Journal of Chromatography, 553 (1991) 373–381
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2132

(*S,S*)-Diphenylethylethanediamine derivatives as chiral selectors

II. Gasparrini-type bound chiral stationary phase with high enantioselectivity for naphthylamides[a]

W. LINDNER*
Institute of Pharmaceutical Chemistry, Karl-Franzens-University of Graz, A-8010 Graz (Austria)
G. URAY
Institute of Organic Chemistry, Karl-Franzens-University of Graz, A-8010 Graz (Austria)
and
U. STEINER
Institute of Pharmaceutical Chemistry, Karl-Franzens-University of Graz, A-8010 Graz (Austria)

ABSTRACT

The synthesis of a chiral stationary phase (CSP) containing the 3,5-dinitrobenzoyl derivative of (*S*,*S*)-1,2-diphenylethanediamine (DPEDA) as a chiral selector by immobilizing the DPEDA derivative onto glycidoxypropyl-modified silica gel and subsequent benzoylation is described. This so-called CSP-II showed high enantioselectivity under normal-phase conditions, in particular for chiral compounds containing a naphthylamide functionality.

INTRODUCTION

In Part I [1], we reported the synthesis of a new chiral stationary phase (CSP-I) derived from (*S*,*S*)-1,2-diphenylethanediamine (DPEDA) as chiral selector (SO). We presented some preliminary results concerning the optical resolution capability of this CSP-I (see Fig. 1) under normal-phase high-performance liquid chromatographic (HPLC) conditions. It was found that CSP-I effectively resolved a wide range of racemic selectands (SAs), probably owing to intermolecular π-donor–acceptor and hydrogen-bonding interactions. As indicated in Fig. 1, CSP-I provides a 3,5-dinitrophenyl group as π-donor and two different amido functions as hydrogen donor–acceptor groups. In this work we investigated the influence of a second 3,5-dinitrobenzoyl function in a similar (*S*,*S*)-DPEDA-derived CSP (CSP-II) on the overall

---

[a] Dedicated to Professor Dr. G. Zigeuner (Karl-Franzens-University of Graz) on the occasion of his 70th birthday.

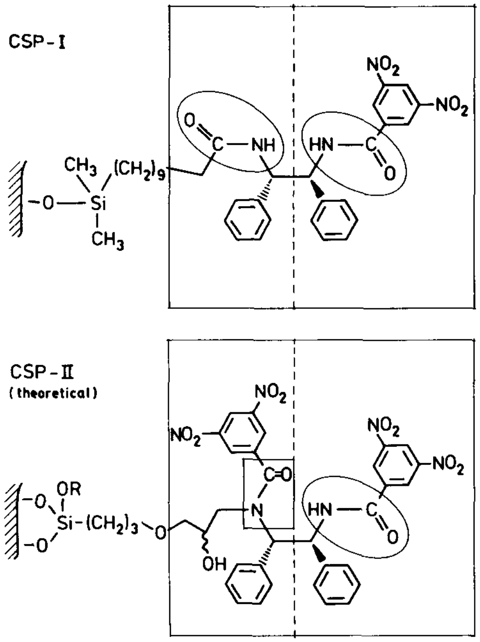


Fig. 1. Structures of CSP-I and CSP-II. The representation of the formulae should assist the visualization of structural elements (*e.g.*, π-acid, hydrogen donor–acceptor, stereogenic centre) responsible for intermolecular interactions with suitable guest molecules. Ellipse = secondary amide; rectangle = tertiary amide; dashed line = quasi-*C*-2 axis.

enantioselectivity. Systematic studies by Pirkle and Pochapsky [2], who evaluated the enantioselectivity of various rationally designed CSPs, have shown that the space filling size, the configuration of the stereogenic centres and hydrogen-donating amido groups must influence the overall selectivity of such modified chiral selectors. Further, the conformation of the immobilized selector molecules is also important for the observed chiral recognition. It should be dependent on the kind and size of the "spacing arm" to the silica surface. Also, as stressed recently by Däppen *et al.* [3], in all discussions of enantioselective chromatographic retention mechanisms it should be mentioned that the observed retention and resolution data are due to the total sum of chiral and non-chiral interactions of the SAs on a given CSP.

The new CSP-II was prepared following a straightforward synthetic route developed by Gasparrini and co-workers [4,5] for *trans*-diaminocyclohexane (DACH). In our particular case we reacted (*S*,*S*)-DPEDA with a glycidoxypropyl-modified silica gel followed by exhaustive benzoylation with 3,5-dinitrobenzoyl chloride (DNB-Cl) (Fig. 2). Surprisingly, this synthetic variation of the Gasparrini approach led to a mixture of mono- and dibenzoylated chiral selectors, as depicted in Fig. 2 and explained under Experimental. Nevertheless, the new CSP-II was found to have interesting separation capabilities, different from those of CSP-I. We made comparative studies of CSP-I and the much easier and more economically prepared CSP-II under similar chromatographic conditions and with the same set of chiral analytes.

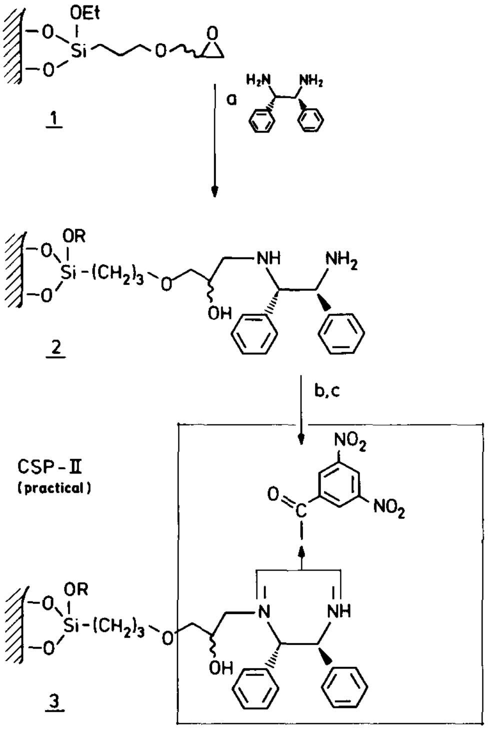


Fig. 2. Reaction scheme for preparing CSP-II. (a) 2 g (*S*,*S*)-DPEDA, 5.8 g epoxide silica gel **1**, 50 ml ethanol (95%), 20 mg phenol, 11 h gentle stirring and reflux. (b) 2 g **2**, 25 ml methylene chloride, 0.5 ml triethylamine, 0.5 g (4 mol equiv.) DNB-Cl, 20 h gentle stirring, room temperature. (c) Methanol-washed silica gel, 20 ml methanol, 0.5 ml concentrated aqueous ammonia, 3 h, room temperature.

EXPERIMENTAL

Apparatus

Chromatography was performed using a Model 410 HPLC pump, a UVIKON UV–VIS detector and an ANACOMP data control station from Kontron (Zürich, Switzerland). A Rheodyne Model 7125 injector with a 20-μl sample loop was connected to a stainless-steel column (125 × 4 mm I.D.). packed with CSP-II using chloroform–dioxane (3:1) as slurry solvent and *n*-heptane as pressurizing solvent.

Chemicals and reagents

Racemic and optically pure drugs were obtained from different pharmaceutical companies. The derivatives (amides, carbamates, ureas) of the various chiral acids, amines and alcohols (obtained from Aldrich, Steinheim, Germany) were prepared by common methods using the respective acid chlorides or isocyanates. Glycidoxypropyltriethoxysilane and 3,5-dinitrobenzoic acid were obtained from Aldrich and LiChrosorb Si 100 (5 μm), HPLC-grade solvents and other common chemicals used for the synthesis of CSP-II from Merck (Darmstadt, Germany). The remaining compounds were available from previous studies [1].

Synthesis of CSP-II

(*S,S*)-DPEDA was synthesized from benzil and ammonia and resolved optically according to a recently published method [6].

Glycidoxypropylsilica was prepared by gently stirring 10 g of silica gel in a refluxing solution of 3 g of glycidoxypropyltriethoxysilane in 200 ml of toluene that had been saturated with water by ultrasonication. After 10 h the modified silica gel was isolated, washed twice with 150 ml of toluene and diethyl ether and dried at 60°C. Elemental analysis gave C 5.34 and H 1.17% (H values are usually not very representative for such a type of analysis).

According to the reaction scheme depicted and briefly described in Fig. 2, the epoxide of the functionalized silica gel reacts with the large excess of (*S,S*)-DPEDA to give the addition product **2**. Elemental analysis gave C 9.55, H 1.54 and N 0.81%. Based on the nitrogen value, a coverage of 280 μM of covalently bonded (*S,S*)-DPEDA per gram of epoxidized silica gel was calculated. The yield of the addition reaction was about 70%.

Subsequently, **2** was exhaustively acylated with excess of DNB-Cl at 60°C and an appropriate base. As judged by the C,N elemental analysis, the benzoylation yield was nearly the same when triethylamine was replaced with pyridine as solvent and HCl scavenger. In order to cleave the formed DNB ester of the secondary hydroxy group (qualitatively judged by DRIFT spectroscopy) the modified silica gel, extensively washed with methanol, was subsequently treated for 3 h with 0.5 ml of concentrated aqueous ammonia in methanol followed by extensive washing with methanol. The content of cleaved DNB acid was measured by UV absorption of the washing solutions. The elemental analyses of the final, vacuum-dried material 3 were C 11.53, H 1.49 and N 1.64%, and for a second batch (acylation in pyridine followed by ammonia cleavage of the material with 0.1 *M* hydrochloric acid in methanol) C 11.33, H 1.46% and N 1.58%. Based on the nitrogen values found for the immobilized compounds **2** and **3**, we calculated a yield of the bis-(N-3,5-dinitrobenzoyl)-DPEDA product of about 60%. This reaction yield is not unusual for a solid-phase type of reaction within pores of varying pore-size distribution.

The final CSP-II material was slurry-packed (chloroform–dioxane) into a 125 × 4 mm I.D. stainless-steel column.

RESULTS AND DISCUSSION

CSP-I and CSP-II are similar; both contain one 3,5-dinitrobenzoyl group and a secondary amido functionality at the same position (see the right-hand boxed parts of the CSP-I and CSP-II molecules depicted in Fig. 1). This part is also identical with the well known "Pirkle phase" derived from 3,5-dinitrobenzoylphenylglycine [7]. In contrast to the right-hand parts, the left-hand parts of the two CSP molecules vary: CSP-I contains two secondary amido groups, whereas CSP-II, which is actually a mixture of mono- and bis-3,5-dinitrobenzoylamides of the DPEDA–epoxide adduct, as symbolized by the formula CSP-II in Fig. 2, partially contains a tertiary amido group. This has no hydrogen-donating capability.

A further distinction between CSP-I and CSP-II is the existence of a new but racemic secondary hydroxyl group as a third stereogenic centre in the CSP-II molecule. Consequently, CSP-II is inhomogeneous in terms of its molecular constitution, including the fact that it is a mixture of diastereoisomers. However, Gasparrini and co-workers' per-3,5-dinitrobenzoylated 1,2-diaminocyclohexane (DACH) [4,5], which could suffer from similar inhomogenities, proved to be highly stereoselective and reproducible in production.

The new CSP-II proved to be enantioselective for a representative range of chiral analytes; the results are summarized in Table I. The chiral acids, amines and alcohols were derivatized to amides, ureas and carbamates, respectively, thus incorporating polar functional groups which might participate as intermolecular interaction sites. As CSP-II has 3,5-dinitrobenzoyl groups serving as π-acids, they should interact with π-bases depending on their basicity. Together with hydrogen-bonding, hydrogen-donating or dipole–dipole stacking sites, paired with the spatial arrangements of the two stereogenic centres (1*S*,2*S*-position), CSP-II, and also CSP-I, display enantioselective multiple contact areas accessible to interaction via complementary binding forces of the chiral analytes.

This is a very generalized statement for explaining particular enantioselectivities. Although CSP-II shows good enantioselectivity it does not seem reasonable at this point to postulate and discuss distinct binding models, as CSP-II is not homogeneous in terms of its chemical constituents (it is also a mixture of diastereoisomers). As can be seen from the data in Table I, various enantiomers and particularly those containing a 1-naphthyl group, are well resolvable on CSP-II. Further, the observed enantioselectivity values (α) for this group of compounds are, with one exception, higher than the corresponding values acquired on CSP-I under comparable mobile phase conditions.

The mean retention time of the compared and resolved pairs was between 10% and 40% lower on CSP-I than on CSP-II, although the two CSPs have similar loadings on a molecular basis (between 280 and 300 μmol of selector per gram of silica gel). Therefore, the degree of benzoylation is higher on CSP-II than on CSP-I, which might influence the retention characteristics of compounds containing strong π-base groups.

However, this trend seems particularly true for well defined and simple compounds having a naphthyl group as a substituent either at the stereogenic centre or at another position of the derivatized chiral analyte.

The more complex the chiral compounds (SAs) are structured, *e.g.*, the oxazolidin-2-ones of beta-blockers but also benzodiazepines, the less (spatially) defined the intermolecular SO–SA interactions might be.

In this context, one should mention that the observed differences in the retentions of stereoisomers on a given CSP are due to a "mixed mode" binding contribution of chiral and non-chiral interactions between the SO and SA molecules [3]. Therefore, by comparing CSP-I with CSP-II, the differences of the spacer parts between the chiral DPEDA molecule part and the silica gel surface should not be ignored (see Fig. 1). The spacer length, for instance, might influence the overall retention and enantioselectivity characteristics to a certain extent. In this respect, it is interesting that the above-mentioned benzodiazepines (Table I, lormetazepam) are significantly less resolved [1] and oxazolidine-2-ones (not shown in Table I) are poorly resolved on CSP-II. On the other hand, the separation capabilities for sulphoxides are not predictable. Simple benzyl phenyl sulphoxide is resolved only on CSP-I, whereas more complicated structures are separated solely with the higher benzoylated CSP-II.

From the present observations and by studying the extensive literature on

TABLE I

ENANTIOSEPARATION OF REPRESENTATIVE ANALYTES ON CSP-II

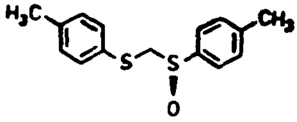
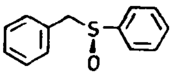
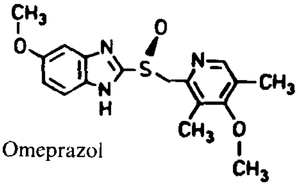
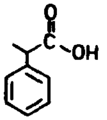
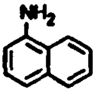
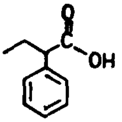
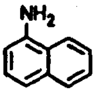
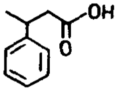
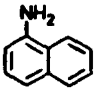
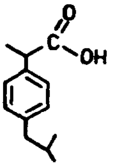
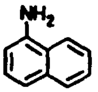
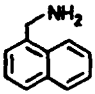
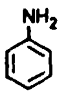
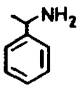
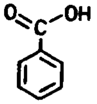
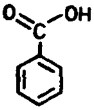
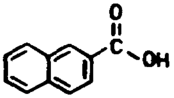
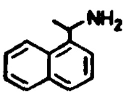
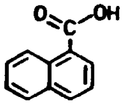
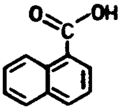
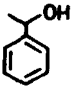
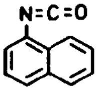
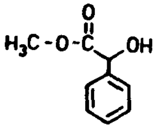
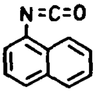
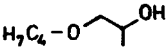
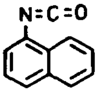
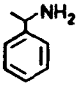
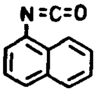
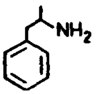
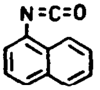
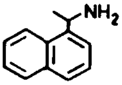
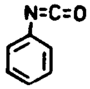
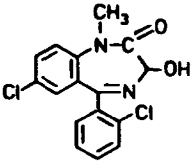
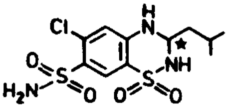
Compound	Derivatization reagent	Mobile phase[a]	k'_1[b]	α[c]	R_s[d]	Elution order	α on CSP-I[e]
Sulphoxides							
		A	3.72	1.27	1.51		1.0
		B	1.79	1.08	0.81		1.0
		A	1.33	1.0	–		1.16
		B	2.12	1.0	–		1.22
 Omeprazol		A	6.33	1.0	–		1.0
		B	7.14	1.12	0.50		1.0
Acids as amides							
		A	2.50	1.66	3.73		1.44
		B	1.33	1.33	2.33		–
		A	2.0	1.65	3.95		1.39
		B	1.0	1.33	2.33		–
		A	2.33	1.09	0.50		1.11
		B	1.72	1.0	–		–
		A	1.72	1.97	4.42	$S>R$	1.36
		B	1.21	1.32	2.57	$S>R$	–
		A	1.33	1.66	3.11	$S>R$	1.34
		B	1.15	1.18	1.37	$S>R$	1.0
		A	2.03	1.09	0.58		1.0

TABLE I *(continued)*

Compound	Derivatization reagent	Mobile phase[a]	k'_1[b]	α[c]	R_s[d]	Elution order	α on CSP-I[e]
Amines as amides							
		A	1.08	1.20	1.15	*R*>*S*	1.18
		B	1.24	1.21	1.74	*R*>*S*	–
		A	3.05	1.65	3.0	*R*>*S*	1.43
		B	2.23	1.47	3.60	*R*>*S*	–
		A	2.0	1.30	2.13	*R*>*S*	1.18
		B	1.66	1.18	1.06	*R*>*S*	–
		A	2.14	2.52	4.90	*R*>*S*	1.72
		B	1.59	1.88	4.21	*R*>*S*	1.09
		A	8.27	3.40	4.78		1.28
		B	3.35	2.26	4.51		1.0
Alcohols as carbamates							
		A	1.72	1.39	2.37		1.37
		B	0.65	1.27	1.17		1.0
		A	2.94	1.07	0.40		1.0
		B	0.94	1.0	–		–
		A	1.53	1.15	1.18		–
		B	0.53	1.01	–		–
Amines as ureas							
		B	4.0	1.32	2.71	*R*>*S*	–
		A	2.44	1.17	1.26		1.25
		B	3.11	1.12	1.18		–

(Continued on p. 380)

TABLE I *(continued)*

Compound	Derivatization reagent	Mobile phase[a]	k'_1[b]	α[c]	R_s[d]	Elution order	α on CSP-I[e]
		A B	2.22 2.33	1.95 1.44	4.06 3.12	R>S R>S	1.26 –
Miscellaneous							
 Lormetazepam		A B	5.88 3.70	1.15 1.10	0.67 1.23		1.37 1.29
Butizide 		C	4.63	1.11	0.56		1.24

[a] Mobile phases (%, v/v): (A) *n*-heptane–2-propanol–diethylamine (DEA) (70:30:0.1); (B) *n*-heptane–dioxane–DEA (70:30:0.1); (C) *n*-heptane–dioxane (50:50); flow-rate 0.9 ml/min; the column was thermostated at 20°C.

[b] Capacity factor: $k' = (t_r - t_0)/t_0$.

[c] Selectivity: $\alpha = k'_2/k'_1$.

[d] Resolution: $R_s = (\alpha - 1)/4\alpha \cdot k'_2/(k'_2 + 1) \cdot \sqrt{N_2}$.

[e] Most of these data have been published previously [1]; the mobile phases used on CSP-II and CSP-I are identical.

chromatographic enantioseparations [8,9], it is clear that the total structure, the configuration of the stereogenic centres and subsequently the conformation of chiral selector molecules are of great importance for chiral recognition. Large parts of the selected molecules (CSP-I and CSP-II) are identical (the substituents at the stereogenic centres, their configurations and the amides as functional groups), as shown in idealistic form in Fig. 1. Realistically we have to deal with a less defined CSP-II structure expressed by the formula 3 in Fig. 2. The new but racemic hydroxyl group in CSP-II, however, seems to influence considerably its overall stereoselectivity compared with CSP-I, although we never observed a reversal of elution order.

Assuming ideal and homogeneous situations, (*S*,*S*)-DPEDA is a molecule with *C*-2 symmetry and the acyl derivatives thereof (see the molecule structures of CSP-I and CSP-II in Fig. 1) still have a quasi-*C*-2 symmetry, as indicated by the dotted line. With the aid of this line it also becomes clear that CSP-I contains two relatively similar secondary amido functions (as indicated by the elliptical lines in Fig. 1), whereas the main proportion of CSP-II contains a secondary and a tertiary amido function.

As mentioned above, the right-hand part of the quasi-*C*-2 symmetrical molecule is equivalent to the chiral 3,5-dinitrobenzoylphenylglycine molecule part of the corresponding CSP introduced by Pirkle *et al.* [7]. Consequently, and assuming a three-point interaction model for chiral recognition, Pirkle *et al.*'s and our two CSPs should resolve selected enantiomers in a similar way. This could be confirmed for, e.g., the simple 1-naphthoyl derivative of (*R,S*)-phenylethylamine which was separated in the same elution order.

In the present (1*S*,2*S*)-DPEDA-type CSPs there are two adjacent stereogenic centres with the same configuration and which are substituted with similar amide functionalities. However, at this point it is not clear whether the "doubling" of similar chiral molecule parts within one chiral molecule will act synergistically in terms of "additively" enlarging the overall enantioselectivity. As we could see from various stick and ball and spacefill models, this seems unlikely owing to predominant conformational changes. Thus, "stereogenic contact areas" get exposed, leading to hardly predictable enantioselectivity models.

CSP-I and CSP-II are starting points for studying more systematically quasi-*C*-2 symmetrical but similar multifunctionalized chiral selector molecules and the contribution of stereoselective increments. This is of considerable interest to us and is the subject of ongoing research in the field of chiral recognition in liquid chromatography.

Consequently, a logical extension of CSP-II is to synthesize a well defined mono-N-3,5-dinitrobenzoyl-(*S,S*)-DPEDA derived CSP. Results on these aspects will be published elsewhere [10].

In conclusion, the described and easily prepared CSP-II is, depending on the method of synthesis, not fully homogeneous in terms of the molecular structure of the chiral selector. However, the synthesis is reproducible. The column efficiency of the CSP-II packing is good and comparable to those of typical π-acid–π-base "Pirkle-type" CSPs. Depending on the structure of the chiral analytes, reduced plate heights (h) between 3 and 8 were observed, leading to plate numbers (N) between 20 000 and 65 000 per metre.

CSP-II proved practically useful and showed higher enantioselectivity towards chiral analytes containing a naphthyl group than CSP-I [1]. CSP-II is chemically and thermally stable; after long-term usage (2000 column volumes of a non-aqueous mobile phase were continuously pumped through the column at 60°C), the retention and the enantioselectivity decreased by only about 5%.

ACKNOWLEDGEMENTS

We are grateful to D. Lindner for her help in preparing the manuscript. This work was partially supported by a grant (P7071) from the Austrian Fonds zur Förderung der wissenschaftlichen Forschung.

REFERENCES

1 G. Uray and W. Lindner, *Chromatographia*, 30 (1990) 323.
2 W. Pirkle and T. Pochapsky, *Chem. Rev.*, 89 (1989) 347.
3 R. Däppen, V. R. Meyer and H. Arm, *J. Chromatogr.*, 464 (1989) 39.
4 G. Gargaro, F. Gasparrini, D. Misiti, G. Palmieri, M. Pierini and C. Villani, *Chromatographia*, 24 (1987) 505.
5 F. Gasparrini, D. Misiti, C. Villani, F. La Torre and M. Sinibaldi, *J. Chromatogr.*, 457 (1988) 235.
6 E. J. Corey, R. Imwinkelried, St. Pikul and Y. B. Xiang, *J. Am. Chem. Soc.*, 111 (1989) 5493.
7 W. Pirkle, C. Welch and M. Hyun, *J. Org. Chem.*, 48 (1983) 5022.
8 W. Lindner and C. Pettersson, in I. Wainer (Editor), *Liquid Chromatography in Pharmaceutical Development*, Aster Publishing, Springfield, 1985, Ch. 3, p. 63.
9 W. Lindner, *Chromatographia*, 24 (1987) 97.
10 W. Lindner, G. Uray and Ch. Leitner, *Chromatographia*, in preparation.