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Stereochemical Elucidation of New Sagittamides C–F from a Didemnid Ascidian

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Four new minor congeners, sagittamides C–F, were isolated from an unidentified Didemnid tunicate that previously afforded sagittamides A and B. The structures were determined by interpretation of spectroscopic data, degradation to amino acids, and comparisons with sagittamide A. An unexpected change in relative configuration of the hexacetoxy C5–C10 stereoelement is present in sagittamides D and F. A tentative assignment of configuration was possible through a systematic deduction based on analysis of ^{13}C NMR data and symmetry considerations.

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Introduction

Previously, we reported the isolation and structure elucidation of long-chain polyacetoxylated α,ω-diamides sagittamides A (**1**) (Fig. 1) and B (**2**)[1,2] from a didemnid tunicate collected in Micronesia. The structures of these natural products embody a C5–C10 stereohexad of six contiguous acetoxyl groups, an oxidation motif not commonly found in other lipid natural products. The structures are suggestive of polyketide (PK) biosynthesis with incorporation of several hydroxymalonate extender units.[1] Elucidation of the relative stereochemistry of the C5–C10 segment in **1**[2] was a difficult, non-trivial problem. The first proposed configuration of *anti-anti-anti-syn-anti* was based on progressive stereochemical analysis and comparison with a series of 16 hexaol peracetates model compounds derived from D-xylose and D-ribose; however, this assignment differed from that of Kishi and coworkers who proposed an *anti-anti-syn-anti-syn* isomer.[3] Kishi's independent assignment of configuration of the stereohexad is based on application of iterative $^{3}J_{HH}$ vicinal coupling constant analysis and total synthesis of **1** and a diastereomer in which both terminal L-amino acid groups were replaced with D-lysine and D-ornithine respectively. The ^{1}H NMR chemical shifts of the C5–C10 hexad were identical to those of the natural product and the absolute configurations (directionality of C5–C10 stereocentres) were determined by matching the correct synthetic diastereomer with the natural product. The 500-MHz ^{1}H NMR spectrum of co-mixtures of natural **1** with each of the two synthetic diastereomers (ratio of 2:1) showed only one discrete set of six OAc singlets for the synthetic L-Lys, L-Orn diastereomer, whereas the mismatched D-Lys, D-Orn diastereomer showed more than six singlets due to slight diastereomeric differences. We re-examined the relative configuration of natural **1** by careful remeasurements of $^{2}J_{CH}$ and $^{3}J_{CH}$ using the HSQC-HECADE[4] measurement of ^{1}H–^{13}C residual dipolar couplings (RDCs) in aligned media and a least-squares fit to the predicted values of RDCs.[5] The outcome of these experiments corrected earlier reported values of $^{2,3}J_{CH}$ and unambiguously confirmed the Kishi assignment.

Further investigations of the tunicate extract have now resulted in the isolation of four additional minor constituent sagittamides C (**3**), D (**4**), E (**5**), and F (**6**), comprising two homologues and two stereoisomers of **1**. Here we report the structures **3**–**6** by analysis of spectroscopic data in comparison with those of **1** and degradation and identification of the constituent amino acids. A tentative stereochemical assignment of the configuration of the C5–C10 stereohexad in the new stereoisomers is provided from ^{13}C NMR data and symmetry considerations, along with an appraisal of ^{1}H NMR J_{HH} analysis in the context of second-order coupling of near-symmetrical polyol stereoelements.

Results and Discussion

High resolution-fast atom bombardment mass spectrometry (HR-FABMS) of sagittamide C (**3**) showed pseudomolecular ions at *m/z* 1016.5901 ($[M + H]^+$) and *m/z* 1038.6 ($[M + Na]^+$) corresponding to a molecular formula of $C_{50}H_{85}N_3O_{18}$ and it is therefore a higher homologue of **1** ($C_{48}H_{81}N_3O_{18}$) differing by two CH_2 units. Inspection of the ^{1}H NMR spectrum of **3** in CD_3OD showed very few differences from **1**, and the ^{13}C NMR data of **3** were almost identical with **1** except for the presence of two additional peaks in the midchain CH_2 cluster at δ 30.5 ppm. Thus, **3** has a longer C_{28} α,ω-dicarboxyl chain compared with C_{26} in **1**. COSY and HMBC analysis of **3** showed the same correlations linking the proximal acetoxylated C5 through to the chain terminus C1 as seen in **1**; consequently, the additional two CH_2 groups were placed in the long-chain segment between C11 and C28.

Sagittamide D (**4**), $C_{48}H_{81}N_3O_{18}$ (*m/z* 988.5605, $[M + H]^+$), was isomeric with **1**. ^{1}H NMR analysis of **4** confirmed the

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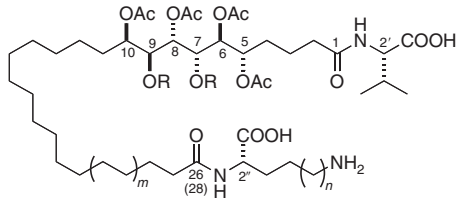
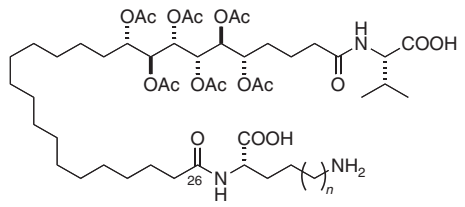
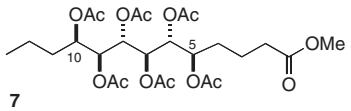


1 R = Ac, *m* = 1, *n* = 1 Sagittamide A
2 R = H, *m* = 1, *n* = 1 B
3 R = Ac, *m* = 2, *n* = 1 C
5 R = Ac, *m* = 1, *n* = 2 E



4 *n* = 1 Sagittamide D
6 *n* = 2 F



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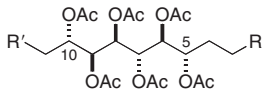
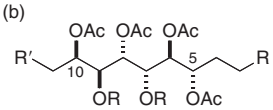
(a)



anti-anti-anti-syn-anti
(5*S*,6*S*,7*S*,8*R*,9*S*,10*S*)
Original assignment

(b)



anti-anti-syn-anti-syn
(5*S*,6*S*,7*S*,8*S*,9*S*,10*R*)
Kishi assignment (correct)

Fig. 1. The absolute stereostructure of sagittamide A (**1**). (a) Original assignment (ref. [2]) and (b) revised assignment (ref. [3]).

presence of the hexad of the contiguous AcO–CH signals and terminal ornithine and valine residues; however, the stereochemistry of the C5–C10 segment was different as indicated by significant changes in the ^{1}H and ^{13}C NMR chemical shifts. The ^{1}H NMR chemical shifts (600 MHz, CD_3OD) of the four internal OCH proton multiplets at δ 5.42, 5.15, 5.13, and 5.11 ppm in **1** were shifted to δ 5.32, 5.30, 5.15, and 5.13 ppm in **4**; similarly, the ^{13}C NMR signals (CD_3OD) for the C5–C10 acetoxylated CH signals in **1** (δ 73.4, 71.4, 69.2, 68.4, 70.4, 71.9 ppm respectively) now appeared as a symmetrical pattern (δ 73.4, 69.03, 71.0, 71.0, 68.99, 73.6 ppm). The same high symmetry was also observed when the ^{1}H and ^{13}C NMR spectra of **4** were recorded in [D_6]DMSO (Table 1).

Sagittamides E (**5**) and F (**6**) were isomeric with each other; pseudomolecular ions observed in HR-FABMS at *m/z* 1002.5764 ($[M + H]^+$) and 1002.5731 ($[M + H]^+$) respectively, confirmed the common formula $C_{49}H_{83}N_3O_{18}$. The mass of compound **5** was greater than **1** by one CH_2 unit, and ^{1}H NMR analysis showed both **1** and **5** had identical signals for the acetoxylated C5–C10 hexad. Consequently, the configuration of **5** is the same as that of **1**. The ^{1}H NMR chemical shifts and vicinal *J* values of the hexaacetate C5–C10 segment in compound **6** were the same as those of sagittamide D (**4**). Therefore, **5** and **6** differ in the configuration of one or more stereocentres in C5–C10 but each shares the configuration of their respective homologues **1** and **4**.

Given that **5** is a homologue of **1** and no methyl branches or *N*- or *O*-methyl groups were present, it appeared the extra CH_2 unit in **5** was associated with a difference in lipid chain length or one of the amino-acid residues. 2D NMR analysis of **5** shows the same signals associated with a Val end group, but careful assessment of the HSQC spectrum revealed a new cross-peak corresponding to an extra CH_2 (δ_C 21.1; δ_H 1.3, m) that is consistent with the γ-CH_2 of lysine.[6] Exhaustive hydrolysis of **5** (6 M HCl, 110°C, 12 h) followed by treatment of the dried hydrolysate with 5-fluoro-2,4-dinitrophenyl-L-alaninamide (L-FDAA, L-Marfey's reagent, 80°C, 10 min) followed by HPLC analysis gave peaks that co-eluted with standard L-FDAA-L-Val and a new peak with a retention time identical with that of standard L-FDAA-L-Lys adduct,[7] proving that **5** and **6** contain L-Lys instead of L-Orn in the distal amide group.

We addressed the configurational relationship between stereoisomeric sagittamides A (**1**) and D (**4**) in the following manner. From biosynthetic considerations, it is likely that the change in configuration in **4** with respect to **1** involves inversion of a minimal number of stereocentres, possibly only one. The salient feature of the ^{1}H and ^{13}C NMR spectra of **4** and **6**, which is lacking in **1**, is the high degree of symmetry for both ^{1}H and ^{13}C NMR spectra, which reduces the C5–C10 spin systems to three sets of almost-degenerate chemical shifts. The chemical shifts of C5, C6, and C7 of **4** (Table 1) differ from each other significantly, but are virtually identical with their counterparts at C10, C9, and C8, in that order. This suggests that the change in configuration produces local symmetry in **4** resulting in either a local *pseudo*-C_2 axis or a mirror plane σ (*pseudo-meso*), in contrast to the lack of symmetry (C_1) present in **1**. Fig. 2 depicts the structures of all possible isomers of **1** (Fig. 2a), bearing symmetrical C5–C10 stereo-hexads, grouped as *pseudo*-C_2 and *pseudo-meso* isomers (Fig. 2b′–h′). An analysis of the configuration of **4**, assuming parsimonious changes from **1**, excludes most of these permutations and all of their respective mirror images because they require inversions of three of more centres. This leaves two possibilities for **4** as an epimer of **1** at either C5 or C10 (Fig. 2b and c respectively).

We favour a C10 epimer of **1** as the configuration for **4** from symmetry considerations and by comparison of **4** with 16 stereoisomeric model compounds used in structure elucidation of sagittamide A (**1**),[2] including **7**, two *pseudo*-C_2 models **8**, **9**, and two *pseudo-meso* isomers **10**, **11**.[8] The ^{13}C NMR signals of **4** and *pseudo*-symmetrical isomers of **8**–**10** were, as expected, almost identical within each matched pair of signals C5/C10, C6/C9, and C7/C8 ($\Delta\delta < 0.1$ ppm), but differed between the pairs. Although all ^{13}C NMR chemical shifts for C5–C10 (see Accessory Publication, Table S1) occurred in the range δ 67.2–71.7 ppm, the range differences for C6/C7 and C8/C9 pairs were consistently larger ($\Delta\delta = 1.9$–2.8 ppm) in *pseudo*-C_2 isomers than in *pseudo-meso* isomers ($\Delta\delta = 0.1$ ppm). Because the chemical shifts differences for C6/C7 and C8/C9 in sagittamide D (**4**) are within the larger range ($\Delta\delta = 2.5$ ppm), we may

Table 1. Partial ^{1}H and ^{13}C NMR data for 1 and 4 (CD_3OD)
See Experimental section for a complete listing of values for **4**

Carbon no.	**1** (ref. [1])		**4**	
	δ_H[A]	δ_C	δ_H[B,C]	δ_C[D]
4a	1.59 (m, 1H)	29.1	1.66 (m, 2H)	28.6
4b	1.63 (m, 1H)			
5	4.92 (m, 1H)	73.4	4.82 (m, 1H)[E]	73.4
6	5.11 (dd, 8.3, 4.0, 1H)	71.4	5.15 (dd, 8.9, 2.8, 1H)[F]	69.03[G]
7	5.15 (dd, 8.3, 1.5, 1H)	69.2	5.33 (dd, 8.9, 1.9, 1H)[H]	71.0
8	5.42 (dd, 9.9, 1.5, 1H)	68.4	5.32 (dd, 8.9, 1.9, 1H)[H]	71.0
9	5.13 (dd, 9.9, 1.8, 1H)	70.4	5.15 (dd, 8.9, 2.8, 1H)[F]	68.99[G]
10	4.82 (td, 6.8, 1.8, 1H)	71.9	4.80 (dt, 10.7, 2.8, 1H)	73.6
11	1.42 (m, 2H)	31.8	1.59 (m, 2H)	28.7

[A] 400 MHz.
[B] 800 MHz.
[C] *J* values from the 800-MHz ^{1}H NMR spectrum. H4 and H11 assignments confirmed from DQFCOSY (500 MHz).
[D] 125 MHz.
[E] Obscured by solvent.
[F,G,H] Interchangeable pairs.

assign **4** as a *pseudo*-C_2 isomer. The isomers depicted in Fig. 2d and e were eliminated because they each would require inversion of three stereocentres of **1** to obtain **4**;[9] this narrows the choice for **4** to a C5 or C10 epimer of **1** (Fig. 2b or c respectively).

Unfortunately, the required models corresponding to Fig. 2b and c were not available for direct comparison. Nevertheless, partial comparison of the C8–C10 stereotriad in the model compounds with those of **1** and **4** suggested a reasonably confident assignment of the stereochemistry. The C7–C10 *anti-anti-syn* relative configuration of **7** matches well for **1** (Fig. 3b) but poorly for the C9–C10 chemical shifts of **4** (Fig. 3a). Therefore, by elimination, it is likely that C10 in sagittamide D (**4**) is inverted with respect to **1**, and **4** has the configuration corresponding to Fig. 2c. The *anti-anti-syn-anti-anti* relative configuration for **4**, corresponding to Fig. 2c, is also supported by the closer match of C8–C10 chemical shifts with an *anti-anti* stereoelement (see **4** and model **11**, Fig. 4d).

An alternative approach draws on Kishi's vicinal coupling constant profile analysis by matching J_{HH} values of contiguous overlapping stereotriads (*syn-anti* relative configurations) in acyclic peracetoxy compounds to databases of averaged J_{HH} ranges obtained from multiple literature sources.[3] At lower field strength (500 MHz, [D_6]DMSO, or CD_3OD, Fig. 5), the ^{1}H NMR chemical shifts of the pairs of signals H7/H8 and H6/H9 in **4** were not resolved. In fact, because of overlapping spin states, corresponding to the frequencies of the upfield and downfield multiplet components in coupled H7 and H8, the H6/H9 multiplets exhibited characteristics of second-order (virtual) coupling and it was not possible to obtain reliable δ and J values under these conditions. It is often overlooked that second-order coupling in ^{1}H NMR spectra of *all* pseudo-symmetrical stereosegments will limit not only the Kishi-type J_{HH} analysis, but also integrated Murata-type J-based analyses.[10] Symmetry places critical demands for NMR signal dispersion that can sometimes be achieved at higher fields. The second-order effects in the ^{1}H NMR spectrum of **4** were removed at 800 MHz (CD_3OD, Fig. 5) and partial separation of all four multiplets observed. Careful measurements of the vicinal coupling constants in **4** for proton signals H5/H6 ($J = 2.8$ Hz), H6/H7 ($J = 8.9$ Hz), H7/H8 ($J = 1.9$ Hz), H8/H9 ($J = 8.9$ Hz), and H9/H10 ($J = 2.8$ Hz) allowed validation of the assignments by the Kishi method;[3] specifically, that configuration of stereotriads may be assigned confidently if the differences of J profiles between the database and **4** exceed the tolerance $\Sigma|\Delta\text{Hz}| \geq 3.3$ Hz. Four sequential overlapped stereotriads were matched to predict the relative stereochemistry of **1**;[3a] however, because of the symmetry in **4**, only a match with the first of two overlapped stereotriads for C5–C6–C7–C8 was required; both remaining stereochemical possibilities (Fig. 2b and c) have *anti-syn-anti* in the second stereotriad, C6–C7–C8–C9. Pleasingly, contiguous J_{HH} analysis of **4** also narrows down to the same two configurations obtained from ^{13}C NMR analysis; unfortunately, the sequential J_{HH} couplings fall within the tolerance of analysis of C5–C6–C7–C8 for both *anti-anti-syn* (Fig. 2c, $\Sigma|\Delta\text{Hz}| = 2.5$ Hz) and *syn-anti-syn* (Fig. 2b, $\Sigma|\Delta\text{Hz}| = 1.6$ Hz).[11] Consequently, interpretation of contiguous J_{HH} values in **4** is equivocal and, in this case, the preceding argument for configuration by ^{13}C NMR chemical shift and symmetry arguments is stronger.

As noted above, the relative configurations of sagittamides D (**4**) and F (**6**) are identical in the C4–C10 region by NMR; therefore, we assign both compounds as (5*S*,6*S*,7*S*,8*S*,9*S*,10*S*). Note this is not a fully rigorous proof because lack of the model compound with exactly the predicted configuration (cf. Fig. 2c) prevents direct comparison with **4**, and the foregoing stereochemical assignments of **4** and **6** must be considered, at best, tentative.

Long-chain lipids that are functionalized at both α,ω-termini are relatively rare in nature. Examples among marine natural products include the 'two-headed' sphingolipids derived from sponges rhizochalin[12] and isorhizochalin[13] from *Rhizochalina incrustata*, oceanapiside from *Oceanapia philipensis*,[14] calyxoside from *Calyx* sp.,[15] and leucettamols from *Leucetta microrhaphis*.[16] A variation on the latter theme is found in rhapsamine, an unusual linear C_{28} polyene, terminally substituted by 1,3-diamino-2-propanol groups, from the Antarctic sponge *L. leptorhapsis*.[17] Long-chain dicarboxylic acid derivatives are even less common but do occur in certain bacteria. For example, very-long-chain dimethyl branched C_{29}–C_{32} α,ω-dicarboxylic acids comprise some 40% of the fatty acyl membrane lipids of the anaerobic bacterium *Thermoanaerobacter*

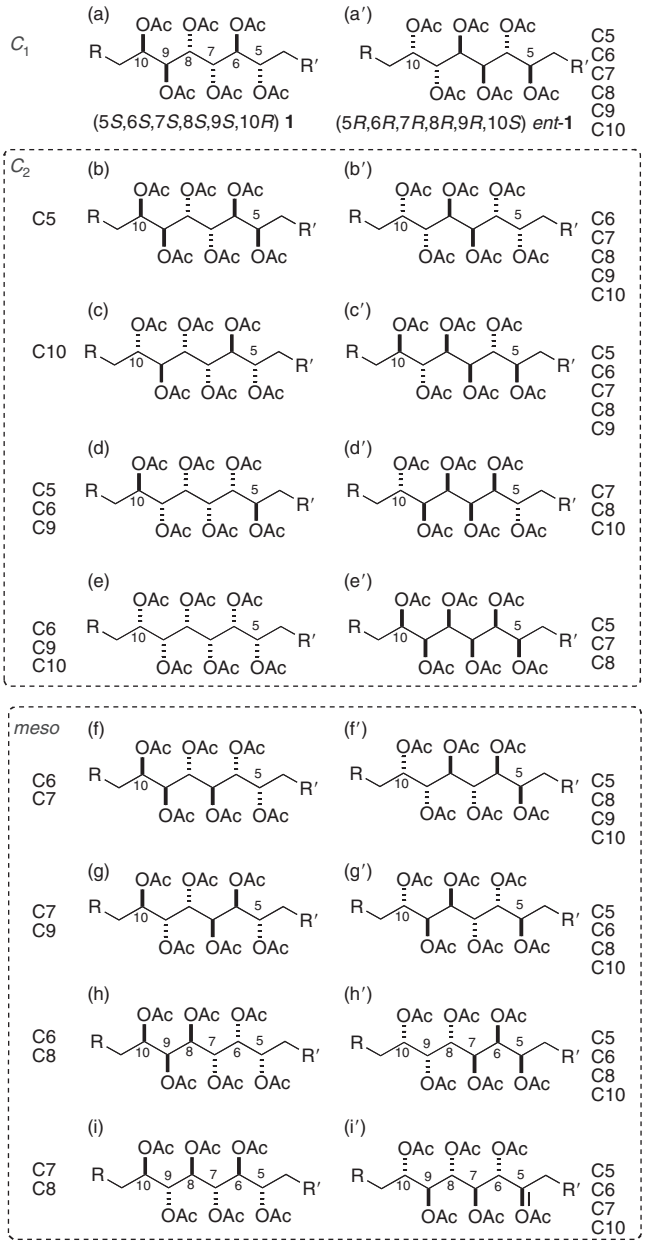


Fig. 2. C5–C10 stereohexads of (a) sagittamide A (**1**) and all stereoisomers possessing *pseudo*-symmetrical properties. (b)–(e) Local two-fold rotation axis (*pseudo*-C_2), or (f)–(h) σ, local mirror plane (*pseudo-meso*). (a′)–(h′) are mirror images of (a)–(h) respectively. Locants next to each structure are the stereocentres in **1** that must be inverted to achieve the depicted configuration.

ethanolicus 39E, and appear to be biosynthesized by a unique transmembrane ω-oxidation-coupling mechanism.[18] The biosynthesis of sagittamides **1**–**6** is unknown; however, the contiguous stereoregular hexad of acetoxyls between C5 and C10 in the structure of **1** and its congeners may have a different origin to post-translationally modified fatty acids. An alternative hypothesis invokes polyketide synthase (PKS) biosynthesis starting with malonyl CoA or acetyl CoA and ketosynthase-catalyzed chain extension with three hydroxymalonyl CoA units, with partial reduction of the keto groups, with the remainder of the chain assembled by malonyl CoA units in the conventional manner.[19] After our disclosure of the structure of **1**,[1] the first experimental evidence for PK chain extension by a hydroxymalonate extender was reported by Thomas and coworkers in

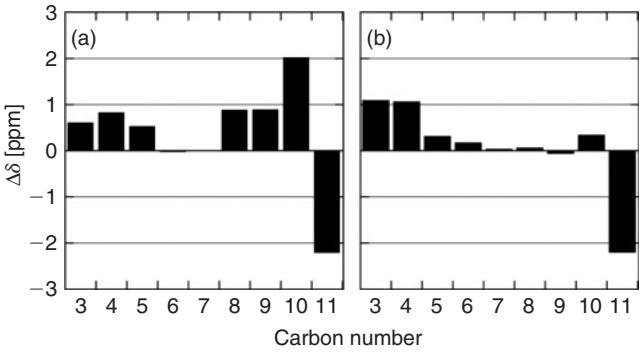


Fig. 3. Comparison of ^{13}C NMR $\Delta\delta$ values for **1**, **4**, and **7** (125 MHz, [D$_6$]DMSO, 298 K). (a) $\Delta\delta = \delta(\mathbf{4}) - \delta(\mathbf{7})$. (b) $\Delta\delta = \delta(\mathbf{1}) - \delta(\mathbf{7})$.

the PK zwittermicin A,[20] and other examples have since been described.[21]

Two explanations may be advanced to explain the epimeric configurations in the minor congeners **4** and **6**. Both substrate control and enzyme stereospecificity of PKS ketosynthase (KS) and ketoreductase KR modules determine 1,3-tacticity and 1,2-relative stereochemistry respectively, in the nascent PK chain (Fig. 6). Generally, the configuration of the β-carbon in PKS chain extension is determined by intrinsic properties of the operative KR domain. If sequential additions of hydroxymalonyl CoA (Fig. 6*i*) are syndiotactic (alternating configurations at the HO-bearing C2 carbon, Fig. 6*ii*), as they appear to be in the major natural product sagittamide A (**1**), stereoregularity in C9–C10 may be disrupted by possible 'slippage' through enolization and partial epimerization at the α-carbon before reduction of the intermediate α-hydroxy-β-ketothioester in the growing chain. Such lack of stereofidelity is perhaps not unexpected as hydroxymalonyl thioesters, with lower pK_as for the α-CH than malonyl- and α-methylmalonyl thioesters, are more prone to spontaneous epimerization. Alternatively, the tacticity of the *first* of the three hydroxy-ketide additions to the growing long-chain acyl thioester may be lax compared with the last two where preset stereocentres impose order. Other explanations for the origin of the (AcO–CH)$_6$ hexad (e.g. cytochrome P450 oxidations of a regular malonate-extended PK) seem less appealing because they do not explain the regular, tight clustering of oxidation states at C5–C10. Further investigations are required to elaborate the details of the intriguing biosynthesis of **1**–**6**.

Conclusion

In conclusion, we have characterized sagittamides C–F (**2**–**6**), four new relatives of sagittamide A (**1**) comprising a pair of higher homologues extended by $(CH_2)_2$ in the long chain and a second pair of homologues in which the L-Orn in **1** is replaced with L-Lys. Sagittamides A–C (**1**–**3**) and E (**5**) share the same relative configuration at C5–C10, whereas sagittamides D (**4**) and F (**6**) are tentatively assigned as epimeric at C10 with respect to **1**.

Experimental

General Procedure

Solvents used for purification of **3**–**6** were of HPLC grade. Fourier-transform (FT)-IR spectra were measured on a Mattson Galaxy FTIR spectrometer and optical rotations were measured on a Jasco DIP-370 polarimeter. NMR spectra were recorded on various instruments: Varian Inova 400 MHz, Bruker DRX

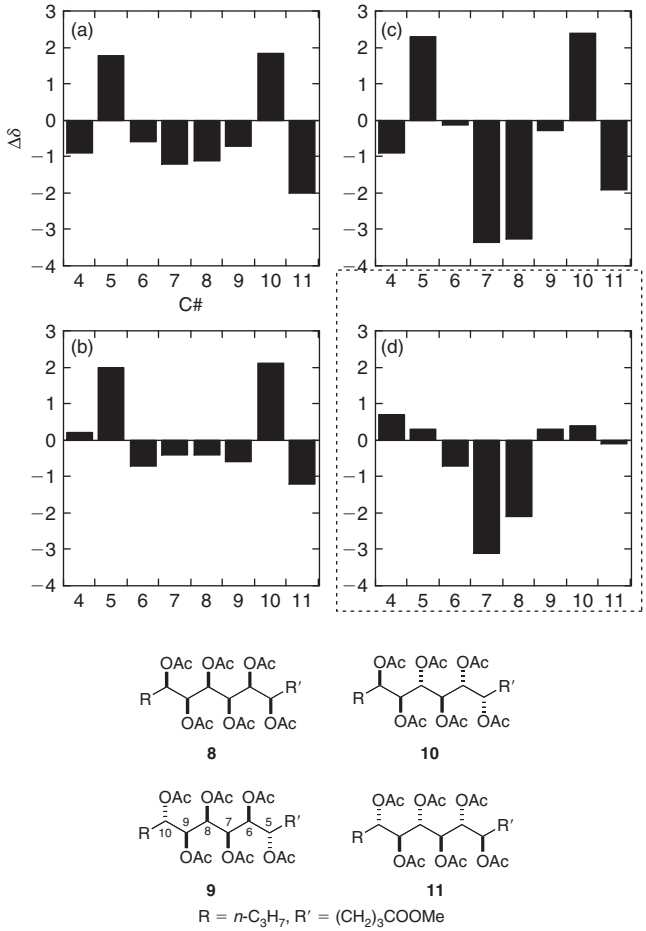


Fig. 4. Comparison of ^{13}C NMR (125 MHz, [D$_6$]DMSO, 298 K) $\Delta\delta$ values for **4** and models (a) $\Delta\delta = \delta(\mathbf{4}) - \delta(\mathbf{8})$. (b) $\Delta\delta = \delta(\mathbf{4}) - \delta(\mathbf{9})$. (c) $\Delta\delta = \delta(\mathbf{4}) - \delta(\mathbf{10})$. (d) $\Delta\delta = \delta(\mathbf{4}) - \delta(\mathbf{11})$.

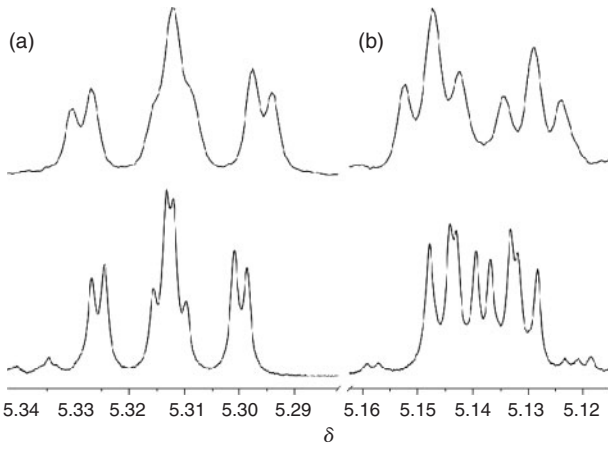


Fig. 5. Partial ^{1}H NMR spectra (CD$_3$OD, 23°C) of sagittamide D (**4**) at 500 MHz (top) and 800 MHz (bottom). (a) H7/H8; (b) H6/H9. See Table 1 for *J* values.

600 MHz with 5-mm cryoprobe (UC Davis), or Varian Xsens 500 and Varian 800 spectrometers (both cryoprobes, UC San Diego) and referenced to internal residual solvent (^{1}H NMR CD$_2$HOD, $\delta = 3.30$ ppm; [D$_6$]DMSO, $\delta = 2.50$ ppm; ^{13}C NMR CD$_3$OD, $\delta = 49.00$ ppm; [D$_6$]DMSO, $\delta = 40.45$ ppm). LR-ESIMS and MS-MS were obtained by direct infusion into a ThermoFinnigan MSQ quadrupole spectrometer. Reversed-phase HPLC purifications were carried out on Phenomenex C$_{18}$ Luna (10 × 250 mm or 4.6 × 150 mm) columns with flow rates of 3–4 mL min^{-1} and coupled to an evaporative light scattering detector or a refractive index detector. HR-MS measurements were obtained from University California, Riverside, Mass Spectrometry Facility.

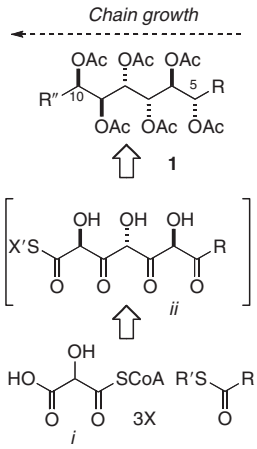


Fig. 6. Hypothetical assembly of sagittamide A (**1**) from polyketide synthase (PKS)-mediated sequential chain extensions of hydroxymalonyl CoA to the growing ketide chain R(C=O)SR′. Direction of chain growth is chosen arbitrarily. Intermediate *ii* does not imply ordering of reduction steps.

Animal Material

An unidentified colonial ascidian (01–17–133) was collected in 2001 by hand from shallow water (~1 m) at Arrow Island, Micronesia, in 2001 and kept frozen until required. The animal had a typical appearance of other colonial *Didemnum* species consisting of thin sheets of dark-brown pigmented tunic with lighter, orange-tan zooids that were less apparent in the frozen material. A crepe-paper like texture was apparent in the frozen material. A preserved type sample (ethanol) is archived at University of California, San Diego.

Extraction and Isolation

The tunicate (261.6 g, frozen) was macerated and suspended in 1:1 MeOH/CH$_2$Cl$_2$ and stirred overnight before filtering. The remaining marc was then ground and re-extracted twice more with 1:1 MeOH/CH$_2$Cl$_2$ and the extracts combined and concentrated to a brown solution that was partitioned against hexanes. The H$_2$O content of the MeOH layer was adjusted to 40% and the mixture extracted twice with CHCl$_3$. The aqueous methanol layer was concentrated to remove all organic solvent and the residual aqueous extract partitioned with *n*-butanol.

The CHCl$_3$ layer was dried, then separated using silica chromatography eluting with a gradient of 5% MeOH/CH$_2$Cl$_2$ to 100% MeOH. The most polar fraction contained nearly pure sagittamides. Concurrently, the *n*-butanol fraction was dried, then separated by size-exclusion chromatography (LH-20), eluting with MeOH. The sagittamide-containing fractions from both silica and LH-20 were finally purified by reversed-phase HPLC (1:1 CH$_3$CN:H$_2$O, 0.5% TFA, 10 × 250 mm C$_{18}$ column) to yield *sagittamide C* (**3**) (5.9 mg, 0.0023% wet weight), *sagittamide D* (**4**) (18.1 mg, 0.0069% wet weight) as a mixture with **1**,

sagittamide E (**5**) (3.0 mg, 0.0011% wet weight), and *sagittamide F* (**6**) (1.3 mg, 0.00050% wet weight). Pure *sagittamide D* (**4**) was obtained by repurification on an analytical C_{18} reversed-phase HPLC column.

*Sagittamide C (**3**)*: colourless glass. δ_H (600 MHz, CD_3OD) 5.42 (dd, 9.9, 1.5, H8), 5.15 (dd, 8.4, 1.8, H7), 5.13 (dd, 9.9, 2.1, H9), 5.11 (dd, 8.4, 3.6, H6), 4.88 (m, H5), 4.84 (m, H10), 4.42 (dd, 8.1, 5.1, H2″), 4.32 (d, 6.0, H2′), 2.95 (br t, 6.6, H5″), 2.25 (m, H2, H2′, H27, H27′), 2.16 (hep, 6.0, H3′), 2.08 (s, OAc), 2.07 (s, OAc), 2.024 (s, OAc), 2.020 (s, OAc), 2.008 (s, OAc), 2.006 (s, OAc), 1.98 (m, H4β′), 1.73 (m, H3β, H3α′, H3β′, H4α′), 1.60 (m, H4α, H4β, H26α, H26β), 1.52 (m, H3α), 1.43 (m, H11α, H11β), 1.37 (m, H12α, H12β), 1.27 (br s, 24H), 0.97 (d, 7.2, H4′), 0.96 (d, 7.2, H5′). δ_C (150 MHz, CD_3OD) 176.5 (C28), 175.8 (C1), 174.9 (C1′), 174.8 (C1″), 172.4 (OAc), 172.3 (OAc), 171.9 (OAc), 171.6 (OAc), 171.5 (OAc), 171.3 (OAc), 73.2 (C5), 71.7 (C10), 71.2 (C6), 70.3 (C9), 69.0 (C7), 68.2 (C8), 59.0 (C2′), 52.7 (C2″), 40.2 (C5″), 36.8 (C27), 36.1 (C2), 31.9 (C11 or C3′), 31.6 (C11 or C3′), 30.8–30.4 (C13 through C26), 29.7 (C3′), 29.0 (C4), 27.0 (C26), 26.2 (C12), 22.9 (C3), 21.1 (OAc), 20.94 (OAc), 20.90 (OAc), 20.86 (OAc), 20.82 (OAc), 20.75 (OAc), 19.7 (C5′), 18.4 (C4′). *m/z* (HR-FABMS) 1016.5901 $[M+H]^+$; Calc. for $C_{50}H_{86}N_3O_{18}^+$, 1016.5901.

*Sagittamide D (**4**)*: colourless glass. $[\alpha]_D^{24}$ −31.0 (*c* 0.92, MeOH). ν_{max} (ATR)/cm^{-1} 2926, 2854, 1748, 1681, 1539, 1434, 1371, 1214, 1139, 1033 cm^{-1}. δ_H (600 MHz, [D_6]DMSO) 8.02 (d, 7.8, NH″), 7.93 (d, 8.4, NH′), 5.25 (dd, 9.0, 1.2, H7 or H8), 5.23 (dd, 8.4, 1.2, H7 or H8), 5.04 (m, H6 or H9), 5.01 (m, H6 or H9), 4.79 (m, H5 or H10), 4.76 (m, H5 or H10), 4.14 (m, H), 4.12 (m, H), 2.77 (t, 6.9, H5″), 2.12 (m, H2, H2, H25, H25, H3′), 2.03 (s, 2 OAc), 2.01 (s, OAc), 2.00 (s, OAc), 1.98 (s, OAc), 1.96 (s, OAc), 1.75 (m, H3″), 1.57 (m, H3, H3, H24, H24, H3″, H4″, H4″), 1.47 (m, H4, H4, H11), 1.34 (m, H11), 1.23 (br s, H13 through H23), 0.86 (d, 6.6, H4′ and H5′). δ_C (150 MHz, [D_6]DMSO) 174.5 (C1′), 174.2 (C1″), 173.2 (C26), 172.8 (C1), 170.9 (OAc), 170.6 (OAc), 170.5 (OAc), 170.4 (OAc), 170.3 (OAc), 170.1 (OAc), 72.3 (C5 and C10), 70.5 (C6 or C9), 70.3 (C6 or C9), 68.0 (C7 and C8), 58.0 (C2′), 52.3 (C2″), 39.4 (C5″), 36.1 (C25), 35.5 (C2), 31.2 (C11), 30.7 (C3′), 30.0–29.6 (C13 through C23), 29.4 (C4), 29.2 (C3″), 26.2 (C24), 25.6 (C4″), 24.8 (C12), 21.7 (C3), 21.55–21.47 (6 OAc), 20.1 (C5′), 19.0 (C4′). δ_H (500 MHz, CD_3OD) 5.31 (m, H7 and H8), 5.14 (m, H6 and H9), 4.81 (m, H10), 4.79 (m, H5), 4.43 (dd, 8.0, 5.2, H2″), 4.32 (d, 5.8, H2′), 2.96 (t, 6.9, H5″), 2.25 (m, H2, H25), 2.16 (m, H3′), 2.070 (s, OAc), 2.066 (s, OAc), 2.03 (s, 2 OAc), 2.00 (s, OAc), 1.98 (s, OAc), 1.96 (m, H3′), 1.73 (m, H3′, H4′), 1.66 (m, H4, H3), 1.61 (m, H24), 1.59 (m, H11), 1.52 (m, H4), 1.32 (m, H12, H12), 1.28 (br s, H13 through H23), 0.97 (d, 6.9, H5′), 0.96 (t, 6.9, H4′). For ^1H NMR (800-MHz partial data and accurate *J* values of H5–H10), see Table 1. δ_C (125 MHz, CD_3OD) 176.5 (C26), 175.8 (C1), 174.8 (C1′), 174.7 (C1″), 172.35 (OAc), 172.33 (OAc), 171.7 (OAc), 171.6 (OAc), 171.49 (OAc), 171.46 (OAc), 73.6 (C10), 73.4 (C5), 71.0 (C6 and C9), 69.03 (C7 or C8), 68.99 (C7 or C8), 59.0 (C2′), 52.7 (C2″), 40.2 (C5″), 36.8 (C25), 36.1 (C2), 31.6 (C3′), 30.8–30.1 (C13 through C23), 29.7 (C3′), 28.7 (C11), 28.6 (C4), 26.9 (C24), 26.4 (C12), 25.2 (C4′), 23.0 (C3), 20.94–20.79 (6 OAc), 19.8 (C4′), 18.4 (C5′). *m/z* (HR-FABMS) 988.5605 $[M+H]^+$; Calc. for $C_{48}H_{82}N_3O_{18}^+$, 988.5588.

*Sagittamide E (**5**)*: colourless glass. δ_H (600 MHz, CD_3OD) 5.42 (dd, 9.9, 1.5, H8), 5.15 (dd, 8.4, 1.8, H7), 5.13 (dd, 10.2, 1.8, H9), 5.11 (dd, 8.4, 3.6, H6), 4.90 (m, H5), 4.84 (m, H10), 4.42 (dd, 8.4, 5.1, H2″), 4.32 (d, 6.0, H2′), 2.95 (t, 6.0, H6″, H6″), 2.25 (m, H2, H2, H25, H25), 2.15 (m, H3′), 2.08 (s, OAc), 2.07 (s, OAc), 2.03 (s, OAc), 2.020 (s, OAc), 2.008 (s, OAc), 2.007 (s, OAc), 1.97 (m, H3β′), 1.73 (m, H3β, H3α″, H5″, H5″), 1.62 (m, H4β, H24, H24), 1.51 (m, H3α), 1.44 (m, H11, H11), 1.38 (m, H12, H12), 1.28 (br s, H13 through H23, H4″, H4″), 0.97 (d, 6.6, H5′), 0.96 (d, 6.6, H4′). δ_C (150 MHz, CD_3OD) 176.5 (C26), 175.8 (C1), 174.9 (C1′), 174.8 (C1″), 172.5 (OAc), 172.3 (OAc), 171.9 (OAc), 171.6 (OAc), 171.5 (OAc), 171.4 (OAc), 73.2 (C5), 71.7 (C10), 71.2 (C6), 70.3 (C9), 69.0 (C7), 68.2 (C8), 59.0 (C2′), 59.0 (C2″), 40.2 (C6″), 36.9 (C25), 36.1 (C2), 31.9 (C11), 31.6 (C3′), 30.8–30.4 (C13 through C23), 29.7 (C3″), 29.0 (C4), 26.9 (C24), 26.2 (C12), 25.2 (C5″), 22.9 (C3), 21.1 (C4″), 21.0 (OAc), 20.9 (OAc), 20.88 (OAc), 20.87 (OAc), 20.83 (OAc), 20.76 (OAc), 19.7 (C5′), 18.4 (C4′). *m/z* (HR-FABMS) 1002.5764 $[M+H]^+$; Calc. for $C_{49}H_{84}N_3O_{18}^+$, 1002.5744.

*Sagittamide F (**6**)*: δ_H (600 MHz, CD_3OD) 5.32 (dd, 8.7, 1.8, H7 or H8), 5.30 (dd, 8.7, 1.8, H7 or H8), 5.15 (t, 3.0, H6 or H9), 5.13 (t, 3.0, H6 or H9), 4.83 (m, H5 or H10), 4.80 (tt, 10.5, 2.4, H5 or H10), 4.43 (dd, 8.4, 5.4, H2″), 4.32 (dd, 5.4, H2′), 2.95 (t, 6.3, H5″, H5″), 2.25 (m, H2, H2, H25, H25), 2.16 (m, H3′), 2.067 (s, OAc), 2.063 (s, OAc), 2.029 (s, 2 OAc), 1.99 (OAc), 1.98 (OAc), 1.73 (m, 5H), 1.65 (m, 2H), 1.62 (m, 2H), 1.52 (m, 4H), 1.28 (br s, H13 through H23, H4″, H4″), 0.97 (d, 7.2, H4′), 0.96 (d, 7.2, H5′). *m/z* (HR-FABMS) 1002.5731 $[M+H]^+$; Calc. for $C_{49}H_{84}N_3O_{18}^+$, 1002.5744.

*Marfey's Amino Acid Analysis of Sagittamide E (**5**)*

Sagittamide E (**5**) was hydrolyzed (6 M HCl, 110°C, overnight) and the dried hydrolysate derivatized with Marfey's reagent[7] according to the previously described protocol.[1] The L-FDAA derivatives co-eluted with those of L-lysine and L-valine.

Accessory Publication

^1H NMR, COSY, HSQC, and HMBC spectra of **3**–**6** are available on the Journal's website.

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