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PERFORMANCE OF A NEW HPLC CHIRAL STATIONARY PHASE DERIVED FROM *N*-(3,5-DINITROBENZOYL)-D-α-PHENYLGLYCINE WITH A QUINOXALINE BRANCHING UNIT

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SUMMARY

A new brush-type HPLC chiral stationary phase (CSP) comprising π-acidic *N*-(3,5-dinitrobenzoyl)-D-α-phenylglycine chiral units bound to the quinoxaline units of modified γ-aminopropyl silica gel by a 1,2-diaminoethane spacer has been prepared by solid-phase synthesis. To test the CSP attempts were made to separate the enantiomers of twenty-two racemates with different functional groups and the results were compared with those obtained by use of three structurally related chiral stationary phases.

INTRODUCTION

Liquid chromatographic resolution of enantiomers on chiral stationary phases (CSPs) is one of the most convenient and accurate methods for determination of optical purity. Brush-type or Pirkle-type CSPs are the most widely investigated CSPs and are present in many commercially available products [1,2]. They contain a low-molecular-weight chiral molecule (chiral selector) covalently bound to the silica gel surface and resolve racemates as a result of enantioselective interactions between the CSP and the analytes [3]. For effective π–π interactions, Pirkle-type CSPs usually contain π-acidic and/or π-basic aromatic groups [4].

Numerous Pirkle-type CSPs are based on derivatives of α-amino acids, inexpensive and readily available enantiomerically pure materials [5–7]. For example, CSPs based on *N*-(3,5-dinitrobenzoyl)-α-amino acids (DNB-AAs), mainly *N*-(3,5-dinitrobenzoyl)-D-α-phenylglycine or *N*-(3,5-dinitrobenzoyl)-L-leucine, have been used for separation of the enantiomers of a variety of analytes [8–11]. Subtle structural modification of the CSP

has often been shown to have remarkable effects on their enantiomer-separation properties [10,12].

Here we report the solid phase synthesis of **CSP-1** (Fig. 1) and a comparative study of the effect of π–π interactions on enantiomer separations by the new CSP and three structurally related chiral stationary phases **CSP-2–CSP-4** (Fig. 1). All the CSPs contain the π-acidic *N*-(3,5-dinitrobenzoyl)-D-α-phenylglycine as a chiral unit, but differ in the group connecting the silica surface and the chiral unit.

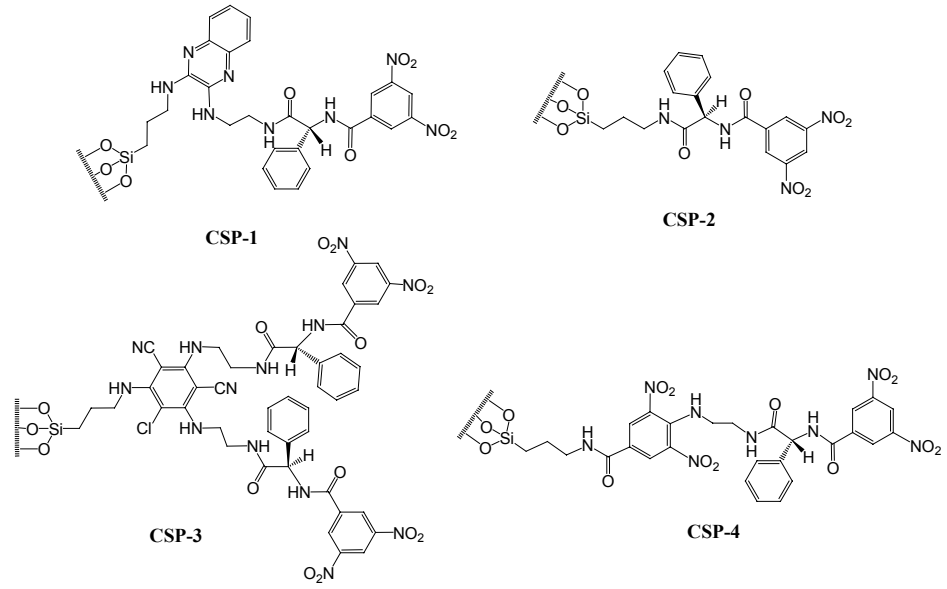

Fig. 1

The chemical structures of chiral stationary phases **CSP-1–CSP-4**

In designing the new CSP reported here we expected that introduction of structurally different π-basic connecting groups would alter the enantiomer-recognition properties. **CSP-1** contains the *N*,*N*′-dialkyl-2,3-diaminoquinoxaline group, an aromatic unit with π-donor character, as a linking structure. **CSP-2** is the known, commercially available Pirkle CSP with no such link. Chiral stationary phases **CSP-3** and **CSP-4** have previously been prepared in our laboratory and proved superior to **CSP-2** for resolution of some arylamides and DNB-AA esters [10,11]. **CSP-3** is a tweezer-type CSP, with two chiral units connected to the silica surface by a π-basic *N*,*N*′,*N*″-trialkyl-1,3-dicyano-2,4,6-triamino-5-chlorophenyl moiety.

In **CSP-4** the link between the silica surface and the chiral unit is the strongly π-acidic *N*,*N*′-dialkyl-3,5-dinitro-4-aminobenzamide moiety.

EXPERIMENTAL

Chemicals

Nucleosil 100-5 NH_2 HPLC silica gel was from Macherey–Nagel (Düren, Germany), 2,3-dichloroquinoxaline was from Sigma–Aldrich (Aldrich Chimica, Milan, Italy), and 1,2-diaminoethane, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), *N*-(3,5-dinitrobenzoyl)-D-α-phenylglycine, and *N,N,N*-triethylamine were from Aldrich. All solvents were purchased from J.T. Baker (Davenport, Holland) and were distilled before use.

The racemates used for evaluation of the columns were *trans*-stilbene oxide (**TR-1**), benzoin (**TR-2**), benzoin methyl ether (**TR-3**), flavanone (**TR-4**), Tröger base (**TR-5**), and 1,1′-bi-2-naphthol (**TR-6**), from Sigma–Aldrich, and 1-(9-anthryl)-2,2,2-trifluoroethan-1-ol (**TR-7**), 7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*f*]1,4-diazepine-3-carboxylic acid ethyl ester (**TR-8**), 7-chloro-1,3-dimethyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*f*]1,4-diazepine-3-carboxylic acid ethyl ester (**TR-9**), *N*-(1-phenylethyl)-2,2-dimethylpropionamide (**TR-10**), *N*-(1-phenylethyl)benzamide (**TR-11**), *N*-(1-phenylethyl)-1-naphthoicamide (**TR-12**), *N*-phenyl-2-[4-(2-methylpropyl)phenyl]propionamide (**TR-13**), *N*-(1-phenylethyl)-3,5-dinitrobenzamide (**TR-14**), and isopropyl esters of *N*-(3,5-dinitrobenzoyl) derivatives of α-amino acids (**TR-15**–**TR-22**), all of which were prepared in our laboratory.

Solutions of the analytes were prepared by dissolving ca. 1 mg of the racemic compound in 1 mL dichloromethane. Analysis was performed with 5 μL of the freshly prepared solution.

Apparatus and Chromatography

Elemental analysis was performed by the Central Analytical Service (CAS) at Rudjer Boskovic Institute.

Chromatography was performed with a Knauer (Berlin, Germany) WellChrom Maxi-Star K-1000 pump and a Knauer six-port-valve HPLC injector with 20-μL loop. UV detection was performed at 254 nm with a Knauer WellChrom K-2500 detector; a Jasco CD-2095 detector was used

to determine the order of elution of the enantiomers. Chromatograms were integrated by means of the Knauer Eurochrom 2000 software package.

Columns packed with **CSP-2**–**CSP-4**, prepared previously in our laboratory [10,11], were compared with the newly developed column.

The retention times of the enantiomers (t_{R1} and t_{R2}) were used to calculate the capacity factors of the first and second eluted enantiomers, k'_1 and k'_2, respectively, by use of the equations $k'_1 = (t_1 - t_0)/t_0$ and $k'_2 = (t_2 - t_0)/t_0$. The selectivity factor was calculated by use of the equation $\alpha = k'_2/ k'_1$ and the resolution factor by use of the equation $R_S = 2(t_2 - t_1)/(w_1 + w_2)$, where w is the baseline peak width, obtained by drawing tangents to the points of inflection of the chromatographic peak.

HPLC columns, dimensions 150 mm × 4.6 mm i.d., purchased from Max Stevenson (Berlin, Germany), were packed by a slurry technique, by use of a Knauer pneumatic HPLC pump.

n-Hexane, isopropanol, and dichloromethane used for chromatography and column packing were analytical grade (J.T. Baker) and were redistilled before use.

The HPLC column dead volume (t_0) was estimated by use of 1,3,5-tri-*tert*-butylbenzene.

Preparation of Stationary Phases

SP-1

A suspension of Nucleosil 100-5 NH_2 silica gel (4.00 g; Anal. 1.36 % N, 3.49 % C, 0.97 mmol NH_2 groups g^{-1}) and 2,3-dichloroquinoxaline (**1**) (0.79 g; 4.00 mmol) in DMF (15 mL) was stirred at room temperature for 24 h. The modified silica gel was collected on a G-4 filter and washed first with DMF then with methanol. After drying for 4 h at 70°C, 4.28 g **SP-1** was obtained, Anal. 5.75% C, 0.98% H, 2.02% N. On the basis of the molar ratio of carbon and nitrogen, 1.00 g **SP-1** contains 0.23 mmol bound organic material.

SP-2

A suspension of **SP-1** (4.00 g) in 1,2-diaminoethane (15 mL) was stirred at ambient temperature for 24 h. The modified silica gel was collected on a G-4 filter and washed first with 4:1 methanol–triethylamine then with methanol. After drying at 70°C for 4 h, 3.96 g **SF-2** was obtained, Anal. 5.60% C, 1.12% H, and 2.59% N. On the basis of the molar ratio of carbon and nitrogen, 1.00 g **SP-2** contains 0.22 mmol bound organic material.

CSP-1

A suspension of **SP-2** (2.00 g), *N*-(3,5-dinitrobenzoyl)-D-α-phenylglycine (0.35 g; 1.00 mmol), and EEDQ (0.25 g; 1.00 mmol) in dry THF (10 mL) was stirred for 24 h at room temperature. After collecting the modified silica on a G-4 filter, washing several times with methanol, and drying at 70°C for 4 h, 2.21 g **CSP-1** was obtained, Anal. 9.56% C, 1.49% H, and 3.51% N. On the basis of the molar ratio of carbon and nitrogen, 1.00 g **CSP-1** contains 0.22 mmol bound organic material.

RESULTS AND DISCUSSION

The objective of this study was to examine selectivity differences toward enantiomers resulting from modification of the structure of aromatic units placed between the silica gel surface and the *N*-(3,5-dinitrobenzoyl)-D-α-phenylglycine chiral unit. One interesting starting material for

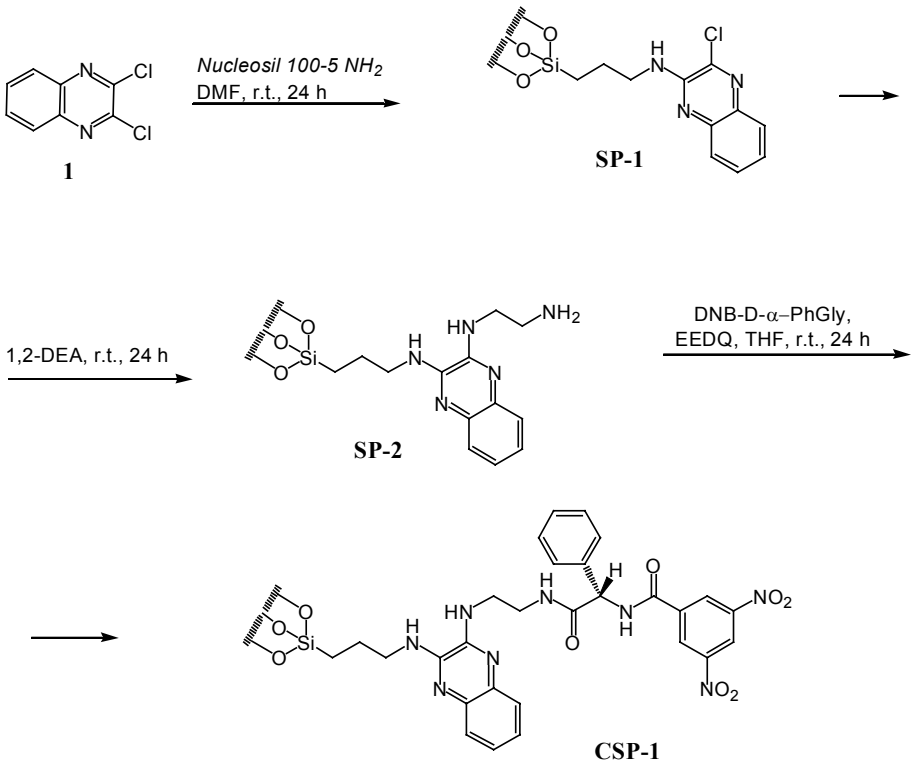

Fig. 2

Synthetic route for preparation of **CSP-1**

this purpose is 2,3-dichloroquinoxaline (**1**). Two reactive chlorine atoms in compound **1** are susceptible to nucleophilic displacement reactions by a variety of nucleophiles and react stepwise [13]. This feature enables the preparation of **CSP-1** by solid-phase synthesis, as outlined in Fig. 2. The procedure for solid-phase synthesis of **CSP-3** has been described elsewhere [10] and worked well, as was confirmed by preparation of soluble analogue of **CSP-3** by use of the same procedure and by recording of its IR, UV, and NMR spectra. We used the same procedure for preparation of **CSP-1**, assuming it works also for this CSP because compound **1** contains two chlorine atoms quite suitable for nucleophilic substitution reactions. In this procedure compound **1** was bound directly to aminopropyl silica gel by substitution of the chlorine atom at the C(2) position. The reaction was conducted with excess **1** to ensure exhaustive arylation of γ-aminopropyl groups. In the next step **SP-1** was reacted with a large excess of 1,2-diaminoethane to replace the chlorine atom at the C(3) position. In the last step the primary amino groups of **SP-2** were acylated with EEDQ-activated *N*-(3,5-dinitrobenzoyl)-D-α-phenylglycine and **CSP-1** was obtained. The prepared CSP was then slurry-packed into 150 mm × 4.6 mm i.d. stainless steel columns.

Screening of the efficacy of the column packed with **CSP-1** was achieved by HPLC of test racemates **TR-1**–**TR-22**, Fig. 3. Chromatographic results are summarized in Table I and compared with results obtained by use of three structurally similar CSP, **CSP-2**–**CSP-4**, under the same chromatographic conditions. Comparable chromatograms obtained for **TR-6** and **TR-19** on columns packed with **CSP-1** and **CSP-2** are shown in Fig. 4.

Silica has favourable physical properties and silica-based CSPs are the most popular in liquid chromatography [14]. One seriously undesirable property, however, is the presence of residual achiral polar groups on the silica surface. In **CSP-1**–**CSP-4** these residual polar groups are slightly acidic silanol groups and strongly basic aminopropyl groups, which remain after incomplete reaction between aminopropyl silica and the organic selector. Because the basicity of free aminopropyl groups is much more strongly expressed than the acidity of silanol groups, the silica surface is basic. One role of the mobile phase is to suppress the deleterious effects of these groups. In our earlier paper [10], in which we described the synthesis of **CSP-3** and comparison of its enantiomer recognition capability with that of **CSP-2**, we reported that use of the ternary mixture *n*-hexane–dichloromethane–methanol, 100:30:1, as mobile phase usually resulted in better

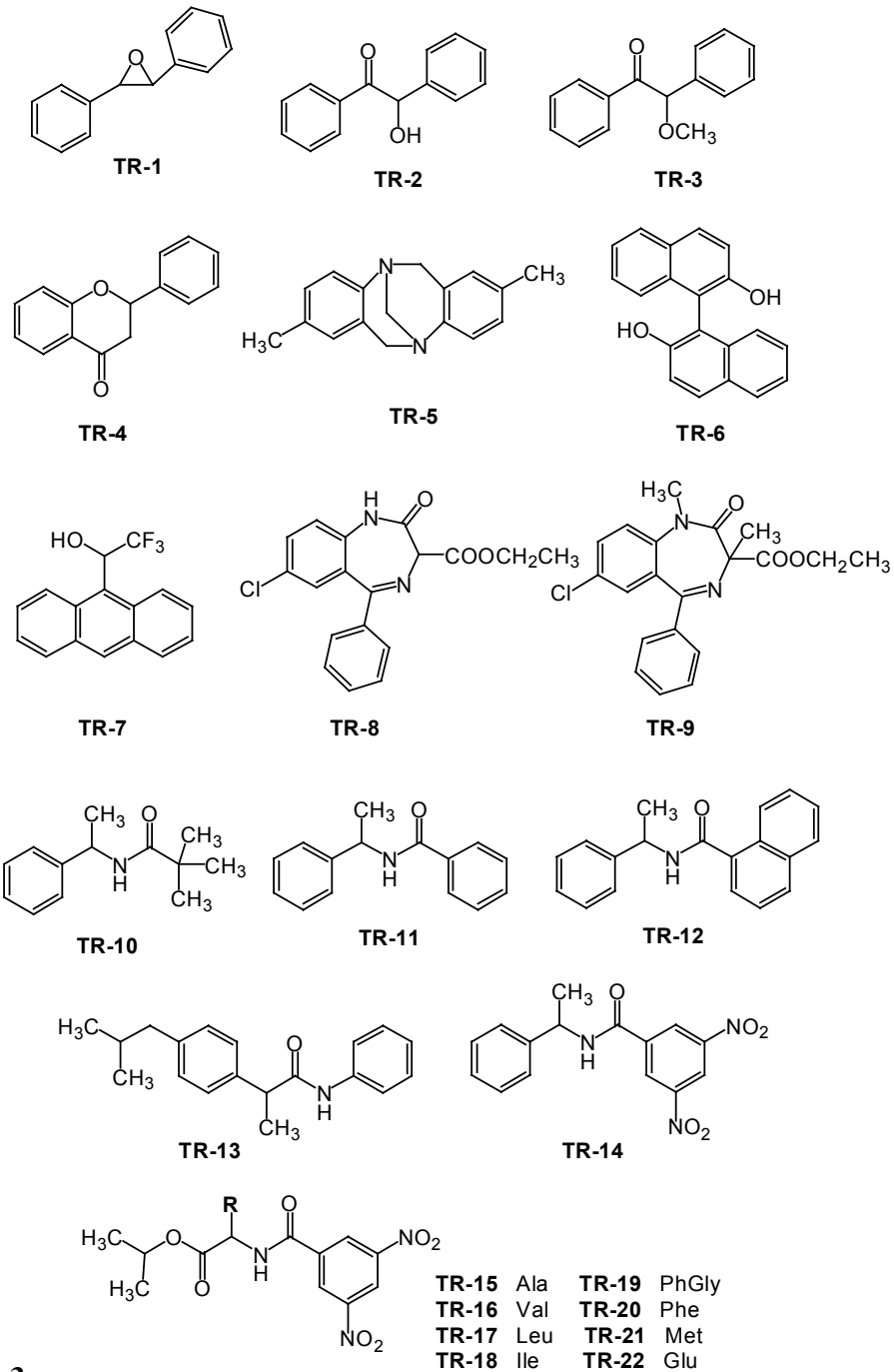

Fig. 3

The structures of the test racemates used for evaluation of the CSPs

Table I

HPLC data obtained for test racemates **TR-1**–**TR-22** by use of columns (150 mm × 4.6 mm i.d.) packed with **CSP-1**–**CSP-4**. The mobile phase was *n*-hexane–dichloromethane–methanol, 100:30:1, the flow rate 1.0 mL min, and detection was by UV absorbance at 254 nm. t_0 was 1.58, 1.45, 1.42, and 1.76 min for **CSP-1**–**CSP-4**, respectively

Test racemate	**CSP-1**			**CSP-2**			**CSP-3**			**CSP-4**		
	k'_1	α	R_S	k'_1	α	R_S	k'_1	α	R_S	k'_1	α	R_S
TR-1	0.36	1.00	0.00	0.42	1.00	0.00	1.59	1.00	0.00	0.42	1.00	0.00
TR-2	3.34	1.00	0.00	2.57	1.00	0.00	2.33	1.00	0.00	2.37	1.00	0.00
TR-3	0.97	1.00	0.00	0.85	1.00	0.00	0.70	1.00	0.00	1.07	1.00	0.00
TR-4	0.85	1.00	0.00	0.80	1.00	0.00	0.69	1.00	0.00	0.99	1.00	0.00
TR-5	1.05	1.00	0.00	0.84	1.00	0.00	0.48	1.11	0.75	1.86	1.10	0.34
TR-6	11.56	1.22	1.33	12.57	1.27	2.08	7.06	1.14	1.88	7.74	1.17	1.88
TR-7	6.52	1.30	1.75	5.94	1.34	2.48	3.57	1.22	2.02	5.45	1.31	2.85
TR-8	54.72	1.00	0.00	11.46	1.17	1.42	19.88	1.16	1.46	35.28	1.27	3.28
TR-9	0.95	1.00	0.00	0.84	1.00	0.00	0.62	1.00	0.00	1.96	1.28	0.91
TR-10	1.66	1.21	1.33	1.22	1.04	0.18	1.43	1.39	1.31	1.86	1.13	0.50
TR-11	3.87	1.11	0.86	2.27	1.00	0.00	0.41	1.00	0.00	4.59	1.11	0.96
TR-12	7.80	1.12	1.04	3.76	1.14	1.56	3.29	1.10	1.22	8.39	1.11	1.07
TR-13	1.77	1.00	0.00	1.72	1.00	0.00	1.32	1.00	0.00	1.89	1.04	0.10
TR-14	14.69	1.05	0.84	10.42	1.00	0.00	8.55	1.05	0.69	18.12	1.08	0.90
TR-15	8.66	1.23	2.03	6.07	1.14	1.69	4.72	1.15	1.70	9.63	1.20	1.98
TR-16	5.26	1.21	1.97	4.68	1.04	0.81	2.85	1.18	1.68	5.38	1.24	1.82
TR-17	6.29	1.24	2.46	5.33	1.04	0.85	2.10	1.13	2.01	6.65	1.22	2.06
TR-18	5.15	1.20	2.66	6.46	1.00	0.00	2.74	1.14	1.78	5.20	1.23	1.84
TR-19	6.49	1.45	4.89	8.73	1.21	1.83	4.77	1.21	2.84	8.89	1.25	2.41
TR-20	7.72	1.34	3.96	7.08	1.10	1.22	4.42	1.16	1.83	8.44	1.24	1.99
TR-21	13.10	1.33	3.66	4.22	1.11	1.49	6.70	1.19	1.98	11.17	1.25	2.86
TR-22	7.50	1.14	1.11	6.79	1.04	0.62	4.68	1.24	2.14	7.71	1.17	2.75

enantiomer separation than the binary mixture *n*-hexane–isopropanol, 9:1. Obviously, isopropanol as modifier, usually the best choice [15] for such stationary phases, does not suppress the effect of the basic amino groups. When the mobile phase with methanol as alcohol modifier was used, the peaks of the enantiomers were not so broad and better separations were obtained between them. The positive contribution of dichloromethane can be ascribed to promotion of hydrogen-bonding interactions, because it is known that dichloromethane competes with the CSP in dipole–dipole and van der Waals interactions [16].

On the basis of these results we also compared these two mobile phase mixtures for evaluation of **CSP-1**–**CSP-4**. The mobile phase *n*-hexane–dichloromethane–methanol, 100:30:1, proved superior to the binary mixture *n*-hexane–isopropanol, 9:1, for all four columns.

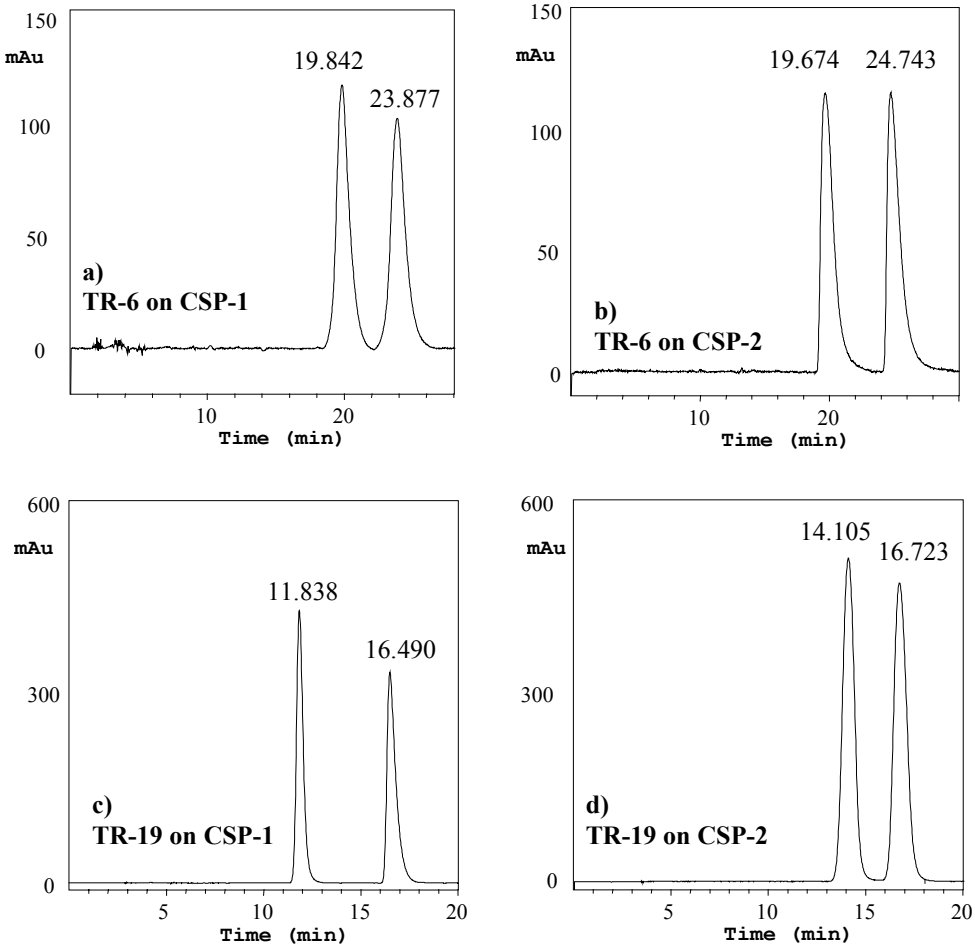

Fig. 4

Chromatograms obtained from **TR-6** and **TR-19** on columns packed with **CSP-1** (a, c) and **CSP-2** (b, d)

Because **CSP-1** is structurally similar to **CSP-2**–**CSP-4** its enantiomer selectivity was expected to be similar to that of these CSP. All four columns failed to resolve aromatic racemates containing "hidden" oxygen functionality (**TR-1**–**TR-4**). **CSP-1** completely resolved the enantiomers of 1,1′-bi-2-naphthol (**TR-6**) and 1-(9-anthryl)-2,2,2-trifluoroethan-1-ol (**TR-7**) with resolution factors 1.33 and 1.75, respectively. On **CSP-1**, however, long retention times were obtained for **TR-6**, **TR-7**, and, especially, the benzodiazepine **TR-9**. This is because of the polar structure of the

chiral selector, i.e. the polarity of the amino groups at the C(2) and C(3) positions of the quinoxaline unit. As shown in Table I, **CSP-1** also resolved arylamines **TR-10**–**TR-12** and **TR-14** and isopropyl esters of *N*-(3,5-dinitrobenzoyl) derivatives of α-amino acids **TR-15**–**TR-22**. **CSP-1** is superior to **CSP-2**–**CSP-4** for resolution of the enantiomers of **TR-18**–**TR-21**, possibly because of the π–π interactions between the π-basic quinoxaline and the 3,5-dinitrobenzoyl unit present in **TR-15**–**TR-22** as a π-acidic site. The order of elution of the enantiomers, monitored by use of a CD detector, was identical for all four columns. This is indicative of similar chiral recognition mechanisms, which depend solely on the chiral centre unit and are independent of other, achiral, units between the silica and the chiral unit. The whole structure of the organic molecule bonded to the silica does, however, affect the extent of complexation between selector and analyte. For this reason **CSP-1**–**CSP-4**, despite having the same chiral selector, separate some pairs of enantiomers by different amounts.

CONCLUSION

The novel chiral stationary phase **CSP-1**, derived from *N*-(3,5-dinitrobenzoyl)-D-α-phenylglycine with a quinoxaline branching unit, proved capable of separating the enantiomers of a broad range of test racemates as a result of stereoselective hydrogen bonding and π-donor–π-acceptor group interactions. These results indicate that insertion of a π-donor or π-acceptor aromatic linking unit between the silica surface and the *N*-(3,5-dinitrobenzoyl)-D-α-phenylglycine chiral unit broadens the chromatographic applicability of the classic Pirkle **CSP-2**.

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