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Bromoetherification-based strategy towards the spirocyclic chromophore of chlorofusin

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Abstract—A short and direct route to the racemic model chromophore of the potent p53-MDM2 antagonist, chlorofusin, is presented. The spirocyclic chromophores were successfully constructed in high yields by an NBS-mediated intramolecular haloetherification. Ring expansion was observed in the subsequent reaction of the bromides with silver nitrate in refluxing acetone and water. Conversion of the bromides to the desired ketones was finally achieved by NMO oxidation. All four possible isomeric chromophores were finally synthesized and unambiguously characterized by X-ray crystallography studies.

The p53 protein plays a key role in apoptosis pathways which help to maintain the genomic integrity of cells by coordinating the cellular response to DNA damage through induction of cell cycle arrest.[1] However, in approximately half of all cancers, p53 is inactivated by mutations and other genomic alterations,[2] and in many of the remaining cancers, it is functionally inactivated by the binding of the cellular MDM2 (HDM2).[3] MDM2 regulates the transcription factor p53 by binding to its transactivation domain and promoting its ubiquitin-dependent degradation.[4] While overexpression of MDM2 has been observed in many tumors,[5] it has been shown that p53 function can be restored by disrupting its interaction with MDM2. Therefore, discovery and development of small molecules to inhibit the p53-MDM2 interaction has emerged as an important field for cancer chemotherapies.[6] Recently, Williams and co-workers reported the isolation of an architecturally complex cyclopeptide chlorofusin (Fig. 1) as a secondary metabolite of *Microdochium caespitosum*.[7] Chlorofusin has been shown to inhibit p53-MDM2 interaction with an IC_{50} of 4.7 μM by direct binding to the N-terminal domain of MDM2.[8] Chlorofusin is, therefore, of potential interest as a lead in developing new anticancer therapeutics.[9]

Structurally, chlorofusin is characterized by a cyclic peptide containing an L-ornithine side-chain that incorporates a densely functionalized chromophore. The originally ambiguously absolute-stereochemistry of the cyclic peptide portion was recently determined by chemical synthesis and NMR studies.[10] However, the absolute stereochemistry of the chromophore still remains unknown, although its relative configuration was deduced from spectroscopic studies, showing that the protons attached to C-8, C-10 and C-13 all lie on the same side of the chromophore (Fig. 1). As part of our studies towards the total synthesis and stereochemical assignment of chlorofusin, we present herein a facile synthesis of azaoxaspirane **1**, a model of chlorofusin

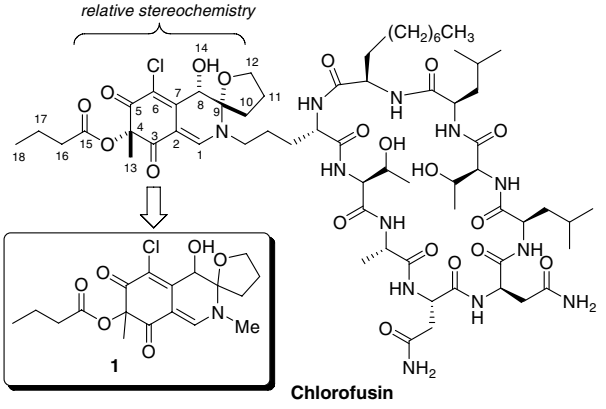

Figure 1. Primary structure of chlorofusin and its model chromophore **1**.

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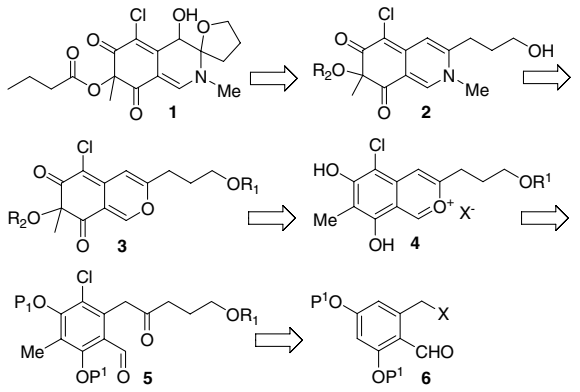
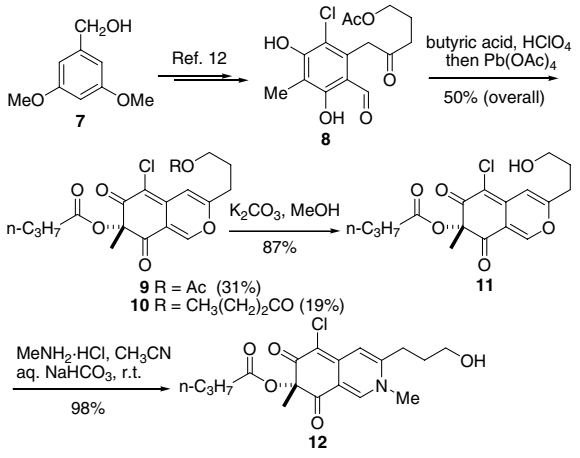


Figure 2. Retrosynthesis of azaoxaspirane **1**.

chromophore, as well as studies on the chemistry of related intermediates.

The retrosynthetic analysis employed in the synthesis of azaoxaspirane **1** is outlined in Figure 2. We envisioned that the five-membered *N*,*O*-spiroketal could be formed from the vinylogous γ-pyridone **2** by a suitable cyclization reaction, such as an oxidative spirocyclization or an intramolecular electrophile-mediated cyclization. Synthesis of the vinylogous γ-pyridone **2** could be easily achieved from tertiary carbinol **3** by insertion of a primary amine.[11,12a] Precursor **3** belongs to a structurally diverse family of natural products called azaphilones,[13] which have spurred the development of several synthetic routes, including recently asymmetric efforts.[14,15] Generally, pyronoquinones and pyrylium salts derived from formyl ketones are highly useful intermediates, which can be transformed into 6*H*-isochromene ring system by oxidative reaction.

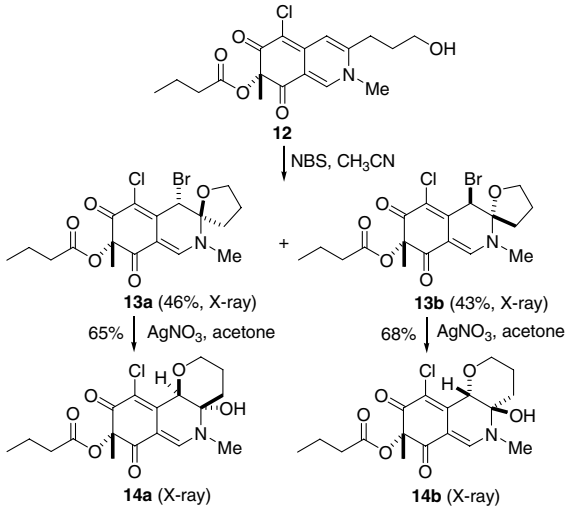
Recently, we reported a practical synthesis of chloroazaphilone and vinylogous γ-pyridone, utilizing $Pb(OAc)_4$ oxidation of the corresponding $HClO_4$/HOAc pyrylium salts in a sequential one-pot operation.[12] Herein, we follow this procedure to prepare the key intermediate **12**, as shown in Scheme 1. The chlorine atom was introduced prior to the formation of azaphilone nucleus, using SO_2Cl_2 to afford aryl chloride **8**. Previous studies on



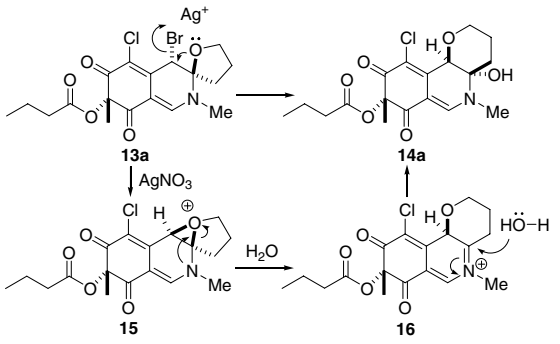
Scheme 1. Synthesis of vinylogous γ-pyridone **12**.

the formation of isochromenes utilized lead tetra-acetate as an oxidant and various carboxylic acids as reactants.[14c] Treatment of formyl ketone **8** with $HClO_4$ at room temperature in butyric acid, followed by lead tetra-acetate oxidation of the resulting pyrylium salt in a one-pot fashion, afforded compound **9** and simultaneously **10** in 50% overall yield. This one-pot process also allows to efficiently install the butyl acyl group. Although the product was a mixture of **9** and **10**, bearing different acyl groups, hydrolysis of the mixture of **9** and **10** was carried out in methanol using potassium carbonate to provide desired alcohol **11** in high yield. Our attention then turned to the construction of the tricyclic core presented in chlorofusin. In order to address the relative stereochemistries produced in the spirocycle formation and to gain additional information for the future total synthesis of chlorofusin, we chose vinylogous γ-pyridone **11** as a substrate to match the characteristics of the natural product. Therefore, vinylogous γ-pyridone **12** was prepared from azaphilone **11** by treatment with methylamine in acetonitrile.

As mentioned above, the most direct approach to the target azaspiroketal having a flanking hydroxyl group would be via the selective epoxidation or dihydroxylation of the 8,9-double bond of vinylogous γ-pyridone **12**. However, all efforts towards this end failed. Thus, our attention was turned to other intramolecular electrophilic cyclizations.[16] To our delight, treatment of vinylogous γ-pyridone **12** with NBS in CH_3CN at ambient temperature afforded the somehow unstable bromides **13a** and **13b** (46% for **13a**, 43% for **13b**) (Scheme 2). Utilizing intramolecular NBS-mediated haloetherification, an efficient method for spirocyclization of precursor **12** was established, giving predominantly a five-membered ring (5-*exo* attack, exclusively). X-ray crystallographic analyses of **13a** and **13b** confirmed the *anti* relative stereochemistry of the spiro cyclization, which was in keeping with the expected results of haloetherification. With this efficient bromoetherification reaction in hand, the azaspirocycles were then sub-



Scheme 2. NBS-promoted spirocyclization and Ag(I)-mediated ring expansion.

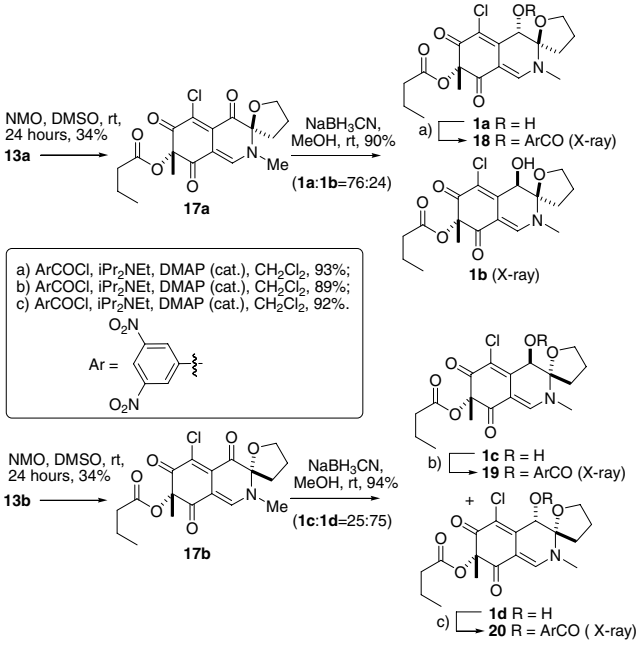


Scheme 3. Proposed mechanism for ring expansion of bromide **13a**.

jected to nucleophilic displacement of the bromide substituents. Aqueous $AgNO_3$ in refluxing acetone provided clean conditions for transformation to the corresponding 'alcohol' species (Scheme 2). Unfortunately, it turned out that the ring expansion products **14a** and **14b** were respectively obtained under such conditions. Both structures (**14a** and **14b**) were confirmed unambiguously by X-ray crystallographic studies.

On the basis of the above experimental results, a possible rationalization of the observed ring expansion[17] is proposed in Scheme 3. Warming of bromide **13a** with aqueous silver nitrate resulted in the formation of a carbon cation, which was stabilized by the neighboring azaspirocycle oxygen atom, forming an oxonium ion **15**. C–O bond fragmentation of the oxonium species under the driving force of the nitrogen lone electron pair would generate an iminium ion intermediate **16**. Finally, nucleophilic attack of hydroxide from the sterically less hindered face of the newly formed 4*H*-pyran ring would result in the formation of the stable ring expansion product **14a**.

To avoid the ring expansion, other milder hydrolysis conditions were also fully investigated. However, despite



Scheme 4. Synthesis of spirocyclic chromophores.

extensive efforts, all attempts to attain this goal failed to give desirable results. To our delight, direct transformation of these bromides to the corresponding spiroketones was achieved in moderate yields using oxidative conditions of NMO in DMSO[18] (Scheme 4). Reduction of **17a** using $NaBH_3CN$ in methanol at rt generated two new products **1a** and **1b** in high yield (90%, **1a**:**1b** = 76:24). Fortunately, these two diastereomers could be easily separated and purified by routine column chromatography on silica gel. Accordingly, exposure of **17b** to the same conditions afforded the analogous isomers **1c** and **1d** (94%, **1c**:**1d** = 25:75). Separation of **1c** and **1d** was achieved by simple recrystallization (hexane/ethyl acetate), relying on the great solubility difference between these two compounds (solubility of **1c** is much lower than that of **1d**). Analytically pure sample of **1d** was finally obtained by semi-preparative reverse phase HPLC. Following the conditions indicated above, all four possible tricyclic cores related to the chlorofusin chromophore were obtained from the vinylogous γ-pyridone **12**. The relative configurations were unambiguously determined by single-crystal X-ray crystallographic analyses, using either direct (isomer **1b**) or indirect (isomers **1a**,**c** and **1d** using their 3,5-di- nitrobenzoate derivatives) measurements (For details, please see Supplementary data.). This allowed the first time a clear correlation of the respective NMR spectra[19–22] to the corresponding chemical structures in a one-to-one fashion. Such information is definitely of great value to help the total synthesis of chlorofusin and its analogues, as well as the spiro-intermediates produced therein.

In conclusion, our recent studies on methodology to construct the unique chromophore of chlorofusin, a natural p53-MDM2 antagonist, are described. An efficient approach to the azaphilone nucleus precursors, and a short and direct route to the typical chromophores characterized our strategy. The desired azaspirocycles were successfully constructed by an intramolecular NBS-mediated haloetherification. These syntheses allowed us to get all four possible isomers of such a chromophore. X-ray crystallographic studies of these isomers and their intermediates allowed the NMR spectra to be clearly assigned to the corresponding chemical structures. Further studies towards the total synthesis of chlorofusin are underway in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.04.062.

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19. Characterization data of **1a**: yellow solid, mp 182–185 °C (dec.). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.64 (1H, s), 4.75 (1H, s), 4.12 (1H, dt, $J = 3.9$ and 8.2 Hz), 3.88 (1H, dt, $J = 1.8$ and 8.6 Hz), 3.23 (3H, s), 2.74 (1H, br s), 2.52–2.43 (2H, m), 2.42 (2H, t, $J = 7.3$ Hz), 2.19–2.16 (1H, m), 2.12–2.08 (1H, m), 1.69–1.64 (2H, m), 1.55 (3H, s), 0.96 (3H, t, $J = 7.4$ Hz) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 190.7, 189.4, 173.0, 150.6, 145.2, 118.2, 102.0, 96.2, 84.7, 69.74, 69.71, 38.9, 35.2, 31.0, 25.7, 23.2, 18.3, 13.1 ppm. IR (KBr): ν_{max} 3386, 1737, 1685, 1641, 1581, 1424, 1080, 1037, 850, 773 cm^{-1}. HRMS (ESI, *m*/*z*) calcd for $C_{18}H_{22}ClNO_6Na$ (M+Na$^+$): 406.1028; found, 406.1043.
20. Characterization data for **1b**: yellow solid, mp 142–144 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.67 (1H, s), 4.80 (1H, s), 4.22–4.16 (2H, m), 3.20 (3H, s), 2.83 (1H, br s), 2.43 (2H, t, $J = 7.5$ Hz), 2.15–2.10 (2H, m), 2.11–2.04 (1H, m), 1.94–1.90 (1H, m), 1.70–1.64 (2H, m), 1.54 (3H, s), 0.98 (3H, t, $J = 7.4$ Hz) ppm. ^{13}C NMR ($CDCl_3$, 75.0 MHz): δ 191.0, 190.5, 174.0, 150.0, 145.1, 118.7, 101.5, 97.7, 85.3, 71.3, 70.0, 37.5, 35.4, 33.8, 26.0, 23.2, 18.4, 13.6 ppm. IR (KBr): ν_{max} 3385, 2966, 1737, 1683, 1641, 1581, 1417, 1359, 1323, 1236, 1084, 1063, 851, 775 cm^{-1}. HRMS (ESI, *m*/*z*) calcd for $C_{18}H_{22}ClNO_6Na$ (M+Na$^+$): 406.1028; found, 406.1041.
21. Characterization data for **1c**: mp 155–157 °C. ^{1}H NMR ($CDCl_3$:CD_3OD = 10:1, v/v, 400 MHz): δ 7.68 (1H, s), 4.74 (1H, s), 4.10–4.07 (1H, dt, $J = 4.1$ and 8.2 Hz), 3.88–3.83 (1H, dt, $J = 6.8$ and 8.7 Hz), 3.39 (1H, br s), 3.23 (3H, s), 2.45–2.41 (2H, m), 2.42 (2H, t, $J = 7.4$ Hz), 2.18–2.14 (1H, m), 2.10–2.04 (1H, m), 1.69–1.64 (2H, m), 1.53 (3H, s), 0.97 (3H, t, $J = 7.4$ Hz) ppm. ^{13}C NMR ($CDCl_3$:CD_3OD = 10:1, v/v, 150 MHz): δ 190.9, 190.7, 173.3, 151.4, 147.0, 117.9, 102.3, 97.0, 85.1, 70.1, 69.8, 39.4, 35.6, 31.6, 26.1, 23.4, 18.7, 14.0 ppm. IR (KBr): ν_{max} 3350, 2850, 1643, 1584, 1080, 1042 cm^{-1}. HRMS (ESI, *m*/*z*) calcd for $C_{18}H_{22}ClNO_6Na$ (M+Na$^+$): 406.1028; found, 406.1028.
22. Characterization data for **1d**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.74 (1H, s), 4.79 (1H, s), 4.26–4.17 (2H, m), 3.23 (3H, s), 2.80 (1H, br s), 2.41 (2H, t, $J = 7.3$ Hz), 2.16–2.10 (2H, m), 2.08–2.01 (1H, m), 1.93–1.88 (1H, m), 1.71–1.61 (2H, m), 1.52 (3H, s), 0.96 (3H, t, $J = 7.4$ Hz) ppm. ^{13}C NMR ($CDCl_3$, 150 MHz): δ 190.4, 189.6, 172.6, 149.5, 145.0, 117.4, 100.9, 97.0, 84.2, 70.8, 69.8, 37.5, 35.1, 33.8, 25.6, 23.4, 18.2, 13.5 ppm. IR (KBr): ν_{max} 3500, 1713, 1683, 1635, 1582, 1417, 1363, 1323, 1222, 1094, 1060 cm^{-1}. HRMS (ESI, *m*/*z*) calcd for $C_{18}H_{22}ClNO_6Na$ (M+Na$^+$): 406.1028; found, 406.1027.