

Alasmontamine A, A First Tetrakis Monoterpene Indole Alkaloid from *Tabernaemontana elegans*

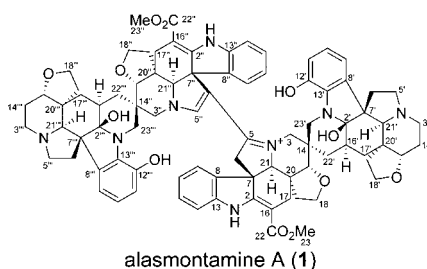
Yusuke Hirasawa,[†] Saori Miyama,[†] Takahiro Hosoya,[†] Koichiro Koyama,[†]
Abdul Rahman,[‡] Idha Kusumawati,[‡] Noor Cholies Zaini,[‡] and Hiroshi Morita^{†,*}

Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41 Shinagawa-ku,
Tokyo 142-8501, Japan, and Faculty of Pharmacy, Airlangga University,
Jalan Dharmawangsa Dalam, Surabaya 60286, Indonesia

moritah@hoshi.ac.jp

Received October 21, 2009

ABSTRACT



A novel tetrakis monoterpene indole alkaloid, alasmontamine A (**1**) consisting of bis-vobtusine-type skeletons, was isolated from the leaves of *Tabernaemontana elegans*. The structure including the relative stereochemistry was elucidated on the basis of spectroscopic data. Alasmontamine A (**1**) exhibited moderate cell growth inhibitory activity against HL-60 cells.

Tabernaemontana elegans Stapf is a member of the Apocynaceae family that occurs in tropical or subtropical regions including Indonesia, Malaysia, and Africa. Traditionally, the roots have been used as a remedy for pulmonary diseases in Africa.¹ *Tabernaemontana* species so far have been shown to produce various skeletal types of indole alkaloids, including iboga-type alkaloids such as ibogamine,² aspidosperma-type alkaloids such as taberhanine,³ and vobasiny-ibogan bisindole alkaloids such as conodiparine A.⁴ Recently, we isolated a new type of bisindole alkaloids as biscarpamontamines A and B from

T. sphaerocarpa.⁵ In our search for structurally and biogenetically interesting alkaloids from tropical plants found in Indonesia, a first tetrakis monoterpene indole alkaloid, alasmontamine A (**1**) consisting of tetrakis aspidosperma-type skeletons, was isolated from the leaves of *T. elegans*, together with vobtusine⁶ and vobtusine lactone.⁷ In this paper, we describe the isolation and structure elucidation of the new alkaloid, alasmontamine A (**1**).

Alasmontamine A (**1**),^{8,9} yellow amorphous solid, $[\alpha]_D^{27}$ –311 (*c* 1.0, MeOH), showed molecular formula, C₈₄H₉₁N₈O₁₂, which was determined by HRESITOFMS [*m/z*

[†] Hoshi University.

[‡] Airlangga University.

(1) van der Heijden, R.; Brouwer, R. L.; Verpoorte, R.; van Beek, T. A.; Harkes, P. A. A.; Svendsen, A. B. *Planta Med.* **1986**, 144–147.

(2) Wenkert, E.; Cochran, D. W.; Gottlieb, H. E.; Hagaman, E. W.; Braz, Filho, R.; Matos, F. J. A.; Madruga, M. I. L. M. *Helv. Chim. Acta* **1976**, 59, 2437–2442.

(3) Kam, T. S.; Pang, H. S.; Lim, T. M. *Org. Biomol. Chem.* **2003**, 1, 1292–1297.

(4) Kam, T. S.; Sim, K. M.; Pang, H. S. *J. Nat. Prod.* **2003**, 66, 11–16.

(5) Zaima, K.; Hirata, T.; Hosoya, T.; Hirasawa, Y.; Koyama, K.; Rahman, A.; Kusumawati, I.; Zaini, N. C.; Shiro, M.; Morita, H. *J. Nat. Prod.* **2009**, 72, 1686–1690.

(6) Schuler, B. O. G.; Verbeek, A. A.; Warren, F. L. *J. Chem. Soc.* **1958**, 4776–4777.

(7) (a) Kunesch, N.; Das, B. C.; Poisson, J. *Bull. Soc. Chim. Fr.* **1970**, 4370–4375. (b) Rolland, Y.; Kunesch, N.; Poisson, J.; Hagaman, E. W.; Schell, F. M.; Wenkert, E. *J. Org. Chem.* **1976**, 41, 3270–3275.

702.3415 ($M + H$)²⁺, $\Delta +0.3$ mmu]. IR absorptions (3440 and 1670 cm⁻¹) implied the presence of NH and/or OH, and conjugated ester carbonyl functionalities. ¹H and ¹³C NMR, HMQC, and HMBC spectra revealed 84 carbon signals due to 18 sp² and 12 sp³ quaternary carbons, 15 sp² and 10 sp³ methines, 27 sp³ methylenes, and 2 methyl groups (303 K by 600 MHz cryo probe). Among them, 8 sp³ methylenes (δ_C 55.0; δ_H 4.11, and 4.26, δ_C 48.6; δ_H 2.37 and 2.88, δ_C 51.6; δ_H 2.31 and 3.09, δ_C 50.7; δ_H 3.71 and 3.83, δ_C 53.5; δ_H 3.82 and 3.92, δ_C 48.6; δ_H 2.37 and 2.88, δ_C 51.6; δ_H 2.31 and 3.09, and δ_C 45.2; δ_H 3.19, and 4.73), 1 sp² methine (δ_C 158.7; δ_H 7.68), 4 sp³ methines (δ_C 76.2; δ_H 4.49, δ_C 64.7; δ_H 2.80, δ_C 72.1; δ_H 4.29, δ_C 64.7; δ_H 2.79), 7 sp² quaternary carbons (δ_C 161.4, 144.8, 168.2, 136.1, 158.7, 144.7, and 136.9), and 2 sp³ quaternary carbons (δ_C 93.6 and 93.2) were ascribed to those bearing a nitrogen atom. Since 18 out of 44 elements of unsaturation were accounted for, **1** was inferred to possess 26 rings.

The gross structure of **1** was elucidated by analyses of 2D NMR data including ¹H–¹H COSY, HOHAHA, HSQC, and HMBC spectra in CD₃OD at 313 K by using a 920 MHz NMR spectrometer (Figure 1). Each pair of the observed ¹H

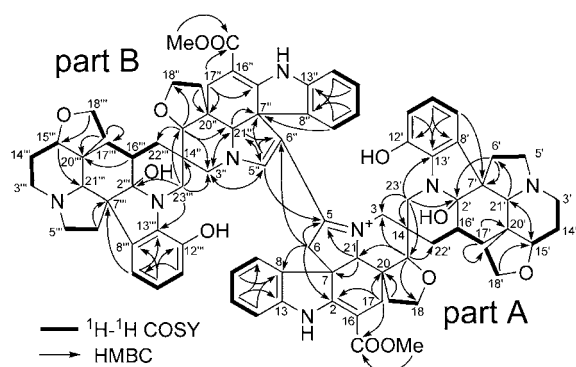


Figure 1. Selected 2D NMR correlations for alasmontamine A (**1**).

and ¹³C NMR signals seemed to be due to each half moiety (parts A and B) of a dimeric compound. In part A, connectivities of C-9–C-12, C-18 to C-19, C-3'–C-15', C-5' to C-6', C-9'–C-11', C-18'–C-19', and C-17'–C-22' were deduced from ¹H–¹H COSY and HOHAHA correlations. In the HMBC spectrum, long-range ¹H–¹³C correlations

(8) The leaves of *T. elegans* collected at Alas Purwo, Indonesia in 2007 were extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted at pH 9 with saturated Na₂CO₃ aq and extracted with CHCl₃. CHCl₃-soluble alkaloidal materials were subjected to a silica gel column (CHCl₃/MeOH) twice followed by an LH-20 column (CHCl₃/MeOH) to afford alasmontamine A (**1**, 0.0005%) together with known alkaloids, vobtusine⁶ and vobtusine lactone.⁷

(9) Alasmontamine A (**1**): yellow amorphous solid; $[\alpha]_D^{27} -311$ (c 1.0, MeOH); IR (KBr) ν_{\max} 3440, 2940, 1670, and 1630 cm⁻¹; UV (MeOH) λ_{\max} 365 (ϵ 17 800), 329 (20 400), 295 (19 700), 259 (17 700), and 218 (55 400) nm; CD (MeOH) λ_{\max} 373 (θ -22 100), 338 (-13 100), 325 (-17 900), 295 (4 500), 280 (4 100), 265 (13 600), 250 (5 600), and 225 (33 100) nm; ¹H and ¹³C NMR (Table 1); ESIMS (pos.) m/z 702 ($M + H$)²⁺; HRESITOFMS m/z 702.3415 ($M + H$)²⁺, calcd for C₈₄H₉₂N₈O₁₂ 1404.6824.

indicated that part A possessed a 12'-*O*-demethylvobtusine-type framework with an iminium functionality at C-5 (δ_C 168.2). The presence of an iminium carbon (C-5) was elucidated by HMBC correlations for H₂-6 and H-21 to C-5. In addition, the ¹³C signals at 6- and 21-positions around the iminium functionality were observed at lower field due to deshielding effects (Table 1) compared with those of vobtusine. The relative configurations at C-7, 15, 20, 21, 7', 15', 16', 20', and 21' in part A were based on NOESY correlations of H-9 and H-19a/H-21, H-15/H₂-17, H-9' and H-18'b/H-21', and H-16'/H-19'b, while the 3,3'-spirobipiperidine (C-3, 14, 15, 20, 21, N, 2', 16', 22', 23', and N) ring adopted a boat–chair conformation that was supported by NOESY correlations as shown in Figure 2. Furthermore the

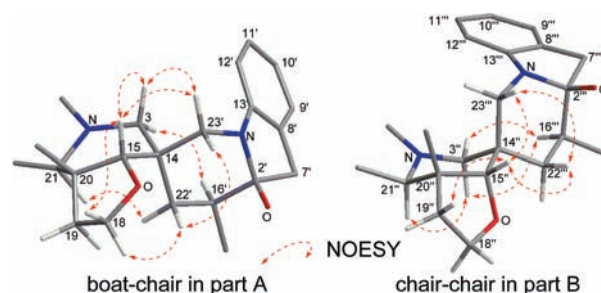


Figure 2. Selected NOESY correlations around two spiro carbons (C-14 and C-14') in parts A and B of **1**.

β -configuration of the OH group at C-2' was deduced from the upfield chemical shift of C-6' (δ_C 30.6) by the γ -gauche effect.^{6,7}

On the other hand, detailed analyses of the HMBC spectrum of **1** indicated that part B possessed a 12'-*O*-

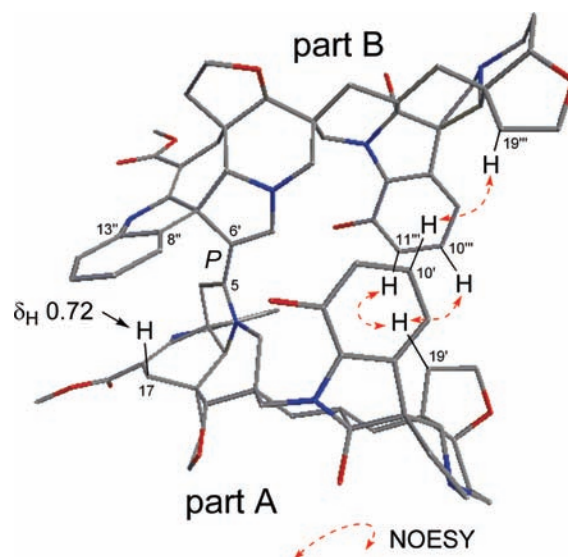


Figure 3. Selected NOESY correlations and the relative stereochemistry for alasmontamine A (**1**).

Table 1. ^1H and ^{13}C NMR Data of Alasmontamine A (**1**) in CD_3OD

unit A				unit B			
position	δ_{H}^a	δ_{C}^b	HMBC ^a	position	δ_{H}^a	δ_{C}^b	HMBC ^a
2		161.4	6b, 17b	2''		158.7	17''
3a	4.11 (1H, d, 13.6)	55.0	22'b, 23' b	3''a	3.82 (1H, m)	53.5	5'', 15'', 22''', b 23'''
3b	4.26 (1H, d, 13.6)			3''b	3.92 (1H, m)		
5		168.2	6a, b 6b, 21, 5'' b	5''	7.68 (1H, s)	158.7	3'', b 21''
6a	2.76 (1H, m)	48.7		6''a		104.6	6a, b 5'', 21'' b
6b	2.95 (1H, d, 18.6)			6''b			
7		52.4 ^{a,c}	6, 21	7''		60.9	5'', 9'', 21'' b
8		134.9	6a, b 6b, 10, 12, 21	8''		135.4	10'', 12'', 21''
9	7.56 (1H, m)	122.6 ^{a,c}	11	9''	7.26 (1H, d, 7.3)	124.4	11''
10	6.91 (1H, dd, 7.5, 7.2)	122.8	12	10''	7.11 (1H, dd, 7.3, 7.3)	123.4	12''
11	7.19 (1H, dd, 7.4, 7.2)	130.0	9	11''	7.33 (1H, dd, 7.6, 7.3)	131.2	9''
12	6.96 (1H, d, 7.4)	111.1	10	12''	7.02 (1H, d, 7.6)	111.6	10''
13		144.8	9, 11	13''		144.7	9'', 11''
14		41.8 ^{a,c}	22'b, 23'b ^b	14''		39.8	3''b, 15'', b 22''b, b 23''b ^b
15	2.79 (1H, s)	87.3	17, b 18b, 19b, b 22'b ^b	15''	3.46 (1H, s)	87.1	3''b, 18'', 22''b, b 23''a
16		92.6	17	16''		98.4	17''
17a	0.72 (1H, d, 16.2)	31.1		17''a	2.36 (1H, d, 14.5)	28.2	15'', b 19''b, b 21'' b
17b	2.51 (1H, d, 16.2)			17''b	2.66 (1H, dd, 14.5, 1.6)		
18a	3.83 (1H, m)	69.2	15 ^b	18''a	3.75 (1H, m)	64.3	19''b ^b
18b	4.05 (1H, dd, 8.4, 8.4)			18''b	3.75 (1H, m)		
19a	1.56 (1H, 11.7, 11.7, 11.7)	35.8	15, b 17, b 21 ^b	19''a	1.43 (1H, ddd, 12.8, 8.5, 4.3)	37.0	17''b, b 18'', b 21'' b
19b	1.62 (1H, m)			19''b	1.60 (1H, m)		
20		50.8	15, b 17, 18b, 19b, b 21 ^b	20''		49.1	17'', 19'' b
21	4.49 (1H, s)	76.2	3b, 15, b 17	21''	4.29 (1H, s)	72.1	3''b, 5'', 15'', 17'', 19'' b
22		169.1	17b, 23	22''		168.6	17'', 23''
23	3.78 (3H, s)	51.7		23''	3.69 (3H, s)	51.9	
2'		93.6 ^{a,c}	6'b, 22'b, b 23'b	2'''		93.2 ^{a,c}	17'''a, 22'''a, 22'''b, b 23'''b
3'a	2.37 (1H, m)	48.6 ^{a,c}	15'	3'''a	2.37 (1H, m)	48.6 ^{a,c}	15'''
3'b	2.88 (1H, m)			3'''b	2.88 (1H, m)		
5'a	2.31 (1H, m)	51.6 ^{a,c}	6'b	5'''a	2.31 (1H, m)	51.6 ^{a,c}	6'''b
5'b	3.09 (1H, m)			5'''b	3.09 (1H, m)		
6'a	1.20 (1H, m)	30.6 ^{a,c}		6'''a	1.21 (1H, m)	31.4 ^{a,c}	
6'b	2.74 (1H, m)			6'''b	2.75 (1H, m)		
7'		57.0	6'b, 9'	7'''		56.0 ^{a,c}	6'''b, 9'''
8'		136.6	10'	8'''		135.9	10'''
9'	6.65 (1H, d, 7.7)	116.3	11'	9'''	6.83 (1H, d, 7.5)	115.9	11'''
10'	5.85 (1H, dd, 7.9, 7.7)	121.6		10'''	6.35 (1H, dd, 7.6, 7.5)	120.9	
11'	6.22 (1H, d, 7.9)	116.5	9'	11'''	6.41 (1H, d, 7.6)	118.9	9'''
12'		142.0	10'	12'''		142.0	10'''
13'		136.1	9', 11', 23'a, 23'b ^b	13'''		136.9	9'', 11'', 23'''a, 23'''b ^b
14'a	1.95 (1H, m)	25.5 ^{a,c}		14'''a	1.95 (1H, m)	25.5 ^{a,c}	
14'b	1.99 (1H, m)			14'''b	1.99 (1H, m)		
15'	3.54 (1H, brt, 2.6)	80.5 ^{a,c}	18'b	15'''	3.51 (1H, brt, 2.7)	80.5 ^{a,c}	18'''b
16'	2.28 (1H, m)	31.2 ^{a,c}		16'''	1.99 (1H, m)	32.3 ^{a,c}	22'''b
17'a	1.07 (1H, brd, 12.9)	31.6 ^{a,c}		17'''a	0.97 (1H, d, 11.6)	32.1 ^{a,c}	
17'b	1.90 (1H, dd, 12.9, 12.9)			17'''b	2.00 (1H, m)		
18'a	4.01 (1H, ddd, 10.1, 8.6, 3.6)	65.1 ^{a,c}		18'''a	3.94 (1H, ddd, 9.9, 8.6, 3.2)	64.8 ^{a,c}	
18'b	4.23 (1H, ddd, 10.1, 8.1, 8.0)			18'''b	4.16 (1H, ddd, 9.9, 8.2, 7.9)		
19'a	2.07 (1H, m)	36.8 ^{a,c}	18'b ^b	19'''a	1.74 (1H, ddd, 10.5, 9.2, 9.4)	36.2 ^{a,c}	18'''b ^b
19'b	2.59 (1H, ddd, 11.8, 8.0, 3.6)			19'''b	2.56 (1H, ddd, 10.5, 7.9, 3.2)		
20'		46.3 ^{a,c}		20'''		46.1 ^{a,c}	16'''', b 19'''b ^b
21'	2.80 (1H, s)	64.7 ^{a,c}		21'''	2.79 (1H, s)	64.7 ^{a,c}	
22'a	1.49 (1H, brd, 13.0)	29.3 ^{a,c}		22'''a	1.67 (1H, brd, 14.4)	36.0	3'', b 23''b ^b
22'b	2.15 (1H, dd, 13.0, 12.8)			22'''b	2.20 (1H, dd, 14.8, 14.4)		
23'a	3.71 (1H, d, 14.2)	50.7	15, 22'a	23'''a	3.19 (1H, dd, 14.5, 1.6)	45.2	15'', 22'''a ^b
23'b	3.83 (1H, m)			23'''b	4.73 (1H, m)		

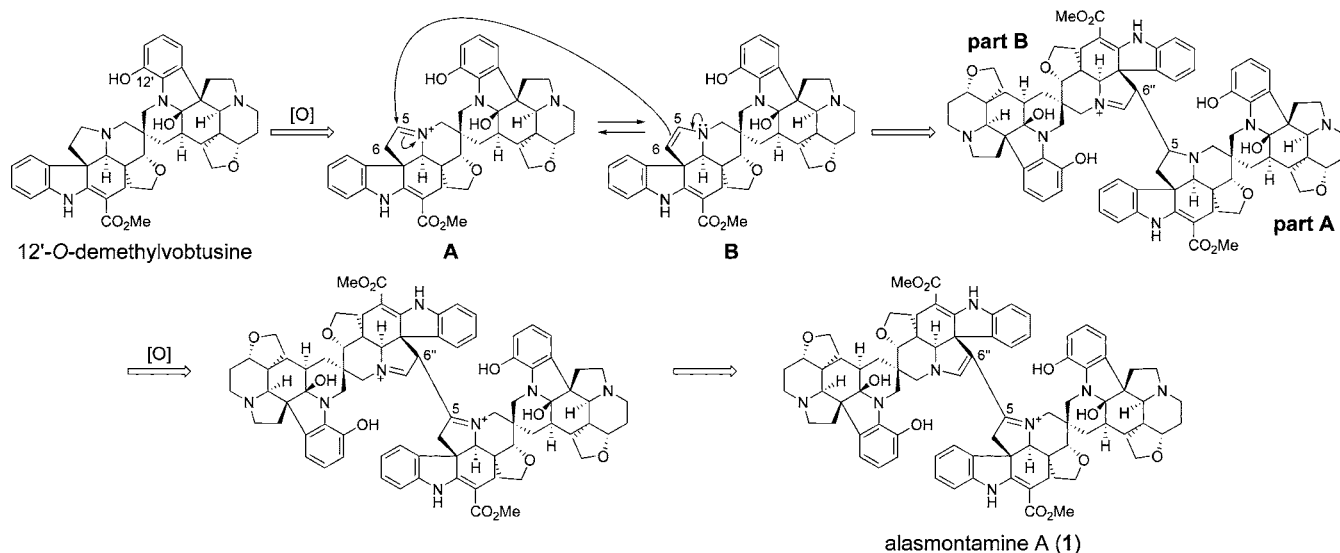
^a Recorded at 313 K by 920 MHz NMR. ^b Recorded at 303 K by 600 MHz cryo probe NMR. ^c Assigned by HSQC or HMBC.

demethylvobtusine type framework with an enamine on C-5'' and C-6''. HMBC correlations for H-3''b and H-5'' to C-21'' (δ_{C} 72.1), H₂-3'' and H-21'' to C-5'' (δ_{C} 158.7), and H-5'' and H-21'' to C-3'' (δ_{C} 53.5) supported connections among C-3'', 5'', and 21'' through a nitrogen atom. And H-5'', H-9'', and H-21'' to C-7'' (δ_{C} 60.9), and H-5'' and H-21'' to C-6'' (δ_{C} 104.6) revealed the presence of an enamine on C-5'' and C-6''. The relative configurations at C-7'', 14'', 15'', 20'', 21'', 7''', 15''', 16''', 20''', and 21''' in part B were the same as corresponding ones of part A. However, the conformation of the 3,3'-spirobipiperidine (C-3'', 14'', 15'', 20'', 21'', N, 2''', 16''', 22''', 23''', and N) ring in part A was different from that in part B. NOESY correlations of H₂-3''/H-16''', H-3''a/H-21'', H-15''/H-22'''b and H-23'''a, and H-22'''b/H-

23'''a suggested the chair–chair conformation of a 3,3'-spirobipiperidine ring in part B. The ^{13}C NMR chemical shifts of part B except for an enamine moiety were in good agreement with those of vobtusine which also possessed a chair–chair conformation assigned by X-ray analysis.⁵ The connection of C-5 and C-6'' between parts A and B was provided by HMBC correlations for H-5'' to C-5 and H-6a to C-6''.

Finally, the relative stereochemistry between parts A and B in **1** was elucidated by the combination of Monte Carlo (MC) search¹⁰ in MacroModel program¹¹ and NOESY correlations. A total of 3000 MC steps were performed to confirm the reproducibility of calculation results. After the MC conformational search, each of the resulting conforma-

Scheme 1. Plausible Biogenetic Path for Alasmontamine A (**1**)



tions was subjected to the energy-minimization calculation by MMFF94s force field.¹² Low-energy conformers belonged to two separate clusters.

The lowest energy one (1234.59 kJ/mol) had an *M* rotation at the C-5–C-6'' axis, and the other one had a *P* rotation (1251.76 kJ/mol) that corresponded with the solution conformer as shown below. Since the latter only satisfied the NOESY correlations of H-10'/H-19'', H-19'/H-10'', and H-19'/H-11'', the relative stereochemistry of **1** was assigned as Figure 3. The allylic proton signal for H-17a was shifted to higher field (δ_{H} 0.72) as compared with that of H-17''a (δ_{H} 2.36). This can be explained by the anisotropic effect of the benzene ring (C-8'' – C-13'') as shown in a computer generated 3D drawing (Figure 3).

Alasmontamine A (**1**) consisting of bis-vobtusine type skeletons is a novel tetrakis monoterpene indole alkaloid from nature. A plausible biogenetic pathway for alasmontamine A (**1**) is proposed as shown in Scheme 1. Tetrakis monot-

erpene indole skeleton might be formed through an iminium-enamine coupling (C-5–C-6) of two vobtusine-type skeletons **A** and **B**, which might be produced through Polonovski-type reaction¹³ from the *N*-oxide of 12'-*O*-demethylvobtusine. Formation of alasmontamine A (**1**) might occur through further oxidation, which was accompanied by enamine formation as shown in Scheme 1.

Alasmontamine A (**1**) showed moderate cell growth inhibitory activity against HL-60 cells (IC_{50} 31.7 μM).

Acknowledgment. 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 and a grant from the Open Research Center Project. We also acknowledge the Nanotechnology Support Project in Central Japan (Institute for Molecular Science), financially supported by the Nanotechnology Network of the Ministry of Education, Culture, Sports, Science and Technology Japan.

Supporting Information Available: 1D and 2D NMR spectra for compound **1**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL902420S

(10) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Cauffield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467.

(11) Molecular modeling experiments employed MacroModel 9.1 equipped with the Maestro 7.5 graphical interface (Schrödinger, LLC, New York, NY, 2005) installed on a Linux Red Hat 9.0 system, and were performed using the MMFF94s force field.

(12) Halgren, T. *J. Am. Chem. Soc.* **1990**, *112*, 4710–4723. Halgren, T. *J. Comput. Chem.* **1999**, *20*, 720–729.

(13) Grierson, D. *Org. React.* **1990**, *39*, 85–295.