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Complete Structure Elucidation of Shishididemniols, Complex Lipids with Tyramine-Derived Tether and Two Serinol Units, from a Marine Tunicate of the Family Didemnidae

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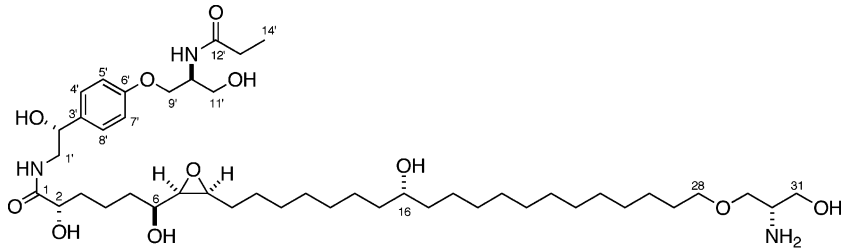
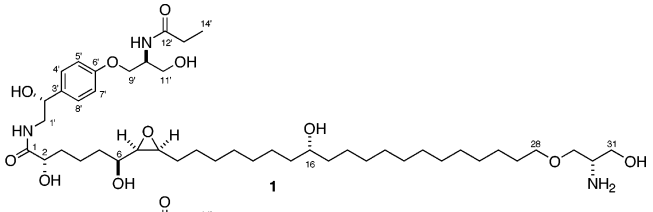


shishididemniol A (**1**)

Two new serinolipid derivatives, shishididemniols A (**1**) and B (**2**), were isolated as antibacterial constituents of a tunicate of the family Didemnidae. The structure of **1** was elucidated by interpretation of spectral data and the application of the modified Mosher method to **1** and its suitable degradation products. Compound **2** was the chlorohydrin of **1**. Compounds **1** and **2** exhibited antibacterial activity against fish pathogenic bacterium *Vibrio anguillarum*.

Introduction

Fish and shellfish diseases are the most serious concerns for aquaculture today.[1] The popular action taken in fish farms is chemotherapy using chemical substances such as antibiotics and synthetic organic compounds to control these diseases.[1] However these chemical substances pose environmental and hygienic problems.[1] We have been searching for antimicrobial metabolites against fish pathogenic bacteria from marine natural products that are expected to be environmentally benign. As a part of the study, we have discovered novel bromotyrosine derivatives from the marine sponge *Hexadella* sp.[2] that exhibited antibacterial activity against the fish pathogenic bacterium *Aeromonas hydrophila*. Subsequently, we found that the extract of a tunicate of the family Didemnidae collected in southern Japan exhibited activity against the bacterium *Vibrio anguillarum*, which causes vibriosis in fish.[3] Bioassay-guided fractionation afforded two novel serinolipid derivatives, shishididemniols A (**1**) and B (**2**). This paper describes the isolation, structure elucidation and antimicrobial activitiy of these compounds.



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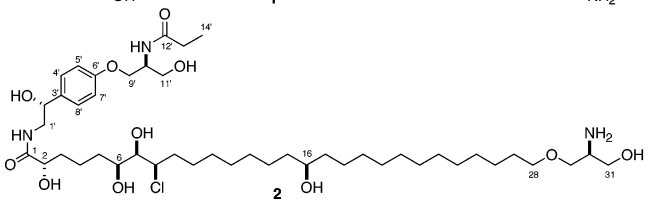

2

Results and Discussion

The EtOH extract of the tunicates (780 g) was partitioned between $CHCl_3$ and H_2O, and the aqueous layer was further extracted with *n*-BuOH. The *n*-BuOH fraction was separated by ODS flash chromatography followed by reversed-phase HPLC to afford shishididemniol A (**1**) and B (**2**) in yields of

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(1) Shao, Z. J. *Adv. Drug. Deliv. Rev.* **2001**, *50*, 229−243.
(2) Matsunaga, S.; Kobayashi, H.; van Soest, R. W. M.; Fusetani, N. *J. Org. Chem.* **2005**, *70*, 1893−1896.
(3) Roberts, R. J. In *Bacterial Disease of Fish*; Inglis, V., Roberts, R. J., Bromage, N. R., Eds.; Blackwell Science: Oxford, 1993; pp 109−121.

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395 mg (5.1×10^{-2} % wet weight) and 152 mg (1.9×10^{-2} % wet weight), respectively.

The molecular formula of shishididemniol A (**1**) was established as $C_{45}H_{81}N_3O_{11}$ on the basis of NMR and HRESIMS data. Interpretation of the ^{1}H NMR spectrum (in DMSO-d_6) together with HSQC data showed the presence of an *N*-substituted methylene (δ_H 3.12 and 3.31, δ_C 46.2), two nitrogenous methines (δ_H 3.23, δ_C 52.0; δ_H 4.02, δ_C 50.2), five oxygenated methylenes (δ_H 3.40 (2H), δ_C 70.7; δ_H 3.44 and 3.50, δ_C 67.5; δ_H 3.48 and 3.56, δ_C 58.7; δ_H 3.93 and 3.95, δ_C 66.5; δ_H 3.46 and 3.49, δ_C 60.1), four oxymethines (δ_H 3.80, δ_C 70.8; δ_H 3.19, δ_C 68.7; δ_H 3.33, δ_C 69.5; δ_H 4.55, δ_C 70.8), two epoxide methines (δ_H 2.69, δ_C 60.5; δ_H 2.87, δ_C 56.2), a triplet methyl (δ_H 0.97, δ_C 9.90), a methylene (δ_H 2.10, δ_C 28.4), two aromatic signals [δ_H 6.89 (2H), δ_C 114.1 (2C); δ_H 7.22 (2H), δ_C 127.1 (2C)], two amide protons (δ 7.51 and 7.74), and seven exchangeable protons (δ 4.19, 4.83, 4.85, 5.24, 5.41, 5.41, and 7.80). The remainder of the ^{1}H NMR signals were attributed to long methylene chains (δ 1.15−1.45). In addition, two non-protonated sp^2 carbon signals (δ 135.6 and δ_C 157.7) and two carbonyl carbon signals (δ 173.0 and 173.9) were observed in the ^{13}C NMR spectrum (Table 1).

Interpretation of 2D NMR data led to substructures **a**−**c** (Figure 1A). In substructure **a**, the propionamide moiety (C-12′ to C-14′) was identified from the COSY and HMBC data. The COSY spectrum revealed spin systems for C-9′-C10′(-NH-10′)-C-11′-OH-11′ and NH-1′-C-1′-C-2′-OH. The connectivity between N-10′ and C-12′ was determined by HMBC cross-peaks, NH-10′/C-12′ and H-10′/C-12′. ^{1}H and ^{13}C NMR chemical shift values clearly indicated the presence of a *para*-substituted phenyl ether moiety. The ether linkage was established by the HMBC cross-peak, H-9′/C-6′, while further HMBC correlations, H-2′/C-3′, H-2′/C-4′(8′), and H-4′(8′)/C-2′, demonstrated the connectivity between C-2′ and C-3′. The connectivities from OH-2 to H_2-4 and from OH-6 to H_2-9 were assigned on the basis of the COSY data. Further analysis of the COSY and TOCSY data indicated that the C-6 oxymethine proton was adjacent to H_2-5, which was in turn correlated with H_2-4. The ^{1}H−^{1}H coupling constant between H-7 and H-8 (J = 4.1 Hz) revealed that C-7 and C-8 were a part of a *cis*-epoxide. HMBC cross-peaks, NH-1′/C-1, H-2/C-1, and OH-2/C-1, showed the connectivity between C-1 and N-1′.

Substructure **b** comprises the terminus of an alkyl chain attached to a serinol ether.[4,5] The serinol unit (O-C-29-C-30-C-31-OH-31) was clearly defined by the COSY and HSQC data. The chemical shift value of C-30 at δ_C 52.0 and the ninhydrin-positive property of **1** implied that C-30 was substituted by a free amino group. The ether linkage was established by HMBC cross-peaks from H_2-28 to C-29.

Substructure **c** contains an oxymethine group placed in the middle of a long methylene chain. The COSY spectrum showed that C-16 oxymethine proton was coupled to OH-16 and two pairs of methylene protons (H_2-15 and H_2-17). Therefore, substructure **c** should be inserted between substructure **a** and **b** via methylene chains. The location of substructure **c** in the alkyl chain was determined by analysis of FAB-MS/MS data of **1**. Notable fragment ions observed at *m*/*z* 258 and 288 allowed us to locate the hydroxyl group at C-16 (Figure 1B).

TABLE 1. ^{1}H and ^{13}C NMR Data for Shishididemniols A (1) and B (2) in DMSO-d_6[a]

	1		**2**	
position	δ_H (mult, *J* Hz)	δ_C (mult[b])	δ_H (mult, *J* Hz)	δ_C (mult[b])
1		173.9 (C)		173.9 (C)
2	3.80 (br)	70.8 (CH)	3.81 (br)	70.9 (CH)
3a	1.42 (m)	34.3 (CH_2)	1.43 (m)	34.4 (CH_2)
3b	1.55 (m)		1.57 (m)	
4a	1.31 (m)	20.4 (CH_2)	1.37 (m)	21.0 (CH_2)
4b	1.42 (m)		1.44 (m)	
5a	1.29 (m)	33.7 (CH_2)	1.31 (m)	32.6 (CH_2)
5b	1.40 (m)		1.38 (m)	
6	3.19 (br)	68.7 (CH)	3.46 (m)	70.9 (CH)
7	2.69 (dd, 4.1, 8.2)	60.5 (CH)	3.27 (t, 4.4)	75.6 (CH)
8	2.87 (ddd, 4.1, 4.1, 8.2)	56.2 (CH)	3.99 (dt, 8.8, 4.4)	66.2 (CH)
9a	1.28 (m)	27.9 (CH_2)	1.67 (m)	34.4 (CH_2)
9b	1.54 (m)		1.77 (m)	
10−14	1.15−1.45 (m)	28.9−29.3	1.17−1.31 (m)	29.4−29.8
15	1.21−1.34 (m)	37.2 (CH_2)	1.22−1.34 (m)	37.7 (CH_2)
16	3.33 (m)	69.5 (CH)	3.33 (m)	69.5 (CH)
17	1.21−1.34 (m)	37.2 (CH_2)	1.22−1.34 (m)	37.7 (CH_2)
18−25	1.15−1.45 (m)	28.9−29.3	1.17−1.31 (m)	29.4−29.8
26	1.27 (m)	25.5 (CH_2)	1.27 (m)	25.5 (CH_2)
27	1.49 (tt, 7.6, 7.6)	28.9 (CH_2)	1.49 (m)	28.6 (CH_2)
28	3.40 (t, 7.6)	70.7 (CH_2)	3.39 (m)	70.7 (CH_2)
29a	3.44 (m)	67.5 (CH_2)	3.43 (m)	67.7 (CH_2)
29b	3.50 (dd, −10.5, 4.8)		3.49 (m)	
30	3.23 (m)	52.0 (CH)	3.20 (m)	52.0 (CH)
31a	3.48 (m)	58.7 (CH_2)	3.48 (m)	59.0 (CH_2)
31b	3.56 (m)		3.55 (dd, 4.7, 10.9)	
1′a	3.12 (m)	46.2 (CH_2)	3.13 (m)	46.1 (CH_2)
1′b	3.31 (m)		3.31 (m)	
2′	4.55 (br)	70.8 (CH)	4.55 (br)	70.9 (CH)
3′		135.6 (C)		135.6 (C)
4′, 8′	7.22 (d, 8.7)	127.1 (CH)	7.22 (d, 8.8)	127.1 (CH)
5′, 7′	6.89 (d, 8.7)	114.1 (CH)	6.89 (d, 8.8)	114.1 (CH)
6′		157.7 (C)		157.7 (C)
9′a	3.93 (dd, −9.9, 5.9)	66.5 (CH_2)	3.93 (dd, −9.8, 6.0)	66.4 (CH_2)
9′b	3.95 (dd, −9.9, 5.7)		3.95 (dd, −9.8, 5.4)	
10′	4.02 (m)	50.2 (CH)	4.03 (m)	50.2 (CH)
11′a	3.46 (m)	60.1 (CH_2)	3.46 (m)	60.1 (CH_2)
11′b	3.49 (m)		3.50 (m)	
12′		173.0 (C)		173.1 (C)
13′	2.10 (q, 7.2)	28.4 (CH_2)	2.10 (q, 7.7)	28.4 (CH_2)
14′	0.97 (t, 7.2)	9.90 (CH_3)	0.97 (t, 7.7)	9.91 (CH_3)
OH-2	5.41 (d, 4.1)		5.48 (br)	
OH-6	4.85 (br)		4.19 (br)	
OH-7			4.82 (br)	
OH-16	4.19 (br)		4.19 (br)	
NH_2-30	7.80 (br)		7.61 (br)	
OH-31	5.24 (br)		4.83 (br)	
NH-1′	7.51 (t, 5.9)		7.50 (t, 5.8)	
OH-2′	5.41 (d, 4.1)		5.42 (br)	
NH-10′	7.74 (d, 7.8)		7.75 (d, 8.3)	
OH-11′	4.83 (br)		4.82 (br)	

[a] 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR. [b] Multiplicity from HSQC experiment.

The stereochemistry of C-6 with respected to that of C-7 in **1** could not be determined by analysis of NMR data.[6,7] This

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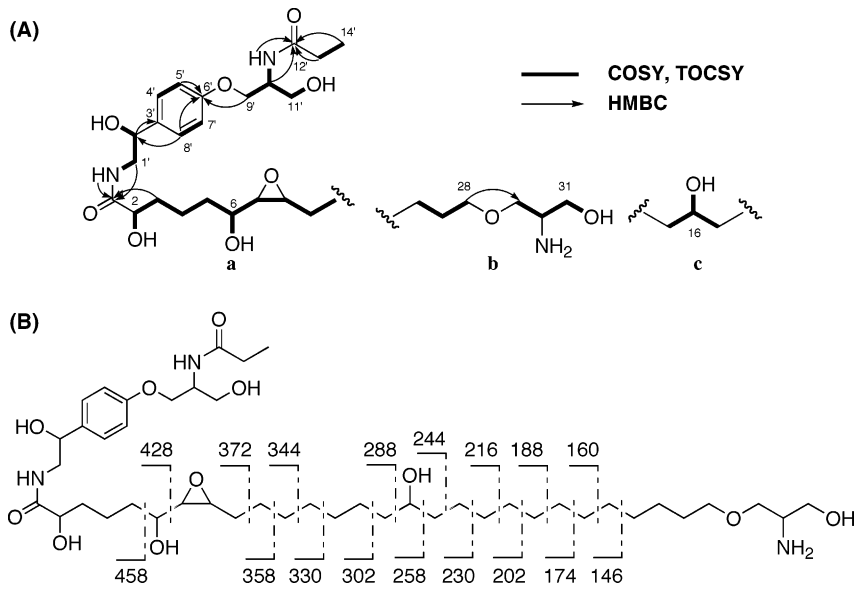
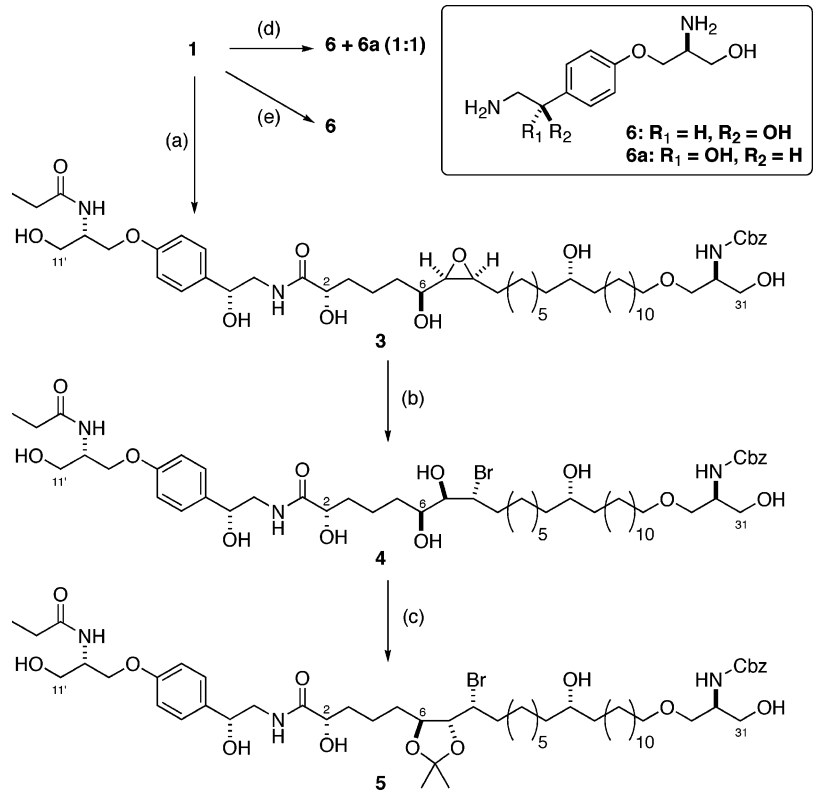


FIGURE 1. (A) Partial structures of shishididemniol A (**1**). (B) FAB-MS/MS analysis of **1**.

SCHEME 1 [a]



[a] Reagents and conditions: (a) Cbz-Cl, Et_3N, $CHCl_3$/MeOH (3:1), rt, 16 h; (b) $MgBr_2$ OEt_2, Et_2O, rt, 3 h; (c) 2,2-dimethoxpropane, PPTS, CH_2Cl_2, rt, 1 h; (d) HCl/MeOH, 100 °C, 4 h; (e) hydrazine, 120 °C, 12 h.

was shown by converting **1** to the 6,7-*O*-isopropylidene derivatives **5**, via the *N*-Cbz-protected bromohydrin **4** (Scheme 1).[8] NOESY cross-peaks observed between one acetonide methyl signal (δ_H 1.40) and H-6 and between another methyl signal (δ_H 1.37) and H-7 showed the *trans*-relationship for H-6 and H-7. Therefore, the relative stereochemistry from C-6 to C-8 in **1** was assigned as *threo-cis*-2,3-epoxy alcohol (6*S**,7*R**,8*S**).

The absolute stereochemistry of C-2′ and C-10′ in **1** was envisaged to be determined by application of the modified Mother's method[9,10] to compound **6**, which was expected to be obtained by acidic hydrolysis of **1**. We used (*R*)-2-amino-1-phenylethanol, which was commercially available, and (*S*)-2-amino-3-phenoxy-1-propanol (**9**) as model compounds to assign the stereochemistry of C-2′ and C-10′ in **6**. Compound **9** was

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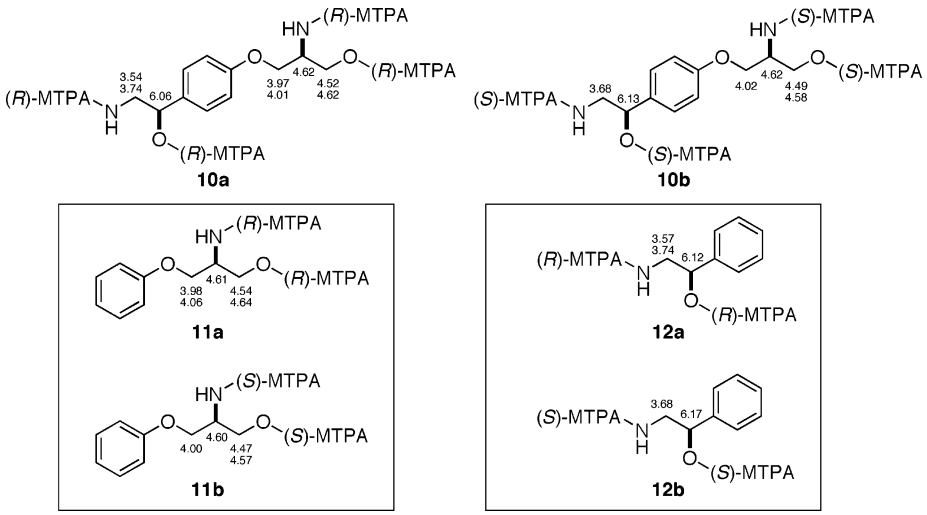
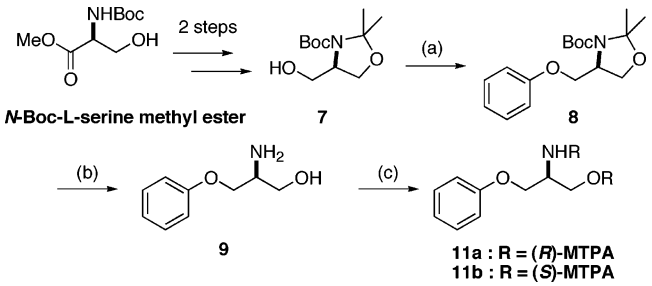


FIGURE 2. ^{1}H NMR chemical shift values for **10a**/**10b**, **11a**/**11b**, and **12a**/**12b**.

SCHEME 2 [a]



[a] Reagents and conditions: (a) phenol, DEAD, PPh_3, toluene, 80 °C, 18 h; (b) CH_2Cl_2/TFA (1:1), rt, 2 h; (c) (*S*)-or (*R*)-MTPA-Cl, pyridine, rt, 30 min.

synthesized as outlined in Scheme 2. *N*-Boc-L-serine methyl ester was converted to a chiral alcohol **7** in two steps according to Garner's procedure.[12] Treatment of **7** with triphenylphosphine and diethyl azodicarboxylate at 80 °C in toluene with phenol provided the phenyl ether **8**,[13,14] which was deprotected with TFA to yield the expected compound **9**. The (*R*)- and (*S*)-MTPA derivatives of (*R*)-2-amino-1-phenylethanol and those of compound **9** gave characteristic ^{1}H NMR profiles, indicating that the stereochemistry at C-2′ and C-10′ in **6** could be determined by the modified Mosher method. Acidic methanolysis product of **1** was separated by ODS column chromatography to afford a fraction that contained UV-absorbing polar material. This material, which gave a single set of ^{1}H NMR signals, was converted to the (*R*)- and (*S*)-MTPA derivatives. To our disappointment H_2-1′ and H-2′ were doubled, implying that the benzyl carbon (C-2′) in **6** was racemized by acid and what we isolated was a 1:1 mixture of **6** and **6a**. As a method to cleave amide bonds under a milder condition, we chose hydrazinolysis. Compound **1** was hydrazinolyzed[11] to yield a fraction that contained **6**, which was then converted to the (*R*)- and (*S*)-MTPA derivatives (**10a** and **10b**, respectively). Only one set of signals were observed in ^{1}H NMR spectra of **10a** and **10b**.

The absolute stereochemistry of C-2′ and C-10′ in **6** was determined by comparison of ^{1}H NMR spectra of **10a** and **10b** with those of **11a** and **11b** and those of (*R*)- and (*S*)-MTPA derivatives of (*R*)-2-amino-1-phenylethanol (**12a** and **12b**, respectively) (Figure 2**)**. ^{1}H NMR chemical shift values of H_2-1′ and H-2′ of **10a** and **10b** were in remarkable agreement with those of the corresponding signals of **12a** and **12b**, respectively, whereas the chemical shift values of H_2-9′, H-10′, and H_2-11′ agreed well with those of the corresponding signals of **12a** and **12b**, respectively. Therefore, the absolute stereochemistry of C-2′ and C-10′ was assigned as *R* and *S*, respectively (Figure 2).

The absolute stereochemistry of C-2, C-6, and C-30 in **1** was determined by application of the modified Mosher method to **1** and **5**. Treatment of **1** with (*S*)-MTPACl and (*R*)-MTPACl yielded the (*R*)-MTPA derivative (**13a**) and (*S*)-MTPA derivative (**13b**), respectively. Analysis of $\Delta\delta_{S-R}$ values between **13a** and **13b** allowed the assignment of 6*S* and 30*S* (Figure 3). The negative $\Delta\delta_{S-R}$ values observed for H_2-3, H_2-4, and H_2-5 implied the 2*S*-stereochemistry. In order to exclude the effect of the C-6 MTPA ester, the acetonide **5** was converted to the (*R*)-MTPA derivative (**14a**) and (*S*)-MTPA derivative (**14b**). The negative $\Delta\delta_{S-R}$ values observed for H_2-3 to H_2-8 signals confirmed the 2*S*-stereochemistry (Figure 3).

Because C-16 was too far from the nearest functionalized carbon, it was not possible to detect the effects of MTPA esters on chemical shifts of assigned proton signals, e.g., H-8 and H_2-9. This problem was hampered by esterification with 2NMA (2-naphthylmethoxyacetic acid), which was reported to exhibit anisotropic effects to more distant protons.[15] In order to avoid confusion in assignments, we intended to introduce one 2NMA group to a derivative of **1**. For that purpose the bromohydrin **4** was treated with $NaIO_4$ followed by reduction with $NaBH_4$ to obtain **15**, whose two primary alcohols were protected with TBDPS groups[16,17] to yield **16** (Scheme 3). The secondary alcohol in **16** was esterified with (*R*)-2NMA or (*S*)-2NMA in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide

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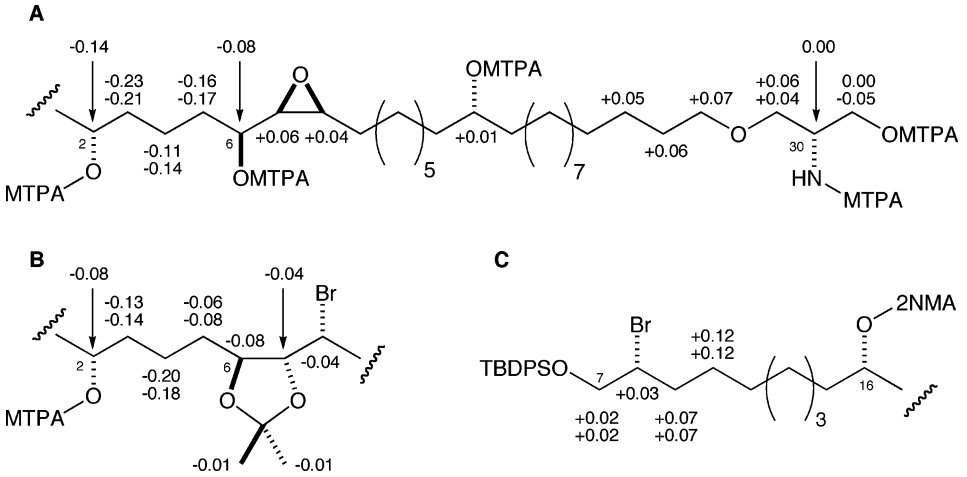
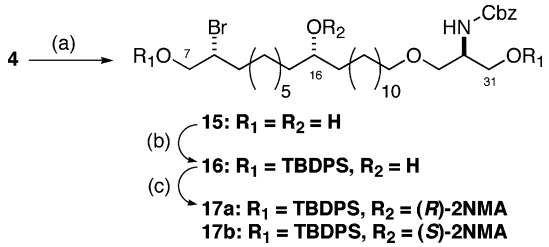


FIGURE 3. (A) Distribution of $\Delta\delta_{S-R}$ values for the MTPA derivatives **13a**/**13b**. (B) Distribution of $\Delta\delta_{S-R}$ values for the MTPA derivatives **14a**/**14b**. (C) Distribution of the 2NMA esters of $\Delta\delta_{R-S}$ values for **17a**/**17b**.

SCHEME 3 [a]



[a] Reagents and conditions: (a) $NaIO_4$, MeOH/H_2O (4:1), rt, 1 h; then $NaBH_4$, rt, 15 min; (b) TBDPSCl, pyridine, rt, overnight; (c) (*R*)- or (*S*)-2NMA, EDC, DMAP, DMAP·Cl, CH_2Cl_2, rt, overnight.

hydrochloride (EDC), DMAP, and DMAP·HCl[18] in CH_2Cl_2 to give (*R*)- or (*S*)-2NMA derivatives (**17a** and **17b**, respectively). Comparison of ^{1}H NMR data of **17a** and **17b** showed that the absolute configuration at C-16 was *R* (Figure 3). Therefore, the stereochemistry of **1** was determined to be 2*S*,6*S*,7*R*,8*S*,16*R*,30*S*,2′*R*,10′*S*.

Shishididemniol B (**2**) had a molecular formula of $C_{45}H_{82}ClN_3O_{11}$ as established by HRESIMS, which implied the presence of one less unsaturation equivalent than **1**. The ^{1}H and ^{13}C NMR spectra of **2** were almost superimposable on those of **1** except for the replacement of the epoxide group by an oxymethine (δ_H 3.27, δ_C 75.6) and a chlorinated methine (δ_H 3.99, δ_C 66.2), suggesting that **2** was a HCl adduct of **1**. Interpretation of NMR and FAB-MS/MS data of **2** confirmed that **2** was the 7-hydroxy-8-chloro-derivative of **1**.

In order to determine the stereochemical relationship between C-6 and C-7 in **2**, its *N*-Cbz derivative **18** was converted to the acetonide **19** (Scheme 4). On the basis of NOESY correlations observed between one singlet methyl signal (δ_H 1.38) and H-6 and between another singlet methyl signal (δ_H 1.37) and H-7, the relative stereochemistry of C-6 and C-7 in **2** was assigned as identical with that in **1**. Treatment of **18** with potassium carbonate[19] in MeOH afforded the epoxide **3** ($[\alpha]^{20}_D$ −17.2 (*c* 0.10 MeOH)), which was indistinguishable from compound **3** derived from **1** ($[\alpha]^{20}_D$ −17.6 (*c* 0.10 MeOH)) in the $[\alpha]_D$ and ^{1}H NMR. Therefore, the absolute stereochemistry of **2** was determined to be 2*S*,6*S*,7*R*,8*R*,16*R*,30*S*,2′*R*,10′*S*.

Two classes of related serinolipid derivatives, didemniserinolipids[4] and cyclodidemniserinol trisulfate,[5] have been reported from tunicates of the genus *Didemnum*. The structure of didemniserinolipid B, whose serinol moiety was in the *S* configuration, was determined by total synthesis.[20] Shishididemniols, didemniserinolipids, and cyclodidemniserinol trisulfate all possess C_{28}-polyketide acid in the central part of the molecule. Unlike didemniserionlipids and cyclodidemniserinol trisulfate, shishididemniols A and B had two serinol and an oxygenated tyramine moieties and were not sulfated.

Shishididemniols A and B showed antibacterial activity in disk agar diffusion assay against the fish pathogenic bacterium *V. anguillarum* (20 μg/ 6.5 mm ϕ disk zone of inhibition; 8 and 7 mm for **1** and **2**, respectively).

Experimental Section

Animal Material. The tunicate was collected by hand using SCUBA at depths of 5−15 m off Kushizaki in Shishijima (32° 15′ N, 130° 14′ E) of the Amakusa Islands and was kept frozen at −20 °C until processed. The tunicate was identified as a member of the family Didemnidae on the basis of the presence of characteristic spicules.[21]

Extraction and Isolation. The frozen tunicates (780 g wet weight) were extracted four times with EtOH, and the combined extracts were concentrated and partitioned between water and $CHCl_3$. The aqueous layer was further extracted with *n*-BuOH. The *n*-BuOH fraction was separated by ODS flash chromatography with aqueous MeOH. The fraction eluted with 70% MeOH was separated by ODS HPLC (72% MeOH containing 0.2 M $NaClO_4$) to afford shishididemniols A (**1**, 395.4 mg, 5.1×10^{-2} % based on wet weight) and B (**2**, 151.6 mg, 1.9×10^{-2} % based on wet weight).

Shishididemniol A (1). Colorless oil; $[\alpha]^{20.5}_D$ −33.7 (*c* 1.00, MeOH); UV (MeOH) λ_{max} 275 nm (ϵ 1195), 281 (1017); IR (film) ν_{max} 3428, 1655 cm^{-1}; HRESIMS *m*/*z* 840.59682 $(M + H)^+$ (calcd for $C_{45}H_{82}N_3O_{11}$, Δ +1.89 mmu). ^{1}H and ^{13}C NMR data, see Table 1. HMBC correlations (DMSO-d_6) H-2/C-1; H-7/C-6, 8; H-8/C-7, 9; H-27/C-26, 28; H-28/C-27, 29; H-29a/C-28, 30, 31; H-29b/C-

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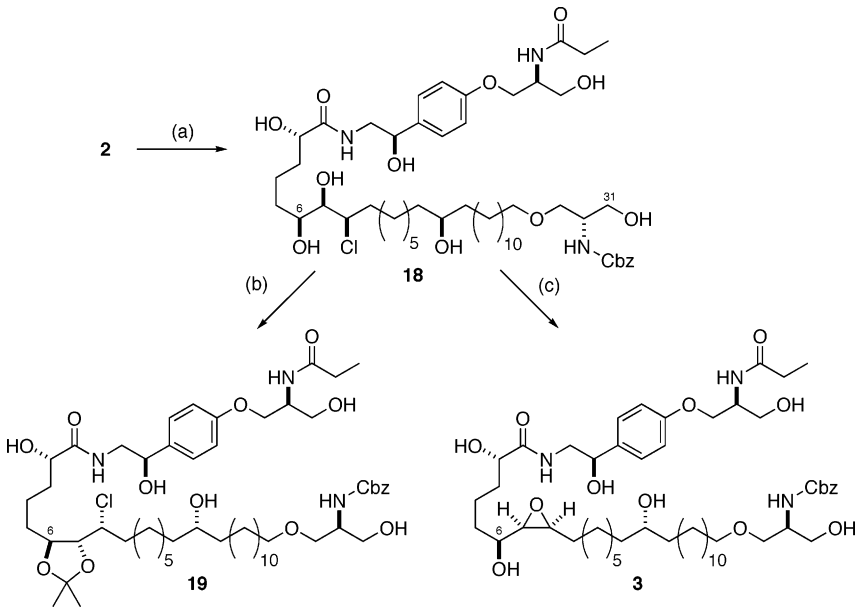
SCHEME 4 [a]



[a] Reagents and conditions: (a) Cbz-Cl, Et_3N, $CHCl_3$/MeOH (3:1), rt, 16 h; (b) 2,2-dimethoxypropane, PPTS, CH_2Cl_2, rt, 1 h; (c) K_2CO_3, MeOH, rt, 1.5 h.

28, 30, 31; H-1′a/C-1, 2′, 3′; H-1′b/C-1, 2′, 3′; H-2′/C-3′, 4′, 8′; H-4′, 8′/C-2′, 4′, 5′, 6′, 7′, 8′; H-5′, 7′/C-3′, 5′, 6′, 7′; H-9′a/C-6′, 10′, 11′; H-9′b/C-6′, 10′, 11′; H-10′/C-9′, 11′, 12′; H-13′/C-12′, 14′; H-14′/C-12′, 13′; NH-1′/C-1, 1′; OH-2′/C-1′, 2′, 3′; NH-10′/C-10′, 11′, 12′.

Shishididemniol B (2). Colorless oil; $[\alpha]^{20.5}_D$ −19.4 (*c* 1.00, MeOH); UV (MeOH) λ_{max} 275 nm (ϵ 1222), 281 (1043); IR (film) ν_{max} 3429, 1642 cm^{-1}; HRESIMS *m*/*z* 876.57161 (M + H)$^+$ (calcd for $C_{45}H_{83}{}^{35}ClN_3O_{11}$, Δ −1.21 mmu). ^{1}H and ^{13}C NMR data, see Table 1. HMBC correlations (DMSO-d_6) H-2/C-1, 3, 4; H-8/C-7, 9; H-27/C-26, 28; H-28/C-26, 27, 29; H-29a/C-28, 30, 31; H-29b/C-28, 30, 31; H-30/C-29, 31; H-31a/C-29, 30; H-1′a/C-1, 2′, 3′; H-1′b/C-1, 2′, 3′; H-2′/C-1′, 3′, 4′, 8′; H-4′, 8′/C-2′, 4′, 5′, 6′, 7′, 8′; H-5′, H-8′/C-3′, 4′, 5′, 6′, 7′, 8′; H-9′a/C-6′, 10′, 11′; H-9′b/C-6′, 10′, 11′; H-10′/C-9′, 11′, 12′; H-11′a/C-9′, 10′; H-11′b/C-9′, 10′; H-13′/C-12′, 14′; H-14′/C-12′, 13′.

Preparation of *N*-Cbz Derivative 3. To a solution of **1** (50 mg, 0.06 mmol) in $CHCl_3$/MeOH (3:1, 0.4 mL) were added triethylamine (66.4 μL) and Cbz-Cl (17 μL). After stirring for 16 h at room temperature, the reaction mixture was evaporated, suspended in H_2O, and extracted with EtOAc to give **3** (45 mg, 77.1%): ESIMS *m*/*z* 974.37505 (M + H)$^+$. ^{1}H NMR (600 MHz, CD_3OD) 7.35−7.27 (m, 5H), 7.29 (d, *J* = 8.7, 2H), 6.93 (d, *J* = 8.7, 2H), 5.09 (d, *J* = −12.3, 1H), 5.06 (d, *J* = −12.3, 1H), 4.71 (dd, *J* = 7.7, 4.6, 1H), 4.22 (m, 1H), 4.05 (dd, *J* = 4.8, 3.3, 2H), 3.99 (m, 1H), 3.78 (m, 1H), 3.69 (d, *J* = 6.0, 2H), 3.57 (m, 2H), 3.51−3.40 (m, 6H), 3.39−3.33 (m, 2H), 2.98 (ddd, *J* = 7.8, 4.4, 4.4 1H), 2.82 (dt, *J* = 4.4, 8.5, 1H), 2.24 (q, *J* = 7.8, 2H), 1.73−1.25 (m, 42H), 1.12 (t, *J* = 7.8, 3H).

Preparation of Bromohydrin 4. To a solution of **3** (40 mg) in dry Et_2O (2 mL) was added $MgBr_2$·OEt_2 (90 mg). The solution was stirred at room temperature for 4 h and was then evaporated. The residue was separated by ODS HPLC (gradient elution of 50−60% aqueous MeCN) to afford **4** (21 mg): ESIMS *m*/*z* 1054.38330 and 1056.35926 (M + H)$^+$. ^{1}H NMR (600 MHz, CD_3OD) 7.36−7.27 (m, 5H), 7.29 (d, *J* = 8.3, 2H), 6.93 (d, *J* = 8.3, 2H), 5.08 (d, *J* = −12.9, 1H), 5.05 (d, *J* = −12.9, 1H), 4.71 (dd, *J* = 7.6, 4.9, 1H), 4.23 (m, 1H), 4.12 (m, 1H), 4.05 (dd, *J* = 5.5, 1.8, 2H), 4.00 (m, 1H), 3.77 (m, 1H), 3.69 (d, *J* = 5.5, 2H), 3.67 (m, 1H), 3.57 (m, 2H), 3.52−3.40 (m, 6H), 3.38−3.31 (m, 2H), 2.24 (q, *J* = 7.6, 2H), 1.94−1.25 (m, 42H), 1.12 (t, *J* = 7.6, 3H).

Preparation of Acetonide 5. To the mixture of **4** (10 mg) and PPTS (pyridinium *p*-toluenesulfonate) (2.4 mg) in CH_2Cl_2 (100 μL) was added 2,2-dimethoxypropane (20 μL). The reaction mixture was stirred at room temperature for 1 h and was then evaporated. The residue was partition between EtOAc and H_2O. The organic layer was purified by ODS HPLC (gradient elution of 80−100% aqueous MeOH) to give **5** (6 mg): ESIMS *m*/*z* 1094.33124 and 1096.36362 (M + H)$^+$. ^{1}H NMR (600 MHz, CD_3OD) 7.39−7.25 (m, 5H), 7.30 (d, *J* = 8.7, 2H), 6.93 (d, *J* = 8.7, 2H), 5.08 (d, *J* = −12.6, 1H), 5.06 (d, *J* = −12.6, 1H), 4.70 (dd, *J* = 7.8, 4.6, 1H), 4.22 (m, 1H), 4.07 (br, 1H), 4.05 (dd, *J* = 5.5, 2.7, 2H), 3.99 (m, 2H), 3.78 (m, 1H), 3.69 (d, *J* = 5.5, 2H), 3.64 (dd, *J* = 7.7, 2.7, 1H), 3.57 (m, 2H), 3.52−3.40 (m, 6H), 3.35 (m, 1H), 2.24 (q, *J* = 7.5, 2H), 1.90−1.24 (m, 42H), 1.40 (s, 3H), 1.37 (s, 3H), 1.12 (t, *J* = 7.5, 3H).

Methanolysis of 1. A 60 mg portion of **1** was dissolved in 5 N HCl/MeOH (1:4), the solution was heated at 100 °C for 4 h, and the reaction mixture was cooled and dried. The residue was separated by ODS flash chromatography with H_2O, 20%, 60%, 80% and MeOH to give the mixture of **6** and **6a** (11.1 mg). The ^{1}H NMR data of **6a** was identical with that of **6** (vide infra).

Hydrazinolysis of 1. A 20 mg portion of **1** was dissolved in hydrazine (100 μL), the solution was heated at 120 °C for 15 h, and the reaction mixture was cooled and freeze-dried. The residue was separated by ODS flash chromatography with 20% aqueous MeOH and MeOH to give crude **6** (5.3 mg): ^{1}H NMR (600 MHz, CD_3OD) 7.34 (d, *J* = 8.7, 2H), 7.07 (d, *J* = 8.7, 2H), 4.41 (dd, *J* = 8.0, 5.7, 1H), 4.27 (dd, *J* = −10.1, 4.2, 1H), 2.52 (dd, *J* = −10.1, 7.1, 1H), 3.88 (dd, *J* = −11.5, 4.6, 1H), 3.81 (dd, *J* = −11.5, 6.0, 1H), 3.66 (m, 1H), 3.06 (d, 7.8, 2H).

Preparation of (*R*)- and (*S*)-MTPA Derivatives of 6 (10a and 10b). To a solution of crude **6** (1.5 mg) in pyridine (30 μL) was added (*S*)-MTPACl (20 μL). The residue was partitioned between EtOAc and H_2O with 0.1 M Na_2CO_3.The organic layer was purified by ODS HPLC (gradient elution of 75−100% aqueous MeOH) to yield (*R*)-MTPA derivative **10a** (0.3 mg). The (*S*)-MTPA derivative **10b** (0.5 mg) was prepared in the same way. **10a**: ^{1}H NMR (600 MHz, CD_3OD) 7.62−7.20 (m, 20H; aromatic), 7.08 (d, *J* = 8.5, 2H; H-4′, H-8′), 6.73 (d, *J* = 8.5, 2H; H-3′, H-5′), 6.06 (dd, *J* = −9.0, 4.8, 1H; H-2′), 4.62 (m, 1H; H-10′), 4.62 (m, 1H; H-11′a), 4.52 (m, 1H; H-11′b), 4.01 (dd, −10.3, 5.2, 1H; H-9′a), 3.97 (dd, −10.3, 5.9, 1H; H-9′b), 3.74 (m, 1H; H-1′a), 3.54 (m, 1H; H-1′b),

3.54 (s, 3H; OMe in MTPA), 3.50 (s, 3H; OMe in MTPA), 3.37 (s, 3H; OMe in MTPA), 3.30 (s, 3H; OMe in MTPA). **10b**: ^{1}H NMR (600 MHz, CD_3OD) 7.64−7.26 (m, 22H; aromatic in MTPA, H-4′, H-8′), 6.87 (d, J = 8.2, 2H; H-5′, H-7′), 6.13 (t, J = 6.9, 1H; H-2′), 4.62 (m, 1H; H-10′), 4.58 (m, 1H; H-11′a), 4.49 (dd, J = −11.0, 6.2, 1H; H-11′b), 4.02 (m, 2H; H_2-9′), 3.68 (d, 6.2, 2H; H_2-1′), 3.39 (s, 3H; OMe in MTPA), 3.35 (s, 3H; OMe in MTPA), 3.34 (s, 3H; OMe in MTPA), 3.20 (s, 3H; OMe in MTPA).

Synthesis of 7. To a solution of *N*-Boc-L-serine methyl ester (550 mg, 2.5 mmol) and *p*-toluenesulfonic acid monohydrate (6.5 mg, 0.03 mmol) in dry toluene (6 mL) was added 2,2-dimethoxypropane (0.6 mL). The reaction mixture was refluxed for 30 min with stirring and evaporated. The residue was subjected to silica gel flash chromatography (*n*-hexane/EtOAc = 4:1) to yield 3-(1,1-dimethylethyl)-4-methyl-*S*-2,2-dimethyl-3,4-oxazolidinedicarboxylate (556 mg, 2.15 mmol, 85.9% yield). To a solution of 3-(1,1-dimethylethyl)-4-methyl-*S*-2,2-dimethyl-3,4-oxazolidinedicarboxylate (556 mg, 2.15 mmol) in THF/MeOH (95:5, 13.2 mL) was added $LiBH_4$ (93 mg, 4.26 mmol). The reaction mixture was stirred at room temperature for 2 h and then evaporated. The residue was purified by silica gel flash chromatography (*n*-hexane/EtOAc = 1:1) to obtain **7** (416 mg, 1.82 mmol, 84.5% yield). **3-(1,1-Dimethylethyl)-4-methyl-*S*-2,2-dimethyl-3,4- oxazolidinedicarboxylate**: ^{1}H NMR (600 MHz, $CDCl_3$) 4.46−3.96 (m, 3H; CH_2-O, C*H*-N), 3.71 (s, 3H; OMe), 1.66−1.34 (m, 15H; five methyls). **7**: ^{1}H NMR (600 MHz, $CDCl_3$) 4.14−3.56 (m, 5H; CH_2-O × 2, C*H*-N), 1.60−1.41 (m, 15H; five methyls).

Preparation of 9. To a mixture of **7** (277 mg, 1.20 mmol), phenol (113 mg, 1.20 mmol), and triphenylphosphine (314 mg, 1.20 mmol) in toluene (3 mL) was added diethyl azodicarboxylate (628 μL (40% solution in toluene), 1.21 mmol). The reaction mixture was stirred for 18 h at 80 °C and evaporated. The residue was subjected to silica gel flash chromatography (*n*-hexane/EtOAc = 4:1) to yield **8** (226 mg, 0.74 mmol, 61.4% yield). To a solution of **8** 150 mg (0.49 mmol) in CH_2Cl_2 (2 mL) was added TFA (1 mL). The reaction mixture was stirred at room temperature for 2 h and then evaporated to yield **9** as a TFA salt (135 mg, 0.48 mmol, 98.0% yield). **8**: ^{1}H NMR (600 MHz, $CDCl_3$) 7.28−6.89 (m, 5H; aromatic), 4.30−3.79 (m, 5H; CH_2-O × 2, C*H*-N), 1.63−1.44 (m, 15H; five methyls). **9**: ^{1}H NMR (600 MHz, CD_3OD) 7.29 (t, J = 7.7, 2H; aromatic), 7.01−6.95 (m, 3H; aromatic), 4.22 (dd, J = −10.5, 3.9, 1H; CH_{2a}-O), 4.16 (dd, J = −10.5, 6.9, 1H; CH_{2b}-O), 3.88 (dd, J = −11.6, 4.6, 1H; CH_{2a}-O), 3.81 (dd, J = −11.6, 5.6, 1H; CH_{2b}-O), 3.63 (m, 1H; C*H*-N).

(*R*)- and (*S*)-MTPA Derivatives of 9 (11a and 11b). To a solution of **9** (8 mg) in pyridine (50 μL) was added (*S*)-MTPACl (40 μL). The reaction mixture was stirred at room temperature for 30 min and then evaporated. The residue was partitioned between EtOAc and H_2O with 0.1 M Na_2CO_3.The organic layer was subjected to silica gel flash chromatography with $CHCl_3$ to yield (*R*)-MTPA derivative **11a** (10.9 mg). The (*S*)-MTPA derivative **11b** (11.0 mg) was prepared in the same way. **11a**: ^{1}H NMR (600 MHz, CD_3OD) 7.58−6.77 (m, 15H; aromatic), 4.06 (m, 1H; CH_{2a}-OPh), 3.98 (m, 1H; CH_{2b}-OPh), 4.61 (m, 1H; C*H*-N), 4.64 (m, 1H; CH_{2a}-OMTPA), 4.54 (m, 1H; CH_{2a}-OMTPA), 3.53 (s, 3H; OMe in MTPA), 3.49 (s, 3H; OMe, in MTPA). **11b**: ^{1}H NMR (600 MHz, CD_3OD) 7.58−6.81 (m, 15H; aromatic), 4.00 (m, 2H; CH_2-OPh), 4.60 (m, 1H; C*H*-N), 4.57 (m, 1H; CH_{2a}-OMTPA), 4.47 (m, 1H; CH_{2a}-OMTPA), 3.52 (s, 3H; OMe in MTPA), 3.48 (s, 3H; OMe, in MTPA).

Preparation of (*R*)- and (*S*)-MTPA Derivatives of (*R*)-2-Amino-1-phenylethanol (12a and 12b). To a solution of the commercially available (*R*)-2-amino-1-phenylethanol (20 mg) in pyridine (50 μL) was added (*S*)-MTPACl (60 μL). The reaction mixture was stirred at room temperature for 30 min and then evaporated. The residue was partitioned between EtOAc and H_2O with 0.1 M Na_2CO_3. The organic layer was subjected to silica gel flash chromatography with $CHCl_3$ to yield (*R*)-MTPA derivative **12a** (14 mg). The (*S*)-MTPA derivative **12b** (18 mg) was prepared in the same way. **12a**: ^{1}H NMR (600 MHz, CD_3OD) 7.46−7.14 (m, 15H; aromatic), 6.12 (m, 1H; C*H*-O), 3.74 (m, 1H; CH_{2a}-N), 3.57 (m, 1H; CH_{2b}-N), 3.38 (s, 3H; OMe in MTPA), 3.30 (s, 3H; OMe, in MTPA). **12b**: ^{1}H NMR (600 MHz, CD_3OD) 7.44−7.32 (m, 15H; aromatic), 6.17 (m, 1H; C*H*-O), 3.68(m, 2H; CH_2-N), 3.40 (s, 3H; OMe in MTPA), 3.20 (s, 3H; OMe, in MTPA).

Preparation of (*R*)- and (*S*)-MTPA Derivatives of 1 (13a and 13b). To a solution of **1** (5 mg) in pyridine (50 μL) was added (*S*)-MTPACl (60 μL). The reaction mixture was stirred at room temperature for 30 min and then evaporated. The residue was partitioned between EtOAc and H_2O with 0.1 M Na_2CO_3.The organic layer was purified by ODS HPLC (gradient elution of 90−100% aqueous MeOH) to yield (*R*)-MTPA derivative **13a** (6 mg). The (*S*)-MTPA derivative **13b** (5.3 mg) was prepared in the same way. **13a**: ^{1}H NMR (600 MHz, CD_3OD) 5.07 (m, 1H; H-2), 1.74 (m, 1H; H_2-3a), 1.86 (m, 1H; H_2-3b), 1.26 (m, 1H; H_2-4a), 1.45 (m, 1H; H_2-4b), 1.62 (m, 1H; H_2-5a), 1.75 (m, 1H; H_2-5b), 5.00 (m, 1H; H-6), 2.90 (dd, J = 9.0, 4.2, 1H; H-7), 2.98 (m, 1H; H-8), 5.07 (m, 1H; H-16), 1.26 (m, 2H; H_2-26), 1.46 (m, 2H; H_2-27), 3.33 (m, 2H; H_2-28), 3.41 (m, 1H; H_2-29a), 3.39 (m, 1H; H_2-29b), 4.39 (m, 1H; H-30), 4.50 (dd, J = −14.4, 7.6, 1H; H_2-31a), 4.39 (m, 1H; H_2-31b), 3.67 (m, 1H; H_2-1′a), 3.56 (dd, J = −14.4, 6,2, 1H; H_2-1′b), 6.01 (t, J = 6.2, 1H; H-2′), 7.14 (d, J = 8.9, 2H; H-4′/8′), 6.67 (d, J = 8.9, 2H; H-5′/7′), 4.11 (dd, J = −9.6, 8.3, 1H; H_2-9′a), 3.97 (dd, J = −9.6, 5.5, 1H; H_2-9′b), 4.76 (m, 1H; H-10′), 4.59 (dd, J = −11.0, 8.3, 1H; H_2-11′a), 3.86 (m, 1H; H_2-11′b), 2.53 (m, 1H, H_2-13′a), 2.35 (m, 1H; H_2-13′b), 0.95 (t, J = 7.3, 3H; H_3-14′). **13b**: ^{1}H NMR (600 MHz, CD_3OD) 4.93 (m, 1H; H-2), 1.51 (m, 1H; H_2-3a), 1.65 (m, 1H; H_2-3b), 1.15 (m, 1H; H_2-4a), 1.31 (m, 1H; H_2-4b), 1.46 (m, 1H; H_2-5a), 1.58 (m, 1H; H_2-5b), 4.92 (m, 1H; H-6), 2.96 (dd, J = 9.3, 4.2, 1H; H-7), 3.02 (m, 1H; H-8), 5.08 (m, 1H; H-16), 1.31 (m, 2H; H_2-26), 1.52 (m, 2H; H_2-27), 3.40 (m, 2H; H_2-28), 3.47 (dd, J = −10.3, 5.5, 1H; H_2-29a), 3.43 (dd, J = −10.3, 6.2, 1H; H_2-29b), 4.39 (m, 1H; H-30), 4.50 (dd, J = −11.0, 4.1, 1H; H_2-31a), 4.34 (dd, J = −11.0, 6.9, 1H; H_2-29a), 3.63 (dd, J = −14.5, 6,2, 1H; H_2-1′a), 3.56 (m, 1H; H_2-1′b), 6.04 (t, J = 6.2, 1H; H-2′), 7.26 (d, J = 8.9, 2H; H-4′/8′), 6.61 (d, J = 8.9, 2H; H-5′/7′), 3.93 (dd, J = −9.3, 6.6, 1H; H_2-9′a), 3.74 (br, 1H; H_2-9′b), 4.70 (m, 1H; H-10′), 4.73 (dd, J = −11.7, 5.5, 1H; H_2-11′a), 4.54 (dd, J = −11.7, 6.2, 1H; H_2-11′b), 2.52 (m, 1H, H_2-13′a), 2.25 (m, 1H; H_2-13′b), 0.97 (t, J = 7.2, 3H; H_3-14′).

Preparation of (*R*)- and (*S*)-MTPA Derivatives of 5 (14a and 14b). To a solution of **5** (2 mg) in pyridine (50 μL) was added (*S*)-MTPACl (40 μL). The reaction mixture was stirred at room temperature for 30 min and then evaporated. The residue was partitioned between EtOAc and H_2O with 0.1 M Na_2CO_3. The organic layer was purified by ODS HPLC (gradient elution of 90−100% aqueous MeOH) to yield (*R*)-MTPA derivative **14a** (3.3 mg). The (*S*)-MTPA derivative **14b** (3.6 mg) was prepared in the same way. **14a**: ^{1}H NMR (600 MHz, CD_3OD) 5.08 (m, 1H; H-2), 1.79 (m, 1H; H_2-3a), 1.55 (m, 1H; H_2-3b), 1.55 (m, 1H; H_2-4a), 1.42 (m, 1H; H_2-4b), 1.53 (m, 1H; H_2-5a), 1.45 (m, 1H; H_2-5b), 3.95 (m, 1H; H-6), 3.62 (m, 1H; H-7), 4.02 (m, 1H; H-8), 5.08 (m, 1H; H-16), 3.38 (m, 2H; H_2-28), 3.41 (m, 2H; H_2-29), 4.06 (m, 1H; H-30), 4.40 (dd, J = −11.2, 5.0, 1H; H_2-31a), 4.34 (dd, J = −11.2, 7.3, 1H; H_2-31b), 3.66 (m, 1H; H_2-1′a), 3.59 (m, 1H; H_2-1′b), 6.00 (dd, J = 7.3, 5.5, 1H; H-2′), 7.15 (d, J = 8.7, 2H; H-4′/8′), 6.68 (d, J = 8.7, 2H; H-5′/7′), 4.11 (dd, J = −10.5, 9.0, 1H; H_2-9′a), 3.97 (dd, J = −10.5, 5.5, 1H; H_2-9′b), 4.77 (m, 1H; H-10′), 4.60 (dd, J = −11.0, 8.5, 1H; H_2-11′a), 3.84 (br, 1H; H_2-11′b), 2.54 (m, 1H, H_2-13′a), 2.37 (m, 1H; H_2-13′b), 0.96 (t, J = 7.4, 3H; H_3-14′a), 5.06 (d, J = −12.4, 1H; CH_2 in Cbz), 5.03 (d, J = −12.4, 1H; CH_2 in Cbz), 1.39 (s, 3H; CH_3 in acetonide), 1.34 (s, 3H; CH_3 in acetonide). **14b**: ^{1}H NMR (600 MHz, CD_3OD) 5.00 (dd, J = 8.7, 4.1, 1H; H-2), 1.66 (m, 1H; H_2-3a), 1.41 (m, 1H; H_2-3b), 1.35 (m, 1H; H_2-4a), 1.24 (m, 1H; H_2-4b), 1.47 (m, 1H; H_2-5a), 1.37 (m, 1H; H_2-5b), 3.87 (m, 1H; H-6), 3.58 (m, 1H; H-7), 3.98 (m, 1H; H-8), 5.08 (m, 1H; H-16), 3.38 (t, J = 6.4, 2H; H_2-28), 3.40

(m, 2H; H_2-29), 4.05 (m, 1H; H-30), 4.42 (dd, $J = -11.0$, 5.0, 1H; H_2-31a), 4.34 (dd, $J = -11.0$, 6.9, 1H; H_2-31b), 3.68 (dd, $J = -14.0$, 6.2, 1H; H_2-1′a), 3.57 (m, 1H; H_2-1′b), 6.05 (dd, $J = 7.3$, 6.5, 1H; H-2′), 7.27 (d, $J = 8.7$, 2H; H-4′/8′), 6.62 (d, $J = 8.7$, 2H; H-5′/7′), 3.93 (dd, $J = -11.6$, 6.4, 1H; H_2-9′a), 3.75 (br, 1H; H_2-9′b), 4.70 (m, 1H; H-10′), 4.74 (dd, $J = -11.5$, 5.5, 1H; H_2-11′a), 3.54 (dd, $J = -11.5$, 5.6, 1H; H_2-11′b), 2.53 (m, 1H, H_2-13′a), 2.26 (m, 1H; H_2-13′b), 0.98 (t, $J = 7.3$, 3H; H_3-14′a), 5.03 (s, 2H; CH_2 in Cbz), 1.38 (s, 3H; CH_3 in acetonide), 1.33 (s, 3H; CH_3 in acetonide).

Preparation of 16. A 10 mg portion of **4** was treated with $NaIO_4$ (20 mg) in MeOH/H_2O (4:1, 1 mL) at room temperature for 1 h, followed by reduction with $NaBH_4$ (23 mg). The reaction mixture was evaporated, and the residue was purified by ODS HPLC (gradient elution of 80−100% aqueous MeOH) to obtain **15** (5.5 mg). To a solution of **15** (4 mg) in pyridine (50 μL) was added *tert*-butyldiphenylchlorosilane (TBDPSCl) (10 μL). After stirring at room temperature overnight, the reaction mixture was evaporated, suspended in H_2O, and extracted with EtOAc. The organic layer was evaporated, and the residue was separated by silica gel flash chromatography (*n*-hexane/EtOAc = 4:1) to yield **16** (4.6 mg). **15**: ESIMS *m*/*z* 644.22893 and 646.23454 $(M + H)^+$. ^{1}H NMR (600 MHz, $CDCl_3$) 7.36−7.28 (m, 5H), 5.43 (m, 1H), 5.09 (s, 2H), 4.12 (m, 1H), 3.85−3.53 (m, 8H), 3.40 (t, $J = 6.7$, 2H), 1.85−1.20 (m, 36H). **16**: ESIMS *m*/*z* 1120.39025 and 1122.40749 $(M + H)^+$. ^{1}H NMR (600 MHz, benzene-d_6) 7.80−7.72 (m, 5H), 7.26−7.20 (m, 16H), 5.10 (s, 2H), 5.03 (d, $J = 8.7$, 1H), 4.20 (m, 1H), 3.97−3.85 (m, 3H), 3.83 (dd, $J = -10.5$, 6.0, 1H), 3.74 (dd, $J = -9.4$, 6.4, 1H), 3.57 (dd, $J = -8.6$, 3.9, 1H), 3.46 (m, 1H), 3.37 (dd, $J = -8.6$, 5.5, 1H), 3.20 (t, $J = 6.4$, 2H), 1.86−1.17 (m, 36H), 1.20 (s, 9H), 1.14 (s, 9H).

Preparation of (*R*)- and (*S*)-2NMA Derivatives (17a and 17b). To a solution of **16** (1.5 mg) in CH_2Cl_2 (300 μL) were added (*R*)-2-naphthylmethoxyacetic acid ((*R*)-2NMA), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) (5 mg), DMAP (0.5 mg), and DMAP·HCl (0.5 mg), and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated and partitioned between EtOAc and H_2O. The organic layer was subjected to silica gel flash chromatography (*n*-hexane/EtOAc = 9:1) to obtain the (*R*)-2NMA derivative **17a** (1.5 mg). The (*S*)-2NMA derivative **17b** (1.5 mg) was prepared in the same way. **17a**: ^{1}H NMR (600 MHz, C_6D_6) 8.67−7.00 (m, 32H, aromatic), 5.37 (s, 1H, -C*H*-O in 2NMA), 5.09 (s, 2H; -CH_2- in Cbz), 5.08 (m, 1H; H-16), 5.01 (d, $J = 8.7$, 1H; NH-30), 4.20 (m, 1H; H-30), 3.95 (m, 1H; H-8), 3.92 (m, 1H; H-7a), 3.89 (m, 1H; H-31a), 3.85 (dd, $J = -10.6$, 5.4, 1H; H-7b), 3.73 (dd, $J = -9.6$, 6.8, 1H; H-31b), 3.57 (br, 1H; H-29a), 3.36 (br, 1H; H-29b), 3.28 (s, 3H; OMe in 2NMA), 3.20 (m, 2H; H_2-28), 1.79 (m, 1H; H-9a), 1.72 (m, 1H; H-9b), 1.46 (m, 1H; H-10a), 1.29 (m, 1H; H-10b), 1.55−0.80 (m, 32H; H_2-11 to H_2-15, H_2-17-H_2-27), 1.19 (s, 9H; *tert*-butyl), 1.13 (s, 9H; *tert*-butyl). **17b**: ^{1}H NMR (600 MHz, C_6D_6) 8.03−7.02 (m, 32H, aromatic), 5.12 (m, 1H; H-16), 5.09 (s, 2H; -CH_2- in Cbz), 5.01 (d, $J = 8.7$, 1H; NH-30), 4.92 (s, 1H, -C*H*-O in 2NMA), 4.20 (m, 1H; H-30), 3.92 (m, 1H; H-8), 3.90 (m, 1H; H-7a), 3.89 (m, 1H; H-31a), 3.83 (dd, $J = -10.6$, 5.4, 1H; H-7b), 3.73 (dd, $J = -9.6$, 6.8, 1H; H-31b), 3.57 (br, 1H; H-29a), 3.36 (br, 1H; H-29b), 3.30 (s, 3H; OMe in 2NMA), 3.20 (m, 2H; H_2-28), 1.72 (m, 1H; H-9a), 1.65 (m, 1H; H-9b), 1.34 (m, 1H; H-10a), 1.17 (m, 1H; H-10b), 1.61−0.78 (m, 32H; H_2-11 to H_2-15, H_2-17-H_2-27), 1.19 (s, 9H, *tert*-butyl), 1.13 (s, 9H, *tert*-butyl).

Preparation of *N*-Cbz Derivative 18. To a solution of **2** (40 mg, 0.046 mmol) in $CHCl_3$/MeOH (3:1, 0.4 mL) were added triethylamine (66.4 μL) and Cbz-Cl (17 μL). After stirring at room temperature for 16 h, the reaction mixture was evaporated, suspended in H_2O, and extracted with EtOAc. The organic layer was purified by ODS HPLC (gradient elution of 70−100% aqueous MeOH) to afford **18** (21.7 mg, 46.7%). **18**: ESIMS *m*/*z* 1010.45044 and 1012.44065 $(M + H)^+$. ^{1}H NMR (600 MHz, CD_3OD) 7.37−7.26 (m, 5H), 7.29 (d, $J = 8.7$, 2H), 6.94 (d, $J = 8.3$, 2H), 5.08 (d, $J = -12.3$, 1H), 5.05 (d, $J = -12.3$, 1H), 4.71 (dd, $J = 7.6$, 4.8, 1H), 4.23 (m, 1H), 4.05 (dd, $J = 5.5$, 2.3, 2H), 4.03−3.99 (m, 2H), 3.78 (m, 1H), 3.70 (d, $J = 5.5$, 2H), 3.67 (m 1H), 3.58 (m, 2H), 3.52−3.39 (m, 7H), 3.35 (m, 1H), 2.24 (q, $J = 7.8$, 2H), 1.89−1.25 (m, 42H), 1.12 (t, $J = 7.8$, 3H).

Preparation of Acetonide 19 from 18. To the mixture of **18** (5 mg) and PPTS (1.7 mg) in CH_2Cl_2 (50 μL) was added 2,2-dimethoxypropane (10 μL). The reaction mixture was stirred at room temperature for 1 h and was then evaporated. The residue was partition between EtOAc and H_2O. The organic layer was purified by ODS HPLC (gradient elution of 80−100% aqueous MeOH) to give **19** (5.2 mg): ESIMS *m*/*z* 1050.45589 and 1052.50406 $(M + H)^+$. ^{1}H NMR (600 MHz, CD_3OD) 7.37−7.26 (m, 5H), 7.30 (d, $J = 8.7$, 2H), 6.93 (d, $J = 8.7$, 2H), 5.08 (d, $J = -12.3$, 1H), 5.06 (d, $J = -12.3$, 1H), 4.70 (dd, $J = 7.5$, 4.8, 1H), 4.22 (m, 1H), 4.05 (dd, $J = 5.6$, 2.7, 2H), 4.04−3.98 (m, 2H), 3.96 (m, 1H), 3.80−3.74 (m, 2H), 3.69 (d, $J = 5.4$, 2H), 3.57 (m, 2H), 3.52−3.39 (m, 6H), 3.35 (m, 1H), 2.24 (q, $J = 7.8$, 2H), 1.84−1.25 (m, 42H), 1.38 (s, 3H), 1.37 (s, 3H), 1.12 (t, $J = 7.8$, 3H).

Preparation of 3 from 18. To a solution of **18** (5 mg) in 500 μL of MeOH was added potassium carbonate (18 mg). The reaction mixture was stirred at room temperature for 1.5 h, quenched with acetic acid, and then evaporated. The residue was purified by ODS HPLC (gradient elution of 50−60% aqueous MeCN) to yield **3** (3.5 mg) whose ^{1}H NMR spectrum and $[\alpha]_D$ value (**3** from **18**: $[\alpha]^{20}_D$ −17.2 (*c* 0.10 MeOH), **3** from **1**: $[\alpha]^{20}_D$ −17.6 (*c* 0.10 MeOH)) were indistinguishable from those of **3** prepared from **1**.

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Supporting Information Available: FAB-MS/MS data of **1** and **2**; ^{1}H NMR, ^{13}C NMR, COSY, TOCSY, HMBC, and HSQC spectra of **1** and **2** in DMSO-d_6; ^{1}H NMR spectra of **3**−**19**; ^{1}H NMR, COSY, and TOCSY spectra of **13a** and **13b** in CD_3OD; ^{1}H NMR and COSY spectra of **14a** and **14b** in CD_3OD; and NOESY spectra of **5** and **19** in CD_3OD. This material is available free of charge via the Internet at http://pubs.acs.org.

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