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Synthesis of Cyclic Oligomers from Histidine-Derived Building Blocks Using Dynamic Combinatorial Chemistry

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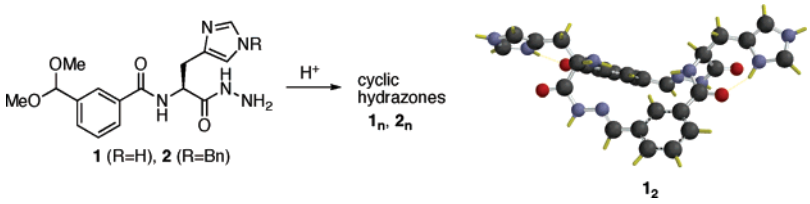

Histidine-derived hydrazide acetal monomers (3-dimethoxymethylbenzoyl)-L-histidine methyl ester **1** and (3-dimethoxymethylbenzoyl)-τ-benzyl-L-histidine methyl ester **2** were prepared from a histidine methyl ester and a τ-benzyl-histidine methyl ester by N-acylation with 3-(dimethoxymethyl)benzoic acid (**3**) followed by hydrazinolysis. Acid-promoted hydrolysis of each acetal hydrazide initially produced a library of cyclic oligomers that eventually converted to a cyclic dimer. The cyclic dimers $\mathbf{1}_2$ and $\mathbf{2}_2$ were spectroscopically characterized and found to direct their imidazole-bearing sidechains outward (exo). No evidence for templating the cyclic oligomers was observed using various metal ions and anionic substrates. The average of pK_{a_1} and pK_{a_2} of dimer $\mathbf{1}_2$ was determined by potentiometric titration to be 6.6. Dimer $\mathbf{1}_2$ was found to catalyze the hydrolysis of *p*-nitrophenylacetate 10 times faster than 4-methyl imidazole.

Introduction

Dynamic combinatorial chemistry, the synthesis of complex molecules from simple building blocks via reversible linkages, can give access to structures that would be difficult or impossible to synthesize by traditional means. In a dynamic combinatorial library (DCL), a species that is otherwise disfavored can be amplified in the presence of a template with which it forms a supramolecular complex.[1−5] This change in equilibrium upon addition of a template has been used by Lehn et al., Sanders et al., Otto and Kubik, and others to produce many complex molecules.[6−9] Especially interesting is the generation of catalytic species using transition-state analogues as templates.[10,11]

Toward the generation of DCLs that produce catalytic species, it would be highly desirable to add to the selection of available building blocks so that these artificial systems might reflect the functional group diversity of the proteinogenic amino acids. Our interest in a histidine-based building block **1** is due to the importance of histidine in enzyme active sites as an acid/base residue[12] and as a ligand for transition metals in metalloenzymes.[13−15] There are a number of examples of dynamic combinatorial chemistry that exploit relatively strong transition metal−ligand interactions to achieve templating.[6,16−24] Our long-

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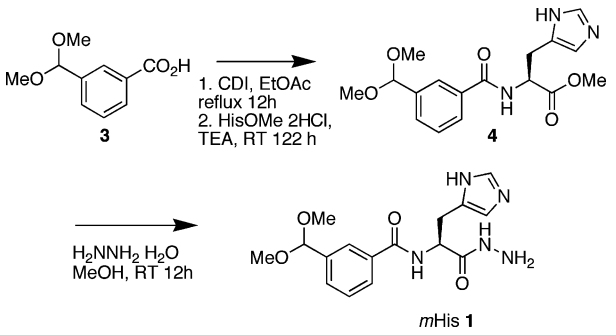

FIGURE 1. Synthesis of *mHis* (**1**).

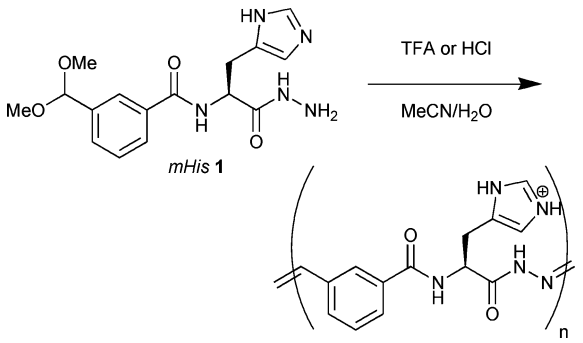

FIGURE 2. Oligomerization of *mHis* (**1**).

term goal is to use histidine-based building blocks to produce metalloenzyme active-site mimics through templating on transition-metal−TSA complexes. Acid-catalyzed hydrazone exchange has been demonstrated to be a versatile reaction for DCL, and the suitability of bifunctional hydrazide/aldehyde building blocks synthesized from amino acids is well-established.[7,18,23−28] Our work incorporates the histidine residue into a DCL building block, providing entry to dynamic libraries of oligo-histidine macrocycles of possible use as imidazole-based catalysts. To our knowledge, this is the first DCL to incorporate histidine or any other imidazole-bearing building block.

Results and Discussion

The histidine-derived pseudo-peptide hydrazide building block *mHis* **1** was synthesized by a modification of literature methods starting from 3-(dimethoxymethyl)-benzoic acid **3** (Figure 1).[26] The acid was coupled to L-histidine methyl ester dihydrochloride using carbonyldiimidazole as a coupling reagent.[29] The crude product amide ester **4** was subjected to room temperature hydrazinolysis to yield the *mHis* monomer **1** that was characterized spectroscopically.

Treatment of monomer **1** (2.5 mM in 3:1 acetonitrile/water, room temperature, 8 h) with an excess of TFA or HCl initially produced a mixture of cyclic oligomers, primarily dimer, trimer, and tetramer, as indicated by HPLC and ESI-MS (Figures 2 and 3). Longer reaction times led to nearly complete conversion of the oligomeric mixture to the cyclic dimer. When a large excess of acid was used, equilibrium was reached in 15 h; with a slight excess of acid, equilibrium was attained in 14 days.

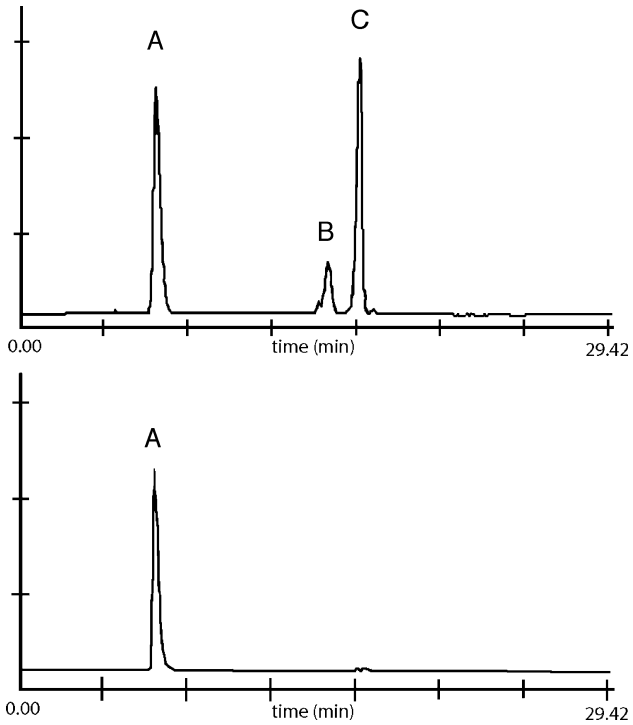

FIGURE 3. HPLC trace of reaction of **1** with 2 equiv of HCl at 8 h (top) and 14 days (bottom). (A) Cyclic dimer; (B) cyclic tetramer; and (C) cyclic trimer. All peaks were identified by ESI-MS.

A time-dependent ^{1}H NMR experiment in which *mHis* (**1**) was treated with excess d-TFA in CD_3CN/D_2O confirmed that the initially formed aldehyde was consumed rapidly, leading to the formation of a mixture of oligomers, with dimers and trimers predominating (Figure 4). The multiplet at 5.7 ppm (labeled B in Figure 4) is assigned to the cyclic dimer α-CH based on NMR characterization of the isolated compound (vide infra); it is observed to steadily increase in magnitude over time. The multiplet at 5.1 ppm (labeled A in Figure 4) is assigned to the cyclic trimer α-CH. It is seen emerging initially before eventually disappearing and is the only discernible α-CH-type signal other than peak B. The presence of a cyclic trimer at this stage is also apparent in the ESI-MS. These oligomeric species were slowly consumed over 15 h to generate almost exclusively cyclic dimers.

The crude cyclic dimer hydrochloride salt $\mathbf{1}_2$ was recovered from an equilibrium mixture by removal of solvent in vacuo and was analyzed by ESI-MS and NMR. NOEs observed

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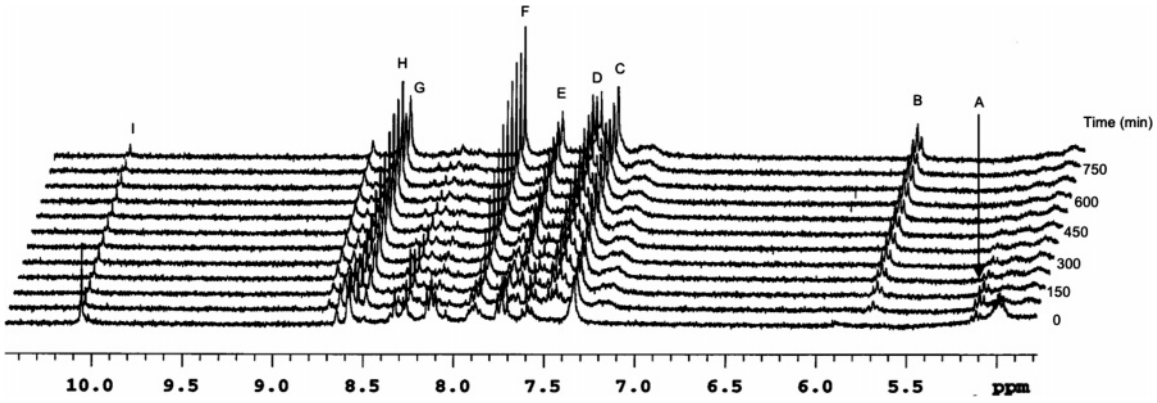

FIGURE 4. Time-dependent ^{1}H NMR spectra showing evolution of macrocycle library derived from **1** to the predominantly dimeric state. (A) Trimer α-CH; (B) dimer α-CH; (C) dimer imidazole C4/5; (D) dimer aryl; (E) dimer aryl; (F) dimer imine CH; (G) dimer aryl; (H) dimer imidazole C2; and (I) linear oligomer aldehyde CH.

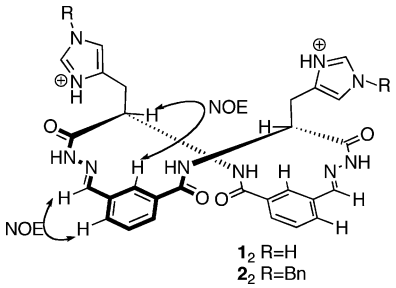

FIGURE 5. Proposed structure of cyclic dimers $\mathbf{1}_2$ and $\mathbf{2}_2$ based on NOESY data.

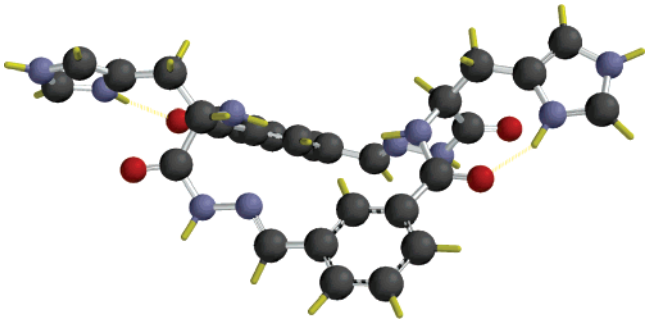

FIGURE 6. PM3 calculated structure of cyclic dimer $\mathbf{1}_2$.

between the ortho-aryl C−Hs and the hydrazone C−H and the α-amino acid C−H suggest that the structure of the dimer is as illustrated in Figure 5. Molecular mechanics and gas-phase PM3 models[30] of the cyclic dimer dication (Figure 6) are in agreement with this proposed structure with the two imidazolium units directed exo. The all-dimer state is most likely favored entropically at these low concentrations. The cyclic dimer also was observed by ESI-MS to form various complexes, with Zn(II) in methanolic solution, specifically, $[(\mathbf{1}_2)_2\mathrm{Zn}]^{2+}$, $[(\mathbf{1}_2)_3(-\mathrm{H})\mathrm{Zn}_2]^{3+}$, and $[(\mathbf{1}_2)_2(-2\mathrm{H})\mathrm{Zn}_2]^{2+}$.

In an attempt to perturb the equilibrium in the *mHis* library away from the all-dimer state, experiments were carried out in which template candidates were added to the oligomer libraries or to the starting mixture of monomer and acid under various conditions. Template candidates that we explored include metal ions (zinc triflate, cadmium chloride, mercuric chloride, copper (II) triflate, cobalt (II) chloride, and iron (III) chloride) and anions (diphenylphosphate and phenylphosphonic acid). These experiments were carried out in a variety of mixtures of acetonitrile and water, using 1.1 equiv of TFA or 100 mM ammonium chloride buffer (pH 3) and analyzed by HPLC. We observed no templating behavior in any of these experiments as the final distribution of oligomeric species (largely dimer) was unperturbed. We believe that in the case of the metal template candidates, interactions between imidazole and metal are suppressed by the fact that under the acidic conditions required for component exchange, the imidazoles are protonated. It is also possible that the thermodynamically favored cyclic dimer forms metal ion complexes that are as (or more) stable than the higher oligomers, so no detectable change in the equilibrium occurs in the presence of metal ion. As noted previously, we found that the cyclic dimer under neutral conditions formed various complexes with zinc that were detected by ESI-MS. In the case of anionic candidates, it is likely that there is no appreciable advantage to the trimeric or tetrameric cationic oligomer over the dimeric cationic oligomer for anion binding, especially considering that all species are strongly solvated in the largely aqueous experimental medium.

To explore the effect of substitution on the histidine imidazole, and to allow for library generation and templating studies in nonaqueous solvent systems, the *N*-benzyl-histidine derivative of the monomer was synthesized according to Figure 7. τ-Benzyl-L-histidine **5** was converted to the methyl ester **6** by refluxing in methanolic hydrogen chloride. The resulting crude ester dihydrochloride was freed with TEA and coupled to 3-(dimethoxymethyl)benzoic acid **3** using EDC and HOBT in THF to give ester **7**. The hydrazide monomer **2** was prepared by hydrazinolysis of the ester **7** overnight in methanol at room temperature. The resulting monomer *mBnHis* **2** was found to be soluble in polar organic solvents such as chloroform.

The benzyl-substituted monomer **2** was found to react to form cyclic oligomers in a similar fashion to the parent monomer. When treated at 5 mM concentration with excess TFA in 75% acetonitrile in water, the monomer produced a library consisting of cyclic dimer, cyclic trimer, and cyclic tetramer, which were detected by LC/ESI-MS. The evolution of the mixture was also followed by proton NMR (Figure 8). Over a 60 h period, the oligomers were consumed to produce only the cyclic dimer.

The *BnHis* dimer $\mathbf{2}_2$ could be produced and isolated on a preparative scale by treating a 3:1 acetonitrile/water solution with excess trifluoroacetic acid at room temperature for 10 days.

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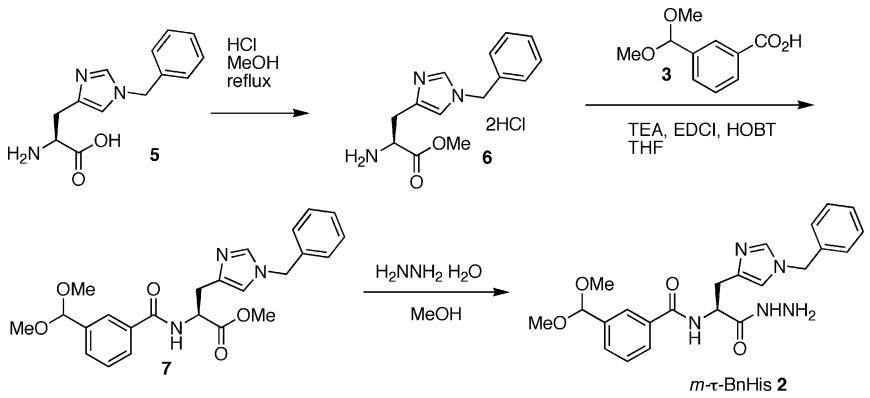

FIGURE 7. Synthesis of **2**.

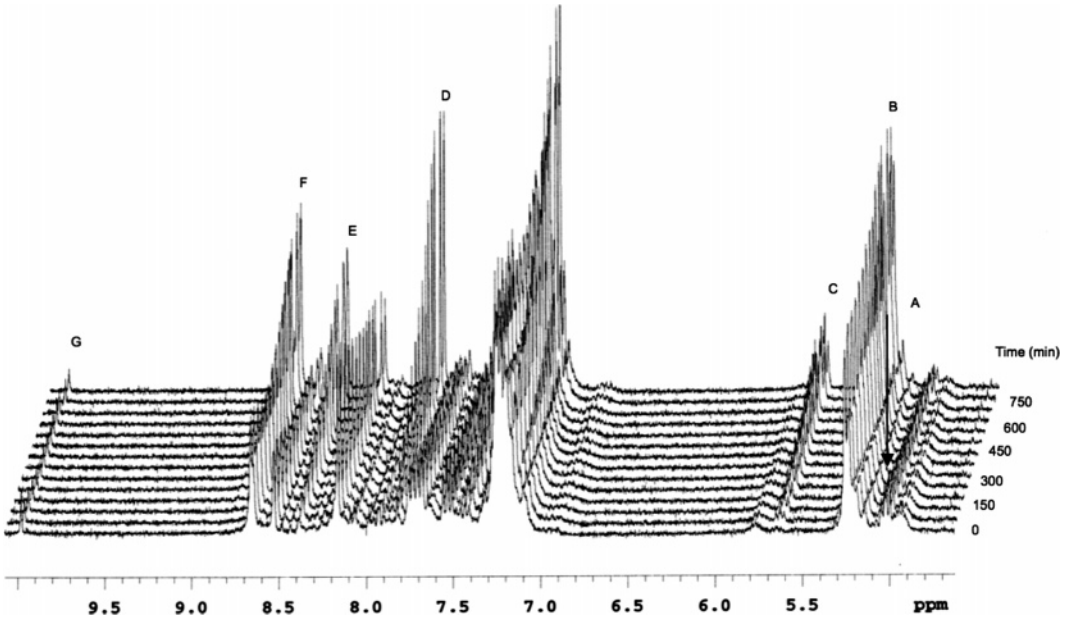

FIGURE 8. Time-dependent ^{1}H NMR of oligomer library derived from **2**. (A) Trimer histidine α proton; (B) benzylic protons; (C) dimer histidine α protons; (D) dimer imine CH; (E) dimer aromatic CH; and (F) dimer imidazole 5 position.

Solvent evaporation left the dimer in moderately high purity as a ditrifluoroacetate salt. NOESY data for the *BnHis* cyclic dimer $\mathbf{2_2}$ (Figure 5) reveal an NOE enhancement between the histidine α proton (5.8 ppm) and the 2 position of the 3-iminyl-benzamide moiety (8.7 ppm), indicating the proximity of the histidine α proton to the aromatic ring 2 position. This suggests that the cyclic oligomer is arranged in such a way as to place the histidine side chain away from the aromatic backbone and the α proton toward the aromatic backbone. In addition, we observe an NOE interaction between the imine CH and the aromatic ring's 4 position, suggesting the aromatic imine conformation illustrated in Figure 5. The structure of the cyclic dimer derived from **2** is therefore analogous to the structure of the non-benzylated *mHis* cyclic dimer.

Templating experiments using the τ-benzyl-L-histidine-derived monomer **2** revealed no templating behavior. These experiments were carried out by adding template candidates to a pre-equilibrated library of cyclic dimer $\mathbf{2_2}$ or combining monomer **2** and template candidates in various solvents and treating with 1.1 equiv of TFA. Samples were monitored by HPLC. Template candidates tested include metal ions and anionic templates analogously with previous experiments using monomer **1**. All mixtures yielded the cyclic dimer at equilibrium. The oligomers are protonated under these experimental conditions, and it is likely that no interaction with metal takes place. In the case of anionic templates, it is likely that the ion−ion interactions we were attempting to exploit are not sufficiently discriminating to select higher oligomers over dimers.

pK_a of *mHis* Cyclic Dimer. We wished to determine if the presence of two imidazole moieties on the macrocycle would lead to any perturbation in basicity. To characterize the acid−base properties of the *mHis* cyclic dimer, a solution of the dimer in dilute aqueous HCl was titrated with sodium hydroxide solution, and the pH was monitored by a potentiometric probe as a function of added base. Over the addition of 2 equiv of sodium hydroxide, there was only one discrete endpoint visible. We interpret this to mean that the pK_a values for first and second protonation are quite close together. The average of the pK_a of the first and second protonation of the cyclic dimer appears to be approximately 6.6 (see Figure 1 in the Supporting Information), as compared to 7.45 for 4-methyl imidazole.[31] This is most likely due to a depression of the pK_a of the second protonation, possibly due to electrostatic interactions between the two imidazole moieties.

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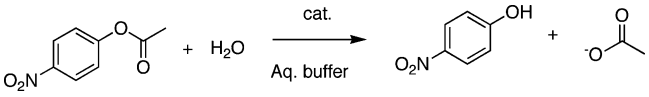

FIGURE 9. Catalytic hydrolysis of *p*-nitrophenylacetate.

TABLE 1. Hydrolysis of *p*-Nitrophenyl Acetate Catalyzed by Dimer 1_2 and 4-Methyl Imidazole

pH	$\mathbf{1_2}$ cyclic dimer, k_2 (M^{-1} 1 s^{-1}1)	4-methyl imidazole, k_2 (M^{-1} s^{-1})	background, k_1 (s^{-1}1)
6.2	0.26	0.022	8.4×10^{-6}
6.6	0.83	0.083	1.7×10^{-5}
7.0	0.88	0.095	1.7×10^{-5}

Hydrolysis of *p*-Nitrophenyl Acetate Catalyzed by *mHis* Cyclic Dimer. Since the *mHis* cyclic dimer possesses two imidazole moieties, we wondered whether it would display hydrolytic activity toward *p*-nitrophenylacetate. Catalysis of *p*-nitrophenylacetate hydrolysis by the *mHis* cyclic dimer was investigated by spectrophotometric assay at 320 nm. Reactions were carried out in 100 mM bis-Tris buffer at pH 6.20, 6.60, and 7.00 at 25 °C. The k_2 values for the *mHis* cyclic dimer at pH 6.2, 6.6, and 7.0 were found to be 0.22, 0.83, and 0.95 s^{-1} M^{-1}, respectively. These values are approximately 5 times the appropriate k_2 values for the electronically similar 4-methyl imidazole, when adjusted for imidazole equivalents (Figure 9 and Table 1). Since the two imidazole units of the dimers are oriented exo, there would be no opportunity for cooperative acid/base or acid/nucleophile catalysis. The relatively modest observed catalytic rate enhancement for the cyclic dimer relative to the simple imidazole could be explained by a greater effective concentration of a free nucleophilic imidazole unit in the dimer at pH 6.2−7.0 due to its greater acidity. Although our results are not directly comparable to existing peptidic artificial esterases incorporating histidines[32,33] due to differences in experimental conditions, qualitatively, the rate enhancements we observed are relatively small.

Conclusion

We have synthesized two histidine-derived pseudo-peptide monomers that undergo thermodynamically controlled oligomerization under acidic conditions. Although the initial distribution of oligomers for both non-benzylated ($\mathbf{1}_n$) and benzylated ($\mathbf{2}_n$) systems included cyclic dimers, trimers, and tetramers, at equilibrium both systems led exclusively to the cyclic dimer. The imidazole units of the cyclic dimers $\mathbf{1_2}$ and $\mathbf{2_2}$ are oriented exo based on solution NMR studies. These results demonstrate that the monomer *mHis* can be incorporated into cyclic oligomers by dynamic combinatorial chemistry. The average pK_a value of cyclic dimer $\mathbf{1_2}$ (6.6) is significantly lower that that of 4-methyl imidazole (7.45). Dimer $\mathbf{1_2}$ also catalyzes the hydrolysis of *p*-nitrophenyl acetate 5 times faster (on a normalized basis) than 4-methyl imidazole. No conditions were found in which the library could be diverted from the all-dimer state by templating. Screening of appropriate template compounds is ongoing, as well as exploration of alternative exchange reactions that might be more compatible with metal−imidazole interactions. In nature, histidine plays many catalytic roles that take advantage of the chemical versatility of imidazole. Monomers of this type may prove useful in establishing DCLs with a biomimetic functional diversity.

Experimental Section

3-(Dimethoxymethyl)-*N*-benzyl-histidine Methyl Ester (4). Carbonyl diimidazole (1.82 g, 11.3 mmol) was dissolved in 45 mL of dry ethyl acetate. 3-Carboxybenzaldehyde dimethoxyacetal (**3**) (2.19 g, 11.2 mmol) was added to this solution, and the mixture was stirred under nitrogen for 3 h. The solution was refluxed overnight and then cooled. L-Histidine methyl ester dihydrochloride (4.06 g, 16.8 mmol) was added, followed by 6 mL (43 mmol) of triethylamine. The mixture was stirred for 2 weeks at room temperature. The mixture was then diluted with 50 mL of chloroform and washed with saturated aqueous sodium bicarbonate (two portions of 50 mL). The resulting organic phase was evaporated in vacuo to produce 3.13 g of 3-(dimethoxymethyl)-benzoyl-histidine methyl ester as a light yellow foaming oil (80% crude yield). This material was sufficiently pure to carry to the next step. ^{1}H NMR: 300 MHz in $CDCl_3$; 7.97 (1H, s), 7.85 (1H, d, J = 6.3 Hz), 7.62−7.64 (3H, m), 7.45 (1H, t, J = 7.5 Hz), 6.87 (1H, s), 5.43 (1H, s), 5.00 (dt, J = 7.5, J = 5.1), 3.73 (3H, s), 3.33 (6H), 3.24 (2H, pseudo-t, J = 9.3); HRMS (ESI): 348.1533, Calcd mass ($C_{17}H_{22}N_3O_5$) 348.1559.

3-(Dimethoxymethyl)-*N*-benzyl-histidine Hydrazide (1) (*mHis*). Crude 3-(dimethoxymethyl)benzoyl-histidine methyl ester (**4**) (3.13 g, 9.00 mmol) was dissolved in 80 mL of methanol, filtered through filter paper, and treated with 2.5 mL of hydrazine monohydrate. After 0.5 h stirring at room temperature, a white precipitate was observed. The mixture was stirred for 72 h, evaporated in vacuo, and triturated 3 times with methanol. The insoluble solid product **1** was collected (2.64 g, 84% yield, 67% over two steps). ^{1}H NMR: 300 MHz in 50% v/v CD_3CN/D_2O: 7.76 (1H, s), 7.73 (1H, d, J = 9.3) 7.54−7.55 (2H, m), 7.45 (1H, t, J = 7.5), 6.87 (1H, s), 5.38 (1H s), 4.70 (1H, dd, J = 8.7, J = 6.0), 3.28 (6H), 3.03 (2H, m); ^{13}C NMR: 75.45 MHz in 50% v/v CD_3CN/D_2O: 172.5, 168.95, 139.8, 136.4, 134.7, 134.5, 131.1, 129.7, 128.6, 126.6, 117.5, 104.0, 54.0, 53.9, 30.1; IR: ν_{max}/cm^{-1} 3280, 3042, 2951, 2834, 1660, 1638, 1547; HRMS (ESI): 348.1663, Calcd mass ($C_{16}H_{22}N_5O_4$) 348.1672.

Synthesis of τ-Benzyl-L-histidine Methyl Ester Dihydrochloride (6). τ-Benzyl-L-histidine (**5**) (6.50 g, 26.5 mmol) was placed in 100 mL of 3 M methanolic HCl and refluxed for 2 h. Solvent was removed under vacuum giving the crude τ-benzyl-L-histidine methyl ester dihydrochloride (**6**) (8.57 g, 97%). ESI-MS: 260.1397, M + H^+ (theoretical, 260.1399). 300 MHz ^{1}H NMR in D_2O: 8.67 (1H, d, J = 1.8 Hz), 7.26−7.36 (6H, m), 5.26 (2H, s), 4.30 (1H, t, J = 6.9 Hz), 3.60, (3H, s), 3.26, (2H, d, J = 7.2 Hz). ^{13}C NMR in D_2O: 169.5, 139.5, 136.1, 130.3, 129.5, 134.5, 128.4, 121.8, 54.7, 53.7, 52.6, 25.9.

Synthesis of 3-(Dimethoxymethyl)benzoyl-τ-benzyl-L-histidine Methyl Ester (7). τ-Benzyl-L-histidine methyl ester dihydrochloride (**6**) (0.85 g, 3.0 mmol), 3-(dimethoxymethyl)benzoic acid (**3**), (0.50 g, 3.0 mmol), and 1-hydroxybenzotriazole hydrate (0.40 g, 3.0 mmol) were placed in 100 mL of dry THF and cooled in an ice bath. Triethylamine (1.18 mL, 9.9 mmol) was added, followed by *N*-(3-diethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.49 g, 3.0 mmol). The reaction mixture was stirred for 4 days. Solvent was removed under vacuum. The crude residue was reconstituted in 200 mL of chloroform and washed with three 10 mL portions of saturated sodium bicarbonate. The organic portion was dried with magnesium sulfate and evaporated to brown crude oil. This was chromatographed on silica eluted with ethyl acetate to give 0.81 g of yellow oil (61.5%). ESI-MS *m*/*z* 438.1937 (theoretical M + H^+ 438.2029). 300 MHz ^{1}H NMR in $CDCl_3$: 8.31 (1H, d J = 7.5 Hz), 7.98 (1H, s), 7.83 (1H, d, J = 8.1 Hz), 7.59 (1H, d, J = 7.8 Hz), 7.48 (1H, s), 7.42 (1H, t, J = 8.1 Hz), 7.24−7.33 (3H,

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m), 7.08−7.11 (2H, m), 6.68 (1H, s), 5.41 (1H, s), 5.04 (2H, s), 4.95 (1H, m), 3.64 (3H, s), 3.31 (6H, s), 3.14 (2H, m). ^{13}C NMR: 172.2, 167.1, 138.8, 138.3, 137.4, 136.1, 134.3, 130.0, 129.3, 128.7, 128.6, 127.6, 127.5, 126.2, 117.3, 102.8, 53.2, 53.0, 52.9, 52.5, 51.1, 29.7.

Synthesis of 3-(Dimethoxymethyl)benzoyl-τ-benzyl-L-histidine Hydrazide (2). 3-(Dimethoxymethyl)benzoyl-τ-benzyl-L-histidine methyl ester (**7**) (0.81 g, 1.85 mmol) was dissolved in 20 mL of dry methanol and treated with 1 mL of hydrazine monohydrate. After stirring for 3 days at room temperature, solvent was removed under vacuum, and the crude product was chromatographed on silica (9:1 dichloromethane/methanol) to give 0.405 g (50%) of **2**. ESI-MS *m*/*z* 438.2112 (theoretical M + H$^+$ 438.2143). 300 MHz ^{1}H NMR in $CDCl_3$: 8.54, (1H, d, J = 6.6 Hz), 7.96 (1H, s), 7.82 (1H, d, J = 7.8 Hz), 7.57 (1H, d, J = 7.5 Hz), 7.37−7.45 (2H, m), 7.24−7.30 (3H, m), 7.07−7.10 (2H, m), 6.74 (1H, s), 5.39 (1H, s), 5.00 (2H, s), 4.84 (1H, q, J = 5.4 Hz), 3.29 (6H, s), 2.90−3.18 (2H, m). ^{13}C NMR: 171.8, 167.2, 138.7, 138.4, 136.9, 135.9, 133.7, 130.1, 129.1, 128.7, 18.4, 127.5, 127.3, 125.8, 117.5, 102.5, 53.0, 52.8, 52.7, 51.1, 29.6.

Oligomerization of 1 (*mHis* Monomer). Analytical scale: monomer **1** (1.0 mg, 2.9 μmol) was placed in 1 mL of 75% acetonitrile v/v aqueous solution (2.88 mM). This was treated with 10 μL of TFA (0.13 mmol, 46-fold excess) or 60 μL of 53.7 mM stock solution (0.2 mL in 50 mL) of TFA in water (1.1 equiv of TFA with respect to **1**). Preparative scale: **1** (100 mg, 288 μmol) was placed in 100 mL of 75% acetonitrile v/v aqueous solution. Concentrated hydrochloric acid (0.05 M) was added, and the solution was stirred for 2 weeks. The mixture was evaporated in vacuo to produce a white amorphous solid (crude cyclic dimer $\mathbf{1_2}$ hydrochloride salt). No further purification was required. ^{1}H NMR 300 MHz in CD_3OD: δ_H 8.75 (1H, s), 8.69 (1H, s), 7.81 (1H, s), 7.69 (1H, pseudo-dt, J = 6.9, 1.8 Hz), 7.35−7.43 (3H, m), 5.76 (1H pseudo-t, J = 7.2 Hz), 3.40 (1H, dd, J = 15.6, J = 6.3 Hz), 3.21 (1H, dd, J = 15.3, 7.5 Hz); ^{13}C NMR:100.57 MHz in CD_3OD: 171.0, 167.1, 143.3, 134.7, 133.1, 132.5, 131.0, 130.6, 128.3, 127.1, 124.2, 117.3, 49.7, 26.0. ESI-MS: *m*/*z* 284.1, 284.6; HRMS (ESI): 567.2278, Calcd mass ($C_{28}H_{27}N_{10}O_4$) 567.2211.

Preparative Synthesis of *BnHis* Cyclic Dimer $\mathbf{2_2}$. 3-(Dimethoxymethyl)benzoyl-τ-benzyl-L-histidine hydrazide **2** (100 mg, 0.23 mmol) was dissolved in 40 mL of 75% acetonitrile/25% water v/v. This solution was treated with 0.5 mL of TFA. After 10 days, solvent was removed under high vacuum, and the residue was dried under sodium hydroxide to give 89 mg (0.091 mmol, 79%) of the *τ-BnHis* cyclic dimer diTFA acid salt.

300 MHz ^{1}H NMR in d_4-methanol: 8.93 (1H, d, J = 1.5 Hz), 8.66 (1H, s), 7.84 (1H, s), 7.56 (1H, dt, J = 8.1 Hz, 0.9 Hz), 7.44−7.41, (2H, m), 7.34, (1H, t, J = 7.2 Hz), 7.28−7.15, (5H, m), 5.83, (1H, m), 5.35, (2H, m), 3.36−3.29, (1H, m), 3.22−3.14, (1H, m). ^{13}C NMR: 172.5, 168.8, 145.0, 136.4, 136.3, 136.0, 135.6, 134.0, 133.1, 130.3, 130.1, 129.91, 129.2, 128.5, 126.1, 121.8, 53.8, 50.8, 27.9. NOESY: strong NOE cross-peaks are present between α proton (5.83) and aromatic 2 position (8.66) and also between imine CH (7.84) and aromatic 4 position (7.41), indicating a conformation in which the imidazole-bearing sidechains are oriented outward from the macrocyclic structure. ESI-MS: *m*/*z* 747.3380; M + H$^+$ (calcd 747.3156).

Templating Experiments for 1 and 2. Solutions of monomer (**1** or **2**, 5 mM) were prepared in 75% acetonitrile/25% water and treated with a stock solution of TFA in acetonitrile to bring the amount of TFA to 1.5 equiv in relation to the monomer. Templating agents were added to these solutions using stock solutions in 0.33, 1, and 10 equiv quantities. Templating agents used included zinc triflate, copper (II) triflate, cobalt (II) chloride, nickel (II) chloride, iron (II) chloride, cadmium (II) chloride, and mercury (II) chloride. Organic templates that were used included diphenylphosphate, trimesic acid, phosphoryl choline, *p*-toluenesulfonic acid, and phosphoric acid. Samples were analyzed over days by diluting in water (10:1) followed by HPLC analysis.

Catalysis of *p*-Nitrophenylacetate Hydrolysis by *mHis* Cyclic Dimer. All reactions were carried out in 100 mM bis-Tris buffer and analyzed spectrophotometrically at 320 nm at 25 °C in a 1 cm cuvette. Assays were carried out at pH 6.20, 6.60, and 7.00. The *p*-nitrophenylacetate concentration was 2.69×10^{-4} M. 4-Methyl imidazole and *mHis* cyclic dimer concentrations were varied. First-order constants k_1 were calculated from initial rates and substrate concentrations. First-order constants k_1 were plotted against the catalyst concentration to give the apparent second-order constant k_2.

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Supporting Information Available: Spectral characterization of all new compounds, including ^{1}H, ^{13}C, and 2-D NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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