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Total Synthesis of (+)-Chaetocin and its Analogues: Their Histone Methyltransferase G9a Inhibitory Activity

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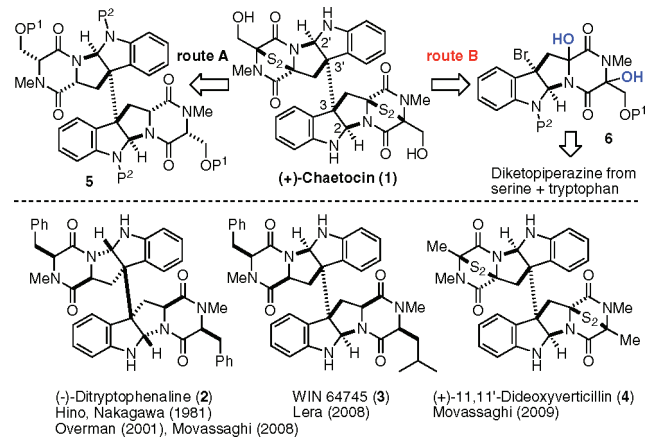
(+)-Chaetocin (**1**) is a complex epidithiodiketopiperazine (ETP) alkaloid produced by *Chaetronium minutum*, which was isolated in 1970.[1] In addition to its antibacterial and cytostatic activity,[1,2] **1** is known to be a potent inhibitor of lysine-specific histone methyltransferases (HMTs).[3] Because methylation of histone proteins plays important roles in controlling gene expression patterns,[4] synthetic study of **1** and its analogues would be helpful in developing biological tools for epigenetic research. However, total synthesis of **1** has not been reported to date. We herein describe the first total synthesis of (+)-**1**, as well as its antipode and sulfur-deficient analogues. We also examined the biological activity of these compounds toward H3K9 HMT G9a, which is a potential therapeutic target in human cancer.[5]

Following the early report by Nakagawa and Hino,[6a] elegant methodologies were developed to synthesize the dimeric octacyclic carbon frameworks characteristic of this family, and several total syntheses of related compounds have been reported (Scheme 1).[6b−d] Although there had been no report on the chemical synthesis of this subclass of the ETP family when we started our project, Movassaghi and co-workers recently completed a landmark total synthesis of dimeric ETP **4**.[7,8] Based on the total synthesis of **2**, they installed disulfide bridges into the octacyclic core through α-hydroxylation with $[Ag(py)_2]MnO_4$ as a stoichiometric oxidant. In spite of their successes, the chemical synthesis of **1** still poses the additional problem of how to prevent facile *β*-elimination of serine units, if serine is used as a starting material. Keeping a proposed biosynthetic route in mind,[7] we initially attempted the α-oxidation of dimeric compound **5** (Scheme 1, route A). We selected a radical bromination reaction[9] for this purpose, since enolate chemistry seemed inappropriate. However, the octacyclic core was found to be extremely unstable under radical conditions, resulting in complete decomposition.[10] Therefore, we decided to test the early stage oxidation of the diketopiperazine (DKP) unit (route B), which is distinct from the bioinspired late-stage oxidation strategy employed in the synthesis of **4**. To our delight, oxidized compound **6** was accessible via bromocyclization of the corresponding DKP, followed by radical α-bromination (*vide infra*). We expected that the octacyclic core structure would be constructed if the compound **6** reacted without problem in Movassaghi's Co(I)-mediated radical dimerization reaction.[11]

According to the early stage oxidation strategy, the total synthesis of (+)-**1** was achieved as shown in Scheme 2. DKP **9** was

Scheme 1. Dimeric DKPs and Retrosynthesis of (+)-Chaetocin (**1**)



synthesized without epimerization in 69% yield (5 steps) starting from known *N*-Cbz-protected *N*-methyl-D-serine (**7**)[12] and commercially available D-Try-OMe•HCl (**8**).[13] The obtained DKP was subjected to the bromocyclization reaction with NBS to afford **10** in 88% yield. This reaction was highly stereoselective, and no appreciable amount of the other stereoisomer was detected.[14] The relative stereochemistry of **10** was unambiguously determined by X-ray analysis after simple conversion, revealing that the bromine atom is positioned *trans* to the α-protons of the DKP ring.[13] Because retention of the stereochemistry is ensured in the Co(I)-mediated radical dimerization reaction,[11] the absolute stereochemistry at the benzylic position would be directly transferred to those at the 3 and 3′ positions of (+)-**1**. Thus, the tetracyclic compound **10** derived from D-amino acids, and not from L-amino acids, can serve as a key precursor to (+)-**1**.

From **10**, the total synthesis was accomplished in an additional three steps. While a standard radical bromination reaction using AIBN at high temperature led to decomposition of **10**, the reaction at room temperature using V-70[15] as a radical initiator gave the desired tribromide **11** cleanly in a stereoselective manner,[16] even though the α-protons of the DKP ring point to the concave face of the fused 5,5-ring system. After simple filtration to remove precipitated succinimide, the obtained crude tribromide was treated with phosphate buffer to afford diol **12** in 47% yield as a major stereoisomer,[17] accompanied with other three minor diastereomers (12% in total).[13] Since the obtained diol **12** was relatively unstable in $CDCl_3$, we thought that the protection of the resulting hemiaminals might be necessary before examining the dimerization reaction. However, to our delight, the hemiaminal was not sensitive

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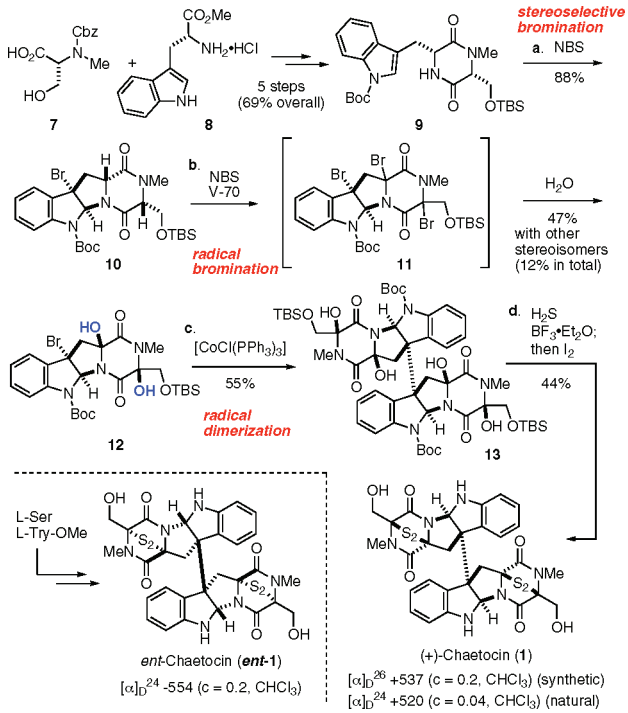
Scheme 2. Total Synthesis of (+)- and *ent*-Chaetocin[a]

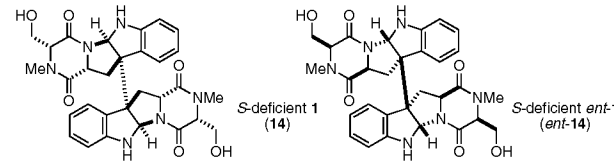


[a] Conditions: (a) NBS, MeCN, −30 °C, 10 h; (b) NBS, V70, CCl_4, rt, 5 h; then pH7 phosphate buffer/MeCN = 1/1, rt, 3 h; (c) $CoCl(PPh_3)_3$, acetone, rt, 1.5 h; (d) H_2S, BF_3•Et_2O, CH_2Cl_2, −78 °C to rt, sealed tube, 1.5 h; then I_2.

to a reductive coupling reaction using a Co(I) complex,[11] so that the unprotected diol **12** could be directly used to furnish the desired octacyclic tetraol **13** as a single isomer in 55% yield.

With the dimeric tetraol in hand, the stage was set for the construction of the disulfide bridges. The reaction of **13** with condensed H_2S (bp −60.7 °C) was carried out at −78 °C in the presence of BF_3•OEt_2. Examination of the ^{1}H NMR spectrum of the crude product indicated that the sulfur nucleophile was delivered to the putative iminium ions stereoselectively, probably mainly from the outer surface of the double-decker structure. After aqueous workup, the crude mixture in ethyl acetate was treated with I_2, and pure (+)-**1** was isolated in 44% yield. The obtained compound was spectroscopically identical to a natural sample of (+)-**1**. It should be noted that no less than ten bond-forming and cleaving events, namely four substitution reactions (OH→SH), deprotection of four Lewis acid-sensitive protecting groups (TBS and Boc groups), and two S−S bond formations, occurred in the final step.

This success prompted us to investigate the influence of the absolute stereochemistry and the sulfur functionality of **1** on its biological activity. Thus, based on the established route, we synthesized the antipode of (+)-**1** (*ent*-**1**) and the sulfur-deficient analogues **14** and *ent*-**14**,[13] and the inhibitory activity of these compounds against G9a was examined using a known ELISA method.[3b] Interestingly, **1** and *ent*-**1** inhibited G9a equally effectively (IC_{50}: 2.4 and 1.7 μM, respectively). In contrast, sulfur-deficient analogues **14** and *ent*-**14** were inactive (IC_{50} >50 μM). These results clearly indicate that the sulfur functionality is crucial for the biological activity, while G9a is not sensitive to the 3D structure of **1**.[13]



In summary, the first total synthesis of (+)-chaetocin has been accomplished in only nine steps starting from the known *N*-Cbz-*N*-Me-serine, taking advantage of two radical reactions as key reactions. The structure−inhibitory activity relationship of the synthesized compounds should be helpful for the future design of biological tools. Further structure−activity studies are under way.

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Supporting Information Available: Complete ref 3c, detailed experimental procedures, full characterization of new compounds, copies of ^{1}H and ^{13}C NMR spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) The stereochemistry was tentatively assigned based on the experimental results described in the Supporting Information (L).

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