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# Stereoselective thermal transfer of fructose from sucrose to cyclodextrins

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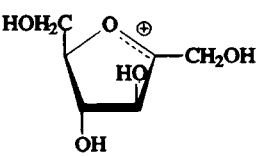
**Abstract**

Branched cyclodextrins (CDs) have been formed by the thermal transfer of a fructosyl group from sucrose to O-6 of one of the glucose residues of cyclomaltohexa- and hepta-ose ($\alpha$-CD and $\beta$-CD). In each case the fructosyl group adds almost entirely in the $\beta$ configuration. The resultant fructosylcyclodextrins (Fru-CDs) show increased solubility in water and, in the case of Fru-$\beta$-CD increased ability to solubilize sparingly soluble compounds by inclusion, relative to the parent cyclodextrins. However, the Fru-CDs have similar abilities to form complexes as their respective parent CDs. Fru-CDs act as inhibitors of invertase.

*Keywords:* Stereoselective thermal transfer; Fructose; Sucrose; Cyclodextrins

## 1. Introduction

During the melt thermolysis of anhydrous, acidified sucrose, the fructosyl cation, **1**, is formed. This cation can react with hydroxyl nucleophiles, present in the melt.

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We have used this reaction to form kestoses [1], higher oligosaccharides and anhydro sugars [2] and a fructoglucan polymer [3]. The thermal transfer of fructose to amylose and amylopectin has been achieved in dimethyl sulfoxide solution (preceding paper). Cyclodextrins have now been chosen as suitable models to initiate our planned studies of the fructosylation of polymers in the melt. Cyclomaltohexaose and cyclomaltoheptaose were allowed to react with anhydrous, acidified sucrose in the melt. These melts were produced at temperatures below the melting points of the crystalline CDs by freeze-drying aqueous solutions of the CDs together with sucrose and the citric acid catalyst. In each case the major product was a branched cyclodextrin resulting from the addition of a β-fructofuranosyl group to O-6 of one of the glucose residues.

Branched cyclodextrins having one or more glucopyranosyl residues as side-chains have been produced using enzymes, see for example Ref. [4]. Branched CDs have similar complexation abilities to the parent CDs, but show increased solubility in water and aqueous methanol and also increased ability to solubilize sparingly soluble compounds by inclusion [5,6] and therefore may have increased usefulness in the food and drug industries. Branched CDs carrying β-D-galactosyl residue(s) may have a possible use as drug carriers that will bind to specific receptors in liver cells [7].

## 2. Results and discussion

By freeze-drying an aqueous solution, a mixture of β-CD with a five-fold excess of sucrose and the citric acid catalyst was obtained that melted to a colorless liquid at 115°C. This mixture was thermolyzed for 30 min at 120°C and the resultant melt examined by LC using two different systems. In an $NH_2$-bonded silica column with acetonitrile–water eluant a product, **2**, was observed; its running more slowly than the parent CD indicated increased molecular size [8]. With a $C_{18}$-bonded silica column and methanol–water eluant, **2** ran more rapidly than the parent CD, indicating greater solubility in water [8] and this system was employed for preparative LC. The yield of **2** based on relative LC peak areas was 21% of the starting CD. Attempts to precipitate **2** from an aqueous solution of the thermolysis product mix using ethanol–acetone or cyclohexane [9] resulted in augmentation of the parent CD in the precipitate, presumably because of the greater solubility of the complexes of **2**.

In addition to **2**, a lesser amount of another faster-running product, **3**, was isolated by preparative LC. A further faster-running peak proved to be a highly-colored polymeric material and was probably an unsaturated fructose degradation product.

Colorimetric assay [3] of **2** and **3** gave glucose:fructose ratios of 6:1 and 2.3:1, respectively (expected 7:1 for mono-substitution and 3.5:1 for di-substitution). Methylation analysis of **2** using mild hydrolysis [3] revealed the presence of a fructofuranosyl group linked at only C-2. Strong hydrolysis [3] of per-*O*-methylated **2** demonstrated the presence of glucopyranosyl residues linked 1,4 and 1,4,6 in the ratio 6:1. A similar treatment of **3** showed the presence of a variety of fructosyl residues and 1,4- and 1,4,6-linked glucopyranosyl residues in the ratio 4.5:1. It was decided that **3** represented a mixed product and so it was not pursued further. It is however very probable that **3**

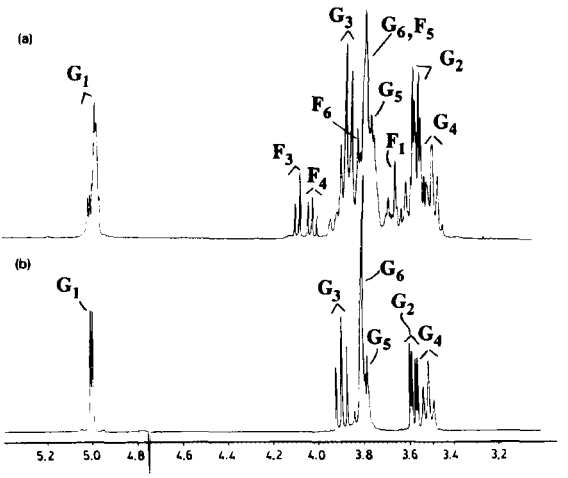


Fig. 1. Comparison of $^1$H NMR spectra of (a) Fru-β-CD and (b) β-CD with partial assignments (G + F indicate protons in glucose and fructose residues, respectively).

contained β-CD units carrying more than one fructose at O-6 of glucose residues or possibly fructosyl chains of two or more units.

Immediately after LC separation and drying, **2** was pale yellow in color, but after treatment with carbon in aqueous solution it could be dried to a colorless glass. It is probable that unsaturated degradation-products complex with **2** and pass with it through the LC column.

FABMS of **2** gave a peak at 1298 $[M + H]^+$ and a series of peaks corresponding to $[M - (162)_n]^+$ confirming the presence of a single fructosyl group on the CD. $^1$H NMR spectroscopy revealed a doublet centered at 4.08 ppm coupled to a triplet centered at 4.02 ppm corresponding to H-3 and H-4 of fructose respectively ($J_{3,4}$ 8.8 Hz). The stronger signals corresponding to the glucose residues could readily be assigned by comparison with underivatized β-CD and by H,H-COSY, although there was line broadening and splitting caused by non-equivalence of the residues. The $^1$H NMR spectra of β-CD and Fru-β-CD are compared in Fig. 1. In Fru-β-CD the signal for H-1 of glucose is considerably broadened and shows a clear doublet on the downfield shoulder which integrates in the ratio 1:6 with the remainder of the signal. This doublet can probably be attributed to the H-1 signal of the 6-*O*-substituted glucose residue. The $^{13}$C NMR spectrum showed a pattern of large and small peaks, the former showing splitting. The large peaks correspond to the signals for the glucose residues and are split by non-equivalence. They can readily be assigned by comparison with the spectrum of underivatized β-CD. The smaller peaks correspond to the signals for fructose and possibly for the 6-*O*-substituted glucose residue. The signals for the carbons of the fructosyl group can readily be assigned by comparison with model compounds [17]. This left unassigned one small signal at 70.9 ppm. An APT spectrum revealed that this was a secondary carbon and it was assigned as the signal for C-5 of the 6-*O*-substituted glucose, shifted upfield from the signal for C-5 of the remaining glucose residues.

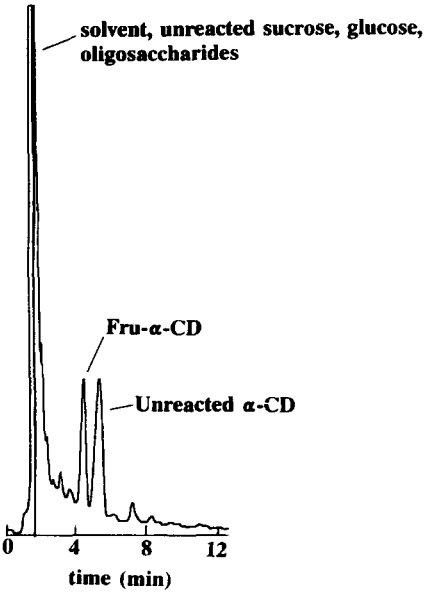


Fig. 2. Thermal transfructosylation to cyclomaltohexaose: LC of crude preparation dissolved in water using LC system (*ii*).

Model 6-*O*-fructofuranosylglucose compounds show that the effect of this substitution is to cause an upfield shift of C-5 and to leave C-6 unaffected [17]. 2D NMR was inconclusive on this point as even at 400 MHz the signals for H-5 and H-6 of glucose were merged and it was not possible to identify the H-5 signal of the substituted residue. The tertiary carbon at 104.1 ppm indicated the presence of a β-linked fructofuranosyl residue. A barely distinguishable signal was observed at the position expected for C-2 of an α-linked fructofuranosyl residue in one preparation, but this was not observed in duplicate preparations.

On the basis of this evidence **2** was assigned as 6-*O*-(β-D-fructofuranosyl)cyclomaltoheptaose. A similar treatment of α-CD yielded 6-*O*-(β-D-fructofuranosyl)cyclomaltohexaose, **4** (34% yield). LC of the crude preparation of **4** is illustrated in Fig. 2.

The solubilities of **2** and **4** were determined as were their respective abilities to solubilize a slightly soluble drug [10]. The results are summarized in Tables 1 and 2.

Table 1
Solubility in water of CDs and Fru-CDs

| Compound | Solubility (mg/mL) [a] |
|---|---|
| Cyclomaltohexaose (α-CD) | 126 |
| 6-*O*-(β-D-Fructofuranosyl)cyclomaltohexaose (Fru-α-CD) | 1054 |
| Cyclomaltoheptaose (β-CD) | 20 |
| 6-*O*-(β-D-Fructofuranosyl)cyclomaltoheptaose (Fru-β-CD) | 1136 |

[a] Determined at 21°C ± 1°C.

Table 2
Solubility of estriol in water in the presence of CDs and Fru-CDs at 20°C.

| Solubility in water ( $\mu$g/mL) | Solubility in $1.5\times10^{-2}$ M CD solution ( $\mu$g/mL) | | | |
|---|---|---|---|---|
| | $\alpha$-CD | Fru-$\alpha$-CD | $\beta$-CD | Fru-$\beta$-CD |
| 23 | 33 | 29 | 747 | 1909 |

The complexation behaviors of **2** and **4** with sodium 4-nitrophenolate were investigated using UV-vis spectrometry. The anion exhibits a spectral shift upon complexation with $\alpha$- or $\beta$-CD [11]. Measurement of spectra in solutions containing various amounts of the $\alpha$- or $\beta$-CD gave isosbestic points at 394 and 405 nm, respectively, indicating 1:1 complexation. A similar experiment with the 6-*O*-( $\beta$-D-fructofuranosyl) analogues, **4** and **2**, gave isosbestic points at 394 and 400 nm, respectively.

Dissociation constants for the complexes with 4-nitrophenolate anion were calculated using a graphical method [12] which is a modification of the method of Hildebrand and Benesi [13]. The relationship used is:

$$\frac{C_{4-\mathrm{NP}}C_{\mathrm{CD}}}{\Delta A} = \frac{K}{\Delta\epsilon} + \frac{C_{\mathrm{CD}}}{\Delta\epsilon} \quad (1)$$

in which $C_{4\text{-NP}}$ and $C_{\mathrm{CD}}$ are the total concentration of 4-nitrophenolate ion and cyclodextrin, $\Delta A$ is the difference between the absorbance of the test solution and one containing the same total concentration of free 4-nitrophenolate ion, $\Delta\epsilon$ is the difference between the extinction coefficients of the complex and free 4-nitrophenolate ion and $K$ is the dissociation constant for the reaction:

$$\text{Complex} \leftrightarrows \text{CD} + 4 - \text{NP} \quad (2)$$

A linear plot results with the slope yielding $\Delta\epsilon$ and the intercept yielding $K$. Values of the respective dissociation constants are listed in Table 3.

The results in Table 3 indicate that the presence of a fructofuranosyl substituent does not significantly alter the complexation ability of the CDs and this concurs with the findings of other studies of glucosyl CDs [5]. NMR studies [16] indicate that in $\alpha$-CD bearing a glucosyl side chain the glucose residue is not situated over the entrance to the cavity and therefore does not affect inclusion behavior. We presume that the same situation occurs with the fructosyl CDs.

Table 3
Dissociation constants for the complexes of CDs and Fru-CDs with 4-nitrophenolate anion

| Compound | $K_{\mathrm{diss}}$ [a] |
|---|---|
| Cyclomaltohexaose ($\alpha$-CD) | $4.30\pm0.16\times10^{-4}$ M (c.f. $4.0\pm0.8\times10^{-4}$ M, [14]) |
| 6-*O*-( $\beta$-D-Fructofuranosyl)cyclomaltohexaose (Fru-$\alpha$-CD) | $4.85\pm0.22\times10^{-4}$ M |
| Cyclomaltoheptaose ( $\beta$-CD) | $1.72\pm0.15\times10^{-3}$ M (c.f. $1.4\times10^{-3}$ M, [15]) |
| 6-*O*-( $\beta$-D-Fructofuranosyl)cyclomaltoheptaose (Fru-$\beta$-CD) | $1.61\pm0.38\times10^{-3}$ M |

[a] Determined at 25°C and pH 11.0.

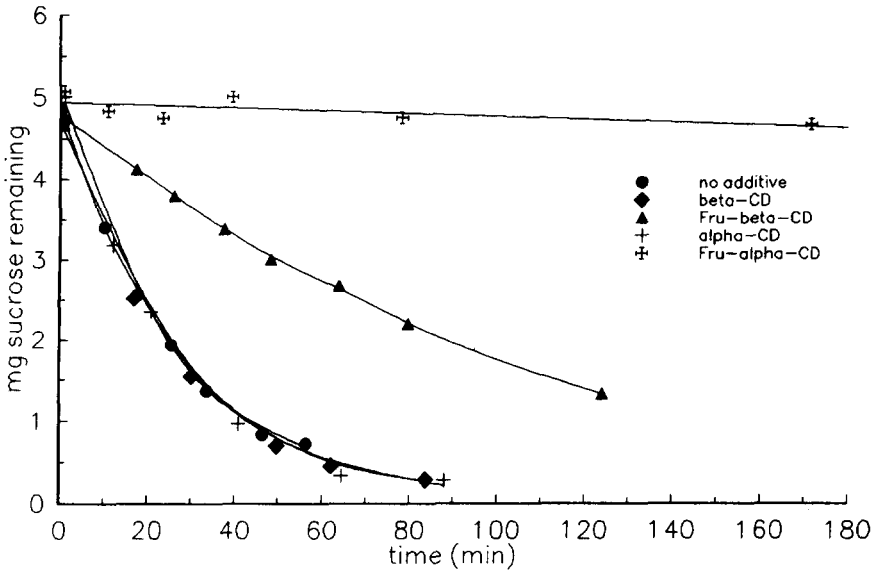


Fig. 3. Action of invertase upon sucrose in the presence of CDs and Fru-CDs.

The solubilities and solubilizing power of the Fru-CDs, Tables 1 and 2, also agreed with the findings of other authors with respect to glucosyl CDs [5,10].

Compounds **2** and **4** were unaffected by invertase under conditions that brought about the complete hydrolysis of sucrose in 30 min. In a 1:1 mole ratio with sucrose **2** and, to a much greater extent, **4** demonstrated inhibition of invertase, Fig. 3. No inhibition was manifested by the parent CDs. A more detailed kinetic study is required to determine the nature of the inhibition, but in the meantime we note that these results indicate that the Fru-$\alpha$-CD appears to be a non-reversible inhibitor of the hydrolysis of sucrose by invertase. Furthermore provided that the Fru-$\alpha$-CD is capable of transport to regions of plants or of plant cells, where invertase action occurs, then it is likely to be a potent plant-growth regulator.

## 3. Experimental

*General methods.*—Cyclodextrins were purchased from Sigma and used without any further purification. LC of CDs and Fru-CDs was performed with either (*i*) a Waters $\mu$-Bondapak $NH_2$, 8 × 100 mm, 10 $\mu$m, Radial-Pak cartridge eluted with 65:35 MeCN–water at 2 mL/min; (*ii*) a Waters Resolve $C_{18}$, 8 × 100 mm 5 $\mu$m, Radial-Pak cartridge eluted with aq 8.5% MeOH at 2 mL/min; or (*iii*) a Phenomenex Rezex RCM-Monosaccharide 300 × 7.8 mm column eluted with water at 0.6 mL/min and 75°C. Preparative LC was performed using three Waters Delta-Pak $C_{18}$ 25 × 100 mm Radial-Pak cartridges in series eluted with aq 8.5% MeOH at 20 mL/min. Methylation

analyses and colorimetric assays were carried out as described [3]. NMR spectra were measured using a Varian Unity *plus* 400 MHz spectrometer in $D_2O$ and referenced to an external standard of $Me_4Si$. FABMS was carried out by J. Sears at the Mass Spectrometry Facility of Montana State University, using xenon atoms at 6 KeV, with a matrix of 1:1 glycerol: Magic Bullet (3:1 dithioerythritol–dithiothreitol) and a mass resolution of 1500. UV-vis spectra were measured with a Hewlett–Packard HP 8452A diode array spectrophotometer at a temperature of 25°C.

*Thermal fructosylation of CDs.*—Sucrose (27.5 g) was dissolved in water (110 mL) and cyclodextrin (5.5 g) added. The mixture was warmed to 60°C to dissolve the CD which dissolved readily in the concentrated sucrose solution and remained in solution upon cooling to 25°C whereupon citric acid (0.275 g) was added. The solution was freeze-dried for 96 h with a final shelf temperature of 45°C, at which point 4.3% residual water was present. Storage in a desiccator over Drierite for a further 72 h reduced the water content to 1.5% although this step was not necessary for the successful outcome of the thermolysis. The dried product was a brittle, white, solid foam which softened at 105°C and was completely liquid at 115°C. The product ( ~ 5.0 g) was ground to a powder and placed in a 500 mL round-bottomed flask. The flask was heated (120°C, 30 min) under vacuum with rotation in an oil bath. The product after heating was a pale-yellow glass which was dissolved in aq $NH_4OH$ ($10^{-2}$ M) and fractionated by preparative LC.

*Isolation and characterization of fructosyl CDs.*—The entire product of the thermal fructosylation of β-CD was examined by LC. Using LC system (*i*) glucose, sucrose, and oligosaccharides, which were formed as side reactions of the sucrose, ran as a group of peaks between 3.1 and 5.2 min, β-CD ran at 9.1 min and **2** ran at 11.6 min. Using LC system (*ii*) glucose, sucrose, and oligosaccharides ran between 1.4 and 1.93 min, **3** (2% overall yield by integrated peak area relative to the summed areas of **2**, **3**, and unchanged CD) ran at 5.5 min, **2** (21% yield) ran at 9.0 min, and β-CD at 11.7 min. A sharp peak at 7.4 min proved, upon isolation, to be a highly-colored, polymeric degradation product. Compounds **2** and **3** were isolated by preparative LC and taken to dryness under vacuum (40°C) to yield pale-yellow glasses. Treatment of an aqueous solution of **2** with decolorizing carbon (8 h) subsequently yielded a glass with no visible color.

Glucose:fructose ratios of **2** and **3** were obtained by colorimetric assay indicating, respectively, 6:1 and 2.3:1. (Expected 7:1 for mono- and 3.5:1 for di-substitution.) Methylation analyses with mild hydrolysis [3] revealed only 2-linked fructofuranosyl (Fru*f*) in **2**. The same procedure when applied to **3** revealed almost equal quantities of 2-linked Fru*f* and fructopyranosyl (Fru*p*) and lesser amounts of 1,2-linked Fru*f* and Fru*p*. Strong hydrolysis [3] of per-*O*-methylated **2** demonstrated the presence of 1,4-linked glucopyranosyl (Glc*p*) and 1,4,6-linked Glc*p* residues in the ratio 6:1. The same residues were present in **3** in the ratio 4.5:1.

The $^{13}C$ NMR spectrum of **2** was assigned as described. Two types of peak were observed; those of greater intensity which were split corresponded to the carbons of the glucose residues and are denoted $C_G$, those of lesser intensity corresponded to the carbons of the fructosyl moiety denoted $C_F$ and to C-5 of the substituted glucose residue denoted $C_{G'}$. Chemical Shifts are expressed in ppm downfield from external $Me_4Si$:

104.1 ($C_F$-2), 102.13, 102.08, 101.99 ($C_G$-1), 81.6 ($C_F$-5), 81.49, 81.38, 81.32, 81.16 ($C_G$-4), 79.9 ($C_F$-3), 75.0 ($C_F$-4), 73.4, 73.3 ($C_G$-3), 72.3, 72.26, 72.1 ($C_G$-2), and ($C_G$-5), 70.9 ($C_{G'}$-5), 62.99 ($C_F$-6), 60.7, 60.6, 60.5, 60.3 ($C_F$-1 and $C_G$-6).

The assignment of the $\beta$ configuration to the fructosyl residue was based upon comparison with Refs. [1,17]. A barely distinguishable signal was observed at 107.6 ppm in one of the preparations which might be attributed to C-2 of an $\alpha$-linked fructofuranosyl group.

FABMS of **2** gave a peak at 1298 ($[M + H]^+$) and a series of peaks: 1135, 973, 811, 649, and 487 corresponding to successive loss of 1–5 residues of mass 162.

Compound **2** was assigned as 6-*O*-($\beta$-D-fructofuranosyl)cyclomaltoheptaose; $[\alpha]_D^{20}$ +114.7° (*c* 8, $D_2O$).

The entire product of the thermal fructosylation of $\alpha$-CD was examined by LC system (*ii*). $\alpha$-CD ran at 5.2 min and **4** (34% yield by integrated peak area) was observed at 4.35 min. Preparative chromatography of **4** was complicated by the presence of the aforementioned unsaturated degradation-product, which coincided with **4** under preparative conditions but not under analytical conditions. Treatment of **4** with decolorizing carbon after isolation effectively removed this contaminant. Methylation analysis of **4** with mild hydrolysis revealed the presence of 2-linked fructofuranosyl groups. Strong hydrolysis of methylated **4** revealed 1,4- and 1,4,6-linked glucose residues in the ratio 4.4:1.

The $^{13}C$ NMR spectrum of **4** was assigned in the same manner as was that of **2**: 104.7 ($C_F$-2), 102.23, 102.2, 102.0 ($C_G$-1), 82.1 ($C_F$-5), 82.05, 82.0, 81.8 ($C_G$-4), 77.5 ($C_F$-3), 75.3 ($C_F$-4), 74.2, 74.1 ($C_G$-3), 72.86, 72.76, 72.6, 72.5 ($C_G$-2) and ($C_G$-5), 71.4 ($C_{G'}$-5), 63.4 ($C_F$-6), 61.24, 61.17, 61.11, 60.98, 60.80 ($C_F$-1 and $C_G$-6).

FABMS of **4** gave a peak at 1136 ($[M + H]^+$) and a series of peaks: 973, 811, 649, and 487 corresponding to the successive loss of 1–4 residues of mass 162.

Compound **4** was assigned as 6-*O*-($\beta$-D-fructofuranosyl)cyclomaltohexaose; $[\alpha]_D^{20}$ +147.6° (*c* 9, $D_2O$).

*Solubility of* **2** *and* **4**.—The solubilities in water of lyophilized **2** and **4** were determined by the method described in Ref. [10]. The respective solubilities of the parent CDs were determined by the same method for the purposes of comparison.

*Solubilization of a slightly soluble drug.*—This was determined using the method described in Ref. [10]. LC conditions for Estriol [Estra-1,3,5(10)-triene-3,16$\alpha$,17$\beta$-triol] were a Waters Resolve $C_{18}$ 8 × 100 mm, 5 $\mu$m Radial-Pak column eluted with aq 55% MeOH at 1 mL/min with UV detection at 280 nm; retention time was 9.3 min.

*Reaction with invertase.*—Compounds **2** and **4** (20 mg in 2 mL water) were treated with invertase (0.03 mg, Sigma, EC 3.2.1.26, from bakers' yeast) and incubated at 37°C (These conditions caused the complete hydrolysis of sucrose in less than 30 min). Compounds **2** and **4** were assayed using LC system (*ii*) and fructose was assayed using LC system (*iii*). After 24 h there was no measurable reduction in **2** and **4** and no free fructose was observed. A 1:1 molar ratio mixture of sucrose (5 mg/mL) and CD or Fru-CD was incubated with invertase (0.002 mg/mL) at 37°C. Sucrose was monitored by LC (Waters Resolve $C_{18}$ 8 × 100 mm, 5 $\mu$m Radial-Pak column eluted with water at 1 mL/min). The addition of **4** gave essentially complete inhibition; a further addition of enzyme followed by incubation for 12 h resulted in loss of ~16% of sucrose.

Compound **4** also displayed inhibition in a 1:2 molar ratio with sucrose but in this case the disappearance of sucrose was more rapid resulting in ~ 15% loss in 2.6 h.

*Calculation of dissociation constants for the complex with 4-nitrophenol anion.*—All solutions were made up in phosphate buffer adjusted to pH 11.0. A solution of 4-nitrophenol (4-NP) was mixed with varying concentrations of CDs and the volume adjusted so that the final concentration of 4-NP was $2.5 \times 10^{-5}$ M. UV-vis spectra were recorded at 25°C. Dissociation constants were obtained by plotting data according to Eq. (1), slope and intercept were obtained by regression analysis. Absorbance was measured at an arbitrary wavelength, 354 nm for $\beta$-CDs and 420 nm for $\alpha$-CDs.

## Acknowledgements

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