Glycolipids from Sponges. Part 17.[1] Clathrosides and Isoclathrosides, Unique Glycolipids from the Caribbean Sponge *Agelas clathrodes*

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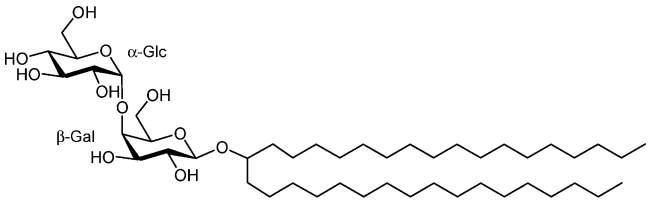
Received September 4, 2005

Two families of unique glycolipids, clathrosides A−C (**2a**−**4a**) and isoclathrosides A−C (**5a**−**7a**) were isolated from the Caribbean sponge *Agelas clathrodes*. Clathrosides and isoclathrosides are glycosides of a very-long-chain alcohol derived from fatty acids, a new class of glycolipids that appears to be characteristic of marine sponges. The six compounds differ in configuration and in the branching of alkyl chains. Stereostructures of the clathrosides were determined by NMR and CD spectroscopy, mass spectrometry, and chemical degradation. Location of the methyl branch on the proper alkyl chain required an exceptional 1-D TOCSY experiment, in which coherence was transferred through as many as 13 vicinal couplings.

Glycolipids are membrane components and occur in all kingdoms of living organisms, i.e., bacteria, plants, and animals including man. They are usually divided into three main groups, glycoglycerolipids, glycosphingolipids, and isoprenoid glycosides, depending on their lipid moiety, which can be, respectively, an acylated glycerol, an acylated sphingosine (ceramide), or a terpene alcohol. A number of glycolipids exist, however, that cannot be classified in any of these groups. These compounds are made of a glycosyl moiety (one or several saccharide units) linked to the hydroxyl group of a fatty alcohol or a hydroxy fatty acid, or to the carboxyl group of a fatty acid (ester linkage). They are typically found in bacteria and yeasts and frequently possess interesting biological properties. Porifera are another source of these "atypical" glycolipids: we recently reported the occurrence in *Plakortis simplex* of simplexides (e.g., **1**), diglycosides of a very-long-chain secondary alcohol, acting



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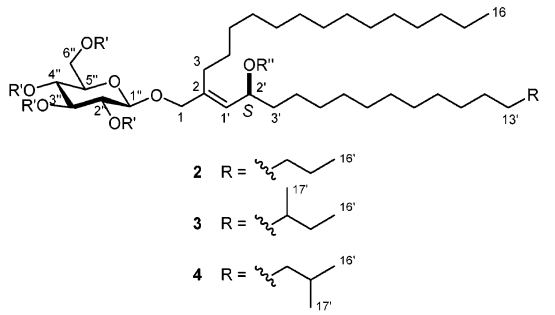
on the immune system.[2] In this paper, we wish to report the isolation and structure elucidation of the new glycolipids clathrosides A−C (**2a**−**4a**) and their stereoisomers isoclathrosides A−C (**5a**−**7a**) from *Agelas clathrodes*. Like simplexides, compounds **2a**−**7a** are glycosides of long-chain alcohols, probably derived from the condensation of two fatty acid molecules. However, the aglycone is quite different in that the glycosylated alcohol function is primary, and an additional double bond and hydroxyl group are present.

Results and Discussion

Specimens of *Agelas clathrodes* (Porifera, class Demospongiae, order Agelasida, family Agelasidae) were collected at Sweeting Cay (Grand Bahamas Island) and extracted with MeOH and $CHCl_3$. Following our usual procedure,[3] the extract was partitioned between H_2O and *n*-BuOH, and the organic phase was subjected to sequential column chromatography with RP-18 and normal silica gel, giving a crude glycolipid mixture that was acetylated with Ac_2O in pyridine.

Repeated direct-phase HPLC separation of this mixture led to a fraction (4.9 mg) entirely composed of homologous peracetylated clathrosides and a fraction (4.3 mg) composed of peracetylated isoclathrosides. Reversed-phase HPLC separation of the former fraction gave clathroside peracetate (**2b**) in the pure form and an

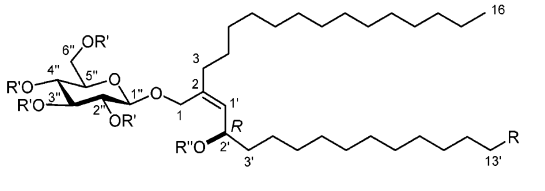


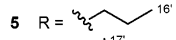
2 R =

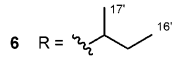
3 R =

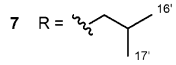
4 R =

a	R' = H	R" = H	**d**	R' = Bz	R" = Bz
b	R' = Ac	R" = Ac	**e**	R' = H	R" = Bz
c	R' = H	R" = Ac	**f**	R' = CD_3CO	R" = CD_3CO



5 R = 

6 R = 

7 R = 

inseparable mixture of peracetylated clathrosides B (**3b**) and C (**4b**). Likewise, reversed-phase HPLC separation of the latter fraction gave isoclathroside peracetate (**5b**) and peracetylated isoclathrosides B (**6b**) and C (**7b**) as a mixture.

All the peracetates **2b**−**7b** were deacetylated with MeONa/MeOH to give the respective natural products **2a**−**7a**. The conditions that we usually employ for glycolipid deacetylation, i.e., methanolysis in MeOH/Et_3N (8:2) at 50−80 °C for 12−24 h, were not effective in this case. For example, deacetylation of compound **2b** gave only small amounts of compound **2a** even at 80 °C, while the main product of the reaction was the monoacetyl derivative **2c**, in which all the acetyl groups on the sugar, but not the acetyl group on the aglycone, had been removed. This seems to be a general trend, since in our previous work we have often observed that deacetylation with this procedure is much faster for two or more nearby acetoxy groups compared to an isolated one.

The ^{1}H NMR spectrum (pyridine-d_5) of clathroside A **2a** showed it to be a glycolipid. The presence of long-chain alkyl groups was indicated by a large peak at δ l.25, and a series of signals in the midfield region of the spectrum suggested a carbohydrate partial structure. However, neither the usual NH doublet around δ 7 of a

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10.1021/np050331v CCC: $33.50
Published on Web 12/24/2005

Table 1. ^{1}H and ^{13}C NMR Data of Clathroside Peracetate **2b** and Isoclathroside Peracetate **5b**

		2b		**5b**	
pos.		δ_H [mult., *J* (Hz)]	δ_C (mult.)	δ_H [mult., *J* (Hz)]	δ_C (mult.)
1	a	4.25 (br d, 12.5)	71.7 (CH_2)	4.47 (br d, 12.7)	64.3 (CH_2)
	b	3.98 (br d, 12.5)		4.12 (br d, 12.7)	
2			139.2 (C)		138.1 (C)
3	a	2.21 (m)	28.3 (CH_2)	2.11 (m)	33.5 (CH_2)
	b	1.99 (m)		1.95 (m)	
4	a	1.40 (m)	28.3 (CH_2)	1.43 (m)	27.4 (CH_2)
	b	1.27 (m)		1.34 (m)	
5−13, 5′-13′		1.32−1.21	29.9−29.4	1.32−1.21	29.9−29.4
14, 14′		1.26 (submerged)	31.9 (CH_2)	1.26 (submerged)	31.9 (CH_2)
15, 15′		1.28 (submerged)	22.7 (CH_2)	1.28 (submerged)	22.7 (CH_2)
16, 16′		0.88 (t, 6.8)	14.1 (CH_3)	0.88 (t, 6.8)	14.1 (CH_3)
1′		5.33 (br d, 9.3)	127.3 (CH)	5.27 (br d, 9.0)	129.2 (CH)
2′		5.47 (ddd, 9.3, 6.8, 6.8)	70.6 (CH)	5.37 (ddd, 9.0, 6.8, 6.8)	70.9 (CH)
3′	a	1.65 (m)	34.9 (CH_2)	1.62 (m)	35.1 (CH_2)
	b	1.48 (m)		1.40 (m)	
4′		1.27 (submerged)	25.2 (CH_2)	1.25 (submerged)	25.1 (CH_2)
1″		4.47 (d, 8.1)	99.1 (CH)	4.53 (d, 8.1)	97.7 (CH)
2″		5.03 (dd, 9.5, 8.1)	71.4 (CH)	5.00 (dd, 9.5, 8.1)	71.4 (CH)
3″		5.20 (t, 9.5)	73.0 (CH)	5.20 (t, 9.5)	73.2 (CH)
4″		5.08(dd, 9.9, 9.5)	68.6 (CH)	5.12 (dd, 9.9, 9.5)	68.2 (CH)
5″		3.64 (ddd, 9.9, 4.0, 2.0)	71.7 (CH)	3.89 (ddd, 9.9, 4.0, 2.3)	71.3 (CH)
6″	a	4.25 (submerged)	62.1 (CH_2)	4.30 (dd, 12.4, 4.0)	61.8 (CH_2)
	b	4.13 (dd, 12.5, 2.0)		4.18 (dd, 12.4, 2.2)	
Ac's	CH_3	2.09, 2.04, 2.02, 2.02, 2.01	21.3−20.5	2.08, 2.03, 2.02, 2.02, 1.99	21.3−20.6
	CO		170.6−169.0		170.8−169.2

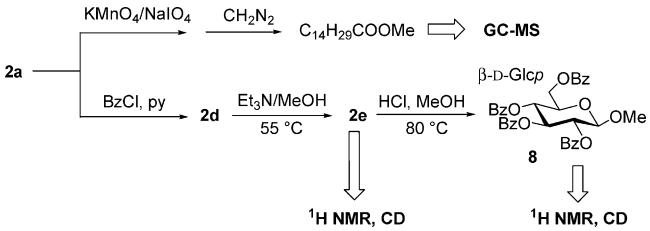
glycosphingolid nor the characteristic quintet around δ 5.2 of H-2 of an acylated glycerol was present. The high-resolution ESI mass spectrum of compound **2a** showed a prominent pseudomolecular ion $[M + Na]^+$ at *m*/*z* 665.5352, corresponding to the molecular formula $C_{38}H_{74}O_7$.

The 1- and 2-D NMR experiments directed at the structural elucidation of clathroside A were performed using the peracetate **2b**, to take advantage of the better signal dispersion in the proton spectrum of this derivative and of the possibility to distinguish between alkoxymethine and acetoxymethine protons on the basis of their chemical shifts.[3] The ^{13}C NMR spectrum showed the presence of one anomeric carbon at δ 99.1 (C-1″), and the relevant anomeric proton at δ 4.47 (H-1″) in the ^{1}H NMR spectrum was identified using the HSQC spectrum. Sequential assignment of the remaining six sugar protons (four CH and one CH_2) was achieved using the COSY spectrum. This demonstrated that (a) the sugar was a hexose and (b) it was in the pyranose form, on the basis of the shielded chemical shift (δ 3.64) of the sole nonacetylated oxymethine proton, i.e., H-5″, compared to the other oxymethine ring protons, all resonating above δ 5.0. The large coupling constants between H-1″ and H-2″, H-2″ and H-3″, H-3″ and H-4″, and H-4″ and H-5″ (Table 1) showed all these protons to be axial and, therefore, that the sugar was a β-glucopyranose.

The structure of the aglycone moiety of clathroside A was established as follows. The ^{13}C NMR spectrum of compound **2b** contained, in addition to the sugar carbon signals, those of one oxymethylene carbon atom at δ 71.7, one oxymethine carbon atom at δ 70.6, two sp^2 carbon atoms (one CH at δ 127.3 and one C at δ 139.2) accounting for one trisubstituted double bond, and several signals of sp^3 carbon atoms.

The oxymethylene protons (δ 4.25, H-1a and δ 3.98, H-1b) were identified using the HSQC spectrum. Using these protons as a starting point, the COSY spectrum permitted the assignment of, in sequence, the olefinic proton at δ 5.33 (H-1′, allylically coupled to the previous protons), the oxymethine proton at δ 5.47 (H-2′), the aliphatic protons at δ 1.65 and 1.48 (H_2-3′), and, finally, protons resonating in the large band at δ 1.25. Another spin system belonged to the aglycone and was composed of the allylic methylene protons at δ 2.21 and 1.99 (H_2-3), those at δ 1.40 and 1.27 (H_2-4), and, once again, protons resonating in the band at δ 1.25. The two spin systems were connected via the HMBC spectrum, which showed correlation peaks of the proton at δ 1.98 (H_2-3) with the carbon atoms at δ 127.3 (C-1′) and 139.2 (C-2). The HMBC also allowed us to locate the sugar unit at C-1 on the basis of the correlation peak between the anomeric proton at δ 4.47 (H-1″) and C-1 (δ 71.7). The configuration of the double bond of clathroside A was determined as *E* by analysis of the ROESY spectrum of compound **2b**. Strong correlation peaks were observed between H-2′ and both protons at C-3 and between H-1′ and the protons at C-1. Finally, compound **2b** showed only a 6H triplet at δ 0.88 in the high-field region of the ^{1}H NMR spectrum and only one methyl signal (apart from those of the acetyl groups) at δ 14.1 in the ^{13}C NMR spectrum. These data indicated that the alkyl chains linked at C-3 and C-3′ were unbranched.

Scheme 1. Degradation Procedure of Clathroside A (**2a**)



Determination of the length of the two alkyl chains of compound **2a** could not be carried out by NMR analysis, but required chemical degradation. A small amount of compound **2a** (100 μg) was subjected to Lemieux oxidation with $KMnO_4$/$NaIO_4$ in *t*-BuOH (Scheme 1). The reaction was expected to cause extensive cleavage of the functionalized part of the molecule, leading to two molecules of fatty acid whose carboxyl carbon atoms originated from C-2 and C-2′, respectively. The fatty acids isolated from the reaction mixture were methylated with CH_2N_2 and subjected to GC-MS analysis, allowing us to identify methyl pentadecanoate as the sole product of the reaction. Accordingly, the alkyl chains have the same length, as indicated in structure **2a**.

To complete the structure elucidation of the aglycone moiety of clathroside A (**2a**), only the configuration at C-2′ remained to be determined. Because of the allylic position of this carbinol, one obvious possibility was the CD-based method developed by Nakanishi for determination of the absolute configuration of allylic benzoates.[4] On the other hand, a selective benzoylation at C-2′

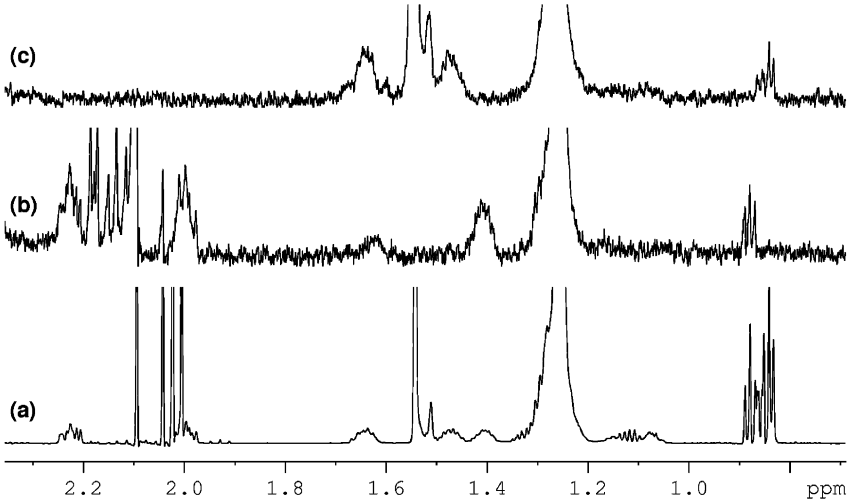


Figure 1. (a) ^{1}H NMR spectrum of peracetylated clathrosides B (**3b**) and C (**4b**); (b) 1-D TOCSY, mixing time 600 ms, selective excitation at δ 2.21, H-3a; the methyl triplet at δ 0.88 (H_3-16) is clearly visible. (c) 1-D TOCSY, mixing time 600 ms, selective excitation at δ 1.65, H-3′a; only the branched chain methyl protons are visible.

appeared difficult, while measurement of the CD spectrum of the perbenzoate would have been meaningless because of the strong interactions between the benzoate chromophores on the sugar. However, the low propensity toward transesterification at C-2′, described earlier, allowed a simple preparation of the desired derivative. Compound **2a** (400 μg) was treated with benzoyl chloride in pyridine to give the perbenzoate **2d**, which was subjected to methanolysis with MeOH/Et_3N (8:2) at 55 °C for 18 h. The benzoate groups on the sugar were selectively removed, leading to the 2′-*O*-benzoyl derivative **2e** (Scheme 1). The CD spectrum of compound **2e** showed a positive Cotton effect at 227 nm, which, according to Nakanishi's model,[4] implies the *S* configuration at C-2′.

Finally, the configuration of the sugar was established as D by subjecting compound **2e** to acidic methanolysis with 1 M HCl in 92% MeOH. The resulting methyl glycosides were perbenzoylated and subjected to HPLC. The chromatogram contained a peak that was identified as methyl β-D-glucopyranoside (**8**) by comparison of its ^{1}H NMR and CD spectra with those of an authentic sample.[5]

Clathrosides B (**3a**) and C (**4a**) could not be separated, not even as their peracetyl derivatives, and were studied as a mixture. The high-resolution ESI mass spectrum of the mixture gave a single $[M + Na]^+$ pseudomolecular ion at *m*/*z* 679.5500 (molecular formula $C_{39}H_{76}O_7$), showing it to be composed of isomers, each with one additional CH_2 compared with clathroside A (**2a**). The ^{1}H NMR spectrum, performed on the peracetates **3b** and **4b**, was very similar to that of compound **2b**, except for the methyl region, where a complex signal was present, whose overall integration accounted for three methyl groups. The HSQC spectrum revealed that these signals originated from the overlapping of four methyl signals: the triplet of an unbranched chain (δ_H 0.88, δ_C 14.1), the doublet of an *iso* chain (δ_H 0.86, δ_C 22.7), and the triplet (δ_H 0.85, δ_C 11.4) and the doublet (δ_H 0.84, δ_C 19.2) of an *anteiso* chain.[6] The mixture of **3a** and **4a** was then subjected to Lemieux degradation, followed by CH_2N_2 methylation as described above. GC-MS analysis of the resulting methyl esters permitted detection of methyl pentadecanoate (49.8%), methyl 13-methylpentadecanoate (40.1%), and methyl 14-methylpentadecanoate (10.1%).

The above data indicated that clathrosides B (**3a**) and C (**4a**) differed from clathroside A (**2a**) only by an additional methyl branch on, respectively, the third to last or penultimate carbon atom of one of the two alkyl chains. The ratio between **3a** and **4a** was estimated as 4:1 on the basis of the integration of the GC peaks. However, these data did not allow us to establish whether the additional alkyl group was on the "northern" (C-3/C16) or the "southern" (C-3′/C16′) alkyl chain, or randomly located on the two chains.

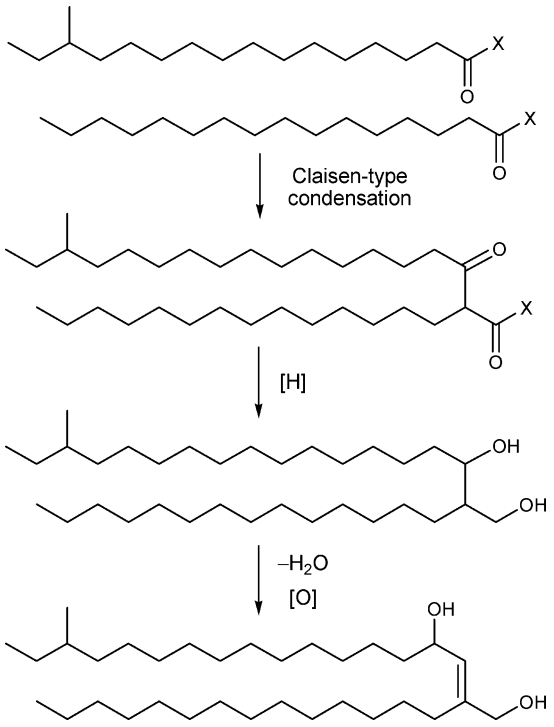
This goal was achieved through two diagnostic 1-D TOCSY experiments. We needed to correlate the terminal methyl groups of the alkyl chains with the protons at C-3 or C-3′, but the presence of a large number of overlapped methylene signals made a sequential assignment impossible. The only way to correlate these protons appeared to be a TOCSY sequence, and even so, coherence had to be transferred through as many as 13 vicinal couplings for the experiment to be successful. Therefore, we used a very long mixing time (600 ms), which turned out to be the best compromise between the increased coherence transfer obtained as the mixing time increases and the overall loss of sensitivity due to relaxation during the mixing time. In the first experiment, selective excitation of H-3a (δ 2.21) was performed, and only the methyl triplet of the unbranched chain (δ 0.88) appeared in the spectrum (Figure 1b); in contrast, when H-3′a (δ 1.65) was excited, only the methyl signals of the *iso* and *anteiso* chains were present in the spectrum (Figure 1c). Therefore, the additional methyl group was in the "southern" chain, i.e., linked to C-13′ in clathroside B (**3a**) and its derivatives, and to C-14′ in clathroside C (**4a**) and its derivatives.

Because clathrosides (**2b**−**4b**) could only be isolated as their peracetyl derivatives, there was a chance that one or more acetyls were already present in the natural glycolipids. This possibility was excluded by subjecting a small amount of the crude glycolipid fraction from *A. clathrodes* to acetylation using trideuteroacetic anhydride instead of acetic anhydride. The isolation procedure used for **2b**−**4b** was repeated, except that the three homologous clathrosides were not separated, and a fraction (0.5 mg) of a mixture of compounds **2f**−**4f** was obtained. The ^{1}H spectrum of the pertrideuteroacetylated clathrosides was very similar to that of compounds **2b**−**4b**, but no acetyl methyl singlet was present, showing that all the acetyl groups had been introduced by the acetylation reaction.

After structure elucidation of peracetylated clathrosides A−C (**2b**−**4b**) was completed, the ^{1}H and ^{13}C NMR spectra of the natural clathrosides (**2a**−**4a**) were assigned on the basis of its COSY and HSQC spectra (see Experimental Section).

Isoclathroside A (**5a**) was isomeric with clathroside A (**2a**), as shown by the high-resolution ESI mass spectrum, giving an $[M + Na]^+$ ion at *m*/*z* 665.5312. The broad features of the ^{1}H and ^{13}C NMR spectra of the respective peracetates **5b** and **2b** were also the same, with the same number of signals with similar multiplicities; however, the chemical shifts of several protons and carbons

Scheme 2



of the aglycone were remarkably different (Table 1). Examination of the COSY, HSQC, and HMBC spectra showed that isoclathroside A peracetate (**5b**) had the same planar structure as clathroside A peracetate (**2b**) and that the sugar moiety was the same, so that the difference must be confined in the geometry of the double bond and/or the configuration at C-2′.

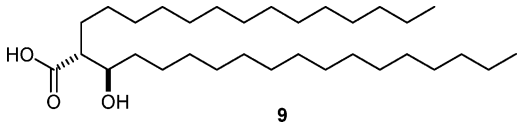
Analysis of the ROESY spectrum demonstrated that the configuration of the double bond was indeed different, that is to say *Z*. In fact, distinct correlations between one of the protons at C-1 (δ 4.47) and H-2′ and between H-1′ and both protons at C-3 were present in the spectrum. The configuration at C-2′ was determined using the same procedure as for compound **2a**, leading to the 2′-*O*-benzoyl derivative **5e**. The CD spectrum of compound **5e** showed a negative Cotton effect at 228 nm, indicating the *R* configuration at C-2′; clathroside A and isoclathroside A differed also in the configuration at C-2′.

Finally, the structures of isoclathrosides B (**6a**) and C (**7a**), isolated as an inseparable mixture, were established in the same way as was done for clathrosides B and C, on the basis of (a) the high-resolution ESI mass spectrum ($[M + Na]^+$ ion at *m*/*z* 679.5505; (b) the ^{13}C NMR spectrum, showing additional methyl signals at δ 22.7, 19.2, and 11.4; (c) Lemieux degradation/methylation, which provided methyl pentadecanoate (53.4%), methyl 13-methylpentadecanoate (36.9%), and methyl 14-methylpentadecanoate (9.7%); and (d) a 1-D TOCSY experiment, showing that upon selective excitation at δ 2.11 (H-3a) only the methyl triplet of the unbranched chain (δ 0.88) appeared in the spectrum, and therefore that the "northern" chain was unbranched.

Conclusion

Clathrosides and isoclathrosides are a further, structurally different example of a new kind of glycolipid from Porifera, namely glycosides of alcohols with a very long chain (C_{32} and C_{33} in this case), functionalized near the middle of the chain. An obvious hypothesis for the biosynthesis of the C_{32} aglycone of clathroside A is the coupling of two molecules of a C_{16} fatty acid. The first step would be a Claisen-type condensation, followed by reduction and dehydration (Scheme 2).

A similar biosynthetic pathway has been proposed, and to some extent demonstrated,[7] for mycolic acids (e.g., **9**) from mycobacteria and related microorganisms. It is worth noting that in the methyl branched compounds **3a**, **4a**, **6a**, and **7a** the methyl branch is located specifically on one of the two chains. This implies a very high substrate selectivity of the enzyme responsible for the condensation step or, alternatively, could suggest that a different biosynthetic mechanism is operating for the biosynthesis of clathrosides and isoclathrosides.



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Because of the structural similarity between clathrosides and the immunoactive simplexides (**1**), the activity of clathroside A (**2a**) and isoclathroside A (**5a**) on the immune system of mammals was assayed (MLR assay on murine cell and production of cytokines by human basophils), but the compounds did not show any significant activity.

Experimental Section

General Experimental Procedures. High-resolution ESIMS spectra were obtained on a Micromass QTOF Micro mass spectrometer. ESIMS experiments were performed on an Applied Biosystem API 2000 triple-quadrupole mass spectrometer. The spectra were recorded by infusion into the ESI source using MeOH as the solvent. Optical rotations were measured at 589 nm on a Perkin-Elmer 192 polarimeter using a 10 cm microcell. CD spectra were recorded on a Jasco J-710 spectrophotometer using a 1 cm cell. NMR spectra were determined on Varian UnityInova 700 and 500 NMR spectrometers; chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_H 7.26, δ_C 77.0; pyridine-d_5: δ_H 8.71, 7.56, and 7.19, δ_C 149.8, 135.3, and 123.4). Homonuclear ^1H connectivities were determined by COSY experiments. Through-space ^1H connectivities were evidenced using a ROESY experiment with a mixing time of 500 ms. The reverse-detected, gradient-enhanced single-quantum heteronuclear correlation (HSQC) spectra were optimized for an average $^1J_{CH}$ of 140 Hz. The gradient-enhanced multiple-bond heteronuclear correlation (HMBC) experiments were optimized for a $^3J_{CH}$ of 8 Hz. GC-MS spectra were performed on a Hewlett-Packard 5890 gas chromatograph with a mass selective detector MSD HP 5970 MS, a split/splitless injector, and a fused-silica column, 25 m × 0.20 mm HP-5 (cross-linked 25% Ph Me silicone, 0.33 mm film thickness); the temperature of the column was varied, after a delay of 3 min from the injection, from 150 °C to 280 °C with a slope of 10 °C min^{-1}; quantitative determination was based on the area of the GLC peaks. High-performance liquid chromatographies (HPLC) were achieved on a Varian Prostar 210 apparatus equipped with an Varian 350 refractive index detector or a Varian 325 UV detector.

Collection. Specimens of *A. clathrodes* (Porifera, class Demospongiae, order Agelasida, family Agelasidae) were collected by scuba (depth 23 m) during the third "Pawlik expedition" (July 2000) along the coast of Sweeting Cay (Grand Bahamas Island) and identified by Prof. M. Pansini, University of Genova. A reference sample (#6-00) has been deposited at the Istituto di Zoologia, Genova, Italy. The samples were stored at −20 °C until extraction.

Extraction and Isolation Procedure. The frozen sponge sample of *A. clathrodes* #6-00 (volume of fresh material 2400 mL, dry weight after extraction 142 g) was blended in MeOH, then extracted in sequence with MeOH (4 × 2.5 L), MeOH/$CHCl_3$ (2:1, 2.5 L), MeOH/$CHCl_3$ (1:2, 2.5 L), and $CHCl_3$ (2 × 3 L). The MeOH and MeOH/$CHCl_3$ extracts were partitioned between BuOH and H_2O. The BuOH phase was concentrated in vacuo and combined with the $CHCl_3$ extract. The total lipophilic extract (16.2 g) was subjected to reversed-phase chromatography on a column packed with RP-18 silica gel, by elution with a gradient of H_2O/MeOH and then $CHCl_3$. The fraction eluted with $CHCl_3$ (5.88 g) was further chromatographed on a column packed with SiO_2, eluted with a gradient of *n*-hexane/EtOAc (9:1) to MeOH. The fraction eluted with EtOAc/MeOH (7:3) (364 mg) was composed only of glycolipids. This fraction was acetylated with Ac_2O in pyridine at 25 °C for 18 h. The acetylated glycolipids were subjected to HPLC separation on an SiO_2 column [eluent: *n*-hexane/EtOAc (6:4)], thus

affording a fraction (50.9 mg) containing peracetylated clathrosides and isoclathrosides, together with larger amounts of other peracetylated glycolipids. Further normal-phase HPLC purification of these fractions [eluent: *n*-hexane/*i*-PrOH (97:3)] gave 4.9 mg of a mixture of peracetylated clathrosides A−C (**2b**−**4b**) and 4.3 mg of a mixture of peracetylated isoclathrosides A−C (**5b**−**7b**). Reversed-phase HPLC separation of the former fraction on an RP-18 column [eluent: MeOH/H_2O (97:3)] gave 1.2 mg of pure clathroside A peracetate (**2b**) and 1.8 mg of an inseparable mixture of clathroside B peracetate (**3b**) and clathroside C peracetate (**4b**). Likewise, reversed-phase HPLC separation of the latter fraction under the same conditions gave 0.9 mg of pure isoclathroside A peracetate (**5b**) and 1.2 mg of an inseparable mixture of isoclathroside B peracetate (**6b**) and isoclathroside C peracetate (**7b**).

Clathroside A peracetate (2b): colorless oil, $[\alpha]_D^{25}$ −9 ($CHCl_3$, *c* 0.1); ^1H and ^{13}C NMR, Table 1.

Mixture of clathroside B peracetate (3b) and clathroside C peracetate (4b): colorless oil; ^1H NMR (500 MHz, $CDCl_3$), same spectrum as **2b**, except for the methyl region, δ 0.88 (**3b** and **4b**, t, J = 7.0 Hz, H_3-16), 0.86 (**4b**, d, J = 6.6 Hz, H_3-16′ and H_3-17′), 0.85 (**3b**, t, J = 7.2 Hz, H_3-16′), 0.84 (**3b**, d, J = 6.5 Hz, H_3-17′); ^{13}C NMR ($CDCl_3$), same signals as for **2b**, plus δ 39.1 (**4b**, CH_2, C-14′), 36.6 (**3b**, CH_2, C-13′), 34.4 (**3b**, CH, C-14′), 27.1 (**3b**, CH_2, C-12′), 22.7 (**4b**, CH_3, C-16′ and C-17′), 19.2 (**3b**, CH_3, C-17′), 11.4 (**3b**, CH_3, C-16′).

Isoclathroside A peracetate (5b): colorless oil; $[\alpha]_D^{25}$ −12 ($CHCl_3$, *c* 0.1); ^1H and ^{13}C NMR, Table 1.

Mixture of isoclathroside B peracetate (6b) and isoclathroside C peracetate (7b): colorless oil; ^1H NMR (500 MHz, $CDCl_3$), same spectrum as **5b**, except for the methyl region, which was the same as in **3b**−**4b**; ^{13}C NMR ($CDCl_3$), same signals as **5b**, plus the same additional signals as in **3b**−**4b**.

Deacetylation of 2b−**7b.** Compound **2b** (1.2 mg) was dissolved in 950 μL of MeOH, and 50 μL of a 0.4 M solution of MeONa in MeOH was added. The reaction was allowed to proceed for 18 h at 25 °C, then the reaction mixture was dried under nitrogen and the residue partitioned between H_2O and $CHCl_3$. The organic layer was washed with H_2O and taken to dryness, giving 1.0 mg of clathroside A (**2a**). Compounds **3b**−**7b** were deacetylated using the same procedure.

Clathroside A (2a): colorless oil; $[\alpha]_D^{25}$ −6 ($CHCl_3$, *c* 0.1); ^1H NMR (500 MHz, pyridine-d_5) δ 7.28 (1H, d, J = 4.4 Hz, OH-2″), 7.16 (1H, d, J = 3.0 Hz, OH-3″), 7.14 (1H, d, J = 4.0 Hz, OH-4″), 6.41 (1H, t, J = 6.3 Hz, OH-6″), 6.15 (1H, d, J = 4.2 Hz, OH-2′), 6.08 (1H, br d, J = 8.9 Hz, H-1′), 4.98 (1H, d, J = 7.8 Hz, H-1″), 4.83 (1H, m, H-2′), 4.70 (1H, br d, J = 12.3 Hz, H-1a), 4.57 (1H, m, H-6″a), 4.40 (2H, m, H-6″b and H-1b), 4.25 (2H, m, H-3″ and H-4″), 4.11 (1H, m, H-2″), 3.95 (1H, m, H-5″), 2.43 (1H, m, H-3a), 2.37 (1H, m, H-3b), 1.92 (1H, m, H-3′a), 1.63 (1H, m, H-3′b), 1.59 (1H, m, H-4a), 1.49 (1H, m, H-4b), 1.33−1.20 (alkyl chain protons), 0.86 (3H, t, J = 6.8 Hz, H_3-16 and H_3-16′); ^{13}C NMR (pyridine-d_5) δ 137.1 (C, C-2), 132.9 (CH, C-1′), 103.7 (CH, C-1″), 78.7 (CH, C-3″), 78.5 (CH, C-5″), 75.3 (CH, C-2″), 72.7 (CH_2, C-1), 71.9 (CH, C-4″), 67.4 (CH, C-2′), 62.9 (CH_2, C-6″), 38.9 (CH_2, C-3′), 32.2 (CH_2, C-14 and C-14′), 30.2−29.7 (several CH_2, alkyl chains), 29.1 (CH_2, C-4), 28.9 (CH_2, C-3), 26.3 (CH_2, C-4′), 23.0 (CH_2, C-15 and C-15′), 14.3 (CH_3, C-16 and C-16′); HRESIMS *m*/*z* 665.5352 ($[M + Na]^+$, calcd for $C_{38}H_{74}NaO_7$ 665.5327).

Mixture of clathroside B (3a) and clathroside C (3a): colorless oil; ^1H NMR (500 MHz, pyridine-d_5), same spectrum as **2a**, except for the methyl region, δ 0.86 (6H, m); HRESIMS *m*/*z* 679.5500 ($[M + Na]^+$, calcd for $C_{39}H_{76}NaO_7$ 679.5483).

Isoclathroside A (5a): colorless oil; $[\alpha]_D^{25}$ −2 ($CHCl_3$, *c* 0.1); ^1H NMR (700 MHz, pyridine-d_5) δ 7.26 (1H, d, J = 3.6 Hz, OH-2″), 7.18 (1H, br s, OH-3″ or OH-4″), 7.11 (1H, br s, OH-4″ or OH-3″), 6.53 (1H, t, J = 5.4 Hz, OH-6″), 6.06 (1H, d, J = 4.2 Hz, OH-2′), 5.87 (1H, br d, J = 8.6 Hz, H-1′), 4.99 (1H, d, J = 7.8 Hz, H-1″), 4.97 (1H, m, H-2′), 4.85 (1H, br d, J = 11.8 Hz, H-1a), 4.62 (br d, J = 11.8 Hz, H-1b), 4.60 (overlapped, H-6″a), 4.37 (1H, ddd, J = 11.6, 5.9, and 5.9 Hz, H-6″b), 4.20 (2H, overlapped, H-3″ and H-4″), 4.10 (1H, m, H-2″), 4.01 (1H, m, H-5″), 2.50 (1H, m, H-3a), 2.30 (1H, m, H-3b), 1.91 (1H, m, H-3′a), 1.71 (1H, m, H-3′b), 1.55 (2H, m, H_2-4), 1.33−1.20 (alkyl chain protons), 0.86 (3H, t, J = 6.8 Hz, H_3-16 and H_3-16′); ^{13}C NMR (pyridine-d_5) δ 135.5 (CH, C-1′), 102.5 (CH, C-1″), 78.7 (CH, C-3″), 78.4 (CH, C-5″), 75.2 (CH, C-2″), 71.9 (CH, C-4″), 67.4 (CH, C-2′), 65.6 (CH_2, C-1), 62.9 (CH_2, C-6″), 39.1 (CH_2, C-3′), 35.1 (CH_2, C-3), 32.2 (CH_2, C-14 and C-14′), 30.2−29.7 (several CH_2, alkyl chains), 28.2 (CH_2, C-4), 26.1 (CH_2, C-4′), 23.0 (CH_2, C-15 and C-15′), 14.3 (CH_3, C-16 and C-16′); HRESIMS *m*/*z* 665.5312 ($[M + Na]^+$, calcd for $C_{38}H_{74}NaO_7$ 665.5327).

Mixture of isoclathroside B (6a) and isoclathroside C (7a): colorless oil; ^1H NMR (500 MHz, pyridine-d_5), same spectrum as **5a**, except for the methyl region, δ 0.86 (6H, m); HRESIMS *m*/*z* 679.5505 ($[M + Na]^+$, calcd for $C_{39}H_{76}NaO_7$ 679.5483).

Oxidative Cleavage of Clathroside A (2a) and Isoclathroside A (5a). Compound **2a** (100 μg) was subjected to oxidative cleavage with $KMnO_4$/$NaIO_4$ as described,[8] and the resulting carboxylic acids were methylated with CH_2N_2. After removal of the solvent, the residue was analyzed by GC-MS and shown to be composed only of methyl pentadecanoate (t_R = 15.08), identified by comparison of its retention time and mass spectrum with those of an authentic sample. Oxidation of isoclathroside A (**5a**) gave the same results.

Oxidative Cleavage of Clathrosides B (3a) and C (4a). A small portion of the mixture of compounds **3a** and **4a** (100 μg) was subjected to oxidative cleavage with $KMnO_4$/$NaIO_4$ as described above. GC-MS analysis of the resulting methyl esters mixture showed it to be composed of methyl pentadecanoate (t_R = 15.08, 49.8%), methyl 14-methylpentadecanoate (t_R = 17.10, 9.5%), and methyl 13-methylpentadecanoate (t_R = 17.34, 40.7%). All the esters were identified by comparison of its retention time and mass spectrum with those of an authentic sample.

Oxidative Cleavage of Isoclathrosides B (6a) and C (7a). A small portion of the mixture of compounds **6a** and **7a** (100 μg) was subjected to oxidative cleavage with $KMnO_4$/$NaIO_4$ as described above. GC-MS analysis of the resulting methyl esters mixture showed it to be composed of methyl pentadecanoate (t_R = 15.12, 53.4%), methyl 14-methylpentadecanoate (t_R = 17.15, 9.7%), and methyl 13-methylpentadecanoate (t_R = 17.39, 36.9%).

Clathroside A Perbenzoate (2d). Clathroside A (**2a**, 400 μg) was benzoylated with benzoyl chloride (10 μL) in pyridine (200 μL) at 25 °C for 16 h. The reaction was then quenched with MeOH and after 30 min was dried under nitrogen. Methyl benzoate was removed by keeping the residue under vacuum for 24 h with an oil pump, and the crude reaction product was purified by HPLC (column: Luna SiO_2, 5 μ; eluent: *n*-hexane/*i*-PrOH (99:1), flow 1 mL/min, λ 280 nm), giving pure clathroside A perbenzoate (**2d**): ^1H NMR ($CDCl_3$) δ 8.03 (2H, d, J = 7.8 Hz, benzoyl *o*-protons), 7.99 (4H, d, J = 7.8 Hz, benzoyl *o*-protons), 7.87 (2H, d, J = 7.8 Hz, benzoyl *o*-protons), 7.82 (2H, d, J = 7.8 Hz, benzoyl *o*-protons), 7.57−7.26 (15H, m, benzoyl protons), 5.85 (1H, t, J = 9.7 Hz, H-3″), 5.66−5.53 (3H, m, H-2′, H-2″, and H-4″), 5.39 (1H, br d, J = 9.0 Hz, H-1′), 4.80 (1H, d, J = 7.9 Hz, H-1″), 4.47 (1H, dd, J = 12.2 and 3.0 Hz, H-6″a), 4.35 (1H, dd, J = 12.2 and 5.6 Hz, H-6″b), 4.29 (1H, br d, J = 12.4 Hz, H-1a), 4.08 (1H, br d, J = 12.4 Hz, H-1b), 3.90 (1H, m, H-5″), 2.11 (1H, m, H-3a), 1.92 (1H, m, H-3b), 1.70 (1H, m, H-3′a), 1.49 (1H, m, H-3′b).

Isoclathroside A perbenzoate (5d) was prepared as described above: ^1H NMR ($CDCl_3$) δ 8.04 (2H, d, J = 7.8 Hz, benzoyl *o*-protons), 7.99 (2H, d, J = 7.8 Hz, benzoyl *o*-protons), 7.94 (4H, d, J = 7.8 Hz, benzoyl *o*-protons), 7.90 (2H, d, J = 7.8 Hz, benzoyl *o*-protons), 7.82 (2H, d, J = 7.8 Hz, benzoyl *o*-protons), 7.60−7.26 (15H, benzoyl protons), 5.91 (1H, t, J = 9.7, H-3″), 5.68 (1H, t, J = 9.7 Hz, H-4″), 5.56 (1H, dd, J = 9.7 and 8.0 Hz, H-2″), 5.53 (1H, m, H-2′), 5.31 (1H, br d, J = 9.3 Hz, H-1′), 4.94 (1H, d, J = 8.1 Hz, H-1″), 4.72 (1H, dd, J = 12.2 and 2.8 Hz, H-6″a), 4.69 (1H, br d, J = 12.9 Hz, H-1a), 4.52 (1H, dd, J = 12.2 and 5.2 Hz, H-6″b), 4.45 (1H, m, H-5″), 4.18 (1H, br d, J = 12.9 Hz, H-1b), 1.91 (1H, m, H-3a), 1.75 (1H, m, H-3b), 1.65 (1H, m, H-3′a), 1.50 (1H, m, H-3′b).

Clathroside A 2′-*O*-benzoate (2e). Compound **2d** was subjected to basic methanolysis by keeping it in 0.5 mL of MeOH/Et_3N (8:2) at 55 °C for 18 h. Removal of the solvent and HPLC purification (column: SiO_2, eluent: *n*-hexane/*i*-PrOH (85:15), flow 1 mL/min, λ 280 nm) gave pure compound **2e**: CD (MeOH) λ_{max} = 227 nm ($\Delta\epsilon$ = +5.1); ^1H NMR ($CDCl_3$) δ 8.03 (2H, br d, J = 7.8 Hz, benzoyl *o*-protons), 7.55 (1H, br t, J = 7.5 Hz, benzoyl *p*-proton), 7.43 (2H, t, J = 7.6 Hz, benzoyl *m*-protons), 5.73 (1H, m, H-2′), 5.51 (1H, br d, J = 9.2 Hz, H-1′), 4.27−4.35 (2H, m, H-1a and H-1″), 4.04 (1H, br d, J = 12.9 Hz, H-1b), 3.85 (1H, dd, J = 12.0 and 3.3 Hz, H-6″a), 3.75 (1H, dd, J = 12.0 and 5.1 Hz, H-6″b), 3.60−3.47 (2H, m, H-3″ and H-4″), 3.38 (1H, t, J = 8.7 Hz, H-2″), 3.30 (1H, m, H-5″).

Isoclathroside A 2′-*O*-benzoate (5e) was prepared as described above: CD (MeOH) λ_{max} = 228 nm ($\Delta\epsilon$ = −5.5); ^1H NMR ($CDCl_3$) δ 8.02 (2H, br d, J = 7.8 Hz, benzoyl *o*-protons), 7.55 (1H, br t, J = 7.6 Hz, benzoyl *p*-proton), 7.43 (2H, br t, J = 7.8 Hz, benzoyl

m-protons), 5.90 (1H, m, H-2′), 5.43 (1H, br d, $J = 9.5$ Hz, H-1′), 4.40 (1H, d, $J = 7.8$, H-1″), 4.35 (2H, br s, H-1a and H-1b), 3.95 (1H, m, sugar proton), 3.82 (1H, m, sugar proton), 3.59 (2H, m, sugar protons), 3.48 (1H, m, sugar proton), 3.35 (1H, m, H-2″).

Absolute Configuration of Glucose. Clathroside A 2′-*O*-benzoate (**2e**) was subjected to acidic methanolysis with 1 M HCl/MeOH. The reaction mixture was kept for 12 h at 80 °C in a sealed tube, then dried under nitrogen and partitioned between $CHCl_3$ and H_2O/MeOH (8:2). The aqueous layer, containing methyl glycosides, was dried and subsequently benzoylated with benzoyl chloride (10 μL) in pyridine (200 μL) at 25 °C for 16 h. The reaction was then quenched with MeOH and after 30 min was dried under nitrogen. Methyl benzoate was removed by keeping the residue under vacuum for 24 h with an oil pump. The residue was purified by HPLC (column: SiO_2; eluent: *n*-hexane/*i*-PrOH (99:1), flow 1 mL/min). The chromatogram contained one peak ($t_R = 11.8$ min), which was identified as methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (**8**) by comparison of its ^{1}H NMR and CD spectra with those of an authentic sample.[5] The procedure was repeated using isoclathroside A 2′-*O*-benzoate (**5e**) as substrate, with the same results.

Acknowledgment. The authors are grateful to Prof. J. R. Pawlik, UNCW, for inviting them to participate in the third "Pawlik Expedition" under the NSF project grant entitled "Assessing the Chemical Defenses of Caribbean Invertebrates", during which the material was collected, and to Prof. M. Pansini, University of Genova, for the sponge taxonomy determination. Mass and NMR spectra were recorded at the "Centro di Servizi Interdipartimentale di Analisi Strumentale", Università di Napoli "Federico II". The assistance of the staff is gratefully acknowledged. This research project is funded by the Italian Government, MIUR PRIN (Italy).

Supporting Information Available: 1-D and 2-D NMR spectra of clathroside A peracetate (**2b**) and isoclathroside A peracetate (**5b**), ^{1}H and ^{13}C NMR spectra of clathroside A (**2a**) and isoclathroside A (**5a**), ^{1}H NMR spectra of compounds **2d**, **2e**, **3b**/**4b**, **5d**, **5e**, and **6b**/**7b**, and CD spectra of compounds **2e**, **5e**, and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP050331V