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NEW CHIRAL, COVALENTLY BONDED, π-DONOR STATIONARY PHASES FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY, BASED ON DERIVATIVES OF OPTICALLY ACTIVE 1-(α-NAPHTHYL)ETHYLAMINE

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SUMMARY

On the basis of optically active 1-(α-naphthyl)ethylamine, a series of chiral naphthylamine derivatives was synthesized. These amines were attached to 3-glycidoxypropyl silica to give chiral stationary phases for high-performance liquid chromatography. Their application for chiral separations was tested with some 3,5-dinitrobenzoyl amides. The most promising of these chiral phases was also synthesized by coupling the chiral naphthylamine derivative with 3-glycidoxypropyl trimethoxysilane before binding to silica. This phase with higher loading allowed the separation of derivatives of various classes of chiral compounds such as amines, alcohols, acids, amino alcohols, amino ketones and amino acids. Best separations are found for derivatives with a strong π-acceptor group such as the 3,5-dinitrophenyl group.

INTRODUCTION

In an earlier paper[1] we described the synthesis of two chiral stationary phases for high-performance liquid chromatography (HPLC) based on optically active 1-phenylethylamine. These phases were suited for the separation of 3,5-dinitrobenzoyl derivatives of enantiomeric amines. Other phases for the separation of enantiomeric π-acceptor compounds and their applications have been published[2–11].

From the chromatographic data[1–10] it can be deduced that a naphthyl group in the sample or in the stationary phase is very useful for a good chiral separation. We therefore adapted the synthesis of our chiral stationary phases[1] to the use of 1-(α-naphthyl)ethylamine as chiral starting material, as shown in Fig. 1. Variation of the amide substituent at the chiral centre allowed us to study the influence of the phase upon the separation factor for various samples. The phase with the pivaloyl substituent (phase IV) turned out to have an enhanced chiral separation effect for several classes of compounds.

EXPERIMENTAL

Materials

LiChrosorb Si 100 (Merck, Darmstadt, F.R.G.) and Spherisorb S5W (Phase

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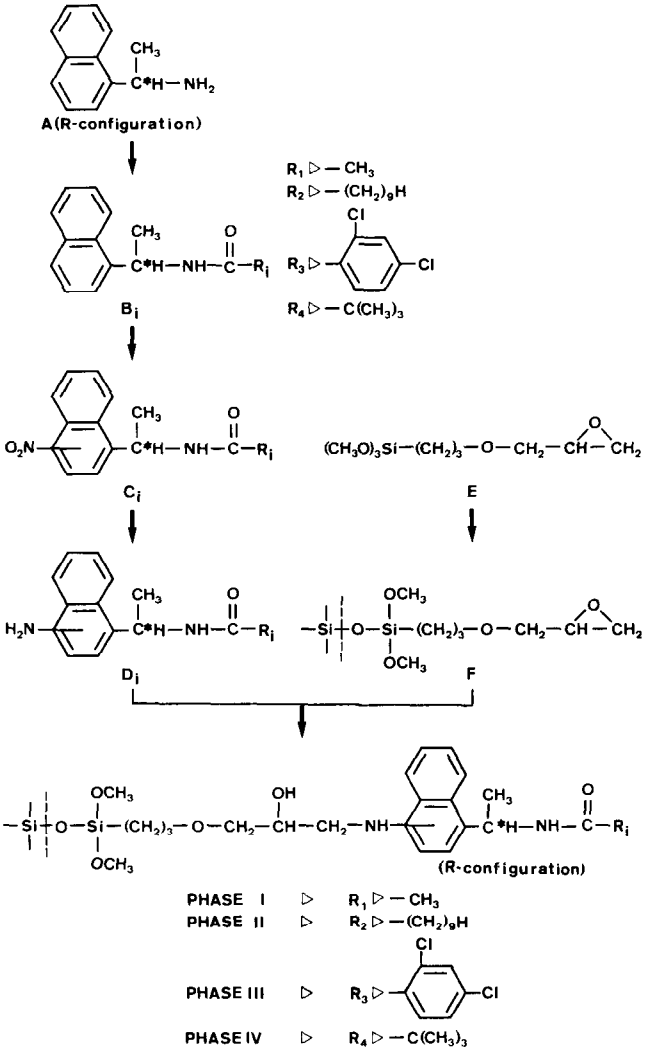


Fig. 1. Preparation of phases I–IV.

Separations, Queensferry, U.K.) with a particle size of 5 μm were used. All other chemicals were purchased from Fluka (Buchs, Switzerland) or from Merck.

Preparation of phases I–V

Phases I–IV were prepared according to the scheme shown in Fig. 1. Only the details for the synthesis of phase I are given in this paper. Phases II–IV were synthesized by the same procedure with small variations in the purification steps and in the yield.

To 4.4 g (56 mmol) of acetyl chloride and 3.2 g (55 mmol) of propylene oxide in 100 ml of dioxane, a mixture of 5.0 g (29 mmol) of (*R*)-1-(α-naphthyl)ethylamine (A) in 50 ml of dioxane was added dropwise at room temperature. After 1 h, the

solvent was partially removed and the crude product was recrystallized from a mixture of ethanol and water. The yield of B_1 was 6.1 g.

A 3.0-g (14 mmol) quantity of B_1 was dissolved in 25 ml of concentrated acetic acid and 10 ml of nitric acid (65%) were added. This mixture was stirred at 65°C for 1 h, hydrolysed with ice and extracted with dichloromethane. The organic phase was washed several times with saturated sodium hydrogen carbonate solution and dried with magnesium sulphate. The crude product was recrystallized from a mixture of dichloromethane and hexane. The yield of C_1 (mixture of positional isomers) was 1.9 g.

A 1.7-g (6.6 mmol) quantity of C_1 was dissolved in 60 ml of dry ethanol and mixed with 1.6 g (32 mmol) of hydrazine hydrate. At 60°C, palladium on activated carbon was added in portions until no more nitrogen was evolved. The mixture was filtered through Celite and, after adding a saturated solution of sodium chloride, extracted with dichloromethane. The organic phase was dried with magnesium sulphate and the solvent was removed. The yield of D_1 (mixture of positional isomers) was 1.4 g. It was identified with mass spectroscopy and ^1H NMR.

To 5.1 g of LiChrosorb Si 100 (dried at 100°C and 0.1 mbar for 4 h), a mixture of 1.8 g (7.6 mmol) of 3-glycidoxypropyl trimethoxysilane (E) in 20 ml of dry toluene was added. The toluene was eliminated at 0.1 mbar and 40°C. The temperature was then raised to 75°C for 4 h. After cooling, the gel was washed with dry toluene. The yield of F was 5.49 g (white powder). Elemental analysis of F resulted in 4.12% C and 0.93% H. From these results, a surface density of 0.9 groups/nm^2 was calculated.

A 1.15-g quantity of F was stirred with 0.25 g (1.1 mmol) of D_1 in 15 ml of dry methanol for 6 h at 65°C. After cooling, phase I was washed with methanol. Phase I (white powder) was packed into the HPLC column as a methanol slurry. Elemental analysis of phase I resulted in 5.74% C, 1.11% H and 0.28% N. From these results, a surface density of the chiral moiety of 0.2 groups/nm^2 can be estimated.

For the preparation of phase V (Fig. 2), 0.15 g (0.6 mmol) of D_4 and 0.13 g (0.6 mmol) of E were stirred in 10 ml of dry toluene for 1 h. A 1.89-g quantity of

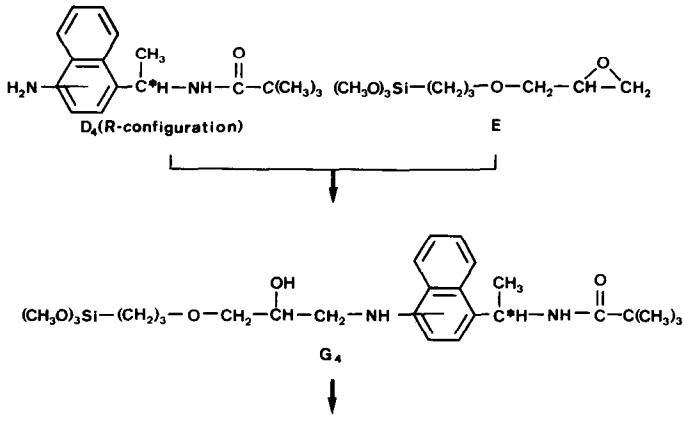
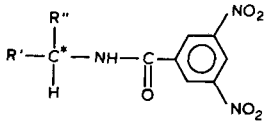


Fig. 2. Preparation of phase V. Phase V corresponds to phase IV but with a higher loading.

TABLE I

RESOLUTION OF SOME 3,5-DINITROBENZOYL TEST COMPOUNDS ON CHIRAL STATIONARY PHASES I–V

Mobile phase: *n*-hexane–isopropanol (78:22); the configuration of all phases is *R*; k'_1 = capacity factor of the first eluted enantiomer; α = separation factor; nr = no resolution.



R'	*R''*	*Phase I*		*Phase II*		*Phase III*		*Phase IV*		*Phase V*		*Last eluted enantiomer for all the phases*
		k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	
C_6H_5	CH_3	5.30	1.82	4.65	1.98	2.93	2.22	10.1	2.10	27.8	1.99	*R*
Naphthyl	CH_3	5.30	2.68	5.09	2.88	3.11	3.53	10.5	4.74	30.8	4.92	*R*
C_2H_5	CH_3	3.35	1.06	2.78	1.20	2.47	1.09	5.52	1.24	12.1	1.29	
C_6H_5	$COOCH_3$	6.83	nr	5.09	1.13	4.43	nr	12.3	1.18	31.3	1.14	*R*

Spherisorb S5W (dried at 100°C and 0.1 mbar for 4 h) was added. This mixture was stirred slowly at 100°C for 6 h. After cooling, phase V was washed with methanol. Phase V (slightly violet powder) was packed into the HPLC column as a methanol slurry. Elemental analysis of phase V resulted in 5.17% C, 0.92% H and 0.3% N. From these results, a surface density of 0.5 groups/nm^2 and a satisfactory C:N ratio can be calculated.

Comments on the preparation of phases I–V

An attempt to prepare phase I *in situ* (preparation of F in a HPLC column packed with silica according to ref. 12 followed by pumping a methanol solution of D_1 through the column) failed. This column had a very low loading and showed a separation factor $\alpha = 1.26$ for the 3,5-dinitrobenzoyl (DNB) derivative of racemic 1-phenylethylamine. Pumping the D_1 solution through a column packed with F increased the separation factor of this same compound to $\alpha = 1.48$. The separation factor of phase I for this compound ($\alpha = 1.82$) was not achieved.

The increase in the carbon content from F to phases I–IV due to the chiral moiety was always *ca.* 1.5%. This is a small value compared to our previous phases[1]. Syntheses according to the scheme of Fig. 2 are to be preferred.

Liquid chromatography

The following equipment was used: 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.). Phases I–IV were packed into stainless-steel tubes 250 mm × 2.3 mm I.D. Phase V was packed into a stainless-steel tube 250 mm × 3.2 mm I.D. In all cases, a methanol slurry was used to pack the columns. They were equilibrated with *n*-hexane–isopropanol (78:22, azeotropic mixture). For both types of columns, a flow-rate of 1 ml/min was chosen.

Various eluents were used with these covalently bonded phases, including water. Basic eluents are not allowed because they cleave the chiral moiety from the phase.

RESULTS AND DISCUSSION

Phases I–IV

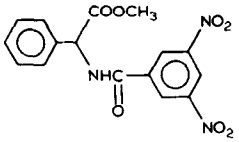
The synthesis of phases I–IV according to the scheme in Fig. 1 allowed us to investigate the effect of the chiral 1-(α-naphthyl)ethylamine derivatives upon chiral separation. The same glycidoxypropyl silica was used as an intermediate for all the phases. A minimal and constant retention of the samples due to interactions with the silica was to be expected. Therefore, the chiral separations of the test compounds on phases I–IV can be compared directly if the same eluent is used.

The phases were tested with 3,5-dinitrobenzoyl (DNB) derivatives of some amines. Due to interactions of the DNB group (π-acceptor, amide) with the stationary phase (π-donor, amide), these samples are strongly retained and, in general, good resolutions of the enantiomers are observed. The results are shown in Table I.

Racemic phenylglycine methyl ester DNB amide was not resolved on phases

TABLE II

SEPARATION OF RACEMIC PHENYLGLYCINE METHYL ESTER DNB AMIDE ON PHASES I AND III FOR ELUENTS OF VARIOUS POLARITIES



Isopropanol in n-hexane (%)	*Phase I*		*Phase III*	
	k'_1	α	k'_1	α
1	52.0	1.13	75.1	1.11
5	14.8	1.10	23.3	1.07
10	7.9	1.07	12.0	1.05
20	4.4	nr	6.8	nr

I and III with 22% isopropanol in *n*-hexane as eluent. But if the polarity of the eluent is lowered, a separation of these enantiomers can be observed. The corresponding data are shown in Table II.

Comments on the chiral separation mechanism of phases I–IV. In ref. 5, the retention of DNB amides on π-donor stationary phases with an amide group is rationalized with two main processes: the dipole-stacking process and the hydrogen-bonding process. This model is also used to rationalize the difference in chiral selectivity of two similar π-donor stationary phases. Using this concept, it can be deduced for our phases that phase III should operate predominantly with the dipole-stacking process (carbonyl group in conjugation with an aromatic ring) and phase IV should operate predominantly with the hydrogen-bonding process (bulky *tert.*-butyl group next to the carbonyl group). Phase III should better resolve compounds that prefer the dipole-stacking process (*e.g.* DNB amides of aryl alkyl amines) than compounds that prefer the hydrogen-bonding process (*e.g.* DNB amides of amino acid esters). Correspondingly, phase IV should show poor separations if the dipole-stacking process is important and good separations if the hydrogen-bonding process is important.

The data in Table I show that among phases I–IV, phase IV is the best one for our test compounds. This cannot be rationalized with the concept of ref. 5 alone. To do that, other parameters must be added to the model. One possible parameter is the rigidity of the stationary phase. Such a concept is discussed in ref. 13.

Phase V

Compared to our previous phases[1], the loading of phases I–IV as determined by elemental analysis was low. Therefore, phase V with the same functional group as phase IV was synthesized according to the scheme of Fig. 2. This synthesis resulted in a higher loading of the chiral moiety and therefore in a striking increase of the capacity factors of the test compounds, as can be seen in Table I. All further experiments were performed with phase V.

Optimization of the mobile phase. For similar separations of enantiomers, var-

TABLE III

OPTIMIZATION OF THE MOBILE PHASE FOR PHENYLGLYCINE METHYL ESTER DNB AMIDE ON PHASE V

Last eluted enantiomer in all cases was *R*.

Mobile phase	k'_1	α
n-Hexane–isopropanol (60:40)	11.6	1.10
n-Hexane–dichloromethane (50:50)	12.8	1.12
n-Hexane–chloroform (50:50)	14.0	1.18
tert.-Butyl methyl ether–*n*-hexane (75:25)	13.7	1.43
n-Hexane–dioxan (75:25)	10.8	1.43
n-Hexane–tetrahydrofuran (75:25)	8.2	1.41

ious mixtures of *n*-hexane and isopropanol are often used in the literature as the mobile phase. After also having done our first experiments with *n*-hexane–isopropanol (see Tables I and II), we tried to find an optimum mobile phase for stationary phase V. According to Snyder's solvent triangle[14], we used mixtures of *n*-hexane with dichloromethane (which is a strong dipole), with chloroform (which is a proton donor) and with ether (*tert.*-butyl methyl ether, which is a proton aceptor). Phenylglycine methyl ester DNB amide was the test compound for these experiments, whose data are shown in Table III. Because *tert.*-butyl methyl ether gave far the best separation factor (α = 1.42 instead of 1.10 with *n*-hexane–isopropanol), two other ethers, dioxan and tetrahydrofuran, were also investigated. As can be seen from Table III, all three ethers can be used equally well. Therefore, we often used mixtures of *n*-hexane and tetrahydrofuran for the following experiments. However, it cannot be generalized that eluents of *n*-hexane and an ether are always the best choice for stationary phase V. The peaks of, for example, 3,5-dinitrobenzoic acid α-phenethyl ester (compound 22 in Table V) were broader with *n*-hexane–tetrahydrofuran than with *n*-hexane–isopropanol, therefore the resolution of the enantiomers was poorer although the separation factor was identical with both mobile phases. It can be concluded that it may be quite worthwhile to search for an optimum mobile phase in the case of a difficult separation of enantiomers.

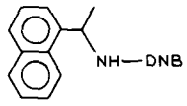
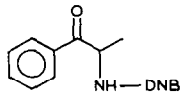
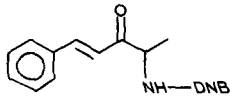
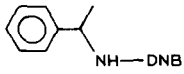
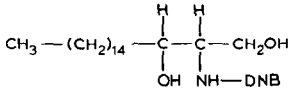
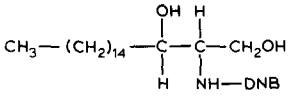
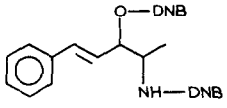
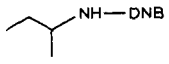
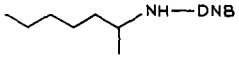
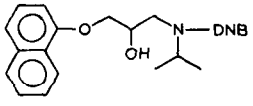
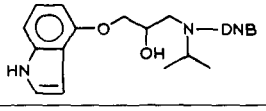
Application range of phase V. Numerous racemates could be separated into the enantiomers with stationary phase V. Table IV lists the DNB derivatives of amines and amino alcohols and Table V the other compounds, including compounds without a π-acceptor group. In all cases where we were able to check the elution order, the *R*-enantiomer was eluted last. The highest separation factor was determined for N-1-(α-naphthylethyl)-3,5-DNB amide: α = 4.92 in *n*-hexane–isopropanol (78:22) (compound 1 in Table IV). It was even possible to separate the enantiomers of 1-(phenylethyl)-3,5-dimethoxy benzoic acid amide, which is a π-donor (compound 27 in Table V). Fig. 3 shows the separation of the di-DNB derivative of merucathine, a β-aminoalcohol. Fig. 4 shows the separation of a mixture of *erythro* and *threo* DNB amides of dihydrosphingosine, also an amino alcohol, into the four isomers.

Reversed-phase separations with phase V. It is also possible to use stationary phase V in the reversed-phase mode. Table VI shows the data for two compounds separated with methanol–water (60:40). For phenylglycine methyl ester DNB amide

TABLE IV

SEPARATION OF DNB AMIDES ON STATIONARY PHASE V

DNB = 3,5-dinitrobenzoyl.

*Compound**	*Structure*	k'_1	α	*Mobile phase***	*Last eluted enantiomer*
1		30.8	4.92	a	*R*
2		4.21	3.10	b	*R*
3		5.20	2.43	b	*R*
4		6.50	2.14	b	*R*
5		14.5	1.64	b	
6		12.2	1.46	b	
7		11.2	1.36	b	3*S*, 4*R*
8		4.33	1.23	b	
9		3.79	1.21	b	
10		3.62	1.09	b	
11		8.67	1.06	b	

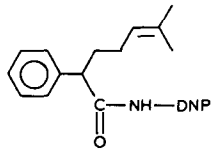
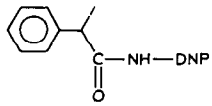
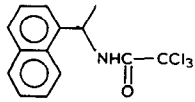
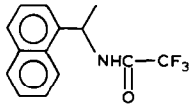
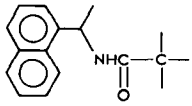
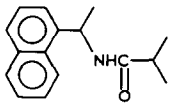
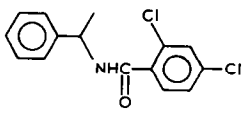
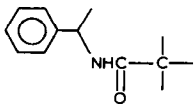
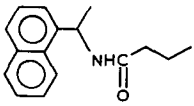
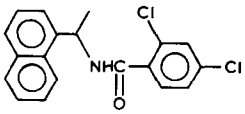
* Identification of compounds: 2 = DNB amide of cathinone, 3 = DNB amide of merucathinone, 5 = DNB amide of *erythro* dihydrosphingosine, 6 = DNB amide of *threo* dihydrosphingosine, 7 = di-DNB derivative of merucathine, 10 = DNB amide of propranolol, 11 = DNB amide of pindolol; the structures of the amino alcohol derivatives (compounds 5, 6, 7, 10, 11) were elucidated with mass spectroscopy.

** Mobile phase: a = *n*-hexane–isopropanol (78:22); b = *n*-hexane–tetrahydrofuran (75:25).

TABLE V

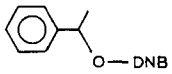
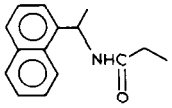
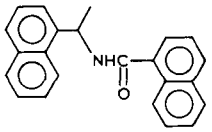
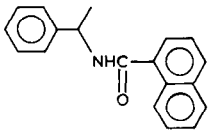
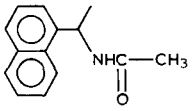
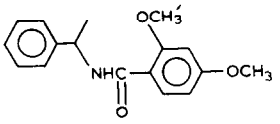
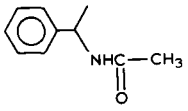
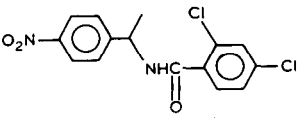
SEPARATION OF OTHER COMPOUNDS ON STATIONARY PHASE V

DNB = 3,5-dinitrobenzoyl; DNP = 3,5-dinitrophenyl.

Compound	*Structure*	k'_1	α	*Mobile phase**	*Last eluted enantiomer*
12		8.25	2.94	b	
13		11.8	2.27	b	
14		1.29	1.20	c	*R*
15		1.35	1.18	c	*R*
16		1.02	1.16	b	*R*
17		3.58	1.14	c	*R*
18		1.46	1.14	b	*R*
19		0.83	1.12	b	*R*
20		2.14	1.12	b	
21		1.67	1.10	b	*R*

(Continued on p. 102)

TABLE V (*continued*)

Compound	*Structure*	k'_1	α	*Mobile phase**	*Last eluted enantiomer*
22		1.67	1.10	a	*R*
23		2.65	1.09	b	*R*
24		2.50	1.08	b	*R*
25		2.38	1.07	b	*R*
26		5.83	1.07	b	*R*
27		2.92	1.07	b	*R*
28		4.79	1.05	b	*R*
29		3.79	1.04	b	*R*

* Mobile phase: a = *n*-hexane–isopropanol (78:22), b = *n*-hexane–tetrahydrofuran (75:25), c = *n*-hexane–tetrahydrofuran (85:15).

(the test compound of Tables II and III), a reversal of the elution order in comparison to the straight-phase mode could be observed. This is the only case where the *S*-isomer was determined to elute last. After re-equilibrating phase V with *n*-hexane–isopropanol or *n*-hexane–tetrahydrofuran, no loss of chiral resolution could be observed. However, we did not test the long-term stability of this stationary phase under aqueous conditions. As already mentioned, basic eluents are not used because of cleavage of the chiral moiety from the stationary phase.

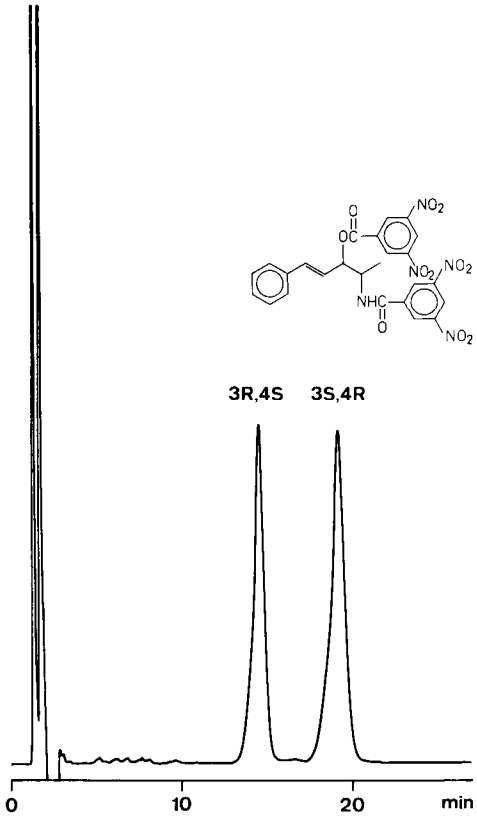
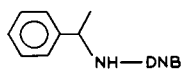
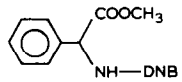


Fig. 3. Separation of (±) merucathine di-DNB derivative on stationary phase V. The mixture was not racemic but had a slight excess of the 3*S*,4*R*-enantiomer. Column: 25 cm × 3.2 mm I.D. Mobile phase: *n*-hexane–tetrahydrofuran (75:25), 1 ml/min. Detector: UV 254 nm.

TABLE VI

SEPARATION OF DNB AMIDES ON STATIONARY PHASE V WITH METHANOL–WATER (60:40)

DNB = 3,5-dinitrobenzoyl.

Compound	*Structure*	k'_1	α	*Last eluted enantiomer*
4		4.67	1.55	*R*
Test		6.46	1.10	*S*

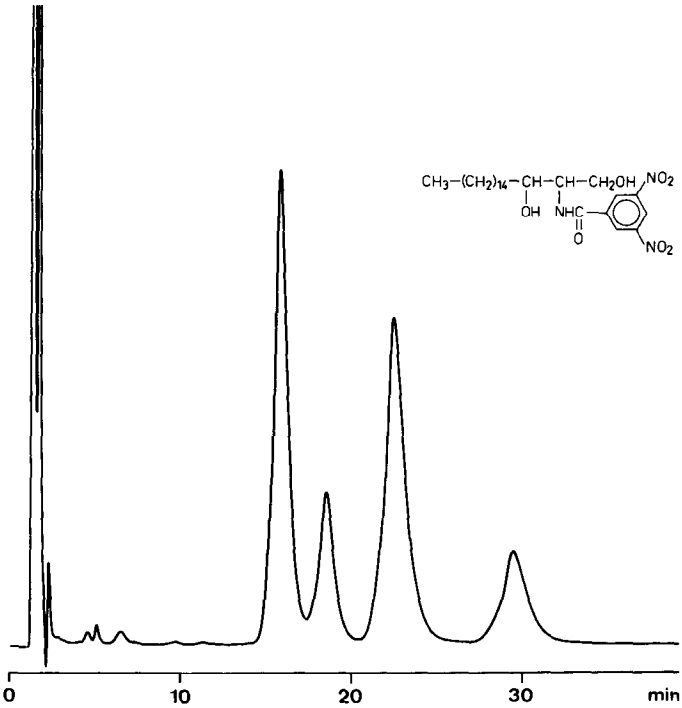


Fig. 4. Separation of a 80:20 mixture of (±)-*threo*- and (±)-*erythro*-dihydrosphingosine DNB amide. Conditions as in Fig. 3.

CONCLUSIONS

Using chiral 1-(α-naphthyl)ethylamine as starting material, chiral stationary π-donor phases are accessible. These π-donor phases are especially suited for the separation of π-acceptor samples. The best π-acceptor group for this application is the 3,5-dinitrophenyl group. Amines and alcohols can be derivatized with 3,5-dinitrobenzoyl chloride using the Schotten-Baumann reaction[15]. For amino acids, special conditions are recommended[4]. Acids can be converted into the acid chlorides and then derivatized with 3,5-dinitroaniline. For this conversion, standard methods (*e.g.* thionyl chloride or thionyl chloride–pyridine at 0°C[16]) can be used. Using these derivatizations with non-chiral reagents to introduce the π-acceptor group, many compounds including cathinone, merucathinone, merucathine, propranolol, pindolol and dihydrosphingosine have been separated into the enantiomers.

A chiral stationary phase based on phase V will soon be available from Serva, Heidelberg, F.R.G.

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