Pergamon

Tetrahedron 57 (2001) 9555–9561

TETRAHEDRON

Condensation reactions of calix[4]arenes with unprotected hydroxyamines, and their resulting water solubilities

Inese Smukste and David B. Smithrud*

Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221-0172, USA

Received 16 July 2001; accepted 18 September 2001

Abstract—In this report, we describe a convenient method to create neutrally charged, water soluble calix[4]arenes that contain hydroxyamides attached to their lower rims. Selective amidation reactions of a diacid calix[4]arene with several unprotected hydroxyamines was achieved using 2-ethoxy-1,2-dihydroquinoline 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 10 hydroxyl groups were as soluble in water as a calix[4]arene that contained two carboxylates.

1. Introduction

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[9] 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 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.

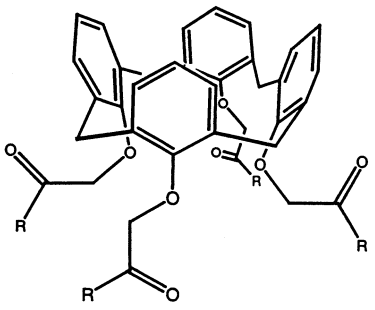
The main purpose of this project was to develop a general method for selectively attaching unprotected polyhydroxyamines to calix[4]arenes through amide bond formation. Loss of material and time from performing protection and deprotection steps would thus be avoided. Conventional wisdom would suggest that the greater number of hydroxyl groups added to a calixarene, the more water-soluble it would become. We determined whether this statement is true by systematically adding hydroxyl groups to a calix[4]arene and measuring its solubility in water and in a 95:5 (v/v) water/DMSO mixture. Hydroxyamines were chosen because many are commercially available, inexpensive, and highly soluble in water. 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 polyhydroxyamines can be added to carboxylic acids positioned at the lower rim of calix[4]arenes without undergoing rearrangements to the esters.

2. Results and discussion

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 **1a**.[5c] Addition of TRIS to the tetra-acyl chloride calix[4]arene **1b** resulted in multiple products as determined by HPLC analysis. There were many more compounds than the expected mono-, di-, tri-, and

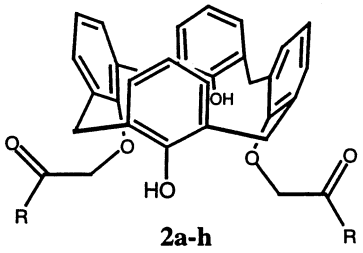
Keywords: calixarenes; solvents; molecular modeling.
* Corresponding author. Fax: +1-513-556-9239;
e-mail: david.smithrud@uc.edu

0040–4020/01/$ - see front matter
PII: S0040-4020(01)00965-6

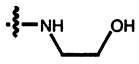
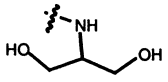
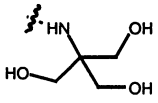
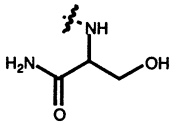
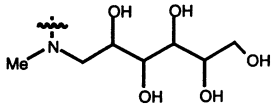
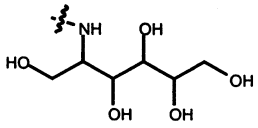
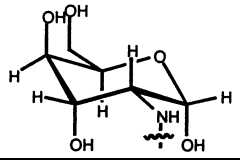


1 a, R = OH
b, R = Cl
c, R = OMe

Table 1. Yields of the condensation reactions of **2a** with hydroxyl amines



2a-h

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

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

tetra-amides. Apparently, acyl chlorides are too reactive, giving a mixture of calix[4]arenes that have various combinations of amides and esters. Attempts to convert methyl esters to amides by refluxing calix[4]arene **1c** 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 to amidate benzoic acids with unprotected TRIS.[12] EEDQ also 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 **1a** 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 calixarenes. Because the di-amides were the major products of the amidation reactions of tetra-acid calix[4]arene **1a**, we assumed that higher yields of the di-amide product would be obtained by using the less hindered diacid calix[4]arene **2a** (Table 1).[5d]

Condensation reactions between calix[4]arene **2a** and ethanol amine, serinol, TRIS, and serine amide in the presence of EEDQ produced calix[4]arene diamides **2b**–**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 unreacted 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 **2e** required the solution to be kept below refluxing temperature (ca 90°C); serine amide appears to decompose with high heat. Furthermore, deprotonation of the starting serine amide salt required the addition of 1 equiv. of KOH (but not NaOH). Counter ion selectivity has been previously observed in calixarene reactions.[13] Another caveat should be mentioned

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

Calix[4]arene	Water[a]	Water/DMSO[b]
2a	3.9±0.1	7.0±0.1
2b	<0.02	<0.02
2c	0.36±0.04	1.49±0.08
2d	0.18±0.03	0.7±0.1
2e	1.60±0.05	3.5±0.4
2f	2.9±0.2	5.2±0.5
2g	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.

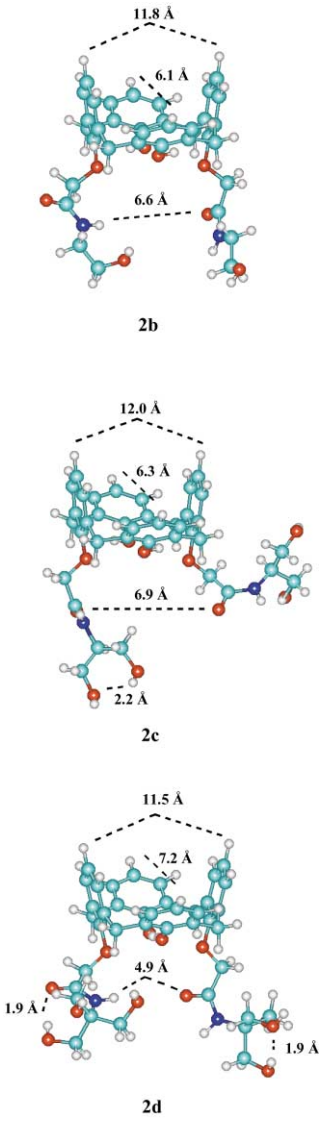


Figure 1. Equilibrium structures of calix[4]arenes **2b**–**d** as determined by molecular dynamic simulations. Dotted lines indicate key interaction distances between functional groups. Weaker interactions between the functional groups of serinol-calix[4]arene **2c** as compared to TRIS-calix[4]-arene **2d**, as indicated by $(C{=})O_{amide}\cdots H(-N)_{amide}$ distances (6.9 and 4.9 Å, respectively), most likely gives calix[4]arene **2c** greater water solubility. Distances between the hydrogen atoms at the *para* positions depend on whether the phenol is functionalized.

for the isolation of the TRIS-calix[4]arene **2d**. 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 **2d** was purified by recrystalization. The other hydroxyamides did not rearrange 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 **2a** produced moderate yields (ca 50%) of the derivatized calix[4]arenes **2f** and **2g**, respectively. These reactions required longer reaction times, the addition of 1 equiv. of $(i\text{Pr})_2\text{EtN}$ 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 **2a** to give calix[4]arene **2h**. We should note that the reaction 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 **2f** 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 calixarene **2g**. 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. Other conformers most likely are 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 have 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 **2b**) did not increase its solubility to any great extent. With four hydroxyl groups added (serinol-calixarene **2c**), the calix[4]arene became soluble in water to about one tenth as soluble as the dicarboxylate salt of calix[4]arene **2a**. But TRIS-calixarene **2d**, with a total of six hydroxyl groups, gave a less water-soluble calixarene than the serinol-calixarene **2c**.

To understand this reversal in solubility, molecular modeling was performed using CVFF forcefield (as presented by Discover), which was shown 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 hydroxyamide was extensively H-bonded to the phenolic moieties of the calix[4]arene, whereas the other was non bonded except for one H-bond ($(C{=})O_{amide}\cdots H(-N)_{amide}$) between the two hydroxyamides. Hydrated calix[4]arenes showed, not surprisingly, that the addition of water disrupted most intramolecular H-bonds (Fig. 1). The structures of calixarenes **2b–d** were similar except that a greater intramolecular network of H-bonds existed for the TRIS-calixarene **2d**. The $(C{=})O_{amide}\cdots H(-N)_{amide}$ interaction distance was shorter for the TRIS-calixarene **2d** than the serinol-calixarene **2c** and ethanolamine-calxiarene **2b** (5.0, 6.9, and 6.2 Å, respectively). Apparently, weaker interactions between the amides of serinol-calixarene **2c** exposes them more to the bulk water as compared to TRIS-calixarene **2d**, which results in a greater water solubility of serinol-calixarene **2c**. Calixarenes **2c** and **2d** also had a single short H-bond between the hydroxyl groups. Only the TRIS-calixarene **2d** 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 reduces 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 Ar*C*H_2Ar in ^{13}C NMR spectra (ca 31 ppm) confirms that all calix[4]arenes (**1a–c**, **2a–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 ArC*H*$_2$Ar protons. Calix[4]arenes **2b–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 (**2f**,**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.

Once ten hydroxyl groups are added to a calix[4]arene (calix[4]arenes **2f** and **2g**), the water solubility approximately matches that of the di-sodium salt of calixarene **2a**. *N*-methyl-D-glucamine and 2-amino-2-deoxy-D-glucitol also form intramolecular H-bonds, but the large number of hydroxyl groups accounts for the greater water solubility. According to the solubility data (Table 2), a single amide (serinamide-calix[4]arene **2e**) proved to be approximately equivalent to four hydroxyl groups (calix[4]arenes **2f** and **2g**) in terms of solubility. The high water solubility of serinamide-calix[4]arene **2e** 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 fifteen times more water soluble than one containing an hydroxyl group ($\pi_{Asn}=-0.60$ and $\pi_{Ser}=-0.04$, out of a total range of $\delta\pi=1.81$ for the natural amino acids). Serinamide-calix[4]arene **2e** is not 15 times more soluble than serinol-calix[4]arene **2b**, because it also had an extensive H-bonded network in water.

3. Conclusion

Condensation reactions between unprotected hydroxyamines and diacid calix[4]arene **2a** 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 gave lower yields. The solubilities of the hydroxyamide derivatized calix[4]arenes depended 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. The highest solubility in water (ca 3 mM) was found for calix[4]arenes containing 10 hydroxyl groups. This value approximately doubles with the addition of 5% (v/v) of DMSO. These values closely match the solubility of a calix[4]arene that is derivatized with two carboxylates.

4. Experimental

4.1. General procedures

^1H and ^{13}C NMR spectra were obtained with a Bruker AC250 spectrometer. ^1H 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 are uncorrected. HPLC grade pyridine was purchased from Aldrich and distilled over CaH_2. All reagents were purchased from Aldrich and utilized without further purification. All reactions were done under an argon atmosphere.

4.2. 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 **2b–e** were solvated by placing them in a 20×20×20 Å periodic box (ca 220 water molecules, 300 K, 1 atm). Complete solvation of calix[4]arenes **2f**,**g** required a box with dimensions of 25×25×25 Å (ca 440 water molecules). Non-bonded interactions were

limited by a switched cutoff of 9.8 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.

4.3. 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 containing water, 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 $A = \varepsilon lc$ 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.

4.3.1. 25,26,27,28-Terakis(methoxycarbonylmethoxy)-calix[4]arene (1c). Calix[4]arene (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 equiv. 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 (2×100 mL). The organic phases were collected, dried over $MgSO_4$, and the solvent was removed in vacuo. Calix[4]arene **1c** was purified by recrystalization from EtOH to give 10.0 g (14.0 mmol, 80% yield) of white crystals. Mp 138–139°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+ calcd mass: $C_{40}H_{40}O_{12}Na^+$ 735.2417, found: 735.2377.

4.3.2. 25,27-Bis[*N*-(2-hydroxyethyl)aminocarbonylmethoxy]-calix[4]arene-26,28-diol (2b). Diacid calix[4]arene **2a**[5d] (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 **2b** as an off-white powder, which was pure by ^{1}H NMR analysis ($> 95\%$) : mp 274–275°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 Hz), 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+ calcd 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.

4.3.3. 25,27-Bis[*N*-(2-hydroxy-1-hydroxymethyl-ethyl)-aminocarbonylmethoxy]-calix[4]arene-26,28-diol (2c). Diacid calix[4]arene **2a** (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 **2c** precipitated to give 0.216 g of a white powder (0.31 mmol, 83% yield): mp 233–234°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+ $C_{38}H_{43}N_2O_{10}$ $(M+H)^+$ calcd 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.

4.3.4. 25,27-Bis[*N*-(2-hydroxy-1,1-bishydroxymethyl-ethyl)aminocarbonylmethoxy]-calix[4]arene-26,28-diol (2d). Diacid calix[4]arene **2a** (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 **2d** as a white powder (0.33 mmol, 90% yield): mp 225–227°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+ $C_{40}H_{46}N_2O_{12}Na^+$ calcd 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.

4.3.5. 25,27-Bis[*N*-(1-amido-2-hydroxyethyl)aminocarbonylmethoxy]-calix[4]arene-26,28-diol (2e). 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 **2a** (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°C for 20 h. Solvents were removed in vacuo, and calix[4]arene **2e** 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°C (begins to darken around 191°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$^+$ $C_{38}H_{41}N_4O_{10}$ $(M+H)^+$ calcd 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.

4.3.6. 25,27-Bis[*N*-methyl-*N*-(2,3,4,5,6-pentahydroxyhexyl)-aminocarbonylmethoxy]-calix[4]arene-26,28-diol (2f). Diacid calix[4]arene **2a** (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°C for 4 days. 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 **2f** as an off-white solid (0.27 mmol, 49% yield): 130°C (dec.). ^{1}H NMR δ 3.13–3.47 (m, signals are overlapped by CH_3OH), 3.68–3.81 (14H, 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$^+$ $C_{46}H_{58}N_2O_{16}Na^+$ calcd 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.

4.3.7. 25,27-Bis[*N*-(2,3,4,5-tetrahydroxy-1-hydroxymethyl-pentyl)-aminocarbonylmethoxy]-calix[4]arene-26,28-diol (2g). Diacid calix[4]arene **2a** (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°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 **2g** as a white powder (0.27 mmol, 48% yield): mp 202–203°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$^+$ $C_{44}H_{55}N_2O_{16}$ $(M+H)^+$ calcd 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.

Acknowledgements

The authors thank the University of Cincinnati for its generous funding of this project. Smukste was supported by the University of Cincinnati Research Council Fellowship and the Stecker Fellowship.

References

1. (a) Gutsche, C. D. In *Calixarenes*; Stoddart, F. J., Ed.; *Monographs in Supramolecular Chemistry*; The Royal Society of Chemistry: Imperial College, 1989. (b) *Calix[4]arenes a Versatile Class of Macrocyclic Compounds*, Vincens, J., Bohmer, V., Eds.; *Topics in Inclusion Science*, Kluwer Academic: Dordrecht, 1991. (c) Gutsche, C. D. In *Calix[4]-arenes Revisited*; Stoddart, F. J., Ed.; *Monographs in Supramolecular Chemistry*; The Royal Society of Chemistry: Imperial College, 1998. (d) *Calix[4]arenes for Separations*, Lumetta, G. J., Rogers, R. D., Gopalan, A. S., Eds.; *ACS Symposium Series 757*, American Chemical Society: Washington DC, 2000. (e) *Calix[4]arenes in Action*, Mandolini, L., Ungaro, R., Eds.; Imperial College, 2000.
2. (a) Ref. 1b, part three (pp 127–171, 211–215). (b) Ref. 1c, chapter 6.1–6.3. (c) Ref. 1d, chapters 2–17.
3. (a) Ref. 1c, chapter 6.6. (b) Ref. 1d, chapters 20–24. (c) Ref. 1e, chapter 3.
4. (a) Hamuro, Y.; Calama, M. C.; Park, H. S.; Hamilton, A. D. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2680–2683. (b) Park, H. S.; Lin, Q.; Hamilton, A. D. *J. Am. Chem. Soc.* **1999**, *121*, 8–13.
5. (a) Arena, G.; Contino, A.; Gulino, F. G.; Magrì, A.; Sansone, F.; Sciotto, D.; Ungaro, R. *Tetrahedron Lett.* **1999**, *40*, 1597–1600. (b) Arena, G.; Casnati, A.; Contino, A.; Sciotto, D.;

Ungaro, R. *Tetrahedron Lett.* **1997**, *38*, 4685–4688. (c) Casanti, A.; Ting, Y.; Berti, D.; Fabbi, M.; Pochini, A.; Ungaro, R.; Sciotto, D.; Lombardo, G. G. *Tetrahedron* **1993**, *49*, 9815–9822. (d) Arena, G.; Casnati, A.; Mirone, L.; Sciotto, D.; Ungaro, R. *Tetrahedron Lett.* **1997**, *38*, 1999–2002.

6. Almi, M.; Adruini, A.; Casanti, A.; Pochini, A.; Ungaro, R. *Tetrahedron* **1989**, *45*, 2177–2182.
7. (a) Shinkai, S.; Shirahama, Y.; Tsubaki, T.; Manabe, O. *J. Am. Chem. Soc.* **1989**, *111*, 5477–5478. (b) Shinkai, S.; Shirahama, Y.; Tsubaki, T.; Manabe, O. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1859–1860. (c) Arimori, S.; Nagasaki, T.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 2* **1995**, 679–683.
8. Grote Gansey, M. H. B.; Steemers, F. J.; Verboom, W.; Reinhoudt, D. N. *Synthesis* **1997**, 643–648.
9. (a) Dondoni, A.; Marra, A.; Scherrmann, M-C.; Casanti, A.; Sansone, F.; Ungaro, R. *Chem. Eur. J.* **1997**, *3*, 1774–1782. (b) Marra, A.; Dondoni, A.; Sansone, F. *J. Org. Chem.* **1996**, *61*, 5155–5158.
10. (a) Zhang, Y. L.; Shen, H. X.; Liu, Y.; Zhang, C. X.; Chen, L. X. *Anal. Lett.* **2000**, *33*, 831–845. (b) Hendel, R. A.; Zhang, L. H.; Janout, V.; Conner, M. D.; Hsu, J. T.; Regen, S. L. *Langmuir* **1998**, *14*, 6545–6549. (c) Jin, T.; Kinjo, M.; Kobayashi, Y.; Hirata, H. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 3135–3140.
11. Haino, T.; Rudkevich, D. M.; Rebek, Jr., J. *J. Am. Chem. Soc.* **1999**, *121*, 11253–11254.
12. Hernandez, B. A.; Chang, V.; Villanueva, I.; Heagy, M. D. *J. Org. Chem.* **1999**, *64*, 6905–6906.
13. (a) Ref. 1c, chapter 4.6. (b) Verboom, W.; Datta, S.; Asfari, Z.; Harkema, S.; Reinhoudt, D. N. *J. Org. Chem.* **1992**, *57*, 5394–5398.
14. Kley, J. T.; Kiedrowski, G. V.; Unger, C.; Massing, U. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 261–264.
15. Moran, J. K.; Georgiev, E. M.; Yordanov, A. T.; Mague, J. T.; Roundhill, D. M. *J. Org. Chem.* **1994**, *59*, 5990–5998.
16. Jaime, C.; de Mendoza, J.; Prados, P.; Nieto, P. M.; Sánchez, C. *J. Org. Chem.* **1991**, *56*, 3372–3376.
17. Fauchère, J.-L.; Charton, M.; Kier, L. B.; Verloop, A.; Pliska, V. *Int. J. Pept. Protein Res.* **1988**, *32*, 269–278.