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CHIRAL COVALENTLY BONDED STATIONARY PHASES FOR THE SEPARATION OF ENANTIOMERIC AMINE DERIVATIVES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

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SUMMARY

On the basis of optically active 1-phenylethylamine, two new chiral stationary phases of the type $R_1HN–C_6H_4–C^*H(CH_3)NHCO–R_2$, bonded to a silica matrix, have been synthesized for application in high-performance liquid chromatography. These phases can be used with either non-polar (*n*-hexane + 2-propanol) or polar (water + methanol) solvents for the resolution of enantiomeric amine derivatives with separation factors up to 1.80.

INTRODUCTION

Many different methods have been developed for the separation of optical isomers[2]. One of these, with many possible applications and perhaps hitherto the best investigated, is the direct chromatographic separation of enantiomers on chiral stationary phases. On account of their high efficiency, capillary gas chromatography and high-performance liquid chromatography (HPLC) are used with preference, the latter with the extra possibility of performing preparative-scale separations.

Although the chromatographic separation of racemic amino acids has been a reliable, routine method for some time[3], only a few suitable stationary phases exist for other classes of compounds[4–13]. To obtain good enantioselectivity, these phases must be synthesized "tailor-made" for a limited range of applications. Phases with high separating powers and a broad range of applications are rare[6–9].

In this paper the preparation and properties of two new chiral bonded phases (Fig. 1) for HPLC are described. They are especially suitable for separations of 3,5-dinitrobenzoyl derivatives of enantiomeric amines and show remarkably high separation factors.

* This paper is part of the dissertation of R. Däppen[1].

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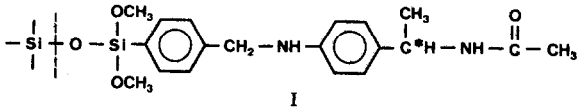

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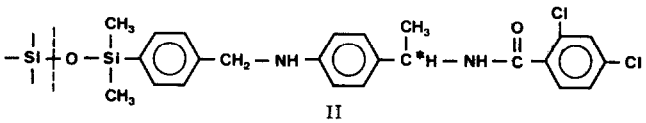

II

Fig. 1. Structures of phases I and II.

EXPERIMENTAL

Preparation of chiral bonded phases

Materials. LiChrosorb Si 100 (E. Merck, Darmstadt, F.R.G.) with a particle size of 5 μm and a specific surface of 320 $m^2\ g^{-1}$ was used as a silica matrix. All other chemicals were purchased from Fluka (Buchs, Switzerland) or E. Merck.

Phase I. This phase was prepared according to the scheme in Fig. 2.

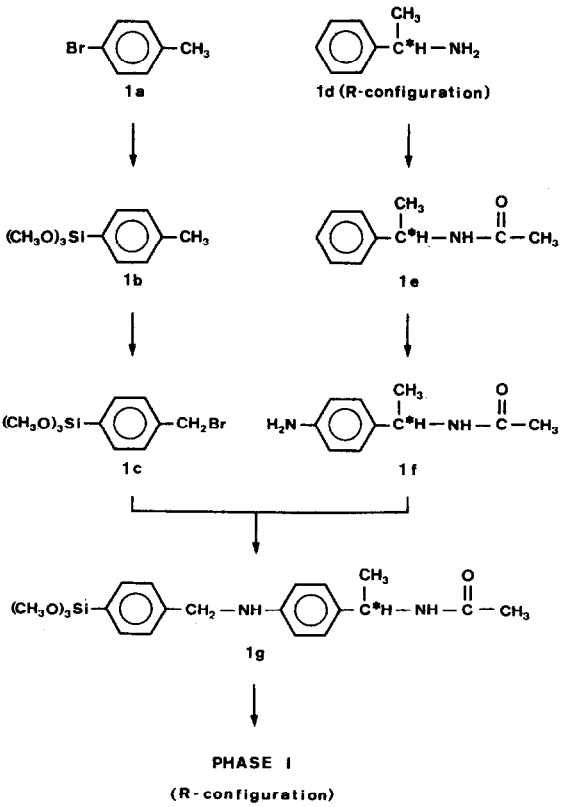

Fig. 2. Preparation of phase I.

To 5.66 g (233 mmol) of magnesium was added a portion of a solution of 38.1 g (223 mmol) of 4-bromotoluene (1a) in 70 ml of dry diethyl ether. After the start of the reaction, 100 ml of dry diethyl ether were added and, while the reaction vessel was cooled in a water-bath, the remaining part of the solution of 1a was added dropwise. The mixture was then heated under reflux for 45 min, 25.8 ml (225 mmol) of silicon tetrachloride were added and the heating was continued for 2 h. While cooling the reaction vessel in a water-bath, 75 ml (1.8 mol) of dry methanol were added, then the mixture was refluxed for 2 h. The raw product 1b was filtered and the filtrate distilled at *ca.* 70°C and 0.1 mbar. The yield of 1b was 17.4 g.

A 17.4-g (82 mmol) amount of 1b, 16.1 g (90.4 mmol) of N-bromosuccinimide, 0.1 g of 2,2'-azobisisobutyronitrile and 80 ml of dry carbon tetrachloride were refluxed for 2 h. The reaction product, 1c, was filtered and distilled twice at *ca.* 110°C and 0.1 mbar. The yield of 1c was 18.4 g.

A 30.6-g (390 mmol) amount of acetyl chloride in 30 ml of dry dioxane were chilled to 0°C, then 31.6 g (260 mmol) of *R*(+)-1-phenylethylamine were added dropwise. The mixture was stirred for 1 h and, after addition of 10 ml of 2 *M* sodium hydroxide solution, stirring was continued for another 30 min. The mixture was then hydrolyzed with ice and an excess of hydrochloric acid and extracted with methylene chloride. The organic phase was dried with potassium carbonate and the methylene chloride was removed by distillation. After recrystallization from a mixture of water and ethanol a yield of 17.7 g of 1e resulted.

To a mixture of 31 ml of nitric acid (65%) and 26 ml of sulphuric acid (98%) at 0°C, 16.4 g (101 mmol) of 1e was added in portions and stirred for 1 h at the same temperature. The nitrated product was separated by addition of ice and water, dissolved in methylene chloride, washed several times with 2 *M* sodium hydroxide solution and dried with magnesium sulphate. The methylene chloride was removed by distillation and the product recrystallized from a mixture of water and ethanol. The yield was 13.5 g.

A 7.24-g (34.8 mmol) amount of the nitrated product was dissolved in dry methanol, 4.1 g (82 mmol) of hydrazine hydrate were added and the mixture was heated to 60°C. Palladium on activated carbon was added in portions until, after about 2 h, no more nitrogen was evolved. The mixture was filtered through Celite, rendered alkaline with 2 *M* sodium hydroxide solution and extracted with methylene chloride. The organic phase was dried with potassium carbonate, the methylene chloride removed by distillation and the product 1f recrystallized with a large amount of diethyl ether. The yield of 1f was 4.55 g.

To a solution of 2.53 g (8.7 mmol) of 1c in 20 ml of dry chloroform, a solution of 3.1 g (17.4 mmol) of 1f in 40 ml of dry chloroform was added and the mixture was heated under reflux for 14 h. After cooling and filtration, the chloroform was removed by distillation. The reaction product was first chromatographed on 70 g of silica with methanol–methylene chloride (5:95), followed by preparative HPLC on silica with methanol–methylene chloride (2:98). The yield of 1g was 1.22 g. The specific rotation was $[\alpha]_D^{20} = +121°$ (ethanol). Identification was effected by ^1H NMR and mass spectrometry.

A 3.4-g amount of LiChrosorb Si 100 (dried at 200°C and 0.1 mbar) was mixed with a solution of 0.85 g of 1g in 20 ml of dry toluene. Toluene was eliminated by heating to 70°C at 0.1 mbar. Then, at the same pressure, the temperature was raised

to 135°C and held there for 6 h. After cooling, phase I was washed successively with toluene, acetone, acetone–water (1:1), acetone and toluene and dried at 130°C and 0.1 mbar. The yield of phase I was 4.01 g (beige powder).

Elemental analysis resulted in 10.9% C, 1.64% H and 1.35% N. From these results a surface density of 1.1 groups per nm^2 and a reasonable C:N ratio could be calculated.

Phase II. This phase was prepared according to the scheme in Fig. 3.

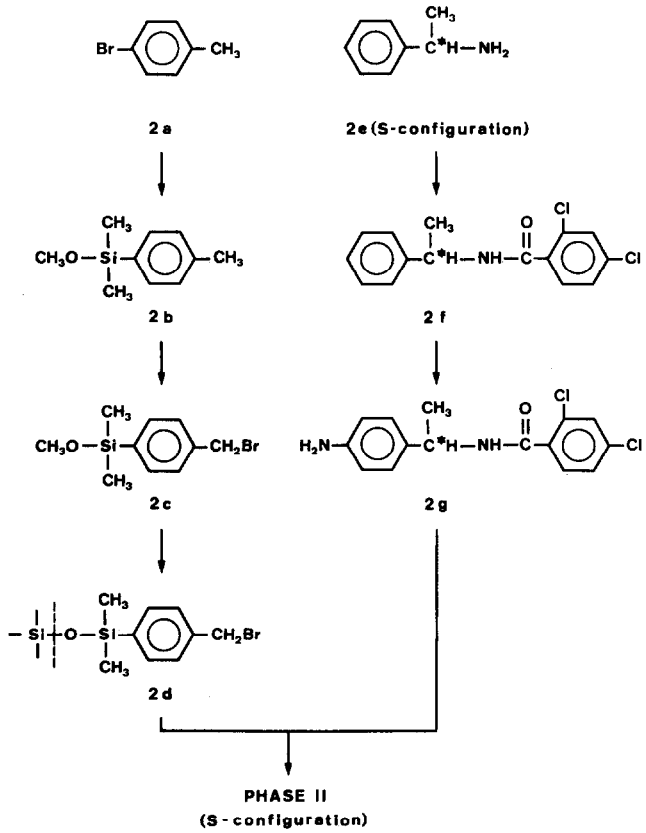

Fig. 3. Preparation of phase II.

A portion of a solution of 21.4 g (125 mmol) of 4-bromotoluene (2a) in 70 ml of dry diethyl ether was added to 3.04 g (125 mmol) of magnesium. After the start of the reaction, 100 ml of dry diethyl ether were added. While the reaction vessel was cooled in a water-bath, the remaining part of the solution of 2a was added dropwise. The mixture was heated under reflux for 45 min, 15 ml (125 mmol) of dichlorodimethylsilane were added and the heating was continued for 2 h. Then 20 ml (480 mmol) of dry methanol were added at 15°C, with intense stirring. Stirring was continued for 1.5 h at room temperature and, after filtration, 11.9 g (150 mmol) of dry pyridine and 20 ml of dry methanol were added. After storing the mixture in the refrigerator overnight, it was filtered and distilled twice at *ca.* 85°C and 0.1 mbar. The yield of 2b was 10.4 g.

A 7.58 g (42.1 mmol) amount of 2b, 9.74 g (54.7 mmol) of N-bromosuccinimide, 0.2 g of 2,2'-azobisisobutyronitrile and 100 ml of dry carbon tetrachloride were refluxed for 2 h. After cooling, the reaction product 2c was filtered and distilled twice at *ca.* 110°C and 0.1 mbar. The yield of 2c was 7.8 g.

A 1.73-g amount of LiChrosorb Si 100 (dried at 200°C and 0.1 mbar) was mixed with a solution of 1.85 g (7.1 mmol) of 2c in 10 ml of dry toluene. Toluene was eliminated by heating to 70°C at 0.1 mbar. Then, at the same pressure, the temperature was raised first to 100°C for 6 h and then to 160°C overnight. The gel 2d was washed with dry acetone. The yield of 2d was 2.13 g. Elemental analysis resulted in 9.94% C, 1.34% H and 5.45% Br. From these results, a surface density of 2.2 groups per nm^2 and a reasonable C:Br ratio could be calculated.

A 50-g (239 mmol) amount of 2,4-dichlorobenzoyl chloride, 80 ml of dry dioxane and 19 g (240 mmol) of dry pyridine were chilled to 0°C. A solution of 30 g (248 mmol) of *S*(−)-1-phenylethylamine in 30 ml of dry dioxane was added dropwise and the mixture was stirred for 2 h. After addition of water, the solid 2f was separated by filtration and recrystallized from ethanol. The yield of 2f was 62.2 g.

To a mixture containing 20 ml of nitric acid (65%) and 16 ml of sulphuric acid (98%) at *ca.* 15°C, 17.0 g (57.8 mmol) of 2f was added in portions and stirred for 1 h at room temperature. The nitrated product was separated by addition of water, dissolved in methylene chloride, washed several times with 2 *M* sodium hydroxide solution and dried with magnesium sulphate. Crystals were obtained from a mixture of methylene chloride and diethyl ether. Recrystallization from a mixture of methylene chloride and *n*-hexane yielded 8.1 g.

To 8.50 g (25.1 mmol) of the nitrated product dissolved in 80 ml of dry methanol, 3.5 g (70 mmol) of hydrazine hydrate were added and heated to 60°C. Palladium on activated carbon was added in portions until, after about 2 h, no more nitrogen was evolved. The mixture was filtered through Celite and placed in a refrigerator for crystallization. Recrystallization from methanol in several steps yielded 5.87 g of 2g. The specific rotation was $[\alpha]_D^{20} = -54°$ (ethanol). Identification was effected by ^1H NMR and mass spectrometry.

A 2.08-g (1.4 mmol Br) amount of 2d was mixed with a solution of 0.98 g (3.2 mmol) of 2g in 40 ml of dry dioxane and the mixture was stirred slowly for 20 h at 100°C. After cooling, phase II was filtered off and washed successively with dioxane–water (1:1), acetone–water (1:1), acetone, methylene chloride and *n*-hexane and dried at 130°C and 0.1 mbar for 4 h. The yield of phase II was 2.17 g (ochre powder).

Elemental analysis resulted in 16.3% C, 1.81% H, 1.05% N, 1.46% Cl and 1.01% Br. From these results, a surface density of 0.9 groups per nm^2 and satisfactory C:N and C:Cl ratios could be calculated.

Comments on preparation and structures of phases I and II. Phase I can also be prepared like phase II according to Fig. 3; on the other hand, phase II cannot be synthesized like phase I according to Fig. 2 because of the chemical instability of 2c under the necessary reaction conditions. It should be possible to synthesize a phase from 1c and 2g in accordance with Fig. 2.

We did not investigate which of the two preparation methods used gives better phases. Preparation according to Fig. 2 should give cleaner phases, but preparation according to Fig. 3 more complete shielding of remaining silanol groups.

TABLE I

PROPERTIES OF COLUMNS PACKED WITH PHASES I AND II

Δp = Pressure drop; t_0 = retention time of an unretained sample (toluene was used); N = plate number for toluene; $\varphi = \Delta p t_0 d_p^2/\eta L^2$ = dimensionless flow parameter according to Bristow and Knox[15]; d_p = particle diameter; η = viscosity; L = column length.

Property	*Column I (phase I)*	*Column II (phase II)*
Δp (bar)	65	80
t_0 (sec)	97.2	102
N	4500	5500
φ	630	820

The calculated C:N and C:Cl ratios are not exactly in accordance with the theoretical values; nevertheless, they indicate that a large part of the desired structure is bonded on to the surface of the gel.

Syntheses of bonded phases described previously[4,5,10–13] always used a nucleophile immobilized on the surface of the silica matrix. As far as we know, our method of preparation of HPLC phases is the first one to use a nucleophile (1f or 2g) in solution attacking immobilized alkyl bromide groups.

The new phases I and II each contain a π-donor group (aniline group) and are, therefore, designed for the chromatographic separation of compounds with π-acceptor groups.

Liquid chromatography

Columns were slurry packed using a method developed in our laboratory[14]. To eliminate fines, phase I was sedimented five times and phase II three times in methanol; the height of the liquid was 5 cm and the sedimentation time was *ca.* 1 h, after which the suspension was decanted.

Stainless-steel tubes (250 × 3.2 mm I.D.) were used as columns. A slurry prepared from 2 g of the phase and 30 ml of methanol–triethylene glycol (1:9) was filled into the column at a pressure of 680 bar. The columns were conditioned with methanol, ethyl acetate and *n*-hexane.

The following equipment and conditions were used in the chromatographic experiments: mobile phase, isopropanol–*n*-hexane (1:4) (viscosity η = 0.4 mPas); flow-rate, 1 ml min^{-1}; pump, Altex 110 Solvent Metering Pump (Altex, Berkeley, CA, U.S.A.); detector, Uvikon LCD 725 UV detector (Kontron, Zürich, Switzerland), detection at 254 nm; and sampling device, Rheodyne 7120 Syringe Loading Sample Injector with a 20-μl loop (Rheodyne, Berkeley, CA, U.S.A.). In the following these conditions are called "standard conditions".

For the two columns filled with phases I and II the values in Table I were determined.

RESULTS AND DISCUSSION

On both columns, *R,S*-N-(1-phenylethyl)-3,5-dinitrobenzoylamide (PEDA), a

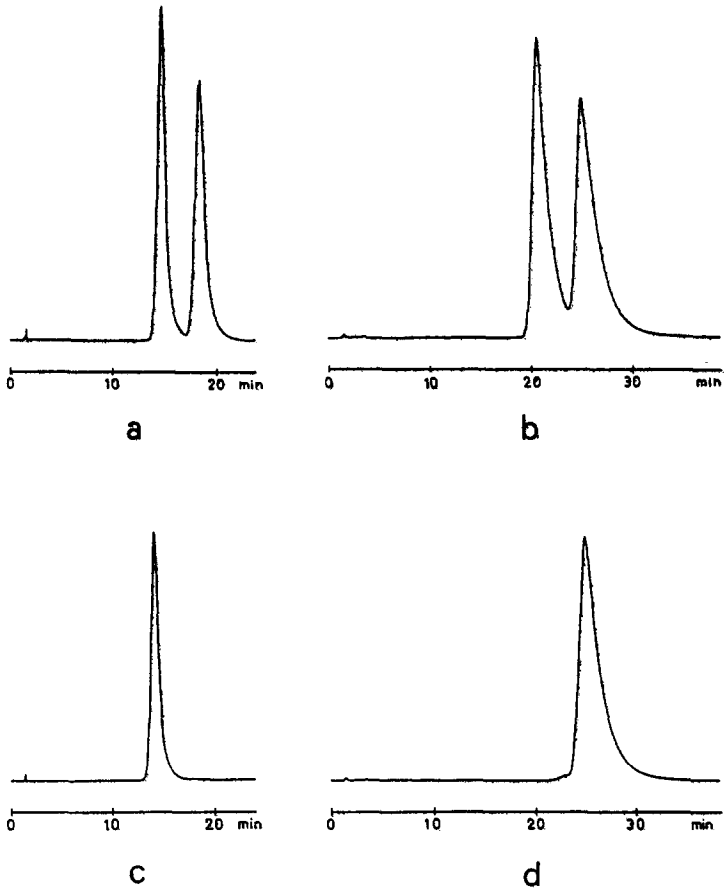

Fig. 4. Resolution of *R,S*-N-(1-phenylethyl)-3,5-dinitrobenzoylamide under the standard chromatographic conditions on (a) column I (*R* configuration) and (b) column II (*S* configuration), and chromatograms of the *S*(+)enantiomer of the same sample under the same conditions on (c) column I and (d) column II.

TABLE II

CHROMATOGRAPHIC DATA FOR SEPARATION OF PEDA ENANTIOMERS

k'_1 = capacity factor of the first eluted peak; $\alpha = k'_2/k'_1$ = separation factor; k'_2 = capacity factor of the second eluted peak.

Property	*Column I*	*Column II*
Configuration of the phase	*R*	*S*
k'_1	8.07	11.0
α	1.31	1.25
First eluted peak	*S*(+)	*R*(−)

TABLE III
SEPARATION OF PEDA ENANTIOMERS WITH METHANOL–WATER (1:3) AS THE MOBILE PHASE

Property	*Column I*	*Column II*
Configuration of the phase	*R*	*S*
k'_1	0.91	9.06
α	1.21	1.08
First eluted peak	*S*(+)	*R*(−)

compound with a π-acceptor group, was resolved into enantiomers. The chromatograms are shown in Fig. 4. Table II lists the chromatographic data for this separation.

The preparative separation of PEDA on column I and polarimetric measurements of the separated components confirmed the above-mentioned elution order.

Under the same experimental conditions, but with water–methanol (1:3) as the mobile phase, the results in Table III were obtained. If the water content of the mobile phase is increased to 50%, k'_1 on column I increases to 5.98, which corresponds to the usual reversed-phase behaviour. The separation factor and elution order remain unchanged.

In another experiment, using as the mobile phase 0.01 *M* hydrochloric acid–methanol (1:3), we tried to protonate the phases in order to convert the π-donor of the stationary phase into a π-acceptor; for PEDA, which is a π-acceptor, a change in the chiral recognition mechanism is very probable. This experiment failed; the k'

TABLE IV
SEPARATION OF ENANTIOMERS WITH THE STRUCTURE R–C*H–NH–C(=O)–R″ ON COLUMNS I AND II

Sample			*Column I*		*Column II*	
No. *R*	*R′*	*R″ (π-acceptor)*	k'_1	α	k'_1	α
1 C_6H_5	C_2H_5	$[3,5\text{-}(NO_2)_2]C_6H_3$	6.87	1.39	13.0	1.25
2	$n\text{-}C_3H_7$		6.25	1.41	13.5	1.22
3	$n\text{-}C_4H_9$		6.18	1.40	12.7	1.23
4	$n\text{-}C_5H_{11}$		5.71	1.45	12.5	1.22
5	$n\text{-}C_7H_{15}$		4.94	1.48	10.8	1.23
6	$n\text{-}C_8H_{17}$		4.71	1.51	10.8	1.26
7	$n\text{-}C_9H_{19}$		4.32	1.55	9.51	1.26
8	$n\text{-}C_{10}H_{21}$		4.09	1.55	8.85	1.25
9	$n\text{-}C_{13}H_{27}$		3.48	1.60	7.75	1.28
10	$n\text{-}C_{15}H_{31}$		3.32	1.63	7.38	1.28
11	$n\text{-}C_{16}H_{35}$		3.01	1.64	6.94	1.30
12 C_6H_5	CH_3	$(2,4\text{-}Cl_2)C_6H_3$	1.72	1.07	2.41	1.05
13 $(4\text{-}NO_2)C_6H_4$	CH_3	$(2,4\text{-}Cl_2)C_6H_3$	4.06	1.05	6.94	1.07
14 $(4\text{-}CH_3O)C_6H_4$	CH_3	$[3,5\text{-}(NO_2)_2]C_6H_3$	9.96	1.40	20.3	1.27
15 $(4\text{-}CH_3O)C_6H_4$	$n\text{-}C_4H_9$	$[3,5\text{-}(NO_2)_2]C_6H_3$	8.95	1.49	19.3	1.26
16 1-Naphthyl	CH_3	$[3,5\text{-}(NO_2)_2]C_6H_3$	7.33	1.80	15.7	1.65

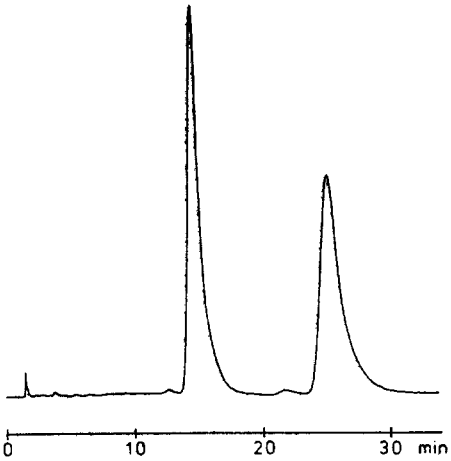

Fig. 5. Resolution of *R,S*-N-[1-(1-naphthyl)ethyl]-3,5-dinitrobenzoylamide on column I under the standard conditions.

values diminished whilst the α-values were almost identical with those obtained with water–methanol as the mobile phase.

Other compounds were chromatographed under the standard conditions; their structures and chromatographic data are given in Table IV. For the homologous series of compounds 1–11, k'_1 decreases with increasing carbon number of the group R'; on column I, α increases with increasing carbon number, but on column II it does not change much. Similar behaviour is observed on comparing compounds 14 and 15 with the corresponding underivatized compounds: for column II α seems to be independent of R' and of the kind of substituent bonded to the aromatic ring of R. The smallest α-values are observed for compounds 12 and 13, which have weaker π-acceptor groups than all other samples in Table IV.

The highest separation factors on both columns were observed for *R,S*-N-[1-(1-naphthyl)ethyl]-3,5-dinitrobenzoylamide (Table IV, compound 16, and Fig. 5), as shown in Table V. The elution order is the same as for the previously investigated phenyl analogue.

No resolution was observed for the π-donors 2,2,2-trifluoro-1-(9-anthracene)-ethanol and N-(1-phenylethyl)-2,4-dimethoxybenzoylamide, or for the π-acceptors N-[1-(4-nitrophenyl)ethyl]acetamide and 1-phenylethyl-3,5-dinitrobenzoate.

TABLE V

SEPARATION OF ENANTIOMERS OF COMPOUND 16

Property	*Column I*	*Column II*
Configuration of the phase	*R*	*S*
k'_1	7.33	15.7
α	1.80	1.65
First eluted peak	*S*(+)	*R*(−)

In comparison with published results[2–13], the enantioselectivity of our stationary phases is very good, in the explored range of applications. In order to broaden this range, it is intended to investigate further the protonation of the stationary phase and, by using bromoalkyl groups as coupling components between the chiral centre and the silica matrix, to synthesize similar phases with even higher separation factors.

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