Published in final edited form as:

Tetrahedron. 2009 ; 65(16): 3281–3284. doi:10.1016/j.tet.2008.09.029.

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.

Introduction

Chlorofusin (**1**, Figure 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^a (IC₅₀ = 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 nonstandard 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 (¹H and ¹³C 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^a or *L*-Ada8^a residue,⁴ and recently Nakata⁵ 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

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^aAbbreviations.

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)

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.

Retrosynthetic Analysis

For the synthesis of the chlorofusin cyclic peptide, the coupling and macrocyclization sites in our original work³ 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 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 Searcey⁴ 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 (Figure 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}

Synthesis of Cyclic Peptide

The synthesis of the cyclic peptide began with debenzylation of **8**³ to form tripeptide **9** (H₂, Pd/C, 93%), Scheme 1. Coupling of **9** and dipeptide **11**³ provided the pentapeptide **7** (EDCI,^a HOAt,^a DMF, 0 to 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 to 23 °C, 73%). The methyl ester in **5** was hydrolyzed with LiOH in THF-H₂O 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 to 23 °C, 0.01 M, 76%).

Final global deprotection of both the SES^a and Trt^a 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-H₂O 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.

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.

Experimental

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 H₂ (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 (SiO₂, 5% MeOH-CH₂Cl₂) afforded **9** as a white foam (93%, 85 mg): ¹H 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); ¹³C 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⁺, C₂₂H₄₅N₄O₉SSi requires 569.2671); [α]_D²³ -16 (*c* 1.0, CHCl₃).

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 CH₂Cl₂ (3 mL) was treated with piperidine (81 μL, 0.82 mmol) and stirred at 23 °C for 100 min. Flash chromatography (SiO₂, 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 NaHCO₃ (2 × 5 mL), water (10 mL), and saturated aqueous NaCl (10 mL). The organic phase was dried (Na₂SO₄) and concentrated under reduced pressure. Flash chromatography (SiO₂, 60% EtOAc-hexanes) afforded **7** as a gray-white solid (87%, 226 mg): ¹H 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); ¹³C 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, 48.37, 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⁺, C₇₅H₉₁N₈O₁₃SSi requires 1371.6190); [α]_D²³ -22 (*c* 1.2, CHCl₃).

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 H₂ (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 N₂. The residue was treated with

Et₂O (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 NaHCO₃ (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 NaHCO₃ (2 × 2 mL), water (2 mL) and saturated aqueous NaCl (4 mL). The organic phase was dried (Na₂SO₄) and concentrated under reduced pressure. Flash chromatography (SiO₂, 3% MeOH-CH₂Cl₂) afforded **5** as a white solid (73%, 49 mg): ¹H 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); ¹³C 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⁺, C₉₅H₁₃₅N₁₂O₁₈SSi requires 1791.9501); [α]_D²³ -20 (c 1.3, CHCl₃).

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-H₂O (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 (Na₂SO₄) 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 N₂. The residue was triturated with Et₂O (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 NaHCO₃ (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 NaHCO₃ (2 × 5 mL), water (10 mL) and saturated aqueous NaCl (10 mL). The organic phase was dried (Na₂SO₄) and concentrated under reduced pressure. Flash chromatography (SiO₂, 2-3% MeOH-CH₂Cl₂) afforded **4** as a viscous oil (76%, 463 mg); ¹H 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⁺, C₈₉H₁₂₂N₁₂O₁₅SSi requires 1681.8534); [α]_D²³ +14 (c 0.34, CHCl₃).

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 μ mol) 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 N₂ and under reduced pressure. The resulting mixture was triturated with Et₂O (3 $\times$ 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

Acknowledgments

We gratefully acknowledge the financial support of the National Institutes of Health (CA41101) and the Skaggs Institute for Chemical Biology. SYL is a Skaggs fellow.

References

1. (a) Duncan SJ, Gruschow S, Williams DH, McNicholas C, Purewal R, Hajek M, Gerlitz M, Martin S, Wrigley SK, Moore M. J. Am. Chem. Soc 2001;123:554–560. [PubMed: 11456567] (b) Duncan SJ, Gruschow S, Williams DH, McNicholas C, Pureval R, Hajek M, Gerlitz M, Martin S, Wrigley SK, Moore M. J. Am. Chem. Soc 2002;124:14503–14503. (c) Duncan SJ, Cooper MA, Williams DH. Chem. Commun 2003:316–317.
2. (a) Fischer PM, Lane DP. Trends Pharmacol. Sci 2004;25:343–346. [PubMed: 15219971] (b) Boger DL, Desharnais J, Capps K. Angew. Chem. Int. Ed 2003;42:4138–4176.
3. Desai P, Pfeiffer SS, Boger DL. Org. Lett 2003;5:5047–5050. [PubMed: 14682761]
4. (a) Malkinson JP, Zloh M, Kadom M, Errington R, Smith PJ, Searcey M. Org. Lett 2003;5:5051–5054. [PubMed: 14682762] (b) Woon ELY, Arcieri M, Wilderspin AF, Malkinson JP, Searcey M. J. Org. Chem 2007;72:5146–5151. [PubMed: 17559272]
5. Tomonori M, Miyagi M, Suzuki K, Shibasaki M, Saikawa Y, Nakata M. Heterocycles 2007;72:275–291.
6. (a) Lee SY, Clark RC, Boger DL. J. Am. Chem. Soc 2007;129:9860–9861. [PubMed: 17637057] (b) Clark RC, Lee SY, Boger DL. J. Am. Chem. Soc 2008;130 in press.
7. Qian W-J, Wei W-G, Zhang Y-X, Yao Z-J. J. Am. Chem. Soc 2007;129:6400–6401. [PubMed: 17472388]
8. (a) Brady SF, Varga SL, Freidinger RM, Schwenk DA, Mendlowski M, Holy FW, Veber DF. J. Org. Chem 1979;44:3101–3105. (b) Rich DH, Bhatnagar P, Mathiaparanam P, Grant JA, Tam JP. J. Org. Chem 1978;43:296–302. [PubMed: 621598]
9. (a) Schroeder, E.; Luebke, K. The Peptides. Vol. 1. Academic Press; New York: 1965. (b) Gisin BF, Merrifield RB. J. Am. Chem. Soc 1972;94:3102–3106. [PubMed: 5032495]
10. (a) Boger DL, Ledebner MW, Kume M. J. Am. Chem. Soc 1999;121:1098–1099. (b) Boger DL, Ledebner MW, Kume M, Searcey M, Jin Q. J. Am. Chem. Soc 1999;121:11375–11383.

Tetrahedron. Author manuscript; available in PMC 2010 January 1.

Figure 2.
Retrosynthetic analysis of the cyclic peptide

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