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Calyceramides A–C: neuraminidase inhibitory sulfated ceramides from the marine sponge *Discodermia calyx*☆

Yoichi Nakao, Kentaro Takada, Shigeki Matsunaga and Nobuhiro Fusetani*

Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

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Abstract—Three new sulfated ceramides, calyceramides A–C (**1**–**3**) were isolated as inhibitors of neuraminidase from the marine sponge *Discodermia calyx*. Their structures were determined by spectroscopic and chemical methods. The ceramides inhibited neuraminidase with IC_{50} values of 0.2–0.8 μg mL^{-1}.

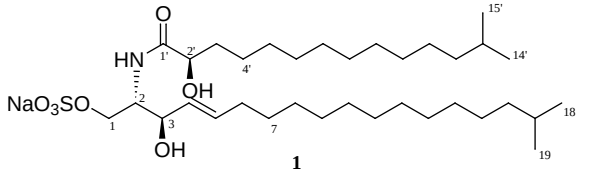
1. Introduction

Neuraminidase cleaves an α-linked terminal *N*-acetylneuraminic acid from glycoproteins, glycolipids, and oligosaccharide.[2] In several viral and bacterial infections, neuraminidase is known to play important roles; influenza virus employs this enzyme to detach itself from the infected cell in the budding stage, thus indicating requirement of neuraminidase for replication of the virus. Therefore, selective inhibitors of neuraminidase are potential therapeutic agents against influenza.[3] In fact, neuraminidase inhibitors, such as zanamivir (GG167)[4] and oseltamirvir (GS4104),[5] which were designed on the basis of the crystal structure of the enzyme–substrate complex, showed a broader antiviral spectra, better tolerance, and induce less resistance than M2 inhibitors such as amantadine and rimantidine.[6]

In our research program to explore potential drug leads from Japanese marine invertebrates, we found significant inhibitory activity against neuraminidase in the hydrophilic extract of the marine sponge *Discodermia calyx* collected off Sikine-jima Island. Bioassay-guided separation of the extract led to the isolation of three active compounds, calyceramides A–C, which were elucidated as sulfated ceramides by spectral and chemical methods. This paper deals with isolation and structure elucidation of those inhibitors.

2. Results and discussion

The combined MeOH and EtOH extracts of the frozen sponge were partitioned between CH_2Cl_2 and H_2O. The aqueous layer was further extracted with *n*-BuOH. The CH_2Cl_2 layer was chromatographed on silica gel, while the *n*-BuOH layer was separated by ODS flash chromatography. The active fractions from each chromatography were combined and purified by repeated reversed-phase HPLC to afford calyceramides A (**1**, 3.0 mg, 2.8×10^{-7}% yield based on wet weight), B (**2**, 1.5 mg, 1.4×10^{-7}%), and C (**3**, 1.5 mg, 1.4×10^{-7}%).



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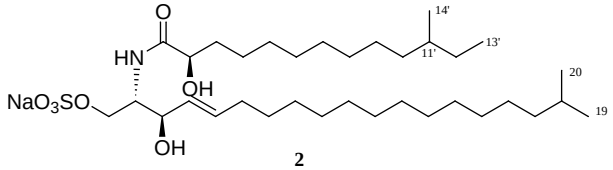

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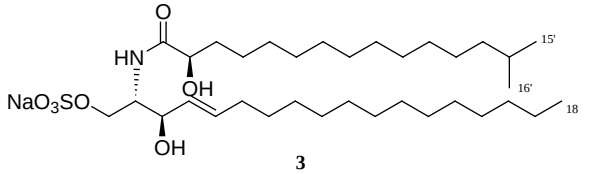

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Calyceramide A (**1**) had a molecular formula of $C_{34}H_{66}NO_7SNa$ which was determined by high-resolution FABMS (*m/z* 632.4590 (Δ −2.1 mmu)). The IR spectrum exhibited bands attributable to sulfate (1230 cm^{-1}) and amide (1644 cm^{-1}) functionalities. The presence of a sulfate group was also supported by the ion peaks at *m/z* 97 $(HOSO_3)^-$ and 80 $(SO_3)^-$ in FABMS. The ^1H NMR spectrum revealed the presence of four secondary methyls at δ

☆ Part 106 of the Bioactive Marine Metabolites series.[1]

Keywords: biologically active compounds; enzyme inhibitors; marine metabolites; sponges.

* Corresponding author. Tel.: +81-3-5841-5299; fax: +81-3-5841-8166; e-mail: anobu@mail.ecc.u-tokyo.ac.jp

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Table 1. NMR data of calyceramide A (**1**) in CD_3OD

C/H No.	δ_C	δ_H (J in Hz)	^1H–^1H COSY	HMBC
1a	73.1	4.15, dd (10.4, 3,4)	H-1b,	C-2, C-3
1b		4.26, dd (10.4, 4.6)	H-1a, H-2	
2	53.9	4.01, ddd (8.1, 4.6, 3.4)	H-1b	C-1, C-3, C-1′
3	72.8	4.12, dd (8.1, 7.3)	H-4	C-2
4	130.6	5.45, dd (15.0, 7.3)	H-3, H-5	C-3, C-6
5	135.4	5.72, dt (15.0, 6.9)	H-4, H-6	C-3, C-6, C-7
6	34.3	2.02, dt (7.3, 6.9)	H-5, H-7	C-4, C-5, C-7
7	30.4	1.34, m	H-6	C-6, C-8
8–15	30–32	1.2–1.4		
16	29.1	1.16, m	H-15, H-17	C-15, C-17, C-18, C-19
17	40.2	1.52, m	H-16, H-18, H-19	C-15, C-16, C-18, C-19
18	23.0	0.87, d (6.5)	H-17	C-16, C-17
19	23.0	0.87, d (6.5)	H-17	C-16, C-17
1′	177.1			
2′	72.9	3.97, dd (8.1, 3.9)	H-3′a, H-3′b	C-1′
3′a	35.8	1.52, m	H-2′, H-3′b	C-2′, C-4′
3′b		1.69, m	H-2′, H-3′a	
4′–11′	30–32	1.2–1.4		
12′	28.5	1.16, m	H-11′, H-13′	C-11′, C-13′, C-14′, C-15′
13′	40.2	1.52, m	H-12′, H-14′, H-15′	C-11′, C-12′, C-14′, C-15′
14′	23.0	0.87, d (6.5)	H-13′	C-12′, C-13′
15′	23.0	0.87, d (6.5)	H-13′	C-12′, C-13′

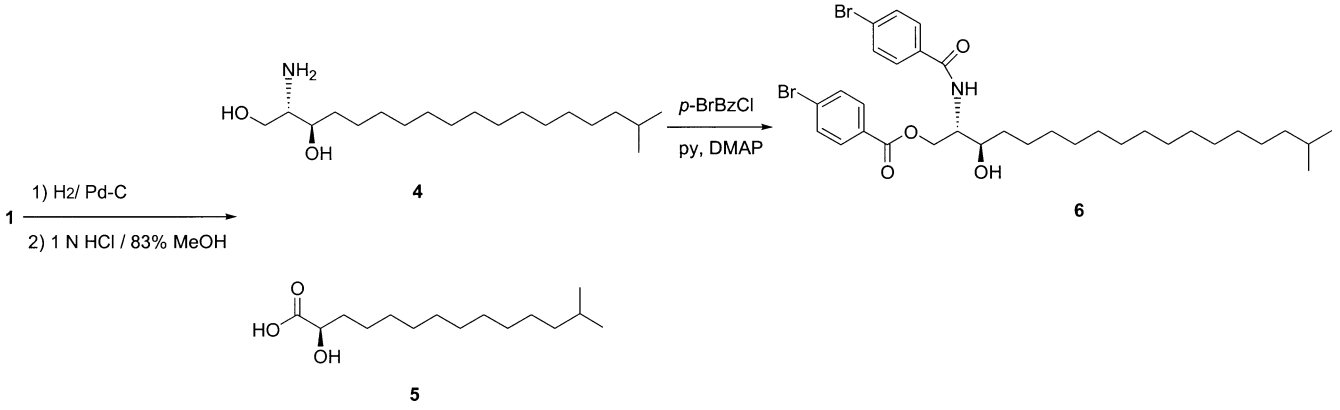
0.87 (12H), three heteroatom-bearing methines at δ 3.97, 4.01, and 4.12, and an oxygenated methylene protons at δ 4.15 and 4.26, two olefinic protons at δ 5.45 and 5.72, and a huge methylene envelope at δ 1.2–1.4 (Table 1). Interpretation of the ^1H–^1H COSY and HMQC[7] spectra resulted in two partial structures (C-1 to C-7 and C-2′ to C-4′), both of which were connected to long aliphatic chains. An HMBC[8] correlation between H-2 and C-1′ not only confirmed the position of this nitrogen atom, but also connected the two partial structures through an amide bond.

To determine the length of each alkyl chain, calyceramide A (**1**) was hydrogenated followed by methanolysis[9,10] to afford sphingosine **4** and α-hydroxy fatty acid **5**. The former was converted to the bis-*p*-bromobenzoate **6** whose molecular formula was deduced to be $C_{33}H_{47}Br_2NO_4$ on the basis of FABMS data, thus implying that the sphingosine had 19 carbons, while the α-hydroxy acid had 15 carbons (Scheme 1).

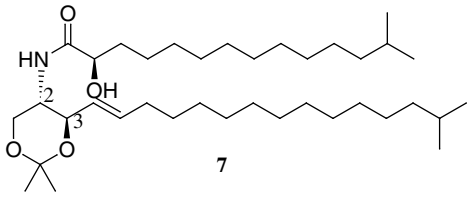
The relative stereochemistry at C-2 and C-3 was determined as follows. Calyceramide A (**1**) was treated with 2,2-dimethoxypropane and a catalytic amount of *p*-TsOH in CH_2Cl_2 to afford acetonide **7**.[9] The *anti*-relationship of C-2 and C-3 in **7** was inferred from the coupling constant between H-2 and H-3 (J=8.6 Hz) as well as from ROESY[11] cross peaks between H-3/H_3-21 and H-1b/H_3-21 (Fig.1).

Absolute configuration of calyceramide A (**1**) was determined by the application of the modified Mosher's method.[12] Treatment of **1** with (*S*)-(+)- and (*R*)-(−)-MTPACl in CH_2Cl_2/pyridine (1:1) to yield the corresponding 3,2′-bis-MTPA esters. The distribution of positive and negative $\Delta\delta$ ($\delta_S-\delta_R$) values around the MTPA esters implied *R*-stereochemistry for both C-3 and C-2′ (Fig. 2). Therefore, the stereochemistry at C-2 was *S*.

Calyceramide B (**2**) had the molecular formula same as that of **1**. Although the low-field region of the ^1H NMR spectrum was superimposable on that of **1**, their methyl signals were apparently different; one of the four doublet methyls in **1** was replaced by a triplet one. Further analysis of the 2D NMR spectra indicated that **2** had an iso- and an anteiso-terminus. To determine their structures, calyceramide B (**2**) was hydrogenated followed by acid hydrolyzed, and the resulting sphingosine moiety was converted to bis-*p*-bromobenzoate **8**. FABMS and ^1H NMR data of **8** were consistent with the C_{20} sphingosine with an iso-terminus, thereby



Scheme 1.



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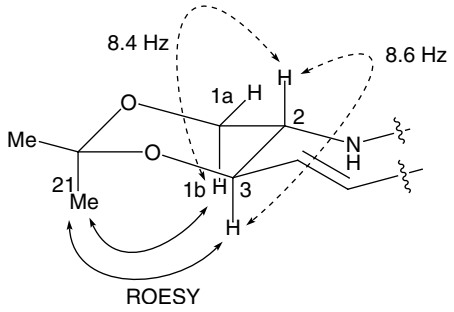


Figure 1. Coupling constants and ROESY correlations of acetonide **7**.

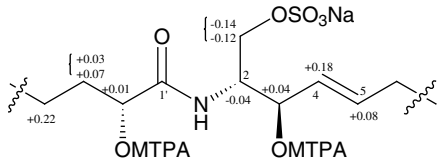
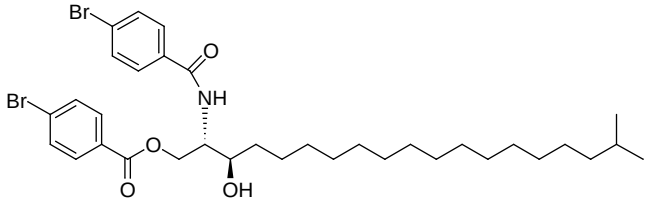


Figure 2. $\Delta\delta$ values for the MTPA esters of **1**.

disclosing that the fatty acid portion was an α-hydroxyl C_{14} acid with an anteiso-terminus. The absolute stereochemistry was determined to be 2*S*, 3*R*, 2′*R* on the basis of both ^{1}H NMR data of the acetonide and the modified Mosher's method as in the case of **1**. The stereochemistry at C-11′ remains to be determined.



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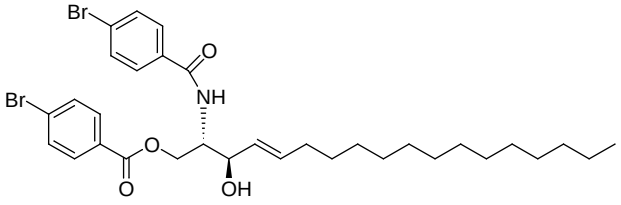

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The molecular formula of calyceramide C (**3**) was also identical with that of **1**. The ^{1}H NMR spectrum contained two doublet [δ 0.86, (6H)] and one triplet [δ 0.88, (3H)] methyl signals, indicating the iso-terminus of either sphingosine or fatty acid portion. Bis-*p*-bromobenzoate **9** derived from **3** exhibited a triplet methyl signal at δ 0.83 in the ^{1}H NMR spectrum, while its FABMS data indicated the presence of a C_{18}-sphingosine portion. Accordingly, the fatty acid portion was α-hydroxy C_{16} iso acid. The 2*S*, 3*R*, 2′*R*-stereochemistry was deduced for **3** from the result of the modified Mosher's analysis and comparison of ^{1}H NMR data with that of **1**.

3. Biological activity

Calyceramides A–C inhibited neuraminidase from the bacterium *Clostridium perfrigens* with IC_{50} values of 0.4, 0.2 and 0.8 μg mL^{-1}, respectively, while the known inhibitor 4-acetylneuraminic acid showed only weak activity in our assay (IC_{50} 1.5 mg mL^{-1}).

4. Conclusions

Three new ceramide 1-sulfates named calyceramides A–C were isolated as neuraminidase inhibitors from the marine sponge *D. calyx*. Their structures were determined by spectroscopic and chemical methods. Our compounds were closely related to ceramide 1-sulfates isolated from the bryozoan *Watersipora cucullata*[9] as inhibitors of DNA topoisomerase I. It is of interest that calyceramides A–C inhibited neuraminidase significantly.

5. Experimental

5.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a JEOL A600 NMR spectrometer. ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent peaks: δ_H 3.30 and δ_C 49.0 for CD_3OD, δ_H 2.04 and δ_C 29.8 for acetone-d_6, δ_H 7.24 and δ_C 77.0 for $CDCl_3$. Optical rotation was measured on a JASCO DIP-1000 digital polarimeter in MeOH. FAB mass spectra were obtained on a JEOL SX102/SX102 tandem mass spectrometer using triethanolamine or *m*-nitrobenzylalcohol as the matrices. IR spectra were recorded on a JASCO FT/IR-5300 spectrometer. Fluorescence for inhibition assay was measured with a Molecular Device Spectra MAX GEMINI apparatus.

5.2. Collection and isolation

Sponge samples were collected by hand using SCUBA at depths of 15–20 m off Sikine-jima Island, 200 km south of Tokyo, immediately frozen, and kept frozen at −20°C until processed. The frozen sponges (10 kg) were homogenized and extracted with MeOH and EtOH. The combined extracts were concentrated and partitioned between CH_2Cl_2 and H_2O. The aqueous layer was further extracted with *n*-BuOH. The CH_2Cl_2 layer was chromatographed on silica gel with a $CHCl_3$/MeOH system, while the *n*-BuOH extract was chromatographed on an ODS column with an aq. MeOH system. The active fractions obtained from both columns were combined and separated by repeated reversed-phase HPLC (Cosmosil 5C_{18}-AR II ϕ 20×250 mm). Final purification was done by recycling reversed-phase HPLC (Develosil C_{30}-VG5, ϕ 20×250 mm) with 96% MeOH containing 0.3 M $NaClO_4$ to afford calyceramides A (**1**, 3.0 mg, 2.8×10^{-7}% yield

based on wet weight), B (**2**, 1.5 mg, 1.4×10^{-7}%), and C (**3**, 1.5 mg, 1.4×10^{-7}%).

5.2.1. Calyceramide A (1). Colorless solid; $[\alpha]_D^{20}=+24.8°$ (*c* 0.10, MeOH); IR (film) 3850, 3563, 1644, 1540, 1472, 1230, 1102 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFABMS (neg.) *m/z* 632.4539 $(M-Na)^-$ [$C_{34}H_{66}NO_7S$ (Δ −2.1 mmu)]

5.2.2. Calyceramide B (2). Colorless solid; $[\alpha]_D^{20}=+14.5°$ (*c* 0.10, MeOH); IR (film) 3600, 3100, 1732, 1651, 1540, 1466, 1377, 1033 cm^{-1}; ^1H NMR (CD_3OD) δ 5.72 (1H, dt, *J*=15.4, 6.6 Hz, H-5), 5.45 (1H, dd, 15.4, 7.7, H-4), 4.27 (1H, dd, 10.4, 5.0, H-1b), 4.11 (1H, dd, 10.4, 3.8, H-1a), 4.10 (1H, dd, 8.1, 7.7, H-3), 4.01 (1H, ddd, 8.1, 5.0, 3.4, H-2), 3.97 (1H, dd, 8.1, 3.8, H-2′), 2.02 (2H, dt, 7.7, 6.6, H-6), 1.69 (1H, m, H-3′b), 1,52 (1H, m, H-3′a), 1.52 (2H, m, H-18, H-11′), 1.38 (2H, m, H-7), 1.2–1.4 (m, H-8-16, H-4′-10′), 1.16 (4H, m, H-17, H-12′), 0.87 (6H, d, 6.5, H-19, H-20), 0.86 (3H, t, 7.1, H-13′), 0.85 (3H, d, 6.5, H-14′); HRFABMS (neg.) *m/z* 632.4590 $(M-Na)^-$ [$C_{34}H_{66}NO_7S$ (Δ +3.0 mmu)]

5.2.3. Calyceramide C (3). Colorless solid; $[\alpha]_D^{20}=+16.9°$ (*c* 0.10, MeOH); IR (film) 3600, 3100, 1732, 1651, 1538, 1466, 1377, 1217, 1024 cm^{-1}; ^1H NMR (CD_3OD) δ 5.72 (1H, dt, *J*=15.4, 6.6 Hz, H-5), 5.44 (1H, dd, 15.4, 7.5, H-4), 4.25 (1H, dd, 10.4, 4.8, H-1b), 4.11 (1H, dd, 10.4, 3.4, H-1a), 4.10 (1H, dd, 8.1, 7.5, H-3), 4.01 (1H, ddd, 8.1, 4.8, 3.4, H-2), 3.97 (1H, dd, 8.1, 3.8, H-2′), 2.02 (2H, dt, 7.3, 6.6, H-6), 1.69 (1H, m, H-3′b), 1,52 (1H, m, H-3′a), 1.52 (1H, m, H-14′), 1.38 (2H, m, H-7), 1.2–1.4 (m, H-8-17, H-4′-12′), 1.16 (2H, m, H-13′), 0.88 (3H, t, 6.9, H-18), 0.86 (6H, d, 6.6, H-15′, H-16′); HRFABMS (neg.) *m/z* 632.4558 $(M-Na)^-$ [$C_{34}H_{66}NO_7S$ (Δ −0.2 mmu)]

5.2.4. Hydrogenation and acid hydrolysis of 1. Methanol solution of calyceramide A (**1**, 0.4 mg in 0.4 mL) was stirred under the atmosphere of H_2 over 10% Pd–C (catalytic amounts) for 4 h at room temperature to give a hydrogenated derivative, which showed an $(M-Na)^-$ peak at *m/z* 634 in FABMS (neg.). This was treated with 1N HCl in 83% MeOH at 70°C for 18 h. The reaction mixture was extracted with *n*-hexane. To the aqueous alcoholic layer were added $CHCl_3$ and H_2O, and the mixture was shaken. The organic phase was evaporated to furnish sphingosine **4**.

5.2.5. Bis-*p*-bromobenzoate 6. *p*-Bromobenzoylchloride (0.5 mg) and DMAP (catalytic amounts) were added to a solution of **4** in CH_2Cl_2/pyridine (1:1, 200 μl), and the mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The organic layer was evaporated and purified by normal phase HPLC (Cosomosil 5SL-2, ϕ 10×250 mm) with $CHCl_3$ to yield **6**, which showed 1:2:1 $(M+H)^+$ peaks at *m/z* 680, 682, 684 in FABMS (pos.).

5.2.6. Acetonide 7. A mixture of **1** (0.8 mg), 2,2-dimethoxypropane (50 μl) and *p*-TsOH (catalytic amounts) in CH_2Cl_2 (100 μl) was stirred at room temperature for 3 h. After addition of triethylamine (5 μl) and H_2O (2 mL), the reaction mixture was extracted with Et_2O (3×2 mL). The Et_2O extract was evaporated and chromatographed on a silica gel column with $CHCl_3$/MeOH (199:1) to yield acetonide **7**.

^1H NMR (acetone-d_6) δ 5.71 (1H, dt, *J*=15.0, 6.9 Hz, H-5), 5.34 (1H, dd, 15.0, 6.9, H-4), 4.32 (1H, dd, 8.6, 6.9, H-3), 3.94 (1H, dd, 7.3, 3.8, H-2′), 3.79 (1H, m, H-2), 3.77 (1H, dd, 8.4, 4.6 H-1b), 3.68 (1H, m, H-1a), 1.99 (2H, m, H-6), 1.66 (1H, m, H-3′b), 1,51 (1H, m, H-3′a), 1.34 (2H, m, H-7); FABMS *m/z* 592 $(M+H)^+$ (pos.).

5.2.7. Bis-*p*-bromobenzoate 8. Calyceramide B (0.4 mg) was hydrogenated and converted to bis-*p*-bromobenzoate as the same manner as that of **1**. FABMS (pos.) *m/z* 694, 696, and 698 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 7.82, 7.78, 7.54, 7.49, 4.59 (H-1b), 4.57 (H-1a), 4.34 (H-2), 4.13 (H-3), 1.33, 1.22, 1.12, 0.83.

5.2.8. Bis-*p*-bromobenzoate 9. Calyceramide C (0.4 mg) was acid hydrolyzed without prior hydrogenation to afford sphingosine. The sphingosine was converted to bis-*p*-bromobenzoate as the same manner as that of **1**. FABMS (pos.) *m/z* 664, 666, and 668 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 7.83, 7.78, 7.54, 7.50, 5.75 (H-5), 5.52 (H-4), 4.58 (H-1b), 4.56 (H-1a), 4.35 (H-2), 4.13 (H-3), 1.33, 1.22, 1.12, 0.84.

5.2.9. 3,2′-Bis-(*R*)-MTPA ester of calyceramide A (1). A mixture of **1** (0.2 mg) and (*S*)-(+)-MTPACl (10 μl) in CH_2Cl_2/pyridine (1:1, 40 μl) was stirred at room temperature for 30 min. The reaction mixture was diluted with H_2O (3 mL) and extracted with $CHCl_3$ (3×3 mL). The organic layer was concentrated under reduced pressure to furnish the 3,2′-bis-(*R*)-MTPA ester, which showed an $(M+2MTPA-H)^-$ peak at *m/z* 1064 in FABMS (neg.). ^1H NMR (CD_3OD) δ 5.83 (1H, dt, *J*=15.8, 6.6 Hz, H-5), 5.55 (1H, dd, H-3), 5.26 (1H, dd, 15.8, 8.9, H-4), 5.12 (1H, dd, H-2′), 4.31 (1H, m, H-2), 4.10 (1H, dd, 10.5, 5.0, H-1b), 4.06(1H, dd, 10.5, 3.5, H-1a), 1.78 (1H, m, H-3′b), 1.41 (1H, m, H-3′a), 1.18 (1H, m, H-4a′).

5.2.10. 3,2′-Bis-(*S*)-MTPA ester of calyceramide A (1). 3,2′-Bis-(*S*)-MTPA ester of **1** was prepared as described above. ^1H NMR (CD_3OD) δ 5.91 (1H, dt, *J*=15.0, 6.9 Hz, H-5), 5.59 (1H, dd, H-3), 5.44 (1H, dd, 15.0, 8.6, H-4), 5.13 (1H, dd, H-2′), 4.27 (1H, m, H-2), 3.98 (1H, dd, 10.5, 3.4, H-1b), 3.92 (1H, dd, 10.5, 5.8, H-1a), 1.85 (1H, m, H-3b′), 1.44 (1H, m, H-3a′), 1.40 (1H, m, H-4a′); FABMS *m/z* 1064 $(M+2MTPA-H)^-$.

5.2.11. 3,2′-Bis-(*R*)-MTPA ester of calyceramide B. ^1H NMR (CD_3OD) δ 5.83, 5.55, 5.26, 5.12 4.31, 4.10, 4.06, 1.78, 1.41, 1.18.

5.2.12. 3,2′-Bis-(*S*)-MTPA ester of calyceramide B. ^1H NMR (CD_3OD) δ 5.91, 5.59, 5.44, 5.13, 4.27, 3.98, 3.92, 1.85, 1.44, 1.40.

5.2.13. 3,2′-Bis-(*R*)-MTPA ester of calyceramide C. ^1H NMR (CD_3OD) δ 5.83, 5.55, 5.26, 5.12 4.31, 4.10, 4.06, 1.78, 1.41, 1.18.

5.2.14. 3,2′-Bis-(*S*)-MTPA ester of calyceramide C. ^1H NMR (CD_3OD) δ 5.91, 5.59, 5.44, 5.13, 4.27, 3.98, 3.92, 1.85, 1.44, 1.40.

5.3. Enzyme inhibition assay

Neuraminidase inhibitory activity was examined using neuraminidase from the bacterium *Clostridium perfringens* (Sigma N-2876). The substrate, 4.45 mM 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (M-8639) in 0.1 M NaOAc buffer (pH 4.2) (90 μl) was added to the mixture of 2.25×10^{-3} unit of the enzyme in the same buffer (90 μl) and a sample in DMSO (20 μl); the mixture was incubated at 37°C for 30 min. The amounts of 4-methylumbelliferone released was measured by fluorescence at 450 nm by an excitation at 360 nm.[13]

Acknowledgements

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References

1. Part 105: Fujita, M.; Nakao, Y.; Itoh, Y.; Seiki, M.; Soest, R. W. M.; Fusetani, N. *Tetrahedron* **2001**, *57*, 1229–1234.
2. Shauer, R. *Trends Biochem. Sci.* **1985**, *10*, 357–360.
3. (a) Gubareva, L. V.; Kaiser, L.; Hayden, F. G. *Lancet* **2000**, *355*, 827–835. (b) Air, G. M.; Ghate, A. A.; Stray, S. J. *Adv. Virus Res.* **1999**, *54*, 375–402.
4. von Itzstein, M.; Wu, W. Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Van Phan, T.; Synthe, M. L.; White, H. F.; Oliver, S. W.; Coleman, P. G.; Varghese, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418–423.
5. Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C. *J. Am. Chem. Soc.* **1997**, *119*, 681–690.
6. Hay, A. J.; Wolstenholme, A. J.; Skehel, J. J.; Smith, M. H. *EMBO. J.* **1985**, *4*, 3021–3024.
7. Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285–4294.
8. Bax, A.; Azolos, A.; Dinya, Z.; Sudo, K. *J. Am. Chem. Soc.* **1986**, *108*, 8056–8063.
9. Ojika, M.; Yoshino, G.; Sakagami, Y. *Tetrahedron Lett.* **1997**, *38*, 4235–4238.
10. Kobayashi, J.; Mikami, S.; Shigemori, H.; Takao, T.; Shimonishi, Y.; Izuta, S.; Yoshida, S. *Tetrahedron* **1995**, *51*, 10487–10490.
11. Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811–813.
12. Othani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
13. Hiraiwa, M.; Uda, Y.; Nishizawa, M.; Miyatake, T. *J. Biochem.* **1987**, *101*, 1273–1279.