MAGNETIC RESONANCE IN CHEMISTRY
Magn. Reson. Chem. **36**, 663–669 (1998)

Coupling of molecular motions through non-bonding interactions: ^{13}C NMR spin–lattice relaxation studies of a host–guest complex

Göran Hilmersson and Julius Rebek, Jr*

Skaggs Institute for Chemical Biology and Department of Chemistry, Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA

Received 18 November 1997; revised 1 April 1998; accepted 2 April 1998

ABSTRACT: ^{13}C NMR spin–lattice relaxation and ^{13}C–$\{^{1}$H$\}$ nuclear Overhauser measurements were performed on the encapsulation complex between [2.2]paracyclophane and a dimeric capsule known as the hydroxy 'softball.' The data were analyzed using the formalism for an isotropically diffusing sphere. The binding constant for the complex is $(3.5 \pm 0.5) \times 10^3$ l mol^{-1} in $CDCl_3$ at 295 K. The average dipole–dipole relaxation time is 0.45 ± 0.04 s for the CH vectors of the encapsulated [2.2]paracyclophane and 0.30 ± 0.03 s for the skeleton of the hydroxy 'softball.' The correlation time for the skeleton of the hydroxy 'softball' is 2.7×10^{-10} s. The corresponding correlation time for the encapsulated [2.2]paracyclophane is calculated to be 1.2×10^{-10} s. This results in an average dynamic coupling constant, χ, of 0.47, indicating shape complementarity and correlated motion between the hydroxy 'softball' and the [2.2]paracyclophane. © 1998 John Wiley & Son Ltd.

KEYWORDS: NMR; ^{13}C NMR; spin–lattice relaxation; inclusion complex; [2.2]paracyclophane

INTRODUCTION

Detailed information about molecular motion processes is important to understand better some of the basic principles governing host–guest chemistry. To the best of our knowledge, there have been only a few detailed studies of the molecular motions of host–guest complexes.[1] These studies include the examination of non-covalent binding interactions in the inclusion complexes formed between cyclodextrin or curbituril and small organic anionic or cationic molecules, which induce ion–dipole attraction(s) and hydrophobic effect(s). The average dynamic coupling constant, χ, taken as the ratio of host correlation time to guest correlation time,[1b,e] has been used for interpreting the molecular motions of these host–guest complexes. This type of molecular motion study has yet to be extended to hydrogen-bonded capsules, and we describe here such a system.

Previously this group has reported the syntheses and studies of large self-complementary molecules capable of assembling into pseudo-spherical capsules ('tennisball,'[2] 'softball'[3] and hydroxy 'softball'[4]). These capsules are dimers of self-complementary, C-shaped molecules that assemble by 8 or 16 hydrogen bonds in a motif that resembles the seam keeping the halves of a tennis ball or softball together. The formation of the dimeric complexes is in part driven by filling the cavity with molecules of suitable size and shape.[3b]

The encapsulation of guests appears to be fast on the human time-scale, although their exchange is slow on the NMR time-scale as separate resonances are observed for free and bound species in the spectra.

This paper describes the molecular dynamics for the inclusion complex formed between the hydroxy 'softball' (Fig. 1) and [2.2]paracyclophane. The guest [2.2]paracyclophane is a small, rigid molecule in which the ^{13}C spin–lattice relaxation is dominated by the fluctuating dipolar interaction between ^{13}C nuclei and directly bonded protons, facilitating the interpretation of NMR results.

THEORY

Measurements of spin–lattice relaxation times (T_1) of resonances of proton-bearing carbons in proton-decoupled ^{13}C NMR spectra have been used extensively for studies of rotational motions of molecules in solution.[5] For a ^{13}C nucleus directly linked to at least one proton, the major relaxation process generally arises from the dipole interaction between the carbon nucleus and attached proton(s). The inverse relaxation time is given by a dipolar coupling constant, *DCC* [Eqn (1)], a parameter related to the strength of the dipolar interaction, and a reduced spectral density function, $J(\omega)$, reflecting the dynamic features.

$$DCC = (\mu_0/4\pi)(h/2\pi)\gamma_C\gamma_H r^{-3} \quad (1)$$

* *Correspondence to*: J. Rebek, Jr, Skaggs Institute for Chemical Biology and Department of Chemistry, Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA
Contract/grant sponsor: National Institutes of Health.
Contract/grant sponsor: Skaggs Research Foundation.

CCC 0749-1581/98/090663–07 $17.50

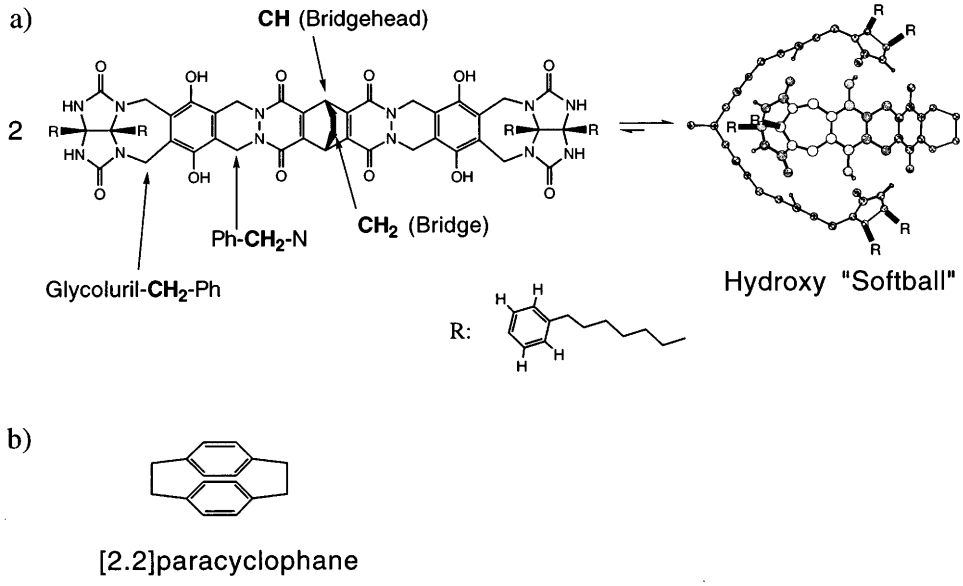

Figure 1. (a) Self-assembly of two identical monomeric units into the hydroxy 'softball' (the R groups, 4-*n*-heptylphenyl, are attached by its aromatic ring to the glycoluril moiety of the hydroxy 'softball') and (b) [2.2]paracyclophane. The ^{13}C T_1 relaxation time was measured for the CH and CH_2 groups of the skeleton as indicated by arrows.

where $r_{CH} = 1.09$ Å (typical C—H bond length obtained from rotational spectroscopy) and γ_H and γ_C are the magnetogyric ratios for proton and carbon, respectively.

The simplest model for molecular motion is that of an isotropically diffusing sphere (the single correlation time theory).[6] The spectral density is given in this case by the equation

$$J(\omega) = \frac{\tau_C}{1 + \omega^2 \tau_C^2} \tag{2}$$

The molecular correlation time, τ_C, is the average time required for the molecule to pass through two orientations (in seconds). This model is a good approximation for rigid, spherical molecules where the correlation time is equal for all carbons.

The measured relaxation parameters, T_1 and NOE, are given by the following expressions:[7,8]

$$\frac{1}{T_{1,\,DD}} = \frac{N}{10} \&DCC)^2$$

$$[J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)] \tag{3}$$

$$\mathrm{NOE} = 1 + \frac{\gamma_H}{\gamma_C}\left[\frac{6J(\omega_H + \omega_C) - J(\omega_H - \omega_C)}{J(\omega_H - \omega_C) + 3J(\omega_H) + 6J(\omega_H + \omega_C)}\right] \tag{4}$$

were N = number of attached protons.

If extreme narrowing conditions exist, such that $\tau_C^2(\omega_H + \omega_C)^2 \ll 1$, Eqns (3) and (4) are simplified to

$$\frac{1}{NT_{1,\,DD}} = (DCC)^2 \tau_C \tag{5}$$

$$\mathrm{NOE} = 1 + \frac{\gamma_H}{2\gamma_C} \tag{6}$$

Furthermore, if the relaxation is dominated by the dipole–dipole (DD) interaction, the maximum NOE ($\mathrm{NOE}_{max} = 2.988$) should be obtained. Any deviation of the NOE from NOE_{max} under these conditions indicates the presence of other relaxation mechanisms.

Another relaxation mechanism encountered for ^{13}C nuclei is the spin–rotation (SR) mechanism. This is particularly important for small molecules and substituents, e.g. methyl groups. For aromatic tertiary carbons the chemical shift anisotropy (CSA) mechanism often contributes to the spin–lattice relaxation; this mechanism is especially important at high field strengths owing to the field dependence of the CSA mechanism.[9]

If cross-correlation phenomena can be neglected, the total relaxation rate, $1/T_1$, is given by the sum of the relaxation rates for the contributing mechanisms:

$$\frac{1}{T_1} = \frac{1}{T_{1,\,DD}} + \frac{1}{T_{1,\,SR}} + \frac{1}{T_{1,\,CSA}} + \cdots \tag{7}$$

RESULTS AND DISCUSSION

The ^1H NMR spectra indicate that the dimeric capsule (the hydroxy 'softball') is the dominant species in $CDCl_3$; the concentration of monomers must be very low as the NH chemical shift is concentration independent (0.02–0.001 M).[3,4a]

The ^1H NMR spectrum of the hydroxy 'softball' with 3.5 equiv. of [2.2]paracyclophane in $CDCl_3$ shows only the resonances for free [2.2]paracyclophane, the assembled hydroxy 'softball' and the encapsulated [2.2]paracyclophane. The CH_2 groups from the complexed [2.2]paracyclophane appear at 1.69 ppm and the aromatic protons of the complexed [2.2]paracyclophane appear at 5.02 ppm, i.e. 1.38 and

1.30 ppm upfield of the resonances for free [2.2]paracyclophane in $CDCl_3$, respectively. The association constant, K_a [Eqn (8)], for the hydroxy 'softball' · [2.2]paracyclophane complex in $CDCl_3$ was determined from the relative NMR intensities to be $(3.5 \pm 0.5) \times 10^3\,l\,mol^{-1}$.

$$\text{'softball'} + \text{[2.2]paracyclophane} \xrightarrow{K} \text{'softball'} \cdot \text{[2.2]paracyclophane}$$

$$K_a = \frac{[\text{'softball'} \cdot \text{[2.2]paracyclophane}]}{[\text{'softball'}][\text{[2.2]paracyclophane}]} \quad (8)$$

This constant is large enough to allow the assumption that the hydroxy 'softball' · [2.2]paracyclophane complex is the major complex of the hydroxy 'softball' in solution.

The observation of only one set of resonances for the hydroxy 'softball' · [2.2]paracyclophane complex indicates either that the reorientation time of the guest inside the capsule is fast on the NMR time-scale, thereby averaging the asymmetric shielding environment provided by the host, or it may be a result of the high symmetries of both the host and the guest.

Obviously the exchange rate between free and encapsulated [2.2]paracyclophane is slow on the NMR time-scale as separate and sharp signals are observed at 295 K. The guest exchange gives rise to very weak cross peaks in the $^1H,^1H$-NOESY spectrum (mixing time 0.5 s). The observed NOE cross peaks between the hydroxy 'softball' and the [2.2]paracyclophane suggests that on the NMR time-scale there are no preferred host-guest orientations.

The proton decoupled ^{13}C NMR spectrum of the mixture of the hydroxy 'softball' and [2.2]paracyclophane also shows only one set of resonances for the hydroxy 'softball' with encapsulated [2.2]paracyclophane and one set for free [2.2]paracyclophane. The resonances from carbons bearing one and two protons were assigned based on the $^1H,^{13}C$-HMQC and $^1H,^1H$-NOESY spectra. The ^{13}C NMR chemical shifts for the encapsulated [2.2]paracyclophane carbons were observed 1.14, 1.44 and 0.74 ppm upfield of the corresponding carbons of the free [2.2]paracyclophane.

Molecular modeling of the hydroxy 'softball' with [2.2]paracyclophane was performed using Macromodel and the Amber* force field.[10] From a Monte Carlo conformational search the most stable structures were found to be a pair of enantiomeric assemblies. The chirality is derived from a slightly unsymmetrical orientation of the guest [2.2]paracyclophane inside the otherwise symmetric hydroxy 'softball.' In solution, however, heteroconversion of the enantiomeric assemblies is expected to be fast. The cavity of the minimized structure was measured to be roughly 10 Å long and 9 Å wide (nucleus to nucleus). The shape of [2.2]paracyclophane is roughly spherical. This molecule fits (based on van der Waals radii) inside a rectangular box with sides of 7.8, 5.5 and 4.3 Å. The lowest energy conformation found of the hydroxy 'softball' · [2.2]paracyclophane complex is shown in Fig. 2.

^{13}C spin lattice relaxation times (T_1)

If extreme narrowing conditions exist (which is very likely for such a small, rigid and roughly spherical molecule) the relaxation is only due to dipolar interactions and one should obtain NOE_{max}. The NOEs were determined by taking the ratios of peak intensities between spectra obtained with ^1H decoupling turned on all the time (to allow for NOE build-up) and from spectra with no proton decoupling prior to acquisition.[12] The relaxation delay in this experiment was 50 s, e.g. about $10T_1$ for the CH_2 and the CH carbons. The quaternary carbons were not measured because of the low signal-to-noise ratio.

The NOEs determined are given in Table 1. Since the measured NOEs are lower than 2.988, other spin–lattice relaxation mechanisms such as spin–rotation and possibly chemical shift anisotropy are important.

A surprising finding is that the aromatic carbon experiences only roughly 50% dipole–dipole relaxation at 150 MHz. What are the other relaxation pathways involved? Spin–rotation is a known relaxation pathway for substituted aromatics. However, a 50% contribution of the spin–rotation mechanism to the spin–lattice relaxation does not seem reasonable, but at a magnetic field of 150 MHz it seems more likely that the major relaxation pathway for the ^{13}C nuclei is the CSA relaxation.[9]

In order to verify this hypothesis, an NOE experiment was also performed at a magnetic field of 100 MHz as CSA is field dependent. At this field the dipole–dipole relaxation was determined to be 78% for the CH carbons and 95% for the CH_2 carbon. This strongly indicates that the CSA is indeed a major relaxation pathway for the CH aromatic carbons in [2.2]paracyclophane at 150 MHz.

The ^{13}C spin–lattice relaxation times for [2.2]paracyclophane in $CDCl_3$ at 295 K were determined from an inversion–recovery experiment with short recovery times and a large number of dummy scans to allow for the development of a steady state.[13]

The CH_2 carbon at 35.8 ppm and the CH carbon at 133.3 ppm have spin–lattice relaxation times of 4.2 and

Table 1. Measured NOEs for the different carbons in [2.2]paracyclophane at 600 MHz[a]

Carbon nuclei	NOE $(1 + \eta)$	Dipole–dipole relaxation (%)
CH_2	2·8	93
CH	1·9	47

[a] The theoretical limit of a nucleus totally dominated by the DD mechanism is 2.988.

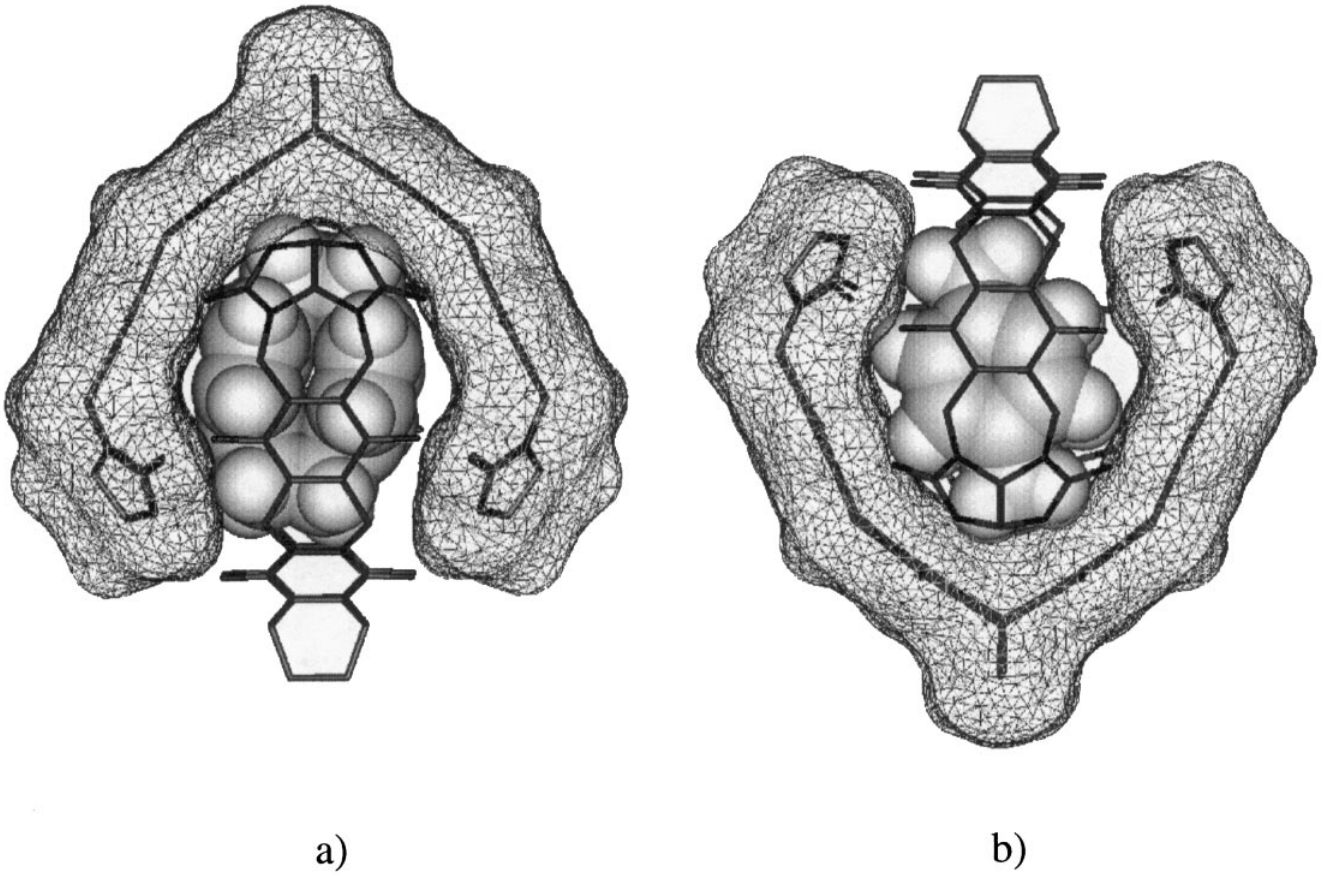

Figure 2. The energy-minimized structures of the hydroxy 'softball' · [2.2]paracyclophane complex. The calculated Connolly surface[11] is shown for the subunit that is in the plane. The guest, [2.2]paracyclophane, is shown as a CPK model. (a) Front view; (b) side view.

5.2 s, respectively. The dipole–dipole relaxation times ($T_{1,\mathrm{DD}}$) for the CH vectors are calculated to be 8.60 s (CH_2) and 11.1 s (CH) from the measured T_1 and the fraction of dipole–dipole relaxation determined from the measured NOE.

At a magnetic field of 14 T the free [2.2]paracyclophane is in the extreme narrowing limit and the correlation time for the molecule is calculated using Eqn (3). This gives correlation times of 4.2×10^{-12} s (CH) and 5.4×10^{-12} s (CH_2).

Altogether, we cannot exclude the possibility that the molecule undergoes an anisotropic motion in solution. However, the difference between the correlation times for the CH vectors is small compared with their respective errors and we therefore suggest that [2.2]paracyclophane mainly undergoes an overall isotropic motion in $CDCl_3$.

The ^{13}C spin–lattice relaxation times for the hydroxy 'softball' · [2.2]paracyclophane complex in $CDCl_3$ at 295 K were determined using the inversion–recovery experiment as performed for the guest above.[15] It is assumed that the spin–lattice relaxation of the complex is dominated by the DD mechanism exclusive of the [2.2]paracyclophane aromatic carbons.

Table 2 gives the ^{13}C NMR spin–lattice relaxation times for selected carbons for free [2.2]paracyclophane and for the hydroxy 'softball' · [2.2]paracyclophane complex.

Since the NT_1 of the different CH vectors from the hydroxy 'softball' skeleton are nearly identical (0.27, 0.30 and 0.31 s), it is reasonable to assume that these carbons represent the overall molecular tumbling. These data also indicate that the hydroxy 'softball' can be described by a simple model in which the 'softball' is taken to be a pseudo-spherical molecule that tumbles isotropically in solution.

The spin–lattice relaxation times are not necessarily in the extreme narrowing limit at the magnetic field used of 14 T. In calculating correlation times, there are two valid solutions to Eqn (3). One is shorter and approaches extreme narrowing conditions while the other is longer and lies far outside the regime where such an approximation is valid.

In order to exclude one of these possibilities, the ^1H–$\{^{13}C\}$ NOEs were determined for the carbons of the hydroxy 'softball.' The measured NOE for the carbons of the skeleton (CH_2 and CH carbons) was found to be in the range 1.9–2.1), indicating that this system is in or just outside the extreme narrowing limit.

The correlation times calculated from the determined $T_{1,\mathrm{DD}}$ for the ^{13}C NMR resonances from the hydroxy 'softball' · [2.2]paracyclophane complex are given in

Table 2. Measured spin–lattice relaxation times (T_1) for the ^{13}C NMR resonances for free [2.2]paracyclophane and for the encapsulation complex formed between hydroxy 'softball' and [2.2]paracyclophane together with their ^{13}C and ^{1}H NMR chemical shifts[a]

Signal	δ ^{13}C (ppm)	δ ^{1}H (ppm)	T_1 (^{13}C) (s)
Free guest, [2.2]paracyclophane			
CH_2	35.8	3.08	4.3
CH	133.3	6.42	5.1
Complexed guest, [2.2]paracyclophane			
CH_2	35.0	1.69	0.23
CH	131.9	5.03	0.22
Hydroxy 'softball'			
CH_2 (bridge)	23.2	0.93	0.15
CH (bridgehead)	32.6	4.02	0.27
Glycoluril-**CH_2**Ph	41.9	6.50	0.16
		4.17	
Ph**CH_2**N	38.0	5.72	0.15
		4.00	
CH (*n*-heptylphenyls)	129.0	7.00	0.22
	128.2	7.11	0.21
	128.0	6.90	0.22
	127.7	7.36	0.22
CH_3 (*n*-heptyl)	14.2	2.69	3.2
CH_3 (*n*-heptyl)	14.2	2.55	3.1

[a] The T_1 of the CH_2 carbons for the *n*-heptylphenyl groups are between 0.3 and 2.6 s.

Table 3. These are significantly more accurate than those calculated from the ^{13}C–$\{^{1}H\}$ NOEs. Based on the 5% error taken from the inversion–recovery experiment, the error in the calculated correlation times is between 10 and 20%. The smaller error is estimated for the carbon with longer T_1 and the larger error is estimated for the carbon with shorter T_1.

Table 3. Calculated spin–lattice relaxation times ($T_{1,DD}$) and correlation times for the CH vectors for free [2.2]paracyclophane and for the encapsulation complex formed between hydroxy 'softball' and [2.2]paracyclophane.

Signal	δ ^{13}C (ppm)	$T_{1,DD}$(^{13}C)[a] (s)	τ_c[a] (s)
Free guest, [2.2]paracyclophane			
CH_2	35.8	8.6	5.4×10^{-12}
CH	133.3	11.1	4.2×10^{-12}
Complexed guest, [2.2]paracyclophane			
CH_2	35.0	0.46	1.2×10^{-10}
CH	131.9	0.44	1.3×10^{-10}
Hydroxy 'softball'			
CH_2 (bridge)	23.2	0.30	2.7×10^{-10}
CH (bridgehead)	32.6	0.27	3.8×10^{-10}
Glycoluril-**CH_2**Ph	41.9	0.31	2.3×10^{-10}
Ph**CH_2**N	38.0	0.31	2.7×10^{-10}

[a] The value for τ_c is calculated based upon the contribution of the ^{1}H,^{13}C dipole–dipole relaxation ($T_{1,DD}$) to the overall measured T_1 (from the inversion–recovery experiment).

The average correlation time of the signals from the skeleton of the hydroxy 'softball' based on NOE data is 3×10^{-10} s [Eqn (4)]. Despite the low accuracy of the measured NOEs, the results offer similar correlation times as the T_1. These values are also close to previously measured correlation times for the overall rotation of other molecules with molecular weights of about 1000.[1b,5j,l]

The aromatic CH carbons of the complexed [2.2]paracyclophane have T_1 of 0.22 s and the corresponding CH_2 has a T_1 of 0.23 s. The short T_1 value for the aromatic CH carbons cannot be explained solely by dipolar relaxation via its bonded proton, and therefore other mechanisms must be important. It is reasonable to assume that there is a contribution to the spin–lattice relaxation time from the ^{13}C–^{1}H dipole relaxation of about 50%, since the dipole–dipole relaxation contribution to the spin–lattice relaxation of the aromatic carbons of the free [2.2]paracyclophane is only about 50% (Table 1). Such an approximation is not unreasonable as both the free and the encapsulated guest are close to the extreme narrowing limit. A 50% contribution of the dipole–dipole mechanism to the overall T_1 gives a correlation time of 1.3×10^{-10} s for the CH carbon of encapsulated [2.2]paracyclophane.

The flexible side-chains of the host glycolurils, the *n*-heptylphenyls, have larger T_1 values owing to additional internal mobility of the alkyl chains compared with the hydroxy 'softball' skeleton. Correlation times of 1×10^{-11}–7×10^{-11} s were determined for the *n*-heptyl groups with shorter correlation times for the methyl groups and longer times for the methylene groups next to the phenyl unit.

The four aromatic CH carbons all have surprisingly short T_1 relaxation times (0.21–0.22 s). Such short spin–lattice relaxation times are probably a result of a contribution from CSA relaxation, thus making the dipole–dipole relaxation time longer than the observed T_1.

The correlation time for [2.2]paracyclophane is found to be slowed by a factor of 20 upon encapsulation by the hydroxy 'softball,' based upon the correlation times of the CH_2 and CH carbons of [2.2]paracyclophane. The dynamic coupling constant, χ, for the complex is calculated to be 0.45 based on the average correlation time for [2.2]paracyclophane (CH_2 and CH carbons) and the average correlation time (2.9×10^{-10}) for the skeleton of the hydroxy 'softball.' This large value indicates that the [2.2]paracyclophane mobility is largely affected by, and probably associated with, that of the hydroxy 'softball.' The large value of the average dynamic coupling constant also indicates the close shape complementarity of the guest to the cavity.

Modelling[11] suggests that the interior of the hydroxy 'softball' is roughly spherical. A guest molecule may either bind/interact with the hydroxy 'softball,' or behave as if it were in a smooth cavity, experiencing free

and fast molecular reorientation without bumping into solvent molecules or protrusions from the walls. However, molecular modeling of the encapsulated [2.2]paracyclophane indicate a slightly preferred orientation in which the alkyl carbons point toward the center of the monomeric units, i.e. the underside of the bridge. In such a conformation the rotation of [2.2]paracyclophane along its longer axis would therefore be expected to be slower. This was not observed in the NOESY spectrum, but the NOE cross peaks from a preferred and 'static' orientation may very well be indistinguishable from those expected from a complex wherein the guest molecule undergoes fast reorientation.

The binding of a guest molecule by a host is entropically disfavored, since translational and possibly rotational entropy is lost. The previous observation of entropy-driven encapsulation was rationalized by a gain in entropy upon the release of two solvent molecules.[4b] The loss of entropy upon complexation could also be compensated for by an increase in enthalpy due to favorable van der Waals interactions between the host and the guest.[15] For a system with perfect shape complementarity such interactions would result in strong binding and the resulting complex would be tight, i.e. the guest would, in essence, become a part of the host.

Based on its binding constant, [2.2]paracyclophane appears to be a good guest. The approximate packing coefficients (PCs) (from the van der Waals volume of the guest, 190 Å, *vs.* that of the host cavity, 313 Å), is 60%, a value close to that of organic liquids and similar host–guest complexes.[3b,16]

The slow molecular motions of [2.2]paracyclophane inside the hydroxy 'softball' confirm that the shape complementarity of guest and host is very high. It is also noteworthy that the tight complex arises in the absence of any strong non-covalent interactions, e.g. ionic, dipolar or hydrogen bonding, between the hydroxy 'softball' and its guest. However, several weak non-bonding phenyl–phenyl interactions are possible, both face-to-face and edge-to-face.[17] We have observed that guest molecules with additional functional groups, which can form electrostatic interactions or hydrogen bonds with the hydrogen bonding network of the hydroxy 'softball,' have larger binding constants. Such complexes may, however, have lower dynamic coupling constants because a number of hydrogen bonding sites exists on the concave surfaces of the softball. We will address these issues in future experiments.

EXPERIMENTAL

For the NMR experiments 29.5 mg (9.4×10^{-6} mol) of hydroxy 'softball' were dissolved in 300 μl of $CDCl_3$ and 9.32 mg (3.23×10^{-5} mol) of [2.2]paracyclophane were added in 200 μl of $CDCl_3$, yielding a solution that was 19 mM in hydroxy 'softball' and 65 mM in [2.2]paracyclophane. Prior to the NMR experiments the samples were degassed by several freeze–pump–thaw cycles.

The NMR spectra were recorded at a constant temperature of 295 K on a Bruker DRX 600 spectrometer with a dual carbon–proton probe, operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C. The chemical shifts were referenced to residual $CHCl_3$; ^{1}H, δ 7.26 ppm; ^{13}C, δ 77.23 ppm.

^{13}C NMR T_1 and NOE measurements

Spectra for the carbon T_1 experiments were acquired using the standard inversion–recovery pulse sequence, with a relaxation delay of 5 s and 16K data points. For each T_1 experiment 32 dummy scans were followed by 2000 scans under continuous proton Waltz decoupling. The following two sets of recovery times were used in two different T_1 experiments: (a) 4.5, 3, 2, 1.1, 0.6, 0.4, 0.2 and 0.1 s; (b) 1.8, 0.9, 0.5, 0.25, 0.12, 0.06, 0.04 and 0.02 s. The spectra were processed with exponential multiplication using a line broadening factor of 4 Hz. Both the T_1 of the guest and the host were measured on the same sample to exclude viscosity differences between different samples. The ^{13}C spin–lattice relaxation times (T_1) were evaluated using a non-linear three-parameter fitting of the following expression to the intensities (peak intensities and integrals):

$$S_\tau = S_\infty\left[1 - A\,\exp\left(-\frac{\tau}{T_1}\right)\right] \qquad (9)$$

where S_τ is the measured signal intensity at time τ and S_∞ is the equilibrium magnetization at very high τ values. In Fig. 3 the resulting curve using Eqn (9) on the intensity *vs.* recovery times for selected ^{13}C resonances are given.

Reported T_1 values have an accuracy estimated to be better than 5% based on the fit to the data. This does not include possible systematic errors.

NOEs were obtained from the increase of the signal intensity when broadband decoupling is used compared with when it is absent and were calculated using the relation NOE $= \eta + 1 = I_E/I_N$, where I_E and I_N are the enhanced and not enhanced peak areas, respectively. A delay time of $10T_1$ was used to ensure complete relaxation of the ^{13}C magnetization. The accuracy of the NOE is better than 20% based on the difference of two experiments for each number.

In all T_1 and NOE experiments, standard pulsed broadband decoupling techniques were used, with the decoupler power attenuated during the recovery time in order to avoid sample heating.

Assignments

Assignment of the ^{1}H and the proton-bearing ^{13}C chemical shifts for the hydroxy 'softball' and [2.2]paracyclophane were made using a ^{1}H,^{13}C-HMQC experiment and a ^{1}H,^{1}H-NOESY experiment.

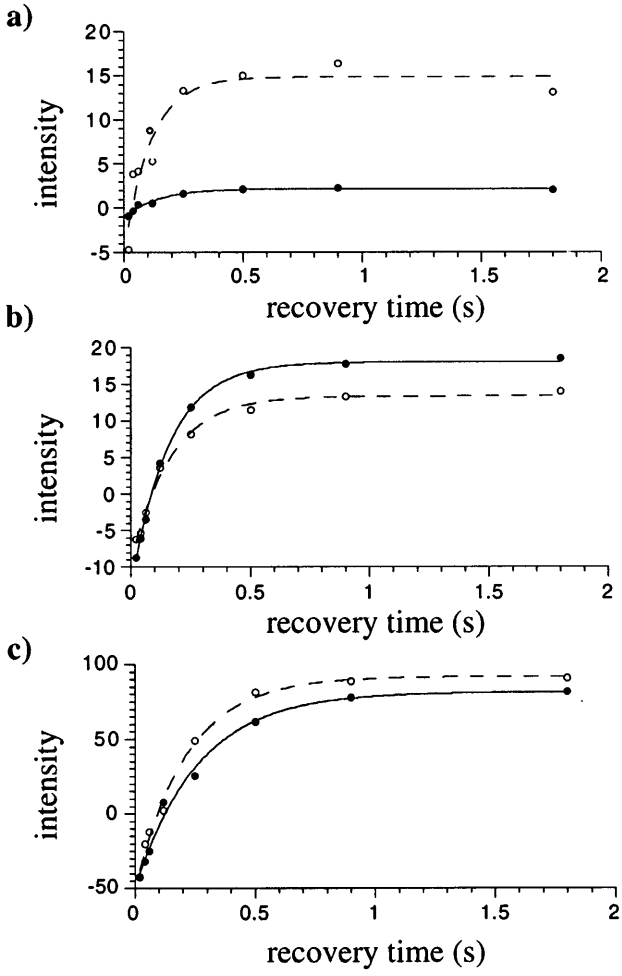

Figure 3. Curve fitting Eqn (9) to the intensities *vs.* recovery times obtained from the inversion–recovery experiment. The closed circles are the peak intensities and the open circles are the corresponding integrals. (a) Glycoluril-CH_2Bz, $T_1 = 0.15$ s; (b) $PhCH_2N$, $T_1 = 0.15$ s; (c) CH (bridge), $T_1 = 0.27$ s.

$^1H,^{13}C$-HMQC. The phase sensitive gradient-selected experiment was run with spectral widths of 8000 Hz in the acquisition domain, F_2, and 30 000 Hz in the evolution domain, F_1. A single transient of 2K data points for each of the 256 increments was recorded, with a relaxation delay of 2 s. The transients were collected using the standard TPPI method. The resulting matrix was multiplied by a 90° phase-shifted square-sine bell function in both dimensions and zero filled to 1K data points in the F_1 dimension before Fourier transformation.

$^1H,^1H$-NOESY. The phase-sensitive experiment was run with spectral widths of 8000 Hz and 2K data points in the F_2 dimension. A total of 16 transients for each of the 192 increments were recorded with a relaxation delay of 2 s and a mixing time of 1.0 s. The transients were collected using the standard TPPI method. The resulting matrix was multiplied by a 90° phase-shifted square-sine bell function in both dimensions and zero filled to 1K data points in the F_1 dimension and 2K data points in the F_2 dimension before Fourier transformation.

Acknowledgments

We are grateful to Dr Lena Mäler for stimulating discussions and valuable advice and to Dr Ulrike Obst for her kind help with molecular modeling. We also thank Mr Kent Pryor for linguistic improvements. Financial support from the National Institutes of Health and the Skaggs Research Foundation and a fellowship from the Knut and Alice Wallenberg Foundation to G.H. are all gratefully acknowledged.

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