ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 329 (2000) 647–653

CARBOHYDRATE RESEARCH

Host–guest complex formation in cyclotrikis-(1→6)-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]

Paola Cescutti [a], Jean-Pierre Utille [b], Roberto Rizzo [a,*]

[a] *Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università di Trieste, Via Licio Giorgieri 1, I-34127 Trieste, Italy*

[b] *Centre de Recherches sur les Macromolécules Végétales (CERMAV), CNRS, BP 53, F-38041 Grenoble, France*

Received 6 April 2000; accepted 12 July 2000

Abstract

The possibility that cyclotrikis-(1→6)-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl] (CGM6) forms inclusion complexes, like cycloamyloses (cyclodextrins), was investigated by means of electrospray mass spectrometry and fluorescence spectroscopy. The complexing ability of both 1-anilinonaphthalene-8-sulfonate (ANS) and 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS), which were already used with cyclodextrins, was investigated. The former showed very little or no tendency to be complexed by CGM6, while the latter produced detectable adducts with CGM6. Fixed 90° angle light scattering experiments supported the findings obtained by molecular modelling calculations, which indicated a polar character for the CGM6 internal cavity. CGM6–TNS complexes were probably formed throughout interaction of the polar regions of the two molecules.

Keywords: Cyclooligosaccharides; Inclusion complexes; Cyclotrikis-(1→6)-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]; Mass spectrometry; Fluorescence

1. Introduction

Bacteria are able to synthesise a number of cyclooligosaccharides starting from either linear oligomers or carbohydrate polymers. Cyclic molecules exhibiting different glycosidic bonds have been isolated and recognised for a long time. Since their first characterisation, cyclomaltoses [1], cyclosophoraoses [2], cycloinulosaccharides [3] and cycloisomaltooligosaccharides [4] have been investigated for both their structural and biological properties. Among these systems, cyclomaltoses (cyclodextrins) have been extensively examined for their ability to form inclusion complexes with both aliphatic and aromatic molecules. As a consequence, a number of technological applications of these inclusion complexes have been exploited mainly aimed at improving product formulations, particularly in the pharmaceutical industry [1].

More recently, synthetic cyclooligosaccharides have been produced both to mime the chemical properties of the natural products and to provide different stereochemical motives, which could possibly confer new interesting complexing properties.

Abbreviations: CGM6, cyclotrikis-(1→6)-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]; ANS, 1-anilinonaphthalene-8-sulfonate; TNS, 2-*p*-toluidinylnaphthalene-6-sulfonate; GuHCl, guanidinium chloride; amu, atomic mass unit; ESMS, electrospray mass spectrometry; OR, orifice voltage; *R*, Rayleigh ratio.

* Corresponding author: Fax: +39-040-6763691.

E-mail address: rizzor@bbcm.univ.trieste.it (R. Rizzo).

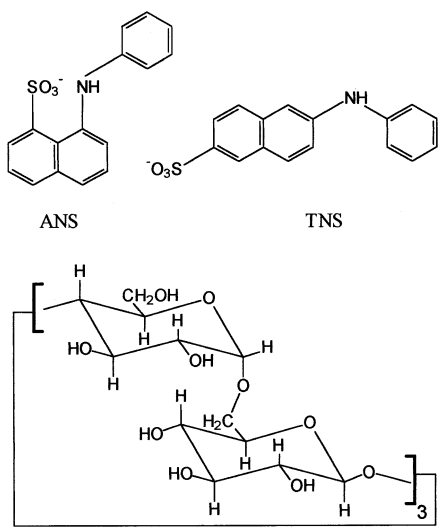
0008-6215/00/$ - see front matter
PII: S0008-6215(00)00217-2

A few years ago, different cyclooligosaccharides exhibiting both the α-(1→4) and the β-(1→6) stereochemical motif were synthesised [5], and the cyclotrikis-(1→6)-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl] (see Scheme 1) was specifically investigated for its conformational properties [6]. Studies on the host–guest complex formation ability of the cyclogentiomaltohexasaccharide (hereafter referred as CGM6) are reported in this paper. The inclusion ability was tested by means of electrospray mass spectrometry and fluorescence spectroscopy using two aromatic molecules exhibiting different molecular shapes: 1-anilinonaphthalene-8-sulfonate (ANS) and 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS) (see Scheme 1) [7,8]. In addition to this, simple fixed 90° angle light scattering experiments suggested some structural characteristics of water–CGM6 interaction.

2. Materials and methods

All chemicals were analytical grade; ANS, TNS and guanidinium chloride (GuHCl) were purchased from Sigma (USA). Solutions were always prepared using double distilled water; CH_3OH and CH_3CN for ESMS experiments were HPLC grade.

Mass spectra were recorded on a API-1 PE SCIEX quadrupole mass spectrometer equipped with an articulated ion spray and connected to a syringe pump for the injection of the sample. The instrument was calibrated using a polypropylene glycol mixture (3.3×10^{-5} M PPG 425, 1×10^{-4} M PG 1000, and 2×10^{-4} M PPG 2000), 0.1% MeCN, and 2 mM ammonium formate in 50% aq MeOH. The samples were injected at a flow rate of 5 μL/min, the ion spray voltage (ISV) was 5000 V and the orifice voltage (OR) was 50 V. The spectra were recorded using a step size of 0.1 amu. Some experiments were performed varying the OR from 50 to 180 V. Samples for mass spectrometry analysis were prepared according to the procedures described in Ref. [8].



CGM6

Scheme 1.

Fluorescence spectra were recorded with a Perkin–Elmer LS 50 B luminescence spectrometer. For ANS analysis, the instrumental parameters were: excitation wavelength 365 nm, fluorescence maximum 515 nm, slits (both excitation and emission) 7.5 nm, scan speed 50 nm/min. For TNS analysis: excitation wavelength 284 nm, fluorescence maximum 469 nm, slits (both excitation and emission) 7.5 nm, scan speed 50 nm/min. Experiments were carried out at 25 °C.

Fixed 90° angle light scattering data were obtained by means of the above described spectrofluorimeter irradiating the solutions at 365 and 284 nm for ANS and TNS solutions, respectively. Scattering intensity (detected at 90°) were obtained measuring the first armonic of the Rayleigh scattering at 730 and 568 nm for irradiation at 365 and 284 nm, respectively. GuHCl solutions for scattering experiments were 0.5 M.

NMR spectra were obtained at 298 K on a UNITY + 500 Varian™ spectrometer equipped with a Sun Spare IPX computer. Samples were dissolved in high quality D_2O. Reference shifts were taken from standard values. ^1H assignments were based on homo- and heteronuclear correlation spectroscopy.

3. Results and discussion

The modelling of the possible three-dimensional structures of different conformers generated by CGM6 has already been published

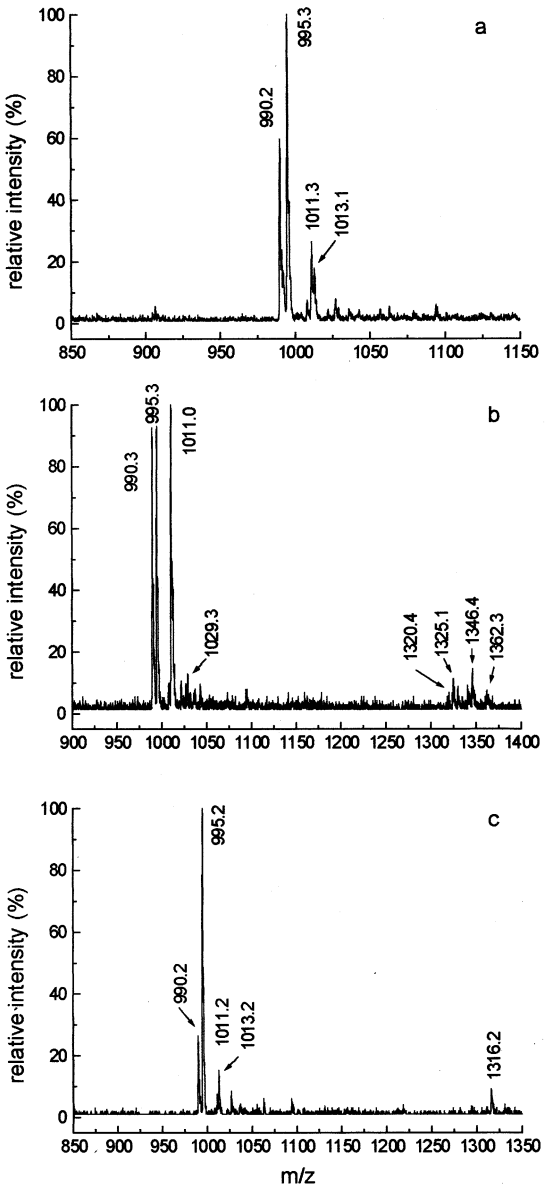


Fig. 1. Positive mode electrospray mass spectra of (a) CGM6; (b) a CGM6–TNS mixture and (c) a CGM6–ANS mixture, recorded at OR = 50 V.

Table 1
Assignment of the observed ions for ESMS experiments carried out on CGM6

m/z	Assignment
990.2	CGM6 + NH_4^+
995.3	CGM6 + Na^+
1011.3	CGM6 + K^+
1013.1	Linear CGM6 + Na^+
1967.5	2CGM6 + Na^+

[6]. By using both NMR measurements and molecular modelling calculations, Spieser et al. were able to define that in aqueous solutions CGM6 molecules gave rise to a number of different conformers that exhibited both symmetrical and non-symmetrical triangular overall shapes. Some of them were able to form a cavity whose diameters were in the 4–6 Å range. In other conformers, both residue tilting and hydroxymethyl group rotation caused fill-in of the cavity. Contrary to cyclomaltoses, the positions of both ring-oxygen atoms and hydroxymethyl groups conferred a polar character on the inner cavity. Therefore, the authors concluded that: "The polar cavity…would be more likely to chelate or coordinate ions or form complexes with polar guest molecules" [6].

Mass spectrometry experiments.—ESMS experiments on CGM6 in the presence of either ANS or TNS were carried out following the similar procedures used for cyclomaltoses [8]. In those experiments, mass spectra were recorded as a function of the orifice voltage set in the 50–130 V range. This methodology allowed us to distinguish between labile non-specific adducts and more stable inclusion complexes.

Mass spectra of CGM6 both alone and in the presence of either ANS or TNS are shown in Fig. 1. The characteristic peaks obtained for CGM6 are reported in Table 1. Besides molecular ions detected as ammonium, sodium and potassium adducts, other minor peaks were present in the spectrum and were assigned as follows. The peak at *m/z* 1013.1 was due to the linear hexamer obtained as side compound during the synthesis, while the peak at *m/z* 1967.5 corresponded to a cluster composed of one sodium ion and two CGM6 molecules, which disappeared by raising the orifice voltage (OR) up to 120 V.

The ESMS peak assignments relative to CGM6 in the presence of TNS are shown in Table 2. The abundance of potassium ion adducts was due to the use of TNS as K^+ salt. At OR = 50 V, a number of peaks produced by CGM6/TNS adducts was present. By raising the OR voltage up to 180 V, the presence of CGM6/TNS adducts was confirmed, as shown in Fig. 2, indicating that they were not labile species but rather stable complexes. It is also worth mentioning that high

OR voltages produced very little CGM6 fragmentation in comparison with that obtained with cyclodextrins under the same experimental conditions.

The investigation of possible CGM6–ANS complexes showed similar results. However, both the low number and the low intensity of the peaks relative to the complex (see Figs. 1 and 2 and Table 3) indicated that ANS was by far less effectively complexed by CGM6 than TNS, as confirmed by fluorescence spectroscopy experiments.

Fluorescence spectroscopy.—It is well known that the fluorescence yield of both ANS and TNS strongly increases when they are either dissolved in apolar solvents or complexed in binding sites not accessible to polar solvent molecules [9]. This behaviour was exploited to evaluate the complexation ability of different cyclomaltoses as a function of both host cavity dimension and guest molecule overall shape [7]. The ANS and TNS complexation ability of either α- and β-cyclodextrin is shown in Fig. 3 (**a** and **b**, respectively), where the increase of fluorescence intensity is plotted as a function of cyclo-oligosaccharide concentration. The increase of fluorescence intensity was higher in the presence of TNS than in the presence of ANS and this finding indicated that ANS was practically not included by α-cyclodextrin and included to a very little extent by β-cyclodextrin, as demonstrated in a previous paper [7] by means of different techniques.

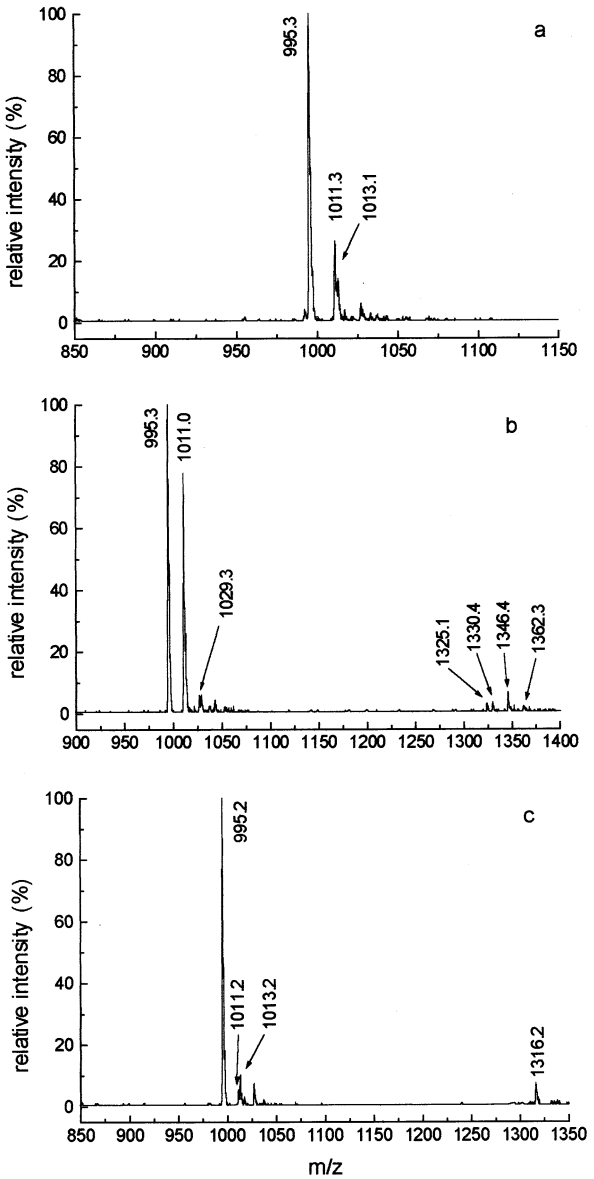


Fig. 2. Positive mode electrospray mass spectra of (a) CGM6; (b) a CGM6–TNS mixture and (c) a CGM6–ANS mixture, recorded at OR = 180 V.

Table 2
Assignment of the observed ions for ESMS experiments on CGM6 in the presence of TNS

m/z	Assignment
990.3	$CGM6+NH_4^+$
995.3	$CGM6+Na^+$
1011.0	$CGM6+K^+$
1029.3	Linear $CGM6+K^+$
1320.4	$CGM6+TNS^-+2NH_4^+$
1325.1	$CGM6+TNS^-+NH_4^++Na^+$
1330.4	$CGM6+TNS^-+2Na^+$
1341.2	$CGM6+TNS^-+NH_4^++K^+$
1346.4	$CGM6+TNS^-+K^++Na^+$
1362.3	$CGM6+TNS^-+2K^+$

Table 3
Assignment of the observed ions for ESMS experiments on CGM6 in the presence of ANS

m/z	Assignment
990.2	$CGM6+NH_4^+$
995.2	$CGM6+Na^+$
1011.2	$CGM6+K^+$
1013.2	Linear $CGM6+Na^+$
1316.2	$CGM6+ANS^-+2Na^+$

The fluorescence behaviour of ANS and TNS in the presence of CGM6 was very similar to that exhibited by α-cyclodextrin, as shown in Fig. 4, where data pertaining for both cyclooligosaccharides are reported. By assuming that both α-cyclodextrin and CGM6 possess an internal cavity, fluorescence experiments indicated that the host-to-guest fit in the presence of TNS could be considered very similar. However, as already mentioned, molecular modelling calculations strongly suggested that CGM6 is a rather flexible molecule, which could give rise to stable conformers exhibiting 'polar' cavity. Therefore, a similarity between the complexation mechanism of cyclomaltohexaose and that of CGM6 was hardly supported.

Some hints about the possible structure of the CGM6–TNS complexes were obtained by means of fixed 90° angle light scattering measurements.

Light scattering experiments.—The usefulness of light scattering experiments was suggested by the behaviour of the first armonic of the Rayleigh scattering as observed in fluorescence experiments. As a matter of fact, while the intensity of the scattered light was rather constant in experiments with α-cyclodextrin, it increased dramatically upon increasing the CGM6 concentration. The experimental find-

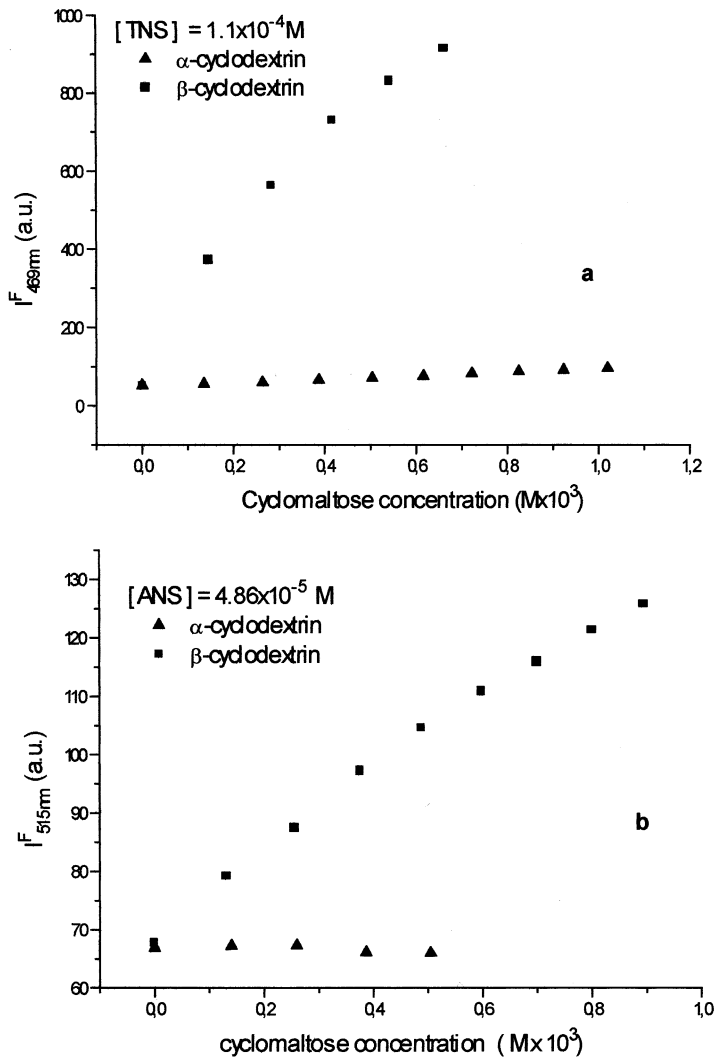


Fig. 3. Fluorescence intensity enhancement of (a) TNS in the presence of increasing amount of α-cyclodextrin (▲) and β-cyclodextrin (■); (b) ANS in the presence of an increasing amount of α-cyclodextrin (▲) and β-cyclodextrin (■).

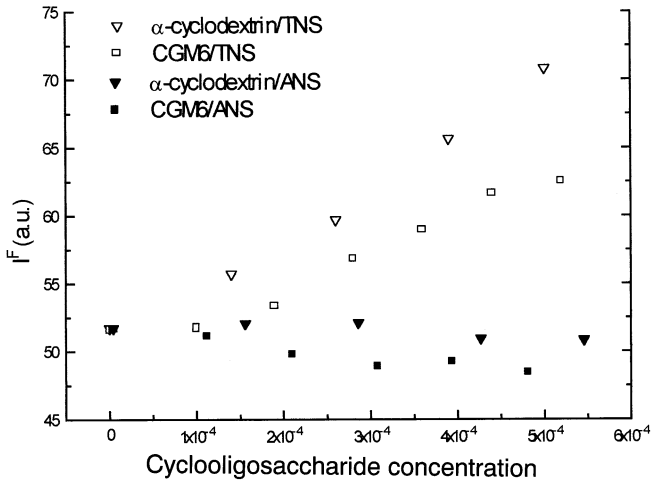


Fig. 4. Fluorescence intensity behaviour of ANS and TNS in the presence of increasing amounts of either α-cyclodextrin or CGM6. ANS and TNS concentrations as in Fig. 3.

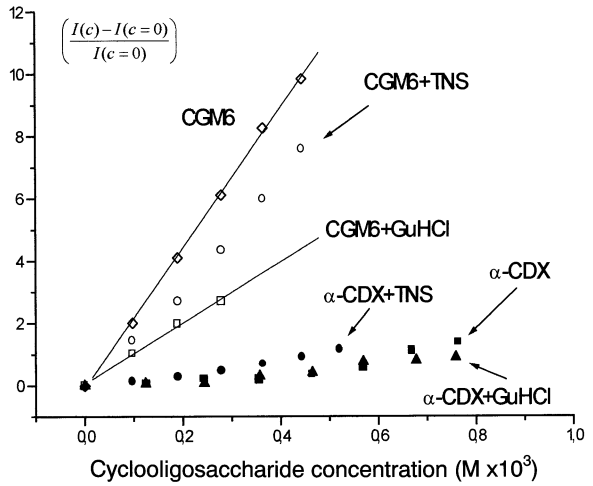


Fig. 5. Relative light scattering increase of CGM6 solutions (unshaded symbols) and α-cyclodextrin (α-CDX) solutions (shaded symbols) in the presence of different co-solutes. GuHCl refers to guanidinium chloride.

ings recorded as a function of cyclooligosaccharide concentration are shown in Fig. 5, where the scattering intensity was reported as relative intensity increase ($I_c - I_{c=0}$), normalised for the scattered intensity of solutions at zero cyclooligosaccharide concentration ($I_{c=0}$). In the following paragraphs, scattering data are strictly examined as a comparison between the behaviour of identical solutions of either α-cyclodextrin or CGM6.

As shown in Fig. 5, the slope of the scattering behaviour of CGM6 solutions was slightly decreased by the presence of TNS molecules and a further decrease was observed when the cahotropic agent guanidinium chloride (0.5 M) was added to a CGM6 solution. Contrary to this, α-cyclodextrin exhibited a similar scattering behaviour both in water and GuHCl solutions (Fig. 5). This observation allowed us to neglect refractive index variations when passing from water to GuHCl solutions.

The different behaviour of CGM6 with respect to α-cyclodextrin could suggest that the dimensions of the solute particles are different either because the two cyclooligosaccharides exhibit different size or because one of them may form rather large aggregates. However, this conclusion could be easily ruled out considering both the chemical similarity of the two systems and the CGM6 geometrical features obtained by molecular modelling. The hypothesis that CGM6 could give rise to multimolecular aggregates (large solute particles) was also ruled out by inspecting its high resolution ^{1}H NMR spectrum (Fig. 6). This was obviously obtained using a solute concentration higher than that used for light scattering experiments; nevertheless, it gave no records of resonance peak splitting.

An alternative explanation for the slope of the scattering curves could be assumed taking into account that the scattering intensity (or the Rayleigh ratio, R) depends on the solute–solvent structure in solution and, in particular, on the possibility that water molecules are structured around the solute. As a matter of fact, R is the sum of three contributions: $R = R_d + R_c + R_o$, where R_d takes into account density fluctuations, R_c accounts for concentration fluctuations and R_o is the anisotropic contribution, which is a measure of the ordering of the molecules [10].

In the system under investigation, the formation of small water cluster around CGM6 molecules may not be excluded taking into account the information acquired by molecular modelling. Many of the hydrophilic groups in the CGM6 are directed towards the inner part of the molecule. Therefore, less polar groups are exposed to the solvent possibly giving rise to a hydrophobic interaction, which could produce a water cluster. As a matter of fact, the addition of guanidinium

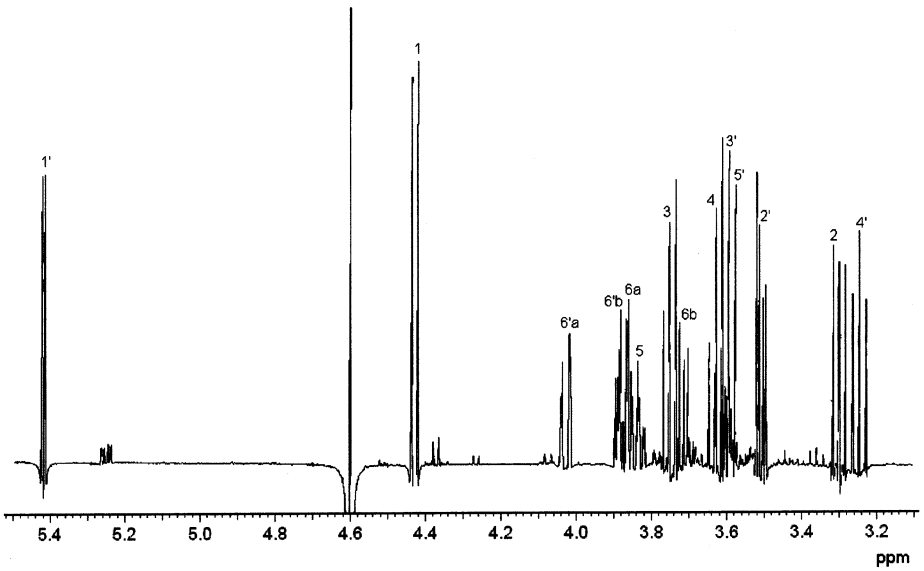


Fig. 6. ^{1}H NMR spectrum of CGM6. Assignments are indicated in the picture: numbers refer to the sugar carbon atom numbering. Primes indicate (1→4)-linked residue carbon atoms; **a** and **b** refer to the two protons of the methylene groups (see Ref. [6]).

chloride, which is known to perturb the water structure, significantly lowered the slope of the scattered light curves obtained as a function of increasing CGM6 concentration.

4. Conclusions

Binding studies carried out by means of both electrospray mass spectrometry and fluorescence spectroscopy indicated that amphyphyllic molecules like TNS are able to interact with CGM6. In addition to this, fixed 90° angle light scattering data are in agreement with the results of molecular modelling calculations, which suggested the presence of a polar cavity in the solution conformation of CGM6. Therefore, it is very realistic that TNS does not interact with CGM6 through its aromatic moiety, as with cyclomaltoses. The complex could be formed rather by inserting the sulfate group, which is directed along the major axis of the molecule, into the polar CGM6 cavity. It is worth stressing that none of the known cyclosaccharidic compounds exhibit complexing properties similar to those of cyclodextrins.

Acknowledgements

This work was carried out with the financial support of the University of Trieste. The 'Centro Servizi Polivalenti di Ateneo' of the University of Trieste is acknowledged for ESMS experiments.

References

[1] V.T. D'Souza, K.B. Lipkowitz, *Eds. Chem. Rev.*, 98 (1998) 1741–2076.
[2] M.W. Breedveld, K.J. Miller, *Microbiol. Rev.*, 58 (1994) 145–161.
[3] M. Kawamura, T. Uchiyama, T. Kuramoto, Y. Tamura, K. Mizutani, *Carbohydr. Res.*, 192 (1989) 83–90.
[4] T. Oguma, T. Horiuchi, M. Kobayashi, *Biosci. Biotech. Biochem.*, 57 (1993) 1225–1227.
[5] H. Driguez, J.-P. Utille, *Carbohydr. Lett.*, 1 (1994) 125–128.
[6] S. Spieser, K. Mazeau, M.C. Brochier, C. Gey, J-P. Utille, F.R. Taravel, *Glycoconjugate J.*, 15 (1998) 511–521.
[7] V. Crescenzi, A. Gamini, A. Palleschi, R. Rizzo, *Gazz. Chim. Ital.*, 116 (1986) 435–440.
[8] P. Cescutti, D. Garozzo, R. Rizzo, *Carbohydr. Res.*, 290 (1996) 105–115.
[9] W.O. McClure, G.M. Edelman, *Biochemistry*, 5 (1966) 1908–1918.
[10] F. Franks, in F. Franks (Ed.), *Water. A Comprehensive Treatise*, Vol. 4, Plenum Press, New York, 1975, p. 54 (Chapter 1).