COMMUNICATION

Chiroptical Sensing of Amino Acids, Amines, Amino Alcohols, Alcohols and Terpenes with π-Extended Acyclic Cucurbiturils

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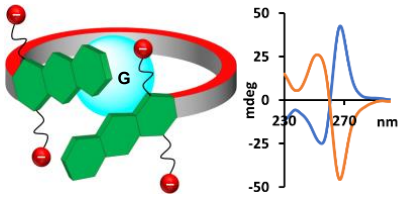
Diandra S. Hassan,[a] Zeus A. De los Santos,[a] Kimberly G. Brady,[b] Steven Murkli,[b] Lyle Isaacs[b] † and Christian Wolf[a] †



The efficiency and scope of two acyclic π-wall extended cucurbiturils, M2 and M3, exhibiting rapidly interconverting helical conformers for chiroptical sensing of amines, amino acids, alcohols, and terpenes at micromolar concentrations in water is evaluated. The formation of 1:1 host-guest complexes results in spontaneous induction of circular dichroism signals that can be used for accurate determination of the absolute configuration and enantiomeric composition of the analyte based on a simple mix-and-measure protocol.

Life processes rely on an intricate web of molecular recognition events and catalytic processes that occur between chiral macromolecules and small molecules.[1] Over the past several decades supramolecular chemists have sought to deepen our understanding of non-covalent interactions (e.g. hydrogen bonds, π–π interactions, metal-ligand interactions, electrostatic interactions, and solvophobic effects) most commonly by studying interactions between achiral hosts and guests.[2] Within this realm, achiral molecular container compounds including calixarenes, cyclophanes, pillararenes, and self-assembled capsules are quite popular and have been used to create drug delivery systems, self-assembled materials, sensing ensembles, and supramolecular catalysts.[3] Cucurbit[n]uril (CB[n], n = 5, 6, 7, 8, 10; Figure 1) molecular containers are achiral (D_{nh}-symmetric) hosts composed of *n* glycoluril rings connected by *2n* CH_2-bridges which define a central hydrophobic cavity and two symmetry equivalent ureidyl C=O portals with a strong dipole moment.[4,5] Accordingly, CB[n] hosts bind strongly to hydrophobic (di)cations in water with K_a values that routinely exceed 10^6 M^{-1}.[4,6] Despite the wealth of molecular recognition studies with supramolecular inclusion complexes,[7] few examples of chirality sensing with achiral macrocyclic hosts that display induced circular dichroism (ICD) signals upon binding of enantiomeric analytes have been introduced[8] and reports with stereodynamic foldamers have remained scarce to date.[9] Because macrocyclic CB[n]s do not possess a good chromophore and are achiral they are poorly suited for (chiroptical) sensing on their own. The Nau group addressed this limitation non-covalently by using CB[n]•dye complexes as the components of indicator displacement assay[10] based sensing arrays to monitor a variety of chemical and biological species and processes.[11] Other groups have covalently attached chromophores to the CB[n] framework for sensing and imaging purposes.[12] To enable chirality sensing within achiral CB[n], the Nau group allowed chiral analytes to bind to CB[8]•dye complexes to create chiral CB[8]•dye•analyte ternary complexes that could be detected by induced circular dichroism.[13]

In the past decade, the Isaacs group has created acyclic CB[n]-type receptors that feature a central glycoluril oligomer that is capped with aromatic sidewalls.[14] These acyclic CB[n] possess a preorganized C-shaped conformation and retain the essential binding properties of macrocyclic CB[n] but with higher water solubility and better optical properties. Acyclic CB[n] have been used to solubilize insoluble drugs in water, as an *in vivo* reversal agent for neuromuscular blockers and drugs of abuse, and as a component of fluorescence sensing arrays.[15]

[a] *Chemistry Department, Georgetown University, Washington, DC 20057, USA*
[b] *Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, USA*
† LIsaacs@umd.edu cw27@georgetown.edu
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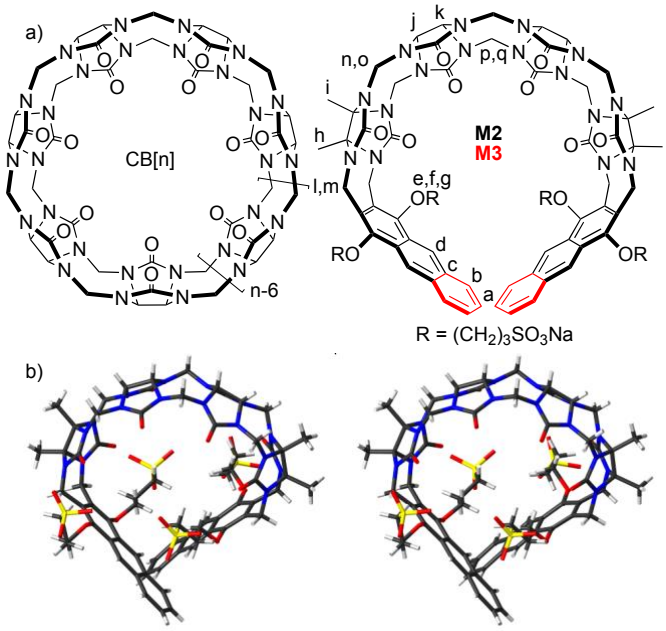

Figure 1. a) Chemical structures of CB[n], **M2**, and **M3**. and b) cross-eyed stereoview of the X-ray crystal structure of **M3**[16] illustrating the helical chirality.

Recently, we reported that the extension of the naphthalene walled **M2** to the anthracene walled **M3** results in a helically chiral structure that is formed to alleviate steric interaction between the walls (Figure 1).[16] Most recently, Biedermann and co-workers utilized the acyclic CB[n]-type receptor **M2** and observed characteristic ICD fingerprints with amino acids, peptides, terpenes, and steroids that can be used for qualitative analyte identification and to monitor racemization processes.[17]

We have evaluated the chiroptical sensing properties of the acyclic CB[n]-type receptors **M2** and **M3** toward a broad selection of chiral compounds that are naturally CD-silent in the region of interest and now show that aromatic wall extension at the termini of the acyclic CB[n] scaffold significantly improves the chiroptical sensing utility, enabling ICD analysis at single digit micromolar concentrations. We found that the anthracene-derived cucurbituril **M3** gives more red-shifted CD maxima compared to **M2** under otherwise identical conditions which is generally desirable because it reduces the likelihood of interference from CD-active impurities that may be present in real-world samples. The remarkable chiroptical sensing capability of **M3** is further highlighted with the determination of the enantiomeric ratio (*er*) of an amine. Several samples covering a wide *er* range were analyzed with an absolute error margin of less than 5%.

As described above, **M2** and **M3** exhibit an out of plane twisting that results in an overall helically chiral structure. Samples of **M2** and **M3** are, of course, a racemic mixture of isoenergetic *M*- and *P*-enantiomers, and can also assume C_{2v}-symmetric structures in a guest dependent manner. Based on previous results,[16] we know that complexes of **M2** and **M3** interconvert rapidly between the two senses of chirality. Upon complexation with a chiral guest compound, the enantiomeric hosts are transformed into a rapidly equilibrating mixture of diastereomeric binary host•guest complexes. The imprinting of the guest chirality onto the stereodynamic host scaffold can be directly observed by the appearance of ICD signals originating from the peripheral aromatic units in **M2** and **M3**. Recently, we showed that extension of the aromatic sidewalls from naphthalene to anthracene units enhances the optical properties by generating a red-shifted UV-Vis signal with increased molar absorptivity, and it also results in stronger host-guest interactions.[16] To examine the usefulness of **M2** and **M3** to differentiate between the enantiomers of chiral guests, we selected a total of 21 ammonium salts, amino acids, alcohols, and terpenes, some carrying a small aromatic ring that could interact with the chromophoric host termini and thus contribute to the induction of CD signals while others, for example compounds **8** and **20**, are purely aliphatic structures (Figure 2).

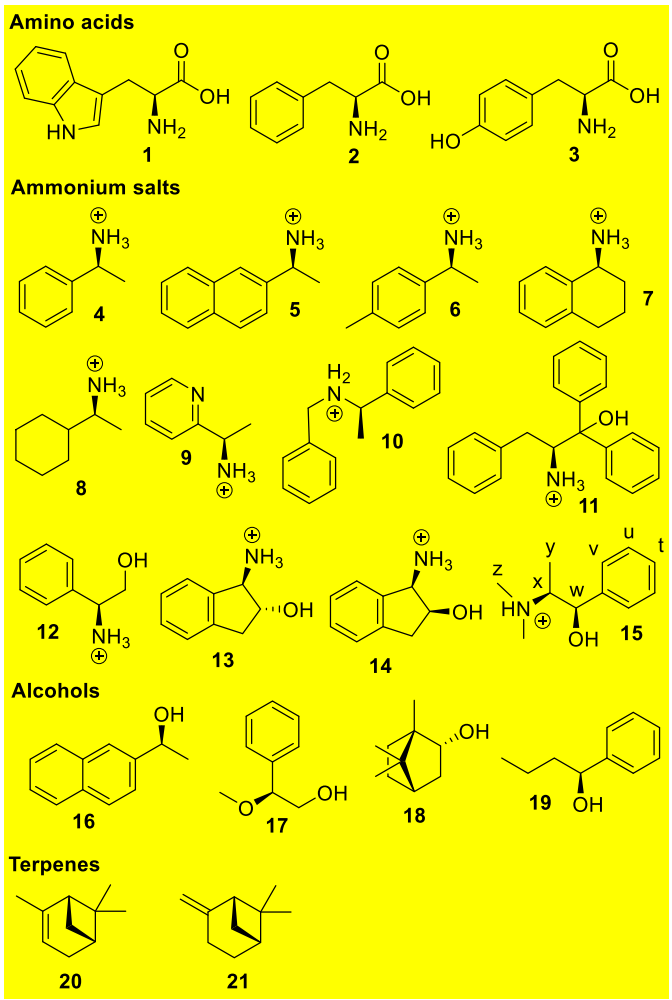

Figure 2. Structures of the amino acids, ammonium salts, alcohols and terpenes **1**-**21**. Only one enantiomer is shown.

To establish a direct comparison between **M2** and **M3**, we tested both sensors with a guest panel (**1** - **21**) which included amines and amino alcohols **5**, **8**, **13**, and **14** under identical conditions (Figure 3 and ESI). The host guest complexation is fast and we were able to collect CD spectra within a few minutes

after mixing of the host and guest at 5.0 µM concentrations. It is noteworthy that both **M2** and **M3** display distinct CD signals at low micromolar concentrations which is not possible with the large majority of chiroptical sensors reported to date. The good performance of **M3**, which has not been employed in CD chirality sensing studies to date, is particularly promising with regard to quantitative *er* analysis, *vide infra*. We found that the CD signals generated by **M3** are more red-shifted than those obtained with **M2** (Figure 3A and 3B) The **M3** host also exhibits a stronger signal than **M2** in most cases, another feature that favors its use for quantitative *er* determination purposes. However, this was not always the case and we observed that the complexation of the amino alcohols **13** and **14** affords larger CD amplitudes when the smaller host **M2** is used (Figure 3C and 3D).

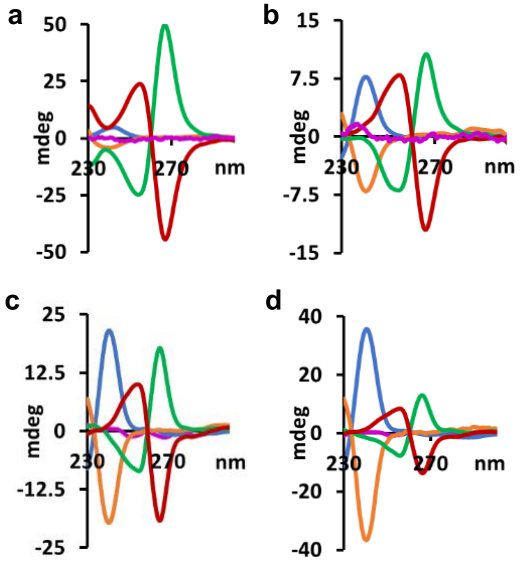

Figure 3. Induced CD signals of the assemblies obtained upon addition of equimolar amounts of **5**, **8**, **13**, and **14** (from left to right) to **M2** (blue and orange) and **M3** (red and green), respectively. a) **M2**•*R*-**5** (blue), **M2**•*S*-**5** (orange), **M3**•*R*-**5** (green), **M3**•*S*-**5** (red); b) **M2**•*R*-**8** (blue), **M2**•*S*-**8** (orange), **M3**•*R*-**8** (green), **M3**•*S*-**8** (red); c) **M2**•*R,S*-**13** (blue), **M2**•*S,R*-**13** (orange), **M3**•*R,S*-**13** (green), **M3**•*S,R*-**13** (red); d) **M2**•*R,R*-**14** (blue), **M2**•*S,S*-**14** (orange), **M3**•*R,R*-**14** (green), **M3**•*S,S*-**14** (red). Control experiments with enantiopure analytes in the absence of sensor gave negligible CD effects in the region of interest (shown in purple). The mixtures were stirred for 15 minutes at 25 °C and CD analysis was conducted at 5.0 µM in deionized water using a quartz cuvette with a 10 mm path length.

With a practical chirality sensing protocol in hand, we further applied **M2** and **M3** to the diverse group of analytes shown in Figure 2. For example when **M3** and guests like *N*-methylpseudoephedrine, **15**, 1-(2-naphthyl)ethanol, **16**, borneol, **18**, and pinene, **21**, which represent a variety of compound classes were mixed at 5.0 µM (Figure 4 and ESI), we observed distinct ICD signals in all cases using stoichiometric amounts of sensor and analyte. We noticed that **M2** generally gives CD maxima at approximately 240 nm at 5.0 µM whereas a new ICD signal appears at 290 nm at 50.0 µM concentration (ESI). A similar trend was observed with **M3** which affords a CD maximum at 270 nm at 5.0 µM but can also produce a relatively small ICD signal at 380 nm when the concentration of the host guest complex is increased to 50.0 µM. Overall, the ability of **M3** to generate strong ICD responses upon binding of a variety of chiral analytes at low micromolar concentrations stands out.

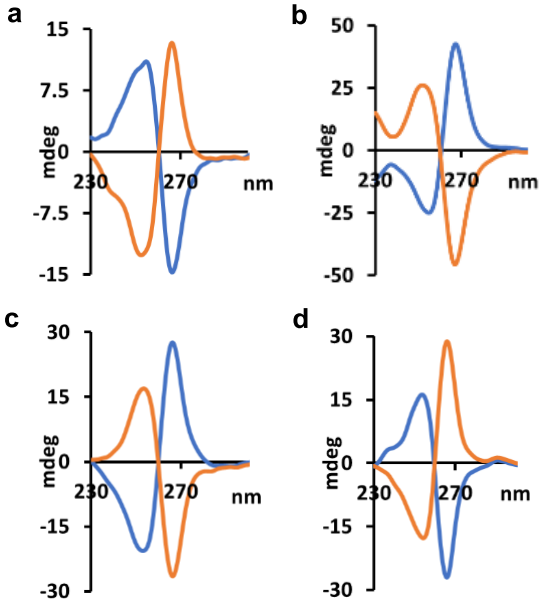

Figure 4. CD Spectra of the assemblies obtained upon addition of equimolar amounts of **15**, **16**, **18** and **21** to **M3**. CD spectra obtained with the *R* enantiomers of **15**, **16** and **18** or with (+)-**21** are shown in blue and with the *S* enantiomers of **15**, **16** and **18** or with (-)-**21** in orange, respectively. The CD analysis was performed at 5.0 µM using a quartz cuvette with a 10 mm path length. See ESI for details.

To glean information regarding the mode of interaction between the hosts and guests, we performed ^{1}H NMR experiments between hosts **M2** and **M3** and selected guests (**2**, **6**, **13**, **15**, **16**, **17**, **20**, and **21**) at 1:1 and 1:2 host:guest stoichiometry. Figure 5a and 5g show the ^{1}H NMR spectra for hosts **M2** and **M3** whereas Figure 5d shows the spectrum for guest **15** (see Figures 1 and 2 for proton assignments). The ^{1}H NMR spectrum of **M2** is well resolved and can be fully assigned (Figure 5a) whereas for **M3** the resonances are broadened due to aggregation at room temperature and the assignments are based on the high temperature measurements reported previously.[16] The ^{1}H NMR spectrum for **M2•15** (Figure 5c) displays a number of interesting features. First, the resonances for guest aromatic protons H_t, H_u, and H_v as well as methyl protons H_y shift substantially (> 1 ppm) upfield upon complexation which indicates that the aromatic ring and methyl group H_y are located in the magnetic shielding region defined by the aromatic walls and cavity of **M2** as expected. At a 1:2 **M2**:**15** stoichiometry (Figure 5b) we observe dramatically broadened but separate resonances for H_t, H_u, H_v, and H_y (free **15**) and $H_{t'}$, $H_{u'}$, $H_{v'}$, and $H_{y'}$ (**M2•15**) which indicates that the guest exchange process is in the intermediate exchange regime on the chemical shift timescale. Conversely, upon the formation of **M2•15**, we observed the downfield shifting and splitting of the resonances for H_c and H_d of the aromatic walls. In the free host **M2**, the aromatic walls engage in edge-to-face π–π interactions with each other and are thus upfield shifted; upon formation of **M2•15** these interactions are disrupted which results in the observed downfield shifting. Host **M2** has time averaged C_{2v}-symmetry and therefore only one set of resonances are observed for H_c and H_d. However, upon formation of the chiral and enantiomerically pure **M2•15** complex the aromatic resonances split into four distinct resonances. This observation is consistent with but does not require that host **M2** adopting a helical conformation in the

M2•15 complex. Unfortunately, the fast dynamics of interconversion of the M- to P-helical forms prevented us from observing the two diastereomeric forms (e.g. M-**M2•15** and P-**M2•15**). Figure 5e shows the ^{1}H NMR spectrum for the **M3•15** complex which similarly displays upfield shifting of the aromatic (H_t, H_u, H_v) and methyl resonances (H_y) upon complexation indicating cavity inclusion of the aryl ring and CH_3-group. At a 1:2 **M3**:**15** ratio only a single set of broadened resonances are observed for guest **15** which indicates the guest exchange process in in the intermediate to fast exchange regime on the chemical shift timescale. Once again, a splitting of the resonances for the anthracene walls (H_a, H_b, H_d) into more than three resonances is consistent with the formation of the chiral **M3•15** complex. Related ^{1}H NMR measurements were made for hosts **M2** and **M3** with guests **2**, **6**, **13**, **16**, **17**, **20**, and **21** (ESI). Similar trends in ^{1}H NMR chemical shifts and multiplicity were observed which indicates that the central hydrophobic portion of the guest resides inside the cavity of the host with the cationic and polar functional groups located at the C=O portals. Overall, the NMR results firmly establish the basic geometrical features of the complexes and also provide evidence for the formation of chiral and potentially helical host guest complexes at the origin of the observed induced circular dichroism signals.

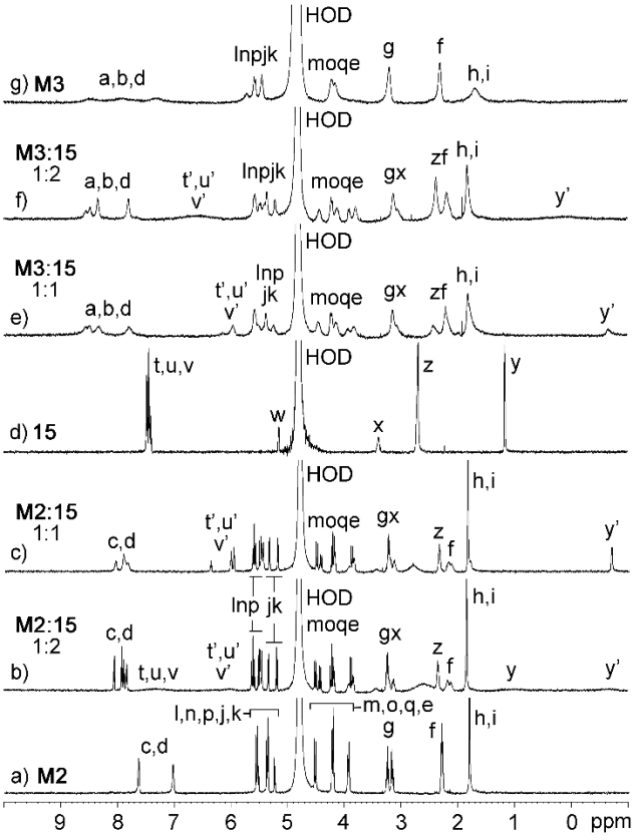

Figure 5. 1H NMR spectra recorded (600 MHz, D_2O, 25 °C) for: a) **M2**, b) 1:2 ratio of **M2**:**15**, c) 1:1 ratio of **M2**:**15**, d) **15**, e) 1:1 ratio of **M2**:**15**, f) 1:2 ratio of **M2**:**15**, g) **M3**. Primed resonances refer to bound guest under slow exchange kinetics.

Using the same protocol, we assessed the utility of **M3** for the determination of enantiomeric ratio of chiral nonracemic samples. Enantiomeric mixtures of **5** were prepared and subjected to chiroptical sensing with **M3**. Plotting of the enantiomeric excess versus the induced CD amplitudes at 268 nm produced a linear response which is in agreement with the formation of 1:1 host•guest complexes (Figure 6). We then applied **M3** to several samples containing **5** in varying enantiomeric compositions (Table 1). The sensing analysis of the ICD spectra with the previously obtained calibration curve showed that our assay correctly identifies the absolute configuration of the major enantiomer and allows quantification of the enantiomeric ratio with good accuracy. The error margin remains within a few percents which is generally considered acceptable for high-throughput applications.[9g] For example, the sensing of a sample having a 95.0:5.0 (*R*:*S*) composition gave almost the same ratio of 95.7:4.3 (*R*:*S*) (entry 2). These sensing results demonstrate the potential of acyclic cucurbiturils for high-throughput *er* analysis which can nowadays be conducted with commercially available plate readers.[18]

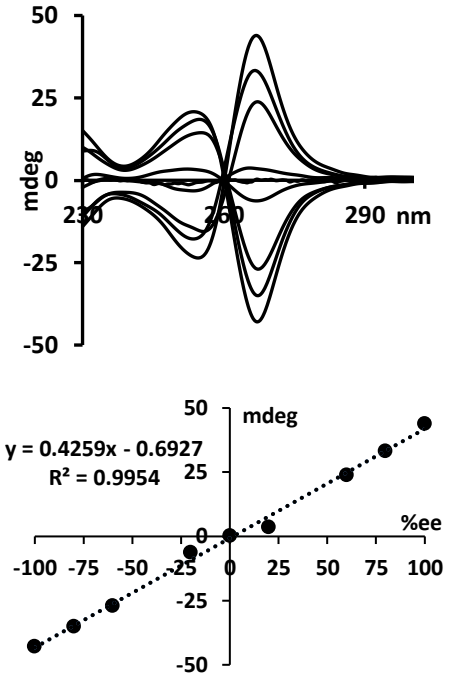

Figure 6. Induced CD signals observed upon addition of samples of amine **5** of different enantiomeric excess values to **M3**, and linear plot of the chiroptical sensor response measured at 268 nm.

Table 1. Determination of the enantiomeric composition of mixtures of 5 with M3.

Entry	Sample composition Absolute config.	(*R*) : (*S*)	CD sensing results Absolute config.[a]	(*R*) : (*S*)[b]
1	(*S*)	30.0 : 70.0	(*S*)	26.2 : 73.7
2	(*R*)	95.0 : 5.0	(*R*)	95.7 : 4.3
3	(*R*)	85.0 : 15.0	(*R*)	82.1 : 17.9
4	(*S*)	45.0 : 55.0	(*S*)	48.4 : 51.6
5	(*S*)	25.0 : 75.0	(*S*)	24.1 : 75.9
6	(*S*)	35.0 : 65.0	(*S*)	39.8 : 60.2

[a]Based on the sign of CD response. [b]Based on the amplitude of the CD response at 268 nm.

Conclusions

In summary, we have demonstrated the ability of acyclic cucurbiturils (**M2** and **M3**) to generate circular dichroism signals upon binding of chiral ammonium salts, alcohols, amino acids, and terpenes in an aqueous environment. Comparison of **M2** and **M3** showed that the extended peripheral aromatic walls in the latter are advantageous because stronger, red-shifted CD effects generated upon stoichiometric analyte complexation are typically observed. This study proves that acyclic CB[n] have a comprehensive chirality sensing scope spanning a variety of compound classes and allow convenient determination of the absolute configuration and enantiomeric composition of chiral samples. The utility and practicality of this approach were demonstrated with the successful quantitative analysis of several samples containing a chiral amine in vastly different enantiomeric compositions.

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Conflicts of interest

L.I. is an inventor on patents held by University of Maryland on acyclic cucurbit[n]urils. The other authors declare no competing financial interest.

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