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Host−[2]Rotaxane: Advantage of Converging Functional Groups for Guest Recognition

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A host−[2]rotaxane was constructed by converting a diaminophenylcalix[4]arene into a [2]rotaxane using the DCC-rotaxane method (Zehnder, D.; Smithrud, D. B. *Org. Lett.* **2001**, *16*, 2485−2486). *N*-Ac-Arg groups were attached to the dibenzo-24-crown-8 ring of the rotaxane to provide a convergent functional group. To demonstrate the advantage provided by the rotaxane architecture for recognition of guests that contain a variety of functional groups, association constants (K_A) for *N*-Ac-Trp, indole, *N*-Ac-Gly, fluorescein, 1-(dimethylamino)-5-naphthalenesulfonate, and pyrene bound to the [2]rotaxane were determined by performing ^{1}H NMR and fluorescence spectroscopic experiments. The host−[2]rotaxane had the highest affinity for fluorescein with a $K_A = 4.6 \times 10^6$ M^{-1} in a 98/2 buffer (1 mM phosphate, pH 7)/DMSO solution. A comparison of K_A values demonstrates that both the aromatic pocket and ring of the host−[2]rotaxane contribute binding free energy for complexation. Association constants were also derived for the same guests bound to the diaminophenylcalix[4]arene and to a diphenylcalix[4]arene that contained arginine residues displayed in a nonconvergent fashion. The host−[2]rotaxane provides higher affinity and specificity for most guests than the host with divergent *N*-Ac-Arg groups of the one that only has an aromatic pocket. For example, the K_A for the complex of the host−[2]rotaxane and fluorescein in the DMSO/water mixture is more than 2 orders of magnitude greater than association constants derived for the other hosts.

Introduction

Mimicry of protein binding domains continues to be an important goal of modern chemistry. Antibodies provide the paradigm for molecular recognition in biology and for the development of synthetic hosts.[2] Small molecules are bound within hydrophobic grooves or pockets on antibody surfaces and make contact with multiple polar side chains displayed on hypervariable loops to give highly specific recognition.[3−5] In theory, mimetic antibodies should be possible if molecules could be made that have a hydrophobic pocket with convergent functional groups. However, developing synthetic hosts that contain this arrangement is not a trivial endeavor. Cyclophanes demonstrated early on the importance of a preformed, rigid, aromatic pocket for strong complex formation.[6] Attaching functional groups to such synthetic pockets does provide for enhanced recognition and function.[7−12] However, in most cases, these groups are not truly convergent; they do not point toward the pocket. Convergent functional groups, such as those found in capped crown ethers or cyclophanes, do enhance association.[13,14] A new powerful method for constructing convergent functional groups is found in the molecular imprint polymers, which have become an intriguing source of artificial receptors and catalysts.[15−17]

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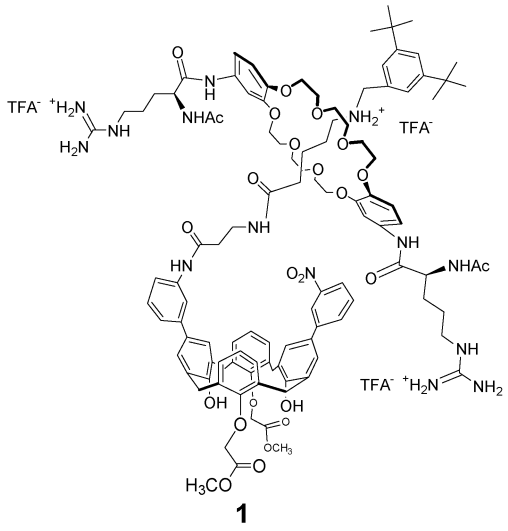
In the preceding paper,[18] we showed that templates based on the rotaxane architecture[19−22] can support convergent functional groups. Amino acid−[2]rotaxanes with opposing guanidinium and carboxylate groups form a strong intramolecular salt bridge, and ones with converging carboxylates associate with *N*-Ac-Arg in DMSO/water mixtures and methanol. As the percentage of water increased in these experiments, the strength of association dropped dramatically.

To improve the performance of [2]rotaxanes as mimics of protein binding domains, one blocking group was replaced with a calix[4]arene. The calix[4]arene provides the necessary hydrophobic pocket for strong association in aqueous solutions, and we envision the rotaxane ring as a crude mimic of an antibody hypervariable loop. Calix[4]arenes were chosen to provide the framework for the hosts because they are readily synthesized in large quantities and derivatized with a variety of functional groups on their upper and lower rims. Acetyl-protected arginines were used as the recognition elements because arginines are a key residue in protein binding domains[23] and contain a chiral center that could potentially provide diastereomeric complexes with chiral guests.

Although some calix[4]arenes form solid-phase inclusion complexes with aromatic compounds,[24,25] simple calix[4]arenes in the solution phase form weak complexes.[26,27] Calix[4]arenes underivatized at the upper rims fail to form even solid-phase inclusion complexes.[25] Therefore, rigid calix[4]arenes with an extended hydrophobic cavity are necessary for effective binding of organic molecules in the solution phase.[28] Several "deep-cavity", aryl-substituted calix[4]arenes have been prepared,[29−40] and some have shown the ability to form complexes. As examples, tetraamidophenyl-substituted calix[4]arenes form capsules around guests,[33] diphenylphosphacalix[4]arenes act as metal catalysts,[34] and a dibenzoate-derivatized calix[5]arene binds two molecules of imidazole.[35]

For our studies, three novel hosts were constructed: host−[2]rotaxane **1**, calix[4]arene **2**, and calix[4]arene **3**. Association constants for complexes of these hosts with guests, which contain a variety of functional groups and aromatic surfaces, were derived and compared to determine whether convergent functional groups do enhance guest recognition.



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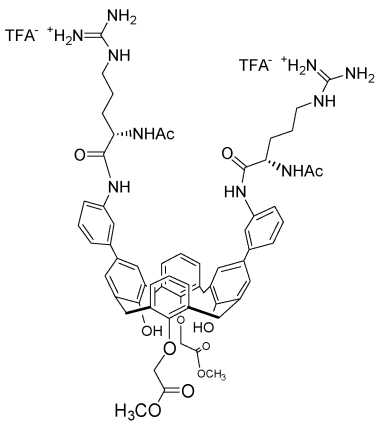

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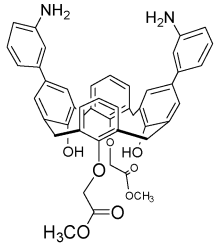

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Results

Design of the Hosts. [2]Rotaxane **1** has arginine residues as recognition elements, which can point toward the pocket. We should note that rotation around the

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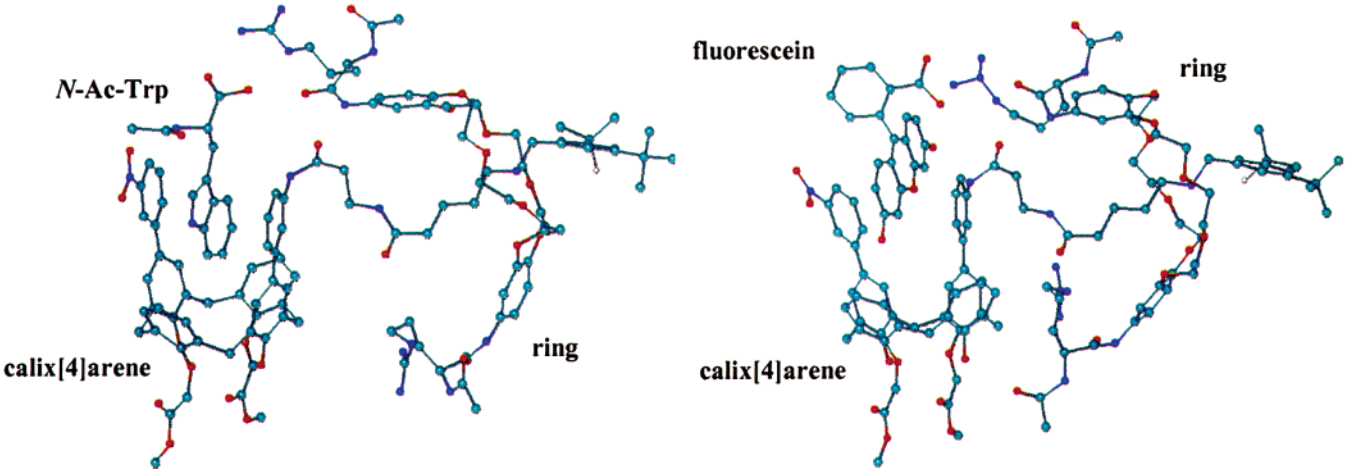

FIGURE 1. Lowest energy structures of [2]rotaxane **1** bound to *N*-Ac-Trp and fluorescein obtained using Monte Carlo simulations at 300 K (molecular mechanics calculations, MM+ force field). H-atoms are removed for clarity.

biaryl bond can give syn and anti isomers of the calix[4]arenes; the syn isomer is shown throughout the text. The arginine residues are not connected to the pocket of [2]rotaxane **1**, but the two domains are held together in a threaded manner. Their participation in the binding event depends on the energy gained through noncovalent bond formation balanced against the energy lost through a reduction in the motion of the arginine moiety and ring. In the preceding paper, we showed that the reduction in ring motion with complex formation does not detract significantly from the binding free energy.[18] Lowest energy structures of [2]rotaxane **1** bound to *N*-Ac-Trp and fluorescein (Figure 1), as determined by Monte Carlo simulations, indicate that the host is appropriately designed to recognize small and large compounds that have aromatic rings and carboxylates.

Calix[4]arene **2** also has arginine residues as recognition elements. However, these groups are held in a more divergent arrangement. A simultaneous interaction of the guest with both the hydrophobic pocket and the arginine side chains of calix[4]arene **2** is unlikely. A large portion of the binding free energy would be used to pay for the loss of entropic energy required to force the arginine side chains to bend toward the pocket. There may also be a substantial loss of enthalpic energy due to geometrical constraints placed on the side chains.

Calix[4]arene **3** contains the same aromatic pocket as [2]rotaxane **1** without the arginine residues. Larger association constants determined for guests complexed to [2]rotaxane **1** as compared to calix[4]arene **3** would show that the arginine residue or the ring of the rotaxane contributes to the binding free energy.

Synthesis of the Hosts. One convenient method for selective functionalization of calix[4]arenes begins with selective dialkylation of the lower rim followed by electrophilic substitution of the phenolic units of the dialkylated calix[4]arenes.[41] The lower rim of calix[4]arene **4** was alkylated with ethyl bromoacetate in a mixture of THF and DMF (Scheme 1).[42] Adding 2 equiv of bromine in $CHCl_3$[43] gave calix[4]arene **5** in high yield, which likely locks it in the cone conformation.[44] *m*-Nitrophenyl rings were successfully attached using a Suzuki coupling reaction of *m*-nitrophenylboronic acid—commercially available—and calix[4]arene **5**. Unfortunately, the aqueous conditions (Na_2CO_3, H_2O, toluene, and methanol) hydrolyzed the ethyl esters. $Pd(PPh_3)_4$-catalyzed coupling reaction between dibromocalix[4]arene **5** and *m*-nitrophenylboronic acid under anhydrous conditions (Cs_2CO_3, DMF) resulted in a mixture of mono- and di-*p*-phenylcalix[4]arenes in low yields. Numerous attempts to employ other Pd(0) catalysts ($Pd(OAc)_2 \cdot 2P(otol)_3$[45] or the Negishi method[46]) resulted in no reaction or a complicated mixture of products. Therefore, the reaction was run under the basic aqueous conditions, and the acids were re-esterified. Product formation was monitored by observing the loss of the bromophenol's aromatic signal at 7.04 ppm and the growth of the *m*-nitrophenyl signal at 7.36 ppm in ^{1}H NMR spectra.

The coupling reaction and hydrolysis provided calix[4]arene **6** in 70−80% yield. Calix[4]arene **6** is poorly soluble in most organic solvents, and thus, it was purified by trituration with ethyl ether to remove excess *m*-nitrophenylboronic acid and then recrystallized from methanol. Refluxing calix[4]arene **6** in a MeOH/$CHCl_3$ solution with a catalytic amount of acid provided calix[4]arene **7** in 70−80% yield. Methyl esters were chosen to simplify the ^{1}H NMR spectra of the binding assays.

Selective reduction of one of the two nitro groups of calix[4]arene **7** was necessary for the synthesis of [2]rotaxane **1**, whereas complete reduction gives calix[4]arene **3** and the material needed for the creation of

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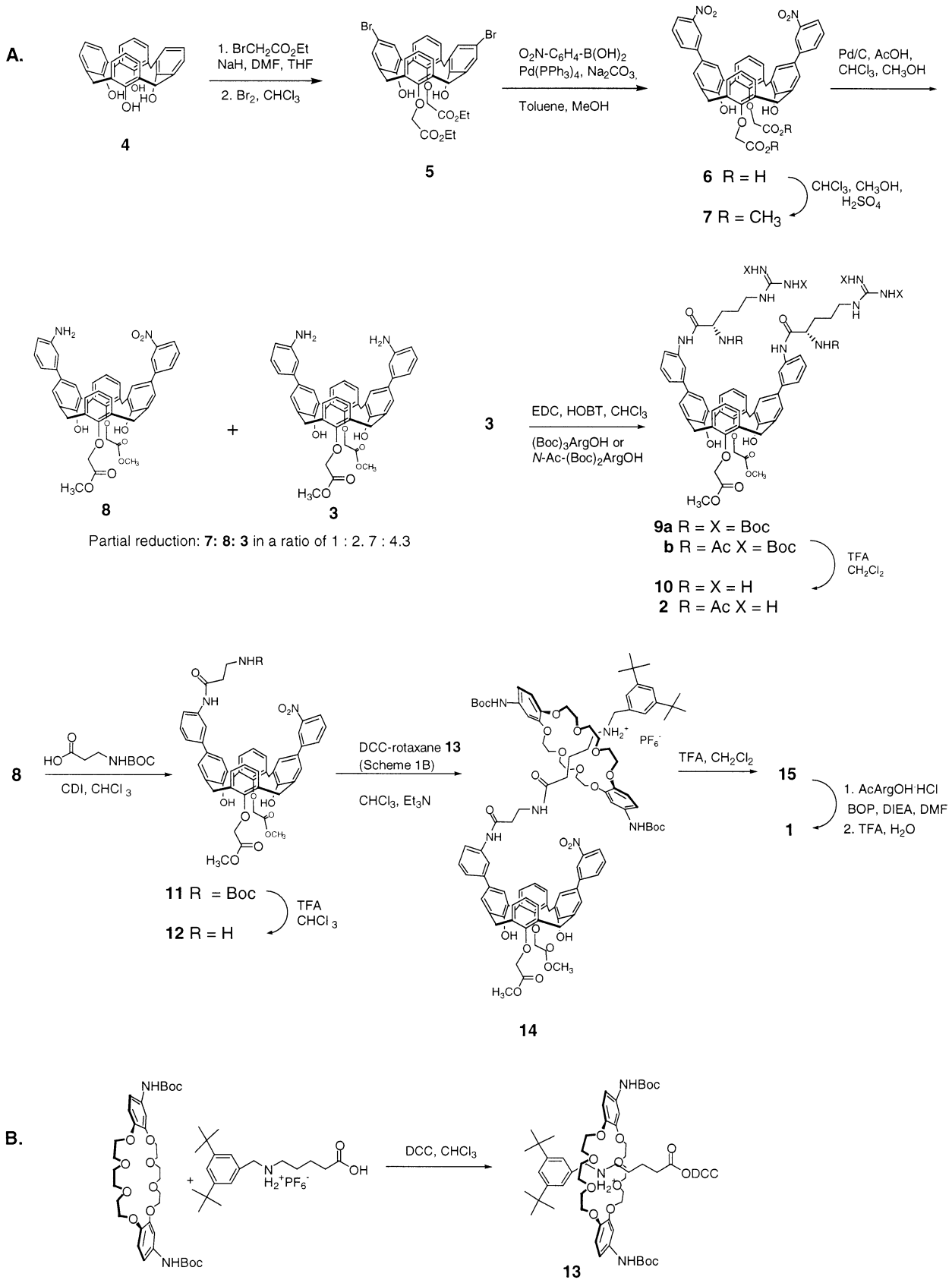
SCHEME 1



calix[4]arene **2**. We have previously used Sn/HCl to reduce dinitrophenylcalix[4]arenes, but this method is unsuitable for monoreduction; the reaction proceeds too quickly, and partial hydrolysis of the esters gives a mixture of products. Other conventional reducing methods (1,4-dicyclohexadiene, Pd/C; H_2, Pd/C, HCO_2NH_4;

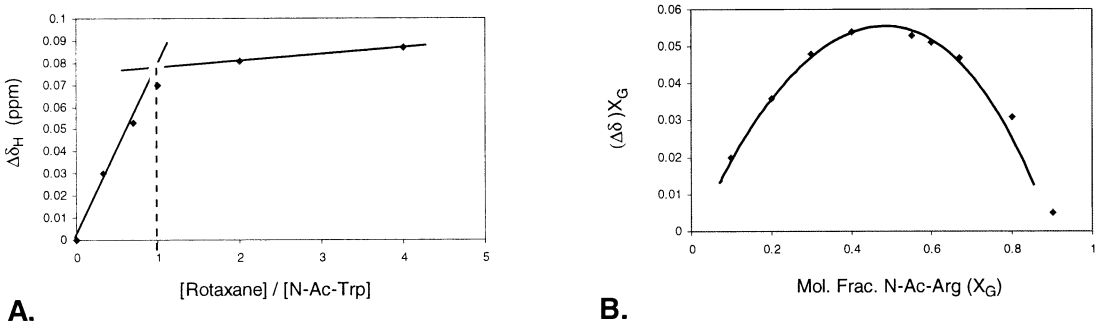


FIGURE 2. (A) Chemical shift dependency of the amide proton of *N*-Ac-Trp, which was held at a constant concentration of 1.0 mM while the concentration of [2]rotaxane **1** was increased. (B) A Job plot[47] where the concentration of the complex ($(\Delta\delta)X_G$) is compared against the mole fraction of *N*-Ac-Trp (X_G). The lines are drawn to overlay the data points.

Pd/C, H_2 in $CHCl_3$ or in a $CHCl_3$/methanol solution) produced only a small yield of calix[4]arenes **8** and **3** even after long reaction times at elevated temperatures. The best method we found for monoreduction involves the addition of acetic or formic acid to a solution of dinitrocalix[4]arene **7** in a $CHCl_3$/methanol mixture (50/50 (v/v)) in the presence of Pd/C under a H_2 atmosphere. The reaction was monitored by TLC and terminated when ∼70−80% of calix[4]arene **7** was consumed, usually within 12−18 h. The crude reaction mixture was separated by column chromatography to give calix[4]arenes **7**, **8**, and **3** in yields of 1, 2.7, and 4.3 ratio, respectively.

To create calix[4]arenes with arginines displayed on their upper rims, e.g., calix[4]arene **2**, synthetic methodology was needed to attach derivatized arginines to diaminophenylcalix[4]arenes. Initial attempts to construct calix[4]arene **10** through the coupling reaction of calix[4]arene **3** with *N*-Boc-Arg-OH with CDI, EDC, or DCC/HOBT as activating agents in DMF—needed to solubilize *N*-Boc-Arg-OH—were unsuccessful. Fully Boc-protected arginine $(Boc)_3$-Arg-OH was attached using EDC/HOBT activation in $CHCl_3$ to provide $(Boc)_3$-Arg-calix[4]arene **9a** in an 84% yield. Complete removal of Boc protecting groups required exposure to a 50−70% TFA solution in CH_2Cl_2 and extended reaction times, which resulted in a moderate yield of calix[4]arene **10**. *N*-Ac-$(Boc)_2$-Arg-OH was attached to calix[4]arene **3** also through EDC/HOBT coupling. Deprotection of the resulting calix[4]arene **9b** required only exposure to a 30% TFA solution in $CHCl_3$ for 1 h for complete removal of the Boc groups to give calix[4]arene **2** in a good yield.

Host−[2]Rotaxane Formation. Synthesis of [2]rotaxane **1** was based on the general method of coupling DCC-[2]rotaxanes to primary amines to give [2]rotaxanes.[1] The addition of a primary amine to calix[4]arene **8**, which is necessary for coupling, was easily accomplished by attaching Boc-*β*-alanine using EEDQ in refluxing pyridine or through CDI coupling in refluxing $CHCl_3$. Subsequent deprotection of Boc-*β*-alanylcalix[4]arene **11** with 20% TFA in CH_2Cl_2 gave *β*-alanylcalix[4]arene **12**. DCC-rotaxane **13**,[18] which contains a Boc-protected ring, was used to make [2]rotaxane **14**. Deprotection with exposure to TFA proceeded smoothly to give the diamino[2]rotaxane **15**. *N*-Ac-Arg was attached to the ring of diamino[2]rotaxane **15** instead of Boc-protected arginines. Experience with the synthesis of rotaxanes in our laboratory showed that the removal of multiple Boc groups requires a large percentage of TFA in CH_2Cl_2 or DMF and long reaction times, which tends to cleave some of the *tert*-butyl groups of the di-*tert*-butylphenyl blocking group. The poor solubility of *N*-Ac-Arg in most organic solvents is problematic. Attempts to couple diamino[2]rotaxane **15** with the sodium salt of *N*-Ac-Arg with activating reagents commonly used for amidation (CDI, DCC/HOBT, or PyBroP, as a suspension in DMF or DMSO) were unsuccessful. [2]Rotaxane **1** was obtained in good yields only when the hydrochloride salt of *N*-Ac-Arg was activated with BOP in the presence of DIEA.

^1H and ^{13}C NMR spectra of the final [2]rotaxanes were too complex for accurate determination of their preferred conformation in solution. However, the precursor calix[4]arenes were clearly locked in a cone conformation[37] (see the Supporting Information), which strongly suggests that the [2]rotaxanes are also locked in a cone conformation.

Monitoring Association through ^1H NMR Spectroscopy. A series of guests were chosen to test the ability of the hosts to recognize charged, polar, or aromatic groups and to determine whether the arginine group of host **1** or **2** and the aromatic pocket of all three hosts interact with a guest. *N*-Ac-Trp was chosen because it contains a variety of functional groups and the tryptophan side chain is a key recognition amino acid in protein binding domains.[23]

[2]Rotaxane **1** strongly interacts with *N*-Ac-Trp in DMSO-d_6 and in DMSO-d_6/buffer (1 mM phosphate, pH 7.0) mixtures. Association was too favorable to derive an accurate K_A from ^1H NMR binding assays. Plots of the changes in the chemical shifts of the aromatic N−H proton of *N*-Ac-Trp versus the change in concentration of [2]rotaxane **1** did show that a 1/1 complex is most likely formed (Figure 2A). To verify this result, a Job plot[47] was constructed by plotting the concentration of the complex versus the mole fraction of *N*-Ac-Trp (Figure 2B). A maximum in the curve is reached at a mole fraction of ca. 0.5, which is consistent with a 1/1 complex. The formation of a 1/1 complex with a single arginine group, according to molecular modeling results, suggests that the syn and anti constitutional isomers of the host−[2]-rotaxane bind with the same association constant.

The proton resonance of the aromatic N−H shifted upfield with an increase in the concentration of [2]-

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TABLE 1. Association Constants for Various Host−Guest Complexes Measured at 25 °C[a]

host	guest	solution	$K_A \times 10^{-4}$ (M^{-1})[b]	$K_{A(1)}/K_{A(2)}$[c]	$K_{A(1)}/K_{A(3)}$
1	*N*-Ac-L-Trp	DMSO	10 ± 0.5	111	11
		DMSO/H_2O (80/20)	10 ± 0.2	420	14
		DMSO/H_2O (50/50)	1.2 ± 0.1	>60	2
		DMSO/H_2O (25/75)	3.75 ± 0.08	>190	>19
		DMSO/H_2O (2/98)	1.9 ± 0.3		
	indole	DMSO	4.2 ± 0.4	>400	10
		DMSO/H_2O (80/20)	1.8 ± 0.1	>180	1
		DMSO/H_2O (50/50)	0.9 ± 0.1	>45	1
		DMSO/H_2O (25/75)	1.1 ± 0.1	>55	1
	N-Ac-Gly	DMSO-d_6	0.16 ± 0.03	4	>16
		DMSO-d_6/H_2O (80/20)	<0.01		
	L-Trp	DMSO	1.0 ± 0.4	20	
		DMSO/H_2O (80/20)	1.5 ± 0.4	> 150	
	D-Trp	DMSO	0.9 ± 0.1		
	1,5-DNS	DMSO/H_2O (2/98)	5.5 ± 0.5	4	10
	fluorescein	DMSO/H_2O (2/98)	470 ± 80	320	480
	pyrene	DMSO/H_2O (2/98)	3.2 ± 0.3	3	3
2	*N*-Ac-L-Trp	DMSO-d_6	0.09 ± 0.01		
		DMSO-d_6/H_2O (80/20)	0.024 ± 0.006		
		DMSO-d_6/H_2O (50/50)	<0.02		
		DMSO-d_6/H_2O (25/75)	<0.02		
	L-Trp	DMSO-d_6	0.05 ± 0.01		
		DMSO-d_6/H_2O (80/20)	NA[d]		
	N-Ac-Gly	DMSO-d_6	0.041 ± 0.005		
		DMSO-d_6/H_2O (80/20)	NA		
	indole	DMSO-d_6	NA		
		DMSO-d_6/H_2O (80/20)	<0.01		
		DMSO-d_6/H_2O (50/50)	<0.02		
		DMSO-d_6/H_2O (25/75)	<0.02		
	1,5-DNS	DMSO/H_2O (10/90)	1.50 ± 0.04		
	fluorescein	DMSO/H_2O (10/90)	1.45 ± 0.05		
	pyrene	DMSO/H_2O (10/90)	1.3 ± 0.1		
3	*N*-Ac-L-Trp	DMSO	0.91 ± 0.05		
		DMSO-d_6/H_2O (80/20)	0.7 ± 0.2		
		DMSO-d_6/H_2O (50/50)	0.5 ± 0.1		
		DMSO-d_6/H_2O (25/75)	<0.2		
	N-Ac-Gly	DMSO-d_6	<0.01		
	indole	DMSO-d_6	0.42 ± 0.05		
		DMSO/H_2O (80/20)	1.2 ± 0.1		
		DMSO/H_2O (50/50)	0.8 ± 0.04		
		DMSO/H_2O (25/75)	0.8 ± 0.1		
	1,5-DNS	DMSO/H_2O (10/90)	0.58 ± 0.06		
	fluorescein	DMSO/H_2O (10/90)	0.98 ± 0.06		
	pyrene	DMSO/H_2O (2/98)	1.22 ± 0.08		

[a] K_A values determined from analysis of fluorescence spectra except for experiments performed in a deuterated solvent, which were determined from analysis of ^1H NMR spectra. Water solutions were buffered with 1 mM phosphate buffer, pH 7.0. [b] Values with less than signs are estimated. [c] A comparison of K_A values, with the numerical subscript indicating a host. [d] NA indicates no association was detected.

rotaxane **1** in DMSO-d_6, suggesting that the indole ring of *N*-Ac-Trp becomes embedded in the electron-rich environment of the aromatic pocket of the host. The signal for some aromatic protons of the indole ring shifted downfield. A large downfield shift ($\Delta\delta$ = 0.3 ppm) was observed for the aromatic protons of *N*-Ac-Trp in the presence of 1 equiv of [2]rotaxane **1** as compared to the guest alone in a 50/50 DMSO/buffer solution. A downfield shift indicates that these parts of the indole ring are held in a face-to-face manner with the phenyl rings of the calix[4]arene pocket, and not in an edge-to-face alignment.[6] This geometrical arrangement is observed in the molecular modeling results vide infra. Only small changes in chemical shifts were observed with the other hosts, which suggests that the aromatic ring of DB24C8 partially encloses the pocket of the rotaxane.

An estimation of the contribution to the binding free energy provided by the functional groups of *N*-Ac-Trp was obtained by comparing the association constants for indole and *N*-Ac-Gly bound to [2]rotaxane **1** (Table 1). The K_A for the complex between indole and [2]rotaxane **1** was too large for accurate determination; however, the K_A for the *N*-Ac-Gly could be determined. A downfield shift in the resonance of the acetyl protons of *N*-Ac-Gly, caused by the increase in concentration of [2]rotaxane **1**, provided for an easily traceable signal. The significantly sized association constant ($K_A = 1.6 \times 10^3$ M^{-1}) in DMSO-d_6 suggests that a salt bridge formed between the carboxylate of *N*-Ac-Gly and the host. Consistent with a salt bridge, adding water to the solution reduced the association constant for the *N*-Ac-Gly−[2]rotaxane **1** complex.

Calix[4]arenes **2** and **3** bound *N*-Ac-Trp weaker than [2]rotaxane **1** in DMSO-d_6. A downfield shift of the N−H proton of the indole ring was observed with an increase in the concentration of either host. In contrast to the [2]-rotaxane **1** complex, the indole ring is not placed in an electron-rich environment of calix[4]arenes **2** and **3**. The

TABLE 2. Fluorescence Properties of the Hosts[a]

host	solvent	$\lambda_{ex,max}$	$\lambda_{em,max}$	F_{host}/F_{indole}[b]
1	DMSO	286	388	0.05
	DMSO/H_2O[c] (25/75)	290	360	<0.005
2	DMSO	287	343	0.45
	DMSO/H_2O (25/75)	290	350	0.01
3	DMSO	280	390	0.36
	DMSO/H_2O (25/75)	272	393	0.29

[a] Fluorescence measured at 25.0 °C. [b] Ratio of a host's fluorescence at its maximum excitation and emission wavelengths versus indole's fluorescence at $\lambda_{ex,max}$ 280 nm and $\lambda_{em,max}$ 333 nm. [c] Water solutions are buffered with 1.0 mM phosphate, pH 7.0.

N−H proton is either held near an electron-poor edge of a host's aromatic ring or involved in a H-bond complex. This result suggests that *N*-Ac-Trp is held in a different binding geometry for [2]rotaxane **1**.

With the addition of water to the solutions (80/20 (v/v) DMSO-d_6/buffer), no shifts could be detected in the signal of the N−H proton of *N*-Ac-Trp's indole ring in the presence of calix[4]arene **2**. There was a small downfield shift for an aromatic proton of *N*-Ac-Trp, and this signal was used to derive the association constant. Apparently, water disrupts a salt bridge, and association occurs via pocket penetration. The similar K_A values observed for *N*-Ac-Trp and *N*-Ac-Gly bound to calix[4]arene **2** in DMSO-d_6 also suggest that a salt bridge provides a significant proportion of the binding free energy for the association of *N*-Ac-Trp. The very weak association between *N*-Ac-Gly and calix[4]arene **3** further demonstrates the importance of the arginine groups for the complexes formed between calix[4]arene **2** and *N*-Ac-Gly. The addition of water to a millimolar solution of calix[4]arene **3** precipitated the host, which prevented further NMR analysis of the calix[4]arene **3**−*N*-Ac-Trp complex.

The chemical shifts of indole or calix[4]arene **2** did not change when they were mixed in the millimolar concentration range in DMSO-d_6, indicating no association occurs in this solvent. With the addition of 20% buffer (1 mM phosphate, pH 7.0) to the solutions, a weak complex formed. The N−H proton of indole shifted upfield with an increase in the concentration of calix[4]arene **2**. The aromatic ring enters a shielding environment, which suggests that the ring becomes buried within the aromatic pocket in a manner similar to that of the complex formed between [2]rotaxane **1** and indole. Because the binding plot was linear with the host and guest combined in the low millimolar range, a $K_A < 100$ M^{-1} was assigned.

Fluorescence Titrations. Fluorescence binding assays were performed to determine the larger association constants and to discover whether complexes form in solutions that contain a large percentage of water. Besides tryptophan derivatives and indole, other fluorophores, having a variety of functional groups, were chosen as guests. The host's were fluorescent as well, albeit weaker than the fluorophores (Table 2). In DMSO, calix[4]arene **2** is the strongest fluorophore, followed closely by calix[4]arene **3**, and [2]rotaxane **1** only fluoresces weakly. The ring or arginine derivative may quench the fluorescence of the biaryl rings of [2]rotaxane **1** in DMSO. As the percentage of water is increased in the solutions, the fluorescence of the hosts drops. In a 25/75 (v/v) DMSO/buffer (1 mM phosphate buffer, pH 7.0) solution, calix[4]arene **3** has only a small drop in fluorescence. On the other hand, the fluorescence intensity of [2]rotaxane **1** was approximately nonexistent, and the intensity was greatly reduced for calix[4]arene **2** with the addition of water to a DMSO solution. The change in fluorescence caused by the addition of water may be due to a greater water solvation of hosts **1** and **2** or a change in their structure.

The reduction in fluorescnece intensity observed with the addition of water was not caused by a precipitation of hosts. For the least soluble host, calix[4]arene **3**, host concentration was verified by removing the fluorescence solution, spinning the sample to condense any precipitate, and comparing the absorbance, and thus concentration, of the solution to a predetermined calibration curve.

Significant quenching of the fluorescence of *N*-Ac-Trp occurs in the presence of [2]rotaxane **1**, allowing an association constant to be determined for the complex. The derived large K_A for association in DMSO (Table 1) is consistent with the fact that an accurate association constant could not be obtained from the ^{1}H NMR experiments. With the addition of a small amount of water to the DMSO solution (20% (v/v)), the K_A for this complex does not change within experimental error. At higher concentrations of water, K_A drops only by a small amount. A 5-fold reduction occurs in an essentially pure buffer solution (98/2 (v/v) buffer/DMSO).

Indole also interacts strongly with [2]rotaxane **1**. The magnitude of its K_A becomes more similar to the values derived for the *N*-Ac-Trp−[2]rotaxane **1** complex for solutions having higher percentages of water. This result indicates that the major driving force for the interaction of *N*-Ac-Trp with [2]rotaxane **1** is the placement of its indole ring into the aromatic pocket. However, the larger K_A observed for *N*-Ac-Trp as compared to indole suggests that either a salt bridge or H-bonds exist between the rotaxane's and guest's functional groups, even in the presence of a high percentage of water.

The advantage of converging functional groups for recognition is clearly evident upon comparing the association constants for [2]rotaxane **1** and calix[4]arene **2** binding the various guests. A 10-fold smaller K_A is observed for the calix[4]arene **2**−*N*-Ac-Trp complex in DMSO, as compared to [2]rotaxane **1**. As the percentage of water is increased in the solutions containing *N*-Ac-Trp and calix[4]arene **2**, the association constant drops by at least a factor of 5, maintaining a 2 orders of magnitude reduction in K_A compared to those of complexes formed with [2]rotaxane **1**. Association constants for complexes of calix[4]arene **2** in solutions containing 50% and 75% water are approximations. A change in fluorescence was observed only with the addition of a large amount of the host. The $K_A < 200$ M^{-1} limiting value was chosen because of the results obtained for the assay performed in the 80/20 (v/v) DMSO/buffer mixture. In this solvent system, the plot of the small change in fluorescence versus host concentration was linear and could not be fitted using a nonlinear least-squares fitting procedure. The association constant in this solvent mixture was thus determined by ^{1}H NMR experiments to give a value of $K_A = 240$ M^{-1}. Binding assays that produced linear plots at a high concentration of hosts (10^{-5} to 10^{-4} M) were assigned K_A values less than 200 M^{-1}.

Calix[4]arene **2** binds indole with K_A values similar to those observed for *N*-Ac-Trp. Because of the relatively weak association, most of the values are estimates. We cannot determine which functional group drives association. The fluorescence titrations were complicated by the fact that calix[4]arene **2** and indole have almost identical excitation and emission spectra. Fortunately at higher concentrations of water, the fluorescence intensity of calix[4]arene **2** is greatly diminished. In the 25/75 (v/v) DMSO/buffer solution, the host's fluorescence is very weak (Table 2), and a high concentration of host (10^{-4} M) was needed to produce a change in the fluorescence intensity of indole. Thus, the limiting value of $K_A = 200$ M^{-1} was assigned. The same K_A was assigned to the complex formed in a 50/50 (v/v) DMSO/buffer solution for the same reasons.

Fluorescence assays of calix[4]arene **3** were complicated by an apparent self-association of the host, especially in solutions containing a high percentage of water. Dimerization would be consistent with the synthetic capsules developed by Rebek.[33] Association constants for guest complexes were estimated by using the Benesi−Hildebrand method[48] for the data obtained with low concentrations of components, which should have had an insignificant amount of the dimer in the formation in the solution. These values were consistent with K_A values derived using all the data through a nonlinear least-squares fitting of the data to a standard binding equation for a 1/1 complex.[49] Thus, if dimerization occurs, only a small percentage of the hosts interact at the concentrations used in the experiment.

Small changes in the fluorescence of *N*-Ac-Trp and indole were observed with the addition of calix[4]arene **3** to a solution containing either guest. For the *N*-Ac-Trp complex, the binding strength diminishes with the addition of water to the solution. A small association constant ($K_A = 5 \times 10^3$ M^{-1}) was derived for complexation in a 50/50 (v/v) DMSO/water solution. In the 25/75 (v/v) DMSO/water solution, the fluorescence plot was linear and could not be fitted. We arbitrarily assigned a K_A value that is half as large as the one derived in the 50/50 (v/v) DMSO/water solution, which is a conservative estimate. The K_A for the indole complex is actually weakest in DMSO and increases with the addition of water to the solution. Overlapping fluorescence spectra did not occur for these experiments. Calix[4]arene **3** fluoresces at 408 nm, leaving the indole and *N*-Ac-Trp window at 334 nm open.

1-(Dimethylamino)-5-naphthalenesulfonate (1,5-DNS), fluorescein, and pyrene form tight complexes with all three hosts, with [2]rotaxane **1** having the strongest interactions. The largest association constant is observed for the complex of [2]rotaxane **1** and fluorescein; its K_A is over 2 orders of magnitude greater than the association constants observed for the other dye complexes. For the weaker complexes, i.e., K_A values of 1×10^4 M^{-1} or smaller, a 10/90 (v/v) DMSO/buffer solution was necessary to solubilize the higher concentrations of hosts that were necessary to obtain curvature in the binding plots. For example, small changes in the fluorescence of fluorescein was observed with the addition of calix[4]arene **2** in the same concentration range as used for [2]rotaxane **1** (10^{-7} to 10^{-6} M), which resulted in a linear binding plot. A higher concentration of calix[4]arene **2** (10^{-6} to 10^{-5} M) did result in significant curvature in the plot. A 10/90 (v/v) DMSO/buffer solution was also used in the calix[4]arene **3** and pyrene study. Calix[4]arene **3** has a slight preference for pyrene as compared to its complexes with the other fluorophores.

The long-wavelength emissions of these dyes do not overlap with the fluorescence of the hosts except for the case of pyrene and calix[4]arene **3**. The fluorescence of calix[4]arene **3** is substantially weaker than that of pyrene (<10%), and thus, the addition of host should not have produced a large error in the fluorescence measurements. This appears to be the case since the shape of pyrene's fluorescence bands did not change with the addition of calix[4]arene **3** and the derived K_A has a small standard deviation.

Discussion

The arginine-derivatized ring of [2]rotaxane **1** contributes favorable binding free energy for most of the complexes investigated in this study. A one order of magnitude enhancement in K_A is observed for the binding of *N*-Ac-Trp by [2]rotaxane **1** as compared to calix[4]arene **3** in DMSO. The ca. 1.5 kcal/mol difference in binding free energy can easily be accounted for if a salt bridge or H-bonds form between the guanidinium moiety of [2]-rotaxane **1** and the amino acid backbone of *N*-Ac-Trp. As the percentage of water is increased, the difference in K_A increases except for the solution containing 50% buffer. The reduction in K_A observed for the calix[4]arene **3**−*N*-Ac-Trp complex with the addition of water is reasonable considering that the polar groups of the guest become strongly solvated with water, which results in a greater desolvation penalty for guest inclusion into the hydrophobic pocket. Apparently, the guanidinium moiety of [2]rotaxane **1** can accommodate the polar groups of the guest and lessen the desolvation penalty.

The importance of desolvation is evident by the 10-fold drop in the K_A for L-Trp association with [2]rotaxane **1**, as compared to the *N*-Ac-Trp complex. The additional charge of the ammonium ion of L-Trp interacts favorably with solvent molecules, which may need to be removed for complexation. Since D-Trp associates with [2]rotaxane **1** as favorably as L-Trp, chiral recognition does not occur for this guest in DMSO.

The importance of the hydrophobic effect is exemplified by the strength of the indole interaction with the hosts. A 10-fold larger K_A is observed for the binding of indole by [2]rotaxane **1** as compared to calix[4]arene **3** in DMSO. A possible H-bond may exist between the arginine of the ring and the N−H of indole. With the addition of water to DMSO, the complex between indole and [2]rotaxane **1** is weakened, which could be explained by the breaking of this H-bond. On the other hand, the presence of water enhances the association between calix[4]arene **3** and indole, which is consistent with the hydrophobic effect. In DMSO/water mixtures, hosts **1** and **3** bind indole with approximately the same large association constant. Furthermore, the N−H proton of indole shifts upfield in the presence of either host. These results suggest that these

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(49) Conners, K. A. *Binding Constants, The Measurement of Molecular Complex Stability*; Wiley: New York, 1987.

hosts have similar hydrophobic cavities—the ring of [2]-rotaxane **1** does not significantly distort its pocket—and the hydrophobic effect is an important component for complex formation. The fact that *N*-Ac-Gly associates very weakly with [2]rotaxane **1** in DMSO/water mixtures suggests that the indole ring is necessary for *N*-Ac-Trp to strongly interact with [2]rotaxane **1**.

Examination of the association constants derived for complexes formed with calix[4]arene **2** shows that it is a poorly designed host. The arginine side chains appear not to operate jointly with the hydrophobic pocket to obtain a highly effective host. For most guests, the K_A values for complexes formed with calix[4]arene **2** are actually smaller than the ones derived for calix[4]arene **3** with the same guests. This result suggests that the pocket of calix[4]arene **2** is closed, possibly through intramolecular interactions between the amino acids on the upper rim. Accordingly, indole does not bind in a DMSO solution, because this solvent does not break interactions between the amino acids. Adding water to the solution weakens the intramolecular interactions and promotes indole binding through the hydrophobic effect. On the other hand for charged guests, the hydrophobic effect does not appear to contribute to the binding free energy. The binding free energy derived for the complex of calix[4]arene **2** and *N*-Ac-Trp (−4 kcal/mol) is consistent with a salt bridge.[50] A similar free energy for association was derived for L-Trp and *N*-Ac-Gly, which suggests that only a salt bridge forms between calix[4]-arene **2** and small, polar guests in DMSO. Consistent with a salt bridge, adding water to the solutions reduces the association constants.

Guests with large aromatic surfaces, however, do strongly bind calix[4]arene **2**. In fact all fluorophores associate with calix[4]arene **2** to the same extent, which suggests that contact between aromatic surfaces provides the favorable binding energy. The other possibility is that the interaction energies between the functional groups of fluorescein and 1,5-DNS just happen to be equal in magnitude to the hydrophobic interaction energies of pyrene. This situation is unlikely since interactions between charged groups of a guest and calix[4]arene **2** are weak even in a high percentage of DMSO. The fact that calix[4]arene **2** interacts strongly with pyrene shows that its pocket can interact with a large aromatic ring better than a small ring such as indole. Besides the greater hydrophobic effect, the pocket of calix[4]arene **2** could also be more accommodating of the fluorophores because intramolecular interactions between the arginine residues should be very weak in solutions containing a high percentage of water.

[2]Rotaxane **1** forms the strongest complexes with the fluorophores. According to the K_A values, [2]rotaxane **1** prefers fluorescein by two orders of magnitude over the other fluorophores. Fluorescein has a large aromatic surface area and a carboxylate moiety; both components were targeted in the design of the host. A 3-fold larger K_A is observed for the association of pyrene by [2]rotaxane **1**, as compared to calix[4]arene **3**, whereas the smaller indole ring binds both hosts with the same association constant within experimental error. Therefore, the ro-

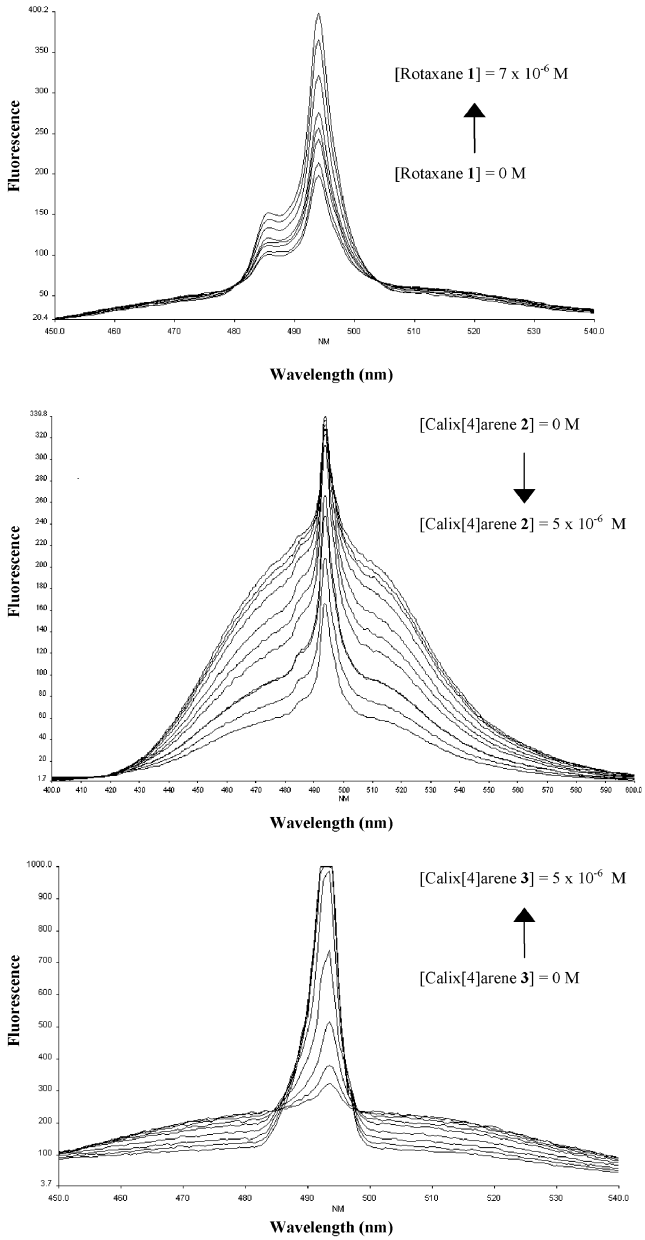

FIGURE 3. Fluorescence titrations of 1,5-DNS and [2]-rotaxane **1**, calix[4]arene **2**, and calix[4]arene **3** in DMSO/buffer (1 mM phosphate, pH 7) mixtures at 25 °C. An enhancement in the fluorescence intensity with the addition of host **1** or **3** suggests that 1,5-DNS enters the hydrophobic pocket of the hosts.

taxane can contribute a greater amount of binding free energy to a complex with a large aromatic guest, most likely, through favorable interactions with the crown ether's aromatic ring or an enhancement in the hydrophobic effect. As also seen with small charged guests, [2]-rotaxane **1** can accommodate the charged fluorophores better than calix[4]arene **3**. For the latter host, the highest affinity is observed for pyrene followed by fluorescein and then 1,5-DNS. This trend is consistent with the number of charged groups; the greater the desolvation penalty, the weaker the complex.

One major difference observed in these studies is that the fluorescence of 1,5-DNS is increased with exposure to [2]rotaxane **1** or calix[4]arene **3**, whereas in the

(50) Thompson, S. E.; Smithrud, D. B. *J. Am. Chem. Soc.* **2002**, *124*, 442−449.

presence of calix[4]arene **2** it is decreased (Figure 3). Enhanced fluorescence suggests that 1,5-DNS is embedded within a hydrophobic environment of hosts **1** and **3**.[51−53] Even in a high concentration of water, it appears that the aromatic pocket of calix[4]arene **2** with divergent functional groups does not associate strongly through the hydrophobic effect.

Conclusion

The convergent arrangement of the functional groups of the host−[2]rotaxane is advantageous for binding guests that have an aromatic ring and a negative charge. For these guests, [2]rotaxane **1** has stronger interactions than a host with divergent functional groups or one that just has an aromatic pocket. This result suggests that the pocket and arginine derivative of [2]rotaxane **1** interact with an aromatic ring and anion, respectively. The aromatic ring of a guest provides the majority of binding free energy in DMSO and DMSO/water mixtures. In the case of indole, the ring of the rotaxane does not appear to help or hinder association. But for larger aromatic guests, the ring does contribute and provide favorable binding free energy. The arginine derivative on the rotaxane's ring makes a significant contribution to the binding free energy for charged guests. Although the strengths of these complexes are diminished in the presence of water, a greater binding free energy is observed for [2]rotaxane **1** binding *N*-Ac-Trp than for its association with indole, which essentially binds through the hydrophobic effect alone. Thus, [2]rotaxane **1** still binds charged guests strongly in solutions with a high percentage of water by forming favorable interactions with its convergent functional group. The divergent groups of calix[4]arene **2** are actually counterproductive for guest binding; the recognition elements and aromatic pocket do not cooperate in complex formation.

Experimental Section

General Information. All air-sensitive reactions were carried out under positive argon pressure, and 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. 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 250 MHz for proton nuclei and 62.9 MHz for carbon nuclei. Chemical shifts are in parts per million and are referenced using an internal TMS standard.

Monitoring Association. For ^{1}H NMR assays, six separate solutions were made that contained a guest at a constant concentration of 1.0 mM. Five of the solutions contained a different amount of a host from 0.2 to 3.0 mM. At this concentration range, K_A values in the range of 10^3 M^{-1} can usually be accurately derived. Weaker complexes will generally provide linear binding plots. Assays that had linear plots were repeated with the guest held at a constant concentration of 5.0 mM and the host varied from 1.0 to 10 mM. If no changes were observed in the chemical shifts of either the host or guest, we assigned a K_A value of zero. One caveat with ^{1}H NMR experiments is that a change in environment must occur upon complexation to produce a change in the chemical shift of a proton. Literature precedence[6] has shown that in most cases the resonances of a guest's aromatic protons will change upon complexation. In these studies, the N−H of the indole ring should be a likely indicator of association. It is highly solvated in DMSO-d_6 or DMSO-d_6/buffer solutions and should experience a dramatic change in environment once bound to a host.

Fluorescence binding assays were performed on hosts and fluorescent guests, including ones that did not have an observable association in the ^{1}H NMR experiments. In the latter cases, we wanted to verify that association does not occur. A known amount of guest, dissolved in a particular solution, was added to a 3.5 mL cuvette (maintained at 25 °C), and aliquots from a concentrated host stock solution were added. The change in volume caused by the addition of host was less than 10%. For assays containing a mixture of DMSO and buffer (1 mM phosphate, pH 7.0), the host stock solution contained the same ratio of solvents except for the calix[4]arene **3**−pyrene experiment. In this study, the stock and assay solutions contained 10/90 and 2/98 (v/v) DMSO/buffer, respectively. Several assays were repeated with different concentrations of host and guest to obtain a curvature in the binding plots. The concentrations of the hosts and guests are given in the Supporting Information. Complexes with large association constants ($K_A > 2 \times 10^4$ M^{-1}) had an observed maximum change in fluorescence equal to 50−75% of the calculated value (ΔF_{max}). Because of solubility problems, weaker complexes had an observed maximum change in fluorescence equal to 30−50% of ΔF_{max}.

Maximum excitation and emission wavelengths were used for the guests except in the cases of a high concentration of the components or an overlap of the emission bands. At high concentration of a fluorophore, a shoulder of the excitation or emission band was used or monitored, respectively. If overlap occurred, λ_{em} was chosen such that the total emission of the host was less than 10% of the emission of the guest. In the case of calix[4]arene **2** mixed with *N*-Ac-Trp and indole in DMSO and DMSO/buffer (80/20 (v/v)), significant overlap in the emission spectra occurred. No quenching occurred in these assays, but there was a slight increase in fluorescence intensity consistent with the amount of host added. At higher concentrations of water, the fluorescence of calix[4]arene **2** is negligible. A small amount of quenching was only observed with a high concentration of host, which is consistent with the degree of association observed by ^{1}H NMR spectroscopic experiments.

Molecular Modeling. Energy-minimized conformers of [2]rotaxane **1** bound to *N*-Ac-Trp and fluorescein were determined using a conjugate gradient method (Polak−Ribiere) to an rms gradient of 0.01 kcal/(mol·Å) (semiempirical calculations, AM1 force field to assign charges, and then molecular mechanics calculations, MM+ force field as presented by HyperChem, Hypercube, Inc., Gainesville, FL). A guest was combined with the host in several different orientations, and the complexes were minimized (Polak−Ribiere) to an rms gradient of 0.1 kcal/(mol·Å). The conformation−energy space of the lowest energy complex was further explored through Monte Carlo simulations. A complex was heated from 0 to 300 K over 10 steps, and the simulations were run for 1000 steps at 300 K. Low-energy complexes were chosen out of the 1000 structures, and a final round of energy minimization was performed using a conjugate gradient method (Polak−Ribiere) to an rms gradient of 0.1 kcal/(mol·Å) (molecular mechanics calculations, MM^+ force field). The lowest energy complexes for host−[2]rotaxane bound to *N*-Ac-Trp and fluorescein are presented.

Compound Synthesis. The mixture of syn and anti isomers of the crown ether derivatives or the sliding of the rotaxane ring complicate most ^{1}H and ^{13}C NMR spectra.

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(52) Li, Y.-H.; Chan, L.-M.; Tyer, L.; Moody, R. T.; Himel, C. M.; Hercules, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 3118−3126.
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Changing the temperature did not significantly reduce the number of conformers.

5,17-Dibromo-25,27-bis(ethoxycarbonylmethoxy)calix[4]arene-26,28-diol (5). To a solution of 6.00 g (10.0 mmol) of calix[4]arene diethyl ester[42] in 40 mL of $CHCl_3$ was slowly added 3.10 g (20.0 mmol) of Br_2 in 20 mL of $CHCl_3$. The reaction mixture was stirred at rt for 3 h, followed by the removal of $CHCl_3$ and residual Br_2 in vacuo. Recrystallization from $CHCl_3$ gave 7.54 g (99%) of calix[4]arene **5** as a white powder: mp 122 °C dec; ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.97 (s, 2H), 7.37 (s, 4H), 7.07 (d, J = 7.6 Hz, 4H), 6.82 (t, J = 7.6 Hz, 2H), 4.75 (s, 4H), 4.34−4.21 (m, 6H), 3.46 (d, J = 13.2 Hz, 4H), 1.27 (t, J = 7.4 Hz, 6H); ^{13}C NMR (63 MHz, DMSO-d_6) δ 168.61, 151.86, 132.77, 130.59, 130.17, 129.24, 125.43, 109.88, 72.23, 60.89, 30.09, 13.89; TOF MS m/z calcd for $C_{36}H_{35}O_8Br_2^+$ 753.0699, found 753.0740.

5,17-Bis(3-nitrophenyl)-25,27-bis(hydroxycarbonylmethoxy)calix[4]arene-26,28-diol (6). A 1.13 g (1.50 mmol) sample of dibromocalix[4]arene **5** and 1.00 g (5.99 mmol) of 3-nitrophenylboronic acid were dissolved in a 55 mL of toluene/methanol (9/1) solution. A 1.11 g (10.5 mmol) sample of Na_2CO_3 was dissolved in 10 mL of water, and this mixture was added to the reaction mixture. The flask was flushed twice with Ar, and charged with 0.15 g (0.15 mol) of $Pd(PPh_3)_4$. After the reaction mixture was stirred at 70 °C for 40 h, the organic phase was separated, diluted with 100 mL of CH_2Cl_2, and extracted with 5% HCl. The organic phases were collected and dried with Na_2SO_4. After the solvents were removed in vacuo, the crude product was purified via column chromatography (SiO_2, $CHCl_3$, CH_3OH) to give 0.91 g (78%) of calix[4]arene **6** as a yellow solid: mp 270 °C dec; ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.40 (s, 2H), 8.09 (d, J = 7.9 Hz, 4H), 7.66 (s, 4H), 7.71−7.64 (m, 2H), 7.17 (d, J = 7.6 Hz, 4H), 6.79 (t, J = 7.6 Hz, 2H), 4.75 (s, 4H), 4.43 (d, J = 12.9 Hz, 4H), 3.59 (d, J = 12.9 Hz, 4H); ^{13}C NMR (63 MHz, DMSO-d_6) δ 170.29, 153.29, 152.78, 148.43, 141.82, 133.12, 132.57, 130.09, 129.23, 128.56, 128.44, 127.13, 125.27, 120.88, 120.13, 72.33, 30.55; TOF MS m/z calcd for $C_{44}H_{35}N_2O_{12}^+$ 783.219, found 783.2256.

5,17-Bis(3-nitrophenyl)-25,27-bis(methoxycarbonylmethoxy)calix[4]arene-26,28-diol (7). A 3.40 g (4.34 mmol) sample of calix[4]arene **6** was refluxed in a solution of $CHCl_3$/CH_3OH (2/1 (v/v)), containing concd H_2SO_4 (2−3%) for 12 h. Upon solvent removal, 3.50 g (99%) of the calix[4]arene **7** was obtained as an off-white solid, which was pure by ^{1}H NMR analysis: mp 151−155 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.37 (s, 2H), 8.11 (d, J = 8.1 Hz, 2H), 8.00 (s, 2H), 7.84 (d, J = 7.8 Hz, 2H), 7.54 (t, J = 8.3 Hz, 2H), 7.33 (s, 4H), 7.02 (d, J = 7.4 Hz, 4H), 6.83 (t, J = 7.4 Hz, 2H), 4.80 (s, 4H), 4.55 (d, J = 13.2 Hz, 4H), 3.92 (s, 6H), 3.52 (d, J = 12.5 Hz, 4H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 169.23, 153.79, 152.36, 148.69, 142.86, 132.87, 132.48, 130.16, 129.66, 129.44, 128.90, 127.26, 125.86, 121.22, 121.00, 77.21, 72.28, 52.31, 31.64; MS HiResESI m/z calcd for $C_{46}H_{38}N_2O_{12}Na^+$ 833.2322, found 833.2300.

Partial Reduction of 5,17-Bis(3-nitrophenyl)-25,27-bis(methoxycarbonylmethoxy)calix[4]arene-26,28-diol. A 2.00 g (2.47 mmol) sample of calix[4]arene **7** was dissolved in 130 mL of a $CHCl_3$/CH_3OH/CH_3CO_2H (10/5/1) solution. The flask was flushed with Ar and charged with 100 mg of Pd/C, H_2 (50 psi), and the reaction mixture was stirred at rt for 21 h. The progress of reduction was monitored by TLC, and the reaction was ended when ca. 80% of dinitrophenylcalix[4]arene **7** was consumed. The reaction mixture was filtered through a plug of Celite, the solvents were removed in vacuo, and the products were separated by column chromatography (SiO_2, $CHCl_3$/CH_3OH) to give 0.23 g (0.28 mmol), 0.60 g (0.77 mmol, 35%), and 0.91 g (1.2 mmol, 56%) of calix[4]arene **7**, **8**, and **3**, respectively (yields are calculated on the basis of the amount of consumed calix[4]arene **7**).

Data for 5-(3-aminophenyl)-17-(3-nitrophenyl)-25,27-bis(methoxycarbonylmethoxy)calix[4]arene-26,28-diol (8): mp 141−145 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, $^1/_2$H), 8.10 (d, J = 8.0 Hz, $^1/_2$H), 8.01 (s, $^1/_2$H), 7.83 (d, J = 7.5 Hz, $^1/_2$H), 7.75 (s, $^1/_2$H), 7.70−7.50 (m, 1$^1/_2$H), 7.32 (s, 2H), 7.25 (s, 2H), 7.17−7.15 (m, 1H), 7.06−6.76 (m, 8H), 6.60−6.50 (m, 1H), 4.79−4.73 (m, 4H), 4.57−4.45 (m, 4H), 3.90 (s, 3H), 3.88 (s, 3H), 3.51−3.42 (m, 4H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 169.25, 152.29, 148.68, 146.29, 133.16, 132.74, 132.53, 132.40, 130.83, 130.21, 129.49, 129.23, 128.94, 128.51, 128.21, 127.25, 125.77, 117.50, 113.45, 110.62, 72.47, 72.25, 52.27, 31.65, 31.45; TOF MS m/z calcd for $C_{46}H_{41}N_2O_{10}Na^+$ 803.2581, found 803.2604.

Data for 5,17-bis(3-aminophenyl)-25,27-bis(methoxycarbonylmethoxy)calix[4]arene-26,28-diol (3): mp 156 °C dec; ^{1}H NMR (250 MHz, $CDCl_3$) 7.68 (s, 2H), 7.25 (s, 4H), 7.16 (t, J = 7.6 Hz, 2H), 6.90−6.98 (m, 6H), 6.83 (s, 2H), 6.76 (t, J = 7.4 Hz, 2H), 6.59 (d, J = 7.6 Hz, 2H), 4.76 (s, 4H), 4.50 (d, J = 13.0 Hz, 4H), 3.87 (6H, s), 3.40−3.80 (br s, 2H), 3.45 (d, J = 13.0 Hz, 4H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 169.23, 152.65, 152.35, 146.58, 142.57, 133.03, 132.52, 129.44, 129.32, 128.26, 127.88, 127.25, 125.67, 117.34, 113.58, 113.24, 77.21, 72.32, 52.19, 31.65; TOF MS ES+ m/z calcd for $C_{46}H_{43}N_2O_8$ (MH^+) 751.3019, found 751.3069.

5,17-Bis((Boc)$_3$arginylaminophenyl)-25,27-bis(methoxycarbonylmethoxy)calix[4]arene-26,28-diol (9a). A 140 mg (0.185 mmol) sample of calix[4]arene **3**, 266 mg (0.560 mmol) of (Boc)$_3$-Arg-OH, and 76 mg (0.560 mmol) of HOBT were dissolved in 10 mL of $CHCl_3$, and the mixture was cooled to 0 °C. After 118 mg (0.616 mmol) of EDC was added, the reaction mixture was warmed to rt and stirred under Ar for 1 h. The reaction mixture was refluxed for 12 h to facilitate coupling. The crude reaction mixture was diluted with ca. 30 mL of $CHCl_3$ and extracted with 5% HCl and then water. The organic layers were collected and dried over $NaSO_4$, and the product was purified by column chromatography (SiO_2, $CHCl_3$, C_2H_5OH) to give 0.26 g (84%) of calix[4]arene **9a** as a yellow oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.60−9.15 (m, 6H), 9.08 (s, 2H), 7.64 (s, 2H), 7.53−7.45 (m, 4H), 7.37−7.27 (m, 8H+solvent), 6.99−6.95 (m, 4H), 6.77 (t, J = 7.5 Hz, 2H), 5.94 (d, J = 8.1 Hz, 2H), 5.36 (d, J = 7.8 Hz, 1H), 4.76 (s, 4H), 4.50 (d, J = 13.3 Hz, 4H), 4.60−4.40 (m, 2H), 3.89 (s, 6H), 4.00−3.80 (m, 2H), 3.80−3.60 (m, 2H), 3.47 (d, J = 13.3 Hz, 4H), 1.93−1.67 (m, 8H), 1.52−1.34 (m, 54H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.67, 169.20, 163.46, 161.15, 155.50, 154.83, 152.96, 152.28, 141.81, 137.86, 132.88, 131.53, 129.35, 129.02, 128.39, 127.75, 127.22, 125.68, 124.57, 124.28, 122.92, 119.22, 118.18, 111.38, 105.07, 84.25, 83.80, 79.69, 72.33, 61.19, 52.21, 44.16, 44.03, 31.56, 31.21, 29.49, 28.43−27.91 (m), 27.02, 24.75, 24.63, 14.18; TOF MS ES+ m/z calcd for $C_{88}H_{114}N_{10}O_{22}Na^+$ 1685.8007, found 1685.8002.

5,17-Bis(*N*-acetyl(Boc)$_2$arginylaminophenyl)-25,27-bis(methoxycarbonylmethoxy)calix[4]arene-26,28-diol (9b). A 200 mg (0.266 mmol) sample of calix[4]arene **3**, 334 mg (0.800 mmol) of Ac-(Boc)$_2$-Arg-OH, and 108 mg (0.800 mmol) of HOBT were dissolved in 15 mL of $CHCl_3$, and the mixture was cooled to 0 °C. A 170 mg (0.880 mmol) sample of EDC was added to the reaction mixture, which was warmed to rt and refluxed under argon for 1 h (TLC analysis indicated complete conversion). The crude reaction mixture was diluted with ca. 30 mL of $CHCl_3$ and extracted with 1% HCl and water. The product was purified by column chromatography (SiO_2, $CHCl_3$, CH_3OH) to give 0.29 g (70%) of calix[4]arene **9b** as a transparent oil: ^{1}H NMR (250 MHz, $CDCl_3$) 9.70−9.40 (br m, 4H), 9.12 (s, 1H), 7.53 (s, 2H), 7.46−7.42 (m, 4H), 7.35−7.27 (m, 8H), 6.70−6.98 (m, 4H), 6.79−6.77 (m, 2H), 6.37 (m, 2H), 5.00−4.51 (m, 2H), 4.76 (s, 4H), 4.50 (d, J = 13.2 Hz, 4H), 4.20−4.00 (m, 2H), 3.90 (6H, s), 3.83−3.80 (m, 1H), 3.70−3.34 (m, 3H), 3.47 (d, J = 13.2 Hz, 4H), 2.60−2.45 (m, 1H), 2.17−2.03 (m, 6H), 1.92−1.67 (m, 8H), 1.51−1.31 (m, 36H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 170.29, 170.20, 169.15, 163.04, 154.72, 152.90, 152.22, 141.76, 138.01, 137.79, 132.86, 132.36, 131.46, 129.32, 128.98, 128.35, 127.16, 125.90, 125.65, 122.90, 119.07, 118.51, 115.05, 84.34, 79.74, 72.27, 53.16, 52.19, 44.05, 31.52, 28.14, 27.93, 24.90, 23.29, 23.12; TOF MS m/z calcd for $C_{82}H_{103}N_{10}O_{20}^+$ 1547.7350, found 1547.7360.

5,17-Bis(3-(2-amino-5-guanidinopentanoylamino)-phenyl)-25,27-bis(methoxycarbonylmethoxy)calix[4]-arene-26,28-diol (2). A 254 mg (0.16 mg) sample of calix[4]-arene **9b** was dissolved in 5 mL of $CHCl_3$, and 1.5 mL of TFA was added to the reaction mixture. Deprotection was complete within 6 h, as determined by TLC analysis. The solvent and TFA were removed in vacuo, and the crude product was purified via column chromatography (SiO_2, $CHCl_3$, CH_3OH) to give 0.15 g (82%) of calix[4]arene **2** as a yellow oil: ^{1}H NMR (250 MHz, $CDCl_3$) 8.00−7.80 (m, 2H), 7.63 (s, 2H), 7.50 (d, J = 7.6 Hz, 2H), 7.36−7.26 (m, 8H), 6.99 (d, J = 7.4 Hz, 4H), 6.77 (t, J = 7.4 Hz, 2H), 4.76 (s, 4H), 4.49−4.45 (m, 2H), 4.43 (d, J = 13.1 Hz, 4H), 3.89 (s, 6H), 3.45 (d, J = 13.1 Hz, 4H), 3.40−3.11 (m, 4H), 2.04 (s, 6H), 2.00−1.40 (m, 8H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 170.02, 169.88, 169.12, 157.73, 153.06, 152.47, 152.19, 150.25, 140.42, 139.22, 132.82, 132.31, 131.46, 130.71, 128.85, 128.28, 128.05, 126.74, 124.91, 120.68, 119.83, 116.34, 78.52, 52.58, 51.69, 47.55, 31.52, 28.14, 24.90, 21.60; TOF MS ES+ m/z calcd for $C_{62}H_{71}N_{10}O_{12}^+$ 1147.5253, found 1147.5204.

5,17-Bis(*N*-acetylarginylaminophenyl)-25,27-bis(meth-oxycarbonylmethoxy)calix[4]arene-26,28-diol (10). A 120 mg (0.071 mg) sample of of calix[4]arene **9a** was dissolved in 5 mL of CH_2Cl_2, and 3.0 mL of TFA was added to the reaction mixture. Complete deprotection required 24 h. The solvent and TFA were removed in vacuo, and the crude product was purified via column chromatography (SiO_2, $CHCl_3$, CH_3OH) to give 0.030 g (41%) of calix[4]arene **10** as a yellow oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.15−7.33 (8H, m), 6.93−7.00 (6H, m), 6.88 (2H, s), 6.78 (2H, t, J = 7.5 Hz), 6.65 (2H, d, J = 8.3 Hz), 4.78 (4H, s), 4.50 (4H, d, J = 13.1 Hz), 4.20−4.40 (2H, m), 3.90 (6H, s), 3.47 (4H, d, J = 13.1 Hz), 1.10−1.90 (6H, m), 0.86−0.89 (4H, m); ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.5, 168.3, 167.2, 153.8, 153.4, 143.2, 139.3, 134.3, 133.2, 130.5, 130.3, 130.0, 128.3, 126.7, 124.0, 119.3, 73.3, 55.1, 52.9, 41.8, 32.1, 29.8, 25.5; TOF MS ES+ m/z calcd for $C_{58}H_{67}N_{10}O_{10}^+$ 1063.5041, found 1063.5146.

5-[3-(3-*tert*-Butoxycarbonylaminopropionylamino)-phenyl]-17-(3-aminophenyl)-25,27-bis(methoxycarbonyl-methoxy)calix[4]arene-26,28-diol (11). To a solution of 436 mg (2.30 mmol) of Boc-β-alanine in 20 mL of $CHCl_3$ was added 374 mg (2.30 mmol) of CDI. The reaction was stirred at rt for 1 h, and then 600 mg (0.768 mmol) of calix[4]arene **8** and 52 mg (0.77 mmol) of imidazole were added to the solution. The reaction mixture was stirred at rt for 30 min and then refluxed for 4 h. The solvents were removed in vacuo. Flash chromatography (SiO_2, $CHCl_3$, C_2H_5OH) afforded 703 mg (96%) of calix[4]arene **11** as a yellow solid: mp 145−150 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.70−7.67 (m, 2H), 7.54−7.26 (m, 8H), 7.05 (d, J = 7.4 Hz, 1H), 6.97−6.91 (m, 4H), 6.90−6.76 (m, 2H), 6.65 (t, J = 7.5 Hz, 1H), 4.75 (s, 4H), 4.53−4.44 (m, 4H), 3.88 (s, 6H), 3.53−3.38 (m, 6H), 2.63 (t, J = 5.5 Hz), 1.44 (s, 9H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 169.78, 169.11, 156.22, 153.66, 152.77, 152.18, 148.47, 142.69, 141.89, 138.22, 132.93, 132.82, 132.68, 132.52, 132.40, 132.18, 131.68, 130.67, 130.08, 129.33, 129.08, 128.81, 128.38, 128.22, 128.01, 127.11, 125.60, 125.48, 122.50, 121.04, 120.83, 119.11, 117.93, 117.77, 79.37, 77.17, 72.14, 52.09, 37.35, 36.46, 31.43, 31.27, 28.27; TOF MS m/z calcd for $C_{54}H_{54}N_3O_{13}^+$ 952.3657, found 952.3703.

5-[3-(3-Aminopropionylamino)phenyl]-17-(3-amino-phenyl)-25,27-bis(methoxycarbonylmethoxy)calix[4]-arene-26,28-diol (12). A 667 mg (0.700 mmol) sample of calix-[4]arene **11** was dissolved in 10 mL of $CHCl_3$, and 3 mL of TFA was added. After the reaction mixture was stirred at rt for 1 h, the solvent and TFA were removed in vacuo, and the product was purified by column chromatography (SiO_2, $CHCl_3$, CH_3OH) to give 504 mg (84%) of calix[4]arene **12**: mp 284 °C dec; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.99 (br s, 1H), 7.72 (s, 1H), 7.47−7.46 (m, 2H), 7.33−7.22 (m, 6H), 7.05 (d, J = 4.5 Hz, 1H), 6.98−6.91 (m, 4H), 6.77−6.73 (m, 2H), 6.66 (t, J = 4.5 Hz, 1H), 4.75 (s, 4H), 4.56−4.44 (m, 4H), 3.88 (s, 6H), 3.51−3.36 (m, 4H), 3.09 (t, J = 3.3 Hz, 2H), 2.49 (t, J = 3.3 Hz, 2H); ^{13}C NMR (63 MHz, DMSO-d_6) δ 169.35, 168.92, 154.20, 153.56, 153.13, 152.48, 151.52, 148.40, 141.96, 140.77, 139.48, 133.18, 132.62, 131.21, 130.14, 129.30, 129.14, 128.98, 128.64, 127.20, 126.83, 125.45, 121.38, 120.92, 120.20, 117.39, 117.00, 79.33, 79.17, 72.24, 51.95, 36.40, 35.78, 31.43, 31.49, 30.81, 30.62; TOF MS m/z calcd for $C_{49}H_{46}N_3O_{11}^+$ 852.3132, found 852.3105.

Calix[4]arene Di[(*tert*-butoxycarbonylamino)benzo]-[24]crown-8 Host [2]Rotaxane (14). A 400 mg (0.589 mmol) sample of di-Boc-crown ether and 275 mg (0.589 mmol) of 5-(3,5-di-*tert*-butylbenzylamino)pentanoic acid hexafluorophosphate were dissolved in 3 mL of $CHCl_3$. The solution was cooled to −10 °C (ice/salt bath) for 10 min, and then 146 mg (0.707 mmol) of DCC in 0.5 mL of $CHCl_3$ was added.[18] After the reaction mixture was stirred for 1.5 h, 153 mg (0.442 mmol) of calix[4]arene **12**, dissolved in 0.3 mL of $CHCl_3$, was added. The reaction was stirred at rt overnight. The solvent was removed in vacuo, the oily residue was dissolved in CH_3CN, and DCU was removed by filtration. CH_3CN was removed by evaporation, and the crude reaction mixture was triturated with ethyl ether to remove cyclic tether. The crude product was purified using rotary chromatography (SiO_2, CH_2Cl_2, CH_3-OH) to give 546 mg (67%) of [2]rotaxane **14**. Using this procedure, [2]rotaxane **14** was obtained in 30−70% yield; the success of the reaction is very sensitive to the purity of di-Boc-crown ether: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.34 (s, 1H), 8.06 (d, J = 8.0 Hz, 1H), 8.01 (s, 1H), 7.84−7.83 (m, 2H), 7.81 (s, 1H), 7.72 (d, J = 6.8 Hz, 1H), 7.50 (dt, J = 8.0, 1.6 Hz, 1H), 7.36−6.74 (m, 25H), 6.40 (q, J = 6.4 Hz, 1H), 4.77−4.76 (m, 4H), 4.55−4.45 (m, 6H), 4.20−4.00 (m, 6H), 4.00−3.93 (m, 4H), 3.89 (s, 6H), 3.60−3.42 (m, 18H), 3.20−3.05 (m, 4H), 3.90 (m or br s, 2H), 2.63 (m, 2H), 1.85 (m, 2H), 1.49 (s, 18H), 1.31−1.22 (m, 4H), 1.23 (s, 18H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 172.65, 170.25, 169.23, 153.83, 153.16, 152.86, 152.19, 151.17, 148.56, 147.37, 143.00, 141.30, 138.94, 133.15, 132.56, 131.75, 131.30, 129.64, 129.44, 129.11, 128.94, 128.35, 127.97, 127.17, 126.96, 125.81, 124.31, 123.07, 121.61, 121.04, 120.85, 117.68, 113.09, 111.59, 104.92, 80.21, 72.26, 70.34, 69.94, 69.67, 68.41, 68.15, 52.63, 52.20, 48.72, 37.12, 35.71, 35.12, 34.77, 31.55, 31.35, 30.59, 29.62, 28.30, 25.74, 22.24, 20.95, 14.14, 13.63; TOF MS m/z calcd for $C_{103}H_{127}N_6O_{24}^+$ 1831.8902, found 1831.8905.

Calix[4]arene Di(aminobenzo)[24]crown-8 Host [2]-Rotaxane (15). A 480 mg (0.243 mmol) sample of calix[4]-arene rotaxane **14** was dissolved in 10 mL of CH_2Cl_2, and 3 mL of TFA was added. After the reaction was stirred at rt for 3 h, volatile materials were removed under high vacuum. To remove traces of TFA, the crude product was dissolved in 10 mL of a CH_2Cl_2/CH_3OH (2/1) solution, which was subsequently removed under high vacuum. This procedure was repeated several times. Crude [2]rotaxane **15** was dissolved in CH_2Cl_2/CH_3OH (9/1) and extracted with 0.1 N $NHCO_3$. The organic phase was collected and dried over Na_2SO_4, and the solvent was removed in vacuo to give 378 mg (89%) of the rotaxane **9**, which was used without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03−7.50 (m, 6H), 7.40−6.84 (m, 17H), 6.70−6.60 (m, 6H), 6.56−6.52 (m, 2H), 6.30−6.20 (m, 2H), 4.71 (s, 4H), 4.53−4.39 (m, 6H), 4.12−3.20 (m, 6H (−OCH_3), 24H (DB24C8), 4H (ArCH_2Ar), 4H (−NH_2)), 3.09−2.80 (m, 6H), 2.75−2.70 (m, 4H), 1.75−1.79 (m, 2H), 1.27 (s, 18H), 1.27−1.14 (4H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.12, 169.53, 169.44, 152.69, 152.57, 151.81, 151.06, 147.82, 141.76, 140.87, 139.74, 132.95, 132.79, 132.41, 131.93, 131.58, 130.29, 129.06, 128.52, 128.14, 126.87, 125.66, 124.54, 123.00, 121.00, 119.27, 117.42, 114.00, 109.94, 103.60, 77.17, 72.16, 70.10, 69.03, 68.65, 68.02, 60.25, 52.27, 48.60, 45.53, 36.10, 34.76, 31.33, 25.77, 22.11, 20.93; TOF MS m/z calcd for $C_{93}H_{111}N_6O_{20}^+$ 1631.7853, found 1631.7805.

Calix[4]arene Di[(*N*-acetylarginylamino)benzo][24]-crown-8 Host−[2]Rotaxane (1). A 370 mg (0.227 mmol) sample of amino[2]rotaxane **15**, 148 mg (0.681 mmol) of Ac-Arg-OH·HCl, and 302 mg (0.681 mmol) of BOP were dissolved in 3 mL of DMF. A 316 μL sample of DIEA was added to the

solution. The reaction mixture was stirred at rt for 12 h. TLC analysis indicated incomplete conversion; therefore, 50 mg (0.23 mmol) of BOP and Ac-Arg-OH·HCl were added. The reaction was continued for another 12 h, and then the solvent and DIEA were removed under high vacuum. Triturating the crude material with an acetone/ether (1/1) solution removed DIEA·HCl, HMPA, and HOBt, which are soluble. The precipitate was dissolved in 20 mL of CH_2Cl_2/CH_3OH (8/2) and extracted with water. The organic phase was separated, the solvents were removed, and the product was purified by column chromatography to give 297 mg (66%) of [2]rotaxane **1** as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.00 (s, 2H), 8.42 (s, 1H), 8.19 (d, J = 8 Hz, 1H), 8.11 (s, 2H), 7.98 (s, 1H), 7.80−7.75 (m, 3H), 7.69 (s, 2H), 7.59−6.97 (m, 28 H), 6.77 (t, J = 7.6 Hz, 4H), 6.73 (d, J = 7.6 Hz, 2H), 6.21 (s, 1H), 6.06 (d, J = 7.6 Hz, 1H), 4.86 (s, 4H), 4.83 (s), 4.56 (br s, 2H), 4.39 (d, J = 12.8 Hz, 4 H), 4.13−4.08 (m, 8H), 3.93−3.91 (m, 2H), 3.82 (s, 6H), 3.81−3.52 (m, 24 H), 3.37−3.10 (m, 10H), 1.87 (s, 6H), 1.75−1.21 (m, 14 H), 1.17 (s, 18 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.39, 170.21, 170.09, 169.49, 169.35, 158.30, 157.98, 156.70, 153.36, 152.73, 152.41, 150.49, 148.40, 140.71, 139.44, 133.11, 132.52, 131.49, 130.94, 130.05, 129.16, 128.96, 128.86, 128.53, 128.12, 127.11, 126.69, 125.17, 123.81, 122.61, 121.17, 120.83, 120.07, 113.74, 111.63, 79.09, 72.35, 69.97, 69.71−67.63 (m), 53.52, 52.93, 51.92, 48.13, 47.60 (several signals are overlaid by the CD_3OD signal), 36.31, 34.98, 34.29, 31.08, 30.94, 30.74, 30.52, 29.19, 25.31, 25.14, 22.35, 21.90; TOF MS m/z calcd for $C_{109}H_{139}N_{14}O_{24}^+$ 2028.0087, found 2027.9873.

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Supporting Information Available: NMR properties of the calix[4]arenes, a representative binding plot, and concentrations of the components in the fluorescence assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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