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Review

Regioselectively modified polysaccharide derivatives as chiral stationary phases in high-performance liquid chromatography

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Abstract

This review summarizes several regioselectively modified polysaccharide derivatives which have been prepared in order to be used for chiral separations chromatography. The goal was to combine the effects of the known tris-arylcarbamate and tris-arylesters of polysaccharides. The use of new materials as chiral stationary phases (CSPs) in liquid chromatography for enantiomeric discrimination was investigated and compared to the homogeneously substituted polysaccharide derivatives. The different works describing the preparations, the performances and the applications of regioselectively modified polysaccharide derivatives have been resumed.

Keywords: Reviews; Chiral stationary phases, LC; Derivatization, LC; Enantiomer separation; Polysaccharides

Contents

1. Introduction

Homogeneously substituted polysacoharide derivatives are common materials and have been used as chiral stationary phases (CSPs) in recent decades. As a result, a number of CSPs based upon cellulose and amylose have been developed and are well known as the commercially available Chiralcel® OD, Chiralpak® AD and AS columns, etc. [1–3]. Some other polysaccharides such as xylan, dextran, chitin, chitosan or amylopectin have also been used as starting materials for the preparation of CSPs. Among them however, the tris-substituted phenylcarbamate derivatives of chitosan [4–6], chitin [7] and amylopectin [8,9] are the more studied as CSPs. Although these polysaccharides give sometimes very good results generally the enantioselectivity behavior is lower than those obtained on cellulose and amylose CSPs [9].

In order to extend the range of applications on a

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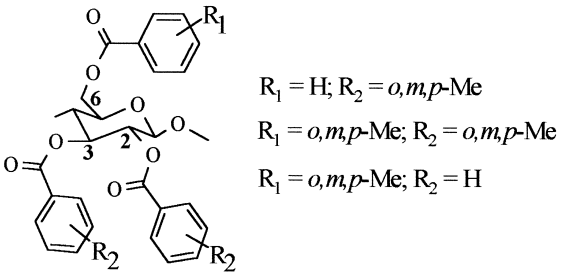
same CSP, several researches have tried to combine the different substituents on cellulose or amylose resulting interesting data from the view points of enantioselectivity. Four possibilities can be used on cellulose and amylose: polysaccharides having different ester or carbamoyl substituents at O–C(2,3) and O–C(6) respectively or the polysaccharide derivatives substituted with benzoates at positions 2 and 3 and with carbamate at position 6 and vice versa.

The regioselective heterosubstitution of polysaccharides was first introduced by Kaida and Okamoto [10]. The chemical pathway of the different heterosubstitutions is resumed in Scheme 1. The different CSPs corresponding to each series and synthesized according to Scheme 1 will be described and their chiral discrimination properties will be reported.

In all cases, complete carbamoylation reaction is observed, but unfortunately, during the removal of the trityl group, some minor decarbamoylation occurred (about 5%) at the position 2 and 3 [11]. Contrary to carbamoylation, complete esterification at positions 2, 3 of 6-trityl cellulose proved to be difficult to achieve. With the different benzoyl chlorides used as reagents, partial detritylation and subsequent esterification at the 6 position occurred, leading to products also benzoylated at position 6 [11,12]. As in the previous cases, the removal of the trityl group gave about 15% of de-esterification [11]



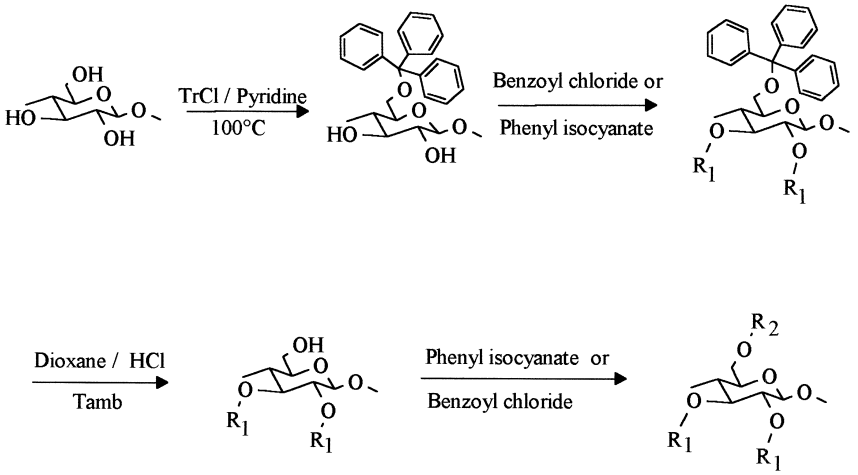
Scheme 2.

because the ester function are more labile than the carbamate ones.

1.1. The different ester substitutions at O–C(2,3) and O–C(6) positions

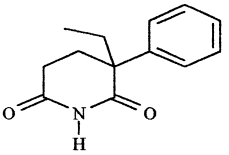
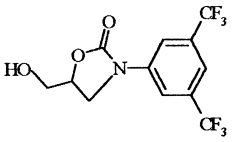
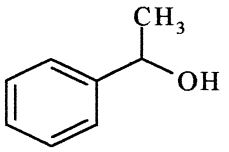
In 1992 Francott and Wolf reported that the enantiomers of 4-*tert*.-butyl-phenyl-3-cyclohexanone are well separated on the *m*-methylbenzoyl cellulose, but not on the *p*-methylbenzoyl cellulose [13]. To study this phenomenon, Francotte and Zhang have synthesized several CSPs (Scheme 2) which combine benzoate, *o, m, p*-methylbenzoate at the 2, 3 and 6 position of the glucose moieties of the cellulose chain respectively [14,15].

The chiral recognition capabilities of the 16 different combinations obtained were tested with racemates listed in Table 1.



Scheme 1.

Table 1
Selectivities on mixed benzoylesters of celluloses[a]

Compounds	Position	6-B	6-OMB	6-MMB	6-PMB
	2.3-B	1.00	1.22	1.21	1.25
	2.3-OMB	1.33	1.52	1.38	1.41
	2.3-MMB	1.29	1.51	1.35	1.49
	2.3-PMB	1.53	1.52	1.33	1.53
	2.3-B	1.00	1.32	1.00	1.00
	2.3-OMB	1.20	1.38	1.22	1.38
	2.3-MMB	1.00	1.16	1.00	1.20
	2.3-PMB	1.00	1.17	1.57	1.23
	2.3-B	1.59	1.00	1.36	1.46
	2.3-OMB	1.00	1.00	1.00	1.00
	2.3-MMB	1.11	1.25	1.00	1.00
	2.3-PMB	1.00	1.00	1.00	1.00

[a] B: benzoate, OMB: *o*-methylbenzoate, MMB: *m*-methylbenzoate, PMB: *p*-methylbenzoate. Column: 250*4.0 I.D. mobile phase: hexane/2-propanol (90/10); flow-rate: 0.7 ml min^{-1} [14].

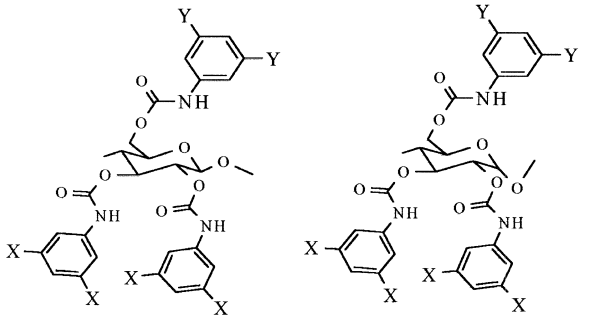
From these results, it appears that in the case of glutethimide, when the 6-position in cellulose is substituted with the *o*-methylbenzoyl group, this position governs chiral recognition and by changing the type of methylbenzoyl group at position 2 and 3, no significant variations in selectivity were detectable. At the same time, it can be seen that when the 2,3 positions are substituted with the *p*-methylbenzoyl group, the substituent in the 6 position seems to have only a minor influence. For the second racemate shown in Table 1, the mixed ester 2,3-*p*-methylbenzoate 6-*m*-methylbenzoate displays the best chiral recognition, whereas the corresponding homosubstituted esters show only poor selectivity or no enantioselectivity at all. For phenylethanol, the best selectivity is clearly obtained with tribenzoylcellulose but again a good selectivity is observed on the mixed 2,3-*m*-methylbenzoate 6-*o*-methylbenzoate ester, whereas the pure esters show no selectivity. The first example shows that it is quite possible that the chiral environment created by the mixed derivative differs from that provided by the respective homoderivatives. This indicates that variations in structure at the molecular level may greatly/significantly exert a great influence on the molecular recognition capacity. The supramolecular structure seems to play an important role in the discrimination.

1.2. The different carbamoyl substitutions at O–C(2,3) and O–C(6) positions

The regioselectively carbomoylated derivatives of cellulose and amylose were first prepared by Kaida and Okamoto [10] who established that the tris-(3,5-dimethylphenylcarbamate) homoderivatives of cellulose (CSP-l) and amylose (CSP-5), and the *tris*-(3,5-dichlorophenylcarbamate) homoderivatives of cellulose (CSP-2) and amylose (CSP-6) show a high optical resolving power for many racemates [16,17]. To study the optical resolving ability, they have prepared two types of regioselectively carbamoylated cellulose and amylose with 3,5-dimethylphenyl or 3,5-dichlorophenylisocyanates (Scheme 3).

Félix and co-workers have extended the work by Okamoto and Kaida to cellulose, amylose and amylopectine derivatives in introducing at the 6 position a chiral phenylcarbamate the 2,3 position being substituted by the classical phenyl, 3,5-dimethylphenyl or 3,5-dichlorophenylcarbametes [12]. The structure of polysaccharide derivatives is presented on Scheme 4.

Recently CSPs 9 and 10 were synthesized and studied by Acemoglu et al. [11] but they don't use the classical racemates (except compound **2**) in order to evalute the recognition power of the CSPs and it is



Cellulose | Amylose

CSP-3 X = Me; Y = Cl | CSP-7 X = Me; Y = Cl

CSP-4 X = Cl; Y = Me | CSP-8 X = Cl; Y = Me

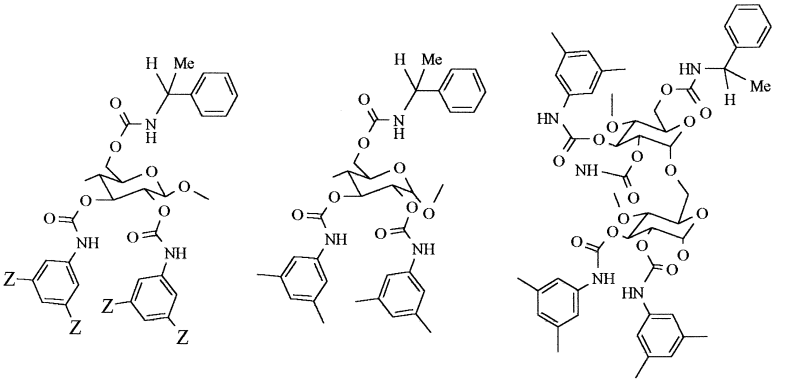
Scheme 3.

difficult to compare the chiral recognition power ability of the CSPs. So, as several racemates used have industrial applications the results will be treated in the application chapter.

The results obtained on the regio selective 3,5-dimethylphenyl/3,5-dichlorophenyl carbamoylated cellulose and amylose derivatives are listed in Table 2. In the case of cellulose derivative, the optical resolving abilities depended greatly on the racemates. For example, in the resolution of *trans*-stilbene oxide (**6**), the (−) isomer eluted first on the CSP-1, but the (+) isomer eluted first on the CSP-2. This result indicates that the mechanism of the chiral recognition of the first one for 6 is different from that of second one. As for the resolution of same compound, the mixed CSP showed a similar enantioselectivity to that of CSP-2, eluting the (+) isomer first. Thus, for **6** a similar discrimination seems to proceed on CSP-2, CSP-3 and CSP-4 showing that the ether oxygen of 6 appears to be the most important site for an interaction with the NH group of the 3,5-dichlorophenylcarbamate residue.

On the other hand, in the resolution of benzoin (**3**) CSP-1 and CSP-4 showed the same enantioselectivity. CSPs-2 and -3 also showed the same enantioselectivity which is in opposite to that of CSPs-1 and -4. These results suggest that the carbamate residue at the 6 position of the CSPs may play an important role for the chiral recognition of **3**.

In the amylose derivatives (Table 2), the enantioselectivity of CSP-5 was similar to that of CSP-7. CSP-6 showed an analogous chiral discrimination to CSP-8. For example, trifluoroanthrylethanol (**1**) was



Cellulose | Amylose | Amylopectin

CSP-9: 6-(*R*); Z = Me | CSP-12: 6-(*R*) | CSP-15: 6-(*R*)

CSP-10: 6-(*S*); Z = Me | CSP-13: 6-(*S*) | CSP-16: 6-(*S*)

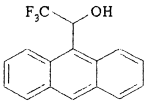
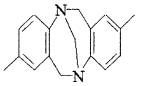
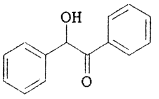
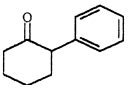
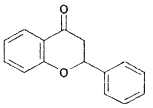
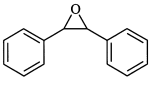
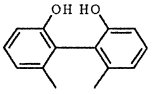
CSP-11: 6-(*R,S*); Z = Me | CSP-14: 6-(*R,S*) | CSP-17: 6-(*R,S*)

CSP-18: 6-(*R*); Z = H

CSP-19: 6-(*R*); Z = Cl

Scheme 4.

Table 2
Optical resolution of racemate on cellulose, amylose and amylopectin (substituted-phenylcarbamates)[a]

Compounds	1		2		3		4		5		6		7	
														
	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α
CSP-1	2.13(−)	2.59	0.97(−)	1.32	2.43(+)	1.58	1.17(+)	1.15	1.47(−)	1.41	0.47(−)	1.68	2.36(−)	1.83
CSP-2(*)	0.28(−)	1.38	0.87(+)	1.65	3.08(−)	1.21	2.65(−)	1.26	1.55(−)	1.20	0.56(+)	1.84	1.62(+)	1.11
CSP-3	0.44(−)	1.37	0.52(+)	1.39	2.64(−)	1.15	0.97(−)	1.28	0.77(−)	1.15	0.35(+)	1.80	1.08(−)	1.30
CSP-4(*)	0.77(−)	1.55	0.82(+)	1.22	2.84(+)	1.13	1.36(−)	1.20	1.21(−)	1.12	0.50(+)	1.33	2.26(−)	1.28
CSP-5	1.30(+)	1.15	0.53(+)	1.58	3.14(−)	1.21	0.61(−)	~1	0.93(+)	1.12	0.42(+)	3.04	2.46(−)	2.11
CSP-6	0.37	1.00	0.84(+)	1.34	6.08(+)	~1	0.63(+)	~1	1.62(+)	1.10	0.50(+)	1.32	1.10(+)	~1
CSP-7	0.73(+)	1.16	0.93(+)	1.42	5.83(−)	1.11	0.85(−)	~1	1.29(+)	1.14	0.71(+)	1.45	3.57(−)	1.51
CSP-8	0.27(+)	~1	0.53	1.43	1.86(+)	1.70	1.53(+)	~1	1.00(+)	1.07	0.40(+)	~1	0.51(+)	1.18
CSP-9	2.05(−)	2.01	0.80(+)	1.72	2.38(+)	1.31	1.17	1.35	1.26	1.25	0.70	1.58	0.92	1.30
CSP-10	2.40(−)	2.19	0.94	1.62	3.09(+)	1.38	1.33	1.29	1.67	1.21	0.86	1.39	1.15	1.32
CSP-11	1.96(−)	1.50	0.60(+)	1.48	2.19(+)	1.23	0.88	1.25	1.33	1.06	0.58	1.00	0.85	1.12
CSP-12	3.41(+)	1.21	0.85	1.13	4.23(+)	1.21	0.95	1.00	1.51	1.37	0.57	1.75	1.14	1.65
CSP-13	1.32(−)	1.33	0.69(+)	1.31	3.10(+)	1.32	1.02	1.14	1.90	1.36	0.49	1.60	0.95	4.22
CSP-14	1.38(−)	1.31	0.72(+)	1.22	3.99(+)	1.14	0.90	1.00	1.78	1.08	0.52	1.78	0.96	3.64
CSP-15			0.10(+)	8.00	1.64	1.00			0.60	2.00	0.27	1.00	0.69	1.00
CSP-16			1.17	1.00	2.40(+)	1.46			1.81	1.00	0.43	1.44	0.85	2.83
CSP-17			0.93	1.00	2.34(+)	1.32			1.04	1.79	0.25	1.80	0.63	3.33
CSP-18	1.76(−)	1.41	0.94(+)	1.45	4.36(+)	1.04	1.83	1.22	2.21	1.07	0.66	1.35	1.72	1.14
CSP-19	1.05	1.00	0.28	1.43	1.21	1.00	0.68	1.29	0.74	1.00	0.16	1.60	0.61	1.00
CSP-20	3.66(−)	1.32	1.12(+)	1.40	3.65	1.00	1.67	1.13	2.52	1.04	0.86	1.06	1.83	1.10
CSP-21	2.66	1.00	1.23(+)	1.39	4.62(−)	1.10	2.22	1.17	2.76	1.00	0.84	1.19	2.56	1.09
CSP-22	1.30	1.00	0.90(+)	1.37	3.03(+)	1.10	1.90	1.19	1.87	1.05	0.60	1.22	1.92	1.04
CSP-23	3.77	1.00	0.66(+)	1.39	3.16	1.00	1.27	1.23	2.15	1.00	0.71	1.10	1.31	1.00
CSP-24	3.20(−)	1.33	1.04(+)	1.37	5.01(+)	1.10	1.98	1.32	3.29	1.00	0.93	1.20	1.99	1.18
CSP-25	1.95	1.00	0.65(+)	1.12	2.86(+)	1.06	1.19	1.10	1.83	1.00	0.59	1.07	1.13	1.00

[a] Eluent: hexane/2-propanol (90/10); (*) hexane/2-propanol (95/5); flow-rate: 0.5 ml min^{-1} [11,12,16–18,22].

resolved on CSP-7 with almost the same α value as that on CSP-5, though neither CSP-6 nor CSP-8 resolved **1**. For most racemates, except for Tröger base (**2**) and benzoin (**3**) CSPs-5 to -7 showed a higher optical resolving ability than did CSP-8. Kaida and Okamoto explained the difference observed between the cellulose and the amylose derivatives by the conformational differences of the helix of the polysaccharide derivatives: left-handed threefold (3/2) helix for cellulose and left-handed fourfold (4/1) helix for amylose [10].

The results obtained on heterosubstituted cellulose, amylose and amylopectin having a chiral carbamate at the 6 position are also listed in Table 2. The only difference between CSPs 9–17, except the nature of the polysaccharide, is the configuration of the phenylethylcarbaniate (PEC) at the 6 position of the glucose unit. All those CSPs reveal themselves as very suitable for chiral recognition but their chromatographic behavior is quite different from the corresponding homosubstituted CSPs. It is not unusual to obtain best enantioselectivity with heterosubstituted CSPs. For example, **5** was separated on CSP-13 with an α value of 1.36 although it was either not or poorly ($\alpha=1.12$) recognized on Chiralpak AS and Chiralpak AD, respectively [19]. This phenomenon was commonly observed with **2** and **4** on cellulose, **1** and **5** on amylose, **3**, **5** and **7** on amylopectin based CSPs. The widest range of separations was obtained with cellulose and amylose derivatives. Although having a higher specificity, amylopectin based CSPs give excellent results since

5 and **6** were better separated on CSP-17. The retention factors obtained with CSP-15 are particularly low. Amylopectin with a *R*-PEC at the 6 position and a 3,5-dimethylphenylcarbamate at the 2,3 position is partially soluble in the eluent. The desorption of some amount of chiral selector leads to fast elution and low resolution in spite of high selectivity. A phenomenon of interest is the complementarity of the three polysaccharides. **1**, **2** and **4** were well recognized on cellulose, **3** and **5** on amylopectin and **7** on amylose. The chromatographic behaviors converge, as the polymeric structures become closer. A first sign is the good resolution of **6** on amylose and amylopectin (α=l.78 on CSP-14 and α=l.80 on CSP-17). The second one is the selectivity increase obtained for **5** both on amylose and amylopectin heterosubstituted CSPs compared to the homosubstituted ones. This behavior could be explained by the similarities between the polysaccharide shapes, more precisely the presence of α-glucosidic linkages in both polymer chains.

It is obvious that the enantioselectivities change when PEC configuration is modified. A dramatic decrease in selectivities is observed with **3** when configuration changes from *S* (CSP-12; α=4.22) to *R* (CSP-13; α=1.65). There seems to be no regular rules for the whole series of CSPs 9–17. On the one-hand, **6** are well recognized on CSP-9 (*R*-PEC; α=1.58) and not at all on CSP-11 (*R,S*-PEC; α=1). On the other hand, **6** is not separated on CSP-15 (*R*-PEC; α=1) and well separated on CSP-17 (*R,S*-PEC; α=1.8). Some trends appear within series prepared with the same polysaccharide. Higher separation factors were obtained with cellulose based CSP-9 and CSP-10, *R*-PEC and *S*-PEC respectively. It is more surprising that the cellulose tris(*R,S*-PEC) results surpass those of cellulose tris(*S*-PEC) [20,21]. Thus, this proves that using chiral substituent at the 6 position only is an effective mean to improve selectivity with cellulose CSPs. Within the amylose series, the α values for CSP-13 having a *S*-PEC as the substituent are the most elevated ones, being quite in accordance with the results obtained with amylose tris(PEC) [19]. Lastly, the behavior of the CSP-17 must be pointed out, i.e. amylopectin substituted at the 6 position with *RS*-PEC, gives the best selectivities. The average results obtained on CSP-15 and -16 have a chiral carbamate at the 6 position reveal the chiral center added on the step of chemical modification is not responsible for the selectivity increase. So it would be simply the positive influence of the steric effect induced by the phenylethylcarbamate [22].

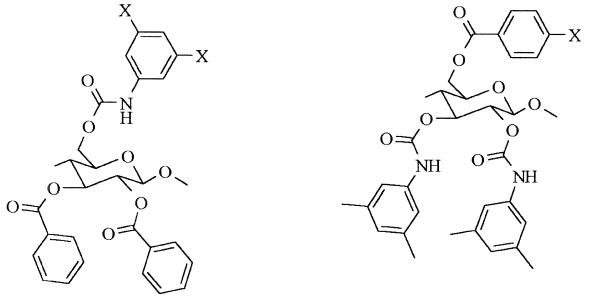
The retention orders are not affected by the absolute configuration of PEC at the 6 position. **1 (−/*S*), 2 (+/5*R*,11*R*)** and **3 (+/*S*)** were eluted first on all the CSPs (except for **1** on CSP-12). Thus, most likely the chiral recognition is controlled by the macromolecular structure of the polysaccharide. The less retained enantiomers were the same on heterosubstituted CSPs 9–11 and on cellulose tris(3,5-dimethylphenylcarbamate) [16]. Conversely, it is between CSPs 12–14 and the corresponding amylose tris(PEC) [20,21] or between CSPs 15–17 and the corresponding amylopectin tris(PEC) that same elution orders were observed. As a consequence, the interactions involving the 6 position seem to be more involved in the chiral discrimination mechanism in the case of amylose and amylopectin were concerned. This common behavior could be explained as discussed above, by the similar conformation of these polysaccharides.

So, using a chiral phenylcarbamate at the 6 position is mainly responsible for a selectivity increase. Yet, the reason of the improvement differs depending on the polysaceharide used. The recognition mechanism is more complicated for amylopectin, which is a branched polysaccharide compared to cellulose and amylose. In that case, it seems most likely that the steric hindrance of the PEC creates the desired effect described.

1.3. Mixed ester and carbamoyl substitutions at O–C(2–3) and O–C(6) positions

These type of polysaccharides CSP were first described by Francotte and Zhang [15] and reinvestigated by Félix and co-workers [12]. 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 CSPs are presented in Scheme 5.

In their work Acemoglu et al. [11] have synthesized several mixed cellulose CSPs already described like CSPs-20, -22, -23 and -24. They have



CSP-20: X = Me
CSP-21 : X = H
CSP-22: X = Cl

CSP-23: X = Me
CSP-24: X = H
CSP-25: X = Cl

Scheme 5.

also completed their work with several new derivatives presented in Scheme 6.

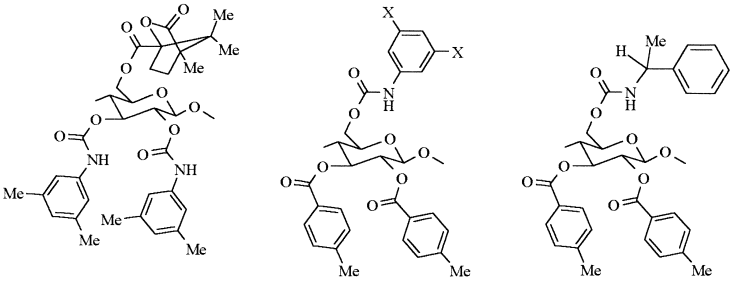
Like for CSPs-9 and -10 (see above) Acemoglu et al. [11] have not used the ''classical'' racemates (except compound **2**) to evaluated the recognition power of the CSPs-26 to -30.

The chromatographic results obtained with CSPs -20 to -25 are reported in Table 2. For CSPs-20 to -22, 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. CSP-21 possesses the most elevated values for most capacity factors. The steric effects caused by the methyl or the chlorine group (CSP-20 and -22) (Van der Waals radius: *r*CH3=2 Å; *r*Cl=1.8 Å; *r*H=1.2 Å) seems to make the approach of the racemat difficult. The destabilization of the complex CSP/solute results in a decrease of k_1. This phenomenon is confirmed with the CSPs-23 to -25. Retentions are always higher on CSP-24 (except for **1**). No correlation exits between capacity factors and enantioselectivities. A high value of k_1 does not necessary lead to a good separation (CSP-20: **1** k=3.66, α=1.32 and **3** k=3.65, α=1). It means that the amount of achiral interactions is predominant compared to the amount of chiral ones. So the separation mechanism and the retention sites 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.

It is interesting to compare the chiral recognition abilities of the mixed CSPs with the commercial ones such as Chiralcel OB [16] and OD [23] containing the same substituents but homogenuously in all 2 and 3 positions. Firstly, best separations were obtained for **2** on 20–21 and 23, 24 CSPs and for **4** on 23, 24 CSPs. This means mixed CSPs are able to surpass commercial ones for some racemates. However CSPs-23–25 shows lower selectivities showing the importance of a carbamate moiety at the 6 position.

2. Applications

It is well know that the composition of the mobile phase plays an important role in the enantiomeric separation on polysaccharides derivative CSPs [24]. The separation of some β-blockers and benzodiaz-



CSP-26

CSP-27: X = Me
CSP-28: X= Cl

CSP-29: 6-(*R*)
CSP-30: 6-(*S*)

Scheme 6.

Table 3
Influence of the mobile phase composition on retention times, enantioselectivities and resolutions of β-blockers and benzodiazepin[a]

Mobile phase compounds	A			B			C		
	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs
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

[a] Conditions: CSP-10, Mobile phase: Hexane/iPrOH–70:30 (A); Hexane/iPrOH80:20 (B); Hexane/tPrOH–90: 10(C); flow-rate 1 ml min^{-1} [12].

epins with various hexane/isopropanol mobile phases were achieved on CSP-10 (Table 3). For β-blockers, retention times and enantioselectivities decrease with increasing amount of isopropanol. For benzodiazepins, the decrease in retention times is not associated with decrease of the α values. The hydroxyl groups of benzodiazepins which may compete with the isopropanol for interaction sites on the CSPs seems to be more involved in the retention than in the chiral discrimination.

In order to optimize the separations on our most promising CSPs, the influence of two organic mobile phase modifiers is presented in Tables 4, 5 and 6, respectively for CSP-9, -10 and -11. The best results were obtained for β-blockers, whatever the CSP used when a small amount (10 m*M*) of diethylamine or octanoic acid was added to the mobile phase. Diethylamine has poor influence on the retention times but notably improves the resolutions. It may act as 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 of α and Rs values (except for tertatolol). As an example, the α value of oxprenolol goes from 4.7 to 10.6 thanks to the addition of acid. It has been reported [25] that adding trifluoroacetic acid to the solvent increases the separation of some racemates on coating cellulose derivative. The same phenomenon was observed with octanoic acid in the mobile phase. In the case of β-blockers apparently the changes occurring in the supramolecular structure of the polymer improve the separations. At the same time, the use of trifluoroacetic acid in combination with triethylamine as ion-pair reagent on the Chiralcel OJ results a decrease of the peak tailing and an increase of the selectivity [26–28]. No real correlations were observed between the mobile phase composition and the α values for benzodiazepins. The mechanism of chiral discrimination seems to be different for the two families of racemates. The best separations for β-blockers and benzodiazepins were obtained with CSP-9 and -1 respectively.

On the other hand, CSP-9 (with a (*R*) configuration of the chiral carbon atom at position 6) has the best enantio recognition power. For example, all the isomers of oxazolam are separated, CSP-10 (with a (*S*) configuration of the chiral carbon atom at position 6) is less powerful. CSP-11 (with (*R*,*S*) configuration of the chiral carbon atoms at position 6) has in general a lower recognition power. With the

Table 4
Separation of β-blockers and benzodiazepin[a]

Mobile phase compounds	A			B			C		
	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs
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	l.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	0.85	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	1.07	3.98	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

[a] Conditions: CSP-9 Mobile phase: Hexane/iPrOH–80:20 (A); Hexane/iPrOH–80:20, Et_2NH 10 m*M* (B); Hexane/iPrOH–80:20, octanoic acid 10 m*M* (C); flow-rate 1 ml min^{-1} [12].

Table 5
Separation of β-blockers and benzodiazepins[a]

Mobile phase Compounds	B			C		
	k_1	α	Rs	k_1	α	Rs
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	0	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

[a] Conditions: CSP-10 Mobile phase: Hexane/iPrOH–80:20, Et_2NH 10 m*M* (B); Hexane/iPrOH–80:20, octanoic acid 10 m*M* (C); flow-rate 1 ml min^{-1} [12].

Table 6
Separation of β-blockers and benzodiazepins[a]

Mobile phase compounds	B			C		
	k_1	α	Rs	k_1	α	Rs
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

[a] Conditions: CSP-11 Mobile phase: Hexane/iPrOH–80:20, Et_2NH 10 m*M* (B); Hexane/iPrOH–80:20, octanoic acid 10 m*M* (C), flow-rate 1 ml min^{-1} [12].

symmetrical cellulose tris(phenylethylcarbamate) CSPs the order of enantio separation ability is given by the following chiral substitution sequence: (*R*)> (*R*,*S*)>(*S*) [20,29]. These results demonstrate that the addition of a chiral carbon at 6 position increases the enantio recognition power without changing the elution order (*R*-(+)-propanolol is always eluted first) which is not the same with the symmetrical by substituted derivatives. These results are in agreement with those of Okamoto and co-workers showing that all the positions (2,3,6) on the glucose unit of cellulose participate in the chiral recognition [20,29]. Only the separation of oxazepam enantiomers was reported on Chiralcel OD previously [30].

Table 8
SEC resolution of ketoconazole on CSP-12[a]

% of modifier	17%	20%	25%
k_1	9.3(+)	6.4(+)	3.7(+)
k_2	12.2(−)	6.6(−)	4.7(−)
α	1.30	1.29	1.27
Rs	0.83	0.73	0.75

[a] Mobile Phase: CO_2 with different percentages of modifier (17, 20 and 25%). Modifier: methanol–ethanol, 1/3–2/3, onoic acid (10 mmol). Column outlet pressure: 3 MPa. Column temperature: 30°C. Flow rate: 2.5 ml min^{-1}. Detection: UV 254 nm [31].

In preliminary studies [32], it was found that the enantiomers of ketoconazole could be separated by HPLC on Chiralpak AD. The chromatography of itraconazole on Chiralcel OD, however, gave only two peaks for the four stereoisomers; the first peak was due to dextrorotatory isomer, while the second peak was levorotatory, since no net rotation would be observed for a racemate.

CSPs-9 and -12 were used to study the separation of ketoconazole and intraconazole in HPLC and SFC [31]. The results are collected in Tables 9–11 showing the retention factors *k*, α, and Rs. In HPLC, for ketoconazole, the resolution factor Rs was 1.7 with an enantioselectivity factor α of 1.5 on CSP-9; while on CSP-12, Rs was 1.53 with α of 117. Thus, CSP-12 is somewhat less enantioselective than CSP-9. For itraconazole, four peaks were obtained, with the following separation parameters (Table 7) only the separation of peaks 2 and 3 was poor.

In SFC increasing the proportion of the modifier in the mobile phase causes only a slight decrease in

Table 7
HPLC separation of ketoconazole and itraconazole[a]

Compound	CSP	k_1	k_2	k_3	k_4	Mobile phase
Ketoconazole	CSP-9	10.8(+)	15.6(−)			A
Ketoconazole	CSP-12	14.2(+)	17.2(−)			B
Itraconazole	CSP-12	24.3(+)	28.3(−)	30(+)	33(−)	C
α		1.16	1.05	1.10		
Rs		1.94	0.68	1.13		

[a] Mobile phases: (A) hexane–isopropanol, 70–30 (v/v), octanoic acid (10 mmol). (B) hexane–methanol–ethanol, 80–05–15 (v/v/v), octanoic acid (10 mmol). (C) hexane–methanol–ethanol, 75–12–13 (v/v/v), octanoic acid (10 mmol). Flow rate: 1 ml min^{-1}. Detection: UV 254 nm [31].

Table 9
SFC separation of itraconazole on CSP-2[a]

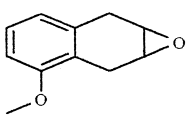
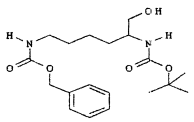
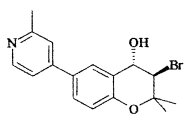
Column outlet pressure		35 MPa	
MeOH–EtOH (v/v)	1/3–2/3	1/2–1/2	2/3–1/3
Octanoic acid (10 mmol)			
k_1	44.8	46.9	49.1
k_2	54.1	65.6	58.2
k_3	58.2	62.8	63.6
k_4	65.1	69.8	70.2
$(\alpha/\mathrm{Rs})_{1,2}$	1.20/2.04	1.20/2.0	1.18/1.84
$(\alpha/\mathrm{Rs})_{2,3}$	1.07/0.74	1.10/1.11	1.09/0.98
$(\alpha/\mathrm{Rs})_{3,4}$	1.12/1.14	1.11/1.31	1.10/1.13

[a] Mobile Phase: CO_2 with 17% modifier: Modifier: variable. Column outlet pressure: 35 MPa. Column temperature: 30°C. Flow rate: 2.5 ml min^{-1}. Detection: UV 254 nm [31].

enantioselectivity and a modest decrease in the separation factor for ketoconazol (Table 8). It may be useful therefore to increase the percentage of the polar modifier to a degree that, while shortening the retention time, does not adversely affect the resolution. It was also found that varying the methanol/ethanol proportion in the polar modifier causes only negligible changes in retention time, enantioselectivity, and resolution. For itraconazole, four peaks were obtained; and, as in the HPLC separations, only peaks 2 and 3 were poorly resolved. A study of the influence of the proportion of methanol and ethanol in the polar modifier was also carried out, and the results are presented in Table 9. It is thus clear that the greater the proportion of methanol in the modifier, the longer the retention times become for itraconazole. One would however expect the opposite of these results, because methanol is more polar than ethanol. This apparent discrepancy might be explained by the reduction in the solubility of the drug as the proportion of ethanol decreases, thereby increasing the retention times. Therefore, the organic modifier must contain a sufficient quantity of ethanol to reduce the retention time while maintaining the quality of the separations.

The results obtained with the mixed cellulose derivatives synthesized according to Ref. [11] are listed in Tables 10 and 11. The resolution properties of CSPs-9, -10, -23, -24 and -26, prepared from different cellulose-2,3-bis-*O*-carbamate-6-*O*-arylester derivatives were evaluated for three racemates (**8–10**) (Table 10). Although none of these CSPs was able to resolve all the three test racemates. CSP-24

Table 10
Separation of some racemates on various CSPs[a]

	8			**9**			**10**					
Compounds										Phase mobile		
Detection (nm)	220			210			280					
	k_2	α	Rs	k_2	α	Rs	k_2	α	Rs			
CSP-9	2.03	1.00	–	4.64	1.00	–	7.08	1.17	0.82		A	
CSP-10	2.19	1.10	0.49	5.15	1.00	–	5.28	1.22	1.21		A	
CSP-20	2.58	1.00	–	2.60	1.00	–	2.38	1.25	0.49	A	B	C
CSP-22	3.13	1.11	0.61	4.18	1.44	1.39	2.20	1.16	0.60	D	B	C
CSP-23	2.32	1.16	0.63	3.75	1.00	–	3.60	1.00	–		A	
CSP-24	3.49	1.17	0.56	4.38	1.00	–	3.64	1.00	–		A	
CSP-26	1.43	1.00	–	3.45	1.00	–	4.69	1.00	–		A	
CSP-27	2.50	1.00	–	2.85	1.00	–	3.62	1.00	–	A	B	B
CSP-28	1.92	1.00	–	5.58	1.55	2.04	6.07	1.26	1.03	A	A	C
CSP-29	3.21	1.00	–	2.71	1.00	–	2.56	1.00	–	A	B	C
CSP-30	3.16	1.00	–	2.81	1.00	–	1.75	1.00	–	A	B	C

[a] Mobile phases: (A) hexane–isopropanol, 90–10 (v/v). (B) hexane–isopropanol, 80–20 (v/v). (C) hexane–isopropanol, 85–15 (v/v). (D) hexane–isopropanol, 95–5 (v/v). Flow rate: 0.5 ml min^{-1} [11].

Table 11
Separation of some racemates on mixed CSPs-22 and 24 k [11]. Mobile phase: hexane–isopropanol[a]

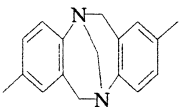
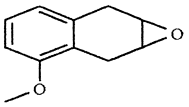
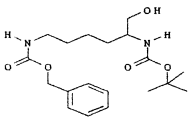
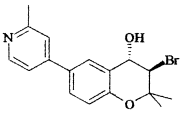
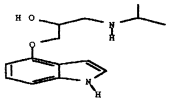
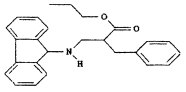
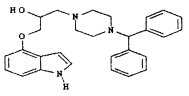
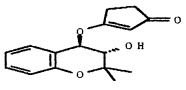
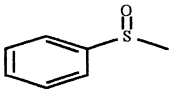
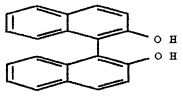
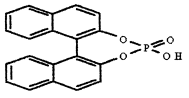
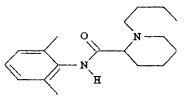
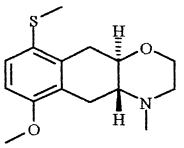
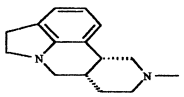
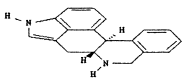
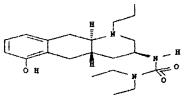
No.	Compounds	CSP-22			T(°C)	% *i*-PrOH	CSP-24			T(°C)	% *i*-PrOH
		k_2	α	Rs			k_2	α	Rs		
2		1.83	1.57	1.00	25	10	1.20	1.95	1.50	20	10
		2.63	1.00	–	15	3	2.36	2.00	1.50	20	10
8		3.94	1.11	0.51	25	10	2.00	1.17	0.59	20	3
		9.79	1.51	1.15	15	3	3.77	1.19	0.67	20	10
9		1.52	1.00	–	25	10	3.31	1.00	–	40	20
10		11.6	1.15	0.69	25	10	3.02	1.00	–	20	10
		2.60	1.77	1.76	15	3	3.22	1.10	0.60	40	20
11		4.83	1.10	1.19	25	10	12.1	1.00	–	20	10
12		10.5	1.00	–	25	10	3.04	1.18	0.56	20	10
		3.74	1.00	–	15	3	9.67	1.17	0.47	20	10
13		9.02	1.08	0.43	25	10	17.6	1.22	0.63	20	10
14		9.49	1.00	–	25	10	13.0	1.00	–	20	10
15		0.31	1.00	–	25	10	3.34	1.00	–	20	10
16		162	1.00	–	25	10	10.7	1.00	–	20	10
17		299	1.00	–	25	10	0.64	1.00	–	20	10

Table 11. Continued

No.	Compounds	CSP-22 k_2	α	Rs	T(°C)	% *i*-PrOH	CSP-24 k_2	α	Rs	T(°C)	% *i*-PrOH
18		0.79	1.00	–	25	10	0.94	1.00	–	20	10
19		5.24 11.7	1.00 1.00	– –	25 15	10 3	1.29 2.70	1.16 1.18	0.48 0.57	20 20	10 10
20		0.79	1.00	–	25	10	0.32	1.00	–	25	10
21		5.24	1.00	–	25	10	7.64	1.00	–	25	10
22		11.7	1.00	–	25	10	6.48	1.00	–	25	10

[a] Flow rate: 1.0 ml min^{-1}. Detection: UV 210 [11].

seems to show highest enantioselectivity for **8** and CSP-9 had almost identical resolution properties to CSP-10. The resolution properties of CSPs-20, -22 and -27 to -30, prepared from different cellulose-2,3-bis-*O*-arylester-6-*O*-carbamate derivatives were evaluated for the same compounds (Table 10). Only CSP-22 exhibited the ability for the separation of all the three racemates.

The authors have fully investigated CSP-22 and -24, the results are summarized in Table 11. Good enantioselectivities are observed for the separations of **2** (α=2.00), **8** (α=1.19), **12** (α=1.18), **13** (α=1.22) and **19** (α=1.18). Nevertheless, the best enantioseparation is achieved for **2**, indicating the excellent suitability of this CSP for the separations of racemates having relatively rigid conformation. The results obtained with CSP-22 (Table 11) show that under those conditions, a chiral separation could be achieved for approximately 50% of all racemates. The most interesting result was obtained in the case of **9** (α=1.51, Rs=1.15). From these results, it seems likely that the CSP-22 can be used for the enantiomeric of separations of amino acid derivatives and racemates containing a heteroatom as stereogenic center.

3. Conclusion

The studies performed on regioselectively substituted cellulose derivatives have shown the importance of the substituent at O–C (6) position. Regioselectively substituted heterofunctional cellulose having 2,3-bis-*O*-carbamate-6-*O*-ester function or vice versa do not combine the resolution power of the known cellulose-tris carbamate and cellulose-tris-benzoate phases.

However, the polysaccharides CSPs having 2,3-bis-*O*-carbamate-6-*O*-chiral carbamate especially, the 6-(*R*-phenylethylcabamate)-2,3-(3,5-dimethylphenylcarbamate) of cellulose and the 6-(*S*-phenylethylcabamate) - 2,3 - (3,5 - dimethylphenylcarbamate) of amylose exhibit a higher enantioselective power than the corresponding cellulose and

amylose-tris carbamate or tris chiral carbamate. When the racemates are not separated on Chiracel OD, Chiralpak AD or AS the use of the 6-(phenylethylcarbamate) - 2,3 - (3,5 - dimethylphenylcarbamate) cellulose or amylose can give the solution like the case for the case itraconazole.

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