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Chiral recognition mechanisms on chiral stationary phases derived from tyrosine

Specific influence of the nature of the asymmetric centre vicinal functional group

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ABSTRACT

Four chiral stationary phases (CSPs) derived from N-(3,5-dinitrobenzoyl)tyrosine were synthesized. They only differ in one potential site of interaction, *viz.*, the functional group directly bound to the asymmetric centre. This was evaluated in terms of its dominant character according to the selectivity parameters χ_e, χ_d and χ_n of an equivalent solvent. For this purpose, the enantiomeric separation of nine racemates using liquid and subcritical fluid chromatographic modes was performed on the four CSPs. The chromatographic data allowed the determination of the nature of the interaction occurring at the modular site of interaction depending on the solute structure. The influence of the nature of the polar modifier on this interaction was also investigated.

INTRODUCTION

Chiral stationary phases (CSPs) derived from N-(3,5-dinitrobenzoyl)amino acids are among the most widely used for enantiomeric chromatographic separations of numerous compounds [1]. They are commonly described as independent CSPs as each chiral graft operates independently in distinguishing the solute enantiomers. Consequently, chiral recognition processes on 3,5-dinitrobenzoyl (DNB) derivative CSPs are better understood than on cooperative CSPs for which chiral entities are acting in concert to afford chiral discrimination. The strong π-acid DNB moiety of the

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chiral selector (CS) is generally involved in the chiral recognition mechanism through a π–π interaction with a solute bearing a complementary π-basic moiety.

The early commercialization of the well known (*R*)-N-(3,5-dinitrobenzoyl)-phenylglycine derived CSP [(*R*)-DNBPG], designed by Pirkle and co-workers [2–4] and the easy and inexpensive preparation of this type of CSP have prompted many researchers to design new π-acid CSPs [5–12]. Although the scopes of application of these CSPs do not vary much, all workers agree with the fact that small structural changes in the CSPs have significant effects on the chromatographic behaviour. For instance, we recently reported the facile enantiomeric separation of a series of N-arylsulphinamoyl acetates on CSPs derived from tyrosine, whereas it was hardly achieved on DNBPG [13].

For several years our laboratories have been involved in the development of CSPs derived from tyrosine [6,14,15]. This amino acid was chosen because of its ability to bind to silica via its hydroxyl group. After derivatization into an allylic ether, the CS could be grafted onto silica according to an anti-Markownikoff addition reaction [6], as described by Rosini *et al.* [16].

This original grafting mode allows the carboxylic acid group of the amino acid to be kept free for further derivatization, whereas most of the time it was used for grafting the CS onto silica. In this manner, two CSPs were obtained: (*S*)-thio-DNBTyr-A (*n*-butylamide, CSP 1, Fig. 1) and (*S*)-thio-DNBTyr-E (methyl ester, CSP 2, Fig. 1) [6,14]. More recently, the introduction of a π-basic moiety has led to two novel "mixed" CSPs: (*S*)-thio-DNBTyr-(*R*)-NEA and (*S*)-thio-DNBTyr-(*S*)-NEA [15]. This present paper deals with two novel CSPs derived from tyrosine, obtained by converting the methyl ester group ($COOCH_3$) of the previously described CS 2 [6] into a primary alcohol (CH_2OH, CSP 3, Fig. 1) and a primary amide ($CONH_2$, CSP 4, Fig. 1). CSPs 1–4 were evaluated with nine racemates (used as test solutes with earlier tyrosine-derived CSPs) (Fig. 2) in both liquid (LC) and subcritical (SubFC) fluid chromatography.

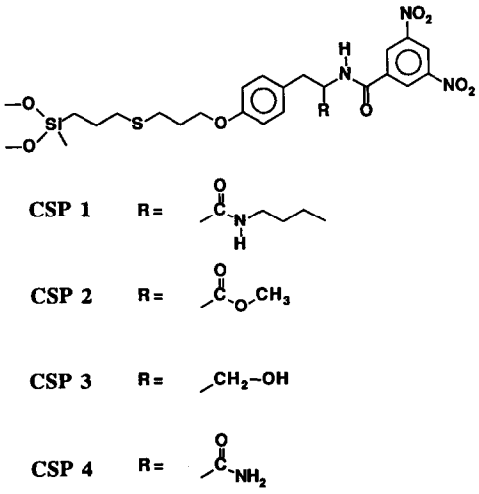


Fig. 1. Structures of CSPs.

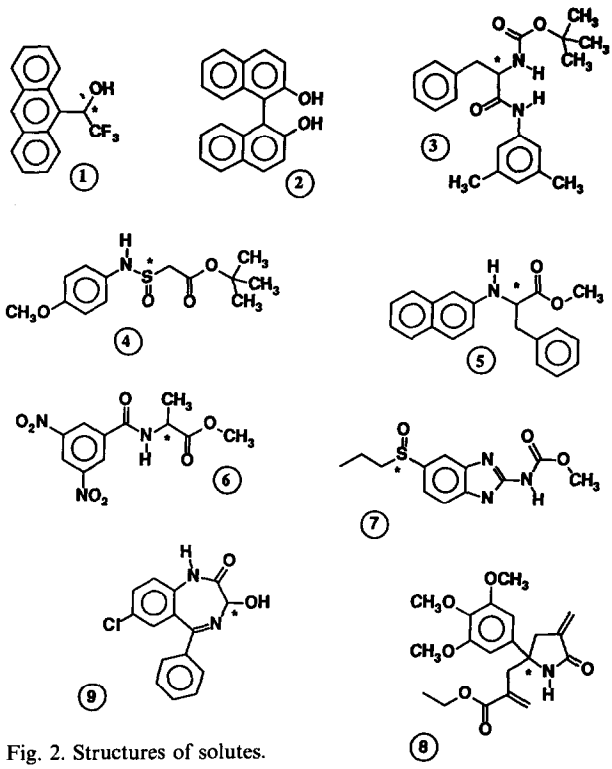


Fig. 2. Structures of solutes.

These four CSPs, only differing in one site of interaction, were expected to be of great interest in the investigation of chiral recognition mechanisms.

EXPERIMENTAL

Apparatus

LC was performed with a modular liquid chromatograph (Gilson, Villiers-le-Bel, France) equipped with a Model 303 pump, a Model 802c manometric module, a Model 811 dynamic mixer (1.5 ml) and a UV-116 variable-wavelength detector (190–350 nm). All results were recorded with a Shimadzu C-R3A integrator (Touzart et Matignon, Vitry-sur-Seine, France). The standard operating conditions were a flow-rate of 2 ml/min at room temperature.

For SubFC, the carbon dioxide, kept in a container with an eductor tube, was passed into a Model 303 pump (Gilson) through an ethanol cooling bath. The pump head (10 SC) was cooled in order to improve its efficiency. The inlet adaptor and cooling jacket were laboratory made. The polar modifier (ethanol) was added using a second Gilson pump and mixed with carbon dioxide through a Gilson mixer (Model 802). A constant-temperature water-bath provided temperature control of the column. A Polychrom 9060 diode-array detector (Varian, Palo Alto, CA, U.S.A.) was used without modification. The pressure was monitored by a back-pressure regulator (TESCOM, Model 26-1700; GEC Composants, Asnières, France) connected in-line

after the detector and maintained at 45°C with a water-bath. All results were recorded with a Shimadzu C-R3A integrator (Touzart et Matignon). The standard operating conditions were average column pressure 150 bar, temperature 25°C and carbon dioxide flow-rate 4.5 ml/min at 0°C.

^{1}H NMR spectra were recorded 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. Chemical shifts are given in parts per million and coupling constants in Hertz.

Optical rotations were measured on a Perkin-Elmer 141 micropolarimeter with a thermostated 1-dm quartz cell.

Silica gel 60F-254 (Merck, Darmstadt, F.R.G.) was used for thin-layer chromatography (TLC).

Melting points were measured on a Büchi-Tottoli hot-stage apparatus (Roucaire, Vélizy-Villacoublay, France) and are uncorrected.

The compounds, listed with their empirical formulae, had elemental analyses consistent with their formulae to within $\pm 0.3\%$ (Service Central de Microanalyse du CNRS, France).

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

Mobile phase

Carbon dioxide was of N-45 grade (99.995% pure) (Air Liquide, Alphagaz, Paris, France). Ethanol and hexane were of LiChrosolv grade, purchased from Merck. Methylene chloride of analytical-reagent grade was purchased from Prolabo (Paris, France).

Solutes

The structures of the solutes used are given in Fig. 2. 2,2,2-Trifluoro-1-(9-anthryl)ethanol (solute 1) and bi-2-naphthol (solute 2) were purchased from Janssen (Pantin, France). The 3,5-dimethylanilide derivative of N-Boc-*t*-phenylalanine (solute 3) was prepared according to Pirkle and McCune [17] starting from commercially available racemic N-*t*-Boc-phenylalanine (Propeptide, Vert-le-Petit, France). Methyl N-(2-naphthyl) phenylalanilate (solute 5) was prepared using a Bucherer reaction according to Pirkle and Pochapsky [18]. The enantiomeric separation of methyl N-3,5-DNB-alanilate (solute 6) on (*S*)-thio-DNBTyr-A was reported previously [19], as also were those of N-arylsulphinamoyl acetate (solute 4), albendazole sulphoxide (solute 7), α-methylene γ-lactam (solute 8) and oxazepam (solute 9) [6,13,20].

Chiral stationary phases

Structures of the investigated CSPs are shown in Fig. 1. (*S*)-thio-DNBTyr-A (CSP 1) and (*S*)-thio-DNBTyr-E (CSP 2) have been described previously [6]. Grafting rates were CSP 1 0.22 mmol/g and CSP 2 0.21 mmol/g (calculation based on N according to elemental analyses).

CSP 3 (Fig. 3)

(S)-2-amino 3-(4-allyloxyphenyl)propan-1-ol (II). This compound was ob-

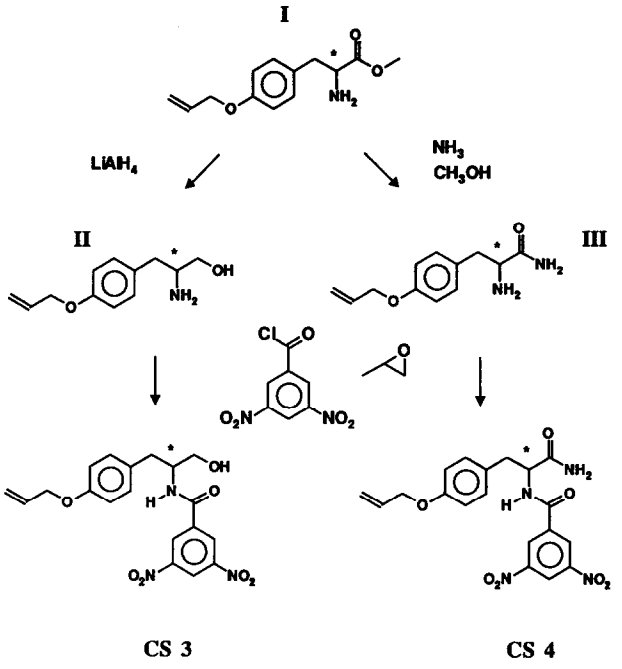


Fig. 3. Synthesis of CSs 3 and 4.

tained by reduction of the previously described (*S*)-tyrosine-O-(2-propen-1-yl) methyl ester (**I**) [6] with aluminium lithium hydride according to Powell *et al.* [21].

In a three-necked 500-ml round-bottomed flask equipped with a mechanical stirrer, 250 ml of anhydrous diethyl ether and 4.8 g (127 mmol) of aluminium lithium hydride were introduced. A solution of **I** (16.8 g, 71.5 mmol) in 50 ml of diethyl ether was then slowly added dropwise with stirring. Completion of the reaction was obtained after stirring overnight. Triethanolamine (20.6 g, 138 mmol) was then added at room temperature during 2 h. The reaction mixture became viscous and water (4.6 ml) was added. Stirring was maintained overnight. The organic phase was decanted, then filtered through a hydrophobic filter and the solvent was stripped off, giving a yellow oil. After crystallization in diisopropyl ether (40 ml), a yellow, slightly sticky solid resulted (6.8 g, 46% yield), pure enough to be used in the following step. TLC monitoring afforded a single spot [methylene chloride–methanol (9:1, v/v)]. M.p.: 77–79°C.

(S)-2-[N-(3,5-dinitrobenzoyl)amino]-3-(4-allyloxyphenyl)propan-1-ol(CS3). 3,5-Dinitrobenzoyl chloride (7.56 g, 32.8 mmol) and propylene oxide (6.6 ml, 98 mmol) were added simultaneously in small portions to a solution of **II** (6.8 g, 32.8 mmol) in 100 ml of anhydrous tetrahydrofuran (THF). The mixture became slightly warm as the reaction proceeded. After stirring overnight under a nitrogen atmosphere, the solvent was removed under reduced pressure. The solid was dissolved in chloroform and the organic solution was washed with water until neutral, then brine. After stripping off of the solvent under reduced pressure, the remaining solid was crystallized in acetonitrile (50 ml) and a yellow solid (3.4 g, 25% yield) resulted. M.p.: 144–146°C. $[\alpha]_D = -75°$,

$[\alpha]_{436} = -180°$ ($c = 1$, THF, 22°C). Elemental analysis corresponded to $C_{19}H_{19}N_3O_7$. ^1H NMR (C^2HCl_3): δ 1.91 (s, 1H, O*H*); 2.98 (d, 2H, CHCH_2OH); 3.86 (m, 2H, CHCH_2Ar); 4.42 (m, 1H, C*H*N); 4.53 (m, 2H, CH_2OAr); 5.25–5.45 (m, 2H, CH_2=CH); 5.95–6.11 (m, 1H, CH_2=C*H*); 6.64 (d, 1H, N*H*CO); 6.87–7.26 (m, 4H, Ar); 8.70–9.20 (m, 3H, Ar).

CSP 4 (Fig. 3)

(S)-tyrosine-O-(2-propen-1-yl)amide (III). A 14.1-g (60-mmol) amount of **I** was added to a saturated solution of gaseous ammonia in methanol at room temperature with magnetic stirring. Completion of the reaction was monitored by TLC [methylene chloride–methanol (95:5, v/v)]. After stirring for 2 days at room temperature, methanol was evaporated under vacuum and the remaining solid was triturated with warm (50°C) diisopropyl ether (60 ml). The pure amide derivative was obtained with 94% yield after filtration. M.p.: 112–114°C. $[\alpha]_D = -33°$ ($c = 2$, THF, 22°C). Elemental analysis corresponded to $C_{12}H_{16}N_2O_2$. ^1H NMR (C^2HCl_3): δ 1.38 (s, 2H, NH_2); 2.67 (dd, 1H, CHCH_2Ar); 3.19 (dd, 1H, CHCH_2Ar); 3.57 (m, 1H, CH_2C*H*N); 4.52 (m, 2H, CH_2OAr); 5.2–5.5 (m, 2H, CH_2=CH); 5.80 (s, 2H, CONH_2); 5.96–6.15 (m, 1H, CH_2=C*H*); 6.65–7.25 (m, 4H, Ar).

(S)-N-(3,5-dinitrobenzoyl)tyrosine-O-(2-propen-1-yl)amide (CS 4). CS 4 was obtained in the same manner as CS 3, starting from 6.9 g (30 mmol) of 3,5-DNB chloride, 6.07 ml (90 mmol) of propylene oxide and 6.6 g of **III** in 100 ml of THF. After cooling of the reaction medium, the solid was filtered, rinsed with diisopropyl ether–THF (3:2, v/v) and dried under vacuum. A 7.8-g amount (63% yield) of pure CS 4 resulted. M.p.: 189–191°C. $[\alpha]_D = -41.2°$ ($c = 1$, methanol, 22°C). Elemental analysis corresponded to $C_{19}H_{18}N_4O_7$. ^1H NMR [$(C^2H_3)_2SO$]: δ 2.89–2.96 (dd, 1H, CHCH_2Ar); 3.09–3.15 (dd, 1H, CHCH_2Ar); 4.48 (m, 2H, CH_2OAr); 4.68 (m, 1H, CH_2N*H*); 5.2–5.4 (m, 2H, CH_2=CH); 6.0 (m, 1H, CH_2=C*H*); 6.83 (d, 2H, Ar); 7.22 (s, 1H, CONH_2); 7.24 (d, 2H, Ar); 7.73 (s, 1H, CONH_2);.8.94–9.04 (m, 3H, Ar); 9.38 (d, 1H, CH_2N*H*CO).

Grafting

CS 3 and 4 were grafted onto 3-mercaptopropylsilica (obtained from 5-μm irregular LiChrosorb Si 60 silica) in the same manner as CS 1 and 2 [6] to afford CSP 3 and 4. Elemental analyses: CSP 3, C 16.09, H 2.67, N 0.9, S 3.66, Si 32.65%, corresponding to 0.21 mmol of chiral moiety per gram of CSP 3 (based on N); CSP 4, C 18.04, H 2.54, N 1.03, Si 29.90%, corresponding to 0.18 mmol of chiral moiety per gram of CSP 4 (based on N).

RESULTS AND DISCUSSION

For the present study, two novel CSPs (CSPs 3 and 4, Fig. 1) were prepared and (*S*)-thio-DNBTyr-A (5-μm irregular LiChrosorb Si 60 silica, 150 × 4.6 mm I.D., CSP 1) was taken as a reference. For each CSP, the enantiomeric separation of solutes 1–9 was performed using LC and SubFC modes. For LC, ethanol and methylene chloride were used as polar modifiers as their different chromatographic behaviours have previously been emphasized [13,19,22]. Because of the low polarity of methylene chloride, the elution of solutes 7–9 was not attempted.

TABLE I

CHROMATOGRAPHIC DATA ON CSPs 1–4 USING HEXANE–ETHANOL AS MOBILE PHASE

Operating conditions: flow-rate, 2 ml/min; room temperature; UV detection at 230 nm. k'_2 is the capacity factor of the second-eluted enantiomer, $k'_2 = (t_{r2} - t_0) - 1$, where t_{r2} is the retention time of the last-eluted enantiomer and t_0 is the retention time of a non-retained solute (t_0 = 0.9 min, heptane). The selectivity, α, between two enantiomers is the ratio of their respective capacity factors (k'_2/k'_1). The resolution factor, R_s, was calculated from $R_s = 2[(t_{r2} - t_{r1})/(\omega_1 + \omega_2)]$, where ω_1 and ω_2 are the band widths of the first- and second-eluted enantiomers, respectively. Low resolutions were estimated. The most retained enantiomer is specified for some solutes in the column EO (elution order). The absolute configurations of solute 4 enantiomers are unknown.

Solute	Ethanol (%, v/v)	CSP 1				CSP 2				CSP 3				CSP 4			
		k'_2	α	R_s	EO	k'_2	α	R_s	EO	k'_2	α	R_s	EO	k'_2	α	R_s	EO
1	5	5.69	*1.25*	*2.1*	(*R*)	*6.19*	1.04	0.4	(*R*)	5.33	1.16	1.2	(*R*)	5.75	1.16	1.3	(*R*)
2	5	12.78	*1.12*	*1.0*		12.51	1	0		10.53	1	0		*13.36*	1.07	0.5	
3	5	*5.36*	*1.39*	*1.9*	(*S*)	3.94	1.19	1.2	(*S*)	3.89	1.27	1.5	(*S*)	4.80	1.16	1.1	(*S*)
4	5	11.53	*1.77*	*5.9*	(−)	9.58	1.19	2.1	(−)	10.14	1.22	2.4	(−)	*12.69*	1.32	2.9	(−)
5	10	*16.03*	*3.83*	*12.5*	(*S*)	12.74	2.50	8.0	(*S*)	10.56	1.73	9.2	(*S*)	14.82	2.94	10.0	(*S*)
6	10	9.53	*1.19*	*1.7*	(*S*)	12.44	1.05	0.5	(*S*)	10.72	1	0	(*S*)	*12.59*	1.09	1.0	(*S*)
7	10	16.50	*1.17*	*1.1*		21.63	1.10	1.0		29.22	1.09	0.7		*34.42*	1.06	0.5	
8	15	8.33	*1.18*	*1.2*		6.74	1	0		6.52	1	0		*11.86*	1.08	0.6	
9	15	13.47	*1.35*	*1.8*		10.02	1	0		14.67	1.12	0.7		*17.17*	1.24	1.2	

TABLE II

CHROMATOGRAPHIC DATA ON CSPs 1–4 USING HEXANE–METHYLENE CHLORIDE AS MOBILE PHASE

UV detection at 254 nm. Other conditions as in Table I.

Solute	CH_2Cl_2 (%, v/v)	CSP 1				CSP 2				CSP 3				CSP 4			
		k'_2	α	R_s	EO	k'_2	α	R_s	EO	k'_2	α	R_s	EO	k'_2	α	R_s	EO
1	30	7.92	*1.37*	*2.7*	(*R*)	*8.61*	1.08	0.5	(*R*)	7.50	1.18	1.3	(*R*)	7.67	1.18	1.5	(*R*)
2	30	6.44	*1.13*	*1.0*		*8.86*	1	0		6.34	1	0		7.83	1.05	>0	
3	30	7.68	1	0		6.65	1.24	1.0	(*S*)	*8.99*	*1.29*	*1.2*	(*S*)	6.99	1	0	
4	50	*7.31*	*2.29*	*2.8*	(−)	4.18	1.28	1.2	(−)	6.92	1.25	1.2	(−)	6.80	1.40	1.4	(−)
5	30	5.97	*8.40*	*10.0*	(*S*)	5.17	3.40	8.1	(*S*)	5.61	3.26	8.6	(*S*)	*6.19*	4.24	8.0	(*S*)
6	30	17.14	*1.64*	*4.3*	(*S*)	*26.92*	1.15	1.0	(*S*)	19.19	1.08	0.9	(*S*)	25.83	1.40	3.2	(*S*)

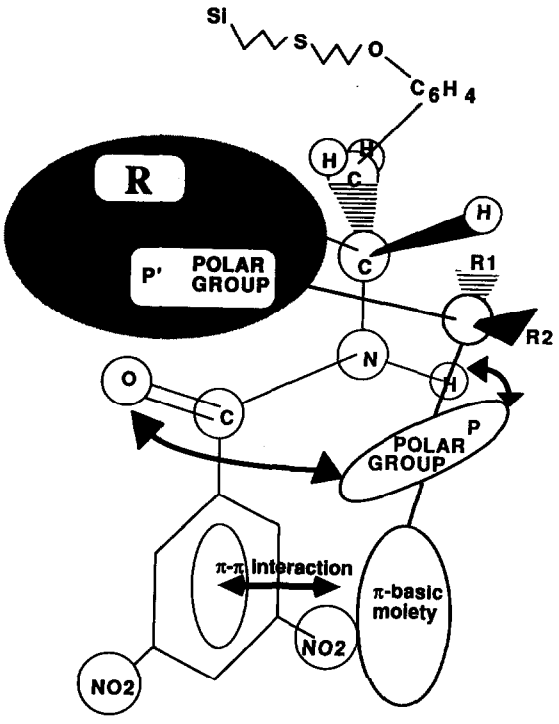


Fig. 4. Schematic representation of chiral recognition mechanisms between CSPs 1–4 and solutes 3, 4, 5, 8 and 9. The difference in chromatographic behaviour is due to the specific interaction between the site of interaction R of the CSP and a polar group of the solute.

Chromatographic data are given in Tables I and II for LC and III for SubFC. For each CSP, the amount of polar modifier in the mobile phase, the capacity factor for the most retained enantiomer (k'_2), the selectivity value (α), the resolution factor (R_s) and the elution order (EO) are reported. For each solute, the highest values of each parameter are given in italics.

From a qualitative point of view, CSP 1 displays a greater enantiorecognition ability towards solutes 1–9. However, data obtained with the other CSPs may be useful for the comprehension of chiral recognition mechanisms. Most of the solutes bear a π-basic moiety which can be involved in a charge-transfer complex with the complementary π-acid 3,5-DNB moiety of the CSP (Fig. 4). Hence, the π–π interaction is assumed to be the driving force in the chiral recognition process. Further, an additional polar group (generally amidic or a related function) located between the π-basic moiety and the asymmetric centre of the solute may participate to a hydrogen bonding or a dipole stacking with the amidic group of the CSP (Fig. 4). These two sites of interaction are common to the four CSPs. Thus, the differences in chromatographic behaviour of these CSPs result from a third potential interaction between the modular site of interaction (R in Fig. 4) of the CSP and a second polar group borne by the solute.

In order to determine the nature of this third interaction, the R group of each

TABLE III

CHROMATOGRAPHIC DATA ON CSPs 1–4 IN THE SubFC MODE

Flow-rate, 4.5 ml/min; temperature, 25°C; average column pressure, 150 bar; UV detection at 229 nm.

Solute	Ethanol (%, w/w)	CSP 1			CSP 2			CSP 3			CSP 4		
		k'_2	α	R_s	k'_2	α	R_s	k'_2	α	R_s	k'_2	α	R_s
1	15	*6.50*	*1.23*	*1.6*	5.76	1.07	0.4	5.41	1.16	1.2	5.64	1.17	1.3
2	15	*16.04*	*1.10*	*1.0*	12.82	1	0	12.22	1	0	15.00	1.07	0.5
3	15	*5.49*	*1.25*	*1.6*	3.70	1.16	1.0	3.96	1.20	1.2	4.57	1.15	1.2
4	7	*15.27*	*1.85*	*7.0*	9.50	1.28	3.1	11.72	1.26	2.8	13.32	1.42	4.7
5	18	*18.31*	*3.29*	*10.7*	14.66	2.34	8.4	11.7	2.22	10.4	17.40	2.76	11.3
6	7	11.22	*1.16*	*1.3*	11.31	1.07	0.5	10.82	1	0	*12.16*	1.10	1.2
7	15	19.57	*1.15*	*1.0*	15.33	1.09	>0	22.90	1.10	0.9	*27.87*	1.10	0.5
8	15	8.02	*1.18*	*1.2*	5.40	1.03	>0	5.75	1	0	*9.06*	1.11	0.4
9	18	17.40	*1.46*	*2.6*	10.44	1.05	>0	14.80	1.14	1.1	*20.94*	1.29	1.3

CSP was assimilated to a solvent possessing the same functional group. Thus, it can be classified according to its dominant character with regard to the selectivity parameters χ_e, χ_d and χ_n (Table IV). These parameters reflect the relative ability of a solvent to act mainly as a proton acceptor (χ_e), a proton donor (χ_d) or a strong dipole (χ_n). The *n*-butylamidic group of CSP 1 was compared with N-methylformamide ($P' = 6.0$: $\chi_e = 0.41$), the methyl ester group of CSP 2 was compared with ethyl acetate ($P' = 4.4$; $\chi_n = 0.43$), the primary alcohol group of CSP 3 was compared with methanol ($P' = 5.1$; $\chi_e = 0.48$) and the primary amidic group of CSP 4 was compared with formamide ($P' = 9.6$).

In order to eliminate the influence of the grafting rate, the capacity factors reported in Tables I–III have been corrected for a theoretical grafting extent of 0.2 mmol per gram of silica (as the magnitude of the variation of the grafting rates was low, it was assumed that capacity factors were proportional to the grafting rate). They were then correlated with the selectivity parameters reported in Table IV.

The retention of solutes possessing a dipole free from steric hindrance was found to fit well with the dipolar character of the CSPs. In Fig. 5 the logarithm of the capacity factor of solutes 3, 5 and 8 is plotted *versus* the proton donor, acceptor and dipolar characters of the CSPs (expressed as the forms $P'\chi_d$, $P'\chi_e$ and $P'\chi_n$, respectively, corresponding to partial polarities) (LC, ethanol as polar modifier). According to the Fig. 5 (good correlation with the dipolar character), a dipole stacking between the R group of the CSP and a dipole borne by the solute [carbamate, ester (1.8 D) and amide (3.7 D) for solutes 3, 5 and 8, respectively] may be advocated.

Fig. 6 shows the correlation between the capacity factor of solute 4 and the proton donor character of the CSPs ($P'\chi_d$) (LC, ethanol as polar modifier). In this instance, the chiral recognition mechanism has been described previously [13]. The *tert.*-butyl ester group of the solute interacts with the R group of the CSP. A good correlation with the dipolar character of the CSP was then expected (represented by a dotted line in Fig. 6). However, according to Fig. 6, it can be assumed that the steric hindrance of the *tert.*-butyl group on the relatively low ester dipole (1.8 D) probably prevents the dipole stacking and rather favours the donor–acceptor mechanism.

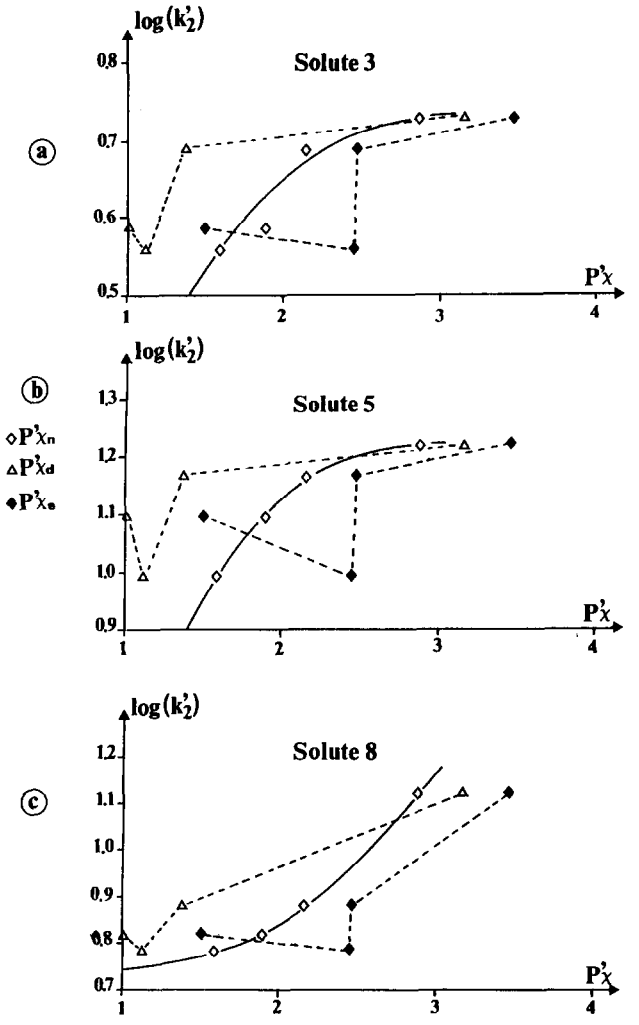


Fig. 5. Logarithm of the capacity factor of solutes (a) 3, (b) 5 and (c) 8 *versus* (◇) the dipolar, (◆) the proton acceptor and (△) the proton donor characters of the CSPs (LC mode). Mobile phases: hexane–ethanol, (a) 95:5, (b) 90:10 and (c) 85:15 (v/v). Flow-rate, 2 ml/min. Room temperature.

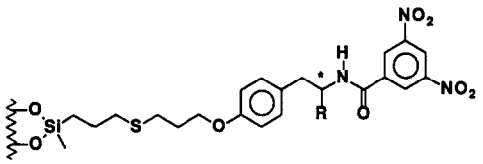
A third type of mechanism may be advocated for solute 9 (oxazepam). The capacity factors of this solute were found to fit well with the proton acceptor character of the CSPs (Fig. 7). The hydroxyl or the amidic proton of solute 9 is probably involved in hydrogen bonding with the basic site of the CSP.

Fig. 7 also illustrates the fact that no significant differences were observed between the LC and SubFC modes when using ethanol as polar modifier. The ethanol content in SubFC was chosen in order to obtain similar capacity factors to those in LC. Under these conditions, comparable selectivity values were observed. The advantage of SubFC lies in higher resolution values per unit time, giving shorter analysis times, as previously emphasized by Macaudière *et al.* [24,25]. As the correlations described

TABLE IV

SELECTIVITY PARAMETERS, AS DEFINED AND CALCULATED BY SNYDER [23] FROM SOLUBILITY DATA REPORTED BY ROHRSCHNEIDER

χ_e, Proton acceptor character; χ_d, proton donor character; χ_n, dipolar character.



CSP	R	Equivalent solvent	P'	χ_e	χ_d	χ_n
CSP 1	CONH–*n*–C_4H_9	N-Methylformamide	6.0	0.41	0.23	0.36
CSP 2	$COOCH_3$	Ethyl acetate	4.4	0.34	0.23	0.43
CSP 3	CH_2OH	Methanol	5.1	0.48	0.22	0.31
CSP 4	$CONH_2$	Formamide	9.6	0.36	0.33	0.30

above in LC were also obtained in SubFC, it can be inferred that the chiral recognition mechanisms are the same in both modes. It can also be assumed that neither hexane nor carbon dioxide affects chiral recognition processes, probably because their low polarities prevent them from solvating the sites of interaction of the CSPs.

On the other hand, significant differences were observed between ethanol and methylene chloride as polar modifiers, as outlined by the investigations on the retention of solute 5 on CSPs 1–4. When ethanol is used as a polar modifier, the retention of solute 5 fits well the dipolar character curve (Fig. 5b). It can therefore be assumed that a dipole stacking occurred between the R group of the CSP and the ester

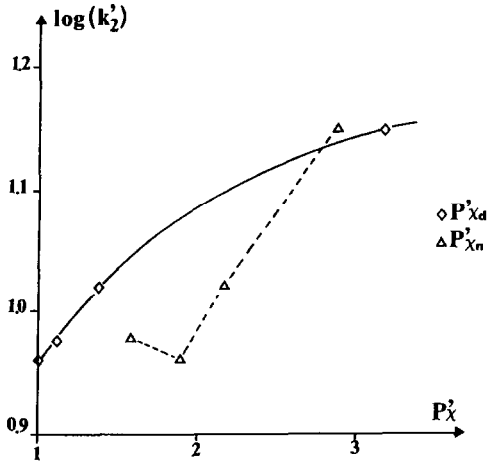


Fig. 6. Logarithm of the capacity factor of solute 4 *versus* (◇) the proton donor and (△) the dipolar characters of the CSPs (LC mode). Mobile phase, hexane–ethanol (95:5, v/v). Other conditions as in Fig. 5.

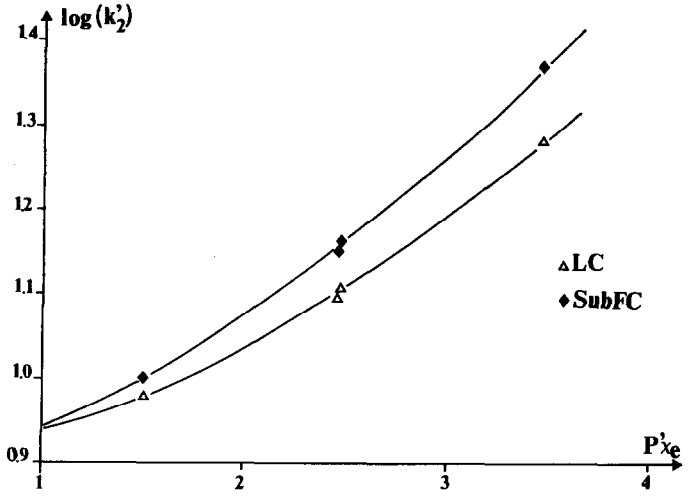


Fig. 7. Logarithm of the capacity factor of solute 9 *versus* the proton–acceptor character of the CSPs in (△) LC and (◆) SubFC modes. LC: flow-rate, 2 ml/min; hexane–ethanol (85:15, v/v); room temperature. SubFC: flow-rate, 4.5 ml/min; carbon dioxide–ethanol (82:18, w/w); average pressure, 150 bar; temperature, 25°C.

function of solute 5. On the other hand, when methylene chloride (strong dipole) is used, the retention of solute 5 may rather be correlated with the proton donor character of the CSPs (Fig. 8), thus indicating a proton donor–acceptor type of mechanism. In fact, the retention mechanism depends on the nature of the polar modifier; with a strong dipolar solvent the retention mechanism rather involves

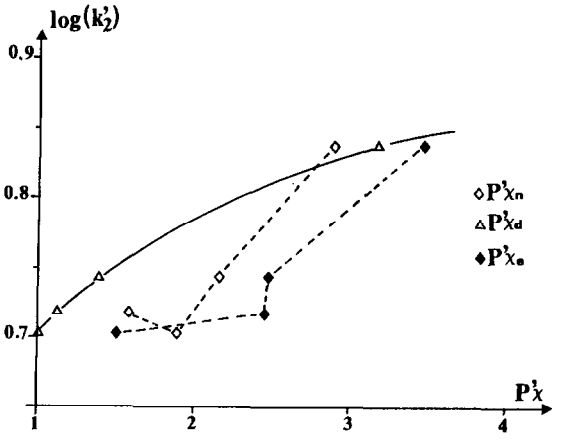


Fig. 8. Logarithm of the capacity factor of solute 5 *versus* (◇) the dipolar, (◆) the proton acceptor and (△) the proton donor characters of CSPs using hexane–methylene chloride (70:30, v/v) as the mobile phase. Other conditions as in Fig. 5. The retention is correlated with the proton donor character instead of the dipolar character as in Fig. 5b.

hydrogen bondings, whereas with a strong donor–acceptor solvent, dipole stackings are favoured. The observed elution orders suggest that the two mechanisms work in the same stereochemical sense.

Another important feature concerning stereoselectivity was observed. Despite the higher retention times, the selectivity values obtained with CSP 4 are lower than those with CSP 1. Equivalent attractive CSP–solute interactions can be advocated for these two CSPs, but repulsive CSP–solute interactions, responsible for chiral discrimination, may result from the bulky *n*-butyl group. The nature of the amidic substituent has been poorly investigated. Nevertheless, these preliminary results suggest that it plays an important role in the chiral recognition process. Further investigations are in progress in order to improve the chiral discrimination power of CSPs derived from tyrosine.

CONCLUSION

The grafting mode chosen for designing CSPs derived from tyrosine has allowed the synthesis of four CSPs based to one pattern. They only differ in one potential site of interaction which may be characterized by its ability to act as a proton donor, a proton acceptor or a strong dipole. For solutes possessing a π-basic moiety and two polar groups such as amide, ester or alcohol in the vicinity of the asymmetric centre, a chiral recognition process based on three simultaneous interactions is advocated. The results give a key to the determination of the nature of the third interaction. For solutes possessing a dipole (ester, amide or carbamate) free from steric hindrance, the dipole stacking constitutes the main attractive interaction. The results of this study also demonstrate that steric effects play an important role in chiral recognition, either by preventing dipole stacking or by generating repulsive interactions, leading to chiral discrimination. Through results obtained with solute 5, the important contribution of the solvent to chiral recognition is emphasized. When using a polar modifier possessing either a strong dipole or a proton donor–acceptor character, the CSP rather acts through the opposite character.

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