

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

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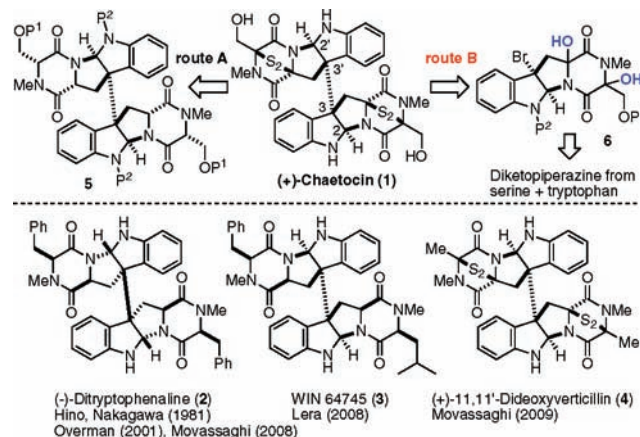
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(+)-Chaetocin (**1**) is a complex epidithiodiketopiperazine (ETP) alkaloid produced by *Chaetronium minutum*, which was isolated in 1970.¹ In addition to its antibacterial and cytostatic activity,^{1,2} **1** is known to be a potent inhibitor of lysine-specific 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]MnO₄ 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 reaction⁹ 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**.

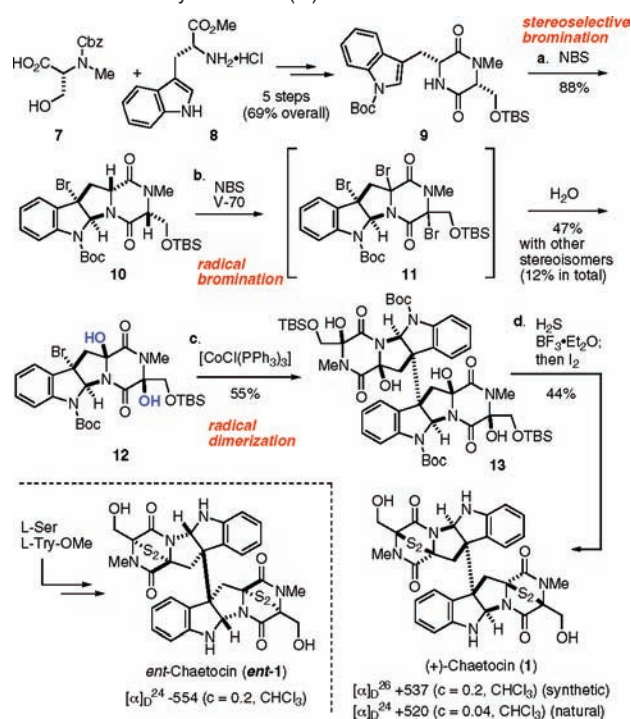
From **10**, the total synthesis was accomplished in an additional three steps. While a standard radical bromination reaction using AIBN at high temperature led to decomposition of **10**, the reaction at room temperature using V-70¹⁵ as a radical initiator gave the desired tribromide **11** cleanly 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 CDCl₃, 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

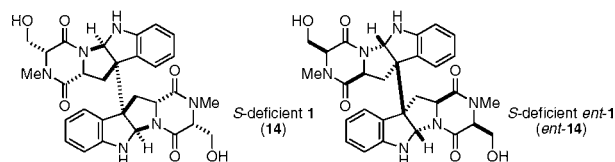
^a Conditions: (a) NBS, MeCN, -30 °C, 10 h; (b) NBS, V70, CCl₄, rt, 5 h; then pH7 phosphate buffer/MeCN = 1/1, rt, 3 h; (c) CoCl(PPH₃)₃, acetone, rt, 1.5 h; (d) H₂S, BF₃·Et₂O, CH₂Cl₂, -78 °C to rt, sealed tube, 1.5 h; then I₂.

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 H₂S (bp -60.7 °C) was carried out at -78 °C in the presence of BF₃·OEt₂. Examination of the ¹H 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 I₂, 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 (IC₅₀: 2.4 and 1.7 μM, respectively). In contrast, sulfur-deficient analogues **14** and *ent*-**14** were inactive (IC₅₀ >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 ¹H and ¹³C 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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