General Approach for Preparing Epidithiodioxopiperazines from Trioxopiperazine Precursors: Enantioselective Total Syntheses of (+)- and (−)-Gliocladine C, (+)-Leptosin D, (+)-T988C, (+)-Bionectin A, and (+)-Gliocladin A

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S *Supporting Information*

ABSTRACT: A common strategy for preparing tryptophan-derived epidithiodioxopiperazine (ETP) natural products containing a hydroxyl substituent adjacent to a quaternary carbon stereocenter is reported. This strategy is exemplified by enantioselective total syntheses of four heptacyclic ETP natural products—gliocladine C (**6**), leptosin D (**7**), T988C (**8**), and bionectin A (**9**)—starting with the di-(*tert*-butoxycarbonyl) derivative **17** of the trioxopiperazine natural product gliocladin C, which is readily available by enantioselective chemical synthesis.

In addition, total syntheses of the enantiomer of gliocladine C (*ent*-**6**) and gliocladin A (**11**), the di(methylthio) congener of bionectin A, are reported. These syntheses illustrate a synthetic strategy wherein diversity in the dioxopiperazine unit of ETP natural products is introduced at a late stage in a synthetic sequence. In vitro cytotoxicity of compounds in this series against invasive human prostate (DU145) and melanoma (A2058) cancer cell lines is described and compared to that of chaetocin A (**4**).
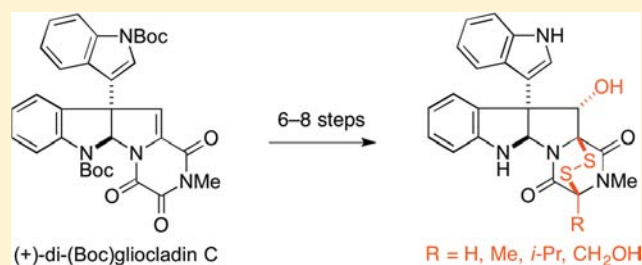
■ INTRODUCTION

Epidithiodioxopiperazine (ETP) toxins are a class of fungal metabolites (Figure 1) that possess unique molecular architectures and potent activities against parasites, viruses, bacteria and cancer cells.[1,2] In recent studies, epidithiodioxopiperzines have shown selectivity against myeloma and solid tumors,[1,3] with a number of molecular targets now identified.[4] The biological activity of these natural products is associated with the di- or polysulfide-bridged dioxopiperazine subunit,[1] which can form covalent adducts with cysteine residues of proteins, generate reactive oxygen species by a redox-cycling mechanism,[5] or sequester zinc from protein targets.[4d] In spite of their promising biological properties, the therapeutic potential of molecules containing ETP fragments is largely unexplored, possibly a result of the scarcity of ETP natural products and the difficulty in preparing this unique fragment.[1]

Both the structure and chemical lability of ETPs pose a number of challenges for chemical synthesis. The ground breaking syntheses of sporidesmin A (**2**)[6] and gliotoxin (**1**)[7] reported by Kishi and co-workers in the 1970s were achieved by introducing the ETP fragment in masked form as a nucleophilic unit.[8] This imaginative method has been employed subsequently to assemble simplified ETP[9a] structures and in the total synthesis of additional ETP natural products.[9b] Beginning

with Movassaghi's pioneering total synthesis of dideoxyverticillin A (**3**) in 2009, enantioselective total syntheses of several dimeric, C_2-symmetric, ETP natural products have been accomplished recently.[10−14] Several of these recent total syntheses improved upon methods introduced by earlier workers for incorporating the disulfide fragment of the epidithiodioxopiperazine by both electrophilic and nucleophilic sulfenylation.[15] In addition, strategies for controlled introduction of 2−4 sulfur atoms into the bridge of these natural products have been developed.[16]

The largest group of ETP toxins is tryptophan-derived and contains an ETP fragment fused to a cyclotryptamine moiety. Many of these naturally occurring compounds possess oxygenation in the pyrrolidine ring adjacent to the quaternary carbon stereocenter (e.g., **5−11**), and a majority of the dimeric structures, illustrated by leptosin K (**5**), are C_1-symmetric. The presence of a hydroxyl substituent adjacent to the quaternary carbon stereocenter markedly increases the lability of molecules of this type to both acids and bases.[17,18] We report herein the first approach for preparing cyclotryptophan ETP natural products containing both hydroxyl substitution at C11 of the
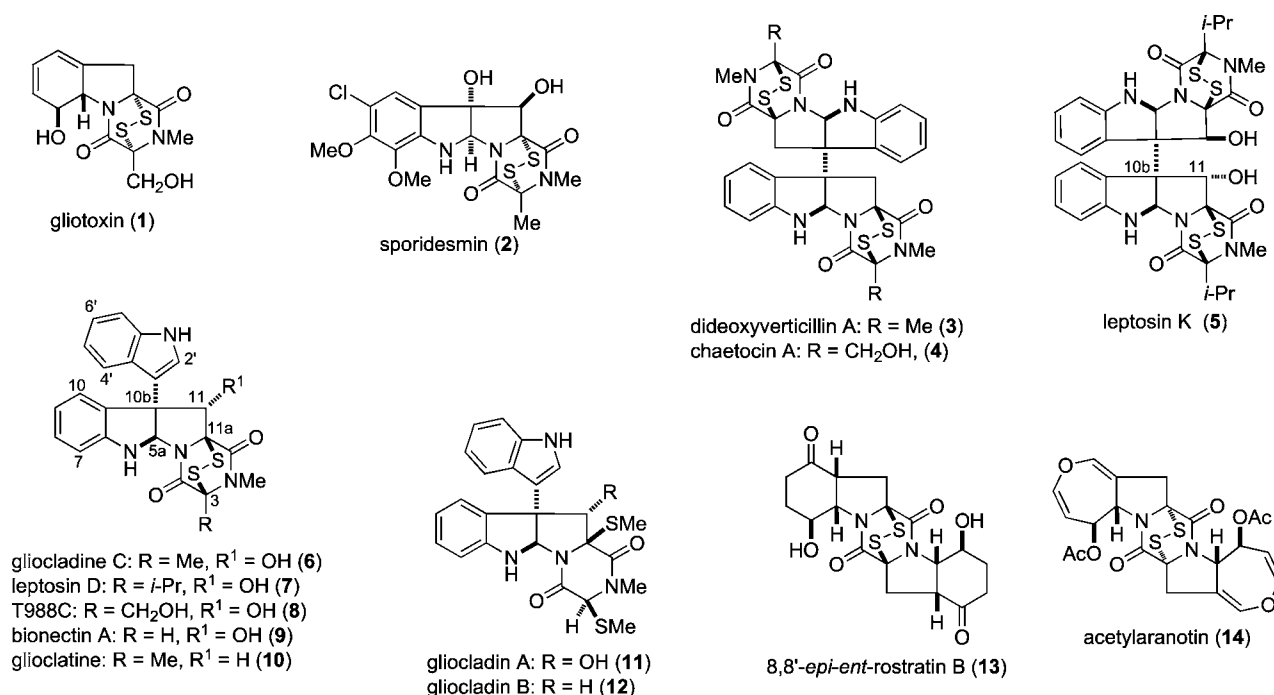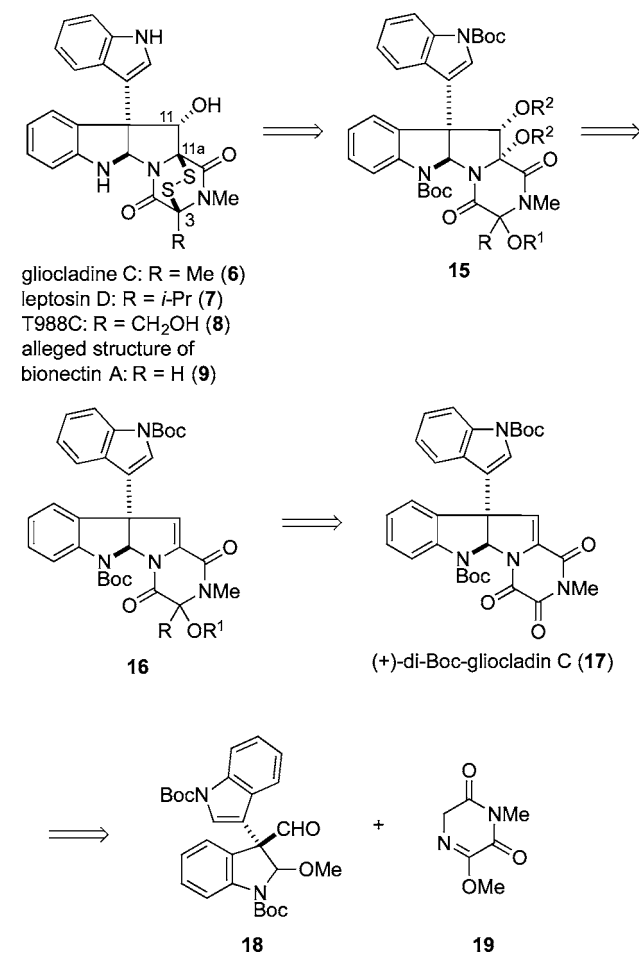
Figure 1. Representative epidithiodioxopiperazine (ETP) and related di(methylthio) dioxopiperazine natural products.

pyrrolidine ring and a C10b quaternary stereocenter. Our strategy is exemplified by enantioselective total syntheses of gliocladine C (6),[2a,19] leptosin D (7),[20] T988C (8),[21] and bionectin A (9).[2b] In addition, the total synthesis of a di(methylthio) congener gliocladin A (11,[22] also called bionectin C[2b]) is reported.[23] In vitro cytotoxicity of compounds in this series against invasive human prostrate and melanoma cancer cell lines is described also and compared to that of chaetocin A.

RESULTS AND DISCUSSION

Synthesis Plan. Central to our synthetic strategy was the recognition that functionality embedded in the di-(tert-butoxycarbonyl) derivative 17[19] of the trioxopiperazine natural product gliocladin C[22,24] could be manipulated to access various members of the family of ETP natural products exemplified by 6−9, in addition to di(methylthio) analogues such as 11 (Scheme 1). We envisaged fashioning the epidisulfide by displacement of the oxygen substituents at C3 and C11a of precursor 15, which in turn should be available from stereoselective dihydroxylation of the C11,C11a double bond of alkylidene dioxoxopiperazine 16.[25] Alternatively, stereoselective epoxidation of the double bond of intermediate 16 could also provide a suitably oxidized precursor for fashioning the ETP fragment. In both cases, we expected the oxidant to approach the double bond from the C11-Re face, as the X-ray structure of (+)-gliocladin C[19] suggests that the fused-indoline ring would shield the C11-Si face (Figure 2). Tertiary alcohol 16 would result from chemoselective addition of an appropriate organometallic reagent to the most electrophilic carbonyl group of trioxopiperazine 17.[26] Recently, we reported an efficient second-generation total synthesis of (+)-gliocladin C that proceeds via the intermediacy of 17.[19] The central feature of this convergent synthesis was the late stage coupling of enantioenriched pro-dielectrophile 18 (or its enantiomer) with pro-dinucleophile 19, which allows access to

Scheme 1

gliocladine C: R = Me (6)
leptosin D: R = i-Pr (7)
T988C: R = CH₂OH (8)
alleged structure of
bionectin A: R = H (9)

15

16

(+)-di-Boc-gliocladin C (17)

18   +   19

gram quantities of either di-(tert-butoxycarbonyl)gliocladin C (17) or its enantiomer (Scheme 1).
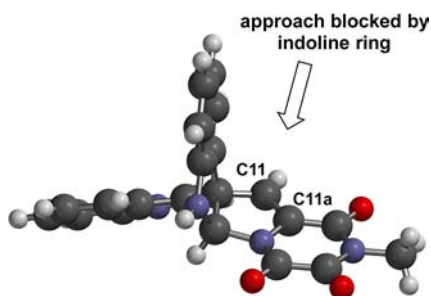
Figure 2. X-ray model of gliocladin C[19] showing steric shielding of the C11,C11a double bond by the fused indoline fragment.
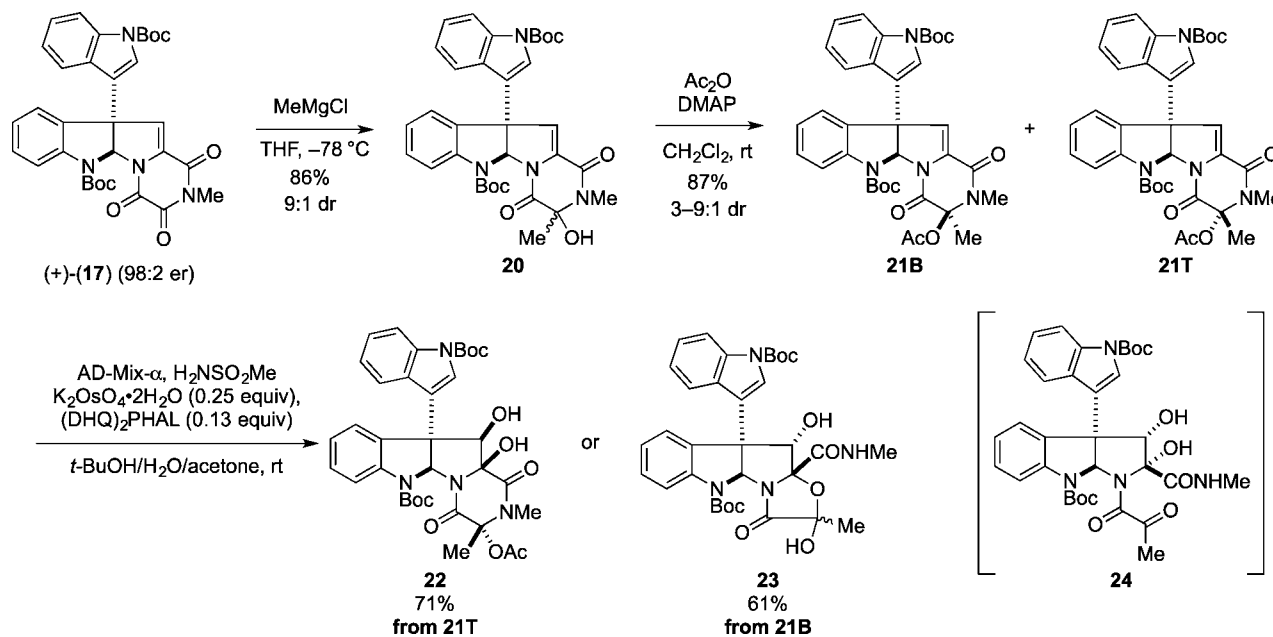
Enantioselective Total Synthesis of (+)-Gliocladine C and ent-Gliocladine C.

We began our synthesis endeavors by targeting gliocladine C (**6**), which contains a methyl substituent at C3.[2a] Selective addition of methylmagnesium chloride to (+)-di-(tert-butoxycarbonyl)gliocladin C (**17**) at −78 °C gave alcohol product **20** as a 9:1 mixture of alcohol epimers (Scheme 2). It was critical in this reaction to maintain the temperature at −78 °C, as over methylation occurred at higher reaction temperatures. When the 9:1 mixture of epimers **20** was exposed to excess Et$_3$N in DMF at room temperature, epimerization of the alcohol stereocenter by reversible opening of the dioxopiperazine ring resulted in a ~2.5:1.0 mixture of alcohol epimers. Acetylation of the 9:1 mixture of alcohol epimers **20** upon reaction with acetic anhydride and 4-dimethylaminopyridine (DMAP) at room temperature provided **21** as a separable mixture of acetate epimers in ratios that ranged from 3:1−9:1. As a distinction is necessary throughout this discussion, the protected alcohol epimers are classified as either top (T) or bottom (B) based upon their corresponding retention factors on silica gel thin layer chromatography, with (T) being the least polar. Dihydroxylation of the alkylidene double bond of the separated acetate epimers **21T** (minor diastereomer) and **21B** was achieved using the Sharpless AD-mix-α conditions.[27] Because of the hindered nature of the double bond, these
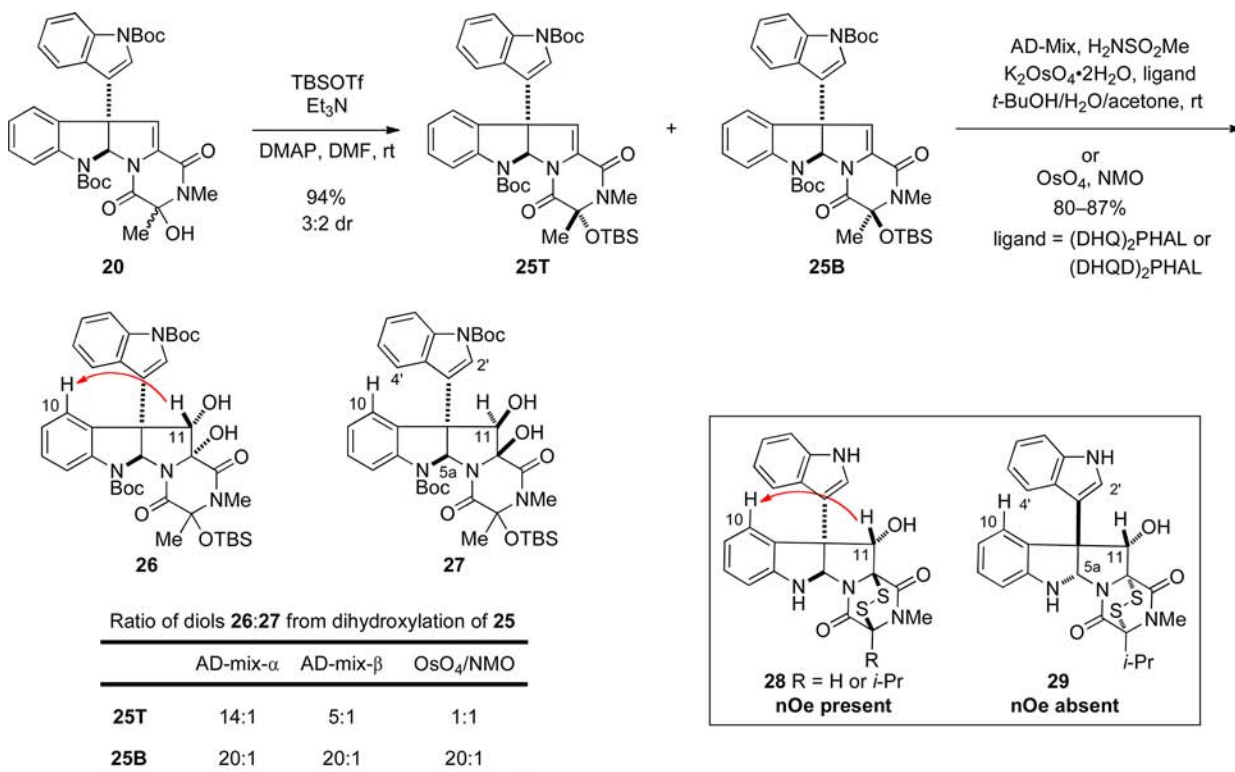
reactions required the presence of additional K$_2$OsO$_4$•2H$_2$O (0.25 equiv) and (DHQ)$_2$PHAL (0.13 equiv) to proceed at a convenient rate. To our surprise, the major product isolated from the oxidation of **21T**, diol **22**, was determined to have the undesired β-configuration (vide infra). In contrast, oxidation of epimer **21B** took place with high facial selectivity from the C11-Re face to give the ring-contracted oxazolidinone **23** (2:1 dr at C3) in 61% yield. This product likely was the result of acetate cleavage under the basic dihydroxylation conditions to generate ketone **24**.[28,29]

We turned to examine a sequence in which the alcohol substituent of intermediate **20** was masked with a base-stable tert-butyldimethylsiloxy group (Scheme 3). Silylation of the 9:1 mixture of alcohol epimers **20** provided a 3:2 mixture of siloxy epimers, the erosion in configuration at C3 undoubtedly arising from triethylamine-promoted epimerization of the tertiary alcohol prior to silylation. In our initial experiments, these siloxy epimers were separated on silica gel to provide pure samples of **25T** (major diastereomer) and **25B**. Epimers **25T** and **25B** were individually dihydroxylated with OsO$_4$/NMO, AD-mix-α or AD-mix-β, with additional K$_2$OsO$_4$•2H$_2$O and (DHQ)$_2$PHAL [or (DHQD)$_2$PHAL] being added to the latter reactions to increase the rate of dihydroxylation. The α-diol product was formed with high selectivity from oxidation of **25B**, independent of the oxidant. In contrast, dihydroxylation of **25T** using AD-mix-α yielded the α-diol selectively (dr = 14:1), whereas both AD-mix-β (dr = 5:1) and OsO$_4$ (dr = 1:1) afforded significant amounts of the β-diol product. The relative configuration of diol products **26** and **27** was assigned from diagnostic nuclear Overhauser enhancement (NOE) of the C11 methine hydrogen, as has been documented for diastereomers such as **28** and **29** (see Scheme 3).[2b,21,30] For example, irradiation of the C11−H of diol **26** leads to an observable NOE signal with the aromatic C10−H resonance. In these rigid structures, a β-hydrogen at C11 is in close proximity to both C10−H and C4′-H, whereas, an α-hydrogen at C11 is held far from the C10−H such that the dominant NOE signals observed upon selective irradiation of this hydrogen occur

Scheme 2

Scheme 3

Ratio of diols 26:27 from dihydroxylation of 25

|	AD-mix-α	AD-mix-β	OsO$_4$/NMO
25T	14:1	5:1	1:1
25B	20:1	20:1	20:1

with the C2′-H, C4′-H, and C5a-H.[31] The diminished facial selectivity seen in dihydroxylation of siloxy epimer **25T** from the C11-*Re* face appears not to be the result of steric interactions with the siloxy substituent, as the lowest selectivity was observed with the smallest oxidant OsO$_4$.[32]

We were now set to address the critical incorporation of the epidithio bridge. Initially, we attempted to access gliocladine C (**6**) directly from diol **26B**. However, the acid lability of a pyrrolidinoindoline containing a C11-hydroxyl substituent proved problematic, with 3,3′-biindole being the only product isolated upon exposure of **26B** to excess H$_2$S and BF$_3$•OEt$_2$ at room temperature. As this fragmentation undoubtedly arises by cleavage via a retro enamine-aldol process of the C10b–C11 bond,[17,18] it was apparent that the C11-hydroxyl substituent would require protection prior to introducing the disulfide (Scheme 4). Therefore, the initially produced 3:2 mixture of siloxy epimers **25** was dihydroxylated with AD-mix-α and the crude diol products were acetylated to provide α-diacetates **30** in 76% yield over the two steps; this intermediate contained ~5% of the corresponding β-diacetates. Diacetate **30B** was transformed into a crystalline intermediate, which allowed assignment of the relative configuration of the siloxy stereocenters of the epimers of intermediates **25**, **26** and **30**.[33]

Diacetates **30** were successfully transformed to (+)-gliocladine C in two additional steps. Exposure of intermediate **30** to 10 equiv of BF$_3$•OEt$_2$ in a 1:1 mixture of condensed H$_2$S and CH$_2$Cl$_2$ initially at −78 °C with slow warming to room temperature, followed by oxidation with either iodine or air provided a single ETP product **32**.[34] Precisely how the oxidation step was carried out proved to be important, because the first step gave rise to a BF$_3$ complex of a dithiol intermediate—presumably **31**—even after aqueous work up.[35] This intermediate was characterized by a combination of low-resolution mass spectrometry, ^{11}B NMR, and ^{19}F NMR

Scheme 4
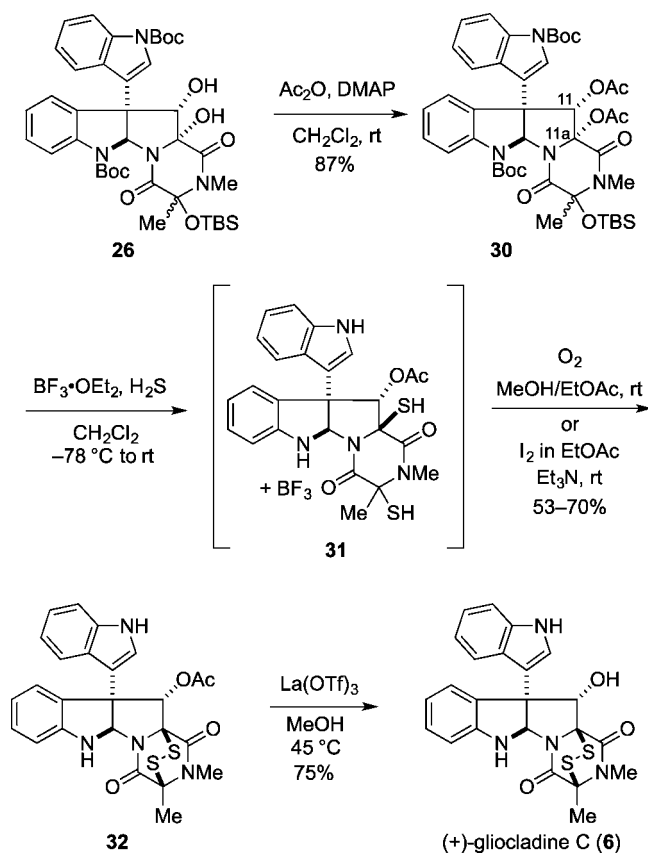
analysis. After some experimentation, it was found that allowing this unpurified intermediate to react at room temperature with

either iodine and Et$_3$N in EtOAc, or an oxygen atmosphere in MeOH/EtOAc, provided ETP **32** in 53−70% yield from diacetates **30**.[36] All that remained was deacetylation, which was best accomplished by heating **32** at 45 °C in methanol in the presence of an excess of La(OTf)$_3$.[37] In this way, enantiopure (+)-gliocladine C (**6**), [α]$^{23}_{D}$ +505 (c 0.47 pyridine), was formed in 75% yield; this product exhibited spectroscopic and optical rotation data in agreement with those reported for the natural sample.[2a,38] Starting with (−)-di-(tert-butoxycarbonyl)-gliocladin C, (−)-**17**,[39] ent-(+)-gliocladine C (ent-**6**), [α]$^{23}_{D}$ −489 (c 0.16 pyridine), was prepared in identical fashion in 24% overall yield over six steps.[40]

The facial selectivity in the introduction of the epidisulfide bridge to form ETP **32** requires further comment. Diastereoselection in the addition of sulfur to C11a of an intermediate N-acyliminium ion intermediate is believed to result from steric shielding by the angular 3-indolyl substituent. An alternative explanation in which the C11 acetate plays a decisive role—for example, by formation of a dioxolanium ion intermediate—appears less likely in light of our earlier report of the transformation summarized in eq 1.[37]


$$\text{(1)}$$
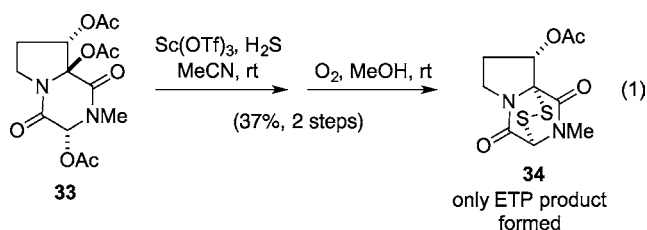
Enantioselective Total Synthesis of (+)-Leptosin D. In order to establish the generality of the synthetic sequence we had developed, as well as to provide samples for biological evaluation, we turned to the synthesis of the ETP natural products **7**−**9** that differ in substitution at C3. Following the chemistry defined for the synthesis of gliocladine C, potential leptosin D precursor **37** was obtained in 46% yield over four steps from (+)-**17** (Scheme 5). In this series, dihydroxylation of both siloxy epimers of intermediate **36** with AD-mix-α gave rise to the α-configured diacetates **37** in high distereoselectivity (20:1 dr). However, upon attempted formation of the corresponding ETP from **37**, only monothiol **38** was obtained.

We postulated that a better leaving group at C3 would be required to overcome the increased A^{1,3} strain associated with generation of the C3 N-acyliminium ion intermediate in the isopropyl series. Thus, tertiary alcohol intermediate **35** was acetylated, providing acetate **39** as a 1.2:1.0 mixture of epimers. Upon dihydroxylation with AD-mix-α, **39T** afforded the β-diol **40** with high stereoselectivity (20:1 dr), whereas **39B** provided the α-diol **41** also with high stereoselectivity (20:1 dr, Scheme 6).[41] Fortunately, the formation of ring-contracted products analogous to **23** was not observed during Sharpless dihydroxylation of **39**, presumably as a result of the larger isopropyl group mitigating cleavage of the C3 acetate. The relative configurations of diols **40** and **41** were assigned using NOE data in the manner previously discussed.[31] Furthermore, NOE enhancements between the C11a hydroxyl proton of **41** and the isopropyl group indicated that these substituents were cis.

The elaboration of diol **41** to leptosin D is summarized in Scheme 7. Diacetylation of **41** with a large excess of acetic anhydride and DMAP provided diacetate **42** in 91% yield. As
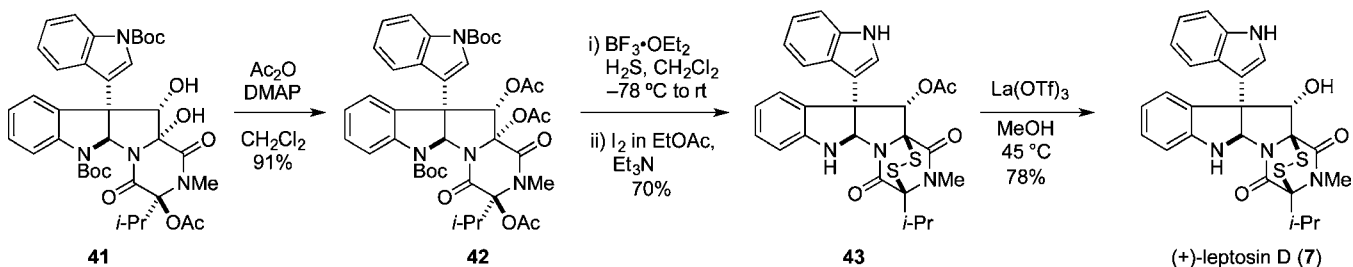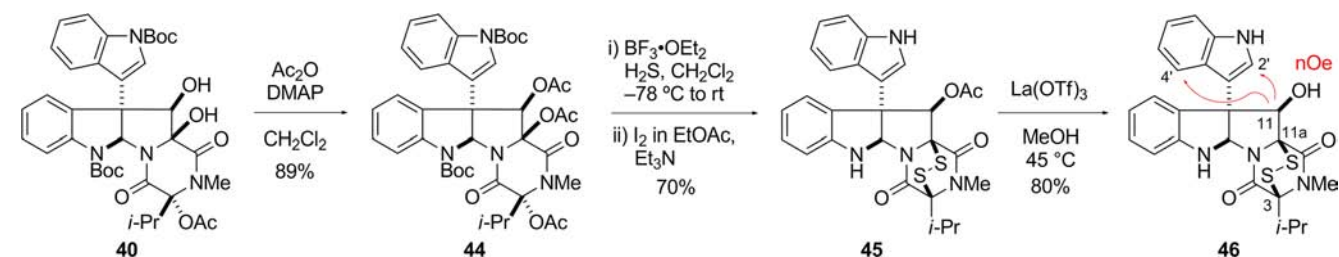
Scheme 5
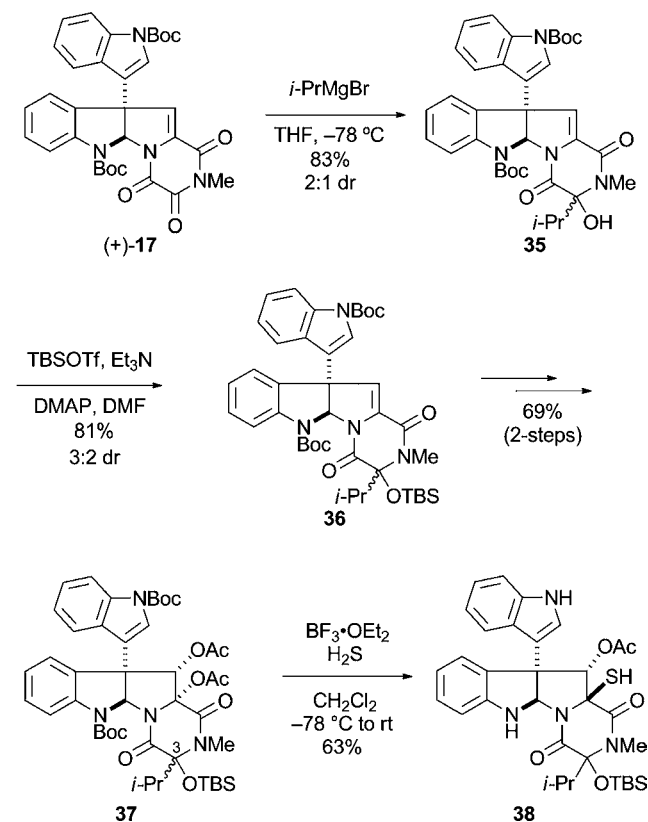
Scheme 6
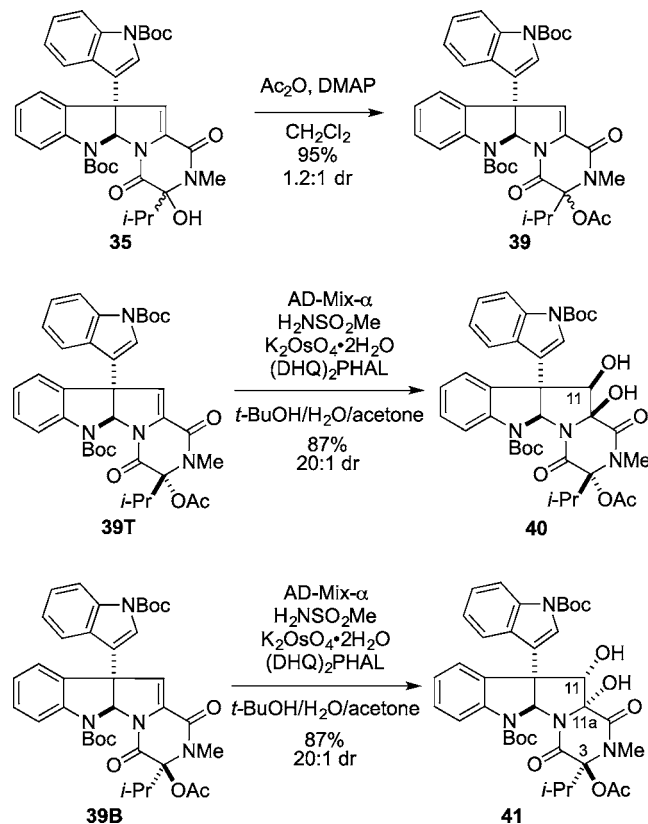
observed in the gliocladine C series, exposure of **42** to excess H$_2$S in the presence of BF$_3$•OEt$_2$ at −78 °C to room

Scheme 7

Scheme 8

temperature, followed by oxidation, provided a single epidisulfide product, **43**, in 70% yield. Lewis acid-promoted deacetylation of **43** gave (+)-leptosin D (**7**) in 78% yield. The optical rotation of synthetic **7**, $[\alpha]^{22}_D$ +423 (c 0. 225, CHCl₃), compared well with the value reported for the natural sample, $[\alpha]^{21}_D$ +436 (c 0.51, CHCl₃), as did all spectroscopic data.[20]

In a similar fashion, β-diol **40** was elaborated to ETP intermediate **45** in 62% overall yield (Scheme 8). Deacetylation of **45** gave 11-*epi*-leptosin D (**46**) in 80% yield. The absence of ¹H NMR NOE enhancement between H11 and H10 and the presence of an NOE between H11 with both H2′ and H4′, confirmed the relative configuration of the secondary alcohol of ETP **46**. ¹H NMR spectral data for synthetic **46** were essentially identical to that reported for **48**, a degradation product of leptosin K₁ (eq 2).[30,42] The absolute configuration
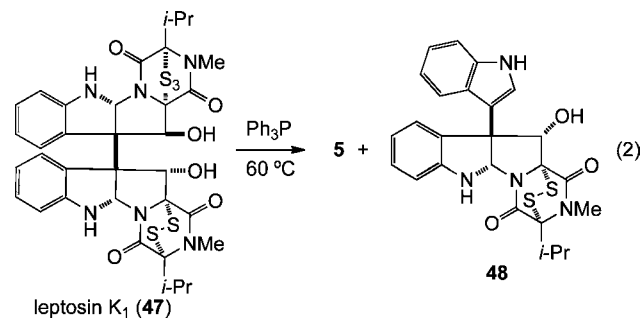
of **48** had been established by CD spectroscopy, thus **48** and synthetic 11-*epi*-leptosin D (**46**) should be enantiomers. However, the rotation of **46**, ($[\alpha]_D^{23}$ +212 (c 0.13, CHCl₃) is of the same sign and similar magnitude to that reported for leptosin K₁ degradation product **48**, $[\alpha]_D^{23}$ +179 (c 0.17, CHCl₃).

We turned to CD spectroscopy to clarify the structural ambiguity associated with synthetic 11-*epi*-leptosin D (**46**) and leptosin K₁ degradation product **48**. Epidisulfides have been the subject of a number of theoretical and experimental CD studies.[30,43] The sign of the bands at approximately 230 and 270 nm—with more emphasis on the 270 nm band—can be used to assign the absolute configuration of the epidisulfide

bridge in ETPs of the type considered here. The contribution of transitions arising from both the disulfide bonding orbitals to the antibonding σ-orbitals of the dioxopiperazine (n to σ* transition) and the peptide n to π* transition in the dioxopiperazine ring dominate at about 230 nm for disulfides with small C–S–S–C dihedral angles, with a positive band typically correlating with the 3S,11aS configuration.[43d,44] Whereas a charge-transfer band arising from an interaction of the bonding disulfide orbitals with the antibonding π-orbitals of the dioxopiperazine (n to π* charge-transfer) dominates at about 270 nm, with a negative band corresponding to the 3S,11aS epidisulfide configuration.[43d]

In our studies, the negative CD band at 265 nm for (+)-11-*epi*-leptosin D (**46**) and the similar band observed for (+)-leptosin D (**7**) provide strong support for our assignment of the 3S,11aS absolute configuration for these products (Figure 3). Similarly, the CD data reported by Numata and co-workers for epidisulfide **48** are in good agreement with the assigned 3R,11aR configuration for this product.[30] As NOE data for synthetic 11-*epi*-leptosin D (**46**) support the relative configuration at C11, we surmise that the optical rotation for **48** was reported incorrectly by the earlier authors.
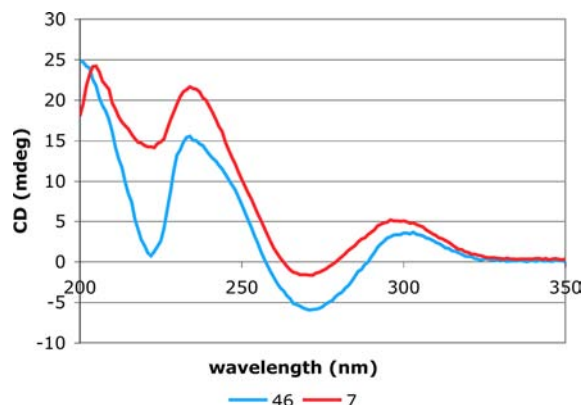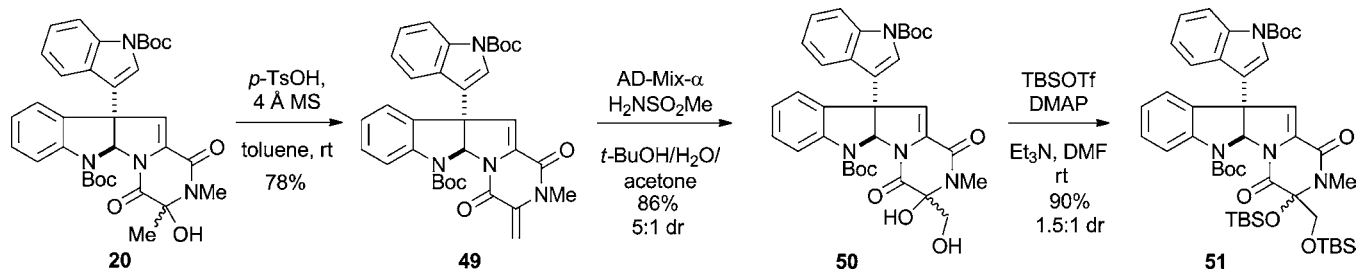

Figure 3. CD spectrum of **7** and **46** in EtOH (c = 2 × 10⁻⁴ M).

Scheme 9

Enantioselective Total Synthesis of (+)-T988C.

The total synthesis of T988C (**8**) posed an additional synthetic challenge: an efficient method for installing the hydroxymethyl side chain, preferably protected as its *tert*-butyldimethylsilyl ether to allow concomitant deprotection during formation of the epidisulfide, would be required. Initial efforts focused on SmI₂-mediated[45] addition of (*tert*-butyldimethylsiloxy)methyl chloride[46] to the C3 carbonyl group of trioxopiperazine **17**. However, addition of this organometallic reagent to **17** was not observed. Therefore, our attention turned to the formation of an exomethylene, which could be oxidized to provide the hydroxymethyl functionality. In this vein, the Wittig reaction of (+)-**17** with methyltriphenylphosphorane was examined and found to be capricious. However, acid mediated dehydration of hemiaminal **20** provided dialkylidene dioxopiperazine **49** in 78% yield (Scheme 9). The exomethylene unit of **49** could be selectively epoxidized using dimethyldioxirane;[47] however, the product was not stable and only diol **50** was isolated in low yield. After some experimentation, selective dihydroxylation of the exocyclic methylidene of **49** was achieved using AD-mix-α without additional potassium osmate and (DHQ)₂PHAL, giving diol **50** in 86% yield.[48] Protection of the 5:1 mixture of epimeric diols **50** as their corresponding *tert*-butyldimethyl-silyl ethers provided **51** as a separable 1:1.5 mixture of disilyl epimers.

Fortunately, both epimeric disilyl ethers **51** could be elaborated to (+)-T988C (**8**). Oxidation of **51T** or **51B** using our augmented AD-mix-α conditions took place with high diastereoselectivity to provide **52** in good yield as predominantly the α-diol stereoisomers (20:1 dr) (Scheme 10). Acetylation of the combined diols **52** (1:1.5 with respect to the C3 center) afforded α-diacetate **53** as a mixture of siloxy epimers.[49] Treatment of this mixture of silylether epimers **53** with excess H₂S in the presence of BF₃•OEt₂ at −78 °C to room temperature, followed by oxidation with iodine and Et₃N, provided a single epidisulfide product, **54**, in 70% yield. To realize this yield, it was essential in the first step to increase the amount of BF₃•OEt₂ to 30 equiv, otherwise a separable mixture of **54** and the corresponding product in which the primary silyl ether had not been removed was isolated. Deacetylation of **54** as before, provided (+)-T988C (**8**). The optical rotation of synthetic **8**, $[\alpha]^{23}_D$ +282 (*c* 0.024, MeOH), compared well with the value reported for the natural sample, $[\alpha]^{21}_D$ +277 (*c* 0.0006, MeOH), as did all spectroscopic data.[21]

Enantioselective Total Synthesis of (+)-Bionectin A and (+)-Gliocladin A.

Focus then turned toward the preparation of bionectin A (**9**)[2b] and gliocladin A (**11**), which both lack an alkyl substituent at C3.[22,2b] Reduction of the trioxopiperazine (+)-**17** with NaBH₄ at −42 °C gave a 1:1 mixture of epimeric alcohol products (Scheme 11). In contrast, reduction with lithium tri-*sec*-butylborohydride (L-selectride) at
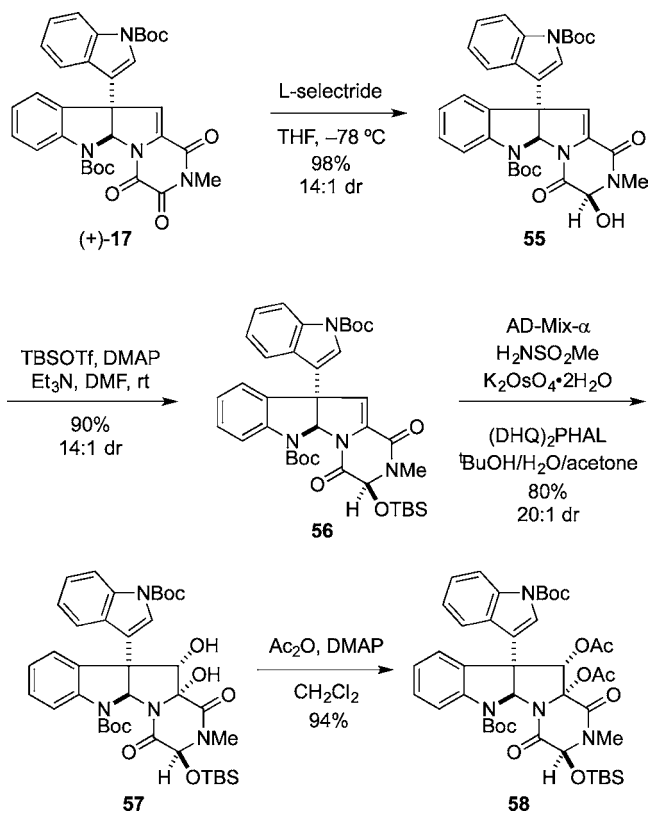
Scheme 10
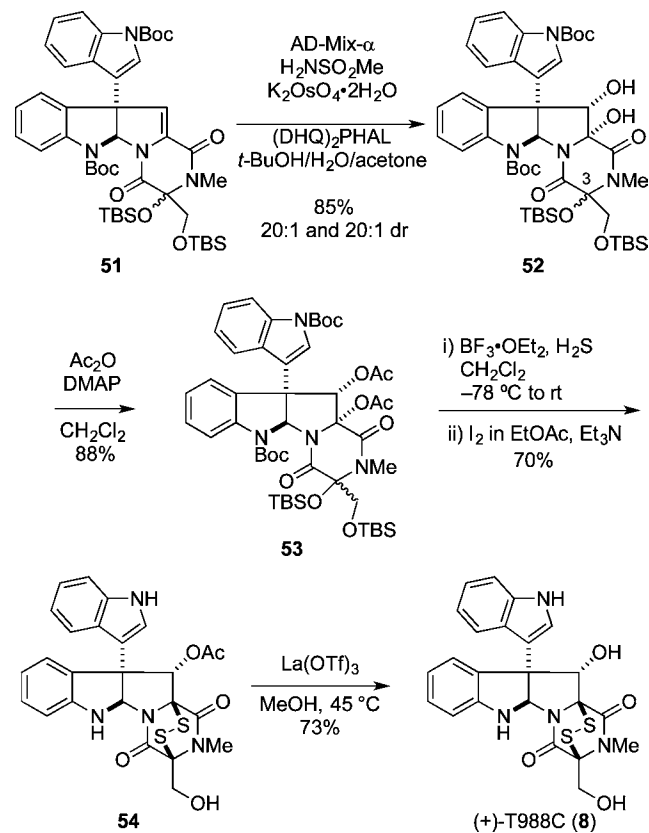
−78 °C was highly stereoselective, providing alcohol **55** as a 14:1 mixture of alcohol epimers. Silylation of this 14:1 mixture of epimers **55** took place without epimerization at C3 to give **56** as a separable 14:1 mixture of C3-siloxy epimers. The relative configurations of these epimers were tentatively assigned by their relative polarities. In general, we have found that epimers possessing a β-oriented C3 oxygen substituent (cf. **25B** and **39B**) were more polar on silica gel. Dihydroxylation of **56** and subsequent diacetylation of the major α-diol product **57** afforded intermediate **58** in 75% yield over two steps.[49]

Intermediate **58** could be employed to introduce either the epidisulfide or the di(methylthio) functionality of **9** and **11**, respectively (Scheme 12). To this end, **58** was exposed to BF₃•OEt₂ and MeSH to afford a separable 2.5:1 mixture of di(methylthio) derivative **61** and a methylthio stereoisomer. It was readily established by 1D NOE analysis that these products differ in configuration at C3, with the C11a methylthio substituent being β-oriented in each stereoisomer.[50] Therefore, a two-step sequence was used to stereoselectively introduce the di(methylthio) functionality. In this sequence, the unpurified

Scheme 11

MeOH), compared well with the value reported for the natural sample, $[\alpha]^{21}_D$ +244 (c 0.2, MeOH), as did spectroscopic data.[2b,22]

Dithiol intermediate **59** was converted also to the corresponding epidisulfide by exposure to iodine and Et$_3$N. To our surprise, the resulting product **60** was somewhat unstable and was best transformed immediately to the corresponding alcohol product. This deacylation had to be carried out under carefully prescribed conditions; judicious choice of both substrate concentration and temperature were paramount, as increasing either afforded significant quantities of 3,3′-biindole. The product **9** isolated after acetyl cleavage exhibited spectroscopic and mass spectrometric data expected for this ETP product. However, NMR data for synthetic bionectin A (**9**) did not agree as closely with the data reported by Zheng and co-workers[2b] as similar comparisons of the other natural products prepared in this study.[52,53] The structural assignment for synthetic **9** was based upon 1D and 2D NMR data, including NOE data confirming that the C11-OH is α-oriented. In addition, a positive CD band at 230 nm and negative band at 270 nm) indicated that the ETP moiety possessed the 3S,11aS configuration (Figure 4). We also
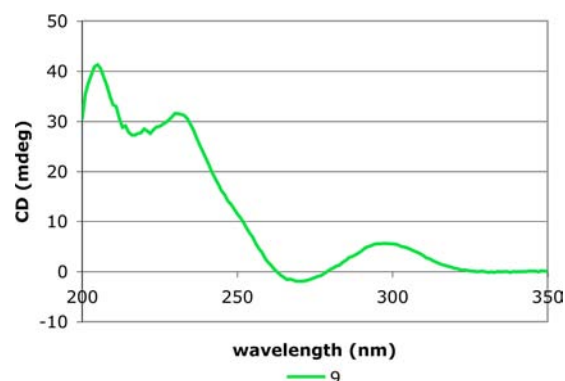

Figure 4. CD spectrum of **9** in EtOH (c = 2 × 10⁻⁴ M).

dithiol intermediate **59** formed from BF$_3$•OEt$_2$-promoted reaction of **58** with H$_2$S was allowed to react with excess MeI and K$_2$CO$_3$ in acetone to yield **61** as a single stereoisomer.[51] The acetyl group was then removed to provide (+)-gliocladin A (**11**). In this step, the addition of DMAP was necessary to increase the rate of acetyl cleavage, otherwise decomposition pathways appeared to dominate because of instability of the product to the reaction conditions. Under the best conditions found, the reaction returned a 1.5:1.5:1.0 mixture of dithioether **61**, gliocladin A (**11**), and 3,3′-biindole. The optical rotation of synthetic **11**, $[\alpha]^{23}_D$ +266 (c 0. 34,

determined that the small differences in ¹H NMR shifts between synthetic **9** and those reported for bionectin A were not an artifact of different protonation states by showing that
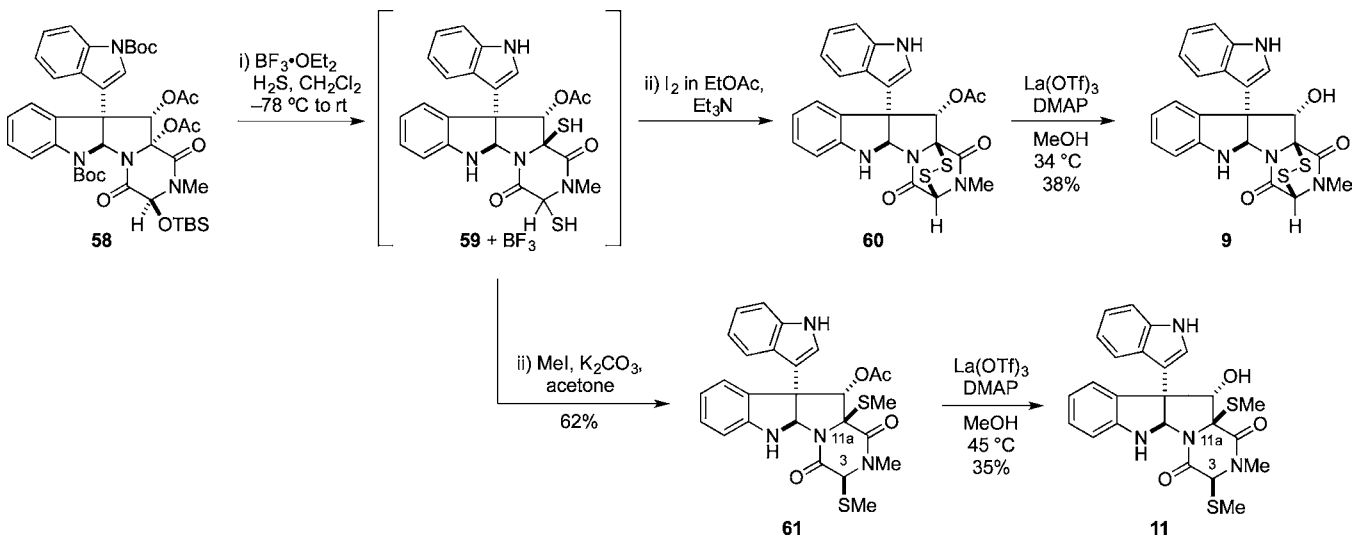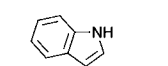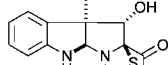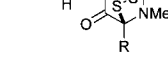
Scheme 12
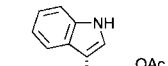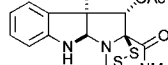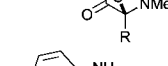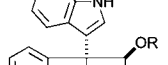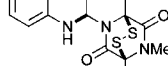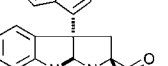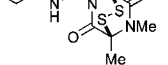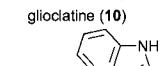
Table 1. Activity against Invasive Prostate Cancer (DU145) and Melanoma (A2508) Cell Lines

Entry	Structure		DU145 IC$_{50}$	A2058 IC$_{50}$
1		R = Me (6) gliocladine C	0.43 μM	0.68 μM
2		R = i-Pr (7) leptosin D	0.29 μM	0.85 μM
3		R = CH$_2$OH (8) T988C	0.53 μM	0.96 μM
4		R = H (9)	0.70 μM	2.0 μM
5		R = Me (32)	0.26 μM	0.96 μM
6		R = i-Pr (43)	0.39 μM	1.0 μM
7		R = CH$_2$OH (54)	0.50 μM	1.2 μM
8		R = H (46)	2.8 μM	4.0 μM
9		R = Ac (45)	2.5 μM	4.2 μM
10	glioclatine (10)		0.85 μM	1.8 μM
11	62		2.3 μM	3.8 μM
12	gliocladin A (11)		>5 μM	>5 μM
13	*ent*-gliocladine C		0.68 μM	0.35 μM
14	chaetocin A (4)		0.073 μM	0.061 μM

the ^{1}H NMR signals of **9** were unchanged upon sequentially adding 0.1−2 equiv of trifluoroacetic acid.[54] As the largest difference in ^{1}H NMR resonan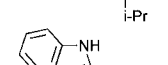ces was seen for C5a-H and N(6)-H ($\Delta\delta$ = 0.21 for each), we speculate that the natural product contained a small amount of a metal impurity bound between N(6) and the proximal carbonyl group.[55]

Biological Evaluation. Having synthesized a number of epidithiodioxopiperazines and related structures, their activities against two invasive cancer cell lines, DU145 (human prostate

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dx.doi.org/10.1021/ja400315y | J. Am. Chem. Soc. 2013, 135, 4117−4128

cancer) and A2058 (melanoma), were determined (Table 1). Analysis of the data reveals a number of trends. Most notably a disulfide is required for activity, with di(methylthio) congener **11** (entry 12) being inactive within the detection limits of the assay.[1,56−58] In regard to the active compounds, potency against the two cancer cell lines varies little whether the C11-hydroxyl group is acetylated (entries 5−7) or not (entries 1−3). However, the relative configuration of the C11-oxygen does affect activity (entries 2 and 6 vs 8 and 9), such that a 4−10 fold decrease in activity is seen when the C11-oxygen is cis to the epi-disulfide (entries 8 and 9 compared with entries 1−7). Furthermore, the data suggest that the relative configuration of the epi-disulfide bridge relative to the C10b quaternary carbon center is important for activity, with glioclatine (**10**) being about 2−3 fold more potent than diastereomer **62** (entries 10 and 11).[59] Absolute configuration does not appear to be important for activity, as gliocladine C and its enantiomer possess similar activities against DU145 and A2058 (entries 1 and 13). The presence of a hydroxyl substituent at C11 has only a minor influence, with glioclatine being about 2-fold less active than gliocladine C (entries 1 and 10). As ETP natural products **6−9** had been isolated by different co-workers and screened in different biological assays, the data in Table 1 allow the first insight into the role—minor as it turns out—of the C3 substituent (entries 1−7). We also had the opportunity to test chaetocin A (**4**), which possesses two epidithiodioxopiperazine fragments, in the same assays. When compared to T988C, which has the same C3 hydroxymethyl substituent, chaetocin A is 7 times more active against DU145 than T988C and 16 times more active against A2058 (entries 3 and 14).[60] However it merits note that chaetocin A is only 3.5 times more active against A2058 melanoma than the acetoxy derivative **32** of gliocladine C or leptosin D (entries entries 2, 5, and 14).

CONCLUSIONS

The first total syntheses of the epidithiodioxopiperazine natural products gliocladine C (**6**), leptosin D (**7**), T988C (**8**), and bionectin A (**9**) are reported, as well as total syntheses of *ent*-gliocladine C and the di(methylthio)-containing natural product gliocladin A (**11**). In these syntheses, the α-ketoimide carbonyl and alkylidene double bond of the trioxopiperazine unit of di-(*tert*-butoxycarbonyl)gliocladin C (**17**) are exploited to incorporate the C3 substituent and stereoselectively introduce the C11 hydroxyl group and the disulfide bridge of the ETP natural products **6−9**. Pivotal steps in the syntheses are (a) introduction of the C3 substituent by nucleophilic addition to the C3 carbonyl group, (b) stereoselective dihydroxylation of the C11−C11a double bond, and (c) stereoselective introduction of sulfur substituents at C3 and C11a by $BF_3 \cdot OEt_2$-promoted reaction of H_2S with precursors containing an acetoxy leaving group at C11a and a siloxy (or acetoxy) leaving group at C3, followed by mild oxidation. Stereoselection in forming the disulfide bridge is suggested to result from initial iminium ion formation at C11a, followed by kinetically controlled trapping with H_2S from the face opposite the angular 3-indolyl substituent.

The concise chemical synthesis of (+)- or (−)-**17** (10 steps and 15% overall yield from isatin)[19] and the divergent sequences developed in this study for elaborating these intermediates (6−8 steps, 14−33% yield) provided ample quantities of each targeted compound for in vitro cytotoxicity evaluations using DU145 prostrate and A2058 melanoma cells. As would be expected, the disulfide was required for activity.[1,56]

As reported for chaetocin A and its enantiomer in inhibiting histone methyltransferase G9a,[11] gliocladine C and its enantiomer exhibited similar activities against both cancer cell lines. The role of the C3 substituent, which was systematically varied in this study, was found to be minor, as was the effect of a C11 oxygen substituent. Synthetic T988C, which has the same C3 hydroxymethyl substituent as the ETP fragments of chaetocin A yet a single ETP fragment, was ∼10 times less active than chaetocin A. However, the presence of two ETP fragments is not essential to realize good activity, because chaetocin A is only 3.5 times more active against A2058 melanoma than the acetoxy derivative **32** of gliocladine C or leptosin D.

ASSOCIATED CONTENT

ⓈSupporting Information

Experimental details for synthesis of new compounds and their cytotoxicity assay, copies of ^{1}H and ^{13}C NMR spectra of new compounds, and CD spectra for ETP products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

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(40) See Supporting Information.

(41) It is of note that dihydroxylation of both **21T** and **39T**, which both have acetate substituents at C3, preferentially provided the β-diol products.

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(50) See Supporting Information for 1D NOE data.

(51) We have observed similar increases in stereoselectivity using this two-step sequence in the synthesis of other structurally related di(methylthio)dioxopiperazines. Whether the increase in stereoselectivity results form thermodynamic equilibration of dithiol intermediates, or involvement of the β-C11a-thiol intermediate in delivery of the second equivalent of H2S, or other factors is unclear at present.

(52) See Supporting Information for spectral comparisons of NMR spectra of synthetic and isolated epidisulfide natural products.

(53) Mean $|\Delta\delta| = 0.073$ ppm (^{1}H NMR) and 0.57 ppm (^{13}C NMR).

(54) See Supporting Information for experimental details and spectral data.

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(57) While this article was in review, Movassaghi, Hergenrother and co-workers reported in vitro cytotoxicity activity against several cancer cell lines (all different from the invasive cell lines employed in our studies) of 50 molecules synthesized in the Movassaghi laboratory, many containing ETP fragments or related dithio substituents.[58] The "monomeric" members of this set were derived largely from sarcosine, thus containing a hydrogen substituent at C3, and none contained oxidation at C11. Although this impressive study and the one we report here largely targeted different sites for variation, two common new themes emerged: the importance of the configuration of the disulfide bridge and the minimal change in activity brought about by variations in the C3 substituent.

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dx.doi.org/10.1021/ja400315y | *J. Am. Chem. Soc.* 2013, 135, 4117−4128