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## Note

# Chiral stationary phases via hydrosilylation reaction of N-acryloyl-amino acids

## I. Stationary phase with one chiral centre for high-performance liquid chromatography and development of a new derivatization pattern for amino acid enantiomers

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During the last decade, efforts have been made to achieve a better resolution of amino acid enantiomers by use of high-performance liquid chromatography (HPLC) with chiral stationary phases (CSPs). These developments are documented in several reviews[1–6]. Especially chiral diamide donor–acceptor CSPs, which have long been known in gas chromatography[7], seem to be very powerful[8].

In previous investigations we studied donor–acceptor CSPs containing the aromatic amino acid phenylglycine[9,10]. We found that these CSPs have a good enantioselectivity for several $N^{\alpha}$-acylamino acid esters. Computer-aided energy calculations[10] indicated that one $\pi$–$\pi$ donor–acceptor interaction and two hydrogen bonds interacting between amino acid derivatives and the CSP are essential for enantioselective recognition. We now report that it is possible to increase the separation factor, $\alpha$, by strengthening one of the hydrogen bonds assumed to be essential for enantioselective molecular recognition.

In the literature, different methods of coupling have been used to link amino acids to a silica matrix. Coupling the amino acid via the C-terminus is more common (Pirkle-type columns), but N-terminal linking has the advantage that standard methods of peptide chemistry can be used to attach further groups at the C-terminus of the amino acid. Up to now coupling via the N-terminus of amino acids to the silica matrix has been achieved, *e.g.*, by derivatization with succinic anhydride and reaction with aminopropylsilica[11] or by derivatization of the amino acid with 10-undecenyl chloride, hydrosilylation of the olefin to give the chlorosilane derivative and coupling with silica[8].

Although the hydrosilylation reaction of olefins is well known[12], to our knowledge there have been no reports of the addition of hydrogensilanes to acryloylamides. For coupling a phenylglycine derivative via the $N^{\alpha}$-terminus to a silica

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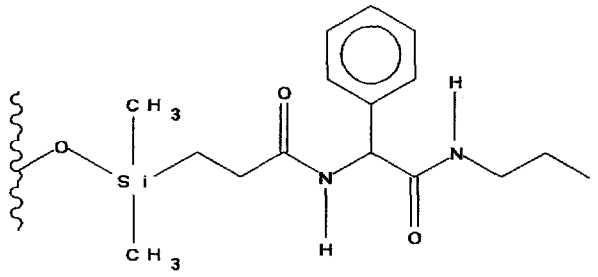

Fig. 1. Structure of the chiral stationary phase

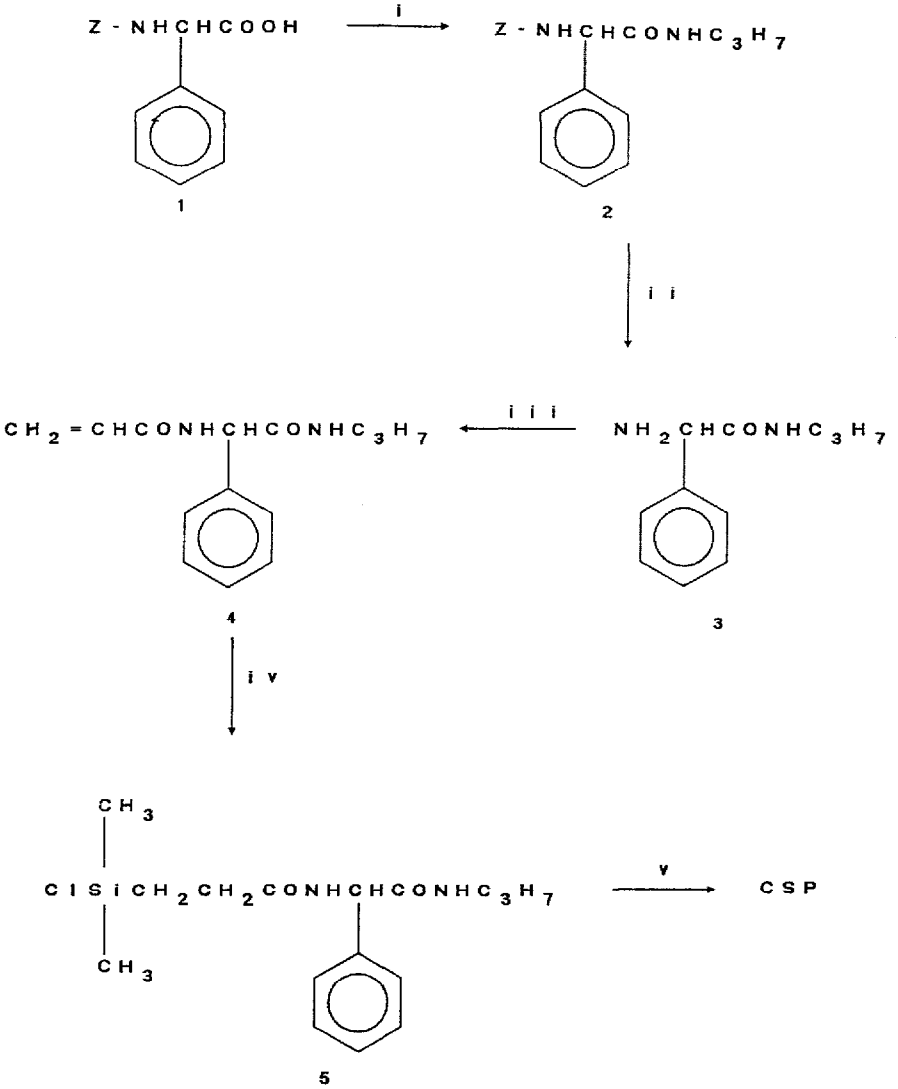

Fig. 2. Preparation of the CSP (according to ref. 15). Z = Benzyloxycarbonyl; i = mixed anhydride synthesis of **1** with propylamine; ii = catalytic hydrogenation of **2** over Pd–C; iii = reaction of **3** with acryloyl chloride; iv = hydrosilylation reaction of **4** with dimethylchlorosilane; v = coupling with Nucleosil Si 100-5 in pyridine.

matrix, the hydrosilylation reaction of acryloylamino acid derivatives is established to generate the CSP shown in Fig. 1.

## EXPERIMENTAL

The scheme for the preparation of the CSP is shown in Fig. 2.

(*R*)-Phenylglycine, acryloyl chloride, dimethylchlorosilane, trimethylchlorosilane, hexachloroplatinic acid and propylamine were purchased from E. Merck (Darmstadt, F.R.G.). Nucleosil Si 100-5 was purchased from Macherey, Nagel & Co. (Düren, F.R.G.).

$N^{\alpha}$-3,5-Dinitrobenzoylamino acid 2-propyl esters were synthesized according to ref. 13. The corresponding $N^{\alpha}$-3,5-dinitrobenzoylamino acid propylamides, dimethylamides and diethylamides were obtained according to ref. 14. The acryloyl acid derivative of D-phenylglycine (**4**) was synthesized as shown in Fig. 2 according to ref. 15.

All new compounds showed the expected analytical and spectroscopic data (elemental analysis, $^{1}H$ NMR, $^{13}C$ NMR spectroscopy).

*Preparation of the chiral stationary phase*

All steps were carried out under a nitrogen atmosphere, avoiding any moisture. A 3.20-g (0.13-mol) amount of acryloyl-D-phenylglycylpropylamide (**4**), 4.50 g (0.48 mol) of dimethylchlorosilane and 100 mg of hexachloroplatinic acid were suspended in 120 ml of chloroform. After refluxing for 2 h, the solution was evaporated to dryness *in vacuo* (0.05 mbar). To a solution of the corresponding dimethylchlorosilyl derivative (**5**) in 50 ml of dry pyridine, 2.4 g of silica gel (dried for 24 h at 160°C and 0.05 mbar) were added and the mixture was stirred for 24 h at 40°C. The modified silica was end-capped by adding 4.0 ml of trimethylchlorosilane to the solution and stirring for 24 h at room temperature. The gel was collected by filtration through a G4 sintered-glass filter funnel, washed successively with methanol, chloroform, dichloromethane and dried *in vacuo* (0.05 mbar). Chromatographic columns (200 × 4.0 mm I.D.) were slurry-packed by conventional techniques. The CSP contained 0.54 mmol of chiral group per gram of gel (determined by elemental analysis).

*Instrumental*

HPLC experiments were carried out with a Perkin-Elmer (Überlingen, F.R.G.) Series 2B liquid chromatograph equipped with a Perkin-Elmer LC 55 variable-wavelength detector. Solvents were distilled and filtered through a G4 sintered-glass filter funnel before use. Solutes (0.2–0.5 mg/ml in ethyl acetate) were injected through a 10-$\mu$l sample loop. Chromatographic runs were performed at a flow-rate of 1 ml/min at room temperature.

## RESULTS AND DISCUSSION

The hydrosilylation of an acryloylamino acid derivative with dimethylchlorosilane was successfully achieved with hexachloroplatinic acid as the catalyst. Dicyclopentadienylplatinum dichloride does not act as a catalyst (no hydrosilylation product). At least 60% of the resulting products were found to be the $\alpha$-adduct to the

TABLE I

ENANTIOMER SEPARATION OF $N^{\alpha}$-3,5-DINITROBENZOYLAMINO ACID 2-PROPYL ESTERS ON THE CSP

$k'_D$ = Capacity factor for the D-isomer; $k'_L$ = capacity factor for the L-isomer; $\alpha$ = separation factor. Mobile phase: 2-propanol–hexane (10:90).

| *Amino acid* | $k'_D$ | $k'_L$ | $\alpha$ |
|---|---|---|---|
| Alanine | 1.62 | 2.68 | 1.65 |
| Valine | 0.75 | 1.97 | 2.63 |
| Leucine | 0.90 | 1.95 | 2.17 |
| Isoleucine | 0.86 | 2.24 | 2.62 |
| Phenylalanine | 1.26 | 2.76 | 2.19 |
| Phenylglycine | 1.09 | 2.00 | 1.84 |
| Tyrosine[a] | 0.66 | 1.46 | 2.21 |
| Serine | 3.03 | 3.60 | 1.19 |
| Threonine | 2.14 | 2.50 | 1.17 |
| Proline | 1.05 | 1.05 | 1.00 |
| Lysine | 9.15 | 12.37 | 1.35 |
| Glutamic acid | 0.81 | 1.29 | 1.60 |

[a] Mobile phase: 2-propanol–hexane (30:70, v/v).

C=C bond according to $^1$H NMR and $^{13}$C NMR spectroscopy. Further, the propionic acid amide derivative was obtained as a result of hydrogenation. The chlorosilylamino acid derivative is sensitive towards moisture and light; its addition to silica should occur spontaneously.

The chromatography of $N^{\alpha}$-3,5-dinitrobenzoylamino acid 2-propyl esters on the new CSP yielded excellent separation factors (Table I). In each instance investigated the L-enantiomer showed a stronger retention than the D-enantiomer. Racemic proline derivatives were not resolved.

TABLE II

ENANTIOMER SEPARATION OF $N^{\alpha}$-3,5-DINITROBENZOYLAMINO ACID N-PROPYLAMIDES ON THE CSP

Mobile phase: 2-propanol–hexane (10:90, v/v).

| *Amino acid* | $k'_D$ | $k'_L$ | $\alpha$ |
|---|---|---|---|
| Alanine | 2.29 | 12.18 | 5.32 |
| Valine | 0.56 | 5.76 | 6.01 |
| Leucine | 0.88 | 5.80 | 6.58 |
| Isoleucine | 0.84 | 5.35 | 6.35 |
| Phenylalanine | 1.95 | 13.22 | 6.77 |
| Phenylglycine | 1.84 | 6.59 | 3.59 |
| Tyrosine | 6.70 | Too strong interaction | |
| Serine | 6.67 | 18.95 | 2.84 |
| Threonine | 3.25 | 10.95 | 3.37 |
| Proline | 3.08 | 2.71 | 0.88 |
| Lysine | 13.70 | 46.66 | 3.40 |
| Tryptophan | 4.53 | 34.46 | 7.60 |
| Glutamic acid | 3.53 | 6.53 | 1.85 |

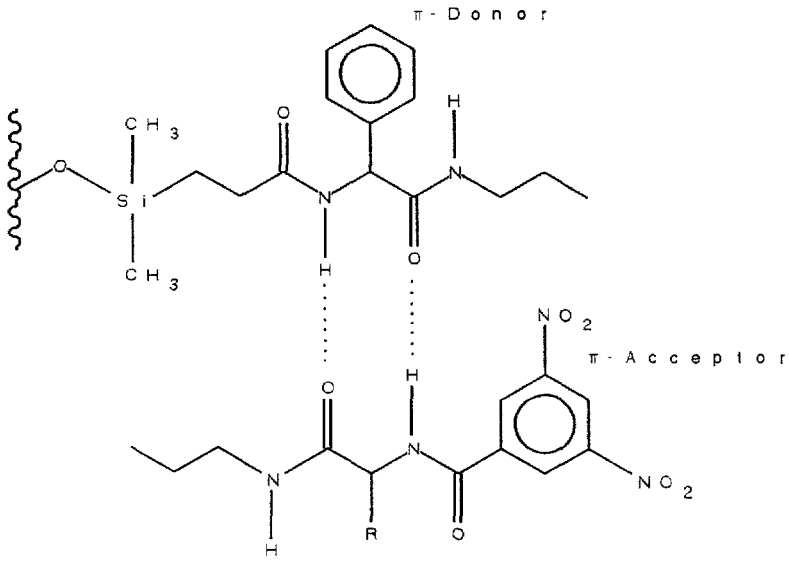


Fig. 3. Proposed adsorption complex of the CSP with N$^{\alpha}$-3,5-dinitrobenzoylamino acid propylamides.

Although the reaction of D-phenylglycinepropylamide with acryloyl chloride does not generate a long spacer, the subsequent hydrosilylation leads to an efficient CSP. Obviously no marked distance between the chiral moiety and the silica matrix is necessary to achieve high enantioselectivity.

To study the influence of the derivatization pattern of the C$^{\alpha}$-terminus of N$^{\alpha}$-3,5-dinitrobenzoylamino acids on enantiomer separation, the chromatographic behaviour of the corresponding 2-propyl esters and propylamides was investigated. As shown in Table II, a dramatic increase in the separation factor was observed when the amides were used instead of the esters.

As indicated in Fig. 3, our previously proposed recognition model[10] gives an explanation of the phenomenon. We postulate that in the chiral diamide donor–acceptor stationary phase investigated, the more strongly retained enantiomer interacts with the CSP at least via two hydrogen bonds and a $\pi$–$\pi$ donor–acceptor complex. The exchange of an ester group for an amide group at the C$^{\alpha}$-terminus of the amino acid derivative increases the Lewis basicity of the carbonyl oxygen atom. As a consequence, the corresponding hydrogen bond between the solute and the CSP is strengthened, especially for the L-enantiomer, which shows a stronger retention than the D-enantiomer.

Changing the derivatization pattern of the analyte from N$^{\alpha}$-3,5-dinitrobenzoylamino acid 2-propyl ester to N$^{\alpha}$-3,5-dinitrobenzoylamino acid propylamide causes such a strong increase in the $k'_L$ value (Tables I and II) that it becomes necessary to increase the concentration of 2-propanol in the mobile phase to 30% (Table III).

Derivatization of N$^{\alpha}$-3,5-dinitrobenzoylamino acids to secondary amides leads to a further increase in the $k'_L$ value, as shown in Table IV. In secondary amides the electron density of the carbonyl carbon atom is higher than in primary amides. The steric hindrance in C$^{\alpha}$-diethylamides, however, results in lower $k'_L$ values (Table V)

TABLE III

ENANTIOMER SEPARATION OF $N^{\alpha}$-3,5-DINITROBENZOYLAMINO ACID N-PROPYLAMIDES ON THE CSP

Mobile phase: 2-propanol–hexane (30:70, v/v).

| *Amino acid* | $k'_D$ | $k'_L$ | $\alpha$ |
|---|---|---|---|
| Alanine | 0.51 | 2.84 | 5.60 |
| Valine | 0.27 | 1.71 | 6.30 |
| Leucine | 0.25 | 1.69 | 6.76 |
| Isoleucine | 0.22 | 1.61 | 7.30 |
| Phenylalanine | 0.54 | 3.54 | 6.60 |
| Phenylglycine | 0.47 | 1.56 | 3.34 |
| Tyrosine | 0.88 | 6.77 | 7.70 |
| Serine | 1.17 | 3.04 | 2.61 |
| Threonine | 0.70 | 2.22 | 3.16 |
| Proline | 0.53 | 0.53 | 1.00 |
| Lysine | 1.50 | 4.49 | 2.99 |
| Tryptophan | 0.63 | 4.56 | 7.27 |
| Glutamic acid | 0.51 | 0.95 | 1.85 |

TABLE IV

ENANTIOMER SEPARATION OF $N^{\alpha}$-3,5-DINITROBENZOYLAMINO ACID DIMETHYLAMIDES ON THE CSP

Mobile phase: 2-propanol–hexane (30:70, v/v).

| *Amino acid* | $k'_D$ | $k'_L$ | $\alpha$ |
|---|---|---|---|
| Alanine | 0.91 | 4.13 | 4.52 |
| Valine | 0.34 | 2.36 | 6.91 |
| Leucine | 0.29 | 2.41 | 8.36 |
| Isoleucine | 0.27 | 2.24 | 8.19 |

than for the corresponding dimethylamides (Table IV). The increase in the $k'_L$ values for the $C^{\alpha}$-amides of dimethylamine emphasizes that the Lewis basicity of the corresponding carbonyl $C^{\alpha}$ atom is important for the strength of the diastereomeric complexes between the enantiomers of an analyte and a chiral selector, whereas the $C^{\alpha}$-amide hydrogen is not involved in the chiral recognition mechanism.

TABLE V

ENANTIOMER SEPARATION OF $N^{\alpha}$-3,5-DINITROBENZOYLAMINO ACID DIETHYLAMIDES ON THE CSP

Mobile phase: 2-propanol–hexane (30:70, v/v).

| *Amino acid* | $k'_D$ | $k'_L$ | $\alpha$ |
|---|---|---|---|
| Alanine | 0.44 | 1.85 | 4.21 |
| Valine | 0.18 | 1.15 | 6.34 |
| Isoleucine | 0.18 | 1.24 | 6.72 |

As shown in Table II, $N^{\alpha}$-3,5-dinitrobenzoyl-D- and -L-prolinepropylamide are resolved with the CSP, but not with a baseline separation. Interestingly, the L-enantiomer is eluted first. As proline bears a secondary amino group, there is a lack of an α-amide proton which might effect an alternative mechanism of enantioselective recognition.

Coupling an additional chiral centre at the C-terminus of the CSP and its influence on the chromatographic behaviour of amino acid derivatives and other enantiomers will be considered in Part II. Further evidence of the mechanism of enantiomeric recognition will be obtained by computer-aided energy calculations for the reversible diastereomeric adsorption complexes.

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