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Mixed cellulose-derived benzoates bonded on allylsilica gel as HPLC chiral stationary phases: influence of the introduction of an aromatic moiety in the fixation substituent

Jordi Garcés,[a,†] Pilar Franco,[a,‡] Laureano Oliveros[b] and Cristina Minguillón[a,*]

[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—Several mixed alkenoxybenzoyl/benzoates and 10-undecenoyl/benzoates of cellulose were fixed onto allylsilica gel by the radical coupling of double bonds. The introduction of an aromatic group in the fixation substituent modifies the chiral recognition ability of the resulting chiral stationary phases (CSPs) in comparison with the 10-undecenoate/benzoate cellulose derivatives. Better enantioselectivity values are achieved when the electronic and geometric characteristics of both substituents, fixating and derivatizing, are similar.

1. Introduction

Chiral stationary phases (CSPs) based on polysaccharide derivatives have been successfully used in liquid chromatography for the separation of enantiomers.[1] However, the solubility of these derivatives in certain solvents limits their applicability in HPLC. The different approaches for the fixation of the polysaccharide derivative to the chromatographic matrix[2] have allowed the use of a broader variety of solvents as mobile phase modifiers, in contrast to the classic hydrocarbon/alcohol mixtures which are compatible with the coated phases.[3–5]

The 10-undecenoate/phenylcarbamates[6] or benzoates[7] of cellulose bonded on chromatographic matrices developed by our research group show certain differences in their chiral discrimination ability from that of the homosubstituted coated supports. These variations in resolving behaviour could be considered as a consequence of two main factors: the lack of homogeneity in the substitution of the cellulose derivative, containing an aliphatic moiety on some of the hydroxyl groups, and the influence that the process of fixation can cause on the discrimination ability. It has been shown that fixation of 10-undecenoyl/phenylcarbamates or benzoates of polysaccharides to allylsilica gel takes place either by heterogeneous coupling of the double bonds, on the chromatographic matrix and on the polysaccharide derivative, or by reticulation of the 10-undecenoyl groups themselves.[8] Both processes occur simultaneously and may affect the chiral discrimination ability of the CSPs by deforming the secondary structure of the polysaccharide. It is generally accepted that this conformation plays an important role in the enantioselectivity of the resulting CSP.[1] Moreover, the presence of a certain number of alkenoyl chains on the cellulose derivative implies a decrease in the total number of aryl residues, usually related with the chiral recognition phenomenon.[9]

In the present study, in order to retain the structural regularity in the polymer and, hence, the secondary helical structure of the polysaccharide, while maintaining the fixing capability, long chain alkenoxybenzoyl groups have been introduced on cellulose instead of the

* Corresponding author. Present address: IRBB-Parc Científic de Barcelona, Josep Samitier, 1-5, E-08028 Barcelona, Spain. Tel.: +34-93-403-7107; fax: +34-93-403-7114; e-mail: cminguillon@pcb.ub.es

† Present address: AlconCusí R&D, Analytical Chemistry Unit, Camil Fabra, 58, E-08320 El Masnou (Barcelona), Spain.

‡ Present address: Chiral Technologies Europe, Parc d'Innovation, Bd. Gonthier d'Andernach, F-67400 Illkirch, France.

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alkenoyl groups formerly used (Fig. 1). The synthesis and the chromatographic results obtained with 4-methylbenzoyl and 4-methoxybenzoyl derivatives of cellulose are here compared to the analogous derivatives fixed through 10-undecenoyl groups.

2. Results and discussion

2.1. Preparation of the chromatographic supports

4-(10-Undecenyloxy)benzoic acid was obtained as described elsewhere.[10] Thus, the 10-undecenyl *p*-toluensulfonate, prepared from 10-undecen-1-ol, was used in the alkylation of ethyl 4-hydroxybenzoate. The obtained ester was later hydrolysed and the resulting acid was transformed into the corresponding acyl chloride **1** by the treatment with thionyl chloride (Scheme 1).

Cellulose derivatives were prepared following the method described for the 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose.[11] Thus, cellulose was allowed to react successively with the prepared alkenoxybenzoyl chloride **1** and the appropriate benzoyl chloride (Scheme 2). Reaction time for the introduction of the first reagent was set to yield substitution degrees of fixing groups in the order of 0.2–0.3 groups per glucose unit. This amount of fixing groups was determined to be the optimal amount to fix 10-undecenoyl derivatives onto 100 Å allylsilica gel.[12] The aromatic reagent **1** showed a reduced reactivity compared to that shown by 10-undecenoyl chloride under the same conditions. Therefore, longer reaction times were needed to obtain similar substitution degrees. Different ratios of substituents were introduced on derivatives **A** and **A′** to assess the effect of an increased reticulation of the derivative on the chromatographic behaviour of the resulting CSP. Derivatives containing 10-undecenoyl groups (**C** and **D**) were prepared for comparative purposes (Scheme 2). All derivatives were characterised by their ^{1}H NMR spectra and elemental analyses (Table 1).[13]

The obtained cellulose derivatives were fixed on allylsilica gel, previously prepared from spherical silica gel (Nucleosil® 100-5) and allyltriethoxysilane. An additional treatment of 'end-capping' with hexamethyldisilazane was performed on the chromatographic matrix before fixation.[7] The resulting CSPs were characterised by their elemental analyses (Table 1). The new aromatic alkenyl group were less efficient than 10-undecenoyl in the fixation of the cellulose derivative. Only **A′**, with a higher content of fixing groups, was immobilised in comparable ratio to **C**. All CSPs obtained were slurry packed into stainless-steel tubes in order to be chromatographically tested.

2.2. Chromatographic results

The resulting HPLC columns were tested in normal phase conditions using heptane/2-propanol and heptane/chloroform mixtures as the mobile phase. A selec-

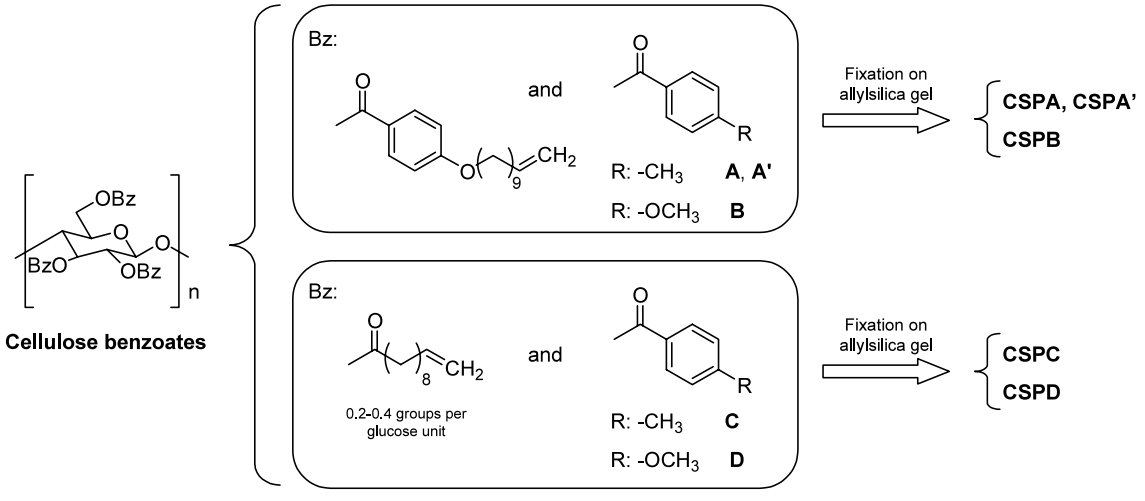
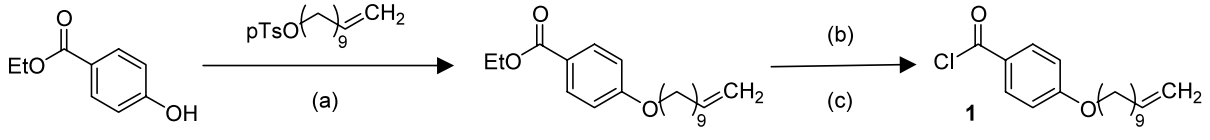


Figure 1. Type of substitution of the synthesised cellulose benzoates.

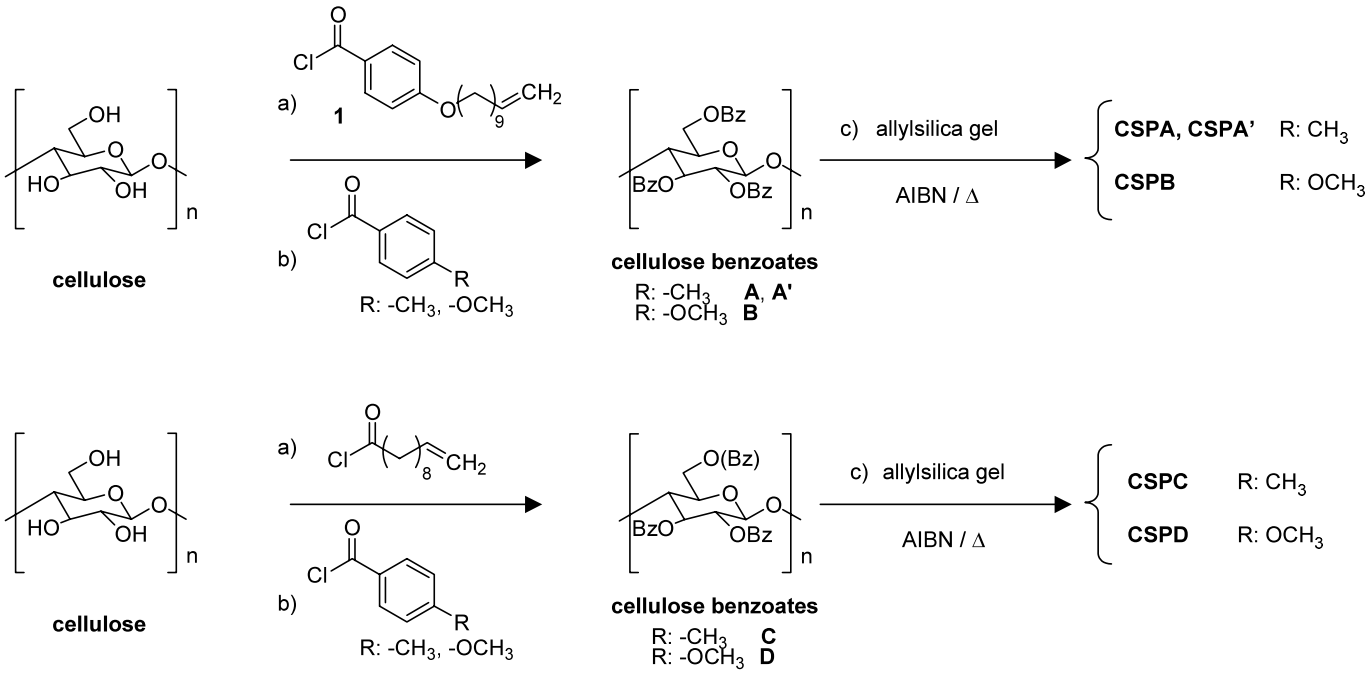


Scheme 1. (a) K_2CO_3, butanone, reflux; (b) KOH, EtOH/H_2O, 75°C; (c) Cl_2SO.

tion of the chromatographic results for a series of racemic compounds (Fig. 2) is presented in Table 2.

As a first approach, **CSPA** was prepared in order to be compared with the already described **CSPC** as cellulose derivative **C** was among the best chiral selectors previously studied.[7] Due to the known strong effect of the substituent position on enantioselectivity,[14] the fact of having a 4-substituted aromatic ring, as in the fixing substituent, was considered an important factor to keep a geometric regularity. In spite of the chemical differences between **A** and **C**, when the chromatographic behaviour of **CSPA** and **CSPC** was compared, no significant changes in enantioselectivity were observed.

Although cellulose derivative **A** had substitution degrees comparable to those of derivative **C** for both types of substituents, the former yielded a support with less cellulose content than the latter. The introduction of more fixing groups on cellulose derivative **A′** allowed the obtention of a CSP with a polymeric content comparable to that of **CSPC**. The higher number of alkenoxybenzoyl groups on the cellulose derivative (**CSPA′** versus **CSPA**) did not seem to have a negative influence on the chiral resolution properties, as it has been observed in previous studies with 10-undecenoyl derivatives. In these latter the increase in the number of fixing moieties on the polysaccharide resulted in an important decrease in enantioselectivity and resolution,[8,11] mainly attributable to the reticulation process undergone by these groups. However, the effect of this enhanced reticulation degree was not noticeable on **CSPA′**. Selectivity values are only slightly reduced on **CSPA′** regarding those on **CSPA** and **CSPC** (Fig. 3). Only compound **6** is affected by a considerable reduction of selectivity in this CSP (Fig. 4). This fact suggests a stabilisation of the secondary structure of the polysaccharide derivative in spite of the reticulation process.



Scheme 2. (a) and (b) pyridine, reflux; (c) 2% w/w AIBN, 2 h, 100°C, without solvent. A different amount of reagent **1** was used to obtain cellulose derivatives **A** and **A′**.

Table 1. Characterisation of cellulose derivatives and chiral stationary phases

Cellulose derivative	Analyses of cellulose derivatives		Substitution of glucose units[a]		Chiral supports	Analyses of the final chiral supports		g cellulose derivative/ 100 g phase[b]
	% C	% H	Unsaturat.	Ar		% C	% H	
A	69.26	5.91	0.23±0.05	2.37±0.16	**CSPA**	11.32	1.65	12.5
A′	71.45	6.80	0.88±0.12	1.98±0.25	**CSPA′**	14.08	2.00	15.9
B	64.41	5.40	0.20±0.05	2.51±0.25	**CSPB**	9.59	1.52	10.4
C	69.11	5.99	0.29±0.06	2.43±0.14	**CSPC**	14.19	1.65	16.2
D	64.87	5.70	0.22±0.05	2.55±0.21	**CSPD**	9.51	1.39	10.5

[a] The maximum substitution degree of a glucose unit being 3 (number of OH). Based on ^{1}H NMR and elemental analyses (calculations as reported in Ref. 13).

[b] Based on the elemental analyses of the chiral supports.

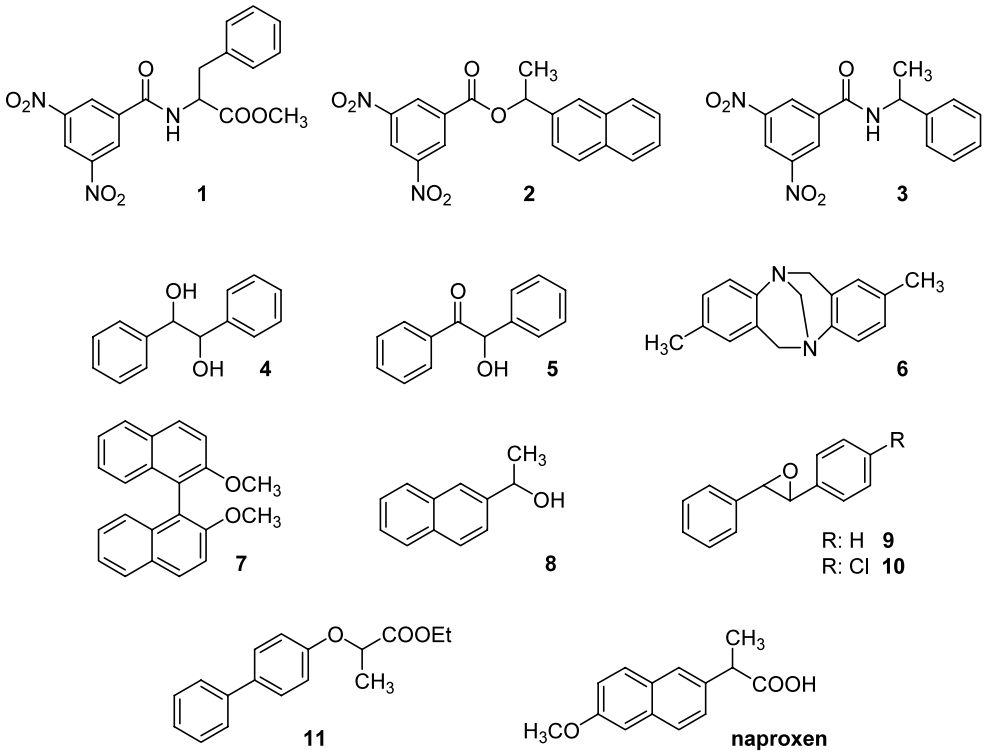


Figure 2. Chemical structures of the racemic test compounds.

Table 2. Chromatographic results

Racemic compd	CSPA′			CSPA			CSPC			CSPB			CSPD			Mobile phase
	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs	
1	8.05	1.15	0.79	6.59	1.13	0.19	4.98	1.18	0.82	12.3	1.00	–	8.49	1.00	–	80:20[a]
2	7.17	1.20	1.22	7.00	1.24	0.33	4.64	1.24	1.31	7.81	1.19	–	7.04	1.00	–	80:20[a]
3	4.77	1.09	–	3.91	1.00	–	3.06	1.10	–	6.65	1.00	–	5.18	1.00	–	80:20[a]
4	8.09	1.06	–	3.57	1.00	–	9.08	1.00	–	13.0	1.24	1.74	10.6	1.15	0.55	98:2[a]
5	3.92	1.19	2.05	3.53	1.21	1.54	3.48	1.23	1.90	4.56	1.18	1.27	3.73	1.19	0.64	98:2[a]
6	3.94	1.46	2.45	2.97	1.89	2.41	3.55	2.21	3.98	3.70	1.34	0.97	2.36	1.00	–	98:2[a]
7	2.79	1.38	1.69	2.29	1.31	–	2.17	1.29	0.85	1.67[d]	1.00	–	1.59	1.00	–	98:2[a]
8	5.22	1.14	1.72	4.64	1.20	1.65	4.71	1.21	2.21	5.85	1.12	0.96	4.79	1.10	0.31	98:2[a]
9	0.71	1.31	1.85	0.69	1.38	1.73	0.67	1.35	1.83	0.71	1.45	1.77	0.63	1.43	0.97	98:2[a]
10	0.89	1.54	3.50	0.78	1.66	2.95	0.74	1.67	2.94	0.79	1.64	2.33	0.63	1.66	1.19	98:2[a]
11	2.06	1.05	–	2.84	1.00	–	3.09	1.06	–	2.61	1.12	–	2.15	1.00	–	98:2[a]
Naproxen	10.0	1.11	1.31	10.4	1.10	–	7.70	1.08	0.87	12.2	1.12	–	11.2	1.13	–	98:2:0.5[b]
1	15.3	1.07	0.86	11.2	1.00	–	9.09	1.05	–	15.2	1.13	0.98	6.39	1.12	0.71	75:25[c]
2	5.46	1.16	1.72	4.24	1.23	1.33	4.82	1.23	2.03	7.83	1.31	1.35	3.35	1.33	1.20	90:10[c]
5	5.08[e]	1.04	–	6.40	1.04	–	6.71	1.04	–	8.82	1.09	–	7.06	1.00	–	95:5[c]
7	3.91	1.17	0.53	3.23	1.09	–	2.31	1.12	0.94	3.89	1.20	–	1.50	1.00	–	95:5[c]
9	0.78	1.20	1.23	1.95	1.13	–	0.52	1.19	0.77	0.87	1.38	1.50	0.47	1.00	–	95:5[c]
10	0.84	1.31	1.81	0.65	1.31	0.65	0.53	1.34	1.40	0.92	1.61	2.17	0.40	1.46	–	95:5[c]

Mobile phase: [a] Heptane/2-propanol, [b] Heptane/2-propanol/TFA, [c] Heptane/chloroform, [d] Heptane/2-propanol (90:10), [e] Heptane/chloroform (90:10).

The presence of the aromatic ring, together with a long enough aliphatic chain, contributes to the maintainance of the structural regularity of the chiral selector. Furthermore, the presence of a higher number of fixing groups led to an increase of cellulose derivative content in the resulting CSP, which might be responsible of the slight increase in retention for **CSPA′** compared to **CSPA**.

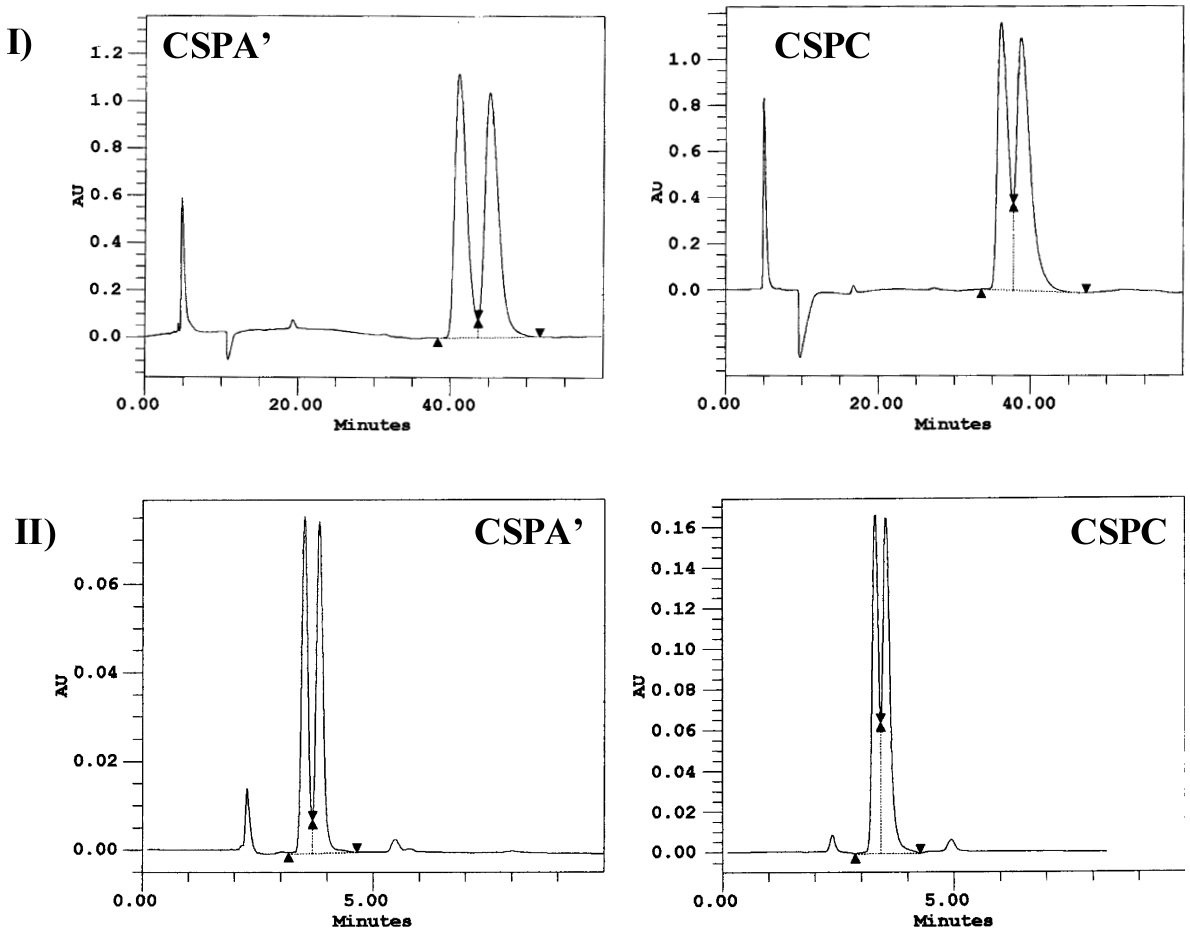


Figure 3. Resolution of (I) **naproxen** (mobile phase: heptane/2-propanol/TFA (98:2:0.5); flow: 0.5 mL/min; λ, 230 nm) and (II) **9** (mobile phase heptane/chloroform (95:5); Flow: 1 mL/min; λ, 254 nm).

In order to obtain polysaccharide derivatives with a higher uniformity in terms of structural features, but also of electronic properties of substituents, the preparation of two new cellulose derivatives **B** and **D** with a 4-alkoxyderivatization was undertaken. Both derivatives were prepared and bonded on allylsilica gel, although the percentage of cellulose on the CSPs was not higher than 10.5 (see Table 1). This lower cellulose content does not seem to be related to the bulkiness of the new fixing agent, as this phenomenon was observed for both derivatives. A reduced fixation degree was already observed for some 10-undecenoate/benzoates of cellulose[7] (i.e. 10-undecenoate/3,5-methoxybenzoate), which were fixed in a lower extent to the matrix than their corresponding methyl-substituted analogues.

When the chromatographic behaviours of **CSPB** and **CSPD** are compared, an improvement in the chiral recognition ability can be observed as a result of the introduction of an aromatic ring in the fixing substituent (Fig. 4). That is, when the electronic and geometric characteristics of the main derivatizing and the fixing agents are the same. This improvement is even more noticeable due to the low recognition ability shown by **CSPD** for some of the test compounds. When comparison was possible, **CSPD** showed notable similarities with the coated material described by Okamoto et al.[15] and prepared with homogeneously substituted 4-methoxybenzoate of cellulose.

In general terms, the improvements in selectivity achieved with **CSPA**/**A**′ and **CSPB** compared to **CSPC** and **CSPD**, respectively, allowed us to make an evaluation of the effect of the use of 10-undecenoyl groups as fixing substituents on selectivity. Although the existence of a negative effect is shown by the obtained data, its relevance depends on the derivative considered. Nevertheless, the ready availability and ease of use of 10-undecenoyl chloride make it still a suitable choice for the fixation of polysaccharide derivatives on chromatographic matrices.

3. Experimental

^{1}H NMR spectra were recorded on a Varian (Palo Alto, CA) GEMINI-300 Spectrometer with pyridine-d_5 at 70°C. Elemental analyses were performed in a CE Instruments apparatus (model EA 1108, Carlo Erba, Milan, Italy) using standard conditions by the Serveis Científico-Tècnics de la Universitat de Barcelona (Spain). The CSPs were packed into stainless-steel tubes

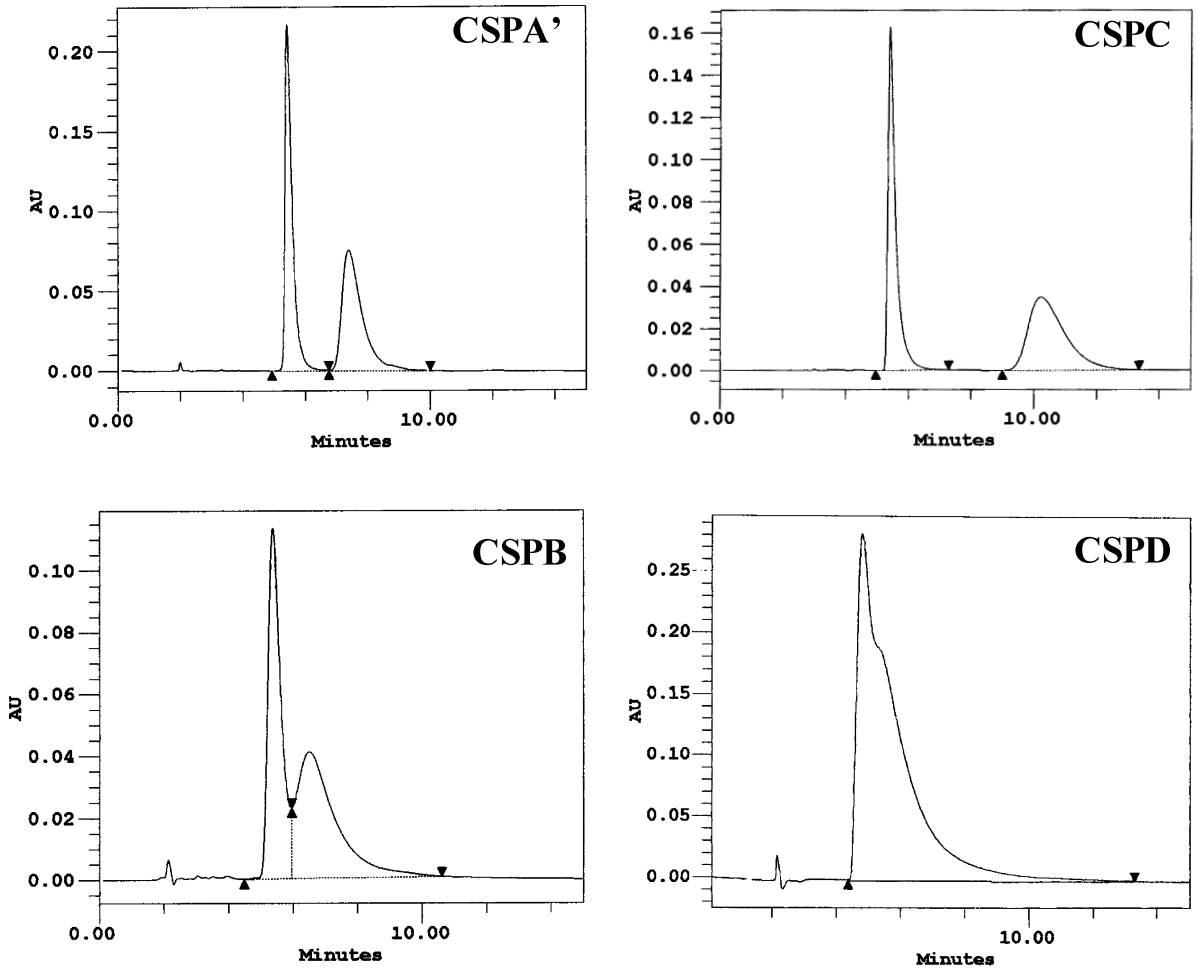


Figure 4. Resolution of **6** using heptane/2-propanol (90:10) as mobile phase (Flow: 1 mL/min; λ, 230 nm).

(150×4.6 mm I.D.) by the slurry method. The chromatographic experiments were performed on an HPLC system consisting of a Waters 600E pump, a Waters 717 autosampler (Millipore, Milford, MA, USA) and equipped with a Waters 996 photodiode 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.

Avicel® and 4-methoxybenzoic acid were purchased from Merck (Darmstadt, Germany). Tosyl chloride (TsCl), 10-undecen-1-ol, 10-undecenoyl chloride, ethyl 4-hydroxybenzoate, thionyl chloride, α,α′-azoisobutyronitrile (AIBN) and 4-methylbenzoic acid were supplied by Fluka (Buchs, Switzerland). Nucleosil 100-5 was a product from Macherey-Nagel (Düren, Germany).

3.1. Preparation of the cellulose derivatives A, A′ and B

Cellulose (Avicel®, 1 g, 6.2 mmol of glucose units) suspended in dry pyridine (50 mL) and 4-(10-undecenyloxy)benzoyl chloride **1** in different proportions were stirred under reflux. Reaction times and molar ratios of reagents are indicated in Table 3. The appropriate benzoyl chloride was added dropwise (4-methylbenzoyl chloride for **A** and **A′**, or 4-methoxybenzoyl chloride for **B**) and the mixture was refluxed (Table 3). The resulting products were isolated as the insoluble fraction in methanol. They were redissolved in chloroform, reprecipitated in methanol and thoroughly washed in this solvent. All derivatives were characterised by their ^{1}H NMR spectra and elemental analyses (Table 1).

Table 3. Molar ratio of reagents and reaction times for the preparation of the cellulose benzoates

Cellulose derivative	Fixing reagent[a]		Benzoate reagent[b]	
	M[c]	t[d] (h)	M[c]	t[d] (h)
A	0.4	24	5.0	48
A′	1.1	4	3.0	24
B	0.4	24	4.0	48
C	0.4	2	4.0	16
D	0.4	2	4.0	16

[a] Compound **1** for **A**, **A′** and **B**; 10-undecenoyl chloride for **C** and **D**.
[b] 4-Methylbenzoyl chloride for **A**, **A′** and **C**; 4-methoxybenzoyl chloride for **B** and **D**.
[c] M = mol of reagent/mol glucose units.
[d] Time of reaction in pyridine at reflux temperature.

4-(10-Undecenyloxy)benzoate/4-methylbenzoate of cellulose **A** ^{1}H NMR (300 MHz, pyridine-d_5, 70°C): δ 1.2–1.9 (m, $C^{3'}H_2$-$C^{8'}H_2$); 2.03, 2.08 and 2.31 (3s+m, 2-Ar-CH_3, 3-Ar-CH_3, 6-Ar-CH_3 and $C^{9''}H_2$); 3.72 (m, $C^{2'}H_2$ and C^5H); 4.12 (m, C^4H); 4.45 (m, C^6H_2); 4.9–5.2 (m, C^1H and $C^{11'}H_2$); 5.62 (m, C^2H); 5.90 (m, C^3H and $C^{10'}H$); 6.80, 7.01 and 7.19 (3d, 2-$C^{3'',5''}H$, 3-$C^{3'',5''}H$ and 6-$C^{3'',5''}H$); 7.84, 7.94 and 7.99 (3d, 2-$C^{2'',6''}H$, 3-$C^{2'',6''}H$ and 6-$C^{2'',6''}H$).

4-(10-Undecenyloxy)benzoate/4-methoxybenzoate of cellulose **B** ^{1}H NMR (300 MHz, pyridine-d_5, 70°C): δ 1.2–1.9 (m, $C^{3'}H_2$-$C^{8'}H_2$); 2.12 (m, $C^{9'}H_2$); 3.56 (s, 2-$ArOCH_3$ and 3-$ArOCH_3$); 3.70 (m, $C^{2'}H_2$ and C^5H); 3.80 (s, 6-$ArOCH_3$); 4.12 (m, C^4H); 4.55 (m, C^6H_2); 4.9–5.2 (m, C^1H and $C^{11'}H_2$); 5.65 (m, C^2H); 5.95 (m, C^3H and $C^{10'}H$); 6.61, 6.78 and 6.99 (3d, 2-$C^{3'',5''}H$, 3-$C^{3'',5''}H$ and 6-$C^{3'',5''}H$); 7.93, 8.06 and 8.08 (3d, 2-$C^{2'',6''}H$, 3-$C^{2'',6''}H$ and 6-$C^{2'',6''}H$).

3.2. Preparation of the cellulose derivatives C and D

Derivatives **C** and **D** were synthesised analogously to **A** and **B** by the reaction of cellulose (Avicel®, 1 g, 6.2 mmol of glucose units) with 10-undecenoyl chloride (2.3 mmol) and 22.3 mmol of the appropriate benzoyl chloride (4-methylbenzoyl chloride for **C**, or 4-methoxybenzoyl chloride for **D**), as described elsewhere.[7]

10-Undecenoyl/4-methoxybenzoate of cellulose **D** ^{1}H NMR (300 MHz, pyridine-d_5, 70°C): δ 0.8–2.40 (m, $C^{2'}H_2$-$C^{9'}H_2$); 3.56 and 3.57(s+s, 2-$ArOCH_3$ and 3-$ArOCH_3$); 3.70 (m, C^5H); 3.80 (s, 6-$ArOCH_3$); 4.20 (m, C^4H); 4.51 (m, C^6H_2); 4.9–5.2 (m, C^1H and $C^{11'}H_2$); 5.65 (m, C^2H); 5.95 (m, C^3H and $C^{10'}H$); 6.61, 6.78 and 6.99 (3d, 2-$C^{3'',5''}H$, 3-$C^{3'',5''}H$ and 6-$C^{3'',5''}H$); 7.93, 8.06 and 8.08 (3d, 2-$C^{2'',6''}H$, 3-$C^{2'',6''}H$ and 6-$C^{2'',6''}H$).

3.3. Preparation of the chiral stationary phases (CSPs)

A 2 g amount of allylsilica gel, prepared as previously described,[7] were added to a solution of 0.4 g of the appropriate cellulose derivative in 20 mL of chloroform, containing a 2% w/w of AIBN (α,α'-azobisisobutyronitrile). After evaporation of the solvent at room temperature, the solid material was allowed to react for 2 h at 100°C. The CSP thus obtained was suspended in chloroform and heated to the refluxing temperature for 2 h. The resulting suspension was filtered off and the solid washed with chloroform, tetrahydrofuran and acetone. The materials thus obtained were characterised by their elemental analyses (Table 1).

Acknowledgements

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