High-Performance Liquid Chromatographic Enantioseparation of Betti Base Analogs on a Newly Developed Isopropyl Carbamate-Cyclofructan6-Based Chiral Stationary Phase

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ABSTRACT The direct separation of the enantiomers of 1-(α-aminoarylmethyl)-2-naphthol, 1-(α-aminoalkyl)-2-naphthol, 2-(α-aminoarylmethyl)-1-naphthol analogs, and 2-(1-amino-2-methylpropyl)-1-naphthol) was performed on a newly developed chiral stationary phase containing isopropyl carbamate-cyclofructan6 as chiral selector, with *n*-heptane/alcohol/trifluoroacetic acid as mobile phase. The effects of the mobile-phase composition, the nature and concentration of the alcoholic and acidic modifiers, and the structures of the analytes on the retention and resolution were investigated. In some cases, separations were carried out at constant mobile-phase compositions in the temperature range 5–40°C. Thermodynamic parameters and T_{iso} values were calculated from plots of ln k' or ln α versus $1/T$. $-\Delta(\Delta H^{\circ})$ ranged from 2.8 to 3.2 kJ mol^{-1}, $-\Delta(\Delta S^{\circ})$ from 7.7 to 10.1 J mol^{-1} K^{-1}, and $-\Delta(\Delta G^{\circ})$ from 0.2 to 0.5 kJ mol^{-1}. It was found that the enantioseparations were enthalpy driven. The sequence of elution of the stereoisomers determined in some cases was (*R*) < (*S*). *Chirality 23:549–556, 2011.*

KEY WORDS: column liquid chromatography; enantiomer separation; 1-(α-aminoarylmethyl)-2-naphthol analogs; 1-(α-aminoalkyl)-2-naphthol analogs; 2-(α-aminoarylmethyl)-1-naphthol analogs; cyclofructan6; isopropyl carbamate

INTRODUCTION

The Mannich reaction is one of the most frequently applied three-component reactions in organic chemistry.[1,2] In a modified Mannich reaction 100 years ago, Betti achieved the straightforward synthesis of 1,3-diphenylnaphthoxazine in methanol. Acidic hydrolysis of the ring compound produced led to 1-aminobenzyl-2-naphthol. The synthesized aminonaphthol became known in the literature as the Betti base, and the protocol as the Betti reaction.[3,4]

Naphthoxazinone derivatives have received considerable attention because of the interesting pharmacological properties associated with this heterocyclic scaffold.[5–7] Screening of a virtual database indicated that the *N*-containing analogs of aminonaphthols, such as 7-[anilino(phenyl)methyl]-2-methyl-8-quinolinol, are a promising new classes of nonpeptide inhibitors of the MDM2-p53 interaction.[8]

The chemical and biological importance of Betti base enantiomers demands precise methods of separation. The resolution of enantiomers may involve classical diastereomeric salt formation, use of chiral auxiliaries, enzymatic resolution, high-performance liquid chromatographic (HPLC) methods, etc. Aromatic- and aliphatic-substituted 1- and 2-naphthol analogs were separated by HPLC on cellulose-based chiral stationary phases (CSPs) by Sztojkov-Ivanov et al.[9,10] and Ilisz et al.[11] and by capillary electrophoresis with the application of cyclodextrins and chiral crown ethers,[12] whereas Berkecz et al.[13] used a β-cyclodextrin-based CSP.

In this study, the use of a newly developed cyclofructan (CF)-based chiral selector is examined. The CFs consist of six or more (usually seven or eight) β-(2→1)-linked D-fructofuranose units.[14–16] Common abbreviations for these compounds are CF6, CF7, CF8, etc. These selectors are members of the macrocyclic oligosaccharides like the cyclodextrins, which are perhaps the best known members of this class. CF6, with six D-fructofuranose units, contains an 18-crown-6 ether core (Fig. 1). The 18-crown-6 ring serves as the skeleton core of CF6, with six fructofuranose units attached on its rim. The fructofuranose units are alternatively pointing toward and away from the molecular center, which are described as "inward inclined" and "outward inclined.[16]" It was recently reported that derivatized-CF-bonded CSPs permitted effective enantiomeric separations for a variety of compounds and especially for chiral molecules containing a primary amine functional group.[17,18] Such columns can be used in normal-phase and polar-organic modes.

Enantioselective retention and separation are influenced by temperature.[19–28] To investigate the thermodynamic functions of enantioselective adsorption, van't Hoff plots were constructed, which may be interpreted in terms of the mechanistic aspects of chiral recognition:

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

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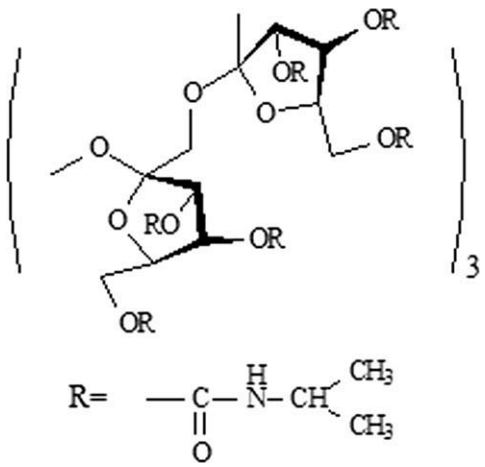


Fig. 1. Molecular structure of the isopropyl-carbamoylated cyclofructan6 chiral selector of the IP-CF6 column.

in which k' is the retention 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. The corresponding $\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$ values for the separated enantiomers can be determined from a modification of eq. 1:

$$\ln \alpha = -\frac{\Delta(\Delta H^o)}{RT} + \frac{\Delta(\Delta S^o)}{R}, \quad (2)$$

where α is the selectivity factor ($\alpha = k_2'/k_1'$). If $\Delta(\Delta H^\circ)$ is invariant with temperature (i.e., a linear van't Hoff plot is obtained), this expression shows that a plot of $R \ln \alpha$ versus $1/T$ has a slope of $-\Delta(\Delta H^\circ)$ and an intercept of $\Delta(\Delta S^\circ)$.

This article describes direct normal-phase HPLC methods for the enantioseparation of racemic Betti base analogs (for the structures, see Fig. 2) on a newly developed isopropyl carbamate-cyclofructan6 (IP-CF6)-based CSP (Fig. 1). The effects of the mobile-phase composition, the nature and concentration of the alcoholic and acidic modifiers, the specific structural features of the selector and analytes (aryl or alkyl substituents), and temperature on the retention are discussed on the basis of the experimental data. The elution sequence was determined in some cases and was compared with the results obtained on polysaccharide-based CSPs.

EXPERIMENTAL

Chemicals and Reagents

The following Betti base analogs were prepared by the aminoalkylation of 2-naphthol with benzaldehyde or other aromatic aldehydes in the presence of NH_3[29] (in some cases, NH_3/MeOH was replaced by ammonium carbamate or ammonium hydrogen carbonate as solid ammonia sources[30]) to obtain 1,3-diaryl-2,3-dihydro-1*H*-naphth[1,2-*e*][1,3]oxazines: **1A–1K** (**1A**, 1-(α-aminobenzyl)-2-naphthol; **1B**, 1-[amino-(4-methylphenyl)methyl]-2-naphthol; **1C**, 1-[amino-(4-methoxyphenyl)methyl]-2-naphthol; **1D**, 1-[amino-(4-fluorophenyl)methyl]-2-naphthol; **1E**, 1-[amino-(4-chlorophenyl)methyl]-2-naphthol; **1F**, 1-[amino-(4-bromophenyl)methyl]-2-naphthol; **1G**, 1-[amino-(4-nitrophenyl)methyl]-2-naphthol; **1H**, 1-[amino-(3-bromophenyl)methyl]-2-naphthol; **1I**, 1-[amino-(3-nitrophenyl)methyl]-2-naphthol; **1J**, 1-[amino-(2-thienyl)methyl]-2-naphthol;

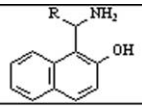
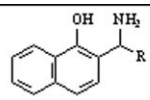
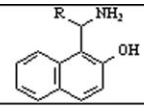
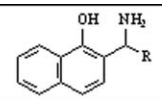
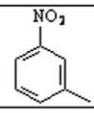
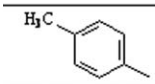
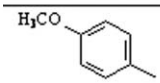
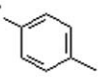
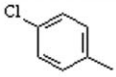
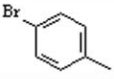
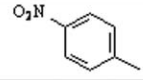
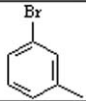
R substituent			R substituent		
	**1A**	**2A**		**1I**	**2I**
	**1B**	**2B**		**1J**	**2J**
	**1C**	**2C**		**1K**	**2K**
	**1D**	**2D**	—CH_3	**1L**	–
	**1E**	**2E**	—CH_2CH_3	**1M**	–
	**1F**	**2F**	—$CH_2CH_2CH_3$	**1N**	–
	**1G**	–		**1O**	**2O**
	**1H**	**2H**		**1P**	–

Fig. 2. Structures of 1-(α-aminoarylmethyl)-2-naphthol, 1-(α-aminoalkyl)-2-naphthol, 2-(α-aminoarylmethyl)-1-naphthol analogs, and 2-(1-amino-2-methylpropyl)-1-naphthol (**2O**).

and **1K**, 1-[amino-(3-thienyl)methyl]-2-naphthol) (Fig. 2). Subsequent acidic hydrolysis gave the desired aminonaphthols. 1-(α-Aminoalkyl)-2-naphthol analogs **1L–1P** (**1L**, 1-(1-aminoethyl)-2-naphthol; **1M**, 1-(1-aminopropyl)-2-naphthol; **1N**, 1-(1-aminobutyl)-2-naphthol; **1O**, 1-(1-amino-2-methylpropyl)-2-naphthol; **1P**, 1-(1-amino-2,2-dimethylpropyl)-2-naphthol; and **2O**, 2-(1-amino-2-methylpropyl)-1-naphthol) were prepared via the reactions of aliphatic aldehydes with 2-naphthol or 1-naphthol in the presence of methanolic NH_3 solution in methanol (MeOH) at 60°C or of ammonium carbamate, yielding dialkylnaphthoxazines. Acidic hydrolysis of these naphthoxazines led to the desired aminonaphthol hydrochlorides (Fig. 2).[30,31]

Syntheses and assignation of absolute configurations of enantiomers of **1A** and **1O** were carried out according to the literature methods. (*S*)-**1A** (ee 97%) was obtained according to the literature protocol, starting from the racemic compound.[32] For the synthesis of (*R*)-**1O**, 1-[(1*R*)-2-methyl-1-{[(1′*R*)-1′-(1-naphthyl)ethyl]-amino}propyl]-2-naphthol was prepared by the literature process.[10,33] This product was then hydrogenated (Pd/C) resulting in (*R*)-**1O**.

2-(α-Aminoarylmethyl)-1-naphthols **2A–F** and **2H–K** (**2A**, 2-(α-aminobenzyl)-1-naphthol; **2B**, 2-[amino-(4-methylphenyl)methyl]-1-naphthol; **2C**, 2-[amino-(4-methoxyphenyl)-methyl]-1-naphthol; **2D**, 2-[amino-(4-fluorophenyl)methyl]-1-naphthol; **2E**, 2-[amino-(4-chlorophenyl)methyl]-1-naphthol; **2F**, 2-[amino-(4-bromophenyl)methyl]-1-naphthol; **2H**, 2-[amino-(3-bromophenyl)methyl]-1-naphthol; **2I**, 2-[amino-(3-nitrophenyl)-methyl]-1-naphthol; **2J**, 2-[amino-(2-thienyl)methyl]-1-naphthol; and **2K**, 2-[amino-(3-thienyl)methyl]-1-naphthol) (Fig. 2) were prepared in a manner similar to that for their regioisomeric 1-(α-aminoarylmethyl)-2-naphthol counterparts.[30,34]

n-Heptane, 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), as were trifluoroacetic acid (TFA), glacial acetic acid (AcOH), and other reagents of analytical reagent grade.

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.

Apparatus and Chromatography

The HPLC measurements were carried out on a Waters Breeze system consisting of a 1525 binary pump, a 487 dual-channel absorbance detector, a 717 plus autosampler, and Breeze data manager software (Waters Chromatography, Milford, MA). The chromatographic system was equipped with a Rheodyne Model 7125 injector (Cotati, CA) with a 20-μl loop.

The column used for direct separations was IP-CF6, 250 mm × 4.6 mm ID, with a particle size of 5 μm (University of Arlington at Texas, TX). The dead time (t_0) of the column was determined by injecting tri-*t*-butyl benzene. The columns were thermostated in a Spark Mistral column thermostat (Spark Holland, Emmen, The Netherlands). The precision of the temperature adjustment was ±0.1°C.

RESULTS AND DISCUSSION

The analytes examined in this study can be classified into two groups (Fig. 2), i.e., 2-naphthol analogs and 1-naphthol analogs, differing in the steric arrangement of the substituted aromatic rings (or aliphatic chains) around the stereogenic center. All compounds in Tables 1 and 2 were evaluated by using minimum of three of the following mobile phases: *n*-heptane/IPA/TFA (65/35/0.1 v/v/v), (70/30/0.1 v/v/v), (75/25/0.1 v/v/v), (80/20/0.1 v/v/v), (90/10/0.1 v/v/v), and (90/10/0.3 v/v/v) and *n*-heptane/EtOH/TFA (90/10/0.1 v/v/v). To simplify the presentation, the chromatographic results for *n*-heptane/IPA/TFA (75/25/0.1 v/v/v) as mobile phase are given for all analytes (Tables 1 and 2). With this eluent, the stereoisomers of all analytes were retained, and a partial or baseline enantiomeric separation was achieved (only exception was **1O**). A small percentage of TFA was applied to protonate the amino groups. The 18-membered crown ethers fit well a protonated primary amino group.[35]

TABLE 1. Chromatographic data, retention factors (k'), separation factors (α), and resolutions (R_S) for the separation of the stereoisomers of 1-(α-aminobenzyl)-2-naphthol and 1-(α-aminoalkyl)-2-naphthol analogs on IP-CF6 CSP

Analyte	Mobile phase: *n*-Heptane/alcohol/TFA (v/v/v)	k_1'	k_2'	α	R_S
1A	65/35/0.1[a]	2.11	2.11	1.00	0.00
	70/30/0.1[a]	3.35	3.65	1.09	0.65
	75/25/0.1[a]	3.64	3.98	1.09	1.40
1B	65/35/0.1[a]	2.28	2.52	1.10	1.15
	70/30/0.1[a]	3.39	3.84	1.13	1.20
	75/25/0.1[a]	4.01	4.57	1.14	1.90
1C	75/25/0.1[a]	5.27	5.96	1.13	1.15
1D	75/25/0.1[a]	4.55	4.81	1.06	1.05
1E	65/35/0.1[a]	2.25	2.43	1.08	0.90
	70/30/0.1[a]	4.14	4.57	1.10	1.00
	70/30/0.3[a]	4.48	4.90	1.10	1.55
	75/25/0.1[a]	4.35	4.81	1.11	1.45
1F	75/25/0.1[a]	4.41	4.96	1.12	1.65
1G	75/25/0.1[a]	7.05	7.30	1.04	0.45
	90/10/0.1[a]	42.14	45.05	1.07	1.70
1H	75/25/0.1[a]	3.60	4.14	1.15	1.65
1I	75/25/0.1[a]	6.99	7.52	1.08	0.95
	90/10/0.1[a]	37.07	40.90	1.10	1.50
	90/10/0.1[b]	31.82	34.61	1.09	1.45
1J	75/25/0.1[a]	5.98	6.10	1.02	0.20
	90/10/0.1[a]	24.93	25.67	1.03	0.35
	90/10/0.1[b]	22.60	23.19	1.03	0.50
1K	75/25/0.1[a]	5.59	5.78	1.03	0.35
	90/10/0.1[a]	20.52	21.34	1.04	0.40
	90/10/0.1[b]	18.84	19.62	1.04	0.85
1L	65/35/0.1[a]	3.11	3.49	1.12	2.00
	70/30/0.1[a]	6.62	7.94	1.20	2.05
	75/25/0.1[a]	7.29	8.86	1.22	2.15
1M	75/25/0.1[a]	5.95	7.19	1.21	2.35
1N	75/25/0.1[a]	5.09	6.00	1.18	1.80
1O	75/25/0.1[a]	4.40	4.40	1.00	0.00
	90/10/0.1[b]	14.46	15.08	1.04	0.70
1P	75/25/0.1[a]	3.09	3.46	1.12	1.55

Chromatographic conditions: column, IP-CF6; flow rate, 0.5 ml min^{-1}; detection, 215 nm; temperature, ambient; and t_0, 6.57 min.
[a]Mobile phase, *n*-heptane/IPA/TFA (v/v/v).
[b]Mobile phase, *n*-heptane/EtOH/TFA (v/v/v).

For all analytes, dependence of chromatographic data on alcohol concentration was measured minimum at three mobile-phase compositions, but to reduce the number of data, results only for analytes **1A**, **2A**, **1B**, **2B**, **1E**, **2E**, and **1L** are presented in Tables 1 and 2. In all cases, k' increased with decreasing alcohol content, which is typical normal-phase behavior. The concentration of the alcohol in most cases exerted a slight effect on the enantioselectivity; a slight increase in α was registered with decreasing IPA content, i.e., the ratio of the nonchiral and chiral interactions between the CSP and the analytes depended only slightly on the concentration of the alcohol and R_S decreased with decreasing k'.

Comparison of the separation performances of the two groups of analytes containing the same substituents revealed that, at constant *n*-heptane/IPA/TFA = 75/25/0.1 (v/v/v)

TABLE 2. Chromatographic data, retention factors (k'), separation factors (α), and resolutions (R_S) for the separation of the stereoisomers of 2-(α-aminobenzyl)-1-naphthol analogs on IP-CF6 CSP

Analyte	Mobile phase: *n*-Heptane/alcohol/TFA (v/v/v)	k_1'	k_2'	α	R_S
2A	65/35/0.1[a]	2.13	2.13	1.00	0.00
	70/30/0.1[a]	4.35	4.90	1.12	1.20
	70/30/0.3[a]	4.42	4.87	1.10	1.80
	75/25/0.1[a]	5.26	5.88	1.12	1.45
	80/20/0.1[a]	6.03	6.81	1.13	1.50
2B	65/35/0.1[a]	2.20	2.47	1.12	2.05
	70/30/0.1[a]	4.01	4.62	1.15	2.15
	75/25/0.1[a]	4.29	4.96	1.16	2.25
2C	75/25/0.1[a]	7.63	8.06	1.06	0.55
	90/10/0.1[a]	23.73	25.62	1.09	0.75
	90/10/0.3[a]	27.19	29.36	1.08	0.80
2D	75/25/0.1[a]	5.52	5.97	1.08	1.20
	75/25/0.1[a,b]	7.06	8.00	1.13	1.50
2E	65/35/0.1[a]	2.59	2.87	1.11	1.20
	70/30/0.1[a]	4.70	5.17	1.11	1.25
	75/25/0.1[a]	5.90	6.61	1.12	1.55
2F	75/25/0.1[a]	5.83	6.80	1.17	2.10
2H	75/25/0.1[a]	4.50	5.50	1.22	3.05
2I	75/25/0.1[a]	9.55	10.32	1.08	1.10
	80/20/0.1[a]	12.34	13.45	1.09	1.15
2J	75/25/0.1[a]	5.78	6.40	1.11	1.25
	75/25/0.1[a,b]	6.64	7.89	1.19	1.70
2K	75/25/0.1[a]	5.79	6.34	1.09	1.45
	90/10/0.1[a]	22.15	24.58	1.11	1.55
2O	75/25/0.1[a]	4.45	4.74	1.06	0.70
	90/10/0.1[a]	13.34	14.27	1.07	0.75
	90/10/0.3[a]	15.38	16.07	1.04	1.05

Chromatographic conditions: column, IP-CF6; flow rate, 0.5 ml min^{-1}; detection, 215 nm; temperature, ambient; and t_0, 6.57 min.
[a]Mobile phase, *n*-heptane/IPA/TFA (v/v/v).
[b]Temperature, 10°C.

eluent composition, similar or slightly larger k', α, and R_S were observed for the 1-naphthol analogs than for the 2-naphthol analogs (exceptions were **2C** and **2D**). The steric arrangement of the aromatic side chain in the 1-naphthol derivatives probably favors interactions with the selector, and therefore, k', α, and R_S were larger. The nature of the alcohol greatly influenced the retention and separation. To investigate the effects of the nature of the alcohol, analytes **1A**, **2A**, **1B**, **2B**, **1E**, **2E**, and **1L**, containing phenyl, an electron-donating methyl group (4-methylphenyl), an electron-withdrawing chloro group (4-chlorophenyl), and methyl substituents, were selected. The application of five alcohols (EtOH, PrOH, IPA, BuOH, and *t*-BuOH) revealed that in the *n*-heptane/alcohol/TFA mobile-phase system containing a constant concentration of alcohol (3.25 M), k_1' was larger for the 1-naphthol analogs than for the 2-naphthol analogs (an exception was *t*-BuOH, Fig. 3). For both the 1-naphthol and 2-naphthol analogs, EtOH, IPA, and *t*-BuOH resulted in greater retention. Wang and Wenslow[36] found that the changes caused in the CSP structure by the different alcohols may affect the retention and chiral selectivity of the CSP, depending on the size and structure of the analyte.

As concerns enantioselectivity and resolution for the 2-naphthol analogs, EtOH, IPA, and *t*-BuOH exhibited some separation ability, whereas for the 1-naphthol analogs IPA and *t*-BuOH did so; α varied between 1.02 and 1.15. The enantiomers of **1L** were practically baseline resolved (α = 1.18–1.28) in all *n*-heptane/alcohol/TFA systems with the exception of the application of BuOH. With the application of IPA or *t*-BuOH as alcoholic modifier, which contain a branched side chain, α was usually and R_S was always larger than in the cases of PrOH and BuOH, where the carbon chains are straight. The alcohols with longer, branched and bulky side chains, such as IPA and *t*-BuOH, generally promoted the chiral interactions between the analyte and the CSP, and higher α and R_S could be observed. The most efficient alcoholic modifiers for the enantioseparation of the Betti base analogs on the IP-CF6 CSP were *t*-BuOH and especially IPA; in most of the experiments, therefore, IPA was applied.

The effects of the nature and content of the acidic modifier on the separation were investigated for analytes **1A**, **1B**, **1E**, and **1L** and **2A**, **2B**, **2E**, and **2O** in a mobile-phase system of *n*-heptane/IPA/acidic modifier = 75/25/0.05–0.3 (v/v/v). A comparison of the chromatographic data revealed that TFA was more suitable than AcOH as the acidic modifier at a constant concentration of 0.30% in terms of retention, enantioselectivity, and resolution. Increase of the TFA content from 0.05 to 0.3% in the mobile-phase system *n*-heptane/IPA/TFA = 75/25/0.05–0.3 (v/v/v) resulted in a small decrease in the retention factor, probably because of the increased ionic or polar–polar interaction between the analytes and the mobile phase.

At constant mobile-phase composition, in spite of the similar retention factors, methyl (Me) substitution on the aromatic ring (**1B** vs. **1A** and **2B** vs. **2A**) led to greater selectivity and resolution, probably because of the steric effects (Tables 1 and 2). The presence of the MeO functional group (**1C** and **2C**) resulted in larger k' values, as a result of nonenantioselective H-bonding interactions, but the selectivity (α) and R_S were lower when compared with those for **1A** and **2A**. Halogen substitution on the aromatic ring increased the bulkiness and steric rigidity of the analytes. The higher polar and steric effects of the halogen-substituted naphthol analogs (**D**, **E**, **F**, and **H**) cause an increase in k_1' and k_2' values compared to the nonsubstituted analogs (with the exception of **H**), and α and R_S were also slightly larger than for **1A** and **2A** (exceptions were **1D** and **2D**). Despite the slight deviation in the tendencies in k' for **D**, **E**, and **F**, the α and R_S values increased slightly in the sequence: fluorine–chlorine–bromine, indicating the importance of the steric and polar effects in chiral discrimination. The retention factors of **1G**, **1I**, and **2I**, containing a nitro group, were the highest among both the 1-(α-aminoarylmethyl)-2-naphthol and the 2-(α-aminoarylmethyl)-1-naphthol analogs, but these large retention factors were not associated with enhanced selectivity.

Conformationally more constrained **H** and **I** analogs were better separated than the less constrained **F** and **G** analogs, respectively. Of the heteroaryl-substituted analogs (**J** and **K**) larger selectivity and resolution were obtained for 1-naphthol analogs than for 2-naphthol ones.

Comparison of the chromatographic behavior of the 2-naphthol analogs containing different alkyl substituents (**1L**-**1P**) at constant mobile-phase composition revealed that the retention factors were larger than those for the 2-naphthol

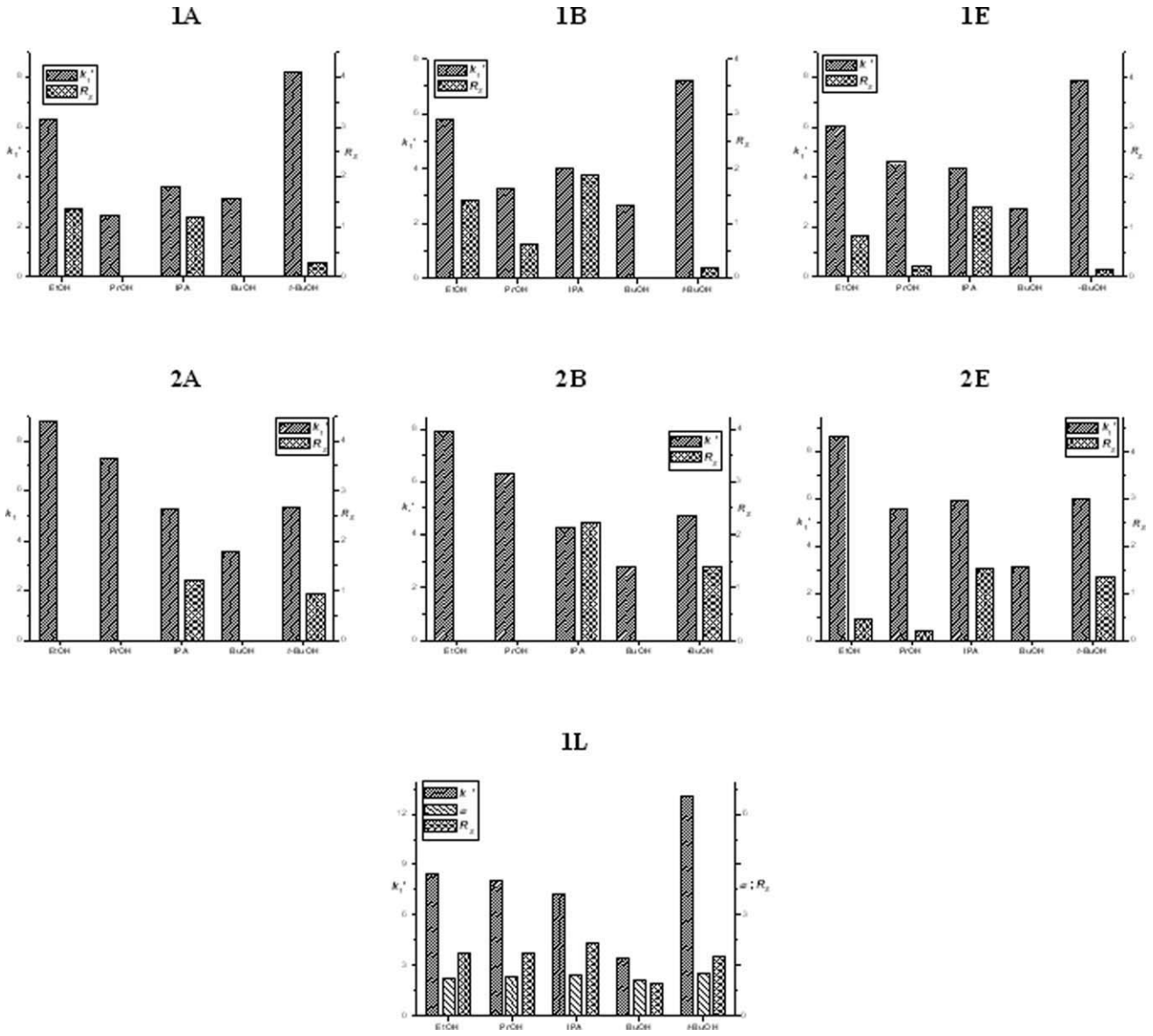


Fig. 3. Effects of the nature of the alcoholic modifier on the retention factor of the first-eluting enantiomer (k_1'), resolution (R_S), and separation factor (α). Chromatographic conditions: column, IP-CF6; mobile phase, *n*-heptane/alcoholic modifier/TFA; alcoholic modifiers, MeOH, EtOH, PrOH, IPA, BuOH, and *t*-BuOH; concentration of alcoholic modifier in *n*-heptane/alcoholic modifier/TFA system, 3.25 M; flow rate, 0.5 ml min^{-1}; and detection, 215 nm.

analogs containing a phenyl ring (**1A**) (the only exception was **1P**) (Table 1). The 2-naphthol analogs containing different alkyl substituents affected the selectivity and resolution to a greater extent. Analytes with a larger or more bulky alkyl chain exhibited smaller retention factors and also smaller α and R_S values (the enantiomers of **1O** were not separable in IPA-containing mobile phase).

To determine the specific structural effects of the alkyl substituents on the chromatographic data, such as k', the Meyer parameters (V^a) of the alkyl groups were applied (Fig. 4). According to Meyer, the steric effects of substituents on reaction rates are characterized by the size descriptor V^a (Meyer parameter).[37] All the plots of V^a versus k' were fitted by straight lines with good correlation coefficients. Linear regression analysis revealed that the retention of the analytes depended strongly on the volume of the substituents. A bulkier substituent inhibited the interaction with the selector; at the same time, the interactions of the two stereoisomers with the selector differed appreciably. Despite the smaller k' values, α and R_S decreased slightly with increasing carbon number of the alkyl side chain (for **1P** somewhat larger decreases in α and R_S were observed). It may be stated that, besides the nature of the substituents (aromatic or aliphatic), steric effects strongly influenced the retention and chiral discrimination of the 1- and 2-naphthol analogs.

The elution sequence for **1A** and **1O** was found to be (*R*) < (*S*), which is reversal compared to results obtained earlier on polysaccharide-based CSPs.[9–11] Selected chromatograms for Betti base analogs are depicted in Figure 5.

Effects of Temperature and Thermodynamic Parameters

To investigate the effect of temperature on the chromatographic parameters, a variable-temperature study was carried out between 5 and 40°C for analytes **1A**, **1L**, and **2A**, which can be considered as base structures in the series of 1- and 2-naphthol analogs. At a mobile-phase composition of *n*-hep-

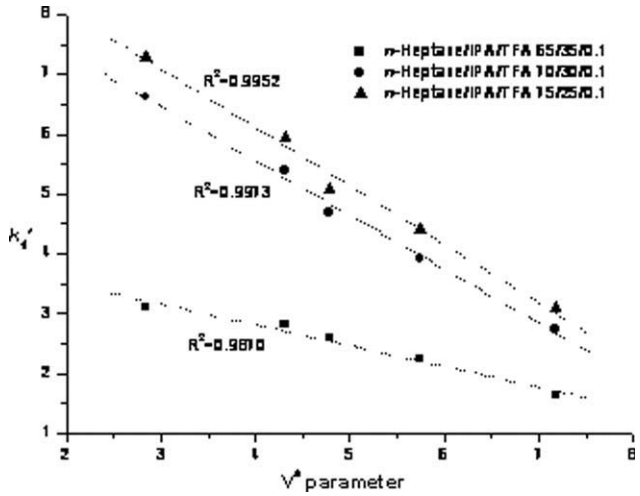


Fig. 4. Dependence of the retention factors of the first-eluting enantiomers (k_1') of 1-(aminoalkyl)-2-naphthol analogs on the Meyer substituent parameter (V^a). Chromatographic conditions: column, IP-CF6; mobile phase, *n*-heptane/IPA/TFA = 65/35/0.1, 70/30/0.1, and 75/25/0.1 (v/v/v); flow rate, 0.5 ml min^{-1}; and detection, 215 nm.

TABLE 3. Retention factor of first-eluting enantiomer (k_1'), separation factor (α), and resolution (R_S) of enantiomers analytes 1A, 1L, and 2A on IP-CF6 CSP as a function of temperature

		Temperature (°C)				
Analyte	k_1', α, R_S	5	10	20	30	40
1A	k_1'	5.16	4.40	3.71	3.18	2.73
	α	1.19	1.16	1.09	1.06	1.02
	R_S	1.49	1.13	0.95	0.63	<0.20
1L	k_1'	7.65	6.88	5.56	4.71	4.05
	α	1.31	1.30	1.24	1.19	1.15
	R_S	2.48	2.05	1.84	1.50	1.23
2A	k_1'	6.72	5.63	3.99	3.12	2.44
	α	1.21	1.20	1.16	1.10	1.06
	R_S	1.87	1.49	1.35	1.06	0.71

Chromatographic conditions: column, IP-CF6; mobile phase, *n*-heptane/IPA/TFA = 70/30/0.1 (v/v/v); flow rate, 0.5 ml min^{-1}; and detection, 215 nm.

tane/IPA/TFA = 70/30/0.1 (v/v/v), the chromatographic data were measured and calculated in 10°C increments over the temperature range studied (Table 3). A comparison of the chromatographic data in Table 3 reveals that the k_1', α, and R_S values decreased with increasing temperature. It is evident that an increase in separation temperature lowered the separation factor, α. The greatest fall in retention factor was for **2A**, but the decline in the values of separation factor and resolution was not so large.

The ΔH° and $\Delta S^{\circ *}$ values calculated from the slopes and intercepts of the plots of eq. 1 for the enantiomers were negative (Table 4). Further, the ΔH° and $\Delta S^{\circ *}$ values for the first-eluting enantiomer were always less negative than those for the second-eluting enantiomer. The slight difference in the $-\Delta H^\circ$ and $-\Delta S^{\circ *}$ values for **1A** and **1L** indicates that the 2-naphthol core probably determines the interactions with the CSP, whereas the largest $-\Delta H^\circ$ and $-\Delta S^{\circ *}$ values for **2A** can be attributed to the favored structure of this 1-naphthol analog promoting the steric/rigid or polar interactions with the CSP.

The data on $\Delta(\Delta H^\circ)$, $\Delta(\Delta S^\circ)$, and $\Delta(\Delta G^\circ)$ are presented in Table 4. The $-\Delta(\Delta H^\circ)$ and $-\Delta(\Delta S^\circ)$ values did not differ considerably for the three analogs, but were more negative for **1A**. 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. The thermodynamic parameter $-\Delta(\Delta G^\circ)_{298\ K}$ suggests that **1L** (containing a methyl group) induces highly efficient binding to the selector, as reflected by the large negative $\Delta(\Delta G^\circ)$ values.

From the data, the temperature, T_{iso}, at which the enantioselectivity balances out and the elution sequence changes was calculated (Table 4). T_{iso} was > 45°C, indicating that lower temperatures are preferable for the best separation of most of the analytes.

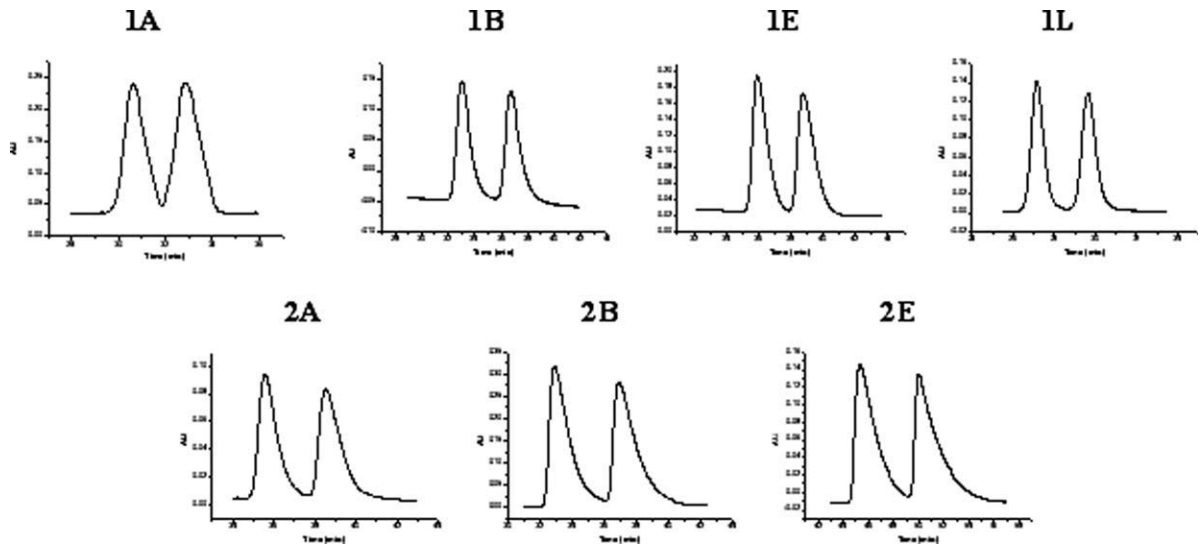


Fig. 5. Selected chromatograms of Betti base analogs. Chromatographic conditions: column, IP-CF6; mobile phase, *n*-heptane/IPA/TFA = 65/35/0.1 (v/v/v) for **1L**, 70/30/0.1 (v/v/v) for **2B**, 75/25/0.1 (v/v/v) for **1A**, **1B**, and **2E**, and 70/30/0.3 (v/v/v) for **1E** and **2A**; temperature, 25°C; flow rate, 0.5 ml min^{-1}; and detection, 215 nm.

TABLE 4. Thermodynamic parameters, ΔH°, ΔS°*, $\Delta(\Delta H^\circ)$, $\Delta(\Delta S^\circ)$, $\Delta(\Delta G^\circ)$, and correlation coefficients (R^2) of analytes 1A, 1L, and 2A on IP-CF6 CSP

Analyte	Stereoisomer	$-\Delta H^\circ$ (kJ mol^{-})	$-\Delta S^\circ$* (J mol^{-1} K^{-1})	Correlation coefficient (R^2)	$-\Delta(\Delta H^\circ)$ (kJ mol^{-1})	$-\Delta(\Delta S^\circ)$ (J mol^{-1} K^{-1})	$-\Delta(\Delta G^\circ)_{298\ K}$ (kJ mol^{-1})	T_{iso} (°C)
1A	1	12.4	30.4	0.9903	3.2	10.1	0.2	45
	2	15.6	40.5	0.9929				
1L	1	13.0	29.2	0.9977	2.8	7.7	0.5	87
	2	15.8	36.9	0.9982				
2A	1	20.5	57.3	0.9969	2.9	8.6	0.3	58
	2	23.4	65.9	0.9981				

Chromatographic conditions: column, IP-CF6; mobile phase, *n*-heptane/IPA/TFA = 70/30/0.1 (v/v/v); R^2, correlation coefficient of van't Hoff plot, ln $k - 1/T$ curves; flow rate, 0.5 ml min^{-1}; and detection, 215 nm.

In summary, the complex formation that involved multiple intermolecular interactions between the analyte and the CSP was generally exothermic, and the corresponding entropic contribution was also negative. As it is evident from the $\Delta(\Delta G^\circ)$ data, the separations for the investigated analytes on this CSP were enthalpically favored. However, the exact chiral recognition mechanism on IP-CF6 is not yet clear. Further investigations are needed to clarify the effects of acidic/alcoholic modifiers and interactions governing the chiral recognition.

CONCLUSIONS

The direct separations of 27 1- and 2-naphthol (Betti base) analogs were performed in normal-phase mode on a newly developed CSP containing IP-CF6 as chiral selector. Applying *n*-heptane/alcoholic modifier/acidic modifier mobile-phase system, IPA and *t*-BuOH as alcoholic modifier and TFA as acid seemed to be most effective in the enantioseparation. By variation of the nature and concentration of the alcoholic and acidic modifiers in most cases, baseline resolution was achieved for the majority of the investigated analytes. Probably because of the steric effect, the 1-naphthol and sterically more constrained analogs exhibited larger retention factors, separation factors, and resolutions. On the basis of thermodynamic data, the separation proved to be enthalpically favored. The elution sequence was reversal compared to results obtained on polysaccharide-based CSPs.

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