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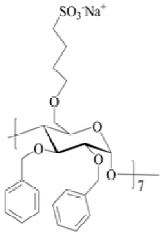

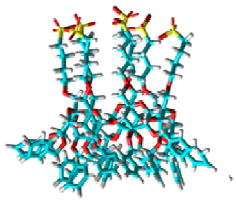

- Amphiphilic cyclodextrin
- Critical Micelle Concentration ~90 µM
- Chiral selector in capillary electrophoresis

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Synthesis of 2,3-dibenzyl-6-sulfobutyl-α and β cyclodextrins: new chiral surfactants for capillary electrophoresis

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ABSTRACT

Amphiphilic sodium hexakis (2,3-O-dibenzyl-6-O-sulfobutyl) cyclomaltohexaose and sodium heptakis (2,3-O-dibenzyl-6-O-sulfobutyl) cyclomaltoheptaose were synthesized in four steps. Pyrene fluorescence studies indicate micelle formation at 90 µM in water for both modified cyclodextrins (CDs). Both CDs offer potential as chiral micellar selectors for enantiomeric separations using capillary electrophoresis. Sodium heptakis (2,3-O-dibenzyl-6-O-sulfobutyl) cyclomaltoheptaose baseline resolved fluorescent cyanobenzylindole (CBI) derivatives of D/L-serine at concentrations of 50-200 µM.

Introduction

Cyclodextrins (CDs) are chiral oligosaccharide macrocycles composed of 6, 7, and 8 α-1, 4-D-glucopyranose units classified as α-, β-, and γ-cyclodextrin, respectively. CDs command great interest not only because of complexation ability of the native macrocycle, but due to possession of two secondary hydroxyls and one primary hydroxyl per glucopyranose unit. Each face of the pocket opening can be tailored with different moieties. Synthetic modification strategies are varied and have been well documented.[1-3] Undersubstitution of these CD hydroxyls is common due to the harsh conditions typically required for full substitution and the extensive purification necessary for isolation of single cyclodextrin isomers.[4] Selectively modified cyclodextrins that are undersubstituted are characterized by an average degree of substitution.

CDs and modified CDs have been used extensively as chiral selectors in capillary zone electrophoresis. In micellar electrokinetic chromatography, the buffer contains a micelle-forming surfactant as a pseudo-stationary phase.[5] The analytes differentially partition into the micelle and are separated. For chiral separation, a micelle-forming surfactant such as sodium dodecyl sulfate is typically used with the chiral cyclodextrin added to establish a secondary equilibrium with the micelle. Sometimes, a single chiral surfactant such as a bile salt is employed.[6]

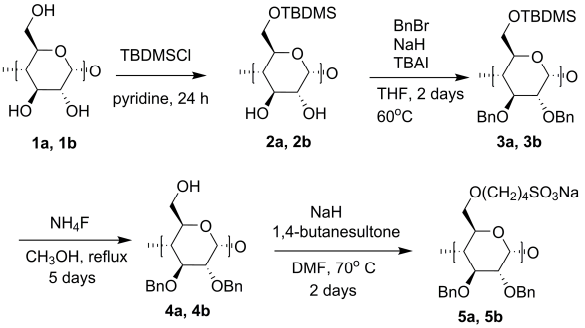
Our interest has been in developing micelle-forming amphiphilic cyclodextrins that can be used as chiral surfactants in capillary electrophoresis. A number of amphiphilic CDs have been previously synthesized, including both ionic and nonionic CDs.[7-15] As far as we are aware, amphiphilic CDs which form micelles have not been used in capillary electrophoresis studies. The intention of this work was to synthesize sodium hexakis (2,3-O-dibenzyl-6-O-sulfobutyl) cyclomaltohexaose (**5a**, Scheme 1) and sodium heptakis (2,3-O-dibenzyl-6-O-sulfobutyl) cyclomaltoheptaose (**5b**, Scheme 1) for this purpose. These cyclodextrin derivatives present 12 and 14 aromatic moieties, respectively, on one side of the cyclodextrin while 6 and 7 anionic sulfobutylethers, respectively, protrude from the other side of the molecule. The synthesis is described here and, as shown below, both CD derivatives form micelles with critical micelle concentrations (CMCs) of ~ 90 µM.

The ability of **5b** to act as a chiral selector in capillary electrophoresis is demonstrated with a fluorescent derivative of D/L-serine. D/L-serine was a chosen as a test analyte because of the biological significance of D-serine as a neuromodulator[16] and the challenge it presents in chiral separation among CBI-D/L-amino acids.[17] Specifically, the D/L-serine is derivatized with naphthalene-2,3-dicarboxaldehyde and cyanide under basic conditions[18] to form a fluorescent cyanobenz[f]isoindole -D/L-serine (CBI-D/L-serine) derivative. We demonstrate that chiral resolution is achieved with 50-200 µM of **5b** in the background electrolyte.

Results and Discussion

Sodium hexakis (2,3-O-dibenzyl-6-O-sulfobutyl) cyclomalto-hexaose (**5a**) and sodium heptakis (2,3-O-dibenzyl-6-O-sulfobutyl) cyclomaltoheptaose (**5b**) were synthesized according to Scheme 1. The primary hydroxyl groups were readily protected using standard procedures.[2] *tert*-Butylammonium iodide (TBAI) in catalytic amounts has been used to quantitatively benzylate sterically hindered hydroxyls on a glucose derivatives at room temperature in the presence of excess sodium hydride in tetrahydrofuran (THF).[19] This

concept was adapted to perbenzylate the secondary hydroxyls of cyclodextrins by using 0.01 equivalents of TBAI, two equivalents of benzyl bromide, excess sodium hydride in THF and heating the reaction to reflux for two days. Larger amounts of benzyl bromide were required for complete benzylation if the reaction was performed at room temperature. The addition of TBAI was found to be critical for the synthesis of both **3a** and **3b**; benzylation in its absence, regardless of amount of benzyl bromide or time allotted, was found to produce only undersubstituted benzyl derivatives as determined by NMR spectroscopy. We hypothesize that the requirement of TBAI is the catalytic *in situ* formation of benzyl iodide, with its better leaving group. Ammonium fluoride was chosen over the more traditional tetrabutylammonium fluoride as a deprotection agent due to ease of workup; **4a** and **4b** are soluble in chloroform whereas ammonium fluoride is not, thus eliminating the need for column chromatography of **4a** and **4b**.



Scheme 1: Synthesis of amphiphilic cyclodextrin derivatives (n = 6,7)

Complete sulfoalkylation of primary hydroxyls in tetrahydrofuran (THF) has been shown previously to be problematic.[20] Kirschner and Green were able to synthesize single isomer 2,3-dialkyl-6-sulfoalkylated cyclodextrin derivatives using a 3-fold molar excess of 18-crown-6 ether in addition to alkanesultone and potassium hydride in THF.[20] 18-crown-6 ether, by complexing with potassium counterion, helps to solubilize the increasing anionic CD. This procedure, while successful, requires addition of large amounts of 18-crown-6 ether, followed by ion-exchange chromatography and numerous extractions for its removal. We find that addition of 1,4 butanesultone (3 eq per hydroxyl), and excess sodium hydride in dry DMF, with mild heating, allows for complete sulfobutylation to **5a** and **5b**. The products were conveniently purified by ultrafiltration. The products were fully characterized by ^{1}H, ^{13}C, COSY, and HMQC NMR spectroscopy as well as ESI-MS.

Pyrene fluorescence has been used to determine CMCs of calixarene-based surfactants.[21] We used a modified procedure from Lopez-Diaz *et al.*[22] to determine the CMCs of **5a** and **5b**. Pyrene solution in methanol (1.25 µL of 2 mM) was pipetted into vials and blown dry with nitrogen. Aqueous solutions of **5a** or **5b** (2.5 mL) ranging from 5 µM to 5 mL were subsequently added and stirred resulting in a pyrene concentration of 1 µM. The emission spectrum of pyrene was obtained using an excitation wavelength of 320 nm. The emission range was set between 350 and 450 nm. Emission intensities were recorded at 373 nm for 1st vibration peak (I_1) and 384 nm for 3rd vibrational peak (I_3).[22]

The ratio of I_1/I_3 in the emission spectrum of pyrene changes in response to the solvent polarity; the I_1/I_3 ratio is a reflection of the local structure in the vicinity of the probe. A change in local dipole moment indicates the equilibrium partitioning from an aqueous environment to a more hydrophobic one.[23] Hydrophobic molecules (*e.g.* pyrene) have a greater affinity for the hydrophobic micellar core than the hydrophilic bulk solution. A plot of the (I_1/I_3) ratio against the log of surfactant concentration produces a sigmoidal shaped curve. The CMCs of the amphiphilic cyclodextrin derivatives were determined from the sharp changes in the slopes as pyrene transitions from an increasing less polar to a micelle hydrophobic environment.[24] The values of the I_1/I_3 ratio were used to estimate the hydrophobicity of the aggregate microenvironment using an empirical scale of the relative band intensities of pyrene in different solvents.[25] Initial I_1/I_3 values at the CMC in Figure 1 indicate a methanol/methylene chloride-like environment inside the aggregates but with the local polarity approaching benzyl alcohol as the concentration of amphiphile increases.[25] This apparent change in the hydrophobicity of the interior with concentration past the CMC may be due to activity effects and/or greater exclusion of water from the interior as concentration of monomer increases. The CMCs of both **5a** and **5b** are determined to be approximately 90 µM.

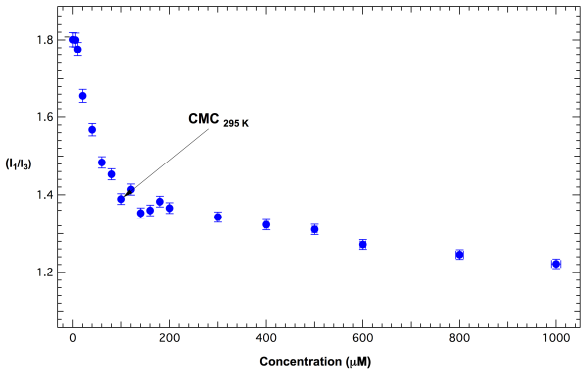


Figure 1. Determination of CMC of **5a** using pyrene fluorescent molecular probe. CMC = 90 ± 2 µM.

We found that **5b** was effective as a chiral selector in capillary electrophoresis studies of fluorescent CBI-derivatives of D,L amino acids. Electrophoresis was performed in reverse polarity mode (anode on detector side) at -25 kV with low pH (2.00) phosphate buffer. Under this condition, electroosmotic flow is minimized and highly charged anionic **5b** migrates toward the detector. CBI-amino acids may interact through their hydrophobic naphthalene group to form inclusion complexes in the CD cavity and/or through interaction with the benzyl groups of the secondary rim of the CD. CBI-amino acids can also interact through H-bonding with the CD. Strong complex formation should result in sweeping of the CBI-amino acids toward the detector.

Average migration times of fluorescent CBI-D/L-serine pair were observed to decrease with increasing concentration of **5b**. This result is expected since, as concentration of **5b** increases, the equilibrium shifts toward complex and, given that the complex is negatively charged (-7), the CBI-D-serine is driven toward the detector more rapidly. Apparent electrophoretic mobilities (average of CBI-D/L-serine pair), μ_i, were calculated from the migration times according to the equation[26]

$$\mu_i = \frac{v_i}{E} = \frac{L_d/t_m}{V/L_t} \qquad (1)$$

where v_i is electrophoretic velocity (cm/s), E is field strength (V/cm), L_d is length of capillary to the detector, L_t is the total length, t_m is the migration time, and V is the applied voltage (V). Mobility depends on charge/size ratio. To obtain actual mobilities, apparent mobilities should be corrected for both (1) electroosmotic flow and (2) viscosity changes in the background electrolyte due to increasing concentration of **5b**. At pH 2.00, however, electroosmotic flow is virtually abolished and is too small to measure. Also, we find that viscosity changes are negligible over the concentration range of **5b** (0-1 mM). Thus the apparent mobilities are the actual mobilities in this study.

Actual mobilities as a function of [**5b**] are shown in Figure 2. Mobility of the analyte increases with [**5b**] as the equilibrium concentration of charged complex increases (higher charge/size

ratio). Maximum mobility is reached at ~800 - 1000 µM of **5b**, which indicates nearly complete complexation of the CBI-serine pair in this concentration range.

Chiral resolution (R_s) reaches a maximum of ~2 (baseline resolution is 1.50) at concentrations of 50-200 µM of **5b** with a migration times of 6-9 min. An electropherogram using 100 µM of **5b** is illustrated in Figure 3. A number of other chiral CBI-amino acids were baseline-resolved at equally low concentrations.

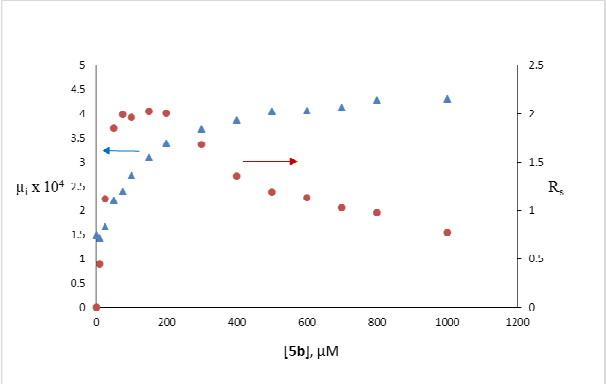


Figure 2. Electrophoretic mobilities, μ_i ($cm^2\ V^{-1}\ s^{-1}$) and chiral resolution (R_s) of CBI-D/L-serine pairs versus concentration of **5b**. Capillary electrophoresis: 50 µm id, 68 cm total, 48 cm to detector, -25 kV, 25 mM phosphate, pH 2.0, 410 nm LIF detection.

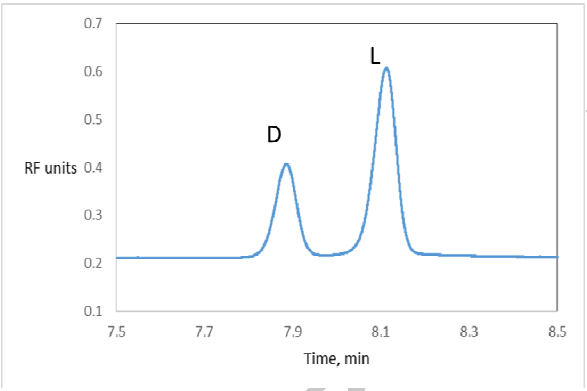


Figure 3. Resolution of CBI-D/L-serine using 100 µM of **5b**. See **Figure 2** for CE conditions.

Most chiral separations of fluorescently-tagged amino acids employ a CD or modified CD in combination with a micelle-forming surfactant such as sodium dodecyl sulfate.[16] Typically, the CD is neutral and the surfactant is negatively charged. The analyte distributes between the CD, the micelle and the aqueous phase to provide separation. In most circumstances, the CD and surfactant are in used concentrations greater than 1 mM. For example, CBI-D/L-serine enantiomers have been separated using 30 mM β-CD/60 mM chiral sodium taurocholate[27] and 10 mM γ-CD/50 mM sodium dodecylsulfate.[28] Other CBI/-DL-amino acids have been separated with hydroxylpropyl-β-CD/50 mM sodium dodecylsulfate, but this mixture failed to resolve the serine enantiomers.[29]

In some cases, the use of a surfactant is not required. Quan *et al*. employed combination of chiral selectors in 20 mM hydroxylpropyl-γ-CD/15% (w/v) D-(+)-glucose to provide resolution of the serine enantiomers.[30] We have optimized resolution using 10 mM commercially-available randomly sulfated β-CD in reverse polarity mode at low pH.[17] All of these examples to point to the usual requirement of high concentrations of cyclodextrin (mM range) in the background electrolyte, either with or without surfactant.

In contrast, we obtain baseline chiral resolution of CBI-D/L-serine with 50 µM of **5b** in the background electrolyte. The ability of **5b** to resolve CBI-D/L-serine enantiomers at such low concentrations must be considered in the context of intermolecular interactions. First, it is clear that the **5b** has unusually high binding to the serine derivatives. This is evident from Figure 2, where the average mobility reaches a plateau at or near 800 µM of **5b**, indicative of nearly complete complexation with CBI-D/L-serine at this concentration. The binding constant, K, assuming 1:1 interaction between **5b** and analyte, was determined by nonlinear curve fitting of the following equation[31]

$$\mu_i = \frac{\mu_f + \mu_c K[CD]}{1 + K[CD]} \qquad (2)$$

where μ_i is the calculated mobility, μ_c is the saturated mobility, μ_f is the mobility with no CD. Both K and μ_c are treated as adjustable parameters in the fit.

The treatment yields a K of 5510 ± 560 M^{-1} and μ_c of 4.89 ± 0.11 x 10^{-4} $cm^2\ V^{-1}\ s^{-1}$. In contrast, a similar treatment of the mobility curve of randomly sulfated β-CD (average degree of sulfation of 9) yields a K of 81 ± 3 M^{-1} and μ_c of 3.91 ± 0.05 x 10^{-4} $cm^2\ V^{-1}\ s^{-1}$ (see Supplementary Data). The much stronger binding of **5b** to serine is most probably due to its well-defined and extended hydrophobic cavity, with its sulfobutyl groups on the primary face and the benzyl groups on the secondary face. The randomly sulfated β-CD has sulfate groups on both faces of the CD and lacks the extended hydrophobic cavity.

Wren and Rowe developed a model relating enantiomer mobility to the concentration of CD chiral selector.[32] Penn et al. extended the treatment[33] and showed that maximum mobility difference (related to resolution) occurs when the CD concentration equals the inverse of the average binding constant, K. For **5b**, this corresponds to 180 µM, consistent with the maximum in R_s achieved at 50-200 µM observed in Figure 2. For randomly sulfated β-CD, maximum mobility difference is calculated to be 12 mM, consistent with that optimized experimentally.[17]

Conclusion

In summary, we have synthesized, for the first time, amphiphilic 2,3-dibenzyl-6-sulfobutyl-α and β-cyclodextrins, sodium salts (**5a** and **5b**). Both molecules form micelles and exhibit CMCs of about 90 µM. **5b** is shown to bind strongly to fluorescent CBI-D/L-serine derivatives while providing chiral resolution at low concentrations. These new CDs have potential as chiral surfactants in capillary electrophoresis studies that employ fluorescence detection.

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Supplementary Data

Supplementary data, including the experimental section, NMR, MS and binding constant data, can be found in the online version at

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