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Synthesis of the chlorofusin cyclic peptide

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ABSTRACT

An efficient and convergent solution-phase synthesis of the cyclic peptide of the natural product chlorofusin, a reported inhibitor of the MDM2–p53 interaction, is detailed.

1. Introduction

Chlorofusin (**1**, Fig. 1) was isolated from the fungal strain *Microdochium caespitosum* and found to disrupt the MDM2–p53 interaction by directly binding to the N-terminal domain of MDM2 (IC_{50}=4.6 μM, K_D=4.7 μM).[1] Thus, chlorofusin represents an exciting lead for antineoplastic intervention that acts by a rare disruption of a protein–protein interaction, although the structural details of the inhibitory MDM2 binding have yet to be established.[2] On the basis of extensive spectroscopic and degradation studies, the chlorofusin structure was proposed to be composed of a densely functionalized, azaphilone-derived chromophore linked through the terminal amine of ornithine to a 27-membered cyclic peptide composed of nine amino acid residues.[1] Two of the cyclic peptide amino acids possess a non-standard or modified side chain and four possess the D-configuration. Although the spectroscopic and degradation studies of chlorofusin permitted the identification of the cyclic peptide structure and connectivity, the stereochemistry of two asparagine residues Asn3 and Asn4 was only established to have opposite stereochemistries (L and D) and their respective assignments were not possible. In 2003, we reported the synthesis of the two cyclic peptide diastereomers bearing either the L-Asn3/D-Asn4 or D-Asn3/L-Asn4 stereochemistry and were able to correlate the former with the spectroscopic properties (^{1}H and ^{13}C NMR) of the natural product.[3] Concurrent with this disclosure, Searcey reported the synthesis of the L-Asn3/D-Asn4 diastereomer incorporating either a D-Ada8 or L-Ada8 residue,[4] and recently Nakata[5] has also reported a synthesis of this cyclic peptide.

Similarly, spectroscopic studies conducted by Williams provided the structure and a relative assignment of the stereochemistry for the unusual azaphilone-derived chromophore, but did not permit an assignment of its absolute stereochemistry.[1] Based on gradient 1D NOE studies, the chromophore's relative stereochemistry was suggested to be 4*R**,8*R**,9*R** in which all three oxygen substituents on the chromophore reside on the same face. Recently, we reported the total synthesis of chlorofusin, which displayed spectroscopic properties indistinguishable from those reported for the natural product,[6a] and its distinguishable seven alternative chromophore diastereomers[6b] that required the reassignment of the chromophore relative stereochemistry and provided an unambiguous assignment of its absolute stereochemistry (4*R*,8*S*,9*R*). This assignment, which differed from that reported near simultaneously by Yao,[7] also confirmed the accuracy of our earlier L-Asn3/D-Asn4 cyclic peptide stereochemical assignment.

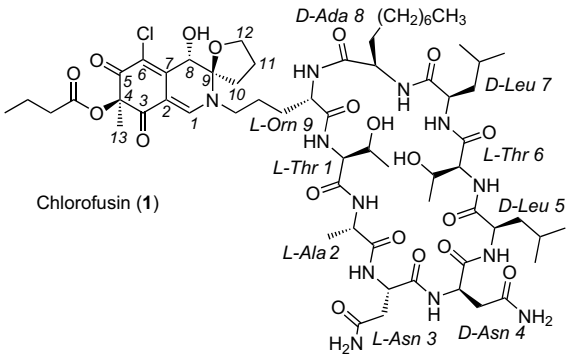

Figure 1. Structure of chlorofusin.

Abbreviations: Ada, 2-aminodecanoic acid; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOAt, 1-hydroxy-7-azabenzotriazole; MDM2, murine double minute 2 protein; p53, protein 53; SES, 2-(trimethylsilyl)ethylsulfonyl; Trt, trityl (triphenylmethyl).

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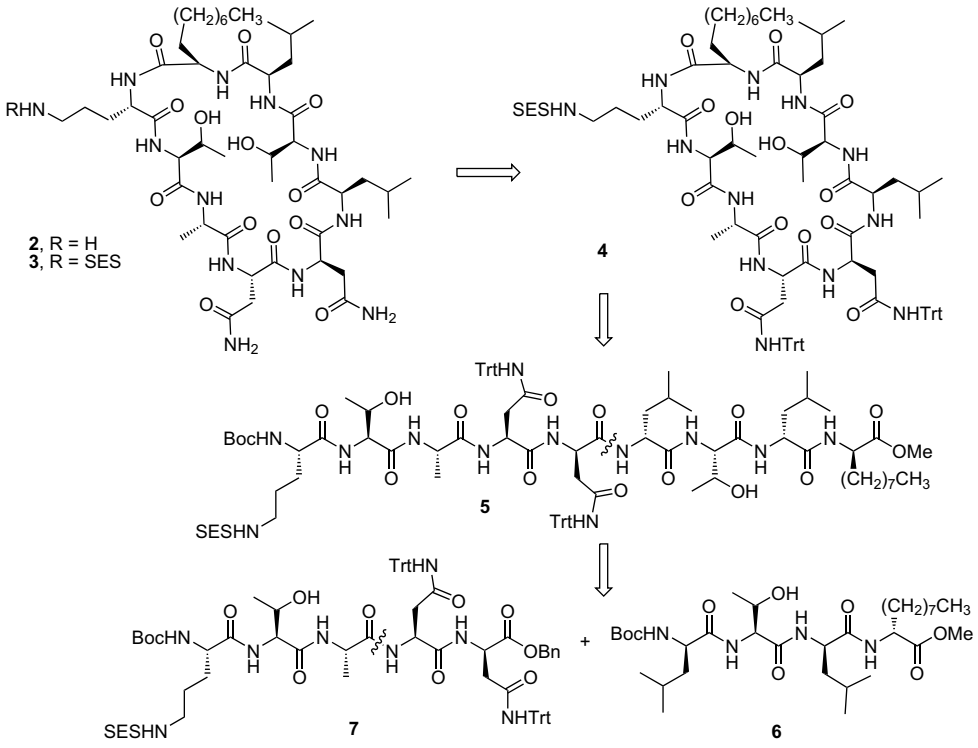
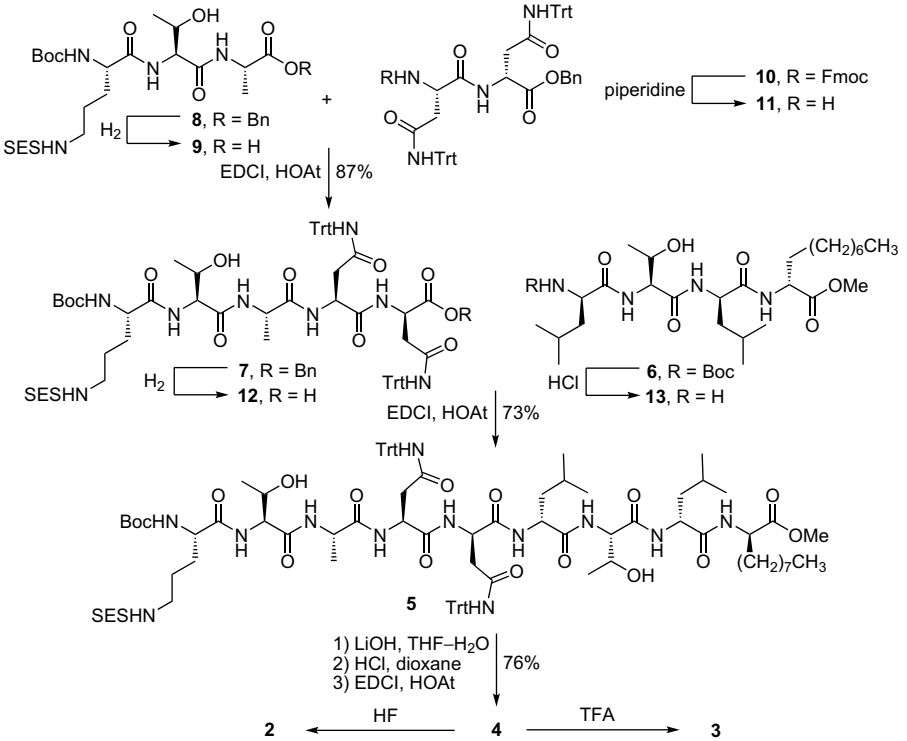


Figure 2. Retrosynthetic analysis of the cyclic peptide.

2. Retrosynthetic analysis

For the synthesis of the chlorofusin cyclic peptide, the coupling and macrocyclization sites in our original work[3] were chosen to minimize the use of protecting groups and to maximize the convergency of the synthesis, while permitting a deliberate late stage incorporation of the subunit bearing the two Asn residues thereby providing convenient access to both diastereomers required to assign the absolute stereochemistry. As this work progressed and facilitating its present continuation, an even more



Scheme 1. Synthesis of cyclic peptide **4**.

convergent synthesis of the cyclic peptide was desired that might facilitate the examination of a wider range of analogs as well as provide the basis for an alternative total synthesis of the natural product itself. Complementary to the solid phase synthesis detailed by Searcey[4] and analogous to the Nakata synthesis of the chlorofusin cyclic peptide,[5] the macrocyclization site chosen was the L-Orn9/D-Ada8 amide, constituting ring closure at a favorable L/D amino acid pair (Fig. 2).[8] Moreover, and distinct from the approach disclosed by Nakata, the approach utilized the same three di-, tri-, and tetrapeptide intermediates (**6**, **8**, and **10**) utilized in our original approach, but avoided our prior late stage coupling and incorporation of **11** that can lead to competitive, but minor, diketopiperazine formation.[3,9]

3. Synthesis of cyclic peptide

The synthesis of the cyclic peptide began with debenzylation of **8**[3] to form tripeptide **9** (H_2, Pd/C, 93%), Scheme 1. Coupling of **9** and dipeptide **11**[3] provided the pentapeptide **7** (EDCI, HOAt, DMF, 0–23 °C, 87%). Benzyl ester cleavage of **7** under hydrogenation conditions provided the carboxylic acid **12**, which was coupled with tetrapeptide **13**[3] to provide the linear peptide **5** (EDCI, HOAt, DMF, 0–23 °C, 73%). The methyl ester in **5** was hydrolyzed with LiOH in THF–H_2O at 0 °C to provide the corresponding carboxylic acid, and subsequent Boc removal with 4 M HCl in dioxane provided the deprotected amino acid, which was used in the next step without purification. The macrocyclization was achieved under dilute reaction conditions to provide the cyclic peptide **4** (EDCI, HOAt, DMF, 0–23 °C, 0.01 M, 76%).

Final global deprotection of both the SES and Trt groups was accomplished with anhydrous HF to provide the fully deprotected cyclic peptide **2**,[10] or the two Trt groups could be selectively removed upon treatment with a 20:1 TFA–H_2O mixture, a procedure used in our previous work,[3] to yield **3**. This second improved route to **2**–**4** has been used to provide large quantities of the cyclic peptide that may be used in the preparation of chlorofusin and its analogs.

4. Conclusion

This alternative synthetic route to **2**–**4** provided a higher overall yield of the cyclic peptide than our original route and required less chromatographic purification in the late stages of the synthesis. Ring closure of the cyclic peptide was accomplished by amide bond formation between an L-amine and a D-carboxylic acid that not only improved the yield, but it also provided material containing smaller amounts of separable minor side products. The cyclic peptide prepared using this improved route is being employed in the preparation of chromophore analogs of chlorofusin and related materials.

5. Experimental

5.1. Boc-L-Orn(SES)-L-Thr-L-Ala-OH (9)

A solution of **8**[3] (106 mg, 0.16 mmol) in EtOH (2 mL) was treated with 10% Pd/C (21 mg). The resulting black suspension was stirred under H_2 (1 atm) for 1 h. The catalyst was removed by filtration through a pad of Celite and washed with EtOAc (5 mL). The filtrate was concentrated under reduced pressure. Flash chromatography (SiO_2, 5% MeOH–CH_2Cl_2) afforded **9** as a white foam (93%, 85 mg). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.97 (d, J=7.0 Hz, 1H), 7.59 (d, J=8.4 Hz, 1H), 7.07 (d, J=7.9 Hz, 1H), 6.93 (t, J=5.7 Hz, 1H), 4.23 (m, 2H), 3.97 (m, 2H), 3.46 (q, J=7.0 Hz, 1H), 2.90 (m, 4H), 1.69 (br m, 1H), 1.48–1.53 (br m, 3H), 1.40 (s, 9H), 1.29 (d, J=7.3 Hz, 3H), 1.07 (m, 4H), 0.88 (m, 2H), 0.05 (s, 9H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 174.0, 172.0, 169.6, 155.6, 78.3, 66.7, 57.7, 56.1, 47.7, 46.9, 42.1, 28.3 (3C), 26.4, 19.6, 18.6, 17.5, 10.1, −1.8 (3C); IR (film) ν_{max} 3316, 2953, 1653, 1526, 1315, 1167, 1139, 842, 756 cm^{-1}; HRESI-TOF m/z 569.2672 (M+H$^+$, $C_{22}H_{45}N_4O_9SSi$ requires: 569.2671); $[\alpha]_D^{23}$ −16 (c 1.0, $CHCl_3$).

5.2. Boc-L-Orn(SES)-L-Thr-L-Ala-L-Asn(Trt)-D-Asn(Trt)-OBn (7)

A solution of **10**[3] (285 mg, 0.27 mmol) in anhydrous CH_2Cl_2 (3 mL) was treated with piperidine (81 μL, 0.82 mmol) and stirred at 23 °C for 100 min. Flash chromatography (SiO_2, 70% EtOAc–hexanes) afforded the unstable free amine **11** as a gray solid, which was employed directly in the next reaction. A flask containing **9** (108 mg, 0.19 mmol), **11** (155 mg, 0.19 mmol), HOAt (78 mg, 0.57 mmol), and EDCI (109 mg, 0.57 mmol) at 0 °C under Ar was slowly treated with anhydrous DMF (2 mL), stirred at 0 °C for 1 h and then stirred at 23 °C for 24 h. The reaction mixture was diluted with EtOAc (30 mL) and washed with aqueous 1 N HCl (2×5 mL), saturated aqueous $NaHCO_3$ (2×5 mL), water (10 mL), and saturated aqueous NaCl (10 mL). The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. Flash chromatography (SiO_2, 60% EtOAc–hexanes) afforded **7** as a gray-white solid (87%, 226 mg). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.76 (s, 1H), 8.54 (s, 1H), 8.21 (d, J=8.0 Hz, 1H), 8.13 (d, J=7.7 Hz, 1H), 7.94 (d, J=7.0 Hz, 1H), 7.65 (d, J=8.2 Hz, 1H), 7.32–7.39 (br m, 5H), 7.12–7.29 (br m, 31H), 6.93 (t, J=5.8 Hz, 1H), 5.09 (m, 2H), 5.00 (d, J=4.7 Hz, 1H), 4.63 (m, 1H), 4.58 (dd, J=13.8, 6.3 Hz, 1H), 4.30–4.36 (br m, 1H), 4.05 (m, 1H), 3.97 (m, 1H), 2.86–2.92 (br m, 4H), 2.80 (m, 2H), 2.58 (br m, 2H), 1.71 (m, 1H), 1.48–1.57 (br m, 3H), 1.41 (s, 10H), 1.23 (d, J=7.0 Hz, 3H), 1.05 (d, J=6.4 Hz, 3H), 0.88 (m, 2H), 0.04 (s, 9H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 172.3, 171.9, 171.0, 170.9, 169.4, 168.8, 168.5, 155.6, 144.8 (3C), 144.7 (3C), 135.9, 128.6 (12C), 128.5 (2C), 128.0, 127.7 (2C), 127.5 (6C), 127.5 (6C), 126.4 (6C), 78.3, 69.5, 69.4, 66.6, 66.1, 60.5, 57.5, 54.3, 50.1, 49.2, 48.4, 46.9, 42.1, 37.5, 28.9, 28.3 (3C), 26.5, 19.3, 18.8, 10.1, −1.86 (3C); IR (film) ν_{max} 3310, 2954, 1655, 1514, 1494, 1448, 1125, 1168, 851, 753, 699 cm^{-1}; HRESI-TOF m/z 1371.6167 (M+H$^+$, $C_{75}H_{91}N_8O_{13}SSi$ requires: 1371.6190); $[\alpha]_D^{23}$ −22 (c 1.2, $CHCl_3$).

5.3. Boc-L-Orn(SES)-L-Thr-L-Ala-D-Asn(Trt)-L-Asn(Trt)-D-Leu-L-Thr-D-Leu-D-Ada-OMe (5)

A solution of **7** (52 mg, 0.038 mmol) in EtOH (1 mL) was treated with 10% Pd/C (16 mg). The resulting black suspension was stirred under H_2 (1 atm) for 2 h. The catalyst was removed by filtration through a pad of Celite and washed with EtOAc (5 mL). The filtrate was concentrated under reduced pressure to provide **12** as a white foam, which was directly employed in the next reaction without further purification. A sample of **6**[3] (24 mg, 0.038 mmol) was treated with 4 M HCl in dioxane solution (0.1 mL), and the resulting mixture was stirred at 23 °C for 1 h. The volatiles were removed with a stream of N_2. The residue was treated with Et_2O (2×2 mL) and concentrated under reduced pressure to afford amine **13** as a white foam, which was directly employed in the next reaction without further purification. A flask containing **12** (49 mg, 0.038 mmol), **13** (20 mg, 0.038 mmol), HOAt (15 mg, 0.11 mmol), EDCI (21 mg, 0.11 mmol), and $NaHCO_3$ (10 mg, 0.11 mmol) at 0 °C was slowly treated with anhydrous DMF (0.4 mL). The reaction mixture was stirred at 0 °C for 1 h and then stirred at 23 °C for 24 h under Ar. The reaction mixture was diluted with EtOAc (10 mL) and washed with aqueous 1 N HCl (2×2 mL), saturated aqueous $NaHCO_3$ (2×2 mL), water (2 mL), and saturated aqueous NaCl (4 mL). The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. Flash chromatography (SiO_2, 3% MeOH–CH_2Cl_2) afforded **5** as a white solid (73%, 49 mg). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.61 (s, 1H), 8.58 (s, 1H), 8.23 (d, J=7.0 Hz, 3H), 7.93 (d,

J=6.7 Hz, 1H), 7.81 (d, J=8.4 Hz, 1H), 7.75 (d, J=7.4 Hz, 1H), 7.67 (app t, J=8.59 Hz, 2H), 7.15–7.29 (br m, 30H), 7.11 (d, J=8.0 Hz, 1H), 6.93 (t, J=5.6 Hz, 1H), 4.98 (d, J=4.5 Hz, 1H), 4.75 (d, J=5.4 Hz, 1H), 4.58 (m, 1H), 4.52 (dd, J=12.7, 8.1 Hz, 1H), 4.31–4.41 (br m, 3H), 4.27 (dd, J=14.6, 8.0 Hz, 1H), 4.14 (br m, 2H), 4.04 (m, 1H), 3.97 (m, 1H), 3.92 (dd, J=10.3, 5.6 Hz, 1H), 3.58 (s, 3H), 2.84–2.94 (br m, 4H), 2.70 (m, 1H), 2.55–2.66 (br m, 3H), 1.57–1.73 (br m, 4H), 1.43–1.56 (br m, 8H), 1.40 (s, 9H), 1.24 (br m, 16H), 1.03 (d, J=6.2 Hz, 3H), 0.98 (d, J=6.3 Hz, 3H), 0.87 (br m, 10H), 0.82 (m, 6H), 0.04 (s, 9H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 172.5, 172.28, 172.26, 172.2, 171.2, 170.9, 169.7, 169.4, 169.0, 168.6, 162.4, 155.6, 144.77 (3C), 144.75 (3C), 128.6 (12C), 127.54 (6C), 127.50 (6C), 126.40 (3C), 126.36 (3C), 78.4, 69.5, 66.7, 59.8, 58.5, 57.5, 54.3, 52.2, 51.9, 51.8, 50.7, 50.6, 50.1, 48.2, 46.9, 42.1, 40.9, 40.6, 36.3, 35.9, 31.3, 30.7, 28.9, 28.70, 28.68, 28.3 (3C), 26.5, 25.4, 24.2, 24.0, 23.2, 23.0, 22.2, 21.7, 21.5, 20.8, 19.6, 19.4, 18.7, 14.2, 14.0, 10.1, −1.9 (3C); IR (film) ν_{max} 3309, 2928, 1650, 1518, 1448, 1251, 1168, 1141, 841, 754 cm^{-1}; HRESI-TOF m/z 1791.9542 (M+H$^+$, $C_{95}H_{135}N_{12}O_{18}SSi$ requires: 1791.9501); $[\alpha]_D^{23}$ −20 (c 1.3, $CHCl_3$).

5.4. *cyclo*-L-Thr-L-Ala-L-Asn(Trt)-D-Asn(Trt)-D-Leu-L-Thr-D-Leu-D-Ada-L-Orn(SES) (4)

A solution of **5** (90 mg, 0.050 mmol) in THF–H_2O (1:1, 2 mL) was treated with LiOH (21 mg, 1.0 mmol) at 0 °C. The mixture was stirred at 0 °C for 18 h before being quenched with the addition of aqueous 2 N HCl (0.5 mL). The mixture was extracted with EtOAc (5×1 mL), and the combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure to provide the hydrolyzed product that was directly employed in the next reaction without further purification. The carboxylic acid was treated with 4 M HCl in dioxane (1 mL) and the resulting solution was stirred at 23 °C for 1 h. The volatiles were removed under a stream of N_2. The residue was triturated with Et_2O (2×2 mL) and concentrated under reduced pressure to afford a gray solid, to which HOAt (27 mg, 0.20 mmol), EDCI (38 mg, 0.20 mmol), and $NaHCO_3$ (17 mg, 0.20 mmol) were added. The combined mixture was cooled to 0 °C and anhydrous DMF (5 mL) was added. The reaction mixture was stirred at 0 °C for 48 h before being diluted with EtOAc (50 mL), washed with aqueous 1 N HCl (2×5 mL), saturated aqueous $NaHCO_3$ (2×5 mL), water (10 mL), and saturated aqueous NaCl (10 mL). The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. Flash chromatography (SiO_2, 2–3% MeOH–CH_2Cl_2) afforded **4** as a viscous oil (76%, 463 mg). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.37 (br s, 2H), 8.88 (s, 1H), 8.64 (s, 1H), 8.21 (d, J=3.2 Hz, 1H), 7.91 (s, 1H), 7.87 (d, J=6.0 Hz, 1H), 7.77 (m, 1H), 7.15–7.31 (br m, 30H), 7.01 (s, 1H), 6.89 (m, 1H), 6.84 (t, J=5.5 Hz, 1H), 6.33 (br s, 1H), 5.35 (d, J=4.0 Hz, 1H), 5.26 (d, J=4.5 Hz, 1H), 5.07 (m, 1H), 4.77 (t, J=11.3 Hz, 1H), 4.54 (m, 1H), 4.17 (m, 3H), 3.93 (m, 1H), 3.87 (m, 1H), 3.80 (m, 1H), 3.73 (m, 1H), 2.79–2.98 (br m, 4H), 2.73 (m, 2H), 1.78–1.95 (br m, 2H), 1.42–1.66 (br m, 8H), 1.38 (d, J=7.3 Hz, 3H), 1.33 (d, J=6.2 Hz, 3H), 1.26 (br m, 16H), 1.00 (d, J=5.9 Hz, 3H), 0.87 (app t, J=6.9 Hz, 6H), 0.82 (m, 3H), 0.77 (m, 6H), 0.30 (d, J=6.3 Hz, 3H), 0.00 (s, 9H); HRMALDI-FTMS (DHB) m/z 1681.8610 (M+Na$^+$, $C_{89}H_{122}N_{12}O_{15}SSi$ requires: 1681.8534); $[\alpha]_D^{23}$ +14 (c 0.34, $CHCl_3$).

5.5. *cyclo*-L-Thr-L-Ala-L-Asn-D-Asn-D-Leu-L-Thr-D-Leu-D-Ada-L-Orn (2)

A sample of **4** (30 mg, 18.1 μmol) was treated with two drops of anisole at 23 °C and attached to an HF (anhydrous) apparatus. HF(g) was condensed in the reaction apparatus at −78 °C for 30 min and then allowed to warm to 0 °C and stirred for 1.5 h. The HF was removed with a stream of N_2 and under reduced pressure. The resulting mixture was triturated with Et_2O (3×2 mL), dissolved in TFA (2 mL), and placed on the lyophilizer for 8 h to provide **2** as a white solid (26.4 mg, 88%) identical in all respects to authentic material.[4]

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