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Structure–activity relationship study on 13-deoxytedanolide, a highly antitumor macrolide from the marine sponge *Mycale adhaerens*

Shinichi Nishimura,[a,b,†] Shigeki Matsunaga,[a] Satoru Yoshida,[a] Yoichi Nakao,[a] Hiroshi Hirota[b] and Nobuhiro Fusetani[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]*Genomic Sciences Center, RIKEN, Tsurumi, Yokohama 230-0045, Japan*

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Abstract—To obtain information of structure–activity relationships (SARs) of 13-deoxytedanolide, its chemical transformation has been carried out, targeting on such functional groups as an epoxide, hydroxyls, ketones, and olefins. A total of 10 derivatives have been prepared and their cytotoxicity against P388 murine leukemia cells and inhibitory activity of polypeptide elongation in yeast cell lysate provided some important SARs; the southern hemisphere comprises the pharmacophore, while the epoxide-bearing side chain is essential for the activity.

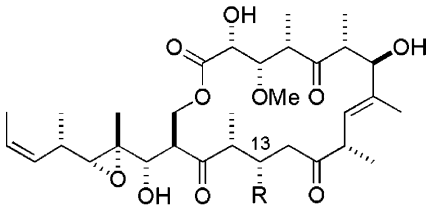
1. Introduction

In our previous paper, we reported that 13-deoxytedanolide (13-DT, **1**, Scheme 1) bound to the 60S large ribosomal subunit of the budding yeast *Saccharomyces cerevisiae*, thus inhibiting polypeptide elongation.[1] Although many metabolites target ribosomes, 13-DT is the first macrolide inhibitor for the eukaryotic ribosome. It should be noted that a small number of ribosomal inhibitors has been reported to exert potent antitumor activity.[2,3] To obtain not only more active and less toxic derivatives but also SARs of this important class of cytotoxins, we attempted to modify such functional groups as an epoxide, hydroxyls, ketones, and olefins. A total of ten derivatives has been prepared and evaluated for cytotoxicity against P388 leukemia cells as well as for inhibitory effects on polypeptide elongation in yeast cell lysate, which provided some important information for SARs. This paper describes the chemical transformation on 13-deoxytedanolide and biological activity of the products.

2. Results and discussion

2.1. Chemical transformation

When exposed to $CDCl_3$, a considerable portion of 13-DT (**1**) was converted to a less polar compound **3** which had a molecular formula of $C_{26}H_{38}O_8$, smaller than **1** by a unit of $C_6H_{12}O$ as established by HRESIMS (Scheme 2). The ^1H NMR spectrum of **3** contained most of the



13-deoxytedanolide (**1**): R = H
tedanolide (**2**): R = OH

Scheme 1.

Keywords: 13-Deoxytedanolide; Antitumor; Marine sponge-derived macrolide; SAR; Cytotoxicity; Protein synthesis inhibition.
* Corresponding author. Tel.: +81 3 5841 5299; fax: +81 3 5841 8166; e-mail: anobu@mail.ecc.u-tokyo.ac.jp
† Present address: Chemical Genetics Laboratory, RIKEN, Hirosawa 2-1, Wako, Saitama 351-0198, Japan

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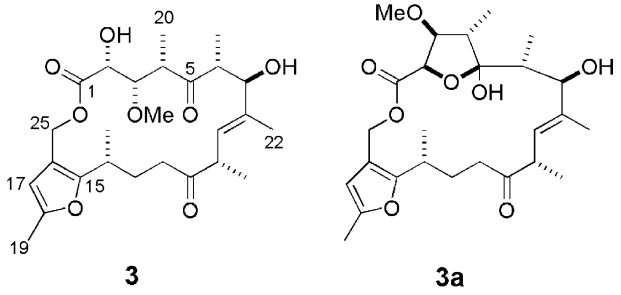


Scheme 2.

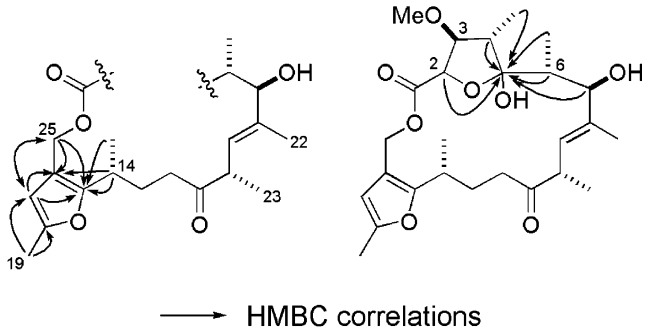


Figure 1. HMBC correlations which confirm presence of the tri-substituted furan ring in **3** or **3a** (left) and the hemiketal ring in **3a** (right).

Table 1. ^{1}H and ^{13}C NMR spectral data for **3** in $CDCl_3$

Compound **3**		
Position	^{1}H (mult., J in Hz)	^{13}C (mult.)
1		172.7 (s)
2	3.74 (d, J = 1.8 Hz)	71.6 (d)
3	3.72 (dd, J = 9.0, 1.8 Hz)	82.8 (d)
4	3.05[a]	48.7 (d)
5		215.9 (s)
6	3.03[a]	50.3 (d)
7	4.17 (d, J = 9.0 Hz)	79.0 (s)
8		138.1 (s)
9	5.35 (dd, J = 9.2, 1.5 Hz)	129.1 (d)
10	3.20[a]	45.6 (d)
11		211.5 (s)
12	2.15–2.30 (m)	39.0 (t)
13	1.75–2.05 (m)	29.4 (t)
14	2.92 (m)	31.2 (d)
15		156.3 (s)
16		115.0 (s)
17	5.93 (d, J = 1.0 Hz)	107.7 (d)
18		150.2 (s)
19	2.21 (d, J = 1.0 Hz)	13.6 (q)
20	1.26 (d, J = 7.0 Hz)	14.5 (q)
21	1.27 (d, J = 6.9 Hz)	15.1 (q)
22	1.64 (d, J = 1.5 Hz)	10.7 (q)
23	1.03 (d, J = 6.9 Hz)	16.2 (q)
24	1.25 (d, J = 7.0 Hz)	20.1 (q)
25a	5.14 (d, J = 12.4 Hz)	59.1 (t)
25b	4.83 (d, J = 12.4 Hz)	
OMe	3.40 (s)	61.0 (q)

[a] Overlapped with other signals.

signals assignable to the macrocyclic portion of **1**, but the signals for the side chain portion were missing (Table 1). Instead, the ^{13}C NMR spectrum showed four new aromatic carbon signals (δ 156.3, 150.2, 115.0, and 107.7 ppm), which were characteristic of the tri-substituted furan ring. HMBC correlations from H_3-19 to C-17 and C-18 and weak COSY correlation ($^{4}J_{H/H}$ = 1.0 Hz) between H-17 and H_3-19 supported the presence of a 2,4,5-tri-substituted ring. HMBC correlations from H-14 and H_3-24 to C-15 connected C-14 and C-15 bond, while those from H_2-25 to C-15, C-16, and C-17 implied the connectivities from C-16 to C-25, thus completing the structural unit in Figure 1. Perhaps, compound **3** was generated by acid-catalyzed fragmentation as illustrated in Figure 2.[4] It should be noted that **3** was present as a mixture of the keto and hemiacetal forms in a ratio of 3:2 in $CDCl_3$, whereas it was exclusively in the keto form in CD_3OD. The structure of the hemiacetal **3a** was confirmed by an HMBC correlation, from H-2 to C-5 (Fig. 1). The stereochemistry at C-5 was assigned to be *R* from NOESY correlations, H-4/H-6, H-4/Me-21, and Me-20/Me21.

Upon treatment with $NaBH_4$, the 11-keto group was preferentially reduced to obtain a pair of diastereomers, **4** and **5** (Scheme 3). Configurations of C-11 in **4** and **5** were assigned by interpretation of NOESY data and coupling constants in the ^{1}H NMR spectrum (Fig. 3). Both compounds exhibited a NOESY cross peak between H-9 and H-12b, thereby indicating that C-9 and C-12 are *gauche* with respect to the C-10/C-11 bond. NOESY cross peaks, H-9/H-11 and H-10/H-12a, implied the 11*S* stereochemistry for **4**, while NOESY cross peaks, H-10/H-11 and H-10/H-12a, were in agreement with the 11*R* stereochemistry for **5**. To confirm these assignments we carried out the modified Mosher's analysis; tetra-MTPA esters of **4** revealed $\Delta\delta$ values as shown in Figure 4, thus implying the 11*S* stereochemistry.[5] The modified Mosher's analysis of tri-MTPA derivatives prepared from 13-DT also supported this assignment.

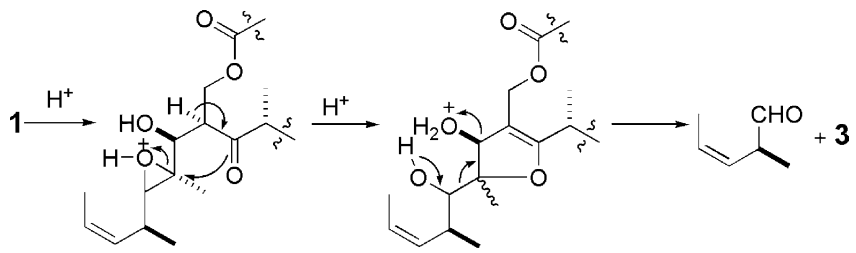
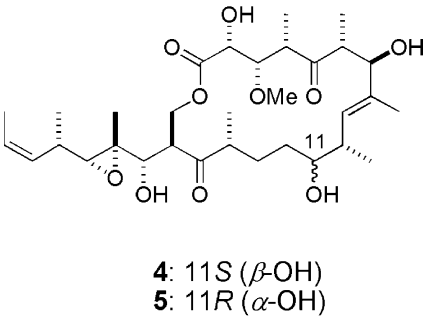


Figure 2. Proposed reaction mechanism for the acid-catalyzed formation of **3** from **1**.



Scheme 3.

Catalytic hydrogenation of **1** over Pd–C afforded the tetrahydro-derivative **6**, whereas hydrogenation over $RhCl(PPh_3)_3$ yielded **7** in which only the Δ^{21}-olefin was reduced (Scheme 4). The configuration of C-8 in **6** was assigned on the basis of the NOESY and coupling constant data, which revealed the zig-zag conformation between C-6 and C-10 (Fig. 5). A NOESY cross peak between H-2 and H-8 supported this assignment.

The three hydroxyl groups displayed different degrees of reactivity toward acylation. We employed 4-pentenyl chloride to acylate **7**; we expected that its bulkiness would allow to differentiate the hydroxyl groups. In fact, the C-2 and C-7 hydroxyl groups were preferentially acylated to afford monoacyl derivatives **8** and **9**, respectively, together with the diacyl derivative **10** (Scheme 5). The C-17 hydroxyl group was not acylated under this condition (see Section 4.7). Treatment with excess acetic anhydride afforded the triacetate **11**.

Oxidation with $OsO_4/NaIO_4$ cleaved the less hindered Δ^{21}-olefin of **1** selectively to afford the aldehyde **12** whose ^1H NMR spectrum in CD_3OD exhibited a pair of hemiacetal signals [δ 4.39 (0.6H, d, $J = 6.3$ Hz) and 4.48 (0.4H, d, $J = 4.2$ Hz)], thus suggesting a facile hydration of the aldehyde group.

2.2. Biological activities

With 10 derivatives of 13-DT in hand, we determined their cytotoxicity and inhibitory activity against polypeptide elongation (Table 2, Fig. 6). The IC_{50} values of their cytotoxicity against P388 murine leukemia cells ranged from 14 pg/mL to higher than 5 μg/mL, while those of their polypeptide elongation inhibition from 0.15 μM to higher than 100 μM. Compound **3**, in which the side chain was incorporated into the furan ring, lost both activities, suggesting the importance of both or either of the 15-keto group and/or the side chain. The significance of the hydrophobic terminus was substantiated by the lost or significantly reduced activity of **12**, in which the terminal aldehyde group was hydrated. Because both **1** and **4** exhibited similar degrees of biological activities, the oxygen atom on C-11 can be either a keto or a hydroxyl group. The oxygen atom might function as a hydrogen bond acceptor. The significant difference in the activity of **4** and **5**, which are epimeric at C-11, implied the strict requirement for spatial alignment of the

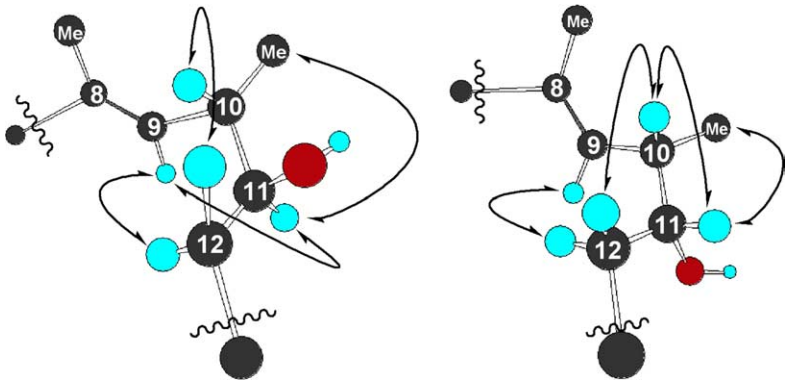


Figure 3. Selected NOESY correlations in dihydro derivatives **4** (left) and **5** (right).

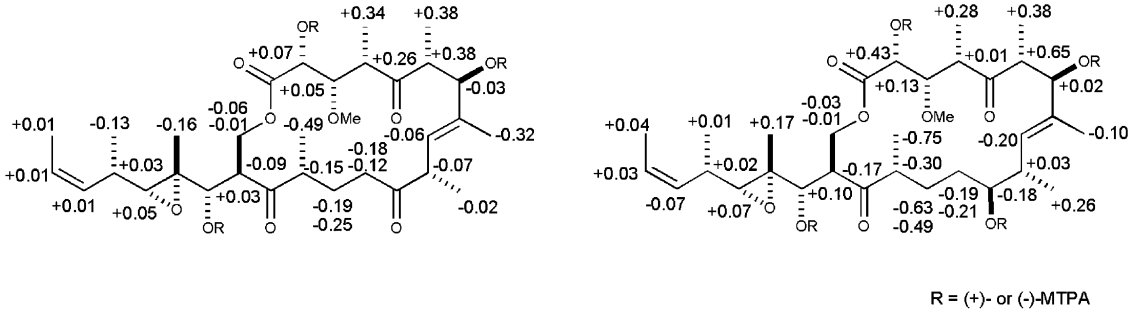


Figure 4. Distributions of $\Delta\delta$ values in 13-DT (left) and in **4** (right).

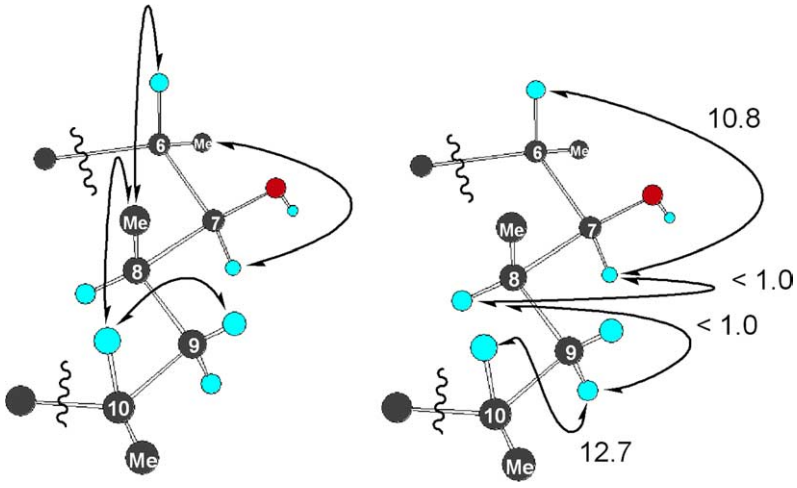
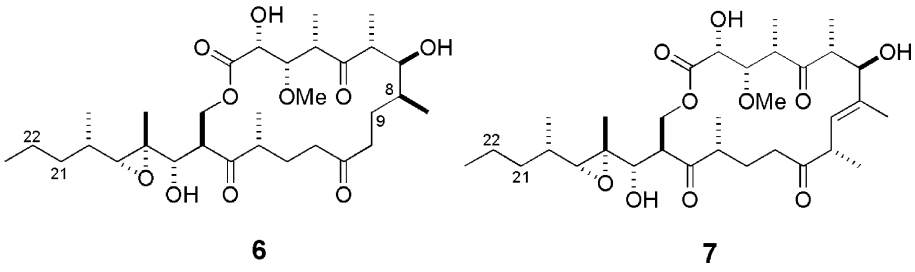
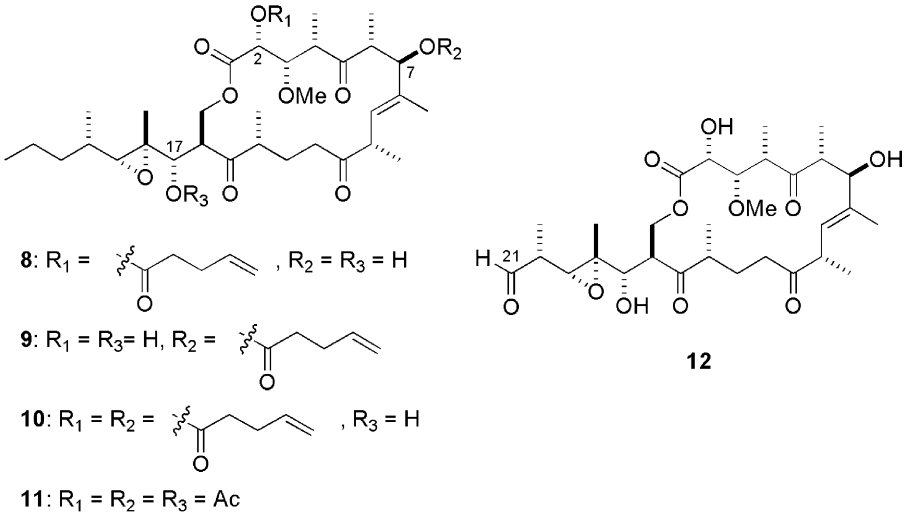


Figure 5. Stereochemistry at C-8 of **6**. The zig-zag conformation from C-6 to C-10 was revealed by NOESY correlations (left) and $^{3}J_{H/H}$ values (right).



6 **7**

Scheme 4.



8: R_1 = , $R_2 = R_3 = H$

9: $R_1 = R_3 = H$, R_2 =

10: $R_1 = R_2$ = , $R_3 = H$

11: $R_1 = R_2 = R_3 = Ac$

12

Scheme 5.

oxygen atom at C-11. The oxygen atom at C-17 may also contribute to the potent activity, which was revealed by the loss of polypeptide elongation inhibition of the derivative **11**. Although **11** showed potent cytotoxicity, this is not contradictory, because retention of the activity in acetates are frequently explained by hydrolysis by endogenous esterases.[6,7]

The contribution of Δ^{8}-olefin functionality to the activity was demonstrated by the significantly reduced activity of **6**, while Δ^{21}-olefin functionality is less important as judged by the fully retained activities of **7**. The conformations of the southern hemisphere of **1** and **6** are considerably different as judged by the following NMR data: (1) C-12 methylene protons resonated at δ

Table 2. Biological activity of 13-DT and **3**–**12**

Compounds	Cytotoxicity	Polypeptide elongation inhibition
	IC_{50} (ng/mL)	IC_{50} (μM)
13-DT (**1**)	0.064	0.15
3	>5000	>100
4	0.014	0.15
5	92	0.80
6	49	1.5
7	0.2	0.4
8	20	0.75
9	9.2	0.65
10	500	>50
11	8	>50
12	>5000	15.0

2.29 in **1**, while they resonated at δ 2.45 and 2.53 in **6**; (2) H-12b exhibited NOESY correlations with CH_3-27 and CH_3-28 in **6**, which were absent in **1**. Therefore, the northern hemisphere and the Δ^8-olefin may play a role for stabilizing the conformation of the southern hemisphere in the active form. Two hydroxyl groups in the northern hemisphere may not be incorporated in the pharmacophore, which was indicated by the decreased but still potent activity of mono-acylated derivatives **8** and **9**. The bulky acyl group may prevent the binding of the compound to the target site. Dipentenoate **10** showed only weak or no activity. Two bulky modifications may generate synergetic effect, to avoid its binding to the ribosome.

In this paper, we are not able to discuss the role of the epoxide, because it was not possible to modify this functional group without touching other part of the molecule.

3. Conclusions

The cytotoxicity and polypeptide elongation inhibition of 13-DT derivatives disclosed the structural features important for the potent cytotoxicity. The strict spatial alignment of C-11 oxygen atom and the side chain with a hydrophobic terminus are important for cytotoxicity. The northern hemisphere appears to play a critical role to maintain the conformation of the southern hemisphere in a suitable form to bind to its cellular target. However, further study is required to disclose the optimal arrangement of functional groups for this class of compounds. Further SAR studies for 13-DT may help to develop potent cytotoxins and hopefully anticancer agents.

4. Experimental

4.1. General experimental procedures

NMR spectra were recorded either on a JEOL α600, a JEOL α500, a Bruker DRX600, or a Bruker DRX500 spectrometer. Chemical shifts were referenced to solvent peaks: δ_H 3.30 for CD_3OD, δ_H 7.27 for $CDCl_3$, and δ_C 77.2 for $CDCl_3$. MS spectra were obtained with a JEOL SX-102 for FAB-MS or a JEOL JMS-T100LC for ESI-MS.

4.2. Preparation of 3

When 13-DT (23.5 mg) was dissolved in $CDCl_3$, the solution immediately turned purple. Separation of the products by ODS-HPLC on Cosmosil AR-II (70% aq MeOH) yielded **3** (2.3 mg) and unreacted 13-DT (3.9 mg). ^1H and ^{13}C NMR data for **3**, see Table 1; HRESIMS (positive) *m/z* 479.2628 (calcd for $C_{26}H_{39}O_8$, 479.2645). Compound **3a**: ^1H NMR ($CDCl_3$): δ 5.84 (d, J = 0.9 Hz, H-17), 5.29 (dd, J = 10.1, 1.1 Hz, H-9), 5.07 (d, J = 12.2 Hz, H-25a), 5.04 (br s, H-7), 4.87 (d, J = 7.3 Hz, H-2), 4.64 (d, J = 12.2 Hz, H-25b), 3.90 (dd, J = 9.0, 7.3 Hz, H-3), 3.42 (s, OMe), 3.23 (overlapped, H-10), 2.78 (m, H-14), 2.25 (overlapped, H-4), 2.22 (d, J = 0.9 Hz, H_3-19), 2.15–2.30 (m, H_2-12), 2.00 (overlapped, H-6), 1.75–2.05 (m, H_2-13), 1.69 (d, J = 1.5 Hz, H_3-22), 1.22 (d, J = 7.0 Hz, H_3-24), 1.19 (d, J = 6.7 Hz, H_3-20), 1.09 (d, J = 6.9 Hz, H_3-23), 0.98 (d, J = 7.3 Hz, H_3-21); ^{13}C NMR ($CDCl_3$): δ 212.0 (C-11, s), 171.0 (C-1, s), 154.0 (C-15, s), 150.7 (C-18, s), 140.3 (C-8, s), 126.9 (C-9, d), 115.2 (C-16, s), 109.5 (C-5, s), 106.5 (C-17, d), 88.1 (C-3, d), 78.2 (C-2, d), 76.1 (C-7, d), 59.5 (C-27, q), 58.7

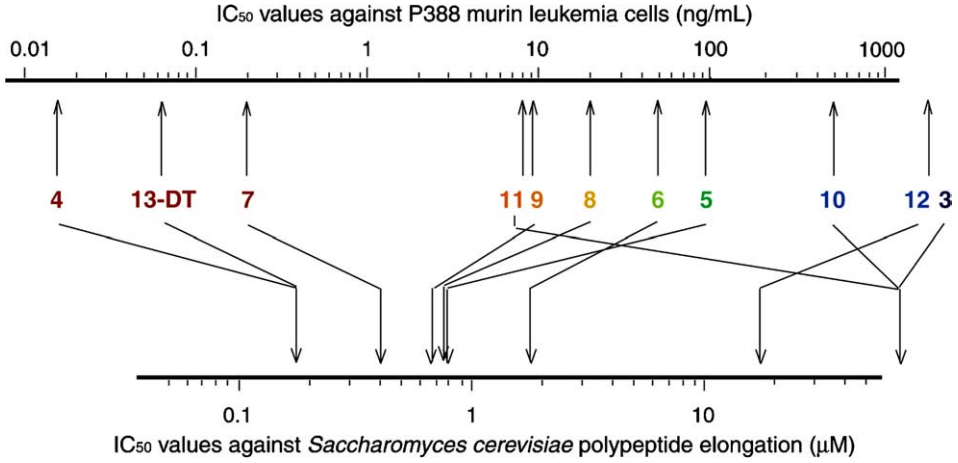


Figure 6. Schematic diagram of SAR for 13-DT.

(C-25, t), 48.3 (C-6, d), 46.4 (C-10, d), 44.2 (C-4, d), 38.2 (C-12, t), 30.8 (C-14, d), 29.1 (C-13, t), 21.1 (C-24, q), 16.4 (C-23, q), 13.8 (C-19, q), 13.6 (C-20, q), 12.6 (C-22, q), 9.3 (C-21, q).

4.3. Preparation of 4 and 5

To a solution of 13-DT (12.0 mg) in MeOH (1.0 mL) was added $NaBH_4$ (20 equiv), and the mixture was stirred at rt for 10 min. The reaction mixture was diluted with 10 mL of water and desalted by passing through a short column of ODS (5 mL). The product was separated by ODS-HPLC on Cosmosil MS with 45% aq MeCN followed by ODS-HPLC on the same column with 65% aq MeOH to afford **4** (2.4 mg) and **5** (4.3 mg). Compound **4**: ^{1}H NMR (CD_3OD): δ 5.54 (ddq, J = 10.8, 10.4, 1.5 Hz, H-21), 5.48 (dq, J = 10.8, 6.9 Hz, H-22), 5.13 (br d, J = 9.2 Hz, H-9), 4.08 (dd, J = 10.4, 4.2 Hz, H-29b), 3.99 (d, J = 10.4 Hz, H-7), 3.98 (dd, J = 10.8, 10.4 Hz, H-29a), 3.65 (d, J = 1.9 Hz, H2), 3.64 (dd, J = 9.2, 1.9 Hz, H-3), 3.34 (s, OMe), 3.29 (m, H-16), 3.26 (overlapped, H-17), 3.19 (dq, J = 9.2, 6.9 Hz, H-4), 3.10 (dq, J = 10.4, 6.9 Hz, H-6), 3.03 (ddd, J = 8.4, 8.4, 1.9 Hz, H-11), 2.66 (m, H-14), 2.60 (d, J = 9.2 Hz, H-19), 2.46 (m, H-20), 2.17 (m, H-13b), 2.15 (m, H-10), 1.60 (dd, J = 6.9, 1.5 Hz, H_3-23), 1.54 (br s, H_3-26), 1.31 (s, H_3-30), 1.30 (m, H-12b), 1.27 (d, J = 6.9 Hz, H_3-25), 1.24 (d, J = 6.9 Hz, H_3-24), 1.18 (d, J = 7.3 Hz, H_3-28), 1.15 (m, H-13a), 1.10 (d, J = 6.5 Hz, H_3-31), 0.96 (d, J = 6.9 Hz, H_3-27), 0.90 (m, H-12a); HRESIMS (positive) m/z 619.3466 (calcd for $C_{32}H_{52}NaO_{10}$, 619.3458); **5**: ^{1}H NMR (CD_3OD): δ 5.52 (br d, J = 9.2 Hz, H-9), 5.49 (ddq, J = 10.9, 6.7, 0.6 Hz, H-22), 5.35 (ddq, J = 10.9, 10.8, 1.7 Hz, H-21), 4.07 (d, J = 10.2 Hz, H-7), 4.03 (overlapped, H-29b), 4.03 (overlapped, H-29a), 3.64 (d, J = 2.3 Hz, H-2), 3.57 (dd, J = 9.4, 2.3 Hz, H-3), 3.39 (m, H-11), 3.35 (s, OMe), 3.32 (overlapped, H-17), 3.28 (overlapped, H-16), 3.19 (dq, J = 9.4, 6.9 Hz, H-4), 3.14 (dq, J = 10.2, 7.1 Hz, H-6), 2.62 (d, J = 9.6 Hz, H-19), 2.60 (m, H-14), 2.46 (m, H-20), 2.24 (m, H-10), 1.73 (m, H-13b), 1.59 (dd, J = 6.9, 1.7 Hz, H_3-23), 1.53 (m, H-13a), 1.52 (br d, J = 1.4 Hz, H_3-26), 1.31 (s, H_3-30), 1.26 (d, J = 7.1 Hz, H_3-25), 1.25 (m, H-12b), 1.22 (d, J = 6.9 Hz, H_3-24), 1.19 (d, J = 7.3 Hz, H_3-28), 1.10 (d, J = 6.7 Hz, H_3-31), 0.98 (d, J = 7.1 Hz, H_3-27), 0.92 (m, H-12a); HRESIMS (positive) m/z 619.3446 (calcd for $C_{32}H_{52}NaO_{10}$, 619.3458).

4.4. MTPA esters of 4

To a stirred solution of **4** (1.0 mg) in dry pyridine (three drops) was added (*R*)-(−) MTPACl (5 mg in 50 μL of dry toluene), and the mixture was left to stand at rt for 2 h. The reaction mixture was dried in vacuo, diluted with water, and extracted three times with EtOAc. The organic layer was evaporated, and the residue was subjected to ODS-HPLC on Cosmosil AR-II using gradient elution system (40–100% MeCN) to afford tetra-[(*S*)-(−)-MTPA] ester of **4**. ^{1}H NMR (CD_3OH): δ 5.61 (d, J = 9.82 Hz, H-7), 5.52 (dq, J = 11.5, 6.42 Hz, H-22), 5.43 (br dd, J = 11.49, 9.89, H-21), 5.34 (d, J = 11.4 Hz, H-17), 5.19 (d, J = 8.35 Hz, H-9), 5.08 (d, J = 6.75 Hz, H-2), 4.66 (m, H-11), 4.61 (br d, J = 11.8 Hz, H-29b), 4.07 (dd, J = 6.75, 3.95 Hz, H-3), 4.03 (dd, J = 11.8, 5.35 Hz, H-29a), 3.59 (3H, s, OMe), 3.57 (6H, s, OMe), 3.55 (3H, s, OMe), 3.42 (m, H-16), 3.38 (overlapped, H-6), 3.35 (3H, s, OMe), 3.00 (d, J = 8.99 Hz, H-19), 2.58 (m, H-10), 2.51 (overlapped, H-4), 2.48 (m, H-29), 2.29 (m, H-14), 1.64 (d, J = 6.42 Hz, H_3-23), 1.48 (d, J = 6.98 Hz, H_3-24), 1.41 (s, H_3-30), 1.37 (s, H_3-26), 1.36 (m, H-13b), 1.22 (m, H-12b), 1.18 (d, J = 7.35 Hz, H_3-25), 1.16 (d, J = 6.76 Hz, H_3-31), 1.15 (m, H-12a), 0.95 (d, J = 6.3 Hz, H_3-27), 0.62 (m, H-13a), 0.21 (d, J = 7.55 Hz, H_3-28); FABMS (positive) m/z 1483 $[M+Na]^+$. Other MTPA esters were prepared in the same manner.

4.5. Preparation of 6

13-DT (1.0 mg) in MeOH (1.0 mg) was hydrogenated over 10% Pd/C (2.0 mg) under an atmosphere of H_2 gas at rt overnight. The reaction mixture was passed through a layer of Celite, washed with MeOH and EtOAc, concentrated, and subjected to RP-HPLC on Develosil C30 UG5 by a gradient elution system (60–80% aq MeOH) to obtain **6** (0.6 mg). ^{1}H NMR (CD_3OD): δ 4.38 (dd, J = 10.4, 3.5 Hz, H-29b), 4.21 (dd, J = 11.2, 10.4 Hz, H-29a), 3.75 (d, J = 1.9 Hz, H-2), 3.74 (dd, J = 9.23, 1.9 Hz, H-3), 3.47 (br d, J = 10.38 Hz, H-7), 3.44 (ddd, J = 11.2, 10.0, 3.5 Hz, H-16), 3.33 (s, OMe), 3.11 (d, J = 10.0 Hz, H-17), 3.06 (dq, J = 9.23, 7.30 Hz, H-4), 2.92 (m, H-14), 2.89 (dq, J = 10.00, 6.54 Hz, H-6), 2.64 (m, H-10), 2.54 (d, J = 9.2 Hz, H-19), 2.53 (ddd, J = 17.3, 11.2, 4.6 Hz, H-12b), 2.45 (ddd, J = 16.9, 11.2, 5.4 Hz, H-12a), 1.77 (m, H-13b), 1.72 (dd, J = 12.7, 12.7 Hz, H-9b), 1.66 (m, H-13a), 1.46 (m, H-20), 1.40 (s, H_3-30), 1.24–1.46 (m, H_2-22), 1.21 (d, J = 7.3 Hz, H_3-24), 1.18–1.36 (m, H_2-21), 1.18 (m, H-9a), 1.15 (d, J = 6.5 Hz, H_3-25), 1.12 (m, H-8), 1.05 (d, J = 6.9 Hz, H_3-28), 1.04 (d, J = 6.2 Hz, H_3-31), 1.02 (d, J = 7.3 Hz, H_3-27), 0.88 (t, J = 6.9 Hz, H_3-23), 0.82 (d, J = 6.54 Hz, H_3-26); HRESIMS (positive) m/z 621.3592 (calcd for $C_{32}H_{54}NaO_{10}$, 621.3615).

4.6. Preparation of 7

13-DT (1.0 mg) in MeOH (1.0 mL) was vigorously stirred in the presence of $RhCl(PPh_3)$ (1.0 mg) and H_2 gas at rt for 1 day. The reaction mixture was concentrated, subjected repeatedly to RP-HPLC on Develosil C-30 UG5 with 70% aq MeOH to obtain **7** (0.7 mg). ^{1}H NMR (CD_3OD): δ 5.29 (dd, J = 9.6, 1.2 Hz, H-9), 4.30 (dd, J = 10.4, 4.2 Hz, H-29b), 4.08 (dd, J = 10.4, 11.9 Hz, H-29a), 3.95 (d, J = 10.4 Hz, H-7), 3.68 (d, J = 1.9 Hz, H-2), 3.62 (dd, J = 9.6, 1.9 Hz, H-3), 3.41 (overlapped, H-16), 3.41 (overlapped, H-10), 3.35 (s, OMe), 3.18 (d, J = 10.0 Hz, H-17), 3.11 (m, H-4), 3.09 (m, H-6), 2.81 (m, H-14), 2.53 (d, J = 9.2 Hz, H-19), 2.31 (t, J = 7.7 Hz, H_2-12), 1.94 (m, H-13a), 1.65 (m, H-13b), 1.63 (d, J = 1.2, H_3-26), 1.42 (m, H-20), 1.35 (s, H_3-30), 1.26 (d, J = 6.9 Hz, H_3-25), 1.22–1.46 (m, H_2-22), 1.22–1.46 (m, H_2-21), 1.22 (d, J = 7.3 Hz, H_3-24), 1.11 (d, J = 7.3 Hz, H_3-28), 1.04 (d, J = 6.2 Hz, H_3-31), 1.02 (d, J = 6.9 Hz, H_3-27), 0.89 (t, J = 6.9 Hz,

H_3-23); HRESIMS (positive) *m/z* 621.3592 (calcd for $C_{32}H_{54}NaO_{10}$, 621.3615).

4.7. Preparation of 8, 9, and 10

To a solution of **7** (2.7 mg) in dry pyridine (1.0 mL) was added 4-pentenoyl chloride dropwise (9.1 M, 8.0 μL), and the mixture was allowed to stand at rt and monitored by MS. When ions corresponding to the diacylated product appeared, the reaction was quenched by adding phosphate buffer (2.0 M, pH 7.0, 2.0 mL) to avoid acid-catalyzed decomposition. The mixture was extracted with EtOAc, dried over $MgSO_4$, and concentrated. The crude product was subjected to ODS-HPLC on Cosmosil AR-II using a gradient elution system (60–96.5% aq MeOH) to obtain **8** (0.8 mg), **9** (0.6 mg), and **10** (0.5 mg) together with **7** (0.8 mg). Compound **8**: ^{1}H NMR (CD_3OD): δ 5.88 (m, H-4′), 5.18 (dd, J = 9.4, 0.96 Hz, H-9), 5.10 (br d, J = 17.1 Hz, H-5′b), 5.02 (br d, J = 10.2 Hz, H-5′a), 4.69 (d, J = 2.3 Hz, H-2), 4.18 (dd, J = 10.8, 10.4 Hz, H-29b), 4.14 (dd, J = 4.2, 10.4 Hz, H-29a), 4.05 (dd, J = 7.7, 2.3 Hz, H-3), 3.97 (d, J = 9.6 Hz, H-7), 3.44 (m, H-16), 3.37 (s, OMe), 3.32 (overlapped, H-10), 3.28 (overlapped, H-17), 3.01 (dq, J = 7.7, 7.3 Hz, H-4), 2.91 (dq, J = 6.9, 9.8 Hz, H-6), 2.78 (m, H-14), 2.57 (m, H_2-2′), 2.55 (d, J = 9.23 Hz, H-19), 2.43 (m, H-12b), 2.39 (m, H_2-3′), 2.31 (m, H-12a), 2.09 (m, H-13b), 1.51 (d, J = 0.96 Hz, H_3-26), 1.44 (m, H-13a), 1.42 (m, H-20), 1.33 (s, H_3-30), 1.29 (d, J = 7.3 Hz, H_3-24), 1.29 (d, J = 6.7 Hz, H_3-25), 1.28–1.43 (m, H_2-22), 1.28–1.43 (m, H_2-21), 1.16 (d, J = 7.1 Hz, H_3-28), 1.04 (d, J = 6.7 Hz, H_3-27), 1.04 (d, J = 6.7 Hz, H_3-31), 0.90 (t, J = 7.1 Hz, H_3-23); HRESIMS (positive) *m/z* 701.3886 (calcd for $C_{37}H_{58}NaO_{11}$, 701.3877); **9**: ^{1}H NMR (CD_3OD): δ 5.80 (m, H-4′), 5.42 (d, J = 10.0 Hz, H-9), 5.32 (d, J = 10.4 Hz, H-7), 5.03 (br d, J = 17.3 Hz, H-5′a), 4.96 (br d, J = 10.0 Hz, H-5′b), 4.27 (dd, J = 10.4, 4.2 Hz, H-29b), 4.10 (dd, J = 10.4, 10.4 Hz, H-29a), 3.68 (d, J = 2.3 Hz, H-2), 3.61 (dd, J = 9.6, 2.3 Hz, H-3), 3.42 (overlapped, H-16), 3.40 (overlapped, H-10), 3.37 (s, OMe), 3.36 (overlapped, H-6), 3.20 (d, J = 8.5 Hz, H-17), 3.17 (dq, J = 9.6, 6.9 Hz, H-4), 2.80 (m, H-14), 2.53 (d, J = 9.2 Hz, H-19), 2.41 (t, J = 6.9 Hz, H_2-2′), 2.33 (dt, J = 6.9 Hz, H_2-3′), 2.28 (m, H-12b), 2.28 (m, H-12a), 1.96 (m, H-13a), 1.62 (s, H_3-26), 1.62 (m, H-13b), 1.43 (m, H-20), 1.35 (s, H_3-30), 1.27–1.43 (m, H_2-22), 1.27–1.43 (m, H_2-21), 1.23 (d, J = 7.3 Hz, H_2-24), 1.16 (d, J = 6.9 Hz, H_3-25), 1.12 (d, J = 7.3 Hz, H_3-28), 1.04 (d, J = 6.2 Hz, H_3-31), 0.97 (d, J = 6.9 Hz, H_3-27), 0.90 (t, J = 6.9 Hz, H_3-23); HRESIMS (positive) *m/z* 701.3890 (calcd for $C_{37}H_{58}NaO_{11}$, 701.3877); **10**: ^{1}H NMR (CD_3OD): δ 5.89 (m, H-4′), 5.80 (m, H-4″), 5.34 (d, J = 10.4 Hz, H-9), 5.34 (d, J = 10.4 Hz, H-7), 5.11 (br d, J = 16.9 Hz, H-5′a), 5.03 (br d, J = 16.9 Hz, H-5″a), 5.02 (br d, J = 10.8 Hz, H-5′b), 4.96 (br d, J = 10.4 Hz, H-5″b), 4.70 (d, J = 2.3 Hz, H-2), 4.19 (dd, J = 10.8, 10.4 Hz, H-29b), 4.15 (dd, J = 10.4, 4.2 Hz, H-29a), 4.04 (dd, J = 7.8, 2.3 Hz, H-3), 3.42 (m, H-16), 3.39 (s, OMe), 3.32 (overlapped, H-10), 3.27 (d, J = 10.0 Hz, H-17), 3.14 (dq, J = 10.4, 6.9 Hz, H-6), 3.07 (dq, J = 7.7, 6.9 Hz, H-4), 2.77 (m, H-14), 2.57 (m, H_2-2′), 2.55 (d, J = 9.2 Hz, H-19), 2.41 (m, H_2-2″), 2.39 (m, H-12b), 2.39 (m, H_2-3′), 2.34 (m, H_2-3″), 2.34 (m, H-12a), 2.00 (m, H-13b), 1.51 (s, H_3-26), 1.43 (m, H-13a), 1.42 (m, H-20), 1.34 (s, H_3-30), 1.30 (d, J = 7.3 Hz, H_3-24), 1.23–1.46 (m, H_2-22), 1.23–1.46 (m, H_2-21), 1.20 (d, J = 6.9 Hz, H_3-25), 1.16 (d, J = 7.3 Hz, H_3-28), 1.04 (d, J = 6.5 Hz, H_3-31), 1.01 (d, J = 6.9 Hz, H_3-27), 0.90 (t, J = 7.3 Hz, H_3-23); HRESIMS (positive) *m/z* 783.4291 (calcd for $C_{42}H_{64}NaO_{12}$, 783.4295).

4.8. Preparation of 11

To a stirred solution of 13-DT (0.8 mg) in dry pyridine (0.5 mL) was added Ac_2O (90 μL), and the mixture was left standing at rt overnight. Reaction was quenched by addition of phosphate buffer (2.0 M, pH 7.0, 2.0 mL), and the reaction mixture was extracted with EtOAc three times, dried over $MgSO_4$, and evaporated. The residue was subjected to ODS-HPLC on Cosmosil AR-II using a gradient elution system (70–100% aq MeOH) to yield **11** (0.9 mg). ^{1}H NMR (CD_3OD): δ 5.50 (dq, J = 10.8, 7.0 Hz, H-22), 5.34 (overlapped, H-9), 5.34 (d, J = 10.0 Hz, H-7), 5.32 (overlapped, H-21), 4.80 (overlapped, H-17), 4.72 (d, J = 2.7 Hz, H-2), 4.22 (dd, J = 10.8, 4.2 Hz, H-29b), 4.14 (dd, J = 10.8, 10.8 Hz, H-29a), 4.04 (dd, J = 8.1, 2.7 Hz, H-3), 3.62 (ddd, J = 10.8, 10.8, 4.2 Hz, H-16), 3.38 (s, OMe), 3.31 (overlapped, H-10), 3.19 (dq, J = 10.0, 7.3 Hz, H-6), 3.07 (dq, J = 8.1, 6.9 Hz, H-4), 2.81 (d, J = 9.2 Hz, H-19), 2.78 (m, H-14), 2.44 (m, H-20), 2.36 (m, H-12b), 2.30 (m, H-12a), 2.17 (s, OAc), 2.03 (s, OAc), 2.00 (m, H-13a), 1.99 (s, OAc), 1.60 (dd, J = 6.9, 1.9 Hz, H_3-23), 1.51 (s, H_3-26), 1.40 (m, H-13b), 1.38 (s, H_3-30), 1.32 (d, J = 6.9 Hz, H_3-24), 1.21 (d, J = 6.9 Hz, H_3-25), 1.14 (d, J = 7.3 Hz, H_3-28), 1.10 (d, J = 6.5 Hz, H_3-31), 1.00 (d, J = 6.9 Hz, H_3-27); HRESIMS *m/z* 743.3655 (calcd for $C_{38}H_{56}NaO_{13}$, 743.3619).

4.9. Preparation of 12

To a solution of 13-DT (4.0 mg) in 50% aq MeOH (1.0 mL) was added 0.1 M OsO_4 aqueous solution (40 μL) and 0.1 M $NaIO_4$ aqueous solution (0.9 mL), and the mixture was allowed to stand at rt overnight. The reaction mixture was dried in vacuo and extracted with EtOAc. The organic extract was concentrated and subjected to ODS-HPLC on Cosmosil AR-II using a gradient elution system (10–45% aq MeCN), followed by ODS-HPLC on Cosmosil AR-II with 50% aq MeOH to yield **12** (1.9 mg). ^{1}H NMR: δ 5.25 (0.5H, d, J = 10.0 Hz, H-9), 5.23 (0.5H, d, J = 10.0 Hz, H-9), 4.57 (0.5H, dd, J = 10.5, 4.0 Hz, H-29b), 4.53 (0.5H, dd, J = 10.6, 4.5 Hz, H-29b), 4.48 (0.5H, d, J = 4.17 Hz, H-21), 4.39 (0.5H, d, J = 6.25 Hz, H-21), 4.1 (0.5H, dd, J = 10.6, 11.6 Hz, H-29a), 4.08 (0.5H, dd, J = 10.3, 11.6 Hz, H-29a), 3.97 (0.5H, d, J = 9.8 Hz, H-7), 3.96 (0.5H, d, J = 10.0 Hz, H-7), 3.71 (0.5H, d, J = 1.8 Hz, H-2), 3.68 (0.5H, d, J = 1.8 Hz, H-2), 3.63–3.66 (1H, overlapped, H-3), 3.41 (1H, m, H-16), 3.4 (0.5H, m, H-10), 3.39 (0.5H, m, H-10), 3.38 (3H, s, OMe), 3.16 (1H, m, H-4), 3.13 (1H, d, J = 10.3 Hz, H-17), 3.11 (1H, m, H-6), 2.77 (1H, m, H-14), 2.75 (0.5H, d, J = 9.25 Hz, H-19), 2.67 (0.5H, d, J = 9.3 Hz, H-19), 2.29 (2H, m, H_2-12), 1.98 (1H, m, H-13b), 1.66 (0.5H, m, H-20), 1.63 (3H, s, H_3-26), 1.62

(0.5H, m, H-20), 1.56 (1H, m, H-13a), 1.34 (3H, s, H_3-30), 1.28 (3H, d, $J = 4.69$ Hz, H_3-25), 1.23 (3H, d, $J = 7.01$ Hz, H_3-24), 1.13 (3H, d, $J = 7.13$ Hz, H_3-28), 1.06 (1.5H, d, $J = 6.7$ Hz, H_3-31), 1.05 (1.5H, d, $J = 5.34$ Hz, H_3-31), 1.02 (3H, d, $J = 6.8$ Hz, H_3-27); HRESIMS *m/z* 605.2933 (calcd for $C_{30}H_{46}NaO_{11}$, 605.2938).

4.10. Cytotoxicity test

Cytotoxicity against P388 cells was evaluated by using MTT assay as described previously.[8]

4.11. In vitro polypeptide elongation

Protein synthesis inhibition was assessed by in vitro polypeptide elongation experiment, which was carried out as described in the accompanying paper.[1]

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