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Isolation, Synthesis, and Radical-Scavenging Activity of Rhodomelin A, a Ureidobromophenol from the Marine Red Alga *Rhodomela confervoides*

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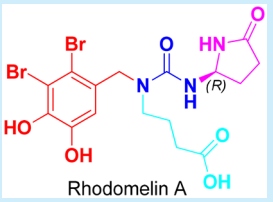
Supporting Information

ABSTRACT: A novel ureidobromophenol, rhodomelin A (**1**), was characterized from *Rhodomela confervoides*. Its structure was elucidated by spectroscopic analysis. Both enantiomers of **1** were synthesized using a convergent strategy starting from D/L-pyroglutamic acids, respectively, allowing assignment of the *R*-configuration for the naturally occurring isomer by chiral HPLC analysis. Rhodomelin A represents the first example of a naturally occurring ureidopyrrolidone alkaloid incorporating a γ-aminobutyric acid unit. The scavenging activity of **1** toward DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate)) radicals was assayed.



The marine red algal species of the genus *Rhodomela* (family Rhodomelaceae, order Ceramiales) have been described as a rich source of bromophenolic compounds.[1] We have previously reported several bromophenols from *Rhodomela confervoides*.[2] Further investigation of the polar fraction of the algal extract has now resulted in the isolation of a novel ureidobromophenol, namely, rhodomelin A (**1**, Figure 1),

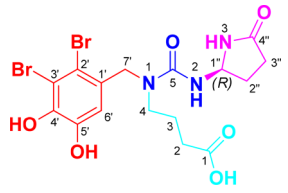


Figure 1. Chemical structure of rhodomelin A (**1**).

which represents the first example of a ureidopyrrolidone alkaloid incorporating γ-aminobutyric acid and 2,3-dibromo-4,5-dihydroxybenzyl units. Compound **1** has a chiral center at C-1″ which is far from the 2,3-dibromo-4,5-dihydroxybenzyl chromophore, thus precluding assignment of its absolute configuration with confidence by ECD methods. Attempts to obtain quality crystals of **1** for X-ray crystallographic analysis were not successful.

We report herein the isolation and structure elucidation of **1**, the synthesis of both possible enantiomers, and subsequent chiral HPLC analysis to determine the absolute configuration of **1**. The ability to scavenge DPPH and ABTS radicals was assayed as well.

An air-dried and ground sample of the marine red alga *R. confervoides* was extracted with 95% EtOH at room temperature. After the solvent was removed under reduced pressure at <40 °C, the residue was suspended in H_2O and then successively partitioned with petroleum ether, EtOAc, and *n*-butanol. The EtOAc extract was purified using a combination of silica gel and Sephadex LH-20 column chromatography steps to yield compound **1**.

Rhodomelin A (**1**), obtained as a brown powder, displayed a characteristic dibrominated pseudomolecular ion cluster at *m*/*z* 510/508/506 (1:2:1) $[M - H]^-$ in the negative ion ESI mass spectrum. The molecular formula was determined to be $C_{16}H_{19}Br_2N_3O_6$ by HRFABMS, indicating eight degrees of unsaturation. The IR spectrum exhibited absorption bands for NH/OH group(s) at 3747 and 3649 cm^{-1}, for carboxy and amide carbonyl groups at 1733 and 1640 cm^{-1}, and for aromatic unit(s) at 1534 and 1448 cm^{-1}.

In examining the ^1H NMR spectrum, signals for two methines (H-6′ and H-1″) and six methylenes (H_2-2 ∼ H_2-4, H_2-7′, H_2-2″, and H_2-3″) as well as two NH protons (NH-2 and NH-3) were observed (Table 1). The ^{13}C NMR data revealed the presence of 16 carbon resonances, which were grouped by DEPT and HSQC spectra into categories of one

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Table 1. ^{1}H and ^{13}C NMR Data for Rhodomelin A (1)[a]

no.	δ_H mult (J, Hz)	δ_C
1		174.5, C
2	2.20, t (7.3)	30.8, CH_2
3	1.68, m	23.2, CH_2
4	3.13, m	45.3, CH_2
5		156.5, C
1′		129.2, C
2′		113.5, C
3′		112.8, C
4′		143.5, C
5′		145.4, C
6′	6.63, s	113.3, CH
7′	4.39, d (16.7)	50.4, CH_2
	4.32, d (16.7)	
1″	5.37, m	62.0, CH
2″	2.29, m	27.5, CH_2
	1.84, m	
3″	2.30, m	29.1, CH_2
	2.05, m	
4″		176.1, C
NH-2	6.98, d (8.1)	
NH-3	7.88, br s	

[a]Recorded in DMSO-d_6 at 500 MHz for ^{1}H and 125 MHz for ^{13}C.

aliphatic and one aromatic methine, six aliphatic methylenes, and eight nonprotonated (five aromatic and three carbonyl) carbon atoms (Table 1). COSY data disclosed two spin systems corresponding to −NHCH(NH)CH_2CH_2− and −$CH_2CH_2CH_2$− substructures (Figure 2). Through iterative

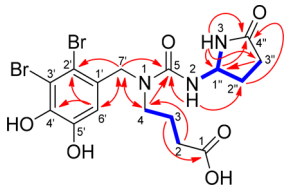


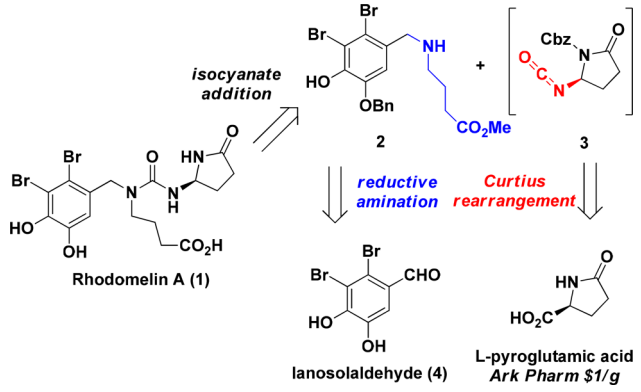
Figure 2. Key HMBC (red arrows) and COSY (blue bold lines) correlations for rhodomelin A (**1**).

analyses of the battery of 1D and 2D NMR spectroscopic data, compound **1** was deduced to have four substructures including a 2,3-dibromo-4,5-dihydroxybenzyl unit,[2] a 4-substituted butanoic acid moiety, a ureido group,[1b] and a pyrrolidone residue. These fragments accounted for all components of the molecular formula and unsaturations. The linkage of one nitrogen atom (N-1) in the ureido unit to C-4 of the butanoic acid moiety and to C-7′ of the 2,3-dibromo-4,5-dihydroxybenzyl unit was supported by HMBC correlations from H-4 to C-5 and from H_2-7′ to C-2′, C-6′, and C-5, respectively, whereas the linkage of another nitrogen atom in the ureido unit (N-2) to the pyrrolidone residue was evidenced by a COSY interaction from NH-2 to H-1″ and by an HMBC correlation from NH-2 to C-2″ (Figure 2). These key correlations enabled assignment of the planar structure of **1** as *N*-(2,3-dibromo-4,5-dihydroxybenzyl)-*N*′-(5-oxopyrrolidin-2-yl)-γ-ureidobutyric acid, which was named rhodomelin A. A hypothetical biosynthetic pathway for **1** is proposed in Scheme S1 (Supporting Information).

Compound **1** has one chiral center at C-1″ and assignment of its absolute configuration was challenging. This chiral center is far from the 2,3-dibromo-4,5-dihydroxybenzyl chromophore and thus ECD calculation was not useful in determining its absolute configuration. Attempts to get quality crystals for X-ray analysis were not successful. We therefore concentrated our efforts toward a total synthesis of both enantiomers of **1**.

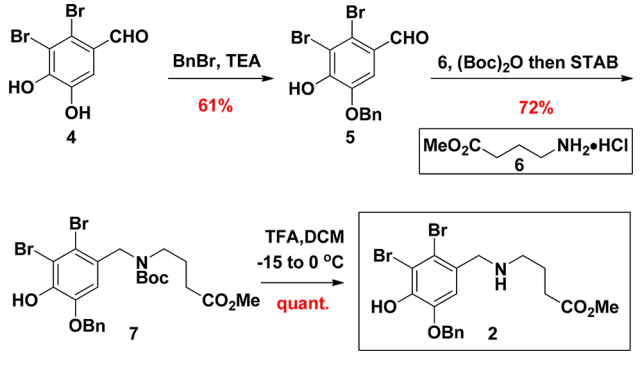
Rhodomelin A (**1**) contains an acid/base-sensitive 5-ureidopyrrolidone unit and a dibromo-substituted phenolic moiety. Retrosynthetically speaking, compound **1** could be disconnected to amino ester **2** and pyroglutamic isocyanate **3** through a nucleophilic 1,2-addition. The former (**2**) could further be derived from lanosolaldehyde (**4**), which could be synthesized from vanillin in two steps,[3] while the latter (**3**) could be generated through Curtius rearrangement[4] from commercially available chiral pyroglutamic acid in a one-pot transformation. This convergent strategy would allow both enantiomers of **1** be accessed through one unified route starting with D/L-pyroglutamic acids (Scheme 1).

Scheme 1. Retrosynthetic Analysis of Rhodomelin A (1)



Our synthesis started with regioselective protection of the known compound lanosolaldehyde **4**,[3] followed by a reductive amination with amino ester salt **6** in 72% yield. The multisubstituted phenolic fragment **2** was obtained readily upon quantitative removal of Boc protecting group (Scheme 2).

Scheme 2. Synthesis of Compound 2



The second required fragment, L-pyroglutamic isocyanate (**3**), was formed diastereoselectively *in situ* through a modified one-pot Curtius rearrangement[4] and was then coupled with fragment **2** to provide a protected version of the conserved chiral fragment **3** in good yield (75%). The Bn- and Cbz-protecting groups were then removed in one step under

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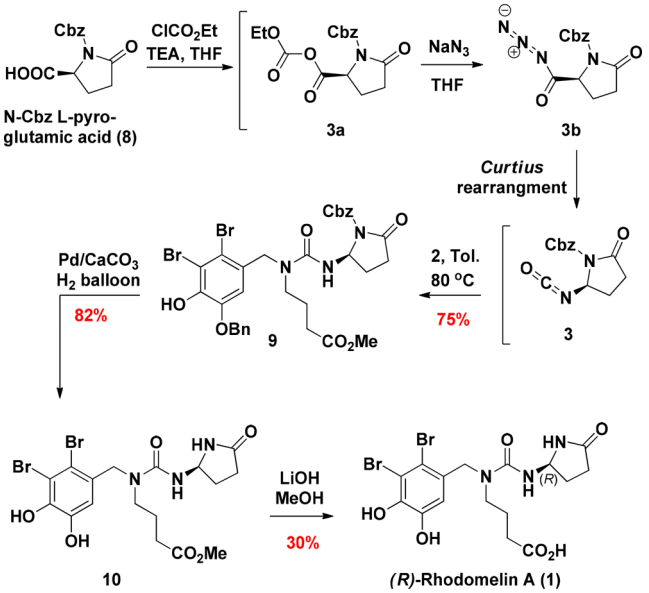
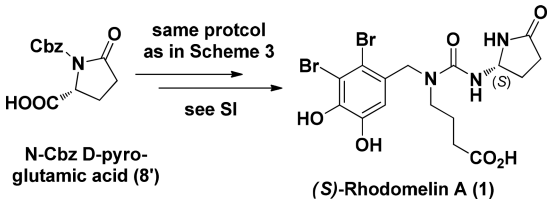
hydrogenation, and an additional LiOH-mediated hydrolysis yielded (*R*)-configured rhodomelin A (Scheme 3).

Scheme 3. Synthesis of (*R*)-Rhodomelin A



The same synthetic sequence was followed starting from D-pyroglutamic acid and yielded (*S*)-rhodomelin A (Scheme 4).

Scheme 4. Synthesis of (*S*)-Rhodomelin A



The NMR data, optical rotations, and chiral HPLC profiles of the natural rhodomelin A (**1**) were compared with those of synthesized (*R*)- and (*S*)-**1**. As shown in Table S1 (Supporting Information), the ^{1}H and ^{13}C NMR data for natural **1** were essentially identical to those of synthesized (*R*)-**1** and (*S*)-**1**. As for specific optical rotation, the value of rhodomelin A ($[\alpha]^{25}_{D}$ +18.5 (*c* 0.09, MeOH)) was in good agreement with that of the synthesized (*R*)-**1** ($[\alpha]^{25}_{D}$ +21.1 (*c* 0.19, MeOH)) and opposite to that of the synthesized (*S*)-**1** ($[\alpha]^{25}_{D}$ −17.9 (*c* 0.28, MeOH)), indicating that the absolute configuration at C-1″ of natural rhodomelin A (**1**) was *R*.

The results from chiral HPLC analysis showed that natural **1** had same retention time and identical UV spectrum as that of synthesized (*R*)-**1**. As expected, natural **1** and synthesized (*S*)-**1** had the same UV profile, but different retention times (Figure 3), further confirming that the absolute configuration at C-1″ of natural **1** is *R*.

The radical-scavenging activities of natural **1** were evaluated using DPPH[5] and TEAC[6] assays following previously reported methods. Compound **1** displayed significant scavenging activity against DPPH with IC_{50} value of 3.82 μM, which is 21.5-fold more potent than that of the positive control BHT (IC_{50} = 82.13 μM). In addition, this compound also exhibited moderate scavenging activity against ABTS radicals with a

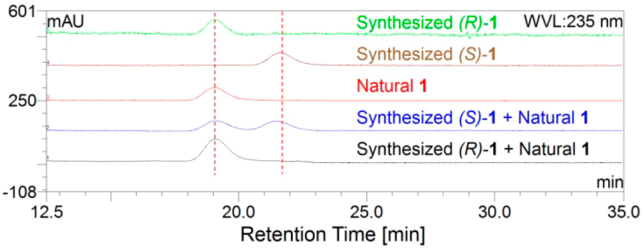


Figure 3. Chiral HPLC profiles of (*R*)-**1**, (*S*)-**1**, natural **1**, the mixture of (*S*)-**1** and natural **1**, and the mixture of (*R*)-**1** and natural **1** (from top to bottom) over a CHIRALPAK AD-H column (eluent: *n*-hexane/2-propanol 75:25, flow rate 1 mL/min), detected at 235 nm.

TEAC value of 4.37 mM (Table 2). The synthetic (*R*)- and (*S*)-**1** were also tested for the activity against DPPH radicals,

Table 2. DPPH and ABTS Radical-Scavenging Activity of 1[a]

compd	DPPH (IC_{50}, μM)	TEAC (mM)
1	3.82 ± 0.01	4.37 ± 0.24
BHT	82.13 ± 0.20	n.t.
ascorbic acid	20.07 ± 0.15	1.02 ± 0.01

[a]Each value is presented as the means ± SD (n = 3). n.t.: not tested.

with the (*R*)-isomer having stronger activity (IC_{50} = 4.60 μM) than that of the (*S*)-isomer (IC_{50} = 8.90 μM).

In conclusion, a novel ureidobromophenol (rhodomelin A, **1**) was isolated and identified from the marine red alga *Rhodomela confervoides*. Its planar structure was determined by analysis of spectroscopic data, and the absolute configuration was unambiguously assigned by total synthesis and chiral HPLC analysis. Rhodomelin A (**1**) represents the first example of a naturally occurring ureidopyrrolidone alkaloid incorporating a γ-aminobutyric unit and showed potent scavenging activity against DPPH and ABTS radicals.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b03716.

Experimental details and spectra for natural and synthesized **1** (PDF)

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Notes

The authors declare no competing financial interest.

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