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Thioether bridged cationic cyclodextrin stationary phases: Effect of spacer length, selector concentration and rim functionalities on the enantioseparation

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ABSTRACT

The preparation and evaluation of four single thioether bridged cationic cyclodextrin (CD) chiral stationary phases (CSPs) with different spacer length, selector concentration and rim functionalities are reported. Mono-6-(1-vinyl/allyl/butenylimidazolium)-β-CDs chloride were synthesized and clicked onto thiol silica to form three novel cationic native-CD-CSPs (**CSP1**, **CSP2** and **CSP3**) and a post-synthetic phenylcarbamoylation of **CSP2** was performed affording **CSP4**. The enantioseparation ability of the as-prepared CSPs were evaluated in high performance liquid chromatography (HPLC) by separating over forty enantiomers including isoxazolines, dansyl amino acids, flavonoids, tröger's base, 4-chromanol, bendroflumethiazide and styrene oxide. Most of the enantiomers were well resolved with the resolution (R_s) of 4NPh-OPr reaching 12.68. The effects of spacer length, selector concentration and rim functionalities on the enantioseparation were investigated. A comparison of the current CSP with a commercial column (Cyclobond I 2000) was also conducted to reveal the superiors enantioselectivity of the as-prepared CSPs.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharide molecules which are widely employed in HPLC as chiral stationary phases (CSPs) [1–4]. CDs have a hydrophobic internal cavity and hydrophilic external surfaces and are able to form inclusion complexes with numerous compounds. In addition, CD hydroxyl groups could be functionalized with alkyl, alkoxyl or phenylcarbamoyl groups to afford dipole-dipole, H-bonding, hydrophobic and π-π interactions [5–9]. Development of structurally well-defined CD-CSPs has been considered as the perpetual goal in this area owing to their good separation ability and batch-to-batch reproducibility. In 2008, the well-known click chemistry (Cu (I) catalytic 1,3-dipolar cycloaddition) was firstly employed for fabrication of a structurally well-defined native CD-CSP and a series of new CD-CSPs were thereafter developed via this robust approach for chiral chromatography [10–17].

In order to avoid the use of metal catalyst and the explosive azide derivatives, very recently, a more versatile click tool, thiol-ene reaction, has attracted increasing attention in fabrication of separation materials due to its very mild reaction conditions and the formed stable thioether bond. Several kinds of zwitterionic stationary phases were successfully fabricated via this approach and applied for achiral hydrophilic interaction liquid chromatography (HILIC) [18–20] or mixed-mode chromatography [21]. Besides, thiol-ene click chemistry was also approved to be suitable for the development of chiral separation materials. Novel crown ether modified quinine CSPs were fabricated by Liang et al. and evaluated for enantioseparation of acids and primary amines [22]. Quinine carbamate based monolith and silica CSPs were prepared in Lämmerhofer's group for efficient enantioresolution [23,24]. Strong cation exchanger (SCX) CSPs were developed by Lindner's group and applied in supercritical fluid chromatography (SFC) for chiral resolution of basic drugs and their analogs [25]. Zou and coworkers reported a novel phenylcarbamoylated β-CD-CSP prepared via thiol-ene click chemistry for enantioseparation of some β-blockers [26]. Encouraged by the effectiveness of thiol-ene reaction, our group reported a stable β-CD-CSP by immobilizing 1-vinylimidazolium-β-CD tosylate to thiol silica via thiol-ene click reaction for efficient resolution of anti-charged analytes [27,28] and two novel multifunctional CD-CSPs with well-defined struc-

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tures were further prepared via a similar approach for ultimate enantioseparation of a large group of enantiomer pairs [29].

It is known that the enantiorecognition ability of CD-CSPs strongly depends on their structure modifications such as CD rim functionalization, linker type and length, surface concentration, etc. Various favorable interactions like H-bonding, dipole-dipole, π-π and electrostatic effects can be introduced to CD rims and CSP linkers to tune the CSPs' enantioselectivity [30–34]. The induced steric effects ascribed from spacer length and CD types can also engender obvious influence on the enantioseparation [6,35].

Considering the effectiveness of thiol-ene click chemistry in development of CD-CSPs, it is necessary to comprehensively investigate their structural effects such as thioether spacer length, surface concentration and CD rim functionalities on their enantioselectivities to provide scientific guidance for such CSPs' practical applications. Herein, four single thioether bridged cationic CD-CSPs with different spacer lengths, surface concentration and rim functionalities were fabricated and their separation performance were evaluated in reversed-phase HPLC using over forty enantiomers including isoxazolines, dansyl amino acids and carboxylic acids, flavonoids, tröger's base, 4-chromanol, bendroflumethiazide and styrene oxide. A comparison study with the commercial column CYCLOBOND I 2000 was also conducted.

2. Experimental

2.1. Chemicals

Azobisisobutyronitrile (AIBN) and pyridine were purchased from Tianjin Chemical Regents (Tianjin, China). HPLC-grade methanol (MeOH) and acetonitrile (ACN) were provided by Guangfu chemical reagents (Tianjin, China). Silica gel was obtained from Fuji silica (Fuji, Japan). 3-Mercaptopropyltrimethoxysilane, 1-allylimidazole, anhydrous *N,N*-dimethylformamide (DMF) and toluene were purchased from Heowns (Tianjin, China). *p*-tolyl isocyanate was purchased from Energy-Chemical (Shanghai, China). Mono-6^A-deoxy-(*p*-tolylsulfonyl)-β-cyclodextrin (TsO-CD) was synthesized according to the reported procedure [12]. All the isoxazoline racemic pairs used were synthesized according to our previously reported procedure [28]. All the other racemates were obtained from Energy-Chemical (Shanghai, China). CYCLOBOND I 2000 was purchased from Sigma-Aldrich (Shanghai, China). The racemates structures and some representative chromatograms are shown in Figs. 1 and 2.

2.2. Instruments and methods

^{1}H NMR were collected on a Bruker ACF400 (400 MHz) supplied by Bruker Biospin (Fällanden, Switzerland). Mass spectra were collected on an Agilent 1200 HPLC-6310 (USA). Elemental analysis was performed on a VarioMICRO CHNOS elemental analyzer (Elementar Analysensysteme, Hanau, Germany). Fourier-transform infrared (FTIR) spectra were performed on an AVATR360 supplied by Thermo Nicolet (USA). Chromatographic analyses were performed on an Agilent 1100 HPLC (USA).

Methanol (MeOH) or acetonitrile (ACN) mixed with ultra-pure water or triethyl ammonium acetate buffer (TEAA) were used as mobile phases (MPs). Samples were dissolved in MeOH/H_2O (v/v = 1:1) at a concentration of 1 mg mL^{-1} and the injection volume was set as 10 μL. All the samples were filtered through a 0.45 μm membrane before usage. UV absorbance was detected at 220–300 nm. Each solution was injected in triplicate and the average value was used. All the separations were performed with a flow rate of 1 mL min^{-1} at 30 °C. Calculations for capacity factor, *k*; selectivity, α and resolution, R_s were performed following USP standards.

2.3. Preparation of novel single bridged cationic native and p-methylphenylcarbamoylated CD CSPs via thiol-ene click chemistry

The synthetic pathway of the novel native and *p*-methylphenylcarbamoylated-CD-CSPs undergoes several steps (Fig. 3). TsO-CD and thiol functionalized silica were prepared using our previous approaches [26,27].

2.3.1. Preparation of mono-6^A-deoxy-6-(1-alkenylimidazolium)-β-CD chloride

TsO-CD (5.24 g, 4.06 mmol) and 1-alkenylimidazole (1.2 mL) were added to anhydrous DMF (30 mL), the reaction solution was heated to 60 °C and kept for 24 h under N_2. The resultant solution was poured into acetone. The white solid collected by filtration was washed with acetone (2 × 20 mL) to afford compounds ***1***, ***2*** and ***3*** with a yield of 70–80%. The obtained mono-6^A-deoxy-6-(1-alkenylimidazolium)-β-CD tosylate ***1***, ***2*** and ***3*** were dissolved in DI water and subjected to Amberlite 900 (Cl) resin fixed column. The resulting filtrate was dried under reduced pressure to afford product ***1′***, ***2′*** and ***3′***.

Analytical data of the synthesized mono-6^A-deoxy-6-(1-alkenylimidazolium)- β-CD chloride

Mono-6^A-deoxy-6-(1-vinylimidazolium)-β-CD chloride (*1′*): ^{1}H NMR (400 MHz, DMSO-d_6): 9.42 (1H), 8.18 (1H), 7.87 (1H), 6.00–5.42 (3H), 4.99–4.84 (7H), 4.58–3.55 (42H), 2.34 (4H). ESI–MS (*m*/*z*): 1212.4 (calculated) and 1212.8 (found) for [M$^+$].

Mono-6^A-deoxy-6-(1-allylimidazolium)-β-CD chloride (*2′*): ^{1}H NMR (400 MHz, DMSO-d_6): 9.15 (1H), 7.76–7.73 (2H), 6.01–5.33 (3H), 4.98–4.85 (7H), 4.57–3.56 (42H), 2.34 (4H). ESI–MS (*m*/*z*): 1225.4 (calculated) and 1225.6 (found) for [M$^+$].

Mono-6^A-deoxy-6-(1-butenylimidazolium)-β-CD chloride (*3′*): ^{1}H NMR (400 MHz, DMSO-d_6): 9.13 (1H), 7.81 (1H), 7.73 (1H), 6.01–5.64 (3H), 5.10–4.75 (7H), 4.55–3.56 (42H), 2.34 (4H). ESI–MS (*m*/*z*): 1239.4 (calculated) and 1239.7 (found) for [M$^+$].

2.3.2. Click coupling of thiol silica and cationic CD

Compound ***1′***, ***2′*** or ***3′*** (3 g) was added to a 30 mL of MeOH and DI water mixture (1:1, v/v) under stirring. Thiol silica (5 g) and AIBN (100 mg) were then added into the clear solution. The reaction was allowed to stir for 24 h at 45 °C. The crude product was obtained by filtration, which was washed subsequently with DMF (2 × 20 mL), DI water (2 × 20 mL), MeOH (2 × 20 mL) and acetone (2 × 20 mL) and vacuum dried (60 °C, 0.1 mbar) to afford the desired **CSP1**, **CSP2** and **CSP3**.

2.3.3. Synthesis of *CSP4*

CSP2 (2.5 g) was dissolved in 30 mL of pyridine and *p*-tolyl isocyanate (3.85 mL) was then added into the mixture and the reaction mixture was allowed to stir at 85 °C for 18 h protected with N_2. The crude product was afforded by filtration and extracted with acetone for 8 h followed by vacuum drying.

2.4. Column packing

The chiral columns were fabricated by packing the prepared CSPs into stainless-steel column (150 mm × 4.6 mm I.D.) using the typical slurry-packing technique with MeOH/$CHCl_3$ as the packing solvent.