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Synthesis and Biological Evaluation of Epidithio-, Epitetrathio- and *bis*-(Methylthio)diketopiperazines. Synthetic Methodology, Enantioselective Total Synthesis of Epicoccin G, 8,8′-*epi*-*ent*-Rostratin B, Gliotoxin, Gliotoxin G, Emethallicin E and Haematocin, and Discovery of New Antiviral and Antimalarial Agents

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Abstract

An improved sulfenylation method for the preparation of epidithio-, epitetrathio- and *bis*-(methylthio)diketopiperazines from diketopiperazines has been developed. Employing NaHMDS and related bases and elemental sulfur or *bis*[*bis*(trimethylsilyl)amino]trisulfide (**23**) in THF, the developed method was applied to the synthesis of a series of natural and designed molecules, including epicoccin G (**1**), 8,8′-*epi*-*ent*-rostratin B (**2**), gliotoxin (**3**), gliotoxin G (**4**), emethallicin E (**5**) and haematocin (**6**). Biological screening of selected synthesized compounds led to the discovery of a number of nanomolar anti poliovirus agents (i.e. **46**, 2,2′-*epi*-**46** and **61**, Table 5) and several low micromolar anti *Plasmodium falciparum* lead compounds (i.e. **46**, 2,2′-*epi*-**46**, **58**, **61** and **1**, Table 5).

INTRODUCTION

2,5-Diketopiperazines are a ubiquitous class of compounds of diverse molecular architectures and biological activities.[1] Numerous have been discovered from natural sources while many more have been synthesized in the laboratory for biological investigations and drug discovery purposes.[1] The 2,5-diketopiperazine structural motif constitutes a unique scaffold upon which three dimensional molecules, including chiral ones, may be constructed,[1,2] thereby providing a useful alternative to the planar structural motifs

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Supporting Information. Experimental procedures and characterization data for key compounds (pdf cif files). This material is available free of charge *via* the Internet at http://pubs.acs.org.

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commonly found in drugs and drug candidates, the latter being often far from ideal in terms of pharmacological properties.[3]

Of particular interest are the naturally occurring epidithiodiketopiperazines and *bis*-(methylthio)diketopiperazines, whose biological activities include antiviral, antibacterial, antiallergic, antimalarial and cytotoxic properties.[1,4] Despite their promising biological profiles, however, these compounds remain largely unexplored, primarily due to their natural scarcity and the synthetic laboratory challenge they pose.[5,6]

In order to alleviate some of these deficiencies and facilitate biological investigations in this area, we recently initiated a research program directed toward the development of improved methods of sulfenylation of 2,5-diketopiperazines and applied them to the total synthesis of natural and designed epidithio-, epitetrathio- and *bis*-(methylthio)diketopiperazines. In preliminary communications we already reported an improved method for the sulfenylation of 2,5-diketopiperazines[7] and the total synthesis[8] of epicoccin G[9] (**1**, Figure 1) and 8,8′-*epi*-*ent*-rostratin B[10] (**2**, Figure 1). In this article we describe further studies in this area that include enantioselective total syntheses of gliotoxin[11] (**3**, Figure 1), gliotoxin G[12] (**4**, Figure 1), emethallicin E[13] (**5**, Figure 1) and haematocin[14] (**6**, Figure 1), as well as the monomeric unit (**7**, Figure 1) of aranotin[15,16] (**8**, Figure 1). We also report our biological evaluation of a number of selected synthesized compounds that led to the discovery of potent anti poliovirus and anti *Plasmodium falciparum* agents.

RESULTS AND DISCUSSION

Methodology Development

Recognizing the deficiencies of the then available sulfenylation methods of 2,5-diketopiperazines, we set as part of our goals the development of improved sulfenylation methods to construct epidithiodiketopiperazines and *bis*-(methylthio)diketopiperazines. Figure 2 depicts a number of selected sulfenylation methods of 2,5-diketopiperazines known at the outset of our investigations. Thus, as early as 1968, Trown,[17] and subsequently Hashimoto[18] (1987), pioneered the use of 3,6-dibromodiketopiperazines (**9**) as substrates and KSAc as a sulfur source to prepare epidithiodiketopiperazines (**18**). In 1971, Poisel and U. Schmidt[19] introduced the use of sodium tetrasulfide (Na_2S_4) as a source of sulfur to produce epidithiodiketopiperazines (**10**→**18**, Figure 2), and in 1972 the classical U. Schmidt method[20] for the synthesis of these compounds from 2,5-diketopiperazines employing sulfur (S_8) and $NaNH_2$ in liq. NH_3 (**11**→**18**, Figure 2) was reported. In 1973, Kishi[21] reported a method of masking 3,6-dithiodiketopiperazines with anisaldehyde and then generating the desired epidithiodiketopiperazines at a later stage (**12**→**18**, Figure 2), a tactic that he elegantly applied to synthesize gliotoxin (**3**).[6h,6i] In 1975, Matsunari[23] utilized 3,6-dimethoxydiketopiperazines as substrates in conjunction with H_2S as a source of sulfur to prepare epidithiodiketopiperazines (**13**→**18**, Figure 2), where-as in 2002 Overman and Sato[24] employed the corresponding *bis*-acetates and H_2S in their quest of similar epidithiodiketopiperazines (**14**→**18**, Figure 2). In 2009, Movassaghi[6e] and Sodeoka[6f] applied the use of 3,6-dihydroxydiketopiperazines (**15** and **16**, respectively, Figure 2) and H_2S to construct the epidithiodiketopiperazine structural motifs (**18**, Figure 2) of their synthetic targets, 11,11′-dideoxyverticillin A and chaetocin, respectively. In 2010, Kim and Movassaghi described the use of potassium trithiocarbonate (K_2CS_3) to generate an epidithiodiketopiperazine moiety from monosilylated 3,6-dihydroxydiketopiperazine intermediate (**17**→**18**, Figure 2) in their elegant synthesis of chaetocins A and C and 12,12′-dideoxychetracin A.[6g]

Inspired by the U. Schmidt method[20] of introducing sulfur atoms into 2,5-diketopiperazines directly using S_8 and $NaNH_2$ in liq. NH_3, we opted to employ S_8 and NaHMDS or LiHMDS

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as the base in THF. Our expectations included not only the convenience of carrying out the sulfenylation reaction in an organic solvent rather than liq. NH_3, but also the possibility of generating more well-defined sulfenylating species to effect the desired reaction more efficiently and with stereocontrol. In retrospect, we realized that the reaction of S_8 with NaHMDS had already been studied by M. Schmidt[24a–c] in the 1960s, a study[24a] that we inadvertently missed in our preliminary communications.[7,8] Our investigations with this reaction are summarized in Scheme 1. Thus, from the reaction of S_8 (**19**) and NaHMDS, we were able to isolate, chromatographically, and characterize three reactive species: tetrasulfide **21** [40% yield, ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 0.26 ppm; ^{13}C NMR ($CDCl_3$, 150 MHz): δ = 2.4 ppm; HRMS $[M+H]^+$: calcd for $C_{12}H_{36}N_2O_2S_4$+H: 449.0911; found: 449.0908], pentasulfide **22** [5% yield, HRMS $[M+H]^+$: calcd for $C_{12}H_{36}N_2O_2S_5$+H: 512.0280; found: 512.0241], and trisulfide **23** [8% yield, HRMS $[M+H]^+$: calcd for $C_{12}H_{36}N_2O_2S_3$+H: 417.1190; found: 417.1186]. Their formation, presumably through intermediate **20**, may be explained as shown in Scheme 1 and it is consistent with the observations of M. Schmidt *et al.*[24] The predominance of the tetrasulfide **21** is most likely due to steric shielding and charge repulsion during the second nucleophilic attack by the $(TMS)_2N^-$ species on the sulfur chain (see **20**, Scheme 1). The reaction of the resulting mixture with 2,5-diketopiperazines as exemplified with substrate **24** in the presence of excess base (NaHMDS) as shown in Scheme 2 is consistent with the presence of these species, although only epidi- and epitetrasulfides were isolated. Reduction of the mixture (presumably containing additional sulfenylated species, such as epitri- and epipentasulfides as well as open-chain oligosulfides) with $NaBH_4$ followed by oxidation of the resulting dithiolate **26** (aq. NH_4Cl; then KI_3) led to a good yield of the epidithiodiketopiperazine **27** (69%). The same result was obtained from the pure tetrasulfide **25** (obtained in 22% yield from **24**, see Scheme 2) upon reduction/oxidation (94%). Reaction of dithiolate **26** with MeI furnished *bis*-(methylthio)diketopiperazine **28** in 72% overall yield from **24**. In support of the proposed mechanism (Scheme 2), we found that only mono-deuteration occurs upon quenching the initially formed species from substrate **24** and NaHMDS (2.2 equiv). Also in support of the intramolecular nature of the second C-S bond formation are the good yields of the epidithio- and epitetrathiodiketopiperazine products observed.

The generality and scope of the sulfenylation reaction was explored with a variety of substrates. These explorations led to a series of epidithiodiketopiperazines (Table 1) and *bis*-(methylthio)diketopiperazines (Table 2). Thus, under the reaction conditions shown in Table 1, 3,6-unsubstituted diketopiperazines such as **29** (entry 1) reacted to form epidithiodiketopiperazines (i.e. **30**, entry 1), albeit in modest yield (40%), the latter observation being attributed to possible unhindered intermolecular reactions of the intermediate sulfur species. This speculation is supported by the higher yields observed with 3,6-mono- and 3,6-disubstituted substrates (e.g. entries 2–5). The relatively low yield of epidithiodiketopiperazine **38** (entry 6) is most likely due to the steric congestion at the sites of sulfenylation (i.e. positions 3 and 6). It is notable that both *syn* (entries 4, 8, 10–12) and *anti* (entries 3 and 9) 3,6-disubstituted diketopiperazine systems enter the reaction equally well. These include monocyclic (entries 3–7) and polycyclic (entries 8–12) systems. The fact that sulfenylation occurs from the same side of the molecule in both the *syn* and the *anti* series provides support for the intramolecular nature of the second C-S bond formation (see **24d**→**25**, Scheme 2). All epidithiodiketopiperazine products shown in Table 1 are racemic as a consequence of the enolate intermediacy in these reactions. Enantiopure compound **45** (entry 10) gave a mixture of enantiopure diastereoisomers (ca. 1.4:1 dr) due to the additional chiral centers within the structure.

Employment of the reaction conditions shown in Table 2 on the indicated substrates led to the corresponding *bis*-(methylthio)diketopiperazines. All products were isolated as single racemic *syn* compounds with the exception of **55** (entry 6), which was formed as a mixture

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of enantiopure diastereoisomers (ca. 1.4:1 dr) due to the additional stereocenters within the substrate. Again, the observation of only the *syn* product provides support for the intramolecularity of the second sulfenylation step. The excellent stereoselectivity and good yields obtained in this sulfenylation reaction and its epidithiodiketopiperazine-forming counterpart (see Table 1) demonstrate the superiority of this method in comparison to the traditional U. Schmidt process that often leads to mixtures of the *syn* and *anti* products in lower yields.

The effect of the alkali metal in the base on the efficiency of the reaction was then examined. Thus, KHMDS, NaHMDS and LiHMDS were used in the sulfenylation protocol shown in Table 1 using diketopiperazine substrates **24**, **41** and 2-*epi*-**43** to generate epidithiodiketopiperazines **27**, **42** and **44**, respectively. As shown in Table 3, the results consistently point to NaHMDS as the preferred base for this reaction, although all three bases gave good yields of the epidithiodiketopiperazine products. As we shall see below, however, this is not always the case, especially with more sensitive substrates (see Table 4).

Previously known[24a] *bis*[*bis*(trimethylsilyl)amino]trisulfide (**23**, Scheme 3) was prepared and investigated for its suitability as a sulfenylating agent of 2,5-diketopiperazines in the presence of base. Thus, pure **23** reacted with diketopiperazine **24** in the presence of NaHMDS in THF at ambient temperature to produce a mixture of epidithiodiketopiperazine **27** (43%) and epitetrathiodiketopiperazine **25** (22%). A speculative mechanism for the formation of these products is shown in Scheme 3.[25] Thus, the initially formed enolate **24b** may react with trisulfide **23** through path a (attack at terminal S) to afford trisulfide intermediate **24e**, which may then suffer in-tramolecular attack by the second enolate (**24f**) to afford epidithiodiketopiperazine **27** and $(TMS)_2NSNa$ (**23b**). The same product (**27**) could be formed from enolate **24b** and trisulfide **23** through path b (attack at the central S) *via* the intermediacy of species **24g** and **24h** by intramolecular attack as shown in the scheme. Alternatively, trisulfide intermediate **24f** may undergo different intramolecular collapse to generate, through path c, epitrithiodiketopiperazine **24i**,[26] whose opening with $(TMS)_2N^-$ as shown may form epitetrathiodiketopiperazine **25** *via* intermediate species **24j**.

Total Syntheses of Epicoccin G (1), 8,8′-*epi*-*ent*-Rostratin B (2), Gliotoxin (3), Gliotoxin G (4), Emethallicin E (5) and Haematocin (6). Empowered with the improved sulfenylation method[7,8] we were able to synthesize a number of biologically active sulfenylated diketopiperazine natural products[8] (Figure 1), including the antiviral agent epicoccin G[9] (1), the 8,8′-*epi*-*ent*-isomer (2) of the cytotoxic agent rostratin B,[10] the antiviral and antibiotic gliotoxin[11] (3), and its epitetrathio counterpart gliotoxin G[12] (4), the immunosuppressant emethallicin E[13] (5), and the antifungal agent haematocin[14] (6). The designed synthetic strategies employed to construct these molecules are exemplified with those depicted for epicoccin G [1, a *bis*-(methylthio)diketopiperazine] and 8,8′-*epi*-*ent*-rostratin B (2, an epidithiodiketopiperazine), in retrosynthetic format, in Scheme 4. Thus, epicoccin G (1) was disconnected retrosynthetically to its *bis*-unsaturated precursor 58 through a *bis*-hydrogenation step. The latter intermediate was then traced to *bis*-endoperoxide 60 through the rarely used Kornblum–DeLaMare rearrangement,[27] anticipating a regioselective rupture of the endoperoxide moieties under basic conditions. Steric control in the latter process was envisioned to furnish the desired regioisomer (58). Through a *bis*-photooxygenation/*bis*-sulfenylation sequence, *bis*-endoperoxide 60 was traced back to *bis*-diene diketopiperazine 45 through the intermediacy of *bis*-diene 55. Similar retrosynthetic analysis of 8,8′-*epi*-*ent*-rostratin B (2) led to the same precursor (45) as shown in Scheme 4. The latter was envisioned to arise from L-*N*-Boc-tyrosine (62) *via* bicyclic intermediate 63[28] (see Scheme 4) through appropriate elaboration and dimerization procedures.

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The synthesis of the *bis*-diene **45** from the known tyrosine-derived hydroxy enone **63**[28] is shown in Scheme 5. Thus, acetylation of **63** followed by treatment with Zn and AcOH in MeOH at 65 °C and exposure to DBU led to the deoxygenation product bicyclic enone **64** possessing the desired *syn* ring junction (51% yield for the three steps). Luche reduction[29] of the latter ($NaBH_4$, $CeCl_3$) gave allylic alcohol **65** (possessing the α configuration as expected on steric grounds; inconsequential) in 92% yield. In preparation for the pending cyclodimerization, key intermediate **65** was separately processed with LiOH and TFA to afford coupling partners **66** (99% yield, TFA salt) and **67** (99% yield), respectively. *N*-Boc carboxylic acid **67** and amine methyl ester TFA salt **66** were coupled in the presence of BOP-Cl and Et_3N to afford amide **68** in 86% yield. Treatment of the latter with TFA followed by exposure to Et_3N led to the formation of pentacyclic diketopiperazine **69** in 77% yield for the two steps. The desired *bis*-dehydration of *bis*-allylic alcohol **69** was achieved through the intermediacy of *bis*-allylic trifluoroacetate **70** formed by treatment of the former with $(CF_3CO)_2O$ in the presence of Et_3N and 4-DMAP (69% yield). The latter intermediate (**70**) was smoothly converted to the targeted *bis*-diene **45** upon exposure to catalytic amounts of $Pd(PPh_3)_4$ in the presence of K_2CO_3 (90% yield).[30]

The advancement of *bis*-diene **45** to the desired sulfenylated intermediates epidithiodiketopiperazine **46** and *bis*-(methylthio)diketopiperazine **55** and their diastereoisomers is summarized in Scheme 6. Thus, sulfenylation of **45** according to the developed procedure [NaHMDS-S_8] furnished a mixture of oligosulfides (**71**) from which emerged epidithiodiketopiperazines **46** and 2,2′-*epi*-**46** and *bis*-(methylthio)diketopiperazines **55** and 2,2′-*epi*-**55** upon reduction/oxidation ($NaBH_4$; KI_3; 55% combined yield for **46** and 2,2′-*epi*-**46**, ca. 1.4:1 dr) and reduction/methylation ($NaBH_4$; MeI; 58% overall yield for **55** and 2,2′-*epi*-**55**, ca. 1.4:1 dr). The stereochemical configurations of these chromatographically separated products were deciphered by NOESY correlations as indicated in Scheme 6 (bottom).

The correct diastereoisomers **55** and 2,2′-*epi*-**46** were elaborated to the target molecules epicoccin G (**1**) and 8,8′-*epi*-*ent*-rostratin B (**2**) through similar pathways as shown in Schemes 7 and 8, respectively. Thus, reaction of *bis*-(methylthio)diketopiperazine *bis*-diene **55** with singlet oxygen (generated from triplet oxygen and UV light in the presence of tetraphenylporphyrin sensitizer)[31] in CH_2Cl_2 at −45 °C furnished *bis*-endoperoxide **60**, which was treated with DBU (−45→0 °C) without isolation to afford *bis*-hydroxy enone **58** as the major product (52% overall yield, Scheme 7). The latter compound was subjected to catalytic hydrogenation [H_2, 20% $Pd(OH)_2$/C] to give smoothly epicoccin G in 86% yield. Processing epidithio *bis*-diene 2,2′-*epi*-**46** with singlet oxygen (0 °C) followed by treatment of the resulting *bis*-endoperoxide (**61**) with Et_3N (0→25 °C) furnished epidithio *bis*-hydroxy enone **59** in 55% overall yield (Scheme 8). The sensitivity of the epidithiodiketopiperazine structural motif within **59** dictated the use of Stryker's reagent[32] $[CuH(PPh_3)]_6$ (as opposed to the hydrogenation conditions employed for the conversion of **58** to epicoccin G, Scheme 7) for the required reduction of the olefinic bonds, followed by re-oxidation with KI_3 to regenerate the partially cleaved epidithio moiety, thereby furnishing 8,8′-*epi*-*ent*-rostratin B (**2**) in 82% overall yield.

As further demonstrations of the applicability of the present improved sulfenylation method, we pursued the enantioselective total synthesis of gliotoxin (**3**) and gliotoxin G (**4**), as well as emethallicin E (**5**) and haematocin (**6**) (Figure 1). The devised synthetic strategy toward these target molecules envisioned bicyclic hydroxy diene **78** (see Scheme 9) as a common intermediate. This key building block was obtained in multi-gram quantities from the tyrosin-derived hydroxy enone *N*-Boc methyl ester **63** as shown in Scheme 9.[33] Thus, Luche reduction ($NaBH_4$, $CeCl_3$) of **63**[28] gave diol **72** stereoselectively (99% yield), which was

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smoothly acetylated to afford hydroxy acetate **73** in 91% yield. The latter was converted to hydroxy diene **74** through palladium-catalyzed elimination [$Pd(OAc)_2$ (cat.), PPh_3 (cat.), Et_3N, 86%]. Photooxygenation of this diene (O_2, TPP, hν, 73%) generated hydroxy endoperoxide **75**, whose reduction with thiourea afforded triol **76** in 84% yield. Selective monosilylation of the latter (TIPSOTf, 96% yield) followed by engagement of the 1,2-diol system into a thionocarbonate moiety [$(im)_2C=S$, 90% yield] furnished intermediate **77**. The latter was deoxygenated [$P(OMe)_3$, 82% yield] and desilylated (aq. HCl, 98% yield) to afford the desired building block hydroxy diene **78**.

The enantioselective total synthesis of gliotoxin (**3**) and gliotoxin G (**4**) from the common building block **78** is summarized in Scheme 10. Thus, hydrolysis of the methyl ester within **78** (LiOH) led to carboxylic acid **79** (99% yield), which was coupled with L-serine derivative **80**[34] (HATU, HOAt, DIPEA) to afford amide **81** in 88% yield. Removal of the Boc group from the latter and exposure of the resulting amino ester to Et_3N furnished tricyclic diketopiperazine **82** (63% overall yield), whose structure was proven beyond doubt through X-ray crystallographic analysis (see ORTEP, Scheme 10). Sulfenylation of the latter required the use of S_8 and LiHMDS, conditions that furnished directly gliotoxin (**3**, 23% yield) and gliotoxin G (**4**, 33% yield) (plus 6% recovered starting material **82**). Interestingly, attempts to effect the sulfenylation of **82** with [NaHMDS-S_8] failed to produce gliotoxin or gliotoxin G, leading instead to aromatization of the cyclohexadiene ring and decomposition. These results underscore the subtle differences in reactivity of the various alkali metal HMDS bases and point to the importance of thorough experimentation in attempting to achieve certain transformations, including the present sulfenylation.

Scheme 11 summarizes the enantioselective total syntheses of emethallicin E (**5**) and haematocin (**6**) from common intermediate **78**. Thus, a three-step sequence involving replacement of the Boc protective group with Alloc (TFA, 95% yield; then AllocCl, 88% yield) followed by saponification of the methyl ester group (LiOH) furnished hydroxy carboxylic acid **83** in high yield. Coupling of building blocks **83** and **84** (obtained in the first step of the above sequence **78**→**83**) under the influence of BOP-Cl and DIPEA led to amide **85** in 83% yield over the two steps. Pentacyclic *bis*-hydroxy diketopiperazine **86** was generated from amide **85** in 84% overall yield upon cleavage of the Alloc protecting group [$Pd_2(dba)_3$ (cat.)] in the presence of Et_2NH. The structure of intermediate **86** was unambiguously confirmed by X-ray crystallographic analysis (see ORTEP, Scheme 11). Sulfenylation of *bis*-hydroxy diketopiperazine **86** with [LiHMDS-S_8] led to tetrasulfide **87** as the major product (46% yield, plus 43% recovered starting material **86**).

As in the case of the gliotoxins discussed above (Scheme 10), the standard [NaHMDS-S_8] conditions failed to produce the sulfenylated product from substrate **86** in satisfactory yield, leading only to 10% yield of epitetrasulfide **87** (Scheme 11). This observation prompted a systematic investigation to optimize the yield of this sulfenylation reaction varying the HMDS base and the solvent. The results of this study, shown in Table 4, revealed LiHMDS in THF as the optimum conditions (entry 5/LiHMDS). The formation of the epitetrasulfide as the predominant product in this case is also of interest. This example underscores once again the importance of careful optimization of conditions to achieve the best results in diketopiperazine sulfenylation reactions. It is also noteworthy that the use of *bis*[*bis*(trimethylsilyl)amino]trisulfide (**23**, Scheme 3) as a sulfenylating reagent in the presence of LiHMDS as a base proved less reactive than the corresponding *in situ* generated species [LiHMDS-S_8], leading to recovery of 80% of starting material (**86**) and no epidisulfide or epitetrasulfide products. The use of NaHMDS or KHMDS and trisulfide **23** led primarily to aromatization under the same sulfenylation conditions.

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The stereochemical configuration of the epitetrasulfide **87** was based on NMR spectroscopic studies and was confirmed by the successful synthesis of the natural products **5** and **6**. Indeed, intermediate **87** served as a common precursor to emethallicin E (**5**) and haematocin (**6**) as shown in Scheme 11. Thus, *bis*-esterification of **87** with phenylacetic acid ($PhCH_2COOH$) in the presence of DCC and 4-DMAP gave *bis*-phenylacetate **88** (71% yield, plus 26% recovered starting material **87**), whose reduction/oxidation (1,3-propane dithiol; then O_2) furnished the desired product emethallicin E (**5**) in 54% overall yield. Alternatively, *bis*-acetylation of **87** (AcOH, DCC, 4-DMAP, 71% yield, plus 24% recovered starting material **87**) followed by reduction/methylation ($NaBH_4$; then MeI) of the resulting *bis*-acetate afforded haematocin (**6**) in 97% overall yield. The use of the DCC/4-DMAP esterification protocol instead of the more conventional acid anhydride or chloride methods was dictated by the sensitivity of the substrate (**87**) and products (**88**, **89**) under the reaction conditions, especially toward aromatization.

As part of a program directed toward the total synthesis of aranotin (**8**) we attempted to construct its monomeric unit (**7**, see Scheme 13) through diazo epoxide precursor **95** as shown in Scheme 12.[35] Thus, bicyclic diene system **90** (for its synthesis see Scheme 9, **77**→**78**, step h) was reacted with *bis*-trichloroethylazodicarboxylate (TrocN=NTroc) to afford Diels–Alder adduct **91** stereoselectively (steric control) and in 93% yield. Desilylation of the latter with TBAF gave hydroxy derivative **92** (92% yield), whose treatment with methyl(trifluoromethyl) dioxirane followed by sequential exposure to Zn and $CuCl_2$ in the presence of NH_4OH afforded diazo epoxide **93** as a single diastereoisomer (76% overall yield). The stereochemical configuration of **93** was established through X-ray crystallographic analysis (see ORTEP, Scheme 12). As expected, this epoxide did not enter the obligatory rearrangement with loss of N_2 by virtue of the *syn* arrangement of the diazo and epoxide moieties that does not allow for the proper orbital orientations.[35a]

Our inability to reach the *anti* diazo epoxide **95** (Scheme 12), whose rearrangement to oxepin **94** was anticipated to be facile, prompted us to pursue the alternative pathway (Scheme 13) involving trichloroethyl nitrosoformate compound **96** (generated from TrocNHOH and $NaIO_4$) as the dienophile. The latter reacted with bicyclic diene **90** to give Diels–Alder adduct **97** (88% yield) diastereo- and regioselectively (presumably due to steric control).[36] The structure of **97** was assigned based on NMR spectroscopic analysis (COSY, NOESY, HMBC, HSQC). This intermediate was epoxidized with methyl(trifluoromethyl) dioxirane to afford directly oxepin system **7** (40% yield), presumably *via* the fleeting epoxide **98** through a retro-Diels–Alder/epoxide opening. Epoxide **98** apparently must be of the *anti* configuration with respect to the N–O bridge, which allows for the facile rearrangement/extrusion of **96** (which undergoes disproportionation with expulsion of oxygen to form TrocN=NTroc).[36]

Biological Evaluation

Having synthesized various types of epidithio- and *bis*-(methylthio)diketopiperazines we selected a number of them for biological evaluation. Specifically, selected compounds were tested against poliovirus and *Plasmodium falciparum*.[37] Table 5 summarizes the results of these biological assays. Thus, in the anti poliovirus assays (carried out in the laboratory of D. F. S. under the auspices of the National Institute of Allergy and Infectious Diseases, NIAID), epidithiodiketopiperazines **46** (code number KCN-19), 2,2′-*epi*-**46** (code number KCN-2,2′-*epi*-19) and epidithio-*bis*-endoperoxide-diketopiperazine **61** (code number KCN-21) proved to be the most potent, exhibiting EC_{50} = 101–115 nM, 107–123 nM, and EC_{50} = 21.4 nM values, respectively, depending on the assay (see Table 5, entries 2, 3 and 7). Table 5 also displays selectivity indices (SI = CC_{50}/EC_{50} or EC_{90}, with CC_{50} = 50% cell-inhibitory, cytotoxic concentration deter-mined in stationary cells and $EC_{50/90}$ = 50%/

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90% poliovirus-inhibitory, effective concentration) for some of these compounds. Compounds **46** (SI = 41–70), 2,2′-*epi*-**46** (SI = 27–75) and **61** (SI = 23–59) were the most impressive in this regard (see Table 5). The anti poliovirus drug Pirodavir(R) (**99**, Table 5, entry 9), used as a control in this poliovirus assay, exhibited EC_{50} = 1.58 μM, underscoring the significant activities of KCN-19 (**46**), KCN-2,2′-*epi*-19 (2,2′-*epi*-**46**), and KCN-21 (**61**).

In the anti *Plasmodium falciparum* assays (carried out in the laboratories of E. A. W. at TSRI), epidithiodiketopiperazines **46** (IC_{50} = 3.6 μM, Table 5, entry 2), 2,2′-*epi*-**46** (IC_{50} = 2.7 μM, entry 3), **59** (IC_{50} = 4.5 μM; not included in the table, for structure see Scheme 4), **61** (IC_{50} = 2.5 μM, entry 7), and *bis*-(methylthio)diketopiperazines **58** (IC_{50} = 1.2 μM, entry 6), 2,2′-*epi*-**58** (IC_{50} = 4.4 μM; not included in the table, for structure see Scheme 4), and epicoccin G (**1**, IC_{50} = 2.5 μM) proved to be the most potent.

CONCLUSION

An improved method for the sulfenylation of 2,5-diketopiperazines based on the use of alkali metal hexame-thyldisilazide bases (i.e. NaHMDS, LiHMDS and KHMDS) and sulfur (S_8) in THF at 25 °C as a means to prepare epidithio-, epitetrathio- and *bis*-(methylthio)diketopiperazines has been developed. A second method involving the use of *bis*[*bis*(trimethylsilyl)amino]trisulfide [$(TMS)_2NSSSN(TMS)_2$] and NaHMDS for the direct preparation of epidithio- and epitetrathiodiketopiperazines has also been developed.

Application of these methods led to the synthesis of an array of sulfenylated diketopiperazine systems, including the natural products epiccocin G (**1**), gliotoxin (**3**), gliotoxin G (**4**), emethallicin E (**5**), haematocin (**6**) and the 8,8′-*epi*-*ent*-isomer (**2**) of rostratin B. With the exception of gliotoxin (**3**),[6h] these accomplishments represent the first enantioselective total syntheses of these natural products and their analogs and feature a number of novel synthetic strategies and reactions, including the [2+2] photooxygenation and the rarely used Kornblum–DeLaMare rearrangement.

Biological investigations of selected members of the synthesized compound libraries led to the discovery of a number of potent anti poliovirus agents (i.e. **46**, 2,2′-*epi*-**46** and **61**) and a series of anti *Plasmodium falciparum* lead compounds (i.e. **46**, 2,2′-*epi*-**46**, **58**, **61** and **1**) that may facilitate biological investigations and drug discovery efforts in the antiviral and anti-malarial areas, respectively.

By blending total synthesis of natural products of biological and medical interest with method development endeavors and chemical biology studies, the work described herein exemplifies the modern paradigm of natural product synthesis and underscores its relevance and importance to chemistry, biology and medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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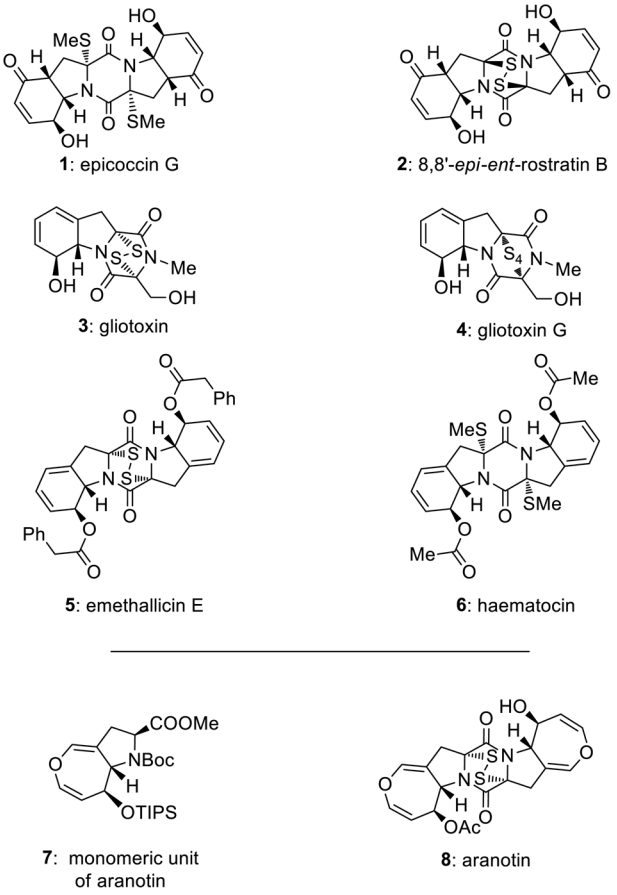

Figure 1.
Selected naturally occurring epidithio-, epitetrathio- and *bis*-(methylthio)diketopiperazines.

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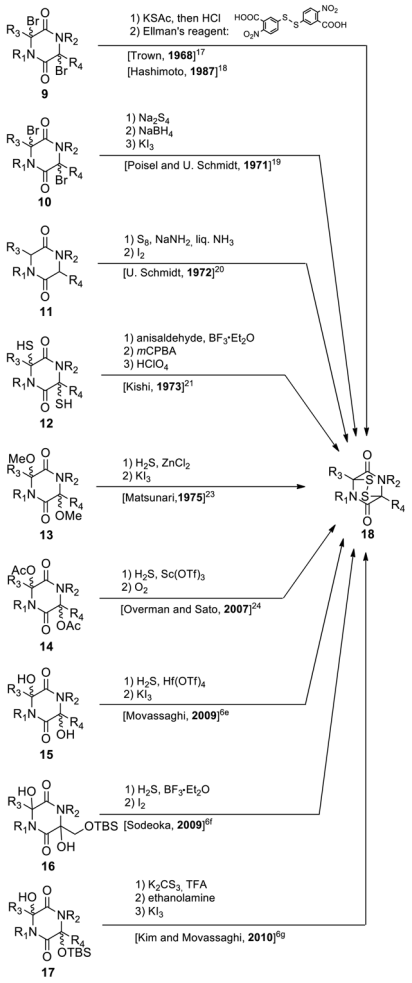

Figure 2.
Selected sulfenylation methods of 2,5-diketopiperazines.

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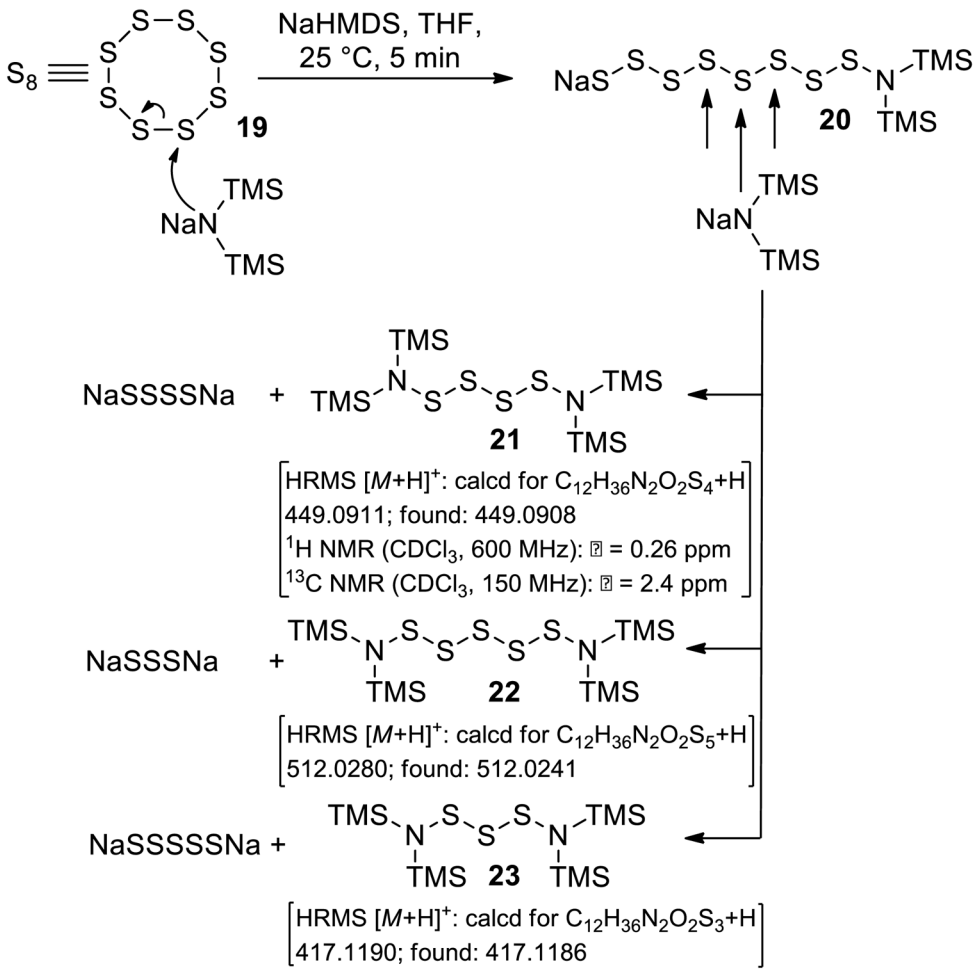

Scheme 1.

Reaction of Sulfur (S_8) with NaHMDS [$NaN(TMS)_2$][a]

[a]Reagents and conditions. NaHMDS (0.6 M in PhMe, 3.0 equiv), S_8 (1.0 equiv), THF, 25 °C, 5 min, **21**: 40%, **22**: 5%, **23**: 8%.

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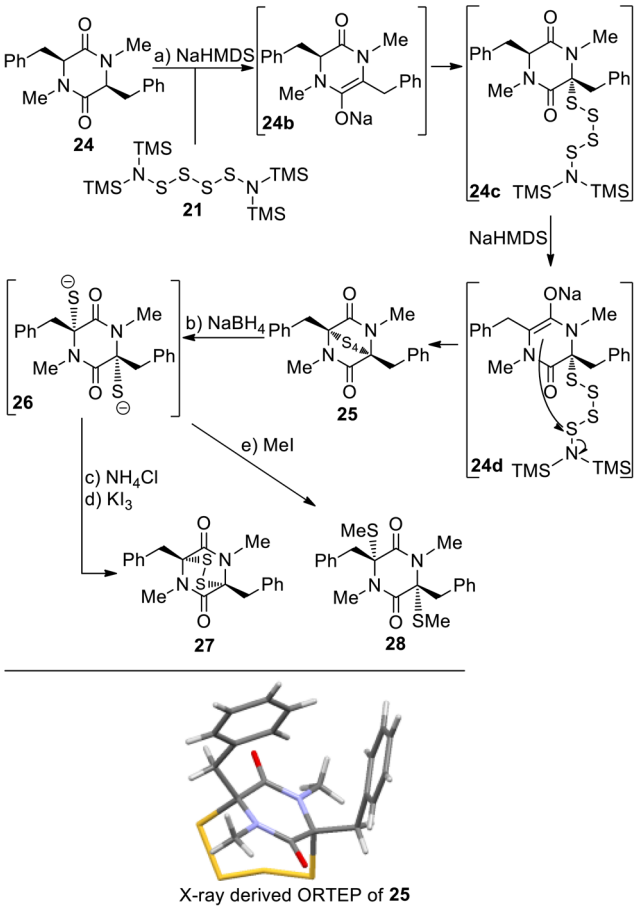

Scheme 2.

Sulfenylation of 2,5-Diketopiperazines with [NaHMDS-S_8]. Preparation of Epitetrathiodiketopiperazine 25, Epidithiodiketopiperazine 27 and *bis*-(Methylthio)diketopiperazine 28[a]

[a]Reagents and conditions. a) NaHMDS (0.6 M in PhMe, 3.0 equiv), S_8 (1.0 equiv), THF, 25 °C, 1 min; then **24** (1 M in THF, 1.0 equiv), 1 min; then NaHMDS (0.6 M in PhMe, 2.0 equiv), 25 °C, 30 min; b) $NaBH_4$ (25 equiv), THF/MeOH (1:1), 0→25 °C, 45 min; c) NH_4Cl aq. (1.0 M), 25 °C; d) KI_3 aq. (1.4 M), 25 °C, 10 min, 69% over the four steps from **24**; e) MeI (50 equiv), 25 °C, 15 h, 72% over the three steps from **24**.

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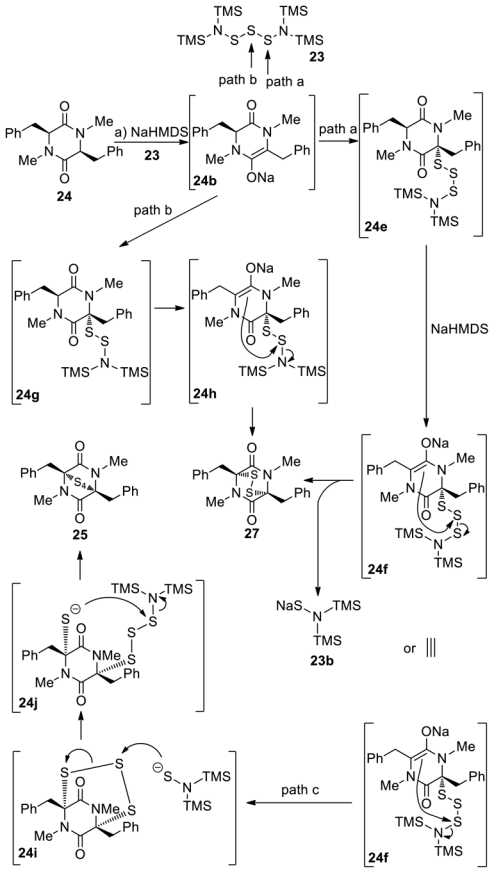

Scheme 3.

Reaction of Diketopiperazine 24 with *bis*[*bis*(trimethylsilyl)amino]trisulfide [$(TMS)_2SSS(TMS)_2$] and NaHMDS and Mechanistic Considerations. Direct Formation of Epidithio- and Epitetrathiodiketopiperazines[a]

[a]Reagents and conditions. a) $(TMS)_2NSSSN(TMS)_2$ (4.0 equiv), NaHMDS (0.6 M in PhMe, 4.0 equiv), THF, 25 °C, 30 min, **25**: 22%, **27**: 43%.

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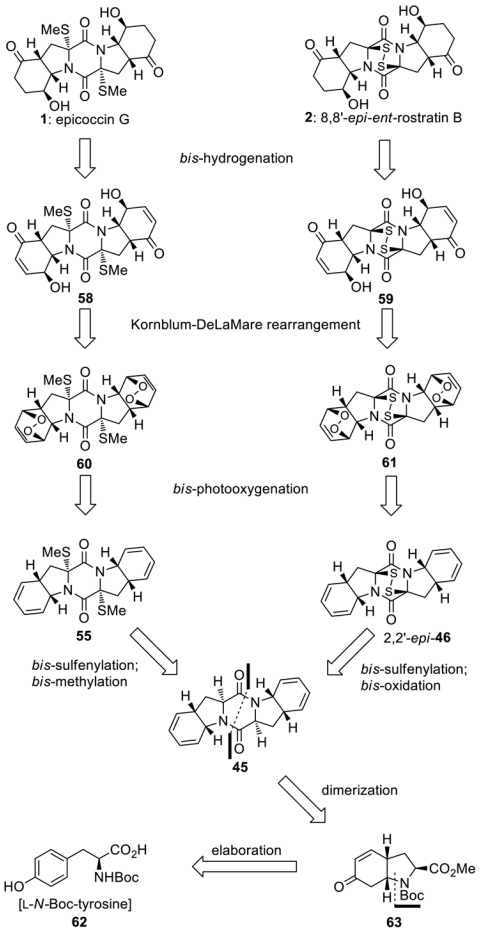

Scheme 4.
Retrosynthetic Analysis of Epicoccin G (1) and 8,8′-*epi*-*ent*-Rostratin B (2)

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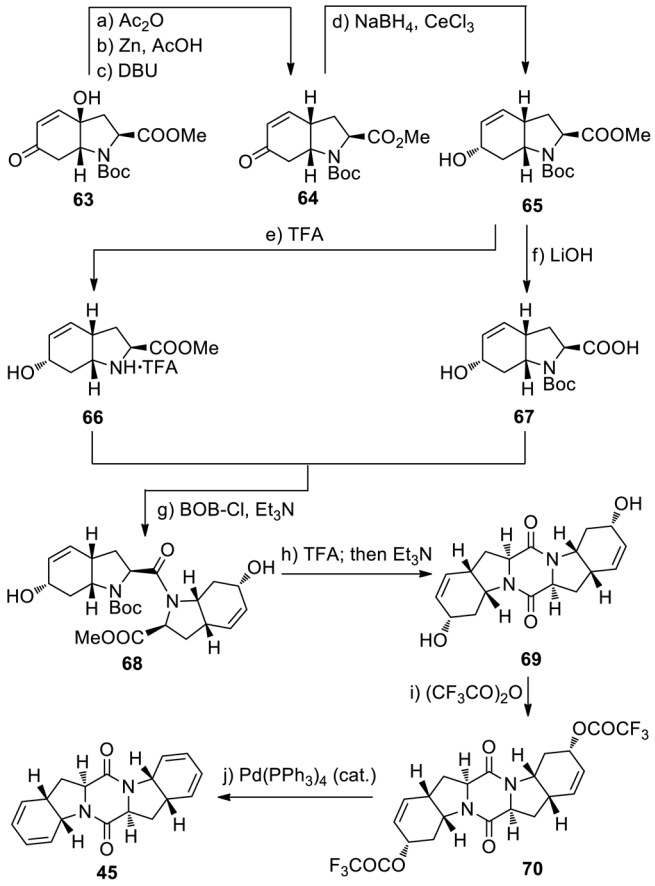
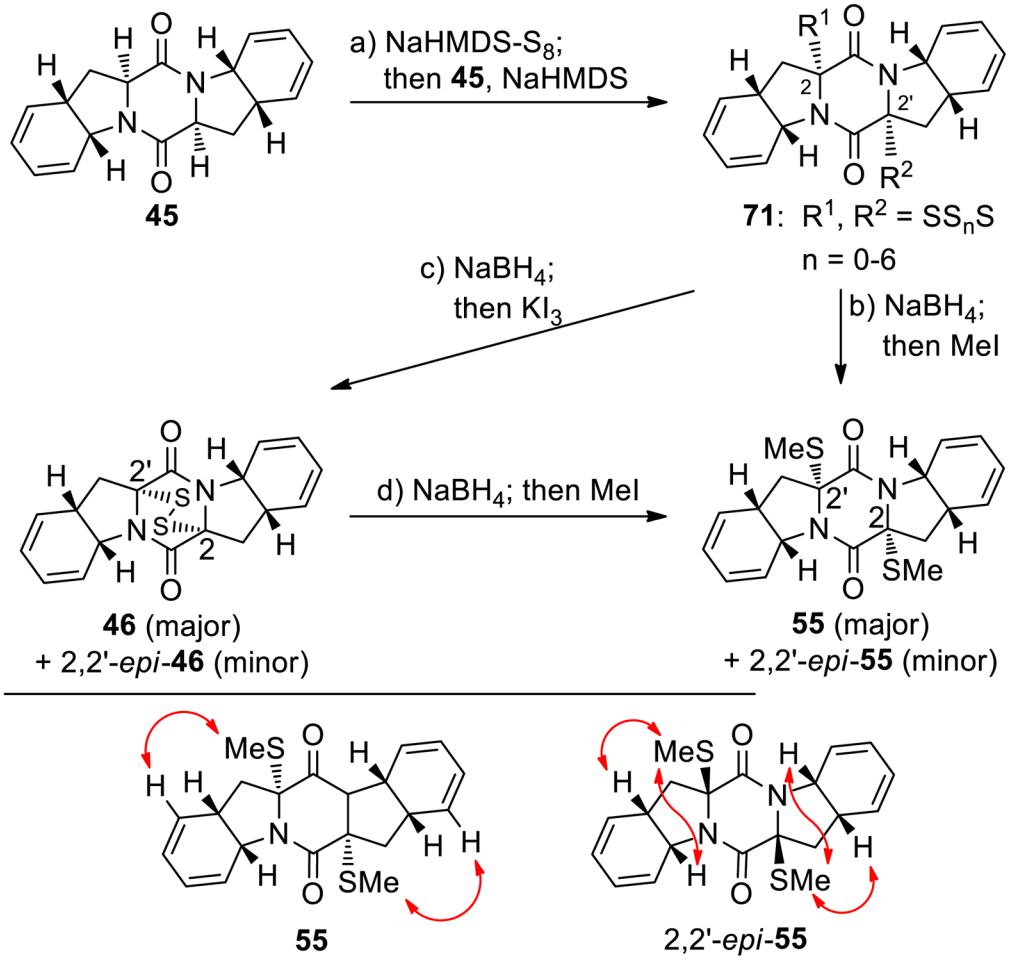


Scheme 5.

Synthesis of *bis*-Diene Diketopiperazine 45[a]

[a]Reagents and conditions. a) Ac_2O (2.0 equiv), Et_3N (3.0 equiv), 4-DMAP (0.2 equiv), CH_2Cl_2, 0→25 °C, 4 h; b) Zn (8.0 equiv), AcOH (2.0 equiv), MeOH, 65 °C, 30 min; c) DBU (5.0 equiv), PhMe, 65 °C, 3 h, 51% for the three steps; d) $NaBH_4$ (1.1 equiv), $CeCl_3 \cdot 7H_2O$ (1.0 equiv), MeOH, −78→0 °C, 1 h, 92%; e) TFA/CH_2Cl_2 (1:1), 0→25 °C, 30 min, 99%; f) aq. LiOH (1.0 M)/THF (4:1), 0→25 °C, 3 h, 99%; g) **66**, **67** (1.0 equiv each), BOP-Cl (1.1 equiv), Et_3N (3.0 equiv), CH_2Cl_2, 0→25 °C, 15 h, 86%; h) TFA (32 equiv), CH_2Cl_2, 0→25 °C, 1.5 h; then Et_3N (5.0 equiv), CH_2Cl_2, 0→25 °C, 15 h, 77% for the two steps; i) $(CF_3CO)_2O$ (4.0 equiv), Et_3N (6.0 equiv), 4-DMAP (0.3 equiv), MeCN, −40→25 °C, 1 h, 69%; j) $Pd(PPh_3)_4$ (0.1 equiv), K_2CO_3 (2.1 equiv), dioxane, 65 °C, 30 min, 90%.



Scheme 6.

Synthesis of Dithiodiketopiperazines 46 and 2,2′-*epi*-46, and *bis*-(Methylthio)diketopiperazines 55 and 2,2′-*epi*-55[a] and Stereochemical Assignments of 55 and 2,2′-*epi*-55 by NOESY Studies (Arrows Designate NOESY Correlations)

[a]Reagents and conditions. a) NaHMDS (0.6 M in PhMe, 3.0 equiv), S_8 (1.0 equiv), THF, 25 °C, 1 min; then **45** (1 M in THF, 1.0 equiv), 1 min; then NaHMDS (0.6 M in PhMe, 2.0 equiv), 25 °C, 30 min; b) $NaBH_4$ (25 equiv), THF/MeOH (1:1), 0→25 °C, 45 min; then MeI (50 equiv), 25 °C, 15 h, 58% over the three steps from **45** (**55**:2,2′-*epi*-**55** ca. 1.4:1 dr); c) $NaBH_4$ (25 equiv), THF/MeOH (1:1), 0→25 °C, 0.75 h; then KI_3 aq. (1.4 M), 25 °C, 10 min, 55% over the three steps from **45** (**46**: 2,2′-*epi*-**46** ca. 1.4:1 dr); d) $NaBH_4$ (25 equiv), THF/MeOH (1:1), 0→25 °C, 45 min; then MeI (50 equiv), 25 °C, 15 h, 65% from **46**(**55**:2,2′-*epi*-**55** ca. 1.4:1 dr).

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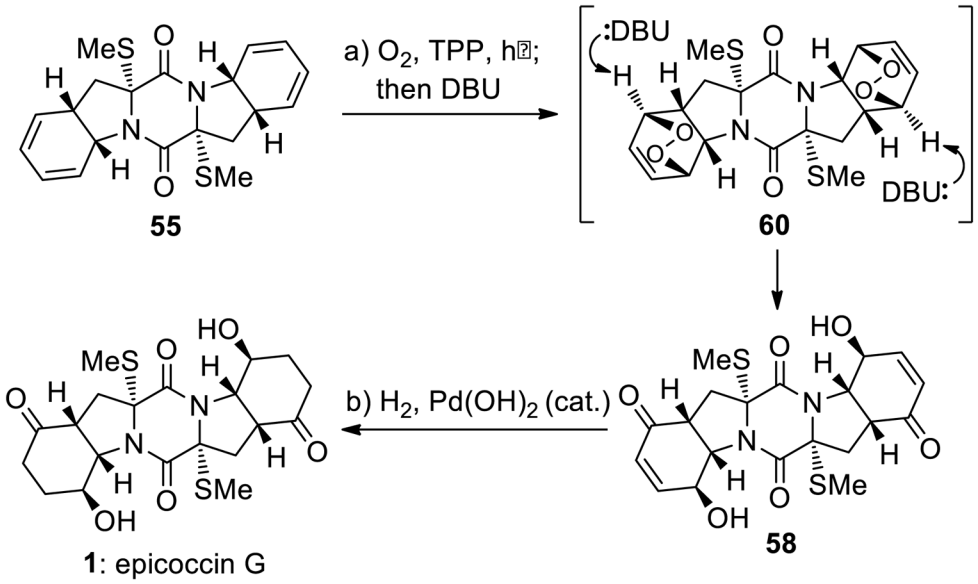
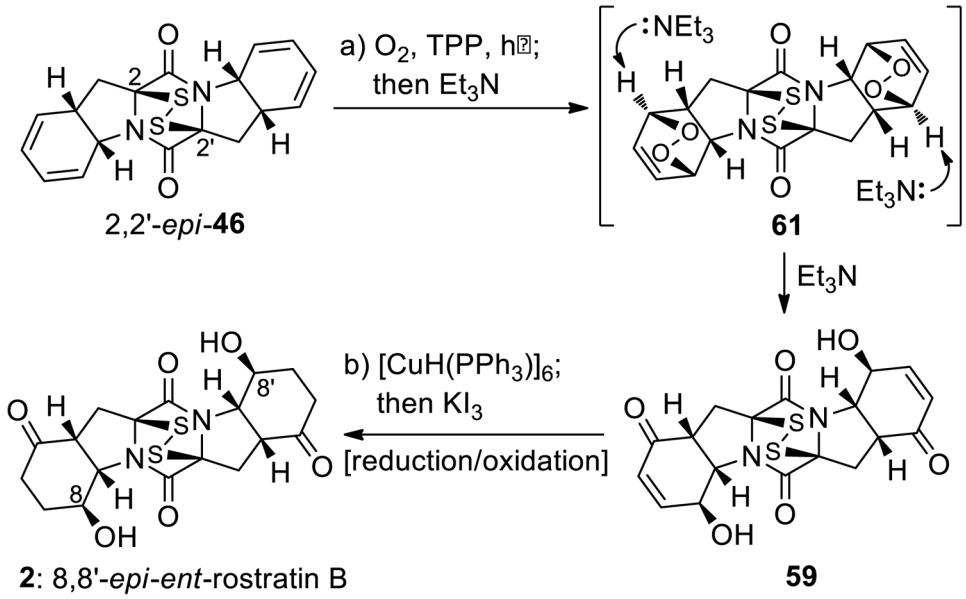


Scheme 7.

Completion of the Total Synthesis of Epicoccin G (1)[a]

[a]Reagents and conditions. a) O_2, TPP (0.02 equiv), CH_2Cl_2, −45 °C, 45 min; then DBU (10.0 equiv), −45→0 °C; 1 h, 52% from **55**; b) H_2, $Pd(OH)_2$/C (20% w/w, 0.4 equiv), MeOH, 25 °C, 1 h, 86%.



Scheme 8.

Completion of the Total Synthesis of 8,8′-*epi*-*ent*-Rostratin B (2)[a]

[a]Reagents and conditions. a) O_2, TPP (0.02 equiv), CH_2Cl_2, 0 °C, 2 h; then Et_3N (5.0 equiv), 0→25 °C, 3 h, 55% for the two steps; b) $[CuH(PPh_3)]_6$ (10.0 equiv), benzene, 25 °C, 30 min; then KI_3 aq. (1.4 M), 25 °C, 10 min, 82%.

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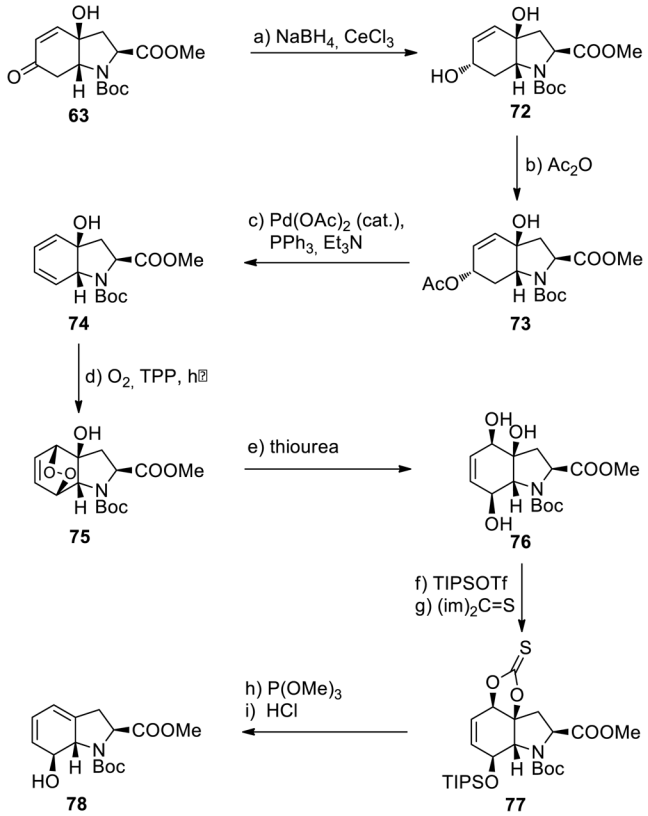

Scheme 9.

Synthesis of Common Key Building Block 78[a]

[a]Reagents and conditions. a) $NaBH_4$ (2.0 equiv), $CeCl_3 \cdot 7H_2O$ (1.3 equiv), MeOH, −20→0 °C, 3 h, 99%; b) Ac_2O (2.0 equiv), Et_3N (3.0 equiv), 4-DMAP (0.2 equiv), CH_2Cl_2, 0 °C, 1 h, 91%; c) $Pd(OAc)_2$ (0.02 equiv), PPh_3 (0.1 equiv), Et_3N (1.2 equiv), PhMe, 25→110 °C, 3 h, 86%; d) O_2, TPP (0.0036 equiv), CH_2Cl_2, 25°C, 24 h, 73%; e) thiourea (2.0 equiv), MeOH, 25 °C, 2 h, 84%; f) TIPSOTf (1.1 equiv), Et_3N (2.0 equiv), CH_2Cl_2, 0 °C, 30 min, 96%; g) $(im)_2C{=}S$ (1.2 equiv), PhMe, 110 °C, 3 h, 90%; h) $P(OMe)_3$, 111 °C, 12 h, 82%; i) HCl aq. (1.0 M), CH_2Cl_2/Et_2O (1:1), 0 °C, 10 min, 98%.

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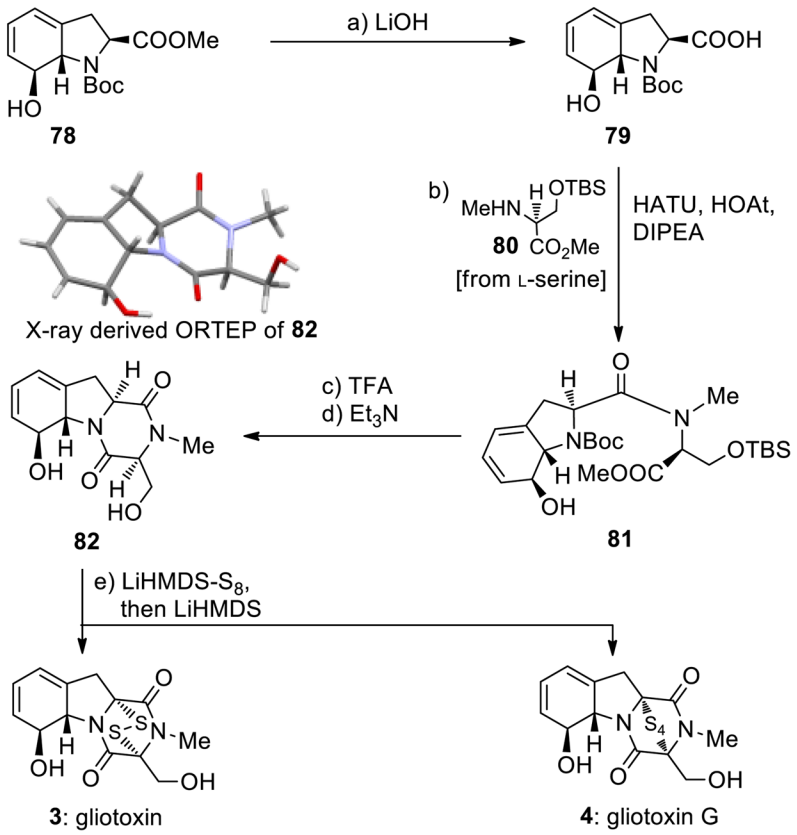

Scheme 10.

Completion of the Enantioselective Total Syntheses of Gliotoxin (3) and Gliotoxin G (4)[a]

[a]Reagents and conditions. a) aq. LiOH (1.0 M)/THF (6:1), 0→25 °C, 5 h, 99%; b) **79** (1.0 equiv), **80** (2.0 equiv), HOAt (1.1 equiv), HATU (1.1 equiv), DIPEA (3.0 equiv), CH_2Cl_2, 0→25 °C, 15 h, 88%; c) TFA/CH_2Cl_2 (1:1), 0→25 °C, 3 h; d) Et_3N (5.0 equiv), CH_2Cl_2, 0→25 °C, 15 h, 63% for the two steps; e) LiHMDS (1.0 M in THF, 4.0 equiv), S_8 (8.0 equiv), THF, 25 °C, 5 min; then **82** (0.06 M in THF, 1.0 equiv) 5 min; then LiHMDS (1.0 M in THF, 4.0 equiv), 25 °C, 1.5 h, **3**: 23%, **4**: 33%, plus 6% recovered starting material **82**.

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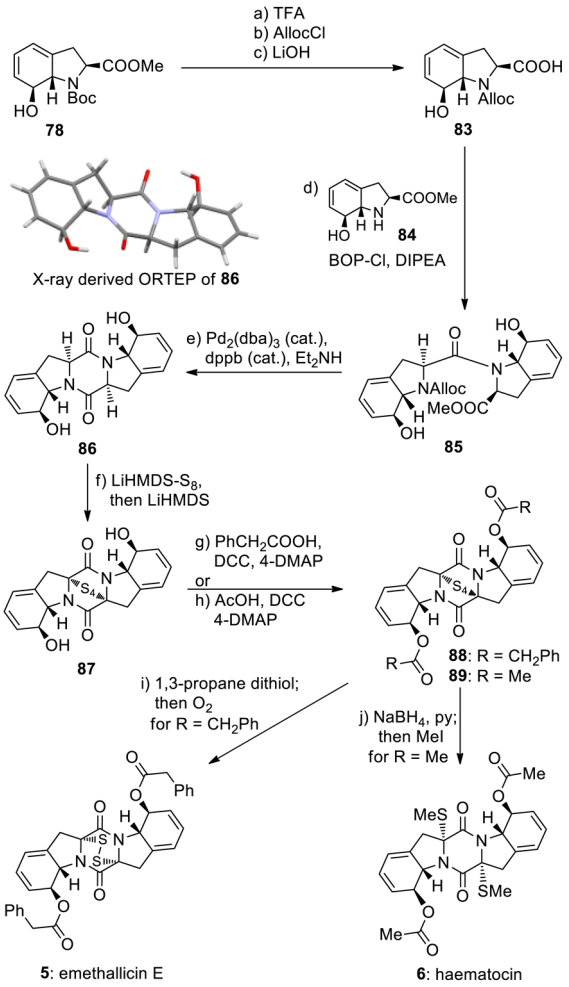
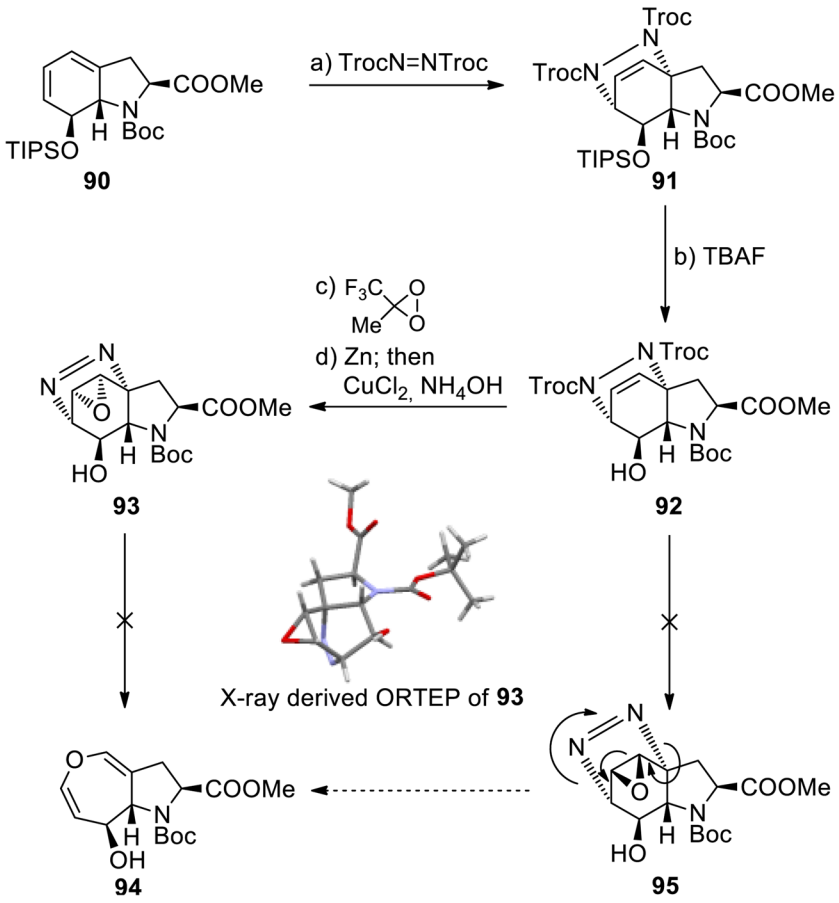


Scheme 11.

Completion of the Enantioselective Total Syntheses of Emethallicin E (5) and Haematocin (6)[a]

[a]Reagents and conditions. a) TFA/CH_2Cl_2 (1:2.5), 25 °C, 4 h, 95%; b) AllocCl (1.7 equiv), $NaHCO_3$ (10.0 equiv), dioxane/H_2O (1:1), 0→25 °C, 3 h, 88%; c) LiOH aq. (1.0 M)/THF (1:1), 0→25 °C, 5 h; d) **83**, **84** (1.0 equiv each), BOP-Cl (1.1 equiv), DIPEA (3.0 equiv), CH_2Cl_2, 0→25 °C, 15 h, 83% for the two steps; e) $Pd_2(dba)_3$ (0.02 equiv), dbbp (0.05 equiv), THF/Et_2NH (2:1), 25 °C, 2 h, 84%; f) LiHMDS (1.0 M in THF, 20 equiv), S_8 (37 equiv), THF, 25 °C, 5 min; then **86** (0.06 M in THF/Et_2O (9:1), 1.0 equiv), 5 min; then LiHMDS (1.0 M in THF, 20 equiv), 25 °C, 5 h, 46%, plus 43% recovered starting material **86**; g) $PhCH_2COOH$ (30 equiv), DCC (30 equiv), 4-DMAP (3.0 equiv), 0→25 °C, 15 h, 71%, plus 26% recovered starting material **87**; h) AcOH (30 equiv), DCC (30 equiv), 4-DMAP (3.0 equiv), 0→25 °C, 15 h, 71%, plus 24% recovered starting material **87**; i) 1,3-propane dithiol (90 equiv), Et_3N (0.32 equiv), MeCN/CH_2Cl_2 (25:1), 25 °C; then concentrate; then O_2, MeOH, 2 h, 25 °C, 54% overall; j) $NaBH_4$ (80 equiv), MeOH/py (1:1), 0 °C; then MeI (485 equiv) 0→25 °C, 4 h; 97% overall.



Scheme 12.

Attempted Synthesis of Oxepin 7 by Ring Expansion of a Diazo Epoxide[a]

[a]Reagents and conditions. a) TrocN=NTroc (1.2 equiv), CH_2Cl_2, 41 °C, 4 h, 93%; b) TBAF (1.0 M in THF, 2.0 equiv), THF, 0 °C, 30 min, 92%; c) 1,1,1-trifluoro acetone (62 equiv), Na_2EDTA, $NaHCO_3$ aq. (15 equiv), oxone(R) (38 equiv), MeCN, 0→25 °C, 15 h; d) Zn (21 equiv), MeOH/NH_4Cl aq. (1.0 M) (4:1), 25 °C, 2 h; then NH_4OH aq. (15 M), $CuCl_2$ aq. (1.0 M), 25 °C, 5 min, 76% for the two steps.

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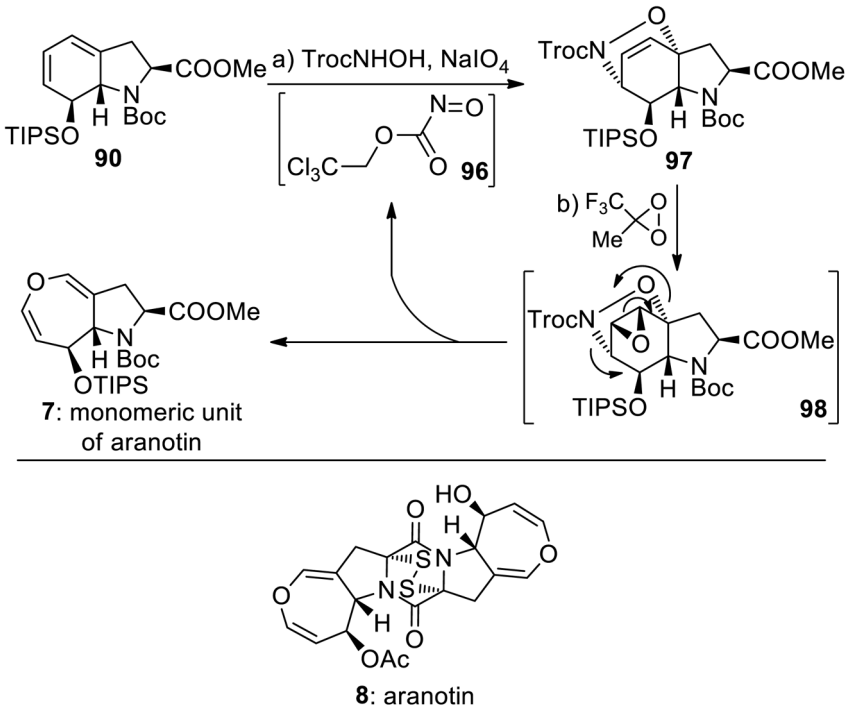

8: aranotin

Scheme 13.

Synthesis of Oxepin (7) by Ring Expansion of Nitroso Epoxide 98[a]

[a]Reagents and conditions. a) TrocNHOH (2.5 equiv), $NaIO_4$ (1.0 equiv), TBAI (1.0 equiv), CH_2Cl_2/H_2O (4:1), 0→25 °C, 10 min, 88%; b) 1,1,1-trifluoro acetone (7.0 equiv), Na_2EDTA, Na-HCO_3 aq. (60 equiv), oxone(R) (20 equiv), $MeCN/H_2O$ (1:1), 0 °C, 15 h, 40%.

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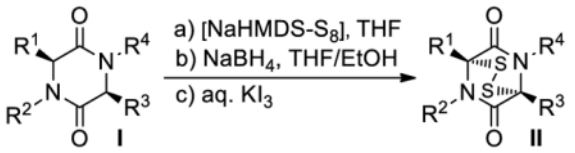
Table 1

Preparation of Epidithiodiketopiperazines[a]



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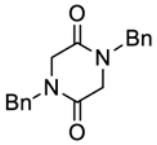
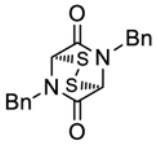
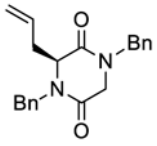
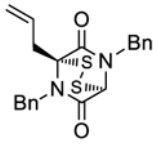
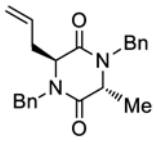
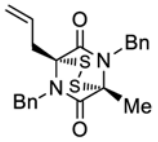
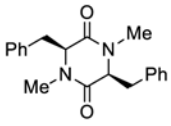
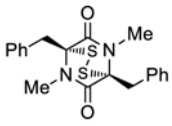
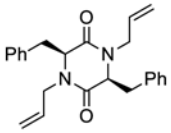
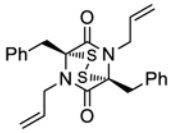
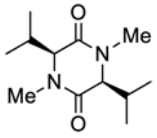
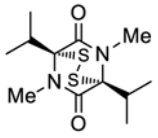
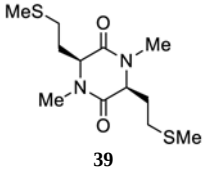
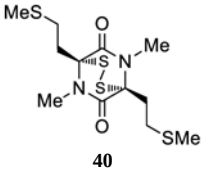
1	 **29**	 **30**	40
2	 **31**	 **32**	63
3	 **33**	 **34**	70
4	 **24**	 **27**	69
5	 **35**	 **36**	65
6	 **37**	 **38**	45
7	 **39**	 **40**	43

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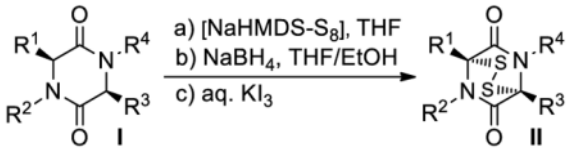
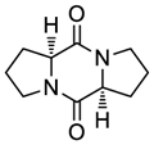
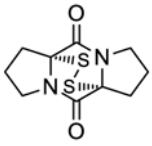
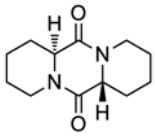
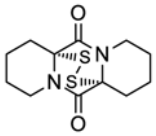
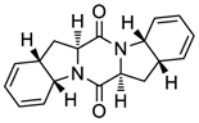
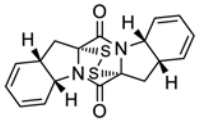
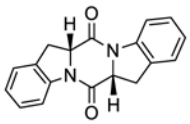
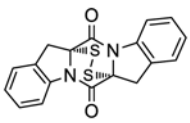
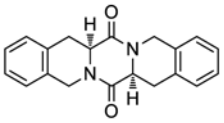
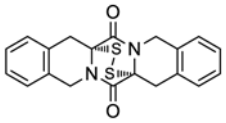


Entry	Substrate	Product[b]	Overall Yield [%][c]
8	 **41**	 **42**	65
9	 **43**	 **44**	68
10	 **45**	 **46**	55[d]
11	 **47**	 **48**	68
12	 **49**	 **50**	70

[a] Reactions were performed on 100 mg scale at 25 °C.

[b] Racemic mixture unless otherwise stated.

[c] Yield of isolated products after chromatography.

[d] ca. 1.4:1 dr.

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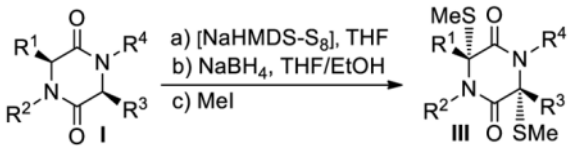
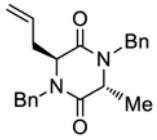
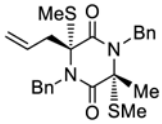
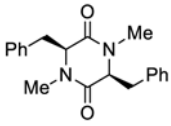
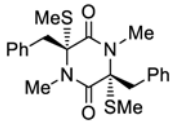
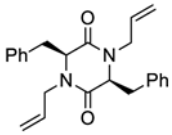
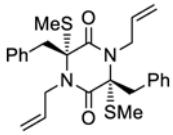
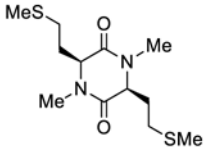
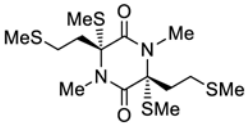
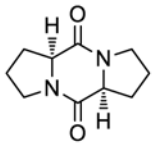
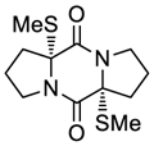
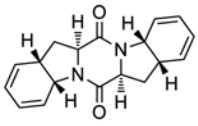
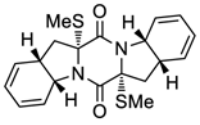
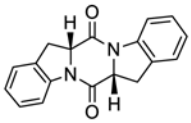
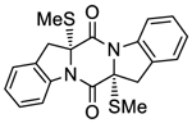
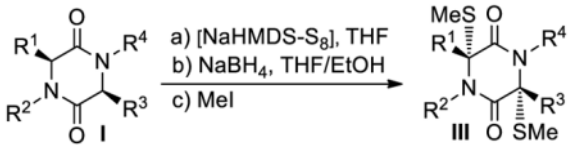
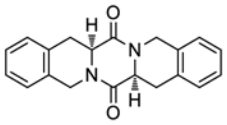
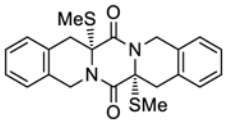
Table 2

Preparation of *bis*-(Methylthio)diketopiperazines[a]



Entry	Substrate	Product[b]	Overall Yield [%][c]
1	 **33**	 **51**	70
2	 **24**	 **28**	72
3	 **35**	 **52**	63
4	 **39**	 **53**	51
5	 **41**	 **54**	64
6	 **45**	 **55**	58[d]
7	 **47**	 **56**	61



Entry	Substrate	Product[b]	Overall Yield [%][c]
8	 **49**	 **57**	67

[a] Reactions were performed on 100 mg scale at 25 °C.

[b] Racemic mixture unless otherwise stated.

[c] Yield of isolated products after chromatography.

[d] ca. 1.4:1 dr.

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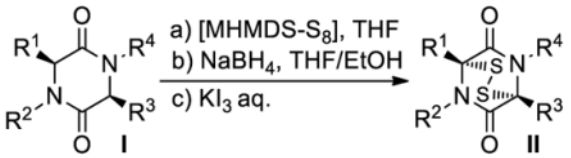
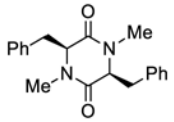
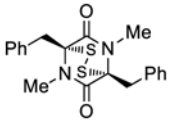
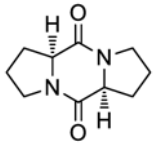
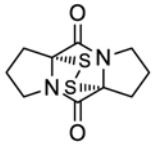
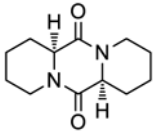
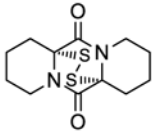
J Am Chem Soc. Author manuscript; available in PMC 2013 October 17.

Table 3

Influence of the Base in the Sulfenylation of Selected Epidithiodiketopiperazines[a]



I II

Entry	Substrate	Product[b]	LiHMDS	NaHMDS	KHMDS
				Yield [%][c]	
1	 **24**	 **27**	45	69	50
2	 **41**	 **42**	50	65	53
3	 **43**	 **44**	53	69	43

[a]Reactions were performed on 50 mg scale at 25 °C.

[b]Racemic mixtures were obtained.

[c]Yield of isolated products after chromatography.

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Table 4

Optimization Study of the Sulfenylation of Diketopiperazine 86[a]

86 87

Entry	Solvent	LiHMDS	NaHMDS	KHMDS
		Yield of 87 (%) [rsm][b]		
1	THF	20 [70]	10 [40]	<5 [35]
2	CH_2Cl_2	<5 [<5]	<5 [<5]	<5 [<5]
3	PhMe	15 [70]	<5 [45]	<5 [30]
4	Et_2O	25 [60]	15 [35]	<5 [35]
5	THF[c]	46 [43]	28 [30]	<5 [40]

[a] Reactions were performed on 5 mg scale at 25 °C.

[b] Yields of product and recovered starting material (rsm) are isolated yields after chromatography, <5% yield refers to no detectable product or starting material as determined by ^{1}H NMR spectroscopic analysis of the crude reaction mixture.

[c] Reverse addition of preformed sulfenylation reagent to substrate and base.

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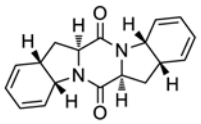
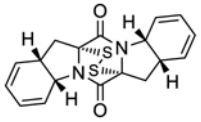
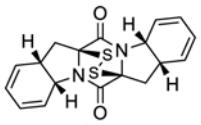
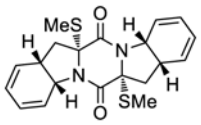
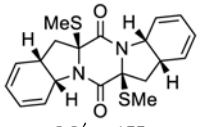
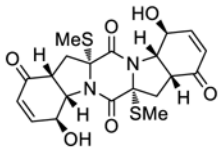
Table 5

Biological Evaluation of Selected Compounds in Poliovirus and *Plasmodium falciparum* Assays[a]

Entry	Structure	Code Number	Poliovirus EC_{50} [visual;[b] neutral red[c]]	Poliovirus EC_{90} [virus yield[d]]	Selectivity Index (Poliovirus) [visual;[b] neutral red;[c] virus yield[d]]	*Plasmodium falciparum* IC_{50}
1	 **45**	KCN-7	>50 μM;[b] n.d.[c,e]	n.d.[d,e]	n.d.[e]	>50 μM
2	 **46**	KCN-19	101±59 nM;[b] 115±59 nM[c]	149±65 nM[d]	70±48;[b] 56±45;[c] 41±23[d]	3.6 μM
3	 2,2′-*epi*-**46**	KCN-2,2′-*epi*-19	107±73 nM;[b] 123±90 nM[c]	177±45 nM[d]	59±59;[b] 75±92;[c] 27±11[d]	2.7 μM
4	 **55**	KCN-18	14.5 μM;[b] 25.6 μM[c]	n.d.[d,e]	5.7;[b] 3.4;[c] n.d.[d,e]	>50 μM
5	 2,2′-*epi*-**55**	KCN-2,2′-*epi*-18	56.9 μM;[b] >50 μM[c]	n.d.[d,e]	1.5;[b] n.d.;[c,e] n.d.[d,e]	>50 μHM
6	 **58**	KCN-20	>50 μM;[b] n.d.[c,e]	n.d.[d,e]	n.d.[e]	1.2 μM

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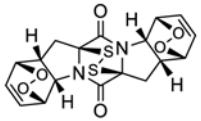
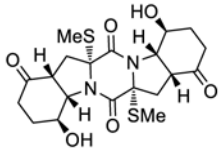
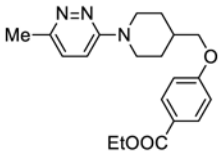
Entry	Structure	Code Number	Poliovirus EC_{50} [visual;[b] neutral red[c]]	Poliovirus EC_{90} [virus yield[d]]	Selectivity Index (Poliovirus) [visual;[b] neutral red;[c] virus yield[d]]	*Plasmodium falciparum* IC_{50}
7	 **61**	KCN-21	21.4±11.9 nM;[b] 21.4±2.4 nM[c]	38.1±7.1 nM[d]	59±33;[b] 41±17;[c] 23±8[d]	2.5 μM
8	 **1**: epicoccin G	KCN-1	>50 μM;[b] n.d.[c,e]	n.d.[d,e]	n.d.[e]	2.5 μM
9	 **99**:Pirodavir®[f]	—	n.d.;[b,e] 1.58 μM[c]	1.55 μM[d]	>18[c]	n.d.[e]

[a] Assays for entries 2, 3 and 7 were carried out as triplicates; mean and standard deviation are given. For experimental details of all assays, see Supporting Information.

[b] Visual assay.

[c] Neutral red assay.

[d] Virus yield reduction assay.

[e] Not determined.

[f] Standard anti poliovirus drug used as a control.

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