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Enantiomer separation and indirect chromatographic absolute configuration prediction of chiral pirinixic acid derivatives: Limitations of polysaccharide-type chiral stationary phases in comparison to chiral anion-exchangers

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ABSTRACT

Chiral α-arylthiocarboxylic acids with different substitution patterns, representing new pirinixic acid derivatives with dual PPARα/γ agonistic activities, have been separated into enantiomers on *tert*-butylcarbamoylquinine and quinidine based chiral anion-exchangers and amylose tris(3,5-dimethylphenylcarbamate) coated silica on analytical and preparative scale. Absolute configurations of individual enantiomers were assigned chromatographically via elution orders on the chiral anion-exchangers and were confirmed by stereoselective syntheses via Ewans auxiliaries that have lead to enantiomeric products with known absolute configurations. The results of both methods were in full agreement. Moreover, the receptor stereoselectivity in PPARα transactivation activities was consistent within the test set of structurally related compounds. Limited correlation (between elution order and substitution) was observed within the set of α-arylthiocarboxylic acids on the amylose tris(3,5-dimethylphenylcarbamate) based chiral stationary phase (CSP), in particular the elution order changed with remote substitution. This clearly demonstrates the risks of chromatographic absolute configuration assignments by prediction from one structural analog to another one, especially with CSPs such as polysaccharide CSPs that are recognized for their broad applicability due to multiple binding and chiral recognition modes. It is therefore of utmost importance that such chromatographic absolute configuration predictions by extrapolation to structural analogs are combined with orthogonal methods for verification of the results.

1. Introduction

Pirinixic acid (Fig. 1) has been proposed as a moderate agonist of alpha and gamma peroxisome proliferator activated receptors (PPAR). It represents a potential lead structure for the development of new chemical entities for treatment of metabolic disorders such as dyslipidemia and type 2 diabetes. Alkyl substitution in α-position to the carboxylic acid group yields chiral pirinixic acid derivatives with enhanced PPAR alpha and gamma activities which can be further optimized by variation of the aryl-substitution pattern [1]. Through α-substitution chiral α-arylthio carboxylic acids are obtained for which enantioselectivities in terms of PPAR activation have to be considered.

Drug discovery of such chiral compounds involves the synthesis of individual enantiomers for biological activity tests and requires the determination of absolute configurations of the enantiomeric compounds as stereochemical descriptors to pinpoint their identity [2]. Preparative liquid chromatography is a viable route for straightforward and rapid access to both enantiomers with little efforts and minimal time for method development [3]. It provides the target enantiomers in high enantiomeric purities and yields. The most common methodologies for absolute configuration assignment nowadays [4–6] are NMR [5,7–13], X-ray diffraction analysis [5,9–11,14–22] and circular dichroism (CD) spectroscopy [19,23,24] (including VCD [4,17,23,25,26]). Single-crystal X-ray diffraction analysis is the most preferred direct method to determine absolute configurations. Yet, it is only amenable for enantiomeric compounds that provide crystals with adequate resonant scattering and has also some caveats especially for compounds which contain only light atoms (as critically reviewed recently by Flack and Bernardinelli [27]). It requires

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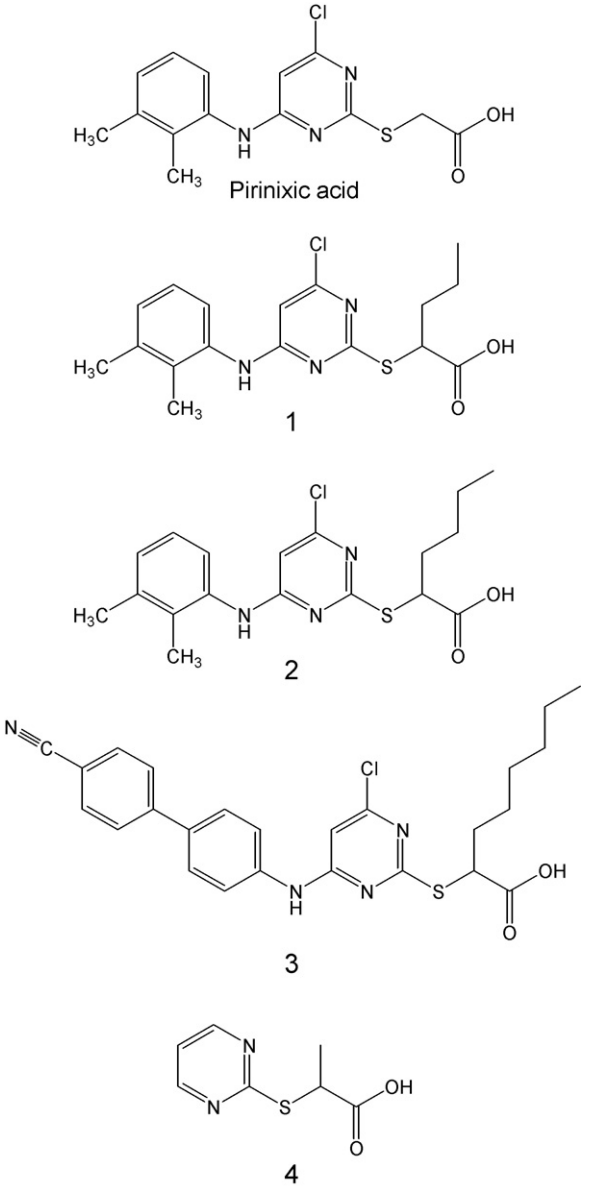


Fig. 1. Pirinixic acid and chiral α-arylthiocarboxylic acid analogs that have been investigated in the present study.

critical evaluation of characteristic parameters such as the Flack parameter which is sometimes not reported yet indicates satisfactory absolute-structure determination, in order to avoid erroneous results [27]. By means of CD and VCD absolute configurations can be determined by comparison of experimental CD or VCD spectra with those that have been calculated by quantum chemistry calculations. Agreement between calculated and experimental spectra is an indication for a correct absolute configuration assignment.

For sake of simplicity indirect methods such as assignment of absolute configurations by chromatographic elution orders have also been frequently employed [4,28–32]. It is based on the knowledge of the absolute configuration of a structurally related reference compound which follows the same chiral recognition mechanism in the chromatographic enantiomer separation process on a given chiral stationary phase [4]. It is a simple, straightforward and cheap methodology which can be employed for a larger set of structurally related compounds, yet requires some deeper understanding on how the chiral stationary phase recognizes and distinguishes enantiomers for a given class of test solutes featuring more or less structural variability. Unfortunately, chiral recognition mechanisms on chiral stationary phases may be sensitive to even minor structural or conditional changes. This becomes clearly evident, for instance, by reversals of elution orders upon minor variations of experimental conditions such as changes of modifier type [33,34] or percentage [34,35]. It has also been reported that elution orders can be reversed upon deposition of polysaccharide selectors onto the silica surface from distinct solvents, i.e. with slightly altered preparation conditions, probably due to altered supramolecular structures of the polymeric selectors [36]. Last but not least, numerous examples can be found in the literature that showed reversals of elution orders on a given CSP with minute structural alterations within a homologous compound series, i.e. of structurally closely related compounds [37,38]. In particular the latter phenomenon poses some serious risks on the chromatographic absolute configuration assignment which is based on consistent elution orders within a series of structural homologs. Therefore, it exists consensus that verification of the chromatographic absolute configuration assignment must be performed by a second independent methodology in order to minimize the risk for false assignments.

Herein we report on a methodology for the chromatographic enantiomer separation of pirinixic acid analogs (Fig. 1) by enantioselective liquid chromatography as well as the absolute configuration assignment after preparative chromatographic resolution. Absolute configuration determinations were confirmed by independent methods such as chemical correlations via stereoselective synthesis and consistency in receptor subtype enantioselectivity. The risk of false assignments is illustrated by use of two distinct types of chiral stationary phases, low molecular brush-type chiral anion-exchangers (Chiralpak QD-AX and QN-AX) and polymeric type CSP based on a polysaccharide selector (Chiralpak AD-H), which differed in their consistencies of chiral recognition mechanisms within the set of investigated solutes, i.e. of chiral 2-arylthiocarboxylic acids.

2. Experimental

2.1. Materials

The pirinixic acid analogs **1**–**3** were synthesized as described elsewhere [1]. Enantiomers of **4** were synthesized as described below. The α-aryloxycarboxylic acid reference compounds **5**–**8** were research samples from former studies. Dichlorprop, 2-(2,4-dichlorophenoxy)propionic acid **9**, was supplied by Aldrich (Sigma–Aldrich, Vienna, Austria).

For enantioselective HPLC 150 mm × 4 mm I.D. Chiralpak QD-AX and QN-AX (5 μm diameter particles) as well as a 250 mm × 4 mm I.D. Chiralpak AD-H (5 μm) from Chiral Technologies Europe (Illkirch, France) were employed as columns. (*S*)-(−)- and (*R*)-(+)-2-bromopropionic acid, caesium carbonate, and 2-mercaptopyrimidine were from Sigma–Aldrich (Vienna, Austria). Methanol (gradient grade), n-heptane and 2-propanol (both HPLC grade) were supplied by Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA) was from Fluka. DMF (purum >99%, Fluka, Vienna, Austria), toluene (>99%, VWR, Vienna) and ethyl acetate (technical quality) were used as solvents for the synthesis.

2.2. Enantioselective HPLC experiments

Chromatographic measurements were carried out on a 1100 Series HPLC system from Agilent Technologies (Waldbronn, Germany) equipped with an autosampler, a binary pump, a degasser for the mobile phase, and a multiple wavelength detector (MWD). In some preliminary runs an optical rotation detector OR-990

Table 1
Chromatographic data.

Compound	Chiralpak QD-AX[a]				Chiralpak QN-AX[a]				Chiralpak AD-H[b]			
	k_1	α	R_S	e.o.	k_1	α	R_S	e.o.	k_1	α	R_S	e.o.
1	2.53	1.33	2.4	(*R*)-(+)<(*S*)-(−)	2.66	1.21	1.8	(*S*)-(−)<(*R*)-(+)	0.49	1.23	1.8	*S*<*R*
2	1.98	1.30	2.3	(*R*)-(+)<(*S*)-(−)	2.52	1.20	1.7	(*S*)-(−)<(*R*)-(+)	0.47	1.25	1.9	*S*<*R*
3	5.04	1.77	6.5	*R*<*S*	5.31	1.83	7.2	*S*<*R*	2.14	1.68	6.4	*R*<*S*
4	8.70[c]	1.03	0.6	*R*<*S*	1.96[d]	1.06	1.0	*S*<*R*	1.25	1.12	1.7	*S*<*R*

[a] Methanol–glacial acetic acid–ammonium acetate (98:2:0.5; v/v/w); 25 °C; 1 mL/min; 150 mm × 4 mm I.D.
[b] Heptane–2-propanol–TFA (80:20:0.1; v/v/v); flow rate, 1 mL/min; temperature, ambient; 250 mm × 4 mm I.D.
[c] Methanol–glacial acetic acid (98:2; v/v); flow rate, 0.25 mL/min; temperature, 25 °C.
[d] Methanol–glacial acetic acid–ammonium acetate (98:2:0.5; v/v/w); 25 °C; flow 0.25 mL/min.

from Jasco (Gross-Umstadt, Germany) was coupled in series with a Corona charged aerosol detector (CAD) (ESA Analytical, Aylesbury, UK) to monitor the sign of optical rotation for the individual enantiomers. For analytical separations the sample was dissolved in methanol (Chiralpak QN-AX and QD-AX) and n-heptane/2-propanol (80:20; v/v) (Chiralpak AD-H), respectively, at a concentration of about 0.5 mg/mL and an aliquot of 2 μL was injected. UV detection was performed at 250 nm. The column temperature was kept constant at 25 °C. The data analysis was performed with the Chemstation chromatographic data software from Agilent Technologies. The chromatographic results are summarized in Table 1.

Preparative chromatography runs were carried out on a VWR EliteChrom HPLC System equipped with a quaternary gradient pump, a UV detector, a manual injector from Rheodyne with a 500 μL loop. The data were acquired and processed by EZ-Chrom software. The column was a 150 mm × 4 mm I.D. Chiralpak QD-AX. Separations were performed at ambient temperature employing an eluent consisting of a mixture of methanol–acetic acid–ammonium acetate (98:2:0.5; v/v/w) and a flow rate of 1 mL/min. The detection wavelength was 250 nm. For preparative scale separations on Chiralpak QD-AX, the sample was dissolved in methanol at a concentration of about 20 mg/mL. Depending on the type of solute and separation factor, about 5–10 mg sample masses per run were injected onto the analytical 150 mm × 4 mm I.D. column. Individual enantiomer fractions were collected separately and the combined fractions were evaporated to dryness. The obtained residues were extracted from slightly HCl-acidic saturated brine solution into ethyl acetate. The combined organic phases were dried over Na_2SO_4, filtrated and evaporated to dryness yielding the final enantiomeric products. Enantiomeric excess values of the obtained enantiomers are summarized in Table 2.

2.3. Synthesis of 2-(pyrimidin-2-ylthio)propionic acid (**4**) enantiomers

2.3.1. Synthesis

First, 0.50 g (4.5 mmol) of 2-mercaptopyrimidine was converted into its caesium salt by addition of 0.5 equiv. of Cs_2CO_3 (methanolic solution, 0.72 g, 2.25 mmol). After stirring for 1 h the solvent was evaporated and the residue dissolved in 40 mL of DMF (turbid solution). 1 equiv. (0.40 mL, 4.5 mmol) of (*S*)- or (*R*)-2-bromopropionic acid was added with a Hamilton syringe. The reaction mixture was stirred at room temperature (r.t.) for 14 h under N_2-atmosphere. The reaction progress was controlled by TLC (eluent: ethyl acetate–methanol (9/1); R_f = 0.49).

After that DMF was evaporated, the residue suspended in toluene and again evaporated to remove all of the DMF. The crude product was extracted with an acidic (pH ∼ 3–4), saturated NaCl-solution and ethyl acetate. The aqueous phase was washed with ethyl acetate (3× 20 mL) and the combined organic phases were dried over $MgSO_4$. After evaporation of the solvent a yellowish or white powder was furnished.

(*R*)-2-(pyrimidin-2-ylthio)-propionic acid (from (*S*)-(+)-2-bromopropionic acid): yield: 88% of a yellowish powder; enantiomeric excess (ee), 57%.

(*S*)-2-(pyrimidin-2-ylthio)-propionic acid (from (*R*)-(+)-2-bromopropionic acid): yield: 95% of a white powder; ee, 89%.

2.3.2. Characterization

^{1}H NMR and ^{13}C NMR were recorded at room temperature with a Bruker DRX400 spectrometer. Spectra were recorded in CD_3OD and the solvent signals were used as reference. Raw data were processed with SpinWorks Version 2.5.5. software. ESI-mass spectra were recorded on a PE Sciex API 365 spectrometer. Analytical thin-layer chromatography (TLC) was performed on Kieselgel 60 F_{254} plates from Merck (Darmstadt, Germany).

NMR and MS spectra were identical for both enantiomers, as expected.

^{1}H NMR [CD_3OD]: δ = 1.60 (3H, d, J = 7.5 Hz), 4.49 (1H, q, J = 22.0 Hz), 7.13 (1H, t, J = 10.0 Hz), 8.56 (2H, d, J = 4.9 Hz); MS (ESI, negative): 182.8 $[M-H]^-$.

3. Results and discussion

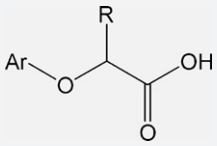
3.1. Analytical and preparative HPLC

Chiral pirinixic acid derivatives and analogs, respectively, are prime candidates to be separated into enantiomers on *O*-9-(*tert*-butylcarbamoyl)quinidine and corresponding quinine-based chiral stationary phases (Fig. 2) [30,31,39–41]. These acidic compounds are retained on such CSPs according to a primary anion-exchange

Table 2
Enantiomeric excess (%) and CSP employed for preparative scale separation of pirinixic acid analogs on Chiralpak QD-AX as well as their PPARα activity [1].

	ee (%)	PPARα activity, EC50 (μM) ± SD (rel. activation compared to control means ± SD)
(*R*)-**1**	94	2.2 ± 0.1 (151 ± 4%)
(*S*)-**1**	93	9.7 ± 0.3 (147 ± 3%)
(*R*)-**2**	91	0.5 ± 0.2 (159 ± 26%)
(*S*)-**2**	93	5.61 ± 0.7 (166 ± 14%)
(*R*)-**3**	99	0.03 ± 0.005 (113 ± 4%)
(*S*)-**3**	97	2.2 ± 0.4 (147 ± 13%)

Table 3
Enantiomer separation data of reference aryloxycarboxylic acids[a].



Compound	R	Ar	Chiralpak QN-AX			Chiralpak QD-AX			Chiralpak AD-H		
			k_1	α	e.o.	k_1	α	e.o.	k_1	α	e.o.
			Mobile phase, methanol–acetic acid–ammonium acetate (98:2:0.5; v/v/w)						Mobile phase, heptane–2-propanol (80:20; v/v) + 0.1% (v/v) TFA		
5	CH_3	2-Naphthyl	2.78	1.17	*S* < *R*	2.73	1.24	*R* < *S*	0.71	1.33	*S* < *R*
6	CH_3	4-Chlorophenyl	1.93	1.08	*S* < *R*	1.90	1.18	*R* < *S*	0.57	1.50	*S* < *R*
7	C_2H_5	4-Chlorophenyl	1.67	1.13	*S* < *R*	1.70	1.19	*R* < *S*	0.54	1.25	*S* < *R*
8	$CH(CH_3)_2$	4-Chlorophenyl	1.52	1.21	*S* < *R*	1.44	1.18	*R* < *S*	0.44	1.10	*R* < *S*
9 (Dichloroprop)	CH_3	2,4-Dichlorophenyl	2.38	1.21	*S* < *R*	2.47	1.42	*R* < *S*	0.40	1.88	*S* < *R*
			Mobile phase, methanol–0.1 M ammonium acetate buffer (80:20; v/v) (pHa = 6.0)								
5	CH_3	2-Naphthyl	11.10	1.11	*S* < *R*	1.61	1.18	*R* < *S*	–	–	–
6	CH_3	4-Chlorophenyl	7.59	1.08	*S* < *R*	1.00	1.15	*R* < *S*	–	–	–
7	C_2H_5	4-Chlorophenyl	7.09	1.09	*S* < *R*	n.d.	–	–	–	–	–
9 (Dichloroprop)	CH_3	2,4-Dichlorophenyl	10.94	1.19	*S* < *R*	7.21	1.29	*R* < *S*	–	–	–

[a] Experimental conditions: flow rate, 1 mL/min; temperature, 25 °C.

retention process. Hence, the eluent must contain a certain amount of chiral acids and counter-ions, respectively, that compete for binding at the ion-exchange site and allow the elution of solutes by their displacement from these primary interaction sites. A methanol-based mobile phase consisting of acetic acid and ammonium acetate providing counter- and co-ions was employed in the first instance for separation of the target solutes. The enantiomer separation results obtained with these two anion-exchangers are summarized in Table 1. It is obvious that arylthiocarboxylic acids with electron-withdrawing chlorine substitution (compounds **1**, **2**, and **3**) are much better separated than the analog which lacks such an electron-withdrawing group (**4**). These trends give rise to the conclusion that the aromatic moiety of the solutes is involved in π–π-interactions with the electron-rich quinoline moiety of cinchona alkaloid derived selectors. This points towards a chiral recognition mechanism resembling that of aryloxy carboxylic acids of the type Ar–X–CH(R_1)–COOH with X being –O– for which the enantioselectivity factor α increased with the electron-withdrawing effect of the aromatic substituents (Table 3) [42]. Fig. 3 depicts chromatograms of separations obtained for **1** and **2** on quinidine and quinine carbamate-based anion-exchangers with charged aerosol detector (CAD) along with optical rotation detector (ORD) traces (corrected for delay times between detectors). It is worth noting that elution orders are reversed when the quinidine based CSP is exchanged for its quinine-based counterpart (Fig. 3). This "pseudo-enantiomeric" behaviour of quinidine- and quinine-derived CSPs which possess opposite configurations at the stereogenic centers of C8 and C9 (Fig. 1), but equal configurations in positions 1, 3, and 4 is also in agreement with what is known from corresponding α-aryloxy carboxylic acid type herbicides. Fig. 4 shows the enantiomer separation of compound **3** on the quinine carbamate CSP.

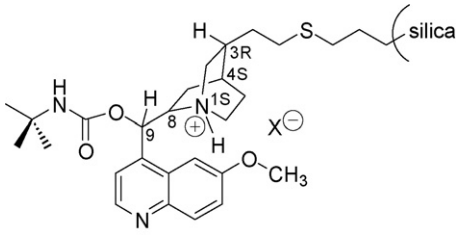


Fig. 2. Quinine and quinidine carbamate-based chiral stationary phases. Chiralpak QN-AX: (8*S*,9*R*), quinine derived; Chiralpak QD-AX: (8*R*,9*S*), quinidine derived.

Chromatographic separations of compounds **1**, **2**, and **3** were finally performed preparatively in 20–100 mg scale employing the quinidine carbamate-based CSP to produce single enantiomers for *in vitro* activity tests on PPARα and PPARγ receptor subtypes [1]. Table 2 summarizes the results in terms of enantiomeric excess that was measured for each enantiomer along with information on employed column type. Throughout single enantiomers with a high enantiomeric excess could be obtained (typically between 90% and 99% ee). Such ee-values are certainly good enough for supporting unambiguous *in vitro* tests to elucidate the receptor subtype stereoselectivities (Table 2).

3.2. Absolute configuration assignment

3.2.1. By correlation with aryloxycarboxylic acids as reference system

Prior information on chromatographic enantiomer separation data and on preferred binding affinities of quinidine and quinine carbamate selectors existed for a wider set of aryloxycarboxylic acids of the type Ar–X–CH(R_1)–COOH with X being –O– as mentioned above [42]. A selection of such data is shown in Table 3. The quinidine CSP displays slightly better enantioselectivities than the quinine CSP which showed reversed elution order. Most importantly, *R*-enantiomers exhibit throughout higher affinities to quinine carbamate selectors, while *S*-enantiomers show consistently higher binding strength to quinidine carbamate selectors.

The thio ether group is isosteric to the ether group. Thus the chiral recognition mechanism is supposed to be identical for these two sets of chiral acids Ar–X–CH(R_1)–COOH with X being –O– and –S–. Enantiomers of 2-arylthiocarboxylic acids eluting first from the quinidine carbamate CSP should therefore have *R*-configuration and the second eluted enantiomers *S*-configuration.

3.2.2. Via chemical correlation

No single enantiomer standards with known absolute configuration were available from the thioether subset to validate that the substitution of the oxygen by a sulphur as well as replacement of the phenyl ring by a pyrimidine ring does not perturb the molecular recognition mechanism of cinchonan carbamate selectors. Thus, we considered to synthesize single enantiomers of

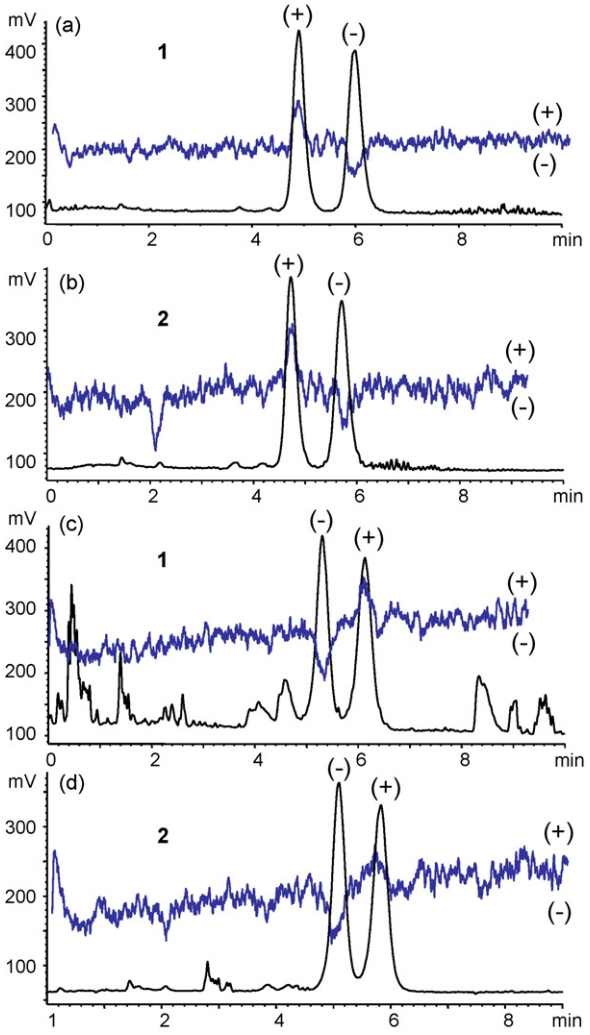


Fig. 3. HPLC-CAD chromatograms with overlaid ORD traces of compounds **1** (a and c) and **2** (b and d) on *O*-9-(*tert*-butylcarbamoyl) quinidine (a and b) and quinine (c and d) based CSPs. Experimental conditions: eluent, methanol–glacial acetic acid–ammonium acetate (98:2:0.5; v/v/w); flow rate, 1 mL/min; temperature, 25 °C. (Note, the extra peaks in chromatogram c represent detector noise/spikes! The ORD traces were shifted for the delay time between the detectors to align the corresponding chromatograms!).

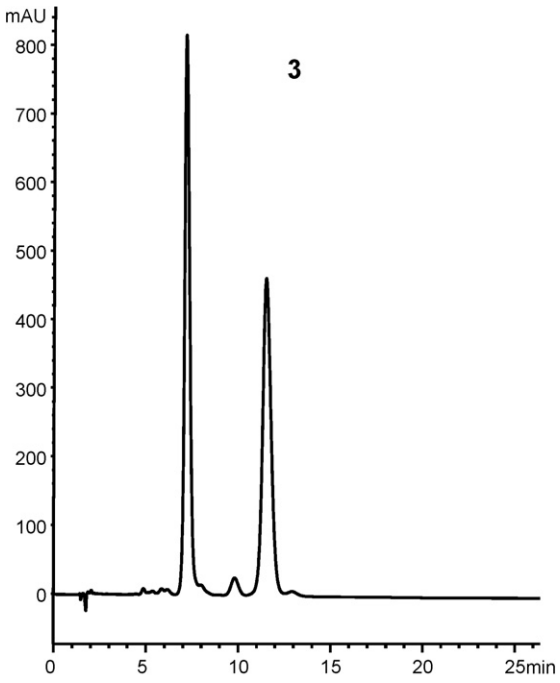


Fig. 4. HPLC enantiomer separations of **3** on *O*-9-(*tert*-butylcarbamoyl) quinine-based CSP. Experimental conditions: Eluent, methanol–glacial acetic acid–ammonium acetate (98:2:0.5; v/v/w); flow rate, 1 mL/min; temperature, 25 °C.

2-(2-pyrimidinylthio)propionic acid. They were readily accessible from (*R*)- and (*S*)-2-bromopropionic acid by nucleophilic substitution (SN_2) with pyrimidine-2-thiol (Fig. 5). Due to inversion of the stereoconfiguration in the course of SN_2, the *S*-enantiomer of the 2-(2-pyrimidinylthio)propionic acid should be obtained from (*R*)-bromopropionic acid and the *R*-enantiomer of the product from (*S*)-bromopropionic acid. It turned out that high enantiomeric excess values of the products are solely afforded if the reaction is carried out with caesium thiolate as nucleophile while nearly racemic products were obtained with thiol as reagent [43]. As can be seen from Fig. 6, relatively high ee values of 57% and 89% for *R*- and *S*-products, respectively, resulted when caesium thiolate was employed for the nucleophilic substitution reaction (vs. ~10% ee with thiol). Moreover, on the quinine carbamate CSP the *S*-enantiomer eluted prior to the *R*-enantiomer (Fig. 6a and b) while the elution order is reversed on the quinidine carbamate CSP (not shown). These results confirm the above chromatographic absolute

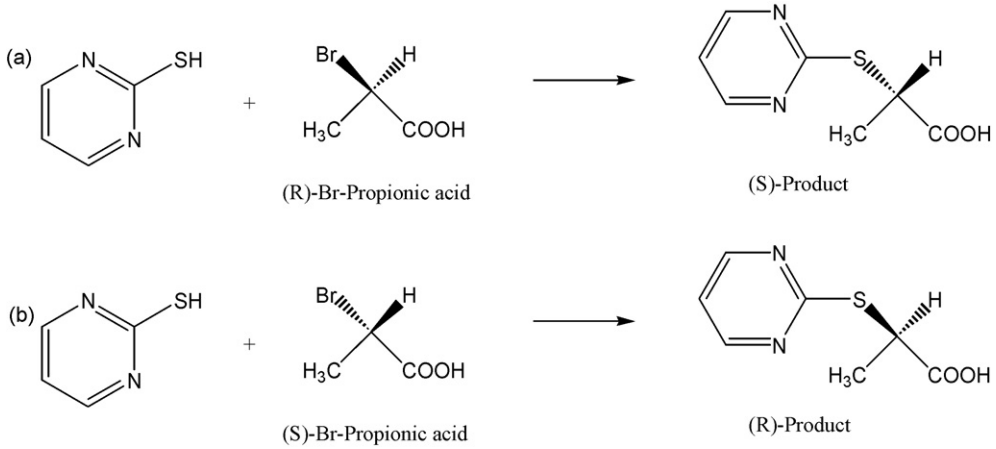


Fig. 5. Reaction scheme for the preparation of 2-(pyrimidin-2-ylthio)propionic acid (**4**) enantiomers. (a) *S*-product from (*R*)-bromopropionic acid, and (b) *R*-product from (*S*)-bromopropionic acid.

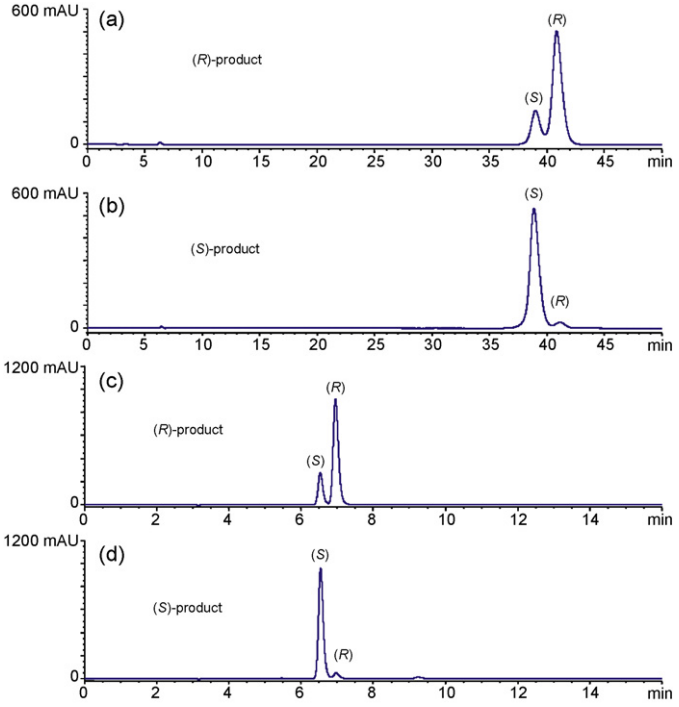


Fig. 6. Analytical quality control of the synthesized enantiomers of 2-(pyrimidin-2-ylthio)propionic acid (**4**) on a *O*-9-(*tert*-butylcarbamoyl) quinine-based CSP (a and b) and an amylose tris(3,5-dimethylphenylcarbamate) type CSP (c and d). Conditions: (a and b) mobile phase, methanol-acetic acid (98:2; v/v); flow rate, 0.25 mL/min; ambient temperature; (c and d) mobile phase, heptane–2-propanol–TFA (80:20:0.1; v/v/v); flow rate, 1.0 mL/min; ambient temperature; UV detection at 254 nm.

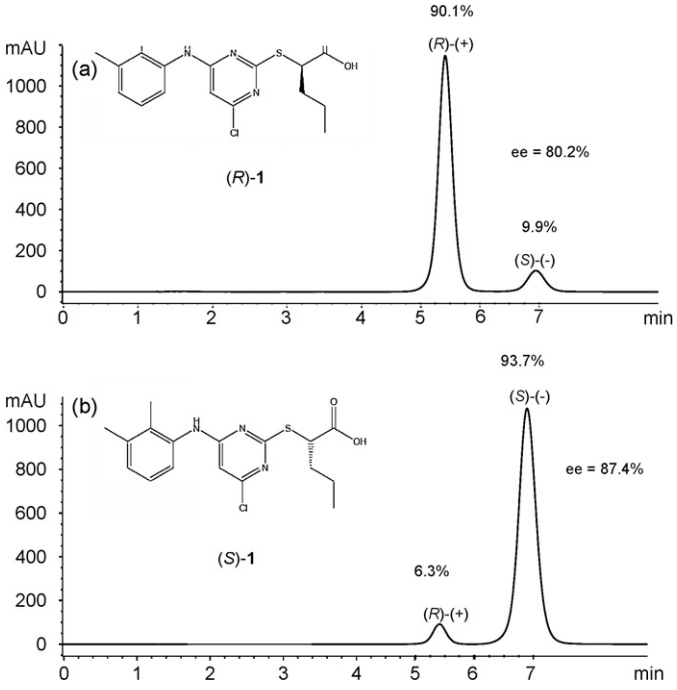


Fig. 8. Enantiomeric purity determination of (*R*)-**1** (a) and (*S*)-**1** (b) obtained from enantioselective synthesis via Evan's auxiliary. Chiral stationary phase, Chiralpak QD-AX; column dimension, 150 mm × 4 mm I.D.; Eluent, methanol–glacial acetic acid–ammonium acetate (98:2:0.5; v/v/w); flow rate, 1 mL/min; temperature, 25 °C; sample, (*R*)-**1** (2.56 mg/0.5 mL in methanol); sample, (*S*)-**1** (2.86 mg/0.5 mL in methanol); injection volume, 1 μL; detection, UV 254 nm.

configuration assignment on basis of elution orders of α-aryloxy alkanoic acids as reference compounds.

3.2.3. *Via stereoselective synthesis employing Evan's auxiliary*

The above chromatographic absolute configuration assignment represents an indirect method that makes use of reference compound(s) with known configuration and assumes consistencies of chiral recognition mechanisms between reference and sample compounds. Since this is not necessarily always the case, verification by an independent method is of utmost importance. In the present study, an enantioselective synthesis method has been devised for preparation of α-substituted carboxylic acid enantiomers employing the established method of Evan's auxiliary [44] (Fig. 7). It starts from the *R*- or *S*-enantiomer of benzyloxazolidinone which was first converted into its *N*-alkanoyl derivative. The following α-substitution reaction involves an intermediary metal-chelated (Z)-enolate in which the C_4-substitutent of the oxazolidinone ring dictates the diastereoface selection of nucleophilic reagent [44]. By starting from (*R*)-4-benzyloxazolidinone primarily *R*-configurated intermediates and end products, respectively, are expected to be obtained (Fig. 7). In fact, (*R*)-**1** could be obtained from (*R*)-benzyloxazolidinone with an ee of about 80% and the resultant enantiomeric product showed the expected elution order on the quinidine carbamate CSP as predicted by above described considerations (Fig. 8a). The corresponding (*S*)-4-benzyloxazolidinone starting material gives rise to formation of the *S*-enantiomers of the target α-arylthio carboxylic acids as demonstrated by the chromatogram for (*S*)-**1** in Fig. 8b (ee = 87%). Similar results could be achieved for **2** (e.g. 90% ee for *S*-enantiomer) with the elution order

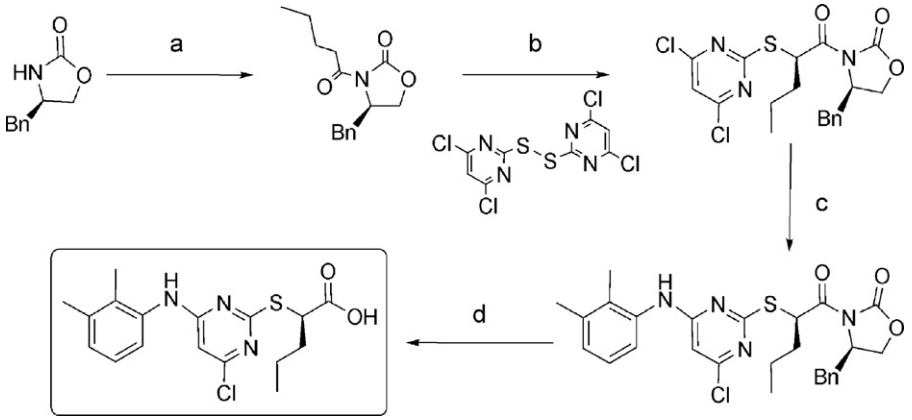


Fig. 7. Reaction scheme for stereoselective synthesis of (*R*)-**1** via Evan's auxiliary (*R*)-benzyloxazolidinone (corresponding (*S*)-**1** can be obtained by starting with (*S*)-benzyloxazolidinone). Reagents and conditions: (a) potassium-*tert*-butoxide, pentanoyl chloride, abs. THF, 0 °C, 1 h (b) LDA, abs. THF, −78 °C, 3 h; (c) 2,3-dimethylaniline, *N*-ethyl isopropyl amine, THF, reflux, 3.5 d; (d) LiOH, THF, H_2O, 0 °C → r.t., 3 h. Experimental details have been described elsewhere [1].

being fully in agreement with predictions of the indirect chromatographic assignment.

3.3. Changes in molecular recognition mechanisms as risks for false assignments exemplified by polysaccharide CSP

Enantioselective liquid chromatography is probably the most important method for absolute configuration assignment of enantiomers via their elution order if it has been previously proven for the same pair of enantiomers under the same conditions. Any CSP can be safely applied for this purpose provided that the enantioselectivity of the CSP is good enough and the elution order has been validated before.

A frequent important task in drug discovery is to determine the absolute configurations of a set of structural congeners synthesized for sake of lead optimization. X-ray and CD spectroscopy may be too time consuming to apply them for all individual members of a test set. Hence, some sort of correlations such as chromatographic assignments may be put in place instead for sake of simplicity. However, one must bear in mind that the prediction of absolute configurations of structural analogs based on elution orders that have been established with a reference system of the same lead structure, but distinct substitution patterns (like herein) is by far more critical. Considerable precaution is of utmost importance as variations in molecular recognition within a congeneric set of compounds may easily occur upon different substitutions of a lead structure. In principle, this holds for all type of CSPs, yet polysaccharide CSPs may be particularly prone to such perturbations and alterations in chiral recognition mechanisms and elution orders in dependence of substitution patterns. This may be attributed to the multiple binding modes that may exist for such polymeric selectors (as opposed to more well-defined receptor-like brush-type selectors). It is also obvious that the risk for perturbations in the chiral recognition mechanism within a congeneric series increases with decreasing similarity of the substitutions around the stereogenic center. The problem will be illustrated hereafter for the current test set with a polysaccharide CSP.

The target test compounds **1**–**4** were also injected on an amylose tris(3,5-dimethylphenylcarbamate) coated polysaccharide-type CSP. This CSP exhibited good enantioselectivities for the target solutes as pointed out above (Table 1). However, it is striking that the elution orders within the test set of α-arylthiocarboxylic acids are less consistent than with quinidine and quinine carbamate CSPs. Most notably, the elution order was reversed for 2-(pyrimidin-2-ylthio)propionic acid derivative **3** as compared to the other α-arylthiocarboxylic acids. A similar observation is found for the α-aryloxycarboxylic acid **8** (see Table 3). Such reversals of elution order with minor structural changes have been frequently reported in the literature for polysaccharide type CSPs (e.g. Ref. [35]). They constitute a serious problem limiting polysaccharide CSPs for the purpose of chromatographic absolute configuration predictions of structural analogs. For example, if the synthesized 2-(pyrimidin-2-ylthio)propionic acid enantiomers were taken as reference compounds to chromatographically assign configurations of pirinixic acid analogs **1**–**3**, one would have run into problems with false absolute configuration assignments for **3**.

Polysaccharide CSPs are widely applicable for enantiomer separations of various types of solutes. However, the molecular recognition mechanisms with which they distinguish between enantiomers remains mostly concealed because of the structural complexity of polymeric selectors. Understanding of interaction mechanisms, though, would be helpful or even necessary for unequivocal indirect chromatographic absolute configuration assignments. Current knowledge states that hydrogen bonding, dipole–dipole interaction as well as π–π-interactions are driving forces for inclusion complexation into the grooves formed by the pendant aryl carbamate residues of the polysaccharide selectors [35,45]. Steric factors may play a major role which enantiomer is the stronger bound one. Such mechanisms are sensitive to structure-induced binding mode alterations, even within a congeneric series. This has been demonstrated, for example, in a recent paper by Ma et al. in which they described the reversal of the elution order for two structural analogs that differed solely in π-acidity/basicity of an aromatic group [35]. Even minor variations of steric factors, π-electron density and substitution patterns may easily lead to perturbation of molecular recognition mechanisms within a series of structural analogs on polysaccharide CSPs leading to altered elution orders. While this may provide the basis for the broad applicability, it makes polysaccharide CSPs virtually useless for chromatographic absolute configuration assignments of structural analogs for which elution orders have not been validated before. In the given case, the reversal of elution order for compound **3** as compared to the other arylthiocarboxylic acids could be triggered by the additional aromatic substitutions on the arylthio ring system ("remote effects"), while the change in elution order of compound **8** in comparison to the remaining set of aryloxycarboxylic acids may have been induced by the steric bulkiness of the R-substituent (see Table 3).

In contrast, on quinine and quinidine carbamate CSPs elution orders were found to be more consistent [39–41,46,47], and this seems to be valid for aryloxy carboxylic acids [42] and arylthiocarboxylic acids likewise. The primary driving force for interaction of acidic solutes with the selectors is an ionic hydrogen bond at the fixed anion-exchange site. Thus, all test analytes are equally oriented towards the quinuclidine ring. They are similarly aligned in the active binding and chiral distinction site of the selector which is spanned by moieties arranged around the C9-stereogenic center [18,46]. Short-range secondary interactions that only become activated if steric dispositions are spatially favorable are then deciding on the preferentially established chiral recognition mechanism and the elution order. For the present aryloxy- and arylthio carboxylic acids π–π-interaction of the α-aryl group with quinoline is tentatively driving enantioselectivity and elution order on the cinchona alkaloid CSPs. The degree of enantioselectivity usually increases significantly with π-acidity, yet also π-basic solutes are separated. The elution order does not change with π-acidity/basicity (see Table 3). Thus, the chiral recognition mechanism is more consistent facilitating the application for absolute configuration assignment. Substitutions farther away from the stereogenic center such as structural decorations at the α-arylthio moiety have usually less effect on the principal chiral recognition mechanism and the elution order, as they are exposed to unoccupied open space of the chiral selector's binding site (see X-ray crystal structures in Refs. [18,46,48]). Scrambling of the enantiorecognition mechanism might be envisaged by acidic groups in the alkyl side chain of the solutes which are, however, not present in the current solute set. Being aware of mechanistic fundamentals on such low molecular anion-exchangers, its use for predictions of absolute configurations of structural analogs is feasible with high confidence, unlike with polymeric polysaccharide CSPs.

4. Conclusion

Enantiomers of pirinixic acid analogs were obtained by preparative chromatography and enantioselective synthesis. Absolute configuration assignments were based, in the first instance, on chromatographic elution orders on cinchona alkaloid derived CSPs taking a set of α-aryloxycarboxylic acids with known absolute configurations as reference system. Since this methodology bears some considerable risks for false assignments, several attempts for verification of the absolute configuration predictions were undertaken. First, 2-(pyrimidin-2-ylthio)propionic acid enantiomers

with established configurations were synthesized stereoselectively. Second, enantiomers of two pirinixic acid derivatives were synthesized by enantioselective synthesis via Evan's auxiliary. Both approaches confirmed the assigned configurations. Moreover, receptor stereoselectivity in terms of preferential activity was consistent within the tested series with significantly lower EC_{50} values at the PPARα receptor for the *R*-enantiomers (more active) of **1**, **2**, and **3** being a further indication for a correct assignment [1].

In general, it turned out that the chiral recognition mechanism is more consistent within the test set of α-arylthiocarboxylic acids on the cinchonan carbamate-based CSPs while limited correlation (between elution order and substitution) was observed with the polysaccharide type CSP. This finding suggests that chromatographic absolute configuration assignments based on elution orders which have been validated for a pair of enantiomers with known configuration is always safely possible for this pair of enantiomers, while predictions for structural analogs assuming an identical chiral recognition mechanism bears a considerable risk for false assignments if the molecular recognition mechanism is unknown. Confirmations by an orthogonal methodology are therefore absolutely required. Yet, configuration predictions of structural analogs based on elution orders on polysaccharide CSPs are strongly discouraged because of the high susceptibility to alterations of the chiral recognition mechanisms even with minute structural changes. CD and X-ray diffraction methodologies appear to be better choices if such technologies are readily available, but are more laborious and time consuming. They are therefore usually applied for one or two members of a congeneric series. For the rest of the molecules indirect assignments are still frequently employed.

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References

[1] H. Zettl, M. Dittrich, R. Steri, E. Proschak, O. Rau, D. Steinhilber, G. Schneider, M. Lämmerhofer, M. Schubert-Zsilavecz, QSAR Comb. Sci. 28 (2009) 576.
[2] H.Y. Aboul-Enein, I.W. Wainer (Eds.), The Impact of Stereochemistry on Drug Development and Use, John Wiley, New York, 1997.
[3] E.R. Francotte, J. Chromatogr. A 906 (2001) 379.
[4] C. Roussel, A. Del Rio, J. Pierrot-Sanders, P. Piras, N. Vanthuyne, J. Chromatogr. A 1037 (2004) 311.
[5] N. Harada, Chirality 20 (2008) 691.
[6] S. Allenmark, J. Gawronski, Chirality 20 (2008) 606.
[7] W.H. Pirkle, J. Chem. Soc. [Sect.] D: Chem. Commun. (1970) 1525.
[8] W.H. Pirkle, K.A. Simmons, J. Org. Chem. 46 (1981) 3239.
[9] J.K. Rugutt, H.H. Yarabe, S.A. Shamsi, D.R. Billodeaux, F.R. Fronczek, I.M. Warner, Anal. Chem. 72 (2000) 3887.
[10] M. Kosaka, T. Sugito, Y. Kasai, S. Kuwahara, M. Watanabe, N. Harada, G.E. Job, A. Shvet, W.H. Pirkle, Chirality 15 (2003) 324.
[11] J. Naito, M. Kosaka, T. Sugito, M. Watanabe, N. Harada, W.H. Pirkle, Chirality 16 (2004) 22.
[12] G.E. Job, A. Shvets, W.H. Pirkle, S. Kuwahara, M. Kosaka, Y. Kasai, H. Taji, K. Fujita, M. Watanabe, N. Harada, J. Chromatogr. A 1055 (2004) 41.
[13] M. Kurosu, K. Li, Org. Lett. 11 (2009) 911.
[14] D. Casarini, L. Lunazzi, F. Gasparrini, C. Villani, M. Cirilli, E. Gavuzzo, J. Org. Chem. 60 (1995) 97.
[15] C. Roberto, F. Rosella, G. Bruno, T. Luciana, B. Adriana, S. Daniela, C. Paola, P. Marco, F. Vincenzo, B. Olivia, L.T. Francesco, Chirality 16 (2004) 625.
[16] C. Danel, C. Foulon, A. Guelzim, C.H.A. Park, J.-P. Bonte, C. Vaccher, Chirality 17 (2005) 600.
[17] J.-V. Naubron, L. Giordano, F. Fotiadu, T. Buergi, N. Vanthuyne, C. Roussel, G. Buono, J. Org. Chem. 2006 (2006) 5586.
[18] W. Bicker, I. Chiorescu, V.B. Arion, M. Lämmerhofer, W. Lindner, Tetrahedron Asymm. 19 (2008) 97.
[19] U. Kiehne, T. Bruhn, G. Schnakenburg, R. Frohlich, G. Bringmann, A. Luetzen, Chem. Eur. J. 14 (2008) 4246.
[20] Y. Zhang, B. Song, P.S. Bhadury, D. Hu, S. Yang, X. Shi, D. Liu, L. Jin, J. Sep. Sci. 31 (2008) 2946.
[21] M.-P. Vaccher, J. Charton, A. Guelzim, D.-H. Caignard, J.-P. Bonte, C. Vaccher, J. Pharm. Biomed. Anal. 46 (2008) 920.
[22] C. Roberto, F. Rosella, L.T. Francesco, B. Anna, F. Vincenzo, C. Mercedes, F. Cristina, R. Dante, M. Antonello, Chirality 21 (2009) 604.
[23] P.J. Stephens, F.J. Devlin, F. Gasparrini, A. Ciogli, D. Spinelli, B. Cosimelli, J. Org. Chem. 72 (2007) 4707.
[24] W. Bicker, K. Kacprzak, M. Kwit, M. Lämmerhofer, J. Gawronski, W. Lindner, Tetrahedron Asymm. 20 (2009) 1027.
[25] S. Abbate, G. Longhi, E. Castiglioni, F. Lebon, P.M. Wood, L.W.L. Woo, B.V.L. Potter, Chirality 21 (2009) 802.
[26] S. Abbate, L.F. Burgi, E. Castiglioni, F. Lebon, G. Longhi, E. Toscano, S. Caccamese, Chirality 21 (2009) 436.
[27] H.D. Flack, G. Bernardinelli, Chirality 20 (2008) 681.
[28] W.H. Pirkle, A. Tsipouras, M.H. Hyun, D.J. Hart, C.S. Lee, J. Chromatogr. 358 (1986) 377.
[29] W. Pirkle, H.L.J. Brice, T.S. Widlanski, J. Roestamadji, Tetrahedron Asymm. 7 (1996) 2173.
[30] E. Zarbl, M. Lämmerhofer, F. Hammerschmidt, F. Wuggenig, M. Hanbauer, N.M. Maier, L. Sajovic, W. Lindner, Anal. Chim. Acta 404 (2000) 169.
[31] M. Lämmerhofer, D. Hebenstreit, E. Gavioli, W. Lindner, A. Mucha, P. Kafarski, P. Wieczorek, Tetrahedron Asymm. 14 (2003) 2557.
[32] S. Coles, D. Davies, M. Hursthouse, S. Yesilot, B. Cosut, A. Kilic, Acta Crystallogr. Sect. B: Struct. Sci. B65 (2009) 355.
[33] K. Balmér, B.-A. Persson, P.-O. Lagerström, J. Chromatogr. A 660 (1994) 269.
[34] B.-A. Persson, S. Andersson, J. Chromatogr. A 906 (2001) 195.
[35] S. Ma, S. Shen, H. Lee, M. Eriksson, X. Zeng, K. Fandrick, N. Yee, C. Senanayake, N. Grinberg, J. Chromatogr. A 1216 (2009) 3784.
[36] E. Francotte, T. Zhang, J. Chromatogr. A 718 (1995) 257.
[37] G. Massolini, G. Fracchiolla, E. Calleri, G. Carbonara, C. Temporini, A. Lavecchia, S. Cosconati, E. Novellino, F. Loiodice, Chirality 18 (2006) 633.
[38] A. Ghanem, J. Chromatogr. A 1132 (2006) 329.
[39] M. Lämmerhofer, W. Lindner, J. Chromatogr. A 741 (1996) 33.
[40] N.M. Maier, L. Nicoletti, M. Lämmerhofer, W. Lindner, Chirality 11 (1999) 522.
[41] A. Mandl, L. Nicoletti, M. Lämmerhofer, W. Lindner, J. Chromatogr. A 858 (1999) 1.
[42] M. Lämmerhofer, PhD thesis, Karl-Franzens University Graz, 1996.
[43] B. Strijtveen, R.M. Kellogg, J. Org. Chem. 51 (1986) 3664.
[44] D.A. Evans, M.D. Ennis, D.J. Mathre, J. Am. Chem. Soc. 104 (1982) 1737.
[45] T.D. Booth, I.W. Wainer, J Chromatogr. A 737 (1996) 157.
[46] C. Czerwenka, M. Lämmerhofer, N.M. Maier, K. Rissanen, W. Lindner, Anal. Chem. 74 (2002) 5658.
[47] C. Czerwenka, M. Lämmerhofer, W. Lindner, J. Sep. Sci. 26 (2003) 1499.
[48] K. Akasaka, K. Gyimesi-Forras, M. Lämmerhofer, T. Fujita, M. Watanabe, N. Harada, W. Lindner, Chirality 17 (2005) 544.