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Novel carbamoyl type quinine and quinidine based chiral anion exchangers implementing alkyne–azide cycloaddition immobilization chemistry

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ABSTRACT

The synthesis and chromatographic evaluation of a series of new *Cinchona* derived chiral weak anion exchangers is presented. Huisgen Cu(I) mediated alkyne–azide cycloaddition, so-called *click chemistry*, was used as an immobilization strategy. In this way it was possible to immobilize about 90% of offered selector via 1,2,3-triazole linker, which displays a more efficient way of binding the selector to modified silica compared to common radical mediated thiol-ene addition. Problems associated with potential radical scavenging properties of chiral selectors thereby could be circumvented. The evaluation of the synthesized chiral stationary phases regarding chromatographic behavior was carried out using polar organic mode mobile phase composition and a set of representative chiral organic acids. Different loading densities revealed an optimum selector density of about 310 μmol/g chiral stationary phase with respect to resolution and selectivity. A decrease of performance was observed for higher loading, indicating mutual spatial influence of selector units leading to sterical hindrance. In addition, we observed that the effect of free azide groups on retention is negligible and the overall chromatographic behavior is comparable to other *Cinchona* derived chiral stationary phases.

1. Introduction

The separation of chiral compounds is an important topic both in analytical science and on industrial scale, including the separation of bioactive compounds [1–3], flavors and fragrances [4,5], environmental pollutants [6–8], food contaminants [9], purity determination of pharmaceutical products [10,11] and drug development [12]. Besides HPLC, various other methods such as supercritical fluid chromatography [13], capillary electrophoresis [14], gas chromatography [15], simulated moving bed technology [16], enzymatic resolution [17] and crystallization [18] are routinely used for the enantiomer separation of enantiomers either on analytical or preparative scale.

Chiral stationary phases (CSPs) for the separation of chiral compounds using liquid chromatography are most commonly based on modified silica materials comprising either immobilized low-molecular mass chiral selectors (e.g. *Cinchona*-based ion exchangers) [19], macromolecular [20] (e.g. biopolymers or synthetic polymers) or macrocylic selectors such as cyclodextrins [21], antibiotics [22] or chiral crown ethers [23]. The broad family of CSPs has enabled separation of almost any racemic mixture of choice, ranging from neutral lipophilic to highly polar hydrophilic compounds. For the latter especially ion exchange CSPs have been found favorable [24,25]. Besides anion and cation exchange materials one can also distinguish zwitterion ion exchange-type CSPs [26–29].

Separation of chiral organic acids is feasible on chiral anion exchange materials. These are based on selectors containing ionizable primary, secondary or tertiary amino group as well as permanently charged quaternary amines [30], whereas the last represents the strongest anion exchanger type. In case of *Cinchona*-based materials retention is primarily driven by long-range electrostatic forces related to the protonated quinuclidine group with a deprotonated acidic group and the formation of an ion-pair. Chiral recognition is enabled by formation of a diastereomeric pair supported by additional directed interactions like intermolecular hydrogen bonds, π–π-interactions, Van der Waals and steric influences [31,32]. A chiral recognition model for carbamoylated *Cinchona*-based selectors has been recently reviewed by Laemmerhofer and Lindner [24].

Commercially available chiral anion exchange materials (Chiralpak® QN-AX and QD-AX), which are used in this study as reference materials, are immobilized via a radical addition reaction

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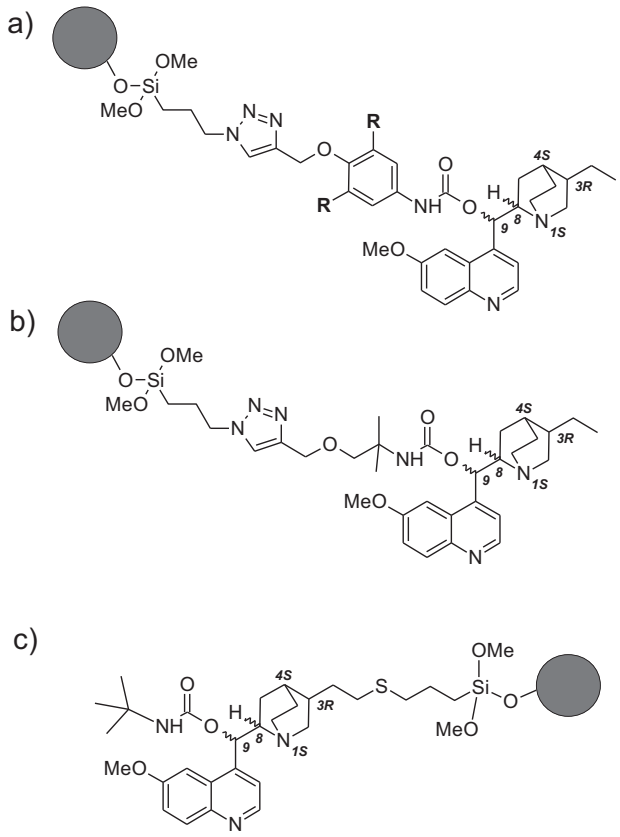

Fig. 1. Structures of the CSPs comprising selectors **A–F** immobilized onto AzPrSi via *click chemistry*. The structures of commercially available QN-AX and QD-AX CSPs are also shown. a) CSP-1 + CSP-2 *R* = Cl (8*S*,9*R*); CSP-3 to CSP-6 *R* = Cl (8*R*,9*S*); CSP-7 + CSP-8 *R* = OMe (8*S*,9*R*); CSP-9 + CSP-10 *R* = OMe (8*R*,9*S*); CSP-13 to CSP-15 *R* = Cl (8*R*,9*S*); b) CSP-11 (8*S*,9*R*); CSP-12 (8*R*,9*S*). For selector density on silica see Table 1. c) QN-AX (8*S*,9*R*), QD-AX (8*R*,9*S*). Selector density of QN-AX and QD-AX: app. 340 μmol/g silica.

concept of a thiol group. Generally, mercaptopropyl-modified silica gel is often used as a backbone material for the immobilization of chiral low molecular mass selectors comprising a double bond such as *Cinchona* alkaloids quinine and quinidine [33]. Since this type of radical mediated immobilization reaction does not work for selectors with radical-quenching activity, an alternative way for immobilization was evaluated in this study – Huisgen 1,3-dipolar cycloaddition, the so-called *click chemistry* [34], which was already applied for the immobilization of *Cinchona* based chiral selectors by Kacprzak et al. [35].

In this reaction 1,2,3-triazoles are formed from azides and terminal alkyne moieties using Cu(I) as a catalyst. Besides copper catalysis, also ruthenium(II)-based complexes can be used for this type of reaction [36,37]. Copper can be used as Cu(I) salts such as CuI, which is however air sensitive, or as $CuSO_4$ precatalyst in combination with a reducing agent such as ascorbic acid, whereas Cu(I) is formed *in situ* [38].

The novel chiral ion exchange materials presented in this work are low-molecular mass selectors based on diverse carbamoylated dihydroquinine derivatives. Because the selectivity of quinine-based chiral ion exchange materials can be modified relatively easily by different substitution pattern on the hydroxy-group at position C9 (see Fig. 1) of the quinine/quinidine building block [39], this concept was applied using different isocyanates for carbamoylation. In contrast to the work of e.g. Kacprzak et al. [31], immobilization took place via a carbamoyl linker and not by the vinyl moiety at the quinuclidine ring, which changes the geometry of the bound selector with respect to the silica surface of the backbone material. By this strategy the overall selectivity of the CSP may be altered (see also Fig. 1). Subsequently the CSPs prepared in this way were evaluated in terms of their chiral separation power.

2. Experimental

2.1. Materials and methods

NMR-spectra were recorded on a Bruker DRX 400 spectrometer (Karlsruhe, Germany) operating at 400 MHz. Either $CDCl_3$ or CD_3OD (both 99.8%, Deutero GmbH, Kastellaun, Germany) was used as a solvent and the solvent signals were used as a reference. The raw data were processed with SpinWorks 2.5 software. The FTIR-measurements were carried out on a Bruker Tensor 27 Diamond ATR spectrometer (Ettlingen, Germany) with Opus 4.2 software. Mass spectrometric measurements of the various selectors were performed using a 4000 QqLIT mass spectrometer equipped with an ESI ion source from Applied Biosystems (Foster City, USA). All mass spectra were measured in positive ionization mode. For data processing Analyst 1.5 software was used. Elemental analyses were operated on a EURO EA 3000 CHNS-O instrument from HEKAtech (Wegberg, Germany). Determination of the chlorine content was performed using potentiometric titration with a Mettler DL 21 titrator (Greifensee, Switzerland). Melting points were determined using heating stage Leica VM TG (Bensheim, Germany). Flash column chromatography was carried out using Normasil 60 Silica Gel from VWR. Daisogel SP-120-5-P from Daiso (Japan) was used as a basis for silica derivatization (spherical particles with a mean particle size of 5 μm, mean pore diameter 120 Å, particle size distribution ≤1.25, pore volume 1.0 mL/g, specific surface area 300 m^2/g).

Analytical grade solvents for synthesis were purchased from DonauChem, Fluka, Merck, ROTH, Sigma-Aldrich and VWR. Reagents and catalysts were obtained from ABCR, Acros Organics, Buchler, Fluka, Merck, Sigma-Aldrich, TCI and VWR. As the bulk mobile phase and additives the following chemicals were used: MeOH (HPLC grade quality, 99.8%, VWR), AcOH (≥99%, Sigma-Aldrich) and NH_4OAc (≥97%, p.a., Fluka). For comparative purposes commercially available Chiralpak® QN-AX and QD-AX columns were used (150 × 4 mm ID, 5 μm), because these two quinine and quinidine derived carbamoylated reference materials show high capabilities in terms of resolution of chiral acidic compounds [32,40–43].

2.2. Chromatography

The chromatographic screening of the columns was carried out on a 1290 series Infinity HPLC system from Agilent Technologies (Waldbronn, Germany) equipped with a column compartment for six columns and a diode array detector (DAD). The concentration of analytes was approximately 1 mg/mL in MeOH and the detection wavelength was 254 nm. The injected volume was set to 5 μL. The columns were thermostated at 25 °C. Elution was performed in the isocratic mode with a flow rate of 1.0 mL/min. The composition of the polar organic eluent was MeOH/AcOH/NH_4OAc = 99/1/0.25 (v/v/w). The mobile phase was degassed by sonication prior to use. Acetone (50 μL/mL in MeOH) was used as a non-retained void volume marker. Data processing was carried out with ChemStation chromatographic data software from Agilent and Excel spreadsheet software from Microsoft Corporation.

2.3. Analytes

The retention on *Cinchona* alkaloids based CSPs is primarily driven by an ion pairing (exchange) mechanism [33]. Due to the large number of CSPs to be screened only a relatively small set of organic acid type analytes (see Fig. 2) was chosen for the evaluation. The test compounds were either commercially available,