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Thiol–ene click chemistry derived cationic cyclodextrin chiral stationary phase and its enhanced separation performance in liquid chromatography

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ABSTRACT

This work is the first demonstration of a simple thiol–ene click chemistry to anchor vinyl imidazolium β-CD onto thiol silica to form a novel cationic native cyclodextrin (CD) chiral stationary phase (CSP). The CSP afforded high enantioseparation ability towards dansyl (Dns) amino acids, carboxylic aryl compounds and flavonoids in chiral HPLC. The current CSP demonstrates the highest resolving ability (selectivity >1.1, resolution >1.5) towards Dns amino acids in a mobile phase buffered at pH = 6.5, with the resolution of Dns-DL-leucine as high as 6.97. 2,4-dichloride propionic acid (2,4-ClPOPA) was well resolved with the selectivity and resolution of 1.37 and 4.88, respectively. Compared to a previously reported native CD-CSP based on a triazole linkage, the current cationic CD-CSP shows a stronger retention and higher resolution towards acidic chiral compounds, ascribed to the propitious strong electrostatic attraction. Stability evaluation results indicated that thiol–ene reaction can provide a facile and robust approach for the preparation of positively charged CD CSPs.

1. Introduction

Enantiopure drugs are always in high demand due to the different biological and pharmacological activities of drug enantiomers. Enantioseparation of chiral compounds will continue to be a key research area in pharmaceutical industry, food additives and life science, to name a few [1–3]. It is well established that direct enantioseparation by high performance liquid chromatography (HPLC) with chiral stationary phases (CSPs) can serve as a robust technique for separation at both analytical and preparative scale.

Cyclodextrins (CDs) are naturally-occurring cyclic oligosaccharides consisting of several (6, 7, 8) glucose units, which are one of the most commonly used chiral selectors for enantioseparation due to their ability to form host–guest inclusion with a large variety of chiral compounds [4,5]. More importantly, enantiorecognition ability of CDs can be finely tuned by modification of their hydroxyl moieties with other functional groups [6,7]. There are numerous reported CD derivatives with different functionality for diverse purposes, such as methylated CDs [8,9], acetylated CDs [10,11], phenylcarbamoylated CDs [12,13], hydroxypropylated CDs [14,15] and sulfated CDs [16,17]. Cationic CDs form an important branch of functionalized CDs and have exhibited great potential in chiral resolution of acidic enantiomers in capillary electrophoresis (CE) [18–20]. The introduction of positive charges endows CD selectors with strong electrostatic attraction sites which are favorable for the "three-point" chiral recognition principle [21,22]. Some typical series of cationic CDs used in CE include amino-CDs [23], alkylamino-CDs [24], imidazolium-CDs [25], pyrrolidinium-CDs [26], etc. However, there are very few studies which demonstrate the resolving power of cationic CD CSPs by the more versatile HPLC technique. Zhou et al. reported the preparation of several positively charged CD CSPs with ether linkages [27]. Ng's group also developed a kind of cationic phenylcarbamoylated-CD CSP via a polymerization reaction [28]. Unfortunately, such approaches cannot afford a controllable CSP structure. The former approach was unable to determine the number of CD hydroxyl groups participating in the reaction and the batch-to-batch reproducibility is poor; while for the latter, it is difficult to control the degree of polymerization. In addition, no acidic enantiomers were separated on these CSPs to affirm the effect of the cationic sites. Therefore, it remains highly challenging to fabricate a stable and structurally well-defined cationic CD CSP in this field.

In recent years, an important segment of "click chemistry", i.e. radical-based thiol–ene reaction, has been attracting great

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interest since it possesses several advantages such as insensitivity to water, oxygen and many functional groups, ability to proceed neat under mild conditions and without the need of a metal catalyst. The thiolether linkage formed serves as a strong and stable covalent bond, which are able to withstand harsh conditions. Thiol–ene reaction has been widely employed for dendrimer synthesis, new polymer synthesis, biomolecules functionalization and surface modification [29–37]. This reaction has also been employed to prepare new chromatographic materials very recently. Liang et al. synthesized a novel zwitterionic stationary phase with high hydrophilicity by thiol–ene reaction between cysteine and vinyl silica [38,39]. Xu et al. developed a new imidazolium-based zwitterionic stationary phase for hydrophilic interaction chromatography (HILIC) [31]. Lindner et al. reported the development of reactive thiol-modified monolithic capillaries and in-column surface functionalization by radical addition of a chromatographic ligand for capillary electrochromatography [40]. The same group also fabricated a strong cation exchange-type chiral stationary phase (CSP) for enantioseparation of chiral amines by subcritical fluid chromatography [30]. These attributes motivate us to employ thiol–ene reaction to prepare stable and structurally well-defined cationic CD CSPs for HPLC enantioseparation.

To the best of our knowledge, there are very few reports on thiol–ene reaction for the fabrication of stable cationic native CD CSPs. In this work, we demonstrate a facile approach to construct a novel native CD CSP bearing cationic imidazolium on the linking bridge via thiol–ene reactions. On one hand, it is a new approach to the preparation of functional stable CD CSPs with thiolether linkage; on the other hand, it is the first demonstration of significant improvement of chiral selectivity towards acidic chiral compounds in HPLC via electrostatic interaction ascribed to the stable and positively-charge imidazolium.

2. Experimental

2.1. Chemicals and materials

Azobisisobutyronitrile (AIBN) were purchased from Tianjin Chemical Reagents (Tianjin, China). 3-Mercaptopropyltrimethoxysilane, 1-allylimidazole were purchased from Energy-Chemical (Shanghai, China). Mono-6^A-deoxy-(*p*-tolylsulfonyl)-β-cyclodextrin (TsOCD) was synthesized according to the reported procedure [41], HPLC-grade methanol (MeOH), acetonitrile (ACN), triethylamine, acetic acid were provided by Guangfu chemical reagents (Tianjin, China). Ultra-pure water was prepared by Milli-Q water purification system (Billerica, MA, USA). Anhydrous *N,N*-dimethylformamide (DMF) and toluene were purchased from Heowns (Tianjin, China). All the acidic racemates were purchased from Sigma-Aldrich (Shanghai, China). All the flavonoid recemates were obtained from Energy-Chemical (Shanghai, China). Kromasil spherical silica gel (5 μm, 100 Å) were obtained from Eka Chemicals (Bohus, Sweden).

2.2. Instruments and chromatographic conditions

Fourier-transform infrared (FTIR) spectra were collected on an AVATR360 supplied by Thermo Nicolet (USA). Mass spectra were collected on LCQ Deca XP MAX system (Thermo Fischer, USA). ^{1}H NMR were recorded on a Bruker ACF400 (400 MHz) supplied by Bruker Biospin (Fällanden, Switzerland). Solid state ^{13}C NMR was performed on a Varian Infinityplus 300 NMR spectrometer (300 MHz, 7.0 T) (USA). Elemental analysis was performed on a VarioMICRO CHNOS elemental analyzer (Elementar Analysensysteme, Hanau, Germany). Chromatographic analyses were performed on a Laballiance HPLC system with a diode array detection (DAD) system (State college, PA, USA).

The mobile phases (MP) were prepared using different amounts of ACN or MeOH mixed with ultra-pure water followed by adding 0.1–1% (v/v) triethylamime and thereafter adjusted to the desired pH with acetate acid (The MP was denoted as MP-TEAA). 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 MP-TEAA and samples were filtered through a 0.22 μm membrane before usage. The detection was performed at 220–300 nm and each solution was injected in triplicate and the average value was used. Calculations for capacity factor, k; selectivity, α and resolution, R_s were performed following USP standards.

2.3. Preparation of the cationic imidazolium CD CSP via thiol–ene click chemistry

The synthetic pathway of the novel CSP undergoes several steps (Fig. 1). TsOCD was synthesized according to our previously reported procedure [41].

2.3.1. Synthesis of mono-6^A-deoxy-6-(1-allylimidazolium)-β-cyclodextrin tosylate (**2**) [28]

To a solution of dry TsOCD (**1**) (5.24 g, 4.06 mmol) in anhydrous DMF (10 mL) was added 1-allylimidazole (1.1 mL, 12.1 mmol). The reaction solution was stirred at 85 °C for 24 h under N_2. After reaction, the reaction solution was poured into acetone (30 mL) and stirred for one hour, filtration. The obtained solid product was washed with acetone (2 × 20 mL) to afford the mono-6^A-deoxy-6-(1-allylimidazolium)-β-cyclodextrin tosylate (**2**) (Yield 92%). ^{1}H NMR(400 MHz, DMSO-d_6): 9.42(1*H*), 7.96(1*H*), 7.89(1*H*), 7.46–7.48(2*H*), 7.10–7.12(2*H*), 5.68–5.69(3*H*), 4.83–4.84(7*H*), 3.55–4.58(42*H*), 2.34(3*H*); ESI-MS(*m/z*): calcd($C_{54}H_{82}N_2O_{37}S$)1382.4 [M$^+$], found 1428.6 [M + 2Na$^+$].

2.3.2. Preparation of thiol functionalized silica

Activated silica (2.94 g, dried at 120 °C for 24 h under vacuum) was suspended in anhydrous toluene (20 mL) followed by adding 3-mercaptopropyltrimethoxysilane (0.75 mL). The reaction system was heated to reflux and kept for 12 h under N_2. After reaction, the crude product was rinsed with acetone for 12 h to afford the desired thiol functionalized silica (**3**).

2.3.3. Click coupling of thiol silica (**3**) and cationic CD (**2**)

To a 20 mL of MeOH and DI water mixture (1:1, v/v) was added **2** (1.5 g) under stirring. After dissolving, thiol silica **3** (3 g) and AIBN (50 mg) was added into the clear solution. The reaction mixture was stirred for 24 h with the protection of N_2. After that, the crude product was obtained by filtration and washed with DI water (2 × 20 mL), MeOH (2 × 20 mL) and acetone (2 × 20 mL) subsequently and vacuum dried at 60 °C (0.1 mbar) to afford the desired CSP.

2.4. Column packing

The prepared CSP was packed into stainless-steel column (150 mm × 4.6 mm I.D.) with the typical slurry-packing technique using MeOH as the solvent. The column efficiency for the studied racemates were between 3000 and 5000 m^{-1} at flow rates of 0.5–0.8 mL min^{-1}.