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Structure−Function Relationship of Amino Acid−[2]Rotaxanes

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Synthetic methodology was developed to construct amino acid−[2]rotaxanes that have phenylalanine and 3,5-di-*tert*-butylbenzene as blocking groups and dibenzo-24-crown-8, derivatized with either *N*-acetylargininyl or a carboxylic group, as the ring. A relative measure of the intramolecular interaction energies between the functional groups in DMSO/water mixtures is obtained by comparing their pK_a values. Rotaxane structures were investigated through 2D NMR analysis and molecular dynamics simulations. Association constants for complexes of amino acids and rotaxanes in various protonation states were determined in a variety of solvent systems by ^{1}H NMR analysis. The unique intracomponent interactions that exist in the rotaxanes and their ability to act as artificial receptors are discussed.

Introduction

The benefit of a convergent arrangement of functional groups for guest recognition—observed in protein binding domains—was demonstrated several years ago by Rebek in a series of classic studies of hosts that contain a U-shaped arrangement of diacids and their derivatives.[1] As a general method, however, it suffers because obtaining a precise alignment of functional groups for guest recognition can either be impossible to achieve or require extensive synthetic trial and error. One way to overcome these obstacles is to relax the rigid, preformed pocket rule[2] and allow artificial receptors to adopt favorable geometries upon guest binding. The loss in binding free energy caused by an entropically unfavorable energy term may not be too devastating,[3] and furthermore, hosts with greater flexibility may be designed to catalyze reactions. Many proteins, such as triosephosphate isomerase[4] and dihydrofolate reductase,[5−9] use mobile peptide loops to recognize substrates or enhance the rate of chemical transformations.[10,11] Some recently developed hosts have relied on a flexible component for guest association. A series of ferrocene-based receptors have a "ball bearing" that reorganizes upon binding,[12,13] and molecular clips and tweezers have been designed to use flexible arms for guest recognition.[14−16]

We present here a novel approach of using the rotaxane architecture to obtain hosts that have flexible, converging functional groups. Rotaxanes contain a cyclic compound threaded onto a linear molecule (axle), which has blocking groups on its ends to keep the ring from dethreading.[17−21] Rings can slide along the axle and perform a pirouette motion, and these unique motions are key features of novel nanoscale devices.[22−28] In our hosts, the ring motion

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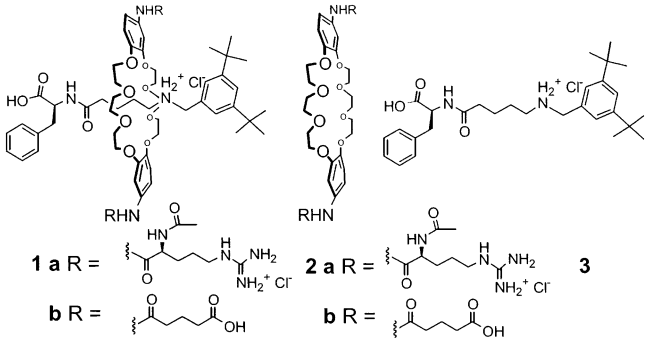
is used as a mimic of the peptide loops that cover protein binding sites. [2]Rotaxanes **1a,b** were constructed to test the ability of rotaxanes to perform as artificial receptors. The dibenzo-24-crown-8 (DB24C8) ring contains amino acids that could interact with the carboxylic acid of the phenylalanine blocking group to give additional structure to the rotaxane or interact with a guest.

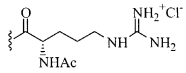
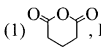
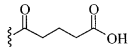
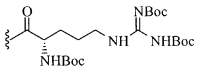
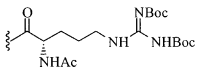


We wanted to determine whether the unique property of the ring, not covalently linked to the axle but with reduced freedom of motion, could provide strong intramolecular (called intracomponent) and intermolecular interactions. A comparison of the pK_a values of the rotaxanes and their separated components, e.g., **2a** + **3a**, is used to provide a relative measure of the strength of intracomponent interactions. Association constants (K_A) of [2]rotaxanes **1a,b** bound to amino acids provide a measure of their ability to form intermolecular interactions.

Results and Discussion

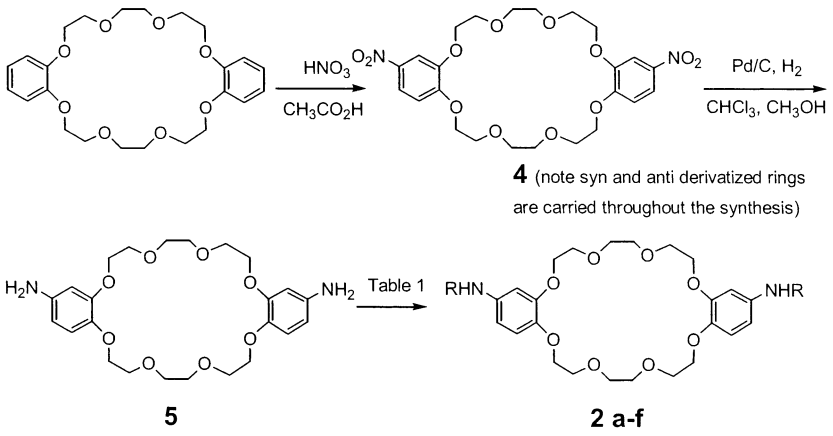
Synthesis of Rotaxanes. Rotaxanes **1a,b** were synthesized in good yields using the DCC-[2]rotaxane method developed in our research group.[29] We demonstrated that DCC-[2]rotaxanes can be constructed by the addition of DCC to pseudorotaxanes composed of DB24C8 rings threaded onto an axle containing an ammonium ion and carboxylic acid. This method needed to be further developed to provide rotaxanes that could be derivatized to give not only the artificial receptors described in this paper, but also other rotaxanes, especially host rotaxanes (see the following paper in this issue).

TABLE 1. Synthesis of Derivatized Crown Ethers

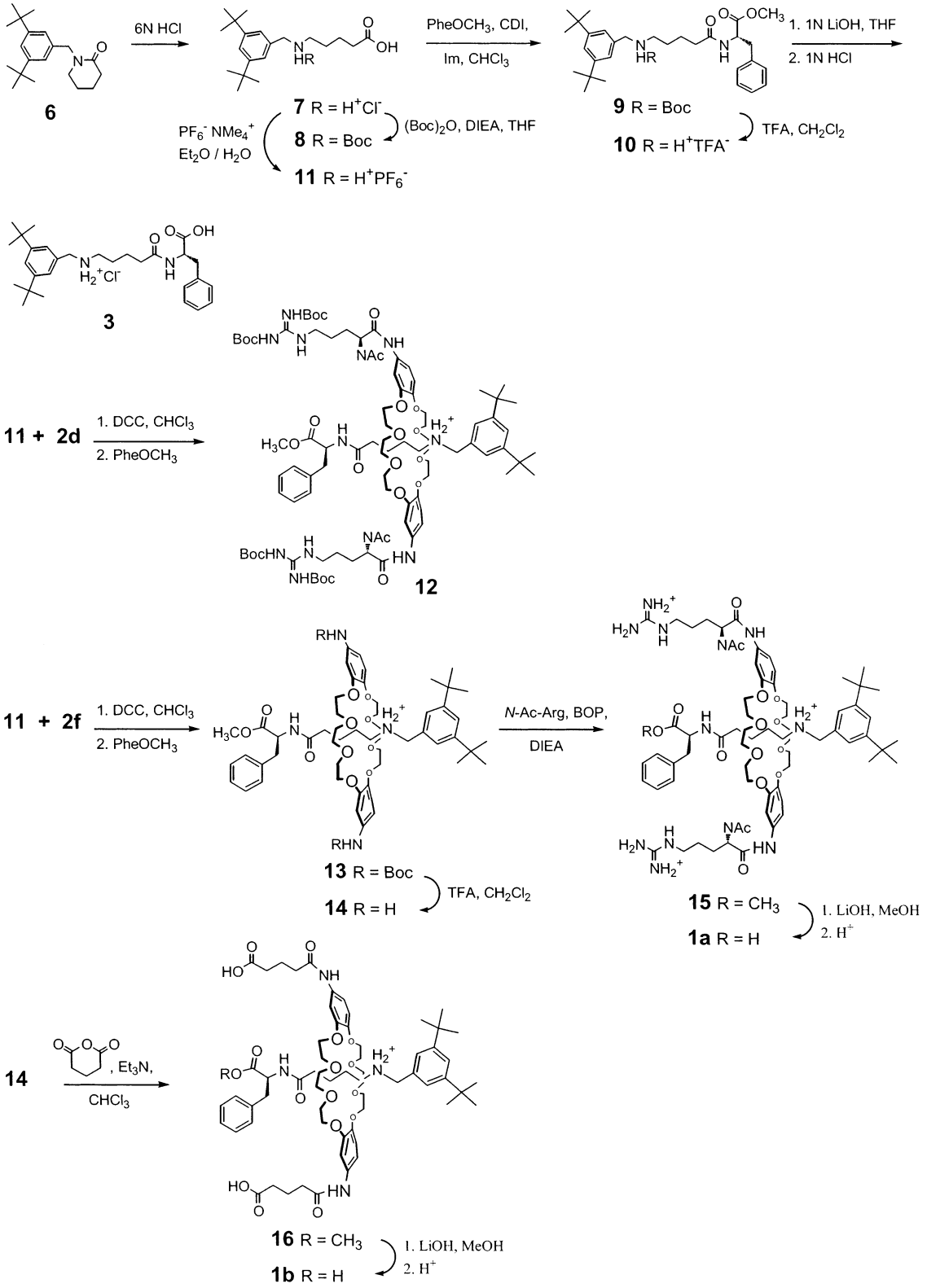
Cmpd	Reagent	R	yield
2a	BOP, N-Ac-ArgOH, DIEA, DMF		50%
2b	(1)  , Et_3N (2) HCl		78%
2c	CDI, $(Boc)_3$ArgOH, $CHCl_3$, reflux		55%
2d	CDI, $Ac(Boc)_2$ArgOH, $CHCl_3$, reflux		69%
2e	$(CF_3CO)_2O$, pyridine, CH_2Cl_2		99%
2f	From **4** $(Boc)_2O$, H_2, Pd/C, DMF		90%

One convenient way to make a variety of rotaxanes would be to use DCC-[2]rotaxanes that have derivatized DB24C8 rings. To test this possibility, a variety of arginine groups were attached to DB24C8 and converted into DCC-[2]rotaxanes. The synthetic route (Scheme 1) began with nitration of DB24C8, which produced a mixture of syn and anti constitutional isomers of dinitro-DB24C8 **4** (the syn isomer is shown throughout the text). Pd/C-catalyzed H_2 reduction of aromatic nitro derivatives is usually performed in a methanol or ethanol solution. However, dinitro-DB24C8 **4** is poorly soluble in these solvents; therefore, reduction was performed in a $CHCl_3$ and methanol solution in the presence of 10 mol % Pd/C under H_2. In this solvent system complete reduction to give diamino-DB24C8 **5** requires extended reaction times (12−36 h), which also results in the partial decomposition of the product. Crown ether **5** also decomposes upon exposure to air, elevated temperatures, and purification using flash column chromatography. Therefore, it was not purified prior to being coupled with the various arginine derivatives (Table 1). CDI-catalyzed coupling reactions with $(Boc)_3$-Arg-OH and Ac-$(Boc)_2$-Arg-OH yielded crown ethers **2c** and **2d**, respectively, in 50−70% overall yields. The minimally protected *N*-Ac-Arg-OH·HCl was attached to give crown ether **2a** in DMF since the amino

SCHEME 1



SCHEME 2



acid is insoluble in $CHCl_3$, tetrahydrofuran, or acetonitrile. In addition, the more reactive coupling reagent BOP was necessary to facilitate the reaction. Crown ether **2e** was obtained in quantitative yield by reacting crown ether **5** with trifluoroacetic anhydride in pyridine. The addition of glutaric anhydride to crown ether **5** gave crown ether **2b**, which was used in comparison studies.

For [2]rotaxane formation (Scheme 2), slight modifications to the previously described method[29] were necessary. A derivatized crown ether was used, and the axle was synthesized through acid hydrolysis of lactam **6**.

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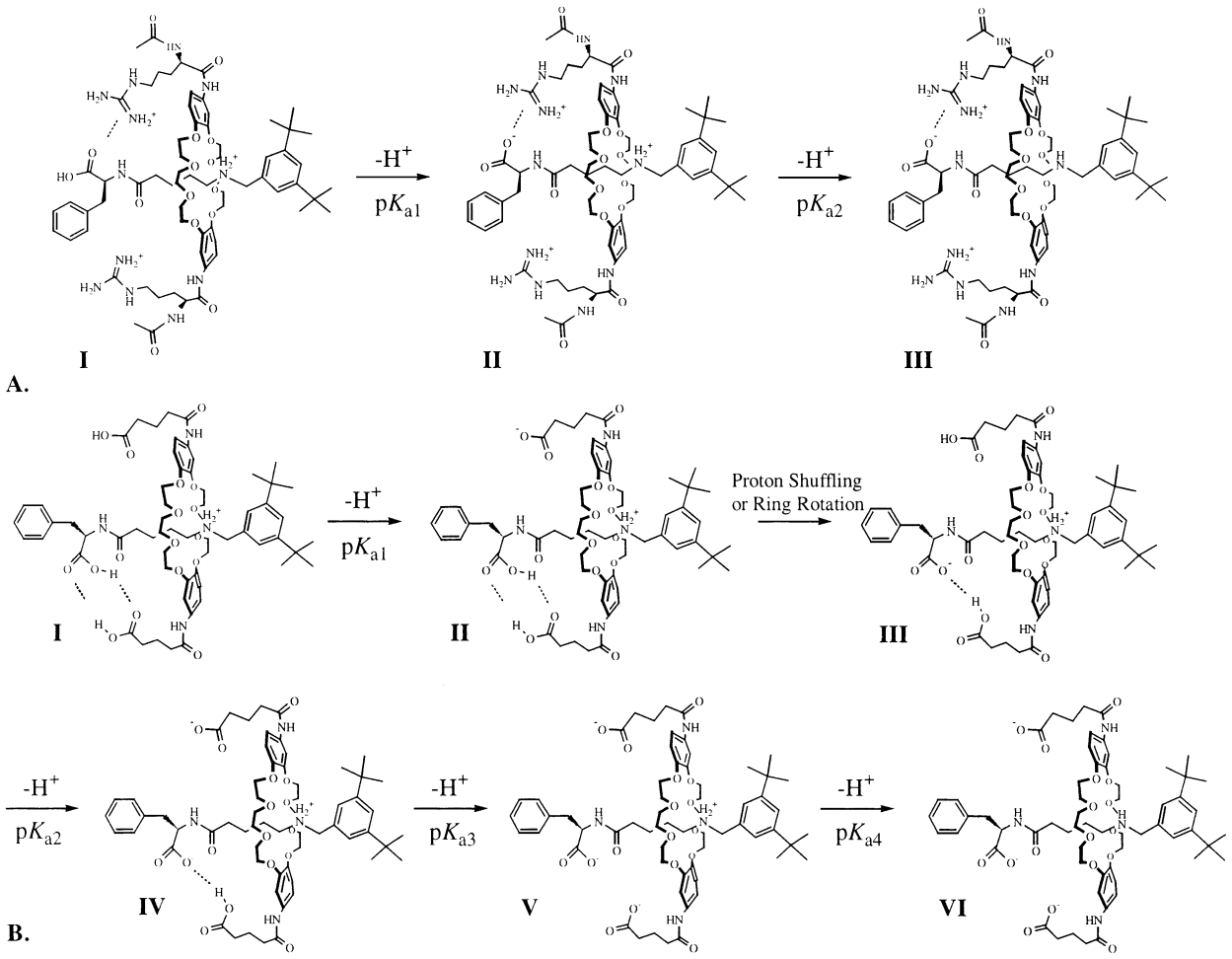

FIGURE 1. (A) Potential intramolecular interactions (indicated by dashed lines) for [2]rotaxane **1a** include a dipole−ionic interaction for its fully protonated form **AI** and a salt bridge for its monocarboxylate state **AII**. (B) Depending on the pH, a variety of potential interactions could exist for [2]rotaxane **1b**. In **BI**, the fully protonated carboxylic acids could exist as a dimer. For the monocarboxylate states **BII** and **BIII**, the rotaxane could still have dimeric acids (**BII**) or, more likely, a single-H-bonded complex (**BIII**), which forms through proton shuffling or rotation of the ring. In **BIV**, a single-H-bonded structure for its dicarboxylate state and, for **BV**, no favorable interactions should occur between the carboxylates.

Amino acid **7** provided the axle component **11** after counterion exchange of Cl^- with PF_6^-. It was also used to construct the axle component **3** by first protecting the amine with Boc, coupling with Phe-OMe, deprotection, and then hydrolysis. According to ^1H NMR analysis, rings **2c,d** were threaded onto axle **11**. However, attempts to make [2]rotaxane **12**, through the addition of DCC to a mixture containing axle **11** and ring **2d**, followed by Phe-OMe and Et_3N, gave only a small amount of the desired product. Apparently, the greater size of the derivatized arginine rings produced extensive steric congestion during the coupling step. A smaller ring that contained TFA-protected amino groups (crown ether **2e**) was tried, but the poor solubility of the ring precluded formation of a DCC-[2]rotaxane. The *N*-Boc-protected ring **2f** was soluble in $CHCl_3$ and gave good yields of DCC-[2]rotaxane (up to 70%), which are similar to the yields obtained for reactions with DB24C8. *N*-Acetylphenylalanine methyl ester was added to the DCC-[2]rotaxane to give the Boc-protected rotaxane **13** in a 72% yield. Deprotection of the amines with 30% TFA in CH_2Cl_2 proceeded smoothly to give [2]rotaxane **14**. The addition of *N*-acetylarginine (activated with BOP) or glutaric anhydride to [2]rotaxane **14**, followed by ester hydrolysis, produced the artificial receptors **1a** (56%) and rotaxane **1b** (76%), respectively.

Investigation of Intramolecular Interactions. Differences in the pK_a values of the functional groups displayed in the rotaxanes and the ones free in solution are used to provide a relative measure of the interaction energies within the rotaxanes. Greater interactions, whether favorable or unfavorable, will produce greater differences.[30−32] Accordingly, the pK_a of the carboxylic acid of the phenylalanine moiety will be lowered if a salt bridge forms in [2]rotaxane **1a** or a H-bond forms in [2]-rotaxane **1b** (Figure 1). In a similar manner, the pK_a of the ammonium ion of the axle should be raised if it forms a complex with the crown ether's oxygen atoms through favorable cation−dipole interactions. The pK_a values of the DB24C8 derivatives **2a,b** and axle **3** were determined to provide baseline pK_a values for the functional groups

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TABLE 2. pK_a Values of the Compounds and Compound Mixtures in Different Ratios of DMSO and Water Measured at 25 °C

compd(s)	acidity constant [a]	DMSO/water 90/10	60/40	50/50	40/60
1a	pK_{a1}	4.0 ± 0.1	4.4 ± 0.1	4.01 ± 0.05	4.10 ± 0.07
1b		8.11 ± 0.04	5.65 ± 0.05	5.1 ± 0.1	4.53 ± 0.06
2b		10.78 ± 0.03	7.29 ± 0.04	6.50 ± 0.03	5.84 ± 0.04
3		9.07 ± 0.06	5.9 ± 0.2	5.17 ± 0.05	4.38 ± 0.07
2a + **3**		9.06 ± 0.05	5.66 ± 0.04	5.09 ± 0.07	4.67 ± 0.05
2b + **3**		8.86 ± 0.07	5.74 ± 0.07	5.2 ± 0.1	4.56 ± 0.05
N-Boc-Phe		7.8 ± 0.2	6.08 ± 0.08	5.6 ± 0.1	4.99 ± 0.04
1a	pK_{a2}	8.4 ± 0.1	10.5 ± 0.5[b]	10.3 ± 0.5[b]	9.5 ± 0.5[b]
1b		9.22 ± 0.03	6.59 ± 0.05	6.34 ± 0.06	5.68 ± 0.02
3		10.8 ± 0.1	9.89 ± 0.03	9.80 ± 0.09	9.66 ± 0.02
2a + **3**		10.70 ± 0.07	8.4 ± 0.2	8.2 ± 0.1	8.1 ± 0.1
2b + **3**		10.52 ± 0.04	7.09 ± 0.01	6.30 ± 0.01	5.62 ± 0.07
1b	pK_{a3}	9.89 ± 0.01	9.5 ± 0.2	9.3 ± 0.2	9.3 ± 0.2
2a + **3**		ND[c]	ND	ND	ND
2b + **3**		10.56 ± 0.05	7.79 ± 0.01	7.16 ± 0.01	7.3 ± 0.3
1b	pK_{a4}	ND	13 ± 0.5[d]	12 ± 0.5[d]	12 ± 0.5[d]
2b + **3**		11.4 ± 0.3	10.1 ± 0.1	10.0 ± 0.1	9.9 ± 0.1

[a] pK_a values were calculated using the BEST program.[33] [b] pH values observed with the addition of 1.5 equiv of base to the assay solutions. [c] ND means not detected, because of the limitation of the electrode in the solvent mixture. [d] pH values observed with the addition of 3.5 equiv of base to the assay solutions.

when they are extensively free in solution. An intramolecular salt bridge could form in axle **3** once its acid is deprotonated. pK_a values were also determined for equal molar solutions of ring **2a** or **2b** with axle **3**. Substantial differences in the pK_a values of the rotaxanes **1a,b**, as compared to the rotaxane parts **2** and **3**, whether alone or combined in a solution, would demonstrate that additional free energy is available for noncovalent bond formation between functional groups held together by the intracomponent interactions of rotaxanes.

The strength of noncovalent interactions is highly dependent on the environment around the groups involved in the complex. For these studies, mixtures of DMSO and water were used as the solvent to solubilize the compounds and, more importantly, to determine the strength of the noncovalent interactions in an aqueous environment. The long-term goal of these studies is to use the rotaxane architecture to create artificial receptors that operate in the biological milieu.

Examination of the pK_a values (Table 2) shows that strong interactions do exist between some of the functional groups of the rotaxanes. The pK_{a1} of [2]rotaxane **1a** is substantially smaller than the pK_{a1} values of [2]-rotaxane **1b**, *N*-Boc-phenylalanine, the derivatized rings **2a,b**, and axle **3** (Figure 2). We assign the pK_{a1} of [2]-rotaxane **1a** and axle **3** to the carboxylic acid of the phenylalanine moiety. The pK_a values of the axle ammonium ions for rotaxanes developed in our laboratory generally are abnormally large because of their favorable interactions with the ring's oxygen atoms. For example, [2]rotaxane **1a**, as a methyl ester, has a pK_a of 14.6 ± 0.1 in a 90/10 (v/v) DMSO/water solution. Moreover, the pK_{a1} of axle **3** is more similar to the pK_a of the acid of *N*-Boc-Phe in this solvent system. Because the pK_{a1} of axle **3** is approximately unchanged in the presence of the derivatized ring **2a** or **2b**, intermolecular interactions between these compounds in DMSO/water mixtures are weak and the intracomponent interactions of [2]rotaxane **1a** are more favorable than the intermolecular interactions between the ring and axle as separate components.

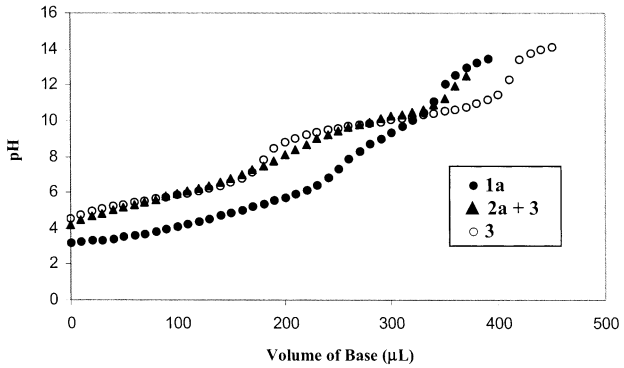

FIGURE 2. Representative potentiometric titration plots in 40/60 (v/v) DMSO/H_2O solutions at 25 °C. The curve of [2]rotaxane **1a** is substantially shifted to lower pH values compared to those of the axle **3** and crown **2a** plus axle **3**, which are more similar. Lowering of the titration curve for [2]rotaxane **1a** is consistent with a strong intramolecular salt bridge. pH values were not corrected.

Thus, the low pK_{a1} of [2]rotaxane **1a** is caused by the incorporation of the functional groups in a rotaxane.

The second pK_a of [2]rotaxane **1a** is assigned to the ammonium ion of the axle, and its magnitude is much lower than the pK_{a1} of [2]rotaxane **1a** as the methyl ester ($\Delta pK_a = 6$ in 90/10 (v/v) DMSO/water). One explanation for the small pK_a values measured for [2]rotaxane **1a** is that the favorable salt bridge (lowers pK_{a1}) forces the ring away from the ammonium ion (lowers pK_{a2}). This structure was obtained in molecular modeling simulations and supported by ^{1}H NMR chemical shift values vide infra. These results suggest that intracomponent interactions may be fine-tuned by adjusting the position of the ammonium ion in the axle or by changing the length of the side chain on the ring.

The pK_{a1} of [2]rotaxane **1a** is approximately unchanged with the addition of water to the solutions, whereas the other pK_a values shown in Table 2 are reduced in the presence of water from 10% to 60%. Water stabilizes the

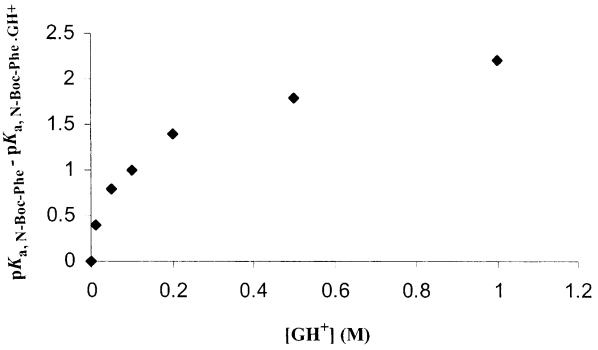

FIGURE 3. pK_a values of *N*-Boc-Phe are reduced by the presence of GH^+ in 90/10 (v/v) DMSO/water solution at 25 °C. This calibration curve was used to derive $\Delta pK_{a,sat}$ of *N*-Boc-Phe for a theoretical saturated solution of GH^+.

ions better than DMSO, which causes the reduction in the pK_a values.[34] The smaller solvent effect observed for [2]rotaxane **1a** is consistent with the ions being confined in a complex, shielding them from solvent molecules. Even though pK_{a1} stays near 4.0, the intracomponent interaction becomes weaker with the addition of more water. The magnitude of ΔpK_{a1} (obtained by subtracting the pK_{a1} of [2]rotaxane **1a** from that of axle **3**) is diminished. A similar insensitivity to a change in solvent is observed for the pK_{a3} of [2]rotaxane **1b**. Accordingly, strong intramolecular interactions should exist for the dicarboxylate state (**BIV**, Figure 1) of this rotaxane. Although studies in pure water were not attempted because of the limited solubility of the rotaxanes, it appears that in water intracomponent interactions between the charged groups would be greatly weakened.

A more quantitative measure of the interaction energy between the carboxylate and the arginine functional groups of [2]rotaxane **1a** was sought. Changes in pK_a values caused by changes in the concentration of ions have been used to provide a measure of the strength of a salt bridge formed between the ions.[30,32] The degree of association between *N*-Boc-Phe and guanidinium chloride (GH^+) was chosen as a model system to provide a measure of the energy difference between the intracomponent interactions of [2]rotaxane **1a** and intermolecular interactions. A 90/10 mixture of DMSO/water proved to be a suitable solution for the experiment because the extent of salt bridge formation changed significantly with the addition of GH^+ from 0.1 to 1.0 M. As $[GH^+]$ increased, the pK_a of *N*-Boc-Phe dropped until a saturation value was reached. At a 1.0 M concentration of GH^+, a pK_a value of 5.8 was measured for *N*-Boc-Phe. Because the pK_{a1} of [2]rotaxane **1a** is lower ($pK_{a1} = 4.0$) in the same solvent mixture, the effective molarity of its guanidinium moiety for salt bridge formation is greater than 1.0 M.

To determine a theoretical pK_a for *N*-Boc-Phe in a saturated solution of GH^+, a calibration curve was generated by plotting the changes in the pK_a of *N*-Boc-Phe against changes in the concentration of GH^+ (Figure 3). The curve was fitted to a standard binding equation for a two-component system using a nonlinear least-squares procedure,[35] giving a K_A of $8 \pm 2\ M^{-1}$ and $\Delta pK_{a,sat}$ of 2.4 ± 0.1 for the salt bridge. Changes in the dielectric constant of the solution with the addition of GH^+ were ignored. A $pK_{a,sat}$ value of 5.4 is derived ($pK_{a,sat} = pK_{a,N\text{-Boc-Phe}} - \Delta pK_{a,sat}$), which is the theoretical pK_a for *N*-Boc-Phe exposed to an infinite concentration of GH^+. The fact that the pK_{a1} of [2]rotaxane **1a** ($pK_{a1} = 4.0$) is 1.4 pK_a units below $pK_{a,sat}$ shows that the unique threaded architecture of the rotaxane greatly reduces the entropic loss of bringing functional groups together.

We use the term intracomponent to describe the interactions between the functional groups of the rotaxanes. The magnitude of this interaction is further revealed once the pK_a of [2]rotaxane **1a** is compared to the pK_a values observed for Phe ($pK_{a1} = 5.9$) and *N*-Boc-Phe ($pK_a = 7.8$); all studies were performed in a 90/10 (v/v) DMSO/water mixture. The pK_a of [2]rotaxane **1a** ($pK_a = 4.0$) is more similar to the one observed for Phe than that for *N*-Boc-Phe. On the other hand, the pK_a of tether **3** alone ($pK_{a1} = 9.1$) or combined with ring **2a** ($pK_{a1} = 9.1$) is more similar to that of *N*-Boc-Phe. The lower pK_a of Phe compared to *N*-Boc-Phe stems from the intramolecular interaction between the functional groups that are held in close proximity via covalent bonds. Therefore, the functional groups of [2]rotaxane **1a** behave more like they are linked covalently than noncovalently.

Examination of the pK_a values of [2]rotaxane **1b** shows that strong intracomponent interactions occur for this rotaxane, as well. The fully protonated state can be stabilized by a H-bonded dimer between two carboxylic acids (Figure 1, **BI**). However, the strength of this interaction is diminished in H-bonding solvents such as DMSO, and especially water.[36,37] If the carboxylic acid dimer does form, then the remaining free acid should have the most acidic proton and be deprotonated first in the potentiometric titrations. If the resulting carboxylate remains free, giving structure **BII**, the pK_{a1} of [2]rotaxane **1b** should match the pK_a of the ring **2b**. Because pK_{a1} is lower (by ca. 1.5 units throughout the solvent mixtures), the carboxylic acid of the rotaxane does not remain free. Deprotonation leads to a more stable state, which is most likely the single-H-bonded complex (**BIII**). This conformer can be reached by either a shuffling of the protons or rotation of the ring. The pK_{a2} of [2]rotaxane **1b** is more similar to the pK_a of ring **2b**, and these values become more similar with a higher percentage of water. Thus, pK_{a2} fits the profile of a free acid (**BIII** going to **BIV**, Figure 1). For [2]rotaxane **1b**, the greatest anomaly is observed for pK_{a3}. It is substantially larger than the other pK_a values of the acids, and its value does not change significantly with a higher percentage of water. Both results are consistent with an acid that forms a stable intracomponent H-bond (**BIV**), as a result of existing in a rotaxane. pK_{a4} is quite large, and assigned to the ammonium ion of the axle. Its acidity is significantly weaker than the ammonium ion observed for [2]rotaxane **1a**. As discussed previously, the large pK_a suggests that

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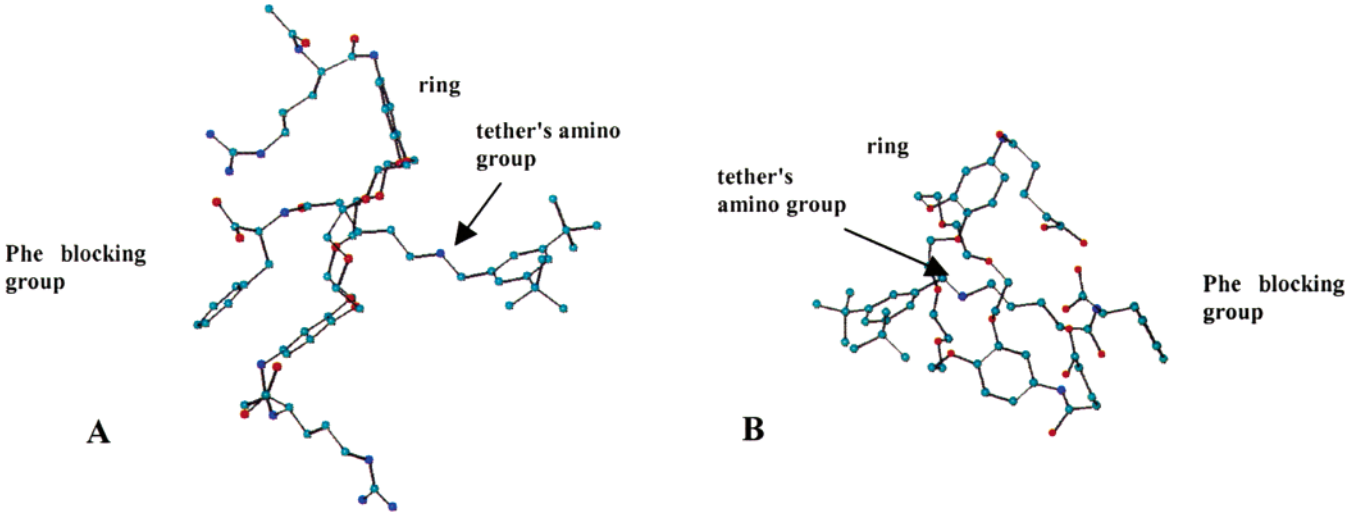

FIGURE 4. Lowest energy structures (molecular dynamics simulations, MM+ force field as presented by Hyperchem[38]) of (A) [2]rotaxane **1a** and (B) [2]rotaxane **1b**, both in a monocarboxylate state. The carboxylate of the phenylalanine blocking group, for [2]rotaxane **1a**, forms a salt bridge with the guanidinium moiety and, for [2]rotaxane **1b**, H-bonds to both carboxylic acids of the ring. Another important feature is that the ring is not centered over the ammonium ion of the tether in [2]rotaxane **1a**, whereas, in [2]rotaxane **1b**, the ring is centered over the ammonium ion. H-atoms are removed for clarity.

the crown ether oxygen atoms interact strongly with the ammonium ion.

Structure Determination. Molecular dynamics simulations were performed (MM+ force field as presented by Hyperchem[38]) to determine whether the functional groups can interact simultaneously; the crown ether is seated over the ammonium ion of the axle while its side chain interacts with the carboxylate of the phenylalanine blocking group. The lowest energy conformations of the rotaxanes (Figure 4), in the monocarboxylate state, showed that both rotaxanes **1a** and **1b** should form the predicted bonds depicted in Figure 1. However, the lowest energy conformation of [2]rotaxane **1b** has both carboxylic acids of the crown ether forming H-bonds with the carboxylate of the axle. Because the pK_{a2} of [2]rotaxane **1b** is only slightly smaller than the pK_a of ring **2b**, if the third carboxylic acid does form a complex, the interactions are weak. In the lowest energy structure of [2]rotaxane **1a**, both arginines of [2]rotaxane **1a** most likely do not interact with the carboxylate simultaneously. Forcing all three functional groups together produced structures with energy values substantially larger than the global minimum.

The modeling results support the hypothesis that the ammonium ion interacts weaker with the crown ether of [2]rotaxane **1a** than that of [2]rotaxane **1b**. In the lowest energy conformer of [2]rotaxane **1a**, the crown ether is not centered over the ammonium ion and the oxygen atoms are too far away for strong interactions ($-(CH_2)_2N^+\cdots O(CH_2)_2-$, 4.8−6.0 Å), whereas, in [2]rotaxane **1b**, the crown ether is centered over the ammonium ion, forming multiple ion−dipole interactions ($-(CH_2)_2-N^+\cdots O(CH_2)_2-$, 2.8−4.5 Å). The lowest energy structure of [2]rotaxane **1b** in the dicarboxylate state has approximately the same structure as the monocarboxylate state (Figure 4B) except that the protonated acid of the ring interacts with the carboxylate of the phenylalanine blocking group. The dicarboxylate structure is relevant to the argument that the larger pK_a determined for the ammonium ion of [2]rotaxane **1b**, as compared to [2]rotaxane **1a**, is due to the favorable interactions between the ammonium ion and the oxygen atoms of the ring.

Further evidence for the different positions of the crown ether ring is obtained by examining the chemical shifts of the axle protons. Threading of the ring produces a dramatic downfield shift of the benzylic protons of the axle, which is caused by interactions between the oxygen atoms of the crown ether and the ammonium ion. In DMSO-d_6, the benzylic protons of the free axle **11** reside at 4.04 ppm. Rotaxane formation shifts this resonance downfield to 4.56 ppm for di-Boc-[2]rotaxane **13** and [2]-rotaxane **1b** in its monocarboxylate state. The benzylic protons lie between 4.36 and 4.49 ppm for [2]rotaxane **1b** in its other protonation states. However, the chemical shift of the benzylic protons of [2]rotaxane **1a** (4.04 ppm, Figure 5B) matches those of the free axle **11**.

The structures of rotaxanes **1a,b** were also investigated in DMSO-d_6 solutions using 2D NMR analysis. We looked particularly for cross-peaks between the crown ether and the axle to find the preferred position of the ring. [2]Rotaxane **1a**, as the carboxylate, existed as a single conformer and had extensive structure as evident by multiple key NOEs found between the crown ether's $-CH_2-$ and the benzylic and aromatic protons of the axle's blocking group (Figure 5). NOEs also existed between the β-protons of the arginine side chain and the α-protons of the axle and between the benzylic protons of the phenylalanine blocking group and the anilinic N−H of the crown ether. These NOEs are consistent with the calculated lowest energy conformation of [2]rotaxane **1a** (Figure 4). [2]Rotaxane **1b** had two conformers in the fully protonated state in a 70/30 ratio. As the pH of the solution increases, one conformer disappears and [2]rotaxane **1b** exists as a single conformer in the tricarboxylate state. The minor conformer had NOEs between the crown ether's $-CH_2-$ and the axle, whereas the major conformer did not have any observable NOEs between the rotaxane components. These results are consistent with

(38) Hypercube, Inc., Gainesville, FL.

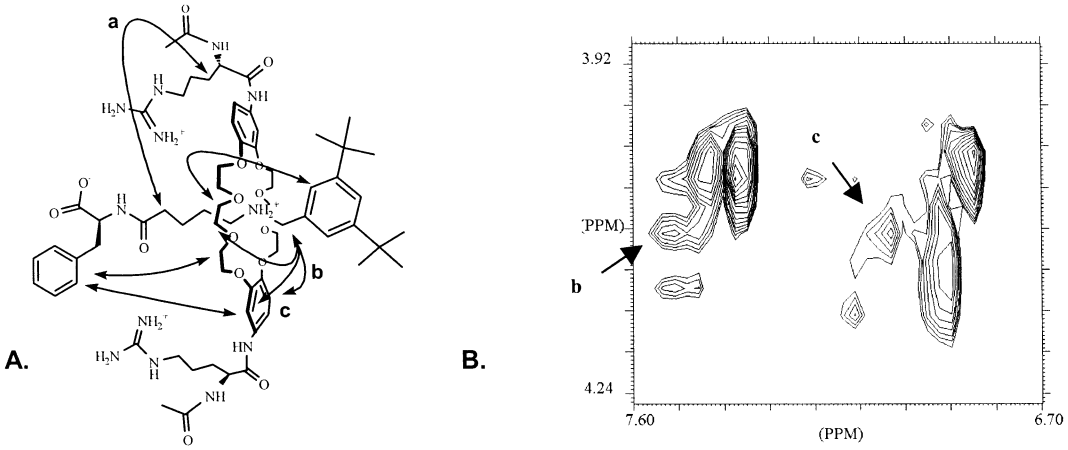

FIGURE 5. (A) Key NOEs between the ring and the axle of [2]rotaxane **1a** observed in DMSO-d_6 are indicated by double-headed arrows. An NOE (a) which exists between the arginine derivative and the axle is a strong indicator of a salt bridge between the carboxylate of the phenylalanine blocking group and the guanidinium moiety of the ring. (B) A portion of the NOESY spectrum that shows the cross-peaks between the benzylic protons of the 3,5-di-*tert*-butylphenyl blocking group and the aromatic protons of the ring (b and c).

TABLE 3. Association Constants of the Hosts Bound to *N*-Ac-Arg-OMe, Determined at 25 °C[a]

host[b]	solvent	counterion	K_A (M^{-1})	host	amt of Me_4NOH (equiv)	K_A (M^{-1})[c]
1b	DMSO-d_6	$(Me)_4N^+$	393 ± 22	**1b**	0	NB
	DMSO-d_6	Li^+	96 ± 14		1	55 ± 7
	DMSO-d_6/H_2O (90/10)	$(Me)_4N^+$	404 ± 27		2	474 ± 27
	DMSO-d_6/H_2O (90/10)	Li^+	66 ± 6		3	404 ± 27
	MeOH-d_4	$(Me)_4N^+$	62 ± 9	$AcCO_2^-$	1	114 ± 17

[a] Association constants were obtained by performing binding titrations using ^1H NMR spectroscopy. [b] Hosts were tricarboxylates except for the assay performed in methanol, where the host was the dicarboxylate. [c] Assay solutions contained DMSO-d_6/water in a 90/10 ratio.

the pK_a results that suggest [2]rotaxane **1b** has weaker intramolecular interactions than [2]rotaxane **1a**.

Rotaxanes as Hosts. [2]Rotaxane **1a** was tested as an artificial receptor for phenylalanine and alanine. These guests were chosen because the charges of their ionized functional groups match the ones displayed on the rotaxane. Phe and Ala were both used to have guests that were soluble in low and high concentrations of water, respectively. No changes were observed in the chemical shifts of Phe, Ala, or [2]rotaxane **1a** in the ^1H NMR spectra of solutions (100% DMSO-d_6, 90/10, 60/40, and 40/60 (v/v) DMSO-d_6/H_2O) containing both components at 5 mM concentrations when compared to the spectra of pure amino acid and [2]rotaxane **1a** in the same solvent systems. Thus, the compounds do not associate or only associate weakly. This result is not surprising. For a guest to bind, the strong internal salt bridge of [2]-rotaxane **1a** would have to be broken. To ensure that the amino acids and rotaxanes were fully ionized, the pH values of the solutions were titrated to approximately the pI of the components ($pH_{exptl} = 8.5$). No buffers were used to reduce the possibility of the buffers competing for the binding sites.

[2]Rotaxane **1b**, as the tricarboxylate with Me_4N^+ counterions, strongly binds *N*-acetylarginine methyl ester (*N*-Ac-Arg-OMe) in DMSO-d_6 (Table 3). Being fully deprotonated, intramolecular interactions between the acids do not exist. Thus, unlike the case for [2]rotaxane **1a**, intramolecular interactions do not have to be broken for guest association. The highly soluble Me_4N^+ also enhances association. Changing the counterion to Li^+ resulted in a pronounced lowering of K_A, which is consistent with a stronger ion pair. The addition of 10% H_2O to the solutions did not significantly change K_A, but at higher concentrations of water, e.g., 60/40 (v/v) DMSO-d_6/H_2O, complex formation could not be detected. Thus, one problem with these rotaxanes is that, as the percentage of water is increased, the extent of complex formation is greatly diminished. These results reveal the necessity of a hydrophobic pocket for high-affinity recognition in water.

The extent of association of [2]rotaxane **1b** and *N*-Ac-Arg-OMe should depend on the protonation state of the rotaxane. Binding assays were performed with [2]rotaxane **1b** existing in the mono-, di-, and tricaboxylate states in 90/10 (v/v) DMSO-d_6/H_2O solutions. No association was detected between *N*-Ac-Arg-OMe and [2]rotaxane **1b** in the fully protonated state. Having a single carboxylate, [2]rotaxane **1b** weakly associated ($K_A = 55\ M^{-1}$) with the guest. [2]Rotaxane **1b**, as the dicarboxylate, has the highest affinity for *N*-Ac-Arg-OMe ($K_A = 474\ M^{-1}$), slightly better than in the tricarboxylate state ($K_A = 404\ M^{-1}$). The additional binding free energy observed for the dicarboxylate state may arise through a H-bonded complex between the protonated acid and the amide of the guest. The N−H amide proton shifted downfield with the addition of [2]rotaxane **1b**, which is consistent with H-bonding. Another possibility is that the additional negative charge of the tricarboxylate species may bring more water to the combining site and weaken the complex.

Further evidence for the structures of [2]rotaxane **1b** in its various protonation states (Figure 1) is obtained by comparing the association constants of [2]rotaxane **1b** and acetate bound to *N*-acetylarginine. [2]Rotaxane **1b** in the di- or tricarboxylate state (the appropriate amount of Me_4NOH was added to the solutions) associates with

N-Ac-Arg-OMe approximately 4 times greater than acetate in 90/10 (v/v) DMSO-d_6/water solution. The larger K_A reveals the energetic advantage for complexation of having the functional groups displayed within a rotaxane versus separated in solution. On the other hand, [2]-rotaxane **1b** as the monocarboxylate associates with *N*-Ac-Arg-OMe only half as likely as acetate. This smaller K_A supports our hypothesis that state **BIII** (Figure 1) is more stable than state **BII**. If the free carboxylate of **BII** predominated in solution, the monocarboxylate form of [2]rotaxane **1b** would have a K_A for *N*-Ac-Arg-OMe similar to the one obtained for acetate. For the dicarboxylate state **BIV**, we suggest that a salt bridge first forms with the guest through its free carboxylate and then a dimeric salt bridge forms. A shuffling of the protons would need to occur after the guest forms the first salt bridge before the second carboxylate interacts with the guest. A more intriguing possibility is that the ring rotates to accommodate the guest in accordance with the machinelike nature of rotaxanes.[22−28]

The question remains as to whether the mobile functional groups of [2]rotaxane **1b** enhance guest recognition by adjusting the groups for maximum contact with the guest or decrease guest recognition through the loss of rotational and translational motion of the ring. One way to answer this question is to compare the association constants obtained in this study to K_A values for complexes of arginine bound to hosts that have rigidly held carboxylates as the recognition elements. Unfortunately, there are only a few hosts designed to bind arginine. This paucity is surprising considering the propensity of arginine in protein domains[39] and the importance of arginine recognition in biological processes, such as in the universal recognition sequence RGD.[40−42] Hosts that bind arginine derivatives through salt bridge formation in DMSO-d_6 with diphosphates or triphosphates as the recognition elements have association constants approximately 5 times larger than that of [2]rotaxane **1b**.[43−45] We have developed a diacid template that bound *N*-Ac-Arg-OMe in DMSO-d_6 with an association constant 5 times weaker ($K_A = 70\ M^{-1}$) compared to that of [2]-rotaxane **1b**.[32] However, the acids of the template were not convergent. A dicarboxylate template developed by Eliseev[46] is probably the best host to use as a comparison. One form of this host has a rigid placement of carboxylates that recognize the guanidinium ion with a $K_A = 170\ M^{-1}$ in MeOH-d_4. A similar association constant was derived for [2]rotaxane **1b** bound to *N*-Ac-Arg-OMe in the same solvent ($K_A = 62 \pm 9\ M^{-1}$). Because a rotaxane can associate with a guest in a manner comparable to that of a highly preorganized, rigid host, there is not a large entropic loss associated with association.

Conclusion

The rotaxanes performed according to their predicted properties. [2]Rotaxane **1a** formed a very strong intramolecular salt bridge, giving it extensive structure. [2]-Rotaxane **1b** had weaker intramolecular interactions, allowing it to perform as an artificial receptor as evident by its association with *N*-Ac-Arg-OMe in methanol, DMSO, and DMSO/water mixtures. The demonstrated properties of the rotaxanes suggest that future rotaxanes may be designed to incorporate both structure and function to make highly selective hosts in the manner of proteins. One further piece that needs to be incorporated is a hydrophobic domain to increase the association constants in aqueous environments. These rotaxanes have been constructed, and their properties are reported in the following paper in this issue.[47]

Experimental Section

General Information. All syntheses were carried out under positive Ar pressure, and the solvents were freshly distilled under vacuum over a suitable drying agent. All reagents were used as purchased. The melting points of the compounds are given uncorrected. For potentiometric titrations, 5 mM solutions of the templates were prepared in freshly distilled DMSO and boiled water. These solutions were titrated under Ar with a 0.10 M solution of Me_4NOH in DMSO. The pK_a values of the templates were calculated using the BEST program.[33]

NMR Experiments. NMR spectra used for product identification and conformational analysis were measured in $CDCl_3$ or DMSO-d_6 using a spectrometer operating at 400.14 MHz for proton nuclei and 100.23 MHz for carbon nuclei or one operating at 62.9 MHz for carbon nuclei and 250 MHz for proton nuclei. Chemical shifts are in parts per million and are referenced using an internal TMS standard. NOESY experiments were obtained at 298 K: mixing time 150 and 300 ms, FID acquisition time 0.15 s, SW = 5800 Hz, 2K data points, 512 increments each with 8 transients per FID. TOCSY experiments were run with a 300 ms mixing time, L1 = 118, and a low-power pulse of 15 dB. ROESY experiments were obtained at 298 K with a cw pulse power of 30 dB and a mixing time of 400 ms. A QSINE window function was applied in both dimensions with ssb = 2, and the data were phased in the usual fashion.

For the structural determination, the rotaxanes were investigated in DMSO-d_6 as the monocarboxylate, obtained by the addition of 1 equiv of Me_4NOH to the solutions. [2]-Rotaxane **1b**'s structure was also investigated with an increase in the pH of a 90/10 (v/v) DMSO/H_2O solution. The pH values of these solutions were set using a potentiometer.

Calculated Structural Determination. Global minimum structures of the rotaxanes were determined through molecular dynamic simulations using the MM+ force field as presented by Hyperchem (Hypercube, Inc., Gainesville, FL). A series of conformers were generated for each template by heating them to 800 K over 1 ps. At this temperature, interactions between the functional groups were periodically broken, and the crown ether and amino acid moved freely. A total simulation time of 6 ps was used with 1 fs steps. Random conformers were chosen (ca. 20) during the simulation process and minimized using the Polak−Ribiere minimization algorithm with a termination condition of an rms gradient of 0.010 kcal/(Å mol) for [2]rotaxane **1a** and 0.015 kcal/(Å mol) for [2]-rotaxane **1b**. For each rotaxane, most starting conformers minimized to the same lowest energy conformer.

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Monitoring Association. Complex formation between [2]-rotaxane **1a** (3.0 mM) and Phe or Ala (up to 20.0 mM) was investigated in various solutions (100% DMSO-d_6, 90/10, 60/40, and 40/60 (v/v) DMSO-d_6/H_2O). No shifts in the proton resonances for either component were observed, indicating that complexes were not formed. Association of the rotaxanes with *N*-Ac-Arg-OMe was investigated in DMSO-d_6, DMSO-d_6/H_2O (the proton resonance of water was suppressed in the experiments), or MeOH-d_4 at 25.0 °C. For assays performed in DMSO-d_6 solutions, the amino acid was held at a constant concentration of 3.0 mM and exposed to 0.5, 1.0, 2.0, 3.0, and 6.0 mM [2]rotaxane **1b** in separate solutions. An appropriate amount of Me_4NOH was added to the rotaxane solutions to obtain the desired protonation state. H_2O was added to these solutions to give a final ratio of 90/10, 80/20, or 60/40 (v/v) DMSO-d_6/water, and the assays were repeated. Similar experiments were performed for $AcCO_2NMe_4$ (1.0, 3.0, 5.0, 10.0, and 20.0 mM) exposed to 3.0 mM *N*-Ac-Arg-OMe. The extent of complex formation was determined by monitoring the changes in the chemical shifts of the amide proton of *N*-Ac-Arg-OMe. For assays performed in MeOH-d_4, [2]rotaxane **1b** was held at a constant concentration of 2.0 mM in separate solutions containing 1.0, 2.0, 5.0, 10.0, 15.0, 26.0, or 37.0 mM *N*-Ac-Arg-OMe. Shifts in the aromatic protons, caused by changes in a guest's concentration, were used to monitor complex formation. Plots of the changes observed in the binding titrations were fitted using a nonlinear least-squares fitting procedure to derive the association constants.[35]

Compound Synthesis. The mixture of syn and anti constitutional isomers of the crown ether derivatives or the sliding of the rotaxane ring, complicate most ^1H and ^{13}C NMR spectra. Changing the temperature did not significantly reduce the number of conformers.

Di(nitrobenzo)[24]crown-8 (4). To a 10 mL $CHCl_3$ solution containing 6.0 g of dibenzo[24]crown-8 (11 mmol) was slowly added dropwise 7 mL of nitric acid dissolved in 20 mL of acetic acid. The reaction mixture was stirred for 2 h, and then refluxed for an additional 1 h. After being cooled to rt, the crude material was washed with H_2O. Recrystallization in $CHCl_3$/EtOH yielded 5.95 g (95%) of crown ether **4** as a white solid: mp 149−150 °C; ^1H NMR (250 MHz, $CDCl_3$) δ 7.87 (d, J = 5.5 Hz, 2H), 7.72 (s, 2H), 6.87 (d, J = 5.5, 2H), 4.22 (br s, 8H), 3.96 (br s, 8H), 3.80 (br s, 8H); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 154.30, 148.42, 141.51, 117.96, 111.33, 108.36, 71.54, 69.79−68.93 (m); TOF MS *m*/*z* calcd for $C_{24}H_{30}N_2O_{12}$-Na^+ [M + Na] 561.1696, found 561.1699.

Di(aminobenzo)[24]crown-8 (5). A 300 mg (0.56 mmol) sample of dinitro-DB24C8 **4** was suspended in 50 mL of absolute ethanol, and 100 mg (10 mol %) of 10% Pd/C was added. The reaction mixture was stirred under H_2 (50 psi) overnight. Pd/C was removed by filtration through Celite, and the solvent was evaporated under reduced pressure to give 267 mg (56%) of crown ether **5** as a yellow foam: ^1H NMR (250 MHz, $CDCl_3$) δ 6.72 (d, J = 8.5 Hz, 2H), 6.26 (d, J = 2.1 Hz, 2H), 6.19 (dd, J = 8.2, 2.3 Hz, 2H), 4.10−4.05 (m, 8H), 3.90−3.78 (m, 16H), 3.45 (br s, 4H); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 150.30, 140.90, 117.44, 107.30, 103.29, 102.72, 71.13−69.14 (m), 61.75; TOF MS *m*/*z* calcd for $C_{24}H_{35}N_2O_8^+$ 479.2395, found 479.2560. Crown ether **5** is prone to decomposition on standing and was used immediately in subsequent reactions.

Di(*N*-acetylarginylaminobenzo)[24]crown-8 (2a). A 200 mg (0.417 mmol) sample of crown ether **5**, 740 mg (1.67 mmol) of BOP, and 362 mg (1.67 mmol) of Ac-Arg-OH·HCl were dissolved in 5 mL of DMF, followed by the addition of 725 μL (4.17 mmol) of DIEA. The reaction mixture was stirred at rt for 12 h. The solvent and excess DIEA were removed under high vacuum. The crude product was triturated with ethyl ether to remove BOP and HMPA. Crown ether **2a** was purified using reversed-phase HPLC with water (1% TFA)/CH_3CN as eluent (50% yield): ^1H NMR (250 MHz, D_2O) δ 7.10 (br s, 2H), 7.07−6.99 (d, J = 4.0 Hz, 2H), 6.96−6.90 (m, 4H), 4.36 (t, J = 7.5 Hz, 2H), 4.20 (br s, 8H), 3.92 (br s, 8H), 3.80 (m, 8H), 3.24 (t, J = 5.8 Hz, 4H) 2.07 (s, 6H), 1.93−1.65 (m, 8H); ^{13}C NMR (63 MHz, DMSO-d_6) δ 170.56, 169.70, 157.59, 148.31, 144.53, 133.08, 114.71, 112.06, 106.67, 70.32, 69.19, 53.00, 40.11, 29.19, 25.13, 22.45; TOF MS *m*/*z* calcd for $C_{40}H_{63}N_{10}O_{12}^+$ 875.4627, found 875.4642.

Di(4-carboxybutyrylaminobenzo)[24]crown-8 (2b). To a solution of 60 mg (0.12 mmol) of crown ether **5** in 2 mL of $CHCl_3$ were added 43 mg (0.38 mmol) of glutaric anhydride and 52 μL (0.38 mmol) of Et_3N. The reaction mixture was stirred at rt for 12 h. After the reaction mixture was dried under vacuum, the crude material was dissolved in CH_2Cl_2/CH_3OH (9/1) and extracted with 1 N HCl (3×). The organic phases were collected and dried over Na_2SO_4. The solvents were removed under vacuum, and the product was purified by column chromatography (SiO_2, CH_2Cl_2/CH_3OH) to give 69 mg (78%) of the diacid crown **2b** as a white solid: mp 156−158 °C; ^1H NMR (250 MHz, $CDCl_3$) δ 9.72 (s, 2H), 7.30 (s, 2H), 7.06 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.6 Hz, 2H), 4.02 (s, 8H), 3.75−3.65 (m, 16H), 3.33 (s, 3H), 2.33−2.24 (m, 8H), 1.82−1.76 (m, 4H); ^{13}C NMR (63 MHz, DMSO-d_6) δ 174.09, 170.21, 148.27, 144.18, 133.34, 114.63, 111.57, 106.33, 70.29, 69.20, 69.08, 68.65, 35.32, 33.00, 20.43; TOF MS *m*/*z* calcd for $C_{34}H_{45}N_2O_{14}^-$ 705.2871, found 705.2898.

Di[(Boc)$_3$arginylaminobenzo][24]crown-8 (2c). A 541 mg (1.14 mmol) sample of tri-Boc-Arg-OH was dissolved in 3 mL of CH_2Cl_2, and 185 mg (1.14 mmol) of carbonyldiimidazole was added. After the evolution of carbon dioxide ceased (15−20 min), 267 mg of freshly prepared crown ether **5**, dissolved in 3 mL of CH_2Cl_2, was added dropwise. The reaction mixture was stirred for 1 h and then evaporated under reduced pressure. The residue was purified by column chromatography, using a 20/80 EtOH/CH_2Cl_2 solution as the eluent to give 630 mg (77%) of crown ether **2c** as a yellow foam: ^1H NMR (400 MHz, $CDCl_3$) δ 9.44 (br s, 2H), 9.29 (br s, 2H), 8.92 (s, 2H), 7.23−7.18 (m, 2H), 6.82−6.78 (m, 4H), 5.91 (br s, 2H), 4.48 (m, 2H), 4.11−3.49 (m, 28H), 1.83−1.62 (m, 8H), 1.50−1.36 (m, 54H); ^{13}C NMR δ 170.4, 162.9, 160.6, 155.4, 154.6, 148.7, 145.4, 131.7, 114.5, 114.4, 113.1, 107.5, 83.8, 79.5, 79.0, 70.8, 69.6, 68.9, 53.9, 43.7, 29.1, 28.1, 27.8, 27.7, 24.5; TOF MS *m*/*z* calcd for $C_{66}H_{107}N_{10}O_{22}$ [M + 1] 1391.7561, found 1391.7513.

Di[*N*-acetyl(Boc)$_2$arginylaminobenzo][24]crown-8 (2d). To a solution of 967 mg (2.32 mmol) of Ac-$(Boc)_2$-Arg-OH in 20 mL of $CHCl_3$ was added 376 mg (2.32 mmol) of CDI. The reaction mixture was stirred for 1 h, and then 400 mg (0.835 mmol) of crown ether **5** was added. After being stirred for 24 h at rt, the mixture was extracted with small portions of 0.1% HCl (pH ≈ 5) and water. The product was purified by column chromatography (Al_2O_3, CH_2Cl_2, CH_3OH) to give 737 mg (69%) of crown ether **2d**: ^1H NMR (400 MHz, $CDCl_3$) δ 9.50 (br s, 1H), 9.36 (br s, 1H), 8.90 (s, 2H), 7.55 (br s, 2H), 7.24 (d, J = 4.0 Hz, 2H), 6.80−6.70 (m, 4H), 4.75−4.70 (m, 2H), 4.10−3.60 (m, 28H), 2.07 (s, 6H), 1.88−1.62 (m, 8H), 1.51 (s, 18H), 1.34 (s, 18H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.14, 170.77, 163.04, 161.05, 154.89, 153.21, 151.22, 129.15, 128.49, 128.17, 113.98, 84.48, 70.18, 68.60, 67.14, 44.08, 31.35, 25.22, 23.87; TOF MS *m*/*z* calcd for $C_{60}H_{94}N_{10}O_{20}Na^+$ [M + Na] 1297.6544, found 1297.6544.

Di(trifluoroacetylaminobenzo)[24]crown-8 (2e). To a solution of 89 mg (0.19 mmol) of crown ether **5** in 5 mL of $CHCl_3$ and 0.5 mL of pyridine was slowly added 0.3 mL of $(CF_3CO)_2O$. The reaction mixture was stirred at rt for 1 h, the solvents were removed under high vacuum, and the crude product was dissolved in 20 mL of $CHCl_3$ and extracted twice with 10 mL of water and 10 mL of 1 N HCl. The organic phase was separated, and the solvent was removed to give 124 mg (99%) of crown ether **2e** (pure by ^1H NMR analysis): ^1H NMR (250 MHz, DMSO-d_6) δ 7.27 (s, 2H), 7.21 (d, J = 8.8 Hz, 2H), 6.96 (d, J = 8.6 Hz, 2H), 4.04 (br s, 8 H), 3.75−3.40 (m, 16H); ^{13}C NMR (63 MHz, DMSO-d_6) δ 148.19, 146.12, 129.75, 113.93, 113.72, 113.55, 107.77, 72.32, 70.35, 69.96, 69.07, 68.93, 68.78, 60.17; TOF MS *m*/*z* calcd for $2(C_{28}H_{32}F_6N_2O_{10})Na^+$ [2M + Na] 1363.3820, found 1363.3315.

Di(*tert*-butoxycarbonylaminobenzo)[24]crown-8 (2f). To a solution of 200 mg (0.37 mmol) of dinitro-DB24C8 in 10 mL of DMF were added 20 mg of 5% Pd/C and 800 mg (3.67 mmol) of di-*tert*-butyl dicarbonate. The mixture was stirred at rt under a H_2 atmosphere (50 psi) for 2 h. The solution was filtered through a plug of Celite, and the solvent was removed under high vacuum. Purification by flash chromatography (SiO_2, ethyl acetate) provided 227 mg (90%) of crown ether **2f** as a white powder: mp 152−154 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (s, 2H), 6.78 (dd, J = 2.0, 8.8 Hz, 2H), 6.72 (m, 2H), 6.35 (s, 2H), 4.13 (dt, J = 3.6, 5.6 Hz, 8H), 3.89−3.87 (m, 8H), 3.81 (s, 8H), 1.50 (s, 18H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 152.91, 149.39, 144.71, 132.66, 115.22, 111.13, 105.98, 80.25, 72.53−68.52 (m), 28.35; TOF MS *m*/*z* calcd for $C_{34}H_{50}N_2O_{12}Na^+$ [M + Na] 701.3261, found 701.3255.

***N*-(Di-3,5-*tert*-butylbenzyl)-δ-valerolactam (6)**. A 5.00 g (50 mmol) sample of δ-valerolactam was dissolved in 20 mL of DMF, and 2.20 g (55.0 mmol) of a 60% suspension of sodium hydride in mineral oil was carefully added (evolution of hydrogen!). After gas evolution ceased (10−15 min), 15.7 g (55.0 mmol) of 3,5-di-*tert*-butylbenzyl bromide in 20 mL of DMF was added dropwise. The reaction mixture was stirred for 1 h, and the solvent was removed in vacuo. Lactam **6** was purified by means of column chromatography, eluting with CH_2Cl_2 to give 11.37 g (76%) of off-white crystals: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 1H), 7.06 (s, 2H), 4.60 (s, 2H), 3.20 (t, J = 5.8 Hz, 2H), 2.48 (t, J = 5.9 Hz, 2H), 1.7−1.9 (m, 4H), 1.31 (s, 18H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 169.7, 150.6, 135.8, 121.7, 120.8, 50.1, 46.8, 34.4, 32.0, 31.1, 22.9, 21.0; TOF MS *m*/*z* calcd for $C_{20}H_{32}NO$ [M + H] 302.2484 found 302.2482.

5-(3,5-Di-*tert*-butylbenzylamino)pentanoic Acid Hydrochloride (7). A 3.00 g (9.95 mmol) sample of lactam **6** was dissolved in a 60 mL solution of 6 N HCl, and the reaction mixture was refluxed for 24 h. The mixture was cooled to rt and extracted with CH_2Cl_2 (3 × 20 mL). The organic phase was dried with Na_2SO_4, and after the solvent was removed in vacuo, pure product was obtained as a transparent oil according to ^{1}H NMR analysis (>95%). The product was recrystallized from dioxane to give 3.12 g (88%) of axle **7** as a white powder: mp 154−155 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (br s, 2H), 7.41 (s, 1H), 7.37 (s, 2H), 4.11 (s, 2H), 2.85−2.80 (m, 2H), 2.30 (t, J = 6.8 Hz, 2H), 1.95−1.82 (m, 2H), 1.63−1.59 (m, 2H), 1.31 (s, 18H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 176.82, 151.72, 129.23, 124.52, 123.20, 51.57, 45.82, 34.89, 33.33, 31.39, 25.27, 21.85; MS *m*/*z* calcd for $C_{20}H_{34}NO_2^+$ 320.2591, found 320.2540.

5-[*tert*-Butoxycarbonyl(3,5-di-*tert*-butylbenzyl)amino]-pentanoic Acid (8). A 240 mg (1.1 mmol) sample of di-*tert*-butyl dicarbonate, dissolved in 2 mL of THF, was added to a solution of 320 mg (0.989 mmol) of amino acid **7** in 30 mL of THF and 2 mL of 2 N NaOH. The reaction mixture was stirred at rt for 1 h. After the solvents were removed under vacuum, an oily residue remained. The crude material was dissolved in 30 mL of ethyl acetate and extracted with 1 N $NaHCO_3$ followed by 1% HCl. Ethyl acetate was removed under high vacuum to give acid **8** in quantitative yield as a transparent oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (s, 1H), 7.06 (s, 2H), 4.42 (br s, 1H), 4.38 (br s, 1H), 3.24 (m, 1H), 3.14 (m, 1H), 2.34 (m, 2H), 1.58−1.46 (m, 13H + water), 1.31 (s, 18H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 178.81, 150.84, 146.74, 121.04, 85.14, 79.61, 46.03, 34.74, 33.62, 31.43, 28.44, 27.38, 21.94; MS *m*/*z* calcd for $C_{25}H_{40}NO_4^-$ 418.2957, found 418.2963.

2-{5-[*tert*-Butoxycarbonyl(3,5-di-*tert*-butylbenzyl)amino]pentanoylamino}-3-phenylpropanoic Acid Methyl Ester (9). A 360 mg (0.858 mmol) sample of acid **8** was dissolved in 5 mL of dried CH_2Cl_2 and activated with 153 mg (0.944 mmol) of CDI for 20 min. A solution of 204 mg (0.944 mmol) of phenylalanine methyl ester hydrochloride and 65 mg (0.94 mmol) of imidazole in 5 mL of CH_2Cl_2 was added to the reaction mixture. After the solution was stirred overnight at rt, it was diluted with 20 mL of CH_2Cl_2 and extracted with 10 mL of 1% HCl. The organic phase was collected and dried over Na_2SO_4. Once the solvent was removed under high vacuum, acid **9** was obtained as a transparent oil in a quantitative yield, which was pure by ^{1}H NMR analysis (>95%); further purification if necessary can be performed by column chromatography using $CHCl_3$/MeOH as eluent: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31−7.21 (m, 4H), 7.10−7.05 (m, 4H), 6.01 (d, J = 6.4 Hz, 0.5H), 5.83 (br s, 0.3 H), 4.88 (dd, J = 6.0, 12.4 Hz, 1H), 4.41−4.36 (m, 3H), 3.75 (s, 3H), 3.21−3.04 (m, 4H), 2.20−2.10 (m, 2H), 1.63−1.52 (m, 4H), 1.45 (s, 9H), 1.31 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 172.06, 155.76, 150.74, 136.96, 135.91, 129.99, 129.11, 128.43, 126.95, 121.38, 120.92, 117.04, 85.52, 79.40, 52.93, 52.15, 50.87, 45.77, 37.77, 35.78, 34.66, 31.39, 28.39, 27.76, 22.62; TOF MS *m*/*z* calcd for $C_{35}H_{53}N_2O_5^+$ 581.3954, found 581.4000.

2-[5-(3,5-Di-*tert*-butylbenzylamino)pentanoylamino]-3-phenylpropionic Acid Methyl Ester (10). A 500 mg (0.861 mmol) sample of ester **9** was dissolved in 5 mL of a $CHCl_3$/TFA (80/20) solution, and the reaction mixture was stirred at rt for 1 h. After the crude material was dried under high vacuum, it was extracted (CH_2Cl_2/H_2O). The organic phases were collected and dried under high vacuum to give 423 mg (85%) of ester **10** as a transparent oil, which was pure by ^{1}H NMR analysis (>95%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.30−9.20 (br s, 2H), 7.42 (s, 1H), 7.28−7.23 (m, 5H), 7.10(d, J = 7.1 Hz, 2H), 6.43 (d, J = 7.8 Hz, 1H), 4.81(q, J = 6.3 Hz, 1H), 4.00−3.90 (m, 2H), 3.71 (s, 3H), 3.14 (dd, J = 5.8, J = 13.7, 1H), 3.01 (dd, J = 7.2,13.7 Hz, 1H), 2.90−2.80 (m, 2H), 2.20 (t, J = 6.2 Hz, 2H), 1.80−1.60 (m, 4H), 1.31 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 173.20, 172.10, 152.02, 135.86, 129.71, 129.15, 128.69, 127.23, 124.00, 123.49, 53.50, 52.43, 52.02, 45.95, 37.67, 34.85, 34.47, 31.27, 25.32, 20.87; TOF MS *m*/*z* calcd for $C_{30}H_{45}N_2O_3^+$ 481.3430, found 481.3333.

2-[5-(3,5-Di-*tert*-butylbenzylamino)pentanoylamino]-3-phenylpropanoic Acid Hydrochloride (3). To a solution of 440 mg (0.740 mmol) of ester **10** in 10 mL of THF was added 1 N LiOH to adjust the pH to 10. The reaction was stirred at rt for 2 h. At this time, TLC analysis indicated complete hydrolysis had occurred. Solvents were removed under high vacuum, and the residue was extracted with CH_2Cl_2/1 N HCl. The organic layers were collected and dried over $NaSO_4$. After the solvent was removed under high vacuum, 356 mg (96%) of acid **3** was obtained as a white solid: mp 75−77 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.21 (br s, 1H), 9.13 (br s, 1H), 7.41 (s, 1H), 7.32−7.19 (m, 7H), 4.52 (dd, J = 3.6, 12.4 Hz, 1H), 4.04 (s, 2H), 3.16 (dd, J = 4.8, 14.0 Hz, 1H), 2.97 (dd, J = 9.2, 14.0 Hz, 1H), 2.90−2.80 (m, 2H), 2.17−2.13 (m, 1H), 1.90−1.80 (m, 1H), 1.70−1.43 (m, 4H), 1.27 (s, 18H); ^{13}C NMR (63 MHZ, $CDCl_3$) δ 174.42, 174.17, 161.52, 151.81, 136.62, 129.53, 129.19, 128.48, 126.83, 124.34, 123.30, 54.47, 52.11, 46.33, 36.83, 34.85, 34.60, 31.43, 31.31, 25.23, 22.11; TOF MS *m*/*z* calcd for $C_{29}H_{43}N_2O_3^+$ 467.3275, found 467.3319.

Phenylalanine Methyl Ester Di(*tert*-butoxycarbonylaminobenzo)[24]crown-8 Rotaxane (13). A 350 mg (0.516 mmol) sample of crown ether **2f** and 240 mg (0.516 mmol) of acid **11**, obtained by partitioning acid **7** and PF_6NMe_4 in ether/water, were dissolved in 3 mL of dried $CHCl_3$. The solution was cooled to −10 °C (ice/salt bath), and 130 mg (0.631 mmol) of DCC dissolved in 0.5 mL of $CHCl_3$ was added. After the reaction was stirred under Ar for 1.5 h, a solution of 93 mg (0.52 mmol) of phenylalanine methyl ester dissolved in 0.3 mL of $CHCl_3$ was added. The reaction was left to stir overnight at rt under Ar. After the solvent was removed under vacuum, an oily residue remained, which was dissolved in CH_3CN. DCU precipitated and was removed by filtration. The crude material was dried, dissolved in CH_2Cl_2, and extracted with 1% HCl to remove the excess phenylalanine methyl ester. The product was purified using rotary chromatography (SiO_2, CH_2Cl_2, CH_3OH) to give 430 mg (72%) of [2]rotaxane **13** as a white solid: mp 104−105 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H), 8.18 (d, J = 7.6 Hz, 1H), 7.30−7.15 (m, 10H), 6.88 (s, 4H), 4.55 (d, J = 5.2, 2H), 4.50−4.45 (m, 1H), 4.10−4.01 (m, 8H), 3.82−3.70 (m, 4H), 3.65−3.42 (m, 14H), 3.22−3.12 (m, 2H),

2.99 (dd, $J = 5.6$ Hz, $J = 14.0$ Hz, 1H), 2.84 (dd, $J = 9.6$ Hz, $J = 13.6$ Hz, 1H), 1.95−1.90 (m, 2H), 1.46 (s, 18H), 1.40−1.20 (m, 4H), 1.14 (s, 18H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 172.35, 172.14, 153.12, 151.21, 147.50, 143.20, 136.62, 133.17, 131.89, 131.09, 130.24, 129.23, 128.43, 126.74, 124.55, 123.16, 113.20, 111.82, 105.35, 105.27, 80.24, 70.03−68.36 (m), 64.30, 53.92, 53.76, 52.73, 52.06, 48.86, 37.60, 34.85, 34.68, 31.39, 30.63, 29.66, 28.31, 25.89, 22.07; TOF MS *m*/*z* calcd for $C_{64}H_{95}N_4O_{15}^+$ 1159.6794, found 1159.6783.

Phenylalanine Methyl Ester Di(aminobenzo)[24]crown-8 Rotaxane (14). A 200 mg (0.167 mmol) sample of [2]-rotaxane **13** was dissolved in 2 mL of CH_2Cl_2, and 0.4 mL of TFA was added. The reaction mixture was stirred under Ar at rt. After 2 h, TLC analysis indicated that the reaction was complete. The solvent and TFA were removed under high vacuum. To ensure that TFA was removed, the residue was dissolved in CH_3Cl/CH_3OH (9/1) and evaporated; this procedure was repeated three times. The crude material was dissolved in CH_2Cl_2 and extracted with 0.1 M $NaHCO_3$. The organic layers were collected and dried over Na_2SO_4. After the solvent was removed under high vacuum, 172 mg (96%) of [2]-rotaxane **14** was obtained, which was pure by ^{1}H NMR analysis (>95%): mp 56−58 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (d, $J = 7.6$ Hz, 1H), 7.39−7.17 (m, 8H), 6.73 (d, $J =$ 8.4 Hz, 1.2 H, 64% of the conformer mixture), 6.63 (d, $J = 8.4$ Hz, 0.7 H, 36% of the conformer mixture), 6.32 (s, 1.2 H, 63% of the conformer mixture), 6.23 (s, 0.6 H, 37% of the conformer mixture), 6.10 (d, $J = 8.4$ Hz, 1.2 H, 64% of the conformer mixture), 6.05 (d, $J = 8.4$ Hz, 0.7 H, 36% of the conformer mixture), 4.80−4.60 (m, 4H), 4.60−4.55 (m, 2H), 4.50−4.45 (m, 1H), 4.20−3.80 (m, 8H), 3.75−3.40 (m, 15H), 3.20−2.95 (m, 3H), 2.86 (dd, $J = 9.6$ Hz, $J = 13.6$ Hz, 1H), 1.90−1.85 (m, 2H), 1.80−1.40 (m, 4H), 1.32−1.05 (m, 18H); ^{13}C NMR (63 MHz, DMSO-d_6) δ 172.06, 171.60, 158.77, 158.19, 150.55, 147.51, 146.54, 137.17, 131.40, 128.95, 128.14, 126.46, 125.28, 123.68, 122.70, 115.28, 113.07, 107.64, 70.13−68.31 (m), 53.43, 52.04, 51.69, 48.19, 36.71, 34.27, 33.86, 31.11, 30.94, 25.29, 21.78; TOF MS *m*/*z* calcd for $C_{54}H_{79}N_4O_{11}^+$ 959.5745, found 959.5749.

Phenylalanine Methyl Ester Di(*N*-acetylarginylaminobenzo)[24]crown-8 Rotaxane (15). A 200 mg (0.203 mmol) sample of [2]rotaxane **14**, 360 mg (0.812 mmol) of BOP, and 176 mg (0.812 mmol) of Ac-Arg-OH·HCl were dissolved in 3 mL of freshly distilled DMF. A 353 μL sample of DIEA was added, and the reaction mixture was stirred under Ar at rt for 24 h. DMF and excess DIEA were removed under high vacuum, and the remaining oily residue was extracted with CH_2Cl_2/CH_3OH (8/2)/ H_2O. The organic phases were collected and dried over Na_2SO_4. The product was purified using rotary chromatography (SiO_2, CH_2Cl_2, CH_3OH; to remove the rotaxane, a 1% TFA solution in CH_3OH was used as eluent) to give 153 mg (56%) of [2]rotaxane **15** as a yellow oil: ^{1}H NMR (400 MHz, CD_3OD) δ 7.41−6.92 (m, 14 H), 4.71 (br s, 2H), 4.60 (m, 1H), 4.46−4.44 (m, 2H), 4.22−4.00 (m, 8H), 3.84−3.48 (m, 25 H), 3.23−3.20 (m, 1H), 3.00−2.85 (m, 1H), 2.02 (s, 6H), 2.00−1.91 (m, 2H), 1.78−1.73 (m, 4H), 1.46−1.15 (m, 26H); ^{13}C NMR (63 MHz, DMSO-d_6) δ 177.62, 172.05, 171.57, 170.17, 169.70, 158.53, 156.96, 150.50, 143.08, 137.17, 134.27, 132.90, 131.40, 128.94, 128.10, 126.41, 123.68, 122.66, 119.24, 111.56, 104.69, 70.06−68.05 (m), 53.42, 53.06, 52.03, 51.65, 51.48, 48.16, 36.69, 34.27, 33.92, 30.90, 29.20, 28.20, 25.20, 22.33, 21.79; TOF MS *m*/*z* calcd for $C_{70}H_{107}N_{12}O_{15}^+$ 1355.7980, found 1355.8617.

Phenylalanine Di(*N*-acetylarginylaminobenzo)[24]-crown-8 Rotaxane (1a). A 100 mg (0.068 mmol) sample of rotaxane **15**·3HCl was dissolved in ca. 3 mL of THF, and 1 N LiOH was added until the pH was adjusted to 10. The reaction mixture was stirred at rt for 12 h, at which time the starting material was consumed according to TLC analysis (SiO_2, CH_2Cl_2, CH_3OH). After the solvents were removed under high vacuum, the crude product was dissolved in CH_3OH to precipitate LiCl, which was removed by filtration. [2]Rotaxane **1a** was obtained as a yellow oil in a greater than 95% yield, according to ^{1}H NMR analysis: ^{1}H NMR (250 MHz, CD_3OD) δ 7.52−6.80 (m, 14 H), 4.76−4.47 (m, 5H (ArCH_2N, 2H; α-*H* of Arg, 2H; α-*H* of Phe, 1H)), 4.30−3.10 (m, 30H (OCH_2CH_2O of crown ether, 24H; NCH_2CH_2 of axle, 2H; NCH_2CH_2 of Arg, 4H), 2.99−2.90 (m, 2H), 2.30−2.20 (m, 2H), 2.09 (s, 6H), 1.88−1.55 (m, 4H), 1.50−0.87 (m, 24H); ^{13}C NMR (63 MHz, DMSO-d_6) δ 182.91, 173.00, 172.00, 167.64, 150.63, 137.72, 134.35, 131.30, 129.07, 128.10, 126.29, 124.22, 122.19, 72.00−69.00 (m), 60.13, 53.34, 48.48, 40.52, 36.70, 34.60, 34.30, 31.19, 31.01, 25.14, 24.68, 22.20; TOF MS *m*/*z* calcd for $C_{69}H_{105}N_{12}O_{15}^+$ 1341.7822, found 1341.7902.

Phenylalanine Methyl Ester Di(4-carboxybutyryl-aminobenzo)[24]crown-8 Rotaxane (16). To a solution of 150 mg (0.14 mmol) of amino[2]rotaxane **14** in 3 mL of $CHCl_3$ were added 68 mg (0.60 mmol) of glutaric anhydride and 30 μL of Et_3N. The reaction mixture was stirred at rt for 2 h. The solvent was evaporated, and the remaining oily residue was triturated with 10 mL of Et_2O to remove excess glutaric anhydride and Et_3N. The solid residue was dissolved in CH_2Cl_2/CH_3OH (9/1) and extracted with 1 N HCl. The organic phases were collected and dried over Na_2SO_4. Solvents were removed in vacuo, and the product was purified via column chromatography (SiO_2, CH_2Cl_2/CH_3OH) to give 130 mg (76%) of [2]rotaxane **16** as an off-white wax: ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.82 (s, 2H), 8.20 (d, $J = 7.6$ Hz, 2H), 7.44 (s, 2H), 7.30−7.15 (m, 8H), 7.04 (d, $J = 8.4$ Hz, 2H), 6.90 (d, $J =$ 9.1 Hz), 4.55 (s, 2H), 4.45−4.40 (m, 1H), 4.20−3.95 (m, 8H), 3.90−3.10 (m, 21H + water), 2.99 (dd, $J = 5.6$ Hz, $J = 13.6$ Hz, 1H), 2.84 (dd, $J = 9.2$ Hz, $J = 13.6$ Hz, 1H), 2.40−2.20 (m, 8H), 2.00−1.90 (m, 2H), 1.90−1.80 (m, 4H), 1.50−1.40 (m, 4H), 1.13 (s, 18H); ^{13}C NMR (CD_3CN, reference 1.32 ppm) δ 174.86, 174.39, 173.11, 172.07, 153.80, 152.28, 148.32, 144.83, 138.09, 134.06, 132.92, 130.26, 129.42, 127.77, 125.15, 124.52, 113.72, 113.38, 106.83, 71.53, 71.20, 71.02, 70.85, 69.39, 69.22, 54.63, 53.67, 52.75, 52.03, 51.40, 49.63, 38.23, 36.56, 35.50, 35.32, 34.02, 33.77, 33.60, 32.58, 32.29, 31.70, 26.68, 26.27, 26.08, 25.67, 25.50, 23.01, 21.62; TOF MS *m*/*z* calcd for $C_{64}H_{91}N_4O_{17}^+$ 1187.6379, found 1187.6388.

Phenylalanine Di(4-carboxybutyrylaminobenzo)[24]-crown-8 Rotaxane (1b). A 130 mg (0.106 mmol) sample of [2]rotaxane **16** was dissolved in 2 mL of CH_3OH, and 1 N LiOH was added to adjust the pH to 10. The reaction mixture was stirred at rt until the starting material was consumed as determined by TLC and ^{1}H NMR analysis. After the solvents were removed under high vacuum, the crude product was dissolved in CH_3OH to precipitate LiCl, which was removed by filtration. [2]Rotaxane **1b** was obtained as a yellow oil in a greater than 95% yield, according to ^{1}H NMR analysis: ^{1}H NMR (250 MHz, CD_3OD) δ 7.88−6.88 (m, 14H), 4.70 (s, 2H), 4.61−4.60 (m, ~$^1/_2$H), 4.45−4.44 (m, ~$^1/_2$H), 4.19−4.13 (m, 8H), 4.00−3.40 (m, 20H), 3.24−3.22 (m, 2H), 3.14−3.07 (m, 1H), 2.97−2.92 (m, 2H), 2.43−2.34 (m, 6H), 2.26 (t, $J = 8.1$ Hz, 2H), 1.98−1.93 (m, 6H), 1.60−1.20 (m, 4H), 1.20 (s, 18H); ^{13}C NMR (63 MHz, CD_3OD, reference 49.0 ppm) δ 181.86, 175.21, 174.82, 174.23, 173.50, 173.38, 152.41, 148.61, 145.34, 139.63, 138.27, 134.27, 134.13, 133.13, 130.56, 130.18, 129.46, 129.08, 127.86, 127.27, 125.43, 124.36, 118.19, 113.86, 107.09, 71.83, 71.40, 71.22, 69.68, 69.45, 53.85, 52.08, 38.37, 37.91, 36.81, 36.22, 35.70, 34.02, 31.87, 27.06, 24.07, 23.64, 22.08; TOF MS *m*/*z* calcd for $C_{63}H_{89}N_4O_{17}^+$ 1173.6223, found 1173.6243.

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Supporting Information Available: NOESY spectrum and assignment table of [2]rotaxane **1a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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