OCH_3), 2.39 (m, 1 H, H-8), 2.30 (dt, 2 H, H-2, J = 7.3 Hz, J = 1.8 Hz), 2.10 (t, 2 H, H-4, J = 7.8 Hz), 2.05 (t, 2 H, H-7, J = 7.8 Hz), 1.68 (dp, 2 H, H-3, J = 7.3 Hz, J = 1.5 Hz), 1.60-1.20 (m, 8 H, H-16, H-17, H-18, H-9), 0.9 (t, 3 H, H-20, J = 6.9 Hz); ^{19}F NMR ($CDCl_3$, 376.2 MHz) ϕ 102.37 (d, $J_{F,F}$ = 192.6 Hz), 130.49 (dm, $J_{F,F}$ = 192.6 Hz).

10,10-Difluoro-9β,11β-thromboxane A_2 Methyl Ester (44). A THF solution of the reducing agent S-1 was prepared by treating $LiAlH_4$ in THF (1.0 M solution in THF) with equimolar amounts of ethanol (1.0 M solution in THF) and optically pure (*S*)-(-)-1,1'-bi-2-naphthol (0.5 M solution in THF) at 0 °C and then stirring at 22 °C for 1 h. Three equivalents of the freshly prepared reducing agent (S-1) (7.5 μmol) was cooled at -78 °C, and to this cold cloudy mixture was added the α,β-unsaturated ketone **43** (1.0 mg, 2.5 μmol, in 100 μL of dry THF). The mixture was stirred at -78 °C for 3 h and then quenched with moist ethyl ether, filtered through a Celite pipet column, and concentrated. Crystallization from hexane with a trace of ethyl ether gave recovered chiral auxillary ligand, (*S*)-(-)-1,1'-bi-2-naphthol. Normal-phase HPLC chromatography of the residue (silica gel, 10 mm i.d. column) with 1% *n*-PrOH/99% hexane as eluant (3 mL/min, detected at 205 nm) gave pure the 15*S* isomer (**44**, 0.6 mg) at 29, 30, and 31 min. This material was found to be identical by ^{1}H and ^{19}F NMR with the slower moving isomer obtained in the $NaBH_4$ reduction.

10,10-Difluoro-9β,11β-thromboxane A_2 (46). To a solution of the methyl ester of 10,10-difluorothromboxane (≈300 μg) in 250 μL of methanol was added 250 μL of 1 N NaOH. The mixture was stirred at 22 °C for 1 h and carefully acidified with 0.1 N HCl to pH 3, and the hydrolysate was extracted with ethyl acetate (10 × 1 mL). The ethyl acetate extract was dried (Na_2SO_4), filtered, and concentrated to give **46** (205 μg): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.88 (dd, 1 H, H-14, $J_{13,14}$ = 15.5 Hz, $J_{14,15}$ = 5.0 Hz), 5.77 (dd, 1 H, H-13, $J_{13,14}$ = 15.5 Hz, $J_{12,13}$ = 7.0 Hz), 5.60 (m, 1 H, H-11), 5.47 (m, 1 H, H-5), 5.43 (m, 1 H, H-6), 4.82 (m, 1 H, H-9), 4.40 (m, 1 H, H-12), 4.29 (m, 1 H, H-15), 2.40 (m, 1 H, H-8), 2.32 (m, 2 H, H-2), 2.20 (m, 2 H, H-4), 2.0 (m, 4 H, H-7, H-3), 1.80-1.20 (m, 8 H, H-16, H-17, H-18, H-19), 0.90 (t, 3 H, H-20, J = 6.5 Hz); ^{19}F NMR ($CDCl_3$, 376.2 MHz) ϕ 102.37 (d, $J_{F,F}$ = 190.7 Hz), 130.34 (d, $J_{F,F}$ = 190.7 Hz).

(15*R*)-10,10-Difluoro-9β,11β-thromboxane A_2 (46A). Following the procedure for the preparation of the 15*S* isomer, the 15*R* isomer **46A** was obtained: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.80 (dd, 1 H, H-14, $J_{13,14}$ = 15.3 Hz, $J_{14,15}$ = 6.5 Hz), 5.73 (dd, 1 H, H-13, $J_{13,14}$ = 15.3 Hz, $J_{12,13}$ = 7.7 Hz), 5.58 (m, 1 H, H-11), 5.48 (m, 1 H, H-5), 5.43 (m, 1 H, H-6), 4.83 (m, 1 H, H-9), 4.36 (m, 1 H, H-12), 4.30 (t, 1 H, H-15, J = 6.5 Hz), 2.35 (m, 4 H, H-8, H-2), 2.15 (m, 2 H, H-4), 2.03 (m, 4 H, H-3, H-7), 1.80-1.20 (m, 8 H, H-16, H-17, H-18, H-19), 0.89 (t, 3 H, H-20, J = 6.3 Hz); ^{19}F NMR ($CDCl_3$, 376.2 MHz) ϕ 102.32 (dd, $J_{F,F}$ = 191 Hz, $J_{H,F}$ = 3.1 Hz), 130.20 (dt, $J_{F,F}$ = 191 Hz).

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Structure and Chemical Properties of Ptilomycalin A

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Abstract: The structure of ptilomycalin A (**1**), a marine alkaloid possessing potent antiviral and antibiotic activites, has been determined on the basis of NMR analyses of its trifluoroacetyl (TFA) derivative (**2**). It has a unique structure consisting of a polycyclic guanidine and a spermidine group, each of which is linked to a 16-hydroxyhexadecanoic acid moiety. The rotational isomerism of the acylated sperimidine moiety of **2** has been studied by comparing the NMR properties of the synthetic trifluoroacetyl derivatives of spermidine (**5**), dipropylenetriamine (**11**), diethylenetriamine (**12**), and pentylamine (**13**). From these experiments, a plausible conformation of **2** and ptilomycalin A (**1**) has been proposed as shown in **2c**, in which an anion is trapped between the guanidine and spermidine moieties. The TFA derivative **2** acts as a phase-transfer agent. NMR analysis of the stability of the complexes formed between **2** and several organic carboxylates in $CDCl_3$ solutions has been carried out.

Introduction

A large number of studies on marine natural products have been carried out in the past two decades, firstly because marine organisms produce pharmaceutically and biologically important substances[1] and secondly because many marine natural products possess unusual chemical structures that are seldom found in the metabolites of terrestrial organisms.[2] Examples are polycyclic ethers such as palytoxin,[3] brevetoxins,[4] okadaic acid,[5] and ciguatoxin.[6] They are of physiological importance and have unique and complex structures.

It should be noted that relatively few alkaloids have been found from marine resources,[7] while a large number of alkaloids have been isolated from terrestrial plants, many of which have been used for a long time as drugs to cure human diseases.

In the course of screening for novel bioactive agents from marine sponges, we have isolated an antitumor, antiviral, and antifungal compound designated ptilomycalin A (**1**) from the Caribbean sponge *Ptilocaulis spiculifer*.[8] The same compound was also isolated from a Red Sea sponge of *Hemimycale* sp. Ptilomycalin A shows cytotoxicity against P388 (IC_{50} 0.1 μg/mL), L1210 (IC_{50} 0.4 μg/mL), and KB (IC_{50} 1.3 μg/mL) and antifungal activity against *Candida albicans* (MIC 0.8 μg/mL) as well as very good

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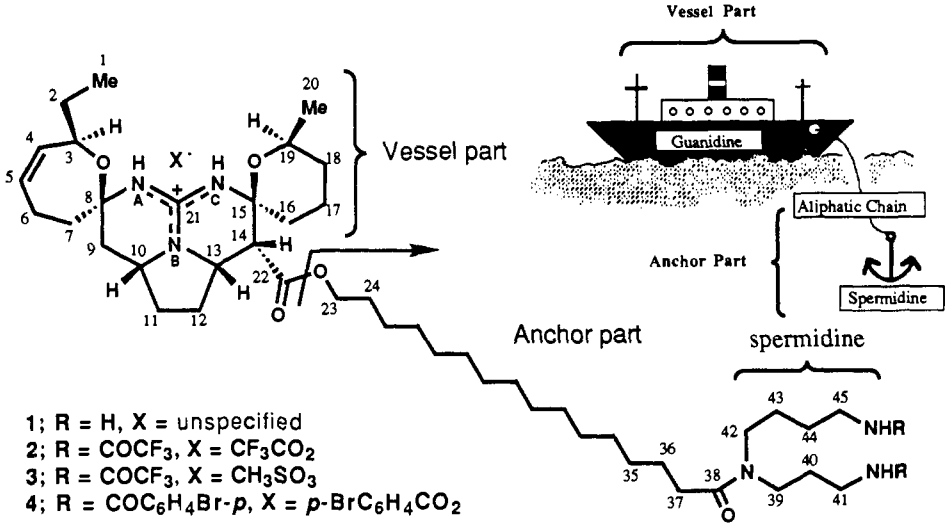
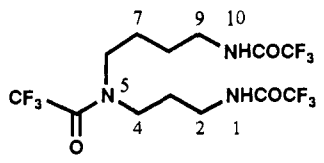
Chart I





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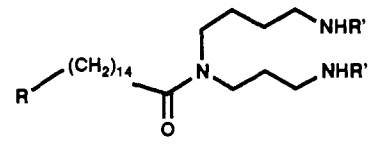
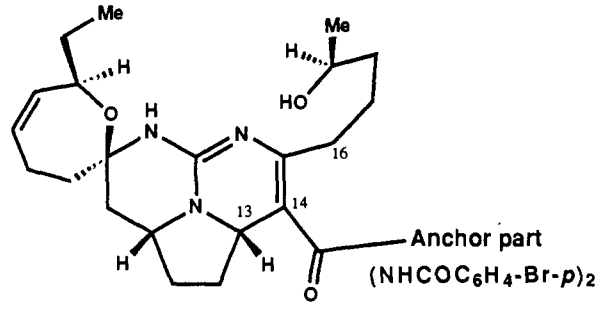


6; R = CH_3, R' = H
7; R = CH_3, R' = $COCF_3$
8; R = CH_2OH, R' = COC_6H_4Br-*p*
9; R = CH_2OAc, R' = $COCF_3$



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antiviral activity (HSV) at a concentration of 0.2 μg/mL.

In this paper, we describe the structure elucidation and chemical properties of ptilomycalin A, which proved to be a novel polycyclic alkaloid.

Results and Discussion

Ptilomycalin A (**1**),[9] $[\alpha]^{25}D$ −2.5° (*c* 0.70, $CHCl_3$), *m/z* 785 (MH^+), exhibits intense IR bands at 3600–2400 (OH or NH), 1730 (ester), and 1650 cm^{-1} (amide). In spite of its relatively high molecular weight and high affinity for TLC silica gel, ptilomycalin A is easily soluble in chloroform. The ^1H NMR spectrum ($CDCl_3$) (Table I),[10] however, gives only broad signals, and the ^{13}C NMR spectrum (Table I), although it exhibits rather sharp signals, affords little information about the molecular composition because many of the signals overlap. The molecular formula $C_{49}H_{78}N_6O_7F_6$ was obtained by HRFABMS of the bis(trifluoroacetyl) derivative **2** (TFA-ptilomycalin A), an oil, $[\alpha]^{25}D$ −15.8° (*c* 0.68, $CHCl_3$), possessing sharper proton and carbon signals in the NMR spectra (Table II). Consequently, structure elucidation work was performed mainly on this derivative.

Initially, chemical degradation such as oxidation, hydrogenation, metal hydride reduction, and acid and alkaline hydrolysis was attempted on **1** as well as on its derivatives. Most reactions, however, only gave intractable mixtures from which no identifiable product was isolated. Thus, structure elucidation had to depend primarily on spectroscopic analyses of **2**.

The ^1H and ^{13}C NMR spectra of **2** are summarized in Table II.[11] Extensive overlapping of the proton signals in the δ 1–2 regions of the ^1H NMR spectrum was the most serious problem in the elucidation of the structure. Also characteristic was the

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(10) Supplementary material.

(11) The NMR spectra were also measured in pyridine-d_5 using 2–65 mg of **2**. The chemical shifts are significantly dependent on the concentration of the solution. The shifts ($CDCl_3$) described herein are those obtained for a 60 mM solution of **2**.

Table II. NMR Properties of 2

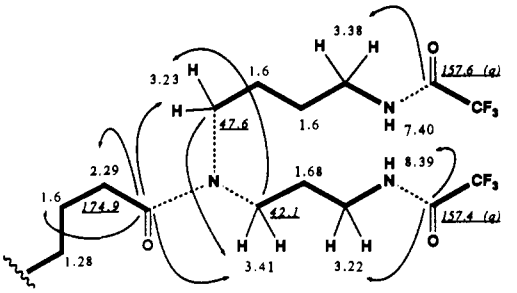
	$CDCl_3$[a]		pyridine-d_5[b]	
no.	^{13}C (δ)	^1H (δ), *m*, *J* (Hz)	^{13}C (δ)	^1H (δ)
1	10.21	0.79, t, 7.5	10.62	0.76
2	29.19	1.51, m	29.66	1.36
		1.42, tq, 14, 7.5		1.36
3	70.89	4.44, m	71.17	4.62
4	133.69	5.47, dt, 11, 2.5	134.01	5.35
5	129.96	5.65, ddt, 11, 8, 2.5	130.37	5.50
6α	23.74	2.15, m	23.96	1.85
6β		2.35, br t, 14		2.17
7α	36.22	2.58, br t, 14	36.57	2.70
7β		1.86, dd, 14, 6		1.65
8	83.86		84.25	
9α	36.89	1.40, t, 12.5	36.96	1.20
9β		2.55, dd, 12.5, 6.5		2.30
10	54.06	4.00, tt, 12.5, 6.5	54.27	3.85
11α	30.65	1.6, m	30.91	1.45
11β		2.19, m		1.98
12α	26.81	1.79, ddt, 5, 3.5, 13	27.02	1.68
12β		2.25, m		2.06
13	52.13	4.29, dt, 10, 5	52.63	4.22
14	50.10	2.94, d, 5	50.44	2.99
15	80.82		81.27	
16α	31.68	1.68, m	32.20	1.72
16β		1.68, m		1.60
17α	18.01	2.15, m	18.51	2.51
17β		1.68, m		1.55
18α	32.06	1.65, m	32.30	1.45
18β		1.18, dq, 5, 12.5		1.03
19	67.12	3.84, ddq, 12.5, 2.5, 6.5	67.21	3.91
20	21.56	1.04, d, 6.5	21.79	0.96
21	149.09		*c*	
22	168.58		169.06	
23	65.53	4.08, dt, 14, 6.5	65.57	4.12
		4.05, dt, 14, 6.5		4.05
24	28.54	1.6, m	28.93	1.51
25	25.87	1.28, br s	26.26	1.25
26				
|	*d*	1.25, br s	*e*	1.2
34				
35	*d*	1.28, br s	29.84	1.25
36	25.74	1.6, m	25.98	1.67
37	33.21	2.29, t, 8	33.20	2.28
38	174.87		173.46	
39	42.13	3.41, t, 6.5	43.11	3.38
40	27.03	1.68, m	27.89	1.77
41	36.13	3.22, q, 6.5[f]	37.57	3.36
42	47.59	3.23, m	47.71	3.17
43	26.07	1.6, m	26.70	1.53
44	26.43	1.6, m	26.86	1.54
45	39.25	3.38, q, 6.5[f]	39.76	3.42
N_AH		10.22, br s		11.20
N_CH		9.87, br s		10.74
41-NH		8.39, t, 6.5		10.05
41-CO	157.35		*g*	
41-CF_3	115.89[h]		*g*	
45-NH		7.40		10.47
45-CO	157.61		*g*	
45-CF_3	116.04[h]		*g*	

[a] For 60 mM solution. [b] ^{13}C and ^1H NMR spectra were measured for 80 and 2.5 mM solutions, respectively. [c] Overlapped with the solvent signal. [d] Carbon signals appear at δ 29.65, 29.63, 29.60, 29.57, 29.51, 29.48, 29.43, 29.15. [e] Carbon signals appear at δ 30.10, 30.06, 30.01, 29.98, 29.91, 29.87, 29.58. [f] Coupling pattern was deduced from decoupling difference spectra. [g] Exact chemical shift was not determined. [h] Assignments may be interchanged.

appearance of a number of methylene carbons in the δ 20–50 region of the ^{13}C NMR spectrum. The large signal at δ_H 1.25 indicated the presence of a long aliphatic chain. If these methylenes were due to a fatty acid (e.g., palmitic acid) moiety, a triplet for the terminal methyl would be observed, but the spectrum lacked this signal.

The molecular structure of ptilomycalin A finally obtained is shown in **1**. Owing to its structural similarity to a ship trailing an anchor, the structure elucidation will be interpreted as an "anchor part" and a "vessel part", successively (see Chart I).

Anchor Part. The ^1H NMR (500 MHz, $CDCl_3$) and H,C COSY spectra of **2** exhibited seven groups of methylene protons, and the HOHAHA[12] spectrum indicated that they could be classified into two separate networks: (i) from CH_2-42 (^1H NMR δ_H 3.23, ^{13}C NMR δ_C 47.6) to CH_2-45 (δ_H 3.38, δ_C 39.3) and (ii) from CH_2-39 (δ_H 3.41, δ_C 42.1) to CH_2-41 (δ_H 3.22, δ_C 36.1). It was deduced that the four terminal methylenes were connected to nitrogen on the basis of their chemical shifts. Two of them (CH_2-45 and CH_2-41) are coupled with the amide protons resonating at δ 7.40 and 8.39, respectively, and they further correlated with the carbonyl carbons (δ 157.6 and 157.4, respectively) in COLOC[13] and HMBC[14] spectra (see **a**). The carbonyl signals



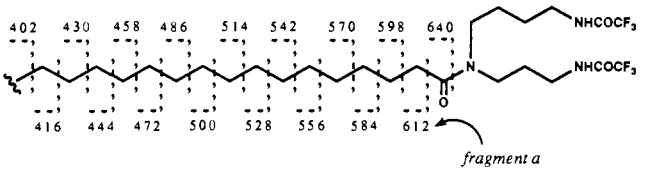
a

(proton decoupled) are split into quartets (J = 37 Hz) by coupling with the fluorine atoms of the trifluoromethyl groups; thus, they must be the carbonyl carbons of the two trifluoroacetyl groups. The 2D spectra also showed correlation of CH_2-42/C-39 and CH_2-39/C-42. These data strongly suggested that the protons were those of a spermidine moiety whose terminal amino groups were blocked by trifluoroacetyl groups.

The ^1H and ^{13}C NMR signals of the seven methylene groups are accompanied by small signals, implying that the spermidine moiety exists in two rotational isomers. Rotational isomerization is usually observed for tertiary amides.[15] Therefore, the secondary amino group of the sperimidine must be connected to a carbonyl carbon. This was confirmed by preparing the tris(trifluoroacetyl)sperimidine **5**, the NMR properties of which indicated that **5** existed in a 3:1 mixture of rotational (around the tertiary amide linkage) isomers. Its ^1H and ^{13}C NMR spectra parallel those of **2** with respect to the signals due to the spermidine moiety. The presence of NOEs (see Figure 1) between H_2-42 and H_2-37 (**2a**, major) as well as H_2-39′ and H_2-37′ (**2b**, minor) suggested the geometry of the major and minor rotational isomers. This isomerism will be discussed in detail below.

The H_2-39 and H_2-42 proton signals of **2** exhibited cross peaks to the carbonyl signals at δ 174.9 (C-38), which is also correlated with H_2-37 (δ 2.29) and H_2-36 (δ 1.6) in the COLOC and HMBC spectra. The latter proton signal is obscured by the large envelope at δ 1.25–1.28 due to the long-chain methylene groups, and further correlation of H_2-36 by homonuclear 2D experiments was impossible.

The mass spectrum of **2** was helpful in deducing the structure of the "anchor". Fragment **a** (*m*/*z* 612) is accompanied by peaks



resulting from the successive loss of methylene units. The frag-

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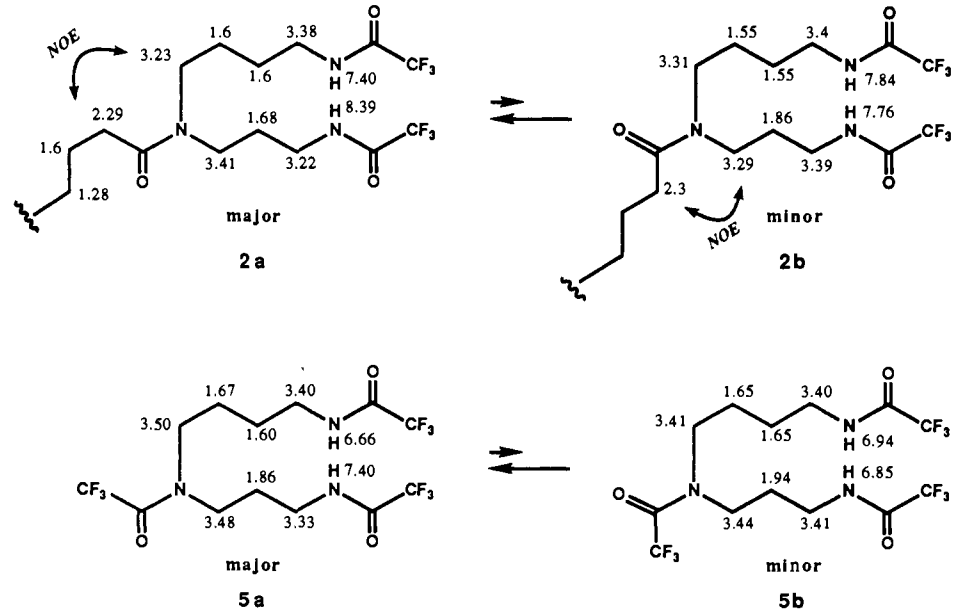


Figure 1. ^1H NMR (500 MHz, $CDCl_3$) assignment of the spermidine part of TFA–ptilomycalin A (**2**) and tris(trifluoroacetyl)spermidine (**5**). The assignment is done for the respective rotational isomers **2a** (major)/**2b** (minor) and **5a** (major)/**5b** (minor). The curved arrows in **2a** and **2b** indicate the NOE observed in the phase-sensitive NOESY spectrum.

mentation indicated that at least 15 methylenes were connected to C-38, although the exact length of the chain was not inferable at this stage.

A clue to the complete structure of the anchor was obtained when **6** was isolated from both sponges as the bis(trifluoroacetyl) derivative **7**. Its mass spectrum, *m/z* 575, showed the presence of hexadecanoyl group, and the structural features of **7** were confirmed by NMR analysis. The structure of the anchor (from C-23 to C-45) was established by methanolysis (MeONa/MeOH) or $LiAlH_4$ reduction of **2** and also by methanolysis of the di-*p*-bromobenzoate **4** (NMR data in Table III)[10] of ptilomycalin A, which resulted in the isolation of the fragments **9** (after acetylation) and **8**, respectively.[16] The main component of these fragments is (16-hydroxyhexadecanoyl)spermidine, and it was deduced that the terminal hydroxy group was connected to the "vessel" moiety of ptilomycalin A.

Vessel Part. The molecular formula corresponding to the anchor is $C_{27}H_{46}N_3O_4F_6$, which leaves the fragment $C_{22}H_{32}N_3O_3$ for the remaining "vessel" subunit of TFA-ptilomycalin A. Subtracting the ^1H signals due to the "anchor" from the whole spectrum of **2** made the analysis of the NMR spectra considerably simpler. The HOHAHA spectrum revealed that the proton signals could be grouped into the following three assemblies: (i) CH_3-1 to CH_2-7, (ii) CH_2-9 to CH-14, and (iii) CH_2-16 to CH_3-20. Besides these proton signals, two D_2O exchangeable signals appear at δ 10.22 and 9.87. Their extreme downfield chemical shifts suggested that these protons were those of an ammonium or imminium salt.[17] The fact that **2** actually exists as a salt was verified by the presence of one set of carbon signals (proton decoupled) [δ 116.8 (q, CF_3, $^1J_{CF}$ = 292 Hz) and 162.7 (q, CO–CF_3, $^2J_{CF}$ = 34 Hz)] assignable to trifluoroacetate anion. TFA-ptilomycalin A (**2**) as a salt is soluble in organic solvents, suggesting that it may act as a phase-transfer agent. In fact, shaking a $CDCl_3$ solution of **2** with an aqueous solution of sodium methanesulfonate resulted in the incorporation of 1 molar equiv of the methanesulfonate anion (**3**, δ_{Me} 2.78) into the organic layer.

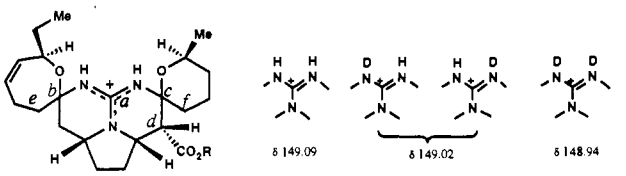
When a $CDCl_3$ solution of **2** was shaken with 0.1 N NaOH solution, the lowfield NH signals (δ 10.22 and 9.87) disappeared, but they regenerated slowly (24–48 h) on standing. These

Table IV. Deuterium Exchange Studies on the ^{13}C NMR Spectrum of **2** in the Presence of 3 Molar Equiv of CD_3OD[a]

	$CDCl_3$		pyridine-d_5	
no.	δ	$\Delta\delta$[b]	δ	$\Delta\delta$[b]
3	70.89	<0.01	71.17	<0.01
7	36.22	0.05	36.57	0.05
8	83.86	0.08	84.25	0.08
13	52.13	0	52.63	<0.01
14	50.10	0.03	50.44	0.03
15	80.82	0.08	81.27	0.08
16	31.68	0.05	32.20	0.05
19	67.12	<0.01	67.21	<0.01
21	149.09	0.15[c]	*e*	
		0.07[d]		
39	42.13	<0.01	43.11	<0.01
40	27.03	<0.01	27.89	<0.01
41	36.13	*f*	37.57	0.13
44	26.43	<0.01	26.86	<0.01
45	39.25	0.13	39.76	0.12

[a] For only the carbon signals that showed significant dueterium-induced shifts. [b] Chemical shift difference induced by partial deuteration of the NH protons. [c] Corresponding to N_AD/N_CD. [d] Corresponding to N_AD/N_CH and N_AH/N_CD. [e] Overlapped with the solvent signals. [f] Obscured by other signals.

properties suggested the presence of a strongly basic moiety in **2**, such as guanidine. It is well-known that a solution of a strongly basic organic compound can absorb carbon dioxide from the air, forming a carbonate.[18] The basic group must possess a carbon whose signal appears at δ 149.09, because all the other carbon



signals were correlated with the protons contained in the networks i–iii and this signal is the only one that remained unrelated (COLOC or HMBC spectrum) with any of the protons.

When the ^{13}C NMR spectra ($CDCl_3$) of **2** were taken in the presence of 3 molar equiv of CD_3OD, the carbon signal at δ 149.09

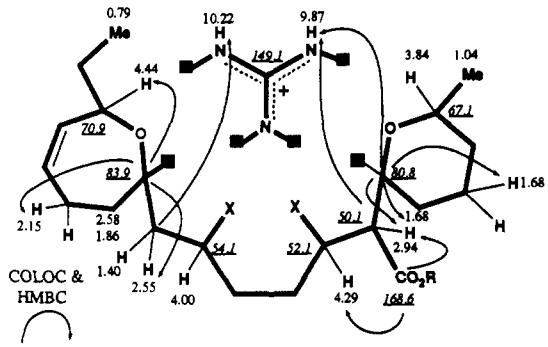
(16) Many attempts to isolate the left-half fragment were done in the reactions described herein. However, no trace of such compound was obtained except for **10**.

(17) Nakamura, H.; Ohizumi, Y.; Kobayashi, J.; Hirata, Y. *Tetrahedron Lett.* **1984**, *25*, 2475. Fedoreyev, S. A.; Utkina, N. K.; Ilyin, S. G.; Reshetnyak, M. V.; Maximov, O. B. *Tetrahedron Lett.* **1986**, *27*, 3177.

(18) Stecher, P. G. Ed. *The Merck Index*, 8th ed.; Merck & Co., Inc.: New Jersey, 1968; p 510.

was split into three peaks (δ 149.09, 149.02, and 148.94) due to an isotopic effect[19] (Table IV). This observation suggests that the carbon is adjacent to the two exchangeable protons, and consideration of its chemical shift together with the fact that three nitrogens exist in the "vessel" led to the conclusion that a guanidine moiety was present in **2**.[20] (The Sakaguchi test[21] was negative for **2**, and such deuterium shift experiments[19] can be a convenient method for detecting a guanidinium moiety.)

Substitution of the exchangeable protons with deuteriums also caused upfield shifts in the signals of two quaternary carbons at δ 83.9 (C-8) and 80.8 (C-15) (both $\Delta\delta = 0.08$). The former had been correlated (COLOC, HMBC) with H-6, H-3, and H-9 and the latter with H-14, H-16, and H-17. The N_AH signal at δ 10.22 showed a cross peak to C-9, and N_CH at δ 9.87 was also correlated with C-15 and C-14 (see **b**). These data allowed us to propose



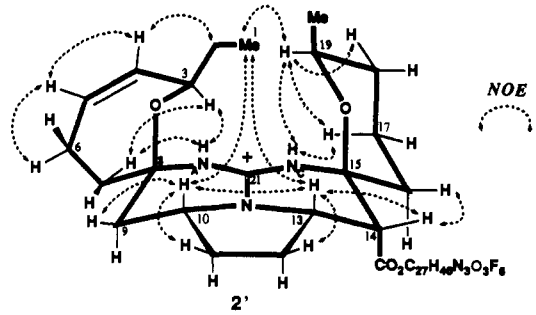
b

the partial structure (planar) for the "vessel" as depicted in **2** except for C-22.

The IR spectrum ($CHCl_3$) of **1** exhibits an intense absorption at 1730 cm^{-1} assignable to an ester group. In the COLOC spectrum of **2**, the ester carbonyl carbon (δ 168.6) showed cross peaks to H-13 (δ 4.29) and H-14 (δ 2.94). Comparison of the chemical shifts of H-13 and H-14 indicated the ester carbonyl group to be attached to C-14. This assumption was verified by taking up the methanolysis product of **4** (see above) in $CDCl_3$ and measuring the ^1H NMR spectrum on the crude material, which mainly consisted of the alcohol **10** accompanied by a small amount of **8**. The ^1H NMR spectrum of **10** (Table III) revealed that (a) the ester linkage O–CH_2 (23) still survived, (b) H-14 was lost, (c) the pattern of H-13 changed from a dt (J = 10.5, 5 Hz) into a dd (J = 10.5, 6 Hz), and (d) H_2-16 were shifted markedly downfield ($\Delta\delta$ = 0.84 and 1.24). The UV maxima (MeOH) of **10** were observed at 237 and 342 nm, the former being ascribable to the *p*-bromobenzoyl group and the latter to the chromophore containing a homocyclic diene.[22] The IR absorption ($CHCl_3$) at 1655 cm^{-1} is consistent with extended conjugation.

The Complete Structure. The COLOC spectrum of **2** confirmed the correlation between the ester carbonyl carbon (C-22, δ 168.6) and H_2-23 (δ 4.08 and 4.05), providing proof that the hydroxy terminus of the anchor was connected to C-22 through an ester linkage. All the above results were consistent with the planar structure **2** for TFA-ptilomycalin A.

The stereochemistry of **2** was determined on the basis of phase-sensitive NOESY and ROESY[23] experiments. The appearance of NOEs between H-3 (δ 4.44) and H-7α (δ 2.58), H-7α and N_AH (δ 10.22), and H-3 and N_AH confirmed the stereochemistry of C-8 as shown in **2'**. This was supported by the large long-range coupling constants (^4J = 2.5 Hz) between H-3 and H-5 (δ 5.65) and between H-4 (δ 5.47) and H-6β (δ 2.35), in which



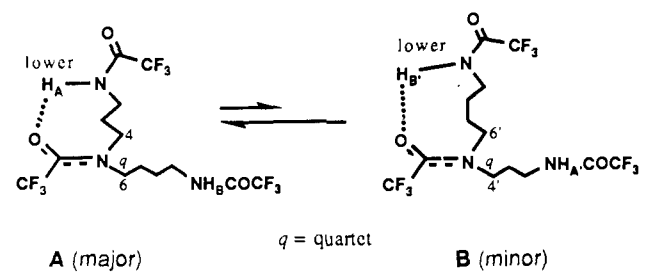
2'

each pair had a dihedral angle of 90°. The large coupling constants (^3J = 12.5 Hz) between H-9α (δ 1.40) and H-10 (δ 4.00) suggested that these two protons were in a diaxial relationship. The NOEs between H-9β (δ 2.55) and H-10, H-10 and H-11β (δ 2.19), H-10 and H-13 (δ 4.29), H-12β (δ 2.25) and H-13, and H-13 and H-14 (δ 2.94) indicated that these protons were all β-oriented, which was supported by the appearance of NOEs between H_3-1 (δ 0.79) and H-10 and between H_3-1 and H-13. The coupling constants ($J_{17\alpha,18\beta}$ = 12.5 Hz, $J_{18\beta,19}$ = 12.5 Hz, $J_{17\beta,18\beta}$ = 5 Hz, and $J_{18\alpha,19}$ = 2.5 Hz) and NOEs (H-19 ↔ H-18α and H-17α) suggested that the tetrahydropyran ring was in a chair conformation. The NOEs between H-19 (δ 3,84) and H-17α (δ 2.15), H-17α and N_CH (δ 9.87), and N_CH and H-19 established the stereochemistry at C-15. From these data the relative stereochemistry as well as the conformation of **2** was determined as shown in **2'**. Because the dihedral angles between C-21 and both H-10 and H-13 were 90°, it seems reasonable that the C-21 signal did not show cross peaks in the HMBC and COLOC spectra. It is worth noting that NOE appears between H-19 and H_3-1. The conformation of **2'** is consistent with this NOE.

NMR Properties of the Spermidine Moiety. As we have shown above, the ^1H and ^{13}C signals of the anchor of **2** are accompanied by small signals (major:minor = 3:1) resulting from rotational isomerism around the amide linkage of C-38. Analysis of the HOHAHA spectrum of **2** enabled us to assign the proton signals of the major and minor isomers as shown in **2a** and **2b** (Figure 1). In the major isomer (**2a**), a $(CH_2)_3$ unit is cis to the carbonyl of the tertiary amide, while a $(CH_2)_4$ unit is cis to the carbonyl in the minor isomer (**2b**).

In order to obtain more detailed information about the isomerism of the spermidine amide, three trifluoro derivatives of spermidine analogues, **5**, **11**, and **12**, were prepared as model compounds and their NMR behavior was examined.

Comparing the proton chemical shifts between **2a** and **5a** and between (**2b** and **5b**) (Figure 1), we noticed that in the major isomers **2a** and **5a** the protons of the secondary amide connected to $(CH_2)_3$ appear at lower field [δ 8,39 (**2a**) and 7.40 (**5a**)] than those of the amide attached to $(CH_2)_4$ [δ 7.40 (**2a**) and 6.66 (**5a**)], although in the minor isomers **2b** and **5b** the chemical shifts of the $(CH_2)_4NH$- are lower [δ 7.84 (**2b**) and 6.94 (**5b**)] than those of the $(CH_2)_3NH$- [δ 7.76 (**2b**) and 6.85 (**5b**)]. These facts may be rationalized by assuming the presence of hydrogen bonds between the amide protons and the tertiary amide carbonyls. From the experimental results, we conclude that the hydrogen bonding which forms the eight-membered ring (**A**) is more stable than that constructing the nine-membered ring (**B**).



The ^1H NMR spectrum ($CDCl_3$) of **5** (Table V) exhibits four amide proton signals at δ 7.40, 6.94, 6.85, and 6.66 (integral ratio,

(19) Christofides, J. C.; Davis, D. B. *J. Am. Chem. Soc.* **1983**, *105*, 5099.
(20) Shimizu, Y.; Hsu, C.; Fallon, W. E.; Oshima, Y.; Miura, I.; Nakanishi, K. *J. Am. Chem. Soc.* **1978**, *100*, 6791. Albizati, K. F.; Faulkner, D. J. *J. Org. Chem.* **1985**, *50*, 4163.
(21) Sakaguchi, S. *J. Biochem.* **1925**, *5*, 25.
(22) Cho, H.; Shima, K.; Hayashimatsu, M.; Ohnaka, Y.; Mizuno, A.; Takeuchi, Y. *J. Org. Chem.* **1985**, *50*, 4227.
(23) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811.

Table V. NMR Properties ($CDCl_3$) of **5**[a]

no.	^{13}C (δ), *m*	^{1}H (δ), *m*
2	36.76, t (38.91, t)	3.33, q, 6 (3.41,[b] m)
3	26.66, t (28.35, t)	1.86, quint, 6 (1.94, quint, 7)
4	43.98, t (45.34, t[c])	3.48, t, 6 (3.44[b])
6	47.30, t[c] (46.61, t)	3.43,[b] m (3.41,[b] m)
7	25.72, t (23.75,[d] t)	1.67, m (1.65, m)
8	26.19, t (26.09,[d] t)	1.60, m (1.65, m)
9	39.05, t (37.23, t)	3.40, q, 6 (3.40[b])
1-NH		7.40, br s (6.85, br s)
1-CO	157.9[e] (157.9[e])	
1-CF_3	115.88[f,g] (116[g])	
5-CO	157[e] (157[e])	
5-CF_3	116.48[g] (116[g])	
10-NH		6.66, br s (6.94, br s)
10-CO	157.9[e] (157.9[e])	
10-CF_3	115.91[f,g] (116[g])	

[a] Minor signals are shown in parentheses. [b] Obscured by other signals. [c] Signal was split into quartet by the coupling with fluorine ($^4J_{NH}$). [d,f] Assignments may be interchanged. [e] Signal was split into quartet by the coupling with fluorine ($^2J_{NH}$). [g] Signal was split into quartet by the coupling with fluorine ($^1J_{NH}$).

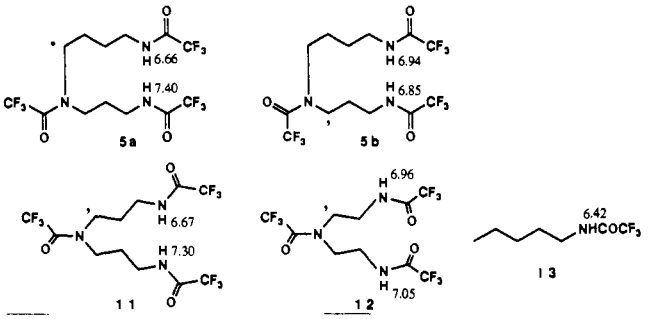


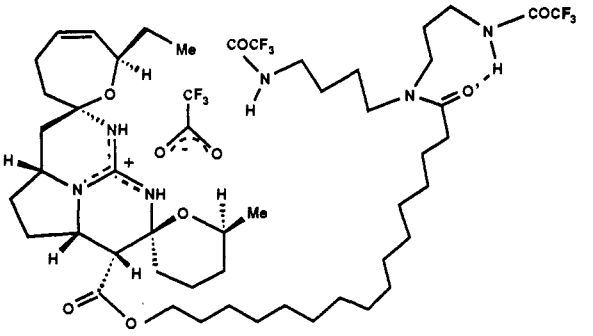
Figure 2. ^1H NMR assignments for trifluoro derivatives of spermidine (major rotatomer **5a**, minor rotatomer **5b**), dipropylenetriamine (**11**), diethylenetriamine (**12**), and pentylamine (**13**). The signals of carbons asterisked (*) appear as quartets owing to coupling ($^4J_{CF}$) with fluorine atoms of trifluoroacetyl group.

1.0:0.3:0.3:1.0). Therefore, **5** must consist of two rotational isomers (**5a** and **5b**) around the tertiary amide bond. (Isomerism around the terminal secondary amides is rapid on the NMR timescale and can be neglected.) In this case it was impossible to assign a structure to each isomer because the homonuclear NOE (between CF_3 and CH_2N-) was not available. In the ^{13}C NMR spectrum (proton decoupled), however, two finely split quartets (J = 3 Hz; $^4J_{CF}$) appear at δ 47.30 and 45.34, the intensity ratio of which is approximately 3:1. In the H,C COSY spectrum, the first (major) is correlated with the proton signal resonating at δ 3.43 [$CH_2(CH_2)_3$-] and the second (minor) with the proton at δ 3.44 [$CH_2(CH_2)_2$-]. Because allyl couplings (^4J) are generally larger for a cisoid isomer than a transoid one,[24] the structures of the major and minor rotational isomers were deduced as shown in **5a** and **5b**, respectively (Figure 1).

Similar isomerization and couplings ($^4J_{CF}$) were also observed for the spermidine analogues **11** and **12**. Although **11** and **12** are symmetrical molecules, the DEPT spectra of **11** and **12** showed six and four methylene signals, respectively. Fine splittings ($^4J_{CF}$ = 3 Hz) were observed for one of the methylene carbons of **11** and **12**. On the other hand, rotational isomerization around the terminal secondary amides was not observed in **5**, **11**, **12**, and **13**.

It should be pointed out that NHs of the "anchor" of **2** exhibited unexpected properties in the deuterium exchange experiments. When CD_3OD was added to a $CDCl_3$ solution of **2**, the ^1H signal of the amide protons cleanly disappeared. On the other hand, deuterium exchange did not occur for the NHs of **5**, **11**, **12**, and **13** in $CDCl_3$ solution. Also, we observed that of the five TFA amides (**2**, **5**, **11**, **12**, **13**) mentioned above the proton chemical shifts of the NH proton of **2** are markedly lower than those of the other compounds (Figures 1 and 2).

Ptilomycalin A (**1**) itself is a strikingly nonpolar substance in spite of the presence of polar moieties such as a guanidinium cation and a spermidine unit. Considering all of these facts, we propose the molecular structure of bis(trifluoroacetyl)ptilomycalin A (and very possibly of ptilomycalin A itself) to be as shown in illustration **2c**, in which the counter anion is encapsulated between the guanidinium and the spermidine moieties.

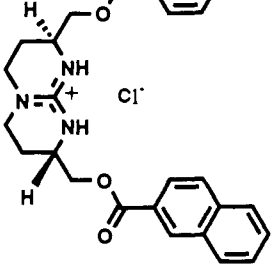


2c

Ptilomycalin A as a Possible Anion Recognizer. NMR spectroscopy is an excellent method for discerning whether an anion is trapped inside a cage as illustrated in **2c**. We studied the qualitative dynamics of the dissociation of the complex formed between ptilomycalin A (**1**) and several organic anions, mainly carboxylates. The ^1H NMR spectra of the complexes of **1** and carboxylates, however, gave only broad signals; consequently, we focused on the ^1H NMR behavior of TFA-ptilomycalin A (**2**) with various organic anions.

A $CDCl_3$ solution of an appropriate amount of **2** (X = CF_3COO) was shaken with an aqueous saturated solution of sodium 2-methylpropionate. The ^1H NMR spectrum revealed that 1 molar equiv of 2-methylpropionate anion moved into the $CDCl_3$ solution to form a complex (**2**/Me_2CHCOO^-). The chemical shift change of the methyl signals of 2-methylpropionate at various concentrations (0.3–0.6 mM) is shown in the supplementary material. It is obvious from these experiments that the chemical shifts of the methyls do not change within 0.01 ppm. The independence of the methyl chemical shifts on the concentration can be ascribed to the "tightness" of the complex. One should note the nonequivalence of the two methyl signals of 2-methylpropionate anion in this complex. This also supports the cagelike nature of **2**, in which the anion is trapped tightly in a chiral environment.

The "vessel" of ptilomycalin (**1**) has a close similarity to Lehn's guanidine compound (**14**),[25] an enantioselective anion recognizer.



14

In order to investigate whether **2** exhibits similar behavior, an NMR study was performed on complexes of **2** and several *N*-

(24) Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd ed.; Pergamon Press: Oxford, 1969; Chapter 4-4.

(25) Echavarren, A.; Galan, A.; Lehn, J.-M.; de Mendoza, J. *J. Am. Chem. Soc.* **1989**, *111*, 4994.

acetylamino acid anions. However, no evidence of enantioselectivity was obtained for **2**. The ^{1}H NMR spectra of the complexes of **2**/D- and **2**/L-*N*-acetylmethionate were completely identical. However, in these experiments we found that **2** exhibited a preference for certain counter anions. When the $CDCl_3$ solution of **2**/L-*N*-acetylalanate was shaken with an aqueous solution of L-*N*-acetylmethionate, the L-*N*-acetylalanate in the organic layer was completely replaced with L-*N*-acetylmethionate. However, L-*N*-acetylalanate never went into the $CDCl_3$ solution of **2**/L-*N*-acetylmethionate. By repeating such experiments, the relative ability of the *N*-acetylamino acid anions to move into the organic solution forming a complex with **2** was estimated to be L-*N*-acetylmethionate ≈ L-*N*-acetylvalinate > L-*N*-acetylalanate ≈ L-*N*-acetylisoleucinate ≫ L-*N*-acetylglycinate. This series may reflect the "fit" of the anions in the cavity of **2**.

Summary

The structure of ptilomycalin A (**1**), composed of a pentacyclic guanidine and spermidine unit linked together by a 16-hydroxyhexadecanoyl chain, has been determined on the basis of spectroscopic analyses of its TFA derivative (**2**). The relative stereochemistry has been deduced by use of NOEs observed in the NOESY spectrum. Rotational isomerism of the acylated spermidine moiety of **2** has been studied by comparing the NMR properties of synthetic trifluoroacetyl derivatives of spermidine (**5**), dipropylenetriamine (**11**), diethylenetriamine (**12**), and pentylamine (**13**).[26] As a result of these experiments, a plausible conformation of **2** and ptilomycalin A (**1**) has been proposed as described in **2c**. Qualitative analysis of the stability of the complexes formed by **2** and several organic carboxylates has been carried out.

Four analogues of ptilomycalin A have been recently obtained from *Crambe crambe* sponges.[27]

Experimental Section

Isolation of 1 from the Sponge *Hemimycale* sp. *Hemimycale* sp. (50 g) was collected in the summer of 1986 from the Red Sea. Specimens were frozen immediately after removal from the sea, freeze-dried (20 g), and then extracted twice to afford after evaporation 900 mg from the petroleum ether extract, 1.5 g from CH_2Cl_2, and 2.7 g from the MeOH–CH_2Cl_2 (15:85) extract. The latter two extracts, which were biologically active (P388, IC_{50} 0.25 μg/mL; A-59, 2 μg/mL; and against *Candida albicans*, MIC 7.8 μg/mL), were separated on a 4.0- × 10-cm columm of NS-gel (Nippon Seimitsu Kagaku 10503) and eluted under reduced pressure with H_2O and an increasing percentage of MeOH. Ptilomycalin A (**1**) was eluted with 60–80% MeOH. The combined fractions were further purified (five times) on the NS-gel column with various mixtures of MeOH–H_2O to obtain ptilomycalin A (**1**, 400 mg, 12% from the crude extract). Although compound **6** could be identified in the extract (TLC), it was not isolated from the *Hemimycale* sponge because of its low concentrations. The TLC systems used with the crude extracts were (1) silica gel (Merck, kieselgel 60 F_{254}), solvent $CHCl_3$–MeOH, 4:1, R_f = 0.1, 0.2, 0.4, 0.5; (2) amino (Merck, HPTLC plates NH_2 F_{254}S), solvent CH_3CN–H_2O–AcOH, 20:1:1, R_f = 0.4, 0.45, 0.5; (3) cyano (Merck, HPTLC plates CN F_{254}S), solvent $CHCl_3$–MeOH–NH_4OH–AcOH, 2:8:4:1, R_f = 0.35, 0.4, 0.6; and (4) reversed phase (Merck, HPTLC plates RP-18 F_{254}S), solvent MeOH, R_f = 0.01.

Isolation of 1 and 6 from the Sponge *Ptilocaulis spiculifer*. *P. spiculifer* was collected in 1986 from the Caribbean (400 g) and was immediately frozen. After freeze-drying, the sponge was extracted with three solvents to afford after evaporation of the three extracts: (a) with EtOAc, 2.4 g, (b) with $CHCl_3$, 3.0 g, and (c) with MeOH–$CHCl_3$ (1:9), 2.1 g. The latter two extracts revealed the same biological and chromatographic behavior as the parallel extracts from *Hemimycale* sp. Both extracts were applied to a 4- × 10-cm column of NS-gel eluted, under reduced pressure, with 50–80% MeOH in H_2O. Ten fractions (60 mL each) were collected. Fractions 3–8 were combined and further purified on an NS-gel column to afford in ca. 90% purity two major fractions, the one (500 mg) containing a mixture of ptilomycalin A (**1**, 85%) and compound **6** (2%) and the other containing the known isoptilocaulin[8a] (30 mg).

The separation of **1** and **6** was achieved after reducing the compounds' polarities by the preparation of trifluoroacetyl or *p*-bromobenzoyl derivatives.

Preparation of Trifluoroacetyl Derivatives. A mixture of **1** and **6** (100 mg) in acetone (25 mL) was treated with trifluoroacetic anhydride (1 mL) and anhydrous K_2CO_3 (50 mg) and refluxed for 3 h. The K_2CO_3 was filtered, most of the solvent was evaporated, and the reaction mixture was diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (2 × 25 mL). The CH_2Cl_2 layer was washed with water (15 mL) and dried over anhydrous $MgSO_4$, and the residue (90 mg) after evaporation was applied to a silica-H column (1.5- × 6-cm). The column was eluted with CH_2Cl_2 followed by CH_2Cl_2–MeOH (1–50%). Compound **7** was eluted with CH_2Cl_2 (2 mg, 0.1% of the crude extract). TLC on silica gel eluted with MeOH–$CHCl_3$ (1:9) gave R_f = 0.7. Compound **2** was eluted with MeOH–CH_2Cl_2, 2:98 (60 mg, 7% of the crude extract, R_f = 0.6 on the same system).

Preparation of *p*-Bromobenzoyl Derivatives. A mixture of **1** and **6** (100 mg) in dry CH_2Cl_2 (25 mL) was treated with a mixture of *p*-bromobenzoyl chloride (Aldrich, 75 mg), Et_3N (50 μL), and (dimethylamino)pyridine (5 mg) and refluxed under nitrogen for 4 h. The reaction mixture was washed with 1 N HCl (5 mL) and then with water (2 × 10 mL). The residue (150 mg) after evaporation was applied to a silica-H column (1.5- × 6-cm). Compound **4** and the di-*p*-bromobenzoate derivative of **6** were eluted from the column in the same way as the TFA derivatives to afford 110 and 4 mg, respectively.

Ptilomyclin A (1): an oil; $[\alpha]^{25}$D −2.5° (*c* 0.70, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600–2400, 2930, 2860, 1730, 1650, 1610, 1450, 1160, 1080, 1010 cm^{-1}; MS *m/z* 785 ($C_{45}H_{81}N_6O_5$, MH^+). ^{1}H and ^{13}C NMR properties are listed in Table I.

Compound 2: an oil; $[\alpha]^{25}$D −15.8° (*c* 0.68, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3200 br, 2920, 2850, 1720, 1650, 1610, 1155 cm^{-1}; HRFABMS *m/z* calcd for $C_{49}H_{79}N_6O_7F_6$ (MH^+) 977.5914, found 977.5915. ^{1}H and ^{13}C NMR properties are listed in Table II.

Compound 4: an oil; UV (MeOH) λ_{max} 237 nm (ε 27 000); IR ($CHCl_3$) ν_{max} 3500–3000 br, 2940, 2850, 1730, 1655, 1610, 1590, 1460, 1300, 1010, 905 cm^{-1}. ^{1}H and ^{13}C NMR properties are listed in Table III.

Compound 7: an oil; EIMS *m/z* 575 ($C_{27}H_{47}N_3O_3F_6$, M^+); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (3 H, t), 1.25 (br s), 1.28 (br s), 1.55 (br s), 1.60 (br s), 1.70 (m), 2.30 (2 H, t), 3.25 (4 H, m), 3.40 (4 H, m), 6.42 (1 H, br s), 8.25 (1 H, br s).

Methanolysis of 4. To a solution of **4** (5.3 mg, 4.6 μmol) in 0.5 mL of MeOH was added 80 μL of 1.7 M MeONa in MeOH, and the solution was allowed to stand at room temperature for 4 h. Another 80 μL of 1.7 M MeONa in MeOH was added, and the mixture was kept at room temperature for 7 days. After removal of the solvent, the residue was dissolved in $CHCl_3$ and filtered. The solvent was evaporated to afford a mixture (3.0 mg) of **8** and **10**. This product was separated by HPLC [Inertsil ODS, 5 μm, Gasukuro Kogyo, CH_3CN–H_2O (13:7) followed by MeOH] to give **10** (2 mg, 0.4 μmol). **10**: UV (MeOH) λ_{max} 237 (ε 29 000), 342 nm (2900); IR (KBr) ν_{max} 1655 cm^{-1}. ^{1}H NMR properties are listed in Table III. **8**: IR (film) ν_{max} 3320 br, 2924, 2853, 1630, 1592, 1550, 1484, 1450, 1072, 1010 cm^{-1}; FABMS *m/z* 790/788/786 [$C_{37}H_{55}N_3O_4Br_2Na$, $(M + Na)^+$], 768/766/764 [$C_{37}H_{56}N_3O_4Br_2$, $(M + H)^+$], 710/708 [$(M + Na + H - Br)^+$]; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25 (18 H, br s), 1.28 (2 H, m), 1.34 (2 H, m), 1.55 (2 H, m), 1.63 (2 H, m), 1.65 (2 H, m), 1.67 (2 H, m), 1.75 (2 H, quint, *J* = 6 Hz), 2.31 (2 H, t, *J* = 7.5 Hz), 2.5 (1 H, br s), 3.31 (2 H, t, *J* = 6.5 Hz), 3.35 (2 H, q, *J* = 6 Hz), 3.48 (2 H, m), 3.49 (2 H, m), 3.62 (2 H, br t, *J* = 6 Hz), 6.24 (1 H, t, *J* = 6 Hz, NH), 7.1–7.8 (8 H, ArH), 7.93 (1 H, t, *J* = 6 Hz, NH).

$LiAlH_4$ Reduction of 2. A solution of **2** (45 mg, 45 μmol) in dry THF (5 mL) was reduced with $LiAlH_4$ (10 mg, 263 μmol) under N_2 at 0 °C for 50 min. Excess $LiAlH_4$ was destroyed with EtOAc, and then a few drops of saturated Na_2SO_4 solution were added. After evaporation, the crude residue was acetylated with Ac_2O/pyridine (1:1, 0.2 mL, 18 h). The residue after evaporation was chromatographed on a silica gel column to give **9** (4 mg, 6.3 μmol) and **2** (20 mg). **9**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.28 (br s), 1.35 (br s), 1.5–1.7 (m), 2.31 (2 H, t), 3.27 (4 H, m), 3.43 (4 H, m), 4.04 (2 H, t), 6.45 (1 H, br s), 8.23 (1 H, br t).

Methanolysis of 2. **2** (20 mg, 20 μmol) was refluxed under N_2 in a 2% MeONa (MeOH) solution for 3 h. The solution was neutralized with HOAc, and the solvent was evaporated. The residue after acetylation as described above was chromatographed on silica gel to give **9** (2 mg, 3.2 μmol) and **2** (10 mg).

Trifluoroacetylation of Spermidine. To a solution of spermidine (27.8 mg, 0.19 mmol) in 160 μL of pyridine (distilled from CaH_2) was added trifluoroacetic anhydride (0.28 mL, 2.0 mmol) at 0 °C, and the mixture was allowed to stand at room temperature for 15 min. Water was added,

(26) As to the NMR behavior of amides, see the following: Gellman, S. H.; Dado, G. P.; Liang, G. B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164. Gellman, S. H.; Dado, G. P. *Tetrahedron Lett.* **1991**, *32*, 7377. Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 3138. We are grateful to Prof. S. H. Gellman for his kind information on these references.

(27) J.-Erijman, E. A.; Sakai, R.; Rinehart, K. L. *J. Org. Chem.* **1991**, *56*, 5712.

and the reaction mixture was extracted with ethyl acetate four times. The organic layers were combined, washed with water, dried over Na_2SO_4, and evaporated to give a yellowish material. The crude product was purified by chromatography on silica gel (EtOAc) to afford pure **5** (75.0 mg, 91.1%): MS m/z 433 (M^+); IR ($CHCl_3$) ν_{max} 3440, 2940, 1720, 1685, 1540, 1440, 1325, 1155 cm^{-1}; ^{13}C NMR (125 MHz, pyridine-d_5) δ 24.5, 26.5, 26.7, 37.8, 39.5, 45.1, 47.6 (q, $^4J_{CF}$), 117, 158 (major conformer)/26.3, 27.1, 29.0, 37.4, 39.6, 45.5 (q, $^4J_{CF}$), 46.6, 117, 158 (minor conformer); ^1H NMR (500 MHz, pyridine-d_5) δ 1.4-1.6 (m), 1.86 (quint, H_2-3'), 1.87 (quint, H_2-3), 3.2-3.4 (m), 10.39 (br s), 10.58 (br s). ^{13}C NMR and ^1H NMR properties ($CDCl_3$) are listed in Table V.

Compound 11 was prepared from dipropylenetriamine (Tokyo Kasei No. D0090) by the same manner described above. **11**: ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.6, 28.5, 36.6, 37.2, 43.9, 45.2 (q, $^4J_{CF}$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.88 (2 H, quint, J = 7 Hz), 1.96 (2 H, quint, J = 7 Hz), 3.33 (2 H, q, J = 7 Hz), 3.42 (2 H, q, J = 7 Hz), 3.46 (2 H, t, J = 7 Hz), 3.48 (2 H, t, J = 7 Hz), 6.67 (1 H, br s), 7.30 (1 H, br s); ^{13}C NMR (125 MHz, pyridine-d_5) δ 27.2, 29.1, 37.4, 37.8, 45.3, 45.8 (q, $^4J_{CF}$ = 2.8 Hz), 117.3 (q, $^1J_{CF}$ = 286 Hz), 117.1 (q, $^1J_{CF}$ = 286 Hz), 156.8 (q, $^2J_{CF}$ = 35 Hz), 158.0 (q, $^2J_{CF}$ = 36 Hz), 158.2 (q, $^2J_{CF}$ = 36 Hz); ^1H NMR (500 MHz, pyridine-d_5) δ 1.89 (2 H, quint, J = 7 Hz), 1.91 (2 H, quint, J = 7 Hz), 3.40 (2 H, q, J = 7 Hz), 3.42 (2 H, q, J = 7 Hz), 3.48 (4 H, t, J = 7 Hz), 10.33 (1 H, br s), 10.54 (1 H, br s).

Compound 12 was prepared from diethylenetriamine (Aldrich No. D9385-6) by the same manner described above. **12**: ^1H NMR (500 MHz, $CDCl_3$) δ 3.6-3.75 (8 H, m), 6.96 (1 H, br s), 7.05 (1 H, br s); ^{13}C NMR (125 MHz, pyridine-d_5) δ 37.0, 38.0, 46.2, 46.9 (q, $^4J_{CF}$ = 2.5 Hz), 117.0 (q, $^1J_{CF}$ = 286 Hz), 117.1 (q, $^1J_{CF}$ = 286 Hz), 157.8 (q, $^2J_{CF}$ = 35 Hz), 158.51 (q, $^2J_{CF}$ = 36 Hz), 158.53 (q, $^2J_{CF}$ = 36 Hz); ^1H NMR (500 MHz, pyridine-d_5) δ 3.70 (4 H, m), 3.80 (4 H, m), 10.84 (2 H, br s).

Compound 13 was prepared from pentylamine (Tokyo Kasei No. A0445) by the same manner described above. **13**: ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.7 (C-5), 22.2 (C-4), 28.5 (C-2), 28.8 (C-3), 40.0 (C-1), 116.0 (q, $^1J_{CF}$ = 285.5 Hz), 157.6 (q, $^2J_{CF}$ = 36.6 Hz); ^1H NMR (500 MHz, $CDCl_3$) δ 0.90 (3 H, t, J = 7 Hz, H_3-5), 1.32 (4 H, m, H_2-3, 4), 1.58 (2 H, quint, J = 7.5 Hz, H_2-2), 3.34 (2 H, q, J = 7.5 Hz, H_2-1), 6.42 (1 H, br s); ^{13}C NMR (125 MHz, pyridine-d_5) δ 14.1 (C-5), 22.5 (C-4), 29.1 (C-2), 29.2 (C-3), 40.2 (C-1); ^1H NMR (500 MHz, pyridine-d_5) δ 0.63 (3 H, t, J = 7 Hz, H_3-5), 1.08 (4 H, m, H_2-3, 4), 1.45 (2 H, quint, J = 7.5 Hz, H_2-2), 3.31 (2 H, q, J = 7.5 Hz, H_2-1), 10.3 (1 H, br s).

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Supplementary Material Available: ^1H NMR spectra of **2** and the complex of **2** and 2-methylpropionate; ^{13}C NMR spectra of **2**, measured in $CDCl_3$ and in the presence of CD_3OD (isotopic shift experiment); and Tables I and III of the NMR properties of ptilomycalin A (**1**) and its *p*-bromobenzoate (**4**) as well as compound **10** (7 pages). Ordering information is given on any current masthead page.

Thiotropocin Biosynthesis. Shikimate Origin of a Sulfur-Containing Tropolone Derivative

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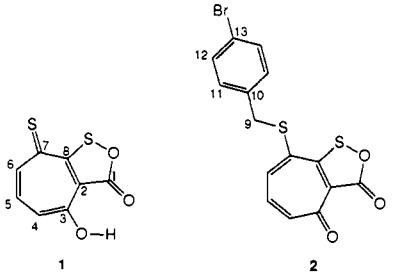
Abstract: Feeding of [U-$^{13}C_6$]glucose to *Pseudomonas* CB-104 gave labeled thiotropocin (**1a** and **1b**). The ^{13}C NMR spectrum of the derived *p*-bromobenzyl thioether (**2a** and **2b**) displayed a pattern of enhancements and couplings consistent with a shikimate origin for thiotropocin by way of a symmetrical phenylpyruvate (**11**) intermediate. The latter metabolite is proposed to undergo oxidative ring expansion followed by further oxidation and oxygen-sulfur exchange. These conclusions were further supported by specific and efficient incorporation of both [3-^{13}C]phenylalanine (**13**) and [1,2-$^{13}C_2$]phenylacetic acid (**12**) into thiotropocin.

Thiotropocin (**1**) is a novel antibiotic first isolated in 1984 from the fermentation broth of *Pseudomonas* sp. CB-104 by Harada and Ono and their colleagues at Takeda.[1] Thiotropocin and its sodium salt were found to exhibit antibacterial, antifungal, and antiprotozoal activities in vitro, with the observed antibiotic activity being strongest at low pH. The antibiotic caused morphological changes in both *Proteus mirabilis* and *Escherichia coli* and displayed immediate growth-inhibitory but only weak lytic activity against *P. mirabilis*.

The structure of thiotropocin was established by a combination of spectroscopic methods and X-ray crystallographic analysis of the *p*-bromobenzyl derivative **2**. Thiotropocin is the first example of a naturally occurring tropothione derivative and has a unique structure containing an -S-O-CO- moiety. Among the previously known naturally occurring aromatic tropolone derivatives are several fungal metabolites, including stipitatic (**4**) and stipitatonic (**5**) acids from *Penicillium stipitatum*.[2] Labeling studies have established the derivation of these latter compounds from acetate and methionine and led to the proposal that the common seven-membered tropolone ring could be formed by oxidative ring expansion of a methylated aromatic tetraketide (**3**) as illustrated in Scheme I.[3] By contrast, the alkaloidal tropolone derivative colchicine (**6**) has been shown to be biosynthesized from phenyl-



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