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Minalemines A-F: Sulfamic acid peptide guanidine derivatives isolated from the marine tunicate *Didemnun rodriguesi*

M. A. Expósito,[a] B. López,[b] R. Fernández,[b] M. Vázquez,[1b] C. Debitus,[d] T. Iglesias,[a] C. Jiménez,[c] E. Quiñoá,[b] and R. Riguera[b]*

[a]*Dpto. de Química Orgánica, Universidad de Vigo. 36200 Vigo. Spain*

[b]*Dpto. de Química Orgánica, Universidad de Santiago. 15706 Santiago de Compostela. Spain*

[c]*Dpto. de Química Fundamental, Universidade da Coruña. 15071 A Coruña. Spain*

[d]*ORSTOM, Centre de Noumea, B. P. A5, Noumea, New Caledonia.*

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Abstract

Six new guanidine compounds, named minalemines A-F[2], were isolated from the marine tunicate *Didemnun rodriguesi* by careful HPLC separation. Their structures were elucidated by MS, 1D- and 2D-NMR spectral analysis, and chemical degradation. These compounds incorporate one agmatine (Agma) and one homoagmatine (Hagma) terminal unit with their guanidine groups free and the primary amino groups linked through a peptidic bond to L-Leu and a very rare β-*N*-carboxymethyl amino acid (Ncma) bearing a saturated long chain. The saturated chains of minalemines A-C are homologs (C7, C8, and C9 respectively). Minalemines D-F are the sulfamic acid derivatives of minalemines A-C and constitute the first examples of such a functional group in a marine organism.

Keywords: *Didemnun rodriguesi*; sulfamic acid; guanidine; marine metabolites.

In recent decades, tunicates have been subjected to very close scrutiny and, as a result, a large number of bioactive nitrogenous metabolites with significant biological activity have been described[1-3]. Acyclic and cyclic peptides — and to a lesser extent depsipeptides — with novel and complex structures constitute the major class among those metabolites.

[1] Present address: SmithKline Beecham Pharmaceuticals PTM, 28760-Tres Cantos, Spain.

[2] Named in memory of Professor Luigi Minale.

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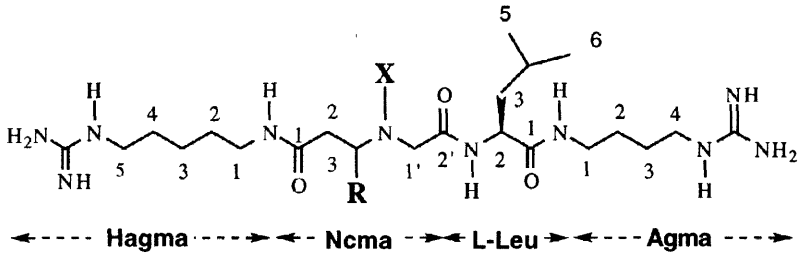
Compounds containing guanidine groups have also been isolated from tunicates[4-7]. In the particular case of the *Didemnun* genus, a number of metabolites derived from amino acids have also been reported[8-9].

In connection with our research on the biologically active natural products from the marine tunicate *Didemnun rodriguesi*[10], we now report the isolation and structural elucidation of six highly nitrogenated peptide guanidine compounds, named as minalemines A-F (**1-6**), that share a common skeleton and differ in the substitution at the central nitrogen and the length of the saturated chain. Compounds **4-6** are the sulfamic acid derivatives of **1-3**.



	X	R
1	H	C_7H_{15}
2	"	C_8H_{17}
3	"	C_9H_{19}
4	SO_3H	C_7H_{15}
5	"	C_8H_{17}
6	"	C_9H_{19}

The tunicate *Didemnun rodriguesi* was collected in the Baie des Citrons (Noumea, New Caledonia) and immediately frozen and lyophilized. The freeze-dried specimens were homogenized and extracted with MeOH at r.t. The MeOH extracts were partitioned into *n*-hexane, CH_2Cl_2, and *n*-BuOH. The *n*-BuOH residue was desalted on an Amberlite XAD-2 column and then submitted to chromatography on Sephadex LH-20 using MeOH:H_2O (2:1) as eluent to afford two major fractions.

The first fraction was purified by reversed-phase HPLC to yield minalemines A (**1**) (9 mg, t_R 7 min), B (**2**) (12 mg, t_R 9.30 min), and C (**3**) (7 mg, t_R 12 min).

The molecular formula of minalemine A (**1**), $C_{29}H_{60}N_{10}O_3$, was established by HRFABMS of its $[M + H]^+$ pseudomolecular ion at *m/z* 597.4930, and indicated the presence of 5 unsaturations in the molecule. The corresponding $[M + Na]^+$ ion was observed at *m/z* 619 when the spectrum was run in the presence of NaCl. Furthermore, FAB MS/MS on the pseudomolecular ion $[M + H]^+$ showed significant fragments at *m/z* 580 and 564 corresponding to $[M - NH_2]^+$ and $[M - 2NH_2]^+$, indicating the easy loss of two primary amino groups.

The peptidic nature of this compound was indicated by the FTIR and NMR spectra. Thus, one- (^1H, ^{13}C, DEPT) and two-dimensional (COSY, TOCSY, HMQC, and HMBC) NMR analysis of minalemine A (**1**) indicated the presence of five spin systems which could be

ascribed to the following four substructures: leucine (Leu), agmatine (Agma), homoagmatine (Hagma), and a *β-N*-carboxymethyl amino acid (Ncma) bearing a saturated long chain. These substructures satisfy the unsaturation requirements suggested by the molecular formula.

The existence of Hagma and Agma units was deduced by ^1H-^1H COSY and TOCSY correlations between the NH amide protons and the NH guanidine protons. The presence of these substructures was confirmed by the HMBC correlations between the C=N group at δ 158.6 and the H-5 and H-4 methylene protons of Hagma and Agma units, respectively. The Leu unit was easily identified by ^1H-^1H COSY, TOCSY, and HMBC correlations from the 1-NH amide proton to H-5 and H-6 methyl protons.

As for the Ncma fragment, the ^1H-^1H COSY spectrum of **1** displayed correlations between the H-3 methine proton at δ 3.61 (C-3 δ 57.5) and the methylene protons H-2 (δ 2.67 & 2.79/C-2 δ 35.0) and H-4 (δ 1.66 &1.84/C-4 δ 31.7), which in turn were coupled to the methylene protons of a long saturated chain [δ 1.38-1.51 m (C5-8 nCH_2, m) and δ 0.97 (CH_3, t)]. In addition, the HMBC correlations between the H-1' isolated methylene protons (δ 3.97 & 4.05, AB system/ C-1' δ 46.5) and the C-3 methine carbon and the C-2' carbonyl group at δ 166.6; and between the C-1 carbonyl group at δ 172.4 and the H-2 methylene protons, confirmed the structure proposed for the Ncma unit as that of a *β-N*-carboxymethyl amino acid.

Table 1.
^1H- and ^{13}C-NMR spectral data for minalemine A (**1**) in CD_3OD. Chemical shifts for minalemines B (**2**) and (**3**) are practically identical to those of **1**.

Position	δ_H mult. *J* (Hz)	δ_C mult.	Position	δ_H mult. *J* (Hz)	δ_C mult.
Hagma			L-Leu		
1	3.22 m/3.35 m	40.1[a] (t)	1		174.5 (s)
2	1.59 m/1.70 m	29.8[d] (t)[d]	2	4.44 t (7.5)	53.8 (d)
3	1.38 m/1.51 m	25.0 (t)	3	1.59 m/1.70 m	42.0 (t)
4	1.59 m/1.70 m	29.4[d] (t)[d]	4	1.75 m	25.9 (t)
5	3.22 m/3.35 m	42.3[b] (t)[b]	5	1.00[e] d (6.5)	21.8[f] (q)
1-NH	8.43 t (5.6)		6	1.02[e] d (6.4)	23.4[f] (q)
5-NH	7.59 t (5.7)		NH	8.66 d (7.4)	
C=N		158.6 (s)			
Ncma			Agma		
1		172.4 (s)	1	3.22 m/3.35 m	39.7[a] (t)
2	2.67 dd (16.4, 7.35)/	35.0 (t)	2	1.59 m/1.70 m	27.5[c] (t)
	2.79 dd (16.4, 4.5)		3	1.59m/1.70 m	27.0[c] (t)
3	3.61 m	57.5 (d)	4	3.22m/3.35 m	42.1[b] (t)
4	1.66 m/ 1.84 m	31.7 (t)	1-NH	8.28 t (5.65)	
5-9	1.38 m/1.51 m	23.6-32.8 (t)	4-NH	7.29 t (5.7)	
10	0.97 t (6.9)	14.4 (q)	C=N		158.6 (s)
1'	3.97 d (15.7)/	46.5 (t)			
	4.05, d (15.7)				
2'		166.6 (s)			

Signals a, b, c, d, e and f can be interchanged.

The connectivities between these substructures were established by HMBC cross peaks displayed by the NH amide protons and carbonyl carbons of minalemine A (**1**), as shown in Figure 1. The linkage between Hagma and Ncma units was corroborated by the fragment ions at *m/z* 411 [M - Hagma - $COCH_2$]$^+$ and 185 [Hagma - $COCH_2$]$^+$ in its (+) LRFABMS, indicative of a favored α-cleavage[11] with respect to the central nitrogen. The equivalent loss of Agma was not observed (see Scheme 1).

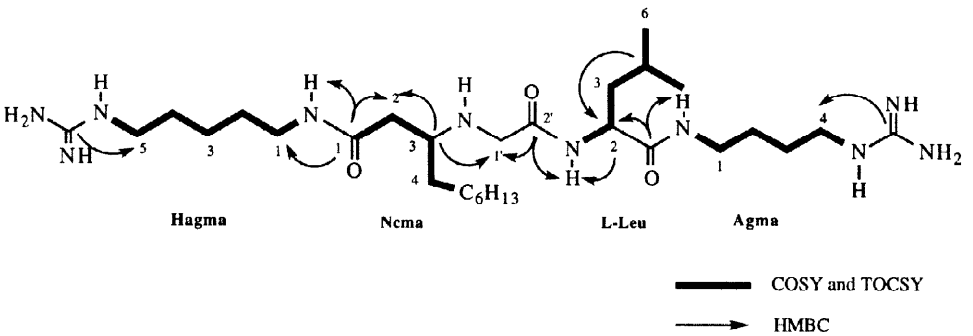
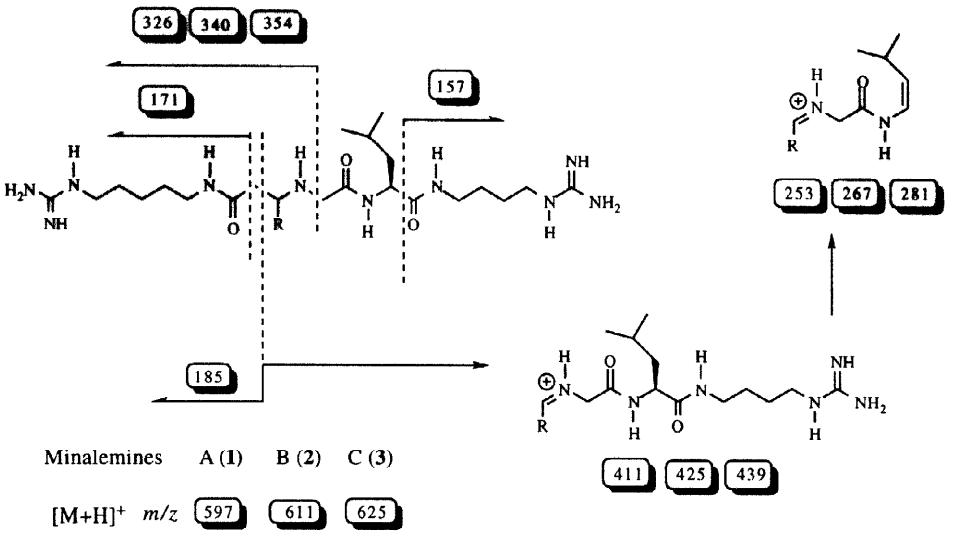


Figure 1. 2D-NMR data of minalemine A (**1**): The partial structures (solid lines) were obtained from ^{1}H-^{1}H COSY and TOCSY correlations, and their connectivities by HMBC.

Minalemine A (**1**) was thus shown to comprise of a central β-*N*-carboxymethyl amino acid (Ncma) residue carrying a saturated chain (R), and bearing a homoagmatine unit at one end and a leucine connected in turn to an agmatine unit at the other. Both Agma and Hagma are bonded to the rest of the molecule by their primary amino groups and the guanidine groups remain free.

The other two minalemines isolated from this fraction, B (**2**) and C (**3**), were found to be one and two CH_2 homologs of minalemine **1**, respectively. This was deduced from their (+) LRFABMS (glycerol), which showed the pseudomolecular [M + H]$^+$ ions at *m/z* 611 in the case of **2** and *m/z* 625 for **3**, and these differ by 14 and 28 amu respectively from **1**. The NMR and IR spectral data of compounds **2** and **3** were practically identical to those of **1**, suggesting that all three compounds contain the same substructures in the same sequence, but differ only in the number of methylene groups in the saturated chain of the Ncma unit.

The second major fraction, obtained from the butanol fraction of *Didemnum rodriguesi*, was purified by reversed-phase HPLC to afford 64 mg of what was apparently a pure compound with an optical rotation of $[\alpha]^{20}_D$ –23 (*c* = 0.002, MeOH). However, the (+) LRFABMS of this material displayed three major [M + H]$^+$ peaks at *m/z* 677, 691, and 705, differing by 14 mass units and suggesting the presence of three homologous compounds. Once again, the corresponding [M – H + Na]$^+$ peaks were observed at *m/z* 698, 712, and 726 when the (+) LRFABMS was obtained after the addition of NaCl to the matrix.



Scheme 1. Fragmentation patterns observed in (+) LRFABMS of minalemines A-C (**1-3**).

After several unsuccessful attempts to isolate the individual components, we were able to separate them by gradient-analytical HPLC into compounds **4-6** in a 7:4.5:4 ratio respectively. HPLC-MS confirmed the presence of these three compounds and gave the MS for each individual component: **4** (t_R 7.6 min, $[M + H]^+$ at *m/z* 677), **5** (t_R 8.0 min, $[M + H]^+$ at *m/z* 691), and **6** (t_R 8.4 min, $[M + H]^+$ at *m/z* 705). The molecular formulas, $C_{29}H_{60}N_{10}O_6S$ for **4**, $C_{30}H_{62}N_{10}O_6S$ for **5**, and $C_{31}H_{64}N_{10}O_6S$ for **6**, differed by one CH_2 unit and, were established by HRFABMS. The (+) LRFABMS and HPLC-MS also show significant ions at *m/z* 597 for **4**, 611 for **5**, and 625 for **6.** HRFABMS indicated that these correspond to the loss of SO_3 from the corresponding pseudomolecular ions $[M + H]^+$ of **4**, **5**, and **6**, suggesting these compounds contain a sulfonic acid group. Their (–) LRFABMS show very intense ions $[M - H]^-$ at *m/z* 675, 689, and 703 (parent ion), indicative of the easy loss of a proton from the FAB generated anion. All this information, along with the IR band at 1238 cm^{-1}, demonstrates the existence of an SO_3H group in **4-6**.

As minalemines D-F (**4-6**) are homologs and differ only in the number of the CH_2 groups, we carried out the structural elucidation directly on the mixture. Thus, one- (^1H, ^{13}C, DEPT) and two- dimensional (COSY, TOCSY, HMQC, and HMBC) NMR analysis of the mixture of minalemines D-F (**4-6**), and comparison with those of minalemine A (**1**), indicated the presence of the same four residues as in **1** and in the same sequence. The molecular formulas of minalemines D-F (**4-6**) differ from those of minalemines A-C (**1-3**) by SO_3, confirming the presence of an additional SO_3H group in the same skeleton.

The location of the SO_3H group linked to the central nitrogen atom in the Ncma residue (Ncma-SO_3H) was established by careful comparison of the ^{13}C- and ^1H-NMR chemical shifts of minalemines D-F (**4-6**) with those of minelamine A (**1**). Thus, while the signals for the leucine, agmatine, and homoagmatine residues of **4-6** resonate at practically identical

chemical shifts as in **1**, substantial differences were found in the chemical shifts of the *β-N*-carboxymethyl amino acid residue (Ncma) and, in particular, in the protons and carbons vicinal to the nitrogen atom. This information indicates that the sulfonic acid group must be linked to that particular nitrogen. The 1D and 2D NMR spectra were run in several solvents (CD_3OD, C_5D_5N, and DMSO-d_6) and this confirmed the location of the sulfonic acid group in the Ncma residue (Ncma-SO_3H). Therefore, minalemines D-F (**4-6**) are the sulfamic acid derivatives of minalemines A-C (**1-3**), as was later confirmed by selective hydrolysis.

Chemical transformations were carried out in order to confirm the proposed structures for all these compounds and to determine the absolute stereochemistry of the leucine unit. The presence of free guanidine functions in minalemines **1-6** was shown by a positive coloration with Sakaguchi[12] reagent and confirmed by the formation of the expected 4,6-dimethylpyrimidine derivative on treatment with 2,4-pentanedione[13] in pyridine.

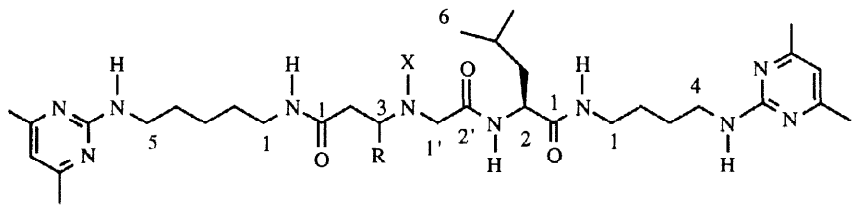


Figure 2. 4,6-dimethylpyrimidine derivatives of minalemines A-F (**1-6**).

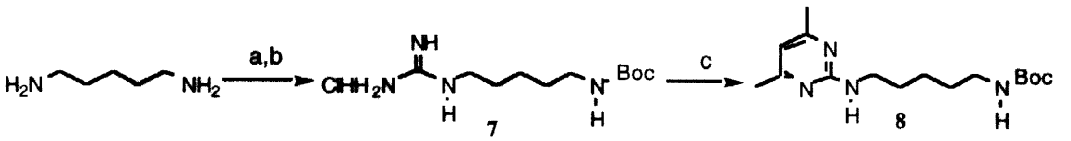
Strong acid hydrolysis of minalemines A-F (**1-6**) led to cleavage of the peptide bonds and also to the loss of the guanidine groups. Chiral GC-MS analysis after derivatization using *n*-BuOH and TFA on the hydrolyzed product showed the presence of L-leucine, 1,4-butanediamine, and 1,5-pentanediamine in a 1:1:1 ratio. Mild acid hydrolysis was shown to be very selective and to produce the exclusive cleavage of the *N*-SO_3H group. In this way, treatment of the sulfamic acid-containing minalemines D-F (**4-6**) with 3M HCl afforded minalemines A-C (**1-3**).

The stability of the 4,6-dimethylpyrimidine derivatives was found to be of great use in the protection of the terminal guanidines during hydrolysis. In this way, when the acid hydrolysis was carried out on the guanidine-protected derivatives of **1** and the resulting product derivatized as the *N*-trifluoroacetyl butyl ester derivatives, the GC-MS showed the presence of agmatine and homoagmatine 4,6-dimethylpyrimidine and L-leucine derivatives in a 1:1:1 ratio. The same results were obtained when the process was repeated with **2**, **3**, and the mixture of minalemines D-F (**4-6**). For this GC-MS comparison we used commercially available D- and L-leucine and agmatine. An authentic sample of the diprotected homoagmatine **8** was prepared[14-15] by the procedure shown in Scheme 2, hydrolyzed and derivatized as the *N*-trifluoroacetyl derivative, before GC-MS injection.

Table 2.
^{1}H- and ^{13}C-NMR spectral data for minalemines D-F (**4-6**)

Position	CD_3OD	C_5D_5N	DMSO-d_6	CD_3OD	C_5D_5N	DMSO-d_6	δ_c
	δ_H mult (*J* Hz)	δ_H mult (*J* Hz)	δ_H mult (*J* Hz)	δ_c	δ_c	δ_c	mult
Hagma							
1	3.32 m/3.04 m	3.59 m/3.29 m	3.06 m/2.96 dd (6.1, 13.4)	38.6	39.0	37.9	t
2	1.61 m	1.83 m/1.69 m	1.40 m	25.4	25.8	25.2	t
3	1.29 m	1.20 m	1.25 m	23.8	24.1	23.3	t
4	1.61 m	1.83 m/1.69 m	1.40 m	25.4	25.8	25.2	t
5	3.14 m	3.39 m	3.06 m	41.2	41.9	40.6	t
1-NH		8.36 m	7.97 br s				
5-NH		8.27 br s	7.43 br s				
NH-gua			6.93 br s/7.25 br s				
C=N				157.6	158.3	156.6	s
Ncma -SO_3H							
1				173.1	172.9	171.1	s
2	2.3 6m/2.13 m	2.74 m/2.46 m	2.22 dd (12.0, 3.3) /2.07 m	39.6	40.6	38.5	t
3	3.94 m	4.52 m	3.76 m	58.2	58.5	56.5	d
4	1.61 m	2.09 m/1.47 m	1.56 m/1.23 m	32.7	33.1	31.7	t
5	1.29 m	1.33-1.12 m	1.33 m	26.6	27.1	28.3	t
6-8	1.29 m	1.33-1.12 m	1.33 m	26.1-32.0	26.7-31.9	27.9-31.2	t
9	1.29 m	1.33-1.12 m	1.33 m	22.7	22.8	22.0	t
10	0.87 t br	0.83 t (7.5)	0.82 t	13.4	14.2	13.9	q
1'	3.64 dd (6.3)	4.26 d (16.8)/ 4.07 d (16.8)	3.47 d (16.4)/ 3.36d (16.4)	47.9	48.3	47.1	t
2'				174.1	173.0	171.5	s
L-Leu							
1				174.5	173.9	172.2	s
2	4.31 m	4.96 m	4.11 m	52.6	52.9	51.1	d
3	1.75 m	2.21 m	1.56 m	39.9	40.6	39.7	t
4	1.75 m	2.09 m	1.40 m	25.2	25.5	24.3	d
5	0.87 br d	1.04[a] d (6.0)	0.82 d	22.7	23.6	23.1	q
6	0.87 br d	1.00[a] d (6.3)	0.82 d	20.4	21.4	21.0	q
NH		9.77 m	8.66 d (7.7)				
Agma							
1	3.35 m/3.04 m	3.70 m/3.15 m	3.06 m/ 2.96 dd (6.1, 17.4)	38.7	39.0	38.1	t
2	1.61 m	1.86 m/1.65 m	1.40 m	25.5	26.1	25.2	t
3	1.61 m	1.86 m/1.65 m	1.40 m	25.5	26.1	25.2	t
4	3.14	3.46 m	3.06 m	41.2	41.9	40.5	t
1-NH		9.11 br s	7.79 br s				
4-NH		8.64 br s	7.67 br s				
NH-gua			6.93 br s/7.25 br s				
C=N				157.6	158.5	156.7	s

[a] Signals can be interchanged.



a) $(BOC)_2O$, THF, r.t.; b) 1*H*-pyrazol-1-carboxamidine hydrochloride, DIEA, DMF, and c) 2,4-pentanedione, Py, 128 °C, 3h.

Scheme 2. Synthesis of 4,6-dimethylpyrimidine derivative of homoagmatine **8**.

The presence of highly nitrogenated compounds and of guanidine groups in tunicates is well documented[1-10], but the minalemines D-F constitute the first report of marine metabolites incorporating a sulfamic acid functionality. Several antibiotic peptides with β-amino acids bearing a long saturated chain have been reported from microorganisms: An illustrative example is iturin A[16], a cyclic peptide which was isolated as an inseparable mixture of CH_2 homologs. As far as we know, the only precedent of a marine peptide containing a similar residue to Ncma, but forming part of a diketopiperazine ring, is etzionin, which was isolated from an unidentified red tunicate collected in the Red Sea[11]. Biogenetically, the minalemines can be envisioned as the result of the condensation of homoagmatine, Ncma (a β-amino acid bearing a long saturated chain), L-Leu, and agmatine. The Ncma residue could be formed from the Michael condensation of glycine and a C_{10}, C_{11} or C_{12}, α,β-unsaturated fatty acid (minalemines A/D, B/E, and C/F respectively).

Although etzionin has been reported to be active against *Candida albicans*[11], minalemines A-F were shown not to be active in this assay, nor against a battery of human and mouse tumoral cells. In spite of our efforts, crystals of **1** suitable for X-ray diffraction analysis could not be obtained and the absolute stereochemistry of the remaining chiral carbon remains unknown. Stereospecific synthesis of Ncma and of minalemines is underway.

EXPERIMENTAL

General: NMR spectra were recorded on a Bruker WM-250 (250.23 MHz for ^1H and 62.89 MHz for ^{13}C), Bruker ARX-4000 (400.13 MHz for ^1H and 100.61 MHz for ^{13}C) and Bruker AMX-500 (500.13 MHz for ^1H and 125.75 MHz for ^{13}C) using CD_3OD, C_5D_5N, and DMSO-d_6. For HMQC experiments $^1J_{CH}$ = 130-140 Hz was used and $^nJ_{CH}$ = 9 Hz for HMBC experiments. FABMS were obtained on a Kratos MS-50 and on a Fisons VG AUTOSPECM. HPLC separations were performed on a reversed-phase μ-Bondapack C_{18} (7.8 mm I.D. x 30 cm) and on a μ-Bondapack-NH_2 (7.8 mm I.D. x 30 cm) columns. Gas chromatography analysis was performed with a Chirasil-Val III capillary column (50 m x 0.25 mm).

Extraction and Isolation: The frozen tunicate was lyophilized and 1 Kg of lyophilizate was extracted with MeOH at r.t. (4 x 4 L). The methanol extract was decanted off and concentrated in vacuum. The viscous concentrate was partitioned between 10% aqueous methanol (1 L) and hexanes (2 x 2 L). The methanolic portion was then made 40% aqueous and extracted twice with CH_2Cl_2 (2 x 2 L). Finally, the MeOH was removed from the aqueous/alcoholic layer and the resulting aqueous portion was extracted twice with *n*-BuOH (2 x 2 L).

The organic layers were concentrated under vacuum to yield 4.7 g from the hexanes, 7.2 g from the CH_2Cl_2 extract and 15.6 g from the *n*-butanol extract. The *n*-butanol extract was desalted by passage through an Amberlite XAD-2 column eluted first with water (3 bed volumes) and then with methanol (2 bed volumes). Concentration of the methanol eluates gave a residue which was chromatographed on a Sephadex LH-20 column (60 x 4 cm) eluting with MeOH:H_2O (2:1). The fraction eluted first (from 450 to 650 mL; 1.320 g) was submitted to HPLC separation on a reversed-phase μ-Bondapack C_{18} HPLC column [30 cm x 7.8 mm, MeOH:H_2O:TFA (60:40:1), flow 3 mL/min] affording minalemines A (**1**) (9 mg, t_R 7 min), B (**2**) (12 mg, t_R 9.30 min), and C (**3**) (7 mg, t_R 12 min). The second fraction (from 700 to 900 mL; 1.95 g) was purified on a reversed phase μ-Bondapack-NH_2 column [30 cm x 7.8 mm, CH_3CN:MeOH:H_2O (20:20:1), flow 3 mL/min] yielding 64 mg of the mixture of **4**, **5**, and **6** as a single peak (t_R 12 min, $[\alpha]_D$ – 23.3 (*c* = 0.002, MeOH). Analytical HPLC and HPLC-MS on a reversed-phase C-18 column (15 cm x 21 mm) using a linear gradient of a mixture of solvents A (H_2O 0.1% TFA) and B (CH_3CN 0.1 % TFA) at 200 μL/min (A/B ratio from 95:5 up to 5:95 in 10 min) and UV detector at 214 nm showed the three components in a 7:4.5:4 ratio: minalemine D (**4**) (t_R 7.6 min.; M^+ at *m/z* 677), minalemine E (**5**) (t_R 8.0 min; M^+ at *m/z* 691), and minalemine F (**6**) (t_R 8.4 min; M^+ at *m/z* 705).

Minalemine A (1).- $[\alpha]_D$ –22.8 (c = 0.002, MeOH); UV (MeOH) λ_{max} 222 nm; IR (KBr) 1667, 2850–3000, and 3100–3500 cm^{-1}; HRFABMS *m/z* 597.4930 $[M + H]^+$ calcd. for $C_{29}H_{61}N_{10}O_3$ (Δ 0.2 mmu); (+) LRFABMS using thioglycerol as matrix *m/z* (rel int): 597 ($[M + H]^+$, 37), 411 (8), 326 (6), 253 (21), 185 (25), 171 (12), 157 (15), 85 (100); (+) LRFABMS using glycerol as matrix + NaCl *m/z* 619 $[M + Na]^+$; FAB MS/MS *m/z* 597 $[M + H]^+$, 580 $[M - NH_2]^+$, 564 $[M - 2NH_2]^+$; ^1H and ^{13}C NMR, see Table 1.

Minalemine B (2).- $[\alpha]_D$ –21.2 (c = 0.001, MeOH); UV (MeOH) λ_{max} 222 nm; IR (KBr) 1667, 2850–3000, and 3100–3500 cm^{-1}; (+) LRFABMS using glycerol as matrix *m/z* (rel int): 611 ($[M + H]^+$, 33), 425 (8), 340 (4), 267 (15), 185 (18), 171 (10), 157 (13), 85 (100); ^1H and ^{13}C NMR, see Table 1.

Minalemine C (3).- $[\alpha]_D$ –23.3 (c = 0.001, MeOH); UV (MeOH) λ_{max} 222 nm; IR (KBr) 1667, 2850–3000, and 3100-3500 cm^{-1}; (+) LRFABMS using glycerol as matrix *m/z* (rel int): 625 [($[M + H]^+$, 58), 439 (12), 354 (8), 281 (16), 185 (18), 171 (23), 157 (30), 85 (100); ^1H and ^{13}C NMR, see Table 1.

Minalemine D (4).- UV (EtOH) λ_{max} 272 nm; IR (KBr) 1039, 1182, 1233, 1653, 2857, 2928, and 3337 cm^{-1}; HRFABMS *m/z* 677.4333 $[M + H]^+$ calcd. for $C_{29}H_{61}N_{10}O_6S$ (Δ –16.3

mmu), 597.4858 [M – SO_3 + H]$^+$ calcd. for $C_{29}H_{61}N_{10}O_3$ (Δ –7.0 mmu); (+)LRFABMS using glycerol as matrix *m/z* 677 [M + H]$^+$, 597 [M – SO_3 + H]$^+$; (+) LRFABMS using glycerol as matrix + NaCl *m/z* 698 [M – H + Na]$^+$; (–)LRFABMS *m/z* 675 [M – H]$^-$. HPLC/MS *m/z* (rel int): 677 (100), 597 (15); ^{1}H and ^{13}C NMR, see Table 2.

Minalemine E (5).- UV (EtOH) λ_{max} 272 nm; IR (KBr) 1039, 1182, 1233, 1653, 2857, 2928, and 3337 cm^{-1}; HRFABMS *m/z* 691.4480 [M + H]$^+$ calcd. for $C_{30}H_{63}N_{10}O_6S$ (Δ 17.2 mmu) and 611.4962 [M – SO_3 + H]$^+$ calcd. for $C_{30}H_{63}N_{10}O_3$ (Δ 12.2 mmu); (+) LRFABMS using glycerol as matrix *m/z* 691 [M + H]$^+$, 611 [M – SO_3 + H]$^+$; (+) LRFABMS using glycerol as matrix + NaCl *m/z* 712 [M – H + Na]$^+$; (–) LRFABMS *m/z* 689 [M – H]$^-$ HPLC/MS *m/z* (rel int): 691 (100), 611 (17); ^{1}H and ^{13}C NMR, see Table 2.

Minalemine F (6).- UV (EtOH) λ_{max} 272 nm; IR (KBr) 1039, 1182, 1233, 1653, 2857, 2928, and 3337 cm^{-1}; HRFABMS *m/z* 705.4666 [M + H]$^+$ calcd. for $C_{31}H_{65}N_{10}O_6S$ (Δ –14.3 mmu), 625.5115 [M – SO_3 + H]$^+$ calcd. for $C_{31}H_{65}N_{10}O_3$ (Δ –12.6 mmu); (+)LRFABMS *m/z* using glycerol as matrix 705 [M + H]$^+$, 625 [M – SO_3 + H]$^+$; (+) LRFABMS using glycerol as matrix + NaCl *m/z* 726 [M – H + Na]$^+$; (–)LRFABMS *m/z* 703 [M – H]$^-$; HPLC/MS *m/z* (rel int): 705 (100), 625 (14); ^{1}H and ^{13}C NMR, see Table 2.

Acid hydrolysis of minalemines A-F and GC/MS analysis. 1 mg of the minalemine dissolved in 1 mL of 6M HCl was heated at 110 °C for 24 h in a sealed tube. The excess HCl was removed in vacuo and the hydrolyzate was dissolved in water (1 mL) and treated with *n*-BuOH (1 mL) at 100 °C for 40 min. The concentrated reaction product was dissolved in CH_2Cl_2 (1 mL) containing trifluoracetic anhydride (1 mL), heated in a sealed tube at 150 °C for 15 min, and then the solvent evaporated in a stream of nitrogen. Capillary GC-MS analysis (Chirasil Val III; He at 1 mL/min as carrier; oven temperature from 50 to 200 °C at 2 °C/min.) showed peaks at 45.37, 62.21 and 63.90 min in a 1:1:1 ratio, which were identified as L-leucine, 1,4-butanediamine, and 1,5-pentanediamine.

Acid hydrolysis of the 4,6-dimethylpyrimidine derivatives of minalemines A–F and GC/MS analysis. A sealed tube containing 3 mg of the minalemine, 0.3 mL of 2,4-pentanedione and 0.5 mL of pyridine was heated at 125 °C for 2.5 h and then evaporated in vacuo. The residue dissolved in 1 mL of 6M HCl was heated at 110 °C for 24 h in a sealed tube. The excess HCl was removed in vacuo and the hydrolyzate dissolved in water (1 mL) and treated with *n*-BuOH (1 mL) at 100 °C for 40 min. The concentrated reaction product was dissolved in CH_2Cl_2 (1 mL) containing trifluoroacetic anhydride (1 mL) and was heated in a sealed tube at 150 °C for 15 min, the solvent evaporated in a stream of nitrogen and injected into the Gas chromatograph. Capillary GC-MS analysis and comparison with authentic samples, [Chirasil-Val III column (50 m x 0.25 mm) ; carrier gas: He; flow rate: 1 mL/min; oven temperature: 50 °C isothermal for 30 min and then heating at 2 °C/min up to 200 °C]showed peaks at t_R 45.4, 72.09, and 76.40 min in a 1:1:1 ratio, corresponding to the TFA amide-butyl ester of L-Leu, and the TFA amides of the 4,6-dimethylpyrimidine derivatives of agmatine and homoagmatine respectively. Standard D and L-leucine, agmatine and homoagmatine were derivatizated by the same procedure. Homoagmatine was prepared as shown below.

Preparation of the 4,6-dimethylpyrimidine derivative of homoagmatine (8). To a stirred solution of 1,5-pentanediamine, (1.34 g, 13.1 mmol) in 25 mL of THF, 0.6 mL (2.6 mmol) of di-*t*-butyldicarbonate was added dropwise at room temperature and under argon. The reaction mixture was then concentrated under reduced pressure and the crude material dissolved in water, acidified with HCl until pH 2–3, and the diprotected amine formed extracted with hexane/ether 1:1 (3 x 25 mL) and discharged. The aqueous layer was basified with 2M NaOH until pH 11–12, and extracted with hexane/ether 1:1 to give 120 mg of the Boc-protected amine [^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ (ppm) 1.23 (m, 2H), 1.33 (s, 9H), 1.34 (m, 2H), 1.36 (m, 2H), 2.58 (m, 2H), 3.02 (t, J=6.4 Hz, 2H), and 4.82 (br s, NH)], used without further purification in the next step.

To a stirred solution of crude mono Boc-protected 1,5-pentanediamine (107 mg, 0.53 mmol) in 0.5 mL of DMF was added, 1*H*-pyrazole-1-carboxamidine hydrochloride and DIEA (0.53 mmol each) under nitrogen. After stirring for 4 h at room temperature, 25 mL of ether were added to complete precipitation of crude **7**, which was collected, washed with ether and dried to give 67 mg of **7**: [^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ (ppm) 1.33 (s, 9H), 1.39 (m, 2H), 1.45-1.53 (m, 4H), 2.98 (m, 2H), 3.18 (m, 2H), 5.23 (br s, NH).^{13}C-NMR (CD_3Cl, 100.61 MHz): δ(ppm) 23.4, 27.9, 28.3, 29.1, 40.1, 41.2, 78.9, 156.3 and 157.5 ppm].

A solution of 67 mg (0.24 mmol) of crude **7** in pyridine (0,5 ml) and 2,4-pentanedione (0.5 mL) were heated in a sealed tube at 125-128°C for 3h. The solvent was removed under reduced pressure to yield 28 mg of compound **8** purified by flash chromatography on silica gel 60 (230-240 mesh) using acetate/hexane 1:3 as eluent. [^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ(ppm) 1.37 (m, 2H), 1.43 (s, 9H), 1.51 (m, 2H), 1.59 (m, 2H), 2.26 (s, 6H), 3.11 (t, J=6.2 Hz, 2H), 3.40 (m, J=6.0 and 6.7 Hz, 2H), 4.55 (br s, NH), 5.08 (br s, NH) and 6.27 (s, 1H). ^{13}C-NMR (CD_3Cl, 100.61 MHz): δ(ppm) 23.8, 24.0, 28.4, 29.7, 40.4, 41.1, 79.0, 109.4, 155.9, 162.2 and 167.3 ppm. EIMS *m/z* (%relative intensity): 308 (M^+, 32), 235 (29), 136 (100), 122 (38) and 107(17)].

Selective conversion of minalemines D-F to (4-6) to minalemines A-C (1-3). 5 mg of the mixture of minalemines D-F (**4-6**) were treated with 3M HCl (2 mL) at r.t. for 24 h. The residue was basified and extracted with $CHCl_3$: ^{i}PrOH (8:2) giving a mixture of minalemines A-F (**1-3**), characterized by NMR and MS.

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