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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. 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. However, harmful effects of TBT on marine organisms such as fish, crustaceans, and especially molluscs were subsequently reported. Nanogram per liter concentrations of TBT induce masculinization of female gastropods and have resulted in the extinction of certain species. As of 2004, approximately 150 gastropod species worldwide have been affected. Consequently, the International Maritime Organization (IMO) prohibited the use of TBT-based antifouling paints on ships in 2008. 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. 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. 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.

Dolastatin 16 (1), a macrocyclic depsipeptide, was first isolated in 1997 from the sea hare Dolabella auricularia as a potential anticaner compound by Pettit and co-workers. 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. 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...
the absolute configurations of the contiguous stereocenters of 2 and 3 were (2R,3R) and (2S,3R), respectively.\textsuperscript{15}

In 2010, Tan’s group reported that 1 effectively inhibited the larval settlement and metamorphosis of the barnacle *Amphibalanus amphitrite* with an EC\textsubscript{50} value of 0.003 \( \mu \)g/mL.\textsuperscript{16}

The LC\textsubscript{50}/EC\textsubscript{50} 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.\textsuperscript{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.\textsuperscript{18} A notable feature of these syntheses is construction of the contiguous stereogenic centers of 2 and 3 with almost complete diastereoselectivity 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)\textsuperscript{19} and subsequent hydrogenolysis to remove the benzyl ester. Amide formation with 13 and 14 in a similar manner in the presence of Et\textsubscript{3}N afforded 11 in 94% yield.

![Scheme 1. Retrosynthetic analysis of dolastatin 16.](image)
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 $^{10}$ 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 $^{8}$ 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 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 epimerization$^{21}$ (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.
In a preliminary study to construct 16, coupling reactions between 8 and TFA salt 17 were conducted (Scheme 5). However, extensive attempts under various reaction conditions provided only low yields of the desired amide 18. In the presence of bromotripyrrolidinophosphonium hexafluorophosphate (PyBroP), the yield of 18 was 28%, but the main product of this reaction was the diketopiperazin e 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. 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) and L-prolinol (21) to afford amide 22 (Scheme 6). Careful optimization 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 investigations with coupling reagents, such as triphosgene, HATU, DECP, or EDCI, for synthesis of 23, PyBroP and 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. 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) 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%. 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. In addition, the conversion of the primary hydroxy group to a carboxylic acid was efficient. These results demonstrate that the use of aminoolcohol 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 protocol was used for macrolactonization to afford 1 in 31% overall yield (two steps), because other procedures such as the Yamaguchi lactonization 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 for the natural and synthetic samples.
Conclusions

In summary, the total synthesis of dolastatin 16 (I) 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 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 (CH$_3$OH), and acetonitrile (CH$_3$CN) were purchased from Kanto Chemical Co. Inc. Dichloromethane (CH$_2$Cl$_2$) and triethylamine (Et$_3$N) were distilled from CaH$_2$. All commercially obtained reagents were used as received.

Analytical TLC was carried out using pre-coated silica gel plates (Merck TLC silica gel 60F$_{254}$). 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.

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

| compound | EC$_{50}$ (μg/mL) | LC$_{50}$ (μg/mL) | Cytotoxicity LC$_{50}$ (μg/mL)
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Synthetic 1</td>
<td>&lt;0.03</td>
<td>&gt;10</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Natural 1 &amp;</td>
<td>0.003</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Synthetic 2</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;100</td>
</tr>
<tr>
<td>25</td>
<td>1.17</td>
<td>&gt;10</td>
<td>10-30</td>
</tr>
<tr>
<td>CuSO$_4$</td>
<td>0.10</td>
<td>&gt;10</td>
<td>8.6</td>
</tr>
</tbody>
</table>

*Soben obtained by Tan, see ref. 16. *obtained by Pettit, see ref. 17. *reference. *against cypris larvae of Amphibalanus amphitrite. *against MCF-7 cell (breast cancer cell) *described as G1$_{50}$ (μg/mL).
in ppm relative to CHCl₃ (δ = 7.26) in CDCl₃ for ¹H NMR, and CDCl₃ (δ = 77.0) for ¹³C NMR. Splitting patterns are designated as s, d, t, q, and m, indicating singlet, doublet, triplet, quartet, and multiple, respectively.

**TFA-H-Dml-Obn (13).** To a solution of N-Boc-dolamethyline (4) (Boc-Dml-Obn) (116 mg, 0.473 mmol) in THF (2.4 mL) were added BnOH (53.9 μL, 0.520 mmol), PPh₃ (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 NaHCO₃, extracted with EtOAc, washed with brine, dried over Na₂SO₄, 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%): [α]标记 = +15.4 (c 0.23, CHCl₃); IR (neat) 3750, 2974, 2876, 2360, 2341, 1716, 1507, 1166, 772, 669 cm⁻¹; ¹H NMR (CDCl₃, 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); ¹³C NMR (CDCl₃, 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 (EI) m/z: [M + Na]⁺ Calcd for C₁₀H₁₇NO₂Na 358.1989; Found 358.1992.

To Boc-Dml-Obn (255 mg, 0.760 mmol) was added TFA/CH₂Cl₂ (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-Dvp-Pro-OH (14).** To a solution of N-Boc-dolaphenylalanine (5) (Boc-Dvp-OH) (208 mg, 0.709 mmol) and HCl-H-Pro-Obn (12) (257 mg, 1.06 mmol) in CH₂CN (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-Dvp-Obn as a transparent solid (205 mg, 0.525 mmol, 97%): [α]标记 = –40.5 (c 10, CHCl₃); IR (neat) 3317, 2967, 2875, 1685, 1649, 1518, 1454, 1152, 754, 701 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 6.79 (1H, d, J = 7.3, 13.4 Hz), 7.08-7.15 (5H, m); ¹³C NMR (CDCl₃, 100 MHz) δ 13.5, 57.8, 60.0, 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, 179.5; HRMS (EI) m/z: [M + Na]⁺ Calcd for C₁₄H₁₄NO₂Na 360.3514; Found 360.3509.

**Boc-o-MeVal-Pro-Dvp-Pro-Obn (9).** To Boc-Dvp-Pro-Obn (11) (70.5 mg, 0.116 mmol) was added TFA/CH₂Cl₂ (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-Dvp-Pro-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 CH₂CN (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): [α]标记 = +13.8 (c 0.43, CHCl₃); IR (neat) 3137, 2967, 2875, 1685, 1649, 1518, 1454, 1152, 754, 701 cm⁻¹; ¹H NMR (CDCl₃, 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); ¹³C NMR (CDCl₃, 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, 126.0, 126.9, 128.0, 128.7, 128.8, 128.16, 128.22, 128.3, 128.56, 128.58, 128.59, 128.53, 129.6, 135.5, 135.6, 140.2, 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: [M + Na]⁺ Calcd for C₃₀H₄₀N₂O₅Na 840.4882; Found 840.4883.
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Boc-Pro-O-Hiv-o-MeVal-Pro-Dpv-Pro-Dml-OBn (15). To 9 (13.9 mg, 17.0 μmol) 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-o-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 μmol) in CH₂CN (1.7 mL) was added DMTMM (9.41 mg, 34.0 μmol) under Ar atmosphere. After 48 h of stirring at room temperature, the mixture was concentrated in vacuo.

To a solution of the crude TFA salt and Boc-Pro-O-Hiv-OH (8) (5.36 mg, 17.0 μmol) in CH₂CN (1.7 mL) was added DMTMM (9.41 mg, 34.0 μmol) under Ar atmosphere. After 48 h of stirring at room temperature, the mixture was concentrated in vacuo.

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To a solution of the crude TFA salt and Boc-Pro-O-Hiv-OH (8) (5.36 mg, 17.0 μmol) in CH₂CN (1.7 mL) was added DMTMM (9.41 mg, 34.0 μmol) under Ar atmosphere. After 48 h of stirring at room temperature, the mixture was concentrated in vacuo.
**Boc-Pro-O-Hiv-o-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-O-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 CH₂CN (1.0 mL) were added Pr₃NEt (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]²⁰S = +32.8 (c 4.20, CHCl₃); IR (neat) 3496, 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, 13.9, 16.9, 19.7, 21.6, 23.2, 24.0, 24.6, 25.4, 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, 58.7, 58.42, 59.8, 60.0, 60.1, 60.5, 67.5, 67.7, 113.0, 113.4, 113.6, 113.7, 113.8, 113.9, 137.7, 137.8, 138.1, 167.8, 169.5, 169.6, 169.8, 169.9, 173.1, 173.4; HRMS (ESI) m/z: [M + Na⁺] Calcd for C₂₉H₅₄N₃O₇Na 534.3150; Found 534.3144.

**BnO-Lac-Pro-O-Hiv-o-MeVal-Pro-CH₂OH (24).** To Boc-Pro-O-Hiv-o-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-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 CH₂CN (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]²⁰S = +2.2 (c 1.25, CHCl₃); IR (neat) 3734, 2970, 2877, 2360, 2341, 1743, 1647, 1456, 1187, 1114, 1013, 751 cm⁻¹; ¹H NMR (CDCl₃, 100 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, 18.9, 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, 54.8, 60.0, 60.1, 60.5, 70.9, 71.5, 75.0, 75.1, 75.35, 75.41, 127.7, 127.8, 129.7, 128.38, 128.40, 135.7, 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 C₃₁H₄₇N₃O₇Na 586.3134; Found 586.3136.

**BnO-Lac-Pro-O-Hiv-o-MeVal-Pro-Dpz-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-Dpz-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-o-MeVal-Pro-Dpz-Pro-Dml-OH (16) (87.0 mg, 0.148 mmol) in CH₂CN (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 CH₂OH (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 until 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): [α]D10 = +11.8 (c 0.38, CH3OH); IR (neat) 3394, 3236, 2965, 2876, 2360, 2341, 1748, 1733, 1652, 1506, 1457, 1428, 1389, 1299, 1184, 1091, 1015, 752, 702 cm⁻¹; 1H NMR (CDCl3, 400 MHz) δ 0.78-0.91 (15H, m), 0.99-1.06 (9H, m), 1.43 (2H, 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 (1H, d, J = 7.3 Hz) 7.68 (1H, d, J = 10.2 Hz); 13C NMR (CDCl3, 100 MHz) δ 15.0, 15.3, 16.2, 19.5, 19.7, 19.8, 20.4, 20.6, 21.2, 23.2, 23.6, 24.7, 25.0, 25.1, 27.4, 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, 171.9, 172.1, 172.4, 174.8; HRMS (ESI) m/z: [M + Na]⁺ Calcd for C24H32N3O4Na 501.2185; Found 501.2181.


Notes and references

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