Solution Structure of the Inclusion Complexes between Cyclodextrins and Dialkylamines: An NMR Study

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Interaction between chemically modified cyclodextrins (CDs) and benzyl(*tert*-butyl)amine (**1**), *tert*-butyl(methyl)amine (**2**) and benzyl(methyl)amine (**3**) has been investigated by NMR spectroscopy. The experimental results revealed that the complexation-induced shifts for the reported amines were more pronounced for the carbon atoms than for the protons. These shift variations were successfully exploited for determining the association constants with heptakis(2,6-*O*-dimethyl)-β-CD, which were found to be 295, 119 and 101 M^{-1} for **1**, **2** and **3**, respectively. Features of the geometries of the amine/DM-β-CD complexes were deduced by measuring the NOE intermolecular interactions between host and guest protons. These data made it possible to elucidate fully the nature of the amine/CD complexes and provided information complementary to that previously obtained by EPR spectroscopy on the complexation of the closely related nitroxide/CD paramagnetic complexes.

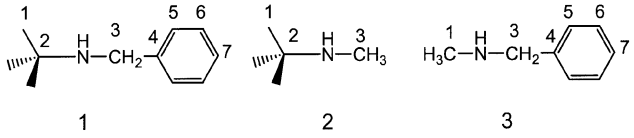
Introduction

The cyclic oligosaccharides known as cyclodextrins (CDs) form complexes with a wide variety of organic molecules, and their properties and applications have been extensively reviewed.[1]

EPR spectroscopy has contributed to the understanding of CDs, by study of CD/radical complexes in aqueous solutions. The majority of these studies have focused on the determination by EPR of the binding constants for inclusion complexes between CDs and persistent radicals such as sterically protected nitroxides.[2,3] In all those cases, however, no more detailed studies on the dynamics of the inclusion process could be performed, because of the similarities of the spectroscopic parameters of the radicals in the two environments.

Some of us have recently reported that benzyl *tert*-butyl nitroxide is a particularly suitable radical probe for studying the dynamics in solution of cyclodextrin (and other complexing agents) inclusion phenomena.[4−6] In this case, radical inclusion manifests in large spectral changes in the EPR spectrum, due both to the decrease in nitrogen hyperfine splitting (a(N)) induced by the less polar environment of the β-CD host cavity, and to the strong reduction in benzylic proton coupling (a(2Hβ)) dictated by the conformational changes occurring upon complexation. The EPR spectra also showed a strong dependence of linewidth on temperature, indicating that the lifetime of the radical in the associated and the free forms is of the order of the EPR timescale. Analysis of the EPR line shape enabled us to measure the rate constants for the association and dissociation processes.

With benzyl *tert*-butyl nitroxide, two modes of accommodation in the larger rim are a priori possible: either from the *tert*-butyl side or from the phenyl side. However, only one species was detected; it was identified as the 1:1 inclusion complex in which the N−O group is quite deeply embedded in the host cavity. This behaviour is different to that found with substituted diphenylmethyl *tert*-butyl nitroxide; here, distinct types of inclusion complexes have been observed.[3] In order to clarify this aspect fully and to obtain a more detailed picture of the geometry of the host-guest complex, we decided to utilise NMR spectroscopy.[7] Since the above nitroxide cannot be studied by NMR, however, we were forced to investigate the interaction with CDs of the corresponding amine − i.e., benzyl(*tert*-butyl)amine (**1**) − and of the structurally related *tert*-butyl(methyl)amine (**2**) and benzyl(methyl)amine (**3**) (Scheme 1).



1 2 3

Scheme 1.

Results

Room temperature ^1H and ^{13}C NMR spectra of amines **1**−**3** (0.01−0.02 M) were recorded in D_2O solutions in the presence of variable amounts ($0-16\times10^{-2}$ M) of two modified β-cyclodextrins: namely heptakis(2,6-*O*-dimethyl)-CD (DM-β-CD) and heptakis(2,3,6-*O*-trimethyl)-CD (TM-β-CD). CD_3OD (10% v/v) was added in each case to improve the amine solubility.

The ^1H NMR (300 MHz) signals of the alkyl protons in amine **1** (10 mM), recorded in the presence of DM-β-CD, showed small differences with respect to those of the free amine (Table 1). In particular, downfield shifts were ob-

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Table 1. Complexation-induced ^{1}H chemical shift differences (ppm) for amines **1**, **2** and **3** in D_2O at 293 K

Amine[a]	Host[b]	H1	H3	H5	H6	H7
1	DM-β-CD	0.013[c]	0.024	−0.052	0.002	−0.052
1	TM-β-CD	0.007	0.008	0.004	0.004	0.004
2	DM-β-CD	0.040	0.052			
3	DM-β-CD	−0.033	[d]	−0.040	−0.040	−0.040

[a] Amine 0.01 M. – [b] Host 0.01 M. – [c] Upfield displacements are negative. – [d] The H3 signal overlaps with those of the CD.

served – of about 0.013 ppm for the *tert*-butyl and 0.024 ppm for the benzyl protons – after treatment with 10 mM DM-β-CD. The aromatic protons, giving rise to an unresolved multiplet centred at 7.37 ppm, showed a different pattern in the presence of DM-β-CD: the *ortho* and *para* hydrogens experienced a shielding of 0.052 ppm while the *meta* hydrogen did not undergo any displacement.[8]

Smaller displacements of proton resonances were observed when the spectrum of amine **1** was recorded in the presence of 0.01 M TM-β-CD; this result was in agreement with the weaker capability of this host for accommodating *tert*-butyl benzyl nitroxide, previously found by EPR spectroscopy.[5]

Complexation of amine **2** (0.01 M) with an equimolar amount of DM-β-CD resulted in downfield shifts of 0.040 ppm and 0.052 ppm for the *tert*-butyl and methyl protons, respectively.

The ^{1}H NMR spectrum of amine **3** in the presence of an equimolar amount of DM-β-CD showed an upfield shift of 0.040 ppm of the aromatic protons and an upfield shift of 0.033 ppm of the methyl protons, similar to what was observed with amine **1**. Evidence for inclusion of amines **1**–**3** in the cyclodextrin cavity is also provided by the observed ^{13}C NMR shift differences, reported in Table 2, for solutions of the same concentrations as used for recording the proton spectra.

The carbons of the *tert*-butyl group in amine **1** experienced a marked deshielding (more than 1 ppm) after complexation by DM-β-CD. The benzyl protons were less sensitive to the presence of cyclodextrin, as indicated by the weak shielding observed for the methylene carbon (−0.13 ppm). The aromatic carbons C5 and C6, possessing the same chemical shift in the spectrum of the free amine, showed separate resonances. With TM-β-CD, the extent of deshielding was markedly reduced (Table 2), similarly to what had been found in the ^{1}H NMR spectrum.

In the case of amine **2**, accommodation in the DM-β-CD cavity also gave rise to displacements of the ^{13}C signal (Table 2), although the shifts were smaller than with **1**. In addition, shifts of opposite sign were observed for the *tert*-butyl carbons (C1 and C2).

In the case of amine **3**, ^{13}C chemical shifts were only slightly affected by the presence of DM-β-CD and the signs of the complexation-induced displacements of C1 and C3 were opposite to those seen with amine **1**.

Since, under the above conditions, the experimental NMR spectra each represent a concentration-weighted average of the spectrum of the amine in water and of that of the amine complexed in CD, the association constant could be calculated by computer-fitting of the concentration dependence of the $\Delta\delta$ values. Since the ^{1}H NMR spectroscopic data show only small differences between the chemical shifts of the free and the complexed amine (generally less than 0.05 ppm), we decided to use the much more pronounced carbon shifts to evaluate the association constants of the amines with DM-β-CD quantitatively. Experiments (from 7 to 11 runs) were performed by keeping the amine concentration constant while increasing that of DM-β-CD. In the case of amine **1** (0.01 M), complexation-induced chemical shifts (δ_i) were measured for both primary (C1) and quaternary (C2) *tert*-butyl carbons. The pattern of titration suggests a 1:1 final stoichiometry for the complex. The binding constant was calculated by nonlinear, least-square fitting of the $\Delta\delta$ values of both carbon species (C1 and C2) of the *tert*-butyl group. These undergo displacements of 1.5 and 1.2 ppm, respectively, when all the amine is complexed. An average value of 295 ± 50 M^{-1} was obtained for the association constant of **1**.

In the case of amine **2** (0.015 M) the titration was carried out by monitoring the shifts of the methyl moiety of the *tert*-butyl group (C1) and of the methyl group bound to the nitrogen atom (C3) (Figure 1). The shift corresponding to 100% complexation was displaced to lower field by 1.82 ppm for C1, while C3 showed a displacement of 1.18 ppm. The average binding constant was calculated as 119 ± 10 M^{-1}.

Titration of compound **3** (0.02 M) with DM-β-CD was monitored using the complexation-induced shifts of the benzyl carbon. The final shift corresponding to 100% complexation was only 0.27 ppm downfield relative to that of the free amine, and the binding constant was calculated as 101 ± 11 M^{-1}.

Features of the geometry of the amine/DM-β-CD complexes can be deduced, in principle, by measuring the NOE intermolecular interactions between host and guest protons. Since these complexes fall near the zero transition between

Table 2. Complexation-induced ^{13}C chemical shift differences (ppm) in D_2O at 293 K

Amine[a]	Host[b]	C1	C2	C3	C4	C5, C6	C7
1	DM-β-CD	+1.16[c]	+0.94	−0.13	+0.39	−0.07, −0.25	+0.04
1	TM-β-CD	+0.35	+0.23	−0.12	+0.26	−0.04	−0.03
2	DM-β-CD	+0.87	−0.42	+0.58			
3	DM-β-CD	−0.28		+0.17	−0.01	−0.20, −0.16	−0.10

[a] Amine 0.01 M. – [b] Host 0.01 M. – [c] Upfield displacements are negative.

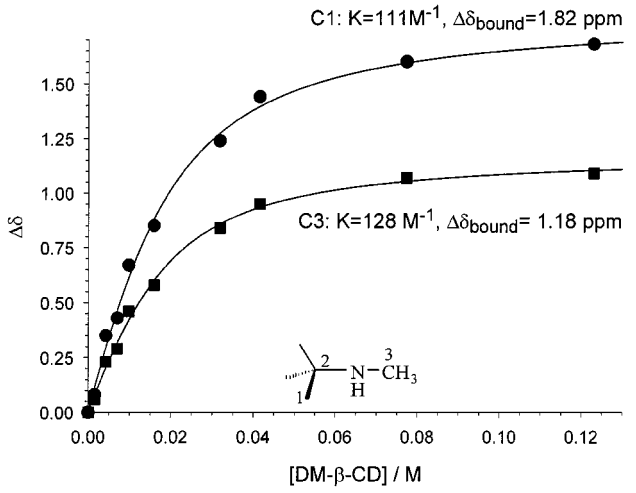


Figure 1. Plot of complexation-induced chemical shift for **2** versus concentration of DM-β-CD.

positive and negative NOEs, a spin-lock technique (ROESY) was employed in order to obtain sizeable NOEs with the spectrometer used for this investigation (400 MHz).[9,10] All the experiments were carried out on 0.03−0.08 M solutions of amines containing an equimolar amount of DM-β-CD, with mixing times varying from 50 to 500 ms.

In the case of amine **1**, the ROESY spectra recorded at low mixing times (50−100 ms) only showed intermolecular cross-peaks between the *tert*-butyl protons of the amine and H3 and H5 of the host, the intensity being stronger for H3 than for H5 (Table 3).

Table 3. NOE (ROESY) effects in DM-β-CD complexes with amines **1−3**

DM-β-CD	1		2		3	
	CMe_3	Aromatic	CMe_3	CH_3	CH_3	Aromatic
H3	+++[a]	−	++	+++	+	+
H5	++	−	+++	++	−	−
H6	−	−	+	−	−	−
6-OMe	−	++	−	−	−	−

[a] +++, strong; ++, medium; +, weak; −, no effect.

Interaction between aromatic protons of the amine and the CD protons was detected at higher mixing time (200 ms), and cross-peaks between CD 6-OCH_3 protons and aromatic protons were also readily observed. The intermolecular NOE interactions between CD and the benzylic protons of **1** could not be unambiguously assigned, since the signal of CD H6 appears at the same chemical shift (3.70 ppm) of the latter.

With amine **2**, cross-peaks were detected between *tert*-butyl protons and the H3, H5, and H6 protons of CD (mixing time 200−400 ms). In this case, in contrast to what had been observed with amine **1**, a stronger interaction was measured for H5 than for H3, while the interaction with H6 was the weakest. An interaction was also evident between the *N*-Me protons and H3 and H5, the cross-peak intensity being stronger for H3 than for H5, while no correlation with H6 was detected.

In the case of benzyl methyl amine (**3**), both aromatic and methyl protons showed cross-peaks with the inner proton H3 of the host, while no other intermolecular cross-peaks relating amine protons with H5 were detected (mixing time 200−400 ms).

Discussion

The first feature evident from our data is that the investigated amines exhibit complexation-induced displacements more pronounced for the carbon atoms than for the protons. This behaviour is quite unusual, since the guest protons are much more exposed to screening effects than the carbons.[7] Although the interpretation of ^{13}C NMR shift changes is difficult, due to the marked sensitivity of carbon shielding towards even minor conformational distortions, these variations were successfully exploited for determining the association constants.

An examination of the data from the ^{13}C NMR titrations confirms what had previously been observed for the accommodation of nitroxide radicals deriving from the amines **1−3** inside the cavity of DM-β-CD.[5] Firstly, the good agreement between experimental data and the theoretical curves (Figure 1), as well as the values of the association constants obtained from different carbon shifts, both confirm the 1:1 stoichiometry assumed for the inclusion complexes. In addition, the most stable complex is that formed by amine **1**, while those from amines **2** and **3** show smaller, although similar, binding constants (Table 4). The better host-guest affinity between **1** and DM-β-CD is presumably due to the presence in this amine of two *N*-substituents (*tert*-butyl and benzyl groups) of substantial hydrophobicity. Indeed, replacement of one of the substituents with the less hydrophobic methyl group results in a lower stability of the corresponding complexes. The stability constants of the amine/CD complexes are comparable to those of the complexes between the corresponding nitroxide radicals and DM-β-CD in water, the largest differences being found for amine **1** (295 M^{-1}) and its nitroxide (1079 M^{-1}). The similarity of the affinity constants suggests a similar inclusion geometry for amines and nitroxide radicals, and therefore the structure of the complex with the former is likely also to reflect the mode of association of the nitroxide radicals.

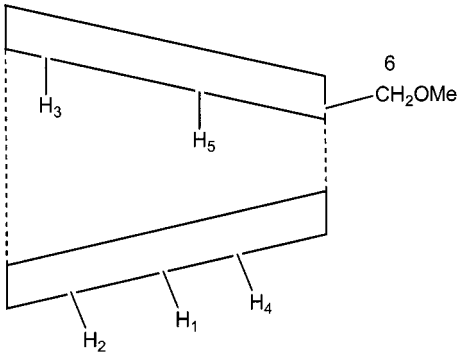
Table 4. Association constants for the accommodation of amines **1−3** and corresponding nitroxides by DM-β-CD

Amine	Host	K/M^{-1}	$K_{Nitroxide}$[a]
1	DM-β-CD	295±50	1079
2	DM-β-CD	119±10	84.8
3	DM-β-CD	101±11	70.0

[a] From ref.[4]

In the case of amine **1**, the ROESY spectra show intermolecular cross-peaks between the *tert*-butyl protons and H3 and H5 of the host; this demonstrates the full involvement of the aliphatic end of the amine in complexation. If

it is taken into account that H3 is situated near the larger rim, while H5 is more deeply embedded in the cavity (Scheme 2), then the stronger intensities of the cross-peaks between H3 and the *N*-*tert*-butyl protons of **1** seem to indicate that the *tert*-butyl group is accommodated in the wider cavity of the host. Moreover, the intense cross-peak between the CD 6-OCH_3 protons and the aromatic protons of amine **1** confirms that the phenyl group is located close to the smaller rim.



Scheme 2

Analysis of the NOE interactions between **2** and DM-β-CD suggests a reversed mode of inclusion of the *N*-*tert*-butyl group in the complex, since in this case the interaction of the *tert*-butyl protons is stronger with H5 than with H3. On the other hand, the NOE observed between the *N*-Me group and H3 is stronger than with H5. These data suggest that amine **2** is situated with the *N*-*tert*-butyl side in the CD smaller rim. The absence of cross-peaks between the *tert*-butyl and the 6-OCH_3 protons of the CD suggests that **2** is less deeply embedded inside the host cavity than amine **1**; this would be in agreement with the general view that a guest molecule seeks the closest contact to the CD host cavity.

The behaviour of benzyl methyl amine (**3**) in the presence of DM-β-CD is more complex. Since NOEs indicate inclusion of both phenyl and methyl groups in the smaller side of the torus-shaped cavity, this may presumably be taken as evidence that the **3**/CD complex exists in two different forms. In addition, the absence of intermolecular interactions between the amine and those CD protons situated close to the smaller rim (H5) suggests that the molecule is not deeply inserted in the cavity. This characteristic would explain the larger association and dissociation rate constants measured by EPR for the complex between DM-β-CD and methyl benzyl nitroxide.

In conclusion, we have shown that the use in parallel of both NMR and EPR spectroscopy may provide complementary information concerning the formation of complexes between CD and closely related paramagnetic and diamagnetic guests, as reported previously for a different type of host cavity.[11]

Experimental Section

^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Gemini 300 (300 MHz) and a Varian Mercury 400 (400 MHz) spectrometer. Amine titrations with DM-β-CD were carried out on a Varian Gemini 300. All NMR experiments were performed in D_2O solutions at 293 K, using residual HOD as an internal standard (4.80 ppm). CD_3OD (10% v/v) was added in order to improve the amine solubility.

NMR determinations of the binding constants were performed on solutions of the guest (**1** 0.0133 M; **2** 0.0154 M; **3** 0.020 M), titrated with increasing amounts of DM-β-CD (0−0.15 M in D_2O). The resulting mixtures were allowed to equilibrate for one hour before measuring the spectrum.

ROESY data were collected on a Varian Mercury 400, without spinning the sample. The spectra were recorded with mixing times of 50−500 ms in the phase-sensitive mode, using a CW spin-lock field of 2 kHz. The instrumental settings were: 90° pulse width 15 μs, spectral width 4000 Hz in each dimension, 256 increments of 2 K data points, 8 scans per t_1 value, repetition time 3 s.

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