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Synthesis of New Trisulfonated Calix[4]arenes Functionalized at the Upper Rim, and Their Complexation with the Trimethyllysine Epigenetic Mark

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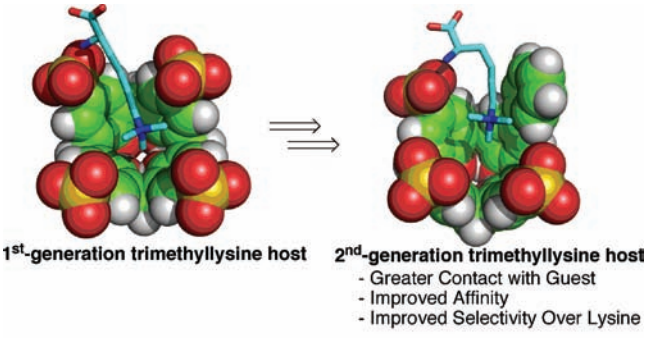
ABSTRACT



A synthetic route to produce a new family of trisulfonated calix[4]arenes bearing a single group, selectively introduced, that lines the binding pocket is reported. Ten examples, including new sulfonamide and biphenyl-substituted hosts, each with additional binding elements, demonstrate the tuning of guest affinities and selectivities. NMR titrations in phosphate-buffered water show that one of the new hosts binds to the modified amino acid trimethyllysine with the highest affinity and selectivity observed to date.

p-Sulfonatocalix[4]arene (PSC, **1**) is a macrocyclic host that has received much attention due to its ability to complex with biologically important guests in aqueous solution.[1−6] Recently we have shown that **1** strongly binds the biologically important post-translationally modified amino acid, trimethyllysine.[7] The trimethyllysine mark is an important signaling site that triggers protein−protein interactions and a variety of downstream cellular events related to gene regulation.[8−10]

Our goal at the outset of this work was to modify the calix[4]arene skeleton to provide a functional handle for synthetic tuning of the affinities for selected biological partners. Previous work in our laboratory (unpublished) indicated to us that synthetic modifications made to the lower rim of **1**[11,12] offer little ability to control the binding of biological partners due to the distance of these modifications from the binding pocket. Despite the dominant position of sulfonated calixarenes among water-soluble supramolecular hosts,[1] we found that examples bearing modifications on the upper rim such as the mono-substituted, trisulfonated calix[4]arenes exemplified by **2** (Figure 1) have not been prepared synthetically or explored

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as supramolecular hosts. Recent work has highlighted the importance of selective functionalization of a variety of other macrocylic systems, such as calixpyrroles and cyclodextrins, in the tuning of binding properties and development of new technologies.[13,14]

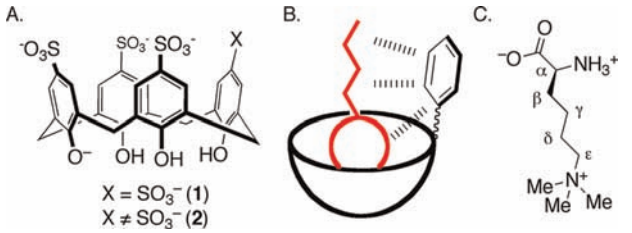
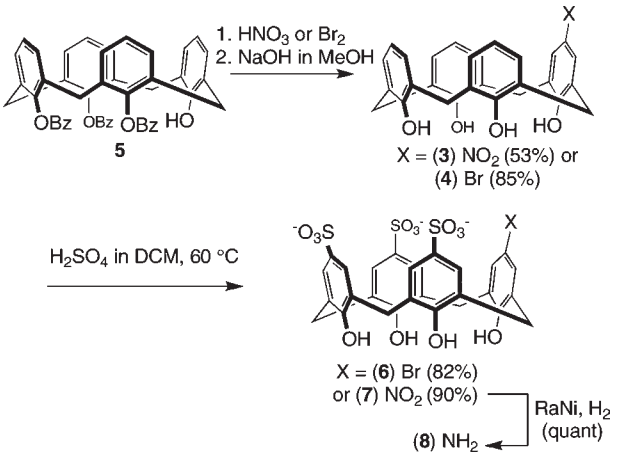


Figure 1. (A) Host, **1**, and exemplary trisulfonate, **2**. (B) Schematic showing potential for additional interactions between host and guest in generic structures such as **2**. (C) Trimethyllysine.

Previous work toward these desymmetrized synthetic modifications to the upper rim of a calix[4]arene scaffold led to compounds **3**[15,16] and **4**[17] (Scheme 1). Compounds **3** and **4** are both accessed from tribenzoylester calix[4]arene, **5**. Nitration of **5** using nitronium tetrafluoroborate and nitric acid was explored, and both supplied **3** in moderate yields. Bromination of **5** proceeded smoothly using Br_2 under literature conditions[17] yielding compound **4**. Our next step was to explore sulfonation conditions starting from the reported calix[4]arenes **3** and **4**. Following literature procedures[18,19] to chlorosulfonate **3** with chlorosulfonic acid followed by hydrolysis to the sulfonate led to an inseparable mixture of decomposition products. Next, we attempted treatment with neat sulfuric acid[20] followed by recrystallization from brine[21] but were unable to isolate product from the solution. One commonly observed problem was *ipso*-sulfonation which displaced the newly installed nitro group to supply symmetric **1** as the major product isolated by HPLC. To address these problems we sought a sulfonation condition that could be applied to both **3** and **4** and that would avoid *ipso*-sulfonation, be amenable to larger scale reactions, and would not require purification. These requirements were met by sulfonation using neat sulfuric acid added to dichloromethane heated at 60 °C; treatment for 1 h for **3** and 3 h for **4** provided clean products that precipitated directly out of the reaction mixtures and were isolated by centrifugation. Subsequent treatment of **7** with Raney nickel under a H_2 atmosphere supplied amino-intermediate **8** in quantitative yield and high purity after filtration and lyophilization of the reaction.

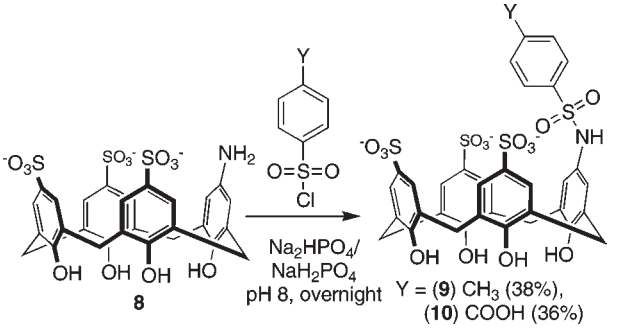
With **6** and **8** in hand we proceeded to develop synthetic conditions to provide additional functionality for contacting our intended guest, trimethyllysine. From **8** we envisioned reaction with sulfonyl chlorides to supply a new set of sulfonamide-containing hosts. Initial coupling conditions consisted of reacting calix[4]arene **8** with an appropriate sulfonyl chloride in DMF and pyridine.

Scheme 1. Synthesis of Trisulfonated Intermediates



While these conditions did provide small amounts of product (isolable by HPLC), useful quantities were not obtained. The low nucleophilicity of the anilino nitrogen atom in **8** led to frequent recovery of unreacted starting material under a large variety of typical reaction conditions used for making sulfonamides. We found that conducting the sulfonamide formation step in 1 M sodium phosphate buffer (pH 8) supplied **9** and **10** in reasonable yields after purification by HPLC (Scheme 2).

Scheme 2. Synthesis of Sulfonamide Calix[4]arenes, **9** and **10**



Aryl bromide **6** was intended to be a precursor for Suzuki couplings to furnish a variety of biphenyl-functionalized sulfonated calix[4]arene hosts. Numerous standard Suzuki reaction conditions were attempted with $PhB(OH)_2$, including using H_2O mixed with DME,

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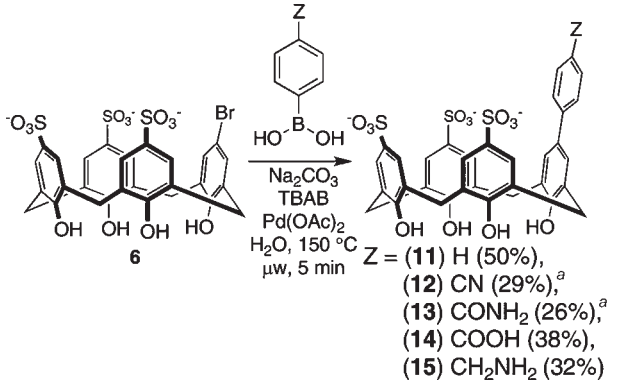
THF, or dioxane as the solvent; $Pd(PPh_3)_4$ or $Pd(OAc)_2$ in the presence of added phosphine ligands (including "Buchwald" ligands such as S-phos) as the catalyst; Na_2CO_3, K_2CO_3, or K_3PO_4 as the base; and varied temperatures and times of reaction. All such conditions provided little or no detectable product, with yields < 5%. Finally, we found $Pd(OAc)_2$, $PhB(OH)_2$, and Na_2CO_3 in water under microwave irradiation supplied **11** in 50% yield after only 5 min[22] (Scheme 3).

These reaction conditions also worked for a variety of aryl boronic acids (compounds **12**–**15**), with yields ranging from 38 to 50% after HPLC purification. The reaction of 4-cyanophenylboronic acid under these conditions generated two products, the desired cyano product (**12**), and its partially hydrolyzed primary amide analog (**13**), which were isolated from a single HPLC purification run in 29% and 26% yields, respectively.

Scheme 3. Synthesis of Extended Biphenyl Calix[4]arenes, **11**–**15**[a]



[a] These two products arise from a single reaction. See text.

NMR titrations were carried out to determine the affinities of the new family of functionalized trisulfonated calix[4]arene hosts for trimethyllysine, as well as their selectivity over unmethylated lysine. Solutions were prepared by dissolving the amino acid in pure, buffered D_2O (40 mM Na_2HPO_4/NaH_2PO_4, pD 7.0 = pH 7.4[23]) to create the receiving phase and adding solid calixarene to a portion of the same solution to create a titrant solution that was perfectly matched in terms of buffer, pH, and amino acid concentrations to the receiving phase. Addition of the titrant solutions caused changes in the chemical shifts of amino acid guests that were fit to 1:1 binding isotherms in order to provide K_{assoc} values for each host–guest pair (Table 1).

The hosts **6**, **7**, and **8**, which have had one sulfonate removed and replaced by a neutral heteroatom, display significantly worsened affinities and selectivities for trimethyllsine relative to the parent compound PSC. The nitro-substituted, electron-poor **7** and amino-substituted, electron-rich **8** have identical binding profiles for lysine and trimethyllysine, demonstrating that the π-electrostatics of the newly substituted calixarene ring have no influence on the binding of these cationic partners under these conditions. The bromo-substituted **6** has 2-fold higher affinities for both amino acids, which we hypothesize arises from improved dispersive interactions and hydrophobic contributions of the bromo substituent relative to amino or nitro. The tosyl sulfonamide host **9** showed the weakest binding for trimethyllysine (570 M^{-1}) with some affinity recovered when the *para*-methyl group is replaced by a charged carboxylate in host **10** (2900 M^{-1} for trimethyllysine). It is likely that the flexibility of the sulfonamide linkage either (a) produces host conformations that do not provide additional contacts with guests or (b) allows hydrophobic collapse of the newly introduced aryl substituent that is detrimental to binding. This type of collapse is impossible for the rigidly linked Suzuki products **11**–**15**. Phenyl-substituted host **11** showed the highest affinity for trimethyllysine (64 000 M^{-1}) that we have observed to date, as well as the highest selectivity over unmethylated lysine (150-fold) among this class of hosts including the parent compound **1**. Isothermal titration calorimetry of host **11** and trimethyllysine (see Supporting Information) provided a similar K_{assoc} of 79 700 M^{-1}. These affinities for trimethyllysine are similar to those of the naturally evolved proteins that bind trimethyllysine-modified proteins and peptides.[8–10] Introduction of cyano, amido, carboxy, and amino substituents (hosts **12**–**15**) to this host skeleton all proved to negatively affect binding.

Table 1. Affinities and Selectivities for Trimethyllysine

host	K_{assoc} (M^{-1})[a] trimethyllysine	K_{assoc} (M^{-1})[a] lysine	selectivity for trimethyllysine
1[b]	37000 ± 18000	520 ± 200	70
6	3900 ± 100	440 ± 110	9
7	1700 ± 90	150 ± 20	11
8	1600 ± 200	180 ± 4	8
9	570 ± 180	30 ± 5	17
10	2900 ± 80	120 ± 8	24
11	64000 ± 13000	420 ± 50	150
11	79700 ± 6300[c]	n.d.	
12	2100 ± 220	210 ± 30	10
13	5900 ± 1600	140 ± 10	42
14	1700 ± 270	110 ± 20	16
15	5200 ± 1300	200 ± 40	26

[a] Determined by ^1H NMR spectroscopy (500 MHz) at 298 K in D_2O (40 mM Na_2HPO_4/NaH_2PO_4, pD 7.0 = pH 7.4) by titration of host (20–50 mM) into a solution of amino acid (2–3 mM). The K_{assoc} values arise from 2 to 4 trackable NMR signals from 2 replicate titrations per guest. Errors reported are standard deviations. [b] Values previously reported.[7] [c] Value determined by ITC. See Supporting Information.

We sought structural clues to help explain the structure/function relations observed. All host–trimethyllysine complexes show maximum upfield shifts for the *N*-methyl and CH_2-ε protons of trimethyllysine, indicating that all form complexes with the methylated ammonium

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functional group buried deep in the calixarene's highly shielding pocket (Table 2).[7] This is unlike the binding of unmethylated lysine, which previous work has shown to form a side-on complex with parent host **1**.[7,24] We also considered chemical shifts of protons at the opposite end of trimethyllysine, with the CH-α resonances of trimethyllysine providing the most easily observed set of diagnostic peaks. We found that shifts of the trimethyllysine CH-α protons do not occur for hosts **1**, **6**, **7**, **8**, as expected; they have no additional functionality able to engage these distal guest protons. Aryl sulfonamide-functionalized hosts **9** and **10** also do not produce shifts of CH-α on trimethyllysine, offering support to our hypothesis that these appended elements do not make significant additional contacts with the full length of the amino acid guests. Finally, the biphenyl-containing hosts **11**–**15** all show similar upfield chemical shifts for CH-α that are indicative of significant CH-aromatic contacts between CH-α and the appended aromatic rings

Table 2. Maximum Chemical Shifts for Trimethyllysine Resonances upon Complexation by Different Hosts[a]

	$-\Delta\delta_{max}$, ppm		
host	*N*-CH_3	CH_2-ε	CH-α
6	2.24	1.33	0.025[b]
7	2.29	1.39	0.045[b]
8	2.49	1.52	0.08[b]
9	2.14	1.26	0[c]
10	2.38	1.38	0[c]
11	2.16	1.38	0.31
12	2.53	1.61	0.29
13	2.40	1.54	0.32
14	2.39	1.66	0.40
15	2.49	1.59	0.32

[a] All resonances shift upfield upon binding. Averaged $|\Delta\delta_{max}|$ values obtained from the K_{assoc} fits (see Table 1 and Supporting Information) are reported unless otherwise noted. [b] These small shifts do not fit the 1:1 binding isotherm, so maximum observed shifts are reported. [c] No measurable change in signal during titration.

If the geometries of the complexes of **11**–**15** with trimethyllysine are generally similar (as revealed by the similarity in $|\Delta\delta_{max}|$ for all trimethyllysine protons), then what is the basis for the weakened binding of cyano, amido, carboxy, and amino hosts **12**–**15** relative to the highly potent phenyl host **11**? The possibilities include CH–π interactions weakened by aryl electron-withdrawing groups, and decreased hydrophobic contributions in **12**–**15** due to the increased polarity and improved solvation of the hosts' appended aryl rings relative to the more hydrophobic **11**. The functionalized aromatic ring in question is engaging the methylenes and zwitterionic functions of the amino acid guest, so it is possible that both the electronic modulation of CH–π contacts between the host and guest and changes in solvation play a role. Whatever the detailed explanation, it is clear that the phenyl-for-sulfonate substitution made between the parent compound **1** and **11** more than compensates for the affinity loss caused by removing a sulfonate and succeeds in producing a highly specific host for the trimethyllysine epigenetic motif that can operate in the medium that matters, pure water.

In summary, we have developed a simple method to access a new family of desymmetrized trisulfonated calix[4]arenes. We have used these novel calix[4]arenes to access sulfonamide-functionalized and biphenyl-functionalized reaction products providing additional contact points for host–guest interactions. Sulfonated calixarenes are increasingly being used in biomedical research, including as medical agents that are active *in vivo*,[25] as critical components of novel enzyme assays,[26] and as protein-binding small molecules.[1,27] The intermediates **6** and **8** offer researchers the ability to tune potencies and selectivities of sulfonated calix[4]arenes by specific installation of a new functionality directly lining the binding pocket and promise to facilitate efforts to optimize molecules for each of these diverse applications.

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Supporting Information Available. Experimental procedures for synthesis and binding studies, spectral data for new compounds, stacked NMR plots and titration curves for all host–guest pairs. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.