CHIRALITY 13:581–587 (2001)

Mechanism of Chiral Recognition in the Enantioseparation of 2-Aryloxypropionic Acids on New Brush-Type Chiral Stationary Phases

VLADIMIR VINKOVIC,[1] DARKO KONTREC,[1] VITOMIR SUNJIC,[1] LUCIANO NAVARINI,[2]*
FLAVIO ZANETTI,[2] AND ORNELLA AZZOLINA[3]

[1]*Ruder Boskovic Institute, Zagreb, Croatia*
[2]*Polytech scarl, Area Science Park, Trieste, Italy*
[3]*Dipartimento di Chimica Farmaceutica, Università di Pavia, Pavia, Italy*

ABSTRACT New brush-type chiral stationary phases (**CSP I–IV**) comprising N-3,5,6-trichloro-2,4-dicyanophenyl-L-α-amino acids (**1–4**) were prepared by binding of chiral selectors **1–4** to γ-aminopropyl silica gel. To check the role of excess free aminopropyl groups, **CSP V** was prepared by binding N-3,5,6-trichloro-2,4-dicyanophenyl-L-alanyl-(3-triethoxysilyl)propylamide to unmodified silica gel. The best separation of racemic 2-aryloxypropionic acids (**TR-1-13**) was obtained with **CSP I;** the -(–)-*S* enantiomer were regularly eluted first, as determined by a CD detector. The mechanism of chiral recognition implies a synergistic interaction of carboxylic acid analyte with the chiral selector and achiral free γ-aminopropyl units on silica. In fact, **CSP V,** which is lacking an achiral aminopropyl spacer, shows a lower separation ability for 2-aryloxypropionic acids, but a similar enantioselective discrimination of esters **TR-19-20,** in comparison with **CSP I. CSP I–IV** retain unaltered separation ability after a few months of continuous work using a large number of various mobile phases. *Chirality 13:581–581, 2001.*

KEY WORDS: chiral stationary phase; 2-aryloxypropionic acids; enantioseparation; HPLC

The group of chiral compounds based on 2-aryloxypropionic acids includes a number of important herbicides widely used for the pre- and postemergent control of broadleaf weeds in field grass, turf grass, and cereal crops. These compounds mimic the action of plant growth hormones (auxins) in certain types of broadleaf weeds causing abnormal growth and development of the plant. This unnatural development ultimately leads to the death of the plant.[1] It was recognized some time ago that for these chiral compounds only the (R)-enantiomers show herbicidal activity, and since the 1980s the enantiopure products have replaced the earlier-used racemic products in Switzerland and other countries.[2]

Several 2-aryloxypropionic acids also possess analgesic-antiinflammatory properties similar to those of ketoprofen or acetylsalicylic acid, but with less ulcerogenic activity.[3,4] In a recent study in which several 2-aryloxypropionic acids were investigated as potential analgesic-antiinflammatory drugs, it has been reported that the activity of one of the enantiomers is superior to that of the racemates.[5] Furthermore, some 2-aryloxyalcanoic acids were found to exhibit noteworthy antilipidemic effects and/or inhibit prostaglandin-dependent human platelet aggregation.[6,7]

Due to the importance of chiral 2-aryloxypropionic acids, both for agrochemical as well as for pharmaceutical applications, it is clear that their enantioseparation is a topic that has stimulated several research groups.

Chiral resolution of racemic 2-aryloxypropionic acids has been achieved by using several methods. In addition to the fractional crystallization of diastereomeric salts with cyinchonidine,[5] the enzymatic stereoselective hydrolysis of methyl ester racemates,[8,9] capillary electrophoresis,[10–12] and chromatographic enantioseparations on chiral stationary phases (CSPs) have been described. In particular, enantioseparations by high-resolution gas chromatography on cyclodextrin derivatives based CSPs[2] as well as high-performance liquid chromatography (HPLC) have been reported. The HPLC CSPs which have been used to separate mainly derivatized but also underivatized 2-aryloxypropionic acids include teicoplanin,[1] N-3,5-dinitrobenzoylphenylglycine,[13–17] brush-type containing π acidic 3,5-dinitrobenzoyl groups and two adjacent stereogenic centers[6,18–20], α_1-acid glycoprotein[14,16] cellulose derivatives[21,22], CSP derived from tartaric acid[23] and quinine derived ion exchange type CSPs.[24]

Herein we report on the preparation and application of some novel and recently described[25] brush-type CSPs for

*Correspondence to: L. Navarini, Polytech scarl, Area Science Park, Trieste, Italy. E-mail: navarini@polytech3.area.trieste.it
Received for publication 17 November 2000; Accepted 10 April 2001

HPLC enantioseparation of 2-aryloxypropionic acids. The mechanism of chiral recognition is discussed and evidence for cooperative effects of the achiral and chiral components of the CSPs in the enantiorecognition process are presented.

MATERIALS AND METHODS

Chemicals

The reagents were supplied as follows: 2,4,5,6-tetrachloro-1,3-benzene dicarbonitrile (TCBDC) by Caffaro S.p.A. (Italy); N,N′-dicyclohexylcarbodiimide (DCC), and 3-aminopropyltriethoxysilane (APTES) by Sigma-Aldrich (Sigma-Aldrich, Italy); Nucleosil 100-5 NH_2 (specific surface area 350 m^2/g, bonded phase coverage 2.5 µmol/m^2, calculated coverage density 1.51 groups/nm^2); and Nucleosil-100-5 from Macherey-Nagel (Delchimica, Italy). All solvents were pro analysis grade, and purchased from J.T. Baker (Deventer, Holland). TLC was performed on Merck's (Darmstadt, Germany) DC-Alufolien with Kieselgel 60_{254}.

IR spectra were obtained on KBr pellets using a Perkin Elmer M 137 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Varian XL-GEM 300 for $CDCl_3$ solutions, if not stated otherwise; shifts are given in ppm downfield from TMS as an internal standard. Melting points were determined with Electrothermal 9100 apparatus. Elemental analyses were carried out at the Central Analytical Service (CAS), Ruder Boškovic Institute.

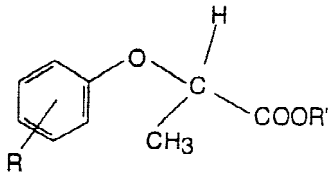
Racemate **TR-1** (Mecoprop) was supplied by IRIS Technologies, L.L.C. (Lawrence, KS, USA), racemates **TR-14–TR-18** were prepared by condensation of the substituted phenol with ethyl (or methyl) 2-bromopropionate and the acids (**TR-2–TR-13**) by hydrolysis of the corresponding esters.[5] Racemates **TR-19** (diclofop methyl) and **TR-20** (fenoxaprop ethyl) were supplied by Macherey-Nagel (Düren, Germany) as a gift. The chemical structures of analyzed racemic samples are reported in Table 1.

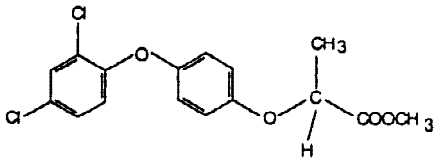
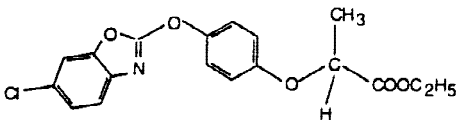
Chromatography

HPLC was performed with a Knauer WellChrom Maxi-Star K-1000 pump (Knauer GmbH, Berlin, Germany) using a Knauer HPLC 6-port-valve injector with a 20 µl loop and with a Waters 510 HPLC pump using a Waters U6K injector (Waters, Milano, Italy). Detection was achieved at 254 nm with a Knauer WellChrom K-2500 detector and with a Jasco CD-1595 circular dichroism (CD) detector permitting simultaneous UV and CD detection (Jasco Europe s.r.l., Lecco, Italy). Integration of the chromatograms was made with the BDS software package (Barspec, Rehovot, Israel) and with the Chromstar LC22 software package, v. 3.12 (Bruker Spectrospin, Milan, Italy). The packing of HPLC columns, purchased from Max Stevenson (Berlin, Germany; dimensions: 250 mm length × 4.6 mm I.D.), was carried out by a slurry technique using a Knauer pneumatic HPLC-pump. n-Hexane, 2-propanol, and other solvents used for HPLC were analytical grade from J. T. Baker and redistilled before use.

The analytic samples were prepared by dissolving 1 mg of racemate in 1 ml of 2-propanol; 5 µl of this solution was used for analysis. The enantioselective efficacy of the columns was evaluated for four racemates, **TR-1–TR-4** (Table 2). On the basis of retention times of the enantiomers (t_{R1} and t_{R2}), the following chromatographic parameters were calculated: retention factors (k'_1 and k'_2), separation factor (α) and resolution factor (R_S). The determination of the dead volume of the columns (t_0) was performed by using 1,3,5-tri-*tert*-butylbenzene.

TABLE 1. Chemical structures of analyzed racemic samples



Test racemate	R′	R
TR-1	H	2-CH_3, 4-Cl
TR-2	H	3-Cl
TR-3	H	3-C_6H_5CO
TR-4	H	4-C_6H_5CO
TR-5	H	2-C_3
TR-6	H	3-C_3
TR-7	H	4-C_3
TR-8	H	4-CH_2-CH_3
TR-9	H	2-Cl
TR-10	H	2,5-$(CH_3)_2$
TR-11	H	2,6-$(CH_3)_2$
TR-12	H	4-$C_6H_5CH_2$
TR-13	H	4-(2,4-$F_2C_6H_3$)
TR-14	-CH_3	3-CH_3
TR-15	-CH_3	4-CH_3
TR-16	-CH_3	2-Cl
TR-17	-CH_2-CH_3	2,6-$(CH_3)_2$
TR-18	-CH_3	4-(2,4-$F_2C_6H_3$)
TR-19		
TR-20		

Syntheses

Preparation of **CSP I** and **CSP II** have been described[25] and repeated for the present study. **CSP I:** Anal. Found: C 6.42, H 1.09, N 2.29%, based on the N-content, 0.221 mmol selector/g silica are bound. **CSP II:** Anal. Found: C 6.12, H 0.99, N 2.11%, based on the N-content, 0.197 mmol selector/g silica are bound.

Preparation of CSP III and CSP IV (Scheme 1).

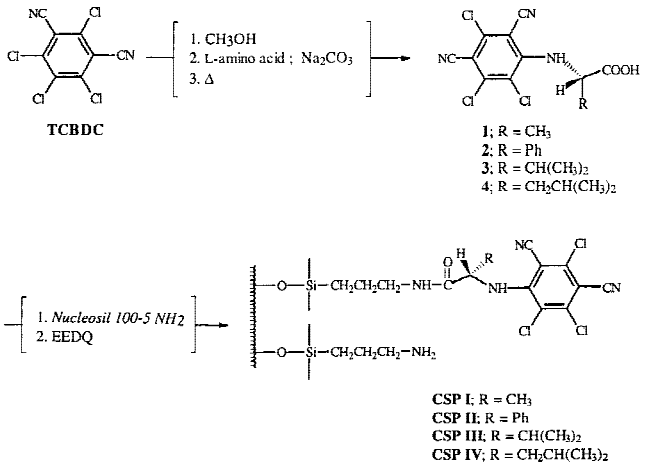
(2S)-3-Methyl-2-[(3,5,6-trichloro-2,4-dicyanophenyl)amino]butanoic acid (3). To a suspension of TCBDC (5.0 g,

TABLE 2. HPLC resolution of analytes (TR-1–TR-4) on columns filled with stationary phases CSP 1–IV*

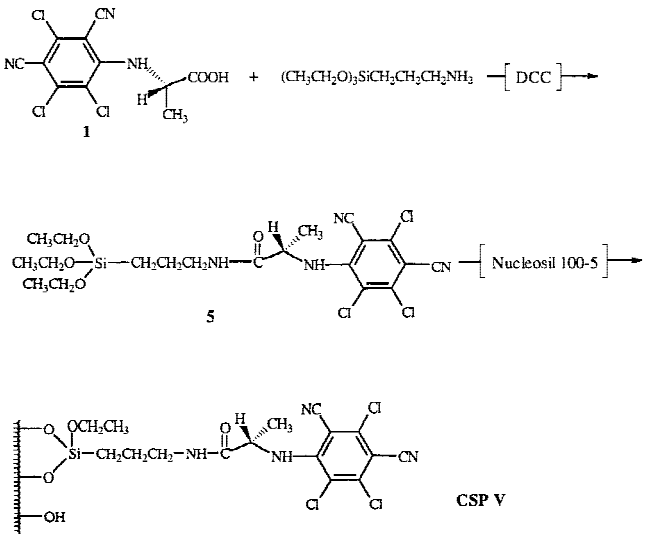
Stationary phase	Compound	k'_1	α	R_s
CSP I	TR-1	0.63	1.43	1.28
	TR-2	4.35	1.16	1.35
	TR-3	4.74	1.11	0.96
	TR-4	6.13	1.21	1.26
CSP II	TR-1	8.03	1.11	0.76
	TR-2	5.53	1.07	0.88
	TR-3	12.18	1.03	0.63
	TR-4	3.87	1.12	0.54
CSP III	TR-1	1.37	1.04	nm
	TR-2	2.68	1	—
	TR-3	6.56	1	—
	TR-4	9.43	1.03	nm
CSP IV	TR-1	1.86	1	—
	TR-2	2.03	1	—
	TR-3	3.95	1	—
	TR-4	6.07	1	—

*Conditions were not optimized.
Mobile phase: 98% n-hexane/2-propanol (8:2 v/v), 2% acetic acid (v/v); flow rate 1 ml/min. nm = not measurable.

18.8 mmol) in MeOH (50 ml) a hot solution of L-valine sodium salt, prepared by dissolution of L-valine (4.40 g, 37.6 mmol) in 50 ml of Na_2CO_3 solution (5.20 g, 37.6 mmol) was added. After 1.5 h of stirring at 90°C (bath temperature), the solution was filtered through a cotton plug, cooled, and washed with CH_2Cl_2 (2 × 50 ml). Aqueous phase was adjusted to pH 1 by adding 200 ml of 1M hydrochloric acid and extracted with CH_2Cl_2 (2 × 50 ml). Obtained was 4.23 g (65%) of pale-yellow oil. IR: 3400, 2960, 2220, 1730, 1640, 1570, 1510, 1210, 1060, 850, 760, 730, 710 cm^{-1}. ^1H NMR (acetone-d_6): 0.97 (3H, d, J = 6.6 Hz), 1.18 (3H, d, J = 6.6 Hz), 5.00-5.15 (1H, m,), 6.71 (2H, d, J = 7.9 Hz), 9.40 (1H, bs). ^{13}C NMR (acetone-d_6): 16.79, 17.20, 31.52, 60.97, 95.89, 103.56, 113.06, 114.11, 120.45, 139.14, 141.69, 149.00, 171.19. Anal. Calcd. for $C_{13}H_{10}N_3O_2Cl_3$ (mw 346.58): C 45.04, H 2.90, and N 12.12%. Found: C 45.21, H 3.14, and N 12.09%.



Scheme 1. Synthetic route for preparation of **CSP I-IV.**



Scheme 2. Synthetic route for preparation of **CSP V.**

(2S)-4-Methyl-2-[(3,5,6-trichloro-2,4-dicyanophenyl)amino]pentanoic acid (4). Starting from L-leucine (4.93 g, 37.6 mmol), the reaction and isolation of the product was performed as described for 3. Obtained was 5.18 g (76%) of pale-yellow oil. IR: 3380, 2960, 2220, 1740, 1705, 1570, 1505, 1380, 1335, 1200, 1160, 1110 cm^{-1}. ^1H NMR (acetone-d_6): 0.97 (3H, d, J = 6.8 Hz), 1.02 (3H, d, J = 6.8 Hz), 1.84-2.10 (2H, m), 5.22-5.62 (1H, m), 6.64 (1H, d, J = 7.6 Hz), 9.10 (2H, bs). ^{13}C NMR (acetone-d_6): 21.41, 22.45, 25.05, 41.33, 55.89, 95.93, 103.71, 113.05, 114.11, 120.35, 139.16, 141.65, 150.01, 172.43. Anal. Calcd. for $C_{14}H_{12}N_3O_2Cl_3$ (mw 360.61): C 46.62, H 3.35, and N 11.65%. Found: C 46.71, H 3.51, and N 11.58%.

Chiral stationary phase ***CSP III.*** Silicagel Nucleosil 100-5 NH_2 (2.15 g; C 3.49%, N 1.36%) was slurried in dry THF (10 ml), then compound **3** (0.30 g, 0.9 mmol), and EEDQ (0.23 g, 0.9 mmol) were added and the reaction mixture stirred for 16 h at room temperature. Solid material was collected on filter, washed with MeOH (50 ml), and dried at 70°C for 4 h. Obtained was 2.27 g of **CSP III.** Anal. Found: C 5.77, H 1.03, N 2.14, and Cl 2.67%. Based on the N-content, 0.186 mmol selector/g silica are bound.

Chiral stationary phase ***CSP IV.*** Starting from 5.1 g of Nucleosil 100-5 NH_2, compound 4 (0.89 g, 2.48 mmol) and EEDQ (0.61 g, 2.48 mmol) in dry THF (25 ml), 5.4 g of **CSP IV** were obtained. Anal. Found: C 5.94, H 1.28, N 2.11, and Cl 2.62%. Based on the N-content, 0.178 mmol selector/g silica are bound.

Preparation of CSP V (Scheme 2).

(2S)-2-[(3,5,6-Trichloro-2,4-dicyanophenyl)amino]-N-(3,3,3-triethoxy-3-silapropyl)propanamide (5). A solution of compound **1** (1.02 g, 3.2 mmol) in CH_2Cl_2 (15.0 ml) was mixed with a solution of DCC (0.66 g, 3.2 mmol) in CH_2Cl_2 (10.0 ml), and then a solution of APTES (0.71 g, 3.2 mmol) in CH_2Cl_2 (85.0 ml) was added dropwise. After 2 h of stirring at room temperature the reaction mixture was filtered through a cotton plug, filtrate evaporated to dryness, and crude product

dissolved in toluene (5.0 ml). Chromatography on silica gel, using toluene-acetone (9:1 v/v), afforded 1.18 g (71%) of pure **5** as a pale-yellow oil. ^{1}H NMR ($CDCl_3$): 0.66 (2H, t, J = 6.6 Hz), 1.23 (9H, t, J = 7.0 Hz), 1.58 (3H, d, J = 6.5 Hz), 1.62-1.81 (2H, m), 2.33 (1H, d, J = 6.6 Hz), 3.31-3.36 (2H, m), 3.84 (6H, q, J = 6.9 Hz), 4.90-5.01 (1H, m), 6.46-6.52 (1H, m). ^{13}C NMR ($CDCl_3$): 7.66, 18.13, 21.31, 22.40, 42.17, 51.85, 58.42, 93.91, 103.15, 112.95, 114.35, 120.29, 125.82, 141.82, 147.92, 170.69. Anal. Calcd. for $C_{20}H_{27}N_4O_4Cl_3Si$ (mw 521.88): C 46.02, H 5.21, and N 10.73%. Found: C 46.18, H 5.56, and N 10.68%.

Chiral stationary phase ***CSP V.*** Nucleosil 100-5 (4.15 g) and compound 5 (0.80 g, 1.5 mmol) were heated under reflux in dry toluene (10 ml) for 24 h. Solid product was collected on filter, washed with toluene (50 ml) and methanol (50 ml), and dried at 70°C. Obtained was 4.46 g of **CSP V.** Anal. Found: C 5.52, H 1.28, N 1.89%. Based on the C, N-content, 0.310 mmol selector/g silica are bound.

RESULTS AND DISCUSSION

Regioselective functionalization of 2,4,5,6-tetrachloro-1,3-dicyanobenzene by nucleophilic substitution of the chlorine atom at C(4) with L-α-amino acid residue was used to prepare chiral selectors **1** and **2.**[25] Their binding to aminopropyl silica gel afforded chiral stationary phases **CSP I** and **CSP II,** respectively (see Scheme 1).

The evaluation of these CSPs on some test racemates[25] revealed the possibility to use N-3,5,6-trichloro-2,4-dicyanophenyl derivatives of α-amino acids as an alternative to the well known chiral selectors based on N-(3,5-dinitrobenzoyl) derivatives (DNB) of α-amino acids developed by Pirkle et al.[26,27] Our chiral selectors differ in the functional group connecting the chiral center of the π-acid substituted aromatic ring. Specifically, the hydrogen donating–accepting amide group between the chiral center and the π-acid substituted aromatic ring of the DNB-α-amino acids-derived CSPs is replaced by a hydrogen-donating, weakly acidic NH group in the N-3,5,6-trichloro-2,4-dicyanophenyl derivatives of α-amino acids based chiral selectors. Since the functional group connecting the chiral center to the aromatic ring in 2-aryloxypropionic acids is an ether oxygen with a free electron pair available for hydrogen bonding, it can be hypothesized that these racemates are more favorable for enantiorecognition by CSPs based on N-3,5,6-trichloro-2,4-dicyanophenyl derivatives of α-amino acid than by DNB-α-amino acid CSPs.[13]

In fact, brush-type CSPs based on DNB-α-amino acids are not able to effectively separate underivatized 2-aryloxypropionic acids[14,18,28] and this may be indirectly explained according to the interaction model for (R)-N-3,5-dinitrobenzoylphenylglycine CSP and an (S)-2-aryloxypropionic acid methyl ester proposed by Dernoncour and Azerad.[13] In order to check the above-mentioned hypothesis, we tested **CSP I** and **CSP II** with the four test racemates **TR-1–4** shown in Table 1. As shown in Table 2, both **CSP I** and **CSP II** separate the underivatized 2-aryloxypropionic acids, the best separations being obtained with **CSP I,** derived from L-alanine (Fig. 1).

These findings prompted us to prepare **CSP III** and **CSP IV** containing as chiral selectors N-3,5,6-trichloro-2,4-dicyanophenyl-L-valine (3) and N-3,5,6-trichloro-2,4-dicyanophenyl-L-leucine (**4**), respectively (Scheme 1). In spite of the fact that in DNB-α-amino acid-derived CSPs phenylglycine generally performs better than valine or leucine for a variety of racemates,[29] it cannot be excluded a priori a different behavior for our CSPs. The experimental results obtained by screening the test racemates **TR-1–4** on **CSP III** and **CSP IV** (Table 2) show that more bulky substituents on the chiral center reduce enantioseparation. We therefore focused our attention on **CSP I** by testing a number of 2-aryloxypropionic acids and some methyl- and ethyl-esters reported in Table 1.

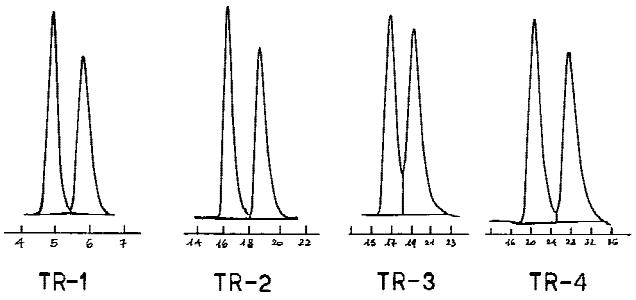


Fig. 1. Chromatograms obtained for samples **TR-1–TR-4** on column filled with **CSP I.** Column dimensions: 250 mm length × 4.6 mm I.D.; mobile phase: 98% n-hexane/2-propanol (8:2, v/v), 2% acetic acid (v/v). Conditions are not optimized.

In order to find a relationship between the elution order and the absolute configuration of the enantiomers, HPLC separation was also monitored by circular dichroism (CD) detector at 254 nm and exemplary chromatograms are given in Figure 2.

It has been previously reported that the CD spectra of (+)-(R)-enantiomers of 2-aryloxypropionic acids are characterized by a positive Cotton effect in the region between 240 and 300 nm.[5] Accordingly, the first eluted peak is the (–)-(S)-enantiomer and the second eluted peak is the (+)-(R)-enantiomer; this elution order has been observed in all cases. Two different mobile phases were used to separate 2-aryloxypropionic acids and their esters: in particular, a weak carboxylic acid was added to the eluent used for the separation of 2-aryloxypropionic acids to suppress the analyte ionization process. The results reported in Table 3 indicate the versatility of **CSP I** in resolving 2-aryloxypropionic acids and 2-aryloxypropionates, the former being systematically better resolved than the latter.

When in the mobile phase used for enantioseparation of 2-aryloxypropionic acids trifluoroacetic acid (TFA) was substituted for acetic acid, the retention times substantially decreased and chiral resolution was completely eliminated.

The low coverage density of **CSP I** (0.38 groups/nm^2 calculated on the basis of the amount of the chiral selector and of the specific surface area of the silica) suggests a specific role of unreacted aminopropyl groups in the enantiorecognition process. It can be assumed that 2-aryloxypropionic acids (pKa 3.2–3.4[11]) protonate the residual primary amino groups of the spacer, which enables the "anchor and align" interactions with the molecules of the analyte and their approach to the chiral selector. This interaction is eliminated when the strong acid trifluoroacetic acid is added; it completely protonates residual aminopro-

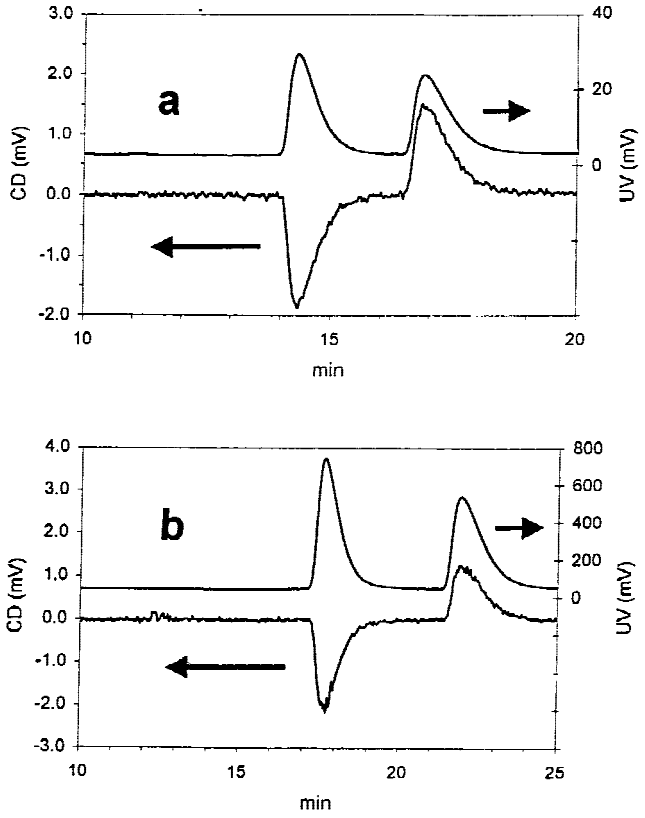


Fig. 2. HPLC resolution of samples **TR-6 (a)** and **TR-13 (b)** monitored by UV and circular dichroism (CD) simultaneous detection. Experimental conditions: see text.

TABLE 3. HPLC resolution of analytes (TR-5–TR-20) on column filled with stationary phase CSP I[1]

Compound	Mobile phase[2]	k'_1	α	Rs
TR-5	A	1.96	1.17	1.30
TR-6	A	3.34	1.23	1.85
TR-7	A	3.55	1.23	1.86
TR-8	A	2.45	1.21	1.76
TR-9	A	4.74	1.10	0.97
TR-10	A	1.48	1.19	1.43
TR-11	A	1.75	1.12	1.07
TR-12	A	3.92	1.13	1.29
TR-13	A	4.34	1.30	2.53
TR-14	B	1.20	1.08	0.89
TR-15	B	1.24	1.09	0.86
TR-16	B	1.37	1.05	nm
TR-17	B	0.74	1.00	—
TR-18	B	1.46	1.16	1.54
TR-19	B	1.87	1.04	0.57
TR-20	B	2.75	1.12	1.15

[1]Conditions were not optimized.
[2]A: 98% n-hexane/2-propanol (8:2 v/v), 2% acetic acid (v/v); flow rate 1 ml/min.
B: n-hexane/2-propanol 99.5:0.5 (v/v), flow rate 1 ml/min. nm = not measurable.

TABLE 4. HPLC resolution of some analytes on columns filled with stationary phases CSP V and CSP I[1]

Stationary phase	Compound	Mobile phase[2]	k'_1	α	R_s
CSP V	TR-1	A	0.59	1	—
	TR-2	A	0.74	1.02	nm
	TR-3	A	0.61	1	—
	TR-4	A	0.66	1.03	0.54
	TR-19	B	1.08	1.04	nm
	TR-20	B	2.12	1.12	1.07
CSP I	TR-19	B	1.87	1.04	0.57
	TR-20	B	2.75	1.12	1.15

[1]Conditions were not optimized.
[2]A: 98% n-hexane/2-propanol (8:2 v/v), 2% acetic acid (v/v); flow rate 1 ml/min.
B: n-hexane/2-propanol 99.5:0.5 (v/v), flow rate 1 ml/min. nm = not measurable.

pyl groups and suppresses their interactions with acidic analytes.

In order to provide further evidence supporting this hypothesis, **CSP V** was prepared by the route presented in Scheme 2.

CSP V contains the same chiral selector as **CSP I,** but no unreacted aminopropyl groups. As reported in Table 4, this CSP exhibits very low chiral separation for the four test racemates **TR-1–4,** whereas esters of some 2-aryloxypropionic acids exhibit enantioseparation similar to **CSP I** (see Fig. 3).

The mechanism of chiral recognition of 2-aryloxypropionic acids on **CSP I** seems to include the following interactions: formation of ion pairs (Coulomb interactions) between analyte carboxylic groups and residual unreacted aminopropyl groups, π–π interactions between π-acid persubstituted benzene ring and π-basic oxyaromatic ring, hydrogen bonding between the amino group of the chiral selector and free electron pair of ethereal oxygen in the analyte, and Van der Waals interactions between the groups on the chiral centers (Fig. 4).

An ionic interaction between analyte carboxylic groups and residual unreacted aminopropyl groups may be suggested as cooperative with chiral recognition, since it drives the analyte in proximity to the chiral selector, as schematically presented in Figure 4a. In **CSP V** such ionic interactions are absent (see Fig. 4b), which makes the enantiorecognition process for 2-aryloxypropionic acids less effective but does not affect the chiral recognition of 2-aryloxypropionic acid esters.

To the best of our knowledge, this is the first example in which an achiral free γ-aminopropyl unit on silica is directly involved in the process of chiral recognition. Several authors reported on the negative role played by residual unreacted polar groups on the silica surface in the chiral recognition process.[30–33] When residual aminopropyl groups have been suggested to be beneficial for enantioselectivity, no mechanism has been proposed.[34]

It is interesting to note that in a recent report Balsells and Walsh[35] demonstrated that achiral ligands can promote chiral catalysis by organometallic complexes. This

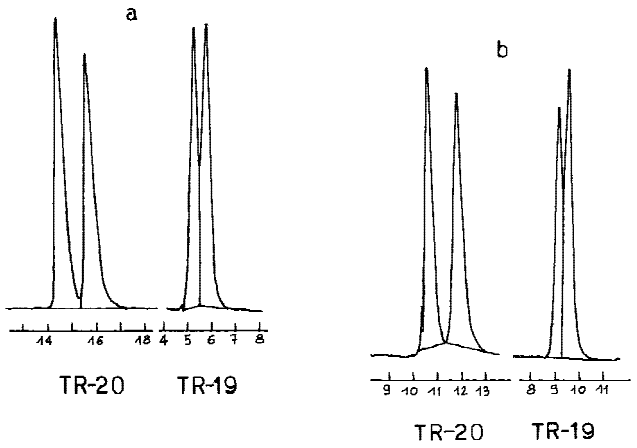


Fig. 3. Chromatograms obtained for samples **TR-19** and **TR-20** on columns filled with **CSP I (a)** and **CSP (b).** Column dimensions: 250 mm length × 4.6 mm I.D.; mobile phase: 99.5% n-hexane/0.5% 2-propanol (v/v); flow rate 1 ml/min. Conditions are not optimized.

strategy, called by the authors "amplification of the chiral environment," seems amenable to broader application, practically whenever chiral recognition is an issue. In this context it is also interesting to mention that the ionic form (Li-salt) of 2-aryloxypropionic acids is more effective for chiral recognition in the lipase-catalyzed enantioselective esterification.[36]

In conclusion, we have shown that resolution efficacy of new CSPs in the enantioseparation of 2-aryloxypropionic acids is based on the "anchoring" of analyte by achiral spacer driving the process of chiral selectivity. We are currently exploring the cooperative effect of various achiral and chiral components in the stationary phases aimed at improving chromatographic enantioseparation.

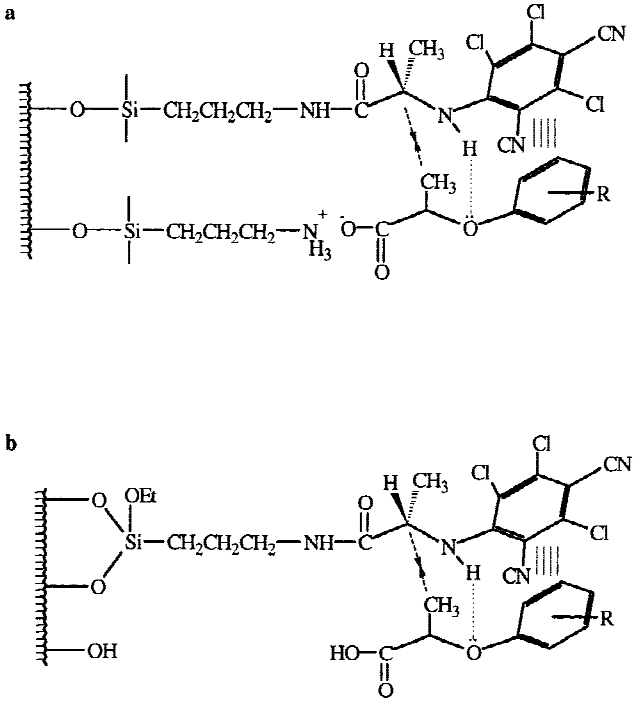


Fig. 4. Possible chiral recognition mechanism for resolution of 2-aryloxypropionic acids on **CSP I (a)** and **CSP V (b).**

ACKNOWLEDGMENTS

We thank Jasco Europe s.r.l. (Lecco, Italy) for giving us the opportunity to use their CD-1595 circular dichroism detector. We thank Ettore Castiglioni (European Chirality Services, Lecco, Italy) and Ahmed Aced (IRIS Technologies L.L.C., Lawrence, KS, USA) for technical advice.

LITERATURE CITED

1. Schneiderheinze JM, Armstrong DW, Berthold A. Plant and soil enantioselective biodegradation of racemic phenoxyalkanoic herbicides. Chirality 1999;11:330–337.
2. Buser HR, Muller MD. Enantioselective analyses of persistent and modern pesticides. A step toward sustainable agriculture. Chimia 1997; 51:694–700.
3. Marzo A, Quadro G, Bruno G, Treffner E, Reali FL, Borrelli G, Stradi R. MR714, a new aryloxypropionic non-steroidal antiinflammatory agent. Arzneim Forsch 1983;33:198–205.
4. Astoin J, Lepage F, Fromantin JP, Poisson M. Proprietes analgesiques et anti-inflammatoires d'acides m-benzoyl phenoxy alcanoiques. Eur J Med Chem Chim Ther 1980;15:457–462.
5. Azzolina O, Collina S, Vercesi D, Ghislandi V, Bonabello A, Galmozzi MR. Chiral resolution, configurational study and pharmacological profile of 2-phenoxypropionic acids. Il Farmaco 1997;52:449–456.
6. Bettoni G, Ferorelli S, Loiodice F, Tangari N, Tortorella V, Gasparrini F, Misiti D, Villani C. Chiral α-substituted α-aryloxy acetic acids: synthesis, absolute configuration, chemical resolution, and direct separation by HPLC. Chirality 1992;4:193–203.
7. Romstedt KJ, Lei LP, Feller DR, Witiak DT, Loiodice F, Tortorella V. Differential eudismic ratios in the antagonism of human platelet function by phenoxy- and thiophenoxyacetic acids. Il Farmaco 1996;51:107–114.
8. Azzolina O, Vercesi D, Collina S, Ghislandi V. Chiral resolution of methyl 2-aryloxypropionates by biocatalitic stereospecific hydrolysis. Il Farmaco 1995;50:221–226.
9. Azzolina O, Collina S, Vercesi D. Stereoselective hydrolysis by esterase: a strategy for resolving 2-(R-R′-phenoxy)propionyl ester racemates. Il Farmaco 1995;50:725–733.
10. Garrison AW, Schmitt P, Kettrup A. Separation of phenoxy acid herbicides and their enantiomers by high-performance capillary electrophoresis. J Chromatogr A 1994;688:317–327.
11. Otsuka K, Smith CJ, Grainger J, Barr JR, Patterson DG, Tanaka N, Terabe S. Stereoselective separation and detection of phenoxy acid herbicide enantiomers by cyclodextrin-modified capillary zone electrophoresis-electrospray ionization mass spectrometry. J Chromatogr A 1998;817:75–81.
12. Schmitt Ph, Garrison AW, Freitag D, Kettrup A. Application of cyclodextrin-modified micellar electrokinetic chromatography to the separations of selected neutral pesticides and their enantiomers. J Chromatogr A 1997;792:419–429.
13. Dernoncour R, Azerad R. High-performance liquid chromatographic separation of the enantiomers of substituted 2-aryloxypropionic acid methyl esters. J Chromatogr 1987;410:355–361.
14. Blessington B, Crabb N, O'Sullivan J. Chiral high-performance liquid chromatographic studies of 2-(4-chloro-2-methylphenoxy)propanoic acid. J Chromatogr 1987;396:177–182.
15. Blessington B, Crabb N. Chiral high-performance liquid chromatographic analysis of phenoxy herbicide mixtures. J Chromatogr 1988; 454:450–454.
16. Blessington B, Crabb N. Proposed primary reference methods for the determination of some commercially important chiral aryloxypropionate herbicides in both free acid and ester forms. J Chromatogr 1989; 483:349–358.

17. Azzolina O, Ghislandi V. Antiphlogistic aryloxypropionic acids: configurational study. Il Farmaco 1993;48:713–724.
18. Pirkle WH, Lee W, Welch CJ. Chromatographic separation of the enantiomers of 2-aryloxypropionic acids, esters and amides. Enantiomer 1997;2:423–431.
19. Lee W. Liquid chromatographic resolution of N-1- and 2-naphtylamide derivatives of 2-aryloxypropionic acids. Bull Korean Chem Soc 1998; 19:913–916.
20. Uray G, Maier NM. Diphenylethanediamine (DPEDA) derivatives as chiral selectors: VI. Enantioseparation of underivatized aryl substituted carboxylic acids on four differently linked N-3,5-dinitrobenzoyl-DPEDA derived CSPs. Enantiomer 1996;1:211–217.
21. Okamoto Y, Aburatani R, Kaida Y, Hatada K. Direct optical resolution of carboxylic acids by chiral HPLC on tris(3,5-dimethylphenylcarbamate)s of cellulose and amylose. Chem Lett 1998:1125–1128.
22. Azzolina O, Collina S, Ghislandi V. Optical resolution of aryloxypropionic acids and their esters by HPLC on cellulose tris-3,5-dimethyltriphenylcarbamate derivative. Il Farmaco 1993;48:1401–1416.
23. Muller MD, Bosshardt HP. Enantiomer resolution and assay of propionic acid-derived herbicides in formulations by using chiral liquid chromatography and achiral gas chromatography. J Assoc Off Anal Chem 1988;71:614–617.
24. Lammerhofer M, Lindner W. Quinine and quinidine derivatives as chiral selectors. Brush type chiral stationary phases for high performance liquid chromatography based on cinchonan carbamates and their application as chiral anion exchangers. J Chromatogr A 1996;741: 33–48.
25. Kontrec D, Vinkovic V, Šunjic V. Preparation and evaluation of the chiral stationary phases based on N-4-(2,5,6-trichloro-1,3-dicyano)-phenyl derivatives of L-α-amino acids. J Liquid Chrom Rel Technol 2000;23:1203–1215.
26. Pirkle WH, House DW, Finn JM. Broad spectrum resolution of optical isomers using chiral high-performance liquid chromatographic bonded phases. J Chromatogr 1980;192:143–158.
27. Pirkle WH, Finn JM, Schreiner JL, Hamper BC. A widely useful chiral stationary phase for high-performance liquid chromatography separation of enantiomers. J Am Chem Soc 1981;103:3964–3966.
28. Perrin SR, Pirkle WH. In: Ahuja S, editor. Chiral separation by liquid chromatography. ACS Symposium Series 1991;471:43.
29. Welch CJ. Evolution of chiral stationary phase design in the Pirkle laboratories. J Chromatogr A 1994;666:3–26.
30. Pirkle WH, Readnour RS. The influence of end-capping on the enantioselectivity of a chiral phase. Chromatographia. 1991;31:129–132.
31. Pirkle WH, Welch CJ. Effect of superfluous remote polar functionality on chiral recognition. J Chromatogr 1992;589:45–51.
32. Liu Y, Svec F, Frechet JMJ, Juneau KN. Polymer- versus silica-based separation media: elimination of nonspecific interactions in the chiral recognition process through functional polymer design. Anal Chem 1997;69:61–65.
33. Kuroda Y, Kato T, Ogoshi H. Liquid chromatography with aminopropylsilica gel modified by heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin derivative. Bull Chem Soc Jpn 1993;66:1116–1120.
34. Ihara T, Sugimoto Y, Asada M, Nakagama T, Hobo T. Influence of the method of preparation of chiral stationary phases on enantiomer separations in high-performance liquid chromatography. J Chromatogr A 1995;694:49–56.
35. Balsells J, Walsh PJ. The use of achiral ligands to convey asymmetry: chiral environment amplification J Am Chem Soc 2000;122:1802–1803.
36. Okamoto T, Ueji S. Drastic enhancement of the enantioselectivity of lipase-catalysed esterification in organic solvents by the addition of metal ions. Chem Commun 1999;939–940.