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Preparation of a new crown ether-based chiral stationary phase containing thioester linkage for the liquid chromatographic separation of enantiomers

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ABSTRACT

A new chiral stationary phase (CSP) containing thioester linkages was prepared by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to mercaptopropylsilica gel. The chiral recognition ability of the new CSP was found to be greater than that of the previously reported CSP containing amide linkages in the resolution of the various α-amino acids that were tested, except for that of Met, Ser and Thr. In the resolution of racemic amines and amino alcohols, the new CSP was always better than the one containing amide linkages in terms of the separation factors (α) and the resolutions (R_S). Given the identical elution orders on the two CSPs, it was concluded that the chiral recognition mechanism is not affected by the change of the linkage type. In addition, the new CSP was found to be quite stable under the acidic mobile phase conditions that were utilized, indicating that the thioester linkage is useful as a tethering group.

1. Introduction

Chiral crown ethers have been successfully utilized as chiral selectors of chiral stationary phases (CSPs) for the liquid chromatographic separation of the two enantiomers of racemic primary amino compounds [1,2]. For example, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (**1**, Fig. 1) has been very successful as a chiral selector of CSPs for the liquid chromatographic resolution of racemic compounds containing a primary amino group [3,4]. Especially, CSP **2** (Fig. 1) developed in our laboratory by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to 3-aminopropylsilica gel was very successful in the resolution of various racemic primary amino compounds including α-amino acids [5], β-amino acids [6], γ-amino acids [7], amino alcohols [8], amines [8], aryl α-aminoketones [9], tocainide and its analogues [10], *di*- and *tri*-peptides [11] and fluoroquinolone antibacterials [12]. CSP **2** was also quite successful in the resolution of racemic non-primary amino compounds including secondary amines [13], secondary amino alcohols [14,15] and *N*-(3,5-dinitrobenzoyl)-α-amino acids [16] and their amides [17].

Even though CSP **2** is quite effective, the unreacted aminopropyl groups remaining on the surface of the stationary phase, which originate from the incomplete process of bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to 3-aminopropylsilica gel, might impair the chiral recognition ability of the stationary phase. The primary ammonium ions resulting from the protonation of the unreacted residual aminopropyl groups under acidic mobile phase conditions compete with those of analytes for the complexation inside the cavity of the crown ether ring of the stationary phase, which is known to be essential for chiral recognition [1,2]. Consequently, removing the unreacted residual aminopropyl groups of the CSP would be expected to improve the chiral recognition ability of the CSP.

In an effort to avoid the problems related to the unreacted residual aminopropyl groups of CSP **2**, in this study, we prepared a new CSP (CSP **3**, Fig. 1) and applied it to the resolution of various α-amino acids, amines and amino alcohols. CSP **3** intrinsically does not contain any unreacted aminopropyl groups, because the thioester linkage is used to connect (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to silica gel and consequently, its chiral recognition ability is expected to be greater than that of CSP **2**.

2. Experimental

2.1. Preparation of CSP 3 and column packing

A slurry consisting of 3 g of Kromasil silica gel (5 μm, surface area 305 m^2/g, Eka Chemicals, Bohus, Sweden) in 100 ml of toluene was refluxed in a 250 ml round bottom flask equipped with a Dean-Stark trap, a condenser and a magnetic stirring bar until the complete azeotropic removal of water was achieved. Then, 3-mercaptopropyltrimethoxysilane (1.23 ml, 6.62 mmol) was added to the slurry. The whole mixture was gently stirred for three days at the reflux temperature and then, cooled to room temperature,

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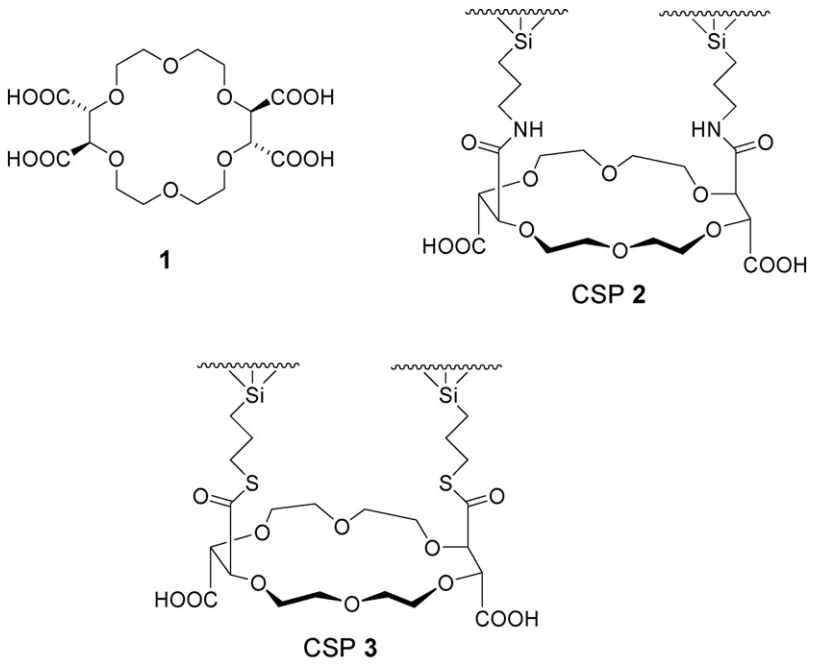

Fig. 1. Structures of (+)-(18-crown-6)-2,3,11,12-tetracarboxylica acid **1**, CSP **2** and CSP **3**.

filtered and washed successively with methanol, acetone, ethyl acetate, methyl chloride, hexane and diethyl ether. The resulting modified silica gel (3-mercaptopropylsilica gel) was dried under high vacuum. The calculation based on the elemental analysis of 3-mercaptopropylsilica gel (found: C, 1.60%; H, 2.80%; S, 1.32%) showed that the surface concentration of 3-mercaptopropyl groups was 1.14 μmol/m^2 based on carbon.

3-Mercaptopropylsilica gel (3.0 g) was slurried into 100 ml of toluene in a 250 ml round bottom flask equipped with a Dean-Stark trap, a condenser and a magnetic stirring bar. The whole mixture was refluxed until the complete azeotropic removal of water was achieved. Then, toluene was removed under reduced pressure. To the residue were added dried methylene chloride (30 ml) and 2,6-lutidine (0.18 ml, 1.55 mmol). The heterogeneous solution was stirred for 30 min at 0 °C. To this cooled solution was slowly added (+)-(18-crown-6)-2,3,11,12-tetracarboxylic dianhydride, which was prepared from (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (325 mg, 0.74 mmol) via the reported procedure [5], in 15 ml of methylene chloride. The whole mixture was stirred for two days at room temperature under an argon atmosphere and then filtered and washed successively with methanol, acetone, ethyl acetate, methylene chloride, hexane and diethyl ether. The resulting modified silica gel (CSP **3**) was dried under high vacuum. The calculation based on the elemental analysis of CSP **3** (found: C, 4.55%; H, 2.60%; S, 1.23%) and the 3-mercaptopropylsilica gel according to the reported method [18] showed that the surface concentration of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid was 0.48 μmol/m^2 based on carbon.

CSP **3** (2.2 g) was slurried in methanol and packed into a 150 mm × 4.6 mm I.D. stainless steel HPLC column by using a conventional slurry packing method with an Alltech slurry packer.

2.2. Chromatography

Chromatography was performed with an HPLC system consisting of a Waters model 515 HPLC pump (Milford, MA, USA), a Rheodyne model 7725i injector (Rohnert Park, CA, USA) with a 20 μl sample loop, a Waters 2487 Dual absorbance detector and a YoungLin Autochro data module (Software: YoungLin Autochro 2000). The temperature of the chiral column was maintained at 20 °C by using a Julabo F30 Ultratemp 2000 cooling circulator (Seelbach, Germany). The void volume was measured by injecting 2,6-lutidine. The racemic and optically active α-amino acids, amines and amino alcohols used in this study as analytes were obtained from Aldrich. The injection samples were prepared by dissolving each analyte in methanol at a concentration of 1.0 mg/ml. The usual injection volume was 3.0 μl.

3. Results and discussion

CSP **3** containing thioester linkages was applied first of all to the resolution of various α-amino acids. The mobile phase used for the resolution of α-amino acids on CSP **2** was 80% methanol containing 10 mM sulfuric acid. Under identical mobile phase conditions, the retention times of the two enantiomers for the resolution of α-amino acids on CSP **3** were quite long. The competition of the primary ammonium ions, resulting from the protonation of the unreacted residual aminopropyl groups of CSP **2** under acidic mobile phase conditions, with the primary ammonium ions of the analytes for complexation inside the cavity of the crown ether ring of the stationary phase might diminish the retention times of the two enantiomers. However, the primary ammonium ions originating from unreacted residual aminopropyl groups are not present on CSP **3** and consequently, the retention times of the enantiomers might be longer on CSP **3** than on CSP **2** under identical mobile phase conditions.

In order to reduce the retention times on CSP **3**, ammonium acetate was added to the mobile phase. The ammonium ions in the mobile phase are expected to compete with the primary ammonium ions of the analytes for complexation inside the cavity of the crown ether ring of the stationary phase and, consequently, the retention times of the enantiomers would be expected to be diminished. As shown in Fig. 2, the retention factor (k_1) decreases continuously as the concentration of ammonium acetate in the mobile phase (80% methanol containing 10 mM sulfuric acid) is increased. The separation factor (α) and the resolution (R_S) also decrease continuously as the concentration of ammonium acetate in the mobile phase is increased. Considering the retention times