Journal of Chromatography, 498 (1990) 67–79
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 966

(*S*)-*thio*-DNBTYR-A AND (*S*)-*thio*-DNBTYR-E AS CHIRAL STATIONARY PHASES FOR ANALYTICAL AND PREPARATIVE PURPOSES

APPLICATION TO THE ENANTIOMERIC RESOLUTION OF ALKYL N-ARYLSULPHINAMOYL ESTERS

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(First received July 3rd, 1989; revised manuscript received September 5th, 1989)

SUMMARY

The enantiomeric separation of *tert.*-butyl N-arylsulphinamoyl esters, which are chiral compounds of biological interest, was investigated on three chiral stationary phases (CSPs): (*R*)-DNBPG, (*S*)-*thio*-DNBTyr-A and (*S*)-*thio*-DNBTyr-E. (*S*)-*thio*-DNBTyr-A exhibits the greatest enantiorecognition ability towards these solutes, allowing the extension of chiral separation to the preparative scale. Chiral recognition mechanisms are discussed. They involve several parameters, *e.g.*, the nature of the polar modifier used in the mobile phase, the π-basicity of the solute, the number of sites of interaction on the CSP and the steric hindrance due to the silica matrix.

INTRODUCTION

Chiral stationary phases (CSPs) derived from N-(3,5 dinitrobenzoyl)amino acids are widely used for chromatographic chiral separations on both analytical and preparative scales[1–3]. They include the well known (*R*)-DNBPG, derived from (*R*)-phenylglycine, designed by Pirkle *et al.*[4]. The wide scope of application of these CSPs probably results from the various potential sites of interactions occurring in the vicinity of the asymmetric centre. Chiral recognition mechanisms involved with these CSPs have been extensively studied[1–3], and the results indicate that small structural changes in the CSPs have significant effects on chromatographic behaviour[5–7].

Recently, we developed two novel π-acid CSPs derived from (*S*)-tyrosine[7–9]. In comparison with (*R*)-DNBPG, the original features of these CSPs result from the

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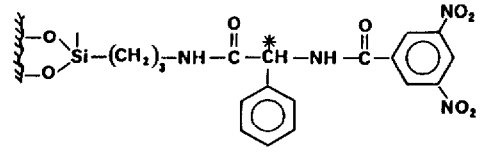
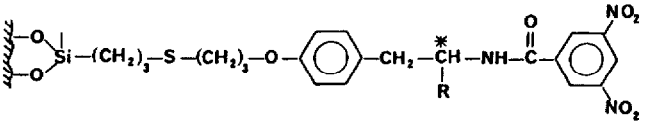
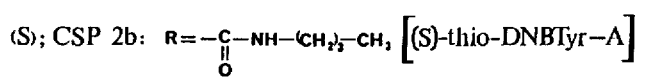


(R); CSP 1 [(R)-DNBPG]



(S); CSP 2a: R = —C(=O)—O—CH_3 [(S)-thio-DNBTyr—E]



(S); CSP 2b: R = —C(=O)—NH—$(CH_2)_3$—CH_3 [(S)-thio-DNBTyr—A]

Fig. 1. Structure of CSPs.

grafting mode: (*S*)-tyrosine is bound to silica gel via its hydroxyl group, thus allowing the introduction of various functional groups on its carbonyl moiety *e.g.*, methyl ester [(*S*)-*thio*-DNBTyr-E] or *n*-butylamide [(*S*)-*thio*-DNBTyr-A](see Fig. 1). Both CSPs exhibit different behaviour. So far, for a few series of compounds including sulphoxides, phosphine oxides and lactams of pharmaceutical interest, we have found that (*S*)-*thio*-DNBTyr-E displays the widest range of application, whereas (*S*)-*thio*-DNBTyr-A exhibits a greater selectivity[7,8]. In comparison with (*R*)-DNBPG, which is bound to silica via an aminopropyl spacer, (*S*)-*thio*-DNBTyr-A possesses a longer spacer arm, the asymmetric centre then being removed further from the silica matrix whose steric effect is minimized. Consequently, the chromatographic behaviour of these CSPs is expected to be different. Very recently, Pirkle and Burke[10] employed the grafting mode chosen for (*S*)-*thio*-DNBTyr-A again in order to design a novel CSP.

By designing these two novel CSPs we aimed to extend the scope of application of Pirkle-type CSPs. This objective was applied to separate a family of compounds with the general formula $ArNHSOCR_1R_2COOC(CH_3)_3$, named *tert.*-butyl N-arylsulphinamoyl esters. These compounds act as specific inhibitors of coniferyl alcohol dehydrogenase (CADH), a zinc metalloenzyme involved in the lignification process of plants[11,12]. The synthesis and physico-chemical properties of these compounds were initially described by Cazaux and co-workers[13–17].

In this paper we report the direct enantiomeric separation of a series of *tert.*-butyl N-arylsulphinamoyl esters on (*R*)-DNBPG, (*S*)-*thio*-DNBTyr-A and (*S*)-*thio*-DNBTyr-E. Some insights into the chiral recognition mechanisms are also given and

the chromatographic and structural parameters that may affect them are evaluated. In addition, an example of preparative chiral chromatography is presented.

EXPERIMENTAL

Apparatus

For liquid chromatography, a modular liquid chromatograph (Gilson, Villiers-le-Bel, France) equipped with a Shimadzu C-R4A integrator (Touzart et Matignon, Vitry-sur-Seine, France), was used. The standard operating conditions were flow-rate 2 ml/min and room temperature.

Preparative chromatography was performed with a Modulprep apparatus (Jobin-Yvon, Longjumeau, France). The chiral stationary phase [200 g of (*S*)-*thio*-DNBTyr-A, $d_p = 7$ μm][7] was packed into the column (260 × 40 mm I.D.) by axial compression under 15 bar. UV detection was carried out at 254 nm with a variable-wavelength detector (190–370 nm) (Jobin-Yvon). The preparative chromatograph was operated at room temperature. The eluent inlet pressure was about 11 bar, which gave a flow-rate of *ca.* 42 ml/min.

Chiral stationary phases

The structures of the CSPs are given in Fig. 1. CSP 1 [(*R*)-DNBPG, $d_p = 5$ μm] is a Pirkle covalent column (250 × 4.6 mm I.D.) commercially available from J. T. Baker (Sochibo, Velizy-Villacoublay, France).

General procedures for the synthesis of CSP 2a and 2b derived from (*S*)-tyrosine were given in a previous paper[7]. The CSPs were obtained starting from LiChrosorb Si-60 silica gel (5 μm) (Merck, Darmstadt, F.R.G.) modified with γ-mercaptopropyltrimethoxysilane (Fluka, Buchs, Switzerland)[7]. They were packed into 150 × 4.6 mm I.D. stainless-steel columns by the classical slurry technique under 400 bar using ethanol as pumping solvent.

Mobile phase

Ethanol, isopropanol and hexane were of LiChrosolv grade (Merck) and chloroform and methylene chloride were of analytical-reagent grade (Prolabo, Paris, France).

Solutes

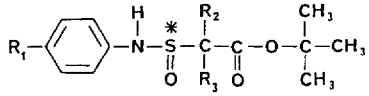
The structures of the *tert.*-butyl N-arylsulphinamoyl esters (**1a–d**, **2a** and **b**) are given in Table I. Samples of these compounds were given by Professor L. Cazaux (Laboratoire de Synthese et de Physicochimie Organique, Université Paul Sabatier, Toulouse, France).

RESULTS AND DISCUSSION

Chromatographic data are given in Table II. For each polar modifier, its concentration (%) in the mobile phase, the selectivity value (α) and the capacity factor for the most retained enantiomer (k'_2) are reported.

TABLE I

STRUCTURE OF TEST SOLUTES



Compound	R_1	R_2	R_3
1a	NO_2	H	H
1b	CF_3	H	H
1c	Cl	H	H
1d	OCH_3	H	H
2a[a]	H	H	CH_3
2b	H	CH_3	CH_3

[a] Two asymmetric centres.

Influence of the nature of the mobile phase

Solvents were chosen according to their dominant character with regard to the selectivity parameters χ_e, χ_d and χ_n (Table III)[18]. These parameters reflect the relative ability of a solvent to act mainly as a proton acceptor (ethanol, isopropanol), a proton donor (chloroform) or a strong dipole (methylene chloride).

According to chromatographic data (Table II), polar modifiers can be classified into two groups: alcohols and chlorinated solvents. Chlorinated solvents exhibit a greater selectivity towards these solutes whereas the efficiency is higher when using alcohols, especially ethanol. This may be correlated with the fact that polar groups on CSPs are more easily solvated by alcohols than by chlorinated solvents. Therefore, CSP–solute interactions are maximized when using chlorinated solvents (higher selectivity) but the adsorption–desorption kinetics of solutes on CSPs are slower (weaker efficiency).

Improvement of the separation of solute **1d** was achieved on CSP 2b by optimizing the mobile phase composition, as reported previously for sulphoxides[19]. A ternary hexane–ethanol–chloroform mobile phase was prepared starting from hexane–ethanol (92:8, v/v; solvent A) and hexane–chloroform (50:50, v/v; solvent B) binary mixtures (Fig.2). The concave profile of the capacity factor curves is in agreement with that obtained by Pescher *et al.*[20], according to whom the following explanations can be suggested to account for this profile: (a) like the phosphine oxides studied by Pescher *et al.*, *tert.*-butyl N-arylsulphinamoyl esters are highly soluble in chloroform; and (b) ethanol molecules are strongly adsorbed on CSPs and are not easily displaced by chloroform molecules.

Starting from a hexane–ethanol binary mixture, when a small amount of chloroform is added to the mobile phase the solubility of the solute increases according to (a), whereas following (b) the chloroform displaces only a few ethanol molecules which still hinder CSP–solute interactions. Hence this leads to a decrease in k' values. This accounts for the descending left-hand part of the curve. On the other hand, starting from a hexane–chloroform binary mixture, according to (b), as soon as a few ethanol molecules are added they will be preferentially adsorbed on the CSP, making

TABLE II

SEPARATION OF *tert*-BUTYL N-ARYLSULPHINAMOYL ESTERS USING DIFFERENT POLAR MODIFIERS

% = Percentage polar modifier; k'_2 is the capacity factor of the second-eluted enantiomer, $k'_2 = (t_{R_2}/t_0) - 1$, where t_{R_2} is the retention time of the last-eluted enantiomer and t_0 the retention time of a non-retained solute. The selectivity, α, between two enantiomers is the ratio of their respective capacity factors (k'_1/k'_2). Operating conditions: flow-rate, 2 ml/min; room temperature; mobile phase, the percentage (v/v) of polar modifier in *n*-hexane is given; UV detection at 260 nm.

Polar modifier	*Solute*	*CSP 1 [(R)-DNBPG]*			*CSP 2a [(S)-thio-DNBTyr-E]*			*CSP 2b [(S)-thio-DNBTyr-A]*		
		(%)	α	k'_2	*(%)*	α	k'_2	*(%)*	α	k'_2
Ethanol	**1a**	20	NR[a]	1.97	15	NR	7.30	5	1.72	27.01
	1b	2.5	1.04	2.72	5	1.05	3.13	5	1.48	3.96
	1c	2.5	1.10	3.46	5	1.12	4.70	5	1.54	6.05
	1d	2.5	1.13	7.13	5	1.17	9.25	5	1.64	12.08
	2a	2.5	1.21	2.59	5	1.14	3.18	5	1.45	3.97
	2b	2.5	1.22	2.26	5	1.24	3.27	5	1.54	3.84
Isopropanol	**1a**	20	NR	2.76	15	NR	13.89	7	1.40	14.80
	1b	4.5	1.09	2.22	7	1.10	2.20	7	1.72	3.61
	1c	4.5	1.13	2.92	7	1.11	3.36	7	1.77	5.76
	1d	4.5	1.17	7.08	7	1.22	7.48	7	1.88	12.21
	2a	4.5	1.30	2.28	7	1.23	2.78	7	1.63	3.73
	2b	4.5	1.32	2.05	7	1.44	3.35	7	1.77	3.98
Chloroform	**1a**	50	NR	5.19	50	NR	6.43	50	1.50	11.86
	1b	20	NR	2.41	50	1.37	2.38	50	1.97	5.55
	1c	20	NR	3.31	50	1.42	3.19	50	2.04	6.28
	1d	50	1.21	7.58	50	1.45	3.66	50	2.06	5.77
	2a	30	1.23	6.38	30	1.42	5.51	30	1.82	8.45
	2b	30	1.23	6.16	30	1.64	4.47	30	1.83	5.10
Methylene chloride	**1a**	50	NR	3.22	50	1.12	7.63	50	1.54	13.15
	1b	30	NR	1.17	50	1.26	2.94	50	2.00	7.18
	1c	30	NR	2.02	50	1.28	4.14	50	2.00	8.88
	1d	30	NR	1.62	50	1.27	5.16	50	2.00	8.44
	2a	50	1.20	7.25	50	1.35	2.33	50	1.89	4.17
	2b	50	1.15	4.16	50	1.55	1.75	50	1.75	2.21

[a] NR = No resolution, $\alpha = 1$

TABLE III

SELECTIVITY PARAMETERS, AS DEFINED AND CALCULATED BY SNYDER[18] FROM SOLUBILITY DATA REPORTED BY ROHRSCHNEIDER

Values in italics indicate the dominant character of the solvent: χ_e (proton acceptor), χ_d (proton donor), χ_n (strong dipole).

Polar modifier	χ_e	χ_d	χ_n
Ethanol	*0.52*	0.19	0.29
Isopropanol	*0.55*	0.19	0.27
Chloroform	0.25	*0.41*	0.33
Methylene chloride	0.29	0.18	*0.53*

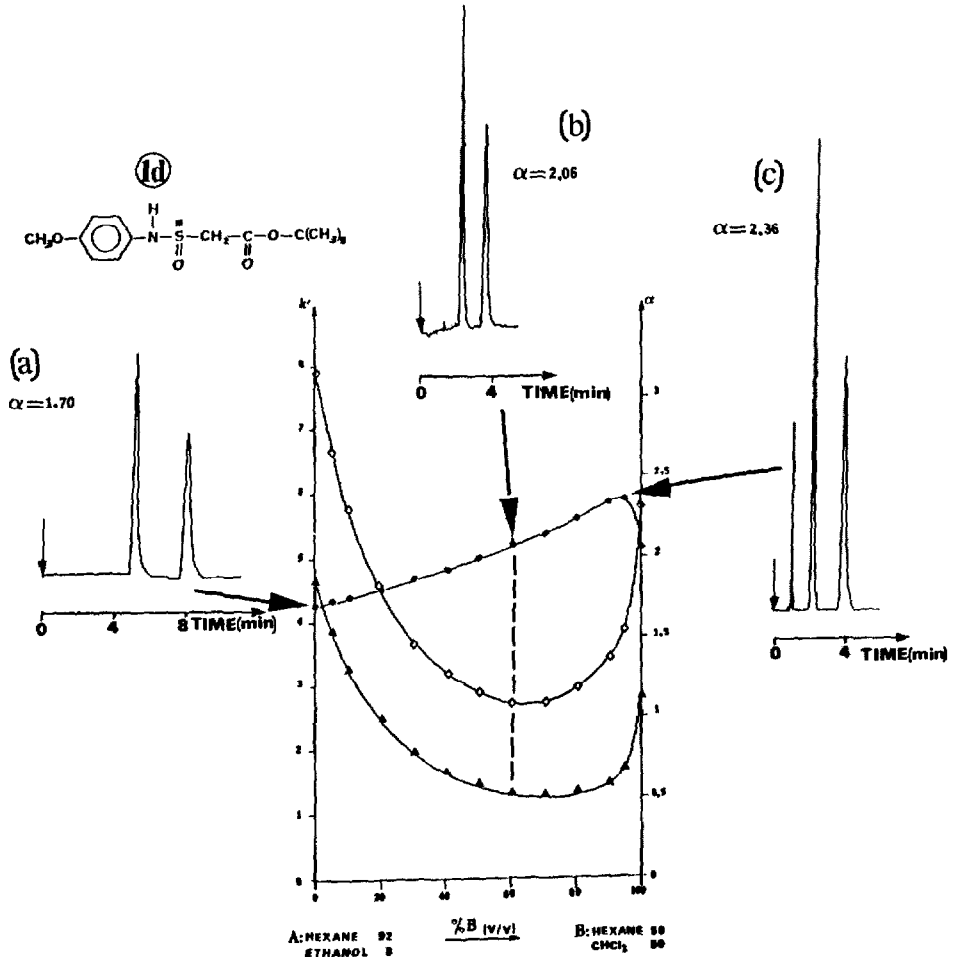


Fig. 2. Ternary optimization for compound **Id** on CSP 2b using hexane–ethanol (92:8, v/v; solvent A) and hexane–chloroform (50:50, v/v; solvent B) binary eluents. The capacity factors, k'_1 (▲) and k'_2 (◇), and the selectivity, α (●), are plotted *versus* the content of binary mixture B in the ternary mixture A–B. Flow-rate, 2 ml/min; room temperature; UV detection at 260 nm. (a) Mobile phase A 100%, $k'_1 = 4.66$, $k'_2 = 7.92$, $\alpha = 1.70$; (b) mobile phase A–B (40:60), $k'_1 = 1.30$, $k'_2 = 2.68$, $\alpha = 2.06$; (c) mobile phase A–B (5:95), $k'_1 = 1.64$, $k'_2 = 3.87$, $\alpha = 2.36$.

CSP–solute interactions more difficult and, as a consequence, decreasing the k' values (right-hand part of the curve). Consequently, the curve showing k' *versus* the ternary mixture composition passes through a minimum value corresponding to the highest resolution value per unit time (Fig. 2b).

The selectivity value shows a linear increase with increasing chloroform content with a maximum corresponding to a mobile phase containing 95% of B.

Influence of the CSP structure

According to the chromatographic data (Table II), CSP 2b exhibits the greatest selectivity towards this family of solutes. As an example, Fig. 3 shows the difference in

selectivity between the three CSPs for compound **1b** when using chloroform in the mobile phase.

CSPs 1 and 2b have the same number of potential sites of interaction. Nevertheless, in order to obtain the same retention times on these CSPs, the elution strength of the mobile phase should be weaker when using CSP 1 (see Table II). CSP–solute interactions are thus weaker on CSP 1 than on CSP 2b. The removal of the chiral centre away from the silica matrix in CSP 2b compared with CSP 1 makes the CSP 2b chiral graft more accessible to the solute. This also accounts for the weaker selectivity values observed on CSP 1 (see Fig. 4).

On the other hand, CSP 2a and CSP 2b differ only in an aliphatic amide dipole (dipole B, framed in Fig. 4) instead of an ester group. The differences in both selectivity and retention between these two CSPs may result from a CSP–solute interaction involving dipole B (acidic character) and the ester group of the solute (basic character) (see Fig. 4, interaction 3).

The elution order for compound **1d** was established on these three CSPs starting from a racemate enriched with the most retained enantiomer on CSP 2b. No inversion of elution order occurred on any CSP on changing the nature of the polar modifier of the mobile phase. This was also true on comparing CSP 2 types, whereas an inversion of elution order was observed on changing from CSP 2b to CSP 1 with

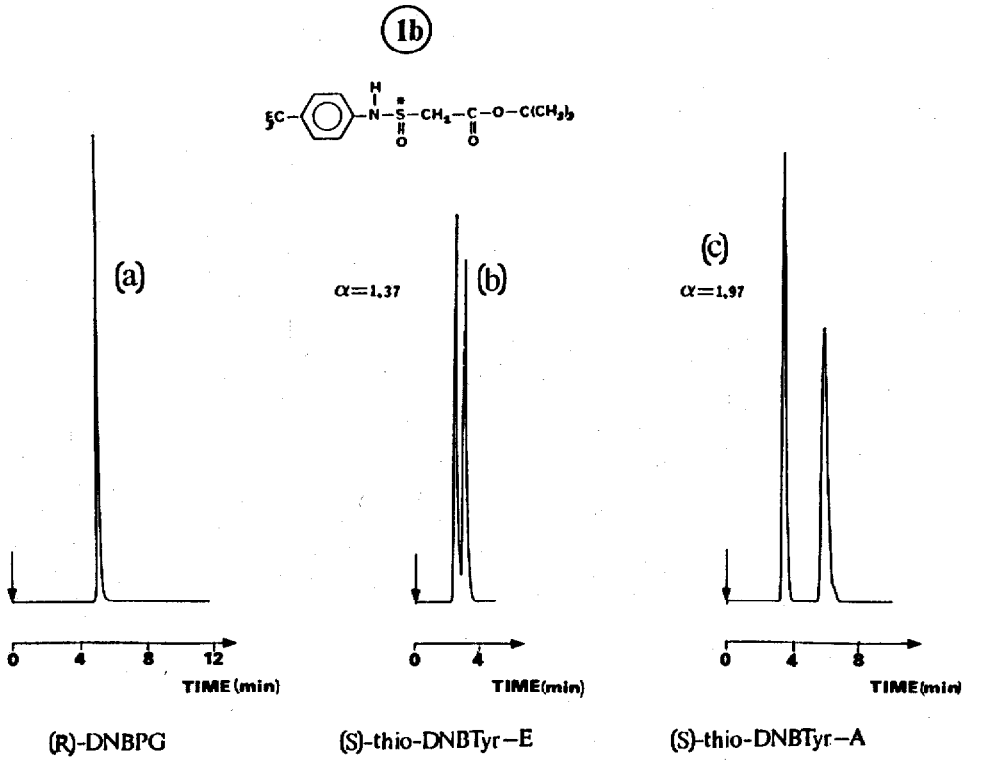


Fig. 3. Comparison of selectivity values for compound **1b** on different CSPs. Operating conditions: mobile phase, hexane–chloroform (50:50) [except for (a) (80:20)]; flow-rate, 2 ml/min; room temperature; UV detection at 260 nm.

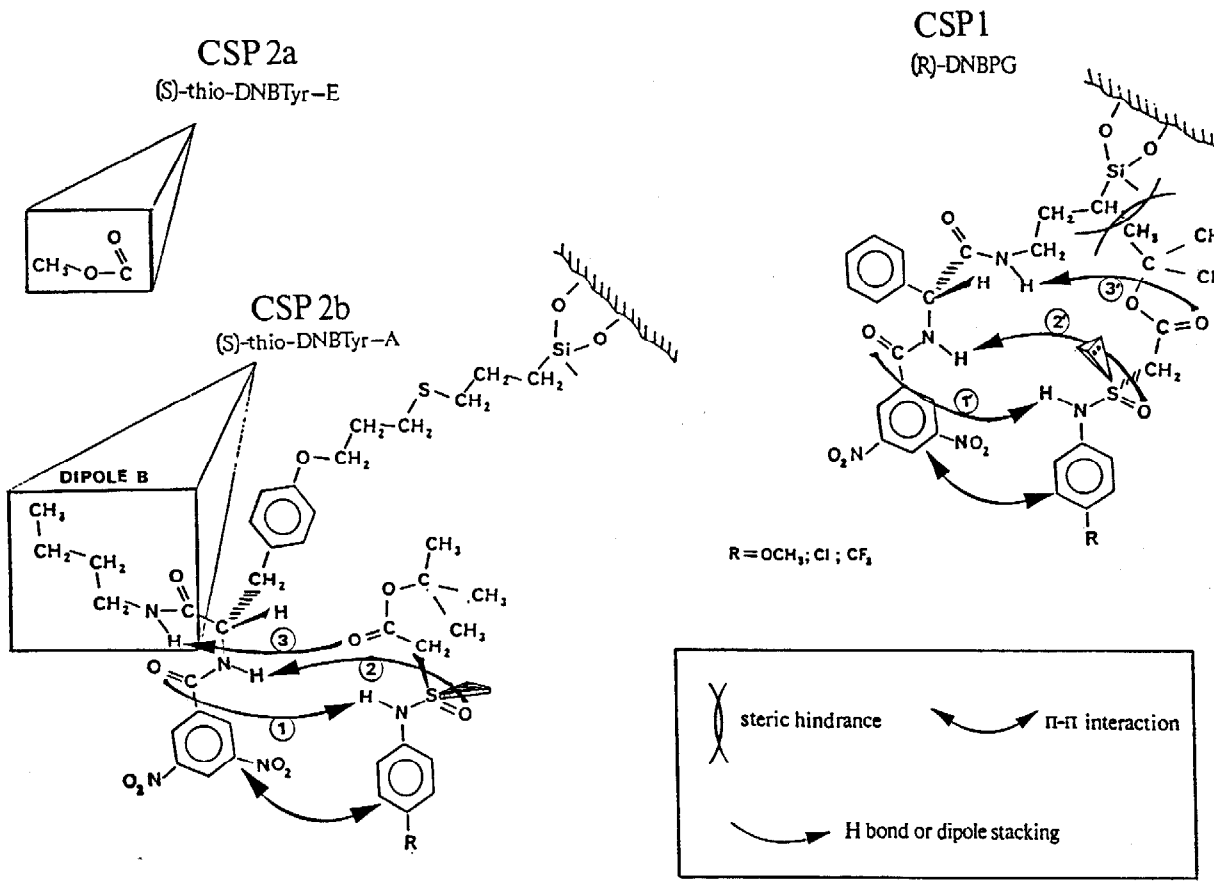


Fig. 4. Proposed chiral recognition model for compounds **1b–d**. (a) CSP 2b. The most strongly retained enantiomer is represented. (b) CSP 2a. The hydrogen bonding between the amide dipole B and the carbonyl moiety of the solute is cancelled, leading to a loss of selectivity. (c) CSP 1. Compared with CSP 2b, the CSP–solute interactions are sterically hindered by the *tert.*-butyl moiety of the solute and the silica matrix. The most retained enantiomer is opposite to that on CSP 2b according to the experimental results.

ethanol, isopropanol or chloroform (no resolution occurred for compound **1d** on CSP 1 when using methylene chloride as polar modifier).

Influence of the aromatic ring substituent

It is now well established that the π–π interaction occurring between the 3,5-dinitrobenzoyl moiety of a Pirkle-type CSP and a racemate possessing a complementary π-basic character may contribute to the chiral recognition process[1–3]. Nevertheless, this is not always the main directional attractive interaction, as the resolution of π-acid solutes on π-acid CSPs has already been reported[8,9], thus emphasizing the importance of dipole stackings and/or hydrogen bondings in the chiral recognition process.

Compounds **1a–d** differ only in the nature of their aromatic ring substituent, which can be either electron-withdrawing (NO_2, CF_3, Cl) or electron-donating (OCH_3). These substituents can be characterized by their Hammet σ_H value. The more electron-withdrawing an aromatic ring substituent is, the higher is the value assigned to it. The Hammet σ_H values given in Table IV were taken from ref. 22.

In Fig. 5 the logarithm of selectivity values of compounds **1a–d** on CSP 2b are plotted *versus* the Hammet σ_H values of their substituents. The selectivity values of compounds **1b–d** show a good linear decrease with σ_H values when using ethanol ($R = 0.9998$) or isopropanol ($R = 0.9975$). The chiral recognition mechanism probably involves a π–π interaction as the difference in energy between the two CSP–enantiomer transient complexes (represented by log α) is proportional to the π-basic character of the solute (represented by σ_H) (see Fig. 4). On the other hand, when using chlorinated solvents, the π–π interaction does not appear to be as stereoselective as with alcohols. The amide dipoles of the CSP are less solvated with chlorinated solvents, and consequently interactions involving these amide dipoles are stronger when using these solvents. It can be assumed that the π–π interaction is of lesser importance in comparison with hydrogen bonding or dipole–dipole interactions.

Compound **1a** displays a different behaviour compared to **1b–d**. The selectivity values obtained for this compound are much weaker except when using ethanol as the polar modifier (Fig. 5). Owing to the very strong electron-withdrawing effect of the nitro substituent, this compound has a π-acid character. A π-donor–acceptor interaction can no longer be considered for this compound on π-acid CSPs. Only dipole–dipole interactions, hydrogen bondings or steric effects govern the chiral recognition process. The chromatographic results are in good agreement with those obtained by Lienne *et al.*[8] on π-acid solutes: CSP 2a, which contains only one amide dipole, displays lower selectivity values than CSP 2b. From Table II, it can be inferred that

TABLE IV

HAMMET σ_H VALUES ACCORDING TO REF. 21

Solute	*Substituent*	σ_H
1a	NO_2	0.778
1b	CF3	0.551
1c	Cl	0.227
1d	OCH_3	−0.268

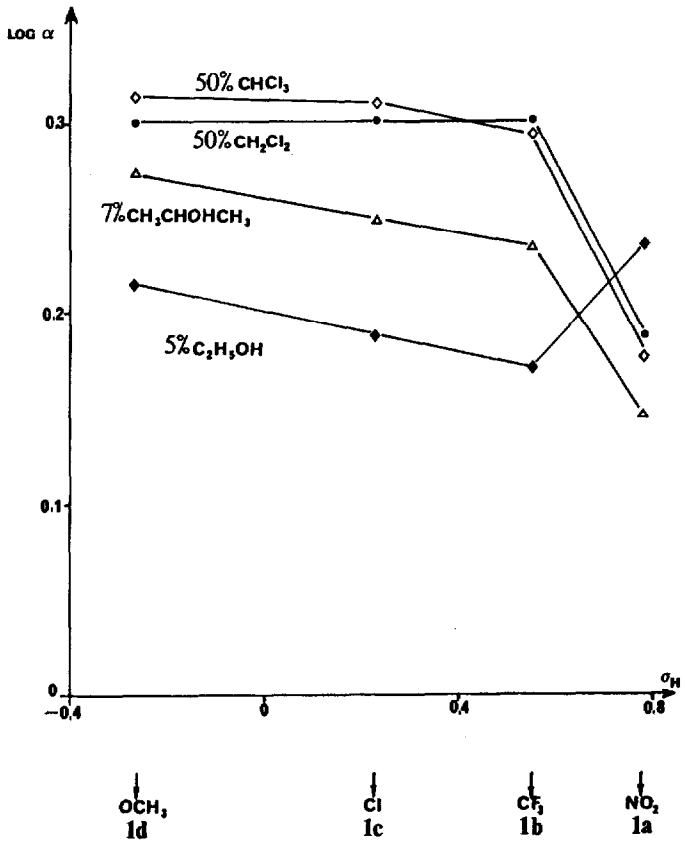


Fig. 5. Variation of log α with Hammet σ_H values for compounds **1a–d** on CSP 2b using hexane–polar modifier mobile phases.

enantiorecognition of compound **1a** is based on an interaction involving dipole B of CSP 2b.

A similar study was carried out by Wainer and Alembik[22], who studied the influence of π-basicity on the separation of aromatic amides on (*R*)-DNBPG. The results were different from those reported above because the electronegativity of the *para* substituent seemed to have very little effect on the stereoselectivity.

Influence of steric bulk at the asymmetric centre

Compounds **2a** and **2b** (Table I) differ only in the number of methyl substituents near the asymmetric centre. Table II shows that, as a general rule, they are less retained on any CSP than compounds **1a–d**. The steric hindrance due to methyl substituents probably weakens the CSP–solute **2a** and **b** interactions.

The variation of selectivity values for compounds **2a** and **b** differs with the CSP structure. According to Table II, the selectivity increases with steric hindrance on CSP 2a whereas it decreases on CSP 2b. The dipole B–solute interaction (3 in Fig. 4) is probably sterically hindered (weaker selectivity values on CSP 2b). On the other hand, steric hindrance which does not alter any CSP–solute interaction of CSP 2a

allows an increase in chiral recognition. Equivalent attractive interactions can be suggested between either the less or the most retained enantiomer and this CSP, but with regard to the less stable diastereoisomeric complex these interactions may confer an energetically disfavoured conformation on the solute enantiomer. Repulsive CSP–less retained enantiomer interactions may account for chiral discrimination.

Application to preparative-scale chromatography

Biological studies on these compounds were previously carried out using racemates as their enantiomeric separation had never been performed. By extending their analytical separation to the preparative scale, we aimed to isolate the optically pure enantiomers in order to allow biological studies to be carried out.

The high stability of this type of CSP allows their use for preparative-scale applications. A preparative separation of compound **1d** was carried out on 200 g of (*S*)-*thio*-DNBTyr-A. The mobile phase composition was determined from analytical optimization data: hexane–isopropanol (85:15, v/v) with $\alpha = 1.83$ and $k'_2 = 5.78$. The selectivity could be improved by using chlorinated polar modifiers ($\alpha = 2$), but the resolution factor was the same as with isopropanol owing to a lower efficiency. These chromatographic conditions allowed the injection of 200 mg of racemate **1d** per run.

Fig. 6 shows a preparative-scale separation of 200 mg of compound **1d**. Fractions 1 and 2 from different runs were pooled, evaporated and the final products were crystallized from hexane–diisopropyl ether. The first-eluted enantiomer was obtained with 100% enantiomeric excess (e.e.), monitored on analytical CSP 2b (Fig. 6, chromatogram 1; m.p. 107°C; $[\alpha]^{22}_D = +144°$ [c = 1, tetrahydrofuran (THF), 22°C]. The

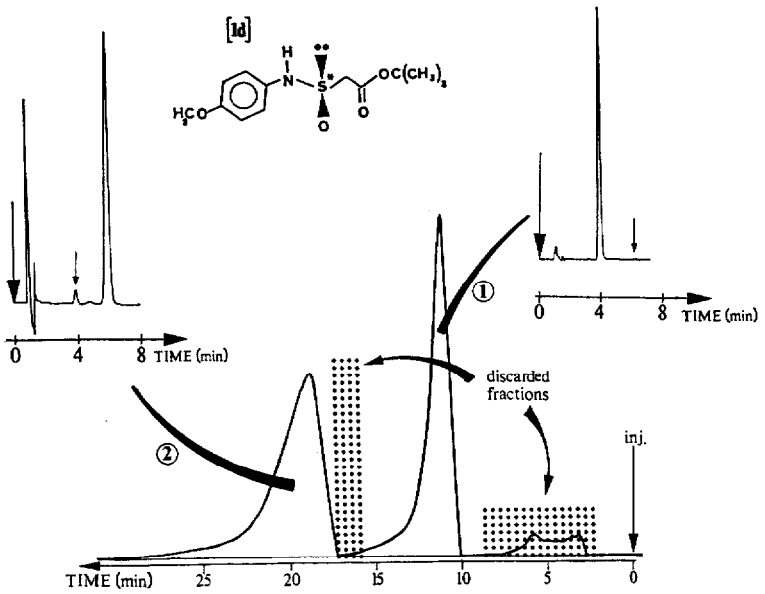


Fig. 6. Preparative-scale resolution of 200 mg of compound **1d** on 200 g of CSP 2b. Operating conditions: mobile phase, hexane–isopropanol (85:15, v/v); flow-rate, 42 ml/min; UV detection at 254 nm. The enantiomeric purity of the collected fractions was checked on an analytical column (CSP 2b). Operating conditions: mobile phase, hexane–ethanol (85:15, v/v); flow-rate, 2ml/min; UV detection at 260 nm.

most retained enantiomer was obtained with 95.8% e.e.; m.p. 102–103°C. Unfortunately, it was impossible to measure the rotatory power of this enantiomer; when it was dissolved in various solvents such as chloroform or THF, a brown precipitate rapidly appeared. For the first-eluted enantiomer, the same effect occurred but more slowly, thus allowing the determination of the rotatory power.

CONCLUSION

(*S*)-*thio*-DNBTyr-A allows the separation of the enantiomers of *tert.*-butyl N-arylsulphinamoyl esters on both analytical and preparative scales. This study shows, once again, that small changes in the chemical structure of the CSP may induce a significant enhancement of enantiorecognition ability. The binding mode of (*S*)-*thio*-DNBTyr-A allows the asymmetric centre to be away from the silica matrix, making the formation of transient CSP–bulky solute complexes easier in comparison with (*R*)-DNBPG.

The differences between alcohols and chlorinated solvents used as polar modifiers in the mobile phase may be correlated with their aptitudes to solvate amide dipoles on CSPs, thus exhibiting either a good efficiency (alcohols) or a good selectivity (chlorinated solvents).

The results also show the increase in selectivity with the π-basic character of the solute and the involvement of dipole B of (*S*)-*thio*-DNBTyr-A in the chiral recognition process. Spectroscopic studies would probably provide complementary information to the chromatographic data.

ACKNOWLEDGEMENT

The authors express their grateful thanks to Dr. L. Cazaux for the gift of the solutes which prompted us to initiate this work.

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