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Enantioselective chromatography on brush-type chiral stationary phases containing totally synthetic selectors Theoretical aspects and practical applications☆

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Abstract

A family of totally synthetic, brush-type chiral stationary phases (CSPs) for HPLC applications are available through a simple and flexible methodology based on the covalent attachment of chiral amines to oxirane-activated silica microparticles. Tailored CSPs for specific classes of compounds can be obtained by a proper choice of the optically active amine. Some typical features of the above phases are illustrated, with new results on the thermodynamics of CSP–solute interactions and on the analysis of stereolabile compounds.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Allyl *p*-nitrophenyl sulfoxide; Benzyl phenyl selenoxide

1. Introduction

Stereochemistry and, in particular, the preparation of enantiopure substances by a variety of methods, are the subject of a multitude of contemporary studies. In this context, the determination of enantiomeric compositions represents an important trend in modern research regarding the synthesis, characterization, transformation and use of chiral compounds.

The field of the separation of racemates can itself be divided in three major domains: (i) crystallization processes (including direct crystallization of racemates, classical separation of diastereomers and crystallization induced asymmetric transformations); (ii) kinetic resolutions (which can be performed either chemically or by means of enzymes); and (iii) chromatographic methods.

Separation science, especially enantioselective chromatography such as liquid (HPLC), gas (HRGC), supercritical fluid (SFC) and capillary electrophoresis (CE), has greatly affected stereochemical practice. High-resolution chromatographic systems based on chiral stationary phases (direct methods) are nowadays widely adopted in

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view of their simplicity, speed of analysis, reproducibility and sensitivity [1–14]; with the introduction of rationally designed chiral stationary phases [15–17] (CSPs) and dedicated chiroptical detection systems [18,19], it is now possible to detect, isolate and characterize a large number of chiral compounds, taking advantage of the inherent performances of HPLC, HRGC, SFC and CE.

Enantiomer separations can be achieved by means of either an intra- or an intermolecular approach: the intramolecular method is based on the preparation of diastereomers followed by separation on non-chiral sorbents, while the intermolecular method involves a chiral medium (i.e. a chiral stationary phase or a chiral mobile phase) and does not involve derivatizations with chiral reagents. In most chromatographic systems, a chiral environment is created by an optically active compound (usually referred to as a chiral selector) bonded to or dissolved in an inert support; this assembly forms the chiral stationary phase and its effectiveness depends on the enantiodiscrimination ability of the chiral selector and on the chromatographic properties of the achiral support. The large opportunities for variations in the nature of the chiral discriminating agents and therefore in the kind of interactions involved in the recognition process have permitted the enantiomer separations of several compounds with central, axial or planar chirality containing many types of functionality. Enantioselective chromatographic techniques have a great impact in various fields of research, e.g., the stereochemical analysis of natural compounds, asymmetric syntheses and bioconversions, precise determination of the stereochemical purity of building blocks and drugs, investigation of the reaction mechanism of conversions of chiral compounds, study of intramolecular rate processes (stereodynamics, enantioconformational and diastereoconformational analysis) and study of the thermodynamic parameters ($\Delta\Delta G$, $\Delta\Delta H$, $\Delta\Delta S$) of the stereoselection process concerning the transient diastereomeric complexes formation.

Whereas the first four issues are part of daily routines in quality control analysis, in organic synthesis, in the pharmaceutical chemistry, etc., the last two are still connected with specialized research areas, and in this context we shall attempt to highlight the potential of different brush-type CSPs, recently developed in our laboratories and containing synthetic selectors, in the fields of enantiomeric purity determination, thermodynamic characterization of the recognition process and investigation of the stereochemical stability of chiral compounds.

2. Experimental

2.1. Chemicals

Solvents used in the chromatographic experiments were of HPLC grade. (*S*)-Glycidol was obtained from Aldrich (Milwaukee, WI, USA); racemic analytes were available from earlier studies.

2.2. Preparation of chiral stationary phases

Syntheses of CSP1 [20] and CSP2, 3 and 4 [21] have been described; diastereomeric CSP2a and CSP2b were prepared starting from (*S*)-glycidoxypropyltrimethoxysilane followed a reported procedure [22].

2.3. Apparatus and chromatography

Chromatographic experiments were performed on a Waters (Milford, MA, USA) chromatograph equipped with a Model U6K injector, two Model M510 solvent-delivery systems and a Model M490 multi-wavelength detector (Waters). Thermodynamic data were obtained from variable-temperature chromatography using an HPLC oven (range 25–75°C for CSP1 and 25–95°C for CSP2, 3 and 4; ΔT ±0.5°C) containing the chiral column and a 1-m long connecting capillary to ensure thermal equilibration of the mobile phase; the same arrangement was used for the dynamic HPLC experiments.

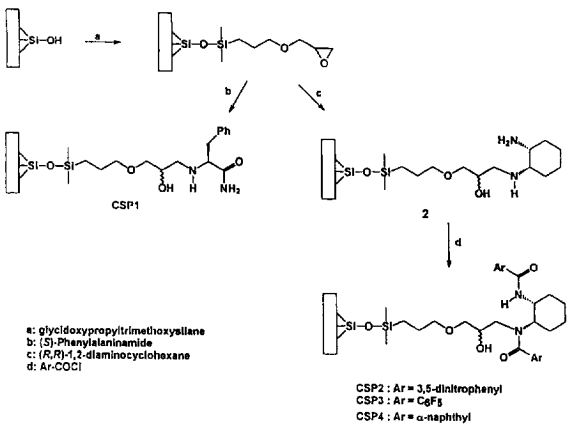


Fig. 1. Synthetic pathways to CSP1–4.

3. Results and discussion

In recent years we have developed a simple synthetic methodology for the synthesis of brush-type CSPs (Fig. 1): after activation of silica gel with glycidoxypropyltrimethoxysilane, ring opening by optically active amines results in the covalent attachment of the chiral selectors to the silica surface; further treatment of the intermediate **2** with aromatic acid chlorides [15–17] gives the final CSPs. Depending on the nature of the aromatic groups on CSP2–4, successful resolutions can be obtained for analytes having π-acid or π-basic groups close to the stereogenic center. As a general rule (Fig. 2), an aromatic moiety near a polar group (C=O, S=O, P=O, NHC=O, OH, etc.) containing or in the vicinity

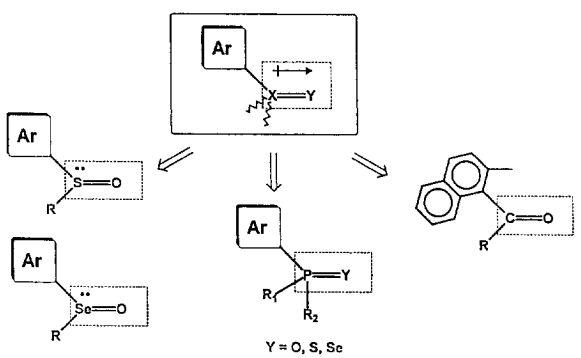


Fig. 2. General structures of chiral compounds resolved on CSP2–4.

of the stereogenic element is required for a successful separation. CSP1, on the other hand, has been employed in enantiomeric resolutions of Cu(II)-complexing species, such as dansyl- and dabsylamino acids [20]. When using the above-reported synthetic scheme, the final selector is actually present as a mixture of diastereomers, because the racemic, commercially available glycidoxypropyltrimethoxysilane is used in the first step; to establish the influence of the side-chain stereogenic center configuration on the recognition ability of our CSPs, we prepared two diastereomeric phases (Fig. 3) (CSP2a and CSP2b; Ar = 3,5-dinitrophenyl) having the same stereochemistry at the carbinol function and opposite stereochemistry at the functionalized diamine framework. Selected examples of racemates (Fig. 4) resolved on the two CSPs are gathered in Table 1 and Fig. 5; clearly, the two stereochemically pure CSPs afford similar results for a large number of racemic compounds, the heterochiral selector (*R,S,S* configuration) showing higher enantioselectivities in a few cases; moreover, the relative elution order on the two CSPs (determined by collecting a single peak on one column and reinjecting it onto the other column) was inverted for all the analytes investigated [23]. In addition to their practical consequences, these observations suggest that in most of the resolutions effected by CSP2, only the cyclic diamide core of the organic moiety is effectively involved in the enantiodifferentiation process.

Selectors available in both the enantiomeric forms offer a distinct advantage over proteins or carbohydrates. CSPs based on the utilization of synthetic selectors show several ideal characteristics of a CSP: thermal and chemical inertness with any mobile phase, broad applicability with high levels of enantioselectivity, efficiency, loadability, and predictability. Moreover, they especially allow the inversion of the elution order of two resolved enantiomers by switching from one chiral column to its enantiomeric version, under otherwise identical conditions; in addition, peak coalescence is observed on the racemic version of the CSP, i.e., the two enantiomers are co-eluted in a single peak with an averaged retention factor

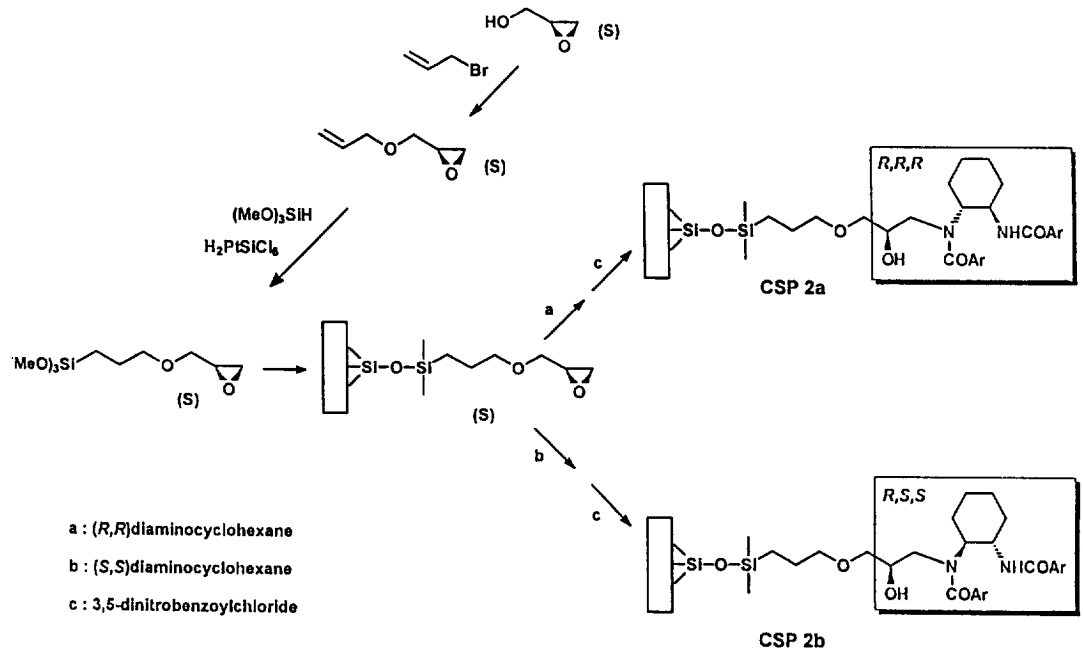


Fig. 3. Synthetic pathways to diastereomeric CSP2a and CSP2b.

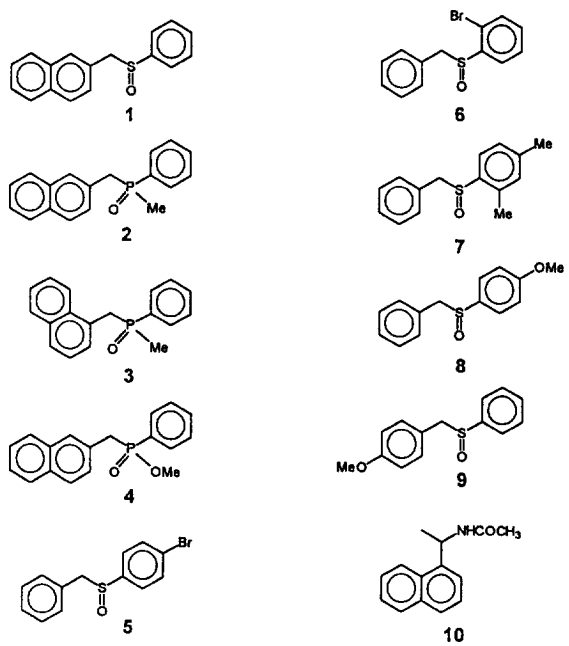


Fig. 4. Structures of racemic solutes resolved on CSP2a and CSP2b.

Table 1
Comparison between homo- and heterochiral CSP2a and CSP2b

Compound[a]	(*R*,*R*,*R*)-CSP		(*R*,*S*,*S*)-CSP	
	k'_1	α	k'_1	α
1	0.78	2.94	0.84	3.22
2	2.43	1.64	2.57	1.68
3	2.17	1.49	2.42	1.53
4	0.73	1.39	0.78	1.39
5	0.39	1.44	0.44	1.42
6	0.33	1.67	0.42	1.69
7	0.96	1.81	1.10	1.79
8	1.02	1.50	1.14	1.59
9	0.80	1.79	0.82	1.80
10	0.65	1.42	0.77	1.48

Column dimensions, 250 × 4 mm I.D.; eluent, 2-propanol–CH_2Cl_2 (3:97, v/v); temperature, 25°C; flow-rate, 1.5 ml/min; UV detection at 280 nm; void volume marker, 1,3,5-tri-*tert.*-butylbenzene.

[a] For compound identification, see Fig. 4.

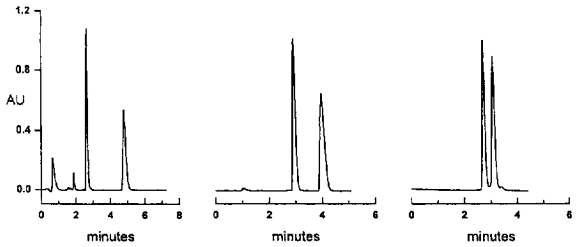


Fig. 5. Chromatographic resolutions of racemic S- and P-chiral compounds on CSP2b. From left to right, compounds **1**, **4** and **7** (see Fig. 4). Experimental conditions as in Table 1.

(Figs. 6 and 7). Verification of peak inversion or coalescence on passing from one CSP to its enantiomeric form or to the racemic version, respectively, represents a proof that the two observed peaks are indeed enantiomerically related; this is particularly useful if racemic or optically enriched reference samples are not available, or if the enantiomers of interest are present in complex matrices. In addition, enantiomeric trace analysis can be performed in a more accurate and precise way if the smaller peak can be eluted before the major peak (Fig. 8).

Under these conditions, the integration is easier and more precise and it is possible to identify and quantify [determination of enantiomeric excess (e.e.)] two enantiomers in a complex mixture without having either of the two enantiomers available and without chiroptical detection. A further application for the racemic version of a given CSP is represented by the tandem arrangement (chiral plus racemic columns connected in series), which can be exploited to increase the chemo- and/or diastereoselectivity of the chromatographic system. However, often a single CSP is sufficient to accomplish a complete, simultaneous chemical and optical fractionation of multi-component samples; in addition to the analytical applications, the broad selectivity of brush-type phases plays

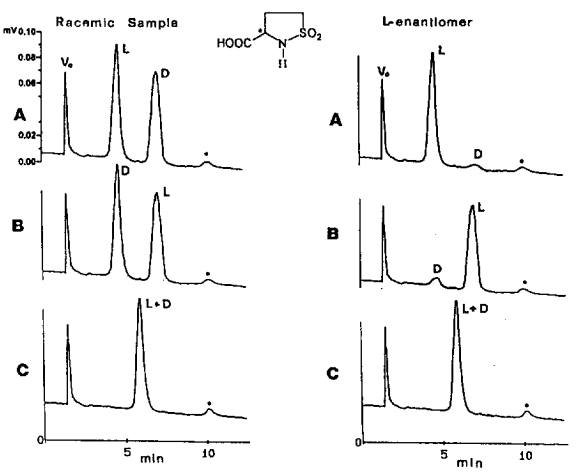


Fig. 6. Examples of enantiomeric and racemic columns switching for racemic and optically active samples [20]. (A) (*S*)-CSP1; (B) (*R*)-CSP1; (C) racemic version of CSP1.

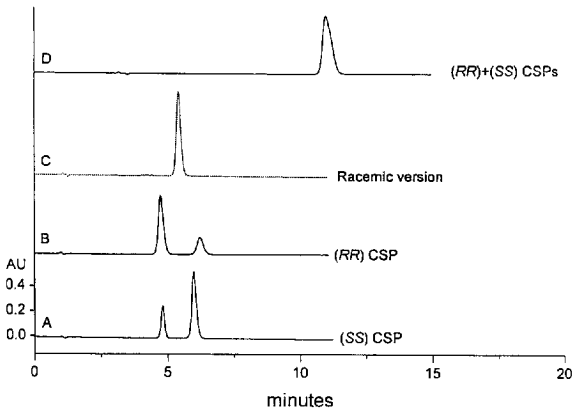


Fig. 7. Examples of racemic and enantiomeric columns switching for an optically enriched sample. Sample: ca. 70:30 (*R*/*S*)-propranolol as oxazolidin-2-one. Eluent, CH_2Cl_2–2-propanol (97:3, v/v); flow-rate, 1.5 ml/min. Columns: (a) (*S*,*S*)-CSP2; (b) (*R*,*R*)-CSP2; (c) racemic version of CSP2; (d) (*S*,*S*)-CSP2 and (*R*,*R*)-CSP2 columns coupled in series.

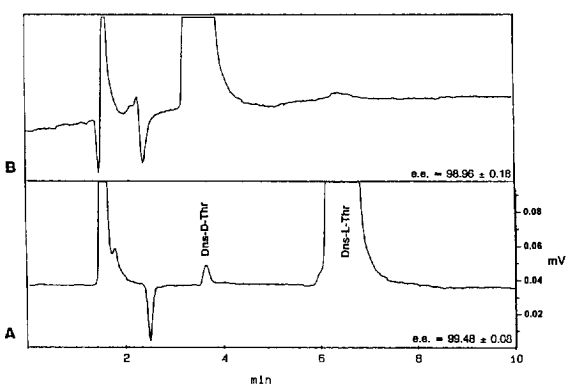


Fig. 8. Column switching in enantiomeric trace analysis [21]. (A) (*S*)-CSP1; (B) (*R*)-CSP1.

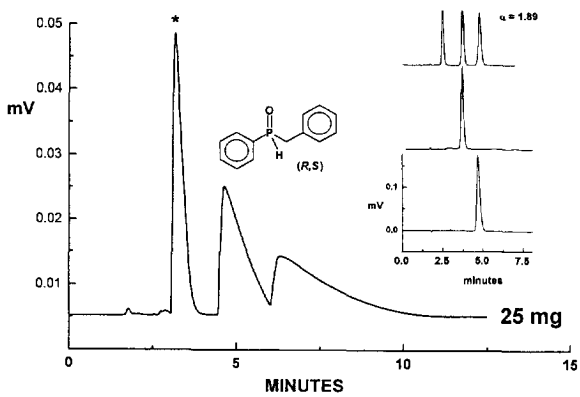


Fig. 9. Semi-preparative resolution on CSP2. Column, 250 × 10 mm I.D.; eluent, *n*-hexane–$CHCl_3$–2-propanol (50:40:10, v/v/v); flow-rate, 9.0 ml/min; temperature, 25°C; detection, refractive index. Asterisk denotes unknown impurity. First-collected fraction, 8 mg, e.e. > 99.9%; second-collected fraction, 8 mg, e.e. = 99.2%.

an important role in preparative chromatography: as shown in Fig. 9, the two enantiomers of a secondary phosphine oxide can be obtained in high chemical and optical purity in a single run, even in the presence of large amounts of impurity in the racemic mixture.

3.1. Thermodynamics of enantiomer separation on CSPs

The enantioselectivity of a given CSP stems from its ability to form diastereomeric adducts, which differ in their stability, with the analyte enantiomers. The retention factor (k') and α_{RS} value for the *R* and *S* enantiomers separated on a CSP are related to thermodynamic quantities by the following equations:

$$k'_{R(S)} = K_{R(S)}\phi \quad (1)$$

$$\ln k'_{R(S)} = \ln K_{R(S)} + \ln \phi = -\Delta G^{\circ}_{R(S)}/RT + \ln \phi$$
$$= -\Delta H^{\circ}_{R(S)}/RT + \Delta S^{\circ}_{R(S)}/R + \ln \phi \quad (2)$$

$$\ln \alpha_{RS} = -\Delta\Delta G^{\circ}_{RS}/RT \quad (3)$$

$$\ln \alpha_{RS} = -\Delta\Delta H^{\circ}_{RS}/RT + \Delta\Delta S^{\circ}_{RS}/R \quad (4)$$

where $K_{R(S)}$ is the adsorption equilibrium constant for the *R* (or *S*) enantiomer onto the CSP, $\Delta G^{\circ}_{R(S)}$, $\Delta H^{\circ}_{R(S)}$ and $\Delta S^{\circ}_{R(S)}$ are the corresponding standard free energy, enthalpy and entropy changes, respectively, and ϕ is the column phase ratio. The difference in the standard free energy of adsorption for an enantiomeric pair is related to α_{RS}, at a given temperature, by Eq. 3; corresponding enthalpic and entropic contributions are given by Eq. 4 [24–27].

From Eq. 3, it follows that enantioselective chromatography is a less demanding process (in terms of $\Delta\Delta G_{RS}$) than other chemical procedures for obtaining optically pure compounds [28], a $\Delta\Delta G^{\circ}_{RS}$ of 100 cal/mol usually being sufficient to accomplish a complete analytical separation.

A three-dimensional graph (Fig. 10) shows Eq. 3 for a temperature range extending from cryo-HPLC (−70°C) to the highest temperature allowed for GC chiral phases (ca. 250°C) and a limiting value of 3.0 kcal/mol for the enantioselectivity of the chromatographic system. The shaded area in Fig. 10 defines the enantioselectivity values observed on CSP1–4 and their accessible temperature range.

From van't Hoff analysis of enantioselectivity, the enthalpy and entropy contributions to $\Delta\Delta G^{\circ}$ are available: in the cases of a single separation mechanism operating over the explored temperature range, van't Hoff plots yield straight lines and enthalpies and entropies are determined from the slopes and intercepts, respectively.

For most enantiomeric separations carried out on our CSPs, $\Delta\Delta H^{\circ}_{RS}$ and $\Delta\Delta S^{\circ}_{RS}$ have the same

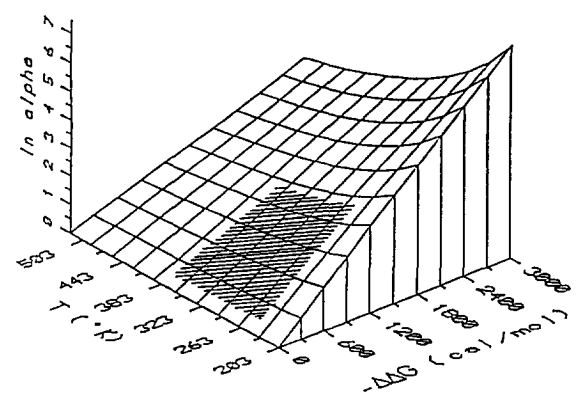


Fig. 10. Enantioselectivity ($\ln \alpha$) as a function of difference in the free energy of adsorption ($\Delta\Delta G$) and temperature.

Table 2
Thermodynamic data

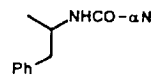
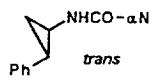
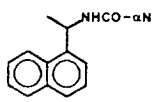
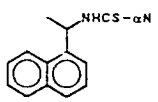
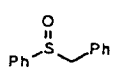
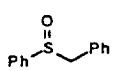
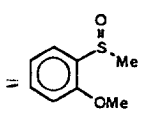
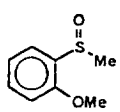
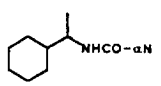
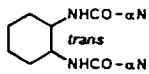
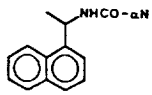
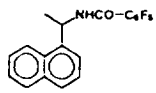
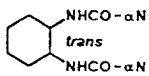
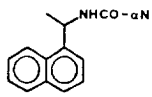
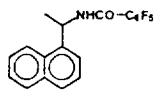
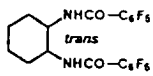
Entry	Compound[a]	CSP	Eluent	$\alpha_{25°C}$	$-\Delta\Delta H$ (cal/mol)	$-\Delta\Delta S$ (cal/mol · K)	Data from Ref.
1	Dns-Ser	1	0.25 m*M* $Cu(OAc)_2$ in CH_3CN–NH_4OAc buffer	5.69	1039	0.03	[20]
2	Dbs-Ser	1	As above	4.24	1139	0.95	[20]
3	Dns-Met	1	As above	2.31	649	0.51	[20]
4	Dbs-Met	1	As above	1.74	572	0.82	[20]
5	Dns-Thr	1	As above	3.79	804	0.05	[20]
6	Dbs-Thr	1	As above	2.62	1080	1.71	[20]
7		2	30% IPA[b] in hexane	1.24	407	0.94	[5]
8		2	As above	2.09	1282	2.86	[5]
9		2	40% IPA in hexane	4.38	2380	5.10	[5]
10		2	As above	1.39	556	1.24	[5]
11		2	20% IPA in hexane	1.58	725	1.55	This work
12		2	45% dioxane in hexane	1.86	1053	2.32	[21]
13		2	20% IPA in hexane	1.32	497	1.12	This work
14		2	45% dioxane in hexane	1.49	695	1.53	This work
15		3	10% IPA in hexane	1.21	284	0.57	This work

Table 2 (*Continued*)

Entry	Compound[a]	CSP	Eluent	$\alpha_{25°C}$	$-\Delta\Delta H$ (cal/mol)	$-\Delta\Delta S$ (cal/mol · K)	Data from Ref.
16		3	As above	4.25	1194	1.10	This work
17		3	As above	2.14	1154	2.37	This work
18		3	As above	1.52	932	2.28	This work
19		3	60% MeOH in H_2O	1.97	1039	2.11	This work
20		3	As above	1.48	594	1.22	This work
21		3	As above	1.19	396	0.97	This work
22		4	As above	1.33	427	0.87	[5]
23	Ala[c]	4	20% IPA in hexane	1.85	910	1.80	This work
24	Val[c]	4	As above	3.58	1933	3.90	This work
25	Val[c]	4	55% MeOH in H_2O	1.55	1045	2.70	This work
26	Leu[c]	4	20% IPA in hexane	3.54	1520	2.60	This work
27	Ile[c]	4	As above	4.44	1576	2.30	This work
28	Met[c]	4	As above	2.35	873	1.20	This work
29	PhGly[c]	4	As above	2.76	1299	2.70	This work
30	Phe[c]	4	As above	2.31	1777	3.90	This work
31	Bis-Val[d]	4	As above	8.30	4300	10.2	This work
32	Bis-Ile[d]	4	As above	10.09	4541	10.6	This work

[a] Dns = densyl; Dbs = dabsyl: αN = α-naphthyl; PhGly = phenylglycine.
[b] IPA = 2-propanol.
[c] As N-3,5-dinitrobenzoyl-*n*-hexyl–diamides.
[d] As N-3,5-dinitrobenzoyl-1,12-dodecan–(bisamides).

(negative) sign and $\Delta\Delta H^{\circ}_{RS}$ is larger than $T\,\Delta\Delta S^{\circ}_{RS}$ in the working temperature range (25–95°C) [20,21], hence ln α decreases linearly with $1/T$: this is the expected behaviour for a bimolecular association process with the second-eluted enantiomer forming a more tightly bound complex with the CSP ($\Delta\Delta H^{\circ} < 0$) whereas the first-eluted enantiomer forms a less structured complex and experiences less restriction of degrees of freedom ($\Delta\Delta S^{\circ} < 0$). Thermodynamic data for a large number of enantiomeric separations carried out with both organic and aqueous eluents are collected in Table 2.

3.2. Dynamic enantioselective HPLC

From the practical point of view, the increased α values observed at low temperatures simplify the study of stereolabile compounds that rapidly undergo reversible isomerizations during chromatography (Fig. 11); for isomerization processes having an energy barrier $\Delta G^{\neq} \approx 18$ kcal/mol (half-lives of the exchanging species are a few seconds at 25°C), the complete resolution of the various solute forms can be achieved if the characteristic time of the interconversion process is kept lower than the chromatographic residence time; sub-ambient temperature, high eluent flow-rates and reduced column lengths can be used for this purpose, the loss in efficiency usually being overcompensated by a substantial gain in selectivity.

Successful applications of low-temperature HPLC on CSP2 include the separation of the atropisomers of naphthyl ketones [32] and sulfoxides [33,34], featuring hindered rotation around the C_{Ar}–CO and C_{Ar}–SO bonds. Extension of the dynamic HPLC technique, in the form of variable-temperature chromatography, to stereochemically stable (at room temperature) compounds has recently been reported for a series of allyl aryl sulfoxides bearing different substituents on the aromatic ring [35]. When allylic sulfoxides are chromatographed on CSP2 under the usual conditions (25°C, flow-rate 2.0 ml/min), two baseline-resolved, symmetrical peaks are observed for the two enantiomers; increasing the column temperature and decreas-

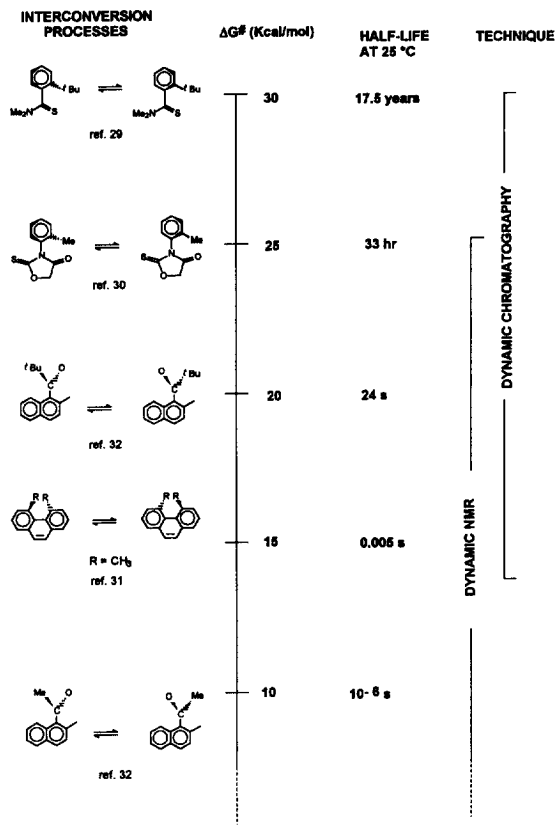


Fig. 11. Free energy of activation ($\Delta G^{\neq}$) and half-lives at 25°C of first-order enantiomerization processes. A comparison between dynamic NMR and dynamic chromatography is shown on the right.

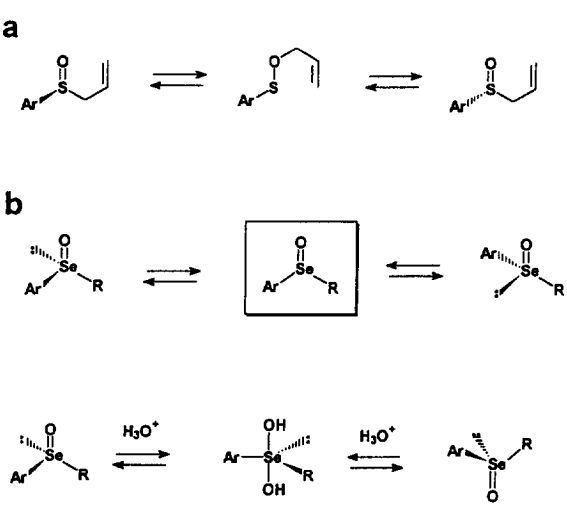


Fig. 12. $R \rightleftharpoons S$ interconversion processes for (a) allylic sulfoxides and (b) selenoxides.

ing the eluent flow-rate (85°C, flow-rate 0.5 ml/min) resulted in the appearance of an interconversion plateau between the resolved peaks because of the increased $R \rightleftharpoons S$ isomerization rate (compared with the separation rate) taking place via the achiral sulfenate (Fig. 12a). Out-of-column kinetic determinations showed the extent of peak deformation to be qualitatively related to the stereochemical stability of the different sulfoxides; a different dynamic HPLC pattern is observed for allyl *p*-nitrophenyl sulfoxide when analysed under the same experimental conditions (Fig. 13): deformations of the chromatographic profile appear at column temperatures around 45°C with a reaction zone preceding the first-eluted peak, and complete peak coalescence occurs at 85°C. These results are in qualitative agreement with earlier findings on related allyl aryl sulfoxides with strong electron-withdrawing groups on the aromatic ring, which were found to have a lower enantiomerization barrier and an increased proportion of the achiral sulfenate at the equilibrium [36,37]. Additional examples of compounds undergoing on-column enantiomeri-

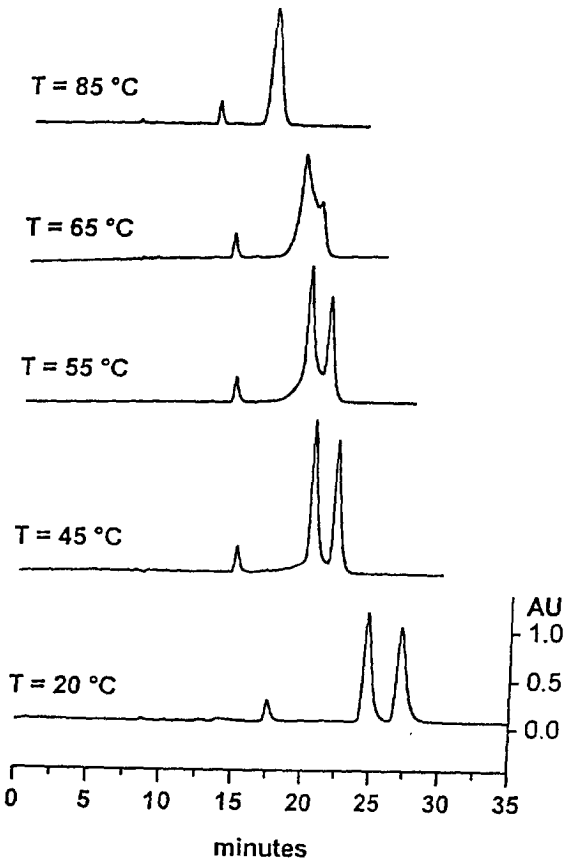


Fig. 13. Variable-temperature HPLC of allyl *p*-nitrophenyl sulfoxide on CSP2 (250 × 4 mm I.D. column); eluent, *n*-hexane–dioxane–methanol (70:30:1, v/v/v); flow-rate, 0.5 ml/min; UV detection at 254 nm.

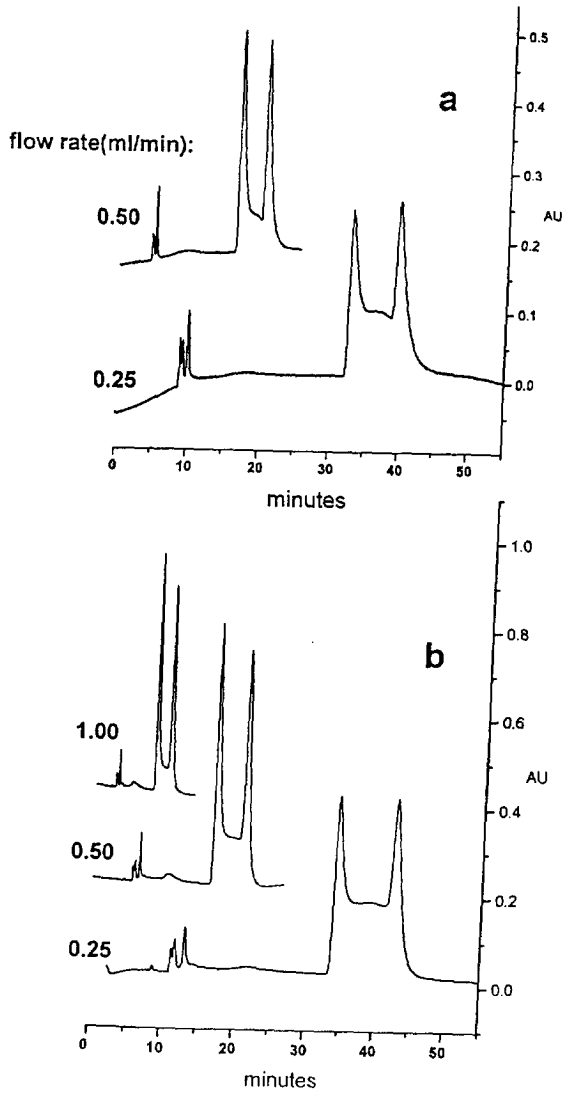


Fig. 14. Variable-flow HPLC of benzyl phenyl selenoxide on CSP2 (250 × 4 mm I.D. column); UV detection at 254 nm.(a) Eluent, chloroform–2-propanol (95:5, v/v); temperature, 100°C; (b) eluent, *n*-hexane–2-propanol–methanol (60:30:10, v/v/v); temperature, 55°C.

zation reactions at elevated temperatures are represented by unsymmetrical selenoxides (Fig. 14); chiral alkyl aryl selenoxides show greater configurational liability than the corresponding sulfoxides: the two possible racemization mechanisms (pyramidal inversion [38] or formation of an achiral hydrate in the presence of moisture [39]; Fig. 12b) have in fact activation barriers ($\Delta G^{\neq}$) between 14.6 and 25.5 kcal/mol. Dynamic HPLC (in the form of variable-flow chromatography, Fig. 14) of benzyl phenyl selenoxide shows that the enantiomerization process is strongly solvent dependent: similar peak deformations are observed when the racemic selenoxide is chromatographed on CSP2 using either chloroform–2-propanol (95:5, v/v) at 100°C or *n*-hexane–2-propanol–methanol (60:30:10, v/v/v) at 55°C, i.e., on-column interconversion is accelerated by large amounts of alcoholic modifiers in the mobile phase. Also in this case, the results obtained from dynamic enantioselective chromatography are in keeping with kinetic results obtained from bulk solution investigations [39].

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