Host–Guest Systems

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A “Through-Shell” Binding Isotope Effect**

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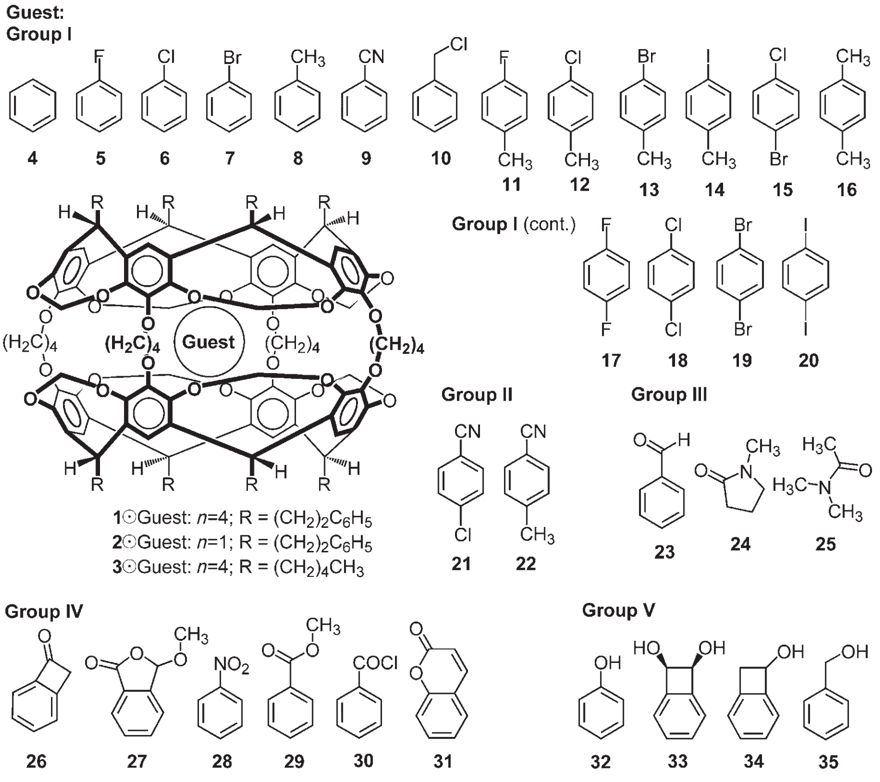
Isotope effects play an important role in chemical and biological sciences.[1] In particular, kinetic isotope effects allow the probing of reaction mechanisms,[2] transition-state geometries,[3] tunneling contributions to reaction rates, and steric requirements in the rapture of mechanical bonds.[4,5] In addition, substantial differences in noncovalent interactions that underlie olfaction,[6] enzyme–cofactor binding,[7] retention on normal and reversed phase HPLC columns,[8,9] and molecular encapsulation have been observed upon deuteration.[10,11] Isotopic substitution also affects the thermodynamic properties of molecules, thus leading to changes in the vapor pressure and melting point, as well as to the phase segregation of polymers.[12–14] However, the origin of binding isotope effects (BIE) and how specific molecular interactions influence the volume, energy, and entropy of a molecule and give rise to the observed changes in properties upon isotopic substitution is not fully understood.[15] It is well established that the molar volume of a molecule decreases upon deuteration as a consequence of the smaller zero point energy (ZPE) of the C–D bond relative to the C–H bond and the anharmonicity of the potential energy surface, which leads to different equilibrium interatomic distances. For example, the difference in the mean C–H and C–D bond lengths of methane is $\Delta l = 0.004$ Å, as determined by gas-phase electron-diffraction studies.[16]

Here we introduce a novel supramolecular approach for the determination of the changes in the bond lengths and volume upon deuteration which allows determination of the differences in the C–H/C–D bond lengths of a single molecule that is isolated inside a highly polarizable medium.[17] A comparison of the apparent differences in the C–H/C–D bond lengths in different media might provide new insights into the magnitude of the intermolecular and intramolecular contributions to the observed molar volume isotope effect.[18] In our approach, a hemicarcerand serves as a sensitive “molecular ruler” that allows the precise determination of small size differences between two incarcerated guests from the chromatographic properties of both hemicarceplexes. We also provide for the first time a quantitative analysis of the chromatographic behavior of hemicarceplexes and an understanding of how the molecular information that is stored in the encapsulated guest (size, shape, chirality, electrostatic potential, and local and global dipole moment) is translated through the hemicarcerand shell into a surface–hemicarceplex interaction.[19,20] A detailed understanding of such inside–outside information transfer[19–21] might shed light on similar processes in biological systems and should be important for applications of container molecules or self-assembled capsules as molecular switches, sensors, information storage devices, or new tools in separation technology.[21a,22]



Scheme 1. Hosts and guests investigated in the study.

In an investigation that addressed how the guest of hemicarceplexes **1** ⊙ guest (guest: **4**–**35**; Scheme 1)[23–25] communicates with an external chromatographic stationary phase we noted a large increase in the HPLC retention factor $k' = (t_R - t_0)/t_0$ (t_R = solute retention time; t_0 = retention time of unretained solute; mobile phase: CH_2Cl_2/0.5% Et_2O;

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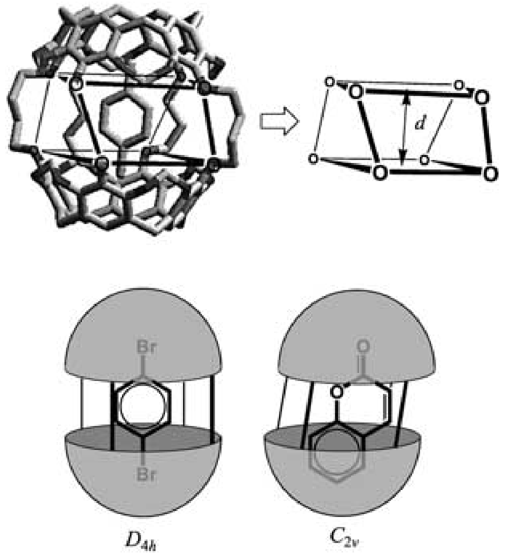


Figure 1. Definition of d for the energy-minimized structure of $\mathbf{1}\odot\mathbf{16}$ (left) and shapes of hemicarceplexes: D_{4h}: guests **4**–**25**, **32**, **35**; C_{2v}: guests **26**–**31**, **33**, **34**.

1 mL min^{-1}) with increasing guest size. The value of k' increased 35-fold between the fastest ($\mathbf{1}\odot$benzene; $k' = 1.5$) and slowest eluting hemicarceplex ($\mathbf{1}\odot$7-methoxyphthalide; $k' = 51.86$). Furthermore, retention did not correlate with guest polarity; for example, the value of k' increased in the order $\mathbf{1}\odot\mathbf{4} < \mathbf{1}\odot\mathbf{5} < \mathbf{1}\odot\mathbf{6} < \mathbf{1}\odot\mathbf{9} < \mathbf{1}\odot\mathbf{8} \approx \mathbf{1}\odot\mathbf{7} \ll \mathbf{1}\odot\mathbf{16}$. The flexible $O(CH_2)_4O$ linkers of **1** allow adaptation of the inner phase of the host to the size and shape of the guest in order to maintain an optimal space occupancy.[26] Thus, it is tempting to assume that the retention times are related to the size and shape of the hemicarceplexes: An increased outer surface should increase the surface–surface interaction. To test this hypothesis we carried out molecular dynamics simulations (MDSs) for each hemicarceplex $\mathbf{1}\odot$guest (guest: **4**–**35**) and chose the distance d between the center of the two squares formed by connecting the four ether oxygen atoms of a cavitand as a measure for the change in the hemicarcerand size (Figure 1). These MDS studies (Amber*, 300 K, 10 ns) provided time-averaged values for d and information about the shape of the hemicarceplexes, which we categorized as D_{4h}- (guests **4**–**25**, **32**, **35**) or C_{2v}-symmetric (guests **26**–**31**, **33**, **34**) according to the relative orientation of the cavitands.[27] We were delighted to find that $\ln k'$, which is proportional to the change in free energy ΔG upon transferring a hemicarceplex from the mobile phase to the stationary phase, correlates almost linearly with the value of d for hemicarceplexes having nearly D_{4h} symmetry (group I hemicarceplexes in Scheme 1; Figure 2).

What is the physical basis behind this correlation? Further analysis of the MDS studies shows that elongation of a hemicarceplex leads to an increased outward rotation of the lone pairs of electrons on the oxygen atoms of the $O(CH_2)_4O$ linker. Since an outward pointing lone pair of electrons on an oxygen atom is able to engage in polar Si−OH⋯O_{host} interactions with the silica surface of the stationary phase, this will increase the retention of the hemicarceplex. In fact, a good linear correlation between $\ln k'$ and the time-averaged sum of all the C_1-O_2-C_3-C_4 dihedral angles (θ) is observed (see Scheme 2 and Supporting Information).

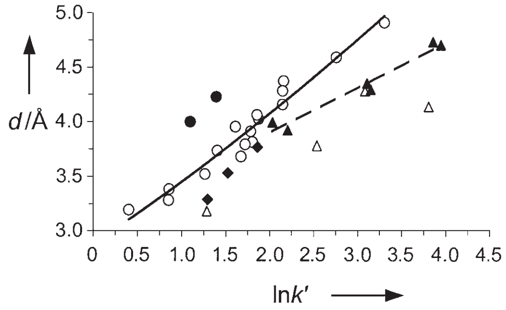
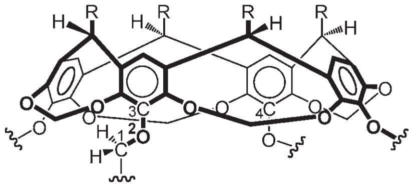


Figure 2. Semilogarithmic plot of hemicarceplex $\mathbf{1}\odot$guest length (d) and retention factor ($\ln k'$): guests of group I (○); guests of group II (●); guests of group III (◆); guests of group IV (▲); guests of group V (△; see Scheme 1); and best fit for the correlation of group I guests. (CH_2Cl_2/0.5% Et_2O; 1 mL min^{-1}; Phenomenax, Luna Si (2), 15 cm×4.6 mm, 5 μm; 22 °C).



Scheme 2. Definition of C_1-O_2-C_3-C_4 dihedral angle θ. Lone pairs of electrons on the oxygen atoms are fully protected (inwards) for $\theta = 180°$ and fully exposed (outwards) for $\theta = 0°$.

Thus, differences in the retention times of the hemicarceplexes with D_{4h} symmetry are primarily controlled by the length of the hemicarcerand through the time-averaged orientation of the lone pairs of electrons on the ether oxygen atoms. However, several hemicarceplexes deviated from this behavior and showed weaker (group II, Scheme 1) and stronger retention (group III, Scheme 1) than predicted based on their length. Even though the exact reason for their different behavior is not fully understood, it might be a consequence of the particularly large dipole moment of the guest, which influences the hemicarceplex–silica interaction. In fact, Cram and co-workers explained the differences in the thin layer chromatography retention factors of hemicarceplexes $\mathbf{2}\odot$DMF, $\mathbf{2}\odot$*N*,*N*-dimethylacetamide, and $\mathbf{2}\odot$DMSO on the basis of such modulations of carceplex–silica dipole–dipole interactions induced by the dipole of the guest.[19] In the group II hemicarceplexes the CN groups are aligned along the polar axis of the host and are "pushed" into the cavitand such that the positively charged C atom is in the same horizontal plane as the cavitand's oxygen belt. We believe that the proximity of the positive pole of the CN group leads to a slight weakening of all Si−OH⋯O_{host} interactions involving these oxygen atoms. A related but opposite effect could explain the retention behavior of the group III hemicarceplexes: their guests possess a carbonyl group, whose negative pole is in proximity to the oxygen atom rim of a cavitand leading to a slight strengthening of local Si–OH···O_{host} and dipole–dipole silica–hemicarceplex interactions by roughly an equal amount in all three hemicarceplexes.

Hemicarceplexes whose guest induces C_{2v} symmetry of the host (group IV, Scheme 1) are retained more strongly than hemicarceplexes of group I with a comparable d value, but still show a linear correlation between $\ln k'$ and d. Because of the limited number of hemicarceplexes in this series and the fact that each guest has a polar functional group, is not fully clear whether the increased hemicarceplex–silica surface interaction is a result of the shape of the hemicarceplex or the presence of the polar functional group.

Finally, hemicarceplexes whose guest has a hydroxy group all show stronger retention, as predicted (group V, Scheme 1). A logical explanation would be through-shell guest-OH···O-silica hydrogen bonds that increase the binding interaction of the hemicarceplex with the surface.

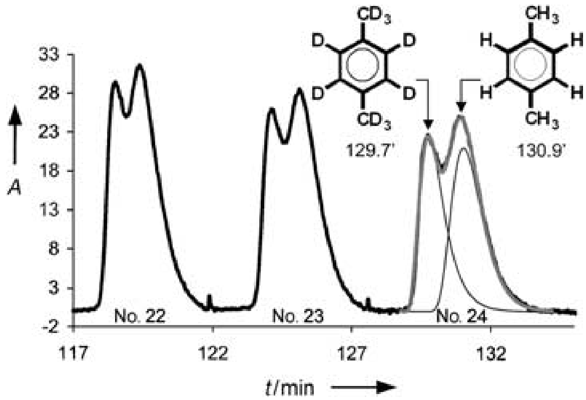
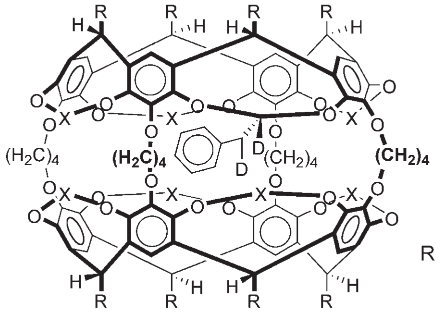
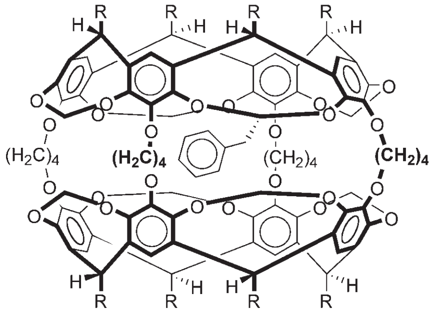


Figure 3. Normal-phase HPLC recycle chromatogram (thick blackline) of a 1:1 mixture of **3**$\odot$*p*-xylene and **3**$\odot$[D_{10}]-*p*-xylene and peak deconvolution of the 24th cycle (mobile phase: A/B, 65:35, A: 2.0 v% Et_2O in CH_2Cl_2, B: CH_2Cl_2; 1 mLmin^{-1}; Phenomenax, Luna Si (2), 25 cm×4.6 mm, 5 µm; 22 °C). Gray line: best fit of the double peak of the 24th cycle; thin black line): peak **3**$\odot$[D_{10}]-*p*-xylene and **3**$\odot$*p*-xylene.



[D_{16}]-**36**

R = pentyl
X=CD_2



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Even though the dipole moment or OH group of a tightly bound guest is able to "communicate" with the silica surface, the length of the hemicarceplex is the key factor that controls retention in all hemicarceplex groups (I–V). If this is indeed true, separation of isotopomeric hemicarceplexes that differ solely in the extent of guest deuteration might be possible. We tested this idea with the **1**$\odot$*p*-xylene/**1**$\odot$[D_{10}]-*p*-xylene pair. Initial attempts to separate both hemicarceplexes failed even with recycling chromatography because of strong tailing of the peaks. A screen of other hemicarceplexes with different appending groups showed reduced tailing for **3**$\odot$**16** and, indeed, the onset of separation of **3**$\odot$**16** and **3**$\odot$[D_{10}]-**16** was observed after 14 chromatographic cycles, with **3**$\odot$[D_{10}]-**16** eluting before **3**$\odot$**16** as predicted from the volume isotope effect of the guest (Figure 3).[28] From the difference in the retention times in the 24th cycle (Figure 3), we determined $\Delta \ln k' = \ln k'_H - \ln k'_D = 0.013(1)$ and a binding isotope effect of $k'_H/k'_D = 1.013(1)$. This binding isotope effect is conceptionally new since it depends primarily on the volume difference between the two isotopomers, which induces a small structural change in the surrounding hemicarcerand that leads to the observed change in the binding energy of $\Delta\Delta G = 7.6(6)$ calmol^{-1}. The following observations rule out participation of the guest's C–H/D bonds in the hemicarceplex–silica interaction: 1) the isotopomeric hemicarceplexes **36** and [D_{16}]-**36**, in which the outward-pointing acetal C–H/D bonds interact with the stationary phase, show a reversed elution order;[8] 2) the absence of separation for the pair **3**$\odot$**16** and **3**$\odot$[D_4]-**16**, in which the aryl protons of the guest more exposed to the bulk phase are labeled, but partial separation for the pair **3**$\odot$**16** and **3**$\odot$[D_6]-**16**, in which the shielded methyl groups of the guest are labeled. The latter experiments also demonstrate that the partial separation of the pair **3**$\odot$**16** and **3**$\odot$[D_{10}]-**16** is a consequence of deuterating the methyl groups of the guest.

If the volume isotope effect of the guest were the sole cause of the difference in the retention time between **3**$\odot$**16** and **3**$\odot$[D_{10}]-**16**, what would be the difference in the C–H/C–D bond lengths? From the $\Delta \ln k'$ value and the slope of the $\ln k'$ versus d plot for hemicarceplexes **3**$\odot$guest (guest = **4**–**8**, **11**–**19**; see Supporting Information), we calculated the hemicarceplex contraction upon guest deuteration as $\Delta d = 0.0098 \pm 0.0009$ Å. MDS studies of *p*-xylene hemicarceplexes in which we systematically constrained the C–H bonds of the guest to a length between 1.0 and 1.1 Å showed that a shortening of the methyl C–H bonds by 0.01 Å decreased d by 0.0204 Å. Thus, the measured Δd value corresponds to a difference in the C–H/C–D bond lengths of $\Delta l = 0.0048 \pm 0.0005$ Å. Bartell and Roskos used $\Delta l = 0.005$ Å in model calculations that accurately predicted the measured volume isotope effect of benzene.[29] Likewise, Bates et al. found a good agreement between the experimentally observed and modeled differences in the unit cell dimensions of crystalline $C_{36}H_{74}$ and $C_{36}D_{74}$ by assuming $\Delta l = 0.005$ Å.[18a] The close match between these and our measured bond lengths suggest that the volume isotope effect of the guest is likely the main reason for the observed chromatographic isotope effect. However, other contributions to the observed difference in the deuteration-induced hemicarceplex length cannot be excluded. For example, the higher polarizability of a C–H bond relative to a C–D bond would result in stronger CH–π interactions between the host and guest and a decrease in the difference in length between $\mathbf{3}\odot\mathbf{16}$ and $\mathbf{3}\odot[D_{10}]$-**16**.[18a,b] Furthermore, Rebek and co-workers showed that a CD_3 group is more strongly attracted to the inner surface of a cavitand than is a CH_3 group.[10] This result has been explained with a blue shift of the C–H/C–D stretching frequency upon formation of a CH/CD–π interaction that leads to an increased ZPE splitting.[15] Since hemicarceplexes are fluxional in solution,[30] a tighter host–guest complex is expected for $\mathbf{3}\odot[D_{10}]$-**16** than $\mathbf{3}\odot\mathbf{16}$, which will increase the difference in length between $\mathbf{3}\odot\mathbf{16}$ and $\mathbf{3}\odot[D_{10}]$-**16** beyond what is expected based on the different dimensions of the C–H and C–D bond.[16]

In conclusion, we have unraveled some of the mechanisms that lead to the observed large differences in the chromatographic behavior of hemicarceplexes. Our investigations show that guest size plays a crucial role in modulating the hemicarceplex–surface interactions and that even such subtle changes resulting from deuteration lead to a measurable change in the retention time. We believe that simple correlations between retention behavior and structural parameters, such as the computed length and shape of the complex also exist for other hemicarcerands and capsules. If indeed such correlations can be found for chiral hemicarceplexes,[18b] novel and powerful methodologies for chiral separation and the determination of the absolute configuration of a chiral guest molecule might become available.

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