NMR analyses of cyclodextrin complexes with substituted benzoic acids and benzoate anions

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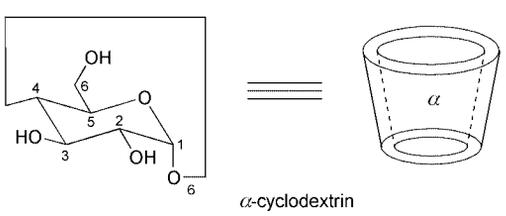
Inclusion modes of α-cyclodextrin with *para*-substituted benzoic acids (X = H, OH, CH_3, CN, NO_2) and the corresponding anions are analyzed with NOEs from ROESY experiments and with ^{1}H NMR shielding variations. The data indicate a predominant deep inclusion of the carboxy group in the complexes with all substituted benzoic acids, irrespective of the nature of the substituents. Protons in *ortho*- and *meta*-positions to the carboxylic group show complexation shifts larger than medium-induced shifts observed for any solvent. Complexation-induced ^{13}C NMR shift (CIS) changes of the phenyl derivatives are also measured, with partial correction of literature signal assignments. Measurements of the same derivatives in several aprotic solvents reveal no correlation with complexation induced shifts, showing that polarity effects resulting from the medium cannot be the major source of the observed CIS values.

Introduction

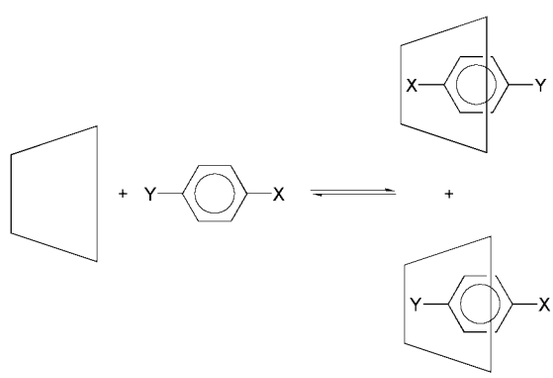
Cyclodextrin (CD, Scheme 1) complexes belong to the most



Scheme 1

often used and widely studied supramolecular systems.[1] Their structural characterization is an essential prerequisite for the development of further applications, and for the understanding of the underlying non-covalent interactions, including their use as enzyme models. Crystal structure analyses have greatly contributed to the elucidation of the basic CD conformation and hydrogen bond networks. Solid state structures are, however, of limited value for the analysis of supramolecular CD complexes, which are most often used in solution, since solvent effects greatly contribute to complex formation, which is moreover often characterized by a multitude of possible conformations. For these reasons NMR-spectroscopy has always played a major role in the analysis of CD complexes,[2] although the complexity of the underlying spin systems posed many problems before the advent of powerful new techniques like 2D NMR and spin lock NOE experiments.[3] The present paper addresses open and until now controversial questions about inclusion modes of benzoic acid derivatives in α-CD. For this we use not only NOE measurements, but also arguments based on the observed shielding changes. In this context we also analyze the until now mostly overlooked shielding effects of the cyclodextrin host on the encapsulated guest compound, and compare also complexation induced ^{13}C NMR shifts with the possibly related medium-induced shifts.

Since the pioneering work of Bergeron and co-workers[2a,b] on the inclusion of benzoic acid in CDs it is known that the carboxylic group binds well within α-CDs, especially in its non-ionized form. However, conflicting views are expressed in the literature regarding the regioselectivity of inclusion of benzoic acids with electron withdrawing substituents such as nitro or cyano groups. Based on the comparison of affinities measured for 1,4-disubstituted benzenes with the corresponding benzoic acids Connors *et al.*[4] concluded that these compounds form two different complexes (Scheme 2), with a clear preference for the



Scheme 2

one with the COOH group inside the cavity. Davies and Savage[5] proposed that the substituent with the larger Hammett σ-value should be located at the narrower end (or on the inside) of the cyclodextrin cavity because of the favorable dipole–dipole interaction energy. Recent studies[6] on the prediction of association constants using artificial neural networks show a better correlation with hydrophobic binding increments and molar refraction indices than with Hammett constants, with a preference for the carboxy group inside the cavity.

Ionization of the carboxylic group leads to much weaker binding. Smaller association constants have been measured and more random association is assumed.[2,7] However, no experimental evidence for the structure of these complexes in solution has been presented[2b] so far with the exception of NMR spectra of the α-cyclodextrin–sodium benzoate complex, however this was only at a low magnetic field.[2]

In the present work we studied several α-cyclodextrin complexes with 4-substituted benzoic acids in the two ionization

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forms. We tried to find unambiguous evidence for the regioselectivity and depth of inclusion, mainly on the basis of intermolecular NOE data. The observed proton complexation-induced NMR shifts are also used to deduce the complex geometries. Medium-induced NMR shifts for both proton and carbon-13 nuclei were also measured in order to shed light on the origin of the complexation-induced NMR shifts of the immersed substrates.

Experimental

Substituted benzene derivatives (reagent grade quality) as well as deuterated solvents were purchased from Aldrich and used without further purification. α-Cyclodextrin (Fluka) was dried prior to use overnight in a drying pistol over phosphorus pentoxide at 90 °C.

NMR spectra were recorded on Bruker AM 400 and on AVANCE 500 NMR spectrometers, operating at 400.1 (500.1) MHz for protons and 100.0 (125.0) MHz for carbon-13. A 5 mm dual (inverse) ^{1}H–^{13}C probehead was used at room temperature 300 ± 1 K. The solvent dependence studies (Tables 4 and 5) were conducted unless otherwise stated at substrate concentration of 5×10^{-3} M for protons and at 5×10^{-2} M for C-13. The pH was adjusted to 1.5 and 7.0, respectively, for the acid and anion series. Proton chemical shifts were measured with external reference acetone-d_6 and then corrected for the differences in the bulk susceptibilities of the solvents. Correction factors were determined from the differences in the measured chemical shifts of internal (2.00 ppm) and external acetone-d_6 in these solvents. They also include some terms for, for example, van der Waals interaction in the liquid state. The following experimentally determined correction factors were used: for $CDCl_3$ 0.87 ppm (theoretical value calculated from the bulk susceptibilities 1.17); for CCl_4 0.79 ppm (0.97); for D_2O 0.83 ppm (1.08); for DMSO-d_6 0.43; for dioxane-d_8 0.53; for CD_3OD 0.19 (0.29). No correction of the carbon-13 NMR chemical shift values was made.

Proton complexation-induced shifts (CIS) values were calculated from two or three experiments with different substrate : ring ratios using known K_A-values from the literature.[8] Agreement between the thus determined CIS values was usually within 0.02 ppm, leading to an estimated accuracy of ±0.02 ppm. Carbon-13 CIS values were taken from ref. 9, but reassigned (see below).

2D ROESY and T-ROESY spectra were measured on samples without degassing at quite different concentrations in D_2O (see below); usually small differences between the relative cross peak intensities were found. For maximizing the intensity of the intermolecular cross peaks concentrations corresponding to a 40 and 60% complexation degree of the host molecule were used. Concentrations were also adjusted to give the best possible separation of the signals in order to minimize second-order effects and prevent high TOCSY transfer peak intensities. Typical concentration ranges used in the acid series were *e.g.* 5×10^{-2} M *p*-nitrobenzoic acid with 3×10^{-2} M cyclodextrin and 2×10^{-1} M sodium benzoate with 5×10^{-2} M cyclodextrin for the anion inclusion experiments. T-ROESY experiments give fewer TOCSY transfer peaks, allow longer spin-locking time and thus higher sensitivity.

The 2D ROESY spectra were measured using the pulse sequence proposed by Griesinger and Enst;[10] as spin-locking field a DANTE[11] pulse sequence consisting of 4000 pulses of 12 μs (60°) with a delay of 63 μs on the transmitter channel was applied. The 2D T-ROESY[12] spectra used for spin locking a sequence of 2000 180° *x* 180° −*x* pulses, each with duration of 200 μs. Typical measuring conditions were: sweep width 5000(2500) Hz; data size 2K/1K in the $\omega 2/\omega 1$ direction, $\pi/3$ shifted squared sine bell windows in both directions; delay between the scans 2 s, 8 to 64 scans, depending on concentration. Phase sensitive spectra were acquired using the TPPI method.

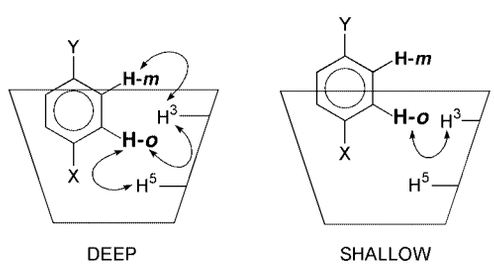
Unambiguous signal assignment in these highly coupled multispin systems within a narrow shift range is not trivial. In the complex with *p*-nitrobenzoic acid assignment of the two aromatic protons was done on the basis of a GHMBC[13] experiment taking into account the well known difference between the geminal and vicinal CH coupling constants ($^{3}J(CH) \gg {}^{2}J(CH)$) in aromatic systems and using the large difference between the chemical shifts of the C-*ipso* and C-*para* atoms. Assignment of the overlapping cyclodextrin protons in some of the complexes was made by GHSQC experiments.[13]

Results and discussion

NOE data

The most informative method for determination of conformations in solution is the measurement of NOEs.[3,14] Typically for host–guest complexes, the spin-lock ROESY version is applied, due to the favorable molecular correlation times in the rotating frame. With ROESY cancellation of distance dependent cross peaks is prevented and ambiguities arising from spin diffusion can be avoided. Problems arising from off-resonance dependence, TOCSY transfer *etc*. and for intermolecular interaction, especially from different correlation times of the host and guest molecules, limit the quantitative use of cross peak intensities for distance information; usually the observed through space connectivities are taken as distance constraints. With cyclodextrin complexes quantitative evaluations are furthermore limited by the fast exchange between the glucose signals within the symmetrical macrocycle, and the very different correlation times of host and guest, posing severe problems for a calibration between NOE intensities and distances. The different correlation times became obvious from the fact that in NOESY spectra the aryl protons showed positive, and the cyclodextrin signals negative cross peaks.

For a benzene derivative, included in the α-cyclodextrin molecule, cross peaks with the H-3 and H-5 protons located deep in the cavity will be observed. Discrimination between deep and shallow inclusion is possible on the basis of the presence or absence of interaction of the benzene protons with the H-5 protons located deep in the cavity (see Scheme 3).



Scheme 3

For *para*-disubstituted benzene derivatives, which often occur *e.g.* in drug molecules,[15–18] two conformers with opposite regioselectivity are possible, usually denoted as 'head-on' or 'tail-on' inclusion. Thus, in principle four different limiting inclusion arrangements are possible. In view of the fast exchange between the free and the complex state, with more than one possible geometry, one can on the basis of the ROESY results firmly discriminate between in- and outside cavity inclusion, in the former case between a particular predominant one (see above), and a random-type inclusion.

Whereas it is generally accepted that phenols and anilines as well as their ionized forms adopt deep inclusion in the cavity with one predominant orientation of the hydroxy or amino group protruding from the secondary rim of the cavity, for

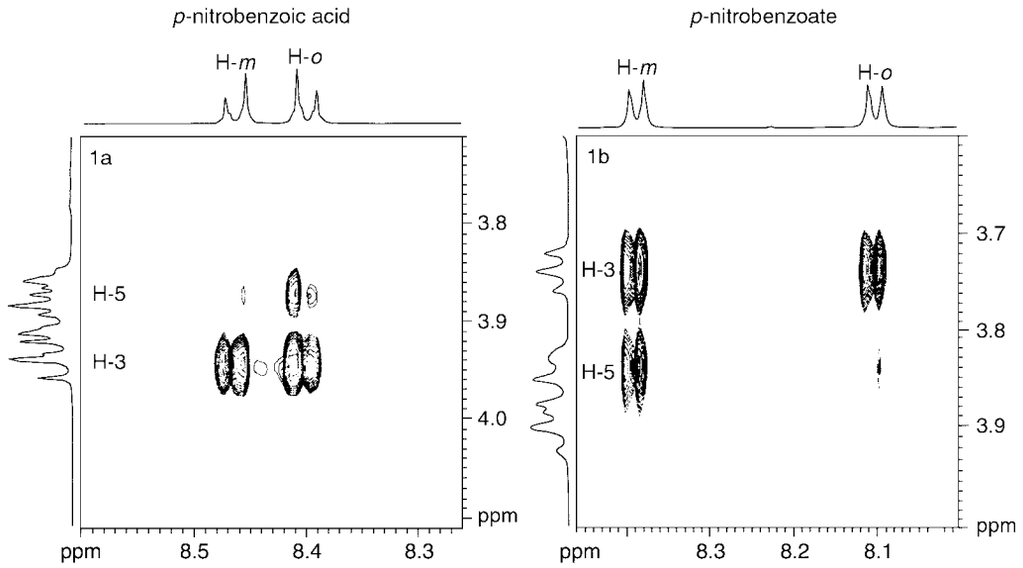


Fig. 1 T-ROESY spectra for a) *p*-nitrobenzoic acid and b) *p*-nitrobenzoate.

Table 1 Observed intermolecular cross peaks in the 2D-ROESY spectra of complexes of X,Y-disubstituted benzenes with the protons located inside the α-cyclodextrin cavity[ab]

K_A/M^{-1}	X	Y	H-3	H-5
33	CH_2COOH[c]	H	—	—
	C_6H_5		+	—
720	COOH	H		
	H-ortho		++	+
	H-meta		+	—
	H-para		+	
1100	COOH	CH_3	—	—
	H-ortho		+	+
	H-meta		+	
1130	COOH	OH	—	—
	H-ortho		+	+
	H-meta		+	
440	COOH	CN		
	H-ortho		++	+
	H-meta		++	
350	COOH	NO_2		
	H-ortho		++	+
	H-meta		++	
13	COO^-	H	++	+
	H-ortho		++	+
	H-meta		++	+
	H-para		+	
6	COO^-	CH_3	++	+
	H-ortho		+	+
	H-meta		++	+
16	COO^-	OH		
	H-ortho		++	+
	H-meta		++	+
79	COO^-	CN		
	H-ortho		++	+
	H-meta		+	+
74	COO^-	NO_2		
	H-ortho		+	
	H-meta		++	++

[a] Relative intensity of the peaks: ++ = strong, + = weak, — = no signal. [b] For typical concentration ranges see the Experimental section. [c] Protons in the substrate corresponding to the cross peaks are given in italics.

substituted benzoic acids and their anions more random orientations have been reported.[19]

The ROESY results (Table 1) show in the case of phenylacetic acid only an interaction of the aromatic protons with the H-3 protons close to the secondary rim, indicating a shallow penetration of the phenyl ring.[20] In contrast, for all benzoic acids a typical pattern of a deep penetration is observed (see *e.g.* Fig. 1a depicting the intermolecular cross peaks for the α-cyclodextrin–*p*-nitrobenzoic acid complex). Both aromatic protons lie near to the H-3 proton as evidenced by strong cross peaks in the ROESY spectrum, whereas an interaction *only* of the protons in the *ortho*-position to the carboxylic group with the H-5 protons of the cavity is observed. This presents clear evidence for the predominant deep inclusion of the carboxy group in all substituted benzoic acids, *irrespective* of the substituent nature. The presence of small amounts of the isomeric 1 : 1 complexes with opposite orientation of the benzoic acid as well as of 1 : 2 complexes as calculated by Connors *et al.*[4] for the nitro- and cyano-substituted acids cannot be excluded since the corresponding cross peaks may not have large enough intensities and will be buried in the noise. Some differences in the relative intensities of the different complexes do exist (Table 1), but a clear-cut pattern of three cross peaks with a signal between H-*meta* and H-5 but not between H-*ortho* and H-5 is observed.

Ionization of the carboxylic group not only weakens the affinity to CD,[4] but also leads to a change of inclusion mode. Preference for the conformation with the carboxylate group outside the cavity cannot be excluded, but comparably strong interactions of the aromatic protons in the *meta* and *ortho* position to the carboxylate group with the H-5 cyclodextrin protons are observed, indicating no preferential binding mode for the anions. Only in the case of the *p*-nitrobenzoate complex is a strong preference for the nitro-group inclusion inside the cavity found, which can be seen from the corresponding T-ROESY spectra in Fig. 1b. This observation strongly suggests that the observed cross peaks in the anion series reflect random inclusion, and are not due to saturation transfer phenomena.[21]

Proton CIS values

The complex geometries deduced from the ROESY spectra are corroborated from the measured complexation-induced chemical shifts for both the cyclodextrin and the guest molecules (Tables 2 and 3). Upon inclusion of aromatic substrates in the cyclodextrin cavity, there are shift changes for many of the cyclodextrin protons. Typically the most sizeable upfield shifts for both benzoic acid and benzoate are observed for the H-3 protons, which are located in the shielding area of the aromatic ring of the guest molecule.[2d,3,22] In contrast, the H-5 protons are more deshielded in the acids than in the anions, although quite a scattering of the values is observed, likely due to their position in the vicinity of the shielding cone edges where

Table 2 Complexation-induced ^{1}H NMR shifts (CIS values) on the α-cyclodextrin protons in complexes with 4-substituted benzoic acid[a]

K_A/M^{-1}	X	Y	H-1	H-2	H-3	H-4	H-5	H-6
720	COOH	H	−0.06	−0.08	−0.45	−0.01	0.17	0.02/−0.05
1100	COOH	CH_3	−0.04	−0.06	−0.29	0.00	0.12	0.02/−0.04
1130	COOH	OH	−0.06	−0.06	−0.39	0.01	0.16	0.01/−0.04
440	COOH	CN	−0.03	−0.05	−0.40	0.02	0.10	0.04/−0.02
350	COOH	NO_2	−0.04	−0.04	−0.13	−0.02	0.00	0.06/0.0
13	COO^-	H	−0.04	−0.07	−0.18	−0.05	0.04	0.0/0.0
6	COO^-	CH_3	−0.05	−0.09	−0.32	−0.05	0.03	0.01/0.03
16	COO^-	OH	−0.07	−0.09	−0.32	−0.05	0.18	0.01/0.03
79	COO^-	CN	−0.01	−0.06	−0.33	0.03	0.11	0.02/0.07
74	COO^-	NO_2	−0.07	−0.10	−0.36	−0.04	−0.03	−0.01/−0.03

[a] CIS values calculated from two or three experiments with different substrate/ring on the basis of the known association constants.[8] All values in ppm, estimated error ±0.02 ppm.

Table 3 Complexation-induced ^{1}H NMR shifts (CIS values) on the aromatic protons in 4-substituted benzoic acid in complexes with α-cyclodextrin[a]

K_A/M^{-1}	X	Y	H-*ortho*	H-*meta*	H-other
33	CH_2COOH	H	0.15	0.11	0.11 (H-*para*); 0.12 (CH_2)
720	COOH	H	0.38	0.14	0.19 (H-*para*)
1100	COOH	CH_3	0.42	0.14	0.12 (CH_3)
1130	COOH	OH	0.30	0.09	
440	COOH	CN	0.35	0.23	
350	COOH	NO_2	0.40	0.24	
13	COO^-	H	0.22	0.11	
6	COO^-	CH_3	0.25	0.19	0.10 (CH_3)
16	COO^-	OH	0.38	0.17	
79	COO^-	CN	0.29	0.16	
74	COO^-	NO_2	0.26	0.25	

[a] See footnote to Table 2.

Table 4 ^{1}H NMR chemical shifts of benzoic acid in different solvents at 1×10^{-3} M concentration

Solvent	ε_r	H-*ortho*	MIS ($\delta_S - \delta_{H_2O}$)	H-*meta*	MIS ($\delta_S - \delta_{H_2O}$)	H-*para*	MIS ($\delta_S - \delta_{H_2O}$)	Δ (H-*o* − H-*m*)
CCl_4	2	7.99	0.15	7.35	0	7.46	−0.03	0.64
[a]		7.95	0.11	7.34	−0.01	7.45	−0.04	0.61
$CDCl_3$	5	7.93	0.09	7.29	−0.06	7.43	−0.06	0.64
[a]		7.92	0.08	7.31	−0.04	7.44	−0.05	0.61
Dioxane	10	8.00	0.16	7.44	0.09	7.55	0.06	0.56
Acetone-d_6	21	7.99	0.15	7.46	0.11	7.58	0.09	0.53
Methanol-d_4	33	7.88	0.04	7.33	−0.02	7.45	−0.04	0.55
DMSO-d_6	47	7.96	0.12	7.48	0.13	7.59	0.10	0.48
D_2O	78	7.84		7.35		7.49		
Complex		8.23	0.39	7.49	0.14	7.68	0.19	0.74

[a] Concentration of 1×10^{-5} M.

shielding changes abruptly to deshielding. The observed shifts are consistent with the deep inclusion of both benzoic acids and benzoates. It should be noted that small upfield shifts are measured also for the H-1 and H-2 protons, whereas most H-4 and H-6 nuclei remain unaffected.

Although the purely aliphatic CD cavity lacks any strong shielding tensors acting on entrapped guest molecules, all protons in the studied guest compounds experience sizeable shifts to lower fields upon complexation (Table 3). These shift changes, which have been largely neglected up till now, can also be of practical use for the determination of affinities by NMR shift titration. They are, however, difficult to rationalize on the basis of known shielding mechanisms.

The shifts on the protons in the *ortho*-position to the carboxylic groups, which are located near the cavity primary rim are larger than of those more distant from the cavity-located *meta*-protons in the guest molecule. Analogous behavior is observed for the benzoate complexes, where the carboxylate group is not the primary binding site. In the *p*-nitrobenzoate complex equal chemical shifts for both the protons located inside the cavity and those near the secondary rim are seen. Additional complexation-induced shifts of *ca*. 0.1 ppm are observed for the aliphatic protons in the complexes, with phenylacetic acid and 4-methylbenzoic acid without a distinct dependence on the position of the proton, either inside or outside the cavity.

It is known that the CD cavity provides a much less polar environment inside than the outside water, which could be one reason for the observed shift changes. We therefore measured the proton chemical shifts of benzoic acid in several solvents. We chose some representative solvents of different polarity, donor and acceptor properties as well as of different ability to form H-bonds. In order to avoid the influence of self-association for the low polarity solvents $CDCl_3$ and CCl_4 concentrations of 1×10^{-5} M have also been measured. As can be seen from Table 4, the complexation-induced shifts are larger than any of the medium-induced shifts (MIS). Some resemblance between the shifts induced by dioxane, acetone and DMSO could be revealed, however, the complexation-induced shifts are twice as large. The induced shifts in the other measured solvents are smaller or of *opposite* direction in comparison to the CIS. Analogously to the CIS no significant dependence

Table 5 ^{1}H NMR chemical shifts of 4-substituted benzoic acids at a concentration of 5×10^{-5} M in various solvents and in the corresponding complexes with α-cyclodextrin, CIS ($\delta_{D_2O(cplx)} - \delta_{D_2O}$)

Y	Protons	$CDCl_3$	CCl_4	CD_3OD	D_2O	D_2O(cplx)	CIS
H	H-*ortho*	7.93	7.99	7.88	7.84	8.23	0.39
	H-*meta*	7.29	7.35	7.33	7.35	7.49	0.14
	H-*para*	7.43	7.46	7.45	7.49	7.68	0.19
CH_3	H-*ortho*	7.82	7.90	7.76	7.72	8.14	0.42
	H-*meta*	7.09	7.14	7.14	7.16	7.30	0.14
	CH_3	2.25	2.37	2.27	2.20	2.32	0.12
OH	H-*ortho*	7.78	[a]	7.74	7.75	8.08	0.30
	H-*meta*	6.67		6.68	6.76	6.85	0.09
CN	H-*ortho*	7.99	8.08	8.03	7.95	8.30	0.35
	H-*meta*	7.58	7.67	7.72	7.70	7.93	0.23

[a] Not soluble.

Table 6 ^{13}C-NMR Shifts of aromatic substrates (X,Y-substituted benzenes) in CD_2Cl_2 and D_2O. Medium induced shifts, MIS ($\delta_{CD_2Cl_2} - \delta_{D_2O}$) and complexation induced shifts (CIS) in α-CD

X	Y	Solvent	C-1	C-2	C-3	C-4	C-5	C-6	C-other
1-COOH	H	CCl_4 [a]	129.51	130.10	127.98	133.13			172.01
		DMSO [a]	130.82	129.28	128.53	132.81			167.38
		CD_2Cl_2 [a]	129.82	130.54	128.98	134.27			172.77
		D_2O [b]	130.91	130.64	129.74	134.79			171.97
		MIS	*−1.09*	*−0.10*	*−0.76*	*−0.52*			*−0.80*
		CIS	*−1.18*	*1.59*	*−0.76*	*0.93*			*−1.70*
1-OH	4-NO_2	CD_2Cl_2	161.90	116.07	126.53	142.18			
		D_2O	163.75	116.77	127.50	141.47			
		MIS	*−1.85*	*−0.70*	*−0.97*	*−0.71*			
		CIS	*1.88*	*0.23*	*0.87*	*−1.21*			
1-COOH	3-NO_2	CD_2Cl_2	131.43	125.46	148.94	128.65	130.24	136.14	169.83
		D_2O [c]	131.48	125.45	149.14	128.67	130.43	136.74	169.32
		MIS	*−0.05*	*0.01*	*−0.20*	*−0.02*	*−0.19*	*−0.60*	*0.51*
		CIS	*−0.49**	*1.70**	*−0.34*	*−0.85**	*−0.97**	*1.40*	*−1.12*
1-OH	3-NO_2	CD_2Cl_2	157.05	110.87	149.78	116.04	130.71	122.33	
		D_2O	157.57	111.29	149.72	116.37	131.49	123.59	
		MIS	*−0.52*	*−0.42*	*0.06*	*−0.33*	*−0.78*	*−1.26*	
		CIS	*1.14*	*0.80*	*−0.71*	*0.18*	*−0.05*	*1.26*	
1-OH	4-CN	CD_2Cl_2	161.22	116.79	134.60	103.53			119.71
		D_2O	161.44	117.39	135.76	102.89			121.59
		MIS	*−0.22*	*−0.60*	*−1.16*	*0.63*			*−1.88*
		CIS	*1.40*	*0.30*	*−0.16*	*−1.66*			*−0.69*
1-OH	3-CN	CD_2Cl_2	157.00	119.13	113.44	124.65	130.97	120.96	119.00
		D_2O	157.07	119.88	112.87	125.54	131.81	122.13	120.61
		MIS	*−0.07*	*−0.75*	*0.57*	*−0.89*	*−0.84*	*−1.17*	*−1.61*
		CIS	*1.22*	*0.21*	*−1.33*	*−0.33*	*0.19*	*1.20*	*−0.27*

[a] Measured with internal TMS as standard, concentration of 5×10^{-2} M. [b] After Gelb *et al.*[9] [c] Reassignment of the values in ref. 9 has been made, some of the values marked with asterisks are still interchangeable.

of the medium-induced shifts on the *para*-substituent in the benzoic acid is visible in the data presented in Table 5.

Carbon CIS values

Complexation-induced ^{13}C NMR shifts of aromatic substrates in α-cyclodextrin have also been used for the analysis of inclusion modes.[23] Komiyama *et al.*[2d,23] proposed a correlation between the penetration depth and the ^{13}C complexation-induced shifts of the proton-bearing carbons. Gelb *et al.*[9] determined shielding by approximately 1 ppm of the included *ipso*-carbons and deshielding by up to 2 ppm for the corresponding *para*-carbons, and explained these opposing shifts with an electric field induced in these aromatic substrates by the CD-cavity. On the other hand, Tabushi and Mizutani,[24] based on early gas-phase force field calculations, dismissed the proposal that the interactions influencing the complexation-induced ^{13}C shifts are mainly of polar nature. Inoue *et al.*[25] introduced a reaction field theory based on semi-empirical MO methods in order to explain the observed complexation-induced ^{13}C shifts.

It should be noted that most of the induced shifts have values lower than 2 ppm (see Table 6), and that the differences between the published data vary by up to 0.65 ppm. The data originate mostly from the older literature and even contain some erroneous assignments, corrected in this work. Thus, it was clear from the ^{13}C spectrum of *m*-nitrobenzoic acid, acquired in CD_2Cl_2, that the assignment in ref. 9 was not correct, since the signal around 125 ppm is definitely not a quaternary one. We assigned the ^{13}C signals using CH-correlation taking into account the characteristic proton pattern; some other assignments in ref. 9 could also be interchanged. We measured the chemical shifts of some representative benzene molecules in CD_2Cl_2, CCl_4, DMSO-d_6 and D_2O with the aim of disentangling the influence of the medium polarity. The difference observed between the shifts in aprotic solvents and D_2O we describe as medium-induced shift (MIS, Table 5). No correlation between the medium-induced and complexation-induced shifts for the compounds studied could be found, indicating that no simple medium polarity change upon complexation is responsible for the observed ^{13}C CIS values.

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