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Synthesis and evaluation of pseudopeptide chiral stationary phases for enantioselective resolution

Huifang Shen, Ganhong Du, Keyuan Liu, Long Ye, Shoulei Xie, Liming Jiang*

MOE Key Laboratory of Macromolecular Synthesis and Functionalization; Department of Polymer Science and Engineering, Zhejiang University, Hangzhou, 310027, China

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ABSTRACT

Poly(2-oxazoline)s are regarded as bioinspired polymers due to their structural relation to polypeptides. In this work, a new kind of poly(2-oxazoline)s containing dipeptide segments in the side chains was synthesized through a bottom-up protocol, which involves ring-opening copolymerization of 2-(*N*-Boc-L-2-pyrrolidinyl)-2-oxazoline (PyOX$_{Boc}$) with 2-(3-butenyl)-2-oxazoline (BuOX) followed by deprotection and amide coupling with *N*-protected L-proline. The resulting vinyl-functionalized polymers were subsequently immobilized onto mercaptopropylated silica bead matrices by means of thio-click chemistry and their potential as the chiral stationary phase (CSP) for high-performance liquid chromatography was preliminarily evaluated with a series of structurally different racemates. The results showed that this class of pseudopeptide CSPs is particularly adapted to the enantiomeric separation of 1,1′-bi-2-naphthol and acyloin compounds (such as benzoin) under normal-phase conditions. Moreover, an increase in the length of polymer main chains is beneficial to the enhancement of both enantioselectivity and resolution ability. The chiral discrimination of analytes by the polymeric selectors stems primarily from hydrogen bonding and π-π interactions as well as steric hindrance.

1. Introduction

Over the past few decades, the increasing demand of enantiomerically pure compounds such as chiral drugs and agrochemicals has favored the rapid development in separation science. Among the diverse techniques for determining the enantiomeric composition and also for obtaining pure enantiomers on analytical and industrial scales [1–14], high-performance liquid chromatography (HPLC) is recognized as one of the most widely used methods [2–12]. Undoubtedly, the preparation of a chiral stationary phase (CSP) possessing an effective enantioselective recognition ability for a variety of racemates with different functionalities is the key to the chromatographic separation technology. Many commercially available CSPs derived from natural cellulose and amylose [15–24] are known to show excellent resolving performance for a large number of racemates, wherein the helical secondary structure of polysaccharide matrices is believed to play a crucial role in the chiral recognition [20–22,24]. Accordingly, a lot of research work related to various synthetic helical polymers have been carried out for elucidating the relationships between the chiral higher-order structure and the enantioselectivity, and further finding more efficient chiral stationary materials [25–28].

Given the rigid cyclic structure of proline as well as the unique PPI and PPII conformations of polyproline peptides [29,30], the use of oligoprolines as chiral recognition materials has attracted considerable attention in the last decade [31–42]. Li and co-workers first utilized non-substituted oligoprolines as CSPs for HPLC giving high and broad-spectrum enantioselectivity [33–35]. Later, Minguillón et al. [36–38] synthesized the oligomers of six to eight (4*R*)-hydroxy-L-proline residues as chiral selectors and covalently bound them to a silica gel chromatographic matrix. The resulting particulate and monolithic chiral columns showed a broader application domain and an increased loading capacity than the corresponding single proline-derived analogues. Considering the fact that long chain peptides require tedious solid-phase synthesis and formation of ragged chains is usually unavoidable, Lao and Gan developed di/triproline CSPs and successfully applied them to chromatographic separation of various acidic, basic, and neutral compounds [39–42]. They demonstrated that three factors including adjacent hydrogen bond acceptor and carbon-donor as well as a rigid proline residue chain are crucial for the excellent enantioselectivity of the oligopeptide CSPs [40].

Recently, we reported a class of polymer-supported biomimetic catalysts in which L-proline is appended to poly(2-oxazoline) back-

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* Corresponding author.
E-mail address: cejlm@zju.edu.cn (L. Jiang).

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bone through a amide linkage [43,44]. The organocatalysts were found to promote the aldol reaction of cyclohexanone and a range of aromatic aldehydes in neat or water environments with high activity and *syn*-selectivity. We reasoned that the peculiar tertiary amide skeleton of poly(2-oxazoline)s, residing in the proximity of the catalytic site, may produce beneficial stereodiscriminating effects for the asymmetric transformation. Based on the previous work and the chiral separation ability of known oligoproline-derived CSPs [36–42], here we attempt to incorporate diproline motifs into poly(2-oxazoline) backbone in order to develop new types of chiral separation materials. Choosing the polymer as a scaffold for anchoring chiral entities is not only due to its plenty of chemistry that would offer a great deal of flexibility with respect to the molecular design, but also to its structural relationship with polypeptides [45–49]. To the best of our knowledge, despite significant interest in this kind of pseudopeptide polymer materials [46,47], so far no reports have explored their potential application in the construction of CSPs and chromatographic separations.

2. Experimental

2.1. Chemicals and materials

2-Chloroethylamine hydrochloride, trifluoroacetic acid (TFA), 1,3,5-tri-*tert*-butylbenzene, *tert*-butoxycarbonyl (Boc)-L-proline, 3,5-dimethylbenzoyl (DMB)-L-proline, 3,5-dinitrobenzoyl (DNB)-L-proline, 3,5-dimethylbenzoyl chloride, 3, 5-dinitrobenzoyl chloride, hexamethyldisilazane (HMDS), *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate (TBTU), and azobisisobutyronitrile (AIBN) were purchased from Energy Chemicals (Shanghai, China). 3-Mercaptopropyl-triethoxysilane, HPLC-grade spherical silica gel (particle size, 5 μm; pore size, 100 Å and surface area 298 m^2/g) was obtained from BOSHI Biotechnology (Shanghai, China). Solvents used for HPLC are of chromatographic grade (J&K China Chemical Ltd., Beijing). Dichloromethane (CH_2Cl_2), triethylamine, acetonitrile and piperidine were distilled from calcium hydride and stored under dry nitrogen atmosphere before use. Scandium triflate [$Sc(OTf)_3$] was prepared as the reported method [50] and used after drying in vacuum at 200 °C for 48 h. The racemic analytes were commercially available or prepared by the usual methods (see the Supporting Information).

2.2. Instrumentation

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III spectrometer at 400 MHz using TMS as the internal standard with DMSO-d_6 or $CDCl_3$ as solvent. FT-IR spectra were recorded using an IR VECTOR-22 spectrometer (Bruker Co., German). Elemental analyses were performed on a Thermo Electron apparatus EA 1110 (CHNS-0, RAL, German) using standard conditions. *D*-Line specific optical rotations ($[\alpha]^{20}D$) were measured in methanol at room temperature using a Perkin-Elmer 341 LC polarimeter. Circular dichroism (CD) spectra were recorded on a Bio-Logic MOS-450 CD spectropolarimeter (France) at ambient temperature (cell length: 1 cm, scanning speed: 10 nm/sec). Gel permeation chromatography (GPC) data were acquired on a Waters-150C apparatus equipped with two PL gel 5 μm MIXED-C (300 mm × 7.5 mm) columns and a differential refractometer detector using tetrahydrofuran (THF) or dimethylformamide (DMF) as the eluent (flow rate 1 mL/min, 40 °C). The number-average molecular weight (M_n) and polydispersity index (PDI) of the polymers were calculated on the basis of a PMMA calibration.

2.3. Synthesis of 2-oxazoline monomers

Monomers 2-(*N*-Boc-L-2-pyrrolidinyl)-2-oxazoline ($PyOX_{Boc}$) and 2-(3-butenyl)-2-oxazoline (BuOX) were synthesized according to a general adapted procedure (see the Supporting Information for details). All the products were characterized by standard spectroscopic methods (Fig. S1–S4), from which satisfactory analysis data were obtained.

$PyOX_{Boc}$: $[\alpha]^{20}D = -87.7°$ (c 1.0, CH_3OH). ^1H NMR (400 MHz, $CDCl_3$): δ/ppm = 4.64 – 4.35 (m, 1H), 4.34 – 4.12 (m, 2H), 3.81 (dt, J = 13.1, 7.8 Hz, 2H), 3.64 – 3.22 (m, 2H), 2.31 – 1.75 (m, 4H), 1.44 (d, J = 16.0 Hz, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ/ppm = 168.80, 154.09, 79.43, 67.74, 54.49, 46.37, 31.32, 28.36, 24.13. MS (ESI+): m/z (%) = 262.8 (100) $[M+Na]^+$, 502.3 (25) $[2M+Na]^+$.

BuOX: ^1H NMR (400 MHz, $CDCl_3$): δ/ppm = 6.04 – 5.46 (m, 1H), 5.13 – 4.91 (m, 2H), 4.22 (t, J = 9.4 Hz, 2H), 3.82 (t, J = 9.5 Hz, 2H), 2.80 (s, 1H), 2.55 – 2.23 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ/ppm = 167.78, 136.86, 115.31, 67.15, 54.35, 29.36, 27.31, 77.04, 76.72, 68.11, 54.08, 49.73, 38.76, 28.30. MS (ESI+): m/z (%) = 126.0 (100) [M].

2.4. Synthesis of polymer chiral selectors (Fig. 1)

The synthesis of chiral selectors began with ring-opening copolymerization of $PyOX_{Boc}$ with BuOX followed by global deprotection then amide coupling with *N*-protected L-proline. Resultant polymer-bound diproline selectors with different *N*-terminal amino functions PPyOX-ProDMB, PPyOX-ProDNB, and PPyOX-ProBoc are hereinafter referred to as CS2, CS3, and CS4, respectively. The detailed preparation procedure is described in the Supporting Information.

2.5. Preparation of mercaptopropyl-silica gel (MPS)

In a 250 mL three-neck round flask with Dean-Stark trap, 10 g acid-washed silica gel and 100 mL dry toluene were placed under nitrogen. The slurry was then refluxed for 4 h to remove azeotropic water, after which 5 mL 3-mercaptopropyltriethoxysilane (27 mmol) was added slowly, and the mixture was heated at 140 °C for 48 h. The silica gel (MPS) was collected by filtration, washed with toluene, methanol, *n*-hexane and CH_2Cl_2, finally dried at 60 °C for 5 h under vacuum to constant weight. The elemental analysis gave 5.01% C, 1.348% H, and 0% N, corresponding to 1.21 mmol/g of mercaptopropyl groups based on the C percentage.

2.6. Preparation of chiral stationary phases (Fig. 1)

PPyOX-ProBoc-MPS (CSP-4): To a solution of PPyOX-ProBoc (CS4; 2.571 g, 5.53 mmol of BuOX monomeric units) in THF (25 mL) was added AIBN (0.328 g, 2 mmol) and MPS (2.327 g, 5.14 mmol of sulfhydryl group) and the resulting slurry was stirred at 60 °C for 24 h under a N_2 atmosphere. The modified silica was collected by filtration, washed subsequently with THF, methanol, and chloroform, and then dried thoroughly to yield the desired CSP-4 (3.059 g). The same procedure was carried out for preparing the other three chiral stationary phases CSP-2, CSP-3, and CSP-1, with the use of PPyOX-ProDMB (CS2), PPyOX-ProDNB (CS3), and **P1** (CS1) as their respective chiral selectors. The CSPs were packed into stainless-steel tube (250 mm × 4.6 mm i.d.) by the conventional high-pressure slurry packing technique using ethanol as packing solvent [51].

2.7. End-capping treatment of CSPs

In-column end-capping treatment of residual silanol groups was carried out according to the documented method [52]. The col-