Journal of Chromatography A, 1218 (2011) 4869–4876

Contents lists available at ScienceDirect

Journal of Chromatography A

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

High-performance liquid chromatographic enantioseparation of 1-(phenylethylamino)- or 1-(naphthylethylamino)methyl-2-naphthol analogs and a temperature-induced inversion of the elution sequence on polysaccharide-based chiral stationary phases

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ARTICLE INFO

Article history:
Available online 18 January 2011

Keywords:
Column liquid chromatography
Enantiomer separation
1-(Phenylethylamino)methyl-2-naphthol analogs
1-(Naphthylethylamino)methyl-2-naphthol analogs
Lux Cellulose-1 column
Lux Cellulose-2 column
Lux Amylose-2 column

ABSTRACT

The stereoisomers of five 1-(phenylethylamino)methyl-2-naphthol analogs or 1-(naphthylethylamino)methyl-2-naphthol analogs containing two chiral centers were directly separated on chiral stationary phases containing the chiral selectors cellulose *tris*-(3,5-dimethylphenyl) carbamate (Lux Cellulose-1), cellulose *tris*-(3-chloro-4-methylphenyl) carbamate (Lux Cellulose-2) and amylose *tris*-(5-chloro-2-methylphenyl) carbamate (Lux Amylose-2). Experiments were performed in normal-phase mode in a wide temperature range −5 to 70 °C. Thermodynamic parameters and T_{iso} values were calculated from plots of $\ln k$ or $\ln \alpha$ vs. $1/T$. $-\Delta(\Delta H^\circ)$ ranged from 1.0 to 4.7 kJ mol^{-1}, $-\Delta(\Delta S^\circ)$ from 1.6 to 11.0 J mol^{-1} K^{-1} and $-\Delta(\Delta G^\circ)$ from 0.1 to 1.5 kJ mol^{-1}. The sequence of elution of the stereoisomers was determined in all cases and in one case a temperature-induced inversion of the elution sequence was observed.

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1. Introduction

The synthesis and application of new chiral ligands in asymmetric transformations is currently a field of great interest in organic chemistry. In the past decade, modified three-component Mannich reactions, based on the aminoalkylation of 2- or 1-naphthol, have become of considerable importance for the formation of a C–C bond under mild experimental conditions [1]. The syntheses of the aminonaphthols obtained in this way differ in the starting nitrogen source: (i) through the use of primary or secondary amines, secondary or tertiary aminonaphthols have been synthetized [1–6]; (ii) with ammonia as source, primary aminonaphthols have been prepared [1]. The areas of application of these aminonaphthols differ with the nature of the amino group; for example the enantiomers of secondary and tertiary aminonaphthols have been widely applied as chiral catalysts in the enantioselective alkylation or arylation of benzaldehyde [1–5].

Since this asymmetric catalytic activity of aminonaphthol analogs depends strongly on their stereochemistry, there is a clear need for the elaboration of precise separation and identification methods by which their configurations can be assigned. One of the most frequently applied techniques is chiral high-performance liquid chromatography (HPLC). HPLC enantioseparations of similar analogs have been performed on cellulose- or amylose-based chiral stationary phases (CSPs) by Sztojkov-Ivanov et al. [7,8], Ilisz et al. [6,9], on β-CD based CSP by Berkecz et al. [10] and by capillary electrophoresis with the application of substituted β-cyclodextrins and chiral crown ethers by Ilisz et al. [11]. Chankvetadze et al. [12–16] proposed chloromethylphenylcarbamate derivatives of cellulose and amylose as useful CSPs for HPLC enantioseparations in 1990s. These CSPs have been recently commercialized and used for enantioseparation of various chiral compounds [17–19]. Alfonsov et al. [20] separated enantiomers of aminonaphthol analogs by crystallization, applying L-(+)-tartaric acid.

Enantioselective retention and separation are influenced by the concentration and nature of the mobile phase components, together with other variables, such as the pH and temperature. In chromatographic enantioseparations, the relationship between the chromatographic data and the column temperature is as follows:

$$\ln k = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \phi \quad (1)$$

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doi:10.1016/j.chroma.2011.01.027

in which k is the retention factor, α is the separation factor, ΔH° is the enthalpy of transfer of the solute from the mobile phase to the stationary phase, ΔS° is the entropy of transfer of the solute from the mobile phase to the stationary phase, R is the gas constant, T is temperature and ϕ is the phase ratio of the column.

Eq. (1) reveals that a plot of $\ln k$ vs. $1/T$ is linear, with slope $-\Delta H^\circ/R$ and intercept $\Delta S^\circ/R + \ln\phi$, if ΔH° is invariant with temperature. Since the value of ϕ is often not known, the $\Delta S^{\circ *}$ values $[\Delta S^{\circ *} = \Delta S^\circ + R\ln\phi]$ calculated from the intercepts of the plots via Eq. (1) are generally used. Any uncertainty in the phase ratio affects all $\Delta S^{\circ *}$ values in the same manner.

$\Delta(\Delta H)^\circ$ and $\Delta(\Delta S)^\circ$ are the difference in ΔH° and ΔS°, respectively, for a given pair of enantiomers:

$$\Delta(\Delta H)^\circ = \Delta H_2^\circ - \Delta H_1^\circ \quad \text{and} \quad \Delta(\Delta S)^\circ = (\Delta S_2^{\circ *} - R\ln\phi) - (\Delta S_1^{\circ *} - R\ln\phi) = \Delta S_2^{\circ *} - \Delta S_1^{\circ *} \qquad (2)$$

In chiral chromatography, however, the van't Hoff plots often deviate from linearity, possibly as a result of the inhomogeneity of the CSP surface, leading to a mixed retention mechanism [21]. Additionally, there are both achiral and chiral contributions to retention that can vary with a wide variety of experimental parameters [22–27].

If $\Delta(\Delta H)^\circ$ and $\Delta(\Delta S)^\circ$ are both negative, the enantioseparation is enthalpically driven, as in the common case. The second-eluted enantiomer forms a more stable complex with the selector than does the first-eluted enantiomer, with a more unfavorable entropy for enantioseparation. The separation factor decreases with increasing temperature. When the column temperature reaches a point referred to as the enantioselective temperature (T_{iso}), the enthalpy contribution is wholly compensated by the entropy term. At this point, $\alpha = 1.0$, and the two enantiomers coelute. T_{iso} can be calculated from $\ln k$ vs. $1/T$ curves. Above T_{iso}, the enantioseparation is entropically driven, and a reversal of the elution sequence for a pair of enantiomers is observed. The separation factor increases with increasing temperature in this domain, and the column efficiency increases concurrently. These two features make entropically driven enantioseparation especially attractive.

The existence of T_{iso} was predicted by Koppenhoefer and Bayer [22]. The first evidence was obtained by Gil-Av and co-workers [28] and Schurig and Betschinger [29] from gas-chromatographic studies.

Because of the narrow temperature range in HPLC, temperature-induced inversion of the elution sequences has rarely been observed. A reversal of the elution sequence was reported for amino alcohols on Chiralcel OD [30], for phenylethylamine analogs [31] on a brush-type column and for sotalol on a cellubiohydrolase-I-based CSP [32]. Schlauch and Frahm [33] observed a reversed elution sequence for some cyclic amino acids on a copper(II)-D-penicillamine CSP, as did Yao et al. [34] for 1,1′-binaphthol analogs on a polysaccharide-type CSP. Stringham and Blackwell [35] reported a reversed elution sequence in supercritical fluid chromatography.

The present paper describes normal-phase HPLC methods for the enantioseparation of new racemic 1-(phenylethylamino)methyl-2-naphthol or 1-(naphthylethylamino)methyl-2-naphthol analogs possessing two chiral centers (Fig. 1). These HPLC methods rely on the use of recently commercialized polysaccharide-based chiral CSPs: *tris*-(3,5-dimethylphenyl)-carbamoylated cellulose (Lux Cellulose-1), *tris*-(3-chloro-4-methylphenyl)-carbamoylated cellulose (Lux Cellulose-2) and *tris*-(5-chloro-2-methylphenyl)-carbamoylated amylose (Lux Amylose-2). The effects of the mobile phase composition, the nature of the alcoholic modifier, the specific structural features of the analytes and selectors and temperature on the retention are discussed on the basis of the experimental data. The temperature-induced inversion of the elution sequence was revealed. The elution sequence was determined in all cases.

2. Experimental

2.1. Materials and methods

Synthesis of the analytes **1** [2,3], **2** [4], **3** [5], **4** and **5** [6], either in racemic form or in enantiopure form, was achieved by heating 2-naphthol, the racemic or nonracemic amine and the aldehyde in question under solvent-free conditions, as described in the literature [2–6].

Starting from the racemic amine, the formation of two diastereomers and four stereoisomers (**a**–**d**) is possible during the reaction in each case. The NMR spectra of the crude product showed the presence of the two diastereomeric pairs **a**,**b** and **c**,**d** (Fig. 1) in different ratios. By recrystallization from di-2-propyl ether ($i\mathrm{Pr_2O}$), the major enantiomer pairs (**1a**,**1b**, **2a**,**2b**, **3a**,**3b**, **4a**,**4b** and **5a**,**5b**) were isolated for all five model compounds. The yields of the reactions were 78% for **1**, 82% for **2**, 65% for **3**, 72% for **4** and 68% for **5**.

To correlate the peaks with the absolute configurations, the synthesis of compounds **1**–**5** has been repeated starting from the nonracemic amines [*S*-(−)-1-phenylethylamine for **1**, **3**–**5** or *R*-(+)-1-(1-naphthyl)ethylamine for **2**]. The major diastereomers were obtained by recrystallization from $i\mathrm{Pr_2O}$. The yields were as follows: 72% for **1**, 85% for **2**, 63% for **3**, 75% for **4** and 60% for **5**. The absolute configurations of C-1 and C-1′, determined by X-ray measurements, were (1*S*,1′*S*) for **1a** [2], (1*R*,1′*S*) for **3a** [5] and (1*R*,1′*R*) for **2b** [4]. The absolute configurations for **4** and **5** were deduced from the close structural analogy with **3**.

n-Heptane, methanol (MeOH), ethanol (EtOH), 1-propanol (PrOH), 2-propanol (IPA), 1-butanol (BuOH) and *tert*-butanol (*t*-BuOH) of HPLC grade were purchased from Sigma–Aldrich (St. Louis, MO, USA), as were trifluoroacetic acid (TFA), glacial acetic acid (AcOH), diethylamine (DEA) and other reagents of analytical reagent grade. The Milli-Q water was further purified by filtration on a 0.45-μm filter, type HV, Millipore (Molsheim, France).

The eluents were degassed in an ultrasonic bath, and helium gas was purged through them during the analyses. Stock solutions of analytes (1 mg ml^{-1}) were prepared by dissolution in the starting mobile phase.

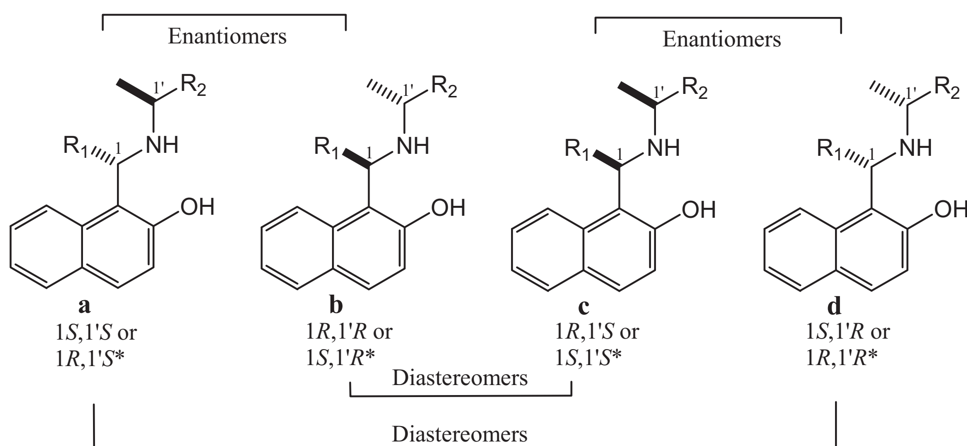
2.2. Apparatus

The HPLC separations were carried out on a Waters HPLC system consisting of an M-600 low-pressure gradient pump, an M-2996 photodiode-array detector and a Millenium[32] Chromatography Manager data system (Waters Chromatography, Milford, MA, USA) equipped with a Rheodyne Model 7125 injector (Cotati, CA, USA) with a 20-μl loop.

The HPLC columns used were cellulose *tris*-(3,5-dimethylphenyl) carbamate (Lux Cellulose-1) or cellulose *tris*-(3-chloro-4-methylphenyl) carbamate (Lux Cellulose-2) and amylose *tris*-(5-chloro-2-methylphenyl) carbamate (Lux Amylose-2) CSP (Phenomenex, Torrance, CA, USA). The dead-time (t_0) of the column was determined by injecting tri-*tert*-butyl benzene. The columns were thermostated in a Spark Mistral column thermostat (Spark Holland, Emmen, The Netherlands). The precision of temperature adjustment was ±0.1 °C.

3. Results and discussion

The experimental conditions, including the nature and concentration of the alcoholic modifier and the temperature in the



Analyte	R_1	R_2	Analyte	R_1	R_2
1a-1d			**3a-3d***	N	
2a-2d			**4a-4d**	N	
-	**-**	**-**	**5a-5d**	N	

* According to C.I.P. convention

Fig. 1. Structures of 1-(phenylethylamino)- or 1-(naphthylethylamino)methyl-2-naphthol analogs.

n-heptane/alcohol/DEA mobile phase were investigated and all data on the three different polysaccharide CSPs are given in Tables 1–3.

The retention factors depended strongly on the alcohol content of the mobile phase. Typical normal-phase behavior was observed at ambient temperature on the Lux Cellulose-1 column: decrease of the IPA content resulted in larger retention factor k' and in most cases larger separation factor, α, and resolution, R_S (Table 1).

The nature of the alcoholic modifier influenced the retention, enantioselectivity and resolution, i.e. the ratio of the nonchiral and chiral interactions between the CSP and the analytes depended on the nature (and also the concentration) of the alcohol. Wang and Wenslow [36] found that the changes caused in the CSP structure by the different alcohols may affect the chiral selectivity of the CSP, depending on the size and structure of the analyte.

For analytes **1a**–**1d** and **5a**–**5d** on the Lux Cellulose-1 and Lux Amylose-2 columns, the application of MeOH, EtOH, PrOH, IPA, BuOH and *t*-BuOH in the same molar concentration of 6.5×10^{-2} M, or 2.6×10^{-2} M, k'_1, α, and R_S increased in the sequence MeOH–*t*-BuOH and were largest in the mobile phase containing IPA and *t*-BuOH, and smaller in the eluent system containing BuOH. These results are consistent with the results obtained on a Kromasil CelluCoat column, containing the same selector [6].

The structures of the analytes influenced the chiral recognition and a structure–retention relationship was observed. At the same eluent composition [*n*-heptane/IPA/DEA = 95/5/0.1 (v/v/v)], the analog with a substituted naphthyl ring resulted in an increase in k' (and sometimes α and R_S values) as compared to that with a substituted phenyl ring, i.e. **2** vs. **1** which can be attributed to the increased steric effect and π–π interactions.

A comparison of the enantioseparation of analytes not containing or containing a *N*-heteroatom (i.e. **1** vs. **3**–**5**) reveals the importance of steric, π–π or polar interactions. The presence of an *N*-atom in the *ortho* position close to the chiral center in most cases hindered the interaction with the CSP, resulting in smaller k' (and sometimes smaller α and R_S values). The presence of an *N*-atom in the *meta* or the *para* position promoted the interaction between the analyte and the CSP.

For comparison of the performances of the three polysaccharide-based columns, separations were carried out with the same mobile phase, *n*-heptane/IPA/DEA = 95/5/0.1 (v/v/v), on Lux Cellulose-1, Lux Cellulose-2 and Lux Amylose-2 (Table 1). Of the two cellulose-based columns Lux Cellulose-1 seemed to be the more effective in the separation of the enantiomers of **1a**,**1b**, **1c**,**1d**, **2a**,**2b**, **3a**,**3b**, and **4a**,**4b**, while Lux Cellulose-2 was better in the separation of **2c**,**2d**, **3c**,**3d**, **4c**,**4d**, **5a**,**5b** and **5c**,**5d**.

Comparison of the cellulose *tris*-(3-chloro-4-methylphenyl) carbamate (Lux Cellulose-2) and amylose *tris*-(5-chloro-2-methylphenyl) carbamate (Lux Amylose-2) selectors demonstrated that k'_1 (and sometimes α and R_S) was generally higher on the amylose-based column (exception was **5**; **5c**,**5d** was not separable on Lux Amylose-2). Interestingly, despite the lower k', **4** exhibited much higher α and R_S values on the Lux Cellulose-2 CSP.

Table 1
Chromatographic data, retention factors (k'), separation factors (α) and resolutions (R_S) of the separation of 1-(phenylethylamino)- or 1-(naphthylethylamino)methyl-2-naphthol analogs.

Analyte	Column	Mobile phase (v/v/v)	k'_1	k'_2	α	R_S	Elution sequence
1a,**1b**	Cellulose-1	80/20/0.1	0.58	0.65	1.12	0.75	*b* < *a*
	Cellulose-1	90/10/0.1	0.86	0.98	1.14	1.00	*b* < *a*
	Cellulose-1	95/5/0.1	0.97	1.11	1.15	1.05	*b* < *a*
	Cellulose-1	98/2/0.1	1.31	1.54	1.18	1.15	*b* < *a*
	Cellulose-2	95/5/0.1	0.70	0.70	1.00	0.00	–
	Amylose-2	95/5/0.1	1.23	1.55	1.26	1.44	*a* < *b*
1c,**1d**	Cellulose-1	80/20/0.1	0.66	0.79	1.21	1.10	*d* < *c*
	Cellulose-1	90/10/0.1	1.00	1.22	1.22	1.45	*d* < *c*
	Cellulose-1	95/5/0.1	1.11	1.39	1.24	1.85	*d* < *c*
	Cellulose-1	98/2/0.1	1.48	1.91	1.29	2.40	*d* < *c*
	Cellulose-2	95/5/0.1	0.70	0.70	1.00	0.00	–
	Amylose-2	95/5/0.1	1.67	2.44	1.46	4.80	*c* < *d*
2a,**2b**	Cellulose-1	80/20/0.1	1.27	1.85	1.45	4.20	*a* < *b*
	Cellulose-1	90/10/0.1	1.77	2.61	1.48	4.20	*a* < *b*
	Cellulose-1	95/5/0.1	2.13	3.20	1.50	4.30	*a* < *b*
	Cellulose-1	98/2/0.1	3.12	4.78	1.53	4.30	*a* < *b*
	Cellulose-2	95/5/0.1	0.87	0.87	1.00	0.00	–
	Amylose-2	95/5/0.1	1.58	1.71	1.09	0.72	*b* < *a*
2c,**2d**	Cellulose-1	80/20/0.1	0.86	0.86	1.00	0.00	–
	Cellulose-1	90/10/0.1	1.16	1.16	1.00	0.00	–
	Cellulose-1	95/5/0.1	1.17	1.18	1.01	0.40	*c* < *d*
	Cellulose-1	98/2/0.1	1.58	1.69	1.07	0.50	*d* < *c*[a]
	Cellulose-2	95/5/0.1	1.05	1.35	1.29	2.90	*d* < *c*
	Amylose-2	95/5/0.1	1.84	2.89	1.58	3.50	*d* < *c*
3a,**3b**	Cellulose-1	80/20/0.1	0.47	0.53	1.13	0.60	*b* < *a*
	Cellulose-1	90/10/0.1	0.65	0.74	1.14	0.75	*b* < *a*
	Cellulose-1	95/5/0.1	0.77	0.88	1.14	0.95	*b* < *a*
	Cellulose-1	98/2/0.1	0.94	1.07	1.14	1.00	*b* < *a*
	Cellulose-2	95/5/0.1	0.81	0.87	1.07	1.13	*b* < *a*
	Amylose-2	95/5/0.1	1.34	1.34	1.00	0.00	–
3c,**3d**	Cellulose-1	80/20/0.1	0.72	0.72	1.00	0.00	–
	Cellulose-1	90/10/0.1	1.08	1.08	1.00	0.00	–
	Cellulose-1	95/5/0.1	1.36	1.36	1.00	0.00	–
	Cellulose-1	98/2/0.1	1.67	1.67	1.00	0.00	–
	Cellulose-2	95/5/0.1	1.16	1.31	1.13	1.64	*d* < *c*
	Amylose-2	95/5/0.1	1.60	1.92	1.20	2.22	*c* < *d*
4a,**4b**	Cellulose-1	80/20/0.1	1.37	2.18	1.58	5.25	*b* < *a*
	Cellulose-1	90/10/0.1	3.96	6.70	1.69	7.85	*b* < *a*
	Cellulose-1	95/5/0.1	4.99	9.05	1.81	8.80	*b* < *a*
	Cellulose-1	98/2/0.1	9.16	17.83	1.95	9.60	*b* < *a*
	Cellulose-2	95/5/0.1	3.81	9.61	2.52	16.85	*b* < *a*
	Amylose-2	95/5/0.1	6.91	9.73	1.41	2.31	*b* < *a*
4c,**4d**	Cellulose-1	80/20/0.1	1.94	2.33	1.20	2.50	*c* < *d*
	Cellulose-1	90/10/0.1	5.86	7.39	1.26	4.00	*c* < *d*
	Cellulose-1	95/5/0.1	8.00	10.44	1.31	4.35	*c* < *d*
	Cellulose-1	98/2/0.1	15.28	21.18	1.39	5.70	*c* < *d*
	Cellulose-2	95/5/0.1	2.43	8.48	3.49	17.12	*d* < *c*
	Amylose-2	95/5/0.1	8.66	12.29	1.42	2.88	*d* < *c*
5a,**5b**	Cellulose-1	80/20/0.1	2.55	2.55	1.00	0.00	–
	Cellulose-1	90/10/0.1	6.34	6.77	1.07	0.80	*b* < *a*
	Cellulose-1	95/5/0.1	8.45	9.78	1.16	1.85	*b* < *a*
	Cellulose-1	98/2/0.1	21.64	31.29	1.45	5.55	*b* < *a*
	Cellulose-2	95/5/0.1	13.62	15.62	1.15	2.02	*a* < *b*
	Amylose-2	95/5/0.1	6.48	9.60	1.48	2.82	*b* < *a*
5c,**5d**	Cellulose-1	80/20/0.1	2.55	3.86	1.51	4.60	*d* < *c*
	Cellulose-1	90/10/0.1	6.47	10.84	1.68	7.65	*d* < *c*
	Cellulose-1	95/5/0.1	9.05	17.42	1.93	9.50	*d* < *c*
	Cellulose-1	98/2/0.1	25.70	52.85	2.06	10.20	*d* < *c*
	Cellulose-2	95/5/0.1	12.44	28.41	2.28	13.77	*d* < *c*
	Amylose-2	95/5/0.1	10.31	10.31	1.00	0.00	–

Chromatographic conditions: columns, Lux Cellulose-1, Lux Cellulose-2, Lux Amylose-2; mobile phase, *n*-heptane/IPA/DEA = 80/20/0.1, 90/10/0.1, 95/5/0.1 and 98/2/0.1 (v/v/v); flow rate, 0.5 ml min^{-1}; detection, 230 nm; temperature, ambient (ca. 25 °C); hold-up times, for Lux Cellulose-1 t_0 = 3.75 min, for Lux Cellulose-2 t_0 = 5.90 min, for Lux Amylose-2 t_0 = 6.50 min.

[a] Above T_{iso} temperature.

In summary, the three newly commercialized CSPs display a complementary character. The elution sequences were determined in all cases and no consistent elution sequence was observed. In most cases, the elution sequences on Lux Cellulose-1 (or Lux Cellulose-2) and Lux Amylose-2 were inverted. Selected chromatograms and the most important chromatographic parameters for **1**–**5** are depicted in Fig. 2 and Table 2.

3.1. Effects of temperature and thermodynamic parameters

In order to investigate the effects of temperature on the chromatographic parameters, a variable-temperature study was carried out on Lux Cellulose-1 column, usually over the temperature range 5–40 °C (Table 3). For **2c**,**2d** the investigated temperature range was −5 °C to 80 °C. Experimental data for the mobile phase

Table 2
Chromatographic data, retention factor (k') and resolution (R_S) for the separation of four enantiomers of aminonaphthol analogs on Lux Cellulose-1 CSP with variation of the type and content of the alcoholic modifier.

Analyte	Mobile phase (v/v/v)	k'_1	k'_2	k'_3	k'_4	R_{S1}	R_{S2}	R_{S3}	Elution sequence
1	**a**	1.16	1.40	1.59	1.77	1.5	1.55	1.4	$b<c<a<d$
2	**b**	0.78	0.93	1.68	2.66	1.35	5.71	5.20	$c<d<a<b$
3	**c**	1.76	2.25	4.01	4.46	2.3	6.48	1.50	$b<a<d<c$
4	**d**	9.16	15.28	17.83	21.18	12.55	3.63	2.83	$b<c<a<d$
5	**e**	21.64	25.70	31.29	52.85	2.35	3.16	9.86	$b<d<a<c$

Chromatographic conditions: mobile phase, **a**, *n*-heptane/*t*-BuOH/DEA = 95/5/0.1 (v/v/v); **b**, *n*-heptane/PrOH/DEA = 99/1/0.1 (v/v/v); **c**, *n*-heptane/*t*-BuOH/DEA = 99/1/0.1 (v/v/v); **d**, *n*-heptane/MeOH/DEA = 98/2/0.1 (v/v/v); **e**, *n*-heptane/IPA/DEA = 95/5/0.1 (v/v/v); temperature, for analytes **1**, **2**, **4** and **5**, 25.0 °C, for analyte **3**, 5.0 °C; flow rate, 0.5 ml min^{-1}; detection, 230 nm.

n-heptane/IPA/DEA = 90/10/0.1 and 98/2/0.1 (v/v/v) are listed in Table 3. A comparison of the retention factors in Table 3 reveals that all of the recorded values decreased with increasing temperature together with the separation factor, α, and the resolution, R_S, while for analyte **2c**,**2d** at high temperature increases in α and R_S were observed.

To shed light on the effect of temperature on the separation, accurate chromatographic data were accumulated from which van't Hoff plots were constructed (Eq. (1)). The ΔH° and $\Delta S^{\circ *}$ values on the Lux Cellulose-1 column were negative (Table 4). The second-eluting enantiomers have more negative $\Delta S^{\circ *}$ values, and it is likely that they have fewer degrees of freedom on the CSP, i.e. they are held at more points or are less able to move or rotate. It is widely accepted that both enantiomers undergo the same nonspecific interactions, whereas the more strongly retained one is subject to additional stereospecific interactions.

Table 3
Temperature dependence of retention factor of first eluting enantiomer (k'_1), separation factor (α) and resolution (R_S) of enantiomers of 1-(phenylethylamino)- or 1-(naphthylethylamino)methyl-2-naphthol analogs.

Analyte	Eluent	k'_1, α, R_S	Temperature (°C)					
			5	10	15	20	30	40
1a,**1b**	**a**	k'_1	1.22	1.10	1.04	0.95	0.78	0.66
		α	1.39	1.32	1.27	1.25	1.17	1.12
		R_S	3.43	2.73	2.48	2.25	1.33	0.99
1c,**1d**	**a**	k'_1	1.48	1.33	1.23	1.10	0.92	0.74
		α	1.29	1.28	1.27	1.26	1.24	1.23
		R_S	2.86	2.79	2.77	2.26	2.21	2.14
2a,**2b**	**a**	k'_1	2.69	2.23	2.09	1.77	1.50	1.23
		α	1.76	1.68	1.65	1.54	1.52	1.41
		R_S	5.14	5.12	4.97	4.70	4.49	4.08
2c,**2d**	**a**	k'_1	1.51	1.33	1.17	1.16	0.98	0.82
		α	1.12	1.06	1.00	1.00	1.00	1.00
		R_S	1.54	0.78	0.00	0.00	0.00	0.00
2c,**2d**	**b**	k'_1	2.89	2.48	–	2.23	1.99	1.70
		α	1.09	1.08	–	1.00	1.03	1.09
		R_S	1.16	0.93	–	0.00	0.60	1.18
3a,**3b**	**a**	k'_1	0.81	0.75	0.68	0.63	0.54	0.47
		α	1.22	1.20	1.18	1.17	1.15	1.13
		R_S	1.88	1.49	1.38	1.24	1.00	0.84
3c,**3d**	**a**	k'_1	1.61	1.43	1.27	1.16	0.98	0.82
		α	1.00	1.00	1.00	1.00	1.00	1.00
		R_S	0.00	0.00	0.00	0.00	0.00	0.00
4a,**4b**	**a**	k'_1	3.50	3.30	3.13	2.91	2.52	2.16
		α	2.07	1.99	1.93	1.85	1.74	1.65
		R_S	7.66	7.64	7.61	7.58	7.53	7.35
4c,**4d**	**a**	k'_1	6.16	5.62	5.15	4.65	3.84	3.17
		α	1.40	1.37	1.35	1.32	1.27	1.23
		R_S	4.11	4.08	4.05	3.96	3.84	3.33
5a,**5b**	**a**	k'_1	8.06	7.50	6.95	6.57	5.45	4.78
		α	1.17	1.16	1.15	1.13	1.11	1.09
		R_S	1.79	1.76	1.74	1.67	1.64	1.06
5c,**5d**	**a**	k'_1	8.92	8.28	7.48	6.82	5.92	5.01
		α	1.88	1.83	1.79	1.75	1.69	1.60
		R_S	9.66	7.66	7.10	7.04	7.00	6.60
			Temperature (°C)					
			−5	0	50	60	70	80
2c,**2d**	**a**	k_1	1.80	1.67	0.69	0.60	0.50	0.43
		α	1.16	1.15	1.00	1.00	1.05	1.12
		R_S	1.75	1.60	0.00	0.00	0.37	0.44
2c,**2d**	**b**	k_1	–	2.97	1.47	–	–	–
		α	–	1.13	1.15	–	–	–
		R_S	–	1.90	1.90	–	–	–

Chromatographic conditions: column, Lux Cellulose-1; mobile phase, **a**, *n*-heptane/IPA/DEA = 90/10/0.1 (v/v/v), **b**, *n*-heptane/IPA/DEA = 98/2/0.1 (v/v/v); flow rate, 0.5 ml min^{-1}; detection 230 nm.

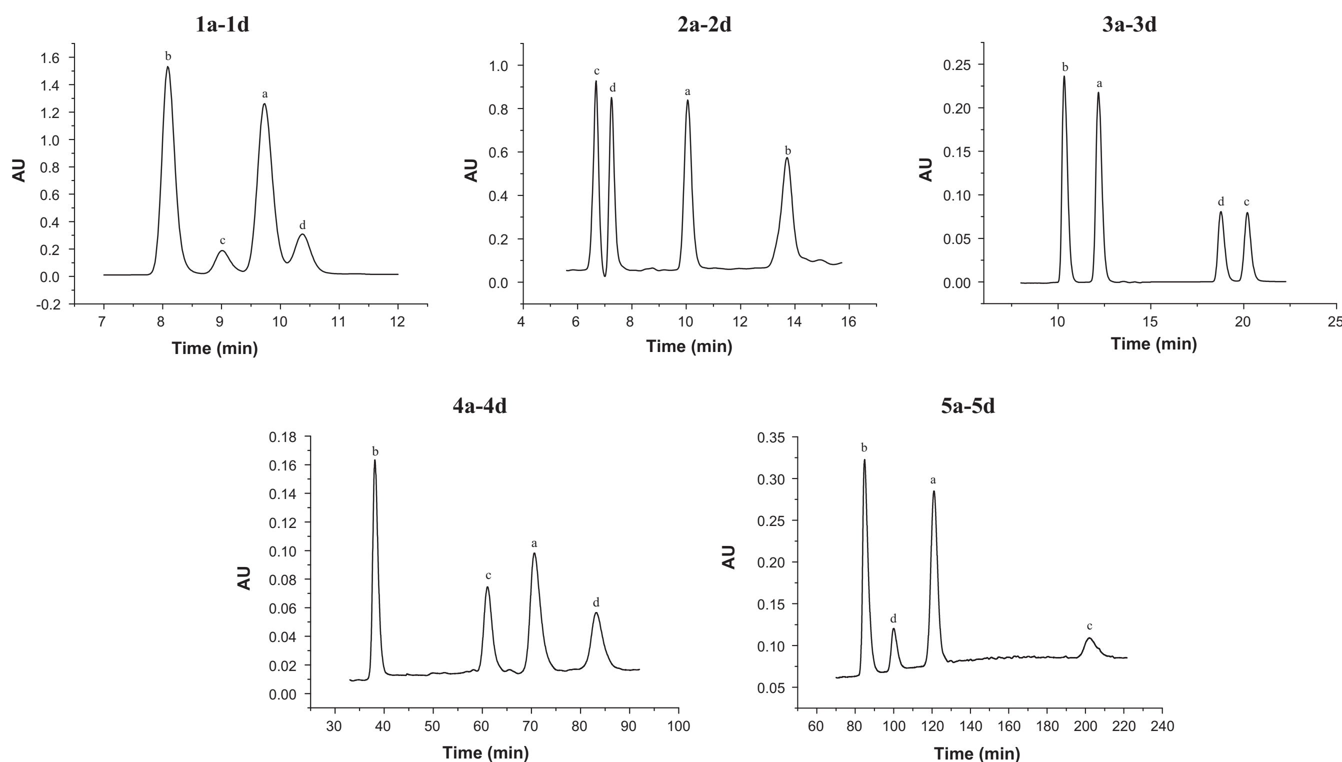


Fig. 2. Selected chromatograms for the separation of four enantiomers of analytes **1**–**5**. Chromatographic conditions: column, Lux Cellulose-1; mobile phase, *n*-heptane/*t*-BuOH/DEA = 95/5/0.1 (v/v/v) for **1**, *n*-heptane/PrOH/DEA = 99/1/0.1 (v/v/v) for **2**, *n*-heptane/*t*-BuOH/DEA = 99/1/0.1 (v/v/v) for **3**, *n*-heptane/MeOH/DEA = 98/2/0.1 (v/v/v) for **4** and *n*-heptane/IPA/DEA = 98/2/0.1 (v/v/v) for **5**; temperature 25 °C, except for analyte **3** 5.0 °C; flow rate 0.5 ml min^{-1}; detection, 230 nm.

In Table 4, the $-\Delta(\Delta H^{\circ})$ values ranged from 1.0 to 4.7 kJ mol^{-1}. The interactions of **4a**,**4b** with Lux Cellulose-1 were characterized by the highest negative $\Delta(\Delta H^{\circ})$ value, while **1c**,**1d** exhibited the least negative $\Delta(\Delta H^{\circ})$. The trends in the change in $-\Delta(\Delta S^{\circ})$ showed that **2a**,**2b** and **4a**,**4b** had the largest negative entropies, $-\Delta(\Delta S^{\circ})$ ranging from 1.6 to 12.7 J mol^{-1} K^{-1} (Table 4). The $\Delta(\Delta S^{\circ})$ values are governed by the difference in the number of degrees of freedom between the stereoisomers on the CSP, and mainly by the numbers of solvent molecules released from the chiral selector and the analyte when the analyte is associated with the CSP. For analyte **2c**,**2d** above T_{iso} temperature both $\Delta(\Delta H^{\circ})$ and $\Delta(\Delta S^{\circ})$ values were positive, indicating an entropically driven separation.

The thermodynamic parameter $-\Delta(\Delta G^{\circ})_{298}$ suggests that **2a**,**2b** (an enantiomer pair containing a naphthalene ring) and **4a**,**4b** and **5c**,**5d** (enantiomer pairs containing a pyridyl group, with an *N*-atom in position 3 or 4) induce highly efficient binding to the selector, as reflected by the large negative $\Delta(\Delta G^{\circ})$ values.

The data were used to calculate the temperature, T_{iso}, at which the enantioselectivity balanced out and the elution sequence changed (Table 4). In most cases, T_{iso} was considerably higher than room temperature; enthalpically driven enantioseparation was obtained. For **2c**,**2d**, T_{iso} at mobile phase composition of *n*-heptane/IPA/DEA = 90/10/0.1 (v/v/v) was 38 °C and at *n*-heptane/IPA/DEA = 98/2/0.1 (v/v/v) 22 °C (Fig. 3). For 1,1′-bi-2-naphthol analog on polysaccharide-based CSP Yao et al. [34] observed a decrease in T_{iso} temperature from 38.8 °C to 11.0 °C by decreasing IPA content in *n*-hexane from 10% to 4%.

At *n*-heptane/IPA/DEA = 90/10/0.1 (v/v/v) mobile phase composition increasing the temperature from −5 °C, α decreased then after a domain where no separation occurred, α increased with increasing temperature (Table 3). In a domain around the isoenantioselective temperature, enantioseparation could not be obtained. This domain may be referred to as a “temperature-induced blind zone” in chiral recognition [34]. Outside the blind zone, enthalpically or entropically-driven enantioseparation can be observed. It should be mentioned that peak inversions by temperature changes were generally only observed for separations with marginal enantioselectivity [30,31,33–35]. For **2c**,**2d** analogs at *n*-heptane/IPA/DEA = 98/2/0.1 (v/v/v) mobile phase composition an inversion of the enantiomeric elution sequence with good resolution was observed (Fig. 4).

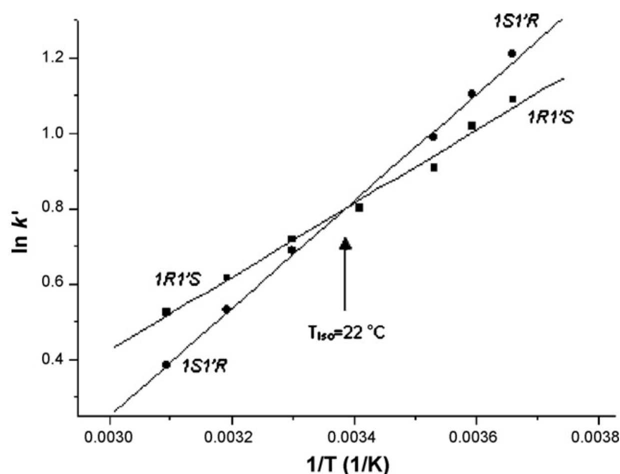


Fig. 3. ln k' vs. $1/T$ curves for **2c**,**2d** in the temperature range 5–50 °C. Chromatographic conditions: column, Lux Cellulose-1; mobile phase, *n*-heptane/IPA/DEA = 98/2/0.1 (v/v/v); flow rate, 0.5 ml min^{-1}; detection, 230 nm.

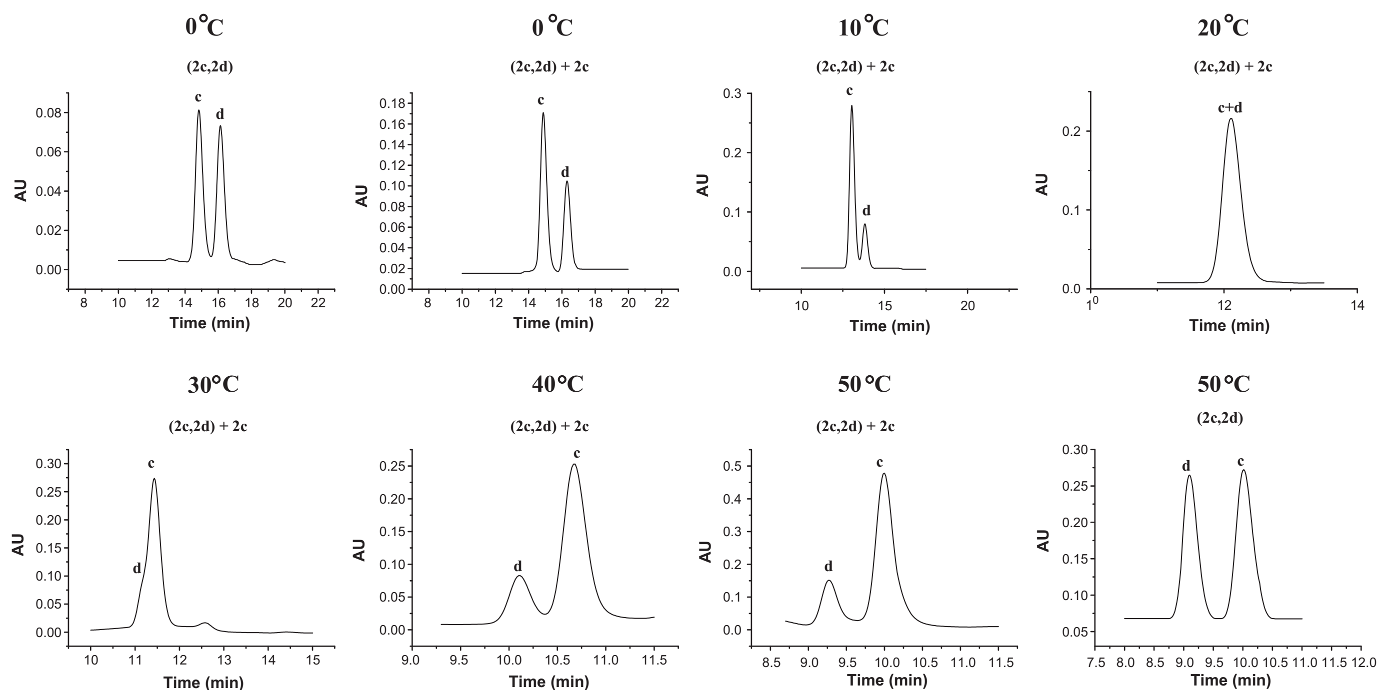


Fig. 4. Temperature-induced inversion of the elution sequence for analyte **2c**,**2d**. Chromatographic conditions: column, Lux Cellulose-1; mobile phase, *n*-heptane/IPA/DEA = 98/2/0.1 (v/v/v); flow rate, 0.5 ml min^{-1}; detection, 230 nm; temperature range 0–50 °C; racemic **2c**,**2d** was spiked with **2c**.

Table 4
Thermodynamic parameters, ΔH°, $\Delta S^{\circ *}$, $\Delta(\Delta H^\circ)$, $\Delta(\Delta S^\circ)$, $\Delta(\Delta G^\circ)$, correlation coefficients (R^2) and T_{iso} temperature of analytes **1**–**5** on Lux Cellulose-1 column.

Analyte	Mobile phase	Stereoisomer	$-\Delta H^\circ$ (kJ mol^{-1})	$-\Delta S^{\circ *}$ (J mol^{-1} K^{-1})	Correlation coefficients (R^2)	$-\Delta(\Delta H^\circ)$ (kJ mol^{-1})	$-\Delta(\Delta S^\circ)$ (J mol^{-1} K^{-1})	$-\Delta(\Delta G^\circ)_{298K}$ (kJ mol^{-1})	T_{iso} (°C)
1	**a**	**b**(1)	14.0	48.2	0.9955	3.0	8.7	0.5	80
		a(2)	17.0	56.9	0.9991				
	a	**c**(1)	14.1	47.2	0.9961	1.0	1.6	0.5	373
		d(2)	15.1	48.8	0.9966				
2	**a**	**a**(1)	15.6	48.1	0.9911	4.3	11.0	1.0	121
		b(2)	20.0	59.1	0.9904				
	a	**c**(1)[a]	12.6[a]	41.8[a]	0.9970	2.0[a]/−2.0[b]	6.5[a]/−6.5[b]	>0.2[a]$_{(273K)}$ 0.1[b]$_{(323K)}$	38
		d(2)[a]	14.6[a]	48.3[a]	0.9997				
	b	**c**(1)[a]	8.1[a]	20.8[a]	0.9908	3.7[a]/−3.7[b]	12.7[a]/−12.7[b]	>0.2[a]$_{(273K)}$ 0.4[b]$_{(323K)}$	22
		d(2)[a]	11.8[a]	33.5[a]	0.9965				
3	**a**	**b**(1)	11.4	42.8	0.9997	1.6	4.0	0.4	117
		a(2)	13.0	46.8	0.9995				
	a	**c**(1)	13.8	45.8	0.9988	–	–	–	–
		d(2)	13.8	45.8	0.9988				
4	**a**	**b**(1)	10.0	25.4	0.9980	4.7	10.8	1.5	160
		a(2)	14.7	36.3	0.9987				
	a	**c**(1)	13.8	34.3	0.9903	2.8	7.2	0.7	114
		d(2)	16.6	41.5	0.9964				
5	**a**	**b**(1)	11.0	22.0	0.9947	1.5	4.1	0.3	93
		a(2)	12.5	26.1	0.9963				
	a	**d**(1)	12.0	24.7	0.9983	3.2	6.1	1.4	244
		c(2)	15.1	30.8	0.9984				

Chromatographic conditions: column, Lux Cellulose-1; mobile phase, **a**, *n*-heptane/IPA/DEA = 90/10/0.1 (v/v/v), **b**, *n*-heptane/IPA/DEA = 98/2/0.1 (v/v/v); flow rate, 0.5 ml min^{-1}; detection, 230 nm; $\Delta S^{\circ *} = \Delta S^\circ + R \ln \Phi$, where Φ is the phase ratio; R^2, correlation coefficient of van't Hoff plot, $\ln k - 1/T$ curves; T_{iso}, temperature of $\ln k - 1/T$ curves where enantioselectivity cancels; (1) and (2) first and second eluted enantiomer.

[a] Below T_{iso} temperature.
[b] Above T_{iso} temperature.

4. Conclusions

Normal-phase HPLC methods were developed for the separation of the stereoisomers of five 1-(phenylethylamino)- or 1-(naphthylethylamino)methyl-2-naphthol analogs, using polysaccharide-based CSPs: cellulose *tris*-(3,5-dimethylphenyl) carbamate (Lux Cellulose-1), cellulose *tris*-(3-chloro-4-methylphenyl) carbamate (Lux Cellulose-2) and amylose *tris*-(5-chloro-2-methylphenyl) carbamate (Lux Amylose-2). The chromatographic parameters depended on the mobile phase composition, the nature and concentration of the alcoholic modifier and temperature. A temperature-induced inversion of the elution sequence was observed. Baseline resolution was achieved in all cases; the three newly commercialized CSPs have a complementary character which leads to successful resolution. The elution sequence was determined in all cases, but no general predictive rule could be found to describe the elution behavior. In most cases, the elution sequences on Lux Cellulose-1 (or Lux Cellulose-2) and

Lux Amylose-2 were inverse, which is advantageous from the aspect of chiral separation.

Acknowledgments

The authors gratefully acknowledge support for this work through Hungarian National Science Foundation grant OTKA K 67563. A.P. is grateful to Gen-Lab Kft (Budapest, Hungary) and Phenomenex (Torrance, CA, USA) for the generous gift of the Lux Cellulose and Lux Amylose columns.

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