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Synthesis of the Chlorofusin Cyclic Peptide: Assignment of the Asparagine Stereochemistry

Pankaj Desai, Steven S. Pfeiffer, and Dale L. Boger*

Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

boger@scripps.edu

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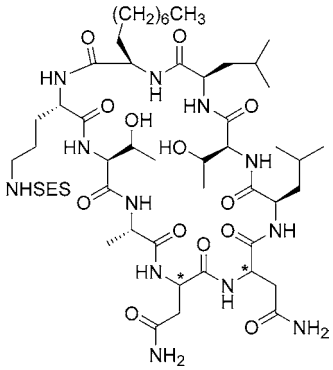
ABSTRACT



An efficient synthesis of two diastereomers of the chlorofusin cyclic peptide bearing either the L-Asn3/D-Asn-4 or D-Asn3/L-Asn4 stereochemistry is detailed. Four key subunits were prepared, sequentially coupled, and cyclized to provide the two diastereomeric macrocycles. The absolute stereochemistry at the asparagine residues 3 and 4 was assigned as L and D, respectively, by correlating the NMR data of the two diastereomers with that reported for the natural product.

Phosphoprotein p53 plays a crucial role in the prevention of cancer.[1−3] It controls cell proliferation and development of genetic abnormalities by inducing G1 arrest or apoptosis upon DNA damage.[4,5] The protein MDM2 (HDM2) interacts with the tumor suppressor p53 to form a stable complex[6] in which the DNA binding domain of p53 is concealed and unable to function as a regulator of cell division.[7−10] The X-ray crystal structure of a p53-MDM2 complex revealed that this interaction is mediated by three key hydrophobic amino acid residues of p53 and a hydrophobic cleft in MDM2.[11] Molecules that bind this MDM2 hydrophobic cleft can disrupt the p53−MDM2 interaction and restore the inhibited function to p53.[12−14]

Chlorofusin is a fungal metabolite isolated as the major component in the fermentation broth of *Fusarium* sp. 22026[15] (updated as *Microdochium caespitosum*).[16] It was found to disrupt the interaction between p53 and MDM2 by directly

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binding to the N-terminal domain of MDM2 (IC_{50} = 4.6 μM, K_D = 4.7 μM).[15,17] Thus, chlorofusin represents an exciting lead for antineoplastic intervention that acts by a rare disruption of a protein−protein interaction.[18]

On the basis of spectroscopic studies, the chlorofusin structure was proposed to be composed of a densely functionalized chromophore linked through the terminal amine of ornithine to a 27-membered cyclic peptide composed of nine amino acid residues (Figure 1).[15] Two of the

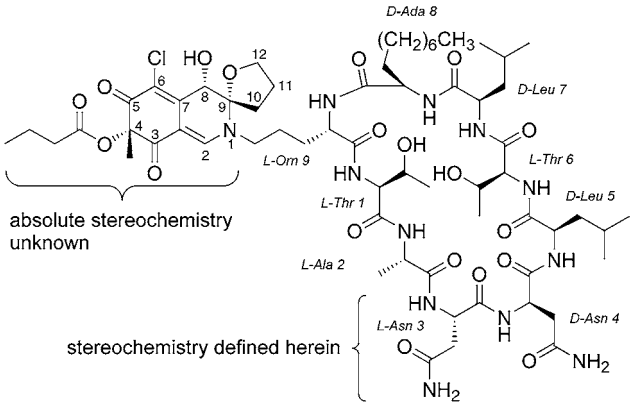


Figure 1. Proposed structure of chlorofusin.

cyclic peptide amino acids possess a nonstandard or modified side chain, and four possess the D-configuration. Although the spectroscopic studies of chlorofusin permitted assignment of the unusual chromophore structure and relative stereochemistry, the absolute stereochemistry at C-4, C-8, and C-9 could not be assigned. Similarly, the two cyclic peptide asparagine residues Asn3 and Asn4 were established to have opposite stereochemistries (L and D), although the respective assignments were not possible.

Herein, we report the synthesis of the two possible Asn diastereomers (L,D and D,L) of the chlorofusin cyclic peptide and the assignment of the natural product stereochemistry as L-Asn3 and D-Asn4. Four key subunits were assembled, sequentially coupled, and cyclized to provide the 27-membered cyclic peptide core (Figure 2). The coupling and macrocyclization sites were carefully chosen to minimize the use of protecting groups and maximize the convergency of the synthesis. Deliberate late-stage incorporation of the subunit bearing the two asparagine residues allowed convenient access to both diastereomers required to assign the absolute stereochemistry.

The D-2-aminodecanoic acid (D-Ada) residue bearing the only nonstandard side chain was prepared by alkylation of

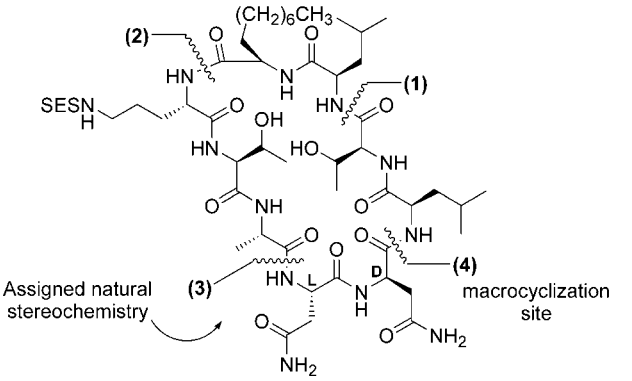
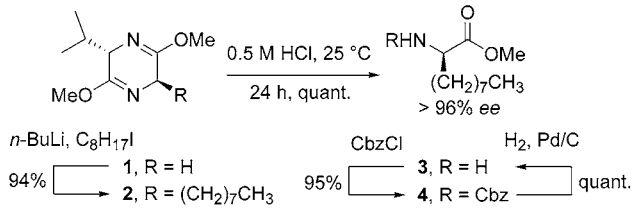


Figure 2. Chlorofusin cyclic peptide.

the Schöllkopf reagent (*S*)-**1**[19] with 1-iodooctane (*n*-BuLi, THF, −78 °C, 94%, >96% de), Scheme 1. Hydrolysis of **2**

Scheme 1



(0.5 M aq HCl, 25 °C, 24 h, quant.) and Cbz amine protection (3 equiv CbzCl, 3 equiv of Na_2CO_3, EtOAc−H_2O 1:1, 0−25 °C, 18 h, 95%) to assist chromatographic purification and permit prolonged storage provided **4** (>96% ee),[20] and subsequent Cbz deprotection (H_2, 10% Pd/C, EtOH, 25 °C, 1 h, quant.) afforded D-Ada-OMe (**3**).

The preparation of the key subunits and assemblage of the heptapeptide **16** common to both diastereomers is detailed in Scheme 2 and the preparation of the two required asparagine dipeptides is summarized in Scheme 3. Dipeptide **5** was obtained by coupling Boc-D-Leu-OH and L-Thr-OBn (EDCI, HOAt, DMF, 0−25 °C, 18 h, 94%), and subsequent debenzylation (H_2, 10% Pd/C, EtOH, 25 °C, 1 h) provided the corresponding free acid **6**. In a similar manner, dipeptide **7** was obtained from Boc-D-Leu-OH and D-Ada-OMe (**3**, EDCI, HOAt, DMF, 0−25 °C, 18 h, 95%), which was further subjected to Boc deprotection (4 M HCl, dioxane, 0 °C, 1 h) to provide **8**. Coupling of **6** and **8** (EDCI, HOAt, $NaHCO_3$, DMF, 0−25 °C, 18 h, 85%) afforded tetrapeptide **9** that was subsequently hydrolyzed (LiOH, THF−H_2O 1:1, 0 °C, 18 h) to provide acid **10**. Dipeptide **11** was prepared by coupling Boc-L-Thr-OH and L-Ala-OBn (EDCI, HOAt, DMF, 0−25 °C, 18 h, 86%) and was followed by Boc deprotection (4 M

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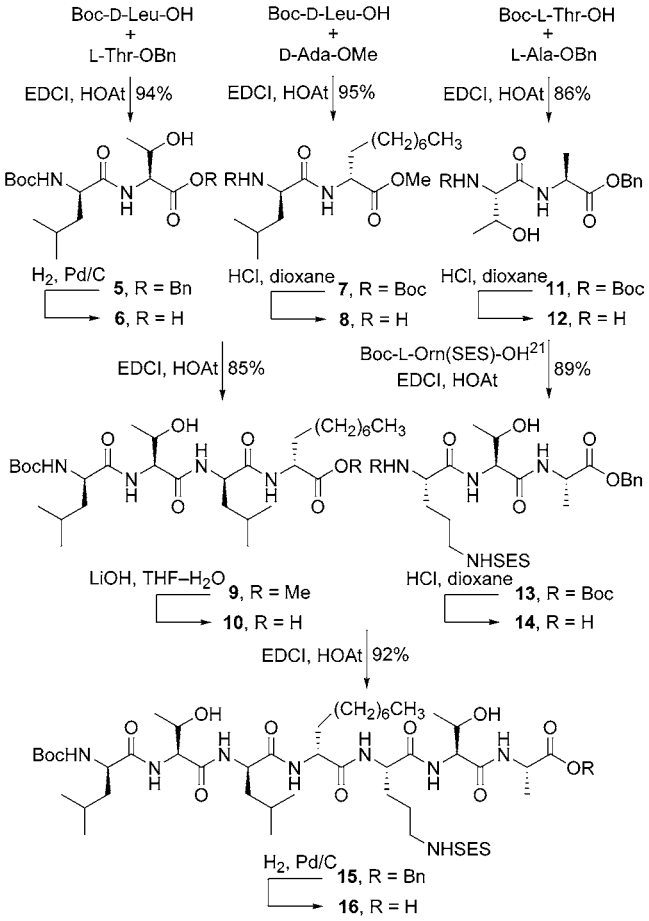
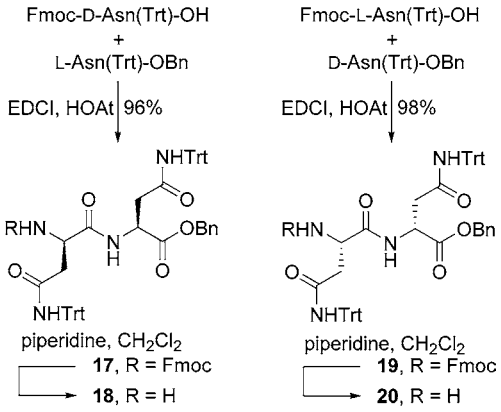
(20) Established by chiral HPLC analysis: CHIRALCEL OD 0.46 × 25 cm column, 12% EtOAc−hexanes, 1 mL/min; retention times = 10.2 min (*S*), 11.3 min (*R*); $[\alpha]_D$ −5.0 (*c* 1.0, $CHCl_3$).

Scheme 2



HCl, dioxane, 0 °C, 1 h) to provide **12**. Subsequent coupling of **12** with Boc-L-Orn(SES)-OH[21] (EDCI, HOAt, $NaHCO_3$, DMF, 0−25 °C, 18 h, 89%) afforded tripeptide **14** after Boc deprotection (4 M HCl, dioxane, 0 °C, 1 h) of compound **13**. The tetrapeptide **10** was coupled with tripeptide **14** (EDCI, HOAt, $NaHCO_3$, DMF, 0−25 °C, 18 h, 92%) to provide the heptapeptide **15**, which was further debenzylated (H_2, 10% Pd/C, EtOH, 25 °C, 1 h) to afford free acid **16**.

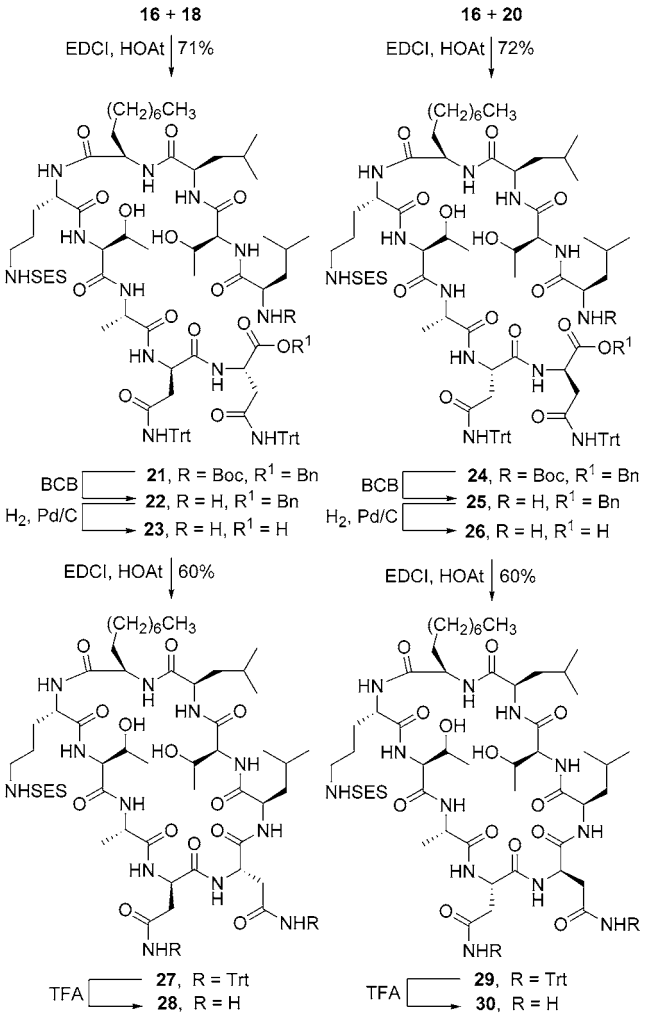
Scheme 3



The two asparagine dipeptides **17** (from Fmoc-D-Asn(Trt)-OH and L-Asn(Trt)-OBn) and **19** (from Fmoc-L-Asn(Trt)-OH and D-Asn(Trt)-OBn) were prepared simultaneously (Scheme 3). Fmoc deprotection immediately prior to use under standard conditions (0 °C, 10% piperidine in CH_2Cl_2) provided **18** and **20**, respectively.

As depicted in Scheme 4, heptapeptide **16** could either be coupled with dipeptide **18** to afford the D,L-linear peptide

Scheme 4



21 in 71% yield or coupled with dipeptide **20** to afford the corresponding L,D-diastereomer **24** in 72% yield. Each diastereomer was further subjected to a sequential deprotection of the Boc group (bromocatecholborane BCB, CH_2Cl_2, 0 °C, 1 h) and benzyl group (H_2, 10% Pd/C, EtOH, 25 °C, 1 h). Subsequent macrocyclization under high dilution conditions (EDCI, HOAt, 0.01 M DMF, 0−25 °C, 24 h) afforded the diastereomeric macrocycles **27** and **29** in 60% yield in each case. TFA-mediated deprotection of the trityl groups (TFA−H_2O 20:1, 25 °C, 5 h) cleanly provided the corresponding cyclic peptides **28** and **30**.

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The data obtained from COSY, HMBC, and HMQC NMR experiments indicated that the proton and carbon chemical shifts for peptide **30** closely matched those reported for the natural product as compared to those for peptide **28** (Figure 3).[22] Characteristic of this similarity in proton chemical shifts,

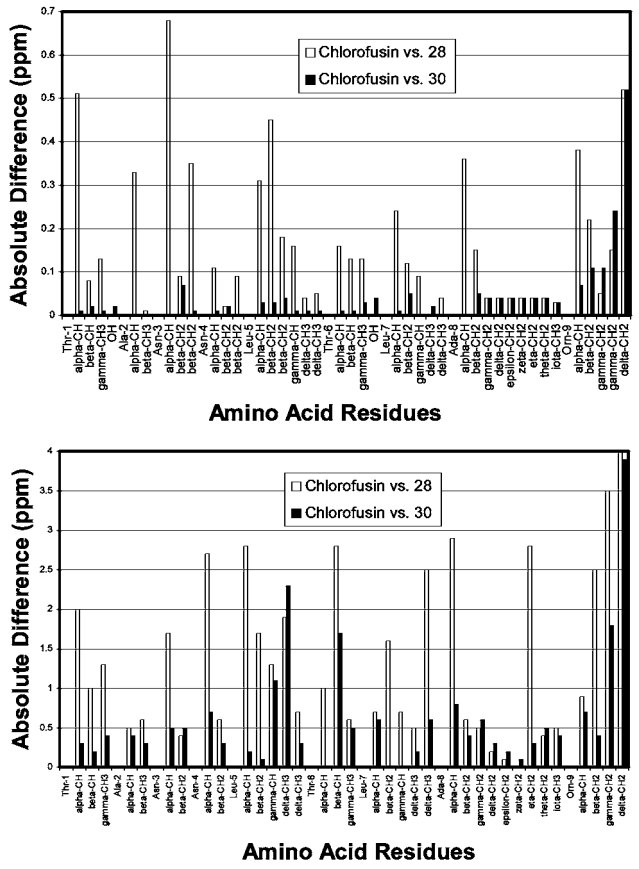

Figure 3. Difference in H^1 (top) and C^{13} (bottom) NMR chemical shifts (DMSO-d_6, 500 MHz).

the nine α-CH chemical shifts of **30** exhibited ≤ 0.02 δ differences from those reported for chlorofusin (av $\Delta\delta$ = 0.016), whereas those of **28** exhibited larger differences of 0.1−0.6 δ (av $\Delta\delta$ = 0.34). Analogous comparisons of the ^{13}C shifts of the α-carbons provide similar distinctions (**28**, $\Delta\delta$ = 0.5−3.0, av $\Delta\delta$ = 1.91 vs **30**, $\Delta\delta$ = 0.0−0.8, av $\Delta\delta$ = 0.47). In addition, Williams et al. reported diagnostic long-range NOEs between the methyl groups of Leu5 and the methyl group of Thr1 as well as the methylene groups of the Ada8 side chain for chlorofusin.[15] A key long-range NOE between both β-methylene hydrogens of Asn3 and the two methines of Thr6 including the α-H (C_2^6−H) was also observed. It was suggested that residues Leu5, Thr1, and Ada8 may fold and cluster over the top of the macrocyclic ring while Asn3 folds under and lies proximal to Thr6 (C_2^6−H). A large geminal coupling constant of the Asn3 methylene (J = 15.2 Hz) indicative of a restricted conformation and the slow exchange of the Asn3 carboxamide was suggested to be potentially derived from intramolecular H-bonding to the Thr6 hydroxyl accounting for the clearly defined Asn3/Thr6 side chain NOEs. To fold in this manner, it was suggested that Asn3 likely has the L-configuration and Asn4 has the D-configuration.[15]

The long-range NOEs observed in the NOESY and ROESY NMR experiments for **28** and **30** were not as extensively defined in our work as those in the Williams study but were sufficient to indicate that peptide **30** satisfies the above key NOEs while **28** does not.[22] Notably, **30** not only exhibited the diagnostic Leu5 side chain NOEs with the Thr1 and Ada8 side chains indicative of their potential clustering on the top of the cyclic peptide but also exhibited the NOEs between the Asn3 methylene and the Thr6 α-H and β-H indicating that they lie proximal to one another potentially beneath the cyclic peptide. In contrast, **28** exhibited a different series of NOEs. Instead of Thr1, it was Thr6 that exhibited an extensive series of NOEs with Leu5. Thus, the Leu5 methyl groups exhibited NOEs with the Thr6 methyl and α-H as well as the Ada8 side chain methylenes, and the Leu5 γ-methine and NH exhibited NOEs with the Thr6 α-H. Both the Asn3 (observed with chlorofusin) and Asn4 (not observed with chlorofusin) methylenes exhibited NOEs with the Thr6 α-H, but the additional NOEs of Asn3 to the Thr6 side chain seen with chlorofusin and **30** were not observed. Interestingly, neither isomer (**28** or **30**) exhibited the chlorofusin Asn4 methylene NOE with the Thr1 α-H. The Asn3 methylene of **30** exhibited the large geminal coupling constant characteristic of the natural product (J = 15.8 vs 15.2 Hz for chlorofusin). This large geminal coupling constant was not observed with the methylene of Asn3 of **28**, whereas that of Asn4 of **28** did exhibit a large geminal coupling (J = 16.3 Hz). These observations permit the assignment of absolute configuration at Asn3 in chlorofusin as L and that of Asn4 as D.[23]

Extensions of these studies to the total synthesis of chlorofusin and structural analogues are in progress and will be reported in due course.[23]

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Supporting Information Available: Full experimental details for the preparation of **2**−**30**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(22) HMBC experiments permitted the unambiguous assignment (connectivity) of the Thr1 vs Thr6, Asn3 vs Asn4, and Leu5 vs Leu7 residues in **28** (Ala2 Me ↔ Ala2 NH ↔ Thr1 α-C.; Leu5 Me ↔ Leu5 β-CH_2 ↔ Leu5 NH ↔ Asn4 α-C). In contrast with **30**, only Leu7 could be unambiguously established (Leu7 β-CH_2 ↔ Leu7 CO ↔ Ada8 NH: weak) and the remaining residue assignments were made on the basis of ^{1}H and ^{13}C NMR chemical shift comparisons reported for chlorofusin.

(23) Both **28** and **30** and a series of Orn 9 derivatives (-NHSES → NH_2, NHCbz. NHFmoc, NHBoc, N-phthalimide, and NH-naphthalene acetamide) were assayed for inhibition of MDM2/p53 binding in an ELISA assay enlisting immobilized p53 and full length MDM2. None were active (IC_{50} > 250 μM), indicating that they are ≥100 times less effective than chlorofusin.