Locating Regions of Maximum Chiral Discrimination: A Computational Study of Enantioselection on a Popular Chiral Stationary Phase Used in Chromatography

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Abstract: The concept of maintaining spatial congruence between substrate binding site and regions of greatest enantiodifferentiation to ensure efficient chiral recognition in host−guest chemistry is described in this paper. Regions of maximum chiral recognition were located by determining Boltzmann-weighted intermolecular energies of chiral probe molecules placed at well-defined grid points around a molecule and then evaluating the magnitude of (dis)-similarity of interaction at each grid point. Sites having little or no energy differences between enantiomeric probes are nondiscriminatory while those of greatest energy difference correspond to regions of maximum chiral discrimination. Seven analyte molecules containing a diverse set of organic functional groups were evaluated when binding to permethylated *β*-cyclodextrin, a popular chiral stationary phase used in chromatography. The preferred binding site for host−guest association is the interior of the cyclodextrin, and the region of maximum discrimination is found to coincide with this location for all analytes studied. Forcing the guests to bind to the exterior of the macrocycle by blocking the interior of the cyclodextrin is predicted to reduce or eliminate resolution. A literature report confirming this prediction is cited.

Introduction

Although the intermolecular forces have been exhaustively studied and are well documented,[1] precisely how these forces act, in concert, to discriminate between molecules has only been studied in a comprehensive manner during the past decade. These studies have taken place under the aegis of research in "molecular recognition".[2] A subset of molecular recognition is chiral recognition.[3] Here, in contrast to more common experiments where one molecule is asked to differentiate between others based on differences in size, shape, charge, or other physicochemical properties, chiral discrimination is far more subtle. In chiral discrimination the molecules being discriminated have the same size, same shape, same molecular electrostatics, etc.; they can only be discerned when giving rise to slightly different diastereomeric responses once they associate with another chiral object or environment. The intermolecular forces responsible for enantiodiscrimination are the same as those in other cases of molecular recognition, but the corresponding differential free energies are usually much smaller in magnitude.

Chiral discrimination is the basis of chiral chromatography.[4] Advances in chromatographic techniques for planar (TLC),[5] gas,[6] liquid,[7] and super- and subcritical fluid phase chromatographies[8] along with more recent developments in capillary

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(1) A well-written introductory textbook exists: Rigby, M.; Smith, E. B.; Wakeham, W. A.; Maitland, G. C. *The Forces Between Molecules*; Oxford Science Publications, Clarendon Press: Oxford, 1986.

(2) For major reviews see: (a) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27* (8), 1009. (b) Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27* (8), 89. (c) Rebek, J. Jr., *Angew. Chem., Int. Ed. Engl.* **1990**, *29* (3), 245. (d) Schneider, H.-J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1417. (e) Atwood, J. L.; Davies, J. E. D.; MacNirol, D. D.; Vögtle, F.; Lehn, J.-M.; Gokel, G. W., Eds. *Comprehensive Supramolecular Chemistry*, Pergamon Press: Oxford, 1996; especially Vols 1−3.

(3) Webb, T. H.; Wilcox, C. S. *Chem. Soc. Rev.* **1993**, 383 and references therein.

(4) (a) Souter, R. W. *Chromatographic Separations of Stereoisomers*; CRC Press: Boca Raton, 1985. (b) Zeif, M.; Crane, L. Eds. *Chromatographic Chiral Separations*; Chromatographic Science Series, Vol. 40; Marcel Dekker: New York, 1987. (c) König, W. A. *The Practice of Enantiomer Separation by Capillary Gas Chromatography*; Hüthig: Heidelberg, 1987. (d) Hinze, W. L.; Armstrong, D. W., Eds. *Ordered Media in Chemical Separations*; ACS Symp. Ser. No. 342; American Chemical Society: Washington, DC, 1987. (e) Allenmark, S. G. *Chromatographic Enantioseparation. Methods and Application*; Ellis Horwood: Chichester, 1988. (f) Stevenson, D.; Wilson, I. D., Eds.; *Chiral Separations*; Plenum: New York, 1988. (g) *Chiral Liquid Chromatography*; W. J., Lough, Ed. Blackie: London, 1989. (h) Stevenson, D.; Wilson, I. D., Eds. *Recent Advances in Chiral Separations*; Plenum: New York, 1990. (i) Ahuja, S., Ed. *Chiral Separations by Liquid Chromatography*; ACS Symp. Ser. No. 471; American Chemical Society: Washington, DC, 1991. (j) Subramanian, G., Ed.; *Chiral Separations by Liquid Chromatography*, VCH: Weinheim, 1994. (k) Ahuja, S., Ed.; *Chiral Separations. Applications and Technology,* American Chemical Society: Washington, DC, 1997.

(5) For reviews see: (a) Ward, T. J.; Armstrong, D. W. *J. Liq. Chromatogr.* **1986**, *9*, 407. (b) Martens, J.; Bhushan, R. *Chem. Ztg.* **1988**, *112*, 367. (c) Martens, J.; Bhushan, R. *Int. J. Peptide Protein Res.* **1989**, *34*, 433. (d) Martens, J.; Bhushan, R. *J. Pharm. Biomed. Anal.* **1990**, *8*, 259. (e) Bhushan, R.; Joshi, S. *Biomed. Chromatogr.* **1993**, *7*, 235.

(6) Pertinent discussions can be found in ref 4; for additional reviews concerning gas chromatocraphic applications of cyclodextrins see: (a) König, W. A. In *Drug Stereochemistry, Analytical Methods and Pharmacology*; Wainer, I. W., Drayer, D. E., Eds.; Marcel Dekker: New York, 1988; pp 113−145. (b) Schurig, V.; Nowotny, H.-P. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 939. (c) Jung, M.; Mayer, S.; Schurig, V. *LC-GC* **1994**, June, 458. (d) Mani, V.; Wooley, C. *LC-GC* **1995**, Sept, 734.

(7) Pertinent discussions can be found throughout ref 4; for additional reviews concerning applications of cyclodextrins in liquid chromatography see: (a) Armstrong, D. W.; Alak, A.; Bui, K.; DeMond, W.; Ward, T.; Riehl, T. E.; Hinze, W. L. *J. Inclusion Phenom.* **1984**, *2*, 533. (b) Armstrong, D. W. *J. Liq. Chromatogr.* **1984**, *7*, 353. (c) Beesley, T. E. *Am. Lab.* **1985**, May, 78. (d) Ward, T. E.; Armstrong, D. W. *J. Liq. Chromatogr.* **1986**, *9*, 407. (e) Armstrong, D. W.; Han, S. *CRC Crit. Rev. Anal. Chem.* **1988**, *19*, 175. (f) Johns, D. *Am. Lab.*, **1987**, *January*, 72. (g) Armstrong, D. W. *Anal. Chem.* **1987**, *59*, 84. (h) Pettersson, C. *Trends Anal. Chem.* **1988**, *7*, 209. (i) Armstrong, D. W.; Hilton, M.; Coffin, L. *LC-GC* **1991**, *Sept*, 646. (j) Husain, N.; Warner, I. M. *Am. Lab.* **1993**, *Oct*, 80.

electrophoresis with chiral mobile phase additives[9] have been impressive. The impact of these analytical techniques in several subdisciplines of the chemical and pharmaceutical sciences has been substantial: one can now assess enantiomer purity as well as purify optical isomers by one or more of these methods in a quick and routine manner. In chiral chromatography one typically uses a chiral stationary phase (CSP) that interacts, in an enantiodiscriminating way, with passing analytes of the mobile phase. A rich history has evolved concerning the conceptual ideas leading to these CSPs, and how they were designed, implemented, and tested for such tasks.[4] At the heart of these chiral resolutions taking place on column is the molecular recognition process itself[10] that has been studied by our group and others using computational chemistry.[11]

One class of materials that has been prominent as a CSP as well as for use as a mobile phase additive is the cyclodextrins,[12] prototypical host−guest complexers that have far more uses than just in chromatography.[13] The cyclodextrins are inherently chiral, being composed of α-D-glucose moieties each containing five stereogenic centers. We recently developed a computational protocol that accurately predicts the relative retention orders and magnitudes of chiral recognition of these CSPs.[14] In that study we also described which forces were most responsible for holding the binary complexes together, and which forces were most responsible for chiral discrimination. A key issue in that study was the following: Where do analytes tend to bind? Do they selectively bind to the interior or to the exterior of the macrocycle, and do they do this at the primary or the secondary rim of the macrocycle? This was an especially important question to answer because the gas chromatographic environment is distinctly different from traditional aqueous environments that are well-known to drive nonpolar guests into the host cavity via the hydrophobic effect. Without that driving force it was not clear where the analytes preferred to bind. Moreover, a detailed extrathermodynamic study of these CSPs in the gas phase by Berthod, Li, and Armstrong showed clear evidence for some analytes binding to the interior but others binding to another domain that was presumed to be the exterior.[15]

The Issue

Knowing where analytes tend to bind in or around a cyclodextrin is important, but this constitutes only part of what leads to effective chiral separations. Another aspect that is critical for effective resolutions is knowing which region of the CSP is most discriminating. This is especially important because if the preferred binding site differs from the site that is most highly discriminating, one is relegated to an inferior region of chiral selection leading to loss of discriminatory power or even no recognition at all. This can be illustrated by considering a cyclodextrin, or any other chiral host−guest complexer for that matter, where if the preferred binding site exists on the interior of the host but the most chiral discriminating region is on the exterior of the host one loses discriminatory power.

This is a general issue that seems not to have been discussed in the literature and one that is important enough to play a major role in, e.g., the design of chiral catalysts, improved chiral stationary phases for chromatography or any other area of science involving chiral discrimination. Having *a priori* knowledge of which region of a host is most discriminatory is especially useful because one could then devise techniques to force the substrate to bind at that less-favored but more discriminatory site. In terms of chiral chromatography with, say, cyclodextrins this could be as simple as adding to the mobile phase a competing substrate that binds exclusively to the interior of the cyclodextrin thus forcing the guest to the less favored and more discriminating exterior of the cyclodextrin. In terms of chromatography this would first reduce the retention time of the analytes on the column and secondly enhance the resolution, both of which are desirable traits. For other systems more complicated means of forcing the substrate to an alternative site could be envisioned.

This issue of finding and using the most chiral region of a molecule for asymmetric induction or for chiral recognition is something that we have yet to see described in the literature. We introduce here the "principle of maximum chiral recognition", which we posit as the case when a guest's binding site is spatially coincident with the receptor's site of greatest enantiodifferentiation. This is important and we use this journal for bringing it to the attention of scientists interested in molecular recognition. How, then, does one define the most enantiodiscriminating region of a molecule?

Method for Defining the Most Enantiodifferentiating Region of a Molecule

Note that we do not focus on "the most chiral molecule" nor on "the most chiral region" of a molecule but rather on the most enantiodiscriminating region of a molecule. The two concepts (most chiral and most enantiodiscriminating) may be interrelated but they are different.

Recently there have been advances in defining the magnitude of chirality of an object.[16] The most chiral molecule, however, need not be the best at discriminating between enantiomers. That will depend individually on the host and the guest involved as well as on environmental effects from solvent and salts. What we have decided to do is to consider each guest as an individual case when binding to the host and to map out the regions of

(8) (a) Armstrong, D. W.; Tang, Y.; Ward, T.; Nichols, M. *Anal. Chem.* **1993**, *65*, 1114. (b) Yi, G.; Bradshaw, J. S.; Rossiter, B. E.; Reese, S. L.; Petersson, P.; Markides, K. E.; Lee, M. J. *Org. Chem.* **1993**, *58*, 2561. (c) Yi, G.; Li, W.; Bradshaw, J. S.; Malik, A.; Lee, M. *J. Heterocycl. Chem.* **1995**, *32*, 1715.

(9) For reviews see: (a) Vespalec, R.; Bocek, P. *Electrophoresis* **1994**, *15*, 755. (b) Nishi, H.; Terabe, S. *J. Chromatogr. A* **1995**, *694*, 246. (c) Guttman, A.; Brunet, S.; Cooke, N. *LC-GC* **1996**, *Jan*, 32.

(10) Jinno, K., Ed. *Chromatographic Separations Based on Molecular Recognition*; Wiley-VCH: New York, 1997.

(11) For reviews of molecular modeling applied to chiral chromatography see: (a) Lipkowitz, K. B. *J. Chromatogr. A* **1994**, *666*, 493. (b) Lipkowitz, K. B. *J. Chromatogr.* **1995**, *694*, 15. (c) Lipkowitz, K. B. In *Theoretical and Computational Chemistry*; Parkanyi, C., Ed.; Elsevier: New York, 1998; Vol. 5.

(12) The most current review of general applications of cyclodextrins in the area of separations science is the following: Snopek, J.; Smolková-Keulemansová, E.; Cserháti, T.; Gahm, K. H.; Stalcup, A. In *Comprehensive Supramolecular Chemistry*; Szejtli, J., Osa, T., Eds.; Pergamon Press: Oxford, 1996; Vol. 3, pp 515−571.

(13) (a) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry, Reactivity and Structure, Concepts in Organic Chemistry*, 6; Springer-Verlag: New York, 1978. (b) Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344. (c) Szejtli, J. *Cyclodextrins and their Inclusion Complexes*; Akadémiai Kaidó: Budapest, 1982. (d) Duchêne, D., Ed. *Cyclodextrins and Their Industrial Uses*; Editions de Sante: Paris, 1987. (e) Szejtli, J. *Cyclodextrin Technology*; Kluwer Academic: Dordrecht, 1988. (f) A thematic issue on the topic of cyclodextrins is in preparation: *Chem. Rev.*, Lipkowitz, K. B., D'Souza, V.; guest editors, 1997.

(14) (a) Lipkowitz, K. B.; Pearl, G.; Coner, B.; Peterson, M. A. *J. Am. Chem. Soc.* **1997**, *119*, 600. (b) Lipkowitz, K. B.; Coner, B.; Peterson, M. A.; Morreale; A. *J. Phys. Org. Chem.* **1997**, *10*, 311.

(15) Berthod, A.; Li, W.; Armstrong, D. W. *Anal. Chem.* **1992**, *64*, 873.

(16) (a) Buda, A. B.; Mislow, K. *J. Am. Chem. Soc.* **1992**, *114*, 6006. (b) Buda, A. B.; Auf der Heyde, T.; Mislow, K. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 989. (c) Zabrodsky, H.; Peleg, S.; Avnir, D. *J. Am. Chem. Soc.* **1992**, *114*, 7843. (d) Zabrodsky, H.; Peleg, S.; Avnir, D. *J. Am. Chem. Soc.* **1993**, *115*, 8278. (e) Zabrodsky, H.; Avnir, D. *J. Am. Chem. Soc.* **1995**, *117*, 462. (f) Weinberg, N.; Mislow, K. *J. Math. Chem.* **1995**, *17*, 35. (g) Mislow, K. *Croat. Chem.* **1996**, *69*, 485. (h) Tratch, S. S.; Zefirov, N. S. *J. Chem. Inf. Comput. Sci.* **1996**, *36*, 448. (i) Katzenelson, O.; Zabrodsky Hel-Or, H.; Avnir, D. *Chem. Eur. J.* **1996**, *2*, 174.
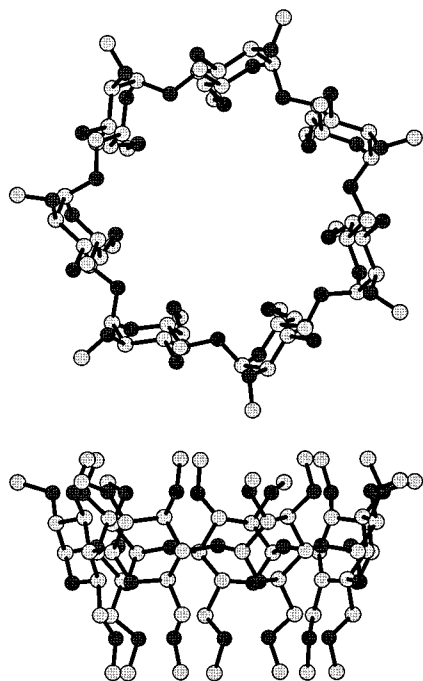
Figure 1. (Top) View looking into the permethyl-β-cyclodextrin chiral cavity. (Bottom) Side view illustrating the typical conical shape of these molecules. The more open end is the 2° rim and the narrower end is the 1° rim. Dark gray tones represent oxygen atoms and light gray tones are carbons. Hydrogen atoms are omitted for clarity. The structure is presented as having a near 7-fold symmetric, time-averaged geometry.

greatest enantiodifferentiation for each substrate as a unique case. This is in contrast to computing the magnitude of chirality of a molecule or a fragment of that molecule and then globally saying molecule A is more chiral than molecule B and should, therefore, be more enantioselective.

In this paper we consider a prototypical host molecule: permethylated β-cyclodextrin, **1**, the most popular CSP used in chiral gas chromatography (Figure 1). In our earlier study we assessed the binding of analyte molecules to this CSP with molecular dynamics simulations using an empirical force field (EFF).[14] While EFFs are not especially well suited for computing small energy differences (typical enantiodifferentiating free energies when binding to CSPs are $<kT$), the success of this approach lies in the fact that the analytes are enantiomers. Because enantiomers are being compared computationally, a cancellation of errors results. If a particular force field overestimates, say, electrostatic effects and underestimate, hydrogen bonding interactions, the errors arising from that force field will be comparable for each analyte (though not exactly the same because the corresponding complexes are diastereomers rather than enantiomers). This is the reason why we and others have been so successful at predicting differential free energies of binding computationally.[11]

The MD simulations we used in our previous study[14] were lengthy (each on the order of 25 ns) and they were averaged over multiple trajectories for ensemble averaging, beginning from different regions on the complex's potential energy surface (to ensure good coverage of phase space). In that study a single enantiomeric analyte molecule was confined to remain in the vicinity of the cyclodextrin with use of a reflective wall so that every time the analyte moved 20 Å from the cyclodextrin it was gently pushed back to further interact with the host molecule. This way we were effectively simulating the millions of collisions an analyte molecule in the gas phase experiences with the stationary phase cyclodextrin as it migrates through the chromatographic column. In all cases studied we found that the preferred binding site is the interior of the macrocycle. Figure 2 depicts an example of this.

In this figure we plot 50 000 points representing the location of an analyte's center of mass (in this case limonene) sampled from equal time periods along the MD trajectories. The points are distributed relative to the center of mass of the cyclodextrin, which is shown in its time averaged, nearly 7-fold symmetric shape. It is evident from this plot that the most probable binding region for both R and S limonene is the interior of the cyclodextrin with a slight preference for the narrower primary rim. The other analytes we studied likewise preferred binding to the interior of the cyclodextrin (to maximize van der Waals interactions).

Although we have located the preferred binding site and we were able to define the intermolecular forces responsible for molecular recognition, we have not identified the most discriminating regions of the CSP. It would be tempting to compare the preferred positions of R vs S analytes by simply subtracting the "dots" in these plots to accomplish this. But, that does not work because the simulations are really not ergodic and consequently differences in these plots (where the larger differences are presumably the regions of greatest discrimination) would be meaningless. In this case one would be mapping out more of a computational artifact than real differences in chiral recognition.

To overcome this problem of inequivalent sampling of phase space we adopt the following procedure. The time averaged, 7-fold symmetric center of mass of cyclodextrins is placed at the origin of a Cartesian coordinate system and a grid is placed over that molecule. At each grid point the center of mass of the analyte molecule is positioned and then systematically rotated about a local coordinate system along three orthogonal axes. The number of grid points and the number of rotations per axis is arbitrary (vide infra). Rather than saving only the lowest energy orientation at each grid point we Boltzmann averaged the energies as the value to be compared. Because we are using a deterministic grid search methodology we note that whatever is being done to the R enantiomer is being done equivalently to the S enantiomer. This way sampling artifacts are removed. The differences at each grid point, between R vs S analytes, indicate discriminatory regions. Those grid points with zero or small energy differences between mirror image isomers are not discriminating while those grid points with the largest differences are most discriminating.

The software we used is an in-house program called *mmodgrid* running on an SGI platform. It is written in C language and is available from one of the authors.[17] Among other features this program allows one to carry out grid scans using different force fields. The AMBER* force field[18] was used in this study with an effective dielectric set to unity and without any cutoffs of any kind. Because of the nature of the systematic grid search being done, the software has been parallelized, and in this study we used simultaneously eight processors of a Cray J90 in addition to 15 available processors on various types of SGI workstations.

The dimensions of the grid surrounding the cyclodextrin are 27 Å $\times$ 23 Å $\times$ 26 Å. We have selected grid spacings of 0.25 Å and 45° rotations per axis. Hence at each grid point we sample 512 unique orientations of guest relative to host. The total number of grid points for the R and also for the S analyte

(17) B. Coner: coner_bob@lilly.com. See also footnote 36 in ref 14a.
(18) This is a modified version of Kollman's original AMBER force field: Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alogona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765.
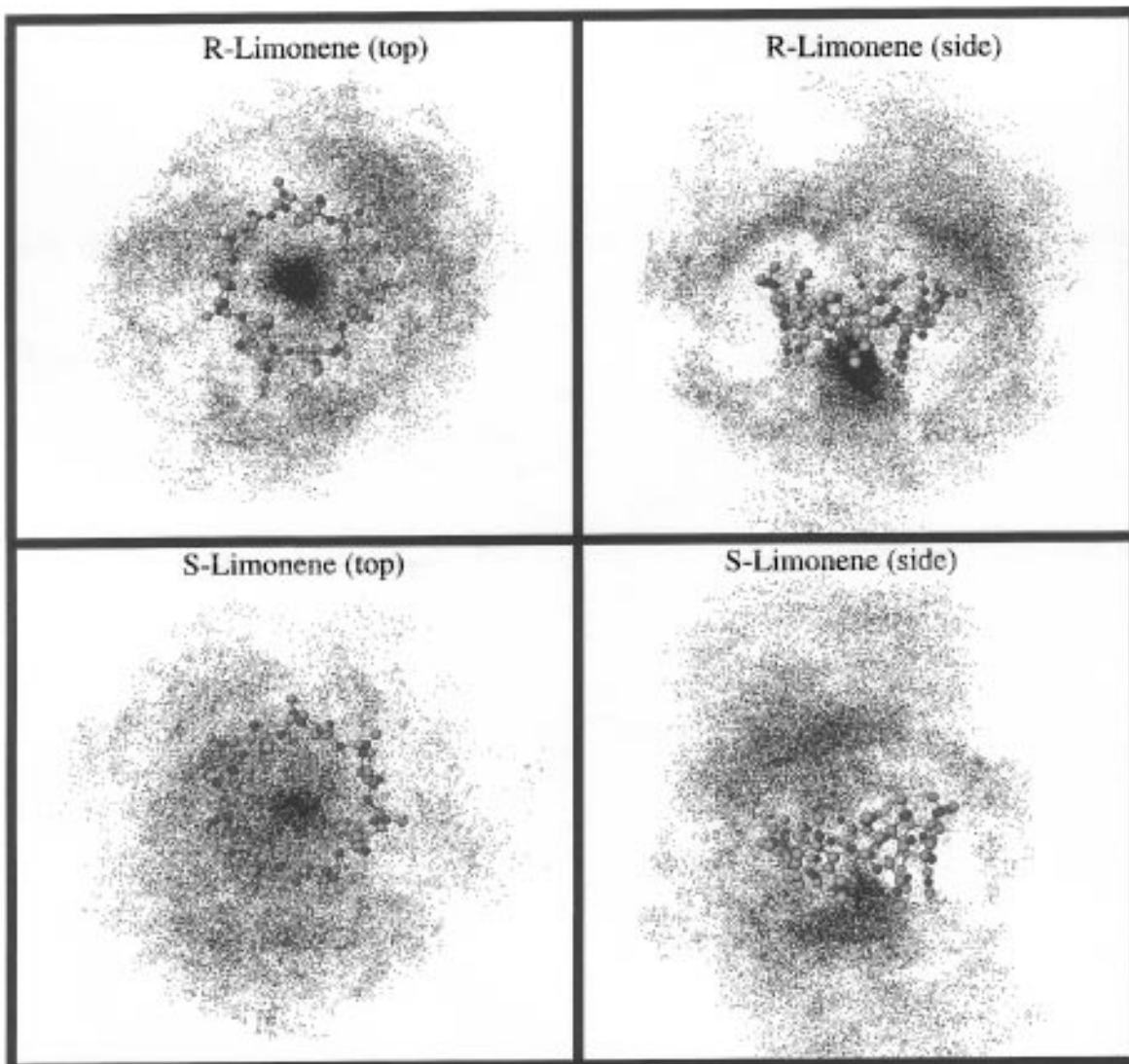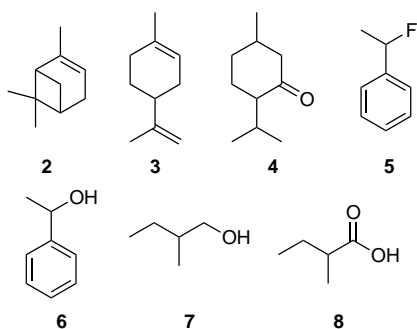
Figure 2. "Dot plot" illustrating the limonene center of mass, relative to the cyclodextrin, over the combined 25-ns simulation. (Top) End-on and side views of the R enantiomer. (Bottom) End-on and side views of the S enantiomer. Original diagrams are also color coded to indicate the intermolecular energy at each point. Lowest energies are at the interior.

Chart 1

is approximately 269 000. Visualization of the results was done with IRIS Explorer.[19]

Systems Studied

The molecules studied include the following: α-pinene (**2**), limonene (**3**), isomenthone (**4**), 1-fluoro-1-phenylethane (**5**), 1-phenylethanol (**6**), 2-methyl-1-butanol (**7**), and 2-methylbutanoic acid (**8**). All of these

molecules have been resolved on CSP **1**[20-25] and have been studied by us using molecular dynamics simulations.[14] These molecules were selected for study here because they are representative of the type of molecules resolved on such cyclodextrin CSPs, but more importantly because they contain a diverse collection of functional groups beginning from the weakly polar hydrocarbons (**2** and **3**) to more polar fluorinated

(20) Several groups have carried out limonene resolutions on this CSP including the following: (a) Schurig, V.; Nowotny, H.-P. *J. Chromatogr.* **1988**, *441*, 155. (b) Schurig, V.; Jung, M.; Schmalzing, D.; Schleimer, M.; Duvekot, J.; Buyten, J. C.; Peene, J. A.; Mussche, P. *J. High Resolut. Chromatogr.* **1990**, *13*, 470. (c) Takeoka, G.; Flath, R. A.; Mon, T. R.; Buttery, R. G.; Teranishi, R.; Güntert, M.; Lautamo, R.; Szejtli, J. *J. High Resolut. Chromatogr.* **1990**, *13*, 202. (d) Askari, C.; Hener, U.; Schmarr, H.-G.; Rapp, A.; Mosandl, A. *Fresenius J. Anal. Chem.* **1991**, *340*, 768. (e) Keim, W.; Köhnes, A.; Meltzow, W.; Römer, H. *J. High Resolut. Chromatogr.* **1991**, *14*, 507. (f) Mosandl, A.; Fischer, K.; Hener, U.; Kreis, P.; Rettinger, K.; Schubert, V.; Schmarr, H.-G. *J. Agric. Food Chem.* **1991**, *39*, 1131. (g) Bicchi, C.; Artuffo, G.; D'Amato, A.; Galli, A.; Galli, M. *Chirality* **1992**, *4*, 125. (h) Kobor, F.; Angermund, K.; Schomburg, G. *J. High Resolut. Chromatogr.* **1993**, *16*, 299.

(21) Several groups have resolved pinene on this CSP, including all the citations in ref 20 and the following: Reinhardt, R.; Steinborn, A.; Engewald, W.; Anhalt, K.; Schulze, K. *J. Chromatogr. A* **1995**, *697*, 475.

(22) Isomenthone: Askari, C.; Mosandl, A.; Schmarr, H.-G. *Arch. Pharm. (Weinheim)* **1992**, *325*, 35.

(23) 1-Fluoro-1-phenylethane: Reinhardt, R.; Engewald, W.; Goj, O.; Haufe, G. *Chromatographia* **1994**, *39*, 192.

(24) 1-Phenylethanol: see ref 20h.

(25) Methylbutanol and Methylbutanoic acid: see ref 20f.

(19) IRIS Explorer Center (North America), Downers Grove, IL 60551-5702 or via <URL http://www.nag.co.uk/1h/Welcome_IEC>.
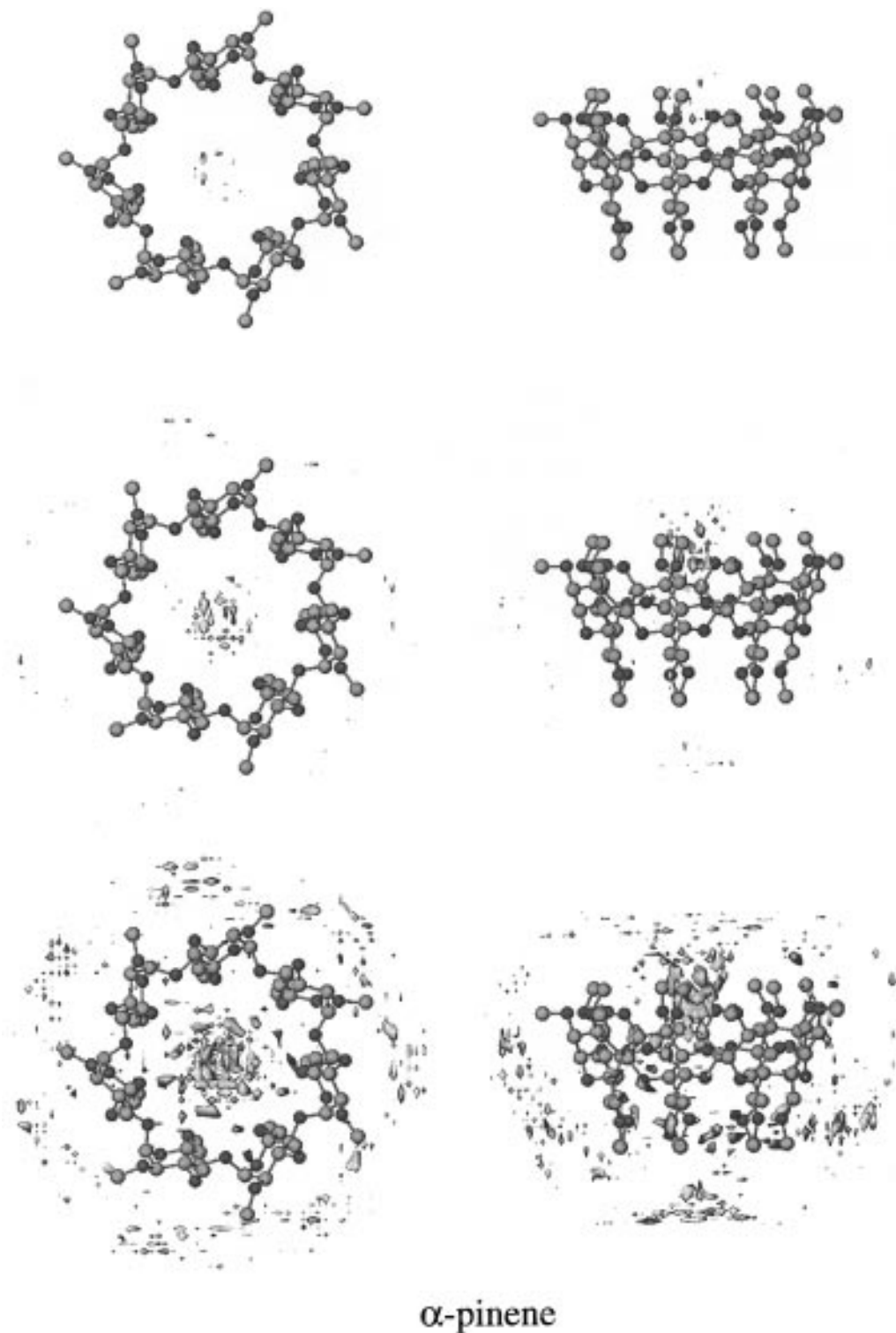
α-pinene

Figure 3. Regions of chiral recognition between a symmetric host, permethyl-β-cyclodextrin, and α-pinene. The cyclodextrins and their gray-tone color codes are the same as in Figure 1. The most discriminating region is depicted at the top panel of the figure. Regions of less chiral discrimination are enclosed in the second panel showing that exterior sites are also discriminatory but to a lesser extent. The bottom panel shows regions of space that are even less discriminatory than above. Inside the macrocycle the region of greatest discrimination is localized near the (wider) secondary rim. At all levels of chiral discrimination the inside of the macrocycle is most cognizant of differences between R and S guests.

and carbonyl containing structures (**4** and **5**) and terminating with polar alcohol and carboxylic acid groups (**6**–**8**).

In this study both the CSP and the analyte were treated as rigid bodies. The cyclodextrin structure corresponds to the time-averaged MD structures derived from our simulations. The probe molecules correspond to the lowest energy conformer determined with the AMBER* force field with the exception of the carboxylic acid. In this case the carboxylic hydrogen was rotated so that the O=C−O−H

dihedral angle is antiperiplanar rather than synperiplanar so that it is in a "productive" orientation for intermolecular hydrogen bonding.

Results and Discussion

The results of our calculations show, uniformly and irrespective of which organic functional group is present, that the greatest difference in interaction energy for R vs S probes exists in the interior of the macrocycle. Three examples of this are
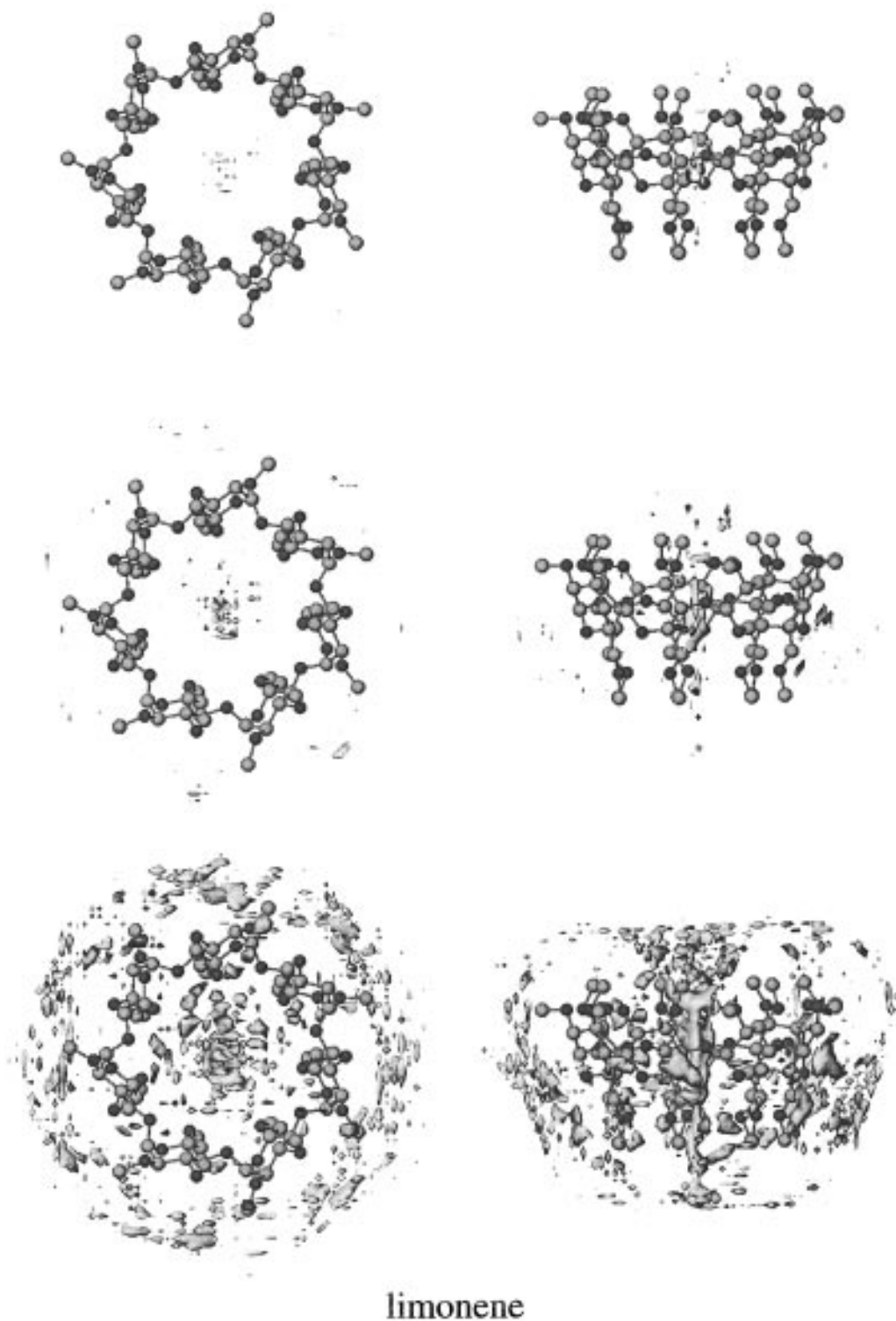
limonene

Figure 4. Same as in Figure 3 for limonene. Note that there exists less clear preference for enantiodifferentiation at the secondary rim; discrimination takes place in a more distributed way throughout the interior of the macrocycle's cavity.

illustrated in Figures 3−5 (others are included as Supporting Information). We show here pinene and limonene because these guests have been resolved by Bradshaw (see below) and 2-methylbutanoic acid for the sake of comparison with a polar guest. In these figures are plotted isoenergy contour surfaces of differences in Boltzmann weighted energies between R and S probe molecules at each grid point. At the top of each figure is the region of greatest energy difference, and it is thus the most enantiodiscriminating part of the CSP. In the next two panels of each figure are regions containing smaller and smaller differential energies, enclosing volumes of space having less

and less enantiodiscrimination. Of course the regions not being rendered are very weakly discriminating or have no chiral recognition at all (at least for that particular probe). The reader should also note that if the cyclodextrin were 7-fold symmetric the results in these figures should show a $1/7$ symmetry since the rotation of the cyclodextrin by $1/7$ turn produces an absolutely identical environment for the guest molecule. This symmetry is difficult to see because the structure of the cyclodextrin we used in these calculations corresponds to an average structure derived from a lengthy MD simulation and is not a symmetric structure.
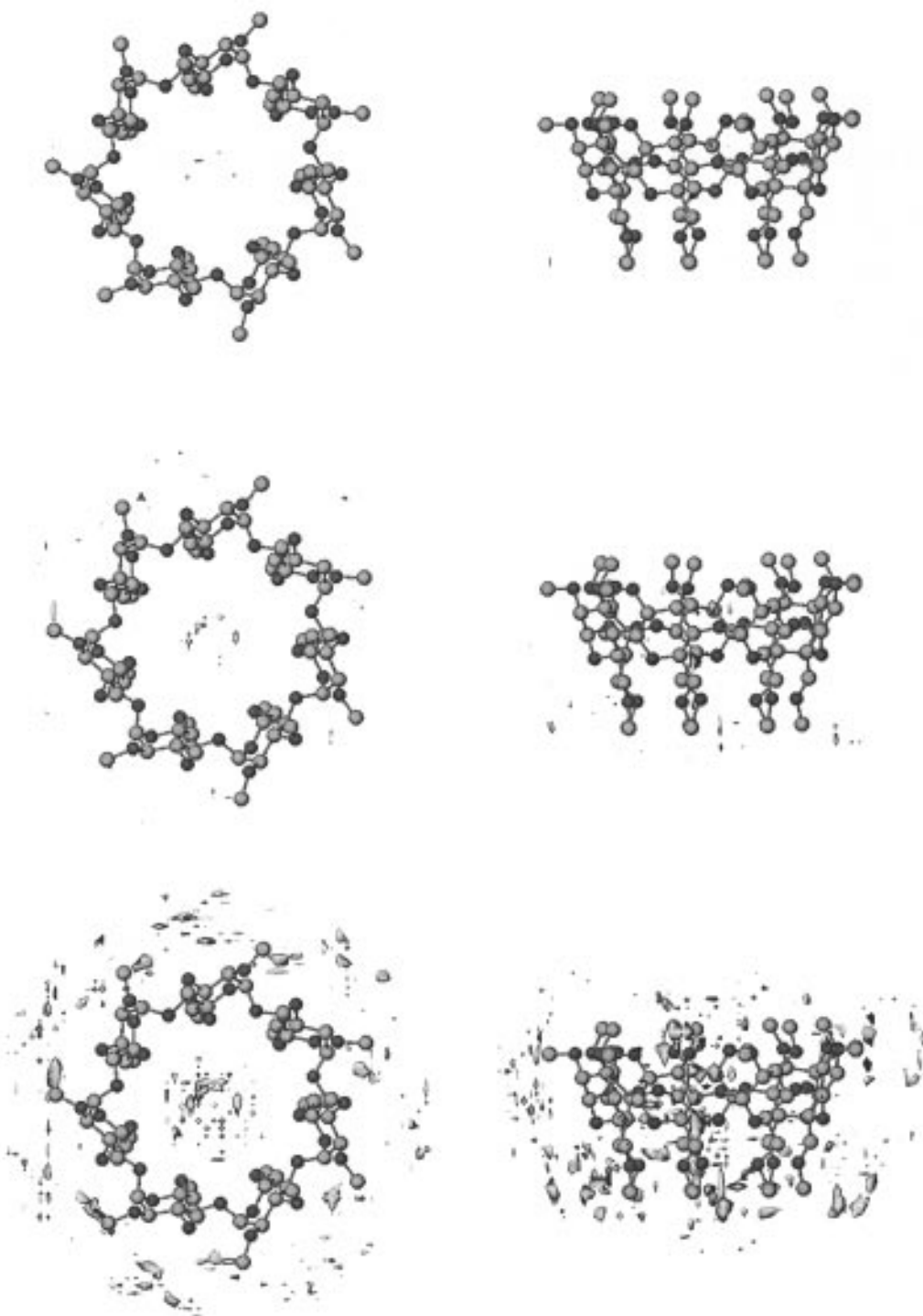
2-methylbutanoic acid

Figure 5. Same as in Figure 3 for a polar guest (2-methylbutanoic acid). While the region of chiral recognition is still the interior of the macrocycle, there exists substantial regions of chiral discrimination on both the interior and exterior of this host CSP. Similar trends exist for the other polar guests (not shown).

What we find, then, is that the most enantiodifferentiating region of the macrocycle (the interior) is also where the analytes prefer to bind (see Figure 2). So, in this case of molecular recognition Nature places the analytes in the vicinity of highest chiral discrimination, but this need not be true for other host−guest systems, and this is the issue we are bringing to the fore in this paper. In fact, for this system, if one could prohibit the analyte from binding to this region of maximum discrimination,

say, by forcing the analyte to reside on the exterior of the cyclodextrin, one would predict reduced or no chiral discrimination at all.

In terms of cyclodextrin discrimination we point out that some preliminary experimental evidence confirms our prediction, albeit with a different (yet very similar) cyclodextrin. In the work of Bradshaw *et al.*,[26] two isomeric cyclodextrin CSPs were created, one possessing a β-cyclodextrin rotaxane having a

benzene-containing chain passing through the macrocycle's cavity and the other having those chains on the exterior of the cyclodextrin. The authors were able to resolve α-pinene (**2**) and limonene (**3**) on the non-rotaxane CSP (the non-rotaxane CSP is capable of forming an inclusion complex with the analyte). But, for the rotaxane CSP that blocks inclusion complexation and forces the analyte to the exterior of the macrocycle, no resolution was observed. This is only a preliminary study and remains inconclusive, but it is supportive of our results presented above.

The reader should also note that two polar alcohols were resolved on both CSPs in that study. It appears from our maps that the *exterior* regions of chiral discrimination are (1) less well-defined and (2) smaller in magnitude for the hydrocarbons than for the alcohols we evaluated. In other words, we find more and better chiral discriminating sites on the outside of the CD cavity when the polar analytes are considered than we do for the simple hydrocarbons. This indicates that as the polarity of the analyte increases new enantiodiscriminating regions around the CSP become more prominent more quickly for the more polar analytes than for the less polar analytes, i.e., alternative enantiodiscriminating regions exist for the alcohols but not for the hydrocarbons. While this is consistent with Bradshaw's preliminary results, it is clear that additional examples are needed.

The energy differences plotted in our figures correspond to the total intermolecular energies between host and guest. We were also curious about the behavior of the component energies, which in this study are the van der Waals (dispersion) and Coulombic (electrostatic) terms computed by the AMBER* force field. As expected, the electrostatic component of the intermolecular energy increases as the polarity of the guest increases, though in all examples studied here the dominant interaction energy is the van der Waals term. The question we wanted to address is the following: Do the electrostatic and van der Waals contributions to the total chiral recognition both have their maximum discriminatory effect in the interior of the cyclodextrin? Or, does the electrostatic component behave in a more discriminating way on the exterior of the macrocycle? *A priori* there is no way of knowing this, but if this were true one might find examples where very polar analytes would be better off binding to the exterior of the cavity rather than the interior to maximize chiral recognition. To assess this we computed the Boltzmann-weighted energy differences between R and S probe molecules as before, but plotted their van der Waals and Coulombic terms separately. Both components were found to be greatest in the interior of the macrocycle for all the analytes studied here. However, in all examples, the van der Waals contribution to the enantiodifferentiation is far more localized to a central region within the interior of the cavity than is the contribution of the electrostatic component. The electrostatic recognition is found to be more diffuse, and having

multiple recognition sites on both the inside and the outside of the macrocycle. However, this effect is overwhelmed by the van der Waals contribution to chiral discrimination that is highly localized to the interior of the host cavity. Finally, the regions of greatest chiral discrimination, summed up as in Figures 2−4, tend to show recognition at the primary rim for some analytes, the secondary rim for others, and occasionally near the equator of the macrocycle for other analytes, but no clear trends are readily apparent concerning exactly where on the interior of the cyclodextrin recognition takes place.

Summary

The point of this paper is to bring to the attention of scientists interested in chiral recognition an issue that has not yet received attention in the literature but which constitutes a major issue in enantiodiscrimination. The issue concerns locating the region of maximum chiral recognition and then ensuring that the substrates interacting with that selector are also positioned at that region to promote maximum differentiation. The system we studied is a typical host−guest complexing system, but the ideas are applicable to any aspect of molecular recognition where chiral discrimination is important.

To discern what region is most discriminating we used a simple grid search method that treats both R and S probes equivalently as they interact with the chiral selector. At each point in space the difference in computed intermolecular energies can be compared; regions with little or no difference in energies are nondiscriminating while regions of larger difference between the enantiomeric probes are more highly enantiodifferentiating. For permethylated β-cyclodextrin, the most popular chiral stationary phase used in gas chromatography, we found the most enantiodifferentiating regions are at the interior rather than the exterior of the macrocycle. Both the van der Waals and the electrostatic components of this enantiodifferentiation are found inside the cyclodextrin, but the (minor) electrostatic component is less localized to this region, having sites outside the cavity. Seven representative analytes were investigated in this study, ranging from nonpolar to polar in nature and containing a diversity of organic functionality. In all cases the regions of maximum chiral discrimination correspond, roughly, to the preferred binding site for those analytes. One may conclude, for these examples, that if the analytes are prohibited from residing in this highly discriminating region that little or no differentiation will take place. A preliminary literature report concerning two of the analytes studied here confirms this hypothesis, but more studies are warranted.

Supporting Information Available: Additional plots for analytes discussed in the paper (5 pages). See any current masthead page for ordering and Internet access instructions.

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(26) Bradshaw, J. S.; Chen, Z.; Yi, G.; Rossiter, B. E.; Malik, A.; Pyo, D.; Yun, H.; Black, D. R.; Zimmerman, S. S.; Lee, M. L.; Tong, W.; D'Souza, V. T. *Anal. Chem.* **1995**, *67*, 4437.