# Bromophenols Coupled with Nucleoside Bases and Brominated Tetrahydroisoquinolines from the Red Alga *Rhodomela confervoides*[⊥]

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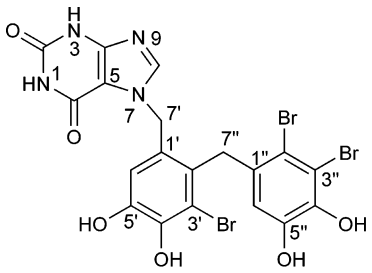
*Received August 12, 2006*

Three new bromophenols C−N coupled with nucleoside base derivatives (**1**−**3**) and three new brominated 1,2,3,4-tetrahydroisoquinolines (**5**−**7**), together with a new brominated tyrosine derivative (**4**), have been isolated from polar fractions of an ethanolic extract of the red alga *Rhodomela confervoides*. By spectroscopic and chemical methods including HRMS and 2D NMR data, their structures were determined as 7-[3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl]-3,7-dihydro-1*H*-purine-2,6-dione (**1**), 7-(2,3-dibromo-4,5-dihydroxybenzyl)-3,7-dihydro-1*H*-purine-2,6-dione (**2**), 9-[3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl]adenine (**3**), (−)-8*S*-(3-bromo-5-hydroxy-4-methoxy)phenylalanine (**4**), (−)-3*S*-8-bromo-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**5**), methyl (−)-3*S*-8-bromo-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**6**), and methyl (−)-3*S*-6-bromo-8-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**7**). Compounds **5**−**7** were semisynthesized by using **4** as the starting material.

As part of a program to systematically assess the chemical and biological diversity of seaweeds distributed along the gulf of the Yellow Sea, 35 bromophenols of diverse structural types, including monoaryl, diaryl, and triaryl bromophenols, and a unique bromophenol sulfoxide, as well as unusual bromophenols coupled with amino acid derivatives and C−N coupled deoxyguanosine nucleoside, together with bromophenol sulfates have been characterized from an EtOAc-soluble fraction of the alcoholic extract of *Rhodomela confervoides* (Huds.) Lamour (Rhodomelaceae).[1−4] Continuing our investigation of the more polar fractions from the same material, we report herein the isolation and structural elucidation of three new bromophenols coupled with nucleoside base derivatives (**1**−**3**), a new brominated tyrosine derivative (**4**), and three new bromosubstituted 1,2,3,4-tetrahydroisoquinoline derivatives (**5**−**7**). The co-occurrence of **4**−**7** and other bromophenols in this alga indicates that bromination of the aromatic amino acids (tyrosine and/or phenylalanine) plays important roles in the biosynthesis of these compounds. This is the first report of bromophenols coupled to nucleoside base derivatives.



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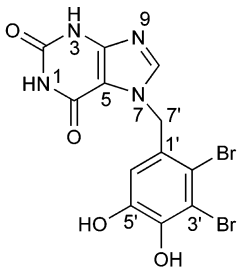

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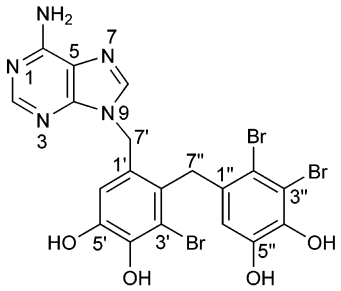

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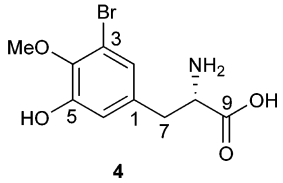
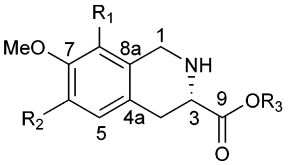


**4**



**5** $R_1$ = Br, $R_2$ = OH, $R_3$ = H
**6** $R_1$ = Br, $R_2$ = OH, $R_3$ = Me
**7** $R_1$ = OH, $R_2$ = Br, $R_3$ = Me

## Results and Discussion

Compound **1** was obtained as a white, amorphous solid. The IR spectrum (KBr) showed absorption bands for hydroxy (3159 $cm^{-1}$), conjugated carbonyl (1693 $cm^{-1}$), and aromatic (1568, 1495 $cm^{-1}$) functional groups. The FABMS spectrum gave a characteristic tribrominated quasi-molecular ion peak cluster at *m*/*z* 631/633/635/637 (1:3:3:1) $[M + H]^+$, and HRFABMS at *m*/*z* 630.8490 $[M + H]^+$ indicated the molecular formula as $C_{19}H_{13}Br_3N_4O_6$ (calcd for $C_{19}H_{14}{}^{79}Br_3N_4O_6$, 630.8463). The NMR spectra of **1** exhibited diagnostic resonances attributed to the 3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl moiety identical to the co-occurring bromophenol derivatives containing nitrogen.[4] In addition, an aromatic singlet at $\delta$ 7.71 (1H, s, H-8) and two broad exchangeable singlets at $\delta$ 11.54 (1H, s, N*H*-1) and 10.83 (1H, s, N*H*-3) in the $^1$H NMR spectrum and a strong fragment ion peak at *m*/*z* 153 in the FABMS spectrum suggested the presence of a xanthine moiety in **1**. This was confirmed by five characteristic $sp^2$ carbon signals including a methine at $\delta$ 142.5 (C-8) and four quarternary carbons at $\delta$ 155.3 (C-6), 151.0 (C-2), 149.1 (C-4), and 106.3 (C-5) in the $^{13}$C NMR and DEPT spectra. The spectroscopic data indicated that **1** is a bromophenol derivative coupled to xanthine. In order to further confirm the presence of the bromophenol and xanthine moieties and determine their

[⊥] Dedicated to the late Dr. Kenneth L. Rinehart of the University of Illinois at Urbana−Champaign for his pioneering work on bioactive natural products.

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10.1021/np060400l CCC: $37.00 
Published on Web 01/19/2007

**Table 1.** NMR Data for Compounds **1**−**3**[a]

| | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|
| no. | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 2 | | 151.0 qC | | 150.8 qC | 8.11 s | 152.2 CH |
| 4 | | 149.1 qC | | 149.8 qC | | 149.2 qC |
| 5 | | 106.3 qC | | 106.1 qC | | 118.4 qC |
| 6 | | 155.3 qC | | 155.0 qC | | 156.1 qC |
| 8 | 7.71 s | 142.5 CH | 7.98 s | 142.7 CH | 7.97 s | 140.4 CH |
| 1-N*H* | 11.54 brs | | | | | |
| 3-N*H* | 10.83 brs | | | | | |
| 6-N*H* | | | | | 7.32 brs | |
| 1′ | | 127.2 qC | | 127.6 qC | | 126.9 qC |
| 2′ | | 126.8 qC | | 113.2 qC | | 127.2 qC |
| 3′ | | 114.6 qC | | 112.4 qC | | 115.3 qC |
| 4′ | | 142.8 qC | | 143.8 qC | | 143.6 qC |
| 5′ | | 144.6 qC | | 144.9 qC | | |
| 6′ | 6.47 s | 113.7 CH | 6.29 s | 113.0 CH | 6.54 s | 114.3 CH |
| 7′ | 5.20 s | 46.8 $CH_2$ | 5.37 s | 49.8 $CH_2$ | 5.11 s | 43.6 $CH_2$ |
| 1″ | | 129.7 qC | | | | 129.3 qC |
| 2″ | | 114.4 qC | | | | 114.2 qC |
| 3″ | | 113.0 qC | | | | |
| 4″ | | 142.5 qC | | | | 143.1 qC |
| 5″ | | 144.8 qC | | | | |
| 6″ | 5.99 s | 113.6 CH | | | 6.05 s | 113.4 CH |
| 7″ | 4.08 s | 38.7 $CH_2$ | | | 4.13 s | 38.3 $CH_2$ |

[a] Data were measured in DMSO-$d_6$, for **1**−**3** at 500 MHz for $^1$H and 125 MHz for $^{13}$C NMR. Assignments are based on DEPT, $^1$H−$^1$H COSY, HMQC, and HMBC experiments. qC represents the quaternary carbon resonance.

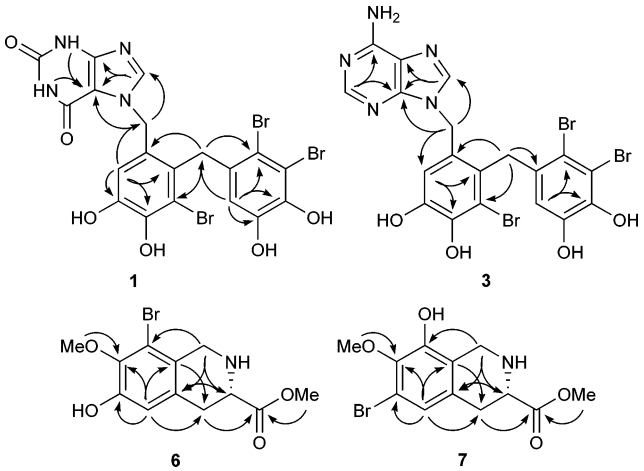



**Figure 1.** Major HMBC correlations of compounds **1**, **3**, **6**, and **7**.

connectivity, HMQC and HMBC experiments of **1** were carried out. The HMQC spectroscopic analysis led to the unambiguous assignment of the proton and carbon resonances (Table 1). In the HMBC spectrum, two- and three-bond correlations (Figure 1) from H-6′ to C-2′, C-4′, C-5′, and C-7′, from H-6″ to C-2″, C-4″, C-5″, and C-7″, from $H_2$-7′ to C-2′ and C-6′, and from $H_2$-7″ to C-1′, C-3′, C-2″, and C-6″, in combination with chemical shifts of these carbons (Table 1), proved unambiguously the presence of the 3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl moiety in **1**. In addition, HMBC correlations from H-8 to C-4 and C-5 and from the two nitrogen protons to C-5, together with the chemical shifts of these protons and carbons, as well as chemical shifts of the two remaining oxygenated carbons C-2 and C-6, confirmed the presence of the xanthine moiety. HMBC correlations from $H_2$-7′ to both C-5 and C-8 demonstrated unequivocally that the 3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl moiety was located at N-7 of the xanthine moiety. Consequently, the structure of **1** was determined as 7-[3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl]-3,7-dihydro-1*H*-purine-2,6-dione.

Compound **2** was obtained as a white powder and showed IR and UV spectroscopic data similar to those of **1**. The FABMS spectrum gave a quasi-molecular ion peak cluster at *m*/*z* 431/433/435 (1:2:1) $[M + H]^+$, and the molecular formula $C_{12}H_8Br_2N_4O_4$ was indicated by HRFABMS at *m*/*z* 430.8968 $[M + H]^+$ (calcd for $C_{12}H_9{}^{79}Br_2N_4O_4$, 430.8990). The NMR spectra of **2** showed resonances (Table 1) of the xanthine moiety identical to that of **1**, in addition to characteristic resonances of a 2,3-dibromo-4,5-dihydroxybenzyl unit.[4] Comparison of the NMR data of **1** and **2** indicated that the 3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl moiety in **1** was replaced by the 2,3-dibromo-4,5-dihydroxybenzyl unit in **2**. Thus, the structure of **2** was established as 7-(2,3-dibromo-4,5-dihydroxybenzyl)-3,7-dihydro-1*H*-purine-2,6-dione.

Compound **3**, a minor component, was obtained as a brown gum. The ESIMS exhibited a characteristic tribrominated quasi-molecular ion peak cluster at *m*/*z* 614/616/618/620 (1:3:3:1) $[M + H]^+$, and the molecular formula $C_{19}H_{14}Br_3N_5O_4$ was indicated by HRESIMS. The $^1$H NMR spectrum of **3** resembled that of **1** except for resonances assignable to an adenine moiety at $\delta$ 8.11 (1H, s, H-2), 7.97 (1H, s, H-8), and 7.32 (2H, brs, exchangeable, N$H_2$) of **3** replaced the resonances of the xanthine moiety of **1**. Due to the quantitative limitations of **3**, its $^{13}$C NMR data could not be directly obtained by a normal-phase NMR probe at 125 MHz. However, by using a reversed-phase nanoprobe, the $^{13}$C NMR data of **3** (Table 1) were obtained from HSQC and HMBC experiments. Chemical shift values of carbons bonded to protons were obtained from correlation peaks in the HSQC spectrum of **3**, while chemical shift values of quaternary carbons were determined from correlation peaks (Figure 1) in the HMBC spectrum of **3**. These data confirmed the presence of 3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl and adenine moieties. HMBC correlations from H-7′ to C-4 and C-8 proved that the bromophenol moiety was located at N-9 of the adenine moiety. Therefore, the structure of **3** was determined as 9-[3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl]adenine.

Compound **4** was obtained as colorless needles, mp 251−252 °C, $[\alpha]_D^{20}$ −19.9 (*c* 0.5, $H_2O$). The IR spectrum showed absorption bands for hydroxy and/or amino (3195 $cm^{-1}$), carbonyl (1637 $cm^{-1}$), and aromatic (1581 and 1529 $cm^{-1}$) functional groups. The positive and negative ESIMS of **4** exhibited monobrominated quasi-molecular ion peak clusters at *m*/*z* 290/292 (1:1) $[M + H]^+$ and 288/290 (1:1) $[M- H]^-$, respectively. The molecular formula $C_{10}H_{12}BrNO_4$ was indicated by HRESIMS at *m*/*z* 287.9871 $[M - H]^-$ (calcd for $C_{10}H_{11}{}^{79}BrNO_4$, 287.9872). In the $^1$H NMR spectrum of **4**, two mutually coupled aromatic protons at $\delta$ 7.09 (1H, d, $J$ = 1.5 Hz, H-2) and 6.86 (1H, d, $J$ = 1.5 Hz, H-6) and an aromatic methoxy singlet at $\delta$ 3.86 (3H, s, O*Me*) indicated the presence of a 1,3,4,5-tetrasubstituted phenyl unit with O-methyl as one of the substituents. In addition, an ABX spin system at $\delta$ 3.95 (1H, dd, $J$ = 8.0 and 5.0 Hz, H-8), 3.18 (1H, dd, $J$ = 14.5 and 5.0 Hz, H-7a), and 3.02 (1H, dd, $J$ = 14.5 and 8.0 Hz, H-7b) indicated the presence of a $CH_2CH$ unit. The $^{13}$C NMR and DEPT spectra of **4** (Table 2) showed 10 carbon resonances corresponding to the above two units in addition to an additional carboxylic carbon at $\delta$ 173.8. The two deshielded quaternary carbons ($\delta$ 144.0 and 150.2) and two shielded quaternary carbons ($\delta$ 133.7 and 117.3) suggested that the aromatic unit is 3-bromo-5-hydroxy-4-methoxy substituted.[2] The chemical shifts and coupling patterns of the $CH_2CH$ unit in combination with the molecular composition of **4** revealed that the methylene carbon must be connected with C-1 of the 3-bromo-5-hydroxy-4-methoxyphenyl unit and that the methane carbon has to be substituted by the carboxyl and amino groups. This deduction was further confirmed by 2D NMR data including $^1$H−$^1$H COSY, HSQC, and HMBC experiments. Therefore, the structure of **4** was determined

**Table 2.** NMR Data for Compounds **4**−**7**[a]

| | **4** | | **5** | | **6** | | **7** | | **5a** | |
|---|---|---|---|---|---|---|---|---|---|---|
| no. | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | | 133.7 qC | 4.05 d (15.5)<br>4.27 d (15.5) | 46.1 $CH_2$ | 3.78 d (16.0)<br>4.03 d (16.0) | 48.3 $CH_2$ | 3.74 d (16.5)<br>4.03 d (16.5) | 43.1 $CH_2$ | 3.50 d (15.5)<br>3.78 d (15.5) | 42.8 $CH_2$ |
| 2 | 7.09 d (1.5) | 125.3 CH | | | | | | | | |
| 3 | | 117.3 qC | 3.74 (11.5, 4.5) | 57.2 CH | 3.65 dd (9.0, 4.5) | 56.1 CH | 3.64 dd (10.0, 4.5) | 56.1 CH | 3.37 m (10.0, 4.5) | 54.6 CH |
| 4 | | 144.0 qC | α 2.98 dd (17.0, 11.5)<br>β 3.20 dd (17.0, 4.5) | 30.5 $CH_2$ | α 2.80 dd (16.0, 9.0)<br>β 2.92 dd (16.0, 4.5) | 31.8 $CH_2$ | α 2.81 dd (16.0, 10.0)<br>β 2.94 dd (16.0, 4.5) | 31.5 $CH_2$ | 2.90 m | 31.5 $CH_2$ |
| 4a | | | | 131.2 qC | | 131.8 qC | | 132.0 qC | | 131.1 qC |
| 5 | | 150.2 qC | 6.70 s | 117.1 CH | 6.60 s | 117.1 CH | 6.79 s | 124.1 CH | 6.65 s | 116.4 CH |
| 6 | 6.86 d (1.5) | 117.3 CH | | 152.0 qC | | 150.7 qC | | 115.3 qC | | 117.2 qC |
| 7 | 3.02 dd (14.5, 8.0)<br>3.18 dd (14.5, 5.0) | 35.8 $CH_2$ | | 146.0 qC | | 145.2 qC | | 144.1 qC | | 143.6 qC |
| 8 | 3.95 dd (8.0, 5.0) | 61.0 CH | | 117.9 qC | | 118.0 qC | | 148.8 qC | | 149.7 qC |
| 8a | | | | 120.0 qC | | 125.5 qC | | 123.3 qC | | 124.2 qC |
| 9 | | 173.8 qC | | 173.1 qC | | 174.0 qC | | 174.2 qC | | 173.6 qC |
| O*Me* | 3.86 s | 55.9 $CH_3$ | 3.74 s | 60.8 $CH_3$ | 3.71 s | 60.7 $CH_3$ | 3.71 s | 61.1 $CH_3$ | 3.68 s | 61.7 $CH_3$ |
| COO*Me* | | | | | 3.71 s | 60.7 $CH_3$ | 3.71 s | 61.1 $CH_3$ | | |

[a] Data were measured at 500 MHz for $^1H$ and 125 MHz for $^{13}C$ NMR, in $D_2O$ for **4**, in methanol-$d_4$ for **5**−**7**, and in DMSO-$d_6$ for **5a**. Coupling constants (*J*) in Hz are given in parentheses. Assignments are based on DEPT, $^1H-^1H$ COSY, HMQC, and HMBC experiments. qC represents the quaternary carbon resonance.

as 3-bromo-5-hydroxy-4-methoxyphenylalanine. The 8*S* absolute configuration was assigned on the basis of the negative optical rotation.[5−7]

Compound **5** was obtained as a white, amorphous powder, $[\alpha]_D^{20}$ −99.1 (*c* 0.11, MeOH). The FABMS of **5** exhibited a quasi-molecular ion peak cluster with one bromine atom at *m*/*z* 302/304 (1:1) $[M + H]^+$, and the molecular formula was determined as $C_{11}H_{12}BrNO_4$ with six degrees of unsaturation by HRFABMS at *m*/*z* 302.0047 $[M + H]^+$ (calcd for $C_{11}H_{13}{}^{79}BrNO_4$, 302.0028). The IR, UV, and NMR features of **5** were similar to those of **4** except that the resonance assigned to H-2 in the $^1H$ NMR spectrum of **4** was replaced by an AB spin system attributed to an isolated methylene unit at $\delta$ 4.27 (1H, d, $J$ = 15.5 Hz, H-1a) and 4.05 (1H, d, $J$ = 15.5 Hz, H-1b) in the spectrum of **5**. In addition, the coupling constants of the ABX spin system changed from 14.5 ($J_{AB}$), 8.0 ($J_{AX}$), and 5.0 ($J_{BX}$) Hz of **4** to 17.0 ($J_{AB}$), 11.5 ($J_{AX}$), and 4.5 ($J_{BX}$) Hz of **5**. In the $^{13}C$ NMR spectrum of **5**, the isolated methylene carbon resonated at $\delta$ 46.1, and a comparison of the $^{13}C$ NMR data of **4** and **5** indicated that the aromatic methine carbon ($\delta$ 125.3, C-2) of **4** was replaced by a quaternary carbon at $\delta$ 120.0 of **5**. The methylene and methine carbons of the ABX spin system of **5** were shielded by $\Delta\delta$ 5.3 and 2.8 ppm, respectively, compared to those of **4**. These spectroscopic data suggested that **5** is 8-bromo-6-hydroxy-7-methoxy-1,2,3,4-tetrohydroisoquinoline-3-carboxylic acid. This deduction and the NMR data assignment were further confirmed by 2D NMR experiments of **5** (Supporting Information). In the $^1H$ NMR spectrum of **5**, the coupling constants of H-3 with H-4α and H-4β (11.5 and 4.5 Hz) indicated that **5** possesses a gauche-(−) conformation.[8,9] The 3*S* absolute configuration is confirmed by the negative optical rotation of **5**.[10,11] Therefore, the structure of **5** is (−)-(3*S*)-8-bromo-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

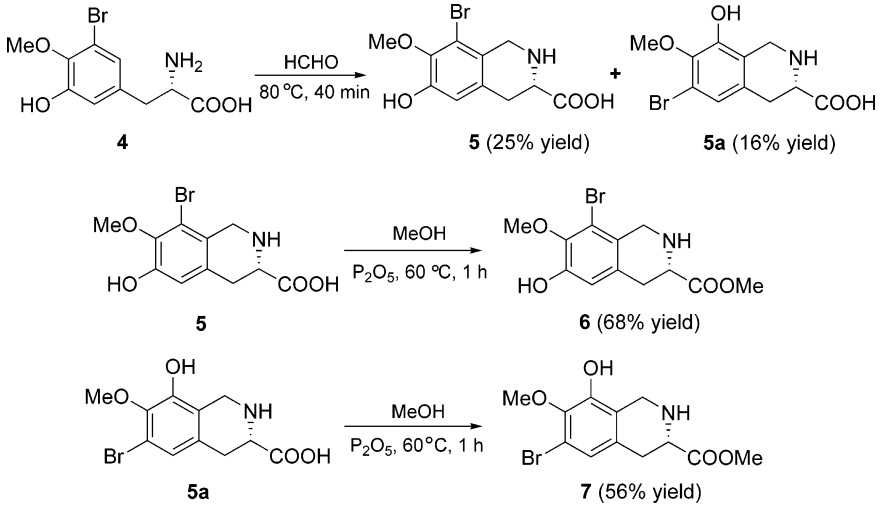
Compound **6** was obtained as a brown gum, $[\alpha]_D^{20}$ −49.6 (*c* 0.07, MeOH). The UV, IR, and NMR features resembled those of **5** except that the NMR spectra of **6** showed two overlapped methoxy resonances at $\delta_H$ 3.71 (6H, s) and $\delta_C$ 60.7, instead of one in **5**. The EIMS exhibited a monobrominated quasi-molecular ion peak cluster at *m*/*z* 315/317 (1:1) $[M]^+$, and HREIMS indicated that it possessed the molecular formula $C_{12}H_{14}BrNO_4$ with a $CH_2$ unit more than that of **5**. These data revealed that **6** is a methylated derivative of **5**, which was confirmed by 2D NMR data of **6** (Supporting Information). In the HMBC spectrum of **6**, three-bond correlations from the methoxy protons at $\delta_H$ 3.71 (6H, s) to C-9 demonstrated unambiguously that **6** is the methyl ester of **5**. Therefore, the structure of compound **6** was determined as methyl (−)-3*S*-8-bromo-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate.

Compound **7** was obtained as a brown gum, $[\alpha]_D^{20}$ −29.0 (*c* 0.07, MeOH). The UV, IR, and EIMS spectra of **7** were very similar to those of **6**. The HREIMS of **7** also indicated that it had the molecular formula $C_{12}H_{14}BrNO_4$, identical to that of **6**. However, in the NMR spectra of **7**, H-5 and C-5 were deshielded, respectively, by $\Delta\delta_H$ 0.19 and $\Delta\delta_C$ 7.0 ppm, while C-1 and C-8a were shielded by, respectively, $\Delta\delta_C$ 5.2 and 2.2 ppm, compared to those of **6**. These spectroscopic data indicated that **7** is an isomer of **6** with hydroxy at C-8 and bromine at C-6. This was confirmed by 2D NMR experiments of **7**. In the HMBC spectrum of **7**, three-bond correlations from $H_2$-1 to C-3, C-4a, and C-8, from H-5 to C-4, C-7, and C-8a, and from the methoxy protons to C-7 (Figure 1) in combination with chemical shifts of these protons and carbons (Table 2) demonstrated unequivocally that bromine, methoxy, and hydroxy were located at C-6, C-7, and C-8 of the 1,2,3,4-tetrahydroisoquinoline parent structure, respectively. Therefore, the structure of **7** was determined as methyl (−)-3*S*-6-bromo-8-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate.

From a biogenetic point of view, **4** may be the precursor of **5**−**7**. Based on this hypothesis a semisynthesis of **5**−**7** was readily accomplished by using **4** as the starting material[12,13] (Scheme 1). Although the isomer of **5** (**5a**) was obtained in the semisynthesis, it was not detectable in the fractions of the extract by TLC and HPLC analyses. The methylation of **5** and **5a** was accomplished in high yields by stirring a methanol solution of **5** or **5a** with $P_2O_5$ at 60 °C for 1 h, but the methylation did not occur under the simulated isolation conditions, by heating a MeOH solution of **5** or **5a**, either with or without silica gel at 45 °C for 48 h. These experiments supported that **4**−**7** are natural products rather than artifacts.

That bromophenols in *Odonthalia floccosa* (Rhodophyceae) are biogenetically derived from the bromination of oxidation products

**Scheme 1.** Semisynthesis of Compounds **5**−**7**




of L-tyrosine (such as 4-hydroxybenzaldehyde) was indicated in several reports.[14−16] Bromoperoxidases have been proven to play a key role in the enzymatic bromination process of marine algae.[17,18] However, our systematic investigation has revealed that bromophenols in *R. confervoides* possess some diversity, such as derivatives of benzoic acid, phenylacetic acid, and phenylpropionic acid, as well as their aldehydic and alcoholic forms. The isolation of **4** indicated that the aromatic ring of L-tyrosine derivatives may be one of the substrates of bromoperoxidases and that the bromination may occur also at the very beginning steps of the L-tyrosine metabolism in this alga. On the basis of the isolation of bromophenols C−N coupled with amino acid, nucleoside, and base derivatives from this alga, we hypothesize that there is an unprecedented enzymatic catalyzed nitrogen alkylation process in the biosynthesis of these metabolites. This was supported by our unsuccessful efforts on the semisynthesis of these metabolites under different conditions. The coupling reaction of the isolated bromophenols such as 2,3-dibromo-4,5-dihydroxybenzyl alcohol and 2,3-dibromo-4,5-dihydroxybenzaldehyde to amino acid or nucleoside base derivatives such as $\gamma$-ureidobutyric acid, *S*-pyroglutamic acid, xanthine, and adenine did not give the expected products.

## Experimental Section

**General Experimental Procedures.** Melting points were determined on an XT-4 micro melting point apparatus. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter. 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 $D_2O$, DMSO-$d_6$, or methanol-$d_4$, with solvent peaks (or TMS, in the case of $D_2O$) being used as references. FABMS and HRFABMS data were measured employing a Micromass Autospec-Ultima ETOF spectrometer, and ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo ionspray source) spectrometer. HRESIMS data were measured using an AccuToFCS JMS-T100CS spectrometer. Column chromatography was performed over normal-phase silica gel (200−300 mesh), RP-18 reversed-phase silica gel (43−60 $\mu$m), and Sephadex LH-20. HPLC separation was performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual $\lambda$ absorbance detector with an Alltima (250 × 22 mm) preparative column packed with $C_{18}$ RP material (10 $\mu$m). TLC was carried out using glass precoated silica gel $GF_{254}$ plates. Plates were visualized under UV light (254 nm) or by spraying with 3% $FeCl_3$ in EtOH followed by heating.

**Plant Material.** This was described in a previous report.[1]

**Extraction and Isolation.** The preliminary separation procedure has been described in a previous report.[1] The fraction XII (6.62 g) obtained from Si gel CC of the EtOAc-soluble portion was subjected to reversed-phase MPLC over $C_{18}$ reversed-phase silica gel (500 g) and eluted with a gradient increasing MeOH in $H_2O$ to give five subfractions, $l_1$−$l_5$. Subfraction $l_3$ (1.12 g) was chromatographed over Sephadex LH-20 eluting with $CHCl_3$−MeOH (1:1) to give three fractions, $l_{3a}$−$l_{3c}$. Fraction $l_{3b}$ (0.11 g) was then separated by reversed-phase preparative HPLC using MeOH−$H_2O$−AcOH (60:40:0.1) as mobile phase to give **1** (17 mg) and **2** (13 mg). Fractions $r_6$ (0.21 g) and $r_7$ (0.16 g) obtained from $C_{18}$ reversed-phase MPLC of the combined fractions XVIII−XXIV[4] were separately chromatographed over Sephadex LH-20 with MeOH as mobile phase to give corresponding subfractions $r_{6a}$ and $r_{6b}$, and $r_{7a}$ and $r_{7b}$. Subfractions $r_{6b}$ (0.05 g) and $r_{7b}$ (0.03 g) were separately purified by reversed-phase HPLC, using a mobile phase of MeOH−$H_2O$ (23:77) for $r_{6b}$, to yield **5** (9 mg), and MeOH−$H_2O$ (70:30) for $r_{6b}$, to yield **3** (0.5 mg). The $H_2O$-soluble portion of the extract was chromatographed over D 101 macroporous resin. Successive elution with $H_2O$, EtOH−$H_2O$ (50:50), and EtOH−$H_2O$ (95:5) gave three corresponding fractions, $w_1$−$w_3$. Fraction $w_2$ (42.9 g) was subjected to reversed-phase MPLC eluting with a gradient of increasing MeOH (0%−100%) in $H_2O$ to give five subfractions, $w_{2a}$−$w_{2e}$. Subfraction $w_{2b}$ (1.1 g) was dissolved by MeOH and then filtered. The insoluble precipitate was washed with MeOH to yield a white, amorphous powder, **4** (302 mg). The filtrate was chromatographed over silica gel, eluting with a gradient of increasing MeOH (1−100%) in $CHCl_3$, to give four subfractions, $w_{2b1}$−$w_{2b4}$. Fraction $w_{2b3}$ (0.04 g) was chromatographed over Sephadex LH-20 eluting with MeOH to give two fractions, $w_{2b3a}$ and $w_{2b3b}$, and then fraction $w_{2b3b}$ (0.02 g) was purified by reversed-phase preparative HPLC using MeOH−$H_2O$ (78:22) as mobile phase to yield **6** (4 mg) and **7** (3 mg).

**7-[3-Bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl]-3,7-dihydro-1*H*-purine-2,6-dione (1):** white, amorphous solid; IR (KBr) $\nu_{max}$ 3159, 2925, 1693, 1568, 1495, 1406, 1329, 1281, 1174, 1132, 1086, 860, 812, 752, 706, 602, 525, 501 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 500 MHz) and $^{13}$C NMR (DMSO-$d_6$, 125 MHz) data, see Table 1; FABMS *m*/*z* 637/635/633/631 (13:35:36:14) [M + H]$^+$, 557 (4), 555 (8), 553 (5), 404 (6), 402 (10), 400 (6), 330 (16), 318 (21), 302 (24), 153 (100), 135 (20), 103 (31), 85 (38); HRFABMS *m*/*z* 630.8490 [M + H]$^+$ (calcd for $C_{19}H_{14}{}^{79}Br_3N_4O_6$, 630.8463).

**7-(2,3-Dibromo-4,5-dihydroxybenzyl)-3,7-dihydro-1*H*-purine-2,6-dione (2):** white powder; IR (KBr) $\nu_{max}$ 3350, 2925, 1695, 1568, 1473, 1385, 1331, 1255, 1198, 1024, 895, 825, 766, 706, 613, 521, 488 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 500 MHz) and $^{13}$C NMR (DMSO-$d_6$, 125 MHz) data, see Table 1; FABMS *m*/*z* 435/433/431 (28:53:24) [M + H]$^+$, 330 (10), 318 (13), 274 (100), 207 (14), 153 (30), 115 (25); HRFABMS *m*/*z* 430.8968 [M + H]$^+$ (calcd for $C_{12}H_9{}^{79}Br_2N_4O_4$, 430.8990).

**9-(3-Bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl)adenine (3):** brown gum; $^1$H NMR (DMSO-$d_6$, 500 MHz) and $^{13}$C NMR (DMSO-$d_6$, 125 MHz) data, see Table 1; ESIMS *m*/*z* 620/618/616/614 (30:98:100:35) [M + H]$^+$; HRESIMS *m*/*z* 613.8642 (calcd for $C_{19}H_{15}{}^{79}Br_3N_5O_4$, 613.8674).

**(−)-8*S*-3-Bromo-5-hydroxy-4-methoxyphenylalanine (4):** colorless needles; mp 251−252 °C; $[\alpha]_D^{20}$ −19.9 (*c* 0.50, $H_2O$); UV ($H_2O$) $\lambda_{max}$ (log $\epsilon$) 203 (4.71), 281 (3.38) nm; IR (KBr) $\nu_{max}$ 3195, 2929, 1637,

1581, 1529, 1427, 1398, 1325, 1296, 1234, 1144, 1005, 879, 837 $cm^{-1}$; $^1H$ NMR ($D_2O$, 500 MHz) and $^{13}C$ NMR ($D_2O$, 125 MHz) data, see Table 2; (+)-ESIMS *m/z* 292/290 $[M + H]^+$ (96:100); (−)-ESIMS *m/z* 581/579/577 (32:60:30) $[2M - H]^-$, 290/288 (90:100) $[M - H]^-$; (−)-HRESIMS *m/z* 287.9871 $[M - H]^-$ (calcd for $C_{10}H_{11}{}^{79}BrNO_4$, 287.9872).

**(−)-3*S*-8-Bromo-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5):** white powder; $[\alpha]_D^{20}$ −99.1 (*c* 0.11, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 207 (4.49), 287 (3.41) nm; IR (KBr) $\nu_{max}$ 3477, 3001, 2943, 1641, 1610, 1570, 1485, 1429, 1396, 1317, 1259, 1115, 991, 837 $cm^{-1}$; $^1H$ NMR (MeOH-$d_4$, 500 MHz) and $^{13}C$ NMR (MeOH-$d_4$, 125 MHz) data, see Table 2; FABMS *m/z* 304/302 (45:45) $[M + H]^+$; HRFABMS *m/z* 302.0047 $[M + H]^+$ (calcd for $C_{11}H_{13}{}^{79}BrNO_4$, 302.0028).

**Methyl (−)-3*S*-8-bromo-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (6):** brown gum; $[\alpha]_D^{20}$ −49.6 (c 0.07, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 205 (4.56), 282 (3.53) nm; IR (KBr) $\nu_{max}$ 3413, 2931, 1715, 1640, 1600, 1570, 1439, 1385, 1281, 1120 $cm^{-1}$; $^1H$ NMR (MeOH-$d_4$, 500 MHz) and $^{13}C$ NMR (MeOH-$d_4$, 125 MHz) data, see Table 2; EIMS *m/z* 317/315 (8:8) $[M]^+$, 302/300 (6:7), 258/256 (90:100), 176 (25); HREIMS *m/z* 315.0123 (calcd for $C_{12}H_{14}{}^{79}BrNO_4$, 315.0106).

**Methyl (−)-3*S*-6-bromo-8-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (7):** brown gum; $[\alpha]_D^{20}$ −29.0 (*c* 0.07, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 206 (4.53), 275 (3.11) nm; IR (KBr) $\nu_{max}$ 3446, 1718, 1644, 1600, 1560, 1421, 1385, 1026 $cm^{-1}$; $^1H$ NMR (MeOH-$d_4$, 500 MHz) and $^{13}C$ NMR (MeOH-$d_4$, 125 MHz) data, see Table 2; EIMS *m/z* 317/315 (9:10) $[M]^+$, 300/298 (5:3), 258/256 (90:100), 176 (20); HREIMS *m/z* 315.0093 (calcd for $C_{12}H_{14}{}^{79}BrNO_4$, 315.0106).

**Semisynthesis of 5−7.** Compound **4** (60.0 mg) was dissolved in $H_2O$ (5.0 mL), and then formaldehyde (2.0 mL) was added. After stirring at 80 °C for 40 min, the reaction mixture was evaporated under reduced pressure to obtain a residue. The residue was dissolved in MeOH (6.0 mL) and filtered. The filtrate was divided into two portions. The first portion (2.0 mL) was isolated by preparative reversed-phase HPLC using MeOH−$H_2O$−HCOOH (15:85:0.2) as mobile phase to yield **5** (5.0 mg) and **5a** (3.2 mg). $P_2O_5$ (0.3 g) was added into the second portion (4.0 mL). After stirring at 60 °C for 1 h, the reaction solution was evaporated to give a residue that was chromatographed over Sephadex LH-20 eluting with $CHCl_3$−MeOH (1:1) to yield a mixture of **6** and **7**. The mixture was further separated by preparative reversed-phase HPLC using MeOH−$H_2O$−HCOOH (30:70:0.2) as mobile phase to yield **6** (6.3 mg) and **7** (3.3 mg). The MS, $^1H$ NMR, and optical rotation data of semisynthesized compounds **5**−**7** were identical to those of the natural products. Compound **5a** was obtained as white needles ($Me_2CO$), mp 213−214 °C; $[\alpha]_D^{20}$ −36.3 (*c* 0.06, MeOH); IR (KBr) $\nu_{max}$ 3471, 2939, 1721, 1611, 1489, 1312, 1255, 1116 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 500 MHz) and $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) data, see Table 2; FABMS *m/z* 304/302 (53:50) $[M + H]^+$.

**Acknowledgment.** The authors are grateful to Prof. A. Zeper for MS measurements, and financial support from the New Century Excellent Talent (NCET) Program of Chinese Ministry of Education, the Natural Sciences Foundation of China (NSFC, Grant No. 20432030), and the National "973" Program of China (Grant No. 2004CB13518906), and the Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT, Grant No. IRT0514).

**Supporting Information Available:** MS and 1D and 2D NMR spectra of compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP060400L