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Dynamic ligand-exchange chiral stationary phases derived from N-substituted (*S*)-phenylglycinol selectors

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Abstract

Six chiral stationary phases based on N-substituted (*S*-phenylglycinol were synthesised and dynamically loaded onto octadecyl-silica gel column. The effect of the alkyl (C_7, C_9, C_{12}) and aryl (methoxybenzyl, naphtylmethyl, anthrylmethyl) anchor moieties and influence of various chromatographic conditions (concentration of copper(II) ions, pH of the mobile phase, column temperature and organic modifier addition) on retention and enantioselectivity of the chiral columns toward selected α-amino acids were studied. The surface concentrations of chiral selectors, determined using the breakthrough method, were found to be in the range of 0.66–0.88 $\mu mol/m^2$. The order of elution of α-amino acid enantiomers was found to be $R<S$ on the N-C_{12}-(*S*)-phenylglycinol and all N-aryl-(*S*)-phenylglycinol phases but a reversed elution order ($S<R$) was observed on the N-C_7- and N-C_9-(*S*)-phenylglycinol phases.

Keywords: Chiral stationary phases, LC; Enantiomer separation; (*S*)-Phenylglycinol; Amino acids

1. Introduction

Chiral ligand-exchange chromatography (LEC) has been widely employed in resolving α-amino acid enantiomers since the pioneering work of Davankov and co-workers in the late 1980s [1,2]. The mechanism of this separation technique is based on the formation of tertiary complexes: analyte–divalent metal cation–chiral selector. The most popular chiral selectors, α-amino acids and their N-substituted derivatives, are usually applied as chiral mobile phase additives [3,4] or as chiral molecules of the stationary phases (CSPs) after binding covalently [5,6] or hydrophobically [7,8] to a solid column support. In the first case, the diastereomeric complexes are formed in the bulk of the mobile phase and then they are adsorbed on the achiral stationary phase. In the second case, enantioselective formation of tertiary complexes between the chiral stationary phase and the analyte plays the main role. In both cases the stereoselective recognition results from small energy differences in the chiral complex stability as is described by 'three-point' interaction rule [9]. The observed enantioselectivity of the chromatographic system depends on the CSPs surface coverage, the kind of the anchor molecule used to attach the chiral ligand to the support surface, and the concentration of a metal ion and other modifiers in the mobile phase [10–12].

In the present paper we report synthesis of six N-substituted-(*S*)-phenylglycinol chiral selectors and their application in HPLC separation of some α-amino acids enantiomers. The efficiency of the chiral columns and the influence of the concentration of copper(II) ions, pH of the mobile phase, column

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temperature and organic modifier addition on the chromatographic system are evaluated.

2. Experimental

2.1. Apparatus

Chromatographic analyses were performed using a Hewlett-Packard liquid chromatograph type HP 1090 equipped with autosampler, thermostated column compartment and diode-array detector. Polarimetric measurements were carried out using a Rudolph Research automatic polarimeter Autopol II. Elemental analysis were obtained using Perkin-Elmer elemental analyser PE 240.

2.2. Chemicals

Racemic and enantiomerically pure α-amino acids and octadecyl-silica gel LiChrosorb RP-18 (5 μm) were obtained from Merck (Darmstadt, Germany). Sodium cyanoborohydride, (*S*)-(+)-2-phenylglycinol, heptyl aldehyde, nonyl aldehyde, dodecyl aldehyde, 2-naphthaldehyde, 4-methoxybenzaldehyde and 9-anthraldehyde were purchased from Aldrich (Steinheim, Germany) and were used as supplied. All solvents used as the reaction media were of HPLC or analytical grade.

2.3. Synthesis of chiral stationary phases

Six chiral stationary phases (Fig. 1) derived from (*S*)-(+)-phenylglycinol were prepared by a one-step reductive N-alkylation of the aminoalcohol in a procedure analogous to that described for alkylation of amino acids [13].

The appropriate aldehyde (11 mmol) was added within 15 min at room temperature to a suspension of (*S*)-(+)-phenylglycinol (10 mmol) and sodium cyanoborohydride (7 mmol) in methanol (15 ml). The reaction mixture was stirred for 24 h at room temperature and then the solvent was removed under reduced pressure. The residue was purified with a radial thin-layer chromatography on silica-gel plates using hexane–isopropanol (8:1, v/v) as the eluent. The analytical data of the obtained products are given below.

N-(*n*-Heptyl)-(*S*)-phenylglycinol [C_7-(*S*)-Phg-ol]: $[\alpha]_D$ +68.6° (*c*=6.72; EtOH). $C_{15}H_{25}NO$ (235.36); requires: C, 76.55%; H, 10.70%; N, 5.95%; found: C, 77.24%; H, 10.99%; N, 5.86%.

^{1}H NMR (C^2HCl_3) δ=0.89 (t, CH_3, 3H), 1.25 (s, CH_2, 10H), 1.52 (m, CH_2, 2H), 3.69 (d, CH_2-(OH), 2H), 2.52 (q, CH_2-(NH), 2H), 3.33 (t, CH-, 1H), 7.32 (m, C_6H_5-, 5H), 1.5 (s, OH-), 3.7 (s, NH-).

FTIR (film) ν=3322 (OH, NH), 3062 (C_6H_5-C), 2926 (CH_2), 2855 (CH_2), 1377 (CH_3), 1026 (CO), 721 (C_6H_5); cm^{-1}.

N-(*n*-Nonyl)-(*S*)-phenylglycinol [C_9-(*S*)-Phg-ol]: $[\alpha]_D$ +37.18° (*c*=6.75; EtOH). $C_{17}H_{29}NO$ (263.42) requires: C, 77.51%; H, 11.10%; N, 5.31%; found: C, 77.24%; H, 10.99%; N, 5.86%.

^{1}H NMR (C^2HCl_3) δ=0.89 (t, CH_3, 3H), 1.25 (s, CH_2, 14H), 1.5 (m, CH_2, 2H), 3.71 (d, CH_2-(OH), 2H), 2.49 (q, CH_2-(NH), 2H), 3.57 (t, CH-, 1H), 7.32 (m, C_6H_5-, 5H), 1.5 (s, OH-), 2.8 (s, NH-).

FTIR (film) ν=3327 (OH, NH), 3062 (C_6H_5-C), 2924 (CH_2), 2853 (CH_2), 1377 (CH_3), 1026 (CO), 721 (C_6H_5); cm^{-1}.

N-(*n*-Dodecyl)-(*S*)-phenylglycinol [C_{12}-(*S*)-Phg-ol]: $[\alpha]_D$ +17.80° (*c*=8.0; EtOH). $C_{20}H_{35}NO$ (305.50) requires: C, 78.63%; H, 11557%; N, 4.58%; found: C, 78.55%; H, 11.52%; N, 4.66%.

^{1}H NMR (C^2HCl_3) δ=0.89 (t, CH_3, 3H), 1.25 (s, CH_2, 20H), 1.55 (m, CH_2, 2H), 3.75 (d, CH_2-(OH), 2H), 2.5 (q, CH_2-(NH), 2H), 3.64 (t, CH-, 1H), 7.29 (m, C_6H_5-, 5H), 2.0 (s, OH-), 3.7 (s, NH-).

FTIR (film) ν=3329 (OH, NH), 3062 (C_6H_5-C), 2923 (CH_2), 2852 (CH_2), 1377 (CH_3), 1026 (CO), 720 (C_6H_5); cm^{-1}.

N-(4-Methoxybenzyl)-(*S*)-phenylglycinol [MB-(*S*)-Phg-ol]: $[\alpha]_D$ +57.38° (*c*=6.0; EtOH). $C_{16}H_{19}NO_2$ (257.33) requires: C, 74.68%; H, 7.44%; N, 5.44%; found: C, 73.89%; H, 7.64%; N, 5.96%.

^{1}H NMR (C^2HCl_3) δ=3.71 (d, CH_2-(OH), 2H), 4.02 (t, CH_2-(NH), 2H), 2.79 (t, CH-, 1H), 7.35 (m, C_6H_5-, 5H), 8.34 (m, $C_{14}H_9$-, 1H), 7.53 (m, $C_{14}H_9$-, 4H), 8.00 (m, $C_{14}H_9$-, 4H).

FTIR (film) ν=3323 (OH, NH), 3028 (C_6H_5-C), 2923 (CH_2), 1611 (C_6H_5), 1310 (COC), 1026 (CO), 702 (C_6H_5); cm^{-1}.

N-(2-Naphthylmethyl)-(*S*)-phenylglycinol [NA-(*S*)-Phg-ol]: $[\alpha]_D$ +52.02° (*c*=4.44; EtOH).

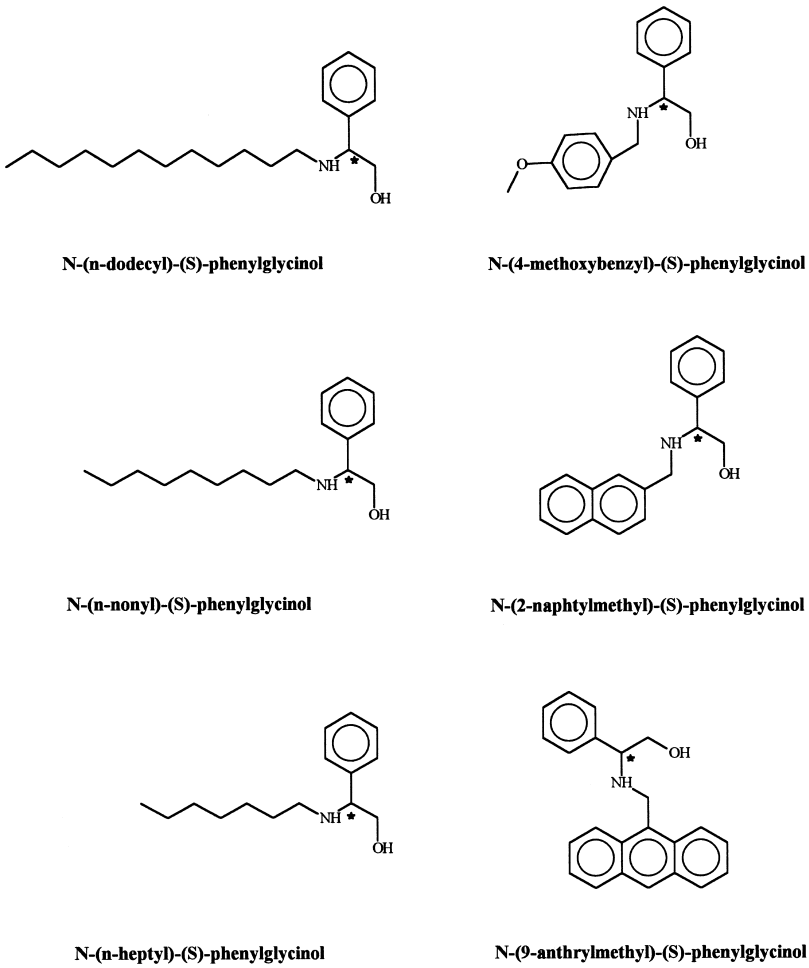

Fig. 1. Chemical structures of the N-substituted-(*S*)-phenylglycinol chiral selectors.

$C_{19}H_{19}NO$ (277.36) requires: C, 82.28%; H, 6.90%; N, 5.05%; found: C, 83.87%; H, 6.94%; N, 5.21%.

^1H NMR (C^2HCl_3) δ=3.72 (d, CH_2-(OH), 2H), 3.90 (t, CH_2-(NH), 2H), 3.65 (t, CH-, 1H), 7.38 (m, C_6H_5-, 5H), 7.81 (m, $C_{10}H_7$-, 7H), 2.32 (s, NH-).

FTIR (film) ν=3320 (OH, NH), 3056 (C_6H_5-C), 2923 (CH_2), 1600 (C_6H_5), 1026 (CO), 701 (C_6H_5); cm^{-1}.

N-(9-Anthrylmethyl)-(*S*)-phenylglycinol [AN-(*S*)-Phg-ol]: $[\alpha]_D$+66.71° (*c*=13.10; EtOH). $C_{23}H_{21}NO$ (327.42) requires: C, 84.37%; H, 6.46%; N, 4.28%; found: C, 83.97%; H, 7.04%; N, 4.11%.

^1H NMR (C^2HCl_3) δ=3.79 (s, CH_3-(alkoksy), 3H), 3.80 (d, CH_2-(OH), 2H), 3.7 (q, CH_2-(NH), 2H), 3.57 (dd, CH-, 1H), 7.39 (m, C_6H_5-, 5H), 7.03 (dd, C_6H_4-, 4H), 3.17 (s, NH-).

FTIR (film) ν=3321 (OH, NH), 3060 (C_6H_5-C), 2963 (CH_2), 1623 (C_6H_5), 1027 (CO), 702 (C_6H_5); cm^{-1}.

2.4. Column packing and column evaluation

Stainless steel columns (150×4.6 mm I.D.) were packed with octadecyl-silica gel (LiChrosorb RP-18, 5 μm) according to a slurry packing procedure [14]. The gel (1.5 g) was ultrasonically dispersed in acetone–isopropanol (1:1, v/v) and the column was

packed using methanol as pressuring agent (500 bar, 30 min). The column efficiency was determined with a test mixture containing uracil (void volume marker), acetophenone, benzene and toluene using methanol–water (85:15, v/v) as the mobile phase at flow-rate of 1.0 ml/min. Only the columns of efficiency at least 35000 plates/m were used for further investigations.

2.5. Dynamic coating of CSPs

The reversed-phase columns were dynamically coated with chiral selectors by passing through a solution of the appropriate CSP in ethanol at flow-rate of 0.5 ml/min. The concentration of coating solutions (C_m) varied depending upon the anticipated properties of the chiral selector (Table 1). The coating process was completed with distilled water used to displace ethanol within the column.

The surface concentration (C_S) of the chiral selector coated onto the RP-18 column was determined by the breakthrough method [15]. The breakthrough volume (V) was indicated by an abrupt rise in UV detector baseline. The surface concentration (C_S) of the chiral selector was calculated according to the equation:

$$C_S = \frac{V \cdot C_m}{A}$$

where A is the surface area of the octadecyl-silica stationary phase in the column.

2.6. Chromatographic conditions

Separations of amino acid enantiomers on the prepared chiral chromatographic columns were achieved using an aqueous solution of sodium acetate (10 mM) and copper acetate as the mobile phase at a flow-rate 1.0 ml/min. The acidity of the mobile phase was adjusted to pH 5.0–6.0 with acetic acid. The UV detector was operated at 235 nm (DAD in single wavelength mode).

3. Results and discussion

The chiral chromatographic columns used in the present study were prepared by loading N-substituted-(*S*)-phenylglycinols onto octadecyl-silica support. The surface concentration of the chiral selectors determined according to the breakthrough method was in the range 0.66–0.88 $\mu m/m^2$ (Table 1). The chiral selectors of such columns are kept in place by adsorption forces and the columns may 'bleed', thus their durability was checked in the first instance. The stability of retention power of the prepared columns was demonstrated by passing through a volume of mobile phase. The values of the capacity factors of Leu and Tyr enantiomers were essentially unchanged after passage of up to 3000 ml of the eluent (Fig. 2). The durability of the column was much less when mobile phase containing methanol was used and capacity factors of the analytes were substantially diminished after passage of about 1000 ml of the eluent.

The examined chromatographic columns displayed appreciable affinity toward amino acids under applied chromatographic conditions (Table 2) with good enantioselectivity, α, in the range 1.05–2.18. However, a striking effect of N-anchor type on the

Table 1
Details of coating solutions and surface coverage

Chiral selector	Concentration of the solution used for coating[a] (mM)	Surface coverage ($\mu m/m^2$)
C_7-(*S*)-Phg-ol	20.03	0.66
C_9-(*S*)-Phg-ol	12.83	0.72
C_{12}(*S*)-Phg-ol	17.45	0.88
MB-(*S*)-Phg-ol	14.09	0.62
NA-(*S*)-Phg-ol	10.63	0.54
AN-(*S*)-Phg-ol	16.59	0.52

[a] In ethanol

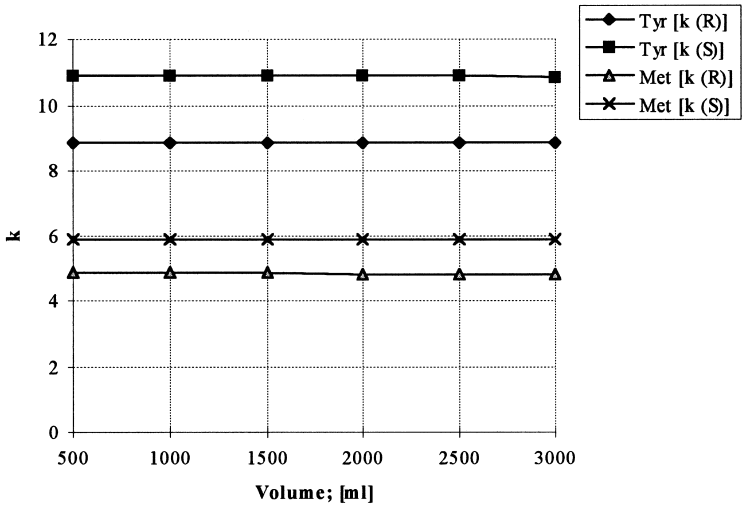

Fig. 2. Stability of a octadecyl-silica column coated with AN-(*S*)-Phg-ol, as indicated by the effect of the volume of the mobile phase on the retention factors of Tyr and Met enantiomers; eluent: [Cu(II)]=1.0 m*M*, pH 5.5; T=40°C; flow-rate=1 ml/min; detection, UV at 235 nm.

elution order was observed. (*S*)-Amino acids were eluted before (*R*)-enantiomers on N-aryl-and C_{12}-(*S*)-phenylglycinol CSPs but C_9-(*S*)-Phg-ol and C_7-(*S*)-Phg-ol bind (*R*)-enantiomers of the investigated amino acids more strongly than the (*S*)-enantiomers. A similar reversal in elution order was noted previously for amino acid separations on CSPs [16] and the possible reason for the observed phenomenon was discussed. Although we cannot at present rationalise our observation, it seems that the changeover in the enantiomer elution order is caused by different predominant conformations of specific chiral selectors forming diastereomeric tertiary complexes with selectand molecules.

In order to establish the thermodynamics of the stereodiscrimination process, the effect of temperature on the chromatographic parameters was investigated. The temperature dependence of capacity factor, k, and resolution, α, is described by the equations:

$$\ln k_i = -\frac{\Delta H_i^0}{RT} + \frac{\Delta S_i^0}{R} + \ln \phi$$

$$\ln \frac{k_i}{k_j} = \ln \alpha = -\frac{\Delta \Delta H_{ij}^0}{RT} + \frac{\Delta \Delta S_{ij}^0}{R}$$

where ΔH^0 and ΔS^0 are the enthalpy and the entropy changes associated with the analyte retention process, and ϕ is the phase ratio. The subscripts i and j

Table 2
Retention of amino acids and enantioselectivity of N-substituted (*S*)-Phg-ol coated onto octadecyl-silica column

Analyte	C_7-(*S*)-Phg-ol			C_9-(*S*)-Phg-ol			C_{12}-(*S*)-Phg-ol			MB-(*S*)-Phg-ol			NA-(*S*)-Phg-ol			AN-(*S*)-Phg-ol		
	k_S	k_R	α	k_S	k_R	α	k_R	k_S	α	k_R	k_S	α	k_R	k_S	α	k_R	k_S	α
Leu	6.88	8.61	1.25	5.96	7.85	1.32	6.24	7.50	1.20	5.59	6.51	1.16	8.30	9.00	1.08	8.91	10.87	1.22
Ile	7.49	8.69	1.16	4.90	7.16	1.46	6.40	8.88	1.39	6.35	7.3	1.15	7.22	7.95	1.10	7.87	8.84	1.12
Met	3.82	5.35	1.39	3.54	4.56	1.29	2.75	3.36	1.22	2.52	4.00	1.58	2.70	5.04	1.87	4.83	5.88	1.22
Val	2.89	3.62	1.25	1.74	2.38	1.36	2.18	2.77	1.27	1.32	2.90	2.18	2.38	4.07	1.71	2.64	4.10	1.55
Tyr	5.02	8.62	1.72	5.80	8.18	1.41	3.75	4.39	1.17	2.31	3.14	1.36	7.67	8.10	1.05	8.87	10.87	1.22

Conditions: eluent [Cu(II)]=1.0 m*M*, aqueous solution, pH 5.5; T=40°C; flow-rate=1 ml/min; detection, UV at 235 nm.

refer to the enantiomeric form of a generic racemic solute. Both equations predict linear inverse relationships between ln k or ln α and the temperature (Van't Hoff plots). The linear regression of ln $k = f(1/T)$ and ln $\alpha = f(1/T)$ over temperature range of 25 to 70°C gave thermodynamic parameters with high correlation coefficients ($R^2 > 0.99$). The results are shown in Table 3.

The distinct trend in $\Delta\Delta H^0$ and $\Delta\Delta S^0$ values was observed as the N-anchor molecule in CSPs was changed. Both thermodynamic parameters diminish in absolute values with elongation of N-alkyl substituent causing a reversal of sign, and therefore of elution order of the amino acid enantiomers, on transition from C_9-(*S*)-Phg-ol to C_{12}-(*S*)-Phg-ol chiral stationary phase. For N-aryl CSPs no such a trend has been observed, although both $\Delta\Delta H^0$ and $\Delta\Delta S^0$ depend on the nature of the aryl substituent. The thermodynamic parameters associated with chiral recognition are comparable for MB-(*S*)-Phg-ol and NA-(*S*)-Phg-ol. Values of $\Delta\Delta H^0$ for AN-(*S*)-Phg-ol are little greater than for the rest of the N-aryl CSPs. Moreover, due to faster mass transfer the best resolution factors, R_S, of the amino acid enantiomers were obtained on this chiral stationary phase (compare Fig. 3).

The retention of an amino acid on the column bearing aminoalcohol moiety is due to formation of labile tertiary complexes involving the analyte molecule, copper(II) cation and the chiral selector. Therefore, it depends on concentration of Cu (II) ion, pH of the mobile phase and addition of organic mobile phase modifier.

An increase in copper(II) acetate concentration from 0.5 to 1.5 m*M* decreased the capacity factors of the separated amino acids. Apparently even small concentration of the Cu(II) ion in the mobile phase saturates the complexing centres of the chiral selector and the surplus of copper cation promotes countercurrent formation of amino acid copper complexes in the mobile phase.

The change of pH of the mobile phase had an even more pronounced effect on the capacity factors of the analytes although the separation factor, α, of the amino acid enantiomers stayed almost the same. It seems that variation in pH of the mobile phase influences formation of both diastereomeric complexes of an analyte to a similar extent. Simultaneously, the resolution factors, R_S, of the amino acid enantiomers increased with pH to reach a plateau at about pH 5.5 due to changes of the peak width.

Addition of an organic modifier (methanol) to the mobile phase accelerated the elution of amino acids. The peak symmetry was also improved but the chiral resolution of the amino acid enantiomers was gradu-

Table 3
Thermodynamic data

Column	Solute	$\Delta\Delta H^0$ (kJ/mol)[a]	$\Delta\Delta S^0$ (J/mol K)[a]	$-\Delta\Delta G^0$ (25°C, kJ/mol)	ΔH_R^0 (kJ/mol)	ΔH_s^0 (kJ/mol)
C_7-(*S*)-Phg-ol	Leu	−3.0±0.8	−6.3±0.5	1.7±0.2	9.2±0.3	12.2±0.9
	Tyr	−5.5±0.8	−13.1±0.5	3.7±0.2	15.9±0.9	21.4±0.9
C_9-(*S*)-Phg-ol	Leu	−1.8±0.9	−3.0±0.5	0.9±0.3	10.2±0.3	11.9±0.6
	Tyr	−4.2±0.8	−10.5±0.4	2.9±0.1	16.1±0.8	20.3±0.4
C_{12}-(*S*)-Phg-ol	Leu	1.5±0.4	3.2±0.3	0.9±0.1	8.2±0.3	9.7±0.6
	Tyr	2.5±0.6	6.9±0.9	1.9±0.1	18.6±0.1	21.2±0.1
MB-(*S*)-Phg-ol	Leu	1.5±0.1	3.4±0.4	1.0±0.1	10.0±0.3	11.5±0.4
	Tyr	2.7±0.6	13.2±0.1	3.7±0.4	17.5±0.7	20.2±0.6
NA-(*S*)-Phg-ol	Leu	1.2±0.2	3.3±0.5	0.9±0.1	10.9±0.1	12.2±0.1
	Tyr	1.2±0.1	3.4±0.1	0.9±0.1	17.8±0.5	19.1±0.5
AN-(*S*)-Phg-ol	Leu	2.2±0.2	5.2±0.6	1.4±0.1	10.3±0.1	12.5±0.2
	Tyr	2.9±0.4	7.7±0.2	2.1±0.1	18.9±0.5	21.9±0.5

[a] $-\Delta\Delta H^0$ and $-\Delta\Delta S^0$ are defined as differences of thermodynamic parameters between (*R*)- and (*S*)-enantiomers of the analytes (in this order).

Conditions: eluent [Cu(II)] = 1.0 m*M*, aqueous solution, pH 5.5; flow-rate = 1 ml/min; detection, UV at 235 nm.

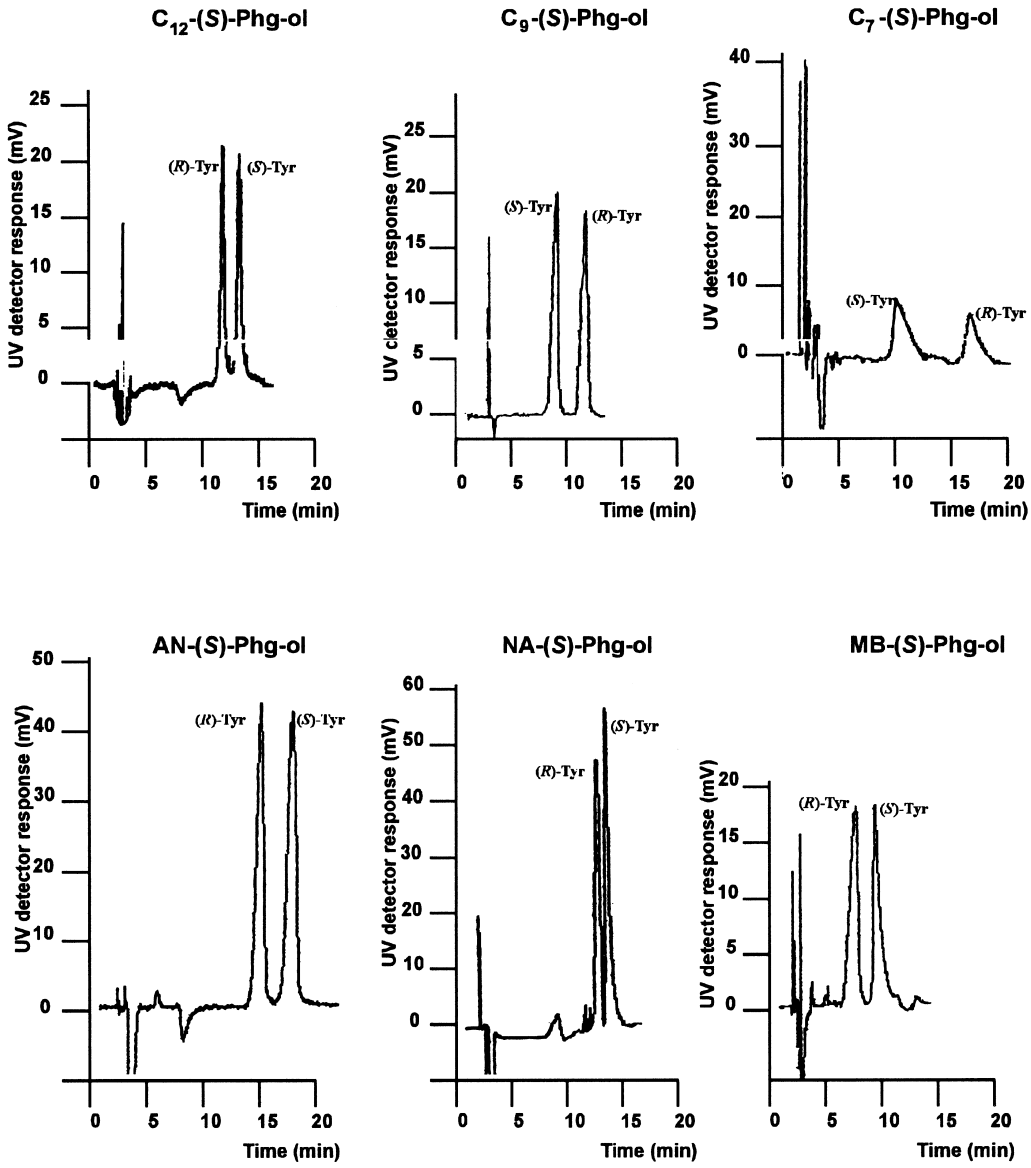

Fig. 3. Chromatograms of Tyr enantiomers separated on an octadecyl-silica column coated with N-substituted-(*S*)-phenylglycinol. Chromatographic conditions: [Cu(II)]=1.0 m*M*, pH 5.5; $T=40°C$; flow-rate=1 ml/min; detection, UV at 235 nm.

ally lost as the capacity factors were dramatically decreased.

4. Conclusions

In this paper we have described a simple method for the preparation of a ligand-exchange chiral stationary phase for the direct resolution of amino acids enantiomers. Using a series of alkyl and aryl substituted (*S*)-phenylglycinoles as a chiral selector, we have studied the effect of N-substituents and influence of various factors (concentration of copper(II) ions, pH of the mobile phase, column tem-

perature and organic modifier addition) on retention and enantioselectivity in chiral chromatography.

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