Tetrahedron 71 (2015) 370–380

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Stereodivergent total synthesis of chlorofusin and its all seven chromophore diastereomers

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ARTICLE INFO

Article history:
Received 17 September 2014
Received in revised form 20 October 2014
Accepted 27 October 2014
Available online 25 November 2014

Keywords:
Chlorofusin
Total synthesis
Stereodivergent synthesis
Azaphilone
p53-MDM2 interaction

ABSTRACT

Chlorofusin (**1**), one of few natural antagonists against p53-MDM2 interactions, is a naturally biogenetic hybrid composed of a 27-membered nonacyclopeptide and a unique chromophore through the hydrocarbon linkage with ornithine. In this article, we describe our recent achievement, in details, of developing a convenient stereodivergent route for parallel total synthesis of chlorofusin (**1**) and its all seven chromophore diastereomers (**1a**–**1g**) in enantiopure forms, starting from a common racemic azaphilone precursor **10**. The newly developed total synthesis shows the great advantages of economy, scalability, stable intermediates, high yields, ease of HPLC-free operations and reduplication.

1. Introduction

The tumor suppressor p53, a tetrameric nuclear transcription factor playing crucial roles in regulating the cell growth, exists at low and unstable level in the normal state of cells.[1] It can be stabilized and activated under stress, resulting in a high cellular concentration and subsequent growth arrest, DNA repair or apoptosis.[2] A number of target genes can be transcriptionally activated by p53, including MDM2 (the human homolog is called HDM2).[3] Basically, both p53 and MDM2 are kept at very low level in unstressed cells, and they form an autoregulatory feedback loop,[4] in which p53 binds to the p2 promoter of MDM2 and activates its expression.[5] Moreover, such balance is regulated mainly by MDM2 due to its strong growth suppressive activity, and overexpression of MDM2 in many human cancers effectively inhibits p53 functions and leads to uncontrolled cell proliferation. Multiple mechanisms[6] are involved in p53-inactivation by MDM2, through 1) directly binding to the N-terminal transactivation domain of p53 and interrupting its transcriptional activity; 2) acting as an E3 ubiquitin ligase to promote p53 ubiquitination and degradation in the proteasome; and 3) enabling the export of p53 out of the nucleus via nuclear export signaling.

X-ray crystal study of the p53-MDM2 complex[7] revealed that a relatively deep hydrophobic cleft exists on the surface of the MDM2 protein, and three key amino acid residues of the p53 peptide (Phe19, Trp23 and Leu26) plays critical roles in the binding between the two proteins. This information of the binding site definitely provides the helpful structural basis for designing small-molecule antagonists, which could bind to MDM2 and block the MDM2-p53 interactions, so as to release p53 and reactivate its functions. As a novel strategy for oncogene therapy, early efforts focused on the small peptides mimicking p53, which showed good binding affinity with MDM2 except for poor bioavailability.[8] Non-peptide inhibitors have also been reported. Among recent achievements, JNJ-26854165[9] and RG7112 are two promising small-molecules undergoing the clinical trials.[10] In addition, three natural products (chlorofusin, chalcone derivatives[11] and hexylitaconic acid[12]) have been reported to exhibit inhibitory activity against the p53-MDM2 interactions.

Chlorofusin (**1**) was discovered as a novel secondary metabolite by isolation from the tropical insect-associated fungal strain *Microdochium caespitosum* during a screening program, and it exhibited low micromolar inhibitory ability by direct binding to the N-terminal domain of MDM2 (IC_{50} 4.6 μM, K_D 4.7 μM).[13] On the basis of the spectroscopic evidences, chlorofusin was proposed to contain a densely functionalized azaphilone-derived chromophore linked through the terminal amine of ornithine to a 27-membered nonacyclopeptide. The cyclic peptide moiety contains two L-threonines, one L-alanine, one L- and D-asparagines, two D-leucines, one

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http://dx.doi.org/10.1016/j.tet.2014.10.062
0040-4020/

D-2-aminodecanoic acid, and one L-ornithine. The two asparagines, Asn3 and Asn4, were suggested to have opposite stereochemistries (with a combination of L-Asn3 and D-Asn4 *or* that of D-Asn3 and L-Asn4). Though absolute chemistry of the chromophore was difficult to determine by spectroscopic methods, all three oxygenated functionalities (C-4, C-8 and C9) were initially suggested to be on the same face of the chromophore by NOE experiments.[13a] Inspired by the unique chemical structure and promising biological properties of chlorofusin, the groups of Boger[14] and Searcey[15] synthesized the cyclic peptide of chlorofusin independently in 2003, and delivered an assignment of the cyclopeptide moiety with a combination of L-Asn3 and D-Asn4. Later, Nakata[16] and our group[17d] also reported the synthesis of the cyclic peptide. In 2007, we revised the relative stereochemistry of the chromophore part and completed an enantioselective total synthesis of the (4*S*,8*R*,9*S*)-diastereomer of chlorofusin using an asymmetric oxidative dearomatization reaction.[17a] Shortly thereafter, Boger's group accomplished the first total synthesis of natural chlorofusin and its seven chromophore diastereomers and reassigned the absolute stereochemistry of the natural chromophore as (4*R*,8*S*,9*R*) (as shown in Fig. 1).[18]

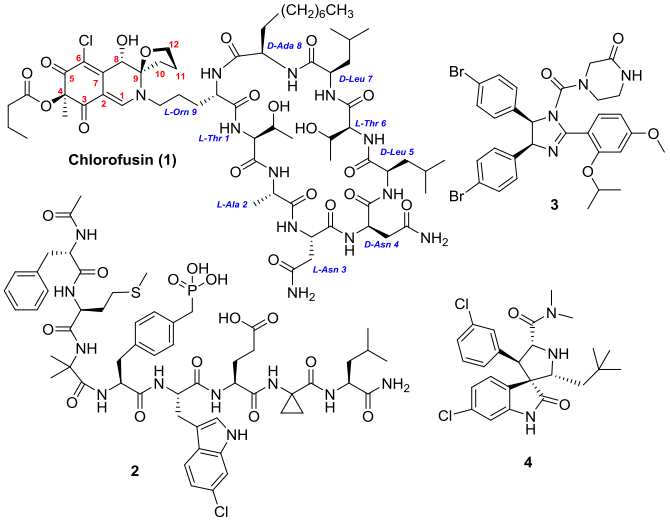

Fig. 1. Several natural and unnatural small-molecule antagonists against p53-MDM2 interactions.

Our previous total synthesis of the (4*S*,8*R*,9*S*)-diasteromer of chlorofusin was successfully disclosed in a convergent fashion (Fig. 2), via mild N-insertion of the cyclic peptide **6** to an optically pure azaphilone (4*S*)-**5** prepared from asymmetric synthesis and one-pot elaboration of the spiro-aminal functionality and reverse-phase HPLC separation at the final step.[17a] Accordingly, semi-preparative chiral HPLC separation of the racemic azaphilone was adopted by Boger's group in their subsequent total synthesis of all eight chromophore diastereomers of chlorofusin, and a one-step oxidation/spirocyclization provided all eight diastereomers of chromophore-dipeptide conjugates.[18] Based on the total synthesis, Searcey tested a number of cyclopeptide derivatives and found they showed little or no inhibitory activity against p53/MDM2 interactions.[19a] Boger also investigated the binding affinity of all eight chlorofusin diastereomers and the synthetic fragments with MDM2, and included that the stereochemistry of chromophore showed little effects on the binding affinity, and molecular integrity was found to be more important and essential for the bioactivity.[19b]

Very recently, a new HPLC-free convergent total synthesis of chlorofusin (**1**) was accomplished in our laboratory.[20] This total synthesis also enabled us to conveniently prepare several unnatural Click hybrids using common azaphilone intermediates. Biological assessment revealed that two chlorofusin-like mimics having

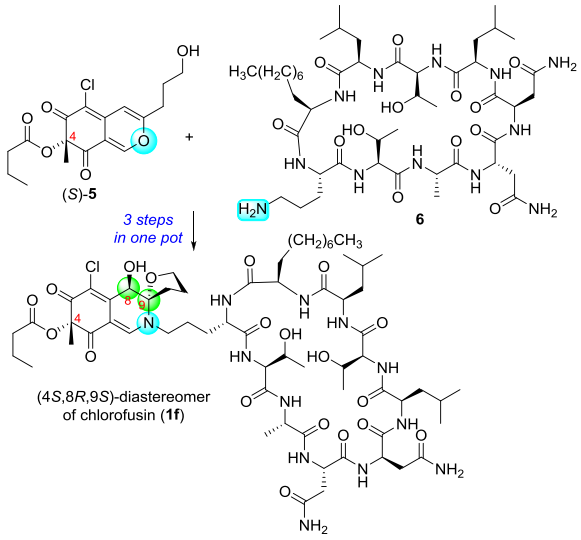

Fig. 2. Key strategies applied in our previous total synthesis of the (4*S*,8*R*,9*S*)-diasteromer of chlorofusin.[17a]

a 1,2,3-triazole linkage exhibited improved inhibitory activities against p53-HDM2 interactions over the natural product.[20] More importantly, the newest synthesis provided us a further opportunity for the total synthesis of all the chromophore diastereomers of chlorofusin. Herein, we wish to report, in details, our recent expansion of this methodology to the stereodivergent total synthesis of natural chlorofusin and its seven chromophore diastereomers starting from a common racemic azaphilone.

2. Results and discussion

2.1. Stereodivergent preparation of fully functionalized chromophores

A key task in the total synthesis of chlorofusin (**1**) is the construction of its chromophore core. It is noteworthy here that few currently available enantioselective methodologies are able to provide both individual *R* and *S* enantiomers of the C-4 quaternary stereocenter.[17a,22] In order to obtain all single-form diastereomers of the chlorofusin chromophore, chirality discrimination of *rac*-azaphilone **10** was considered by us as a useful protocol in this synthesis, utilizing our previously established azaphilone–amination reaction and an economically available chiral amine.[17b] Such a protocol would be advantageous in material-raising in a multi-step total synthesis. We found that Ag(I)-catalyzed oxo-alkyne cycloisomerization of *o*-alkynylbenzaldehyde **7** followed by IBX oxidation[17a,21] was scalable to afford sufficient quantity of *rac*-**8** for the following synthesis (Scheme 1). The reaction[17,23] was slightly optimized (scaled up to 10 g) to provide *rac*-**8** in one-pot (80% yield). The newly born tertiary alcohol **8** was then transformed into the corresponding ester **9** with butyryl chloride and DMAP in pyridine. Site-selective chlorination was carried out with SO_2Cl_2 in dichloromethane, giving the racemic azaphilone **10**. Condensation of azaphilone *rac*-**10** with commercially available (*R*)-(+)-4-methoxyl-α-methylbenzylamine **11** in CH_3CN and aq $NaHCO_3$,[17b,24] affording two flash chromatography-separable diastereomeric vinylogous γ-pyridones **12** and **13** (1:1 dr) at room temperature. The absolute stereochemistry of the C-4 carbon of each single diastereomer was determined by circular dichroism (CD) spectroscopy[17,18] (Fig. 3). To facilitate the establishment of C8/C9 stereochemistry, the chiral auxiliary was removed with (*i*-Pr)$_3$SiH in TFA and water, and replaced with an *N*-allyl with allyl bromide in the presence of catalytic amount of $Pd(PPh_3)_4$ and