Journal of Chromatography A, 666 (1994) 27–40
Elsevier Science B.V., Amsterdam

CHROM. 25 398

Evaluation of Pirkle-type chiral stationary phases by liquid and supercritical fluid chromatography

Influence of the spacer length and the steric hindrance in the vicinity of the stereogenic centre

N. Bargmann-Leyder

Laboratoire de Chimie Analytique (unité de recherche associé au CNRS n° 437), École Supérieure de Physique et Chimie Industrielles de Paris, 10 Rue Vauquelin, 75231 Paris Cedex 05 (France)

J.-C. Truffert and A. Tambuté*

Centre d'Études du Bouchet, B.P. 3, Le Bouchet, 91710 Vert-le-Petit (France)

M. Caude

Laboratoire de Chimie Analytique (unité de recherche associé au CNRS n° 437), École Supérieure de Physique et Chimie Industrielles de Paris, 10 Rue Vauquelin, 75231 Paris Cedex 05 (France)

ABSTRACT

The scope of applications of novel chiral stationary phases (CSPs) derived from (*S*)-phenylalanine or (*R*)-phenylglycine and bearing a long spacer in normal-phase liquid chromatography is reviewed with regard to the similar corresponding commercially available brush-type CSPs. The parameters studied were the spacer length and the steric hindrance in the vicinity of the stereogenic centre. The direct separation of β-blockers in supercritical fluid chromatography (SFC) was also carried out in order to elucidate the influence of the steric decompression. The chromatographic properties of one of the novel CSPs and the commercially available CSP ChyRoSine-A, exhibiting both a long spacer and a weak steric hindrance in the vicinity of the stereogenic centre, were compared. The design and synthesis of three novel CSPs derived from phenylalanine or phenylglycine and starting from the concept of ChyRoSine-A allowed an optimized CSP to be proposed. This CSP possesses both reduced steric hindrance in the vicinity of the stereogenic centre and a long spacer. Its main advantage is that it combines the fields of application of various CSPs (DNBPG, ChiraChrom A1, ChyRoSine-A). In addition, this CSP allows the resolution of β-blockers, where both DNBPG and ChiraChrom A1 fail.

INTRODUCTION

Brush-type chiral stationary phases (CSPs) bearing a π-electron-acceptor group in the vicinity of the chiral centre [the most commonly introduced is the N-(3,5-dinitrobenzoyl) group] are among the most widely used CSPs for enantiomeric separations [1]. These CSPs are able to give with a chiral solute a π–π interaction with a complementary π-electron-donor group, to form a charge-transfer complex.

Since the commercialization of the well known CSP (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine [(*R*)-DNBPG] by Pirkle and co-workers [2–4],

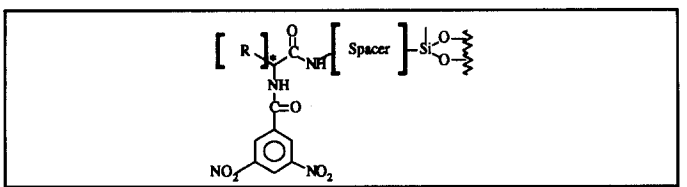
* Corresponding author.

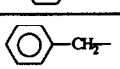
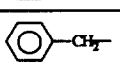
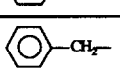
0021-9673/94/$07.00
SSDI 0021-9673(93)E0759-N

numerous brush-type CSPs have been designed and marketed. Although their scope of applications does not vary very much, it is well known that small changes in the structure of a given CSP may have significant effects on its chromatographic behaviour. Accordingly, the choice of the most effective CSP for a given separation is difficult and this work was aimed at the design of a CSP with a wider range of applications.

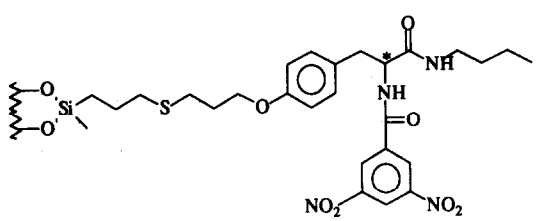
For a few years, our laboratories have been involved in the design and synthesis of brush-type CSPs derived from tyrosine using an efficient and original (with regard to that of the commercial CSPs, which always contains an amide function) grafting mode. Starting from the concept of the CSP ChyRoSine-A (grafting mode and steric decompression), three novel CSPs were designed, two derived from phenylalanine and one from phenylglycine (Fig. 1).

In order to evaluate the chromatographic properties of these new CSPs, ten solutes (Fig.



R	Spacer	CSP
	—$(CH_2)_3$—	1 (DNBPG)
	—$(CH_2)_{11}S(CH_2)_3$—	2
	—$(CH_2)_3$—	3 (ChiraChrom A1)
	—$(CH_2)_3S(CH_2)_3$—	4
	—$(CH_2)_{11}S(CH_2)_3$—	5

CSP 6: ChyRoSine A



CSP 5:

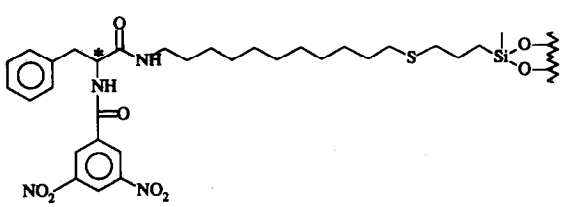


Fig. 1. Structures of the CSPs used.

2), representative of various classes of compounds (alcohols, atropoisomers, N-protected amino acid derivatized 3,5-dimethylanilide, sulphinamoyl esters, N-naphthyl- or 3,5-dinitrobenzoyl amino esters, phosphonorbornadiene oxides, albendazole sulphoxide, lactams, benzodiazepines), which are well separated on ChyRoSine-A, were separated by liquid chromatography (LC). As a complement, 1,2-amino alcohol β-blockers (which are widely used important drugs) (Table 1) were also separated using supercritical fluid chromatography (SFC). The ranges of application of these novel CSPs were compared with that of commercially available CSPs exhibiting similar structures, *viz.*, DNBPG and ChiraChrom A1 (all CSPs used in this study are shown in Fig. 1).

EXPERIMENTAL

Apparatus

^{1}H NMR spectra were recorded at 360 MHz on a Bruker-360 AMX spectrometer or at 200 MHz on a Bruker-WP 200 spectrometer, at 296 K, using tetramethylsilane (TMS) as internal standard and [^{2}H]chloroform or [$^2H_6$]dimethyl sulphoxide as solvent.

Optical rotations were recorded on a Perkin-Elmer Model 141 micropolarimeter equipped with a 1-dm flow cell at 589 (sodium D-line), 436 or 365 nm (Hg).

Analytical LC was performed with a Gilson (Villiers-le-Bel, France) modular liquid chromatograph equipped with a Model 303 pump, a

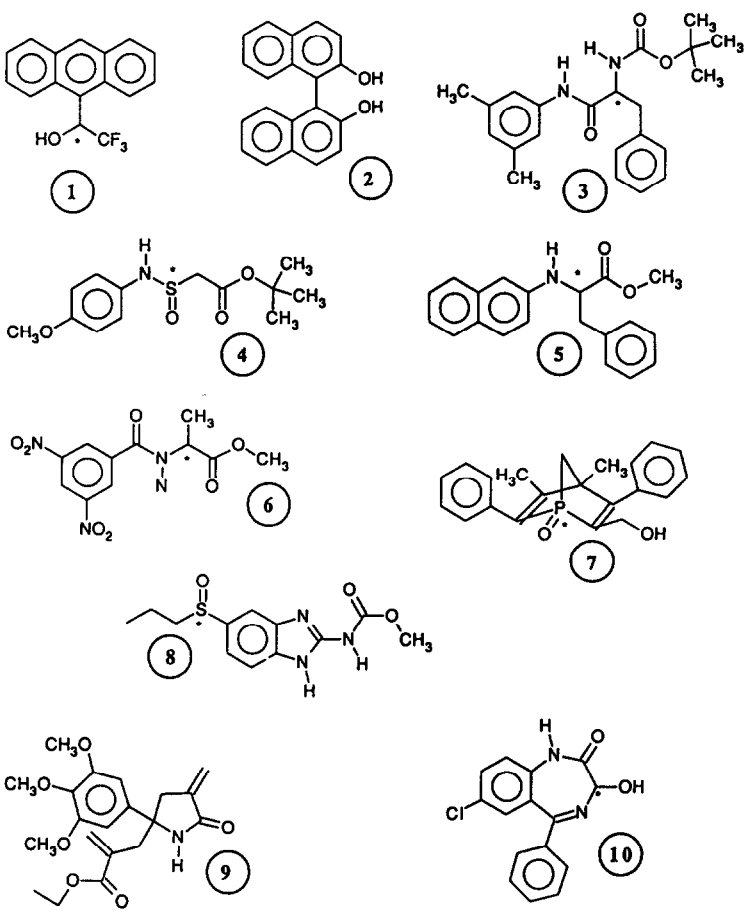


Fig. 2. Structures of the ten test solutes used in LC.

Model 802 manometric module, a Model 811 dynamic mixer (1.5 ml) and a UV-116 variable-wavelength detector. All results were recorded with a Shimadzu C-R3A integrator (Touzart et Matignon, Vitry-sur-Seine, France). The standard operating conditions were flow-rate 2 ml min^{-1} and room temperature.

Analytical SFC was performed with a Gilson SF3 modular supercritical chromatograph. Carbon dioxide (kept in a container with an eductor tube) was passed into a Model 308 pump through an ethanol cooling bath (−5°C). The pump head was cooled at −5°C in order to improve its efficiency. The polar modifier was added using a Model 306 pump and mixed with carbon dioxide in a Model 811 dynamic mixer. A Model 831 temperature regulator and a Model 821 pressure regulator provided temperature and pressure control, respectively. A Model 117 UV variable-wavelength detector equipped with a high-pressure cell was used. Injections were performed using a Model 231-401 autosampling injector. All results were recorded with a Shimadzu C-R3A Integrator.

Silica gel $60F_{254}$ (Merck, Darmstadt, Germany) was used for thin-layer chromatography (TLC).

Melting points were measured on a Büchi–Tottoli hot-stage apparatus and are uncorrected.

Elemental analyses were consistent with the formulae within ±0.3% (Service Central de Microanalyse du CNRS).

CSPs were packed into 150 × 4.6 mm I.D. stainless-steel columns by the usual slurry technique under 400 bar pressure using ethanol as pumping solvent.

Mobile phase

For LC, ethanol and *n*-hexane were of LiChrosolv grade, purchased from Merck (Darmstadt, Germany). Chloroform [stabilized with 0.6% (w/w) of ethanol] of analytical-reagent grade was purchased from Prolabo (Paris, France).

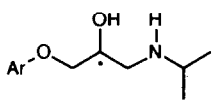
For SFC, carbon dioxide was of N-45 grade (99.995% pure) (Air Liquide, Alphagaz, Paris, France). Methanol of LiChrosolv grade was purchased from Merck and *n*-propylamine (>99% pure) from Fluka (Buchs, Switzerland).

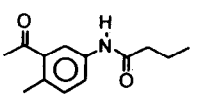
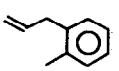
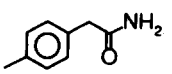
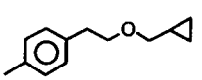
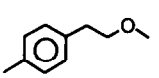
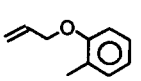
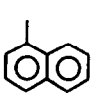
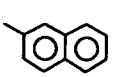
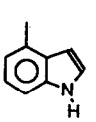
Solutes

The ten solutes (Fig. 2) separated by LC have been previously described [5]. β-Blockers (Table I) were purchased from various suppliers. They were used either as tartrate or hydrochloride salts or as the free base (the results being the same because the mobile phase contained *n*-propylamine and consequently is a basic medium) and simply dissolved in ethanol prior to injection.

TABLE I

STRUCTURES OF THE 1,2-AMINO ALCOHOL β-BLOCKERS USED



Solute	β-Blocker	Ar
1	Acebutolol	
2	Alprenolol	
3	Atenolol	
4	Betaxolol	
5	Metoprolol	
6	Oxprenolol	
7	Propranolol	
8	β-Propranolol[a]	
9	Pindolol	

[a] This compound is an analogue of β-blockers.

Chiral stationary phases

CSPs 3 and 6 are commercially available. CSP 3 (ChiraChrom A1) was purchased from SFCC (Neuilly-Plaisance, France) and CSP 6 (ChyRoSine-A) from SEDERE (Alfortville, France). CSP 1 (DNBPG) was synthesized according to Pirkle *et al.* [2].

The synthesis of CSPs 2, 4 and 5 will be described (the synthetic pathway is shown in Fig. 3). The physico-chemical data of the compounds synthesized and the experimental conditions and physical data are given in Table II.

Synthesis of chiral selectors (CSs) 2, 4 and 5

Preparation of B-2 [(R)-N-(tert-butyloxycarbonyl)phenylglycine (10-undecen-1-yl)amide], B-4 [(S)-N-(tert-butyloxycarbonyl)phenylalanine (2-propen-1-yl)amide] and B-5 [(S)-N(tert-butyloxycarbonyl)phenylalanine (10-undecen-1-yl)-amide]. The synthesis of B-2, B-4, B-5 was carried out starting from (*R*)-N-(*tert*-butyloxycar-

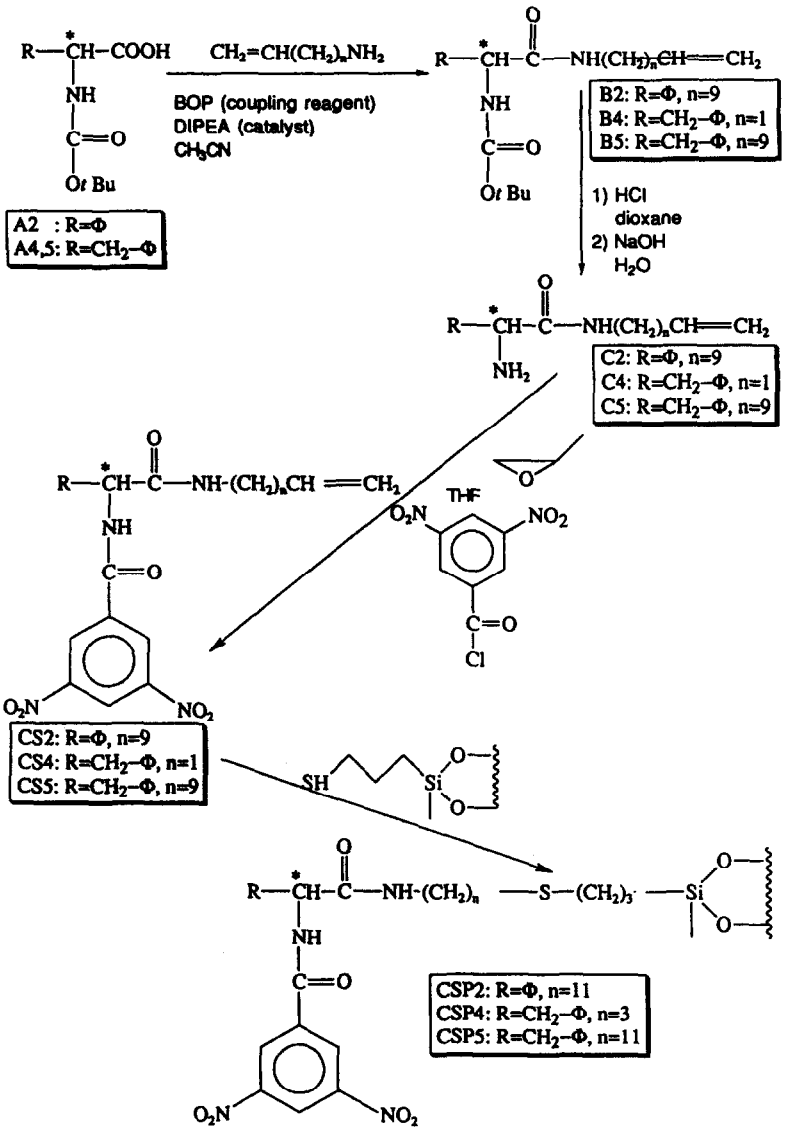


Fig. 3. Synthesis of CSPs 2, 4 and 5.

TABLE II

PHYSICAL DATA (MELTING POINT, ROTATORY POWER), EXPERIMENTAL CONDITIONS (ELUENT USED FOR TLC AND FOR PREPARATIVE LC) AND YIELDS OF THE REACTIONS FOR THE THREE CHIRAL SELECTORS AND THE CORRESPONDING INTERMEDIATES

Compound	M.p. (°C)	$[\alpha]_D^{22}$ (°)	TLC	Preparative LC	Yield (%)
B-2	Viscous oil	−57.1 (MeOH)	CH_2Cl_2–AcOEt (95:5)	CH_2Cl_2–AcOEt (95:5)	86
B-4	105–107	+1 (MeOH)	CH_2Cl_2–AcOEt (95:5)	CH_2Cl_2–AcOEt (95:5)	74
B-5	70–72	+3.8 (MeOH)	CH_2Cl_2–AcOEt (95:5)	CH_2Cl_2–AcOEt (95:5)	73.5
C-2	Viscous oil crystallizing on standing (low m.p.)	−57.1 (MeOH)	CH_2Cl_2–MeOH (90:10)	CH_2Cl_2–MeOH (95:5)	95
C-4	Viscous oil	+17.7 (MeOH)	CH_2Cl_2–MeOH (95:5)	Isolated pure from hydrochloride salt	87.6
C-5	45	+19.5 (MeOH)	CH_2Cl_2–MeOH (90:10)	Isolated pure from hydrochloride salt, m.p. 110–112°C	86.5
CS-2	175–177	−78.6 (THF)	CH_2Cl_2–AcOEt (95:5)	–	69.5
CS-4	187–189	−29.8 (THF)	CH_2Cl_2–MeOH (95:5)	–	68.3
CS-5	138–140	−19.5 (THF)	CH_2Cl_2–MeOH (95:5)	–	66

bonyl)phenylglycine (A-2) and (*S*)-N-(*tert*-butyloxycarbonyl)phenylalanine (A-4,5) respectively, both of which are commercially available (purchased from Propeptide, Vert-le-Petit, France). A 40-mmol amount of BOP[a] (17.7 g) and 40 mmol of alkylamine (2.28 g of allylamine, 6.76 g of undecenylamine, synthesized according to Dobashi and Hara [6]) were added successively to a solution of 40 mmol of A (10.0 g of A-2 and 10.6 g of A-4,5) in acetonitrile (200 ml) with magnetic stirring under a nitrogen atmosphere. Stirring was maintained for 30 min and diisopropylethylamine (DIPEA) (92 mmol, 11.9 g, 16 ml) was added dropwise. Completion of the reaction was achieved after 24 h under magnetic stirring [TLC with dichloromethane–ethyl acetate (95:5)]. The solvent was evaporated under vacuum and the crude product taken up in 150 ml of ethyl acetate. The organic solution was washed successively twice with 200-ml portions of water, 1 *M* hydrochloric acid and water, then brine, and dried by filtration through a hydrophobic filter. A crude oil (B-2) or a crude solid (B-4 and B-5) resulted, which was treated with diisopropyl ether. The pure compound was obtained after chromatography on silica gel using a dichloromethane–ethyl acetate mixture as eluent and after treatment with heptane. Physical data are given in Table II.

B-2: ^1H NMR {[$^2H_6$]dimethyl sulphoxide (Me_2SO-d_6)}, δ 1.19 (s, 9H), 2 (m, 2H), 1.1–1.45 (s, 14H), 3.03 (m, 2H), 4.92 (m, 1H), 4.99 (m, 1H), 5.16 (d, 1H), 5.79 (ddt, 1H), 7.14 (d, 1H), 7.2–7.45 (m, 5H), 8.12 (t, 1H).

B-4: ^1H NMR (Me_2SO-d_6), δ 1.30 (s, 9H),

[a] The synthesis of the amide derivative of an N-protected amino acid was usually performed by using 1-ethoxycarbonyl-2-ethoxy-1,2 dihydroquinoline (EEDQ) as coupling agent. Unfortunately, that method gives low yields with ramified amines such as *tert*-butylamine. However, the synthesis can be achieved starting from the promising reagent BOP [benzotriazol-1-oxytris(dimethylamino)phosphonium hexafluorophosphate]. BOP is no more expensive than EEDQ and no racemization occurs during the reaction process.

2.75 (dd, 1H), 2.94 (dd, 1H), 3.70 (m, 2H), 4.16 (dt, 1H), 5.02 (dm, 1H), 5.09 (dq, 1H), 5.75 (ddt, 1H), 6.90 (d, 1H), 7.15–7.30 (m, 5H), 8.03 (d, 1H). Analysis: calculated for $C_{17}H_{24}N_2O_3$, C 67.10, H 7.89, N 9.21; found, C 67.32, H 7.99, N 9.47%.

B-5: ^{1}H NMR (Me_2SO-d_6), δ 1.1–1.3 (m, 9H), 1.3–1.4 (m, 14H), 2.0 (dd, 2H), 3.0 (t, 2H), 3.1 (m, 2H), 4.3 (dd, 1H), 4.9 (m, 1H), 5.0 (m, 1H), 5.1 (d, 1H), 5.7 (t, 1H), 5.8 (ddt, 1H), 7.1–7.3 (m, 5H). Analysis: calculated for $C_{25}H_{40}N_2O_3$, C 72.12, H 9.62, N 6.73, O 11.54; found, C 71.95, H 9.68, N 6.67, O 11.51%.

Preparation of C-2 [(R)-phenylglycine (10-undecen-1-yl)amide], C-4 [(S)-phenylalanine (2-propen-1-yl)amide] and C-5 [(S)-phenylalanine (10-undecen-1-yl)amide]. A 17-mmol amount of compound B-2 (6.83 g), B-4 (5.10 g) or B-5 (7.07 g) was dissolved in an anhydrous dioxane solution (200 ml) saturated with hydrogen chloride and magnetically stirred for 24 h at room temperature. Completion of the reaction was monitored by TLC (dichloromethane–methanol; see Table II). The solvent was removed *in vacuo* and the residue was dissolved in water (100 ml). The aqueous solution was then made alkaline with 2.5 *M* sodium hydroxide solution and extracted twice with 50-ml portions of dichloromethane. The organic fraction was then washed successively twice with 100-ml portions of water and brine and dried by filtration through a hydrophobic filter. A viscous oil (C-2, C-4) or a solid (C-5) resulted after stripping of the solvent, pure enough to be used in the following step.

C-2: ^{1}H NMR (Me_2SO-d_6), δ 1.08–1.46 (m, 14H), 2 (dt, 2H), 2.17 (s, 2H), 3.03 (dt, 2H), 4.29 (s, 1H), 4.93 (ddt, 1H), 4.99 (ddt, 1H), 5.79 (ddt, 1H), 7.14–7.44 (m, 5H), 8.01 (t, 1H).

C-4: ^{1}H NMR (Me_2SO-d_6), δ 1.7 (s, 2H), 2.64 (dd, 1H), 2.95 (dd, 1H), 3.42 (dd, 1H), 3.63–3.77 (m, 2H), 5.01 (dq, 1H), 5.05 (dq, 1H), 5.76 (ddt, 1H), 7.15–7.30 (m, 5H), 8.00 (t, 1H).

C-5: ^{1}H NMR (Me_2SO-d_6), δ 1.22–1.31 (m, 14H), 1.61 (s, 2H), 1.98 (q, 2H), 2.61 (dd, 1H), 2.92 (dd, 1H), 3.01 (q, 2H), 3.34 (dd, 1H), 4.94 (ddt, 1H), 4.96 (ddt, 1H), 5.86 (ddt, 1H), 7.13–7.29 (m, 5H), 7.77 (t, 1H). Analysis: calculated for $C_{20}H_{33}ClN_2O_3$ (salt), C 68.09, H 9.36, Cl 10.07, N 7.94; found, C 67.87, H 9.42, Cl 10.23, N 7.93%.

Preparation of CS-2 [(R)-N-(3,5-dinitrobenzoyl)phenylglycine (10-undecen-1-yl)amide], CS-4 [(S)-N-(3,5-dinitrobenzoyl)phenylalanine (2-propen-1-yl)amide] and CS-5 [(S)-N-(3,5-dinitrobenzoyl)phenylalanine (10-undecen-1-yl)amide]. Small portions of 3,5-dinitrobenzoyl chloride (12 mmol, 2.71 g) and propylene oxide (36 mmol, 2.05 g) were added simultaneously to a solution of C (12 mmol, 3.60 g of C-2; 2.40 g of C-4; 3.79 g of C-5) in THF (70 ml). Magnetic stirring was maintained overnight at room temperature under a nitrogen atmosphere. The solvent was removed *in vacuo*. The corresponding CS was obtained after trituration with acetonitrile and washing with diethyl ether (see Table II for the physical data).

CS-2: ^{1}H NMR (Me_2SO-d_6), see Table III. Analysis: calculated for $C_{26}H_{32}N_4O_6$, C 62.9, H 6.45, N 11.29; found, C 63.12, H 6.51, N 11.16%.

CS-4: ^{1}H NMR (Me_2SO-d_6): see Table III. Analysis: calculated for $C_{19}H_{18}N_4O_6$, C 57.29, H 4.52, N 14.07; found, C 57.35, H 4.51, N 13.51%.

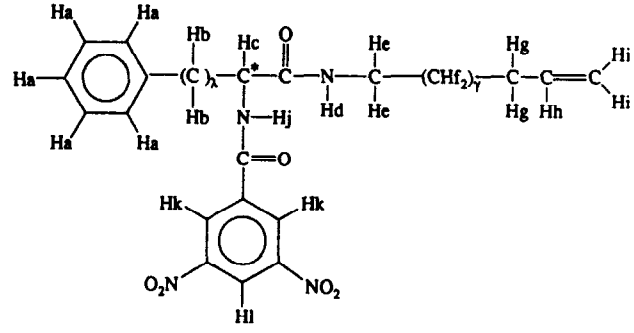
CS-5: ^{1}H NMR (Me_2SO-d_6), see Table III. Analysis: calculated for $C_{27}H_{34}N_4O_6$, C 63.53, H 6.67, N 10.98; found, C 63.40, H 6.86, N 10.82%.

Preparation of 3-mercaptopropylsilanized silica [7]. Into a three-necked 3000-ml round-bottomed flask equipped with a reflux condenser, a mechanical stirrer and a Dean–Stark trap, Kromasil-100A silica (5 μm) (78 g) (EKA-Nobel, supplied by Touzart et Matignon), previously dried at 120°C for 24 h, and 1400 ml of anhydrous toluene were introduced. To avoid any traces of water, 500 ml of distillate were discarded. Under a nitrogen atmosphere the following were successively added to the slurry via a dropping funnel: freshly distilled pyridine (168 ml dropwise) and pure 3-mercaptopropyltrimethoxysilane (344 ml, 1.84 mol). The slurry was refluxed for 39 h with stirring. Modified silica gel was filtered through a fine-pored sintered-glass funnel and washed successively twice with 150-ml portions of toluene, THF, diethyl ether and hexane, then dried in air. The modified silica gel (85 g) was texturally equivalent to the starting material.

Elemental analysis indicated 2.34% of sul-

TABLE III

NMR DATA FOR CHIRAL SELECTORS 2, 4 AND 5



CS	λ	γ	Parameter	H_a	H_b	$H_{b'}$	H_c	H_d	H_e	H_f	H_g	H_h	H_i	$H_{i'}$	H_j	H_k	H_l
2	0	7	δ (ppm)	7.24–7.56		–	5.70	8.36	3.08	1.06–1.48	1.99	5.78	4.92	4.99	9.79	9.13	8.96
				m			d	t	m	m	m	ddt	m	m	d	d	d
			J (Hz)	–		–	7.22	5.41	–	–	–	6.61, 10.22, 17.14	10.22	17.14	7.22	2.41	–
4	1	0	δ (ppm)	7.16–7.34	3.02	3.17	4.80	8.41	–	–	3.74	5.78	5.05	5.11	9.46	9.02	8.93
				m	dd	dd	ddd	t			m	ddt	m	m	d	d	t
			J (Hz)	–	4.70–10.80, 13.60		–	5.60	–	–	5.60	4.90, 10.30, 17.10	10.30, 17.10		8.20	2.10	–
5	1	7	δ (ppm)	7.13–7.33	3.01	3.13	4.76	8.21	3.09	1.22–1.37	1.98	5.75	4.92	5.00	9.48	9.03	8.93
				m	dd	dd	ddd	t	m	m	m	ddt	m	m	d	d	s
			J (Hz)	–	5.05–9.02, 13.30		–	5.40	–	–	–	4.97, 10.58, 17.23	10.58	17.23	8.27	1.40	–

phur, corresponding to 0.73 mmol of sulphur per gram of modified silica gel.

Preparation of CSPs 2, 4 and 5. A mixture of 9 g of 3-mercaptopropylsilanized silica described above, chiral selector (2.7 mmol, 1.34 g of CS-2, 1.075 g of CS-4, 1.38 of CS-5, respectively, corresponding to 0.3 mmol of CS per gram of modified silica gel), radical initiator 2,2'-azobis-(2-methylpropionitrile) (AIBN) (118 mg, 80 mmol per kilogram of modified silica gel) and chloroform freshly distilled onto diphosphorus pentoxide (120 ml) was refluxed with mechanical stirring under a nitrogen atmosphere for 40 h. After cooling, the solid was washed successively twice with 50-ml portions of chloroform, methanol, acetonitrile and diethyl ether (the supernatant being discarded after each washing step). After drying in the presence of diphosphorus pentoxide, CSP 2 (10.0 g), CSP 4 (10.0 g) and CSP 5 (10.1 g) were obtained.

CSP 2: analysis: found, C 25.87, H 3.17, N 1.29, S 1.58, Si 26.37%, corresponding to 0.23 mmol of chiral moiety per gram of modified silica (based on N).

CSP 4: analysis: found, C 29.24, H 3.84, N 1.01, S 1.39, Si 24.27%, corresponding to 0.18 mmol of chiral moiety per gram of modified silica (based on N).

CSP 5: analysis: found, C 26.62, H 2.85, N 1.21, S 2.50, Si 30.10%, corresponding to 0.216 mmol of chiral moiety per gram of modified silica (based on N).

RESULTS AND DISCUSSION

Liquid chromatography

For each CSP, the enantiomeric separation of a series of ten test solutes was performed using hexane–ethanol and hexane–chloroform mixtures as mobile phases (because of the polarity of chloroform, the elution of solute 8 was not attempted). For each solute, the selectivity (α), the capacity factor of the most retained enantiomer (k'_2) and the ethanol or chloroform contents in the mobile phase are reported in Table IV. In order to compare the separation capabilities, the composition of the hexane–ethanol mobile phase was kept unchanged for each solute tested on the six CSPs giving rise to the normalized data reported in the first row. The separations were optimized on each CSP, and the corresponding values are reported in the second row for each solute. For the hexane–chloroform mobile phase, only the optimized values are reported.

Influence of the nature of the mobile phase

It is well known [8,9] that chlorinated solvents exhibit a greater selectivity but a lower efficiency than alcohols. These characteristics may be correlated with the fact that alcohols can be considered as both proton donors and acceptors and hence may interact through hydrogen bonding with the basic and/or the acidic site of the CSP amide dipoles. In contrast, chloroform (mainly a proton donor) only interacts with a unique site. Therefore, the CSP–solute interactions are maximized using chlorinated solvents (resulting in higher selectivity) but the adsorption–desorption kinetics of the solutes on CSP are slower (resulting in weaker efficiency).

Considering Table IV and making a comparison between the optimized values obtained with ethanol or chloroform as polar modifier, it can be stated that most of the solutes tested (1, 4, 5, 6, 7, 9 and 10) exhibit classical behaviour, *i.e.*, the use of chloroform (instead of ethanol) as a polar modifier improves the selectivity. However, there are two exceptions: solute 2, for which the selectivity remains unchanged whatever the polar modifier nature, and solute 3, for which the use of chloroform is unfavourable. The abnormal behaviour of solute 3 is probably due to the presence of two amide dipoles in both the solute and the CSP; the multiplicity of possible sites of interaction may then favour the occurrence of competitive opposite-sense chiral recognition mechanisms, depending on the nature of the mobile phase [10]. The case of solute 2 is particular because it exhibits an atropoisomeric chirality and therefore may be less sensitive to the solvation.

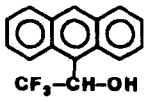
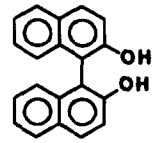
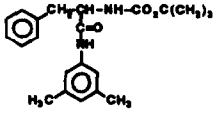
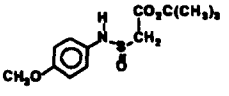
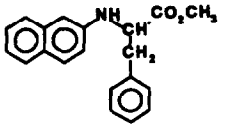
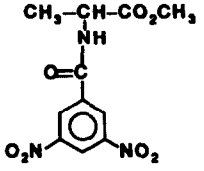
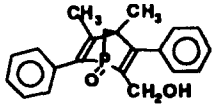
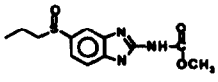
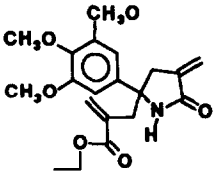
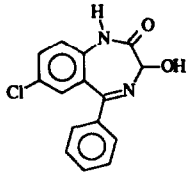
Influence of spacer length

In order to obtain further insight into the influence of the spacer length, two graphs were plotted using the normalized data (see Fig. 4), the first for CSPs 1 and 2 (R = ϕ) and the second

TABLE IV

LIQUID CHROMATOGRAPHIC RESULTS FOR THE RESOLUTION OF THE TEN TEST SOLUTES ON CSP 1–6

Experimental conditions: column, 150 × 4.6 mm I.D.; mobile phase, hexane–ethanol or chloroform with the percentage of polar modifier as indicated; flow-rate, 2 ml min^{-1}; UV detection at 254 nm; room temperature.

Solute	Polar modifier	α/k'_2 (% of polar modifier)					
		CSP 1	CSP 2	CSP 3	CSP 4	CSP 5	CSP 6
	EtOH	1.38/5.0(5)	1.32/7.22(5)	1.35/4.5(5)	1.24/5.6(5)	1.33/4.8(5)	1.24/6.4(5)
	EtOH[a]	1.44/9.4(2.5)	s[b]	1.41/8.4(2.5)	1.29/9.8(2.5)	1.37/8.0(2.5)	s
	$CHCl_3$[a]	1.50/15.3(30)	1.41/13.1(30)	1.62/11.8(40)	1.38/13.9(30)	1.44/10.7(10)	1.38/15.2(25)
	EtOH	1.34/8.9(5)	1/6.31(5)	1.22/12.9(5)	1.19/22.3(5)	1.13/11.4(5)	1.14/14.5(5)
	EtOH[a]	s	s	s	s	1.18/15(2.5)	s
	$CHCl_3$[a]	1.30/20.4(30)	1.20/11.3(30)	1.14/21.5(40)	1.19/7.7(50)	1.18/14.6(10)	1.13/12.4(25)
	EtOH	1.49/5.6(2.5)	2.02/9.3(2.5)	1.33/6.3(2.5)	1.25/8.9(2.5)	1.48/6.7(2.5)	1.34/8.1(2.5)
	EtOH[a]	s	2.09/6.1(5)	s	s	s	1.38/5.8(5)
	$CHCl_3$[a]	1.32/4.9(30)	1.44/4.1(30)	1.06/20.8(20)	1/7.4(30)	1.21/9.1(10)	1.05/10.5(25)
	EtOH	1.14/8.3(5)	1.26/6.5(5)	1.77/8.4(5)	1.73/11.3(5)	1.79/11.2(5)	1.90/13.0(5)
	EtOH[a]	s	s	s	s	1.87/14(2.5)	s
	$CHCl_3$[a]	1.29/14.2(40)	1.39/19.6(15)	2.15/13.3(40)	2.04/22.2(30)	2.11/13.8(20)	2.17/13.1(40)
	EtOH	1.84/7.2(10)	2.40/10.1(10)	4.75/12.2(10)	4.49/17.7(10)	3.82/11.7(10)	4.49/19.9(10)
	EtOH[a]	s	s	s	s	4.64/22.8(5)	s
	$CHCl_3$[a]	1.95/3.7(40)	3.31/9.4(15)	10.5/10.7(40)	11.32/17(30)	5.80/10.1(20)	9.27/21.1(20)
	EtOH	1/28.7(5)	1.06/18.1(5)	1.19/10.2(5)	1.09/28.7(5)	1.24/12.5(5)	1.27/21.5(5)
	EtOH[a]	1/17(15)	s	s	1.17/9.9(10)	s	1.20/10.5(10)
	$CHCl_3$[a]	1/13.9(40)	1.10/9.2(40)	1.64/11.8(40)	1.90/34.7(30)	1.59/22.8(20)	1.57/12.6(40)
	EtOH	1.08/8.7(10)	1.17/4.6(10)	1.29/7.0(10)	1.23/10.7(10)	1.33/9.24(10)	1.37/9.1(10)
	EtOH[a]	s	s	s	s	1.37/13.7(5)	s
	$CHCl_3$[a]	1.18/19.5(60)	1.37/5.9(40)	1.46/17.7(60)	1.52/20.0(50)	1.55/19.2(20)	1.31/11.9(60)
	EtOH	1/24.1(10)	1.07/11.9(10)	1.03/14.7(10)	1.10/19.1(10)	1.23/14.6(10)	1.18/19.1(10)
	EtOH[a]	s	s	1.09/8.9(15)	1.10/10.9(15)	s	s
	EtOH	1/6.6(15)	1/15.9(15)	1.15/4.8(15)	1.48/12.1(15)	1.24/6.3(15)	1.18/8.2(15)
	EtOH[a]	1/9.9(10)	s	1.17/8.0(10)	s	s	s
	$CHCl_3$[a]	1/22.6(40)	1.20/9.2(30)	1.44/25.9(40)	1.41/8.9(50)	1.47/13.4(30)	1.24/15.2(45)
	EtOH	1.23/15.1(15)	1.32/8.0(15)	1.53/10.5(15)	1.45/11.3(15)	1.64/8.7(15)	1.50/11.0(15)
	EtOH[a]	s	s	s	1.47/8.2(20)	s	s
	$CHCl_3$[a]	1.35/28.3(90)	1.99/12.7(70)	2.55/28.0(90)	2.84/18.5(90)	3.13/20.1(70)	1.83/21.1(90)

[a] These data correspond to optimized values.

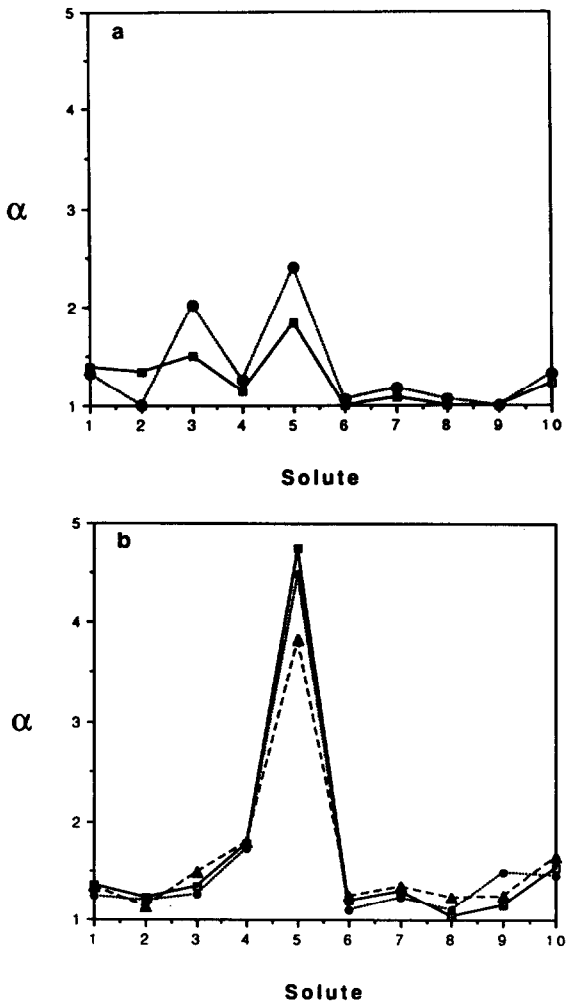


Fig. 4. Influence of the spacer length. The normalized selectivity is plotted for each of the ten solutes using (a) CSPs (□) 1 and (●) 2 (R = ϕ) and (b) CSPs (□) 3, (●) 4 and (▲) 5 (R = CH_2-ϕ).

for CSPs 3, 4 and 5 (R = CH_2-ϕ). For the CSPs with low steric hindrance (CSPs 3, 4 and 5) in the vicinity of the chiral centre, the length of the spacer has no influence on the selectivity, except with solute 5 for which a good selectivity is obtained using the CSP having the shortest spacer (however, in that particular case, a similar selectivity can be obtained with a long spacer by optimizing the separation). In contrast, for the CSPs having higher steric hindrance, some discrepancies can be observed. In fact, solutes 3 and 5 are better resolved on CSP 2 (long spacer) than on CSP 1 (short spacer), *i.e.*, in that case the enhancement of the spacer length improves the selectivity. Solutes 3 and 5 are both (1) strongly π-electron donor and consequently the prevailing interaction that occurs during the discrimination process between the solute and the CSP is a $\pi-\pi$ interaction, and (2) highly hindered in the vicinity of the chiral centre. These two reasons lead to them being better resolved on a CSP that is less bulky because the $\pi-\pi$ interaction is thereby favoured. This observation is in good agreement with previous results [11] showing that solutes bearing bulky substituents are better resolved on a CSP that is less sterically hindered, the attractive and repulsive interactions being better equilibrated.

Influence of steric hindrance in the vicinity of the chiral centre

In order to explain the influence of the steric hindrance in the vicinity of the chiral centre (R = ϕ or CH_2-ϕ), two graphs were plotted using the normalized data (see Fig. 5), the first for CSPs 1 and 3 (short spacer) and the second for CSPs 2 and 5 (long spacer). As a general rule it can be observed that steric hindrance has a greater effect than the spacer length on the selectivity values.

Using a CSP with a reduced steric hindrance (R = CH_2-ϕ), the selectivity is slightly improved for solutes 6, 7, 8, 9 and 10 and more for solutes 4 and 5 (especially 5). A decrease in steric hindrance is especially favourable for π-electron-donor solutes (4 and 5), particularly with a bulky molecule (5), as observed above. However, the behaviour of solutes 1, 2 and 3 is less clear; with a short spacer the selectivity increased slightly with steric hindrance, whereas with a long spacer the selectivity decreased slightly with steric hindrance for solutes 1 and 2 and increased for solute 3, although the last is a π-electron donor (like 4 and 5). The structure of solute 3 is particular in that it contains two amide dipoles and consequently many interaction sites. It appears that its separation is improved by using a CSP with a longer spacer or with weak steric hindrance. However, the separation becomes more difficult on a CSP having these two characteristics at the same time. It can therefore be

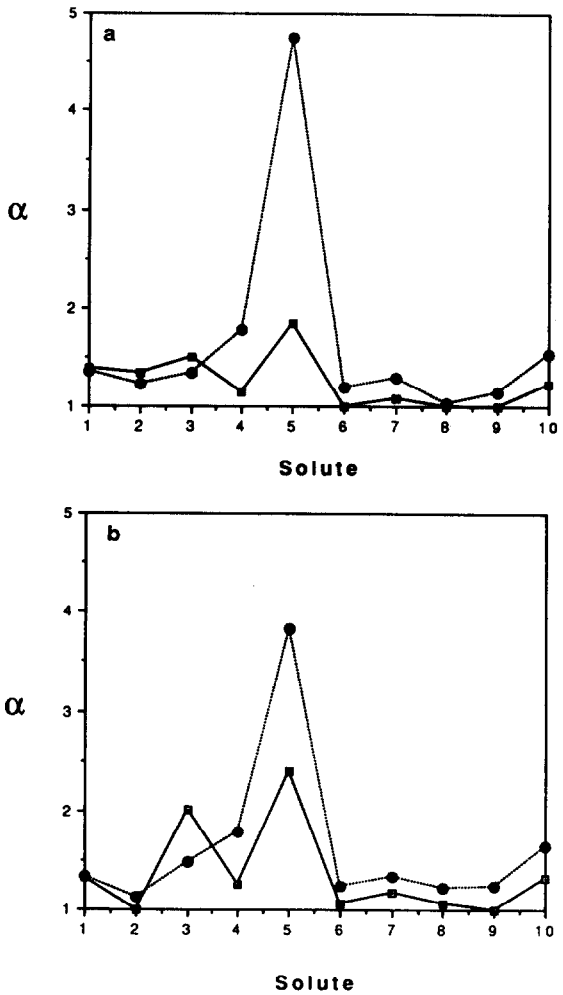


Fig. 5. Influence of steric hindrance in the vicinity of the stereogenic centre (nature of the R substituent). The normalized selectivity is plotted for each of the ten solutes using (a) CSPs (□) 1 and (●) 3 (short spacer) and (b) CSPs (□) 2 and (●) 5 (long spacer).

inferred that relative bulkiness is needed for the effective establishment of all stereoselective interactions needed for the chiral discrimination to occur. The resolution of solutes 1 and 2 is less affected by the steric hindrance, probably because they possess a few interaction sites.

Using these CSPs in LC, it can be stated that the chiral resolution of most of the solutes is slightly affected by the steric decompression (steric hindrance or spacer length). However, the class of solutes bearing a strong π-electron donor group is much better resolved using a less bulky CSP. However, in some instances (presence of two amide dipoles in the solute) medium bulkiness is preferable for good discrimination.

Supercritical fluid chromatography

The direct enantiomeric separation of β-blockers was recently achieved on tyrosine-derived CSPs (ChyRoSine-A and its improved version) [12] using SFC. It has been shown that carbon dioxide acts as a complexing agent toward amino alcohols by setting up a bridge with the hydroxyl and the amine protons of the solute. In this way, the resulting complex possesses lower acid–base properties and a higher conformational rigidity, responsible for chiral discrimination.

The ability of class I CSPs to separate such enantiomers of high polarity was investigated in this study. For each CSP, the enantiomeric separation of some β-blockers was performed using supercritical carbon dioxide–methanol [containing 1% (v/v) of *n*-propylamine] binary mixtures as mobile phase. For each solute, the selectivity (α), the capacity factor of the most retained enantiomer (k'_2) and the amount of polar modifier in the mobile phase are reported in Table V.

β-Blockers are not resolved on CSP 1, which is the most bulky, and are poorly resolved on CSP 2 and 3 (both having medium bulkiness). On the other hand, the selectivity values of all β-blockers increase from CSP 3 to 5 (*i.e.*, as the length of the spacer increases), on which very good separations are achieved. These results clearly demonstrate the importance of steric decompression for the resolution of β-blockers. The high conformational rigidity and the relatively large size of the transient complex formed between the 1,2-amino alcohol and the molecule of carbon dioxide may explain this importance. As was observed in LC for the resolution of the ten test solutes, a bulky and rigid solute is better resolved on a CSP that is less bulky. Thus CSP 5 appears to be the most effective for this application.

An inversion of the enantiomeric elution order was observed for propranolol between CSPs 2 and 3, whereas no inversion occurred between

TABLE V

SUPERCRITICAL FLUID CHROMATOGRAPHIC RESULTS FOR THE RESOLUTION OF β-BLOCKERS ON CSP 1–6

Experimental conditions: column, 150 × 4.6 mm I.D.; mobile phase, carbon dioxide–methanol [containing 1% (v/v) of *n*-propylamine], with the percentage of polar modifier as indicated; flow-rate at 0°C, 4 ml min^{-1}; UV detection at 224 nm; temperature, 25°C; average column pressure, 180 bar. The enantiomeric elution order is given for propranolol. Some interesting optimized values are given in italics.

	α/k'_2 (% of polar modifier)					
β-Blocker	CSP 1	CSP 2	CSP 3	CSP 4	CSP 5	CSP 6
Acebutolol	1/28.2(5)	1/29.6(5)	1/49.3(5)	1.06/31.0(5)	1.08/19.0(5)	1.11/20.6(5)
Alprenolol	1/11.2(4)	1/11.3(4)	1.01/16.0(4)	1.12/11.5(4) *1.14/16.2(3)*	1.18/13.1(4)	1.22/8.3(4)
Atenolol	1/39.4(6)	1/22.5(6)	1/46.8(6)	1.05/33.3(6)	1.10/30.3(6)	1.11/21.0(6)
Betaxolol	1/19.9(4)	1/15.9(4)	1.05/27.6(4)	1.14/22.0(4)	1.32/22.2(4)	1.17/12.8(4)
Metoprolol	1/16.1(4)	1.05/16.2(4)	1.03/24.0(4)	1.14/17.5(4)	1.16/15.3(4) *1.22/32.3(2.5)*	1.17/12.8(4)
Oxprenolol	1/16.4(4)	1/12.5(4)	1.01/21.5(4)	1.11/17.1(4)	1.20/17.3(4)	1.20/10.1(4)
Pindolol	1/28.7(10)	1/23.3(10)	1.16/35.0(10)	1.33/32.4(10)	1.57/28.7(10)	1.71/18.3(10)
Propranolol	1/12.2(10)	1.15/16.3(10) *1.16/17.3(9)* (*R–S*)	1.15/16.8(10) *1.18/31.9(7)* (*S–R*)	1.36/15.9(10) (*S–R*)	1.52/20.3(10) (*S–R*)	1.71/10.8(10) (*S–R*)
β-Propranolol	1/17.3(7)	1.12/22.1(7)	1.10/24.5(7)	1.21/17.4(7)	1.36/36.2(7)	1.25/13.2(7)

CSPs 3, 4 and 5. This observation suggests the existence of two opposite chiral recognition mechanisms which are in competition whether R = φ or CH_2-φ [13,14]. The steric decompression in the direct vicinity of the stereogenic centre is a very important factor (more important than the spacer length), as has been previously evidenced in LC.

Comparison of CSP 5 and ChyRoSine-A

CSP 5 was compared with CSP 6 (ChyRoSine-A). Both these CSPs are slightly bulky and similar in structure, except that they are grafted on the opposite side.

In LC, the selectivity values are similar on both of these CSPs, but the solutes are as a general rule less retained on CSP 5 than on ChyRoSine-A. It appears that the non-stereoselective interactions are minimized on CSP 5; CSP 5 and ChyRosine-A probably exhibit very different conformations according to the side on which they are grafted to silica.

In contrast, for the resolution of β-blockers in SFC, the tendency is reversed: the amino alcohols are more retained on CSP 5 than on ChyRoSine-A. The selectivities are almost the same (at a constant elution time) except for the best resolved solutes: pindolol (α = 1.57 on CSP 5 and 1.71 on ChyRoSine-A) and propranolol (α = 1.52 on CSP 5 and 1.71 on ChyRoSine-A). The same elution order has been observed for propranolol on CSP 5 and ChyRoSine-A, suggesting a similar chiral recognition mechanism.

CONCLUSIONS

The design and synthesis of three novel CSPs derived from phenylalanine or phenylglycine and starting from the concept of ChyRoSine-A allowed us to propose an optimized CSP (CSP 5). This CSP possesses both less steric hindrance in the vicinity of the stereogenic centre and a long spacer. Its main advantage is that it combines the fields of application of various CSPs (DNBPG, ChiraChrom A1, ChyRoSine-A). In addition, this CSP allows the resolution of β-blockers, where both DNBPG and ChiraChrom A1 fail.

This study also allowed us to confirm some practical aspects concerning the choice of the optimum class I CSP. They are based on the necessary equilibrium between repulsive and attractive interactions during the chiral recognition process. As example, a solute having numerous possible interaction sites and high steric hindrance in the vicinity of the stereogenic centre will be better resolved on a CSP with a few interaction sites and weak steric hindrance.

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