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Synthesis of Histidinoalanine: A Comparison of β-Lactone and Sulfamidate Electrophiles

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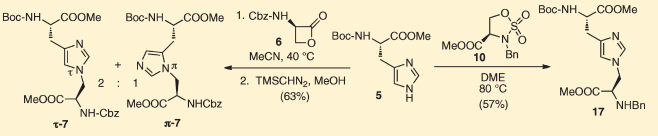
Supporting Information

ABSTRACT: Previous syntheses of histidinoalanine (HAL) have led to mixtures of regioisomers and/or stereoisomers. For example, opening of *N*-Cbz-D-serine-β-lactone (**6**) with Boc-L-His-OMe (**5**) gave a 2:1 mixture of τ- and π-regioisomers. The sulfamidate **10**, derived from *N*-benzyl-D-serine methyl ester (**11**), was reacted with Boc-L-His-OMe (**5**) to give the τ-HAL derivative **17** as a single isomer in 57% yield. A similarly prepared τ-HAL **19**, bearing protecting groups that were all hydrogenolytically labile, led to the free bis-amino acid, τ-L-histidinyl-D-alanine (***τ*-4**), as a salt-free standard for amino acid analysis.



INTRODUCTION

The histidinoalanine (HAL, Figure 1) protein cross-link[1] was first isolated from a protein in 1982;[2] both regioisomers have since been identified from a number of sources. Histidinoalanines are produced in milk products that have been heated and/or treated with alkali;[3] the nutritional consequences are unknown. These bis-amino acids occur naturally in human tissues (connective tissue,[2] dentin,[4] and eye cataracts[5]) where levels appear to correlate with age and disease state. Moreover, HALs have been identified in phosphoproteins of bivalve mollusks[6] where they are integral to the process of mineralization.

Histidinoalanines presumably arise by analogy to the formation of lanthionine and lysinoalanine[7] (Scheme 1) in which thiol and ε-amino nucleophiles respectively add to dehydroalanine. The theonellamides[8] (Figure 1c) are the only example of a HAL residue in a well-defined, small molecule. As such, these antifungal agents are the imidazole analogues of the much-studied lantibiotics, of which the best known is nisin.[9]

Previous syntheses of HAL, typified by that of Fujimoto (Scheme 2),[2] involved a biomimetic, conjugate addition of a histidine to a dehydroalanine.[10] This approach necessarily leads to mixtures of regioisomers and stereoisomers. Tohdo et al. addressed the stereochemical issue by employing an enantiopure serine β-lactone as a synthon for the β-alanine cation.[11] Our pursuit of τ-histidinoalanine began with a reinvestigation of this approach.

RESULTS AND DISCUSSION

The histidine building block **5** was prepared according to Abdo et al.[12] D-Lactone (*R*)-**6** was prepared according to Vederas and co-workers;[13] the procedure involves an intramolecular Mitsunobu reaction of Cbz-Ser-OH and ideally employs DMAD, to give **6** in 47% yield. Unfortunately, DMAD is no longer readily available, and substitution with DIAD led to a product mixture from which it was difficult to chromatographically separate the

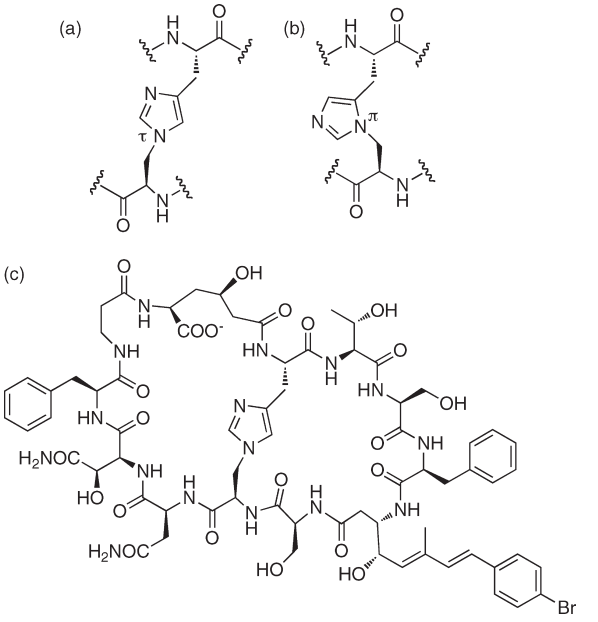


Figure 1. (a) τ-HAL (b) π-HAL, and (c) theonellamide C (**1**).

β-lactone from the ^{i}PrOOCNHNHCOO^{i}Pr. Prolonged exposure to silica, necessary to achieve separation, led to decomposition of the β-lactone and isolated yields of only 15−20%. Sufficient quantities of the β-lactone were nevertheless prepared. Gentle heating of lactone **6** with a 5-fold excess of Boc-L-His-OMe (**5**) and trapping of the intermediate carboxylic acid with trimethylsilyldiazomethane led to a mixture of the two regioisomers of **7** (Scheme 3). The ratio (~2:1 in favor of the τ-isomer) was similar to that reported by Tohdo et al.[14] for the corresponding *tert*-butyl esters (**8**).

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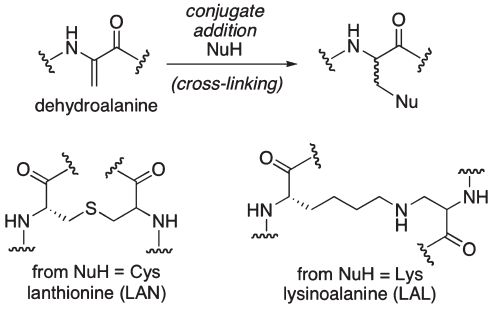
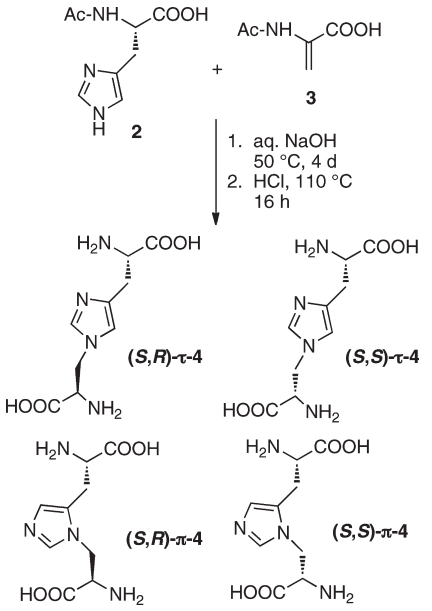
Scheme 1. Cross-Linking Amino Acids Derived from Dehydroalanine



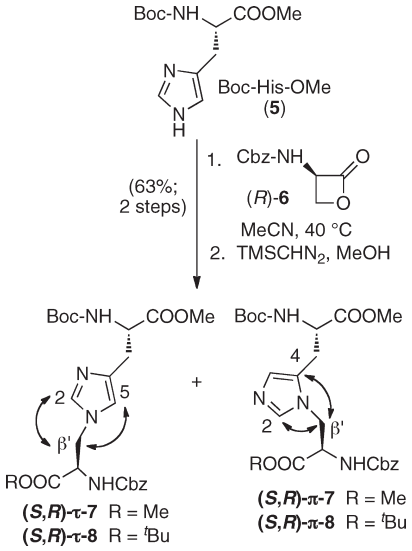
Scheme 2. Conjugate Addition to a Dehydroalanine



We expected that the τ-regioisomer would be the major product of the reaction. Nevertheless, it was important to distinguish the two compounds unambiguously by NMR spectroscopy. Proton NMR spectra were assigned on the basis of COSY correlations and then the ^{13}C NMR on the basis of HMQC and DEPT spectra. Detailed assignments are given in the Experimental Section, and spectra are included in the Supporting Information. The two regioisomers were distinguished on the basis of long-range correlations in their HMBC spectra (Scheme 3). In the case of the τ-regioisomer (**τ-7**), Hβ′ (δ 4.33) showed correlations to both C2 (δ 137.5) and C5 (δ 116.9) of the imidazole ring but not to C4 (δ 138.1). For the π-isomer (**π-7**), Hβ′ (δ 4.33) showed correlations to both C2 (δ 138.0) and C4 (δ 126.5) of the imidazole ring, but not to C5 (δ 128−129). Sass and Marsh reported ^{13}C chemical shifts for both regioisomers of **4**,[6] isolated from bivalve molluscs. Boschin et al. prepared and separated the two regioisomers of **4** (as mixtures of diastereomers) and reported ^{13}C NMR data in D_2O.[10b] While our compounds **τ-7** and **π-7** are fully protected, and our spectra acquired in different solvents, our assignments are consistent with the published data for the free amino acids.

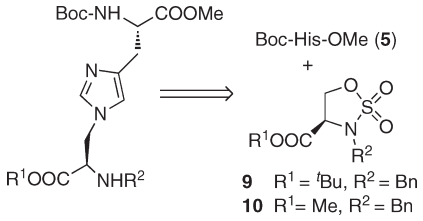
While we had successfully prepared these HAL derivatives, this route was plagued by the difficulty in preparing **6** and the

Scheme 3. Tohdo's Approach to HAL[a]



[a] Key HMBC correlations for compounds **τ-7** and **π-7** are depicted by double-headed arrows.

Scheme 4. Second Generation Retrosynthetic Analysis



separation of the two regioisomers of **7**. Flash chromatography on silica gel gave some pure **τ-7** but mostly mixed fractions. We considered other alanine β-cation synthons, and it seemed that sulfamidates,[15] as introduced by Baldwin and co-workers,[16] might provide a viable solution (Scheme 4).[17] Baldwin's original report looked at the opening of **9** with a variety of heteroatom nucleophiles. Indeed, there have been two reports of the regioselective opening of sulfamidates with unsubstituted imidazole.[18,19] Lubell and co-workers have used *N*-9-phenylfluorenyl[19] and *N*-fluorenylmethoxycarbonyl (Fmoc) sulfamidates.[20] The groups of Vederas[21] and Halcomb[22] focused on the use of *p*-methoxybenzyl (PMB) protected sulfamidates to produce lanthionine and thioglycoside derivatives, respectively. Our early investigations indicated that the *N*-benzyl derivatives were more robust than the analogous *N*-PMB compounds.

Two sulfamidates were prepared, according to Scheme 5. Attempts to convert **13** to sulfamidate **10** directly, with sulfuryl chloride, were unsuccessful, presumably due to aziridine formation.[23]

Our first attempt at opening sulfamidate **10** followed the conditions of Wei and Lubell,[19] employing Boc-His-OMe (**5**) as the nucleophile (Scheme 6), in order to make a meaningful comparison with the β-lactone route. After 4 h, TLC analysis showed that all the sulfamidate **10** had been consumed, and we isolated τ-HAL **17**. Presumably, sulfamidate **10** is less reactive than lactone **6** and thus more regioselective for Nτ. The yield of

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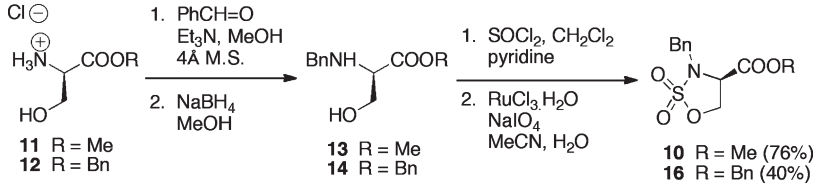
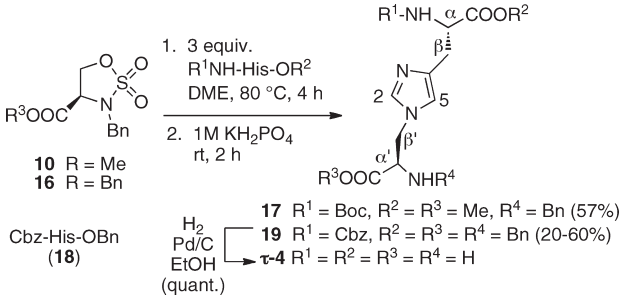
Scheme 5. Synthesis of Sulfimidates 10 and 16



Scheme 6. Formation of τ-HAL



this reaction depended heavily on the purity of the sulfamidate reaction partner; once recrystallized according to Pilkington and Wallis,[24] this gave reproducible results. Alternative conditions for hydrolysis of the intermediate aminosulfamic acid, using propanethiol and a Lewis acid, according to Kim and So,[18] led to more complex product mixtures and lower yields of **17**.

While the regioselective formation of τ-HAL **17** was a pleasing result, this compound has limited utility. The removal of all protecting groups, to produce an amino acid standard for the identification of τ-HAL in peptide/protein hydrolysates, would require several steps. In this regard, we prepared sulfamidate **16** (Scheme 5) and reacted it with commercially available Cbz-L-His-OBn (**18**) to give **19**, again as a single compound (Scheme 6). The yields of this reaction ranged from 20 to 57%; the significant fluctuations are attributed to an inability to purify sulfamidate **16** by recrystallization. Hydrogenolytic cleavage of all protecting groups gave salt-free τ-HAL (**4**). Matsunaga et al. reported NOEs between H5 of the imidazole and Cα and Cβ of both residues, but H2 of the imidazole showed NOEs only to Hα′ and Hβ′ of the "Ala unit."[8a] We observed analogous cross-peaks in the NOESY spectrum, affording evidence for the τ-regioisomer.

CONCLUSIONS

In summary, we report experimental details and characterization data for the reaction of Boc-His-OMe (**5**) with a serine β-lactone (**6**) to produce two regioisomers of HAL, as communicated previously by Tohdo et al.[11] We produced a single regioisomer, τ-HAL, by invoking a cyclic sulfamidate as the electrophile. Moreover, we have produced the free bis-amino acid **4** that can be used as an authentic standard to identify this cross-link in other peptides and proteins. To maximize the potential of a τ-HAL building block for peptide synthesis, efforts are ongoing in our laboratory to produce an orthogonally protected τ-HAL for complex molecule synthesis, including the theonellamides.

EXPERIMENTAL SECTION

General Methods. Triethylamine and pyridine were dried and distilled from CaH_2 and stored over KOH pellets. Methanol was distilled from Mg turnings and stored over 4 Å molecular sieves. Acetonitrile, tetrahydrofuran, and dichloromethane were dried using a solvent purification system. The compounds were visualized by UV fluorescence or by staining with PMA, ninhydrin, or $KMnO_4$ stains. Proton NMR data is reported in ppm downfield from TMS or disodium 3-trimethylsilyl-1-propanesulfonate (DSS) as internal standards. High resolution mass spectra were recorded using either time-of-flight or electrospray ionization.

***Nα-tert*-Butoxycarbonyl-L-histidine Methyl Ester (5).**[12] Triethylamine (11.48 mL, 8.28 g, 82.6 mmol, 2.0 equiv) and $(Boc)_2O$ (21.74 g, 94.98 mmol, 2.3 equiv) were added to a suspension of HCl·L-His·OMe (10.0 g, 41.3 mmol, 1.0 equiv) in methanol (150 mL). The reaction mixture was stirred for 2 h at rt and monitored by TLC until no starting material was detected. The reaction mixture was concentrated and then partitioned between ethyl acetate (500 mL) and water (500 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was suspended in methanol (100 mL), and K_2CO_3 (571 mg, 4.13 mmol, 0.1 equiv) was added. The mixture was heated at 67 °C for 4 h. When all of the starting material was consumed, the mixture was concentrated and partitioned between ethyl acetate (500 mL) and water (500 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography on silica gel, eluting with 2:1 EtOAc−heaxanes→100% EtOAc→95:5 EtOAc−MeOH, affording **5** as a colorless solid. (6.766 g, 61%); R_f 0.27 (9:1 CH_2Cl_2−MeOH); $[\alpha]_D^{25}$ −9.70 (*c* 1.00, $CHCl_3$). ^1H NMR (CD_3OD, 400 MHz) δ 1.40 (s, 9H), 2.94 (dd, *J* = 14.7, 8.5 Hz, 1H) 3.06 (dd, *J* = 14.7, 5.4 Hz, 1H), 3.67 (s, 3H), 4.38 (dd, *J* = 8.5, 5.4 Hz, 1H), 6.85 (s, 1H), 7.5 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 26.7, 28.3, 50.6, 53.3, 54.5, 78.6, 134.3, 155.8,172.0; HRMS (+TOF) calcd for $C_{12}H_{20}N_3O_4$ $(M + H)^+$ 270.1448, obsd 270.1450.

***N*-(Benzyloxycarbonyl)-D-serine-β-lactone (6).**[13]. Diisopropyl azodicarboxylate (1.87 mL, 1.80 mg, 8.36 mmol, 1.0 equiv) was added dropwise, over 10 min, to a solution of triphenylphosphine (2.19 g, 8.36 mmol, 1.0 equiv) in THF (60 mL) at −78 °C. A solution of Cbz-D-Ser-OH (2.0 g, 8.36 mmol, 1.0 equiv) in THF (13 mL) was added dropwise to the mixture over 30 min. The mixture was stirred at −78 °C for 20 min, the cooling bath removed, and the mixture slowly warmed, with stirring, to rt over 2.5 h. The mixture was concentrated, and the residual pale yellow syrup was purified by flash chromatography on silica gel, eluting with 4:1 hexanes−EtOAc, to give (*R*)-**6** as a colorless solid (300 mg, 17%). R_f 0.41 (1:1 Hex−EtOAc); mp 125−128 °C, lit.[13b] mp 133−134 °C; $[\alpha]_D^{29}$ +23.6 (*c* 1.00, CH_3CN), lit.[13b] $[\alpha]_D^{22}$ +26.5 (*c* 1, MeCN), lit.[13d] $[\alpha]_D^{26}$ +34.2 (c 0.5, MeCN). ^1H NMR (acetone-d_6, 400 MHz) δ 4.42−4.48 (m, 2H), 5.11 (br s, 2H), 5.28 (dd, *J* = 6.7, 5.0 Hz, 1H), 5.30 (dd, *J* = 6.7, 5.0 Hz, 1H), 7.30−7.38 (m, 5H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 60.4, 66.4, 67.4, 128.5, 128.7, 128.8, 129.2, 137.3, 156.3, 170.3; HRMS (+TOF) calcd for $C_{11}H_{12}NO_4$ (MH^+) 222.0766, obsd 222.0764.

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Dimethyl *N*α-*tert*-Butoxycarbonyl-*N*α′-carbobenzyloxy-τ-L-histidino-D-alaninate (*S,R*)-τ-7 and (*S,R*)-π-7. Boc-His-OMe (**5**) (3.0 g, 11 mmol, 5 equiv) was added to a solution of *N*-Cbz-D-serine-β-lactone (**6**) (490 mg, 2.26 mmol, 1 equiv) in MeCN (30 mL). The mixture was stirred at 40 °C for 4 h, cooled, and concentrated. Analysis of the crude product mixture by ^{1}H NMR, and integration of the imidazole H5 signals in each isomer, estimates the τ:π ratio at 3.7:1.0 The residue was subjected to flash chromatography on silica gel, eluting with 2% MeOH in CH_2Cl_2 to afford some pure (*S,R*)-τ-7 (83 mg), many fractions that contained both isomers (150 mg), and a trace of (*S,R*)-π-7 (6 mg) for a total yield of 213 mg (63%).

(*S,R*)-τ-7 R_f 0.52 (9:1 CH_2Cl_2−MeOH); $[\alpha]_D^{29}$ −23.3 (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (s, 9H, Boc ^{t}Bu), 2.95 (dd, *J* = 14.5, 4.5 Hz, 1H, Hβa His), 3.04 (dd, *J* = 14.5, 4.5 Hz, 1H, Hβb His), 3.68 (s, 3H, $COOCH_3$ His), 3.78 (s, 3H, $COOCH_3$ Ala), 4.33 (br s, 2H, Hβ′ Ala), 4.52−4.54 (m, 1H, Hα His), 4.60 (m, 1H, Hα′ Ala), 5.14 (s, 2H, CH_2Ph), 5.59 (d, *J* = 6.0 Hz, 1H, NH His), 5.88 (d, *J* = 8.1 Hz, 1H, NH Ala) 6.56 (s, 1H, H5 His), 7.23 (s, 1H, H2 His), 7.33−7.40 (m, 5H, Cbz Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.3 (Boc ^{t}Bu), 30.2 (Cβ His), 47.9 (Cβ′ Ala), 52.1 ($COOCH_3$ Ala), 53.1 ($COOCH_3$ His), 53.5 (Cα His), 54.9 (Cα′ Ala), 67.4 (Cbz CH_2), 79.6 (Boc CMe_3), 116.9 (C5, His), 128.2 (Cbz *C*H), 128.4 (Cbz *C*H), 128.6 (Cbz *C*H), 135.8 (Cbz 4 °C), 137.5, (C2 His) 138.1 (C4, His), 155.6 (Boc, Cbz, 2 × C═O), 169.4 (*C*OOMe Ala), 172.5 (*C*OOMe, His); HRMS (+TOF) calcd for $C_{24}H_{33}N_4O_8$ (MH^+) 505.2292, obsd 505.2289.

(*S,R*)-π-7. R_f 0.41 (9:1 CH_2Cl_2−MeOH); $[\alpha]_D^{26}$ +5.2 (*c* 0.3, $CHCl_3$). ^{1}H NMR (CD_3OD, 400 MHz) δ 1.40 (s, 9H), 2.92−3.00 (m, 1H), 3.11−3.17 (m, 1H), 3.71 (s, 3H), 3.75 (s, 3H), 4.26 (dd, *J* = 14.2, 9.2 Hz, 1H), 4.41−4.51 (m, 2H), 4.55−4.58 (m, 1H), 5.09 (s, 2H), 6.75 (s, 1H), 7.29−7.34 (m, 5H), 7.52 (s, 1H). On the time scale of the ^{13}C NMR experiment, two species were observed in an approximately 2:1 ratio. Where distinct signals were observed for the minor species, these are indicated in parentheses. ^{13}C NMR (CD_3OD, 100 MHz) δ 25.0 (25.1), 26.7, 44.1 (44.2), 50.9, 51.2, 52.3 (52.2), 53.8 (53.7), 65.9, 78.8, 125.8, 126.8, 127.1 127.2, 127.5, 136.0, 137.4 (137.5), 155.8, 156.2, 169.3, 171.4; HRMS (+TOF) calcd for $C_{24}H_{33}N_4O_8$ (MH^+) 505.2292, obsd 505.2289.

***N*-Benzyl-D-serine Methyl Ester (13).** Dry methanol (30 mL) was added to flame-dried, powdered 4 Å molecular sieves (3 g) in a round-bottomed flask. To this suspension were added, sequentially, HCl·D-Ser-OMe (**11**) (3.0 g, 19.3 mmol, 1 equiv), triethylamine (2.69 mL, 1.95 g, 19.3 mmol, 1 equiv) and benzaldehyde (1.95 mL, 2.05 g, 19.3 mmol, 1 equiv). The mixture was stirred for 16 h at rt, filtered through Celite, washed well with methanol, and concentrated. The acid-labile imine could not be visualized by TLC, so the success of the reaction was gauged by ^{1}H NMR: aldehyde starting material δ 10.03; imine product δ 8.43. Provided there was negligible aldehyde, the material was carried directly into the reduction reaction. The oily yellow imine was redissolved in MeOH (30 mL) and treated portion-wise with $NaBH_4$ (729 mg, 19.3 mmol, 1 equiv) at 0 °C. After 4 h at 0 °C, water (10 mL) and EtOAc (10 mL) were added. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a yellow oil (3.268 g, 81%). This compound was typically utilized without further purification. However, for the purposes of characterization, flash chromatography on silica gel, eluting with 100% EtOAc, provided **13** as a pale yellow oil (897 mg, 67%); R_f 0.62 (9:1 CH_2Cl_2−MeOH); $[\alpha]_D^{29}$ +1.22 (*c* 1.00, MeOH). ^{1}H NMR (CD_3OD, 400 MHz) δ 3.38 (app. t, *J* = 5.0 Hz, 1 H), 3.68 (d, *J* = 12.8 Hz, 1H), 3.72 (s, 3H), 3.74 (d, *J* = 5.0 Hz, 1H), 3.75 (d, *J* = 5.0 Hz, 1H), 3.82 (d, *J* = 12.8 Hz, 1H), 7.22−7.35 (m, 5H); ^{13}C NMR (CD_3OD, 100 MHz) δ 50.4, 50.7, 61.4, 61.7, 126.3, 127.5, 127.6, 138.5, 172.8; HRMS (+TOF) calcd for $C_{11}H_{16}NO_3$ (MH^+) 210.1124, obsd 210.1126.

Methyl 4*R*,3-Benzyl-2-oxo-1,2,3-oxathiazolidine-4-carboxylate (10). Pyridine (6.32 mL, 6.18 g, 78 mmol, 5 equiv) was added to a solution of Bn-D-Ser-OMe (**13**) (3.268 g, 15.6 mmol, 1.0 equiv) in CH_2Cl_2 (45 mL) at −78 °C. Thionyl chloride (1.92 mL, 2.23 g, 18.7 mmol, 1.2 equiv) was added dropwise over 10 min, and the solution was stirred at −78 °C for 45 min and then allowed to warm to rt over 1 h. During this time, there was some precipitate formed. The reaction mixture was quenched by the addition of 1% aq HCl (90 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 150 mL), and the combined organic layers were washed with brine (300 mL), dried over $MgSO_4$, filtered, and concentrated. The orange residue was dissolved in MeCN (160 mL), and the solution was cooled to −5 °C. Ruthenium(III) chloride trihydrate (41% Ru, 193 mg, 0.8 mmol, 0.05 equiv) was added, followed by $NaIO_4$ (4.01 g, 18.7 mmol, 1.2 equiv) and H_2O (160 mL). The black reaction mixture was stirred for 20 min at −5 °C and a further 20 min at rt. The mixture was partitioned between CH_2Cl_2 (600 mL) and saturated $NaHCO_3$ (250 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 150 mL), and the combined blue-green-black organic layers were washed with brine (150 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography on silica gel, eluting with 2:1 hexanes−EtOAc, afforded **10** as a colorless oil (3.125 g, 74% over two steps). The material solidified on storage in the refrigerator and could be recrystallized from diethyl ether. R_f 0.44 (1:1 Hex−EtOAc); $[\alpha]_D^{29}$ +1.7 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.72 (s, 3H), 4.07 (dd, *J* = 7.4, 4.6 Hz, 1H), 4.50 (d, *J* = 2.2 Hz, 2H), 4.60 (dd, *J* = 9.0, 4.0 Hz, 1H), 4.66 (dd, *J* = 9.0, 4.6 Hz, 1H), 7.33−7.42 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 50.3, 53.1, 58.1, 67.4, 128.6, 128.8, 129.1, 133.5, 168.2; HRMS (−TOF) calcd for $C_{11}H_{12}NO_5S$ $(M - H)^+$ 270.0441, obsd 270.0439.

Dimethyl *N*α-*tert*-Butoxycarbonyl-*N*α′-benzyl-τ-L-histidino-D-alaninate (17). A solution of sulfamidate **10** (102 mg, 0.38 mmol, 1 equiv) and Boc-His-OMe (**5**) (312 mg, 1.16 mmol, 3 equiv) in anhydrous DME (10 mL) was stirred for 5 h at 80 °C. During this time, the reaction mixture became cloudy, and by the end, a yellow oil separated out from the solvent. The mixture was cooled to rt, and then a solution of 1 M KH_2PO_4 (25 mL) was added to quench the reaction (by hydrolysis of the sulfamic acid) and stirred vigorously for 2 h. More water (10 mL) was added, the mixture was extracted with EtOAc (3 × 25 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography on silica gel, eluting with 4% MeOH in CH_2Cl_2, to give **17** as a colorless solid (77 mg, 57%). R_f 0.50 (9:1 CH_2Cl_2−MeOH); $[\alpha]_D^{27}$ +56 (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (s, 9H), 2.99 (dd, *J* = 7.3, 5.0 Hz, 1H), 3.07 (dd, *J* = 7.3, 5.0 Hz, 1H), 3.49−3.53 (m, 1H), 3.66 (d, *J* = 10.9 Hz, 1 H), 3.68 (s, 3H), 3.72 (s, 3H), 3.83 (d, *J* = 13.3 Hz, 1H), 4.08 (d, *J* = 5.6 Hz, 1H), 4.09 (d, *J* = 5.6 Hz, 1H), 4.53 (d, *J* = 4.8 Hz, 1H), 5.82 (br d, *J* = 8.1 Hz, 1H), 5.89 (brd, *J* = 8.1 Hz, 1H), 6.67 (d, *J* = 6.3 Hz, 1H), 7.23−7.35 (m, 5 H), 7.39 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.3, 30.1, 49.4, 52.0, 52.1, 52.2, 52.4, 53.5, 60.9, 61.0, 79.5, 117.1, 127.4, 128.1, 128.5, 137.5, 138.9, 155.6, 172.5, 172.6; HRMS (+TOF) calcd for $C_{23}H_{33}N_4O_6$ (MH^+) 461.2394, obsd 461.2404.

Benzyl 4*R*,3-Benzyl-2-oxo-1,2,3-oxathiazolidine-4-carboxylate (16). *N-Benzyl-D-serine Benzyl Ester (**14**).* Flame-dried 4 Å molecular sieves (1.5 g) were added to a solution of HCl·D-Ser-OBn (**12**) (1.0 g, 4.37 mmol, 1.0 equiv) in anhydrous MeOH (10 mL) at rt under N_2. Triethylamine (618 μL, 450 mg, 4.37 mmol, 1.0 equiv) and benzaldehyde (436 μL, 458 mg, 4.37 mmol, 1.0 equiv) were added sequentially. The mixture was stirred for 24 h at RT, filtered through Celite, washing well with methanol, and concentrated. The acid-labile imine could not be visualized by TLC, so the success of the reaction was gauged by ^{1}H NMR: aldehyde starting material δ9.86; imine product δ8.21. Provided there was negligible aldehyde, the material was carried directly into the reduction reaction. The oily yellow product was redissolved in MeOH (10 mL) and treated portion-wise with $NaBH_4$ (0.242 mg, 6.43 mmol, 1.0 equiv) at 0 °C. After 4 h, H_2O and EtOAc (25 mL each) were added, and the organic layer was separated, washed with brine, dried over $MgSO_4$, filtered, and concentrated to give Bn-D-Ser-OBn (**14**) that was utilized without further purification.

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The residue was dissolved in CH_2Cl_2 (24 mL). Pyridine (1.75 mL, 1.71 g, 21.6 mmol, 5.0 equiv) was added and the reaction mixture cooled to −78 °C. Thionyl chloride (375 μL, 615 mg, 5.18 mmol, 1.2 equiv) was added dropwise over 30 min, and the solution was stirred at −78 °C for 45 min and then allowed to warm to rt over 1 h. The reaction mixture was quenched by the addition of 1% HCl (38 mL). The aqueous layer was extracted with CH_2Cl_2 (150 mL), and the combined organic layers were washed with saturated $NaHCO_3$ (38 mL), dried over $MgSO_4$, filtered, and concentrated to give a yellow residue.

The residue was dissolved in MeCN (75 mL) and cooled to −5 °C. Ruthenium(III) chloride hydrate (35−40% Ru, ∼45 mg, 0.21 mmol, 0.05 equiv), $NaIO_4$ (1.11 g, 5.18 mmol, 1.2 equiv), and H_2O (75 mL) were then added sequentially, and the reaction mixture was stirred for 20 min at −5 °C and a further 20 min at rt. The mixture was partitioned between CH_2Cl_2 (300 mL) and saturated $NaHCO_3$ (75 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 75 mL), and the combined organic layers were washed with brine (75 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography on silica gel, eluting with 3:1 hexanes−EtOAc, to afford **16** as a colorless solid (643 mg; 40% over three steps). R_f 0.57 (2:1 Hex−EtOAc); $[\alpha]_D^{29}$ +54.7 (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.05 (dd, *J* = 7.4, 4.5 Hz, 1H), 4.41 (d, *J* = 14.3 Hz, 1H), 4.46 (d, *J* = 14.3 Hz, 1H), 4.55 (dd, *J* = 8.9, 7.5 Hz, 1H), 4.62 (dd, *J* = 8.9, 4.5 Hz, 1H), 5.10 (d, *J* = 12.1 Hz, 1H), 5.13 (d, *J* = 12.1 Hz, 1H), 7.28−7.38 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 50.2, 58.2, 65.0, 67.3, 67.9, 126. 8, 127.4, 128.4, 128.5, 128.61, 128.68, 128.98, 133.4, 134.4, 167.5; HRMS (+TOF) calcd for $C_{17}H_{17}NO_5NaS$ $(M + Na)^+$ 370.0719, obsd 370.0725.

Dibenzyl *N*α-*tert*-Carbobenzyloxy-*N*α′-benzyl-τ-L-histidino-D-alaninate 19. Cbz-His-OBn (**18**) (2.11 g, 5.55 mmol, 3 equiv) was added to a solution of sulfamidate **16** (643 mg, 1.85 mmol, 1 equiv) in anhydrous DME (52 mL) and stirred for 5 h at 80 °C. The mixture was cooled to rt, and then a solution of 1 M KH_2PO_4 (50 mL) was added to quench the reaction (by hydrolysis of the sulfamic acid). The mixture was extracted with EtOAc (3 × 100 mL), and the combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography on silica gel, eluting with 2:1 EtOAc−hexanes, to afford **19** as a colorless solid (637 mg, 57%). R_f 0.61 (9:1 CH_2Cl_2−MeOH); $[\alpha]_D^{29}$ +10.2 (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.07 (br s, 1H), 2.95 (dd, *J* = 14.6, 3.1 Hz, 1H), 3.03 (dd, *J* = 14.6, 3.1 Hz, 1H), 3.44 (dd, *J* = 11.5, 5.6 Hz, 1H), 3.58 (d, *J* = 13.2 Hz, 1H), 3.75 (d, *J* = 13.2 Hz, 1 H), 3.88−3.99 (m, 2H), 4.61−4.64 (m, 1H), 5.02−5.16 (m, 6H), 6.26 (d, *J* = 8.9 Hz, 1 H), 6.41−6.43 (m, 1H), 7.15−7.36 (m, 21H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.6, 49.0, 51.8, 51.9, 53.9, 60.6, 60.7, 66.6(2C), 67.2, 116.9, 117.1, 127.2, 127.8, 127.9, 128.0, 128.17, 128.24, 128.26, 128.32, 128.6, 134.8, 135.6, 136.4, 136.9, 137.4, 138.7, 156.1, 171.3, 171.8; HRMS (+TOF) calcd for $C_{38}H_{39}N_4O_6$ (MH^+) 647.2864, obsd 647.2870.

τ-L-Histidino-D-alanine (4). To a solution of protected histidinoalanine **19** (247 mg, 0.38 mmol, 1 equiv) in a 1:1 mixture of EtOAc−MeOH (10 mL) was added Pd/C (50% wet with water, 10 wt %, 800 mg). The reaction vessel was evacuated, opened to an atmosphere of H_2, and stirred for 48 h. The suspension was filtered through Celite, washing well with methanol and water, concentrated to remove the organic solvents, and then freeze-dried to provide compound **τ-4** (81 mg, 88%). R_f 0.46 (3:3:3:1 ^{n}BuOH:EtOH:NH_3:H_2O); $[\alpha]_D^{29}$ +12.6 (*c* 1.00, 1.0 N HCl), lit.[8a] for a 1.2:1.0 mixture of diastereomers: $[\alpha]_D^{23}$ +9.8 (*c* 0.07, 1.0 N HCl). ^{1}H NMR (D_2O, 400 MHz) δ 3.16−3.29 (m, 2H, His Hβ), 3.97 (app. t, *J* = 5.5 Hz, 1H, His Hα), 4.19 (app. t, *J* = 5.0 Hz, 1H, Ala Hα′), 4.65 (d, *J* = 5.0, 2H, Ala Hβ′), 7.33 (s, 1H, H5), 8.44 (s, 1H, H2); ^{13}C NMR (D_2O, 100 MHz) δ 29.3 (Cβ His), 50.9 (Cβ Ala), 56.4 (Cα His), 56.9 (Cα′ Ala), 122.8 (C5 His), 133.8 (C4 His), 139.6 (C2 His), 173.1, 175.5; HRMS (+TOF) calcd for $C_9H_{15}N_4O_4$ (MH^+) 243.1091, obsd 243.1087.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra for all compounds, along with ^{1}H−^{1}H COSY, HMQC, and HMBC for **τ-7** and **π-7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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