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Brevianamide J, A New Indole Alkaloid Dimer from Fungus *Aspergillus versicolor*

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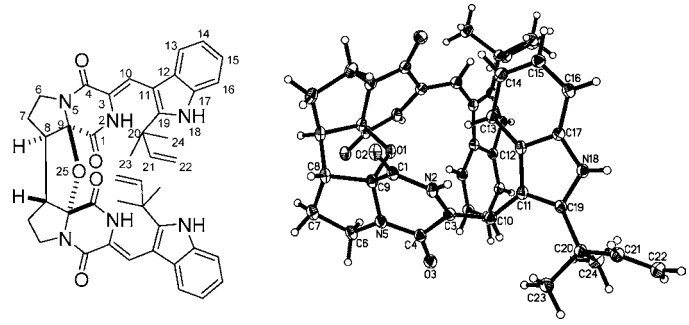
ABSTRACT



Brevianamide J (1), a new indole alkaloid dimer, was isolated together with four new diketopiperazine alkaloids (brevianamide K−N, 2−5) from the solid-state fermented culture of *Aspergillus versicolor*. Their structures were elucidated on the basis of spectral data. X-ray crystallographic analysis confirmed the structures of 1 and 4.

Diketopiperazine alkaloids, a class of important secondary metabolites, are widely found in fungi such as *Aspergillus*,[1] *Penicillium*,[2] *Pestalotiopsis*,[3] and *Chromocleista*.[4] This class of alkaloids are derived from different amino acids and one or more isoprene units. Most of them are characteristic of diverse ring systems and possess diverse biological activities,[5] which attracted much attention of synthetic chemists.[6] Species of *Aspergillus* are important medically and commercially. Members of the genus are sources of natural products that can be potentially used to treat human diseases.[7] In the course to investigate the alkaloids from the fungus *Aspergillus versicolor*, a new alkaloid dimer (**1**), together

(1) (a) Kato, H.; Yoshida, T.; Tokue, T.; Nojiri, Y.; Hirota, H.; Ohta, T.; Williams, R. M.; Tsukamoto, S. *Angew. Chem., Int. Ed.* **2007**, *46*, 2254. (b) Tsukamoto, S.; Kato, H.; Greshock, T. J.; Hirota, H.; Ohta, T.; Williams, R. M. *J. Am. Chem. Soc.* **2009**, *131*, 3834. (c) Wang, F. Z.; Fang, Y. C.; Zhu, T. J.; Zhang, M.; Lin, A. Q.; Gu, Q. Q.; Zhu, W. M *Tetrahedron* **2008**, *64*, 7986.

(2) (a) Capon, R. J.; Stewart, M.; Ratnayake, R.; Lacey, E.; Gill, J. H. *J. Nat. Prod.* **2007**, *70*, 1746. (b) Ding, Y. S.; Gruschow, S.; Greshock, T. J.; Finefield, J. M.; Sherman, D. H.; Williams, R. M. *J. Nat. Prod.* **2008**, *71*, 1574. (c) Kozlovsky, A. G.; Vinokurova, N. G.; Adanin, V. M.; Burkhardt, G.; Dahse, H.-M.; Grfe, U. *J. Nat. Prod.* **2000**, *63*, 698.

(3) Ding, G.; Jiang, L. H.; Guo, L. D.; Chen, X. L.; Zhang, H.; Che, Y. S. *J. Nat. Prod.* **2008**, *71*, 1861.

(4) Park, Y. C.; Gunasekera, S. P.; Lopez, J. V.; McCarthy, P. J.; Wright, A. E. *J. Nat. Prod.* **2006**, *69*, 580.

(5) (a) Shinohara, C.; Hasumi, K.; Takei, Y.; Endo, A. *J. Antibiot.* **1994**, *47*, 163. (b) Nuber, B.; Hansske, F.; Shinohara, C.; Miura, S.; Hasumi, K.; Endo, A. *J. Antibiot.* **1994**, *47*, 168. (c) Fukuyama, T. F.; Chen, X.; Peng, G. *J. Am. Chem. Soc.* **1994**, *116*, 3127. (d) Schkeryantz, J. M.; Woo, J. C. G.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1995**, *117*, 7025. (e) Wulff, J. E.; Herzon, S. B.; Siegrist, R.; Myers, A. G. *J. Am. Chem. Soc.* **2007**, *129*, 4898.

(6) (a) Depew, K. M.; Danishefsky, S. J.; Rosen, N.; Sepp-Lorenzino, L. *J. Am. Chem. Soc.* **1996**, *118*, 12463. (b) Schkeryantz, J. M.; Woo, J. C. G.; Siliphaivanh, P.; Depew, K. M.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 11964. (c) Stocking, E. M.; Williams, Robert, M.; Sanz-Cervera, J. F *J. Am. Chem. Soc.* **2000**, *122*, 9089. (d) Herzon, S. B.; Myers, A. G. *J. Am. Chem. Soc.* **2005**, *127*, 5342. (e) Artman, G. D.; Grubbs, A. W.; Williams, R. M. *J. Am. Chem. Soc.* **2007**, *129*, 6336.

(7) Fenical, W.; Jensen, P. R.; Cheng, X. C. Avrainvillamide, a Cytotoxic Marine Natural Product, and Derivatives there of U.S. Patent 6066635, 2000.

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with four new diketopiperazine alkaloids (**2**−**5**), was isolated from the solid-state fermented culture of *Aspergillus versicolor*. Compound **1** was a new alkaloid dimer. It may be derived from the corresponding monomer (**2**). Compound **1** represented a unique structure of indole alkaloid. Compound **3** is the first type of oxepin-containing alkaloid with phenylalanine residue. Here, the isolation, structure elucidation, and biological activities of compounds **1**−**5** are described (Figure 1).

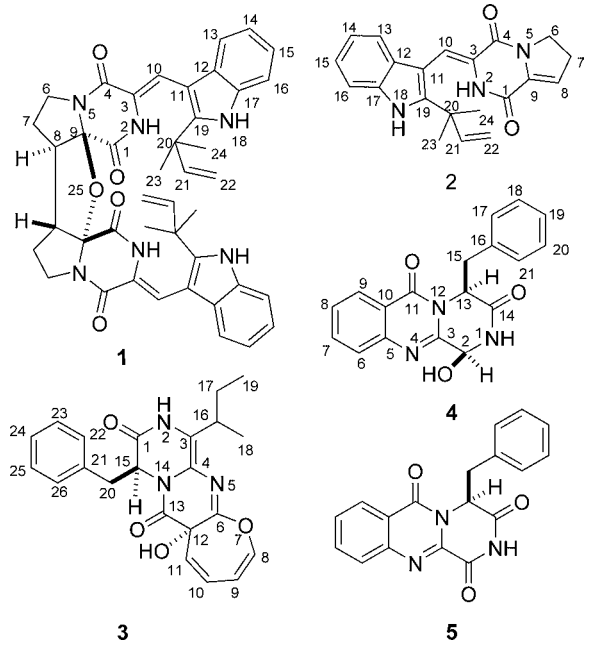

Figure 1. Structures of compounds **1**−**5**.

Brevianamide J (**1**) was obtained as colorless cubic crystals.[8] The UV spectrum with λ_{max} in methanol at 201 (4.44), 223 (4.49), 261 (4.18), and 349 (4.04) nm was indicative of indole functionality with an extended conjugation.[9] High-resolution ESIMS analysis of **1** suggested a molecular formula of $C_{42}H_{42}N_6O_5$. The NMR spectra of **1** revealed 21 protons and 21 C-atoms, suggesting **1** to be a symmetric, homodimer (Table 1). The IR absorption bands at 1633, 1695, 3353, and 3423 cm^{-1} are characteristic of amides or lactams. The ^{13}C NMR signals at δ 163.4 (C-1) and 161.4 (C-4) confirmed the presence of lactam carbonyls. The ^1H NMR signals at δ 4.96 (1H, d, J = 10.7 Hz, H-22), 4.97 (1H, d, J = 17.4 Hz, H-22) and 5.98 (1H, dd, J = 17.4, 10.7 Hz), and the HMBC correlations of methyls at δ 1.39 and 1.40 (each 3H, s, H-23 and H-24) with the C-atoms at δ 39.3 (C-20), 105.3 (C-19) and 145.2 (C-21) suggested the moiety of $-C(CH_3)_2CH{=}CH_2$ at C-19 (Table 1). A dehydrotryptophan moiety could be concluded from the ^1H NMR signals at δ 8.02 and 7.03 (each d, J = 8.0 Hz, H-13, H-16), 7.52 and 6.96 (each t, J = 8.0 Hz, H-14, H-15), and the key HMBC correlations of H-18 with C-11, C-12 and C-19, and H-10 with C-3, C-4, C-11 and C-12. Besides the signals for five C-atoms of isoprene unit and eleven C-atoms of dehydrotrytophan moiety, there were five ^{13}C NMR signals left for a proline moiety. The α-C (C-9) of proline residue resonated at δ 102.2, suggesting that C-9 was oxygenated. The β-C (C-8) presented to be a methylidyne, indicative of a substitute at C-8. Therefore, it could be supposed that two monomers were connected at C-8 and C-9. The structure of compound **1** can not be determined only with NMR data. The structure of **1** was finally determined to be a 2-fold dimer as opposed to the mirror dimer on the basis of X-ray single crystallographic analysis (Figure 2).

Table 1. NMR Data of **1** and **2** (^1H: 600 MHz; ^{13}C: 150 MHz)[a,b,c]

	1		**2**	
no.	δ_H(mult., J Hz)	δ_C	δ_H(mult., J Hz)	δ_C
1		163.4		154.4
2	11.29 (s)		8.77 (1H, s)	
3		127.1		126.3
4		161.4		155.1
6	4.17 (dd, 9.0, 8.8) 4.02 (t, 9.0)	45.0	4.00 (2H, t, 8.9)	46.0
7	2.29 (1H, m) 2.15 (1H, m)	29.3	2.75 (2H, td, 8.9, 2.9)	28.1
8	3.65 (1H, d, 10.2)	52.6	6.09 (1H, d, 2.9)	119.2
9		102.2		134.2
10	7.80 (1H, s)	115.0	6.89 (1H, s)	110.3
11		105.3		103.7
12		127.5		126.4
13	8.02 (1H, d, 8.0)	121.0	7.41 (1H, d, 7.9)	119.2
14	7.52 (1H, t, 8.0)	120.4	7.00 (1H, t, 7.9)	119.4
15	6.96 (1H, t, 8.0)	122.9	7.06 (1H, t, 7.9)	121.3
16	7.03 (1H, d, 8.0)	111.0	7.17 (1H, d, 7.9)	112.1
17		135.8		135.6
18	11.44 (s)		11.06 (1H, s)	
19		144.4		144.6
20			39.3	39.5
21	5.98 (1H, dd, 17.4, 10.7)	145.2	6.05 (1H, dd, 15.8, 9.1)	145.6
22	4.97 (1H, d, 17.4) 4.96 (1H, d, 10.7)	111.7	5.01 (1H, d, 15.8) 5.03 (1H, d, 9.1)	112.1
23	1.39 (3H, s)	27.5	1.45 (3H, s)	27.9
24	1.40 (3H, s)	27.8	1.45 (3H, s)	27.9

[a] Assignments were based on HSQC and HMBC experiments. [b] Only half of the NMR signal data of **1** was presented here. [c] The NMR spectra **1** and **2** were recorded in C_5D_5N and in DMSO-d_6, respectively.

Brevianamide K (**2**) was isolated as yellow needle crystals. The molecular formula $C_{21}H_{23}N_3O_2$ was inferred from the quasi-molecular ion peak at m/z 372.1679 $[M + Na]^+$ in the HRESIMS spectrum. The IR, UV, and NMR spectra were very similar to those of compound **1**. Signals for 21 proton and 21 C-atoms were observed in the NMR spectra,[10] indicating that compound **2** could be the monomer of **1**. Comparison of the ^{13}C NMR spectra of compounds **1** and **2**, it was found that two C-atoms in **2** resonanted at δ 119.2

(8) Compound **1**: colorless cubic crystals; mp 226−227 °C; $[\alpha]^{20}_D$ +45.0° (*c* 0.10, acetone); UV (MeOH). λ_{max} (log ε). 201(4.44), 223 (4.49), 261 (4.18), 349 (4.04). nm; IR(KBr). υ_{max}: 3423, 3353, 2969, 2930, 1695, 1633, 1576, 1455, 1385, 749 cm^{-1}; 1H and 13C NMR data, see Table 1; (+)-HRESIMS m/z 733.3115 $[M + Na]^+$ (calcd for $C_{42}H_{42}N_6O_5Na$, 733.3109).

(9) Dillman, R. L.; Cardellina, J. H., II *J. Nat. Prod.* **1991**, *54*, 1056.

(10) Compound **2**: yellow needles; mp 157−158 °C; UV (MeOH). λ_{max} (log ϵ). 201 (4.36), 225 (4.38), 283 (4.24), 367 (4.14). nm; IR(KBr). υ_{max}: 3427, 3367, 2968, 1673, 1638, 1617, 1424, 740 cm^{-1}; 1H and 13C NMR data, see Table 1; (+)-HRESIMS (positive mode). m/z 370.1516 $[M + Na]^+$ (calcd for $C_{21}H_{21}N_3O_2Na$, 370.1226).

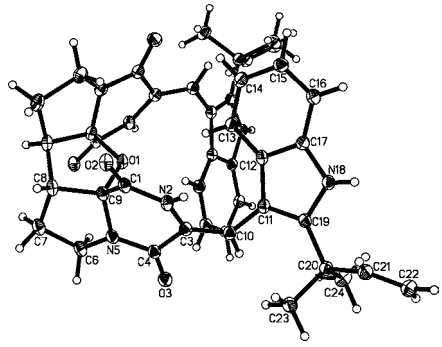

Figure 2. ORTEP diagram of compound **1**.

(C-8) and 134.2 (C-9) instead at δ 52.6 (C-8) and 102.2 (C-9) as those in **1**. Thus, the presence of a bouble bond at C-8 and C-9 in **2** could be resumed. The above postulation was confirmed by the HMBC correlations of H-8 with C-1 and C-9, and H-6 and H-7 with C-9. The structure of compound **2** was determined by HSQC and HMBC experiments.

Brevianamide L (**3**) was obtained as colorless cubic crystals with a molecular formula $C_{22}H_{23}N_3O_4$ from the quasi-molecular ion peak at *m/z* 416.1576 $[M + Na]^+$ in the HRESIMS. The IR peak at ν_{max} 3420 cm^{-1} suggested the presence of hydroxyl group. The presence of amides could be concluded from the IR peaks at ν_{max} 3261, 1684, and 1667 cm^{-1}, and the ^{13}C NMR signals at δ 166.1 and 163.2. The ^{13}C NMR spectrum of **3** showed 22 signals.[11] An oxepin moiety could be concluded from ^1H NMR signals of H-8, H-9, H-10, and H-11, and the HMBC correlations of H-8/C-6, H-10/C-12, and H-11/C-6, C-12, and C-13. Meanwhile, the connections among C-3, C-4, C-16, C-17 and C-19 could be deduced from the coupling system of H-18/H-16/H-17/H-19, and the HMBC correlations of H-17 and H-18 with C-3 (δ 120.7), and H-16 with C-3 and C-4 (δ 117.0). The above information revealed that compound **3** was oxepin-containing compound. Detailed comparison of the NMR data of **3** with those of the A-C rings of oxepinamide A and cinereain supported this conclusion.[12] Compound **3** was hydrolyzed in 6 N HCl (aq.) for 12 h at 100 °C to afford L-phenylalanine, $[\alpha]^{20}_D$-34.0 (*c* 0.1, H_2O), which was determined by comparing with an authentic sample. A benzyl group was located at C-15 in view of the HMBC correlations of H-20 with C-1, C-15, and C-21. A double bond between C-3 (δ 120.7) and C-4 was determined from the HMBC correlations of H-17 and H-18/C-3, and H-16/C-3 and C-4 (δ 117.0). The ^{13}C NMR signal at δ 70.4 could be assigned to C-12 from the HMBC correltions of H-10 and H-11 with C-12. A double bond between N-5 and C-6 was suggested by the HMBC correlations of H-8 and H-11 with C-6 (δ 153.4). The structure of compound **3** was elucidated by the analysis of HSQC and HMBC spectra (Figure 3).

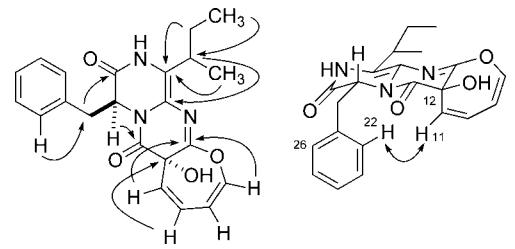

Figure 3. Key HMBC and NOESY correlations of **3**.

The NOESY correlation between H-22/H-26 (δ 7.07, 2H, m) and H-11 (δ 5.83, 1H, d, J = 10.3 Hz) suggested that relative orientation of H-11 and H-22 or H-26. L-Phenylalanine was obtained from the hydrolysis of compound **3**. Thus, the absolute configurations of C-12 and C-15 were determined respectively as *S* and *R* (Figure 3). It was unsuccessful to obtain single crystal of compound **3**. The stereochemistry at C-16 was not determined so far.

The molecular formula $C_{18}H_{15}N_3O_3$ of brevianamide M (**4**) was provided by the quasi-molecular ion peak at *m/z* 344.1014 $[M + Na]^+$ in the HRESIMS.[13] Its IR spectrum showed the presence of hydroxyl group (υ_{max} 3421 cm^{-1}). The ^{13}C NMR signal at δ 169.6 and 160.4, and the IR peaks at υ_{max} 3362, 1694, and 1674 cm^{-1} suggested the presence of amide carbonyls. A phenylalanine residue could be concluded from the ^1H NMR signals at δ 7.61 (2H, d, J = 7.4 Hz), 7.26 (2H, t, J = 7.4 Hz), 7.20 (1H, t, J = 7.4 Hz), 5.93 (1H, dd, J = 9.1, 6.2 Hz), 3.83 (1H, dd, J = 13.3, 6.2 Hz) and 4.09 (1H, dd, J = 13.3, 9.1 Hz), and the HMBC correlation of H-15/C-14 (169.6), C-16 (137.6), C-17 (130.1), and C-21 (130.1). Another ortho-substituted phenyl ring was recognized from the ^1H NMR signals at δ 8.40 and 7.88 (each 1H, d, J = 8.1 Hz), and 7.75 and 7.45 (each 1H, t, J = 8.1 Hz). The connection of C-10/C-11/N-12/C-13 was deduced from the HMBC correlation of H-9 and H-13/C-11. The structure of compound **4** was finally confirmed by X-ray crystallographic analysis (Figure 4). Compound **4** was hydrolyzed in 6 N HCl (aq.) for 12 h at 100 °C to afford L-phenylalanine. Therefore, the absolute configuration was determined as 2*S* and 13*S*.

(11) Compound **3**: colorless cubic crystals; mp 182−183 °C; $[\alpha]^{20}_D$ +190.0° (*c* 0.10, acetone); UV (MeOH). λ_{max} (log ε). 201(4.03), 223 (4.12), 261 (3.15), 349 (3.76). nm; IR(KBr). υ_{max}: 3420, 3261, 2960, 1684, 1667, 1642, 1598, 1390, 1290, 700 cm^{-1}; 1H NMR (600 MHz, $CDCl_3$). δ 8.40 (1H, s, H-2), 7.20 (3H, m, H-23, 24 and 25), 7.07 (2H, m, H-22 and 26), 6.61 (1H, d, 7.3, H-8), 6.19 (1H, dd, 10.3, 7.3, H-10), 5.83 (1H, d, 10.3, H-11), 5.52 (1H, t, 7.3, H-9), 5.30 (1H, t, 5.3, H-15), 3.21(1H, dd, 13.8, 5.3, H-20), 3.09, (1H, dd, 13.8, 5.3, H-20), 3.03 (1H, m, H-16), 1.42 (1H, m, H-17), 1.51 (1H, m, H-17), 0.77 (3H, t, 7.2, H-19), 0.75 (3H, d, 7.2, H-18);13C NMR (150 MHz, $CDCl_3$). δ 165.8 (C-13), 164.7 (C-1), 153.4 (C-6), 144.3 (C-8), 135.4 (C-21), 129.9 (C-22 and 26), 128.9 (C-10), 128.2 (C-23 and 25), 126.9 (C-24), 120.7 (C-3), 117.0 (C-4), 105.2 (C-9), 132.0 (C-11), 70.2 (C-12), 56.3 (C-15), 36.9 (C-20), 31.9 (C-16), 26.3 (C-17), 16.8(C-18), 11.1 (C-19); (+)-HRESIMS *m/z* 416.1576 $[M + Na]^+$ (calcd for $C_{22}H_{23}N_3O_4Na$, 416.1581).

(12) (a) Belofsky, G. N.; Anguera, Jensen, M. P. R.; Fenical, W.; Köck, M. *Chem.—Eur. J.* **2000**, *6*, 1355. (b) Cutler, H. G.; Springer, J. P.; Arrendale, R. F.; Arison, B. H.; Cole, P. D.; Roberts, R. G. *Agric. Biol. Chem.* **1988**, *52*, 1725.

(13) Compound **4**: colorless cubic crystals; mp 206−207 °C; $[\alpha]^{20}_D$ −147.7° (*c* 0.13, acetone); UV (MeOH). λ_{max} (log ϵ). 208 (4.54), 222 (4.55), 269 (4.02), 304 (3.64). nm; IR(KBr). υ_{max}: 3325, 3082, 2970, 1694, 1622, 1646, 1599, 1436, 1385, 918 cm^{-1}; 1H and 13C NMR data, see table 2 (+)-HRESIMS *m/z* 344.1014 $[M + Na]^+$ (calcd for $C_{18}H_{15}N_3O_3Na$, 344.1006).

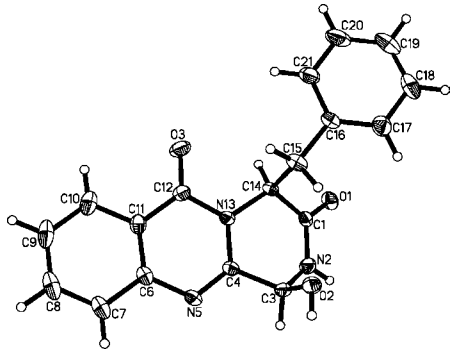

Figure 4. ORTEP diagram of compound **4**.

The molecular formula of brevianamide N (**5**) was established as $C_{18}H_{15}N_3O_3$ from the quasi-molecular ion peak at *m*/*z* 342.0853 $[M + Na]^+$ in the HRESIMS, one more unsaturated degree than **4**.[14] The NMR spectra and UV absorptions at λ_{max} at 221 (4.33), and 307.6 (3.89) nm of compound **5** were close to those of **4**. However, a ketonic C-atom at δ 155.3 (C-2) presented in compound **5** rather than an acetal C-atom as in **4** (Table 2). The structure of compound **5** was finally elucidated by comparing the NMR data with those of **4** and by HSQC and HMBC experiments. The hydrolysis of compound **5** in 6 N HCl (aq.) yielded L-phenylalanine, indicating that the absolute stereochemistry of C-13 was *S*. The moiety of anthranilic acid in compounds **4** and **5** was present in some other diketopiperazines.[15]

(14) Compound **5**: colorless needles; 239−240 °C; $[\alpha]^{20}_D$ −359.3° (*c* 0.14, acetone); UV (MeOH). λ_{max} (log ε). 221 (4.33), 307.6 (3.89). nm; IR(KBr). υ_{max}: 3420, 2922, 2854, 1739, 1710, 1690, 1595, 1467, 1326, 779 cm^{-1}; 1H and 13C NMR data, see table 3; (+)-HRESIMS *m*/*z* 342.0853 $[M + Na]^+$ (calcd for $C_{18}H_{13}N_3O_3Na$, 342.0849).

(15) (a) Penn, J.; Mantle, P. G.; Bilton, J. N.; Sheppard, R. N. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1495. (b) Fujimoto, H.; Negishi, E.; Yamaguchi, K.; Nishi, N.; Yamazaki, M. *Chem. Pharm. Bull.* **1996**, *44*, 1843. (c) Chou, T.-C.; Depew, K. M.; Zheng, Y.-H.; Safer, M. L.; Chan, D.; Helfrich, B.; Zatorska, D.; Zatorski, A.; Bornmann, W.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 8369.

Table 2. NMR data of **4** and **5** (^{1}H: 600 MHz; ^{13}C: 150 MHz)[a,b]

	4		**5**	
no.	δ_H (m, J = Hz)	δ_C	δ_H (m, J = Hz)	δ_C
1	10.70 (1H, d, 4.9)		8.54 (1H, brs)	
2	6.40 (1H, d, 4.9)	76.8		155.3
3		151.2		138.9
5		147.8		146.0
6	7.88 (1H, d, 8.1)	127.6	7.99 (1H, d, 8.1)	129.8
7	7.75 (1H, t, 8.1)	134.5	7.91 (1H, t, 8.1)	135.6
8	7.45 (1H, t, 8.1)	127.2	7.72 (1H, t, 8.1)	130.0
9	8.40 (1H, d, 8.1)	126.8	8.40 (1H, d, 8.1)	127.1
10		121.2		121.5
11		160.4		159.7
13	5.93 (1H, dd, 9.1, 6.2)	58.0	5.91 (1H, dd, 5.5, 3.0)	58.0
14		169.6		166.8
15	3.83 (1H, dd, 13.3, 6.2)	40.6	3.46 (1H, dd, 14.1, 5.5)	38.4
	4.09 (1H, dd, 13.3, 9.1)		3.59 (1H, dd, 14.1, 3.0)	
16		137.6		132.4
17	7.61(1H, d, 7.4)	130.1	6.76 (1H, d, 7.5)	129.4
18	7.26 (1H, t, 7.4)	128.4	7.15 (1H, t, 7.5)	129.2
19	7.20 (1H, t, 7.4)	126.8	7.24 (1H, t, 7.5)	127.1
20	7.26 (1H, t, 7.4)	128.4	7.15 (1H, t, 7.5)	129.2
21	7.61(1H, d, 7.4)	130.1	6.76 (1H, d, 7.5)	129.4

[a] Assignments were based on HSQC and HMBC experiments. [b] The NMR spectra of **4** and **5** were recorded in C_5D_5N, $CDCl_3$, respectively.

Compounds **1**−**5** exhibited no cytotoxicity against human breast cancer (Bre04), human lung (Lu04) or human neuroma (N04) cell lines ($GI_{50} > 10$ μg/mL), and no inhibitory activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, or *Candida albicans* at a concentration of 100 μg/mL.

Acknowledgment. This work was supported by the West Light Foundation of the Chinese Academy of Sciences.

Supporting Information Available: HRESIMS, 1D and 2D NMR spectra of **1**−**5**, and X-ray crystallographic data of **1** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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