Total Syntheses of Thiocoraline and BE-22179 and Assessment of Their DNA Binding and Biological Properties

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Abstract: Full details of the total syntheses of thiocoraline (**1**) and BE-22179 (**2**), C_2 symmetric bicyclic octadepsipeptides possessing two pendant 3-hydroxyquinoline chromophores, are described in which their relative and absolute stereochemistry were established. Key elements of the approach include the late-stage introduction of the chromophore, symmetrical tetrapeptide coupling, macrocyclization of the 26-membered octadepsipeptide conducted at the single secondary amide site following disulfide formation, and a convergent assemblage of the tetradepsipeptide with introduction of the labile thiol ester linkage in the final coupling reaction under near racemization free conditions. By virtue of the late-stage introduction of the chromophore and despite the challenges this imposes on the synthesis, this approach provides ready access to a range of key chromophore analogues. Thiocoraline and BE-22179 were shown to bind to DNA by high-affinity bisintercalation analogous to echinomycin, but with little or no perceptible sequence selectivity. Both **1** and **2** were found to exhibit exceptional cytotoxic activity (IC_{50} = 200 and 400 pM, respectively, L1210 cell line) comparable to echinomycin and one analogue, which bears the luzopeptin chromophore, was also found to be a potent cytotoxic agent.

Thiocoraline (**1**, Figure 1) is a potent antitumor antibiotic[1] isolated from *Micromonospora* sp. L-13-ACM2-092. It constitutes the newest member of the class of naturally occurring, 2-fold symmetric bicyclic octadepsipeptides which include BE-22179[2] (**2**), triostin A[3] (**3**), and echinomycin[4] (**4**), which bind to DNA with bisintercalation.[5,6] Unlike BE-22179, thiocoraline does not inhibit DNA topoisomerase I or II, but it does inhibit DNA polymerase α at concentrations that inhibit cell cycle progression and clonogenicity.[7] It was found to unwind double-stranded DNA[7] and was suggested to bind to DNA with bisintercalation analogous to triostin, echinomycin, and members of the larger cyclic decadepsipeptides including sandramycin,[8,9] the luzopeptins,[9,10] and the quinoxapeptins.[11] The initial studies on thiocoraline as well as BE-22179 established their two-dimensional structures but not their relative and absolute stereochemistry.[1,2] Triostin A and echinomycin possess a D-stereochemistry at the α-position of the amide linkage to the quinoxaline chromophore (D-Ser) and L-stereochemistry at the remaining stereogenic centers. We have shown that the analogous centers of sandramycin[8] and the quinoxapeptins,[11] like the luzopeptins,[10] also incorporate D-Ser. Moreover, it was reported that a synthetic analogue of **3** possessing an all L-stereochemistry showed no appreciable DNA binding.[12] Thus, we anticipated that **1**, as well as the structurally related **2**, might possess a similar stereochemistry including the incorporation of an unusual chromophore bearing D-Cys. Herein, we report full details of the first total syntheses of thiocoraline and BE-22179 which

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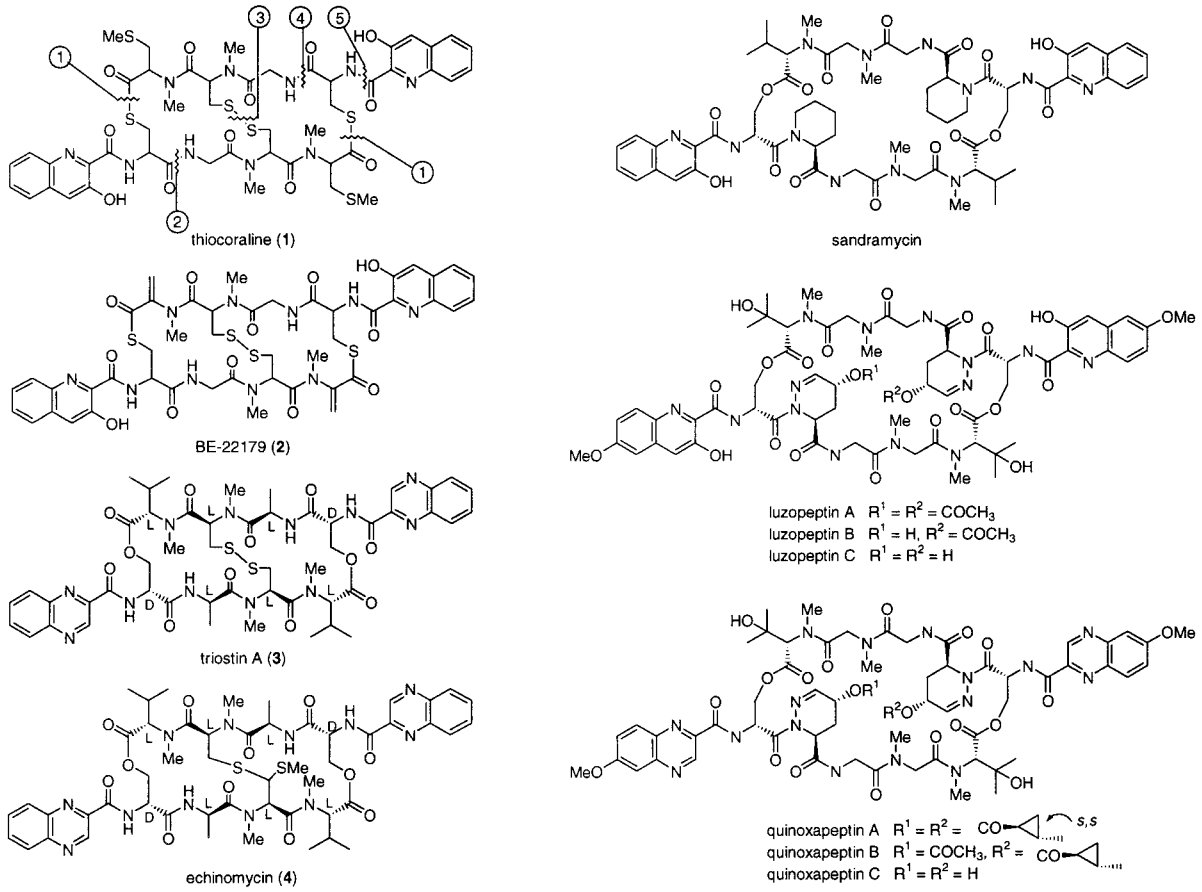


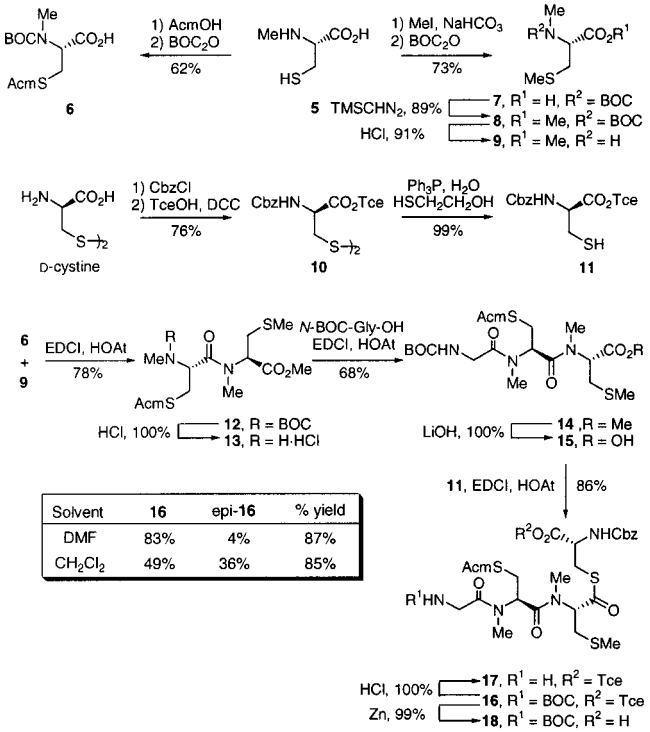
Figure 1.

established their relative and absolute stereochemistry,[13] the preparation of sufficient material with which further studies could be conducted, and the extension of these studies to the preparation of several key chromophore analogues.

Key elements of the approach include the late-stage introduction of the chromophore, symmetrical tetrapeptide coupling, macrocyclization of the 26-membered octadepsipeptide conducted at the single secondary amide site following disulfide formation, and a convergent assemblage of the tetradepsipeptide with introduction of the labile thiol ester linkage in the final coupling reaction under near racemization free conditions. By virtue of the late-stage introduction of the chromophore and despite the challenges this imposes on the synthesis because of a potential intramolecular *S*−*N* acyl transfer with cleavage of the macrocyclic thiol ester, this approach provided ready access to a range of chromophore analogues.

Tetradepsipeptide Synthesis. The convergent assemblage of key tetradepsipeptide **16** from tripeptide **15** and *N*-Cbz-D-Cys-OTce (**11**) along with the preparation of the three suitably functionalized Cys residues found in **1** are summarized in Scheme 1. Sequential *S*- and *N*-protection of *N*-Me-Cys-OH (**5**)[14] with an acetamidomethyl (Acm) group (1.5 equiv of *N*-hydroxymethylacetamide, H_2SO_4) and BOC group (BOC_2O, 62%) gave **6**, the precursor to the bridging disulfide Cys residue. Selective *S*-methylation of *N*-Me-Cys-OH (**5**,[14] MeI, $NaHCO_3$) followed by BOC protection (BOC_2O, NaOH, 73%) provided **7**. Esterification of **7** ($TMSCHN_2$, 89%) followed by BOC deprotection of **8** (3 M HCl−EtOAc, 91%) provided **9**, the precursor to the second functionalized L-Cys residue. Alternative attempts to esterify **7** under basic conditions (MeI, $NaHCO_3$, DMF) or the exposure of **8** or **9** to tertiary amines (Et_3N, CH_2Cl_2) led to occasional extensive β-elimination of MeSH to provide the dehydro amino acid. Compound **11**, constituting the chromophore-bearing D-Cys residue, was prepared by the reduction of its disulfide precursor **10** (Ph_3P, 2-mercaptoethanol,

Scheme 1



Solvent	16	epi-16	% yield
DMF	83%	4%	87%
CH_2Cl_2	49%	36%	85%

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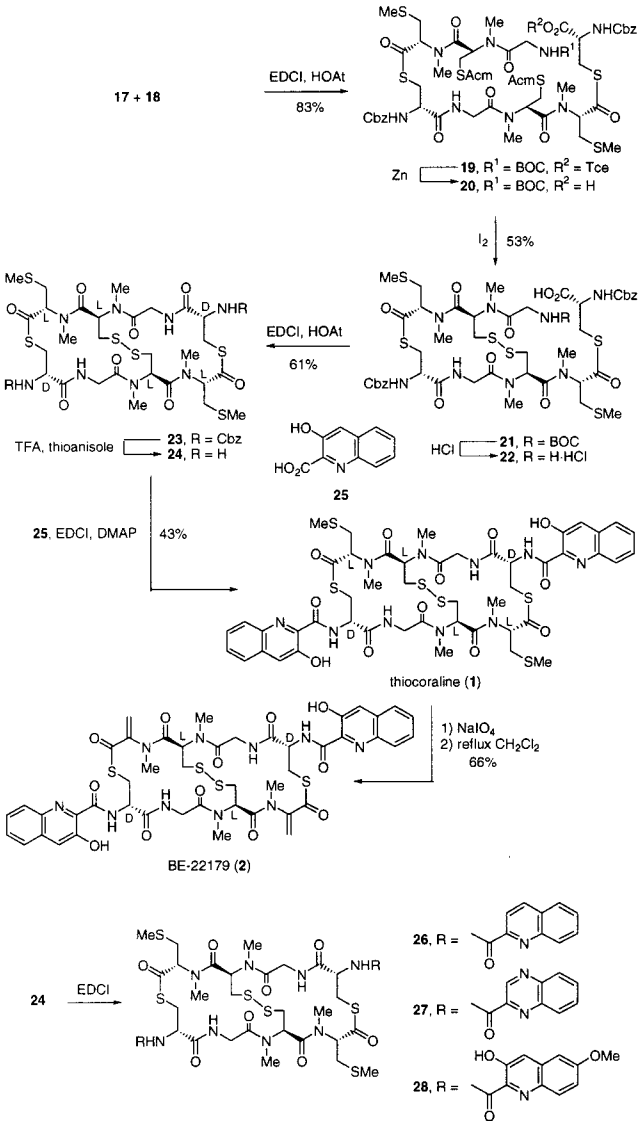
99%) which in turn was obtained by stepwise Cbz (CbzCl, $NaHCO_3$) and Tce (trichloroethanol, DCC,[15] HOBt,[15] 76%) protection of D-cystine. The esterification reaction with trichloroethanol proved sensitive to racemization and when conducted to the absence of HOBt (33% de vs 100% de) or in the presence of DMAP (33% de) led to extensive racemization. Coupling of **6** with **9** (EDCI,[15] HOAt,[15] 78%) provided **12** and slightly lower conversions were obtained with HOBt versus HOAt. BOC deprotection of **12** (3 M HCl−EtOAc, 100%), coupling with *N*-BOC-Gly-OH (EDCI, HOAt, 68%) and methyl ester hydrolysis of **14** (LiOH, 100%) provided **15**.

The key thiol esterification reaction linking the D-cysteine derivative **11** and the tripeptide **15** was accomplished under near racemization free conditions with use of EDCI−HOAt (83%) in the absence of added base to afford the depsipeptide **16** (de 95:5). Much lower conversions were observed using DPPA[16] or DEPC[16] and Et_3N due in part to competitive base-catalyzed formation of disulfide **10**. Analogous to prior reports,[16] near complete racemization was observed (**16**:*epi*-**16** = 58:42) when the nonpolar solvent CH_2Cl_2 was used. In addition, the use of base in all reactions following formation of the thiol ester **16** was found to lead to competitive β-elimination or direct cleavage of the thiol ester and was necessarily avoided.

Cyclic Octadepsipeptide Formation and Completion of the Total Syntheses of Thiocoraline and BE-22179. Linear octadepsipeptide formation was accomplished by deprotection of the amine (3 M HCl−EtOAc, 100%) and carboxylic acid (Zn, 90% aqueous AcOH, 99%) of **16** to provide **17** and **18**, respectively, which were coupled with formation of the secondary amide in the absence of added base (EDCI, HOAt, CH_2Cl_2, 83%) to obtain **19** (Scheme 2). Cyclization of **19** to provide the 26-membered cyclic octadepsipeptide **23** with ring closure conducted at the single secondary amide site was accomplished by sequential Tce ester deprotection (Zn, 90% aqueous AcOH), disulfide bond formation[17] (I_2, CH_2Cl_2−MeOH, 25 °C, 0.001 M, 53% for two steps), and BOC deprotection (3 M HCl−dioxane) followed by treatment with EDCI−HOAt (0.001 M CH_2Cl_2, −20 °C, 6 h, 61% for two steps). Reversing the *N*-BOC deprotection and disulfide bond formation steps in this four-step sequence resulted in lower conversions (13% overall for four steps). To date, all attempts to effect ring closure followed by disulfide bond formation have not been successful. Even though the 26-membered ring macrocyclization reaction unconstrained by the disulfide bond proceeds exceptionally well (>50%), the subsequent disulfide bond formation (I_2, CH_2Cl_2−MeOH, 25 °C) within the confines of the 26-membered ring failed to occur. Thus, the order of steps enlisted for formation of **23** was not to improve macrocyclization via the constrained disulfide, but rather to permit disulfide bond formation. While it is possible this may be due to constraints within the macrocycle destabilizing the disulfide, the lack of similar observations with **3** and **4** suggest the origin of the difficulties may lie with competitive intramolecular cleavage of the adjacent thiol ester by the liberated bridging thiol within the 26-membered macrocycle.

Scheme 2



Removal of the Cbz-protecting group under mild conditions[18] (TFA−thioanisole, 25 °C, 4 h) and coupling of the resulting amine **24** with 3-hydroxyquinoline-2-carboxylic acid (**25**,[19,20] EDCI, DMAP, 43%) without protection of the chromophore phenol provided (−)-**1**, $[\alpha]^{25}_D$ −180 (*c* 0.11, $CHCl_3$) [lit[1] $[\alpha]^{25}_D$ −191 (*c* 1.1, $CHCl_3$)], identical in all respects with the properties reported for natural material.[1] Under these conditions, a problematic intramolecular *S*−*N* acyl migration of the liberated amine with cleavage of the thiol ester was minimized. Treatment of **1** with $NaIO_4$ served to provide the corresponding bissulfoxide as a mixture of diastereomers which was warmed in CH_2Cl_2 (reflux, 6 h, 66% overall) to promote elimination and provide (−)-BE-22179 (**2**), $[\alpha]^{25}_D$ −89 (*c* 0.01, $CHCl_3$) [lit[2] $[\alpha]^{25}_D$ −94 (*c* 0.44, $CHCl_3$)], identical in all respects with the properties reported for the natural material.[2] The correlation of synthetic and natural **1** and **2** confirmed the two-dimensional structure assignments and established their relative and absolute stereochemistries as those shown in Scheme 2.

Interestingly, both **23** and thiocoraline (**1**) as well as the related natural product analogues **26**−**28** adopt a single solution conformation that is observed by ^1H NMR in well-defined

(15) DCC = dicyclohexylcarbodiimide; EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; HOBt = 1-hydroxybenzotriazole; HOAt = 1-hydroxy-7-azabenzotriazole.

(16) DPPA = diphenyl phosphorazidate; DEPC = diethyl phosphorocyanidate. (a) Yamada, S.; Yokoyama, Y.; Shioiri, T. *J. Org. Chem.* **1974**, *39*, 3302. (b) Yokoyama, Y.; Shioiri, T.; Yamada, S. *Chem. Pharm. Bull.* **1977**, *25*, 2423.

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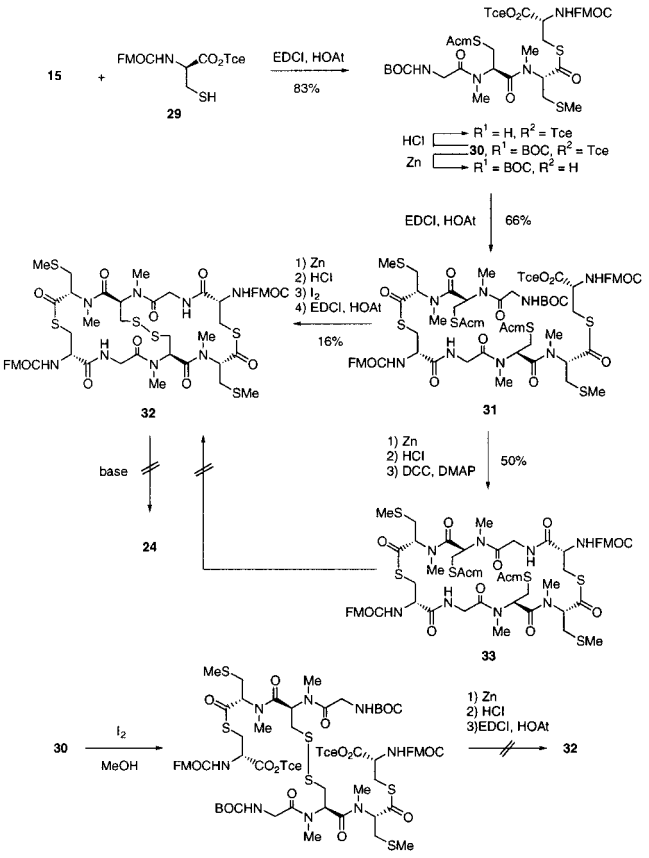
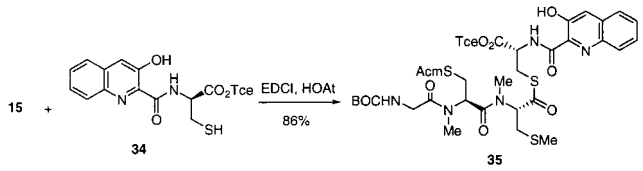
(19) Prepared from methyl 3-hydroxyquinoline-2-carboxylate[20] by treatment with LiOH, THF−MeOH−H_2O 3/1/1, 25 °C, 2 h (71%).

(20) Boger, D. L.; Chen, J.-H. *J. Org. Chem.* **1995**, *60*, 7369.

Scheme 3



Scheme 4



spectra. That of synthetic **1** proved identical to the published ^{1}H NMR spectrum of natural **1**.[1] In contrast, BE-22179 exhibits a more complex, but still well defined, ^{1}H NMR spectrum consistent with its adoption of two unsymmetrical or four symmetrical conformers in near equal proportions. The NMe signals (2 NMe) and the two olefin signals (C═CHH) appear as eight, near 1:1, well resolved singlets in the ^{1}H NMR spectrum. Importantly, the ^{1}H NMR spectrum of synthetic **2** proved identical to that published for natural **2**.[2]

Alternative Approaches. Prior to implementing the successful sequence, preliminary studies[21] were first conducted enlisting an FMOC-protecting group and basic deprotection conditions versus a Cbz-protecting group on **23** (Scheme 3). Thus, tetradepsipeptide **30** and octadepsipeptide **31** were prepared by the procedures described for the synthesis of **16** and **19**. Cyclization of **31** to provide the bridged 26-membered cyclic octadepsipeptide **32** was accomplished by sequential Tce ester deprotection (Zn, 90% aqueous AcOH), BOC deprotection (3 M HCl−dioxane), and disulfide bond formation (I_2, CH_2Cl_2−MeOH, 25 °C, 0.001 M) followed by treatment with EDCI−HOAt (0.001 M CH_2Cl_2, −20 °C, 6 h, 16% for four steps). However, exposure of **32** to Et_2NH or piperidine led to decomposition of the macrocycle rather than clean FMOC deprotection. Alternative treatment of **32** with other amines including dicyclohexylamine, Et_3N, or DMAP also failed to provide the cyclic amine **24** which we attributed to the sensitivity of the thiol ester to nucleophiles, the competitive β-elimination induced by the deprotonation of the α-position of the Cys residues, and a potential intramolecular *S*−*N* acyl transfer to the liberated amine with cleavage of the thiol ester. However, efforts to trap the liberated amine in situ to obtain **1** directly (**25**, EDCI, DMAP) or a protected derivative of **24** (BOC_2O or CbzCl, Et_3N) were also unsuccessful.

We also examined the approach in which the bridged 26-membered macrocycle is formed via simultaneous formation of both secondary amides. However, intermolecular disulfide bond formation (I_2, MeOH) and sequential deprotection of Tce and BOC group and the treatment of the resulting symmetrical disulfide with EDCI and HOAt gave complex mixtures of products including a range of oligomers and higher order macrocycles in which the formation of **32** was not observed (Scheme 3).

Finally, we also examined an approach in which the pendant chromophore was introduced at the initial stages of the synthesis. Thus, the coupling reaction of **15** and **34**[21] (EDCI, HOAt, 86%) gave tetradepsipeptide **35** which possesses the substituted quinoline chromophore (Scheme 4). However, elimination of thiol ester was problematic under the conditions of BOC deprotection (HCl or 90% aqueous TFA, 0 °C) or Tce ester hydrolysis (Zn, 90% aqueous HOAc, 0 °C) and the following coupling reaction which gave only a trace of the desired linear octadepsipeptide. Presumably, this may be attributed to the increased acidity of the α-proton of the activated *N*-acyl-D-Cys derivative bearing an amide- versus carbamate-protecting group.

Analogue Synthesis. The late-stage generation of amine **24** followed by introduction of the pendant chromophore provided the opportunity to examine chromophore analogues of **1** and **2**. Thus, the amine **24** was also coupled with quinoline-2-carboxylic acid, quinoxaline-2-carboxylic acid (which is the chromophore found in echinomycin and triostin A), and 3-hydroxy-6-methoxyquinoline-2-carboxylic acid[10,20] (which is the chromophore found in the luzopeptins) to afford the key chromophore analogues **26**−**28** (Scheme 2).

DNA Binding Affinity. Apparent absolute binding constants and apparent binding site sizes were obtained by measurement of the fluorescence quenching upon titration of **1** and **2** with calf thymus (CT) DNA. The excitation and emission spectra for thiocoraline and BE-22179 were determined in aqueous buffer (Tris-HCl, pH 7.4, 75 mM NaCl). Both thiocoraline and BE-22179, which have the same chromophore, exhibited an intense fluorescence in solution with enhanced excitation (380 nm) and emission (510 nm) maxima which was quenched upon DNA binding. Moreover, the intensity of this fluorescence greatly facilitated the measurement of fluorescence quenching and allowed measurements to be carried out at low initial agent concentrations of 1−10 μM where the compounds are soluble. Analogous measurements with echinomycin could not be conducted because of its less intense fluorescence emission and low solubility. For the titrations, small aliquots of CT-DNA (320 μM in base pair) were added to 2 mL of a solution of the agent (2 μM) in Tris−HCl (pH 7.4), 75 mM NaCl buffer. Additions were carried out at 15-min intervals to allow binding equilibration. Scatchard analysis[22] of the titration results was conducted using the equation $r_b/c = Kn - Kr_b$, where r_b is the number of molecules bound per DNA nucleotide phosphate, c is the free drug concentration, K is the apparent binding constant, and n

(21) Experimental details and characterization may be found in the Supporting Information.

(22) Scatchard, G. *Ann. N. Y. Acad. Sci*. **1949**, *51*, 660.

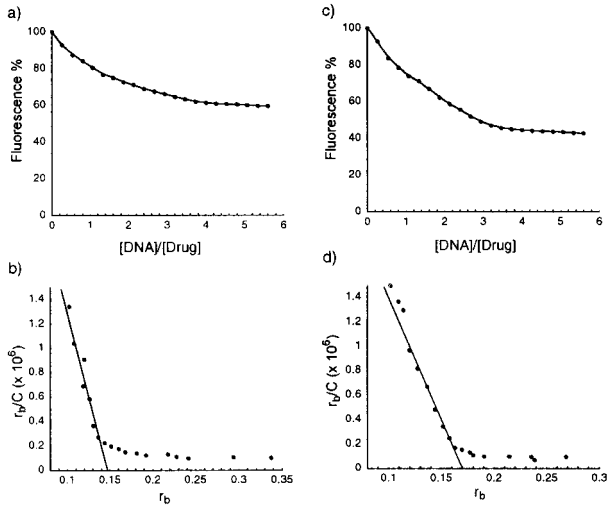


Figure 2. (a) Fluorescence quenching of thiocoraline (excitation at 380 nm and emission at 510 nm in Tris−HCl (pH 7.4) and 75 mM NaCl buffer solution) with increasing CT-DNA concentration. (b) Scatchard plot of fluorescence quenching of part a. (c) Fluorescence quenching of BE-22179 (excitation at 380 nm and emission at 510 nm in Tris−HCl (pH 7.4) and 75 mM NaCl buffer solution) with increasing CT-DNA concentration. (d) Scatchard plot of fluorescence quenching of part c.

Table 1. Comparative DNA Binding Properties

property	thiocoraline (**1**)	BE-22179 (**2**)	echinomycin (**4**)
K_B,[a] M^{-1}	2.6×10^6 (1:6.5)	2.2×10^6 (1:5.8)	(2.2×10^6)[d]
(−)-unwinding [c][b]	0.11	1.1	0.044
(+)-winding [c][c]	0.44	>2.2	0.22

[a] Calf thymus DNA, K_B = apparent binding constant determined by fluorescence quenching. The value in parentheses is the agent/base pair ratio at saturated high-affinity binding and may be considered a measure of the selectivity of binding. [b] Agent/base pair ratio required to unwind negatively supercoiled ΦX174 DNA (form I to form II gel mobility, 0.9% agarose gel). [c] Agent/base ratio required to induce complete rewinding or positive supercoiling of ΦX174 DNA (form II to form I gel mobility, 0.9% agarose gel). [d] Binding constant established by footprinting at a 5′-CCGC site, Figure S1 in Supporting Information.

is the number of the agent binding sites per nucleotide phosphate. A plot of r_b/c versus r_b gives the association constant (slope) and the apparent binding site size (*x*-intercept) for the agents (Figure 2 and Table 1).

Thiocoraline was found to exhibit a relatively high affinity for duplex DNA ($K_B = 2.6 \times 10^6$ M^{-1}) with a saturating stoichiometry of high affinity binding at a 1:6.5 agent to base pair ratio. BE-22179, which is structurally distinct possessing two exocyclic olefins, also displayed a similar affinity and binding site size with CT-DNA. The high affinity binding of one molecule per 5.8−6.5 base pairs approaches that of the saturated limit of four base pairs assuming bisintercalation spanning two base pairs, suggesting thiocoraline and BE-22179 bind to DNA with limited selectivity among available sites. This proved consistent with attempts to establish a selectivity of DNA binding by DNase I[23] and MTE footprinting[24] on w794 DNA,[25] using protocols we successfully applied to sandramycin[8] and echinomycin, which failed to reveal a distinguishable selectivity for **1** (Supporting Information Figures 1 and 2). Our previous studies of sandramycin, the luzopeptins, and quinoxapeptins, which are larger symmetrical cyclic decadepsipeptides, revealed that they exhibit a higher affinity for CT-DNA ($K_B = 1.0-3.4 \times 10^7$ M^{-1}). Since thiocoraline and BE-22179 possess the same chromophore as sandramycin ($K_B = 3.4 \times 10^7$ M^{-1}), this indicates that the differing ability to bind duplex DNA arises from the cyclic depsipeptide, its ring size, and differing peptide backbone and not the structure of the chromophore.

Similarly, echinomycin and triostin A bind to DNA by bisintercalation and are the most extensively studied natural products in these series. In contrast to sandramycin and the luzopeptins which bind 5′-PyPuPyPu sites and exhibit the highest affinity for 5′-CATG spanning a two-base pair 5′-AT site,[9,10] the quinoxalines bisintercalate preferentially at 5′-CG sites (i.e., 5′-GCGT or 5′-PuPyPuPy) also spanning two base pairs with each intercalation occurring at a PuPy versus PyPu step. The structural distinctions between **1** and **2** versus triostin A (**3**) are subtle. Beyond the different chromophores, they include the conservative side chain CH_2SCH_3 versus NMe-Val $CH(Me)_2$ alteration and the more significant Gly versus L-Ala (H vs Me) substitution, and the thioester versus ester (S vs O) backbone alteration. Nonetheless, these changes abolished the DNA binding selectivity and, as shown below, may reduce the stability of the bisintercalation complexes.

Bifunctional Intercalation. Confirmation that thiocoraline and BE-22179 bind to DNA with bisintercalation was derived from their ability to induce the unwinding of negatively supercoiled DNA. This was established by their ability to gradually decrease the agarose gel electrophoresis mobility of supercoiled ΦX174 DNA (unwinding) at increasing concentrations followed by a return to normal mobility (rewinding) at even higher concentrations. Under the conditions we employed, echinomycin unwound ΦX174 DNA at a 0.044 agent/base pair ratio (Figure 3 and Table 1). Thiocoraline completely unwound ΦX174 DNA at a higher 0.11 agent/base pair ratio, whereas BE-22179 required even higher concentrations, producing the unwinding at an agent/base pair ratio of 1.1. Complete rewinding of the supercoiled DNA occurred at an agent/base pair ratio of 0.44 for thicoraline versus 0.22 for echinomycin, whereas BE-22179 failed to produce the rewinding of ΦX174 DNA at the concentrations examined. The thiocoraline analogue **27**, which bears the quinoxaline chromophore of echinomycin, was found to behave in a manner indistinguishable from thiocoraline itself. Thus, the distinctions in **1** and **2** and echinomycin detected here appear to be related to the nature of the cyclic depsipeptide and not the structure of the chromophore. Under these conditions, ethidium bromide, a monointercalater, does not unwind supercoiled DNA although it can unwind supercoiled DNA under conditions which prevent dissociation of the bound agent during electrophoresis. Thus, the unwinding of negatively supercoiled DNA and the subsequent positive supercoiling of the DNA by thiocoraline and **27**, indicative of bisintercalation, were similar although slightly less effective than echinomycin, whereas that of BE-22179 was substantially less effective. This suggests that BE-22179 binds with a smaller unwinding angle, with lower stability, or with faster off-rates than echinomycin and thiocoraline.

We also briefly examined the ability of **1** or **2** to cleave, alkylate, or cross-link DNA. In particular, the electrophilic unsaturation found in BE-22179 might be expected to serve as an alkylation site for covalent attachment to DNA, especially following bisintercalation binding. No evidence was found to suggest that either **1** or **2** cleave DNA in simple assays monitoring the conversion of supercoiled ΦX174 DNA (Form

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(25) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661.

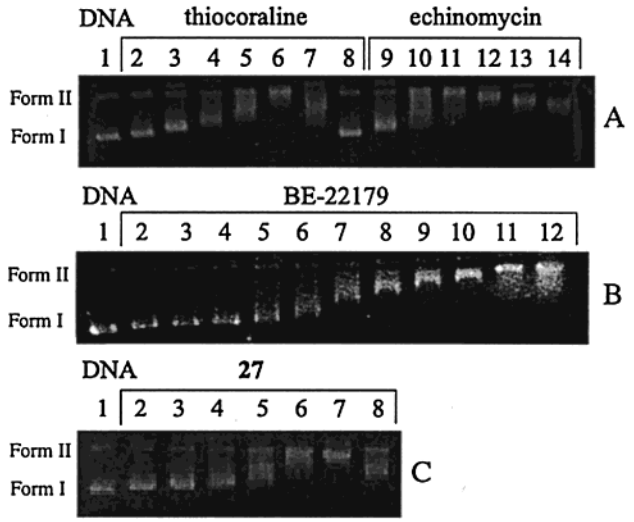

Figure 3. Agarose gel electrophoresis. (A) Lane 1, untreated supercoiled ΦX174 DNA, 95% form I and 5% form II; lanes 2−8, thiocoraline-treated ΦX174 DNA; lanes 9−14, echinomycin-treated ΦX174 DNA. The [agent]-to-[base pair] ratios were 0.022 (lanes 2 and 9), 0.033 (lanes 3 and 10), 0.044 (lanes 4 and 11), 0.066 (lanes 5 and 12), 0.11 (lanes 6 and 13), 0.22 (lanes 7 and 14), and 0.44 (lane 8). (B) Lane 1, untreated supercoiled ΦX174 DNA, lanes 2−12, BE-22179-treated ΦX174 DNA. The [agent]-to-[base pair] ratios were 0.022 (lane 2), 0.033 (lane 3), 0.044 (lane 4), 0.066 (lane 5), 0.11 (lane 6), 0.22 (lane 7), and 0.44 (lane 8), 0.44 (lane 9), 0.66 (lane 10), 1.1 (lane 11), and 2.2 (lane 12). (C) Lane 1, untreated supercoiled ΦX174 DNA, 95% form I and 5% form II; lanes 2−8, thiocoraline analogue (**27**)-treated ΦX174 DNA. The [agent]-to-[base pair] ratios were 0.022 (lane 2), 0.033 (lane 3), 0.044 (lane 4), 0.066 (lane 5), 0.11 (lane 6), 0.22 (lane 7), and 0.44 (lane 8).

Table 2. Comparative Deoxyoligonucleotide Binding Properties

	thiocoraline (**1**) K_B (10^6 M^{-1})	sandramycin K_B (10^6 M^{-1})
5′-GCGCGC	7.0	145
5′-GCATGC	4.3	230
5′-GCCGGC	5.9	85
5′-GCTAGC	3.0	85

I) to relaxed (Form II) or linear (Form III) DNA under a range of conditions. Similarly, sequencing cleavage studies conducted with w794 DNA enlisting the thermal depurination and cleavage detection of adenine N3 or N7 or guanine N3 or N7 alkylation sites did not reveal alkylation by **2**. However, these studies do not exclude alkylation at nonthermally labile sites including the guanine C2 amine. Additional assays conducted with w794 DNA following established protocols[25] provided no evidence of DNA interstrand cross-linking. These studies would detect both thermally labile and nonthermally labile alkylation sites, but only those engaged in interstrand cross-linking. Given the C_2 symmetric nature of **2**, bisintercalation analogous to echinomycin and triostin A places the two electrophilic sites in positions to react only with the complementary strands of duplex DNA (interstrand DNA cross-linking) and would preclude intrastrand DNA cross-linking. Thus, these studies safely excluded DNA cross-linking by **2** even with reaction of nonthermally labile sites (e.g., G C2 amine), but do not rule out monoalkylation events at nonthermally labile sites.

DNA Binding Selectivity. The preceding studies suggested that thiocoraline binds to DNA with high affinity, but with little or no selectivity. Consequently, we examined the binding of **1** with a set of four duplex deoxyoligonucleotides, 5′-GCGC-3′ where = TA, AT, GC, CG, incorporating the high affinity intercalation sites of the related bisintercalators echinomycin (5′-PuCGPy),[4] sandramycin (5′-CATG),[9] and the luzopeptins (5′-CATG).[10] The binding constants were established by titration using the fluorescence quenching that is observed upon DNA binding. The excitation and emission spectra for thiocoraline and BE-22179 were recorded in aqueous buffer (Tris−HCl, pH 7.4, 75 mM NaCl). To minimize fluorescence decrease due to dissolution or photobleaching, the solutions were stirred in 4-mL cuvettes in the dark with the minimum exposure to the excitation beam necessary to obtain a reading. The titrations were carried out with a 15-min equilibration time after each deoxyoligonucleotide addition. Scatchard plots of thiocoraline binding to the deoxyoligonucleotides exhibited a downward convex curvature which we interpreted to indicate a high-affinity bisintercalation and a lower affinity binding potentially involving monointercalation. Using the model described by Feldman[26] which assumes one ligand with two binding sites, we were able to deconvolute the curves according to the equation

$$\frac{r_b}{c} = \frac{1}{2}(K_1(n_1 - r_b) + K_2(n_2 - r_b)) + \sqrt{(K_1(n_1 - r_b) - K_2(n_2 - r_b))^2 + 4K_1K_2n_1n_2} \quad (1)$$

where K_1 and K_2 represent the association constants for high- and low-affinity binding, and n_1 and n_2 represent the number of bound agents per duplex for the separate binding events. Scatchard plots of the data revealed 1:1 binding in each case. That of the high affinity binding is consistent with binding of a single molecule with bisintercalation surrounding a central two base pair site. A small preference was observed for GC-rich binding with 5′-GCGCGC and 5′-GCCGGC exhibiting the tightest binding, but the differences are small ranging from 3 to 7 × 10^6 M^{-1} for the four deoxyoligonucleotides (Table 2). Thus, consistent with the results of footprinting and other related studies herein, the binding of **1** with the deoxyoligonucleotides exhibited little selectivity.

Table 3. Biological Activity

	compound	L1210[a] (nM)	HIV-RT[b] (μM)
1	thiocoraline	0.2	900
2	BE-22179	0.4	
23		>10^5	
26		20	
27		40	
28		0.4	
32		>10^5	
	echinomycin	0.08	700
	sandramycin	0.001	2
	luzopeptin A	0.008	6
	luzopeptin B	30	3
	luzopeptin C	>100	0.4
	quinoxapeptin A	0.3	0.6
	quinoxapeptin B	2	0.9
	quinoxapeptin C	>100	0.3

[a] L1210 mouse leukemia cytotoxic assay. [b] HIV-1 reverse transcriptase inhibition.

Biological Properties. Table 3 summarizes the biological properties of echinomycin, thiocoraline, and BE-22179 along with those of precursor **23** and their analogues. Thiocoraline and BE-22179 exhibit exceptionally potent cytotoxic activity in the L1210 assays (IC_{50} = 200 and 400 pM, respectively) being slightly less potent than echinomycin. Compounds **23** and **32** lacking both chromophores and containing the Cbz- and FMOC-protecting groups were inactive and >10^5 times less

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potent than thiocoraline. Analogue **28**, which bears the same chromophore as the luzopeptins, also exhibited potent activity while **26**, lacking the quinoline C3 phenol, and **27**, bearing the quinoxaline chromophore of echinomycin and triostin A, exhibited less potent cytotoxic activity. In addition, thiocoraline, like echinomycin, was found to be only a weak inhibitor of HIV-1 reverse transcriptase.

Most notable of these observations is that both thiocoraline and BE-22179 are exceptionally potent cytotoxic agents joining the small group of compounds that exhibit IC_{50}'s at subnanomolar or low picomolar concentrations (200−400 pM).

Experimental Section

***N*-BOC-NMe-L-Cys(Acm)-OH (6).** A solution of NMe-L-Cys-OH hydrochloride salt (**5**, 1.35 g, 10.0 mmol) and acetamidomethanol (13.4 g, 15 mmol) in water (5 mL) was treated with concentrated HCl (0.64 mL), and the reaction mixture was stirred at 25 °C for 12 h. The reaction mixture was concentrated in vacuo. The residue was dissolved in 100 mL of THF−H_2O (1:1), and the resulting solution was brought to pH 8 by adding 1 N aqueous NaOH. Di-*tert*-butyl dicarbonate (2.62 g, 12.0 mmol) was added, and the reaction mixture was stirred at 25 °C for 12 h, maintaining a pH 8. The reaction mixture was poured onto 1 N aqueous HCl (150 mL) and extracted with $CHCl_3$ (3 × 100 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 15 cm, 4% MeOH−$CHCl_3$ eluent) afforded **6**[21] (1.89 g, 6.21 mmol, 62%) as a white foam.

***N*-BOC-NMe-L-Cys(Me)-OH (7).** A solution of NMe-L-Cys-OH hydrochloride salt (**5**, 1.35 g, 10.0 mmol) in 100 mL of THF−H_2O (1:1) was sequentially treated with $NaHCO_3$ (1.68 g, 20.0 mmol) and MeI (0.65 mL, 10.5 mmol), and the reaction mixture was stirred at 25 °C for 3 h. The reaction mixture was brought to pH 8 by adding 1 N aqueous NaOH. Di-*tert*-butyl dicarbonate (2.62 g, 12.0 mmol) was added, and the reaction mixture was stirred at 25 °C for 12 h, maintaining a pH 8. The reaction mixture was poured onto 1 N aqueous HCl (150 mL) and extracted with $CHCl_3$ (3 × 100 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 15 cm, 2% MeOH−$CHCl_3$ eluent) afforded **7**[21] (1.89 g, 7.63 mmol, 76%) as a colorless oil.

***N*-BOC-NMe-L-Cys(Me)-OMe (8).** Trimethylsilyl diazomethane (2.0 M hexane solution, 3.70 mL, 0.74 mmol) was added dropwise to a solution of **7** (1.86 g, 7.40 mmol) in 100 mL of benzene−MeOH (5:1) at 0 °C. Following the addition, the reaction mixture was concentrated in vacuo. Flash chromatography (SiO_2, 3 × 15 cm, 20% EtOAc−hexane eluent) afforded **8**[21] (1.77 g, 6.73 mmol, 91%) as a colorless oil.

NMe-L-Cys(Me)-OMe (9). Compound **8** (1.32 g, 5.0 mmol) was treated with 5 mL of 3 M HCl−EtOAc and the mixture was stirred at 25 °C for 30 min before the volatiles were removed in vacuo. The residual HCl was removed by adding Et_2O (10 mL) to the hydrochloride salt followed by its removal in vacuo. The residue was dissolved in $CHCl_3$ (200 mL), and the organic layer was washed with saturated aqueous $NaHCO_3$ (100 mL) and saturated aqueous NaCl (100 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated in vacuo to give **9**[21] (746 mg, 91%) as a colorless oil which was used directly in the next reaction without further purification.

(*N*-Cbz-D-Cys-OTce)$_2$ (10). A solution of D-cystine (500 mg, 2.1 mmol) and NaOH (352 mg, 8.4 mmol) in 20 mL of THF−H_2O (1:1) was treated with CbzCl (0.63 mL, 4.4 mmol), and the reaction mixture was stirred at 25 °C for 1 h. The reaction mixture was diluted with water (50 mL) and washed with $CHCl_3$ (3 × 50 mL). The aqueous phase was acidified with 6 N aqueous HCl (50 mL) and extracted with $CHCl_3$ (3 × 50 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in pyridine (20 mL), and HOBt (840 mg, 6.3 mmol) and trichloroethanol (0.69 mL, 5.3 mmol) were added. The mixture was cooled to −20 °C and treated with DCC (1.29 g, 6.3 mmol), and the resulting mixture was stirred at −20 °C under Ar for 24 h. The white precipitate of DCU was removed by filtration, and the filtrate was concentrated in vacuo. The residue was diluted with EtOAc (100 mL), and the organic phase was washed with 1 N aqueous HCl (100 mL), saturated aqueous $NaHCO_3$ (100 mL), and saturated aqueous NaCl (50 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 15 cm, 20% EtOAc−hexane eluent) afforded **10**[21] (1.23 g, 1.6 mmol, 76%) as a colorless oil.

***N*-Cbz-D-Cys-OTce (11).** A solution of **10** (771 mg, 1.0 mmol) in 10 mL of THF was treated with Ph_3P (262 mg, 1.0 mmol), 2-mercaptoethanol (70 μL, 1.0 mmol), and water (180 μL, 10 mmol), and the reaction mixture was stirred at 50 °C for 5 h before being concentrated in vacuo. Flash chromatography (SiO_2, 3 × 18 cm, 20% EtOAc−hexane eluent) afforded **11**[21] (764 mg, 1.98 mmol, 99%) as a colorless oil.

***N*-BOC-NMe-L-Cys(Acm)-NMe-L-Cys(Me)-OMe (12).** A solution of **6** (l.75 g, 5.74 mmol) in CH_2Cl_2 (57 mL) was treated sequentially with HOAt (781 mg, 5.74 mmol) and EDCI (1.10 g, 5.74 mmol), and the mixture was stirred at 0 °C for 15 min. A solution of **9** (935 mg, 5.74 mmol) was added, and the reaction mixture was stirred for an additional 12 h. The reaction mixture was poured onto 1 N aqueous HCl (100 mL) and extracted with EtOAc (2 × 100 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ (100 mL) and saturated aqueous NaCl (50 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 15 cm, EtOAc eluent) afforded **12**[21] (2.01 g, 4.46 mmol, 78%) as a white foam.

***N*-BOC-Gly-NMe-L-Cys(Acm)-NMe-L-Cys(Me)-OMe (14).** A sample of **12** (2.01 g, 4.46 rnmol) was treated with 4.5 mL of 3 M HCl−EtOAc and the mixture was stirred at 25 °C for 30 min before the volatiles were removed in vacuo. The residual HCl was removed by adding Et_2O (10 mL) to the hydrochloride salt **13** followed by its removal in vacuo. After this procedure was repeated three times, 1.96 g of **13** (100%) was obtained and used directly in the following reaction without further purification.

A solution of *N*-BOC-Gly-OH (773 mg, 4.46 mmol) and hydrochloride salt **13** (1.96 g, 4.46 mmol) in CH_2Cl_2 (45 mL) was treated sequentially with HOAt (909 mg, 6.69 mmol), EDCI (1.26 g, 6.69 mmol), and $NaHCO_3$ (549 mg, 6.69 mmol), and the reaction mixture was stirred at 0 °C for 12 h. The reaction mixture was poured onto 1 N aqueous HCl (100 mL) and extracted with EtOAc (2 × 100 mL). The combined organic phase was washed with saturated aqueous $NaHCO_3$ (100 mL) and saturated aqueous NaCl (50 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 5 × 14 cm, 20% acetone−EtOAc eluent) afforded **14**[21] (1.54 g, 3.03 mmol, 68%) as a white foam.

***N*-BOC-Gly-NMe-L-Cys(Acm)-NMe-L-Cys(Me)-OH (15).** Lithium hydroxide monohydrate (92 mg, 2.31 mmol) was added to a solution of **14** (394 mg, 0.77 mmol) in 10 mL of THF−MeOH−H_2O (3:1:1) at 0 °C, and the resulting reaction mixture was stirred at 25 °C for 1.5 h. The reaction mixture was poured onto 1 N aqueous HCl (100 mL) and extracted with $CHCl_3$ (3 × 50 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo to give **15**[21] (393 mg, 100%) as a white foam which was used without further purification.

***N*-Cbz-D-Cys[*N*-BOC-Gly-NMe-L-Cys(Acm)-NMe-L-Cys(Me)]-OTce (16).** A solution of **15** (393 mg, 0.77 mmol) in DMF (8 mL) was treated sequentially with HOAt (150 mg, 0.92 mmol) and EDCI (183 mg, 0.92 mnol), and the mixture was stirred at −20 °C for 15 min. A solution of **11** (300 mg, 0.77 mmol) was added, and the reaction mixture was stirred for an additional 4 h. The reaction mixture was poured onto 1 N aqueous HCl (100 mL) and extracted with EtOAc (100 mL). The combined organic phase was washed with saturated aqueous $NaHCO_3$ (100 mL) and saturated aqueous NaCl (50 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 15 cm, 33% EtOAc−hexane eluent) afforded **16**[21] (551 mg, 0.64 mmol, 83%) as a white foam and *epi*-**16** (28 mg, 0.032 mnol, 4%) as a white foam.

***N*-Cbz-D-Cys[*N*-Cbz-D-Cys(*N*-BOC-Gly-NMe-L-Cys(Acm)-NMe-L-Cys(Me))-Gly-NMe-L-Cys(Acm)-NMe-L-Cys(Me)]-OTce (19).** Compound **16** (432 mg, 0.5 mmol) was treated with 5.0 mL of 3 M HCl−EtOAc, and the mixture was stirred at 25 °C for 30 min before the volatiles were removed in vacuo. The residual HCl was removed by adding Et_2O (10 mL) to the hydrochloride salt **17** followed by its removal in vacuo. After repeating this procedure three times, 429 mg

of **17** (100%) was obtained and used directly in the following reaction without further purification.

A solution of **16** (432 mg, 0.5 mmol) in 90% aqueous AcOH (15 mL) was treated with Zn (1.62 g, 25 mmol), and the resulting suspension was stirred at 0 °C for 2 h. The zinc was removed by filtration, and the filtrate was concentrated in vacuo. The residue was poured onto 1 N aqueous HCl (50 mL) and extracted with $CHCl_3$ (3 × 50 mL). The combined organic phase was dried (Na_2SO_4), filtered, and concentrated in vacuo to give **18** (430 mg, 100%) as a white foam which was employed directly in the next reaction without further purification.

A solution of **17** (429 mg, 0.5 mmol) and **18** (430 mg, 0.5 mmol) in CH_2Cl_2 (5.0 mL) was treated sequentially with HOAt (98 mg, 0.6 mmol) and EDCI (119 mg, 0.6 mmol), and the reaction mixture was stirred at 0 °C for 6 h. The reaction mixture was poured onto 1 N aqueous HCl (50 mL) and extracted with EtOAc (2 × 50 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ (50 mL) and saturated aqueous NaCl (30 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 4 × 15 cm, 20% acetone−EtOAc eluent) afforded **19**[21] (613 mg, 0.42 mmol, 83%) as a white foam.

***N*-Cbz-D-Cys[*N*-Cbz-D-Cys(*N*-BOC-Gly-NMe-L-Cys-NMe-L-Cys-(Me)]-Gly-NMe-L-Cys-NMe-L-Cys(Me)]-OH (21).** A solution of **19** (500 mg, 0.34 mmol) in 90% aqueous AcOH (15 mL) was treated with Zn (1.08 g, 17.0 mmol), and the resulting suspension was stirred at 0 °C for 2 h. The zinc was removed by filtration, and the filtrate was concentrated in vacuo. The residue was poured onto 1 N aqueous HCl (100 mL) and extracted with $CHCl_3$ (3 × 50 mL). The combined organic phase was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue in CH_2Cl_2 (100 mL) was added dropwise to a solution of iodine (868 mg, 3.4 mmol) in 340 mL of CH_2Cl_2−MeOH (10:1), and the reaction mixture was stirred at 25 °C for 2 h. The reaction mixture was cooled in an ice bath, and 5% aqueous $Na_2S_2O_3$ was added until the color of iodine disappeared. The mixture was washed with 1 N aqueous HCl (50 mL) and saturated aqueous NaCl (30 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 16 cm, 10% MeOH−$CHCl_3$ eluent) afforded **21**[21] (201 mg, 0.17 mmol, 49%, typically 49−53%) as a pale yellow foam.

[*N*-Cbz-D-Cys-Gly-NMe-L-Cys-NMe-L-Cys(Me)]$_2$ (Cysteine Thiol) Dilactone (23). A sample of **21** (180 mg, 0.15 mmol) was treated with 1.5 mL of 3 M HCl−dioxane, and the mixture was stirred at 25 °C for 30 min before the volatiles were removed in vacuo. The residual HCl was removed by adding Et_2O (5 mL) to the hydrochloride salt followed by its removal in vacuo. The residue in CH_2Cl_2 (150 mL) was treated sequentially with HOAt (122 mg, 0.75 mmol) and EDCI (149 mg, 0.75 mmol), and the reaction mixture was stirred at 0 °C for 6 h. The reaction mixture was poured onto 1 N aqueous HCl (50 mL) and extracted with EtOAc (2 × 50 mL). The combined organic phase was washed with saturated aqueous $NaHCO_3$ (50 mL) and saturated aqueous NaCl (30 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 4 × 15 cm, 25% EtOAc−hexane eluent) afforded **23**[21] (84 mg, 77 μmol, 52%, typically 52−61%) as a white solid.

Thiocoraline (1). A sample of **23** (14.0 mg, 12.9 μmol) was treated with 2 mL of TFA−thioanisole (10:1) and the reaction mixture was stirred at 25 °C for 6 h before being concentrated in vacuo. The residue was treated with 3 M HCl−EtOAc, and the volatiles were removed in vacuo to give the hydrochloride salt.

A solution of **25** (11.9 mg, 64.5 μmol) and DMAP (7.7 mg, 64.5 μmol) in CH_2Cl_2 (1 mL) was treated with EDCl (12.6 mg, 64.5 μmol), and the reaction mixture was stirred at 25 °C for 30 min. The hydrochloride salt **24** was added, and the reaction mixture was stirred at 25 °C for 3 d. The reaction mixture was poured onto 1 N aqueous HCl (5 mL) and extracted with EtOAc (2 × 5 mL). The combined organic phases were washed with saturated aqueous NaCl (3 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. PTLC (SiO_2, $CHCl_3$:EtOAc:HOAc = 10:20:0.3 eluent) afforded **1** (6.5 mg, 5.5 μmol, 43%) as a white solid which exibited a ^{1}H NMR spectrum identical to the chart published for authentic **1**.[1,21]

BE-22179 (2). A sample of **1** (1.0 mg, 0.85 μmol) in 30% aqueous acetone (400 μL) was treated with $NaIO_4$ (0.4 mg, 8.5 μmol), and the reaction mixture was stirred at 25 °C for 12 h before being quenched by adding aqueous $Na_2S_2O_3$. The mixture was concentrated in vacuo, and the residue was extracted with EtOAc (2 × 2 mL). The combined organic phases were washed with saturated aqueous NaCl (3 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo to give the crude sulfoxides. A solution of the crude sulfoxides in CH_2Cl_2 (400 μL) was warmed at reflux for 6 h, and the volatiles were removed in vacuo. PTLC (SiO_2, $CHCl_3$:EtOAc:HOAc = 10:20:0.3 eluent) afforded **2** (0.6 mg, 0.56 μmol, 66%) as a pale yellow solid which exibited a ^{1}H NMR spectrum identical to the chart published for authentic **2**.[2,21]

[*N*-(2-Quinoline carboxyl)-D-Cys-Gly-NMe-L-Cys-NMe-L-Cys-(Me)]$_2$ (Cysteine Thiol) Dilactone (26). In the same manner as that described for **1**, the reaction of **23** (5.0 mg, 4.6 μmol) with quinoline-2-carboxylic acid (4.0 mg, 23.0 μmol), EDCI (4.5 mg, 23.0 μmol), and DMAP (2.8 mg, 23.0 μmol) in CH_2Cl_2 (300 μL) and purification by PTLC (SiO_2, $CHCl_3$:EtOAc:HOAc = 10:20:0.3 eluent) afforded **26**[21] (2.8 mg, 2.4 μmol, 52%) as a white foam.

[*N*-(2-Quinoxaline carboxyl)-D-Cys-Gly-NMe-L-Cys-NMe-L-Cys-(Me)]$_2$ (Cysteine Thiol) Dilactone (27). In the same manner as that described for **1**, the reaction of **23** (5.0 mg, 4.6 μmol) with quinoxaline-2-carboxylic acid (4.0 mg, 23.0 μmol), EDCI (4.5 mg, 23.0 μmol), and DMAP (2.8 mg, 23.0 μmol) in CH_2Cl_2 (300 μL) and purification by PTLC (SiO_2, $CHCl_3$:EtOAc:HOAc = 10:20:0.3 eluent) afforded **27**[21] (2.0 mg, 2.2 μmol, 47%) as a white foam.

[*N*-(3-Hydroxy-6-methoxy-2-quinoline carboxyl)-D-Cys-Gly-NMe-L-Cys-NMe-L-Cys(Me)]$_2$ (Cysteine Thiol) Dilactone (28). In the manner similar to that described for **1**, the reaction of **23** (5.0 mg, 4.6 μmol) with 3-hydroxy-6-methoxy-quinoline-2-carboxylic acid[10,20] (4.0 mg, 23.0 μmol), EDCI (4.5 mg, 23.0 μmol), and DMAP (2.8 mg, 23.0 μmol) in CH_2Cl_2 (300 μL) and purification by PTLC (SiO_2, $CHCl_3$:EtOAc:HOAc = 10:20:0.3 eluent) afforded **28**[21] (2.5 mg, 2.4 μmol, 51%) as a white foam.

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Supporting Information Available: Characterization data for **6**−**12**, **14**−**16**, **19**, **21**, **23**, **1**, **2**, **26**−**28**, experimental details and characterization data on **29**−**35**, and experimental details for the DNA binding studies are provided including two figures illustrating the DNase footprinting of **1** and echinomycin (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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