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High Resolution ^{1}H-NMR Study on Self-Complexation Phenomena in Cyclodextrin Dimers.

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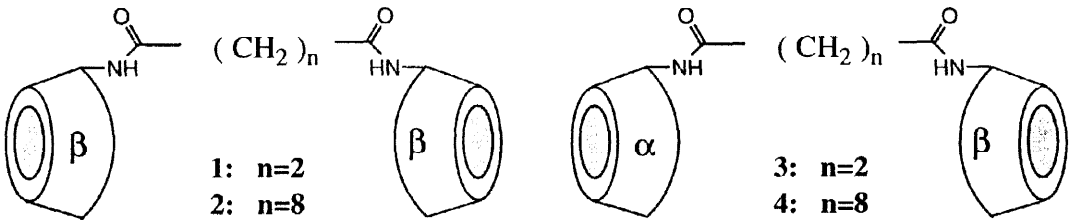
Abstract : The unusual conformational behaviour of a series of new cyclodextrin (CD) dimers is studied by dedicated ^{1}H-NMR techniques. In water some CD-dimers form stable self-inclusion complexes, for which the aliphatic linker is partially included in one of the two CD cavities. This phenomenon affects the conformation of the substituted sugar ring of one CD moiety. In the case of the α–β heterodimer, the linker appears to be preferentially in the cavity of β–CD, in contrast to what is known for intermolecular complexes where at ambient temperature a preference for α–CD is observed.

Studying the conformational changes and deformations that can occur during the inclusion of guests in host molecules is of great importance in understanding molecular recognition processes. In this respect, liquid-state NMR is a valuable tool to obtain information on the main geometrical and dynamic features of host-guest binding[1]. In the present paper we show that specific ^{1}H-NMR sequences, providing enhanced resolution, can help overcome the problem of spectral overcrowding thereby giving detailed information about local conformations and self-complexation phenomena in cyclodextrin (CD) dimers.



Scheme 1

As part of a program aimed at the development of host molecules with improved binding properties, the four CD dimers **1-4**, containing a flexible spacer constituted by either a C2 or C8 aliphatic chain were synthesized[2,3]. These chains link the positions 3 of the cyclodextrin secondary sides by means of covalent amide bonds.

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Complexation experiments revealed that the two cavities of compounds **1-4** were capable of cooperative binding to some guest molecules. This was concluded from the fact that the measured binding constants were much larger than those involving the monomers[2,4]. It was also found that the binding constants involving compounds **1** and **3** were larger than those obtained for compounds **2** and **4**. In order to get more information about the complexation behaviour of **1-4**, ^{1}H-NMR spectra of these compounds were recorded in D_2O and in organic solvents[3] (Fig. 1).

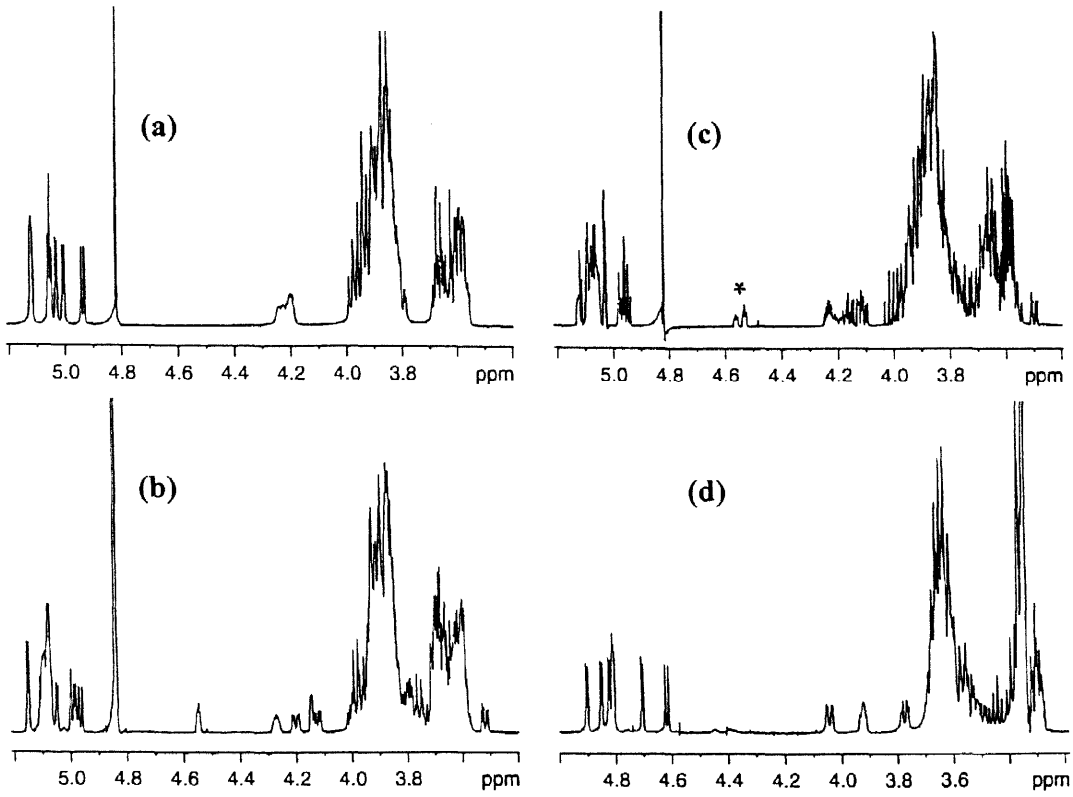


Fig. 1. 600 MHz ^{1}H-NMR spectra of compounds **1** (a), **2** (b) and **4** (c) in D_2O, and of compound **2** in DMSO-d_6 (hydroxyl proton signals have been removed by the addition of one drop of D_2O) (d). Temperature: 298 K, concentration: 5 mM.

For the dimers containing the octamethylene spacer (compounds **2** and **4**), surprisingly, examination of the anomeric region revealed more proton signals than expected *a priori* from the symmetrical chemical structure. As this feature was not observed in less polar solvents (compare the anomeric region of **2** in D_2O, Fig. 1b, and in DMSO-d6, Fig. 1d), we postulated that in aqueous solution the long and flexible hydrophobic linker in **2** and **4** could be partially included in one of the two CD cavities. This could explain the lower binding constants found for the complexes with **2** and **4** as well as the unequivalence of the ^{1}H (and ^{13}C, not shown here) resonances of the C8 linker[3]. It is worthnoting that the ^{1}H-NMR spectrum in DMSO excludes totally the possibility of rotaxans-type structures. We felt however that more detailed ^{1}H-NMR studies, in particular on local conformation aspects, were necessary to validate this proposition. These studies are presented below.

SPECTRAL ASSIGNMENT

In order to get further insight into the conformations of the CD-dimers, a systematic 2D ^{1}H-NMR study was undertaken on compound **2**. Chemical mono-functionalization breaks the C_7 symmetry of the parent natural molecule and, as it is observed in Fig. 1, the spectra become extremely complicated even at high magnetic field. In comparison to the spectra of other classes of biomolecules such as peptides and nucleic acids, cyclodextrin protons resonate in a limited bandwidth: 0.2 ppm for H1 and 0.5 ppm for the set of protons H2, H3, H4, H5 and H6,6'. The extreme complexity of the spectrum due to high similarity of the glucose units therefore precluded a full assignment by classical 2D (and even 3D) methods. Particularly, the necessity to separate the 14 anomeric signals of **2** - starting point for a stepwise assignment of the cyclodextrin protons - led us to perform 2D semi-soft experiments.

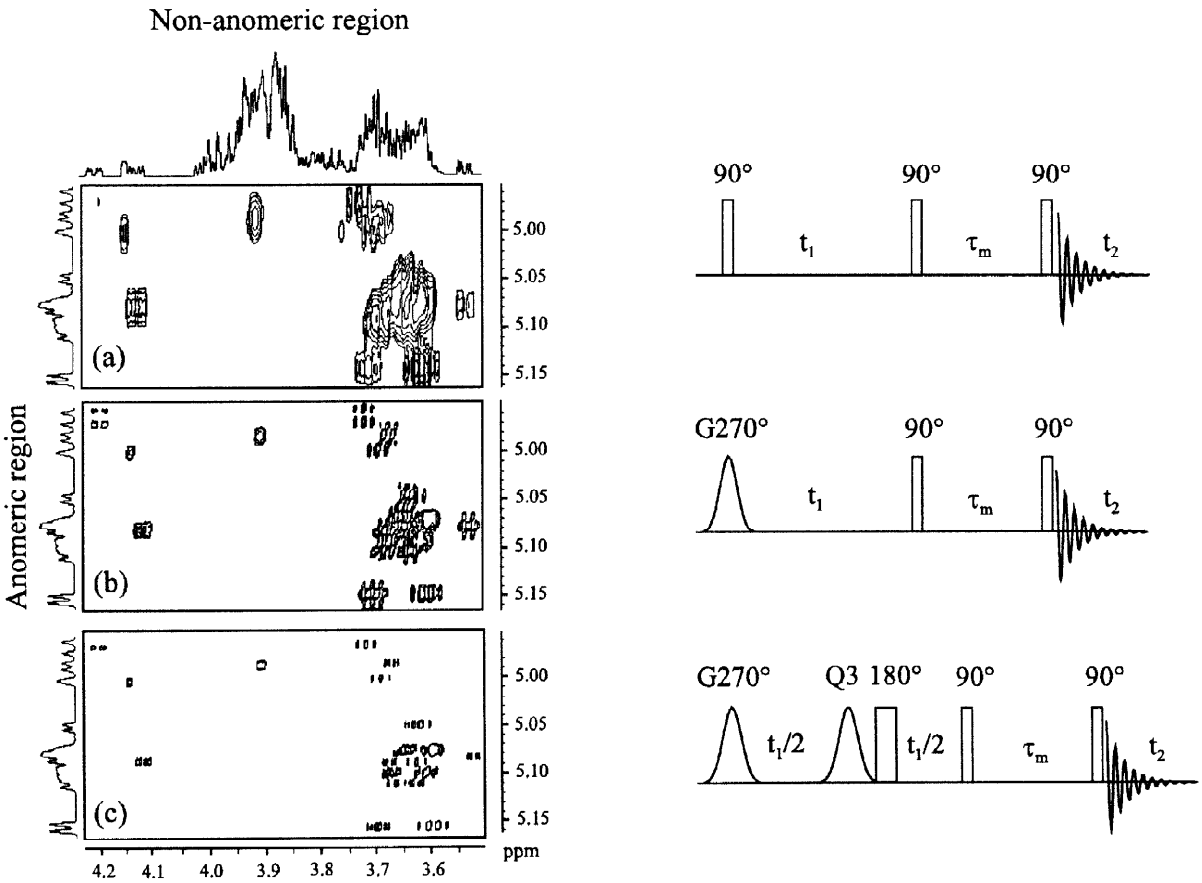


Fig. 2. 600 MHz 2D ^{1}H-NMR maps of compound **2** in D_2O. Parts of the conventional NOESY (a), semi-soft NOESY (b) and semi-soft NOESY experiment decoupled in F1 dimension (c); mixing time 200 ms. The corresponding pulse sequenses are presented schematically. For selective excitation and refocusing, 270° gaussian (12 ms) and Q3 gaussian cascade pulses (20 ms) - centered on the H1 region - were used respectively.

Solutions to improve the effective resolution and the separation of the cross-peaks in the indirect dimension (F1) of homonuclear 2D maps have been proposed some years ago[5] and applied to the field of cyclodextrins[6]. For example, the use of refocusing pulses during the evolution delay allows cancellation of the homonuclear couplings in F1. Fig. 2, displaying classical and semi-soft contour plots, shows the spectacular gain in resolution obtained by these methods in the case of the NOESY[7] experiment used for the sequential assignment (connectivities of the type $H1_i \rightarrow H2_i$ and $H1_i \rightarrow H4_{i+1}$). The same techniques were also applied in the case of COSY[8], RELAYs[9] and TOCSY[10] experiments, used for spin-system identification.

^{1}H assignment of two glucose units (**m** and **n**) for which the anomeric signals were equivalent, was achieved via semi-soft double quantum correlation[11] (Fig. 3), semi-soft DREAM[12] and semi-soft pseudo-3D ROESY-TOCSY pulse sequences.

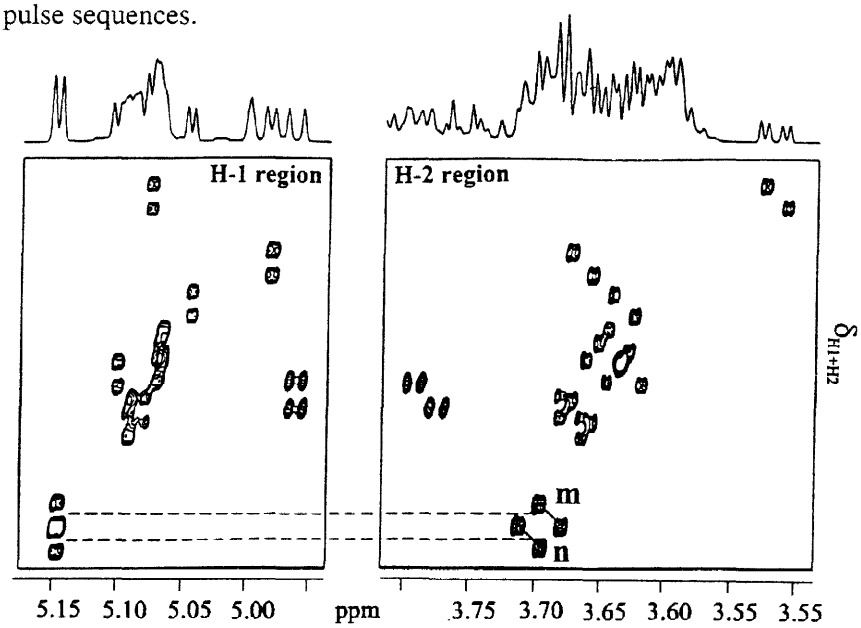
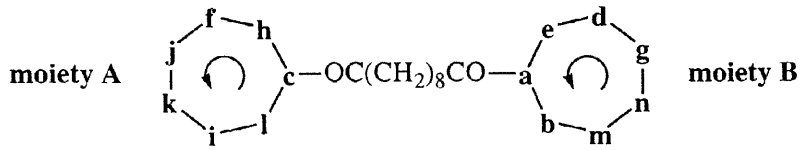


Fig. 3. Part of the semi-soft double quantum correlation used for the separation of two fully overlapped anomeric protons in compound **2**.

By the above mentioned methods, all the H1 to H4 protons (plus some H5, H6,6' protons) were unequivocally assigned (Table 1) and sequencing of the glucose units was carried out (Scheme 2). The 12 glucose and the 2 amino-modified altrose units were named **a**, **b**, **c**... according to the relative order of their anomeric proton chemical shifts (from highfield to downfield). Their disposition along the two cyclodextrins of the compound 2 is presented in the following top-view scheme (the arrows show the direction of the α 1→4 glucosidic bonds) .



Scheme 2

Table 1. Chemical Shifts (in ppm) of Compound **2** CD protons in D_2O, at 298K.

Pyranoside Unit (Moiety)	*H1*	*H2*	*H3*	*H4*	*H5*	*H6,6'*	*NH*
a (B)	4.961	3.785	4.203	3.909	4.273	3.812	8.063
b (B)	4.981	3.664	4.002	3.606	3.939	-	
c (A)	4.999	4.146	4.552	4.124	3.952	3.791	7.503
d (B)	5.047	3.630	3.994	3.597	3.898	-	
e (B)	5.069	3.634	3.934	3.710	3.698	-	
f (A)	5.070	3.643	3.860	3.600	-	-	
g (B)	5.076	3.653	3.977	3.621	-	-	
h (A)	5.079	3.516	3.666	3.595	-	-	
i (A)	5.085	3.674	3.853	3.662	3.706	3.910	
j (A)	5.093	3.666	3.861	3.614	3.700	-	
k (A)	5.095	3.672	3.852	3.626	3.701	3.896	
l (A)	5.104	3.625	3.766	3.688	3.759	3.924	
m (B)	5.149	3.687	3.956	3.598	-	-	
n (B)	5.151	3.703	3.937	3.588	3.837	-	

STRUCTURAL ANALYSIS

Careful examination of the CD chemical shifts revealed a peculiar behaviour of some of the non-anomeric protons. Neglecting the modified altrose units bearing the amide groups, all H3 resonances of one CD-moiety (we denote this moiety by A) are upfield shifted in comparison to those of the other CD-moiety (B). Such an upfield shift is often observed when aliphatic guest molecules are encapsulated in unmodified cyclodextrins[13] and constitutes a strong indication that the aliphatic chain linking the two moieties is included in one of the two cavities as depicted in Fig. 4.

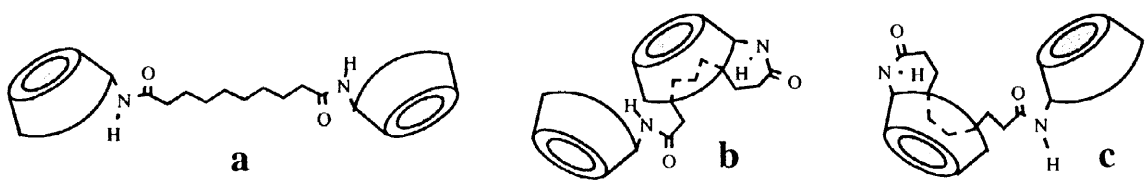
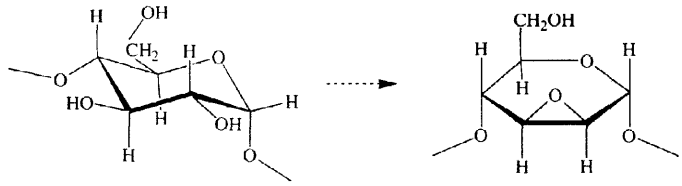


Fig. 4. Possible conformations of the cyclodextrin dimers in solution. Note that for the homodimers (β–β or α–α), cases (b) and (c) cannot be distinguished.

This conclusion was confirmed by off-resonance ROESY experiments[14] (the mixing time was 150 ms), which revealed the presence of through-space interactions between some aliphatic protons and some H3 protons of moiety A, whereas no cross-peak between aliphatic protons and H3 protons of moiety B was observed. It should be noted that in other CD-dimers studied by ^{1}H-NMR, the two cyclodextrin units were reported to be magnetically equivalent[15], which can be explained by the rigid structure of the spacer, thereby preventing its inclusion in either cavity.

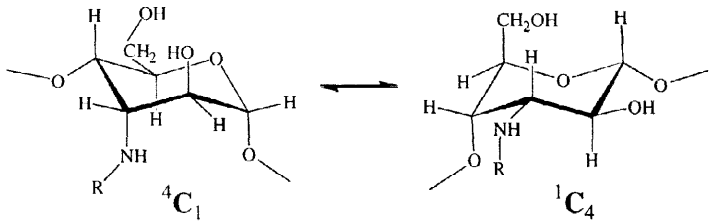
It is well known that in α-, β- and γ-cyclodextrins, the α-*D*-glucopyranose units adopt the $^4C_1$ conformation **I**. This conformation is maintained in protected and unprotected mono(2-*O*-tosyl)-β-cyclodextrin, the first intermediates in the synthetic routes to CD-dimers **1-4**.



I **II**

Scheme 3

In subsequent steps, the tosylated β-cyclodextrin is first transformed into the 2,3-manno-epoxide **II** which is then subjected to a nucleophilic attack by ammonia, leading to diaxial opening[16] and the introduction of an amine function on position 3. The resulting $^4C_1$ amino-modified α-*D*-altrose unit immediately changes to the more stable $^1C_4$ conformation shown below, as confirmed by examination of the coupling constants[16-18]. In this way, both the OH2 and the substituent at C3 are in a favourable equatorial position.



$^4C_1$ $^1C_4$

Scheme 4

In the final steps of the syntheses of **1-4**, two cyclodextrin units are coupled to the activated dicarboxylic acid functions of the spacers and deprotected: the altrose units are not further modified.

Examination of the coupling constants of **2** revealed that all non-substituted glucose units are in the $^4C_1$ conformation. The modified altrose unit of the empty cavity (moiety B) exhibited coupling constants similar to those of mono(3-amino-3-deoxy)-β-cyclodextrin[17] ($J_{1,2}$ = 7.2 Hz, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.8 Hz), suggesting that this unit is in the $^1C_4$ conformation. The other modified altrose unit (of moiety A) showed completely different coupling constants ($J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 4.3 Hz, $J_{4,5}$ = 10.4 Hz) indicating that this unit is in $^4C_1$ conformation. Its amide group is in axial position and directed towards the interior of the cavity, thus favouring the entrance of the aliphatic chain into this moiety. The free energy gain due to this kind of self-complexation in water should be sufficient to compensate for the conversion of the altrose unit to the less stable $^4C_1$ conformation. As this self-complexation does not occur in DMSO ($J_{1,2}$ = 7.1 Hz for the altrose unit) we may conclude that solvophobic forces push the linker into one of the cavities and this subsequently pulls the adjacent altrose unit into the less favourable $^4C_1$ conformation.

To identify the two amide protons at position 3 of the altrose units, separate NMR experiments were carried out in the mixture H_2O:D_2O (95:5). In the ^{1}H-NMR spectrum of **2**, two doublets were observed in the amide region, indicating that the magnetic non equivalence also involved the NH groups. The measured temperature coefficients of the two signals were quite different, -10.1 ppb/K moiety B) and -3.4 ppb/K (moiety A), suggesting that the latter proton is protected from the solvent[19], due to its location in the interior of the cavity. Both H3-NH coupling constants were quite large (8.8 Hz and 9.0 Hz) which is characteristic of a rather rigid local conformation around the amide bond. A similar behaviour was observed for compound **4**.

DYNAMICS BEHAVIOUR

Increase of the temperature up to 90° C did not essentially modify the ^{1}H NMR spectral characteristics of compound **2** in D_2O, suggesting that a quite stable self-inclusion complex is formed in this solvent. This invariance of the signals with temperature could lead to the erroneous interpretation that the two possible conformations of compound **2** (cases b and c in Fig. 4) do not exchange on the NMR time scale. The respective signals of the two conformations are all the same, making it impossible to establish whether exchange between the two forms takes place, as each observed line corresponds to both the initial and the final states. Any exchange, if present, should be slow on the NMR time scale, as no line broadening was observed whatever the temperature of the experiment was. An absence of exchange would obviously imply that the two forms are separated by a very high energy barrier, which for symmetry reasons is impossible to verify. A study of the dynamic behaviour of the C8 heterodimer **4** was expected to be more informative in this respect. Indeed, temperature variation was found to lead to a change of the relative intensities of the ^{1}H NMR signals. By comparing the ^{1}H and ^{13}C spectra of **4** with those of **2**, it was possible to assign a number of the signals to the self-included moiety of the former compound. By following the separate signals belonging to the two forms of **4** (such as those marked with an asterisk in Fig. 1c) as a function of temperature, we could monitor the variation of the peak areas, and determine the thermodynamical parameters of the exchange. If it is assumed that the lifetimes of all other conformations (e.g the "extended" form with both cavities empty, Fig. 4a) are negligibly short, this process can be seen as a two-site exchange $\alpha \rightleftarrows \beta$ where α denotes the form in which the aliphatic linker is complexed with the α–CD cavity and β the form in which this linker is complexed with the β–CD cavity.

It is then possible to write for the two-site exchange from α to β: $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -RT \ln \frac{[\beta]}{[\alpha]}$

which can be rewritten as : $-R \ln \frac{[\beta]}{[\alpha]} = \Delta H^\circ \left(\frac{1}{T}\right) - \Delta S^\circ$

An Arrhenius plot of $-R\ln\frac{[\beta]}{[\alpha]}$ versus $\frac{1}{T}$ gives direct access to the enthalpic and entropic terms $\Delta H°$ and $\Delta S°$ (Fig. 5). In our case $[\alpha]$ and $[\beta]$ were determined by integrating the H2 peaks of the altrose units belonging to the occupied cavities at different temperatures. Least square fitting afforded the following values :

$$\Delta H° = 2.7 \pm 0.1 \text{ kcal·M}^{-1} \text{ and } \Delta S° = 10.1 \pm 0.4 \text{ cal·M}^{-1}\text{·K}^{-1}$$

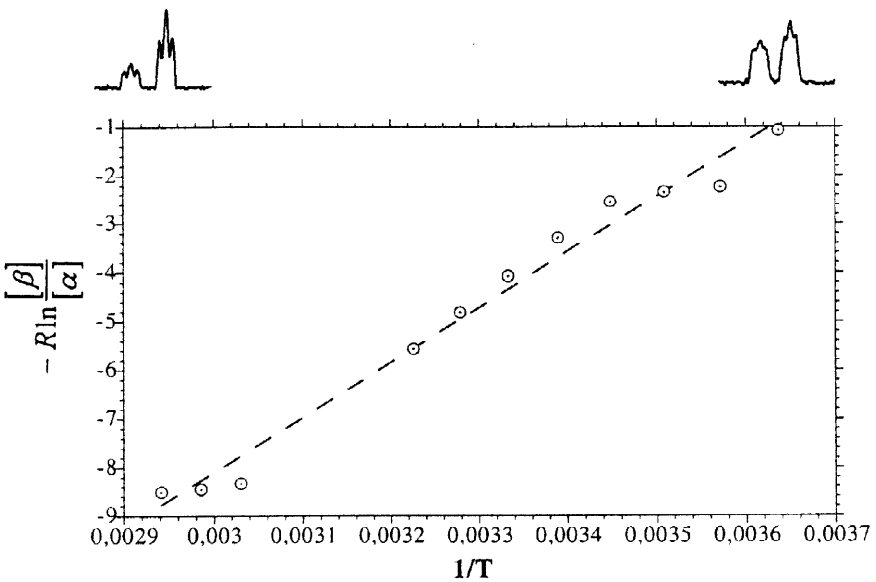


Fig. 5. Arrhenius plot for the exchange $\alpha \rightleftharpoons \beta$. The concentration ratios $[\alpha]$ and $[\beta]$ have been measured by integration of the signals marked with an asterisk in Fig.1c, which corresponds to the H2 protons of the two altrose units.

From the results presented above the following remarks can be made.

(i) Inclusion of the C8 spacer into one of the two cavities of the homo or hetero-dimer is a slow process relative to ^{1}H chemical shift timescale. It involves an exchange between the two "folded" conformations of the CD-dimers.

(ii) At ambient temperature there is a preference of the aliphatic chain to be included in the β-CD moiety rather than in the α-CD moiety. This is in contrast to what is observed when long aliphatic carbon chains are bound in cyclodextrins[13]. In these cases the α-CDs are the preferred host molecules. We attribute the observed phenomenon to the fact that the linker has to be curved in order to be included, thus requiring more space.

(iii) At high temperature the equilibrium is shifted towards inclusion of the spacer in the β-CD suggesting that the larger cavity of the latter cyclodextrin enables that the encapsulated hydrocarbon chain is more mobile.

In conclusion, we have demonstrated that selective excitation techniques combined with refocusing procedures can be a way to overcome the intrinsic limitations in resolution of 2D NMR. These powerful tools have enabled us to perform an in-depth study of the structure and dynamic behaviour of the new CD-dimers **2** and **4**. Two important results have been obtained. Firstly, it is shown that for **2** and **4** self-complexation of the linking aliphatic chain takes place through occupation of alternatively one of the two CD-cavities. Secondly, it is proven that such an inclusion process induces a structural modification of the involved cavity, the substituted altrose unit changing from a $^{1}C_{4}$ to a $^{4}C_{1}$ conformation. This transition to an energetically higher local conformational state which is compensated by the inclusion process, shows that cyclodextrins are not as rigid templates as usually assumed. The study of the C8 heterodimer has revealed some interesting dynamic aspects of the self-inclusion process, namely the preference of the aliphatic linker to be included in the β-CD cavity instead of in the α-CD cavity (an entropy driven process), which is in contrast to what is observed for pure intermolecular host-guest complexes, at ambient temperature.

Since self-complexation of the $(CH_2)_8$ chain of **2** and **4** competes with the binding of a ditopic guest molecule, incorporation of polar groups in the linker which prevents such a folding might afford a better host molecule. Studies are underway to test this hypothesis.

Experimental Part

^{1}H-NMR spectra were acquired at 600 MHz using a Bruker AMX-600 spectrometer equipped with a bath cooling unit. Band selective excitation and inversion were carried out via gaussian[20] 90° or 270° and gaussian cascade[21] Q3 pulses respectively, created by the SHAPE software package. COSY (COrrelation SpectroscopY), TOCSY (TOtal Correlation SpectroscopY), NOESY (Nuclear Overhauser Effect SpectroscopY) and ROESY (Rotating frame nuclear Overhauser Effect SpectroscopY) experiments were performed in phase sensitive mode using the time proportional phase incrementation method[22] (TPPI).

Acknowledgements

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References

1. Wenz, G. *Angew. Chem. Int. Ed. Engl.* **1994**, 33, 803-822.
2. Venema, F.; Baselier, C.M.; van Dienst, E.; Ruel, B.H.M.; Feiters, M.C.; Engbersen, J.F.J.; Reinhoudt, D.N.; Nolte, R.J.M. *Tetrahedron Lett.* **1994**, 35, 1773-1776.
3. Venema, F.; Baselier, C.M.; Feiters, M.C.; Nolte, R.J.M. *Tetrahedron Lett.* **1994**, 35, 8661-8664.
4. Venema, F.; Rowan, A.E.; Nolte, R.J.M. *J. Am. Chem. Soc.* **1996**, 118, 257-258.
5. Brüschweiler, R.; Griesinger, C.; Sörensen, O. W.; Ernst, R. R. *J. Magn. Reson.* **1988**, 78, 178-185.

6. Berthault, P.; Desvaux, H.; Perly, B. *Magn. Reson. Chem.* **1993**, 31, 259-265.
7. Macura, S.; Ernst, R.R. *Mol. Phys.* **1980**, 41, 95.
8. Aue, W.P.; Bartholdi, E.; Ernst, R.R. *J. Chem. Phys.* **1976**, 64, 2229-2246.
9. Eich, G.; Bodenhausen, G.; Ernst, R.R. *J. Am. Chem. Soc.* **1982**, 104, 3731-3732.
10. Bax, A.; Davis, D.G. *J. Magn. Reson.* **1985**, 65, 355-360.
11. Mareci, T.H.; Freeman, R. *J. Magn. Reson.* **1982**, 48, 158-163.
12. Berthault, P.; Perly, B. *J. Magn. Reson.* **1989**, 81, 631-634.
13. Guo, Q.-X.; Li, Z.Z.; Ren, T.; Zhu, X.-Q. *J. Incl. Phenom.* **1994**, 17, 149-156.
14. Desvaux, H.; Berthault, P.; Birlirakis, N.; Goldman, M.; Piotto, M. *J. Magn. Reson.* **1995**, A113, 47-52.
15. Jiang, T.; Sukumaran, D.K.; Soni, S.; Lawrence, D.S. *J. Org. Chem.* **1994**, 59, 5149-5155.
16. Breslow, R.; Czarnik, A.W.; Lauer, M.; Leppkes, R.; Winkler, J.; Zimmerman, S. *J. Am. Chem. Soc.* **1986**, 108, 1969-1979.
17. Ikeda, H.; Nagano, Y.; Du, Y.; Ikeda, T.; Toda, F. *Tetrahedron Lett.* **1990**, 31, 5045-5048.
18. Ogino, H. *J. Am. Chem. Soc.* **1981**, 103, 1303-1304.
19. Zimmermann, G.R.; Legault, P.; Selsted, M.E.; Pardi, A. *Biochemistry* **1995**, 34, 13663-13671.
20. Emsley, L.; Bodenhausen, G. *J. Magn. Reson.* **1989**, 82, 211-221.
21. Emsley, L.; Bodenhausen, G. *Chem. Phys. Lett.* **1990**, 165, 469.
22. Marion, D.; Wüthrich, K. *Biochem. Biophys. Res. Comm.* **1983**, 113, 967.