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Total Synthesis and Biological Activity of Dolastatin 16

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The total synthesis of dolastatin 16, a macrocyclic depsipeptide first isolated from the sea hare *Dolabella auricularia* as a potential antineoplastic metabolite by Pettit *et al.*, was achieved in a convergent manner. Dolastatin 16 was reported by Tan to exhibit strong antifouling activity, and thus shows promise for inhibiting the attachment of marine benthic organisms such as *Amphibalanus amphitrite* to ships and submerged artificial structures. Therefore, dolastatin 16 is a potential compound for a new, environmentally friendly antifouling material to replace banned tributyltin-based antifouling paints. The synthesis of dolastatin 16 involved the use of prolinol to prevent formation of a diketopiperazine composed of L-proline and N-methyl-D-valine during peptide coupling. This strategy for the elongation of peptide chains allowed the efficient and scalable synthesis of one segment, which was subsequently coupled with a second segment and cyclized to form the macrocyclic framework of dolastatin 16. The synthetic dolastatin 16 exhibited potent antifouling activity similar to that of natural dolastatin 16 toward cypris larvae of *Amphibalanus amphitrite*.

Introduction

Biofouling is the accumulation of organisms on immersed artificial structures such as ship hulls, jetty pilings, aquaculture net cages, and seawater intake pipes, and results in significant economic and environmental problems. For example, the settlement of marine benthic organisms on a ship's surface increases fuel consumption by as much as 40% due to friction.¹ In addition, frequent dry-docking and maintenance to remove biofouling organisms is an expense. Antifouling paints have been used to address this problem and minimize the associated economic costs. Tributyltin (TBT)-based antifouling paint was developed during the 1960s and was so efficient against a broad range of fouling organisms that it became the leading solution, adopted by approximately 70% of the world's shipping fleets.² However, harmful effects of TBT on marine organisms such as fish,³ crustaceans,⁴ and especially molluscs⁵ were subsequently reported. Nanogram per liter concentrations of TBT induce masculinization of female gastropods and have resulted in the extinction of certain species.⁶ As of 2004, approximately 150 gastropod species worldwide have been affected.⁷ Consequently, the International Maritime Organization (IMO) prohibited the use of TBT-based antifouling paints on ships in 2008.⁸ Currently, TBT-based paints have been replaced by

copper-based antifouling agents, but these require a high concentration of copper and a co-biocide to achieve the same efficacy. Concerns about copper toxicity have led several countries to review their existing copper environmental risk assessments in coastal waters, and a number of countries have already banned copper-based antifouling paints in areas with a high density of boats.⁹ Thus, the development of antifouling agents without heavy metals is highly desired.

Marine organisms prevent fouling of their outer surfaces through the use of natural chemical defense substances with antifouling properties without causing serious environmental problems.¹⁰ Therefore, natural antifouling products, especially those with potent settlement-inhibiting activities but without biocidal properties, are potential candidates as non-biocide-based and environmentally friendly antifouling agents. Several marine antifouling natural products have been reported over the past decade, resulting from the search for nontoxic and environmentally benign active components for antifouling paints.^{11,12}

Dolastatin 16 (**1**), a macrocyclic depsipeptide, was first isolated in 1997 from the sea hare *Dolabella auricularia* as a potential anticancer compound by Pettit and co-workers.¹³ This unique depsipeptide proved to strongly inhibit the growth of a variety of human cancer cell lines and thus was a candidate for further development as an anticancer drug. Gerwick *et al.* also described the isolation of **1** from a Madagascan cyanobacterium, *Lyngbya majuscula*, in 2002.¹⁴ The unique structural feature of **1** is the presence of the unusual amino acids dolamethylleucine (**2**) and dolaphenvaline (**3**). The stereostructures of **2** and **3** were not assigned in the first report, but subsequent X-ray crystallographic studies of the natural product showed that

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[†] Electronic Supplementary Information (ESI) available: Preparation of **7**, **8**, **10**, **17** and **20**, optimizations for **18**, **22** and **23**, and ¹H and ¹³C NMR spectra for all compounds. See DOI: 10.1039/x0xx00000x

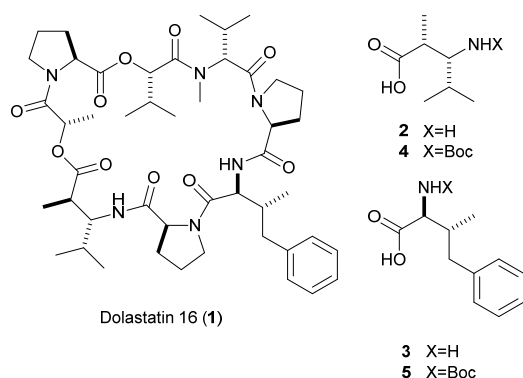


Figure 1. Structure of dolastatin 16 (**1**) and the unusual amino acids **2** and **3**.

the absolute configurations of the contiguous stereocenters of **2** and **3** were (2*R*,3*R*) and (2*S*,3*R*), respectively.¹⁵

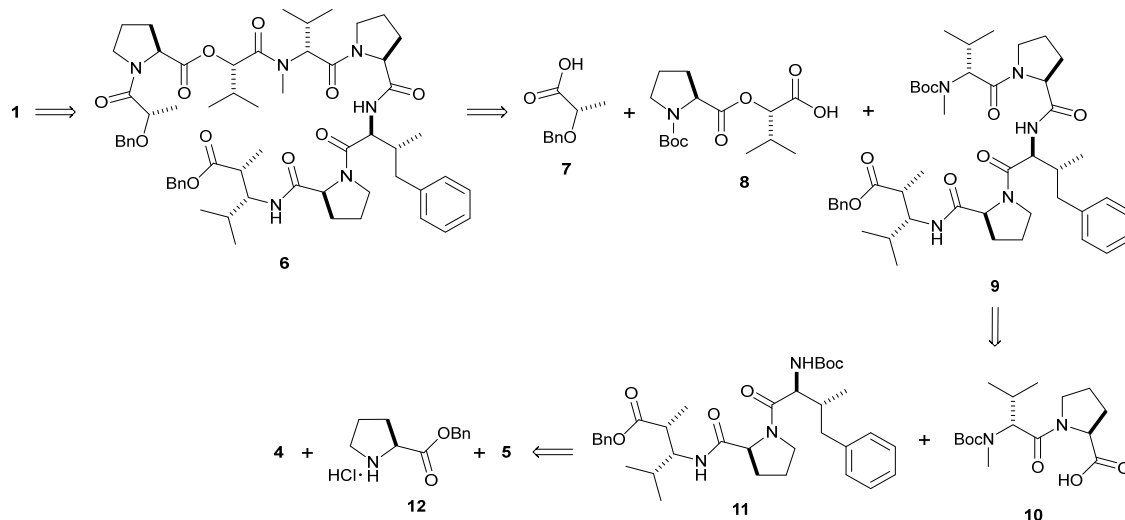
In 2010, Tan's group reported that **1** effectively inhibited the larval settlement and metamorphosis of the barnacle *Amphibalanus amphitrite* with an EC₅₀ value of 0.003 µg/mL.¹⁶ The LC₅₀/EC₅₀ ratio of **1** is 6000, and, therefore, **1** was expected to be a promising lead compound alternative to the heavy-metal-based antifouling agents currently used. Pettit's group completed the first total synthesis of **1** in 2015.¹⁷ Surprisingly, **1** isolated from natural sources exhibited impressive activity against several human cancer cell lines, whereas synthetic **1** did not possess significant activity. This discrepancy raises the question of whether the antifouling properties of synthetic **1** are also much lower than those of **1** isolated from natural sources. Concise and scalable syntheses of *N*-Boc-dolamethylleuine (**4**) and *N*-Boc-dolaphenvaline (**5**), the *N*-Boc-protected unusual amino acids in **1**, have been developed using

asymmetric Mannich reactions.¹⁸ A notable feature of these syntheses is construction of the contiguous stereogenic centers of **2** and **3** with almost complete diastereo- and enantioselectivity by employing chiral organocatalysts. With adequate amounts of the unusual amino acids synthesized, attention was focused on the assembly of the macrocyclic framework of **1**. Herein, the synthetic details of the total synthesis of **1** are described and the significant biological activities of synthetic **1** are reported.

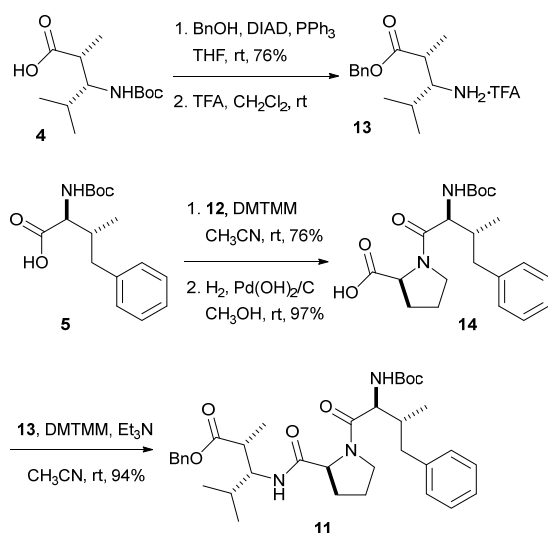
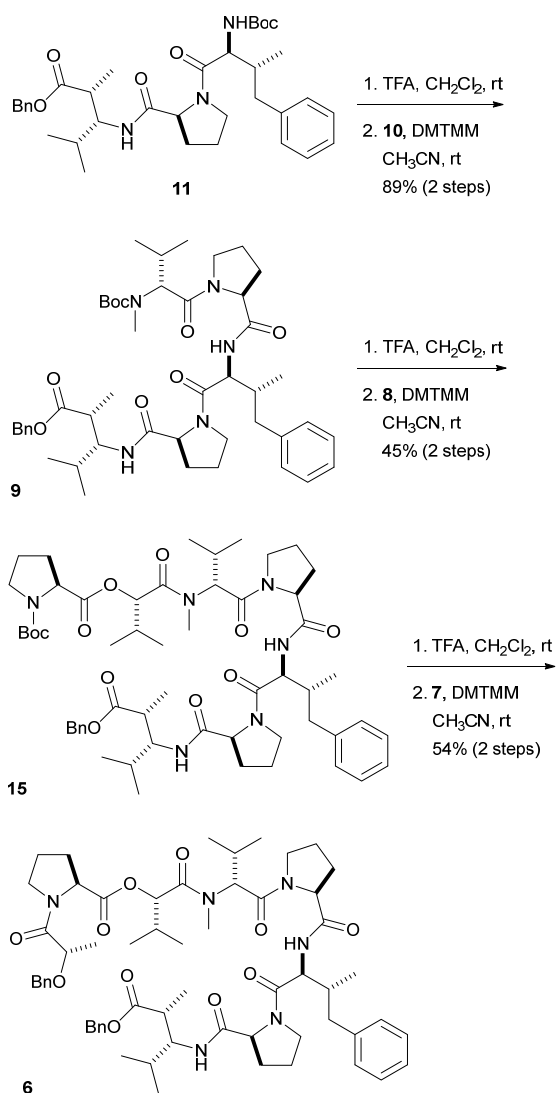
Results and discussion

A retrosynthetic analysis for the synthesis of dolastatin 16 using a build-up approach is presented in Scheme 1. The synthesis of **1** was envisioned via macrolactonization between the hydroxy group in lactate and the carboxylic acid in dolamethylleuine of **6**. Synthesis of **6** was designed by condensation of *O*-benzyl-L-lactic acid (**7**) and peptide fragments **8** and **9**. Fragment **9** would be prepared from carboxylic acid **10** and southern segment **11**. The southern segment **11** was traced back from L-proline benzyl ester hydrochloride (**12**) and the two unusual amino acid units **4** and **5**.

Preparation of **11** is shown in Scheme 2. Dolamethylleuine benzyl ester (**13**) was obtained from **4** through a Mitsunobu reaction with benzyl alcohol, followed by deprotection of the Boc group with TFA. The carboxylic acid **14** was prepared in 74% yield (over two steps) by condensation between **5** and **12** in the presence of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM)¹⁹ and subsequent hydrogenolysis to remove the benzyl ester. Amide formation with **13** and **14** in a similar manner in the presence of Et₃N afforded **11** in 94% yield.

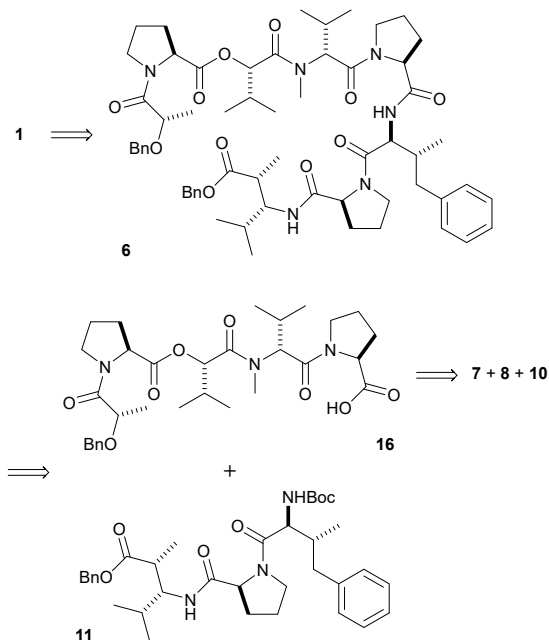


Scheme 1. Retrosynthetic analysis of dolastatin 16 (**1**).

Scheme 2. Synthesis of southern segment **11**.Scheme 3. Synthesis of **6**.

Next, the peptide backbone of **1** was completed as shown in Scheme 3. After removal of the Boc protecting group of **11**, coupling reaction with **10**²⁰ in the presence of DMTMM furnished **9** in high yield (89% over two steps). Using the same protocol, amide **15** was synthesized from **9** and **8**²⁰ in 45% yield (over two steps). Finally, all fragments of **1** were assembled by removal of the Boc group of **15** with TFA, followed by coupling with **7**²⁰ in 54% yield (over two steps). Further optimizations resulted in moderate yields of **15** and **6**. Obviously, the modest yields afforded in the last two coupling reactions (45% and 54%, respectively) were not acceptable in this advanced stage of the synthesis.

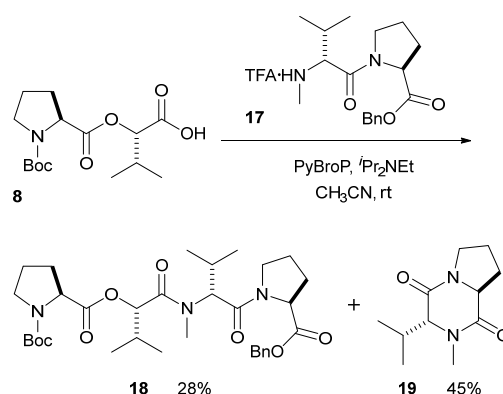
To develop a more efficient total synthesis, an alternative convergent route was strategized. The improved retrosynthetic analysis for the convergent route is illustrated in Scheme 4. A coupling reaction between the northern segment **16** and the southern segment **11** would access **1** via **6**, because the above-mentioned peptide formation reaction between **10** and **11** proceeded in high yields (89% over two steps) without epimerization²¹ (Scheme 3), although this concept is the same as Pettit's synthesis. This strategy was considered best for the total synthesis of dolastatin **16** because **11**, which contains the unusual amino acid units, could be used as the most advanced intermediate for the preparation of **6**. The northern segment **16** would be obtained from **7**, **8**, and **10** by stepwise fragment condensations.

Scheme 4. Improved retrosynthetic analysis of dolastatin **16** (**1**).

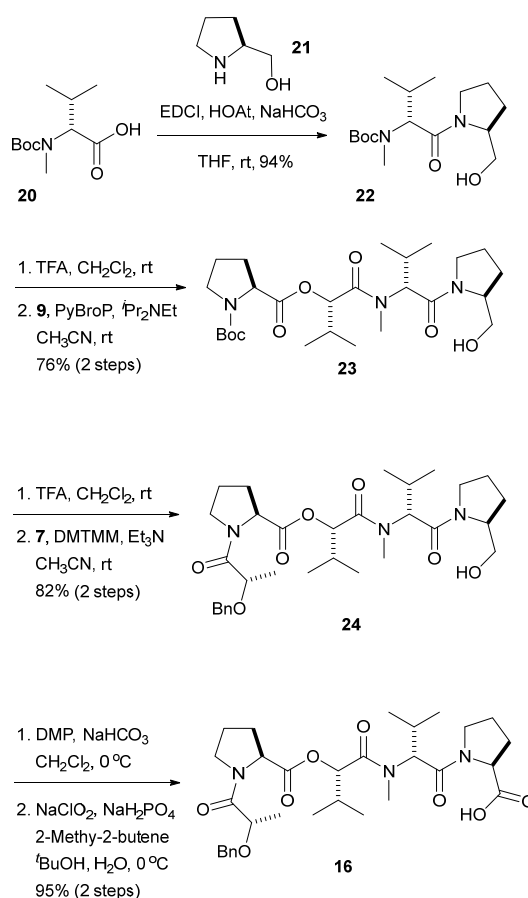
In a preliminary study to construct **16**, coupling reactions between **8** and TFA salt **17**²⁰ were conducted (Scheme 5). However, extensive attempts under various reaction conditions provided only low yields of the desired amide **18**.²² In the presence of bromotripyrrolidinophosphonium hexafluorophosphate (PyBroP),²³ the yield of **18** was 28%, but the main product of this reaction was the diketopiperazine **19** composed of L-proline and *N*-methyl-D-valine. Formation of diketopiperazine is a well-known side reaction in the synthesis of dipeptide esters containing *N*-methyl or prolyl-type amide linkages.²⁴ To minimize the tendency of dipeptide **17** to cyclize into diketopiperazine, prolinol was used in place of proline ester as the C-terminal amino acid.

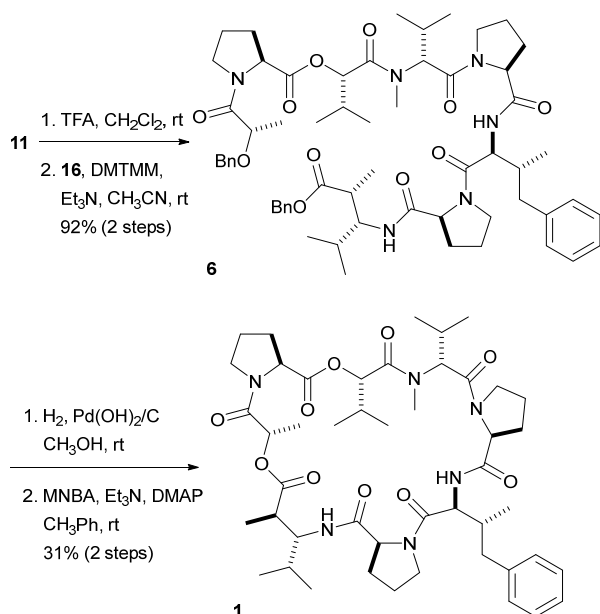
The synthesis of **16** commenced with a condensation reaction between *N*-Boc-*N*-methyl-D-valine (**20**)²⁰ and L-prolinol (**21**) to afford amide **22** (Scheme 6). Careful optimization²² allowed a high yield and scale-up (up to 2.6 mmol of **21**) for this reaction with the EDCI/HOAt system. After TFA-promoted cleavage of the Boc group of **22**, coupling reaction of the resulting TFA salt with **9** produced amide **23**. After extensive investigations²² with coupling reagents, such as triphosgene,²⁵ HATU,²⁶ DECP,²⁷ or EDCI, for synthesis of **23**, PyBroP and *i*Pr₂NEt were found to provide **23** in 76% yield without epimerization. The effectiveness of PyBroP in facilitating the coupling reactions of *N*-methylated amino acids is well recognized.²³ To complete the components of the northern segment, the Boc group of **23** was deprotected using TFA, and the resulting TFA salt was condensed with *O*-benzyl-L-lactic acid (**7**)²⁰ to give amide **24** in 82% yield (two steps). Finally, successive Dess-Martin and Pinnick oxidation of **24** afforded **16** with an overall yield of 95%.²⁸ The synthetic steps leading to **16** demonstrated high yields for amide bond formation with various secondary amines in the presence of the unprotected primary alcohol, resulting from the judicious choice of coupling reagents.²⁹ In addition, the conversion of the primary hydroxy group to a carboxylic acid was efficient. These results demonstrate that the use of aminoalcohol instead of the corresponding α -amino acid ester is an effective strategy for chain elongation of peptide frameworks.

With segments **11** and **16** synthesized, the total synthesis of **1** was completed as shown in Scheme 7. Cleavage of the Boc group from **11** with TFA, followed by a coupling reaction with **16** using DMTMM furnished the linear precursor **6** in 92% yield (over two steps). Global deprotection of the benzyl groups of **6** afforded the desired seco acid. Lastly, the Shiina protocol³⁰ was used for macrolactonization to afford **1** in 31% overall yield (two steps), because other procedures such as the Yamaguchi lactonization³¹ did not provide the desired product. All data (¹H and ¹³C NMR, HRMS, and optical properties) for synthetic **1** were identical to those reported by Pettit and co-workers^{13,17} for the natural and synthetic samples.



Scheme 5. Diketopiperazine formation.

Scheme 6. Synthesis of northern segment **16**.

Scheme 7. Total synthesis of dolastatin 16 (**1**).

Next, the biological activities of **1** and of synthetic intermediates **16** and **25**, the latter being the amine derivative of **11**, were evaluated. Antifouling activity was evaluated as 50% effective concentration (EC₅₀) of each compound against the settlement of the cypris larvae of *Amphibalanus amphitrite* after a 48h incubation period (Table 1). Despite the concerns that synthetic **1** would possess much lower antifouling activity compared to its natural counterpart (as shown by Pettit), the synthetic sample was found to be highly potent (EC₅₀ < 0.03 µg/mL) and more effective than CuSO₄ as a fouling inhibitor. The EC₅₀ values of **16** and **25** indicated moderate to weak activity. These results demonstrate that all components of **1** and/or the cyclic structure are essential for strong antifouling activity. The 50% lethal concentrations toward the same larvae (LC₅₀ > 10 µg/mL) as well as MCF-7 cells (LC₅₀ > 30 µg/mL)³² were much greater than the EC₅₀ value, and thus **1** is a novel candidate for an environmentally friendly antifouling material. In addition, the MCF-7 cell results agreed with those in the report by Pettit (GI₅₀ > 10 µg/mL).¹⁷ The LC₅₀ value of **16** on MCF-7 cells (LC₅₀ > 100 µg/mL) was much greater than the value produced by **25** (LC₅₀ between 10 and 30 µg/mL) containing unusual amino acids.

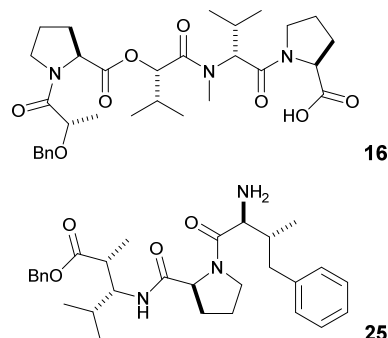
Conclusions

In summary, the total synthesis of dolastatin 16 (**1**) was achieved using a convergent process. The synthesis involved scalable and concise preparation of the southern and northern segments **11** and **16**, and efficient assembly of the two segments to construct the macrocyclic skeleton of **1** after considering unsuccessful results. The synthetic sequences

Table 1. Biological activity of synthetic dolastatin 16 (**1**) and segments **16** and **25**

compound	EC ₅₀ (µg/mL) ^d	LC ₅₀ (µg/mL) ^d	Cytotoxicity LC ₅₀ (µg/mL) ^e
Synthetic 1	< 0.03	> 10	> 30
Natural 1 ^a	0.003	20	—
Synthetic 1 ^b	—	—	> 10 ^f
16	> 10	> 10	> 100
25	1.17	> 10	10–30
CuSO ₄ ^c	0.10	> 10	8.6

^aobtained by Tan, see ref. 16. ^bobtained by Pettit, see ref. 17. ^creference. ^dagainst cypris larvae of *Amphibalanus amphitrite*. ^eagainst MCF-7 cell (breast cancer cell) ^fdescribed as GI₅₀ (µg/mL)



for **11** and **16** provided subgram amounts for overall yields of 56% (7 steps) and 69% (3 steps), respectively. The synthesis of **16** was characterized using prolinol to prevent formation of diketopiperazine containing L-proline and N-methyl-D-valine. The product, **1**, obtained from this synthesis also allowed confirmation of its significant antifouling activity, yet low toxicity. Detailed investigation of the structure-activity relations of **1** and the preparation of molecular probes for elucidating a mechanism of action are currently underway.

Acknowledgements

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Experimental

General Methods. Tetrahydrofuran (THF), methanol (CH₃OH), and acetonitrile (CH₃CN) were purchased from Kanto Chemical Co. Inc. Dichloromethane (CH₂Cl₂) and triethylamine (Et₃N) were distilled from CaH₂. All commercially obtained reagents were used as received.

Analytical TLC was carried out using pre-coated silica gel plates (Merck TLC silica gel 60F₂₅₄). Wakogel 60N 63-212 µm was used for column chromatography. IR spectra were recorded on a JASCO FTIR-4100 Type A spectrometer using a NaCl cell. ¹H and ¹³C NMR spectra were recorded using a JNM-EX 400 (400 MHz and 100 MHz) spectrometer. Chemical shifts are reported

in ppm relative to CHCl_3 ($\delta = 7.26$) in CDCl_3 for ^1H NMR, and CDCl_3 ($\delta = 77.0$) for ^{13}C NMR. Splitting patterns are designated as s, d, t, q, and m, indicating singlet, doublet, triplet, quartet, and multiplet, respectively.

TFA-H-Dml-OBn (13). To a solution of *N*-Boc-dolamethylleuine (**4**) (Boc-Dml-OH) (116 mg, 0.473 mmol) in THF (2.4 mL) were added BnOH (53.9 μL , 0.520 mmol), PPh_3 (186 mg, 0.710 mmol), and DIAD (0.373 mL, 0.710 mmol) at 0°C under Ar atmosphere. The mixture was stirred at room temperature for 16 h, quenched with saturated NaHCO_3 , extracted with EtOAc, washed with brine, dried over Na_2SO_4 , and concentrated *in vacuo*. The crude product was purified using column chromatography (5% EtOAc in hexane) to afford Boc-Dml-OBn as a colorless oil (120 mg, 0.358 mmol, 76%): $[\alpha]^{23}_{\text{D}} = +15.4$ (c 0.23, CHCl_3); IR (neat) 3750, 2974, 2876, 2360, 2341, 1716, 1507, 1166, 772, 669 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 0.86–0.90 (6H, m), 1.20 (3H, d, $J = 7.3$ Hz), 1.40 (9H, s), 1.57–1.64 (1H, m), 2.78–2.85 (1H, m), 3.35–3.41 (1H, m), 5.05–5.12 (2H, m), 5.23 (1H, d, $J = 10.8$ Hz), 7.31–7.37 (5H, m); ^{13}C NMR (CDCl_3 , 100 MHz) δ 15.7, 19.2, 19.9, 28.4, 31.8, 40.5, 58.6, 66.3, 78.8, 128.1, 128.3, 128.6, 135.7, 156.4, 175.6; HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{19}\text{H}_{29}\text{NO}_4\text{Na}$ 358.1989; Found 358.1992.

To Boc-Dml-OBn (255 mg, 0.760 mmol) was added TFA/ CH_2Cl_2 (1:4 v/v, 25 mL). After 1 h of stirring at room temperature, the solution was concentrated *in vacuo* to afford crude **13**, which was used in the next step without further purification.

Boc-Dpv-Pro-OH (14). To a solution of *N*-Boc-dolaphenvaline (**5**) (Boc-Dpv-OH) (208 mg, 0.709 mmol) and HCl-H-Pro-OBn (**12**) (257 mg, 1.06 mmol) in CH_3CN (3.5 mL) was added DMTMM (294 mg, 1.06 mmol) under Ar atmosphere. After 16 h of stirring at room temperature, the mixture was concentrated *in vacuo*. The residue was purified using column chromatography (10% EtOAc in hexane) to afford Boc-Dpv-Pro-OBn as a transparent solid (259 mg, 0.539 mmol, 76%): $[\alpha]^{23}_{\text{D}} = -31.8$ (c 2.21, CHCl_3); IR (neat) 3734, 3308, 2976, 2360, 2341, 1746, 1709, 1647, 1497, 1433, 1169, 752, 700 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 0.79 (3H, d, $J = 6.8$ Hz), 1.37 (9H, s), 1.77–1.86 (3H, m), 1.96–2.02 (1H, m), 2.04–2.14 (1H, m), 2.34 (1H, dd, $J = 6.8$, 13.4 Hz), 2.72 (1H, dd, $J = 6.8$, 13.4 Hz), 3.10–3.20 (1H, m), 3.24–3.31 (1H, m), 3.34–4.40 (1H, m), 4.46–4.50 (1H, dd, $J = 6.3$, 8.8 Hz), 5.06 (2H, s), 5.22 (1H, d, $J = 9.3$ Hz), 7.10–7.28 (10H, m); ^{13}C NMR (CDCl_3 , 100 MHz) δ 14.1, 24.9, 28.3, 28.9, 38.4, 40.0, 46.4, 54.0, 58.8, 66.8, 79.5, 126.0, 128.1, 128.2, 128.3, 128.49, 128.50, 128.54, 129.4, 135.5, 140.4, 155.9, 170.9, 171.8; HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{28}\text{H}_{36}\text{N}_2\text{O}_5\text{Na}$ 503.2516; Found 503.2515.

To a solution of Boc-Dpv-Pro-OBn (259 mg, 0.539 mmol) in CH_3OH (2.7 mL) was carefully added 20% $\text{Pd}(\text{OH})_2/\text{C}$ (25.9 mg, 10 wt%) under Ar atmosphere. The solution was purged with H_2 gas and stirring was continued under H_2 at room temperature for 16 h. The solution was filtered through celite and concentrated *in vacuo*. The crude product was purified using column chromatography (30% EtOAc in hexane) to afford **14** as a transparent solid (205 mg, 0.525 mmol, 97%): $[\alpha]^{23}_{\text{D}} = -40.5$ (c 1.10, CHCl_3); IR (neat) 3734, 3302, 2977, 2360, 2341, 1715, 1647, 1615, 1507, 1455, 1168, 754, 702 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 0.89 (3H, d, $J = 6.8$ Hz), 1.40 (9H, s), 1.83–1.91 (2H, m),

2.02–2.08 (2H, m), 2.09–2.18 (1H, m), 2.39 (1H, dd, $J = 6.8$, 13.4 Hz), 2.74 (1H, dd, $J = 7.3$, 13.4 Hz), 3.13–3.19 (1H, m), 3.28–3.36 (1H, m), 4.38–4.44 (1H, m), 4.52 (1H, dd, $J = 4.4$, 8.3 Hz), 5.31 (1H, d, $J = 9.3$ Hz), 7.15–7.28 (5H, m); ^{13}C NMR (CDCl_3 , 100 MHz) δ 14.3, 24.9, 27.9, 28.3, 38.4, 39.9, 46.9, 54.0, 59.3, 79.8, 126.2, 128.3, 129.4, 140.1, 155.9, 172.7, 174.0; HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{21}\text{H}_{30}\text{O}_5\text{N}_2\text{Na}$ 413.2047; Found 413.2047.

Boc-Dpv-Pro-Dml-OBn (11). To a solution of crude TFA-H-Dml-OBn (**13**) and **14** (253 mg, 0.648 mmol) in CH_3CN (3.2 mL) were added Et_3N (0.542 mL, 3.89 mmol) and DMTMM (179 mg, 0.648 mmol) under Ar atmosphere. After 16 h of stirring at room temperature, the mixture was concentrated *in vacuo*. The residue was purified using column chromatography (10% EtOAc in hexane) to afford **11** as a transparent solid (372 mg, 0.612 mmol, 94%): $[\alpha]^{23}_{\text{D}} = +17.6$ (c 2.05, CHCl_3); IR (neat) 3734, 3417, 3311, 2973, 2876, 2360, 2341, 1715, 1507, 1245, 1171, 752, 700 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 0.76–0.86 (9H, m), 1.13 (3H, d, $J = 7.3$ Hz), 1.37–1.45 (10H, m), 1.78–1.90 (2H, m), 1.92–2.10 (2H, m), 2.14–2.26 (1H, m), 2.40 (1H, dd, $J = 6.8$, 13.4 Hz), 2.73 (1H, dd, $J = 7.3$, 13.2 Hz), 2.77–2.85 (1H, m), 3.18–3.30 (2H, m), 3.64 (1H, dt, $J = 3.4$, 9.8 Hz), 4.45–4.50 (2H, m), 4.90–5.00 (2H, m), 5.31 (1H, d, $J = 9.2$ Hz), 6.79 (1H, d, $J = 10.2$ Hz), 7.08–7.15 (1H, m), 7.18–7.34 (9H, m); ^{13}C NMR (CDCl_3 , 100 MHz) δ 14.0, 15.9, 19.5, 19.8, 24.9, 28.3, 29.1, 31.9, 38.3, 39.7, 40.5, 46.7, 53.9, 57.0, 60.6, 66.3, 79.5, 126.1, 128.0, 128.2, 128.3, 128.6, 129.5, 135.6, 140.3, 155.9, 171.5, 171.9, 175.9; HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{35}\text{H}_{49}\text{N}_3\text{O}_6\text{Na}$ 630.3514; Found 630.3509.

Boc-D-MeVal-Pro-Dpv-Pro-Dml-OBn (9). To Boc-Dpv-Pro-Dml-OBn (**11**) (70.5 mg, 0.116 mmol) was added TFA/ CH_2Cl_2 (1:4 v/v, 3.9 mL). After 1 h of stirring at room temperature, the solution was concentrated *in vacuo* to afford crude TFA-H-Dpv-Pro-Dml-OBn, which was used in the next step without further purification.

To a solution of the crude TFA salt and **10** (38.1 mg, 0.116 mmol) in CH_3CN (1.5 mL) were added DMTMM (32.1 mg, 0.116 mmol) under Ar atmosphere. After 16 h of stirring at room temperature, the mixture was concentrated *in vacuo*. The residue was purified using column chromatography (20% acetone in hexane) to afford **9** as a colorless oil (84.3 mg, 0.103 mmol, 89% for 2 steps): $[\alpha]^{23}_{\text{D}} = +13.8$ (c 0.43, CHCl_3); IR (neat) 3317, 2967, 2875, 1685, 1649, 1518, 1454, 1152, 754, 701 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz, mixture of rotamers) δ 0.70–0.90 (15H, m), 1.15 (3H, d, $J = 7.3$ Hz), 1.30–1.50 (10H, m), 1.79–2.43 (12H, m), 2.58–2.80 (4H, m), 3.10–3.29 (2H, m), 3.60–3.77 (4H, m), 4.26–4.80 (3H, m), 4.90–5.00 (2H, m), 6.80 (1H, d, $J = 10.2$ Hz), 6.92–7.30 (10H, m); ^{13}C NMR (CDCl_3 , 100 MHz, mixture of rotamers) δ 14.3, 15.86, 15.93, 17.86, 17.92, 18.3, 19.5, 19.80, 19.82, 20.1, 24.8, 25.1, 26.8, 27.0, 28.1, 28.2, 28.3, 28.35, 28.4, 29.1, 29.3, 29.5, 31.8, 31.9, 38.5, 39.6, 39.7, 40.4, 40.6, 46.6, 46.7, 47.4, 52.4, 52.5, 57.0, 59.3, 60.1, 60.2, 60.6, 60.7, 61.2, 61.6, 62.6, 66.3, 76.6, 79.8, 80.1, 80.2, 126.03, 126.09, 128.0, 128.07, 128.08, 128.16, 128.22, 128.3, 128.56, 128.58, 129.50, 129.53, 129.6, 135.5, 135.6, 140.26, 140.33, 155.2, 156.3, 156.8, 169.0, 169.7, 170.2, 170.5, 171.1, 171.2, 171.3, 171.47, 171.50, 171.54, 172.5, 175.87, 175.92; HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{46}\text{H}_{67}\text{N}_5\text{O}_8\text{Na}$ 840.4882; Found 840.4883.

Boc-Pro-O-Hiv-D-MeVal-Pro-Dpv-Pro-Dml-OBn (15). To **9** (13.9 mg, 17.0 μ mol) was added TFA/CH₂Cl₂ (1:4 v/v, 0.60 mL). After 1 h of stirring at room temperature, the solution was concentrated *in vacuo* to afford crude TFA-H-D-MeVal-Pro-Dpv-Pro-Dml-OBn, which was used in the next step without further purification.

To a solution of the crude TFA salt and Boc-Pro-O-Hiv-OH (**8**) (5.36 mg, 17.0 μ mol) in CH₃CN (1.7 mL) was added DMTMM (9.41 mg, 34.0 μ mol) under Ar atmosphere. After 48 h of stirring at room temperature, the mixture was concentrated *in vacuo*. The residue was purified using column chromatography (20% acetone in hexane) to afford **15** as a colorless oil (7.82 mg, 7.70 μ mol, 45% for 2 steps): $[\alpha]^{23}_D = +15.2$ (c 0.52, CHCl₃); IR (neat) 3800, 2969, 2876, 2318, 1746, 1684, 1647, 1541, 1508, 1456, 1396, 1171, 1088, 1011, 754, 701 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz, mixture of rotamers) δ 0.73–1.20 (24H, m), 1.37–1.44 (10H, m), 1.74–2.60 (16H, m), 2.70–2.86 (2H, m), 2.99 (3H, s), 3.31–3.65 (8H, m), 4.28–4.32 (0.4H, m), 4.39–4.48 (2.6H, m), 4.74–5.12 (4H, m), 6.76–6.82 (1.6H, m), 6.89 (0.4H, d, *J* = 8.3 Hz), 7.12–7.40 (10H, m); ¹³C NMR (CDCl₃, 100 MHz, mixture of rotamers) 15.9, 16.06, 16.11, 16.2, 18.0, 18.1, 19.49, 19.53, 19.58, 19.7, 19.80, 19.82, 24.8, 24.9, 25.0, 26.4, 26.5, 28.3, 28.35, 28.43, 28.5, 28.88, 28.93, 29.2, 30.2, 31.9, 38.5, 39.7, 40.3, 46.7, 46.9, 47.3, 52.8, 57.0, 58.5, 60.0, 60.7, 60.8, 66.26, 66.31, 75.2, 76.6, 77.2, 79.69, 79.73, 126.0, 126.1, 127.9, 127.98, 128.0, 128.16, 128.17, 128.25, 128.34, 128.6, 129.5, 129.6, 135.5, 140.2, 140.5, 153.8, 168.6, 169.2, 169.5, 171.1, 171.5, 171.57, 171.62, 172.4, 172.8, 173.1, 175.9 δ ; HRMS (ESI) *m/z*: [M + Na]⁺ Calcd for C₅₆H₈₂N₆O₁₁Na 1037.5934; Found 1037.5926.

BnO-Lac-Pro-O-Hiv-D-MeVal-Pro-Dpv-Pro-Dml-OBn (6). To **15** (8.2 mg, 8.10 μ mol) was added TFA/CH₂Cl₂ (1:4 v/v, 0.30 mL). After 1 h of stirring at room temperature, the solution was concentrated *in vacuo* to afford crude TFA-H-Pro-O-Hiv-D-MeVal-Pro-Dpv-Pro-Dml-OBn, which was used in the next step without further purification.

To a solution of the crude TFA salt and BnO-Lac-OH (**7**) (1.6 mg, 8.90 μ mol) in CH₂Cl₂ (0.20 mL) were added Et₃N (1.13 μ L, 8.10 μ mol) and DECP (1.23 μ L, 8.10 μ mol) under Ar atmosphere. After 36 h of stirring at room temperature, the mixture was concentrated *in vacuo*. The residue was purified using column chromatography (20% acetone in hexane) to afford **6** (4.7 mg, 4.40 μ mol, 54% for 2 steps): $[\alpha]^{23}_D = +10.0$ (c 1.74, CHCl₃); IR (neat) 3734, 3413, 3309, 2969, 2876, 1735, 1646, 1508, 1428, 1183, 1101, 752 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz, mixture of rotamers) δ 0.69–1.10 (24H, m), 1.30–1.45 (4H, m), 1.60–2.85 (20H, m), 2.94 (3H, s), 3.10–3.70 (5H, m), 4.05–5.10 (11H, m), 6.74 (1.5 H, d, *J* = 10.2 Hz), 6.80 (0.5 H, d, *J* = 9.2 Hz), 7.00–7.20 (1H, m), 7.24–7.29 (14H, m); ¹³C NMR (CDCl₃, 100 MHz, mixture of rotamers) δ 13.8, 14.3, 15.89, 15.92, 16.2, 16.7, 17.1, 17.3, 18.0, 18.1, 19.4, 19.5, 19.7, 19.77, 19.82, 20.2, 22.4, 24.8, 24.9, 24.99, 25.01, 25.7, 26.5, 28.1, 28.3, 28.7, 29.0, 29.1, 29.2, 29.9, 30.2, 31.6, 31.86, 31.93, 38.4, 38.6, 39.6, 39.7, 40.4, 40.6, 46.5, 46.7, 46.8, 46.9, 47.2, 52.5, 53.2, 56.97, 56.99, 58.7, 59.1, 59.2, 60.1, 60.3, 60.67, 60.70, 66.2, 66.3, 70.9, 71.2, 75.0, 75.3, 75.6, 75.8, 126.00, 126.04, 127.66, 127.71, 127.9, 127.95, 127.99, 128.17, 128.22, 128.3, 128.4, 128.58, 128.59, 129.5, 129.6, 135.5, 135.6, 137.76, 137.81, 140.4, 140.5, 167.7, 168.8, 169.4,

169.9, 171.0, 171.1, 171.2, 171.3, 171.5, 171.6, 171.7, 172.5, 175.9; HRMS (ESI) *m/z*: [M + Na]⁺ Calcd for C₆₁H₈₄N₆O₁₁Na 1099.6090; Found 1099.6082.

Boc-Pro-O-Hiv-D-MeVal-Pro-OBn (18) and cyclo(-D-MeVal-Pro-) (19). To a solution of crude **17** and Boc-Pro-O-Hiv-OH (**8**) (41.0 mg, 0.130 mmol) in CH₃CN (0.65 mL) were added *i*Pr₂NEt (136 μ L, 0.780 mmol) and PyBroP (60.6 mg, 0.130 mmol) under Ar atmosphere. After 16 h of stirring at room temperature, the mixture was concentrated *in vacuo*. The residue was purified using column chromatography (20% acetone in hexane) to afford **18** (22.4 mg, 0.036 mmol, 28% for 2 steps) and **19** (12.4 mg, 0.059 mmol, 45% for 2 steps).

Amide 18. Colorless oil; $[\alpha]^{23}_D = +6.5$ (c 0.62, CHCl₃); IR (neat) 2971, 2876, 1744, 1700, 1649, 1397, 1254, 1169, 1088, 999, 752, 699 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz, mixture of rotamers) δ 0.65–1.00 (12H, m), 1.31–1.40 (9H, m), 1.70–2.30 (10H, m), 2.61 (0.82H, s), 2.67 (1.50H, s), 2.85 (0.24H, s), 2.88 (0.44H, s), 3.24–3.51 (4H, m), 4.20–4.71 (3H, m), 4.83–5.14 (3H, m), 7.18–7.31 (5H, m); ¹³C NMR (CDCl₃, 100 MHz, mixture of rotamers) δ 15.9, 16.0, 16.2, 17.9, 19.4, 19.7, 19.9, 20.1, 22.1, 23.1, 23.2, 23.8, 24.0, 24.9, 25.0, 26.2, 26.4, 28.4, 28.5, 28.7, 28.9, 28.97, 29.02, 29.6, 29.8, 29.9, 30.4, 30.5, 30.6, 30.7, 46.1, 46.2, 46.3, 46.4, 46.5, 46.7, 58.3, 58.5, 58.7, 58.7, 59.1, 59.2, 59.8, 60.0, 60.1, 66.8, 67.1, 75.0, 75.5, 76.7, 77.0, 77.3, 79.6, 79.8, 128.08, 128.14, 128.3, 128.4, 128.5, 135.57, 135.59, 135.6, 135.7, 153.9, 167.76, 167.83, 168.1, 168.9, 169.2, 169.5, 171.8, 172.2, 172.4, 172.7, 173.4; HRMS (ESI) *m/z*: [M + Na]⁺ Calcd for C₃₃H₄₉N₃O₈Na 638.3412; Found 638.3409.

Diketopiperazine 19. Colorless oil; $[\alpha]^{23}_D = +3.90$ (c 0.19, CHCl₃); IR (neat) 3734, 3479, 2965, 1651, 1456, 1403, 1296, 669 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 1.02 (3H, d, *J* = 6.8 Hz), 1.09 (3H, d, *J* = 6.8 Hz), 1.80–2.00 (3H, m), 2.15–2.22 (1H, m), 2.38–2.44 (1H, m), 3.00 (3H, s), 3.45–3.52 (2H, m), 3.59–3.66 (2H, m), 4.06–4.12 (1H, m); ¹³C NMR (CDCl₃, 100 MHz) δ 18.8, 19.7, 22.5, 29.7, 32.1, 34.6, 45.7, 58.9, 71.2, 165.0, 167.7; HRMS (ESI) *m/z*: [M + Na]⁺ Calcd for C₁₁H₁₈N₂O₂Na 233.1261; Found 233.1262.

Boc-D-MeVal-Pro-CH₂OH (22). To a solution of Boc-D-MeVal-OH (**20**) (608 mg, 2.63 mmol) and L-prolinol (**21**) (H-Pro-CH₂OH) (266 mg, 2.63 mmol) in THF (13 mL) were added NaHCO₃ (221 mg, 2.63 mmol), HOAt (358 mg, 2.63 mmol), and EDCI (504 mg, 2.63 mmol) under Ar atmosphere. After 16 h of stirring at room temperature, the mixture was concentrated *in vacuo*. The residue was purified using column chromatography (10% acetone in hexane) to afford **22** as a colorless oil (773 mg, 2.46 mmol, 94%): $[\alpha]^{23}_D = +78.2$ (c 1.28, CHCl₃); IR (neat) 3734, 3445, 2966, 2874, 2360, 2341, 1626, 1541, 1472, 1391, 1257, 1150, 1051, 930, 882, 769, 669 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz, mixture of rotamers) δ 0.83 (3H, d, *J* = 6.8 Hz), 0.89 (3H, d, *J* = 6.4 Hz), 1.43–1.47 (9H, m), 1.50–1.60 (1H, m), 1.76–1.93 (2H, m), 1.98–2.07 (1H, m), 2.20–2.35 (1H, m), 2.75 (3H, s), 3.38–3.56 (2H, m), 3.57–3.70 (2H, m), 4.20–4.29 (1.3H, m), 4.53 (0.7 H, d, *J* = 10.7 Hz), 4.79 (0.3H, d, *J* = 7.8 Hz), 4.96 (0.7 H, d, *J* = 7.3 Hz); ¹³C NMR (CDCl₃, 100 MHz, mixture of rotamers) δ 18.0, 18.3, 19.7, 20.1, 24.4, 24.5, 26.8, 26.9, 28.2, 28.3, 28.4, 28.9, 29.4, 47.5, 47.9, 58.1, 61.2, 61.5, 61.6, 63.1, 67.5, 67.7, 80.0, 80.3, 155.3, 156.3, 171.2, 172.0; HRMS (ESI) *m/z*: [M + Na]⁺ Calcd for C₁₆H₃₀N₂O₄Na 337.2098; Found 337.2101.

Boc-Pro-O-Hiv-D-MeVal-Pro-CH₂OH (23). To **22** (50.6 mg, 0.161 mmol) was added TFA/CH₂Cl₂ (1:4 v/v, 5.4 mL). After 1 h of stirring at room temperature, the solution was concentrated *in vacuo* to afford crude TFA·H-D-MeVal-Pro-CH₂OH, which was used in the next step without further purification.

To a solution of the crude TFA salt and Boc-Pro-O-Hiv-OH (**8**) (50.8 mg, 0.161 mmol) in CH₃CN (1.0 mL) were added ⁱPr₂NEt (0.280 mL, 1.61 mmol) and PyBroP (113 mg, 0.242 mmol) under Ar atmosphere. After 16 h of stirring at room temperature, the mixture was concentrated *in vacuo*. The residue was purified using column chromatography (20% acetone in hexane) to afford **23** as a colorless foam (63.0 mg, 0.123 mmol, 76% for 2 steps): [α]_D²³ = +32.8 (c 4.20, CHCl₃); IR (neat) 3446, 2971, 2877, 2360, 2341, 1747, 1699, 1637, 1399, 1366, 1167, 1121, 1088, 1011, 754, 666 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz, mixture of rotamers) δ 0.76–1.05 (12H, m), 1.35–1.41 (9H, m), 1.65–2.40 (10H, m), 2.99 (3H, s), 3.22–3.80 (6H, m), 4.10–4.25 (1H, m), 4.26–4.40 (1H, m), 4.92–5.00 (2H, m); ¹³C NMR (CDCl₃, 100 MHz, mixture of rotamers) δ 16.2, 16.7, 16.9, 17.9, 18.1, 18.2, 19.1, 19.3, 19.6, 19.65, 19.72, 21.6, 23.2, 24.0, 24.46, 24.50, 26.3, 26.5, 27.88, 27.89, 28.1, 28.2, 28.3, 28.39, 28.42, 28.7, 29.1, 29.4, 29.5, 29.95, 29.99, 30.04, 30.07, 30.4, 46.0, 46.2, 46.4, 47.8, 47.9, 57.8, 58.2, 58.37, 58.42, 59.8, 60.0, 60.1, 60.5, 60.7, 65.1, 65.3, 66.0, 66.5, 75.3, 75.5, 79.7, 79.8, 153.7, 154.4, 167.8, 169.5, 169.6, 169.8, 169.9, 173.1, 173.4; HRMS (ESI) m/z: [M + Na]⁺ Calcd for C₂₆H₄₅N₃O₇Na 534.3150; Found 534.3144.

BnO-Lac-Pro-O-Hiv-D-MeVal-Pro-CH₂OH (24). To Boc-Pro-O-Hiv-D-MeVal-Pro-CH₂OH (**23**) (696 mg, 1.36 mmol) was added TFA/CH₂Cl₂ (1:4 v/v, 45 mL). After 1 h of stirring at room temperature, the solution was concentrated *in vacuo* to afford crude TFA·H-Pro-O-Hiv-D-MeVal-Pro-CH₂OH, which was used in the next step without further purification.

To a solution of the crude TFA salt and **7** (245 mg, 1.36 mmol) in CH₃CN (6.8 mL) were added Et₃N (1.14 mL, 8.16 mmol) and DMTMM (376 mg, 1.36 mmol) under Ar atmosphere. After 16 h of stirring at room temperature, the mixture was concentrated *in vacuo*. The residue was purified using column chromatography (20% acetone in hexane) to afford **24** as a colorless foam (635 mg, 1.11 mmol, 82% for 2 steps): [α]_D²³ = –2.60 (c 1.05, CHCl₃); IR (neat) 3734, 3446, 2966, 2875, 2360, 2341, 1744, 1636, 1456, 1188, 1112, 1013, 750, 699 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz, mixture of rotamers) δ 0.77–1.09 (12H, m), 1.38–1.43 (3H, m), 1.61–2.50 (10H, m), 2.88 (0.3H, s), 2.95 (0.1 H, s), 3.02 (2.6 H, s), 3.34–3.80 (6H, m), 4.03–4.16 (1H, m), 4.19 (1H, q, *J* = 6.3 Hz), 4.30–4.69 (3H, m), 4.94–5.09 (2H, m), 7.24–7.32 (5H, m); ¹³C NMR (CDCl₃, 100 MHz, mixture of rotamers) δ 16.4, 17.1, 17.26, 17.30, 18.0, 18.1, 18.3, 19.0, 19.2, 19.6, 19.7, 19.8, 21.4, 24.5, 24.7, 25.16, 25.24, 26.4, 26.5, 27.9, 28.0, 28.1, 29.0, 29.5, 30.0, 30.2, 31.4, 46.2, 46.6, 47.8, 48.0, 58.3, 58.8, 59.1, 59.9, 60.0, 60.2, 60.8, 65.1, 65.6, 66.8, 69.7, 71.0, 74.5, 75.0, 75.3, 75.9, 77.6, 127.4, 127.7, 127.8, 128.2, 128.4, 128.9, 137.7, 137.8, 138.1, 167.8, 169.5, 169.8, 170.0, 171.1, 171.4, 171.5, 172.2, 172.7; HRMS (ESI) m/z: [M + Na]⁺ Calcd for C₃₁H₄₇N₃O₇Na 596.3306; Found 596.3301.

BnO-Lac-Pro-O-Hiv-D-MeVal-Pro-OH (16). To a solution of **24** (635 mg, 1.11 mmol) in CH₂Cl₂ (22 mL) at 0 °C were added NaHCO₃ (336 mg, 4.00 mmol) and DMP (1.22 g, 2.89 mmol)

under Ar atmosphere. The mixture was stirred for 16 h at 0 °C, quenched with copious amount of saturated Na₂S₂O₃ solution, then saturated NaHCO₃, extracted with Et₂O, washed with brine, dried over Na₂SO₄, and concentrated *in vacuo*. The crude aldehyde was used in the next step without further purification.

To a solution of the crude aldehyde in *t*BuOH/H₂O (3:1 v/v, 14 mL) at 0 °C were added 2-methyl-2-butene (3.5 mL, 33.0 mmol), NaH₂PO₄ (266 mg, 2.22 mmol), and NaClO₂ (301 mg, 3.33 mmol). The mixture was stirred for 16 h at 0 °C, quenched with saturated NH₄Cl, extracted with EtOAc, washed with brine, dried over Na₂SO₄, and concentrated *in vacuo*. The crude product was purified using column chromatography (20% acetone in hexane) to afford **16** as a colorless foam (624 mg, 1.06 mmol, 95% for 2 steps): [α]_D²³ = +12.2 (c 1.25, CHCl₃); IR (neat) 3734, 2970, 2877, 2360, 2341, 1743, 1647, 1456, 1187, 1114, 1013, 751 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz, mixture of rotamers) δ 0.68–1.01 (12H, m), 1.33–1.42 (3H, m), 1.70–2.32 (10H, m), 2.83 (0.1H, s), 2.92 (1.9H, s), 2.97 (1.0H, s), 3.30–3.72 (4H, m), 4.10–4.35 (2H, m), 4.40–4.75 (3H, m), 4.90–5.10 (2H, m), 7.19–7.31 (5H, m); ¹³C NMR (CDCl₃, 100 MHz, mixture of rotamers) δ 16.6, 17.1, 17.4, 17.8, 18.1, 18.2, 18.8, 19.4, 19.5, 19.8, 22.1, 24.9, 25.1, 25.7, 26.5, 26.6, 27.8, 28.07, 28.13, 29.3, 29.6, 29.9, 30.4, 31.4, 46.6, 46.8, 47.0, 47.4, 58.8, 60.0, 60.1, 60.5, 70.9, 71.5, 75.0, 75.1, 75.35, 75.41, 127.7, 127.8, 127.9, 128.38, 128.40, 137.5, 137.7, 167.8, 169.1, 169.9, 171.5, 171.9, 173.6, 173.7; HRMS (ESI) m/z: [M – H][–] Calcd for C₃₁H₄₄N₃O₈ 586.3134; Found 586.3136.

BnO-Lac-Pro-O-Hiv-D-MeVal-Pro-Dpv-Pro-Dml-OBn (6). To **11** (90.0 mg, 0.148 mmol) was added TFA/CH₂Cl₂ (1:4 v/v, 5.0 mL). After 1 h of stirring at room temperature, the solution was concentrated *in vacuo* to afford crude TFA·H-Dpv-Pro-Dml-OBn, which was used in the next step without further purification.

To a solution of the crude TFA salt and BnO-Lac-Pro-O-Hiv-D-MeVal-Pro-OH (**16**) (87.0 mg, 0.148 mmol) in CH₃CN (1.5 mL) were added Et₃N (0.124 mL, 0.888 mmol) and DMTMM (41.0 mg, 0.148 mmol) under Ar atmosphere. After 16 h of stirring at room temperature, the solution was concentrated *in vacuo*. The residue was purified using column chromatography (40% acetone in hexane) to afford **6** as a transparent solid (146 mg, 0.136 mmol, 92% for 2 steps).

Dolastatin 16 (1). To a solution of **6** (13.0 mg, 12.0 μmol) in CH₃OH (1.5 mL) was carefully added 20% Pd(OH)₂/C (2.60 mg, 20 wt%) under Ar atmosphere at room temperature. The reaction mixture was stirred under H₂ atmosphere (3 atm) for 16 h. The solution was filtered through celite and concentrated *in vacuo* to afford the crude seco acid, which was used in the next step without further purification.

To a solution of MNBA (20.7 mg, 60.0 μmol) and DMAP (14.7 mg, 12.0 μmol) in toluene (6.4 mL) was added a solution of the crude seco acid and Et₃N (1.7 μL, 12.0 μmol) in toluene (1.3 mL) for a period of 4 hours and 20 minutes under Ar atmosphere. The solution was stirred for 16 h at room temperature, concentrated *in vacuo*, extracted with EtOAc, washed sequentially with 1 N HCl, saturated NaHCO₃ and brine, dried over Na₂SO₄, and concentrated *in vacuo*. The crude product was purified using column chromatography (20% acetone in hexane) to afford dolastatin 16 (**1**) as a transparent solid (3.30 mg, 3.70

μmol , 31% for 2 steps): $[\alpha]_{\text{D}}^{23} = +11.8$ (c 0.38, CH_3OH); IR (neat) 3394, 3326, 2965, 2876, 2360, 2341, 1748, 1733, 1652, 1506, 1457, 1424, 1388, 1299, 1184, 1091, 1015, 752, 702 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 0.78–0.91 (15H, m), 0.99–1.06 (9H, m), 1.43 (3H, d, $J = 6.8$ Hz), 1.45–1.60 (2H, m), 1.65–2.44 (15H, m), 2.45–2.55 (2H, m), 2.78–2.90 (2H, m), 3.08 (3H, s), 3.35–3.50 (2H, m), 3.60–3.70 (2H, m), 3.85–3.92 (1H, m), 4.44 (1H, d, $J = 6.8$ Hz), 4.54 (1H, d, $J = 7.8$ Hz), 4.60–4.64 (1H, m), 4.94 (1H, d, $J = 8.8$ Hz), 5.12–5.20 (2H, m), 5.41 (1H, d, $J = 2.9$ Hz), 6.72 (1H, d, $J = 8.8$ Hz), 7.12–7.19 (1H, m), 7.20–7.30 (2H, m), 7.34 (2H, d, $J = 7.3$ Hz) 7.68 (1H, d, $J = 10.2$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 15.0, 15.3, 16.2, 17.4, 17.9, 19.86, 19.88, 19.93, 20.5, 21.9, 24.9, 25.2, 25.6, 25.8, 28.4, 29.8, 30.9, 31.0, 32.5, 38.8, 41.0, 41.1, 46.1, 46.6, 47.7, 50.7, 56.5, 58.0, 59.0, 59.6, 61.5, 66.8, 76.5, 126.3, 128.5, 129.7, 140.7, 169.2, 169.5, 169.7, 171.1, 171.19, 171.21, 172.4, 174.8; HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{47}\text{H}_{70}\text{N}_6\text{O}_{10}\text{Na}$ 901.5046; Found 901.5042. Spectroscopic data were identical with natural dolastatin 16 (**1**) ($[\alpha]_{\text{D}}^{23} = +15.5$ (c 0.20, CH_3OH)).¹³

H-Dpv-Pro-Dml-OBn (25). To a solution of Boc-Dpv-Pro-Dml-OBn (**11**) (23.1 mg, 38.0 μmol) was added TFA/ CH_2Cl_2 (1:4 v/v, 1.3 mL). After 1 h of stirring at room temperature, the solution was concentrated *in vacuo* to afford crude TFA·H-Dpv-Pro-Dml-OBn, which was used in the next step without further purification.

To a solution of the crude TFA salt in CH_2Cl_2 (0.190 mL) was added 4 M NaOH (0.190 mL, 0.760 mmol). After 1 h of stirring at room temperature, the solution was quenched with H_2O (5.0 mL), extracted with CH_2Cl_2 , dried over Na_2SO_4 , and concentrated *in vacuo*. The crude product was purified using column chromatography (30% EtOAc in hexane) to afford **25** as a colorless oil (14.2 mg, 28.0 μmol , 74%): $[\alpha]_{\text{D}}^{23} = -74.5$ (c 1.00, CHCl_3); IR (neat) 3410, 2964, 2876, 1717, 1653, 1508, 1362, 1173, 753, 701 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 0.75–0.88 (9H, m), 1.16 (3H, d, $J = 7.3$ Hz), 1.45–1.51 (1H, m), 1.70–1.78 (1H, m), 1.80–1.88 (2H, m), 1.89–2.08 (1H, m), 2.62 (1H, dd, $J = 6.3$, 13.1 Hz), 2.10–2.18 (1H, m), 2.76 (1H, dd, $J = 8.3$, 13.4 Hz), 2.83–2.86 (1H, m), 3.03 (1H, dd, $J = 7.8$, 16.8 Hz), 3.20–3.26 (1H, m), 3.36–3.48 (1H, m), 3.68 (1H, dt, $J = 3.4$, 9.8 Hz), 4.53 (1H, dd, $J = 3.4$, 8.3 Hz), 5.00 (1H, d, $J = 12.2$ Hz), 5.04 (1H, d, $J = 12.2$ Hz), 6.84 (1H, d, $J = 10.2$ Hz), 7.10–7.19 (1H, m), 7.20–7.40 (9H, m); ^{13}C NMR (CDCl_3 , 100 MHz) δ 12.9, 13.2, 13.4, 14.0, 15.8, 19.5, 19.7, 19.8, 22.0, 24.9, 25.1, 29.0, 31.7, 31.8, 38.1, 39.7, 40.3, 40.6, 46.3, 52.5, 53.9, 57.0, 57.2, 60.7, 66.2, 66.5, 126.0, 127.9, 128.17, 128.24, 128.3, 128.40, 128.44, 128.5, 128.9, 129.26, 129.29, 135.3, 135.6, 140.6, 171.7, 175.1, 175.8; HRMS (ESI) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{30}\text{H}_{42}\text{N}_3\text{O}_4$ 508.3170; Found 508.3166.

Antifouling Assay.^{12a,12d} The adult barnacles, *Amphibalanus amphitrite*, procured from oyster farms in Lake Hamana and a pier of Shimizu bay, Shizuoka were kept in an aquarium at 20 °C and were fed on *Artemia salina* nauplii. Broods were released as I-II stage nauplii upon immersion in seawater after drying overnight. The nauplii thus obtained were cultured in filtered natural seawater (salinity 28) containing penicillin G (20 $\mu\text{g}/\text{mL}$) and streptomycin sulfate (30 $\mu\text{g}/\text{mL}$) and were fed on the diatom *Chaetoceros gracillis* at concentrations of 40×10^4 cells/mL. Larvae reached the cyprid stage in 5 days. The cyprids were collected, then stored at 4 °C until use (0-day-old).

The test compounds were dissolved in ethanol and aliquots of the solution were transferred to wells of a 24-well polystyrene culture plates and air-dried. Four wells were used for each concentration. To each well were added filtered seawater (2.0 mL, salinity 28) and six 2-day-old cyprids. The plates were kept in the dark at 25 °C for 48 h. The numbers of cyprids that attached, metamorphosed, died, or did not settle were counted under a microscope. Three or four trials were done for each concentration. Probit analysis was used to calculate the EC_{50} values.

Cytotoxicity Assay.³³ The cytotoxicity of dolastatin 16 (**1**) was determined by a standard MTT assay. First, MCF-7 breast cancer cells (Culture Collections, Public Health England) were maintained in RPMI-1640 medium with 10% fetal bovine serum (FBS). Afterwards, the cells were seeded in wells of a 96-well plate at a density of 1.0×10^4 cells per well. After 24 h at 37 °C with 5% CO_2 , the cells were incubated with increasing concentrations of **1** under the same conditions. The medium was removed after 72 h and replaced with a 100 μL solution of MTT in RPMI-1640 with 10% FBS (0.5 mg/mL) and incubated for 3 h at 37 °C with 5% CO_2 . The MTT solution was aspirated and replaced by 100 μL of DMSO. After 15 min of incubation, the optical density was measured at 570 nm using a microplate reader. The results are expressed as the mean percentage of cytotoxicity relative to untreated cells. Each sample concentration of **1** and negative control (1% EtOH in medium) was tested four times, except for the positive control (cisplatin), which was tested twice.

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