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Regioselective carbamoylated and benzoylated cellulose for the separation of enantiomers in high-performance liquid chromatography

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Abstract

The preparation of new chiral stationary phases (CSPs) based on mixed substituted cellulose is reported. The first type of CSP has a 3,5-dimethylphenylcarbamate at the 2 and 3 positions and a phenylethylcarbamate at the 6 position. The second one has a benzoate at the 2 and 3 positions and a *meta*-substituted phenylcarbamate at the 6 position. The last one has a 3,5-dimethylphenylcarbamate at the 2 and 3 positions and a *para*-substituted benzoate at the 6 position. This work shows that the substituent present at the 6 position is very important for chiral recognition. Some of the new CSPs exhibit a better enantioselective power for several racemates compared to the corresponding Chiralcel columns. The resolution of some β-adrenergic blockers and benzodiazepines is also reported.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Enantioselectivity; Benzodiazepines; Cellulose derivatives; Beta-blockers

1. Introduction

Over the last ten years, optical resolution by high-performance liquid chromatography (HPLC) has become a practical and useful method, not only for determining optical purity, but also for obtaining pure optical isomers. Many publications have already reported the abilities of chiral stationary phases (CSPs), based on cellulose and amylose derivatives coated on macroporous silica gel [1–3]. Recently, we have discussed the resolution power of amylopectin derivatives [4–6]. Kaida and Okamoto [7] have prepared CSPs based on cellulose regioselectively derivatized with two kinds of substituted phenylisocyanate. The coated polysaccharides have a 3,5-dimethylphenylcarbamate at the 6 position or the 2,3 positions, and a 3,5-dichlorophenylcarbamate at the 2,3 or the 6 position, respectively. In this study, we made a contribution to the interesting work reported by Kaida and Okamoto. We have developed their concept of mixed substituted polysaccharides in two ways. Firstly, we have introduced a chiral benzylcarbamate moiety at the 6 achiral position of the glucose unit and the 2 and 3 chiral positions have been substituted with an achiral phenylcarbamate.

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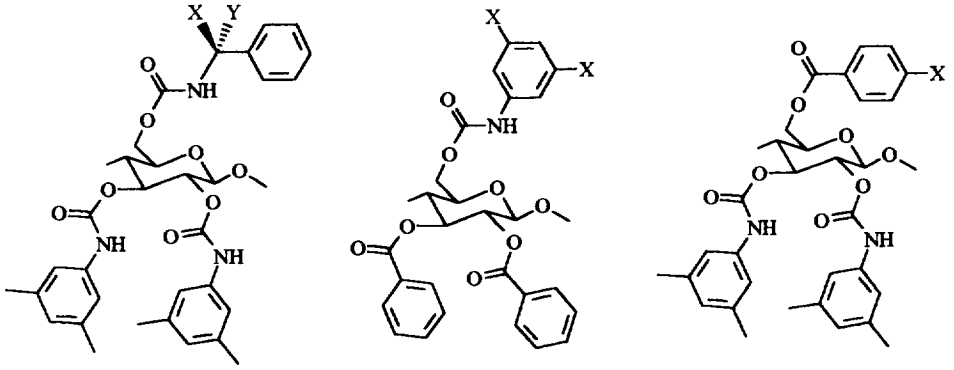
Secondly, two functional groups of different nature have been introduced on the same glucose unit; a benzoate at the 6 position or the 2,3 position and a phenylcarbamate at the 2,3 or the 6 position, respectively. The chromatographic behavior of these polysaccharide CSPs has been studied in detail.

The different types of cellulose-based columns synthesized are represented below (Scheme 1).

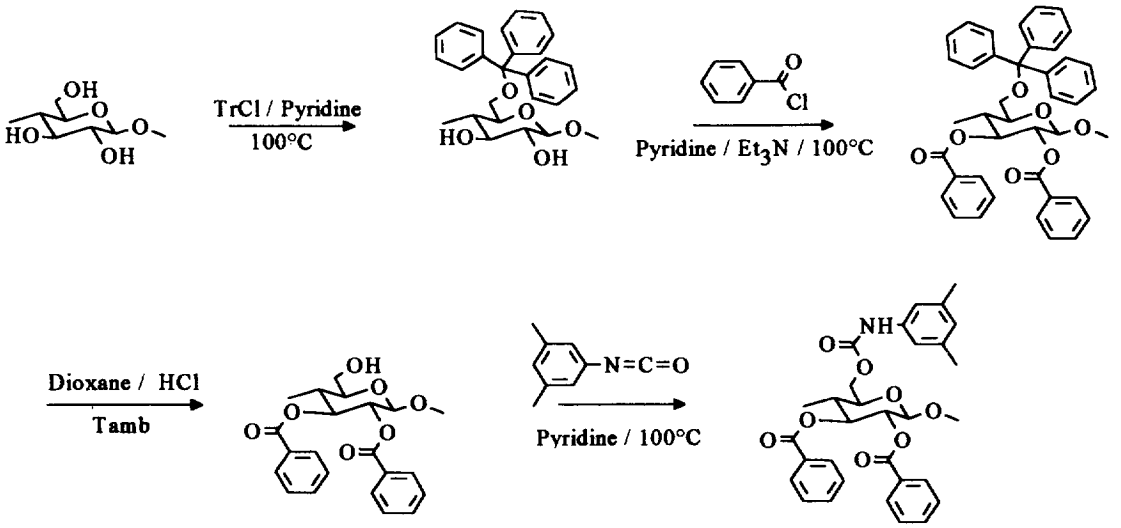


1: (R): X=H, Y=CH3 **4**: X=CH3 **7**: X=CH3

2: (S): X= CH3, Y=H **5**: X=H **8**: X=H

3: (R,S): X, Y=H, CH3 **6**: X=Cl **9**: X=Cl

Scheme 1.



Scheme 2.

The first type of CSP (**1–3**) has a 3,5-dimethylphenylcarbamate at the 2 and 3 positions and a phenylethylcarbamate (PEC) at the 6 position. The second one (**4–6**) has a phenylbenzoate at the 2 and 3 positions and a substituted phenylcarbamate at the 6 position. The last one (**7–9**) has a 3,5-dimethylphenylcarbamate at the 2 and 3 positions and a *para*-substituted benzoate at the 6 position. CSP **10**, not represented here, has a 3,5-dimethylphenylcarbamate at the 2 and 3 positions and an acetate group at the 6 position.

2. Experimental

The synthesis procedure used was almost the same as that described by Kaida and Okamoto [7]. The chemical pathway for CSP **1** is presented in Scheme 2.

Cellulose was allowed to react with a large excess of triphenylmethylchloride in pyridine at 100°C until complete solubilization was attained [8]. A three-fold excess of benzoylchloride, with an equimolar amount of triethylamine, was added to react with the free remaining hydroxyl functions at the 2 and 3 positions over 24 h at 100°C. The product obtained was dissolved in dioxane and a small amount of hydrochloric acid was added. The solution was then stirred for 32 h at room temperature in order to remove the protecting group at the 6 position. Finally, a three-fold excess of the corresponding phenylisocyanate was allowed to react with the remaining free hy-

Table 1
Elemental analysis on the different CSPs

		C%	H%	N%
1	Found	66.12	6.00	6.81
	Calculated	65.78	5.98	6.98
2	Found	66.20	6.13	7.13
	Calculated	65.78	5.98	6.98
3	Found	65.95	6.54	6.89
	Calculated	65.78	5.98	6.98
4	Found	67.49	5.34	2.88
	Calculated	67.31	5.22	2.71
5	Found	66.34	4.85	2.92
	Calculated	66.26	4.70	2.86
6	Found	57.93	3.76	2.50
	Calculated	58.06	3.76	2.51
7	Found	65.76	5.90	4.71
	Calculated	66.90	5.92	4.88
8	Found	66.57	6.85	5.09
	Calculated	66.43	5.71	5.00
9	Found	62.33	5.32	4.52
	Calculated	62.57	5.21	4.71
10	Found	62.41	6.05	5.58
	Calculated	62.65	6.02	5.62

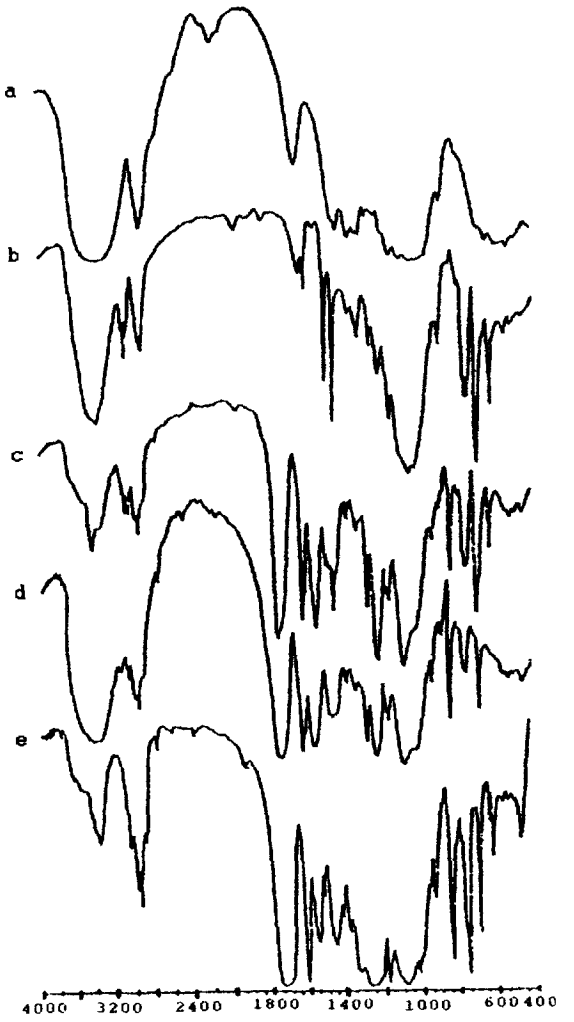


Fig. 1. Infrared spectra of cellulose following the different steps of synthesis of CSP **8**. *a*, Cellulose; *b*, 6-tritylethercellulose; *c*, 6-tritylether-2,3-(3,5-dimethylphenylcarbamate)cellulose; *d*, 2,3-(3,5-dimethylphenylcarbamate)cellulose; *e*, 6-benzoate-2,3-(3,5-dimethylphenylcarbamate)cellulose.

droxyl functions at the 6 position, according to the classical method [2]. After each step, the product was precipitated in methanol and washed several times with the same solvent. 6-Triphenylmethylether-2,3-benzoate cellulose was dissolved in pyridine and precipitated again in methanol.

These products were identified by elemental analysis (Table 1), IR spectrometry and ^{1}H NMR. The IR spectrum evolution is given as an example, for CSP **8** in Fig. 1. The packing chiral materials were prepared as reported elsewhere [9]. Each chiral material was packed in stainless-steel tubes (250× 4.6 mm I.D.) by a slurry method.

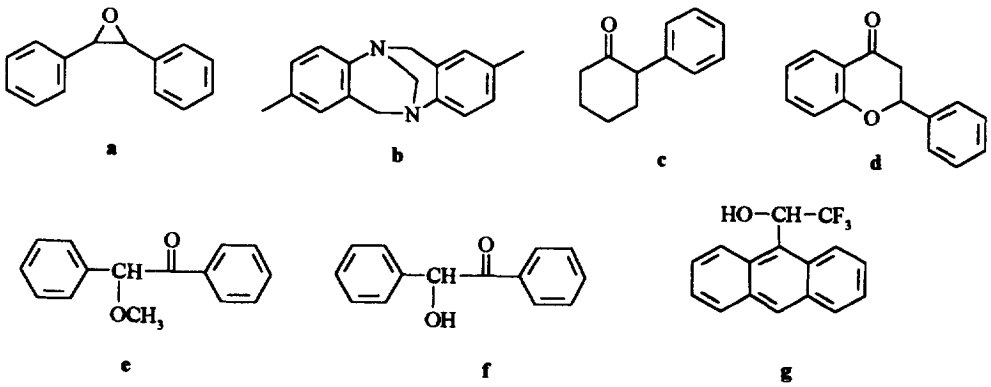
Chromatographic resolutions were carried out with an HPLC system consisting of a Jasco UV-975 UV–Vis detector and a Jasco PU-980 HPLC pump. IR spectra were measured with a Nicolet 205 FT-IR spectrometer. The ^{1}H NMR spectra were measured with a Bruker DPX 400 (400 Mhz) spectrometer, using tetramethylsilane as an internal standard.

3. Results and discussion

The ^{1}H NMR spectra of cellulose derivatives **4** and **8** are given respectively in Fig. 2. In the spectrum of the tris(3,5-dimethylphenylcarbamate) of cellulose reported elsewhere [7], three peaks which were assigned to the two methyl groups on the phenyl groups at the 2,3 and 6 positions were observed at about 2 ppm. In the spectrum of **4,** the peaks due to the methyl groups at the 2 and 3 positions at 2 ppm and 2.1 ppm were smaller than the peak due to the methyl group of the 6 position at 2.3 ppm. In the spectrum of **8,** the peak due to the methyl group at the 6 position at 2.3 ppm was small compared with the peaks due to the methyl groups of the 2 and 3 positions at 2 and 2.1 ppm. These results suggest that the three hydroxyl groups were regioselectively converted into 3,5-dimethylphenylcarbamate groups or benzoate groups. The regioselectivity at the 6 position, calculated according to the ^{1}H NMR spectra and the elemental analysis results, for series **7–10** and **4–6**, were 81 and 75%, respectively. For example, this means that 81% of the hydroxyl groups at the 6 position of **8** were converted into benzoate moieties and 19% were 3,5-dimethylphenylcarbamate moieties, also that 90% of the hydroxyl groups at the 2 and 3 positions were converted into the 3,5-dimethylphenylcarbamate moiety and 10% were converted to the benzoate moiety.

Fig. 3 shows a chromatogram of the resolution of Tröger's base on CSP **2.** The dead-time of the column (t_0) was determined by injection of 1,3,5-tri-*tert.*-butylbenzene. The capacity factors (k'_1 and k'_2), are defined as $(t_1-t_0)/t_0$ and $(t_2-t_0)/t_0$ where t_1 and t_2 are the retention times of the enantiomers, were 0.94 and 1.52, respectively. The separation factor ($\alpha=k'_2/k'_1$) and the resolution factor [$R_s=2(t_2-t_1)/(w_1+w_2)$] were 1.62 and 3.71, respectively.

The chromatographic results obtained with columns **1–10**, for the separation of seven classical racemates **a–g** (see Scheme 3), used as test compounds on polysaccharide CSPs, are reported in Table 2.



Scheme 3.

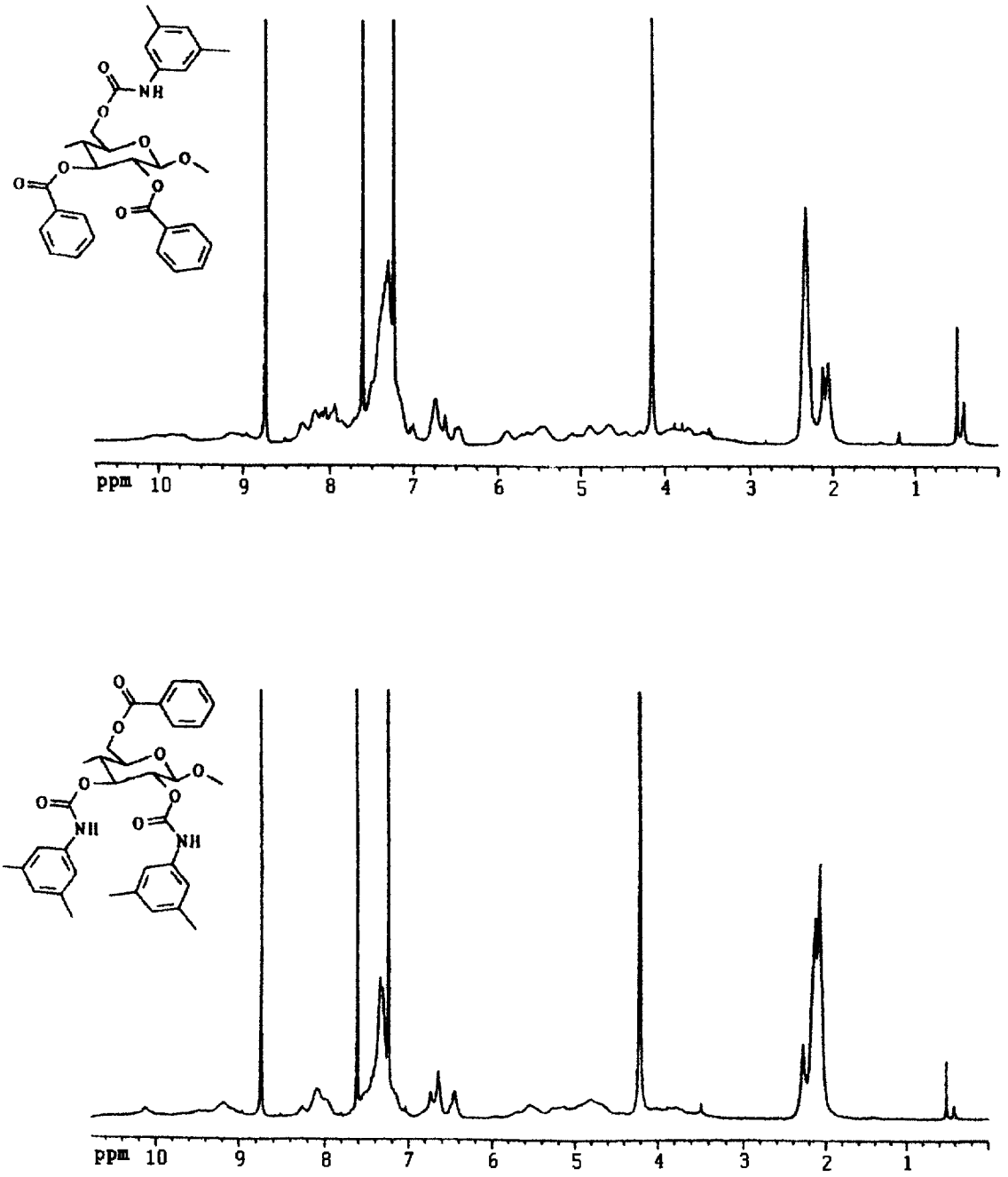


Fig. 2. ^{1}H NMR spectra of cellulose derivatives **4** (top) and **8** (bottom) at 400 MHz (pyridine-d_5, 80°C).

The first goal of this work was to study the influence of the carbamate or ester moiety at the 6 position of the glucose unit. The same derivatization was then carried out at the 2 and 3 positions for the same group of CSPs. Although the initial hydroxyl function at the 6 position was not supported by an

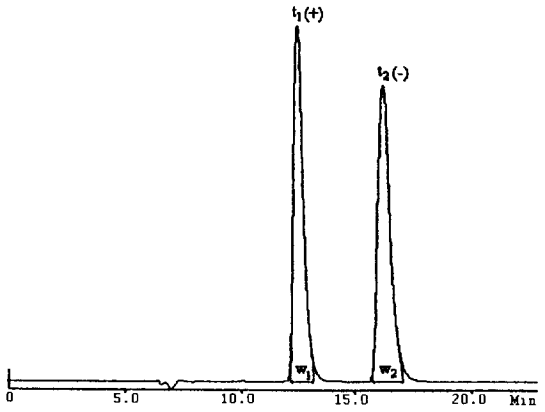


Fig. 3. Optical resolution of Tröger's base on CSP 2. Mobile phase, hexane–isopropanol (90:10, v/v); Flow-rate, 0.5 ml/min.

asymmetric carbon, dramatic effects on enantioselectivity were observed when changing the nature of its substituent. **a** has an α value of 1.58 on **1** and was not resolved on **3**. **g** was well resolved on **4** (α= 1.32) and has an α value of 1 on **5** and **6**. This means that the interactions induced by the 6 position are crucial for the mechanism of chiral discrimination.

The best results were obtained with the first series (**1–3**). CSPs **1** and **2** with *R*-PEC and *S*-PEC at the 6 position, respectively, give the higher separation factors whereas the results obtained with classical tris(*R*-PEC) and tris(*R*/*S*-PEC) cellulose CSPs surpass those of tris(*S*-PEC) [10,11]. It is not obvious that the group at the 6 position is able to modulate the chirality of the discriminating sites. **b**(+), **f**(+) and **g**(−) were eluted first on CSPs **1**, **2** and **3**, which means that the recognition mechanism is the same on the three columns for each racemate. As a result, the 6 position is not responsible for the absolute configuration of the active sites. So, it is not one independent function supported by an asymmetric carbon which controls the selectivity, but the whole chirality of the cellulose macrostructure.

For the second series (**4–6**), the retention mechanism seems to be controlled more by the steric hindrance than by the electronic effects, both induced by the substituent of the phenyl group. Column **5** possesses the most elevated values for most capacity factors. The volume increase caused by the chlorine or the methyl group (**4** and **6**) (Van der Waals radii: r_{CH_3} = 2Å; r_{Cl} = 1.8 Å; r_H = 1.2 Å) seems to make the approach of the racemate difficult. The destabilization of the complex CSP–solute results in a decrease in k'_1. This phenomenon is confirmed with the third series (**7–9**). Retentions are always higher on 8 (except for **g**). No correlation exists between capacity factors and enantioselectivities. A high value of k'_1 does not necessary lead to a good separation (**4**: k'_f = 3.65, α_f = 1 and k'_g = 3.66, α_g = 1.32). It means that the amount of achiral interaction is predominant compared to the amount of chiral interaction. Therefore, the separation mechanism and the retention mechanism are quite independent. The volume of the phenyl substituent on the carbamate or ester moiety seems to modify the retention, but changes in electronic effects have poor influence on it.

In the third series (**7–9**), the results were quite surprising for **9**, which shows lower selectivities. For **1–6**, the CSP–CSP interactions were essentially hydrogen-bonding and dipole–dipole interactions. For **9**, the chlorine induces a lack of electrons in the vicinity of the phenyl moiety at the 6 position and the methyl group induces a delocalized negative charge on the phenyl groups at the 2 and 3 positions. As a consequence, $\pi-\pi^*$ acceptor–donor interactions may occur between cellulose chains, leading to a compact structure that is unable to accurately resolve racemates. An entirely organized structure, where the polymer takes a crystalline form, is not suited for chiral discrimination, as amorphous parts are required.

Finally, CSP **10** only shows good resolution power for **b** and **g**. Phenyl groups seem to be required for obtaining a wide panel of separations.

The carbamate and ester groups used for building our CSPs are often included in the composition of commercial CSPs. It is interesting to compare the abilities of our mixed columns with the classical ones. Histograms 1, 2 and 3 (Fig. 4) compare the enantioselectivity of each mixed column with that of Chiralcel OB [1] and OD [2], corresponding to the moieties present at the 2 and 3 positions. Firstly, best separations were obtained for **b** on **1–3**, **4–6** and **7–8** CSPs, for **c** on **1–3** and **7–8** and for **f** on **2** and **3**, respectively. It means that mixed CSPs are able to surpass commercial ones for some racemates.

Secondly, **7–9** shows lower selectivities. For our future work, a carbamate moiety at the 6 position will be used in preference.

Table 2
Optical resolution of the racemates **a–g** on CSPs **1–10**

CSPs	**a**			**b**			**c**			**d**			**e**			**f**			**g**		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
1	0.7	1.58	3.14	0.80 (+)	1.72	4.07	1.17	1.35	3.6	1.26	1.25	2.26	0.92	1.3	2.14	2.38 (+)	1.31	3.53	2.05 (−)	2.01	7.27
2	0.86	1.39	2.67	0.94 (+)	1.62	3.71	1.33	1.29	2.45	1.67	1.21	1.90	1.15	1.32	2.63	3.09 (+)	1.38	3.92	2.40 (−)	2.19	7.08
3	0.58	1.00	0	0.60 (+)	1.48	2.61	0.88	1.25	1.48	1.33	1.06	0	0.85	1.12	0.86	2.19 (+)	1.23	1.73	1.96 (−)	1.50	3.68
4	0.86	1.06	0.44	1.12 (+)	1.40	1.78	1.67	1.13	0.85	2.52	1.04	0.26	1.83	1.10	0.87	3.65	1.00	0	3.66 (−)	1.32	2.24
5	0.84	1.19	1.15	1.23 (+)	1.39	1.00	2.22	1.17	1.17	2.76	1.00	0	2.56	1.09	0.42	4.62 (−)	1.10	0.93	2.66	1.00	0
6	0.60	1.22	1.45	0.90 (+)	1.37	1.60	1.90	1.19	1.05	1.87	1.05	0.50	1.92	1.04	0.27	3.03 (+)	1.10	1.28	1.30	1.00	0
7	0.71	1.10	0.35	0.66 (−)	1.39	1.00	1.27	1.23	1.20	2.15	1.00	0	1.31	1.00	0	3.16	1.00	0	3.77	1.00	0
8	0.93	1.20	1.38	1.04 (+)	1.37	1.71	1.98	1.32	1.81	3.29	1.00	0	1.99	1.18	1.50	5.01 (+)	1.10	1.03	3.20 (−)	1.22	1.35
9	0.59	1.07	0.20	0.65 (+)	1.12	0.36	1.19	1.10	0.57	1.83	1.00	0	1.13	1.00	0	2.86 (+)	1.06	0.42	1.95	1.00	0
10	0.32	1.00	0	0.32 (+)	1.41	1.20	0.64	1.09	0	0.97	1.00	0	0.64	1.06	0	1.67 (+)	1.00	0	1.23 (−)	1.24	1.06

Mobile phase, hexane–isopropanol (90:10, v/v); flow-rate, 0.5 ml/min. The sign in parentheses shows the optical rotation of the first eluted enantiomer. The configurations of **b**(+), **f**(+) and **g**(+) are (5*R*,11*R*), *S* and *R*, respectively.

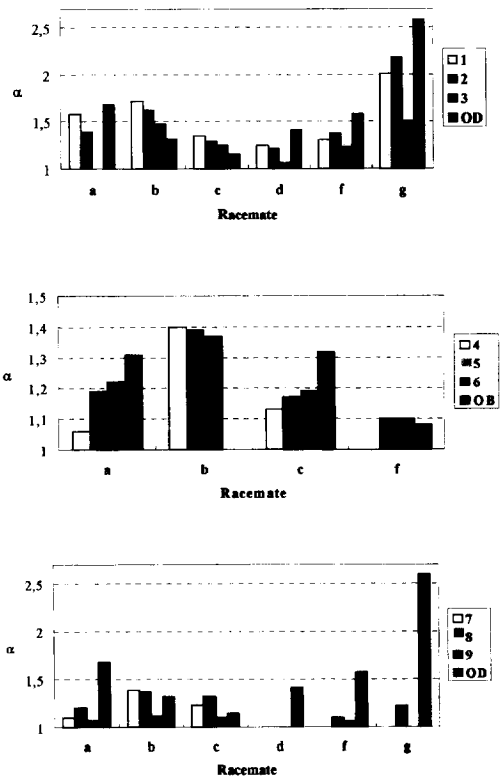


Fig. 4. Comparison of the selectivities of racemates **a–g** on CSPs **1–9** and on Chiracel OB and OD.

4. Applications

The separation of some β-blockers and benzodiazepines with different hexane–isopropanol mobile phases were achieved on CSP **2** (Table 3). For β-blockers, retention times and enantioselectivities decrease as soon as the amount of isopropanol increases. For benzodiazepines, the fall in retention times does not lead to a decrease of the α values. The hydroxyl function of benzodiazepines, which competes with the isopropanol one, seems to be more involved in the retention mechanism than in chiral discrimination.

In order to optimize the separations on our most promising CSPs, the influence of two organic mobile phase modifiers is presented in Table 4 and Table 5 and Table 6, respectively, for **1**, **2** and **3**. The best results were obtained for β-blockers, whatever the CSP considered, due to the addition of a small amount (10 m*M*) of diethylamine or octanoic acid. Diethylamine has little influence on the retention times but notably improves the resolutions. It may act as a silanol suppressor and competitor with the secondary amino group of the β-blocker, thus leading to sharper peaks. The use of octanoic acid leads to a remarkable increase in α and R_s values (except for tertatolol). As an example, the α value of oxprenolol goes from 4.7 to 10.6 when acid is added. It has been reported [12] that adding trifluoroacetic acid to the solvent, when coating cellulose derivatives, increases the separation of some racemates. The same phenomenon may happen with octanoic acid in the mobile phase. In the case of β-blockers, changes occurring in the polymer suprastructure improve the separations. No real correlations were observed between the mobile phase composition and the α values observed for benzodiazepines. The mechanism of chiral discrimination seems to be different for the two families of racemates. As a whole, the best separations for β-blockers and benzodiazepines were obtained with CSP **1** and **3**, respectively.

On the other hand, CSP **1** [with a (*R*) chiral carbon atom at position 6] has the highest enantioselectivity power; for example, all the isomers of oxazolam are separated. The enantioselectivity of CSP **2** [with a (*S*) chiral carbon atom at position 6] is a little lower. CSP **3** [with (*R,S*) chiral carbon atoms at position 6] has, in general, a lower recognition power. The following sequence, (*R*)>(*S*)>(*R,S*), is obtained. With the symmetrical cellulose tris(phenylethylcarbamate) CSPs, the order of enantioselective ability is given by the following chiral substitution sequence: (*R*)>(*R,S*)>(*S*) [10,11]. These results demonstrate that the addition of a chiral carbon at position 6 increases the enantioselective power, without changing the elution order [*R*-(+)-propanolol is always eluted first], which is not the same as with the symmetrical ones. However, these results are in agreement with those of Okamoto and coworkers, showing that all the positions (2, 3 and 6) on the glucose unit of cellulose participate in the chiral mechanism [10,11].

Only the separation of oxazepam enantiomers was reported on Chiracel OD [13]. The other benzodiazepines are not resolved on Chiralcel OD and OB. Fig. 5 shows the resolution of temazepam on CSP **3**.

Table 3
Influence of the mobile phase composition on retention times, enantioselectivities and resolutions of β-blockers and benzodiazepines

Compound	Mobile phase								
	A			B			C		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
Alprenolol	0.23	2.00	1.35	0.32	2.03	1.53	0.53	2.45	2.21
Metoprolol	0.38	1.79	1.53	0.56	1.77	1.62	0.85	2.02	1.85
Oxprenolol	0.47	3.66	6.34	0.64	3.94	7.00	1.07	5.09	9.86
Tertatolol	0.5	3.06	5.33	0.73	3.11	4.54	1.13	3.95	5.79
Nadolol	0.65	1.92	~	1.34	2.04	~	4.84	2.02	~
Propranolol	0.69 (+)	1.54	2.58	0.96 (+)	1.59	2.44	1.47 (+)	2.07	5.30
Acebutolol	0.95	1.00	0	2.07	1.00	0	8.27	1.00	0
Pindolol	1.29	3.86	9.79	2.77	4.81	12.2	10.2	–	–
Sotalol	1.34	1.00	0	2.72	1.00	0	9.73	1.00	0
Labetalol	1.39	1.00	0	2.53	1.00	0	9.21	1.00	0
Isoproterenol	1.42	1.00	0	2.73	1.00	0	10.2	1.00	0
Atenolol	1.98	1.36	2.17	4.27	1.42	1.91	17.6	1.34	1.96
Oxazolam	0.56	1.00	0	0.74	1.00	0	1.37	1.00	0
Clorazepate	1.49	1.00	0	2.20	1.00	0	4.35	1.00	0
Oxazepam	2.40	1.40	1.75	4.08	1.42	2.08	10.8	1.40	2.07
Lorazepam	2.61	1.13	0.57	4.53	1.13	0.70	12.4	1.13	0.69
Temazepam	3.46	1.22	1.93	5.31	1.23	2.00	11.34	1.24	1.95
Lormetazepam	4.25	1.20	1.58	6.42	1.20	1.54	14.22	1.16	1.20

Conditions: CSP **2**; mobile phase, hexane–isopropanol (70:30, v/v) (A), (80:20, v/v) (B) or (90:10, v/v) (C);
Flow-rate, 1 ml/min.

Table 4
Separation of β-blockers and benzodiazepines

Compound	Mobile phase								
	A			B			C		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
Alprenolol	0.27	2.44	2.55	0.24 (+)	2.51	2.24	0.41	3.83	4.50
Metoprolol	0.52	1.81	1.76	0.49 (+)	1.83	2.52	0.59	5.50	5.95
Oxprenolol	0.62	4.70	9.48	0.56 (+)	4.77	10.8	0.77	10.6	11.3
Tertatolol	0.69	3.70	7.81	0.61 (+)	3.74	8.35	1.59 (+)	2.93	6.12
Nadolol	1.33	1.85	2.42	1.15	1.98	3.33	1.49	1.30	0.89
							3.63	1.35	1.70
Propranolol	0.82 (+)	2.00	4.10	0.83 (+)	1.82	4.14	1.06 (+)	3.09	6.37
Acebutolol	1.69	1.00	0	1.73	1.00	0	2.23	1.35	1.00
Pindolol	2.81	5.05	12.9	2.42 (+)	4.32	13.4	3.88	8.89	12.7
Sotalol	2.54	1.00	0	2.35	1.00	0	2.11	1.27	0.87
Labetalol	2.65	1.00	0	2.68	1.00	0	1.50	1.15	~
Isoproterenol	3.02	1.00	0	2.53	1.00	0	2.03	1.00	0
Atenolol	5.22	1.21	1.30	4.76 (+)	1.23	1.68	4.41	2.07	3.30
Oxazolam	0.62	1.17	0.83	0.65 (−)	1.17	0.82	0.62	1.17	0.81
	0.86	1.25	1.47	0.89 (−)	1.24	1.45	085	1.24	1.43
Clorazepate	2.10	1.00	0	2.13	1.00	0	2.06	1.00	0
Oxazepam	3.95	1.36	2.26	3.96 (−)	1.34	1.97	3.85	1.34	2.23
Lorazepam	3.98	1.07	0.55	4.06 (+)	1.08	0.58	3.86	1.09	0.61
Temazepam	5.06	1.21	2.20	5.22 (−)	1.21	2.24	4.78	1.21	2.29
Lormetazepam	6.19	1.07	0.80	6.29 (−)	1.09	0.79	5.96	1.08	0.86

Conditions: CSP **1**; mobile phase, hexane–isopropanol (80:20, v/v) (A), hexane–isopropanol (80:20) containing 10 m*M* Et_2NH (B), hexane–isoproanol (80:20, v/v) containing 10 m*M* octanoic acid (C).
Flow-rate, 1 ml/min.

Table 5
Separation of β-blockers and benzodiazepines

Compound	Mobile phase					
	B			C		
	k'_1	α	R_s	k'_1	α	R_s
Alprenolol	0.26	2.42	2.34	0.40	3.35	3.97
Metoprolol	0.5	1.94	2.97	0.57	5.39	6.57
Oxprenolol	0.62	4.21	10.4	0.77	8.54	13.08
Tertatolol	0.63	3.52	8.00	1.44	2.55	6.00
Nadolol	1.20	2.03	~	1.42	1.17	0.61
				3.21	1.17	0.96
Propranolol	0.91 (+)	1.66	3.73	1.07 (+)	2.68	5.56
Acebutolol	1.96	1.00	0	2.38	1.68	2.04
Pindolol	2.53	4.75	14.1	3.50	8.37	13.2
Sotalol	2.61	1.00	0	2.16	1.20	0.70
Labetalol	2.74	1.00	0	1.60	1.00	0
Isoproterenol	2.82	1.00	0	2.12	1.00	0
Atenolol	4.19	1.38	2.54	4.26	2.20	4.52
Oxazolam	0.73	1.00	0	0.78	1.00	0
				0.94	1.20	~
Chlorazepate	2.23	1.00	0	2.18	1.00	0
Oxazepam	3.88	1.44	2.02	4.09	1.41	2.43
Lorazepam	4.31	1.14	0.73	4.40	1.15	0.99
Temazepam	5.20	1.24	2.18	5.03	1.24	2.35
Lormetazepam	6.62	1.20	1.65	6.53	1.19	1.68

Conditions: CSP **2**; mobile phase; hexane–isopropanol (80:20, v/v) containing 10 m*M* Et_2NH (B); hexane–isopropanol (80:20, v/v) containing 10 m*M* octanoic acid (C).
Flow-rate, 1 ml/min.

Table 6
Separation of β-blockers and benzodiazepines

Compound	Mobile Phase					
	B			C		
	k'_1	α	R_s	k'_1	α	R_s
Alprenolol	0.25	1.00	0	0.44	1.64	0.61
Metoprolol	0.51	1.00	0	0.69	2.39	1.75
Oxprenolol	0.41	1.00	1.43	0.69	3.90	2.88
Tertatolol	0.56	1.33	1.03	1.17	1.99	1.63
Nadolol	1.50	1.00	0	2.05	1.45	0.95
Propranolol	0.56	1.15	0.38	0.73	2.05	1.82
Acebutol	2.16	1.00	0	3.02	1.39	0.94
Pindolol	2.25	1.34	2.72	4.13	2.80	2.68
Sotalol	3.47	1.00	0	3.23	1.13	~
Labetolol	3.50	1.00	0	2.19	1.00	0
Isoproterolol	4.75	1.00	0	3.92	1.00	0
Atenolol	5.08	1.09	0.60	5.72	1.64	1.59
Oxazolam	0.82	1.00	0	0.86	1.00	0
	1.09	1.22	1.47	1.13	1.23	1.41
Chlorazepate	2.61	1.00	0	2.40	1.00	0
Oxazepam	6.37	1.39	0.93	5.33	1.46	2.77
Lorazepam	6.55	1.12	0.45	5.29	1.17	1.19
Temazepam	5.95	1.40	3.70	5.68	1.41	3.97
Lormetazepam	7.59	1.32	2.68	6.94	1.34	3.21

Conditions: CSP **3**; mobile phase, hexane–isopropanol (80:20, v/v) containing 10 m*M* Et_2NH (B); hexane–isopropanol (80:20, v/v) containing 10 m*M* octanoic acid (C). Flow rate, 1 ml/min.

5. Conclusion

Ten regioselectively derivatized cellulose-coated columns were synthesized. They were deemed suitable for chiral discrimination and sometimes gave better results than those obtained using commercial

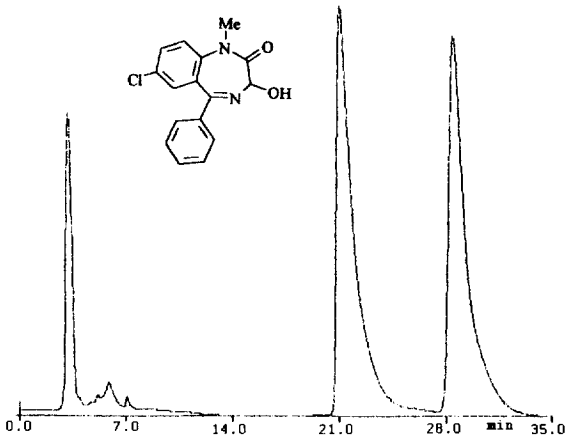


Fig. 5. Optical resolution of temazepam on CSP **3**. Mobile phase, hexane–isopropanol (80:20, v/v), 10 m*M* octanoic acid; flow-rate, 1 ml/min.

columns. The crucial role of the carbamate or ester moiety at the 6 position has been pointed out. Most of the racemic compounds shown in this paper were resolved most effectively on the 6-(*R*-phenylethylcarbamate)-2,3-(3,5-dimethylcarbamate) cellulose CSP. It will be of interest to study the influence of the substitution at the 2 and 3 positions.

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