Chiral-bonded silica gel stationary phases obtained from chiral silanes for high-performance liquid chromatography

Comparison of performance with that of stationary phases obtained from γ-aminopropylsilica gel[☆]

Laureano Oliveros

Conservatoire National des Arts et Métiers, Laboratoire de Chimie Générale (CNRS URA 1103), 292 Rue Saint-Martin, 75141 Paris Cédex 03 (France)

Cristina Minguillón

Laboratorio de Química Farmacéutica, Facultad de Farmacia, Universidad de Barcelona, Avd. Diagonal s/n, 08028 Barcelona (Spain)

Bernard Desmazières and Paul-Louis Desbène

CNRS, URA 455, Université Pierre et Marie Curie, Laboratoire de Chimie Organique Structurale, 4 Place Jussieu, 75230 Paris Cédex 05 and Université de Rouen, LASOC, 43 Rue Saint Germain, 27000 Evreux (France)

ABSTRACT

Several chiral triethoxysilanes, easily prepared from acidic or basic chiral compounds and aminopropyltriethoxysilane or isocyanatepropyltriethoxysilane were bonded onto silica gel. In the chiral stationary phases thus obtained, only silanol groups and the chiral selector can interact with solutes. To deactivate these silanol groups, an end-capping treatment with hexamethyldisilazane was applied. Unlike chlorotrimethylsilane, this reagent does not destroy the chemically bonded chiral silica gel. The performance of these stationary phases was evaluated and compared with that of bonded silica gel phases with the same chiral selector but fixed on a γ-aminopropylsilica gel. Chiral stationary phases obtained from chiral silanes and an end-capping treatment showed the best resolution and the shortest retention time for most of the compounds tested.

INTRODUCTION

The chiral stationary phases used in high-performance liquid chromatography (HPLC) which belong to the acceptor–donor type are generally prepared by condensation of the chiral compound on silica gel previously treated with a silane bearing, in most instances, a primary amino group [1] or an epoxide [2,3] or, rarely, a sulphide group [4]. These condensations are not quantitative. The chiral stationary phases obtained in this way always have some unreacted groups remaining which are able to interact in a non-stereoselective way with the racemic mixtures to be resolved.

There are various ways of preparing stationary

Correspondence to: Dr. L. Oliveros, Conservatoire National des Arts et Métiers, Laboratoire de Chimie Générale (CNRS URA 1103), 292 Rue Saint-Martin, 75141 Paris Cédex 03, France.

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phases without using previously functionalized silica gel. Some of these are easy to carry out; for instance, the condensation of an alkoxysilane [5–7] or a chlorosilane [8,9] bearing a chiral radical on the silica gel. Others are rarely used; for instance, the cross-linking or chemically bonding chiral polysiloxanes with Si–H groups linked to the silica gel via silanol groups [10]. All these methods used for the preparation of chiral stationary phases should be able to overcome the disadvantages inherent in the use of previously functionalized silica. The condensation of chiral silanes on silica gel was therefore chosen as a method for the preparation of chiral stationary phases with the aim of obtaining stationary phases able to establish the minimum of achiral interactions with solutes. If the pursued objective is the resolution of racemic compounds, the reduction of achiral interactions contributes to a reduction in the retention time and to an increase in the selectivity factor [11]. The selectivity factor, α, for a racemic mixture is the ratio between the two capacity factors of the two enantiomers. These are a function of the sum of achiral interactions, common to both enantiomers, and chiral interactions, different in each enantiomer. Thus α will increase when achiral interactions become smaller.

The preparation of such stationary phases from chiral silanes provides the opportunity to compare performances with those of stationary phases bearing the same chiral entity but obtained by condensation on a γ-aminopropylsilica gel [12]; both stationary phases are prepared from the same silica gel.

This paper describes the preparation of six chiral silanes, their condensation on silica gel and the evaluation of the chiral stationary phases thus obtained. One of the six chiral selectors used belongs to the π-acceptor type and the others have a π-donor character. The π-acceptor chiral selector is 3,5-dinitrobenzoylphenylalanine, which has been used previously [13,14]. The π-donor chiral selectors chosen previously [12] were N-(3,5-dimethoxybenzoyl), N-(3,5-dimethylbenzoyl) and 3,5-dimethylanilido derivatives of (S)-phenylalanine and (S)-naproxen (Fig. 1). The last compound has been used by Doyle and co-workers [15,16] to prepare a chiral stationary phase by a different method. Silanes were obtained for this study by the reaction of chiral entities with acidic groups on 3-aminopropyltriethoxysilane and the chiral selector with an amino group on 3-isocyanatopropyltriethoxysilane (Fig. 2). The synthetic scheme for the preparation of chiral stationary phases is given in Fig. 3 and their structures in Fig. 4. Formulae of racemic compounds used as test compounds are given in Fig. 5.

EXPERIMENTAL

NMR spectra were measured using a Brucker AC200 spectrometer. Tetramethylsilane (TMS) was
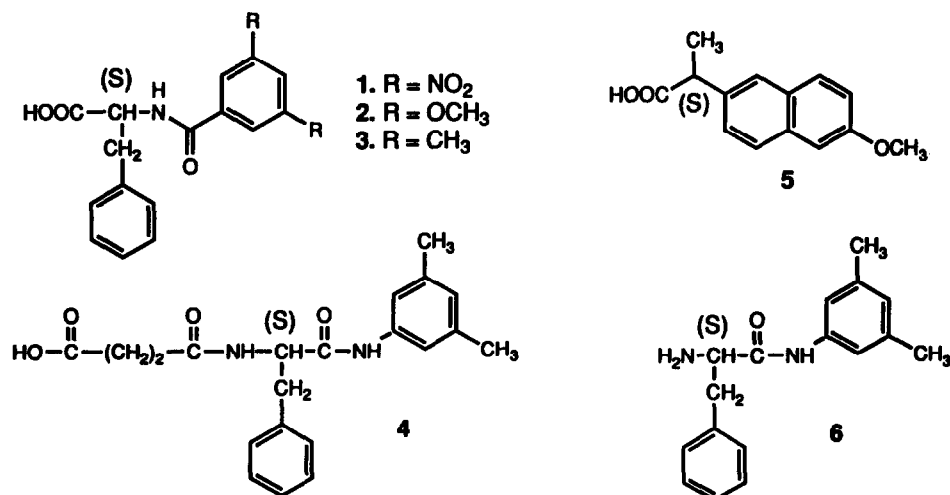

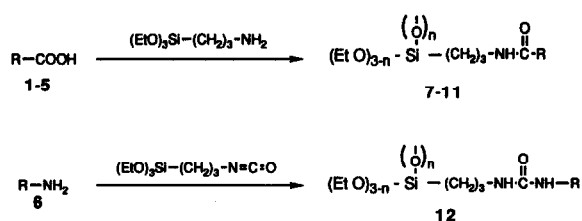Fig. 1. Structures of chiral selectors.

Fig. 2. Synthetic scheme for the preparation of chiral silanes 7 to 12.

used as the internal standard and the chemical shift, δ, is measured in ppm. Rotatory power was measured with a Perkin-Elmer Model 241 polarimeter. Elemental analyses were performed by the Service Central de Microanalyse du CNRS (Vernaison, France). The chromatographic experiments were carried out on an HP 1090 liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a PU4020 UV detector (Philips, Cambridge, UK). The chiral stationary phases were packed into stainless-steel tubes (100 × 4.6 mm I.D.) by the slurry method according to Coq et al. [17]. The volume of sample injected was 5 μl. The flow-rate of the pump was 1 ml/min. The detection wavelength was 254 nm. The mobile phases consisted of various mixtures of n-heptane, chloroform and methanol.

Chemicals and reagents

Compounds 1–4 (Fig. 1) were prepared by the

method described previously [12]. Compound 5 was purchased from Fluka. Compounds 13–15 (Fig. 5) were obtained by treating the methyl ester of each amino acid with 3,5-dinitrobenzoyl chloride. Compounds 16 and 17 (Fig. 5) and 3-aminopropyltriethoxysilane were purchased from Aldrich. 3-Isocyanatopropyltriethoxysilane was purchased from Hüls-Petrarch.

To prepare phenylalanyl(3,5-dimethyl)anilide (6) (Fig. 1), 27 g (73.3 mmol) of (S)-tert.-butoxycarbonylphenylalanyl-3,5-dimethylphenylamine, prepared by the method described previously [12], were dissolved in 250 ml of glacial acetic acid and cooled in an ice-bath. Hydrogen chloride was passed through for 45 min and the solution was left to stand at room temperature for 3 h. The solvent was removed *in vacuo* and 350 ml of 1 M NaOH were added to the residual white solid. The mixture was extracted with chloroform. Evaporation of the solvent gave 19.4 g (98.6% yield) of 6 as a viscous liquid. ^{1}H NMR (200 MHz): δ (C^{2}HCl$_3$) 1.56 (s, 2H, NH$_2$), 2.32 (s, 6H, CH$_3$), 2.78 (m, 1H, CH$_a$Ar), 3.35 (m, 1H, CH$_b$Ar), 3.68 (m, 1H, CH), 6.78 (s, 1H, C^{4}H), 7.27 (m, 7H, aromatics), 9.40 (ba, 1H, NH). ^{13}C NMR (50.3 MHz): δ (C^{2}HCl$_3$) 21.3 (CH$_3$), 40.6 (CH$_2$), 56.7 (CH), 117.2 (C^{2}H and C^{6}H), 125.8 and 126.8 (C^{4}H and C$^{4'}$H), 128.7 and 128.2 (C$^{2',6'}$H and C$^{3',5'}$H), 137.5 and 137.7 (C^1 and C$^{1'}$), 138.5 (C^{3,5}), 172.3 (CO). $[\alpha]_D^{23} = -61°$ (c = 1.5, pyridine).
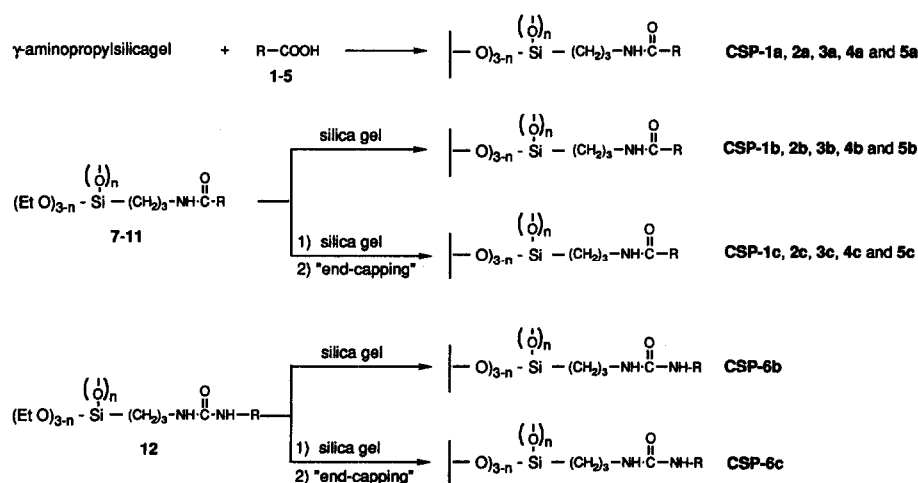
The hydrochloride had a melting point of 230°C

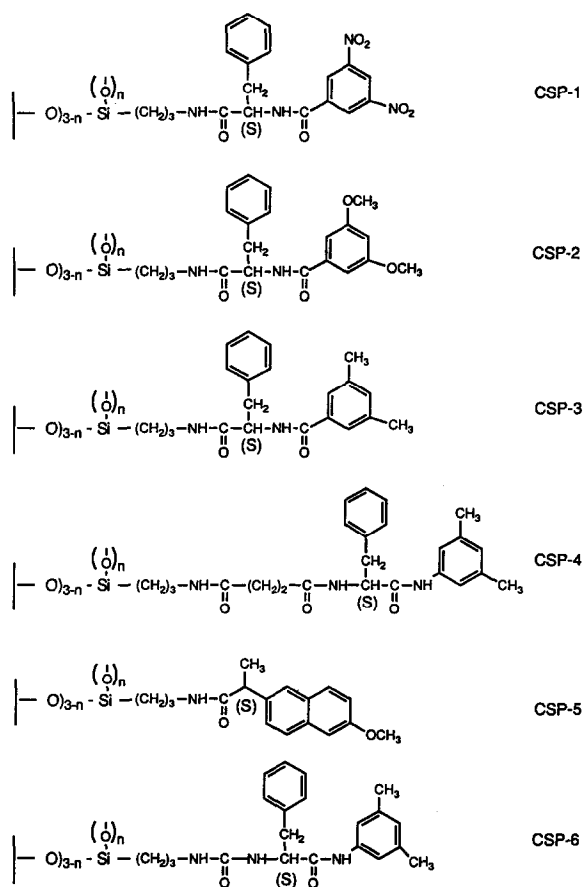Fig. 3. Synthetic scheme for the preparation of chiral stationary phases.

Fig. 4. Structures of chiral stationary phases.

(ethanol absolute). $[\alpha]_D^{23} = +113.5°$ ($c = 1.6$, ethanol 96°). Analysis: calculated for $C_{17}H_{21}ClN_2O$, C 66.98, H 6.94, Cl 11.68, N 9.19%; found, C 66.89, H 6.86, Cl 11.74, N 9.14%.

N-Acyl-3-aminopropyltriethoxysilane (**7, 8, 9, 10** and **11**) (Fig. 2) was prepared as follows. To a
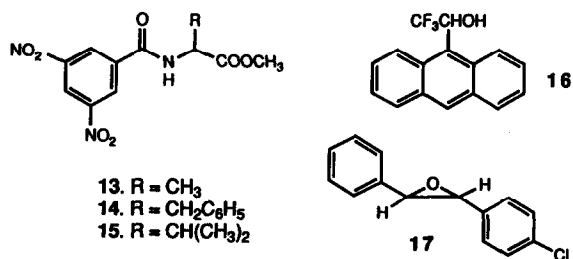

Fig. 5. Structures of test compounds.

solution of 6 mmol of the appropriate chiral acidic compound (**1–5**) in 18 ml of pyridine, 1.7 ml (7.2 mmol) of 3-aminopropyltriethoxysilane in 12 ml of pyridine were added. The mixture was stirred under reflux for 1.5 h. The solvent and the excess of 3-aminopropyltriethoxysilane were removed under reduced pressure (0.1 mmHg) and the residue was used in the following step without further purification.

When silanes were prepared by heating at reflux temperature a variable loss of ethoxy groups was observed. The extent of this loss depended on the silane and on the time of heating. Silane **12** was the most stable in the series prepared here and silane **8** was the least stable. However, more stable phases can be obtained when these compounds are prepared at high temperatures. These can be explained if a partial polymerization takes place in this step. It was therefore decided to prepare these compounds by heating under the same experimental conditions. (In spite of the difference of stability in silanes, the amount of bonded chiral moiety per gram of stationary phase is almost the same in all comparable stationary phases CSP-1b to 5b.)

N-[N-(3,5-Dinitrobenzoyl)-(S)-phenylalanyl]-(3-triethoxysilyl)propylamide (7). ^{1}H NMR (200 MHz): δ (C^2HCl_3) 0.64 (t, 2H, CH_2Si), 1.17 (t, 9H, CH_3), 1.77 (m, 2H, $CH_2CH_2CH_2$), 2.87 (t, 2H, CH_2N), 3.21 (m, 2H, CH_2Ar), 3.77 (q, 6H, CH_2O), 4.58 (m, 1H, CH), 6.68 (bb, 1H, NH), 7.13 (m, 5H, C_6H_5), 8.21 (bb, 1H, NH), 8.85 (d, 2H, C^2H and C^6H), 8.98 (d, 1H, C^4H). ^{13}C NMR (50.3 MHz): δ (C^2HCl_3) 7.5 (CH_2Si), 18.2 (CH_3), 21.8 (CH_2), 37.6 (CH_2Ar), 42.0 (CH_2N), 56.8 (CH), 58.6 (CH_2O), 120.7 (C^4H), 126.6 ($C^{4'}H$), 127.4, 128.3 and 129.1 ($C^{2,6}H$, $C^{2',6'}H$ and $C^{3',5'}H$), 137.7 and 138.8 (C^1 and $C^{1'}$), 148.3 ($C^{3,5}$), 162.4 (ArCONH), 176.5 (CONH).

N-[N-(3,5-Dimethoxybenzoyl)-(S)-phenylalanyl]-(3-triethoxysilyl)propylamide (8). ^{1}H NMR (200 MHz): δ (pyridine-d_5) 0.93 (m, 2H, CH_2Si), 1.24 (t, 9H, CH_3), 1.93 (m, 2H, CH_2), 2.22 (m, 2H, CH_2N), 3.32 (m, 2H, CH_2Ar), 3.72 (s, 6H, CH_3O), 3.89 (q, 6H, CH_2O), 5.35 (m, 1H, CH), 5.61 (bb, 1H, NH), 6.74 (d, 1H, C^4H), 7.16–7.71 (m, 7H, C_6H_5, C^2H and C^6H), 8.32 (bb, 1H, NH). ^{13}C NMR (50.3 MHz): δ (pyridine-d_5) 10.6 (CH_2Si), 19.2 (CH_3CH_2O), 23.7 (CH_2), 38.8 (CH_2Ar), 42.7 (CH_2N), 55.3 (CH_3O), 57.3 (CH_2O), 58.5 (CH), 104.1 (C^4H), 106.2 ($C^{2,6}H$), 126.7 ($C^{4'}H$), 128.7 and

129.9 ($C^{2',6'}H$ and $C^{3',5'}H$), 137.2 and 138.1 (C^1 and $C^{1'}$), 161.2 ($C^{3,5}$), 167.0 (ArCONH), 172.4 (CONH).

N-[N-(3,5-Dimethylbenzoyl)-(S)-phenylalanyl]-(3-triethoxysilyl)propylamide (9). ^{1}H NMR (200 MHz): δ (C^2HCl_3) 0.54 (m, 2H, CH_2Si), 1.16 (t, 9H, CH_3), 1.64 (m, 2H, $CH_2CH_2CH_2$), 2.18 (s, 6H, CH_3Ar), 2.67 (m, 2H, CH_2N), 3.21 (m, 2H, CH_2Ar), 3.73 (q, 6H, CH_2O), 4.53 (m, 1H, CH), 6.98 (bb, 1H, NH), 7.11 (m, 10H, aromatics and NH). ^{13}C NMR (50.3 MHz): δ (C^2HCl_3) 7.4 (CH_2Si), 18.2 (CH_3), 20.9 (CH_3Ar), 23.1 (CH_2), 37.6 (CH_2Ar), 42.6 (CH_2N), 56.5 (CH), 58.2 (CH_2O), 124.6 ($C^{2,6}H$), 126.1 (C^4H), 127.9 and 129.4 ($C^{2',6'}H$ and $C^{3',5'}H$), 132.7 (C^4H), 137.8 ($C^{3,5}$), 134.3 and 138.2 (C^1 and $C^{1'}$), 167.1 (ArCONH), 176.7 (CONH).

(S)-N-[1-(3,5-Dimethylphenylaminocarbonyl)-2-phenylethyl]-N'-(3-triethoxysilylpropyl)succinamide (10). ^{1}H NMR (200 MHz): δ (pyridine-d_5) 0.49 (m, 2H, CH_2Si), 0.91 (t, 9H, CH_3), 1.51 (m, 2H, $CH_2CH_2CH_2$), 1.79 (s, 6H, CH_3Ar), 2.51 (m, 4H, CH_2CO), 2.92 (m, 2H, CH_2N), 3.20 (m, 2H, CH_2Ar), 3.48 (t, 6H, CH_2O), 5.02 (m, 1H, CH), 6.28 (s, 1H, NHAr), 6.6–7.4 (m, 8H, aromatics), 9.15 (bb, 1H, CONH), 10.31 (bb, 1H, CONH). ^{13}C NMR (50.3 MHz): δ (pyridine-d_5) 7.5 (CH_2Si), 18.5 (CH_3), 20.7 (CH_3Ar), 22.8 (CH_2), 33.0 and 33.9 (CH_2CO), 38.3 (CH_2Ar), 42.2 (CH_2N), 55.6 (CH), 56.7 (CH_2O), 117.9 ($C^{2,6}H$), 125.1 and 126.1 (C^4H and $C^{4'}H$), 128.0 and 129.0 ($C^{2',6'}H$ and $C^{3',5'}H$), 137.6 ($C^{3,5}$), 137.6 and 138.8 (C^1 and $C^{1'}$), 170.7, 173.5 and 178.3 (CONH).

(S)-2-(6-Methoxy-2-naphthyl)propionyl-3-triethoxysilylpropylamide (11). ^{1}H NMR (200 MHz): δ (pyridine-d_5) 0.49 (m, 2H, CH_2Si), 0.83 (t, 9H, CH_3), 1.37 (d, 3H, CH_3), 1.55 (m, 2H, $CH_2CH_2CH_2$), 3.16 (m, 2H, CH_2N), 3.41 (s, 3H, CH_3O), 3.51 (q, 6H, CH_2O), 6.93 (m, 2H, C^3H and C^7H), 7.46 (m, 4H, C^1H, C^4H, C^5H and C^8H), 8.36 (bb, 1H, CONH). ^{13}C NMR (50.3 MHz): δ (pyridine-d_5) 9.0 (CH_2Si), 18.2 (CH_3CH_2O), 19.3 (CH_3), 23.5 (CH_2), 42.4 (CH_2N), 46.6 (CH), 54.9 (CH_3O), 58.1 (CH_2O), 105.9 and 116.7 (C^7H and C^5H), 126.0, 126.8, 127.5 and 129.2 (C^3H, C^1H, C^4H and C^8H), 129.3, 133.7 and 138.3 (C^{8a}, C^{4a} and C^2), 157.5 (C^6), 174.1 (CO).

(*S*)-N-(3-Triethoxysilyl)propyl-N'-[1-(3,5-dimethylphenyl)aminocarbonyl-2-phenyl]ethylurea (**12**) was prepared as follows. To a solution of 6 mmol (1.6 g)

TABLE I

ELEMENTAL ANALYSES OF CHIRAL STATIONARY PHASES

Chiral stationary phase	Elemental analysis (%)			Ratio of carbon atoms per nitrogen atom		Bonded chiral moieties per gram of stationary phase (mmol)		α_{exp} (μmol/m^2) from %C[a]
	C	H	N	Analytical	Theoretical	From %C	From %N	
CSP-1b	8.35	1.40	2.22	4.39	4.75	0.36	0.39	1.20
CSP-1c	10.36	1.36	2.11	5.73	—	—	0.38	—
CSP-2b	9.74	1.44	1.22	9.31	10.50	0.39	0.44	1.30
CSP-2c	10.79	1.49	1.20	10.43	—	—	0.43	—
CSP-3b	9.74	1.49	1.30	8.74	10.50	0.39	0.46	1.28
CSP-3c	11.12	1.76	1.27	10.22	—	—	0.45	—
CSP-4b	10.93	1.68	1.80	7.08	8.00	0.38	0.43	1.29
CSP-4c	12.27	1.94	1.72	8.32	—	—	0.41	—
CSP-5b	7.32	1.17	0.62	13.77	17.00	0.36	0.44	1.14
CSP-5c	9.48	1.55	0.65	17.20	—	—	0.46	—
CSP-6b	4.70	1.07	0.83	6.45	7.00	0.19	0.20	0.58
CSP-6c	7.15	1.52	0.89	9.37	—	—	0.21	—

[a] Surface concentration of surface-bonded chiral entities, $\alpha_{exp} = \dfrac{w}{M}\left(\dfrac{10^6}{S_{BET}(1-w)}\right)$, where w = weight of functional group (grams per gram of adsorbent), M = molar weight of the bonded functional group (g/mol) and S_{BET} = specific surface area of the starting support (m^2/g) [18].

of the amine **6** in 18 ml of pyridine, 7.2 mmol of 3-isocyanatopropyltriethoxysilane in 8 ml of pyridine were added. The mixture was stirred under reflux for 1.5 h. The solvent and the excess of iso-cyanatopropyltriethoxysilane were removed under reduced pressure (0.1 mmHg) and the residue was used in the next step without further purification. ^{1}H NMR (200 MHz): δ (pyridine-d_5) 0.72 (m, 2H, CH_2Si), 1.17 (t, 9H, CH_3), 1.78 (m, 2H, $CH_2CH_2CH_2$), 2.13 (s, 6H, CH_3), 3.30 (m, 2H, CH_2Ar), 3.41 (m, 2H, CH_2N), 3.81 (q, 6H, CH_2O), 5.18 (s, 2H, NH), 5.37 (m, 1H, CH), 6.8–7.6 (m, 8H, aromatics). ^{13}C NMR (50.3 MHz): δ (pyridine-d_5) 8.1 (CH_2Si), 16.5 (CH_3CH_2O), 21.3 (CH_3), 24.4 (CH_2), 39.7 (CH_2Ar), 43.2 (CH_2N), 56.6 (CH), 58.4 (CH_2O), 118.4 ($C^{2,6}H$), 125.6 and 126.7 (C^4H and $C^{4'}H$), 128.6 and 129.9 ($C^{2',6'}H$ and $C^{3',5'}H$), 138.3 ($C^{3,5}$), 138.5 and 139.5 (C^1 and $C^{1'}$), 159.2 (NHCONH), 172.2 (CONH).

Chiral stationary phases

Chiral stationary phases CSP-1a, -2a, -3a, -4a and -5a were obtained from the appropriate chiral acidic compound (**1–5**) as described previously [12].

Chiral stationary phases CSP-1b, -2b, -3b, -4b, -5b and -6b were prepared as follows. A 6-g mass of spherical silica (5 μm, 100 Å, Nucleosil 100-5; Macherey–Nagel) was slurried with toluene and then the water was removed azeotropically using a Dean-Stark trap. After the complete removal of water, toluene was removed by distillation and a solution of 6 mmol of the appropriate chiral silane (**7–12**) freshly prepared in 50 ml of pyridine was added. The mixture was stirred under reflux for 1.5 h. The resulting bonded silica was collected by filtration and washed exhaustively with pyridine, ethanol, water, ethanol, acetone and diethyl ether, and dried *in vacuo* at room temperature.

Chiral stationary phases CSP-1c, -2c, -3c, -4c, -5c and -6c were obtained by the procedure just described but 3 ml of hexamethyldisilazane were added after the refluxing period and the mixture was allowed to react for an additional hour at reflux temperature. The stationary phases thus obtained were washed as described.

The elemental analyses and the surface concentration of surface-bonded chiral entities (μmol/m^2), according to Unger *et al.* [18], of the new stationary phases are given in Table I.

RESULTS AND DISCUSSION

Chromatographic results are shown in Table II. Results relating to stationary phases obtained from γ-aminopropylsilica gel (CSP-Xa) are from Oliveros *et al.* [12].

Comparison of performance of stationary phases with the same chiral selector obtained either by condensation of a chiral silane on the silica gel (CSP-Xb) or by condensation of the chiral entity on γ-aminopropyl-silica gel (CSP-Xa)

In many instances stationary phases obtained by the action of chiral silanes on the silica gel (CSP-Xb) have slightly lower selectivity factors than those of stationary phases obtained by condensation of chiral entities on γ-aminopropylsilica gel (CSP-Xa). The CSP-Xb gels show retention times longer than CSP-Xa in most instances. Although there are no free aminopropyl groups in this kind of stationary phase (these are always present in stationary phases obtained from γ-aminopropylsilica gel), the presence of free silanol groups is possible. Silanol groups may be left over from the silica gel or come from the hydrolysis of unreacted ethoxysilyl groups on the silane. The remaining silanol groups on the stationary phases are considered to be strong adsorption sites [19]. This could be the reason for the increased retention of compounds by stationary phases.

Comparing the elemental analyses of CSP-Xa [12] and CSP-Xb (Table I) the calculated values of the amount (mmol) of chiral selector per gram of stationary phase in CSP-Xb are generally 20% lower than those in CSP-Xa (calculated from the percentage carbon). In the last instance, the presence of free aminopropyl groups has not been considered, but the presence of such groups cannot explain the difference in analytical values. In any case, and although the amount of chiral selector per gram of stationary phase would be the same, the lack of aminopropyl groups on CSP-Xb makes them much more polar than CSP-Xa and increases the retention of test compounds. This inconvenience would be overcome by adding an end-capping step in the preparation of stationary phases.

Comments on the end-capping treatment of silicas obtained from chiral silanes

Taking into account these results and the aim to

TABLE II

CAPACITY FCATORS k' AND SELECTIVITY FACTORS α IN COLUMNS TESTED

Chiral stationary phase	13			14			15			16			17		
	k'_1	k'_2	α	k'_1	k'_2	α	k'_1	k'_2	α	k'_1	k'_2	α	k'_1	k'_2	α
CSP-1a	1.84 (R)[a]	2.20	1.19	1.27 (R)	1.54	1.21	1.03 (R)	1.26	1.22	2.18 (S)	2.59	1.19	0.52	0.83	1.60
CSP-1b	2.14 (R)	2.62	1.22	1.05 (R)	1.26	1.19	0.89 (R)	1.05	1.18	3.92 (S)	4.66	1.19	0.39	0.60	1.55
CSP-1c	1.32 (R)	2.18	1.65	0.90 (R)	1.50	1.67	0.84 (R)	1.40	1.66	2.94 (S)	5.27	1.79	0.40	0.56	1.41
CSP-2a	1.66 (R)	3.80	2.29	0.75 (R)	1.66	2.21	0.56 (R)	1.12	2.00	2.06	2.06	1.00	0.04	0.15	3.33
CSP-2b	3.13 (R)	5.34	1.71	4.20 (R)	6.69	1.59	1.29 (R)	2.20	1.71	2.18	2.18	1.00	0.20	0.38	1.93
CSP-2c	1.38 (R)	3.33	2.41	0.68 (R)	1.91	2.80	0.61 (R)	1.42	2.35	1.86	1.86	1.00	0.17	0.17	1.00
CSP-3a	2.02 (R)	3.29	1.63	0.90 (R)	1.71	1.90	0.62 (R)	1.12	1.81	2.91	2.91	1.00	0.14	0.21	1.50
CSP-3b	3.31 (R)	5.15	1.56	1.12 (R)	2.35	2.11	0.92 (R)	1.71	1.84	2.29	2.29	1.00	0.14	0.24	1.70
CSP-3c	0.79 (R)	2.12	2.69	0.44 (R)	1.23	2.79	0.38 (R)	0.92	2.44	1.98	1.98	1.00	0.10	0.10	1.00
CSP-4a	1.90 (R)	4.40	2.32	0.91 (R)	1.91	2.10	0.65 (R)	1.61	2.47	1.50	1.50	1.00	0.14	0.24	1.70
CSP-4b	3.48 (R)	6.86	1.97	1.61 (R)	3.10	1.93	1.26 (R)	2.55	2.02	2.46	2.46	1.00	0.15	0.32	2.09
CSP-4c	1.82 (R)	4.45	2.44	1.13 (R)	2.40	2.12	1.00 (R)	2.14	2.14	2.21	2.21	1.00	0.12	0.18	1.56
CSP-5a	5.53 (S)	7.40	1.34	2.37 (S)	3.57	1.51	1.37 (S)	2.07	1.51	2.10	2.17	1.03	0.22	0.33	1.50
CSP-5b	9.96 (S)	11.34	1.14	2.86 (S)	3.42	1.19	2.54 (S)	2.97	1.17	2.49	2.49	1.00	0.24	0.50	2.06
CSP-5c	3.00 (S)	5.29	1.76	1.29 (S)	2.43	1.89	1.00 (S)	1.84	1.84	1.51	1.58	1.05	0.12	0.12	1.00
CSP-6b	5.35 (R)	7.72	1.44	2.03 (R)	3.78	1.86	1.75 (R)	2.91	1.67	2.21	2.21	1.00	0.18	0.47	2.57
CSP-6c	0.97 (R)	3.35	3.45	0.47 (R)	1.53	3.24	0.37 (R)	1.13	3.04	1.05	1.05	1.00	0.08	0.08	1.00
Mobile phase chloroform–0.5% methanol/heptane	70:30			70:30			70:30			70:30			25:75		

[a] Absolute configuration of first-eluted enantiomer.

prepare stationary phases able to establish the weakest achiral interactions with solutes, the silanol groups were deactivated. This is a common treatment consisting most often in bonding trimethylsilyl radicals onto the silanol groups. This can be achieved by several reagents [20–22]. Trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS) are often used as deactivating reagents. Buszewski [23] has suggested that HMDS liberates ammonia during the end-capping treatment which leads to a partial loss of the bonded silane. The end-capping treatment was therefore carried out first with TMCS, one of the most reactive reagents towards residual silanol groups.

The elemental analyses of the resulting stationary phases showed that this reagent causes the loss of the chiral entity. This degradation has been studied with CSP-1b and CSP-3b, whose chiral selectors are 3,5-dinitrobenzoylphenylalanine and 3,5-dimethylbenzoylphenylalanine, respectively. Elemental analyses after the treatment with TMCS are: C 4.86,

H 1.54 and N 1.11% for CSP-1b; and C 4.60, H 1.55 and N 1.10% for CSP-3b. These values are different from those of the same phases before the treatment (Table I), but similar to each other and also similar to those of the γ-aminopropylsilica gel obtained by the action of 3-aminopropyltriethoxysilane on the same silica gel (C 3.10, H 1.05, N 0.90% [13]). This observation suggests that a breakage between the chiral entity and the spacer takes place on the amide group between them.

Methoxytrimethylsilane has also been used as a silylating agent. In this instance degradation of the bonded stationary phases has also been observed, but to a lesser degree than that observed using TMCS.

Finally, the end-capping treatment was carried out using HMDS. As can be seen in Table I, the amount of chiral compound per gram of stationary phase is nearly the same before and after the end-capping. Therefore, there is no noticeable degradation of the stationary phases.

Comparison of stationary phases with CSP-Xc or without CSP-Xb end-capped silanol groups

The consequences of the end-capping treatment on the chromatographic behaviour of stationary phases obtained by condensation of chiral silanes on silica gel are very important.

First, selectivity factors are clearly larger in end-capped stationary phases. Only compound **17**, resolved by CSP-2b, -3b, -5b and -6b, with selectivity factors from 1.70 to 2.57, is not resolved by end-capped chiral stationary phases with the same chiral selector. At present, we cannot explain this loss of selectivity in chiral stationary phases by the end-capping treatment.

Secondly, in general, retention times shown by these silicas are shorter than those shown by silicas which have not been end-capped. CSP-6b and CSP-6c are good examples: the reduction of the retention time of the first eluted enantiomer and the increase in selectivity factors in CSP-6c relating to CSP-6b is remarkable.

Moreover, if the retention time of the last eluted enantiomer is sometimes longer in CSP-Xc than in CSP-Xb, then the selectivity factor is more strongly enhanced in CSP-Xc than in CSP-Xb. Figs. 6 and 7 show these situations. Chromatograms of the reso-
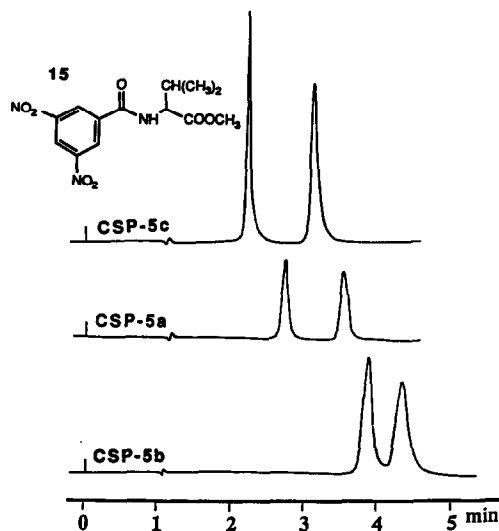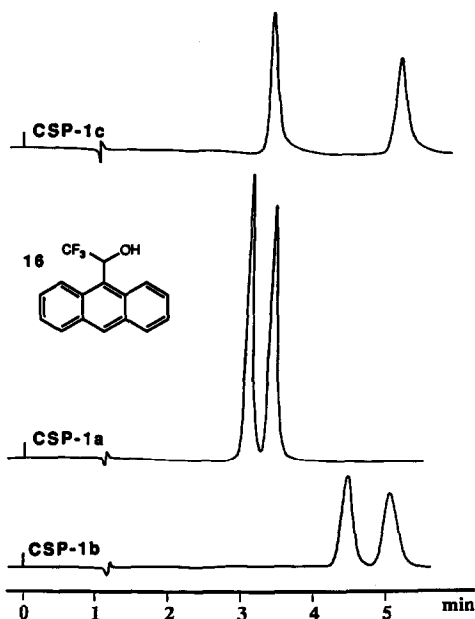
Fig. 7. Chromatograms of resolution of compound **16** on CSP-1a, CSP-1b and CSP-1c. Experimental conditions: column 100 × 4.6 mm I.D.; eluent: chloroform–0.5% methanol/heptane (80:20, v/v); flow-rate 1 ml/min.

Fig. 6. Chromatograms of resolution of compound **15** on CSP-5a, CSP-5b and CSP-5c. Experimental conditions: column 100 × 4.6 mm I.D.; eluent: chloroform–0.5% methanol/heptane (70:30, v/v); flow-rate 1 ml/min.

lution of compounds **15** and **16** on the three kinds of stationary phases (CSP-Xa, CSP-Xb and CSP-Xc) can be compared.

Pirkle and Readnour [24] have reported the influence of end-capping treatment with three trimethylsilyl donors on the enantioselectivity of chiral stationary phases obtained by condensation of a chiral triethoxysilane with π-donor character on silica gel. The differences observed by these workers between end-capped silica and silica which has not been end-capped are less remarkable than those observed in this study. In particular, the retention time of the first-eluted enantiomer is almost insensitive to the end-capping treatment when hexamethyldisilazane is used as the silylating reagent.

Certainly, the method of end-capping treatment is not the same in both studies (end-capping before or after packing the column) but, in our opinion, the differences in results cannot be explained in these terms. The cause of these discrepancies may be the diverse origins of the silica gel used in the two studies. Dobashi and Hara [11] have already observed the influence of silanol groups on the reten-

tion time and selectivity factors in a different way, but with stationary phases obtained from the silica gel Nucleosil 100-5 (100 Å, 5 μm). To verify this hypothesis stationary phases analogous to CSP-1b (non-end-capped) and CSP-1c (end-capped) were prepared from Spherisorb S5W (80 Å, 5 μm) (Phase Separations) silica, possibly the same quality of Spherisorb silica gel used by Pirkle *et al.* [25]. The results confirm that the influence of end-capping treatment on the chromatographic behaviour of chiral stationary phases depends on the origin of the silica gel used. Phases obtained from Spherisorb show only minor differences between end-capped and non-end-capped silicas. Thus values $k'_1 = 4.64$ and $\alpha = 1.32$ and $k'_1 = 4.64$ and $\alpha = 1.34$ are obtained for **16** with chiral stationary phases analogous to CSP-1b and CSP-1c, respectively, prepared from Spherisorb S5W.

Stationary phases CSP-1b and CSP-1c obtained from Nucleosil 100-5 have nearly 1.20 μmol of chiral entity per m² (Table I). Analogous stationary phases obtained by condensation of the same chiral silane on silica gel Spherisorb S5W have a lower specific surface coverage (1.01 μmol/m²) of the same chiral entity (C 4.65, H 0.51, N 1.10% before end-capping treatment and C 5.10, H 0.60, N 1.06% after treatment). Differences in the physical properties of silica, *i.e.*, the amount of residual surface silanols, from manufacturer to manufacturer [26] may cause this reduction. However, the lesser influence of end-capping treatment on stationary phases obtained from Spherisorb S5W cannot be explained by the lower amount of chiral entity grafting on this silica. In fact, CSP-6b and -6c have 0.60 μmol of chiral entity per m² of stationary phase, but there are large differences in chromatographic behaviour between them.

CONCLUSIONS

The method described here to prepare and condense chiral silanes on silica gel is easy to carry out and leads to chiral stationary phases with a good performance. The decrease in achiral interactions in chiral stationary phases often improves, sometimes significantly, the resolution of racemic compounds.

This procedure, condensation of a chiral silane on the silica gel followed by an end-capping treatment, is advantageous compared with the same chiral entity on γ-aminopropylsilica gel.

The influence of end-capping on the selectivity of a chiral stationary phase depends on the origin of the silica gel used to prepare it.

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