Crystallographic evidence for the proposed host behaviour of ptilomycalin A

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The X-ray crystal structures of the model compounds 3, 4 and 6 are determined and used as evidence to support proposed anionic recognition in the alkaloid ptilomycalin A 1.

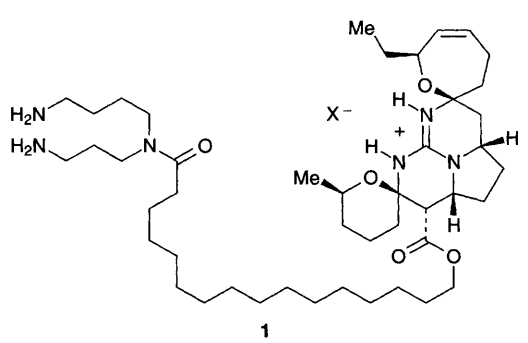
The hydrogen-bond mediated interaction of guanidinium ions with phosphate and carboxylate containing biomolecules is of considerable interest in bioorganic chemistry.[1] Not least of these are the key interactions of the guanidine-containing side chains of arginine residues involved in substrate recognition at enzyme active sites.[2] In addition the guanidinium motif has also been utilised in synthetic host receptors for phosphate and carboxylate containing host molecules, which are of considerable current interest.[1,3]

Recently, a potent antitumor, antiviral and antifungal metabolite, designated ptilomycalin A was isolated from the Caribbean sponge *Ptilocaulis spiculifer* and its structure determined as **1**.[4] The same compound was also isolated from a Red Sea sponge of *Hemimycale sp.*[5] and also from a Caribbean sponge of the *Batzeela sp.*,[6] and can be considered as the parent molecule of a growing class of related metabolites.[6,7]

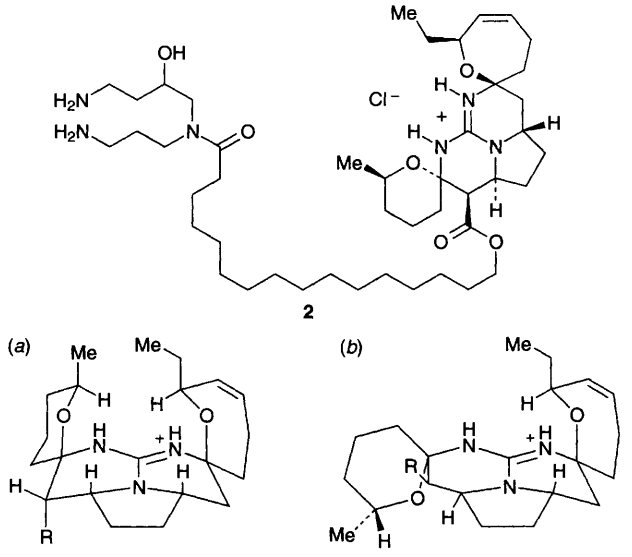
Ptilomycalin A **1** possesses an intriguing structure consisting of a pentacyclic guanidine moiety linked by a long-chain ω-hydroxy acid to a spermidine unit. These features have led to some speculation[8] as to the exact biological role of ptilomycalin A which has centred on its structural similarities to abiotic guanidine based anionic receptor molecules.[9] Indeed Kashman and Kusumi[5] investigated the complexing ability of a derivative of **1** with various organic carboxylates and have determined a scale of binding ability for *N*-acetylamino acids which was estimated to be as follows: L-*N*-acetylmethionate ≈ L-*N*-acetylvalinate > L-*N*-acetylalanate ≈ L-*N*-acetylisoleucinate >> *N*-acetylglycinate. In addition, despite the presence of several polar functional groups, ptilomycalin A behaves as if it were non-polar and is freely soluble in organic solvents such as chloroform;[4] this property also suggests an anionic binding capability possibly tied to strong lipophillic behaviour.[9]

These observations are further supported by analysis of ptilomycalin A **1** which illustrates the presence of an enclosed ionic pocket at the central guanidine sub-unit; this pocket may be acting as a specific recognition site and conferring much of the biological activity found in these compounds [Fig. 1(*a*)]. In relation to this, it is interesting to note that the subsequently isolated 13,14,15-isocrambescidine 800 **2** is substantially less cytotoxic to L1210 cells than other crambescidins and has no observed antiviral activity against HSV-1;[10] this drop in activity may be due to the lack of this structural feature, [Fig. 1(*b*)].

With the above considerations in mind it is not surprising that there is considerable synthetic activity[9,11] towards ptilomycalin A and its structural relatives. Our own research has centred on a biomimetic approach to **1** and we have succeeded in preparing a range of synthetic analogues **3**–**6** using a tandem Michael addition–spirocyclisation sequence (Fig. 2).[12]

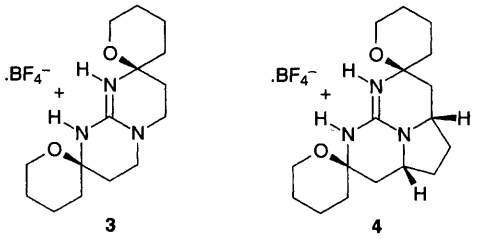


1

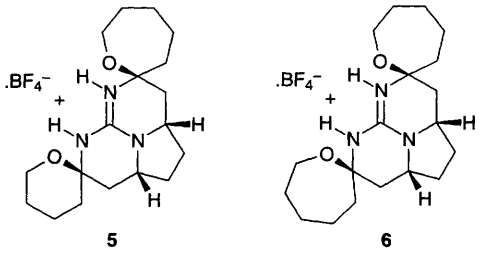


2

Fig. 1



3 **4**



5 **6**

Fig. 2

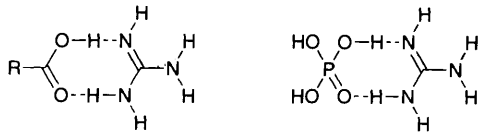


Fig. 3

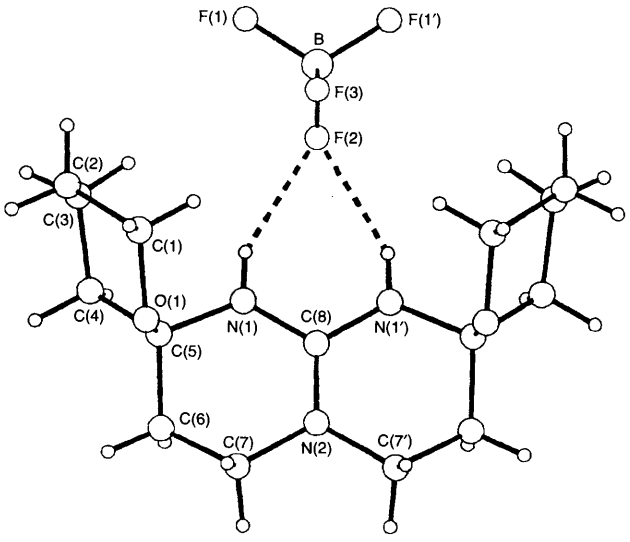


Fig. 4

As part of our structural assignment procedure for these molecules the crystal structures of **3**, **4** and **6** were determined† and from these determinations we observed some interesting behaviour in the interaction of the guanidinium moieties with the fluoroborate counter ions. The fluoroborate anion can undergo[13] a similar interaction with a guanidinium ion to the bidentate ligating interaction that is observed with a carboxylate[14] or a phosphate[15] anion (Fig. 3).

It was thus surprising to find that in the crystal structure of the tetracyclic 6,6,6,6 model compound **3** (Fig. 4) only one of the fluorine atoms of the fluoroborate anion was in fact involved in a strong hydrogen bonding interaction to both N–H bonds of the guanidinium ion (N–H···F hydrogen bonds; H···F, N···F distances and N–H–F angles, respectively, are 2.14, 2.90 Å and 146°).[17] This observation led us to suppose that the guanidinium cavity of **3** was not of sufficient size to accommodate the fluoroborate anion. This behaviour was however not observed in the pentacyclic 6,6,5,6,6 model compound **4** which corresponds more closely to the structure of ptilomycalin A. In this case the fluoroborate anion was involved in two separate non-symmetrical hydrogen bonding interactions but is unable to achieve co-planarity with the guanidinium ions (Fig. 5) (N–H···F hydrogen bonds; H···F, N···F distances and N–H–F angles, respectively, 2.04/2.21, 2.94/3.09 Å and 155/153°). However co-planarity is nearly achieved in the case of the pentacyclic 7,6,5,6,7 model **6** which undergoes an identical hydrogen bonding pattern to **4** (Fig. 6) (N–H···F hydrogen bonds; H···F, N···F distances and N–H–F angles, respectively, 2.03/2.22, 2.88/3.07 Å and 174/170°).

These observations may offer some support to a theory of prebiotic design acting upon ptilomycalin A and related molecules, in that the cavity in **1** which is modelled to an extent by the molecules **4** and **6** is involved in the efficient recognition of a carboxylate species. The similarities between bond lengths in a guanidinium carboxylate[13] (N–H···O distances: 0.909/2.007 and 1.024/1.805 Å), a guanidinium phosphate[14] (N–H···O) distances: 0.863/1.960 and 0.885/2.026 Å) and in the fluoroborate ions found in our model compounds (N–H···F distances 0.86/2.03 and 0.86/2.22 Å typically) are close and despite obvious structural differences, support a theory of the

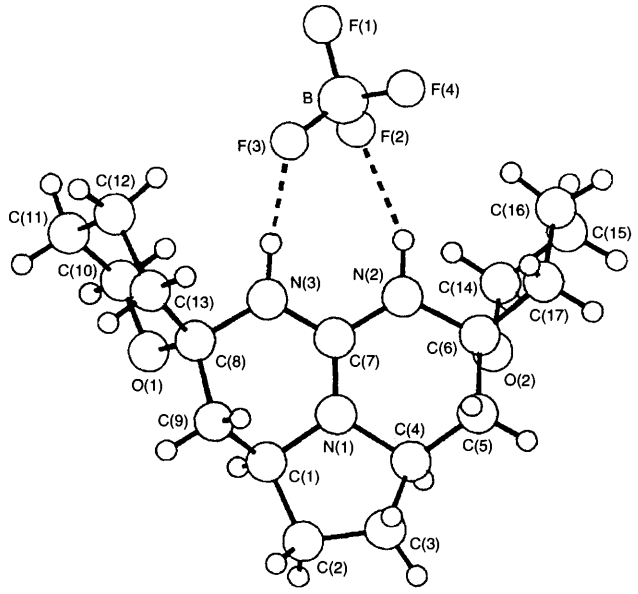


Fig. 5

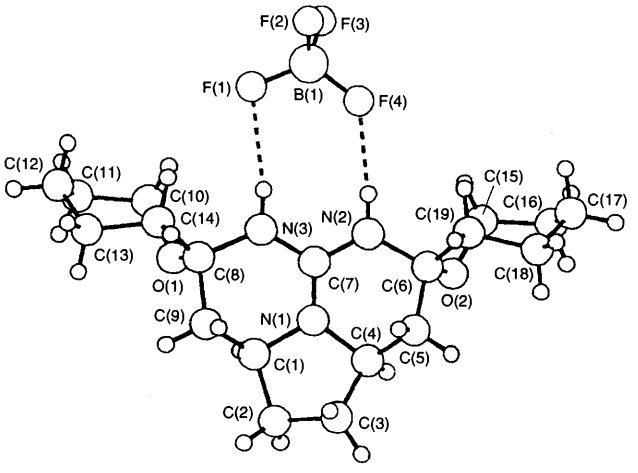


Fig. 6

cavity portion of ptilomycalin A being involved in a carboxylate binding process. Even taking into consideration differences between **1** and **4** or **6** (namely the methyl and ethyl substituents on the spirocyclic rings and the unsaturation present in the 7-membered ring of **1**) it appears that as our model compounds approach **1** in structure, the degree of co-planarity in the fluoroborate-guanidinium interaction increases; this observation may suggest that the structure of **1** represents an optimum host design for an as yet undetermined guest molecule.

This intriguing molecule is receiving considerable synthetic attention which is indeed warranted by its unique structural features, however, possibly of more importance is an in depth study of its biological mode of action concentrating on its ability to recognise specific biomolecules.

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Footnote

† *Crystal data* for **3**: $C_{15}H_{26}N_3O_2{\cdot}BF_4{\cdot}CCl_4$, M_r = 521.01, orthorhombic, a = 19.752(6), b = 11.713(4), c = 9.551(4) Å, V = 2209.7(14) Å^3, space group *Pnma*, Z = 4, D_c = 1.566 g cm^{-3}, $F(000)$ = 1072, μ(Mo-Kα) = 5.9 cm^{-1}, crystal size 0.25 × 0.15 × 0.08 mm, T = 150 K. θ = 2.06–25.08°, $-23 \leq h \leq 9$, $-10 \leq k \leq 12$, $-10 \leq l \leq 10$, total data collected 6426, unique 1808 (used in refining 168 parameters). For **4**: $C_{17}H_{28}N_3O_2{\cdot}BF_4{\cdot}CHCl_3$, M_r = 512.60, triclinic, a = 10.3616(11), b =

10.6317(9), c = 11.5276(12) Å, α = 76.64(3), β = 81.84(5), γ = 69.927(13)°, V = 1157.7(2) Å^3, space group $P\bar{1}$, Z = 2, D_c = 1.470 g cm^{-3}, $F(000)$ = 532, μ(Mo-Kα) = 4.5 cm^{-1}, crystal size 0.35 × 25 × 0.15 mm, T = 150 K, θ = 1.82–24.76°, $-8 \leqslant h \leqslant 11$, $-6 \leqslant k \leqslant 12$, $-12 \leqslant l \leqslant 12$, total data collected 3611, unique 3135 (used in refining 341 parameters). For **6**: $C_{19}H_{32}N_3O_2{\cdot}BF_4{\cdot}CHCl_3$, M_r = 540.65, triclinic, a = 10.043(5), b = 10.800(5), c = 13.461(8) Å, α = 75.06(3), β = 73.12(3), γ = 67.26(3)°, V = 1270.9(11) Å^3, space group $P\bar{1}$, Z = 2, D_c = 1.413 g cm^{-3}, $F(000)$ = 564, μ(Mo-Kα) = 4.1 cm^{-1}, crystal size 0.30 × 15 × 0.12 mm, T = 150 K. θ = 2.82–25.07°, $11 \leqslant h \leqslant 10$, $-11 \leqslant k \leqslant 12$, $-13 \leqslant l \leqslant 15$, total data collected 5188, unique 3420 (used in refining 358 parameters).

Crystallographic measurements for all compounds (**3**, **4** and **6**) were made at 150 K using a FAST area detector diffractometer and Mo-Kα radiation (λ = 0.71069 Å), following previously described procedures.[17] The structures were solved by direct methods and refined on Fo^2 by full-matrix least-squares to final wR_2/R values of 0.1951/0.1079 (**3**), 0.2055/0.1091 (**4**) and 0.1083/0.0886 (**6**) for all unique data above background; the corresponding wR_2/R values for the observed data [$I > 2\sigma(I)$] were 0.1623/0.0665 (**3**), 0.15590/0.0685 (**4**) and 0.0997/0.0467 (**6**). In all cases, the non-hydrogen atoms were anisotropic. Hydrogen atoms were included in idealised positions with U_{iso}s being refined (**3** and **4**) or tied to the U_{eq}s of the parents **6**. The hydrogen on $CHCl_3$ in (**4** and **6**) was ignored. Some atoms in both the BF_4 anions and $CHCl_3$ molecules (**4** and **6**) and CCl_4 molecule (**3**) were disordered; these were assigned partial site occupancies and refined successfully. All calculations were done on a 486DX2/66 personal computer using the programs SHELX-S[18] (solution), SHELXL-93[19] (refinement) and SNOOPI[20] (diagrams). The atom scattering factors were as in SHELXL-93.[19] Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

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