NMR Studies of Cyclodextrins and Cyclodextrin Complexes

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A. Introduction. History, Aims, and Scope

NMR spectroscopy has become the most important method for structural elucidation of organic compounds, particularly in the solution state. The method is of increasing significance for most cyclodextrin (CyD) *applications*, but is also increasing in popularity for controlling the intricate *synthetic modifications* of the cycloamyloses by modern preparative methods.[1−6] Synthetic variations usually lead to much more complicated spin systems than those in the underlying, highly symmetrical frameworks. There are few alternatives to NMR spectroscopy in the study of CyDs. As with many carbohydrates it is often difficult, or too time consuming, to obtain single crystals of CyD derivatives and then to analyze them by X-ray crystallography, and even more so by neutron diffraction. Other techniques such as fluorescence, UV/vis spectroscopy, calorimetry, etc. play a major role in measuring complexation energetics with CyDs, but usually provide only very indirect and qualititative information about inclusion modes and geometries. Structural characterization is of particular significance for supramolecular host−guest complexes, which are the basis of most CyD applications in medicine, catalysis, or in food chemistry, separation and sensor technology. Pharmaceutical uses of CyDs for drug protection or targeting now legally require structural characterization of the administered compounds. NMR spectroscopy is also becoming an important tool for in vitro, in future perhaps even for in vivo, studies of CyD interactions with biological macromolecules such as nucleic acids, proteins, or cell membranes. The most obvious incentive, however, to use NMR techniques for the investigation of CyD complexes is the interest to understand the driving forces and binding modes in

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After studying at Universities in Tübingen, München, and Berlin (TU), H.-J. Schneider obtained his Diploma and in 1969 his Ph.D. in the group of Professor Michael Hanack. He then worked as Postgraduate Research Fellow at the University of California, San Diego, 1967–1969 with Professor R. C. Fahey, and 1969–1971 with Professor Walter Hückel at the University in Tübingen. In 1972, he became professor for organic chemistry at the Universität des Saarlandes where he is presently. Professor Schneider has published more than 180 papers, including several book chapters and invited reviews. His research interests include conformational analysis, NMR spectroscopy, strain–reactivity relations in reactions of aliphatic systems, and selective functionalization of paraffins, including steroids. In the last decade his group has worked mostly on mechanisms of molecular recognition, analyzing binding contributions and conformations of supramolecular complexes, and on new enzyme and receptor analogues. The topics include synthetic allosteric complexes, polyamines, complexes with nucleotides and nucleic acids, with peptides, as well as artificial esterases and nucleases.

Frank Hacket was born in Losheim, Germany, in 1966. He studied chemistry at the University of Saarbrücken and received his Diploma in 1994 under the supervision of H.-J. Schneider. Currently he works as Ph.D. student in the group of Professor Schneider. After studies about the complexation of carbohydrates and alcohols by simple organic anions, his current research interests focuse on molecular recognition with functionalized cyclodextrins.

these noncovalent associations, and then to make optimal use of these factors for new applications. It should be remembered, that the driving force for CyD inclusion often is of solvophobic nature and that most CyD applications involve action in a liquid matrix, which emphasizes again the role of NMR spectroscopy as the most important method applicable in solution. As an example of how misleading it can be to rely only on taken-for-granted assumptions on intracavity inclusion as the major factor behind CyD interactions we cite the well-known deacylation acceleration of *p*-nitrophenylacetate by α-CyD. Tee et al. have convincingly demonstrated that opposite to

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Hiroshi Ikeda was born in 1956 in Ichikawa, Chiba, Japan. He received a B.S. in 1979 from Tokyo Institute of Technology and obtained a Ph.D. in 1988 for his studies of artificial hydrolases using modified cyclodextrins from Tokyo Institute of Technology. He is currently an Assistant Professor at Tokyo Institute of Technology. His research interests focus on the design, synthesis, and characterization of supramolecular systems as artificial enzymes and chemosensors for molecular recognition.

popular earlier views, this does not imply intracavity complexation of the substrate.[7,8]

After the first publications on NMR spectra of CyDs appeared decades ago,[1,2,9] and after the pioneering NMR investigations of Bergeron, Komiyama, Demarco and others,[10−16] there has been a virtual explosion of such studies. *Chemical Abstracts* presently (September 1997) lists more than 900 corresponding sources; perusal of the literature shows that in fact there are far more than 1000 pertinent papers. The older work was restricted to the observation of few CyD protons, mostly at the anomeric centers, which were sufficiently separated from the strongly coupled other signals. The advent of high-field instruments, and in particular of 2D methods, which were applied to cyclodextrins from 1987 on,[17−19] has completely changed the situation, and the possibilities of the now available NMR techniques are far from being exploited. Nuclear Overhauser effects

(NOEs) have already become a major tool in structural studies of complex biomolecules. The earlier measurements with CyDs were limited to steady-state NOEDIF methods and have now been succeeded by much more powerful two-dimensional and spin-lock techniques (see sections B.4 and C.4). Another factor greatly facilitating the elucidation of complex structures is the development of computer-aided molecular modeling methods, based on reliable force fields. NMR spectroscopy is a much too indirect method to derive complex three-dimensional structures without the use of realistic models, which then must be tested against experimentally observed NMR data. This fitting step also is now significantly improved by the availability of modern computer techniques. Only for selected simpler questions can one safely proceed without using more rigorous computational methods. Thus, evidence for intracavity inclusion of guest molecules with strong shielding tensors like those from phenyl derivatives has been deduced quite early[10,11] by often more qualitative observations of upfield shifts of the CyD protons inside the cavity.

The spectacular advances of NMR techniques during the last years has led to a much more detailed structural elucidation of cyclodextrins and their complexes. These tasks represent a fascinating challenge for the NMR spectroscopist in view of the high complexity of the underlying cycloamylose ^{1}H NMR spin systems. These are characterized by signals which—apart form the anomeric proton—absorb in a range of only 0.5 ppm and are strongly coupled. In addition, the shielding effects of the CyD cavity on entrapped guest molecules are limited to few tenths of a ppm at most, as a consequence of a host framework being built up entirely of single, less polar and polarizable bonds and thus weak shift tensors.

Cyclodextrins have played a major role in the development of supramolecular chemistry. Such studies should always involve the characterization of their structures, besides the energetics of complex formation. The latter also can be significantly helped by NMR shift titrations, for which modern high-field instruments usually allow to follow several signals, yielding independent data on equilibrium constants. Progress in the empirical quantification of noncovalent forces, and their use for the design of new chemical technologies will essentially depend on knowledge of both thermodynamics and structures of supramolecular complexes in the same state of matter.

The aim of the present review cannot be to discuss or even to mention all of the more than presently 1000 publications referring to the use of NMR spectroscopy for cyclodextrins. Instead we set out to give, as much as possible, a comprehensive overview about the most important approaches to structural problems with CyDs, mainly in solution. An effort is made to cover most of the literature from 1985 to mid-1997, with fewer references to earlier work. Until now there is only one review by Inoue devoted exclusively to the NMR spectroscopy of cyclodextrins,[20] listing already 220 references. Two recent reviews by Perly and Djedaini et al., both with about 25 references, provide an excellent introduction into modern NMR techniques useful for CyD studies.[4,21] Other recent reviews[22,23] or monographs[3] illustrate the use of NMR techniques in CyD research with interesting examples, yet without a special focus on this method. It is hoped that the present overview will encourage CyD researchers to make increasing use of the now available modern NMR techniques, as well as help to convince NMR specialists that in particular supramolecular CyD complexes present a promising challenge for further development.

In view of the excellent introductions in modern NMR techniques available in several monographs and reviews[4,24−26] we discuss these methods only along their uses to solve specific problems in cyclodextrin research. We first describe conformational properties of free (unsubstituted) and of substituted CyDs (section B), providing also reference data for substituted derivatives which are gaining more and more importance for many present and future applications. In this context we discuss more qualitative interpretations of ^{1}H and ^{13}C NMR shifts, the use of vicinal HCCH coupling constants for conformational analyses with emphasis on rotamers around the C5−C6 bond in CyDs, and the application of two-dimensional techniques for substituted CyDs with more complicated spin systems. Promises and problems in the application of NOE techniques are dealt with in section B.4, together two-dimensional NMR methods. The quantitative application of NMR shift changes is introduced in the context of structural characterization of supramolecular CyD complexes in section C, which also refers to the role of computer-aided molecular modeling and to the dynamics of complexation, including relaxation methods. Sections C.8.a to C.8.h highlight applications of NMR methods to many CyD complexes, embracing classical as well as most recent supramolecular systems. This should illustrate the enormous diversity of cyclodextrin chemistry as well as of the most important NMR methods to control it. Application of nuclei other than ^{1}H and ^{13}C, and of solid-state methods (sections D and E) is kept relatively short in view of the more limited applications and of already available pertinent reviews.

B. ^{1}H and ^{13}C NMR Spectra of Free and of Substituted Cyclodextrins

B.1. Unsubstituted Cyclodextrins

At magnetic fields above 9.4 T, corresponding to 400 MHz for ^{1}H NMR spectra, the dispersion is already high enough to locate in conventional one-dimensional spectra most of the protons, eased by the high symmetry of the macrocycles (Figure 1a,b).

Proton shielding differences among α-, β-, and γ-CyD amount only to 0.1 ppm at the anomeric H-1, and are even smaller at other positions (Table 1). At fields around 400 MHz, only the anomeric protons are separated well enough from the others for an approximate first-order analysis of the ^{1}H NMR spin system. Coupling constants (Table 2) for the other glucose protons can only be accurately obtained from spin system simulations of the high-order multi-

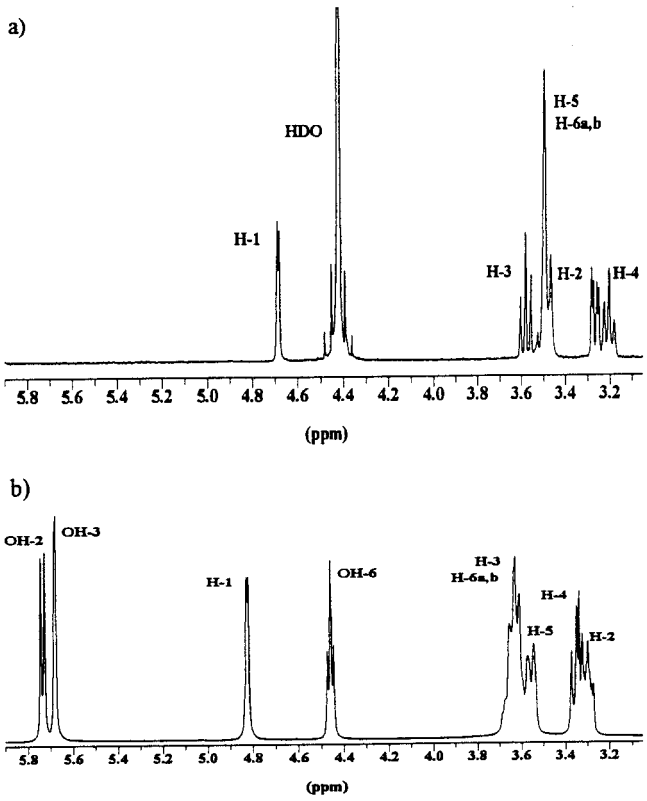


Figure 1. ^{1}H NMR spectra (400 MHz) of β-CyD at 298 K: (a) in D_2O and (b) in DMSO-d_6.

Table 1. ^{1}H NMR Chemical Shifts, δ (ppm), of C−H Protons in Unsubstituted CyD's in D_2O and in DMSO-d_6[a]

	H-1	H-2	H-3	H-4	H-5	H-6a,b
			D_2O			
α-CyD[b]	4.60	3.19	3.57	3.08	3.39	3.44
β-CyD[b]	4.68	3.26	3.58	3.19	3.47	3.49
γ-CyD	4.53	3.08	3.35	3.00	3.26	3.30
			DMSO-d_6			
α-CyD	4.79	3.29	3.78	3.40	3.59	3.65
β-CyD	4.82	3.29	3.64	3.34	3.59	3.64
γ-CyD	4.89	3.32	3.65	3.36	3.56	3.65

[a] From ref 27 unless noted otherwise. [b] From ref 32.

plets,[28] or with 2D techniques (see section B.4). Often, however, more qualitative conclusions are based on first-order approximations. The vicinal H−C−C−H coupling constants (Table 2) are in line with a classical $^4C_1$ chair conformation of the pyranose ring as found with corresponding monomeric 1,4 glycosides of β-glucose. The couplings differ little between free and C-6-substituted CyDs (see below), with the exception of the J_{5-6} values: these, however,

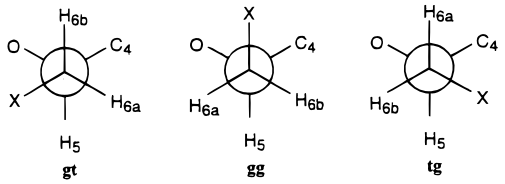


Figure 2. Rotamers in CyD around the C-5−C-6 bond.

Table 3. ^{13}C NMR Chemical Shifts, δ (ppm), of Unsubstituted CyDs in D_2O[a]

	C-1	C-2	C-3	C-4	C-5	C-6
α-CyD	102.19	72.61	74.21	82.07	72.91	61.37
β-CyD	102.58	72.67	73.89	81.94	72.89	61.17
β-CyD[b]	102.58	72.80	73.80	81.55	72.53	63.09
γ-CyD	102.42	73.19	73.82	81.33	72.69	61.21
δ-CyD	100.96	73.08	73.74	79.26	72.34	61.29
ϵ-CyD	99.74	72.65	73.68	78.03	71.78	61.49
ζ-CyD	99.83	72.56	73.66	78.31	71.74	61.53
η-CyD	100.17	72.53	73.67	78.87	71.82	61.54
θ-CyD	100.34	72.51	73.64	78.97	71.90	61.43

[a] From ref 31, unless noted otherwise. [b] From ref 32.

are often not sufficiently resolved due to the nearly isochroneous shifts of the nonequivalent methylene protons A and B at C-6. A more detailed analysis of α-CyD in D_2O does show a shift difference of 0.02 to 0.06 ppm between the nonequivalent H-6 a and b protons as a function of temperature.[28] The then observable J_{5-6a} and J_{5-6b} values indicate for the macrocycle an enhanced contribution of gg conformer (Figure 2), with the C6−OH group pointing into the pyranose ring, whereas in the corresponding monomer [29] the gt conformer, with the C6−OH group gauche to the pyranose oxygen atom, contributes significantly. Substantial differences in the rotamer populations around the C5−C6 bond are expected with different solvents, and in particular with substitution at C6 as discussed below (section B.5).

Cyclooligosaccharides composed of pyranoses with, for instance, an axial 3-OH group, such as in α-cycloaltrin, dominate in pseudo-equilibrating mixtures of $^4C_1/^1C_4$ conformers; this is borne out by the observed vicinal J_{HCCH} coupling constants, which agree with computer-aided molecular modeling, and is supported by solid-state X-ray studies.[30]

^{13}C NMR shifts extend over a much larger scale than proton shifts and are particularly suited to identify cyclodextrins, even in mixtures. The recently available ^{13}C NMR shifts of higher cycloamyloses [31] (Table 3) show differences among the α- to θ-CyDs of up to 4 ppm for C-4, and up to 2 ppm for C-1, with smaller variations at the other carbon atoms. It should be noted that some of the earlier shifts and assignments show considerable deviations.

Table 2. Coupling Constants J (Hz) of C−H Protons in Unsubstituted CyDs[a]

	H-1−H-2	H-2−H-3	H-3−H-4	H-4−H-5	H-5−H-6a	H-5−H-6b	H-6a−H-6b
				In D_2O			
α-CyD[b]	3.5	10.0	8.7	9.3	2.3	3.5	−12.6
				In DMSO-d_6			
α-CyD	3.3	9.5	9.0	9.5	2.0		
β-CyD	3.5	9.3	9.0	9.0	2.0		
γ-CyD	3.5	9.5	9.5	9.5	3.0		

[a] From ref 27, unless noted otherwise. [b] From ref 28.

Solvents have a profound influence not only on CyD complexation free energies but also on CyD ^{1}H NMR spectra, in particular if they either lead to fast exchange of OH protons, as in the case of water, or do not so, as in the case of the frequently used DMSO. In such media one can obtain detailed insight into the intramolecular CyD hydrogen-bond network (see section B.2), and often observes better resolved signals for other protons. Another way to reach a higher dispersion of overlapping CyD signals is the admixture of—mostly aromatic—substrates,[33,34] which are known to form intracavity complexes and have strong shielding tensors affecting mostly the H-3 and H-5 signals (see section C).

As the hydrophobic binding contribution diminishes with decreasing water content, apparent shift changes can be just due to a decrease of complexation constants. Only few studies were devoted to the change of complexation-induced *intrinsic* shifts with the reaction medium. In most cases, these will be the result of conformational changing as a consequence of the change of binding forces, for instance from loose binding with predominating hydrophobic effects in water to tighter binding of guest parts with high polarizibility and electron density in less polar solvents. With larger substituents attached to the CyD moiety, the change of solvent will induce a larger difference in the flexible parts, as is visible in larger shift changes.[35] Other interpretations [20,36−38] in particular of ^{13}C shift changes were based on solvation theory,[39] assuming also strong dipole−multipole interactions as the driving force for complexation. Medium effects on ^{13}C NMR shifts of aromatic cyclodextrin guest molecules, however, show quite different magnitudes and also partially signs opposite those observed by complexation with CyD.[32]

B.2. OH Signals and Hydrogen Bonding in Unsubstituted Cyclodextrins

NMR spectroscopy has been used from the very beginning to identify the hydrogen-bonding network in CyDs.[40] The secondary hydroxyl groups, at the wider rim of the cyclodextrin, form intramolecular bonds in which the OH-3 group of one glucose is interacting with the OH-2 group of the neighboring glucose unit. This leads to a belt of hydrogen bonds around the secondary CyDs side that gives the whole molecule a rather rigid structure, especially in the case of β-cyclodextrin.[41] The primary OH-6 functions placed at the smaller rim's torus are not participating in intramolecular hydrogen bonds, and therefore can rotate so as to block partially the cavity.[10] Many of these findings were first observed in solid-state structures and later confirmed by NMR methods which give also a detailed picture of conformational equilibria in solution.

Protons involved in hydrogen bonds are much more deshielded than "free" protons; this kind of resonance displacement in the range of about 1 ppm has been ascribed previously to hydrogen bonds between secondary OH groups in cyclodextrins.[10,27,40,43] In protic solvents such as water, intermolecular exchange between solute and solvent is too fast on the NMR time scale for the observation of separate OH signals.

Table 4. Chemical Shifts of the Anomeric H-1 and the Hydroxyl Protons of some CyDs in DMSO-d_6 at 35 °C,[42] (Dimethyl-CyDs (DMCyD) and methyl α-D-glucoside (MG) are inserted for comparison.)

	H(1)	OH(2)	OH(3)	OH(6)
α-CyD	4.6	5.32	5.26	4.28
β-CyD	4.68	5.52	5.48	4.26
γ-CyD	4.74	5.53	5.57	4.32
α-DMCyD[a]	4.77		4.45	
β-DMCyD	4.77		4.70	
MG[b]	4.32	4.41	4.46	4.12

[a] DMCyD = 2,6-di-*O*-methylcyclodextrin. [b] MG = methyl α-D-glucoside.

Therefore the observations discussed here are restricted to solvents such as DMSO, where separate signals for the OH groups as well as their couplings to the vicinal C−H protons can well be analyzed. The resonances of OH-3 and OH-2 were found to appear in DMSO between 5.2 and 5.6 ppm, clearly separated from the signals of the free, more shielded OH-6 groups between 4.2 and 4.4 ppm (see Table 4). The strength of the intramolecular hydrogen bonds is also visible by hydrogen−deuterium exchange rate constants in D_2O for the equilibrium:

$$2\text{CyD-OH} + D_2O \rightleftharpoons 2\text{CyD-OD} + H_2O$$

The exchange rate constants for the secondary CyD-OH hydroxy functions of cyclodextrin as measured by ^{1}H NMR are considerably smaller than that for the same reaction with the not macrocyclic amylose (k = 0.75 (α-CyD), 0.65 (β-CyD), 0.85 (amylose)).[44] According to these NMR-derived exchange rates, β-CyD has the strongest hydrogen bond network at its wider rim, making it less flexible in comparison to α-CyD and γ-CyD. This is also in line with the particular low solubility of β-CyD in water.

More detailed information is available from temperature dependence studies of cyclodextrin hydroxy proton resonances.[42,45,46] In DMSO as hydrogen bonding acceptor the following hydrogen-bonding equilibrium is present:

$$-\text{OH}\cdots\text{OH}- \rightleftharpoons -\text{OH}\cdots\text{O}{=}\text{S(CH}_3)_2$$

The left side stands for intramolecular hydrogen bonds in the cyclodextrin, the right side for solvent−solute association. The signals of all hydroxy protons shift almost linearly upfield with increasing temperature, indicating that the hydrogen bonds with the solvent is weakened, and that the equilibrium is shifted toward the left side. The temperature coefficient ($\Delta\delta/\Delta T$) is equally large for all CyD-OH-6 protons, and is in the same range as that for OH-6 methyl α-D-glucoside (MG), which has no possibility to form such intramolecular hydrogen bonds. The temperature effect on the secondary hydroxy protons is much smaller. This observation suggests a strong exposition of only OH-6 to the solvent, in agreement with the secondary hydroxy protons being strongly involved in intramolecular hydrogens. The size of ($\Delta\delta/\Delta T$) increases in the order α-CyD < β-CyD < γ-CyD, which is in some contradiction to the deuterium exchange rates mentioned above.

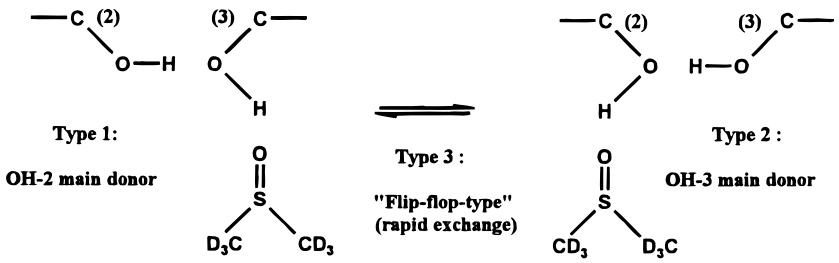


Figure 3. Schematic representation of the intramolecular hydrogen bonding in cyclodextrins: type 1, OH-2 major donor; type 2, OH-3 major donor; type 3, "flip−flop-type" rapid exchange.[42]

Table 5. Observed Vicinal Coupling Constants $^3J_{HOCH}$[a] and Calculated Dihedral Angles of Secondary Hydroxyl Groups of Some CyDs in DMSO-d_6 at 35 °C[42] (Dimethyl-CyDs (DMCyD) and methyl-α-D-glucoside (MG) are inserted for comparison.)

	OH(2)			OH(3)		
	J, Hz	Φ, deg		*J*, Hz	Φ, deg	
α-CyD	7.0	±27.9	±137.7	2.8	±54.7	±115.7
β-CyD	6.7	±30.0	±136.1	2.5	±56.8	±113.8
γ-CyD	7.0	±27.9	±137.7	2.5	±56.8	±113.8
α-DMCyD				ca 0.0	ca ±85.9	
β-DMCyD				ca 0.0	ca ±85.9	
MG	6.6	±30.7	±135.7	5.1	±40.3	±135.7

[a] $^3J_{HOCH} = 10.4 \cos^2 \varphi - 1.5 \cos \varphi + 0.2$.

Three different modes for intramolecular hydrogen bonds are possible in cyclodextrins dissolved in DMSO (see Figure 3)[42]

Analysis of $^3J_{OHCH}$ coupling constants [47] (Table 5) as well as studies of the ^2H isotope effect on ^{13}C and ^1H shifts [48,49] confirms a geometry of the type 2 with OH-2 as donor and OH-3 as receptor of the intramolecular H bonds. In agreement with the temperature dependence of the OH shifts mentioned above the OH-3 group seems to be the major donor in the hydrogen bond with OH-2.[42]

The factor R in eq 1, which was introduced by Inoue

$$R = ((\Delta\delta)_{CyD})/\Delta T/((\Delta\delta)_{MG})/\Delta T \qquad (1)$$

et al.[42] to define a relative value of the temperature-induced shift, changes for cyclodextrins hydroxy protons in comparison to MG (methyl α-D-glucoside). For OH-6, a *R* value nearly equal to 1 was observed, indicating intermolecular hydrogen bonds with the solvent as in the case of MG. Much smaller *R* values for OH-2 and OH-3 are calculated in line with their intramolecular hydrogen-bond formation. The smaller value for OH-3 again suggests this to be the dominant hydrogen-bond donor.

The vicinal $^3J_{HO-CH}$ coupling constants show only in the case of OH-3—remarkable changes between the monomeric glucose ($^3J = 5.1$ Hz) and the macrocyclic CyD ($^3J = 2.5-2.8$ Hz) structure.[42] This implies that conformational changes by cyclization, necessary to form hydrogen bonds of the kind described above, occur only at the 3-position. The finding also supports the dominating hydrogen-bonding donor capacity of OH-3. No variation in the vicinal coupling constants were observed in a temperature range between 25 and 75 °C, showing that the orientation of the secondary hydroxy groups is temperature independent.[42] The torsional HOCH angles as given in Table 5 vary considerably between the free and persubstituted CyD only for OH-3, as calculated on the basis of a suitable parametrization[46]of the Karplus equation (eq 2)

$$^3J_{HOCH} = 10.4 \cos^2 \phi - 1.5 \cos \phi + 0.2 \qquad (2)$$

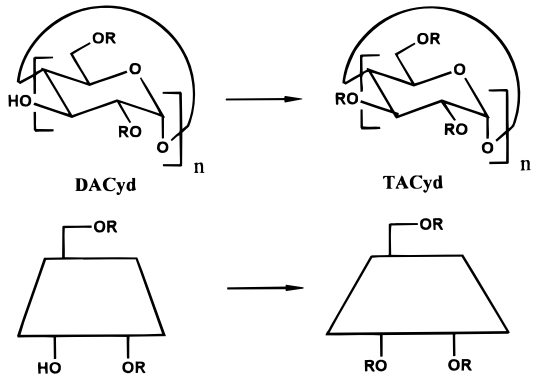


Figure 4. Possible flattening of the CyD torus due to complete derivatization.[50]

The strong hydrogen-bonding network at the wide rim of the cyclodextrin molecule influences conformation and the shape of the whole CyD torus, which can be followed using NMR techniques. Comparison of heptakis(2,6-di-*O*-alkyl)-*β*-cyclodextrin (DACyD), in which the formation of a hydrogen-bonding network is still possible due to a free OH-3 function, and heptakis(2,3,6-tri-O-alkyl)-*β*-cyclodextrin (TACyD) without such hydrogen bond show remarkable differences.

Both coupling constants as well as $^{13}C-T_1$ relaxation times indicate no appreciable change of the glucose $^4C_1$ conformation for DACyD in comparison to native cyclodextrin.[51] In these derivatives the whole, relatively rigid, macrocyclic structure is maintained as result of the participation of OH-3 in a strong hydrogen bond with O-2 of the next glucose. This is also confirmed by the temperature dependence of the hydroxyl resonances. The experiments show that hydrogen bonds in derivatives with disubstituted glucose units are often even stronger than those in not modified CyDs.[42]

The situation changes in the case of persubstituted derivatives (see also section B.3). Significant ^{13}C upfield shifts of the C-4 and C-1 carbon signals going from DACyD to TACyD (Figure 4) are believed to indicate a conformational change in the cyclodextrin macrocyle . The loss of the hydrogen bond between free 3-OH and O-2 of a neighboring glucose unit can lead to a widened rim at the cyclodextrin's secondary

side. As a consequence, the torus can become somewhat more flattened (Figure 4).[50]

B.3. Substituted Cyclodextrins with Relatively Simple Spin Systems

B.3.a. Symmetrical Cyclodextrins with Substituents in All 6-Positions

Tables 6 and 7 contain ^{1}H and ^{13}C NMR reference data for some synthetically easily accessible substituted derivatives with frequently used functions. The substituent-induced proton shifts do not follow a regular pattern (see e.g. Table 6, R = NH−CH_3). The ^{13}C shifts are much better indicators for the structural variations. Thus, the observed ^{13}C shift differences between the starting compound with R = OH and the product with R = X at the primary 6-position vary for instance for X = I and X = NHMe from 52.0 to 10.5 ppm at C-6, and from 2.5 to 3.3 ppm at C-5. These ^{13}C shifts show only very approximate correlation with other substituent induced shifts, or SIS values, which are, however, mostly derived form pure alkane frameworks. The ^{13}C shifts at C-4 reflect syn and anti orientations of the C−X bond with respect to the C5−C4 bond; they agree within ±0.3 ppm with the SIS calculation if one uses *syn*- and *anti*-γ-SIS derived from cyclohexanes and takes into account the weighted average of the gg and gt populations (Figure 2) discussed in sections B.1 and B.5.[52] The vicinal coupling constants except those for the methylene protons H-6 (see below) show within 0.5 Hz no dependence on substituents; they agree with the classical $^4C_1$ glucose chair conformation which remains, in the time-averaged picture, independent of the substituents at C6. The same conformation is seen by NOE within the glucose.

B.3.b. Per-O-methylated Cyclodextrins

Persubstituted cyclodextrins are again relatively easy to prepare and possess interesting properties for many applications.[3] Due to their high symmetry the NMR spectra of permethyl CyDs (PM CyD) are straightforward to analyze; the corresponding data (Tables 8−10) follow essentially the pattern discussed for other substituted CyDs. Botsi, Yannakopoulou, Hadjoudis, and Perly[54] have recently provided clear evidence for a higher flexibility of permethylated CyDs, depending on their ring size. In contrast to the unsubstituted CyDs, there are relatively large differences between the CyDs of different macrocycle size in chemical shifts of carbons C-1 and C-4, which are known to reflect changes around the interglucose bond particularly well, and also of the 3-Me group which is directed toward the cavity. This indicates

Table 6. ^{1}H NMR Chemical Shifts, δ (ppm), of C−H Protons in CyDs with Substituents in All 6-Positions[a]

R (solv.)	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	R
				α-CyD				
OTs[b,c] (DMSO-d_6)	4.61 (d, 3,5)	3.28 (m)	3.80−90 (ov.)[d]	3.38 (t, 9.0)	3.80−90 (ov.)	4.37 (dd, 11.0,4.0)	4.24 (dd, 11.0,4.0)	CH_3: 2.88 (s), H-2,6$_{arom}$ 7.78 (d, 8.5), H−3,5$_{arom}$ 7.41 (d, 8.5)
				β-CyD				
OTs (DMSO-d_6)	4.65 (d, 3.2)	3.21 (m)	3.57 (t, 9.2)	3.29 (t, 9.2)	3.76 (d, 8.8)	4.33 (m)	4.2 (m)	CH_3: 2.42 (s), H-2,6$_{arom}$: 7.76 (d, 8.0), H−3,5$_{arom}$: 7.44 (d, 8.0)
NH−CH_3 (D_2O)	4.92 (d, 3.2)	3.51 (dd, 16.0, 3.4)	3.84 (ov.)	3.34 (t, 9.4)	3.84 (ov.)	2.85 (d, 8.8)	2.64 (dd, 13.2, 8.2)	CH_3: 2.24 (s)
NH−$CH_2\varphi$ (DMSO-d_6)	4.84 (d, 3.2)	3.33 (ov. H_2O)	3.60 (ov.)	3.47 (t, 9.2)	3.66 (ov.)	2.75 (m)	2.69 (m)	NH: 1.67 (br. s.), CH_2: 3.55 (d, 4.4), H_{arom}: 7.09 − 7.27 (m)

[a] Measured at 300 K; against TMS (DMSO-d_6) or sodium (3-trimethylsilyl)propion-1-sulfonate (D_2O) as internal reference unless noted otherwise; values in brackets = J (Hz). [b] OTs = *p*-toluenesulfonate. [c] From ref 53. [d] ov. = overlapping.

Table 7. ^{13}C NMR Shifts, δ (ppm), of CyDs with Substituents in All 6-Positions[a]

R (solv.)	C-1	C-2	C-3	C-4	C-5	C-6	R
				α-CyD			
OTs[b,c] (DMSO-d_6)	103.0	71.1	73.1	82.4	74.1	69.7	CH_3: 21.7, C:$_{arom}$:145.9, 134.3, 130.9, 128.8
				β-CyD			
OTs[d] (DMSO-d_6)	101.80	79.30	61.60	81.00	72.30	68.60	CH_3: 21.00, $C_{arom.}$: 144.7, 132.7, 129.8, 127.5
Br[d] (DMSO-d_6)	102.12	72.06	72.29	84.64	71.03	34.42	
I[d] (DMSO-d_6)	101.87	71.67[e]	71.91[e]	85.69	70.70	9.17	
NH-CH_3[f] (D_{2O})	101.00	72.30[e]	71.50[e]	82.60	69.70	50.70	CH_3: 34.60
NH−$CH_2\varphi$[d] (DMSO-d_6)	102.30	73.10[e]	72.70[e]	83.10	70.80	53.40	CH_2: 48.50, C_{arom}: 141.0, 128.1, 127.8, 126.5

[a] Measured at 300 K. [b] OTs = *p*-toluenesulfonate. [c] From ref 53. [d] Relative to internal DMSO-d_6 (δ = 39.51 ppm). [e] Assignment not sure. [f] Relative to internal sodium (3-trimethylsilyl)propion-1-sulfonate.

Table 8. ^{1}H NMR Chemical Shifts, δ (ppm), of Permethylated CyDs (PMCyDs) in D_2O[a]

	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	CH_3-2	CH_3-3	CH_3-6
PM α-CyD	5.27	3.36	3.78	3.76	3.91	3.90	3.76	3.56	3.67	3.45
PM β-CyD	5.34	3.41	3.74	3.81	3.92	3.91	3.70	3.57	3.66	3.44
PM γ-CyD	5.44	3.43	3.78	3.82	3.99	3.79	3.74	3.60	3.62	3.45

[a] From ref 54.

Table 9. Coupling Constants, *J* (Hz), in Permethylated CyDs (PMCyDs) in D_2O[a]

	H-1−H-2	H-2−H-3	H-3−H-4	H-4−H-5	H-5−H-6a	H-5−H-6b	H-6a−H-6b
PM α-CyD	3.4	9.6	8.6	8.6	4.4	0.9	−10.0
PM *β*-CyD	3.5	9.6	9.3	8.8	4.0	<1	−10.0
PM *γ*-CyD	3.5	9.5	9.0	9.0	3.8	2.4	−10.5

[a] From ref 54.

Table 10. ^{13}C NMR Chemical Shifts, *δ* (ppm), of Permethylated CyDs (PMCyDs) in D_2O[a]

	C-1	C-2	C-3	C-4	C-5	C-6	2-CH_3	3-CH_3	6-CH_3
PM α-CyD	100.42	82.67	83.45	81.92	73.32	73.71	60.41	62.64	61.05
PM *β*-CyD	99.67	82.71	83.61	79.65	73.03	73.46	60.74	62.38	61.04
PM *γ*-CyD	98.29	82.92	83.19	76.74	72.12	73.40	61.14	60.62	61.16

[a] From ref 54.

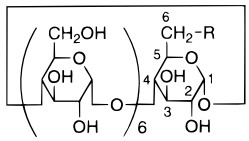
progressive distortions of the interglucose torsional angles: the observed vicinal $J_{5,6,}$ coupling constants yield by application of the Karplus equation, modified after Haasnot and Altona, for the C-5−C-6 bond the gg conformer as the more stable isomer for all permethylated CyDs, in contrast to the free CyDs (see below).

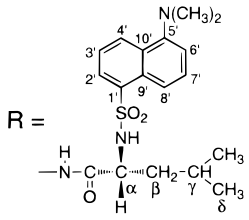
B.4. Substituted Cyclodextrins with More Complicated Spin Systems. Application of Modern 2D NMR Techniques Including NOE Methods

Monosubstituted cyclodextrins, along with many other CyD derivatives, lack the high symmetry of free or symmetrically substituted CyDs and therefore exhibit substantially more complex spin systems. Depending on the site and on the kind of substitution, this holds also for di- and polysubstituted derivatives, requiring the application of more advanced 1D and in particular of 2D methods. Two-dimensional NMR techniques[24,25] now allow the analysis of even very complex structures in great detail and are discussed here with a typical example.[55,56] The combination of several methods has for instance allowed the assignment of signals of a total of 49 magnetically nonequivalent protons in the modified *β*-cyclodextrin compound **1**.

Whereas ^1H resonances for unmodified or for symmetrically substituted CyDs can often be assigned using COSY (COrrelated SpectroscopY) spectra, ^1H resonances for modified CyDs are in most cases too complex to assign using only COSY. It should also be noted that COSY spectra can be quite demanding both in terms of acquisition times and data storage, if one needs high resolution. Dynamic problems arising from signals that are too large for instance from methyl groups or from solvents, can be reduced by multiple quantum filter techniques, in particular with DQF (Double Quantum Filter). For these as for other sequences, such as COLOC (COrelated spectroscopy for Long rang Couplings) or HMBC (Heteronuclear Multiple Bond Correlation) which are especially suited for the connectivity analysis via long-range coupling, we refer to corresponding monographs[24,25] and reviews.[44]

Since ^1H resonances for the anomeric protons of modified CyDs always appear around 4.8 ppm, it is in principle possible to assign all ^1H resonances





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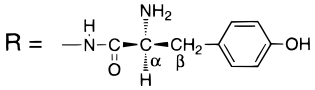

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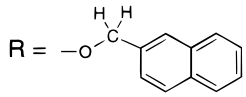

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within each glucose residue of a modified CyD by tracing the spin-coupling relationships starting from the ^1H resonances for the anomeric protons. If the ^1H resonances for the anomeric protons of the modified CyDs overlap, it is difficult to assign the ^1H resonances of each glucose residue, and one has to resort to more time-consuming methods such as HETCOR (HETeronuclear $^{13}C-^1H$ CORorrelation) or less time-consuming HMQC experiments.

Typically applied procedures (Figure 5) are illustrated here in detail with the ^1H NMR spectra of *N*-dansyl-D-leucine appended *β*-CyD (DnsLeu-CyD, **1** as example,[55,56] which also in the time-averaged symmetry has seven different glucose units (Figure 6).

In this case the NMR spectra are expected to provide also information about the self-inclusion state, since analysis of the fluorescence decay of the dansyl moiety indicates that the dansyl moieties of DnsLeu-CyD are located in the cavity (see below). First, the overlapping signals of the ^1H NMR spectra of DnsLeu-CyD are separated into sets of proton resonances belonging to the same D-glucose residue using TOCSY (TOtal Correlation SpectroscopY). The

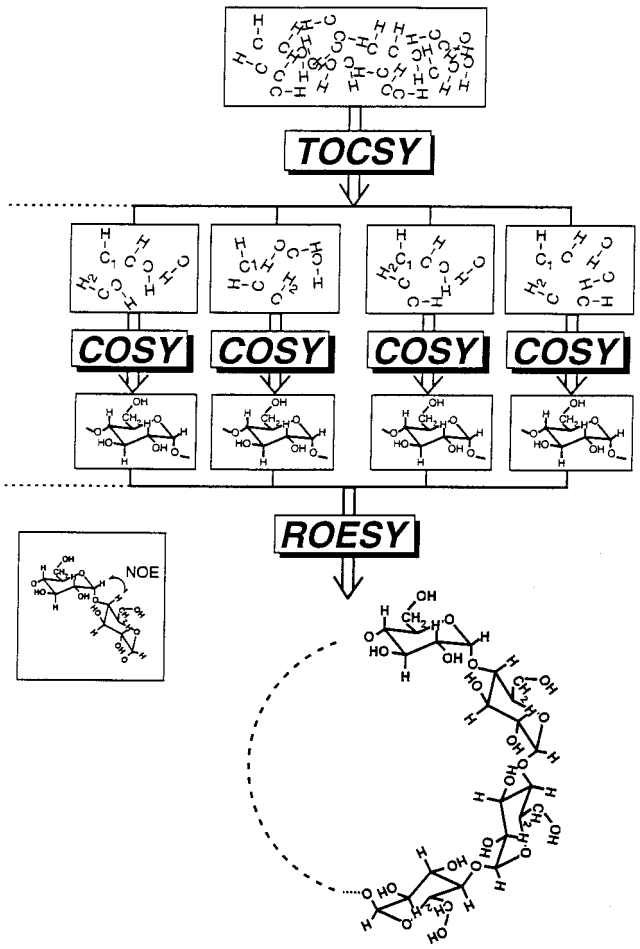


Figure 5. Starting strategy for proton NMR assignments in complicated spin systems.

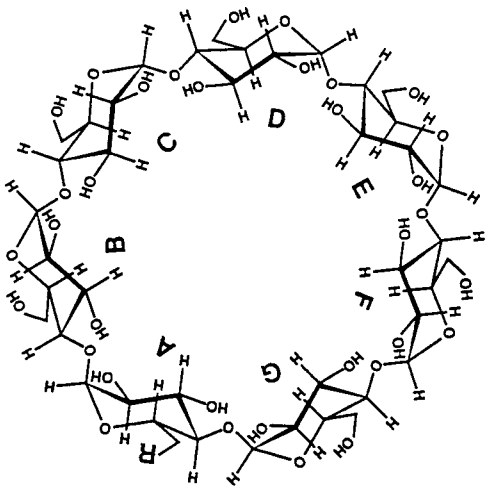


Figure 6. Structure of β-CyD monosubstituted at C-6 with glucose ring labels A to G. (Reprinted with permission from ref 56. Copyright 1997 American Chemical Society.)

resonances of DnsLeu-CyD **1** can thus be grouped into seven sets of resonances for the glucose rings A to G. By using TOCSY and COSY spectra, all resonances groups are then assigned to the protons of one particular glucose residue; however, one still needs to distinguish the different glucose units from each other. Modern instruments allow the use of 1D TOCSY spectroscopy,[57] providing a more detailed assignment. The efficiency of the magnetization

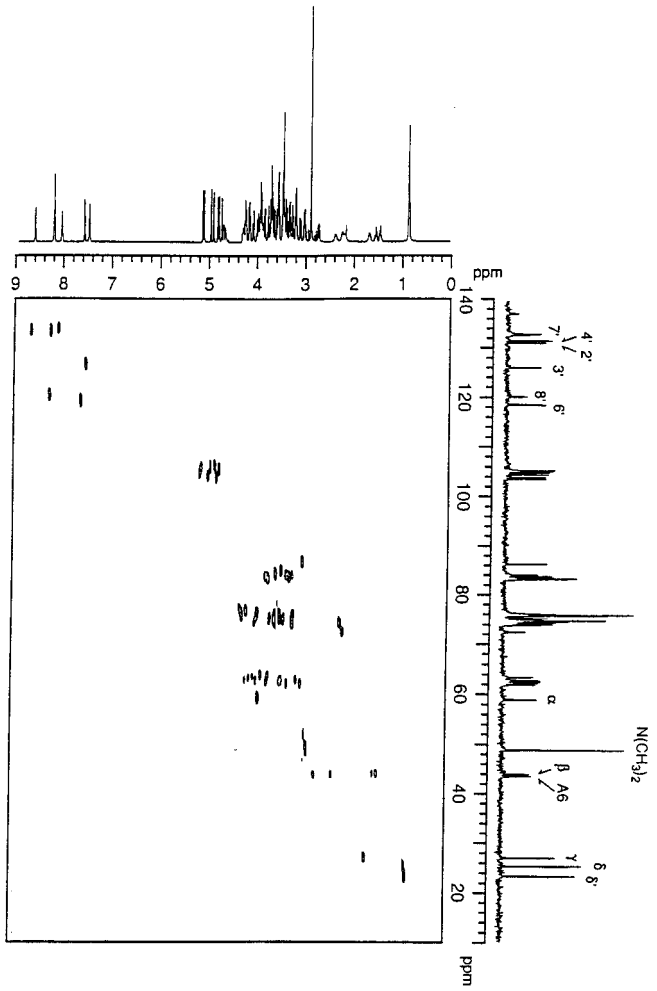


Figure 7. $^{1}H-^{13}C$ HSQC spectrum (400 MHz) of compound **1**. (Reprinted with permission from ref 56. Copyright 1997 American Chemical Society.)

transfer in TOCSY spectra depends on the magnitude of the vicinal coupling constants and on the applied mixing time. With a short mixing time after selective excitation of the H-1 proton resonance of a glucose residue the magnetization of the H-1 proton transfers only to the H-2 proton. With increased mixing times the magnetization transfers to more distant protons (H-3, H-4, etc.), and with much longer mixing times as far as to H-6. Thus, the 1D TOCSY spectrum with varied duration of the propagation period can give not only subspectra of within each D-glucose residue, but also assignments of the different glucose units. The same can be achieved with 2D NMR COSY spectra or 2D TOCSY. 2D TOCSY sequences offer advantages for complicated spin systems, as this technique gives correlations for protons of the same glucose unit on the same line in the TOCSY spectrum, and correlations for signals in the different glucose units can be easily distinguished.

In addition, $^{13}C-^{1}H$ correlation spectra can be used in order to assign the ^{1}H resonances, e.g. for the glucose residue A which bears the dansyl leucine residue at C-6 position as a pendant. There are several methods to obtain $^{13}C-^{1}H$ connectivities. HETCOR can be used with instruments lacking inverse capabilities, inverse techniques such as HSQC (Heteronuclear Single Quantum Coherence spectros-

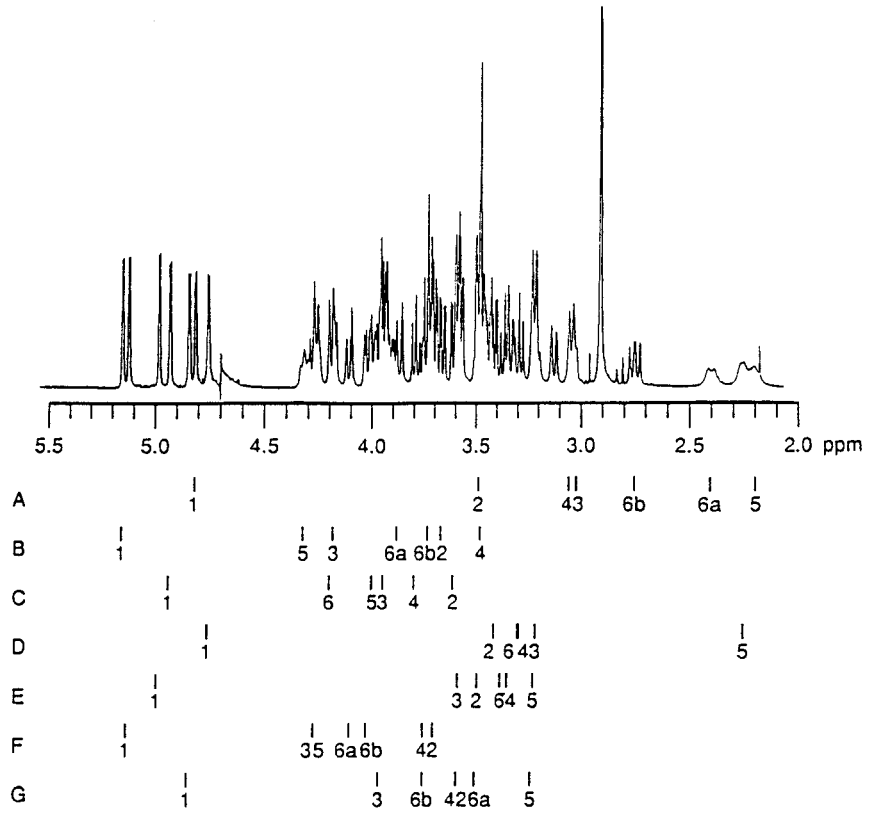


Figure 8. Part of the 500-MHz spectrum with the CyD resonances of compound **1** with signal assignments. (Reprinted with permission from ref 56. Copyright 1997 American Chemical Society.)

copy) or HMQC (Heteronuclear Multiple Qantum Coherence spectroscopy) are particularly useful for small amounts of samples. Since the anisotropic shielding effect of the carbonyl group of the leucine pendant part shifts the resonances of the nearby H-6 protons, it is difficult to assign the resonances of the H-6 protons of the glucose residue A of DnsLeu-CyD. On the other hand, the C-6 carbon resonance of the glucose residue A is found at 43.5 ppm in the ^{13}C NMR spectrum. Thus, the resonances of the H-6 protons of the glucose residue A are assigned by a $^{1}H-^{13}C$ HSQC spectrum as shown in Figure 7.

Finally, the sequence of the glucose residues are determined by a ROESY spectrum (for details on NOE applications see the discussion below). The distance between the H-1 proton of a glucose residue and H-4 of the adjacent glucose residue is short enough to always give a sizable NOE, which can be best observed by ROESY. The glucose residue of which the H-4 resonance has a cross-peak with the H-1 resonance of the glucose residue A in the ROESY spectrum is identified as the glucose residue B. In a similar way, the sequences of all the glucose residues are determined successively. The results of the assignments are shown in Figure 8.

Nuclear Overhauser Effects. Nuclear Overhauser effects[26] are of paramount importance for conformational analysis in solution. Multidimensional methods such as NOESY are usually adequate for the highly complex CyD spin systems. New pulse sequences are constantly being developed, for instance for the suppression of diagonal signals in NOESY spectra. [58] Intramolecular NOEs can help in signal assignments (see above); they also can shed light on deformations of cyclodextrins and in particular on the location of substituents relative to the cavity (see section B.5 on self-inclusion). Intermolecular NOEs provide information about through-space interactions and association modes of host–guest complexes. Besides the analysis of chemical shift changes, NOEs therefore represent the most promising method for the analysis of the most frequently used CyD property to form supramolecular complexes (see section C.4).

Application of traditional NOE difference methods is limited by the unfavorable tumbling rates τ_c of compounds with molecular masses around 1000–2000 Da. The critical cross relaxation rate depends—besides on the viscosity of the solvent—on the product of τ_c and the spectrometer frequency, which for the necessary high signal dispersion should be around 400 MHz or higher. In consequence, the conventional steady-state NOE is positive only for molecular masses well below 1000, and sizable—although negative—only for those well above 5000 Da, placing synthetic host compounds and their complexes near the unfortunate zero transition between positive and negative NOEs. Spin-lock experiments such as the rotating frame NOE (ROESY) or the one-dimensional CAMELSPIN sequence (now often referred to as 1D ROESY) overcome this serious limitation by application of an additional excitation pulse; after this one observes the transverse instead of the conventional longitudinal magnetization enhancements. The spin lock techniques, which have been applied previously to CyD complexes,[59] require high stability and fast frequency switching, which is now standard with digitally controlled NMR instruments.

In the two-dimensional NOE experiment, necessary for many CyD analyses, the interesting parameter is the integrated intensity of the cross-peaks between two protons. Such volume integration is possible with modern instruments and software, and may be directly correlated with the internuclear distance r of the two observed protons via the known r^{-6} dependence. However, the underlying relaxation constants also depend on the tumbling and the shape of the molecule. Spin diffusion and contributions from third nuclei as well as processes other than transverse relaxation during spin-locking also complicate a rigorous quantitative interpretation. ROESY experiments as well as improved versions[60] may give rise to coherent exchange of magnetization in J-coupled networks by the TOCSY mechanism, leading to further serious complications.[61] TOCSY cross-peaks have opposite signs of ROESY cross-peaks, and make distance calibration with vicinal or geminal protons difficult, although the TOCSY peaks are distinguishable in a phase-sensitive spectrum. The recently proposed transverse ROESY experiment[62] almost completely eliminates the TOCSY magnetization transfer by application of a modified spin-locking field, using a periodic multiple-pulse sequence along the y axis.

More detailed studies may require measurements of build-up rates, variation of solvent viscosity and of magnetic field dependence for securing a dipole−dipole DD relaxation mechanism. ROESY cross-peaks can also originate from cross-relaxation with other coupled spins. Such "false positives" are misleading since they have the same sign as the ROESY cross-peaks which one wants to measure. Nevertheless, ROESY cross-peak volumes offer at least semiquantitative information about internuclei distances, or allow the introduction of upper limits for distances between protons separated by more than about 4 Å; protons beyond this cutoff distance give no or only very weak NOE signals. By calibration with fixed and known intramolecular distances r, between vicinal aromatic or olefinic, or between nonequivalent methylene protons one can deduce via the correlation with r^{-6} inter- and intramolecular distances r with some confidence. The inherent approximation of a completely rigid molecule which furthermore undergoes isotropic rotations implies the neglect of intramolecular motions, which is particularly questionable for the most often loose CyD complexes. It has been shown that these problems lead to substantial errors even for intramolecular proton−proton distance calculations in more rigid peptide frameworks.[63] Off-resonance ROESY techniques, employing spin-lock fields with frequencies shifted far away from the observed spectral region, seem to hold much promise not only for more realistic interproton distance evaluations, but also for the determination of individual proton correlation times and dynamics of supramolecular complexes.[64,65]

B.5. Cyclodextrins with Covalently Bound "Pendant" Ligands with Self-Inclusion Properties

Covalent binding of pendant residues to CyDs has provided many systems of great promise for analytical applications, in particular if the pendant group bears chromophores for optical signal sensing.[66,67] In aqueous solution, pendant groups of modified CyDs are most often flexible and may be inside or outside the CyD cavity. In the case of CyDs modified with a fluorophore as a pendant, the analysis of the fluorescence decay of the fluorophore pendant provides useful information with respect to the conformational features, because the fluorescence lifetimes of usual fluorophores are in the range of a measurable time scale (nanoseconds) for the conformations, and the fluorophores are sensitive to the environment, with different lifetimes when located inside or outside the CyD cavity.[55,68,69] The fluorescence decays of the dansyl moiety of *N*-dansyl-leucine-appended β-CyD **1** indicate that there are two conformational isomers, the dominating one (A) with the pendant group inside, the other one (B) with the pendant outside the cavity (see Figure 9).

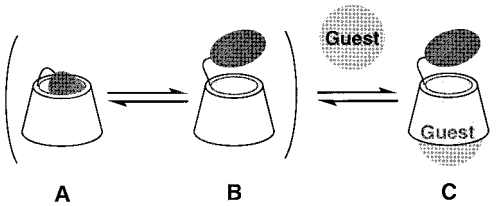


Figure 9. Cyclodextrins with pendant substituents inside and outside the cavity. (Reprinted with permission from ref 56. Copyright 1997 American Chemical Society.)

A NOE cross-peak between a proton of the pendant group and a proton of the CyD moiety will be observed in a ROESY spectrum, if the protons are closer than 4 Å through space.[26] Therefore, if the pendant group is included in the CyD cavity, NOE correlations between the protons of the pendant group and the protons (H-3, H-5, or H-6) of the CyD moiety will be observed, and it is possible to estimate the orientation of the pendant group within the CyD cavity. Figure 10 shows the ROESY spectrum of DnsLeu-CyD with assignments.[55] Strong cross-peaks were observed between the CyD H-3 and H-5 protons, and the naphthyl and methyl protons of the dansyl moiety. A weaker NOE correlation was observed between the protons of CyD and the δ protons of the leucine moiety. The higher mobility of the part bearing the δ protons, allowing a multitude of relaxation pathways, makes this NOE weaker. The NOE data also exclude the possibility of bimolecular complexes: although the NOE correlation between the protons of the dansyl moiety and the protons of CyD moiety may be also interpreted as a head-to-head bimolecular complex in which each dansyl moiety is included in the cavity of another CyD, the NOE correlation between the δ protons of the leucine moiety and the CyD protons is not in line with a head-to-head bimolecular complex, because some additional NOE correlation between the δ protons and the CyD protons should be observed in this case. The head-to-tail bimolecular complex is also ruled out by the NOE correlation since the position of the dansyl moiety in the cavity in the head-to-tail bimolecular complex is in the opposite direction compared to the self-inclusion complex. The dansyl moiety in

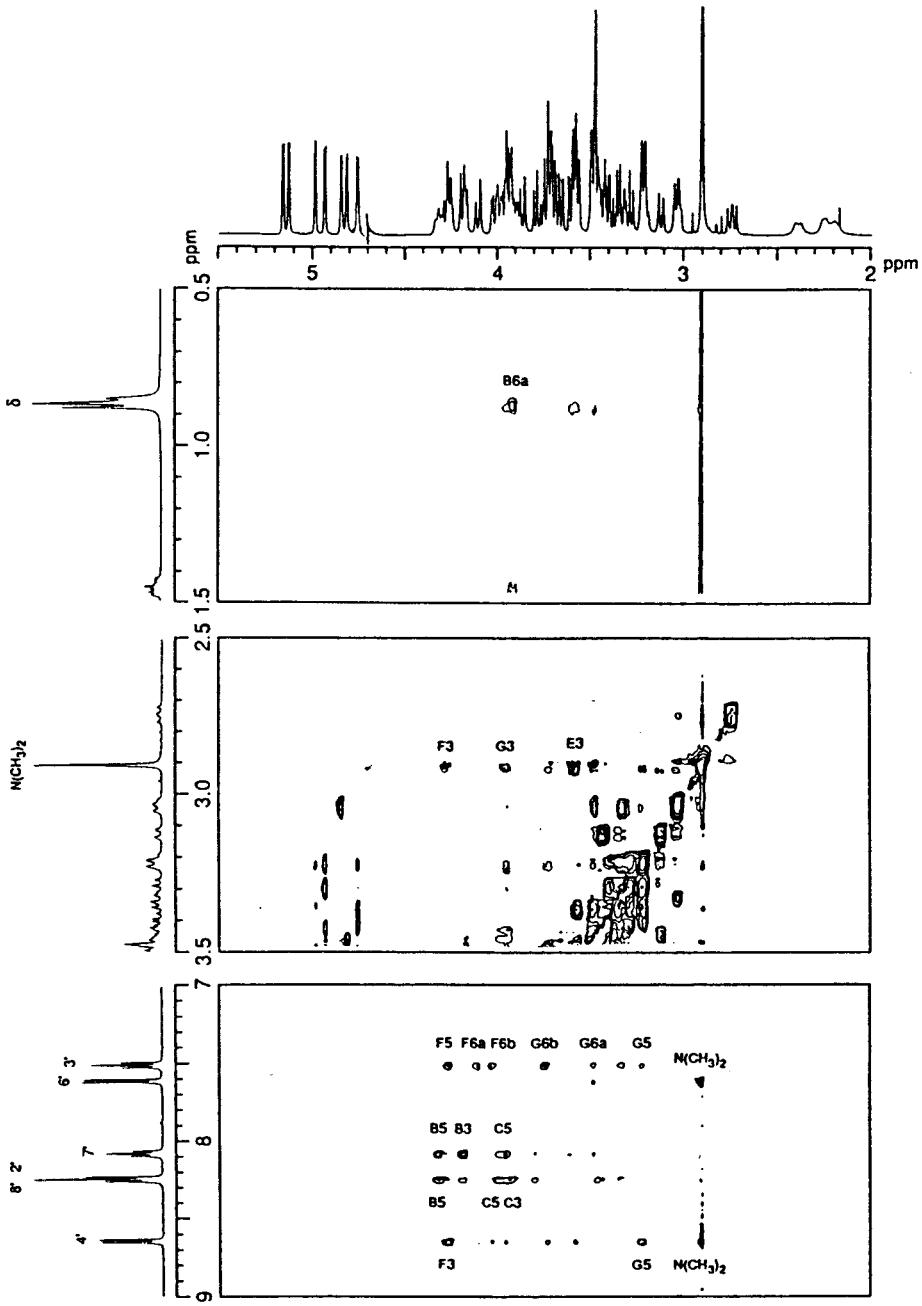


Figure 10. Portion of the 500-MHz ROESY spectrum of the dansyl-leucine-β-CyD **1** in D_2O at 25 °C with mixing times of 300 ms, with cross-peaks between protons of the dansyl and the CyD moieties. (Reprinted with permission from ref 56. Copyright 1997 American Chemical Society.)

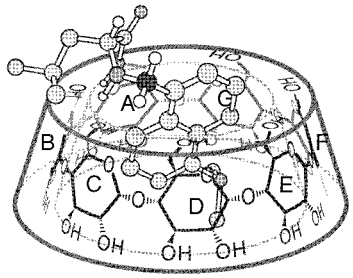


Figure 11. Self-inclusion structure of the dansyl-leucine-β-CyD **1** as deduced from NMR experiments.

the head-to-tail bimolecular complex should exhibit NOEs between different CyD protons compared to that in the self-inclusion complex. Figure 11 shows the structures of DnsLeu-CyD evaluated from both the NOE data and from the observed shielding by the anisotropic ring current effect from the dansyl moiety on the CyD parts.

More quantitative structural data can be obtained by the combined use of NOE data and Molecular Dynamics (MD) and Molecular Mechanics (MM) calculations, which are illustrated with the tyrosine-appended β-CyD.[68] About 50 structures were generated by the simulated annealing (SA) procedure under NOE constraints; and 42 of them, having no distance violation within a tolerance of ± 0.3 Å, were considered further. The position of the tyrosine ring relative to the cyclodextrin units was the same in all 42 structures. Starting from one of the best structures, a 250 ps molecular dynamics (MD) simulation was carried out in a waterbox without constraints.

During the MD simulation, the structure did not deviate from that obtained by the SA procedure. In the SA structures, the aromatic moiety completely fills the cavity; the cyclodextrin part takes an elliptical shape, which tightly fits the aromatic moiety. The simulation data are in agreement with all NOE data, and qualitatively also with ring current effects exerted by the aromatic tyrosine moiety.

Grafting of amino acids or peptides to CyDs opens the way to new vector-carrying molecules with the potential of for instance drug targeting. Corresponding structures have been analyzed earlier[70−72]and recently[73] with a large variety of amino acids. Coupling constants, shifts, and ROESY data indicate that the conformations are largely independent of the amino acid N-terminus protonation; they also indicate self-inclusion of all aromatic amino acid residues. Self-inclusion can be reversed by DMSO as solvent, or by adding better cavity binders. Phase-sensitive COSY or DQF was used to resolve the coupling constants of the amino acid Cα-H and Cβ-H protons. These vary substantially with the solvent, indicating significant changes in the conformer populations around the Cα−Cβ bond of the amino acid residues upon complexation by CyD.

Recently the structure of dansyldiaminoethane-appended β-CyD was determined simultaneously by NMR and X-ray analysis. The X-ray analysis shows that the dansyl group is fully encapsulated within the cyclodextrin cavity, with the dimethylamino and sulfonyl groups emerging from opposite sides. The CyD cavity is considerably deformed, since O-4−O-4 distances parallel to the naphthalene ring were found to be longer than the others. NMR data suggested that the orientation of the dansyl moiety observed in the solid state was retained in solution The circular dichroism spectrum was also consistent with an axial complexation model.

The temperature dependence of NMR spectra provides further information on the population of the inside−outside isomers of the pendant-modified CyDs. The observed temperature effects with naphthyl-appended β-CyD **3** suggest that the naphthyl group as the pendant moves out of the cavity with temperature increase.[74,75] Obviously, at ambient temperature the inside conformer dominates, characterized by upfield shifts of the naphthyl protons. With increase of temperature up to 80 °C, the resonances are shifted downfield, characteristic of a water-exposed naphthyl group outside the cavity.

Cyclodextrins bearing large lipophilic substituents at all OH positions such as shown in Figure 12 exhibit either exchange-broadened or even separate ^{1}H NMR signals at ambient temperature.[76] From the number of signals above coalescence one can derive that there is a symmetrical and a unsymmetrical form slowly interconverting on the NMR time scale. Consideration of the free energy difference variations with the substituent size and molecular mechanics calculations led to the conclusion that the NMR spectra depict here two conformers with the large naphthyl group oriented, or semiencapsulated, into the cavity either all from one side (symmetric conformer (a) in Figure 12), or from opposite sides,

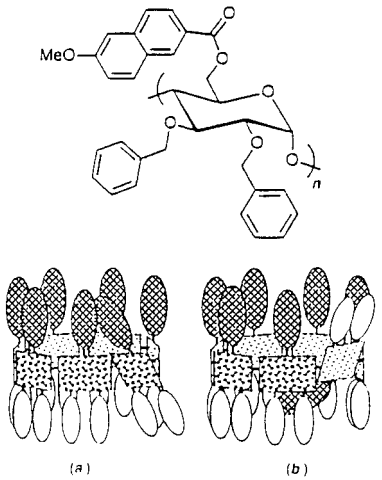
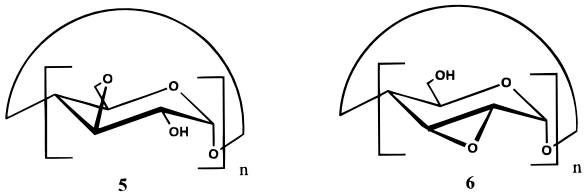


Figure 12. Structure of a naphthoyl- and dibenzyl-substituted β-CyD **4** with time averaged symmetric conformation (a) and an unsymmetrical conformation (b). (Reprinted with permission from ref 76. Copyright 1997 The Royal Society of Chemistry.)

yielding the unsymmetrical orientation (b).

Other Substituted CyDs. Many other asymmetrically substituted CyD derivatives of synthetic importance, such as 3,6-anhydro derivatives **5**[77] or epoxides **6,**[78] sulfobutyl ethers,[79] and various sulfonyl derivatives[80] have been analyzed with modern high-resolution ^{1}H and ^{13}C NMR techniques. Monotosy-



lated CyDs are particularly useful intermediates for the synthesis of functionalized CyD. Methods for regioselective tosylation were developed and the position of tosylation were determined by ^{1}H and ^{13}C NMR technique.[81−84] In D_2O the resonances are at 4.35 and 4.42 ppm for H-2′ of 2-mono-tosylated α-CyD and β-CyD, respectively. The ^{13}C shift for C-2′ of 2-mono-tosylated β-CyD is at 79.6 ppm in DMSO, whereas that for C-6′ of 6-mono-tosylated β-CyD is at 68.8 ppm. Tosylated CyD at C-2 position can be converted to the 3,2-*manno*-epoxide, whereas tosylated CyD at C-3 position is converted to 3,2-*allo*-epoxide.[81] The epoxides show a singlet at 5.15 ppm and a doublet at 5.22 ppm ($J_{1,2}$ = 3.7 Hz) for the *manno*-epoxide and *allo*-epoxide, respectively.

Conversion of 6-*O*-sulfonylated α-cyclodextrins into corresponding 3,6-anhydro derivatives **5** leads to deshielding of the different protons in the bicyclic glucose units *and* to conformational changes of the glucose chair. ^{1}H−^{1}H DQF, COSY, and HOHAHA (Homonuclear Hartmann-Hahn−often called TOCSY instead) techniques were used for the determination of the regioisomeric structures of the anhydroderivatives,[85] which is a convenient way to establish the substitution site of preceding sulfonations. NMR and molecular modeling made evident that a full explora-

Table 11. Calculated Populations of Rotamers around the C5−C6 Bond (in mole fractions), and Observed Coupling Constants ($J_{5,6a}$, $J_{5,6b}$) for the Glucose Residues A and B of Bipyridino-Modified ($CyDEV^{2+}$), Pyridino-Modified ($CyDP^{+}$), and Nicotinamido-Modified ($CyDNA^{+}$) β-Cyclodextrin at 25 °C in D_2O and of 6-*O*-α-D-Glucopyranosyl-α-cyclodextrin (G1-α-CyD) at 30 °C in D_2O

	glucose unit	P_{gg}	P_{gt}	P_{tg}	$J_{5,6a}$	$J_{5,6b}$
$CyDEV^{2+}$	A	0.14	0.86	∼0	1.8	9.8
	B	0.58	0.42	∼0	1.8	5.5
$CyDP^{+}$	A	0.16	0.84	∼0	1.8	9.6
	B	0.76	0.23	0.01	1.8	3.7
$CyDNA^{+}$	A	0.12	0.84	0.04	2.2	9.6
	B	0.78	0.21	0.01	1.9	3.5
G1-α-CyD	A	0.66	0.32	0.02	1.8	4.6

tion of the conformational space in 3,6-anhydro-α-cyclodextrin is still impossible. The potential of long-range carbon−proton correlation experiments has been recognized for conformational analysis of carbohydrates in solution.[86,87] For the 3,6-anhydro-α-cyclodextrins the coupling constants found by these experiments confirm in combination with the results obtained from 1D NOE and COSY spectra some of the geometrical arrangements obtained from molecular modeling.[88] A full spin system as well as conformational analysis for a cyclodipeptide-substituted β-CyD was performed with 2D techniques, including selective excitation sequences (1D TOCSY).[89] The numerous dimeric CyDs prepared by Breslow and his group[90,91] as intriguing receptor and enzyme models were all characterized by NMR data supporting the expected symmetry; the same holds for mono- and bifunctional CyD derivatives bearing for instance imidazoles.

^{13}C NMR spectroscopy gives convenient insight into the distribution of the substituents in modified cyclodextrins. Different water-soluble polymers with very high molecular weight (MW > 10^6) obtained from different cyclodextrins, and e.g. ephedrine,[92] by changing the reaction conditions can be analyzed by this method, as well as the substituent pattern of the reaction products after *O*-carboxymethylation.[93] The calculation of the substitution degree is eased by comparison of the signal integration values of introduced groups with those of the cyclodextrin torus protons.[94] Capped cyclodextrins, obtained from reactions of CyDs with α,ω-disulfonyl chlorides, provide the most important entry into disubstituted CyD derivatives,[95] but are difficult to assign unambiguously even with modern high field and 2D NMR techniques.[19,85,96−99] ^1H NMR, and also partially ^{13}C NMR, were used to secure the structure of cyclodextrin-containing porphyrin derivatives, which can serve as oxygenase models.[100]

Analysis of Rotamers around the C5−C6 Bond in Substituted CyDs. The analysis of conformations around the C5−C6 bond in a modified CyD describe the position of the pendant group with respect to the cavity. The ^1H NMR resonances for H-5 and H-6 usually overlap with other resonances, and the coupling constants $J_{5,6}$ cannot be measured directly. In some special cases, the ^1H resonances for H-5 or H-6 are observed separately, and the conformation can be analyzed with the coupling constants $J_{5,6}$. Three staggered rotamers (gg, gt, and tg) around the C5−C6 bond (see Figure 2 in section B.1) and their populations[101] are considered for analyzing the time averaged coupling constants by[101]

$$J_{5,6a} = 1.7P_{gg} + 1.9P_{gt} + 11.1P_{tg} \quad (3a)$$

$$J_{5,6b} = 1.5P_{gg} + 11.1P_{gt} + 2.1P_{tg} \quad (3b)$$

Here, P_{gg}, P_{gt}, and P_{tg} are the mole fractions of the three rotamers gg, gt, and tg, respectively. The coupling constants $J_{5,6a}$, and $J_{5,6b}$ of each rotamer are calculated with a modified Karplus eq 4, assigning fixed values to the individual conformers.[101]

$$J_{H,H'} = (6.6 - 1.0\cos\phi + 5.6\cos 2\phi)(1 - \sum_{i=1}^{4} f_i \Delta x_i) \quad (4)$$

Table 11 shows the results of such an analysis with the glucose units A and B of a β-CyD modified with a pyridine, a nicotinamide, and a glucose residue.[102−104] Both in the glucose unit A and B, the rotamer tg scarcely exists, as the result of steric or stereoelectronic interactions. It is well-known that the rotamer tg of the D-glucopyranose monomer is less stable because of unfavorable parallel 1,3-interactions between C4−O and C6−O.[101] The rotamer gt is the major component in the glucose unit A of all of these modified CyDs. This means the pendants' residues face the glucose unit B. These conformations agree with the observed NMR shielding, showing that the H-6 resonances of the glucose unit B are shifted by approximately 2.7 ppm.

The populations of gg and gt are almost the same in the glucose unit B of $CyDEV^{2+}$; the rotamer gg, however, is the major component in the glucose unit B of $CyDP^{+}$. This difference causes a shift of about 2.7 ppm of the H-6 resonances in the glucose unit B. The hydroxyl group at C6 in the glucose unit B of $CyDEV^{2+}$ is located between two pyridinium cations, whereas the pyridinium cation in $CyDP^{+}$ is located on one side of the hydroxyl group. The "pendant" sugar residue in 6-*O*-α-D-glucopyranosyl-α-cyclodextrin[104] has on the basis of the observed coupling constants the populations around the C-5-C6 bond as shown in Table 11. Here the rotamer gg is the major component, indicating that the hydrophilic sugar residue is oriented away from the cavity. The static analyses based on contributions of different fixed ground states can be expanded by coupling molecular dynamics calculations with NMR couplings or NOEs, as will be discussed below[105] for exchange in CyD complexes.

C. Cyclodextrin Complexes

C.1. NMR Shift Titrations

Measurements of chemical shift changes as a function of concentrations—so-called NMR titrations—have, compared to most other methods of equilibrium determinations, the advantage of providing several independent signals for the evaluation of association constants *K*. The applied methodology, which should

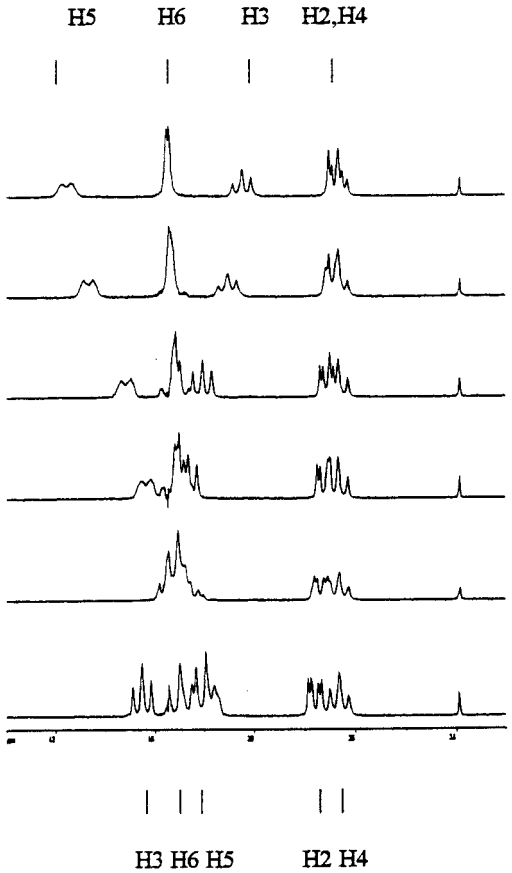


Figure 13. NMR shift titration of α-CyD and *p*-iodophenolate in D_2O at ambient temperature; the CyD H-5 signals shift to lower field, H-3 to higher field, resulting in frequently observed signal crossing during titration.

secure measuring ranges between about 20 and 80% complexation degree, if possible, is described in detail elsewhere.[106−108]

Nonlinear least-squares fitting of the shifts yields individual apparent *K* values; if these differ by more than approximately 10%, and/or if the fitting to a 1:1 stoichiometric model shows systematical deviations, this will require (and allow) investigation of additional equilibria. Another advantage of NMR titrations is that the observed shift changes provide at the same time insight into the conformation of the formed supramolecular complexes, which is difficult to extract e.g. from UV/vis titrations, and impossible for instance from calorimetric data. The availability of high-field instruments allows shift changes of the cyclodextrins themselves or of symmetrically substituted derivatives with most of the anisochroneous signals of the sugar moieties to be followed, provided the encapsulated guest molecule G develops a shielding tensor of sufficient size. This is always the case for aromatic substrates, but barely so for aliphatic compounds. Figure 13 illustrates the spectra of such a typical NMR titration; Figure 14 shows a nearly perfect computer fit obtained from these shifts. The absence of systematic deviation and the agreement between the association constants *K* from all six CyD signals in this case secures the assumed 1:1 intracavity complexation.

NMR shift titrations have thus become one of the most often used methods to determine association

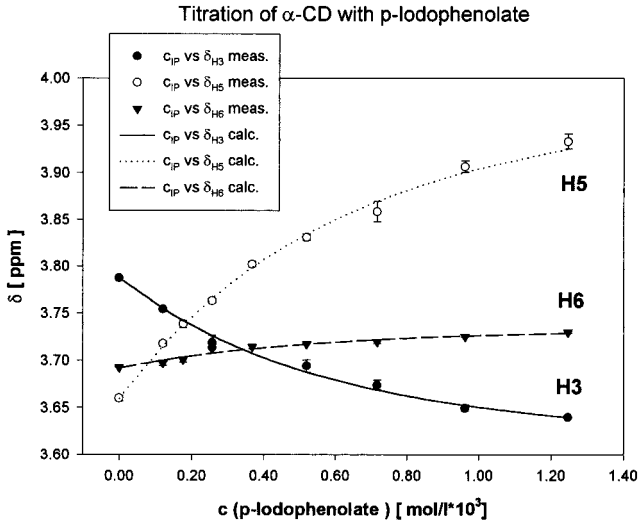


Figure 14. Nonlinear least-squares fitting of NMR shifts caused by *p*-iodophenolate in D_2O to H-5, H-3, and H-6 α-CyD signals.

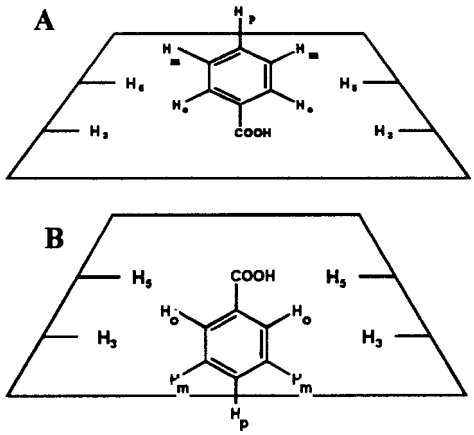


Figure 15. Possible inclusion geometries of α-CyD with benzoic acid.

constants with cyclodextrins.[104,109−111] Complexes with α-CyD and benzoic acid are probably the most thoroughly studied,[112−115] using a variety of methods: They generally indicate that the carboxylic group is closer to the smaller rim (conformation B in Figure 15). Similar conclusions emerge from computer-aided NOE analyses[116] of complexes with β-CyD and benzoic acid, showing a remarkably distinct preference for conformation B: for B the best root-mean-square values (rms) deviations of intermolecular aryl-CyD H-3/ H-5 distances between force field and NOE-calculated distance ratios were rms = 0.143. In contrast, the best value obtained for conformer A yielded only a poor rms = 0.383. Molecular mechanics calculations show a large range of metastable conformations, and however also conformer B as the most stable one.[116] Similar geometries were deduced for benzoate−β-CyD complexes.[117,118] The complex geometry with the hydrophilic carboxylic group inside the hydrophobic cavity was already made evident by Bergeron et al.,[119,120] who also rationalized the results by consideration of the microenvironment effects of the CyD cavity, which was compared before to *p*-dioxane. Intracavity inclusion of the even the more polar benzoate anion was as expected characterized by less pronounced

Table 12. ^{1}H NMR CIS Values from NMR Shift Titrations for α-CyD Complexes with Phenyl Derivatives[a]

proton	7	8	9	10	11
1 (CyD)	−0.04	−0.03	−0.02	−0.01	−0.03
2 (CyD)	−0.08	−0.04	−0.05	−0.03	−0.06
3 (CyD)	−0.25	−0.35	−0.25	−0.26	−0.13
4 (CyD)	0.00	0.01	0.00	0.01	−0.01
5 (CyD)	0.02	−0.05	0.35	0.36	0.14
6 (CyD)	0.00	0.00	0.03	0.03	0.01

[a] Measuring conditions see Figure 14.

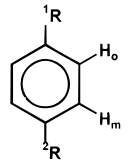
orientation and binding, but believed to be helped by partial hydration also inside the cavity.

Another case studied for a long time are complexes between α-CyD and nitrophenols or the corresponding phenolate.[10,11,104,121,122] Such disubstituted phenyl compounds present a particularly interesting problem, as insertion may occur from two alternative sites. In agreement with NOE data (see below), the observed shielding effects indicate that it is generally the nitro group which is inserted first, and oriented toward the narrow side of the cavity.

Complexation of substituted cylohexanes in α- and *β*-CyD was studied in detail by calorimetry, with NMR data indicating, as expected, deeper penetration into the wider *β*-CyD cavity.[109,123] A comparison of affinities from calorimetric and NMR titrations with ephedrine derivatives shows good agreement between these methods; it was also claimed that the magnitude of the upfield shifts of the cyclodextrin's H-3 and H-5 protons can be used as a measure of the complex stability as well as of the depth of inclusion.[124] ^{13}C NMR chemical shifts changes are also observable for very weak complexes such as in the case of monosaccharides and α-CyDs.[125]

Often used internal reference compounds such as tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) were reported to show significant downfield shifts (by 0.63 ppm in aqueous solution) during NMR titration experiments with cyclodextrins. This was supposed to be the result of an inclusion complex, and external references were recommended for obtaining more reliable association constants.[126] More recent studies, however, show that shift changes of these reference compounds in the presence of cyclodextrins are not the consequence of inclusion in the cyclodextrin cavity, but the result of changes in the magnetic susceptibilities of water due to hydrogen bonding with the glucose units of the cyclodextrins. Better results were then obtained by using internal reference compounds such as tetramethylammonium salts (TMA).[127]

The shifts induced by 100% complexation (CIS values, Table 12) are intrinsic constants which characterize the nature of the complex. Without NMR titration and line fitting they can be obtained from single measurements only, if the dissociation constant is about 10 times larger than the concentration of the (not observed) excess partner, which in the case of cyclodextrins most often will be an aromatic guest molecule. If a full titration and subsequent line fitting is not possible, one can take advantage of the almost unlimited dispersion attainable in 2D NMR spectra. In principle it is sufficient to measure one COSY NMR spectrum at CyD and guest concentrations securing at least for instance 20% degree of complexation, which can be calculated with the known concentrations as long as the association constant *K* is known from other, for instance from optical, from calorimetric, or from competition, measurements. This procedure is of particular importance for substrates such as aliphatic compounds which generate only very small shifts on the CyD host. It also allows determination of ^{13}C CIS values which in view of the long accumulation times are difficult to obtain by direct ^{13}C shift titration.



7	^{1}R = O$^-$	;^{2}R = NO_2
8	^{1}R = OH	;^{2}R = NO_2
9	^{1}R = O$^-$	;^{2}R = I
10	^{1}R = OH	;^{2}R = I
11	^{1}R = CH_2COOH	;^{2}R = I

Aromatic guest compounds for α-CyD.

C.2. The Quantitative Analysis of Complexation-Induced ^{1}H NMR Shift

The distinct shifts induced by guest molecules with strong shielding tensors, such as aromatic systems, can be subjected to quantitative calculations on the basis of, for instance, modified McConnell equations. For aromatic ring current anisotropies the literature provides several computational models (for a review see ref 128). These and the underlying parametrizations were recently evaluated on the basis of several cyclophanes with several protons situated also *above* the ring plane, a geometric disposition typical also for CyD complexes.[129] The best agreement between experiment and calculation with such covalent and therefore better defined models was found with the Johnson−Bovey[130] double loop model, with ring parameters close to the original values. The anisotropy effects $\Delta\chi$ in Table 13 are obtained with the computer program SHIFT,[131] which allows the effect of shielding tensors on all desired nuclei to be calculated and all signals which are in fast exchange on the NMR time scale to be averaged. The underlying CyD complex geometries were obtained after MD simulations[132] with quenched dynamics[133] gas-phase force field energy minimization (CHARMm, Kar-

Table 13. Calculated and Experimental NMR Shielding Effects (in ppm; positive signs indicate upfield shifts) of *p*-Nitrophenol (PNP) and Benzoic acid (BA) on the Inside Protons H3 and H5 of α-Cyclodextrin[a]

		$\Delta\chi$	LEF	$\Delta\chi$ + LEF	exp	lit.$_{opt}$
PNP	H3	0.36	0.06	0.42	0.35	0.26
	H5	0.03	0.04	0.07	0.05	−0.08
BA	H3	0.40	0.07	0.47	0.45	0.40
	H5	−0.01	−0.05	−0.06	−0.17	−0.09

[a] $\Delta\chi$, calculated anisotropy effects; LEF, linear electric field effects. SHIFT calculation based on CHARMm generated geometries, without the conformation being fitted to observed shifts[131] lit.$_{opt}$ calculations by Komiyama et al.,[135] neglecting LEF, and with conformations fitted to observed shifts.

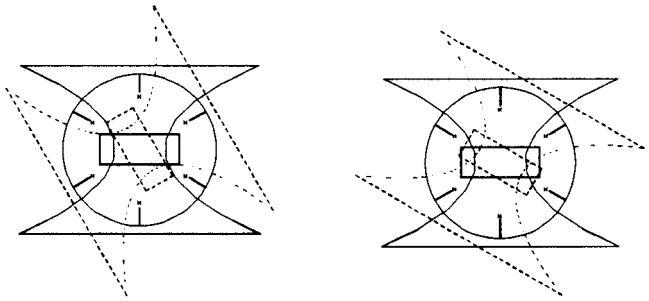


Figure 16. Orientation of phenyl rings and ring current anisotropy cones within the α-CyD cavity: model a, with local minima avoiding repulsions between host and guest protons and, model b, without local minima.

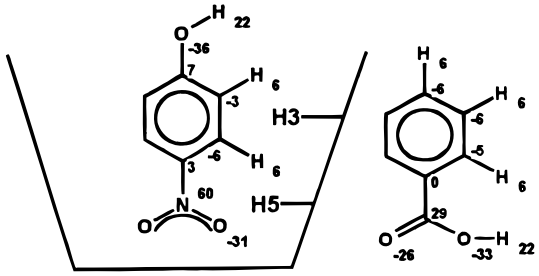


Figure 17. Charge distribution used for SHIFT calculations.

plus[134]). The SHIFT calculations were based on averaged single minimum energy conformations as shown in Figure 16a, except for earlier work from Komiyama et al.,[10,11,51,135] where no distinct minima were taken into account.

In contrast to earlier calculations,[51,135] SHIFT also takes into account the polarization of C−H bonds by the linear electric field effect LEF, which has been shown before to contribute significantly to proton NMR shift changes even within uncharged molecules such as halosteroids.[136] The results in Table 13 [137] were obtained by placing partial charges obtained from the Gasteiger model[138] at the different positions of the phenyl guest molecules as given in Figure 17. The use of local charges derived from CNDO calculations or of charge templates, as supplied for instance within the CHARMm force field, was leading to larger deviations between experiment and calculation. The results indicate that the LEF contributions indeed cannot be neglected even with uncharged compounds. As in molecular mechanics calculations the problem here is the realistic choice of local charges and dielectrics. The situation becomes more difficult with charged compounds, where polarization effects can influence charge distribution significantly. Another complication stems from possible deformations of host and guest upon complexation, which can give rise to intramolecular screening differences particularly with carbon nuclei. Earlier NMR shielding calculations[139,140] have considered such deformations in cyclodextrins, however, without taking into account the sizable LEF contributions.

Proton shifts induced by the CyD moiety **on** the guests are as a consequence of the weak shielding tensors in the alicyclic CyD skeleton usually small, and may be predominantly due to changes of the

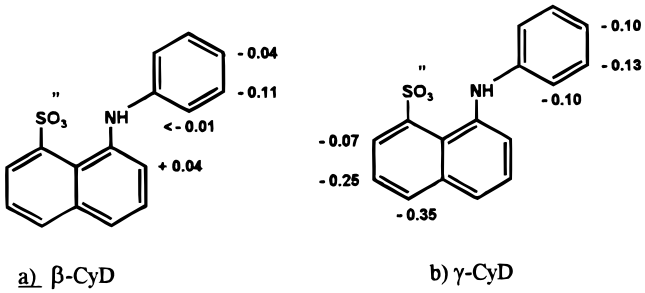


Figure 18. Complexation induced shifts by CyDs on guest molecules (in ppm), in D_2O.

microenvironment, as proposed by Inoue et al. for corresponding ^{13}C NMR shifts.[20,34−38] Nevertheless, these shielding effects can be large enough to be used for NMR titrations, as illustrated in Figure 18 with CyD-induced shifts on a fluorescence dye.[141] This example also demonstrates that shielding exerted by the CyD can be useful not only in cases, where the guest itself has weak shielding tensors, but also for the identification of those parts of the guest which are inside the cavity. Unfortunately, with few exceptions such as with azulene complexes[142] the CyD induced shifts have been until now mostly neglected. De Marco et al. noticed deshielding effects on complexed guest molecules decades ago;[12] recent examples with, for instance, indomethacin inclusion (see section C.10.a) in *β*-CyD show deshielding by up to 0.3 ppm, which, however, might be due to guest conformation changes upon complexation (see section C.8.f)[143]

C.3. Complexation-Induced ^{13}C NMR Shifts

Complexation-induced ^{13}C NMR shifts have been used previously to obtain information on corresponding binding modes.[144−146] Gelb, Lauffer, et al.[144] observed, with benzoic acid, larger shielding effects of, for instance, −0.39 ppm to −0.44 ppm at the α-CyD signals C-5 and C-6, respectively; this was taken as evidence for insertion with the carboxylic group at the narrow rim, in contrast to prevalent views based mostly on NOE data (see below). On the other hand, significant CIS values with deshielding of 0.46 ppm were observed also at C-1. Even larger effects were found with permethylated α-CyD and *p*-biphenylcarboxylate, varying from −0.23 ppm shielding to +1.01 ppm shielding.[147]

Generally carbon atoms of guest molecules which are deeper in the cavity seem to undergo shielding, whereas those closer to the wider cavity of α-CyD tend to be deshielded by complexation.[20,148] Inoue et al.[20,37] have, on the basis of reaction field theory,[39] interpreted these findings as consequence of the difference in dielectric environment. However, as mentioned above[32] solvent effects on aromatic substrates are quite different from those observed by complexation in CyDs.

Interpretation of ^{13}C NMR screening changes is difficult in view of the pronounced sensitivity of carbon shielding toward even minor conformational distortions. In contrast, protons, being exposed much more than the "inside" carbon atoms to through-space screening effects of shielding tensors, exhibit CIS

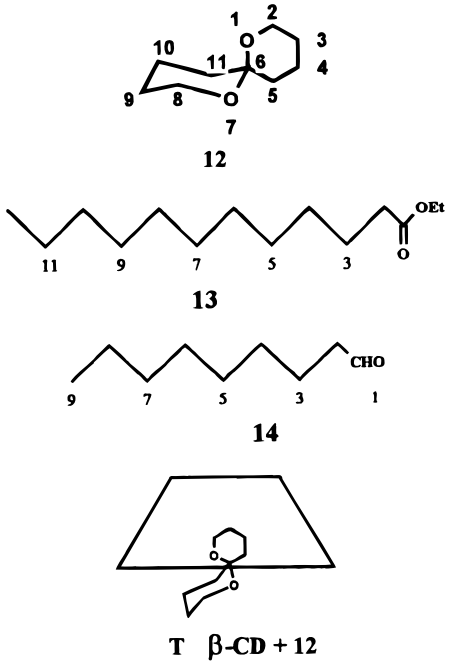


Figure 19. Aliphatic compounds (pheromones) and their inclusion mode.[61]

values which often are in the range of 10% of the total observable shielding range, whereas for carbon shifts the corresponding number is in the order of less than 1%. As a consequence, carbon shifts are also much less accessible to quantitative evaluation of the corresponding intermolecular anisotropy effects. In summary, ^{13}C NMR shift changes are believed now mostly to reflect complexation induced conformational changes in cyclodextrins, such as that of the tilt angle of the pyranoses toward the macrocycle plane (see below).

Complexes with aliphatic guest molecules lacking strong shielding tensors show only small and difficult to rationalize shielding effects on both host and guest. Nevertheless, the shift changes can be used not only to determine association constants, but also may allow one to at least decide if there is intracavity inclusion or not. As an illustrative example we cite studies with the aliphatic pheromones **12**−**14**, which with permethylated tri-*O*-methyl-CyDs (TMCyDs) associate with constants of up to 8000 mol^{-1} in water (D_2O).[61] With α-CyDs also 2:1 complexes were observed, whereas the larger *β*- or *γ*-CyDs allow enough contact to the guests in 1:1 complexes, eventually with coiled conformations in the case of the long alkyl chain derivatives.

Intermolecular ROESY peaks of the CyD H-3 and H-5 protons, in the case of a TM-α-CyD also with of the 3-O-Me protons with guest protons secured (in all cases there) are intracavity inclusion modes of the kind illustrated in Figure 19; however they also partially contact the CyD outside. Cross-peaks observed in some cases also with the outside CyD proton H-4 were ascribed to intramolecular scalar spin saturation transfers from the CyD protons H-3 and/or H-5. Shielding variations were reported not as CIS values from titration fit, but from measurements with excess host or guest. They demonstrate nevertheless the smallness particularly of ^1H NMR shielding effects. These are, indicative of intracavity inclusion, relatively large for the inside CyD protons H-3 and H-5, with irregular shielding (e.g. at H-3 with α-CyD −0.03 ppm for **12**, and −0.023 ppm for **13**), or—more often—deshielding, particularly with the TM hosts with values up to +0.36 ppm. Host protons outside the cavity show smaller effects, with upper limits of 0.05 ppm at H-1, 0.10 ppm at H-2, 0.08 ppm at H-4, 0.05 ppm at H-6, and 0.05 ppm at Me signals (always deshielding). More pronounced on a absolute, but not on a relative scale (see above) are the observed ^{13}C NMR shielding changes, which are all characterized by upfield shifts by up to (−) 2.3 ppm, except for the C-6, 2-O-Me, and 6-O-Me carbon atoms (deshielded by up to 0.58 ppm). The particularly strong effects on C-4 and C-1 were attributed to conformational changes upon complexation.

C.4. NOE Measurements for Conformational Analysis of CyD Complexes

A typical NOE application is illustrated with α-CyD complexes, which basically can occur in three different inclusion modes[105,149] (Figure 20). Type **I** has a para-substituted phenol partially immersed into the cavity, leading to sizable contacts only between the guest H_o and the host H-3 proton. Type **II**, with deeper immersion, has similar contacts between H_m and the CyD protons H-3/H-5, whereas H_o can only "feel" H-3. Type **III** has the opposite immersion mode with the phenolic group inside the cavity. Obviously, all three modes should lead to quite different NOEs and can be distinguished this way. The 2D-ROESY spectrum of the *p*-iodophenolate with α-CyD (Figure 22) shows only those cross-peaks discussed for binding mode **I**.[149]

Volume integration of the cross-peaks shows intensities which at least semiquantitatively correlate with distances calculated for mode **I** by molecular mechanics (Table 14), if one sets the intramolecular

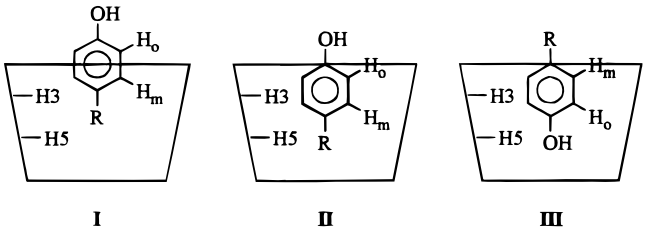


Figure 20. Binding modes of para-disubstituted phenyl compounds in cyclodextrins, with interactions sites of the protons observable by NOEs.

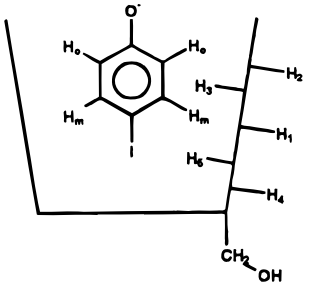


Figure 21. Geometry of the complex between α-CyD and *p*-iodophenolate based on NOE measurements.[149]

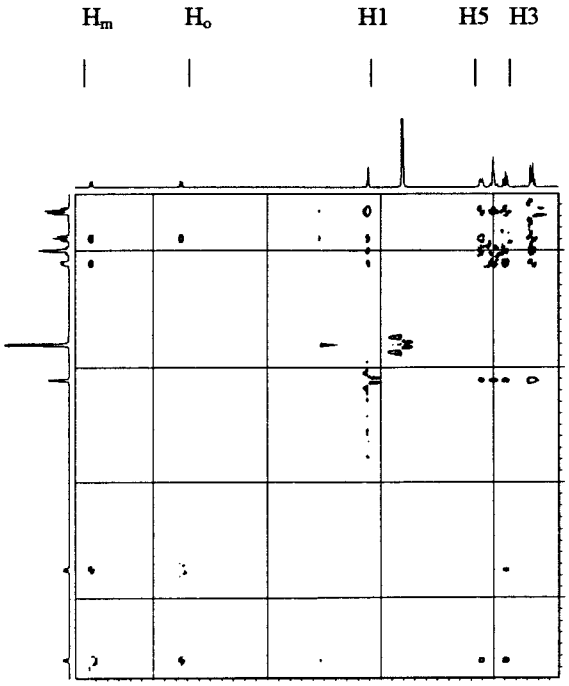


Figure 22. 2D ROESY spectrum of the complex between α-CyD and *p*-iodophenolate (in D_2O, pD = 10,4; relaxation delay 2 s, spinlock time 300 ms, spinlock field 4 kHz).

Table 14. Relative ROESY Integrals (in %) for the Complex between α-CyD and *p*-Iodophenolate, with Distances (Å) from Molecular Mechanics Calculations (CHARMm) in *Italic*[a]

Proton	H_m		H_o	
CyD-H-3	70	*2.32*	35	*3.00*
CyD-H-5	59	*2.37*	<2	*>4.0*

[a] The NOE between the vicinal protons H_o and H_m (r = 2.48 Å) is set equal to 100%.

Table 15. NOE (ROESY) Effects in α-CyD Complexes with Compounds 7−11

H[a]	**7**		**8**		**9**		**10**		**11**	
	o	m	o	m	o	m	o	m	aro	CH_2
H3(CyD)	+[b]	+	+	+	++	++	+	++	+	−
H5(CyD)	−	+	−	+	−	++	−	++	−	−

[a] Observed proton; [b] ++, strong; +, medium; −, no NOE effects. [c] [α-CyD] = 5 mM, [phenyl derivative] = 10 mM.

NOE between the vicinal protons H_o and H_m equal to 100%. Even with biopolymers, where much smaller tumbling rates and stronger directional forces lead to significantly larger (negative) NOEs, it is a common practice to use NOEs only as experimental cutoff parameter for interproton distances beyond 4 Å. In synthetic supramolecular complexes the NOEs intensities are further weakened by rapid exchange between many binding modes with similar internucleus distances. Therefore one often represents the observed NOEs only as strong, medium, or weak (Table 15). The data shown in Table 15 indicate for all parasubstituted phenyl derivatives an inclusion mode between type **I** or **II**, clearly with the more hydrophilic group outside the cavity. The large and well-polarizable iodo group in structures **9**, **10**, and **11** obviously provides for deeper insertion (mode II),

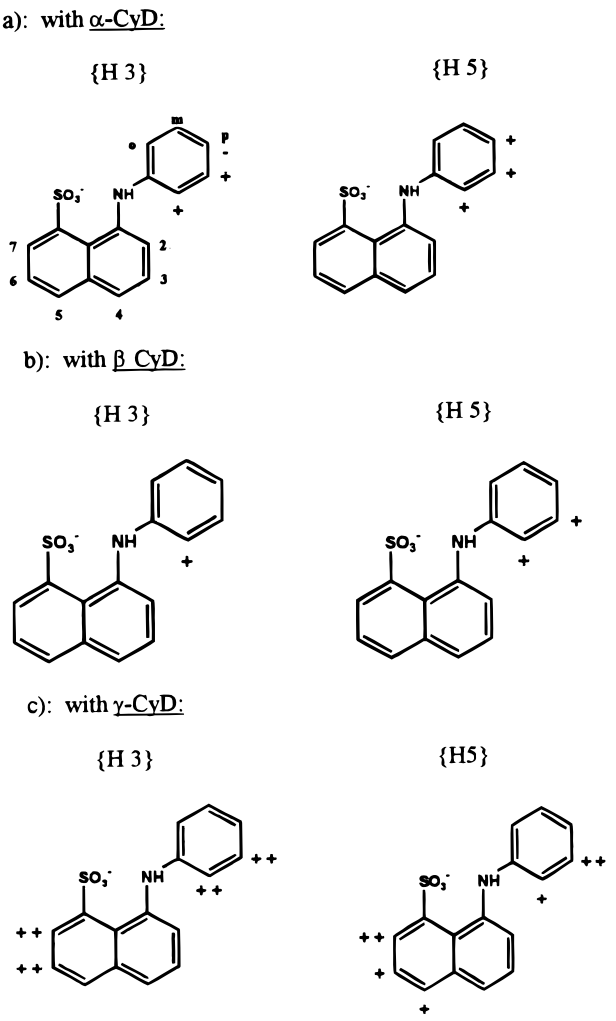


Figure 23. Intermolecular ROESY cross-peaks of complexes between cyclodextrins and ANS (++, strong; +, moderate; others, absent).[141]

as expected by here predominating dispersive interactions as driving force.

Hydrophobic instead of a dispersive binding mechanisms are expected to lead to less tight intracavity inclusion. This is indeed borne out by ROESY measurements with complexes between cyclodextrins and less polarizable substrates[141] shown in Figure 23. The α-CyD intracavity protons must show intermolecular NOEs with the phenyl part of the substrate in view of the host−guest size matching. The wider cavity of *β*-CyD would match the naphthyl part of the dye, which, however, in contrast to the phenyl residue shows **no** intermolecular NOEs. Even the large *γ*-CyD shows relaxation pathways not only with the naphthyl, but also with the phenyl part. The same conclusions emerge from an analysis of the complexation induced shifts, and demonstrate that strong complexation may require loose binding in the case of predominantly solvophobic interactions. Related observations were made with the fluorescence dye TNS (6-*p*-toluidinyl naphthalene-2-sulfonate) and CyDs, which, however, are complicated by formation of different complexes with *β*-CyD. In general, the combination of calorimetric and NMR spectroscopic methods is most promising for providing insight into CyD binding mechanisms.[123,124,149,150]

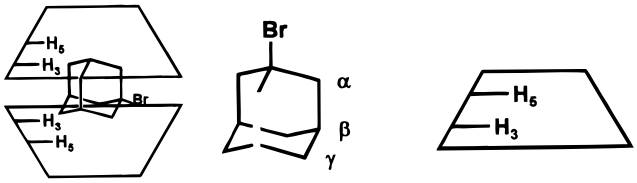


Figure 24. Geometry of the inclusion of 1-bromoadamantane in α-CyD.

Jaime et al. have demonstrated in several papers possibilities and limits of molecular mechanics and dynamics calculations for the prediction and evaluation of CyD complexes in combination with NMR analyses.[105,151−153] 1-Bromoadamantane was shown by a Job plot with NMR shifts to form predominantly a 2:1 complex with α-CyD. The geometry shown in Figure 24 is predicted to have practically the same energy as a complex with two CyD units and the adamantane guest outside, based on molecular mechanics energy minimization (MacroModel) after sampling about 40 different conformers obtained from MD simulations with AMBER. Sizable intermolecular NOEs—up to 2.85% in 1D-ROESY experiments—were observed for H-3/Hα and H-3/Hγ, for which the MM calculated distances are 2.1 to 2.2 Å in conformer A. Smaller values for the other intermolecular NOEs (distances 2.3−2.6 Å) were again in agreement only with conformer A and exclude the alternative complex with the guest molecule outside. The complex of bromoadamantane with β-CyD [151−153] was characterized by a combination of molecular mechanics calculations and NOE measurements as a 1:1 complex with a more favorable orientation of the bromo atom pointing to the wider rim. The preference, however, was found to be not very pronounced; this seems even more to be the case with the complex of β-CyD and the unsubstituted, almost ball-shaped isotropic adamantane itself.

C.5. Dynamics of Complex Formation

With larger and strongly complexing substrates such as decalines,[154] acenaphtene—with formation of a 1:2 complex,[155] dimethylnaphthalene, phenanthrene,[156] or substituted bicyclo[2.2.2]octanes[157] dissociation rates of cyclodextrin complexes are so small below or close to room temperature, that they can be followed by classical NMR line-shape analysis. Thus, a dimer formed by complexation of reduced tetracyanoquinodimethane (TCNQ-) with β-CyD shows separate ^{1}H and ^{13}C NMR signals for free and bound CyD. ^{2}D−^{1}H exchange spectroscopy yields exchange rates from 0.9 s^{-1} at 30 °C to 5.6 s^{-1} at 42 °C.[158] Line-shape analysis also allowed measurement of the complexation/decomplexation rates of CyDs and substituted viologen derivatives.[158,159]

In some cases ^{1}H and ^{13}C NMR spectra at room temperature exhibit only minor shift changes of the signals upon complexation due to only shallow inclusion of large guest molecules such as e.g. 1,2,3-tri-*tert*-butylnaphthalene in the γ-CyD cavity. In these cases line broadening of ^{13}C NMR signals at lower temperatures helps to manifest complexation dynamics.[160] Temperature-dependent ^{1}H NMR spectroscopy also gives information about inside−outside isomerism of chromophores covalently attached to cyclodextrins.[74] The binding schemes discussed above on the basis of fluorescence measurements can be supported this way by NMR.

C.6. Relaxation Times

The rotational correlation times of guest compounds in supramolecular complexes are usually so increased that the corresponding change of relaxation rates may be used to obtain this way also equilibrium constants. If as usual the minor component, say the guest G is observed and one works with excess host H, the transverse relaxation time T_2, which may for simplicity taken from the half-height width ($W_{1/2} = T_2^{-1}$) of a Lorentzian-shaped NMR signal, is the weighted average of the times for the free and bound guest, $T_{2\text{free}}$ and $T_{2\text{bound}}$, respectively:

$$T_{\text{obs}} = aT_{2\text{free}} + b\,T_{2\text{bound}} \qquad (5)$$

As usual a and b reflect the concentrations of free and bound guest molecule, which by application of mass law allow to derive the corresponding equilibrium constant K. If one describes the relaxation time change before and after complexation as ΔT_2, the association constant K for a 1:1 complex can be obtained from the slope of a linear plot described for [H] ≫ [G] by[106,161]

$$[\text{G}]\,\Delta T_2^{-1} = T_{2\text{bound}}K + T_{2\text{bound}}[\text{H}] \qquad (6)$$

Obviously the method is of particular value for complexes not providing NMR shift, or optical signal changes. It was used with ^{81}Br relaxation not only to measure K for bromide itself, but by competition also the association constants of many anions with cyclodextrins.[162]

Relaxation times can provide significant information about mobilities in dynamic ranges not accessible to line-shape analysis, as well as on underlying geometries. New off-resonance ROESY techniques (see section B.4) not only provide more realistic static structures, but also quantification of internal motions.[105] Changes in longitudinal proton relaxation times T_1 upon complexation by cyclodextrins have successfully been used as well to measure association constants with nitramine-type explosives; this represents an interesting alternative for complexes with too small shielding changes for NMR titration.[163]

^{13}C NMR relaxation times in CyDs are for nonquaternary carbon atoms as expected usually small.[164] Spin-label-induced ^{13}C nuclear relaxation rates were used to determine guest molecule positions in α-CyD with di-*tert*-butyl nitroxide complexes.[165] Analysis of dipole−dipole contributions, assuming isotropic reorientation, leads for the naphthoyl-CyD **CDNA** (Figure 25) to correlation times agreeing well with fluorescence-derived values and suggest a tight host−guest binding. Complexes of such CyD derivatives with a merocyanine dye are the basis of light-harvesting antenna systems.[166,167]

Complexes of methylorange with TM-β-CyD were reported to show *increased* T_1 of the CyD carbon signals, whereas with the DM-βCyD derivatives an

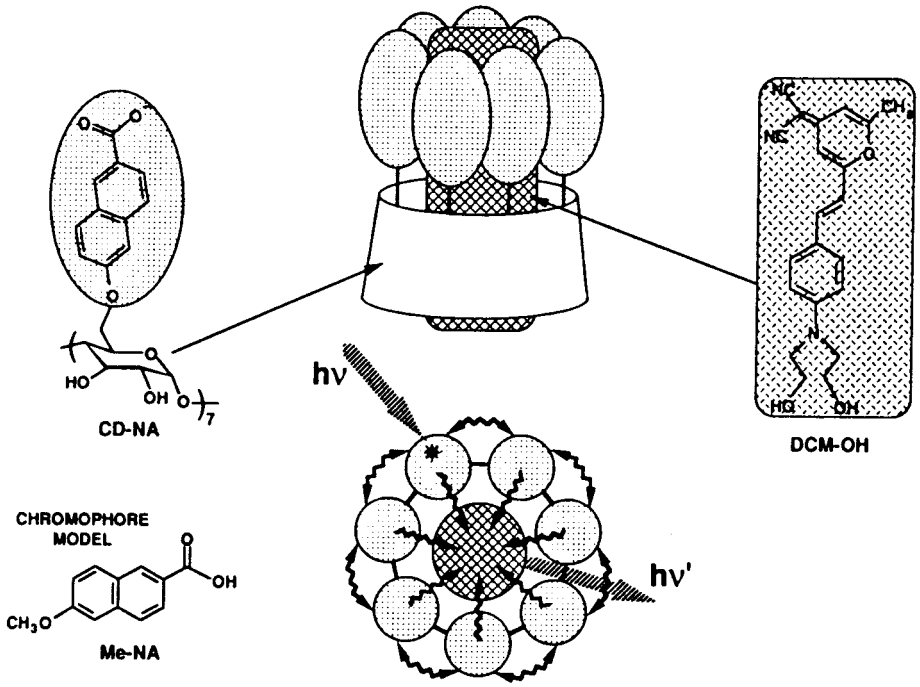


Figure 25. Complex between β-cyclodextrin−heptanaphthoate **CDNA** and the merocynanine laser dye **DCM**-OH.[166] (Reprinted with permission from ref 166. Copyright 1996 American Chemical Society.)

opposite T_1 *decrease* was found.[168] Addition of α-pinene to TM-β-CyD causes not only shift changes of the CyD carbon signals, indicative of complexation-induced variations of the CyD tilt angles,[61,169] but causes also signal broadening as a function of the applied after-excitation pulse delay.[170,54] This was interpreted as evidence for faster internal motion of the pyranose rings as the result of complexation. At the same time, concentration-independent separate signals for free and complexed pinene indicate slow exchange at 500 MHz field strength.

C.7. Inclusion with Modified Cyclodextrins

C.7.a. General Features

Substitution on the CyD rims can greatly enhance the binding of many lipophilic host compounds.[3,54] NMR shifts, relaxation times, and ^{13}C NMR rotational correlation times support the idea that lengthening of the hydrophobic torus by substituting the CyD with methyl groups leads to increased interactions with the hydrophobic parts of substituted azo dyes.[171] Permethylation seems to generate higher flexibility of α-CyD, thus allowing a NMR-evidenced inclusion of *o*-toluic acid.[172] The predominating lipophilic effects generated by methylation of cyclodextrins is obvious from NMR studies with a series of pheromones.[61]

C.7.b. Cyclodextrin Modifications with Amino Groups for Anion and Metal Binding, Etc.

The introduction of one or several amino groups at the rim of CyDs can provide for increased association with anionic substrates, or for complexation with transition metal ions etc. Positively charged amino groups at the rim of cyclodextrins can lead to *K* values around 10^6 with nucleotides.[173] Chemical shifts as well as NOEs between CyD protons H-3 and H-5 and ribose signals show evidence of intracavity inclusion of the sugar moiety, even stronger for the more hydrophilic ribose in comparison to deoxyribose derivatives.[173] Similar observations are made with metal complexes of complementary charges.[174]

Aminoalkylcyclodextrins allow complexation of lanthanide ions directly, or can be varied to yield EDTA-type metal-binding sites. ^{13}C NMR titrations were carried out to study copper complexation and protonation of 6-diethylenetriamine derivatives of β-CyD.[175] Aminoalkyl substituents also lead to a preferred binding of nitrophenoxides over their conjugated acids which causes a decrease in the pK_A values of nitrophenol. The complex formation is observable by changes in both host and guest NMR signals.[176] Reaction of diethylenetriamine pentaacetic dianhydride with 6-mono- and 2- or 6-CyD-mono(ethylenediamine) give derivatives, which allow evaluation of the geometry of the complex with dysprosium(III) by analysis of pseudocontact shifts.[177] Such complexes are also useful to examine optically active NMR shift reagents for chiral discrimination (see section C.8.b). A CyD complex with an attached bipyridyl unit for binding rhenium [178] is discussed in section C.10.c.

With mono[6-(1-pyridinio)-6-deoxy]-α-cyclodextrin the addition of alkali salts of chaotropic anions such as Br^-, I^-, SCN^-, N_3^-, NO_3^-, and ClO_4^- causes a downfield shift in the signal of the C(5)−H located in the CyD interior, and a marked upfield shift in the signal of one of the C(6)−H's of the unsubstituted glucopyranoses, indicating intracavity inclusion of these anions. Nonchaotropic anions such as F^-, Cl^-, SO_4^{2-}, $H_2PO_4^-$, and HPO_4^{2-} have negligible shift effects.[179,180] *N*-Peralkylaminocyclodextrins with lipophilic side chains aggregate strongly in solution and form micelles. This leads to significant line broadening of the glucose and *N*-alkyl group's signals of the cyclodextrin derivatives in NMR, depending

on the solvent used.[181]

C.7.c. Complexation with Pendant-Modified Cyclodextrins

The spectrum of the pendant-substituted CyD **3** (see section B.5) changes significantly upon addition of 1-adamantanecarboxylic acid at 25 °C. The naphthyl region in 1D NMR spectra shows significant broadening and downfield shifts compared to the spectrum of the host **3** alone, indicating a moderate increase in water exposure of the naphthyl unit. The observed spectral broadening is temperature-dependent, consistent with rather slow equilibration between different conformations on the NMR time scale. Generally, the spectra of the pendant group of a modified CyD in the self-inclusion state are different from those of the pendant group itself. These differences essentially disappear upon addition of a guest, indicating that the pendant group is pushed out of the cavity to the bulk solution by the guest molecule.

The NMR spectra of the CyD parts also change upon addition of a guest. The ^{1}H resonances for H-5 of the glucose residue B, C, and F of *p*-(dimethylamino)benzoyl-modified β-CyD are shielded by around 3.3 ppm compared to the unmodified CyD, due to the benzene ring current effect, and are also broadened.[182] NOEs between the *p*-(dimethylamino)benzoyl residue and the H-3 and H-5 CyD protons are also observed. These facts support that the pendant group is included in the cavity, and that the inside−outside isomerism is not always fast on the NMR time scale. Upon addition of 1-adamantanol, the CyD resonances shift downfield, and new upfield shifted resonances (H-6 of the glucose residue B) appear. The CyD protons exhibit no NOE with the *p*-(dimethylamino) benzoyl residue in the presence of the guest, indicating an outside orientation of this pendant group.

In general, the resonances of the anomeric protons are well dispersed, if self-inclusion of a pendant group occurs. If a globular guest molecule is added and the pendant group moves out of the cavity, this spectral dispersion is largely lost, probably due to a removal of the cavity distortion. Therefore the degree of spectral dispersion for the anomeric protons may be used as another indication of self-inclusion of the pendant. Formation of a complex with an added guest also leads to changes of the rotamer distribution. The coupling constants ($J_{5,6a}$ and $J_{5,6b}$) of glucose units A of *p*-nitrophenol-modified CyDs were analyzed as described for the host molecules alone by the presence of a guest like 1-adamantanol (Table 16).[183] The rotamers gt are the major component in the glucose unit A both of the host alone and in the presence of 1-adamantanol.

Table 16. Calculated Populations *P* (in mole fractions) of Rotamers around the C5−C6 Bond in the Modified Glucose Units, with Observed Coupling Constants ($J_{5,6a}$, $J_{5,6b}$) of *p*-Nitrophenol-Modified β-Cyclodextrin alone, and in the Presence of 1-Adamantanol at 25 °C in D_2O[183]

	P_{gg}	P_{gt}	P_{tg}	$J_{5,6a}$	$J_{5,6b}$
CyD alone	14.4	78.7	6.9	2.5	9.1
CyD + 1-adamantanol	19.0	81.0	~0	1.5	9.3

C.8. Chemistry and Application of Various Cyclodextrin Inclusion Complexes

C.8.a. Inclusion Complexes for Chiral Recognition

Enantioselective recognition by cyclodextrins can be most easily analyzed by observation of distinct signals from nonequivalent nuclei[170,184−191] with the advantage that these shift reagents can be applied in aqueous solution. On the other hand, spin system analysis of CyDs can be helped by the use of traditional shift reagents.[33] Often more effective chiral discrimination is observed with substituted, for instance permethylated CyDs,[192−196]or with monoacetylamino-β-CyDs,[197] which is thus also applicable in less polar media. Addition of lanthanide ions may enhance the observed nonequivalence.[156,192,198] With an anhydro cyclomaltoheptaose, a certain reduction of cyclodextrin symmetry is achieved, leading to large differences of affinities and of NMR shielding effects.[199] Perphenylcarbamate residues at β-CyD also lead to larger shift differences by complexation with drugs such as atenolol.[200.] Chiral benzhydrylamines were found by NOE/ROESY experiments to form 2:1 complexes with inclusion of the phenyl rings into separate β-CyD cavities.[201,202] NMR shows the dinitrophenyl ring of racemic peptide derivatives to be inserted into CyD cavities as major driving force, with secondary interactions between the amino acid alkyl residues and the CyD rim providing the stereoselection.[192,203] NMR detection of chiral discrimination, often in combination with chromatographic separations, were described for various phenethylamines.[204]

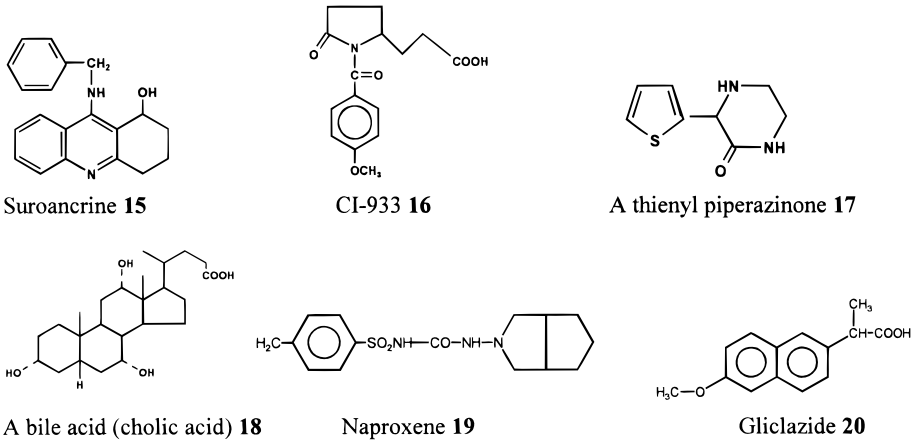
Some muscarinic antagonists, owing their chirality to atrop- or biphenyl type of isomerism also show appreciable shift differences with cyclodextrins.[190] With chiral dinaphthylmethanes, ^{1}H NMR shift changes particularly with the wide enough γ-CyD cavity show enantioselectivity and insertion of one naphthyl unit inside the cavity, with the other one located at the narrow rim of the primary hydroxyl groups.[202] The analysis of chiral binaphthyls show that the more flexible methylated β-CyD leads to higher enantioselectivity than that of the more rigid unsubstituted β-CyD.[202]

Cyclodextrins induce chiral discrimination with many pharmaceutically interesting molecules (see below), like propanolol hydrochloride.[184] Antihistaminic and analgetic agents have been analyzed by NMR spectroscopy; in addition to the determination of optical purity, the results can contribute to the design of CyD-based chiral phases for chromatographic separations (see section C.8.b).[184,205] Complexation of 1,7-dioxaspiro[5.5]undecane shows spectacular high yields in solid-state separations, but no detectable preferential binding in racemic solution due to the—as often—too fast complex dissociation on the NMR time scale.[206]

C.8.b. NMR Analyses for the Design of Chiral Phases for Chromatography and of Sensors

NMR studies in solution can be of substantial help for the design for chromatographic separations, which is one of the most important CyD applications also

Chart 1



in industry.[207−212] Dinitrophenyl amino acids not only show enantioselectivity in their binding constants, but for instance, for the D configurations, usually a stronger ^{1}H NMR shift change at the CyD intracavity H-5 proton.[201] In line with circular dichroism data this indicates that the L isomers are characterized by a shallower insertion, leading also to 2:1 complexes with a second CyD. A NMR and GC investigation with 18 chiral fluoroether anesthetics yielded a rough correlation between chromatographic separation factors and diastereotopic shielding differences.[213,214] ^{13}C NMR shifts show, with some antidepressant azepine-type drugs, larger shift differences for chiral discrimination than proton shifts.[215] The selectivity for ephedrine derivatives with chiral electrodes based on cyclodextrins substituted with long chains was compared to the corresponding NMR shielding differences.[216] Best enantioselectivity factors for O-alkylated β-cyclodextrins as stationary phase were found in the case in which the internal mobility of these derivatives studied by ^{13}C relaxation times analysis and NOE determinations reaches its maximum.[46] Similar conclusions emerge from ^{13}C−CP MAS studies of immobilized CyD derivatives used for HPLC separations.[217] With anionic CyD derivatives good correlations were found between complexation-induced NMR shift nonequivalence and enantiomeric separations of the anti-schistosomiasis drug oxamniquine by capillary electrophoresis (CE), whereas neutral CyD compounds showed neither shift effects nor enantioseparation.[218] On the other hand, studies with the antihistaminic drug dimethindene and various CyD derivatives did not always show a correlation between CE separation and complexation induced NMR shifts.[219]

C.8.c. Complexation of Drugs and Other Biologically Active Compounds

The ability of CyDs to complex a large variety of biologically active substrates has led early to NMR characterization of complexes for instance with aromatic amino acids;[221,221] they invariably show as expected the aryl part to be inserted in the cavity. The potential use of CyDs for drug protection and delivery has led to a particularly large number of NMR studies in this field. NOE and computer-aided molecular modeling was used to characterize the inclusion of many pharmaceutically important compounds[222−231] (Chart 1) such as thymol or carvacrol,[222] naproxen **19**,[226,232] salbutamol,[233] cephalosporin,[234] nifedipin,[235] prostaglandins,[222,228] anthracycline antibiotics,[230,231] or phenylethylamines.[236] The interaction of naproxen **19** with different CyDs was also studied by ^{13}C NMR, indicating formation more than 1:1 complexes. The observed CIS values were found to parallel roughly the shielding difference measured for naproxen between dioxane and aqueous medium, indicating a dioxane-like microenvironment in the cavity.[226] Other studies involved indomethacine,[237] sulfadimethoxine (SDM)[238,239] bile acids such as **18** and its derivatives[224, 240] and CyD complexes with the anti-diabetes drug gliclazide **20**; NOE data indicate that the azabicyclooctyl group of this compound is inside the β-CyD cavity and not the phenyl part.[241] Various histamine derivatives show by NMR inclusion of the imidazol unit in CyD cavities.[242] The antiinflammatory agent indomethacin **21** (see Figure 28) shows interesting conformational changes upon complexation which are discussed under section C.8.f.[243]

The anti-Alzheimer acridine derivative suroancrine **15** has been shown by ROESY to have the benzyl ring inside the β-CyD cavity, with diastereotopic duplication of the benzyl CH_2 signals.[223,232,243,244] Similar enantioselective complexation (see section C.8) was found with other chiral drugs such as the antiamnesic thienylpiperazinone **17**,[245] or the cognitive agent CI-933[246] **16**. These and other more recent studies on drug-CyD interactions demonstrate the significant progress by accompanying molecular mechanics calculations, which at least provide the most likely selection of structures to be then tested against experimental NMR data. The often decisive contributions of solvophobic forces in supramolecular CyD complexes may require water-box MD simulations in order to understand and to sort out, for instance, conflicting NMR results for complexes of β-CyD with acridine derivatives as a function of their substitution.[243,247] Thus, NOE data indicated inclusion only in the presence of a benzyl group in the 9-position of acridines such as **16**, against "common" sense expectations based on space-filling models, but in line with MD simulations in water.[243]

NMR spectroscopy has been previously used to establish inclusion modes, stoichiometries, and association constants of steroids. The NMR data indicate that bile acid inclusion with β-CyD results primarily from interactions with the hydrophilic side chains, whereas those with the wider γ-CyD cavity shows interactions with the polycylic parts.[223,240,248] Similar conclusions emerge from corresponding ^{13}C NMR data.[224,225] Substituents such as the 12-OH group of the steroid skeleton lead to significant changes between the isomers.[223,249] ^1H NMR spectroscopy reveals the competition of binding between bile acids such as **18** and some vitamins in β-CyD.[249] Extensive NMR analyses of flavonols and their glycosides showed the phenol group inside the β-CyD cavity, with hydrogen bonds between the 4-oxo and 5-OH hydrogen to the secondary hydroxyl groups at the wider CyD rim.,[250−252] Cyclodextrin complexes of linoleic and arachidonic acids show by relaxation time and NOE measurements that the carboxyl arms of both acids are inside the cavity, with double bonds partially exposed or buried.[224]

C.8.d. Complexation with Other Organic Guest Compounds

NMR and electrochemical studies of complexes between CyDs and ferrocene derivatives containing alkyl chains suggest that α-CyD interacts with the aliphatic region of the substrates, whereas β-and γ-CyD interact with the ferrocene subunit.[253] Similar inclusion patterns were found with alkyl cyanocobalamines.[254] Tetraaryl porphyrins show insertion of the aryl parts into CyDs,[255−258] however, only with anionic substituents at the aryls, and not with the corresponding cationic ammonium derivatives. Particularly with the more flexible methylated CyD derivatives ^1H NMR shifts and comparison of binding constants suggest a deep insertion of the anionic group into the cavity, with the porphyrin ring interacting with the wider rim of the CyD.[259] The example by Nolte et al.,[258] shown in Figure 26, demonstrates

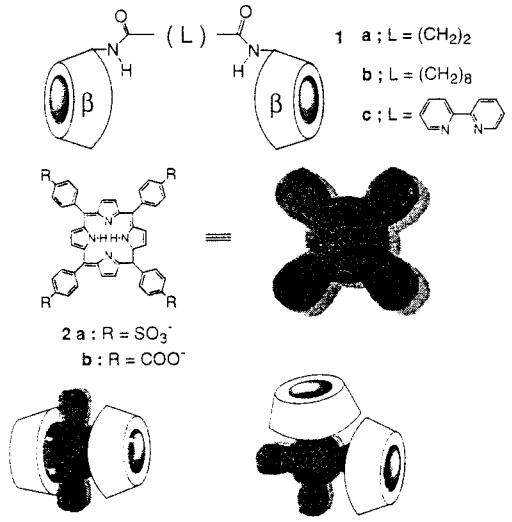


Figure 26. Schematic representation of alternative complex conformations from *meso*-tetraphenylporphyrin and CyD units connected with spacers of different length. (Reprinted with permission from ref 258. Copyright 1996 American Chemical Society.)

how straightforward symmetry arguments can be used to deduce structural alternatives from the observed number of signals. For the anti arrangement one expects two porphyrin signals, for the syn complexation mode four signals. With short spacers between the CyD units, indeed four peaks are visible; with long spacers, two additional signals speak for the presence also of the anti conformation, which is in slow exchange on the NMR time scale. With iron(III) porphyrin CyD complexes the CyD protons show significant broadening which are indicative of selective complexation.[256]

Alkylated phenothiazine−viologen compounds show by ^1H NMR inclusion of the long spacer chain into the CyD cavity.[260] The ^{13}C shifts induced by azodyes[253,255−259] on CyDs were taken as evidence that the dyes protrude from the cavity.[261] The same conclusion was previously reached also on the basis of ^{13}C NMR relaxation times, and broadening or doubling of NMR signals.[261] Alkyl-substituted hydroxyphenylazo derivatives of sulfanilic acid show with α- and β-CyDs in their NMR spectra evidence for multistep inclusion,[262] similar to naphthyl azodyes.[263]

The inclusion of fullerenes C_{60} in γ-CyD in water was recently characterized also by NMR spectroscopy.[264−267] Such lipophilic compounds often require the use of organic solvents, which even can act as cosubstrate. The proton shifts of azulene show with β-CyD in the presence of propanol distinct upfield shifts; these were smaller for those protons assumed to be exposed more to the solvent than to the cavity.[142]

The binding of carbohydrates in CyDs is not only very weak, but difficult to detect by NMR spectroscopy. Ribose in contrast to glucose leads to still weak association, which was reported to be accompanied by small ^{13}C NMR shift changes.[268] With alkyl glycosides, ^1H and ^{13}C NMR shifts as well as relaxation times measured in D_2O indicate inclusion of the alkyl chains and strong demicellation effects.[269] The loose complexes between CyDs and myoinositol phosphate were studied by a combination of ^{31}P and ^1H NMR spectroscopy including NOEs.[270]

Alkanedicarboxylates were on the basis of NOE and shift data reported to form through-ring pseudorotaxane-type complexes with α-CyDs.[271] Similar techniques were applied to CyD complexes with linoic and arachidonic acids.[251] ^1H NMR shifts of a 1:2 complex of 6-bromo-2-naphthol and α-CyD indicate that the first α-CyD accommodates the bromine atom on the naphthalene ring, and the second CyD the hydroxyl group.[272] Flavanole and other polyphenol CyD complexes with association constants of up to about 140 M^{-1} were measured and characterized by ^1H NMR measurements.[273] Self-association by inclusion of a substituent covalently bound to CyDs in the cavity of another CyD derivative has been identified by shielding variations and by intramolecular NOEs with 6-deoxy-6-L-tyrosinylamido-β-CyD, supported by extensive MD simulations in a water box with NOE constraints.[68]

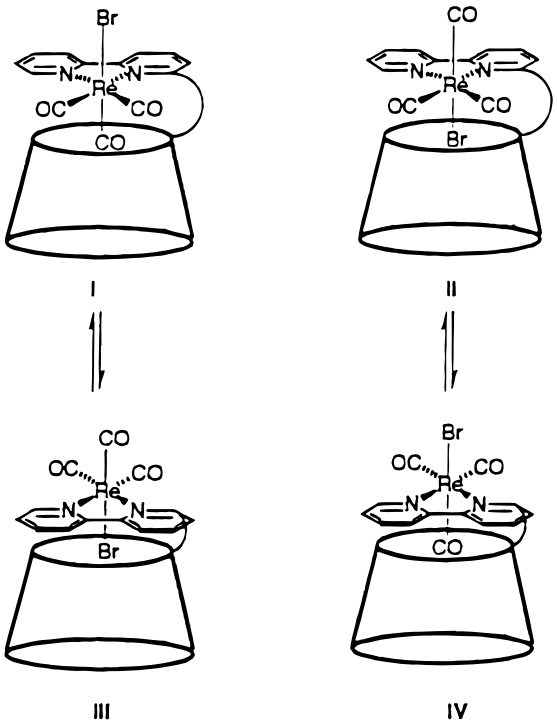


Figure 27. Diastereomeric second shell complexes of rhenium with bipyridylsubstituted CyD. (Reprinted with permission from ref 178. Copyright 1993 The Royal Society of Chemistry.)

C.8.e. Second Shell Complexes/Shift and Relaxation Reagents

Coordination of metals with macrocyclic organic ligands can lead to second shell complexes, in particular with larger hosts such as γ-CyD. With lanthanide ions paramagnetic induced shifts (LIS) and spin−lattice relaxation rate enhancements in the proton resonances were used to define the position of the guest within the host cavity.[274,275] Pseudocontact shifts also allow evaluation of the geometry of these associations.[276] Bimodal inclusion of alkyl side chains of ferrocenyl derivatives was demonstrated by high-field NMR studies.[253]

With copper(II) one can observe complexes formed directly with CyDs by partial deprotonation of the cycloamylose and then use spin−lattice relaxation time (T_1) measurements to localize the copper(II) ion, which seems to be present almost in the center of the CyD cavity.[277] The addition of CyDs to gadolinium complexes used in NMR tomography has a substantial effect on the relaxation times.[274]

A recent paper illustrates the power of modern 2D methods for structural elucidation of highly asymmetrical metal−CyD complexes. Deschenaux et al.[178] have attached a bipyridyl unit to permethyl-β-CyD which serves to bind rhenium, and thus allows for the study electron transfer in the resulting luminescent complex. The ^{1}H NMR signals of these complexes were assigned using at 600 MHz mainly HOHAHA as well as ROESY experiments; in combination with circular dichroism measurements two conformations of the two possible diastereoisomers were made evident (Figure 27).

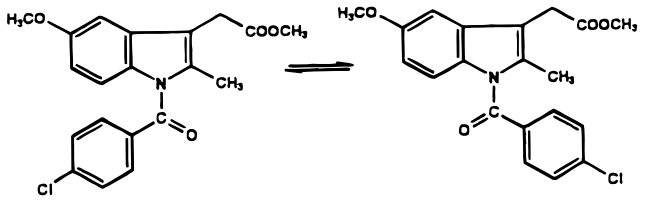


Figure 28. *E*/*Z* equilibrium in indomethacine **21**.

C.8.f. Inclusion Effects on Conformations and on Isomer Distribution of Guest Molecules

Encapsulation of flexible substrates in CyD cavities can have sizable influence on the conformational or other equilibria of the guest compound. The antiinflammatory agent indomethacin **21** occurs in an *E*/*Z* mixture of amide-type stereoisomers (Figure 28) which interconvert rapidly on the NMR time scale. NOE experiments in line with molecular mechanics calculations indicate predominantly a *Z* (cis) configuration upon complexation.[143]

Similarly, preferential binding of the cis isomer was observed with the peptide phenylalanylproline as guest molecule by NMR shift and by diffusion ordered NMR spectroscopy (DOSY).[278] Related changes toward more stable CyD complexes with cisoid amide bonds were observed with L-phenyl-D-proline; these findings were found to be in line with molecular mechanics calculations.[279] The chair−chair interconversion of cis-decaline was found to be strongly affected by complexation in β-CyD.[154] The preference binding of one of the decaline enantiomers by 1.7 kJ/mol established by ^{13}C NMR measurement is in agreement with force field prediction.[280]

Tautomeric equilibria between e.g. the lactim and lactam forms of 3-hydroxypyridine has been found to be markedly affected by binding to β-CyD, with a preferential inclusion of the former, less polar tautomer in the cavity.[281] NMR supports the finding that only the large cavity of γ-CyD shifts the monomer−dimer equilibrium of the tetracyanoquinodimethane anion radicals ($TCNQ^-$).[282]

C.8.g. Interactions with Biopolymers

NMR spectroscopy is increasingly used to elucidate interactions of cyclodextrins with biopolymers. ^{1}H and ^{31}P NMR spectra show for phospholipids with different headgroups, such as for phosphatidylcholine and phosphatidylinositol, different association behavior with α-CyD,[283,284] a process also believed to be involved in CyD-induced hemolysis.[283]

Amphiphilic transporters to be included in organized phases such as micelles or liposomes can be obtained by grafting long chain carboxylic acids onto a modified cyclodextrin.[285] The observed chain-length dependence of the NMR spectra was explained by possible formation of autoinclusion complexes. Water mobility in hydrogels from oxidized CyDs by coupling these via the corresponding aldehyde reactions to chitosan (Figure 29) was studied by measuring longitudinal and transverse proton relaxation.[286] NMR spectroscopy is beginning to be used also for the characterizing of CyD interactions with proteins, such as with the amylose-degrading enzymes.[287] ^{1}H

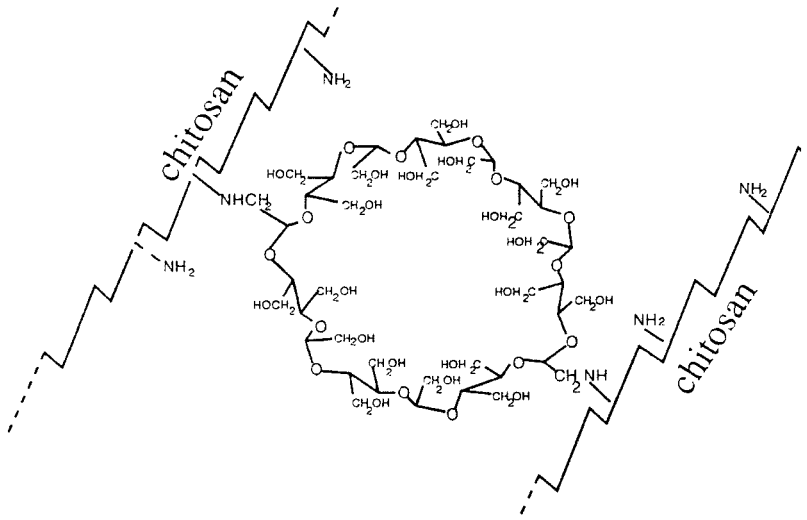


Figure 29. Hydrogel from chitosan and oxidized CyDs. (Reprinted with permission from ref 286. Copyright 1997 Elsevier Science Ltd.)

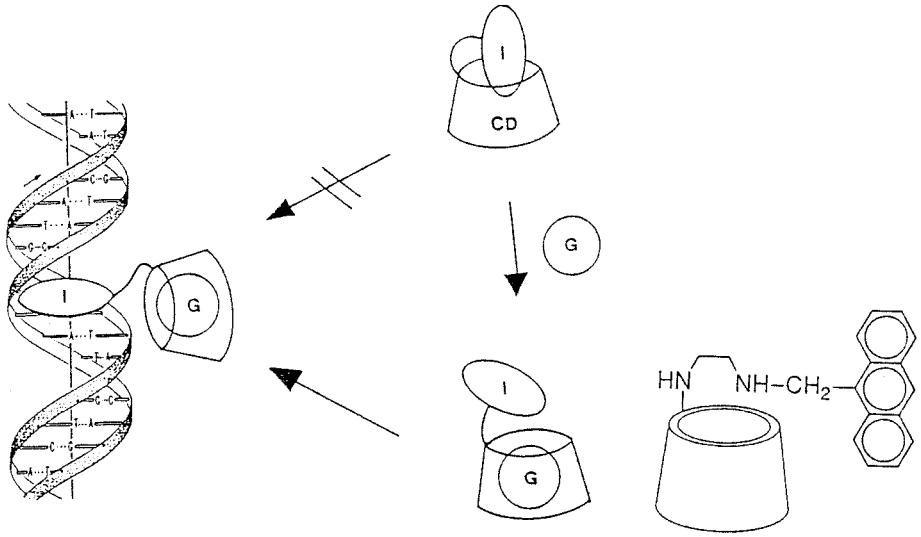


Figure 30. Allosteric interaction between double-stranded DNA, an anthryl unit I attached to β-CyD, and adamantanol as guest G. (Reprinted with permission from ref 289. Copyright 1995 American Chemical Society.)

NMR also gives evidence of the inclusion of lipophilic insulin side chains into cyclodextrins.[288]

A cyclodextrin equipped with an anthryl unit **I** via an ethylenediamine spacer shows binding to double-stranded DNA, if self-inclusion of the aromatic unit into the CyD cavity is relieved by addition of a typical CyD complexer G such as adamantanol (Figure 30).[289] Distinct upfield shifts by the anisotropy effects of the nucleobases and line broadening of the anthryl unit signals demonstrate how the CyD derivative binds by intercalation of the aryl part to DNA, thus providing a chemically switched allosteric system for interactions with double-stranded nucleic acids.

C.8.h. Interactions with Synthetic Polymers and Surfactants; CyD-Based Rotaxanes

The structure and NMR spectrum of a typical CyD-based rotaxane is shown in Figure 31. The spectrum shows that the symmetry of the cycloamylose is retained, whereas the mirror symmetry of the "dumbbell" guest is not. The large shielding difference particularly between the benzylic γ-protons indicates that the movement of the guest is remarkably slow on the NMR time scale.[6,290]

NMR spectroscopy provides an ideal technique to follow rotaxane formation with cyclodextrins.[291,292] ^{13}C relaxation measurements with polyrotaxanes formed from poly (ethylene glycol) and lipophilic CyD derivatives show molecular tubes with particularly fast reorientational motion.[293] The CyD inclusion of long-chain alkanethiols, used in sensor technology for immobilization on gold surfaces, was also characterized by NMR spectroscopy.[294]

With α-CyD the compound **22** as a monomeric model for the so-called ionenes **23** shows a very slow association rate with $k = 0.036\ s^{-1}\ M^{-1}$ (activation barrier 63 kJ/mol), which could be followed by measuring the intensity changes from signals of complexed and uncomplexed material.[295] The slow

$Me_3N^+-(CH_2)_{10}-{}^+NMe_3$

22

$[-{}^+NMe_2(CH_2)_{10}-{}^+NMe_2(CH_2)_{10}-]_n$

23

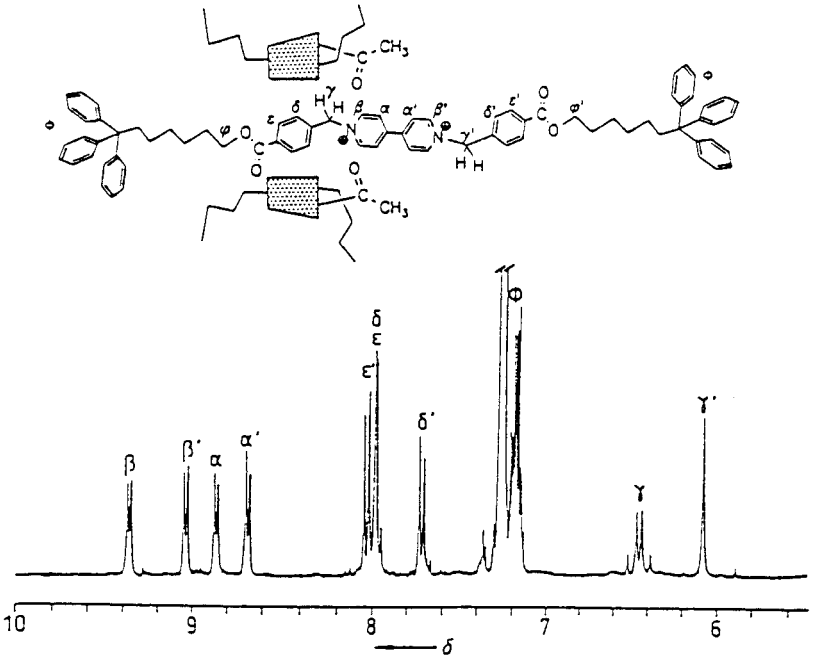


Figure 31. Structure and ^{1}H NMR spectrum of a rotaxane from a substituted β-CyD and a long-chain methyl viologen derivative.[290] Low-field signals are assigned to the complexed site. (Reprinted with permission from ref 290. Copyright 1992 Wiley-VCH Verlag GmbH.)

complexation, which is due to the steric hindrance with the terminal NMe_3 groups, takes days, months, or even years, depending on the temperature used, with the corresponding polymers **23**. This can be rationalized with slow consecutive hopping processes from one alkyl part to the next. Similar measurements including line-shape analyses for the dynamics and NOE for the structure elucidation were reported for carbazole−viologen compounds linked by spacers with different chain length.[159] Binding conditions of various technically important surfactants to CyDs were also followed by NMR spectroscopy.[296,297] For a long-chain surfactant, NOE measurements indicate that complexation with β-CyD can lead to a change in micelle formation.[298,299]

D. Nuclei Other than ^{1}H and ^{13}C

Nuclei other than ^{1}H and ^{13}C have been used if occurrence of these in cyclodextrin derivatives and/or the substrates allows this.[283] Interaction of different phospholipids, such as phoshatidylinositol with cyclodextrins were studied by ^{2}H and by ^{31}P NMR spectroscopy.[270,283] Cyclodextrins were reacted with 1,3,2-dioxaphospholanyl chloride in order to analyze ^{31}P NMR spectra of the derivatives, giving well-resolved signals in the range 138−132 ppm.[300] Per-substitution of CyDs by nitro groups allowed measurement of corresponding ^{15}N NMR spectra.[301] ^{19}F NMR has been applied to complexes with fluoro-containing polymers,[302] with fluorinated tensides,[302,303] and with fluorinated amino acid derivatives.[304] Xenon inclusion into α-CyD was studied by NOE and by measuring ^{129}Xe shifts.[305,306] The ^{129}Xe shifts were reported to show a linear correlation between the radius of the free space available to the Xe atom also in solid CyD complexes.[307]

Deuterated cyclodextrin and *N*-acetyl-L-tryptophan as guest was used with a combination of suitable pulse sequences to analyze via deuterium NMR spectroscopy the structure and the degree of dynamic coupling between host and guest.[308] The deuterium relaxation data indicate that the mobility of both the CyD host and the tryptophan guest remain essentially unaffected by complexation. The latter finding is in line with an only very weak dynamic coupling between host and guest, and is at variance with some early conclusions.[309]

The line-broadening of ^{81}Br NMR signals in the presence of CyDs was used to evaluate the binding abilities toward a series of inorganic anions, as discussed already in section C.6.[162] ^{15}N NMR spectroscopy gives also structural information about amino acids in their cyclodextrins complexes in aqueous solutions.[221]

E. Solid-State NMR

Solid-state ^{13}C cross polarization magic angle spinning NMR measurements also allow binding modes also in crystalline complexes to be identified.[310−312] The subject has aptly been reviewed by Ripmeester et al., [310] which allows us to restrict ourselves here to few typical examples. With solids from β-CyD and acetophenone, one observes for some carbon atoms two carbon signals instead of one. One of these peaks disappears when the temperature is increased, whereas the unshifted peak remains; this indicates a complex with the substrate alternatively inside or outside the cavity.[313] Deuterium exchange and deuterium NMR quadrupole splitting has been used to measure orientation and the influence of external fields in CyD complexes with azodyes.[314] For *p*-nitrophenol complexes with α-CyD an activation energy of 50 kJ/mol was obtained for the motion of the guest in the cavity from temperature-dependent ^{13}C NMR line widths; this motion was confirmed by

^{2}H NMR spectroscopy to be a 2-fold flip of the *p*-nitrophenol molecule about the C-1−C-4 axis.[315]

^{13}C NMR shifts in solid glucose polymers provided insight into glycosidic conformations of the CyDs.[316] Solid-state ^{13}C NMR spectroscopy was also used to follow the dehydration of β-cyclodextrin hydrate.[311,317,318] Comparison of C-1 and C-4 shifts in solution and in crystals of cyclodextrin hydrates were correlated with the conformation about the 1.4-linkage, whereas the C-6 resonances also seem to be sensitive to hydrogen-bonding interactions.[317] ^{2}H NMR spectra and spin-relaxation data of perdeuterated β-CyD hydrate suggest that the macrocycles do not move as a rigid entity.[311]

The effects of benzaldehyde complexation with cyclodextrins on the mobility of the guest were analyzed in terms of nuclear relaxation parameters such as ^{13}C spin−lattice relaxation, ^{1}H spin−lattice relaxation in the rotating frame, and cross polarization transfer (TCH). It was proposed that the large variations observed in the TCH values of the guest in three complexes may be interpreted in terms of motions with correlation times in the 0.1−0.5 ms range.[319] Ferrocene enclathrated in β- and γ-CyD was measured by one-dimensional switching-angle sample spinning NMR spectroscopy, giving data on the dynamics of rotation of the ferrocene inside the CyD cavity.[320,321] Several internal motion processes were identified in solid cyclodextrin inclusion complexes with ferrocenes and ruthenocenes.[322,323]

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