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ARTIFICIAL RECEPTORS FOR MOLECULAR RECOGNITION OF AMINO ACIDS, PEPTIDES AND CARBOHYDRATES

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ARTIFICIAL RECEPTORS FOR MOLECULAR RECOGNITON OF AMINO ACIDS, PEPTIDES AND CARBOHYDRATES

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ABSTRACT

A convenient method for creating neutrally charged, water soluble calix[4]arenes that contain hydroxyamides attached to their lower rims has been developed; selective amidation reactions of a diacid calix[4]arene with several unprotected hydroxyamines was achieved using EEDQ as the coupling agent. The solubilities of the derivatized calix[4]arenes depended on the structure of the hydroxyamide, as well as, the number of hydroxyl groups. Molecular simulations of the derivatized compounds in water revealed that intramolecular H-bond formation is an important component of solubility. Calix[4]arenes containing ten hydroxyl groups were as soluble in water as a calix[4]arene that contained two carboxylates.

Dialkylammonium templated self-assembled association has been used to synthesize DCC-rotaxanes; *N*-Boc protected diaminobenzo[24]crown-8 and 5-(3,5-di-*tert*-butyl-benzylamino)-pentanoic acid hexafluorophosphate salt form a self-assembled complex in chloroform, which is mechanically locked by reacting with DCC. *N*-Boc protected DCC-rotaxane was used to synthesize phenylalanine and di-*p*-phenylcalix[4]arene host-[2]rotaxanes. Upon deprotection, corresponding amino host-[2]rotaxanes were obtained in high yields and used for synthesis of artificial receptors. *N*-acetyl-arginine derivatized di-*p*-phenylcalix[4]arenes and *N*-acetyl-arginine derivatized host-[2]rotaxanes were prepared and their ability to perform as artificial receptors was studied. Rotaxane receptors bound *N*-acetyl protected amino acid carboxylates much stronger than calix[4]arene receptors containing same functional groups. *N*-acetyl-arginine and glutaric acid derivatized phenylalanine host-[2]rotaxanes were prepared and their structure-function relationship was studied in detail. A relative measure of the

intramolecular interaction energies between the functional groups in DMSO-water mixtures was obtained by comparing their pK_a's. Rotaxane structures were investigated through 2D-NMR analysis and molecular dynamics simulations. Association constants for complexes of these rotaxanes and amino acids were determined in a variety of solvent systems by ^{1}H NMR analysis.

PREFACE

"In the recent years supramolecular chemistry has established itself as one of the most actively pursued fields in science. Its implications now reach from the basis of molecular recognition in natural and artificial complexes to exciting new applications in chemical technologies, in new materials and in biology or medicine."

Hans-Jörg Schneider and Anatoly Yatsimirsky

"Although supramolecular chemistry has now become a major field of chemistry, one may wonder why it emerged and developed so late, in the last quarter of this century. There are three main reasons for this; first, supramolecular chemistry requires a solid basis of synthetic methodologies of molecular chemistry for producing building blocks of the supramolecular entities; second, the supramolecular entities are in principal of greater complexity and lability than molecular species, so that their study presents novel challenges; third, the development of supramolecular chemistry requires the availability of powerful methods for the investigation of the structural , dynamic and physiochemical features of the supramolecular entities."

Jean-Marie Lehn

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LIST OF ABBREVIATIONS

Ac	acetyl
Arg	arginine
Boc	*t*-butoxycarbonyl
BOP	benzotriazoly-1-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate
DB24C8	dibenzo[24]crown-8
CDI	*N,N'*-carbonyldiimidazole
CH_2Cl_2	methylene chloride
CH_3Cl	chloroform
DCC	*N,N'*-dicyclohexylcarbodiimide
DCU	dicylcohexyl urea
DIEA	diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMF	dimethylforamide
DMSO	dimethyl sulfoxide
EDC	1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide
EEDQ	2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
HOBT	1-hydroxybenzotriazole
Ms	mesyl (methanesulfonyl)
Mts	2,4,6-trimethylbenzenesulfonyl
Phe	phenylalanine
Py	pyridine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Ts	tosyl (*p*-toluenesulfonyl)

INTRODUCTION

Molecular recognition events are vital to the very existence of life. The storage and replication of genetic information, signal transduction, oxygen uptake, and cell growth rely on host-guest interactions. Enormous work is being conducted to reveal the nature of noncovalent interactions in biological system, and to develop artificial receptors. Besides acting as biomimetics of natural complexation and providing deeper insight into natural recognition events, these hosts may have therapeutic benefits in the field of drug transportation, protein inhibition or can be used as biosensors.

The first concepts of supramolecular chemistry were introduced with the development of modern chemistry and biology; in 1893 Alfred Werner described coordination chemistry, in 1894 Emil Fischer proposed the lock and key principle for the enzyme catalysis, in 1906 Paul Ehrlich defined the concept of a receptor, and in 1952 Watson and Crick proposed the double helical structure of DNA that is based on complementary assembly of the two polynucleotide strands. However, the modern supramolecular chemistry fully developed with the progress in instrumental analysis and developments in macrocyclic chemistry in 1960s.

Non-covalent, multi-site complexation is very general in nature; it ensures high affinity and specificity of the enzyme-substrate, antigen-antibody, and protein-protein complexation. Electrostatic interactions between ion pairs, hydrogen bonds, cation-π-electron and C-H- π -electron interactions, and hydrophobic interactions are among the most commonly encountered non-covalent forces. Many natural receptors have a hydrophobic binding pocket with several convergent functional groups. Therefore, cleft-

like and concave-shaped, pre-organized synthetic hosts, such as cyclodextrins, crown ethers, porphyrines and cyclophanes, are often used as artificial receptors

Herein is described the design, synthesis, and structure-function studies of water soluble host-rotaxane artificial receptors for amino acids, peptides and carbohydrates. Successful artificial receptors must be soluble in aqueous solutions and selectively recognize the target compound. This dissertation describes a method to create water soluble hosts through selective amidation reaction of polyhydroxy amines and the synthesis and properties of novel artificial receptors that demonstrate the unique properties of host-rotaxanes.

Chapter 1

CONDENSATION REACTIONS OF CALIX[4]ARENES WITH UNPROTECTED HYDROXYAMINES, AND THEIR RESULTING WATER SOLUBILITIES

Introduction

Calix[4]arenes are cyclic tetramers of phenol, that can adopt a cone conformation and form a hydrophobic cavity (Figure 1). Derivatized calixarenes have proven to be very versatile hosts[1] capable of selectively binding metals,[2] organic compounds,[3] and recently protein surfaces.[4] Their unique architecture makes them very adaptable. A polar lower rim composed of phenolic oxygens has been extensively derivatized to bind and transport metals.[2] The upper rim is a shallow bowl lined with aromatic rings that can encapsulate small organic molecules or apolar parts of larger compounds.[4] The aromatic rich nature of calixarenes makes them essentially insoluble in water, which limits their use as biomimetic hosts. To increase their water solubility, sulfonate groups have been added to the lower rims of calix[4]arenes.[5] Other water-soluble calix[4]arenes have been obtained by adding phosphinic[6] acid or trialkylammonium functional groups.[7] Only a few neutral, water-soluble calix[4]arenes have been reported. Polyhydroxyl sulfonamides[8] and glycosylated calix[4]arenes[9a,b] have been constructed, but in these latter compounds, multistep deprotection of the glucosyl calix[4]arenes lowered their yields considerably. Neutrally charged, water soluble calixarenes may prove advantageous for binding certain ions and transporting compounds through membranes.[10] Furthermore, derivatized calixarenes that are used as artificial receptors may require the

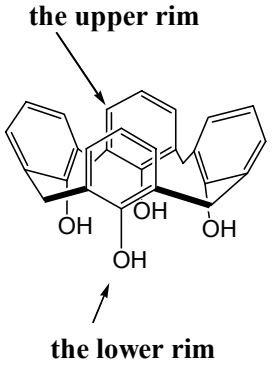


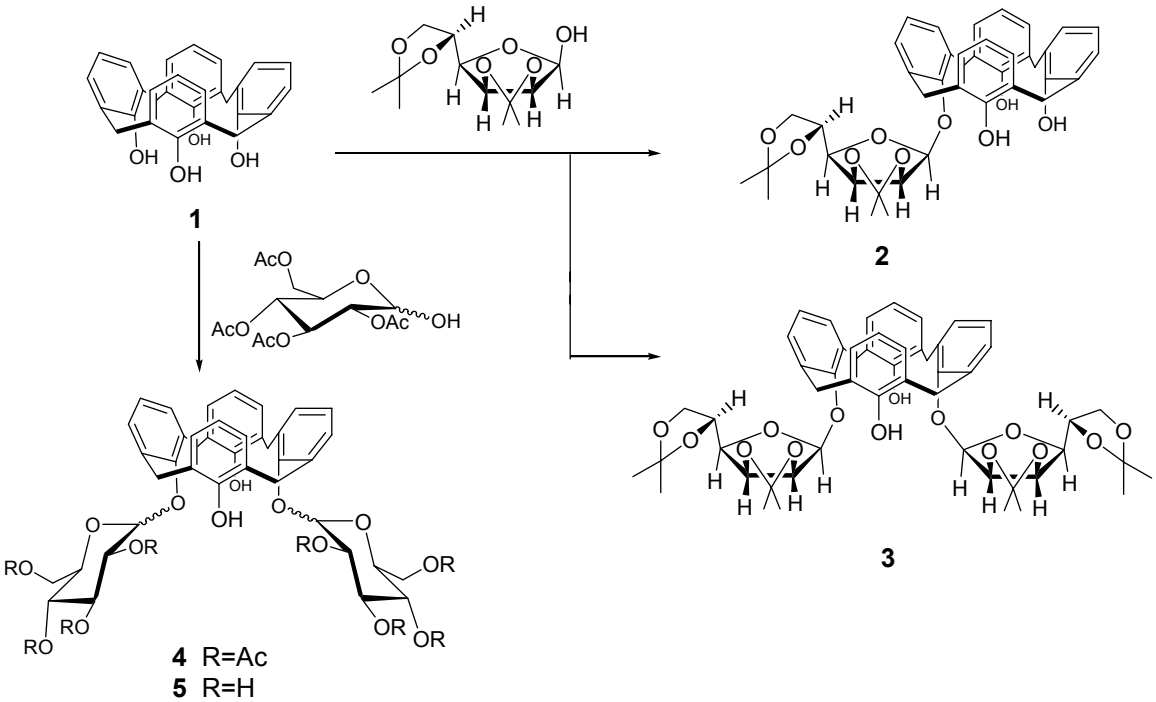
Figure 1. Calix[4]arene

existence of uncharged, polar groups at their lower rims that do not interfere with the binding event that occurs at their appropriately decorated upper rims.

The main purpose of this project was to develop a general method for selectively attaching unprotected polyhydroxy- functional groups at the lower rim of calix[4]arenes. Loss of material and time from performing protection and deprotection steps would thus be avoided.

Ungaro *et al.* reported mono- and bis- addition of α-D-mannofuranose diacetonide and bis- addition of tetraacetyl-α,β-D-glucopyranose[9a] to the lower rim of calix[4]arene under Mitsunobu conditions (Scheme 1). The full deacetonization of calix[4]arenes **2** and **3** was unfeasible[9a,b], while the reaction of calix[4]arene **1** with tetraacetyl-α,β-D-glucopyranose provided complicated product mixtures.

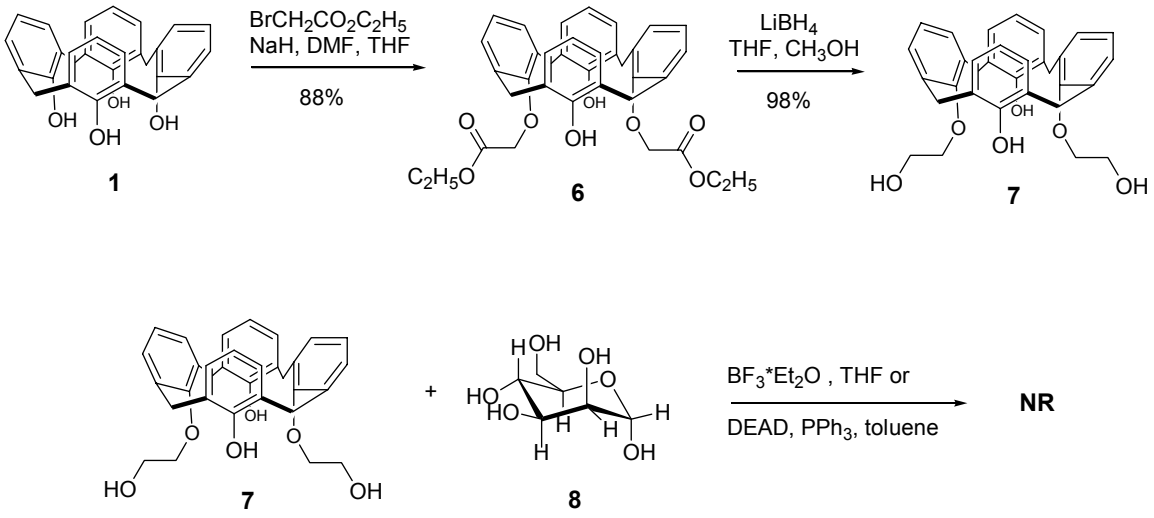
Scheme 1.



Synthesis Polyhydroxyamide Derivatized Calix[4]arenes

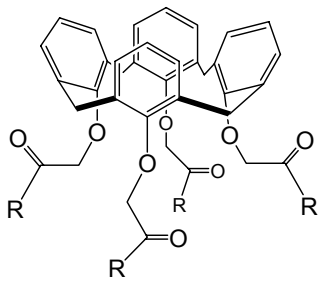
Our initial approach was to attach unprotected glycosides to calix[4]arenes and thus avoid protection/deprotection steps in the synthesis of glycosyl calix[4]arenes. We proposed that decreased steric congestion at the lower rim of calix[4]arene and higher nucleophilicity of hydroxyl group in calix[4]arene **7** would facilitate condensation reaction with unprotected carbohydrates (Scheme 2). Calix[4]arene **7** was synthesized using conventional synthetic methodology; calix[4]arene **1** was reacted with 2.2 equivalents of NaH and 2.2 equivalents of ethylbromoacetate in THF/DMF (9/1) to give diester **6** in 88% yield; the spectral analysis of the product was consistent with reported in literature values. [5(d)] Diester **6** was reduced using $LiBH_4$ to give diol **7** in high yields. The reaction of diol **7** with unprotected D-mannose under Mitsunobu conditions, or in the presence of $BF_3{\cdot}Et_2O$ did not provide the desired glycosides. More forcing reaction conditions resulted in decomposition of sugars.

Scheme 2.



Therefore, more stable hydroxyamines were chosen to provide the desired functional groups. Tris(hydroxylmethyl)aminomethane (TRIS), an inexpensive buffer, has been used to increase the water solubility of hosts. *Tert*-butyl-dimethylsilyl (TBDMS) protected TRIS was added to chlorosulfonyl groups positioned at the upper rim of calix[4]arene,[8] and TRIS-esters have been used to enhance the water solubility of cavitands.[11] In this latter study, deprotection of TBDMS protected TRIS-amides in HCl (aq) resulted in a rapid N- to O-rearrangement to give the TRIS-esters. For our studies, we needed to develop a method whereby unprotected polyhdroxy amines can be added to carboxylic acids positioned at the lower rim of calix[4]arenes without undergoing rearrangements to the esters.

Our initial set of experiments was designed to determine if unprotected TRIS could be added to the carboxylic acids of tetra-acid calix[4]arene **9a**.[5(c)] Addition of TRIS to the tetra-acyl chloride calix[4]arene **9b** resulted in multiple products as determined by HPLC analysis. There were many more compounds than the expected mono-, di-, tri-, and tetra-amides. Apparently, acyl chlorides are too reactive, giving a mixture of calix[4]arenes that have various combinations of amides and esters.



9 a R=OH
b R=Cl
c R=OCH_3

Attempts to convert methyl esters to amides by refluxing calix[4]arene **9c** and TRIS in toluene failed; only the starting materials were recovered. Mixed anhydrides, which are more reactive than esters and less reactive than acyl chlorides, gave the desired selective amidation. The reagent of choice was the pseudobase 2-ethoxy-1,2-dihydroquinoline (EEDQ), which has been used by Heagy *et al.* to amidate benzoic acids with unprotected TRIS.[12] EEDQ does not react appreciably with amino alcohols, and thus, it can be used in excess to drive the reaction to completion.

Condensation reactions of calix[4]arene **9a** and TRIS using EEDQ as the coupling reagent provided mainly the four TRIS-amide derivatives, according to mass spectral analyses. The reaction was also compatible with L-serinamide and serinol, producing the corresponding mono-, di-, tri-, and tetra-amide products. Unfortunately, the products of these reactions could not be analytically purified by HPLC using a C-18 reversed phase column or column chromatography using silica or alumina as the solid support. Furthermore, only about 30% of the desired tetra-amide calix[4]arenes was obtained (according to HPLC analysis) even with prolonged reaction times, heating the solutions, and using a large excess of the hydroxyamines.

The low yields were most likely caused by the steric congestion at the lower rim of the calix[4]arenes that occurs when the four carboxylic acids interact with EEDQ or when the resulting mixed anhydrides interact with the hydroxyamines. Considering that the product ratios of variously substituted calix[4]arenes (TRIS, serinamide, and serinol) were approximately the same, whereas the sizes of these hydroxyamines are substantially different, suggests that the large size of EEDQ limits the yield of the tetra-amide calix[4]arenes. Because the di-amides were the major products of the amidation

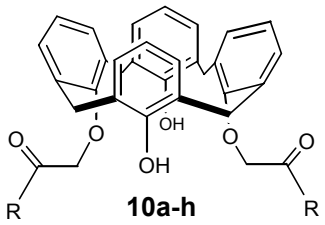
reactions of tetra-acid calix[4]arene **9a**, we assumed that higher yields of the di-amide product would be obtained by using the less hindered diacid calix[4]arene **10a**[5(d)] (Table 1).

Condensation reactions between calix[4]arene **10a** and ethanol amine, serinol, TRIS, and serine amide in the presence of EEDQ produced calix[4]arene diamides **10b-e** respectively, in high yields (Table 1). A convenient one-pot procedure was followed that involved combining the material in pyridine and letting the reaction mixture stir overnight at refluxing temperature. Excess EEDQ was removed by washing with ethyl ether. The hydroxyamines were separated from the calix[4]arene derivatives by extracting the crude material with water/chloroform. Further product purification, if necessary, can be achieved by either recrystalizing in EtOH or by performing column chromatography with silica as the solid support. High yields of the serine amide-calix[4]arene **10e** required the solution to be kept below refluxing temperature (ca. 90 °C); serine amide appears to decomposes with high heat. Furthermore, deprotonation of the starting serine amide salt required the addition of 1 equivalent of KOH (but not NaOH). Counter ion selectivity has been previously observed in calixarene reactions.[13] Another caveat should be mentioned for the isolation of the TRIS-calix[4]arene **10d**. This compound is so highly susceptible to acid catalyzed N-O rearrangement to the ester that a mixture of products was obtained upon extracting with 1N HCl as the aqueous phase. Therefore, TRIS-calix[4]arene **10d** was purified by recrystallization. The other hydroxyamides were not prone to rearrangement under mildly acidic conditions.

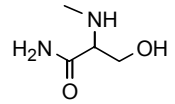
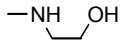
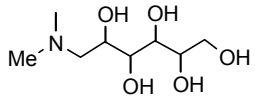
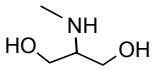
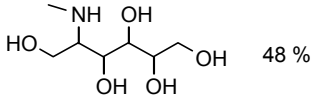
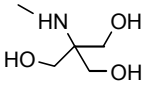
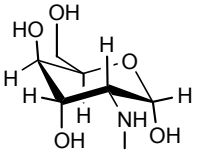
Condensation reactions of larger polyhydroxylamines gave mixed results. The addition of *N*-methyl-D-glucamine and 2-amino-2-deoxy-D-glucitol[14] to the diacid-

calix[4]arene **10a** produced moderate yields (ca. 50%) of the derivatized calix[4]arenes **10f** and **10g**, respectively. These reactions required longer reaction times, the addition of 1 equivalent of $(iPr)_2EtN$ per hydroxyamine, and a large excess of reagents. On the other hand, no detectable product was obtained for the condensation reaction of glucosamine and diacid-calix[4]arene **10a** to give calix[4]arene **10h**. We should note that the reaction

Table 1. Yields of the condensation reactions of **10a** with hydroxyl amines[a]



10a-h

Calix.	R	Yield	Calix.	R	Yield
a	OH	–	**e**		94 %
b		93 %	**f**		49 %
c		83 %	**g**		48 %
d		90 %	**h**		N. D.[b]

[a]EEDQ used as the coupling agent,
[b]N.D. means not detected

mixture containing glucosamine could not be heated in pyridine because of extensive sugar decomposition. Being a secondary amine, the lower yield of N-methyl-D-glucamine calix[4]arene **10f** could be attributed to the greater steric hindrance near the nucleophilic site. 2-Amino-2-deoxy-D-glucitol, however, is a primary amine, and thus, we predicted that its condensation reaction should produce a high yield of 2-amino-2-deoxy-D-glucitol calix[4]arene **10f**. Obtaining a yield around 50% was unexpectedly low considering that the addition of TRIS, whose α-carbon is trisubstituted, gives about 90% product. One possible reason for the lower yield of 2-amino-2-deoxy-D-glucitol (possibly for *N*-methyl-D-glucamine as well) is that the greater flexibility of its long alkyl chain gives only a small percentage of a reactive conformer. Nonreactive conformers most likely have various intramolecular H-bonded structures. Longer reaction times resulted in considerable decomposition of staring materials and products, according to TLC and HPLC analyses. Although a moderate yield of product is obtained for condensation reactions of long polyhydroxyamines, these studies show that EEDQ is an ideal coupling agent for unprotected hydroxyamines, even for ones with extended branching at their α-carbons.

To the best of our knowledge, solubility studies in water of a series of hydroxyl- and polyhydroxyl substituted calix[4]arenes had not been previously investigated. We assumed that the more hydroxyl groups added to the calix[4]arenes, the more soluble they would be in water. Therefore, we were surprised to find that the structure of the hydroxyamine can be more important than the number of hydroxyl groups (Table 2). The addition of two hydroxyl groups to calix[4]arene (ethanolamine-calixarene **10b**) did not increase its solubility to any great extent. With four hydroxyl groups added (serinol-

calixarene **10c**), the calix[4]arene became soluble in water to about one tenth as soluble as the dicarboxylate salt of calixarene **10a**. But TRIS-calixarene **10d**, with a total of six hydroxyl groups, gave a less water-soluble calixarene than the serinol-calixarene **10c**.

Table 2. Solubilities (x 10^4 M) of the calixarenes at ambient temperature

Calix[4]arene	Water[a]	Water / DMSO[b]
10a	3.9 ± 0.1	7.0 ± 0.1
10b	< 0.02	< 0.02
10c	0.36 ± 0.04	1.49 ± 0.08
10d	0.18 ± 0.03	0.7 ± 0.1
10e	1.60 ± 0.05	3.5 ± 0.4
10f	2.9 ± 0.2	5.2 ± 0.5
10g	2.1 ± 0.1	5.9 ± 0.2

[a] Phosphate buffer (10 mM, pH=6.88);

[b]95:5 Phosphate buffer (10 mM, pH=6.88) /DMSO.

To understand this reversal in solubility, molecular modeling was performed using CVFF forcefield (as presented by Discover), which was shown by Roundhill *et al*. to be a suitable method for calix[4]arenes.[15] Energy minimized structures showed that each hydroxyamide had extensive intramolecular H-bonding networks in vacuo. Once these groups are attached to calix[4]arene, however, a new H-bonding pattern emerged. One hydroxyamine was extensively H-bonded to the phenolic moieties of the calix[4]arene, whereas the second hydroxyamine had only one intramolecular H-bond

($(C=)O_{amide}\cdots H(-N)_{amide}$). Molecular simulations of hydrated calix[4]arenes showed, not surprisingly, that the addition of water disrupted most intramolecular H-bonds (Figure 2). The structures of calixarenes **10b-d** were similar except that a greater intramolecular network of H-bonds existed for the TRIS-calixarene **10d**. The $(C=)O_{amide}\cdots H(-N)_{amide}$ interaction distance was shorter for the TRIS-calixarene **10d** than the serinol-calixarene **10c** and ethanolamine-calxiarene **10b** (5.0 Å, 6.9 Å, and 6.2 Å, respectively). Apparently, weaker interactions between the amides of serinol-calixarene **10c** exposed them more to the bulk water as compared to TRIS-calixarene **10d**, which resulted in a greater water solubility of serinol- calixarene **10c**. Calixarenes **10c** and **10d** also had a single short H-bond between the hydroxyl groups. Only the TRIS-calixarene **10d** had a H-bond between a hydroxyl group and an amide carbonyl ($(C=)O_{amide}\cdots H(-O)_{hydroxyl}$, 1.9 Å), which most likely further reduced its water solubility.

Another interesting structural feature obtained from the theoretical structures, which is consistent for all the calixarenes, is that the upper rim of the two phenolic rings are placed closer together than the derivatized phenolic rings, adopting a "flattened cone" conformation. Thus, it may be possible to fine-tune the juxtaposition of upper rim functional groups through selective derivatization of the lower rim. Chemical shifts of the $ArCH_2Ar$ in ^{13}C NMR spectra (ca. 31 ppm) confirm that all calix[4]arenes (**9a-c, 10a-f**) adopt a cone conformation.[16] To determine, whether the cones were slightly flattened, as expected from the molecular modeling results, we examined the chemical shift difference ($\Delta\delta$) of H_{exo} and H_{endo} for $ArCH_2Ar$ protons. Calix[4]arenes **10b-e** had $\Delta\delta$ values consistent with a cone conformation (ca 0.8-0.9 ppm)**.**[1a] Unfortunately, these resonances for calix[4]arenes with larger substituents (**10f**,**g**) are obscured by an overlap

in proton signals from the hydroxyamides. Still, it is reasonable to assume that bulky groups attached to the lower rim of a calix[4]arene would tend to force those aromatic rings closer together at the upper rim.

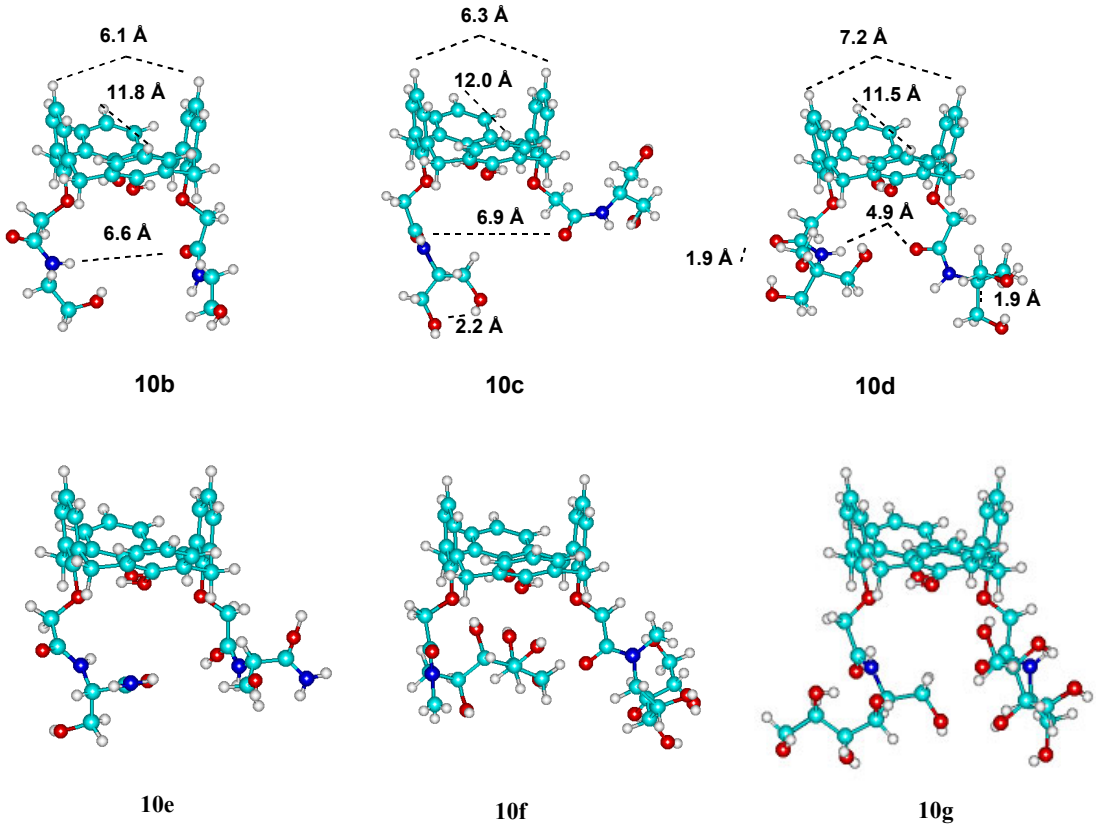


Figure 2. Equilibrium structures of calix[4]arenes **10b-g** as determined by molecular dynamic simulations. Dotted lines indicate key interaction distances between functional groups. Weaker interactions between the functional groups of serinol-calix[4]arene **10c** as compared to tris-calix[4]arene **10d**, as indicated by $(C=)O_{amide}\cdots H(-N)_{amide}$ distances (6.9 Å and 4.9 Å, respectively), most likely give calix[4]arene **10c** greater water solubility. Distances between the hydrogen atoms at the para positions depend on whether the phenol is functionalized.

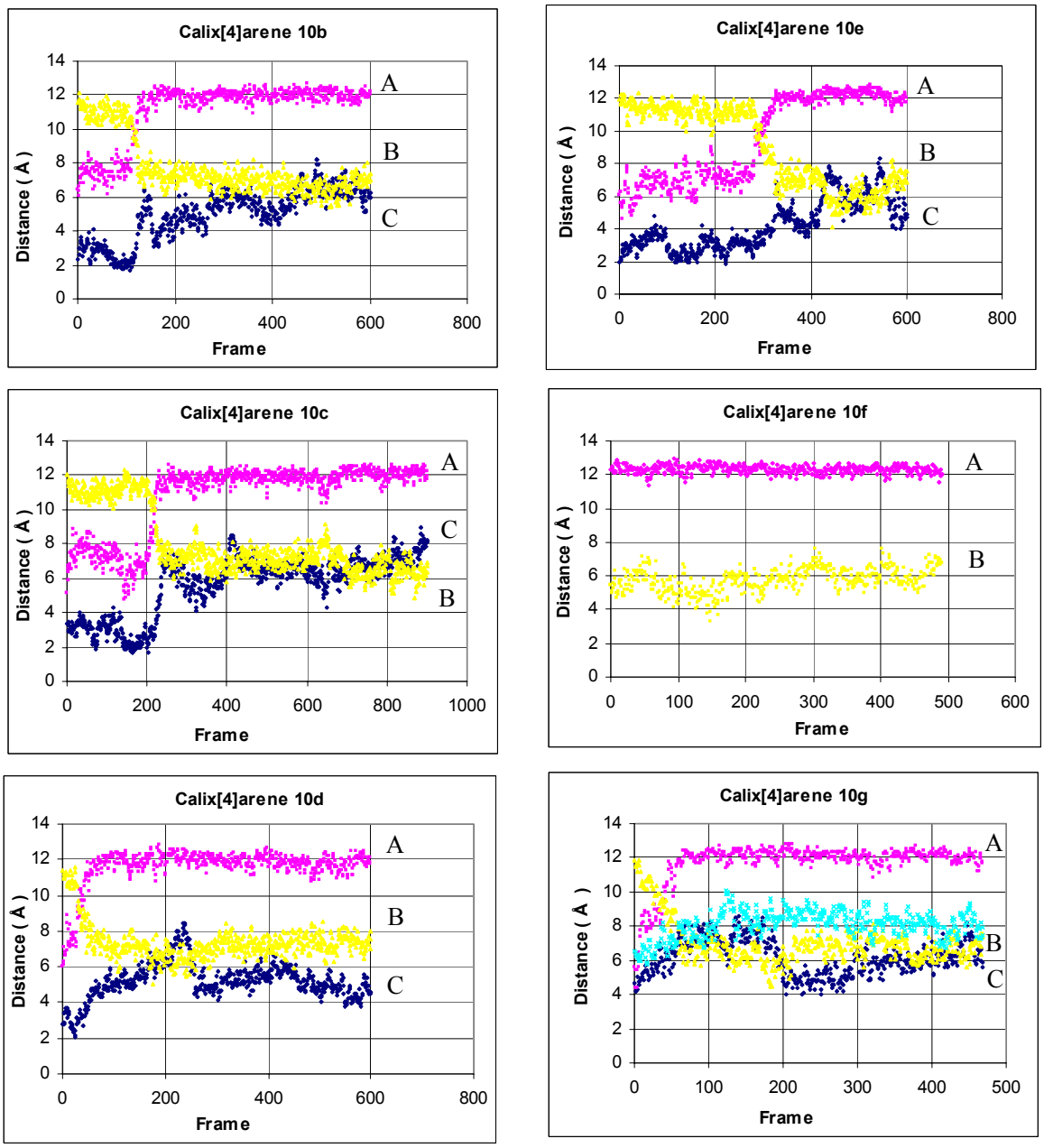


Figure 3. Trajectories of distance between atoms in calix[4]arenes **10 b-g**
Line **A** (red) - distance between para-H of the unsubtituted phenols in calix[4]arenes **10 b-g**
B (yellow)- distance between para-H of the subtituted phenols in calix[4]arenes **10 b-g**
C (blue)- distance between C=O_{amide1} and H-N_{amide2} of the subsituents at the lower rim in calix[4]arenes **10 b-g**

Once ten hydroxyl groups are added to a calix[4]arene (calix[4]arenes **10f** and **10g**), their water solubility approximately matches that of the di-sodium salt of calixarene

10a. Although *N*-methyl-D-glucamine and 2-amino-2-deoxy-D-glucitol also form intramolecular H-bonds, the remaining hydroxyl groups account for the greater water solubility. According to the solubility data (Table 2), a single amide (serinamide-calix[4]arene **10e**) proved to be approximately equivalent to four hydroxyl groups (calix[4]arenes **10f** and **10g**) in terms of solubility. The high water solubility of serinamide-calix[4]arene **10e** can be explained by the nature of the amide and hydroxyl side chains. Hydrophobicity parameters (π)[17] provide a relative measure of the hydrophobicity of amino acid side chains (π (side chain) = log P(Ac-amino acid-NH_2) – log P(Ac-Gly-NH_2)) in terms of Log P values (partition coefficients of a compound in octanol/water). π values indicate that a compound containing an amide group is 15 times more water soluble than one containing a hydroxyl group (π_{Asn} = –0.60 and π_{Ser} = –0.04, out of a total range of $\delta\pi$ = 1.81 for the natural amino acids). We found that serinamide-calix[4]arene **10e** is only ~4.4 times more soluble than serinol-calix[4]arene **10b**, presumably because of an extensive H-bonded network at the lower rim of **10e**.

Conclusion

Condensation reactions between unprotected hydroxyamines and diacid calix[4]arene **10a** in the presence of EEDQ gives moderate to high yields of calix[4]arene diamides. Although EEDQ gives selective amidation, its relatively large size limits its effectiveness at sterically hindered sites. With conversion to the mixed anhydride, the steric hindrance at the α-carbon of the nucleophile does not appear to be important. However, amines with long alkyl chains give lower yields. The solubilities of the hydroxyamide derivatized calix[4]arenes depend on the number of hydroxyl groups and the structure of the hydroxyamides. Intramolecular H-bonds within the derivatized

calix[4]arenes limits the number of favorable intermolecular interactions with water molecules, and thus, reduces their solubilities. Calix[4]arenes containing 10 hydroxyl groups have the highest solubility in water (ca. 3 mM). This value approximately doubles with the addition of 5% (v/v) of DMSO.

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Chapter 2

DI-*P*-PHENYLCALIX[4]ARENES WITH EXTENDED HYDROPHOBIC CAVITIES

Introduction

p-tert-Butylcalix[4]arenes form solid phase inclusion complexes with aromatic compounds; toluene[1], benzene, anisole, and xylene[2], but only weak associations are observed with neutral molecules in solution phase. This phenomenon is explained by the conformational flexibility of tetrahydroxy- or tetraalkoxy- calix[4]arenes (Figure 4), which rapidly equilibrate between two *flattened cone* conformations.

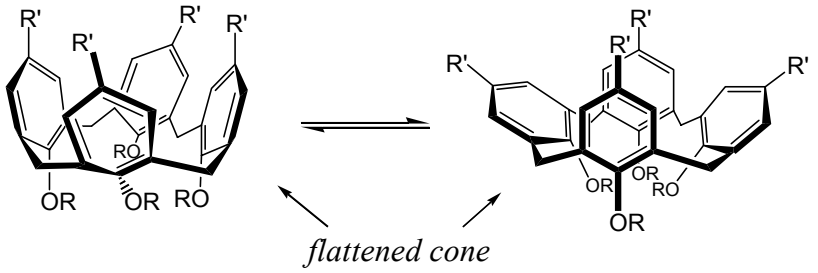
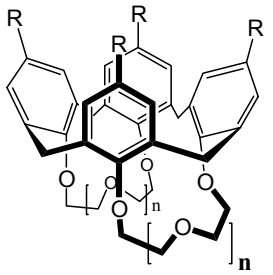


Figure 4. Equilibrium structures of the calix[4]arene C_{2v} conformers

The resulting shallow cavity of the *p-tert*-butylcalix[4]arene does not encapsulate the guests. Calix[4]arenes with underivatized upper rims fail to form even solid phase inclusion complexes.[2] Therefore, rigid calix[4]arenes with an extended hydrophobic cavity are necessary for effective binding of organic molecules in the solution phase.[3]

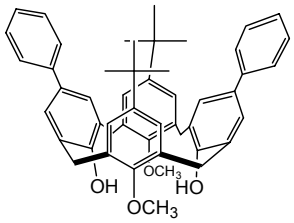
Ungaro *et al.* have prepared a series of rigid calix[4]arene cavitands by linking the proximal phenyl units with short polyethylene glycol chains.[4] Complexation studies with nitromethane showed that more rigid bis-crown-3 calix[4]arene **11b** was a better binder

than bis-crown-4 derivative **11e**, while bis-crown-5 **11f** did not form complexes. The cavitands with deeper hydrophobic pocket **11 b-c** had higher association constants than **11a**. Surprisingly, the *p*-phenylcalix[4]arene bis-crown-3 **11d** did not complex. It is considered that tetra substituted *p*-phenylcalix[4]arenes adopt "pinched" $C_{2\gamma}$ conformation and close the hydrophobic cavity, thus preventing complexation event.



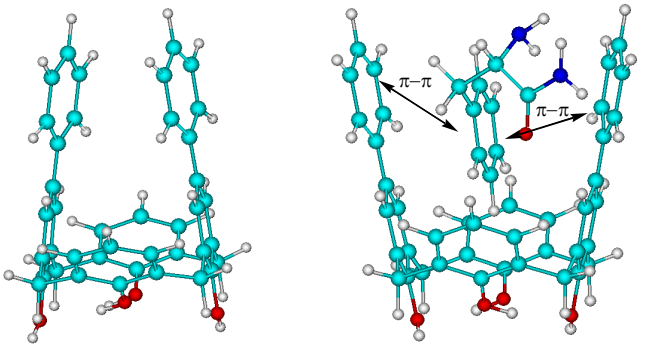
11 a R= H, n=1
b R= *t*Bu, n=1
c R= cyclohexyl, n=1
d R= phenyl, n=1
e R= *t*Bu, n=2
f R= *t*Bu, n=3

We proposed that di-*p*-phenylcalix[4]arene **12** would provide the necessary rigidity and depth of the pocket for binding aromatic amino acids (Figure 5), while avoiding partial closure of the cavity. The crystal structure of 5,17-di-tertbutyl-26,28-dimethoxy-11,23-diphenylcalix[4]arene **13** shows that the cavitand adopts distorted cone conformation with biphenyl units pointing out, thus leaving the hydrophobic pocket exposed.[5]



13

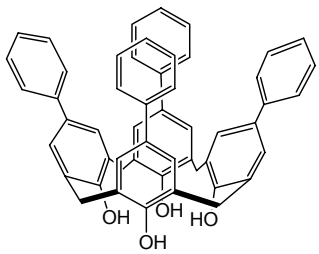
Energy minimized structures using molecular mechanics MM+ force field (as presented by HyperChem) of di-*p*-phenylcalix[4]arene **12** and its complex with phenylalanine amide confirmed the sufficient size of the di-*p*-phenylcalix[4]arene **12** cavity. They also suggested possible π-π interactions between biphenyl units of calix[4]arene **12** and aromatic side chain of the amino acid. Additional functional groups can be easily introduced to tailor affinity and selectivity of the di-*p*-phenylcalix[4]arene hosts for specific guests. The lower rim of the calix[4]arene receptors can be derivatized with polar functional groups using previously described methodology.[6]



12 **12** complex with $PheNH_2$

Figure 5. Energy minimized structures of 5,17-diphenylcalix[4]-arene **12** and 5,17-diphenylcalix[4]-arene **12** complex with phenylalanine amide were obtained using Molecular mechanics MM+ force field Polak-Ribiere algorithm.

Gutsche *et al*. prepared the first "deep-cavity" *p*-phenylcalix[4]arene **14** by stepwise condensation of *p*-phenylphenol and formaldehyde in low overall yield.[7] Arduini *et al*. synthesized calix[4]arene **14** by photolysis of the *p*-iodocalix[4]arene in benzene in 15% yield, or using mercury- and thallium-containing calix[4]arenes.[8] Direct synthesis of calix[4]arene **14** in 10%



p-phenylcalix[4]arene **14**

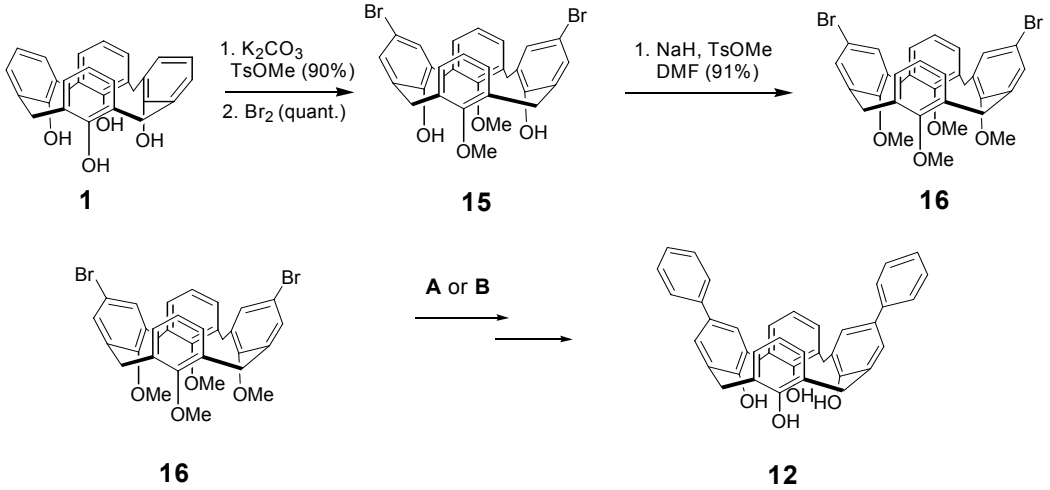
yield has been recently reported.[9] Diverse mono-, di-, and tetra- *p*-arylcalix[4]arenes have been prepared employing Suzuki type coupling[10] of *p*-bromocalix[4]arenes with arylboronic acids using Pd(0) catalysts,[11] or Negishi type cross coupling[12] reactions of arylzinc chlorides to the *p*-iodocalix[4]arenes in the presence of $Ni(PPh_3)_4$ or $Pd(PPh_3)_4$ catalysts in high yields.[13]

Synthesis of 5,17-Diphenylcalix[4]arene

One of the methods for selective functionalization of calix[4]arenes relies on selective dialkylation of the lower rim, and subsequent selective electrophilic substitution of the phenolic units of dialkylated calix[4]arenes.[14] For our studies, 11,23-dibromo-26,28-dimethoxycalix[4]arene **15** was prepared according to literature procedures (Scheme 3).[5] Phenylboronic acid **20a** was obtained from iodobenzene via reaction of benzyllithium intermediate with trimethyl borate, and subsequent hydrolysis of the benzylborate.[15] Calix[4]arene **15** was poorly soluble in most of organic solvents. Initial attempts to convert calix[4]arene **15** into 5,17-diphenylcalix[4]arene failed. To increase the solubility of calix[4]arene **15**, we fully protected the lower rim of **15** by reacting it with excess of toluene-4-sulfonic acid methyl ester and sodium hydride in dimethylforamide. 11,23-Dibromo-25,26,27,28-tetramethoxycalix[4]rene **16**[16] was obtained in 91% yield. Our early efforts to employ Suzuki type cross coupling using literature reported $Pd(OAc)_2 \cdot 2P(otol)_3$ as the catalyst[11(f)] resulted in no reaction. Therefore, Negishi type coupling of the calix[4]arene zinc chloride **17** with iodobenzene in the presence of $Pd(PPh_3)_4$ was performed to give 5,17-diphenyl-25,26,27,28-tetramethoxycali[4]arene **18**. Methoxy groups were removed by reacting calix[4]arene **18**

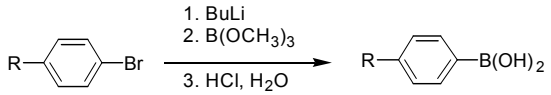
with sodium hydride and ethane thiol in refluxing DMF to provide 5,17-diphenylcalix[4]arene **12** in 22% overall yield.

Scheme 3.



A (i)BuLi**,** THF, -78^{0}C, 1h (ii) $ZnCl_2$, RT, 30 min (iii) CH_6H_5I, $Pd(PhP_3)_4$**,** 18h (iv) NaH, C_2H_2SOH, DMF, reflux , overall (22%)

B (i) C_6H_5-$B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3,Toluene, CH_3OH (98%) (ii) $AlCl_3$, C_2H_5SH, CH_2Cl_2(83%)



19 a R=H
b R= OCH_3

20 a R=H
b R= OCH_3

The fairly low yields of *p*-phenylcalix[4]arene **12** result from facile hydro-de-lithiation of calix[4]arene in the presence of traces of water, which produces considerable amounts of 25,26,27,28-tetramethoxycalix[4]arene as a side product. Moreover, during deprotection step decomposition of calix[4]arene **18** was caused by elevated reaction temperatures, and large excess of sodium hydride that was necessary for complete removal of methoxy groups. In the search for efficient synthetic routes, we repeated Suzuki type coupling of dibromocalix[4]arene **16** with phenylboronic acid **20a** in the presence of $Pd(PPh_3)_4$. Calix[4]arene **18** was obtained in 98% yield. Much milder

reaction conditions for the deprotection step ($AlCl_3$, C_2H_5SH, CH_2Cl_2, RT) provided 5,17-diphenylcalix[4]arene **12** in a 83% yield. In addition, this procedure simplified the purification of the calix[4]arene **12** - ethanethiol is readily removed under vacuo, and $AlCl_3$ is separated by extraction with CH_2Cl_2 / 5% HCl.

Binding Studies of 5,17-Diphenylcalix[4]arene with Amino Acids and Nitrobenzene

5,17-diphenylcalix[4]arene **12** adopts a cone conformation in chloroform due to an extensive hydrogen bond network at the lower rim of calix[4]arene. Ar-$\underline{CH_2}$-Ar proton signals are a typical set of two doublets with $\Delta\delta \sim 0.8$ ppm.[17] In DMSO, which weakens hydrogen bonding and lowers the coalescence temperature, Ar-$\underline{CH_2}$-Ar proton signals at room temperature become a broad singlet at ~3.9 ppm or a pair of broad doublets. In DMSO/CD_3OD solvent system Ar-$\underline{CH_2}$-Ar proton signal is a singlet at 3.9 ppm.

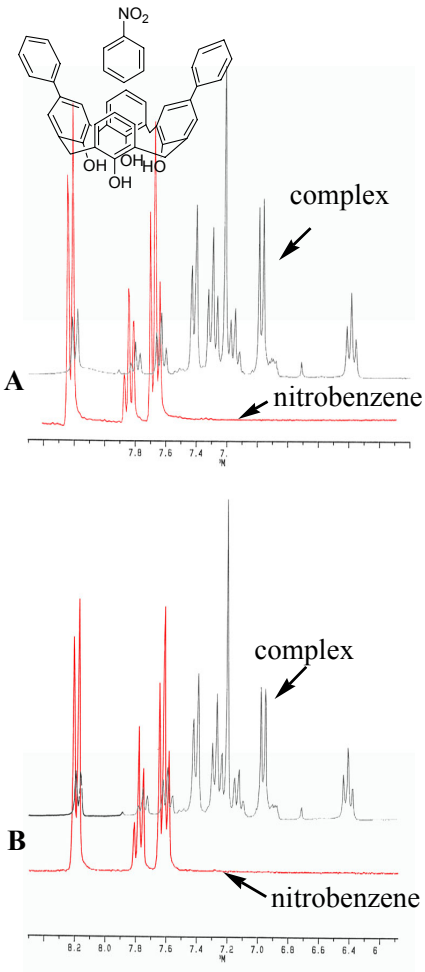


Figure 6. 5,17-*p*-di-phenylcalix[4]arene complex with nitrobenzene in **A**: DMSO-*d6* and **B:** DMSO-*d6* and D_2O (9:1) solution

The hydrophobic nature of calix[4]arene **12** made it essentially insoluble in an aqueous media. It was poorly soluble in methanol as well. Consequently, preliminary complexation studies of calix[4]arene **12** with a variety of guests were done in the following polar solvent systems: CD_3CN, DMSO-*d6*, DMSO-*d6*/D_2O, and DMSO-*d6*/CD_3OD. No association was detected in acetonitrile between the host **12** and

phenylalanine amide **21** at 20 mM concentrations of both components. In DMSO-*d6* one of the phenylalanine amide's $N\underline{H_2}C(O)$ proton signals moved upfield , while the other moved downfield. Upfield shifts were observed for aromatic proton signals of phenylalanine amide and calix[4]arene **12**. The association could be insured through H-bonds between phenylalanine's amides and -OH groups at the lower rim of calix[4]arene **12**, or between the aromatic side chain of phenylalanine amide with the hydrophobic pocket of the host. To determine if hydrophobic interactions occurred, a binding assay of nitrobenzene **22** with 5,17-diphenylcalix[4]arene **12** was performed. Upfield shifts were observed for all of nitrobenzene protons suggesting the interaction of these protons with the π electrons of the calix[4]arene cavity (Figure 6).

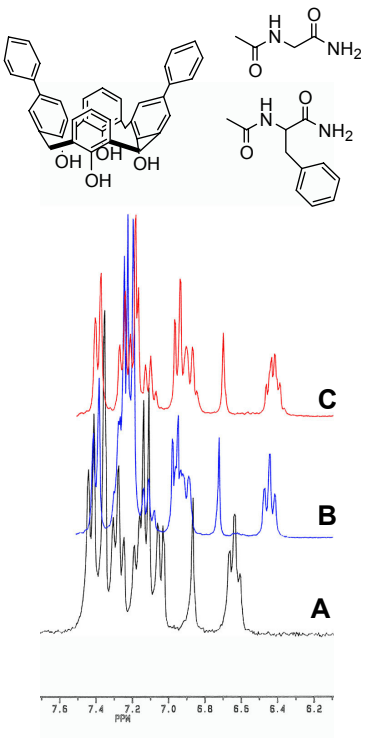


Figure 7. A-calix[4]arene **12**
B-phenylalanine diamide and **12**
C- glycine diamide and **12**
in DMSO-*d6* and CD_3OD (7:3)

We wanted to construct receptors that are selective for aromatic amino acids. Therefore, more polar solvent system (DMSO-*d6*/CD_3OD (3/7)) was chosen to diminish the role of hydrogen bonding in the binding event, and increase the significance of the hydrophobic interactions. To test this hypothesis, the K_A of phenylalanine diamide **23,** glycine diamide **24**, which lacks aromatic side chain, and nitrobenzene **22**, which does not have amide functional groups, bound to the host **12** were compared. No association between calix[4]arene **12** and nitrobenzene **22** was observed in this solvent system. Conversely, upfield shifts were observed for aromatic protons of

phenylalanine diamide **23**, as well as for calix[4]arene **12** aromatic protons. Upon addition of glycine diamide **24,** aromatic protons of calix[4]arene **12** moved upfield (Figure 7). We also observed gradual conversion of the Ar-$\underline{CH_2}$-Ar proton signal from singlet into a set of doublets upon addition of guests **23** or **24**, which would suggest changes in the conformation of the calix[4]arene **12**. Attempts to add water to the solvent system resulted in precipitation of **12** out of the solution.

5,17-diphenylcalix[4]arene **12** associates with nitrobenzene, phenylalanine diamide, and glycine diamide in various polar solvents. The upfield shifts for nitrobenzene protons upon binding with the host **12** in DMSO-*d6*, as well as upfield shifts for calix[4]arene **12** and aromatic protons of phenylalanine diamide suggest that guests interact with the host's hydrophobic cavity. However, the aromatic nature of calix[4]arene **12** limits its solubility in aqueous media. In polar solvent systems, calix[4]arene **12** conformational flexibility decreases its binding potency. In order to obtain selectivity for aromatic amino acids, the hydrophobic cavity of the diphenylcalix[4]arene receptors needs to be rigid and their water-solubility improved.

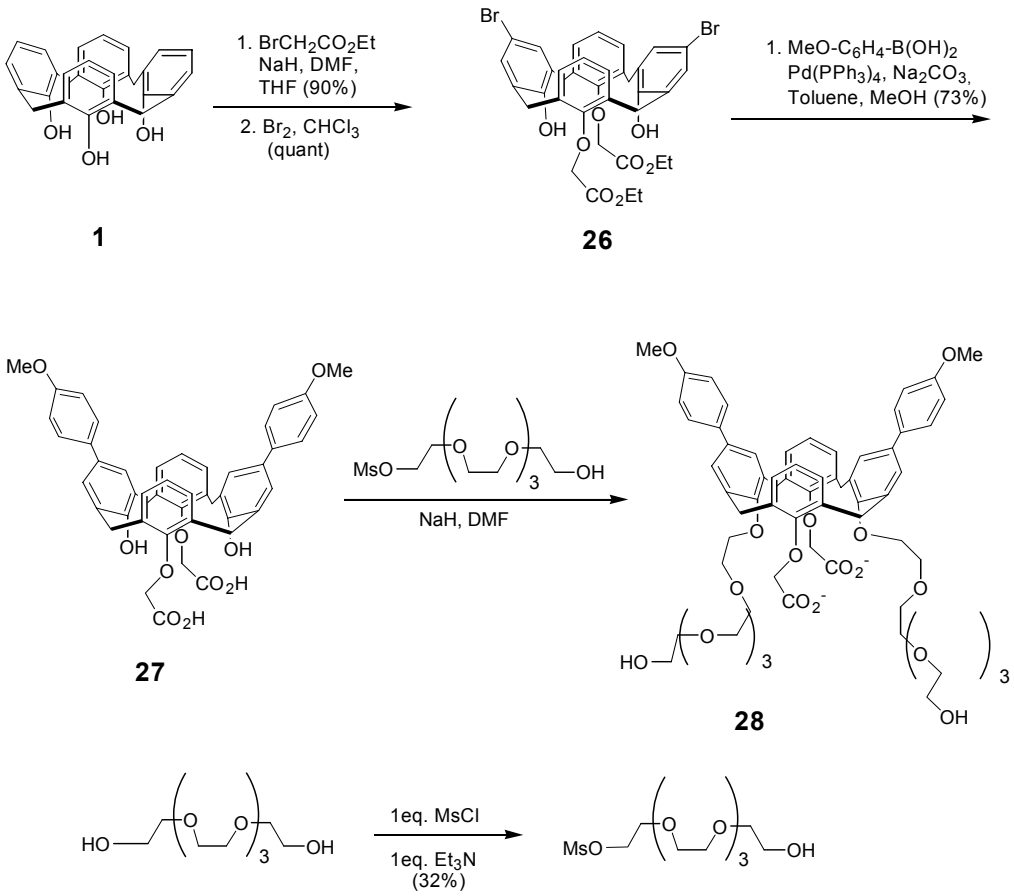
The solubility of the receptors in polar solvent systems was increased by introducing polar substituents at the lower rim, which, in addition, permanently locked calix[4]arenes in the cone conformation.[17] *p*-Phenyl groups were substituted with 4-methoxy phenyl functional groups to further enhance the calix[4]arene host's solubility in polar solvent systems (Scheme 4).

Synthesis of 5,17-Di-[4-methoxyphenyl]- calix[4]arene 28

Di-ethylester calix[4]arene **25** was prepared by reacting calix[4]arene **1** with 2.2 equivalents of sodium hydride and 2.2 equivalents of ethylbromoacetate in THF and

DMF. Spectroscopic analysis was consistent with literature reported values.[18] Calix[4]arene **25** with two ethoxycarbonylmethoxy groups attached to the lower rim is permanently locked in the cone conformation.[17] Calix[4]arene **25** was selectively di-brominated by the addition of two equivalents of bromine to the solution of calix[4]arene **25** in chloroform to give 5,17-dibromo-26,28-bis (ethoxycarbonylmethoxy)-calix[4]arene **26** in quantitative yields. 4-methoxyphenyl-boronic acid **20b** was obtained from 4-bromoanisole **19b** via reaction with butyl lithium, trimethyl borate, and subsequent hydrolysis of dimethyl borate in aqueous HCl (Scheme 3). Suzuki coupling of dibromocalix[4]arene **26** with 4-methoxyphenylboronic acid **20b** provided 5,17-di-[4-methoxyphenyl]-26,28- bis(hydroxycarbonylmethoxy)-calix[4]arene **27** in a 73% yield.

Scheme 4.



Under Suzuki reaction conditions, ethyl esters of calix[4]arene **27** are completely hydrolyzed. Teraethylene glycol mono mesylate was synthesized following literature procedure.[19] Calix[4]arene **27** was reacted with NaH and excess of teraethylene glycol mono mesylate. The crude product was refluxed in ethanol and 2 M Na_2CO_3 solution to hydrolyze possible teraethylene glycol esters. Calix[4]arene **28** was purified by column chromatography, and recrystallized from chloroform.

Binding Studies of 5,17-Di-[4-methoxyphenyl]calix[4]arene 28 with Amino Acids

Unfortunately, calix[4]arene **28** was only sparingly soluble in an aqueous media (ca. $5x10^{-4}$M in 90% / 10% H_2O / DMSO), and no association was observed between it and *N*-acetyl-L-phenylalanine methyl ester **29** at this concentration by UV-Vis titrations. On the other hand, changes in ^{1}H NMR spectrum were observe of a sample containing phenylalanine **29** and calix[4]arene **28** in CD_3OD compared to spectra of pure compounds. The aromatic region of the calix[4]arene **29** and phenylalanine **28** was complicated, however, we did observe shifts in aromatic signals. Additionally, new peaks occurred in the region of the phenylalanine's benzylic protons. It appears that these new peaks arise at the expense of one of the benzylic protons of phenylalanine.

The interactions between calix[4]arene **28** and phenylalanine **29** were observed in methanol. However, the introduction of the polyether tails at the lower rim of **28** substantially lowered the overall yield of the receptor, considerably complicated ^{1}H NMR spectrum, but did not facilitate solubility of **28** in an aqueous media. In addition, the upper rim of calix[4]arene **28** was not appropriately derivatized for facile introduction of different binding groups.

Conclusions

Diphenylcalix[4]arenes provide an extended hydrophobic cavity. 5,17-Diphenylcalix[4]arene **12** form complexes with nitrobenzene, phenylalanine derivatives and glycine diamide in various solvent systems. In order to selectively bind aromatic amino acids, the upper rim of diphenylcalix[4]arenes needs to be derivatized with adequate binding groups. An appropriate functional groups at the lower rim permanently lock calix[4]arenes in the cone conformation. To increase the hosts' solubility in an aqueous media the lower rim needs to be derivatized with polar functional groups. 5,17-di-[4-methoxyphenyl]calix[4]arene **28** with polyether tails at the lower rim is poorly soluble in an aqueous media and do not provide functionality for introduction of binding groups. Additionally, calix[4]arene **28** is obtained in low yields.

To obtain a convenient synthetic method for selective receptors, the design of calix[4]arene hosts was substantially revised. Host-rotaxanes *vide infra* were used for the synthesis of novel artificial receptors.

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Chapter 3

HOST-ROTAXANES

Introduction

Rotaxanes are molecules that consist of two or more non-covalently linked components: a macrocycle(s) threaded on a dumbbell-shaped linear component(s). The number in a bracket indicates the number of non-covalently linked components in the rotaxane, for example, **30** is a [2]rotaxane, **31** and **32** are [3]rotaxanes. A term "pseudorotaxane" refers to the supramolecular assembly **33** that lacks the blocking groups and de-threading is possible (Figure 8).

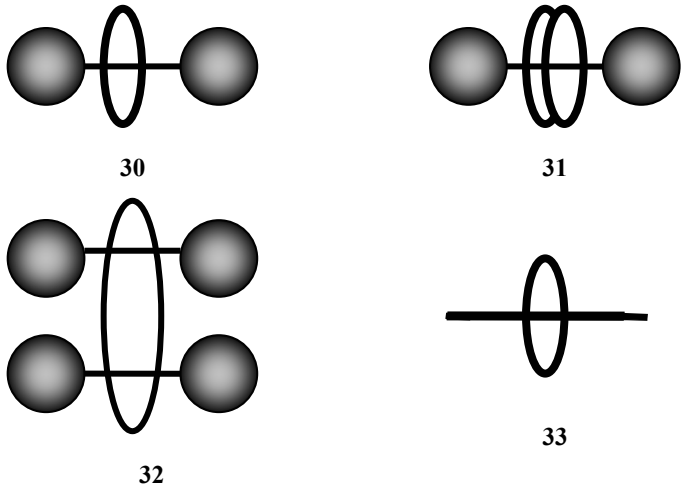
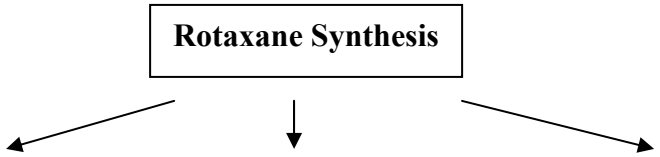


Figure 8. Rotaxane and pseudorotaxane structure

In early 1960s Frisch and Wasserman[1] reported the first experimental evidence of catenanes and rotaxanes. In 1967 rotaxanes were independently synthesized by Harrison and Harrison[2] and Schill and Zöllenkopf[3] research groups using different synthetic methodology, statistical and directed synthesis, respectively (Figure 9). The statistical threading and blocking is entropically unfavorable, while directed rotaxane formation

requires laborious multistep synthetic routes, thus both methodologies result in low over all yields. Only with the development of the supramolecular chemistry and templated synthesis, the first efficient synthetic routes for catenanes and rotaxanes were reported by Sauvage and Dietrich-Buchecker [4] in 1983.

Figure 9.



Statistical threading
Entropically driven threading of the cyclic component and subsequent blocking

Directed synthesis
Synthesis of pre-rotaxane which incorporates covalently linked cyclic and linear components

Templated synthesis
Enthalpically driven self-assembly and stabilization of the pseudorotaxane and subsequent blocking

Currently, templated rotaxane synthesis is used almost exclusively in the construction of low molecular weight rotaxanes.[5,6] It relies on the formation of the host-guest complex between the macrocycle or pre-macrocyclic constituent and the linear component followed by introduction of blocking groups in the threading method or closure of the pre-macrocyclic component in the clipping method (Figure10).

Cation-π, π-π, and hydrophobic interactions, as well as hydrogen bonding and metal-templated self-assembly of pre-rotaxanes have been exploited. Several classes of rotaxanes and pseudo-rotaxanes are based on the above listed non-covalent interactions: dialkylammonium salt complexes with macrocyclic polyethers[7], complexes that use π-π stacking between π-electron rich and deficient aromatic components[8], non-charged

rotaxanes that are formed due to an extensive hydrogen bonding network of polyamides[9], and cyclodextrin-based supramolecular systems.[10]

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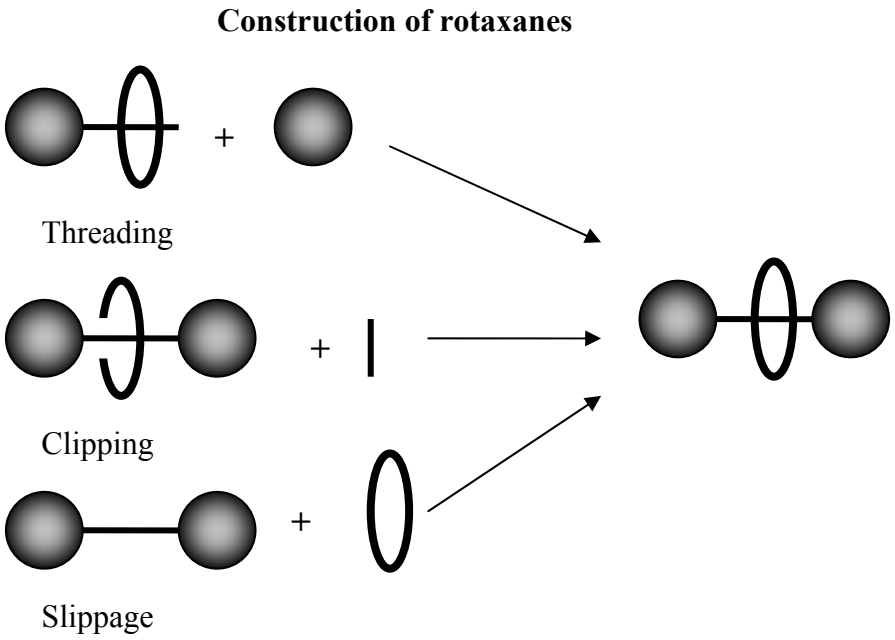
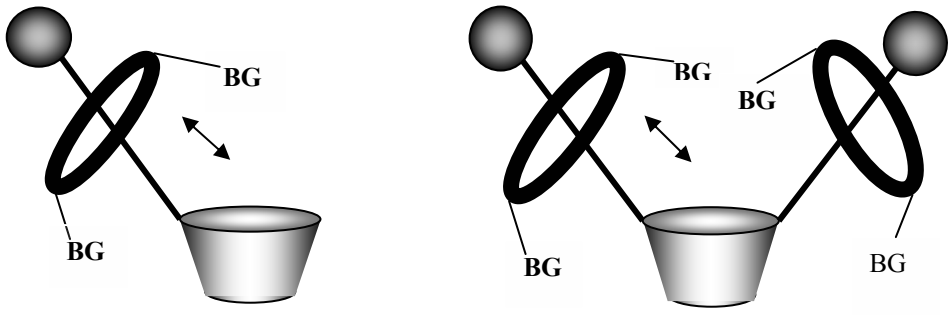


Figure 10. Methods for rotaxane construction

Although rotaxane chemistry emerged from a purely academic interest in the topology of the organic compounds, their unique molecular recognition features and dynamic properties are presently utilized to construct nanoscale molecular devices: molecular machines[11], photo- and electro-active sensitizers[12], chemoselective sensors[13], and information storage devices.[14] Polyrotaxanes have also been incorporated in polymeric materials.[15]

Host-Rotaxane Architecture

Our research was aimed to design and develop host-[2]rotaxanes and host-[3]rotaxanes for molecular recognition of amino acids and carbohydrates. The first generation of calix[4]arene host-rotaxane receptors was constructed for selective recognition of aromatic amino acids in aqueous media. The host-rotaxane consists of three components. First is di-*p*-phenylcalix[4]arene blocking group. The di-*p*-phenylcalix[4]arene is locked in the cone conformation by introducing substituents at its lower rim, while the aromatic nature of the calix[4]arene provides hydrophobic pocket and aromatic π-interactions for the binding event. Second is the dibenzo[24]crown-8 (DB24C8) based macrocyclic component that carries binding domains, and third is the axle with the blocking group - tether (Figure 11). We also constructed phenylalanine host-[2]rotaxanes, where di-*p*-phenylcalix[4]arene blocking group is replaced with phenylalanine. The latter compound's structure-to-function relationships were studied in detail.



Host-[2]rotaxane Host-[3]rotaxane

BG- Binding groups

Figure 11. Host-rotaxane architecture

The unique architecture of rotaxanes allows the ring to slide along the tether and to perform pirouette-type motion; the conformational and motional mobility of the

binding domains creates a novel self-adjusting host for molecular recognition. Thus, host-rotaxanes comply with the induced-fit model proposed for protein-ligand interactions, where association of ligand with protein induces conformational changes in the protein resulting in stronger binding. In addition, host-rotaxanes are composed of interchangeable parts: a tether, a blocking group and a ring. Such make-up of the molecule provides efficient convergent synthetic routes and potential combinatorial approach in the optimization of the particular component of the rotaxane (Figure 12). Furthermore, we developed methodology where the binding groups are attached to the host-rotaxanes in the very last step, thus allowing to obtain a vide variety of the host-rotaxanes from a single precursor. Calix[4]arene host-rotaxane receptors were compared to di-p-phenylcalix[4]arenes, which had the same binding groups directly attached to the calix[4]arene scaffold (Figure 12). The main purpose of this study was to determine the advantages afforded by hosts with converging functional groups for guest association.

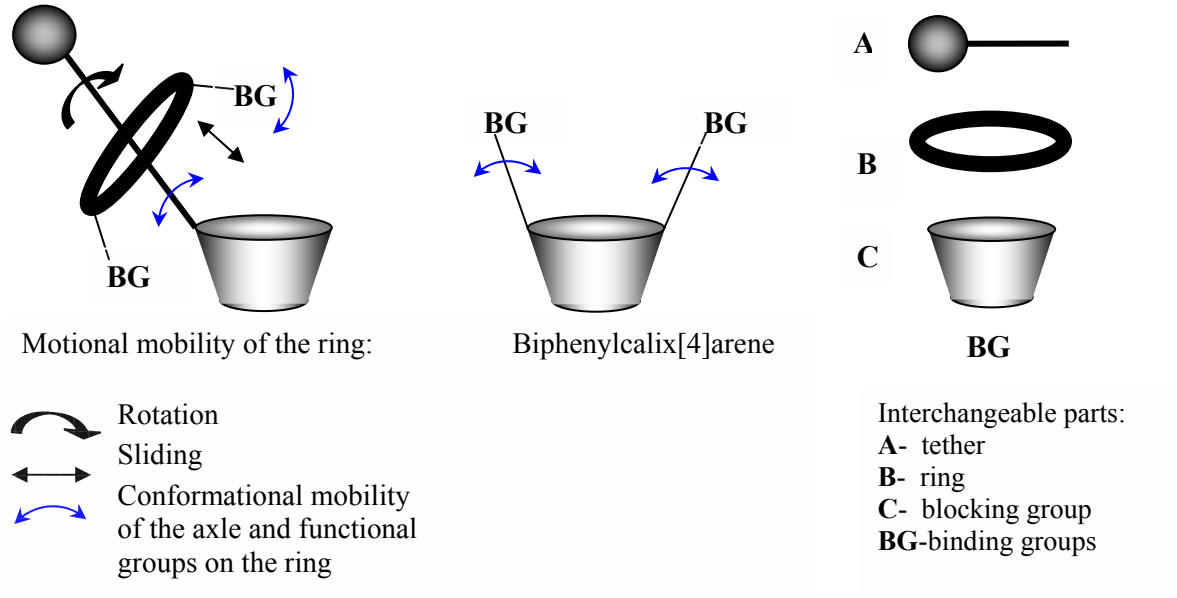


Figure 12. Host-rotaxane motional and conformational properties

Threading method was employed in construction of the host-rotaxanes (Figure 10). The first step is self-assembly of the linear component carrying one blocking group with the DB24C8 derivative[16], which is temporarily blocked with DCC.[17] DCC-blocked/activated pseudo-[2]rotaxane then is reacted with amine functional group containing a second blocking group to provide host-rotaxane. The complexation of DB24C8 with dialkylammonium is driven by [R_2NH^+-H····OR] hydrogen bonds between the crown ether oxygen atoms and the hydrogen atoms of the ammonium ion. In addition, the aromatic rings of the crown ether should have π-π stacking interactions with the

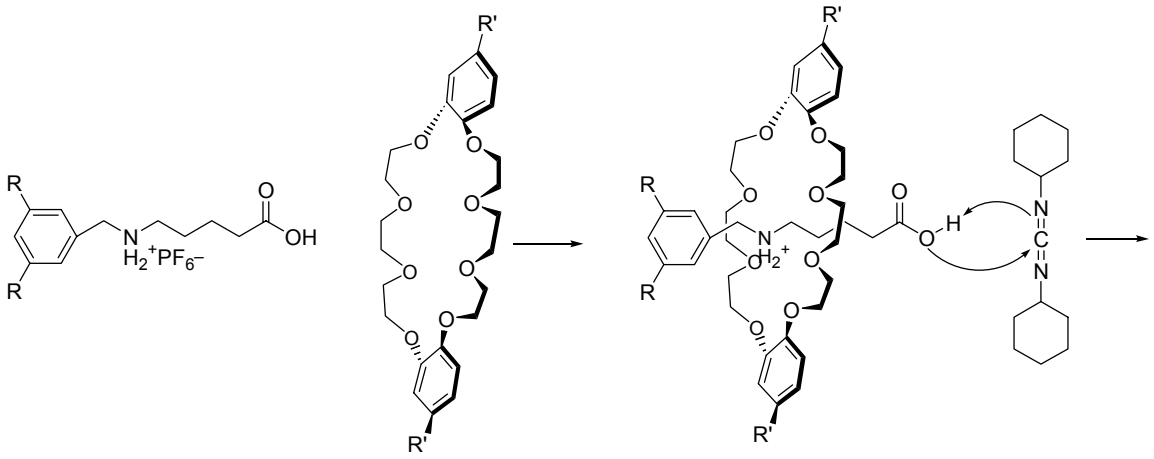


Figure 13. Synthesis of host-[2]rotaxanes

aromatic ring of the tether, according to detail studies of solvent effects on the complexation of DB24C8 with dialkylammonium containing linear components.[18]

Herein, we describe the stepwise approach towards the construction of host-rotaxanes. (i) Synthetic methodology was developed for the creation of diphenylcalix[4]arene blocking group. (ii) A variety of derivatized DB24C8 were constructed and tested for the efficient formation of the DCC-pseudo-[2]rotaxane. (iii) The reaction conditions were optimized for host-rotaxane formation, and their further functionalization. (iv) Finally, novel host-rotaxanes were tested for their ability to bind various guests.

Design and Synthesis of Di-*p*-phenylcalix[4]arene, a Scaffold for Host -Rotaxanes

Preliminary studies (Chapter 2) confirmed the advantages of having calix[4]arenes with the extended hydrophobic pocket for binding organic molecules. Di-p-phenylcalix[4]arene template was preserved in the design of the calix[4]arene host-rotaxanes. The synthetic route for construction of host-rotaxanes required incorporation of an amino functional group on the di-*p*-phenylcalix[4]arene scaffold, which could react with DCC-pseudo-[2]rotaxane (Figure 13). A less sterically hindered amine would be more reactive. On the other hand, the axle of the rotaxane would have to be fairly short to provide a pre-organized host; the binding groups of the crown ether need to be close to the hydrophobic pocket of the calix[4]arene template. We expected that calix[4]arenes **34** and **35** (Figure 14) would provide appropriate templates for the construction of the host[3]-rotaxanes and host[2]-rotaxanes respectively (Figure 11).

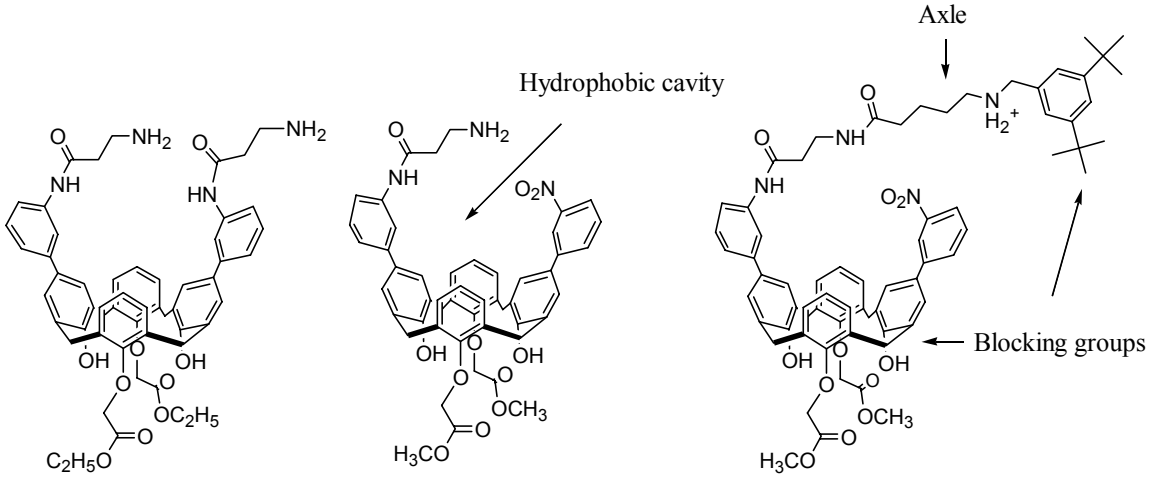


Figure 14. The functional groups at the lower rim lock di- *p*-phenylcalix[4]arenes **34** and **35** in the cone conformation. The β-alanine is introduced at the m-position of biphenyl unit of the calix[4]arene to provide amino functionality. The resulting calix[4]arene axle **36** has the optimal length for succesfull rotaxane formation. Energy minimazed structures of host-rotaxanes with amino acids suggest that the axle is sufficiantly short to reduce enthalpic costs for receptors' association with guests.

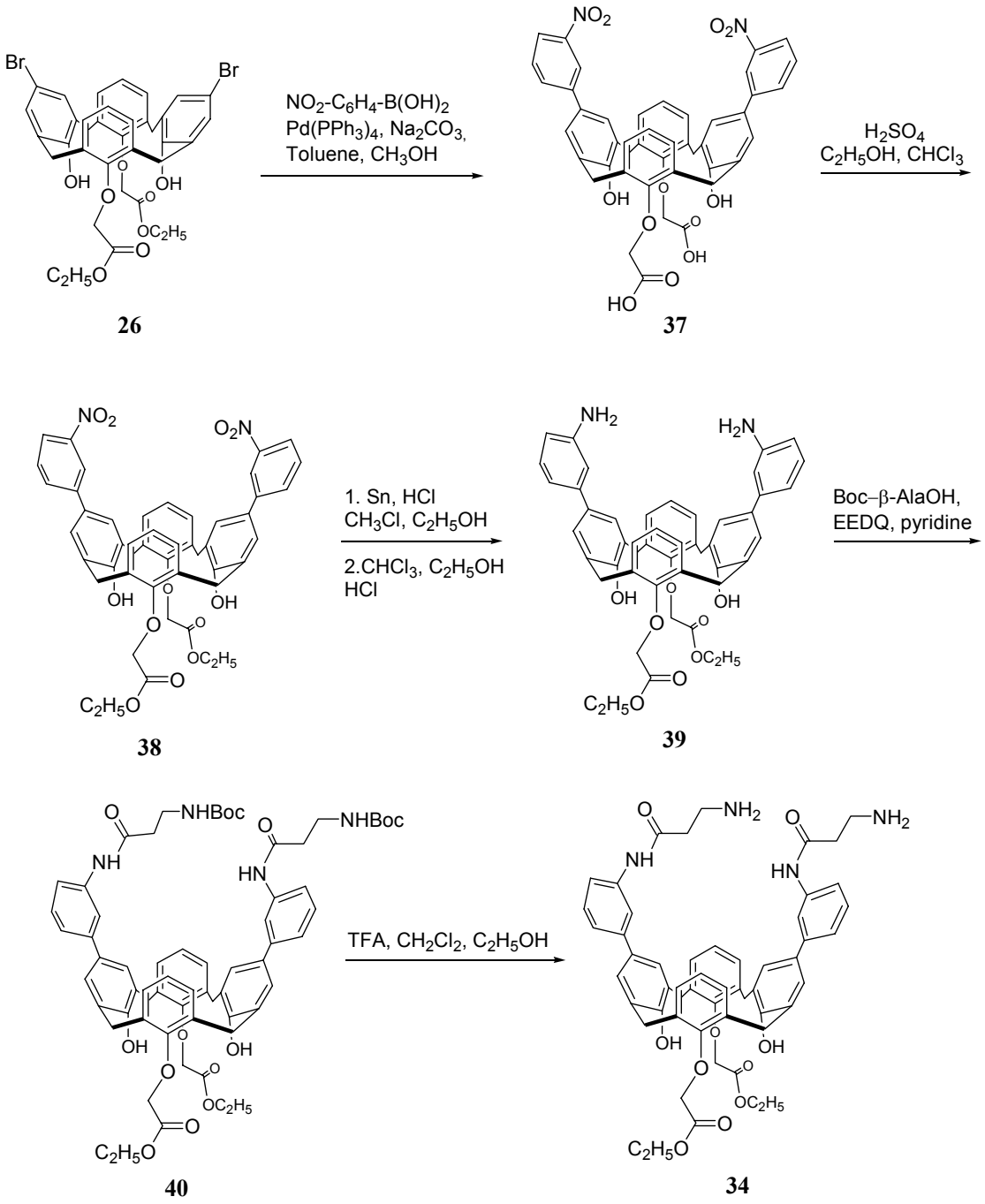
To facilitate the design of rotaxanes, molecular modeling studies (using molecular mechanics calculations as presented by HyperChem) of the host-rotaxanes were performed. Host-rotaxane complexes with phenylalanine and its derivatives suggested that the hydrophobic pocket of the rotaxanes is suitable to accommodate the side chain of the amino acid, while functional groups on the crown ether form hydrogen bonds with the amino- and carboxy- functionalities of phenylalanine.

Synthesis of 5,17-Di-[3-(3-amino-propionylamino)-phenyl]-calix[4]arene 34

Suzuki type coupling provides the most convenient route for synthesis of the *p*-phenylcalix[4]arenes; di-bromo calix[4]arenes can be prepared in high yields and reacted with a variety of phenylboronic acids (Chapter 2). Di-bromo calix[4]arene **26** was coupled with 3-nitrophenylboronic acid to create the calix[4]arene blocking group. In our hands $Pd(PPh_3)_4$ catalyst (Scheme 5) provided consistently high yields of the *p*-phenylcalix[4]arenes. One major disadvantage of the convenient $Pd(PPh_3)_4$ catalyzed Suzuki cross-coupling reaction - performed under aqueous conditions (aq Na_2CO_3, toluene and methanol) - was the complete hydrolysis of the ethyl esters at the lower rim of the calix [4]arene **26**. Numerous attempts to employ other Pd(0) catalysts resulted in no reaction or complicated mixtures of products. $Pd(PPh_3)_4$ catalyzed coupling reaction between di-bromo calix[4]arene **26** and 3-nitrophenylboronic acid under anhydrous conditions (Cs_2CO_3, DMF) resulted in mixture of mono- and di-*p*-phenylcalix[4]arenes in low yields. Therefore, $Pd(PPh_3)_4$ and aq Na_2CO_3 were used in toluene and methanol, and the resulting carboxylic acids were re-esterified. The coupling reaction provided the calix[4]arene **37** in 70-80% yields. Formation of the *p*-phenyl calix[4]arene **37** was monitored by ^1H NMR; aromatic protons' signal of bromophenolic unit in calix[4]arene

is a singlet at ca. 7.04 pm, and the aromatic protons of *p*-phenylphenolic unit is a singlet at ca. 7.36 pm. Additional portions of 3-nitrophenylboronic acid can be added to the reaction mixture in order to force its completion. The resulting calix[4]arene **37** was poorly soluble in most organic solvents, thus impeding purification via column chromatography. Therefore, the crude product was triturated with ethyl ether to remove the excess of 3-nitrophenylboronic acid, and recrystallized from methanol. Di-nitrophenylcalix[4]arene **37** was converted into the ethyl ester **38** by refluxing diacid calix[4]arene **37** in an acidic (2-3% H_2SO_4 or HCl) anhydrous ethanol. Calix[4]arene **38** was reduced to di-*p*-(3-aminophenyl)-calix[4]arene **39** using Sn and hydrochloric acid in an ethanol and methylene chloride solution. Partial hydrolysis of the ethyl esters was observed. Crude reduction products were separated from inorganic impurities, dried and re-esterified by refluxing in chloroform/ethanol mixture in the presence of conc. H_2SO_4 (2-3%). Resulting di-aniline calix[4]arene **39** was coupled with Boc-β–alanine. Initially, CDI was used as activating agent. The poor nucleophilicity and steric hindrance of aniline **39** caused a very slow reaction at room temperature. When the coupling reaction of calix[4]arene **39** with Boc-β-alanine was performed in the presence of EEDQ in refluxing pyridine, calix[4]arene **40** was obtained in high yields. Later we found that calix[4]arene **40** can be successfully prepared using Boc-β-alanine acylimidazole at elevated reaction temperatures (refluxing $CHCl_3$). Subsequent deprotection of Boc-β-alanine calix[4]arene **40** with 20% TFA in CH_2Cl_2 gave β-alanine calix[4]arene **34**.

Scheme 5.



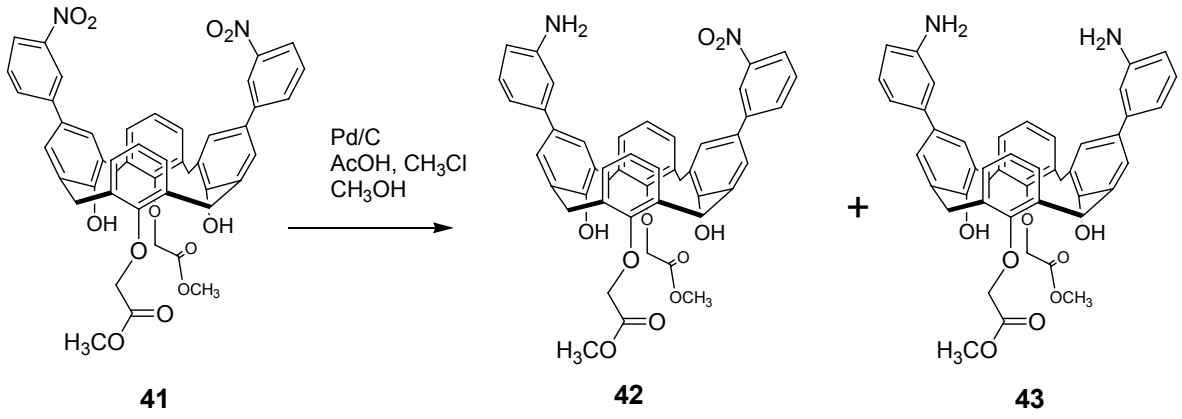
26 **37** **38** **39** **40** **34**

Synthesis of 5-[3-(3-Amino-propionylamino)-phenyl]-17-(3-nitrophenyl)-calix[4]-arene 35

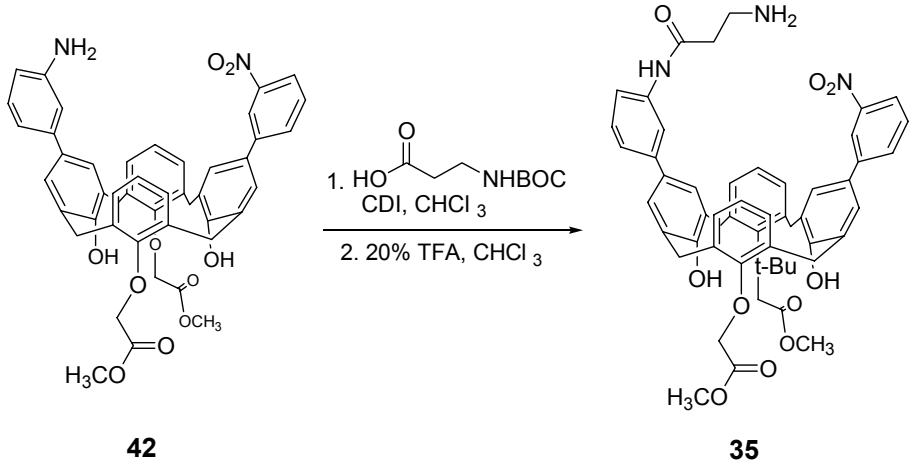
In the synthesis of the calix[4]arene scaffold for host-[2]rotaxane we substituted ethyl esters at the lower rim of the calix[4]arene with methyl esters. This modification simplified the spectral analysis of the resulting rotaxanes and reduced the size of hydrophobic alkyl chains (Scheme 6). Dimethyl ester **41** was obtained upon refluxing diacid calix[4]arene **37** in a methanol and chloroform mixture in the presence of conc. H_2SO_4 (2-3%). Selective reduction of one of the nitro groups in calix[4]arene **41** was required for the construction of the calix[4]arene **35**. Previously employed reduction procedure using Sn/HCl was unsuitable for this purpose; the reaction proceeded too quickly, and partial hydrolysis of the esters gave a mixture of products. Other conventional reducing agents; 1,4-dicyclohexadiene, Pd/C, or H_2, Pd/C, HCO_2NH_4 , or Pd/C, H_2 in chloroform or in chloroform and methanol solution did not facilitate reduction either. Only a small fraction of dinitrocalix[4]arene **41** was converted into calix[4]arenes **42** and **43** even after long reaction times at elevated temperatures. A series of solvent and reagent systems were tried. We finally found that addition of acetic or formic acids to solution of dinitrocalix[4]arene **41** in a chloroform and methanol mixture in the presence of Pd/C under H_2 atmosphere facilitate reduction of cali[4]arene **41** over ca. 12-18 h time period. Reaction was monitored by TLC and terminated when ~70-80% of calix[4]arene **41** were consumed. The crude reaction mixture was separated by column chromatography to give compounds **41**, **42** and **43** in 1, 2.7 and 4.3 ratio respectively. Calix[4]arene **42** was coupled with Boc-β-alanine acylimidazole to give mono-Boc-β-alanine calix[4]arene **44**. Elevated reaction temperatures were necessary to achieve

complete coupling. *tert*-Butoxycarbonyl protecting group was removed using 20% TFA in CH_2Cl_2 to give β-alanine calix[4]arene **35**.

Scheme 6.



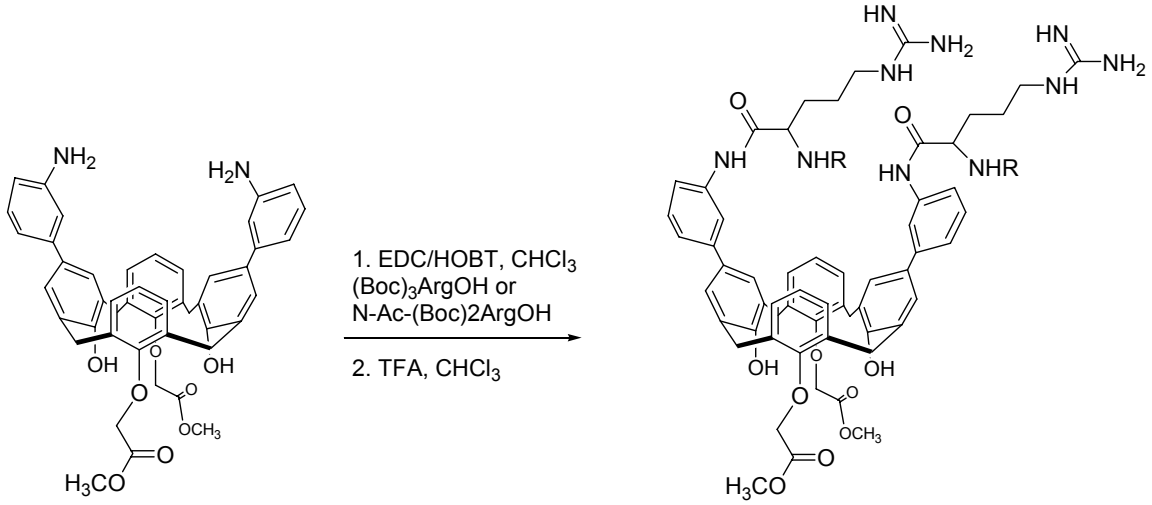
Partial reduction: **41 : 42 : 43** in ratio 1 : 2. 7 : 4.3



Calix[4]arene **43** was used for synthesis of arginine-derivatized calix[4]arenes **45** and **46** (Scheme 7). At first *N*-Boc-ArgOH was employed in the synthesis of calix[4]arene **45**. Coupling reactions were done in DMF due to restricted solubility of *N*-Boc-ArgOH in organic solvents. However, CDI, EDC and DCC/HOBT activating agents did not facilitated reaction. On the other hand, EDC/HOBT efficiently catalyzed the reaction between fully protected $(Boc)_3$ArgOH and calix[4]arene **43** in chloroform to

provide $(Boc)_3$Arg-calix[4]arene **47** in a 84% yield. EDC was used for the coupling reaction, since excess EDC can be easily removed upon extracting organic solvent with water. Complete removal of *tert*-butoxycarbonyl protecting groups required 50-70% TFA solution in methylene chloride and extended reaction times. Calix[4]arene **45** was obtained in moderate yields. Calix[4]arene **46** was obtained via coupling **43** with N-Ac-$(Boc)_2$ArgOH and subsequent removal of the protecting groups. In this case 30% TFA solution in chloroform was sufficient for complete removal of *tert*-butoxycarbonyl groups. Calix[4]arene **46** was obtained in a good yield.

Scheme 7.



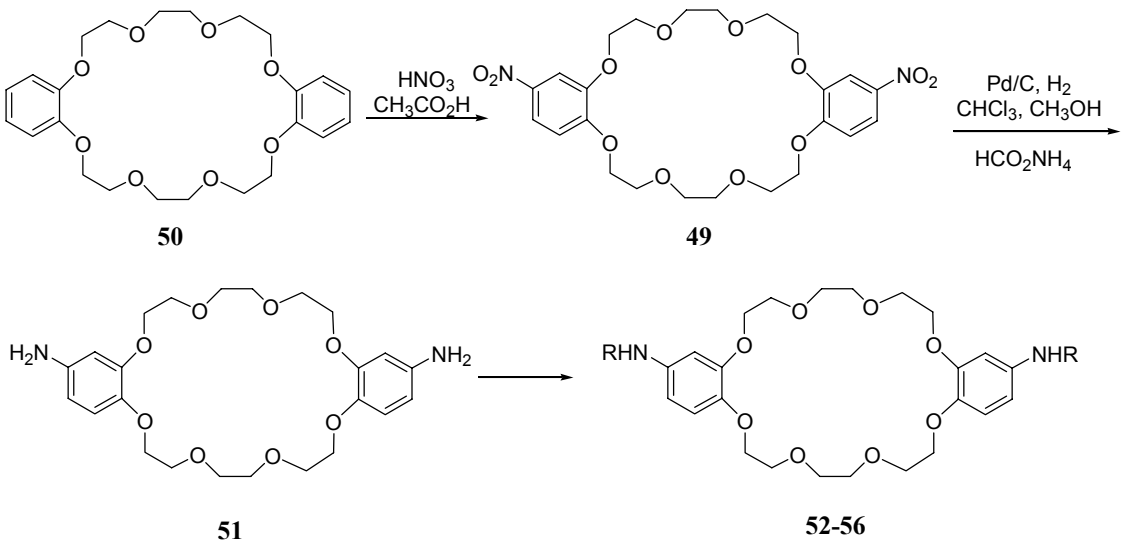
43

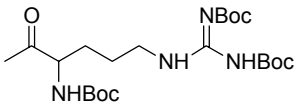
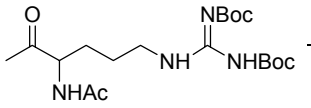
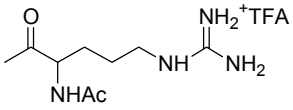
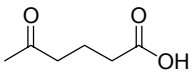
45 R=H
46 R=Ac

Synthesis of Derivatized Dibenzo[24]crown-8 Ethers

Various derivatized DB24C8 were used for construction of the host-rotaxanes. Di-nitrobenzo[24]crown-8 **49** was obtained upon treatment of dibenzo[24]crown-8 (DB24C8) **50** with HNO_3 in a chloroform and acetic acid solution (Scheme 8). Pd/C catalyzed reductions of aromatic nitro derivatives usually are done in methanol or ethanol. DB24C8 **49** was poorly soluble in both solvents, therefore, reduction of **49** was done in chloroform and methanol solution in the presence of 10 mol% of Pd/C and ammonium formate under H_2 atmosphere (50-70 psi). In this solvent system complete reduction required extended reaction times (12-36 h), which resulted in partial decomposition of the di-aminobenzo[24]crown-8 **51.** Amino DB24C8 is sensitive towards exposure to air and elevated temperatures. Purification of **51** using flash column chromatography further facilitated decomposition of the product. Therefore, unpurified di-aminobenzo[24]crown-8 **51** was used for the CDI catalyzed coupling reactions with $(Boc)_3$ArgOH and Ac$(Boc)_2$ArgOH. Crown ethers **52** and **53** were obtained in ~50-70% overall yields. Crown ether **54** was obtained upon deprotection of DB24C8 **53** using 20% TFA in methylene chloride. Trifluoroacetyl DB24C8 **55** was prepared by reacting **51** with trifluoroacetic anhydride in chloroform/ pyridine solution. Diacid DB24C8 **56** was obtained upon reaction of **51** with glutaric anhydride in the presence of triethylamine.

Scheme 8.

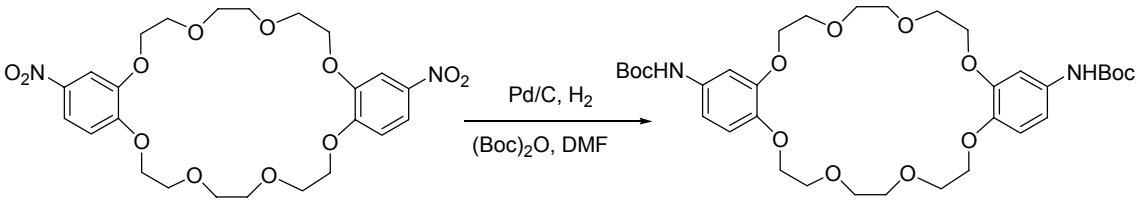


Reaction conditions		DB24C8
CDI, $(Boc)_3$ArgOH, $CHCl_3$, reflux	**52** R=	
CDI, $Ac(Boc)_2$ArgOH, $CHCl_3$, reflux	**53** R=	
20% TFA, CH_2Cl_2	**54** R=	
$(CF_3CO)_2O$, pyridine, CH_2Cl_2	**55** R=	
glutaric anhydride, Et_3N, $CHCl_3$	**56** R=	

Considerable improvement was achieved using the one-pot procedure: Pd/C catalyzed reduction under H_2 atmosphere and protection with di-*tert*-butyl dicarbonate *in*

situ in chloroform/methanol solution, giving a 70-80% overall yield of di-(*tert*-butoxycarbonylaminobenzo)[24]crown-8 **57** (Scheme 9). The major drawback of this procedure was the poor solubility of di-nitrobenzo[24]crown-8 in chloroform/methanol solution. Higher percentage of chloroform facilitated the solubility of **49**, but considerably reduced the solubility of H_2 in the reaction mixture thus slowing the reaction rate (12-24 h). When dimethylforamide was used as the solvent, di-nitrobenzo[24]crown-8 **49** was quantitatively converted into di-*N*-(*tert*-butoxycarbonyl)-aminobenzo [24]crown-8 **57** in 2 hours. DB24C8 **57** is easily purified by column chromatography using silica gel and ethyl acetate as eluent. Since deprotection of DB24C8 **57** using 20% TFA in CH_2Cl_2 gives a quantitative yield of di-aminobenzo[24]crown-8 **51**, now crown ether **57** is used as a stable precursor of the amino crown **51.**

Scheme 9.

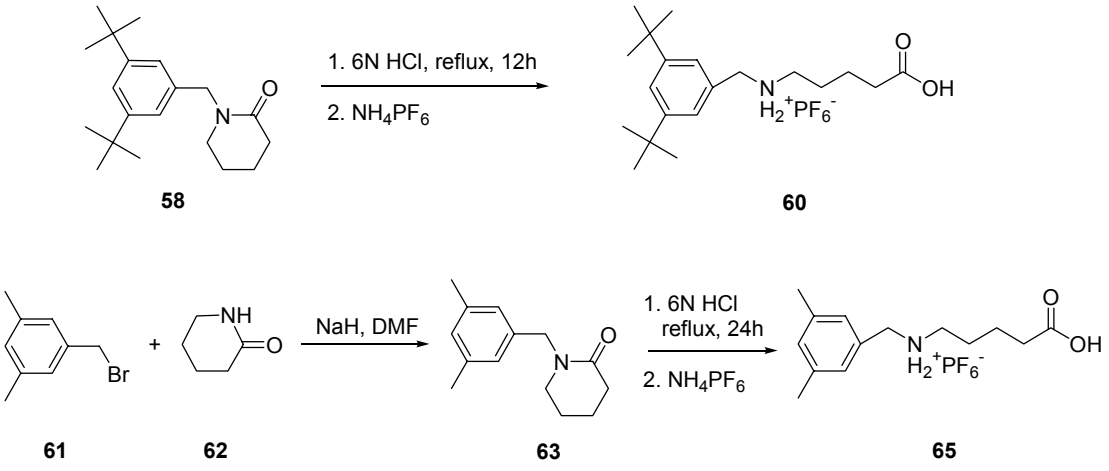


49 **57**

Synthesis of the Tethers

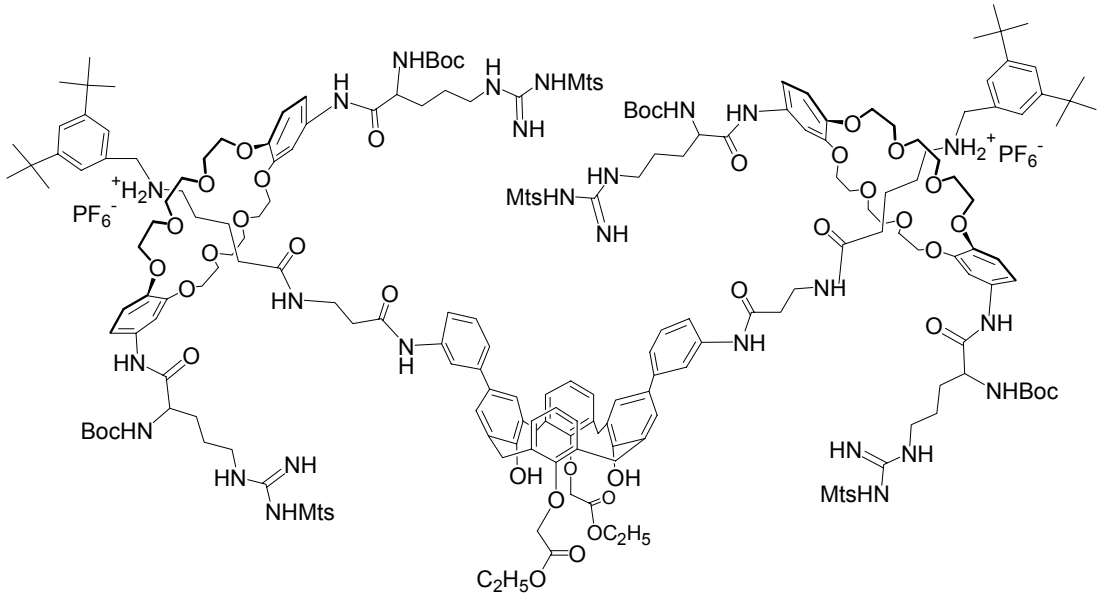
1-(3,5-Di-*tert*-butyl-benzyl)-piperidin-2-one **58**[19] was refluxed in 6 N HCl for 12h and extracted with chloroform to provide 5-(3,5-Di-*tert*-butyl-benzylamino)-pentanoic acid hydrochloride salt **59** in a 88% yield. Dialkylammonium chloride **59** was converted into the hexafluorophosphate salt **60** by extracting its suspension in ethyl ether with solution of 2-4 equivalents of NH_4PF_6 in water. 1-Bromomethyl-3,5-dimethyl-benzene **61** was reacted with piperidin-2-one **62** in the presence of sodium hydride to provide 1-(3,5-dimethyl-benzyl)-piperidin-2-one **63**. Lactam **63** was hydrolyzed by refluxing in 6 N HCl. Resulting 5-(3,5-dimethyl-benzylamino)-pentanoic acid hydrochloride salt **64** is soluble in water, therefore, excess HCl was removed under vacuum, and solution of dialkylammonium hydrochloride **64** in chloroform was extracted with small amounts of saturated NH_4PF_6 solution to provide hexafluorophosphate salt **65**.

Scheme 10.



Scope and Limitation of DCC-Rotaxane Formation

The methodology for DCC-rotaxane formation with underivatized DB24C8 was developed in our research group.[17] At the same time, we encountered considerable limitations when derivatized crown ethers were used instead of DB24C8. The yields of DCC-rotaxanes significantly diminished with an increase in the size of groups on the crown ether. Tether **60** with four methylene long alkyl chain was necessary for the formation of rotaxanes. Initially, DB24C8 **66**[19] and diamino calix[4]arene **34** were used to construct host-[3]rotaxne **67** (Figure 15). Formation of the host-[3]rotaxane **67** was detected by high resolution mass spectroscopy. Unfortunately, host-[3]rotaxane **67** was formed in less than a 5% yield and required laborious purification procedures.



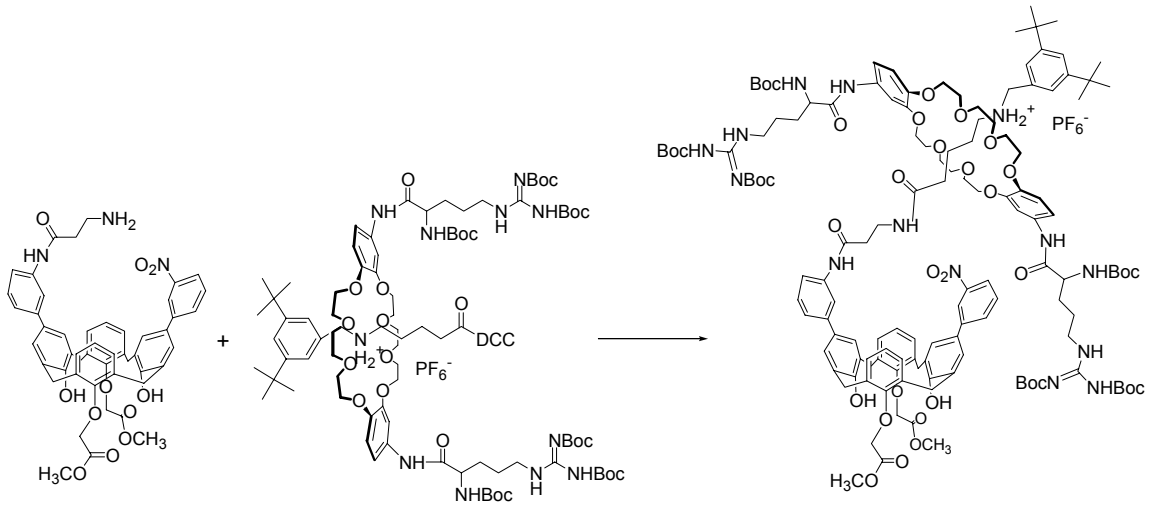
67

Figure 15. Calix[4]arene host-[3]rotaxane

Calix[4]arene **34** had to react with sterically hindered DCC-rotaxane twice, which resulted in an extremely low yield of host-[3]rotaxane **67**. In order to develop synthetic

methodology, calix[4]arene **35** (Scheme 11) with one amino functional group was used for a construction of the host-[2]rotaxanes. Methyl esters were introduced at the lower rim of calix[4]arene **35** to simplify spectroscopic analysis of the rotaxanes. BD24C8 **52** was employed instead of *N*-Boc-Mts-arginine DB24C8 **66**, since *tert*-butoxycarbonyl protecting group can be easily removed under acidic conditions.[20] Upon reaction of calix[4]arene **35** with DCC-rotaxane **68**, host-[2]rotaxane **69** was obtained in ~30% yields. However, the deprotection of hexa- (*tert*-butoxycarbonyl)-rotaxane **69** required high concentration of TFA as well as long reaction times and resulted in considerable decomposition of the product. It appears that *tert*-butoxycarbonyl protecting groups are easily removed from guanidine functionality, while the amino functionality of arginine remains protected even after prolonged reaction times. In addition, we observed removal of the *tert*-butyl groups from the blocking group of the tether and subsequent de-threading of the ring.

Scheme 11.

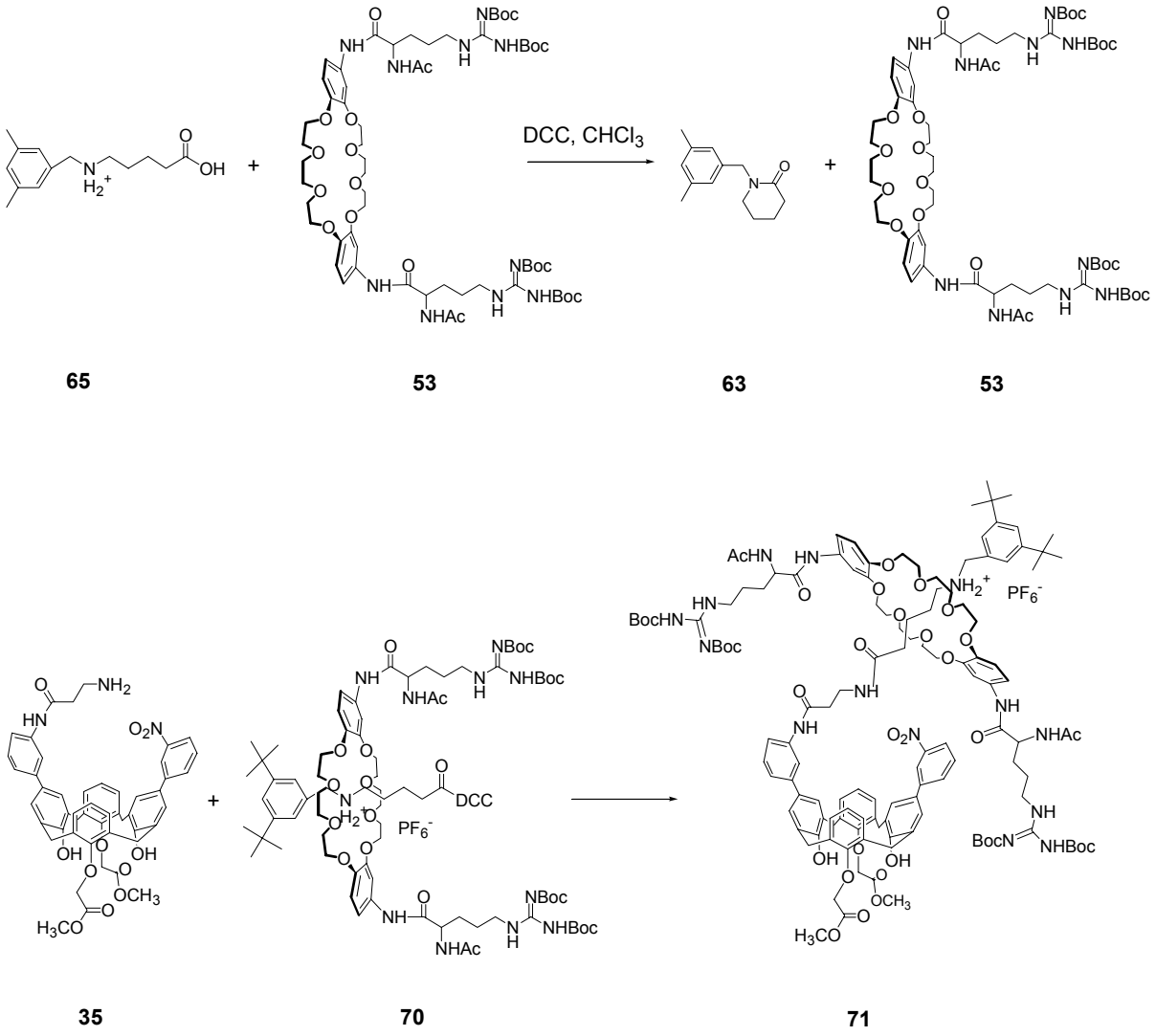


35 **68** **69**

In synthesis of the second generation of DCC-rotaxanes, we used *N*-Ac-$(Boc)_2$Arg DB24C8 **53**, where an amino functional group of arginine would stay protected, and 5-(3,5-dimethyl-benzylamino)-pentanoic acid **65,** which would not be prone towards decomposition under highly acidic conditions. Although ^{1}H NMR studies of the reaction showed that crown ether **53** formed a complex with the tether, upon the addition of DCC, DCC-pseudo rotaxane formation was not detected (Scheme 13). We propose that the flat structure of 5-(3,5-dimethyl-benzylamino)-pentanoic acid **65** allowed the complexation of this tether without threading of DB24C8 **53**. When 5-(3,5-di-tert-butyl-benzylamino)-pentanoic acid **60** was used, DCC-rotaxane **70** was formed and the corresponding calix[4]arene host-[2]rotaxane **71** was obtained in low yields (5-10%). The substitution of calix[4]arene **35** for phenylalanine methyl ester **72** did not increase the yields of the host-[2]rotaxane, suggesting that amine structure is not a limiting factor in this reaction.

The above mentioned obstacles forced us to substantially modify the synthetic strategy for the host-[2]rotaxanes. We expected to improve the formation of DCC-rotaxane by decreasing the steric congestion on the crown ether: the binding groups would be introduced after formation of the host-[2]rotaxane. Using this method, a single host[2]rotaxane precursor can lead to a large variety of the rotaxane receptors.

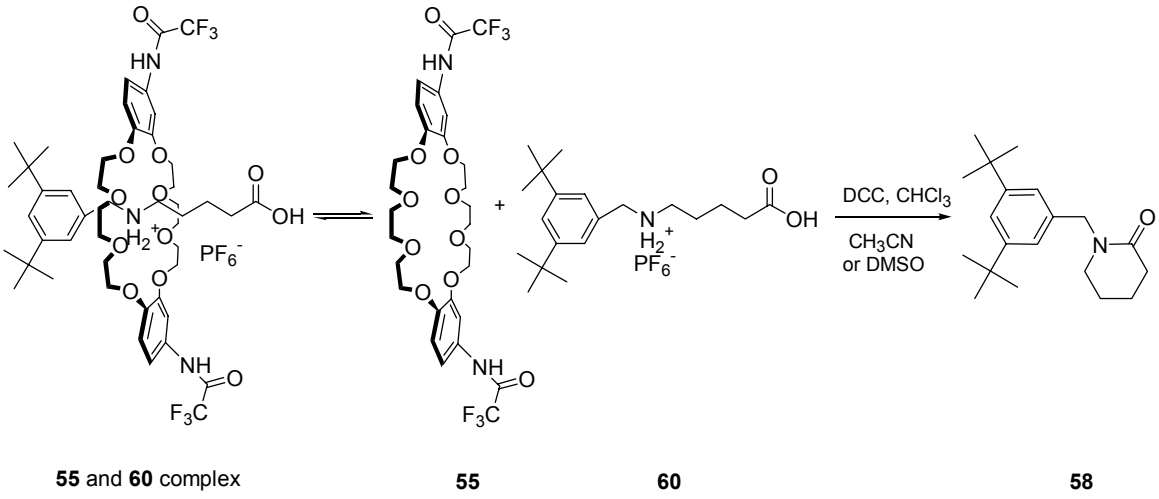
Scheme 12.



Initially, trifluoroacetyl protected crown ether **55** was used. However, DB24C8 **55** was poorly soluble in chloroform. The tether-crown ether complex is formed when the chloroform-soluble crown ether solubilize chloroform-insoluble tether **60**. Since both components were poorly soluble in chloroform, it was necessary to add small amounts (ca. 2-5%) of acetonitrile or dimethylsulfoxide to dissolve compounds **55** and **60**. Unfortunately, acetonitrile and dimethylsulfoxide facilitated fast threading / de-threading

rates of DB24C8. DCC activated the less sterically hindered acid functional group of the uncomplexed tether and only 1-(3,5-di-*tert*-butyl-benzyl)-piperidin-2-one **58** was formed.

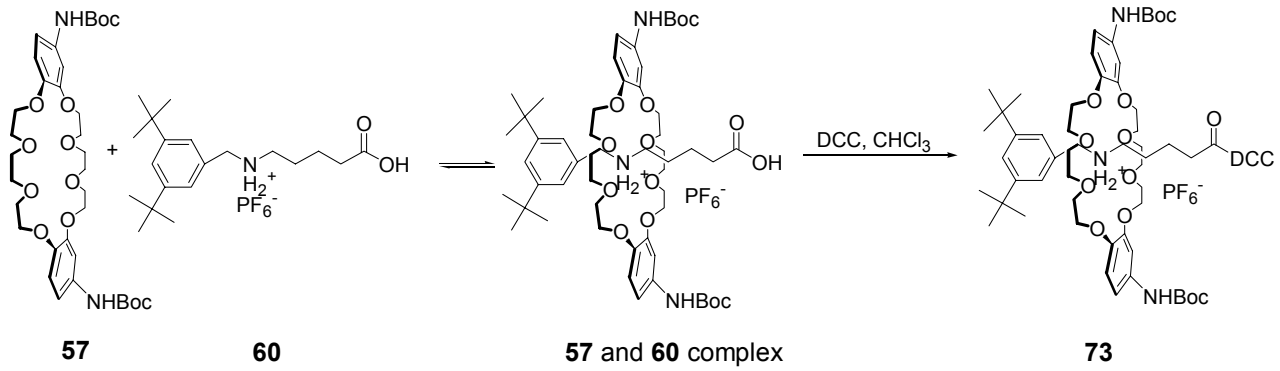
Scheme 13.



55 and **60** complex **55** **60** **58**

When chloroform-soluble di-(*tert*-butoxycarbonylaminobenzo)[24]crown-8 **57** was used, the DCC-rotaxane **73** was formed in 50-80% yields. Analysis of the crude reaction mixture using ^{1}H NMR spectroscopy allows to estimate the yield of DCC-rotaxane. Subsequently, only 0.5-0.8 equivalents of an amine are added, which result in 50-75% yields of host-[2]rotaxanes. Unthreaded crown ether can be easily recovered using column chromatography, providing a convenient and efficient one-pot reaction for synthesis of host-rotaxanes. The removal of *tert*-butoxycarbonyl groups with TFA proceeds smoothly at room temperature, and within 2 hours. Quantitative yields of di-aminobenzo[24]crown-8 host-[2]rotaxanes were obtained. Subsequent coupling of the amino-rotaxane with *N*-acetyl-arginine or glutaric anhydride provided the corresponding host-[2]rotaxanes in good yields.

Scheme 14.

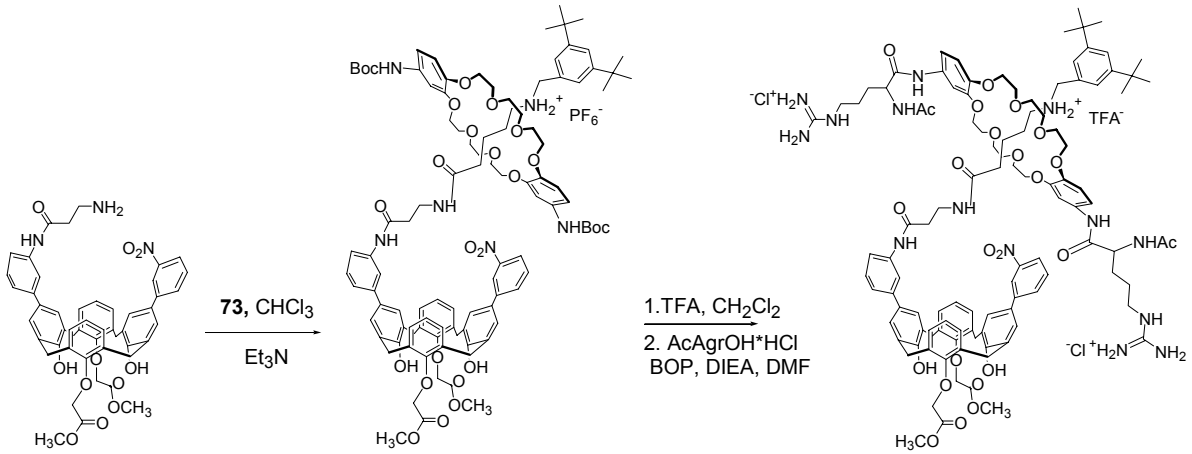


Synthesis of Calix[4]arene Host-[2]rotaxanes

Calix[4]arene host-[2]rotaxane **74** was obtained by reacting calix[4]arene **35** with DCC-rotaxane **73**. We developed efficient step-wise purification methodology; (i) chloroform and triethylamine were removed under vacuum. (ii) The crude reaction product was dissolved in acetonitrile and acetonitrile-insoluble DCU was filtered off. (iii) Filtrate was concentrated under vacuum and an oily residue was triturated with an ethyl ether / acetone (1/1) solution to remove 1-(3,5-di-*tert*-butyl-benzyl)-piperidin-2-one **58** and triethylamine salt. (iv) Rotaxane **74** was separated from crown ether **57** using rotary column chromatography. The removal of *tert*-butoxycarbonyl- protecting groups was accomplished upon treatment of rotaxane **74** with 20% TFA in CH_2Cl_2 to give amino host-[2]rotaxane **75** in a quantitative yield. During the synthesis of arginine-rotaxane **76** we encountered several problems. The usual coupling reagents for amidation (CDI, DCC / HOBT, and PyBroP) did not provide the desired product. Another caveat is poor solubility of *N*-acetyl-arginine in most of the solvent systems. Attempts to activate *N*-acetyl-arginine sodium carboxylate suspension in DMF, or DMSO and couple it with

amino-rotaxane **75** were unsuccessful. Arginine-rotaxane **76** was obtained in good yields only when an arginine hydrochloride salt was used in DMF with BOP as an activating agent in the presence of DIEA.

Scheme 15.



Derivatized Di-*p*-Phenylcalix[4]arene and Calix[4]arene Host-[2]Rotaxane Receptors

To examine advantages afforded by host-rotaxanes, the binding ability of calix[4]arene host-[2]rotaxane **76** for *N*-acetyl amino acids was compared to di-*p*-(3-aminophenyl)-calix[4]arene **43** and arginine derivatized calix[4]arenes **45** and **46** in DMSO and in a (90/10) DMSO/water solution. ^{1}H NMR binding titrations were performed by adding aliquots of a stock host solution to a solution of *N*-acetyl amino acid sodium carboxylates. The change in solution volume was less than ten percent. Association constants were derived by solving the binding equation[22] for plots of the changes in the chemical shifts of the α-proton or aromatic protons of guests against changes in a host's concentration.

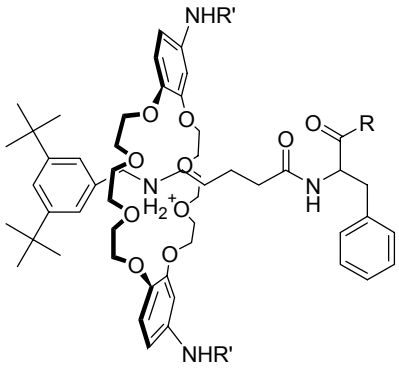
Calix[4]arene host-[2]rotaxane **76** gave the largest association constants in both solvent systems. Binding titrations were performed using 3 mM and 1 mM guest concentrations, however, high association constants ($K_A >> 10^3$) could not be estimated accurately using NMR spectroscopy. Therefore, binding affinity of rotaxane **76** for N-acetyl-tryptophan sodium salt was determined using fluorescence spectroscopy; in 100% DMSO solution $K_A = (1.6 \pm 0.1) \cdot 10^5$.

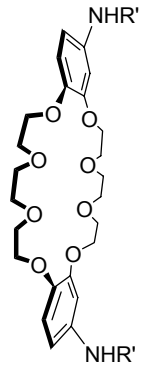
Calix[4]arenes **45** and **46** had association constants on the order 10^3 (M^{-1}), while amino calix[4]arene **43** proved to be a poor host: at 3 mM concentrations of guests no association was detected, when 15 mM concentration of guests were used, we observed down-field shifts for the aromatic and α-proton signals of the guests.

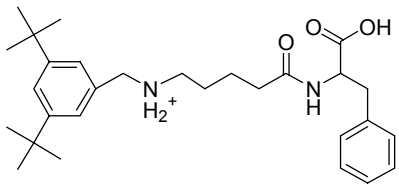
Preliminary studies have shown superiority of calix[4]arene host-[2]rotaxane receptors for binding of amino acids. More detailed investigation of rotaxane receptor properties using fluorescence and two-dimensional NMR spectroscopy need to be done.

Model Studies of Phenylalanine Rotaxanes Self-Adjusting Properties

Phenylalanine rotaxanes **77**, **78** and **79** were constructed to study conformational properties of host-[2]rotaxanes and their ability to serve as self-adjusting receptors. We investigated if the structure of host-rotaxanes **78** and **79** can be controlled by favorable pseudo-intramolecular interactions between the side chains of DB24C8 and the carboxylate of the axle. A relative measure of the strength of pseudo-intramolecular interactions was obtained by comparing the changes of pK_a's of functional groups held within rotaxanes **78** and **79** to ones that are attached to free crown ethers **54**, **56** and axle **80**. Additionally, we investigated the ability of rotaxanes **78** and **79** to bind various guests. Cooperativity effects in the binding event between rotaxanes **78** and **79** and appropriate guests would indicate that a small entropic loss occurs upon alignment of the ring for structure and function, and conformationally mobile rotaxanes have a suitable architecture for construction of highly efficient receptors.

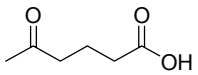




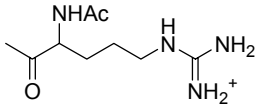


77 R=OCH_3, R'=Boc

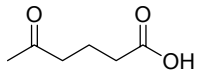
78 R=OH, R'=



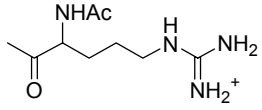
79 R=OH, R'=



56 R'=



54 R'=

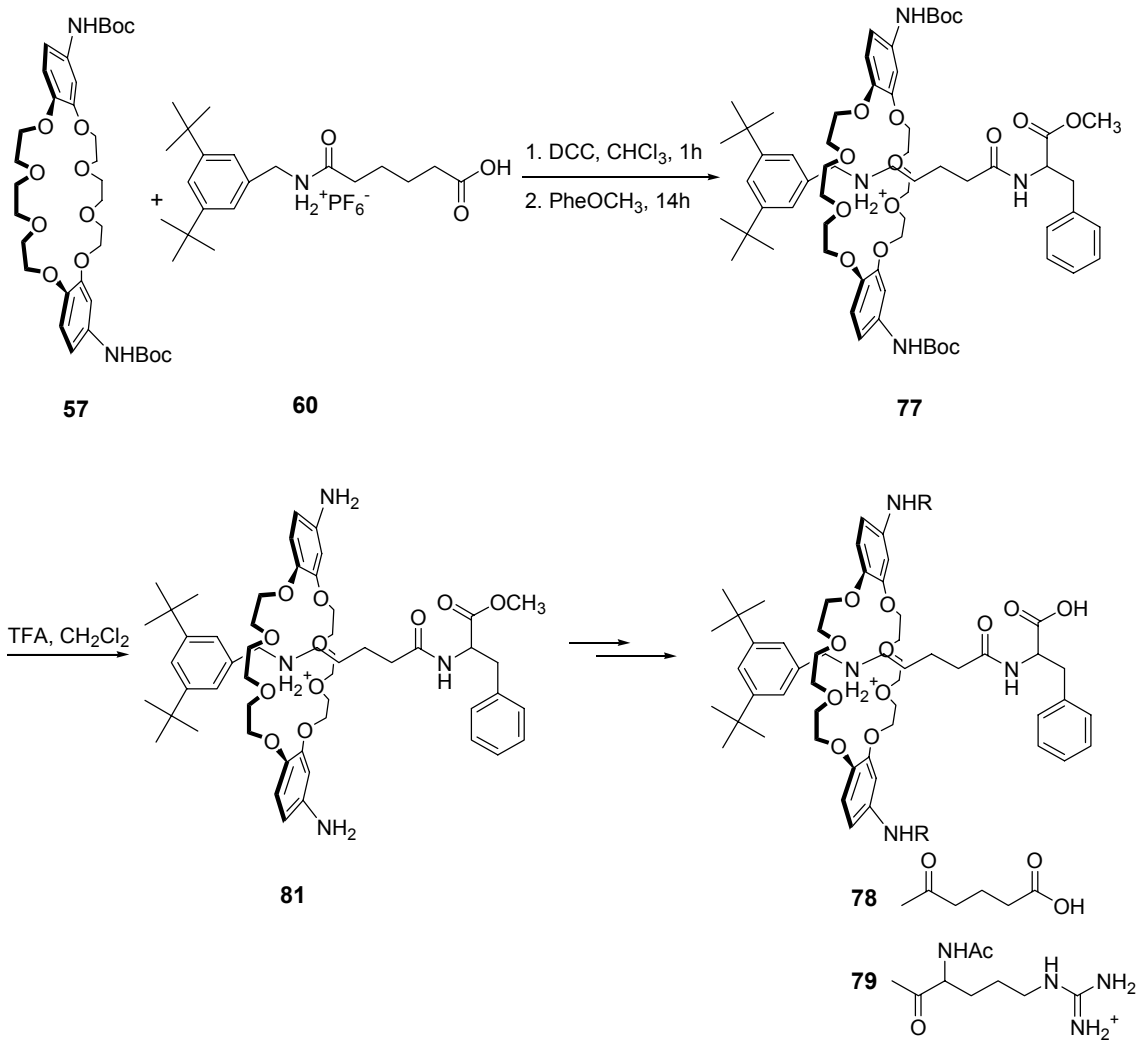


80

Synthesis of Phenylalanine Rotaxanes

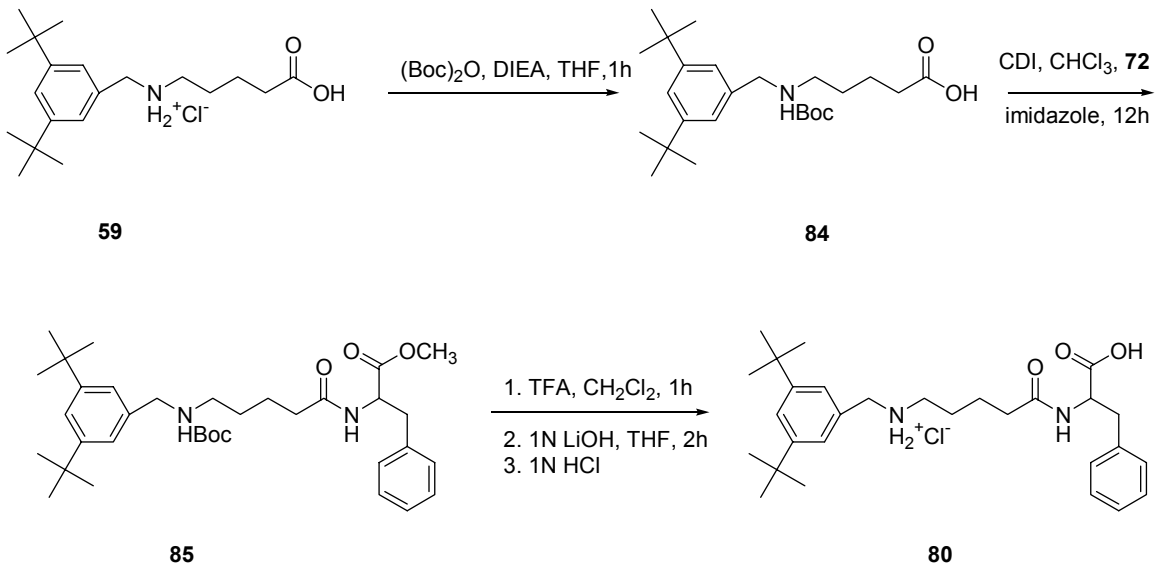
Phenylalanine methyl ester **72** was reacted with DCC-rotaxane **73** to provide rotaxane **77** in a 72% yield. The removal of *tert*-butoxycarbonyl- protecting groups was accomplished upon treatment of rotaxane **77** with 20% TFA in CH_2Cl_2 to give amino rotaxane **81** in high yields. Amino rotaxane **81** was reacted with glutaric anhydride to provide diacid-rotaxane **82**. Arginine-rotaxane **83** was obtained by coupling *N*-acetyl arginine with diamine **81** in the presence of BOP. Lithium catalyzed hydrolysis of methyl ester-rotaxanes **82** and **83** in an aqueous THF provided host-[2]rotaxanes **78** and **79**.

Scheme 16.



Axle **80** was synthesized to compare pseudo-intramolecular interactions in host-rotaxanes **78** and **79** with intermolecular interactions between the free axle and crown ethers. 5-(3,5-Di-*tert*-butyl-benzylamino)-pentanoic acid **60** was reacted with di-*tert*-butyl dicarbonate to give 5-[*tert*-butoxycarbonyl-(3,5-di-*tert*-butyl-benzyl)-amino]-pentanoic acid **84** in a quantitative yield. Acid **84** was coupled with phenylalanine methyl ester **72** to provide compound **85**. The methyl ester of axle **86** was obtained in a 85% yield via TFA catalyzed removal of *tert*-butoxycarbonyl protecting group. LiOH catalyzed hydrolysis of methyl ester **86** in an aqueous THF provided axle **80**.

Scheme 17.



Studies of Pseudo-Intramolecular Interactions

Potentiometric titrations were performed to obtain a relative measure of the interaction energies that exist between functional groups of the rotaxanes. The formation of H-bond in rotaxane **78** or a salt bridge in rotaxane **79** (Figure 16) would lower the pK_a's of the carboxylic acid of the Phe-blocking group. 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 cation-induced dipole interactions. The pK_a's of crown ethers **54**, **56** and axle **80** were determined to provide reference pK_a values for the functional groups when they are extensively free in solution. pK_a's were also determined for crown ethers **54** or **56** combined with axle **80** in equimolar ratios to compare the pseudo-intramolecular interactions to intermolecular interactions. DMSO/ water solutions were used to solubilize compounds and to determine the strength of noncovalent interactions in an aqueous environment.

T**able 3.** pKa's of the compounds and compound mixtures in different ratios of DMSO and water measured at 298 K.

Compound(s)	Acidity Constant[a]	DMSO / Water			
		90/10	60/40	50/50	40/60
	pK_{a1}				
79		4.0 ± 0.1	3.72 ± 0.1	3.50 ± 0.02	3.75 ± 0.08
78		8.11 ± 0.04	5.65 ± 0.05	5.1 ± 0.1	4.53 ± 0.06
56		10.78 ± 0.03	7.29 ± 0.04	6.50 ± 0.03	5.84 ± 0.04
80		9.07 ± 0.06	5.9 ± 0.2	5.17 ± 0.05	4.38 ± 0.07
54 + 80		9.06 ± 0.05	5.66 ± 0.04	5.09 ± 0.07	4.67 ± 0.05
56 + 80		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
	pK_{a2}				
79		ND[b]	ND	ND	ND
78		9.22 ± 0.0.03	6.59 ± 0.05	6.34 ± 0.06	5.68 ± 0.02
80		10.8 ± 0.1	9.89 ± 0.03	9.80 ± 0.09	9.66 ± 0.02
54 + 80		10.70 ± 0.07	8.4 ± 0.2	8.2 ± 0.1	8.1 ± 0.1
56 + 80		10.52 ± 0.04	7.09 ± 0.01	6.30 ± 0.01	5.62 ± 0.07
	pK_{a3}				
78		9.89 ± 0.01	9.5 ± 0.2	9.25 ± 0.2	9.3 ± 0.2
54 +80		ND	ND	ND	ND
56 + 80		10.56 ± 0.05	7.79 ± 0.01	7.16 ± 0.01	7.3 ± 0.3
	pK_{a4}				
78		ND	ND	ND	ND
56 + 80		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, [b]ND means not detected.

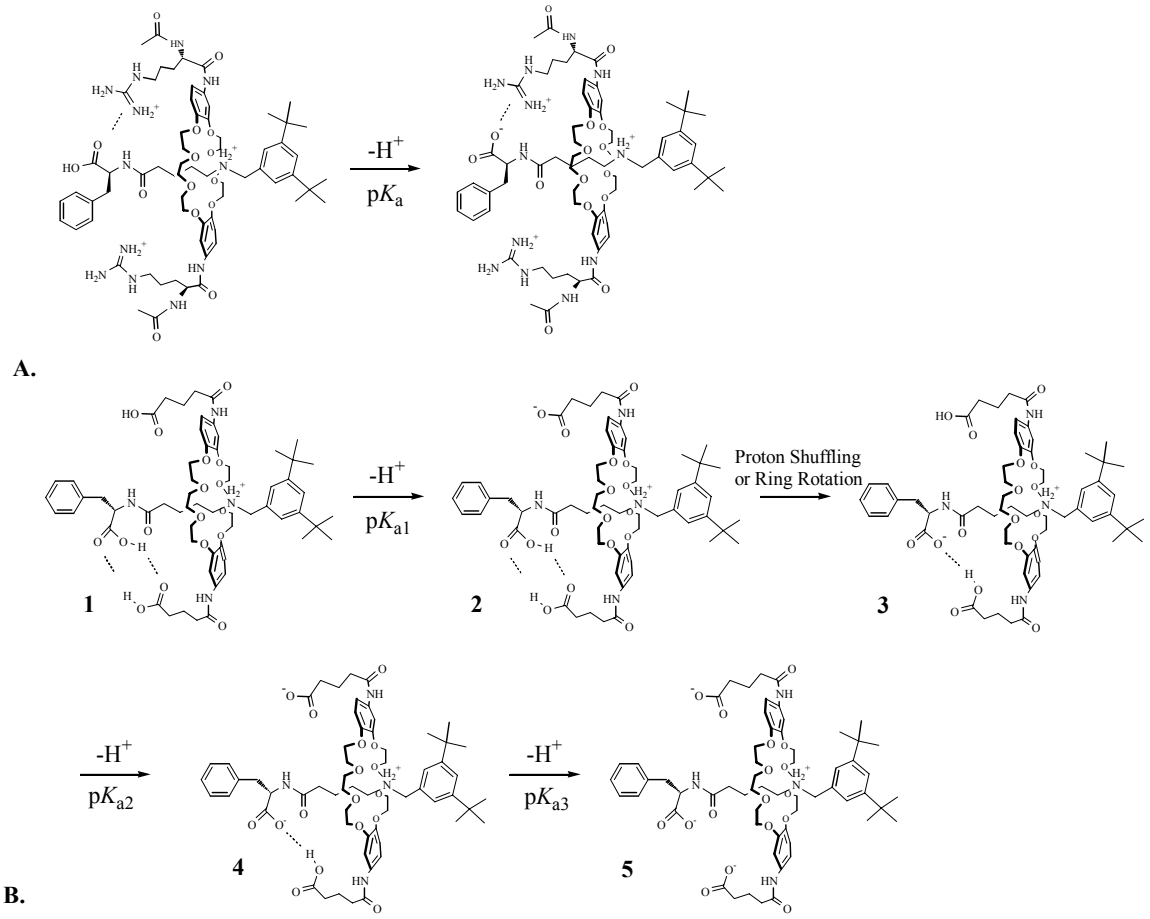


Figure 16. Potential intramolecular interactions (indicated by dashed lines) for the rotaxane **79** include **A.** a dipole-ionic interaction for the fully protonated form of rotaxane **79** and a salt bridge for its monocarboxylate state. **B.** Depending on the pH, a variety of potential interactions could exist for rotaxane **78**. For example, **B1** dimeric carboxylic acids for the fully protonated form. For the dicarboxylate states **B2** and **B3**, the rotaxane could still have dimeric acids **B2** or, more likely, or a single H-bonded complex forms **B3** between the carboxylates through proton shuffling or rotation of the ring. **B4** a single H-bonded structure for its dicarboxylate state, and for **B5**, no interactions should occur between the carboxylates.

Analysis of structure of rotaxane 79 in 90/10 DMSO to water mixture. We assign pK_{a1} of rotaxane **79** (pK_a=4.0), axle **80** (pK_a = 9.1), and 1:1 mixture of axle **80** and DB24C8 **54** (pK_a = 9.1) to the carboxylic acid of the phenylalanine moiety. Much lower pK_{a1} for rotaxane **79** suggest salt-bridge formation between guanidinium ion and carboxylate. Similar pattern in pKa's was observed for *N*-Boc-phenylalanine (pK_a = 7.8) and phenylalanine (pK_{a1} = 5.9). Intramolecular salt formation between ammonium ion

and carboxylate within PheOH considerably lowers pK_a of the acid. The fact that pK_{a1} of axle **80** is not substantially changed in the presence of DB24C8 **54** (compounds **80** + **54**, in Table 3) shows that the pseudo-intramolecular interactions of rotaxane **79** are more favorable than the intermolecular interactions between these groups when the ring and axle are separate components.

A more quantitative measure of the interaction energy between the carboxylate and the arginine side chains of rotaxane **79** was obtained by analyzing model system comprised of *N*-Boc-Phe and guanidinium chloride (GH^+). Changes in pK_a's 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.[21] The degree of association between *N*-Boc-Phe and guanidinium chloride (GH^+) provide a measure of the energy difference between the pseudo-intramolecular interactions of rotaxane **79** compared to intermolecular ones. A 90/10 mixture of DMSO to water proved to be a suitable solution for the experiments because the extent of salt bridge formation changed significantly with the addition of GH^+ from 0.1 M to 1.0 M. As [GH^+] increased, the pK_a of *N*-Boc-Phe dropped until a saturation value was reached.

At the 1.0 M concentration of GH^+, a pK_a value of 5.8 was measured for *N*-Boc-Phe. Because the pK_a of rotaxane **79** is lower (pK_{a1} = 4.0) in the same solvent mixture, the effective concentration of its guanidinium moiety for salt bridge formation is greater than 1.0 M. To determine the 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 17). The curve was fitted to a standard binding equation for a two component system using a non linear least square procedure,

giving an association constant (K_A) of 8 ± 2 M^{-1} and $\Delta pK_{a,sat}$ of 2.4 ± 0.1 for the salt bridge. $pK_{a,sat}$ is the theoretical value obtained for *N*-Boc-Phe exposed to an infinite concentration of GH^+. pK_{a1} of rotaxane **79** (pK_{a1} = 4.0) is 1.4 pK_a units below the value determined for the *N*-Boc-Phe · GH^+ complex ($pK_{a,sat}$ = 5.4). The greater ΔpK_a value observed for rotaxane **79** compared to $\Delta pK_{a,sat}$ indicate strong pseudo-intramolecular interactions, which are insured by architecture of the rotaxane.

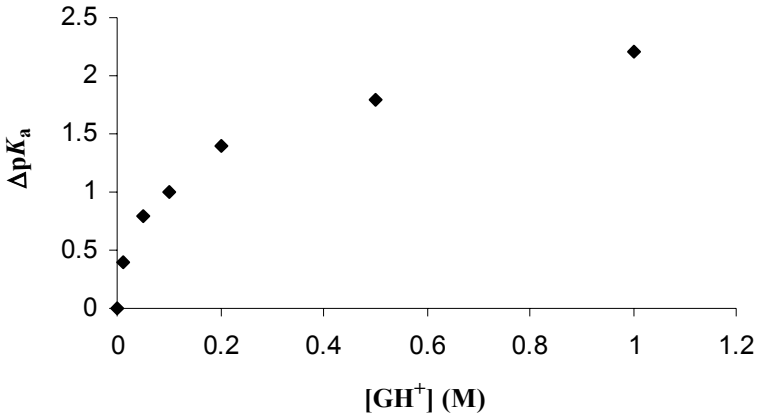


Figure 17. Plot of ΔpK_a of *N*-Boc-Phe caused by the addition of GH^+ in 90/10 DMSO to water solution at 25 °C. This calibration curve was used to derive $\Delta pK_{a,sat}$ for a theoretical saturated solution of GH^+.

Addition of water to the solution of compounds **79**, **80**, **80+54** and *N*-Boc-Phe resulted in a substantially lowered pK_a's for all compounds except rotaxane **79** (Table 3). The observer smaller solvent effect for pK_a's of rotaxane **79** implicates, once again, strong intramolecular interactions.

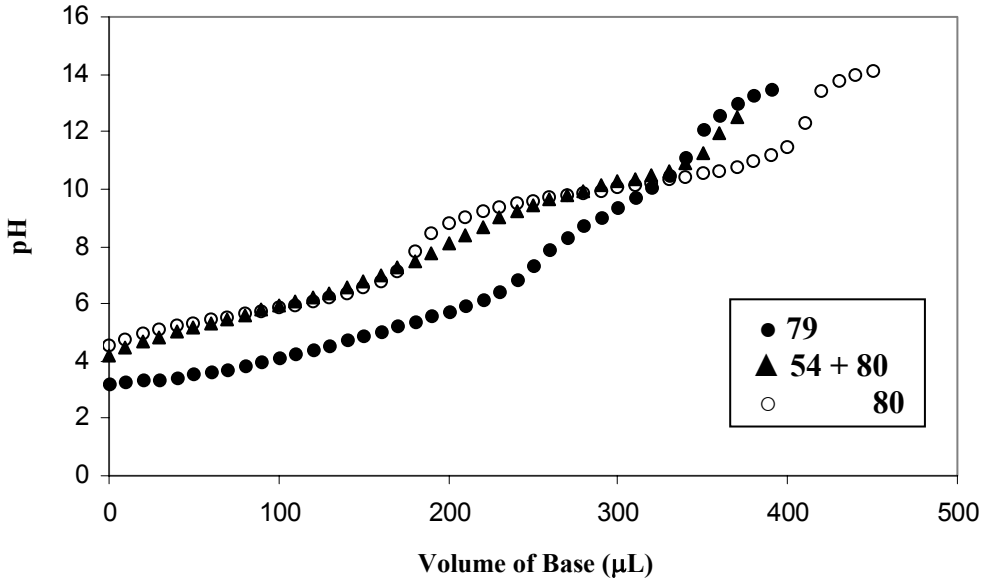


Figure 18. Representative potentiometric titration plots in 40/60 DMSO to H_2O solutions at 25 °C are given. The curve of rotaxane **79** is substantially shifted to lower pH values compared to axle **80** and crown plus axle (**54** + **80**), which are more similar. pH values were not corrected.

Consequently, strong internal salt bridges prevent rotaxane **79** to function as an artificial receptor: no association was detected between alanine or phenylalanine and arginine rotaxane **79** in various DMSO/water solvent systems (100% DMSO-d_6, 90/10, 60/40, and 40/60 DMSO-d_6 to H_2O, 5mM concentration of guests, pH=8.5).

Analysis of structures of rotaxane 78. Examination of the pK_a's of rotaxane **78** shows that pseudo-intramolecular interactions occur for this rotaxane, as well. The fully protonated state can be stabilized by a H-bonded dimer between two carboxylic acids (Figure 16 **B1**). If the carboxylic dimer does form, then the free acid should have the most acidic proton and be deprotonated first. The resulting carboxylate can remain free, giving structure **B2**. In this case pK_{a1} of rotaxane **78** should match the pK_a of DB24C **56**. On the other hand, if H-bonded complex **B3** is formed, the pK_a of rotaxane **78** would drop. The lower pK_{a1} for rotaxane **58** compare to DB24C8 **56** (ca. of 1.5 units throughout

the solvent mixtures), suggest that carboxylate form H-bonded complex **B3**. pK_{a2} of rotaxane **78** is more similar to the pK_a of DB24C8 **56**, and these values become closer with a higher percentage of water - most likely the second carboxylate does not form intramolecular H-bonds (**3B** going to **4B**, Figure 16). pK_{a3} of rotaxane **78** changes only slightly upon addition of water (Table 3) which suggests that the dicarboxylate state (**B4**) has strong pseudo-intramolecular interactions.

Relatively weaker strength of H-bonding within acid rotaxane **78** compare to salt-bridge interactions in rotaxane **79** allows rotaxane **78** act as an artificial receptor. Binding assays were performed with *N*-acetyl arginine and rotaxane **78** existing in the mono-, di-, and tricaboxylate state with Li^+ and Me_4N^+ as counterions in pure DMSO-d_6 and 90/10 DMSO-d_6 to H_2O solutions (Table 4):

(i) Fully protonated host did not associate with *N*-acetyl arginine.

(ii) Receptors with Li^+ counterions exhibited much lower K_As than receptors with Me_4N^+ counterions, which is consistent with a stronger ion pair formation between carboxylates of rotaxane **78** and Li^+ .

(iii) At higher concentrations of water (e.g., 60/40 DMSO-d_6 to H_2O) no association could be detected.

(iv) Having a single carboxylate, rotaxane **78** only weakly associated (K_A = 55 M^{-1}) with the guest.

(v) Rotaxane **78**, as the dicarboxylate, bound *N*-acetyl arginine the strongest (K_A = 474 M^{-1}); slightly tighter than in the tricarboxylate state (K_A = 404 M^{-1}). The additional binding energy observed for the dicarboxylate state may arise through a H-bonded complex between the remaining protonated acid and the amide of *N*-acetyl arginine. The

N-H amide proton shifted downfield with the addition of rotaxane **78**, which is consistent with H-bonding.

Further evidence for the structures of rotaxane **78** in its various protonation states (Figure 16) is obtained by comparing the association constants of rotaxane **78** and acetate bound to *N*-acetyl arginine. Rotaxane **78** in the di- or tricarboxylate state associates with *N*-acetyl arginine approximately four times better than acetate in 90/10 DMSO-d_6. The larger K_A reveals the energetic advantage for complexation of having the functional groups displayed within a rotaxane. On the other hand, rotaxane **78** as the monocarboxylate associates with *N*-acetyl arginine weaker than acetate. This smaller K_A supports our hypothesis that state **B3** (Figure 16) is more stable than state **B2**, and for the host to interact with the guest pseudo-intramolecular interactions within **78** should be interrupted. For the strongest binding state **B4**, we suggest that a salt bridge forms with the free carboxylate and then a dimeric salt bridge forms. A shuffling of the protons would need to occur after *N*-acetyl arginine forms the first salt bridge to have the second carboxylate interact with the guest. A more intriguing possibility is that the ring rotates to accommodate the guest in accordance with the machine-like nature of rotaxanes.

Table 4. Association constants determined at 298 K.

Host	Solvent	Counter ion	K_A (M^{-1})	Host	Eq. Base	K_A (M^{-1})
78	DMSO-d_6	$(Me)_4N^+$	393 ± 22	**78***	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
				$AcCO_2^-$	1	114 ± 17

78* binding studies were done in DMSO-d_6/H_2O (90/10) solution

Structure Determination. Molecular dynamics calculations were performed (MM+ forcefield as presented by Hyperchem) to determine if the functional groups could interact in the predicted manner; the crown ether seated over the ammonium ion of the axle and its side chain interacting with the carboxylate of the phenylalanine blocking group. Lowest energy conformation of the rotaxanes, in the monocarboxylate state, showed that both rotaxanes **78** and **79** should form the predicted H-bonds (Figure 16). However, the lowest energy conformation of rotaxane **78** has both carboxylic acids of the crown ether forming H-bonded complexes with the carboxylate of the axle. Because the pK_{a2} of rotaxane **78** is only slightly smaller ($\Delta pK_a = 0.3$) than pK_a of ring **56**, if the third carboxylic acid does form a complex, the interactions are weak. .

The structures of rotaxanes **78** and **79** were also investigated using 2D-NMR analysis in DMSO-d_6. We looked particularly for cross peaks between the crown ether and the axle to find the preferred position of the ring. Rotaxane **79**, 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 19). NOE's 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 ring. These NOE's are consistent with the calculated lowest energy conformation of rotaxane **79**.

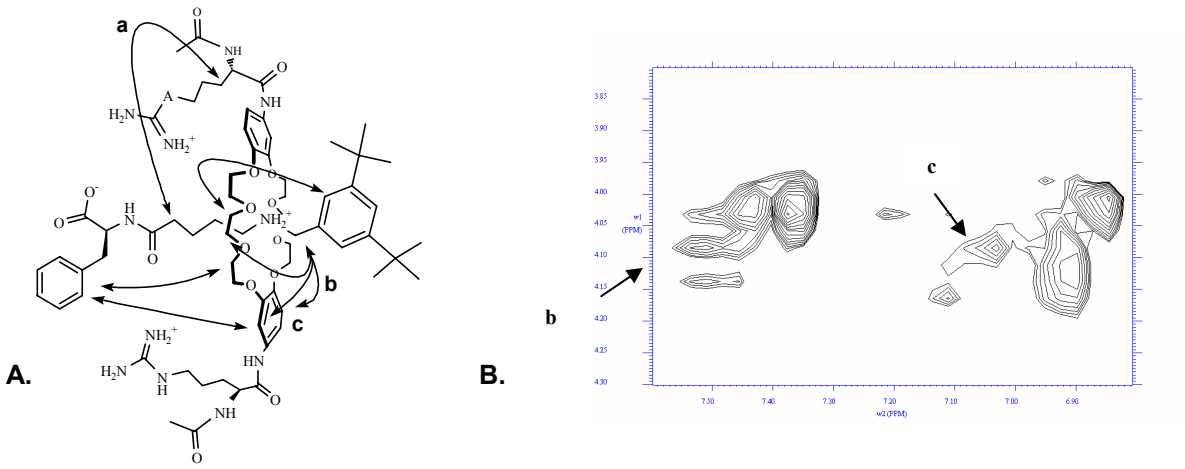


Figure 19. **A.** Key NOE's between the ring and the axle of rotaxane **79** observed in DMSO-d_6 are indicated by double headed arrows. NOE (**a**) between the arginine derivative and the tether is a strong indicator of a salt bridge between the carboxylate of the phenylalanine blocking group and the guanidinium moiety of the ring. **B.** A portion of the NOESY experiment that shows the cross peaks between the benzylic protons of 3,5-di-tert-butylphenyl blocking group and the aromatic protons of the ring (**b** and **c**) is given.

Conclusions

The phenylalanine rotaxanes performed according to their predicted properties. Rotaxane **79** formed a very strong intramolecular salt bridge, which gives it extensive structure. Rotaxane **78** had weaker intramolecular interactions, allowing it to perform as an artificial receptor by binding N-acetyl arginine. The ability to control the structure and function of rotaxanes by incorporating appropriately derivatized functional groups implies a possibility to construct efficient self-adjusting artificial receptors. Superiority of calix[4]arene host-[2]rotaxane receptors confirm the importance of the hydrophobic cavity element in the design of artificial receptors, thus laying the path for future explorations in host-rotaxane chemistry.

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EXPERIMENTAL DATA

Chapter 1

General Procedures ^{1}H and ^{13}C NMR spectra were obtained with a Bruker AC250 spectrometer operating at 250.13 MHz for proton and 62.90 MHz for carbon nuclei and a Bruker AMX400 spectrometer operating at 400.14 MHz for proton and 100.23 MHz for carbon nuclei. ^{1}H NMR chemical shifts were referenced to TMS (0.00 ppm), or d_6-DMSO (2.49 ppm), and ^{13}C NMR chemical shifts to d_6-DMSO (39.5 ppm). UV-VIS spectra were recorded on a Hewlet Packard Kayak XA series spectrometer. HPLC analysis was performed on a Shimadzu 10A series HPLC using a C-18 reversed phase column with water/ CH_3CN as eluent. Melting points were determined on and a MEL-TEMP (Laboratory Devices) apparatus are uncorrected. Pyridine was purchased HPLC grade from Aldrich and distilled form CaH_2. All reagents were purchased from Aldrich and utilized without further purification. All reactions were done under argon atmosphere.

Theoretical Structures Molecular modeling was run on a Silicon Graphics work station using the CVFF forcefield as part of the Discover software (*Insight II* 95.0 Biosym/Molecular Simulations). Energy minimized structures of each hydroxyamine and calix[4]arene were obtained through a steepest descent method with the convergence set at 0.01 kcal/(mol·Å) for the rms gradient. Calix[4]arenes **10b-e** were solvated by placing them in a 20 x 20 x 20 Å periodic box (ca 220 water molecules, 300 K, 1 atm). Complete solvation of calix[4]arenes **10f,g** required a box with dimensions of 25 x 25 x

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Synthetic procedures are given in the sequence that corresponds to the numbering of compounds. This sequence, therefore, sometimes does not follow the sequence in the multistep synthesis.

25 Å (ca 440 water molecules). Nonbonded interactions were limited by a switched cutoff of 9.8 Å and 12.4 Å, respectively. To obtain stable trajectories, the entire systems were minimized using a conjugate gradient method (Polak-Robiere) to a rms gradient of 0.01 kcal/(mol·Å) or to a maximum of 15,000 steps. Equilibrium structures were then obtained by performing molecular dynamic simulations of 1 fs steps for a total of 60,000 steps (300 K). Conformers were stored every 100 steps. By 40,000 steps, the systems had reached equilibrium, and the equilibrium structures were calculated from the last 200 frames.

Solubility Studies

Samples for solubility studies were purified by HPLC, except for the ethanolamine derivative, which was purified by column chromatography. Three separate samples were prepared for each derivatized calix[4]arene in buffered water (10 mM phosphates pH 6.8) and in a 95:5 buffer (10 mM phosphates pH 6.8) / DMSO (v/v) solution by vigorously stirring the solutions that contained an excess amount of calix[4]arene for 24 h. The suspensions were centrifuged at 10,000 rpm for 30 min to remove the insoluble material from the liquid phase. Each clear solution was transferred to another vial and centrifuged for an additional 10 min. Aliquots of these solutions were added to UV-vis cells, and the absorption at 275 nm were recorded. 275 nm was chosen as λ_{obs} because the same molar absorption coefficients were obtained for both solvent systems; DMSO does not strongly absorb light at this wavelength. Only a trace of DMSO existed in the cells (< 0.2 %). The absorption (A) intensities were converted to concentrations (c) using

the A = εlc relationship. Molar absorption coefficient (ε) for each calix[4]arene derivative was obtained by constructing calibration curves of absorption intensities at 275 nm versus known concentrations of pure compounds. To ensure that all insoluble materials were removed, the solutions were centrifuged again for 10 min and the calix[4]arenes concentrations were determined. The differences in the absorbances measured after centrifuging twice and three times were within experimental errors.

25,27-Bis(hydroxyethoxy)calix[4]arene-26,28-diol (7)

Calix[4]arene **6** (0.9 g, 1.5 mmol) was dissolved in 15 ml THF/ 2 ml C_2H_5OH, and 2.0 M $LiBH_4$ (1.5 ml, 3.0 mmol) was slowly added to the reaction mixture. The reaction was stirred at RT overnight, and quenched with 5% HCl. THF and C_2H_5OH were removed under vacuum, and an aqueous layer was extracted with CH_2Cl_2. Organic phase was separated and upon removal of solvent 0.76 g of the title product were obtained. ^{1}H NMR (250.13 MHz, $CDCl_3$) δ 9.16 (2H, s), 7.07 (4H, d, *J*=7.8 Hz), 7.06 (4H, d, *J*=7.4 Hz), 6.87 (2H, t, *J*=7.7 Hz), 6.68 (2H, t, *J*=7.4 Hz), 4.95 (2H, br s), 4.36 (4H, d, *J*=13.3 Hz), 4.28 (8H, s), 3.46 (4H, d, *J*=13.0 Hz); ^{13}C NMR (100.23 MHz, $CDCl_3$) δ 151.58, 150.51, 134.17, 129.39, 128.74, 128.43, 126.35, 120.56, 77.94, 62.15, 31.62.

25,26,27,28-Terakis(methoxycarbonylmethoxy)calix[4]arene (9c)

Calix[4]arene **1** (7.47 g, 17.6 mmol) was dissolved in 9:1 THF/DMF (200 ml), and NaH (60% in mineral oil) (3.54 g, 88.5 mmol) was added in parts to this solution (exothermic reaction). The white suspension was stirred for 20 min. Methyl bromoacetate (10.0 ml, 105 mmol) was slowly added in 1 ml portions (overheating is possible), and the reaction

was refluxed for 24 h. At this time, starting material still existed. Thus, another eq of NaH (60%) (0.70 g, 18 mmol) and methyl bromoacetate (1.7 ml, 18 mmol) were added, and reaction was refluxed for an additional 48 h. Solvents were removed in vacuo, and the crude material was dissolved in $CHCl_3$ (150 ml) and extracted with water (2 x 100 mL). The organic phases were collected, dried over $MgSO_4$, and the solvent was removed in vacuo. Calix[4]arene **9c** was purified by recrystallization from EtOH to give 10.0 g (14.0 mmol, 80% yield) of white crystals. mp 138–139 ^{0}C ^{1}H NMR δ 3.24 (4H, d, *J*=13.6 Hz), 3.76 (12H, s), 4.75 (8H, s), 4.85 (4H, d, *J*=13.6 Hz), 6.64 (12H, s); ^{13}C NMR δ 31.3, 51.5, 71.1, 122.9, 128.6, 134.5, 155.8, 170.6; TOF MS ES+ calc. mass: $C_{40}H_{40}O_{12}Na^+$ 735.2417, found: 735.2377.

25,27-Bis[N-(2-hydroxyethyl)aminocarbonylmethoxy]-calix[4]arene-26,28-diol (10b)

Diacid calix[4]arene **10a** (0.30 g, 0.56 mmol) and EEDQ (0.55 g, 2.2 mmol) were dissolved in pyridine (10 ml) and stirred at a room temperature for 30 min. Ethanolamine (0.27 ml, 4.4 mmol) was added, and the reaction mixture was refluxed for 12 h. Solvents were removed in vacuo, and excess EEDQ was removed by washing with ethyl ether. The crude material was dissolved in 5:1 $CHCl_3/CH_3OH$ (20 ml) and extracted with 5% HCl. The organic phases were collected, dried over $MgSO_4$, and the solvent was removed in vacuo, giving 0.33 g (0.53 mmol, 93% yield) of calix[4]arene **10b** as an off-white powder, which was pure by ^{1}H NMR analysis (> 95%): mp 274 –275 ^{0}C. Additional purification can be accomplished by column chromatography (SiO_2, 10:1 $CHCl_3/CH_3OH$). ^{1}H NMR δ 3.18-3.56 (12H, m), 4.24 (4H, d, *J* = 13.0 Hz), 4.53 (4H, s), 4.68 (2H, br s), 6.63 (2H, t, *J* = 7.3 Hz), 6.82 (2H, t, *J* = 7.4), 7.07 (4H, d, *J* = 7.5 Hz),

7.18 (4H, d, J = 7.3 Hz), 8.34 (2H, s), 8.57 (2H, s); ^{13}C NMR δ 30.5, 41.2, 59.7, 74.2, 119.4, 125.6, 127.4, 128.7, 129.1, 133.6, 151.9, 152.2, 167.9; TOF MS ES+ calc. mass: $C_{36}H_{39}N_2O_8$ $(M+H)^+$ 627.2706, found: 627.2711; Anal. Calcd for $C_{36}H_{38}N_2O_8$: C, 68.99; H, 6.11; N, 4.47. Found C, 69.36; H, 6.33; N, 4.46.

25,27-Bis[N-(2-hydroxy-1-hydroxymethyl-ethyl)aminocarbonylmethoxy] - calix[4]arene-26,28-diol (10c)

Diacid calix[4]arene **10a** (0.20 g, 0.37 mmol) and EEDQ (0.28 g, 1.1 mmol) were dissolved in pyridine (5 mL) and stirred at a room temperature for 1 h. Serinol (0.14 g, 1.1 mmol) was added and the mixture was refluxed for 12 h. Solvent was evaporated in vacuo, and the remaining oily residue was washed with ethyl ether to remove the excess of EEDQ. The solid residue was dissolved in 95:5 acetone/methanol mixture (10 ml), and upon addition of ethyl ether, calix[4]arene **10c** precipitated to give 0.216 g of a white powder (0.31 mmol, 83% yield): mp 233-234 ^{0}C. Further purification can be performed by recrystalization from ethanol. ^{1}H NMR δ 3.44-3.55 (12H, m), 3.95 (2H, br s), 4.27 (4H, d, J = 12.4 Hz), 4.55 (4H, s), 4.77 (4H, s), 6.62 (2H, t, J = 6.6 Hz), 6.80 (2H, t, J = 6.6 Hz), 7.03 (4H, d, J = 6.4 Hz), 7.16 (4H, d, J = 6.4 Hz), 8.16 (2H, s), 8.54 (2H, s); ^{13}C NMR δ 30.8, 52.8, 60.0, 74.6, 119.3, 125.5, 127.4, 128.7, 129.2, 133.4, 152.3, 152.7, 167.9; UV max 203, 223, 266, 313 nm; TOF MS ES+ $C_{38}H_{43}N_2O_{10}$ $(M+H)^+$ calc. mass 687.2918, found 687.2885. Anal. Calcd for $C_{38}H_{42}N_2O_{10}$: C, 66.46; H, 6.16; N, 4.08. Found C, 64.72; H, 6.74; N, 4.32.

25,27-Bis[N-(2-hydroxy-1,1-bishydroxymethyl-ethyl)aminocarbonylmethoxy] -calix[4]arene-26,28-diol (10d)

Diacid calix[4]arene **10a** (0.20 g, 0.37 mmol) and EEDQ (0.28 g, 1.1 mmol) were dissolved in pyridine (5 ml) and stirred at a room temperature for 1 h. TRIS (0.18 g, 1.5 mmol) was added and the mixture was refluxed for 12 h. Solvent was removed in vacuo, and the remaining oily residue was washed with ethyl ether to remove excess EEDQ. Removal of excess TRIS was accomplished by dissolving the crude material in 10:1 $CHCl_3/CH_3OH$ (20 ml) and extracting with water. Organic layers were collected and dried over $MgSO_4$. Solvent was removed in vacuo, and the remaining material was washed with ethyl ether, leaving 0.25 g of calix[4]arene **10d** as a white powder (0.33 mmol, 90 % yield): mp 225-227 ^{0}C. ^{1}H NMR δ 3.43 (4H, d, *J* = 13.1 Hz), 3.69 (12H, d, *J* = 5.5 Hz), 4.31 (4H, d, *J* = 13.0 Hz), 4.50 (4H, s), 4.74 (6H, t, *J* = 5.5 Hz), 6.61 (2H, t, *J* = 7.4 Hz), 6.77 (2H, t, *J* = 7.4 Hz), 6.99 (4H, d, *J* = 7.5 Hz), 7.15 (4H, d, *J* = 7.5 Hz), 7.93 (2H, s), 8.09 (2H, s); ^{13}C NMR δ 31.0, 60.5, 62.8, 74.7, 119.4, 125.6, 127.6, 128.7, 129.2, 133.3, 152.4, 152.9, 168.9. UV max 204, 266, 313 nm. TOF MS ES+ $C_{40}H_{46}N_2O_{12}Na^+$ calc. mass 769.2948, found 769.2938. Anal. Calcd for $C_{40}H_{46}N_2O_{12}$: C, 64.33; H, 6.21; N, 3.75. Found C, 64.15; H, 6.77; N, 3.71.

25,27-Bis[N-(1-amido-2-hydroxyethyl)aminocarbonylmethoxy]-calix[4]arene-26,28-diol (10e)

Serine amide hydrochloride (0.41 g, 2.9 mmol) was dissolved in water (2 ml) and KOH (0.16 g, 2.9 mmol in 2 ml H_2O) was added. Solvent was removed in vacuo, and the solid was dried exhaustively under high vacuum. This material was dissolved in DMSO (5

mL), and it was added to a 1:1 pyridine/DMSO (10 ml) solution containing diacid calix[4]arene **10a** (0.20 g, 0.37 mmol), DMAP (0.045 g, 0.37 mmol), and EEDQ (0.72 g, 2.9 mmol). The reaction mixture was heated at 90 ^{0}C for 20 h. Solvents were removed in vacuo, and calix[4]arene **10e** was purified by column chromatography (SiO_2, $CHCl_3/CH_3OH$), giving 0.25 g of product as an off-white powder (0.35 mmol, 94% yield): mp 201-202 ^{0}C (begins to darken around 191 ^{0}C). ^{1}H NMR δ 3.43 (4H, d, *J* = 13.1 Hz), 3.75-3.65 (4H, m), 4.30 (4H, d, *J* = 13.6 Hz), 4.42-4.44 (2H, m), 4.61 (4H, s), 4.72 (4H, br s or m), 6.60 (2H, t, *J* = 7.5 Hz), 6.78 (2H, t, *J* = 7.5 Hz), 7.02 (4H, d, *J* = 7.5 Hz), 7.14 (4H, d, *J* = 7.5 Hz), 7.43 (2H, s), 8.10 (1H, s), 8.68 (1H, d, *J* = 7.6 Hz); ^{13}C NMR δ 30.9, 53.6, 55.1, 61.8, 74.7, 119.3, 125.5, 127.6, 127.8, 128.8, 129.3, 133.6, 152.6, 152.9, 168.3, 172.0. UV max 203, 276, 281 nm. TOF MS ES$^+$ $C_{38}H_{41}N_4O_{10}$ $(M+H)^+$ calc. mass 713.2823, found 713.2825. Anal. Calcd for $C_{38}H_{40}N_4O_{10}$: C, 64.04; H, 5.66; N, 7.86. Found C, 64.07; H, 6.18; N, 7.06.

25,27-Bis[N-Methyl-N-(2,3,4,5,6-pentahydroxy-hexyl)-aminocarbonylmethoxy]-calix[4]arene-26,28-diol (10f)

Diacid calix[4]arene **10a** (0.30 g, 0.56 mmol), DMAP (0.134 g, 1.12 mmol) and EEDQ (1.11 g, 4.48 mmol) were dissolved in pyridine (15 ml). After stirring for 30 min, N-methylglucamine (0.87 g, 4.5 mmol) was added, and the reaction mixture was heated at 90 ^{0}C for 4 d. Solvents were removed in vacuo, excess EEDQ was removed with ethyl ether, and the crude product was extracted with 9:1 $CHCl_3/CH_3OH$ / 1N HCl. Organic phases were collected, dried over $MgSO_4$, and the solvents were removed in vacuo. Product was purified by column chromatography (SiO_2, $CHCl_3/CH_3OH$) to give 0.24 g of

calix[4]arene **10f** as an off-white solid (0.27 mmol, 49% yield): 130 ^{0}C (dec.). ^{1}H NMR δ 3.13-3.47 (m, signals are overlapped by CH_3OH), 3.68-3.81 (14 H, m), 4.00-4.25 (4H, m), 4.34 (4H, d, *J*=12.3 Hz), 4.83-4.98 (signals are overlapped by H_2O), 6.67-6.74 (4H, m), 6.87-6.91, (4H, m), 7.10 (4H, d, *J*=7.1 Hz) ; ^{13}C NMR δ 30.7, 33.5, 51.2, 63.4, 69.8, 70.2-73.3 (m), 118.5, 124.8, 127.5-128.9 (m), 133.8, 152.8, 153.8, 168.4. TOF MS ES$^+$ $C_{46}H_{58}N_2O_{16}Na^+$ calc. mass 917.3684, found 917.3676. Anal. Calcd for $C_{46}H_{58}N_2O_{16}$: C, 61.73; H, 6.53; N, 3.13. Found C, 59.57; H, 6.84; N, 3.14.

25,27-Bis[N-(2,3,4,5-Tetrahydroxy-1-hydroxymethyl-pentyl)-aminocarbonylmethoxy]-calix[4]arene-26,28-diol (10g)

Diacid calix[4]arene **10a** (0.30 g, 0.56 mmol) and EEDQ (0.55 g, 2.2 mmol) were dissolved in pyridine (15 ml) and stirred at a room temperature for 1 h. A DMSO solution (10 ml) of 2-amino-2-deoxy-glucitol (0.96 g, 4.4 mmol) and $(iPr)_2EtN$ (1.55 ml, 8.90 mmol) was added to the reaction mixture. The reaction was heated at 70 ^{0}C for 36 h. Solvents were removed under high vacuum, and the remaining oily residue was dissolved in 9:1 $CHCl_3/CH_3OH$ (20 ml) and extracted with water. Solvents were evaporated and the solid residue was washed with ethyl ether to remove the excess of EEDQ, and recrystalized from ethanol to give 0.23 g of calix[4]arene **10g** as a white powder (0.27 mmol, 48% yield): mp 202 –203 ^{0}C. ^{1}H NMR δ 3.16-3.54 (22H, m), 3.99-4.04 (4H, m), 4.28-4.75 (16H, m), 6.60 (2H, t, *J* = 7.3 Hz), 6.78 (2H, t, *J* = 7.4 Hz), 7.02 (4H, d, *J* = 7.5 Hz), 7.14 (4H, d, *J* = 7.4 Hz), 8.07 (2H, s), 8.40 (1H, d, *J* = 7.5 Hz); ^{13}C NMR δ 30.7, 53.8, 60.3, 63.4, 67.7, 71.4, 74.6, 119.2, 125.3, 127.6, 128.5, 128.9, 129.1, 133.4, 152.3, 152.8, 168.3. TOF MS ES$^+$ $C_{44}H_{55}N_2O_{16}$ $(M+H)^+$ calc. mass 867.3552, found 867.3570.

Anal. Calcd for $C_{44}H_{54}N_2O_{16}$: C, 60.96; H, 6.28; N, 3.23. Found C, 60.59; H, 7.13; N, 2.96.

Chapter 2

5,17-Diphenylcalix[4]arene-25,26,27,28-tetraol (12)

To the solution of 0.50 g (0.79 mmol) of calix[4]arene **18** in 40 ml of CH_2Cl_2 at 0°C were added 4 ml C_2H_5SH and 1.00 g of $AlCl_3$ in small portions. Reaction mixture was stirred at room temperature for 12 h. TLC analysis indicated incomplete deprotection, therefore, 1.00 g of $AlCl_3$ was added, and reaction was continued for another 6 h. Solvents were removed, crude reaction mixture was carefully neutralized by slowly adding 5% HCl solution (exothermic reaction), and extracted with CH_2Cl_2. Product was purified using column chromatography (SiO_2, pentane, CH_2Cl_2) to give 0.38 g (83%) of the title compound as a white solid. ^{1}H NMR (250 MHz, DMSO-*d6*) δ 7.42 (d, *J*=7.5 Hz, 4H), 7.30 (t, *J*=7.4 Hz, 4H), 7.21 (s, 4H), 7.15 (t, *J*=7.1 Hz, 2H), 6.97 (d, *J*=7.3 Hz, 4H), 6.38 (t, *J*=7.4 Hz, 2H), 4.28 (br s, 4H), 3.32 (br s, 4H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 148.82, 148.44, 140.76, 135.48, 129.07, 128.98, 128.82, 128.10, 127.84, 126.91, 126.75, 122.35, 122.24, 31.94. MS calc for $C_{40}H_{32}O_4$ 576.2301, found 576.2316.

5,17-Diphenyl-25,26,27,28-tetramethoxy-calix[4]arene (18)

1.52 g (2.39 mmol) of calix[4]arene **16** and 1.17 g (9.56 mmol) of phenylboronic acid **20a** were dissolved in the mixture of 60 ml of toluene and 10 ml of methanol. 2.54 g (23.98 mmol) of Na_2CO_3 were dissolved in 20 ml of water and added into the reaction flask: white precipitate formed. The reaction flask was flushed with argon, 10 mol% of $Pd(PPh_3)_4$ were added, and the reaction mixture was refluxed for 12 h. The solution was cooled to room temperature, neutralized carefully using 5% HCl, diluted with CH_2Cl_2 and then extracted with 5% HCl and water. Organic phase was separated, solvents were

removed, and the crude product was purified using column chromatography (SiO_2, pentane, CH_2Cl_2) to give 1.47 g (97%) of the title compound. ^{1}H NMR (250.13 MHz, $CDCl_3$) δ 7.61-6.59 (m, 20 H), 4.42 (d, *J*=12.9 Hz, 2H), 4.11-3.72 (m, 14H), 3.29-3.09 (m, 4H); ^{13}C NMR (62.90 MHz, $CDCl_3$) δ 157.71, 135.44, 134.56, 128-122 (m), 60.80, 36.12, 30.79.

5,17-Dibromo-25,27-bis-(ethoxycarbonylmethoxy)-calix[4]arene-26,28-diol (26)

To the solution of 6.00 g (10.0 mmol) of calix[4]arene **25** in 40 ml $CHCl_3$ were slowly added 3.10 g (20.0 mmol) of Br_2 in 20 ml of $CHCl_3$. Reaction mixture was stirred at RT for 3 h, and then $CHCl_3$ and residual Br_2 were removed. Product was recrystallized from $CHCl_3$ to give 7.54 g (100%) of the title product as a white powder. ^{1}H NMR (250 MHz, $DMSO_{d6}$) 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); (63 MHz, $DMSO_{d6}$) 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: calc for $C_{36}H_{35}O_8Br_2^+$ 753.0699, found 753.0740.

5,17-Di-(4-methoxyphenyl)-25,27-bis-(hydroxycarbonylmethoxy)-calix[4]arene-26,28-diol (27)

10 mg of $Pd(PPh_3)_4$ and 500 mg (0.716 mmol) of calix[4]arene **26** were dissolved in 15 ml of toluene and stirred under argon for 10 min, then 5 ml of 2M Na_2CO_3 and solution of 300 mg (2.148 mmol) of 4-methoxyphenylboronic acid **20b** in10 ml of CH_3OH were added. Reaction mixture was heated at 70-75°C for 12 h. Crude reaction mixture was neutralized with 5% HCl and extracted with $CHCl_3$. Organic phase was separated,

solvents were removed under vacuum and product was purified using column chromatography (SiO_2, pentane, CH_2Cl_2 and CH_3OH) to give 394 mg (73%) of the title product. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.35 (d, *J*=8.6 Hz, 4H), 7.24-7.03 (m, 8H), 6.89 (d, J=8.5 Hz, 4H), 6.75-6.66 (m, 2H), 4.90 (s, 4H), 4.52-4.31 (m, 4H), 3.79 (s, 6H), 3.56-3.49 (m, 4H).

Polyether calix[4]arene (28)

300 mg (0.399 mmol) of calix[4]arene **27** were dissolved in 15 ml of dry DMF, and 40 mg (1.000 mmol) of NaH were added to the solution. Reaction mixture was stirred at RT for 5 min, then 300 mg (1.100 mmol) of tetraethyleneglycol methylsulfonate ester were added, and the reaction mixture was heated at 70°C overnight. Solvent was removed under high vacuum, and product was purified using column chromatography (SiO_2, CH_2Cl_2 : EtOAc, EtOAc and EtOAc : CH_3OH). ^{1}H NMR (250.13 MHz, DMSO-*d6*) δ 7.50-6.50 (m, 18H), 4.80-4.20 (m, 10H), 4.10-3.80 (m, 2H), 3.76-3.73 (m, 6H), 3.55-3.40 (m, 28H, proton signals are overlapped by proton resonance of water), 3.21 (d, *J*=14.0 Hz, 4H).

Chapter 3

General Procedures ^{1}H and ^{13}C NMR spectra were obtained with a Bruker AC250 spectrometer operating at 250.13 MHz for proton and 62.90 MHz for carbon nuclei and a Bruker AMX400 spectrometer operating at 400.14 MHz for proton and 100.23 MHz for carbon nuclei. ^{1}H NMR chemical shifts were referenced to TMS (0.00 ppm) unless noted otherwise. ^{13}C NMR chemical shifts were reference to NMR solvent signals: CD_3CN (1.4 ppm), DMSO-*d6* (39.5 ppm), $CDCl_3$ (77.0 ppm), CD_3OD (49.0 ppm) unless noted otherwise.

The following are typical parameters used: NOESY were obtained at 298 K; mixing time = 150 and 300 ms; and FID acquisition time was 0.15 s. Other parameters were SW = 5800 Hz, 2 K data points and 512 increments each with 8 transients per FID. TOCSY experiments were run with a 300 ms mixing time, L1 = 118 and low power pulse = 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 was phased in the usual fashion.

Binding assays with calix[4]arenes **43** were performed by adding equivalents of calix[4]arene **43** to the 3 mM and 15 mM solutions of *N*-Ac-Gly-ONa, *N*-Ac-Phe-ONa, *N*-Ac-Tyr-ONa and *N*-Ac-Trp-ONa in DMSO-d_6 and DMSO-d_6/D_2O solutions. No associations were detected between calix[4]arene **43** and *N*-Ac-Tyr-ONa in 4/1 and 7/3 solutions of DMSO-d_6/D_2O at 3 mM concentrations of the guest. Some changes in proton signals for all amino acid carboxylates were observed in 100% DMSO-d_6 and 9/1 DMSO-d_6/D_2O solutions at 15 mM concentrations of the guests and 4 to 60 mM concentrations of calix[4]arene **43**. For calix[4]arene **45** and **46** hosts we observed

changes in amino acid proton signals even at 1 mM concentrations of guests in various DMSO-d_6/D_2O solutions, giving association constants $K_A>10^3$ M^{-1}. However, the high association constants make ^1H NMR titrations methods unsuitable for these particular compounds. Studies will be repeated using fluorescence titrations. In preliminary fluorescence titration studies association constant $K_A=1.6\pm1\cdot10^5$ M^{-1} was obtained for calix[4]arene host-[2]rotaxane **76** interactions with *N*-Ac-Trp-ONa in DMSO-d_6.

For the structural determination, the phenylalanine rotaxanes **78** and **79** were investigated in DMSO-d_6 as the monocarboxylate, obtained by the addition of one equivalence of Me_4NOH or LiOH to the solutions. Rotaxane **78**'s structure was also investigated with an increase in the pH of a 90/10 DMSO to H_2O solutions. The pH's of these solutions were set using a potentiometer.

For potentiometric titrations, 5 mM solutions of the compounds were prepared in freshly distilled DMSO and boiled water. These solutions were titrated under argon with a 0.10 M solution of Me_4NOH in DMSO. The pK_a values of the compounds were calculated using the BEST program.

Calculated Structural Determination. Global minimum structures of the phenylalanine rotaxanes were determined through molecular dynamic simulations using the MM+ force field as presented by Hyperchem (Hypercube, Inc. Gainesville, FA). 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 (RMS gradient of 0.005 kcal/(Å mol)). Most conformers minimized to a single conformer that was over 1 kcal/mol lower in energy than the other minimized structures.

Monitoring Association: Association of the phenylalanine rotaxane **78** with *N*-Ac-Arg-OMe was investigated in DMSO-d_6, DMSO-d_6/water solutions (proton resonance of water was suppressed in the experiments) 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 mM, 1.0 mM, 2.0 mM 3.0 mM, and 6.0 mM of rotaxane **78** in separate solutions. An appropriate amount of Me_4NOH was added to the rotaxane solutions to obtain the desired protonation state. Extent of complex formation was determined by monitoring the changes in the chemical shifts of amide proton of *N*-Ac-Arg-OMe.

Association of the rotaxane **79** with *N*-Ac-Ala-ONa and *N*-Ac-Phe-ONa was investigated in DMSO-d_6, DMSO-d_6/water solutions (proton resonance of water was suppressed in the experiments) at 25.0 ˚C. No shifts in the proton resonances for either component were observed for solutions (100% DMSO-d_6, 90/10, 60/40, and 40/60 DMSO-d_6 to H_2O) containing rotaxane **79** and amino acids when compared to solutions containing either the guests or host.

All syntheses were carried out under positive argon pressure. DMF was freshly distilled under vacuum over $MgSO_4$. THF was freshly distilled from sodium metal and benzophenone. Triethylamine and DIEA were distilled from CaH_2. K_2CO_3 was powderized and dried under vacuum for 1 h prior to use. All other solvents and reagents were used as purchased. Melting points were determined on a MEL-TEMP (Laboratory

Devices) apparatus and are uncorrected. HPLC analysis was performed on a Shimadzu 10A series HPLC using a C18 reversed phase column and water / CH_3CN as the eluent. Water for the HPLC assays was purified on a Millipore water purification system.

1.1 SYNTHESIS OF THE CALIX[4]ARENE SCAFFOLDS

5,17-Bis-[3-(3-amino-propionylamino)-phenyl]-25,27-bis-(ethoxycarbonylmethoxy)-calix[4]arene-26,28-diol (34)

0.61 g of calix[4]arene **40** were dissolved in 10 ml of CH_2Cl_2, and 4 ml of TFA were added. The reaction was stirred at RT for 1.5 h, solvent and TFA were removed under high vacuum. The cured product was neutralized with 2N NaOH. However, partial hydrolysis of the ethyl esters was observed. Calix[4]arene was re-esterified by refluxing in anhydrous ethanol in the presence of 1-2 drops of conc. HCl.

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.70 (s, 2H), 7.47 (d, *J*=7.7 Hz, 2H), 7.33-7.13 (m, 8H), 6.96 (d, *J*=7.5 Hz, 4H), 6.90-6.74 (m, 4H), 6.59 (d, *J*=7.5 Hz, 2H), 4.75 (s, 4H), 4.52 (d, *J*=13.1 Hz, 4H), 4.34 (q, *J*=7.0 Hz, 4H), 3.88 (s, 2H), 3.70 (br s, 2H), 3.46 (d, *J*=13.1 Hz, 4H), 3.03 (t, *J*=4.2 Hz, 4H), 2.45 (t, *J*=5.1 Hz, 4H), 1.36 (t, *J*=7.1 Hz, 6H).

5-[3-(3-Amino-propionylamino)-phenyl]-17-(3-aminophenyl)-25,27-bis-(methoxycarbonylmethoxy)-calix[4]arene-26,28-diol (35)

667 mg (0.700 mmol) of calix[4]arene **44** were dissolved in 10 ml $CHCl_3$ and 3 ml TFA were added. Reaction mixture was stirred at RT for 1h, solvent and TFA were removed and product was purified by column chromatography (SiO_2, $CHCl_3$, CH_3OH) to give 504

mg (84%) of the title compound. ^{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). TOF MS: calc for $C_{49}H_{46}N_3O_{11}^+$ 852.3132, found 852.3105.

5,17-Bis-(3-nitrophenyl)-25,27-bis-(hydroxycarbonylmethoxy)-calix[4]arene-26,28-diol (37)

1.13 g (1.50 mmol) of di-bromo calix[4]arene **26** and 1.00 g (5.99 mmol) of 3-nitro-phenylboronic acid were dissolved in 55 ml of toluene/methanol (9/1) solution. 1.11 g (10.49 mmol) of Na_2CO_3 were dissolved in 10 ml water and added to the reaction mixture, the flask was flushed with argon two times, and charged with 0.15 g (0.15 mol) of $Pd(PPh_3)_4$. The reaction was stirred at 70°C for 40 h. Organic phase was separated, diluted with 100 ml of CH_2Cl_2, and extracted with 5% HCl and water. Organic phase was separated, solvents were removed and the crude product was purified via column chromatography (SiO_2, $CHCl_3$, CH_3OH) to give 0.91 g (78%) of calix[4]arene **37** as a yellow solid. ^{1}H NMR (250 MHz, DMSO-*d6*) 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); (63 MHz, DMSO-*d6*) 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: calc for $C_{44}H_{35}N_2O_{12}^+$ 783.219, found 783.2256.

5,11-Bis-(3-nitrophenyl)-25,27-bis-(ethoxycarbonylmethoxy)-calix[4]arene-26,28-diol (38)

2.20 g (2.81 mmol) of calix[4]arene **37** were refluxed in the solution of C_2H_5OH and $CHCl_3$ (2:1) saturated with anhydrous HCl for 12 h. Solvents were removed, and a crude product was recrystallized from ethanol to give 1.95 g (89%) of the title compound as a bright yellow solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.37 (s, 2H), 8.12 (d, *J*=7.5 Hz, 2H), 8.06 (s, H), 7.84 (d, *J*=7.5 Hz, 2H), 7.54 (t, *J*=8.1 Hz, 2H), 7.33 (s, 4H) 7.02 (d, *J*=7.5 Hz, 4H), 6.83 (t, *J*=7.5 Hz, 2H), 4.78 (s, 4H), 4.57 (d, *J*=13.3 Hz, 4H), 4.37 (q, *J*=7.2 Hz, 4H), 3.51 (d, *J*=13.3 Hz, 4H), 1.38 (t, *J*=7.1 Hz, 6H).

5,11-Bis-(3-aminophenyl)-25,27-bis-(ethoxycarbonylmethoxy)-calix[4]arene-26,28-diol (39)

2.6 g (21.0 mmol) of Sn were partially dissolved in 13 ml of conc. HCl, then 10 ml of $CHCl_3/C_2H_5OH$ (1/1) and 1.84 g (2.19 mmol) of calix[4]arene **38** were added. The reaction mixture was refluxed for 6h, then it was cooled to RT and diluted with 20 ml of $CHCl_3$. Organic phase was separated and extracted with water, 0.1 N $NaCO_3$, and dried over $MgSO_4$. Under the reaction conditions a partial hydrolysis of the ethyl esters at the lover rim of the calix[4]arene **39** was observed. Therefore, the crude reaction products were refluxed in $CHCl_3$ and C_2H_5OH solution in the presence of conc. H_2SO_4 (2-3%) for 2-3h. Then solvents were removed to give 1.54 g (90%) of the title compound. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.75 (s, 2H), 7.25 (s, 4), 7.16 (t, *J*=7.5 Hz, 2H), 6.98-6.90 (m, 6H), 6.83 (s, 2H), 6.77 (t, *J*=7.5 Hz, 2H), 6.59 (d, *J*=8.2 Hz, 2H), 4.74 (s, 4H), 4,52 (d, *J*=13.1 Hz, 4H), 4.33 (q, *J*=7.1 Hz, 4H), 3.69 (br s, 4H), 3.45 (d, *J*=13.1 Hz, 4H), 1.35 (t, *J*=7.1 Hz, 6H); (63 MHz, $CDCl_3$) δ 168.68, 152.52, 152.30, 146.65, 142.31, 132.86, 132.40,

129.27, 129.16, 128.10, 127.09, 125.48, 116.96, 113.33, 113.09, 77.21, 72.36, 61.26, 31.54, 13.92.

5,17-Bis-[3-(3-*tert*-butoxycarbonylamino-propionylamino)-phenyl]-25,27-bis-(ethoxycarbonylmethoxy)-calix[4]arene-26,28-diol (40)

To the solution of 613 mg (3.24 mmol) of N-Boc-β-alanine in pyridine were added 801 mg (3.24 mmol) of EEDQ and 630 mg (0.81 mmol) of **39**. The reaction mixture was stirred at 70°C overnight. Solvents were removed in vacuo, crude reaction mixture was dissolved in CH_2Cl_2 and extracted with diluted HCl (pH=4). Calix[4]arene **40** was purified using rotary chromatography (SiO_2 plate, $CHCl_3$, CH_3OH) to give 610 mg (67%) of the title compound. . ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.52 (s, 2H), 7.86-7.52 (m, 6H), 7.29-7.20 (m, 6H), 7.06-6.96 (m, 6H), 6.78-6.73 (m, 2H), 5.40 (br s , 1H), 5.15 (br s, 1H), 4.76 (s, 4H), 4.53 (d, *J*=13.1 Hz, 4H), 4.33 (q, *J*=7.1 Hz, 4H), 3.48-3.37 (m, 8H), 2.65-2.64 (m, 2H), 2.55-2.48 (m, 4H), 1.43 (s, 18H), 1.35 (t, *J*=7.1 Hz, 6H).

5,17-Bis-(3-nitrophenyl)-25,27-bis-(methoxycarbonylmethoxy)-calix[4]arene-26,28-diol (41)

3.40 g (4.344 mmol) of calix[4]arene **37** were refluxed in the solution of $CHCl_3$ and CH_3OH (2/1) in the presence of conc. H_2SO_4 (2-3%) for 12 h. Upon solvent removal, 3.50 g (99%) of the title compound were obtained. ^{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: calc 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

2.00 g (2.467 mmol) of calix[4]arene **41** were dissolved in the 130 ml of $CHCl_3/CH_3OH/CH_3CO_2H$ (10/5/1). The flask was flushed with argon, charged with 100 mg of Pd/C, H_2 (50 Psi), and stirred at RT (21 h). The progress of reduction was monitored by TLC, and the reaction was ended, when ~80% of dinitro calix[4]arene **41** were consumed. The reaction mixture was filtered through a plug of diatomaceous earth, solvents were removed and the products were separated by column chromatography (SiO_2, $CHCl_3$ and CH_3OH); 0.23 g (0.284 mmol) of calix[4]arene **41** were recovered, 0.60 g (0.7684 mmol) of **42**, and 0.91 g (1.212 mmol) of **43** were obtained. Partial reduction resulted in compounds **41** : **42** : **43** in ratio 1 : 2.7 : 4.3 respectively, and provided 35.2% of calix[4]arene **42** and 55.5% of calix[4]arene **43** (yields are calculated based on the consumed calix[4]arene **41**).

5-(3-aminophenyl)-17-(3-nitrophenyl)-25,27-bis-(methoxycarbonylmethoxy)-calix[4]arene-26,28-diol (42)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, ½ H), 8.10 (d, J=8.0 Hz, ½ H), 8.01 (s, ½ H), 7.83 (d, *J*=7.5 Hz, ½ H), 7.75 (s, ½ H), 7.70-7.50 (m, 1½ 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: calc for $C_{46}H_{41}N_2O_{10}^+$ 781.2761, found 781.3635.

5,17-Bis-(3-aminophenyl)-25,27-bis-(methoxycarbonylmethoxy)-calix[4]arene-26,28-diol (43)

^{1}H NMR (250 MHz, $CDCl_3$) δ 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*=12.9 Hz, 4H), 3.87 (6H, s), 3.40-3.80 (bs, 2H), 3.45 (d, *J*=13.1 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+ calc. mass: $C_{46}H_{43}N_2O_8$ (MH^+) 751.3019, found: 751.3069.

5-[3-(3-*tert*-Butoxycarbonylamino-propionylamino)-phenyl]-17-(3-aminophenyl)-25,27-bis-(methoxycarbonylmethoxy)-calix[4]arene-26,28-diol (44)

To the solution of 436 mg (2.305 mmol) of Boc-ß-alanine in 20 ml of $CHCl_3$ were added 374 mg (2.305 mmol) of CDI. The reaction was stirred at RT for 1h, then 600 mg (0.768 mmol) of calix[4]arene **42** and 52 mg (0.768 mmol) of imidazole were added into the flask. The reaction was stirred at RT for 30 min, and then refluxed for 4 h. Solvents were removed in vacuo. Flash chromatography (SiO_2, $CHCl_3$, C_2H_5OH) afforded 703 mg (96%) of calix[4]arene **44** as a yellow solid. ^{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: calc for $C_{54}H_{54}N_3O_{13}^+$ 952.3657, found 952.3703.

1.2 SYNTHESIS OF THE DIBENZO[24]CROWN-8 DERIVATIVES

**** Syn and anti substituted DB24C8 were obtained and used without the separation of individual isomers. Only one of the isomers is shown in the reaction schemes.***

Di-(aminobenzo)[24]crown-8 (51)

100 mg (0.147 mmol) of **57** were dissolved in 2 ml of CH_2Cl_2 and 0.4 ml TFA. Reaction mixture was stirred at RT for 1h. Solvent and TFA were removed under high vacuum, the crude product was dissolved in CH_2Cl_2:CH_3OH (9:1) and extracted with 0.1 N $NaHCO_3$. Organic phase was separated and solvent was removed to give 68 mg (97%) of the title compound. ^{1}H 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: calc for $C_{24}H_{35}N_2O_8^+$ 479.2395, found 479.2560.

Di-[((Boc)$_3$-arginylamino)benzo][24]crown-8 (52)

DB24C8 was obtained upon condensation of amino DB24C8 **51** with CDI activated (Boc)$_3$ArgOH (for a procedure see the synthesis of crown ether **53**). Flash chromatography provided crown ether **52** in a 55% yield.

^{1}H 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). TOF MS: calc for $C_{66}H_{106}N_{10}O_{22}Na^+$ 1413.7381, found 1413.7466.

Di-[(*N*-Ac-(Boc)$_2$-arginylamino)benzo][24]crown-8 (53)

To the solution of 967 mg (2.321 mmol) of Ac(Boc)$_2$ArgOH in 20 ml of $CHCl_3$ were added 376 mg (2.321 mmol) of CDI. The reaction was stirred for 1h, and then 400 mg (0.835 mmol) of crown ether **51** were added. The reaction was continued for 24 h at RT, then the reaction mixture was extracted with small portions of 0.1% HCl (pH >5), and water. Flash column chromatography (Al_2O_3, CH_2Cl_2, CH_3OH) provided 737 mg (69%) of crown ether **53**. ^{1}H 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). TOF MS: calc for $C_{60}H_{94}N_{10}O_{20}Na^+$ 1297.6544, found 1297.7802.

Di-[(*N*-Ac -arginylamino)benzo][24]crown-8 (54)

200 mg of crown ether **53** were dissolved in 2-3 ml of CH_2Cl_2, and ~0.5 ml of TFA were added. The reaction was stirred at RT for 3 h, solvents were evaporated under high

vacuum. The crude product was contaminated with TFA, therefore, is was dissolved in methanol, and dried under high vacuum. This procedure was repeated 4-5 times to give pure by ^{1}H NMR title compound. HPLC purified samples were used for pK_a studies.

Di-[(2,2,2-trifluoro-acetylamino)-benzo][24]crown-8 (55)

To the solution of 89 mg (0.186 mmol) of DB24C8 **51** in 5 ml of $CHCl_3$ and 0.5 ml of pyridine were slowly added 0.3 ml of $(CF_3CO)_2O$. Reaction mixture was stirred at RT for 1h, solvents were removed under high vacuum, the crude product was dissolved in 20 ml of $CHCl_3$, and extracted twice with 10 ml of water and 10 ml of 1N HCl. Organic phase was separated and solvent was removed to give 124 mg (99%) of crown ether **55** (pure by ^{1}H NMR). ^{1}H NMR (250 MHz, DMSO-*d6*) 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-*d6*) 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: calc for $2\cdot(C_{28}H_{32}F_6N_2O_{10})Na^+$ 1363.3820, found 1363.3315.

Di-[(4-carboxy-butyrylamino)benzo][24]crown-8 (56)

To the solution of 60 mg (0.125 mmol) of crown ether **51** in 2 ml of $CHCl_3$ were added 43 mg (0.376 mmol) of glutaric anhydride and 52 µl (0.376 mmol) of Et_3N. The reaction was stirred at RT for 12 h, solvent and Et_3N were removed under vacuum, and crude product was extracted with CH_2Cl_2:CH_3OH/ 1N HCl. Organic phase was separated, solvents were removed under vacuum, and product was purified by column chromatography (SiO_2, CH_2Cl_2, CH_3OH) to give 69 mg (78%) of the title compound,

m.p. 156-158°C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.72 (s, 2H), 7.30 (s, 2H), 7.06 (d, *J*= 8.4Hz, 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, $CDCl_3$) δ 174.09, 170.21, 148.23, 144.18, 133.34, 114.63, 111.57, 106.33, 70.29, 69.20, 69.08, 68.65, 35.32, 32.20, 20.43. TOF MS: calc for $C_{34}H_{45}N_2O_{14}^-$ 705.2871, found 705.2898.

Di-(*tert*-butoxycarbonylaminobenzo)[24]crown-8 (57)

To a solution of 200 mg (0.37 mmol) of di-nitrobenzo[24]crown-8 **49** in 10 ml DMF were added 20mg of Pd/C and 800 mg (3.67 mmol) of di-*tert*-butyl dicarbonate. The mixture was stirred at room temperature under H_2 atmosphere (50 psi) for 2h. The solution was filtered through a plug of diatomaceous earth and solvent was removed under high vacuum. Purification by flash chromatography (SiO_2, ethyl acetate) provided 227 mg (90%) of the title compound as white powder, m.p. 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, 80.25, 72.53-68.52 (m), 28.35. TOF MS: calc for $C_{34}H_{50}N_2O_{12}Na^+$ 701.3261, found 701.3255.

1.3 SYNTHESIS OF THE TETHERS

5-(3,5-Di-tert-butyl-benzylamino)-pentanoic acid hydrochloride (59)

3.00 g (9.95 mmol) of 1-(3,5-di-*tert*-butyl-benzyl)-piperidin-2-one **58** were refluxed in 60 ml of 6N HCl for 24h. The solution was cooled to RT and extracted with CH_2Cl_2 (3x20ml). Organic phases were combined and extracted with 20 ml of H_2O, and then

solvent was evaporated to give pure by H^1 NMR product as light brown oil. Acid **59** was recrystallized from dioxane to give 3.12g (88.1%) of the title compound as a white powder. ^{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), 2.95-2.82 (m, 2H), 1.63-159 (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, 25.71, 21.85. TOF MS: calc for $C_{20}H_{34}NO_2^+$ 320.2591, found 320.2540.

5-(3,5-Di-tert-butyl-benzylamino)-pentanoic acid hexafluorophosphate (60) was obtained by extracting a suspension of hydrochloride salt **59** in ethyl ether with a solution of NH_4PF_6 in water. Ether layer was separated and dried under vacuum to provide acid **60** in an almost quantitative yield as a white solid (m.p. 154-155°C).

1-(3,5-dimethyl-benzyl)-piperidin-2-one (63)

2.00 g (20 mmol) of piperidine-2-one were dissolved in 10 ml of dry DMF, then 0.88 g (22 mmol) of NaH were added into the flask. The reaction was stirred at RT for 5-10 min, and then 4.00 g (20 mmol) of 3,5-dimethylbenzyl bromide were added. The reaction was stirred at RT for 2 h, the solvent was removed under high vacuum. An oily residue was dissolved in CH_2Cl_2 and extracted with 5% HCl, water and brine. The organic phase was dried under vacuum to provide 4.34 g (a quantitative yield) of the title compound. ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.90 (s, 1H), 6.86 (s, 2H), 4.52 (s, 2H), 3.18 (t, *J*=5.6 Hz, 2H), 2.45 (t, *J*=6.3 Hz, 2H), 2.29 (s, 6H), 1.79-1.76 (m, 4H).

5-(3,5-Dimethy-benzylamino)-pentanoic acid hydrochloride (64)

400 mg (1.8 mmol) of lactam **63** were refluxed in 6 N HCl for 24 h. Solvent was removed under vacuum, the residue was dissolved in CH_2Cl_2 and extracted with small amounts of water. Organic phase was dried over $MgSO_4$, and solvent was removed to give 150 mg (30%) of acid **64** as a white solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.16 (s, 2H), 6.98 (s, 1H), 4.03 (br s, 2H), 2.84-2.82 (m, 2H), 2.30 (s, 6H), 1.88-1.86 (m, 2H), 1.65-1.63 (m, 2H). TOF MS: calc for $C_{14}H_{22}NO_2^+$ 236.1651, found 236.1653.

1.4 SYNTHESIS OF CALIX[4]ARENE HOST-[2]ROTAXANES

Calix[4]arene di-[(*tert*-butoxycarbonylamino)-benzo][24]crown-8 host-[2]rotaxane (74)

400 mg (0.589 mmol) of di-(*tert*-butoxycarbonylaminobenzo)[24]crown-8 **57** and 275 mg (0.589 mmol) of 5-(3,5-Di-tert-butyl-benzylamino)-pentanoic acid hexafluoro phosphate **60** were dissolved in 3 ml of $CHCl_3$. The solution was cooled at -10°C (ice/salt bath) for 10 min, and 146 mg (0.707 mmol) of DCC in 0.5 ml of $CHCl_3$ were added. The reaction was stirred under argon atmosphere for 1.5 h, then 153 mg (0.442 mmol) of calix[4]arene **35** solution in 0.3 ml of $CHCl_3$ were added, and the reaction was continued at RT overnight. Solvent was evaporated, the oily residue was dissolved in CH_3CN, and DCU was filtered off. CH_3CN was evaporated; product was triturated with ethyl ether to remove cyclic tether **58**. Rotaxane **74** was separated from DB24C8 **57** using rotary chromatography (SiO_2, CH_2Cl_2, CH_3OH) to give 546 mg (67%) of the rotaxane. Using this procedure calix[4]arene rotaxane **74** is obtained in 30-70% yields. The outcome of the reaction is very sensitive to the purity of DB24C8 **57**. ^{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: calc for $C_{103}H_{127}N_{14}O_{24}^+$ 1831.8902, found 1831.8905.

Calix[4]arene di-(aminobenzo)[24]crown-8 host-[2]rotaxane (75)

480 mg (0.243 mmol) of calix[4]arene rotaxane **75** were dissolved in 10 ml CH_2Cl_2, and 3ml TFA were added. The reaction was stirred at RT for 3h, solvents were evaporated under high vacuum. The crude product was re-dissolved in ~10ml of CH_2Cl_2/CH_3OH (2/1) and solvents were removed under high vacuum several times. Then rotaxane **75** was dissolved in CH_2Cl_2/CH_3OH (9/1) and extracted with 0.1N $NHCO_3$. Organic phase was separated; solvent was removed to give 378 mg (89%) of the title product. Rotaxane **75** was used for the next reaction without further purifications. ^{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 –O<u>CH</u>$_3$, 24H DB24C8, 4H Ar-<u>CH</u>$_2$-Ar, 4H -<u>NH</u>$_2$), 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. δ TOF MS: calc for $C_{93}H_{111}N_6O_{20}^+$ 1631.7853, found 1631.7805.

Calix[4]arene di-[(*N*-Ac-arginylamino)benzo][24]crown-8 host-[2]rotaxane (76)

370 mg (0.227 mmol) of rotaxane **75** , 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, then 316 µl of DIEA were added to the solution. The reaction mixture was stirred at RT for 12 h. TLC analysis indicated incomplete conversion, therefore 50 mg (0.227 mmol) of BOP and Ac-Arg-OH·HCl were added. The reaction was continued for 12 h, then solvent and DIEA were removed under high vacuum, crude product was triturated with acetone/ether (1/1) to remove the DIEA·HCl, HMPA, and HOBt, which are soluble in the acetone/ether solution. The precipitate was dissolved in 20 ml of CH_2Cl_2:CH_3OH (8:2) and extracted with water. Organic phase was separated, solvents were removed, and product was purified by column chromatography to give 297 mg (66%) of rotaxane **76**. ^{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);

13C NMR (101 MHz, CDCl3) 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 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.

1.5 SYNTHESIS OF PHENYLALANINE [2]ROTAXANES

Phenylalanine methyl ester- di-(*tert*-butoxycarbonylaminobenzo)[24]crown-8 rotaxane (77)

350 mg (0.516 mmol) of di-(*tert*-butoxycarbonylaminobenzo)[24]crown-8 **57** and 240 mg (0.516 mmol) of 5-(3,5-Di-tert-butyl-benzylamino)-pentanoic acid hexafluoro phosphate **60** were dissolved in 3 ml of $CHCl_3$. The solution was cooled at -10°C (ice/salt bath) for 10 min, and 130 mg (0.631 mmol) of DCC in 0.5 ml of $CHCl_3$ were added. The reaction was stirred under argon atmosphere for 1.5 h, then 93 mg (0.516 mmol) of phenylalanine methyl ester solution in 0.3 ml of $CHCl_3$ were added, and the reaction was continued at RT overnight. Solvent was evaporated, the oily residue was dissolved in CH_3CN, and DCU was filtered off. CH_3CN was evaporated; product was re-dissolved in CH_2Cl_2, and extracted with 1% HCl to remove the excess of phenylalanine methyl ester. Product was purified using rotary chromatography (SiO_2, CH_2Cl_2, CH_3OH) to give 430 mg (72%) of the rotaxane, m.p. 104-105°C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.13 (s, 1H), 8.18 (d, *J*=7.6Hz, 1H), 7.30-7.15 (m, 10H), 6.88 (s, 4H), 4.55 (d, J=5.2, 2H), 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, 8H), 1.14 (s, 18H); ^{13}C NMR ($CDCl_3$) 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: calc. mass for $C_{64}H_{95}N_4O_{15}^+$ 1159.6794, found 1159.6783.

Phenylalanine di-[(4-carboxy-butyrylamino)-benzo][24]crown-8 rotaxane (78)

130 mg (0.106 mmol) of rotaxane **82** were dissolved in CH_3OH, and 1N LiOH was added till pH=10. Reaction mixture was stirred at RT for 4h, and crude product was analyzed by ^{1}H NMR (to verify that hydrolysis was completed, reaction was continued overnight and proton spectra were compared, indicating no changes). ^{1}H NMR (250 MHz, CD_3OD) δ 7.88-6.88 (m, 14H), 4.70 (s, 2H), 4.61-4.60 (m, ~ ½H), 4.45-4.44 (m, ~ ½H), 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 49.0ppm) 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, 127.29, 125.43, 124.36, 118.19, 113.86, 107.09, 71.83, 71.40, 69.68, 69.45, 55.16, 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: calc. for $C_{63}H_{89}N_4O_{17}^+$ 1173.6223, found 1173.6243.

Phenylalanine di-(*N*-acetyl-arginylamino-benzo)[24]crown-8 rotaxane (79)

100 mg (0.068 mmol) of rotaxane **83**·3HCl were dissolved in 2-3 ml THF, and 1N LiOH was added till pH=10-11. The reaction mixture was stirred at RT for 12 h. The hydrolysis was monitored by TLC (SiO_2, CH_2Cl_2, CH_3OH). Solvents were removed under high vacuum. The crude product was dissolved in CH_3OH, and insoluble LiCl was filtered off. The title compound (pure by ^1H NMR and ^{13}C NMR) was obtained in quantitative yield. TOF MS: calc for $C_{69}H_{105}N_{12}O_{15}^+$ 1341.7822, found 1341.7770.

Phenylalanine methyl ester di-(3-aminobenzo)[24]crown-8 rotaxane (81)

200 mg (0.167 mmol) of rotaxane **77** were dissolved in 2 ml of CH_2Cl_2, and 0.4 ml of TFA was added. Reaction mixture was stirred under argon at RT and monitored using TLC analysis. After 2h reaction was completed. Solvent and TFA were removed under high vacuum. The residue was several times re-dissolved in CH_3Cl/CH_3OH (9/1), and evaporated to remove the residual TFA. Then product was dissolved in CH_2Cl_2, extracted with 0.1M $NaHCO_3$, and dried over anhydrous Na_2SO_4. Upon solvent removal 172 mg (96%) of the title product were obtained, m.p. 56-58°C. ^1H NMR (400 MHz, DMSO-*d6*) δ 8.18 (d, *J*= 7.6Hz, 0.6 H), 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*=9.6Hz, *J*=13.6Hz, 1H), 1.90-1.85 (m, 2H), 1.80-1.40 (m, 4H), 1.32-1.05 (m, 18H); ^{13}C NMR 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, 38.49, 36.71, 34.27, 33.86, 31.11, 30.94, 25.29, 21.78. TOF MS: calc for $C_{54}H_{79}N_4O_{11}^+$ 959.5745, found 959.5749.

Phenylalanine methyl ester di-[(4-carboxy-butyrylamino)-benzo][24]crown-8 rotaxane (82)

To the solution of 150 mg (0.14 mmol) of amino rotaxane **81** in 3 ml of $CHCl_3$ were added 68 mg (0.60 mmol) of glutaric anhydride and 30 µl of Et_3N. The reaction was stirred at RT for 2h. Solvent was evaporated and an oily residue was triturated with 10 ml of Et_2O to remove the excess of glutaric anhydride and Et_3N. The solid residue was dissolved in CH_2Cl_2/CH_3OH (9/1) and extracted with 1N HCl. Organic phase was separated, solvent was removed and crude product was purified using column chromatography (SiO_2, CH_2Cl_2/CH_3OH) to give 130 mg (76%) of the title compound. ^{1}H NMR (250 MHz, $DMSO_{d6}$) 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, 19H+water), 2.99 (dd, *J*=5.6 Hz, *J*=13.6 Hz, 1H), 2.84 (dd, *J*=9.2Hz, *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 (63 MHz, CD_3CN, reference 1.32 ppm) δ 174.86, 174.39, 173.11, 172.07, 160.76, 160.17, 153.80, 152.28, 148.32, 144.83, 138.09, 134.06, 132.92, 130.26, 129.42, 127.77, 125.15, 124.52, 122.74, 114.79, 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: calc for $C_{64}H_{91}N_4O_{17}^+$ 1187.6379, found 1187.6388.

Phenylalanine methyl ester di-(*N*-Ac-arginylamino-benzo)[24]crown-8 rotaxane (83)

200 mg (0.203 mmol) of amino-rotaxane **81**, 360 mg (0.812 mmol) of BOP and 176 mg (0.812 mmol) of AcArgOH·HCl were dissolved in 3ml of DMF, then 353 µl of DIEA were added, and reaction mixture was continued at RT for 24 h. DMF and excess of DIEA were removed under high vacuum, and an oily residue was extracted: CH_2Cl_2:CH_3OH (8:2)/H_2O. Organic phase was separated and rotaxane was purified using rotary chromatography (SiO_2, CH_2Cl_2, CH_3OH, 1% TFA was added to CH_3OH to remove rotaxane **83** from the plate) to give 153 mg (56%) of the title product. ^{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, 22H); ^{13}C NMR (63 MHz, CD_3OD, reference 39.50ppm) δ 172.05, 171.57, 170.17, 169.00, 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, 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: calc for $C_{70}H_{107}N_{12}O_{15}^+$ 1355.7980, found 1355.8617.

1.6 SYNTHESIS OF THE AXLE 80

2-[5-(3,5-Di-*tert*-butyl-benzylamino)-pentanoylamino]-3-phenyl-propanoic acid hydrochloride (80)

The solution of 440 mg (0.740 mmol) of methyl ester **86** in 10 ml of THF was added 1N LiOH (pH=10). The reaction was stirred at RT for 2h. TLC analysis indicated completed hydrolysis. Solvents were removed under high vacuum, and the residue was extracted CH_2Cl_2 / 1N HCl. Organic phase was separated and dried under high vacuum to give 356 mg (96%) of the title compound, m.p. 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: calc for $C_{29}H_{43}N_2O_3^+$ 467.3275, found 467.3319.

5-[*tert*-Butoxycarbonyl-(3,5-di-*tert*-butyl-benzyl)-amino]-pentanoic acid (84)

To the solution of 320 mg (0.989 mmol) of 5-(3,5-di-*tert*-butyl-benzylamino)-pentanoic acid **59** in 30 ml THF were added 2 ml 2N NaOH and 240 mg (1.1 mmol) of di-*tert*-butyl dicarbonate. Reaction was stirred at RT for 1h. Solvents were removed under vacuum and oily residue was dissolved in 30 ml of ethyl acetate and extracted with 1N $NaHCO_3$ and 1% HCl. Ethyl acetate was removed under high vacuum to give **84** in a quantitative yield as transparent oil. ^{1}H NMR (400MHz, $CDCl_3$) 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, 34.73, 33.62, 31.43, 28.44, 27.38, 21.94. MS: calc for $C_{25}H_{40}NO_4^-$ 418.2957, found 418.2963.

2-{5-[*tert*-Butoxycarbonyl-(3,5-di-*tert*-butyl-benzyl)-amino]-pentanoylamino}-3-phenyl-propanoic acid methyl ester (85)

360 mg (0.858 mmol) of 5-[tert-Butoxycarbonyl-(3,5-di-tert-butyl-benzyl)-amino]-pentanoic acid **84** were dissolved in 5 ml of CH_2Cl_2 and activated with 153 mg (0.944 mmol) of CDI for 20 min. Then the solution of 204 mg (0.944 mmol) of phenylalanine methyl ester hydrochloride **72** and 65 mg (0.944 mmol) of imidazole in 5 ml CH_2Cl_2 was added. The reaction was stirred at RT overnight, then it was diluted with 20 ml of CH_2Cl_2 and extracted with 10 ml of 1% HCl. Organic phase was separated, and solvent was removed to give pure by ^{1}H NMR product as a transparent oil in a quantitative yield. ^{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 (63 MHz, $CDCl_3$) 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: calc for $C_{35}H_{53}N_2O_5^+$ 581.3954, found 581.4000.

2-[5-(3,5-Di-*tert*-butyl-benzylamino)-pentanoylamino]-3-phenyl-propionic acid methyl ester (86)

500 mg (0.861 mmol) of compound **85** were dissolved in 5 ml of $CHCl_3$/TFA (80/20) and stirred at room temperature for 1h. Solvents were removed under high vacuum and product was extracted CH_2Cl_2/H_2O. Organic phase was separated and dried under high vacuum to give 423 mg (85%) of the product as a transparent oil. ^{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 (63 MHz, $CDCl_3$) δ 173.20, 172.10, 152.02, 135.86, 129.71, 129.15, 128.69, 127.23, 123.00, 123.49, 53.50, 52.43, 52.02, 45.95, 37.67, 34.47, 31.27, 25.32, 20.87. TOF MS: calc for $C_{30}H_{45}N_2O_3^+$ 481.3430, found 481.3333.