Bromophenols Coupled with Derivatives of Amino Acids and Nucleosides from the Red Alga Rhodomela c onfervoides

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · JUNE 2005

Impact Factor: 3.8 · DOI: 10.1021/np040234m · Source: PubMed

11 AUTHORS, INCLUDING:

Sujuan Wang
Chinese Academy of Medical Sciences
30 PUBLICATIONS **678** CITATIONS

SEE PROFILE

Li Shuai
Chinese Academy of Medical Sciences
67 PUBLICATIONS **827** CITATIONS

SEE PROFILE

Nianjun Xu
Ningbo University
20 PUBLICATIONS **296** CITATIONS

SEE PROFILE

Bromophenols Coupled with Derivatives of Amino Acids and Nucleosides from the Red Alga *Rhodomela confervoides*

Jielu Zhao,[†] Ming Ma,[†] Sujuan Wang,[†] Shuai Li,[†] Peng Cao,[†] Yongchun Yang,[†] Yang Lü,[†] Jiangong Shi,*[,†] Nianjun Xu,[‡] Xiao Fan,[‡] and Lan He*[,§]

Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Bejing 100050, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, and Department of Chemistry, Beijing Normal University, Beijing 100875, People's Republic of China

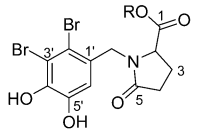
Received December 15, 2004

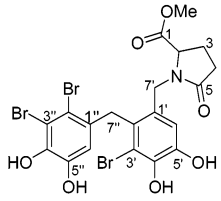
Three new bromophenols coupled with pyroglutamic acid derivatives and one bromophenol coupled with deoxyguanosine were obtained from the red alga *Rhodomela confervoides*. By spectroscopic methods including 2D NMR and single-crystal X-ray structure analysis their structures were elucidated as *N*-(2,3-dibromo-4,5-dihydroxybenzyl)methyl pyroglutamate (**1**), *N*-(2,3-dibromo-4,5-dihydroxybenzyl)pyroglutamic acid (**2**), *N*-[3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl]methyl pyroglutamate (**3**), and 2-*N*-(2,3-dibromo-4,5-dihydroxybenzylamino)deoxyguanosine (**4**), respectively. Compounds **1**−**4** were evaluated against several microorganisms and human cancer cell lines, but found inactive. To our knowledge this is the first report of bromophenols coupled with amino acid or nucleoside derivatives through the C−N bond.

Studies on the red algae of the Rhodomelaceae family have yielded a number of monoaryl and diaryl bromophenols with potent bioactivities.[1−14] Continuing our recently initiated program to assess systematically the chemical and biological diversity of seaweeds distributed in the gulf of the Yellow Sea, China,[15−18] we have found more than 20 bromophenols with a variety of structural types[19,20] from the EtOAc-soluble fraction of the alcoholic extract of *Rhodomela confervoides* (Huds.) Lamour. collected at the coast of Qingdao, China. Although they were inactive against several microorganisms (MIC_{50} > 100 μg/mL) and human tumor cell lines (IC_{50} > 10 μg/mL), the characterization of some unusual bromophenols, such as a sulfoxide,[20] prompted us to further investigate the remaining fractions from the same extract. We report herein the isolation and structural elucidation of four unique bromophenols. Among them, three are coupled with pyroglutamic acid derivatives (**1**−**3**) and one coupled with deoxyguanosine (**4**). They were evaluated against several human cancer cell lines (IC_{50} > 10 μg/mL) including lung adenocarcinoma (A549), stomach cancer (BGC-823), breast cancer (MCF-7), hepatoma (Bel7402), and human colon cancer (HCT-8) cell lines, as well as three microorganisms (MIC_{50} > 100 μg/mL), *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. To our knowledge this is the first report of bromophenols coupled with amino acid or nucleoside derivatives through the C−N bond.

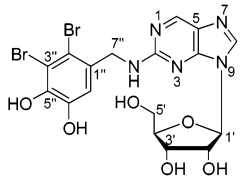
Results and Discussion

Compound **1** was obtained as colorless crystals, mp 183−185 °C, $[\alpha]^{20}_D$ +7.0° (*c* 5.0, Me_2CO). The IR spectrum showed strong absorption bands for hydroxy (3408 cm^{-1}) and carbonyl (1749 cm^{-1}) groups and an aromatic ring (1597 and 1502 cm^{-1}). The FABMS exhibited a characteristic quasi-molecular ion peak cluster with two bromine atoms at *m/z* 422/424/426 (1:2:1) $[M + H]^+$, and the molecular formula $C_{13}H_{13}O_5Br_2N$ was determined by





3



4

HRFABMS at *m/z* 421.9261 (calcd for $C_{13}H_{14}O_5{}^{79}Br_2N$, 421.9239). The ^1H NMR spectrum of **1** showed characteristic signals assignable to a 2,3-dibromo-4,5-dihydroxybenzyl moiety[19,20] at δ_H 6.85 (1H, s, H-6′), 4.86 (1H, d, J = 15.0 Hz, H-7′a), and 4.14 (1H, d, J = 15.0 Hz, H-7′b), which was unambiguously confirmed by the ^{13}C NMR data (see Experimental Section) and the strong fragment cluster at *m/z* 279/281/283 (1:2:1) in the FABMS of **1**. In addition, the NMR spectra showed signals attributed to a methoxyl at δ_H 3.68 (3H, s, H_3-O*Me*) and δ_C 52.5, a methine at δ_H 4.11 (1H, dd, J = 9.0 and 3.0 Hz, H-2) and δ_C 59.6, and two methylenes at δ_H 2.35 and 2.08 (each 1H, m, H-3a and H-3b) and δ_C 23.5 (C-3) and δ_H 2.37 (2H, m, H_2-4) and δ_C 29.6 (C-4), as well as two carbonyls at δ_C 173.1 (C-1) and 175.2 (C-5). The chemical shift values and coupling patterns of these signals in combination with the molecular composition of **1** ($C_{13}H_{13}O_5Br_2N$) suggested that there was a methyl pyroglutamate moiety in **1**. This was confirmed by 2D NMR experiments of **1**. The $^1H-^1H$ COSY spectrum displayed vicinal coupling correlations between H-2 and H_2-3, which in turn correlated with H_2-4, while the HMBC spectrum demonstrated correlations of C-1 with H-2, H_2-3, and the methoxyl protons, and C-5 with H_2-3 and H_2-4. Furthermore, in the HMBC spectrum strong correlations from H_2-7′ to C-2 and C-5 established that the 2,3-dibromo-4,5-dihydroxybenzyl moiety was located at the nitrogen

* To whom correspondence should be addressed. Tel: 86-10-83154789. Fax: 86-10-63017757. E-mail: shijg@imm.ac.cn.
† Institute of Materia Medica.
‡ Institute of Oceanology.
§ Beijing Normal University.

10.1021/np040234m CCC: $30.25
Published on Web 04/16/2005

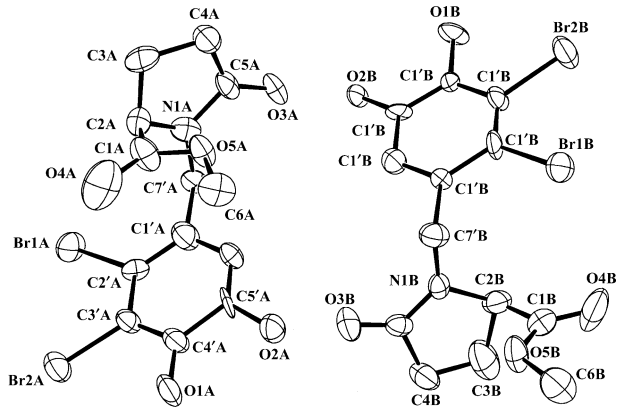


Figure 1. ORTEP drawing of the two isomers (A and B) of compound **1**.

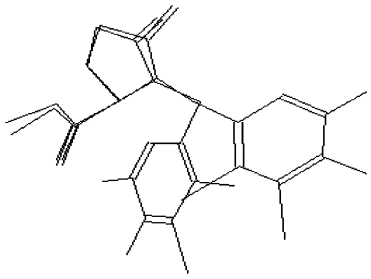


Figure 2. Computer-generated overlapping of two isomers (A and B) of compound **1**.

atom of the methyl pyroglutamate moiety. Therefore, the structure of **1** was determined as *N*-(2,3-dibromo-4,5-dihydroxybenzyl)methyl pyroglutamate. The structural assignment of **1** was further confirmed by an X-ray structure analysis showing that the crystal unit cell contained two conformational isomers (A and B) with different configurations at the nitrogen atoms (*N*1A and *N*1B). The ORTEP drawings of the two isomers, with the atom-numbering scheme indicated, as well as the computer-generated overlapping of the two isomers are given in Figures 1 and 2, respectively.

Compound **2** was obtained as colorless crystals, mp 172−174 °C, $[\alpha]^{20}_D$ +24.0° (*c* 5.7, Me_2CO). The FABMS exhibited a dibrominated quasi-molecular ion peak cluster at *m/z* 408/410/412 (1:2:1) $[M + H]^+$, and the molecular formula $C_{12}H_{11}O_5Br_2N$ was determined by HRFABMS at *m/z* 407.9071 (calcd for $C_{12}H_{12}O_5{}^{79}Br_2N$, 407.9082), which is a CH_2 unit less than that of **1**. The IR and NMR spectra of **2** (Experimental Section) resembled those of **1**, except for the disappearance of the methoxyl signal in the NMR spectra of **2**, indicating that **2** is the acid form of **1**. This was confirmed by the HMBC experiment of **2** and the acidic hydrolysis of **1** yielding **2**. Accordingly, **2** was determined as *N*-(2,3-dibromo-4,5-dihydroxybenzyl)pyroglutamic acid.

Compound **3** was obtained as colorless crystals, mp 196−198 °C, $[\alpha]^{20}_D$ −30.9° (*c* 9.7, Me_2CO) and showed IR absorption bands for hydroxy (3369 cm^{-1}) and carbonyl (1741 cm^{-1}) groups and aromatic rings (1610, 1583, 1505, and 1496 cm^{-1}). The FABMS of **3** gave a characteristic tribromonated quasi-molecular ion peak cluster at 620/622/624/626 (1:3:3:1) $[M - H]^-$, and the molecular formula $C_{20}H_{18}O_7Br_3N$ was determined by HRFABMS at *m/z* 619.8536 $[M - H]^-$ (calcd for $C_{20}H_{17}O_7{}^{79}Br_3N$, 619.8555). The ^1H NMR spectrum of **3** displayed signals assignable to two tetrasubstituted benzyl units[19] at δ 6.80 (1H, s, H-6′), 5.99 (1H, s, H-6″), 4.91 (1H, d, J = 15.0 Hz, H-7′a), 3.69 (1H, d, J = 15.0 Hz, H-7′b), and 4.05 (2H, s, H-7″), besides similar signals attributed to the methyl pyroglutamate

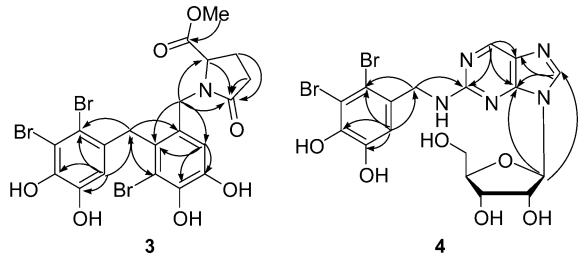


Figure 3. Main HMBC correlations of compounds **3** and **4**.

moiety of **1** at δ 3.87 (1H, dd, J = 9.0 and 3.0 Hz, H-2), 3.66 (3H, s, OC*H*$_3$), and 1.80−2.30 (4H, m, H_2-3 and H_2-4). These data suggested that **3** was an analogue of **1** with an additional tetrasubstituted benzyl unit, which was confirmed by the ^{13}C NMR and DEPT spectra showing 20 carbon signals consisting of 12 quaternary sp^2 carbons [two carbonyls (δ >170 ppm)], three methines, four methylenes, and one methyl (Experimental Section). The substitution pattern and connectivity of these structural units were further demonstrated by 2D NMR techniques including $^1H-^1H$ COSY, HMQC, and HMBC experiments. The NMR signals of protons and protonated carbons were unequivocally assigned by the HMQC experiment (Experimental Section). Homonuclear correlations of H_2-3 with H-2 and H_2-4 in the $^1H-^1H$ COSY spectrum and long-range correlations of C-1 (δ_C 173.0) with H-2, H_2-3, and H_3-6, and C-5 (δ_C 174.2) with H_2-3 and H_2-4 in the HMBC spectrum (see Figure 3), confirmed the presence of the methyl pyroglutamate moiety. In combination with the molecular formula $C_{20}H_{18}O_7Br_3N$, HMBC correlations from H-6′ to C-2′, C-4′, C-5′, and C-7′ (δ_C 128.3, 129.6, 143.7, 145.0, and 43.9, respectively), and from H_2-7′ to C-1′, C-2′, and C-6′ (δ_C 117.0), as well as from H-6″ to C-2″, C-4″, C-5″, and C-7″ (δ_C 116.4, 143.3, 145.1, and 39.9, respectively) and from H_2-7″ to C-1″, C-2″, C-6″, C-1′, and C-3′, unequivocally established the presence of a 3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl moiety. In addition, HMBC correlations from H-7′ to both C-2 (δ_C 58.7) and C-5 in combination with the chemical shift value of C-7′ demonstrated the linkage between C-7′ and the nitrogen atom. Therefore, the structure of **3** was determined as *N*-[3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl]methyl pyroglutamate.

Compound **4** was obtained as a brown amorphous solid, mp 205−207 °C, $[\alpha]^{20}_D$ +7.0° (*c* 4.4, DMSO). The IR spectrum (KBr) showed absorption bands for hydroxy groups (3446 and 3359 cm^{-1}) and aromatic rings (1581 and 1487 cm^{-1}). The FABMS gave a dibrominated quasi-molecular ion peak cluster at *m/z* 546/548/550 (1:2:1) $[M + H]^+$, and the molecular formula was determined as $C_{17}H_{17}O_6Br_2N_5$ by HRFABMS at *m/z* 545.9638 (calcd for $C_{17}H_{18}O_6{}^{79}Br_2N_5$, 545.9623). The ^1H NMR showed characteristic signals attributed to a β-D-ribofuranosyl dexoyguanosine moiety at δ 8.42 (1H, brs, H-8), 8.19 (1H, s, H-6), 5.90 (1H, d, J = 6.0 Hz, H-1′), 4.64 (1H, brs, H-2′), 4.14 (1H, brs, H-3′), 3.96 (1H, brs, H-4′), 3.66 (1H, brd, J = 12.0 Hz, H-5′a), and 3.54 (1H, brd, J = 12.0 Hz, H-5′b) besides typical signals for the 2,3-dibromo-4,5-dihydroxybenzyl moiety at δ 6.73 (1H, s, H-6″) and 4.58 (2H, s, H-7″). The ^{13}C NMR data (Experimental Section) further indicated that **4** consisted of β-D-ribofuranosyl dexoyguanosine and 2,3-dibromo-4,5-dihydroxybenzyl moieties. Although most of the NMR signals were broad, as usually observed for nucleosides, the presence of these moieties was confirmed by 2D NMR techniques including $^1H-^1H$ COSY, HMQC, and HMBC experiments. The HMQC experiment enabled the assignment of the NMR signals of protons and their

Table 1. NMR Data for Compounds **1**−**4**[a]

	1		**2**		**3**		**4**	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		173.1 s		173.2 s		173.0 s		
2	4.11 dd (9.0, 3.0)	59.6 d	4.10 dd (9.0, 3.0)	59.2 d	3.87 dd (9.0, 3.0)	58.7 d		154.3 s
3	(a) 2.35 m	23.5 t	(a) 2.30 m	23.4 t	(a) 1.94 m	22.6 t		
	(b) 2.08 m		(b) 2.04 m		(b) 1.90 m			
4	2.35 m	29.6 t	(a) 2.44 m	30.2 t	(a) 2.20 m	30.4 t		148.6 s
			(b) 2.37 m		(b) 1.91 m			
5		175.2 s		175.6 s		174.2 s		119.9 s
6							8.19 s	152.4 d
8							8.42 s	140.2 d
1′		129.1 s		128.8 s		128.3 s	5.90 d (6.0)	87.8 d
2′		115.8 s		115.5 s		129.6 s	4.64 brs	73.3 d
3′		113.8 s		113.5 s		115.3 s	4.14 brs	70.6 d
4′		144.9 s		144.6 s		143.7 s	3.96 brs	85.9 d
5′		145.8 s		145.4 s		145.0 s	(a) 3.66 brd (12.0)	61.6 t
							(b) 3.54 brd (12.0)	
6′	6.85 s	116.4 d	6.88 s	116.1 d	6.80 s	117.0 d		
7′	(a) 4.86 d (15.0)	47.1 t	(a) 4.91 d (15.0)	46.9 t	(a) 4.91 d (15.0)	43.9 t		
	(b) 4.14 d (15.0)		(b) 4.15 d (15.0)		(b) 3.69 d (15.0)			
1″						132.1 s		130.1 s
2″						116.4 s		112.4 s
3″						113.7 s		113.1 s
4″						143.3 s		143.1 s
5″						145.1 s		145.0 s
6″					5.99 s	114.3 d	6.73 s	113.2 d
7″					4.05 s	39.9 t	4.58 s	44.3 t
O*Me*	3.68 s	52.5 q			3.66 s	52.5 q		

[a] Data were measured in acetone-d_6 for **1**−**3** and DMSO-d_6 for **4**, at 500 MHz for proton and 125 MHz for carbon. Proton coupling constants (*J*) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

attaching carbons (Experimental Section). The vicinal coupling chain of the ribofuranosyl unit was readily traced from the anomeric proton (H-1′) through H-2′, H-3′, and H-4′ to H_2-5′. In the HMBC spectrum (Figure 3) correlations from H-8 to C-4 and C-5, from H-6 to C-2 and C-4, and from H-1′ to C-4 and C-8, in combination with chemical shift values of these protons and carbons, as well as the molecular composition $C_{17}H_{17}O_6Br_2N_5$, confirmed the presence of the deoxyguanosine moiety. Meanwhile, correlations from H-6″ to C-2″, C-4″, and C-5″, from one hydroxyl proton at δ 9.84 to C-3″, C-4″, and C-5″, and from another hydroxyl proton at δ 9.38 to C-4″, C-5″, and C-6″ confirmed the presence of the 2,3-dibromo-4,5-dihydroxybenzyl moiety. Additionally, the three-bond HMBC correlation from H_2-7″ to C-2 and ^{1}H−^{1}H COSY correlation between H-7″ and the exchangeable amino proton at δ 8.42 (overlapped with H-8) demonstrated that the 2,3-dibromo-4,5-dihydroxybenzyl moiety was linked with the 2-amino group of deoxyguanosine. Consequently, the structure of **4** was established as 2-*N*-(2,3-dibromo-4,5-dihydroxybenzyl)deoxyguanosine.

Compound **2** may be an artifact from the hydrolysis of **1**, since **1** can be converted into **2** by keeping the aqueous methanol solution of **1** at room temperature for 3 days. However, the methylation of **2** and the hydrolysis of **3** did not occurr under the simulated isolation conditions, by heating a methanolic solution of **2** or **3**, either with or without silica gel at 45 °C for 48 h. The absolute configuration at the chiral center (C-2) of compounds **1**−**3** has not been determined yet. Compounds **1**−**4** were tested for their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* and showed no growth inhibition at 100 μg/mL. Cytotoxicities of compounds **1**−**4** were evaluated using the MTT method[21,22] against several human cancer cell lines including lung adenocarcinoma (A549), stomach cancer (BGC-823), breast cancer (MCF-7), hepatoma (Bel7402), and human colon cancer (HCT-8) cell lines, but found inactive at 10 μg/mL.

Experimental Section

General Experimental Procedures. Melting points were determined on an XT-4 micro melting point apparatus and are uncorrected. IR spectra were recorded as KBr disks on a Nicolet Impact 400 FT-IR spectrophotometer. 1D and 2D NMR spectra were obtained at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, on an Inova 500 MHz spectrometer in DMSO and acetone-d_6 with solvent peaks as references. EIMS, FABMS, and HRFABMS data were measured with a Micromass Autospec-Ultima ETOF spectrometer. Column chromatography was performed with silica gel (200−300 mesh), Bio-Beads SX3 (200−400 mesh), RP-18 reversed-phase silica gel (43−60 μm), and Sephadex LH-20. TLC was carried out with glass precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 3% $FeCl_3$ in EtOH. HPLC was performed using an Alltima C18 10 μm preparative column (22 × 250 mm).

X-ray diffraction intensity data of **1** were collected on a Rigaku R-AXIS RAPID diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71067 Å) by the ω scan technique, scan width 0−180°, $2\theta \leq 50°$, and were corrected by Lorentz and polarization. A total of 1734 reflections were collected, of which 1707 with $|F|^2 \geq 8\sigma|F|^2$ were observed. The structure of **1** was solved by direct methods and refined by block-matrix least-squares procedure to $R = 0.042$, $R_w = 0.046$ [$w = 1/\sigma|F|^2$]. Hydrogen positions were found from difference Fourier maps and geometric calculations. All calculations were carried out on a PC computer by using the NOMCSDP program system.

Materials. The red alga *Rhodomela confervoides* was collected at the coast of Qingdao, China, in May 2001, and identified by Professor B.-M Xia. A voucher specimen (No. 200102) was deposited at the Department of Marine Algae Chemistry, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071.

Extraction and Isolation. Air-dried *R. confervoides* (14.4 kg) was extracted with EtOH at room temperature for 3 × 48 h. After the solvent was removed under reduced pressure at <40 °C, a dark residue was obtained. The residue was suspended in water and then partitioned with EtOAc. The

EtOAc fraction (594.6 g) was chromatographed over silica gel (1200 g) eluting with a gradient of increasing MeOH (0−100%) in $CHCl_3$ and separated into 24 fractions (I−XXIV) on the basis of TLC analyses. Fraction XI (13 g) was chromatographed over Sephadex LH-20 eluting with petroleum ether−$CHCl_3$−MeOH (5:5:1) to give three subfractions. The second subfraction (2.7 g) was decolored by column chromatography over Bio-Beads SX3 using $CHCl_3$−EtOAc (1:1) and then further separated into five fractions by reversed-phase flash chromatography using MeOH−H_2O−AcOH (50:50:0.1). The subsequent purification of the third fraction (0.12 g) by reversed-phase preparative HPLC using MeOH−H_2O−AcOH (60:40:0.1) as mobile phase yielded compounds **1** (30 mg) and **2** (35 mg). Fraction XII (21 g) was chromatographed by reversed-phase MPLC eluting with a gradient of increasing MeOH in H_2O to give six subfractions. The third subfraction (0.46 g) was decolored by column chromatography over Sephadex LH-20 eluting with $CHCl_3$−MeOH (1:1) and then purified by reversed-phase preparative HPLC using MeOH−H_2O−AcOH (60:40:0.1) as mobile phase to yield **3** (21 mg) and **4** (17 mg).

***N*-(2,3-Dibromo-4,5-dihydroxybenzyl)methyl pyroglutamate (1):** colorless crystals (H_2O−MeOH, 1:1), mp 183−185 °C; $[\alpha]^{20}_D$ +7.0° (*c* 5.0 acetone); IR (KBr) ν_{max} 3408, 2949, 2542, 1749, 1645, 1597, 1464, 1402, 1275, 1190, 1167, 1045, 947, 854 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 1; FABMS *m/z* 422, 424, 426 $[M + H]^+$ (16, 32, 19), 344 (15), 342 (13), 318 (30), 283 (17), 281 (34), 279 (17), 244 (24), 242 (21), 144 (100), 133 (43), 93 (41), 84 (53), 41 (55); HRFABMS *m/z* 421.9261 $[M + H]^+$ (calcd for $C_{13}H_{14}O_5{}^{79}Br_2N$ 421.9239).

Crystal data of 1: $C_{13}H_{13}O_5Br_2N$, M_r 423.06, triclinic, space group *P*1, $a = 8.236(1)$ Å, $b = 10.022(1)$ Å, $c = 10.485(1)$ Å, $\alpha = 63.94(4)°$; $\beta = 85.07(4)°$; $\gamma = 74.59(3)°$; $V = 749.0(1)$ Å^3, $Z = 2$, $D_c = 1.883$ g/cm^3; crystal dimensions 0.20 × 0.30 × 0.30 mm; see Supporting Information for more details.

***N*-(2,3-Dibromo-4,5-dihydroxybenzyl)pyroglutamic acid (2):** colorless crystals (H_2O−MeOH, 1:1), mp 172−174 °C; $[\alpha]^{20}_D$ +24.0° (*c* 5.7, acetone); IR (KBr) ν_{max} 3608, 3429, 2941, 2538, 1718, 1635, 1593, 1502, 1464, 1400, 1346, 1269, 1188, 953, 854, 800, 650 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 1; FABMS *m/z* 412, 410, 408 $[M + H]^+$ (50, 100, 52), 332 (18), 331 (20), 330 (30), 329 (18), 318 (16), 299 (10), 281 (5), 275 (26), 274 (78), 272 (14), 250 (12), 230 (18), 223 (11), 207 (15), 184 (3), 142 (8), 133 (52), 130 (33), 123 (7), 115 (10), 84 (15); HRFABMS *m/z* 407.9071 $[M + H]^+$ (calcd for $C_{12}H_{12}O_5{}^{79}Br_2N$ 407.9082).

Acidic Hydrolysis of 1. A solution of **1** (7 mg) in 2 N HCl (2.5 mL) was stirred at 50 °C for 4 h and then evaporated to dryness under reduced pressure. The residue was dissolved with acetone (1.0 mL) and analyzed by TLC and reversed-phase HPLC together with **2**, indicating that the hydrolysis product of **1** is identical to **2**. The developing solvent system was $CHCl_3$−MeOH−AcOH (5:1:0.1) for TLC. The HPLC analysis was carried out with a YMC-Pack ODS-AM column (3 μm, 100 × 4.6 mm) and a DAD detector (254 nm) using methanol−water (6:4) as mobile phase (0.5 mL/min).

***N*-[3-Bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl]methyl pyroglutamate (3):** colorless crystals (H_2O−MeOH, 1:1), mp 196−198 °C, $[\alpha]^{20}_D$ −30.9° (*c* 9.7, acetone); IR (KBr) ν_{max} 3479, 3369, 2954, 1741,1651, 1605, 1583, 1502, 1460, 1437, 1406, 1275, 1217, 1163, 1092, 955, 860, 812 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 1; FABMS *m/z* 626, 624, 622, 620 $[M - H]^-$ (23, 57, 50, 18), 546 (19), 545 (21), 544 (35), 543 (29), 542 (35), 541 (15), 540 (14), 465 (9), 464 (16), 463 (15), 462 (12), 461 (9), 401 (27), 399 (54), 397 (25), 275 (17), 183 (100), 91 (78); HRFABMS *m/z* 619.8536 $[M - H]^-$ (calcd for $C_{20}H_{17}O_7{}^{79}Br_3N$ 619.8555).

2-*N*-(2,3-Dibromo-4,5-dihydroxybenzyl)-9-β-D-ribofuranosyldeoxyguanosine (4): brown powder, mp 205−207 °C; $[\alpha]^{20}_D$ +7.0° (*c* 4.4, DMSO); IR (KBr) ν_{max} 3446, 3359, 3140, 2937, 1643, 1581, 1597, 1537, 1487, 1417, 1334, 1281, 1173, 1107, 1038, 856, 818 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 1; FABMS *m/z* 550, 548, 546 $[M + H]^+$ (6, 11, 5), 415 (11), 318 (16), 282 (13), 275 (18), 274 (100), 155 (10), 135 (18), 119 (31), 103 (23), 85 (34); HRFABMS *m/z* 545.9638 $[M + H]^+$ (calcd for $C_{17}H_{18}O_6{}^{79}Br_2N_5$ 545.9624).

Acknowledgment. The authors are grateful to A. Zeper for mass spectra measurements. Financial support is from the NSF (Grant No. 20432030) and National "863" program (Grant No. 2001AA620403 and No. 2001AA234021).

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

References and Notes

(1) Katsui, N.; Suzuki, Y.; Kitamura, S.; Irie, T. *Tetrahedron* **1967**, *23*, 1185−1188.
(2) Saenger, P.; Pedersen, M.; Rowan, K. S. *Phytochemistry* **1976**, *15*, 1957−1958.
(3) Kurata, K.; Amiya, T.; Nakano, N. *Chem. Lett.* **1976**, 821−822.
(4) Kurata, K.; Amiya, T. *Chem. Lett.* **1977**, 1435−1438.
(5) Pedersen, M. *Phytochemistry* **1978**, *17*, 291−293.
(6) Kurata, K.; Amiya, T. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 2020−2022.
(7) Aknin, M.; Samb, A.; Mirailles, J.; Costantino, V.; Fattorusso, E.; Mangoni, A. *Tetrahedron Lett.* **1992**, *33*, 555−558.
(8) Kurata, K.; Taniguchii, K.; Takashima, K.; Hayashi, I.; Suzuki, M. *Phytochemistry* **1997**, *45*, 485−487.
(9) Lundgren, L.; Olsson, K.; Theander, O. *Acta Chem. Scand. B* **1979**, *33*, 105−108.
(10) Pederson, M.; Saenger, P.; Fries, L. *Phytochemistry* **1974**, *13*, 2273.
(11) Kurihara, H.; Mitani, T.; Kawabata, J.; Takahashi, K. *J. Nat. Prod.* **1999**, *62*, 882−884.
(12) Craigie, J. S.; Gruenig, D. E. *Science* **1967**, *157*, 1058−1059.
(13) Kamikawa, T.; Yamagiwa, Y.; Arizuka, M. *J. Nat. Prod.* **1990**, *53*, 50−56.
(14) Shoeib, N. A.; Bibby, M. C.; Blunden, G.; Linley, P. A.; Swaine, D. J.; Wheelhouse, R. T.; and Wright, C. W. *J. Nat. Prod.* **2004**, *67*, 1445−1449.
(15) Song, F.-H.; Fan, X.; Xu, X.-L.; Zhao, J.-L.; Yang, Y.-C.; Shi, J.-G. *J. Nat. Prod.* **2004**, *67*, 1644−1649.
(16) Xu, X.-L.; Fan, X.; Song, F.-H.; Zhao, J.-L.; Han, L.-J.; Yang, Y.-C.; Shi, J.-G. *J. Asian Nat. Prod. Res.* **2004**, *6*, 217−221.
(17) Xu, X.-L.; Song, F.-H.; Wang, S.-J.; Li, S.; Fan, X.; Zhao, J.-L.; Yang, Y.-C.; Shang, S.-Q.; Lu, Y.; Shi, J.-G. *J. Nat. Prod.* **2004**, *67*, 1661−1666.
(18) Song, F.-H.; Fan, X.; Xu, X.-L.; Zhao, J.-L.; Han, L.-J.; Shi, J.-G.*J. Asian Nat. Prod. Res.* **2005** (in press).
(19) Fan, X.; Xu, N.-J.; Shi, J.-G. *J. Nat. Prod.* **2003**, *66*, 455−458.
(20) Zhao, J.-L.; Fan, X.; Wang, S.-J.; Li, S.; Shang, S.-Q.; Yang, Y.-C.; Xu, N.-J.; Lu, Y.; Shi, J.-G. *J. Nat. Prod.* **2004**, *67*, 1032−1035.
(21) Mosumann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.
(22) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936−942.

NP040234M