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Total Synthesis, Assignment of Absolute Stereochemistry, and Structural Revision of Chlorofusin

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Chlorofusin (**1**, Figure 1), a novel fungal peptide isolated from the fermentation broth of *Microdochium caespitosum* in 2001,[1] has been found to antagonize p53-MDM2 interactions at micromolar concentrations, by directly binding to the N-terminal domain of MDM2.[2] Association of phosphoprotein p53 with the oncogene product MDM2 results in formation of a stable complex that blocks the DNA-binding domain of p53. This renders p53 incapable of activating transcription of the genes required for induction of G1 arrest or apoptosis.[3] Therefore, by disrupting these protein−protein interactions, chlorofusin represents an attractive lead for anticancer therapy developments. On the basis of the spectroscopic evidence, chlorofusin was proposed to have a densely functionalized chromophore linked through the terminal amine of an ornithine residue to a 27-membered cyclic peptide (composed of nine amino acid residues). The absolute stereochemistry of the peptide was assigned through the total synthesis of both possible cyclopeptides by Boger and Searcey in 2003.[4] Relying on our previous studies of the chlorofusin chromophore,[5−6] we detail herein the first total synthesis of chlorofusin, together with a revision of its previously reported structure and assignment of the absolute stereochemistry.

With a consideration toward convergence, chlorofusin is disconnected into an azaphilone (**2**) and cyclic peptide (**3**) components (Figure 1). Coupling of these two constituents can be achieved utilizing our previously developed conditions for the highly efficient conjugation of azaphilone with primary amines.[5] Elaboration of the azaphilone spiro-aminal functionality is approached through intramolecular bromo-etherification combined with an in situ hydrolysis. This provides for a fast-assembly that has proven to be highly efficient.

Synthesis of racemic **2** was previously reported by us.[6a] Preparation of (*S*)-**2** started from the known aldehyde **4** (Scheme 1).[6b] Copper-mediated asymmetric oxidation[8] of **4** with 1.1 equiv of Cu_2-[(−)-sparteine]$_2O_2$ followed by treatment of aqueous NH_4Cl in CH_3-CN afforded azaphilone **6** (55%, 91% ee). The tertial alcohol in **6** was then converted to its butanoate ester **7** (70%). Selective chlorination of **7** was carried out by treatment with SO_2Cl_2 at room temperature. Removal of acetate in **8** was accomplished using K_2-CO_3 in MeOH, affording (*S*)-2 in 80% yield.

Coupling of (*S*)-**2** with the peptide segment **3b** was performed in facile fashion employing our previously developed conditions,[5] giving **9a** in 80% yield (Scheme 2). The parallel reaction of *rac*-**2** with **3b** afforded two HPLC-separable diastereomers (**9a** and **9b**, 1:1). ^{1}H NMR of these two compounds showed a significant difference in the chemical shifts of their C-13 methyls. Of the two products, (4*S*)-**9a** (δ 1.43) provided a good match with the natural product (δ 1.41), while (4*R*)-**9b** was different (δ 1.29). This suggests that the stereogenic center at the C-4 position of chlorofusin should be the *S* configuration.

The most demanding aspect of this total synthesis is the elaboration of the spiro-aminal moiety under favorable stereochemical control. Considering the insufficiency of reliable methodology to direct the stereochemical outcome of spiro-aminal formation, it was decided to make use of the reversible ring-opening/-closing properties frequently observed in similar cases, and allow the natural peptide environment of chlorofusin to affect the final formation of its spiro aminal in a favorable direction. Toward this end, we sought to optimize our previous methodology,[6] in which bromoetherifi-

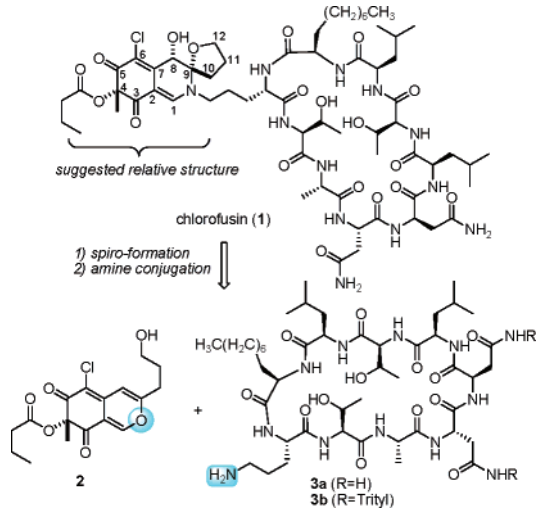
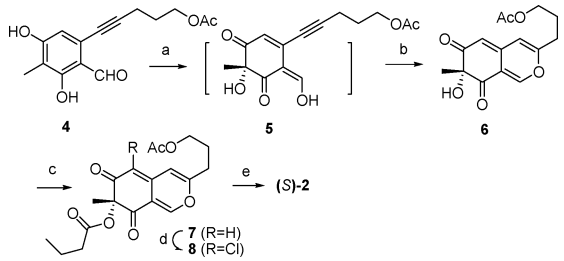


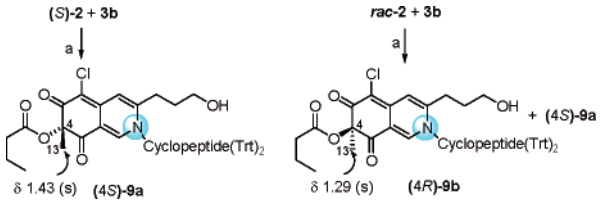
Figure 1. Retrosynthetic analysis of chlorofusin (**1**).

Scheme 1 [a]



[a] Reaction conditions: (a) (−)-sparteine, O_2, $Cu(CH_3CN)_4PF_6$, DIEA, DMAP, CH_2Cl_2; (b) CH_3CN, aq NH_4Cl, 55%, 91% ee (two steps); (c) ^{n}PrCOCl, catalyst DMAP, pyridine, CH_2Cl_2, 70%; (d) SO_2Cl_2, CH_2Cl_2, room temp, 66%; (e) K_2CO_3, MeOH, 80%.

Scheme 2 [a]



[a] Reaction conditions: (a) aq $NaHCO_3$, MeCN, room temp, 1 h, 80%.

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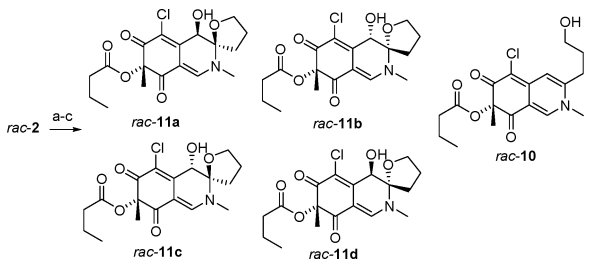
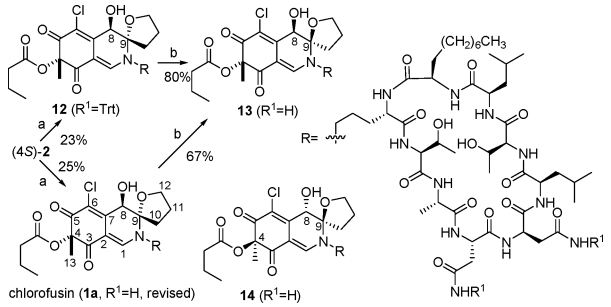
Scheme 3 [a]



[a] Reaction conditions: (a) NBS, CH_3CN, room temp; (b) $MeNH_2HCl$, aq $NaHCO_3$, CH_3CN, room temp; (c) Ag_2O, MeCN, room temp, 70% (**11a**:**11b**:**11c**:**11d** =1:1:2.5:4, by HPLC analysis).

Scheme 4 [a]



[a] Reaction conditions: (a) (4*S*)-**2** and **3a** or **3b**, same conditions as in Scheme 3; (b) TFA-H_2O (10: 1), $^iPr_3SiH$ (2 equiv), room temp.

cation and subsequent in situ mild hydrolysis serve as the key reactions to quickly generate a hydroxylated spiro aminal. Using *rac*-**2** and *rac*-**10** as substrates, various conditions were examined (Scheme 3). In most cases, all four possible products (**11a**−**d**) were generated in different yields and ratios. These four separable compounds were unambiguously characterized by X-ray single crystal analyses.[6a] By comparison of NMR data, **11d** was found to be the closest to the chromophore of chlorofusin (see the Supporting Information). This indicated that the originally proposed stereochemical assignment for the chromophore might need to be revised. To confirm our initial findings, work focused on the synthesis of **11d**. Eventually, one-pot sequential treatment of *rac*-**2** in MeCN with NBS, $MeNH_2$, and Ag_2O was found to work best, both in terms of the product ratio and the overall yield. This sequential treatment protocol greatly reduced the complexity of handling a complex molecule as large as chlorofusin and presented the advantage of ease of operation.

Encouraged by these results, coupling of (4*S*)-**2** with peptide **3b** was initially attempted using a sequential protocol, giving the expected compound **12** in 23% yield (Scheme 4). Unfortunately, final trityl-deprotection using TFA−H_2O (10:1) in the presence of $^iPr_3SiH$ (2 equiv) afforded the unexpected stereoisomer of chlorofusin, **13** (80%). Evidently, the spiro aminal in **12** opened and reformed under the acidic conditions used. This was confirmed when it was shown that *rac*-**11d** could be fully converted to *rac*-**11a** under similar conditions (detection by HPLC). A mechanism was proposed in Figure 2.

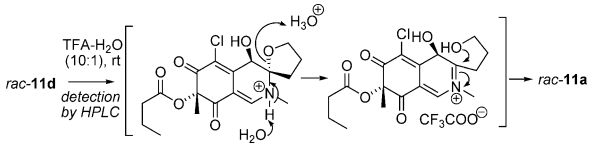


Figure 2. A proposed mechanism for conversion of **11d** to **11a**.

To avoid this side reaction, direct treatment of (4*S*)-**2** with free peptide **3a** was employed, successfully affording the expected compound **1a** (25% after purification by HPLC). The spectroscopic data of **1a** match those reported for the natural sample. To confirm these results, reaction of *rac*-**2** with free peptide **3a** was also examined, giving a 1:1 mixture of **1a** and **14**. The ^1H NMR spectrum of this mixture showed distinct chemical shift differences in the chromophore region as comparison to pure **1a** (see Supporting Information). On the basis of the above evidence, the configuration of the chlorofusin chromophore[1] was revised as (4*S*,8*R*,9*S*)-**1a**.

In summary, the first total synthesis of chlorofusin was accomplished in a convergent fashion. By comparison with all four unambiguous diastereomeric model chromophores, the absolute stereochemistry of the chlorofusin chromophore was finally determined as (4*S*,8*R*,9*S*). This allows the complete structure of natural chlorofusin to be assigned for the first time. The enantioselective copper-mediated oxidation of **4** followed by mild coupling of azaphilone **2** with the amine-bearing cyclopeptide **3** in a cascade fashion, and a final one-pot spiro-aminal formation were achieved with high efficiency to yield the correct stereochemical product. Further biological evaluation of chlorofusin and its isomers, as well as synthetic model chromophores, are underway in this laboratory and will be reported in due course.

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Supporting Information Available: Experimental procedures and characterizations of new compounds, ^1H NMR and ^{13}C NMR spectra, and tables of NMR data and HPLC comparisons and CIF files for compounds **11a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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