Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron 63 (2007) 8282–8285
Tetrahedron

Briarenolides A–C, briarane diterpenoids from the gorgonian coral *Briareum* sp.

Jui-Hsin Su,[a] Ping-Jyun Sung,[b,c] Yao-Haur Kuo,[d] Chi-Hsin Hsu[a,e] and Jyh-Horng Sheu[a,e,*]

[a]*Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan*
[b]*National Museum of Marine Biology and Aquarium, Checheng, Pingtung 944, Taiwan*
[c]*Institute of Marine Biotechnology, National Dong Hwa University, Checheng, Pingtung 944, Taiwan*
[d]*National Research Institute of Chinese Medicine, Taipei 112, Taiwan*
[e]*Asian Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan*

Received 27 April 2007; revised 24 May 2007; accepted 25 May 2007
Available online 2 June 2007

Abstract—Chemical investigation on the gorgonian coral *Briareum* sp. has led to the isolation of six oxygenated briaran diterpenes **1**–**6**, including three new compounds briarenolides A–C (**1**–**3**). The structures of **1**–**3** were determined on the basis of extensive spectroscopic analysis and by comparison of their spectral data with those of related metabolites. Among these metabolites, **1** and **2** are rarely found 9-ketobriaranes. Also, **1** is the first briarane derivative possessing a 20-hydroxy group.

1. Introduction

During the course of our search for bioactive metabolites from marine invertebrates of Taiwanese waters, several briarane-type diterpenoids have been isolated from gorgonian corals *Briareum* sp.[1,2] and *Briareum excavatum*.[3–6] In a previous study, we reported three new diterpenoids with briarane carbon skeleton from *Briareum* sp.[1] Because of our interest in the chemistry of biologically active natural products, the continuing investigation on the chemical constituents of the gorgonian coral *Briareum* sp. was carried out and resulted in the isolation of three new briarane-type diterpenoids, briarenolides A–C (**1**–**3**), along with three known metabolites **4**–**6** (Fig. 1). This paper deals with the isolation and structure elucidation of these new compounds.

2. Results and discussion

The sliced bodies of the gorgonian coral *Briareum* sp. were extracted exhaustively with EtOAc. Purification of the crude extract by Si gel column chromatography followed by repeated normal phase HPLC yielded new metabolites **1**–**3** as well as known compounds including milolide B (**4**),[7]

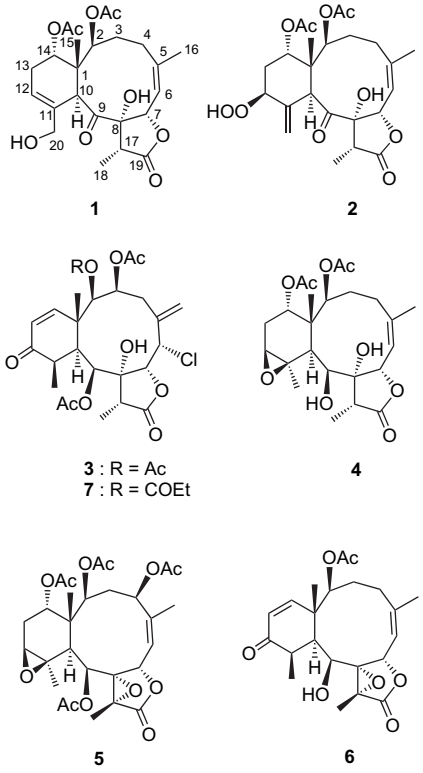

Figure 1. Structures of metabolites **1**–**7**.

Keywords: Briarane-type; *Briareum*.
* Corresponding author. Tel.: +886 7 5252000x5030; fax: +886 7 5255020; e-mail: sheu@mail.nsysu.edu.tw

0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.05.103

stecholide C acetate (**5**),[8] and briaexcavatolide T (**6**)[6]. All compounds were obtained as white powder.

The HRESIMS spectrum of **1** exhibited a molecular ion peak at *m/z* 487.1942 ([M+Na]$^+$), with a molecular formula $C_{24}H_{32}O_9$ and implying nine degrees of unsaturation. The IR absorptions were observed at 3462, 1770, 1734, and 1710 cm^{-1}, suggesting the presence of hydroxy, γ-lactone ester, and ketone groups in **1**. The structure of this compound was deduced from its ^{13}C NMR and DEPT spectra, which showed that the compound has 24 carbons, including five methyls, four sp^3 methylenes (including one oxymethylene), two sp^2 methines, five sp^3 methines (including three oxymethines), two sp^3 quaternary carbons, and six sp^2 quaternary carbons (including one ketone). From ^{1}H and ^{13}C NMR spectra, **1** was found to possess two acetoxy groups and one γ-lactone moiety [δ_H 2.03 (6H, s), δ_C 176.5 (C), 171.1 (C), 170.2 (C)], in addition to two trisubstituted olefins [δ_H 5.79 (1H, s), 5.58 (1H, d, J=10.0 Hz), δ_C 143.4 (C), 133.5 (C), 127.2 (CH), 119.2 (CH)] (Table 1). Moreover, the ^{13}C NMR data (Table 1) designated a ketone carbonyl (δ_C 216.1) in **1**. The gross structure of **1** was determined by a detailed analysis of 1D and 2D NMR spectra. From the ^{1}H–^{1}H COSY spectrum of **1**, it was possible to identify four different structural units, which were assembled with the assistance of an HMBC experiment (Fig. 2). Key HMBC correlations between H-2 to C-1 and C-10; H_2-4 to C-5 and C-6; H-7 to C-8 and C-9 (carbonyl carbon); H-14 to C-10; H_3-15 to C-1, C-2, C-10, and C-14; H_3-16 to C-4, C-5, and C-6; H-17 to C-8 and C-19; H_3-18 to C-8, C-17, and C-19; and H_2-20 to C-10, C-11, and C-12, permitted the connection of the carbon skeleton. Furthermore, the acetoxy groups positioned at C-2 and C-14 were confirmed from the HMBC correlations from δ 5.05 (H-2) and 4.83 (H-14) to the ester carbonyl carbons appeared at δ 171.1 (C) and 170.2 (C), respectively. Thus, the remaining hydroxy group had to be positioned at C-8, an oxygen-bearing quaternary carbon resonating at δ 90.5 ppm.

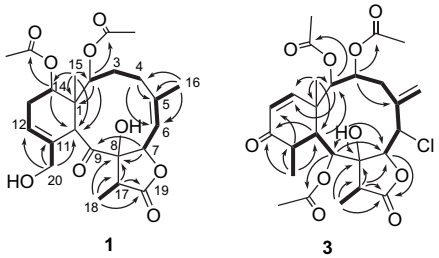

Figure 2. ^{1}H–^{1}H COSY and HMBC correlations for **1** and **3**.

The relative structure of **1** was elucidated by the analysis of NOE, as shown in Figure 3. The NOE correlations between the H-10 and H-2 indicated that both protons are situated on same face and were arbitrary assigned as α protons since the C-15 methyl group is β-oriented and H_3-15 did not show correlation with H-10. H-14 was found to exhibit NOE response with H_3-15 but not with H-10, revealing the β-orientation of this proton. One of the methylene protons at C-3 (δ 1.60, m) exhibited NOE correlation with H-10 and was assigned as

Table 1. ^{1}H and ^{13}C NMR spectral data for **1**–**3**

C/H	**1**		**2**		**3**	
	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]
1		45.7 (C)[d]		47.8 (C)		44.5 (C)
2	5.05 d (7.0)[c]	73.0 (CH)	4.95 d (6.5)	73.9 (CH)	5.11 s	82.9 (CH)
3	α: 1.60 m; β: 1.40 m	34.0 (CH_2)	α: 1.54 m; β: 1.65 m	32.3 (CH_2)	5.15 dd (10.5, 1.5)	75.7 (CH)
4	α: 1.84 m; β: 2.45 m	28.1 (CH_2)	α: 1.99 m; β: 2.40 m	28.7 (CH_2)	α: 2.57 br d (15.5); β: 2.30 m	35.1 (CH_2)
5		143.4 (C)		146.2 (C)		137.0 (C)
6	5.58 d (10.0)	119.2 (CH)	5.47 d (10.0)	117.7 (CH)	4.82 s	66.7 (CH)
7	5.04 d (10.0)	80.3 (CH)	5.01 d (10.0)	80.8 (CH)	5. 92 d (2.5)	77.9 (CH)
8		90.5(C)		89.5 (C)		82.8 (C)
9		216.1 (C)		211.1 (C)	5.09 d (5.5)	74.2 (CH)
10	4.60 s	44.3 (CH)	4.49 s	47.7 (CH)	2.95 t (10.5)	39.2 (CH)
11		133.5 (C)		139.4 (C)	2.62 m	46.7 (CH)
12	5.79 s	127.2 (CH)	4.61 dd (13.0, 4.0)	81.2 (CH)		201.7 (C)
13	2.40 m; 2.17 m;	27.9 (CH_2)	2.19 dt (14.5, 4.0); 1.65 m	32.6 (CH_2)	6.05 d (10.5)	126.5 (CH)
14	4.83 d (2.5)	70.2 (CH)	4.92 s	73.2 (CH)	6.38 d (10.5)	152.9 (CH)
15	1.03 s	12.3 (CH_3)	1.15 s	12.2 (CH_3)	1.14 s	19.3 (CH_3)
16	1.99 s	27.7 (CH_3)	1.99 s	28.4 (CH_3)	5.82 d (2.0); 5.77 s	122.0 (CH_2)
17	3.66 q (7.5)	45.0 (CH)	3.65 q (7.5)	44.5 (CH)	2.44 q (7.5)	41.7 (CH)
18	1.18 d (7.5)	7.5 (CH_3)	1.12 d (7.5)	7.6 (CH_3)	1.29 d (7.5)	10.1 (CH_3)
19		176.5 (C)		176.1 (C)		176.2 (C)
20	4.06 d (13.0); 3.82 d (13.0)	67.3 (CH_2)	5.39 s; 4.64 s	110.6 (CH_2)	1.30 d (7.0)	14.9 (CH_3)
OAc	2.03 s	21.0 (CH_3)	1.99 s	21.0 (CH_3)	2.32 s	21.2 (CH_3)
		170.2 (C)		170.5 (C)		168.9 (C)
	2.03 s	21.4 (CH_3)	2.10 s	21.3 (CH_3)	2.13 s	21.2 (CH_3)
		171.1 (C)		170.2 (C)		169.6 (C)
					2.07 s	21.7 (CH_3)
						170.0 (C)
12-OOH			8.25 br s			
8-OH					3.42 s	

[a] Spectra recorded at 500 MHz in $CDCl_3$.
[b] Spectra recorded at 125 MHz in $CDCl_3$.
[c] J values in parentheses (Hz).
[d] Attached protons were deduced by DEPT experiments.

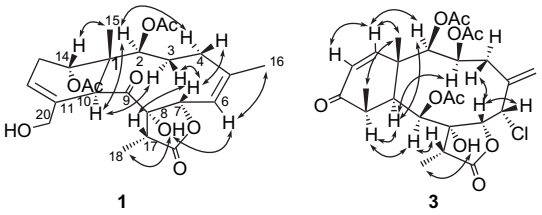

Figure 3. Selective NOE correlations for **1** and **3**.

H-3α while the other (δ 1.40, m) was denoted as H-3β. The NOE correlations observed between H-3β and H-7, and H-7 and H-17 reflected the β-orientations of both protons at C-7 and C-17. NOESY spectrum showed correlation of H_3-16 with H-6 revealing the *Z* geometry of the C-5/C-6 double bond. From the above evidences and the other key correlations of H-7 with H-4β (δ 2.45), H-2 with H-4α (δ 1.84), and due to the fact that 9-ketobriarane, like **1**, might be the product afforded from the oxidation of 9-hydroxy briaranes, like **4**, thus it was suggested that **1** possessed the same configurations at C-1, C-2, C-7, C-8, C-10, C-14, and C-17, as those in compound **4**.[7] The configuration of C-8, however, is not confirmed at this stage by NOE correlations as the 8-hydroxy of **1** did not show signal in ^{1}H NMR spectrum measured in $CDCl_3$. Further study by measuring the ^{1}H NMR spectra (including NOESY spectrum) in pyridine-d_5 fortunately revealed that 8-OH (δ 8.36, s) showed NOE interaction with H_3-18 (δ 1.45, d, *J*=7.2 Hz), but not with H-17 (δ 4.06, q, *J*=7.2 Hz). Thus, the α-orientation of 8-OH could be unambiguously confirmed. From the above results, the relative structure of **1** was established.

The HRESIMS spectrum of **2** exhibited a molecular ion peak at *m/z* 503.1890 ([M+Na]$^+$), with a molecular formula $C_{24}H_{32}O_{10}$ and implying nine degrees of unsaturation. The IR absorptions of **2** showed the presence of hydroxy (3433 cm^{-1}), γ-lactone (1770 cm^{-1}), ester carbonyl (1738 cm^{-1}), and ketone (1714 cm^{-1}) groups. The ^{13}C NMR spectrum of **2** at δ 211.1 (C), 176.1 (C), 170.5 (C), and 170.2 (C) (Table 1), also confirmed the presence of one ketone, one γ-lactone, and two esters in the molecule. From the ^{1}H NMR spectrum of **2** (Table 1), two methyl acetates (δ 2.10, 3H, s; 1.99, 3H, s) and one hydroperoxy proton resonating as a broad singlet at δ_H 8.25 were observed.[9] Furthermore, the spectral data (IR, ^{1}H, and ^{13}C NMR) of **2** were found to be similar to those of **1**. By comparison of NMR data of **2** with those of **1** (Table 1), it was found that a C-11/C-12 double bond with 11-hydroxymethyl substituent in **1** was replaced by an exocyclic sp^2 methylene (δ_H 5.39 and 4.64, each 1H, s; δ_C 110.6, CH_2) with a neighboring secondary hydroperoxy methine in **2**. The relative stereochemistry of all stereocenters except C-12 of **2** was confirmed to be the same as that of **1** by comparison of the proton shifts, coupling constants, and NOE correlations. The hydroperoxy group at C-12 was assigned the β-configuration primarily due to the NOE correlation between H-10 and H-12. Thus, the structure of diterpenoid **2** was established.

The molecular formula $C_{26}H_{33}O_{10}Cl$ for **3** was proposed by the LRESIMS pseudomolecular [M+Na]$^+$ ions at *m/z* 563/565 (in a ratio ca. 1:0.3) and was verified by HRESIMS. Its IR spectrum exhibited the broad OH stretch at 3373 cm^{-1}, a γ-lactone carbonyl at 1780 cm^{-1}, and ester carbonyls at 1743 cm^{-1}. In addition, the UV spectrum (MeOH) showed λ_{max} 224 nm (log ε=3.8), indicating the presence of a conjugated enone.[10] The complete assignment of the ^{1}H and ^{13}C NMR spectra for compound **3** was achieved by a combination of DEPT, COSY, HMBC, and HMQC data. Analysis of the ^{1}H NMR spectrum suggested that three of the six methyl groups were due to acetate moieties, which occurred at δ_H 2.32 (s), 2.13 (s), and 2.07 (s). One of the three remaining methyl groups appeared as singlet at δ_H 1.14 (C-15), while two methyls showed doublet at δ_H 1.29 (C-18, *J*=7.5 Hz) and δ_H 1.30 (C-20, *J*=7.0 Hz). The planar structure of **3** was proposed by the assistance of extensive 2D NMR study (Fig. 2). The location of the OH group was confirmed by the HMBC experiment, which showed a correlation between the OH signal at δ_H 3.42 and C-7, C-8, and C-9. The positions of three acetoxy groups at C-2, C-3, and C-9 were also confirmed by the correlations between the methine protons at δ_H 5.11 (H-2), 5.15 (H-3), and 5.09 (H-9) with the ester carbonyls at δ_C 170.0 (C), 169.6 (C), and 168.9 (C), respectively, in the HMBC spectrum of **3**. The relative stereochemistry of **3** was mostly confirmed to be the same as that of **7** by comparison of the proton chemical shifts and coupling constants for Hs-2, -3, -6, -7, -9, -10, -11, -17 with published values.[10] Furthermore, the relative stereochemistry was also confirmed by observed NOESY correlations (Fig. 3). On the basis of above analysis, the structure of **3** was established.

It is noteworthy to mention that metabolite **1** represents the first example of a briarane possessing a 20-hydroxy group, and **2** is the second hydroperoxybriarane ever discovered.[9] Also, **1** and **2** are rarely found 9-ketobriaranes.[11] The cytotoxicity of **1**–**6** toward Hela (human cervical epitheloid carcinoma), Hepa59T/VGH (human liver carcinoma), Med (human medulloblastoma), and KB (human oral epidermoid carcinoma) was assayed. It was found that all of the six metabolites were inactive (ED_{50}'s>20 μg/mL) toward the above cancer cell lines.

3. Experimental

3.1. General experimental procedures

Melting points were determined using a Fisher–Johns melting point apparatus. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a Hitachi I-2001 infrared spectrophotometer. Ultraviolet spectra were recorded on a Hitachi U-3210 UV spectrophotometer. NMR spectra were recorded on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C. LRMS and HRMS spectral data were obtained by ESI on a Bruker APEX II mass spectrometer. Silica gel (Merck, 230–400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC. High-performance liquid chromatography was performed on a Hitachi L-7100 apparatus with the Merck Hibar Si-60 column (250×21 mm, 7 μm).

3.2. Organism

The organism *Briareum* sp. was collected by hand using SCUBA off the coast of Kenting, Taiwan, in October

1991, at depths of 5 m and stored in a freezer until extraction. The *Briareum* sp. is a species with encrusting, purplish, sheet-forming colonies that overgrow and cover substratum, most of which were dead corals. A voucher specimen was deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University (specimen no. KTSC-101).

3.3. Extraction and separation

The marine organism (4.24 kg fresh wt) was collected and freeze-dried. The freeze-dried material (970 g) was minced and extracted exhaustively with EtOAc (8 L×6). The organic extract was evaporated to give a dark-green residue (34.5 g). The EtOAc solution of the residue was stored at 0 °C to give a solid (3.5 g) that was found to be the mixture of long-chained esters formed from saturated fatty acids and alcohols and was discarded. The remaining mixture was concentrated and was triturated with hexane to yield a hexane-soluble fraction (17.4 g), which was found to be the mixture of low polar cembranes, steroids, and fatty acids. The remaining hexane-insoluble but ethyl acetate-soluble fraction (13.2 g) was separated by Si gel column chromatography, using *n*-hexane and *n*-hexane–EtOAc mixtures of increasing polarity to yield 34 subfractions. Fraction 28 eluted with *n*-hexane–EtOAc (3:2 to 2:3) to afford subfractions 28A and 28B. Subfraction 28A was further separated by normal phase HPLC, using CH_2Cl_2–MeOH (100:1) to yield **3** (2.1 mg), **5** (15.5 mg), and **6** (9.5 mg). Subfraction 28B was also purified by normal phase HPLC using CH_2Cl_2–MeOH (50:1) to yield **1** (2.9 mg), **2** (2.7 mg), and **4** (6.8 mg).

3.3.1. Briarenolide A (1). White powder (2.9 mg); mp 139–142 °C; $[\alpha]_D^{28}$ +40.6 (*c* 0.32, $CHCl_3$); IR (neat) ν_{max} 3462, 1770, 1734, 1710, 1635, 1373 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; ^1H NMR (pyridine-d_5, 300 MHz) δ_H: 8.52 (1H, br s, 20-OH), 8.36 (1H, s, 8-OH), 5.93 (1H, d, *J*=9.6 Hz, H-6), 5.81 (1H, s, H-12), 5.41 (1H, d, *J*=9.6 Hz, H-7), 4.17 (2H, m, H-10), 4.06 (1H, q, *J*=7.2 Hz, H-17), 2.08 (3H, s, OAc), 2.04 (3H, s, OAc), 1.83 (3H, s, H-16), 1.45 (3H, d, *J*=7.2 Hz, H-18), 1.21 (3H, s, H-15); ESIMS *m/z* 487 (40, $[M+Na]^+$); HRESIMS *m/z* 487.1942 $[M+Na]^+$ (calcd for $C_{24}H_{32}O_9Na$ 487.1944).

3.3.2. Briarenolide B (2). White powder (2.7 mg); mp 98–100 °C; $[\alpha]_D^{28}$ –10 (*c* 1.1, $CHCl_3$); IR (neat) ν_{max} 3433, 1770, 1738, 1714, 1645, 1375 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; ESIMS *m/z* 503 (100, $[M+Na]^+$); HRESIMS *m/z* 503.1890 $[M+Na]^+$ (calcd for $C_{24}H_{32}O_{10}Na$ 503.1893).

3.3.3. Briarenolide C (3). White powder (2.1 mg); mp 258–260 °C; $[\alpha]_D^{28}$ +42.5 (*c* 0.4, $CHCl_3$); IR (neat) ν_{max} 3373, 1780, 1743, 1653, 1373 cm^{-1}; UV (MeOH) λ_{max} 224 (log ε=3.8); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; ESIMS *m/z* 563 (80, $[M+Na]^+$); HRESIMS *m/z* 563.1657 $[M+Na]^+$ (calcd for $C_{26}H_{33}O_{10}ClNa$ 563.1660).

3.4. Cytotoxicity testing

Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of the test compounds **1**−**6** were performed using MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[12,13]

Acknowledgements

Financial support was provided by Ministry of Education (C030313) and National Science Council of Taiwan (NSC 95-2323-B-110-002) awarded to J.-H. Sheu.

References and notes

1. Sheu, J.-H.; Sung, P.-J.; Huang, L.-H.; Lee, S.-F.; Wu, T.; Chang, B.-Y.; Duh, C.-Y.; Fang, L.-S.; Soong, K.; Lee, T.-J. *J. Nat. Prod.* **1996**, *59*, 935–938.
2. Sung, P.-J.; Hu, W.-P.; Fan, T.-Y.; Wang, J.-J.; Fang, L.-S. *Nat. Prod. Res.* **2005**, *19*, 689–694.
3. Sung, P.-J.; Chao, C.-H.; Chen, Y.-P.; Su, J.-H.; Hu, W.-P.; Sheu, J.-H. *Tetrahedron Lett.* **2006**, *47*, 167–170 and references cited therein.
4. Sung, P.-J.; Chen, Y.-P.; Hwang, T.-L.; Hu, W.-P.; Fang, L.-S.; Wu, Y.-C.; Li, J.-J.; Sheu, J.-H. *Tetrahedron* **2006**, *62*, 5686–5691.
5. Chen, Y.-P.; Wu, S.-L.; Su, J.-H.; Lin, M.-R.; Hu, W.-P.; Hwang, T.-L.; Sheu, J.-H.; Fan, T.-Y.; Fang, L.-S.; Sung, P.-J. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1900–1905.
6. Wu, S.-L.; Sung, P.-J.; Su, J.-H.; Sheu, J.-H. *J. Nat. Prod.* **2003**, *66*, 1252–1256.
7. Kwak, J. H.; Schmitz, F. J.; Williams, G. C. *J. Nat. Prod.* **2001**, *64*, 754–760.
8. Bloor, S. J.; Schmitz, F. J.; Hossain, M. B.; van der Helm, D. *J. Org. Chem.* **1992**, *57*, 1205–1216.
9. Aoki, S.; Okano, M.; Matsui, K.; Itoh, T.; Satari, R.; Akiyama, S.; Kobayashi, M. *Tetrahedron* **2001**, *57*, 8951–8957.
10. Bowden, B. F.; Coll, J. C.; Vasilescu, I. M.; Alderslade, P. N. *Aust. J. Chem.* **1989**, *42*, 1727–1734.
11. Mootoo, B. S.; Ramsewak, R.; Sharma, R.; Tinto, W. F.; Lough, A. J.; McLean, S.; Reynolds, W. F.; Yang, J.-P.; Yu, M. *Tetrahedron* **1996**, *52*, 9953–9962.
12. Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.
13. Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827–4833.