Isolation and Synthesis of 4-Bromopyrrole-2-carboxyarginine and 4-Bromopyrrole-2-carboxy-*N*(ε)-lysine from the Marine Sponge *Stylissa caribica*§

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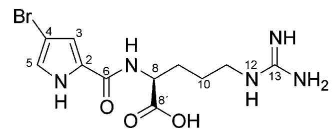
Two new bromopyrrole alkaloids were isolated from the Caribbean sponge *Stylissa caribica*. The new natural products, 4-bromopyrrole-2-carboxyarginine (**1**) and 4-bromopyrrole-2-carboxy-*N*(ε)-lysine (**2**), are derivatives of amino acids linked with a 4-bromopyrrole-2-carboxylic acid. The structures were elucidated on the basis of NMR and MS/MS data and their absolute configurations assigned via synthesis.

The common structural motifs of the pyrrole-imidazole alkaloid family are the bromopyrrole and the aminoimidazole rings. The most prominent member of this group is oroidin,[1] which is the biosynthetic precursor of many complex natural products.[2] In a hypothetical biosynthetic pathway of oroidin the last step is the formation of the amide bond between the bromopyrrole-2-carboxylic acid and the aminopropylimidazole moiety.[3] Some years ago, we have isolated the first pyrrole-imidazole alkaloid with a guanidine function instead of the aminoimidazole (**3**) from the sponge *Agelas wiedenmayeri*.[4] This compound and its decarboxylated derivate laughine (**4**)[5] could be an alternative biosynthetic precursor. Here, we describe the isolation and structure elucidation of two new related compounds. They only differ from **3** by replacement of homoarginine by arginine in **1** and by lysine in **2**. In the lysine derivative the central amide bond is formed with the side chain amino group N(ε) of lysine.

The sponge material was extracted with CH_2Cl_2/MeOH (1:1), and the resulting crude extract was analyzed by HPLC/HRMS. Comparison of the experimental masses with the literature revealed two unknown substances with an isotopic pattern of a singly brominated molecule. The crude extract was partitioned by liquid/liquid extraction. The resulting *n*-BuOH fraction was purified by Sephadex LH-20 chromatography and reversed-phase HPLC.

The structures of 4-bromopyrrole-2-carboxyarginine (**1**) and 4-bromopyrrole-2-carboxy-*N*(ε)-lysine (**2**) were elucidated by 1D and 2D NMR data (Tables 1 and 2) and MS analysis. The positive electrospray mass spectrum of **1** displayed clusters of ion peaks $[M + H]^+$ at *m/z* 346/348. The high-resolution mass of *m/z* 346.0487 indicated the molecular formula $C_{11}H_{17}N_5O_3Br$ ($[M + H]^+$). Examination of the ^1H NMR data revealed the presence of a 4-bromopyrrole-2-carboxamide moiety. The ^{13}C NMR signal at 173.5 ppm and two additional oxygens suggested a carboxyl group. The HMBC correlation from H-8 to C-8′ and the COSY correlation from H-7 to H-8 indicated an *N*-terminal connection between 4-bromopyrrole-2-carboxylic acid and arginine. A positive Sakaguchi reaction[6] and the loss of a guanidine group and ammonia under MS/MS conditions supported the presence of a free guanidine group. The positive electrospray mass spectrum of **2** displayed clusters of ion peaks $[M + H]^+$ at *m/z* 318/320. The high-resolution mass of *m/z* 318.0445 indicated the molecular formula $C_{11}H_{16}N_3O_3Br$ ($[M + H]^+$). Similar to **1** the ^1H NMR data revealed the presence of a 4-bromopyrrole-2-carboxamide moiety. The HMBC correlations from H-7 to C-8 and C-9 as well as a positive ninhydrin reaction[6] on free amino acids proved the connectivity between the side chain amino group of lysine with the 4-bromopyrrole-2-carboxylic acid.

Chart 1. Structural Formulas of 4-Bromopyrrole-2-carboxyarginine (**1**), 4-Bromopyrrole-2-carboxy-*N*(ε)-lysine (**2**), 4-Bromopyrrole-2-carboxyhomoarginine (**3**), and Laughine (**4**)



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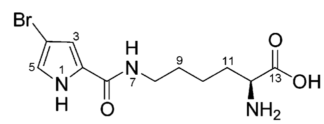

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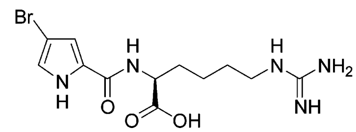

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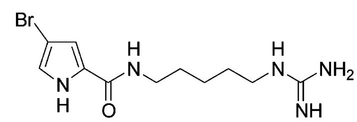

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To assign the absolute configuration of **1** and **2**, it was attempted to hydrolyze the compounds in order to apply Marfey's method. Even with HCl (36%, 18 h, 80 °C) no hydrolysis was observed. Therefore, the syntheses of **1** and **2** were carried out. Reaction of N^G-2,2,5,7,8-pentamethylchroman-6-sulfonyl-L-arginine and 4-bromopyrrol-2-yl trichloromethyl ketone (**5**)[8] at room temperature yielded the protected 4-bromopyrrole-2-carboxyarginine (**6**). Hydrolysis with TFA gave **1** (Scheme 1). The configuration of **1** was obtained by measuring the specific rotation. The natural product appears as L-4-bromopyrrole-2-carboxyarginine since the sign of specific rotation of natural and synthetic product is identical. Reaction of L-lysine ethyl ester with 4-bromopyrrol-2-yl trichloromethyl ketone (**5**)[8] and subsequent ester hydrolysis regioselectively gave **2**.[4b] The configuration of **2** was determined by comparison of optical rotation of natural and synthetic product and additionally by HPLC using Marfey's method.[7] Comparison of

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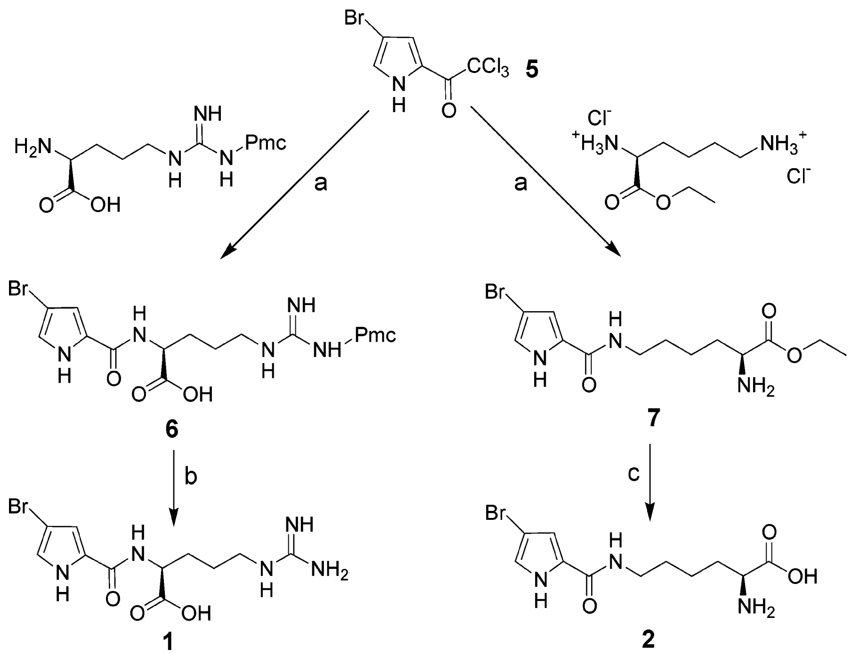
Scheme 1. Solution Phase Synthesis of **1** and **2**[a]



[a] (a) i-Pr$_2$NEt, MeCN, room temperature, 6 h; (b) TFA (98%), 45 min, room temperature; (c) HCl (32%), 22 h, room temperature.

Table 1. NMR Data for 4-Bromopyrrole-2-carboxyarginine (**1**) Recorded in DMSO-d_6[a]

position	δ (^{13}C)/δ(^{15}N)[b] [ppm]	δ (^{1}H) [ppm]	^{1}H,^{1}H-COSY	^{1}H,^{13}C-HMBC
1	(161)	11.84 (1H, s)	3, 5	3, 4
2	126.3			
3	112.1	6.98 (1H, m)	1	2, 5
4	94.9			
5	121.4	7.00 (1H, m)	1	2, 3, 4
6	159.5			
7	(112)	8.20 (1H, d, $J = 8.1$ Hz)	8	6, 8, 9
8	51.5	4.35 (1H, m)	7, 9	6, 8′, 9, 10
8’	173.5			
9	28.0	1.85 (1H, m), 1.70 (1H, m)	8, 10	8, 8′, 10, 11
10	25.3	1.55 (2H, m)	9, 11	8, 9, 11
11	40.3	3.12 (2H, m)	10, 12	9, 10, 13
12	(85)	7.61 (1H, t, $J = 5.5$ Hz)	11	11, 13
13	156.7			

[a] ^{1}H and ^{13}C chemical shifts are referenced to the DMSO-d_6 signal (2.50 and 39.5 ppm, respectively). ^{15}N NMR spectra were not calibrated with an external standard. The δ value has an accuracy of about 1 ppm in reference to NH_3 (0 ppm). [b] For positions no. 1, 7, and 12 δ(^{15}N) is given in parentheses.

Table 2. NMR Data for 4-Bromopyrrole-2-carboxy-*N*(ϵ)-lysine (**2**) Recorded in DMSO-d_6[a]

position	δ (^{13}C)/δ(^{15}N)[b] [ppm]	δ (^{1}H) [ppm]	^{1}H,^{1}H-COSY	^{1}H,^{13}C-HMBC
1	(161)	11.80 (1H, s)	3, 5	2, 3, 4, 5
2	127.0			
3	111.3	6.82 (1H, m)	1, 5	2, 5, 6
4	94.8			
5	121.0	6.96 (1H, m)	1, 3	2, 3, 4, 6
6	159.5			
7	(108)	8.09 (1H, t, $J = 5.7$ Hz)	8	6, 8, 9
8	38.2	3.19 (2H, dd, $J = 6.4, 6.2$ Hz)	7, 9	6, 9, 10
9	28.8	1.48 (2H, m)	8	8, 10, 11
10	21.9	1.39 (2H, m)	11	8, 9, 11, 12
11	29.9	1.77 (2H, m)	10, 12	9, 10, 12, 13
12	52.3	3.77 (1H, t, $J = 6.0$ Hz)	11	10, 11, 13
12-NH$_2$		8.15 (br)		
13	171.1			

[a] ^{1}H and ^{13}C chemical shifts are referenced to the DMSO-d_6 signal (2.50 and 39.5 ppm, respectively). ^{15}N NMR spectra were not calibrated with an external standard. The δ value has an accuracy of about 1 ppm in reference to NH_3 (0 ppm). [b] For positions no. 1 and 7 δ(^{15}N) is given in parentheses.

retention times of natural and synthetic product derivatives and the same sign of specific rotation revealed the *S*-configuration for **2**.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer at 25 °C. The DQF-^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HSQC, ^{1}H,^{13}C-HMBC, ^{1}H,^{15}N-HSQC, and ^{1}H,^{15}N-HMBC experiments were carried out using standard parameters. HPLC-MS analyses were performed with an Agilent 1100 HPLC system and a Bruker Daltonics microTOF$_{LC}$ mass spectrometer. Separation was achieved by a Waters XTerra RP$_{18}$ column (3.0 × 150 mm, 3.5 μm) applying a MeCN/H_2O/HCOOH gradient. UV detection was performed with a DAD (Agilent) at a wavelength of 280 nm. ESI-MS/MS spectra were recorded with an Esquire 3000+ ion trap (Bruker Daltonics). Optical rotation was measured with a Perkin-Elmer 214 MC polarimeter at 23 °C.

Animal Material. The sponge *Stylissa caribica* was collected by scuba diving at Little San Salvador in the Bahamas (74 ft depth, July 2000). The samples were immediately frozen after collection and kept at −20 °C until extraction. The sponge material was compared with previously investigated material of *S. caribica*[8] and was found to match closely (Dr. Michael Assmann, personal communication). The specimens form erect wedged-shaped, thick-bladed columns with irregularly corrugated lengthwise grooves and ridges, subdivided in places to form honeycomb-like depressions. The surface in the depressions is shiny smooth, looking fleshy. The color in life is orange-brown, turning rather dark red-brown in EtOH. A detailed taxonomic description of the sponge is given in ref 9.

Extraction and Isolation. The freeze-dried sponge samples of *S. caribica* (94.7 g) were crushed with a mill and extracted at room temperature exhaustively in a 1:1 mixture of CH_2Cl_2/MeOH. The orange-colored crude extract of *S. caribica* was partitioned between *n*-hexane (4 × 400 mL) and MeOH (300 mL). The MeOH extract was then partitioned between *n*-BuOH (3 × 500 mL) and H_2O (300 mL). The resulting *n*-BuOH (15.9 mg) fraction from the solvent partitioning scheme was purified by gel chromatography on Sephadex LH-20 (Pharmacia) using MeOH as mobile phase. Final purification of the isolated compounds was achieved by preparative RP_{18} HPLC on a Kromasil RP_{18} column (16 × 250 mm, 10 μm) applying a MeCN/TFA (0.1% in H_2O) gradient to afford **1** (8.9 mg, 0.009% of dry weight) and **2** (10.3 mg, 0.011% of dry weight).

1-Fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA) Derivatization and Absolute Configuration of 2 (Marfey's method[7]). To 10 μL (130 μg) of amino acid solution were added 100 μL of 0.1 M $NaHCO_3$ and 100 μL of 3 mM 1-fluoro-2,4-dinitrophenyl-5-L-alanine. The solution was heated to 80 °C for 5 min. Then 50 μL of 0.2 M HCl and 40 μL of 50% aqueous MeCN containing 0.1% formic acid were added to the reaction mixture. Separation was achieved by a Waters XTerra RP_{18} column (3.0 × 150 mm, 3.5 μm) applying an MeCN/H_2O/HCOOH gradient (0 min: 10% MeCN/90% HCOOH (0.1% in H_2O), 30 min: 60% MeCN/40% HCOOH (0.1% in H_2O)) with a flow rate of 0.4 mL/min. UV detection was performed with a DAD (Agilent) at a wavelength of 340 nm. Retention times: natural product, 23.82 min; synthetic compound, 23.80 min.

4-Bromopyrrole-2-carboxyarginine (1): light yellow oil; $[\alpha]^{23}_D$ −16 (*c* 0.25, MeOH); UV (DAD) λ_{max} 271 nm; HPLC-HRESI-(+)MS: t_R = 7.1 min, *m/z* 346.0487 $[M + H]^+$ (calcd for $C_{11}H_{17}N_5O_3{}^{79}Br$, *m/z* 346.0509, Δm = 6.4 ppm).

4-Bromopyrrole-2-carboxy-*N*(ε)-lysine (2): light yellow oil; $[\alpha]^{23}_D$ +5.2 (*c* 0.50, MeOH); UV (DAD) λ_{max} 268 nm; HPLC-HRESI-(+)MS: t_R = 8.1 min, *m/z* 318.0445 $[M + H]^+$ (calcd for $C_{11}H_{17}N_3O_3{}^{79}Br$, *m/z* 318.0448, Δm = 0.9 ppm).

4-Bromopyrrol-2-yl Trichloromethyl Ketone (5). Synthesis was performed according to Kitamura et al.[10c] based on the method of Bailey et al.[10b] A solution of Br_2 (308 μL, 6 mmol) in 20 mL of glacial HOAc was added slowly to a stirred solution of pyrrol-2-yl trichloromethyl ketone (1266 mg, 6 mmol) in 5 mL of glacial HOAc. Pyrrol-2-yl trichloromethyl ketone was synthesized according to Bailey et al.[10a] from pyrrole and trichloroacetyl chloride. After 4 h 30 mL of H_2O was added and the solution was extracted twice with 50 mL of DCM. The combined DCM solutions were dried (Na_2SO_4), and the solvent was evaporated. The crude products were purified by preparative HPLC (Prontosil Eurobond C18 (20 × 250 mm, 5 μm)) applying a gradient containing MeCN/TFA (0.1% in H_2O) to yield **5** as a white powder (754 mg, 44%): ^1H NMR (DMSO-d_6, 400 MHz) δ 12.84 (1H, br s, H-1), 7.54 (1H, dd, H-5), 7.32 (1H, dd, H-3); ^{13}C NMR (DMSO-d_6, 100.7 MHz) δ 171.6 (C-6), 129.0 (C-5), 122.0 (C-2), 121.5 (C-3), 97.6 (C-4), 94.5 (C-7); HRESI-(−)MS *m/z* 287.8364 $[M - H]^-$ (calcd for $C_6H_2NO^{35}Cl_3{}^{79}Br$, *m/z* 287.8380, Δm = 5.6 ppm).

(2*S*)-2-{[1-(4-Bromo-1*H*-pyrrol-2-yl)methanoyl]amino}-5-[*N*-Pmc-guanidino]pentanoic Acid (6). A 159 mg (0.55 mmol) amount of **5**, 190 mg (0.43 mmol) of N^G-2,2,5,7,8-pentamethylchroman-6-sulfonyl-L-arginine, and 150 μL (0.91 mmol) of *N*,*N*-diisopropylethylamine were suspended in MeCN (3 mL). After stirring for 6 h at room temperature the solvent was evaporated. The crude residue was purified by preparative HPLC (Prontosil Eurobond C18 (20 × 250 mm, 5 μm)) applying a gradient containing MeCN/TFA (0.1% in H_2O) to yield **6** as a colorless oil (80 mg, 23.7%). For this compound no NMR data were measured. HRESI-(+)MS: *m/z* 612.1464 $[M + H]^+$ (calcd for $C_{25}H_{35}N_5O_6S^{79}Br$, *m/z* 612.1486, Δm = 3.5 ppm).

(2*S*)-2-{[1-(4-Bromo-1*H*-pyrrol-2-yl)methanoyl]amino}-5-guanidinopentanoic Acid (1). A solution of **6** (60 mg, 0.098 mmol) in TFA (98%, 3 mL) was stirred for 45 min at room temperature. The solvent was evaporated, and the crude residue was purified by preparative HPLC (Prontosil Eurobond C18 (20 × 250 mm, 5 μm)) applying a gradient containing MeCN/TFA (0.1% in H_2O) to yield **1** as a colorless oil (22 mg, 65.3%). The NMR data were identical to the natural product. $[\alpha]^{23}_D$ −8 (*c* 0.15, MeOH); HRESI-(+)MS *m/z* 346.0501 $[M + H]^+$ (calcd for $C_{11}H_{17}N_5O_3{}^{79}Br$, *m/z* 346.0509, Δm = 2.3 ppm).

(2*S*)-2-Amino-6-[1-(4-bromo-1*H*-pyrrole-2-ylmethanoyl)amino]-hexanoic Acid Methyl Ester (7). To a solution of L-lysine methyl ester hydrochloride (100 mg, 0.40 mmol) in MeCN (3 mL) were added 4-bromopyrrole-2-yl trichloromethyl ketone (**5**, 159 mg, 0.55 mmol) and *N*,*N*-diisopropylethylamine (150 μL, 0.91 mmol). After 6 h at room temperature the solvent was evaporated. The crude residue was purified by preparative HPLC (Prontosil Eurobond C18 (20 × 250 mm, 5 μm)) applying a gradient containing MeCN/TFA (0.1% in H_2O) to yield **7** as a colorless oil (55.6 mg, 29.2%). For this compound no NMR data were measured. HRESI-(+)MS: *m/z* 346.0745 $[M + H]^+$ (calcd for $C_{13}H_{21}N_3O_3{}^{79}Br$, *m/z* 346.0761, Δm = 4.5 ppm).

(2*S*)-2-Amino-6-[1-(4-bromo-1*H*-pyrrole-2-ylmethanoyl)amino]-hexanoic Acid (2). A 55.6 mg portion of **7** was dissolved in HCl (32%). After 22 h at room temperature the solvent was evaporated. The crude residue was purified by preparative HPLC (Prontosil Eurobond C18 (20 × 250 mm, 5 μm)) applying a gradient containing MeCN/TFA (0.1% in H_2O) to yield **2** as a colorless oil (17.8 mg, 34.9%). The NMR data were identical to the natural product. $[\alpha]^{23}_D$ +5.4 (*c* 0.41, MeOH); HRESI-(+)MS *m/z* 318.0435 $[M + H]^+$ (calcd for $C_{11}H_{17}N_3O_3{}^{79}Br$, *m/z* 318.0448, Δm = 4.0 ppm).

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