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SYNTHESIS OF A POTASSIUM SENSING AZOCROWN CALIX[4]ARENE

A Major Qualifying Project Report

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By

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Approved:

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Abstract

Calix[4]arenes are a versatile class of supramolecular host compounds, and when derivatized with the proper chemical functionality they are capable of interacting with a wide variety of guests in solution. An azocrown-calix[4]arene in the 1,3-alternate confirmation was derivatized for use as a potassium ion sensor in a microfluidics blood analysis device.

Acknowledgements

First of all, I would like to thank Professor McGimpsey for allowing me to undertake this project in his lab. None of this work would have been possible without the guidance of Professor Lambert and the assistance of Eftim Milkani and the other graduate students in the lab. Additionally, I would like to extend my thanks to Victor Kiryak and Professor Dittami's lab for their assistance as well.

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Calixarenes are a versatile class of molecules which participate in host-guest chemistry. Consisting of anywhere from 4 to 20 aromatic moieties circularly linked with methylene bridges, calix[n]arenes were one of the first supramolcules to be extensively researched in the late 1970's as enzyme mimics due to the deep π-electron rich basket with distinct hydrophobic upper rim and hydrophilic lower rim, capable of forming inclusion complexes with a wide range of guest molecules. Depending on the chemical moieties attached to the rims, calixarenes are capable of strongly binding many different species in solution, from ions to small molecules, and have been widely used in supramolecular chemistry to create self-assembling structures such as cavitands, capsules, and nanotubes. However due to their exceptional selectivity for divalent metal cations, much recent research has been directed towards developing calixarenes (especially calix[4]arenes) for use as ionophores.

Here, a potassium sensing azocrown calix[4]arene in the 1,3-alternate conformation, developed previously[1] has been adapted for use in a Surface Plasmon Resonance (SPR) based microfluidics blood analysis device. The unique structural and conformational properties of the calixarene framework are responsible for the high specificity of the sensor for potassium ions, and accordingly the synthetic procedure employed has been optimized to achieve the proper conformation of the molecule and the azocrown bridge.

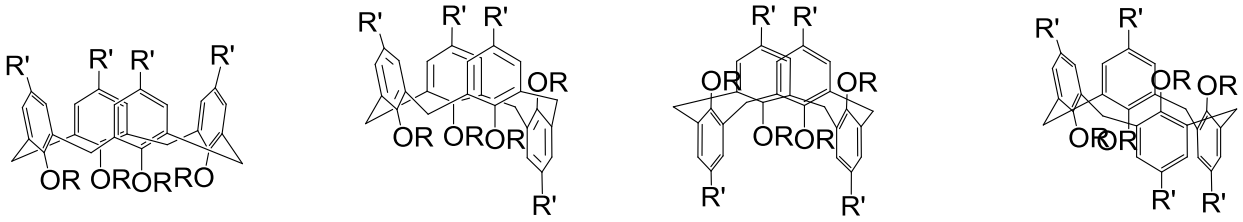
Structural Properties of Calix[4]arenes

The best characterized calix[4]arene is t-butylcalix[4]arene (**1**, R=H, R'=t-butyl), because it was the first synthetically accessible calixarene.[2] Currently, (**1**, R=H, R'=t-butyl) and its *de-tertbutylated* analogue calix[4]arene (**1**, R=H, R'=H) are commercially available from major chemical suppliers and there have been a large number of studies and review works published on the chemistry of these

[1] J. S. Benco, H. A. Nienaber, K. Dennen, W. G. McGimpsey, *J. Photochem. Photobiol. A* **2002**, 152, 33.
[2] A. Zinke, E. Ziegler, *Chemische Berichte* **1944**, 77, 264, 1729.

materials.[3] Upon the discovery of (**1**, R=H, R'=*t-butyl*) it was initially thought that during formation of the molecule the aryl groups were conformationally locked and could not rotate through the central cavity of the ring, explaining the appearance of four separate regioisomers (**2**-**4**, R=H, R'=t-butyl) and explaining the apparent differences in the compounds isolated.[4] However with the assistance of temperature dependant NMR studies, it was shown that the conformational energy barriers were such that (**1**-**4**) were not capable existing independently in solution, and in fact slowly interconvert at room temperature.[5]

Cone | Partial Cone | 1,3-Alternate | 1,2-Alternate



1 **2** **3** **4**

Modifying the groups on the rims of the calixarene can alter its conformational mobility in solution by hindering the interconversion rate between conformers,[6] or depending on the steric bulk of the group introduced, prohibiting interconversion altogether.[7] This is due to hindrance in the two available pathways for conformational interconversion in the calixarene molecule (see Figure 1): the "endo rim through the annulus" pathway where the hydroxyl groups rotate through the center of molecule, or the "exo rim through the annulus" pathway where the pendant aryl groups rotate through the center.[8] Although, in calix[4]arenes the *exo* pathway is so sterically hindered that even when the t-

[3] C. D. Gutsche, Calixarenes: An Introduction (Cambridge, UK: RSC, 2008); Z. Asfari, Calixarenes 2001 (Dordrecht, Holland: Kluwer Academic, 2001); among many others.
[4] J. L. Ballard, W. B. Kay, E. I. Kropa, *J. Paint Technology* **1966**, 38, 251.
[5] H. Kammerer, G. Happel, F. Caesar, *Makromol Chem.* **1977**, 178, 69.
[6] C. D. Gutsche, L. J. Bauer, *J. Am. Chem. Soc.* **1985**, 107, 6052.
[7] V. Bocchi, D. Foina, A. Pochini, R. Ungaro, *Tetrahedron* **1982**, 38, 373.
[8] W.P. van Hoorn, M. G. H. Morshuis, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1998**, 102, 1130.

butyl group is removed (**1**, R=H, R'=H), the molecule cannot interconvert in this way.[9] The only other pathway available is endo through the annulus, and it was found that attaching bulky enough (n-propyl and larger) groups to the hydroxyl moieties will conformationally lock the molecule, causing it to permanently adopt either the cone, partial cone, 1,2-alternate or 1,3-alternate (**1-4**, R=$CH_2CH_2CH_3$, R'=H) conformation in solution.[10]

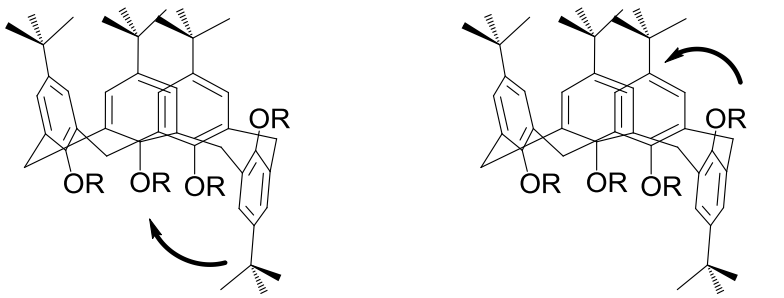


Figure 1: Calixarene Conformational Interconversion

When the groups appended to the endo rim of the calixarene are of requisite steric bulk to prevent conformer interconverson, the major product formed is controlled by the reaction conditions used to introduce the group. The solvent, temperature, and reactivity of the alkylating agent all have some effect on the distribution of conformers recovered, although the choice of base was found to have the greatest effect on the outcome of the reaction.[11] When locked conformers were synthesized, the strength of the base was found to effect the conformational distribution due to the differing pKa values for each of the calixarene hydroxyl groups and rate of oxyanion formation.[12] Also the size of counter-ion with the base significantly affected conformational outcome, suggesting a templating effect.[13]

[9] K. Iwamoto, K. Araki, S. Shinkai, *J. Org. Chem.* **1991**, 56, 4955.
[10] *Ibid.*
[11] *ibid*.
[12] A. Arduini, E. Ghidini, A. Pochini, R. Ungaro, *J. Inclusion Phenom.* **1988**, 6, 119.
[13] S. Pappalardo, *New J. Chem.* **1996**, 20, 465.

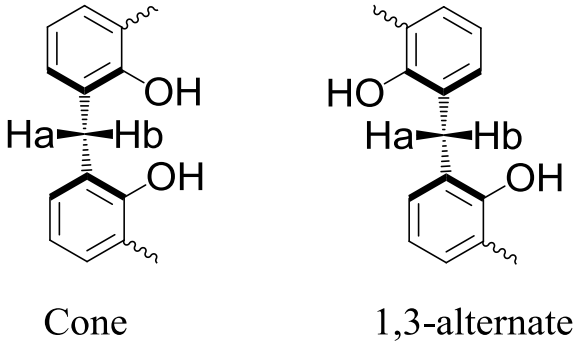


Figure 2: Adapted from C. D. Gutsche, Calixarenes, 2008.

As mentioned earlier, differentiating between the conformers can be accomplished through monitoring either the ^{1}H or the ^{13}C NMR chemical shifts of the methylene bridging moieties. Figure 2 shows the chemical environments experienced by the methylene protons depending on the conformation. It has been shown in conformationally locked calixarenes the chemical shifts differ by 0.9 ± 0.2 ppm (at room temperature) from the cone to the 1,3-alternate conformer, whereas conformationaly mobile calixarenes will demonstrate a broadening effect.[14] For similar reasons the ^{13}C NMR chemical shift of the methylene carbons can be monitored, and consequentially, it has been determined that these carbons will give a signal around 31 ppm if the calixarene is in the cone conformation or around 37 ppm if it is in the 1,3-alternate conformation.[15] Several 2D NMR techniques such as COSY and HMQC have also been shown to be useful in determining the conformation of alkylated calix[4]arenes when other methods fail.[16]

[14] G. Ferguson, A. Notti, S. Pappalardo, M. F. Parsi, A. L. Speck, *Tetrahedron Lett.* **1998**, 39, 1965.
[15] J. DeMendoza, C. Jaime, P. Nieto, P. Prados, C. Sanchez, *J. Org. Chem.* **1991**, 56, 3372.
[16] J. O. Magrans, J. DeMendoza, M. Pons, P. Prados, *J. Org. Chem.* **1997**, 62, 4518.

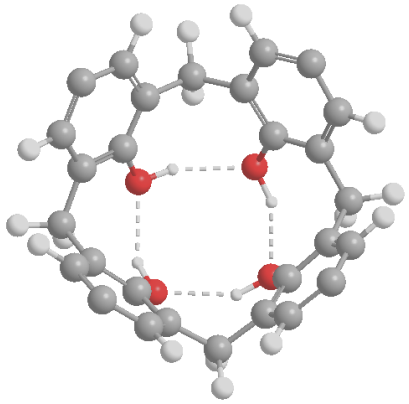
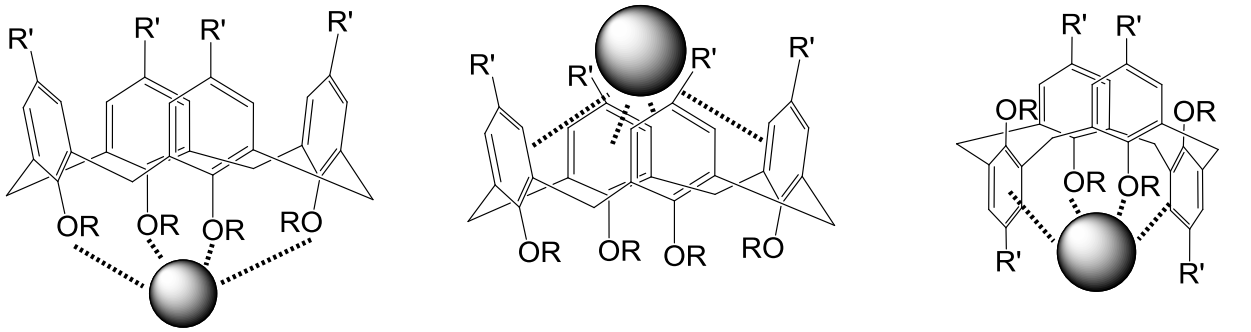


Figure 3: MM2 Optimized (1, R=H, R'=H)

In the calixarene molecule, the different orientations of the π-systems and oxygen atoms among the conformers control which guest molecules the calixarene tends to form the strongest inclusion complexes with. It is well known that (**1**, R=H, R'=t-butyl) in the cone conformation is capable of forming solid state complexes with neutral molecules like chloroform, benzene, toluene, and xylene that have been characterized through X-ray crystallography.[17] The stability of these complexes is due to the intraannular hydrogen bonding between the endo rim hydroxyls in the cone conformation (see Figure 3), causing the π-electron clouds to orient for optimum binding of the neutral guest within the cavity.[18] However in solution, when the stabilizing hydrogen bonding capability of the hydroxyl is disrupted through alkylation (**1**-**4**, R=CH_3 or CH_2CH_3, R'=H), the rotational energy barriers are lowered and the molecule will readily adopt any of the four conformations in solution to form the most stable host-guest interactions with the species present in solution at room temperature, typically favoring metal cations.

[17] C. D. Gutsche, B. Dhawan, K. H. No, *J. Am. Chem. Soc.* **1981**, 103, 3782.
[18] C. D. Gutsche, *Acc. Chem. Res.* **1983**, 16, 161.



Small Cations (Li^+, Na^+)
Cone, lone pair interations

Large Cations ($R"CH_2NH_3^+$)
Cone, pi-system interactions

Medium Cations (K^+, Ag^+)
1,3-alternate, mixed

Figure 4: Adapted from Shinkai, *Chem. Rev.* 1997, 97, 1713

The best studied conformationally mobile calix[4]arene is tetra-O-methylcalix[4]arene (**1**-**4**, R=Me, R'=t-butyl), and it has been shown by ^{1}H NMR that it adopts different conformations in solution (shown in Figure 4) to form complexes with lithium, sodium, potassium, silver, and quaternary ammonium ions through maximizing the coordination with the lone pairs of the oxygen or the π-interactions with the aryl system depending on the size of the cation[19]. Though the guest binding specificity is closely related to the conformation of the molecule, the groups attached to the rims of the calixarene have a much greater effect on binding chemistry especially when they are large enough to conformationally lock the calixarene[20].

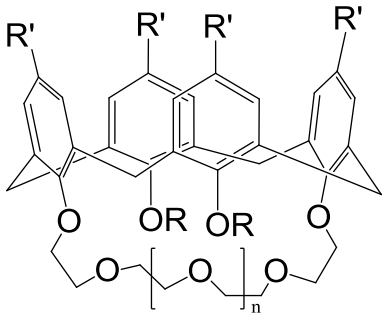
Through the addition of chemical functionality to the rims it is possible to design highly specific binding pockets for a wide variety of chemical species. For instance, adding carboxylic acid functional groups to the endo rim (**1**, R=CH_2COOH, R'=t-butyl) increases binding specificity for divalent metal cations,[21] while (carbamoylmethyl)-phosphine oxide groups bound to the exo rim (**1**, R=Me, R'=$NHCOCH_2P(O)Ph_2$) greatly increases the affinity for trivalent transition metal ions,[22] among many

[19] A. Ikeda, S. Shinkai, *Chem. Rev.* **1997**, 97, 1713.
[20] G. Talanova, V. Talanov, H. Hwang, C. Park, K. Surowiec, R. Bartsch, *Org. Biomol. Chem.* **2004**, 2, 2585.
[21] A. Arduini, A. Pochini, S. Reverberi, R. Ungaro, *J. Chem. Soc. Chem. Commun.* **1984**, 981.
[22] S. Barboso, A. G. Carrera, S. E. Matthews, J. Arnaud-Neu, V. Bohmer, M. J. Schwing-Weill, *J. Chem. Soc. Perkin Trans. 2* **1999**, 719.

other possibilities.[23] In addition to adding simple functional groups, it is also possible to create more complex 3-dimensional structures (*i.e.* crown ethers like (**5**)) to achieve further guest binding specificity by means of preorganization.[24]

Calixarene Ionophores

There are two crucial elements required to develop the potassium sensing molecule: 1) it must be highly selective for potassium ions in blood, and 2) it must be capable of forming a monolayer on the gold sensing surface required for SPR analysis. Conformationally mobile calix[4]arenes have shown that the strongest host-guest binding interactions between postassium and the calixarene occur when it is in 1,3-alternate conformation, implying that calix[4]arenes synthetically locked in the 1,3-alternate conformation may have a higher overall binding specificity for potassium since they are unable to alter their conformation to strongly bind other ions.[25] This evidence combined with the knowledge that crown ethers on their own also had high binding specificities for metal ions, lead researchers to attempt to combine the two structures in one binding pocket, resulting in a molecule called a *calixcrown* (**5**).



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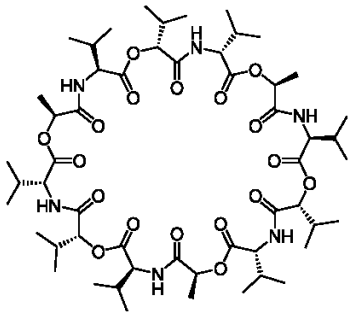


6

Initial molecular modeling and synthetic work indicated that distal bridging of (**1**, R=H, R'=t-butyl) with both rigid and flexible ligands was capable of producing a wide variety of ion selective

[23] H. Otsuka, S. Shinkai, *Supramol. Sci.* **1996**, 3, 189.
[24] J.D. van Loon, W. Verboom, D. N. Reinhoudt, *Org. Prep. Proc. Int.* **1992**, 24, 437.
[25] S. Shinkai, K. Fujimoto, T.Otsuka, H. L. Ammon, *J. Org. Chem.* **1992**, 57, 1516.

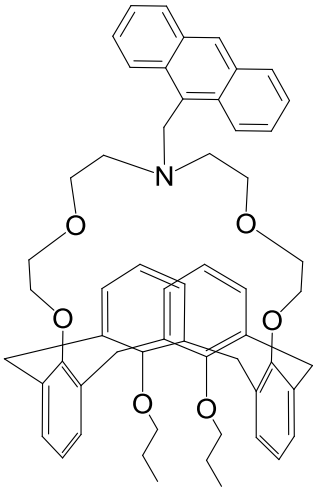
binding pockets from the base calix[4]arene structure.[26] Further synthesis and x-ray crystallography studies on the calixcrowns showed that (**5**, R=H, R'=t-butyl, n=1) can be conformationally locked if anything bulkier than a methyl group is added after the bridge is formed, and specifically that (**5**, R=C_2H_5, R'=t-butyl, n=1) in the partial cone conformation was found to have the highest K+/Na+ selectivity of any synthetic ionophore at the time.[27] However, later work indicated that when the *t-butyl* groups were removed (**5**, R=C_2H_5, R'=H, n=1) the molecule demonstrated the highest K+/Na+ selectivity known, exceeding both that of prior ionophores and even the natural ionophore valinomycin(**6**).[28]

Furthermore, several studies have indicated that calix[4]arenes retain their ion complexation capability when assembled in a monolayer and additionally, it is possible to form a well ordered self assembled monolayer on a gold surface with appropriately derivatized calixarenes. Calix[4]rene (**1**, R=H, R'=t-butyl) is completely insoluble in water, although when the tetra-ester derivatives were synthesized (**1**, R=CH_2COOR", R'=t-butyl) the molecules formed an Langmuir-Blodgett film at the air-water interface. Upon the addition of different metal cations the concentration of calixarene at the interface was observed to change, leading the authors to conclude that calix[4]arene monolayers would retain their ion sensing properties.[29] Derivatized calix[4]arenes have also been deposited in a well characterized monolayer (**1**, R=$CH_2(CH_2)_{10}$SH, R'=t-butyl or H) on a gold surface,[30] and more recently through synthetic modification of the exo rim[31] (**1**, R=*i*-Pr, R'=AcSH), indicating that calixarenes are well suited for SPR applications.

[26] P. Dijkstra, J. Brunink, D. N. Reinhoudt, R. Ungaro, *et. al*, *J. Am. Chem. Soc.* **1989**, 111, 7567.
[27] E. Ghidini, F. Ugozzoli, R. Ungaro, D. N. Reinhoudt, *et. al*, *J. Am. Chem. Soc.* **1990**, 112, 6979.
[28] A. Casnati, A. Pochini, R. Ungaro, *et. al*, *Eur. Chem. J.* **1996**, 2, 436.
[29] Y. Ishikawa, T. Kunitake, T. Matsuda, T. Otsuka, and S. Shinkai, *J. Chem. Soc. Chem. Comm.* **1989**, 736.
[30] B.Huisman, E. van Velzen, F. van Veggel, J. Engbersen, D. N. Reinhoudt, *Tetrahedron Lett.* **1995**, 36, 3273.
[31] B.Genorio, T. He, A. Meden, S. Polanc, J. Jamnik, J. M. Tour, *Langmuir* **2008**, 24, 11523.

Retrosynthetic Analysis

Since calixcrowns locked in the 1,3-alternate conformation had been shown to be extraordinarily selective for potassium ions in solution, the goal of the synthesis was to form the calixcrown in the correct conformation with thiol groups for surface attachment. Earlier research carried out in the McGimpsey Lab indicated that (**7**) was an excellent fluoroionophore for potassium in solution,[32] so it was theorized that the same molecular framework could be adapted for use in the SPR based microfluidics system and the novel synthetic target (**8**) was proposed.



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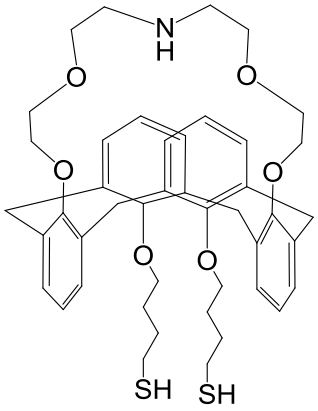
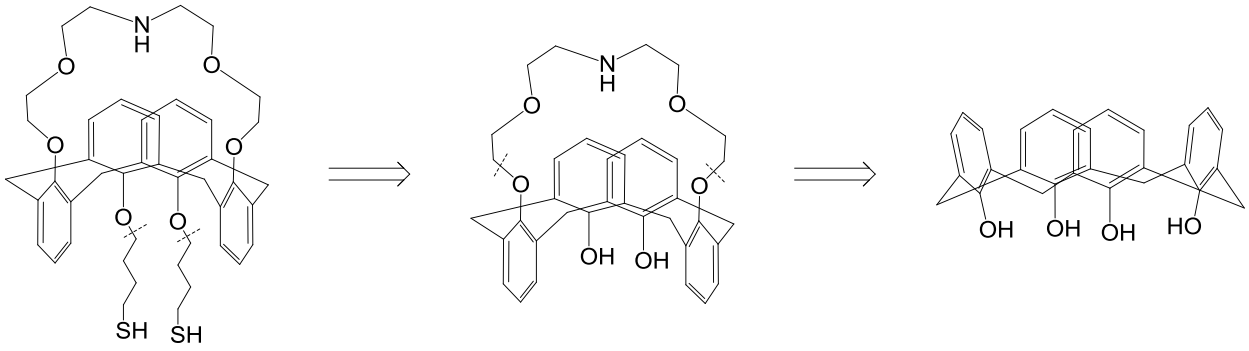


8

There are several possible synthetic routes to the target molecule (**8**). A primary concern was controlling the alkylation of the hydroxyl groups to ensure that the molecule had the proper functionality for both the bridging azocrown ring and the thiol groups for surface attachment. Since both functionalities in the molecule must be achieved through ether linkages which are larger than n-propyl and conformationally lock the molecule, they must be introduced to provide the product calixarene in the proper conformation. Therefore the simplest approach would be to attach the azocrown in a single reaction; leaving only two possible sites for attachment of the thiol linkages (see Scheme 1). The first

[32] J. S. Benco, H. A. Nienaber, K. Dennen, W. G. McGimpsey, *J. Photochem. Photobiol. A* **2002**, 152, 33.

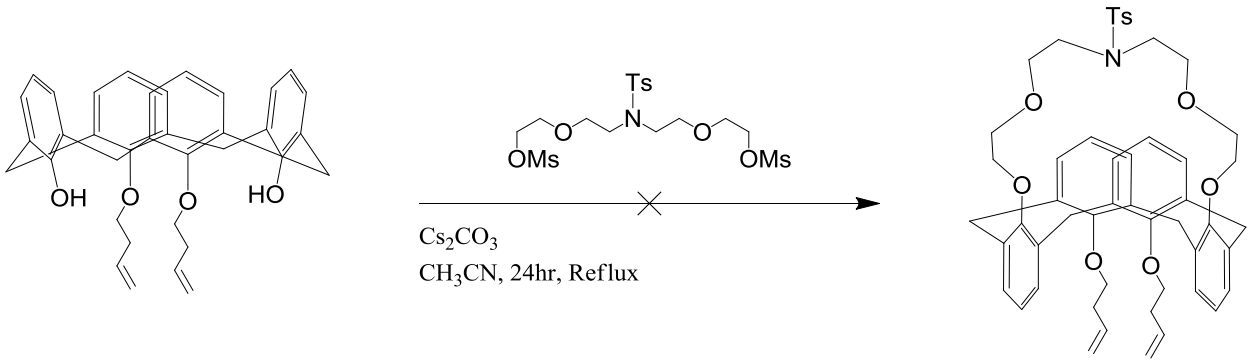
calixcrown studied (**5**, R=H, R'=t-butyl, n=1) was synthesized in a similar manner through the use of a di-tosylate,[33] and many other calixcrowns have been synthesized in this way[34].



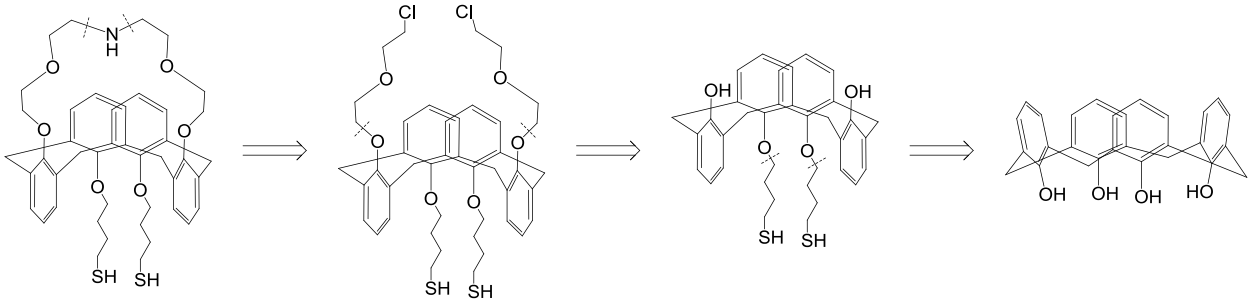
Scheme 1

In this case, attaching the azocrown ring first presents a special challenge because the nitrogen is more reactive than the calixarene hydroxyls and must be protected while the calixarene is further alkylated. Even so, when the synthetic procedure proposed in Scheme 1 was attempted using a tosylate protecting group on the nitrogen and the more reactive mesyl leaving groups on the end of the azocrown fragment, a dimeric calixarene structure was formed and the expected azocrown calixarene was not isolated (see Scheme 2).[35] Instead the authors of that study installed the reactive nitrogen connecting the azocrown after the calixarene had been completely alkylated (see Scheme 3), preventing the formation of calixarene dimers.

[33] C. Alfieri, E. Dradi, A. Pochini, R. Ungaro, G. Andreetti, *J. Chem. Soc., Chem. Commun.* **1983**, 1075.
[34] J.D. van Loon, W. Verboom, D. N. Reinhoudt, *Org. Prep. Proc. Int.* **1992**, 24, 437.
[35] J. S. Kim, O. J. Shon, J. W. Ko, M. H. Cho, I. Y. Yu, J. Vicens, *J. Org. Chem.* **2000**, 65, 2386.



Scheme 2



Scheme 3

However this necessitates that the first alkylation performed on the calixarene must be extremely selective; it must distally di-alkylate the calixarene while leaving the other two hydroxyls available for subsequent alkylation or the correct azocrown bridge will not be formed later. Fortunately it has been shown that mild alkylating conditions will produce the distally di-alkylated product in almost quantitative yields due to the stabilizing effects of hydrogen bonding between the distal hydroxyl groups.[36] Additionally the thiol group is highly reactive under the conditions needed to alkylate the calixarene and install the azacrown, thus it must be protected until the end of the synthesis.

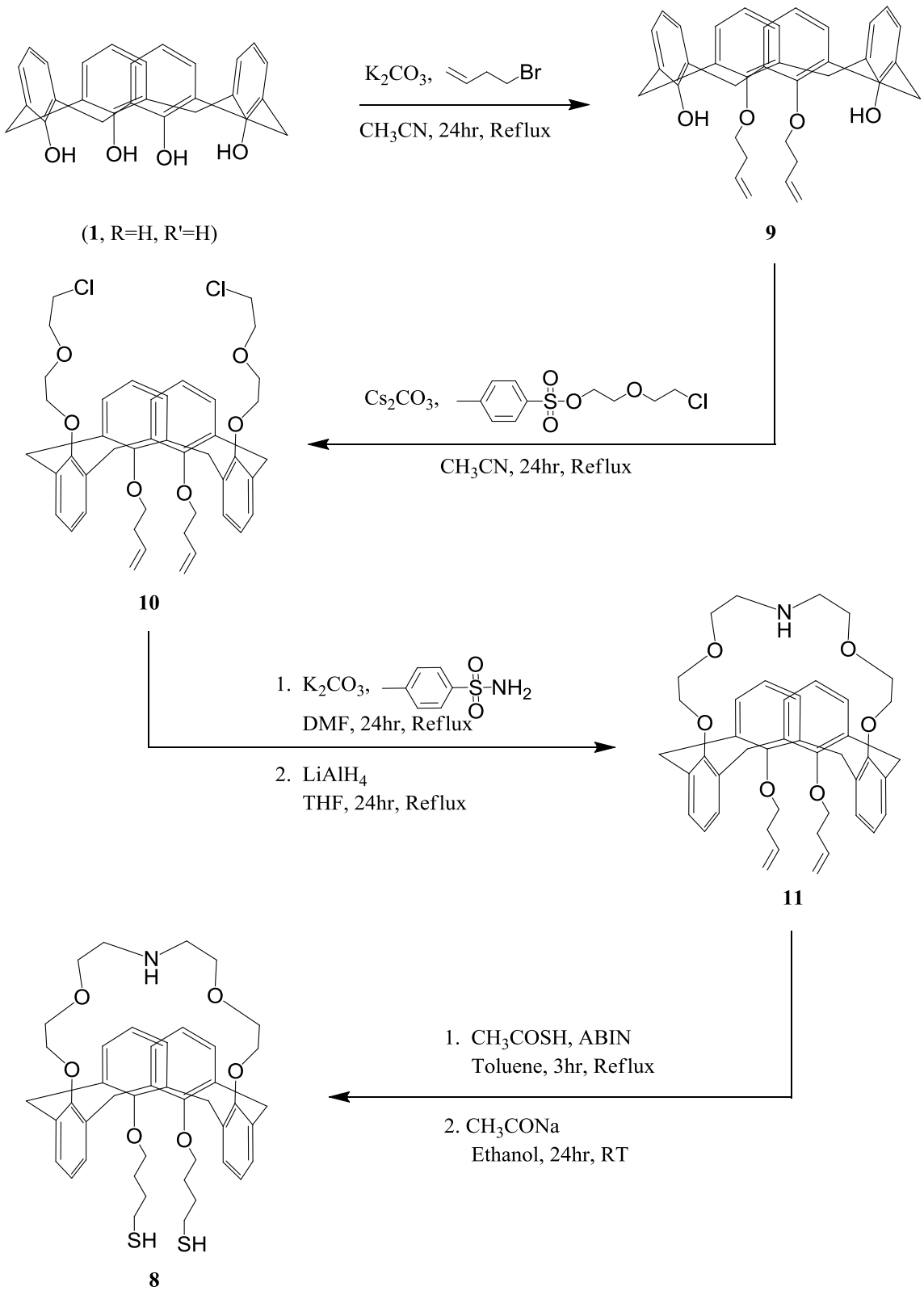
Synthetic Procedure

With the aforementioned retrosynthetic considerations, the synthesis of (**8**) was developed from procedures readily available in the literature (see Scheme 4 for overview). First selective distal di-

[36] J. D. Van Loon, A. Arduini, L. Coppi, W. Verboom, A. Pochini, R. Ungaro, S. Harkema, D. N. Reinhoudt, *J. Org. Chem.* **1990**, 55, 5639.

alkylation was used to add the thiol linkage synthons,[37] followed by a second round of alkylation to attach the foundations of the azocrown bridge with conditions optimized to lock the calixarene in the 1,3-alternate conformation.[38] Then the bridging nitrogen was added as the sulfonamide and subsequently reduced to an amine.[39] Finally, the thiol groups were added to arrive at the final product.[40]

[37] M. Pitarch, J. Browne, M. Kervey, *Tetrahedron*. **1997**, 53, 16195.
[38] J. S. Kim, O. J. Shon, J. W. Ko, M. H. Cho, I. Y. Yu, J. Vicens, *J. Org. Chem.* **2000**, 65, 2386.
[39] J. S. Benco, H. A. Nienaber, K. Dennen, W. G. McGimpsey, *J. Photochem. Photobiol. A* **2002**, 152, 33.
[40] B. Huisman, E. van Velzen, F. van Veggel, J. Engbersen, D. N. Reinhoudt, *Tetrahedron Lett.* **1995**, 36, 3273.

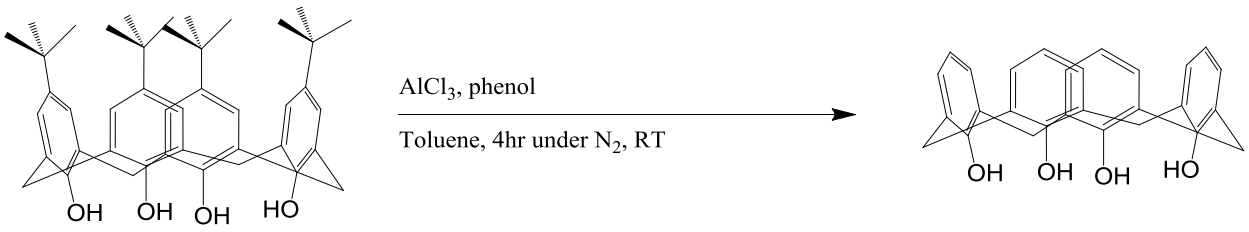

OH
OH
OH
HO
(1, R=H, R'=H)
K2CO3,
Br
CH3CN, 24hr, Reflux
OH
O
O
HO
9
Cl
Cl
O
O
O
O
O
O
10
Cs2CO3,
O
S
O
O
O
Cl
CH3CN, 24hr, Reflux
1. K2CO3,
O
S
NH2
O
DMF, 24hr, Reflux
2. LiAlH4
THF, 24hr, Reflux
N
H
O
O
O
O
O
O
11
1. CH3COSH, ABIN
Toluene, 3hr, Reflux
2. CH3CONa
Ethanol, 24hr, RT
N
H
O
O
O
O
O
O
SH
SH
8

Scheme 4

Results and Discussion

Among other difficulties, the unique chemical properties of the calixarene molecule were at the root of nearly every challenge encountered during the synthesis. Purifying the proper 1,3-alternate conformational isomer of (**10**) was the greatest obstacle in obtaining the final product; and since the conformation of the calixarene is key to the potassium binding ability, extreme care was taken to develop a method for obtaining an analytically pure sample of (**10**) before continuing the synthesis. On the route to (**10**), several planned reaction conditions were altered to improve initially low synthetic yields. However, the generally low solubility of calix[4]arene species in most every solvent complicated many procedures and may have been at the root of the separation difficulties as well as the low yields.

While awaiting the delivery of the initial starting material (**1**, R=H, R'=H), some t-butylcalix[4]arene (**1**, R=H, R'=t-butyl) was discovered in the lab and several attempts were made to remove the t-butyl groups. Following established procedure,[41] (**1**, R=H, R'=t-butyl) was subjected to reverse Friedel-Crafts conditions as per Scheme 5.



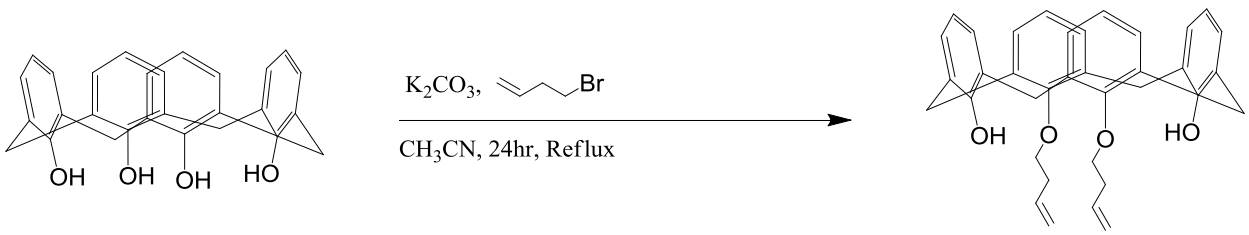
Scheme 5

Since this reaction was not initially planned, the reagents were of questionable age and purity. During the first attempt, crude phenol and aluminum chloride were used without any verification of purity or reactivity. After recrystallization, preliminary ^{1}H NMR analysis indicated that there was still a large amount of starting material present, and column chromatography was used to isolate a very small amount of analytically pure product. Each manually collected fraction was individually analyzed by NMR

[41] C. D. Gutsche, J. A. Levine, P. K. Sujeeth, *J. Org. Chem.* **1985**, 50, 5802.

to confirm the lack of the t-butyl absorbance; however only a single fraction contained analytically pure product.

It was theorized that the age of the reagents, especially the aluminum chloride, were responsible for the low yields. Therefore, to increase the yield of the reaction the reagents were purified by conventional methods. Both the phenol and the aluminum chloride were sublimated under vacuum to improve their purity and produce anhydrous material, and the toluene was dried over 4Å molecular sieves before being used in the reaction. Together, these steps dramatically improved the outcome of the reaction (0.187g, 88% yield) and after initial recrystallization the product was determined to be acceptably pure by NMR without the need for column chromatography.



Scheme 6

The first planned synthetic step (see Scheme 6) was successfully accomplished with the proper reaction conditions and solvent choices. Since the reaction is a standard nucleophillic addition, the hydroxyl groups on the calixarene must be deprotonated by a base in order to react. This poses a special challenge because the inorganic bases strong enough to effect alkylation are completely insoluble in many organic solvents, whereas the calixarene is only soluble in a select few organic solvents. Also since the type of base used controls the distribution of regioisomers produced during the reaction,[42] potassium carbonate was required to promote the formation of the A,C-distally alkylated calix[4]arene

[42] J. D. Van Loon, A. Arduini, L. Coppi, W. Verboom, A. Pochini, R. Ungaro, S. Harkema, D. N. Reinhoudt, *J. Org. Chem.* **1990**, 55, 5639.

(**9**). Thus the reaction was performed in acetonitrile because of its high dielectric constant and ability to dissolve potassium carbonate, even though the calixarene is insoluble.

At first, several experiments were attempted with the planned reaction conditions. The first experimental conditions produced an unexpectedly low yield (0.0372g, 11% yield and 0.0413g, 14% yield). It was observed that during the reaction workup a precipitate formed and remained at the dichloromethane/water interface without seeming to readily solubilize in either layer. After reviewing the work up procedures in the literature, it was theorized that the calixarene may have poor solubility in dichloromethane, and chloroform was tested as the extracting agent. The switch to chloroform along with an increase in the concentration of 4-bromobutene considerably improved the yield (0.3342g, 35% yield), although it did not reach the desired level for the first step in a multistep synthesis.

Further research indicated that a phase transfer catalyst had the potential to dramatically improve yields when the calixarene could be dissolved in solution; and a synthetic phase transfer catalysis reaction utilizing polyethylene glycol, was adapted from the literature.[43] This certainly improved the results (0.5328g, 56% yield) although the polyethylene glycol formed emulsions with the chloroform and water that took days to separate during the reaction workup. In addition, the resultant crude product was highly contaminated with polyethylene glycol and would not crystallize, requiring purification by column chromatography to obtain (**9**).

[43] W. Wang, Q. Zheng, Z. Huang, *Synth. Commun.* **1999**, 29, 3711.

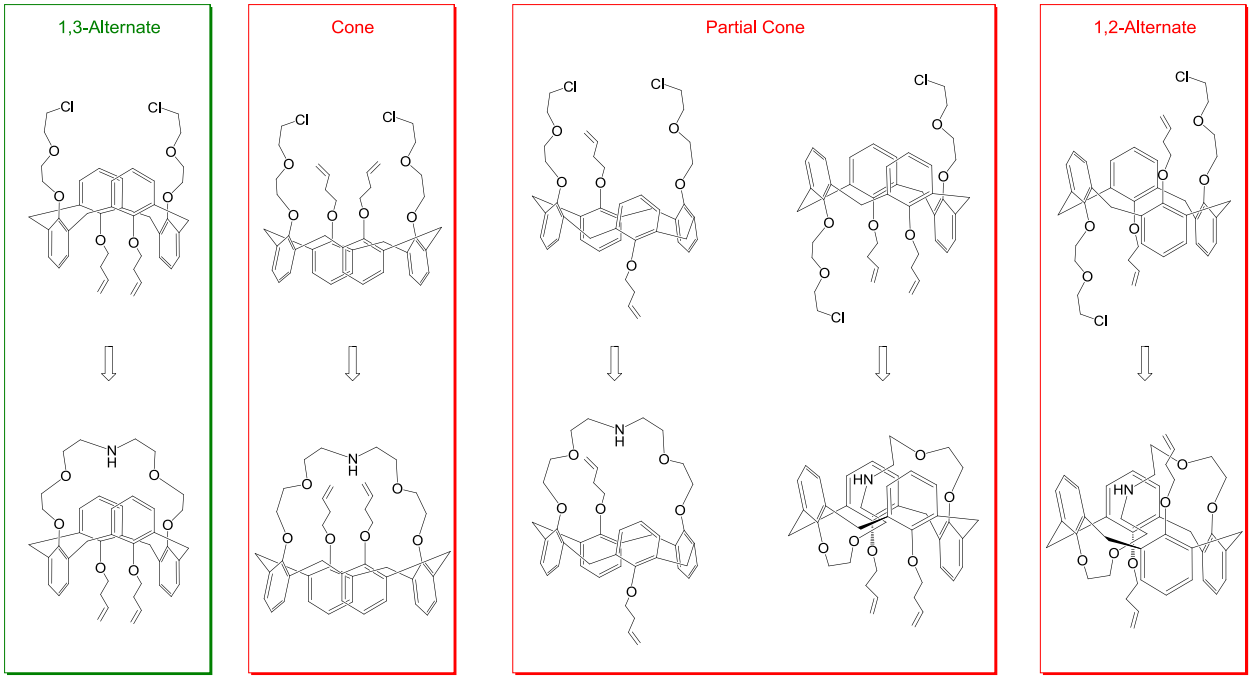


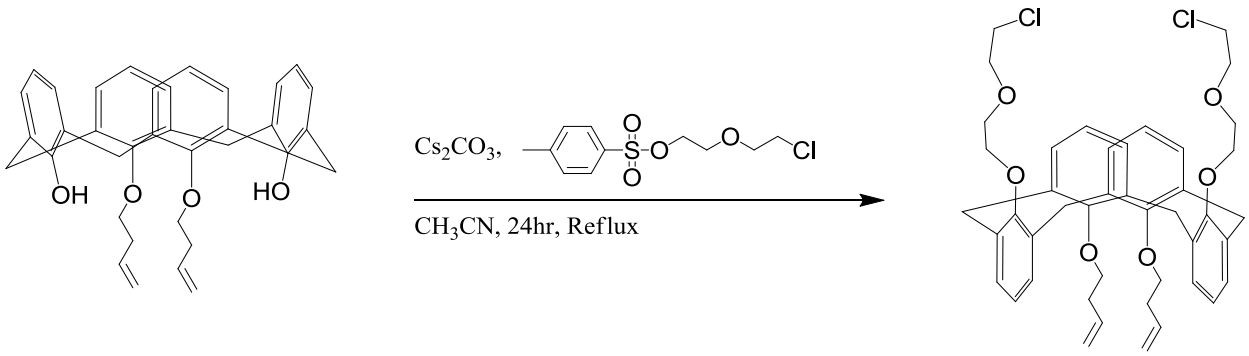
Figure 5: Effect of conformation on azocrown formation

After a sufficient amount of (**9**) had been synthesized, the greatest obstacle in obtaining the final product was purifying the 1,3-alternate conformational isomer of (**10**). Since the target ionophore is known to have the highest potassium binding capability in the 1,3-alternate conformation and (**10**) is a conformationally locked calixarene, it is essential to obtain the precursuor to the azocrown ring in the correct conformation so no cone, partial cone, or 1,2 alternate azocrown calixarene conformers are formed during the ring closing reaction (see Figure 5).

As a result the reaction conditions used to synthesize (**10**) were selected to enhance production of the locked 1,3-alternate conformer through the ion templating effect. Since the calixarene will alter its conformation to achieve the strongest interactions with the ions in solution, a templating effect has been observed in the synthesis of tetra-alkylated conformationally locked calixarenes[44] and especially

[44] K. Iwamoto, K. Fujimoto, T. Matsuda, S. Shinkai, *Tetrahedron Lett.* **1990**, 31, 7169.

calixcrowns[45] depending on the specific base employed. Therefore, cesium carbonate was used as the alkylating base instead of potassium carbonate to bias the final product distribution towards the locked 1,3-alternate conformer.[46]



Scheme 7

When (**9**) was reacted with 2-(2-chloroethoxy)ethanol p-toluenesulfonate to form (**10**) (see Scheme 7), the all of the conformational isomers depicted in the top half of Figure 5 were expected with the majority being the 1,3-alternate conformer. Although upon recrystallization of the crude product, NMR analysis of the methylene region (see Figure 6) indicated the crystalline product to be a mixture of the locked conformers of (**10**), and further purification was attempted.

[45] E. Ghidini, F. Ugozzoli, R. Ungaro, D. N. Reinhoudt, *et. al*, *J. Am. Chem. Soc.* **1990**, 112, 6979.
[46] K. Iwamoto, K. Araki, S. Shinkai, *J. Org. Chem.* **1991**, 56, 4955.

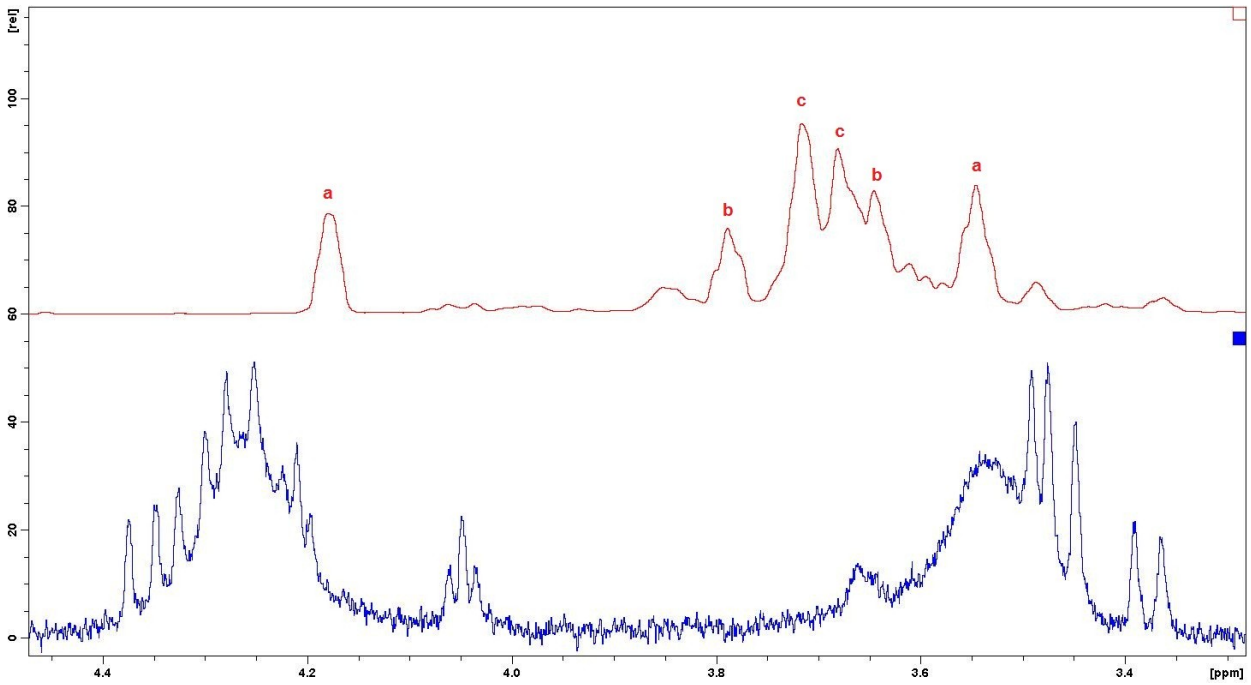


Figure 6: Methylene region comparison of (9) (lower spectra) and the reaction mixture of (10) (upper spectra). Peaks are labeled according to conformer (a = cone, b = partial cone and 1,2-alternate, c = 1,3-alternate).

The most typical procedure specified in the literature for obtaining pure calixarene conformational isomers during the reaction work up is column chromatography; however despite repeated attempts under varying conditions, conventional column chromatography techniques were not able to isolate a pure sample of (**10**) in the 1,3-alternate conformation by NMR. Furthermore, preparative scale TLC was also mentioned as a purification technique, so efforts were made to correlate the TLC data to the results obtained from the column. The separation observed from the initial TLC showed a wide band of calixarene conformers indicating that separation was taking place. However, even combined with column chromatography these conditions failed to provide an analytically pure sample of the 1,3-alternate conformers via NMR. Therefore, given that column chromatography was observed by TLC to improve the separation between conformers, it was theorized that running a significantly longer column under pressure would be the best method to produce a pure sample of (**10**) in the 1,3-alternate conformation.

Conclusions

If the calix[4]arene (**8**) is to be developed into a potassium sensor for use in a SPR based microfluidics device, a reliable procedure for isolating the 1,3-alternate conformer must be implemented. Central to this challenge is discovering a solvent system capable of providing definitive separation of the calixarene conformers when used in either preparative scale thin layer or conventional silica gel column chromatography. Additionally, expanding the conformer characterization methods to include a HPLC method would dramatically improve the ease of identifying reasonably pure fractions obtained from TLC and column chromatography methods.

The efficiency of the chromatographic techniques used to separate conformationally locked calixarenes depends on the ability to reliably identify the correct calixarene conformer. In this synthesis, monitoring the methylene region of the ^{1}H and ^{13}C NMR was the sole analytical method employed to differentiate between the conformational isomers. The primary drawback of this technique is the inability to determine the relative concentrations of the different conformers with any degree of certainty. Although there has been a set of "rules" developed for determining the conformation of a calixarene through the chemical shifts of the methylene protons[47] or carbons[48], the shift observed is too small and the region on the spectra is too crowded to rely on the ^{1}H NMR integral ratios as an indicator of relative concentrations. Furthermore, due to the generally low solubility of the calixarene in all solvents, the sample was too dilute in $CDCl_3$ to obtain high resolution ^{13}C NMR spectra. Thus, it was very difficult to determine if the chromatographic purifications were having any impact on increasing the concentration of the desired 1,3-alternate conformer. Additionally, analysis of the fractions collected from column chromatography was extraordinarily time consuming because the column eluent had to be evaporated and the residue taken up with $CDCl_3$ before a purity determination could be made.

[47] G. Ferguson, A. Notti, S. Pappalardo, M. F. Parsi, A. L. Speck, *Tetrahedron Lett.* **1998**, 39, 1965.
[48] J. DeMendoza, C. Jaime, P. Nieto, P. Prados, C. Sanchez, *J. Org. Chem.* **1991**, 56, 3372.

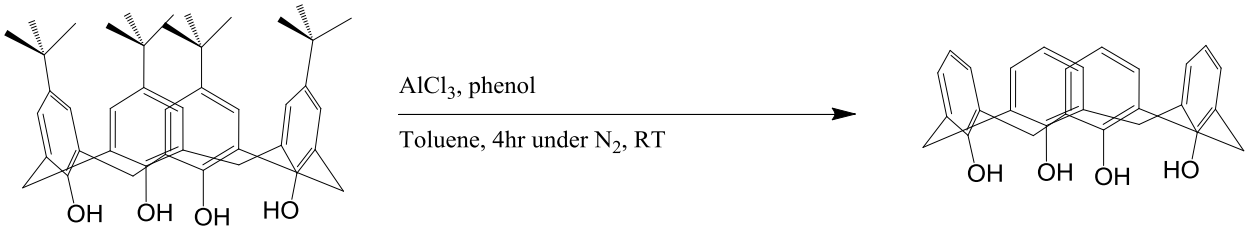
Since (**8**) is a novel compound, it is imperative to isolate products (**10**, **11**, and **8**) in an analytically pure form for spectral characterization if this work is to be published. While conventional column chromatography and TLC have been shown effective in the literature for separating mixtures of calixarene conformers, the solvent system used to effect these separations is highly specific to the functionality of the specific calixarene involved, and the current work indicates that these conditions cannot be easily generalized to effect separations differently functionalized calixarenes. Consequently, to reliably separate a pure sample of the 1,3-alternate conformer of (**10**) a new solvent system must be developed.

Employing an HPLC method, as used by several groups to determine the distribution of conformers produced in synthetic reactions,[49,50] would be an effective method to rapidly determine the relative concentrations of the different conformers and evaluate the performance of the separatory method. Furthermore, an HPLC method would be capable of providing an analytically pure sample of any conformer obtained for further characterization by NMR. If it is not viable to implement an HPLC method, simply employing flash chromatography may significantly increase the performance of the solvent system due to the elevated pressure in the column, as well as the automated UV-based fraction collector capable of separating fractions by their chromatographic signature. Otherwise, the only method to monitor fraction composition will be the very time consuming and generally inaccurate column chromatography coupled with NMR analysis, thus impeding the attainment of (**8**) and its subsequent incorporation into the SPR based microfluidics device.

[49] S. Shinkai, K. Fujimoto, T.Otsuka, H. L. Ammon, *J. Org. Chem.* **1992**, 57, 1516.
[50] W. Verboom, S. Datta, Z. Asfari, S. Harkema, D. N. Reinhoudt, *J. Org. Chem.* **1992**, 57, 5394.

Experimental Section

Procedure 1



Experiment 1A

A slurry of *p-tert*butylcalix[4]arene (1.5 g, 2 mmol), crude phenol (0.94 g, 10 mmol) and $AlCl_3$ (1.5 g, 11 mmol) was stirred in toluene (30 mL) at room temperature for 4 h under nitrogen. After, the mixture was poured slowly onto cooled 0.2 N hydrochloric acid (250 mL) and the aqueous phase was extracted with dichloromethane (2 x 100 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The crude product was further purified by crystallization from a mixture of dichloromethane and methanol.

1H-NMR of the resultant product (0.722g, 85% yield) showed that recrystallization did not produce a pure product. The product was further purified on a silica gel column (DCM:MeOH 9:1) yielding 0.0832g of analytically pure product, as verified through the lack of a t-butyl resonance in NMR.

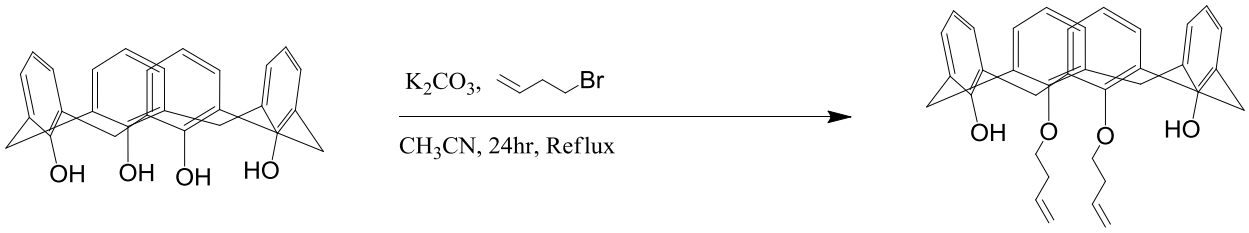
Experiment 1B

A slurry of *p-tert*butylcalix[4]arene (0.324 g, 0.5 mmol), sublimated phenol (0.94 g, 10 mmol) and anhydrous $AlCl_3$ (0.75 g, 5.6 mmol) was stirred in anhydrous toluene (30 mL) at room temperature for 12 h under nitrogen. After, the mixture was poured slowly onto cooled 0.2 N hydrochloric acid (250 mL) and the aqueous phase was extracted with dichloromethane (2 x 100 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The crude product was further purified by crystallization from a mixture of dichloromethane and methanol.

1H-NMR of the resultant product (0.187g, 88% yield) showed that recrystallization had produced acceptably pure product.

$_1$H NMR ($CDCl_3$) 3.55 (br s, 4H), 4.26 (br s, 4H), 6.73 (t, 4H), 7.07 (d, 8H), 10.21 (s, 4H)

Procedure 2



Experiment 2A

Calix[4]arene (0.2576g, 0.6 mmol), 4-bromobutene (0.164g, 1.2 mmol) and potassium carbonate (0.20g, 1.5 mmol) were refluxed in anhydrous acetonitrile (50mL) for 24hrs under anhydrous conditions. The solvent was removed under reduced pressure and the reaction mixture was resuspended in 1 N HCl (20mL) and DCM (20mL). The aqueous phase was separated and extracted with DCM (2x10mL), and the combined organic phases were washed with brine (20mL) and dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure, leaving a brown waxy residue. The product was recrystallized from boiling MeOH/DCM (5:1) with a final mass of 0.0372g (11% yield).

Experiment 2B

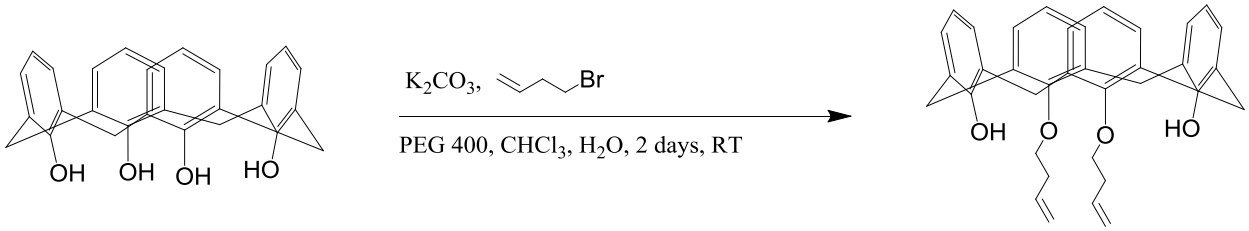
Calix[4]arene (0.2523g, 0.6 mmol), 4-bromobutene (0.165g, 1.2 mmol) and potassium carbonate (0.196g, 1.5 mmol) were refluxed in anhydrous acetonitrile (50mL) for 24hrs under anhydrous conditions. The solvent was removed under reduced pressure and the reaction mixture was resuspended in 1 N HCl (20mL) and DCM (20mL). The aqueous phase was separated and extracted with DCM (2x10mL), and the combined organic phases were washed with brine (20mL) and dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure, leaving a brown waxy residue. The product was recrystallized from boiling MeOH/DCM (5:1) with a final mass of 0.0413g (14% yield).

Experiment 2C

Calix[4]arene (0.7521g, 1.7 mmol), 4-bromobutene (0.447g, 3.5 mmol) and potassium carbonate (0.41g,

3 mmol) were refluxed in anhydrous acetonitrile (100mL) for 24hrs under anhydrous conditions. The solvent was removed under reduced pressure and the reaction mixture was resuspended in 1 N HCl (50mL) and chloroform (50mL). The aqueous phase was separated and extracted with chloroform (2x20mL), and the combined organic phases were washed with brine (50mL) and dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure, leaving a brown waxy residue. The product was recrystallized from boiling MeOH/$CHCl_3$ (5:1) with a final mass of 0.3342g (35% yield).

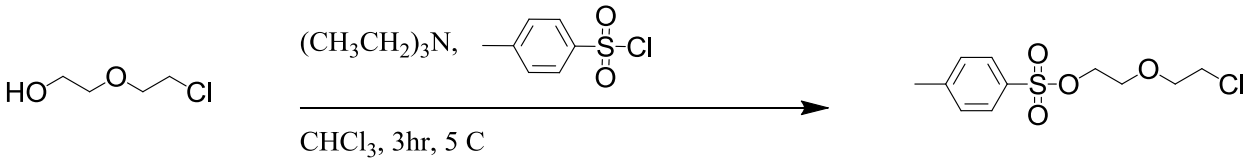
Experiment 2D



Calix[4]arene (0.7536g, 1.7 mmol), 4-bromobutene (0.447g, 3.5 mmol) and potassium carbonate (0.41g, 3 mmol) were combined with deionized water (40mL), chloroform (40mL) with PEG 400 (3g) as a phase-transfer agent. After 2 days of stirring at room temperature, the mixture was neutralized with the addition of 1 N HCL (10mL) and the aqueous layer was discarded. The organic layer was extracted with water (5x20mL) and dried over anhydrous magnesium sulfate. Excess chloroform was removed under reduced pressure, leaving 1.0231g of an oily yellow residue. This crude product was further purified by column chromatography (Silica Gel, MeOH:$CHCl_3$, 3:1) to obtain 0.5328g of pure product (56% yield)

1H NMR (CDCl3): 3.37 (d, 4H) 3.55 (br s, 2H), 4.26 (br s, 2H), 4.34 (d 4H), 5.06 (m, 4H), 5.18 (m, 4H) 6.60-7.05 (m, 12H), 10.21 (s, 2H)

Procedure 3

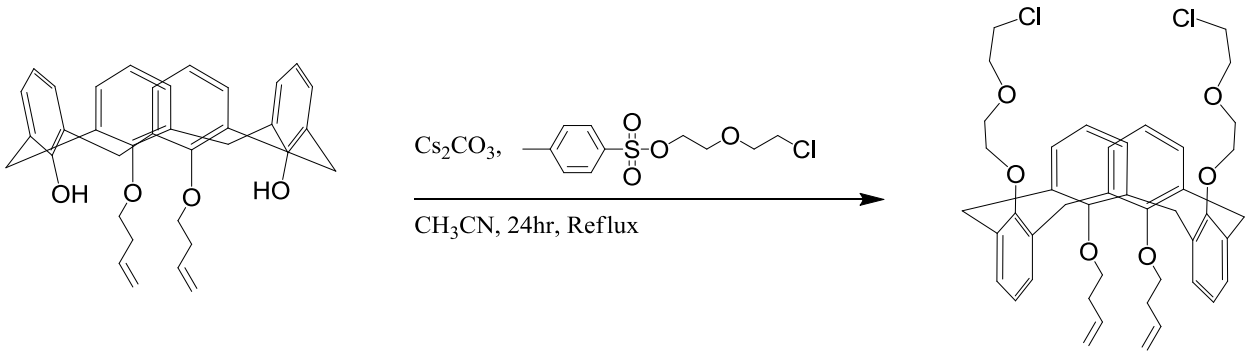


Experiment 3

p-toluenesulfonylchloride (7.626g, 40 mmol) and 2-(2-chloroethoxy)ethanol (3.737g, 30 mmol) were dissolved in DCM (100mL) chilled to 2.5°C. Triethylamine (13.91mL, 100 mmol) was added dropwise, and the solution was allowed to stir with chilling. After 24 hrs, the reaction was quenched by the addition of 1 N HCl (25mL), allowed to warm to room temperature and the aqueous phase was separated and extracted with DCM (2x25mL). The combined organic phases were washed with deionized water (2x25 mL), brine (25mL), and dried over anhydrous magnesium sulfate. Removal of the solvent under reduced pressure gave 9.722g of product (87% yield).

1H NMR (CDCl3): 2.36 (s, 3H), 3.47 (t, 2H), 3.58 (t, 2H) 3.63 (t, 2H) 2.89 (t, 2H), 7.44-7.72 (m, 4H)

Procedure 4



Experiment 4A

A slurry of dialkylated calix[4]arene (0.1327g, 0.25 mmol), 2-(2-chloroethoxy)ethanol p-toluenesulfonate (0.1393g, 0.5 mmol), and cesium carbonate (0.8172g, 2.5 mmol) was suspended in anhydrous acetonitrile (50mL) and heated to reflux. After 24 hours, the reaction mixture was allowed to cool to room temperature and the solvent was removed under reduced pressure. The reaction mixture was then resuspended in $CHCl_3$ (30mL) and 1 N HCl (30mL). The aqueous phase was separated and extracted with chloroform (2x20mL) and the combined organic phases were dried over anhydrous magnesium sulfate. Removal of the solvent and recrystallization from boiling MeOH/$CHCl_3$ (5:1) gave 0.1732g of a colorless crystalline solid (92% yield). Analysis by NMR showed the crystalline solid to exist as a homogeneous mixture of locked product conformers. The crystalline product was further purified by column chromatography (Silica Gel, EtOAc:Hexane, 1:8) although the 1,3-alternate conformer was not isolated when the fractions were examined by NMR. After recombining the fractions, the resultant 0.1354g of product was subjected to a second round of column chromatography (Silica Gel, MeOH:$CHCl_3$, 3:1) yet no pure product was isolated. Recombination of the fractions left a trace amount of product (0.0117g).

Experiment 4B

A slurry of dialkylated calix[4]arene (0.1307g, 0.25 mmol), 2-(2-chloroethoxy)ethanol p-toluenesulfonate

(0.1417g, 0.5 mmol), and cesium carbonate (0.8203g, 2.5 mmol) was suspended in anhydrous acetonitrile (50mL) and heated to reflux. After 24 hours, the reaction mixture was allowed to cool to room temperature and the solvent was removed under reduced pressure. The reaction mixture was then resuspended in $CHCl_3$ (30mL) and 1 N HCl (30mL). The aqueous phase was separated and extracted with chloroform (2x20mL) and the combined organic phases were dried over anhydrous magnesium sulfate. A small aliquout of the light brown solid was analyzed by TLC (EtOAc:Hexane, 1:8) showing by UV light the sulfonate (Rf = 0.83) and a band assumed to be the calixarene conformation isomers (Rf = 0.41) enveloping the starting material (Rf = 0.37). Separate TLC analysis (MeOH:$CHCl_3$, 3:1) separated the sulfonate (Rf = 0.96) and a wider calixarene band (Rf = 0.64) enveloping the starting material (Rf = 0.58). The resulting product (0.1830g, 97% yield) was purified by column chromatography (Silica Gel, MeOH:$CHCl_3$, 3:1) and the fractions obtained were analyzed by TLC to identify those containing calixarene. Pooling these 8 fractions and evaporating the solvent resulted in 0.1231g of colorless crystals (65% yield), however when analyzed by NMR the solid was not a pure conformer.

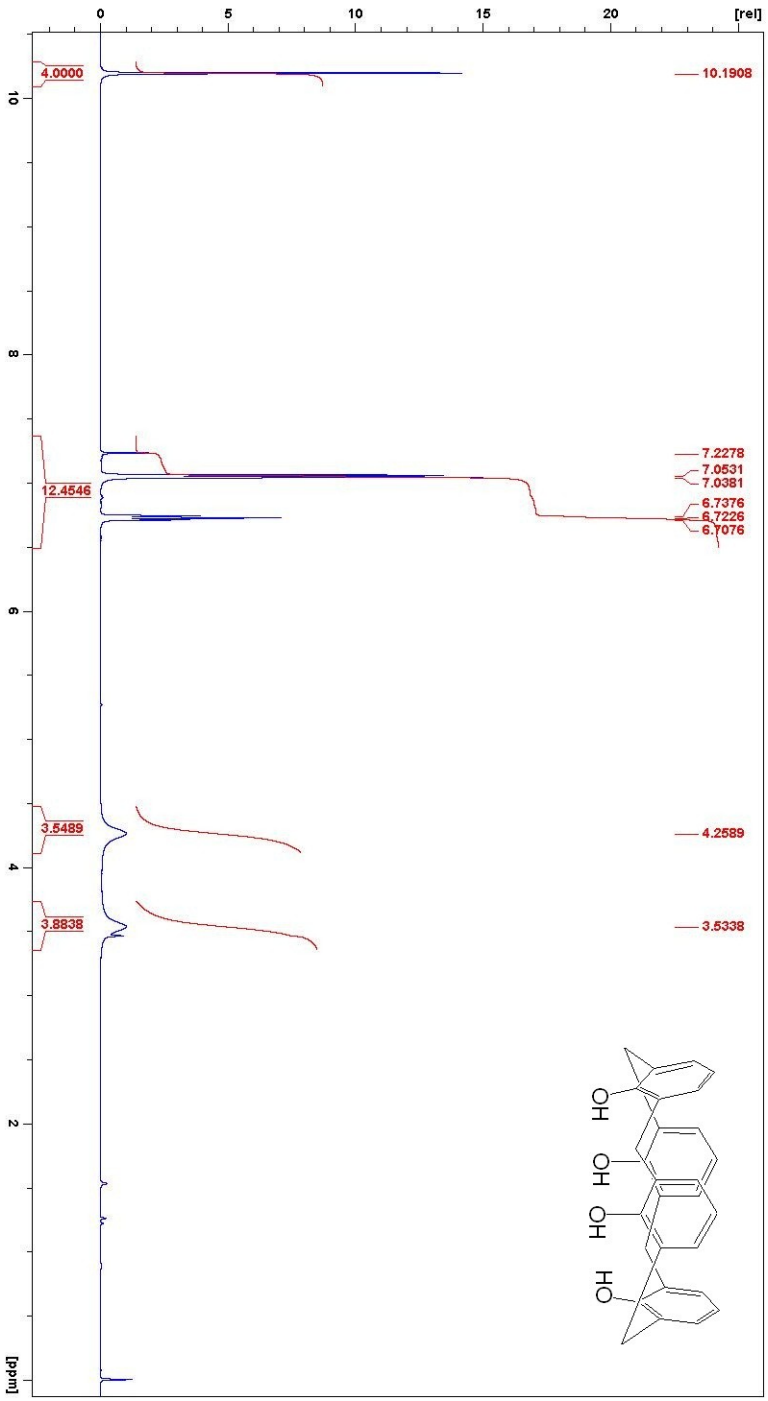
Expected NMR Data:

1H NMR (CDCl3): 3.37 (d, 4H), 3.68 (s, 2H), 3.65 (t, 4H), 3.71 (s, 2H), 3.79 (t, 4H), 3.83 (t, 4H), 4.31 (t, 4H), 4.34 (d 4H), 5.06 (m, 4H), 5.18 (m, 4H) 6.60-7.05 (m, 12H)

Spectral Data

All NMR spetra were acquired in $CDCl_3$ on a 400Mhz Bruker Avance NMR spectrometer.

Procedure 1



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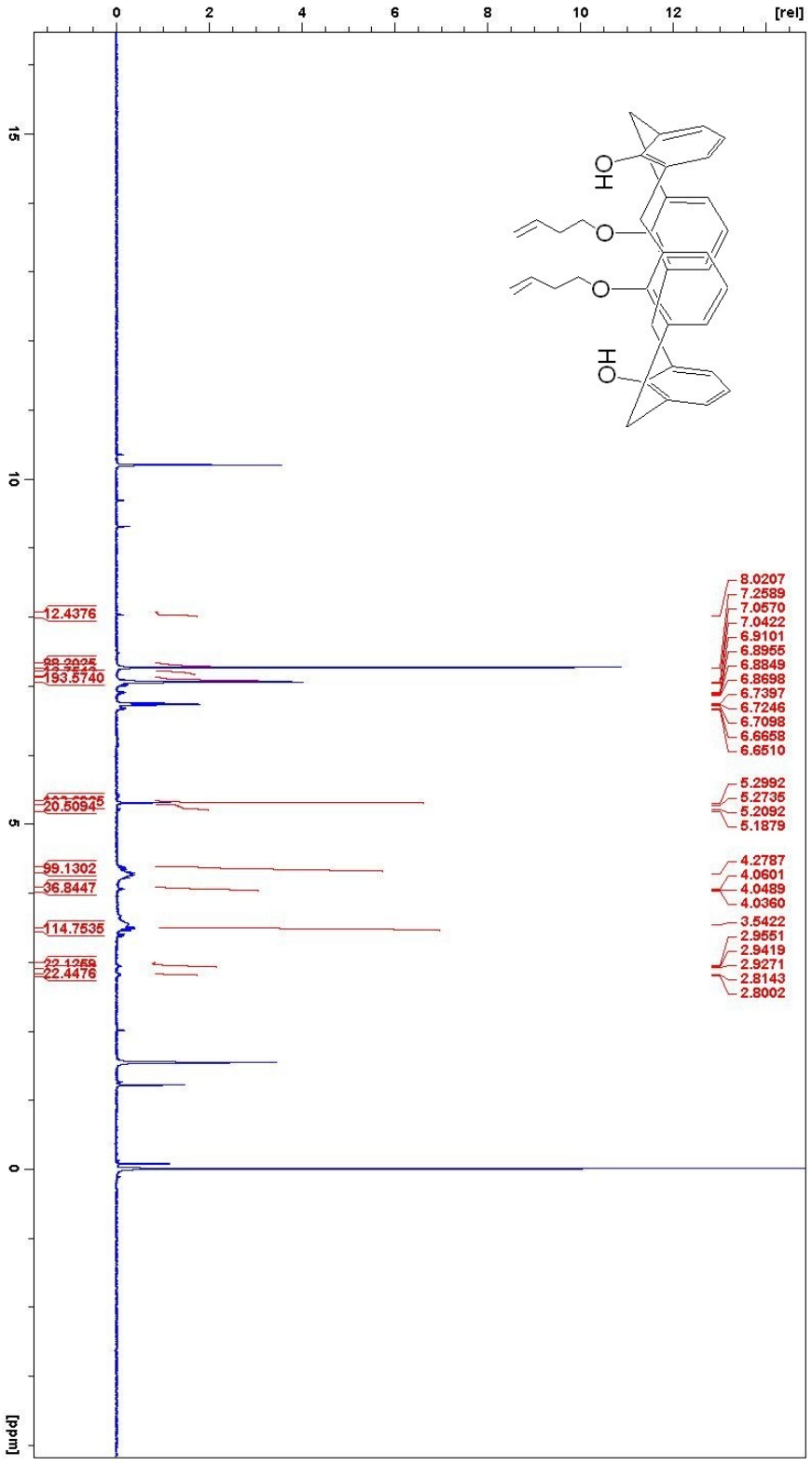
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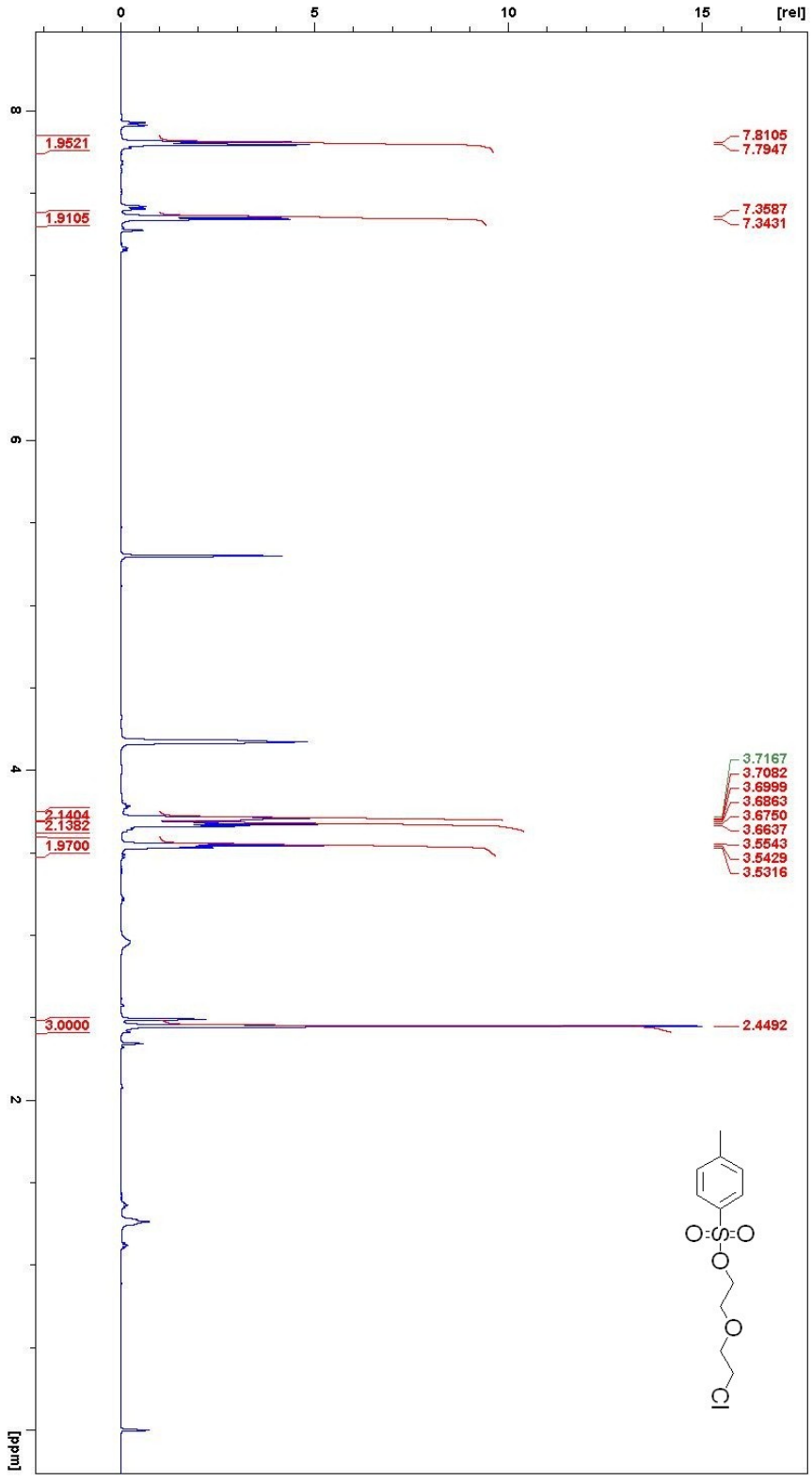
Procedure 3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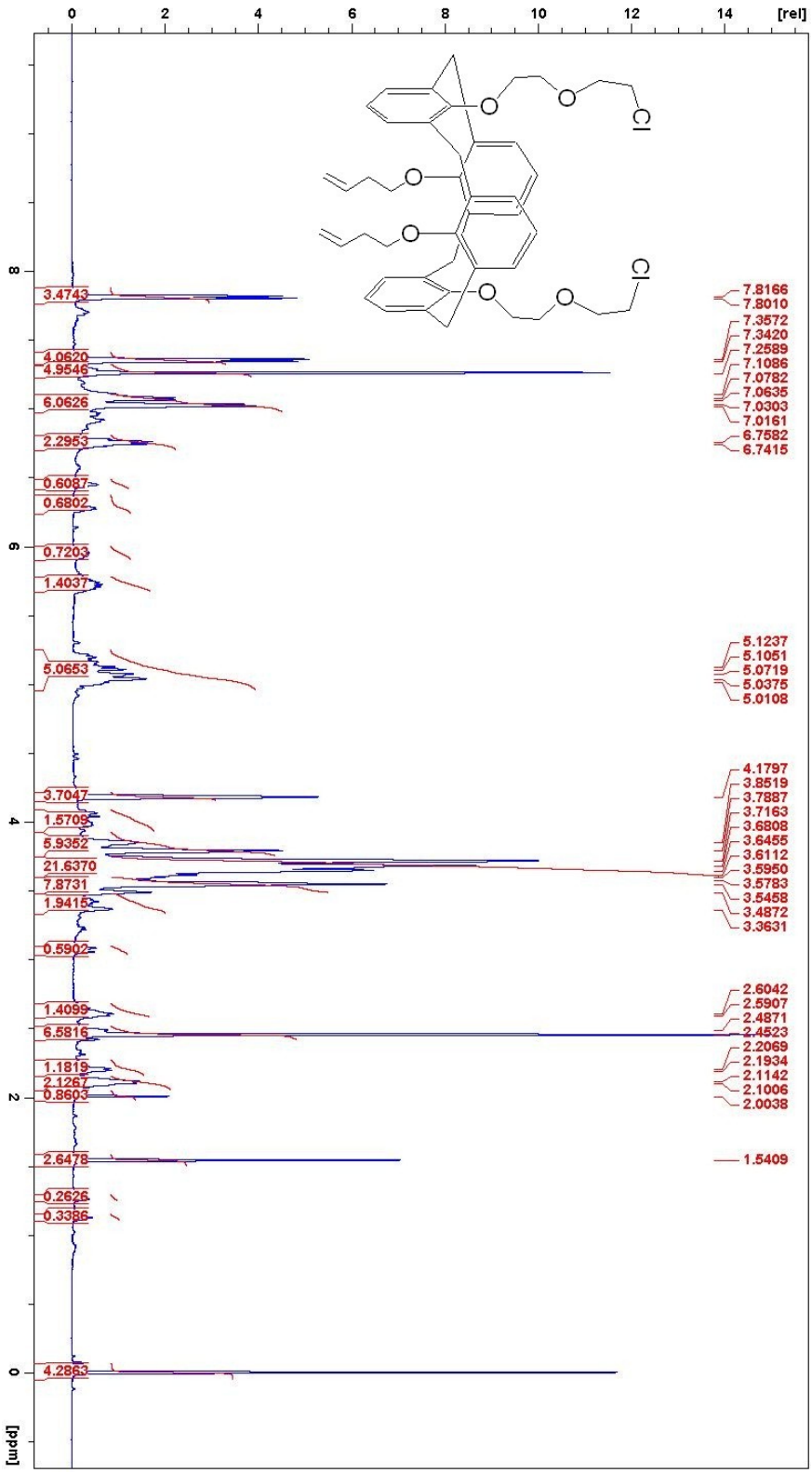
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0.8603
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0.3386
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7.0303
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