Total Synthesis of Bleomycin A_2 and Related Agents. 1. Synthesis and DNA Binding Properties of the Extended C-Terminus: Tripeptide S, Tetrapeptide S, Pentapeptide S, and Related Agents

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Abstract: Full details of concise, diastereocontrolled syntheses of **2–5** and their incorporation into tri-, tetra-, and pentapeptide S, the C-terminus of bleomycin A_2, are described. The extension of the studies to the synthesis of a complete set of tri- and tetrapeptide S structural analogs **29a,b** and **43b–j** is detailed, and their DNA binding constants (apparent K_B, calf thymus DNA) and apparent binding site sizes were determined. Consistent with past observations, the studies highlight the fact that the majority of the DNA binding affinity for bleomycin A_2 (1.0×10^5 M^{-1}) and deglycobleomycin A_2 (1.1×10^5 M^{-1}) is embodied within *N*-BOC-tripeptide S (0.26×10^5 M^{-1}). The additional comparisons of **29a** (0.18×10^5 M^{-1}), *N*-BOC-tetrapeptide S (0.21×10^5 M^{-1}), **43h** (0.20×10^5 M^{-1}), and *N*-BOC-pentapeptide S (0.23×10^5 M^{-1}) versus *N*-BOC-dipeptide S (0.10×10^5 M^{-1}) indicate productive stabilizing binding interactions for the tripeptide S L-threonine subunit and substituent, illustrate that the entire pentanoic acid subunit of tetrapeptide S and its substituents do not significantly contribute to DNA binding affinity, and indicate that the entire β-hydroxy-L-histidine subunit of pentapeptide S does not contribute to DNA binding affinity. With the exception of the L-threonine side chain substituent, the observations suggest that the tri- and tetrapeptide S substituent effects on the bleomycin A_2 DNA cleavage reaction are not due to substantial stabilizing binding interactions with duplex DNA. In addition, the measured apparent binding site sizes for bleomycin A_2 (3.8 base pairs), deglycobleomycin A_2 (3.9 base pairs), *N*-BOC-tripeptide S (3,6 base pairs), *N*-BOC-tetrapeptide S (3.7 base pairs), **43h** (3.5 base pairs), and *N*-BOC-pentapeptide S (4.2 base pairs) versus *N*-BOC-dipeptide S (2.2 base pairs) and **29a** (2.7 base pairs) suggest that it is the tripeptide S subunit of bleomycin A_2 that is fully bound to duplex DNA, that the tripeptide S L-threonine hydroxyethyl substituent detectably affects the agent interaction with duplex DNA, but that the presence or absence of the other tetrapeptide S and pentapeptide S backbone substituents do not substantially alter the binding site size or tripeptide S binding mode.

Bleomycin A_2 (**1**), the major naturally occurring constituent of the clinical antitumor drug blenoxane, is thought to derive its therapeutic effects from the ability to mediate the oxidative cleavage of double-stranded DNA[1-11] or RNA[12-14] by a process that is metal ion and oxygen dependent (Figure 1). Consequently bleomycin A_2,[15] its naturally occurring congeners,[16] and its degradation products[17-23] and semisynthetic derivatives[24-26] as well as synthetic analogs[27-30] have been the subject of extensive

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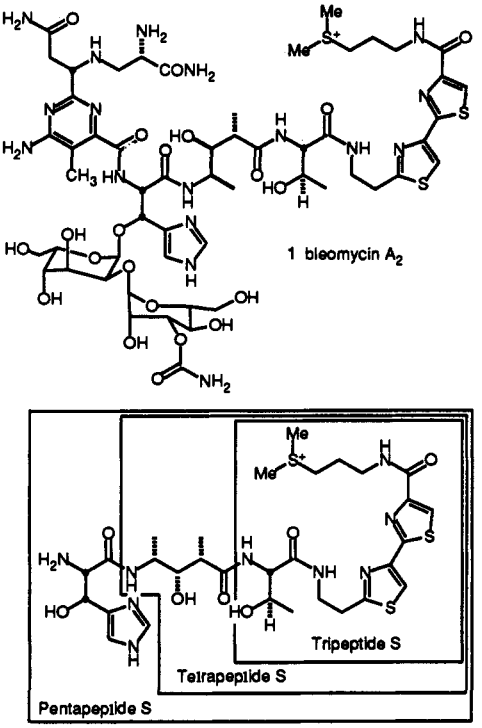

Figure 1.

and continued examination in efforts to define the fundamental functional roles of the individual subunits. The pyrimidoblamic acid subunit along with the adjacent *erythro*-β-hydroxy-L-histidine provide the metal chelation coordination sites required for Fe(II) complexation and molecular oxygen activation responsible for the subsequent DNA cleavage. The small contribution that the metal binding domain makes to the bleomycin A_2 DNA binding affinity has long been recognized,[31] and the potential contribution that this segment may make in polynucleotide recognition has been recently addressed.[14,28,30] The C-terminus tri- and tetrapeptide S subunits including the terminal sulfonium cation and the bithiazole provide the majority of the bleomycin A_2 DNA binding affinity[31] and may contribute to polynucleotide recognition[28,32-34] and the DNA cleavage selectivity. Despite the extensive studies on the bleomycins, the nature of the relevant bleomycin A_2 bithiazole binding with duplex DNA remains unresolved and has been proposed to involve intercalation[35,36] or minor-groove binding.[32,33] Two independent proposals have been advanced which suggest that the key recognition element responsible for the characteristic 5′-GC/5′-GT DNA cleavage of bleomycin A_2 lies in the C-terminus subunits with bithiazole minor-groove binding and hydrogen bonding to a guanine C2 NH_2.[32,33] In this model, the agent adopts a bound conformation possessing a β-turn at the tripeptide S–tetrapeptide S juncture with the tripeptide S subunit fully bound in the minor groove covering 2.5–3.5 base pairs.[32] The position, number, and absolute stereochemistry of the substituents on the chain linking the bithiazole subunit with the metal chelation segment have been suggested to play important or subtle roles in the DNA binding selectivity or subsequent cleavage selectivity and efficiency including a potential contribution to the agent's adoption of this bent DNA bound conformation.[1,37] Representative of such subtle effects, Umezawa and co-workers have shown that the presence and absolute configuration of the C4 methyl substituent of the 4-amino-3-hydroxy-2,4-dimethylbutanoic acid subunit potentiates the cytotoxic potency (>10×) and DNA cleavage efficiency (>10×) and have suggested that such backbone substituents may serve to effectively orient the metal chelation subunit in or adjacent to the minor groove.[1,37] On the other hand, the complementary studies of Hecht and co-workers have demonstrated that the nature and length of the linking chain do not significantly alter the DNA cleavage selectivity of the agents but do alter the relative DNA cleavage efficiency.[14,28] Although the bithiazole has been shown to be essential for observation of the characteristic bleomycin A_2 5′-GC, 5′-GT cleavage reaction, affinity cleavage agents based on dipeptide S albeit with a diffusable oxidant failed to detect a binding selectivity for the agent.[28e] The results of these studies have been interpreted to suggest that the nature of the metal chelate interaction with duplex DNA or its accessible sites of reactivity is responsible for the DNA cleavage selectivity and that such effects override or dominate the contribution from the C-terminus.[14,28] Consequently, the origin of the bleomycin 5′-GC, 5′-GT DNA cleavage selectivity remains unresolved. Although the metal chelation subunit of bleomycin A_2 itself is incapable of DNA cleavage above background Fe(II),[28] the recent demonstration that the Fe(II) complex of P-3A is an effective but nonselective DNA cleaving agent[30] and the observation that simplified Fe-PMA models of the metal chelation subunit exhibit an altered DNA cleavage selectivity[29] indicate that the metal binding domain alone is insufficient to reproduce the characteristic selectivity of the bleomycin A_2 DNA cleavage reaction. In efforts that continue to address the unresolved questions on the origin of the bleomycin A_2 sequence-selective cleavage of duplex DNA, we have initiated efforts to prepare by chemical synthesis a complete set of structural analogs that may permit an assessment of the role of each subunit and substituent.

Complementary to the past efforts of Umezawa and Hecht, herein we report full details of concise, diastereocontrolled

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syntheses of the bleomycin A_2 subunits **2–5** and optically active tripeptide S (**22**), tetrapeptide S[38,39] (**27**), and pentapeptide S (**50**) amenable to incorporation into the large-scale preparation of bleomycin A_2,[40] its naturally occurring congeners, and synthetic analogs.[41,42] Inherent in the design of the approach is the ability to control the relative and absolute stereochemistry of seven of the acyclic stereogenic centers found in **1** in a concise manner which reduces the logistical challenges of the synthesis to a manageable problem readily adaptable to the concurrent preparation of structural analogs. In realization of this objective, we herein detail the extension of the studies to the preparation of the tri- and tetrapeptide structural analogs **29a,b** and **43b–j** (Figure 2). Measurement of their absolute DNA binding constants (apparent K_B, calf thymus DNA) provided a direct assessment of the effect of the linking chain substituents on DNA binding affinity and the apparent binding site size of the agent. Consistent with past observations, the studies illustrate that the majority of the DNA binding affinity for bleomycin A_2 (1.0×10^5 M^{-1}) and deglycobleomycin A_2 (1.1×10^5 M^{-1}) is contained within *N*-BOC-tripeptide S (**21**, 0.26×10^5 M^{-1}). The additional comparisons of **29a** (0.18×10^5 M^{-1}), *N*-BOC-tetrapeptide S (**26**, 0.21×10^5 M^{-1}), **43h** (0.20×10^5 M^{-1}), and *N*-BOC-pentapeptide S (**50**, 0.23×10^5 M^{-1}) versus *N*-BOC-dipeptide S (**55**, 0.10×10^5 M^{-1}) indicate a productive binding role for the tripeptide S L-threonine subunit and substituent and illustrate that the entire pentanoic acid subunit of tetrapeptide S and the entire β-hydroxy-L-histidine subunit of pentapeptide S do not contribute productively to DNA binding affinity. The observations suggest that their effects on the bleomycin A_2 DNA cleavage selectivity or efficiency are not due to substantial stabilizing binding interactions with duplex DNA. In addition, the measured apparent binding site sizes for bleomycin A_2 (3.8 base pairs), deglycobleomycin A_2 (3.9 base pairs), *N*-BOC-tripeptide S (**21**, 3.6 base pairs), *N*-BOC-tetrapeptide S (**26**, 3.7 base pairs), **43h** (3.5 base pairs), and *N*-BOC-pentapeptide S (**50**, 4.2 base pairs) versus that of *N*-BOC-dipeptide S (**55**, 2.2 base pairs) suggest that it is the tripeptide S subunit of bleomycin A_2 that is fully bound to duplex DNA, that the tripeptide S L-threonine substituent detectably affects the agent interaction with duplex DNA, but that the presence or absence of the remaining backbone substituents does not substantially alter the binding site size or tripeptide S binding mode. The incorporation of tetrapeptide S (**27**) into the synthesis of bleomycin A_2, deglycobleomycin A_2, and structural analogs is detailed in the following articles.

***erythro*-β-Hydroxy-L-histidine Subunit.** An appropriately protected derivative of the *erythro*-β-hydroxy-L-histidine subunit[43] that links the tetrapeptide S and pyrimidoblamic acid subunits

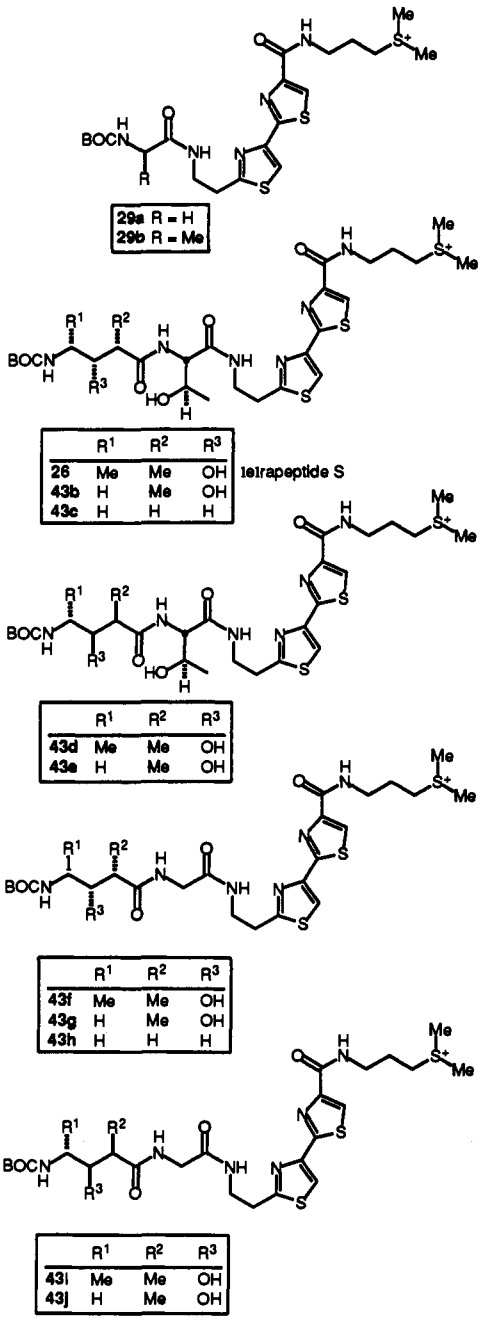

Figure 2.

was prepared as detailed in Scheme 1. The derivative **2** was prepared from **8** through adaptation of the approach detailed by Ohno and co-workers[43a] with modifications in which the competitive retro aldol reaction was suppressed during the azide displacement reaction (5 equiv of NaN_3, DMF, 45 °C, 1.5 h, 94%) and which provided an appropriately protected derivative suitable for direct coupling with the pyrimidoblamic acid subunit without further functionalization (1.1 equiv of $NaOCH_3$, CH_3OH, 0 °C, 5 min, 60%; H_2S, H_2O–CH_3OH, 25 °C, 48 h, 84%). Higher reaction temperatures, longer reaction times, or the use of LiN_3 in the displacement of the bromide suffered from the observance of a competitive retro aldol reaction. The corresponding chloride could be similarly prepared in a diastereoselective aldol addition, but low yields were observed in the subsequent azide displacement reaction. In addition, the reaction

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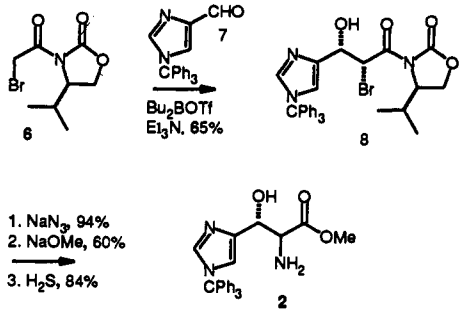
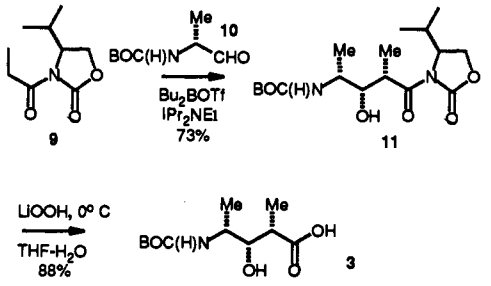
Scheme 1



Scheme 2

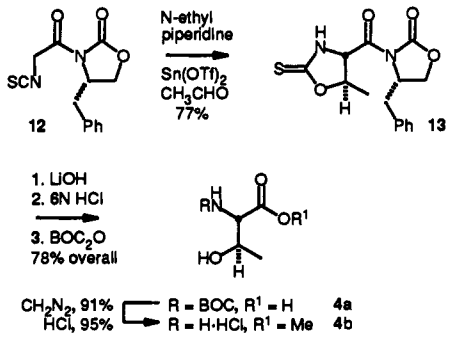


time and temperature for methanolysis of the oxazolidinone proved critical for the conversion, and longer reaction times (15–30 min) or higher reaction temperatures led to generation of competitive retro aldol products. Finally, alternative methods for azide reduction including the use of Ph_3P, THF–H_2O; Bu_3SnH; $Na_2S{\cdot}9H_2O$, Et_3N; and $NiCl_2{\cdot}6H_2O$, $NaBH_4$ proved less successful while careful hydrogenation (H_2, 10% Pd-C, EtOH–EtOAc, 23 °C, 2 h, 80%) was found to provide **2** without trityl deprotection. In initial studies, the stereochemical integrity of **2** was verified through hydrolysis of the methyl ester (LiOH) and removal of the trityl protecting group (H_2, 10% Pd-C) to provide *erythro*-β-hydroxy-L-histidine which proved identical in respects with the properties reported for authentic material.[43]

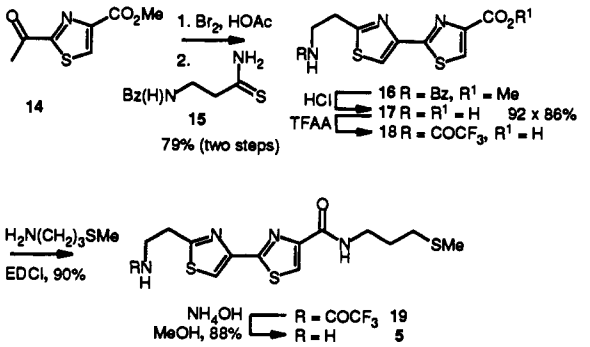
(2*S*,3*S*,4*R*)-4-Amino-3-hydroxy-2-methylpentanoic Acid Subunit. Diastereoselective syn aldol addition of the boron (*Z*)-enolate derived from **9**[44] (1.1 equiv of Bu_2BOTf, 1.2 equiv of iPr_2NEt, CH_2Cl_2, 0 °C, 45 min) with *N*-BOC-D-alaninal (**10**)[45] provided **11** (73%, –78 °C to 25 °C, 24 h; Scheme 2). Hydrolysis of the chiral auxiliary afforded subunit **3**[46] in two steps (88%, 2 equiv of LiOH, 6 equiv of H_2O_2, THF–H_2O 3:1, 0 °C, 3 h) and appropriately protected for subsequent carboxylate coupling.

L-Threonine Subunit. Similarly, the L-threonine subunit **4** was prepared through diastereoselective syn aldol addition of the stannous (*Z*)-enolate derived from **12** (1.2 equiv of *N*-ethylpiperidine, 0.9 equiv of $Sn(OTf)_2$, THF, –78 °C, 1.5 h) with acetaldehyde to provide **13** (77%, 1.5 h, –78 °C) following the procedure detailed by Evans and co-workers[47] (Scheme 3). Exhaustive deprotection of **13** (1.1 equiv of LiOH, THF–H_2O,

Scheme 3



Scheme 4



0 °C, 30 min; 0.5 N aqueous HCl, reflux, 14 h) followed by BOC protection of the amine (1.1 equiv of BOC_2O, 1 N aqueous $NaHCO_3$–THF 3:2, 25 °C, 20 h, 78% overall) provided synthetic *N*-BOC-L-threonine (**4a**) suitably protected for carboxylate coupling. Subsequent methyl ester formation (91%, CH_2N_2, Et_2O, 0 °C, 30 min) and *N*-BOC deprotection (95%, 3 N HCl–EtOAc, 25 °C, 1 h) provided synthetic L-threonine methyl ester (**4b**) suitable for coupling at the amine terminus.

Bithiazole Subunit. The bithiazole **5** was prepared following the approach detailed by Sakai and co-workers[48] with minor modifications (Scheme 4). The reaction of **15** with the crude α-bromo ketone derived from **14** conducted in absolute EtOH (79% from **14**) proved superior to that conducted in DMF (62%), as reported. Similarly, the direct carbodiimide-promoted condensation of **18** with 3-(methylthio)propylamine (90%) proved more convenient than the reported two-step procedure with use of the corresponding acid chloride, and the deprotection of **19** was conducted as detailed by Hecht[40f] to provide the free base **5** directly.

Tripeptide S. Coupling of **4a** with the free base of bithiazole **5** provided **20**, which proved to be a convenient, stable storage intermediate (63%, 1.5 equiv of DCC, 1.2 equiv of NHS; 2.0 equiv of $NaHCO_3$, DME, 25 °C, 24 h; Scheme 5). *S*-Methylation of **20** (96%, 50 equiv of CH_3I, CH_3OH, 25 °C, 72 h) provided **21**, the *N*-BOC derivative of tripeptide S, and subsequent acid-catalyzed deprotection afforded tripeptide S (**22**, 95%, 3 N HCl–EtOAc, 25 °C, 1.5 h) identical in all respects with authentic material.

Tetrapeptide S. Although a linear synthesis of tetrapeptide S based on the coupling of tripeptide S and **3** has been detailed in the independent efforts of Umezawa and Hecht,[39,40] an alternative and convergent preparation was employed for the work detailed herein. Coupling of **3** with **4b** (71%, 1.05 equiv of EDCI, 1.0 equiv of HOBt, 4 equiv of $NaHCO_3$, DMF, 25 °C, 24 h) followed by hydrolysis of the methyl ester **23** (91%, 4 equiv of LiOH, THF–CH_3OH–H_2O 3:1:1, 25 °C, 3 h) provided **24** (Scheme 6).

(44) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127. Evans, D. A.; Nelson, J. V.; Vogel, E.; Taber, T. R. *J. Am. Chem. Soc.* **1981**, *103*, 3099.

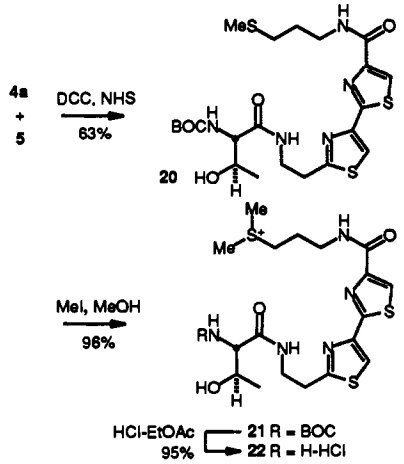
(45) (a) Prepared from *N*-BOC-D-alanine through the following sequence: (1) 1.0 equiv of MeN(OMe)H·HCl, 1.05 equiv of EDCI, 1.0 equiv of HOBt, 4 equiv of $NaHCO_3$, DMF, 25 °C, 15 h, 83%; (2) 5.0 equiv of $LiAlH_4$,[45b] THF, 0 °C, 1 h, 76%. For **10**: mp 85–86 °C, $[\alpha]^{20}_D$ –43.6 (*c* 1.05, $CHCl_3$) (lit[45c] mp 88 °C, $[\alpha]^{20}_D$ –43.2 (*c* 1.05, $CHCl_3$)). (b) Fehrentz, J. A.; Castro, B. *Synthesis* **1983**, 676. (c) Narita, M.; Otsuka, M.; Kobayashi, S.; Ohno, M.; Umezawa, Y.; Morishima, H.; Saito, S.; Takita, T.; Umezawa, H. *Tetrahedron Lett.* **1982**, *23*, 525.

(46) (2*S*,3*S*,4*R*)-4-Amino-3-hydroxy-2-methylpentanoic acid: DiPardo, R. M.; Bock, M. G. *Tetrahedron Lett.* **1983**, *24*, 4805. Narita, M.; Otsuka, M.; Kobayashi, S.; Ohno, M.; Umezawa, Y.; Morishima, H.; Saito, S.; Takita, T.; Umezawa, H. *Tetrahedron Lett.* **1982**, *23*, 525. Ohgi, T.; Hecht, S. M. *J. Org. Chem.* **1981**, *46*, 1232. Levin, M. D.; Subrahamanian, K.; Katz, H.; Smith, M. B.; Burlett, D. J.; Hecht, S. M. *J. Am. Chem. Soc.* **1980**, *102*, 1452. Yoshioka, T.; Hara, T.; Takita, T.; Umezawa, H. *J. Antibiot.* **1974**, *27*, 356.

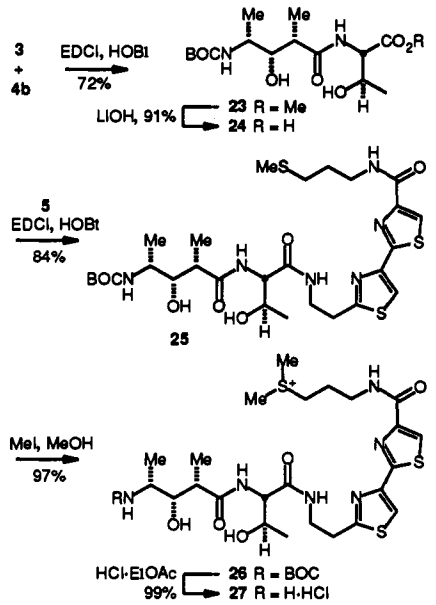
(47) Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1986**, *108*, 6757.

(48) Sakai, T. T.; Riordan, J. M.; Booth, T. E.; Glickson, J. D. *J. Med. Chem.* **1981**, *24*, 279. Houssin, R.; Bernier, J.-L.; Henichart, J.-P. *J. Heterocycl. Chem.* **1984**, *21*, 681. Zee-Cheng, K. Y.; Cheng, C. C. *J. Heterocycl. Chem.* **1970**, *78*, 1439.

Scheme 5



Scheme 6

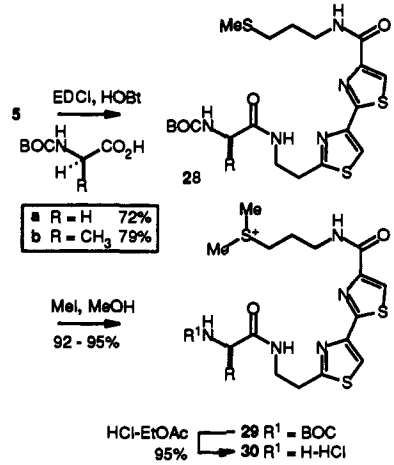


Coupling of **24** with **5** (85%, 1.05 equiv of EDCI, 1.0 equiv of HOBt, 4 equiv of $NaHCO_3$, DMF, 25 °C, 72 h) afforded **25**, which has proven to be a stable storage intermediate in our synthetic efforts. Subsequent *S*-methylation (97%, 50 equiv of CH_3I, CH_3OH, 25 °C, 80 h) provided the *N*-BOC derivative of tetrapeptide S (**26**), and acid-catalyzed deprotection of **26** (99%, 3 N HCl–EtOAc, 25 °C, 1.5 h) provided tetrapeptide S (**27**). Because of the sensitivity of **21–22** and **26–27** to prolonged storage, they are prepared from **20** and **25** immediately prior to use. While the introduction of the terminal sulfonium salt in preceding efforts[40] was postponed to a latter stage, we have found that its earlier introduction provides a more convergent synthesis of bleomycin A_2, further simplifies the purification of subsequent coupling products from neutral reagents (*i.e.*, DCC,DCC) or its coupling partners, and does not introduce additional competitive side reactions.[30]

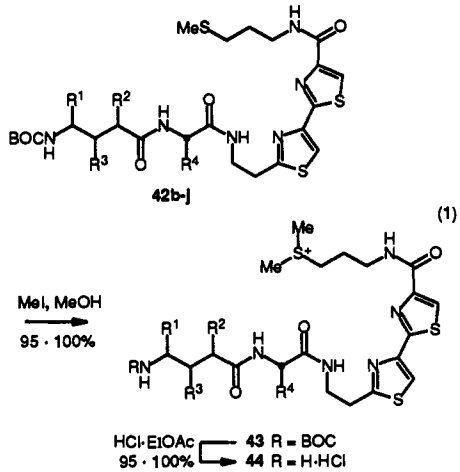
Structural Analogs of Tri- and Tetrapeptide S. In efforts to directly assess the role of the hydroxyethyl substituent within the L-threonine subunit of tripeptide S, the BOC derivatives of the analogs **30a,b** were assembled by coupling **5** with *N*-BOC-glycine or D-*N*-BOC-alanine followed by sequential *S*-methylation of **28a,b** (Scheme 7).

In order to assess the role of the substituents within the 4-aminopentanoic acid subunit of tetrapeptide S potentially acting in concert with the L-threonine hydroxyethyl substituent, the tetrapeptide analogs **43b–j** were prepared. Two-step synthesis of

Scheme 7



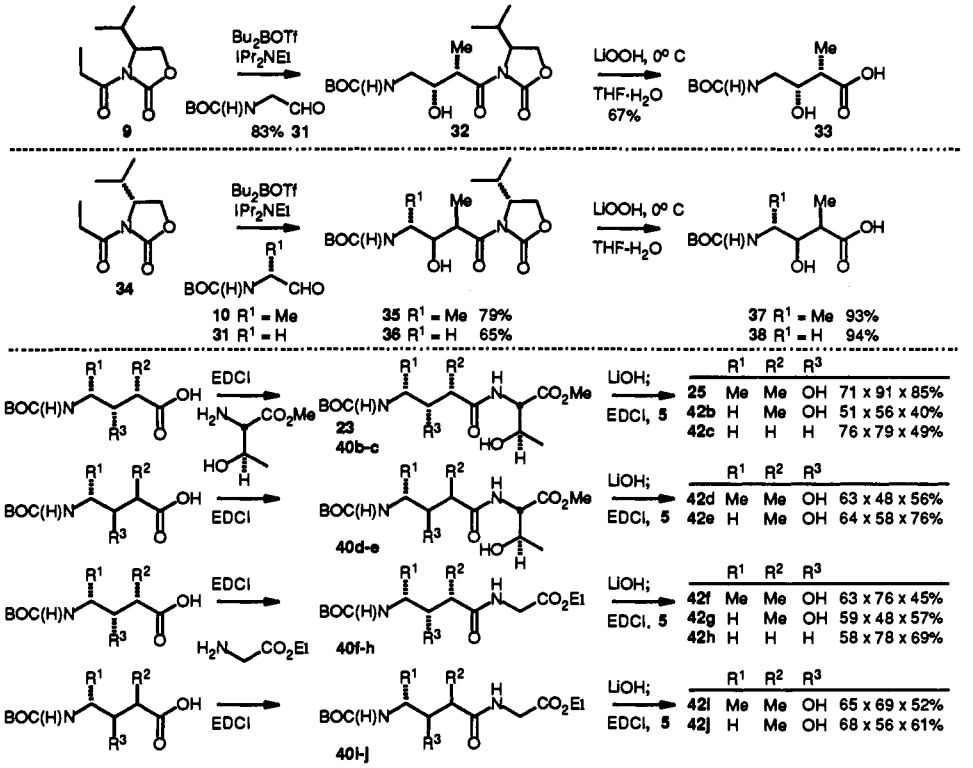
the substituted *N*-BOC-4-aminopentanoic acids **33** and **37–38** following the protocol employed for the preparation of **3** proved straightforward and was based on the diastereoselective syn aldol addition of the boron (*Z*)-enolates derived from **9** or its enantiomer **34**[44] with *N*-BOC-glycinal (**31**)[45b] or *N*-BOC-D-alaninal (**10**)[45] (Scheme 8). Without any effort on the optimization of the conversions, coupling of **3**, **33**, **37–38**, and *N*-BOC-4-aminobutanoic acid ($R^1 = R^2 = R^3 = H$) with L-threonine methyl ester or glycine ethyl ester followed by sequential ester hydrolysis of **40b–j**, coupling of the resultant carboxylic acids **41b–j** with **5** provided the key sulfides **42b–j**. Sequential *S*-methylation of **42b–j** provided **43b–j**, the *N*-BOC derivatives of the tetrapeptide analogs **44b–j** (eq 1).



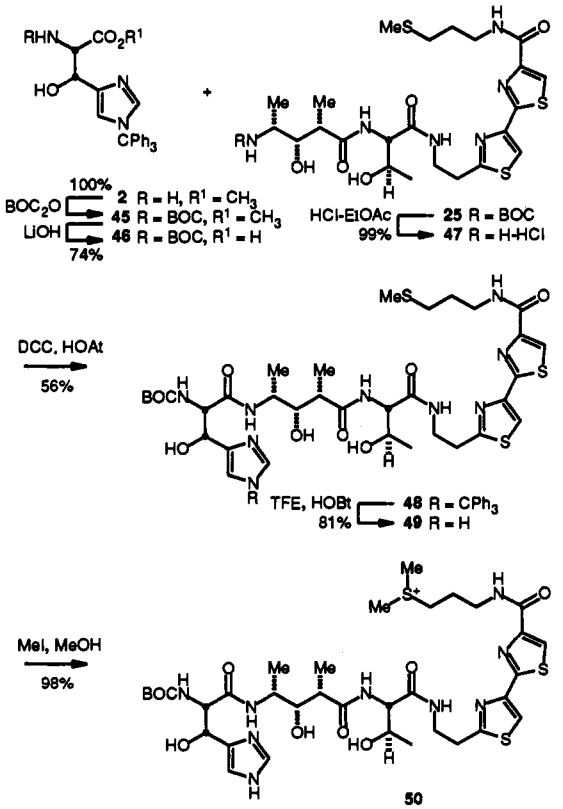
Pentapeptide S. Sequential *N*-BOC protection and methyl ester hydrolysis of **2** provided **46**, which was coupled (1.1 equiv of DCC, 1.1 equiv of HOAt, 1.5 equiv of $NaHCO_3$, DMF, 23 °C, 56 h) with demethyltetrapeptide S (**47**) to provide **48** in good yield (56%) (Scheme 9). Removal of the trityl protecting group under mild acidic conditions (0.5 M HOBt, CF_3CH_2OH, pH 5, 23 °C, 2.5 h, 81%) cleanly afforded **49** and shorter reaction times (45 min; 52% **49** + 40% **48**), or the attempted use of hydrogenolysis (H_2, Pd-C, CH_3OH) for this deprotection reaction proved less satisfactory. *S*-Methylation of **49** (98%, 200 equiv of CH_3I, CH_3OH, 25 °C, 66 h) cleanly provided *N*-BOC-pentapeptide S (**50**).

Pertinent to ongoing efforts to complete the total synthesis of bleomycin A_2 itself, we took this opportunity to examine the coupling of the fully functionalized tetrapeptide S (**27**) lacking any protecting groups with the β-hydroxy-L-histidine derivative **53** also incorporating a minimal number of protecting groups. The intention of the study was to determine whether this coupling at the tetrapeptide S–pentapeptide S juncture could function as

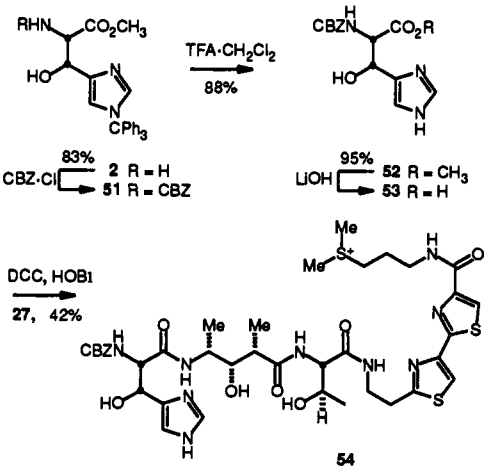
Scheme 8



Scheme 9



Scheme 10



the penultimate reaction for assemblage of the natural product without deliberate protection of the imidazole. Sequential *N*-CBZ protection, acid-catalyzed trityl deprotection, and methyl ester hydrolysis of **2** provided **53** in good overall yield (Scheme 10). Direct coupling of **53** with tetrapeptide S (**27**) effected by treatment with DCC (2.0 equiv, 1.0 equiv of HOBt, DMF, 25 °C, 48 h) afforded **54** (42%) in acceptable conversion without deliberate optimization efforts. Notably, the conversion of **53** + **27** to provide **54** was diminished if HOBt was omitted, if catalytic DMAP was added, or if $NaHCO_3$ (1.5 equiv) was employed in the reaction mixture, and added HOAt proved equally effective as HOBt. Although not detected in the conduct of the reaction, we had anticipated that the unprotected imidazole may react intramolecularly with the carbodiimide activated carboxylate to provide an *N*-acyl imidazole which in turn may serve as the reactive acylating agent or as a precursor to the *N*-hydroxybenzotriazole activated ester.

DNA Binding Properties. The important question to be addressed with the examination of **29a,b** and **43b–j** was the potential role the linking chain substituents may play in the expression of the properties of bleomycin A_2 and the origin of these effects. Central to any interpretation of their role or effect is the impact the tri- and tetrapeptide substituents may have on the DNA binding affinity and selectivity of bleomycin A_2. In conjunction with efforts to address such questions, the apparent DNA binding constants (apparent K_B) and the apparent binding site size for bleomycin A_2, deglycobleomycin A_2, the BOC derivatives of di-, tri-, tetra-, and pentapeptide S (**55**, **21**, **26**, and

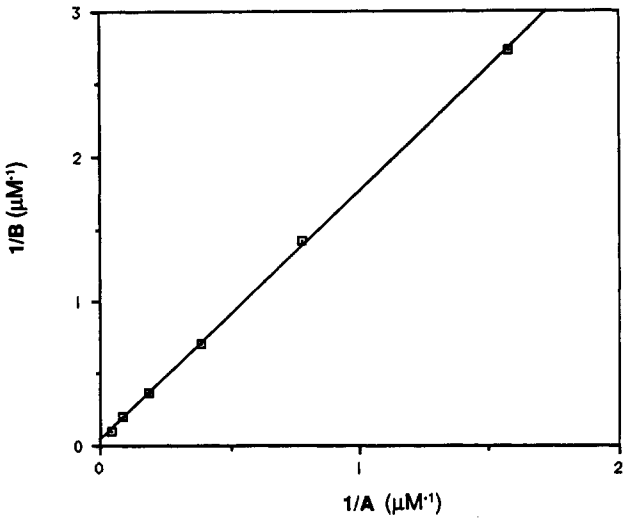


Figure 3. Double-reciprocal plot of 1/[A] versus 1/[B] used to determine the DNA binding affinity (slope, K_{app}) and stoichiometry of binding ($1/n$, y-intercept) of *N*-Boc-tripeptide S.

50), and their analogs (**29a,b** and **43b–j**) with calf thymus DNA were determined through measurement of the characteristic quenching of the bithiazole fluorescence by DNA upon binding.[31] The selection of the BOC derivatives **43** versus the free amines **44** for study was made on the basis of the well-established electrostatically enhanced binding of the free amines, cf. Table 2 for **22**. The BOC derivatives **43** versus the free amines **44** were expected to more closely reflect the properties of the subunits as they are found in the natural product. In the course of our studies, we have found that the photochemical isomerization of the bithiazole[23] and its susceptibility to bleaching were sufficient that the agents were not stable to sustained or repeated UV irradiation. The fluorescence emission was found to decrease with time and after 30 min of continuous UV excitation was reduced to zero. The UV spectrum of the material at this point indicated it was no longer the original bithiazole. Consequently, we modified the protocol of Horwitz[31] to minimize exposure of the agents to the UV light. We prepared premixed solutions of the agents in buffer and the agents in buffer with calf thymus DNA at various agent concentrations and conducted a one time measurement of the fluorescence directly, rather than conducting a traditional titration with repeated fluorescence measurements. Two solutions were prepared, one containing serially diluted concentrations of agent, 0.165 mM calf thymus DNA (base molarity), 1.25 mM NaCl, and 2.5 mM Tris-HCl buffer (pH 8.4) and the other containing the identical concentrations of agents, NaCl, and Tris-HCl buffer but lacking the DNA. The well-established dependence of the measured binding constants on the solution salt concentration has been detailed in the work of Horwitz.[31] The protocol employed herein followed their use of standard low salt concentrations approximating physiological conditions where the apparent binding is maximal. The fluorescence intensities were measured at 355 nm (5-nm slit width) following excitation at 300 nm (5-nm slit width). Double-reciprocal plots of free and bound agents were utilized to determine the apparent equilibrium binding constants and the stoichiometry of binding (apparent binding site size) (Figure 3 and Tables 1 and 2).

In this protocol, it is assumed that all binding sites on DNA are equivalent and noninteracting and that binding of one molecule of agent to DNA does not effect binding of a second molecule. Apparent equilibrium binding constants can be determined from the relationship $1/[B] = 1/nk[D_0][A] + 1/n[D_0]$, which was derived from the equilibrium constant $K = [B]/n[B][A]$ and the relation between [B], [D], and $[D_0]$ where $[B] = n([D_0]-[D])$. In these equations, [A], [B], [D], and $[D_0]$ represent the concentration of free agent, bound agent, free DNA, and total DNA, respectively, n represents the number of bound agent molecules per DNA nucleotide, and K is the apparent equilibrium constant. [A] and [B] are obtained directly from the fluorescence measurements. The solution lacking DNA gives a fluorescence intensity of free agent (F_a) in buffer. The fluorescence emission intensity of agent was found to be quenched by 86–60% in the presence of excess DNA (F_b) (Table 1), and the solution containing agent and DNA gives a composite fluorescence intensity (F) for the free and bound agent in solution. [A] and [B] were calculated from $[A] = F - F_b/F_a - F_b[A_0]$ and $[B] = F_a - F/F_a - F_b[A_0]$, where $[A_0]$ represents the total concentration of agent. The apparent equilibrium constant (K_B) and number of ligand sites per DNA base (n) were determined from the slope and y-intercept, respectively, of plots of 1/[A] against 1/[B] (Figure 3).

Table 1. Fluorescence (F) Quenching of DNA Bound Agent and F_b[a]

agent	% F quenched (F_b)	agent	% F quenched (F_b)
bleomycin A_2	83% (0.17)	**43c**	70% (0.30)
deglycobleomycin A_2	86% (0.14)	**43d**	68% (0.32)
55	77% (0.23)	**43e**	68% (0.32)
21	67% (0.33)	**43f**	67% (0.33)
26	67% (0.33)	**43g**	66% (0.34)
50	65% (0.35)	**43h**	70% (0.30)
29a	80% (0.20)	**43i**	60% (0.40)
29b	73% (0.27)	**43j**	73% (0.27)
43b	62% (0.38)	**56**	63% (0.37)

[a] Determined at an agent–base pair ratio of 1:963, calf thymus DNA.

Table 2. Apparent Binding Constants for Calf Thymus DNA

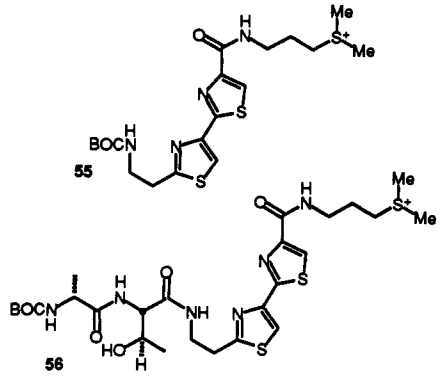
agent	apparent binding constant (K_B, 10^5 M^{-1})	apparent binding site size (base pairs)
bleomycin A_2	1.0 (1.1)[31]	3.8
deglycobleomycin A_2	1.1	3.9
22, tripeptide S	4.5	2.5
55, *N*-BOC-dipeptide S	0.10	2.2
21, *N*-BOC-tripeptide S	0.26	3.6
26, *N*-BOC-tetrapeptide S	0.21	3.7
50, *N*-BOC-pentapeptide S	0.23	4.2
29a	0.18	2.7
29b	0.30	3.0
43b	0.22	3.5
43c	0.22	3.7
43d	0.23	3.9
43e	0.18	3.6
43f	0.21	3.9
43g	0.26	3.5
43h	0.20	3.5
43i	0.27	3.1
43j	0.28	3.5
56	0.16	3.9

The results of the measurements are summarized in Table 2. In these studies, *N*-BOC-tripeptide S (**21**), *N*-BOC-tetrapeptide S (**26**), and *N*-BOC-pentapeptide S (**50**) exhibited near identical DNA binding constants ($K_B = 0.26 \times 10^5$, 0.21×10^5, and 0.23×10^5 M^{-1}, respectively) slightly greater than that of *N*-BOC-dipeptide S (**55**, 0.10×10^5 M^{-1}) and only slightly lower than that of bleomycin A_2 (**1**, 1.0×10^5 M^{-1}) or deglycobleomycin A_2 (1.1×10^5 M^{-1}), indicating that the disaccharide subunit of **1** is not contributing to binding affinity, that the majority of the DNA affinity is derived from tripeptide S, and that the substituted pentanoic acid subunit of tetrapeptide S and the β-hydroxy-L-histidine subunit of pentapeptide S are not contributing to DNA binding affinity. The addition of the natural pentanoic acid subunit to tripeptide S (**21**) or **29a** does not appreciably increase or alter the K_B of **26** or **43f**, respectively, indicating that it does not contribute to binding affinity. The observation that removal of the hydroxyethyl substituent of *N*-BOC tripeptide S (**29a**,

0.18×10^5 M^{-1}) perceptibly lowers the binding affinity while the removal of all the remaining tetrapeptide S substituents (**43h**, 0.20×10^5 M^{-1}) does not additionally affect the DNA binding affinity suggests that the L-threonine subunit productively interacts with duplex DNA, that the L-threonine hydroxethyl substituent may attenuate the binding, and that the remaining linking chain substituents do not contribute to DNA binding affinity. With the possible exception of the L-threonine substituent, this suggests that their effects on the bleomycin A_2 DNA cleavage efficiency or selectivity are not due to measureable stabilizing binding interactions with duplex DNA. In addition, the estimated binding site size for bleomycin A_2 (**1**, 3.8 base pair), deglycobleomycin A_2 (3.9 base pair), *N*-BOC-dipeptide S (**55**, 2.2 base pair), *N*-BOC-tripeptide S (**21**, 3.6 base pair), *N*-BOC-tetrapeptide S (**26**, 3.7 base pair), *N*-BOC-pentapeptide S (**50**, 4.2 base pair), and the corresponding agents lacking the backbone substituents **29a** (2.7 base pair), and **43h** (3.5 base pair) corresponds nicely to the estimated binding site size of bleomycin A_2 independently established by other techniques.[32] Consistent with conclusions drawn from the comparison of the binding constants, the observations suggest that it is the tripeptide S subunit of bleomycin which is fully bound to duplex DNA, that the L-threonine substituent may detectably affect the interaction with duplex DNA, but that the absence or presence of the remaining tetrapeptide S or pentapeptide S linking chain substituents does not substantially alter the binding site size or tripeptide S binding mode. Notably, alternative substitutions of the tetrapeptide S pentanoic acid subunit within agents containing the natural substitution of tripeptide S (**43b–e**) had little effect on the DNA binding affinity ($K_B = 0.18–0.23 \times 10^5$ versus 0.21×10^5 M^{-1}) or binding site size (3.5–3.9 versus 3.7 base pairs). A greater relative variation in the binding affinity and binding site size was observed with the agents lacking the tripeptide S hydroxyethyl substituent (**43f–j**) with some exhibiting slightly higher binding affinities and a more varied range of binding site sizes. Although a number of explanations may accommodate the observations, it is notable that the data agrees surprisingly well with the Sugiura model of bleomycin A_2 DNA binding[32] and would be consistent with the L-threonine hydroxyethyl significantly restricting the conformational properties of the bound agents. In the Sugiura model, bleomycin A_2 has been proposed to adopt a bound conformation possessing a β-turn at the tripeptide S–tetrapeptide S juncture with only the tripeptide S subunit fully bound in the minor groove covering up to 3.5 base pairs and with the backbone substituents contributing to the agent's ability to adopt this bent bound conformation.

In addition, the observations suggest that the significant reductions in the DNA cleavage efficiencies observed with agents incorporating **43h** detailed in the accompanying work are not the result of an altered DNA binding affinity or altered tripeptide S binding mode but rather may be related to such orientational (conformational) effects of the linking chain substituents. The apparent enhanced binding affinity of the tripeptide S analog **29b** incorporating D-alanine in place of L-threonine and the tetrapeptide analogs **43g,i,j** lacking the L-threonine hydroxyethyl substituent appears especially interesting, and these were the only agents to exhibit binding affinities higher than those of the natural subunits. Consequently, efforts on their incorporation into analogs of bleomycin A_2 for further study are underway.

The incorporation of tetrapeptide S (**27**) and its analog **44h** into the total synthesis of bleomycin A_2, deglycobleomycin A_2, and structurally related agents is detailed in the accompanying articles. The extension of these studies to include the additional tetrapeptide analogs are in progress and will be reported in due course.



Experimental Section

(4*R*,3′*R*,2′*R*)-3-[2′-Bromo-3′-hydroxy-3′-(*N*-(triphenylmethyl)imidazol-4″-yl)propanoyl]-4-isopropyl-2-oxazolidinone (8). A solution of **6**[49] (1.42 mmol, 356 mg) in dry CH_2Cl_2 (2 mL) under N_2 at −78 °C was treated with Bu_2BOTf (1.1 equiv, 1.6 mmol, 0.35 mL) followed by Et_3N (1.4 equiv, 2.0 mmol, 0.28 mL), and the mixture was stirred at −78 °C (30 min) and 20 °C (2 h). The mixture was recooled to −78 °C, and **7**[50] (1.0 equiv, 1.42 mmol, 481 mg) in CH_2Cl_2 (2 mL) was added. The mixture was stirred at −78 °C (30 min) and 0 °C (1.5 h). The reaction mixture was diluted with Et_2O–CH_2Cl_2 (20 mL, 2:1) and extracted with 1 N aqueous $KHSO_4$ (1 × 10 mL) and saturated aqueous NaCl (1 × 10 mL), and the organic phase was concentrated in vacuo. The crude oil was dissolved in CH_3OH (4 mL) at 0 °C, and 30% aqueous H_2O_2 (2 mL) was added slowly. The mixture was stirred at 0 °C (1 h) before being concentrated in vacuo to remove most of the CH_3OH. Water was added (5 mL), and the residue was extracted with Et_2O–CH_2Cl_2 (2:1, 2 × 25 mL). The combined organic phase was washed with 5% aqueous $NaHCO_3$ (1 × 15 mL) and saturated aqueous NaCl (1 × 15 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (SiO_2, 2 × 6 cm, 50–100% EtOAc–hexane gradient elution) gave **8** (544 mg, 837 mg theoretical, 65%) as a white solid: R_f 0.28 (SiO_2, 50% EtOAc–hexane); mp 116–117 °C (lit[43a] mp 116.5–118.5 °C, dec); $[\alpha]^{25}_D$ −44 (*c* 0.14, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.39 (d, 1H, J = 1.0 Hz), 7.30 (m, 9H), 7.12 (m, 6H), 6.88 (d, 1H, J = 1.0 Hz), 5.99 (d, 1H, J = 6.0 Hz), 5.16 (d, 1H, J = 6.0 Hz), 4.40 (m, 1H), 4.25 (m, 2H), 4.00 (m, 1H), 2.35 (m, 1H), 0.93 (d, 3H, J = 6.0 Hz), 0.89 (d, 3H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.2, 153.2, 142.2, 140.0, 138.2, 130.2, 128.5, 120.2, 76.0, 69.0, 64.0, 58.2, 50.1, 28.0, 18.0, 14.2; IR (neat) ν_{max} 3463, 3064, 2966, 1773, 1735, 1719, 1490, 1446, 1388, 700, 639 cm^{-1}; FABHRMS (DTT/DTE) *m/e* 588.1490 (M^+ + H, $C_{31}H_{30}N_3O_4Br$ requires 588.1498).

***erythro*-N^{im}-(Triphenylmethyl)-β-hydroxy-L-histidine Methyl Ester (2).** A solution of **8** (0.136 mmol, 80 mg) in dry DMF (5 mL) was treated with NaN_3 (5 equiv, 0.68 mmol, 44 mg), and the mixture was stirred at 45 °C (1.5 h). The solution was poured onto H_2O (5 mL), and the aqueous phase was extracted with EtOAc (3 × 10 mL). The combined EtOAc phase was washed with H_2O (3 × 10 mL) and saturated aqueous NaCl (1 × 10 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (SiO_2, 2 × 5 cm, 50–100% EtOAc–hexane gradient elution) afforded the azide (70 mg, 75 mg theoretical, 94%) as a clear solid: mp 55–57 °C; $[\alpha]^{25}_D$ −55 (*c* 0.10, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.42 (d, 1H, J = 1.0 Hz), 7.32 (m, 9H), 7.12 (m, 6H), 6.90 (d, 1H, J = 1.0 Hz), 5.59 (d, 1H, J = 10.0 Hz), 4.91 (dd, 1H, J = 10.0, 10.0 Hz), 4.48 (m, 1H), 4.25 (m, 2H), 3.55 (d, 1H, J = 10.0 Hz), 2.38 (m, 1H), 0.93 (d, 3H, J = 6.0 Hz), 0.89 (d, 3H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.1, 154.2, 143.0, 140.0, 139.8, 130.1, 129.0, 120.2, 76.0, 70.0, 64.0, 62.5, 59.2, 28.2, 18.0, 14.5; IR (neat) ν_{max} 3374, 2962, 2112, 1781, 1701, 1491, 1445, 1388, 1129, 702 cm^{-1}; CIHRMS (2-methylpropane) *m/e* 551.2418 (M^+ + H, $C_{31}H_{30}N_6O_4$ requires 551.2407).

A solution of the azide (0.27 mmol, 150 mg) in CH_3OH (0.5 mL) under N_2 at 0 °C was treated with $NaOCH_3$ (1.1 equiv, 0.30 mmol, 0.07 mL of a 25 wt % solution in CH_3OH), and the mixture was stirred at 0 °C (5 min). The reaction mixture was quenched with the addition of pH 7 phosphate buffer (1 mL), and the mixture was partitioned between

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saturated aqueous NaCl–NH_4Cl (1:1, 1 mL) and CH_2Cl_2 (2 mL). The aqueous layer was further extracted with CH_2Cl_2 (3 × 2 mL). The combined organic phase was dried ($MgSO_4$) and concentrated in vacuo. Chromatography (SiO_2, 2 × 5 cm, EtOAc) afforded the methyl ester (74 mg, 122 mg theoretical, 60%) as an oil: R_f 0.55 (SiO_2, EtOAc); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.45 (s, 1H), 7.30 (m, 9H), 7.10 (m, 6H), 6.79 (s, 1H), 5.01 (d, 1H, J = 7.0 Hz), 4.32 (d, 1H, J = 7.0 Hz), 3.71 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.0, 142.5, 139.2, 138.8, 130.5, 128.2, 119.6, 76.0, 69.2, 66.4, 52.5; IR (neat) ν_{max} 3152, 2860, 2111, 1747, 1560, 1508, 1205, 1037, 870 cm^{-1}; FABHRMS (DDT/DTE) *m/e* 454.1869 (M^+ + H, $C_{26}H_{23}N_5O_3$ requires 454.1879).

A solution of the methyl ester (0.17 mmol, 78 mg) in H_2O–CH_3OH (1:1, 0.5 mL) at –78 °C under N_2 was treated with liquified H_2S gas (3 mL), and the mixture was stirred in a sealed tube at 25 °C (48 h). The reaction mixture was concentrated in vacuo to give an oily solid. Chromatography (SiO_2, 2 × 5 cm, 10% CH_3OH–$CHCl_3$) gave **2** (62 mg, 73 mg theoretical, 84%) as a pale-yellow solid: R_f 0.35 (SiO_2, 10% CH_3OH–$CHCl_3$); mp 95–97 °C; $[\alpha]^{25}_D$ +37 (c 0.095, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.42 (s, 1H), 7.30 (m, 9H), 7.10 (m, 6H), 6.68 (s, 1H), 4.95 (d, 1H, J = 6.0 Hz), 3.88 (d, 1H, J = 6.0 Hz), 3.62 (s, 3H), 2.50 (br s, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 174.2, 143.0, 140.8, 139.2, 130.5, 128.5, 119.5, 75.8, 69.4, 60.2, 52.2; IR (neat) ν_{max} 3630, 3375, 3050, 1735, 1654, 1560, 1491, 1445, 747 cm^{-1}; FABHRMS (DDT/DTE) *m/e* 428.1982 (M^+ + H, $C_{26}H_{25}N_3O_3$ requires 428.1974).

Anal. Calcd for $C_{26}H_{25}N_3O_3$: C, 73.05; H, 5.89; N, 9.83. Found: C, 72.67; H, 5.83; N, 9.56.

Alternatively, a solution of the methyl ester azide (0.15 mmol, 69 mg) in 20% EtOH–EtOAc (4 mL) at 23 °C was combined with 10% Pd-C (20% by weight, 14 mg), and the mixture was vacuum-purged with H_2 (10×). The reaction mixture was stirred under a H_2 atmosphere at 23 °C for 2 h (TLC, 10% MeOH–CH_2Cl_2). The mixture was filtered, the catalyst was washed with CH_2Cl_2, and the filtrate was concentrated in vacuo. Flash chromatography (SiO_2, 2 × 7 cm, CH_2Cl_2, 5–25% CH_3OH–CH_2Cl_2 gradient elution) provided **2** (52 mg, 65 mg theoretical, 80%) as a white foam.

(4*S*,2′*S*,3′*S*,4′*R*)-3-[4′-(*N*-((*tert*-Butyloxy)carbonyl)amino)-3′-hydroxy-2′-methylpentanoyl]-4-isopropyl-2-oxazolidinone (11). A solution of **9**[44] (2.98 mmol, 552 mg) in dry CH_2Cl_2 (3 mL) under N_2 at 0 °C was treated with Bu_2BOTf (1.1 equiv, 3.28 mmol, 899 mg, 0.71 mL) followed by iPr_2NEt (1.2 equiv, 3.58 mmol, 463 mg, 0.62 mL). After allowing 45 min for complete enolization, the reaction mixture was cooled to –78 °C and kept at –78 °C (15 min). A solution of **10**[45] (1.1 equiv, 3.28 mmol, 568 mg) in CH_2Cl_2 (2 mL) was added, and the mixture was allowed to warm from –78 to 25 °C overnight with stirring (24 h). The reaction was quenched with the addition of pH 7 phosphate buffer (5 mL), the mixture was extracted with Et_2O (3 × 10 mL), and the combined organic phase was washed with saturated aqueous NaCl (1 × 10 mL) and concentrated in vacuo. The crude oil was dissolved in CH_3OH (10 mL) at 0 °C, and 30% aqueous H_2O_2 (3 mL) was added slowly. The mixture was stirred at 0 °C for 4 h before H_2O (5 mL) was added and the milky mixture was concentrated in vacuo to remove most of the CH_3OH. The residue was extracted with Et_2O (3 × 10 mL), and the combined organic phase was washed with 5% aqueous $NaHCO_3$ (1 × 10 mL) and saturated aqueous NaCl (1 × 10 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (SiO_2, 3 × 6 cm, 2:1:7 EtOAc–CH_3OH–hexane) gave **11** (772 mg, 1.06 g theoretical, 73%) as a pale-yellow solid: R_f 0.45 (SiO_2, 2:1:7 EtOAc–CH_3OH–hexane); mp 97–98 °C (EtOAc–hexane, pale-yellow powder); $[\alpha]^{20}_D$ +84 (c 0.26, CH_3OH); ^1H NMR ($CDCl_3$, 200 MHz) δ 4.52 (br d, 1H), 4.45 (m, 2H), 4.25 (m, 1H), 4.00 (br q, 1H), 3.75 (m, 2H), 2.92 (br s, 1H), 2.35 (m, 1H), 1.43 (s, 9H), 1.30 (d, 3H, J = 6.0 Hz), 1.20 (d, 3H, J = 6.0 Hz), 0.89 (d, 3H, J = 7.0 Hz), 0.85 (d, 3H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 178.2, 156.5, 154.2, 78.0, 75.2, 63.6, 58.5, 40.0, 28.6, 28.5, 19.0, 14.9, 14.8, 11.0; IR (neat) ν_{max} 3385, 2975, 2934, 1784, 1697, 1512, 1380, 1367, 1247, 1150, 737 cm^{-1}; CIHRMS (2-methylpropane) *m/e* 359.2182 (M^+ + H, $C_{17}H_{30}N_2O_6$ requires 359.2182).

Anal. Calcd for $C_{17}H_{30}N_2O_6$: C, 56.97; H, 8.44; N, 7.82. Found: C, 56.77; H, 8.65; N, 7.76.

(2*S*,3*S*,4*R*)-4-(((*tert*-Butyloxy)carbonyl)amino)-3-hydroxy-2-methylpentanoic Acid (3). A solution of **11** (2.08 mmol, 745 mg) in THF–H_2O (3:1, 40 mL) was treated at 0 °C with H_2O_2 (6 equiv, 1.75 mL of 30% aqueous H_2O_2) and LiOH (2.0 equiv, 4.16 mmol, 217 mg). The resulting mixture was stirred at 0 °C (3 h), and the excess peroxide was quenched at 0 °C with the addition of 1.5 N aqueous Na_2SO_3 (10 mL). The pH was adjusted to 9–10 with the addition of saturated aqueous $NaHCO_3$, and the oxazolidinone was removed by extraction with CH_2Cl_2 (3 × 10 mL). The aqueous phase was acidified to pH 1–2 with the addition of 1 N aqueous HCl and extracted with EtOAc (3 × 10 mL). The combined organic phase was dried ($MgSO_4$) and concentrated in vacuo. Chromatography (SiO_2, 3 × 7 cm, EtOAc) gave **3** (450 mg, 514 mg theoretical, 88%) as a clear oily solid: R_f 0.8 (SiO_2, EtOAc); $[\alpha]^{25}_D$ +8.4 (c 1.0, CH_3OH); ^1H NMR ($CDCl_3$, 200 MHz) δ 6.00 (br s, 1H), 4.82 (br d, 1H), 3.75 (m, 2H), 2.62 (dq, 1H, J = 6.0, 6.0 Hz), 1.43 (s, 9H), 1.24 (d, 3H, J = 6.0 Hz), 1.16 (d, 3H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 180.6, 156.2, 75.5, 49.0, 42.2, 28.6, 28.5, 17.0, 12.2; IR (neat) ν_{max} 3340, 2980, 2938, 1697, 1523, 1394, 1368, 1166, 1026, 734 cm^{-1}; CIHRMS (2-methylpropane) *m/e* 248.1488 (M^+ + H, $C_{11}H_{21}NO_5$ requires 248.1498).

Anal. Calcd for $C_{11}H_{21}NO_5$: C, 53.43; H, 8.56; N, 5.66. Found: C, 53.19; H, 8.53; N, 5.69.

(4*S*)-3-[((4′*S*,5′*R*)-5′-Methyl-2′-thioxo-4′-oxazolidinyl)carbonyl]-4-(phenylmethyl)-2-oxazolidinone (13). *N*-Ethylpiperidine (3.29 mmol, 0.45 mL) and **12**[47] (2.6 mmol, 718 mg) were added to a suspension of $Sn(OTf)_2$ (2.19 mmol, 913 mg) in THF (10 mL) at –78 °C, and the resulting mixture was stirred at –78 °C (1.5 h). Acetaldehyde (2.41 mmol, 2.41 mL of a 1.0 M solution in THF) was added, and the mixture was stirred for an additional 1.5 h at –78 °C. The reaction mixture was poured onto pH 7 phosphate buffer (40 mL) and extracted with Et_2O (5 × 100 mL). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo. Chromatography (SiO_2, 3 × 7 cm, 30% Et_2O–hexane) afforded two diastereomeric products (11:1): major diastereomer **13** (591 mg, 771 mg theoretical, 77%) and a minor diastereomer (52 mg, 7%). For **13**: oil; $[\alpha]^{25}_D$ +140 (c 0.7, CH_2Cl_2); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.80 (br s, 1H), 7.28 (m, 5H), 5.37 (m, 1H), 4.89 (d, 1H, J = 4.0 Hz), 4.75 (m, 1H), 4.35 (m, 2H), 3.27 (dd, 1H, J = 3.5, 13.5 Hz), 2.82 (dd, 1H, J = 9.5, 13.5 Hz), 1.58 (d, 3H, J = 6.5 Hz); IR (KBr) ν_{max} 3271, 2989, 1767, 1711, 1509, 1402, 1358, 1244, 1118, 1106, 1067, 1030, 993, 861, 717, 701, 622 cm^{-1}; CIMS (2-methylpropane) *m/e* 321 (M^+ + H, base); EIHRMS *m/e* 320.0831 ($C_{15}H_{16}N_2O_4S$ requires 320.0831). For the minor diastereomer: ^1H NMR ($CDCl_3$, 300 MHz) δ 8.00 (br s, 1H), 7.20 (m, 5H), 5.10 (m, 1H), 5.00 (d, 1H, J = 4.0 Hz), 4.75 (m, 1H), 4.15 (m, 2H), 3.20 (dd, 1H, J = 3.5, 13.5 Hz), 2.70 (dd, 1H, J = 9.5, 13.5 Hz), 1.50 (d, 3H, J = 6.5 Hz).

***N*-((*tert*-Butyloxy)carbonyl)-L-threonine (4a).** A solution of LiOH (2.05 mmol, 2.05 mL of 1 N aqueous LiOH) was added to a solution of **13** (1.85 mmol, 591 mg) in THF (7.5 mL) at 0 °C, and the resulting mixture was stirred at 0 °C (0.5 h). The reaction mixture was washed with Et_2O (5 × 3 mL), and the aqueous layer was acidified to pH 1–2 with the addition of 6 N aqueous HCl. The resulting mixture (0.45 N HCl) was warmed at reflux (14 h). The reaction mixture was diluted with THF (2 mL) and treated sequentially with $NaHCO_3$ (4.07 mmol, 4.07 mL of 1 N aqueous $NaHCO_3$) and di-*tert*-butyl dicarbonate (2.03 mmol, 0.46 mL), and the resulting mixture was stirred at 25 °C (20 h). The reaction mixture was poured onto 5% aqueous HCl and extracted with Et_2O (5 × 25 mL). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo. Chromatography (SiO_2, 2 × 7 cm, 70% Et_2O–hexane) afforded **4a** (317 mg, 405 mg theoretical, 78%) as a clear oil: $[\alpha]^{25}_D$ –2.49 (c 0.059, CH_3OH) (lit[51] $[\alpha]^{25}_D$ –2.52 (c 0.98, CH_3OH)); ^1H NMR ($CDCl_3$, 300 MHz) δ 5.68 (d, 1H, J = 9.0 Hz), 4.40 (m, 1H), 4.29 (d, 1H, J = 9.0 Hz), 1.46 (s, 9H), 1.26 (d, 3H, J = 6.0 Hz); IR (neat) ν_{max} 3854, 3754, 3384, 2980, 2936, 1700, 1522, 1458, 1394, 1368, 1254, 1166, 1118, 1070, 1004 cm^{-1}.

L-Threonine Methyl Ester (4b). An Et_2O solution of CH_2N_2 (excess) was transferred into a solution of **4a** (2.24 mmol, 490 mg) in Et_2O (2 mL) at 0 °C. The reaction mixture was allowed to stand at 0 °C (3 h) and concentrated in vacuo. Chromatography (SiO_2, 2 × 5 cm, EtOAc) afforded the methyl ester (475 mg, 521 mg theoretical, 91%) as an oil: ^1H NMR ($CDCl_3$, 200 MHz) δ 5.40 (br d, 1H), 4.25 (m, 2H), 3.75 (s, 3H), 2.55 (br s, 1H), 1.43 (s, 9H), 1.25 (d, 3H, J = 6.0 Hz); IR (neat) ν_{max} 3434, 2979, 2935, 1745, 1718, 1509, 1456, 1438, 1368, 1069, 997, 879 cm^{-1}.

A solution of *N*-((*tert*-butyloxy)carbonyl)-L-threonine methyl ester (0.65 mmol, 152 mg) was treated with 3 N HCl–EtOAc (3 mL), and the mixture was stirred at 25 °C (1 h). The solvent was evaporated in vacuo, and the oily solid was triturated with Et_2O (3 × 1 mL) to give **4b** (105 mg, 111 mg theoretical, 95%) as a hygroscopic solid: $[\alpha]^{23}_D$ –15.2 (c 0.17, 5 N aqueous HCl) (lit $[\alpha]^{20}_D$ –14.5 (c 0.5, 5 N aqueous HCl, Fluka)); ^1H NMR (CD_3OD, 400 MHz) δ 4.28 (m, 1H), 3.91 (d, 1H, J = 4.0 Hz), 3.83 (s, 3H), 1.30 (d, 3H, J = 6.0 Hz); IR (neat) ν_{max} 3423, 2968, 2964, 1743, 1642, 1444, 1296, 1206, 1039 cm^{-1}.

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3-[2′-(2-Aminoethyl)-2,4′-bithiazole-4-carboxamido]propyl Methyl Sulfide (5). The preparation of **18** followed the procedure detailed by Sakai and co-workers[48] with the exception that the reaction of **15** with the crude α-bromo ketone derived from **14** conducted in absolute EtOH (70 °C, 15 min, 79% from **14**) proved superior to that conducted in DMF (62% from **14**) as reported. Similarly, the direct condensation of **18** with 3-(methylthio)propylamine[52] (1.5 equiv of EDCI, 0.5 equiv of HOBt, THF–DMF 9:1, 25 °C, 14 h, 90%) proved more convenient than the reported two-step procedure with use of the corresponding acid chloride.[48] Finally, deprotection of **19** was conducted as detailed by Hecht[40f] in a manner to provide the free base **5** directly. Concentrated aqueous NH_4OH (1 mL of 28–30%) was added to a solution **19** (0.46 mmol, 200 mg) in CH_3OH (1 mL), and the resulting mixture was stirred at 25 °C for 18 h. The reaction mixture was concentrated in vacuo, and the residue was taken up in saturated aqueous $NaHCO_3$ (2 mL). The aqueous phase was extracted with EtOAc (5 × 5 mL), and the combined organic phase was dried ($MgSO_4$) and concentrated in vacuo. Chromatography (SiO_2, 1 × 2 cm, EtOAc) afforded **5** (137 mg, 156 mg theoretical, 88%) as an oil: R_f 0.25 (SiO_2, EtOAc); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.10 (s, 1H), 7.91 (s, 1H), 7.56 (t, 1H, J = 5.0 Hz), 6.45 (br s, 2H), 3.55 (dt, 2H, J = 5.0, 7.0 Hz), 3.25 (m, 4H), 2.61 (t, 2H, J = 7.0 Hz), 2.13 (s, 3H), 1.98 (tt, 2H, J = 7.0, 7.0 Hz); IR (neat) ν_{max} 3300, 3112, 2921, 1656, 1544 cm^{-1}; CIMS (2-methylpropane) m/e 343 (M^+ + H, base); FABHRMS (NBA–CsI) m/e 474.9697 (M^+ + Cs, $C_{13}H_{18}N_4OS_3$ requires 474.9697).

3-[2′-(2″-((*N*-((*tert*-Butyloxy)carbonyl)-L-threonyl)amino)ethyl)-2,4′-bithiazole-4-carboxamido]propyl Methyl Sulfide (20). *N*-Hydroxysuccinimide (1.2 equiv, 3.23 mmol, 371 mg) was added to a solution of *N*-BOC-L-threonine (**4a**, 2.69 mmol, 590 mg) in DME (10 mL) followed by DCC (1.5 equiv, 4.04 mmol, 840 mg), and the resulting mixture was allowed to stir at 25 °C (12 h). The reaction mixture was concentrated in vacuo, the residue taken up in Et_2O (5 mL) and filtered through a pad of Celite, and the filtrate concentrated in vacuo. Chromatography (SiO_2, 3 × 5 cm, 50% Et_2O–hexane) afforded the *N*-hydroxysuccinate ester of **4a** (660 mg, 850 mg theoretical, 78%) as a white solid: mp 125–126 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.00 (m, 1H), 6.22 (m, 1H), 2.84 (s, 4H), 2.81 (m, 1H), 1.90 (d, 3H, J = 7.0 Hz), 1.47 (s, 9H); IR (KBr) ν_{max} 3344, 2980, 1806, 1774, 1740, 1654, 1498, 1430, 1368, 1252, 1204, 1165, 1068, 1014, 956, 880, 786, 766 cm^{-1}; CIMS (2-methylpropane) m/e 317 (M^+ + H); CIHRMS m/e 317.1554 (M^+ + H, $C_{13}H_{20}N_2O_7$ requires 317.1348).

A solution of 1 N aqueous $NaHCO_3$ (2.0 equiv, 0.088 mmol, 0.088 mL) was added to a solution of **5** (0.044 mmol, 15 mg) in DME (1 mL) followed by the *N*-hydroxysuccinate ester of **4a** (1.5 equiv, 0.066 mmol, 21 mg), and the resulting mixture was allowed to stir at 25 °C (24 h). The reaction mixture was poured onto H_2O and extracted with EtOAc (4 × 10 mL). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo. Chromatography (SiO_2, 2 × 5 cm, Et_2O) afforded **20** (15 mg, 24 mg theoretical, 63%) as a clear solid: mp 105–106 °C (EtOAc–hexane) (lit mp[40d] 106–107 °C (EtOAc–*i*Pr_2O)); $[\alpha]^{23}_D$ –19.2 (*c* 0.66, CH_3OH) (lit[40d] $[\alpha]^{24}_D$ –20 (*c* 1.0, CH_3OH)); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.12 (s, 1H), 7.85 (s, 1H), 7.65 (br t, 1H), 7.25 (br t, 1H), 5.60 (br d, 1H, J = 8.0 Hz), 4.40 (m, 1H), 4.15 (m, 1H), 3.75 (m, 2H), 3.59 (dt, 2H, J = 7.0, 7.0 Hz), 3.30 (t, 2H, J = 6.0 Hz), 2.61 (t, 2H, J = 7.0 Hz), 2.13 (s, 3H), 1.98 (tt, 2H, J = 7.0, 7.0 Hz), 1.40 (s, 9H), 1.17 (d, 3H, J = 7.0 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.8, 170.3, 162.8, 162.0, 157.2, 151.8, 149.0, 124.2, 118.0, 80.5, 68.0, 61.5, 40.0, 39.1, 33.5, 32.0, 29.1, 28.5, 20.0, 15.5; IR (neat) ν_{max} 3386, 2973, 1785, 1718, 1654, 1550, 1484, 1370, 1297, 1212, 1162, 1086, 1049, 912, 880, 806, 733 cm^{-1}; UV (H_2O) λ_{max} 292 (ε 11 900), 210 (ε 27 000) nm; CIMS (2-methylpropane) m/e 544 (M^+ + H); FABHRMS (NBA–CsI) m/e 676.0697 (M^+ + Cs, $C_{22}H_{33}N_5O_5S_3$ requires 676.0698).

[3-[2′-(2″-((*N*-((*tert*-Butyloxy)carbonyl)-L-threonyl)amino)ethyl)-2,4′-bithiazole-4-carboxamido]propyl]dimethylsulfonium Iodide (*N*-BOC-Tripeptide S, 21). A solution of **20** (0.023 mmol, 12.3 mg) in dry CH_3OH (0.3 mL) was treated with CH_3I (50 equiv, 0.07 mL), and the mixture was stirred at 25 °C (72 h). The solvent was evaporated in vacuo and the oily solid was triturated with $CHCl_3$ (3 × 1 mL) to give pure **21** (14.8 mg, 15.4 mg theoretical, 96%) as a clear hygroscopic solid: $[\alpha]^{23}_D$ –17 (*c* 0.1, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.20 (s, 1H), 8.17 (s, 1H), 4.20 (m, 1H), 3.92 (d, 1H, J = 4.5 Hz), 3.75 (m, 1H), 3.68 (m, 1H), 3.61 (t, 2H, J = 7.0 Hz), 3.41 (t, 2H, J = 7.5 Hz), 3.28 (t, 2H, J = 7.0 Hz), 2.96 (s, 6H), 2.16 (tt, 2H, J = 7.0, 7.0 Hz), 1.42 (s, 9H), 1.14 (d, 3H, J = 6.5 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.7, 170.9, 164.2, 164.1, 157.9, 151.3, 149.4, 125.4, 118.8, 80.9, 68.4, 61.6, 42.6, 40.0, 38.6, 33.6, 28.6, 25.6, 25.5, 20.3; IR (neat) ν_{max} 3444, 3360, 1634, 1605, 1456, 1393, 1310, 858, 745 cm^{-1}; UV (H_2O) λ_{max} 291 (ε 10 800), 223 (ε 30 200) nm; FABHRMS (NBA) m/e 558.1860 (M^+ – I, $C_{23}H_{36}N_5O_5S_3$ requires 558.1879).

Additionally the salt could be purified by reverse-phase chromatography (C-18, 0.5 × 3.0 cm, 5–40% CH_3OH–H_2O gradient elution): R_f 0.45 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH).

[3-[2′-(2″-(L-Threonylamino)ethyl)-2,4′-bithiazole-4-carboxamido]propyl]dimethylsulfonium Chloride Hydrochloride (Tripeptide S, 22). The solid **21** (0.0092 mmol, 6.3 mg) was treated with 3 N HCl–EtOAc (1 mL) and the mixture stirred at 25 °C (1.5 h). The solvent was evaporated in vacuo, and the oily solid was triturated with $CHCl_3$ (3 × 1 mL) to give pure **22** (4.6 mg, 4.8 mg theoretical, 95%) as a clear hygroscopic solid identical to a sample of authentic material: $[\alpha]^{23}_D$ –16.5 (*c* 0.04, 0.1 N aqueous HCl) (lit[40d] $[\alpha]^{23}_D$ –15 (*c* 0.75, 0.1 N aqueous HCl)), authentic sample $[\alpha]^{23}_D$ –16.2 (*c* 0.04, 0.1 N aqueous HCl); ^1H NMR (CD_3OD, 400 MHz) δ 8.27 (s, 1H), 8.26 (s, 1H), 4.05 (dt, 1H, J = 13.0, 6.5 Hz), 3.82 (ddd, 1H, J = 14.0, 7.0, 7.0 Hz), 3.72 (ddd, 1H, J = 14.0, 7.0, 7.0 Hz), 3.66 (m, 3H), 3.49 (t, 2H, J = 7.0 Hz), 3.38 (m, 2H), 3.00 (s, 6H), 2.18 (tt, 2H, J = 7.0, 7.0 Hz), 1.25 (d, 3H, J = 6.5 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 171.1, 168.8, 163.8, 163.5, 151.2, 148.8, 125.5, 119.4, 67.3, 60.4, 42.5, 40.3, 38.6, 33.3, 25.5, 25.4, 20.4; IR (neat) ν_{max} 3361, 2925, 1653, 1544, 1446, 1366, 1167, 1007, 755 cm^{-1}; UV (H_2O) λ_{max} 291 (ε 10 700), 211 (ε 23 000) nm; FABHRMS (NBA) m/e 459.1442 (M^+ – Cl and HCl, $C_{18}H_{29}N_5O_3S_3$ requires 459.1433).

Additionally the salt could be purified by reverse-phase chromatography (C-18, 0.5 × 3.0 cm, 5–40% CH_3OH–H_2O gradient elution): R_f 0.45 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH).

(2′*S*, 3′*S*, 4′*R*)-*N*-[4′-(((*tert*-Butyloxy)carbonyl)amino)-3′-hydroxy-2′-methylpentanoyl]-L-threonine Methyl Ester (23). A solution of **3** (0.40 mmol, 100 mg) in DMF (3 mL) was treated with **4b** (1.1 equiv, 0.45 mmol, 75 mg), HOBt (1 equiv, 0.40 mmol, 55 mg), $NaHCO_3$ (4 equiv, 1.63 mmol, 137 mg), and EDCI (1.05 equiv, 0.425 mmol, 81 mg), and the reaction mixture was stirred at 25 °C (24 h). The mixture was poured onto H_2O (10 mL) and extracted with EtOAc (5 × 20 mL). The combined EtOAc extracts were washed with H_2O (1 × 10 mL), 10% aqueous HCl (1 × 10 mL), saturated aqueous $NaHCO_3$ (1 × 10 mL), and saturated aqueous NaCl (1 × 10 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (SiO_2, 2 × 5 cm, EtOAc) afforded **23** (105 mg, 145 mg theoretical, 72%) as an oily solid: R_f 0.65 (SiO_2, EtOAc); $[\alpha]^{25}_D$ +32 (*c* 0.25, CH_3OH); ^1H NMR ($CDCl_3$, 200 MHz) δ 6.50 (br d, 1H), 4.80 (br s, 1H), 4.59 (dd, 1H, J = 10.0, 4.4 Hz), 4.35 (m, 1H), 3.80 (s, 3H), 3.78 (m, 1H), 3.65 (m, 1H), 2.52 (m, 1H), 1.43 (s, 9H), 1.30 (d, 3H, J = 7.0 Hz), 1.25 (d, 3H, J = 7.0 Hz), 1.19 (d, 3H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 178.0, 172.2, 157.0, 80.5, 75.5, 68.5, 58.2, 53.0, 48.5, 44.0, 28.9, 28.5, 21.0, 13.0; IR (neat) ν_{max} 3367, 2978, 2936, 1741, 1686, 1656, 1526, 1367, 1166, 732 cm^{-1}; CIHRMS (2-methylpropane) m/e 363.2131 (M^+ + H, $C_{16}H_{30}N_2O_7$ requires 363.2131).

(2′*S*,3′*S*,4′*R*)-*N*-[4′-(((*tert*-Butyloxy)carbonyl)amino)-3′-hydroxy-2′-methylpentanoyl]-L-threonine (24). A solution of **23** (0.44 mmol, 160 mg) in THF–H_2O–CH_3OH (3:1:1, 10 mL) was treated with LiOH (4 equiv, 1.76 mmol, 74 mg) and was allowed to stir at 25 °C (3 h). The organic solvents were evaporated in vacuo, and the mixture was extracted with CH_2Cl_2 (1 × 5 mL). The aqueous phase was acidified with the addition of 10% aqueous HCl (pH 1) and was extracted with EtOAc (5 × 10 mL). The combined organic phases were dried ($MgSO_4$) and concentrated in vacuo to give **24** (140 mg, 153 mg theoretical, 91%), which was carried into the subsequent coupling with **5** without further purification: ^1H NMR ($CDCl_3$, 200 MHz) δ 7.10 (br d, 1H), 5.60 (br d, 1H), 4.55 (dd, 1H, J = 10.0, 4.4 Hz), 4.35 (m, 1H), 3.80 (m, 1H), 3.65 (m, 1H), 2.52 (m, 1H), 1.43 (s, 9H), 1.30 (d, 3H, J = 7.0 Hz), 1.25 (d, 3H, J = 7.0 Hz), 1.19 (d, 3H, J = 7.0 Hz); IR (neat) ν_{max} 3375, 2975, 2930, 1741, 1685, 1654, 1518, 1165, 968, 830, 733 cm^{-1}; FABHRMS (NBA–NaI) m/e 349.1965 (M^+ + H, $C_{15}H_{28}N_2O_7$ requires 349.1975).

Anal. Calcd for $C_{15}H_{28}N_2O_7$: C, 51.70; H, 8.10; N, 8.04. Found: C, 52.08; H, 8.33; N, 7.96.

(2″*S*,3″*S*,4″*R*)-3-[2′-(2‴-((*N*-[4″-(((*tert*-Butyloxy)carbonyl)amino)-3″-hydroxy-2″-methylpentanoyl]-L-threonyl)amino)ethyl)-2,4′-bithiazole-4-carboxamido]propyl Methyl Sulfide (25). A solution of **24** (1.3 equiv, 0.115 mmol, 40 mg) in DMF (1 mL) was treated with **5** (0.088 mmol, 30 mg), HOBt (1 equiv, 0.088 mmol, 12 mg), EDCI (1.05 equiv, 0.093 mmol, 17.8 mg), and $NaHCO_3$ (4 equiv, 0.35 mmol, 30 mg) at 0 °C, and

(52) Commercially available from Pfaltz and Bauer, Inc., Waterbury, CT 06708.

the mixture was stirred at 25 °C (50 h). Saturated aqueous NaCl (3 mL) was added, and the mixture was extracted with EtOAc (5 × 5 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ (1 × 3 mL) and saturated aqueous NaCl (1 × 3 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (SiO_2 PCTLC, 1 mm, 2:1 CH_2Cl_2–CH_3CN, 3:6:0.2 and 3:6:0.5 CH_3CN–CH_2Cl_2–CH_3OH) gave **25** (50 mg, 59 mg theoretical, 85%): $[\alpha]^{23}_D$ −12 (*c* 0.13, $CHCl_3$), $[\alpha]^{23}_{365}$ −50 (*c* 0.13, $CHCl_3$), $[\alpha]^{25}_D$ +16 (*c* 0.05, CH_3OH, concentration dependent), $[\alpha]^{23}_{405}$ +46 (*c* 0.05, CH_3OH, concentration dependent); ^1H NMR ($CDCl_3$, 200 MHz) δ 8.12 (s, 1H), 7.85 (s, 1H), 7.65 (br t, 1H), 7.45 (br t, 1H), 7.02 (br d, 1H, *J* = 5.0 Hz), 4.90 (br d, 1H), 4.35 (br d, 1H, *J* = 5.0 Hz), 4.20 (m, 2H), 3.70 (m, 2H), 3.58 (m, 5H), 3.25 (t, 2H, *J* = 6.5 Hz), 2.60 (t, 2H, *J* = 7.0 Hz), 2.59 (br m, 1H), 2.12 (s, 3H), 1.95 (tt, 2H, *J* = 7.0, 7.0 Hz), 1.43 (s, 9H), 1.22 (d, 3H, *J* = 7.0 Hz), 1.11 (d, 6H, *J* = 6.0 Hz); ^{13}C NMR (CD_3OD, 50 MHz) δ 178.0, 171.6, 169.3, 162.9, 161.8, 156.2, 151.3, 148.9, 124.2, 117.2, 80.5, 75.8, 66.9, 57.3, 48.2, 43.0, 38.9, 38.7, 32.9, 31.8, 29.1, 28.5, 19.0, 17.0, 15.6, 12.5; IR (neat) ν_{max} 3324, 2976, 2933, 1654, 1649, 1546, 1453, 1391, 1366, 1248, 1165, 731 cm^{-1}; UV (H_2O) λ_{max} 292 (ε 9500), 210 (ε 21 700) nm; FABHRMS (DTT/DTE) *m/e* 673.2491 (M^+ + H, $C_{28}H_{44}N_6O_7S_3$ requires 673.2512).

(2″*S*,3″*S*,4″*R*)-[3-[2′-(2‴-((*N*-[4″-(((*tert*-Butyloxy)carbonyl)amino)-3″-hydroxy-2″-methylpentanoyl]-L-threonyl)amino)ethyl)-2,4′-bithiazole-4-carboxamido]propyl]dimethylsulfonium Iodide (*N*-BOC-Tetrapeptide S, 26). A solution of **25** (0.019 mmol, 12.9 mg) in dry CH_3OH (0.3 mL) was treated with CH_3I (50 equiv, 0.06 mL), and the mixture was stirred at 25 °C (80 h). The solvent was evaporated in vacuo, and the oily solid was triturated with $CHCl_3$ (3 × 1 mL) to give pure **26** (15 mg, 15.5 mg theoretical, 97%) as a clear hygroscopic solid: $[\alpha]^{25}_D$ +22 (*c* 0.06, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.26 (s, 1H), 8.20 (s, 1H), 4.38 (d, 1H, *J* = 4.5 Hz), 4.15 (m, 1H), 3.70 (m, 4H), 3.62 (t, 2H, *J* = 7.0 Hz), 3.45 (t, 2H, *J* = 7.5 Hz), 3.34 (m, 2H), 2.99 (s, 6H), 2.62 (dq, 1H, *J* = 7.0, 7.0 Hz), 2.16 (tt, 2H, *J* = 7.0, 7.0 Hz), 1.46 (s, 9H), 1.24 (d, 3H, *J* = 7.0 Hz), 1.18 (d, 3H, *J* = 6.5 Hz), 1.17 (d, 3H, *J* = 6.5 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 178.2, 172.8, 170.8, 164.2, 164.1, 158.0, 151.3, 149.5, 125.4, 118.7, 80.8, 76.2, 68.4, 59.8, 44.3, 42.6, 40.1, 38.6, 33.6, 28.8, 25.6, 25.4, 20.2, 16.6, 12.9; IR (neat) ν_{max} 3311, 2978, 2933, 1650, 1644, 1544, 1445, 1361, 1294, 1250, 1161, 1061, 1022 cm^{-1}; UV (H_2O) λ_{max} 292 (ε 12 100), 215 (ε 31 200) nm; FABHRMS (NBA) *m/e* 687.2668 (M^+ − I, $C_{29}H_{47}N_6O_7S_3$ requires 687.2668).

Additionally the salt could be purified by reverse-phase chromatography (C-18, 0.5 × 3.0 cm, 5–40% CH_3OH–H_2O gradient elution): R_f 0.45 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH).

(2″*S*, 3″*S*, 4″*R*)-[3-[2′-(2‴-((*N*-(4″-Amino-3″-hydroxy-2″-methylpentanoyl)-L-threonyl)amino)ethyl)-2,4′-bithiazole-4-carboxamido]propyl]dimethylsulfonium Chloride Hydrochloride (Tetrapeptide S, 27). The solid **26** (0.0038 mmol, 3.1 mg) was treated with 3 N HCl–EtOAc (1 mL) and the mixture stirred at 25 °C (1.5 h). The solvent was evaporated in vacuo, and the oily solid was triturated with $CHCl_3$ (3 × 1 mL) to give pure **27** (2.5 mg, 2.52 mg theoretical, 99%) as a clear hygroscopic solid: $[\alpha]^{23}_D$ −25 (*c* 0.04, 0.1 N aqueous HCl), $[\alpha]^{23}_{365}$ −63 (*c* 0.04, 0.1 N aqueous HCl), $[\alpha]^{23}_{365}$ −54 (*c* 0.50, 0.1 N aqueous HCl) (lit[40e] $[\alpha]^{20}_{365}$ −52 (*c* 0.5, 0.1 N aqueous HCl)); ^1H NMR (CD_3OD, 400 MHz) δ 8.27 (s, 1H), 8.25 (s, 1H), 4.26 (d, 1H, *J* = 4.5 Hz), 4.12 (m, 1H), 3.80 (m, 4H), 3.66 (t, 2H, *J* = 7.0 Hz), 3.44 (t, 2H, *J* = 7.5 Hz), 3.34 (m, 2H), 3.00 (s, 6H), 2.65 (dq, 1H, *J* = 7.0, 7.0 Hz), 2.20 (tt, 2H, *J* = 7.0, 7.0 Hz), 1.34 (d, 3H, *J* = 7.0 Hz), 1.33 (d, 3H, *J* = 6.0 Hz), 1.19 (d, 3H, *J* = 6.5 Hz); IR (neat) ν_{max} 3386, 2928, 2851, 1642, 1546, 1499, 1438, 1367, 1298, 1247, 1163, 1016 cm^{-1}; UV (H_2O) λ_{max} 291 (ε 11 600), 211 (ε 31 500) nm; FABHRMS (NBA) *m/e* 587.2144 (M^+ − Cl and HCl, $C_{24}H_{39}N_6O_5S_3$ requires 587.2144).

Additionally the salt could be purified by reverse-phase chromatography (C-18, 0.5 × 3.0 cm, 5–40% CH_3OH–H_2O gradient elution): R_f 0.45 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH).

***erythro*-N^{α}-((*tert*-Butyloxy)carbonyl)-N^{im}-(triphenylmethyl)-β-hydroxy-L-histidine Methyl Ester (45).** A solution of **2** (0.012 mmol, 5.1 mg) in THF–H_2O (3:1, 0.12 mL) was treated with di-*tert*-butyl dicarbonate (0.013 mmol, 3 μL) at 23 °C, and the mixture was allowed to stir for 1.5 h. The reaction mixture was extracted quickly with 10% aqueous HCl (0.5 mL, 2×) and EtOAc (0.5 mL, 2×). The organic phase was dried (Na_2SO_4) and concentrated in vacuo. Chromatography (SiO_2, 50% EtOAc–hexane) afforded **45** (6.3 mg, 6.3 mg theoretical, 100%): R_f 0.28 (SiO_2, 1:1 EtOAc–hexane); $[\alpha]^{23}_D$ +33 (*c* 0.03, $CHCl_3$); ^1H NMR (CD_3OD, 400 MHz) δ 8.62 (s, 1H), 7.47 (m, 9H), 7.28 (s, 1H), 7.20 (m, 6H), 5.05 (d, 1H, *J* = 5.5 Hz), 4.47 (d, 1H, *J* = 5.5 Hz), 3.70 (s, 3H), 1.36 (s, 9H); IR (neat) ν_{max} 3395, 2933, 1723, 1687, 1492, 1364, 1159, 1051, 862, 703 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 660.1474 (M^+ + Cs, $C_{31}H_{33}N_3O_5$ requires 660.1474).

***erythro*-N^{α}-((*tert*-Butyloxy)carbonyl)-N^{im}-(triphenylmethyl)-β-hydroxy-L-histidine (46).** A solution of **45** (0.012 mmol, 6.3 mg) in THF–CH_3OH–H_2O (3:1:1, 0.3 mL) was cooled to 0 °C and treated with aqueous 1 N LiOH (1.5 equiv, 0.018 mmol, 18 μL). The reaction mixture was stirred at 0 °C (2 h). After the THF and CH_3OH were evaporated under a N_2 stream, the aqueous phase was extracted with EtOAc (1 × 0.5 mL). The aqueous phase was acidified to pH 4 with the addition of aqueous 1.2 N HCl. The aqueous phase was thoroughly extracted with 30% *i*PrOH–$CHCl_3$ (5 × 0.5 mL) until no more UV activity was detected in the H_2O layer. The combined organic phases were concentrated in vacuo to provide **46** (4.5 mg, 6.1 mg theoretical, 74%): $[\alpha]^{23}_D$ +15 (*c* 0.10, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.58 (s, 1H), 7.37 (m, 9H), 7.15 (m, 6H), 6.95 (s, 1H), 4.92 (d, 1H, *J* = 5.5 Hz), 4.44 (d, 1H, *J* = 5.5 Hz), 1.40 (s, 9H); IR (neat) ν_{max} 3344, 2964, 2923, 1703, 1605, 1487, 1441, 1390, 1364, 1246, 1164, 1128, 1051 cm^{-1}; FABHRMS (NBA) *m/e* 514.2348 (M^+ + H, $C_{30}H_{31}N_3O_5$ requires 514.2342).

(2″*S*,3″*S*,4″*R*)-3-[2′-(2‴-((*N*-(4″-Amino-3″-hydroxy-2″-methylpentanoyl)-L-threonyl)amino)ethyl)-2,4′-bithiazole-4-carboxamido]propyl Methyl Sulfide Hydrochloride (47). The solid **25** (0.0074 mmol, 5 mg) was treated with 4 N HCl–EtOAc (0.5 mL) and the mixture stirred at 23 °C (1 h) under Ar. The white heterogenous reaction mixture was concentrated under a N_2 stream to provide pure **47** as a white powder (4.2 mg, 4.26 mg theoretical, 99%): $[\alpha]^{23}_D$ −4 (*c* 0.2, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.17 (s, 1H), 8.15 (s, 1H), 4.22 (d, 1H, *J* = 4.5 Hz), 4.05 (dq, 1H, *J* = 4.0, 6.5 Hz), 3.75 (dd, 2H, *J* = 2.5, 10.0 Hz), 3.69 (m, 1H), 3.61 (m, 1H), 3.51 (t, 2H, *J* = 7.0 Hz), 3.34 (m, 2H), 3.28 (m, 2H), 2.56 (dq, 1H, *J* = 6.5, 6.5 Hz), 2.10 (s, 3H), 1.92 (tt, 2H, *J* = 7.0, 7.0 Hz), 1.28 (d, 3H, *J* = 7.0 Hz), 1.27 (d, 3H, *J* = 6.5 Hz), 1.14 (d, 3H, *J* = 6.5 Hz); IR (neat) ν_{max} 3277, 3070, 2938, 1643, 1544, 1435, 1293, 1249, 1118, 1057 cm^{-1}; FABHRMS (NBA) *m/e* 573.1985 (M^+ + H, $C_{23}H_{36}N_6O_5S_3$ requires 573.1988).

3-[2′-(2‴-((*N*-(4″(*R*)-((*N*$^{\alpha}$-((*tert*-Butyloxy)carbonyl)-*N*^{im}-(triphenylmethyl)-*erythro*-β-hydroxy-L-histidyl)amino)-3″(*S*)-hydroxy-2″(*S*)-methylpentanoyl)-L-threonyl)amino)ethyl)-2,4′-bithiazole-4-carboxamido]propyl Methyl Sulfide (48). A solution of **46** (0.0031 mmol, 1.6 mg), HOAt (1.1 equiv, 0.0034 mmol, 0.47 mg), $NaHCO_3$ (1.5 equiv, 0.0047 mmol, 0.39 mg), and **47** (1.5 equiv, 0.0047 mmol, 2.7 mg) in DMF (45 μL) at 0 °C was treated with DCC (1.1 equiv, 0.0034 mmol, 0.71 mg) under Ar, and the reaction mixture was stirred at 0 °C (1.5 h). The reaction mixture was warmed to 23 °C and allowed to stir for 56 h. The DMF was evaporated in vacuo, and the residue was purified by chromatography (SiO_2, 0.5 × 3.0 cm, 0–20% CH_3OH–CH_2Cl_2 gradient elution) to afford **48** (1.8 mg, 3.2 mg theoretical, 56%): R_f 0.35 (SiO_2, 10% CH_3OH–CH_2Cl_2); $[\alpha]^{23}_D$ +11 (*c* 0.06, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.13 (s, 2H), 7.41 (s, 1H), 7.35 (m, 9H), 7.11 (m, 6H), 6.91 (s, 1H), 4.59 (m, 1H), 4.33 (d, 1H, *J* = 4.0 Hz), 4.10 (dq, 1H, *J* = 4.5, 7.0 Hz), 3.93 (dd, 1H, *J* = 5.0, 5.0 Hz), 3.66 (t, 2H, *J* = 6.5 Hz), 3.65 (m, 1H), 3.49 (t, 2H, *J* = 7.0 Hz), 3.33 (m, 2H), 3.27 (m, 2H), 2.57 (m, 1H), 2.08 (s, 3H), 1.90 (tt, 2H, *J* = 7.0, 7.0 Hz), 1.39 (s, 9H), 1.12 (d, 3H, *J* = 6.0 Hz), 1.11 (d, 3H, *J* = 6.0 Hz), 1.06 (d, 3H, *J* = 7.0 Hz); IR (neat) ν_{max} 3333, 2923, 1656, 1641, 1544, 1492, 1441, 1390, 1159, 1128 cm^{-1}; FABHRMS (NBA–NaI) *m/e* 1068.4140 (M^+ + H, $C_{53}H_{65}N_9O_9S_3$ requires 1068.4146).

3-[2′-(2‴-((*N*-(4″(*R*)-((*N*$^{\alpha}$-((*tert*-Butyloxy)carbonyl)-*erythro*-β-hydroxy-L-histidyl)amino)-3″(*S*)-hydroxy-2″(*S*)-methylpentanoyl)-L-threonyl)amino)ethyl)-2,4′-bithiazole-4-carboxamido]propyl Methyl Sulfide (49). The solid **48** (0.0004 mmol, 0.4 mg) was treated with a 0.5 M solution of HOBt in CF_3CH_2OH (*ca.* 11 equiv of HOBt, 10 μL, pH 5) at 23 °C under Ar, and the solution was stirred for 2.5 h. The solvent was evaporated under a N_2 stream and in vacuo. Chromatography (SiO_2, 0.5 × 1.0 cm, 0–30% CH_3OH–CH_2Cl_2 gradient elution) afforded **49** (0.25 mg, 0.31 mg theoretical, 81%): R_f 0.15 (SiO_2, 10% CH_3OH–CH_2Cl_2); $[\alpha]^{23}_D$ +29 (*c* 0.01, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.18 (s, 1H), 8.15 (s, 1H), 7.69 (s, 1H), 7.04 (s, 1H), 4.31 (d, 1H, *J* = 4.0 Hz), 4.13 (dq, 1H, *J* = 4.0, 6.5 Hz), 3.92 (dd, 1H, *J* = 5.0, 5.0 Hz), 3.69 (m, 3H), 3.52 (t, 2H, *J* = 7.0 Hz), 3.32 (m, 2H), 3.31 (m, 2H), 2.46 (m, 1H), 2.10 (s, 3H), 1.95 (tt, 2H, *J* = 7.0, 7.0 Hz), 1.41 (s, 9H), 1.17 (d, 3H, *J* = 6.5 Hz), 1.14 (d, 3H, *J* = 6.5 Hz), 1.09 (d, 3H, *J* = 6.5 Hz); IR (neat) ν_{max} 3385, 1682, 1641, 1390, 1354, 1148, 1097 cm^{-1}; FABHRMS (NBA) *m/e* 826.3060 (M^+ + H, $C_{34}H_{51}N_9O_9S_3$ requires 826.3050).

[3-[2'-(2'''-((*N*-(4''(*R*)-((N^{in}-((*tert*-Butyloxy)carbonyl)-*erythro*-β-hydroxy-L-histidyl)amino)-3''(*S*)-hydroxy-2''(*S*)-methylpentanoyl)-L-threonyl)amino)ethyl)-2,4'-bithiazole-4-carboxamido]propyl]dimethylsulfonium Iodide (*N*-BOC-Pentapeptide S, 50). A solution of **49** (0.0005 mmol, 0.4 mg) in dry CH_3OH (20 μL) was treated with CH_3I (200 equiv, 6 μL), and the reaction mixture was stirred at 23 °C (66 h). The solvent was evaporated, and the residue was triturated with neutralized $CHCl_3$ (3 × 0.2 mL) to give **50** (400 μg, 407 μg theoretical, 98%): R_f 0.05 (SiO_2, 20% CH_3OH–CH_2Cl_2); $[\alpha]^{23}_D$ +40 (*c* 0.02, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.85 (s, 1H), 8.21 (s, 1H), 8.15 (s, 1H), 7.50 (s, 1H), 4.31 (d, 1H, *J* = 4.0 Hz), 4.15 (dq, 1H, *J* = 4.0, 6.5 Hz), 4.01 (m, 1H), 3.70 (m, 3H), 3.61 (t, 2H, *J* = 7.0 Hz), 3.48 (m, 2H), 3.30 (m, 2H), 2.98 (s, 6H), 2.58 (m, 1H), 2.18 (tt, 2H, *J* = 7.0, 7.0 Hz), 1.39 (s, 9H), 1.19 (d, 3H, *J* = 6.5 Hz), 1.16 (d, 3H, *J* = 6.5 Hz), 1.12 (d, 3H, *J* = 6.5 Hz); IR (neat) ν_{max} 3385, 2923, 1651, 1636, 1544, 1415, 1364, 1246, 1159, 1062 cm^{-1}; UV (H_2O) λ_{max} 291 (ε 4300), 225 (ε 28 000) nm; FABHRMS (NBA) *m/e* 840.3210 (M^+, $C_{35}H_{54}N_9O_9S_3$ requires 840.3207).

N^{in}-((Benzyloxy)carbonyl)-N^{im}-(triphenylmethyl)-*erythro*-β-hydroxy-L-histidine Methyl Ester (51). $NaHCO_3$ (1.1 equiv, 0.013 mmol, 1.0 mg) and $ClCO_2CH_2Ph$ (1.0 equiv, 0.012 mmol, 2.4 mg) were successively added to a solution of **2** (0.012 mmol, 5.0 mg) in THF–H_2O (3:1, 0.2 mL) at 0 °C under Ar, and the mixture was stirred for 20 min at 0 °C. The reaction mixture was poured into a two-layer solution of EtOAc and saturated aqueous $NaHCO_3$ with vigorous stirring. The organic layer was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 0.5 × 2 cm, 30% EtOAc–hexane) gave **51** (5.4 mg, 6.5 mg theoretical, 83%) as a white film: R_f 0.34 (50% EtOAc–hexane); $[\alpha]^{25}_D$ +13.4 (*c* 0.03, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.38 (br s, 1H), 7.35–7.00 (m, 20H), 6.69 (br s, 1H), 6.31 (d, 1H, *J* = 8.8 Hz), 5.11 (s, 2H), 5.09 (m, 1H), 4.83 (dd, 1H, *J* = 4.4, 8.8 Hz), 3.59 (s, 3H), 3.30 (d, 1H, *J* = 7.2 Hz); IR ($CHCl_3$) ν_{max} 3446, 1723, 1703, 1605, 1517, 1420, 1220, 1041, 923 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 694.1329 (M^+ + Cs, $C_{34}H_{31}N_3O_5$ requires 694.1318).

N^{in}-((Benzyloxy)carbonyl)-*erythro*-β-hydroxy-L-histidine Methyl Ester (52). The solid **51** (0.006 mmol, 3.2 mg) was treated with 20% TFA–CH_2Cl_2 (1 mL), and the mixture was stirred at 0 °C (1.5 h) under Ar. The solvent was evaporated in vacuo to give an oily solid. Chromatography (SiO_2, 0.5 × 1.5 cm, 10% CH_3OH–CH_2Cl_2) afforded **52** (1.6 mg, 1.8 mg theoretical, 88%) as a film: R_f 0.45 (20% CH_3OH–CH_2Cl_2); $[\alpha]^{25}_D$ +1.5 (*c* 0.18, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.85 (br s, 1H), 7.30–7.10 (m, 5H), 7.08 (br s, 1H), 5.04 (br s, 2H), 5.02 (d, 1H, *J* = 7.2 Hz), 4.61 (d, 1H, *J* = 7.2 Hz), 3.69 (s, 3H); IR (neat) ν_{max} 3446, 1728, 1712, 1600, 1490, 1382, 1129 cm^{-1}; FABHRMS (NBA) *m/e* 320.1250 (M^+ + H, $C_{15}H_{17}N_3O_5$ requires 320.1246).

N^{in}-((Benzyloxy)carbonyl)pentapeptide S (54). A solution of **52** (0.015 mmol, 4.7 mg) in THF–CH_3OH–H_2O (3:1:1, 0.3 mL) at 0 °C was treated with aqueous 1 N LiOH (1.5 equiv, 0.022 mmol, 22 μL), and the mixture was stirred for 1.5 h. After evaporation of most of the THF–CH_3OH, the aqueous phase was acidified to pH 4–5 with the addition of aqueous 1.2 N HCl, and the solvent was evaporated in vacuo to afford crude **53**, which was used without further purification: $[\alpha]^{25}_D$ + 7.3 (*c* 0.05, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.19 (br s, 1H), 7.40–7.20 (m, 5H), 7.14 (br s, 1H), 5.05 (d, 1H, *J* = 7.0 Hz), 5.02 (br s, 2H), 4.35 (d, 1H, *J* = 7.0 Hz); IR (neat) 3428, 1718, 1518, 1230, 1041, 920 cm^{-1}.

A solution of **53** (0.002 mmol, 0.6 mg) in DMF (20 μL) was treated sequentially with DCC (2.0 equiv, 0.004 mmol, 0.8 mg), HOBt (1.0 equiv, 0.002 mmol, 0.3 mg), and **27** (1.5 equiv, 0.003 mmol, 1.8 mg) dissolved in DMF (20 μL), and the mixture was stirred under Ar at 25 °C (48 h). After removal of the solvent in vacuo, the crude residue was dissolved in CH_3OH (0.5 mL), and the insoluble inorganic salts were removed by centrifugation. The CH_3OH solution was evaporated, and the sample was triturated with $CHCl_3$ (3 × 0.3 mL). Chromatography (reverse-phase C-18, 10–90% CH_3OH–H_2O gradient elution) afforded **54** (0.7 mg, 1.7 mg theoretical, 42%) as a thin film: R_f 0.5 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{25}_D$ +20 (*c* 0.05, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.55 (br s, 1H), 8.19 (s, 1H), 8.10 (s, 1H), 7.28 (m, 5H), 7.08 (br s, 1H), 4.91 (m, 2H), 4.30 (d, 1H, *J* = 4.0 Hz), 4.11 (m, 1H), 3.60–3.50 (m, 5H), 3.40–3.10 (m, 5H), 3.56 (m, 1H), 2.05 (m, 2H), 1.18 (d, 1H, *J* = 6.8 Hz), 1.13 (d, 1H, *J* = 5.6 Hz), 1.11 (d, 1H, *J* = 6.8 Hz); IR (neat) 3441, 1723, 1600, 1541, 1251, 1093 cm^{-1}; FABHRMS (NBA) *m/e* 874.3072 (M^+, $C_{38}H_{52}N_9O_9S_3$ requires 874.3050).

General Procedure for Measurement of Apparent DNA Binding Constants (K_B). Calf thymus DNA was purchased from the Sigma Chemical Co. as the type 1 highly polymerized sodium salt. A solution of 0.524 mM calf thymus DNA was prepared (sonication for 2–4 h to effect dissolution) in a buffer composed of 2.5 mM Tris-HCl and 1.25 mM NaCl, pH 8.4, as stock solution, which was diluted to 0.165 mM (base molarity) upon preparation of the solutions for fluorescence measurement. Two solutions (2 mL) were prepared, one containing serially diluted concentrations of agent (1–64 μM), 0.165 mM calf thymus DNA, 1.25 mM NaCl, and 2.5 mM Tris-HCl buffer (pH 8.4) and the other containing the identical concentrations of agent, NaCl, and Tris-HCl buffer but lacking DNA. The fluorescence intensities were measured on a JASCO FP-777 fluorescence spectrometer in a 1-cm cuvette at 25 °C, 355 nm (5-nm slit width), following excitation of 300 nm (5-nm slit width). Regression analysis of the slopes of double-reciprocal plots of free and bound agents was utilized to determine the apparent equilibrium binding constants (K_B), and the stoichiometry of binding was obtained from the *y*-intercept, as described in the discussion.

The extent of fluorescence quenching of each agent upon DNA binding and the determination of F_b was conducted in a similar fashion. A solution of 2.89 mM calf thymus DNA was prepared (sonication for 4 h to effect dissolution) in a buffer composed of 2.5 mM Tris-HCl and 1.25 mM NaCl, pH 8.4. Two solutions (2 mL) were prepared, one containing 3 μM agent, 2.89 mM calf thymus DNA, 1.25 mM NaCl, and 2.5 mM Tris-HCl buffer (pH 8.4) and the other containing the identical concentrations of agent and Tris-HCl buffer but lacking DNA. The fluorescence intensities were measured at 355 nm (5-nm slit width) following excitation of 300 nm (5-nm slit width). The ratio of fluorescence intensity with (*ca.* 1:1000 agent–base pair ratio) and without DNA represented the fluorescence quenching of DNA bound agent (Table 1).

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Supplementary Material Available: Full experimental details and characterization for **28a,b, 29a,b, 32, 33, 35–38, 40–43b–j, 44h, 55,** and **56** (24 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.