JOURNAL OF CHEMICAL EDUCATION

Laboratory Experiment

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# Synthesis and Characterization of Calixarene Tetraethers: An Exercise in Supramolecular Chemistry for the Undergraduate Organic Laboratory

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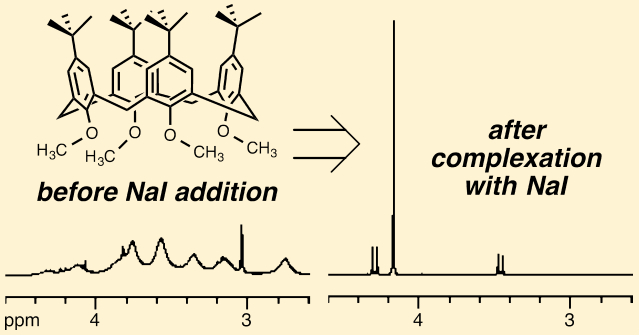
Supporting Information

**ABSTRACT:** In this experiment for an introductory undergraduate organic chemistry lab, students tetraalkylate *tert*-butylcalix[4]arene, a bowl-shaped macrocyclic oligophenol, and examine the supramolecular chemistry of the tetraether product by proton nuclear magnetic resonance (NMR) spectroscopy. Complexation with a sodium ion reduces the conformational mobility of the macrocycle through host–guest interactions, greatly simplifying the $^1$H NMR spectrum of the macrocycle and providing an excellent example of geminal coupling between the diastereotopic protons of the methylene bridges. By dealing explicitly with the organic chemistry of large molecules and host–guest complementarity, this experiment provides a useful pedagogical bridge from small-molecule organic chemistry to the biochemistry of macromolecules such as enzymes.




**KEYWORDS:** *Second-Year Undergraduate, Laboratory Instruction, Hands-On Learning/Manipulatives, Organic Chemistry, Alkylation, Phenols, Conformational Analysis, Noncovalent Interactions, NMR Spectroscopy*

## INTRODUCTION

Supramolecular chemistry, which involves molecular assemblies held together by noncovalent forces, plays significant roles in nanoscience, analytical chemistry, biochemistry, and many other fields.[1] Host–guest interactions are typically covered extensively in biochemistry classes (i.e., in the study of enzyme kinetics). They are less widely studied in organic chemistry classes, although supramolecular catalysts, such as crown ethers (Figure 1a), which sequester cations and allow anions to serve as "free" nucleophiles in substitution reactions, are often discussed. In this *Journal*, laboratory exercises that explore host–guest chemistry are largely confined to the physical chemistry laboratory; these have employed UV–visible spectrophotometry to explore the ability of cyclodextrins (cyclic oligosaccharides, Figure 1b) and crown ethers to bind to small guest ions and molecules.[2,3] Proton nuclear magnetic resonance (NMR) spectroscopy was used in one of these exercises to note the changes in the spectrum of a guest molecule upon complexation with cyclodextrin.[2c]

Supramolecular chemistry is an important pedagogical bridge between organic chemistry and biochemistry; to emphasize this point in our second-year undergraduate organic chemistry classes, a synthetic organic lab experiment was developed that uses $^1$H NMR spectroscopy to explore the host–guest chemistry of the macrocyclic oligophenols known as calixarenes (Figure 1c).[4] These macrocycles present an attractive entry to the study of host–guest interactions for an upper-division undergraduate organic chemistry class. Like cyclodextrins, these cyclic oligophenols have well-defined molecular structures and are readily available. However, calixarenes can be more easily and specifically derivatized than cyclodextrins, on both the wide and narrow rims. As such, they serve as excellent scaffolds for the design of ion sensors and enzyme mimics.[5−7]

An experiment was developed in which a calixarene is tetraalkylated via a straightforward $S_N2$ reaction, proceeding under nonanhydrous, biphasic refluxing conditions in a few hours under phase transfer catalysis (Scheme 1). Upon workup and isolation, the ion-sensor potential of the product tetraether is assessed by $^1$H NMR spectroscopy; dramatic changes in the $^1$H NMR spectrum of the host calixarene are seen upon complexation with a cation of complementary size.

One of the goals in developing this experiment was to emphasize to students that the principles of reactivity they learn in a small-molecule context are also applicable to macromolecules; in this way, calixarenes can form a conceptual bridge from small molecules to very large, complex molecules such as enzymes. Other goals were to underscore the role of symmetry in simplifying NMR spectra and to reinforce the concept of diastereotopicity. Finally, by leading students to think about

**Received:** August 3, 2015
**Revised:** December 16, 2015
**Published:** January 15, 2016





DOI: 10.1021/acs.jchemed.5b00641
*J. Chem. Educ.* 2016, 93, 372−375

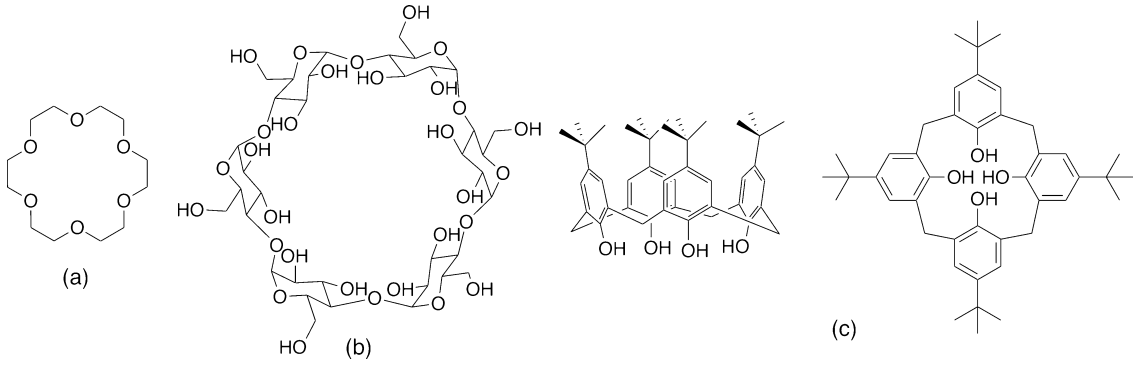
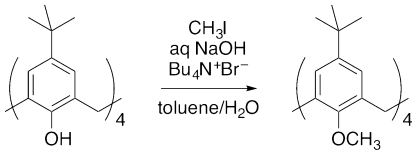



**Figure 1.** Organic macrocycles useful in host–guest chemistry. (a) 18-Crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane). (b) $\alpha$-Cyclodextrin. (c) *tert*-Butylcalix[4]arene (two views).

**Scheme 1. Tetraalkylation of *tert*-Butylcalix[4]arene under Phase Transfer Conditions**




shape and ion complementarity, the basics of macromolecular host–guest interactions were introduced.

## LABORATORY PROCEDURES

### Calixarene Tetraalkylation

The full procedure for this experiment can be found in the Supporting Information. Students prepare a suspension of *tert*-butylcalix[4]arene (220 mg, 0.30 mmol), iodomethane (0.4 mL, 6.0 mmol, 5 equiv per OH), and tetrabutylammonium bromide (29 mg, 0.09 mmol) in toluene (8 mL). The suspension is heated until reflux begins; at this point, aqueous sodium hydroxide solution (50% w/w, 1 mL) is added. The reaction is refluxed for the remainder of the lab period, which is about 3 h; another lab exercise can be carried out while the reaction refluxes. At the end of the lab period, students allow their alkylation reactions to cool and then cap and label their flasks, which are kept in a fume hood until the next lab period.

In the next lab period, students perform a typical aqueous workup, dry their toluene solutions over sodium sulfate, and remove the solvent by rotary evaporation. The white solid is triturated with methanol and obtained by filtration as a white powder.

### NMR Spectroscopy

Students obtain two $^1$H NMR spectra. A known quantity of their tetramethyl product (60 mg, 0.085 mmol) is dissolved in a mixture of chloroform-*d*/acetonitrile-$d_3$ (3:1, v/v). This solution is divided into two parts, one of which is then saturated with sodium iodide and refiltered. The $^1$H NMR spectra of the two solutions are then acquired and compared.

## HAZARDS

*tert*-Butylcalix[4]arene has no hazards listed on its Safety Data Sheet, and the product calixarene tetraether has not been tested for safety; nevertheless, these compounds should be assumed to be toxic irritants, handled only by those with personal protective equipment such as gloves and goggles, and disposed of according to proper hazardous waste procedures. Toluene is highly flammable, irritating to the skin and eyes, harmful to aquatic life, and harmful if inhaled; it should be used only in a fume hood. Iodomethane is toxic with effects on the central nervous system, liver, and kidneys. It is a suspected carcinogen and may be harmful or fatal if swallowed, inhaled, or absorbed through skin. Tetrabutylammonium bromide is irritating to the skin, eyes, and respiratory tract. Concentrated sodium hydroxide solutions are severely caustic to the skin and eyes. Chloroform-*d* is a cancer suspect agent, a mutagen, and a hazard to the skin, eyes, kidneys, and liver. Acetonitrile-$d_3$ is flammable, causes eye and skin irritation, and is harmful if ingested or absorbed through the skin. Sodium iodide and potassium iodide are eye irritants and hazardous when ingested.

## RESULTS AND DISCUSSION

*tert*-Butylcalix[4]arene is commercially available, but expensive (~$20/g from Aldrich); it can also be easily made on a large scale from *tert*-butylphenol and aqueous formaldehyde.[8] An

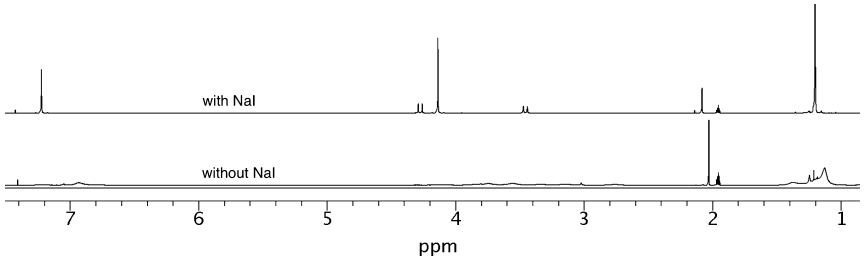



**Figure 2.** $^1$H NMR spectra ($CDCl_3$/$CD_3CN$) of *tert*-butylcalix[4]arene tetramethyl ether before and after saturation with sodium iodide (top and bottom, respectively). Representative student data shown; peaks for $CHCl_3$ and $CH_3CN$ are seen at 7.4 and 2.0 ppm, respectively.



DOI: 10.1021/acs.jchemed.5b00641
J. Chem. Educ. 2016, 93, 372–375

instructor has carried out the latter reaction numerous times to obtain *tert*-butylcalix[4]arene with an occluded toluene molecule that does not affect the alkylation reaction.

Different conditions for the tetraalkylation were tested, and it was found that phase-transfer conditions allowed for consistent yields under nonanhydrous conditions.[9] These conditions also provided an opportunity to discuss phase-transfer catalysis with students and to reinforce concepts such as solubility and intermolecular forces. Alternatively, this alkylation has also been carried out with an oil suspension of sodium hydride as the base and a mixture of tetrahydrofuran and dimethylformamide (10:1) as the solvent.[10] While this reaction typically gave somewhat higher yields in our hands, it sometimes failed completely if students were not able to keep their reactions anhydrous or if the bottle of solvent itself was not kept sufficiently dry. By using the phase-transfer catalytic method, the median purified yield for the class in 2015 was modest, but acceptable (75 mg, 35%, $n$ = 20 pairs); the range of yields, after discarding the reported yields that exceeded 100%, was 2%−74%.

The conformational mobility of this tetraether product at room temperature is evident in its $^1$H NMR spectrum (Figure 2). By preparing NMR samples of this product with and without added metal ions, students assessed how well ions of different sizes can complex to the macrocycle. They found that saturation of the NMR sample with NaI caused the calixarene to adopt the *cone* conformer preferentially,[11] in which all four alkoxy substituents are found on the same "rim" of the macrocycle; this is due to complexation of $Na^+$ to the four methoxy groups on the lower rim. The simplification of the $^1$H NMR spectrum upon addition of NaI was dramatic (Figure 2); no such effect was seen when, for example, KI was substituted for NaI. Previous work[12] using a 1:1 mixture of chloroform and acetonitrile as solvent indicated that this tetraether formed a 1:1 complex with $Na^+$, with an equilibrium constant of formation of $(9.3 \pm 0.7) \times 10^2$ at 320 K. In this experiment, students used a 3:1 mixture of chloroform-*d* and acetonitrile-*d* as a more cost-effective solvent.

Students are given the $^1$H NMR spectrum of the calixarene starting material (see Supporting Information) and acquire $^1$H NMR spectra of the tetraether product and its complex with a sodium ion. The spectra of both the starting material and the tetraether-$Na^+$ complex are simplified by the symmetry of their cone conformers (e.g., one, 8H singlet was seen in the aromatic region), but students are often initially puzzled by the two 4H doublets around 4 ppm. These are the diastereotopic methylene protons of the calixarene bridges, geminally coupled to each other and differing in chemical shift by ∼1 ppm. This large difference in chemical shift underscored the shielding effect that the aromatic rings imposed on the four "inner" protons. At this point of the course, students have previously studied diastereotopicity in other contexts, but this experiment may serve as their first experience with the phenomenon in the lab.

Although calixarenes may, at first, appear large and complex to the typical introductory undergraduate organic chemistry student, their chemistry is typically that of a typical phenol. Simple reactions, such as $S_N2$ *O*-alkylations and electrophilic aromatic substitutions, mainstays of an undergraduate organic chemistry class, can be used to functionalize these macrocycles efficiently,[13] profoundly affecting their shape and conformational flexibility. Metal ions of the appropriate size to complex to all four oxygen atoms simultaneously can bias conformationally mobile calixarenes toward the *cone* conformer. Although it was not explored in these experiments, these ions can also show a "template effect" in the alkylation reactions themselves; when larger alkyl groups are added, the product is obtained as a mixture of noninterconverting conformers. In that case, the choice of base (i.e., NaH vs KH) may dictate the ratio of conformers found in the product mixture.

Feedback from students has been generally positive; most students that responded to an online survey indicated that they agreed that they enjoyed this exercise as much as, if not more than, any other lab exercise they encountered in that organic chemistry class (4.0 average on a five-point Likert scale, $n$ = 28). They also generally agreed that the exercise helped them better understand NMR spectral interpretation (4.6 average), host−guest interactions (3.8 average), and the relationship between small-molecule reactivity and macromolecular reactivity (3.8 average). Some students expressed excitement about leaving "small molecules" behind for a week to work on larger, more complex-looking compounds; students were also impressed by the striking difference in the $^1$H NMR spectrum of the tetramethyl ether upon addition of NaI to the sample. Considering this feedback, we consider this experiment to have met our pedagogical goals in introducing students to the basics of macromolecular host−guest interactions.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00641.

Student laboratory handouts, instructor's notes, student survey results, sample NMR spectra, list of chemicals used (PDF, DOCX)

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

The authors thank the Chemistry 252 (Organic Chemistry II) students at Lawrence University (LU) who carried out this lab and offered helpful advice as well as the LU Chemistry department and the McNair Foundation (B.D.H.) for their support. This material is based upon work supported by the National Science Foundation under CHE-0923473 ("MRI: Acquisition of a 400 MHz NMR Spectrometer to Support Undergraduate Research and Research Training at Lawrence University and the Fox Valley Region of Wisconsin").

## ■ REFERENCES

(1) Lehn, J.-M. *Supramolecular Chemistry: Concepts and Perspectives; A Personal Account*; VCH: Weinheim, Germany, 1995.

(2) (a) Diaz, D.; Vargas-Baca, I.; Gracia-Mora, J. beta-Cyclodextrin inclusion complexes with iodine: an advanced and inexpensive undergraduate chemistry experiment. *J. Chem. Educ.* **1994**, *71* (8), 708−714. (b) Haldar, B.; Mallick, A.; Chattopadhyay, N. Supramolecular inclusion in cyclodextrins: a pictorial spectroscopic demonstration. *J. Chem. Educ.* **2008**, *85* (3), 429−432. (c) Tardajos, G.; González-Gaitano, G. Chemical equilibrium in supramolecular systems as studied by NMR spectrometry. *J. Chem. Educ.* **2004**, *81* (2), 270−274. (d) Baker, G. A.; Crane, N. J.; Mayrhofer, R. C.; Betts, T. A.



DOI: 10.1021/acs.jchemed.5b00641
*J. Chem. Educ.* 2016, 93, 372−375

Cyclodextrin inclusion complexes with a solvatochromic fluorescent probe. *J. Chem. Educ.* **2002**, *79* (10), 1261−1263. (e) Valero, M.; Rodriguez, L. J.; Velázquez, M. M. Determination of thermodynamic parameters of the cyclodextrin inclusion processes: an undergraduate physical chemistry lab experiment. *J. Chem. Educ.* **1999**, *76* (3), 418−419. (f) Hernández-Benito, J.; García-Santos, M. P.; O'Brien, E.; Calle, E.; Casado, J. A practical integrated approach to supramolecular chemistry III. Thermodynamics of inclusion phenomena. *J. Chem. Educ.* **2004**, *81* (4), 540−544.

(3) Peters, S. J.; Stevenson, C. D. The complexation of the $Na^+$ by 18-crown-6 studied via nuclear magnetic resonance. *J. Chem. Educ.* **2004**, *81* (5), 715−717.

(4) (a) Gutsche, C. D. *Calixarenes: An Introduction*; RSC Publishing: Cambridge, UK, 2008. (b) *Calixarenes in the Nanoworld*; Vicens, J., Harrowfield, J., Eds.; Springer: Dordrecht, The Netherlands, 2007.

(5) Sénèque, O.; Rager, M.-N.; Giorgi, M.; Reinaud, O. Supramolecular stabilization of a tris(imidazolyl) Zn−aqua complex evidenced by X-ray analysis: a structural model for mono-zinc active sites of enzymes. *J. Am. Chem. Soc.* **2001**, *123* (34), 8442−8443.

(6) Le Poul, N.; Campion, M.; Douziech, B.; Rondelez, Y.; Le Clainche, L.; Reinaud, O.; Le Mest, Y. Monocopper center embedded in a biomimetic cavity: from supramolecular control of copper coordination to redox regulation. *J. Am. Chem. Soc.* **2007**, *129* (28), 8801−8810.

(7) Izzet, G.; Douziech, B.; Prange, T.; Tomas, A.; Jabin, I.; Le Mest, Y.; Reinaud, O. Calix[6]tren and copper(II): a third generation of funnel complexes on the way to redox calix-zymes. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (19), 6831−6836.

(8) Gutsche, C. D.; Iqbal, M. *p-tert*-Butylcalix[4]arene. In *Organic Syntheses*; White, J. D., Ed.; John Wiley & Sons: New York, 1990; Vol. *68*, pp 234−237.

(9) Bitter, I.; Grün, A.; Ágai, B.; Tôke, L. An easy access to tetra-*O*-alkylated calix[4]arenes of cone conformation. *Tetrahedron* **1995**, *51* (28), 7835−7840.

(10) Iwamoto, K.; Araki, K.; Shinkai, S. Conformations and structures of tetra-*O*-alkyl-*p-tert*-butylcalix[4]arenes. How is the conformation of calix[4]arenes immobilized? *J. Org. Chem.* **1991**, *56* (16), 4955−4962.

(11) Scully, P. A.; Hamilton, T. M.; Bennett, J. L. Synthesis of 2-alkyl- and 2-carboxy-*p-tert*-butylcalix[4]arenes via the lithiation of tetramethoxy-p-*tert*-butylcalix[4]arene. *Org. Lett.* **2001**, *3* (17), 2741−2744.

(12) (a) Blixt, J.; Detellier, C. Kinetics and mechanism of the sodium cation complexation by 5,11,17,23-tetra-p-tert-butyl-25,26,27,28-tetramethoxycalix[4]arene in solution. *J. Am. Chem. Soc.* **1995**, *117* (33), 8536−8540. (b) Blixt, J.; Detellier, C. Conformational dynamics of calixarenes; kinetics of conformational interconversion in 5,11,17,23-tetra-*p-tert*-butyl-25,26,27,28-tetramethoxycalix[4]arene under entropic control. *J. Am. Chem. Soc.* **1994**, *116* (26), 11957−11960.

(13) Thondorf, I.; Shivanyuk, A.; Böhmer, V. Chemical modification of calix[4]arenes and resorcarenes. In *Calixarenes 2001*; Asfari, Z., Böhmer, V., Harrowfield, J., Vicens, J., Eds.; Kluwer Academic Press: Dordrecht, The Netherlands, 2001; pp 26−53.



DOI: 10.1021/acs.jchemed.5b00641
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