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Enantiomeric separation of biaryl atropisomers using cyclofructan based chiral stationary phases

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ABSTRACT

Normal phase chiral HPLC methods are presented for the enantiomeric separation of 30 biaryl atropisomers including 18 new compounds recently produced *via* a novel synthetic approach. Three new cyclofructan based chiral stationary phases were evaluated. Separations were achieved for all but six analytes and the LARIHC™ CF6-P alone provided 15 baseline separations. Effects of polar modifiers and temperature effects also were studied. Apparent thermodynamic parameters were determined by van't Hoff plots. Preparative scale methods were developed and employed resulting in the first ever isolation of these novel atropisomers in their pure enantiomeric form. Insights into the mechanism of retention and chiral discrimination are presented.

1. Introduction

Substituted biaryls in which the rotation around the aryl–aryl single bond is hindered are referred to as atropisomers. They represent a major class of axially chiral molecules that have found use in many applications including privileged ligands in asymmetric synthesis [1–4], chiral resolving agents [5], and as pharmaceutical compounds [6,7]. Recently, a variety of novel 2,2′-diamino-1,1′-binaphthalenes [8] as well as 2,2′-aminohydroxy-1,1′-biaryls were synthesized using a transition metal free direct arylation method [9]. These new compounds have the potential to be used as chiral ligands in asymmetric synthesis and may possess unique biological activities including antitumor and antimicrobial activities [10]. The chiral analytes considered herein are 1,1′-biaryls and fall into one of three groups: 2,2′-diol, 2-amino-2′-ol and 2,2′-diamine. Probe analytes also differ in aryl type and type/position of substituents on the aryl groups.

Chiral molecules are often needed as pure enantiomers for evaluation in the aforementioned applications and thus the need for methods to determine the enantiomeric excess (%ee) of newly synthesized molecules is ever present [11–16]. There is also a need to develop preparative HPLC methods to purify milligram to gram scale amounts of enantiomerically pure compounds [17,18]. HPLC combined with chiral stationary phases (CSP's) has proven to be an excellent technique for the separation of axially chiral molecules [19–22]. A wide variety of CSP's have been used to separate biaryl atropisomers including bonded cyclodextrins [23], 1,3,5-triazine based CSP's [24], quaternized brucine-based CSP's [25], derivatized cyclofructans [26,27] and immobilized polysaccharide-based CSP's [28]. Chiral HPLC is also useful for preparing single enantiomers as instrumental methods and HPLC column dimensions are easily scaled from analytical to semi-preparative and preparative capacities [17].

A new class of CSP's based upon derivatized cyclofructans, which are cyclic oligosaccharides consisting of six or more β(2→1)-linked D-fructofuranose units has recently been introduced [29]. In this study, three functionalized cyclofructan CSP's were evaluated for use as HPLC CSP's. The first, the LARIHC CF6-P (isopropylcarbamate derivatized cyclofructan-6) has shown exceptional selectivity for racemates with a primary amine moiety [30] while the LARIHC CF6-RN (R-naphthylethylcarbamate derivatized cyclofructan-6) and LARIHC CF7-DMP (dimethyphenylcarbamate derivatized cyclofructan-7) CSP's have shown broad selectivity and applicability for a variety of classes of molecules [31–35].

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In this paper, 30 biaryl atropisomers were screened with three CSP's under normal phase and polar organic HPLC conditions to elucidate potential interactions governing retention and enantioselectivity on cyclofructan based chiral selectors. The three commercially available binapthyl analytes, BINAM, BINOL and NOBIN as well as 1-(2-aminophenyl)naphthalen-2-amine were selected for further analysis to study the effect of the three different 2,2′-substituents and type of aryl groups on retention and enantioselectivity. For normal phase separations, the effects of both the type and composition of the polar modifier was investigated as well as the effect of column temperature on enantioselectivity. The effect of acidic and basic additives also was investigated. A preparative separation of 1-(2-amino-3,4,5-trichlorophenyl)naphthalen-2-ol is presented allowing the pure enantiomers of this novel molecule to be evaluated for antimicrobial/antibiotic activity (data not reported), showing the separations reported herein are both scalable and necessary for future applications involving these new biaryls. This is the first report on the enantiomeric separation of many of these important analytes which, in turn, provides insights into the mechanism of retention and chiral recognition for cyclofructan based CSP's.

2. Experimental

2.1. Materials

HPLC grade heptane, ethanol, acetonitrile and ACS grade hexanes (5% methylpentanes) were purchased from Fisher Scientific (Waltham, MA). HPLC grade 1-propanol, 2-propanol 1-butanol, ACS grade trifluoroacetic acid and triethylamine were purchased from Sigma–Aldrich (St. Louis, MO). 1,1′-binaphthyl-2,2′-diamine (BINAM, Table 1 no. 1), 1,1′-bi-2-naphthol (BINOL, no. 2), 2-amino-1,1′-binaphthalen-2′-ol (NOBIN, no. 3), 6,6′-dibromo-[1,1′-binaphthalene]-2,2′-diol (no. 23), 3,3′-bis(3,5-dimethylphenyl)-5,5′,6,6′,7,7′,8,8′-octahydro-[1,1′-binaphthalene]-2,2′-diol (no. 24), 3,3′-diphenyl-[2,2′-binaphthalene]-1,1′-diol (VANOL, no. 25), 3,3′-dibromo-5,5′,6,6′,7,7′,8,8′-octahydro-[1,1′-binaphthalene]-2,2′-diol no. 26, 3,3′-dibromo-[1,1′-binaphthalene]-2,2′-diol (no. 27), 3,3′-bis(triphenylsilyl)-[1,1′-binaphthalene]-2,2′-diol (no. 28), 3,3′-di(anthracen-9-yl)-[1,1′-binaphthalene]-2,2′-diol (no. 29), and 2,2′-dimethoxy-1,1′-binaphthalene (no. 30) were purchased from Sigma–Aldrich (St. Louis, MO). LARIHC CF6-P, CF6-RN and CF7-DMP were obtained from AZYP L.L.C. (Arlington, TX). Analytes 4-22 (Table 1) were synthesized as reported in Ref. [9].

2.2. HPLC methods

All analytical analyses were performed on an Agilent© 1260 Infinity HPLC system utilizing a degasser, quaternary pump, autosampler, column thermostat and diode array detector. Data analysis was carried out using OpenLAB CDS Chemstation© Edition Rev. C.01.04. Samples were prepared at approximately 0.5 mg mL^{-1} in ethanol. Analytical column dimensions were 250 mm × 4.6 mm with 5 μm particle diameter. All injections were 5 μL unless otherwise noted. Flow rates were held at 1 mL min^{-1} unless otherwise noted. Wavelengths monitored were 254 nm and 280 nm. Separations were performed at ambient temperature unless otherwise noted. Normal phase mobile phases consisted of heptane with a polar modifier. Ethanol, 1-propanol, 2-propanol and 1-butanol were evaluated as polar modifiers in the range of 1–50% (v/v). Polar organic mobile phases consisted of acetonitrile with 0–10% methanol as a modifier. Void volumes were determined by the first disturbance in the baseline resulting from unretained diluent. Resolutions (R_s) and peak symmetries (PS) were calculated using Chemstation© software.

Thermodynamic experiments were carried out at 25 °C, 29 °C, 33 °C, 37 °C and 41 °C to determine the enthalpic and entropic contributions using the equation: $\ln k = -(\Delta H^\circ/RT) + (\Delta S^\circ/R) + \ln\phi$ where ΔH° and ΔS° represent the change in standard molar enthalpy and entropy, respectively, R is the universal gas constant and T is the absolute temperature (K) of the column, ϕ is the ratio of stationary phase and mobile phase volumes, V_s and V_m, respectively. $\Delta S^{\circ *}$ is used in place of $(\Delta S^\circ/R) + \ln\phi$ as the chromatographic phase ratio is not easily determined. All thermodynamic values are stated as apparent rather than absolute due to the inability to distinguish between enantioselective and non-enantioselective interactions. Thermodynamic values were calculated using Microsoft© Excel.

Preparative scale analyses were conducted on a Shimadzu© preparative LC system consisting of an LC-20AP pump, SPD-20AV detector, SIL-10AP autosampler and FRC-10A fraction collector. Data analysis was conducted using LabSolutions© Ver. 5.54 SP1. The LARIHC CF6-P preparative column dimensions were 250 mm × 21.2 mm with 5 μm particle diameter (AZYP, LLC). Sample 19 (Table 1) was dissolved in 50:50 hexanes: ethanol at 60 mg mL^{-1}. The mobile phase consisted of 98:2 hexanes: ethanol with a flow rate of 30 mL min^{-1}. Stacked injections of 200 μL (12 mg) were performed at 15 min intervals. The wavelength used was 254 nm. Fractions containing each enantiomer were pooled and solvent removed under reduced pressure. For determining enantiomeric excess of the collected fractions, detector linearity was confirmed at 0.4–20 μg (on column, $R^2 = 0.998$, $n = 5$). Samples were prepared at 1 mg mL^{-1}. S/N for the minor enantiomer peak was >100 with the major enantiomer peak < 0.5 A.U.

3. Results and discussion

3.1. Separations obtained and insights to retention and chiral recognition

Table 1 shows the analyte structures, optimized separation conditions and chromatographic data for 30 biaryl atropisomers. Under normal phase conditions, the LARIHC CF6-P stationary phase showed enantioselectivity toward 22 out of the 30 analytes with 15 baseline separations ($R_s \geq 1.5$). The CF6-RN and CF7-DMP showed enantioselectivity for 15 analytes each with 8 and 10 baseline separations, respectively. The CF6-RN column best complemented the CF6-P column in that it was able to provide two unique separations (Table 1, compounds 9, 24) which were not obtained on the CF6-P column. Further, the CF6-RN provided one additional baseline separation (Table 1, compound 26) which was only partially separated by the CF6-P. Though the CF7-DMP phase did not provide any unique separations, it did on occasion yield excellent resolutions such as a R_s value of 7.6 (Table 1, compound 6). In all, enantioselectivity was observed for 24 of 30 analytes with 17 baseline separations using a heptane mobile phase with ethanol as a polar modifier. Clearly, the CF6-P is the most useful CSP studied in the separation of this set of atropisomers.

The common normal phase additives triethylamine (TEA) and trifluoroacetic acid (TFA) were evaluated at various concentrations. Peak symmetry was improved by using TEA but retention and selectivity were decreased. No significant increase in resolution was observed when TEA concentrations ranged from 0.05% to 0.2%. A large decrease in retention was observed when using 0.2% TEA. Using TFA in the mobile phase caused a decrease in retention and no significant improvement in resolution.

Both retention and selectivity varied considerably for different analyte and CSP combinations. The lack of aromatic functionality

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