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Diphenylethanediamine derivatives as chiral selectors

III☆. Comparison of four new diastereomeric chiral stationary phases prepared by addition of mono-3,5-dinitrobenzoyldiphenylethanediamine derivatives to optically pure epoxy silica

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ABSTRACT

The synthesis and normal-phase HPLC evaluation of four diastereomeric chiral stationary phases (CSPs) based on mono-3,5-dinitrobenzoylated 1,2-diphenylethane-1,2-diamine (DPEDA) as chiral selector is described. Addition of the free amino function to an optically pure epoxide bound to silica resulted in CSPs having an amino alcohol function additionally to the π-acidic dinitrobenzoylamido group. Efficient separation of the 1-naphthyl derivatized enantiomers of aromatic amines, alcohols and carboxylic acids could be accomplished. The main difference in enantioseparation capabilities is found between (*R*,*R*)- or (*S*,*S*)-DPEDA derived CSPs I and II and the *meso*-DPEDA derived CSP III and IV, the latter being only more effective in the case of increased steric hindrance of the analytes.

INTRODUCTION

(*S*,*S*)-1,2-Diphenylethane-1,2-diamine (DPEDA) derivatives have been shown to function as highly efficient chiral selectors (SOs) for enantioselective HPLC. Saigo *et al.* [2] prepared polyamide-based chiral stationary phases (CSPs) with aliphatic and aromatic diacid chlorides, the adipic acid analogue being able to separate 1,1′-binaphthol enantiomers with a good but rather unique resolution.

N-3,5-Dinitrobenzoyl (DNB) derivatives of DPEDA have been shown by us [1,3] to be useful, efficient and broadly applicable "brush-type" chiral selectors utilizing hydrogen bonding together with π-acid–π-base interactions similar as the classical Pirkle-type amino acid-based CSPs [4]. The chiral selectors have been bound to the silica surface in two different ways: one [3] used a nitrogen bonded undecanoyl group as spacer showing a broad enantioseparation capability for many amides and urethanes as well as some alcohols including binaphtol ($R = 0.98$ —which is not as good as Saigos polymer phase with $R = 1.65$, however), some sulphoxides and various drugs. For the second type [1] we used a

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☆ For Part II, see ref. 1.

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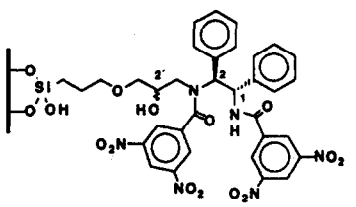
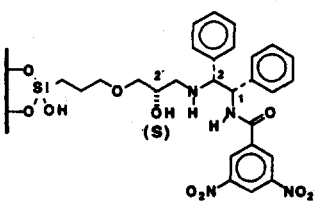
binding technique similar as Gasparrini *et al.* [5]; these authors immobilized (*R,R*)-diaminocyclohexane (DACH) derivatives by an addition reaction of the diamine to a silica which was prefunctionalized with an epoxysilane and performed subsequently an exhaustive 3,5-dinitrobenzoylation of the addition product. This (*R,R*)-DACH derived CSP separates exceptionally well enantiomers of sulphoxides [6] and amino alcohols, derivatized as oxazolidinones [7]. The authors claim [7] that this rather special separation capability is due to a spatial locking of the selectand (SA) in close parallel planes to the DNB groups thus enabling further intermolecular hydrogen bond and dipole interactions between SO and SA. We have found that replacing the chiral (*R,R*)- or (*S,S*)-DACH selector by the similar, but more flexible (*R,R*)- or (*S,S*)-DPEDA molecule, leads to the separation characteristics changing rather unexpectedly resulting in a CSP (see Fig. 1) exposing high enantioselectivity for naphthylamides [1], moderate for oxazolidinones but with poor enantioselectivity for sulphoxides.

This prompted us to prepare a new type [8] of well defined mono-3,5-dinitrobenzoylated CSPs, I–IV (Fig. 1) from (*R,R*)-, (*S,S*)- and *meso*-DPEDA instead of the dibenzoylated version [1]. These novel diastereomeric CSPs have besides the features of typical Pirkle-type CSPs an additional secondary amino group directly bound to an asymmetric carbon (C2) as well as an optically pure carbinol function at C2′. The aim of the following paper was to study specific chiral effects derived from the single well investigated dinitrobenzoylamido group and possible additional effects provided by the adjacent two asymmetric centres.



bis -3,5-dinitrobenzoylated (S,S) DPEDA derived CSP



abs. configuration at position	1	2
CSP I	R	R
CSP II	S	S
CSP III	R	S
CSP IV	S	R

mono -3,5-dinitrobenzoylated DPEDA derived CSPs I-IV

Fig. 1. Chiral stationary phases based on DPEDA as chiral selector.

EXPERIMENTAL

Apparatus

Chromatography was performed using a series 1050 pump and variable-wavelength detector (Hewlett-Packard) and a CHROMA integration equipment and software from Fa. PAAR (Graz, Austria). The injector with a 20-μl loop was connected to stainless-steel columns (125 × 4 mm I.D.), packed with the different CSPs using chloroform–dioxane (3:1) as slurry solvent and *n*-heptane as pressurizing solvent. Column packing was performed by Forschungszentrum Seibersdorf, Austria.

Chemicals and reagents

Racemic and optically pure drugs were obtained from different pharmaceutical companies. Other analytes were from Aldrich (Steinheim, Germany) or prepared by standard procedures. The derivatives (amides, carbamates, ureas) of the various chiral analytes were prepared by common methods using acid chlorides and isocyanates. Allyl bromide, 3,5-dinitrobenzoyl chloride, (*R*)-glycidol, trimethoxysilane and hexachloroplatinic acid were purchased from Aldrich. HPLC-grade solvents and LiChrosorb Si 100, 5 μm, were from Merck (Darmstadt, Germany).

(1*R*,2*R*)- and (1*S*,2*S*)-N-mono-3,5-dinitrobenzoyl-1,2-diphenylethane-1,2-diamine (see Fig. 2, **5a** and **5b**) (DNB-(1*R*,2*R*)-DPEDA and DNB-(1*S*,2*S*)-DPEDA) were prepared according to the literature [3]. The (*R,R*) enantiomer **5a** has not yet been described (m.p. 165–168°C; $[\alpha]_{546}$ + 45.6 (c = 1, MeOH).

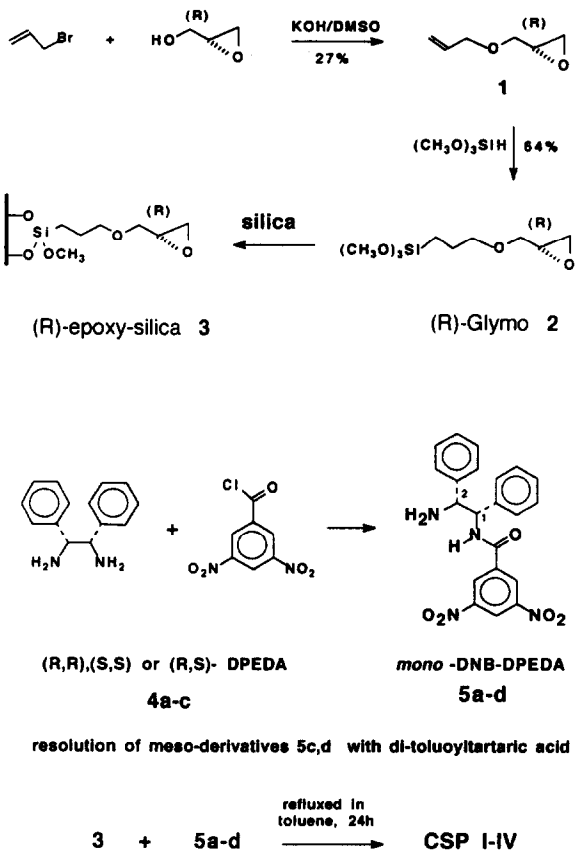


Fig. 2. Synthesis of CSPs I–IV.

Synthesis of (1R,2S) and (1S,2R)-N-mono-3,5-dinitrobenzoyl-1,2-diphenylethane-1,2-diamine, 5c and 5d (DNB - (1R,2S)-DPEDA and DNB-(1S,2R)-DPEDA

Monoacylation of *meso*-DPEDA [9] with 3,5-dinitrobenzoyl chloride was performed similarly as described [3] for the (*S,S*) derivative (yield 57% after Soxhlet extraction of the free base with dichloromethane, m.p. 179–180°C; $C_{21}H_{18}N_4O_5$; NMR in [$^2H_6$]dimethyl sulphoxide, 200 MHz, δ in ppm: 2.0 broad, NH_2; 4.3 d, 9,4 Hz CH; 5.2 d, broad, CH; 7.1–7.6 m, phenyl; 8.8 m, 2H and 8.9 m, 1H dinitrobenzoyl; 9.4 ppm broad, NH).

Resolution of the resulting racemic mixture was performed as follows: the racemic material (20 g, 49.2 mmol) and L-ditoluoyltartaric acid (19 g, 49.2 mmol) were dissolved in 1500 ml ethanol (96%) and the resulting crystals were collected after 48 h at room temperature. After two recrystallizations from ethanol 4.9 g (27%) optically pure (de > 98%) diastereomeric salt was obtained (m.p. 180–185°C, dec). From 4 g of this salt 1.5 g free base was isolated after treatment with a solution of 5 g NaOH in 500 ml water and extraction of the product [the (1*S*,2*R*) derivative **5d**, see below] with dichloromethane (4 × 100 ml). An analytically pure sample was recrystallized from toluene: m.p. 203–205°C, $[\alpha]_{546}$ + 32.2 (c = 1, MeOH). From the collected mother liquids the optically enriched base was isolated and treated similarly with the enantiomeric D-ditoluoyltartaric acid, affording finally 1.6 g optically pure base **5c**, the (1*R*,2*S*) enantiomer, m.p. 203–205°C, $[\alpha]_{546}$ − 32.3 (c = 1, MeOH). Optical purity of **5a–5d** was found to be > 98% enantiomeric excess (ee) according to NMR analysis of the Mosher amides. The absolute configuration of **5c** and **5d** of was established comparing the complete [10] 360 MHz NMR spectra of the Mosher amides of all four diastereomers, whereby the absolute configuration of the (*R,R*) and (*S,S*) derivatives **5a** and **5b** has been known previously [11].

Preparation of (R)-epoxysilica **3**

(*R*) - [3 - (Oxiranylmethoxy)propyl]trimethoxysilane **2** [(*R*)-glymo, see Fig. 2) was prepared from (*R*)-glycidol in two steps in a stereochemically unambigous way [12] using the following procedure.

To a vigorously stirred, cooled (4°C) suspension of pulverized KOH (14.8 g, 0.26 mol) [13] in 100 ml dimethyl sulphoxide was added allyl bromide (88 ml, 1.0 mol) followed by slow addition of (*R*)-glycidol (15.0 g, 2.0 mol). The mixture was allowed to reach room temperature and stirred for 4 h. After addition of water (250 ml) the separated organic phase was washed with water (2 × 100 ml) and dried with magnesium sulphate. After removal of the low-boiling components at normal pressure the residue was distilled at 25–30 mbar, yielding 6.25 g (27%) (*R*)-allyloxymethyloxiran (**1**) as colorless oil (b.p. 66°C/30 mbar, Lit. [14] 43°C/0.4 mbar), $[\alpha]_{546}$ + 13.7 (neat); Mikkilineni *et al.* [14] give a

value $[\alpha]_D + 9.6$ ($c = 0.94$, EtOH), but their correctly drawn structure was named obviously by an error as (*S*) configurated. However, their epoxide was prepared from (*S*)-O-allylglycerol *without inversion step at the secondary carbinol*, therefore the nominal change to (*R*) is due to the CIP-rules only. Hydrosilylation of allyl epoxide **1** (6.0 g, 53 mmol) with trimethoxysilane (6.4 g, 53 mmol) was performed using 2 mg of hexachloroplatinic acid in 5 μl isopropanol as catalyst (60°C, 3 h). Distillation yielded 7.9 g (64%) colorless (*R*)-glymo **2** (b.p. 90°C/0.12 mbar; $[\alpha]_{546} + 9.7$ (neat). (*R*)-Epoxysilica **3** was prepared by refluxing 3.3 g of the silane **2** and 11.1 g of azeotropically dried silica in toluene (100 ml) for 24 h. CH analysis of the washed (toluene) and dried (0.1 mbar, 60°C) silica material gave C 5.71%, H 1.30%, equivalent to 678 μmol epoxide/g silica.

Synthesis of CSPs I–IV

Each of the mono-DNB-derivatives **5a–5d** (1.2 g, 2.95 mmol) was refluxed with 2.15 g (*R*)-epoxysilica **3** in toluene (50 ml) containing phenol (5 mg) for 24 h. The modified silica was washed with toluene, methanol and ethyl ether and dried at 0.1 mbar, 60°C. CHN analysis for CSP I: C 10.61, H 1.52, N 1.20 (corresponds to 214 μmol selector **5a**/g, based on nitrogen); CSP II: C 10.98, H 1.50, N 1.36 (242 μmol selector **5b**/g, based on nitrogen); CSP III: C 11.99, H 1.71, N 1.57 (280 μmol selector **5c**/g, based on nitrogen); CSP IV: C 12.08, H 1.64, N 1.59 (283 μmol selector **5d**/g, based on nitrogen).

RESULTS AND DISCUSSION

Optically pure epoxidized silica was easily prepared in two steps starting from (*R*)-glycidol and allylbromide (Fig. 2). The optical configuration at the epoxidized silica is unambigous (*R*), because a possible intramolecular addition of the alcoholate at the epoxy function would be degenerate [12].

Synthesis of the four different diastereomeric chiral selectors was straightforward (Fig. 2): monoacylation [3] of easily prepared *meso*-1,2-diphenyl-1,2-diaminoethane **4c** and the two commercially available enantiomeric (*R*,*R*)- and (*S*,*S*)-DPEDAs **4a** and **4b** followed by resolution of the (*R*,*S*) enantiomers resulted in two diastereomeric pairs of selectors **5a–5d**. By the addition of the primary amino function of the SOs to the optically pure epoxide **3** the resulting four different diastereomeric chiral stationary phases CSP I–IV (Fig. 1) contain three stereogenic centres.

In order to compare the stereoselectivity of these CSPs and the resulting HPLC columns in a straightforward manner, we decided to use first a single solvent system, *n*-heptane–isopropanol–diethylamine (70:30:0.1). Thus we were able to measure most of the analytes under equal conditions, although accepting that in many cases retention times were unnecessarily long, particularly in the case of the *meso*-DPEDA derived CSPs III and IV.

A series of structurally homologous or similar analytes was chosen as chiral probes (SAs) to contrast differences in enantioseparation characteristics of the four diastereomeric mono-DNB-DPEDA selectors interacting with similarly structured amines (**6–13**), carboxylic acids (**14–19**), alcohols (**20–27**), tetrahydropyrimidines (**28–30**) and sulphoxides (**31–33**). Propranolol (**34**) (derivatized as oxazolidinone), oxazepam and lormetazepam (**35**, **36**) were included in the list of SAs to depict the scope of the new CSPs.

All of the chromatographic data are summarized in Table I. It shows the capacity factors, separation coefficients, resolution values and in some cases the elution order by assigning the absolute configuration of the most retained enantiomer. Similar as for most of the other known Pirkle-type CSPs, the first three classes of analytes could only be reasonably well separated after their derivatization to phenyl and/or naphthylamides, ureas and carbamates.

In the following it will be tried to interpret the chromatographic data listed in Table I with respect to (a) capacity factors representing the sum of chiral and non-chiral interactions and (b) chiral recognition, but separately for the different classes of analytes.

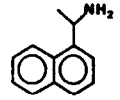
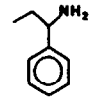
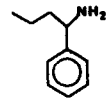
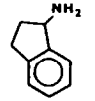
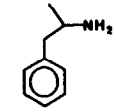
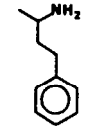
Capacity factors

Clearly CSPs I and II are at the same mobile phase composition less retentive than CSPs III

TABLE I

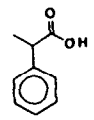
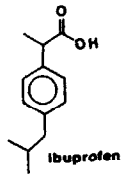
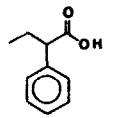
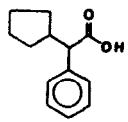
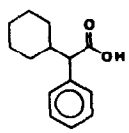
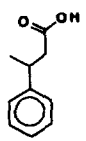
CAPACITY FACTORS, SEPARATION COEFFICIENTS AND RESOLUTION OF DERIVATIZED ENANTIOMERS OF AMINES, CARBOXYLIC ACIDS, ALCOHOLS, SULPHOXIDES AND TETRAHYDROPYRIMIDINE-2-ONES ON CSPs I–IV

Heptanol–isopropanol–diethylamine (70:30:0.1); flow-rate 1 ml/min; 20°C; UV detection at 254 nm.

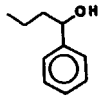
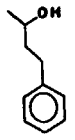
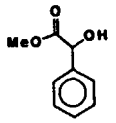
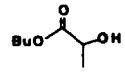
No.	Analyte	Derivative[a]	CSP I (1*R*,2*R*,2'*S*)				CSP II (1*S*,2*S*,2'*S*)				CSP III (1*R*,2*S*,2'*S*)				CSP IV (1*S*,2*R*,2'*S*)			
			k'_1	α	R_s	mre[b]	k'_1	α	R_s	mre	k'_1	α	R_s	mre	k'_1	α	R_s	mre
Amines as amides and ureas																		
6		B	1.55	1.19	0.89	*S*	1.66	1.21	1.07	*R*	4.96	n.r.[c]			4.86	n.r.		
		1-N	4.59	1.44	2.18	*S*	5.51	1.52	2.15	*R*	26.10	1.13	0.88	*S*	30.60	1.05		*R*
		2-N	3.04	1.23	1.66	*S*	3.39	1.31	2.24	*R*	13.80	1.12	0.63	*R*	13.30	1.18	1.06	*S*
		PU	1.49	1.15	1.43	*S*	1.55	1.19	1.25	*R*	5.04	n.r.			5.10	n.r.		
		NU	3.79	1.64	2.76	*S*	4.42	1.57	2.51	*R*	24.30	1.31	2.58	*S*	28.00	1.25	1.93	*R*
7		B	2.93	2.47	5.84	*S*	3.39	2.37	4.97	*R*	16.4	1.17	1.12	*R*	17.50	1.14		*S*
		PU	2.61	2.02	5.19	*S*	3.21	1.96	4.21	*R*	20.10	1.58	3.26	*R*	25.20	1.16	1.08	*S*
8		B	1.38	1.21	1.25		1.43	1.24	1.50		4.34	1.05	–		4.08	1.08	–	
		1-N	4.25	1.45	2.29		5.05	1.53	2.66		20.30	1.25	1.51		21.30	1.21	1.43	
		PU	1.26	1.23	2.17		1.32	1.17	2.49		4.15	1.14	1.12		4.02	1.18	1.37	
		NU	3.33	1.69	4.34		3.85	1.61	2.55		20.80	1.42	2.59		21.70	1.34	2.39	
9		B	1.26	1.18	0.83		1.39	1.17	1.00		1.67	n.r.	–		4.08	n.r.	–	
		1-N	4.02	1.35	2.16		4.76	1.42	1.98		20.70	1.26	1.50		20.90	1.22	1.34	
		PU	1.26	1.14	3.74		1.32	1.13	3.74		3.76	1.12	0.97		3.77	1.14	1.11	
		NU	3.27	1.60	3.26		3.73	1.55	2.99		19.70	1.45	2.87		19.40	1.34	2.15	
10		1-N	5.05	1.11	0.78		6.08	1.10	0.80		20.70	1.07	–		21.00	1.17	0.95	
		NU	4.76	n.r.	–		5.57	n.r	–		22.80	1.16	0.94		22.80	1.15	1.07	
11		1-N	5.22	1.08	–		5.31	1.05	–		23.10	1.06	–		23.30	1.18	1.01	
		NU	4.76	n.r.	–		5.68	n.r.	–		24.40	1.17	1.14		17.60	1.16	1.19	
12		1-N	3.50	n.r.	–		4.25	n.r.	–		13.8	1.22	1.30		13.8	1.17	1.12	
		NU	3.04	1.23	1.50		5.52	1.20	1.32		14.83	1.31	2.90		17.17	1.26	1.96	
13		1-N	4.02	1.06			4.94	1.05	–		17.0	n.r.	–		16.6	n.r.	–	
		NU	3.21	1.18	1.25		3.62	1.17	0.98		16.30	1.29	1.82		16.10	1.26	1.93	

(Continued on p. 46)

TABLE I *(continued)*

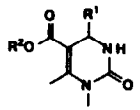
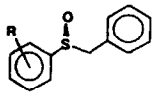
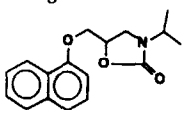
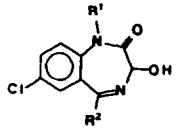
No.	Analyte	Derivative[a]	CSP I (1*R*,2*R*,2′*S*)				CSP II (1*S*,2*S*,2′*S*)				CSP III (1*R*,2*S*,2′*S*)				CSP IV (1*S*,2*R*,2′*S*)			
			k_1'	α	R_s	mre[b]	k_1'	α	R_s	mre	k_1'	α	R_s	mre	k_1'	α	R_s	mre
Carboxylic acids as amides																		
14		A	1.09	1.16	–	*R*	1.09	1.26	–	*S*	2.67	n.r.	–		2.86	n.r.		
		1-NA	3.33	1.63	3.32	*R*	3.67	1.77	2.91	*S*	18.90	1.41	2.23	*R*	20.40	1.40	2.53	*S*
15		A	0.75	1.23	3.74	*R*	0.80	1.21	3.75	*S*	1.47	n.r.			1.50	n.r.		
		1-NA	2.30	2.07	4.87	*R*	2.53	2.07	3.08	*S*	11.30	1.17	1.23	*R*	11.60	1.26	1.85	*S*
		NMA	1.84	1.44	4.99	*R*	2.01	1.46	2.49	*S*	7.10	n.r.			7.78	n.r.		
16		1-NA	2.75	1.75	3.90		3.10	1.70	3.16		13.90	1.50	2.47		14.90	1.50	3.07	
17		1-NA	2.58	1.51	2.87		2.87	1.52	2.49		9.53	1.62	2.84		9.85	1.54	3.01	
18		1-NA	2.53	1.45	2.49		2.81	1.51	2.83		8.62	1.93	3.86		9.21	1.80	3.64	
19		1-NA	3.62	1.08	–		4.07	1.06	–		11.20	1.11			12.54	1.10	0.70	

Alcohols as carbamates

20		PC	1.03	n.r.	–		1.03	n.r.	–		1.38	n.r.	–		1.38	n.r.	–	
		NC	1.95	1.44	3.12	*S*	2.18	1.39	2.87	*R*	6.07	1.44	2.93	*S*	6.35	1.41	2.67	*R*
21		NC	1.72	1.57	3.06		1.89	1.55	2.49		4.99	1.64	3.05		4.93	1.55	3.08	
22		NC	1.55	1.52	1.94		1.72	1.47	2.49		4.41	1.69	2.93		4.54	1.57	2.92	
23		NC	3.43	n.r.			2.98	n.r.			7.67	n.r.			6.75	1.06	–	
24		NC	2.75	n.r.			3.10	n.r.			7.44	1.12	0.75		7.56	1.11	0.76	
25		NC	0.75	1.23	2.50		2.12	1.08	–		4.80	1.19	1.16		4.67	1.18	1.48	
26		PC	1.61	n.r.			1.61	n.r.			1.75	1.15	1.00		1.68	1.19	2.12	
		NC	3.04	1.11	0.83	*S*	3.39	1.12	0.87	*R*	6.48	1.29	1.81	*S*	6.35	1.27	1.61	*R*
27		NC	1.43	1.16	2.50		1.61	1.14	1.00		2.93	1.29	1.80		2.72	1.38	2.49	

(Continued on p. 48)

TABLE I *(continued)*

No.	Analyte	Derivative[a]	CSP I (1*R*,2*R*,2′*S*)				CSP II (1*S*,2*S*,2′*S*)				CSP III (1*R*,2*S*,2′*S*)				CSP IV (1*S*,2*R*,2′*S*)			
			k_1'	α	R_s	mre[b]	k_1'	α	R_s	mre	k_1'	α	R_s	mre	k_1'	α	R_s	mre
Tetrahydropyrimidines																		
28	R^1 = 2-naphthyl R^2 = benzyl		4.19	1.18	1.62		4.48	1.26	2.08		9.10	1.78	2.58		10.50	1.50	2.49	
29	R^1 = 2-naphthyl R^2 = *tert.*-butyl		1.95	1.18	2.14		2.01	1.29	2.49		3.78	1.62	2.43		4.29	1.36	1.50	
30	R^1 = 3,4,5-trimethoxy R^2 = *tert.*-butyl		5.18	n.r.	–		5.51	1.06	–		10.42	1.11	0.62		10.85	n.r.		
Sulphoxides																		
31	R = H		1.61	n.r.			1.78	n.r.			3.72	1.11	0.87		3.59	1.08	0.83	
32	R = 4-Cl		1.43	n.r.			1.49	n.r.			2.67	1.11	0.66		2.70	1.09	0.80	
33	R = 2-MeO		2.24	n.r.			2.47	n.r.			5.67	1.24	1.76		5.70	1.24	2.60	
Drugs																		
34	Propranolol-oxazolidinone		7.29	n.r			8.44	1.10	–	*S*	20.50	1.24	1.44	*R*	17.00	1.25	1.69	*S*
35[d]			4.48	1.23	1.12		4.88	1.18	0.83		5.39	n.r			5.61	n.r.		
36[e]			5.91	1.47	2.22		6.60	1.42	1.93		10.30	1.08	0.54		9.2	n.r.		

[a] B = N-Benzoyl; 1-N = N-1-naphthoyl; 2-N = N-2-naphthoyl; A = anilide; 1-NA = 1-naphthylamide; 2-NA = 2-naphthylamide; NMA = (1-naphthyl)methylamide; 1NU = 1-naphthylurea; PU = phenylurea; NU = 1-naphthylurea; PC = phenyl carbamate; NC = 1-naphthyl carbamate.

[b] mre = Most retained enantiomer.

[c] n.r. = Not resolved.

[d] Oxazepam: R^1 = H; R^2 = phenyl.

[e] Lormetazepam: R^1 = Me; R^2 = 2-Cl-phenyl.

and IV for most of the analytes. The capacity factors for the first eluted enantiomer differ usually by a factor of 2–5, which is rather large if one considers that the chiral selectors are diastereomers and the loading of the silica with the SOs differs by not more than 30%. Non-enantiospecific interactions of analytes with SOs derived from *meso*-DPEDA seem to be often more favourable resulting in much larger capacity factors. Surprisingly, almost equal k' values were observed for the seven membered-ring drugs oxazepam (**35**) and lormetazepam (**36**), the latter being well resolved only by CSPs I and II [15]. The increment on retardation of analytes caused by residual silanol groups is difficult to extract, but it should be noted that all investigated CSPs stem from the same batch of epoxidized silica. Therefore the observed large differences in retention behaviour should mainly be attributed to interactions with the SO groups regarding their shape and accessibility.

Chiral recognition

Amines as amides and ureas. In general, acyl derivatives of amines [16] can be well resolved on all four CSPs. As it has been previously observed for Pirkle-type CSPs, also on CSPs I–IV direct separation of basic amines could not be achieved without derivatization, at least not under normal-phase conditions. Enantioseparation of β-blockers has recently been shown to be feasible using supercritical CO_2 [17] on a 3,5-dinitrobenzoyltyrosine-based CSP. However, simple derivatization of amines with phenyl groups (benzoate, phenylurea) is in some cases sufficient to get baseline resolution on CSPs I–IV. A distinctive case are the derivatives of analyte **7**, which contains already a π-basic naphthyl group; elution order and hence chiral recognition are in this case very special and will be treated below. As expected [18] for typical π-acidic type CSPs, analytes containing a π-basic naphthyl group *per se* or via the derivatization reagent are in general more effectively stereodifferentiated. Comparing analytes **6–9** having the asymmetric carbon next to the aromatic ring and to the NH of the amido group it becomes evident that although amides and ureas have comparable separation coefficients, the urea derivatives give sharper peaks and hence better resolution values. If there are special steric requirements as in cyclic analytes **10** and **11**, amides in contrast to ureas can be better, but not sufficiently resolved on CSPs I and II. However, CSPs III and IV show better resolution capabilities not only for these two analytes but also for compounds **12** and **13**. Comparing the analytes **6**, **12** and **13** which have the amino group one, two or three carbons remote from the phenyl ring one can notice that the enantioselective power decreases markedly for CSPs I and II but not for CSPs III and IV. Conformational differences between these SOs resulting in more or less hindered or spatially directed intermolecular $\pi-\pi$ interactions of the various aromatic groups, also with respect to the (chiral) carbinol group at C2′ must account for these phenomena. However, such strong interactions must not necessarily lead to pronounced stereodifferentiation, but they can be responsible for strong retention (see above).

Carboxylic acids as amides

A wide range of chiral carboxylic acids, derivatized as aromatic amides can be separated. Neglecting the too large capacity factors for CSPs III and IV, all four types of mono-DNB-DPEDA derived CSPs function as effective chiral selectors for naphthylcarbamates, but CSPs III and IV are somewhat less selective for simple acids **20–22**. Note the small capacity factor of 0.75 (0.80) for the well separated ibuprofen (**15**) anilide [19] on CSPs I and II. Again and as found with derivatized amines, sterically more crowded analytes **17** and **18** are better separated by CSPs III and IV.

Very recently enantiomers of underivatized 2-arylpropanoic acids such as ibuprofen (**15**) α = 1.12 and especially naproxen have been separated on a "tailor-made" dinitrobenzoylated CSP [20] and also separation of chiral α-substituted α-aryloxy acetic acids has been achieved on an exhaustively 3,5-dinitrobenzoylated diaminocyclohexane (DNB-DACH) derived CSP [21]. The potential capabilities of CSPs I–IV for non-derivatized acids using special mobile phase additives remain to be tested.

Alcohols as carbamates

In contrast to our previously published undecanoylamide bound mono-DNB-DPEDA derived CSP [3] and the standard Pirkle phenylglycine derived CSP [22], both having two amido functions within the molecule, the new phases CSPs I–IV expose only poor capabilities to separate directly anthryltrifluoroethanol or binaphthol (data not shown). This finding suggests that interaction of a second amido group could be necessary for the recognition of the alcohol function either via direct hydrogen bonding to the amido carbonyl or the second amido function is responsible for a unique *intra*molecular hydrogen bonding of the chiral selector, thus exposing a differently shaped "chiral selector surface", which must be recognized by the chiral carbinol. However, even simple alcohols derivatized as phenyl- and better as α-naphthylcarbamates [18] can be resolved with CSPs I–IV. Again, and as found for the amides of chiral amines and carboxylic acids, the sterically more crowded cyclopentanols and cyclohexanols **23** and **24** are preferably discriminated by the *meso*-DPEDA derived CSPs III and IV. Interestingly, phenyl and naphthylcarbamates of methyl mandelate **26** are also separated more efficiently with these two CSPs and with comparable retention times to CSPs I and II. One could suggest a different mechanism of chiral and non-chiral interaction, because the capacity factors are in most cases much higher for the *meso*-DPEDA derived CSPs III and IV than for CSPs I and II. This observation is also strongly supported by a different elution order (see below).

Tetrahydropyrimidines

A polyamide-based CSP capable of separating nifedipine analogues has been published recently [23]. Remarkably, there is hardly any comparable literature about enantioseparation of aza analogues of nifedipine, some of them exhibiting potent Ca channel blocker capabilities [24]. Racemic 5-benzyl- and *tert.*-butylesters of 4-naphthyltetrahydropyrimidine-2-ones (**28–30**) which have still an NH group in position 3, next to the chiral centre, can be separated well by all four types of CSP. After N-methylation these derivatives could not be resolved any more (data not shown). This could be an explanation why a chiral stationary phase based on this N-methylated type of tetrahydropyrimidine derivative did not work [25]. An efficient π-basic group in position 4 seems to be necessary, because derivatives having a simple phenyl group in that position could also not be separated (data not shown).

Sulphoxides

In comparison to highly dinitrobenzoylated (*R*,*R*)-DACH phases [6] the separation capabilities of the new stationary phases are somewhat limited. However, there is a clear difference between CSPs I and II and the *meso*-DPEDA derived CSPs II and IV. The latter separate very well the sterically more hindered 2-methoxy derivative **33**. However, the important antiulcer drug (±)-omeprazole (data not shown) could not be resolved ($k' = 10.7$ on CSP I).

Elution order

As far as the absolute configurations of the most retained enantiomers are concerned (data indicated in Table I), there is a clear pattern found for CSPs I and II, strongly determined by the absolute configuration of the carbon atom C1 bearing the 3,5-dinitrobenzoylamido function. For all amino derivatives (amides and ureas) the (*S*) enantiomers are most retained by CSP I and the (*R*) enantiomer by CSP II derived from (*S*,*S*)-DPEDA. It is very interesting to note that with our CSP derived from the identical (*S*,*S*)-DPEDA selector, but with *two* dinitrobenzoylated amino centres (Fig. 1) [1] the *same* elution order is found. Therefore the dominating enantioselective interaction must occur due to the configuration at carbon 1 with its primary amido group. The identical pattern is found for the naphthylcarbamate of simple phenylethanol **20** replacing the NH group in the urea of phenylethylamine (**6**) by an oxygen. However, the naphthylcarbamate of methyl mandelate **26**, where a methyl group from analyte **20** is replaced by a carboxymethyl group shows the *same formal pattern* but a sterically seen *different* elution order: note the change of nomenclature due to a different priority. The elution order is

also reversed if the centre of chirality is shifted from the amino part to a carboxylic acid, most retaining the (*R*)-acid amide (*e.g.* **14**, **15**) in the case of CSP I and the (*S*)-acid amide in the case of CSP II. This pattern does not change if the aromatic naphthyl residue is separated by a methylene group from the amido function as demonstrated with ibuprofen (**15**) derivatized as 1-naphtylethylamide. Consistency of the elution order clearly suggests that the chiral recognition occurs in CSPs I and II predominantly due to conventional π-acid–π-base SO–SA interactions combined with strong interactions at the amido hydrogen. Note the strikingly identical elution order for all derivatives of analytes **6** and **7** with CSPs I and II.

This picture is by far not as clear comparing the elution order found with CSPs III and IV. There is a difference between the 1-naphthoylated and the 2-naphthoylated analyte **6** which is not easy to explain. The latter derivative together with the benzoate and the 1-naphthylurea of analyte **7** have the same "reversed" elution order in common. In these special cases stacking of the 3,5-dinitrobenzoyl ring of the SO and the π-basic analytes seems to be different for CSPs III and IV in comparison with CSPs I and II. Interestingly the same deviation for analyte **7** [the (*S*)-enantiomer being most retained] was found with our previously published [3] undecanoylamido DNB-(*S*,*S*)-DPEDA derived CSP. However, with this and also with the *meso*-DPEDA derived CSPs III and IV most other analytes show a consistent pattern of elution order as chiral recognition is dominated by the obviously strongest enantioseparating force, the 3,5-dinitrobenzoylamido group positioned at carbon 1 and reflecting its absolute configuration. The enantioselective interaction of the analytes with the free carbinol group at C2′ of the SO is too weak to induce in any observed case a deviating elution order.

Overall performance of CSPs I–IV

The new CSPs proved practical usefulness and showed broad separation capabilities for amines, carboxylic acids and alcohols, derivatized as naphthylamides, ureas or carbamates. In the course of this study the separation coefficients were not optimized but harmonized using a uniform solvent mixture as mobile phase. However, aprotic solvent mixtures as adressed by

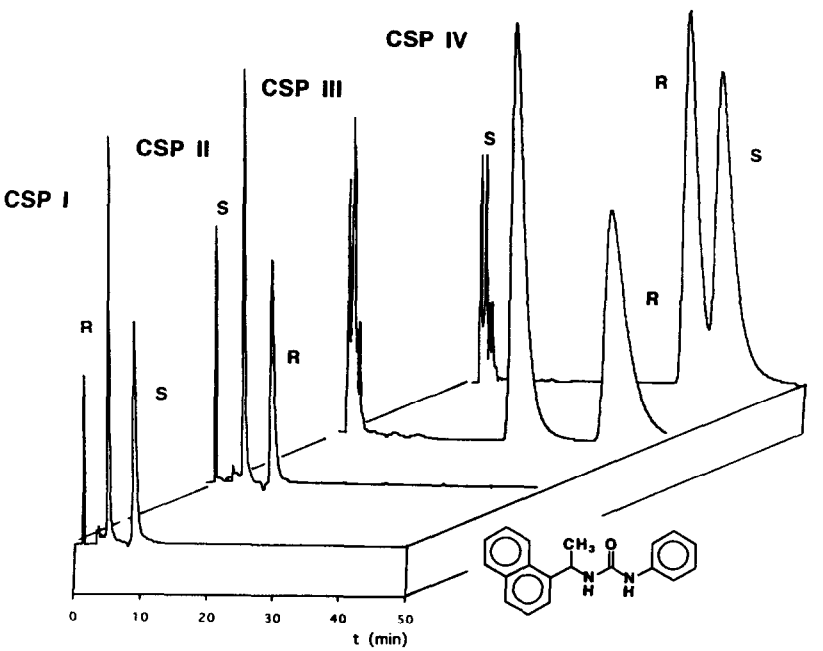


Fig. 3. Chromatograms and elution order of the phenylurea derivative of analyte **7** on CSPs I–IV.

Caude *et al.* [26] might be in some cases advantageous. Also, for practical purposes and especially for CSPs III and IV the preparation of a less heavily selector-loaded silica in combination with specific endcapping resulting in diluted CSPs could be of advantage. From Table I it can easily be extracted, that many of the chromatographic data are very similar for the (*R*,*R*)-DPEDA and (*S*,*S*)-DPEDA derived CSPs I and II as well as for the *meso*-DPEDA derived CSPs III and IV, but the two pairs are fairly different from each other. However, this is not true in all cases: naphthylethylamine (**7**) derivatized as phenylurea shows the strongest effects which can be deduced to the influence of the absolute configuration of the hydroxy groups at C2′ which represents the only structural difference between CSPs III and IV (Fig. 3). Clearly CSP IV does not separate well the enantiomers of this analyte, sharply in contrast to the other three CSPs.

The new CSPs are synthetically easy accessible and the presented data show that the third stereogenic centre substituted with a hydroxy group obviously does not influence significantly the overall stereoselectivity in CSPs I–IV. Important effects are only found at very special occasions (*e.g.* analyte **7**). Therefore this centre could be usually left racemic. This might be different after a derivatization of this alcohol function with groups capable of stronger enantiospecific interactions such as an additional 3,5-dinitrobenzoylester [6] or a carbamate.

CONCLUSIONS

It could clearly be shown that also for the newly synthesized multifunctional CSPs the main chiral discrimination mechanism is driven by π-acid–π-base interactions. These forces are promoted by the primary 3,5-dinitrobenzoylamido group which is directly linked to a centre of chirality. CSPs I and II resolved in many cases the investigated chiral analytes more efficiently than their *meso*-DPEDA derived counterparts; thus they may be somewhat broader applicable for drugs. However, higher steric requirements of certain analytes allow surprisingly better separations with CSPs III and IV, which have obviously "easier" accessible sites for chiral and non-chiral interactions.

The chiral selector units are easily prepared. (*R*,*R*)- and (*S*,*S*)-DPEDA are commercially available, the *meso* compound can be easily prepared in two steps from benzaldehyde and ammonium acetate.

Considering the high similarity of the investigated class of chiral selectors one can still not easily predict which chiral selector of the Pirkle type is the most promising one for a given problem. This becomes even more complicated if one considers open chained chiral selectors with various stereogenic centres leading to conformers which are difficult to characterize. However, the rational development of any new type of chiral selector [20] can be greatly facilitated utilizing knowledge from systematic investigations.

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