ELSEVIER

Journal of Chromatography A, 859 (1999) 143–151

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Convenient synthesis of π-acceptor chiral stationary phases for high-performance liquid chromatography from halogen-substituted 3,5-dinitrobenzoylamides

Oleg R. Malyshev*, Maxim G. Vinogradov

N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky prospect 47, 117913 Moscow, Russia

Received 12 January 1999; received in revised form 4 August 1999; accepted 13 August 1999

Abstract

A convenient method for the ''in column'' synthesis of chiral stationary phases for high-performance liquid chromatography was elaborated. It involves preparation of chiral amides of 2-bromo- or 4-chloro-substituted 3,5-dinitrobenzoic acids followed by nucleophilic substitution of the halogen in the aromatic moiety with 3-aminopropyl groups of silanized silica gel at ambient temperature. A series of π-donor compounds, such as amides and alkyl aryl carbinols, were chromatographed on the prepared chiral stationary phases. The results were compared with data reported for chiral separations of the same substrates on similar (*R*)-*N*-(3,5-dinitrobenzoyl)-α-phenylglycine-derived CSP. An example of indirect enantioseparation of racemic α-phenylethylamine was also described using (*R*)-2-(2-bromo-3,5-dinitrobenzoylamino)-2-phenylethanol as a chiral derivatizing reagent.

Keywords: Chiral stationary phases, LC; π-Acceptors; Enantiomeric separation; 3,5-Dinitrobenzoylamides; α-Phenylethylamine

1. Introduction

Numerous enantioseparations using high-performance liquid chromatography (HPLC) have been reported to be specific for a key role in the π-interaction of π-donors with π-acceptor groups both in a chiral stationary phase (CSP) and in an analyzed compound [1]. It should, however, be noted that the attention was mostly focused on the effect of the CSP π-donor fragment structure on the enantioseparation selectivity [1]. At the same time only a few of the π-acceptor groups are known to have been used for the design of CSPs (Fig. 1). 2-(2,4,5,7-Tetranitro-9-fluorenylideneaminooxy)propionic acid (**A**), its homologs [2] and *N*-(2,4-dinitrophenyl)-(L)-phenylalanin (B) [3] had been studied among the first π-acceptor structural fragments.

More recently chiral amides and esters of 3,5-dinitrobenzoic acid along with anilides, carbamates and uretanes (**C** and **D**) containing a *N*-3,5-dinitrophenyl fragment have been used as π-acceptor CSPs for chiral recognition of π-donor compounds [4].

Two alternative types of mutual arrangement of CSP fragments **A** and **B** (Fig. 2) may be used for the design of π-acceptor CSPs. In one case (type **A**), a CSP chiral fragment is located between the support

*Corresponding author. Fax: +7-095-135-5328.
E-mail address: pvlv@cacr.ioc.ac.ru (O.R. Malyshev)

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00871-7

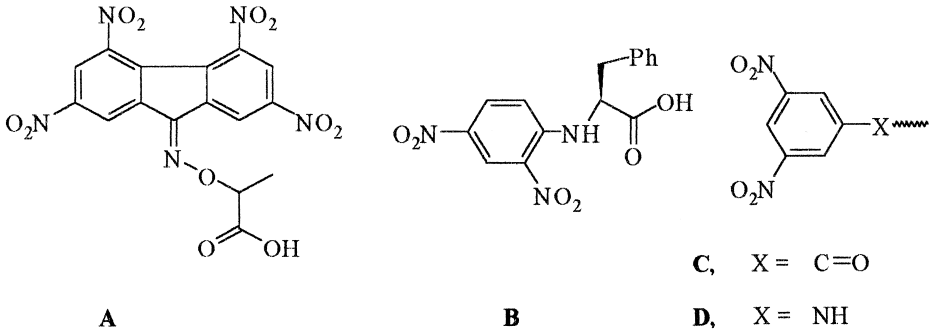


Fig. 1. Typical π-acceptor fragments of CSPs.

and the π-acceptor group. Here a CSP chiral fragment should have at least two functional groups to form chemical bonds with both the modified-silica gel and the CSP π-acceptor component. Alternatively, the π-acceptor group can serve as a connecting link (type **B**) (Fig. 2) between the support and CSP chiral fragment. An advantage of the **B** type chiral fragment arrangement is that it requires only one link-forming functional group, e.g. the amino-group, for including a chiral fragment into CSP. A wide range of various chiral amines may, therefore, be used for preparing π-acceptor CSPs of the **B** type. It is surprising that, in contrast to the **A** type linking, there are only a few examples of immobilization of a chiral selector in the **B** manner for the preparation of CSPs [5,6]. Moreover, to our knowledge, no data regarding the synthesis of CSPs of such type involving a 3,5-dinitrobenzoyl fragment (3,5-DNB) have been reported.

In this connection it was of interest to synthesize 3,5-DNB-containing CSPs arranged in the manner shown by the scheme **B** (Fig. 2). For this purpose (*R*)-phenylglycinol-derived chiral amides **I** and **II** containing a halogen atom in the 3,5-DNB group were obtained and subjected to an ''in column'' reaction with 3-aminopropylsilanized silica gel (APS-silica). **CSP-1** and **CSP-2** were prepared as a result of halogen substitution by amine groups of APS-silica in amides **I** and **II** (Fig. 3).

Another aspect of our investigation was connected with the influence of additional substituents in the CSP π-acceptor aromatic moiety on the efficiency of chiral recognition. This problem is obviously of particular interest for CSPs to be synthesized through the **B** sequence (Fig. 2). Only few findings have been known with regard to how the incorporation of an additional substituent in 3,5-DNB fragment affects the chiral recognition of analytes. The influence of the nature of a substituent and its position in the aromatic moiety of 3,5-dinitrobenzoylamides on the

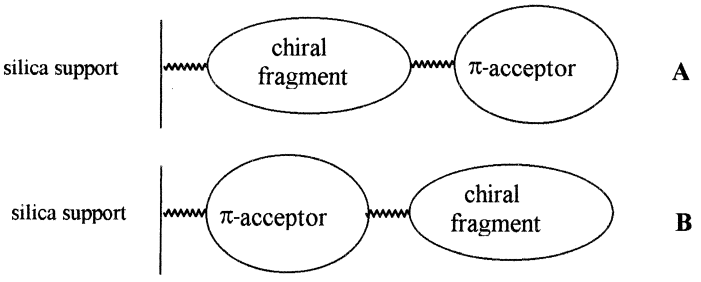


Fig. 2. Principal structural types of π-acceptor CSPs.

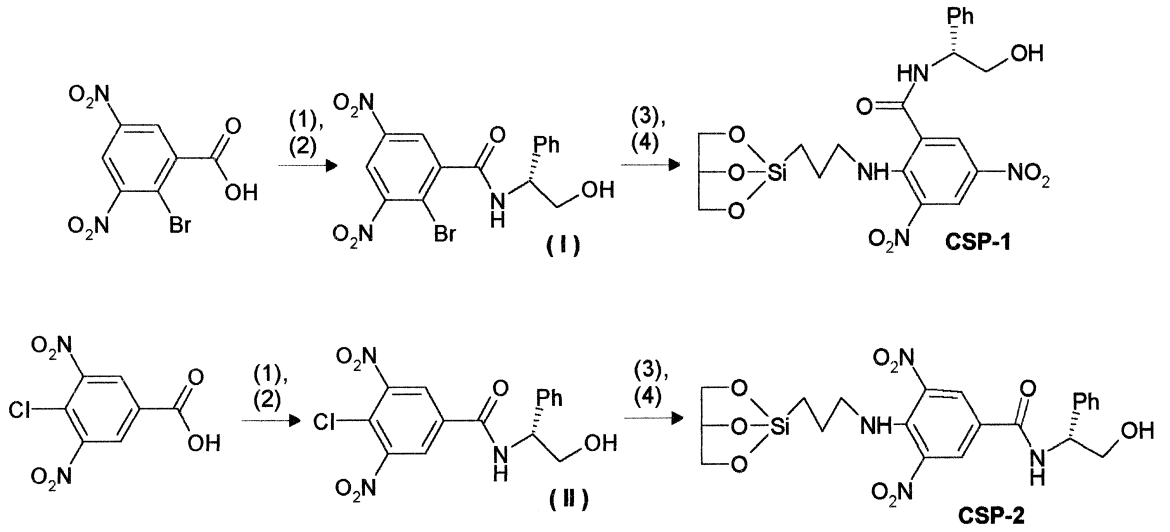


Fig. 3. Syntheses of **CSP-1** and **CSP-2**: (1) $SOCl_2$; (2) (*R*)-phenylglycinol, toluene–H_2O–Na_2CO_3; (3) APS–silica; (4) 6.5% $HClO_4$ in 2-propanol.

efficiency of their separation on the π-donor CSP was studied [7]. It was shown that the incorporation of a substituent in the *ortho* position to the amide group of the analyte moiety (no matter whether it has an electron-donor (CH_3) or an acceptor (NO_2) character) resulted in a decrease of the separation factor value. Dinitrobenzoyl fragments of CSPs described below also contain an additional substituent in the aromatic moiety (the alkylamino group) and, therefore, the results obtained with these CSPs would be of interest for correlation with those Ref. [7]. It was also interesting to compare the results of enantioseparation of standard analytes on the synthesized chiral phases (**CSP-1** and **CSP-2**) with the data known for separation of the same compounds on Pirkle's (*R*)-phenylglycine-derived CSP containing the unsubstituted 3,5-DNB π-acceptor group [8].

Since the halogen in the aromatic ring of CSP precursors **I** and **II** is easily substituted by *N*-nucleophiles, compounds **I** and **II** appear to be suitable derivatizing reagents for indirect enantioseparation of primary or secondary amines. From this point compounds **I** and **II** may be considered as analogs of *N*-(2,4-dinitro-5-fluorophenyl)-L-α-alaninamide, Marfey's derivatizing reagent [9,10].

2. Experimental

2.1. Chemicals and reagents

Amides **1–13** were synthesized from the corresponding amines and acyl chlorides in the presence of aqueous alkaline solution followed by crystallization of the products from EtOH–H_2O. Alkyl aryl carbinols **14–25** were prepared by reduction of the corresponding ketones with $NaBH_4$. 4-Chloro-3,5-dinitrobenzoic acid and compound **26** were obtained from Aldrich (Milwaukee, WI, USA); 2-bromo-3,5-dinitrobenzoic acid was prepared by nitration of 2-bromobenzoic acid; (*R*)-2-phenyl-2-aminoethanol((*R*)-phenylglycinol) was obtained by reduction of (*R*)-phenylglycine with $LiAlH_4$. Compound **27** was prepared by the reaction of 9-formylanthracene with butyl lithium in hexane solution. NMR

spectra of all the prepared analytes were in accordance with their structures. The other chemicals used were obtained from Reachim (Moscow, Russia), and were of purum or puriss grades.

2.2. Instrumental

HPLC experiments were carried out on a Laboratory pristroje Praha chromatograph using 150×3.3 mm glass cartridge columns packed with separon–NH_2 (5μ) or separon (5μ). The void volume of the column was determined with toluene as an unretainable compound. An absorption detector was operated at 254 nm to detect enantiomers of compounds **1–28** and at 420 nm to detect diastereoisomers of compound **29**. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM-250-instrument.

2.3. Preparation of chiral stationary phases *CSP-1* and *CSP-2*

Syntheses of **CSP-1** and **CSP-2** were performed following schemes in Fig. 3.

2.3.1. (R)-2-(2-Bromo-3,5-dinitrobenzoylamino)-2-phenylethanol (*I*)

2-Bromo-3,5-dinitrobenzoic acid (0.875 g, 3 mmol) was mixed with $SOCl_2$ (0.6 g, 6 mmol) and DMF (0.01 g) was added as a catalyst. The mixture was heated under reflux until the reaction mixture becomes homogeneous (1.5 h). The excess of $SOCl_2$ was removed in vacuo (10 torr), the residue was diluted with toluene (30 ml) and the solvent was distilled off. the remaining crude 2-bromo-3,5-dinitrobenzoyl chloride was dissolved in toluene (30 ml) and this solution was added to a vigorously stirred two-phase system consisting of 50 ml toluene solution of (*R*)-2-phenyl-2-aminoethanol((*R*)-phenylglycinol) (0.411 g, 3 mmol) and aqueous 2% solution of Na_2CO_3 (50 ml). The mixture was further stirred for 10 min and allowed to stand for 1 h at room temperature. The white crystalline precipitate that formed was filtered off, washed with water and toluene–heptane (1:1) and dried. Yield of the product was 1.12 g (91%), m.p. 178–180°C; ^{1}H NMR (δ, ppm, 2:1 $CDCl_3$–CD_3OD): 3.8 (m, 2H, CH_2O), 4.35 (s, 2H, NH and OH) 5.2 (q, 1H, NCH), 7.35 (m, 5H, Ph), 8.45 (d) and 8.6 (d) (2H, 2-bromo-3,5-DNB).

NMR $^{13}C\{^1H\}$ (δ, ppm, 2:1 $CDCl_3$–CD_3OD): 56.16 (NCH), 64.55 (CH_2O), 118.60, 119.90, 125.10, 126.65, 127.57, 128.32 (CH-aromatic); 138.24, 142.82, 146.51 (aromatic), 164.98 (C=O); $[\alpha]_D^{20} = -13.1°$ (c=1.0, THF). Analysis for C_{15} H_{12} N_3 O_6 Br: calculated C=43.92; H=2.95; N=10.24; Br=19.48; found C=44.32; H=3.24; N=9.97; Br=18.54.

2.3.2. (R)-2-(4-Chloro-3,5-dinitrobenzoylamino)-2-phenylethanol (*II*)

The synthesis was realized using 4-chloro-3,5-dinitrobenzoic acid (0.740 g, 3 mmol) in a manner similar to that described above for the preparation of compound **I**. Yield of compound **II**: 0.76 g (69%), light yellow crystals, m.p. 164–166°C; ^{1}H NMR (δ, ppm) in (2:1 $CDCl_3$–CD_3OD): 3.8 (m, 2H, CH_2O), 4.35 broad (s, NH and OH), 7.35 (m, 5H, Ph), 8.5 (s, 2H, 4-chloro-3,5-DNB). NMR$^{13}C\{^1H\}$ (δ, ppm) in 2:1 $CDCl_3$–CD_3OD: 56.63 (NCH), 64.85 (CH_2O), 123.0, 126.73, 126.70, 127.67, 128.67, 134.74, 138.40, 149.32 (aromatic), 162.58 (C=O); $[\alpha]_D^{20} = +44.5°$ (c=1.0, THF) Analysis for C_{15} H_{12} N_3 O_6 Cl: calculated C=49.25, H=3.30, N=11.48, Cl=9.69. Found C=48.91, H=3.82, N=11.50, Cl=9.10.

2.3.3. Rate evaluation for model reactions of *I* and *II* with (3-aminopropyl)triethoxysilan

The title reactions were carried out in NMR tubes at 23°C. (3-Aminopropyl)triethoxysilan [$(EtO)_3SiCH_2CH_2CH_2NH_2$] (0.1mmol) was added to a solution of reagent **I** or **II** (0.05 mmol) in d_6-DMSO (0.5 ml), with solution becoming yellow-orange. The reaction course was monitored by ^{1}H NMR. A significant change in the spectra of the reaction mixtures was observed. Quantitative substitution of the halogen in **I** and **II** by the (3-aminopropyl)triethoxysilanaminogroup already took place during the course of the NMR spectra recording (4 min). After further exposure of the reaction solution for 1h at room temperature the ^{1}H NMR spectra of the reaction mixtures remained unchanged.

2.3.4. Chiral stationary phases *CSP-1* and *CSP-2*

A glass column (void volume=1.1 ml) containing

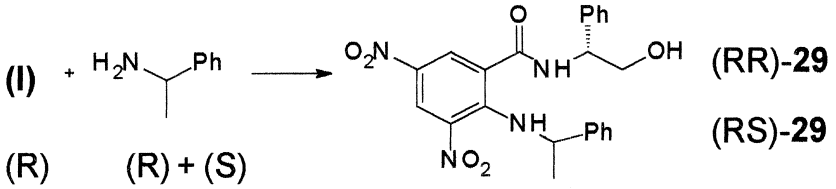


Fig. 4. Reaction between the chiral derivatizing reagent **I** and racemic α-phenylethylamine.

APS-silica was washed with THF (10 ml) and filled up with a solution of compound **I** (0.3 g) in THF–(2,4,6-trimethylpyridine) (1:1, v/v) (3 ml). The filled up column was kept at room temperature for 1 h, then 1.5 ml of the above solution was pumped through the column and kept under this solution for 3h. After that the column was washed with THF and 2-propanol and then examined for the separation of compound **1**. The column was then filled up repeatedly with the same solution of compound **I** and kept at room temperature for three days. After washing with *i*-PrOH, 6.5% $HClO_4$ in *i*-PrOH and pure *i*-PrOH, the column containing **CSP-1** was ready for use.

A procedure for the preparation of **CSP-2** was similar to the procedure for **CSP-1** above, except that the contact time of APS-silica with the base-contaning solution of compound **II** was 20 h.

2.4. Indirect enantioseparation of racemic α-phenylethylamine

The two diastereomeric compounds of **29** (Fig. 4) were obtained using a reaction of the chiral derivatizing reagent (**I**) with racemic α-phenylethylamine. A solution of compound **I** (10 μmol) and racemic α-phenylethylamine (10 μmol) in acetonitrile (0.1 ml) was kept at room temperature for 1 h. The yellow reaction mixture was concentrated and the residue was diluted with *i*-PrOH (0.2 ml) and chromatographed on a column packed with silica gel (separon, 5 μ). The mobile phase was heptane–isopropanol (98:2, v/v), the flow-rate was 1 ml/min. The retention factors for the separation of the diastereomers of **29** were $k_1'=9.98$ and $k_2'=11.37$, and the separation factor $\alpha=1.14$.

3. Results and discussion

In order to evaluate time that is required for the formation of **CSP-1** and **CSP-2** in nucleophilic substitution of the halogen in **I** and **II** by the aminopropyl groups of APS-silica, the ^1H NMR monitoring of the model reactions of **I** and **II** with two equivalents of (3-aminopropyl)triethoxysilan was performed using d_6-DMSO as a solvent. Both reagents (**I** and **II**) proved to react quantitatively for 4 min at room temperature to give the alkyl amino-substituted products. Nevertheless, to ensure that **I** and **II** react with APS-silica as completely as possible, the duration of these reactions was increased until three days for **CSP-1** and 20h for **CSP-2**. In fact, the substitution reactions were principally over for a much shorter time than indicated the as a degree of separation of compound **1** on **CSP-1** was the same when this CSP was prepared either for 3 h or for 3 days (Table 1).

Tables 1 and 2 present the results of chromatographic enantioseparation of test compounds, such as amides and alkyl aryl carbinols, on the prepared CSPs. In a row of amides derived from α-phenylethylamine the marked enantioseparation on both **CSP-1** and **CSP-2** was observed only for the *N*-(1-naphthoyl)-derivative **1** ($\alpha=1.2–1.3$). Much better separation on the same CSPs was observed for ibuprofen amides **8–11**. Analysis on the recognition ability of **CSP-1** and **CSP-2** shows that both phases do operate like those of a π-acceptor character. So, with a decreasing π-donor ability in the row of amides: **8**, **9**, **10**, **11** the α-values obtained on **CSP-1** regularly diminished from $\alpha=1.48$ for compound **8** to $\alpha=1.0$ for compound **11**.

A characteristic structural feature of **CSP-1** and **CSP-2** is that the aromatic moiety of their π-accep-

Table 1
Enantioseparation of amides **1–13** on **CSP-1** and **CSP-2**

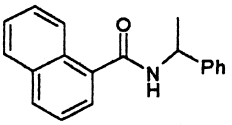
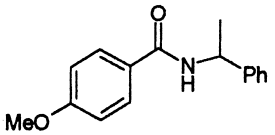
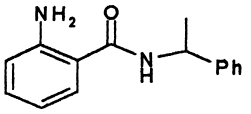
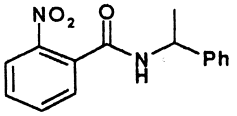
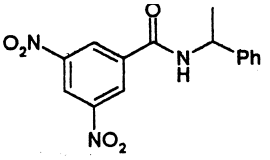
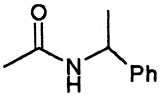
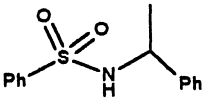
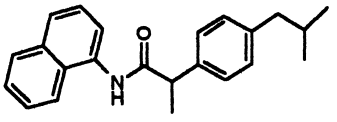
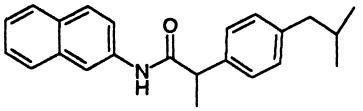
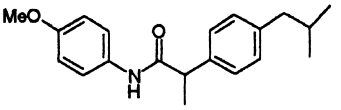
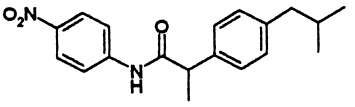
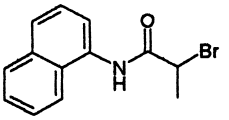
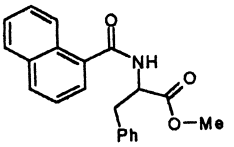
No.	Compound structure	**CSP-1**[a]		**CSP-2**[a]	
		α[b]	k_2'[c]	α	k_2'
1		1.27 1.29[e]	22.80 (*S*)[d] 19.40[e] (*S*)[d]	1.20	9.80
2		1.00	8.19	1.00	6.15
3		1.00	7.62	1.00	6.10
4		1.09	17.67	1.00	10.40
5		1.00	4.00	1.00	2.70
6		1.00	2.10	1.00	2.40
7		1.00	3.02 (*S*)[d]	1.09	10.51
8		1.48	7.48	1.34[f]	16.57[f]

Table 1 (*continued*)

No.	Compound structure	CSP-1[a]		CSP-2[a]	
		α[b]	k_2'[c]	α	k_2'
9		1.22	3.67	1.28[f]	18.09[f]
10		1.15	3.31	1.39[f]	3.24[f]
11		1.00	2.50	1.05[f]	3.30[f]
12		1.08[f]	7.60[f]	1.08[g]	1.08[g]
13		1.04[f]	23.50[f]	1.25[f]	21.42[f]

[a] The mobile phase is *n*-heptane–2-propanol, 80:20 (v/v), unless otherwise indicated.
[b] $\alpha = k_2'/k_1'$ = Enantioseparation factor, a flow-rate of the mobile phase is 1 ml/min.
[c] k_1' and k_2' = Capacity factors for the first- and the second-eluted enantiomers, respectively.
[d] Configuration for the second-eluted enantiomer. This was determined using a commercial (*S*)-α-phenylethylamine.
[e] The values were obtained using the column prepared after 3h exposure of APS-silica with a solution of compound (**I**) (see Section 2.3).
[f] 90:10, v/v.
[g] 95:5, v/v.

tor fragments contain, in addition to nitro groups, the alkylamino substituent. As the latter is an electron-donor type, the role of the π–π-interaction in the mechanism of chiral recognition of selectants should obviously diminish. This fact, however, proved not to be of great importance. So, the capacity and separation factor values for the prepared CSPs and those for Pirkle's CSP (*N*-3,5-DNB-(*R*)-phenylglycine) are fairly similar with a reference to the used analytes. Moreover, in some instances we found a marked improvement in the enantioresolution efficiency when the prepared CSPs were used, in contrast to the conclusion made in [7] that the incorporation of the amino substituent into the 3,5-DNB fragment lowers the efficiency of chiral recognition involving the π-donor–π-acceptor interaction. Thus the result of enantioseparation of 9-anthryl trifluoromethylcarbinol **26** on **CSP-1** ($\alpha = 1.63$) proved superior to that obtained on *N*-3,5-DNB-(*R*)-phenylglycine CSP ($\alpha = 1.33$ [8]). We may suggest that the amino group of the 3,5-DNB fragment due to its acidic character is capable of participating in complexation between CSP and some analytes as a result of the the intermolecular hydrogen bonds

Table 2
Enantioseparation of alkyl aryl carbinols (**14**–**28**) on **CSP-1** and **CSP-2**

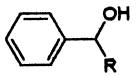
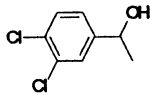
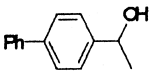
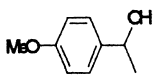
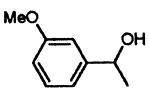
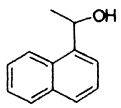
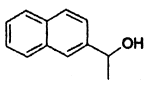
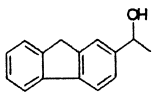
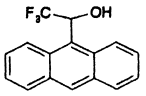
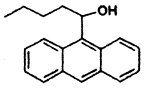
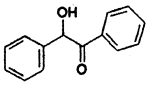
No.	Compound Structure	**CSP-1**[a]		**CSP-2**[a]		α from Ref. [8][a,e]
		α	k'_2	α	k'_2	
						
14	R=Me	1.07	4.75 (*R*)[d]	1.00	2.22	1.05
15	R=Et	1.06	4.20	1.00	2.12	1.05
16	R=Pr	1.07	4.00	1.00	1.90	1.04
17	R=*i*-Pr	1.06	2.33 (*R*)[d]	1.00	1.90	1.08
18	R=*n*-Bu	1.07	3.92 (*R*)[d]	1.00	1.90	–
19		1.08	5.44	1.00	4.22	–
20		1.03	9.40	1.00	7.62	1.03
21		1.06	11.80	1.00	8.46	–
22		1.07	11.00	1.00	8.22	–
23		1.12	11.20 (*R*)[d]	1.00	1.78	1.14
24		1.08	10.30	1.10	10.12	–
25		1.00[c]	3.60[c]	1.08[c] (*R*)[d]	4.85[c]	–

Table 2 (*continued*)

26		1.63[c]	4.46[c] (*S*)[d]	1.30[c]	4.50[c] (*S*)[d]	1.33[c]
27		1.47[c]	3.00[c]	1.31[c]	3.05[c]	1.48[c]
28		1.10[b]	4.75[b] (*S*)[d]	1.00[b]	5.66[b]	–

[a] Flow-rate of the mobile phase is 0.5 ml/min. The mobile phase is *n*-heptane–2-propanol, 99:1 (v/v), unless otherwise indicated.
[c] 95:5, v/v.
[d] Configuration for the second eluted enantiomer. This was determined using a chiral sample prepared by the asymmetric synthesis [11]. For **26** pure enantiomers from Aldrich were used.
[e] For 3,5-DNB-(*R*)-phenylglycine on APS-silica from Ref. [8].
[b] 98:2, v/v.

formation. Another explanation of relatively high α-values achieved with **CSP-1** and **CSP-2** is that the NH group in the π-acceptor fragment forms intramolecular hydrogen bonds with the amide carbonyl group in **CSP-1** or with the NO_2 group in **CSP-2** to afford the planar conformation of the π-acceptor fragment that was postulated [7] to favor to the $\pi-\pi$-interaction between the CSP and a selectant.

While comparing the chromatographic results obtained with **CSP-1** and **CSP-2**, it should be noted that both phases, as a rule, show a similar chiral recognition ability, though **CSP-1** seems to be more universal, as it is most pronounced for separation of alkyl aryl carbinols (Table 2). So, using **CSP-2** instead of **CSP-1** only strong π-donor carbinoles, e.g. compounds **25**, **26** and **27**, could be separated. It is of interest that **CSP-1** shows better separation for *N*-naphthyl amides **8** and **9**, as compared with amide **10** and, on the contrary, **CSP-2** was found to be a preferable stationary phase for amide **10** resolution. It should be noted that **CSP-1** displays a higher structural conformity with respect to analytes containing the bulky 1-naphthyl amide group, whereas **CSP-2** fits better structurally for selectants bearing more stretched π-donor fragments, such as 2-naphthylamide and *p*-methoxyanilyde ones. Thus the availability of a structural conformity between π-donor (analyte) and π-acceptor (CSP) favors better enantioseparation on the prepared CSPs.

Hence, the mechanism of chiral recognition on the prepared CSPs appears to be governed by several factors, i.e. the $\pi-\pi$-complexation, the formation of hydrogen bonds and a steric conformity factor.

References

[1] C.J. Welch, J. Chromatogr. A 666 (1994) 3–26.
[2] F. Mikes, G. Boshart, E. Gil-Av, J. Chromatogr. 122 (1976) 205.
[3] C.H. Lochmuller, R.J. Rayll, J. Chromatogr. 150 (1978) 511.
[4] S. Allenmark, Chromatographic Enantioseparation: Methods and Applications, 2nd edition, Ellis Horwood, New York, 1991.
[5] G. Manecke, G. Günzel, Makromol. Chem. 51 (1962) 199.
[6] H. Brückner, M. Leitenberger, Chromatographia 42 (1996) 683.
[7] R. Däppen, H.R. Karfunkel, F.J. Leusen, J. Chromatogr. 469 (1989) 101.
[8] W.H. Pirkle, J.M. Finn, J.L. Shreiner, B.C. Hamper, J. Am. Chem. Soc. 103 (1981) 3964.
[9] H. Brückner, C. Keller-Hoehl, Chromatographia 30 (1990) 621.
[10] M. Calmes, J. Daunis, A. Hanouneh, R. Jacquier, Tetrahedron Asymmetry 4 (1993) 2437.
[11] R. Noyori, I. Tomino, Y. Tanimoto, M. Nishizawa, J. Am. Chem. Soc. 106 (1984) 6709.