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Evaluation of perphenylcarbamated cyclodextrin clicked chiral stationary phase for enantioseparations in reversed phase high performance liquid chromatography

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ABSTRACT

In this study, perphenylcarbamated cyclodextrin clicked chiral stationary phase (CSP) was developed with high column efficiency. The characteristics of the column were evaluated in terms of linearity, limit of detection and limit of quantification. The enantioselectivity of the as-prepared clicked CSP was explored with 26 recemates including aryl alcohols, flavanoids and adrenergic drugs in reversed phase high-performance liquid chromatography. The effect of separation parameters including flow rate, column temperature, organic modifier and buffer on the enantioselectivity of the clicked CSP was investigated in detail. The correlation study of the analytes structure and their chiral resolution revealed the great influence of analytes' structure on the enantioseparations with cyclodextrin CSP. Methanol with 1% of triethylammonium acetate buffer (pH 4) was proved to be the best choice for the chiral separation of basic enantiomers.

1. Introduction

Chiral compounds may significantly differ from each other in their biological, pharmacological and toxicological effects. A notorious example would be thalidomide which was debuted as a racemic drug [1]. Chiral separation has thus gained wide attention from both academia and industry. To date, chromatographic techniques have evolved as routine approaches for chiral separation, which feature high efficiency, quick analysis and precise resolutions. The most widely used techniques include high performance liquid chromatography (HPLC), gas chromatography, capillary electrophoresis, supercritical fluid chromatography and stimulated moving bed.

By affording direct enantioseparation, HPLC coupled with chiral stationary phases (CSPs) has developed as one of the most important techniques for both detection of enantiomeric purity and quick preparation of pure enantiomers [2,3]. The CSPs include Pirkle-type, macrocyclic antibiotic, crown ethers, imprinted polymers and chiral ligand exchange CSPs as well as polysaccharide-, protein- and cyclodextrin (CD)-based CSPs [4–13]. The CD based CSPs have thus been extensively explored for the enantioseparation by functionalizing the CD rims to construct additional interactions such as π–π stacking, dipole–dipole, ion-paring, electrostatic and steric repulsive effects between analytes and the resulted CDs. Moreover, CD based CSPs are especially attractive for their versatility and durability under various conditions [8–13]. The CD-CSPs can be used in three modes including reversed phase (RP), normal phase (NP) and polar organic modes [13]. RP is the most popular mode in which the solute molecules are distributed between the relatively polar mobile phase and the non-polar stationary phase to afford separation. Under RP conditions, the enantioselectivity of CDs is dependent upon the analyte structure, CD's type and the functionalities on the CD rims. The extent of host–guest inclusion generally depends on CD's cavity dimension and the structure of the enantiomers. According to the concept of size-fit for inclusion complexation, better enantioselectivity for the CSP-enantiomers pairs generally occur when the size of hydrophobic portions of analytes matches with the CD cavity [14].

"Click chemistry" was first proposed by Sharpless and the [3 + 2] dipolar cycloaddition between azides/alkynes catalyzed by copper

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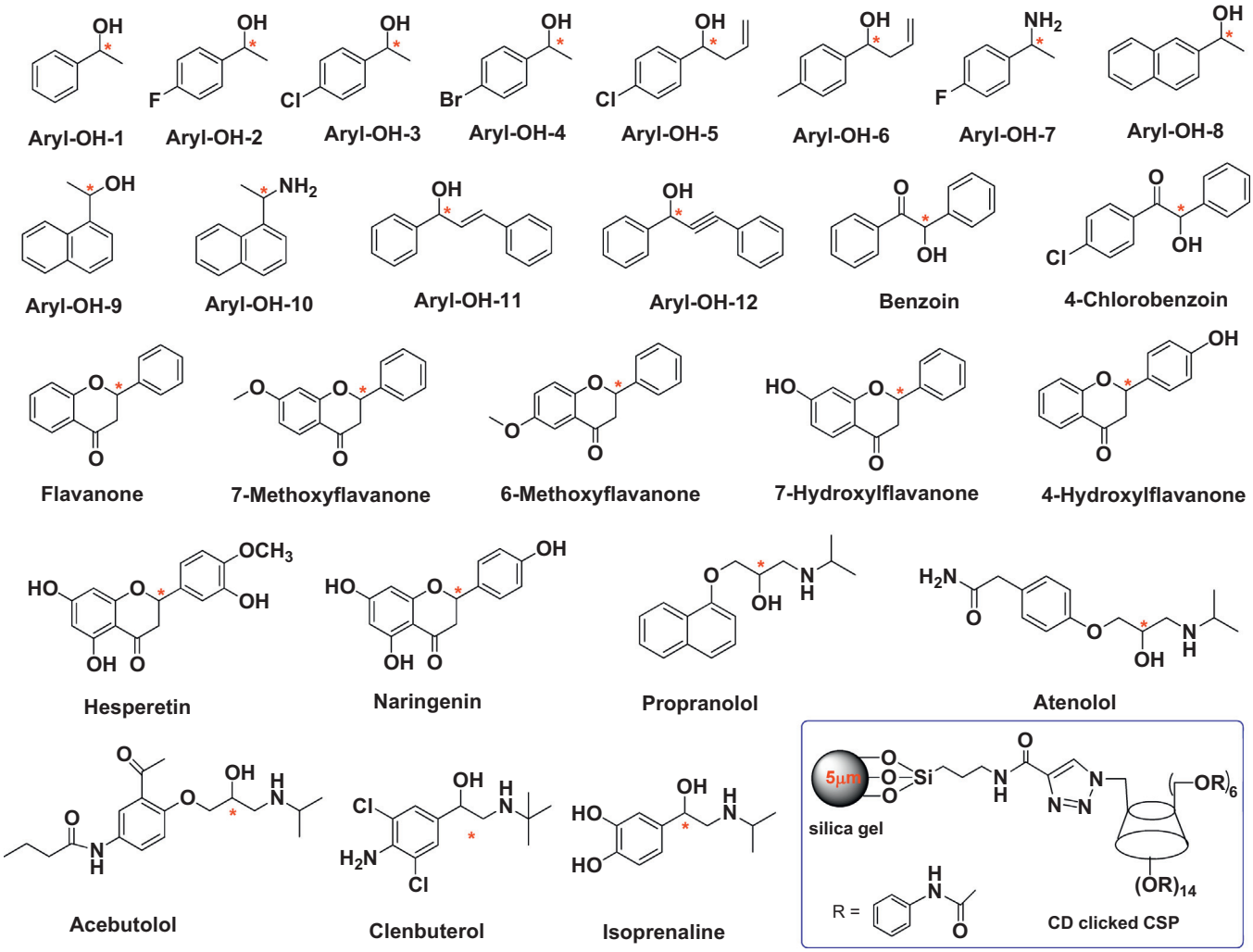

Fig. 1. Structures of racemate studied.

can be regarded as the primary one [15]. By affording high selectivity towards reagents and excellent flexibility in various solvents, click reaction has dramatically burgeoning in the application of immobilization of ligands to solid and polymer supports [16,17]. As a continuation of our long-term research project, our recent studies have demonstrated that the CD based CSPs prepared via click chemistry can afford good enantioselectivities and durability in broad separation conditions in HPLC [5,7,9,13,18]. In order to explore the potential of triazolyl-linked CD CSPs for enantioseparation in HPLC, the perphenylcarbamated CD clicked CSP (structure in Fig. 1) was prepared to pack chiral column with greatly improved column efficiency and CD surface loading. The column efficiency greatly enhanced CSP afforded significant improvement in enantioselectivities to larger library of racemates in comparison to our first report [18].

We herein report the detailed characteristics of column. The enantioseparation capability of the clicked CD CSP was further evaluated with 26 racemates including aryl alcohols, aryl amines, flavanoids, and adrenergic drugs. The effect of the separation conditions including flow rate, column temperature, mobile phase composition, and organic modifiers on the enantioseparation of the model analytes were thoroughly investigated in RPLC. The correlation of analytes' structures with their chiral resolutions by the clicked CD CSP was also described.

2. Experiments

2.1. Chemicals and materials

All reagents such as β-CD and phenyl isocyanate were purchased from Tokyo Chemical Industry (TCI, Japan). HPLC-grade acetonitrile (ACN) and methanol (MeOH) were purchased from Tedia (USA). HPLC-grade acetic acid, phosphoric acid and triethylamine (TEA) were obtained from J&K (Shanghai, China). Deionized water for the experiments was purified by Milli-Q system (Millipore, Bedford, MA, USA). All racemic analytes were purchased from the Meryer (Shanghai, China) (structures in Fig. 1). Kromasil spherical silica gel (5 μm, 100 Å) was purchased from Eka Chemicals (Bohus, Sweden). All other chemicals used were of analytical reagent grade without purification prior to use.

2.2. Apparatus

All enantioseparations with CD clicked CSP were performed on an Agilent HPLC system, which was comprised of an Agilent 1260 system consisting of with G1315D diode array detection (DAD) system, G1329B quaternary pump, a G1331C automatic injector, a G1316A temperature controller and Agilent Chem Station data manager software (Agilent Technologies, Palo Alto, CA, USA).