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High-performance liquid chromatographic enantioseparation of isopulegol-based ß-amino lactone and ß-amino amide analogs on polysaccharide-based chiral stationary phases focusing on the change of the enantiomer elution order

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ABSTRACT

The enantioselective separation of newly prepared, pharmacologically significant isopulegol-based ß-amino lactones and ß-amino amides has been studied by carrying out high-performance liquid chromatography on diverse amylose and cellulose *tris*-(phenylcarbamate)-based chiral stationary phases (CSPs) in *n*-hexane/alcohol/diethylamine or *n*-heptane/alcohol/ diethylamine mobile phase systems. For the elucidation of mechanistic details of the chiral recognition, seven polysaccharide-based CSPs were employed under normal-phase conditions. The effect of the nature of selector backbone (amylose or cellulose) and the position of substituents of the *tris*-(phenylcarbamate) moiety was evaluated. Due to the complex structure and solvation state of polysaccharide-based selectors and the resulting enantioselective interaction sites, the chromatographic conditions (*e.g.*, the nature and content of alcohol modifier) were found to exert a strong influence on the chiral recognition process, resulting in a particular elution order of the resolved enantiomers. Since no prediction can be made for the observed enantiomeric resolution, special attention has been paid to the identification of the elution sequences.

The comparison between the effectiveness of covalently immobilized and coated polysaccharide phases allows the conclusion that, in several cases, the application of coated phases can be more advantageous. However, in general, the immobilized phases may be preferred due to their increased robustness.

Thermodynamic parameters derived from the temperature-dependence of the selectivity revealed enthalpically-driven separations in most cases, but unusual temperature behavior was also observed.

1. Introduction

β-Amino acid derivatives such as β-amino lactones and β-amino amides have remarkable pharmacological importance. Lactones of natural β-amino acids, obtained from sesquiterpene-type α,β-unsaturated lactones, *e.g.*, alantolactone, isoalantolactone or ambrosin, possess significant biological activities, such as increasing the proportion of cells in the G2/M and S phase [1]. Their water-soluble derivatives, in turn, exhibit cytotoxic activity through a prodrug mechanism for different human cancer cell lines [2]. In addition, ring opening of β-amino lactones with different amines results in β-amino amides, which are well-known subunits of biologically important compounds, such as α-hydroxy-β-amino amide bestatin, a potent aminopeptidase B. Its usefulness in the treatment of cancer through its ability to enhance the cytotoxic activity of known antitumor agents was described in the literature [3]. β-Amino amides exhibit other important biological activities as well. For example, pinane-based β-amino amides and similar bicyclic, norbornene-based amides with *N*-heteroaryl substituents possess tyrosine kinase inhibitor properties or even antibiotic activity [4,5]. Sitagliptin, a novel antidiabetic drug (Januvia®) bearing

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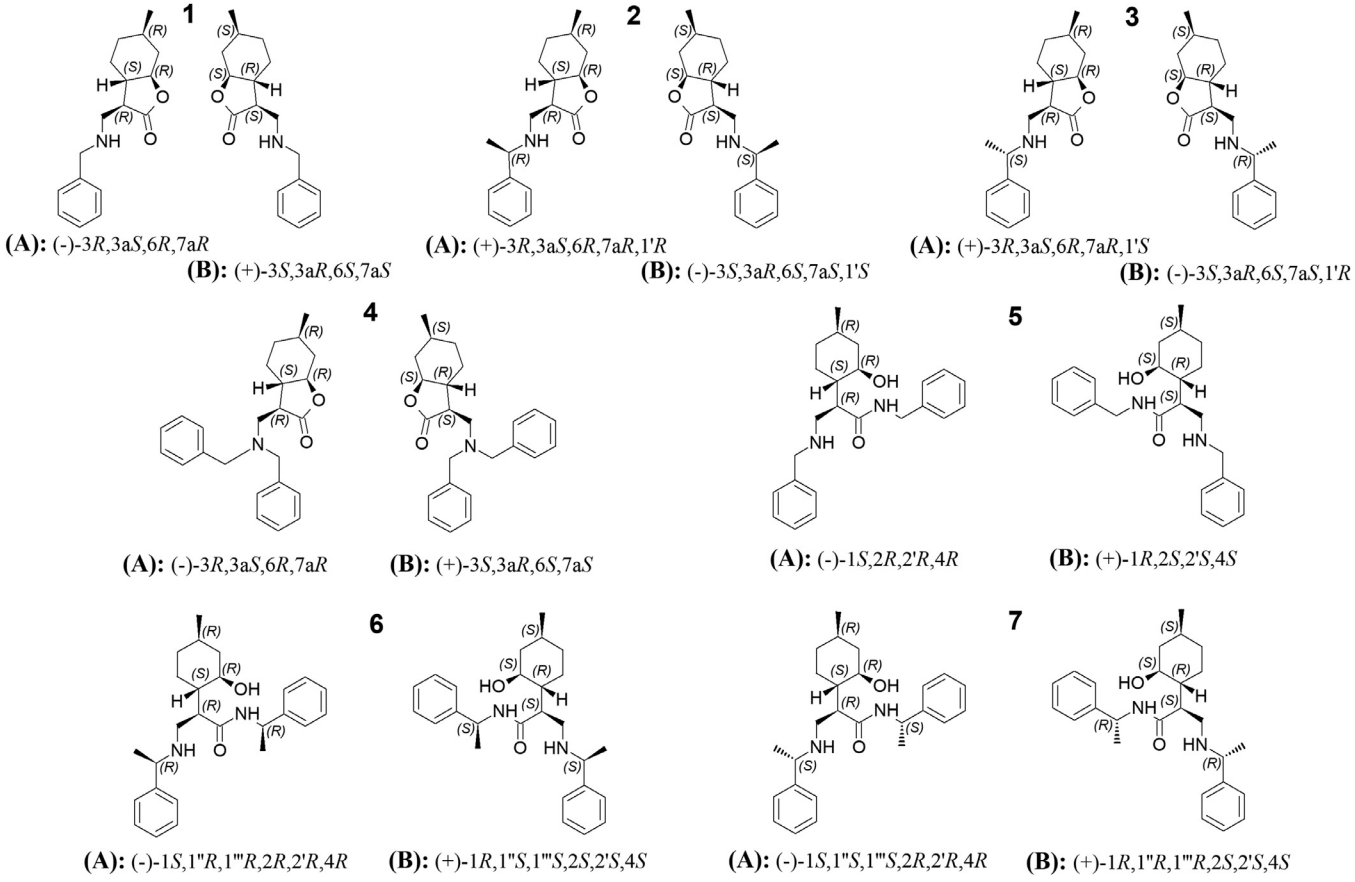

Fig. 1. Structure of isopulegol-based ß-amino lactones and ß-amino amides

a β-amino amide moiety, is a lead antidiabetic agent [6]. Furthermore, some hydroxyl-substituted β-amino amides have remarkable HIV protease or renin inhibitor activities [7]. The determination of enantiomeric and diastereoisomeric purity of β-amino lactones and hydroxyl-substituted β-amino amides is of high significance, because these synthons are excellent starting materials for the synthesis of other families of bioactive building blocks, including aminodiols (by reduction of amino lactones), diamino alcohols (by reduction of hydroxyl-substituted β-amino amides), and their heterocyclic derivatives.

There are several proposed chiral high-performance liquid chromatographic (HPLC) methods for assaying the stereoisomers of different α-, ß-, γ- and δ-lactones [8–12]. However, to the best of our knowledge, no data are available about the enantioseparation of ß-amino lactones. An achiral separation of ß-amino amides was performed by Paulsen *et al.* [13], while a few papers described the separation of ß-amino amide enantiomers [14–16]. It should be noted that enantioseparation of different lactones and amino amides were performed mostly on coated polysaccharide-based chiral stationary phases (CSPs) [8–10,14–16].

Polysaccharide-based selectors represent the most frequently applied CSPs for enantiomeric separations [17–20]. After the first report by Okamoto *et al.* [21], polysaccharide-based CSPs went through a very dynamic development. Chankvetadze *et al.* further extended the applicability of polysaccharide-based phases by incorporating halomethyl *N*-phenylcarbamate moieties to the cellulose and amylose chains [22–25]. Immobilization of amylose- or cellulose-based *tris*-(phenylcarbamate) selectors onto silica resulted in very robust CSPs [26–29], which were successfully applied, *e.g.*, for the enantioseparation of different lactones [11,12].

The main objective of the present paper is to reveal possible structure–separation relationships of the pharmacologically interesting ß-amino lactones and ß-amino amides. Our interest is based on the information that, to the best of our knowledge, no separation has been reported for ß-amino lactone enantiomers so far, and only a few cases were described for the enantiorecognition of ß-amino amides. Investigations were carried out on amylose- and cellulose-based *tris*-(phenylcarbamate)-type CSPs, due to their wide applicability and robust behavior described often in the literature. The study focused on exploring various effects observed with the variation of mobile phase composition, the nature and concentration of the alcohol modifier, the structure of chiral selectors and analytes, and the temperature on retention, selectivity, and resolution of stereoisomers. Elution sequences were determined in all cases.

2. Materials and methods

2.1. Chemicals and reagents

β-Amino lactones (−)-1, (+)-2, (+)-3, and (−)-4 as well as β-amino amides (−)-5, (+)-6, and (−)-7 were prepared from (−)-isopulegol according to a method described earlier. All physical and chemical properties of these compounds were identical with those reported therein [30]. (−)-Isopulegol, purchased from Merck (Darmstadt, Germany), was applied as starting material to prepare key intermediate (+)-α-methylene-γ-butyrolactone with a regioselective hydroxylation, followed by two-step oxidation and ring closure. Michael addition of primary and secondary amines towards lactones afforded β-amino lactones in a highly stereose-

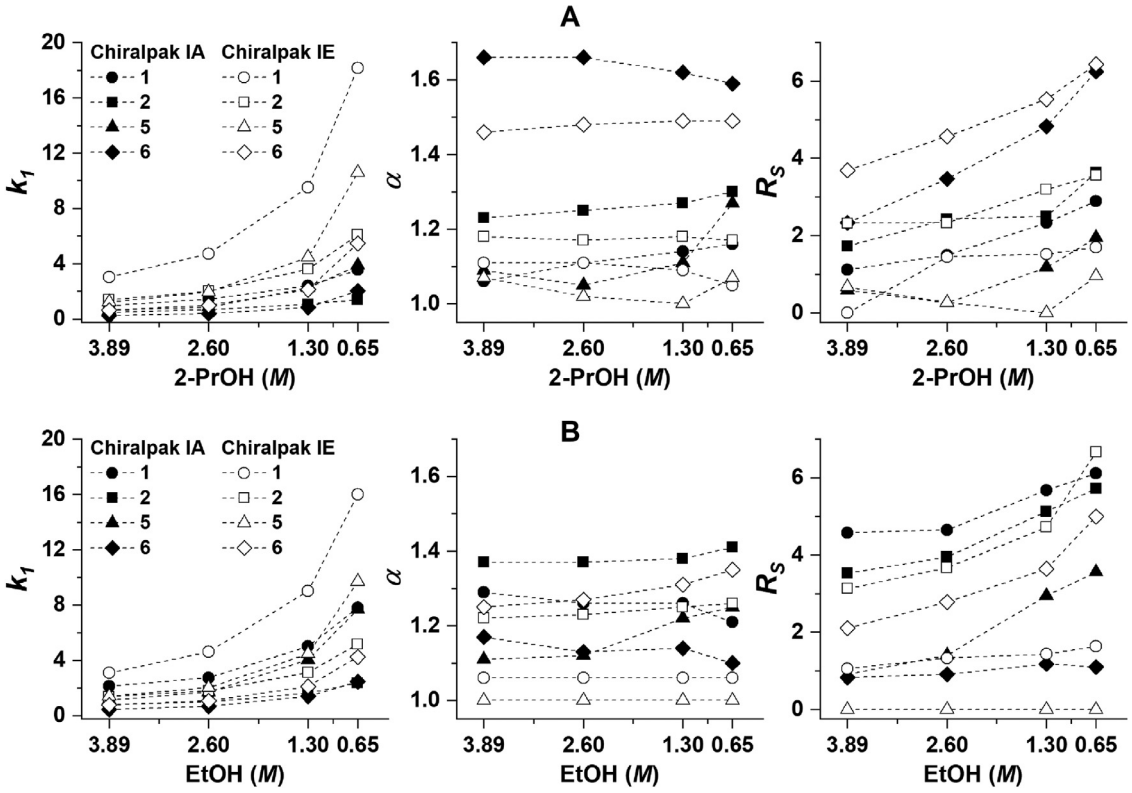

Fig. 2. Effect of mobile phase composition on chromatographic parameters, retention factor (k), separation factor (α) and resolution (R_S) for the separation of analytes 2 and 6 on Chiralpak IA and IE columns Chromatographic conditions: columns, Chiralpak IA, and Chiralpak IE; mobile phase, A, *n*-hexane/2-PrOH/DEA, B, *n*-hexane/EtOH/DEA all containing 20 mM DEA; the concentration of alcohols: 3.893, 2.596, 1.298 and 0.649 M; flow rate 1.0 ml min^{-1}; detection at 220 nm; temperature, 25 °C.

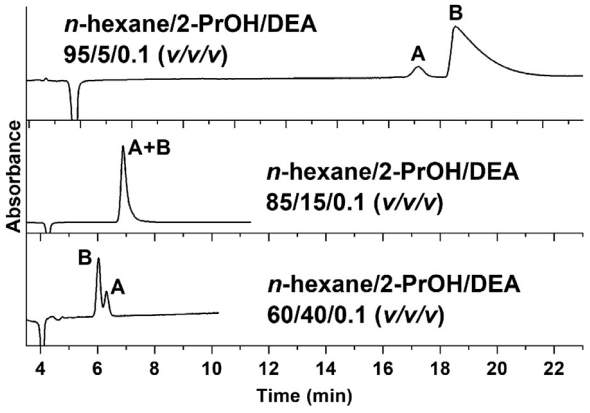

Fig. 3. Effect of mobile phase composition on the elution order of the enantiomers of analyte 5 Chromatographic conditions: column, Chiralpak IA; eluent, *n*-hexane/2-PrOH/DEA (95/5/0.1, 85/15/0.1 and 60/40/0.1 *v/v/v*); flow rate, 1.0 ml min^{-1}; detection at 220 nm; temperature, 25 °C.

lective reaction. Ring opening of β-amino lactones with different amines furnished β-amino amides in excellent yields.

(+)-Isopulegol was prepared according to literature procedures and all spectroscopic data were similar to those described therein [31]. The synthesis of enantiomeric (+)-1, (−)-2, (−)-3, and (+)-4 as well as β-aminoamides (+)-5, (−)-6, and (+)-7 was started from (+)-isopulegol according to the method reported recently. All physical and chemical properties of the enantiomeric pairs of 1–7 were identical with those reported therein [32]. Analytical data of the newly synthesized compounds are presented in Supplementary Information (Fig. S1).

n-Hexane, *n*-heptane, methanol (MeOH), ethanol (EtOH), 1-propanol (1-PrOH), 2-propanol (2-PrOH), 1-butanol (BuOH), diethylamine (DEA) of HPLC grade were provided by VWR International (Radnor, PA, USA).

2.2. Apparatus and chromatography

Liquid chromatographic measurements were performed with the use of two chromatographic systems. The Waters Breeze system consisted of a 1525 binary pump, a 2996 photodiode array detector, a 717 plus autosampler, and Empower 2 data manager software (Waters Corporation, Milford, MA, USA). A Lauda Alpha RA8 thermostat (Lauda Dr. R. Wobser Gmbh, Lauda-Königshofen, Germany) was used to maintain constant column temperature.

The 1100 Series HPLC system from Agilent Technologies (Waldbronn, Germany) contained a solvent degasser, a pump, an autosampler, a column thermostat, and a multiwavelength UV–Vis detector. Data acquisition and analysis were carried out with ChemStation chromatographic data software from Agilent Technologies.

All analytes were dissolved in 2-PrOH or EtOH in the concentration range 0.5–1.0 mg ml^{-1} and injected in a volume of 20 µL. The dead times of the columns were determined by injection of tri-*t*-butylbenzene.

Polysaccharide-based columns amylose *tris*-(3,5-dimethylphenylcarbamate) [Chiralpak IA and Chiralpak AD-H (coated)], amylose *tris*-(3-chlorophenylcarbamate) (Chiralpak ID), amylose *tris*-(3,5-dichlorophenylcarbamate) (Chiralpak IE), amylose *tris*-(3-chloro-4-methylphenylcarbamate) (Chiralpak IF), and amylose *tris*-(3-chloro-5-methylphenylcarbamate) (Chiralpak IG), as well as cellulose *tris*-(3,5-dimethylphenylcarbamate) [Chiralpak IB and Chiralcel OD-H, (coated)] and cellulose *tris*-

Table 1
Chromatographic data, k_1, α, R_S and elution sequences of ß-amino lactones and ß-amino amides on polysaccharide-based chiral stationary phases in normal-phase mode

Analyte	Column	k_1	α	Rs	Elution sequence
1	IA	3.55	1.18	2.89	$A < B$
	IB	2.54	1.17	2.71	$B < A$
	IE	18.16	1.05	1.19	$B < A$
	IC	14.02	1.20	4.22	$B < A$
	IF	12.70	1.13	2.26	$A < B$
	IG	14.83	1.15	2.68	$A < B$
	ID	11.75	1.04	0.70	$B < A$
2	IA	1.55	1.30	3.63	$B < A$
	IB	1.50	1.07	1.20	$B < A$
	IE	8.09	1.17	2.56	$B < A$
	IC	7.65	1.09	2.00	$B < A$
	IF	3.95	1.28	4.79	$B < A$
	IG	4.41	1.26	4.05	$B < A$
	ID	3.59	1.25	4.07	$B < A$
3	IA	1.42	1.06	0.98	$B < A$
	IB	1.36	1.06	0.88	$B < A$
	IE	5.79	1.20	2.61	$B < A$
	IC	5.88	1.55	9.65	$B < A$
	IF	3.99	1.08	1.33	$B < A$
	IG	4.52	1.28	4.10	$B < A$
	ID	3.54	1.33	5.27	$B < A$
4	IA	1.75	1.05	0.57	$A < B$
	IB	1.89	1.06	1.04	$B < A$
	IE	5.00	1.18	1.56	$A < B$
	IC	6.40	1.08	1.71	$A < B$
	IF	3.94	1.19	3.36	$A < B$
	IG	5.36	1.08	0.88	$A < B$
	ID	3.82	1.15	2.76	$A < B$
5	IA	3.87	1.27	1.95	$A < B$
	IB	1.61	1.40	1.06	$A < B$
	IE	10.61	1.07	0.95	$B < A$
	IC	5.18	1.24	2.93	$A < B$
	IF	7.67	1.18	1.77	$A < B$
	IG	12.13	1.10	1.00	$A < B$
	ID	13.53	1.02	0.32	$B < A$
6	IA	2.03	1.59	6.25	$A < B$
	IB	1.03	1.36	2.48	$B < A$
	IE	5.47	1.49	4.44	$A < B$
	IC	3.80	1.37	2.69	$B < A$
	IF	2.77	1.49	3.45	$A < B$
	IG	5.45	1.67	4.85	$A < B$
	ID	5.77	1.04	0.35	$B < A$
7	IA	3.25	1.12	1.86	$B < A$
	IB	0.79	1.00	0.00	- -
	IE	6.21	1.48	4.17	$A < B$
	IC	3.65	1.25	3.05	$A < B$
	IF	4.26	1.65	6.14	$A < B$
	IG	7.01	1.34	2.82	$A < B$
	ID	4.82	2.38	6.71	$A < B$

Chromatographic conditions: columns, Chiralpak IA, IB, IC, ID, IE, IF, and IG; mobile phase,
n-hexane/2-PrOH/DEA (95/5/0.1 v/v/v); flow rate, 1.0 ml min^{-1}; detection at 220 nm; temperature, 25 °C

(3,5-dichlorophenylcarbamate) (Chiralpak IC) all with the same size (250 mm × 4.6 mm I.D., 5 μm particle size) were generous gifts from Chiral Technologies Europe (Illkirch, France). Except for Chiralpak AD-H and Chiralcel OD-H, all CSPs employed in this study are immobilized phases. The structures of selectors are presented in Supplementary Information (Fig. S2).

3. Results and discussions

The ß-amino lactones and ß-amino amides as summarized in Fig. 1 are isopulegol-based analytes with benzyl, methylbenzyl or dibenzyl moieties attached to the *N*-atoms. Opening the ß-lactone ring (analyte 5, 6, and 7) modifies the structural characteristics of the molecules and may influence their interactions with chiral selectors.

3.1. The effect of mobile phase composition

Polysaccharide-based CSPs are most frequently employed in normal-phase mode (NPM), applying mixtures of a nonpolar hydrocarbon (typically *n*-hexane or *n*-heptane) and an alcohol of low molecular weight (*e.g.*, EtOH, 1-PrOH, 2-PrOH, BuOH) as mobile phase [19,20]. The variation of the nature and concentration of alcohol serves most often for the modulation of the chromatographic behavior (*i.e.*, retention and stereoselectivity) in NPM [33–36].

To study the effect of the nature of alcohol modifier on chromatographic parameters, analytes 1, 2, 4, and 6 were selected as representatives of the complete set of analytes of this study. To avoid the generation of an unnecessary large data set among the nine polysaccharide-based CSPs, four of them were selected on the basis of structural similarities. These are amylose- and cellulose-based *tris*-(3,5-dimethylphenylcarbamate) (Chiralpak IA and IB) and *tris*-(3,5-dichlorophenylcarbamate) (Chiralpak IE and IC). For the purpose of a reliable comparison, the studied alcohols, namely EtOH, 1-PrOH, 2-PrOH, and BuOH, were used at the same molar concentration of 1.298 M. This corresponds to a different volume ratio of each alcohol in the mobile phase as follows: EtOH: 7.6 v%, 1-PrOH: 9.7 v%, 2-PrOH: 10.0 v%, and BuOH: 11.9 v%.

Data obtained with the change of the alcohol are presented in Supplementary Information (Table S1). Under normal phase conditions, increasing the apolar character of the alcohol usually results in enhanced analyte retention; however, opposite observations have also been described [35,36]. Under the applied conditions, no general trends can be observed in retention factors: k increased with alcohol apolarity unequivocally only for Chiralpak IE in the case of analyte 1 and 2. Interestingly, separation factors, in most cases, changed only slightly (<10%) with the variation of the nature of alcohol. From a practical point of view, it is important to note that unlike selectivity, resolution is much more dependent on the nature of the alcohol modifier. Depending on the structure of the analyte and the chiral selector, R_S values were higher with EtOH or 2-PrOH, however, in some cases, the highest R_S values were registered in the presence of BuOH. The changein enantioselectivity caused by changing the alcohol modifier was previously rationalized as a result of alteration of the steric environment of the chiral cavities within the chiral polymer material induced by different alcohol modifiers [17,18]. Taking into account all results obtained with respect to the effect of the nature of alcohol on chromatographic parameters in NPM, the use of 2-PrOH and, in some cases, EtOH was favored for this class of compounds. Consequently, these two solvents were chosen for further studies.

Besides studying how the nature of alcohol affects the chiral recognition ability, comparing *n*-hexane and *n*-heptane as the most frequently applied NP solvents is of scientific interest. (It is worth mentioning that *n*-heptane is less toxic compared to *n*-hexane.) Previous works have shown improvements in selectivity with the use of *n*-heptane over *n*-hexane [37]. Applying Chiralpak IB with mobile phases of *n*-hexane/2-PrOH/DEA and *n*-heptane/2-PrOH/DEA and analytes 2 and 4, *n*-heptane showed no improvements over *n*-hexane: retention times, in most cases, were slightly shorter, but α and R_S were significantly lower in mobile phases containing *n*-heptane. It should be noted here that this is only a limited data set (Fig. S3).

For the study of the effects of modifier concentration on chromatographic parameters, two pairs of isopulegol-based ß-amino lactone and ß-amino amide (analytes 1, 5 and 2, 6) were chosen. The mobile phase systems were *n*-hexane/2-PrOH/DEA and *n*-hexane/EtOH/DEA containing 2-PrOH and EtOH at the same molar concentration (3.893, 2.596, 1.298, and 0.649 M), all containing 20 mM DEA, as the usual mobile phase additive used for the chromatography of basic analytes. Chiralpak IA and Chiralpak IE, as the best performing CPSs, were selected for this study. Regarding the

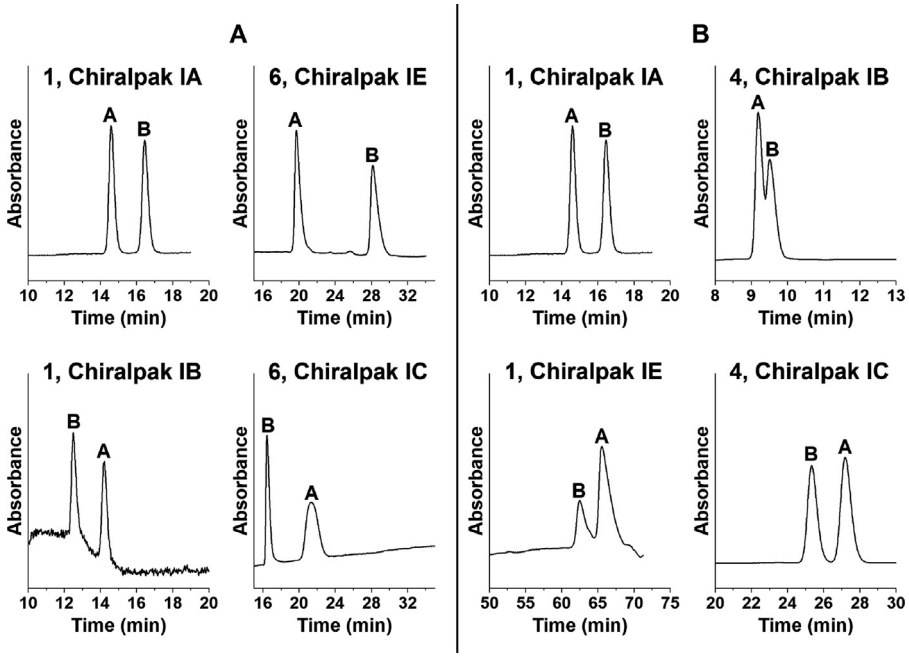

Fig. 4. Effect of backbone and nature of the carbamate substituent of polysaccharide-based CSPs on the elution order A, analytes 1 and 6; chromatographic conditions: column, Chiralpak IA *vs.* IB and Chiralpak IE *vs.* IC; eluent, *n*-hexane/2-PrOH/DEA (95/5/0.1 *v*/*v*/*v*); flow rate, 1.0 ml min^{-1}; detection at 220 nm; temperature, 25 °C; B, analytes 1 and 4; chromatographic conditions: column, Chiralpak IA *vs.* IE and Chiralpak IB *vs.* IC; eluent, *n*-hexane/2-PrOH/DEA (95/5/0.1 *v*/*v*/*v*); flow rate, 1.0 ml min^{-1}; detection at 220 nm; temperature, 25 °C.

retentive characteristics, a typical NP behavior was observed for both alcohol modifiers studied: increasing the apolar *n*-hexane to alcohol ratio resulted in an increased k_1 (Fig. 2). Enantioselectivity exhibited only a small change with increasing *n*-hexane content. Most notably, R_S, in most cases, increased significantly, in particular, for analyte 6 in mobile phase systems containing 2-PrOH. It is worth mentioning that the change in the chromatographic performance caused by the alcohol modifier depended on the structure of the chiral selector as well. Specifically, on Chiralpak IA, slightly higher k_1, α, and R_S were observed for analytes 1, 2, and 6 with the use of EtOH, while on Chiralpak IE, 2-PrOH had a similar effect for analytes 1, 5, and 6.

Not only the nature of the alcohol modifier, but also its concentration in a given mobile phase may affect the elution sequence as observed in several cases on polysaccharide-based CSPs [29,34,38]. In the present study, the reversal of elution order for analyte 5 on Chiralpak IA was registered by changing the composition of *n*-hexane/2-PrOH/DEA mobile phase from 95/5/0.1 *v*/*v*/*v* to 60/40/0.1 (Fig. 3), which probably due to the change in the solvation state of the chiral selector.

3.2. The effect of the structure of selectors

The amylose- and cellulose-based selectors are constructed of α or β 1,4-linked glucopyranose units, respectively. The different linkage is responsible for a difference in the secondary structure of these polysaccharides and of their derivatives. Due to these differences, the interactions between analyte and selector may change and this results in different chromatographic behaviors. Table 1 summarizes chromatographic data for the seven ß-amino lactones and ß-amino amides obtained on seven polysaccharide phases at the same mobile phase composition of *n*-hexane/2-PrOH/DEA (95/5/0.1 *v*/*v*/*v*).

The effect of the polysaccharide backbone can be evaluated by the comparison of the chromatographic data of amylose and cellulose *tris*-(3,5-dimethylphenylcarbamate) (Chiralpak IA *vs.* Chiralpak IB) and *tris*-(3,5-dichlorophenylcarbamate) (Chiralpak IE *vs.* Chiralpak IC), respectively. According to data in Table 1, in most cases, k_1, α, and R_S were higher on amylose- than on cellulose-based CSPs. It appears that, with a few exceptions, the studied analytes fit better to the amylose- than to the cellulose-based polymeric CSP, especially in the case of ß-amino amides with the ß-lactone ring opened. The structural differences between amylose- and cellulose-based *tris*-(3,5-dimethylphenylcarbamate) or *tris*-(3,5-dichlorophenylcarbamate) were found to be reflected in the chiral recognition pattern toward some analytes. Reversal of elution order between amylose- and cellulose-based CSPs, containing the same substituents was registered for analytes 1, 4, and 6 on Chiralpak IA and IB, and for analytes 5 and 6 on Chiralpak IE and IC (Table 1 and Fig. 4A). Examples of reversed elution orders of analytes on amylose- or cellulose-based columns have been described previously [29,34].

The effect of the nature of the phenylcarbamate moiety can be estimated by comparing amylose *tris*-(3,5-dimethylphenylcarbamate) (Chiralpak IA) and amylose *tris*-(3,5-dichlorophenylcarbamate) (Chiralpak IE) or cellulose *tris*-(3,5-dimethylphenylcarbamate) (Chiralpak IB) and cellulose *tris*-(3,5-dichlorophenylcarbamate) (Chiralpak IC). Data in Table 1 reveal that much higher retentions were registered for all analytes on CSPs with *tris*-(3,5-dichlorophenylcarbamate) moiety than on CSPs possessing the *tris*-(3,5-dimethylphenylcarbamate) moiety. Higher retentions were generally accompanied with higher α and R_S values showing that dichloro rather than dimethyl substitution favored the enantioselective interactions, probably through enhanced $\pi-\pi$ interactions. In a few cases lower α and R_S were registered on Chiralpak IE than on Chiralpak IA, but these differences were not significant. In this study, the reversal of elution order was registered for analytes 1, 5, and 7 in the case of Chiralpak IA and IE and for analyte 4 in the case of Chiralpak IB and IC (related examples are depicted in Fig. 4B). The reversal of elution sequence by the change of the chemical structure of substituents on the *tris*-(phenylcarbamate) moiety was also mentioned in earlier publications [29,34,39,40].

Table 2
Effect of mobile phase composition on k_1, α, and R_S of isopulegol-based β-amino lactones and β-amino amides

Analyte	Column	Eluent	t_{R1}	t_{R2}	k_1	α	R_s	Elution order
1	IA	70/30	5.84	6.16	0.96	1.06	1.12	$A < B$
		80/20	7.24	7.70	1.43	1.11	1.50	$A < B$
		90/10	10.06	11.05	2.41	1.14	2.33	$A < B$
		95/05	14.59	16.44	3.55	1.16	2.89	$A < B$
	IE	70/30	12.82	14.23	3.02	1.11	0.55	$B < A$
		80/20	18.84	20.27	4.73	1.11	1.45	$B < A$
		90/10	33.54	36.17	9.52	1.09	1.52	$B < A$
		95/05	62.46	65.51	18.16	1.05	1.69	$B < A$
2	IA	70/30	4.41	4.75	0.48	1.23	1.73	$B < A$
		80/20	4.96	5.44	0.67	1.25	2.43	$B < A$
		90/10	6.10	6.94	1.07	1.27	2.50	$B < A$
		95/05	7.70	9.05	1.40	1.30	3.63	$B < A$
	IE	70/30	7.59	8.35	1.38	1.18	2.32	$B < A$
		80/20	9.61	10.68	2.02	1.17	2.33	$B < A$
		90/10	14.70	16.82	3.61	1.18	3.20	$B < A$
		95/05	23.10	26.42	6.09	1.17	3.56	$B < A$
5	IA	70/30	4.86	5.02	0.63	1.09	0.59	$B < A$
		75/25	5.10	5.27	0.71	1.08	0.35	$B < A$
		80/20	5.52	5.65	0.86	1.05	0.26	$B < A$
		85/15	6.90	-	1.33	1.00	0.00	- -
		90/10	9.56	10.29	2.24	1.11	1.18	$A < B$
		95/05	15.65	19.06	3.87	1.27	1.95	$A < B$
	IE	70/30	7.08	7.34	1.22	1.07	0.67	$B < A$
		80/20	9.42	9.54	1.96	1.02	0.27	$B < A$
		90/10	17.41	17.41	4.46	1.00	0.00	- -
		95/05	37.86	40.35	10.61	1.07	0.95	$B < A$
6	IA	70/30	3.69	4.17	0.24	1.66	2.33	$A < B$
		80/20	4.12	4.87	0.39	1.66	3.47	$A < B$
		90/10	5.42	6.95	0.84	1.62	4.83	$A < B$
		95/05	8.99	12.55	2.03	1.59	6.25	$A < B$
	IE	70/30	5.27	6.24	0.65	1.46	3.69	$A < B$
		80/20	6.33	7.86	0.99	1.48	4.57	$A < B$
		90/10	10.01	13.34	2.14	1.49	5.53	$A < B$
		95/05	21.09	29.74	5.47	1.49	6.44	$A < B$

Chromatographic conditions: columns, Chiralpak IA and IE; eluent, *n*-hexane/2-PrOH/DEA (70/30/01–95/5/0.1 *v/v/v*); flow rate, 1.0 ml min^{-1}; detection, 220 nm; temperature, 25 °C.

The effect of the position of the methyl substituent in the phenylcarbamate moiety on the chromatographic performance was investigated by comparing chromatographic data obtained on amylose *tris*-(3-chloro-4-methylphenylcarbamate) (Chiralpak IF) and amylose *tris*-(3-chloro-5-methylphenylcarbamate) (Chiralpak IG). For all analytes, higher retentions were obtained on Chiralpak IG than on Chiralpak IF, but higher retention was accompanied with higher selectivity and resolution only for half of the studied analytes. It shows that the methyl substituent in position 5 offers stronger retentive interactions, but enantioselectivity may be reduced, probably for steric reasons.

The new generation of covalently immobilized polysaccharide phases are very robust and can be applied in different modalities with different bulk solvents [28,29,41,42]. A comparison of separation performances of covalently immobilized and coated polysaccharide CSPs were performed for analytes 1, 2, and 6 by applying immobilized and coated amylose *tris*-(3,5-dimethylphenylcarbamate) (Chiralpak IA *vs.* Chiralpak AD-H) and cellulose *tris*-(3,5-dimethylphenylcarbamate) (Chiralpak IB *vs.* Chiralcel OD-H) with the same mobile phase composition of *n*-hexane/2-PrOH/DEA (95/5/0.1 *v/v/v*) and *n*-hexane/ethanol/DEA (95/5/0.1 *v/v/v*) (Table 2). Data in Table 2 revealed that in almost all cases higher k_1, α, and R_S values were registered on coated CSPs than on the immobilized CSPs. Interestingly, a reversal of elution sequence was registered for analyte 6 on Chiralpak IA *vs.* Chiralpak AD-H in the *n*-hexane/ethanol/DEA (95/5/0.1 *v/v/v*) mobile phase system (Fig. 5A). A similar change was reported by Chankvetadze *et al.* [29]. Moreover, for analyte 6 on Chiralpak AD-H, the change of EtOH to 2-PrOH in *n*-hexane also resulted in a reversed elution sequence (Fig. 5B).

The strong dependence of the elution order of the individual enantiomers on the applied conditions calls particular attentions to the need of identification of each enantiomer in the case of polysaccharide-based CSPs. The complex structure of polysaccharide-based selectors and their applied conditions depending on solvation status do not allow to predict chiral recognition and elution order at these times.

3.3. The effect of the structure of analyte

Analytes 1–4 are ß-amino lactones, while 5–7, the ring-opened analogs of 1–3, are ß-amino amides. These structural differences may affect chromatographic behavior and chiral recognition. Analyte 4, compared to analyte 1, contains two benzyl moieties instead of a single benzyl group. According to chromatographic data (Table 1), more bulky analyte 4 fits less well into the cavity of amylose or cellulose backbone resulting in a significantly shorter retention.Among the studied CSPs selectivity and resolutions were higher with Chiralpak IE, IF, and ID, probably due to enhanced π–π interactions of analyte 4. Analytes 2 and 3 possess an extra methyl moiety compared to analyte 1. This structural difference has marked influences on the chromatographic behavior. Analyte 2 and 3 are much less retained by each CSP, but in several cases, their enantiomers exhibited better resolution, possibly due to steric reasons. Analytes 5, 6, and 7, ring-opened analogs of analytes 1, 2, and 3, contain an extra hydroxyl and a secondary amino group capable of hydrogen bonding interactions with the carbamate moiety. Furthermore, the additional benzyl ring may be involved in π–π interactions. The presence of extra interaction sites, in most cases, led to enhanced enantioselectivity, while retention

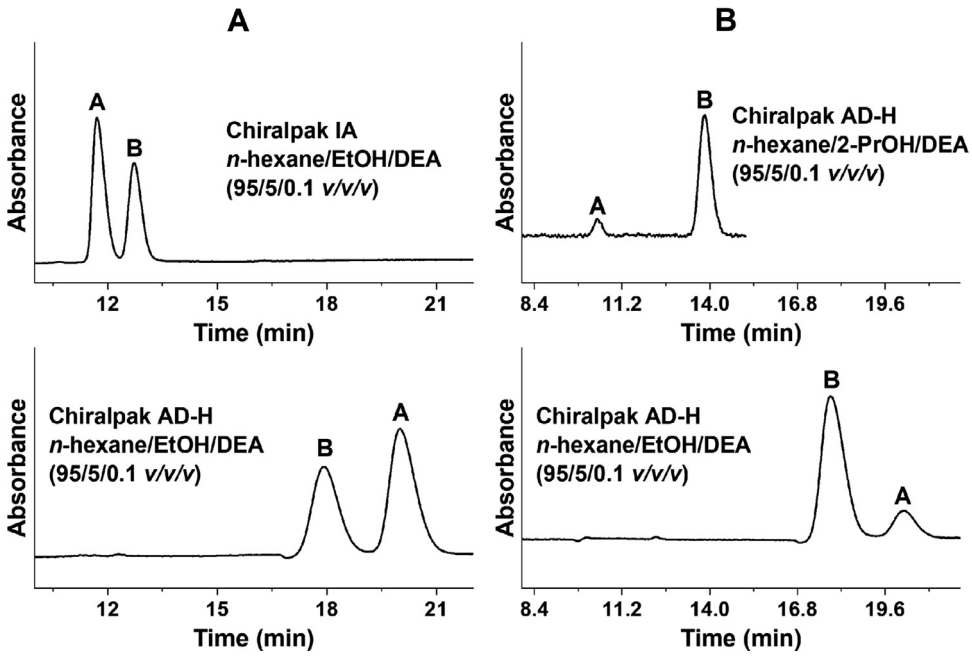

Fig. 5. Effect of selector coating and alcohol modifier on the elution order for analyte 6 on Chiralpak IA and Chiralpak AD-H column Chromatographic conditions: column, A, Chiralpak IA and Chiralpak AD-H, B, Chiralpak AD-H; mobile phases, A, *n*-hexane/EtOH/DEA (95/5/0.1 *v/v/v*), B, *n*-hexane/2-PrOH/DEA (95/5/0.1 *v/v/v*) and *n*-hexane/ EtOH/DEA (95/5/0.1 *v/v/v*); flow rate, 1.0 ml min^{-1}; detection at 220 nm; temperature, 25 °C.

was generally smaller for the amino amide analogs, suggesting reduced nonselective interactions for these compounds.

It is interesting to examine how the structure of analyte affects the elution sequence. In case of analyte 1 the elution sequence depends strongly on the applied CSP, while no changes in elution order were observed for analytes 2 and 3 (Table 1). This draws attention how a simple methyl substitution by creating a new chiral center can affect the chiral recognition. It is important to highlight that the methyl substitution in the same position in case of the amides (5 *vs* 6 and 5 *vs* 7) did not result in a consistent change in the elution sequences. On the basis of this limited data set no clear trend can be suggested how the structure of analytes affect the elution sequence.

For the quantitative characterization of the optimized methods, limits of both detection (LOD) and quantitation (LOQ) were determined for analytes 2 and 6 on Chiralpak IA and Chiralpak IE columns. Due to the better peak shapes sligthly lower LOD and LOQ values were obtained on Chiralpak IE, where LOD and LOQ values for analyte 2 were 6.9 pmol and 23.2 pmol, respectively, while these values for analyte 6 were 4.9 pmol and 16.3 pmol, respectively. Fig. 6 depicts the chromatograms obtained on Chiralpak IE for analytes 2 and 6 for the minor enantiomer in the presence of the major one.

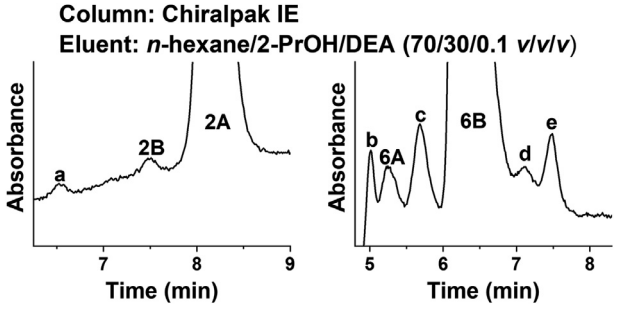

Fig. 6. Chromatograms of analytes 2 and 6 for the determination of enantiomeric and chemical impurities Chromatographic conditions: column, Chiralpak IE; eluent, *n*-hexane/2-PrOH/DEA (70/30/0.1 *v/v/v*); flow rate, 1.0 ml min^{-1}; detection at 220 nm; temperature, 25 °C; the ratio of minor component to major one, 1:10.000; a, b, c, d, e, unknown impurities.

3.4. Effect of temperature and thermodynamic parameters

By careful interpretations of the van't Hoff equation, the studies of temperature dependence of retention and enantioselectivity may offer valuable information on the chiral recognition process. For the enantiomeric pairs, the difference in the change in standard enthalpy $\Delta(\Delta H^\circ)$ and entropy $\Delta(\Delta S^\circ)$ can be obtained on the basis of the van't Hoff equation, not forgetting about the limitations of the simplified approach applied in this study (*i.e.*, not differentiating between chiral and achiral contributions, which may vary in their magnitude) [43–46].

In order to investigate the effects of temperature on the chromatographic parameters, a variable temperature study was carried out for analytes 1, 2, 5, and 6 on Chiralpak IA, Chiralpak AD-H, and Chiralpak IE columns in the temperature range 5–50 °C (at 5 or 10 °C increments). Mobile phases *n*-hexane/2-PrOH/DEA (70/30/0.1 *v/v/v*) and *n*-hexane/ethanol/DEA (70/30/0.1 *v/v/v*) were applied under the same set of experimental conditions, as highlighted their importance by Sepsey *et al* [46]. The corresponding experimental data are summarized in Table S2. Transfer of the analyte from the mobile phase to the stationary phase can commonly be described as an exothermic process. Because of this reason, retention decreases with increasing temperature. On the three studied columns with both mobile phase systems, k and α decreased with increasing temperature in most cases. However, for analyte 1 on Chiralpak IE and for analyte 6 on Chiralpak IA in *n*-hexane/ethanol/DEA (70/30/0.1 *v/v/v*), k decreased, but α increased with increasing temperature (Table S2 and Fig. S4).

From the chromatographic data on the basis of Eq. 1,

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

where R is the universal gas constant, T is temperature in Kelvin, and α is the apparent selectivity factor, ln α *vs.* $1/T$ plots were constructed. As a general trend, linear plots were obtained as indicated

Table 3
Thermodynamic parameters, $\Delta(\Delta H^\circ)$, $\Delta(\Delta S^\circ)$, $T\Delta(\Delta S^\circ)_{298K}$, $\Delta(\Delta G^\circ)_{298K}$, correlation coefficients, (R^2), Q values, and T_{iso} temperatures of isopulegol-based β-amino lactones and ß-amino amides on Chiralpak IA, Chiralpak AD-H, and Chiralpak IE columns.

Analyte	-Δ(ΔH°) (kJ mol^{-1})	-Δ(ΔS°) (J mol^{-1} K^{-1})	Correlation coefficients (R^2)	-TxΔ(ΔS°)$_{298K}$ (kJ mol^{-1})	-Δ(ΔG°)$_{298K}$ (kJ mol^{-1})	Q	T_{ISO} (°C)
1	Chiralpak IA						
	-	-	-	-	-	-	-
	*4.0	*11.4	*0.949	*3.4	*0.6	*1.2	77
2	Chiralpak IA						
	2.3	6.0	0.988	1.8	0.5	1.3	109
	Chiralpak AD-H						
	2.5	6.3	0.994	1.9	0.6	1.3	114
	Chiralpak IA						
	*3.2	*7.9	*0.973	*2.4	*0.8	*1.4	127
5	Chiralpak IA						
	3.7	11.9	0.986	3.6	0.2	1.1	39
	*4.7	*14.4	*0.996	*4.3	*0.4	*1.1	51
6	Chiralpak IA						
	2.5	3.8	0.993	1.1	1.3	2.2	367
	Chiralpak AD-H						
	2.4	3.8	0.993	1.1	1.3	2.2	361
	Chiralpak IA						
	*−2.0	*−7.9	*0.965	*−2.4	*0.3	*0.8	−17
	Chiralpak IE						
1	0.8	1.7	0.997	0.5	0.3	1.6	175
	*−0.9	*−3.3	*0.814	*−1.0	*0.1	*0.9	−5
2	1.2	2.8	0.999	0.8	0.4	1.4	169
	*2.0	*4.8	*0.998	*1.4	*0.5	*1.4	137
5	2.2	6.7	0.984	2.0	0.2	1.1	47
	*1.9	*6.3	*0.933	*1.9	*0.1	*1.1	31
6	2.4	4.9	0.981	1.5	1.0	1.8	226
	*1.1	*1.6	*0.998	*0.5	*0.6	*2.3	385

Chromatographic conditions: columns, Chiralpak IA, Chiralpak AD-H, and Chiralpak IE; mobile phase, *n*-hexane/2-PrOH/DEA (70/30/0.1 *v/v/v*), Q = Δ(ΔH°)/298 x Δ(ΔS°).
* *n*-hexane/EtOH/DEA (70/30/0.1 *v/v/v*); flow rate, 1.0 ml min^{-1}; detection at 220 nm; correlation coefficient (R^2) of *van't Hoff* plot, ln α *vs 1/T* curves;

by the correlation coefficients listed in Table 3. In most cases, differences in the changes in standard enthalpy and entropy, -Δ(ΔH°) and -Δ(ΔS°), in both mobile phases were more negative on Chiralpak IA than on Chiralpak IE (Table 3) indicating a stronger adsorption process. Interestingly, -Δ(ΔH°) and -Δ(ΔS°) values for Chiralpak IA and Chiralpak AD-H were very similar. The two CSPs possess the same selector in covalently bonded or coated form and, consequently, a retention mechanism independent of the immobilization of the selector can be suggested.

According to the data of Table S2, retention decreases in every case, but selectivity increases with increasing temperature in two cases, as reported previously in chromatographic systems applying polysaccharide-type phases [28,29,34,38,47]. The T_{iso} value (the temperature where the enantioselectivity cancels), in most cases, were above room temperature (Table 3). To estimate the enthalpy/entropy contribution to the free energy, Q [$Q = \Delta(\Delta H^\circ)/[298 \times \Delta(\Delta S^\circ)]$ values were calculated. According to data in Table 3, Q values, in most cases, were higher than 1.0, indicating the relatively higher contribution of the enthalpy to the free energy. For the systems in which analytes possess negative T_{iso}, $Q < 1$ suggests a predominantly entropic contribution to the free energy. That is, enantiodiscrimination was driven by entropy in these cases.

4. Conclusions

Enantioseparations of newly prepared ß-amino lactones and ß-amino amides were carried out on amylose- and cellulose-based *tris*-(phenylcarbamate) stationary phases in *n*-hexane/alcohol/DEA and *n*-heptane/alcohol/DEA mobile phases. Regarding mobile phase composition, in case of the studied compounds, applications of 2-propanol and ethanol in the mobile phase seem to be more advantageous, while changing between *n*-hexane and *n*-heptane leads to only slight differences in separation performances. The nature and content of alcohol modifier may have a significant influence on the elution sequence.

The nature of the chiral selector backbone (amylose or cellulose) together with the nature of substituents of the phenylcarbamate moiety influence not only the separation performance but also the elution sequence in several cases. In the applied chromatographic systems in general, much higher retentions were registered for all analytes on CSPs with *tris*-(3,5-dichlorophenylcarbamate) moiety than on CSPs possessing *tris*-(3,5-dimethylphenylcarbamate) moiety, probably due to π-π acceptor type of interactions. The chemical structure of the substituent on the amylose or cellulose backbone may influence not only retention and selectivity but also the elution sequence.

The study of the effect of the position of the substituents of the phenylcarbamate moiety on the chromatographic performance in the case of amylose-based CSPs revealed that *tris*-(3-chloro-5-methylphenylcarbamate) is more efficient regarding the chiral interaction between selector and the investigated analytes than that on *tris*-(3-chloro-4-methylphenylcarbamate).

The new generation of covalently immobilized polysaccharide phases are very robust. However, regarding separation performances for the analytes studied, higher k_1, α, and R_S were registered on coated CSPs than on the comparable immobilized ones. Rarely reported so far, but it is worth highlighting that the change between the two types of CSPs may result in a reversal of the elution sequence.

The structure of selector and analyte, the mobile phase composition (nature and content of bulk solvent and alcohol modifier), and temperature may affect the observed elution order. Consequently, the identification of enantiomers is mandatory for a valid interpretation of data.

Regarding the effect of the nature of analytes, it can be concluded that enantiodiscrimination of ß-amino amides were generally more pronounced, despite their shorter retention times.

The temperature-dependence study revealed enthalpically driven recognition in most cases, but entropy-controlled separation in *n*-hexane/ethanol mobile phase system was also observed under the chromatographic conditions employed in this study.

Declaration of Competing Interest

Authors declare no conflict of interest.

CRediT authorship contribution statement

Dániel Tanács: Methodology, Investigation, Visualization, Writing - original draft. **Tímea Orosz:** Methodology, Investigation, Visualization, Writing - original draft. **Zsolt Szakonyi:** Writing - original draft, Data curation. **Tam Minh Le:** Data curation. **Ferenc Fülöp:** Writing - original draft. **Wolfgang Lindner:** Writing - original draft. **István Ilisz:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing - original draft, Writing - review & editing. **Antal Péter:** Conceptualization, Writing - original draft.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2020.461054.

References

[1] N.J. Lawrence, A.T. McGown, J. Nduka, J.A. Hadfield, R.G. Pritchard, Cytotoxic michael-type amine adducts of α-methylene lactones alantolactone and isoalantolactone, Bioorg. Med. Chem. Lett. 11 (2001) 429–431, doi:10.1016/S0960-894X(00)00686-7.

[2] E. Hejchman, R.D. Haugwitz, M. Cushman, Synthesis and cytotoxicity of water-soluble ambrosin prodrug candidates, J. Med. Chem. 38 (1995) 3407–3410, doi:10.1021/jm00017a025.

[3] W.H. Pearson, J.V. Hines, Synthesis of beta-amino-alpha-hydroxy acids via aldol condensation of a chiral glycolate enolate. A synthesis of (−)-bestatin, J. Org. Chem. 54 (1989) 4235–4237, doi:10.1021/jo00278a050.

[4] L. Kiss, F. Fülöp, Synthesis of carbocyclic and heterocyclic β-aminocarboxylic acids, Chem. Rev. 114 (2014) 1116–1169, doi:10.1021/cr300454h.

[5] L. Kiss, F. Fülöp, Selective synthesis of fluorine-containing cyclic β-amino acid scaffolds, Chem. Rev. 18 (2018) 266–281, doi:10.1002/tcr.201700038.

[6] J.E. Kowalchick, B. Leiting, K.D. Pryor, F. Marsilio, J.K. Wu, H. He, K.A. Lyons, G.J. Eiermann, A. Petrov, G. Scapin, R.A. Patel, N.A. Thornberry, A.E. Weber, D. Kim, synthesis Design, and biological evaluation of triazolopiperazine-based β-amino amides as potent, orally active dipeptidyl peptidase IV (DPP-4) inhibitors, Bioorg. Med. Chem. Lett. 17 (2007) 5934–5939, doi:10.1016/j.bmcl.2007.07.100.

[7] B.R. Lagu, D.C. Liotta, Diastereoselective synthesis of the key lactone intermediate for the preparation of hydroxyethylene dipeptide isosteres, Tetrahedron Lett. 35 (1994) 547–550, doi:10.1016/S0040-4039(00)75834-5.

[8] A. Kamal, M. Sandbhor, A.A. Shaik, Application of a one-pot lipase resolution strategy for the synthesis of chiral γ- and δ-lactones, Tetrahedron 14 (2003) 1575–1580, doi:10.1016/S0957-4166(03)00281-7.

[9] S. Chen, M. Mondal, A.A. Ibrahim, K.A. Wheeler, N.J. Kerrigan, Phosphine-catalyzed asymmetric synthesis of β;-lactones from disubstituted ketenes and aldehydes, J. Org. Chem. 79 (2014) 4920–4929, doi:10.1021/jo500486e.

[10] B. Hu, L. Deng, Direct catalytic asymmetric synthesis of trifluoromethylated γ-amino esters/lactones via umpolung strategy, J. Org. Chem. 84 (2019) 994–1005, doi:10.1021/acs.joc.8b02893.

[11] Z.-H. You, Y.-H. Chen, Y.-K. Liu, From racemic precursors to fully stereocontrolled products: one-pot synthesis of chiral α-amino lactones and lactams, Org. Biomol. Chem 14 (2016) 6316–6327, doi:10.1039/C6OB00953K.

[12] Z. Jie, D. Qiuzheng, Z. Suzhen, S. Fang, L. Xinyu, Z. Zhenzhong, Enantioseparation of three important intermediates of tanikolide with immobilized cellulose chiral stationary phase, J. Chromatogr. Sci. 53 (2015) 959–962, doi:10.1093/chromsci/bmu158.

[13] M.H. Paulsen, M. Engquist, D. Ausbacher, M.B. Strom, A. Bayer, Efficient and scalable synthesis of α,α-disubstituted ß-amino amides, Org. Biomol. Chem. 14 (2016) 7570–7578, doi:10.1039/C6OB01219A.

[14] C.G. Goodman, J.S. Johnson, Asymmetric synthesis of ß-amino amides by catalytic enantioconvergent 2-aza-cope rearrangement, J. Amer. Chem. Soc. 137 (2015) 14574–14577, doi:10.1021/jacs.5b09593.

[15] T. Heck, D. Seebach, S. Osswald, M.K.J. ter Wiel, H-P.E Kohler, B. Geueke, Kinetic resolution of aliphatic ß-amino acid amides by ß-aminopeptidases., Chem. Bio. Chem. 10 (2009) 1558–1561, doi:10.1002/cbic.200900184.

[16] B.V.R. Reddy, N. Raman, B.S. Kumar, C. Rambabu, Chiral separation of sitagliptin phosphate enantiomer by HPLC using amylose based chiral stationary phase, J. Pharm. Res. 7 (2013) 546–550, doi:10.1016/j.jopr.2013.06.014.

[17] G.K.E. Scriba, Chiral recognition in separation sciences. Part I: polysaccharide and cyclodextrin selectors, TrAC Trends Anal. Chem. 120 (2019) 115639, doi:10.1016/j.trac.2019.115639.

[18] G.K.E. Scriba, Chiral recognition in separation science – an update, J. Chromatogr. A 1467 (2016) 56–68, doi:10.1016/j.chroma.2016.05.061.

[19] B. Chankvetadze, Recent developments on polysaccharide-based chiral stationary phases for liquid-phase separation of enantiomers, J. Chromatogr. A 1269 (2012) 26–51, doi:10.1016/j.chroma.2012.10.033.

[20] B. Chankvetadze, Recent trends in preparation, investigation and application of polysaccharide-based chiral stationary phases for separation of enantiomers in high-performance liquid chromatography, TrAC Trends Anal. Chem. 122 (2020) 115709, doi:10.1016/j.trac.2019.115709.

[21] Y. Okamoto, M. Kawashima, K. Hatada, Useful chiral packing materials for high-performance liquid chromatographic resolution of enantiomers: phenylcarbamates of polysaccharide coated on silica gel, J. Amer. Chem. Soc. 106 (1984) 5357–5359, doi:10.1021/ja00330a057.

[22] B. Chankvetadze, E. Yashima, J.Y. Okamoto, Chloromethylphenylcarbamate derivatives of cellulose as chiral stationary phases for high-performance liquid chromatography, J. Chromatogr. A 670 (1994) 39–49, doi:10.1016/0021-9673(94)80278-5.

[23] B. Chankvetadze, E. Yashima, J.Y. Okamoto, Dimethyl-, dichloro- and chloromethylphenylcarbamates of amylose as chiral stationary phases for high-performance liquid chromatography, J. Chromatogr. A 694 (1995) 101–109, doi:10.1016/0021-9673(94)00729-S.

[24] B. Chankvetadze, L. Chankvetadze, Sh. Sidamonidze, E. Kasashima, E. Yashima, Y. Okamoto, 3-Fluoro-, 3-bromo- and 3-chloro-5-methylphenylcarbamates of cellulose and amylose as chiral stationary phases for high-performance liquid chromatographic enantioseparation, J. Chromatogr. A 787 (1997) 67–77, doi:10.1016/S0021-9673(97)00648-1.

[25] B. Chankvetadze, L. Chankvetadze, Sh. Sidamonidze, E. Yashima, J.Y. Okamoto, High performance liquid chromatography enantioseparation of chiral pharmaceuticals using tris(chloro-methylphenylcarbamate) of cellulose, J. Pharm. Biomed. Anal. 14 (1996) 1295–1303, doi:10.1016/S0731-7085(96)01748-7.

[26] Y. Okamoto, R. Aburatani, S. Miura, K. Hatada, Chiral stationary phases for HPLC: cellulose tris(3,5-dimethylphenylcarbamate) and tris(3,5-dichlorophenylcarbamate) chemically bonded to silica gel, J. Liquid. Chromatogr. 10 (1987) 1613–1628, doi:10.1080/01483918708066791.

[27] E. Francotte, T. Zhang, Preparation and evaluation of immobilized 4-methylbenzoylcellulose stationary phases for enantioselective separations, J. Chromatogr. A 1467 (2016) 214–220, doi:10.1016/j.chroma.2016.08.006.

[28] I. Matarashvili, G. Kobidze, A. Chelidze, G. Dolidze, N. Beridze, G. Jibuti, T. Farkas, B. Chankvetadze, The effect of temperature on the separation of enantiomers with coated and covalently immobilized polysaccharide-based chiral stationary phases, J. Chromatogr. A 1559 (2019) 172–179, doi:10.1016/j.chroma.2019.04.024.

[29] M. Maisuradze, G. Sheklashvili, A. Chokheli, I. Matarashvili, T. Gogatishvili, T. Farkas, B. Chankvetadze, Chromatographic and thermodynamic comparison of amylose tris(3-chloro-5-methylphenylcarbamate) coated or covalently immobilized on silica in high-performance liquid chromatographic separation of the enantioemrs of select chiral weak acids, J. Chromatogr. A 1602 (2019) 228–236, doi:10.1016/j.chroma.2019.05.026.

[30] T.M. Le, P. Bérdi, I. Zupkó, F. Fülöp, Z. Szakonyi, Synthesis and transformation of (-)-isopulegol-based chiral β-aminolactones and β-aminoamides, Int. J. Mol. Sci. 19 (2018) 3522, doi:10.3390/ijms19113522.

[31] Y. Nakatani, K. Kawashima, Highly Stereoselective preparation of l-sisopulegol, Synth.-Stuttg. 2 (1978) 147–148, doi:10.1055/s-1978-24696.

[32] D. Friedrich, F. Bohlmann, Total synthesis of various elemanolides, Tetrahedron 44 (1988) 1369–1392, doi:10.1016/S0040-4020(01)85916-0.

[33] F. Ianni, S. Scorzoni, P.L. Gentili, A.D. Michele, M. Frigoli, E. Camaioni, F. Ortica, R. Sardella, Chiral separation of helical chromenes with chloromethyl phenylcarbamate polysaccharide-based stationary phases, J. Sep. Sci. 41 (2018) 1266–1273, doi:10.1002/jssc.201701293.

[34] I. Matarashvili, L. Chankvetadze, S. Fanali, T. Farkas, B. Chankvetadze, HPLC separation of enantiomers of chiral arylpropionic acid derivatives using polysaccharide-based chiral columns and normal-phase eluents with emphasis on elution order, J. Sep. Sci. 36 (2013) 140–147, doi:10.1002/jssc.201200885.

[35] R. Sardella, F. Ianni, A. Lisanti, M. Marinozzi, S. Scorzoni, B. Natalini, The effect of mobile phase composition in the enantioseparation of pharmaceutically relevant compounds with polysaccharide-based stationary phases, Biomed. Chromatogr. 28 (2014) 15 9–167, doi:10.1002/bmc.3015.

[36] T. Wang, R.M. Wenslow, Effects of alcohol mobile-phase modifiers on the structure and chiral selectivity of amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phase, J. Chromatogr. A 1015 (2003) 99–110, doi:10.1016/S0021-9673(03)01262-7.

[37] O. Buddrick, O.A.H. Jones, P.D. Morisson, D.M. Small, Heptane as less toxic option than hexane for the separation of vitamin E from food products using normal phase HPLC, RSC Adv. 3 (2013) 24063–24068, doi:10.1039/C3RA44442B.
[38] C. Xiang, G. Liu, S. Kang, X. Guo, B. Yao, W. Weng, Q. Zeng, Unusual chromatographic enantioseparation behavior of naproxen on an immobilized polysaccharide-based chiral stationary phase, J. Chromatogr. A 1218 (2011) 8718–8721, doi:10.1016/j.chroma.2011.10.014.
[39] L. Chankvetadze, N. Ghibradze, M. Karchkhadze, L. Peng, T. Farkas, B. Chankvetadze, Enantiomer elution order reversal of fluorenylmethoxycarbonyl-isoleucine in high-performance liquid chromatography by changing the mobile phase temperature and composition, J. Chromatogr. A 1218 (2011) 6554–6560, doi:10.1016/j.chroma.2011.06.068.
[40] T. Ikai, Y. Okamoto, Structure Control of Polysaccharide Derivatives for Efficient Separation of Enantiomers by Chromatography, Chem. Rev. 109 (2009) 6077–6101, doi:10.1021/cr8005558.
[41] T. Zhang, P. Franco, D. Nguyen, R. Hamasaki, S. Miyamoto, A. Ohnishi, T. Murakami, Complementary enantiorecognition patterns and specific method optimization aspects on immobilized polysaccharide-derived chiral stationary phases, J. Chromatogr. A 1269 (2012) 178–188, doi:10.1016/j.chroma.2012.09.071.
[42] T. Zhang, P. Franco, Common approaches for efficient method development with immobilised polysaccharide-derived chiral stationary phases, J. Chromatogr. B 875 (2008) 48–56, doi:10.1016/j.jchromb.2008.06.051.
[43] S. Allenmark, V. Schurig, Chromatography on chiral stationary phases, J, Mater. Sci. 7 (1977) 1955–1963, doi:10.1039/a702403g.
[44] G. Gotmar, T. Fornstedt, G. Guiochon, Apparent and true enantioselectivity in enantioseparations, Chirality 12 (2000) 558–564, doi:10.1002/1520-636X(2000)12:7⟨558::AID-CHIR2⟩3.0.CO;2-2.
[45] T. Fornstedt, P. Sajonz, G. Guiochon, Thermodynamic study of an unusual chiral separation. Propranolol enantiomers on an immobilized cellulose, J. Amer. Chem Soc. 119 (1997) 1254–1264, doi:10.1021/ja9631458.
[46] A. Sepsey, É. Horváth, M. Catani, A. Felinger, The correctness of van't Hoff plots in chiral and achiral chromatography, J. Chromatogr. A 1611 (2020) 460594, doi:10.1016/j.chroma.2019.460594.
[47] A. Aranyi, I. Ilisz, Z. Pataj, I. Szatmári, F. Fülöp, A. Péter, 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, J. Chromatogr. A 1218 (2011) 4869–4876, doi:10.1016/j.chroma.2011.01.027.