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SYNTHETIC STUDIES ON CHLOROFUSIN: SYNTHESIS OF THE CYCLIC PEPTIDE PORTION

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Dedicated to Professor Yoshito Kishi on the occasion of his 70th birthday

Abstract – The cyclic peptide portion of chlorofusin was synthesized by condensation of the five segments, D-Ada-OTMSE, Boc-L-Orn(Cbz), Boc-L-Thr-L-Ala, L-Asn(Tr)-D-Asn(Tr)-D-Leu-OTMSE, and Boc-L-Thr-D-Leu, followed by cyclization at the amide bond between D-Ada and L-Orn.

INTRODUCTION

Phosphoprotein p53 plays a crucial role in the regulation of cell proliferation and controls the cell growth and development of genetic abnormalities by inducing G1 arrest or apoptosis in response to DNA damage. The oncoprotein MDM2 forms a stable complex with tumor suppressor p53 and conceals the DNA-binding domain of p53. As a result, p53 does not function as a regulator of cell proliferation. It seems possible that molecules that inhibit the complex formation of p53 and MDM2 would restore the normal function to p53.[1,2] Chlorofusin (**1**) was isolated from the fermentation broth of a tropical insect-associated fungal strain *Microdochium caespitosum* and antagonizes the p53–MDM2 interaction with IC_{50} of 4.6 μM; therefore, chlorofusin (**1**) has the potential as a lead in cancer therapy (Figure 1).[3] Chlorofusin (**1**), most of the structure of which was determined by spectroscopic means and chemical degradation studies, consists of a chromophore and a cyclic peptide portion. The absolute stereochemistry of the chromophore has not been determined. The cyclic peptide portion includes two L-threonines, an L-alanine, both an L- and D-asparagine, two D-leucines, a D-2-aminodecanoic acid, and an L-ornithine. The two asparagine residues have opposite stereochemistries (L and D), but the respective assignments have not been determined. Nevertheless, from the NOE experiments of the NMR studies, it was suggested

that Asn3 likely has the L-configuration and Asn4 has the D-configuration.[3a] Recently, the Boger group[4] and the Searcey group[5] independently succeeded in the synthesis of the cyclic peptide portion (**2**) of chlorofusin (**1**). Boger et al. synthesized the two cyclic peptide portions, each of which contains L-Asn3/D-Asn4 or D-Asn3/L-Asn4.[4] By the precise NMR studies including chemical shift differences between the natural product and the two synthetic diastereomers, they assigned Asn3 and Asn4 as L and D, respectively.[4] They incorporated the subunit bearing the two asparagine residues during the late stage of the synthesis in order to effectively secure the two diastereomers.[4] Searcey et al. succeeded in the solid-phase synthesis of the cyclic peptide portion (**2**) bearing the L-Asn3/D-Asn4 subunit starting from N^{α}-Fmoc-L-aspartic acid immobilized onto a Rink amide MBHA-derivatized polystyrene resin via a linear elongation synthesis.[5] They also described that the chemical shifts of the synthetic cyclic peptide were in good agreement with those of chlorofusin (**1**).[5] On the other hand, the Yao group succeeded in the synthesis of the racemic model chromophore of chlorofusin (**1**).[6] We now report the synthesis of the cyclic peptide portion (**2**) having the L-Asn3/D-Asn4 subunit. Since our objective is the total synthesis of chlorofusin (**1**), it is reasonable to incorporate L-Orn9, which is linked to the chromophore portion, in the late stage of the synthesis of **2**. Along this line, we selected five segments **3** – **7** each of which has Boc or 2-(trimethylsilyl)ethyl (TMSE) protecting groups on the α-amine or carboxylic acid functionalities, respectively (Figure 2). Condensation of these segments and subsequent macrocyclization at the amide bond between D-2-aminodecanoic acid and L-ornithine would afford the cyclic peptide portion (**2**).

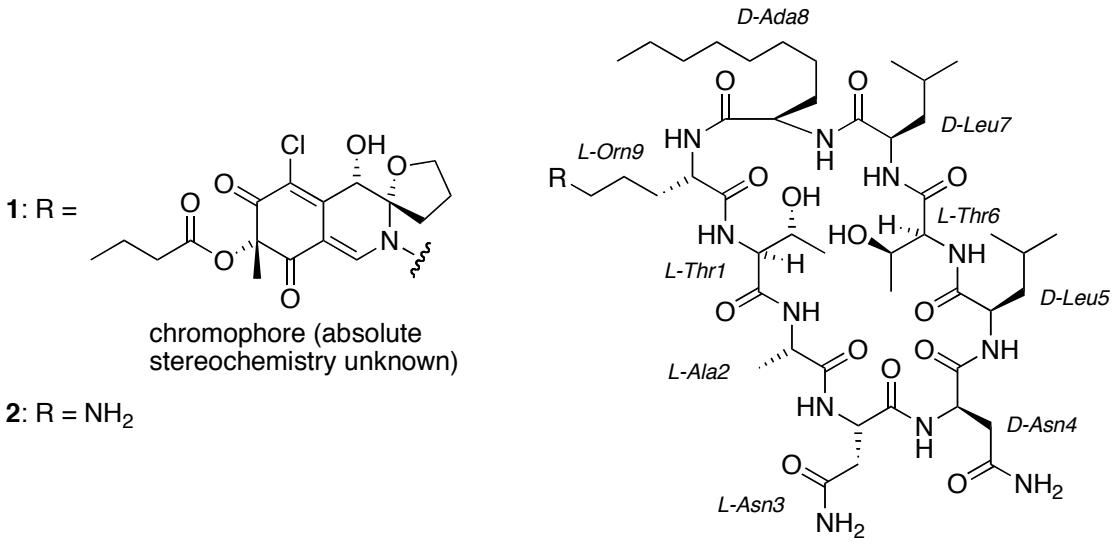


Figure 1. The structures of chlorofusin (**1**) and its cyclic peptide portion (**2**).

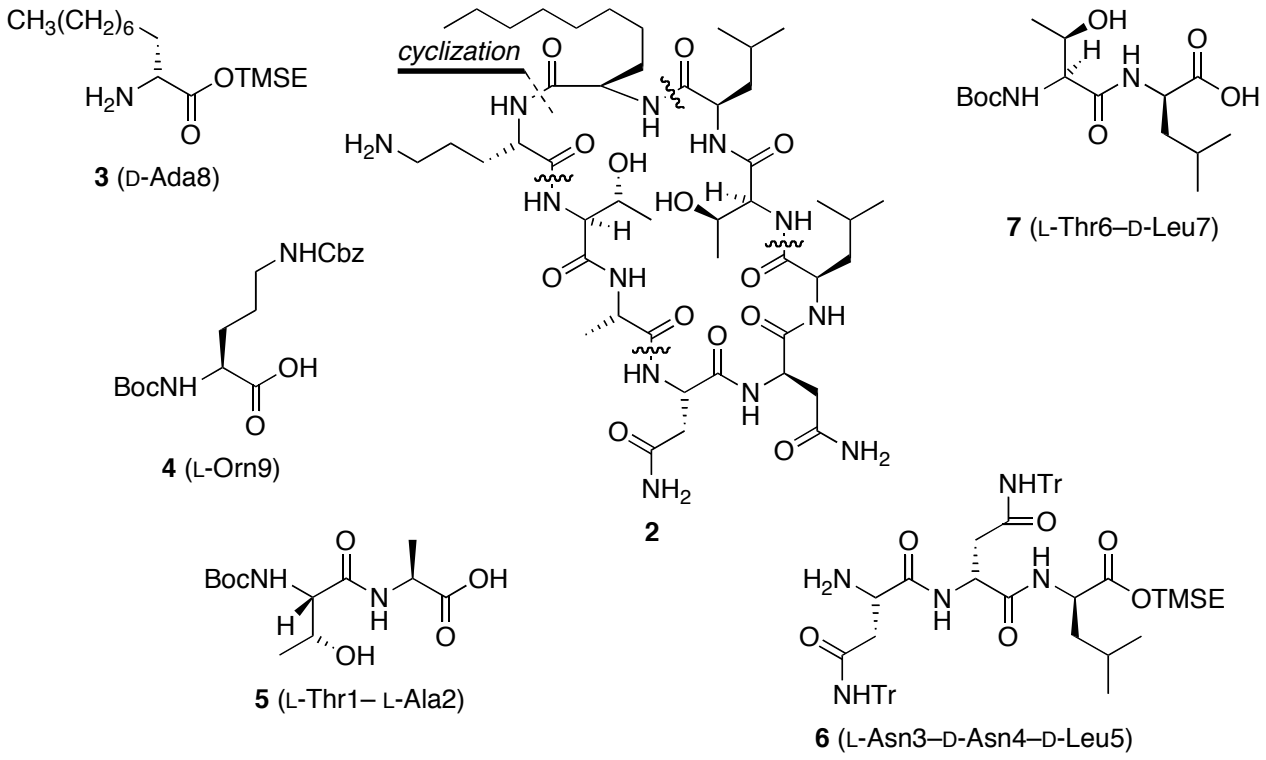
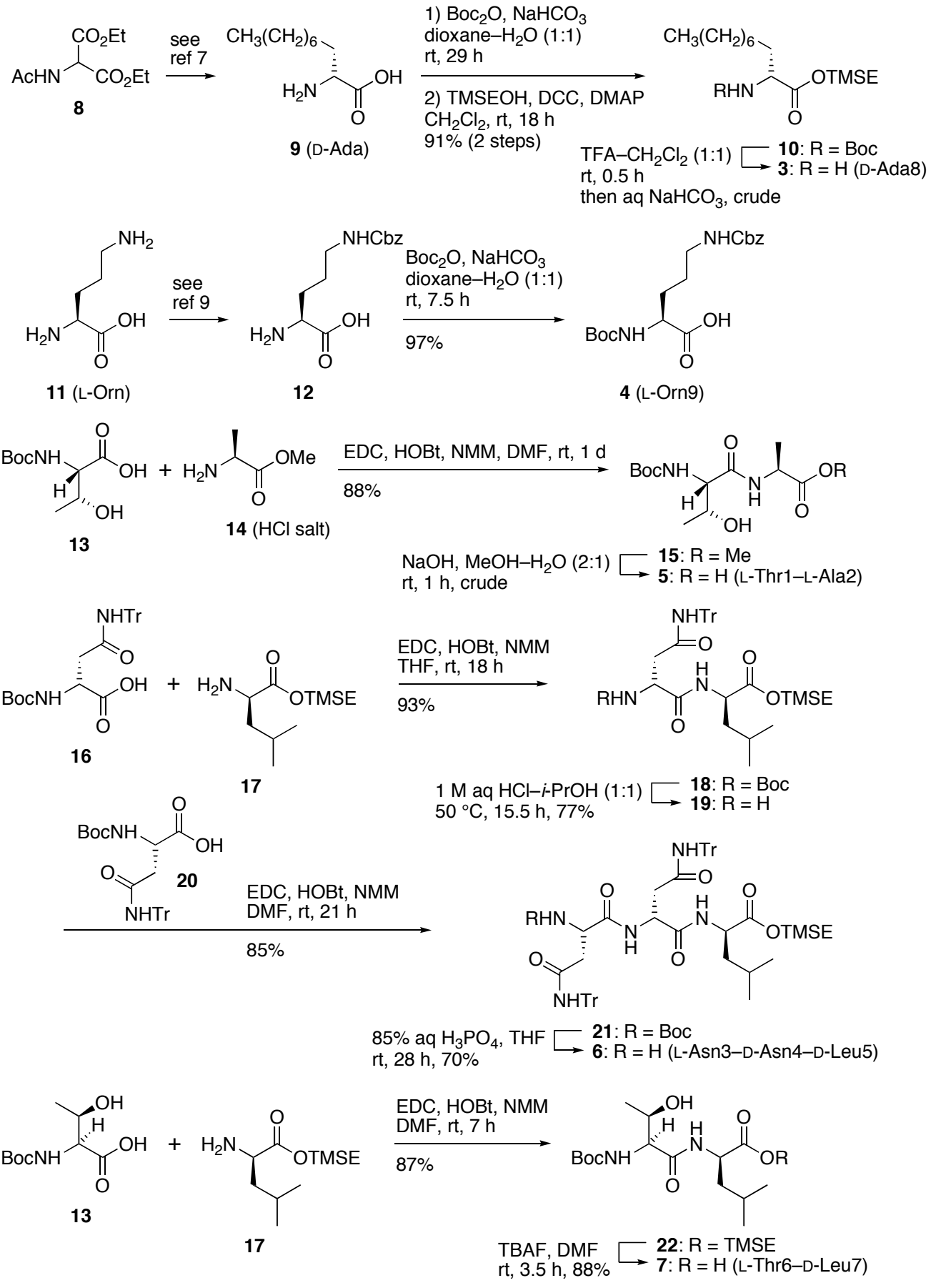


Figure 2. Five segments (**3** – **7**) for the synthesis of **2**.

RESULTS AND DISCUSSION

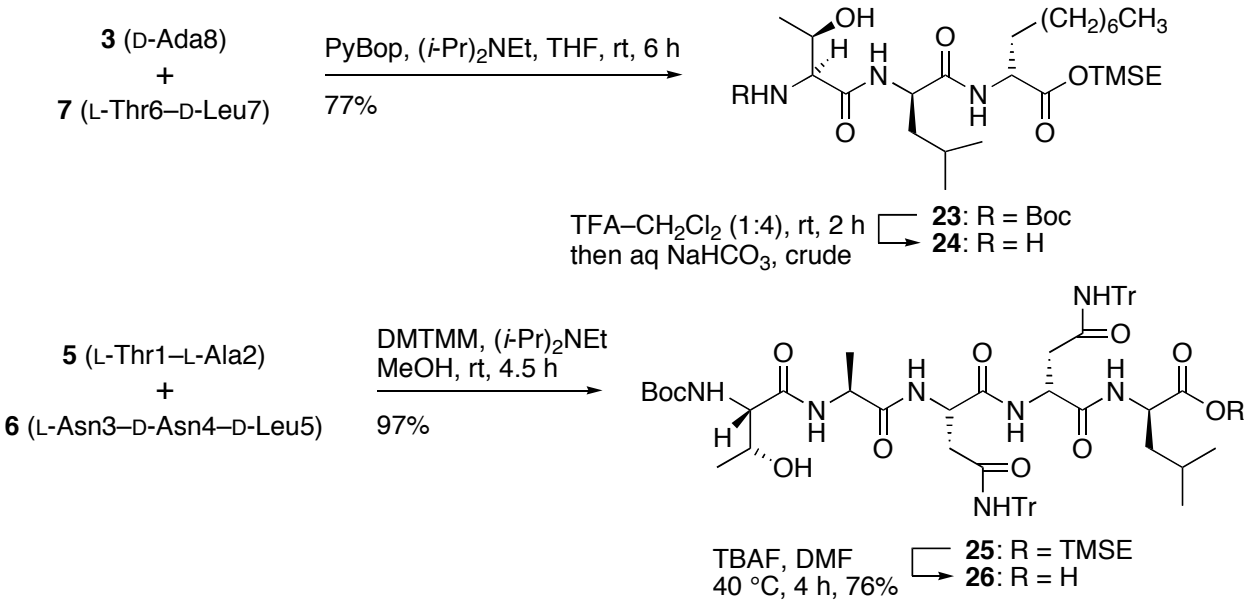
The syntheses of the segments (**3** – **7**) are shown in Scheme 1. D-2-Aminodecanoic acid (D-Ada) (**9**) was prepared from diethyl acetamidomalonate (**8**) by Mori's enzymatic resolution procedures after octylation.[7] Boc protection of **9** followed by esterification of the resulting Boc-D-Ada[8] with trimethylsilylethanol and DCC in CH_2Cl_2 afforded **10** in 91% yield, which was subjected to Boc deprotection with 1:1 TFA–CH_2Cl_2 to provide D-Ada-OTMSE (**3**) (D-Ada8). Boc-L-Orn(Cbz) (**4**) (L-Orn9) was prepared from L-ornithine (**11**) through L-Orn(Cbz) (**12**) by the literature procedure[9] or is commercially available. Dipeptide (**5**)[10] (L-Thr1–L-Ala2) was prepared by the coupling (88% yield) of Boc-L-Thr (**13**) and L-Ala-OMe•HCl (**14**) with EDC–HOBt–NMM (EDC = 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide hydrochloride, HOBt = 1-hydroxybenzotriazole, NMM = *N*-methylmorpholine) in DMF followed by ester hydrolysis of the resulting **15**.[11] The synthesis of tripeptide (**6**) (L-Asn3–D-Asn4–D-Leu5) began with the coupling of Boc-D-Asn(Tr) (**16**) and D-Leu-OTMSE (**17**), the latter of which was derived from D-leucine through the following three-step sequence: (1) Boc protection, (2) TMSE esterification, and (3) Boc deprotection. The coupling of **16** and **17** was realized under the conditions of EDC–HOBt–NMM in THF, giving **18** in 93% yield. After deprotection of the Boc group in **18** with 1:1 aqueous HCl–*i*-PrOH (77% yield),[12] the resulting **19** was coupled with Boc-L-Asn(Tr) (**20**)[12] using EDC–HOBt–NMM in DMF to give **21** in 85% yield. Careful treatment of **21** with aqueous H_3PO_4 in THF[13] afforded the desired tripeptide (**6**) in 70% yield.



Scheme 1. Syntheses of the segments (**3** – **7**).

Dipeptide (**7**) (L-Thr6–D-Leu7) was synthesized by the coupling of Boc-L-Thr (**13**) and D-Leu-OTMSE (**17**) (EDC–HOBt–NMM in DMF, 87% yield) followed by treatment of the resulting **22** with tetrabutylammonium fluoride (TBAF) in DMF (88% yield).

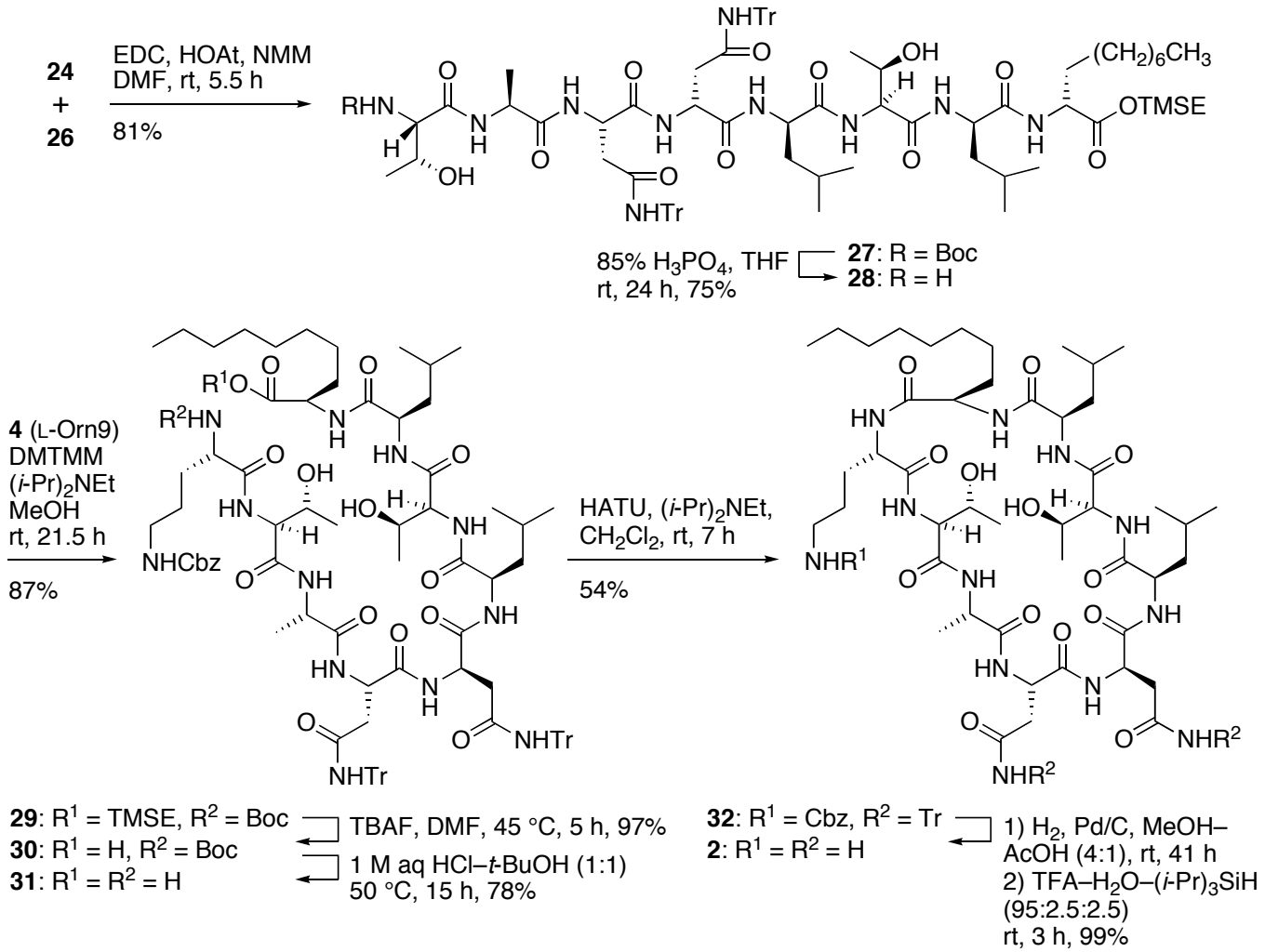
With all the segments in hand, we next focused on their coupling and cyclization. Condensation of **3** (D-Ada8) and **7** (L-Thr6–D-Leu7) was realized using PyBop–$(i\text{-Pr})_2$NEt[14] (PyBop = benzotriazolyloxy-tris(pyrrolidino)-phosphonium hexafluorophosphate) in THF to afford pentapeptide (**23**) in 77% yield, which was deprotected with 1:4 TFA–CH_2Cl_2, giving the crude amine (**24**) (Scheme 2). Condensation of **5** (L-Thr1–L-Ala2) and **6** (L-Asp3–D-Asp4–D-Leu5) was realized with DMTMM–$(i\text{-Pr})_2$NEt[15] (DMTMM = 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride) in MeOH to afford pentapeptide (**25**) in 97% yield, which was deprotected with TBAF in DMF to give carboxylic acid (**26**) in 76% yield.



Scheme 2. Segment couplings.

The coupling of **24** and **26** with EDC–HOAt–NMM (HOAt = 1-hydroxy-7-azabenzotriazole) in DMF provided octapeptide (**27**) in 81% yield, which was deprotected with aqueous H_3PO_4 in THF to give amine (**28**) in 75% yield (Scheme 3). The incorporation of **4** (L-Orn9) into **28** with DMTMM–$(i\text{-Pr})_2$NEt in MeOH afforded the protected cyclization precursor (**29**) in 87% yield. Deprotection of **29** with TBAF provided **30** in 97% yield, which was subjected to aqueous HCl to give the seco amino acid (**31**) in 78% yield. Cyclization of **31** was carried out under a variety of condensation conditions: e.g., EDC–HOAt–NMM (in DMF, rt, 5.5 h, 35% yield), PyBop–$(i\text{-Pr})_2$NEt (in THF, rt, 22 h, 17% yield), and DPPA–$(i\text{-Pr})_2$NEt[16] (DPPA = diphenylphosphoryl azide, in CH_2Cl_2, rt, 15.5 h, 6% yield). Finally, we found that the best conditions were HATU[17] (HATU = *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-

tetramethyluronium hexafluorophosphate, 5 equiv) and $(i\text{-Pr})_2$NEt (5 equiv) in CH_2Cl_2 (0.001 M for **31**) at rt for 7 h, affording the cyclization product (**32**) in 54% yield. Deprotection of the Cbz and Tr groups in **32** was realized by hydrogenolysis (H_2, Pd/C, 4:1 MeOH–AcOH) and the acid treatment (95:2.5:2.5 TFA–H_2O–$(i\text{-Pr})_3$SiH),[5] giving the cyclic peptide portion (**2**) in 99% yield. The ^{1}H NMR spectrum of our synthetic **2** was identical to that of the Searcey's cyclic peptide portion.[5]



Scheme 3. Synthesis of the cyclic peptide portion (**2**)

In summary, the cyclic peptide portion (**2**) of chlorofusin (**1**) was synthesized by condensation of the five segments, (**3** – **7**), followed by cyclization at the amide bond between **3** and **4**. The synthesis of the chromophore portion and its incorporation into the cyclic peptide portion toward the total synthesis of chlorofusin (**1**) are now in progress.

EXPERIMENTAL

General Procedures: The melting points were determined using a micro hot-stage Yanaco MP-S3 and

were uncorrected. The optical rotations were measured by a JASCO DIP-360 polarimeter. The IR spectra were recorded using a JASCO FT IR-200 spectrometer. The ^{1}H and ^{13}C NMR spectra were measured by a JEOL GSX-270 spectrometer, a JEOL LAMBDA 300 spectrometer, a Varian MERCURY plus 300 spectrometer, or a JEOL ALPHA 400 spectrometer. Chemical shifts of the ^{1}H NMR spectra are expressed in ppm relative to the solvent residual signal = 7.26 in $CDCl_3$, 2.50 in $(CD_3)_2SO$, or 3.31 in CD_3OD as the internal standard unless otherwise noted. Chemical shifts of the ^{13}C NMR spectra are expressed in ppm relative to the solvent signal = 77.00 in $CDCl_3$ or 39.52 in $(CD_3)_2SO$ as the internal standard unless otherwise noted. The low and high resolution mass spectra were recorded by a JEOL the Accu TOF JMS-T100LCS (ESI). Silica-gel TLC and preparative TLC (PTLC) were performed using a Merck 60F-254. Silica-gel column chromatography was performed by a Fuji-Davison PSQ100B. The air- and/or moisture-sensitive reactions were carried out under an atmosphere of argon with oven-dried glassware. In general, the organic solvents were purified and dried by appropriate procedures, and evaporation and concentration were carried out under reduced pressure below 30 °C.

Boc-D-ADA-OTMSE (10): To a stirred solution of **9**[7] (491 mg, 2.62 mmol) in 1:1 dioxane–H_2O (52.5 mL) were added at 0 °C $NaHCO_3$ (551 mg, 6.56 mmol) and Boc_2O (1.51 mL, 6.56 mmol). After 29 h at rt, the reaction mixture was extracted with Et_2O (50 mL X 1). The aqueous layer was acidified (pH 3) with 1 M aqueous HCl and the mixture was extracted with EtOAc (50 mL X 3). The combined extracts were dried over Na_2SO_4 and filtered with Celite. The filter cake was washed with ethyl acetate and the combined filtrate and washings were concentrated to afford the crude product[8] (754 mg). This was dissolved in dry CH_2Cl_2 (13.1 mL) and to this were successively added at rt TMSEOH (0.940 mL, 6.56 mmol), DMAP (32.0 mg, 0.262 mmol), and DCC (541 mg, 2.62 mmol). After 18 h at rt, the reaction mixture was filtered with Celite and the filter cake was washed with ethyl acetate. The combined filtrate and washings were concentrated. To the residue were added EtOAc and saturated aqueous $NaHCO_3$. After separation, the aqueous layer was extracted with EtOAc (15 mL X 2) and the combined organic layers were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (51 g) with 19:1 hexane– EtOAc to afford **10** (923 mg, 91%) as a colorless oil: R_f = 0.90 (9:1 $CHCl_3$–MeOH); $[\alpha]_D^{30}$ –6.3 (*c* 0.86, $CHCl_3$); IR (neat) 3370, 2960, 2925, 2860, 1720, 1500, 1458, 1390, 1365, 1352, 1256, 1170, 1046, 938, 860, 840, 760 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.99 (1H, d, *J* = 9.2 Hz), 4.21 (1H, m), 4.19 (2H, m), 1.75 (1H, m), 1.60 (1H, m), 1.43 (9H, s), 1.40–1.13 (12H, m), 0.99 (2H, m), 0.86 (3H, t, *J* = 5.6 Hz), 0.22 (9H, s); ^{13}C NMR ($CDCl_3$) δ 173.14, 155.33, 79.64, 63.51, 53.51, 32.75, 31.79, 29.31, 29.16, 29.13, 28.29, 25.15, 22.62, 17.31, 14.08, –1.55. Anal. Calcd for $C_{20}H_{41}NO_4Si$: C, 61.97; H, 10.66; N, 3.61%. Found: C, 61.79; H, 10.55; N, 3.60%.

D-ADA-OTMSE (3): To a stirred solution of **10** (5.68 g, 14.7 mmol) in CH_2Cl_2 (146 mL) was added at 0 °C TFA (146 mL). After 0.5 h at rt, the mixture was concentrated and to the residue were added EtOAc and saturated aqueous $NaHCO_3$. After separation, the aqueous layer was extracted with EtOAc (20 mL X 2) and the combined organic layers were dried over Na_2SO_4 and filtered with Celite. The filter cake was washed with EtOAc and the combined filtrate and washings were concentrated. The crude residue (**3**) (4.21 g) was directly used for the next step [^{1}H NMR ($CDCl_3$) δ 4.26 (2H, m), 3.90 (1H, br t, *J* = 6.6 Hz), 1.90 (2H, m), 1.52–1.16 (12H, m), 1.02 (2H, m), 0.87 (3H, t, *J* = 7.2 Hz), 0.05 (9H, s)].

Boc-L-Thr–L-Ala (5): To a stirred solution of **13** (505 mg, 2.30 mmol) and **14** (385 mg, 2.76 mmol) in dry DMF (23.0 mL) were successively added at rt HOBt•H_2O (423 mg, 2.76 mmol), NMM (0.633 ml, 5.76 mmol), and EDC (529 mg, 2.76 mmol). After 24 h at rt, saturated aqueous $NaHCO_3$ (20 mL) was added to the reaction mixture and the new mixture was extracted with EtOAc (45 mL X 3). The extracts were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (35 g) with 2:3 hexane– EtOAc to afford **15** (617 mg, 88%) as colorless solids [^{1}H NMR ($CDCl_3$) δ 7.05 (1H, d, *J* = 7.1 Hz), 5.49 (1H, br d, *J* = 7.8 Hz), 4.56 (1H, dq, *J* = 7.2 and 7.2 Hz), 4.34 (1H, dq, *J* = 2.0 and 6.2 Hz), 4.10 (1H, br d, *J* = 7.8 Hz), 3.76 (3H, s), 3.46 (1H, br s), 1.46 (9H, s), 1.41 (3H, d, *J* = 7.2 Hz), 1.20 (3H, d, *J* = 6.2 Hz)]. To a stirred solution of **15** (20.8 mg, 0.0683 mmol) in 2:1 MeOH–H_2O (0.684 mL) was added at 0 °C 1 M aqueous NaOH (0.102 mL). After 1 h at rt, the reaction mixture was acidified (pH 3) with 1 M aqueous HCl at 0 °C and the new mixture was extracted with EtOAc (1 mL X 3). The extracts were dried over Na_2SO_4 and filtered with Celite. The filter cake was washed with ethyl acetate and the combined filtrate and washings were concentrated. The crude residue (**5**)[10] (17.1 mg) was directly used for the next step [^{1}H NMR ($CDCl_3$, TMS = 0.00) δ 7.30 (1H, br d, *J* = 7.2 Hz), 5.65 (1H, br d, *J* = 7.8 Hz), 4.56 (1H, dq, *J* = 7.2 and 7.2 Hz), 4.30 (1H, m), 4.18 (1H, br d, *J* = 7.8 Hz), 4.12–3.40 (2H, br), 1.52–1.38 (12H, br s), 1.18 (3H, d, *J* = 6.3 Hz)].

D-Leu-OTMSE (17): To a stirred solution of D-leucine (1.00 g, 7.62 mmol) and $NaHCO_3$ (1.60 g, 19.0 mmol) in H_2O (38.1 mL) was added at 0 °C Boc_2O (2.10 mL, 9.14 mmol). After 18 h at rt, the reaction mixture was extracted with Et_2O (100 mL X 1). The aqueous layer was acidified with 1 M aqueous HCl (pH 3) and the mixture was extracted with EtOAc (75 mL X 3). The extracts were dried over Na_2SO_4 and filtered with Celite. The filter cake was washed with EtOAc and the combined filtrate and washings were concentrated. A part (1.59 g) of the crude residue [^{1}H NMR ($CDCl_3$, TMS = 0.00) δ 4.88 (1H, br d, *J* = 8.4 Hz), 4.31 (1H, m), 1.86–1.36 (3H, m), 1.45 (9H, s), 0.95 (6H, d, *J* = 6.0 Hz)] was dissolved in dry CH_2Cl_2 (34.4 mL). To this were successively added at rt TMSEOH (1.18 mL, 8.23

mmol), DMAP (83.9 mg, 0.687 mmol), and DCC (1.58 g, 7.66 mmol). After 0.5 h at rt, the reaction mixture was filtered with Celite. The filter cake was washed with EtOAc and the combined filtrate and washings were concentrated. To the residue were added EtOAc and saturated aqueous $NaHCO_3$. After separation, the aqueous layer was extracted with EtOAc (50 mL X 2) and the combined organic layers were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (118 g) with 30:1 hexane– EtOAc to afford Boc-D-Leu-OTMSE (1.93 g, 85%) as a colorless oil [R_f = 0.84 (3:2 hexane–EtOAc); $[\alpha]_D^{30}$ +8.47 (*c* 1.21, $CHCl_3$): IR (neat) 3363, 2958, 1718, 1506, 1470, 1459, 1392, 1365, 1251, 1163, 1122, 1046, 978, 940, 860, 840, 760, 697 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.88 (1H, br d, *J* = 8.0 Hz), 4.27 (1H, m), 4.20 (2H, m), 1.80–1.46 (3H, m), 1.44 (9H, s), 1.00 (2H, m), 0.95 (3H, d, *J* = 6.0 Hz), 0.94 (3H, d, *J* = 6.2 Hz), 0.04 (9H, s); ^{13}C NMR ($CDCl_3$) δ 173.60, 155.37, 79.63, 63.45, 52.09, 41.88, 28.27, 24.71, 22.84, 21.85, 17.27, –1.56]. To a solution of Boc-D-Leu-OTMSE (5.81 g, 17.5 mmol) in CH_2Cl_2 (158 mL) was added at 0 °C TFA (17.5 mL). After 2 h at rt, saturated aqueous $NaHCO_3$ (100 mL) was added to the cooled (0 °C) reaction mixture. The mixture was extracted with EtOAc (100 mL X 3) and the extracts were dried over Na_2SO_4 and filtered with Celite. The filter cake was washed with EtOAc and the combined filtrate and washings were concentrated. The crude residue **17** (4.06 g) was directly used for the next step [^1H NMR ($CDCl_3$) δ 4.27 (2H, m), 3.96 (1H, m), 1.90–1.67 (3H, m), 1.02 (2H, m), 0.95 (6H, br s), 0.44 (9H, s)].

Boc-D-Asn(Tr)-D-Leu-OTMSE (18): To a stirred solution of **16** (5.88 g, 12.4 mmol) and **17** (4.06 g, 17.5 mmol) in dry THF (124 mL) were successively added at 0 °C HOBt•H_2O (2.28 g, 14.9 mmol), NMM (1.63 ml, 14.8 mmol), and EDC (4.28 g, 22.3 mmol). After 18 h at rt, saturated aqueous $NaHCO_3$ (100 mL) was added to the reaction mixture and the new mixture was extracted with EtOAc (250 mL X 3). The extracts were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (256 g) with 3:1 hexane–EtOAc to afford **18** (7.93 g, 93%) as colorless solids: R_f = 0.50 (7:3 hexane–EtOAc); $[\alpha]_D^{26}$ +23.2 (*c* 0.80, $CHCl_3$); mp 63.6–64.2 °C (not recrystallized); IR (KBr) 3330, 3060, 2960, 1698, 1662, 1520, 1498, 1450, 1398, 1394, 1262, 1178, 1042, 938, 860, 840, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36–7.13 (16H, m), 7.06 (1H, br s), 6.28 (1H, d, *J* = 7.0 Hz), 4.47 (2H, m), 4.20 (2H, m), 3.00 (1H, dd, *J* = 16.0 and 3.4 Hz), 2.60 (1H, dd, *J* = 16.0 and 6.4 Hz), 1.70–1.51 (2H, m), 1.46 (1H, m), 1.42 (9H, s), 1.01 (2H, m), 0.89 (6H, br d, *J* = 5.8 Hz), 0.05 (9H, s); ^{13}C NMR ($CDCl_3$) δ 172.48, 171.28, 170.52, 156.04, 144.30, 128.63, 128.47, 127.89, 126.98, 80.05, 70.69, 63.49, 51.06, 40.98, 37.49, 28.22, 24.67, 22.75, 21.74, 17.27, –1.57; HRMS *m/z* calcd for $C_{39}H_{53}N_3NaO_6Si$ ($[M+Na]^+$) 710.3601, found 710.3590.

Boc-L-Asn(Tr)-D-Asn(Tr)-D-Leu-OTMSE (21): A solution of **18** (276 mg, 0.401 mmol) in *i*-PrOH (12.4 mL) and 1 M aqueous HCl (12.4 mL) was heated at 50 °C for 15.5 h. The reaction mixture was neutralized at 0 °C with 1 M aqueous NaOH and the new mixture was extracted with EtOAc (40 mL X 3). The extracts were dried over Na_2SO_4 and filtered with Celite. The filter cake was washed with EtOAc and the combined filtrate and washings were concentrated. The residue was chromatographed on silica gel (12 g) with 3:7 hexane–EtOAc to afford **19** (182 mg, 77%) as colorless solids [^{1}H NMR ($CDCl_3$) δ 7.40–7.04 (17H, m), 4.48 (1H, dt, *J* = 5.2 and 8.0 Hz), 4.20 (2H, m), 3.70 (1H, dd, *J* = 7.6 and 4.2 Hz), 2.75 (1H, dd, *J* = 15.0 and 4.2 Hz), 2.63 (1H, dd, *J* = 15.0 and 7.6 Hz), 1.70–1.40 (3H, m), 1.00 (2H, m), 0.90 (3H, d, *J* = 6.0 Hz), 0.88 (3H, d, *J* = 6.0 Hz), 0.04 (9H, s)]. To a stirred solution of **19** (182 mg, 0.310 mmol) and **20** (146 mg, 0.308 mmol) in dry DMF (3.1 mL) were successively added at 0 °C HOBt•H_2O (56.8 mg, 0.371 mmol), NMM (0.0408 mL, 0.371 mmol), and EDC (71.1 mg, 0.371 mmol). After 21 h at rt, saturated aqueous $NaHCO_3$ (3 mL) was added to the reaction mixture and the new mixture was extracted with EtOAc (5 mL X 3). The extracts were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (16 g) with 1.2:1 hexane–EtOAc to afford **21** (273 mg, 85%) as colorless solids: R_f = 0.60 (3:2 hexane–EtOAc); $[\alpha]_D^{26}$ –3.5 (*c* 0.96, $CHCl_3$); mp 106.4–108.1 °C (not recrystallized); IR (KBr) 3340, 3060, 3030, 2958, 1680, 1493, 1448, 1396, 1368, 1250, 1168, 1040, 940, 900, 860, 840, 756, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.67 (1H, d, *J* = 6.6 Hz), 7.33–7.13 (31H, m), 6.91 (1H, br s), 6.89 (1H, m), 5.78 (1H, d, *J* = 7.8 Hz), 4.73 (1H, m), 4.45–4.28 (2H, m), 4.20 (2H, m), 3.14–3.06 (2H, m), 2.64 (2H, m), 1.64–1.22 (3H, m), 1.40 (9H, s), 1.04 (2H, m), 0.85 (3H, d, *J* = 6.0 Hz), 0.82 (3H, d, *J* = 6.0 Hz), 0.07 (9H, s); ^{13}C NMR ($CDCl_3$) δ 172. 39, 170.72, 170.58, 169.68, 155.63, 144.26, 144.22, 128.65, 128.57, 127.93, 127.89, 127.04, 126.96, 80.29, 70.74, 63.26, 51.49, 49.12, 39.73, 38.24, 28.19, 24.70, 22.60, 21.69, 17.28, –1.55; Anal. Calcd for $C_{62}H_{73}N_5O_8Si$•$2H_2O$: C, 68.93; H, 7.18; N, 6.48%. Found: C, 68.78; H, 6.90; N, 6.49%.

L-Asn(Tr)-D-Asn(Tr)-D-Leu-OTMSE (6): To a stirred solution of **21** (2.42 g, 2.32 mmol) in THF (11.6 mL) was added at rt 85% aqueous H_3PO_4 (10.8 mL, 159 mmol). After 28 h at rt, H_2O (58 mL) and 10 M aqueous NaOH were added to the reaction mixture to adjust pH to 8.0. The mixture was extracted with EtOAc (80 mL X 3) and the extracts were dried over Na_2SO_4 and filtered with Celite. The filter cake was washed with ethyl acetate and the combined filtrate and washings were concentrated. The residue was chromatographed on silica gel (109 g) with 1:4 hexane–EtOAc to afford **6** (1.53 g, 70%) as colorless solids [^{1}H NMR ($CDCl_3$) δ 8.26 (1H, d, *J* = 7.0 Hz), 7.38 (1H, s), 7.38–7.07 (31H, m), 7.03 (1H, s), 4.70 (1H, m), 4.34 (1H, m), 4.19 (2H, m), 3.62 (1H, br t, *J* = 5.6 Hz), 3.00 (1H, dd, *J* = 15.6 and 3.0 Hz), 2.68 (2H, br d, *J* = 5.6 Hz), 2.64 (1H, dd, *J* = 15.6 and 7.0 Hz), 1.60–1.24 (3H, m), 1.02 (2H, m), 0.82 (6H, br s), 0.05 (9H, s)].

Boc-L-Thr-D-Leu-OTMSE (22): To a stirred solution of **13** (126 mg, 0.575 mmol) and **17** (160 mg, 0.691 mmol) in dry DMF (5.75 mL) were successively added at rt HOBt•H_2O (106 mg, 0.692 mmol), NMM (0.158 ml, 1.44 mmol), and EDC (132 mg, 0.689 mmol). After 7 h at rt, saturated aqueous $NaHCO_3$ (6 mL) was added to the reaction mixture and the new mixture was extracted with EtOAc (15 mL X 3). The extracts were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (12 g) with 7:3 hexane–EtOAc to afford **22** (217 mg, 87%) as a colorless oil: R_f = 0.30 (7:3 hexane–EtOAc); $[\alpha]_D^{29}$ –22.5 (*c* 1.13, $CHCl_3$); IR (neat) 3350, 2960, 1835, 1718, 1665, 1500, 1395, 1368, 1253, 1170, 1062, 938, 860, 840, 758 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.88 (1H, d, *J* = 8.0 Hz), 5.52 (1H, d, *J* = 8.0 Hz), 4.51 (1H, m), 4.36 (1H, m), 4.18 (2H, m), 4.06 (1H, d, *J* = 8.0 Hz), 3.53–3.07 (1H, br), 1.72–1.48 (3H, m), 1.44 (9H, s), 1.19 (3H, d, *J* = 7.4 Hz), 0.99 (2H, m), 0.93 (6H, d, *J* = 6.0 Hz), 0.03 (9H, s); ^{13}C NMR ($CDCl_3$) δ 172.98, 171.57, 156.28, 80.23, 66.93, 63.81, 58.64, 51.02, 40.76, 28.19, 24.81, 22.83, 21.60, 18.35, 17.24, –1.61; HRMS *m*/*z* calcd for $C_{20}H_{40}N_2NaO_6Si$ ([M+Na]$^+$) 455.2553, found 455.2547; Anal. Calcd for $C_{20}H_{40}N_2O_6Si$•$0.5H_2O$: C, 54.39; H, 9.36; N, 6.34%. Found: C, 54.36; H, 9.66; N, 6.31%.

Boc-L-Thr-D-Leu-D-Ada-OTMSE (23): To a stirred solution of **22** (69.1 mg, 0.160 mmol) in dry DMF (1.6 mL) was added at 0 °C 1.0 M TBAF in THF (0.240 mL, 0.240 mmol). After 3.5 h at rt, H_2O (1.5 mL) and 1 M aqueous HCl was added to the cooled (0 °C) reaction mixture to adjust pH to 3.0. The mixture was extracted with EtOAc (3 mL X 3) and the extracts were dried over Na_2SO_4 and filtered with Celite. The filter cake was washed with EtOAc and the combined filtrate and washings were concentrated. The residue was chromatographed on silica gel (2.7 g) with 7:3 hexane–EtOAc to afford **7** (46.8 mg, 88%) as colorless solids [^1H NMR ($CDCl_3$) δ 7.00 (1H, d, *J* = 8.6 Hz), 5.61 (1H, d, *J* = 7.8 Hz), 4.55 (1H, m), 4.47 (1H, m), 4.09 (1H, d, *J* = 7.8 Hz), 1.79–1.55 (3H, m), 1.46 (9H, s), 1.21 (3H, d, *J* = 6.2 Hz), 0.96 (3H, d, *J* = 5.8 Hz), 0.94 (3H, d, *J* = 5.6 Hz)]. To a stirred solution of **7** (175 mg, 0.526 mmol) and **3** (151 mg, 0.525 mmol) in dry THF (5.25 mL) were added at 0 °C (*i*-Pr)$_2$NEt (0.229 mL, 1.31 mmol) and PyBOP (328 mg, 0.630 mmol). After 6 h at rt, H_2O (5 mL) was added to the reaction mixture and the new mixture was extracted with EtOAc (5 mL X 3). The extracts were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (16 g) with 3:2 hexane–EtOAc to afford **23** (242 mg, 77%) as a colorless viscous syrup: R_f = 0.30 (7:3 hexane–EtOAc); $[\alpha]_D^{28}$ –11.5 (*c* 0.72, $CHCl_3$); IR (KBr) 3300, 2960, 2928, 2860, 1720, 1698, 1648, 1550, 1470, 1459, 1395, 1368, 1254, 1165, 1062, 938, 860, 840, 762, 698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.98 (1H, d, *J* = 7.0 Hz), 6.79 (1H, d, *J* = 7.6 Hz), 5.53 (1H, d, *J* = 7.5 Hz), 4.53–4.38 (2H, m), 4.34 (1H, m), 4.17 (2H, m), 4.06 (1H, br d, *J* = 7.5 Hz), 4.00–3.70 (1H, br), 1.83-1.50 (5H, m), 1.42 (9H, s), 1.35–1.10 (15H, m),

0.97 (2H, m), 0.92 (3H, br d, *J* = 5.8 Hz), 0.89 (3H, br d, *J* = 5.4 Hz), 0.85 (3H, br t, *J* = 6.5 Hz), 0.04 (9H, s); ^{13}C NMR ($CDCl_3$) δ 172.73, 171.82, 156.01, 80.28, 67.14, 63.83, 59.45, 52.30, 52.17, 40.62, 32.28, 31.73, 29.23, 29.13, 29.10, 28.16, 25.11, 24.73, 22.94, 22.57, 21.67, 19.01, 17.24, 14.03, –1.62; HRMS *m*/*z* calcd for $C_{30}H_{59}N_3NaO_7Si$ ([M+Na]$^+$) 624.4020, found 624.4020; Anal. Calcd for $C_{30}H_{59}N_3O_7Si•0.5H_2O$: C, 58.98; H, 9.90; N, 6.88%. Found: C, 59.01; H, 9.90; N, 6.52%.

L-Thr-D-Leu-D-Ada-OTMSE (24): To a solution of **23** (1.33 g, 2.21 mmol) in CH_2Cl_2 (35.4 mL) was added at 0 °C TFA (8.85 mL). After 2 h at rt, the reaction mixture was evaporated. To the residue was added saturated aqueous $NaHCO_3$ and the mixture was extracted with EtOAc (20 mL X 3). The extracts were dried over Na_2SO_4 and filtered with Celite. The filter cake was washed with EtOAc and the combined filtrate and washings were concentrated. The crude residue **24** (1.11 g) was directly used for the next step (^{1}H NMR (CD_3OD) δ 4.44 (1H, t, *J* = 8.0 Hz), 4.31 (1H, dd, *J* = 9.4 and 5.6 Hz), 4.22 (2H, m), 4.00 (1H, dq, *J* = 6.8 and 6.8 Hz), 3.64 (1H, br d, *J* = 7.2 Hz), 1.89–1.56 (3H, m), 1.63 (2H, br d, *J* = 8.0 Hz), 1.48–1.25 (12H, m), 1.27 (3H, d, *J* = 6.8 Hz), 1.06–0.86 (11H, m), 0.06 (9H, s)).

Boc-L-Thr-L-Ala-L-Asn(Tr)-D-Asn(Tr)-D-Leu-OTMSE (25): To a stirred solution of **5** (340 mg, 1.17 mmol) and **6** (1.11 g, 1.18 mmol) in MeOH (11.7 mL) were added at 0 °C (*i*-Pr)$_2$NEt (0.225 mL, 1.29 mmol) and DMTMM (357 mg, 1.29 mmol). After 4.5 h at rt, H_2O (15 mL) was added to the reaction mixture and the new mixture was extracted with EtOAc (15 mL X 3). The extracts were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (71 g) with 7:3 $CHCl_3$–acetone to afford **25** (1.38 g, 97%) as colorless solids: R_f = 0.25 (4:1 $CHCl_3$–acetone); $[\alpha]_D^{30}$ –10.5 (*c* 1.01, $CHCl_3$); mp 129.1–130.2 °C (not recrystallized); IR (KBr) 3320, 3060, 2958, 1670, 1494, 1450, 1392, 1366, 1250, 1173, 1062, 1039, 1002, 938, 901, 860, 840, 758, 700, 639, 624 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.64–7.51 (2H, m), 7.40–7.12 (33H, m), 7.01 (1H, br), 5.60 (1H, d, *J* = 8.4 Hz), 4.72 (2H, m), 4.40 (1H, m), 4.22 (2H, m), 4.16–4.04 (2H, m), 3.96 (1H, m), 3.01 (1H, m), 2.74 (3H, m), 1.69–1.35 (3H, m), 1.47 (9H, s), 1.18 (3H, d, *J* = 7.6 Hz), 1.08–0.98 (5H, m), 0.87 (3H, br d, *J* = 5.6 Hz), 0.84 (3H, br d, *J* = 5.8 Hz), 0.07 (9H, s); ^{13}C NMR ($CDCl_3$) δ 172.29, 172.17, 171.56, 170.54, 170.00, 156.22, 144.19, 144.16, 128.72, 128.66, 127.89, 127.86, 126.96, 79.82, 70.76, 67.85, 63.46, 58.56, 51.45, 50.19, 40.19, 38.67, 28.30, 24.68, 22.64, 21.75, 18.78, 17.29, 16.93, –1.54; HRMS *m*/*z* calcd for $C_{69}H_{85}N_7NaO_{11}Si$ ([M+Na]$^+$) 1238.5974, found 1238.5948; Anal. Calcd for $C_{69}H_{85}N_7O_{11}Si•2H_2O$: C, 66.16; H, 7.16; N, 7.83%. Found: C, 66.01; H, 7.11; N, 7.72%.

Boc-L-Thr-L-Ala-L-Asn(Tr)-D-Asn(Tr)-D-Leu-L-Thr-D-Leu-D-Ada-OTMSE (27): To a stirred solution of **25** (1.52 g, 1.25 mmol) in dry DMF (12.5 mL) was added at 0 °C 1.0 M TBAF in THF (3.12

mL, 3.12 mmol). After 4 h at 40 °C, H_2O (15 mL) and 1 M aqueous HCl was added to the cooled (0 °C) reaction mixture to adjust pH to 3.0. The mixture was extracted with EtOAc (25 mL X 3) and the extracts were dried over Na_2SO_4 and filtered with Celite. The filter cake was washed with EtOAc and the combined filtrate and washings were concentrated. The residue was chromatographed on silica gel (70 g) with 1:9 hexane–EtOAc to afford **26** (1.05 g, 76%) as colorless solids. To a stirred solution of **26** (65.8 mg, 0.0589 mmol) and **24** (36.3 mg, 0.0723 mmol) in dry DMF (0.589 mL) were successively added at rt HOAt (13.4 mg, 0.0984 mmol), NMM (0.0162 mL, 0.147 mmol), and EDC (13.6 mg, 0.0709 mmol). After 5.5 h at rt, saturated aqueous $NaHCO_3$ (1 mL) was added to the reaction mixture and the new mixture was extracted with EtOAc (1.5 mL X 3). The extracts were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (4.7 g) with 1:1 $CHCl_3$–EtOAc to afford **27** (76.2 mg, 81%) as colorless solids: R_f = 0.40 (2:3 $CHCl_3$–EtOAc); $[\alpha]_D^{28}$ –5.2 (*c* 0.89, $CHCl_3$); mp 119.1–121.2 °C (not recrystallized); IR (KBr) 3310, 3060, 2958, 2930, 2875, 2860, 1660, 1520, 1448, 1366, 1252, 1170, 1092, 1063, 1039, 1002, 938, 860, 840, 764, 756, 700, 639, 627 cm^{-1}; ^1H NMR ($(CD_3)_2SO$, 80 °C) δ 8.34 (1H, s), 8.28 (1H, s), 8.02–7.88 (3H, m), 7.77 (1H, d, *J* = 7.0 Hz), 7.63 (1H, d, *J* = 7.6 Hz), 7.56 (1H, d, *J* = 8.0 hz), 7.46 (1H, d, *J* = 7.8 Hz), 7.31–7.08 (30H, m), 6.15 (1H, br), 4.69–4.46 (4H, m), 4.44–4.33 (1H, m), 4.33–4.20 (3H, m), 4.20–4.08 (4H, m), 4.08–3.88 (2H, m), 2.78–2.54 (4H, m), 1.78–1.44 (6H, m), 1.41 (9H, s), 1.36–1.16 (17H, m), 1.07 (3H, d, *J* = 6.0 Hz), 1.01 (3H, d, *J* = 6.4 Hz), 0.94 (2H, m), 0.89–0.78 (15H, m), 0.04 (9H, s); ^{13}C NMR ($(CD_3)_2SO$, 80 °C) δ 171.85, 171.64, 171.59, 171.38, 170.69, 170.42, 169.85, 169.26, 168.66, 168.36, 155.05, 144.44, 128.22, 127.02, 127.00, 125.97, 125.92, 78.12, 69.38, 66.56, 66.19, 61.94, 59.46, 58.33, 51.90, 50.62, 50.19, 50.02, 48.13, 40.73, 38.21, 37.87, 30.79, 30.49, 28.28, 28.15, 28.11, 27.81, 24.80, 23.85, 23.66, 22.62, 22.46, 21.57, 21.33, 21.29, 19.24, 19.03, 17.90, 16.63, 13.36, –1.92; HRMS *m*/*z* calcd for $C_{89}H_{122}N_{10}NaO_{15}Si$ ($[M+Na]^+$) 1621.8758, found 1621.8749.

Boc-L-Orn(Cbz)-L-Thr-L-Ala-L-Asn(Tr)-D-Asn(Tr)-D-Leu-L-Thr-D-Leu-D-Ada-OTMSE (29): To a stirred solution of **27** (1.67 g, 1.04 mmol) in THF (1.67 mL) was added at rt 85% aqueous H_3PO_4 (1.62 mL, 23.7 mmol). After 24 h at rt, H_2O (8 mL) and 10 M aqueous NaOH were added to the cooled (0 °C) reaction mixture. The new mixture was extracted with EtOAc (15 mL X 3) and the extracts were dried over Na_2SO_4 and filtered with Celite. The filter cake was washed with EtOAc and the combined filtrate and washings were concentrated. The residue was chromatographed on silica gel (78 g) with 95:5 $CHCl_3$–MeOH to afford **28** (1.17 g, 75%) as white solids. To a stirred solution of **28** (43.4 mg, 0.0289 mmol) and **4** (10.6 mg, 0.0289 mmol) in MeOH (0.289 mL) were successively added at 0 °C (i-Pr)$_2$NEt (0.0055 mL, 0.032 mmol) and DMTMM (8.8 mg, 0.032 mmol). After 21.5 h at rt, H_2O (0.5 mL) was added to the reaction mixture and the new mixture was extracted with EtOAc (1 mL X 3). The extracts

were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (2.7 g) with 7:3 $CHCl_3$–acetone to afford **29** (46.7 mg, 87%) as colorless solids; R_f=0.45 (7:3 $CHCl_3$–acetone); $[\alpha]_D^{28}$ –9.4 (*c* 0.97, $CHCl_3$); mp 125.1–126.2 °C (not recrystallized); IR (KBr) 3320, 3060, 3038, 2958, 2930, 2874, 1660, 1520, 1450, 1367, 1256, 1174, 1015, 1002, 938, 860, 840, 768, 756, 700, 640, 625 cm^{-1}; ^1H NMR ($(CD_3)_2SO$, 80 °C) δ 8.34 (1H, s), 8.29 (1H, s), 8.00–7.88 (3H, m), 7.74 (1H, d, *J* = 7.4 Hz), 7.62 (1H, d, *J* = 7.6 Hz), 7.57 (1H, d, *J* = 8.0 Hz), 7.48 (1H, d, *J* = 8.4 Hz), 7.45 (1H, d, *J*=8.2 Hz), 7.39–7.28 (5H, m), 7.28–7.08 (30H, m), 6.90 (1H, br), 6.76 (1H, br), 5.02 (2H, s), 4.63–4.48 (3H, m), 4.40 (1H, m), 4.35–4.20 (2H, m), 4.20–4.08 (4H, m), 4.06 (1H, m), 4.02–3.88 (2H, m), 3.33–2.94 (2H, br), 3.01 (2H, dt, *J* = 6.0, 6.0 Hz), 2.77–2.59 (4H, m), 1.78–1.44 (10H, m), 1.40 (9H, s), 1.34–1.18 (17H, m), 1.04 (3H, d, *J* = 6.0 Hz), 1.01 (3H, d, *J* = 6.0 Hz), 0.94 (2H, t, *J* = 8.2 Hz), 0.91–0.79 (15H, m), 0.08 (9H, s); ^{13}C NMR ($(CD_3)_2SO$, 80 °C) δ 171.91, 171.79, 171.62, 171.57, 171.38, 170.65, 170.33, 169.24, 169.19, 168.70, 168.33, 155.68, 155.05, 144.44, 144.42, 137.01, 128.22, 127.86, 127.20, 127.14, 127.02, 126.99, 125.95, 125.91, 78.76, 78.06, 69.37, 66.18, 64.82, 61.93, 58.25, 57.37, 54.30, 51.87, 50.60, 50.16, 48.05, 40.77, 30.78, 30.48, 28.67, 28.26, 28.13, 28.09, 27.83, 25.63, 24.78, 23.85, 23.64, 22.60, 22.43, 21.55, 21.34, 21.30, 19.01, 18.89, 17.82, 16.61, 13.35, –1.93; HRMS *m/z* calcd for $C_{102}H_{138}N_{12}NaO_{18}Si$ ($[M+Na]^+$) 1869.9919, found 1869.9942.

L-Orn(Cbz)-L-Thr-L-Ala-L-Asn(Tr)-D-Asn(Tr)-D-Leu-L-Thr-D-Leu-D-Ada (31): To a stirred solution of **29** (1.44 g, 0.779 mmol) in DMF (7.8 mL) was added at 0 °C 1.0 M TBAF in THF (2.34 mL, 2.34 mmol). After 5 h at 45 °C, H_2O (2.5 mL) and 1 M aqueous HCl were added to the cooled (0 °C) reaction mixture to adjust pH to 3.0. The new mixture was extracted with EtOAc (15 mL X 3) and the combined extracts were dried over Na_2SO_4 and filtered with Celite. The filter cake was washed with EtOAc and the combined filtrate and washings were concentrated. The residue was chromatographed on silica gel (68 g) with 95:5 $CHCl_3$–MeOH to afford **30** (1.32 g, 97%) as colorless solids. To a stirred solution of **30** (1.32 g, 0.755 mmol) in *t*-BuOH (54 mL) was added at 0 °C 1 M aqueous HCl (54 mL). After 15 h at 50 °C, 1 M aqueous NaOH was added to the reaction mixture to adjust pH to 8.0. The new mixture was extracted with EtOAc (120 mL X 3) and the combined extracts were dried over Na_2SO_4 and filtered with Celite. The filter cake was washed with EtOAc and the combined filtrate and washings were concentrated. The residue was chromatographed on silica gel (64 g) with 95:5 $CHCl_3$–MeOH to afford **31** (968 mg, 78%) as colorless solids: R_f=0.70 (4:1 $CHCl_3$–MeOH); $[\alpha]_D^{29}$ +14.5 (*c* 1.20, $CHCl_3$); mp 146.8–147.1 °C (not recrystallized); IR (KBr) 3310, 3060, 3038, 2958, 2925, 2859, 1660, 1523, 1450, 1410, 1260, 1139, 1024, 1000, 753, 700, 639, 622 cm^{-1}; ^1H NMR ($(CD_3)_2SO$, 80 °C) δ 8.56 (1H, d, *J* = 7.6 Hz), 8.38 (1H, s), 8.27 (1H, s), 8.01 (1H, d, *J* = 6.8 Hz), 7.96–7.82 (2H, m), 7.72 (1H, d, *J* = 8.0 Hz), 7.50–7.28 (7H, m), 7.28–7.04 (31H, m), 6.92 (1H, br), 5.03 (2H, s), 4.67 (1H, m), 4.47 (1H, m), 4.34 (2H,

m), 4.24–4.08 (4H, m), 4.08–3.94 (3H, m), 3.70–3.11 (2H, br), 3.00 (2H, m), 2.88–2.58 (4H, m, 4H), 1.78–1.38 (12H, m), 1.38–1.16 (15H, m), 1.03 (3H, d, J = 6.0 Hz), 1.00 (3H, d, J = 6.2 Hz), 0.92–0.77 (15H, m); ^{13}C NMR ($(CD_3)_2SO$, 80 °C) δ 174.03, 171.86, 171.70, 171.38, 170.65, 170.58, 169.85, 169.64, 168.62, 168.46, 155.69, 144.53, 144.43, 137.03, 128.23, 128.20, 127.85, 127.19, 127.12, 126.97, 126.94, 125.84, 69.37, 69.28, 66.26, 65.50, 64.79, 58.37, 57.08, 54.08, 53.06, 51.38, 50.70, 50.00, 48.79, 38.20, 31.74, 31.53, 30.80, 28.50, 28.42, 28.16, 25.41, 24.83, 23.86, 22.54, 22.45, 21.55, 21.29, 21.10, 19.36, 19.13, 17.57, 13.35; HRMS m/z calcd for $C_{92}H_{117}N_{12}Na_2O_{16}$ ($[M-H+Na_2]^+$) 1691.8506, found 1691.8494.

cyclo(-L-Orn(Cbz)-L-Thr-L-Ala-L-Asn(Tr)-D-Asn(Tr)-D-Leu-L-Thr-D-Leu-D-Ada-) (32): To a stirred solution of **31** (5.1 mg, 0.0031 mmol) in dry CH_2Cl_2 (3.10 mL) were added at 0 °C (i-Pr)$_2$NEt (0.0027 mL, 0.0155 mmol) and HATU (5.9 mg, 0.0155 mmol). After 7 h at rt, H_2O (3 mL) was added to the reaction mixture and the new mixture was extracted with $CHCl_3$ (3 mL X 3). The extracts were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was purified on preparative TLC (silica gel) with 95:5 $CHCl_3$–MeOH to afford **32** (2.7 mg, 54%) as colorless solids: R_f=0.40 (1:4 hexane–EtOAc): $[\alpha]_D^{27}$ +23.2 (c 0.89, $CHCl_3$); mp 175.2–177.0 °C (not recrystallized); IR (KBr) 3320, 3060, 3028, 2959, 2923, 2860, 1658, 1536, 1448, 1260, 1100, 1040, 764, 748, 700 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$, 80 °C) δ 9.00 (1H, br d, J = 6.0 Hz), 8.83 (1H, br d, J = 3.7 Hz), 8.43 (1H, br s), 8.32 (1H, s), 8.03 (1H, br d, J = 4.8 Hz), 7.84 (1H, s), 7.74 (1H, br d, J = 8.1 Hz), 7.38–7.09 (36H, m), 7.02 (1H, br s), 6.85 (1H, br d, J = 9.3 Hz), 6.59 (1H, br s), 6.48 (1H, br d, J = 9.0 Hz), 5.16 (1H, d, J = 4.5 Hz), 4.98 (2H, s), 4.96–4.72 (3H, m), 4.54 (1H, m), 4.38–4.20 (2H, m), 4.20–4.04 (2H, m), 4.00–3.80 (3H, m), 3.75 (1H, m), 3.16 (1H, m), 2.96–2.74 (2H, m), 2.57–2.43 (1H, m), 1.99–1.80 (3H, m), 1.80–1.52 (5H, m), 1.48–1.21 (17H, m), 1.15–1.05 (2H, m), 1.05 (3H, d, J = 5.1 Hz), 0.96–0.84 (9H, m), 0.84–0.76 (6H, m), 0.54 (3H, d, J = 6.3 Hz); ^{13}C NMR ($(CD_3)_2SO$, 80 °C) δ 174.72, 173.33, 173.02, 172.87, 172.48, 171.80, 171.51, 171.03, 170.66, 168.07, 167.10, 155.46, 144.38, 144.33, 136.90, 128.29, 128.20, 127.82, 127.65, 127.08, 127.00, 125.97, 125.81, 69.31, 69.11, 64.83, 64.60, 63.72, 62.26, 53.26, 52.41, 52.30, 50.90, 50.43, 48.48, 48.36, 37.44, 30.80, 29.79, 29.23, 28.30, 28.14, 28.02, 25.65, 24.95, 23.77, 23.60, 22.92, 22.59, 21.53, 20.30, 20.06, 19.70, 15.93, 13.34; HRMS m/z calcd for $C_{92}H_{116}N_{12}NaO_{15}$ ($[M+Na]^+$) 1651.8581, found 1651.8565

cyclo(-L-Orn-L-Thr-L-Ala-L-Asn-D-Asn-D-Leu-L-Thr-D-Leu-D-Ada-) (2): To a solution of **32** (18.2 mg, 0.0112 mol) in dry MeOH (0.16 mL) and AcOH (0.04 mL), 10% Pd on carbon (9.1 mg) was added under an argon atmosphere. After the atmosphere was replaced with H_2, the suspension was vigorously stirred at rt for 41 h. The resulting mixture was filtered through glass filter and the filtrate was evaporated to afford the crude product (18.3 mg). A portion (4.9 mg) was dissolved in 95:2.5:2.5

TFA–H_2O–(i-Pr)$_3$SiH (0.131 mL). After 3 h at rt, the mixture was evaporated and the residue was washed with Et_2O (2 mL X 5). The residue was dissolved in 1:1 AcOH–H_2O (0.20 mL) and the solution was lyophilized to afford **2** (3.0 mg, 99%) as colorless solids: R_f=0.65 (3:11:13 H_2O–MeOH–$CHCl_3$): $[\alpha]_D^{30}$ +3.1 (*c* 0.22, MeOH); mp 170.6–171.9 °C (not recrystallized); IR (KBr) 3325, 3073, 2960, 2932, 2862, 1664, 1540, 1458, 1437, 1318, 1207, 1183, 1138, 840, 802, 724 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$, rt) δ 9.05 (1H, br s), 8.70 (1H, br s), 8.57 (1H, s), 7.79 (1H, br s), 7.69 (1H, d, *J* = 8.1 Hz), 7.61 (2H, br s), 7.50 (1H, d, *J* = 9.2 Hz), 7.24 (1H, s), 7.13(1H, s), 7.09 (1H, s), 6.99 (1H, s), 6.95 (2H, br s), 6.92 (1H, s), 6.68 (1H, br s), 5.29 (1H, d, *J* = 4.4 Hz), 4.98 (1H, d, *J* = 3.3 Hz), 4.76 (1H, m), 4.56 (1H, m), 4.45 (1H, m), 4.35 (1H, m), 4.05–3.85 (6H, m), 3.63 (1H, m), 3.00 (2H, br), 2.90 (1H, br d, *J* = 15.0 Hz), 2.78 (2H, m), 2.70 (1H, dd, *J* = 15.0 and 9.0 Hz), 1.85–1.68 (5H, m), 1.60–1.40 (4H, m), 1.35 (2H, m), 1.30–1.18 (13H, m), 1.13 (3H, d, *J* = 6.4 Hz), 1.06 (3H, br d, *J* = 5.0 Hz), 0.89 (3H, d, *J* = 6.4 Hz), 0.85–0.82 (6H, m), 0.80 (3H, d, *J* = 6.4 Hz), 0.75 (3H, d, *J* = 5.8 Hz), 0.74 (3H, d, *J* = 5.8 Hz); HRMS *m/z* calcd for $C_{46}H_{82}N_{12}NaO_{13}$ ([M+Na]$^+$) 1033.6022, found 1033.6049.

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