Structure and dynamic stability of cyclodextrin inclusion complexes with 1,4-disubstituted bicyclo[2.2.2]octanes

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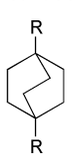
The properties of inclusion complexes of 1,4-di-R-bicyclo[2.2.2]octanes (R = H (**1**), Me (**2**), Cl (**3**), Br (**4**), OH (**5**), Me,OH (**6**)) with cyclodextrins (Cdx) have been studied by various NMR-techniques, microcalorimetry and molecular mechanics and dynamics computations. Compounds **2**, **3** and, possibly, **4** (but not the other compounds) gave dynamically stable 1 : 2 guest–host complexes with α-Cdx, but did not show any indication of a 1 : 1 complex. Microcalorimetry of **5** in water indicates a moderately strong 1 : 1 complex with β-Cdx but at best very weak complexes with α- or γ-Cdx. The unsymmetrically substituted compound **6** behaved similarly to **5**. The behavior depends on the subtle interplay of size, polarity, hydrophobicity, type of solvent and temperature. The origin of the unusually high barrier for formation of the 1 : 2 complex is proposed to be unsynchronized entropy and enthalpy development, originating in the requirement for strong preorganization in the formation of the complex. A slow exchange between dissolved and dispersed **2** was observed and characterized in the solution in the same temperature range.

Naturally occurring cyclodextrins (Cdx's) are composed of six, seven or eight α-(1→4)- linked D-glucopyranose units, traditionally given the names α-, β- and γ-Cdx, respectively. They act as hosts for a variety of small molecules in aqueous solution and have found use in many fields, such as chromatography, the pharmaceutical industry, building blocks in supramolecular chemistry, and as potential enzyme mimics,[1] and their inclusion complexes have proven to be excellent model systems for studying the nature of noncovalent bonding in aqueous solution. In particular, the so called "hydrophobic effect"[2] has been analyzed by the calorimetric determination of heat capacity data for the processes in which hydrophobic moieties are transferred from a nonpolar environment to aqueous solution or *vice versa*. Considerable attention has been devoted to the driving force of the inclusion,[2–4] but much less to the kinetics[5] and its dependence upon variations in solvent, concentration, salt addition *etc.* In order to study the structure and dynamic stability of such inclusion complexes 1,4-disubstituted bicyclo[2.2.2]octanes were used and turned out to possess interesting properties as guest molecules. Thus, the 1,4-dimethyl derivative was found to give a 1 : 2 complex with α-Cdx in D_2O–CD_3OD–DMF-d_7 (5 : 3 : 3), which has a remarkably high barrier to exchange with "free" species.[6,7] We present here an extended study of a series of analogues in order to gain information on the origin of the unusual dynamic stability of some of the complexes and on their structures and stabilities as a function of the 1,4-substituent and the type of Cdx. The complexes of the analogues, shown below, with α-, β- and γ-Cdx have been examined by both experimental and computational methods.



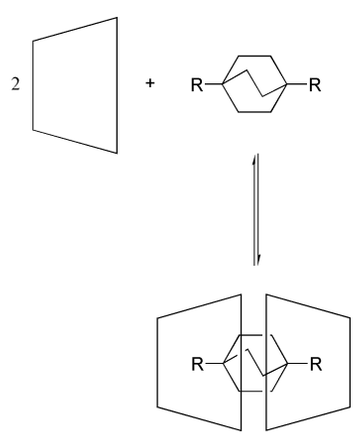
R = H (**1**), CH_3 (**2**),
Cl (**3**), Br (**4**),
OH (**5**), CH_3,OH (**6**)

Results

Dynamic NMR spectroscopy

The ^{1}H NMR study of **2** with α-Cdx in D_2O–CD_3OD–DMF-d_7 exhibited broad singlets at room temperature for the methyl and methylene protons, respectively. On lowering the temperature the signals broadened further and at *ca.* 10 °C decoalesced to three sets of signals (Fig. 1).[6] With **3**, and even more pronounced with **4**, crystallization occurred at low temperatures preventing a detailed study, but apart from that they behaved as **2**. The rate constants for the observed exchanges were evaluated by bandshape simulations,[8] and the corresponding free energies of activation were calculated as 13.4 ± 0.2 kcal mol^{-1} for **2** and 13.2 ± 0.3 kcal mol^{-1} for **3**. The limited available temperature range and possible non-extreme narrowing conditions made the splitting of $\Delta G^{\ddagger}$ into its enthalpy and entropy components impossible. The phenomenon was studied for different concentrations and no significant effect on the rate was observed.

None of the other bicyclooctanes showed similar behavior, nor did any of the compounds **1**–**6** with β- or γ-Cdx. We interpreted the phenomenon as a slow exchange between "free" species and 1 : 2 guest–host complex (eqn. (1)). Evidence in



(1)

terms of peak intensities, ROESY spectrum, behavior of α-Cdx 2-monotosylate (toluene-*p*-sulfonate) and computations have been presented.[6] The ROESY spectrum of **2** allowed a precise determination of the structure of the complex in which half of the guest molecule has penetrated into each of the Cdx cavities

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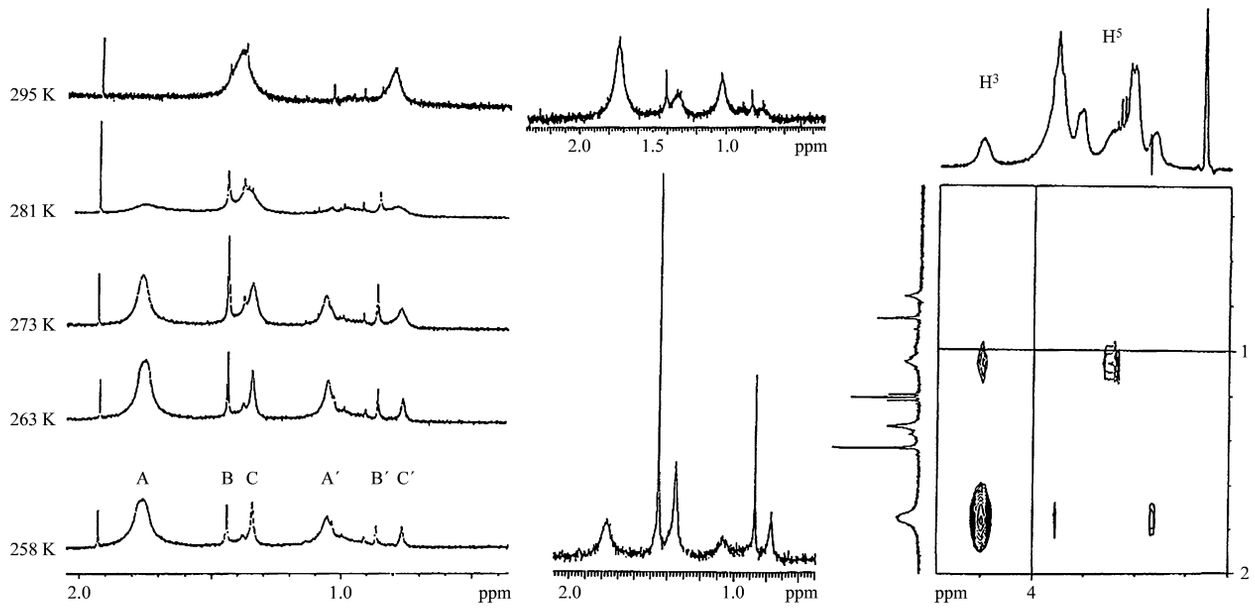


Fig. 1 a) 500 MHz ^{1}H NMR spectra of a solution of **1** (6 mM) and α-Cdx (7 mM) at various temperatures. Solvent: D_2O–CD_3OD–DMF-d_7 (5 : 3 : 3). Signals from impurities at δ *ca.* 1.0, 1.48, and 1.92. b) 300 MHz ^{1}H NMR spectra in the same solvent at the 1 : α-Cdx concentration ratios 1 : 0.5 (lower spectrum) and 1 : 1.2 (upper spectrum); temperature: 258 K. c) Part of the ROESY contour map of the same solution as in Fig. 1a. The α-Cdx protons H^3 and H^5 in the complex, identified by a COSY spectrum, are indicated. Residual protons from CD_3OD give a signal at δ 3.32, and an impurity has peaks at δ 1.20 and 3.63.

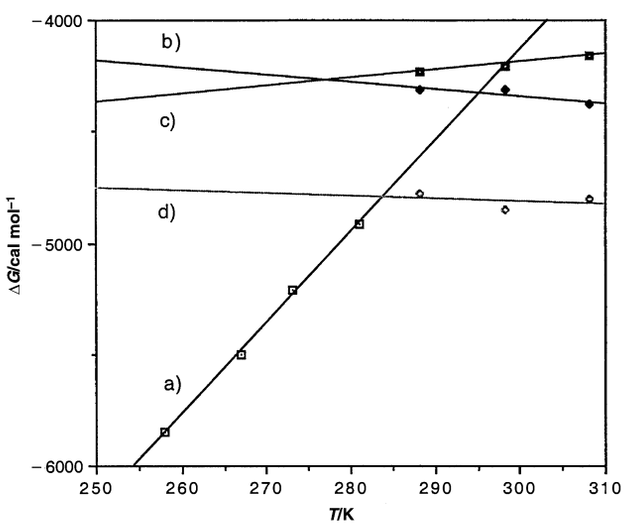


Fig. 2 Temperature dependence of ΔG. a) **2** and α-Cdx from the NMR experiment; b) **5** with β-Cdx from microcalorimetric titration; c) octane-1,8-diol and α-Cdx from microcalorimetric titration; d) nonane-1,9-diol and α-Cdx from microcalorimetric titration. See text for experimental details.

from the side of the secondary rim. Remarkably, there was no sign of the existence of a 1 : 1 complex at any concentration ratio between **2** and α-Cdx in the NMR experiments. The ROESY spectrum also showed negative cross peaks for the exchanging protons. Assuming an equilibrium as in eqn. (1), apparent thermodynamic parameters of **2** for the process, obtained from NMR peak intensities, could be derived: $\Delta H^\circ_{app} = -16.3 \pm 1.0$ kcal mol^{-1} and $\Delta S^\circ_{app} = -41 \pm 5$ cal K^{-1} mol^{-1} (Fig. 2). These values are far from the pattern usually shown for the classical hydrophobic effect.

The third set of signals was interpreted as dispersed compound in slow exchange with dissolved **2**. We could prove that the rate of the Cdx complex formation did not interfere with this equilibrium in that the feature was maintained when the experiments were repeated without Cdx in the solution.[9] Thus, a solution of **2** in the same solvent mixture gave rise to one set of signals above room temperature. At *ca.* 25 °C new signals began to appear at the same chemical shifts as the third signals in the experiment with Cdx, and below *ca.* −15 °C this new set of signals were the only signals present from **2** (Fig. 3). The temperature interval within which this feature was observed was very sensitive to the concentration of the solute as well as solvent composition. The intensity of the signal from the dispersed phase varied with concentration ratios and solvent composition (Fig. 1), but the appearance of this signal could not be avoided at the lower temperatures. A saturation transfer–saturation recovery study revealed a first order rate constant of 0.23 s^{-1} (dispersed → dissolved) at 22 °C for the exchange between the dispersed and dissolved phases. Thus, the rate for complex formation is 10^3 to 10^4 times larger than the rate of exchange between the two phases, and the former process can be studied without interference from the latter.

Microcalorimetry

Isothermal microcalorimetric titration of **5** with cyclodextrins in water gave the results shown in Table 1.[10] The calorimetric data could be fitted to a 1 : 1 model using nonlinear regression methods but not to a 1 : 2 model. A complex of moderate stability was found with β-Cdx ($K_c = 1460 \pm 72$ M^{-1}), whereas complexation with α- and γ-Cdx was very weak. Due to the low solubility of **2** in water or mixtures of water and methanol this compound could not be studied by calorimetric methods.

Diffusion edited NMR

This technique has proven valuable for the study of molecular interactions in mixtures of molecules some of which give complexes with different diffusion coefficients.[11,12] This technique was applied to mixtures of guests with α-Cdx or β- Cdx in order to establish semiquantitatively the strength of the complexes.[13,14] For example, there was ambiguous evidence of complexation of compounds **5** and **6** with α-Cdx from other techniques.

Fig. 4a shows a competitive gradient field experiment between **5** and **6** with β-Cdx in water at room temperature together with a plot of $\ln A_g/A_o$ *versus* g^2, where A_g/A_o is the ratio between signal intensities in the presence and absence of pulse gradients, respectively, according to the relation given in eqn. (2),

$$\ln A_g/A_o = -\gamma^2 g^2 \delta^2 (\Delta - \delta/3) D \qquad (2)$$

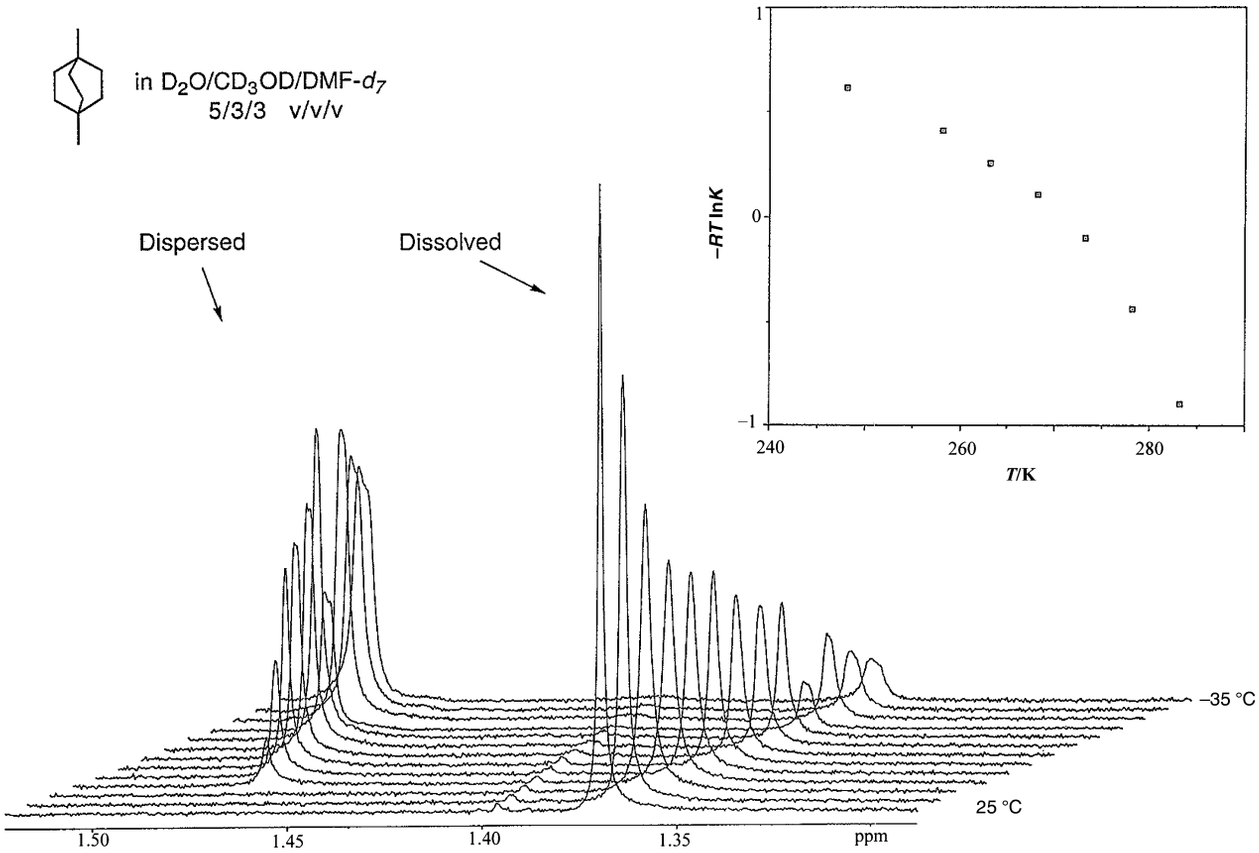


Fig. 3 Temperature dependence of the intensity ratio of the NMR peaks from dispersed and dissolved **2**.

Table 1 Results from the microcalorimetric titration of **5** at 25.01 °C assuming a 1 : 1 model[10]

Cdx	$K_{c/M^{-1}}$	$\Delta G°$/kcal mol^{-1}	$\Delta H°$/kcal mol^{-1}	$\Delta S°$/cal K^{-1} mol^{-1}	ΔC_p/cal K^{-1} mol^{-1}
α	<100	—	—	—	—
β	1460 ± 72	−4.32 ± 0.03	−4.27 ± 0.04	0.17 ± 0.13	−74.8
γ	<50	—	—	—	—

where γ is the magnetogyric ratio (rad s G^{-1}), g is the strength of the pulse gradient fields (G cm^{-1}), δ the length of the diffusion gradients (s) and Δ is the time separation between the pulsed gradients (s).[15] D is the diffusion coefficient (cm^2 s^{-1}). Assuming the same magnitude of D for the complexes and no 1 : 2 complex, as verifed by the calorimetric studies, D will directly reflect the equilibrium constant after compensation for the small differences in diffusion of uncomplexed molecules ($D°$) according to eqn. (3). D^{exp} is the observed "diffusion constant" for the signal representing the equilibrium between free substrate and complex. The estimated equilibrium constants are shown in Table 2. Diffusion studies of **5** and **6** with α-Cdx confirm the weak complexation found in the microcalorimetric study. Interestingly, **5** a showed slightly larger diffusion effect than **6**.

$$K_1/K_2 = (D_1° - D_1^{exp})D_2°/(D_2° - D_2^{exp})D_1° \qquad (3)$$

A gradient field experiment was also performed on **2** in the absence of Cdx under conditions where the two phases appear, showing the expected slow diffusion of the species ascribed to the dispersed phase (Fig. 4b).

Table 2 Equilibrium constants in D_2O evaluated from the diffusion measurements at 295 K

Compound	K/M^{-1} α-Cdx	β-Cdx
2	—	3600
5	35	(1460)[a]
6	30	2500

[a] Reference value from the calorimetric measurements, taken from ref. 10.

Force-field modelling

The 1 : 1 and 1 : 2 complexes of the compounds **2**, **5** and **6** with α- and β-Cdx were studied by molecular mechanics computations using several force fields in Macromodel version 6.5. Default models were used for electrostatic interactions and both the GB/SA water model and the explicit introduction of 150 TIP3P water molecules were applied in addition to vacuum calculations. For the 1 : 1 α-Cdx complexes in the gas phase, Monte Carlo searches using the AMBER* force field and allowing for translation and rotation of the guest molecule followed by energy minimization resulted in a stable and unique inclusion complex only for **2**. For **5** and **6** structurally different minima within a few kcal mol^{-1} were found, the global minima being externally hydrogen bonded complexes. Using β-Cdx as host, global minima were 1 : 1 inclusion complexes with all substrates. Penetration simulations (Fig. 5) revealed major differences between α-Cdx and β-Cdx complexes. For α-Cdx two distinct energy minima corresponding to binding from both sides of the Cdx were found and the barrier for penetration was higher than 25 kcal mol^{-1}. About half of the guest molecule was exposed to the exterior enabling complexation with another host molecule. In the case of β-Cdx a shallow part surrounds a minimum in which the guest molecule is placed, in the center of the cavity. The energy approached that of the

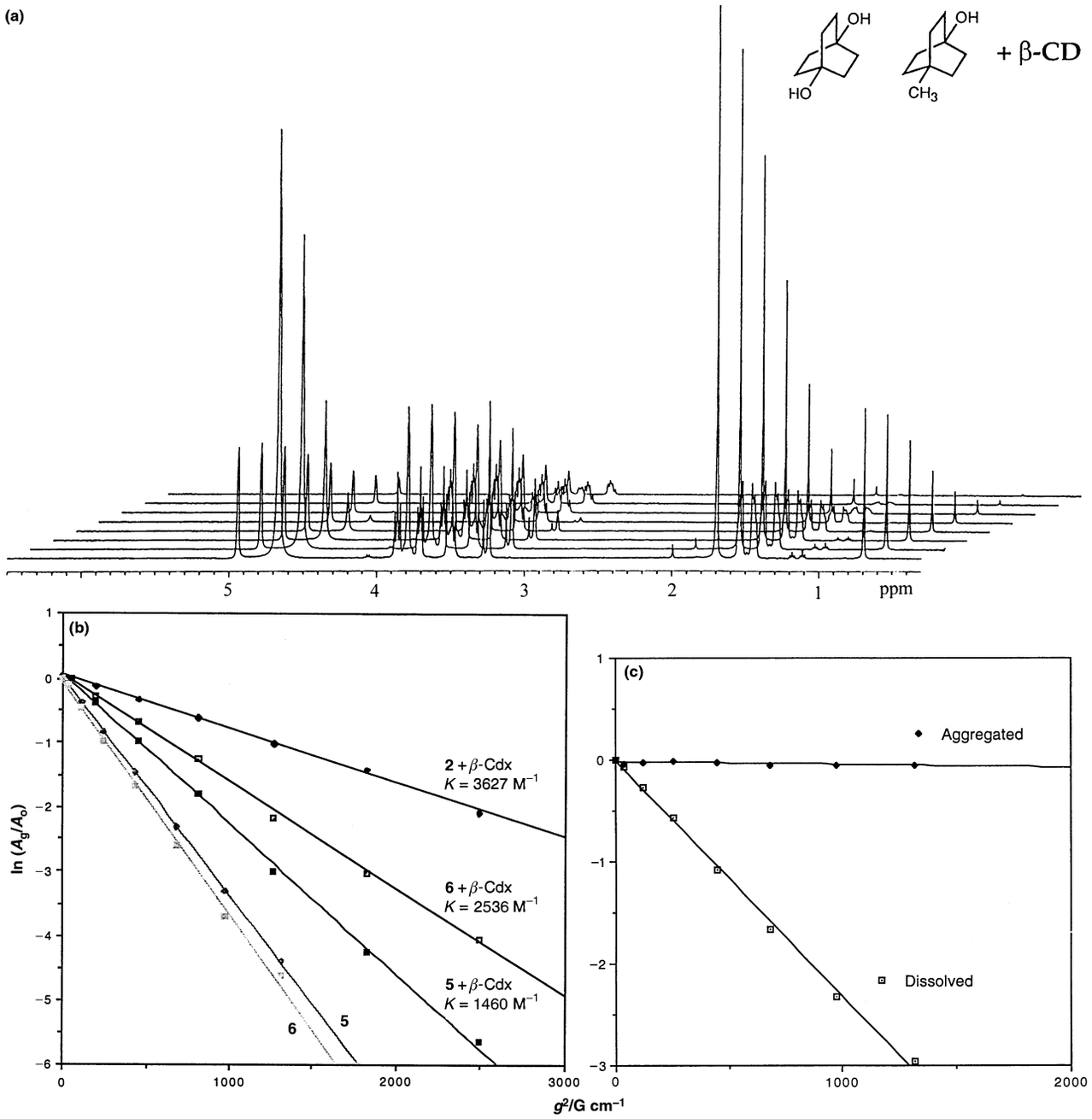


Fig. 4 a) Diffusion edited ^{1}H NMR spectra of **5** and **6** with β-Cdx (7 mM of each component) in D_2O. The gradient was varied from 0 (bottom) to 50 (top) G cm^{-1}. b) ln A_g/A_o as a function of g^2 for **2**, **5** and **6** with β-Cdx at 295 K. c) Diffusion edited ^{1}HNMR spectra of **2** without Cdx at 278 K.

sum of the isolated molecules as the distance increased without passing any noticeable barrier.

In the GB/SA water model all substrates gave stable 1 : 1 complexes with both host molecules, but the stability was much larger with β-Cdx in gratifying agreement with experiment, although the magnitude is overestimated. 1 : 2 Complexes were constructed by docking another cyclodextrin molecule to the 1 : 1 complexes followed by energy minimization. 1 : 2-Complexation was only simulated with α-Cdx. All three substrates gave stable complexes with large negative ΔH-values of similar magnitude. The lower experimental stability of the 1 : 2 complexes of **5** and **6** is not reproduced by the calculations. These substrates penetrate slightly deeper with their OH-group than methyl and halogen substituents (*vide infra*). On the other hand, **1** penetrates too deep into the cavity to allow for 1 : 2-complexation. The results are presented in Table 3 and Fig. 6 shows the energy minimized structure of the 1 : 2 complex of **2**.

A comparison of the distances between the inner protons (H^3 and H^5) of α-Cdx and the methyl and methylene protons of **2** with the NOE effects of the ROESY spectrum indicates that the calculated and solution structures are very similar, and that there is room for two α-Cdx molecules symmetrically disposed around the guest molecule in the 1 : 2 complex (Fig. 5 and 6).[16] The calculated average distances between CH_2 and the closest H^3 and H^5, respectively, are 2.88 and 4.32 Å, and the corresponding values for CH_3 are 3.91 and 3.59 Å. In the 1 : 2 complexes of **2**, **5** and **6** there are 4–6 intermolecular hydrogen bonds between the secondary hydroxy groups in addition to hydrogen bonds between the 2- and 3-hydroxy groups within each cyclodextrin unit.

Molecular dynamics simulations

Obviously, simple molecular mechanics energy minimization cannot account for all aspects of the behavior of the cyclodextrin complexation of the bicyclo[2.2.2]octane derivatives. In the hope of achieving more information, the dynamic behavior

Table 3 Force-field calculations (AMBER*) for docking of 1,4-di-R-bicyclo[2.2.2]octanes **2**, **5** and **6** with α-Cdx and β-Cdx in the gas phase and using the GB/SA water solvent model. Default electrostatic potentials were used

	ΔH_{calc}/kcal mol^{-1}					
	α-Cdx				β-Cdx	
	1 : 1-Complex		1 : 2-Complex		1 : 1-Complex	
Compound	Vacuum	GB/SA	Vacuum	GB/SA	Vacuum	GB/SA
2	−2.89	−1.19	—	−36.9	−1.96	−32.6
5	—[a]	−3.95	—	−49.8	−1.24	−20.5
6	—[a]	−3.49[b]	—	−39.2	−0.80	−29.2[b]

[a] Lowest energy structure externally hydrogen bonded to primary hydroxy group. [b] The methyl group oriented towards the secondary rim in the low energy structure.

Table 4 Results from the molecular dynamics calculations (AMBER*) for the 1 : 2 complexes of 1,4-di-R-bicyclo[2.2.2]octanes **2** and **5** with α-Cdx in the gas phase and using the GB/SA water solvent model. Default electrostatic potentials were used. Computational details in the text

	2				**5**			
	Vacuum		GB/SA		Vacuum		GB/SA	
	250 K	300 K	250 K	300 K	250 K	300 K	250 K	300 K
Mean Cdx distance[a]	7.05	—	6.97	7.03	6.06	—	6.11	6.12
Standard dev.	0.13	—	0.24	0.15	0.11	—	0.18	0.17
Mean dihedral angle[b]	—	—	3	6	—	—	6	7
Standard dev.	—	—	27	23	—	—	10	12

[a] The distance in Å is from the center of the cavity of one Cdx unit, defined as the geometrical center of all glycosidic oxygens, to the corresponding center in the other Cdx unit. [b] The diheral angle (degrees) between the connection of the centres of cavities and the 1,4-bridgehead connection.

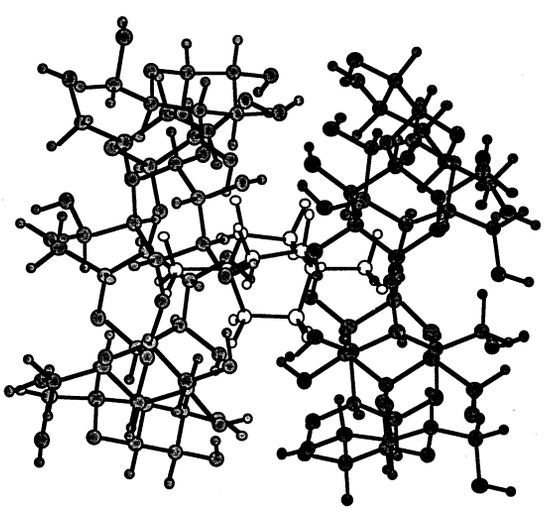


Fig. 5 Amber* minimized structure of the 1 : 2 complex of α-Cdx with **2**

of the complexation, particularly the 1 : 2 complexes with α-Cdx was considered in molecular dynamic simulations on **2**, **5** and **6**. Since the behavior of **6** falls between **2** and **5** only the latter two compounds will be discussed. Starting from energy minimized structures, dynamics simulations were performed of isolated complexes as well as of complexes in the GB/SA solvation models. The results using the two force fields, AMBER* and the OPLS, were essentially identical so only the AMBER* results are given. In the first computations the system was heated up to the desired temperature and then equilibrated there at 200–1000 ps. Simulations were carried out at different temperatures, but two temperatures are presented, 250 K and 300 K, since this is the experimentally relevant interval and also computationally instructive. Some key values are shown in Table 4.

At 300 K complexes of both **2** and **5** decomposed after a few tenths of picoseconds in the gas phase but not using the solvent model. In the water solvent model the complexes were stable even at 350 K. Fig. 7 shows the variation of the distance between the two cyclodextrin rings illustrating the dynamic properties of the complexes using the gas phase and GB/SA models, respectively. The mean distance between the cyclodextrin rings is *ca.* 1 Å larger for **2** than for **5**, reflecting the larger size of CH_3 compared to OH. There is also a tendency for the hydroxy groups of **5** to form a hydrogen bond to the glycosidic oxygen atoms. The variation in the distance between the Cdx rings is also larger for **2** than for **5**, as reflected by the standard deviations. The symmetry axes of the Cdx units and the long axes of the guest molecules are essentially parallel, but **2** is tumbling considerably more in the cavity than **5** (Fig. 8). The average number of intermolecular Cdx–Cdx hydrogen bonds was 3.2 for **2** and 5.7 for **5** at 300 K.

Although, these simulations give a better picture of the behavior of the 1 : 2 complexes, they fail to explain the striking difference in dynamic stability between **2** and **5** (or **6**). An important factor for the equilibrium in eqn. 1 is the property of the uncomplexed guest molecules in water solution or solvent mixtures. For that reason we undertook computations of the guest molecules **2**, **5** and **6**. The heat of transfer from the gas phase to water was calculated as 2.5 kcal mol^{-1} for **2** and −5.4 kcal mol^{-1} for **5** using the GB/SA model. The value for **6** is −1.41 kcal mol^{-1}. The difference of *ca.* 8 kcal mol^{-1} between **2** and **5** is certainly an important factor for the different apparent stabilities of the 1 : 2 complexes of these compounds.

Molecular dynamics simulations of **2**, **5** and **6** in a small box of 150 TIP3P water molecules demonstrate the difference. Whereas **2** was squeezed out of the water drop within 20 ps, **6** and **5** remained in the drop of water for more than 60 and at least 300 ps, respectively. These systems were not further investigated due to lack of periodic boundary conditions in the Macromodel v. 6.5 program.

Discussion

The substrate molecules in this study were chosen due to their size, varied hydrophobicity and limited conformational

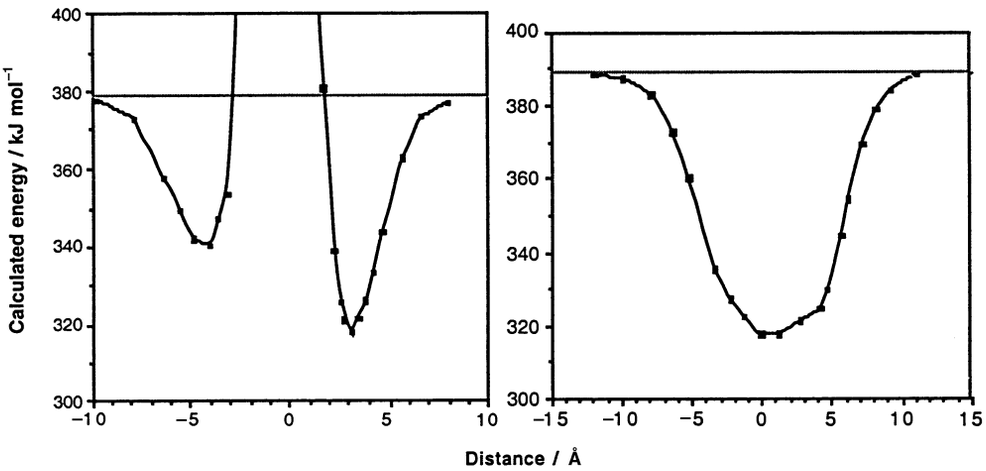


Fig. 6 .Steric energy of the interaction between **5** and α-Cdx as a function of the distance between the center of the cavity of α-Cdx and **5**, and minimum energy structure of the 1 : 1 complex of **5** and α-Cdx. The minimum energy structure is obtained after relaxation of all degrees of freedom. The other structures are given by the following constrained minimization. The center of the cavity is defined by the geometrical center of the 6 glycosidic oxygen atoms. The constrained distance is defined as the distance from this point to the rear methyl carbon atom. This distance is the only constrained parameter in the minimization. The horizontal line represents the sum of the energies of **5** and α-Cdx at long distance.

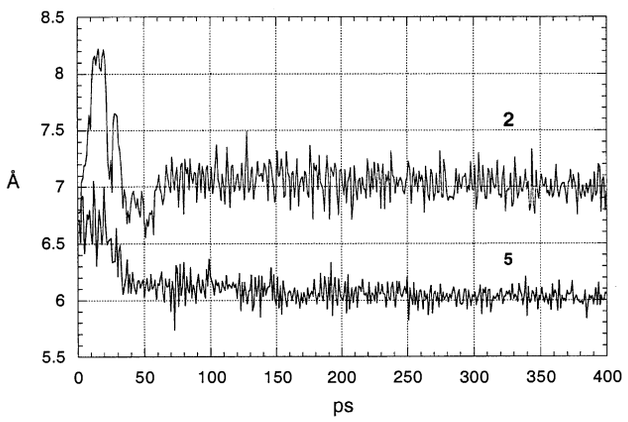


Fig. 7 Molecular dynamics simulations of the 1 : 2 complex of **2** or **5** and α-Cdx using the the GB/SA water model at 250 K. The distance is the distance between the cyclodextrin rings as described in Fig. 6.

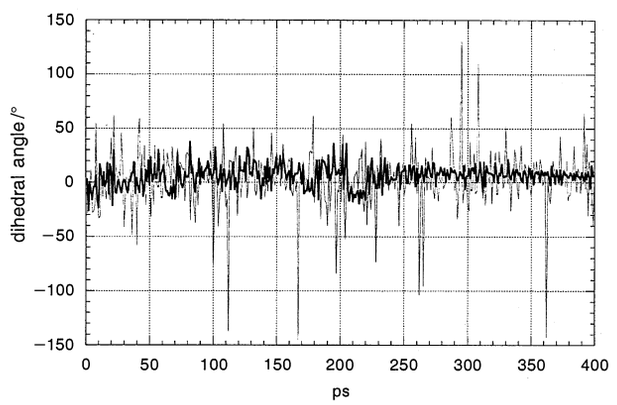


Fig. 8 Molecular dynamics simulations of the 1 : 2 complex of **2** or **5** and α-Cdx at 250 K. The angle refers to the line between the two Cdx centers (as defined in Fig. 6) and the long axis of the substrate. Pale trace **2**, dark trace **5**.

flexibility. From an experimental point of view, they suffer from the disadvantage of being transparent in the easily available part of the UV-spectrum. However, a combined experimental and computational approach gives a good picture of the complexation of the studied bicyclooctane derivatives with cyclodextrins. Considering the substrates **2**, **5** and **6**, all three give weak 1 : 1-complexes ($K \leq 50$ M^{-1}) with α-Cdx, significantly stronger 1 : 1-complexes with β-Cdx with equilibrium constants following the order of hydrophobicity **2** > **6** > **5**. Compound **2** is the only substrate in this series giving a dynamically stable 1 : 2 complex with α-Cdx. Scheme 1 gives a schematic representation of the exchange processes observed by NMR for the 1 : 2 complex with α-Cdx.

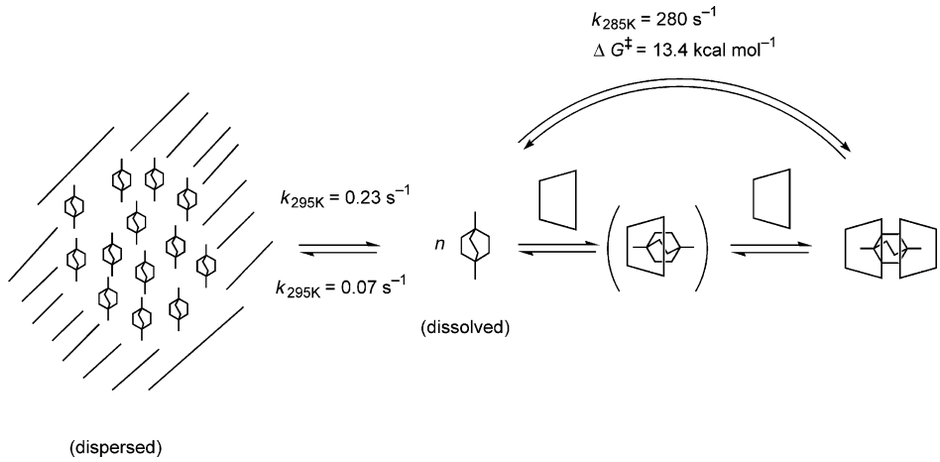
The computations using the GB/SA solvent model give a good picture of the structure and relative stability of the 1 : 1-complexes with α- and β-Cdx, respectively. With α-Cdx the substrates can only partially penetrate the Cdx, exposing a large part of the molecule to the solvent (except to some extent for **1**). Similar results have been reported in the case of bicyclo[2.2.2]octane-1-carboxylate ($K = 45$ M^{-1} with α-Cdx, and $K = 6300$ M^{-1} with β-Cdx).[3d] As a contrast, β-Cdx easily houses a guest molecule maintaining considerable conformational and translational freedom for both guest and host (Fig. 6). The computations also clarify the difference in stability of the 1 : 2-complexes between the the two hosts. The guest molecules are esentially totally buried in the cavity of β-Cdx, whereas a snug fit of the α-Cdx 1 : 2-complexes is obtained with all guests except **1**. The induced fit of the α-Cdx is calculated as 2–4 kcal mol^{-1}. By comparing experimental with calculated ΔH-values for the 1 : 1-β-Cdx : **5** complex and the 1 : 2-α-Cdx : **2** complex it is evident that the calculations exaggerate the magnitude of the interaction by more than a factor two, using the GB/SA water model. This can be explained by the neglect of the dynamic situation, leading to overestimation of both dispersion interaction and hydrogen bonding.

The temperature dependence of $\Delta G°$ for the 1 : 2 complex of **2** with α-Cdx is quite important and a linear relation is obtained within the studied temperature range (Fig. 2). Still $\Delta H°$ and $\Delta S°$ are in excellent agreement with the well-established enthalpy–entropy compensation, which for α-Cdx has been given the form in eqn. (4).[3b] The dihydroxy analogue **5**, on the other

$$T\Delta S° = 0.90\Delta H° + 13.0 \text{ (kJ mol}^{-1}\text{)} \qquad (4)$$

hand, behaves very much as α,ω-disubstituted *n*-alkanes according to the calorimetric data: weak temperature dependence of $\Delta G°$ and a strongly negative ΔC_p, resulting mainly from dehydration of the hydrophobic parts of the solute. Expulsion of cavity-bound water does not seem to be associated with any significant heat capacity change.[17]

The appearance of a dispersed phase is, of course, not unique for **2**. We have found that other highly hydrophobic compounds behave similarly.[18] Apart from the slowly exchanging dispersed phase, there is ample evidence of microheterogeneity in solutions of hydrophobic substances in water and in solvent mixtures.[19] Recently, Marmur argued that the hydrophobic



Scheme 1

effect is due to self-assembly of solute molecules into molecular aggregates, leading to entropy loss and to a high value of molar excess free energy.[20] We have shown that the exchange rate between dispersed and dissolved substance is 10^{-3}–10^{-4} times the exchange rate between dissolved and complex-bound guest, but the "dissolved" molecules might well be assembled in low-entropy aggregates, which exchange fast on the NMR time scale with single molecules, which are possibly necessary for complex formation.

Two major questions emerge from the experimental and computational results. (1) What is the reason for the high dynamic stability of the 1 : 2-complex of **2** as compared to the 1 : 1-complex and compared to the 1 : 2-complexes of **5** or **6**? (2) What is the origin of the high barrier to exchange of the 1 : 2 complex of **2**?

Both questions probably find their answers in the difference in hydrophobicity of the three substrate molecules. The computations suggest that the sizes of the substrates are promoting 1 : 2-complexes governed by dispersion forces and hydrogen bonding between the secondary hydroxy groups of the cyclodextrin and, in the cases of **5** and **6**, between substrate hydroxy and glycocidic oxygen atoms. Computations and experiments, however, indicate an opposite trend in enthalpy of formation of the three 1 : 2-complexes, in the case of **5** corroborated by the calorimetric data. An explanation could be found in a comparably low enthalpy of dissolved **5** and **6** not fully accounted for by the computations. However, the low entropy of **2**, aggregated or not, as compared to **5** and **6** should work in the opposite direction. Unfortunately, a calorimetric study of **2** was not possible.

Small uncharged molecules notoriously exchange at a much higher rate with cyclodextrins, in contrast to the behavior of **2**. The computations did not suggest any origin to the high barrier to exchange of the 1 : 2 complex of **2** to be related to any enthalpy barrier. Since the 1 : 1-complexes obviously are very unstable, an ordered and partly desolvated arrangement of one substrate molecule and two cyclodextrin molecules of very low entropy can be envisaged, preceding the enthalpy gain obtained only when the complex is nearly totally formed. We suggest that the high barrier is due primarily to this entropy related preorganization.

Conclusion

This work illustrates that minor structural modifications can totally change affinity, structure and dynamics of binding of related molecules to cyclodextrins. Whereas the **5**-1 : 1-complex with β-Cdx exhibits normal hydrophobic expressions, **2** behaves quite differently.

The molecular mechanics computations indicate a favorable interaction between the guests and α-Cdx, in the cases of **1** and **2** solely as a result of dispersion forces. The calculated structure of the **2**-α-Cdx 1 : 2-complex is in excellent agreement with the ROESY data and with the stability of the 1 : 2 complexes for **2** and **3** observed in the NMR experiments. Computations indicate strong 1 : 1 β-Cdx complexes in water. Calorimetric experiments of **5** in water, however, show a different pattern: weak interactions with α-Cdx and γ-Cdx, but a relatively strong 1 : 1 binding to β-Cdx.

We have observed 1 : 2 complexes only with **2** and **3** (and possibly **4**) with α-Cdx, which is reasonable considering the degree of penetration of the guests in the cavity (Fig. 6). Less obvious is the origin of the high dynamic stability of these complexes; small uncharged molecules notoriously exchange at a much higher rate. Considering the available data, we propose that the main cause of the high barrier to complex formation is the dissymmetrical development of enthalpy and entropy along the reaction coordinate. The entropy contribution ($-T\Delta S$) increases earlier than the enthalpy term decreases. Probably, the solvent mixture also plays a crucial role, the finer details of which we have not so far been able to settle, although we know that further addition of methanol or DMF increases the exchange rate.

Experimental

Syntheses

Compounds **1**–**6** have been described earlier and had physical data in agreement with those published.[7,21,22] The purity was checked by GC chromatography and spectroscopy. Cyclodextrins were used as delivered. Deuterated cyclodextrins were prepared by two dissolution–evaporation cycles from D_2O or methanol-d_4.

NMR experiments

^{1}H NMR spectra were recorded on Varian XL-300 or Brucker DRX 400 MHz or AMX 500 MHz spectrometers, using the solvent peak as internal shift standard. In the dynamic NMR experiments[23] a typical solution was 7 mM in guest, 3.5–20.0 mM in Cdx and the solvent mixture had the composition 0.5 mL D_2O, 0.3 mL methanol-d_4, and 0.3 mL DMF-d_7.

The ROESY experiment was performed with cw spin lock for mixing at a field strength of 5.6 kHz and a mixing time of 20 ms. The interscan delay was 2.2 s and 64 scans per increment were used. The spectral region F2 × F1, 4590 × 9180 Hz, was digitized to 2k × 256 data points. Fourier transformation to 2k × 256 data points was performed after application of a shifted sine window in F2 and shifted sine^2 window in F1 dimension. Relaxation of the bound ligand protons in the rotating frame, $T_1\rho$, was estimated by application of a non-selective 90° pulse followed by a spin-locking pulse of variable

duration t and acquisition. At a time $t = 20$ ms approximately 50% of the original signal intensity remained.

For the gradient-edited spin-echo experiments the gradient pulse strength was 0–78 G cm^{-1}, the gradient pulse duration time was 0.2 ms and the diffusion delay time was 150 ms. A sweep width of 4672 Hz was used.

Saturation transfer–saturation recovery experiments were performed as earlier described.[24]

Mass spectra were recorded with a JEOL SX-102 mass spectrometer.

The microcalorimetric titration technique has been described earlier.[10,25]

The molecular mechanics calculations were performed using the MM2(91) force field implemented in the MacMimic program package,[26] and the MM3*, AMBER* and OPLS force fields of the Macromodel program version 6.5, using default parameters.[27] The calculations were carried out on a Silicon Graphics O2 workstation. The Polak-Ribiere conjugate gradient algorithm was applied in all minimizations with 1000 iterations limit and a cut-off of 12 Å was used for the non-bonded interactions. Molecular dynamics simulations were performed under NVT ensemble conditions, using 1 or 1.5 fs time step and a bath relaxation time of 0.2 ps at all temperatures.

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