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Clathramides, Unique Bromopyrrole Alkaloids From the Caribbean Sponge *Agelas clathrodes*

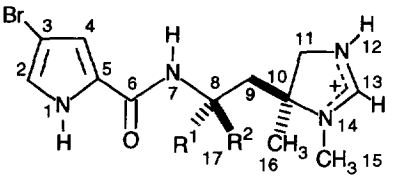
Francesco Cafieri, Ernesto Fattorusso,* Alfonso Mangoni, Orazio Taglialatela-Scafati

Dipartimento di Chimica delle Sostanze Naturali, via D. Montesano 49, I-80131 Napoli, Italy

Abstract: Clathramide A (**1a**) and B (**1b**) are two novel isomeric bromopyrrole alkaloids containing the uncommon N-methylimidazolinium moiety, isolated from the Caribbean sponge *Agelas clathrodes*. Their structures, elucidated on the basis of a detailed spectroscopic analysis, appear not biosynthetically related to oroidin and its derivatives. In addition, **1a-1b** exhibited a mild antifungal activity tested in an agar disk assay on *Aspergillus niger*. Copyright © 1996 Elsevier Science Ltd

Studies to date have amply demonstrated that marine sponges belonging to the genus *Agelas* are an excellent source of novel biologically active compounds. Most of the 12 well-established species of this genus[1] have been subjected to extensive chemical investigation, and the results of such a study may be summarized grouping the *Agelas* typical metabolites into three different families on the basis of their chemical features: bromopyrrole alkaloids (oroidin[2] derivatives), α-glycosphingolipids, and derivatized terpenoids attached to adenine or hypotaurocyamine moieties (agelasine[3] and agelasidine[4] series, respectively). Among them, bromopyrrole alkaloids, which are apparently related each to the other through a common biogenetic origin, probably form the most various and intriguing class. As a matter of fact, the structures and the pharmacological activities of more than 20 compounds of the "oroidin family" have been determined until now. The most interesting examples include the linear keramadine[5] (antiserotonergic), the policyclic agelastatin[6] (cytotoxic), and the dimeric sceptrins[7,8] (antibacteric, antiviral).

As a part of our ongoing search for novel bioactive substances from marine invertebrates, we previously described the occurrence in the polar extracts of some Caribbean *Agelas* sponges of the antiserotonergic pyridinium alkaloid agelongine,[9] and of the potent and selective antihistamine bromopyrrole alkaloids dispacamides.[10] Further investigation on the secondary metabolites composition of one of these sponges (*A. clathrodes*) resulted in the isolation of two novel diastereomeric bromopyrrole alkaloids, clathramide A (**1a**) and B (**1b**), which are the object of the present paper. These compounds are particularly interesting because they, to the best of our knowledge, represent the first bromoalkaloids from an *Agelas* sponge which appear not biosynthetically related to oroidin and its derivatives, in spite of a rather similar structure.



1a R^1= H, R^2= COO^-
1b R^1= COO^-, R^2= H

Specimens of *Agelas clathrodes* (order Poecilosclerida, family Agelasidae) were collected by hand during an expedition to the Caribbean Sea, and immediately frozen. After homogenization, the organisms (110 g dry wt.) were exhaustively extracted first with methanol and then with chloroform. Combined extracts were partitioned between *n*-BuOH and water. The *n*-BuOH soluble material was initially subjected to chromatography over a column packed with reversed phase (RP18) silica gel and eluted with MeOH/H_2O 1:1 (polar alkaloids fraction), MeOH/$CHCl_3$ 9:1 (glycosphingolipid fraction), and then with $CHCl_3$. The "alkaloid fraction" was further purified by a medium pressure liquid chromatography (MPLC) over silica gel (230-400 mesh) using a system of eluants with a gradient of polarity. Fractions eluted with MeOH/EtOAc (85:15), subjected to preliminary spectral analyses, resulted mainly composed by a mixture of the known agelongine[9] with the novel compounds **1a** and **1b**. A subsequent HPLC purification on an RP18 column (eluant H_2O/MeOH 6:4), allowed us to obtain a fraction composed only by **1a** and **1b** in the ratio 4:1. The resolution of the mixture into the individual components was achieved with repeated HPLC separations on an RP18 column using H_2O/CH_3CN 1:1 as eluant, allowing to obtain 4.2 mg of **1a** ($[\alpha]_D$ - 5°) and 1.1 mg of **1b** ($[\alpha]_D$ + 11°) in the pure state, both as colorless solids.

Table 1. ^{13}C (125 MHz) and ^1H (500 MHz) NMR Data of Compounds **1a** and **1b** in CD_3OD.

Position	1a		1b	
	δC, mult.	δH, mult., int., *J* in Hz	δC, mult.	δH, mult., int., *J* in Hz
1-NH		9.55[a] (br.s,1H)		9.62[a] (br.s, 1H)
2	125.4,CH	7.06 (d, 1H, 1.7)	125.6,CH	7.08 (d, 1H, 1.7)
3	97.2,C		97.3,C	
4	118.6,CH	6.94 (d, 1H, 1.7)	118.3,CH	6.89 (d,1H, 1.7)
5	124.0,C		124.1,C	
6	160.3,C		160.4,C	
7-NH		7.85[a] (br.s, 1H)		7.68[a] (br.s, 1H)
8	51.4,CH	4.09 (m, 1H)	52.4,CH	4.18 (m, 1H)
9	33.4,CH_2	2.33 (m, 2H)	35.1,CH_2	2.67 (dd, 1H,14.2, 5.2)
				1.97 (dd, 1H,14.2, 11.4)
10	58.6,C		58.3,C	
11	66.2,CH_2	4.40 (d, 1H, 12.1)	67.1,CH_2	4.34 (d, 1H 12.1)
		4.59 (d, 1H, 12.1)		4.63 (d, 1H, 12.1)
12-NH		11.83[a] (br.s, 1H)		11.92[a] (br.s, 1H)
13	154.0,CH	8.00 (br.s, 1H)	153.9,CH	8.02 (br.s, 1H)
15	36.4,CH_3	3.25 (s, 3H)	38.4,CH_3	3.27 (s, 3H)
16	20.7,CH_3	1.47 (s, 3H)	19.9,CH_3	1.51 (s, 3H)
17	174.3,C		174.4,C	

a. D_2O exchangeable signals recorded in DMSO-d_6.

The FAB mass spectrum (positive ion mode) of **1a** showed prominent pseudomolecular ion peaks at *m/z* 357 and 359 $[M+H]^+$ in the ratio 1:1, suggesting the presence of a bromine atom. The molecular composition of **1a** was determined as $C_{13}H_{17}BrN_4O_3$ by HR-FABMS (measured mass 357.0496; calculated 357.0484 for $C_{13}H_{17}{}^{79}BrN_4O_3$), in accordance with ^1H and ^{13}C NMR data (Table 1). The absorptions of **1a** at λ_{max} 208 (ε 14900), 230 (ε 13000) and 265 (ε 10900) in the UV (CH_3CN) spectrum are reported as typical values for pyrrole chromophores having a carboxamide function at position 2.[11] The presence of this functional group was also suggested by the intense band in the IR (KBr) spectrum at ν_{max} 1640 cm^{-1}. In addition, a 4-bromopyrrole-2-carboxamide nucleus was strongly indicated by the signals at δ 7.06 (H-2, d, J = 1.7 Hz) and 6.94 (H-4, d, J = 1.7 Hz) in the ^1H NMR spectrum (CD_3OD) and by the ^{13}C NMR pattern of resonances (δ 125.4, C-2; δ 97.2, C-3; δ 118.6, C-4; δ 124.0, C-5; δ 160.3, C-6), which appeared very similar to the values reported in the literature for other *Agelas* bromopyrrole alkaloids.[12,13] The two D_2O exchangeable protons of this system were observed in the ^1H NMR spectrum recorded in DMSO-d_6 (see Table 1 and Experimental section).

The ^1H NMR spectrum (CD_3OD) also showed a methine signal at δ 8.00, two methyl singlets at δ 1.47 and 3.25, two coupled doublets at δ 4.40 and 4.59 (AB system), and finally a multiplet at δ 4.09, whose coupling with a methylene signal centered at δ 2.33 was indicated by an ^1H-^1H COSY experiment. Unambiguous association of all the proton signals with the resonances of the relevant carbon atoms was established by using a 2D NMR HMQC experiment. In addition, in the ^{13}C NMR spectrum (CD_3OD) two quaternary carbon atoms resonating at δ 58.6 and 174.3 were detected; the latter signal was attributed to a carboxylate group, further supported by the IR absorption band at ν_{max} 1680 cm^{-1}.

The long range coupling of C-6 with the methine H-8 (δ 4.09), detected through the HMBC spectrum, provided decisive information for extending the determined structure allowing us to connect the pyrrolocarboxamide to C-8, which in turn is linked with the methylene group at δ 2.33, as the above reported COSY data indicated. For the structural determination of compound **1a** to be completed, only the carboxylate group and a $C_5H_{10}N_2$ fragment, accounting for all the remaining atoms of the molecule, had to be located. The $C_5H_{10}N_2$ moiety was determined as a 1,5-dimethylimidazolinium ring, linked to the rest of the molecule as depicted in structure **1a** on the basis of a series of HMBC correlations. The most informative long range couplings were those detected between H-8 and C-10 (δ 58.6); C-16 (δ 20.7) and H_2-9; C-16 and H_2-11 (δ 4.40 and 4.59); C-13 (δ 154.0) and H_2-11; and between C-15 (δ 36.4) and the deshielded singlet at δ 8.00, an appropriate resonance for a methine between two nitrogen atoms. In addition, the methyl group resonating at δ 3.25 could be located on N-14 on the basis of its prominent HMBC cross-peak with C-10, while the NH-12 signal was observed in the ^1H NMR spectrum recorded in DMSO-d_6 (see Table 1 and Experimental section). Finally, the carboxylate group must be located on C-8, as indicated by the ^3J HMBC correlation between H_2-9 (δ 2.33) and C-17 (δ 174.3). These data fully established the structure of clathramide A, apart from the stereochemistry. Key HMBC correlations are shown in fig 1.

Having completed the gross structural determination of **1a**, we repeated for compound **1b** all the MS, UV, IR, and NMR experiments. The analysis of the obtained results was conducive with the same structure as **1a**, which is characterized by two stereogenic centers, namely C-8 and C-10. In the light of the above evidence, we concluded that **1a** and **1b** must differ only for the absolute stereochemistry of one of these centers.

In order to define the relative configuration of clathramides A and B, we performed a series of n.O.e. difference measurements on both compounds **1a** and **1b**. These experiments gave us some interesting information, since it was possible to point out a series of specific n.O.e. enhancements.

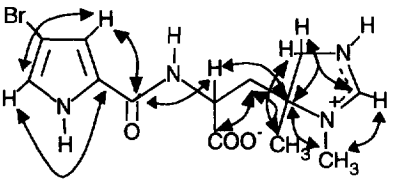


Figure 1. Key HMBC correlations

In particular, for compound **1a**, enhancement of H-8 (δ 4.09) was observed upon irradiation of the methyl protons resonating at δ 3.25 (H_3-15), while irradiation of the methyl at δ 1.47 (H_3-16) caused enhancement of the signal at δ 4.59 (H-11a). These dipolar couplings were not detectable for compound **1b**. On the contrary, the saturation of the multiplet resonating at δ 4.18 (H-8) in the ^{1}H NMR spectrum of **1b** caused the intensity of H-11a (δ 4.63) to significantly increase (8%).

Since the molecular structure of clathramides appears quite flexible, an unambiguous interpretation of the obtained n.O.e. results required some insight about the conformational behavior of the molecules. This was achieved by a molecular dynamics/molecular mechanics conformational study of the two possible diastereomers in the CHARMm force field. A set of 200 conformations for each isomer was generated by an high temperature (1500 K) molecular dynamics (HTMD) simulation (see the Experimental section), and the resulting structures were optimized by molecular mechanics. These simulations showed that the conformational behavior of the molecules is strongly driven by the electrostatic attraction between the charged groups, forcing the imidazolinium ring to lie near the carboxyl group, and by the hydrogen bond between NH-1 and one carboxyl oxygen atom (fig. 2). Consequently, both isomers appeared to exist practically in only one conformation at room temperature. In fact, the 47 lowest energy structures obtained for the *SR/RS* diastereomer showed virtually identical conformations and potential energies. Any other generated conformation possessed an energy at least 3.76 Kcal/mol higher, so that they could be considered not significantly populated at room temperature. In the lowest energy conformer, the distance H-8/H-11a was 2.20 Å, while the distance H-8/H_3-15 (the average position of the three methyl protons is considered) was much longer (5.17 Å). Likewise, for the *SS/RR* diastereomer, the lowest energy conformation (showed by 40 of the 200 generated structures) is lower by at least 2.06 Kcal/mol than any other. In this conformer, the distance H-8/H_3-15 was 2.96 Å, while the distance H-8/H-11a was significantly longer (4.13 Å).

On the basis of the above results, the n.O.e. enhancements detected between H-8 and H_3-15 are well explained by assuming for **1a** the *SS/RR* relative stereochemistry, whereas the dipolar coupling of H-8 with H-11a is in accordance with the *SR/RS* relative configuration of **1b**.

To deduce the absolute stereochemistry of clathramides A-B, we first attempted to hydrolyze the amide linkage and study the free aminoacids. Unfortunately, using different conditions of acid hydrolysis we could only recover a mixture of degradation products, that could not be characterized because of the small amounts. Even circular dicroism, that has long been employed for the assignment of absolute stereochemistry of α-aminoacids, proved to be inconclusive in our case. In fact, the CD spectra of **1a** and **1b** appeared too broadened and weak to give decisive informations.

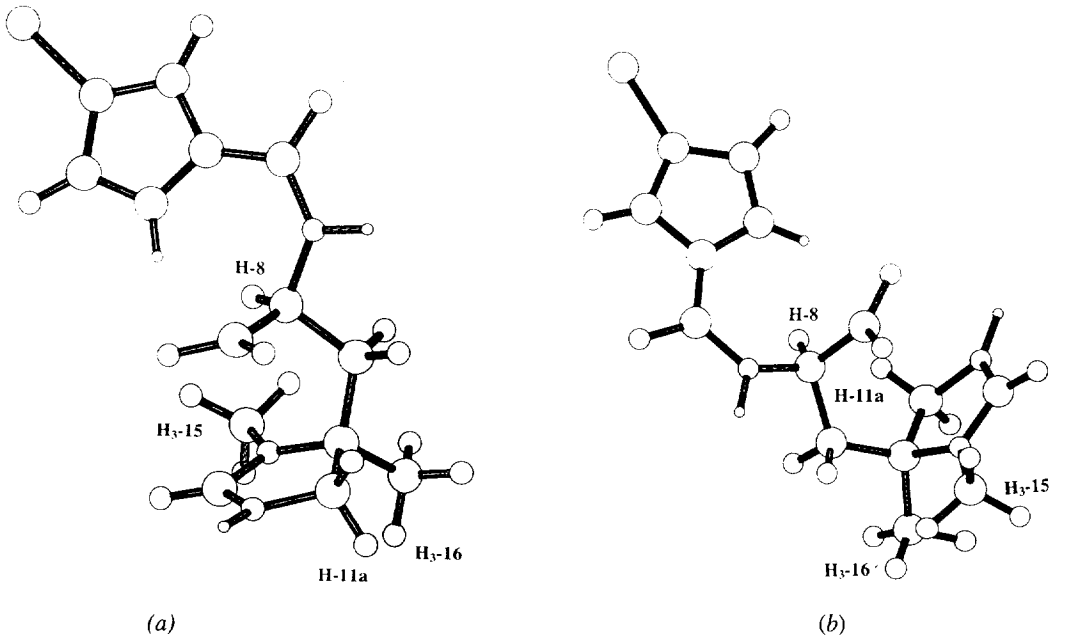
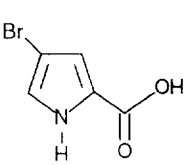


Figure 2. CHARMm lowest energy conformation of clathramide A (*a*) and B (*b*).

Finally, to get some indications on the stereochemistry of the aminoacid stereogenic centers, we attempted an enzymatic reaction with the use of Acylase I (aminoacylase, EC 3.5.1.14), an enzyme that catalyzes the enantioselective hydrolysis of N-acyl-L-aminoacids. Several examples of this reaction have been reported in the literature,[14,15] using a great range of substrates with aliphatic or aromatic acids attached to natural or modified aminoacids. In all these examples the enantioselectivity of the enzyme revealed to be very high, while in some cases, for unfavourable charge interactions or for steric hindrance, a failure of Acylase I in the hydrolysis of some N-acyl-L-aminoacids has been reported.[16]

In our experiments we used two types of Acylase I: PKA (porcine kidney acylase) and AA (*Aspergillus oryzae* acylase), both commercially available and stable in solution. An H_2O/DMSO 9:1 solution of **1a** was added to PKA or AA (in the latter case, $CoCl_2$ 1mM was also added), pH was buffered, and the reaction was allowed to proceed for 30 hours at 40°C. After work-up (see Experimental section), in both cases we obtained a fraction whose NMR spectra contained signals which appeared appropriate for compound **3**, even if every attempt to obtain it in the pure form failed. Another fraction contained the already described compound **2**, as evidenced by ^{1}H NMR and MS spectroscopy. For **1a**, the PKA hydrolytic reaction had much better yields than that of AA. On the contrary, the same experiments, under the same conditions, left product **1b** completely unchanged.



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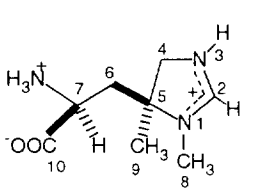

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These results suggest that the diastereomeric compounds **1a** and **1b** differ in the configuration at C-8, in particular indicating the *L* (i.e. *S*) configuration for this carbon **1a**, and the *D* (i.e. *R*) in **1b**. Therefore we propose for **1a** the 8*S*,10*S* configuration and the 8*R*,10*S* for **1b**.

To the best of our knowledge, clathramides A and B are the first examples of *Agelas* bromoalkaloids which, even though structurally similar, do not show a close biogenetic relationship with bromine-containing alkaloids of the oroidin[2] family. Although biosynthetic experiments have not yet been performed, many authors[1] admit that ornithine is the common precursor of these nitrogenous derivatives. One ornithine molecule is supposed to cyclize giving proline (and subsequently pyrrole-2-carboxylic acid), while another one should form an aminopropylimidazole moiety by addition of a guanidine molecule. The two units (which have been also isolated separately, strengthening the biogenetic hypothesis) should join together through an amide bond, giving rise to a final structure with two aromatic rings linked by a three-carbon chain. It appears very difficult to hypothesize that clathramides originate following this biogenetic pathway; most likely, **1a-b** derive from a direct coupling of one proline (or pyrrole carboxylic acid) and one histidine residue, with the subsequent inclusion of two methyl groups.

Clathramides A-B were evaluated for antihistaminic, antiserotonergic, and anticholinergic activities, but, in spite of the structural similarity with other active alkaloids from *Agelas*,[5,10,17] no activity worthy of note has been observed. However, preliminary *in vitro* assays showed that compounds **1a-1b** possess a moderate antifungal activity against *Aspergillus niger*. In an agar disk diffusion assay, 100 μg of the mixture **1a-1b** caused a zone of inhibition of almost 8 mm.

EXPERIMENTAL SECTION

General methods. Optical rotations were measured in MeOH on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (λ = 589 nm) and a 10-cm microcell. IR (KBr) spectra were recorded on a Bruker model IFS-48 spectrophotometer. Ultraviolet spectra were obtained in CH_3CN using a Beckman DU70 spectrophotometer. Low and high resolution FAB mass spectra (CsI ions) were performed in a glycerol matrix on a VG Prospec (FISONS) mass spectrometer equipped with a FAB source. ^1H (500.14 MHz) and ^{13}C (125.03 MHz) NMR spectra were determined on a Bruker AMX-500 spectrometer; chemical shifts are referenced to the residual solvent signal (CD_3OD: $\delta_H = 3.34$, $\delta_C = 49.0$; DMSO-d_6: $\delta_H = 2.50$). Methyl, methylene, and methine carbons were distinguished by DEPT experiments. Homonuclear ^1H connectivities were determined by using COSY experiments. One bond heteronuclear ^1H-^{13}C connectivities were determined with the Bax-Subramanian[18] HMQC pulse sequence using a BIRD pulse 0.50 s before each scan in order to suppress the signals originating from protons not directly bound to ^{13}C (interpulse delay set for $^1J_{CH} = 140$ Hz). During the acquisition time, ^{13}C broad band decoupling was performed using the GARP sequence. Two and three bond ^1H-^{13}C connectivities were determined by HMBC experiments optimized for a $^{2,3}J$ of 10 Hz. Medium-pressure liquid chromatography (MPLC) was performed using a Büchi 861 apparatus with an SiO_2 (230-400 mesh) column. High performance liquid chromatography (HPLC) separations were achieved on a Waters 501 apparatus equipped with an UV detector (λ 245 nm) and with Hibar RP18 LiChrospher (250 x 4 mm) columns.

Collection, extraction and isolation. Specimens of *Agelas clathrodes* were collected in the Summer of 1992 along the coasts of Little San Salvador Island at a depth of 15 m, and identified by Prof. M. Pansini (Università di Genova). They were frozen immediately after collection and kept frozen until extraction. A reference specimen

has been deposited at the Istituto di Zoologia, Università di Genova, Italy. The sponge (110 g of dry weight after extraction) was homogenized and extracted with methanol (4 x 500 mL) and with chloroform (4 x 500 mL). The combined extracts were initially partitioned between H_2O and *n*-BuOH. The organic layer was concentrated *in vacuo* affording 8.2 g of a brown-colored viscous oil. This was subjected to chromatography on a column packed with a RP-18 silica gel. Three fractions (A-C) were collected [eluants: MeOH/H_2O 1:1, MeOH/$CHCl_3$ 9:1 and $CHCl_3$, respectively]. Fraction A (3.5 g), containing polar alkaloids, was further chromatographed by MPLC (SiO_2 column; solvent gradient system of increasing polarity from EtOAc to MeOH). Fractions eluted with MeOH/EtOAc (85:15), rechromatographed by HPLC (eluant H_2O/MeOH, 6:4, RP18 column, flow 0.8 mL/min) gave a fraction composed by **1a** and **1b** in the ratio 4:1. Repeated HPLC separations on a RP-18 column, using H_2O/CH_3CN 1:1 as eluant (flow 0.3 mL/min), afforded pure compounds **1a** (4.2 mg) and **1b** (1.1 mg).

Clathramide A (**1a**). Colorless amorphous solid. $[\alpha]_D^{25}$ -5° (c = 0.001 in MeOH); IR (KBr) ν_{max} 2450, 1680, 1640 cm^{-1}; UV (CH_3CN) λ_{max} 208(ε 14900), 230 (ε 13000), 265 (ε 10900) nm. ^1H and ^{13}C NMR (CD_3OD): see Table 1. ^1H NMR (300 K, DMSO-d_6): δ 11.83 (1H, br.s, NH-12), 9.55 (1H, br.s, NH-1), 7.85 (1H, s, NH-7), 7.94 (1H, br.s, H-13), 7.14 (1H, d, *J* = 1.5, H-2), 6.83 (1H, d, *J* = 1.5, H-4), 4.43 (1H, d, *J* = 10.3, H-11a), 4.21 (1H, d, *J* = 10.3, H-11b), 4.04 (1H, m, H-8), 3.13 (3H, s, H_3-15, partially submerged), 2.20 (2H, m, H_2-9), 1.36 (3H, s, H_3-16). FABMS (positive ions, glycerol matrix) m/z 357 and 359 (1:1) $[M+H]^+$; HRFABMS m/z 357.0496 $[M+H]^+$, calcd. for $C_{13}H_{17}{}^{79}BrN_4O_3$, 357.0484. HMBC correlations: see fig. 1.

Clathramide B (**1b**). Colorless amorphous solid. $[\alpha]_D^{25}$ +11° (c = 0.001 in MeOH); IR (KBr) ν_{max} 2450, 1680, 1640 cm^{-1}; UV (CH_3CN) λ_{max} 208, 230 (ε 13000), 265 (ε 10900) nm. ^1H and ^{13}C NMR (CD_3OD): see Table 1. ^1H NMR (300 K, DMSO-d_6): δ 11.92 (1H, br.s, NH-12), 9.62 (1H, br.s, NH-1), 7.68 (1H, s, NH-7), 7.90 (1H, br.s, H-13), 7.20 (1H, d, *J* = 1.5, H-2), 6.93 (1H, d, *J* = 1.5, H-4), 4.38 (1H, d, *J* = 12.0, H-11a), 4.22 (1H, d, *J* = 12.0, H-11b), 4.03 (1H, m, H-8), 3.16 (3H, s, H_3-15, partially submerged), 2.00 (1H, m, H-9a; H-9b was completely submerged by the solvent signal), 1.37 (3H, s, H_3-16). FABMS (positive ions, glycerol matrix) m/z 357 and 359 (1:1) $[M+H]^+$; HRFABMS m/z 357.0496 $[M+H]^+$, calcd. for $C_{13}H_{17}{}^{79}BrN_4O_3$, 357.0484. HMBC correlations: see fig. 1.

Molecular modeling. Computer modeling studies were carried out using the Quanta/CHARMm 4.0 program (Molecular Simulations Inc. 200 Fifth Avenue, Waltham, MA 02154) on a Silicon Graphics Personal Iris 4D-35G computer. No explicit solvent molecules were included in these calculations, so a distance dependent dielectric constant (RDIE) was used to partially compensate for the absence of solvent. Molecular dynamics (MD) simulations involved a heating period of 5.1 ps, followed by a 5.0 ps equilibration period and then 100 ps of dynamics simulation. The time step of integration was 1 fs. During MD calculations bond lengths involving hydrogen atoms were kept fix using the SHAKE algorithm. The coordinates produced by the simulation were saved every 0.5 ps, giving 200 structures. Each of them was subjected to energy minimization using the conjugated gradient protocol.

Enzymatic reactions. Porcine kidney (grade I) acylase (PKA) and *Aspergillus oryzae* acylase (AA) both supplied from Sigma were used. 1.2 mg (3.3 μmol) of **1a** were dissolved in H_2O/DMSO 9:1, 0.10 M potassium phosphate buffer, pH 7.5. The solution was degassed and 0.7 mg of PKA were added, with a final assay volume of 1.00 mL. To another sample of **1a** (1.2 mg, 3.3 μmol) in H_2O/DMSO 9:1 (0.10 M potassium phosphate buffer), were added 1 mM $CoCl_2$ and 4 mg of AA were added, with a final assay volume of 1.20 mL. Both reactions were allowed to proceed for 30 hours at 40 °C in reaction vials, then were subjected to a common treatment. In particular, the reaction mixture was adjusted to pH 5.0 with concentrated hydrochloric acid, heated to 60 °C x 5

min and filtered. The filtrate was extracted with EtOAc and then with Et_2O. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated *in vacuo*, to afford a fraction which showed to contain compound **2**. The aqueous layer was then evaporated to dryness and the obtained fraction was subjected to NMR analysis, showing signals which appeared appropriate for compound **3**. When we repeated the PKA assay using 0.8 mg of clathramide B (**1b**), the ^1H NMR spectrum of the aqueous layer showed the signals of unchanged **1b**.

Compound 2. FABMS (negative ions, glycerol matrix) m/z 188 and 190 (1:1) $[M-H]^-$; UV and ^1H NMR (CD_3OD) identical with those reported in the literature.[19]

Compound 3. ^1H NMR (CD_3OD): δ 8.14 (1H, s, H-2), 5.06 (1H, m, H-7, partially submerged), 4.85 (1H, d, J = 12.1, H-4a), 4.68 (1H, d, J = 12.1, H-4b), 3.12 (3H, s, H_3-8, partially submerged), 2.65 (2H, m, H_2-6), 1.55 (3H, s, H_3-9).

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