Chiral Molecular Recognition in a Tripeptide Benzylviologen Cyclophane Host

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A cationic chiral cyclophane was synthesized and studied as a host for chiral and racemic π-donor molecules. The cyclophane host has a rigid binding cavity flanked by (*S*)-(valine-leucine-alanine) and *N*,*N*′-dibenzyl-4,4′-bipyridinium subunits, which allow for hydrogen-bonding and π-stacking interactions with included aromatic guest molecules. ^{1}H NMR binding titrations were performed with several different pharmaceutically interesting guest molecules including β-blockers, NSAIDs, and amino acids and amino acid derivatives. The host−guest complexation constants were generally small for neutral and cationic guests (0−39 M^{-1} at 20 °C in water/acetone mixtures). However, a (*R*)/(*S*) enantioselectivity ratio of 13 ± 5 was found for DOPA, a strongly π-donating cationic guest. Two-dimensional NOESY ^{1}H NMR spectra confirm that (*R*)-DOPA binds inside the cavity of the host and that there is no measurable interaction of the cavity with (*S*)-DOPA under the same conditions.

Introduction

Cyclophanes, which are cyclic molecules containing aromatic groups in the ring, have interesting molecular recognition properties.[1] Because they are macrocycles, these hosts have built into them a measure of preorganization that enhances their affinity for guest molecules of the appropriate size and shape.[2] The hydrophobicity and π-stacking interactions of their aromatic groups also contribute to host−guest affinity, which can be very high. For these reasons, they have been widely studied as synthetic receptors[3] and as components of supramolecular assemblies.[4]

Chiral cyclophanes are of particular interest as the active components of stationary phases for chiral separations. Cram and co-workers prepared the first cyclophane of this type, a crown ether incorporating a chiral binaphthol unit.[5] This host was later tethered to chromatographic silica to produce a commercially available stationary phase that has been used for the analysis of chiral hydrogen bond donors, including amines, amino alcohols, amino acids, and amino esters.[6] More recently, Armstrong et al.[7] have immobilized several antibiotic macrocycles onto silica and have used these materials in the analysis of a wide variety of enantiomeric and diastereomeric guests. Recent work in our laboratory has shown that the intercalation of chiral cationic host molecules into α-zirconium phosphate, a lamellar cation exchanger, provides a useful medium for batchwise resolution of racemic mixtures.[8] The scale of this process is more than an order of magnitude higher than it is with conventional "brush"-type chiral stationary phases, but expansion and contraction of the solid and concomitant host preorganization effects have precluded its use in chromatographic applications. Replacing the linear chiral host that was used in those experiments with a cyclophane-type host containing a rigid, preorganized binding cavity could solve the problem of host preorganization and motivates the study reported in this paper.

Several groups[9−11] have now incorporated chiral amino acids into cyclophane hosts. There are three significant advantages to this approach. First, the synthesis is modular and employs well-established peptide coupling methods. Second, each amide bond potentially provides two hydrogen-bonding contacts to the guest, in close proximity to an asymmetric carbon atom. Third, because there is a large inventory of natural and unnatural amino acids from which to choose, a very large number of structurally similar host molecules can be prepared. For hosts containing more than one amino acid, the synthesis can be done in combinatorial fashion, and so in principle one can make and test a diverse library of host molecules.

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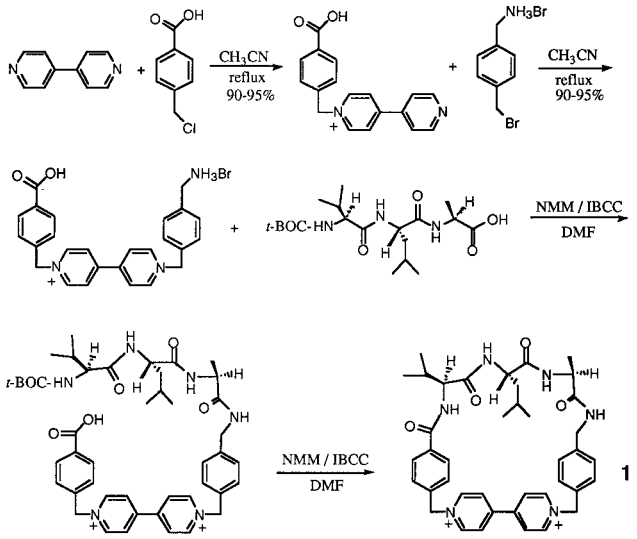

Figure 1. Synthetic scheme for the (*S*)-valine-leucine-alanine cyclophane **1**.

There have been several papers that describe this kind of combinatorial approach, although in practice it has not yet been used to find the best host molecule for a given enantioseparation. The closest examples are in the work of Still and co-workers, who screened a library of peptidosteroid hosts against chiral enkephalin guests[12] and a library of peptide guests against a single cyclophane host.[9] In related work, we recently prepared a small combinatorial library of dipeptide-containing cyclophanes. The affinity of those cyclophanes for chiral guests was unfortunately too low for combinatorial screening of their host−guest interactions.[10]

The cationic chiral cyclophane **1** that is reported here was designed to contain the powerfully π-accepting benzylviologen group[13] along with a tripeptide chiral barrier for enantioselective interactions. The aromatic portion of **1** contains an amine and a carboxy terminus for ease of inclusion of the tripeptide, (*S*)-valine-leucine-alanine, which can be made by standard solid-phase coupling methods. The synthesis is modular, and in principle any tripeptide can be substituted for (*S*)-valine-leucine-alanine. In this paper, we report the complexation of this chiral cyclophane host in aqueous media with NSAIDS, β-blockers, and amino acids and amino acid derivatives, which are interesting analytes for enantioseparations.

Results and Discussion

Synthesis. The synthesis of the (*S*)-valine-leucine-alanine cyclophane **1** is represented in Figure 1. The synthesis is convergent, in the sense that the bipyridinium and tripeptide fragments are made separately and then combined by peptide coupling. The bipyridinium fragment is itself an ω-amino acid, which is made in two separate high-yield quaternization steps from 4,4′-bipyridine and the appropriate benzyl halide derivatives. The bipyridinium part of the molecule is made by reflux of 4-(chloromethyl)benzoic acid with 4,4′-bipyridine in acetonitrile to give the chloride salt of *N*-[(*p*-carboxyphenyl)methyl]4,4′-bipyridine. The amine end of the bipyridinium unit derives from 4-(bromomethyl)benzylamine hydrobromide, which was made in three steps from *p*-bromotolunitrile, in an overall yield of ca. 37%. Briefly, bromotolunitrile was refluxed in water with barium carbonate to give *p*-cyanobenzyl alcohol, which was reduced with lithium alumium hydride in refluxing ether to give *p*-(hydroxymethyl)benzylamine hydrochloride, followed by reflux in hydrogen bromide to give 4-(bromomethyl)benzylamine hydrobromide. *N*-[(*p*-Carboxyphenyl)methyl]4,4′-bipyridine]$^{1+}$(PF_6^-) and 4-(bromomethyl)benzylamine hydrobromide were combined and refluxed in acetonitrile to give the complete bipyridinium unit, {*N*-[(*p*-carboxyphenyl)methyl]-*N*′-[(*p*-(aminomethyl)-phenyl)methyl]-4,4′-bipyridine}$^{2+}$ ($2Br^-$).

The tripeptide unit was synthesized by standard solid-phase methods. The cesium salt of the first amino acid was obtained by reacting the *N*-*t*-Boc-protected amino acid with cesium carbonate. The cesium salt of the *N*-*t*-BOC-amino acid was coupled to Merrifield resin and was deprotected with trifluoroacetic acid in chloroform. Subsequent coupling of *N*-*t*-BOC-amino acids was performed using dicyclohexylcarbodiimide in dichloromethane and dimethylformamide. Finally, the tripeptide was cleaved from the resin using a phase-transfer catalyst and isolated with the N-*t*-BOC protecting group intact.

The bipyridinium unit was then coupled to the protected tripeptide using *N*-methylmorpholine, triethylamine, and isobutyl chlorocarbonate in dry dimethylformamide. Deprotection with trifluoroacetic acid in chloroform and a second coupling with *N*-methylmorpholine, triethylamine, and isobutyl chlorocarbonate in dimethylformamide resulted in the product cyclophane **1**. Despite the large size of the cyclophane ring, the final coupling reaction proceeds in 40% yield under conditions of moderate dilution (6 mM concentration). Compound **1** and the precursors to **1** were characterized by ^{1}H and ^{13}C NMR, mass spectrometry, and elemental analysis. Thermal analysis showed that most of the salts and peptide-containing compounds decomposed significantly before melting.

Compound **1** was also characterized by amino acid analysis. During the hydrolysis step of the amino acid analysis, the cyclophane was cleaved at each of the amide bonds. The bipyridinium portion of the cyclophane did not interfere with the HPLC method for determining the amino acids present in the mixture. The three amino acids in the cyclophane, alanine, leucine, and valine were detected in approximately equimolar quantities (1428, 1496, and 1497 nmol, respectively) in hydrolyzed samples prepared from 1500 nmol of cyclophane **1**.

Determination of Binding Constants. ^{1}H NMR titrations were performed in mixed water/acetone solvents using the bromide salt of **1** at room temperature. The host concentration was kept in the range of 5−10 mM for 1:1 binding experiments with analytes. Self-association of the cationic cyclophane does not occur to any measurable extent from 1 to 13 mM. The concentration of **1** was kept constant in the titrations, and the guest concentration was varied in the range of 1−500 mM depending on solubility. Equilibrium concentrations of the guest molecules, association constants, and errors

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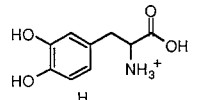
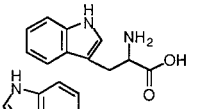
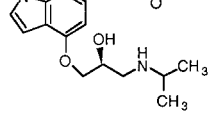
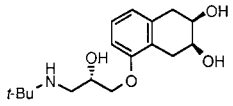
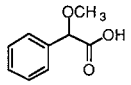
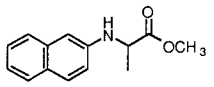
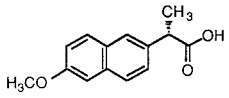
Table 1. Results of NMR Titrations Using the Dibromide Salt of 1. Association Constants Represent the Average of Two or More Proton Chemical Shifts

Guest	K (M^{-1})	Solvent System	Structure
Self-association	not detected	17 D_2O: 1 acetone-d_6: 1 DCl	
(R)-DOPA	39 ±6	"	
(S)-DOPA	3 ±1*	"	
(R)-Tryptophan	5 ±1	"	
(S)-Tryptophan	6 ±1	"	
Pindolol	6 ±1	"	
Nadolol	23 ±3	10 acetone-d_6: 3 D_2O	
(R)-(-)-α-Methoxy phenylacetic acid	5 ±1	1 acetone-d_6: 1 D_2O	
(S)-(+)-α-Methoxy phenylacetic acid	8 ±6	"	
N-(2-naphthyl) alaninate	10 ±1	9 acetone-d_6: 4 D_2O	
(S)-6-methoxy-α-methyl-2-naphthalene acetic acid	9 ±1	29 D_2O: 11 acetone-d_6: 1 MeOD	

*Determined by titration of racemic DOPA.

were calculated using the HOSTEST (v.5.0) program.[14] A 1:1 stoichiometry was found for all complexes in which there was detectable host−guest binding. The results of NMR titrations with several guest molecules are collected in Table 1. Weak binding (1−39 M^{-1}) was found between the guest molecules and **1** in aqueous solutions. This observation is consistent with previous studies of simple acceptors such as methylviologen or 1-methylnicotinamide,[15] as well as β-cyclodextrin derivatives,[16] and pyrogallol or resorcinol cyclic tetramers[17] with amino acids in aqueous media. Apparently, the free energy penalty incurred in desolvating the ionic host is almost equal to the attractive (hydrogen bonding and π-stacking) interactions in water. Stoddart et al. have shown that stronger affinity for amino acids in aqueous media can be achieved using stronger π-accepting hosts such as a box-like cyclophane containing two bipyridinium acceptor subunits instead of the single bipyridinium group in **1**.[18]

Of the guest molecules studied, racemic nadolol and DOPA showed significant association with **1**. The complexation of **1** with the individual enantiomers of DOPA was measured. However, the enantioselectivity of binding to nadolol was not measured, because pure enantiomers were not available. Figure 2a shows two cyclophane ^1H NMR peaks (from the $-CH_2$ groups which reside between the viologen and benzyl units of the cyclophane) in an expanded region in the spectrum of the cyclophane/(*R*)-DOPA complex. While the shifts in these and other cyclophane peaks is small ($\Delta\delta \approx 0.015$ ppm between the free and fully complexed host), the spectra are sufficiently well resolved that they can be accurately measured. Figure 2b shows binding isotherms, in which the change in chemical shift is plotted against the analytical concentration of (*R*)- and (*S*)-DOPA. The solid line is calculated for 1:1 host:guest complexation. Only a slight shift in the cyclophane ^1H NMR peaks was observed at the highest (*S*)-DOPA concentration, 0.338 M. No shifts were observed at lower concentrations. UV−vis spectra showed a negative deviation from Beer's law in the absorption peak at 302 nm for DOPA concentrations above 0.3 M, implying DOPA self-association at high concentration. Therefore, ^1H NMR data obtained at concentrations above 0.3 M were not used in the determination of the DOPA association constants. Self-association was not significant for the other guest molecules in the concentration ranges used. The (*R*)-enantiomer binds to the cyclophane with an association constant of 39 ± 6 M^{-1}. Because there were no observable NMR shifts below 0.3 M concentration with (*S*)-DOPA, its association constant with **1** could not be determined directly. However, it was possible to estimate this association constant through an indirect measurement, by using racemic DOPA.

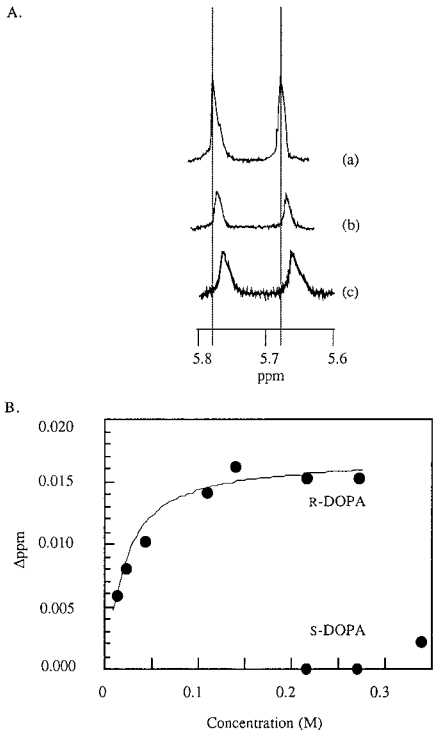


Figure 2. (A) Changes in chemical shift of cyclophane protons ($-CH_2$ groups between bipyridinium and phenyl) in ^1H NMR spectra as a function of (*R*)-DOPA concentration; [*R*] = (a) 0, (b) 0.111, and (c) 0.272 M. (B) Change in chemical shift plotted against the analytical concentration of (*R*)- and (*S*)-DOPA. The solid line is calculated for 1:1 host:guest complexation.

Complexation with racemic DOPA gave a single chemical shift for each of the protons of **1**, implying rapid

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exchange between free **1** and its complexes (**1**·*R* and **1**·*S*) with (*R*)-DOPA and (*S*)-DOPA. The concentration of free **1** ([**1**]) and the total concentration of **1** ($[\mathbf{1}]_T$) are related to the observed chemical shift (δ_{obs}), the chemical shift at saturation (δ_{sat}), and the chemical shift of free **1** (δ_o) by eq 1. This approximation is valid as long as [**1**·*R*] is large compared to [**1**·*S*], which is the case here. K_S, the association constant of (*S*)-DOPA with **1**, can be calculated for each observed chemical shift in the titration curve with (*R*/*S*)-DOPA, using the experimentally derived value of 39 ± 6 M^{-1} for K_R, along with δ_{obs}, δ_{sat}, δ_o, $[\mathbf{1}]_T$, and the total concentrations of the two DOPA enantiomers ($[R]_T$ and $[S]_T$). The equilibrium expressions needed to calculate K_S are given in eqs 2 and 3. Five

$$[\mathbf{1}] = [\mathbf{1}]_T\left(\frac{\delta - \delta_{sat}}{\delta_o - \delta_{sat}}\right) \quad (1)$$

$$K_S = \frac{[\mathbf{1}]_T - [\mathbf{1}] - [\mathbf{1}\cdot R]}{[\mathbf{1}]\,([S]_T - [\mathbf{1}]_T + [\mathbf{1}] + [\mathbf{1}\cdot R])} \quad (2)$$

$$[\mathbf{1}\cdot R] = \frac{[\mathbf{1}]K_R[R]_T}{([\mathbf{1}]K_R + 1)} \quad (3)$$

titration points with (*R*/*S*)-DOPA in the concentration range of 0.028−0.212 M gave $K_S = 3 \pm 1$ M^{-1}, resulting in an enantioselectivity ratio of 13 ± 5 for the association of **1** with the DOPA enantiomers.

Variable-temperature NMR was used to determine thermodynamic parameters for the complexation of (*R*)-DOPA with **1**. Binding experiments were performed at 5, 15, and 25 °C. Although there were slight shifts in the NMR peaks of the cyclophane-only solution with temperature (downfield shift at lower temperature), no variations in the Δppm values for each concentation of (*R*)-DOPA were found with temperature. Therefore, $\Delta H° = 0.0 \pm 0.1$ kcal/mol, and $\Delta S° = +7.3 \pm 1.2$ cal/mol K.

It is interesting to compare cyclophane **1** with cage-like cationic hosts synthesized by Murakami et al., which also contain (*S*)-alanine, -leucine, and -valine groups.[11] Murakami et al. observed much higher association constants (on the order of 10^5−10^6 M^{-1}), which may be attributed to the more hydrophobic nature of the cage and the fact that the guest molecules were anions. While Murakami et al. did not directly measure enantioselectivity ratios for chiral guests, they did determine that the chiral cage imposes its helicity on "hinged" aromatic guests.[11,19] This behavior is consistent with the high enantioselectivity we observe with **1**. The enantioselectivity ratio of DOPA with **1** compares favorably with bowl-shaped cyclophane hosts that also contain three amino acid groups[9] and with commercially available chiral separations media.

2-D NMR Experiments. To further characterize the cyclophane/(*R*)-DOPA host−guest interaction, two-dimensional NOESY ^{1}H NMR experiments were performed on solutions containing both compounds. In addition to the expected strong intramolecular NOE's for the ^{1}H resonances in (*S*)-DOPA, (*R*)-DOPA, and **1**, weak intermolecular NOE's were observed for the solution containing (*R*)-DOPA and **1**, confirming that the cyclophane binds (*R*)-DOPA with rapid exchange between bound and free forms. In Figure 1 of the Supporting Information, horizontal rows from the 2-D NOESY NMR spectra show weak NOE's among the (*R*)-DOPA CH_2 resonance at 3.0 ppm and cyclophane aromatic protons (9.0, 8.4, 7.2), cyclophane CH_2's next to the viologen rings (5.9), the amino acid chiral centers (4.3−3.8, 3.6), and the aliphatic side chains of the amino acids (0.8 ppm); and weak NOE's from the aromatic protons of (*R*)-DOPA to cyclophane resonances at 8.4, 7.2−7.9, 5.9, 5.8, 3.6, and 0.7−1.2 ppm. From the intensity of the NOE peaks, it appears that the aromatic region of **1** is closer to the aromatic region of (*R*)-DOPA compared to the (*R*)-DOPA CH_2 protons. Because of the fast exchange of the NH_3^+ protons of the (*R*)-DOPA, monitoring by NMR is not possible. Therefore, no information about the location of the positive charge relative to the positive charges on **1** can be implied from the 2-D NOESY NMR experiments, although the principles of electrostatics suggest that the NH_3^+ group of the (*R*)-DOPA zwitterion should be as far as possible from the viologen dication region of **1**. By contrast, the equivalent rows from the 2-D NOESY spectrum of an (*S*)-DOPA/cyclophane solution show under identical conditions one extremely weak NOE from the (*S*)-DOPA CH_2 resonances to a cyclophane resonance at 1.0 ppm and two extremely weak NOE's from the (*S*)-DOPA aromatic peaks to cyclophane resonances at 5.9 and 5.8 ppm (Figure 2a,b of the Supporting Information). The near absence of intermolecular NOE's for the latter solution suggests that only incidental contact occurs between (*S*)-DOPA and the exterior of the cyclophane ring. The presence of many intermolecular NOE's between (*R*)-DOPA and the cyclophane supports the hypothesis that there is relatively strong and enantiomerically selective binding of (*R*)-DOPA within the cyclophane cavity.

Intercalation of 1 into α-Zirconium Phosphate. Because the cyclophane is cationic, it was easily intercalated in the lamellar solid acid α-zirconium phosphate by cation exchange for tetra(*n*-butyl)ammonium ions, as previously described.[8]

Attempts to Separate DOPA in 1-Intercalated Solid. Enantioseparation of DOPA using the cyclophane-intercalated α-zirconium phosphate was attempted by means of a batch-mode procedure as previously described.[23] Briefly, 100 mM racemic DOPA, in the same solution mixture that was used for the NMR binding experimnents, was added to the cyclophane-intercalated solid in a 4:1 molar guest:host ratio. By chiral HPLC analysis, there was no enantiomeric excess in the supernatant after 12 h of stirring and filtration of the solid. UV−vis spectroscopy of the supernatant showed that there was no change in DOPA concentration despite this long equilibration period. Thus, no DOPA intercalated into the solid to complex with the cyclophane.

Related work in this laboratory has shown that the association constants of other benzylviologen−cyclophane intercalation compounds with π-donors are about a factor of 5 lower than they are in solution.[20] A possible

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explanation for this effect is that electron donation by the negatively charged α-ZrP sheets reduces the π-acidity of the cyclophane host. A reduction of the association constant of **1** with DOPA by the same factor of 5 would explain the fact that it does not intercalate into **1**/α-ZrP at 100 mM concentration. Interestingly, this effect was not observed in the complexation/intercalation reactions of a "Pirkle phase" chiral host in α-ZrP, because in that case the center of positive charge on the molecule is relatively remote from the π-accepting dinitrobenzoyl group.[8]

Summary and Conclusions

The new tripeptide-containing cyclophane reported here is the first member of a potentially diverse group of hosts which might be prepared by substituting other amino acids for Val-Leu-Ala and other π-acceptors for bipyridinium. Because the binding pocket contains the relatively hydrophilic bipyridinium dication, it binds π-donor analytes weakly in water, and even more weakly when it is intercalated into (anionic) α-zirconium phosphate. Although amino acids with small aliphatic side groups were used to define the binding cavity, the complexation of this host with DOPA is quite enantioselective. Efforts are currently underway to redesign this cyclophane to contain a more hydrophobic and more powerfully π-accepting binding pocket, for more effective complexation of hydrophobic π-donor guests. The fact that this cyclophane, which represents only a single member of a potentially large library, shows good enantioselectivity suggests that a combinatorial synthesis/screening approach could be effective in identifying useful chiral selectors. Solid-phase synthesis and screening techniques of these and related hosts are currently being developed in this laboratory to evaluate the utility of this approach.

Experimental Section

Materials. All starting materials were used as received from Aldrich Chemical Co., J. T. Baker, Sigma, or VWR Scientific. Solvents for high-performance liquid chromatography (HPLC) experiments were of HPLC grade and were degassed with He for 30 min before use.

Instrumentation. ^{1}H NMR spectroscopy was carried out using either a Bruker AM-300 or a Bruker AC-E−200 spectrometer. ^{13}C NMR spectroscopy was carried out using a Bruker AMX-360 spectrometer. 2-D NOESY NMR spectra were acquired on a Bruker AMX-2−500 spectrometer. Analytical HPLC experiments were performed on a Waters 600E system with a Waters 991 photodiode array detector using a Crownpak CR+ column purchased from Chiral Technologies Inc. UV−vis was carried out on a Hewlett-Packard 8452A Diode Array Spectrophotometer for solution samples and on a Varian DMS 300 fitted with a diffuse reflectance attachment for solid samples. Powder X-ray diffraction was performed on a Philips X′Pert MPD diffractometer. Elemental analysis (CHN) was performed by Atlantic Microlabs, Inc., P.O. Box 2288, Norcross, GA 30091. Amino acid analysis was performed by Commonwealth Biotechnologies, Inc. 911 E. Leigh St., Richmond, VA 23219.

Synthesis. {*N*-[(*p*-Carboxyphenyl)methyl]4,4′-bipyridine}$^+$PF$_6^-$. 4,4′-Bipyridine (4.00 g, 25.6 mmol) and 4-(chloromethyl)benzoic acid (2.90 g, 17.1 mmol) were combined and heated to reflux in 400 mL of acetonitrile for 24 h. The product, which precipitated as a white solid, was vacuum-filtered and washed with acetonitrile and diethyl ether. The solid was then dissolved in water with the addition of saturated potassium carbonate (dropwise to pH 8) and precipitated with an excess of ammonium hexafluorophosphate followed by addition of hexafluorophosphoric acid (60 wt % solution in deionized water, dropwise to pH 4). After cooling the solution at 3 °C for several hours, the white solid was vacuum-filtered and washed with deionized water to yield the product (7.1 g, 98%): mp 166 °C; ^{1}H NMR (CD$_3$CN) δ 8.85 (d, J = 6.5 Hz, 4H), 8.33 (d, J = 6.5 Hz, 2H), 8.08 (d, J = 6.5 Hz, 2H), 7.80 (d, 7.0 Hz, 2H), 7.55 (d, 7.0 Hz, 2H), 6.80 (2H, s); ^{13}C NMR ((CD$_3$)$_2$SO) δ 166.26 (carbonyl), [150.53, 144.95, 140.211, 130.92, 125.99, 121.80] (dipyridyl carbons), [150.32, 131.46, 130.30, 130.00] (phenyl carbons), 63.795 (methylene carbon); positive ion FABMS M$^+$ = 291 (M without PF6 counterions). Anal. Calcd (found): C, 49.54% (49.41%); H, 3.44% (3.57%); N, 6.42% (6.40%).

4-(Bromomethyl)benzylamine Hydrobromide. The preparation of this compound is described in the literature.[22] Modifications described below were made to the synthesis to simplify as well as cut the time necessary for the purification in half while increasing the overall yield of the reaction (21% vs 37% overall yield with modifications). Bromotolunitrile (10.0 g, 51.0 mmol) was refluxed in deionized water (300 mL) with barium carbonate (20.0 g) for 2 h. The solid byproduct was removed by filtration. Chloroform extraction of the filtrate and removal of solvent by rotary evaporation yielded a white solid, *p*-cyanobenzyl alcohol (5.10 g, 75.2%). *p*-Cyanobenzyl alcohol (6.54 g, 49.3 mmol) was dissolved in anhydrous ether and added dropwise to LiAlH$_4$ (3.27 g) in ether over 15 min at 0 °C under argon. After the addition, the reaction was refluxed for 3 h. Sodium hydroxide solution (0.10 M) was added followed by chloroform extraction. The chloroform solution was then extracted with 0.1 M HCl. Solvent was removed using rotary evaporation to yield a white solid, *p*-(hydroxymethyl)benzylamine (3.50 g, 51.8%). *p*-(Hydroxymethyl)benzylamine (3.50 g, 25.5 mmol) was refluxed in HBr (100 mL of a solution prepared from 60 mL of concentrated HBr andwater) for 3 h. Solvent was removed by rotary evaporation and the residue was washed with acetone to yield the product as a white solid (6.80 g, 95.6%): mp above 300 °C (dec); ^{1}H NMR ((CD$_3$)$_2$SO) δ 8.18 (3H, broad s), 7.45 (ABq, J_{AB} = 8.15 Hz, $\Delta\upsilon_{AB}$ = 13.8 Hz, 4H), 4.71 (s, 2H), 4.03 (m, 2H); ^{13}C NMR ((CD$_3$)$_2$SO) δ 138.67, 134.22, 129.81, 129.75, 42.15, 34.42; electron ionization MS M$^+$ = 199 (M − HBr). (Caution: product may cause allergic reaction.)

{*N*-[(*p*-Carboxyphenyl)methyl]-*N*′-[(*p*-(aminomethyl)-phenyl)methyl]-4,4′-bipyridine}$^{2+}$(2PF$_6^-$). {*N*-[(*p*-Carboxyphenyl)methyl]4,4′-bipyridine}$^+$PF$_6^-$ (4.25 g, 9.74 mmol) and 4-(bromomethyl)benzylamine hydrobromide (2.72 g, 9.74 mmol) were combined and refluxed in 500 mL of acetonitrile for 48 h. The product, which precipitated as a yellow solid, was vacuum-filtered while hot and washed with acetonitrile and diethyl ether. The solid was then dissolved in distilled water, filtered, and precipitated with ammonium hexafluorophosphate. After cooling the solution at 3 °C for several hours, the white solid was vacuum-filtered and washed with deionized water, methanol, and diethyl ether to yield the product (4.93 g, 50.9%): decomposes without melting above 175 °C; ^{1}H NMR (CD$_3$CN) δ 9.05 (d, J = 6.5 Hz, 4H), 8.45 (d, J = 6.5 Hz, 4H), 8.13 (d, J = 7.0 Hz, 2H), 7.60 (m, 6H), 5.93 (s, 2H), 5.87 (s, 2H), 4.18 (s, 2H); ^{13}C NMR ((CD$_3$)$_2$SO) δ 166.26 (carbonyl), [150.24, 150.19, 145.50, 145.38, 127.31, 127.27] (dipyridyl carbons), [150.32, 136.95, 133.80, 131.46, 130.30, 130.00, 129.48, 129.07] (phenyl carbons), 63.80 (two methylene carbons), 42.81 (C−NH$_2$); positive ion FABMS M$^+$ = 702 (M without PF$_6$ counterions). Anal. Calcd (found): C, 36.8% (37.24%); H, 3.07% (3.16%); N, 4.96% (4.97%).

Cesium Salts of *N*-*t*-BOC-amino Acids.[23] The cesium salt of the first amino acid to be coupled to the resin was obtained by dissolving 2 g of the *N*-*t*-BOC-protected amino acid in 15 mL ethanol/deionized water 2:1 (v/v) and adding aqueous cesium carbonate to pH 7. After rotary evaporation, the cesium salt was obtained as a white solid.

Solid-Phase Peptide Synthesis.[24] In a typical coupling reaction, 5.0 g of Merrifield peptide resin (1.2 mequiv/g), 6.0

mmol of *N*-*t*-BOC-amino acid, Cs^+ salt, and 40 mL dimethylformamide were combined and stirred at 50 °C overnight. The resin was filtered and washed with dimethylformamide, dimethylformamide/deionized water 9:1 (v/v), dimethylformamide, and ethanol. Amino acid loading was determined by elemental analysis. Values for nitrogen content ranged between 0.98 and 1.08% depending on the specific amino acid being coupled. Deprotection of the first amino acid was accomplished by stirring the resin in 25% trifluoroacetic acid in chloroform for 30 min. After filtration, the resin was washed with chloroform, neutralized with 10% triethylamine in chloroform for 10 min, and washed again with chloroform. In subsequent coupling steps, a 3-fold excess of *N*-*t*-BOC-amino acid and dichyclohexylcarbodiimide (caution: DCC may cause allergic reaction.) were added to the peptide resin in 100 mL of dimethylformamide/dichloromethane 1:1 (v/v). After the mixture was stirred for several hours, the peptide resin was filtered and washed with dimethylformamide, dichloromethane, and ethanol. A standard ninhydrin test verified complete coupling after each amino acid addition.[25]

Cleavage of Protected Peptide from Merrifield Resin. Cleavage of the *N*-*t*-BOC-protected amino acids was performed as described previously with changes as noted below.[26] In a typical cleavage reaction, peptide resin (1.00 g) was suspended in tetrahydrofuran (50 mL). Saturated aqueous potassium carbonate (5.0 mL) and tetra(*n*-butyl)ammonium hydrogen sulfate (0.68 g) were added, and the mixture was stirred for 2−3 h. Deionized water (20 mL) was added, and the resin was vacuum filtered and washed with deionized water. The combined filtrates were transferred to a round-bottom flask, and the organic phase was removed by rotary evaporation. The aqueous residue was filtered through a 0.2 μm filter and then acidified to pH 1 with aqueous potassium hydrogen sulfate. The precipitated solid was filtered and washed with deionized water to yield the *N*-*t*-BOC-protected amino acid. In the original synthetic procedure, the resin was washed with both deionized water and ethyl acetate. It was found that the smaller oligomers of the polystyrene resin were soluble in the ethyl acetate and therefore were not filtered properly from the solution prior to acidification. Also, a significant amount of the resin was able to pass through a fine glass fritted filter. Initial attempts at the cleavage procedure resulted in elemental analysis showing a very low nitrogen content, which was attributed to the sample containing up to 80% resin with only 20% peptide. Filtering the solution through a 0.2 μm filter prior to acidification removed any residual resin pieces, resulting in a pure peptide product as shown by elemental analysis.

***(S)*-Ala-Leu-Val-*N*-*t*-BOC.** The solid-phase synthesis and cleavage procedures described above were utilized to yield the product as a white solid (93% yield): mp dec above 158 °C; ^{1}H NMR (C_2D_6OS) δ 8.13 (d, J = 6.71 Hz, 1H), 7.75 (d, J = 7.94 Hz, 1H), 6.75 (d, J = 8.24 Hz, 1H), 4.38 (m, 1H), 4.15 (m, 1H), 3.73 (m, 1H), 1.90 (m, 1H), 1.62 (m, 1H), 1.45 (m, 2H), 1.33 (s, 9H), 1.22 (d, J = 7.01 Hz, 3H), 0.81 (m, 12H); ^{13}C NMR ($(CD_3)_2SO$) δ [175.35, 171.97, 171.43, 155.753] (carbonyl), [78.37, 28.47] (BOC carbons), [60.31, 50.78, 46.92] (chiral center carbons), [24.24, 23.462, 21.862, 19.561, 18.513, 17.462] (amino acid side chains); positive ion FABMS M^+ = 401. Anal. Calcd (found): C, 56.90% (56.90%); H, 8.70% (8.76%); N, 10.50% (10.43%).

{*N*-[(*p*-*(S)*-Ala-Leu-Val-phenyl)methyl]-*N*′-[(*p*-(aminomethyl)phenyl)methyl]-4,4′-bipyridine}$^{2+}$(2Br$^-$). A standard peptide coupling procedure was used to connect {*N*-[(*p*-carboxyphenyl)methyl]-*N*′-[(*p*-aminomethylphenyl)methyl]-4,4′-bipyridine}$^{2+}$ to the tripeptide.[27] (*S*)-Ala-Leu-Val-*N*-t-BOC (0.444 g, 1.11 mmol) was dissolved in dry dimethylformamide (100 mL), cooled to −15 °C under argon, and neutralized with *N*-methylmorpholine (0.122 mL, 1.11 mmol). {*N*-[(*p*-Carboxyphenyl)methyl]-*N*′-[(*p*-(aminomethyl)phenyl)methyl]-4,4′-bipyridine}$^{2+}$(PF_6^-)$_2$ (0.765 g, 1.11 mmol) was dissolved in dry dimethylformamide (100 mL) and treated with triethylamine (0.155 mL, 1.11 mmol). Isobutyl chlorocarbonate (0.144 mL, 1.11 mmol) was added to the peptide solution, followed, about a minute later, by the bipyridine solution. The mixture was allowed to warm to room temperature under argon. The argon purge was removed, and the solution was stirred in air overnight. The solvent was removed by slow evaporation at 50 °C at atmospheric pressure.[28] The residue was dissolved in acetonitrile (100 mL), filtered, and precipitated with tetra(*n*-hexyl)ammonium bromide. After cooling the solution at 3 °C for several hours, the solid was vacuum-filtered and washed with acetonitrile and diethyl ether. The *N*-*t*-BOC protecting group was removed by addition of 25% trifluoroacetic acid in chloroform (100 mL). Solvent was removed by evaporation at room temperature and atmospheric pressure. The residue was then dissolved in acetonitrile, filtered, and precipitated with tetrahexylammonium bromide. After cooling the solution at 3 °C for several hours, the solid was vacuum-filtered and washed with acetonitrile and then diethyl ether to yield the off-white product (0.765 g, 80.8%): mp dec above 100 °C; ^{1}H NMR (C_2D_6OS) δ 9.55 (d, J = 6.5 Hz, 4H), 8.79 (d, J = 6.5 Hz, 4H), 8.45 (m, 2H), 8.22 (d, J = 6.70 Hz, 1H), 8.13 (m, 1H), 8.04 (d, J = 7.0 Hz, 2H), 7.71 (d, J = 7.0 Hz, 2H), 7.60 (d, J = 7.0 Hz, 2H), 7.33 (d, J = 7.0 Hz, 2H), 6.08 (s, 2H), 5.93 (s, 2H), 4.40 (m, 1H), 4.28 (m, 2H) 3.60 (m, 1H), 2.80 (m, 1H), 2.05 (m, 1H), 1.60 (m, 1H), 1.45 (m, 2H), 1.20 (d, J = 7.01 Hz, 3H), 0.85 (m, 12H); ^{13}C NMR ($(CD_3)_2SO$) δ [174.21, 171.69, 167.86] (carbonyl), 167.06 (carboxylic acid), [149.53, 146.20, 138.88, 131.96, 128.02, 127.66] (dipyridyl carbons), [149.60, 135.89, 130.324, 130.121, 129.51, 129.44, 128.47, 128.41] (phenyl carbons), [62.80, 62.78] (methylene groups next to dipyridyl), [57.41, 51.38, 47.90] (chiral center carbons), 41.03 (C−NH_2), [24.24, 23.44, 21.98, 19.21, 18.31, 17.35] (amino acid side chains); positive ion FABMS M^+ = 695 (M without Br counterions). Anal. Calcd (found): C, 56.21% (56.30%); H, 5.85% (5.72%); N, 9.84% (10.01%).

***(S)*-Ala-Leu-Val-Cyclophane^{2+}·2Br$^-$.** {*N*-[(*p*-(*S*)-Ala-Leu-Val-phenyl)methyl]-*N*′-[(*p*-(aminomethyl)phenyl)methyl]-4,4′-bipyridine}$^{2+}$(2Br$^-$) (0.765 g, 0.777 mmol) was converted to the 2PF_6^- salt as described above and dissolved in dry dimethylformamide (130 mL, 6 mM solution) at −15 °C under argon with stirring. *N*-Methylmorpholine (0.095 mL, 0.855 mmol) and isobutyl chlorocarbonate (0.111 mL, 0.855 mmol) were added to the solution followed, about a minute later, by triethylamine (0.119 mL, 0.855 mmol). The mixture was allowed to warm to room temperature and stirred in air overnight. The reaction was cooled to −15 °C again followed by addition of *N*-methylmorpholine (0.095 mL, 0.855 mmol), isobutyl chlorocarbonate (0.111 mL, 0.855 mmol), and triethylamine (0.119 mL, 0.855 mmol) to ensure complete coupling. After stirring the solution for an additional 48 h, the solvent was removed by evaporation at 50 °C at atmospheric pressure. The residue was dissolved in acetonitrile (100 mL), filtered, and precipitated with tetra(*n*-hexyl)ammonium bromide. After the solution was cooled at 3 °C for several hours, the solid was vacuum-filtered and washed with acetonitrile and diethyl ether to yield the pale yellow product (0.262 g, 40.4%): mp dec above 120 °C; ^{1}H NMR (C_2D_6OS) δ [9.60 (d, J = 6.5 Hz, 4H), 8.80 (d, J = 6.5 Hz, 4H)] (dipyridyl), [8.45 (2H, d), 8.20 (1H, d),7.92 (1H, d)] (four −NHCO), [8.02 (d, J = 7.0 Hz, 2H), 7.77 (d, J = 7.0 Hz, 2H), 7.62 (d, J = 7.0 Hz, 2H), 7.31 (d, J = 7.0 Hz, 2H)] (phenyl), [6.04 (s, 2H), 5.98 (s, 2H)] (two −N^+CH_2), 4.28 (m, 2H) (Ph−CH_2−NH), [4.40 (m, 1H), 3.60 (m, 1H), 2.80 (m, 1H)]

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(−CH amino acid chiral centers), 2.05 (m, 1H) (−CH valine), 1.60 (m, 1H) (−CH leucine), 1.42 (m, 2H) ($-CH_2$ valine), 1.17 (d, $J = 7.01$ Hz, 3H) ($-CH_3$ alanine), 0.80 (m, 12H) (two $-CH_3$ valine and two $-CH_3$ leucine); ^{13}C NMR (MeOD-d_4) δ [172.32, 170.08, 169.83, 169.56] (carbonyl), [152.38, 151.91, 148.88, 148.46, 128.31, 127.97] (dipyridyl carbons), [145.43, 144.35, 134.92, 134.50, 132.08, 131.82, 129.87, 129.35] (phenyl carbons), [66.82, 62.48] (methylene groups next to dipyridyl), [57.41, 51.38, 47.90] (chiral center carbons), 40.31 ($C-NH_2$), [31.92, 26.28, 23.14, 19.68, 19.03, 18.51] (amino acid side chains); positive ion FABMS $M^+ = 676.5$ (M without Br counterions). Anal. Calcd (found): C, 57.4% (57.0%); H, 5.74% (5.83%); N, 10.0% (9.93%).

The counterion was changed from Br^- to PF_6^- by dissolving the solid in water and precipitating with ammonium hexafluorophosphate. Anal. Calcd (found): C, 36.8% (37.23%); H, 3.07% (3.16%); N, 4.96% (4.97%).

Determination of Association Constants. The complexation of each guest was monitored by measuring the change in the chemical shift of the cyclophane protons upon mixing of the two components. To determine the association constants, a 5−10 mM stock solution of **1** was made in an appropriate deuterated solvent or mixture. An amount of solid guest was weighed out and dissolved in 1−5 mL of the cyclophane stock solution, depending on solubility. The guest solution was then titrated with aliquots from the cyclophane stock solution in order to keep the concentration of **1** constant while varying the guest concentration. Titrations were continued until no further changes were observed in the chemical shifts. A constant volume of 1.0 mL was used in the NMR tube for each titration. Reported association constants were calculated from the average of at least two (often 4) independent chemical shifts. The approximate equilibrium concentrations of each guest were calculated by the HOSTEST (v.5.0) program, and an error function was defined. The Newton method was then used to optimize the calculated concentrations and minimize the error function. Error limits for the association constants are determined by the program using Monte Carlo methods.

NOESY Experiments.[28] 2-D NOESY ^1H NMR experiments were performed at 300 K on a Bruker AMX-2−500 spectrometer operating in the quadrature mode at a ^1H resonance frequency of 500.13 MHz. ^1H 1-D reference spectra were obtained for 0.026 M (*R*)-DOPA, 0.026 M **1**, and 0.026 M (*S*)-DOPA, each prepared in a solvent mixture of deuterated acetone, DCl, and D_2O in 2:1:17 mole ratio. In all cases, the spectra were obtained with selective presaturation of the large residual HDO peak. The residual ^1H signal of deuterated acetone ($\delta(^1H) = 2.04$ ppm) was used as an internal reference. ^1H 1D spectra and phase sensitive 2D NOESY spectra were also obtained (with presaturation of the residual HDO peak) for solutions of 0.026 M cyclophane/0.026 M (*R*)-DOPA and 0.026 M cyclophane/0.026 M (*S*)-DOPA in the same deuterated solvent mixture. A mixing time of 500 ms was used in the NOESY experiments for each of the two solutions.

Intercalation Experiments. α-Zirconium phosphate was synthesized and intercalated with tetra(*n*-butyl)ammonium hydroxide (TBA^+OH^-) as previously described.[29] Elemental analysis showed 0.80 mmol of TBA^+ per gram of solid (C, 15.03%; H, 3.76%; N, 1.14%). In a typical intercalation procedure, 1.0 g of **1**·$2PF_6$ (1.0 mmol) in 20 mL of acetonitrile was added to 1.3 g of TBA-intercalated solid and shaken for 24 h. The solid was then filtered and washed with acetonitrile to ensure removal of excess **1**. Because there is a 2+ charge on **1**, each molecule of **1** should displace two TBA^+ ions. Elemental analysis showed complete displacement of TBA^+ by **1** to give 0.40 mmol **1** per gram of solid (C, 17.82%; H, 2.84%; N, 3.04%). X-ray powder diffraction (XRD) and diffuse reflectance UV−vis spectroscopy were also used to observe the intercalation of **1** into the solid. XRD showed a layer spacing of 14.5 Å with the intercalation compound of **1**. UV−vis of the cyclophane-intercalated solid showed a peak at 260 nm; the same peak was observed in the UV−vis spectrum of **1** in acetonitrile.

HPLC Separation of DOPA. The enantiomers of DOPA were separated on an analytical scale using a Crownpak CR-(+) column at room temperature. The mobile phase was a mixture of 30 mM perchloric acid and methanol (9:1 v/v) with a detection wavelength of 200 nm and a flow rate of 0.5 mL/min. The DOPA enantiomers were baseline resolved (6.61 min and 10.54 min, $\alpha = 1.59$, $R_s = 1.98$).

Acknowledgment. We thank Prof. Craig Wilcox of the University of Pittsburgh for providing a copy of the HOSTEST (5.0) program. This work was supported by a grant from the National Institutes of Health (GM 43844).

Supporting Information Available: ^1H NOESY data (3 pages). This material is contained in 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.

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