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The application of selective ROE experiments to study solution structures of cyclomaltooligosaccharide derivatives and complexes

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Abstract

Selective one-dimensional ROE experiments were applied to study the host-guest interactions of cyclomaltohexaose with *p*-nitrophenol and the solution structure of 3^A-(4-methylamino-3-nitrobenzyl)-cyclomaltoheptaose. The line selective excitation of the aromatic signals of *p*-nitrophenol gave intense ROE enhancements on the cyclomaltohexaose multiplets (H3 and H5), indicating deep complexation inside the cavity. The results on 3^A-(4-methylamino-3-nitrobenzyl)-cyclomaltpheptaose showed that the aromatic moiety covers the wider base of the cyclomaltoheptaose cone. The rotational correlation time for this compound was calculated to be 2.9×10^{-10} s using carbon-13 spin–lattice relaxation data. Quantitative analysis of the measured enhancements was performed and proton–proton distances were obtained between the aromatic and cyclomaltheptoase protons as well as interglycosidic distances inside the cyclomaltheptaose moiety.

Keywords: Cyclomaltooligosacharides; ROE; Selective NMR experiments

1. Introduction

The ability to bind organic molecules has catapulted cyclomaltooligosaccharides, the cyclic oligosacharides of glucose (also known as cyclodextrins), into prominence in the last 3 decades [1–3]. This property enables these molecules to play a crucial role in drug delivery, enzyme mimics, phase transfer and other frontiers of chemistry [4]. The orientation of the organic guest molecule within the cyclomaltose host is a crucial element of this host–guest interaction and dictates critical properties such as stability of the complex and the catalytic activity of the guest [5]. Deriving this information through X-ray crystallography is tedious and can be applied to only those compounds which can be crystallized and gives information regarding solid state structures [6]. The most important tool for deducing solution phase structures, NOE experiments [7,8], has had limited

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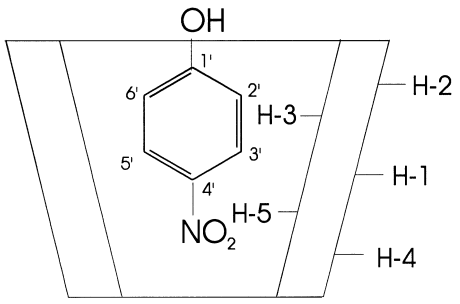

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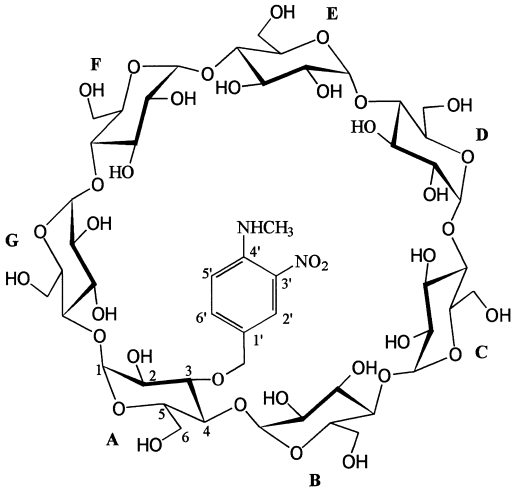

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applications in determination of inter-spatial relationships between the host and the guest in cyclomaltooligosaccharide complexes. The major obstacle in this endeavor is that enhancements in steady-state NOE experiments go through zero [8] as the molecular weight increases and sometimes no enhancement can be observed for medium sized molecules like cyclomaltooligosaccharides and their derivatives. Rotating-frame Overhauser experiments can overcome this problem because the enhancements are always positive [9]. Two-dimensional ROESY experiments [10–12] have been used successfully in the elucidation of structures of chemically modified cyclomaltooligosaccharides [13,14] and recently, of host–guest interactions [15]. Sometimes the resolution of these spectra is not high enough for reliable evaluation and the information under identical conditions (concentration, spectral resolution and data collection time) is less pronounced than the selective experiments that are presented here.

In this investigation, selective rotating-frame Overhauser experiments were applied in order to extract valuable information regarding spatial

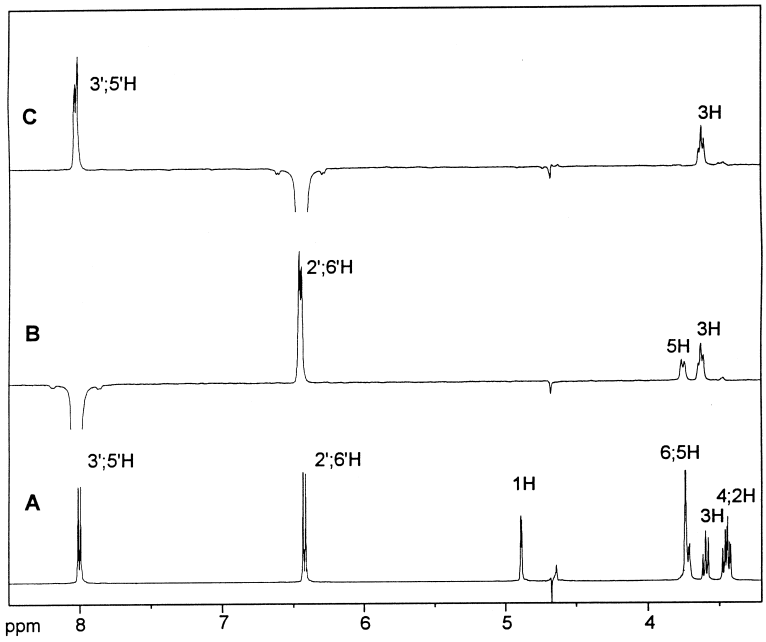

Fig. 1. Spectra of the *p*-nitrophenol-cyclomaltohexaose complex (**1**). A: ^{1}H-NMR spectrum with low power water presaturation. B, C: One-dimensional selective ROE spectra with selective excitation of the 3′,5′ and 2′,6′ doublets. The spin–lock time was 750 ms and 2000 transients were collected in each spectrum.

arrangements in a cyclomaltooligosaccharide complex (*p*-nitrophenol and cyclomaltohexaose (**1**) [16]) as well as a cyclomaltoheptaose derivative (3^A-(4-methylamino-3-nitrobenzyl)-cyclomaloheptaose (**2**)—for the synthesis and complete NMR assignment of **2**, see ref [17]). Quantitative analysis of the ROE enhancements in **2** was performed and proton–proton distances were extracted.

2. Results and discussion

The aromatic signals in the ^{1}H NMR spectrum are well separated from the signals of cyclomaltooligosaccharide in both systems and can be irradiated easily to probe spatial relationships with cyclomaltooligosaccharide protons. Selective excitation is applied routinely in NMR spectroscopy using either hard pulse trains [18–20] or an elegant but hardware dependent method using shaped pulses [21,22]. The aromatic signals (2′,6′H and 3′,5′H in **1**; 2′H,5′H and 6′H in **2**), the $NHCH_3$ signal in **2** as well as two anomeric protons (A-H1 and B-H1 in **2**) were excited in selective one-dimensional ROE experiments using DANTE-Z pulse train.

Cyclomaltohexaose-p-nitro-phenol complex (**1**).—When the 3′;5′ proton doublet was excited in a selective one-dimensional rotating frame Overhauser experiment, 2′;6′ as well as 3H and 5H protons gave very intense ROE signals (B in Fig. 1). The ROE enhancement on both H5 and

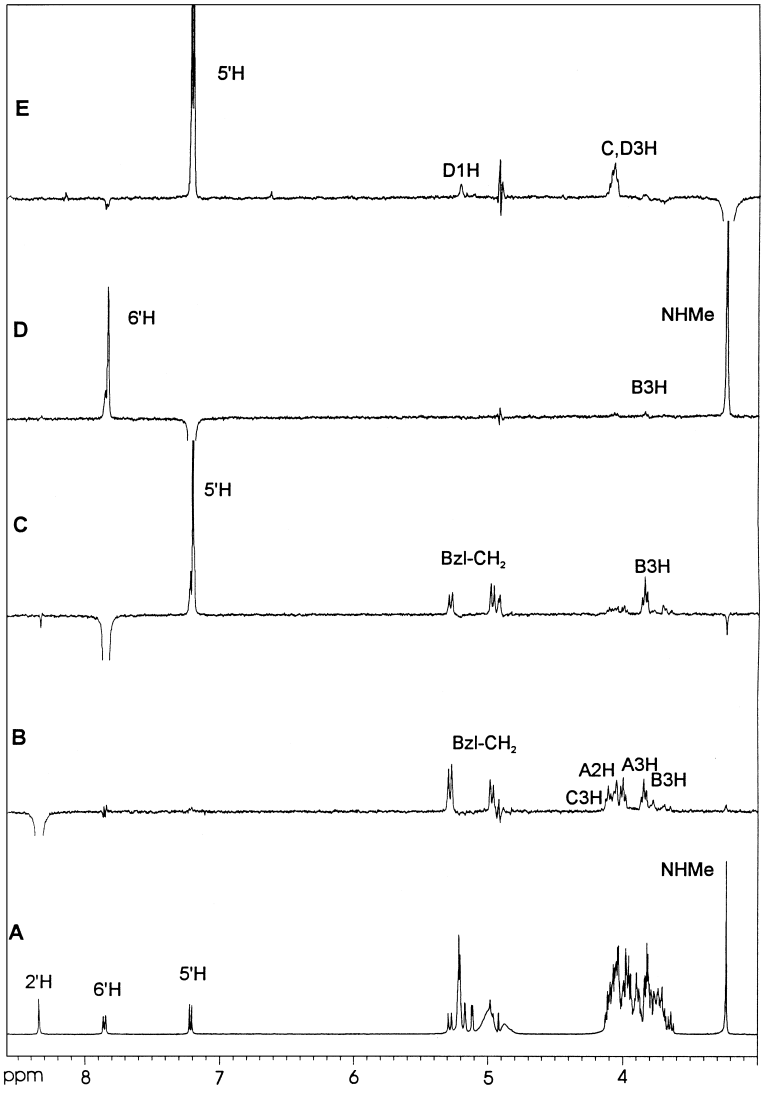

Fig. 2. Spectra of the 3^A-(4-methylamino-3-nitrobenzyl)-cyclomalto-heptaose (**2**). A: ^{1}H-NMR spectrum with low power water presaturation. B–E: One-dimensional ROE spectra with selective excitation of 2′, 6′, 5′ and NHMe. The spin–lock time was set to 500 ms and 2000 transients were collected in each spectrum.

H3 protons gave strong evidence that the substrate is complexed inside the cyclomaltohexaose cavity because these protons are placed in the hydrophobic pocket of the molecule. However H5 is deep inside the cavity and is close to the smaller diameter base. This base is covered by CH_2OH residues of the glucose units and during the complexation the nitro group of the guest molecule is placed close to the narrower base. The selective excitation of the 2′;6′ proton doublet gave an intense ROE peak with the 3′;5′ protons and only with the 3H signal of the cyclomaltohexaose molecule (C in Fig. 1). The H3 proton in the cyclomaltohexaose hydrophobic cavity is close to the wider base. The ROE signal with only H3 indicates that the 3′,5′ part of the substrate is close to the wider base and the OH group is presumably pointing out of the cavity.

3^A-(4-methylamino-3-nitrobenzyl)-cyclomalto heptaose (**2**).—The aromatic and $NHCH_3$ signals are well separated and the selective excitation of these signals in a selective ROE experiment gave a correlation to cyclomaltoheptaose ring protons. Excitation of H2′ gave ROE peak with H3-s in the A, B and C glucose, with H2 in the A ring and the benzyl CH_2 (B in Fig. 2). The selective ROE experiment on the H6′ (C in Fig. 2) shows enhancement on H3 in the B glucose ring besides the expected H5′ and benzyl CH_2. Excitation of H5′ (D in Fig. 2) gave a very small ROE peak on H3 in the B ring and a very intense enhancement on the NHMe signal. The N-methyl signal (E in Fig. 2) had noticeable ROE interactions with H5′, with two of the cyclomaltoheptaose H3 proton in the C and D glucose rings at the opposite side of the molecule, and a small enhancement was observed on the anomeric proton in the D ring. These results can be explained by a structure where the methylamino-nitrobenzyl moiety is capped on the rim of the cyclomaltoheptaose molecule. No ROE peaks were observed with any of the H5 protons in the glucose rings, indicating that the methylamino-nitrobenzyl moiety is not immersed as deeply into the cavity as the para-nitro-phenol into the cyclomaltohexaose.

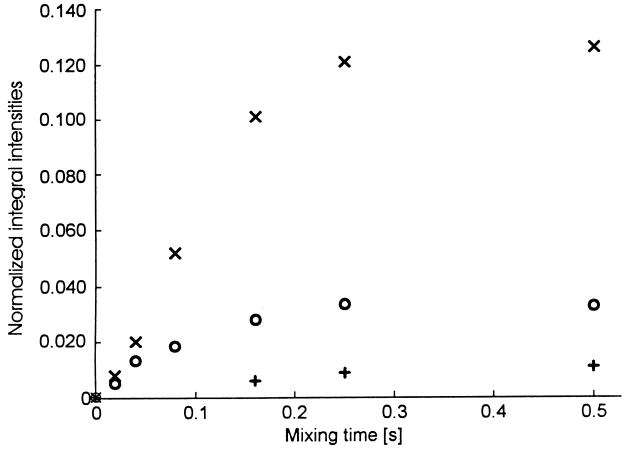

Fig. 3. ROE buildup curves measured on three different multiplets. The "x" symbol represents the integrated signal intensity of G4H as a function of the mixing time when A1H was selectively inverted. The open circles show the buildup curve of A4H with selective inversion of B1H. The third curve (with + marks) shows the buildup curve of B3H with inversion of 2′H.

Quantitative analysis of the observed ROE interactions in **2**.—The rotating frame cross-relaxation rate (σ_{tr}) between two protons (i and j) can be given as follows [8]:

$$\sigma_{tr} = \left(\frac{\mu_0}{4\pi}\right)^2 \frac{\hbar^2\gamma^4}{10r_{i,j}^6}\left(\frac{3}{1+\omega_0^2\tau_c^2}+2\right)\tau_c$$

This formula is valid only for an isotropically tumbling molecule when the source of the relaxation process is purely dipolar. The cross relaxation rate can be extracted from the initial, linear part of the ROE buildup curves (Fig. 3) measured with different mixing times. Since σ_{tr} has a distance dependence parameter ($r_{i,j}$), the unknown distance can be calculated if the correlation time (τ_c) is known. The τ_c values were evaluated from carbon spin–lattice relaxation time measurements. Because of the size of the molecule, molecular motions are expected to be slow and the complete dipolar

Table 1
^{13}C spin–lattice relaxation data of **2**[a]

Glucose unit	^{13}C T_1 (s)							
	C_1	C_2	C_3	C_4	C_5	C_2'	C_5'	C_6'
A	0.24	0.25	0.26	0.25	0.26[1]	0.23	0.22	0.24
B	0.25	0.26[1]	0.24	0.24	0.22			
C	0.26	0.24	0.27[3]	0.25	0.24[6]			
D	0.25	0.25	0.29	0.25	0.25[5]			
E	0.22[2]	0.30	0.30	0.26	0.25			
F	0.25	0.25[5]	0.27	0.28	0.27[4]			
G	0.22[2]	0.27[4]	0.27[3]	0.24	0.24[6]			

[a] Averaged ^{13}C spin–lattice relaxation data were measured for $^1BC_2$–AC_5, $^2EC_1$–GC_1, $^3CC_3$–GC_3, $^4GC_2$–FC_5, $^5FC_2$–DC_5 and $^6GC_5$–CC_5 because of several overlapping signals in the carbon spectrum. The experimental error was ±0.01 s on the measured ^{13}C spin–lattice relaxation times. No spin–lattice relaxation data were measured for CH_2, CH_3 and quaternary carbons since the INEPT sequence was optimized to CH polarization transfer.

Table 2
Cross relaxation rates (σ_{ij}) and proton–proton distances (r_{ij}) obtained from the selective ROE buildup curves

Analyzed proton pair	Cross-relaxation rate (s^{-1})	Distance (Å)
A1H–G4H	0.651	2.1
B1H–A4H	0.168	2.7
2′H–C3H	0.013	4.1
2′H–A2H	0.026	3.6
2′H–A3H	0.032	3.5
2′H–B3H	0.030	3.6
6′H–B3H	0.030	3.6

formula [23] without any simplification was used to calculate the correlation time. The measured values for carbon spin–lattice relaxation times were found to be between 0.22 s and 0.29 s throughout the molecule (Table 1). The measured spin–lattice relaxation time values on the three protonated aromatic carbon signals were 0.23 s (C_2'), 0.22 s (C_5') and 0.24 s (C_6'); these values do not differ from the relaxation times of the carbons in the glucose units, which indicates that the aromatic ring does not have any additional fast rotational freedom over the cyclomaltohexaose moiety. The calculated correlation time was 2.9×10^{-10} s and this value was used in the equation above to obtain proton–proton distances. The formula given by Debye [24] provided a similar result (at 25°C, with the viscosity of 0.89 cP and a molecular radius of 7 Å, $\tau_c = 3.0 \times 10^{-10}$ s) for native cyclomaltoheptaose in water solution. The product of the correlation time with the proton Larmor frequency ($\omega_H\tau_c$) in this case is 0.91 and this value is very close to the border line where no NOE enhancements can be observed (1.118). However it is a little lower which indicates the presence of positive NOE enhancements with very low intensity. The obtained rotating frame cross-relaxation rates and the corresponding proton–proton distances are summarized in Table 2. The highest cross relaxation rate (0.651 s^{-1}) was measured between A1H and G4H, which gave a distance of 2.1 Å between these two protons. Solid state neutron diffraction experiments [25] on the native cyclomaltoheptaose show the same interglycosidic distance for the H1–H4 proton pair. The B1H–A4H distance was determined to be longer (2.7 Å), which can be explained by the distortion of the cone by the methylamino-nitrobenzyl group on the A ring. Selective two-dimensional ROESY experiments showed that the anomeric protons in the C, D, E, F and G rings are also close to the H4 protons in the neighboring glucose units. An interesting observation is that the 6′H–B3H and 2′H–B3H distance was measured to be the same, 3.6 Å. Since H2′ and H6′ are on the opposite side of the aromatic ring, this arrangement is possible only when the plane of the aromatic ring is perpendicular to the base of the cyclomaltoheptaose. Based on both qualitative and quantitative analysis of the observed ROE signals in **2**, it can be summarized that 2′H and the nitro group in the methylamino-nitrobenzyl moiety are pointing towards the cavity.

3. Conclusion

Selective one-dimensional rotating-frame Overhauser spectroscopy was applied to study the solution structure of an inclusion complex of cyclomaltohexaose with *p*-nitrophenol. The data indicated that the substrate is deeply complexed into the cyclomaltohexaose cavity. The spectra of a chemically modified cyclomaltoheptaose derivative (3^A-(4-methylamino-3-nitrobenzyl)-cyclomaltoheptaose) showed similar behavior, however, the deep complexation is prevented by the methylamino substitution on the phenyl ring. Quantitative analysis of the ROE interactions was performed and, proton–proton distances were extracted through rotating-frame cross-relaxation rates. The interactions of two out of the seven anomeric protons with the neighboring H4 protons were studied and it was found that the substitution on the glucose ring has a significant effect on the interglycosidic distances. The quantitative analysis of the ROE signals with the aromatic protons demonstrated that the plane of the phenyl ring is perpendicular to the base plane of the cyclomaltoheptaose molecule. Carbon spin–lattice relaxation times indicated that the phenyl ring does not have an additional fast motional freedom compared to the cyclomaltoheptaose cone.

4. Experimental

NMR spectroscopy.—All spectra were recorded on a Bruker ARX-500 NMR spectrometer using a 5 mm inverse probe. Selective one dimensional rotating-frame experiments were set up using a DANTE-Z pulse train. The following pulse sequence was used to run these spectra: $\Delta_{rel}-[\Phi_{(\phi1)}$

$-\Delta-\Phi_{(\phi 2)}-\Delta]_n-\pi/2_{(\phi 3)}-Sl_{(\phi 4)}-ACQ_{(\phi 5)}$ with the following phase cycles: $\phi_1=x$; $\phi_2=x, -x$; $\phi_3=x, x, y, y, -x, -, x, -y, -y$; $\phi_4=y, y, -x, -x, -y, -y, x, x$; $\phi_5 = x, -x, y, -y, -x, x, -y, y$. The relaxation time ($\Delta_{rel}$) was 2.0 s between each individual transient. The length of the DANTE pulses (Φ) inside the pulse train was 0.8 μs separated by 120 μs delays, and the loop counter (n) was set to 250. The spin-lock pulse strength (Sl) was 2.44 kHz (90 degree pulse length was 102.5 μs).

p-*Nitrophenol-cyclomaltohexaose complex* (**1**).—Cyclomaltohexaose (7.8 mg) and *p*-nitrophenol (PNP, 5.6 mg) were dissolved in 0.8 mL deuterated water to give a molar ratio PNP/α-CD = 0.05/0.01. The pH of the solution was set to 9.7 using 5% NaOD solution.

3^A-(4-methylamino-3-nitrobenzyl)-cyclomaltoheptaose (**2**).—The same selective one-dimensional ROE sequence was used as in the case of the inclusion complex. The acquisition parameters were set to the same values because of the spectral similarities. The only difference was that a routine low power presaturation sequence was inserted to suppress the water signal during the relaxation delay. The data processing procedure was the same as for **1**. ROE buildup curves were measured at five different mixing times: 40, 80, 160, 250 and 500 ms by selective irradiation of the H2′, H6′, AH1 and BH1 signals. The ROE peaks were integrated and the integrals were normalized to the inverted zero mixing time signals and were offset corrected [26]. The cross-relaxation rates were obtained by fitting the initial, linear part of the buildup curves. The average correlation coefficient was 0.95 and $\pm 0.003\ s^{-1}$ error was introduced in the cross-relaxation rates. This $\pm 0.003\ s^{-1}$ error caused ± 0.1 Å error in the longest measured distance (4.1 Å). An INEPT-enhanced inversion recovery experiment [27] was used to measure ^{13}C spin–lattice relaxation times.

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