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3,5-Dimethylphenylcarbamates of amylose, chitosan and cellulose bonded on silica gel
Comparison of their chiral recognition abilities as high-performance liquid chromatography chiral stationary phases

Pilar Franco[a], Antonio Senso[a], Cristina Minguillón[a,*], Laureano Oliveros[b]

[a]*Laboratori de Química Farmacèutica, Facultat de Farmàcia, Universitat de Barcelona, Avda. Diagonal s/n, E-08028 Barcelona, Spain*

[b]*Laboratoire de Chimie Générale, Conservatoire National des Arts et Métiers, 292, rue Saint-Martin, F-75141 Paris Cedex 03, France*

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Abstract

Mixed 10-undecenoyl/3,5-dimethylphenylaminocarbonyl derivatives of amylose and chitosan were prepared and immobilized on allylsilica gel. The enantioselectivities of the resulting supports were compared to those of an analogous cellulose-derived chiral stationary phase previously prepared. © 1998 Elsevier Science B.V.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Amylose; Chitosan; Cellulose; 3,5-Dimethylphenylcarbamate

1. Introduction

During the last two decades polysaccharide derivatives have been widely used in the resolution of racemic compounds by HPLC. Some chiral stationary phases (CSPs) containing phenylcarbamate derivatives of several polysaccharides, such as xylan, dextran or chitosan among others, have been described [1]. However, chiral selectors based on cellulose and amylose derivatives have been the most commonly used, and some of them have led to commercially available CSPs [2].

The recently reported method [3] of fixing polysaccharide derivatives on chromatographic matrices, which results in their insolubilization, represents an improvement over existing coated CSPs. Thus, the bonded polysaccharide-derived CSPs allow the use of a wider choice of solvents in the mobile phase than the coated ones.

Once the validity of the method was established, several variables, such as the number of fixing groups on the polysaccharide [4] or the porosity of the chromatographic matrix used [5], were optimized on the 10-undecenoyl/3,5-dimethylphenylaminocarbonyl derivative of cellulose. The high chiral recognition ability of this derivative with respect to other benzoate [6] and carbamate [7] derivatives of cellulose bonded on the matrix, suggested the interest of a comparison with analogous derivatives of other polysaccharides. Although amylose derivatives have been extensively used in coated CSPs, to our knowledge, there are not many references of the chromato-

*Corresponding author.

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graphic use of chitosan derivatives as chiral selectors. Okamoto et al. [1] described the use of triphenylcarbamate of chitosan, among other polysaccharides, and recently, Cass and co-workers [8] reported the chiral discrimination ability of some arylcarbamate derivatives of chitin. However, in the case of the bonded polysaccharide derivatives developed in our laboratory, the presence of an aliphatic chain, and the fact that it is polymerized, modify their selectivity in comparison with the polysaccharide derivatives that are simply coated. Furthermore, in the case of chitosan derivatives the differences with the coated ones can be even more noticeable, since changes in the starting chitosan led to changes in selectivity [8].

In the present study, 10-undecenoyl/3,5-dimethylphenylaminocarbonyl derivatives of amylose and chitosan analogous to that of cellulose previously described, are fixed on allylsilica gel. Their chromatographic behaviour, using either heptane–2-propanol or heptane–chloroform mixtures as mobile phase, are evaluated and compared to that of a cellulose-derived CSP.

2. Experimental

^{1}H NMR spectra were measured using a Varian GEMINI-300 spectrometer. Elemental analyses were performed on a CE Instruments apparatus Mod. EA 1108 (Carlo Erba Instruments, Milan, Italy) using standard conditions by the Serveis Científico-Tècnics de la Universitat de Barcelona (Spain). The CSPs were packed into stainless-steel tubes (150×4.6 mm I.D.) by the slurry method. The chromatographic experiments were performed on a HPLC system consisting of a waters 600E pump, a Waters 717 auto sampler (Millipore, Milford, MA, USA), equipped with a Waters 996 photo-diode array detector and a Perkin–Elmer 241LC polarimetric detector (Perkin–Elmer, Uberlingen, Germany). The volume of sample injected was 3 μl. The void volume was determined using tri-*tert*.-butylbenzene.

2.1. Preparation of the polysaccharide derivatives

The chiral selectors and CSPS were prepared as indicated in Fig. 1. The amylose derivative **2** was prepared in the same way as the previously described 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose **1** [4].

Amylose (95% purity, DP 300, ICN Biochemicals Inc., CA, USA) was treated with 10-undecenoyl chloride (0.4 mol per mol of glucose units) in pyridine. The mixture was allowed to react at about 100°C for 2 h and then 3.6 mol of 3,5-dimethylphenyl isocyanate per mol of glucose unit was added. After 24 h at 100°C, the product was isolated as the insoluble fraction in methanol, redissolved in chloroform and reprecipitated in methanol.

The chitosan derivative **3** was prepared from low molecular mass chitosan (M_r 70 000, Fluka, Buchs, Switzerland) previously purified by dissolving and reprecipitation. The resulting precipitate was dispersed and washed in methanol and diethyl ether [9]. After this treatment, the polysaccharide was suspended in pyridine and treated with 3,5-dimethylphenyl isocyanate (6 mol per mol of glucosamine unit). The mixture was vigorously stirred at about 100°C until a clear viscous solution was obtained. Then, 0.4 mol of 10-undecenoyl chloride per mol of glucosamine unit were added and the mixture was allowed to react for 24 h. The modified chitosan was also isolated as the insoluble fraction in methanol, redissolved in pyridine and reprecipitated in methanol.

Both amylose and chitosan derivatives were thoroughly washed in hot ethanol in order to remove the N,N′-bis(3,5-dimethylphenyl)urea formed as a by-product. The chiral selectors thus obtained were characterized by their ^{1}H NMR spectra and elemental analyses. The degree of substitution per monosaccharide unit (DS) was calculated from elemental analyses (Table 1).

Amylose derivative **2** (^{1}H NMR, pyridine-d_5, 300 MHz, 70°C) δ: 0.80–1.65 (m, $C^{3'}H_2$–$C^{8}H_2$); 1.99, 2.05 and 2.35 (3s+m, $ArCH_3$, $C^{2'}H_2$ and $C^{9'}H_2$); 4.24 (m, C^4H); 4.63 (m, C^5H); 4.90 (m, C^6H_a); 5.05 (m, C^6H_b and $C^{11'}H_2$=); 5.61 and 5.67 (2m, C^2H and C^1H); 5.87 (m, C^3H and $C^{10'}H$=); 6.46, 6.51 and 6.84 (3s, ArH_b); 6.97, 7.05 and 7.71 (3s, ArH_a); 9.34 and 9.69 (ba+ba, NH).

Chitosan derivative **3** (^{1}H NMR, pyridine-d_5, 300 MHz, 70°C) δ: 1.0–2.6 (m, $C^{2'}H_2$–$C^{9'}H_2$); 2.11 (s+s, 2-$ArCH_3$ and 3-$ArCH_3$); 2.30 (s, 6-Ar–CH_3); 3.65 (m, C^2H); 4.0 (m, C^5H); 4.50 (m, C^6H_2); 4.70 (m, C^3H); 4.85 (m, C^2H); 5.10 (m, $C^{11'}H_2$=) 5.50 (m, C^1H); 5.87 (m, C^3H); 5.90 (m, $C^{10'}H$=); 6.50 and

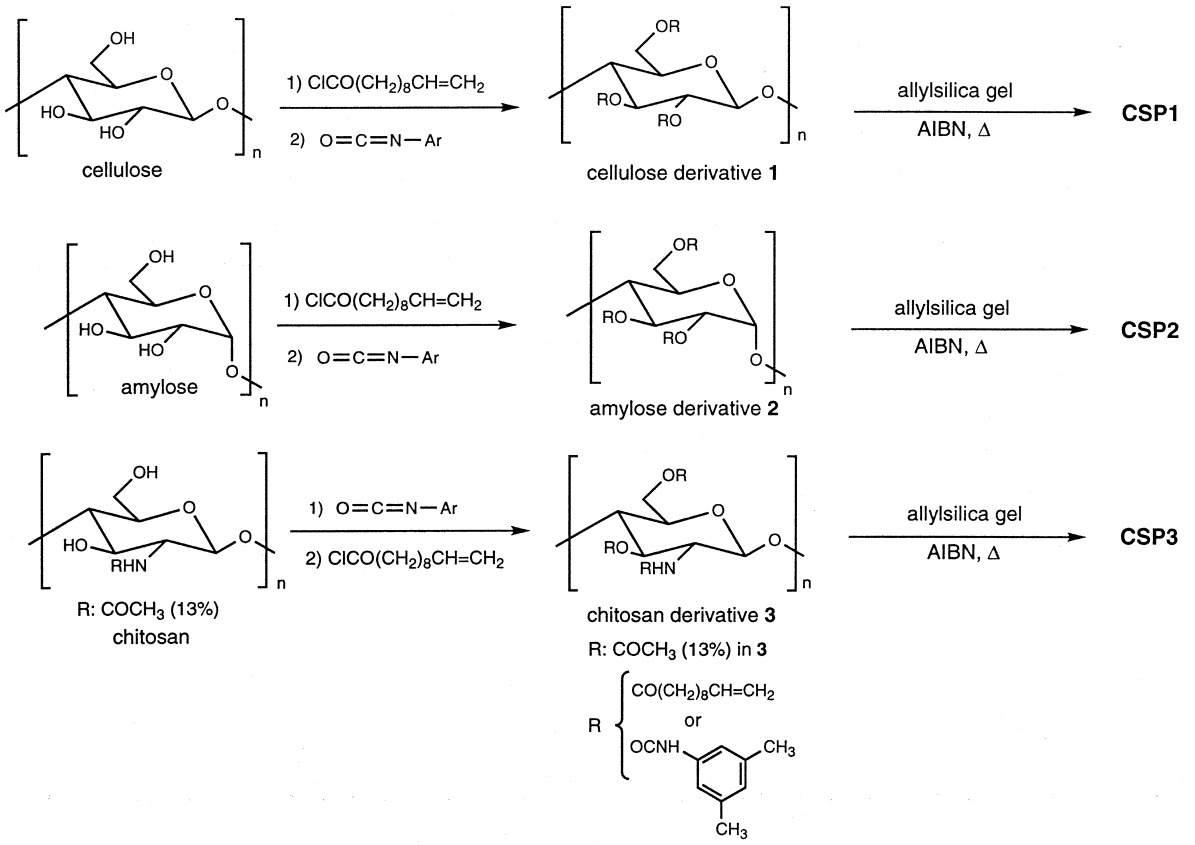

Fig. 1. Preparation of chiral stationary phases.

6.55 (2s, 2-ArH_b and 3-ArH_b); 6.72 (s, 6-ArH_b); 7.30 (s, 6-ArH_a); 7.36 (s+s, 2-ArH_a and 3-ArH_a); 9.30, 9.45, 10.0 and 10.15 (4 ba, NH).

2.2. Preparation of chiral stationary phases

The mixed polysaccharide derivatives were fixed on silica gel (5 μm, Nucleosil 100-5, Macherey-Nagel, Düren, Germany), previously modified by the introduction of allyl groups [6]. The fixation method, based on the radical reaction of the 10-undecenoyl groups on the polysaccharide derivative and the allyl groups on the silica surface, described for the cellulose derivative **1** [4], was applied. The CSPs obtained were suspended in chloroform at reflux temperature for 2 h, filtered off and washed with chloroform and acetone. The resulting CSPs were characterized by elemental analysis (Table 2).

Table 1
Characterization of polysaccharide derivatives

Polysaccharide	Derivative	Elemental analysis			Degree of substitution (DS)[a]	
		%C	%H	%N	10-Undec.	Carbam.
Cellulose	1[b]	65.80	6.79	5.97	0.35±0.02	2.44±0.07
Amylose	2	65.19	6.40	6.57	0.10±0.05	2.63±0.17
Chitosan	3	65.21	6.25	8.01	0.31±0.06[c]	2.43±0.18[c]

[a] Calculated from elemental analysis.
[b] Cellulose derivative B in Refs. [4,5].
[c] The number of acetyl groups (13%) has been taken into account in the calculation.

Table 2
Characterization of CSPs

Polysaccharide	CSP	Elemental analysis[a]			Weight of polysaccharide derivative/ 100 g phase[b]	HETP (cm)[c]
		%C	%H	%N		
Cellulose	CSP1[d]	13.40	2.10	1.00	16.8	8.90×10^{-3}
Amylose	CSP2	9.50	1.42	0.90	13.8	6.31×10^{-3}
Chitosan	CSP3	11.45	1.78	1.21	14.5	3.19×10^{-3}

[a] Referred to final material, including the amount of polysaccharide derivative and the organic content in the modified silica used as a matrix.
[b] Calculations based on %N from the elemental analyses.
[c] Calculated using 1,3,5-tri-*tert*.-butylbenzene.
[d] CSPB in Ref. [4].

3. Results and discussion

In order to obtain a polysaccharide derivative as similar as possible to the one obtained from cellulose, amylose with a degree of polymerization (DP ca. 300) similar to that of microcrystalline cellulose was used as starting product. The preparation of 10-undecenoate/3,5-dimethylphenylcarbamate of amylose was carried out in the same conditions as the cellulose derivative, resulting in a highly substituted amylose derivative (Table 1).

Chitosan is less swollen in pyridine than amylose and cellulose. This feature limits the introduction of a small number of 10-undecenoyl groups. Some attempts were made to obtain the analogous chitosan derivative following the method used with amylose

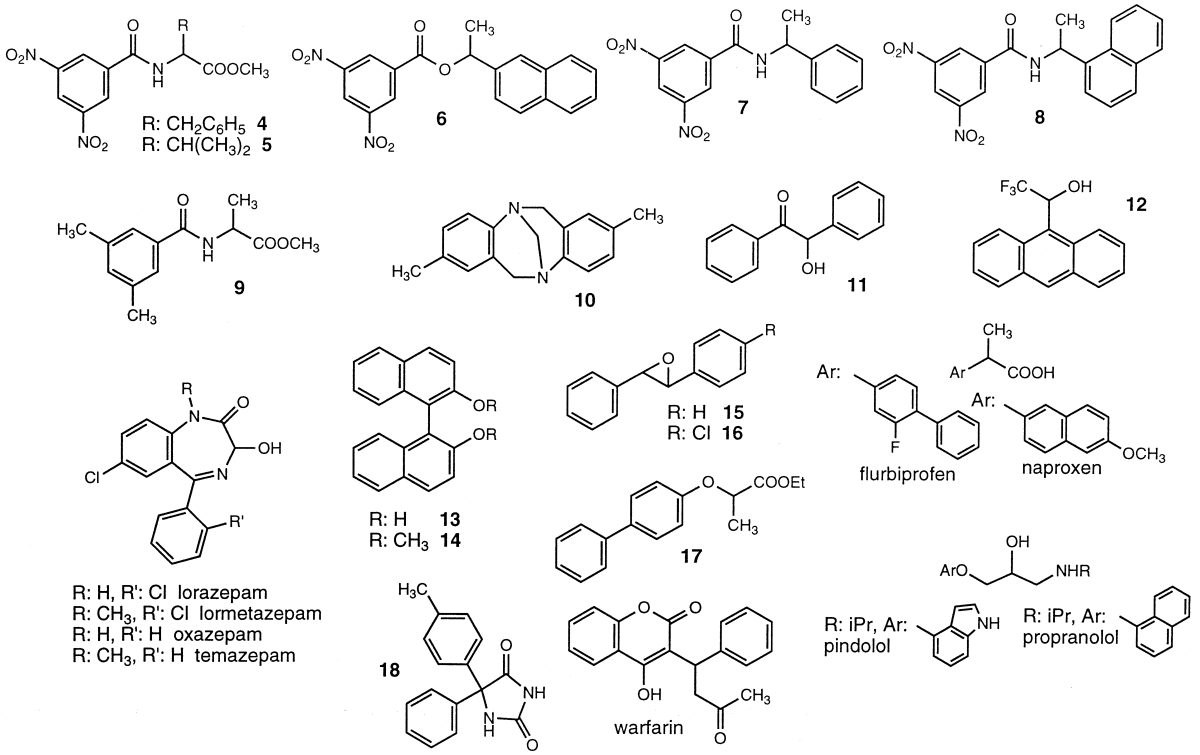

Fig. 2. Chemical structures of racemic test compounds.

Table 3
Chromatographic results obtained using heptane–2-propanol as mobile phase

Racemic compound	CSP1			CSP2			CSP3			Heptane–2-propanol
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	
4	10.2(*R*)	1.30	0.94	4.64	1.00	–	4.72(*S*)	1.10	–	80:20
5	10.4	1.00	–	4.05	1.09	–	4.76(*S*)	1.25	1.41	90:10
6	7.02	1.17	0.51	2.35	1.00	–	4.08(*R*)	1.30	1.63	90:10
7	14.0	1.00	–	4.49(*R*)	1.17	0.64	8.32	1.00	–	90:10
8	5.36(*R*)	1.46	1.16	1.80	1.00	–	3.66	1.00	–	80:20
9	7.79	1.22	0.89	6.49	1.00	–	6.32(+)	1.14	0.97	98:2
10	1.42(+)	1.24	0.88	1.50	1.09	–	1.10	1.00	–	90:10
11	1.92(+)	1.28	1.20	1.95	1.00	–	1.96(+)	1.19	2.51	90:10
12	1.34(*R*)	2.20	2.71	0.76	1.00	–	1.30(*R*)	1.51	3.81	90:10
13	2.98	1.25	0.74	2.33	1.07	–	1.45(+)	1.27	1.78	90:10
14	1.28	1.11	–	0.57	1.00	–	0.98	1.00	–	98:2
15	0.58(−)	1.34	0.93	0.28(+)	1.87	1.96	0.31(+)	1.14	–	90:10
16	0.90[a]	1.00	–	0.31(+)	1.96	2.10	0.35(+)	1.12	–	90:10
17	0.58(+)	1.16	–	0.38	1.00	–	0.56(−)	1.57	3.11	90:10
18	2.49	1.15	–	3.29	1.00	–	1.76(+)	1.41	2.31	90:10
Lorazepam	8.74(−)	1.56	1.35	6.09(+)	1.52	1.96	6.60	1.06	–	90:10
Lormetazepam	7.10	1.00	–	3.81(+)	2.10	4.18	5.07(−)	1.80	4.39	80:20
Oxazepam	8.62	1.09	–	6.26	1.19	0.90	6.89(−)	1.33	1.84	90:10
Temazepam	5.32	1.00	–	4.03	2.23	4.17	4.17(−)	1.80	3.74	80:20
Warfarin	5.13(−)	1.28	0.48	0.86(−)	1.98	3.03	2.40	1.33	1.16	80:20
Pindolol	3.79	4.11	4.11	3.63	1.00	–	4.30	1.00	–	80:20[b]
Propranolol	3.01(+)	1.42	1.33	4.92	1.00	–	2.95	1.00	–	90:10[c]
Naproxen	5.64(−)	1.22	1.19	5.35	1.00	–	3.88	1.00	–	98:2[d]
Flurbiprofen	2.64	1.00	–	2.80	1.25	1.01	1.88	1.00	–	98:2[d]

k'_1, capacity factor for the first eluted enantiomer; α, selectivity factor; R_s, resolution. The configuration or the optical rotation sign of the first eluted isomer is shown in parentheses. Column: 15×0.46 cm. Flow-rate: 1 ml/min.

[a] Heptane–2-propanol (98:2, v/v).

[b] 0.1% of DEA was added to the mobile phase.

[c] 0.05% of DEA was added to the mobile phase.

[d] 0.5% of TFA was added to the mobile phase. flow-rate 0.5 ml/min.

and cellulose. However, the introduction of the alkenoyl groups in the first step failed. The inversion of the derivatizing steps and the use of a large excess of 3,5-dimethylphenyl isocyanate allowed us to obtain a chitosan derivative with a degree of substitution (DS)[1] in the same order as the other polysaccharide selectors prepared.

Both amylose and chitosan derivatives were satisfactorily fixed on allylsilica gel. The resulting chromatographic supports were tested against a set of structurally diverse racemic compounds. Some of them are shown in Fig. 2. The obtained chromatographic results are presented in Tables 3 and 4. The optimal chromatographic conditions for one column should not be suitable for another, when different selectors are in study. Therefore, the mobile phase compositions that are indicated in the tables for every compound are those that allowed the comparison between all three columns, but not always the optimal ones for a particular compound in each column.

The comparison of the chromatographic results obtained with **CSP2** and those of cellulose and chitosan derivatives (**CSP1** and **CSP3**, respectively) showed that the amylose derivative has the most restricted application domain. Its discrimination abil-

[1] Chitosan is obtained by alkaline hydrolysis of the N-acetyl groups of chitin. Nevertheless, a certain degree of N-acetylation remains in commercially available chitosan, which should be taken into account [10] in the calculation of the DS of the final chitosan derivative.

Table 4
Chromatographic results obtained using heptane–chloroform as mobile phase

Racemic compound	CSP1			CSP2			CSP3			Heptane–chloroform
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	
5	2.87(*R*)	1.25	0.97	9.37(*R*)[a]	1.13	–	1.35(*S*)	1.24	2.12	50:50
6	3.93[b]	1.00	–	3.11	1.00	–	1.53(*R*)	1.29	2.39	75:25
7	6.31	1.00	–	17.91[a]	1.24	1.23	3.38(*S*)	1.17	1.93	50:50
8	1.27(*R*)	1.23	–	14.40[a]	1.00	–	2.61(*S*)	1.18	1.66	50:50
9	5.60	1.26	1.19	8.97	1.00	–	9.13	1.06	–	90:10
11	2.20[b]	1.15	0.85	0.44	1.00	–	1.77(+)	1.15	1.48	75:25
12	2.39(*R*)	2.29	3.75	4.63(*R*)[a]	1.25	1.61	1.93(*R*)	1.26	2.62	50:50
13	3.98[c]	1.31	1.39	5.25	1.00	–	5.28	1.13	1.16	75:25
14	1.80	1.15	–	1.51	1.05	–	1.47(+)	1.14	1.10	95:5
15	1.14(−)	1.83	2.92	0.39(+)	1.46	1.11	0.51(+)	1.21	0.93	90:10
16	1.30	1.22	0.80	0.38(+)	1.49	1.13	0.50(+)	1.30	1.25	90:10
17	1.73	1.17	0.73	1.68	1.00	–	2.27(−)	1.51	3.43	95:5
Lorazepam	13.8	1.18	0.72	>45			15.8	1.26	2.43	50:50
Oxazepam	14.1	1.24	1.05	>45			14.4	1.33	2.73	50:50
Temazepam	2.38	1.00	–	6.29	1.00	–	1.49(−)	1.46	3.70	50:50
Warfarin	1.32(+)	2.15	1.74	4.73	1.37	1.38	6.01	1.43	3.17	50:50

k^1, capacity factor for the first eluted enantiomer; α, selectivity factor; R_s, resolution. The configuration or the optical rotation sign of the first eluted isomer is shown in parentheses. Column: 15×0.46 cm. Flow-rate 1 ml/min.
[a] Heptane–chloroform (75:25, v/v).
[b] Heptane–chloroform (80:20, v/v).
[c] Heptane–chloroform (70:30, v/v).

ity was even lower when heptane–chloroform mixtures were used as a mobile phase. However, when using 2-propanol as a mobile phase modifier, **CSP2** was able to resolve some compounds, such as **7** and flurbiprofen, not resolved on the other CSPs in this study. In these conditions **CSP2** showed higher α values for the separation of benzodiazepines and warfarin, among others, than **CSP1** and **CSP3**.

The application domain of **CSP3**, whose chiral selector is the chitosan derivative, was remarkable. With the exception of amino alcohols, such as propranolol or pindolol, easily resolved on **CSP1**, but not in **CSP3**, the chiral discrimination ability of these two CSPs was comparable. Nevertheless, it is worth noting that selectivity factors or resolution values often increased in **CSP3** when chloroform was used as mobile phase modifier (Fig. 3). In this case, **CSP3** led to better separations than **CSP1**. This improvement in the chromatographic results in the presence of chloroform for **CSP3** is opposite to the behaviour observed for **CSP2** in the presence of the same solvent (Fig. 4, Tables 3 and 4). This latter showed lower capacity factors than the other two columns in these conditions and this fact can be associated with the worse enantioselectivity displayed by **CSP2**.

The differences observed either in the discrimination ability or in the elution order for certain enantiomers (benzodiazepines between **CSP2** and **CSP3**, **15** between **CSP1** and **CSP2**/**CSP3** or **17** between **CSP1** and **CSP3**) seems to indicate that different recognition mechanisms can take place in the resolution of the racemic compounds on the three polysaccharide-derived CSPs.

4. Conclusions

All three polysaccharides considered in this study led to derivatives with a remarkable stereoselectivity for a number of the racemic compounds tested. The chitosan derived CSP (**CSP3**) showed a particularly interesting discrimination ability either using heptane–2-propanol or heptane–chloroform mixtures as mobile phase. Due to the ease of preparation of these bonded polysaccharide-derived CSPs and their high stereoselectivity, the development of new CSPs

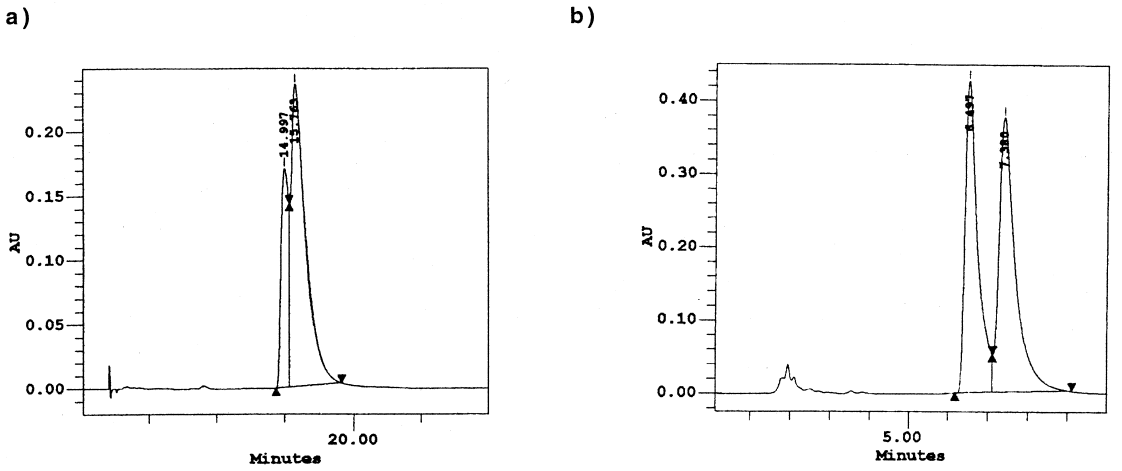

Fig. 3. Resolution of lorazepam on **CSP3** using as mobile phase (a) heptane–2-propanol (90:10, v/v) (k'_1: 6.60; α: 1.06), (b) 100% chloroform (k'_1: 2.38; α: 1.19; R_s: 1.40).

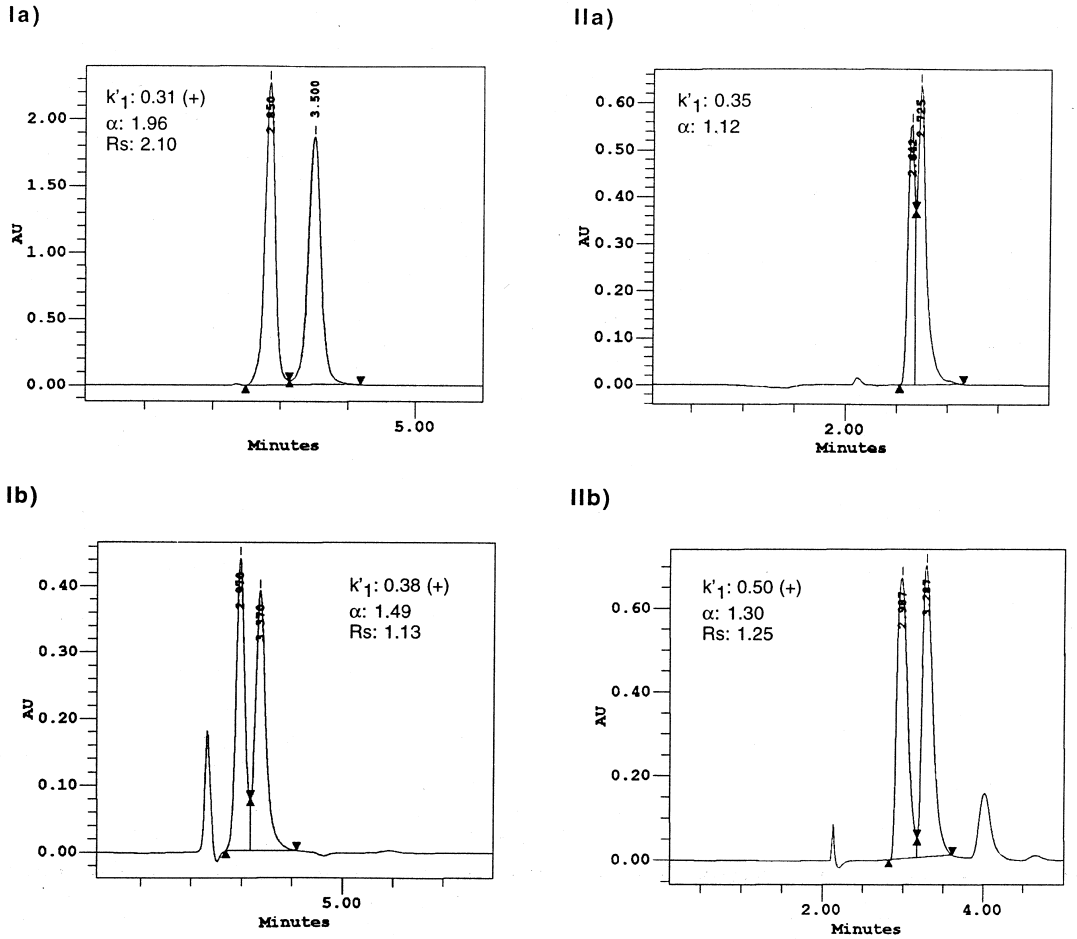

Fig. 4. Comparison of the chromatographic separation of **16** on **CSP2** (**I**) and **CSP3** (**II**). (a) Heptane–2-propanol (90:10, v/v), (b) heptane–chloroform (90:10, v/v).

based on these polysaccharides becomes a promising field of study.

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