ORGANIC LETTERS

2011
Vol. 13, No. 22
5948–5951

Cyclic Bipyridine Glycosides from the Marine-Derived Actinomycete *Actinoalloteichus cyanogriseus* WH1-2216-6

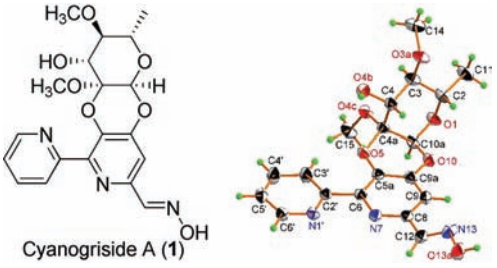
Peng Fu,[†] Peipei Liu,[†] Xia Li,[‡] Yi Wang,[†] Shuxia Wang,[†] Kui Hong,[§] and Weiming Zhu*,[†]

Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, P. R. China, Marine College, Shandong University at Weihai, Weihai 264209, P. R. China, and College of Pharmacy, Wuhan University, Wuhan 430071, P. R. China

weimingzhu@ouc.edu.cn

Received August 18, 2011

ABSTRACT



Cyanogrisides A–D (1–4), four new glycosidic derivatives of bipyridine featuring a novel cyclic glycoside generated by vicinal hydroxyl groups of an aglycone with both the anomeric center and the adjacent carbonyl of a keto sugar, were isolated from the marine-derived actinomycete *Actinoalloteichus cyanogriseus* WH1-2216-6. The structures of 1–4 were elucidated by spectroscopic analysis, X-ray single crystal diffraction, CD spectra, and chemical methods.

Marine natural products are a significant source for drug discovery and have attracted the attention of biologists and chemists for over five decades. To date, more than 20 000 marine natural products have been isolated from marine organisms, approximately one-third of which were *N*-containing compounds.[1] Among all 46 marine drugs, 33 contain nitrogen,[2,3] indicating that nitrogen was an important factor for drug discovery. Recently, obligate marine actinomycetes represent a new resource for structurally diverse secondary metabolites.[4−7]

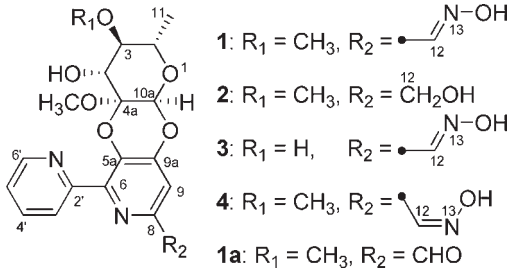
As part of our ongoing research on structurally novel and bioactive *N*-containing compounds from marine-derived actinomycetes, *Actinoalloteichus cyanogriseus* WH1-2216-6 was isolated from marine sediment[8] and was found to produce alkaloids by TLC visualizing with Dragendorff's reagent in a saline culture.[8] The EtOAc extract showed significant cytotoxicity on K562 cells and strong potency to increase the cytotoxicity of adriamycin toward K562/A02 cells. This indicates that the EtOAc

[†] Ocean University of China.
[‡] Shandong University at Weihai.
[§] Wuhan University.

(1) Blunt, J. W.; Copp, B. R.; Hu, W.; Munro, M. H. G.; Northcote, P. T.; Rrinsep, M. R. *Nat. Prod. Rep.* **2009**, *26*, 170–244.
(2) Molinski, T. F.; Dalisay, D. S.; Lievens, S. L.; Saludes, J. P. *Nat. Rev. Drug Discovery* **2009**, *8*, 69–85.
(3) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216–1238.
(4) Laatsch, H. *Frontiers in Marine Biotechnology*; Proksch, P., Muller, W. E. G., Eds.; Horizon Bioscience: Norfolk, 2006; pp 225–288.
(5) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666–673.
(6) Feling, R. H.; Buchanan, G. O.; Mincer, T. J.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Angew. Chem., Int. Ed.* **2003**, *42*, 355–357.
(7) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Rrinsep, M. R. *Nat. Prod. Rep.* **2010**, *27*, 165–237.
(8) Fu, P.; Wang, S. X.; Hong, K.; Li, X.; Liu, P. P.; Wang, Y.; Zhu, W. M. *J. Nat. Prod.* **2011**, *74*, 1751–1756.

10.1021/ol202245s
Published on Web 10/25/2011

extract can reverse the multiple drug resistance of tumor cells which severely limits the effectiveness of chemotherapy in a variety of common malignancies and is responsible for the overall poor efficacy of cancer chemotherapy.[9] Five new bipyridine alkaloids have been identified from the fermentation broth of *A. cyanogriseus* WH1-2216-6;[8] further study resulted in the isolation and identification of four novel bipyridine cyclic glycosides which we named cyanogrisides A–D (**1**–**4**). The cyclic glycosides generated by a glycosidation and an aldol condensation between vicinal hydroxyl groups of an aglycone and both the anomeric center and the adjacent carbonyl of a keto sugar were very infrequent in nature. Only two examples were present in the literature, including a bipyridine glycoside, caerulomycin D (Figure S3).[10,11]



Cyanogriside A (**1**) was assigned a molecular formula of $C_{19}H_{21}N_3O_7$ on the basis of HRESIMS,[12] requiring 11 degrees of unsaturation. Its UV spectrum showed characteristic peaks of a bipyridine chromophore at λ_{max} 233 and 269 nm.[8,10] The IR spectrum indicated that **1** possesses a hydroxyl (3333 cm^{-1}), an aromatic system (1572 cm^{-1}), and ether groups (1126 cm^{-1}). The ^{13}C NMR resolved 19 carbon signals, which were classified by DEPT and HMQC spectra as three methyl carbons (two oxygenated), ten methine carbons (six olefinic ones), and six quaternary carbons (one oxygenated and five olefinic carbons). Moreover, the ^1H NMR spectrum (Table 1) showed four signals at δ_H 5.52–3.25, and the ^{13}C NMR spectrum (Table 1) showed five signals at δ_C 95.6–70.7, which suggested the presence of a glycosyl group. The signals at $\delta_{H/C}$ 3.57/61.2 and 3.64/51.8 suggested the presence of two methoxy groups. ^1H–^1H COSY from H-2 to H-4 through H-3 and the key HMBC correlations of H-11 to C-2 and C-3, H-10a to C-2, and 4a-OCH_3 to C-4a identified the structure of the glycosyl fragment (A) (Figure 1). Five proton signals at δ_H 8.62–7.33 and ten carbon signals at δ_C 154.9–108.6 (Table 1) suggested the presence of a bipyridine nucleus.[8,10] ^1H–^1H COSY correlations of H-3′/H-4′/H-5′/H-6′ and HMBC correlations of H-3′ to C-5′/C-6, H-4′ to C-2′/C-6′, H-9 to C-5a, and H-12 to C-8/C-9 constituted the bipyridine aldoxime moiety (B) (Figure 1). These data suggested that **1** is an analogue of caerulomycin D.[10] The differences in the ^{13}C NMR data in DMSO-d_6 at C-5a and C-9a between **1** and caerulomycin D could be explained by the interchange of two glycosidic linkages. This conjecture has been confirmed by single crystal X-ray diffraction analysis (Figures 2 and S2), and its relative configuration was also determined. Bearing on seven oxygen atoms in the molecule, the final refinement on the Cu Kα data resulted in a Flack parameter of 0.03 (13), allowing an unambiguous assignment of the complete absolute configurations of all the chiral centers as 2*S*, 3*R*, 4*R*, 4a*S*, and 10a*S*.

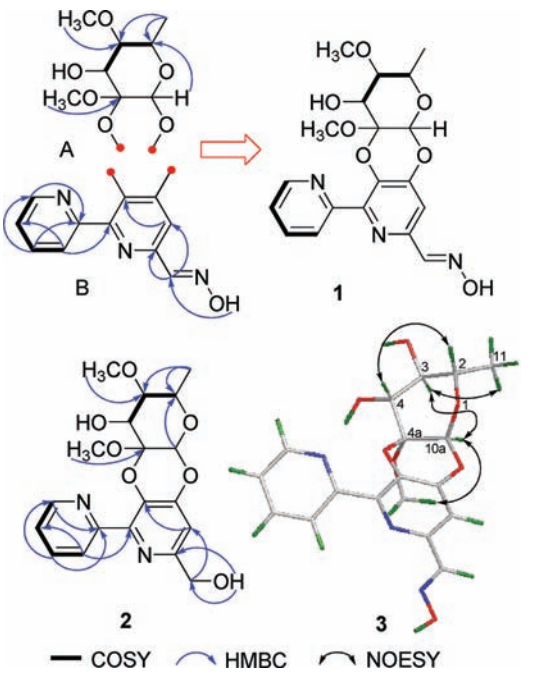


Figure 1. Selected two-dimensional NMR correlations for **1**–**3**.

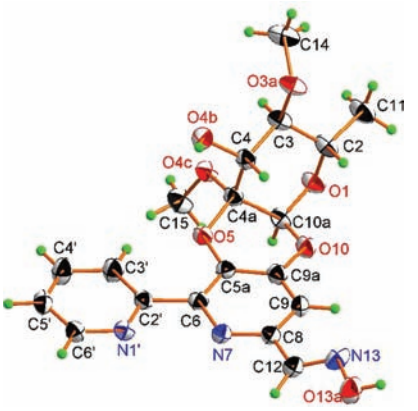


Figure 2. X-ray crystal structure of **1** (Cu Kα radiation).

(9) Li, X.; Sun, B.; Zhu, C. J.; Yuan, H. Q.; Shi, Y. Q.; Gao, J.; Li, S. J.; Lou, H. X. *Toxicol. in Vitro* **2009**, *23*, 29–36.

(10) Mcinnes, A. G.; Smith, D. G.; Walter, J. A.; Wright, J. L. C.; Vining, L. C.; Arsenault, G. P. *Can. J. Chem.* **1978**, *56*, 1836–1842.

(11) Hoeksema, H.; Argoudelis, A. D.; Wiley, P. F. *J. Am. Chem. Soc.* **1962**, *84*, 3212–3213.

(12) Cyanogriside A (**1**): colorless needle (MeOH), mp 235 °C, $[\alpha]_D^{17}$ −68 (*c* 0.1, MeOH); UV(MeOH) λ_{max} (log ε): 233 (3.46), 269 (3.31) nm; CD (MeOH) λ_{max} (Δε) 306 (+1.4), 252 (−5.6), 226 (+8.0), 205 (+0.9) nm; IR (KBr) ν_{max}: 3333, 2906, 1572, 1458, 1417, 1358, 1215, 1126, 1047, 989, 797 cm^{-1}; for ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 404.1464 $[M + H]^+$ (calcd for $C_{19}H_{22}N_3O_7$, 404.1458) .

Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1**–**4**[a]

position	**1**[b]		**1**[c]		**2**[c]		**3**[c]		**4**[b]	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	71.6, CH	3.91, dq, (9.3, 6.2)	70.1, CH	3.86, dq (8.3, 6.4)	70.6, CH	3.84, dq (8.3, 6.5)	72.3, CH	3.82, dq (8.4, 6.1)	71.8, CH	3.86, dq (9.4, 6.3)
3	82.7, CH	3.25, dd (9.4, 9.4)	83.3, CH	3.07, dd (8.1, 8.0)	84.1, CH	3.08, dd (8.3, 8.2)	74.0, CH	3.27, td (8.6, 6.3)	82.4, CH	3.26, dd (9.4, 9.3)
3-OCH$_3$/OH	61.2, CH$_3$	3.57, s	59.5, CH$_3$	3.42, s	60.0, CH$_3$	3.42, s		5.26, d (6.0)	61.2, CH$_3$	3.59, s
4	70.7, CH	3.96, d (9.9)	69.2, CH	3.69, t (6.8)	69.6, CH	3.69, t (6.8)	70.6, CH	3.54, t (5.0)	71.0, CH	3.93, d (9.3)
4-OH		5.61, brs		5.60, d (6.8)		5.60, d (6.8)		5.41, d (4.6)		5.48, brs
4a	95.6, C		96.0, C		96.1, C		96.8, C		95.7, C	
4a-OCH$_3$	51.8, CH$_3$	3.64, s	50.4, CH$_3$	3.42, s	50.8, CH$_3$	3.41, s	51.1, CH$_3$	3.42, s	51.9, CH$_3$	3.63, s
5a	136.7, C		136.7, C		135.2, C		137.0, C		136.8, C	
6	150.9, C		148.6, C		155.2, C		149.6, C		152.3, C	
8	147.0, C		145.9, C		145.9, C		146.4, C		145.5, C	
9	108.6, CH	7.33, s	107.4, CH	7.39, s	108.9, CH	7.09, s	108.0, CH	7.40, s	111.7, CH	7.04, s
9a	147.2, C		146.1, C		149.4, C		146.5, C		146.5, C	
10a	92.8, CH	5.52, s	91.0, CH	5.69, s	91.6, CH	5.64, s	92.1, CH	5.68, s	93.2, CH	5.57, s
11	17.6, CH$_3$	1.42, d (6.6)	17.7, CH$_3$	1.29, d (6.4)	18.3, CH$_3$	1.29, d (6.4)	18.2, CH$_3$	1.27, d (6.0)	17.6, CH$_3$	1.42, d (6.6)
12	148.6, CH	8.16, s	148.2, CH	8.03, s	64.3, CH$_2$	4.51, d (2.7)	148.7, CH	8.04, s	141.5, CH	7.58, s
12-/13-OH				11.60, s		5.45, brs		11.58, s		15.19, s
2′	154.9, C		154.2, C		156.3, C		154.9, C		153.6, C	
3′	124.5, CH	7.97, d (7.7)	124.6, CH	7.92, dd (5.5, 1.3)	125.2, CH	7.90, dd (7.8, 1.3)	125.1, CH	7.94, dd (8.0, 1.4)	124.5, CH	7.91, dd (6.6, 1.4)
4′	137.6, CH	7.87, td (7.7, 1.1)	136.3, CH	7.91, ddd (5.5, 5.0, 1.8)	136.8, CH	7.89, ddd (7.8, 6.2, 1.7)	137.4, CH	7.92, ddd (7.8, 7.8, 1.5)	137.9, CH	7.90, ddd (6.8, 6.8, 1.6)
5′	123.9, CH	7.35, t (5.5)	123.5, CH	7.45, ddd (6.7, 4.9, 2.0)	123.8, CH	7.42, ddd (6.2, 4.8, 2.5)	124.1, CH	7.45, t (5.0)	124.0, CH	7.42, ddd (6.9, 5.0, 2.1)
6′	150.1, CH	8.62, d (4.4)	149.2, CH	8.70, dd (4.8, 1.3)	149.6, CH	8.67, d (4.6)	149.4, CH	8.67, d (3.5)	148.9, CH	8.65, d (4.8)

[a] Spectra were recorded at 600 MHz for ^{1}H and 150 MHz for ^{13}C using TMS as an internal standard. [b] Measured in $CDCl_3$. [c] Measured in DMSO-d_6.

HRESIMS analysis of cyanogriside B (**2**) revealed a molecular formula of $C_{19}H_{22}N_2O_7$.[13] The ^{1}H and ^{13}C NMR data of **2** (Table 1) were similar to those of **1** except that the oxime methine signal at $\delta_{C/H}$ 148.6/8.16 was replaced by a methylene signal at $\delta_{C/H}$ 64.3/4.51. The HMBC correlations from H-12 to C-9 and from 12-OH (δ_H 5.45) to C-8 also supported this change in **2** (Figure 1). The X-ray diffraction analysis (Figure 3) suggested that **2** and **1** have the same relative configuration. The CD Cotton effects at 295 nm ($\Delta\varepsilon$ +2.3) and 257 nm ($\Delta\varepsilon$ −0.8) (Figure S1)

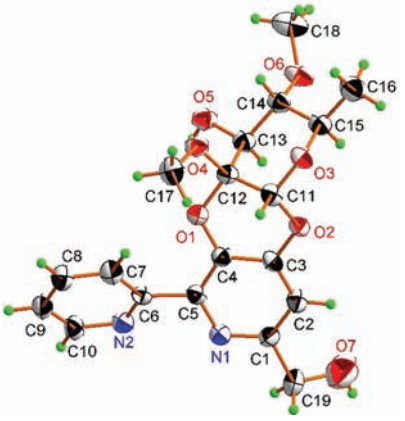


Figure 3. X-ray crystal structure of **2** (Mo Kα radiation).

of **2** were similar to those of **1** [306 nm ($\Delta\varepsilon$ +1.4) and 252 nm ($\Delta\varepsilon$ −5.6)], and the specific rotation of **2** ($[\alpha]_D^{17}$ −30, *c* 0.1, MeOH) was similar to that of **1** ($[\alpha]_D^{17}$ −68, *c* 0.1, MeOH), indicating that **2** has the same absolute configuration of **1**. This deduction was further confirmed by the chemical transformation of **1** into **2** through hydrolysis followed by reduction of **1** (Scheme 1).[14]

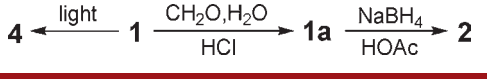
The molecular formula of cyanogriside C (**3**) was assigned to be $C_{18}H_{19}N_3O_7$ based on the HRESIMS,[15] which

(13) Cyanogriside B (**2**): colorless needle (MeOH), mp 197 °C, $[\alpha]_D^{17}$ −30 (*c* 0.1, MeOH); UV(MeOH) λ_{max} (log ε): 218 (3.16), 295 (2.63) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 295 (+2.3), 257 (−0.8), 246 (+0.4), 229 (−1.8), 211 (+0.6), 204 (−2.3) nm; IR (KBr) ν_{max}: 3291, 2986, 2952, 2826, 1583, 1487, 1212, 1128, 1045, 844, 798, 745 cm^{-1}; for ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 413.1319 $[M + Na]^+$ (calcd for $C_{19}H_{22}N_2O_7Na$, 413.1325).

(14) A mixture of **1** (11.8 mg), 37% aqueous formaldehyde solution (380 μL), H_2O (172 μL), and 5 N HCl (44 μL) was heated under reflux for 30 min. After cooling to room temperature, the solution was neutralized with 5 N NaOH and filtered, and the filtrate was extracted three times each with 4 mL of CH_2Cl_2. The combined organic layer was evaporated to dryness to yield the corresponding carbaldehyde **1a** (10.1 mg, 89% yield). Carbaldehyde **1a**, yellow oil, $[\alpha]_D^{17}$ −29 (*c* 0.1, MeOH); UV(MeOH) λ_{max} (log ε): 216 (3.85), 281 (3.50) nm; IR (KBr) ν_{max}: 3381, 2920, 2844, 1703, 1650, 1589, 1459, 1426, 1383, 1255, 1105, 1043, 952 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ = 10.00 (s, 1 H), 8.75 (d, *J* = 4.9 Hz, 1 H), 8.14 (d, *J* = 8.7 Hz, 1 H), 8.06 (t, *J* = 8.7 Hz, 1 H), 7.61 (s, 1 H), 7.53 (t, *J* = 7.1 Hz, 1 H), 5.52 (s, 1 H), 3.95 (d, *J* = 9.3 Hz, 1 H), 3.92 (m, 1 H), 3.62 (s, 3 H), 3.58 (s, 3 H), 3.23 (t, *J* = 9.4 Hz, 1 H), 1.42 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ = 191.8, 152.9, 150.9, 148.4, 147.7, 147.3, 140.2, 139.5, 125.2, 124.6, 110.7, 96.6, 92.6, 82.8, 71.8, 70.8, 61.1, 51.9, 17.6; HRESIMS *m*/*z* 389.1357 $[M + H]^+$ (calcd for $C_{19}H_{21}N_2O_7$, 389.1349). Carbaldehyde **1a** (3.0 mg) was dissolved in CH_3CN (1 mL) and 5 N HOAc (50 μL), and the solution was added to 10 mg of $NaBH_4$ under stirring in small amounts. Stirring was continued for another 20 min, and then the mixture was evaporated to dryness. The residue was dissolved in 200 μL of H_2O and then extracted 5 times each with 4 mL of CH_2Cl_2. The organic layers were combined and evaporated to dryness to yield **2** (1.9 mg, 63% yield) that was identified by HPLC and TLC analysis, ESIMS, and specific rotation.

(15) Cyanogriside C (**3**): yellow oil, $[\alpha]_D^{17}$ −28 (*c* 0.2, MeOH); UV(MeOH) λ_{max} (log ε): 226 (3.91), 260 (3.78) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 308 (+0.5), 252 (−1.1), 227 (+2.0), 203 (+0.3) nm; IR (KBr) ν_{max}: 3279, 2980, 2937, 1574, 1483, 1418, 1215, 1128, 1040, 976, 800, 759 cm^{-1}; for ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 412.1129 $[M + Na]^+$ (calcd for $C_{18}H_{19}N_3O_7Na$, 412.1121).

Scheme 1. Chemical Transformation of **1** into **2** and **4**

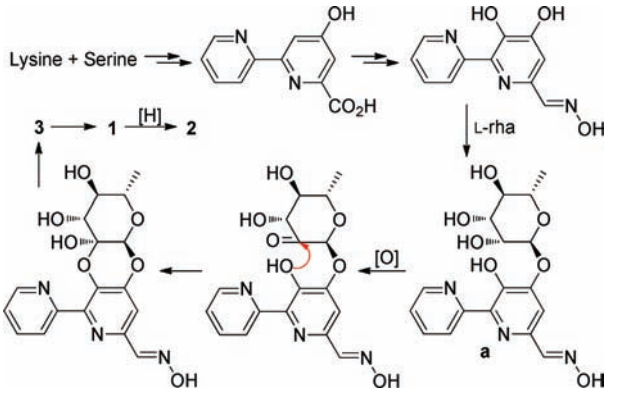


was only one CH_2 less than that of **1**. Careful comparison of its ^1H and ^{13}C NMR spectra (Table 1) with those of **1** showed that 3-OH (δ_H 5.26) in **3** replaced the 3-OCH_3 ($\delta_{C/H}$ 61.2/3.57) in **1**. The NOESY spectrum of **3** (Figure 1) also showed a similar correlation pattern to that of **1**, indicating the same relative configurations. The CD Cotton effects at 308 nm ($\Delta\varepsilon$ + 0.5) and 252 nm ($\Delta\varepsilon$ −1.1) (Figure S1) and the specific rotation ($[\alpha]_D^{17}$ −28, *c* 0.2, MeOH) of **3** suggested that **3** has the same absolute configuration as compound **1**.

Cyanogriside D (**4**) is an isomer of **1** based on HRESIMS.[16] The ^1H and ^{13}C NMR data of **4** (Table 1) were similar to those of **1** except some small differences in the aldoxime group, indicating that **4** and **1** are a pair of geometric isomers. Moreover, due to compound **4** being unstable in the solvent, the *Z*-configuration of the aldoxime group in **4** was assumed, which was confirmed by photoisomerization of **1** into **4** (Scheme 1).[17,18] Because **4** could not be detected in the HPLC profile of the crude extract, it was thought cyanogriside D (**4**) may have been formed during subsequent isolation steps by the photoreaction of **1**.

A plausible biogenetic pathway of cyanogrisides A−C (**1**−**3**) was postulated (Scheme 2). The bipyridine aldoxime moiety was probably derived from lysine, serine, and polyketide,[8,19,20] while the glycosyl moiety was developed from L-rha. The glycosidation of 4-OH of the bipyridine of didemethylcaerulomycin C with L-rha produces glycoside **a** that serves as a key biosynthetic precursor, undergoing oxidation, aldol condensation, and methylation to yield **1** and **3**. Compound **2** possibly results from the reduction of **1**.

Scheme 2. Plausible Biosynthetic Pathways of **1**−**3**



Compounds **1**−**3** were tested for cytotoxic effects on the K562, KB, MCF-7, and HL-60 cell lines using the MTT method[21] and on the A549 cell lines using the SRB method.[22]

Compound **1** showed moderate cytotoxicity against the K562, KB, and MCF-7 cells, with IC_{50} values of 1.2, 4.7, and 9.8 μM, respectively. The moderate cytotoxicities for compound **3** against K562 and KB cells were also observed with IC_{50} values of 0.73 and 4.7 μM, respectively (Table S1). Compounds **1**−**3** were also assayed for the ability to reverse the adriamycin-induced resistance of K562/A02 and MCF-7/Adr cells and the vincristine-induced resistance of KB/VCR cells. The results showed that compound **2** can reverse the multiple drug resistance of K562/A02, MCF-7/Adr, and KB/VCR cells at a concentration of 10 μM, with reversal fold values of 1.7, 1.2, and 3.6, respectively.

Acknowledgment. This work was supported by grants from the National Basic Research Program of China (No. 2010CB833800), the National Natural Science Foundation of China (Nos. 21172204, 30973680, 30670219, and 31070045), and the Special Fund for Marine Scientific Research in the Public Interest of China (No. 2010418022-3).

Supporting Information Available. Experimental details, NMR spectra of **1**−**4**, CD spectra of **1**−**4**, X-ray data for **1** and **2**, and bioassay protocols used. This material is available free of charge via the Internet at http://pubs.acs.org.

(16) Cyanogriside D (**4**): white powder, $[\alpha]_D^{17}$ −67 (*c* 0.2, MeOH); UV(MeOH) λ_{max} (log ε): 233 (3.55), 269 (3.39) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 306 (+0.8), 255 (−2.4), 227 (+2.6), 209 (+0.2) nm; IR (KBr) ν_{max}: 3231, 2929, 1572, 1450, 1213, 1130, 1047, 750 cm^{-1}; for ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 404.1465 $[M + H]^+$ (calcd for $C_{19}H_{22}N_3O_7$, 404.1458).

(17) Zheng, J.; Zhu, H.; Hong, K.; Wang, Y.; Liu, P.; Wang, X.; Peng, X.; Zhu, W. *Org. Lett.* **2009**, *11*, 5262–5265.

(18) A solution of **1** (6.0 mg) in 2.0 mL of acetone was stirred under light at room temperature for 15 h. The acetone was removed under vacuum, and the residue was purified by semipreparative HPLC (50% MeOH−H_2O, 4.0 mL/min) to yield **4** (3.2 mg, 53% yield, t_R 15.5 min) that was identified by HPLC analysis, NMR spectra, and specific rotation.

(19) Vining, L. C.; Mcinnes, A. G.; Mcculloch, A. W.; Smith, D. G.; Walter, J. A. *Can. J. Chem.* **1988**, *66*, 191–194.

(20) Mcinnes, A. G.; Smith, D. G.; Walter, J. A.; Vining, L. C.; Wright, J. L. C. *Can. J. Chem.* **1979**, *57*, 3200–3204.

(21) Mosmann, T. *Immunol. Methods* **1983**, *65*, 55–63.

(22) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R.; Tan, N. H.; Zhou, J. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.