Bioactive Marine Metabolites. Part 122. Schulzeines A—C, New α-Glucosidase Inhibitors from the Marine Sponge Penares schulzei

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · FEBRUARY 2004

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Schulzeines A−C, New α-Glucosidase Inhibitors from the Marine Sponge *Penares schulzei*[1]

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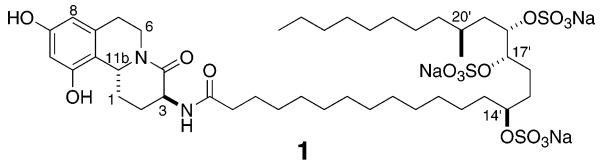
Abstract: Three new α-glucosidase inhibitors, schulzeines A−C (**1**−**3**), were isolated from the marine sponge *Penares schulzei*. Their structures were elucidated by spectral analysis and chemical degradations to be the isoquinoline alkaloids, encompassing two amino acids, and C_{28} fatty acid, the last of which was sulfated. Absolute stereochemistry of schulzeines was determined by application of the modified Mosher analysis to fragments obtained by chemical degradation. Schulzeines A−C inhibit α-glucosidase with IC_{50} values of 48−170 nM.

Introduction

α-Glucosidases not only process protein glycosylation, which is involved in a wide range of biological processes, including promotion of protein folding in the endoplasmic reticulum and stabilization of cell-surface glycoproteins, but also control oligosaccharide metabolism. Thus, inhibitors of α-glucosidases are potential therapeutics for the treatment of such diseases as viral diseases, cancer, and diabetes.[2−4] In fact, imino sugars, inhibitors of these enzymes, showed considerable promise in the treatment of type B hepatitis, while a nojirimycin analogue has been approved for the treatment of noninsulin-dependent diabetes. In the course of our continuing search for potential drug leads from Japanese marine invertebrates, we have previously reported isolation of penarolide sulfates A_1 and A_2, novel sulfated macrolides encompassing a proline residue, as α-glucosidase inhibitors from a marine sponge *Penares* sp.[5] Subsequently, we found potent α-glucosidase-inhibitory activity in the hydrophilic extract of the marine sponge *Penares schulzei*, and bioassay-guided isolation afforded three new tetrahydroisoquinoline alkaloids named schulzeines A−C (**1**−**3**). Here, we report the isolation, structure elucidation, and biological activity of these new metabolites.



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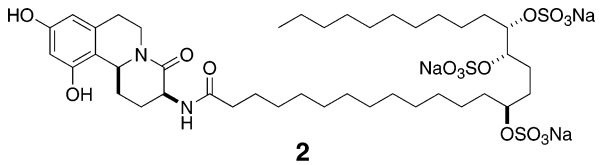

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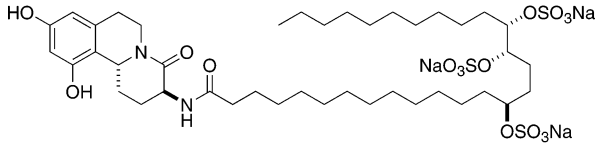

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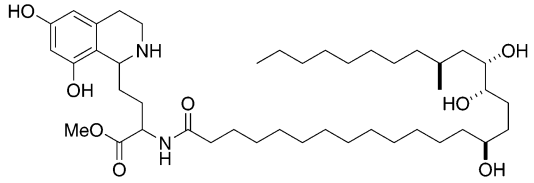

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Results and Discussion

The combined MeOH, EtOH, and acetone extracts of the frozen sponge (600 g, wet weight) were concentrated and partitioned between H_2O and $CHCl_3$. The aqueous layer was further extracted with *n*-BuOH; the *n*-BuOH layer was separated by ODS flash chromatography, ODS MPLC, and repetitive reversed-phase HPLC to afford schulzeines A−C (**1**−**3**).

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(1) Part 122 of the Bioactive Marine Metabolites series. Part 121: Nishimura, S.; Matsunaga, S.; Shibazaki, M.; Suzuki, K.; Furihata, K.; van Soest, R. W. M.; Fusetani, N. *Org. Lett.* **2003**, *5*, 2255−2257.
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Table 1. ^{1}H and ^{13}C NMR Chemical Shift of Schulzeine A (**1**) in CD_3OH and B (**2**) in CD_3OD

	schulzeine A (**1**)		schulzeine B (**2**)	
	^{1}H	^{13}C	^{1}H	^{13}C
1α	1.39[a]	29.3	1.39[c]	29.0
1β	3.06 (dq, 11.9, 3.5)		2.55 (m)	
2α	2.10 (m)	28.3	1.55 (m)	26.0
2β	1.93 (m)		2.26 (m)	
3	4.28 (dt, 11.8, 7.5)	51.9	4.63[d]	49.8
3-NH	8.21 (d, 8.1)			
4		170.4		171.8
6α	4.79[b]	40.6	4.62[d]	40.4
6β	2.62 (dt, 12.3, 2.3)		2.72 (m)	
7α	2.69 (dt, 2.3, 15.4)	30.9	2.60 (dt, 2.5, 12.3)	30.5
7β	2.52 (dt, 15.4, 2.3)		2.71 (m)	
7a		138.4		138.4
8	6.07 (d, 2.3)	107.5	6.11 (d, 2.3)	107.3
9		157.6		157.9
10	6.17 (d, 2.3)	102.2	6.18 (d, 2.3)	101.9
11		156.6		156.1
11a		115.8		115.0
11b	4.77[b]	57.0	4.84 (dd, 11.1, 3.8)	51.6
1′		176.0		176.1
2′	2.22 (dt, 4.2, 7.5)	37.0	2.27 (dt, 2.0, 7.7)	37.0
3′	1.61 (m)	26.7	1.61 (m)	26.8
4′−11′	1.3[a]	30[b]	1.3[c]	30[b]
12′	1.40 (m)	25.8	1.40 (m)	25.9
13′	1.71 (m)	35.3	1.72 (m)	35.4
	1.62 (m)		1.62 (m)	
14′	4.35 (dt 10.8, 6.5)	81.1	4.33 (dt, 10.4, 6.2)	81.2
15′	1.95 (m)	31.6	1.95 (m)	31.7
	1.71 (m)		1.70 (m)	
16′	1.94 (m)	25.4	1.94 (m)	25.8
	1.60 (m)		1.59 (m)	
17′	4.70 (dt, 9.6, 3.4)	79.8	4.65[e]	80.0
18′	4.84 (ddd, 11.9, 3.4, 2.5)	77.5	4.64[e]	80.0
19′	1.59 (m)	36.8	1.74 (m)	29.8
	1.40 (m)		1.54 (m)	
20′	1.75 (m)	29.5	1.3[c]	30[b]
21′	1.16 (m)	39.0	1.3[c]	30[b]
	1.30 (m)			
22′−26′	1.3[a]	30[b]	1.3[c]	30[b]
27′	1.28 (m)	23.6	1.28 (m)	23.7
28′	0.87 (t, 6.9)	14.3	0.87 (t, 7.1)	14.4
29′	0.93 (d, 6.5)	19.4		

[a−e] Signals overlapped with each other. [b,d,e] Interpretation of the co-occurring signals was conducted with the data in pyridine-d_5.

Schulzeine A (**1**) had a molecular formula of $C_{42}H_{69}N_2O_{16}S_3Na_3$ established on the basis of NMR and HRFABMS data. The presence of the sulfate group was evident from fragment ions at *m*/*z* 97 (HSO_4^-) and 80 (SO_3^-) in the negative ion mode FABMS, which was supported by an IR band at 1223 cm^{-1}. Initial analysis of NMR data indicated the presence of two carbonyl carbons (δ_C 176.0 and 170.4), six aromatic carbons, two of which were protonated (δ_H 6.17 and 6.07; δ_C 157.6, 156.6, 138.4, 115.8, 107.5, and 102.2), a nitrogenous methylene (δ_H 4.79 and 2.62; δ_C 40.6), two nitrogenous methines (δ_H 4.77 and 4.28; δ_C 57.0 and 51.9), three oxygenated methines (δ_H 4.84, 4.70, and 4.35; δ_C 81.1, 79.8, and 77.5), a methylene envelope (δ_H 1.30−1.20), a secondary methyl (δ_H 0.93), a terminal methyl (δ_H 0.87), and an exchangeable proton (δ_H 8.21) (Table 1).

Interpretation of 2D NMR data[6] gave rise to substructures **a**−**c** (Figure 1A). Substructure **a** was composed of three proton spin systems; interpretation of COSY data starting from the exchangeable proton (3-NH) led to connectivities of 3-NH−C-3−C-2−C-1−C-11b, the last methine (C-11b) linked to a nitrogen atom. The second spin system consisted of an ethylene unit (C-6 and C-7) flanked by a nitrogen atom and an aromatic carbon, which was readily inferred from the ^{1}H and ^{13}C NMR chemical shift values (Table 1). The third spin system was a pair of *meta*-coupled aromatic protons (H-8 and H-10); the UV absorption at 284 nm (ϵ 1390)[7] and ^{13}C NMR chemical shift values were consistent with a *meta*-dihydroxylated benzene ring. HMBC data not only advocated this assignment but also connected the spin systems described above; HMBC cross-peaks H-7/C-7a, C-8, and C-11a and H-8/C-11a connected the nitrogenous ethyl unit to C-7a, while those between H-6/C-11b and H-11b/C-11a were consistent with the presence of a tetrahydroisoquinoline substructure. The chemical shift values of C-9 (δ 157.6) and C-11 (δ 156.6) placed hydroxyl groups at these carbons. Further HMBC correlations, H-6/C-4 and H-3/C-4, allowed the formation of an amide bond between N-5 and C-4 which was in turn connected to C-3 on the basis of an HMBC cross-peak, H-3/C-4, thus indicating that the tetrahydroisoquinoline unit was fused with a δ-lactam. Additional HMBC cross-peaks, 3-NH/C-1′ and H_2-2′/C-1′, together with HOHAHA data, demonstrated the attachment of an acyl group to 3-NH. Analysis of ^{1}H−^{1}H coupling constants and ROESY data established the conformation of the tricyclic portion and the relative stereochemistry at C-3 and C-11b as shown in Figure 1B.

Substructure **b** contains three oxymethine groups and a secondary methyl group. Downfield shifts of the proton and carbon signals of the oxymethines indicated that they were all sulfated, which was in agreement with the molecular formula. The connectivities within this unit were substantiated by analysis of COSY, HOHAHA, and HSQC data. The C-20′-methine proton, which was coupled to a doublet methyl signal, was also correlated with two pairs of methylene protons in the COSY spectrum. One of the methylene pairs (H_2-19′) was correlated with H-18′, which was in turn coupled with another oxymethine (H-17′); H-17′ was correlated with the third oximethine (H-14′) via two methylenes in HOHAHA data. Substructure **c** was a terminal *n*-propyl group. 2D NMR data implied that substructure **b** was accommodated in the middle of a long acyl group which was terminated at substructure **c**, while the carbonyl moiety, the other terminus of the alkyl chain, was formed by an amide linkage with 3-NH of substructure **a**. The location and orientation of substructure **b** in the alkyl chain was determined by analysis of FAB-MS/MS data of **4**, one of the methanolysis products of **1**.[8] Prominent fragment ions observed at *m*/*z* 475, 505, 533, 563, 593, 607, and 635 permitted us to determine the positions of hydroxyl and methyl groups (Figure 1C).

In addition to **4**, methanolysis of **1** afforded **5**, **6**, and **7**, which arose from cleavage of the amide bond in the side chain (Scheme 1). Relative stereochemistry at C-3 in **5** was unaltered from that of **1** as shown by 1D NOE difference experiments; irradiation of H-3 enhanced H-1α (δ 1.39) and H-2α (δ 2.10) signals, while irradiation of H-11b enhanced H-1β (δ 3.06) and H-2β (δ 1.93)

(6) 1D and 2D NMR spectra including COSY, HOHAHA, ROESY, HSQC, and HMBC were obtained in CD_3OD, CD_3OH, and pyridine-d_5 (see Supporting Information).

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(8) While the structure of **1** or **2** was confirmed by extensive analysis of 2D NMR data except for the location of substructure **b** in the side chain, the structural assignment of **4** was carried out by FAB-MS data, which was in full agreement with FAB-MS/MS data.

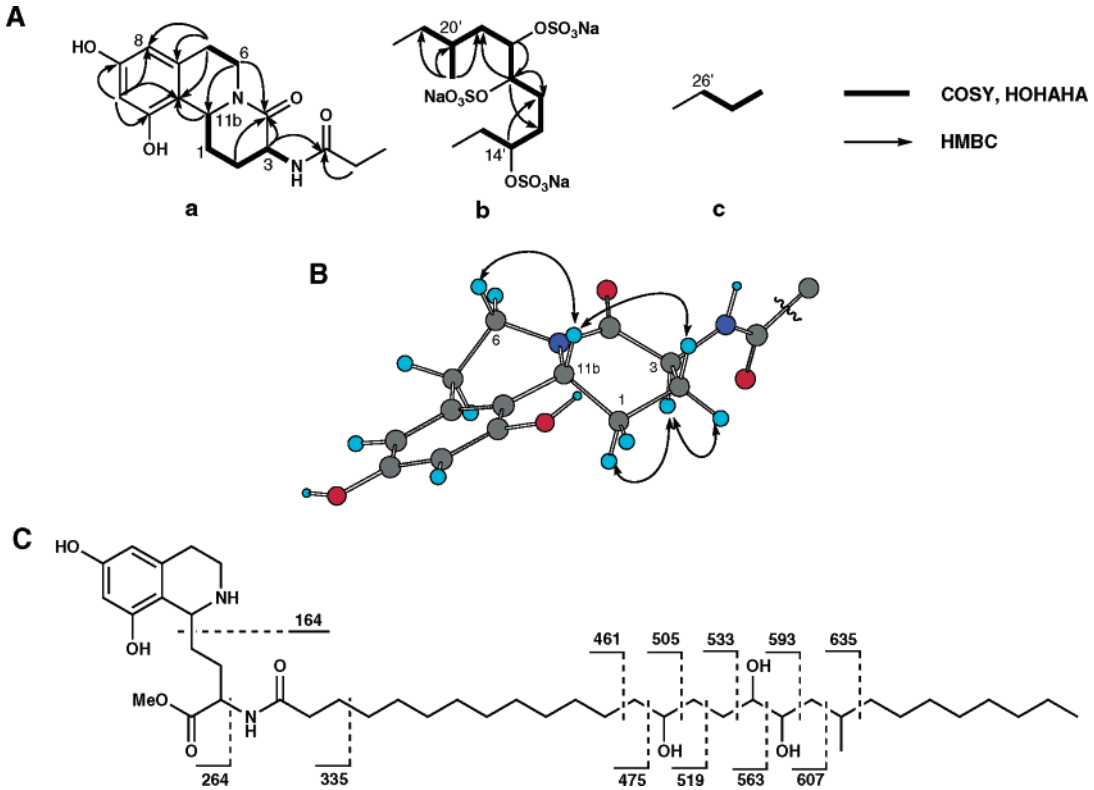

Figure 1. (A) Partial structures of schulzeine A (**1**). (B) Key ROESY cross-peaks of **1** in C_5D_5N. (C) FAB-MS/MS analysis of **4**.

signals. The absolute stereochemistry at C-3 was determined by application of the Mosher analysis.[9,10] Compound **5** was first converted to tris-(*R*)-MTPA (α-methoxy-α-trifluoromethylphenylacetic acid) and tris-(*S*)-MTPA derivatives, which were then saponificated to afford the (*R*)-MTPA and (*S*)-MTPA amides (**8** and **9**, respectively) (Figure 2A). The distribution of $\Delta\delta$ values indicated the 3*S*-configuration. Thus, the absolute configuration of C-11b was *R*.[11]

Compound **6** was found to have the structure of the acyl unit in **1** as analyzed by NMR and FABMS data. By taking advantage of the presence of a vicinal diol in **6**, the configuration of C-14′ was determined by Mosher analysis.[13] The triol **6** was converted to the 17′,18′-*O*-isopropylidene derivative **10** followed by MTPA esterifications to afford the (*R*)- and (*S*)-MTPA esters (**11** and **12**, respectively). The *trans*-relationship for H-17′ and 18′ in **12** was determined by ROESY data; cross-peaks were observed between one acetonide methyl (δ 1.34) and H-17′ and between another acetonide methyl (δ 1.32) and H-18′. Analysis of ^{1}H NMR data of the MTPA esters (**11** and **12**) led to the 14′*S*-configuration (Figure 2b). Compound **7** had a molecular weight that was 18 mass units smaller than that of **6**. Deshielded carbon chemical shift values for C-14′ and C-17′, which were obtained by an HMQC experiment, suggested the formation of an ether linkage between these carbons. However, it was not possible to assign the relative stereochemistry of this compound by interpretation of NMR data. Fortuitously, carbon chemical shift values of the C-14′ to C-18′ in **7** coincided well with those of a synthetic compound,[14] which had 14′*R**, 17′*S**, 18′*S**-stereochemistry and differed significantly from those of three other diastereomeric compounds.[15] Mosher analysis of **7** established the 18′*S*-configuration, thereby assigning 14′*R*, 17′*S*-stereochemistry (Figure 2C). The 14′*R*-configuration contradicted the assignment in **6**, which can be explained because the vicinal sulfate groups of **1** were hydrolyzed faster and the liberated hydroxyl group on C-17′ underwent an S_N2 reaction with C-14′ which still retained a sulfate group, resulting in inversion of the C-14′-stereochemistry. To determine the stereochemistry at C-20′, schulzeine A (**1**) was desulfated with TsOH[16] followed by $NaIO_4$ oxidation and $NaBH_4$ reduction to afford **16**, which was converted to the (*R*)-MTPA ester (**17**). The ^{1}H NMR spectrum of **17** was compared to those of the (*S*)-MTPA and (*R*)-MTPA esters[17,18] of (*S*)-3-methyl-1-pentanol (**18** and **19**, respectively), which disclosed the 20′*S*-configuration

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(11) This assignment was consistent with the CD spectrum of **1**, which gave a positive Cotton effect at 270 nm. The CD spectra of this class of compounds are dependent on the conformation of the central six-membered ring; our analysis of the conformation of **1** and its CD spectrum was in agreement with a closely related compound **11** in ref 12.
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(15) ^{13}C NMR data for model compounds corresponding to C-2 to C-5 and C-1″ were as follows.[15] Inconsistency of chemical shifts for C-17′ and C-18′ between **7** and the 14′*R**, 17′*S**, 18′*S** isomer may be due to γ ($\Delta\delta$ −2.5) and δ (+0.3) effects by a methyl substitute at C-20′. 14′*R**, 17′*S**, 18′*S** isomer [δ 79.3 (C-14′), 32.4 (C-15′), 28.4 (C-16′), 81.9 (C-17′), 74.2 (C-18′)], 14′*S**, 17′*S**, 18′*S** isomer [δ 79.9 (C-14′), 31.4 (C-15′), 27.8 (C-16′), 82.2 (C-17′), 74.5 (C-18′)], 14′*R**, 17′*S**, 18′*R** isomer [δ 80.2 (C-14′), 32.3 (C-15′), 25.0 (C-16′), 81.5 (C-17′), 72.0 (C-18′)], 14′*R**, 17′*R**, 18′*R** isomer [δ 79.6 (C-14′), 31.4 (C-15′), 23.9 (C-16′), 82.1 (C-17′), 71.6 (C-18′)].
(16) Singh, S. B. *Tetrahedron Lett.* **2000**, *41*, 6973−6976.
(17) Eguchi, T.; Kobayashi, K.; Uekusa, H.; Ohashi, Y.; Mizoue, K.; Matsushima, Y.; Kakinuma, K. *Org. Lett.* **2002**, *4*, 3383−3386.
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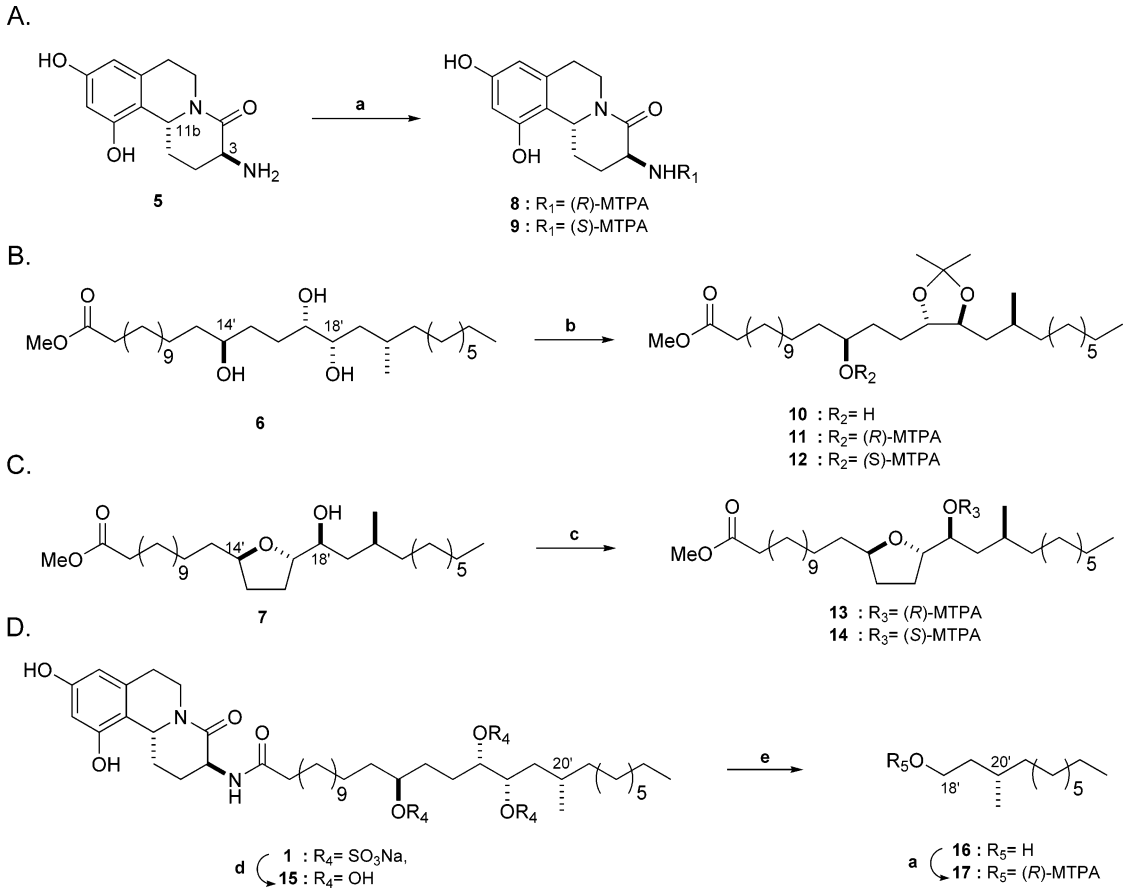
Scheme 1 [a]



[a] Reagents and conditions: (a) (i) (*S*)- or (*R*)-MTPACl, Py, (ii) 2 N NaOH, 90% aqueous MeOH; (b) (i) 2,2-dimethoxypropane, PPTS, MeOH, (ii) (*S*)- or (*R*)-MTPACl, DCM/py (1:1); (c) (*S*)- or (*R*)-MTPACl, DCM/py (1:1); (d) *p*-TsOH, MeOH; (e) (i) $NaIO_4$, MeOH, and then $NaBH_4$, (ii) (*S*)- or (*R*)-MTPACl, DCM/py (1:1).

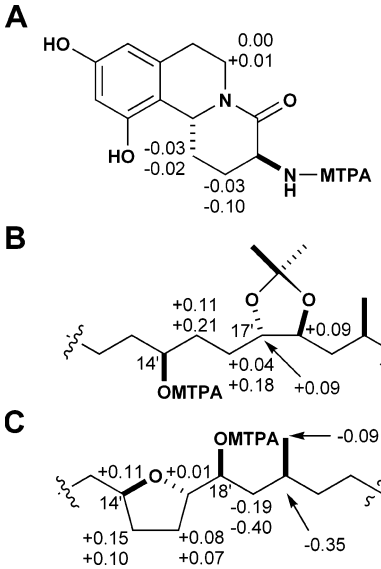


Figure 2. (A) The distribution of $\Delta\delta$ values for the MTPA amide of **5**. (B) The distribution of $\Delta\delta$ values for the MTPA ester of **6**. (C) The distribution of $\Delta\delta$ values for the MTPA ester of **7**.

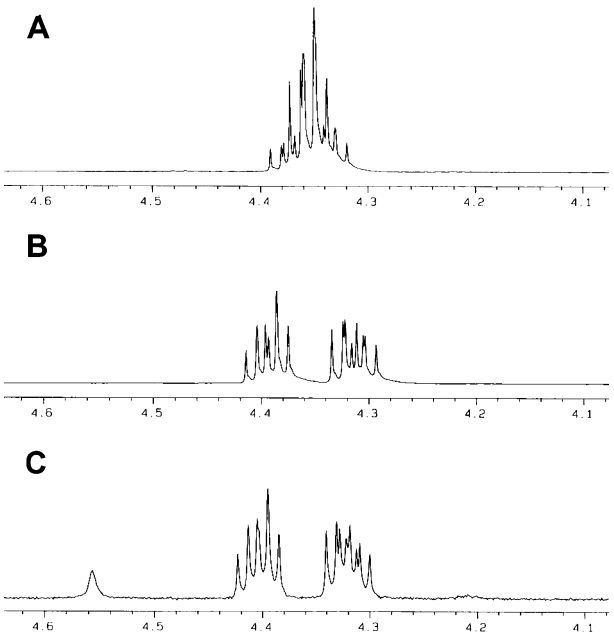


Figure 3. Comparison of proton signals for H_2-1 among (A) (*S*)-MTPA ester of (*S*)-3-methyl-pentanol (**18**), (B) (*R*)-MTPA ester of (*S*)-3-methyl-pentanol (**19**), and (C) (*R*)-MTPA ester **17**.

(Figure 3). Therefore, the stereochemistry of the acyl moiety was determined to be 14′*S*, 17′*S*, 18′*S*, 20′*S*.

Schulzeine B (**2**) had a molecular formula smaller than that of **1** by a CH_2 unit. The ^{1}H NMR spectrum of **2** was almost superimposable on that of **1** except for the absence of the methyl doublet. Interpretation of 2D NMR data of **2**[6] and FAB-MS/MS analysis of a desulfation product **20** led to a gross structure which was the 20′-desmethyl derivative of **1**.[8] ^{1}H NMR data

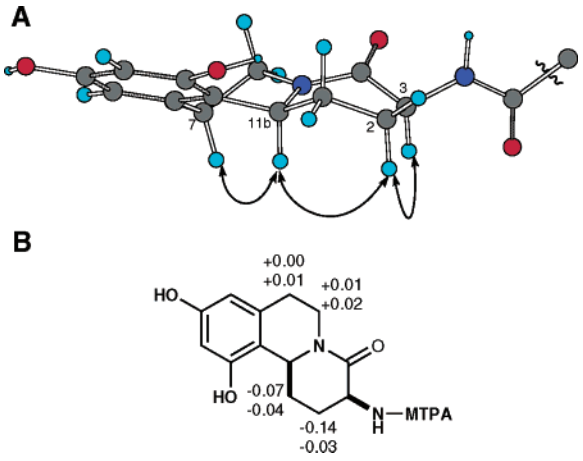
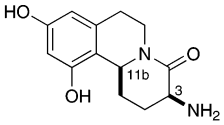


Figure 4. (A) Key ROESY cross-peaks of **2** in C_5D_5N. (B) The distribution of $\Delta\delta$ values for the MTPA amide of **19**.

revealed significant differences in chemical shifts for the fused δ-lactam portion between **1** and **2**. The ROESY correlations between H-3 and H-2α and between H-11b and H-2α in **2** indicated that H-3 and H-11b were on the same face of the six-membered ring (Figure 4A). The stereochemistry of **2** was determined by the same strategy used for **1**. Methanolysis of **2** afforded fragments **21**−**23**, which were converted to MTPA derivatives. NMR analysis of these derivatives led to 3*S*, 11b*S*, 14′*S*, 17′*S*, and 18′*S*-stereochemistry for **2**.[19]



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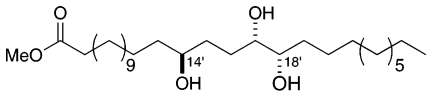

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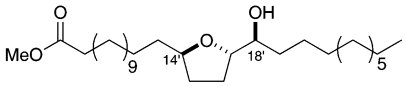


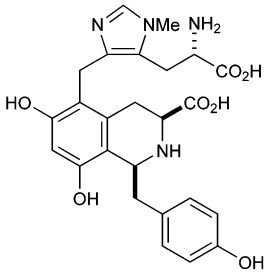
23

The molecular formula of schulzeine C (**3**) was identical to that of **2**. NMR data of the tricyclic part were superimposable to those of **1**, while those of the fatty acid portion were indistinguishable from those of **2**. FAB MS/MS analyses of the desulfated product indicated that the planar structure of **3** was identical to that of **2**. Therefore, **3** is an epimer of **2** at C11b.[20]

Schulzeines A−C inhibited α-glucosidase with IC_{50} values of 48−170 nM. It should be noted that desulfated schulzeines A and B still retained activity (IC_{50} values of 2.5 and 1.1 μM, respectively). Thus, the detergent-like nature of the schulzeines may not be important for the activity, although it contributes to the activity to some extent. Schulzeines were also inhibitory against viral neuraminidase with IC_{50} values of 60 μM.

(19) The 11b*S*-configuration was consistent with the CD spectrum of **2**.[11,12]
(20) Because of a limited amount of **3**, its degradation studies were not performed.

Schulzeines encompass the 9,11-dihydroxyisoquinoline constellation which is reminiscent of imbricatine (**24**)[21,22] and fuscusine (**25**),[23] both isolated from starfishes. The most notable structural difference of schulzeines from these metabolites is the presence of a long alkyl chain whose central portion was functionalized with three sulfate groups. Therefore, schulzeines are a new class of marine natural products.



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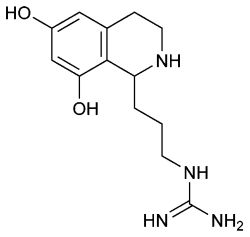

25

Biogenetically, schulzeines are likely derived from phenylalanine (or tyrosine), L-glutamic acid, and C_{28} fatty acid. Condensation of two amino acids after decarboxylation or reduction is proposed for the biosynthetic route to terrestrial isoquinoline alkaloids.[24] Intramolecular lactamization followed by amide bond formation may lead to schulzeines. It is interesting to speculate the true producers of these novel alkaloids.

Experimental Section

General Procedure. ^1H, ^{13}C NMR, and all 2D NMR spectra were recorded on a JEOL A600 NMR spectrometer at 315 K for **1** and 308 K for **2**. ^1H and ^{13}C NMR chemical shifts were referenced to the solvent peaks: δ_H 4.78 for D_2O, δ_H 3.30 and δ_C 49.0 for CD_3OD, δ_H 7.24 and δ_C 77.0 for $CDCl_3$, δ_H 7.19 and δ_C 123.3 for C_5D_5N. Optical rotation was measured on a JASCO DIP-1000 digital polarimeter in MeOH. FAB mass spectra were obtained on a JEOL MS-700T tandem mass spectrometer using either triethanolamine, *m*-nitrobenzyl alcohol, or glycerol as the matrix. Negative mode HR-FABMS spectra were obtained at a resolution of 5000 using PEG 1000 sulfate as a marker. IR spectra were recorded on a JASCO FT/IR-5300 spectrometer. CD spectra were recorded in MeOH using a JASCO J-820 spectropolarimeter.

Animal Material. The sponge was collected by hand using SCUBA at depths of 15−20 m off Hachijo-kojima Island, 300 km south of Tokyo, was immediately frozen, and was kept frozen at −20 °C until processed. The sponge was identified as *Penares schulzei* Thiele (order Astrophorida, class Ancorinidae). A voucher specimen (ZMA POR 16998) was deposited at the Zoological Museum, University of Amsterdam.

Isolation and Purification of Schulzeines A−C. The frozen sponge (600 g) was homogenized and extracted with MeOH, EtOH, and acetone. The combined extracts were partitioned between H_2O and $CHCl_3$, and the aqueous layer was further extracted with *n*-BuOH. The *n*-BuOH layer was separated by ODS flash chromatography with aqueous MeOH. The 70% MeOH eluate was separated by ODS MPLC (50 × 1000 mm) using a stepwise gradient from 45% to 50% MeCN containing 0.2 M $NaClO_4$. The active 45% MeCN fractions were

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purified by reversed-phase HPLC (Develosil C_{30}-UG5; 20 × 250 mm) with MeCN/PrOH/H_2O (25:10:65) containing 0.2 M $NaClO_4$ to afford schulzeines A (**1**, 40 mg, 6.7×10^{-3}% based on wet weight) and B (**2**, 25 mg, 4.2×10^{-5}% based on wet weight). Schulzeine C (**3**, 1.8 mg, 3.0×10^{-6}% based on wet weight) was finally obtained by reversed-phase HPLC (Phenomenex LUNA 5μ PHENYL-HEXYL; 10 × 250 mm) with MeCN/PrOH/H_2O (25:10:65) containing 0.2 M $NaClO_4$.

Schulzeine A (1): white powder; $[\alpha]^{22}_D$ +40° (*c* 0.10, MeOH); IR (film) ν_{max} 3584, 2924, 2852, 1631, 1548, 1464, 1361, 1222, 1103, 949, 839, 625 cm^{-1}; UV (MeOH) λ_{max} 209 nm (ϵ 27 500), 284 (1390); CD $\Delta\epsilon$ (MeOH) −(220 nm), +(240 nm), +(270 nm); ^{1}H NMR data, see Table 1; HR-FABMS *m*/*z* 999.3608 (M − Na)$^-$ [$C_{42}H_{69}N_2O_{16}S_3$-Na_2 (Δ +0.3 mmu)]. HMBC correlations (CD_3OH) H-1α/C-3, 11a, 11b; H1*β*/C-2, 3, 11b; H-2α/C-1, 4, 11b; H-2*β*/C-1, 3, 11b; H-3/C-2, 4, 1′; H-6α/C-4, 7, 11b; H-6*β*/C-4, 7; H-7α/6, 7a, 8; H-7*β*/ C-6, 7a, 8, 11a; H-8/C-7, 9, 10, 11a; H-10/C-8, 9, 11, 11a; H11b/C-1, 7a, 11a; H-2′/C-1′, 3′, 4′; H-3′/C-1′, 2′, 4′; H-12′/C-11′, 13′; H-13′/C-12′, 14′; H-14′/C-12′, 13′, 15′, 16′; H-15′/C-13′, 14′, 16′, 17′; H-16′/C-15′, 17′; H-17′/C-15′, 16′, 18′, 19′; H-18′/16′, 17′, 19′, 20′; H-19′/C-17′, 18′, 20′, 21′, 29′; H-21′/C-20′, 22′; H-27′/C-26′, 28′; H-28′/C-26′, 27′; H-29′/C-19′, 20′, 21′; ROESY (C_5D_5N) H-1α/H-1*β*, 3; H-1*β*/H-2α, 2*β*; H-2α/H-2*β*, 3; H-2*β*/H-11b; H-6α/H-6*β*, 7α, 7*β*; H-7α/H-8; H-7*β*/H-8; H-2′/H-3′; H-20′/H-29′.

Schulzeine B (2): white powder; $[\alpha]^{22}_D$ −23° (*c* 0.10, MeOH); IR (film) ν_{max} 3584, 2924, 2852, 1631, 1548, 1467, 1365, 1221, 1099, 937, 839, 625 cm^{-1}; UV (MeOH) λ_{max} 209 nm (ϵ 20 800), 281 (1500); CD $\Delta\epsilon$ (MeOH) −(225 nm), −(270 nm); ^{1}H NMR data, see Table 1; HR-FABMS *m*/*z* 985.3477 (M − Na)$^-$ [$C_{41}H_{67}N_2O_{16}S_3Na_2$ (Δ +2.9 mmu)]. HMBC correlations (CD_3OH) H-1α/C-3, 11a, 11b; H1*β*/C-2, 3, 11b; H-2α/C-3, 4, 11b; H-2*β*/C-3, 4, 11b; H-3/C-2, 4, 1′; H-6α/C-4, 7, 11b; H-6*β*/C-4, 7, 7a; H-7α/6, 7a, 8, 11a; H-7*β*/ C-6, 7a, 8, 11a; H-8/C-7, 9, 10, 11a; H-10/C-8, 9, 11, 11a; H11b/C-1, 2, 7a, 11, 11a; H-2′/C-1′, 3′, 4′; H-3′/C-1′, 2′, 4′; H-12′/C-11′; H-13′/C-12′, 14′; H-14′/C-15′, 16′; H-15′/C-13′, 14′, 16′, 17′; H-16′/C-17′; H-17′/C-16′, 18′, 19′; H-18′/16′, 17′, 19′; H-19′/C-17′, 18′, 20′; H-27′/C-26′, 28′; H-28′/C-26′, 27′; ROESY (C_5D_5N) H-1α/H-1*β*, 3, 11b; H-2α/H-2*β*, 3, 11b; H-3/H-11b; H-6α/H-6*β*, 7α, 7*β*; H-7α/H-8; H-7*β*/H-8; H-19′/H-20′.

Schulzeine C (3): white powder; $[\alpha]^{22}_D$ +33° (*c* 0.10, MeOH); IR (film) ν_{max} 3584, 2924, 2852, 1612, 1548, 1464, 1363, 1224, 1101, 951, 839 cm^{-1}; UV (MeOH) λ_{max} 209 nm (ϵ 27 800), 284 (1500); ^{1}H NMR (CD_3OD) δ 6.18 (d, 2.3, H-10), 6.08 (d, 2.3, H-8), 4.80 (H-6), 4.78 (H-11b), 4.67 (H-18′), 4.66 (H-17′), 4.35 (q, 5.76, H-14′), 4.26 (dt, 11.9, 7.3, H-3), 3.06 (dq, 13.5, 3.5, H-1a), 2.70 (dt, 15.0, 2.3, H-7a), 2.62 (dt, 12.3, 2.3, H-6b), 2.52 (dt, 15.0, 2.3, H-7b), 2.22 (dt, 4.2, 7.5, H-2′), 2.09 (m, H-2a), 1.97 (m, H-16′a), 1.95 (m, H-15′a), 1.93 (m, H-2b), 1.75 (m, H-19′a), 1.72 (m, H-15′b), 1.71 (m, H-13′a), 1.65 (m, H-13′b), 1.61 (m, H-3′), 1.60 (m, H-16′b), 1.54 (m, H-19′b), 1.42 (m, H-20′), 1.38 (m, H-4′b), 1.3 (H-5′−H-12′, H-21′−H-27′), 0.87 (t, 7.1, H-28′); ^{13}C chemical shifts (HMQC) δ 107.6 (C-8), 102.2 (C-10), 81.3 (C-14′), 80.0 (C-17′), 80.0 (C-18′), 57.1 (C-11b), 52.0 (C-3), 40.7 (C-6), 37.1 (C-2′), 35.4 (C-13′), 31.8 (C-15′), 31.0 (C-7), 29.4 (C-1), 28.3 (C-2), 26.7 (C-3′), 25.8 (C-12′), 25.7 (C-16′), 23.9 (C-27′), 14.4(C-28′). HR-FABMS *m*/*z* 985.3426 (M − Na)$^-$ [$C_{41}H_{67}N_2O_{16}S_3Na_2$ (Δ −2.2 mmu)].

Methanolysis of 1. A 10 mg portion of **1** was dissolved in 5 N HCl/MeOH (1:4), the solution was heated at 100 °C for 1 h, and the reaction mixture was cooled and dried in a stream of N_2 gas. The residue was separated by ODS flash chromatography with 20%, 60%, and 100% MeOH containing 0.1% TFA, followed by reversed-phase HPLC with aqueous MeOH containing 0.1% TFA to afford **4**, **5**, **6**, and **7**.

5: ^{1}H NMR (D_2O) δ 6.25 (d, 2.3), 6.24 (d, 2.3), 4.78 (dd, 10.5, 1.4, H-11b), 4.56 (dd, 8.8, 2.7, H-6a), 3.94 (dd, 12.7, 6.6, H-3), 3.06 (ddd, 14.2, 7.3, 3.5, H-1a), 2.66 (m, H-6b), 2.66 (m, H-7a), 2.56 (m, H-7b), 2.25 (m, H-2a), 1.92 (ddd, 13.4, 12.3, 2.7, H-2b), 1.42 (ddd, 13.9, 11.5, 2.7, H-1b); FABMS (NBA) *m*/*z* 249 (M + H)$^+$.

6: ^{1}H NMR (CD_3OD) δ 3.63 (OMe), 3.51 (H-14′), 3.50 (H-18′), 3.32 (H-17′), 2.30 (H-2′), 1.70 (H-16′a), 1.68 (H-15′a), 1.68 (H-20′), 1.59 (H-3′), 1.50 (H-19′a), 1.41 (H-15′b), 1.40 (H-16′b), 1.16 (H-19′b), 1.30−1.20 (H-4′−H-12′, H-21′−H-27′), 0.89 (H-29′), 0.89 (H-28′); FABMS (NBA) *m*/*z* 501 (M + H)$^+$, 523 (M + Na)$^+$.

7: ^{1}H NMR ($CDCl_3$) δ 3.85 (H-14′), 3.75 (H-17′), 3.63 (OMe), 3.34 (H-18′), 2.28 (H-2′), 1.99 (H-15′a), 1.93 (H-16′a), 1.73 (H-20′), 1.58 (H-3′), 1.56 (H-13′a), 1.55 (H-16′b), 1.47 (H-15′b), 1.41 (H-19′a), 1.38 (H-13′b), 0.98 (H-19′b), 0.88 (H-29′), 0.85 (H-28′); ^{13}C NMR δ 82.5 (C-17′), 79.3 (C-14′), 72.0 (C-18′), 51.4 (OMe), 40.6 (C-19′), 35.7 (C-13′), 34.2 (C-2′), 32.4 (C-15′), 29.0 (C-20′), 28.5 (C-16′), 25.0 (C-3′), 19.0 (C-29′), 14.0 (C-28′); FABMS (NBA) *m*/*z* 483 (M + H)$^+$, 505 (M + Na)$^+$.

Preparation of (*R*)- and (*S*)-MTPA Amides of 5 (8 and 9). A half portion of **5** was treated with (*S*)-MTPACl in pyridine (50 μL) at room temperature for 1 h, and the reaction mixture was partitioned between H_2O and $CHCl_3$ to give tris-(*R*)-MTPA derivative, which was saponified with 2 N NaOH in MeOH/H_2O (9:1) for 1 h. The reaction mixture was desalted with GL-Pak PLS-2 and purified by RP-HPLC (Develosil C30UG-5; 10 × 250 mm) with aqueous MeOH to afford (*R*)-MTPA amide **8**. The (*S*)-MTPA amide **9** was prepared in the same way.

8: ^{1}H NMR (CD_3OD) δ 7.64, 7.44, 6.19 (H-8), 6.13 (H-10), 4.81 (H-5), 4.79 (H-13a), 4.34 (H-2), 3.44 (OMe), 3.09 (H-4a), 2.70 (H-13b), 2.64 (H-12a), 2.54 (H-12b), 2.08 (H-3a), 2.04 (H-3b), 1.41 (H-4b); FABMS *m*/*z* 465 (M + H)$^+$.

9: ^{1}H NMR (CD_3OD) δ 7.64, 7.42, 6.19 (H-8), 6.13 (H-10), 4.79 (H-13a), 4.78 (H-5), 4.34 (H-2), 3.44 (OMe), 3.06 (H-4a), 2.71 (H-13b), 2.64 (H-12a), 2.54 (H-12b), 2.07 (H-3a), 1.94 (H-3b), 1.39 (H-4b); FABMS *m*/*z* 465 (M + H)$^+$.

Preparation of the Acetonide 10 from 6. The mixture of 0.9 mg of **6**, 2,2-dimethoxypropane (1 mL), and PPTS (pyridinium *p*-toluenesulfonate) (cat.) in CH_2Cl_2 (2 mL) was stirred at 0 °C for 1 h, and the reaction mixture was dried in a stream of N_2 gas. The residue was purified on silica gel with $CHCl_3$ to yield the acetonide **10**.

10: ^{1}H NMR (C_5D_5N) δ 3.92 (H-14′), 3.92 (H-18′), 3.81 (H-17′), 3.61 (OMe), 2.32 (H-2′), 2.18 (H-16′a), 2.07 (H-15′a), 1.85 (H-20′), 1.83 (H-15′b), 1.83 (H-16′b), 1.66 (H-19′a), 1.61 (H-3′), 1.50 (3H, Me), 1.50 (3H, Me), 1.29 (H-19′b), 1.30−1.20 (H-4′ to H-12′, H-21′ to H-27′), 0.95 (H-29′), 0.84 (H-28′); FABMS *m*/*z* 541 (M + H)$^+$.

Preparation of (*R*)- and (*S*)-MTPA Ester of the Acetonide 10 (11 and 12). A half portion of the acetonide **10** was treated with (*S*)-MTPACl in CH_2Cl_2/pyridine (1:1, 100 μL), and the reaction mixture was partitioned between H_2O and CH_2Cl_2 (2 mL × 3). The organic layer was purified by RP-HPLC (Cosmosil 5C_{18}-ARII; 10 × 250 mm) with aqueous MeOH to afford the (*R*)-MTPA ester **11**. The (*S*)-MTPA ester **12** was prepared in the same way.

11: ^{1}H NMR (CD_3OD) δ 7.42, 7.30, 5.12 (H-14′), 3.64 (OMe), 3.57 (H-18′), 3.54 (OMe), 3.42 (H-17′), 2.30 (H-2′), 1.82 (H-16′a), 1.61 (H-20′), 1.59 (H-15′a), 1.45 (H-19′a), 1.59 (H-3′), 1.31 (6H, Me), 1.39 (H-15′b), 1.31 (H-16′b), 1.30−1.20 (H-4′b to H-12′, H-21′ to H-27′), 1.08 (H-19′b), 0.89 (H-29′), 0.89 (H-28′); FABMS *m*/*z* 779 (M + Na)$^+$.

12: NMR (CD_3OD) δ 7.42, 7.28, 5.14 (H-14′), 3.66 (H-18′), 3.64 (OMe), 3.54, (OMe), 3.51 (H-17′), 2.30 (H-2′), 1.86 (H-16′a), 1.70 (H-15′a), 1.63 (H-20′), 1.60 (H-15′b), 1.49 (H-16′b), 1.51 (H-19′a), 1.59 (H-3′), 1.34 (3H, Me), 1.32 (3H, Me), 1.19 (H-19′b), 1.30−1.20 (H-4′ to H-12′, H-21′ to H-27′), 0.89 (H-29′), 0.89 (H-28′); FABMS *m*/*z* 779 (M + Na)$^+$.

Preparation of (*R*)- and (*S*)-MTPA Ester of 7 (13 and 14). A half portion of **7** was treated with (*S*)-MTPACl in CH_2Cl_2/pyridine (1:1, 100 μL), and the reaction mixture was partitioned between H_2O and CH_2Cl_2 (2 mL × 3). The organic layer was purified by RP-HPLC (Cosmosil 5C_{18} ARII; 10 × 250 mm) with aqueous MeOH to yield (*R*)-MTPA ester **13**. The (*S*)-MTPA ester **14** was prepared in the same way.

13: NMR (CD_3OD) δ 7.41, 7.30, 5.16 (H-18′), 3.95 (H-17′), 3.78 (H-14′), 3.64 (OMe), 3.54 (OMe), 2.30 (H-2′), 1.97 (H-16′a), 1.88 (H-15′a), 1.72 (H-19′a), 1.59 (H-3′), 1.52 (H-16′b), 1.46 (H-20′), 1.38 (H-15′b), 1.27 (H-19′b), 1.30−1.20 (H-4′ to H-12′, H-21′ to H-27′), 0.93 (H-29′), 0.89 (H-28′); FABMS *m*/*z* 483 $(M + H)^+$.

14: NMR (CD_3OD) δ 7.39, 7.30, 5.15 (H-18′), 3.96 (H-17′), 3.89 (H-14′), 3.64 (OMe), 3.54 (OMe), 2.30 (H-2′), 2.05 (H-16′a), 2.03 (H-15′a), 1.59 (H-16′b), 1.59 (H-3′), 1.53 (H-19′a), 1.48 (H-15′b), 1.30−1.20 (H-4′ to H-12′, H-21′ to H-27′), 1.11 (H-20′), 0.89 (H-28′), 0.87 (H-19′b), 0.84 (H-29′); FABMS *m*/*z* 483 $(M + H)^+$.

Preparation of (+)-MTPA Ester of 3-Methyl-1-undecanol 17. A 2.0 mg portion of **1** was desulfated with *p*-TsOH (cat.) in MeOH (2 mL) at room temperature for 1 h. After neutralization with 10% $NaHCO_3$, the reaction mixture was desalted on GL-Pak PLS-2. The triol **15** was treated with $NaIO_4$ (cat.) in 75% MeOH/H_2O at room temperature for 1 h, followed by reduction with $NaBH_4$ (50 mg), and the reaction mixture was quenched with CH_3COOH and extracted with CH_2Cl_2 to afford 3-methyl-1-undecanol **16**, which was treated with (*S*)-(+)-MTPACl in CH_2Cl_2/pyridine (1:1, 100 μL). The reaction mixture was partitioned between H_2O and CH_2Cl_2, and the organic phase was purified by RP-HPLC (Cosmosil 5C_{18}-ARII; 10 × 250 mm) with aqueous MeOH to afford the (*R*)-(+)-MTPA ester **17**.

17: ^{1}H NMR (CD_3OD) δ 7.48, 7.44, 4.44 (H-1), 3.52 (OMe), 1.69 (H-3), 1.46 (H-2a), 1.28−1.24 (H-5 to H-10), 1.24 (H-4a), 1.12 (H-4b), 0.89 (H-11), 0.87 (H-12); FABMS (NBA) *m*/*z* 403 $(M + H)^+$.

Preparation of (*R*)-(+)- and (*S*)-(−)-MTPA Ester of (*S*)-3-Methyl-1-pentanol 18 and 19. The commercially available (*S*)-3-methyl-1-pentanol was converted to (*R*)-(+)- and (*S*)-(−)-MTPA esters following the same procedure used for the preparation of **17**.

Methanolysis of 2. A 10 mg portion of **2** was hydrolyzed with 5 N HCl/MeOH (1:4). Following the same procedure for the methanolysis of **1**, compounds **20**, **21**, **22**, and **23** were prepared.

21: ^{1}H NMR (D_2O) δ 6.26 (d, 2.3), 6.25 (d, 2.3), 4.72 (dd, 11.5, 3.9, H-11b), 4.41 (dt, 11.7, 3.5, H-6a), 4.10 (dd, 10.7, 7.3, H-3), 2.34 (m, H-1a), 2.71 (m, H-7a), 2.63 (m, H-6b), 2.63 (H-7b), 2.34 (m, H-1a), 2.34 (m, H-2a), 1.66 (m, H-2b), 1.41 (m, H-1b); FABMS (NBA) *m*/*z* 249 $(M + H)^+$.

23: ^{1}H NMR ($CDCl_3$) δ 3.84 (H-14′), 3.75 (H-17′), 3.64 (OMe), 3.34 (H-18′), 2.28 (H-2′), 1.99 (H-15′a), 1.93 (H-16′a), 1.58 (H-16′b), 1.58 (H-3′), 1.56 (H-13′a), 1.48 (H-20′), 1.48 (H-15′b), 1.38 (H-13′b), 1.35 (H-19′), 0.85 (H-28′); ^{13}C NMR δ 82.0 (C-17′), 79.3 (C-14′), 74.2 (C-18′), 51.4 (OMe), 35.7 (C-13′), 34.1 (C-2′), 33.3 (C-19′), 32.5 (C-15′), 28.5 (C-16′), 25.8 (C-20′), 25.0 (C-3′), 14.0 (C-28′); FABMS (NBA) *m*/*z* 469 $(M + H)^+$, 491 $(M + Na)^+$.

Enzyme Inhibition Assay. α-Glucosidase inhibition assay was performed according to the method of Cannell et al.[25] with a slight modification. To the mixture of α-glucosidase (Sigma Type V from Yeast, G0660, 90 μL: 5.0×10^{-2} unit/mL in 0.1 M potassium phosphate including 3.2 mM $MgCl_2$, pH 6.8) and a sample (20 μL in MeOH) was added *p*-nitrophenyl α-D-glucopyranoside (90 μL: 5.0×10^{-2} mg/mL in the same buffer as described above), and the mixture was incubated at 37 °C for 30 min. The absorbance at 410 nm of the reaction mixture was measured.

Acknowledgment. We thank Prof. Yasuo Suzuki for supplying influenza virus A/Memphis/71 (H3N2). This work was partly supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Supporting Information Available: ^{1}H, ^{13}C, and all 2D NMR spectra for both **1** and **2**, ^{1}H NMR for **3**, FAB-MS/MS data for **1**−**3**, MTPA derivatives from **2**, and a summary of the modified Mosher's method for **2** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA037368R

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