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Synthesis and Structure Revision of Symplocin A

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Abstract

Symplocin A, a linear peptide possessing N-terminal *N,N*-dimethylisoleucine, statine, and valic acid residues, has been synthesized for the first time employing our previously established ‘one-pot intramolecular tandem protocol’. Moreover, the stereochemistry of natural symplocin A was unambiguously revised through the confirmation by 1D NMR, 2D NMR, and HPLC comparisons with authentic natural product.

Introduction

Secondary metabolites, derived from marine cyanobacteria displaying a variety of physiological activities, including antimicrobial, antimalarial, antifungal, cytotoxic and neurotoxic properties¹ biological activities, are a promising class of compounds for drug discovery. In particular, the linear depsipeptides, bearing characteristic N-terminal *N,N*-dimethylamino acid (DMAA) and internal statine residues, have attracted significant attention in recent years;² for example, dolastatin 10 (**1**)³ isolated from the sea hare *Dolabella auricularia*, is a highly cytotoxic compound that inspired design of auristatin as the potent ‘warhead’ component of antibody conjugates (ABC) that have been approved as antineoplastic agents for cancer chemotherapy.⁴ Grassystatins A, B (**2a**, **2b**) and C, isolated from *Lyngbya cf. confervoides* collected at Grassy Key and Key Largo, FL, are potent inhibitors of proteases, cathepsin D and E.⁵

Symplocin A (proposed structure **3a**) is a sub-nanomolar cathepsin E inhibitor (IC₅₀ 300 pM), isolated by Molinski and co-workers⁶ in 2012 from the Bahamian cyanobacterium *Symploca* sp. The planar structure of symplocin A - comprising seven α -amino acid residues, along with valic acid and statine subunits - was secured from spectroscopic analysis, including MS and 2D NMR. The absolute configurations of seven residues,

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including (3*R*,4*S*)-statine, were assigned L-configurations by degradation-Marfey's analysis.⁷ An uncommon D-configuration was proposed for both the DMAA and valic acid residues from chiral phase HPLC of the corresponding *O*-2-naphthacyl esters and comparison with standards. The unexpected finding of a terminal D-DMAA, in contrast to L- for all other known linear peptides of this class, and the potent protease inhibitory properties of symplocin A, provoked sufficient interest to explore the structure in more depth.

As a continuation of our interest in developing divergent syntheses of natural products⁸ isolated from cyanobacteria and investigating their structure-activity relationships, together with unusual features of the structure, we decided to develop an asymmetric synthesis of symplocin A. Herein, we present the synthesis of symplocin A, along with those of two non-natural diastereomers, and a stereochemical revision of the natural product.

Results and discussion

The retrosynthetic analysis of **3a** (Figure 2) converged on five subunits: *N,N*-dimethyl ester fragment **4**, Tyr-OBn (**5**), *N*-Boc-Ser (**6**), *N*-Boc-(3*R*,4*S*)-statine (**7**), and tetrapeptide **8**. Fragment **8** could be prepared by conventional condensations of the constituent amino acids. The γ -amino acid, *N*-Boc-(3*R*,4*S*)-statine (**7**), could be obtained using our 'one-pot intramolecular tandem protocol'.⁹ Importantly, the well-known β,γ -epimerization of statine residues could be avoided through an alternate condensation sequence of the five subunits.

The synthesis of tetrapeptide fragment **8** is outlined in Scheme 1. D-Boc-Phe-OH (**9**) was *N*-methylated (MeI, NaH, THF) to provide D-Boc-*N*-Me-Phe-OH (**10**) in quantitative yield:¹⁰ the latter was directly coupled with L-Pro-OMe (HATU, DIPEA) to produce peptide **11** in 62% overall yield.¹⁰ Removal of Boc group in **11** (TFA) and subsequent condensation with Boc-Gly-OH (HATU-DIPEA) afforded tripeptide **12** (80%, two steps). Finally, treatment of compound **12** with TFA and the coupling of the resulting crude salt with L-Boc-Val-OH (HOBt, EDCI, NMM) gave tetrapeptide fragment **8** in 62% overall yield.¹²

Next, we turned our attention to prepare the key (3*R*,4*S*)-statine subunit **7** of **3a** (Scheme 2). The most practicable approach to *N*-Boc-(3*R*,4*S*)-statine subunit **7** is the ring-opening of lactam **13**, which could be prepared easily according to our previously described one-pot intramolecular tandem protocol.⁹ Thus, removal of TBS group in *N*-Boc-lactam **13** (TBAF) gave secondary alcohol **14** (50%). The subsequent ring-opening of **14** (LiOH·H₂O, H₂O₂) generated the **7** in quantitative yield¹³.

The synthesis of subunit **4** and its subsequent coupling with subunit **5** is shown in Scheme 3. Coupling of D-Boc-Ile-OH (**15**) and (*R*)-allyl 2-hydroxy-3-methylbutanoate (**16**, DCC, DMAP) produced ester **17** (76%). Removal of Boc group in **17** (TFA) and subsequent *N,N*-dimethylation by reductive amination [40% HCHO aq., NaBH₃(CN)] generated the desired *N,N*-dimethyl amino ester **4** (64%, two steps).¹⁴ The Pd-catalyzed deprotection of the *O*-allyl group in **4** [Pd(PPh₃)₄, *N*-methylaniline, NMA] afforded the corresponding free acid,¹⁵ which was used to acylate Tyr-OBn (**5**, HATU, HOAt, DIPEA) and deliver linear peptide **18** (45%, two steps).^{10a,d} Deprotection of the benzyl ester **18** (H₂, 1 atm, Pd/C) gave the free acid **19** in quantitative yield.

With fragments **8**, **7** and **19** in hand, we turned our attention to final assembly of **3a** through successive coupling reactions. The crude amine salt, prepared by removal of Boc group in **8** (TFA), was directly coupled with **7** (HATU, HOAt, DIPEA) to give the pentapeptide **20** (59%, two steps). Deprotection of **20** (TFA) afforded a crude amine salt, which, without further purification, was used to acylate **6** (HOBt, EDCI, NMM) and deliver hexapeptide **21** (70%). Finally, removal of the Boc group in **21** (TFA) and direct coupling of the product with the free acid **19** (HOBt, EDCI, NMM), afforded (50*R*, 51*R*)-**3a** {[α]_D^{22.7} +9.6 (*c* 0.25, MeOH), lit⁶ symplocin A: [α]_D^{22.5} +16.0 (*c* 2.18, MeOH)} in 38% overall yield.

To our surprise, the analytical data of optical rotation and NMR spectra generated from synthetic **3a** were substantially different from those reported for symplocin A.⁶ Through comparison of the respective ¹H NMR data of **3a** with the natural product, we observed perceptible differences in the signals of the DMAA residue, and suspected that the stereochemical assignment of the *N,N*-dimethylisoleucine residue might be incorrect. The configurational assignment of DMAA residues can be equivocal. An assertion, “that all marine natural products containing an *N,N*-dimethyl terminal amino acid residue possess the L-configuration at this center”,^{16a} is belied by the finding of both L- and D- variants of *N,N,O*-trimethyl-Ser in aplyronines A-C.^{16g}

Nevertheless, we suspected the correct configuration of the *N,N*-dimethylisoleucine residue of symplocin A may indeed be 2*S*,3*S* or possibly an *allo* diastereomer. To test this hypothesis, we synthesized the symplocin A diastereomer **3b** with (2*S*, 3*S*)-*L,N,N*-dimethylisoleucine at the N-terminus (Scheme 5). Following the synthetic sequence described above, L-Boc-Ile-OH **22** was converted to its *N,N*-dimethylamino ester (2*S*,3*S*)-**4** (Scheme 5) in 37% overall yield. Removal of allyl group in (2*S*, 3*S*)-**4** [Pd(PPh₃)₄, NMA] and transformation of the product to (50*S*,51*S*)-symplocin A (**3b**) {[α]_D^{22.7} +21.2 (*c* 0.25, MeOH)} was achieved in 8% overall yield under conditions similar to those that led to **3a**.

Having successfully synthesized (50*S*, 51*S*)-symplocin A (**3b**), we turned our attention to the comparison of their optical rotation and NMR data. Although the ¹H NMR spectra of our synthetic **3b** showed closer similarity than **3a** to natural symplocin A, subtle differences in chemical shifts and multiplicities remained.

Closer comparisons of the ¹H and ¹³C NMR data of synthetic (50*R*, 51*R*)-**3a** and (50*S*, 51*S*)-**3b** with that of the authentic sample revealed fewer differences between authentic symplocin A and (50*S*,51*S*)-**3b** for the region corresponding to C-49 ~ C-56 (*N,N*-Ile), but larger differences in the domain of C-24 ~ C-31 (statine) (see the Supporting Information). Specifically, the (50*S*,51*S*)-**3b** appeared to be a better match with authentic symplocin A in the DMAA domain, but the smaller chemical shift differences in the statine domain suggested an inversion of one or both of the C-26 and C-27 asymmetric centers. Although it was unlikely the latter stereocenter was in error, given the conditions of hydrolysis-Marfey's analysis,⁷ separate SAR studies by indicated that the 3*S* configuration (statine numbering), associated with pepstatin-derived inhibitors of aspartic proteases, is crucial for tight binding to the enzyme active sites.¹⁷ From the latter report, along with the known difficulties associated with assignment of the C-3 stereocenter in statine residues,^{18,19} we surmised that

the native configuration in symplocin A – which potently inhibits cathepsin E⁶ – may also be 3*S*,4*S*; namely a 26*S* epimer of **3b**.

To test this proposal, we synthesized the diastereomeric (26*S*, 50*S*, 51*S*)-symplocin A (**3c**, Scheme 6). Inversion of the carbinol center in **14** was straight-forward through Swern oxidation²⁰ followed by reduction (NaBH₄, MeOH) to afford (2*S*, 3*S*)-**14** (67%, two steps). ^{8c} Hydrolysis of (2*S*, 3*S*)-**14** (LiOH·H₂O, H₂O₂) gave ring-opened (3*S*, 4*S*)-**7** in quantitative yield. Following the synthetic sequences described above, diastereomeric (26*S*, 50*S*, 51*S*)-symplocin A (**3c**) { $[\alpha]_D^{21.1} +27.6$ (*c* 1.00, MeOH)} was obtained in 7% overall yield from (3*S*,4*S*)-**7**.

To our delight, the NMR data of synthetic **3c** (see Supporting Information) were in excellent agreement with those reported for natural symplocin A. In analytical reversed phase HPLC (see the Supporting Information), both synthetic **3a** and **3b** showed different retention times compared to authentic symplocin A, while synthetic **3c** and the natural sample were identical by retention time, and co-eluted under two different sets of conditions.

Based on the successful synthesis of **3c**, we conclude that the terminal *N,N*-dimethylisoleucine residue of natural symplocin A should be revised to the L-configuration (50*S*,51*S*), and one stereocenter in the statine residue should be changed from 26*R* to 26*S*.

Conclusions

In summary, the first total synthesis of symplocin A (**3c**) and two of its diastereomers, **3a** and **3b**, has been accomplished using our previously established ‘one-pot intramolecular tandem protocol’. This synthetic strategy avoids tedious protecting group interchanges during the sequential amide condensation reactions of five peptide subunits. In addition, the configuration of the natural product was corrected to **3c**,²¹ and unambiguously confirmed by careful comparisons of all four samples by 1D NMR, 2D NMR and HPLC.

Experimental

General

THF was distilled from sodium/benzophenone. DCM was distilled from phosphorus pentoxide. Reactions were monitored by thin layer chromatography (TLC) on glass plates coated with silica gel with fluorescent indicator. Flash chromatography was performed on silica gel (300–400 mesh). Optical rotations were measured on a polarimeter with a sodium lamp. HRMS were measured on a Thermo Scientific LTQ Orbitrap XL apparatus. IR spectra were recorded using film on a Fourier Transform Infrared Spectrometer. NMR spectra were recorded at 400 MHz or 600 MHz, and chemical shifts are reported in δ (ppm) referenced to the appropriate residual solvent peaks unless otherwise noted.

(S)-Methyl 1-((R)-2-(tert-butoxycarbonyl)-3-phenylpropanoyl)pyrrolidine-2-carboxylate (11)—To a stirred solution of D-Boc-Phe-OH (31.83 g, 120.00 mmol) and MeI (74.70 mL, 1.20 mol) in THF (250 mL) under argon atmosphere, was slowly added NaH (60% in mineral oil; 24.00 g, 600.00 mmol) in portions over a period of 2 h at 0 °C.

After being stirred for overnight at room temperature, ice was added to quench the reaction. After removal of THF in vacuo, the residue was dissolved in H₂O (100 mL) and extracted with EtOAc (50 mL). The aqueous layer was acidified to pH = 3 with an aqueous solution of 1N HCl and extracted with EtOAc (50 mL × 3). The combined organic layers were washed with saturated Na₂S₂O₃ followed by brine, then dried over MgSO₄, filtered and concentrated to give **10** as a colorless oil (32.20 g, quant.), which was used for the next step without further purification. To a stirred solution of **10** (30.73 g, 110.0 mmol), Pro-OMe (14.21 g, 110.0 mmol) and HATU (62.74 g, 165.0 mmol) in DCM (450 mL) under argon atmosphere, was added DIPEA (57.5 mL, 330 mmol) at 0 °C. After being stirred overnight at room temperature, saturated NH₄Cl (100 mL) was added to quench the reaction, and the mixture was extracted with EtOAc (100 mL × 3). The combined organic layers were washed with saturated NaHCO₃, followed by brine, dried over MgSO₄, filtered and concentrated. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc = 1:1) to give **11** as a white solid (26.63 g, 62%). [α]_D^{22.5} +74.25 (*c* 2.00, CHCl₃); IR (film) ν_{\max} 2976, 2933, 2868, 1748, 1696, 1653, 1436, 1388, 1170 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.32–7.10 (m, 5H), 5.25–5.10 (m, 0.5H), 4.95–4.70 (m, 0.5H), 4.60–4.35 (m, 1H), 3.73 (s, 3H), 3.58–3.36 (m, 2H), 3.25–3.16 (m, 0.5H), 3.15–3.07 (m, 0.5H), 3.03–2.81 (m, 4H), 2.30–2.10 (m, 1H), 2.05–1.75 (m, 3H), 1.40–1.31 (m, 4.5H), 1.25–1.16 (m, 4.5H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 172.6, 172.2, 168.9, 168.6, 155.2, 154.5, 138.0, 137.5, 129.4, 129.3, 128.2, 127.9, 126.2, 126.0, 79.8, 79.7, 59.3, 59.1, 58.8, 57.4, 52.0, 51.9, 46.3, 46.0, 34.65, 34.57, 29.4, 28.7, 28.0, 27.8, 25.0 ppm; HRMS (ESI) *m/z* calcd for C₂₁H₃₁N₂O₅⁺ (M+H)⁺ 391.2228, found 391.2227.

(S)-Methyl 1-((R)-2-(2-(tert-butoxycarbonyl)-N-methylacetamido)-3-phenylpropanoyl) pyrrolidine-2-carboxylate (12**)**—

To a stirred solution of **11** (22.7 g, 58.0 mmol) in DCM (116 mL) was added TFA (58 mL) at 0 °C, and the mixture stirred for 3 h at room temperature. After removal of the solvent in vacuo, the residue was dissolved in toluene (5 mL) and evaporated. Repeation of this process three times gave the corresponding crude amine TFA salt, which was treated with Boc-Gly-OH (12.19 g, 69.60 mmol) and HATU (28.7 g, 75.4 mmol) in DCM (200 mL) and DIPEA (50.5 mL, 290 mmol) at 0 °C under an argon atmosphere. After being stirred for overnight at room temperature, saturated NH₄Cl (50 mL) was added to quench the reaction, and the mixture was extracted with EtOAc (50 mL × 3). The combined organic layers were washed with saturated NaHCO₃, followed by brine, dried over MgSO₄, filtered and concentrated, the residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc = 1:1) to give **12** as a colorless oil (20.8 g, 80%). [α]_D^{22.7} +63.20 (*c* 2.00, MeOH); IR (film) ν_{\max} 3363, 2982, 1750, 1712, 1648, 1431, 1165 cm⁻¹; ¹H NMR (400 MHz, CD₃OD) δ 7.34–7.11 (m, 5H), 5.65–5.48 (m, 1H), 4.42–4.31 (m, 1H), 3.97 (d, *J* = 17.1 Hz, 1H), 3.74 (d, *J* = 17.1 Hz, 1H), 3.71 (s, 3H), 3.52–3.33 (m, 2H), 3.25–3.12 (m, 1H), 3.01 (s, 3H), 2.88–2.77 (m, 1H), 2.26–2.12 (m, 1H), 1.98–1.77 (m, 3H), 1.43 (s, 9H) ppm; ¹³C NMR (100 MHz, CD₃OD) δ 173.9, 171.3, 170.2, 158.3, 138.6, 130.5, 129.4, 127.6, 80.5, 60.6, 57.8, 52.6, 48.3, 43.0, 35.7, 30.4, 29.9, 28.7, 25.9 ppm; HRMS (ESI) *m/z* calcd for C₂₃H₃₄N₃O₆⁺ (M+H)⁺ 448.2442, found 448.2443.

(S)-Methyl 1-((R)-2-(2-((S)-2-(tert-butoxycarbonyl)-3-methylbutanamido)-N-methylacetamido)-3-phenylpropanoyl) pyrrolidine-2-carboxylate (8)—To a stirred solution of **12** (2.60 g, 5.80 mmol) in DCM (12 mL) was added TFA (6 mL) at 0 °C and stirred for 3 h at room temperature. After removal of the solvent in vacuo, the residue was dissolved in toluene (3 mL) and evaporated. This process was repeated three times to give a crude amine, which was dissolved in DMF and treated, sequentially, with a solution of L-Boc-Val-OH (1.12 g, 6.38 mmol) in DMF (20 mL) and HOBt (862 mg, 6.38 mmol) at -15 °C under an argon atmosphere. The mixture was and stirred for 30 min at the same temperature before adding EDCI (1.22 g, 6.38 mmol) and stirring an additional 1.5h at -15 °C before adding NMM (1.28 mL, 11.6 mmol). After being stirred for overnight at room temperature, saturated NH₄Cl (30 mL) was added to quench the reaction, and the mixture was extracted with EtOAc (30 mL × 3). The combined organic layers were washed with saturated NaHCO₃ followed by brine, dried over MgSO₄, filtered and concentrated. The residue was purified by flash chromatography on silica gel (DCM/MeOH = 40:1) to give **8** as a white foam (1.96 g, 62%). [α]_D^{22.8} +34.3 (*c* 2.00, MeOH); IR (film) ν_{\max} , 3315, 2958, 1750, 1717, 1641, 1451, 1370, 1175 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.30–7.23 (m, 3H), 7.22–7.10 (m, 2H), 6.86 (br s, 1H), 5.62–5.45 (m, 1H), 5.14–5.00 (m, 1H), 4.46–4.38 (m, 1H), 4.19–4.08 (m, 1H), 4.07–3.99 (m, 1H), 3.94–3.86 (m, 1H), 3.73 (s, 3H), 3.44–3.32 (m, 2H), 3.31–3.24 (m, 1H), 3.00 (s, 3H), 2.88–2.80 (m, 1H), 2.21–2.11 (m, 2H), 2.08–2.04 (m, 1H), 1.97–1.87 (m, 2H), 1.44 (s, 9H), 0.97–0.93 (m, 3H), 0.91–0.86 (m, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 172.6, 171.6, 168.01, 167.97, 155.9, 137.1, 129.5, 128.5, 126.8, 80.0, 59.8, 59.1, 56.4, 52.4, 46.9, 41.3, 35.1, 31.2, 29.8, 28.9, 28.4, 25.2, 19.4, 17.5 ppm; HRMS (ESI) *m/z* calcd for C₂₈H₄₃N₄O₇⁺ (M+H)⁺ 547.3126, found 547.3126.

(2S,3R)-tert-Butyl 3-hydroxy-2-isobutyl-5-oxopyrrolidine-1-carboxylate (14)—To a stirred solution of **13** (372 mg, 1.00 mmol) in THF (10 mL) was added TBAF (1.0 M in THF, 2.00 mL, 2.00 mmol), dropwise, at 0 °C, and the mixture allowed to stir for 2 h while warming to room temperature. Saturated NH₄Cl (5 mL) was added to quench the reaction, and the mixture was extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated, and the residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc = 2:1) to give **12** as colorless crystals (129 mg, 50 %). Mp 114 – 116 °C, [α]_D^{22.9} +56 (*c* 0.50, MeOH); IR (film) ν_{\max} 3415, 2955, 2933, 2870, 1770, 1717, 1367, 1290, 1152, 1042, 1022 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 4.16 (d, *J* = 5.1 Hz, 1H), 4.07 (dd, *J* = 10.9, 3.1 Hz, 1H), 2.84 (dd, *J* = 18.1, 5.3 Hz, 1H), 2.77 (brs, 1H), 2.44 (d, *J* = 18.1 Hz, 1H), 1.76–1.65 (m, 1H), 1.53 (s, 9H), 1.52–1.46 (m, 1H), 1.28 (ddd, *J* = 13.5, 11.0, 4.3 Hz, 1H), 1.04–0.93 (m, 6H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 173.0, 149.9, 83.2, 67.8, 66.1, 41.1, 41.0, 28.2, 25.5, 23.8, 21.9 ppm; HRMS (ESI) *m/z* calcd for C₁₃H₂₄NO₄⁺ (M+H)⁺ 258.1700, found 258.1695.

(3R,4S)-4-(tert-Butoxycarbonyl)-3-hydroxy-6-methylheptanoic acid (7)—To a stirred solution of **14** (103 mg, 0.40 mmol) in THF/H₂O (4:1) (6 mL) at room temperature, aqueous H₂O₂ (30% v/v, 0.1 mL) and LiOH·H₂O (50 mg, 1.20 mmol) were added sequentially. Stirring was continued at room temperature for 12 h. After removal of THF in vacuo, the basic aqueous residue was acidified by the addition of 10% citric acid (to pH = 4), and the mixture extracted with EtOAc (10 mL × 3). The combined organic layers were

washed with saturated NaHCO₃, followed by brine, dried over MgSO₄, filtered and concentrated to give **7** as a white powder (110 mg, quant.), which was used for the next step without further purification.

(2R,3R)-((R)-1-(Allyloxy)-3-methyl-1-oxobutan-2-yl) 2-(tert-butoxycarbonyl)-3-methylpentanoate (17)—To a stirred solution of D-Boc-Ile-OH (925 mg, 4.00 mmol) and **15** (791 mg, 5.00 mmol) in dry DCM (8 mL) were added DCC (2.47 g, 12.00 mmol) followed by DMAP (489 mg, 4.00 mmol) at 0 °C, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc and then filtrated, and the filtrate was washed with saturated NH₄Cl, NaHCO₃, followed by brine, dried over MgSO₄, filtered and concentrated, the residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc = 20/1) to give **17** as a colorless oil (1.13 g, 76%). [α]_D^{22.6} +13.50 (*c* 2.00, CHCl₃); IR (film) ν_{\max} 2970, 2934, 2880, 1748, 1719, 1502, 1367, 1159, 1020 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 5.96–5.80 (m, 1H), 5.37–5.29 (m, 1H), 5.27–5.21 (m, 1H), 4.97 (d, *J* = 9.4 Hz, 1H), 4.86 (d, *J* = 4.3 Hz, 1H), 4.64–4.60 (m, 2H), 4.46 (dd, *J* = 9.4, 3.4 Hz, 1H), 2.30–2.20 (m, 1H), 2.08–1.98 (m, 1H), 1.51–1.45 (m, 1H), 1.43 (s, 9H), 1.30–1.21 (m, 1H), 1.03–0.99 (m, 3H), 0.99–0.93 (m, 6H), 0.91–0.88 (m, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 172.7, 169.1, 155.9, 131.6, 119.1, 79.9, 77.4, 65.9, 56.5, 37.8, 30.2, 28.4, 26.3, 18.9, 17.3, 14.3, 11.9 ppm; HRMS (ESI) *m/z* calcd for C₁₉H₃₄NO₆⁺ (M+H)⁺ 372.2381, found 372.2381.

(2R,3R)-((R)-1-(Allyloxy)-3-methyl-1-oxobutan-2-yl) 2-(dimethylamino)-3-methylpentanoate (4)—To a stirred solution of **17** (929 mg, 2.50 mmol) in DCM (10 mL) was added TFA (5 mL) at 0 °C and stirred for 3 h at room temperature. After removal of the solvent in vacuo, the residue was dissolved in toluene (3 mL) and evaporated. Repeated this process three times to give a crude amine. To a stirred solution of the above amine and aqueous HCHO (5 mL, 40%) in MeCN (10 mL) was added Na(BH₃)CN (471 mg, 7.50 mmol) at 0 °C. The mixture was stirred for 1 h at room temperature, and then AcOH (0.2 mL) was added dropwise until the solution was neutral. Stirring was continued for overnight, AcOH being added occasionally to maintain the pH near neutrality. The solvent was removed in vacuo and then the residue was dissolved in EtOAc. The solvent was washed with saturated NaHCO₃, followed by brine, dried over MgSO₄, filtered and concentrated, and the residue purified by flash chromatography on silica gel (petroleum ether/EtOAc = 15:1) to give **4** as a colorless oil (479 mg, 64%). [α]_D²² +37.3 (*c* 1.00, CHCl₃); IR (film) ν_{\max} 2967, 2935, 2877, 1758, 1735, 1455, 1273, 1196, 1124, 1020 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 5.98–5.85 (m, 1H), 5.38–5.31 (m, 1H), 5.28–5.23 (m, 1H), 4.85 (d, *J* = 4.4 Hz, 1H), 4.67–4.63 (m, 2H), 2.94 (d, *J* = 10.8 Hz, 1H), 2.34 (s, 6H), 2.30–2.23 (m, 1H), 1.88–1.79 (m, 1H), 1.51–1.41 (m, 1H), 1.15–1.06 (m, 1H), 1.04–1.01 (m, 3H), 1.01–0.96 (m, 6H), 0.94–0.88 (m, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 171.5, 169.5, 131.7, 119.1, 76.8, 73.0, 65.9, 41.3, 34.0, 30.1, 26.3, 19.2, 17.5, 15.3, 11.2 ppm; HRMS (ESI) *m/z* calcd for C₁₆H₃₀NO₄⁺ (M+H)⁺ 300.2169, found 300.2167.

(2R,3R)-((R)-1-((S)-1-(Benzyloxy)-3-(4-hydroxyphenyl)-1-oxopropan-2-ylamino)-3-methyl-1-oxobutan-2-yl) 2-(dimethylamino)-3-methylpentanoate (18)—To a stirred solution of **4** (359 mg, 1.20 mmol) in THF (24 mL) containing Pd(PPh₃)₄

(116 mg, 0.12 mmol) under argon atmosphere was dropped *N*-Methylaniline (0.32 mL, 3.00 mmol) at room temperature under. After being stirred for 2 h, the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (DCM/MeOH = 10/1) to give a free acid. To a stirred solution of the acid, Tyr-OBn (390 mg, 1.44 mmol) and HATU (821 mg, 2.16 mmol) in DCM (10 mL) under argon atmosphere, was added DIPEA (1.25 mL, 7.20 mmol) at 0 °C. After being stirred for overnight at room temperature, saturated NH₄Cl (10 mL) was added to quench the reaction, and the mixture was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with saturated NaHCO₃, followed by brine, dried over MgSO₄, filtered and concentrated, and the residue purified by flash chromatography on silica gel (petroleum ether/EtOAc/NH₄OH = 4/1/0.025) to give **18** as a colorless oil (277 mg, 45%). [α]_D²² +32.50 (*c* 1.00, CHCl₃); IR (film) ν_{\max} 3344, 2958, 2925, 2877, 1736, 1660, 1512, 1455, 1213, 1165, 1113, 1003 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.41–7.25 (m, 5H), 6.86–6.81 (m, 2H), 6.67–6.61 (m, 3H), 5.21–5.15 (m, 1H), 5.13–5.07 (m, 2H), 4.88–4.80 (m, 1H), 3.13–3.04 (m, 1H), 3.02–2.94 (m, 1H), 2.88 (d, *J* = 10.4 Hz, 1H), 2.29 (s, 6H), 2.28–2.22 (m, 1H), 1.84–1.75 (m, 1H), 1.32–1.27 (m, 0.7H), 1.24–1.20 (m, 0.3H), 1.04–0.97 (m, 1H), 0.96–0.91 (m, 9H), 0.86–0.80 (m, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 171.3, 170.9, 169.4, 155.3, 135.0, 130.5, 128.8, 128.7, 127.0, 115.6, 77.9, 73.4, 67.5, 53.5, 41.6, 37.1, 34.3, 30.8, 26.5, 19.1, 17.1, 15.4, 11.4 ppm; HRMS (ESI) *m/z* calcd for C₂₉H₄₁N₂O₆⁺ (M+H)⁺ 513.2959, found 513.2959.

(S)-Methyl 1-((R)-2-(2-((S)-2-((3R,4S)-4-(tert-butoxycarbonyl)-3-hydroxy-6-methylheptanamido)-3-methylbutanamido)-*N*-methylacetamido)-3-phenylpropanoyl)pyrrolidine-2-carboxylate (20)—

To a stirred solution of **8** (219 mg, 0.40 mmol) in DCM (4 mL) was added TFA (2 mL) at 0 °C and stirred for 3 h at room temperature. After removal of the solvent in vacuo, the residue was dissolved in toluene (1 mL) and evaporated. Repeated this process three times to give a crude amine. To a stirred solution of the amine, (*3R,4S*)-Boc-statine **7** (110 mg, 0.40 mmol), HATU (304 mg, 0.80 mmol) and HOAt (65 mg, 0.48 mmol) in DMF (2 mL) under argon atmosphere, was added DIPEA (0.35 mL, 2.00 mmol) at 0 °C. After being stirred for overnight at room temperature, saturated NH₄Cl (10 mL) was added to quench the reaction, and the mixture was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with saturated NaHCO₃, followed by brine, dried over MgSO₄, filtered and concentrated, and the residue purified by flash chromatography on silica gel (DCM/MeOH = 30:1) to give **20** as a colorless oil (165 mg, 59%). [α]_D^{22.8} +14.40 (*c* 1.00, MeOH); IR (film) ν_{\max} 3424, 1631, 1180 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.27–7.18 (m, 5H), 5.51–5.09 (m, 1H), 4.80–4.60 (m, 2H), 4.44–4.38 (m, 1H), 4.34–4.24 (m, 1H), 4.14–4.04 (m, 1H), 3.98–3.88 (m, 2H), 3.73 (s, 3H), 3.71–3.66 (m, 1H), 3.43–3.31 (m, 2H), 3.30–3.23 (m, 1H), 2.93 (s, 3H), 2.90–2.82 (m, 1H), 2.49–2.41 (m, 3H), 2.39–2.35 (m, 1H), 2.30–2.22 (m, 1H), 2.19–2.12 (m, 1H), 2.03–1.93 (m, 1H), 1.93–1.87 (m, 1H), 1.86–1.79 (m, 1H), 1.72–1.62 (m, 1H), 1.43 (s, 9H), 1.35–1.29 (m, 1H), 1.01–0.96 (m, 3H), 0.95–0.92 (m, 6H), 0.92–0.89 (m, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 173.4, 172.9, 172.0, 168.9, 168.2, 156.9, 137.0, 129.5, 128.6, 126.9, 80.0, 72.4, 59.3, 53.2, 52.6, 47.1, 41.3, 39.6, 35.0, 29.9, 28.9, 28.5, 25.2, 24.9, 23.8, 21.7, 19.5, 17.7 ppm; HRMS (ESI) *m/z* calcd for C₃₆H₅₈N₅O₉⁺ (M+H)⁺ 704.4229, found 704.4228.

(S)-Methyl 1-((R)-2-(2-((S)-2-((3R,4S)-4-((S)-2-(tert-butoxycarbonyl)-3-hydroxypropanamido)-3-hydroxy-6-methylheptanamido)-3-methylbutanamido)-N-methylacetamido)-3-phenylpropanoyl)pyrrolidine-2-carboxylate (21)—To a stirred solution of **20** (134 mg, 0.19 mmol) in DCM (2 mL) was added TFA (1 mL) at 0 °C and stirred for 3 h at room temperature. After removal of the solvent in vacuo, the residue was dissolved in toluene (1 mL) and evaporated. Repeated this process three times to give a crude amine. To a solution of Boc-Ser-OH **6** (47 mg, 0.23 mmol) in DMF (2 mL) was added HOBt (36 mg, 0.27 mmol) at –15 °C under argon atmosphere and stirred for 30 min at the same temperature. EDCI (51 mg, 0.27 mmol) was added and stirred at –15 °C for 1.5 h, then NMM (0.084 mL, 0.76 mmol) and a solution of the above amine in DMF was added. After being stirred for overnight at room temperature, saturated NH₄Cl (10 mL) was added to quench the reaction, and the mixture was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with saturated NaHCO₃, followed by brine, dried over MgSO₄, filtered and concentrated, and the residue purified by flash chromatography on silica gel (DCM/MeOH = 10:1) to give **21** as a colorless oil (105 mg, 70%). [α]_D^{22.8} +7.40 (*c* 1.00, MeOH); IR (film) ν_{\max} 3301, 2949, 1646, 1531, 1455, 1360, 1175, 1070 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.51–7.42 (m, 1H), 7.27–7.10 (m, 5H), 7.03–6.87 (m, 1H), 5.78–5.72 (m, 1H), 5.54–5.43 (m, 1H), 4.88–4.77 (m, 1H), 4.46–4.33 (m, 2H), 4.22–4.14 (m, 1H), 4.14–4.08 (m, 1H), 4.02–3.95 (m, 1H), 3.94–3.87 (m, 2H), 3.87–3.80 (m, 1H), 3.73 (s, 3H), 3.48–3.35 (m, 1H), 3.33–3.24 (m, 1H), 3.19–3.10 (m, 1H), 3.07 (s, 3H), 2.89–2.78 (m, 1H), 2.52–2.39 (m, 2H), 2.39–2.30 (m, 1H), 2.24–2.19 (m, 3H), 2.11–2.03 (m, 1H), 1.97–1.81 (m, 2H), 1.80–1.70 (m, 1H), 1.67–1.51 (m, 2H), 1.43 (s, 9H), 0.98–0.85 (m, 12H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 173.4, 172.8, 171.8, 171.7, 169.2, 168.5, 156.3, 136.9, 129.5, 128.6, 126.9, 80.6, 72.1, 62.5, 58.9, 58.5, 56.4, 55.4, 52.5, 52.4, 46.9, 41.3, 39.6, 38.4, 35.4, 30.3, 30.0, 29.0, 28.4, 25.0, 24.9, 23.8, 21.6, 19.6, 17.4 ppm; HRMS (ESI) *m/z* calcd for C₃₉H₆₃N₆O₁₁⁺ (M+H)⁺ 791.4549, found 791.4549.

(S)-Methyl 1-((R)-2-(2-((S)-2-((3R,4S)-4-((S)-2-((S)-2-((R)-2-((2R,3R)-2-(dimethylamino)-3-methylpentanoyloxy)-3-methylbutanamido)-3-(4-hydroxyphenyl)propanamido)-3-hydroxypropanamido)-3-hydroxy-6-methylheptanamido)-3-methylbutanamido)-N-methylacetamido)-3-phenylpropanoyl)pyrrolidine-2-carboxylate (3a)—To a stirred solution of **21** (103 mg, 0.13 mmol) in DCM (2 mL) was added TFA (1 mL) at 0 °C and stirred for 3 h at room temperature. After removal of the solvent in vacuo, the residue was dissolved in toluene (1 mL) and evaporated. Repeated this process three times to give a crude amine. Compound **18** (87 mg, 0.17 mmol), 10% Pd/C (9 mg) were stirred in MeOH (20 mL) at room temperature under H₂ atmosphere (1 atm). After being stirred for 3 h, the reaction mixture was filtered carefully, and the filtrate was concentrated in vacuo to give a crude acid. The crude acid was suspended in anhydrous DCM (3 mL) and cooled to –15 °C under argon atmosphere, HOBt (27 mg, 0.20 mmol) was added and stirred for 30 min at the same temperature. EDCI (38 mg, 0.20 mmol) was added and stirred at –15 °C for 1.5 h, then NMM (0.057 mL, 0.52 mmol) and a solution of the above amine in DCM was added. After being stirred for overnight at room temperature, saturated NH₄Cl (5 mL) was added to quench the reaction, and the mixture was extracted with EtOAc (5 mL × 3). The combined organic layers were

washed with saturated NaHCO₃, followed by brine, dried over MgSO₄, filtered and concentrated, and the residue was purified by flash chromatography on silica gel (DCM/MeOH = 10:1), and then by semi-preparative reversed-phase HPLC (C₁₈, 2 mL/min, gradient, 40:60 to 100:0 (CH₃CN + 0.1% TFA(aq.)) – (H₂O + 0.1% TFA(aq.)) over 40 min) to give **3a** as a colorless amorphous solid (54 mg, 38%). [α]_D^{22.7} +9.60 (*c* 0.25, MeOH); IR (film) ν_{\max} 3386, 1669, 1436, 1203, 1141, 1037 cm⁻¹; ¹H NMR (600 MHz, Acetonitrile-*d*₃) δ 8.16–8.10 (m, 1H), 8.10–8.04 (m, 1H), 7.68–7.55 (m, 1H), 7.55–7.45 (m, 1H), 7.25–7.19 (m, 4H), 7.19–7.16 (m, 1H), 7.06 (d, *J* = 8.4 Hz, 2H), 6.84–6.76 (m, 1H), 6.70 (d, *J* = 8.4 Hz, 2H), 5.42 (t, *J* = 7.2 Hz, 1H), 4.89 (d, *J* = 4.2 Hz, 1H), 4.61–4.55 (m, 1H), 4.29 (s, 1H), 4.27 (dd, *J* = 8.2, 5.9 Hz, 1H), 4.16–4.09 (m, 1H), 4.08–4.02 (m, 1H), 4.01–3.95 (m, 1H), 3.95–3.89 (m, 1H), 3.84–3.80 (m, 1H), 3.78–3.75 (m, 2H), 3.74–3.69 (m, 1H), 3.66 (s, 3H), 3.38–3.33 (m, 1H), 3.33–3.27 (m, 1H), 3.22–3.17 (m, 1H), 3.15–3.10 (m, 1H), 2.94 (s, 3H), 2.86 (s, 6H), 2.81–2.75 (m, 2H), 2.41–2.37 (m, 2H), 2.15–2.07 (m, 3H), 2.03–1.98 (m, 1H), 1.89–1.86 (m, 1H), 1.81–1.78 (m, 1H), 1.78–1.74 (m, 1H), 1.62–1.55 (m, 1H), 1.52–1.47 (m, 1H), 1.44–1.33 (m, 2H), 1.15–1.10 (m, 1H), 1.09 (d, *J* = 6.8 Hz, 3H), 0.94–0.87 (m, 12H), 0.87–0.82 (m, 6H), 0.68 (d, *J* = 6.7 Hz, 3H) ppm; ¹³C NMR (600 MHz, Acetonitrile-*d*₃) δ 174.0, 173.5, 173.0, 172.5, 171.7, 169.9, 169.4, 168.7, 167.4, 156.5, 138.5, 131.0, 130.2, 128.9, 128.9, 127.1, 115.8, 81.0, 73.2, 72.2, 62.5, 60.3, 59.9, 57.6, 57.0, 56.1, 52.4, 52.4, 47.3, 42.1, 41.4, 39.9, 38.8, 37.1, 35.1, 34.0, 31.0, 30.7, 30.5, 29.3, 25.5, 25.2, 24.4, 23.7, 21.6, 19.3, 18.7, 18.1, 16.6, 15.4, 11.1 ppm; HRMS (ESI) *m/z* calcd for C₅₆H₈₇N₈O₁₄⁺ (M+H)⁺ 1095.6336, found 1095.6314.

(2S,3S)-((R)-1-(Allyloxy)-3-methyl-1-oxobutan-2-yl) 2-(tert-butoxycarbonyl)-3-methylpentanoate ((2S,3S)-17)—Following the same procedure used for the preparation of **17** as described above, *L*-Boc-Ile-OH **22** (1.16 g, 5.00 mmol), instead of D-Boc-Ile-OH, was employed to produce the desired compound as a colorless oil (873 mg, 47%). [α]_D²² +18.30 (*c* 1.00, CHCl₃); IR (film) ν_{\max} 2968, 2935, 2873, 1741, 1707, 1508, 1365, 1156, 1013 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 6.00–5.80 (m, 1H), 5.40–5.30 (m, 1H), 5.30–5.25 (m, 1H), 5.03 (d, *J* = 8.8 Hz, 1H), 4.88 (d, *J* = 4.4 Hz, 1H), 4.70–4.60 (m, 2H), 4.39 (dd, *J* = 8.9, 4.6 Hz, 1H), 2.32–2.23 (m, 1H), 1.99–1.90 (m, 1H), 1.53–1.47 (m, 1H), 1.45 (s, 9H), 1.23–1.14 (m, 1H), 1.03–1.00 (m, 3H), 1.00–0.96 (m, 6H), 0.96–0.91 (m, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 172.0, 169.1, 155.5, 131.6, 119.1, 79.9, 77.5, 65.9, 58.2, 38.1, 30.3, 28.5, 25.0, 19.0, 17.3, 15.6, 11.8 ppm; HRMS (ESI) *m/z* calcd for C₁₉H₃₄NO₆⁺ (M+H)⁺ 372.2381, found 372.2383.

(2S,3S)-((R)-1-(Allyloxy)-3-methyl-1-oxobutan-2-yl) 2-(dimethylamino)-3-methylpentanoate ((2S,3S)-4)—Following the same procedure used for the preparation of **4** as described above, (*2S,3S*)-**17** (743 mg, 2 mmol), instead of **17**, was employed to produce the desired compound (*2S,3S*)-**4** as a colorless oil (470 mg, 78%). [α]_D²² +15.10 (*c* 2.00, CHCl₃); IR (film) ν_{\max} 2966, 2936, 2876, 1757, 1736, 1456, 1272, 1195, 1125, 1015 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 5.98–5.83 (m, 1H), 5.39–5.31 (m, 1H), 5.28–5.23 (m, 1H), 4.86 (d, *J* = 4.8 Hz, 1H), 4.67–4.63 (m, 2H), 2.94 (d, *J* = 10.2 Hz, 1H), 2.35 (s, 6H), 2.32–2.22 (m, 1H), 1.91–1.82 (m, 1H), 1.70–1.62 (m, 1H), 1.20–1.12 (m, 1H), 1.04–1.01 (m, 3H), 1.01–0.98 (m, 3H), 0.93–0.90 (m, 3H), 0.90–0.87 (m, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 171.8, 169.6, 131.7, 119.1, 76.7, 72.4, 72.4, 65.8, 41.6, 33.4, 30.2, 25.2,

19.1, 17.5, 15.8, 10.6 ppm; HRMS (ESI) m/z calcd for $C_{16}H_{30}NO_4^+$ (M+H)⁺ 300.2169, found 300.2167.

(2S,3S)-((R)-1-((S)-1-(Benzyloxy)-3-(4-hydroxyphenyl)-1-oxopropan-2-ylamino)-3-methyl-1-oxobutan-2-yl) 2-(dimethylamino)-3-methylpentanoate ((2S,3S)-18)—Following the same procedure used for the preparation of **18** as described above, (2*S*,3*S*)-**4** (449 mg, 1.50 mmol), instead of **4**, was employed to produce the desired compound (2*S*,3*S*)-**18** as a colorless oil (110 mg, 31%). $[\alpha]_D^{22} +24.90$ (*c* 1.00, CHCl₃); IR (film) ν_{max} 3320, 2968, 2930, 2873, 1731, 1660, 1512, 1451, 1175, 1118, 1037 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.48–7.22 (m, 5H), 6.88–6.76 (m, 2H), 6.72–6.56 (m, 3H), 5.26–5.07 (m, 3H), 4.87–4.80 (m, 1H), 3.19–3.10 (m, 1H), 3.04–2.96 (m, 1H), 2.90 (d, *J* = 10.0 Hz, 1H), 2.33–2.24 (m, 1H), 2.19 (s, 6H), 1.88–1.77 (m, 1H), 1.69–1.59 (m, 1H), 1.18–1.09 (m, 1H), 0.96–0.91 (m, 6H), 0.91–0.86 (m, 3H), 0.84–0.78 (m, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 171.3, 170.9, 169.4, 155.3, 135.0, 130.5, 128.80, 128.78, 127.1, 115.7, 77.7, 72.5, 67.6, 53.6, 41.5, 36.8, 33.5, 30.9, 25.3, 19.0, 17.1, 16.0, 10.6 ppm; HRMS (ESI) m/z calcd for $C_{29}H_{41}N_2O_6^+$ (M+H)⁺ 513.2959, found 513.2959.

(S)-Methyl 1-((R)-2-(2-((S)-2-((3R,4S)-4-((S)-2-((S)-2-((R)-2-((2S,3S)-2-(dimethylamino)-3-methylpentanoyloxy)-3-methylbutanamido)-3-(4-hydroxyphenyl)propanamido)-3-hydroxypropanamido)-3-hydroxy-6-methylheptanamido)-3-methylbutanamido)-*N*-methylacetamido)-3-phenylpropanoyl)pyrrolidine-2-carboxylate (3b)—Following the same procedure used for the preparation of **3a** as described above, (2*S*,3*S*)-**18** (90 mg, 0.18 mmol), instead of **18**, was employed to produce the desired compound **3b** as a colorless amorphous solid (17 mg, 26%). $[\alpha]_D^{22.7} +21.20$ (*c* 0.25, MeOH); IR (film) ν_{max} 3367, 1636, 1403, 1037 cm⁻¹; ¹H NMR (600 MHz, Acetonitrile-*d*₃) δ 7.95–7.87 (m, 1H), 7.87–7.78 (m, 1H), 7.40–7.32 (m, 1H), 7.32–7.26 (m, 1H), 7.26–7.21 (m, 4H), 7.19–7.14 (m, 1H), 7.06 (d, *J* = 8.4 Hz, 2H), 6.95–6.82 (m, 1H), 6.70 (d, *J* = 8.4 Hz, 2H), 5.44 (t, *J* = 7.6 Hz, 1H), 4.85 (d, *J* = 4.4 Hz, 1H), 4.56 (m, 1H), 4.35–4.29 (m, 1H), 4.27 (dd, *J* = 8.4, 5.9 Hz, 1H), 4.15 (t, *J* = 6.5 Hz, 1H), 4.09–4.03 (m, 1H), 4.00–3.93 (m, 1H), 3.91 (d, *J* = 4.3 Hz, 1H), 3.89–3.85 (m, 1H), 3.85–3.79 (m, 1H), 3.79–3.69 (m, 2H), 3.65 (s, 3H), 3.38–3.32 (m, 1H), 3.33–3.26 (m, 1H), 3.22–3.16 (m, 1H), 3.16–3.11 (m, 1H), 2.94 (s, 3H), 2.82 (s, 6H), 2.81–2.78 (m, 1H), 2.78–2.74 (m, 1H), 2.43–2.36 (m, 2H), 2.17–2.09 (m, 3H), 2.01 (m, 1H), 1.90–1.86 (m, 1H), 1.81–1.73 (m, 2H), 1.64–1.55 (m, 1H), 1.53–1.47 (m, 1H), 1.45–1.36 (m, 2H), 1.36–1.31 (m, 1H), 0.99–0.88 (m, 15H), 0.84 (d, *J* = 6.8 Hz, 6H), 0.71 (d, *J* = 6.7 Hz, 3H) ppm; ¹³C NMR (600 MHz, Acetonitrile-*d*₃) δ 173.9, 173.4, 172.6, 172.3, 171.6, 169.7, 169.4, 168.7, 168.3, 156.6, 138.6, 130.9, 130.2, 128.9, 128.9, 127.1, 115.9, 81.2, 72.3, 71.7, 62.6, 59.9, 59.8, 57.4, 56.7, 56.0, 52.7, 52.3, 47.3, 42.7, 41.4, 39.9, 39.1, 36.7, 35.1, 33.9, 30.9, 30.6, 30.5, 29.4, 27.3, 25.5, 25.2, 23.7, 21.6, 19.3, 18.8, 17.9, 16.7, 14.1, 11.5 ppm; HRMS (ESI) m/z calcd for $C_{56}H_{87}N_8O_{14}^+$ (M+H)⁺ 1095.6336, found 1095.6326.

(2S,3S)-tert-Butyl 3-hydroxy-2-isobutyl-5-oxopyrrolidine-1-carboxylate ((2S,3S)-14)—To a stirred solution of oxalyl chloride (0.43 mL, 5.00 mmol) in dry DCM (10 mL) was dropped DMSO (0.71 mL, 10.00 mmol) over 15 min at –78 °C under argon atmosphere, and stirred for 1 h. A solution of **14** (643 mg, 2.50 mmol) in dry DCM (5.0 mL)

was dropped and the mixture was stirred for another 2 h, then TEA (2.08 mL, 15.00 mmol) was dropped and the mixture was allowed to warm to room temperature. Saturated NH₄Cl (20 mL) was added to quench the reaction, and the mixture was extracted with DCM (15 mL × 3). The combined organic layers were washed with saturated NaHCO₃, followed by brine, dried over MgSO₄, filtered and concentrated to give a crude product as a colorless oil which was used in the next step without further purification. The crude mixture was dissolved in MeOH (15 mL) and cooled to 0 °C, then NaBH₄ (236 mg, 6.25 mmol) was added in three portions. After being stirred for 3 h at 0 °C to room temperature, the reaction was quenched with saturated NaHCO₃ and extracted three times with DCM. The combined organic layers were washed with brine and dried over MgSO₄. Filtered and concentrated, the residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc = 2:1) to give (2*S*, 3*S*)-**14** as colorless crystals (431 mg, 1.67 mmol, 67%). Mp 89 – 91 °C, [α]_D^{23.2} +65.50 (*c* 1.00, MeOH); IR (film) ν_{\max} 3445, 2957, 2929, 2866, 1773, 1720, 1358, 1295, 1256, 1154 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 4.60–4.50 (m, 1H), 4.25–4.15 (m, 1H), 2.69 (dd, *J* = 17.1, 7.3 Hz, 1H), 2.59 (dd, *J* = 17.1, 8.0 Hz, 1H), 1.87–1.73 (m, 2H), 1.53 (s, 9H), 1.50–1.43 (m, 1H), 1.01–0.94 (m, 6H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 172.2, 150.0, 83.4, 65.7, 59.9, 40.4, 37.3, 28.2, 25.2, 23.6, 22.6 ppm; HRMS (ESI) *m/z* calcd for C₁₃H₂₄NO₄⁺ (M+H)⁺ 258.1700, found 258.1700.

(3*S*,4*S*)-4-(*tert*-Butoxycarbonyl)-3-hydroxy-6-methylheptanoic acid ((3*S*,4*S*)-7)

—Following the same procedure used for the preparation of **7** as described above, (2*S*, 3*S*)-**14** (335 mg, 1.30 mmol), instead of **14**, was employed to produce the desired compound (3*S*,4*S*)-**7** as a white powder (358 mg, quant.), which was used for the next step without further purification.

(*S*)-Methyl 1-((*R*)-2-(2-((*S*)-2-((3*S*,4*S*)-4-(*tert*-butoxycarbonyl)-3-hydroxy-6-methylheptanamido)-3-methylbutanamido)-*N*-methylacetamido)-3-phenylpropanoyl)pyrrolidine-2-carboxylate ((3*S*,4*S*)-20)

—Following the same procedure used for the preparation of **20** as described above, (3*S*,4*S*)-**7** (358 mg, 1.30 mmol), instead of **7**, was employed to produce the desired compound (3*S*,4*S*)-**20** as a colorless oil (795 mg, 87%). [α]_D^{22.8} +7.40 (*c* 1.00, MeOH); IR (film) ν_{\max} 3323, 2957, 2929, 2866, 1745, 1639, 1525, 1436, 1366, 1278, 1173, 1046 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.27–7.09 (m, 5H), 6.91–6.73 (m, 1H), 6.65–6.52 (m, 1H), 5.61–5.52 (m, 1H), 4.85–4.79 (m, 1H), 4.46–4.39 (m, 1H), 4.38–4.32 (m, 1H), 4.19–4.14 (m, 1H), 4.14–4.07 (m, 1H), 4.02–3.94 (m, 1H), 3.94–3.85 (m, 1H), 3.73 (s, 3H), 3.65–3.55 (m, 1H), 3.40–3.32 (m, 2H), 3.32–3.24 (m, 1H), 3.00 (s, 3H), 2.87–2.78 (m, 1H), 2.53–2.44 (m, 1H), 2.43–2.34 (m, 1H), 2.21–2.08 (m, 2H), 1.99–1.90 (m, 1H), 1.87–1.78 (m, 1H), 1.71–1.61 (m, 1H), 1.60–1.50 (m, 1H), 1.44 (s, 9H), 1.39–1.29 (m, 1H), 0.96–0.90 (m, 12H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 172.9, 172.6, 171.0, 168.01, 167.96, 156.5, 137.0, 129.5, 128.5, 126.9, 79.4, 70.6, 59.1, 58.4, 56.4, 52.42, 52.38, 46.9, 41.7, 41.3, 40.4, 35.1, 31.1, 29.9, 29.0, 28.5, 25.2, 24.9, 23.2, 22.3, 19.4, 18.0 ppm; HRMS (ESI) *m/z* calcd for C₃₆H₅₈N₅O₉⁺ (M+H)⁺ + 704.4229, found 704.4229.

(*S*)-Methyl 1-((*R*)-2-(2-((*S*)-2-((3*S*,4*S*)-4-((*S*)-2-(*tert*-butoxycarbonyl)-3-hydroxypropanamido)-3-hydroxy-6-methylheptanamido)-3-

methylbutanamido)-N-methylacetamido)-3-phenylpropanoyl) pyrrolidine-2-carboxylate ((3*S*,4*S*)-21)—Following the same procedure used for the preparation of **21** as described above, (3*S*,4*S*)-**20** (704 mg, 1.00 mmol), instead of **20**, was employed to produce the desired compound (3*S*,4*S*)-**21** as a colorless oil (290 mg, 37%). $[\alpha]_{\text{D}}^{23.0} +14.50$ (*c* 1.00, MeOH); IR (film) ν_{max} 3442, 2958, 1748, 1645, 1533, 1454, 1367, 1173, 1056 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.36–7.30 (m, 1H), 7.26–7.17 (m, 5H), 7.15–7.08 (m, 1H), 5.79–5.72 (m, 1H), 5.58–5.49 (m, 1H), 4.60–4.49 (m, 1H), 4.43–4.38 (m, 1H), 4.38–4.32 (m, 1H), 4.22–3.87 (m, 6H), 3.73 (s, 3H), 3.68–3.59 (m, 1H), 3.42–3.34 (m, 1H), 3.33–3.26 (m, 1H), 3.04 (s, 3H), 2.88–2.76 (m, 1H), 2.65–2.53 (m, 1H), 2.43–2.34 (m, 1H), 2.25–2.16 (m, 1H), 2.14–2.06 (m, 2H), 1.98–1.76 (m, 3H), 1.75–1.56 (m, 2H), 1.43 (s, 9H), 0.98–0.86 (m, 12H) ppm; ^{13}C NMR (100 MHz, CDCl_3) δ 172.7, 172.3, 172.2, 171.9, 168.5, 168.1, 156.2, 137.0, 129.5, 128.5, 126.9, 80.6, 70.6, 62.9, 59.0, 58.7, 56.5, 56.0, 52.4, 52.0, 46.9, 41.3, 41.0, 40.1, 35.2, 30.7, 30.1, 29.0, 28.4, 25.1, 24.9, 23.3, 22.2, 19.5, 18.0 ppm; HRMS (ESI) m/z calcd for $\text{C}_{39}\text{H}_{63}\text{N}_6\text{O}_{11}^+$ (M+H) $^+$ 791.4549, found 791.4550.

(S)-Methyl 1-((R)-2-(2-((S)-2-((3*S*,4*S*)-4-((S)-2-((S)-2-((R)-2-((2*S*,3*S*)-2-(dimethylamino)-3-methylpentanoyloxy)-3-methylbutanamido)-3-(4-hydroxyphenyl)propanamido)-3-hydroxypropanamido)-3-hydroxy-6-methylheptanamido)-3-methylbutanamido)-Nethylacetamido)-3-phenylpropanoyl)pyrrolidine-2-carboxylate (3c)—Following the same procedure used for the preparation of **3a** as described above, (2*S*,3*S*)-**18** (187 mg, 0.36 mmol), instead of **18**, and (3*S*,4*S*)-**20** (240 mg, 0.30 mmol), instead of **20**, were employed to produce the desired compound **3c** as a colorless amorphous solid (70 mg, 21%). $[\alpha]_{\text{D}}^{21.1} +27.6$ (*c* 1.00, MeOH); IR (film) ν_{max} 3450, 2965, 1745, 1654, 1545, 1444, 1203, 1132, 1052 cm^{-1} ; ^1H NMR (600 MHz, Acetonitrile- d_3) δ 7.69–7.62 (m, 1H), 7.62–7.58 (m, 1H), 7.32–7.26 (m, 1H), 7.26–7.19 (m, 5H), 7.19–7.14 (m, 1H), 7.05 (d, $J = 8.2$ Hz, 2H), 7.02–6.94 (m, 1H), 6.70 (d, $J = 8.2$ Hz, 2H), 5.47 (t, $J = 7.4$ Hz, 1H), 4.82 (d, $J = 4.5$ Hz, 1H), 4.65–4.58 (m, 1H), 4.36–4.30 (m, 1H), 4.27 (dd, $J = 8.5, 5.7$ Hz, 1H), 4.22–4.15 (m, 1H), 4.07–4.01 (m, 1H), 3.98–3.89 (m, 3H), 3.87–3.79 (m, 2H), 3.78–3.69 (m, 1H), 3.65 (s, 3H), 3.36–3.32 (m, 1H), 3.32–3.27 (m, 1H), 3.21–3.17 (m, 1H), 3.17–3.12 (m, 1H), 2.93 (s, 3H), 2.85 (s, 6H), 2.83–2.78 (m, 1H), 2.78–2.74 (m, 1H), 2.42–2.31 (m, 2H), 2.20–2.07 (m, 3H), 2.03–1.97 (m, 1H), 1.89–1.84 (m, 1H), 1.82–1.73 (m, 2H), 1.64–1.56 (m, 1H), 1.54–1.45 (m, 2H), 1.39–1.28 (m, 2H), 1.00–0.94 (m, 6H), 0.93–0.85 (m, 12H), 0.83 (d, $J = 6.8$ Hz, 3H), 0.72 (d, $J = 6.7$ Hz, 3H) ppm; ^{13}C NMR (600 MHz, Acetonitrile- d_3) δ 173.3, 172.4, 172.4, 172.2, 171.6, 169.4, 169.3, 168.8, 168.0, 156.5, 138.6, 130.9, 130.1, 128.9, 128.8, 127.0, 115.9, 81.2, 71.6, 71, 62.7, 59.8, 59.5, 57.1, 56.7, 55.7, 52.3, 52.3, 47.4, 42.8, 41.5, 41.1, 40.7, 36.7, 35.1, 33.9, 31.0, 30.9, 30.3, 29.4, 27.3, 25.5, 25.2, 23.3, 22.1, 19.4, 18.8, 17.9, 16.8, 14.0, 11.5 ppm; HRMS (ESI) m/z calcd for $\text{C}_{56}\text{H}_{87}\text{N}_8\text{O}_{14}^+$ (M+H) $^+$ 1095.6336, found 1095.6340.

Supplementary Material

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Acknowledgements

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21. The errors in stereoassignment of symplocin A, specifically pertaining to the HPLC analysis of *O*-(2'-naphthacyl)- α -DMAA esters, is under investigation in one of our laboratories (TFM).

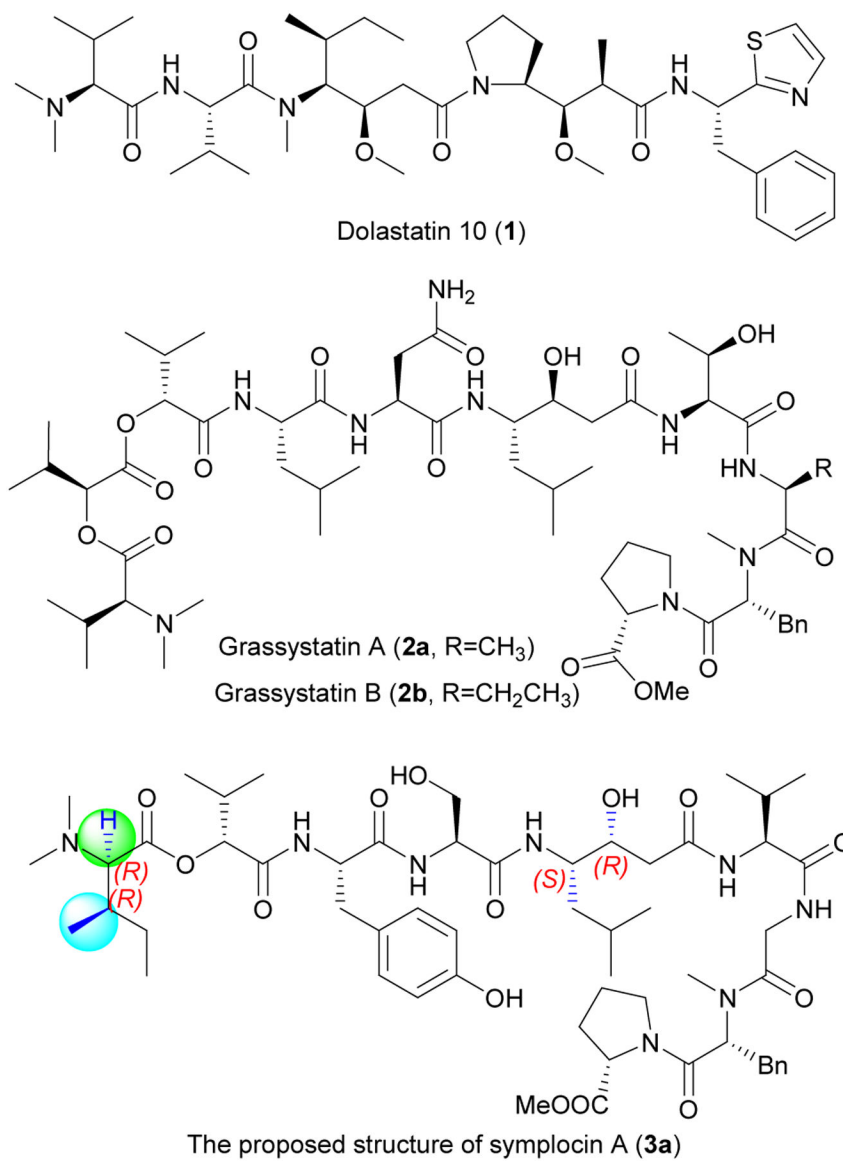


Figure 1.
The Structures of DMAA Linear Peptides.

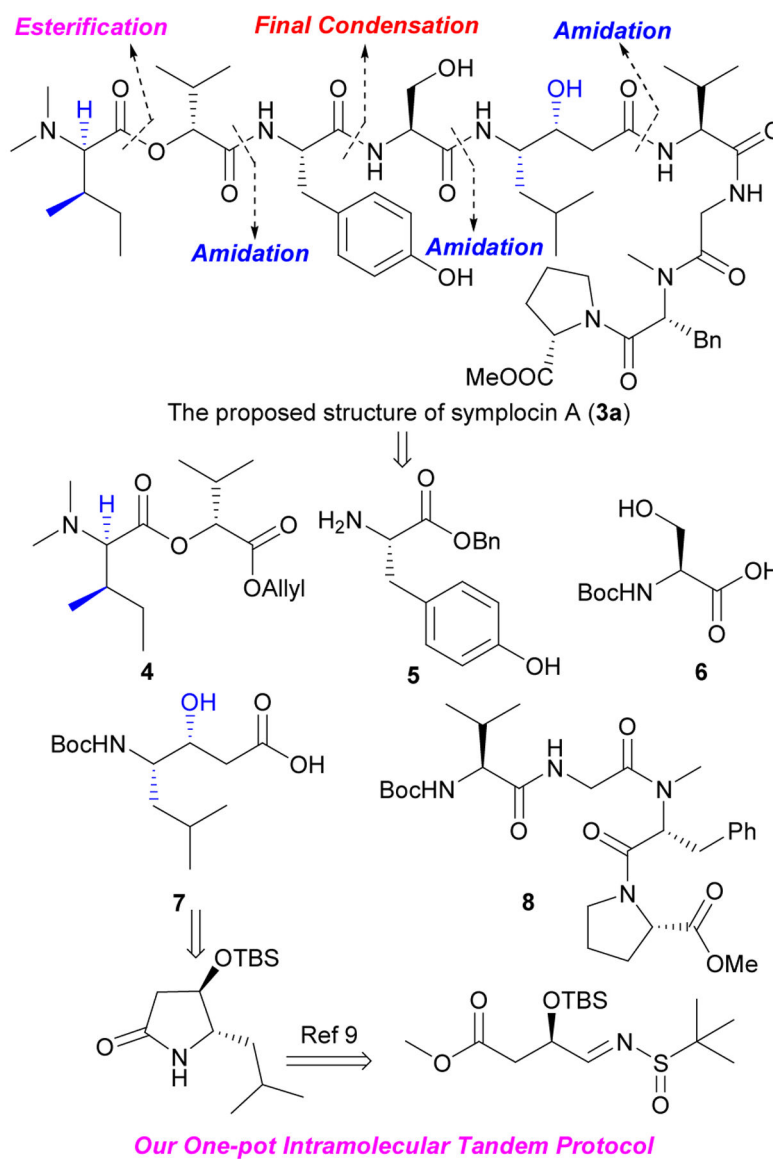
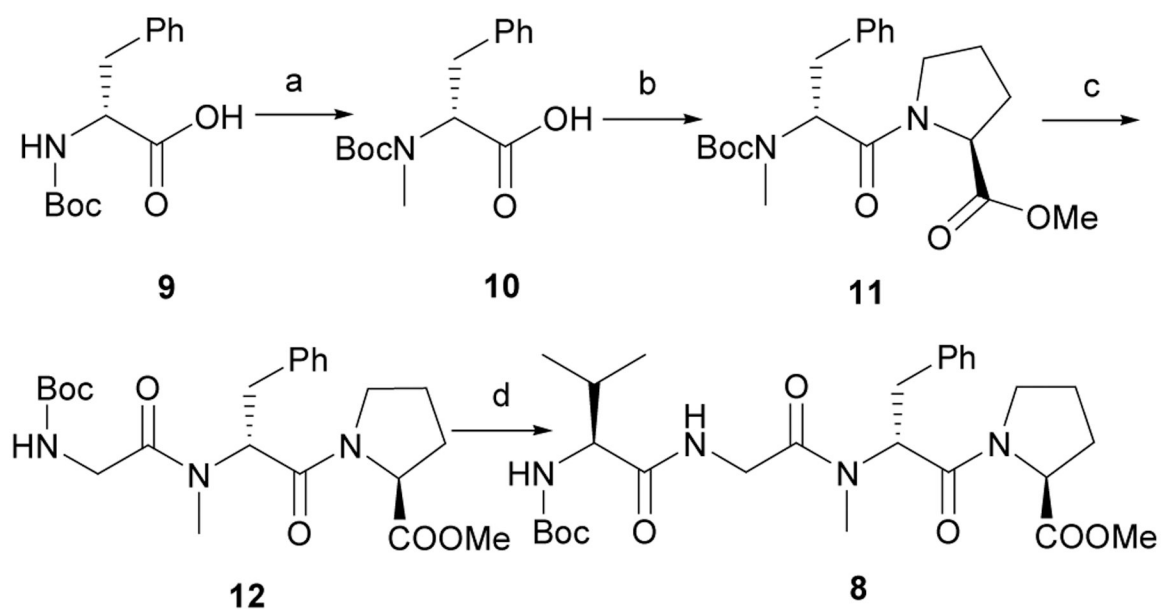
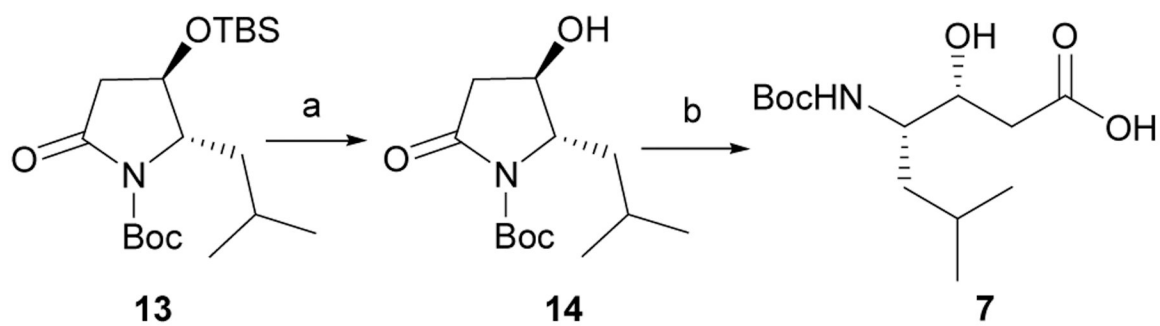


Figure 2.
Retrosynthetic analysis of **3a**.

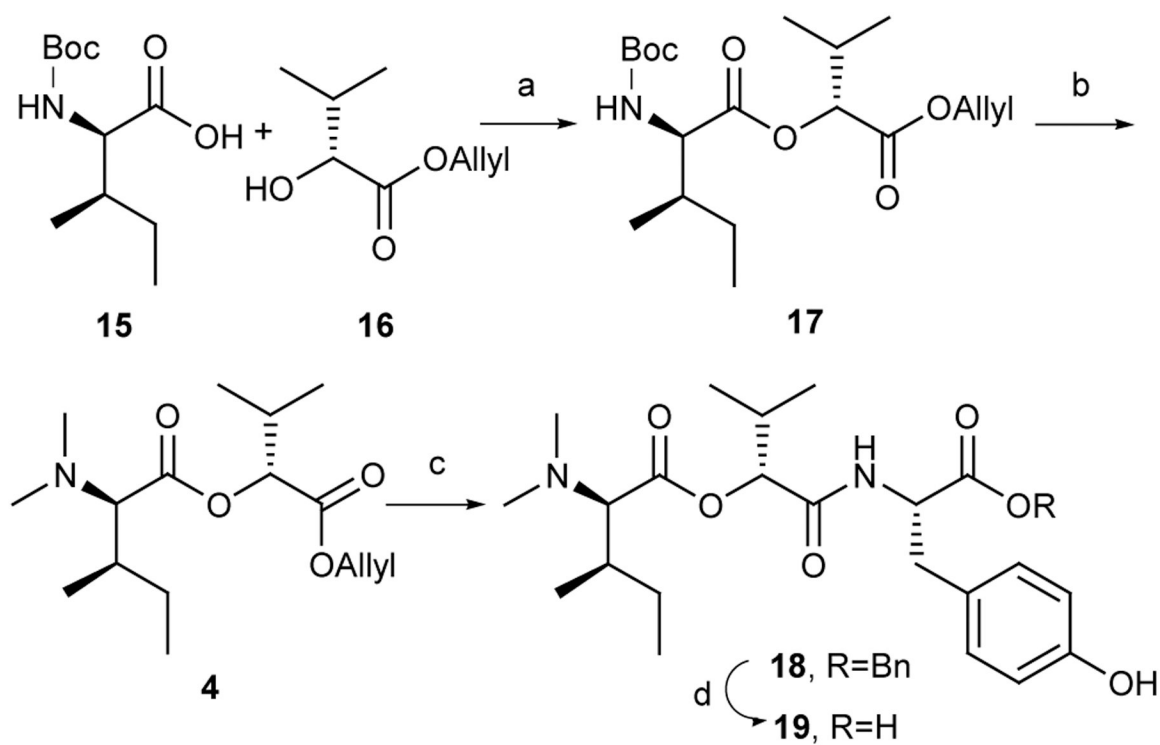
**Scheme 1.**

Preparation of Tetrapeptide Fragment 8. Reagents and conditions: (a) NaH, MeI, THF; (b) L-Pro-OMe, HATU, DIPEA, DCM, 62% (2 steps); (c) (1) TFA, DCM; (2) Boc-Gly, HATU, DIPEA, DCM, 80% (2 steps); (d) (1) TFA, DCM; (2) Boc-Val-OH, HOBt, EDCI, DMF, 62% (2 steps).

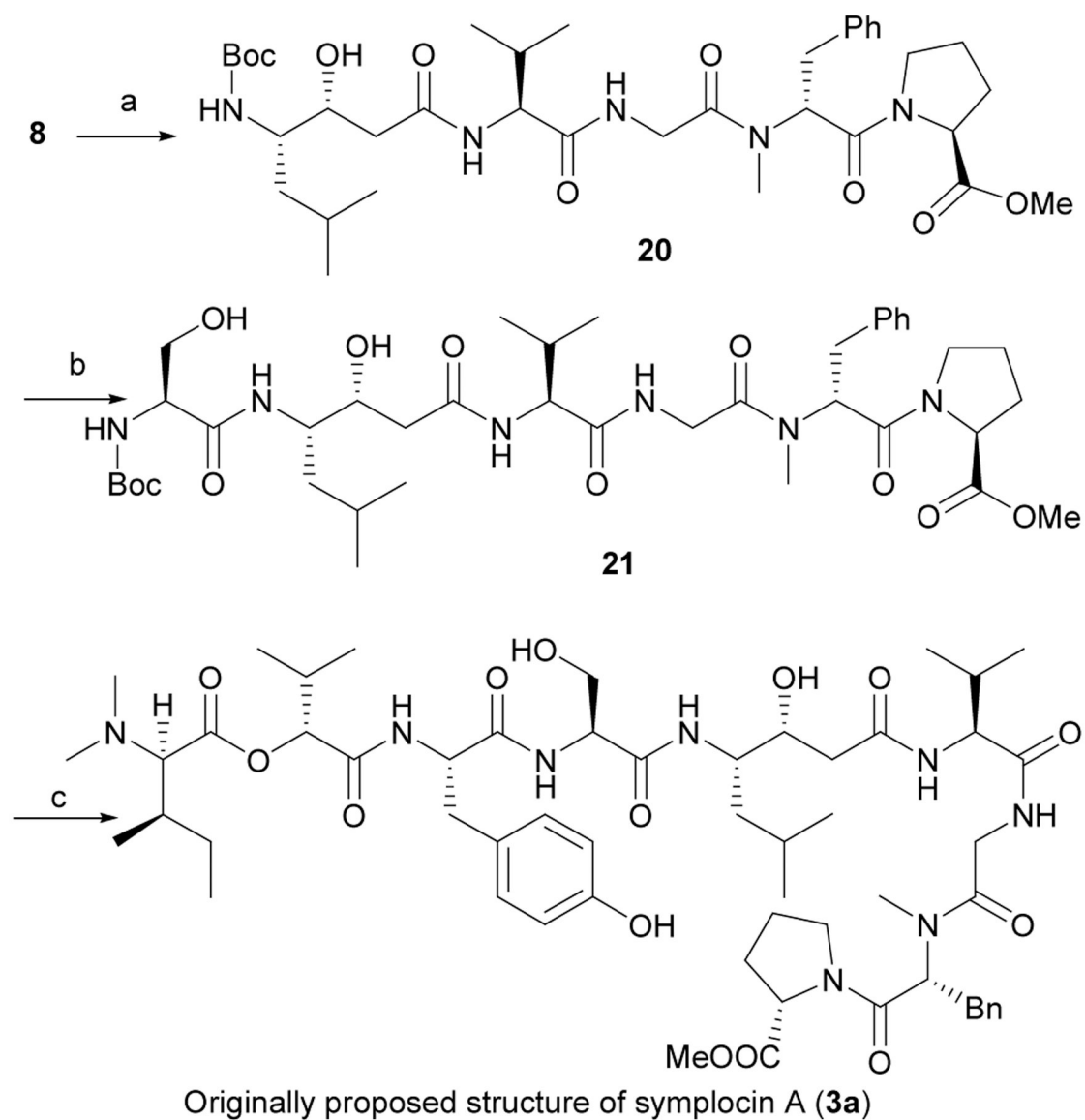
**Scheme 2.**

Preparation of (3*R*,4*S*)-Statine Subunit **7**. Reagents and conditions: (a) TBAF, THF, 50%;

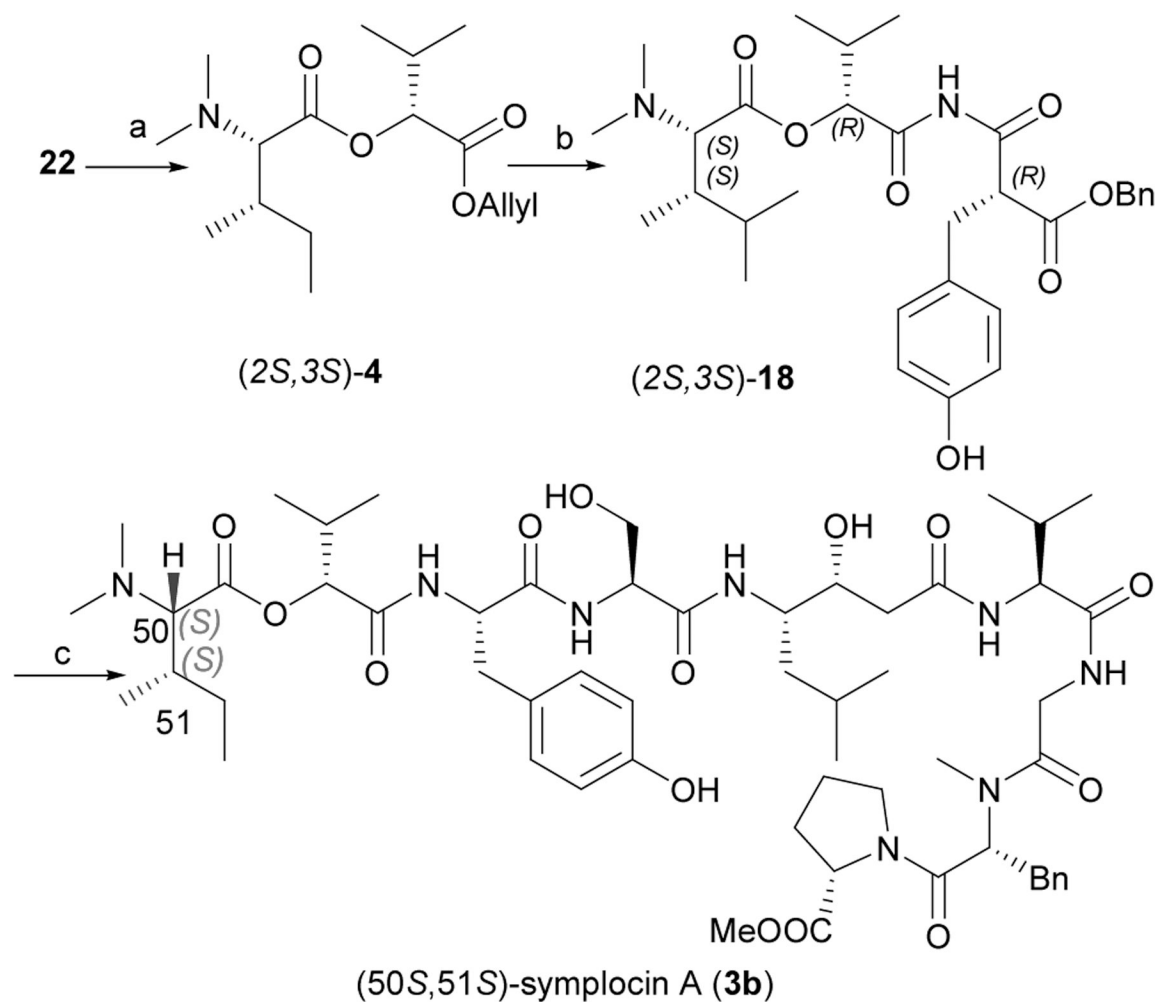
(b) LiOH•H₂O, H₂O₂, THF/H₂O, 100%.

**Scheme 3.**

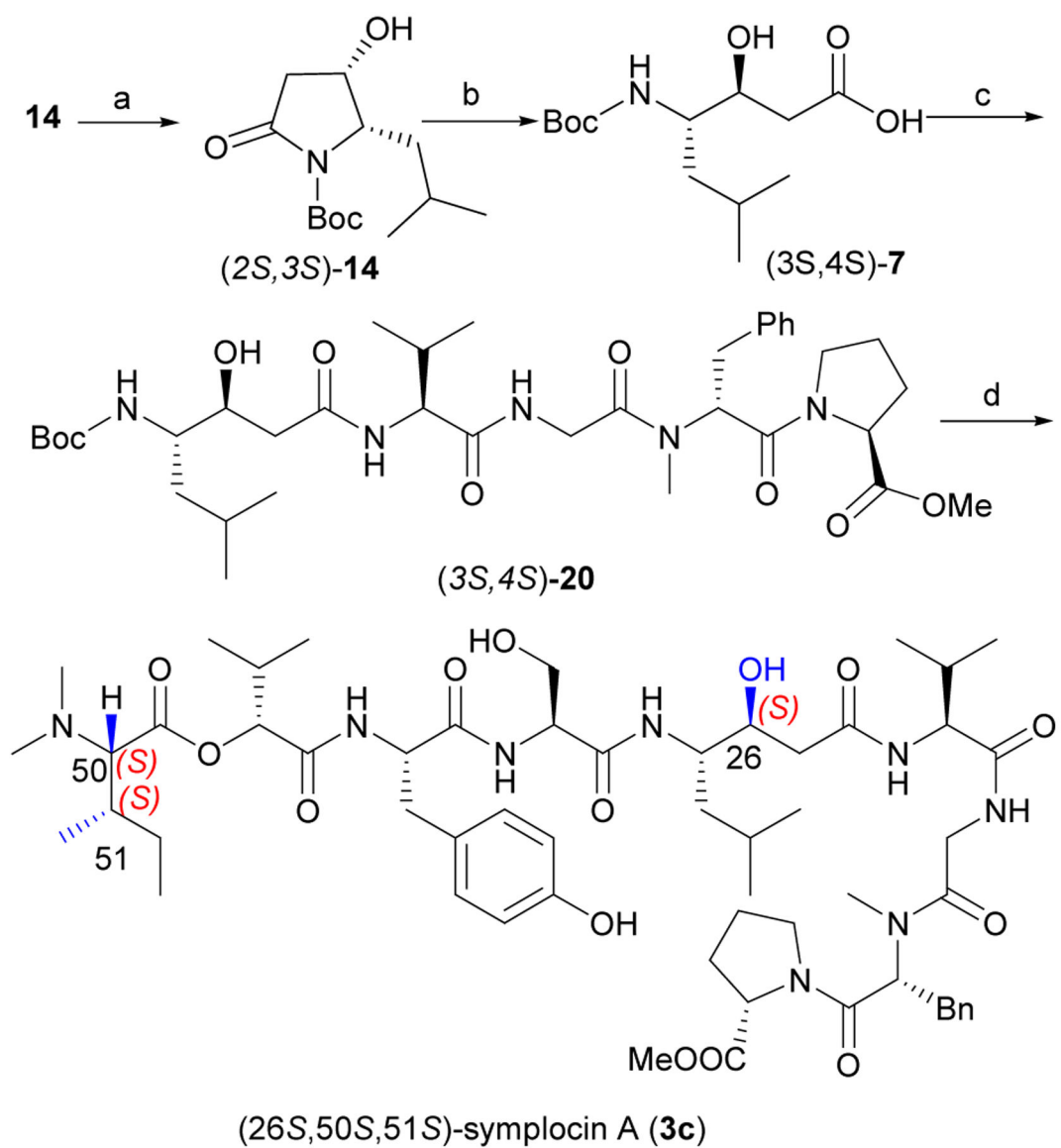
Preparation of Depsipeptide Fragment **19**. Reagents and conditions: (a) DCC, DMAP, DCM, 76%; (b) (1) TFA, DCM; (2) HCHO, NaBH₃(CN), CH₃CN, 64% (2 steps); (c) (1) Pd(PPh₃)₄, *N*-methylaniline, NMA, THF; (2) **5**, HATU, HOAt, DIPEA, DCM, 45% (2 steps); (d) H₂, 1 atm, Pd/C, MeOH, 100%.

**Scheme 4.**

Preparation of Symplocin A (**3a**). Reagents and conditions: (a) (1) TFA, DCM; (2) **7**, HATU, HOAt, DIPEA, DMF, 59% (2 steps); (b) (1) TFA, DCM; (2) **6**, HOBt, EDCI, NMM, DMF, 70% (2 steps); (c) (1) TFA, DCM; (2) **19**, HOBt, EDCI, NMM, DCM, 38% (2 steps).

**Scheme 5.**

Preparation of (50*S*, 51*S*)-Symplocin A (**3b**). Reagents and conditions: (a) (1) **16**, DCC, DMAP, DCM; (2) TFA, DCM; (3) HCHO, NaBH₃(CN), CH₃CN, 37% (3 steps); (b) (1) Pd(PPh₃)₄, *N*-methylaniline, NMA, THF; (2) **5**, HATU, HOAt, DIPEA, DCM, 31% (2 steps); (c) (1) H₂, 1 atm, Pd/C; (2) free amine of **21**, HOBT, EDCI, NMM, DCM, 26% (2 steps).

**Scheme 6.**

Preparation of (26*S*, 50*S*, 51*S*)-Symplocin A (**3c**). Reagents and conditions: (a) (1) $(\text{COCl})_2$, DMSO, DCM; (2) NaBH_4 , MeOH, 67% (2 steps); (b) $\text{LiOH}\cdot\text{H}_2\text{O}$, H_2O_2 , THF/H₂O, 100%; (c) free amine of **8**, HATU, HOAt, DIPEA, DMF, 87%; (d) (1) TFA, DCM; (2) **6**, HOBt, EDCI, NMM, DMF, 37% (2 steps); (3) TFA, DCM; (4) (2*S*,3*S*)-**19**, HOBt, EDCI, NMM, DCM, 21% (2 steps).