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Enantiomeric Separation of Biaryl Atropisomers Using Cyclofructan Based Chiral Stationary Phases

Ross M. Woods¹, Darshan C. Patel¹, Yeeun Lim¹, Zachary S. Breitbach¹, Hongyin Gao³, Craig Keene³, Gongqiang Li³, László Kürti³, and Daniel W. Armstrong^{1,2}

¹The University of Texas at Arlington, 700 Planetarium Place, Arlington, TX, 76019

²AZYP LLC, 700 Planetarium Place, Arlington, TX, 76019

³Division of Chemistry, Department of Biochemistry, UT Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas TX 75390

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.

Keywords

Cyclofructans; Atropisomers; Biaryls; Chiral HPLC; Preparative HPLC

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

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Correspondence to: Daniel W. Armstrong.

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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 $\beta(2\rightarrow1)$ -linked D-fructofuranose units has recently been introduced[29]. ENREF 9 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-naphthyethylcarbamate 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].

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 binaphthyl 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'-binaphthol (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 × 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 (PAF) 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:

$\ln k = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \phi$ where H° and S° 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. $S^{*\circ}$ is used in place of $\frac{\Delta S^\circ}{R} + \ln \phi$ 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 × 21.2 mm with 5 µm particle diameter (AZYD, 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 (12mg) were performed at 15 minute 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, R² = 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 AU.

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 towards 22 out of the 30 analytes with 15 baseline separations (R_s ≥ 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 0.05-0.2%. 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 on the CF6-P column indicates that π-π interactions play no role in either retention or enantioselectivity when using this CSP. The potential exists for π-π interactions when using the aromatic functionalized CF6-RN and CF7-DMP CSP's. However, no pronounced trend showing increased retention was observed under similar mobile phase conditions for these CSP's vs. the CF6-P. Under polar organic conditions, no retention was observed for any analyte when using 100% acetonitrile indicating that retention due to hydrogen bonding between the analyte and CSP is not substantial in the presence of that solvent. The potential exists for hydrogen bonding to play

a role in chiral recognition, but only under conditions that favor analyte retention *i.e.* high heptane content in the mobile phase. Given the apparent lack of strong π - π and H-bonding interactions, the primary mechanism for analyte retention is proposed to be dipole-dipole interactions between the polar 2,2' moieties of the biaryls and the polar groups present on the derivatized cyclofructans. Previous studies using linear free energy relationship (LFER) models have shown that dipolarity/polarizability plays an important role in both retention and chiral recognition when using derivatized cyclofructans under normal phase conditions. [33] This is further validated by the lack of retention observed for analytes with steric hindrance at the 2,2' positions (Table 1, compound 28, 29, 30). This indicates that retention is highly dependent on the ability of the polar 2,2' moieties to form a strong interaction with the CSP.

The retention and selectivities observed for the various analytes varied considerably depending on both the type of aryl groups present as well as the type and location of substituents on the aryl rings. As can be seen in table 2, when using the CF6-P CSP with 10% ethanol in heptane, the binaphthyl diamine BINAM retained longer and showed greater enantioselectivity ($k_1 = 3.4$, $\alpha = 1.22$) than the 2,2'-diol BINOL ($k_1 = 2.6$, $\alpha = 1.12$), with the 2-amino-2'-ol (NOBIN) showing intermediate retention and selectivity ($k_1 = 2.7$, $\alpha = 1.14$). This indicates that the amine group plays an integral role in both retention and enantioselectivity on this CSP. When comparing BINAM ($k_1 = 3.4$, $\alpha = 1.22$) to 1-(2-aminophenyl)naphthalen-2-amine ($k_1 = 3.0$, $\alpha = 1.12$), retention and selectivity were also increased indicating that selectivity is greatly improved when both aryl groups are naphthyl.

With respect to the types of substituents on the aryl groups, the general trend was that increasing the number of electron withdrawing substituents had the effect of increasing retention and selectivity. As can be seen in Fig. 1, a pronounced increase in selectivity is observed (1.15 *vs.* 1.01) on the CF6-P stationary phase when comparing 1-(2-amino-3,4,5-trichlorophenyl)naphthalen-2'-ol (Table 1, compound 19) *vs.* 1-(2-amino-3-chlorophenyl)naphthalen-2'-ol (Table 1, compound 8) with the dichloro substituted analyte showing intermediate selectivity. Possible explanations for the enhanced chiral recognition include altering of the dihedral angle of the aryl-aryl bond as well as induced differences in the hybridization of the amine group. The majority of analytes that showed poor selectivity were either of the naphthyl-phenyl type with only a single substituent present on the phenyl ring or had the 2,2' moieties hindered by bulky substituents at the 3,3' positions. Modeling studies to determine the effect of substituents on the dihedral angle and to elucidate the types of interactions between the derivatized cyclofructans and biaryl analytes is ongoing.

3.2 Effect of Polar Modifier

By increasing the alkyl chain length of the polar modifier in the mobile phase, retention was increased. Minor improvements in selectivity were observed when going from ethanol to 1-propanol or 1-butanol (Table 2). However, both efficiencies and peak symmetries were diminished. Significant tailing was observed when using 1-butanol as a polar modifier and observable band-broadening occurred when switching away from ethanol in all cases. This provides further evidence for a dipolarity/polarizability mechanism of retention as using lower polarity solvents increased retention and slowed on/off kinetics between analyte and

CSP. The effect of varying the composition of polar modifier was an increase in retention and selectivity when the % ethanol was changed from 16% to 4% (Table 3). Based upon the combined results, a mobile phase composition of 10% ethanol in heptane is recommended for screening new biaryl atropisomers in the normal phase mode.

3.3 Temperature Effect

Excellent linearity was observed for van't Hoff plots in the range of 25–41°C indicating that the CSP was not altered significantly in this temperature range. All $S^{\circ*}$ values were negative suggesting enthalpy-driven enantioselectivity (Table 4). The absolute H_2° values were in the range of 14–19 kJ mol⁻¹ indicating a strong interaction with the CSP. The trend in absolute values for the H_2° energies of the 1',1'-binaphthyls matches the trend in retention and selectivity observed for the three types of 2',2' substituents, *i.e.* 2,2'-diamine > 2-amino-2'-ol > 2,2'-diol. This indicates that analyte adsorption to the CSP is more exothermic for 2,2'-diamines and 2-amino-2'-ol analytes than for 2,2'-diols. Absolute values for $H^{\circ*}$ ranged from 1.5–2.5 kJ mol⁻¹ vs. 14–19 kJ mol⁻¹ for H_2° , indicating much of the analyte-CSP interaction is common to both enantiomers. However, the differences in $H^{\circ*}$ were of sufficient magnitude to provide adequate selectivity at room temperature. No significant differences in thermodynamic parameters were observed when switching from ethanol to 1-propanol or 1-butanol (data not shown). Previous studies utilizing the immobilized polysaccharide CSP Chiralpak IA have reported thermodynamic parameters for BINOL using 90:10 hexane: propanol (v/v)[28]. Under those conditions, BINOL showed entropy driven enantioselectivity vs. enthalpy driven for the CF6-P using 90:10 heptane: ethanol. Clearly the driving forces for enantiomeric separation on these two CSP's are different with respect to BINOL, with the CF6-P showing enthalpy driven enantioseparation and the Chiralpak IA showing entropy driven enantioseparation.

3.4 Preparative Scale Separations

Analyte no. 19 (1-(2-amino-3,4,5-trichlorophenyl)naphthalen-2-ol) was initially selected for antimicrobial/antibiotic activity screening (data reported elsewhere) and was needed in an enantiomerically pure form. A preliminary loading study was conducted on a 250 × 4.6 mm CF6-P column with a flow rate of 2 mL min⁻¹ and a resolution of 1.4 was obtained when injecting 100 µg at a concentration of 10 mg mL⁻¹ (Fig. 2). By switching from heptane to hexanes, significant cost savings were realized without a loss of selectivity. Acceptable selectivity was observed at a flow rate of 30 mL min⁻¹ using 2% ethanol in hexanes. Injection volume was increased until baseline resolution was lost. Acceptable resolution was observed on the preparative column when injecting 200 µL at a concentration of 60 mg mL⁻¹ (Fig. 3). By stacking injections every 15 minutes, a total of 24 mg of each enantiomer was collected per hour at a cost of 1.8 L of hexanes. After combining fractions and removing solvent, the %ee of each sample was determined to be 98%

4. Conclusions

New chiral HPLC methods were presented for the enantiomeric separation of a variety of biaryl atropisomers using heptane with ethanol as a polar modifier. The primary mechanism of retention is likely dipolarity/polarizability interactions between the 2,2' functionalities of

the biaryls and polar groups present on the derivatized cyclofructans. Selectivity was observed for 24 out of 30 probe analytes with 17 baseline separations using three different CSP's. The CF6-P CSP was the most successful with 15 baseline separations using simple mobile phases without the need for additives. When ethanol was used as a polar modifier, high efficiencies and good peak symmetries were observed. When propanol and butanol were used, selectivity was improved but band broadening and peak tailing were increased. All probes studied showed enthalpy-driven patterns and the trend in absolute enthalpies of the second eluting enantiomer matched the trend in selectivities observed for the three types of 2',2'-binaphthyls. Future work will involve determining the energy barriers to racemization as well as modeling studies to determine the effects of different substituents on molecular hybridization and chiral recognition.

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- First chiral HPLC methods for recently synthesized biaryl atropisomers
- Preparative scale HPLC methods presented
- Thermodynamic parameters for enantiomeric separations determined
- Insights into forces driving retention and chiral recognition presented

Fig 1. Effects of additional halogen substituents on retention and selectivity. Conditions: column, CF6-P; mobile phase: 95:5 (v/v) heptane: ethanol; detector, UV 254 nm

Fig 2.

Analytical loading study for CF6-P. Conditions: probe no. 19 prepared in 50:50 heptane: ethanol at 10 mg mL⁻¹, injection volumes, 10, 15, 20 µL; mobile phase, 95:5 heptane: ethanol; flow rate, 2 mL min⁻¹; UV 254 nm

Fig. 3.

Preparative scale enantioseparation on CF6-P. Conditions: sample no. 19 prepared in 50:50 hexanes: ethanol at 60 mg mL⁻¹; column dimensions, 250 × 21.2 mm; mobile phase, 98:2 (v/v) hexanes: ethanol; flow rate, 30 mL min⁻¹; UV 254 nm; stacked injections of 200 μL every 15 minutes

Table 1
Structures and chromatographic data for enantiomeric separations

Analyte no.	Structure	Column	Hept: EtOH	α	R _S	k ₁	k ₂
1	251658240 (1,1'-Naphthylidene)-2,2'-diamine	CF6-P	90:10	1.26	4.8	4.1	5.2
		CF6-RN	90:10	1.21	4.6	5.0	6.1
		CF7-DMP	70:30	1.35	6.8	3.1	4.2
2	251658240 (1,1'-Naphthylidene)-2,2'-diol	CF6-P	90:10	1.10	2.6	2.8	3.1
		CF6-RN	95:5	1.06	1.0	5.5	5.9
		CF7-DMP	95:5	1.09	1.5	5.2	5.7
3	251658240 (2'-amino-1,1'-Naphthylidene)-2,2'-diol	CF6-P	90:10	1.10	2.7	3.0	3.4
		CF6-RN	95:5	1.14	1.6	5.5	6.3
		CF7-DMP	90:10	1.27	5.5	5.8	7.4
4	251658240 (1,1'-Naphthylidene)-2,2'-diamine	CF6-P	90:10	1.16	2.9	3.5	4.1
		CF6-RN	90:10	1.10	2.6	4.0	4.5
		CF7-DMP	95:5	1.2	5.3	4.5	5.5
5	251658240 (1,1'-Naphthylidene)-2,2'-diamine	CF6-P	90:10	1.15	3.0	5.1	5.9
		CF6-RN	90:10	1.10	2.7	7.0	7.8
		CF7-DMP	50:50	1.27	3.2	2.6	3.3

Analyte no.	Structure	Column	Hept: EtOH	α	R _S	k ₁	k ₂
6	<p>251658240 1,7-bis(methoxy-4-aminophenyl)-2,2'-biphenyl</p>	CF6-P	95:5	1.1	1.7	8.0	9.0
		CF6-RN	95:5	1.1	1.9	8.6	9.4
		CF7-DMP	70:30	1.5	7.6	5	7.5
7	<p>251658240 1,4-diamino-3-bromophenyl (naphthalen-2-yl)</p>	CF6-P	90:10	1.06	1.0	1.5	1.7
		CF6-RN	90:10			1.7	
		CF7-DMP	99:1			3.7	
8	<p>251658240 1,4-diamino-3-chlorophenyl (naphthalen-2-yl)</p>	CF6-P	90:10	1.06	1.0	1.55	1.67
		CF6-RN	90:10			1.8	
		CF7-DMP	99:1			3.7	
9	<p>251658240 2-amino-3-bromo-6-methoxy-1,1'-biphenyl-2-yl</p>	CF6-P	90:10			5.1	
		CF6-RN	99:1	1.07	1.9	8.3	8.9
		CF7-DMP	99:1			5.6	
10	<p>251658240 1,4-diamino-3-chloro-5-fluorophenyl (naphthalen-2-yl)</p>	CF6-P	99:1	1.03	1.1	17.0	17.6
		CF6-RN	90:10			2.7	
		CF7-DMP	95:5	1.04	1.0	7.4	7.7

Analyte no.	Structure	Column	Hept: EtOH	α	R _S	k ₁	k ₂
11	251658240 1-(2-amino-3,6-dibromophenyl)pyrrolidine-2-ol	CF6-P	90:10	1.13	2.3	2.1	2.4
		CF6-RN	99:1			13.6	
		CF7-DMP	99:1	1.04	1.5	7.4	7.8
12	251658240 1-(2-amino-3-bromo-4-methylphenyl)pyrrolidine-2-ol	CF6-P	99:1			4.8	
		CF6-RN	90:10			1.5	
		CF7-DMP	99:1			3	
13	251658240 1-(2-amino-3-chloro-4-methylphenyl)pyrrolidine-2-ol	CF6-P	90:10	1.03	0.4	3.5	3.6
		CF6-RN	90:10			1.5	
		CF7-DMP	99:1			3.0	
14	251658240 1-(2-amino-3-chloro-5-methylphenyl)pyrrolidine-2-ol	CF6-P	90:10			1.2	
		CF6-RN	90:10			1.5	
		CF7-DMP	75:25			1.9	
15	251658240 1-(2-amino-3-chloro-4-methylphenyl)pyrrolidine-2-ol	CF6-P	99:1	1.01	0.3	5.32	5.39
		CF6-RN	90:10			1.5	
		CF7-DMP	99:1			3.1	

Analyte no.	Structure	Column	Hept: EtOH	α	R _S	k ₁	k ₂
16	251658240 1-(2-amino-3,6-dichlorophenyl)pyrrolidine-2-ol	CF6-P	95:5	1.10	2.1	4.5	4.9
		CF6-RN	90:10			2.5	
		CF7-DMP	99:1	1.04	1.2	7.1	7.4
17	251658240 1-(2-amino-3,5-dichlorophenyl)pyrrolidine-2-ol	CF6-P	95:5	1.1	2.3	3.7	4.1
		CF6-RN	90:10			2.1	
		CF7-DMP	99:1	1.03	1.1	6.5	6.8
18	251658240 1-(2-amino-3,4,5-trichlorophenyl)pyrrolidine-2-ol	CF6-P	95:5	1.09	2.0	3	3.3
		CF6-RN	90:10			1.8	
		CF7-DMP	99:1	1.05	1.6	5.4	5.7
19	251658240 1-(2-amino-3,4,5-trichlorophenyl)pyrrolidine-2-ol	CF6-P	95:5	1.15	3.6	4.8	5.6
		CF6-RN	90:10	1.02	0.5	5.8	5.9
		CF7-DMP	99:1	1.09	2.5	9.1	9.9
20	251658240 1-(2-amino-3-bromophenyl)pyrrolidine-2-ol	CF6-P	90:10				5.4
		CF6-RN	90:10				2.4
		CF7-DMP	99:1				10

Analyte no.	Structure	Column	Hept: EtOH	α	R _S	k ₁	k ₂
21	<p>251658240 1-(2-amino-3,4,5-trichlorophenyl)-6-methoxynaphthalen-2-ol</p>	CF6-P	90:10	1.3	5.8	2.9	3.9
		CF6-RN	90:10	1.06	2.2	7.9	8.4
		CF7-DMP	95:5	1.2	3.6	4.4	5.0
22	<p>251658240 3,3'-di(phenyl[1,1'-bi(naphthalen-2,2'-diylamino)]-2,2'-diamine</p>	CF6-P	99:1	1.13	1.5	1.1	1.2
		CF6-RN	95:5				0.8
		CF7-DMP	95:5				0.5
23	<p>251658240 6,6'-dibromo-1,1'-bi(naphthalen-2,2'-diol</p>	CF6-P	95:5	1.15	2.7	7.3	8.4
		CF6-RN	95:5	1.12	2.2	8.3	9.3
		CF7-DMP	95:5	1.07	1.5	5.8	6.2
24	<p>251658240 1,3-bis(2,4-dihydroxyphenyl)-4,4'-biphenyl</p>	CF6-P	95:5			0.1	
		CF6-RN	99:1	1.07	0.6	0.8	0.9
		CF7-DMP	95:5				0.18
25	<p>251658240 3,3'-di(phenyl[2,2'-bi(naphthalen-1,1'-diol</p>	CF6-P	95:5	1.18	2.1	1.7	2
		CF6-RN	99:1	1.09	1.3	5.6	6.1
		CF7-DMP	95:5	1.17	2.0	1.2	1.4

Analyte no.	Structure	Column	Hept: EtOH	α	R _S	k ₁	k ₂
26	<p>251658240 3,3'-dibromo-4,4'-dihydroxy-1,1'-binaphthalene-2,2'-diol</p>	CF6-P	95:5	1.07	0.8	1.4	1.5
		CF6-RN	99:1	1.19	1.7	4.7	5.6
		CF7-DMP	95:5				1.14
27	<p>251658240 3,3'-dibromo-4,4'-dihydroxy-1,1'-binaphthalene-2,2'-diol</p>	CF6-P	95:5	1.05	0.9	4.4	4.6
		CF6-RN	95:5	1.06	0.9	4.9	5.2
		CF7-DMP	95:5	1.03	0.9	2.9	3.0
28	<p>251658240 3,3'-bis(trimethylsilyl)-4,4'-dihydroxy-1,1'-binaphthalene-2,2'-diol</p>	CF6-P	95:5				0.1
		CF6-RN	95:5				0.95
		CF7-DMP	95:5				0.42
29	<p>251658240 3,3'-bis(trimethylsilyl)-4,4'-dihydroxy-1,1'-binaphthalene-2,2'-diol</p>	CF6-P	95:5				2.3
		CF6-RN	99:1				0.34
		CF7-DMP	95:5				2.0

Analyte no.	Structure	Column	Hept: EtOH	α	R _S	k ₁	k ₂
30	 251658240 2,2'-dimethoxy-1,1'-binaphthalene	CF6-P	95:5			0.57	
		CF6-RN	99:1			1.5	
		CF7-DMP	95:5				0.8

Table 2

Effect of polar modifier on retention and enantioseparation

modifier analyte no.	ethanol				1-propanol				1-butanol			
	k ₁	α	R _s	PAF	k ₁	α	R _s	PAF	k ₁	α	R _s	PAF
1	3.4	1.22	4.1	0.82	4.5	1.27	4.2	0.66	5.7	1.27	3.4	0.59
2	2.6	1.12	2.2	0.85	2.8	1.14	2.1	0.77	3.0	1.12	1.6	0.72
3	2.7	1.14	2.6	0.93	3.2	1.21	3.2	0.66	3.9	1.28	3.5	0.60
4	3.0	1.12	2.6	0.76	4.0	1.17	2.5	0.58	5.4	1.17	1.7	0.54

Conditions: column, CF6-P; mobile phase, 90:10 heptane; modifier (v/v); detector, UV 254 nm; column temperature, 25 °C

Table 3
Effect of polar modifier composition on retention and selectivity

% ethanol analyte no.	16%			13%			10%			7%			4%		
	k ₁	α	R _s	k ₁	α	R _s	k ₁	α	R _s	k ₁	α	R _s	k ₁	α	R _s
1	2.1	1.19	3.3	2.6	1.22	3.7	3.4	1.22	4.1	4.6	1.24	4.9	7.1	1.27	6.1
2	1.5	1.11	1.8	1.9	1.12	2.0	2.6	1.12	2.2	3.7	1.13	2.4	6.2	1.12	2.6
3	1.7	1.12	1.9	2.1	1.13	2.2	2.7	1.14	2.6	3.7	1.16	3.2	5.7	1.20	4.2
4	1.9	1.12	1.8	2.3	1.14	2.3	3.0	1.12	2.6	4.0	1.15	3.0	5.8	1.17	4.0

Conditions: column, CF6-P; mobile phase, heptane: ethanol (v/v); detector, UV 254 nm; column temperature, 25 °C

Table 4**Thermodynamic parameters for enantiomeric separations**

Analyte #	H°_1 (kJ/mol)	$S^{\circ}*_1$ (J/mol*K)	R^2	H°_2 (kJ/mol)	$S^{\circ}*_2$ (J/mol*K)	R^2	H° (kJ/mol)	S° (J/mol*K)
1	-16.1	-39.7	0.999	-18.6	-45.9	0.999	-2.5	-6.2
2	-12.1	-31.6	0.999	-14.6	-38.8	0.999	-2.4	-7.2
3	-15.2	-40.3	0.999	-16.9	-44.1	0.999	-1.7	-3.8
4	-17.4	-44.3	0.999	-18.8	-47.6	0.999	-1.4	-3.4

Conditions: column, CF6-P; mobile phase, 90:10 heptane: ethanol (v/v); detector, UV 254 nm