Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 417–420

Aspermytin A: a new neurotrophic polyketide isolated from a marine-derived fungus of the genus *Aspergillus*

Sachiko Tsukamoto,* Shunsuke Miura, Yuko Yamashita and Tomihisa Ohta

Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-0934, Japan

Received 25 August 2003; accepted 27 October 2003

Abstract—A new polyketide, aspermytin A (**1**), was isolated from a cultured marine fungus, *Aspergillus* sp., which was separated from the mussel, *Mytilus edulis*. Its structure was elucidated on the basis of their spectral data, and the absolute configuration of **1** was determined by the CD spectrum. Aspermytin A induced neurite outgrowth in rat pheochromocytoma (PC-12) cells at concentration of 50 μM.

1. Introduction

Neurons cannot proliferate and regenerate as the terminally differentiated cells, and neurotrophic factors are essential for functional maintenance and organization of neurons. Among a family of neurotrophic factors, such as nerve growth factor (NGF),[1] brain-derived neurotrophic factor (BDNF),[2] neurotrophin 3 (NT-3),[3] and glia-derived neurotrophic factor (GDNF),[4] NGF has been extensively investigated to show pleiotrophic effects such as induction of neuronal differentiation, neural cell survival, and prevention of apoptosis of neurons in both the central and peripheral nervous systems.[1] Rat pheochromocytoma (PC-12) cells have been used as a good in vitro model of neuronal differentiation. After stimulation with NGF, PC-12 cells differentiate to extend neurites and to develop the characteristics of sympathetic neurons.[5] Marine-derived fungi have proven to be rich sources of structurally novel and biologically active secondary metabolites, which are emerging as a significant new chemical resource for drug discovery.[6] As a continuation of our interest in the discovery of new secondary metabolites from marine organisms,[7–9] we report here the isolation and structure elucidation of a new polyketide, aspermytin A (**1**), produced in culture by a marine-derived fungus identified as the genus *Aspergillus*.

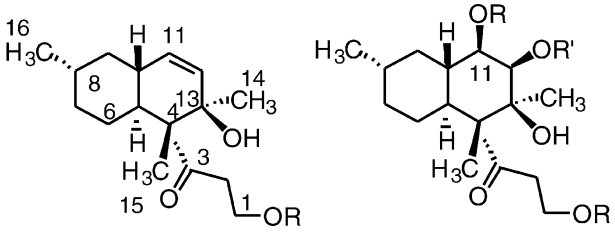
2. Results and discussion

2.1. Isolation of aspermytin A (1)

The fungus was separated from the mussel, *Mytilus edulis*, collected in Toyama Bay in the Japan Sea. The mycelium grown in the culture (2 L) was extracted with MeOH. The EtOAc soluble part (2.41 g) of the extract was purified by ODS chromatography and reversed-phase HPLC to afford aspermytin A (**1**, 9.8 mg) as a neurotrophic compound (Chart 1).

2.2. Structure elucidation of aspermytin A (1)

The FABMS of **1** showed a quasi molecular ion peak at m/z 267 $[M+H]^+$ and the molecular formula was determined as $C_{16}H_{26}O_3$ on the basis of its HRFABMS, requiring four degrees of unsaturation. The IR spectrum of **1** displayed broad hydroxyl absorption at 3300 cm^{-1} and an intense carbonyl peak at 1676 cm^{-1}.



1: R=H
2: R=OAc

3: R=R'=H
4: R=*p*-BrBz; R'=H
5: R=R'=*p*-BrBz

Chart 1.

Keywords: Polyketide; Neurotrophic; *Mytilus edulis*; *Aspergillus* sp.
* Corresponding author. Tel.: +81-76-234-4469; fax: +81-76-234-4417; e-mail: sachiko@p.kanazawa-u.ac.jp

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.10.053

The ^{1}H NMR spectrum in pyridine-d_5 (Table 1) revealed two singlet methyl signals at δ 1.49 and 1.54, and a doublet methyl signal at δ 0.84 (d, J = 6.8 Hz), two hydroxymethyl signals at δ 4.30 (2H, m), two methylene signals bearing a carbonyl carbon at δ 3.05 (dt, J = 18.1, 6.4 Hz) and 3.65 (dt, J = 18.1, 6.4 Hz), and two mutually coupled olefinic signals at δ 5.37 (dd, J = 9.8, 1.0 Hz) and 5.71 (dd, J = 9.8, 2.9 Hz). The ^{13}C NMR data of **1** (Table 1) showed a ketone carbon (δ 214.1), a 1,2-disubstituted double bond (δ 129.5, d and 135.5, d), three methyl carbons (δ 12.6, 22.5, and 28.5), two quaternary carbons (δ 57.7 and 73.2), and two methine carbons (δ 43.7 and 38.7). The hydroxymethyl signals at δ 4.30 (2H, m, H_2-1) were spin coupled with H_2-2 (δ 3.05 and 3.65) (fragment a) (Fig. 1), and the presence of a 1,2,4-trisubstitued cyclohexane ring was indicated by the ^{1}H–^{1}H COSY spectrum. The doublet methyl signal at δ 0.84 (H_3-16) was coupled to a multiplet at δ 1.35 (H-8), and long-range correlations between a ddd signal at δ 1.77 (H-10) and two olefinic signals at δ 5.37 (H-11) and δ 5.71 (H-12) suggested that a 1,2-disubstituted *cis* double bond was attached to the C-10 methine carbon (fragment b). HMBC correlations between a singlet methyl signal H_3-14 and C-4, C-12, and C-3 and between a singlet methyl signal H_3-15 and C-3, C-4, and C-5 showed that the fragment b was comprised of a decaline ring. The *trans* juncture of the decaline ring was indicated by the coupling constant with the magnitude of J = 10.9 Hz between H-5 and H-10. HMBC correlations were observed from H_2-2 to a carbonyl carbon C-3 (δ 214.1) and a quaternary carbon C-4 (δ 57.5), which suggested the fragment a linked to C-4 of the decaline ring via a carbonyl carbon C-3. Thus, the gross structure of **1** was disclosed. The relative stereochemistry of **1** was established by its NOESY spectrum. NOE correlations between H-10 and three signals H-6, H-8, and H_3-15 indicated that they were oriented on the same side (Fig. 2), while the correlations between H-5 and three signals H-7, H-9, and H_3-14 and between H-7 and H-9 revealed that they were on the opposite side of H-6, H-8, H-10, and H_3-15. These NOE data confirmed the *trans* junction of the decaline ring system. The absolute configur-

Table 1. NMR spectral data for aspermytin (**1**) in pyridine-d_5

	δ_H	J (Hz)	δ_C	COSY	HMBC
1	4.30 (2H)	m	57.6 t	H-2, H-2′, 1-OH	
2	3.05	dt, 18.1, 6.4	45.6 t	H-1, H-2′	C-1, C-3, C-4
	3.65	dt, 18.1, 6.4		H-1, H-2	C-1, C-3, C-4
3			214.1 s		
4			57.7 s		
5	2.07	dt, 2.1, 10.9	43.7 d	H-6, H-6′, H-10	C-4, C-6, C-7, C-9, C-15
6	1.00	dq, 2.0, 10.9	27.8 t	H-5, H-6′	
	1.82	m		H-5, H-6	C-8, C-10
7	1.02	dq, 2.0, 10.9	35.7 t	H-7′, H-8	C-5
	1.63	m		H-7	C-9
8	1.35	m	33.4 d	H-7, H-9, H-16	
9	0.78	q, 12.2	41.9 t	H-8, H-9′, H-10	
	1.66	m		H-9	
10	1.77	ddd, 12.2, 10.9, 2.1	38.7 d	H-5, H-9, H-11,[a] H-12[a]	C-9
11	5.37	dd, 9.8, 1.0	129.5 d	H-10,[a] H-12	C-5, C-9, C-13
12	5.71	dd, 9.8, 2.9	135.5 d	H-10, [a] H-11	C-4, C-10, C-13
13			73.2 s		
14	1.49 (3H)	s	28.5 q		C-4, C-12, C-13
15	1.54 (3H)	s	12.6 q		C-3, C-4, C-5
16	0.84 (3H)	d, 6.8	22.5 q	H-8	C-7, C-8, C-9
1-OH	5.94	t, 5.4		H-1	
13-OH	6.46	s			

[a] Long-range correlations.

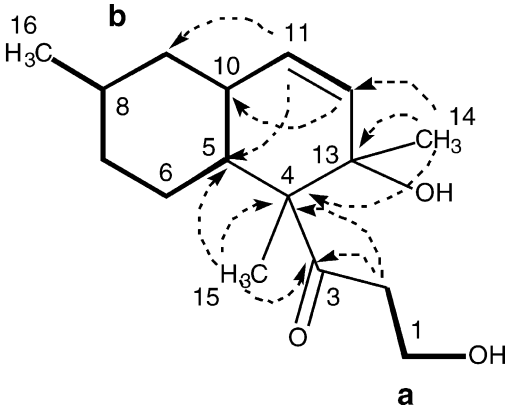


Figure 1. COSY (bold lines) and selected HMBC (dashed arrows) correlations observed for **1**.

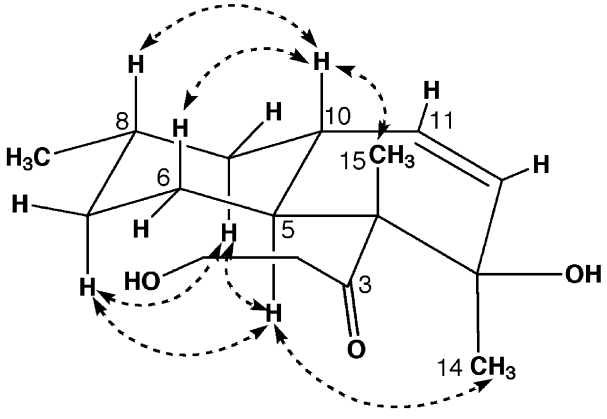


Figure 2. NOE correlations observed for **1**.

ation of **1** was determined by the CD spectrum using a dibenzoate derived from **1**. After acetylation of **1**, the 1-monoacetate **2** was subjected to osmium tetroxide oxidation to afford the 11,12-diol **3**, which was then treated with *p*-bromobenzoyl chloride in pyridine to furnish a mixture of 11-*p*-bromobenzoate **4** and 11,12-di-*p*-bromobenzoate **5** (Chart 1). The magnitudes of the coupling constants of H-11 [δ 5.02 (dd, $J = 11.0$, 3.3 Hz)] and H-12 [δ 5.44 (d, $J = 3.3$ Hz)] of **5** confirmed that the *p*-bromobenzoyl groups of C-11 and C-12 were equatorial and axial, respectively. The CD spectrum of **5** showed a negative maximum ($\Delta\varepsilon = -1.3$) at 236 nm and a positive maximum ($\Delta\varepsilon = +4.7$) at 252 nm, indicating 11*R*,12*R*-configuration of **5**. Thereby, the absolute configuration of **1** was established as represented in Chart 1.

Aspermytin A (**1**) is a heptaketide with a *trans* decalin framework. Several polyketides bearing a *trans* decalin ring have been obtained from microorganisms so far, trichoharzin, an octaketide produced by the marine-derived fungus *Trichoderma harzianum*,[10] tanzawaic acid B isolated from *Penicillium citrinum*,[11] and beta-enone B, a phytotoxin produced from the culture of *Phoma betae* Fr.[12] On the other hand, phomopsidin, which was recently isolated from a marine-derived fungus *Phomopsis* sp. and exhibited anti-microtuble activity, contained a *cis* decalin structure.[13]

2.3. Neurite growth assay

Aspermytin A (**1**) showed significant neurotrophic effect on the rat pheochromocytoma (PC-12) cells (Fig. 3). More than 50% of the cells revealed neurites by 2 days treatment with 50 μM **1**, and the effect was comparable to that by 50 ng/mL NGF.

3. Experimental

3.1. General

Optical rotations were determined with a Horiba SEPA-300 high sensitive polarimeter. UV spectra were measured on a Shimadzu UV-1600 UV–visible spectrophotometer. IR spectra were recorded on a Shimadzu IR-460 infrared spectrophotometer. CD spectra were measured on a Jasco J-780 spectropolarimeter in MeOH. NMR spectra were recorded on a Jeol GSX500 in $CDCl_3$ or pyridine-d_5. All chemical shifts were reported with respect to $CDCl_3$ (δ_H 7.26, δ_C 77.0) or pyridine-d_5 (δ_H 7.18). Mass spectra were measured on a Jeol SX-102 mass spectrometer.

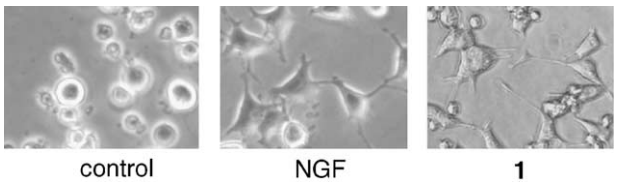


Figure 3. Effects of **1** and NGF on neurite outgrowth in PC-12 cells. PC-12 cells were incubated for 2 days without drug (control) or with 50 ng/mL NGF (NGF) as positive control or 50 μM **1**.

3.2. Fungal strain

Strain of a fungus, *Aspergillus* sp., was isolated from the mussel, *M. edulis*, in Toyama Bay in the Japan Sea and was identified based on the morphological evaluation by NCIBM Japan Co., Ltd (Shizuoka, Japan). A voucher specimen is deposited at Kanazawa University with the code MF297.

3.3. Culture conditions

The fungus was grown in a fermentation broth composed of 1:1 artificial seawater/deionized water with 2.0% malt extract and 0.5% peptone at 28 °C for 21 days.

3.4. Extraction and isolation

The cultured broth (2 L) was filtered. The mycelium was extracted with MeOH. The extract was concentrated under reduced pressure and extracted with EtOAc. The EtOAc layer (2.41 g) was partitioned between hexane and 90% MeOH–H_2O. The aq MeOH fraction (405.5 mg) was subjected to ODS chromatography. The fraction eluted with 80% MeOH–H_2O was purified by reversed-phase HPLC with 75% MeOH–H_2O and then with 50% CH_3CN–H_2O to afford aspermytin A (**1**, 9.8 mg).

3.5. Aspermytin A (1)

$[\alpha]_D^{25}$ +1.2° (*c* 0.104, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 201.5 nm (3.0); IR (film) ν_{max} 3300, 2955, 1676, 1370, 1041 cm^{-1}. ^1H and ^{13}C NMR (pyridine-d_5), see Table 1. FABMS (positive, glycerol matrix) *m*/*z* 267 $[M+H]^+$; HRFABMS *m*/*z* 267.1953 ($C_{16}H_{27}O_3$, Δ −0.7 mmu).

3.6. Acetylation of aspermytin A (1)

A solution of **1** (3.3 mg) in pyridine (0.5 mL) was added to acetic anhydride (0.4 mL), and the mixture was kept at room temperature overnight. The solvent was evaporated, and the residue was purified by silica gel chromatography with hexane/EtOAc (2:1) to afford the monoacetate (**2**, 3.0 mg).

3.6.1. 2. ^1H NMR ($CDCl_3$) δ 0.81 (1H, q-like, $J = 12.2$ Hz, H-9), 0.90 (3H, d, $J = 6.8$ Hz, H_3-16), 0.98 (1H, m, H-6), 1.06 (1H, dq, $J = 3.4$, 11.7 Hz, H-7), 1.12 (3H, s, H_3-14), 1.28 (3H, s, H_3-15), 1.46 (1H, m, H-8), 1.58 (1H, m, H-6), 1.72 (1H, m, H-7), 1.78 (1H, m, H-9), 1.80 (1H, m, H-5), 2.01 (3H, s, 1-OAc), 2.74 (1H, ddd, $J = 18.6$, 6.9, 5.9 Hz, H-2), 3.18 (1H, ddd, $J = 18.6$, 6.9, 5.9, H-2), 4.32 (1H, ddd, $J = 11.2$, 6.9, 5.9 Hz, H-1), 4.37 (1H, ddd, $J = 11.2$, 6.9, 5.9 Hz, H-1), 5.32 (1H, dd, $J = 9.8$, 2.0 Hz, H-11), 5.38 (1H, d, $J = 9.8$ Hz, H-12); FABMS (positive, glycerol matrix) *m*/*z* 309 $[M+H]^+$.

3.7. OsO_4 oxidation of 2

To a solution of **2** (12.7 mg) in *t*-BuOH (0.8 mL) was added OsO_4 (50 μL of 10% aqueous solution) and *N*-methylmorpholine *N*-oxide (12.0 mg) in water (240 μL).

The mixture was stirred at room temperature for 5 days. To the solution was added saturated aqueous Na_2SO_3 (3 mL), and the mixture was stirred for 30 min. The product was extracted with EtOAc, and the organic layer was subsequently washed with 1 N HCl and brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel chromatography with hexane/EtOAc (1:3) to afford **2** (3.8 mg) and its diol (**3**, 7.9 mg).

3.7.1. 3. ^{1}H NMR ($CDCl_3$) δ 0.71 (1H, q, J = 12.7 Hz, H-9), 0.91 (3H, d, J = 6.9 Hz, H_3-16), 0.99 (2H, m, H-6 and H-7), 1.01 (3H, s, H_3-14), 1.34 (1H, m, H-8), 1.35 (3H, s, H_3-15), 1.38 (1H, m, H-6), 1.56 (1H, dq, J = 3.4, 11.7 Hz, H-9), 1.66 (1H, m, H-7), 1.77 (1H, dt, J = 2.9, 11.7 Hz, H-5), 2.01 (3H, s, 1-OAc), 2.13 (1H, dq, J = 12.7, 3.4 Hz, H-9), 2.75 (1H, dt, J = 18.1, 5.9 Hz, H-2), 3.19 (1H, ddd, J = 18.1, 7.3, 5.9 Hz, H-2), 3.32 (1H, dd, J = 11.7, 3.4 Hz, H-11), 3.65 (1H, d, J = 3.4 Hz, H-12), 4.30 (1H, dt, J = 11.2, 5.9 Hz, H-1), 4.34 (1H, ddd, J = 11.2, 7.3, 5.9 Hz, H-1); FABMS (positive, glycerol matrix) m/z 365 $[M+Na]^+$.

3.8. Preparation of *p*-bromobenzoyl esters (4 and 5) from 3

A solution of **3** (6.0 mg) in pyridine (1.0 mL) was treated with *p*-bromobenzoyl chloride (38.5 mg) in the presence of 4-(dimethylamino)pyridine (2.0 mg), and the mixture was stirred at 50 °C overnight. After cooling, the reaction mixture was poured into ice-water, and the whole was extracted with EtOAc. The EtOAc extract was successively washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine, then dried over Na_2SO_4, and concentrated. The residue was purified by silica gel chromatography with hexane/EtOAc (2:1, 1:1) to afford 11-monoester (**4**, 8.4 mg) and 11,12-diester (**5**, 1.9 mg).

3.8.1. 4. ^{1}H NMR ($CDCl_3$) δ 0.76 (1H, q, J = 12.5 Hz, H-9), 0.86 (3H, d, J = 6.4 Hz, H_3-16), 1.00 (1H, q, J = 13.2 Hz, H-7), 1.05 (1H, q, J = 13.2 Hz, H-6), 1.37 (1H, m, H-8), 1.14 (3H, s, H_3-14), 1.41 (3H, s, H_3-15), 1.45 (1H, m, H-6), 1.68 (1H, d-like, J = 12.6 Hz, H-7), 1.85 (1H, d-like, J = 12.7 Hz, H-9), 1.90 (1H, dt, J = 1.7, 11.6 Hz, H-5), 2.02 (1H, m, H-10), 2.02 (3H, s, 1-OAc), 2.77 (1H, dt, J = 18.2, 5.5 Hz, H-2), 3.21 (1H, dt, J = 18.2, 5.5 Hz, H-2), 3.86 (1H, d, J = 2.9 Hz, H-12), 4.32 (1H, dt, J = 5.5, 11.0 Hz, H-1), 4.36 (1H, dt, J = 5.5, 11.0 Hz, H-1), 4.90 (1H, dd, J = 10.7, 2.9 Hz, H-11), 7.61 (2H, d, J = 8.3 Hz), 7.92 (2H, d, J = 8.3 Hz); FABMS (positive, NBA matrix) m/z 547/549 (intensity, 1:1) $[M+Na]^+$.

3.8.2. 5. ^{1}H NMR ($CDCl_3$) δ 0.79 (1H, q, J = 13.2 Hz, H-9), 0.88 (3H, d, J = 6.4 Hz, H_3-16), 1.06 (1H, q, J = 13.2 Hz, H-7), 1.33 (3H, s, H_3-14), 1.12 (1H, m, H-6), 1.42 (1H, m, H-8), 1.49 (1H, m, H-6), 1.50 (3H, s, H_3-15), 1.72 (1H, d-like, J = 12.6 Hz, H-7), 1.89 (1H, d-like, J = 12.7 Hz, H-9), 2.01 (3H, s, 1-OAc), 2.02 (1H, m, H-10), 2.07 (1H, m, H-5), 2.76 (1H, dt, J = 18.2, 5.5 Hz, H-2), 3.14 (1H, dt, J = 18.2, 5.5 Hz, H-2), 4.32 (1H, dt, J = 5.5, 11.0 Hz, H-1), 4.36 (1H, dt, J = 5.5, 11.0 Hz, H-1), 5.02 (1H, dd, J = 11.0, 3.3 Hz, H-11), 5.44 (1H, d, J = 3.3 Hz, H-12), 7.47 (2H, d, J = 8.3 Hz), 7.65 (2H, d, J = 8.3 Hz), 7.70 (2H, d, J = 8.3 Hz), 7.83 (2H, d, J = 8.3 Hz); FABMS (positive, NBA matrix) m/z 707/709/711 (intensity, 1:2:1) $[M+H]^+$.

3.9. Neurite outgrowth assay

Neurite outgrowth assay was carried out with rat pheochromocytoma (PC-12) cells.[5] PC-12 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 5% horse serum, penicillin (50 units/mL), and streptomycin (50 μg/mL) in an incubator containing 5% CO_2 at 37 °C. PC-12 cells were seeded onto 24-well multiplates (1×10^5 cells/mL) and cultivated for a day. The medium was replaced with that containing 50 μM aspermytin A or 5 ng/mL NGF as positive control, and then PC-12 cells were cultivated for 2 days and observed under a phase-contrast microscope. The percentage of the cells with neurites was determined by counting 200 cells.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan and the Sumitomo Foundation.

References and notes

1. Levi-Montalcini, R. *Science* **1987**, *237*, 1154.
2. Barde, Y. A.; Edger, D.; Thoenen, H. *EMBO J.* **1982**, *1*, 549.
3. Maisonpierre, P. C.; Belluscio, L.; Squinto, S.; Ip, N. Y.; Furth, M. E.; Lindsay, R. M.; Yancopoulos, G. D. *Science* **1990**, *247*, 1446.
4. Lin, L.-F. H.; Doherty, D. H.; Lile, J. D.; Bektesh, S.; Collins, F. *Science* **1993**, *260*, 1130.
5. Greene, L. A.; Tischler, A. S. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2424.
6. Blunt, J. W.; Copp, B. R.; Munro, M. H.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2003**, *20*, 1.
7. Tsukamoto, S.; Tane, K.; Ohta, T.; Matsunaga, S.; Fusetani, N.; van Soest, R. W. M. *J. Nat. Prod.* **2001**, *64*, 1576.
8. Tsukamoto, S.; Miura, S.; van Soest, R. W. M.; Ohta, T. *J. Nat. Prod.* **2003**, *66*, 438.
9. Tsukamoto, S.; Tatsuno, M.; van Soest, R. W. M.; Yokosawa, H.; Ohta, T. *J. Nat. Prod.* **2003**, *66*, 1181.
10. Kobayashi, M.; Uehara, H.; Matsunami, K.; Aoki, S.; Kitagawa, I. *Tetrahedron Lett.* **1993**, *34*, 7925.
11. Kuramoto, M.; Yamada, K.; Shikano, M.; Yazawa, K.; Arimoto, H.; Okamura, T.; Uemura, D. *Chem. Lett.* **1997**, 885.
12. Ichihara, A.; Oikawa, H.; Hayashi, K.; Sakamura, S.; Furusaki, A.; Matsumoto, T. *J. Am. Chem. Soc.* **1983**, *105*, 2907.
13. Kobayashi, H.; Meguro, S.; Yoshimoto, T.; Namikoshi, M. *Tetrahedron* **2003**, *59*, 455.