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Review

Chiral stationary phases derived from tyrosine

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ABSTRACT

Numerous chiral stationary phases (CSPs) have been developed in the last decade to provide an efficient and economic means for separating optical isomers. Among them, Pirkle-type CSPs have been designed according to a rational investigation of chiral recognition mechanisms. Following a similar approach, new CSPs possessing specific properties were designed starting from tyrosine.

The special features of CSPs derived from tyrosine are surveyed. These CSPs are characterized by the way in which the chiral selector (CS) is grafted onto silica gel, which allows the preparation of an entire family of CSPs based on the same starting material. This entails a wide scope of application including numerous racemates such as phosphine oxides, sulphoxides, lactams, benzodiazepinones and amino acid derivatives. This is reviewed either for analytical or preparative purposes. Chiral recognition mechanisms involved with tyrosine-derived CSPs are discussed.

Owing to the high stability of their grafting mode, tyrosine-derived CSPs are suited for all types of mobile phase nature, either liquid (LC) (reversed- or normal-phase), or supercritical fluid chromatography (SFC). A convenient method for optimizing the mobile phase (suitable for all Pirkle-type and related CSPs) is proposed. By using SFC, very high resolutions per unit of time are achieved, either on an analytical or on a preparative scale.

CONTENTS

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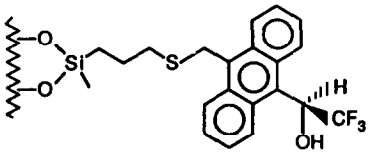
1. INTRODUCTION

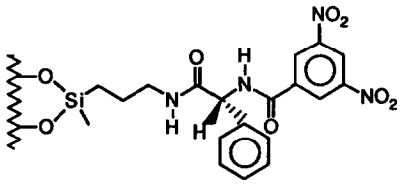
In the last decade, chromatographic enantioseparations have given rise to widespread interest, directly connected with the growing attention paid to stereochemistry in drug design. Chiral high-performance liquid chromatography (HPLC) provides a powerful tool for monitoring enantiomeric ratios (analytical scale) and/or for preparing highly optically pure enantiomers (preparative scale).

Accordingly, there has been a tremendous increase in the design and synthesis of chiral stationary phases (CSPs) [1–4]. They are usually divided into two classes according to the chiral recognition process they are assumed to involve [5]. Independent CSPs (class 1) [6] are obtained by grafting an optically pure moiety (chiral selector, CS) on an achiral matrix (modified silica gel such as γ-aminopropyl, γ-glycidyloxypropyl or γ-mercaptopropyl [2]). Each CS operates independently in distinguishing the solute enantiomers. The chiral discrimination results from the formation of transient CSP–solute enantiomer diastereoisomeric complexes differing in internal energy [7]. They are opposed to cooperative CSPs (class 2), for which chiral entities are acting in concert to achieve chiral discrimination through complex chiral recognition mechanisms (inclusion phenomena). Class 2 includes natural [8–11] (cellulose derivatives, proteins) or synthetic polymers [8,12]. Cyclodextrin-bonded CSPs [13] are located between the two previous classes as each oligosaccharide acts independently and inclusion complex formation is required.

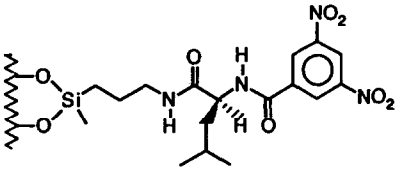
The major contribution to the design of class 1 CSPs was given by Pirkle and co-workers, who proposed a rational approach starting from NMR studies of diastereomeric solvates [14]. The grafting of 2,2,2-trifluoro-1-(9-anthryl)ethanol (initially used as a chiral solvating agent for NMR investigations) onto silica gave rise to the first Pirkle-type CSP (CSP A, Fig. 1) [15,16]. The elucidation of CSP A–solute chiral recognition mechanisms led to the development of a second generation of Pirkle-type CSPs. Numerous 3,5-dinitrobenzoyl (3,5-DNB) derivatives of amines, alcohols and related compounds have been resolved on CSP A [16,17]. The 3,5-DNB group provides both a π-acid (suitable for interacting through a π–π interaction with the strong π-basic 9-anthryl group of CSP A) and a proton acceptor site. By grafting N-(3,5-DNB) amino acids *via* an amidic linkage onto γ-aminopropyl silica, π-acid CSPs were prepared. According to the "reciprocality concept" [17] (if an optically active entity A resolves the enantiomers of B, then optically active B is expected to resolve the enantiomers of A), secondary aromatic alcohols were early resolved. A wide range of π-basic racemates (naturally occurring or prepared after derivatization) were also separated on these CSPs. The most popular π-acid CSPs were prepared from phenylglycine (DNBPG; CSP B, Fig. 1) [17–19] and leucine (DNBLeu; CSP C, Fig. 1) [18,20]. Their early commercialization has extended their scope of application, especially among pharmaceutical compounds.



CSP A



CSP B
(R)-DNBPG



CSP C
(S)-DNBLeu

Fig. 1. Structures of Pirkle-type CSPs.

The rational design success of class 1 CSPs has prompted many researchers to develop new π-acid CSPs (*e.g.*, [21–24]). Although their scopes of application did not greatly differ, their chemical structure has resulted in specific chromatographic behaviours. The chiral recognition ability of a chromatographic system (CSP–solute–mobile phase) requires to a large extent a balance between the potential attractive interactions (π–π interactions, hydrogen bondings or dipole stackings) and steric hindrance, closely related to the conformational rigidity [25]. Hence, in order to extend the scope of application of Pirkle-type CSPs and to improve the knowledge of chiral recognition mechanisms, the development of new concepts is required. Literature data [1–4] indicate that π-basic CSPs are more frequently prepared and studied than the π-acid type, even if their scope of application is narrower (natural enantiomers scarcely show a π-acid character). Hence, the design and preparation of π-acid CSPs still remain a challenge.

For a few years, our laboratories have been involved in the synthesis and evaluation of CSPs derived from tyrosine. Their design is based on a rational approach taking into account the additional functional group of tyrosine (hydroxyl group) with regard to conventional Pirkle-type CSP precursors (phenylglycine, leucine, valine, etc.). This paper is organized into six sections, each emphasizing the originality and the properties of tyrosine-derived CSPs.

2. DESIGN

The design of CSPs derived from tyrosine results from the "reciprocality concept" introduced by Pirkle and House [17]. Our experience with Pirkle-type CSPs started with the enantiomeric separation of a series of tertiary phosphine oxides by Pescher and co-workers [26,27]. These solutes were easily resolved on a preparative scale [28] using a DNBPG CSP. In that manner, chiral aromatic tertiary phosphine oxides were prepared and grafted onto silica in order to provide π-basic CSPs with a chiral phosphorus atom [29,30].

For this purpose, one enantiomer of 1-(4-methoxynaphthyl)methylphenylphosphine oxide was chosen as chiral selector for the following reasons: the racemic mixture was well resolved on the DNBPG CSP (α = 1.61 [27]) and thus could easily be prepared as a single enantiomer with a high enantiomeric purity. Further, the absolute configuration of each enantiomer could readily be deduced by chemical correlation from those of methylnaphthylphenylphosphine oxide enantiomers, which had previously been established by Luckenbach [31].

The methoxy substituent was of great interest. Indeed, after treatment with boron tribromide, the resulting phenol could react either with various electrophilic reagents such as ethyl bromoacetate, allyl bromide or epibromohydrin (Fig. 2). In this way, starting from one chiral selector, three CSPs differing in the grafting mode were obtained [29,32]. The way of coupling 2, previously described by Rosini *et al.* [33], avoids generating a polar linkage in the spacer far from the chiral centre (amino alcohol in way 1 and amide in way 3, Fig. 2); such polar groups may induce non-stereoselective interactions. Accordingly, this grafing mode was selected for synthesizing tyrosine-derived CSPs [34].

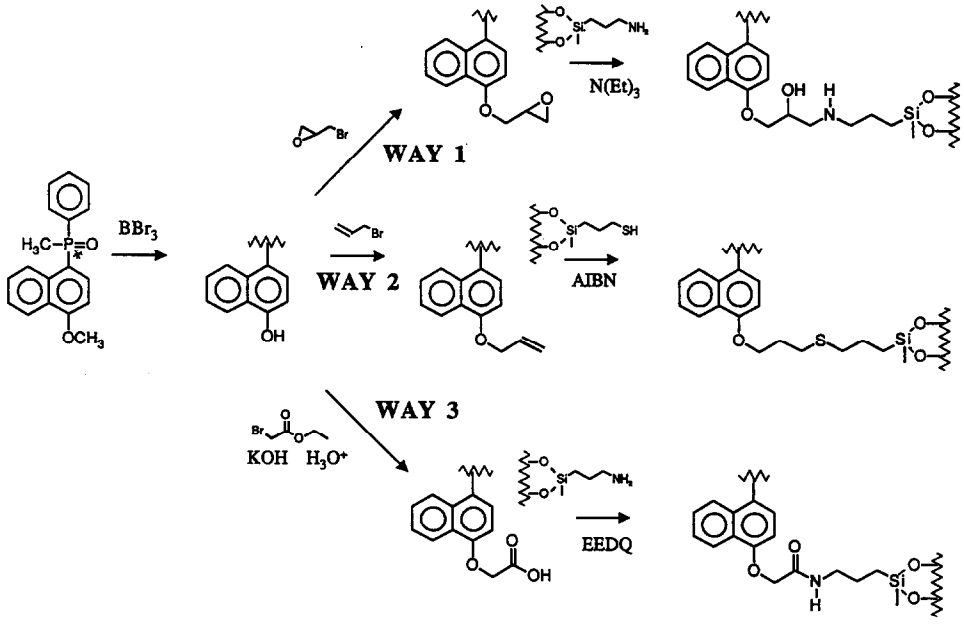


Fig. 2. The three different grafting modes investigated for the CSPs derived from phosphine oxides.

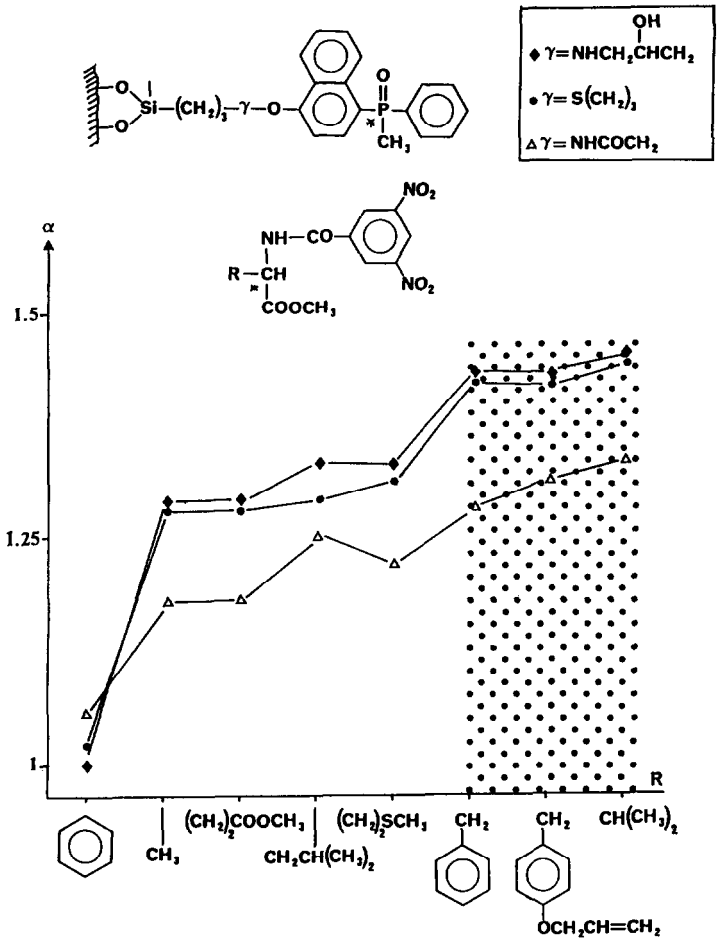


Fig. 3. Enantiomeric separation of 3,5-DNB amino esters on π-basic CSPs derived from phosphine oxides. Operating conditions: column, 150 × 4.6 mm I.D.; mobile phase, *n*-hexane–ethanol [(◆) 83:17; (●) 78:22; (△) 90:10, v/v]; flow-rate, 2 ml/min; temperature, 40°C; UV detection at 254 nm.

The enantiomeric separation of a series of 3,5-DNB amino methyl ester racemates (Fig. 3) was investigated on these CSPs [29,32]. Although the 3,5-DNB derivative of phenylglycine was the reciprocal precursor of these CSPs, it was surprising that this compound was the worst resolved (Fig. 3). This result illustrated the limitations of the "reciprocality concept" insofar as it does not take into account the chiral selector environment (spacer arm, silica matrix, mobile phase nature).

On the other hand, 3,5-DNB derivatives of valine [35], phenylalanine [21,35] and O-allyltyrosine were among the best resolved amino methyl ester derivatives (Fig. 3). Tyrosine was chosen because of its hydroxyl group, which could conveniently be turned into an allylic ether (site 1 in Fig. 4). This was suitable for reaction according to an anti-Markownikoff addition with the thiol group of a γ-mercaptopropyl-modified silica (way 2 in Fig. 2). In this manner, the chiral centre is removed from the silica matrix (with regard to the DNBPG CSP), the steric hindrance of which is minimized.

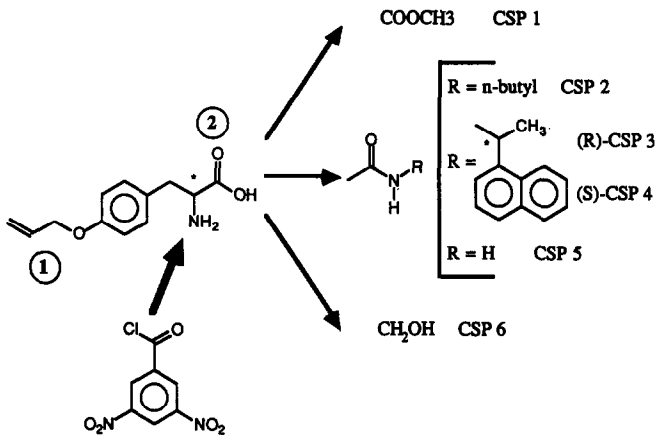


Fig. 4. Design of CSPs derived from tyrosine. The allylic functional group is employed for grafting the CS onto silica. The remaining carboxylic acid group is then converted into ester (CSP 1), amide (CSPs 2–5) or alcohol (CSP 6).

Moreover, supercritical fluid chromatographic (SFC) experiments indicated significant leaching of chiral grafts for CSPs displaying an amidic grafting mode [25]. This was related to the presence of unstable ionically bonded CSs. Such leaching was never evidenced with CSs grafted with a mercapto linking group, thus emphasizing their high stability.

Above all, using the hydroxyl group as the anchorage point, the carboxylic acid group (site 2 in Fig. 4) remains free for further derivatization. It can be converted into various functional groups (ester, amide, alcohol), allowing numerous interaction opportunities. Thus, two CSPs were early prepared [25,34,36,37]: CSP 1 (Fig. 4) for which the carboxylic acid group has been conveniently converted into a methyl ester and CSP 2 bearing an *n*-butyl amide group. They were termed (*S*)-thio-DNBTyr-E and (*S*)-thio-DNBTyr-A, respectively. Then, while keeping the amidic function and the 3,5-DNB group of CSP 2, the additional introduction of a π-basic moiety was considered. The replacement of the *n*-butylamine reagent (CSP 2) with chiral (*R*)- or (*S*)-1-(1-naphthyl)ethylamine afforded two novel "mixed" (*i.e.*, bearing both π-acid and π-basic moieties) CSPs [38] (CSP 3 and 4 in Fig. 4). Although mixed CSPs had been described previously [39,40], these CSPs were the first examples of CSPs suitable for the simultaneous separation of π-basic and π-acid racemates. Finally, very recently two novel CSPs bearing a primary amide (CSP 5 in Fig. 4) or a primary alcohol (CSP 6 in Fig. 4) were prepared [41].

3. SYNTHESIS

Syntheses of CSPs 1 and 2 [34], 3 and 4 [38] and 5 and 6 [41] were reported previously.

The starting material [(*S*)-Boc-tyrosine] belongs to the chiral pool. It is commercially available in large amounts and is fairly cheap. The various steps leading to CSPs 1–6 (Fig. 5) are classical and give satisfactory overall yields. The grafting rates

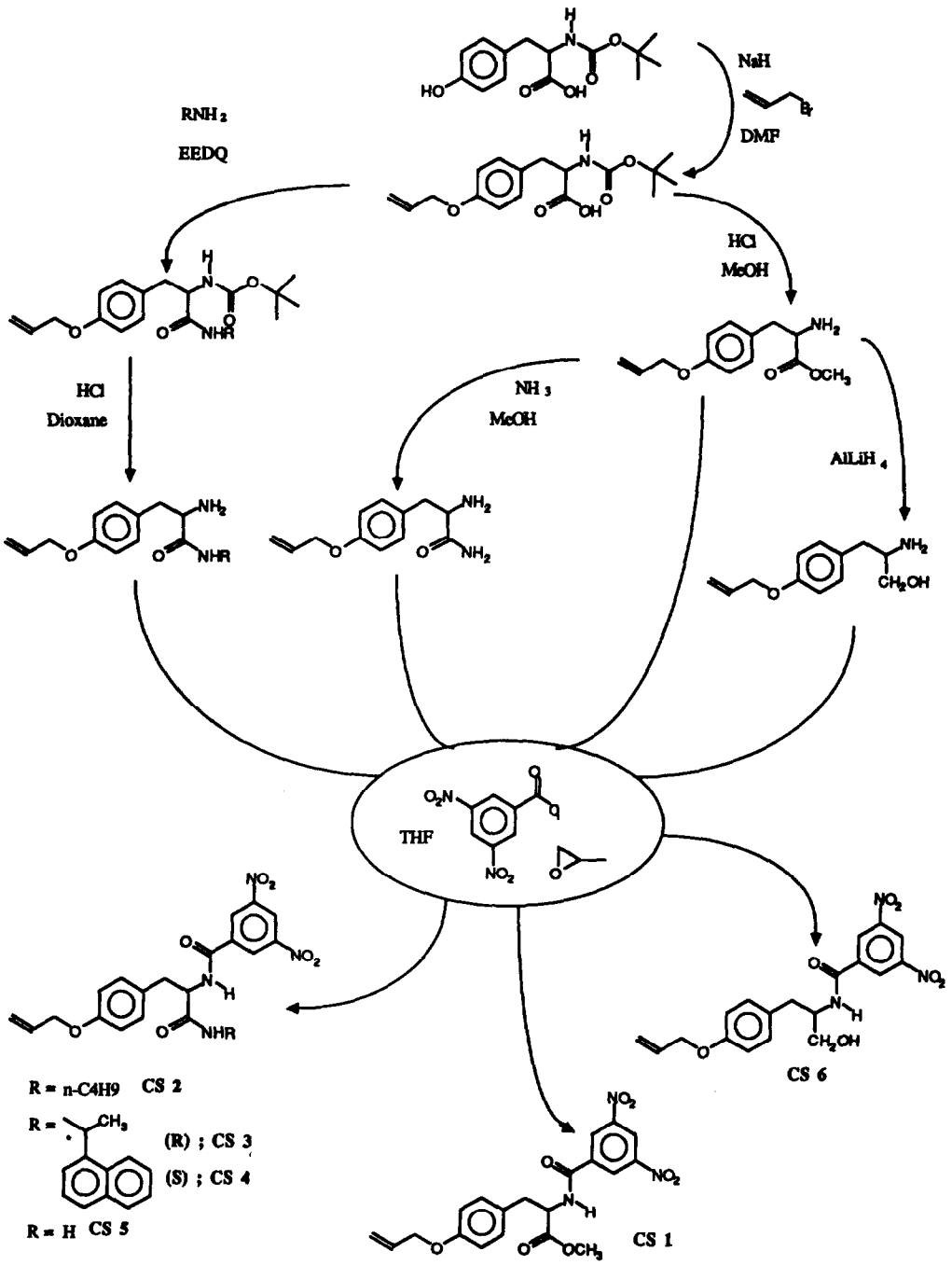


Fig. 5. The various pathways used for the synthesis of CSs derived from tyrosine.

are in the region of 0.2 mmol per gram of modified silica, corresponding to a 20% coverage of the initial mercaptopropyl groups.

4. MOBILE PHASE OPTIMIZATION

Normal-phase (NPLC) and reversed-phase (RPLC) modes are used with CSPs derived from tyrosine. Although the NPLC mode is far more used, recent separations have been carried out under RPLC conditions (Fig. 6) [42]. Recently, super- and sub-critical fluid chromatography (SFC and SubFC) were introduced and a section will be devoted to this new technique.

4.1. Influence of solvent nature in NPLC

n-Hexane is a typical apolar solvent used in the mobile phase and generally polar modifiers are chosen according to their selectivity parameters (χ_e, χ_d, χ_n, which reflect the ability of a solvent to act mainly as a proton acceptor, a proton donor or a strong dipole, respectively) as defined and calculated by Snyder [43,44] from solubility data reported by Rohrschneider [45].

4.1.1. Binary mixtures. From our experience with Pirkle-type and related CSPs the main effective polar modifiers can be classified into two groups: alcohols and chlorinated solvents. Retention times and selectivity values cannot be directly related to the mobile phase polarity (P') but rather depend on the polar modifier chemical structure and selectivity group. For an equivalent mobile phase polarity, differences observed in k' and α values when using alcohols belonging to the same selectivity group can be ascribed to the relative bulkiness of their alkyl moiety. Bulky alcohols give the highest selectivities and retentions, whereas a small linear alcohol such as ethanol exhibits both efficiency (plate number) and solvent strength [27].

Chlorinated solvents exhibit a greater selectivity than alcohols but a lower

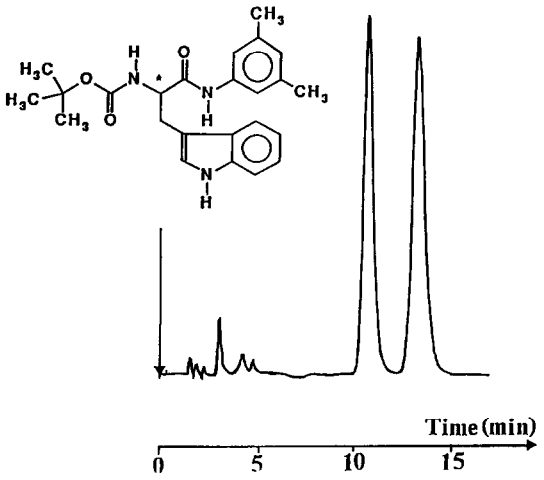


Fig. 6. Reversed-phase separation of the 3,5-dimethylanilide derivative of N-*tert.*-Boc-tryptophan on CSP 2. Operating conditions: column, 150 × 4.6 mm I.D.; mobile phase, methanol–water (80:20, v/v); flow-rate, 1 ml/min; room temperature; UV detection at 230 nm.

efficiency [46]. These characteristics may be correlated with the fact that alcohols can be considered as both proton donors and acceptors. Hence they may interact through hydrogen bondings with the basic and/or the acid site of the CSP amide dipoles. In contrast, chloroform (proton donor) and methylene chloride (strong dipole) only interact with a single site. Therefore, CSP–solute interactions are maximized when using chlorinated solvents (resulting in a higher selectivity) but the adsorption–desorption kinetics of solutes on CSP are slower (resulting in a weaker efficiency). Consequently, it is of interest to use a mixture of both a chlorinated solvent (in order to achieve a high selectivity) and a linear alcohol (in order to obtain a high efficiency and eluting strength) as polar modifier.

4.1.2. Ternary mixtures. Very often, a ternary mixture, *n*-hexane–alcohol–chlorinated solvent, turns out to be the optimum mobile phase for the resolution of enantiomers on Pirkle-type and related CSPs. Pescher *et al.* [27] first observed that phosphine oxides were much more soluble in chloroform than in an isoeluotropic *n*-hexane–alcohol mixture, predicting stronger solute–mobile phase interactions. Plotting α and k' values *versus* the concentration of binary mixture B (*n*-hexane–chloroform) in the ternary mixture A–B (A = *n*-hexane–alcohol), they obtained curves as shown in Fig. 7, displaying a concave profile for k'_2 and a regular increase in α from alcohol to chloroform.

This profile can be explained as follows: the capacity factor, k', can be expressed as

$$k' = \Sigma \text{ CSP–solute interactions} / \Sigma \text{ mobile phase–solute interactions}$$

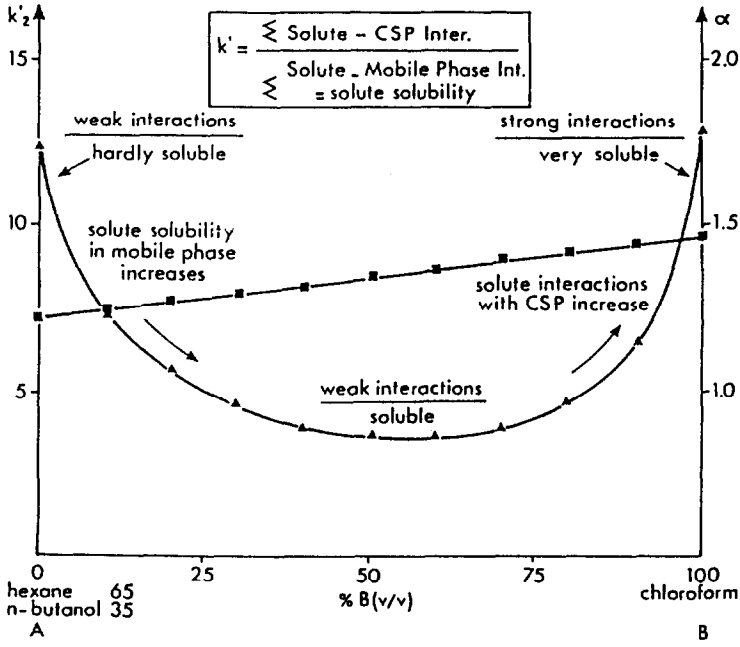


Fig. 7. Dependence of (▲) the capacity factor k' [for the most retained enantiomer of methyl(9-phenanthryl)phenylphosphine oxide] and (■) selectivity α on the chloroform content in the ternary mobile phase *n*-hexane–alcohol–chloroform (%B). Schematic explanation of the concave profile of k' *versus* %B.

To a first approximation, the solute solubility can be considered as a measure of the solute–mobile phase interactions. When the solubility of methyl(9-phenanthryl)-phenylphosphine oxide is plotted *versus* the chloroform content in *n*-hexane–chloroform–alcohol mixtures, a linear increase is observed. Consequently, in these mixtures, two phenomena may be assumed: (a) a higher solubility of solute molecules in the mobile phase when chloroform is added; and (b) the substitution of some alcohol molecules (which are strongly adsorbed on CSP) by chloroform molecules (which are easily displaced from CSP by solute molecules). At first, the CSP–solute interactions can be considered as weak with *n*-hexane–alcohol mobile phases because of the strong adsorption of alcohol molecules on the CSP. When a small amount of chloroform is added to the mobile phase, the solubility of the solute increases according to (a); with regard to (b), the chloroform can displace only a few alcohol molecules fixed on the CSP; the increase in solubility overshadows the phenomenon (b), leading to a decrease in k' values. This accounts for the descending left-hand part of the curve. Alternatively, at higher chloroform contents, phenomenon (b) becomes predominant (mass law). This entails an increase in the CSP–solute interactions and consequently in k', yielding the ascending right-hand part of the curve. In the concave minima region of the k' curve, the selectivity and the resolution per unit of time are significantly increased.

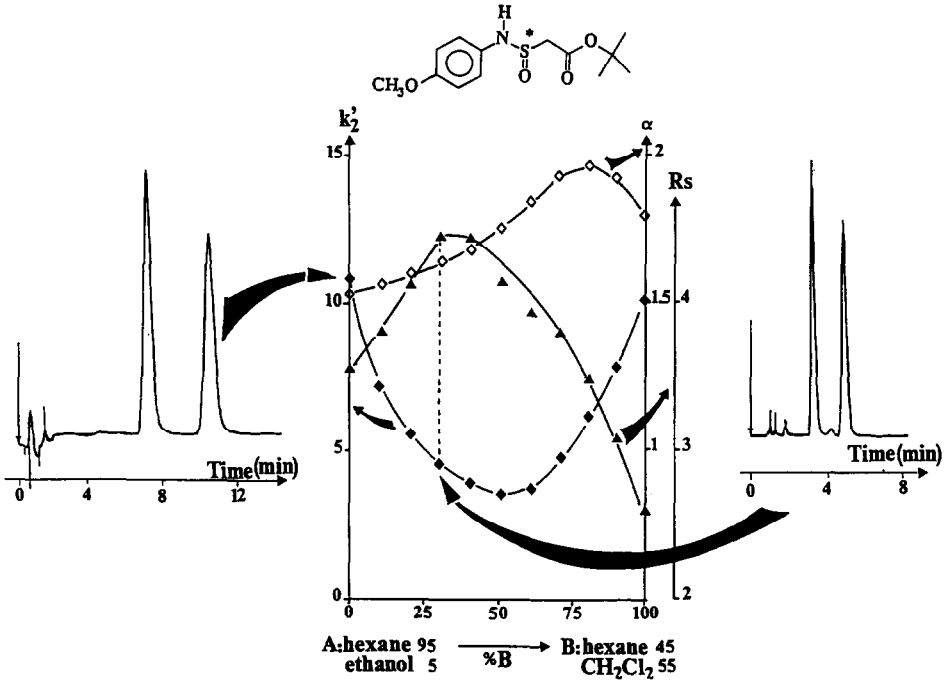


Fig. 8. Ternary optimization for *tert.*-butyl-N-(4-methoxyphenyl)sulphinamoyl acetate on CSP 3 using *n*-hexane–ethanol (95:5, v/v) (solvent A) and *n*-hexane–methylene chloride (45:55, v/v) (solvent B) as eluents. The capacity factor of the most retained enantiomer k'_2 (◆), the selectivity value α (◇) and the resolution factor R_s (▲) are plotted *versus* the content of solvent B in the ternary mobile phase A–B. Operating conditions: column, 150 × 4.6 mm I.D.; flow-rate, 2 ml/min; room temperature; UV detection at 254 nm.

This mobile phase optimization is now currently used [38,46]. As an example, Fig. 8 shows the ternary optimization of the enantiomeric separation of *tert.*-butyl N-(4-methoxyphenyl)sulphinamoyl ester on CSP 2.

In one instance, reversal of the elution order of the enantiomers using an *n*-hexane–ethanol–methylene chloride (or chloroform) ternary mixture as mobile phase has been reported (Fig. 9) [47]. Such a phenomenon is very difficult to explain. The reversal of elution order between ethanol and methylene chloride (or chloroform) can be ascribed to a change in the dominant interaction nature during the transient diastereomeric complex formation. The solvatation of both the solute and the CSP polar groups is affected by the nature of the solvent and is partly responsible for their conformation. The reversal of elution order can be the consequence of two separate causes: (a) both the solute and CSP contain too many sites of interaction of the same nature (two amide dipoles) and a low steric hindrance at the asymmetric centre; and (b) the lack of a strong driving force (such as a π–π overlapping) which could orientate the two molecules preferentially inside the diastereomeric complex, whatever the mobile

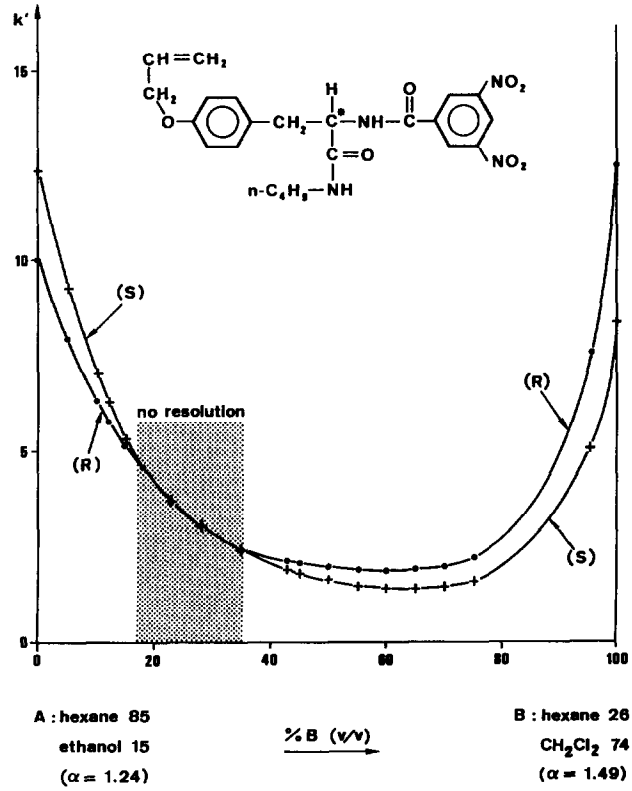


Fig. 9. Reversal of elution order of solute **20a** enantiomers (Table II) on CSP 2 on changing from *n*-hexane–ethanol (85:15, v/v) (solvent A) to *n*-hexane–methylene chloride (26:74, v/v) (solvent B) binary mixtures. The capacity factors *k'* of (+) (*S*)-**20a** and (●) (*R*)-**20a** are plotted *versus* the content of binary mixture B in the ternary mixture A–B. Operating conditions: column, 150 × 4.6 mm I.D.; flow-rate, 2 ml/min; temperature, 25°C; UV detection at 254 nm.

phase nature. As no driving, planar π–π attraction can be advocated, the solute can approach the CS in various orientations; multiple chiral recognition processes can thus be expected, some of them even working in opposite stereochemical senses and involving deeply conformational criteria directly connected to the mobile phase nature. We consider that ethanol favours dipole stacking mechanisms whereas chlorinated solvents rather favour hydrogen bondings.

4.1.3. Influence of the water content in the mobile phase. Generally, the addition of a small amount of water to the mobile phase does not lead to a significant increase in selectivity but gives an improvement of efficiency, leading to a higher resolution (Fig. 10). We assume that the water molecules are preferentially adsorbed on the residual silanols, thus cancelling non-stereoselective silica-solute interactions and leading to better kinetics of exchange [48].

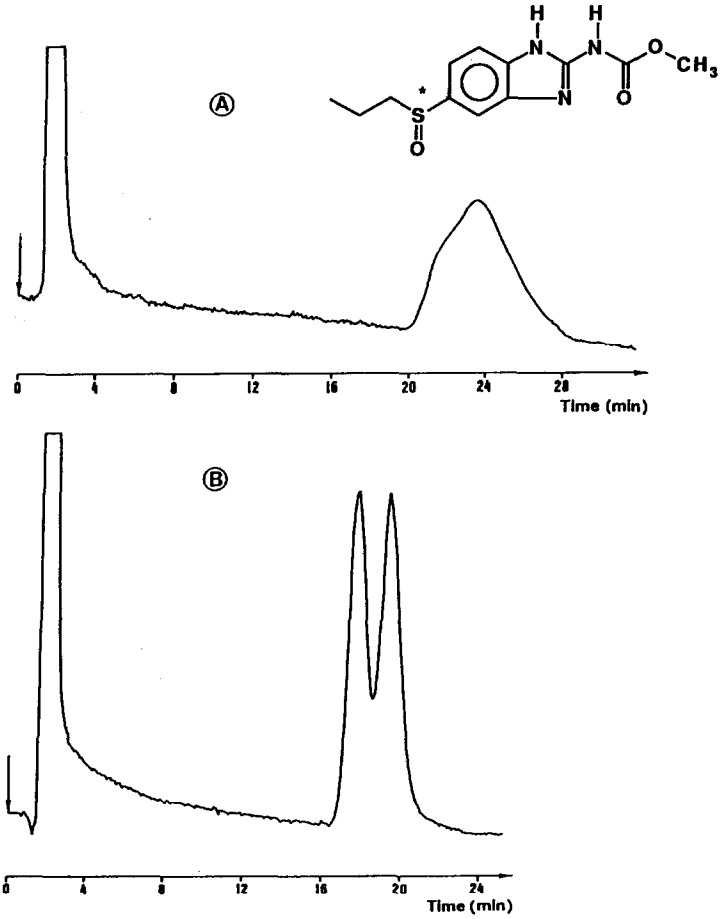


Fig. 10. Influence of the addition of water to an *n*-hexane–2-propanol mobile phase on the resolution of albendazole sulphoxide (solute 13, Table II). Mobile phase: (A) *n*-hexane–2-propanol (80:20, v/v); (B) *n*-hexane–2-propanol containing 2% of water (80:20, v/v). Column: CSP 1 (250 × 4.6 mm I.D.; d_p = 5 μm); flow-rate, 2 ml/min; temperature, 25°C; UV detection at 220 nm.

4.2. Supercritical fluid chromatography

As stated previously, chiral recognition on CSPs derived from tyrosine is mainly based on the setting of polar attractive CSP–solute interactions. Hence, the polarity of carbon dioxide (the main fluid used in SFC) is not sufficient to elute the enantiomers. Accordingly, polar modifiers should be added to the mobile phase. Their properties emphasized in NPLC are equivalent in SFC (modifiers which usually induce a high selectivity such as methylene chloride or chloroform also display a lower efficiency). No significant influence of the average column pressure on selectivity has been observed, meaning that carbon dioxide does not play a major role in the chiral recognition process. For a given racemate, LC and SFC afford the same elution order and a similar stereoselectivity. Similar chiral recognition processes can then be proposed for LC and SFC [49].

Further, the inherent advantages of SFC over LC are maintained (diffusion coefficients, D_m, and thus optimum velocities are 5–10 times greater in SFC than in LC). Consequently, the resolution per unit time in SFC is greater than that in LC. The superiority of SFC is twofold: for a given analysis time resolutions are significantly improved, and for a given resolution, analysis times are greatly reduced (Fig. 11)

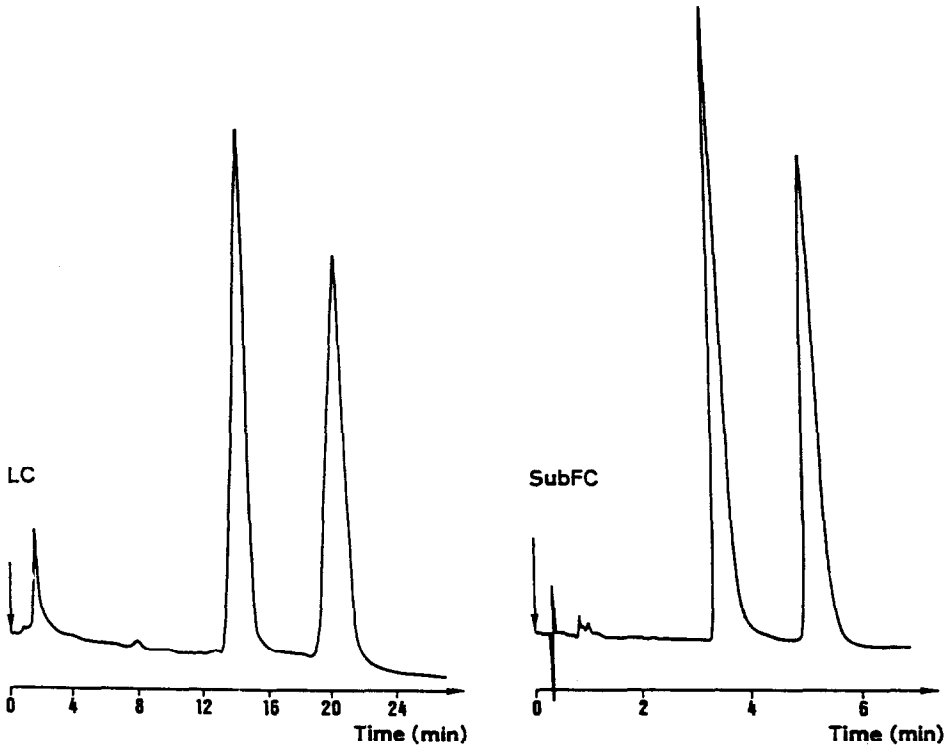


Fig. 11. Comparison of the analysis times measured at constant resolution factor in LC and SFC for oxazepam (solute **10**, Table II). Column: CSP 2 (150 × 4.6 mm I.D.; 5 μm). LC: mobile phase, *n*-hexane–ethanol (90:10, v/v); flow-rate, 2 ml/min; temperature, 25°C. SFC: mobile phase, carbon dioxide–ethanol (92:8, w/w); flow-rate, 6 ml/min at 0°C; average pressure, 200 bar; temperature, 25°C; UV detection at 229 nm.

[50,51]. All these results are valid for the resolution of π-basic solutes on π-acid CSPs, *i.e.*, in the case where a driving π–π interaction occurs in the CSP–solute diastereomeric complex formation. When this planar interaction does not exist, several chiral recognition processes may occur and carbon dioxide, owing to its induced dipolar character, behaves similarly to methylene chloride [47].

5. CHIRAL RECOGNITION MECHANISMS

CSPs derived from tyrosine belong to class 1 CSPs. Each modified tyrosine graft operates independently towards the solute enantiomers through the formation of transient CSP–solute bimolecular complexes.

According to the three-point rule proposed by Dalgliesh [7], to achieve chiral recognition the mechanism requires a minimum of three simultaneous interactions, one of which is stereochemically dependent. Nevertheless, one- or two-contact point models have been proposed to account for chiral discrimination [52–54]. The CSP–solute complex formation results from the occurrence of attractive CSP–solute interactions (π–π interaction, hydrogen bonding, dipole stacking).

In the case of tyrosine-derived CSPs the sites of interaction (amide or ester dipoles and 3,5-dinitrophenyl) are located only in two directions from the asymmetric centre. Accordingly, two-contact-point chiral recognition processes may be advocated for tyrosine-derived CSPs. The two CSP sites of interaction may interact with either two (Fig. 12A) or three (Fig. 12B) solute sites of interaction according to an M1 or M2 mechanism. These mechanisms are referred to as (2–2) or (3–2) mechanisms, respectively, according to Finn [55] (classified by the number of the four groups of the chiral tetrahedral centres involved in the chiral recognition process). For the M1

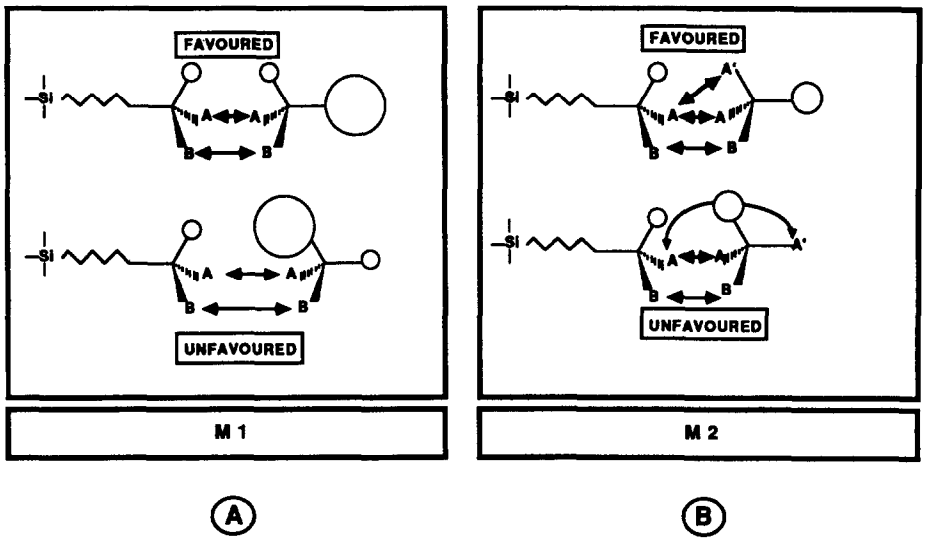


Fig. 12. Schematic representation of the most frequently encountered mechanisms with tyrosine-derived CSPs.

mechanism, equivalent attractive interactions are advocated for the two complexes, and the difference in stability results from the sterically unfavoured conformation of one CSP–solute complex (according to Topiol [54], this may be related to a difference in the distance matrices of the two complexes). This mechanism is frequently encountered [25,48], but higher selectivities are usually achieved with the M2 mechanism. Thus, the enantiomeric separation of N-aryl sulphinamoyl acetates which leads to high selectivity values on CSP 2 effectively obeys an M2 mechanism [46]. This is depicted in Fig. 13. The π–π interaction was evidenced by correlating the selectivity value with the π-acidity of the solute (expressed by means of its Hammet σ value) [46]. The hydrogen bonding HB 3 was evidenced by correlating the retention with the proton donor character of the CSP (CSPs 1, 2, 5 and 6 were tested) [41].

The conformational state of CSPs 3 and 4 results from a potential intra-

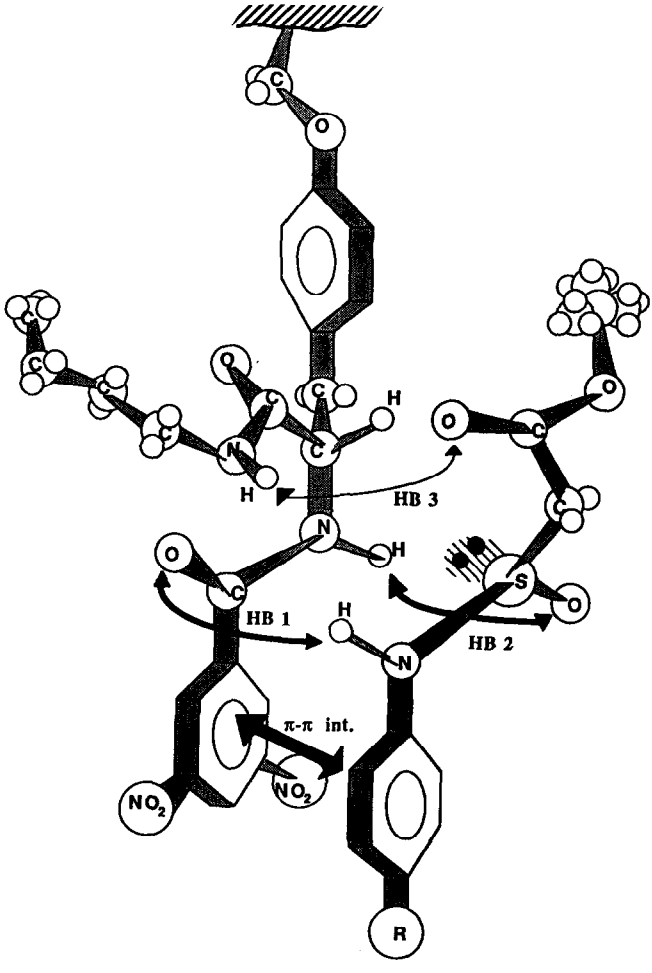


Fig. 13. Proposed chiral recognition model for N-aryl sulphinamoyl esters on CSP 2.

molecular π–π interaction strengthened by an intramolecular dipole stacking [38]. These CSPs bear two asymmetric centres, each located in the vicinity of either a π-basic or a π-acid centre. Accordingly, they may generate π–π complexes either with π-acid or π-basic solutes. So far, no indication had been given of the possibility of such a "mixed" CSP acting independently according to either a π-acid or a π-basic mode. Indeed, the resulting CSPs 3 and 4 display two distinct moieties: the π-acid moiety, in the vicinity of the 3,5-DNB moiety, is structurally similar to CSP 2 and thus exhibits a similar chromatographic behaviour toward π-basic racemates. Nevertheless, lower selectivities are generally observed as the intramolecular π–π interaction weakens to a certain extent the π-acceptor properties of the dinitrophenyl moiety. Further, the presence of the π-basic moiety allows the separation of π-acid racemates. It has been shown that both π-acid and π-basic modes do not interfere with each other [38]. For instance, the separation mechanism of 3,5-dinitroanilide derivatives of substituted 2-arylpropionic acid (APA) and related compounds only involves the π-basic moiety of CSP 3. This is shown in the Fig. 14 (the most retained enantiomer is represented). For APA derivatives (X = H), a typical M1 mechanism may be advocated; the chiral discrimination results from the aromatic group steric hindrance which prevents the

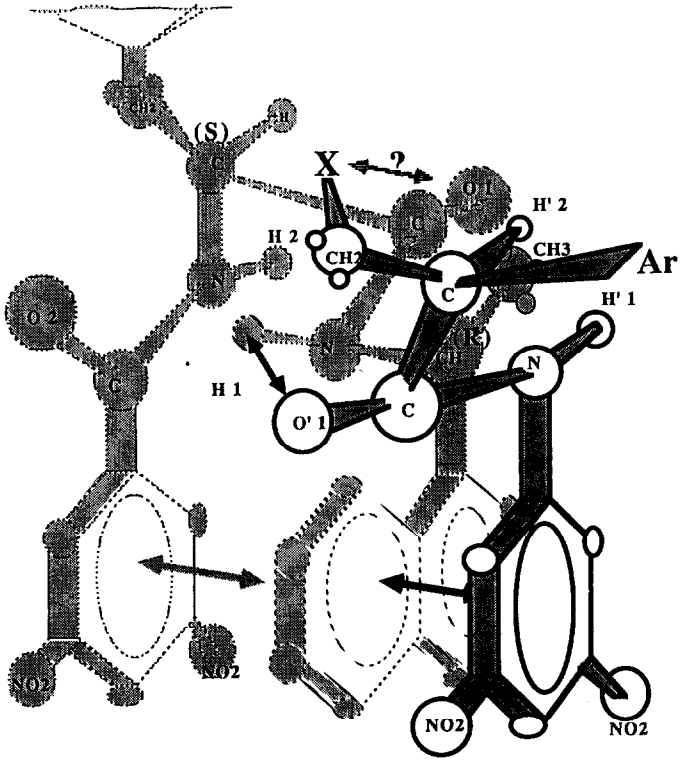


Fig. 14. Proposed chiral recognition mechanism for 3,5-dinitroanilide derivatives of aryl-2-propionic acids (APAs, X = H) and tropic acid (X = OH) on CSP 3. The most retained enantiomer is represented (according to chromatographic data), *S*-form for the APA derivative and *R*-form for the tropic acid derivative.

R enantiomer from approaching the CSP closely. For the tropic acid derivative (X = OH), the occurrence of a third interaction (hydrogen bonding between the hydroxyl group of the solute and the oxygen atom O-1 of the CSP) results in an M2 mechanism. The methyl substituent of the CSP hinders hydrogen bonding with the *S*-enantiomer and thus leads to chiral discrimination.

6. SCOPE OF APPLICATION

Numerous racemates of medium polarity containing aromatic substituents can be resolved on tyrosine-derived CSPs. So far, no racemate containing strong polar groups such as carboxylic acid or amine groups has been resolved. Such functional groups should be converted into functional groups of lower polarity (ester or amide) prior to injection. The scope of application of tyrosine-derived CSPs is focused on racemates containing asymmetric centres (carbon, phosphorus or sulphur). Nevertheless, some atropoisomers such as binaphthol derivatives may also be resolved [34]. Some specific functional groups of medium polarity are required in the vicinity of the asymmetric centre: amide or ester dipoles, carbamate, urea or hydroxyl groups. The chirality may also be borne by a dipole such as phosphine oxide, sulphoxide or sulphinamoyl.

Table I illustrates the scope of application of CSP 1. The main application of CSP 1 lies in the separation of solutes **3** enantiomers. These compounds play a prominent role in asymmetric catalysis [56]. So far, only CSP 1 allows the enantiomeric preparative-scale separation of such compounds [56,57], which are not or only poorly resolved on commercially available Pirkle-type CSPs (DNBPG and DNBLeu). Other miscellaneous racemates are resolved on CSP 1 (solutes **4**–**7**).

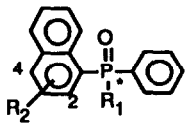
CSP 2 and the DNBPG CSP possess a complementary scope of application (Table II). Some typical Pirkle-type solutes such as the 2,2,2-trifluoro-1-(9-anthryl)-ethanol (TFAE, solute **8**), binaphthol (solute **9**) and oxazepam (solute **10**) are also resolved on CSP 2. Like DNBPG CSP, CSP 2 displays high selectivity values towards N-naphthylamino esters, previously described by Pirkle and Pochapsky [58] (solutes **15**). In addition, CSP 2 allows the enantiomeric separation of compounds of pharmaceutical or biological interest which were not or only poorly resolved on DNBPG CSP. This is the case for the anthelmintic drug albendazole sulphoxide (SOABZ) [48] (solute **13**), α-methylene γ-lactam cytotoxic agents [34] (solutes **18**) and sulphinamoyl CADH inhibitors [46] (solutes **11**). Contrary to all expectations, CSP 2 is also suitable for the separation of some π-acid derivatives of amino amides and esters (solutes **20**) [47]. This phenomenon has been taken into account in order to monitor the enantiomeric purity of CSs 1 and 2 prior to grafting them onto silica. Owing to the mild operating conditions, the racemization of CSs 1 and 2 had never occurred during their multi-step synthesis. A similar behaviour was expected for the other CS 3–6 candidates.

With CSPs 3 and 4, the scope of application of CSP 2 is extended from π-basic to π-acid racemates [38]. The initial scope of application of CSP 2 is maintained, but with lower selectivity owing to an intramolecular π–π interaction occurring within CSPs 3 and 4. Therefore, the CSP–solute π–π interaction is weakened, leading to lower selectivity (about a 10% decrease on average). Nevertheless baseline resolution are still achieved for compounds **8**–**19** [38]. In addition to their ability to resolve π-basic

TABLE I

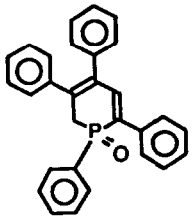
SCOPE OF APPLICATION OF CSP 1

The selectivity values indicated for each solute were obtained with *n*-hexane–ethanol mobile phases, otherwise the polar modifier is specified. Ref. 34 for solutes 1, 3, 4, 5 and 6; ref. 42 for solutes 2 and 7.

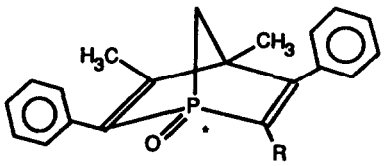


1 Phosphine oxides

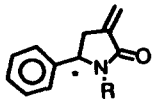
R_1 = methyl	R_2 = H
a R_2 = H 1.24	**h** R_1 = H 1.11
b R_2 = 2-methyl 1.22	**i** R_1 = methoxy 1.05
c R_2 = 4-methyl 1.32	**j** R_1 = *n*-propyl 1.23
d R_2 = 2-methoxy 1.27	**k** R_1 = allyl 1.26
e R_2 = 4-methoxy 1.23	
f R_2 = 2-OH 1.11	
g R_2 = 4-OH 1.26	



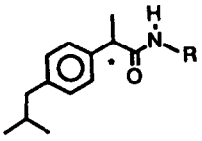
2 1.11



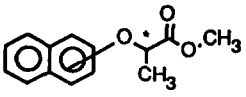
3 Phosphonorbornadiene oxides
a R = H 1.09
b R = methyl 1.33
c R = phenyl 1.07
d R = $COOC_2H_5$ 1.24



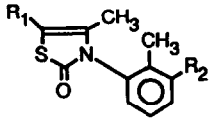
4 **a** R = phenyl 1.06
b R = 2-ethoxyphenyl 1.15
c R = 2,4-dimethoxyphenyl 1.24



5 Ibuprofen derivatives
a R = *p*-anisyl 1.10
b R = naphthyl 1.12



6 **a** 1-naphthyl 1.21 (CH_2Cl_2)
b 2-naphthyl 1.15 (CH_2Cl_2)

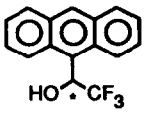


7 **a** R_1 = H, R_2 = methyl 1.08
b R_1 = H, R_2 = H 1.07
c R_1 = methyl, R_2 = H 1.07
d R_1 = methyl, R_2 = methyl 1.09

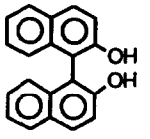
TABLE II

SCOPE OF APPLICATION OF CSP 2

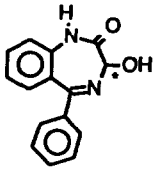
Mobile phase conditions as in Table I. Ref. 34 for solutes 8, 9, 10, 14, 16 and 18; ref. 46 for solutes 11; ref. 42 for solutes 12, 15 and 19; ref. 48 for solute 13; ref. 63 for solutes 17; ref. 47 for solutes 20.



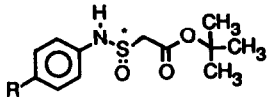
8 TFAE 1.26



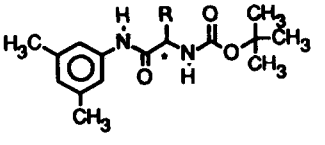
9 Binaphthol 1.13



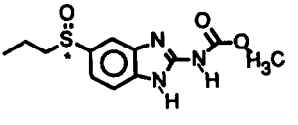
10 Oxazepam 1.53



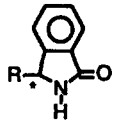
11 N-Aryl sulphinamoyl acetates
- **a** R = NO_2 1.72
- **b** R = CF_3 2.00 ($CHCl_3$)
- **c** R = Cl 2.00 ($CHCl_3$)
- **d** R = OCH_3 2.00 ($CHCl_3$)



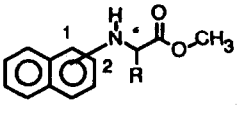
12 3,5-Dimethylanilide derivatives of *tert.*-Boc-amino acids
- **a** Alanine 1.31
- **b** Phenylalanine 1.35
- **c** Tryptophan 1.30
- **d** Tyrosine 1.32



13 Albendazole sulphoxide (anthelmintic) 1.18



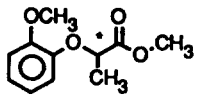
14 Lactams
- **a** R = *p*-anisyl 1.45
- **b** R = 1-naphthyl 1.07
- **c** R = isopropyl 1.07
- **d** R = cyclohexyl 1.15



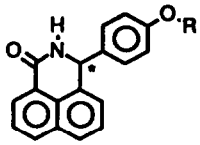
15 N-Naphthyl amino esters
- **a** 2-naphthylphenylalanine 8.83 (CH_2Cl_2)
- **b** 2-naphthylvaline 7.24 (CH_2Cl_2)
- **c** 1-naphthylvaline 9.69 (CH_2Cl_2)

(Continued on p. 376)

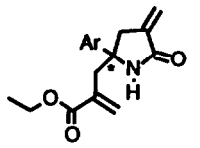
TABLE II *(continued)*



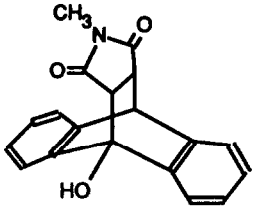
16 *o*-Anisyloxypropionic methyl ester
1.10 (CH_2Cl_2)



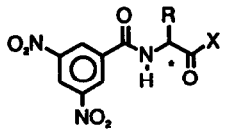
17 **a** R = methyl 1.57
b R = H 1.47
c R = glycidyloxy 1.61



18 α-Methylene γ-lactams (cytotoxic agents)
a Ar = phenyl 1.35
b Ar = 3,4,5-trimethoxyphenyl 1.24



19 1.25



20

X = NH-*n*-C_4H_9 3,5-DNB derivatives of amino *n*-butylamides	X = OCH_3 3,5-DNB derivatives of amino methyl esters
a O-allyltyrosine 1.51 ($CHCl_3$)	**f** leucine 2.01 (CH_2Cl_2)
b phenylalanine 1.40	**g** O-allyltyrosine 1.71 (CH_2Cl_2)
c phenylglycine 3.43 ($CHCl_3$)	**h** phenylglycine 1.18
d methionine 1.35	
e leucine 1.51	

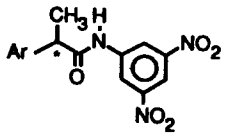
racemates, CSPs 3 and 4 afford good selectivity toward numerous π-acid racemates (Table III). It has been shown that the conformations of CSPs 3 and 4 result in CSPs displaying two distinct moieties (π-acid and π-basic), each suitable for discriminating enantiomers bearing a complementary π-character: a reversal of elution order occurred between CSP 3 and 4 (the absolute configuration of the asymmetric centre bearing the naphthyl group is inverted) for π-acid solutes, whereas no inversion occurred for π-basic racemates [38].

CSPs 5 and 6 were designed in order to obtain further insight into chiral recognition mechanisms [41]. With regard to CSP 1 and 2, these CSPs differ only in one potential site of interaction: the functional group directly bound to the asymmetric centre. The chromatographic data obtained with CSPs 1, 2, 5 and 6 allow the determination of the nature of the CSP–solute interaction occurring at this functional group [41].

TABLE III

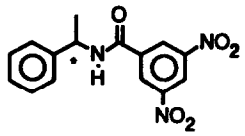
SCOPE OF APPLICATION OF CSPs 3 AND 4

Mobile phase conditions as in Table I. Ref. 38 for all solutes.

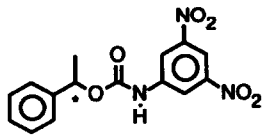


CSP 3

21 3,5-Dinitroanilide derivatives of 2-arylpropionic acids (anti-inflammatory drugs)
- **a** 2-phenylpropanoic acid 1.52
- **b** ibuprofen 1.80
- **c** naproxen 1.48
- **d** flurbiprofen 1.49
- **e** ketoprofen 1.29

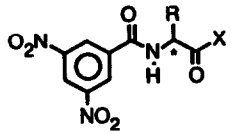


22 3,5-Dinitrobenzoyl derivative of phenylethylamine 1.32



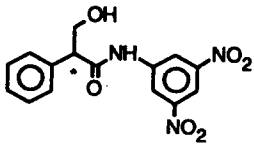
23 3,5-Dinitrophenylcarbamate derivative of phenylethanol 1.17

CSP 4

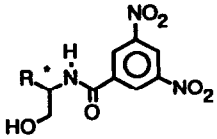


24 X = OCH_3
3,5-Dinitrobenzoyl derivatives of amino methyl esters
- **a** alanine 1.21
- **b** leucine 1.17
- **c** methionine 1.27
- **d** phenylalanine 1.34
- **e** tyrosine 1.31
- **f** valine 1.24

25 X = NH-*n*-C_4H_9
3,5-Dinitrobenzoyl derivatives of amino *n*-butyl amides
- **a** phenylalanine 1.15
- **b** phenylglycine 2.14



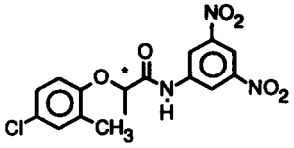
26 3,5-Dinitroanilide derivative of tropic acid 2.10



27 3,5-Dinitrobenzoyl derivatives of amino alcohols
- **a** R = phenyl 1.48
- **b** R = ethyl 1.13

(Continued on p. 378)

TABLE III *(continued)*

	**28**	3,5-Dinitroanilide derivative of CMPP (herbicide) 1.13

7. PREPARATIVE-SCALE CHROMATOGRAPHY

The direct preparative resolution of enantiomers by LC using chiral stationary phases provides a useful alternative to enantioselective synthesis or fractional crystallization of diastereomeric salts. Pirkle-type CSPs, owing to their easy preparation from readily available chiral materials and their good mechanical properties, are attractive candidates for use in large-scale preparative systems [59,60].

Preparative scale separations were carried out on CSPs 1 and 2 according to a linear behaviour [61,62], corresponding to the usual injection conditions in analytical chromatography. This method was chosen for difficult separations ($\alpha < 1.2$) where it is necessary to use a small particle size ($d_p = 7$ μm) to maintain the column efficiency. The main preparative applications performed in our laboratories are given in Table IV. An example of a preparative-scale separation is given in Fig. 15 [57].

The relatively low sample capacity of these CSPs (with respect to microcrystalline cellulose triacetate for instance) is expected to be partly overcome by using SFC. The high diffusion coefficients allow a satisfactory production rate to be maintained.

8. CONCLUSION

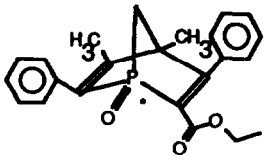
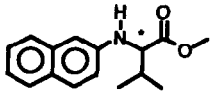
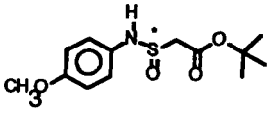
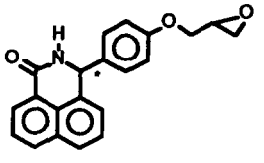
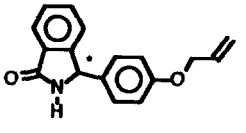
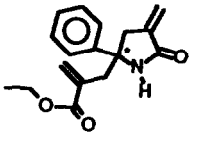
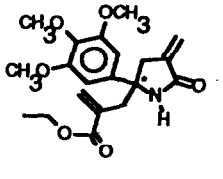
Starting from tyrosine, an entire family of CSPs was synthesized. Their properties mainly result from their specific grafting mode:

(1) With regard to conventional π-acid Pirkle-type CSPs (amidic grafting mode), the asymmetric centre of tyrosine is removed further from the silica matrix, thus limiting its unwanted steric hindrance contribution. Accordingly, the scopes of application of tyrosine-derived CSPs are different from those of DNBPG or DNBLeu, but these CSPs display the same type of interaction. It can be assumed that solutes bearing bulky groups are generally better resolved on tyrosine-derived CSPs than on DNBPG, which however displays a higher enantiorecognition ability toward small molecules. This can be closely related to the conformational flexibility–enantiorecognition ability relationships proposed by Lienne *et al.* [25].

(2) The carboxylic acid moiety of the amino acid, which was previously conventionally used for grafting the CS onto γ-aminopropylsilica remains free for further derivatization. Thus, so far six different CSPs have been prepared and evaluated [34,38,41]. This entails a wide potential scope of application: so far, more than twenty different families of solutes have been resolved. Further, a specific functional group may be readily introduced in the CSP in order to maximize the

TABLE IV

PREPARATIVE-SCALE APPLICATIONS OF CSPs 1 and 2

Solute	CSP	Injection	Ref.
	CSP 1 (200 g)	150 mg	56, 57
	CSP 2 (200 g)	1 g enrichment, initial S/R ratio = 92:8	42
	CSP 2 (200 g)	200 mg	46
	CSP 2 (200 g)	300 mg	63
	CSP 2 (200 g)	300 mg	57
	CSP 2 (200 g)	120 mg	25
	CSP 2 (200 g)	150 mg	57

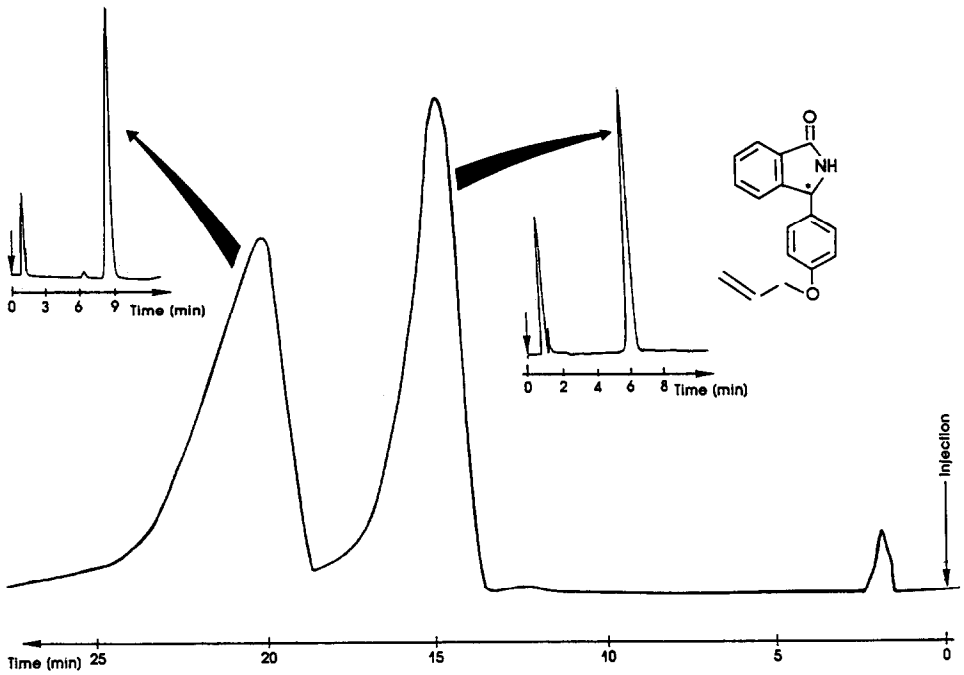


Fig. 15. Preparative-scale resolution of 300 mg of a derivative of solute **14a** (Table II) on 200 g of CSP 2, d_p = 7 μm. Operating conditions: mobile phase, *n*-hexane–ethanol (90:10, v/v); flow-rate, 42 ml/min; UV detection at 254 nm. The enantiomeric purity was checked on an analytical column (CSP 2) using the same mobile phase at 2 ml/min.

resolution of a given racemate. Thus, the introduction of chiral 1-(1-naphthyl)ethylamine has allowed the scope of application of earlier π-acid tyrosine-derived CSPs to be extended to π-acid racemates while keeping their enantiorecognition ability toward π-basic racemates [38].

(3) Very often, a ternary mobile phase optimization results in high resolutions per unit time. This can be very useful for decreasing retention times and increasing the throughput in preparative LC.

(4) Owing to the high stability of their grafting mode, tyrosine-derived CSPs are suited to classical mobile phases used with a silica matrix. The direct reversed-phase separation of pharmaceutical molecules is now under investigation in our laboratories. In addition, the use of modified carbon dioxide SFC allows higher resolutions per unit time to be achieved than with LC.

(5) The high efficiency and stability of tyrosine-derived CSPs allow their use for preparative-scale separations. Enantiomers of high purity have been prepared in the gram range by LC. Preparative applications using SFC are under investigation.

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