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The structures of three new shishididemniols from a tunicate of the family Didemnidae

Hirotsugu Kobayashi,[a] Yoshinari Miyata,[a] Kohtaro Okada,[a] Tsuyoshi Fujita,[b] Takashi Iwashita,[b] Yoichi Nakao,[a] Nobuhiro Fusetani[a] and Shigeki Matsunaga[a,*]

[a]*Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan*
[b]*Suntory Institute for Bioorganic Research, Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618-8503, Japan*

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Abstract—Three new serinolipid derivatives, shishididemniols C (**1**), D (**2**), and E (**3**), were isolated as antibacterial constituents of a tunicate of the family Didemnidae. Their planar structures were elucidated by interpretation of NMR and MS data, whereas the absolute stereochemistry was determined by chemical conversions. Shishididemniols C (**3**), D (**4**), and E (**5**) exhibited antibacterial activity against the fish pathogenic bacterium *Vibrio anguillarum*.

1. Introduction

Several polyoxygenated C_{28}-carboxylic acid derivatives with a serinol ether moiety, such as didemniserinolipids[1,2] and cyclodidemniserinol trisulfate,[3] have been reported from tunicates of the family Didemnidae. Recently, we reported the structures of shishididemniols A (**1**) and B (**2**), which are octopamyl amide of the C_{28}-polyoxygenated carboxylic acid whose both termini are each etherified by a serinol moiety.[4] Further separation of the antibacterial fraction of the extract of the tunicate afforded three new congeners, shishididemniols C (**3**), D (**4**), and E (**5**) (Fig. 1). In the present paper we report the isolation, structure elucidation, and antimicrobial activity of these new metabolites.

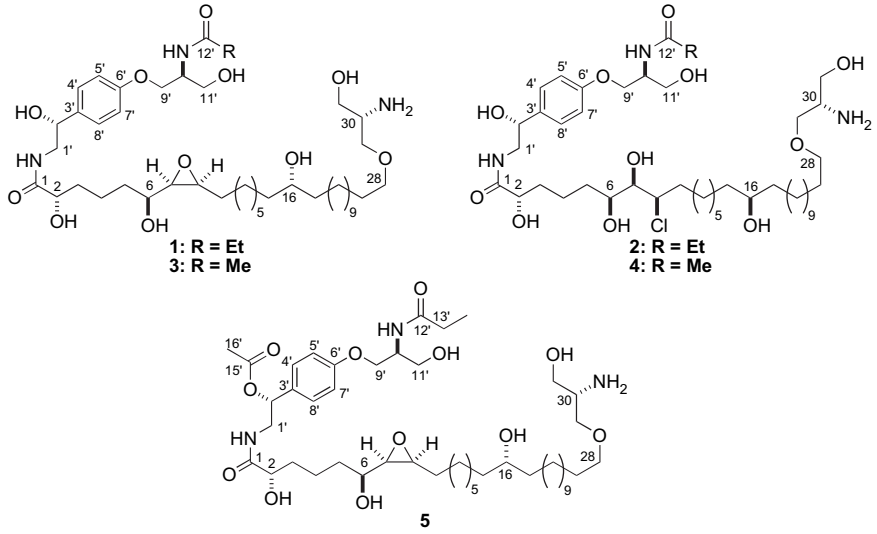

Figure 1. Structures of shishididemniols A (**1**)—E (**5**).

Keywords: Antibacterial; Serinolipid; Tunicate; *Vibrio anguillarum*.
* Corresponding author. Fax: +81 3 5841 8166; e-mail: assmats@mail.ecc.u-tokyo.ac.jp

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2. Results and discussion

ODS HPLC of an antibacterial fraction obtained by ODS flash chromatography[4] afforded two peaks in addition to the peaks that contained **1** and **2**: one of the peak contained shishididemniol C (**3**), whereas another peak was a mixture of two compounds. The latter HPLC peak was further separated by ODS HPLC using MeCN as the organic component of the mobile phase to give shishididemniols D (**4**) and E (**5**).

Shishididemniol C (**3**) had a molecular formula of $C_{44}H_{79}N_3O_{11}$, which was smaller than that of shishididemniol A (**1**) by a CH_2 unit. The ^1H and ^{13}C NMR spectra of **3** were almost superimposable on those of **1** except for the replacement of the ethyl group by a methyl group (δ_H 1.82 and δ_C 22.7). Interpretation of the NMR and FABMS/MS data of **3** led to its gross structure as the lower homolog of shishididemniol A (Fig. 2).

The relative stereochemistry of the part from C-6 to C-8 in **3** was assigned as *threo*,*cis* (6*S**,7*R**,8*S**) by comparison of ^1H and ^{13}C NMR data with those of **1** (Tables 1 and 2, and Ref. 4). The absolute stereochemistry of C-2, C-6, C-30, C-2′, and C-10′ in **3** was determined by application of the modified Mosher's method.[5,6] Treatment of **3** with (*S*)- and (*R*)-MTPACl yielded the (*R*)- and (*S*)-MTPA derivatives (**6a** and **6b**, respectively). Analysis of $\Delta\delta_{S-R}$ values between **6a** and **6b** allowed the assignment of 6*S* and 30*S*.

The absolute stereochemistry of C-2, C-2′, and C-10′ in **3** was determined by comparison of ^1H NMR spectra of **6a** and **6b** with those of (*R*)- and (*S*)-MTPA derivatives of **1**. ^1H NMR chemical shift values of H_2-3, H_2-4, and H_2-5 of **6a** and **6b** were in good agreement with those of the corresponding signals of (*R*)- and (*S*)-MTPA derivatives of **1**, respectively, indicating that the absolute stereochemistry of C-2 was *S*. Absolute stereochemistry of C-2′ and C-10′ was assigned as *R* and *S*, respectively, because the chemical shift values of H_2-1′, H-2′, H-4′/8′, H-5′/7′, H_2-9′, H-10′, and H_2-11′ were similar to those of the corresponding signals of (*R*)- and (*S*)-MTPA derivatives of **1** (Fig. 3).[4]

Table 1. ^{13}C NMR data for shishididemniols C (**3**), D (**4**), and E (**5**) in DMSO-d_6[a]

Position	3	4	5
	δ_C (mult.[b] *J* in Hz)		
1	173.9 (s)	173.9 (s)	174.2 (s)
2	70.8 (d)	70.9 (d)	70.8 (d)
3	34.4 (t)	34.4 (t)	34.4 (t)
4	20.4 (t)	21.0 (t)	20.3 (t)
5	33.7 (t)	32.6 (t)	33.7 (t)
6	68.7 (d)	70.9 (d)	68.6 (d)
7	60.5 (d)	75.6 (d)	60.5 (d)
8	56.2 (d)	66.3 (d)	56.2 (d)
9	27.9 (t)	34.4 (t)	27.8 (t)
10–14	28.9–29.3	29.1–29.3	28.9–29.3
15	37.2 (t)	37.2 (t)	37.2 (t)
16	69.6 (d)	69.5 (d)	69.5 (d)
17	37.2 (t)	37.2 (t)	37.2 (t)
18–25	28.8–29.3	29.1–29.3	28.9–29.3
26	25.3 (t)	25.5 (t)	25.7 (t)
27	28.9 (t)	28.9 (t)	28.9 (t)
28	70.8 (t)	70.7 (t)	70.4 (t)
29	67.5 (t)	68.5 (t)	72.9 (t)
30	52.0 (d)	52.1 (d)	52.5 (d)
31	58.8 (t)	59.6 (t)	63.7 (t)
1′	46.2 (t)	46.2 (t)	43.0 (t)
2′	70.8 (d)	70.8 (d)	73.4 (d)
3′	135.6 (s)	135.7 (s)	130.5 (s)
4′,8′	127.1 (d)	127.1 (d)	127.8 (d)
5′,7′	114.1 (d)	114.0 (d)	114.4 (d)
6′	157.7 (s)	157.6 (s)	158.3 (s)
9′	66.5 (t)	66.4 (t)	66.5 (t)
10′	50.3 (d)	50.3 (d)	50.2 (d)
11′	60.1 (t)	60.0 (t)	60.0 (t)
12′	169.4 (s)	169.3 (s)	173.1 (s)
13′	22.7 (q)	22.7 (q)	28.4 (t)
14′			9.90 (q)
15′			169.7 (s)
16′			20.9 (q)

[a] ^{13}C NMR: 150 MHz.
[b] Multiplicity from HSQC experiment.

The absolute stereochemistry of C-16 in **3** was assigned by using the same method that was applied to **1**. For that purpose **3** was converted to its *N*-Cbz derivative **7**, which was treated with $MgBr_2 \cdot Et_2O$ in THF to yield the bromohydrin

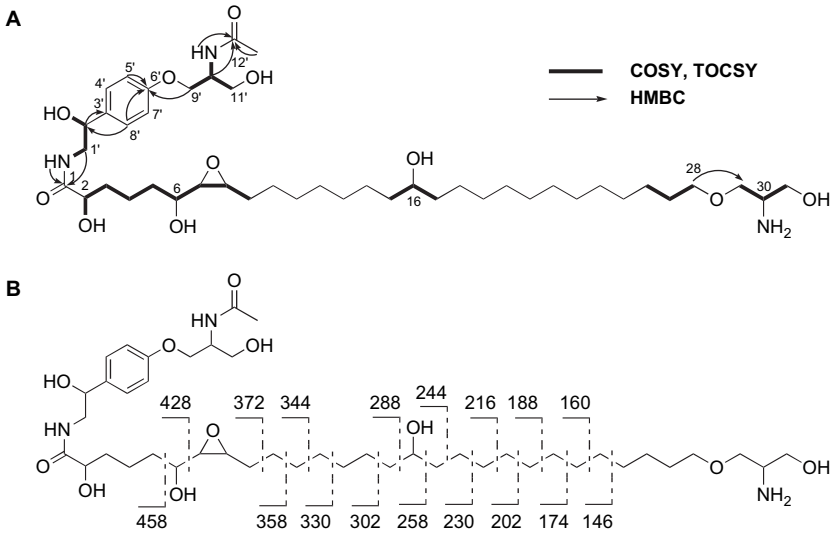


Figure 2. (A) Key 2D NMR correlations of **3**. (B) FABMS/MS analysis of **3**.

Table 2. ^{1}H NMR data for shishididemniols C (**3**), D (**4**), and E (**5**) in DMSO-d_6[a]

Position	3	4	5
	δ_H (mult. J in Hz)		
2	3.80 (br)	3.80 (br)	3.79 (br)
3a	1.42 (m)	1.43 (m)	1.42 (m)
3b	1.55 (m)	1.56 (m)	1.52 (m)
4a	1.32 (m)	1.31 (m)	1.33 (m)
4b	1.42 (m)	1.43 (m)	1.42 (m)
5a	1.30 (m)	1.30 (m)	1.28 (m)
5b	1.41 (m)	1.37 (m)	1.40 (m)
6	3.19 (br)	3.45 (m)	3.19 (br)
7	2.69 (dd, 4.6, 8.5)	3.27 (t, 4.4)	2.69 (dd, 4.4, 8.3)
8	2.87 (ddd, 4.6, 4.6, 8.5)	3.99 (m)	2.87 (m)
9a	1.29 (m)	1.67 (m)	1.28 (m)
9b	1.53 (m)	1.77 (m)	1.53 (m)
10–14	1.15–1.45 (m)	1.17–1.40 (m)	1.15–1.45 (m)
15	1.19–1.35 (m)	1.20–1.34 (m)	1.19–1.35 (m)
16	3.33 (m)	3.33 (m)	3.33 (m)
17	1.19–1.35 (m)	1.20–1.34 (m)	1.19–1.35 (m)
18–25	1.15–1.45 (m)	1.17–1.40 (m)	1.15–1.45 (m)
26	1.27 (m)	1.26 (m)	1.25 (m)
27	1.49 (m)	1.49 (m)	1.45 (m)
28	3.39 (m)	3.38 (m)	3.32 (m)
29a	3.43 (m)	3.45 (m)	3.14 (m)
29b	3.50 (m)	3.51 (m)	3.26 (m)
30	3.22 (m)	3.14 (m)	2.77 (m)
31a	3.48 (m)	3.45 (m)	3.20 (m)
31b	3.56 (dd, −11.5, 4.6)	3.51 (m)	3.32 (dd, −11.5, 4.6)
1′a	3.11 (m)	3.12 (m)	3.32 (m)
1′b	3.31 (m)	3.31 (m)	3.49 (m)
2′	4.55 (br)	4.55 (br)	5.72 (br)
4′,8′	7.22 (d, 8.7)	7.22 (d, 8.6)	7.23 (d, 8.0)
5′,7′	6.88 (d, 8.7)	6.89 (d, 8.6)	6.92 (d, 8.0)
9′a	3.93 (dd, −9.6, 6.0)	3.93 (m)	3.94 (m)
9′b	3.95 (dd, −9.6, 5.5)	3.95 (m)	3.95 (m)
10′	4.02 (m)	4.02 (m)	4.02 (m)
11′a	3.46 (m)	3.46 (m)	3.45 (m)
11′b	3.49 (m)	3.50 (m)	3.49 (m)
13′	1.82 (s)	1.82 (s)	2.10 (q, 7.6)
14′			0.97 (t, 7.6)
16′			1.99 (s)
OH-2	5.49 (d, 4.1)	5.48 (d, 4.1)	5.48 (d, 4.1)
OH-6	5.22 (br)	4.19[b] (br)	4.88 (br)
OH-7		4.85[b] (br)	
OH-16	4.17 (br)	4.19[b] (br)	4.20 (br)
NH_2-30	7.78 (br)	6.97 (br)	7.77 (br)
OH-31	5.22 (br)	4.85[b] (br)	4.46 (br)
NH-1′	7.52 (dd, 6.5, 5.5)	7.50 (dd, 6.1, 5.5)	7.76 (dd, 6.1, 5.8)
OH-2′	5.40 (d, 3.2)	5.42 (br)	
NH-10′	7.85 (d, 7.8)	7.86 (d, 7.7)	7.79 (d, 7.7)
OH-11′	4.83 (br)	5.10[b] (br)	4.90 (br)

[a] ^{1}H NMR: 600 MHz.
[b] These signals may be interchangeable.

8.[7,8] Compound **8** was treated with $NaIO_4$ followed by reduction with $NaBH_4$ to yield **9**, whose two primary alcohols were protected by TBDPS groups to afford **10**. The secondary alcohol in **10** was converted to (*R*)-2NMA ester **11** (Scheme 1).[9] The ^{1}H NMR chemical shift values of **11** derived from **3** were indistinguishable from those of **11** derived from **1** indicating that the absolute configuration at C-16 was *R*.[4] Therefore, the stereochemistry of **3** was 2*S*,6*S*,7*R*,8*S*,16*R*,30*S*,2′*R*,10′*S*.

Shishididemniol D (**4**) had a molecular formula of $C_{44}H_{80}ClN_3O_{11}$ as established by HRESIMS. ^{1}H and ^{13}C

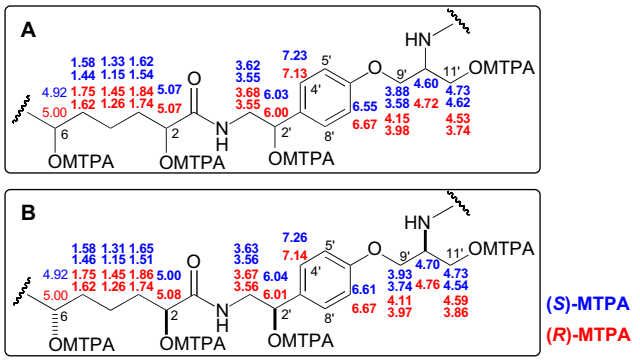


Figure 3. (A) ^{1}H NMR chemical shifts values for **6a** (lower numbers in red)/**6b** (upper numbers in blue). (B) ^{1}H NMR chemical shift values for (*R*)-MTPA derivative of **1** (lower numbers in red)/(*S*)-MTPA derivative of **1** (upper numbers in blue).

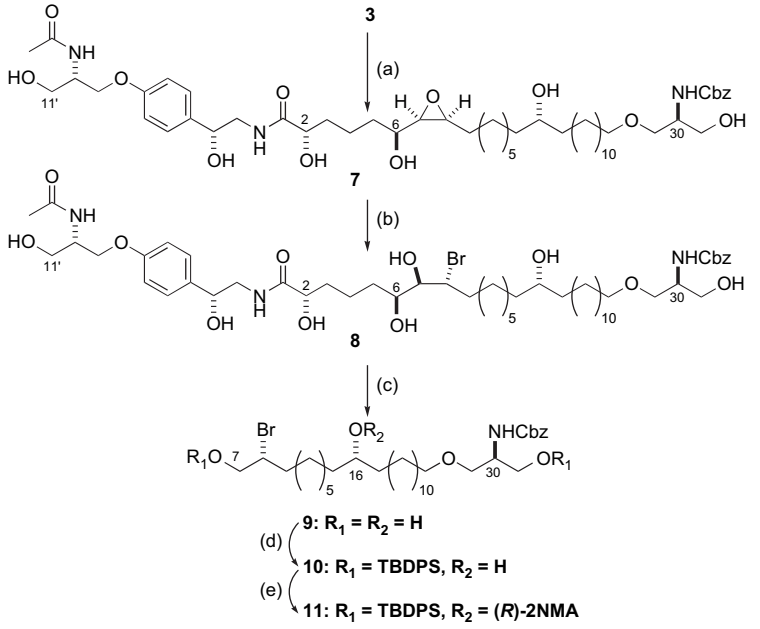
NMR spectra of **4** coincided well with those of **3** except for the replacement of the epoxide group by an oxymethine and a chlorinated methine, suggesting that **4** was a HCl adduct of **3**. The planar structure of **4** was determined on the basis of analysis of NMR and FABMS/MS data (Tables 1 and 2).

The relative stereochemistry from C-6 to C-8 in **4** was assigned as 6*S**,7*R**,8*R** by comparison of the ^{1}H and ^{13}C NMR data with those of **2** (Tables 1 and 2, and Ref. 4). In order to determine the absolute stereochemistry of **4**, its *N*-Cbz derivative **12** was treated with potassium carbonate in MeOH. The optical rotation and the NMR data for the product ($[\alpha]_D^{19}$ −43.3 (*c* 0.10, MeOH)) were indistinguishable from those of compound **7** derived from **3** ($[\alpha]_D^{19}$ −43.7 (*c* 0.10, MeOH)) (Scheme 2).

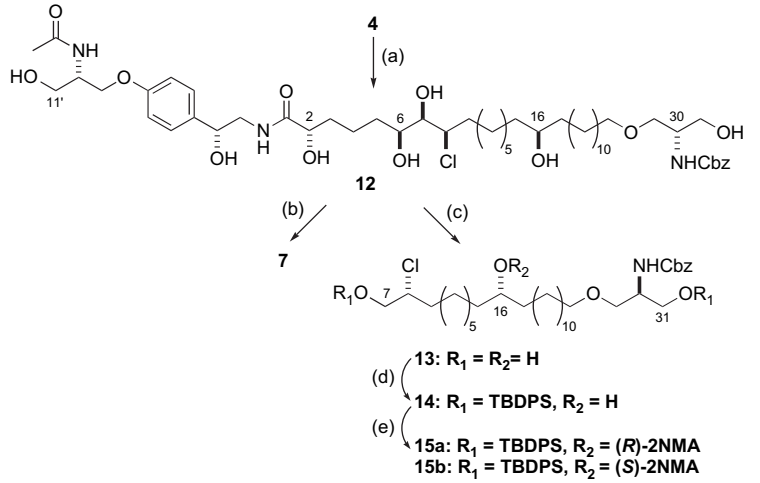
In order to confirm the stereochemistry of C-16, which was remote from other stereogenic centers, **12** was converted to the (*R*)- or (*S*)-2NMA esters (**15a** and **15b**, respectively) in three steps (Scheme 2).[9] The positive $\Delta\delta_{R-S}$ values observed for H_2-7 to H_2-10 signals confirmed the 16*R* stereochemistry (Fig. 4). Therefore, the absolute stereochemistry of **4** was determined to be 2*S*,6*S*,7*R*,8*R*,16*R*,30*S*,2′*R*,10′*S*.

Shishididemniol E (**5**) had a molecular formula of $C_{47}H_{83}N_3O_{12}$ as established by HRESIMS, indicating that a hydrogen in **1** was replaced by an acetyl group. The ^{1}H and ^{13}C NMR spectra of **5** were almost superimposable on those of **1** except for the presence of an additional singlet methyl signal (δ_H 1.99 and δ_C 20.9) and deshielding of H-2′ signal (δ 5.72). The structure of **5** was determined as C-2′ acetyl shishididemniol A (**1**), on the basis of 2D NMR and FABMS/MS data (Tables 1 and 2).

We sought to determine the stereochemistry of **5** by converting both **1** and **5** to a common derivative. Compound **1** was treated with Ac_2O in the presence of triethylamine to yield the peracetyl derivative **16**. However, acetylation of **5** under the same condition afforded a complex mixture. Therefore, we took an alternative method of acetylation. Compound **5** was reacted with acetic acid in the presence of EDC, DMAP, and DMAP·HCl in CH_2Cl_2 to give **16** (Scheme 3): the optical rotation value of **16** derived from **5** and **1** were identical within experimental



Scheme 1. Reagents and conditions: (a) Cbz–Cl, Et_3N, $CHCl_3$/MeOH (3:1), rt, 16 h; (b) $MgBr_2 \cdot OEt$, THF, rt, 3 h; (c) $NaIO_4$, MeOH/H_2O (4:1), rt, 1 h then $NaBH_4$, rt, 15 min; (d) TBDPSCl, pyridine, rt, overnight; and (e) (*R*)-2NMA, EDC, DMAP·HCl, CH_2Cl_2, rt, overnight.



Scheme 2. Reagents and conditions: (a) Cbz–Cl, Et_3N, $CHCl_3$/MeOH (3:1), rt, 16 h; (b) K_2CO_3, MeOH, rt, 2.5 h; (c) $NaIO_4$, MeOH/H_2O (4:1), rt, 1 h then $NaBH_4$, rt, 15 min; (d) TBDPSCl, pyridine, rt, overnight; and (e) (*R*)- or (*S*)-2NMA, EDC, DMAP, DMAP·HCl, CH_2Cl_2, rt, overnight.

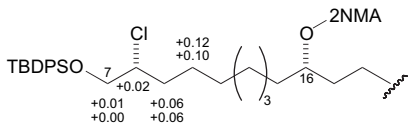
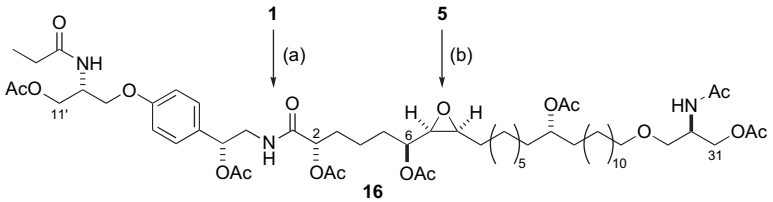


Figure 4. Distribution of $\Delta\delta_{R-S}$ values for the 2NMA derivatives **15a/15b**.

errors. Therefore, **5** was the 2′-acetate of shishididemniol A (**1**).

Shishididemniols C (**3**), D (**4**), and E (**5**) exhibited antibacterial activity in disk agar diffusion assay against the fish pathogenic bacterium *Vibrio anguillarum* (20 μg/6.5 mm ϕ disk, zone of inhibition; 7.5, 7.0, and 7.0 mm for **3**, **4**, and **5**, respectively).



Scheme 3. Reagents and conditions: (a) Ac_2O, Et_3N, CH_2Cl_2, rt, overnight and (b) AcOH, EDC, DMAP, DMAP·HCl, rt, 24 h.

3. Experimental

3.1. Extraction and isolation

The antibacterial *n*-BuOH fraction was separated by ODS flash chromatography as described previously.[4] The fraction eluted with 70% MeOH was separated by ODS HPLC (72% MeOH containing 0.2 M $NaClO_4$) to afford shishididemniols A (**1**), B (**2**), C (**3**, 165 mg), and a mixture of D (**4**) and E (**5**). The mixture of **4** and **5** was purified by ODS HPLC (45% MeCN containing 0.2 M $NaClO_4$) to obtain shishididemniols D (**4**, 40 mg) and E (**5**, 10 mg).

3.1.1. Shishididemniol C (3). Colorless oil; $[\alpha]_D^{18}$ −19.0 (*c* 1.00, MeOH); UV (MeOH) λ_{max} 275 nm (*ε* 870), 281 (770); HRESIMS *m/z* 848.5542 $(M+Na)^+$ (calcd for $C_{44}H_{79}N_3NaO_{11}$, *Δ* −7.1 mmu). For ^1H and ^{13}C NMR data, see Tables 1 and 2. HMBC correlations (DMSO-d_6) H-2/C-3; H-7/C-6; 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-31/C-29, 30; 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′, 8′; H-9′a/C-6′, 10′, 11′; H-9′b/C-6′, 10′, 11′; H-10′/C-9′, 11′, 12′; H-13′/C-12′; NH-1′/C-1, 1′; NH-10′/C-10′, 11′, 12′.

3.1.2. Shishididemniol D (4). Colorless oil; $[\alpha]_D^{19}$ −16.6 (*c* 1.00, MeOH); UV (MeOH) λ_{max} 275 nm (*ε* 1100), 281 (900); HRESIMS *m/z* 884.5379 $(M+Na)^+$ (calcd for $C_{44}H_{80}{}^{35}ClN_3NaO_{11}$, *Δ* −6.5 mmu). For ^1H and ^{13}C NMR data, see Tables 1 and 2. HMBC correlations (DMSO-d_6) H-2/C-1, 3, 4; H-27/C-26, 28; H-28/C-27, 29; H-29a/C-28, 30, 31; H-29b/C-28, 30, 31; 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′, 7′/C-3′, 4′, 5′, 6′, 7′, 8′; H-9′a/C-6′, 10′; H-9′b/C-6′, 10′; H-10′/C-9′, 11′, 12′; H-13′/C-12′; NH-1′/C-1, 1′; NH-10′/C-10′, 11′, 12′.

3.1.3. Shishididemniol E (5). Colorless oil; $[\alpha]_D^{19}$ −30.7 (*c* 0.50, MeOH); UV (MeOH) λ_{max} 275 nm (*ε* 760), 281 (650); HRESIMS *m/z* 904.5874 $(M+H)^+$ (calcd for $C_{47}H_{83}N_3NaO_{12}$, *Δ* −7.1 mmu). For ^1H and ^{13}C NMR data, see Tables 1 and 2. HMBC correlations (DMSO-d_6) H-2/C-3; 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-1′a/C-1, 2′, 3′; H-1′b/C-1, 2′, 3′; H-2′/C-3′, 4′, 8′, 15′; H-4′, 8′/C-2′, 4′, 5′, 6′, 7′, 8′; H-5′, 7′/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′; H-13′/C-12′, 14′; H-14′/C-12′, 13′; H-16′/C-15′; NH-1′/C-1, 1′; NH-10′/C-10′, 12′.

3.2. Preparation of (*R*)- and (*S*)-MTPA derivatives of 3 (6a and 6b)

To a solution of **3** (4 mg) in pyridine (50 μL) was added (*S*)-MTPACl (60 μL). The reaction mixture was stirred at rt for 30 min and then concentrated. 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% MeOH to MeOH) to yield (*R*)-MTPA derivative **6a** (2.6 mg). The (*S*)-MTPA derivative **6b** (2.2 mg) was prepared in the same way. Compound **6a**: ^1H NMR (600 MHz, CD_3OD) 5.07 (m, 1H; H-2), 1.74 (m, 1H; H-3a), 1.84 (m, 1H; H-3b), 1.26 (m, 1H; H-4a), 1.45 (m, 1H; H-4b), 1.62 (m, 1H; H-5a), 1.75 (m, 1H; H-5b), 5.00 (m, 1H; H-6), 2.90 (dd, *J*=8.9, 4.1, 1H; H-7), 2.98 (m, 1H; H-8), 5.08 (m, 1H; H-16), 1.27 (m, 2H; H_2-26), 1.46 (m, 2H; H_2-27), 3.32 (m, 2H; H_2-28), 3.42 (m, 1H; H-29a), 3.38 (m, 1H; H_2-29), 4.39 (m, 1H; H-30), 4.50 (m, 1H; H-31a), 4.38 (m, 1H; H-31b), 3.68 (m, 1H; H-1′a), 3.55 (m, 1H; H-1′b), 6.00 (m, 1H; H-2′), 7.13 (d, *J*=8.7, 2H; H-4′/8′), 6.67 (d, *J*=8.7, 2H; H-5′/7′), 4.15 (dd, *J*=−9.9, 7.8, 1H; H-9′a), 3.98 (dd, *J*=−9.9, 4.2, 1H; H-9′b), 4.72 (m, 1H; H-10′), 4.53 (dd, *J*=−11.4, 5.5, 1H; H-11′a), 3.74 (m, 1H; H-11′b), 2.24 (s, 3H, H_3-13′). Compound **6b**: ^1H NMR (600 MHz, CD_3OD) 5.07 (m, 1H; H-2), 1.54 (m, 1H; H-3a), 1.62 (m, 1H; H-3b), 1.15 (m, 1H; H-4a), 1.33 (m, 1H; H-4b), 1.44 (m, 1H; H-5a), 1.58 (m, 1H; H-5b), 4.92 (m, 1H; H-6), 2.96 (dd, *J*=9.0, 4.2, 1H; H-7), 3.03 (m, 1H; H-8), 5.08 (m, 1H; H-16), 1.33 (m, 2H; H_2-26), 1.52 (m, 2H; H_2-27), 3.39 (m, 2H; H_2-28), 3.47 (dd, *J*=−9.6, 5.5, 1H; H-29a), 3.43 (dd, *J*=−9.6, 5.9, 1H; H-29b), 4.39 (m, 1H; H-30), 4.50 (dd, *J*=−11.0, 4.2, 1H; H-31a), 4.34 (dd, *J*=−11.0, 6.4, 1H; H-29a), 3.62 (m, 1H; H-1′a), 3.55 (m, 1H; H-1′b), 6.03 (m, 1H; H-2′), 7.23 (d, *J*=8.7, 2H; H-4′/8′), 6.55 (d, *J*=8.7, 2H; H-5′/7′), 3.88 (dd, *J*=−9.2, 6.4, 1H; H-9′a), 3.58 (br, 1H; H-9′b), 4.60 (m, 1H; H-10′), 4.73 (dd, *J*=−11.4, 5.5, 1H; H-11′a), 4.62 (m, 1H; H-11′b), 2.22 (s, 3H, H_3-13′a).

3.3. Preparation of *N*-Cbz derivative 7

To a solution of **3** (30 mg, 0.036 mmol) in $CHCl_3$/MeOH (3:1, 0.4 mL) were added triethylamine (50 μL) and Cbz–Cl (25.6 μL). After stirring for 16 h at rt, the reaction mixture was concentrated, suspended in H_2O, and extracted with EtOAc. The organic layer was concentrated and the residue was separated by ODS HPLC (gradient elution of 50% MeCN to 60% MeCN) to give **7** (11.3 mg). Compound **7**: ESIMS *m/z* 960.4252 $(M+H)^+$. ^1H NMR (600 MHz, CD_3OD) 7.37–7.26 (m, 5H), 7.29 (d, *J*=8.7, 2H), 6.93 (d, *J*=8.7, 2H), 5.08 (d, *J*=–12.3, 1H), 5.05 (d, *J*=–12.3, 1H), 4.71 (dd, *J*=7.4, 4.6, 1H), 4.22 (m, 1H), 4.05 (m, 2H), 3.99 (m, 1H), 3.78 (m, 1H), 3.69 (d, *J*=5.9, 2H), 3.57 (m, 2H), 3.51–3.40 (m, 6H), 3.39–3.33 (m, 2H), 2.98 (ddd, *J*=8.2, 4.1, 4.1, 1H), 2.82 (dd, *J*=4.4, 8.2, 1H), 1.97 (s, 3H), 1.73–1.25 (m, 42H).

3.4. Preparation of bromohydrin 8

To a solution of **7** (5 mg) in dry THF (0.5 mL) was added $MgBr_2 \cdot OEt_2$ (9 mg). The solution was stirred at rt for 3 h and was then concentrated. The residue was separated by ODS HPLC (gradient elution of 50% MeCN to 60% MeCN) to afford **8** (2.8 mg). Compound **8**: ESIMS *m/z* 1062.1648 and 1064.1862 $(M+Na)^+$. ^1H NMR (600 MHz, CD_3OD) 7.37–7.26 (m, 5H), 7.30 (d, *J*=8.7, 2H), 6.94 (d, *J*=8.7, 2H), 5.08 (d, *J*=−12.9, 1H), 5.06 (d, *J*=−12.9, 1H), 4.71 (dd, *J*=7.3, 5.1, 1H), 4.22 (m, 1H), 4.12 (m, 1H), 4.05 (m, 2H), 4.00 (m, 1H), 3.78 (m, 1H), 3.69 (d, *J*=5.5, 2H), 3.67 (m, 1H), 3.57 (m, 2H), 3.52–3.40 (m, 6H), 3.38–3.32 (m, 2H), 1.97 (s, 3H), 1.94–1.25 (m, 42H).

3.5. Preparation of (*R*)-2NMA derivative 11 from 8

A 2.8 mg portion of **8** was treated with $NaIO_4$ (5 mg) in MeOH/H_2O (28:5, 0.33 mL) at rt for 1 h, followed by

reduction with $NaBH_4$ (20 mg). The reaction mixture was concentrated, suspended in H_2O, and extracted with EtOAc. The organic layer was concentrated and the residue was separated by silica gel flash chromatography ($CHCl_3$/MeOH=19:1) to yield **9** (1.4 mg). To a solution of **9** (1.4 mg) in pyridine (40 μL) was added *tert*-butyldiphenylchlorosilane (TBDPSCl) (10 μL). After stirring at rt overnight, the reaction mixture was concentrated, suspended in H_2O, and extracted with EtOAc. The organic layer was concentrated and the residue was separated by silica gel flash chromatography (*n*-hexane/EtOAc=4:1) to yield **10** (2.1 mg). To a solution of **10** (2 mg) in CH_2Cl_2 (200 μL) were added (*R*)-2-naphthylmethoxyacetic acid ((*R*)-2NMA) (1.5 mg), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) (5 mg), DMAP (1 mg), and DMAP·HCl (1 mg) and the mixture was stirred at rt overnight. The reaction mixture was concentrated 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 **11** (2.2 mg). ^{1}H NMR spectra of **9**, **10**, and **11** described above were indistinguishable from those of **9**, **10**, and **11** prepared from **1** described in Ref. 4.

3.6. Preparation of *N*-Cbz derivative 12

To a solution of **4** (20 mg, 0.024 mmol) in $CHCl_3$/MeOH (3:1, 0.4 mL) were added triethylamine (25 μL) and Cbz–Cl (17 μL). After stirring at rt for 16 h, the reaction mixture was concentrated, suspended in H_2O, and extracted with EtOAc. The organic layer was purified by ODS HPLC (gradient elution of 50% MeCN to 60% MeCN) to afford **12** (14.8 mg). Compound **12**: ESIMS *m/z* 996.3154 and 998.2731 $(M+H)^+$. ^{1}H NMR (600 MHz, CD_3OD) 7.37–7.26 (m, 5H), 7.30 (d, *J*=8.2, 2H), 6.93 (d, *J*=8.2, 2H), 5.08 (d, *J*=−12.8, 1H), 5.06 (d, *J*=−12.8, 1H), 4.71 (dd, *J*=7.6, 4.9, 1H), 4.22 (m, 1H), 4.05 (m, 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), 3.52–3.33 (m, 8H), 1.97 (s, 3H), 1.89–1.25 (m, 42H).

3.7. Preparation of 7 from 12

To a solution of **12** (4 mg) in 400 μL of MeOH was added potassium carbonate (6 mg). The reaction mixture was stirred at rt for 2.5 h, quenched with acetic acid, and concentrated. The residue was purified by ODS HPLC (gradient elution of 50% MeCN to 60% MeCN) to yield **7** (2.1 mg) whose ^{1}H NMR spectrum and $[\alpha]_D$ value (**7** from **4**: $[\alpha]_D^{20}$ −43.3 (*c* 0.10, MeOH), **7** from **3**: $[\alpha]_D^{20}$ −43.7 (*c* 0.10, MeOH)) were indistinguishable from those of **7** prepared from **3**.

3.8. Preparation of (*R*)- and (*S*)-2NMA derivatives (15a and 15b) from 12

A 10 mg portion of **12** was treated with $NaIO_4$ (20 mg) in MeOH/H_2O (50:1, 1.02 mL) at rt for 1 h, followed by reduction with $NaBH_4$ (20 mg). The reaction mixture was concentrated, suspended in H_2O, and extracted with EtOAc. The organic layer was concentrated and the residue was separated by silica gel flash chromatography ($CHCl_3$/MeOH=19:1) to yield **13** (6 mg). To a solution of **13** (5.5 mg) in pyridine (50 μL) was added TBDPSCl (10 μL). After stirring at rt overnight, the reaction mixture was concentrated, suspended in H_2O, and extracted with EtOAc. The organic layer was concentrated and the residue was separated by silica gel flash chromatography (*n*-hexane/EtOAc=4:1) to yield **14** (9.3 mg). To a solution of **14** (4 mg) in CH_2Cl_2 (200 μL) were added (*R*)-2NMA (2 mg), EDC (5 mg), DMAP (1 mg), and DMAP·HCl (1 mg), and the mixture was stirred at rt overnight. The reaction mixture was concentrated 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 **15a** (4.2 mg). (*S*)-2NMA derivative **15b** (4.2 mg) was prepared in the same way. Compound **13**: ESIMS *m/z* 600.2347 and 602.2281 $(M+H)^+$. ^{1}H NMR (600 MHz, $CDCl_3$) 7.36–7.27 (m, 5H), 5.45 (d, *J*=6.6, 1H), 5.09 (s, 2H), 3.99 (m, 1H), 3.84–3.53 (m, 8H), 3.40 (t, *J*=6.6, 2H), 1.88–1.18 (m, 36H). Compound **14**: ESIMS *m/z* 1076.2973 and 1078.3937 $(M+H)^+$. ^{1}H NMR (600 MHz, benzene d_6) 7.80–7.03 (m, 25H), 5.09 (s, 2H), 5.04 (d, *J*=8.7, 1H), 4.20 (m, 1H), 3.91–3.71 (m, 5H), 3.57 (dd, *J*=−9.4, 3.3, 1H), 3.47 (m, 1H), 3.37 (dd, *J*=−9.4, 6.3, 1H), 3.20 (t, *J*=6.6, 2H), 1.78–1.17 (m, 36H), 1.19 (s, 9H), 1.14 (s, 9H). Compound **15**a: ^{1}H NMR (600 MHz, benzene d_6) 8.67–7.00 (m, 32H, aromatic), 5.38 (s, 1H, –C*H*–O in 2NMR), 5.10 (s, 2H; –CH_2– in Cbz), 5.08 (m, 1H; H-16), 5.02 (d, *J*=8.6, 1H; NH-30), 4.21 (m, 1H; H-30), 3.89 (m, 1H; H-29a), 3.87 (m, 1H; H-8), 3.84 (dd, *J*=−10.5, 5.5, 1H; H-7a), 3.78 (dd, *J*=−10.5, 5.5, 1H; H-7b), 3.74 (dd, *J*=−9.6, 6.3, 1H; H-29b), 3.58 (br, 1H; H-31a), 3.37 (br, 1H; H-31b), 3.29 (s, 3H; OMe in 2NMA), 3.21 (br, 2H; H_2-28), 1.72 (m, 1H; H-9a), 1.61 (m, 1H; H-9b), 1.45 (m, 1H; H-10a), 1.27 (m, 1H; H-10b), 1.55–0.80 (m, 32H; H_2-11 to H_2-15, H_2-17 to H_2-27), 1.20 (s, 9H; *tert*-butyl), 1.14 (s, 9H; *tert*-butyl). Compound **15b**: ^{1}H NMR (600 MHz, benzene d_6) 8.03–7.02 (m, 32H, aromatic), 5.13 (m, 1H; H-16), 5.10 (s, 2H; –CH_2– in Cbz), 5.02 (d, *J*=8.8, 1H; NH-30), 4.93 (s, 1H, –C*H*–O in 2NMR), 4.21 (m, 1H; H-30), 3.89 (m, 1H; H-29a), 3.85 (m, 1H; H-8), 3.83 (dd, *J*=−10.2, 5.5, 1H; H-7a), 3.78 (dd, *J*=−10.2, 5.3, 1H; H-7b), 3.74 (dd, *J*=−9.3, 7.3, 1H; H-29b), 3.58 (br, 1H; H-31a), 3.37 (br, 1H; H-31b), 3.31 (s, 3H; OMe in 2NMA), 3.21 (br, 2H; H_2-28), 1.66 (m, 1H; H-9a), 1.55 (m, 1H; H-9b), 1.33 (m, 1H; H-10a), 1.17 (m, 1H; H-10b), 1.55–0.80 (m, 32H; H_2-11 to H_2-15, H_2-17 to H_2-27), 1.20 (s, 9H; *tert*-butyl), 1.14 (s, 9H; *tert*-butyl).

3.9. Preparation of 16 from 1

To a solution of **1** (30 mg) in CH_2Cl_2 (1 mL) were added triethylamine (450 μL) and Ac_2O (300 μL). The solution was stirred at rt overnight and was then concentrated. The residue was partitioned between EtOAc and H_2O with 0.1 M $NaHCO_3$. The organic layer was separated by ODS HPLC (gradient elution of 70% MeOH to MeOH) to afford **16** (18 mg). Compound **16**: ^{1}H NMR (600 MHz, CD_3OD) 7.29 (d, *J*=8.7, 2H), 6.94 (d, *J*=8.7, 2H), 5.80 (dd, *J*=8.5, 4.8, 1H), 4.90 (dd, *J*=7.6, 4.8, 1H), 4.83 (m, 1H), 4.78 (dt, *J*=4.6, 8.2, 1H), 4.46 (m, 1H), 4.28 (dd, *J*=−11.0, 5.0, 1H), 4.25–4.16 (m, 3H), 4.09–4.01 (m, 3H), 3.55 (dd, *J*=−13.7, 4.6, 1H), 3.51–3.40 (m, 5H), 3.04–2.98 (m, 2H), 2.23 (q, *J*=7.3, 2H), 2.10 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.033 (s, 3H), 2.028 (s, 3H), 2.01 (s, 3H), 1.94 (s, 3H), 1.80–1.23 (m, 42H), 1.12 (t, *J*=7.3, 3H).

3.10. Preparation of 16 from 5

To a solution of **5** (2 mg) in CH_2Cl_2 (200 μL) were added acetic acid (10 μL), EDC (10 mg), DMAP (1 mg), and DMAP·HCl (1 mg), and the mixture was stirred at rt for 24 h. The reaction mixture was concentrated and the residue was separated by ODS HPLC (gradient elution of 70% MeOH to MeOH) to afford **16** (0.6 mg) whose ^{1}H NMR spectrum and $[\alpha]_D$ value (**16** from **5**: $[\alpha]_D^{17}$ −44 (*c* 0.04, MeOH), **16** from **1**: $[\alpha]_D^{17}$ −47 (*c* 0.04, MeOH)) were indistinguishable from those of **16** prepared from **1**.

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