# *Notes*

## (*Z*)-Sarcodictyin A, a New Highly Cytotoxic Diterpenoid from the Soft Coral *Bellonella albiflora*

Yoichi Nakao, Satoru Yoshida, Shigeki Matsunaga, and Nobuhiro Fusetani*

*Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan*

*Received November 25, 2002*

A new cytotoxic diterpene, (*Z*)-sarcodictyin A, was isolated from the Japanese soft coral *Bellonella albiflora*.[1] Its structure was elucidated on the basis of 1D and 2D NMR analysis and chemical degradation. (*Z*)-Sarcodictyin A showed strong cytotoxicity against HeLa human cervix cells.

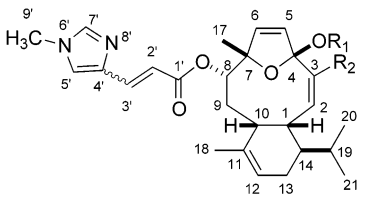
Sarcodictyin A (**2**) was first isolated from the Mediterranean stolonifer *Sarcodictyon roseum*[2] and then from the South African soft coral *Eleutherobia aurea* along with two glycosidated congeners, eleuthosides A and B.[3] Eleutherobin (**3**), a desacetyl congener of the eleuthosides, was isolated as a potent cytotoxic compound from an Australian *Eleutherobia* sp. It was found to stabilize microtubules by competing with the paclitaxel binding site on microtubule polymers.[4] Evaluation of the biological activities of this class of compounds using synthetic compounds[5] led to a common pharmacophore for the microtubule stabilizing activity of the sarcodictyins, eleutherobins, paclitaxel, epothilones, and discodermolide.[6]

In our ongoing program of searching for potential cancer chemotherapeutics from Japanese marine invertebrates, the soft coral *Bellonella albiflora* from southern Japan showed potent cytotoxicity against HeLa human cervix cell lines. Bioassay-guided isolation yielded (*Z*)-sarcodictyin (**1**), along with five known compounds, sarcodictyins A (**2**) and B (**3**), eleutherobin (**4**), (*Z*)-eleutherobin (**5**), and eleutherobin aglycon (**6**). In this paper, the isolation, structure elucidation, and biological activity of this new compound (**1**) are described.

The EtOH extract of the frozen samples (1.5 kg, wet weight) was concentrated and partitioned between $H_2O$ and $Et_2O$, and the aqueous layer was further extracted with *n*-BuOH. The combined $Et_2O$ and *n*-BuOH layers were subjected to the modified Kupchan procedure[7] to yield *n*-hexane, $CHCl_3$, and 60% MeOH layers. The $CHCl_3$ layer showed potent cytotoxicity and was successively separated by ODS flash chromatography, gel filtration, and ODS HPLC to yield (*Z*)-sarcodictyin A (**1**; 1.0 mg, $6.7 \times 10^{-5}$% yield based on wet weight), along with sarcodictyins A (**2**; 14.0 mg, $9.3 \times 10^{-4}$%) and B (**3**; 7.4 mg, $4.9 \times 10^{-4}$%), eleutherobin (**4**; 6.8 mg, $4.5 \times 10^{-4}$%), (*Z*)-eleutherobin (**5**; 0.3 mg, $2.0 \times 10^{-5}$%), and eleutherobin aglycon (**6**; 2.5 mg, $1.7 \times 10^{-4}$%).

The molecular formula of (*Z*)-sarcodictyin A (**1**) was established as $C_{28}H_{36}N_2O_6$ by HRFABMS. The $^1$H NMR spectrum of **1** was almost superimposable on that of sarcodictyin A (**2**), except for the olefinic protons in the urocanyl moiety. These latter protons resonated at $\delta$ 5.75 ($J$ = 12.7 Hz, H2′) and 6.92 ($J$ = 12.7 Hz, H3′), while those of **2** were at $\delta$ 6.33 ($J$ = 15.5 Hz, H2′) and 7.50 ($J$ = 15.5 Hz, H3′). The coupling constant of 12.7 Hz between H2′ and H3′ in **1** indicated its *Z*-geometry. Similar upfield shifts of the comparable olefinic protons were also seen in (*Z*)-eleutherobin.[8]

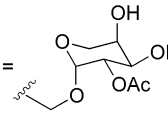
Analysis of the ROESY spectrum established the relative stereochemistry of (*Z*)-sarcodictyin A (**1**). ROESY cross-

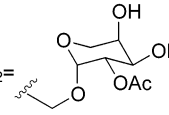


**1**: $R_1$=H, $R_2$=$COOCH_3$, $\Delta^{2'3'}$ (*Z*)

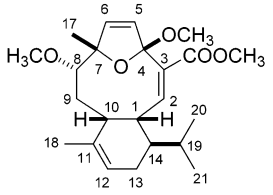
**2**: $R_1$=H, $R_2$=$COOCH_3$, $\Delta^{2'3'}$ (*E*)

**3**: $R_1$=H, $R_2$=$COOC_2H_5$, $\Delta^{2'3'}$ (*E*)

**4**: $R_1$=$CH_3$, $R_2$=  , $\Delta^{2'3'}$ (*E*)

**5**: $R_1$=$CH_3$, $R_2$=  , $\Delta^{2'3'}$ (*Z*)

**6**: $R_1$=$CH_3$, $R_2$=$CH_2OH$, $\Delta^{2'3'}$ (*E*)



**7**

* To whom correspondence should be addressed. Tel: +81-3-5841-5299. Fax: +81-3-5841-8166. E-mail: anobu@mail.ecc.u-tokyo.ac.jp.

10.1021/np0205452 CCC: $25.00 
Published on Web 03/13/2003

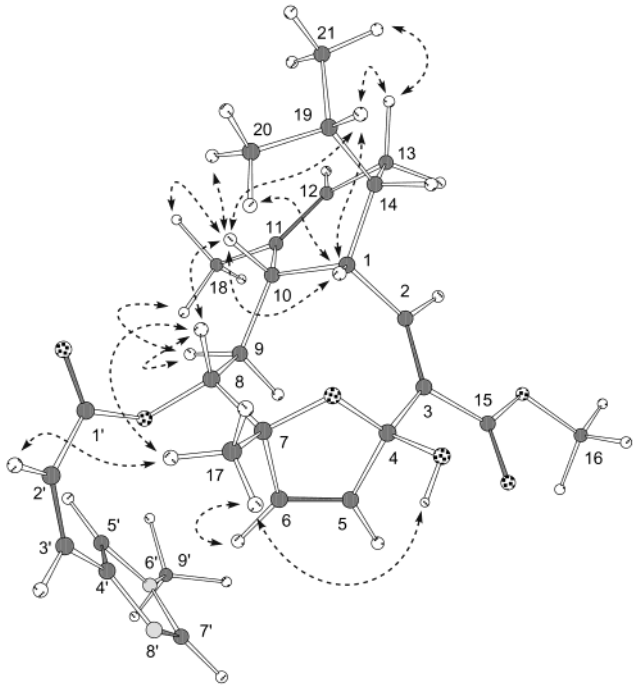



**Figure 1.** Selected NOEs of (*Z*)-sarcodictyin A (**1**).

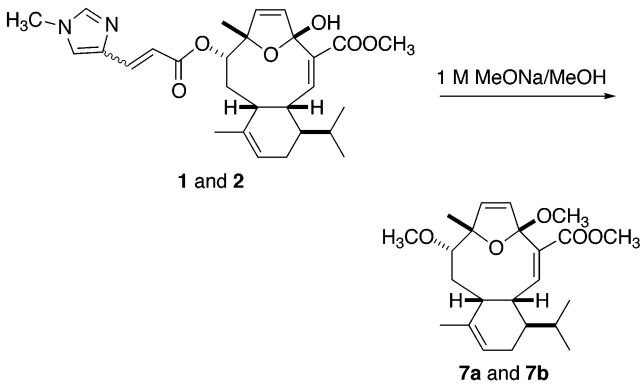



**Figure 2.** Conversion of **1** and **2** to **7a** and **7b**.

peaks between $H_3$-20 ($\delta$ 0.95−0.97)/H-1 ($\delta$ 4.17) and H-10 ($\delta$ 4.17) demonstrated that the isopropyl group, H-1, and H-10 were on the same face of the molecule. A strong ROESY correlation from $CH_3$-17 to 4-OH ($\delta$ 6.58) indicated their *cis*-relationship. ROESY cross-peaks between H-8 ($\delta$ 4.63), H-10 ($\delta$ 4.17), and H-17 ($\delta$ 1.34) correlated both relative stereochemistries above, thus confirming that (*Z*)-sarcodictyin A (**1**) had the same relative stereochemistry of the ring portion as that of sarcodictyin A (**2**).

Optical rotations of (*Z*)-sarcodictyin A (**1**) and sarcodictyin A (**2**) showed the opposite signs ($[\alpha]_D$ +40° for **1** and −15.2° for **2**).[2a] Similar inversions of signs were seen for sarcodictyins C (−16.5°, EtOH, *c* 0.085) and E (+15.6°, MeOH, *c* 0.42),[2b] suggesting the same absolute stereochemistry for **1** and **2**. To confirm the absolute stereochemistry, (*Z*)-sarcodictyin A (**1**) and sarcodictyin A (**2**) were subjected to transesterification with MeONa/MeOH, in which the urocanyl moieties were removed; **1** and **2** were thus converted to **7a** and **7b** (Figure 2). Although the hydroxyl groups of **7a** and **7b** were unexpectedly methylated, FABMS and $^1$H NMR data indicated they had identical planar structures. The CD spectra for **7a** and **7b** both showed negative Cotton effects (231 nm/205 nm), which indicated that they had the same absolute stereochemistry (Figure 3).

(*Z*)-Sarcodictyin A (**1**) was cytotoxic against HeLa cells with an $IC_{50}$ value of 90 ng/mL. The known compounds **2**−**6** showed cytotoxicity against HeLa cells with $IC_{50}$'s of 17−90 ng/mL. (*Z*)-Sarcodictyin A (**1**) showed cytotoxicity against HeLa cells comparable to that of sarcodictyin A (**2**), thereby indicating the geometry of the double bond was not important for activity. This is also the case for eleutherobin (**4**, $IC_{50}$ value 17 ng/mL) and (*Z*)-eleutherobin (**5**, $IC_{50}$ value 17 ng/mL).[8] Altering the C-3 substituent from a methoxycarbonyl group in **2** to an ethoxycarbonyl group in sarcodictyin B (**3**, $IC_{50}$ value 90 ng/mL) has little effect. Eleutherobin aglycon (**6**) is considerably less active than **4** and **5** ($IC_{50}$ values; **6** = 90 ng/mL, **4**/**5** = 17 ng/mL), so the loss of sugar decreases potency in the eleutherobin series.[5c,8]

## Experimental Section

**General Experimental Procedures.** Optical rotation was measured on a JASCO DIP-1000 digital polarimeter. UV spectra were recorded on a Shimadzu BioSpec-1600. CD spectra were recorded in MeOH using a JASCO J-820 spectropolarimeter. NMR spectra were recorded on a JEOL A600 NMR spectrometer operating at 600 MHz for $^1$H and 150 MHz for $^{13}$C. $^1$H and $^{13}$C NMR chemical shifts were referred to DMSO-$d_6$ ($\delta_H$ 2.49 and $\delta_C$ 39.5). FAB mass spectra were measured on a JEOL JMS700 tandem mass spectrometer using NBA with NaCl as a matrix.

**Animal Material.** The animal specimens were collected by hand using scuba off Shishi-jima Island in the Amakusa Islands (32°17′ N; 130°12′ E) in July 1999. They were kept frozen at −20 °C until processed.

**Isolation.** Frozen animals (1.5 kg) were exhaustively extracted with EtOH (3 × 3 L), and the combined extract was concentrated and partitioned between $H_2O$ and $Et_2O$. The aqueous layer was further extracted with *n*-BuOH, which was combined with the $Et_2O$ layer. The combined organic layers were subjected to the modified Kupchan procedure:[7] the layers were first partitioned between *n*-hexane and MeOH/$H_2O$ (90:10), then the MeOH/$H_2O$ (90:10) layer was diluted with $H_2O$ to make MeOH/$H_2O$ (60:40), which was extracted with $CHCl_3$. The $CHCl_3$ layer, showing potent cytotoxicity, was separated by ODS flash chromatography using MeOH/$H_2O$ (5:5 and 7:3), MeCN/$H_2O$ (7:3 and 9:1), MeOH, and $CHCl_3$/MeOH/$H_2O$ (70:30:5). Fractions eluted with MeOH/$H_2O$ (7:3) and MeCN/$H_2O$ (7:3) were combined, concentrated, and gel filtered on a Sephadex LH-20 column (3 × 90 cm) using MeOH. Cytotoxic fractions were collected and separated by reversed-phase HPLC [COSMOSIL 5$C_{18}$ ARII, 20 × 250 mm] using a linear gradient elution from MeOH/$H_2O$ (60:40) to MeOH. The cytotoxic fraction was further purified by reversed-phase HPLC [COSMOSIL 5$C_{18}$ ARII, 10 × 250 mm] using MeCN/$H_2O$ (50:50) to afford pure compounds **1** (1.0 mg), **2** (14.0 mg), **3** (6.8 mg), **4** (0.3 mg), **5** (7.4 mg), and **6** (2.5 mg).

**(*Z*)-Sarcodictyin A (1):** white powder; $[\alpha]^{20}_D$ +40° (*c* 0.1, EtOH); UV (MeOH) $\lambda_{max}$ 208 ($\epsilon$ 16000), 217 ($\epsilon$ 14000), 296 ($\epsilon$ 9900) nm; $^1$H and $^{13}$C NMR, see Table 1; FABMS *m*/*z* 497 [M + H]$^+$, 519 [M + Na]$^+$; HRFABMS *m*/*z* 497.2635 (calcd for $C_{28}H_{37}N_2O_6$ 497.2651).

**Transesterification of 1 and 2.** A portion (0.3 mg) of **1** was dissolved in 0.3 mL of 1 M MeONa/MeOH, and the solution was stirred for 6 h at room temperature. The reaction mixture was applied onto a $SiO_2$ short column (1 × 3 cm) and eluted with $Et_2O$. After evaporating $Et_2O$, the resulting material was dissolved in MeOH and separated by ODS HPLC [COSMOSIL 5$C_{18}$-ARII, 10 × 250 mm] using MeOH as the solvent to give **7a**. Sarcodictyin A was similarly treated to furnish **7b**.

**7a:** colorless solid; CD (MeOH) $\Delta\epsilon$ − (231 nm), + (205 nm); $^1$H NMR ($CD_3OD$, 600 MHz) $\delta$ 4.17 (1H, m, H-1), 6.73 (1H, d, *J* = 9.6, H-2), 6.37 (1H, d, *J* = 5.8, H-5), 6.23 (1H, d, *J* = 5.8, H-6), 3.60 (1H, d, *J* = 7.7, H-8), 1.76 (1H, dd, *J* = 1.9, 14.6, H-9a), 1.24 (1H, m, H-9b), 2.44 (1H, bd, *J* = 12.3, H-10), 5.34 (1H, bs, H-12), 2.38 1H, (bd, *J* = 18.8, H-13a), 2.09 (1H, bd, *J*

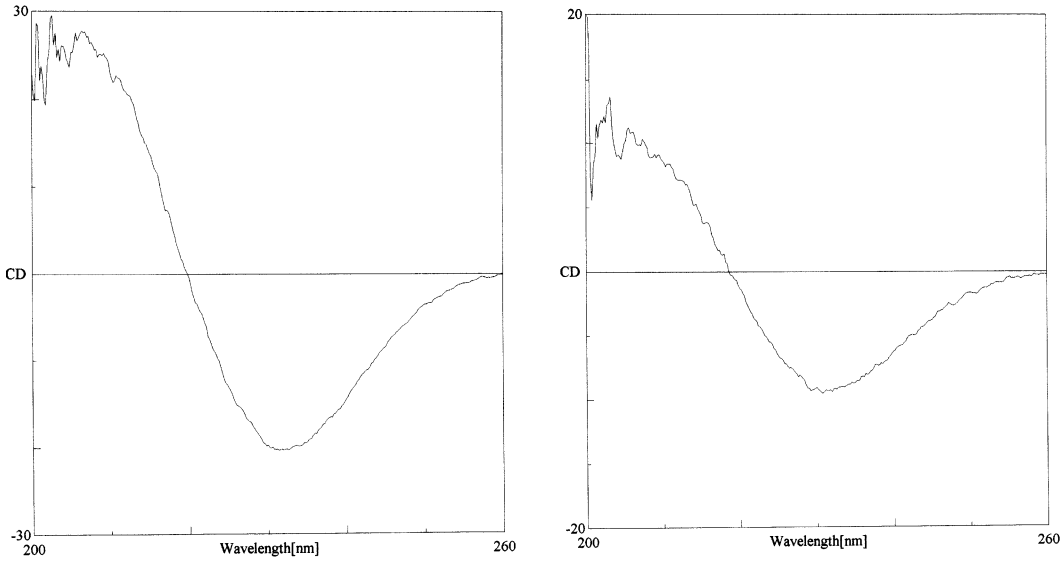



**Figure 3.** CD spectra of **7a** (right) and **7b** (left).

**Table 1.** NMR Spectral Data for (*Z*)-Sarcodictyin A (**1**) in DMSO-$d_6$

| C# | $^{13}$C | $^{1}$H mult., *J* (Hz) | HMBC | ROSEY |
|---|---|---|---|---|
| 1 | 34.0 | 4.17 m | | H-8, H-9b, H-10, H-20, H-21 |
| 2 | 142.6 | 6.44 s | C-4, C-14, C-15 | H-10, H-13a, H-13b, H-14, H-16, H-19 |
| 3 | 134.3 | | | |
| 4 | 114.0 | | | |
| 5 | 133.5 | 6.43 d, 5.4 | C-4, C-6, C-7 | H-6, H9b |
| 6 | 132.3 | 6.09 d, 5.4 | C-5, C-6, C-7 | H-5, H-9b, H-17 |
| 7 | 88.5 | | | |
| 8 | 80.4 | 4.63 d, 7.3 | C-5, C-7, C-9, C-10, C-17 | H-1, H-9b, H-10, H-20, H-21 |
| 9 | 31.1 | 1.57 d, 3.5 | C-7, C-8, C-10, C-11 | H-14 |
| | | 1.25 m | C-7, C-8, C-10 | H-1, H-5, H-6, H-8 |
| 10 | 38.5 | 2.55 m | | H-1, H-2, H-8, H-18, H-20, H-21 |
| 11 | 133.3 | | | |
| 12 | 121.2 | 5.31 m | | H-13a, H-13b, H-14, H-18 |
| 13 | 23.8 | 2.30 m | | H-2, H-12, H-14 |
| | | 2.04 m | | H-2, H-12, H-20, H-21 |
| 14 | 41.5 | 1.22 m | | H-2, H-12, H-13a |
| 15 | 166.7 | | | |
| 16 | 52.7 | 3.63 s | C-15 | H-2 |
| 17 | 25.3 | 1.34 s | C-6, C-7, C-8 | H-6, H-8 |
| 18 | 20.3 | 1.47 s | C-10, C-11, C-12 | H-10, H-12, OH |
| 19 | 28.5 | 1.25 m | | H-2 |
| 20 | 21.9 | 0.97 d, 6.9 | C-14, C-19, C-21 | H-1, H-8, H-10, H-13a |
| 21 | 21.8 | 0.95 d, 6.5 | C-14, C-19, C-20 | H-1, H-8, H-10, H-13a |
| 1′ | 165.4 | | | |
| 2′ | 112.3 | 5.75 d, 12.7 | C-4′ | H-3′ |
| 3′ | 138.9 | 6.92 d, 12.7 | C-1′, C-5′ | H-2′ |
| 4′ | 136.1 | | | |
| 5′ | 125.9 | 8.22 s | C-4′ | H-9′ |
| 7′ | 138.4 | 7.65 s | C-4′ | H-9′ |
| 9′ | 33.5 | 3.70 s | C-5′, C-7′ | H-5′, H-7′ |
| OH | | 6.58 s | C-3, C-4 | H-17 |

= 20.0, H-13b), 1.32 (1H, m, H-14), 3.70 (3H, s, H-16), 1.51 (3H, s, H-17), 1.60 (3H, s, H-18), 1.56 (1H, d sept, *J* = 10.0, 6.5, H-19), 0.97 (3H, d, *J* = 6.5, H-20), 0.95 (3H, d, *J* = 6.9, H-21), 3.19 (3H, s, OMe-4 or -8); ($CDCl_3$, 600 MHz) $\delta$ 4.13 (1H, ddd, *J* = 2.7, 3.8, 9.2, H-1), 6.70 (1H, d, *J* = 10.0, H-2), 6.46 (1H, d, *J* = 5.8, H-5), 6.20 (1H, d, *J* = 5.8, H-6), 3.70 (1H, d, *J* = 8.5, H-8), 1.70 (1H, dd, *J* = 2.3, 15.0, H-9a), 1.3 (1H, m, H-9b), 2.43 (1H, bd, *J* = 12.7, H-10), 5.32 (1H, bs, H-12), 2.40 (1H, bd, *J* = 21.2, H-13a), 2.03 (1H, bd, *J* = 20.0, H-13b), 1.40 (1H, m, H-14), 3.71 (3H, s, H-16), 1.56 (3H, s, H-17), 1.58 (3H, s, H-18), 1.53 (1H, d sept, *J* = 9.5, 6.7, H-19), 0.932 (3H, d, *J* = 6.5, H-20), 0.927 (3H, d, *J* = 6.5, H-21), 3.48 (3H, s, OMe-4 or -8), 3.23 (3H, s, OMe-4 or -8); FABMS *m*/*z* 391 $[M + H]^+$, 413 $[M + Na]^+$.

**7b:** colorless solid; CD (MeOH) $\Delta\epsilon$ − (231 nm), + (205 nm); $^{1}$H NMR ($CD_3OD$, 600 MHz) $\delta$ 4.17 (1H, m, H-1), 6.73 (1H, d, *J* = 10.0, H-2), 6.37 (1H, d, *J* = 6.2, H-5), 6.23 (1H, d, *J* = 6.2, H-6), 3.59 (1H, d, *J* = 7.7, H-8), 1.76 (1H, dd, *J* = 1.9, 14.6, H-9a), 1.24 (1H, m, H-9b), 2.44 (1H, bd, *J* = 12.3, H-10), 5.34 (1H, bs, H-12), 2.38 (1H, bd, *J* = 19.2, H-13a), 2.09 (1H, bd, *J* = 19.6, H-13b), 1.31 (1H, m, H-14), 3.69 (3H, s, H-16), 1.53 (3H, s, H-17), 1.60 (3H, s, H-18), 1.56 (1H, d sept, *J* = 10.0, 6.5, H-19), 0.97 (3H, d, *J* = 6.9, H-20), 0.95 (3H, d, *J* = 6.9, H-21), 3.19 (3H, s, OMe-4 or -8); ($CDCl_3$, 600 MHz) $\delta$ 4.13 (1H, ddd, *J* = 3.5, 4.2, 10.4, H-1), 6.70 (1H, d, *J* = 10.0, H-2), 6.46 (1H, d, *J* = 5.8, H-5), 6.20 (1H, d, *J* = 5.8, H-6), 3.70 (1H, d, *J* = 8.5, H-8), 1.70 (1H, dd, *J* = 1.6, 14.9, H-9a), 1.3 (1H, m, H-9b), 2.43 (1H, bd, *J* = 12.7, H-10), 5.32 (1H, bs, H-12), 2.38 (1H, bd, *J* = 21.5, H-13a), 2.03 (1H, bd, *J* = 20.4, H-13b), 1.40 (1H, m, H-14), 3.71 (3H, s, H-16), 1.56 (3H, s, H-17), 1.58 (3H, s, H-18), 1.53 (1H, d sept, *J* = 10.0, 6.2, H-19), 0.931 (3H, d, *J* = 6.2, H-20), 0.926 (3H, d, *J* = 6.5, H-21), 3.48 (3H, s, OMe-4 or -8), 3.23 (3H, s, OMe-4 or -8); FABMS *m*/*z* 391 $[M + H]^+$, 413 $[M + Na]^+$.

**Acknowledgment.** This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, and Science and Japan Society for the Promotion of Science. Thanks are also due to the crew of R/V Toyoshio-maru of Hiroshima University for assistance in collection of the samples.

## References and Notes

(1) Bioactive Marine Metabolites 116. Part 115: Phuwapraisirisan, P.; Matsunaga, S.; Fusetani, N.; Chaitanawisuti, N.; Kritsanapuntu, S.; Menasveta, P. *J. Nat. Prod.* **2003**, *66*, 289−291.

(2) (a) D'Ambrosio, M.; Guerriero, A.; Pietra, F. *Helv. Chim. Acta* **1987**, *70*, 2019−2027. (b) D'Ambrosio, M.; Guerriero, A.; Pietra, F. *Helv. Chim. Acta* **1988**, *71*, 964−976.

(3) Ketzinel, S.; Rudi, A.; Schleyer, M.; Benayahu, Y.; Kashman, Y. *J. Nat. Prod.* **1996**, *59*, 873−875.

(4) Lindel, T.; Jensen, P. R.; Fenical, W.; Long, B. R.; Casazza, A. M.; Carboni, J.; Fairchild, C. R. *J. Am. Chem. Soc.* **1997**, *119*, 8744−8745.

(5) (a) Nicolaou, K. C.; Xu, J. Y.; Kim, S.; Ohshima, T.; Hosokawa, S.; Pfefferkorn, J. *J. Am. Chem. Soc.* **1997**, *119*, 11353−11354. (b) Nicolaou, K. C.; van Delft, F.; Oshima, T.; Vourloumis, D.; Xu, J.-Y.; Hosokawa, S.; Pfefferkorn, J.; Kim, S.; Li, T. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2520−2524. (c) Nicolaou, K. C.; Kim, S.; Pfefferkorn, J.; Xu, J.; Oshima, T.; Hosokawa, S.; Vourloumis, D.; Li, T. *Angew. Chem., Int. Ed.* **1998**, *37*, 1418−1421. (d) Hamel, E.; Sackett, D. L.; Vourloumis, D.; Nicolaou, K. C. *Biochemistry* **1999**, *38*, 5490−5498.

(6) (a) Ojima, I.; Chakravarty, S.; Inoue, T.; Lin, S.; He, L.; Horwitz, S. B.; Kuduk, S. D.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4256−4261. (b) Giannakakou, P.; Gussio, R.; Nogales, E.; Downing, K. H.; Zaharevitz, D.; Bollbuck, B.; Poy, G.; Sackett, D.; Nicolaou, K. C.; Fojo, T. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2904−2909.

(7) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178−179.

(8) Cinel, B.; Roberge, M.; Behrisch, H.; van Ofwegen, L.; Castro, C. B.; Andersen, R. J. *Org. Lett.* **2000**, *2*, 257−260.

NP0205452