Journal of Chromatography A, 1363 (2014) 128–136

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Influence of the nature of alkyl substituents on the high-performance liquid chromatography enantioseparation and retention of new atropisomeric 1,1′-bibenzimidazole derivatives on amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phase

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ARTICLE INFO

Article history:
Received 14 May 2014
Received in revised form 14 August 2014
Accepted 16 August 2014
Available online 23 August 2014

Keywords:
Enantiomer separation
Chiral stationary phase
Chiralpak AD-3
Enantiomer elution order
Isoenantioselective temperature
Thermodynamic parameters

ABSTRACT

Six new atropisomeric heteroarenes were synthesized by connecting two 2-alkylbenzimidazole fragments via N–N junction. They differ by the substituent nature (methyl, ethyl, propyl, butyl, pentyl and hexyl) of the aliphatic function. The novel atropisomeric compounds were used as chiral probes to study the chromatographic behavior of the amylose tris(3,5-dimethylphenyl carbamate) (Chiralpak AD-3) chiral stationary phase (CSP) under normal phase mode. The pivotal role of the length and flexibility of the 2,2′-alkyl groups on retention, enantioselectivity and enantiomer elution order was demonstrated by enantioselective HPLC analysis. Additional information on the chiral recognition mechanism was obtained from the evaluation of the correlated thermodynamic data.

1. Introduction

Amylose tris(3,5-dimethylphenylcarbamate) (ADMPC) is a versatile and effective selector for high performance liquid chromatography (HPLC). Commercial chiral stationary phases (CSPs) based on ADMPC have been prepared by either coating or immobilizing the polysaccharide derivative on macroporous silica gel [1–4]. The reasons of the widespread use of the ADMPC in enantioselective HPLC may be related to its ability to: (i) exhibit enantiodiscrimination under very different mobile phase conditions (normal-phase, polar organic, reversed-phase and hydrophilic interaction mode) [5–10]; (ii) resolve a wide range of racemic compounds of synthetic and pharmaceutical interest [11–15]; (iii) easily scale-up the production of enantiomers from analytical to preparative scale [16–18].

The skeleton of the polymeric selector consists of D-glucose units linked by an α-(1,4)-D-glucose linkage and it is regularly arranged to form a left-handed helix conformation [19].

The 3,5-dimethylphenyl carbamate side chains surround the helical grooves and delimit the nanosized chiral environments in which the enantiomers enter and interact with its binding sites [1,2]. The most important adsorbing sites for retention and chiral discrimination processes are the polar carbamate and the hydrophobic aromatic groups. The former allows H-bonding and dipole–dipole interactions while the latter π−π and repulsive interactions with the analytes. As shown in previous studies [20,21], the size and geometry of chiral cavities, and then the accessibility of enantiomers to adsorbing sites, may be changed by modifying the mobile phase composition and temperature.

Chirality of ADMPC results from the structural chirality of the glucopyranose units, the chirality inherent to the periodical presence of helical grooves in the polymeric backbone and the supramolecular chirality in the regions between adjacent polymer rods. However the chiral recognition is not a simple consequence of molecular asymmetry but it is the outcome of intermolecular selector–selectand interactions. Formation of transient diastereomeric structures between enantiomers and selector occurs by

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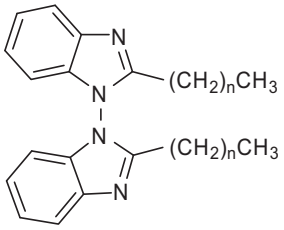
http://dx.doi.org/10.1016/j.chroma.2014.08.060
0021-9673/



Compound	n
1	0
2	1
3	2
4	3
5	4
6	5

Fig. 1. Chemical structure of the chiral analytes **1**–**6**.

preferential orientations of the interacting molecules which minimize short-range repulsive forces and allow the establishment of non-covalent and reversible attractive forces.

Although great attention has been dedicated to the study of attractive intermolecular interactions involved in chiral recognition mechanisms ADMPC CSP [1,2], the role of repulsive effects on the overall enantioselective and retention process has often been underestimated.

Toward that objective, the aim of the present paper was to examine the chromatographic behavior of the series of chiral atropisomers shown in Fig. 1 (compounds **1**–**6**) on the coated-type ADMPC-based Chiralpak AD-3 CSP. Compounds **1**–**6** bear a linear alkyl chain varying in length from 1 to 6 carbon atoms. In addition, the incorporation of an atropisomeric axis as the element of chirality provides the design of a highly dissymmetric and rigid scaffold in which the planes of the two aromatic rings are approximately perpendicular and the 2,2′-alkyl groups are expected sufficiently bulky to hinder the rotation around the N–N junction at room temperature. Consequently, a pair enantiomers is expected to be separable at room temperature. Compounds **1**–**6** were synthesized in the idea that, increasing the length of the 2,2′-alkyl groups, different stereospecific inclusions in the chiral cavities may occur as a consequence of their differences in steric bulkiness, flexibility and hydrophobicity. Mixtures of n-hexane with ethanol or 2-propanol as mobile phases were tested in this study. Further investigations focused on the influence of temperature on retention, enantioselectivity and enantiomeric elution order of **1**–**6**.

Based on the obtained thermodynamic data, some aspects of the chiral recognition mechanism will be discussed.

2. Experimental

2.1. Enantioselective HPLC

Analytical HPLC of **1**–**6** were performed using the commercially available 250 mm × 4.6 mm I.D. Chiralpak AD-3 column (Chiral Technologies Europe, Illkirch, France).

The analytical HPLC apparatus consisted of a PerkinElmer (Norwalk, CT, USA) 200 lc pump equipped with a Rheodyne (Cotati, CA, USA) injector, a 20-μL sample loop, a HPLC Dionex CC-100 oven (Sunnyvale, CA, USA) and a Jasco (Jasco, Tokyo, Japan) Model CD 2095 Plus UV/CD detector. For semi-preparative separations, a commercially available 250 mm × 10 mm I.D. Chiralpak AD column, a PerkinElmer 200 LC pump equipped with a Rheodyne injector, a 500 μL sample loop, a PerkinElmer LC 101 oven and Waters 484 detector (Waters Corporation, Milford, MA, USA) were used.

HPLC grade solvents were purchased from Aldrich (St. Louis, MO, USA).

In analytical enantioseparations, standard solutions were prepared by dissolving about 1 mg of sample, into 5 mL of the mixture n-hexane–ethanol 100:10. The injection volume was 10 μL.

2.2. Circular dichroism

The CD spectra of the enantiomers of **1**–**6** dissolved in ethanol were measured by using a Jasco Model J-700 spectropolarimeter. The optical path and temperature were 0.1 mm and 25 °C, respectively. The spectra are average computed over three instrumental scans and the intensities are presented in terms of ellipticity values (mdeg).

2.3. Synthesis of *1–6*

All the 2-alkyl-1,1′-bibenzimidazoles (**2**–**6**) were prepared according to a general method already described for the 2,2′-dimethyl-1,1′-bibenzimidazole (**1**), starting from 2,2′-azoaniline through a three-step scheme [22]. The general scheme for the synthesis of (**2**–**6**) is reported in supporting information (SI) section.

2,2′-*Diethyl*-1,1′-*bibenzimidazole* **(2)**: white solid with m.p. = 118–119 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (2H, d, ^3J (H,H) = 7.80 Hz), 7.38 (2H, t, ^3J (H,H) = 7.65 Hz), 7.28 (2H, t, ^3J(H,H) = 7.65 Hz), 6.90 (2H, d, ^3J (H,H) = 8.10 Hz), 2.72–2.50 (4H, m), 1.38 (6H, s). ^{13}C NMR (300 MHz, $CDCl_3$): δ 155.55 (s), 140.51 (s), 133.61 (s), 124.16 (s), 123.76 (s), 120.37 (s), 108.23 (s), 19.68 (s), 11.10 (s).

MS (F.A.B.$^+$): *m*/*z* 291 (100%) (M^+).

2,2′-*Di*-(*n*-*propyl*)-1,1′-*bibenzimidazole* **(3)**: white solid with m.p. = 131–132 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (2H, d, ^3J (H,H) = 8.10 Hz), 7.38 (2H, t, ^3J (H,H) = 7.65 Hz), 7.26 (2H, t, ^3J (H,H) = 7.80 Hz), 6.87 (2H, d, ^3J (H,H) = 7.80 Hz), 2.66–2.44 (4H, m), 1.92–1.77 (4H, m), 0.96 (6H, t, ^3J (H,H) = 7.50 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 154.53 (s), 139.95 (s), 140.42 (s), 133.54 (s), 124.17 (s), 123.83 (s), 120.32 (s), 108.34 (s), 28.02 (s), 20.26 (s), 13.84 (s). MS (F.A.B.$^+$): *m*/*z* 319 (100%) (M^+).

2,2′-*Di*-(*n*-*butyl*)-1,1′-*bibenzimidazole* **(4)**: white solid with m.p. = 110 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90 (2H, d, ^3J (H,H) = 8.10 Hz), 7.41 (2H, t, ^3J (H,H) = 7.80 Hz), 7.29 (2H, t, ^3J (H,H) = 7.80 Hz), 6.90 (2H, d, ^3J (H,H) = 8.10 Hz), 2.71–2.51 (4H, m), 1.87–1.75 (4H, m), 1.43–1.29 (4H, m), 0.86 (6H, t, ^3J (H,H) = 7.20 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 154.68 (s), 139.59 (s), 133.22 (s), 124.54 (s), 124.25 (s), 120.15 (s), 108.40 (s), 28.89 (s), 25.78 (s), 22.36 (s), 13.62 (s). MS (F.A.B.$^+$): *m*/*z* 347 (100%) (M^+).

2,2′-*Di*-(*n*-*pentyl*)-1,1′-*bibenzimidazole* **(4)**: white solid with m.p. = 88 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90 (2H, d, ^3J (H,H) = 8.10 Hz), 7.42 (2H, t, ^3J (H,H) = 7.65 Hz), 7.28 (2H, t, ^3J (H,H) = 7.65 Hz), 6.90 (2H, d, ^3J (H,H) = 8.10 Hz), 2.70–2.48 (4H, m), 1.88–1.76 (4H, m), 1.38–1.18 (4H, m), 0.84 (6H, t, ^3J (H,H) = 7.20 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 154.74 (s), 140.52 (s), 133.58 (s), 124.06 (s), 123.75 (s), 120.33 (s), 108.31 (s), 31.36 (s), 26.53 (s), 26.14 (s), 22.17 (s), 13.74 (s). MS (FAB$^+$): *m*/*z* 375 (100%) (M^+).

2,2′-*Di*-(*n*-*hexyl*)-1,1′-*bibenzimidazole* **(6)**: white solid with m.p. = 63–65 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (2H, d, ^3J (H,H) = 8.05 Hz), 7.37 (2H, t, ^3J (H,H) = 7.68 Hz), 7.28–7.22 (2H, m), 6.86 (2H, d, ^3J (H,H) = 7.99 Hz), 2.67–2.45 (4H, m), 1.85–1.74 (4H, m), 1.35–1.18 (12H, m), 0.83–0.78 (6H, m). ^{13}C NMR (300 MHz, $CDCl_3$): δ 154.70 (s), 139.95 (s), 133.37 (s), 124.31 (s), 124.01 (s), 120.21 (s), 108.35 (s), 31.21 (s), 28.86 (s), 26.81 (s), 26.09 (s), 22.31 (s), 13.87 (s). MS (EI$^+$): *m*/*z* 403 (10.8%) $(M+1)^+$, 402 (32.5%) (M^+), 345 (43.4%), 262 (25.1%), 201 (88.8%), 132 (100%).

M.p. were determined on a Büchi B-540 instrument. NMR spectra were recorded on Bruker AC 300 spectrometer. Chemical shifts are given in ppm and coupling constants in Hz. Mass