Carbohydrate Research, 237 (1992) 1–10
Elsevier Science Publishers B.V., Amsterdam

Circular dichroism and ultraviolet spectroscopy of complexes of amylose *

Günter Wulff and Stefan Kubik

Institut für Organische Chemie und Makromolekulare Chemie, Heinrich-Heine Universität Düsseldorf, Universitätsstr. 1, 4000 Düsseldorf (Germany)

(Received December 10th, 1991; accepted April 9th, 1992)

ABSTRACT

The circular dichroism of the complexes formed between slightly hydroxypropylated amylose and cyclomalto-hexaose, -heptaose, and -octaose (α-, β-, and γ-cyclodextrin) with nine different achiral ketones and phenolphthalein has been investigated. In the complexes with the ketones, the amylose helix has a conformation with six glucose units per turn, whereas, in the complex with phenolphthalein, it has seven.

INTRODUCTION

In recent years, complex molecules have been synthesised which are used as hosts for certain guest molecules[1]. Typical natural host–guest systems are enzymes, amylose, and cyclomalto-oligosaccharides (cyclodextrins, CDs). Amylose, a (1 → 4)-α-D-glucan, can adopt a helical conformation with the hydroxyl groups of the glucose units on the outer surface. CDs are formed from amylose by enzymic digestion[2].

CDs and amylose form complexes with the guest molecules included in the mainly non-polar cavity of the helix or the ring, respectively. Whereas CDs have rather rigid molecules, the amylose chain is flexible. Electron diffraction measurements, using crystallised amylose complexes, indicate that, depending on the size of the guest molecule included, a helix with six, seven, or eight glucose units per turn can be formed[3].

C.d. spectroscopy is useful for the study of complexes where an achiral molecule with a suitable chromophore is included in the chiral cavity of a CD, because of the induced Cotton effects[4]. The blue iodine–amylose complex has been studied in

Correspondence to: Professor G. Wulff, Institut für Organische Chemie und Makromolekulare Chemie, Heinrich-Heine Universität Düsseldorf, Universitätsstr. 1, 4000 Düsseldorf, Germany.

* Helical Amylose Complexes with Organic Complexands, Part II. For Part I, see ref. 8.

a similar manner[5]. Cotton effects occur when Congo Red and other azo-dyes form complexes with amylose[6], but they have been interpreted in terms of an adsorption of the dye molecule on the surface of the helix.

The inclusion complexes of amylose with organic molecules usually precipitate from solution and there have been few reports about their structures in solution[7]. However, the complexes of slightly hydroxypropylated amylose are soluble and can be studied by c.d. spectroscopy[8]. The hydroxypropylated amylose used, and referred to subsequently as amylose, had a degree of substitution of 0.075 (i.e., only one substituent per thirteen glucose units). This substitution did not alter the complexing behaviour significantly but markedly improved the solubility of the complexes. By comparing the c.d. spectrum of the 4-*tert*-butylphenol complex of amylose with those of the corresponding CD complexes, it was concluded that the amylose forms a helix with seven or eight glucose units per turn[8]. We now report the c.d. spectra of complexes of amylose and CDs with some achiral ketones and phenolphthalein.

RESULTS AND DISCUSSION

When an achiral chromophore is introduced into the chiral cavity of a CD molecule, it is usually possible to observe an induced Cotton effect[4]. The structure of the complex between the CD and the guest molecule is influenced by the size of the CD cavity, the shape of the guest molecule, and the interactions of the hydroxyl groups of the glucose units with the guest molecule. C.d. spectroscopy allows the geometry of the insertion of the guest molecules to be investigated because the induced Cotton effects depend, for example, on whether the electronic transition moment of the guest molecule is arranged parallel or orthogonal to the central axis of the CD molecule[9].

C.d. spectra of complexes with aliphatic ketones.—The carbonyl group of an aliphatic ketone usually exhibits[10] a Cotton effect at the wavelength of the $n \rightarrow \pi^*$ absorption when included in a CD. The c.d. spectra for the complexes of nine different ketones with cyclomalto-hexaose (αCD), -heptaose (βCD), and -octaose (γCD) have been measured. The ketones were chosen to test a range of molecular shapes. Furthermore, the c.d. spectra of the same ketones with the slightly hydroxypropylated amylose have been recorded in solution. The inclusion complexes of underivatised amylose with these ketones are insoluble. Fig. 1 shows the results for 2-hexanone. The spectra are presented as recorded. Since no attempt was made to shift the equilibrium to the side of the complex, the molecular ellipticity could not be calculated.

The complex of 2-hexanone with αCD has a negative Cotton effect at 272 nm, whereas that with βCD has a positive effect. The c.d. spectrum of the amylose complex is analogous to that of the αCD complex. The results for the other ketones are summarised in Table I. The shapes of the spectra are essentially the same as those in Fig. 1.

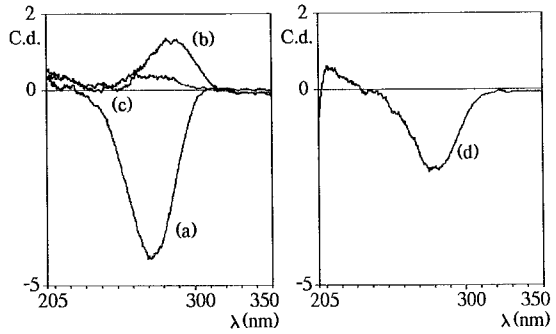


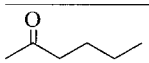
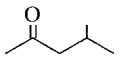
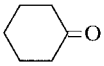
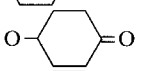
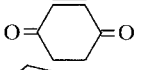
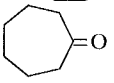
Fig. 1. C.d. spectra of 2-hexanone complexed with αCD (a), βCD (b), γCD (c), and amylose (d).

The data in Table I show that only the acyclic ketones and 4-methylcyclohexanone yield different c.d. spectra for αCD and βCD. Only four of the ketones showed Cotton effects when included in amylose, and the spectra were analogous to those of the αCD complexes.

C.d. spectra of complexes with phenolphthalein.—None of the amylose–ketone complexes exhibited a c.d. spectrum analogous to that of the βCD complex. However, since 4-*tert*-butylphenol has a c.d. spectrum analogous to that of the

TABLE I

Sign [a] of the observed Cotton effects at the wavelength of the $n \rightarrow \pi^*$ absorption of each ketone complexed with amylose and CDS

Ketone	αCD	βCD	γCD	Amylose
	−	+	+	−
	−	+	+	−
	−	+	0	0
	−	+	+	−
	−	0	0	0
	−	0	0	0
	+	−	−	0
	−	−	0	−
	−	−	−	0

[a] +, positive; −, negative; 0, no Cotton effect.

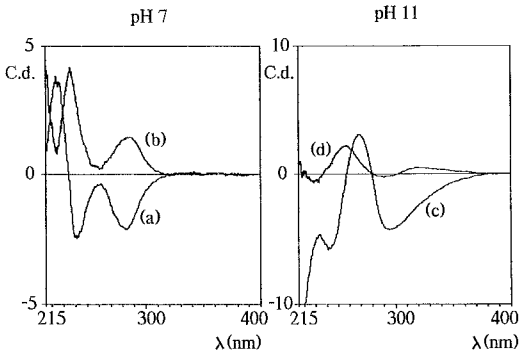


Fig. 2. C.d. spectra of phenolphthalein complexed with βCD at pH 7 (a) and 11 (c), and with γCD at pH 7 (b) and 11 (d).

βCD complex when included in amylose[8], other phenols were investigated. Phenolphthalein forms stable complexes with βCD at pH 7 and 11 which exhibit rather complex c.d. spectra[11]. The purple colour of a solution of phenolphthalein at pH 11 disappears after the addition of βCD. This effect has been interpreted in terms of a strong interaction of phenolphthalein and βCD, which forces the aromatic rings into a non-planar conformation, probably due to the formation of a lactone ring which normally is hydrolysed in alkaline medium[12].

Fig. 2 shows the different c.d. spectra of the complexes of phenolphthalein with βCD and γCD at pH 7 and 11; no Cotton effects were observed with αCD.

Fig. 3 shows the c.d. spectra of the complexes of phenolphthalein with slightly hydroxypropylated amylose, which are comparable only with the c.d. spectra of the βCD complex.

The disappearance of the purple colour during the formation of the βCD–phenolphthalein complex at pH 11 allows the stability constant k_s to be determined. By measuring the absorption of a series of solutions with a fixed concentration of

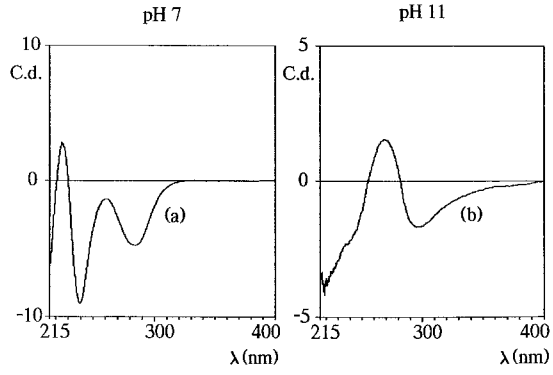


Fig. 3. C.d. spectra of phenolphthalein complexed with amylose at pH 7 (a) and 11 (b).

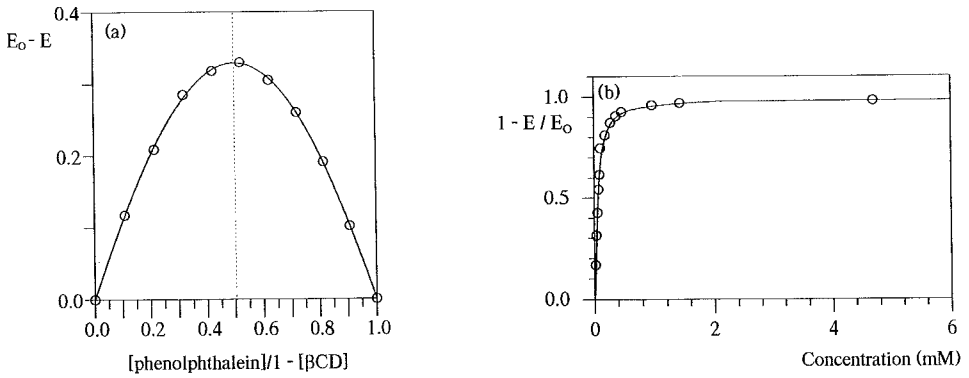


Fig. 4. Determination of (a) the stoichiometry and (b) the stability constant, of the βCD-phenolphthalein complex at pH 11.

phenolphthalein and increasing concentrations of βCD, a graph can be obtained from which k_s can be calculated by non-linear regression[13]. The stoichiometry of the complex was determined by the method of continuous variations[14]. The results are shown in Fig. 4.

The ratio of host (H) and guest (G) molecules in the complex is 1:1; and k_s is given by:

$$k_s = \frac{[HG]}{[H] \cdot [G]}$$

With $[H] = [H]_0 - [HG]$, $[G] = [G]_0 - [HG]$, and $[HG]/[G]_0 = (E - E_0)/(E_{max} - E_0)$, a quadratic equation is obtained which can be solved, namely,

$$\frac{E - E_0}{E_{max} - E_0} = \frac{x + k + 1}{2} - \sqrt{\frac{(x + k + 1)^2}{4} - x}$$

$$x = [H]_0/[G]_0;\ k = 1/k_s[G]_0,$$

where $[H]_0$ and $[G]_0$ are the initial concentrations of the host and guest, respectively, and E, E_0, and E_{max} are the absorption, the absorption of the guest, and the absorption of the complex, respectively. E_{max} was assumed to be zero since the colour of the solution of phenolphthalein completely disappeared[15] after the addition of a large excess of βCD. By variation of k_s, the theoretical binding-isotherms can be calculated. The stability constant is given by the curve which best fits the observed curve. Hence, k_s was determined to be 33 000. This result is in good agreement with reported values (31 000[15] and 37 000[16]).

The stoichiometry of the amylose–phenolphthalein complex can be obtained by the method of continuous variations[14]. Fig. 5 shows the Job plot for an amylose of $\overline{M}_n$ 3.7×10^5. In contrast to the βCD complex, the ratio of host to guest is 1:2. For amyloses with $\overline{M}_n$ 0.8×10^5 and 4.9×10^5, similar results were obtained. Hence, only two molecules of phenolphthalein are included in the amylose chain.

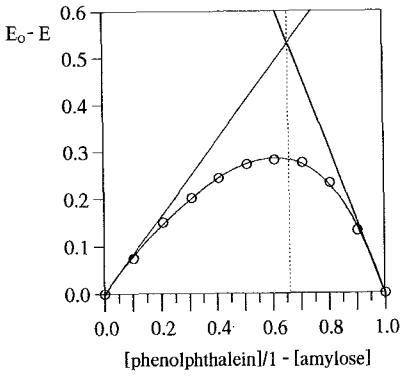


Fig. 5. Determination of the stoichiometry of the amylose–phenolphthalein complex at pH 11.

The stability constant for the amylose–phenolphthalein complex was determined for three amyloses of different molecular weight.

Fig. 6 shows that the stability constants for the amyloses of high molecular weight are of the same order. The complex of the amylose with $\overline{M_n}$ 0.2×10^5 is less stable. Due to the different stoichiometry compared with the βCD complex, another equation for the determination of k_s was used. The formation of a 1:2 complex is a two-step reaction with the stability constants k_{s1} and k_{s2}.

$$H + G \rightleftharpoons HG \qquad k_{s1}$$

$$HG + G \rightleftharpoons HG_2 \qquad k_{s2}$$

The overall stability constant k_s for the reaction $H + 2\,G \rightleftharpoons HG_2$ is given by:

$$k_s = k_{s1} \cdot k_{s2} = \frac{[HG_2]}{[H] \cdot [G]^2}$$

With $[H] = [H]_0 - [HG_2]$, $[G] = [G]_0 - 2[HG_2]$, and $2[HG_2]/[G]_0 = (E - E_0)/(E_{max} - E_0)$, a cubic equation is obtained. At the beginning of the host–guest titration, $[HG_2] \ll 1$. Hence, $[HG_2]^3 \approx 0$ and the equation can be solved by

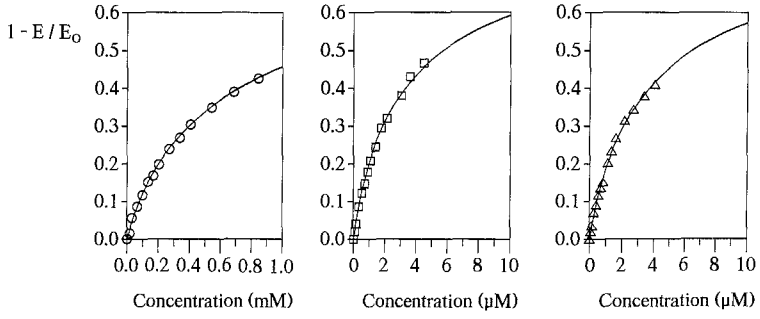


Fig. 6. Determination of the stability constant of the amylose–phenolphthalein complex at pH 11 (○, $\overline{M_n}$ 0.2×10^5; □, $\overline{M_n}$ 3.7×10^5; △, $\overline{M_n}$ 4.9×10^5).

neglecting the cubic term. However, this approximation is valid only when the concentration of the complex is low.

$$\frac{E - E_0}{E_{max} - E_0} = \frac{x + k + 4}{4x + 4} - \sqrt{\frac{(x + k + 4)^2}{(4x + 4)^2} - \frac{1}{x + 1}}$$

$x = [G]_0/[H]_0$; $k = 1/k_s[G]_0[H]_0$

The stability constants for the amylose–phenolphthalein complexes were determined to be 1.9×10^9 for $\overline{M_n}$ 4.9×10^5, 2.1×10^9 for $\overline{M_n}$ 3.7×10^5, and 9.4×10^7 for $\overline{M_n}$ 0.2×10^5, with an error of $\pm 5\%$. The calculated and observed curves showed good correlation and indicated that no large error was made by neglecting the cubic term in the calculation and that a 1:2 complex of amylose with phenolphthalein is reasonable.

The acyclic ketones and 4-methylcyclohexanone exhibit Cotton effects with opposite sign when included into αCD or βCD (Table I), which could reflect different modes of inclusion.

For complexes of substituted benzene and naphthalene derivatives[9], positive Cotton effects are observed if the electronic-transition moment of the chromophore is arranged parallel to the central axis of the CD. On the other hand, if the transition moment is arranged orthogonal to the axis, then negative effects are obtained; there is no effect if it forms an angle of 30° with the axis[9]. However, to our knowledge, no such rules have been assigned for the carbonyl group.

There are mainly two geometries for the arrangement of a carbonyl group in the cavity of a CD as presented in Fig. 7. Since the carbonyl group has a weak electronic-transition moment parallel to the C–O bond[17], geometries A and B should lead to positive and negative Cotton effects, respectively. If only steric factors are taken into account (size of the guest molecule, diameter of cavity), it seems reasonable that the acyclic ketones should exhibit negative Cotton effects with αCD (geometry B) and positive effects with the larger βCD (geometry A). In contrast, 4-methylcyclohexanone seems to be included into αCD with geometry A and in βCD with a geometry more like B.

For the interpretation of the c.d. spectra of the other cyclic ketones, the conformation of the guest molecules also has to be taken into account. X-ray

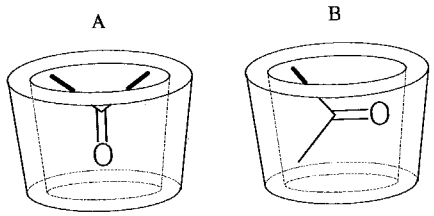


Fig. 7. Possible geometries for the inclusion of a carbonyl group in cyclomalto-oligosaccharides.

analysis of the αCD–cyclopentanone complex showed that the guest molecule adopts a chiral twist conformation in the cavity of the CD, which leads to a negative Cotton effect[18]. This situation may apply to the other cyclic ketones since each exhibited a negative peak in the induced c.d. spectra. The different behaviour of 4-methylcyclohexanone could be due to the fact that the energy barrier between the chair and the twist conformations is too high. In the twist conformation, the methyl group is in a more axial, hence, unfavourable position.

The inclusion complexes of 2-hexanone, 4-methyl-2-pentanone, dicyclopropyl ketone, and 1,4-cyclohexanedione with amylose exhibited negative Cotton effects in their induced c.d. spectra. Each spectrum was analogous to that of the corresponding αCD complex. Thus, it is concluded that amylose has a conformation with six glucose units per helix turn in these complexes. Not even the bulkier ketones seem to be able to force the amylose into a conformation with a larger cavity. However, in the complex with phenolphthalein, amylose is analogous to βCD, as can be seen by comparing the c.d. spectra. In this complex, the amylose helix should have seven glucose units per turn.

The main difference in the complexes of amylose and βCD with phenolphthalein is the stoichiometry, with one molecule of phenolphthalein included in the latter and two molecules in the former. This stoichiometry is independent of the chain length of the amylose. Even amylose with a $\overline{M_n}$ as high as 4.9×10^5 can include only two molecules of phenolphthalein, which may reflect its size. It is unlikely that such a large molecule could be complexed in the middle of the helical domain, and inclusion occurs only at each end of the amylose chain. Probably only part of the phenolphthalein molecule enters the helix. With smaller guest molecules, complexation along the whole helical domain should be possible, so that the number of complexed guest molecules should increase with increasing chain length.

The overall stability constants (k_s) for the phenolphthalein complexes with the amyloses of high molecular weight are rather large. It is assumed that k_{s1} and k_{s2} for each step of complex formation are of the same order. It is unlikely that the inclusion of one phenolphthalein molecule in the amylose helix influences the inclusion of the second one at the other end of the chain. With this assumption, the relation $k_{s1} \approx k_{s2} \approx 45\,000$ is obtained. This value is even larger than the stability constant of the corresponding βCD complex. Therefore, the amylose–phenolphthalein complex seems to be somewhat more stable.

Amylose with a $\overline{M_n}$ of 0.2×10^5 forms a much less stable complex with phenolphthalein. This dependence of the stability constant on the molecular weight of amylose accords with the results of microcalorimetry[8]. However, using microcalorimetry, the formation of a complex of amylose and a guest molecule which induced a helix with more than six glucose units per turn could be detected only for amylose with $\overline{M_n}$ higher than 0.4×10^5. In contrast, phenolphthalein is complexed by an enlarged helix of amylose with $\overline{M_n}$ 0.2×10^5, due to the fact that the complexation occurs only at the more flexible chain ends of the helix.

EXPERIMENTAL

Amylose (Aldrich) and the cyclodextrins (Roth) were commercial products. Slightly hydroxypropylated amylose was synthesised as described[8]. The ketones were purified by distillation before use, except for 1,4-cyclohexanedione and phenolphthalein which were recrystallised twice.

C.d. spectroscopy.—Spectra were recorded with a JASCO J-600 c.d. spectropolarimeter with 1-cm cuvettes. The following aqueous stock solutions were prepared: 0.102 M 2-hexanone, 0.089 M 4-methyl-2-pentanone, 0.087 M 2,2-dimethyl-3-butanone, 0.043 M dicyclopropyl ketone, 0.124 M cyclopentanone, 0.122 M cyclohexanone, 0.126 M 4-methylcyclohexanone, 0.079 M 1,4-cyclohexanedione, 0.100 M cycloheptanone, and 1.38×10^{-4} M phenolphthalein.

CD (50 mg) or hydroxypropylated amylose (200 mg) was dissolved in 1 mL of phosphate buffer (pH 7.0) and 5 mL of the corresponding stock solution. For phenolphthalein, carbonate buffer (pH 11.0) was used for the alkaline solutions. The volume was made up to 10 mL. The solution was stored overnight, and c.d. spectra were recorded.

Host–guest–titration.—Amylose with $\overline{M_n}$ 4.9×10^5 was obtained by fractionation of potato starch. Commercial amylose had $\overline{M_n}$ 3.7×10^5. Amylose with $\overline{M_n}$ 0.2×10^5 was obtained by treatment of commercial amylose (20 g) at 90° in 0.2 M HCl (1 L). After 1 h, the suspension was neutralised, and the product was filtered off, washed repeatedly with water, and dried in vacuo. The amylose with $\overline{M_n}$ 0.8×10^5 was synthesised enzymically[19]. Molecular weight distribution was determined by gel-permeation chromatography on a Waters hydrogel column.

Increasing amounts of hydroxypropylated amylose were dissolved in 8 mL of water with gentle heating. After cooling, 1 mL of a 8.2×10^{-6} M phenolphthalein stock solution (pH 11, carbonate buffer) was added, and the solution was made up to 10 mL. The ratio glucose units : phenolphthalein was in the range 250–10 000. The absorption was measured at 553 nm after 2 h in 1-cm cuvettes. The absorption for complete complexation was assumed to be zero. The stability constant of the βCD complex was determined in the same way. Here, the glucose units : phenolphthalein ratio was in the range 2–500 (3.7×10^{-4} M phenolphthalein).

ACKNOWLEDGMENT

This work was supported by Bundesminister für Forschung und Technologie under the registry number 0319057A.

REFERENCES

1 D.J. Cram, *J. Inclusion Phenom.*, 6 (1988) 387–413.
2 M. Florkin and E.H. Stolz (Eds.), *Comprehensive Biochemistry*, Vol. 13, Elsevier, Amsterdam, 1973, p 141.

3 Y. Yamashita, *J. Polym. Sci., Part A*, 3 (1965) 3251–3260; Y. Yamashita and N. Hirai, *J. Polym. Sci., Part A-2*, 2 (1966) 161–171; Y. Yamashita and K. Monobe, *ibid.*, 9 (1971) 1471–1481.
4 K. Sensse and F. Cramer, *Chem. Ber.*, 102 (1969) 509–521; K. Takeo and T. Kuge, *Staerke*, 24 (1972) 281–320.
5 B. Pfannemüller and G. Ziegast, *Int. J. Biol. Macromol.*,4 (1982) 9–17.
6 R.C. Schulz and J.A. Trisnadi, *Makromol. Chem.*, 177 (1976) 1771–1778; M.K. Pal and A. Roy, *Makromol. Chem. Rapid Commun.*, 6 (1985) 749–754.
7 A. Hayashi, K. Kinoshita, and Y. Kotani, *Agric. Biol. Chem.*, 47 (1983) 1705–1709; J.F. Robyt, J.L. Jane, and D.H. Huang, *Carbohydr. Res.*, 140 (1985) 21–35.
8 G. Wulff and S. Kubik, *Makromol. Chem.*, 193 (1992) 1071–1080.
9 K. Harata and H. Uedaira, *Bull. Chem. Soc. Jpn.*, 48 (1975) 375–378; H. Shimuzu, A. Kaito, and M. Hatano, *ibid.*, 52 (1979) 2678–2684; M. Kajtar, C. Horvath-Toro, E. Kuthi, and J. Szejtli, *Acta Chem. Acad. Sci. Hung.*, 110 (1982) 327–355.
10 M. Otagiri, K. Ikeda, K. Uekama, O. Ito, and M. Hatano, *Chem. Lett.*, (1974) 679–682.
11 L. Barcza, A. Buvari-Barcza, and M. Kajtar, *J. Chem. Soc., Perkin Trans. 2*, (1988) 1687–1690.
12 N. Yoshida, T. Shirai, and M. Fujimoto, *Carbohydr. Res.*, 192 (1989) 291–304.
13 G. Wenz and E. von der Bey, in O. Huber and J. Szejtli (Eds.), *Proc. Symp. Cyclodextrins, 4th*, Kluwer Academic Press, Dordrecht, 1988, p. 133–138.
14 K.A. Connors, *Binding Constants*, 1st ed., Wiley, New York, 1987.
15 T. Okubo and M. Kuroda, *Macromolecules*, 22 (1989) 3936–3940.
16 K. Taguchi, *J. Am. Chem. Soc.*, 108 (1986) 2705–2709.
17 J.W. Sidman, *Chem. Rev.*, 58 (1958) 689–713; C.N.R. Rao, G.K. Goldman, and A. Balasubramanian, *Can. J. Chem.*, 38 (1960) 2508–2513.
18 G. Tsoucaris, G. Le Bas, N. Rysanek, and F. Villain, *J. Inclusion Phenom.*, 5 (1987) 77–84.
19 G. Ziegast and B. Pfannemüller, *Carbohydr. Res.*, 160 (1987) 185–204.