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Direct Resolution of Optically Active Isomers on Chiral Packings Containing Ergoline Skeletons. 5. Enantioseparation of Amino Acid Derivatives

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A new procedure for ergot alkaloid-based chiral stationary phase preparation is described. Synthesis is based on bonding the allyl derivative of terguride to mercaptopropylsilanized silica gel. The packing exhibits higher content of chiral selector, stability, reproducibility, and enantioselectivity toward amino acids compared to that previously studied. The chromatographic behavior of amino acids with different side chains and substituent groups is investigated in order to obtain a deeper insight into the enantiodiscriminative mechanism as well as to determine the limitations and strengths of terguride as a chiral selector for this class of compounds. A variety of factors, including mobile phase parameters such as pH, ionic strength, content and nature of organic modifier, and temperature, are examined.

Enantioseparation of amino acids (AAs) is of great interest in many fields of study concerning the life sciences, including biomedical research, food production, geochronological and archaeological dating procedures, etc. While the literature abounds with techniques for the resolution of free and derivatized AAs, new procedures are welcome whenever advantages in terms of selectivity, cost of the analysis, and reproducibility are offered with respect to current methodology. Accordingly, in previous work we dealt with the use of a semisynthetic ergot alkaloid derivative, 1-(3-aminopropyl)-(5*R*,8*S*,10*R*)-terguride, which, bonded to silica gel, showed good enantioselectivity for a number of carboxylic group-containing racemates, including dansylamino acid derivatives.[1] Proton NMR spectrometry of the chiral agent and an arylcarboxylic acid (naproxen) in solution allowed us to identify two types of interactions, $\pi-\pi$ and electrostatic, that are thought to lead to the enantiodiscriminative process; we were also able to exclude any contribution of the aminopropyl chain to the formation of diastereoisomeric adducts.[2]

These findings and the fact that the synthesis of aminopropylterguride is a relatively complicated procedure led us to develop a new method for the preparation of terguride-based chiral stationary phases (CSPs) and to extend the study to a series of AAs having different side chains and derivatizing groups. This study should also provide a deeper insight into the resolution mechanism, since an additional, steric-type interaction is hypothesized to determine the stability of the two diastereoisomeric complexes. The limitations and strengths of terguride as a chiral selector for this class of compounds are considered. The preparation of a stationary phase based on 1-allyl-(5*R*,8*S*,10*R*)-terguride as chiral selector is described. On this sorbent, the chromatographic behavior of a number of AAs with aliphatic (valine), aromatic (tryptophan), acidic (aspartic and glutamic acids), and polar (serine and threonine) side chains as their dansyl, *N*-(2,4-dinitrophenyl), *N*-(3,5-dinitrobenzoyl), *N*-benzoyl, and *β*-naphthoyl derivatives is examined.

EXPERIMENTAL SECTION

Apparatus. The liquid chromatographic system consisted of a Series 400 (Perkin Elmer, Norwalk, CT) solvent delivery pump equipped with a Model 7125 (Rheodyne) injection valve and connected to a Model 2550 (Varian, Walnut Creek, CA) UV detector. The chromatograms were monitored by a Chromatopac CR3A (Shimadzu, Kjoto, Japan) integrator.

Synthesis of the Chiral Packing. A solution of (5*R*,8*S*,10*R*)-terguride (4 g, 11.7 mM) in CH_2Cl_2 (160 mL) was mixed with a solution of 20% (v/v) tetraethylammonium hydroxide (8 mL) in 24 mL of 50% (w/v) NaOH, and allyl bromide (5 mL, 58.6 mM) was added dropwise with vigorous swirling. After the addition was completed, the swirling was continued for 5 min. The separated organic layer was washed with water (2 × 200 mL) and concentrated under reduced pressure. The residue was chromatographed on a silica gel column (40 g) with CH_2Cl_2 as eluent. The fractions containing allylterguride were evaporated, and the pure compound was crystallized from diethyl ether−petroleum ether (yield 2.5 g of 1-allyl-(5*R*,8*S*,10*R*)-terguride).

The structure was verified by EI-MS and ^{1}H and ^{13}C NMR spectroscopy (data are summarized in Table 1).

EI mass spectra were measured on a Finnigan MAT 90 (double-focusing, BE geometry) instrument under the following conditions: ionization energy 70 eV; source temperature, 250 °C; emission current, 1 mA; accelerating voltage, 5 kV; direct inlet, DIP; temperature, 170 °C.

EI-MS data for 1-allyl-(5*R*,8*S*,10*R*)-terguride [*m*/*z* (relative intensity)]: 381 (10), 380 (39), 308 (6), 307 (15), 265 (6), 264 (24), 263 (36), 249 (8), 221 (5), 220 (5), 209 (7), 208 (21), 207 (100), 195 (12), 194 (18), 100 (4), 74 (3).

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(1) Sinibaldi, M.; Flieger, M.; Cvak, L.; Messina, A.; Pichini, A. *J. Chromatogr. A* **1994**, *666*, 471.
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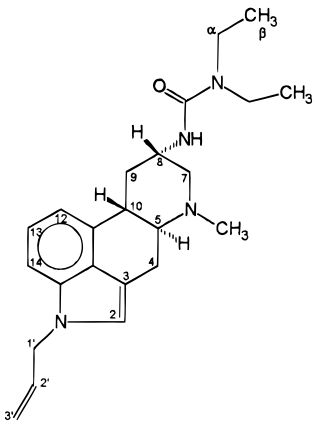
Table 1. NMR Data for 1-Allyl-(5*R*,8*S*,10*R*)-terguride (400 and 100 MHz, $CDCl_3$, 25 °C)



	^{13}C NMR		^{1}H NMR			
atom	δ	mult.	δ	n_H	mult.[a]	J (Hz)
2	121.73	d	6.774	1	d	1.7
3	110.84	s				
4	26.91	t	2.667	1	ddd	14.6, 11.1, 1.7
			3.379	1	dd	14.6, 4.3
5	67.61	d	2.205	1	ddd	11.1, 9.7, 4.3
7	61.89	t	2.485	1	dd	11.7, 2.6
			2.874	1	ddd	11.7, 2.6, 2.4
8	44.95	d	4.282	1	m	
9	32.55	t	1.636	1	ddd	13.2, 13.0, 3.3
			2.796	1	dddd	13.2, 4.3, 2.6, 2.6
10	36.52	d	3.052	1	m	
11	133.52	s				
12	112.83	d	6.888	1	ddd	6.9, 1.3, 0.9
13	122.73	d	7.158	1	dd	8.3, 6.9
14	107.12	d	7.100	1	ddd	8.3, 0.9, 0.8
15	133.68	s				
16	126.71	s				
N—CH_3	43.39	q	2.416	3	s	
N—C=O	156.57	s				
1′	48.91	t	4.674	2	ddd	5.5, 1.7, 1.5.
2′	133.87	d	5.990	1	ddt	17.0, 10.2, 5.5
3′	117.00	t	5.124	1	ddt	17.0, 1.3, 1.7
			5.188	1	ddt	10.2, 1.3, 1.5
α	41.06	t	3.250	1	dq	14.5, 7.1
			3.347	1	dq	14.5, 7.1
β	13.85	q	1.152	3	t	7.1
N—H			5.572	1	d	8.2

[a] Abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

Table 2. Mean Value of the Surface Coverage, Derived from Elemental Analysis and Determined for Four 1-Propyl-(5*R*,8*S*,10*R*)-terguride Silica Gel (CSPII) Materials, Which Were Prepared under the Same Reaction Conditions (See Experimental Section)[a]

CSP	mmol/g (CV, %)[b]	k'_D [c] (SD)	α[d]	N[e]
allyl-TER	0.051 (4.8)	17.7 (±0.65)	1.29	5090

[a] The chromatographic properties of the CSPII were obtained by a series of injections of Dns DL valine. [b] Coefficient of variation. [c] Chromatographic conditions: column size: 150 mm × 4.6 mm i.d.; eluent, 0.02 M potassium phosphate buffer (pH 3.6)/ACN (60:40 v/v); flow rate, 0.8 mL/min; detection, UV, 254 nm; room temperature. k'_D, capacity factor of the more retained enantiomer. [d] Selectivity factor (k'_D/k'_L). [e] Plate number, calculated for the more retained enantiomer and related to a column of 150 mm length.

Mercaptopropyltrimethoxysilane (5 mL) (Serva, Heidelberg, Germany) was coupled with 5 g of vacuum-dried Exsil-100 silica gel (particle size, 5 μm; average pore diameter, 100 Å) (Scientific Glass Engineering, Milton Keynes, UK) by swirling gently in 150 mL of anhydrous toluene at 100 °C for 12 h under dry nitrogen. After cooling, the modified silica was collected by filtration, washed exhaustively with toluene, *n*-hexane, 2-propanol, and diethyl ether, and dried under vacuum. Surface coverage, determined by elemental analysis (C, 2.60%; H, 0.50%; S, 1.69%), corresponded to 0.73 mmol/g.

To the slurry of derivatized silica gel (5 g) in chloroform (50 mL) were added 1-allyl-(5*R*,8*S*,10*R*)-terguride (500 mg, 0.102 mmol) and α,α′-azobis(isobutyronitrile) (22 mg). The mixture was refluxed with gentle stirring at 60 °C for 15 h. The resulting material was filtered out, washed successively with chloroform, methanol, acetone, 2-propanol, and diethyl ether, and dried under vacuum at 50 °C. Elemental analysis (N, 0.31; C, 3.88; H, 0.60; and S, 1.60) corresponded to 0.045 mmol/g.

The sorbent, slurred in methanol (3 g/20 mL), was packed in a stainless steel tube (150 mm × 4.6 mm i.d.), using methanol as the pressurizing solvent.

Material and Samples. All solvents were of HPLC grade and were obtained from Merck (Darmstadt, Germany). Other chemicals of analytical grade were purchased from Carlo Erba (Milan, Italy).

Phosphate buffers were prepared using phosphoric acid and sodium hydroxide, monitoring the pH by means of a Crison 2000 pH micrometer. Water was filtered through Millipore (Bedford, MA) type GS (0.22 μm) filter disks.

Free amino acids and their derivatives, where not otherwise indicated, were purchased from Sigma (St. Louis, MO).

The following DL- and L-amino acid derivatives were examined: dansyl (Dns) derivatives of valine (Val), serine (Ser), aspartic acid (Asp), tryptophan (Trp), methionine (Met), threonine (Thr), phenylalanine (Phen), leucine (Leu), proline (Pro), and alanine (Ala); *N*-(3,5-dinitrobenzoyl) (DNB) derivatives of glutamic acid (Glu), Val, Ala, and Trp; β-naphthoyl (NPT) derivatives of Ala, Val, Ser, and Trp; benzoyl (BNZ) derivatives of Val, Ala, and Trp; and *N*-(2,4-dinitrophenyl) (DNP) derivatives of Val, Trp, Glu, and Ser.

NPT and BNZ derivatives of Trp and Ala,[3] DNP derivatives of Val and Ser,[4] and DNB derivatives[5] were prepared according to the procedures reported in the literature. They were all of a purity sufficient for the purposes of the work.

Sample solutions were prepared by dissolving the AA derivatives in the eluent to obtain a concentration of ~1 mg/mL.

RESULTS AND DISCUSSION

Packing Properties. The new procedure for the preparation of ergot alkaloid-based chiral stationary phases starting from 1-allyl-(5*R*,8*S*,10*R*)-terguride (CSPII, allyl-TER) makes it possible, in comparison to the aminopropyl derivative (CSPI, AM-TER), to increase the surface concentration of the chiral selector by a factor of 1.875.[6] Data obtained from repeated syntheses of CSPII and the corresponding chromatographic properties are reported in Table 2. This resulted in an overall improvement in selectivity

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Table 3. Chromatographic Data of Dns-Amino Acids on CSPI and CSPII[a]

	AMP-TER		allyl-TER	
Dns-AA	k'_D	α	k'_D	α
threonine	9.19	1.21	10.9	1.49
serine	7.96[b]	1.17	11.6[b]	1.47
valine	8.06	1.12	17.7	1.29
leucine	10.1	1.06	20.3	1.27
methionine	11.6	1.07	16.9	1.12
phenylalanine	16.8	1.12	35.4	1.14
glutamic acid	25.5	1.06	51.8	1.28
aspartic acid	38.1	1.14	60.2	1.34
tryptophan	23.4	1.30	74.6	1.29

[a] Conditions: column size, 150 mm × 4.6 mm i.d. eluent, 0.02 M potassium phosphate buffer (pH 3.6)/AcCN (6:4 v/v); flow rate, 0.8 mL/min; detection, UV at 254 nm; room temperature. [b] CSPI, N = 10 544, Rs = 6.6; CSPII, N = 4050, Rs = 9.4.

for dansylamino acids, with the exception of tryptophan. The chromatographic data for a series of Dns-amino acid derivatives on CSPI and CSPII are summarized in Table 3. Comparing the results leads to some immediate conclusions. CSPI exhibits a notably higher efficiency: the plate number (N) is doubled relative to CSPII. This could be explained in terms of polymerization occurring on the surface of the allylterguride-derived support. Since radical reactions of monofunctional thiols with allyl compounds do not lead to the formation of polymers,[7] polymerization of the silica surface has to be ascribed to the mercaptosilanization of the sorbent, yielding a layer rich in terguride moieties. This layer is probably responsible for the decreased selectivity found for Dns-tryptophan. Because of the large size of the molecules, the tryptophan enantiomers could be partially inhibited to interact through stereoselective repulsions between the imidazole group of the amino acid and the urea side chain of terguride,[2] explaning the low resolution of Dns-tryptophan enantiomers on CSPII. However, the large improvement in the enantioselectivities observed with the other compounds more than compensates for the lower efficiencies of the packing, resulting in an overall increase in the resolution (Rs) values (Table 3). Furthermore, the packing exhibited a very high stability, even after 2000 injections. Comparative enantioseparation of Dns-DL-serine on the two sorbents is shown in Figure 1.

To study the enantioselectivity mechanism of the chiral packing, the retention and resolution were examined for a series of amino acid derivatives (Figure 2) as functions of the mobile phase parameters (viz., pH, content and nature of organic modifier, ionic strength of the buffer), the temperature, and the substituent groups.

Effect of pH. Two equilibria are influenced by the pH of the buffer: protonation of basic groups present in both the chiral selector and the analytes and dissociation of the carboxylic groups of the analytes.

The capacity factor (k') and the enantioselectivity (α) values as functions of the pH of phosphate buffer are reported in Figure 3, parts a and b, respectively. The plots show that k' and α values increase with increasing pH (until 4), corresponding to a higher dissociation of the carboxylic functions and, consequently, to a stronger stereoselective "ion pairing" effect exerted by the selector. In the pH range between 4 and 5, the enantioselectivity remains constant, although the retention rapidly decreases. At pH above 5, a decrease in the enantioselectivity was also observed, probably due to the lower protonation of the nitrogen groups present in the selector. Figure 3b shows that better resolution is achieved within the pH range 3.0−6.0, in agreement with the

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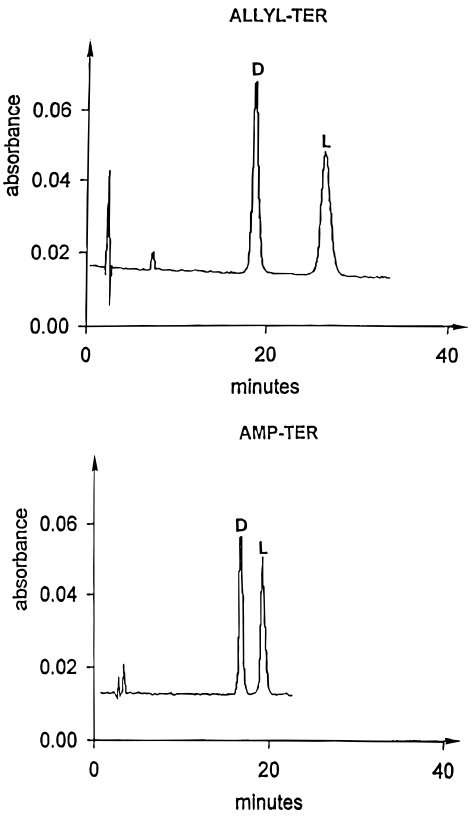

Figure 1. Chromatographic resolution of DL-dansylserine on aminopropyl- and allylterguride CSPs. Chromatographic conditions: column size, 150 mm × 4.6 mm i.d.; eluent, 0.02 M potassium phosphate buffer (pH 3.6)/acetonitrile (6:4 v/v); flow rate, 0.8 mL/min; detection, UV at 254 nm; room temperature.

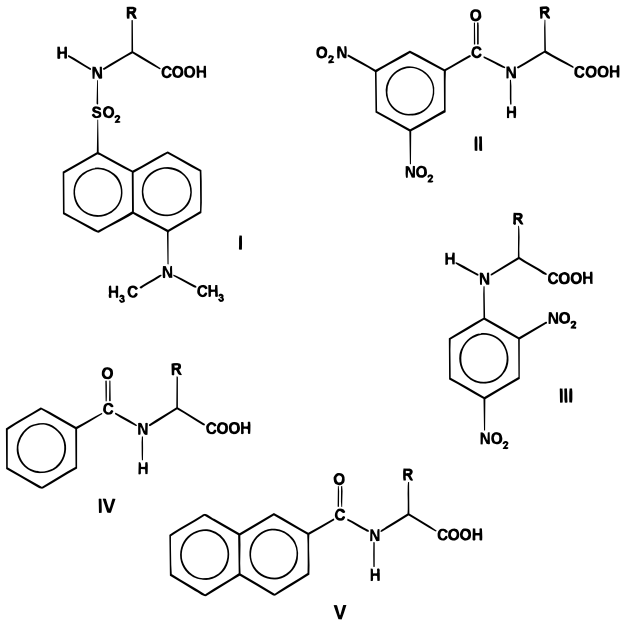

Figure 2. Molecular structures of the examined amino acid derivatives: **I**, dansyl; **II**, *N*-(3,5-dinitrobenzoyl); **III**, *N*-(2,4-dinitrophenyl); **IV**, *N*-benzoyl; and **V**, β-naphthoyl.

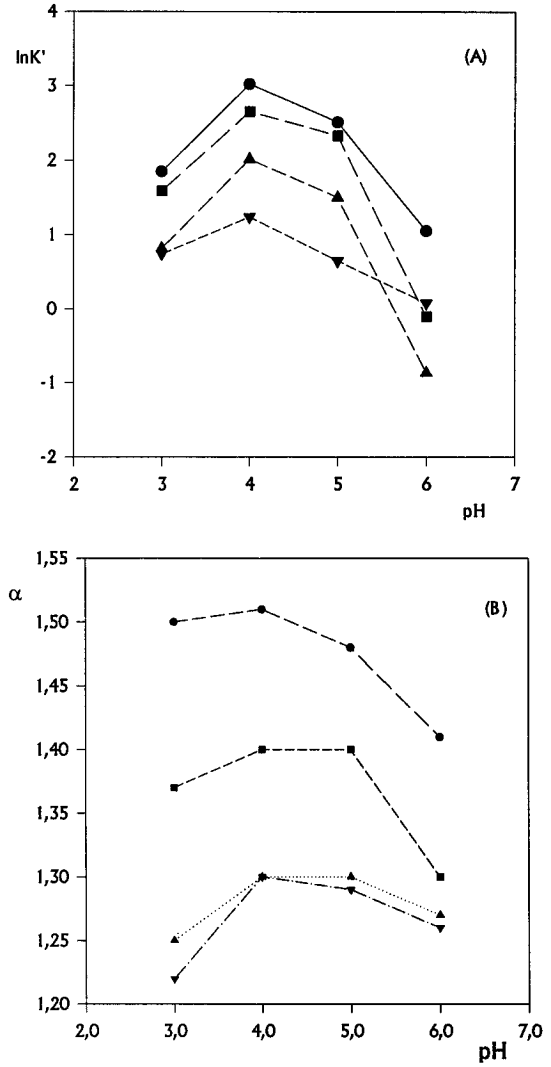

Figure 3. Influence of pH on (a) retention (ln k'_L) and (b) enantiomeric separation factors (α) of the following Dns-amino acid derivatives: valine (▼), serine (▲), aspartic acid (■), and tryptophan (●). Chromatographic conditions: column, 150 mm × 4.6 mm i.d., packed with silica gel based with allylterguride; eluent, acetonitrile/0.05 M potassium phosphate buffer (50:50 v/v); flow rate, 0.8 mL/min; detection, UV at 254 nm; room temperature.

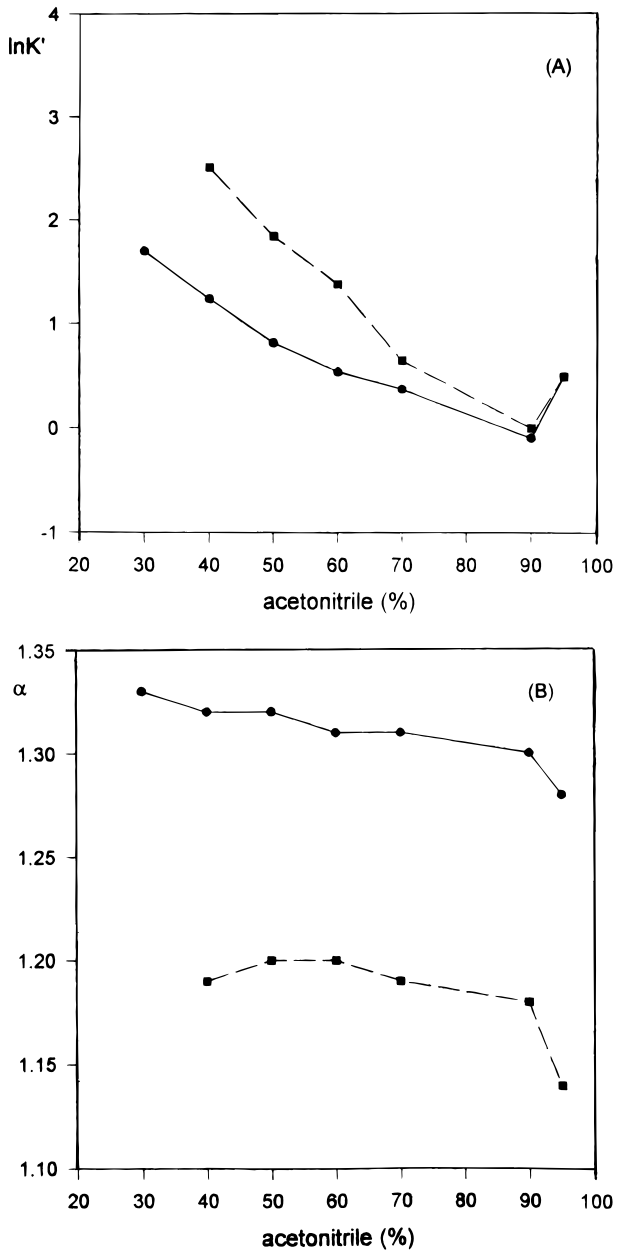

Figure 4. Influence of the organic modifier content (acetonitrile) on (a) retention (k') and (b) enantiomeric separation factors (α) of tryptophan (■) and serine (●) dansyl derivatives. Eluent, 0.05 M potassium phosphate at pH 3.0. Other chromatographic conditions as in Figure 3.

previously reported findings.[8]

Organic Modifier Effect. Terguride-based stationary phases act on a series of structurally similar compounds (Dns-AA) as a reversed-phase chromatographic material. This behavior is illustrated in Figure 4, which shows the retention sequence that parallels the hydrophobicity of the compounds.[9] Plots of k' and α values versus acetonitrile content show that, in the range 10−90% acetonitrile, retention decreases nonlinearly as the amount of organic modifier increases. This indicates that more than one interaction contributes to the retention mechanism. Indeed, the ergoline skeleton, having nonpolar moieties and positively charged nitrogen atoms, retains the samples by a combination of hydrophobic and ionic exchange modes. The anomalous retention trend observed at high acetonitrile contents (95%) may be ascribed to a predominance of the electrostatic attraction in this region, which is favored by decreasing of the aqueous polar solvation shell around the interacting groups.[10] It should be noted that high organic content in the mobile phase can also induce structural changes,[11] giving rise to rearrangements of the diastereoisomeric complexes. Accordingly, changes in retention and enantiodiscrimination have to be taken into account. Thus, both the electrostatic and the hydrophobic interactions determine a constant enantioselectivity in a wide range of acetonitrile levels.

The effect of the nature of the organic modifier has also been investigated. Solvents with different proton-acceptor capacity and eluent strength,[12] such as MeOH, THF, and ACN, have been considered. Table 4 summarizes the capacity and selectivity

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(11) Martin, D.; Weise, A.; Niclas, H. J. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 318.

Table 4. Capacity (k') and Enantioselectivity (α) Factors for a Series of Dansyl Derivatives as a Function of the Organic Modifier[a]

	phosphate buffer/ACN		phosphate buffer/MetOH[b]		phosphate buffer/THF[b]	
Dns-AA	k'_D	α	k'_D	α	k'_D	α
Dns-Val	2.45	1.18	8.06	1.20	0.66	1.0
Dns-Ser	3.00	1.32	8.67	1.34	0.97	1.0
Dns-Trp	7.64	1.20	36.2	2.31	1.37	1.0
Dns-Asp	7.09	1.44	16.5	1.30	1.24	1.17

[a] Chromatographic conditions: column, 150 mm × 4.6 mm i.d.; buffer, 0.05 M potassium acetate (pH 3.0); flow rate, 0.8 mL/min; detection, UV at 254 nm; room temperature. [b] Values obtained with a flow rate of 0.6 mL/min.

factors for a number of Dns derivatives as a function of the organic modifier. The eluent strength was THF < ACN < MeOH. A significant difference in the selectivities was observed between ACN and MeOH only in the case of Trp, while the use of THF did not allow any resolution for Dns-DL-Val, Dns-DL-Ser, and Dns-DL-Trp.

Ionic Strength of the Buffer. A study of the effect of ionic strength on the retention and selectivity in the range 0.01−0.05 M at constant pH and organic solvent percentage (Figure 5) shows that, at a certain buffer strength, there exists a minimal α value exhibited by the CSP. The data may be interpreted as a net effect of the buffer strength on the two main types on binding interactions. The rapid decrease of retention with buffer concentration up to 0.03 M should mainly reflect the competition of ion balancing by the mobile phase with the electrostatic attraction, while the slower decrease in k' should be ascribed to the increasing role of binding in terms of hydrophobic interaction, i.e., by a "salting out" effect.[13] Indeed, the plots demonstrate the role of this last interaction in stabilizing the diastereoisomeric complexes.

Temperature Effect. High column temperatures are commonly used in HPLC to improve the rate of mass transfer and to reduce the elution times. Since in the present system both equilibria controlling the retention are temperature dependent, the influence of this parameter was also investigated. The effect of temperature on the retention and selectivity for some Dns-amino acids with the same mobile phase composition was examined at 25, 35, 45, 55, and 65 °C (data not reported). As expected,[14] increasing temperature brought about an overall linear decrease in the retention and a reduction of the enantioselectivity, which could probably be ascribed to changes of the structural conformation of the chiral selector and to a greater degree of freedom of simultaneous bonding in the diastereomeric adsorbate.[15]

Solute Structural Effect. The structure of the Dns-amino acids strongly affects both retention and selectivity, the elution order paralleling the hydrophobicity of the side chain. Thus, aromatic amino acids (Phe, Trp) are more strongly retained than aliphatic ones (Val, Ala). A different behavior is evident for polar amino acid derivatives (Glu, Asp, Ser, Thr) that can interact with the ureo chain of the chiral selector by hydrogen bonding (Ser, Thr) or by Coulombic attraction (Glu, Asp). These additional interactions reflect the increased enantioselectivity and, at a low concentration of the buffer, an inversion of the elution sequence between Asp and Trp (see Figure 5). Two possible diastereoisomeric associations between terguride and the (I) D- (more retained) and (II) L-forms of Dns-derivatives are illustrated in Figure 6.

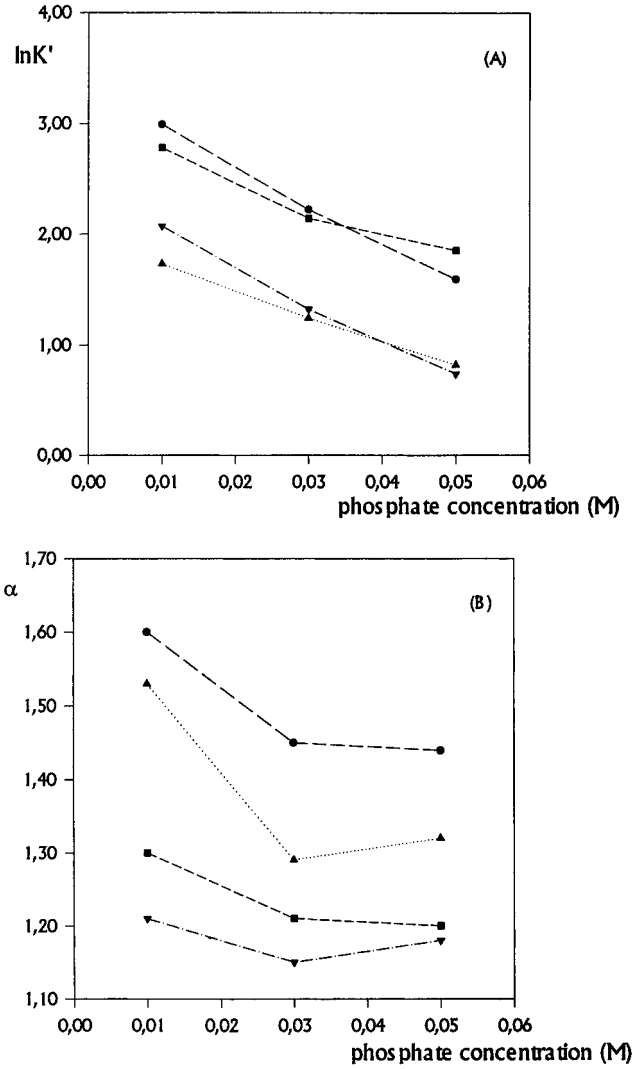

Figure 5. Influence of the buffer ionic strength on (a) retention (ln k') and (b) enantiomeric separation factors (α) of Dns derivatives of valine (▼), serine (▲), aspartic acid (■), and tryptophan (●). Eluent, acetonitrile/potassium phosphate buffer at pH 3.0 (50:50 v/v). Other conditions as in Figure 3.

Chiral resolution was obtained for all the examined derivatives, with the exception of proline, in which the rigid ring present in the molecule probably hinders the formation of differentiated "stacked" adducts with the selector. The enantiomeric separations of a mixture of four Dns derivatives by isocratic elution is shown in Figure 7. However, elutions with pH or organic solvent gradient could be used to improve the enantiomeric analysis of more complex mixtures. The present method may be of interest in the study of racemization reactions,[16] because it offers the ability of resolving aspartic acid ($\alpha = 1.34$) enantiomers in a column zone clear from other constituents of proteins.

To obtain additional information on the recognition mechanism, the influence of the derivatizing group on the retention and

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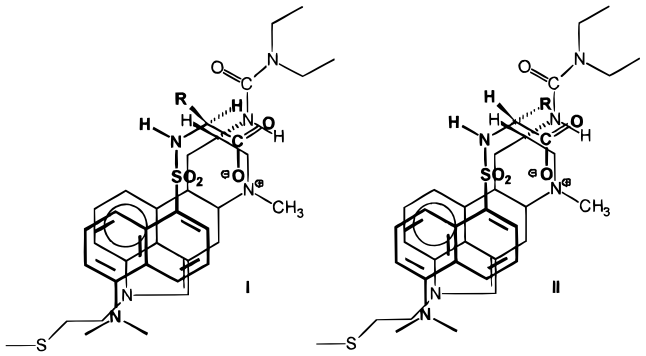

Figure 6. Possible diastereoisomeric adducts between terguride and the (**I**) D- and (**II**) L-forms of a Dns-amino acid derivative.

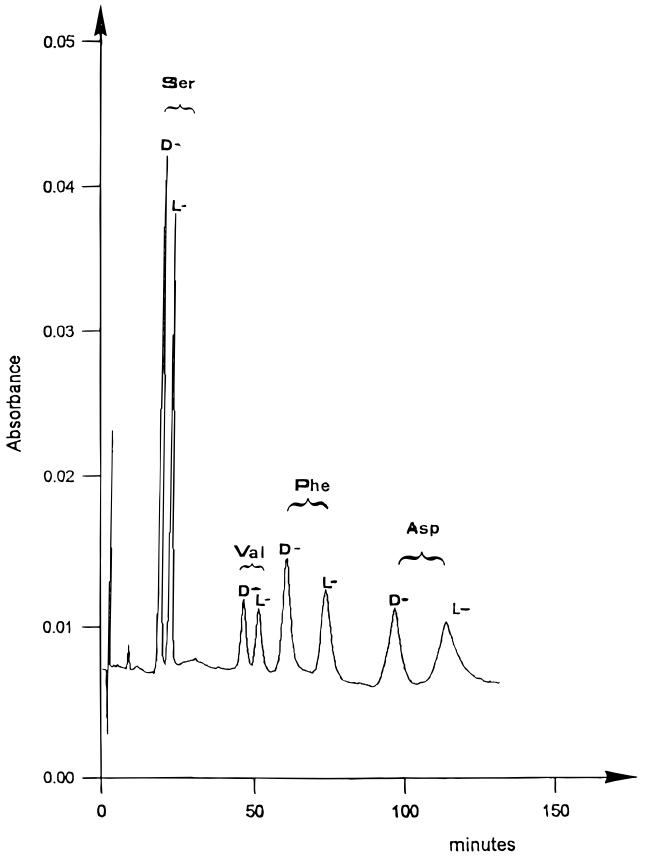

Figure 7. Enantiomer separation of a standard mixture of Dns-amino acid derivatives.

selectivity has also been investigated. Table 5 summarizes the selectivity (α) factors of five (Dns, DNB, DNP, BNZ, and NPT) derivatives of a series of amino acids. The retention order of the amino acids, determined on the basis of the derivatizing group, was

Table 5. Enantioselectivity Factors ($\alpha = k'_D/k'_L$) for a Series of Amino Acids as a Function of the Derivatizing Group[a]

	derivatizing group				
amino acid	Dns	DNB	DNP	benzoyl	naphthoyl
Val	1.25	1.24	1.30	1.00	1.00
Ser	1.35		1.10		1.00
Trp	1.22	1.38	1.94	1.35	1.44
Ala	1.26	1.26		1.00	1.00
Glu	1.30	1.20	1.15		

[a] Mobile phase: 0.05 M phosphate buffer (pH 3.0)/acetonitrile (1:1 v/v). Other conditions as in Table 3.

$$k'_{BNZ} < k'_{DNB} < k'_{NPT} < k'_{Dns} < k'_{DNP}$$

It appears that resolution has to be ascribed mainly to the ability of the compound to favor the formation of "stacking" adducts upon the ergoline skeleton of the selector, involving simultaneous Coloumbic and $\pi-\pi$ interactions. The aromatic moieties in the molecules of dansyl, *N*-benzoyl, *β*-naphthoyl, and *N*-(3,5-dinitrobenzoyl) derivatives possess a lower freedom of rotation around the bond axis than the *N*-(2,4-dinitrophenyl) one, the amide bond providing two fixed directions for associations with the selector. Consequently, the reciprocal position of the *π*-electron acceptor groups in the sample and the imidazole (*π*-electron donor) in the selector with respect to the chiral centers seems to be critical for the resolution. Thus, *N*-benzoyl and *β*-naphthoyl derivatives are not resolved, with the exception of tryptophan, which can provide $\pi-\pi$ interactions with its side chain. Indeed, resolution of all the dinitro derivatives confirms that the enantiodiscriminative process is in good agreement with the NMR assumptions.[2]

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