75 (1H, m)	37.8	t
18	2.01(1H, m)	37.0	d	17b	1.58 (1H, m)		
19a	3.93 (1H, dd, 15.6, 6.8 Hz)	67.6[b]	t	18	1.52 (1H, m)	28.7	d
19b	3.42 (1H, dd, 15.6, 1.4 Hz)			19	0.91 (3H, d, 6.3 Hz)[c]	21.0	q
20	0.97 (3H, d, 6.9 Hz)	16.6	q	20	0.91 (3H, d, 6.9 Hz)[c]	21.0	q
21	1.04 (3H, s)	21.4	q	21	0.82 (3H, s)	21.0	q
22		176.2	s	22		174.8	s
23	3.66 (3H, s)	51.0	q	23	3.64 (3H, s)	51.6	q

[a] Assignments are interchangeable. [b] Assignments are interchangeable. [c] Assignments are interchangeable.

(C-1 to C-8). Thus, calyciphylline N (**1**) was assigned as the 21-deacetoxy derivative of daphmanidin A.

The molecular formula of calyciphylline O (**2**) was determined as $C_{23}H_{37}NO_3$ by HRESIMS [*m/z* 376.2848, $(M + H)^+$, Δ −0.4 mmu]. The IR spectrum (3359 and 1736 cm^{-1}) suggested the presence of a hydroxyl group and an ester carbonyl functionality, respectively. Inspection of the ^{1}H and ^{13}C NMR data (Table 1) suggested that **2** consists of one ester carbonyl, three sp^3 quaternary carbons, seven sp^3 methines, eight sp^3 methylenes, one methoxy, and three methyls. Comparison of the ^{1}H and ^{13}C NMR data of calyciphylline O (**2**) with those of methyl homosecodaphniphyllate[9] indicated that calyciphylline O (**2**) is a hydroxylated analogue of methyl homosecodaphniphyllate. The $^{1}H-^{1}H$ COSY and TOCSY spectra of **2** disclosed four partial structures, **a** (C-2 to C-4, C-2 to C-18, and C-18 to C-19 and C-20), **b** (C-6 to C-7 and C-12, and C-11 to C-12), **c** (C-15 to C-9 and C-17), and **d** (C-13 to C-14). The connectivities of these partial structures, **a**−**d**, were revealed on the basis of HMBC correlations as shown in Figure 3. The NOESY correlations shown in Figure 4 indicated that calyciphylline O (**2**) possesses the same relative stereochemistry as that of methyl homosecodaphniphyllate and an α-orientation of the hydroxy group at C-12. Thus, calyciphylline O (**2**) was assigned as 12-hydroxymethyl homosecodaphniphyllate.

The molecular formula of calyciphylline P (**3**) was established as $C_{30}H_{49}NO_4$ by HRESIMS [*m/z* 488.3717, $(M + H)^+$, Δ −2.3 mmu]. The IR band at 3170 cm^{-1} suggested the presence of hydroxyl group absorption. The ^{1}H and ^{13}C NMR data (Table 2) and the HMQC spectra demonstrated that **3** consists of five sp^3 quaternary carbons, eight sp^3 methines, 12 sp^3 methylenes, and five methyls. The chemical shifts of the ^{1}H and ^{13}C NMR data (Table 2) implied that **3** is a congener of daphnimacropine.[10] The $^{1}H-^{1}H$ COSY and TOCSY spectra of **3** revealed the presence of five structural units, **a** (C-1 to C-4, C-2 to C-18, and C-18 to C-19 and C-20), **b** (C-6 to C-7 and C-12, and C-11 to C-12), **c** (C-15 to C-9 and C-17), **d** (C-14 to C-13 and C-22), and **e** (C-26 to C-28). Inspection of the HMBC spectrum of **3** disclosed the connectivities

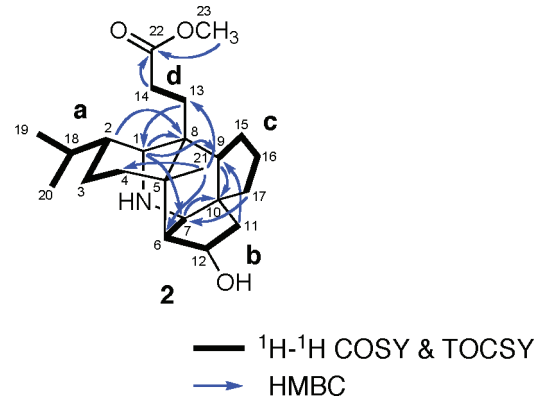


Figure 3. Selected 2D NMR correlations for calyciphylline O (**2**).

of five partial structures, **a**−**e**, as shown in Figure 5. NOESY correlations as shown in Figure 6 verified that the relative stereochemistry of the daphnane skeleton moiety (C-1 through C-21) and the 2,8-dioxabicyclo[3.2.1]octane moiety (C-23−C-30) of calyciphylline O (**3**) were the same as those of daphnimacropine. Thus, calyciphylline P (**3**) was assigned as dihydrodaphnimacropine.

Calyciphyllines N−P (**1**−**3**) did not show cytotoxicity against P388 murine leukemia, L1210 murine leukemia, and KB human epidermoid carcinoma cells (IC_{50} > 5 μg/mL).

Experimental Section

General Methods. Optical rotations were recorded on a JASCO P-1030 polarimeter. IR and UV spectra were recorded on a JASCO FT/IR-230 spectrometer and a Shimadzu UV-1600PC spectrophotometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX-600 and JEOL ECA-500 NMR spectrometers. The 7.26 and 77.0 ppm resonances of residual $CDCl_3$ were used as internal references

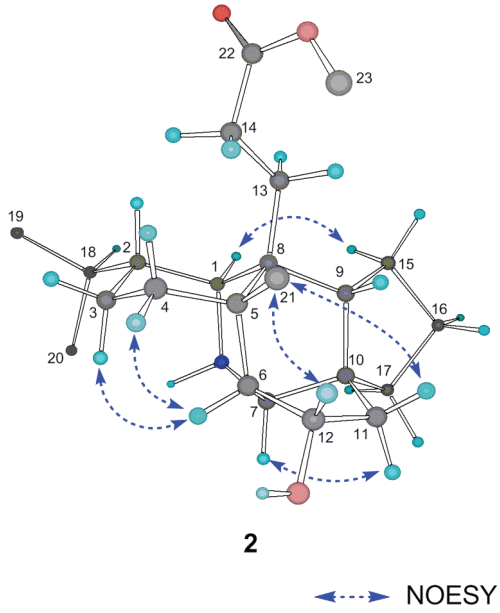


Figure 4. Selected NOESY correlations and relative stereochemistry of calyciphylline O (**2**) (hydrogen atoms of methyl groups are omitted).

Table 2. ^{1}H and ^{13}C NMR Data of Calyciphylline P (**3**) in $CDCl_3$.

position	δ_H	δ_C	
1	3.44 (1H, m)	65.3	d
2	1.53 (1H, m)	37.4	d
3a	1.93 (1H, m)	28.1	t
3b	1.54 (1H, m)		
4a	1.96 (1H, m)	36.4	t
4b	1.58 (1H, m)		
5		35.0	s
6	1.83 (1H, m)	45.5	d
7	5.69 (1H, s)	80.8	d
8		45.8	s
9	2.38 (1H, m)	51.5	d
10		77.0[a]	s
11a	2.73 (1H, m)	28.1	t
11b	1.53 (1H, m)		
12a	2.03 (1H, m)	16.3	t
12b	1.80 (1H, m)		
13a	1.93 (1H, m)	28.1	t
13b	1.54 (1H, m)		
14a	1.68 (1H, m)	29.6	t
14b	1.22 (1H, m)		
15a	2.03 (1H, m)	29.0	t
15b	1.42 (1H, m)		
16a	1.93 (1H, m)	28.1	t
16b	1.54 (1H, m)		
17a	2.12 (1H, m)	39.6	t
17b	1.84 (1H, m)		
18	1.68 (1H, m)	29.6	d
19	0.92 (3H, d, 6.6 Hz)	20.5	q
20	1.04 (3H, d, 6.6 Hz)	21.4	q
21	1.01 (3H, s)	26.1	q
22	3.37 (1H, d, 10.8 Hz)	74.2	d
23		38.1	s
24	1.08 (3H, s)	14.9	q
25	3.39 (2H, m)	66.3	t
26	4.10 (1H, d, 6.6 Hz)	81.7	d
27a	1.93 (1H, m)	24.8	t
27b	1.87 (1H, m)		
28a	2.06 (1H, m)	33.3	t
28b	1.78 (1H, m)		
29		104.9	s
30	1.47 (3H, s)	23.7	q

[a] Overlapping with signal of residual $CDCl_3$.

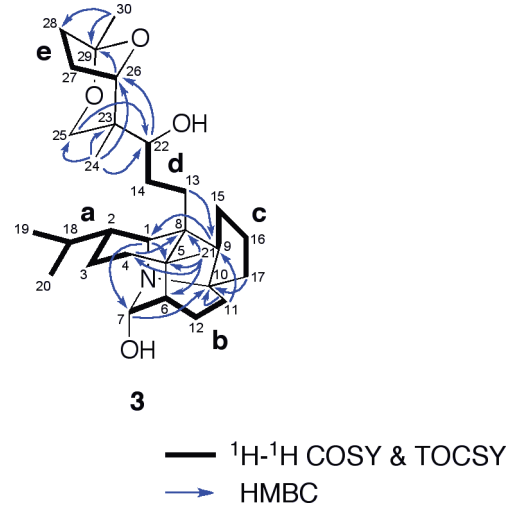


Figure 5. Selected 2D NMR correlations for calyciphylline P (**3**).

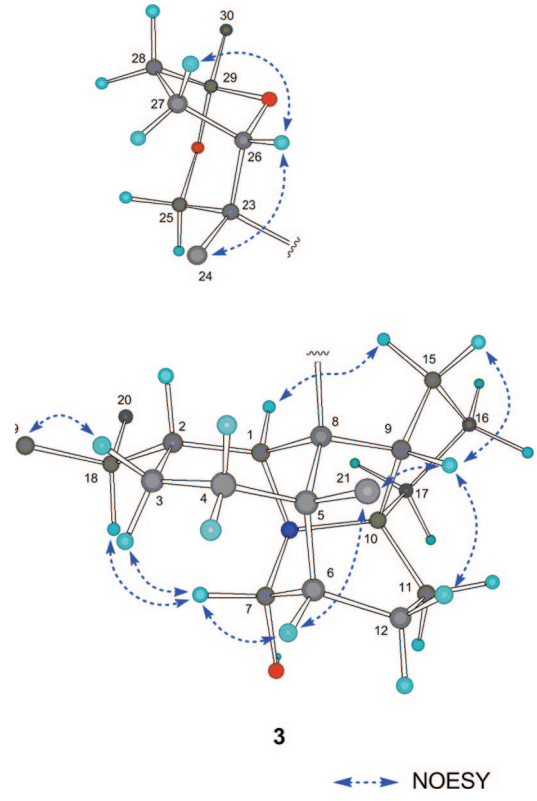


Figure 6. Selected NOESY correlations and partial relative stereochemistry of calyciphylline P (**3**) (hydrogen atoms of methyl groups are omitted).

for ^{1}H and ^{13}C NMR spectra, respectively. ESI mass spectra were obtained on a JEOL JMS-700TZ mass spectrometer.

Plant Material. The leaves and stems of *Daphniphyllum calycinum* were collected from Yunnan Province of China in 2005 by Dr. Huiping Zhang, Department of Chemistry of Natural Drugs, School of Pharmacy, Fudan University. A voucher specimen was deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The leaves (1.8 kg) and stems (11.0 kg) of *D. calycinum* were extracted separately with MeOH, and each MeOH extract (347.4 g from the leaves and 418.1 g from the stems) was partitioned between EtOAc and 3% tartaric acid. The water-soluble extracts, adjusted to pH 10 with saturated Na_2CO_3, were extracted with $CHCl_3$ to give crude alkaloidal fractions (4.3 g from leaves and 13.1 g from stems). The crude alkaloidal fraction prepared from the leaves was subjected to an amino silica gel column (hexane/EtOAc, 1:0 →

4:6, and then $CHCl_3$/MeOH, 1:0 → 0:1), followed by a silica gel column ($CHCl_3$/MeOH, 1:0 → 0:1) to give calyciphyllines N (**1**, 0.00031% yield) and O (**2**, 0.00010%). A part of the crude alkaloidal fraction prepared from the stems (6.7 g) was separated by the same procedure as described above to yield calyciphylline P (**3**, 0.00002%).

Calyciphylline N (1): colorless, amorphous solid; $[\alpha]^{18}_D$ −138.4 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3420, 2936, 1733, 1169, 755 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 370.2375 (M + H; calcd for $C_{23}H_{32}NO_3$, 370.2382).

Calyciphylline O (2): colorless, amorphous solid; $[\alpha]^{23}_D$ −72.5 (*c* 0.47, $CHCl_3$); IR (neat) ν_{max} 3359, 2947, 1736, 1173 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 376.2848 (M + H; calcd for $C_{23}H_{38}NO_3$, 376.2852).

Calyciphylline P (3): colorless, amorphous solid; $[\alpha]^{22}_D$ −14.4 (*c* 0.31, $CHCl_3$); IR (neat) ν_{max} 3170, 2959, 1662, 1200, 1139, 752 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS *m/z* 488.3717 (M + H; calcd for $C_{30}H_{50}NO_4$, 488.3740).

Acknowledgment. The authors thank Dr. Huiping Zhang, Department of Chemistry of Natural Drugs, School of Pharmacy, Fudan University, for her help with collection of the plant, and Ms. S. Oka, Center for Instrumental Analysis, Hokkaido University, for measurements of ESIMS. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References and Notes

(1) For a review of *Daphniphyllum* alkaloids see:Kobayashi, J.; Morita, H. In *Modern Alkaloids: Structure, Isolation, Synthesis and Biology*; Fattorusso, E., Taglialatela-Scafati, O., Eds.; Wiley-VCH: Weinheim, 2008; pp 541−589, and references therein.

(2) (a) Morita, H.; Takatsu, H.; Kobayashi, J. *Tetrahedron* **2003**, *59*, 3575–3579. (b) Kobayashi, J.; Takatsu, H.; Shen, Y.-C.; Morita, H. *Org. Lett.* **2003**, *5*, 1733–1736. (c) Morita, H.; Kobayashi, J. *Org. Lett.* **2003**, *5*, 2895–2898. (d) Morita, H.; Takatsu, H.; Shen, Y.-C.; Kobayashi, J. *Tetrahedron Lett.* **2004**, *45*, 901–904. (e) Takatsu, H.; Morita, H.; Shen, Y.-C.; Kobayashi, J. *Tetrahedron* **2004**, *60*, 6279–6284. (f) Morita, H.; Ishioka, N.; Takatsu, H.; Shinzato, T.; Obara, Y.; Nakahata, N.; Kobayashi, J. *Org. Lett.* **2005**, *7*, 459–462. (g) Kubota, T.; Matsuno, Y.; Morita, H.; Shinzato, T.; Sekiguchi, M.; Kobayashi, J. *Tetrahedron* **2006**, *62*, 4743–4748. (h) Morita, H.; Ishioka, N.; Takatsu, H.; Iizuka, T.; Kobayashi, J. *J. Nat. Prod.* **2006**, *69*, 418–420. (i) Saito, S.; Kubota, T.; Fukushi, E.; Kawabata, J.; Zhang, H.; Kobayashi, J. *Tetrahedron Lett.* **2007**, *48*, 1587–1589. (j) Saito, S.; Kubota, T.; Fukushi, E.; Kawabata, J.; Zhang, H.; Kobayashi, J. *Org. Lett.* **2007**, *9*, 1207–1209. (k) Saito, S.; Kubota, T.; Kobayashi, J. *Tetrahedron Lett.* **2007**, *48*, 3809–3812. (l) Saito, S.; Kubota, T.; Kobayashi, J. *Tetrahedron Lett.* **2007**, *48*, 5693–5695. (m) Saito, S.; Yahata, H.; Kubota, T.; Obara, Y.; Nakahata, N.; Kobayashi, J. *Tetrahedron* **2008**, *64*, 1901–1908.

(3) Bitar, H. E.; Nguyen, V. H.; Gramain, A.; Sévenet, T.; Bodo, B. *Tetrahedron Lett.* **2004**, *45*, 515–518.

(4) Di, Y.-T.; He, H.-P.; Lu, C.-S.; Tian, J.-M.; Mu, S.-Z.; Li, S.-L.; Gao, S.; Hao, X.-J. *J. Nat. Prod.* **2006**, *69*, 1745–1748.

(5) Li, Z.-Y.; Chen, P.; Xu, H.-G.; Yang, Y.-M.; Peng, S.-Y.; Zhao, Z.-Z.; Guo, Y.-W. *Org. Lett.* **2007**, *9*, 477–480.

(6) Fan, C.-Q.; Yin, S.; Xue, J.-J.; Yue, J.-M. *Tetrahedron* **2007**, *63*, 115–119.

(7) (a) Wallace, 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.; Kath, J. C.; Ruggeri, R. B. *J. Org. Chem.* **1995**, *60*, 1120–1130. (e) Heathcock, C. H. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 665–681, and references therein.

(8) Kobayashi, J.; Ueno, S.; Morita, H. *J. Org. Chem.* **2002**, *67*, 6546–6549.

(9) (a) Irikawa, H.; Toda, M.; Yamamura, S.; Hirata, Y. *Tetrahedron Lett.* **1969**, *10*, 1821–1824. (b) Sasaki, K.; Hirata, Y. *J. Chem. Soc., B* **1971**, 1565–1568. (c) Toda, M.; Hirata, Y.; Yamamura, S. *Tetrahedron* **1972**, *28*, 1477–1484.

(10) Kamijo, N.; Nakano, T.; Terao, Y.; Osaki, K. *Tetrahedron Lett.* **1966**, *25*, 2889–2892.

NP800515S