Electrophoretic Resolution of Monosaccharide Enantiomers in Borate–Oligosaccharide Complexation Media

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Abstract: Monosaccharide enantiomers have been directly separated by capillary electrophoresis as complexes with borate and linear or cyclic dextrins of the general poly(gly-(1-4)-α-D-Glu) structure. Their relative electrophoretic migration and their separation selectivity were mainly influenced by the type and concentration of the chiral additive, the concentration of boric acid, and the chemical nature of a fluorescence-tagging (derivatizing) agent. The analytes were detected by laser-induced fluorescence. All sugar enantiomers were resolved using β-cyclodextrin as the electrolyte additive. Their migration order could be adjusted by a type of chiral additive, a derivatizing agent, or the surface property (chemical modification) of the separation capillary. Under certain electrolyte conditions, base-line separation of the sugar enantiomers could be completed in times as short as 30 s. The complexation-induced shifts observed in the ^{1}H NMR and fluorescence spectroscopy experiments were in agreement with the results obtained by capillary electrophoresis. A possible mechanism for the chiral recognition is proposed.

Introduction

Biochemical versatility of glycoconjugates has its origin in the structural diversity of even the simplest carbohydrate molecules. Since various glycoconjugates have recently received considerable attention with regard to important biochemical processes,[1-4] increasingly more sensitive and informative analytical methodologies are being sought for this class of compounds in terms of composition and primary structure. However, questions pertaining to the chirality of carbohydrates seem to have been largely neglected.

While most naturally occurring sugars are assumed to have the D-configuration, the less usual L-forms have also been found, L-glucose in the leaves of the jute and *Grindelia* species and L-galactose in agar and a number of algal mucilages.[5] L- and D-talose, L-mannose, L-galactose, D- and L-fucose, and L-galacturonic acid are the uncommon forms found in bacterial polysaccharides.[5] The possibility of varying the configuration of carbohydrate entities in biopolymers and synthetic analogs is thought to have significant pharmaceutical potential.[6-9]

During the last several years, high-performance capillary electrophoresis (HPCE)[10-12] has been developed as a new powerful tool for separating various glycoconjugates. In addition, the suitable tagging of carbohydrates with fluorescent moieties[13,14] has aided detection of the separated carbohydrates at extremely small quantities. While HPCE has also been successful in the separation of enantiomeric drugs,[15-17] there are no literature reports on the separation of sugar enantiomers.

The present investigation is aimed at the resolution of carbohydrate enantiomers through their complexation with buffer additives in open tubular HPCE. In one effective variant of HPCE, capillary zone electrophoresis, with or without the electroosmotic buffer movement,[15] solutes are separated strictly according to their charge-to-mass ratio. The possibilities of utilizing secondary equilibria have further been enhanced in micellar electrokinetic capillary chromatography,[18] the second highly effective variant of HPCE. Electromigratory separation of carbohydrates can further be influenced by complex formation with charged species like borate[13,14] or metal ions.[19] Since carbohydrates are weakly acidic (pK_a values ≃12–14), they can also be separated as anions at strongly alkaline pHs[20-22] or as ion pairs with cationic detergents and polymers.[22] To visualize carbohydrates for the appropriate HPCE detection, introduction of a fluorophore into their molecules is essential.[13,14] Such a derivatization step can also introduce a charged moiety for improved migration behavior.

We demonstrate here, for the first time, the analytical resolution of fluorescently labeled monosaccharide enantiomers through complexation with borate and dextrins. Linear and cyclic dextrins of various molecular sizes were investigated with respect to their complexation capability. Complexations with borate and other anions were also explored to optimize enantiomeric resolution and to gain further insight into the separation mechanisms

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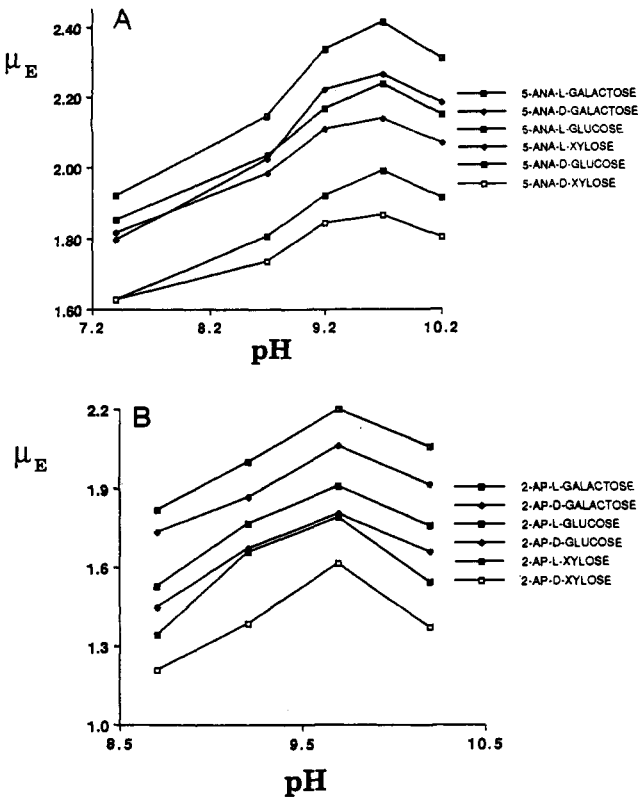

Figure 1. Effect of pH on electrophoretic mobility for (A) ANA-derivatized and (B) AP-derivatized sugar enantiomers. For conditions, see text.

Table I. Effect of pH on Separation Selectivity α for ANA and AP Sugar Enantiomer Derivatives[a]

	α				
	pH				
solute	7.40	8.70	9.20	9.70	10.2
		2-Aminopyridine–			
glucose		1.076	1.080	1.083	1.084
xylose		1.136	1.143	1.147	1.147
mannose		0.949	0.950	0.943	0.942
galactose		1.071	1.105	1.108	1.118
		5-Amino-2-naphthalenesulfonate–			
glucose	1.216	1.202	1.202	1.196	1.196
xylose	1.176	1.224	1.228	1.227	1.230
mannose	0.975	0.968	0.964	0.966	0.968
galactose	1.110	1.096	1.101	1.110	1.096

[a] Capillary: 45/60 cm. Selectivity defined as $\alpha = (t_m(L) - t_0)/(t_m(D) - t_0)$.

involved. The proposed mechanisms were also verified by ^{1}H NMR and fluorescence measurements.

Experimental Section

Apparatus. The experimental setup for capillary electrophoresis/laser-induced fluorescence detection has been described elsewhere.[23] Various lengths of fused silica capillaries (Polymicro Technologies, Phoenix, AZ) of 50-μm i.d. (187-μm o.d.) were used as separation columns. A high-voltage power suppy (Spellman High Voltage Electronics, Plainview, NY) capable of delivering 0–40 kV was employed. Different polarity modes had to be used for uncoated and coated capillaries, the latter being connected in a cathodic mode. On-column fluorescence detection was performed with a Model 56X helium/cadmium laser (Omnichrome, Chino, CA) operating at 325 nm, while fluorescence was measured at 375 nm in the case of the 2-aminopyridine (AP) derivative and 475 nm when 5-aminonaphthalene-2-sulfonic acid (ANA) or 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid (AHNS) were used as derivatizing agents.

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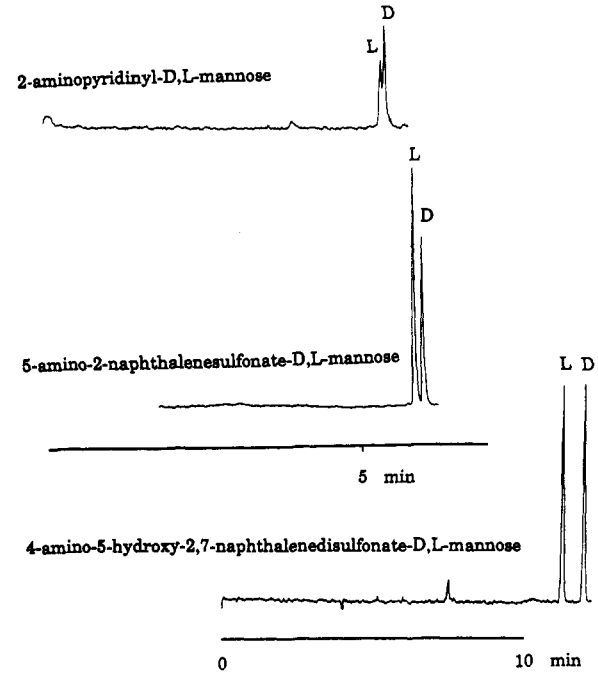

Figure 2. Separation of mannose enantiomers, derivatized with (A) AP, (B) ANA, and (C) AHNS (the other peaks are due to excess of reagent used in the derivatization). Electrolyte: 12.5 mM β-CD in 0.1 M borate, pH = 9.2. Power: 333 V/cm and 34 μA. Capillary: 45/60 cm.

Static fluorescence measurements were carried out with a Perkin-Elmer (Norwalk, CT) Model 650 spectrofluorimeter equipped with a xenon lamp, powered by a Perkin-Elmer Model 150 power supply.

The ^{1}H NMR measurements were performed with a Model AM500 instrument (Bruker, Karlsruhe, Germany) at 22 °C. The concentrations are as follows: 0.5 mM ANA-D- or -L-galactose, 5.0 mM cyclodextrin, and 10–100 mM borate, pD = 9.2.

Materials. The fluorogenic reagents, 2-aminopyridine, 5-aminonaphthalene-2-sulfonic acid, and 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid, and sodium cyanoborohydride were obtained from Aldrich (Milwaukee, WI). The inner surface of fused silica capillaries was modified with an organic layer according to Hjertén[24] or Dolnik *et al.*[25] All sugar enantiomers and polysaccharides, except for Dextrin 10 (Fluka AG, Chemische Fabrik, Buchs, Switzerland), and all other chemicals were obtained from Sigma (St. Louis, MO) and used without further purification.

Preparation of Fluorescent Carbohydrate Derivatives. The sugars were derivatized by a Schiff-base formation between the aromatic amine of a reagent and the aldehyde form of a sugar followed by reduction of the Schiff base to a stable product.[12] The individual sugars were dissolved in distilled water (1 M) and stored in a freezer prior to derivatization. To 10 μL of the reagent solution (1 M, alternatively a saturated solution, adjusted to pH = 6.8 with 10 M HCl) was added 10 μL of the sugar solution. The mixture was heated at 90 °C for 10 min. Next, 4 μL of the reducing agent, sodium cyanoborohydride (0.3 mg/μL), was added, and the mixture was heated for an additional 60 min at 90 °C. Finally, the samples were diluted with water to a concentration of 5–10 mM and stored at –20 °C. All injected samples were prepared from these stock solutions and diluted further before use. The hydrodynamic technique of sample introduction was employed.

Results and Discussion

Effects of pH, Derivative Type, and Borate Concentration. The D- and L-forms of the derivatized sugars were readily separated in several buffer media. Carbohydrates are known to form negatively charged complexes with boronic acids at pH > 7.[26] Within the pH range of 7.4–10.2, both the ANA derivatives and the AP derivatives increased their electrophoretic migration up

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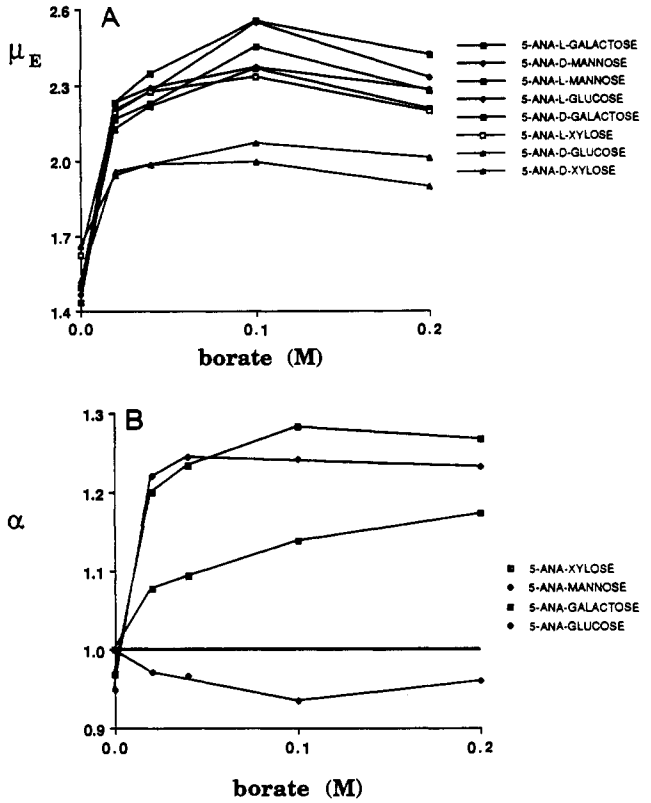

Figure 3. Dependence of (A) electrophoretic mobility ($cm^2/V\,s \times 10^{-4}$) and (B) separation selectivity on borate concentration. Electrolyte: 15 mM β-CD in 0.1 M borate buffer, pH = 9.2. Power: 20 kV and 35 μA. Capillary: 45/60 cm.

Table II. Separation Selectivities α for Different Cyclodextrins[a]

	α		
solute	50 mM α-CD	15 mM β-CD	50 mM γ-CD
	2-Aminopyridine–		
glucose	1.000	1.014	0.925
xylose	1.000	1.101	1.023
mannose	0.934	0.900	0.931
galactose	1.000	1.087	1.000
	5-Amino-2-naphthalenesulfonate–		
glucose	1.000	1.240	1.061
xylose	1.058	1.260	1.124
mannose	1.020	0.969	0.945
galactose	1.051	1.152	1.022
fucose	1.073	1.149	1.075
lyxose	1.013	0.949	0.950
arabinose	0.977	0.802	0.858
ribose	1.068	1.277	1.219
	4-Amino-5-hydroxy-2,7-naphthalenedisulfonate–		
glucose	1.000	0.967	1.000
xylose	1.027	0.988	0.982
mannose	1.015	0.918	0.939
galactose	0.986	0.854	0.933

[a] Capillary: 45/60 cm. Electrolyte: 0.1 M borate, pH = 9.2, and cyclodextrin. Power: 20 kV and 27–32 μA.

to the maximum values at pH = 9.7 (Figure 1). The negatively charged boronate ions are considered to be responsible for the formation of the anionic adducts with sugars. This concentration was kept constant under varying pH by adding the appropriate amount of uncharged borate. At a field strength of 250 V/cm, a constant current of 22 μA was observed. The background electrolyte also contained a 15 mM concentration of β-cyclodextrin (β-CD). A decreased electrophoretic mobility at lower pH values was due to a partial ionization of the amino groups in the derivatives. No changes in the complexation mechanisms could be observed as evidenced by the relatively constant selectivity of the enantiomeric separations (Table I). The selectivity α is defined

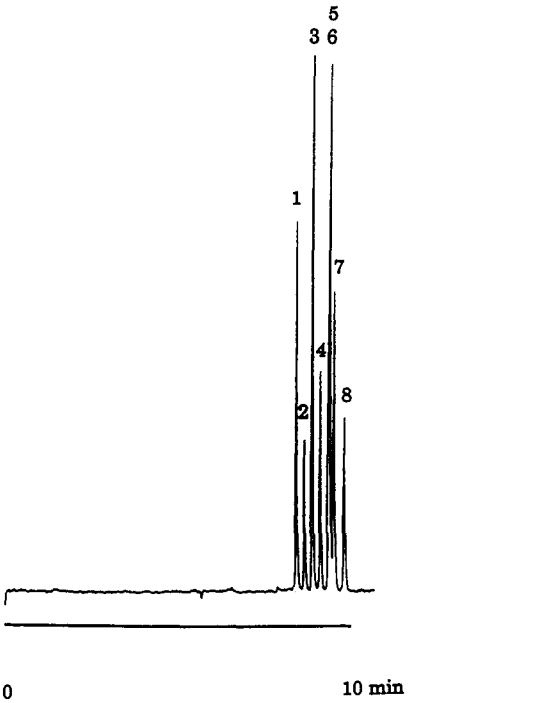

Figure 4. Separation of ANA-derivatized sugar enantiomers: (1) D-xylose, (2) D-glucose, (3) D-galactose, (4) L-mannose, (5) L-xylose, (6) D-mannose, (7) L-glucose, and (8) L-galactose. Electrolyte: 7.5 mM β-CD in 0.2 M borate, pH = 8.7. Capillary: 85/100 cm. Power: 35 kV and 35 μA.

here as the ratio of differences for the migration time t_m minus the time for an electroosmotic marker (a neutral molecule) to reach the detector t_0 expressed for both enantiomers: $\alpha = (t_m(L) - t_0)/(t_m(D) - t_0)$. In general, the ANA derivatives displayed the highest selectivities of all the tested derivatives. Typical separation results are demonstrated in Figure 2. ANA derivatives were thus generally favored in all the remaining investigations.

Recently, thermodynamic studies through calorimetric titrations have been reported on the complexation of naphthalenesulfonates with different cyclodextrins.[27] While pronounced associations were found in aqueous solutions for all cyclodextrins, β-CD yielded consistently more stable complexes with the naphthalene guest molecules than either α-CD or γ-CD.

The effects of borate complexation on the migration time, electrophoretic mobility, and separation selectivity (α-values) were investigated under a constant ionic strength (I = 0.1) by the addition of phosphate. Within the studied borate concentration (0–200 mM), the migration times of both optically active forms of the ANA derivatives of xylose, mannose, galacose, and glucose gradually increased, albeit not at the same rate. The dependences of electrophoretic mobility and selectivity on borate concentration (Figure 3) indicate that a complete complexation of all sugars occurs at roughly 100 mM borate concentration (i.e., at 50 mM of the negatively charged borate ion). The migration times and the enantiomeric separation selectivities initially increase relatively fast with increasing borate concentration, reaching a plateau at 20–100 mM. However, at higher values of borate concentration, a slight decrease in migration times and some α-values was noticed. This could be tentatively attributed to the increasing presence of the polymeric forms of boronates that might compete for the sugars, decreasing their charge-to-mass ratio.

Complexation Effects of Additional Anions. Interestingly, some of the sugar enantiomers (ribose, xylose, fucose, galactose, and glucose) were slightly separated in a phosphate buffer which contained 15 mM β-CD (but no borate), with the α-values yielding

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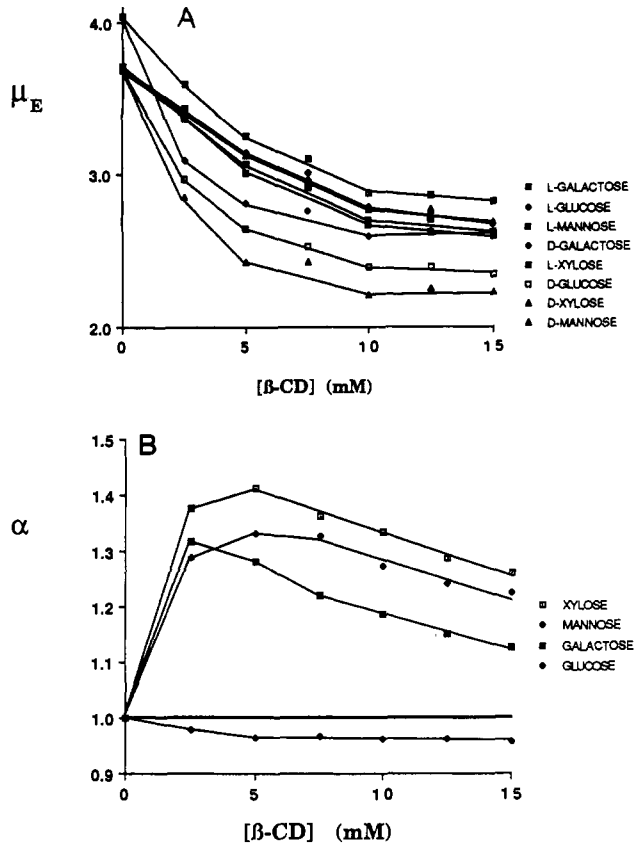

Figure 5. Effects of β-CD concentration on (A) electrophoretic mobility and (B) separation selectivity. Electrolyte: 0.1 M borate, pH = 9.2. Power: 333 V/cm and 34 μA.

Table III. Separation Selectivity for ANA-Derivatized Sugar and Glyceraldehyde Enantiomers in an Optimized System Using 250 V/Cm[a]

sugar	α	sugar	α
glyceraldehyde	1.060	fucose	1.201
ribose	1.589	galactose	1.343
erythrose	1.114	lyxose	0.947
threose	0.739	mannose	0.916
glucose	1.510	allose	1.152
xylose	1.303	talose	0.862
arabinose	0.751	idose	0.761

[a] The electrolyte was 12.5 mM β-CD in 0.5 M borate, pH = 8.2, and 2% tetrahydrofuran.

0.972, 0.978, 0.976, 0.989, and 0.957, respectively. When borate was added, the migration order for all of the solutes changed. In the buffer containing 0.1 M ammonia and 0.05 M acetic acid (pH = 9.2), no enantiomeric pair was resolved. This seems to indicate that phosphate forms a weak complex with the sugars. Presumably, as the sugars are normally associated with β-CD as ternary complexes with borate, the hydrogen-bonding network and steric features of these complexes were altered in the phosphate medium. The reversal in selectivities supports the notion that more than one chiral mechanism operates in β-CD complexation, unless the phosphate anion binds *only* to the sugar/β-CD complex and, thus, increases its electrophoretic migration rate.

Different oxyacids, including molybdate, tungstate, arsenate, and germanate, have been used previously for the separation of carbohydrates and polyols in thin-layer chromatography[28] or paper electrophoresis.[29] When we attempted to use them as complexing agents in this study, extremely broad peaks and/or poor resolution were observed Encouraged by the above results obtained with phosphate, we also tested for phosphorous acid and phenylphosphonic acid as complexing agents (0.1 M) and observed a partial separation of a sugar mixture (ribose, lyxose, xylose, and mannose) at pH = 9.4. None of these results, however, compared with the effectiveness of borate complexation.

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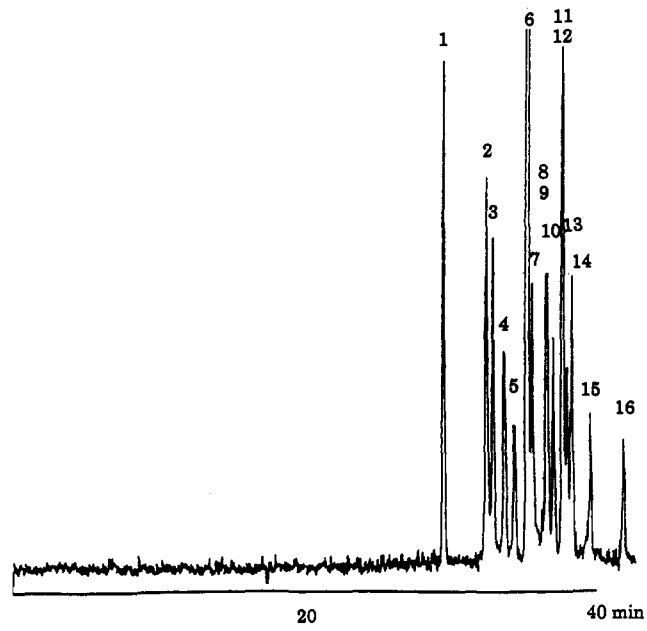

Figure 6. High-efficiency separation of a complex enantiomeric mixture, using 12.5 mM β-CD, 2% tetrahydrofuran, and 0.5 M borate (pH = 8.2) as the electrolyte in a 85/100-cm fused silica capillary and at 25 kV and 38 μA. Peak assignments: (1) D-ribose, (2) D-xylose, (3) L-arabinose, (4) D-fucose, (5) D-glucose, (6) L-xylose and reagent (ANA), (7) L-ribose, (8) D-galactose, (9) L-mannose, (10) D-lyxose, (11) L-xylose, (12) D-mannose, (13) L-glucose, (14) D-arabinose, (15) L-fucose, and (16) L-galactose.

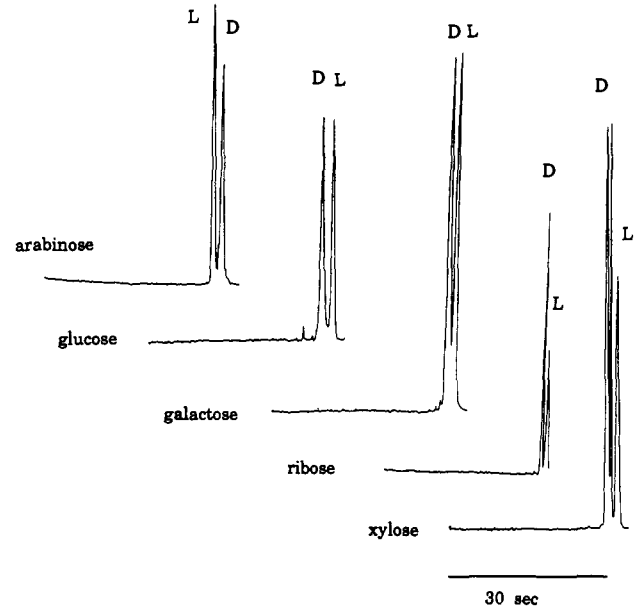

Figure 7. High-speed separations (~30 s) of ANA derivatives. The time constant for detection was 1 ms. Electrolyte: 5 mM β-CD in 20 mM borate, pH = 10. Capillary: 25/37 cm. Power: 30 kV (30 μA). The extra peak in the xylose separation is reagent.

Effects of Cyclodextrin Type and Concentration. Different cyclodextrin derivatives, such as α-CD, β-CD, γ-CD, di-*O*-methyl-β-CD, tri-*O*-methyl-β-CD, and hydroxypropyl-β-CD, were investigated here as chiral selectors. Furthermore, three derivatizing agents differing in size and charge (AP, ANA, and AHNS) were employed to study the influence of a fluorescent tag on the chiral discrimination. The summary of obtained results is shown in

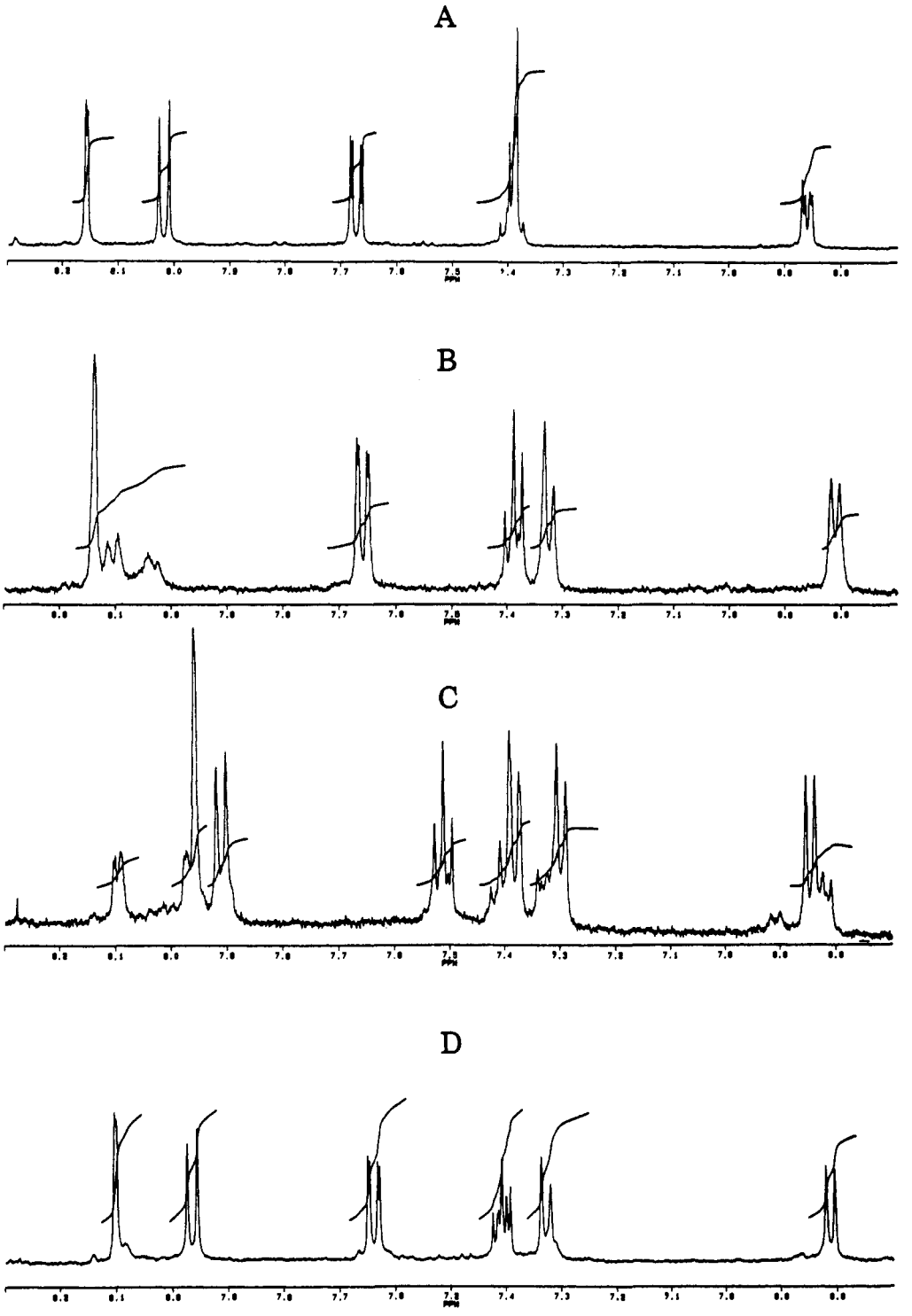

Figure 8. ^{1}H NMR spectra of the 5-amino-2-naphthalenesulfonate tag. Conditions: (A) 0.5 mM ANA–D-galactose in D_2O, (B) 0.5 mM ANA–D-galactose in 0.1 M borate (pD = 9.2), (C) 0.5 mM ANA–D-galactose in 10 mM borate (pD = 9.2) and 5.0 mM β-CD, and (D) 0.5 mM ANA–L-galactose in 10 mM borate (pD = 9.2) and 5.0 mM β-CD.

Table II. Enantioselectivity was observed only with the underivatized CDs and hydroxypropyl-β-CD, indicating that the hydroxyl groups in the CD are important for chiral recognition. In general, the selectivity increased in the following order: $\alpha < \gamma < \beta$. A base-line resolution was obtained for all sugar enantiomers and all derivatives (except for AP–glucose) only with β-CD. The sugar chain is believed to become less flexible upon complexation with borate, possibly facilitating the chiral recognition. The migration order appears to be dependent on the CD used, the tag type, or, in many cases, both. Consequently, desirable selectivity can be obtained by tuning the electrophoretic conditions and through a choice of derivatizing agent. An example of the separation of an eight-component sugar enantiomeric mixture is shown in Figure 4. The ANA derivatives seem generally preferable to analytical work, as the AHNS reagent and the formed derivatives suffer from easy oxidation. In fact, they start to degrade within minutes if left in light. Subsequently, we minimized further use of AHNS derivatives.

The effects of β-CD concentration in 0.1 M borate (pH = 9.2) on the electrophoretic mobility and α-values for the ANA sugar enantiomers are shown in Figure 5. As the extent of complexation increases with the elevation of β-CD concentration, a gradual decrease in electrophoretic migration is experienced. Undoubtedly, this is due to a decrease in the charge-to-mass ratio. When

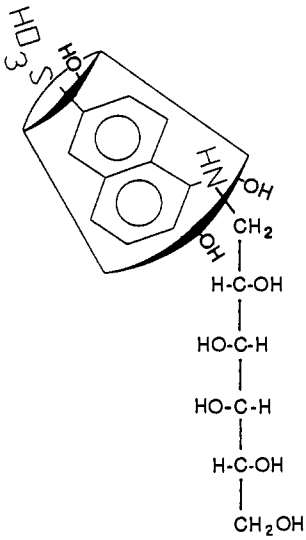

Figure 9. Proposed complex between ANA–D-galactose and β-CD. For simplicity, the hydroxyl groups of one glucose unit only are shown.

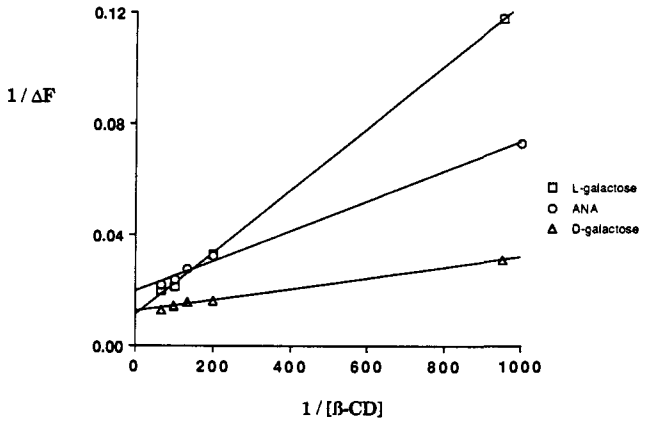

Figure 10. Benesi–Hildebrand plot for ANA and ANA–L- and –D-galactose in 0.1 M borate (pH = 9.2) and β-CD. Solute concentration: 0.1 mM (λ_{ex} = 300 nm; λ_{em} = 475 nm).

a β-CD concentration becomes equal to or larger than 10 mM, the solutes should be fully complexed, and any further concentration increases have only minor effects on migration. Moreover, the electrophoretic mobilities were higher than those recorded in the absence of borate, supporting further the notion of the sugar/borate/cyclodextrin complexation. The selectivity of enantiomeric separation apparently reaches a maximum at about 5 mM β-CD concentration (Figure 5B), except for mannose. The apparent electrophoretic migration of the sugar enantiomer $\mu_{app(E)}$ can be described as the sum of the fractions migrating as the borate complex Θ_B and the borate/β-CD complex $\Theta_{B/\beta\text{-CD}}$. Through introduction of the apparent thermodynamic stability constants for the respective complexes, i.e., K'_B and $K'_{B/\beta\text{-CD}}$, the following expression is obtained

$$\mu_{app(E)} = (\mu_{app(B)} + \mu_{app(B/\beta\text{-CD})}K'_B[\beta\text{-CD}])/(1 + K'_B[\beta\text{-CD}]) \quad (1)$$

where $\mu_{app(B)}$ and $\mu_{app(B/\beta\text{-CD})}$ are the apparent electrophoretic mobilities for the sugar enantiomer as borate and borate/β-CD complexes, respectively. Hence, a selectivity decrease at greater β-CD concentrations is anticipated, as the less bound enantiomer is being compensated by an increasing concentration of β-CD, moving the equilibrium toward the complexed form and, thereby, decreasing the difference in the degree of complexation between the two enantiomers. Very small amounts of uncharged organic buffer modifiers (methanol, ethanol, acetonitrile, tetrahydrofuran, and dioxane) were further capable of modifying the electrophoretic

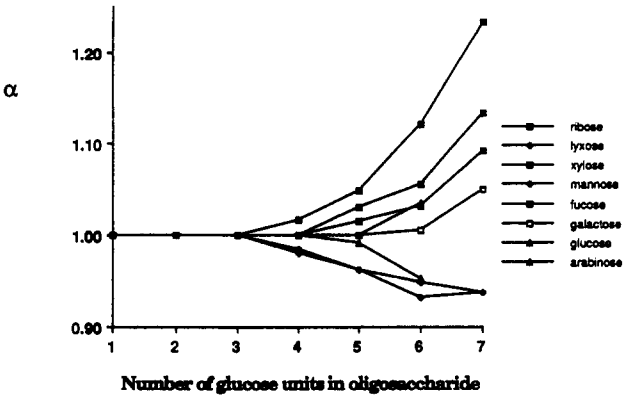

Figure 11. Influence of maltooligomer chain length on enantioselectivity. Conditions: 0.4 M borate, pH = 8.3, and 405 V/cm.

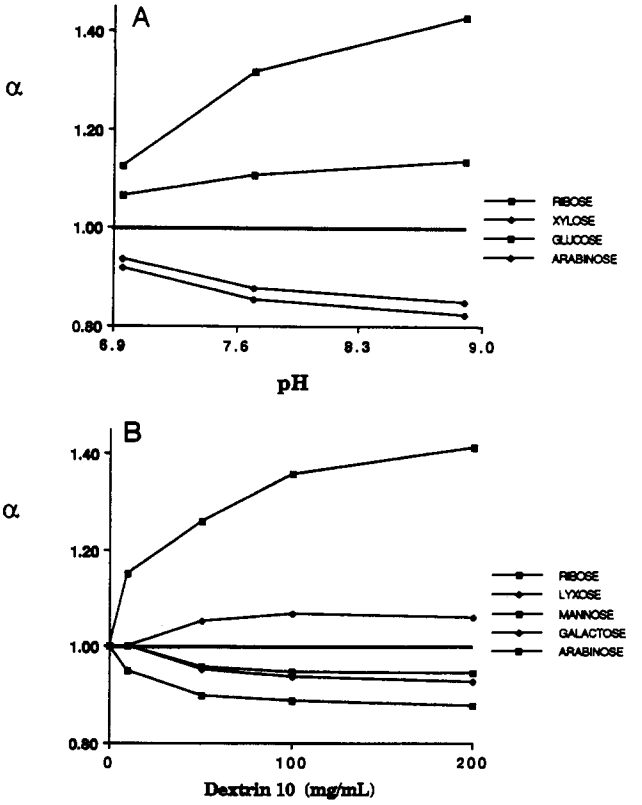

Figure 12. Effect of (A) pH and (B) Dextrin 10 concentration on enantioselectivity. Conditions: (A) 100 mg of Dextrin 10/mL in borate buffer with constant [$B(OH)_4^-$] = 10 mM and (B) 0.2 M borate, pH = 8.3. Field strength: 405 V/cm.

migration toward the optimized separation of enantiomes with either ANA or AP derivatives. An example is shown in Figure 6 with a high-efficiency separation (~400 000 theoretical plates per m) of a complex enantiomeric mixture. Most likely, the organic modifier molecules compete with the analyte molecules for complexation to the CD molecule. Consequently, the electrophoretic mobilities increase, reflecting their preferential migration as borate complexes. A summary of the separation selectivities for glyceraldehyde, tetraoses, and hexaoses is shown in Table III.

Through the appropriate choice of a separation length and electrolyte conditions, extremely fast separations of certain enantiomers become possible (Figure 7). A reversal of the solute migration behavior is feasible through a modification of the capillary surface with an organic layer.[24,25] Alternatively, the electroosmotic flow can be tuned through a dynamic modification of the column's ζ-potential with a cationic detergent, a divalent cation (e.g., spermine or cadaverine), or a cationic polymer. Choice

of migration order can be of value when one of the enantiomers is present in a large excess, e.g., in the determination of the optical purity of a bulk sample.

^{1}H NMR and Fluorescence Spectroscopy: Complexation-Induced Shifts. Interestingly, the migration order of enantiomers seemed related to the orientation of the hydroxyl groups on a sugar derivative molecule. All sugars having the same configuration as D-glucose on C-2 were less strongly bound to CD and, hence, migrated at a higher electrophoretic mobility as borate complexes. Consequently, the chiral center closest to the naphthyl structure appears to determine complex stability when such a naphthyl moiety becomes encapsulated in the hydrophobic cavity of a CD. This effect was ascertained by ^{1}H NMR studies in D_2O. The migration of sugar enantiomers in D_2O were identical to the ones in H_2O, indicating no change in complexation behavior. Spectra were first recorded for individual compounds and then for mixtures of appropriate components. The results are demonstrated in Figure 8. The spectrum of β-CD in D_2O was found identical to that reported in the litature,[30] and no evidence for complexation with borate (100 mM at pD = 9.2) was observed. The hydroxyl groups located at C-2 and C-3 in the cyclodextrin are in a *trans* position and probably positioned too far apart to form a complex. The spectra of ANA-D- and -L-galactose were, however, severely broadened and shifted upfield in the 3–4 ppm region, corresponding to the sugar protons. Interestingly, the H-7 proton (d at δ 8.02 and 8.04) in the naphthyl ring and adjacent to the sulfonic acid group was broadened and shifted downfield, giving two doublets at δ 8.02, 8.04 and 8.09, 8.11, respectively (Figure 8A,B), when borate was added. Furthermore, H-3 and H-4 were resolved due to a greater shift for the H-4 proton.

When β-CD was added to the borate/sugar mixtures, relatively complex spectra were obtained, while an excess of β-CD interfered with most sugar resonances. The H-5 and H-6 protons of β-CD (δ 3.70–3.75) appeared somewhat resolved and shifted upfield. Similar assignments have been correlated to the shielding of protons inside the β-CD cavity for inclusion complexes with aromatic compounds.[31] The H-1, H-2, and H-4 protons (d at 4.95, 2 d at ~3.48, and t at 3.42 ppm, respectively) were essentially unaffected. However, the H-3 proton was shifted upfield (t, from δ 3.82 to 3.75) in the presence of L-galactose. As a consequence of stereospecific recognition, this must involve one of the hydroxyl groups or protons in the sugar derivative. Furthermore, the H-2 proton on L-galactose (2 d at δ 3.54–3.57) was resolved from the large β-CD peaks and shifted upfield to δ 3.52–3.55. Tentatively, the hydroxyl group at C-2 in these sugars (which was also found to determine the migration order in the β-CD concentration study above) may determine the strength of complexation between the sugar–borate/β-CD complex. The shift on the H-2 proton in L-galactose might then be due to a steric hindrance within the complex, as the D-form gave a stronger complex than the L-form.

Shifts in the aromatic part of the spectrum were quite different for the D- and L-galactose derivatives (Figure 8C,D). H-3 was downshifted compared to the situation in Figure 8A,B. For the D-form, H-8 and, especially, H-5 were dramatically upshifted and recorded as triplets instead of two doublets for each proton. The peaks for H-7 were basically restored in both the D- and L-forms upon complexation with β-CD, indicating that the naphthyl moiety was protected from the borate. The naphthyl groups thus seemed to be positioned deep in the CD cavity, with the sulfonic group situated relatively close to or inside the torus. Intracavity complexation of a sulfonated napththalene ring has been observed previously[32] for 6-toluidino-2-naphthalenesulfonate. The carbohydrate chain protruded outwards, interacting with the C-3 hydroxyl group on the CD molecule via its C-2 hydroxyl group, which appears to govern this chiral recognition. A proposed model of the complex between ANA-D-galactose and β-CD is shown in Figure 9. The hydroxyl groups on the sugar chain are consequently available for complex formation with borate.

The complexation mechanisms with β-CD were further verified through fluorescence spectroscopy and observed as an increase in fluorescence intensity and an emission spectral shift. No shifts in the excitation spectra were observed while using 475 nm (λ_{max}) as the emission wavelength. The excitation wavelength was 300 nm. When β-CD was added to mixtures of a sugar derivative (0.1 mM) and borate (0.1 M, pH = 9.5), the λ_{max} shifted to 440 and 450 nm for D- and L-galactose, respectively, indicating complexation between the molecular species. An increase in fluorescence and a shift in λ_{max} were directly related to the complex stability, with D-galactose showing the stronger interaction of the two enantiomers. The change in fluorescence ΔF can be related to the β-CD concentration according to[33,34]

$$\Delta F^{-1} = k_1[\beta\text{-CD}]^{-1} + k_2^{-1} \qquad (2)$$

The linear relationship between ΔF^{-1} and $[\beta\text{-CD}]^{-1}$ (the Benesi–Hildebrand equation) is shown for ANA and ANA-D- and ANA-L-galactose in Figure 10. The straight lines indicate that a 1:1 complex between the sugar derivatives and CD is being formed. An increase in the stability constant together with an increase in the slope appears consistent with the results obtained through the measurements of electrophoretic mobility.

Complexation with Linear Oligosaccharides and Polysaccharides. Cyclodextrins are cyclic oligosaccharides with six to eight glucose units linked in α(1–4)-positions. As shown in this work, they can be successfully used in the separation of sugar enantiomers. Consequently, certain water-soluble polysaccharides were investigated in order to determine which structural features are essential to chiral discrimination. The buffer additives investigated were Dextrin 10 (Glu-(1–4)-α-D-Glu), dextran (Glu-(1–6)-α-D-Glu, with an average molecular weight of 18 300), laminarin (mainly Glu-(1–3)-β-D-Glu), and the anionically charged alginic acid (linear anhydro-β-D-mannuronic acid). Also included were maltose oligomers (Glu-(1–4)-α-D-Glu) ranging from glucose up to maltoheptaose. None, except for Dextrin 10 and the maltooligomers, which had the same glycosidic bonds as the CDs, displayed enantioselectivity. Within the series of maltooligomers, chiral separation was first observed with maltotetraose. The separation selectivity increased with the increasing size of the maltooligomer chain (Figure 11). Here, 0.4 M borate at pH = 8.3 was used as the electrolyte.

Hydrophobic fluorescent probes are known to complex to maltooligosaccharide,[35] while the amphiphilic nature of dextrins has also been reported.[36] Even monosaccharides were shown to form complexes with β-CD in water, although their stability constants were very low.[37] The dextrin chains appear flexible, and depending on the size of a guest molecule, a helix with a different number of glucose units can readily be formed.[38]

Dextrin 10, defined by the manufacturer as an oligomeric mixture containing 10% of reducing matter, was first investigated regarding its potential in chiral recognition. An increase in enantioselectivity was observed by increasing the pH or concentration of Dextrin 10, as can be seen in Figure 12. The gain in selectivity was, however, counteracted by a substantial loss in peak narrowness. Probably, the kinetics of complexation due to the helix formation were slow, as the observed peaks were broad

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(but symmetrical) and the band-broadening effect was more pronounced with the more strongly bound enantiomer. Variation in the borate concentration between 50 and 200 mM gave no visible improvement in peak width and separation efficiency. Contrary to the case of β-CD, the borate seemed to bind to the dextrin as the electrophoretic mobilities increased with the dextrin concentration. A decrease in electrophoretic mobility at >100 mg/mL of dextrin could be due to the depletion of borate, as this roughly corresponded to 0.5 M glucose units. Furthermore, at this high concentration, the dextrin might start to polymerize and if it was held together by bridging borate bonds, the charge-to-mass ratio could diminish. (At pH = 11, the electroosmotic flow was reduced by 67% and the solutes had to be run in the cathodic mode; there was also an improvement in peak narrowness.) The dextrins should preferably be used at a lower pH compared to that of the borate systems, and the ratio of borate to dextrin has to be determined for a particular case, ensuring high peak efficiency as the necessary parameter for both the separation selectivity and the sensitivity of detection. With Dextrin 10, no separation was observed using phosphate as the buffer.

Conclusions

Fluorescent derivatives of sugar enantiomers were for the first time directly separated by capillary electrophoresis as complexes with borate and linear or cyclic dextrins. The selectivity can be adjusted by concentrations of borate and a dextrin. Additionally, the fluorescent tag influenced both selectivity and electrophoretic mobilities, with 5-amino-2-naphthalenesulfonate being the most effective substituent. The migration order could be controlled by a reduction of the electroosmotic flow or a choice of cyclodextrin type. However, no changes in migration order were observed when maltooligomers with a different degree of polymerization were used as chiral selectors. We have also demonstrated that phosphoric, phosphorous, and phenylphosphonic acids complexed with the sugars, providing a partial separation.

The complexation effect between D- and L-galactose derivatives, borate, and β-CD was studied by ^{1}H NMR and fluorescence spectroscopy, and differences in the binding mechanism between the two enantiomers were observed. The naphthyl ring of a derivatized sugar appears inserted into the interior of the cyclodextrin cavity, so that the chiral recognition is most likely governed by the interaction of the C-2 hydroxyl group on the sugar derivative and the C-3 hydroxyl group of the β-CD molecule. The complexation between a sugar–borate assembly and β-CD was shown to be 1:1, as evidenced by the linear relationships in the Benesi–Hildebrand plots.

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