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Sagittamides A and B. Polyacetoxy Long-Chain Acyl Amino Acids from a Didemnid Ascidian

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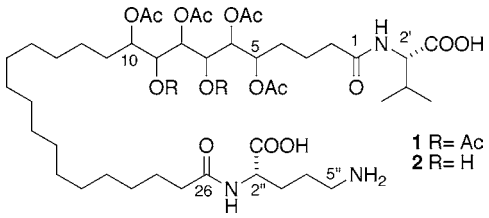
ABSTRACT



An unidentified tunicate from Pohnpei, Micronesia, yielded sagittamides A and B—compounds comprising a long-chain C_{26} dicarboxylic acid that acylates terminal L-valine and L-ornithine groups. The structures, which contain an unprecedented internal *O*-hexacetyl-1,2,3,4,5,6-hexaol moiety, were determined by combined spectroscopic analysis including mass spectrometry and 1D and 2D NMR and chemical degradation. The partial absolute stereochemistry of the new compounds was addressed by Marfey's analysis.

Ascidians (subphylum Urochordata, class Ascidiacea) produce a variety of natural products, typically highly modified peptides.[1] Bioactive ascidian metabolites include the antitumor alkaloids ecteinascidin-743 from *Ecteinascidia turbinata*,[2] diazonamide A from *Diazona angulata* (cf. *chinensis*),[3] the highly cytotoxic depsipeptides didemnin A and B,[4] and the antiviral eudistomins.[5] Through the course of screening of Micronesian marine invertebrates for the presence of inhibitors of histone deacetylases (HDACs), we found that the extract of an unidentified Didemnid ascidian elicited significant inhibition of HDAC (98% inhibition at 4.0 μg/mL) and contained a series of unprecedented polyacetoxylated long-chain acyl amino acids. The major compounds, which we named sagittamides A (**1**) and B (**2**), are characterized by an α,ω-dicarboxylic acid that is acylated at each terminus by a different amino acid. In this paper, we describe the isolation and structural characterization of **1** and **2**, including the absolute configuration of the amino acids.

An unidentified Didemnid compound ascidian, collected from Arrow Island, Pohnpei, in 2001 (Federated States of Micronesia), was exhaustively extracted with 1:1 CH_2Cl_2/MeOH and the concentrated extract sequentially partitioned against hexanes, $CHCl_3$, and *n*-BuOH. Examination of both the $CHCl_3$- and *n*-BuOH-soluble fractions by ^{1}H NMR revealed a large component consisting of acetylated polyhydroxylipids. Purification of the polyacetate containing fractions by Sephadex LH-20 chromatography, followed by reversed-phase HPLC (C_{18}, 1:1 CH_3CN/H_2O, 0.05% TFA), gave sagittamide A (**1**) and B (**2**) as optically active colorless glasses.[6]

Compound **1** was soluble only in polar solvents (MeOH, DMSO) and gave a strong ninhydrin-positive reaction (pink color) indicating the presence of a primary amine. Acid

(1) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Princep, M. R. *Nat. Prod. Rep.* **2005**, *22*, 15−61 and earlier reviews in this series.
(2) Rinehart, K. L., Jr.; Holt, T. G.; Fregeau, N. L.; Stroh, J. G.; Keifer, P. A.; Sun, F.; Li, L. H.; Martin, D. G. *J. Org. Chem.* **1990**, *55*, 4512−4515.
(3) (a) Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 2303−2304. (b) Lindquist, N. L. Ph.D. thesis, University of California, San Diego, 1989.
(4) Rinehart, K. L., Jr.; Gloer, J. B.; Cook, J. C., Jr.; Mizsak, S. A.; Schill, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 1857−1859.
(5) Kobayashi, J.; Harbor, G. C.; Gilmore, J.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1984**, *106*, 1526−1528.

(6) The name "sagittamide" is derived from the Latin word for "arrow".

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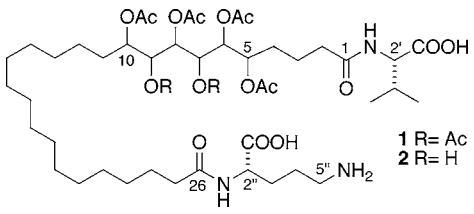
hydrolysis of **1** liberated 1 equiv each of the amino acids, ornithine and valine (vide infra).



MALDI-HRMS of **1**[7] gave an *m*/*z* 988.5585 ([M + H]$^+$, Δmmu −0.3) corresponding to a molecular formula of $C_{48}H_{81}N_3O_{18}$ for the parent compound. The UV spectrum of **1** showed only weak end absorption; however, the IR spectrum showed ester and amide carbonyl stretching (ν 1750, 1654 cm^{-1}, respectively). The ^{1}H NMR (CD_3OD, Table 1) indicated six acetate methyl singlets, a pair of methyl doublets, and multiple oxygenated methines. ESIMS of **1** under conditions of deuterium exchange (CD_3OD) gave *m*/*z* 995.6 [M_D + D]$^+$ indicating six exchangeable hydrogens. The remaining unresolved ^{1}H NMR signals (δ 1.28, bs) were attributable to long unbranched CH_2 chains.

Analysis of HSQC, HMBC, and COSY correlations in methanol-d_4 provided the partial structures **1a**−**c** shown in Figure 1. HMBC correlations (DMSO-d_6, see Supporting

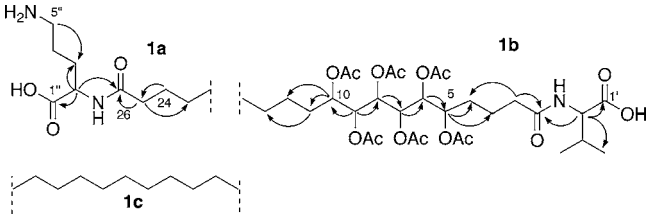


Figure 1. Partial structures of **1** with selected HMBC correlations.

Information) from the amide NH protons to C=O groups allowed placement of the valinyl and ornithinyl residues at the respective carboxyl groups at opposite ends of the long chain.

The presence of two free carboxyl groups in **1** was confirmed by treating the compound with excess diazomethane to give a monomethyl ester (^{1}H NMR, δ 3.71 s, 3H), instead of an expected diester. ESIMS measurement of the product (*m*/*z* 1006.5, [M + Na]$^+$) indicated a net increase of mass of only 4 amu instead of the expected 28 amu. HRMS-MALDI (*m*/*z* 1006.5467, [M + Na]$^+$) revealed the formula of the product, $C_{49}H_{81}N_3O_{17}$, and confirmed that the expected diester product had cyclized to give **3** with loss of the elements of MeOH. Comparison of ESIMS measurements of **3** prepared in CH_3OH (*m*/*z* 1006.5, [M + Na]$^+$) and CD_3OD (*m*/*z* 1009.6, [M_D + Na]$^+$) confirmed the expectation of only three exchangeable hydrogens. The presence of three amide protons (δ 6.01, d, J = 8.8 Hz, 1H; 6.02, br s, 1H; 6.35, br s, 1H) in the ^{1}H NMR spectrum ($CDCl_3$, Table 1) was consistent with a monomethylester-triamide structure **3**. Evidently, the incipient dimethylester ***i*** (Figure 2), formed

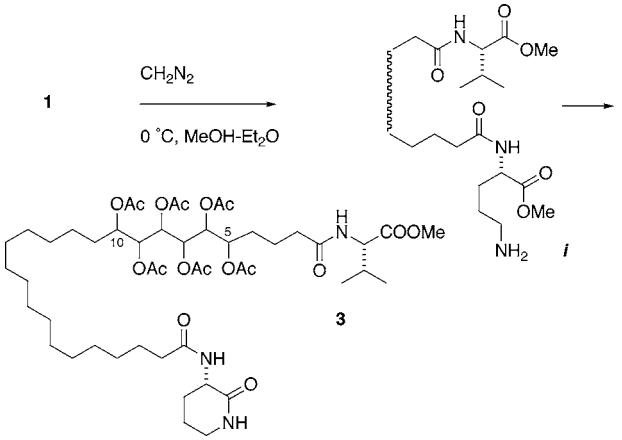


Figure 2. Reaction of **1** with diazomethane.

by addition of diazomethane at both C1′ and C1″ carboxyl groups, undergoes spontaneous intramolecular addition−elimination at the ornithine terminus by the free gamma-NH_2 group to generate lactam **3**.

The second major component, **2**, was shown to be di-desacetyl-**1** from the following analysis. MALDI-HRMS of **2**[8] gave an [M + Na]$^+$ of 926.5237 (Δmmu +3.5) corresponding to the molecular formula $C_{44}H_{77}N_3O_{16}$ for **2**. The difference in mass of 84 amu from **1** and only four AcO methyl singlets in the ^{1}H NMR spectrum indicated that **2** was a didesacetyl derivative of **1**. ESIMS under conditions of deuterium exchange (CD_3OD) gave an [M_D + D]$^+$ of *m*/*z* 913.6 indicating the presence of eight exchangeable hydrogens. Two CH−O signals in the ^{1}H NMR of **2** (δ 3.95, dd, J = 9.7, 1.5 Hz; 4.15, dd, J = 10.1, 1.4 Hz) now appeared ∼1−1.5 ppm upfield with respect to the clustered CH−OAc signals in **1**. These upfield-shifted signals, thus, belonged to the two unesterified secondary CH−OH groups in **2** and were subsequently assigned to H7 and H9 by the following sequential vicinal coupling analysis. ^{1}H−^{1}H ^3J couplings between H5−H6 (J = 2.6 Hz), H6−H7 (J = 10.1 Hz), H7−H8 (J = 1.4 Hz), H8−H9 (J = 9.7 Hz), and H9−H10 (J = 1.5 Hz) were identified from a small-*J*-optimized delayed COSY experiment (J = 2.0 Hz) and supported by HSQC-TOCSY from H5 to C3 and C4 and H10 to C11 and C12.

(7) Sagittamide A (**1**): clear glass; [α]$^{25}_D$ −22.0 (*c* 0.599, MeOH); IR (ZnSe) ν_{max} 3314, 2925, 2853, 1750, 1654, 1540, 1371, 1220, 1032 cm^{-1}; UV (MeOH) λ_{max} 214 nm (ϵ +0.38), 228 (5481), 276 (1060); CD (MeOH) λ_{max} 212 nm ($\Delta\epsilon$ 2301.7), 242 (−0.03); ^{1}H NMR and ^{13}C NMR (Table 1); HRMALDIMS *m*/*z* 988.5585 (M + H$^+$), calcd for $C_{48}H_{82}N_3O_{18}$ 988.5588. The compound, most likely, is in the form of the TFA salt after HPLC purification.

(8) Sagittamide B (**2**): clear glass; [α]$^{25}_D$ −19.7 (*c* 0.173, MeOH); IR (ZnSe) ν_{max} 3319, 3061, 2926, 2854, 1722, 1680, 1543, 1373, 1242, 1207, 1140, 1034 cm^{-1}; UV (MeOH) λ_{max} 214 (ϵ 1534), 256 (702); ^{1}H and ^{13}C NMR (Table 1); HRMALDIMS *m*/*z* 926.5237 [M + Na]$^+$, calcd for $C_{44}H_{77}N_3O_{16}Na$ 926.5202.

Table 1. ^{1}H and ^{13}C NMR Data for **1** and **2** in CD_3OD and ^{1}H NMR Data for **3** in $CDCl_3$

	1		**2**		**3**
no.	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[c]	δ_H[j]
1		175.9			
2	2.27 (2H, m)	36.3	2.30 (2H, m)	36.0	2.27 (2H, m)
3a	1.52 (1H, m)	23.0	1.58 (1H, m)	22.7	1.65 (2H, m)
3b	1.67 (1H, m)		1.69 (1H, m)		
4a	1.59 (1H, m)	29.1	1.73 (2H, m)	27.8	1.65 (2H, m)
4b	1.63 (1H, m)				
5	4.92 (1H, m)	73.4	5.22 (1H, m)	74.1	4.81 (1H, m)
6	5.11 (1H, dd, 8.3, 4.0)	71.4	4.94 (1H, dd, 10.1, 2.6)	72.0	5.11 (1H, dd, 8.8, 2.8)
7	5.15 (1H, dd, 8.3, 1.5)	69.2	4.15 (1H, dd, 10.1 1.4)	68.2	5.15 (1H, dd, 8.8, 1.7)
8	5.42 (1H, dd, 9.9, 1.5)	68.4	4.99 (1H, dd, 9.7, 1.4)	71.2	5.41 (1H, dd, 9.8, 1.7)
9	5.13 (1H, dd, 9.9, 1.8)	70.4	3.95 (1H, dd, 9.7, 1.5)	69.0	5.13 (1H, dd, 9.8, 2.0)
10	4.82 (1H, td, 6.8, 1.8)	71.9	4.76 (1H, td, 6.8, 1.5)	73.2	4.81 (1H, m)
11	1.42 (2H, m)	31.8	1.42 (2H, m)	31.7	1.35 (2H, m)
12	1.32 (2H, m)	26.3	1.32 (2H, m)	26.3	1.26 (2H, m)
13−23	1.28 (22H, br s)	30.5	1.28 (22H, br s)	30.4	1.20 (22H, br s)
24a	1.63 (1H, m)	27.1	1.60 (2H, m)	26.8	1.65 (2H, m)
24b	1.59 (1H, m)				
25	2.24 (2H, m)	37.0	2.24 (2H, m)	36.8	2.27 (2H, m)
26		176.6			
1′		175.0			
2′	4.31 (1H, d, 5.6)	59.1	4.31 (1H, d, 6.0)	59.0	4.53 (1H, dd, 8.8, 4.8)
3′	2.15 (1H, hep, 6.8)	31.8	2.17 (1H, hep, 6.8)	31.3	2.15 (1H, m)
4′	0.96 (3H, d, 6.8)	18.5	0.97 (3H, d, 6.8)	18.2	0.92 (3H, d, 6.8)
5′	0.97 (3H, d, 6.8)	19.8	0.98 (3H, d, 6.8)	19.2	0.89 (3H, d, 7.2)
1″		174.9			
2″	4.42 (1H, dd 8.2, 5.0)	52.9	4.43 (1H, dd, 8.0, 5.2)	53.2	4.26 (1H, dt, 11.3, 5.6)
3a″	1.75 (1H, m)	29.9	1.75 (1H, m)	29.9	1.50 (1H, m)
3b″	1.98 (1H, m)		1.98 (1H, m)		2.57 (1H, m)
4″	1.72 (2H, m)	25.3	1.75 (2H, m)	25.0	1.93 (2H, m)
5″	2.95 (2H, t, 7.2)	40.4	2.95 (2H, t, 7.0)	39.9	3.34 (2H, m)
5-OAc	2.01 (3H, s)	21.0, 172.4	1.95 (3H, s)[f]	20.5, 171.5[g]	2.11 (3H, s)[h]
6-OAc	2.02 (3H, s)	21.1[d], 171.7	1.97 (3H, s)[f]	20.5, 171.7[g]	2.09 (3H, s)[h]
7-OAc	2.07 (3H, s)	20.9[e], 172.0			2.05 (3H, s)[h]
8-OAc	2.01 (3H, s)	21.0, 171.5	1.98 (3H, s)[f]	20.5, 171.8[g]	2.02 (3H, s)[h]
9-OAc	2.08 (3H, s)	21.0[e], 171.7			2.02 (3H, s)[h]
10-OAc	2.03 (3H, s)	21.2[d], 172.6	1.99 (3H, s)[f]	20.5, 172.0[g]	2.01 (3H, s)[h]
OMe					3.71 (3H, s)
NH-2′					6.01 (1H, d, 8.8)
NH-2″					6.35 (1H, br s)
NH-5″					6.02 (1H, br s)

[a] Spectra recorded at 400 MHz referenced to residual CD_2HOD $\delta = 3.30$. [b] Spectra recorded at 100 MHz and referenced to CD_3OD $\delta = 49.0$. [c] Assigned from HSQC and HMBC (400 MHz). [d−h] Signals are interchangeable. [j] Recorded in $CDCl_3$ (400 MHz) and referenced to $CHCl_3$ $\delta = 7.24$.

The identities and absolute stereochemistry of the amino acid residues in **1** were determined by Marfey's analysis.[9] Acid hydrolysis of **1** (6 M HCl, 16 h, 110 °C) and derivatization of the products with 2,4-dinitrophenyl-5-fluoro-L-alaninamide (Marfey's reagent) under standard conditions,[10] followed by analysis (C_{18} HPLC−MS, UV detection), gave two peaks. The early-eluting peak (t_R = 20.9 min) matched the Marfey's derivative of L-valine, while the late-eluting peak (t_R = 24.7 min) was identified as the Marfey's derivative of L-ornithine.

Sagittamides represent an unexpected departure from the structures of other α,ω-long-chain bifunctionalized natural products from marine organisms.[11] The biosynthesis of the contiguous, stereoregular hexaol segment that constitutes C5−10 is unknown. Two possible explanations for the origin of the hexaol segment in **1** and **2** are polyketide synthase mediated chain extension by three iterative additions of hydroxymalonyl CoA, followed by ketone reductions to the growing lipid chain or incorporation of an unidentified C_6 carbohydrate unit. The latter is unprecedented while the former is rare. Hydroxymalonyl CoA (HMCoA) polyketide chain extension is known in the biosynthesis of avermectin[12] and genetic evidence supports its involvement in zwittermicin A production by *Bacillus cereus*,[13] but we are unaware of examples of polyketide assembly from *iterative* HMCoA

(9) Marfey, P..*Carlsberg Res. Commun.* **1984**, *49*, 591−596.

chain extensions. The stereochemistry of the hexa-acetoxylated stereoelement at C5−C10 of **1** and **2** is presently unassigned and is clearly a challenging problem.[14]

Pure **1** was not active in an antitumor cell assay (human colon tumor, HCT-116, IC_{50} > 100 μg/mL[15]) and did not inhibit HDAC's (IC_{50} > 100 μg/mL). The active fraction from the original extract is now under investigation.

Acknowledgment. We thank the Federated States of Micronesia for permission to collect in territorial waters and Stam for assistance with collections. We are grateful to Mark Leed and Mark Zabriskie (Oregon State University) for HDAC assay results, Jeff de Ropp (UC Davis NMR Facility) and Evan Rogers for assistance with 600 MHz NMR measurements, and Jorge Jimenez (AgraQuest, Inc.) for assistance with ^{13}C NMR (100 MHz). HRMS measurements were carried out by Ron New of the UC Riverside MS Facility. The 600 and 400 MHz NMR spectrometers and LCMS were purchased with funds provided by instrument grants NIH RR11973, NSF CHE-9808183, and RR14701-01, respectively. This work was supported by a grant from the NIH (CA 85602).

Supporting Information Available: Color image of the Didemnid ascidian (01-133, underwater), 1D ^{1}H NMR for **1**−**3**, ^{13}C NMR for **1**, and COSY, HSQC, and HMBC spectra for **1** (CD_3OD and DMSO-d_6) and **2** (CD_3OD). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) A solution of **1** (0.50 mg) in deionized water (200 μL, MilliQ) and hydrochloric acid (12 M, 200 μL) was sealed in a screw-thread pressure vial and heated at 110 °C for 16 h. After removal of the volatiles, the residue was redissolved in deionized water (100 μL), and the solution centrifuged to remove insoluble material. The supernatant was mixed with Marfey's reagent (200 μL, 1% solution of 2,4-dinitrophenyl-5-fluoro-L-alaninamide in acetone) and aqueous 1.0 M $NaHCO_3$ (20 μL) and then heated at 85 °C for 12 min and cooled before quenching (1.0 M HCl, 10 μL). LCMS of this solution (4 μL injection, Phenomenex Luna C_{18} column, 2.0 × 100 mm) used a solvent gradient (90:10 H_2O/CH_3CN to 35:65 H_2O/CH_3CN over 40 min, flow rate = 0.4 mL/min), and monitoring was done by UV (λ 210, 254, 285, and 340 nm) and ESIMS (negative ion). Two peaks, corresponding to Marfey's peaks of L-valine and L-ornithine, eluted at t_R = 20.9 and 24.7 min, respectively. Marfey's derivatives of paired standards of L- and D-amino acids (1 μg/μL), prepared in the same way, eluted at the following respective retention times: D- and L-lysine (t_R = 26.2, 27.4 min) D- and L-ornithine (t_R = 23.3, 24.9 min), L- and D-proline (t_R = 16.8, 17.8 min), and L- and D-valine (t_R = 20.9, 24.3 min).

(11) Recent examples of long-chain α,ω-bifunctionalized lipids include the C_{28} sphingolipids from sponges [oceanapiside from *Oceanapia phillipensis*: (a) Nicholas, G. M.; Hong, T. W.; Molinski, T. F.; Lerch, M. L.; Cancilla, M. T.; Lebrilla, C. B. *J. Nat. Prod.* **1999**, *62*, 1678−1681. (b) Nicholas, G. M.; Molinski, T. F. *J. Am. Chem. Soc.* **2000**, *122*, 4011−4019], rhizochalin from *Rhizochalina incrustata* [(c) Makarieva, T. N.; Denisenko, V. A.; Stonik, V. A. *Tetrahedron Lett.* **1989**, *30*, 6581−6584. (d) Molinski, T. F.; Makarieva, T. N.; Stonik, V. A. *Angew. Chem. Intl. Ed.* **2000**, *39*, 4076−4079], calyxoside from a *Calyx* sp. [(e) Zhou, B.-N.; Mattern, M. P.; Johnson, R. K.; Kingston, D. G. I. *Tetrahedron* **2001**, *57*, 9549−9554], and ene−yne lipids from *Haliclona* sp. [(f) Zhou, G−X.; Molinski, T. F. *Mar. Drugs* **2003**, *1* (1), 46−53 and references cited within].

(12) Ikeda, H.; Nonomiya, T.; Usami, M.; Ohta, T.; Omura, S. *Proc. Nat. Acad. Sci. U.S.A.* **1999**, *96*, 9509−9514.

(13) Emmert, E. A. B.; Klimowicz, A. K.; Thomas, M. G.; Handelsman, J. *App. Env. Micro.* **2004**, *70*, 104−113.

(14) Should the biosynthesis of the hexaol derive from a carbohydrate fragment, it is tempting to speculate that the relative configuration of C5−C10 in **1** and **2** may follow the stereogenicity of a natural aldohexose.

(15) Viability assays were carried out on cultured HCT-116 tumor cells using a *soluble* MTS formazan dye end point. For details, see ref 11f.