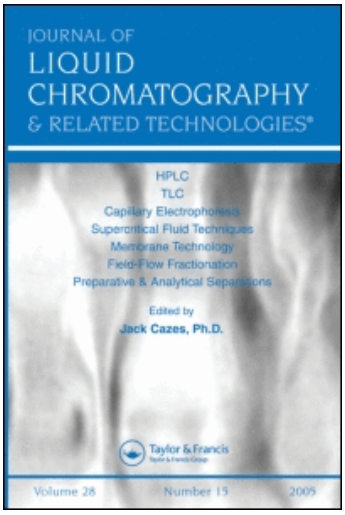


## Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Chiral Chromatographic Discrimination Ability of a Cellulose 3,5-Dimethylphenylcarbamate/10-Undecenoate Mixed Derivative Fixed on Several Chromatographic Matrices

L. Oliveros[a]; P. Lopez[a]; C. Minguillon[b]; P. Franco[b]

[a] Laboratoire de Chimie Générale Conservatoire National des Arts et Métiers 292, Paris Cédex 03, France [b] Laboratori de Química Farmacèutica Facultat, de Farmàcia Universitat de Barcelona Avd., Barcelona, Spain

**To cite this Article** Oliveros, L. , Lopez, P. , Minguillon, C. and Franco, P.(1995) 'Chiral Chromatographic Discrimination Ability of a Cellulose 3,5-Dimethylphenylcarbamate/10-Undecenoate Mixed Derivative Fixed on Several Chromatographic Matrices', Journal of Liquid Chromatography & Related Technologies, 18: 8, 1521 — 1532

**To link to this Article: DOI:** 10.1080/10826079508009292

**URL:** http://dx.doi.org/10.1080/10826079508009292

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(8), 1521-1532 (1995)

# CHIRAL CHROMATOGRAPHIC DISCRIMINATION ABILITY OF A CELLULOSE 3,5-DIMETHYL-PHENYLCARBAMATE/10-UNDECENOATE MIXED DERIVATIVE FIXED ON SEVERAL CHROMATOGRAPHIC MATRICES#

**L. OLIVEROS[1]*, P. LOPEZ[1], C. MINGUILLON[2], AND P. FRANCO[2]**

[1]*Laboratoire de Chimie Générale*
*Conservatoire National des Arts et Métiers*
*292, rue Saint-Martin*
*75141 Paris Cédex 03, France*
[2]*Laboratori de Química Farmacèutica*
*Facultat de Farmàcia*
*Universitat de Barcelona*
*Avd. Diagonal s/n*
*08028 Barcelona, Spain*



## ABSTRACT

The properties as chiral selector in HPLC chiral stationary phases (CSPs) of a cellulose derivative bearing simultaneously 3,5-dimethylphenylamino carbonyl and 10-undecenoyl groups are described. This polysaccharide is reticulated (or bonded) on chromatographic supports such as silica gel, previously treated or not, graphite or alumina. The chiral stationary phases thus obtained are resistant to the usual solvents used in liquid chromatography and can be used on normal or reversed phase conditions.

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# Presented at the 5th International Symposium on Chiral Discrimination (Stockholm, October 1994).

The results obtained on the resolution of racemic compounds with these stationary phases depend on the nature of the support. The best results were obtained with the chiral stationary phase in which the cellulose derivative is bonded on allyl silica gel.

## INTRODUCTION

Lactose, starch and cellulose are the oldest chiral chromatographic supports used in the resolution of racemic compounds[1]. Regarding polysaccharide derivatives, the first use of cellulose acetate as stationary phase was reported in 1967[2]. However, the development of polysaccharide derivatives in chiral HPLC was marked by the studies of Hesse and Hagel, published in 1973, on cellulose triacetate[3]. These authors show that the secondary structure of the polymer has an important role in the enantioselectivity of the stationary phase.

Since 1984 Okamoto et al. described the preparation of chiral stationary phases (CSPs) for HPLC constituted by macroporous γ-aminopropylsilica gel on which phenylcarbamates of cellulose and other polysaccharides had been adsorbed[4,5]. These CSPs are now widely used because of their ability in the resolution of a very large range of racemic compounds, and they are commercially available[6]. Nevertheless, the chiral selector in these CSPs is soluble in a number of organic solvents. This solubility limits the choice of eluant.

Okamoto and co-workers described the preparation of stationary phases in which the phenylcarbamates of cellulose are not absorbed but bonded to silica gel[7,8]. Although the optical resolving power of these CSPs is, in certain cases, slightly lower than that of the same cellulose derivatives absorbed on silica gel, their enantioselectivity is still good enough. In spite of the advantage of their stability in the presence of solvents, these bonded CSPs are not commercially available. This is probably a consequence of the relative complexity of the preparation described for these CSPs.

In this study the chromatographic behavior of CSPs whose chiral selector is a cellulose derivative, which is resistant to solvents usually used in liquid chromatography, is described. This property has been obtained from a cellulose derivative in which the glucose units bear 3,5-

dimethylphenylaminocarbonyl and 10-undecenoyl groups at the same time. This compound can undergo reticulation on chromatographic supports (silica gel, modified silica gel, graphite, alumina). When the matrix used is allyl silica gel this reticulation results in the covalent bonding of the chiral selector to the matrix surface. The characteristics and the performances of the resulting CSPs are discussed.

## EXPERIMENTAL SECTION

Elemental analyses were performed by the Service Central de Microanalyses du CNRS (Vernaison, France). The chromatographic experiments were performed on an HPLC system consisting of a Waters 600E pump, a Waters 717 auto sampler (Millipore, Milford, MA, USA) and equipped with a Waters 996 photo diode array detector and a Perkin-Elmer 241LC polarimetric detector (Perkin-Elmer, Uberlingen, Germany). The chiral stationary phases were packed into stainless-steel tubes (150 x 4.6 mm ID) by the slurry method. The volume of sample injected was 1 ml. The flow-rate of the pump was 1 ml/min. The detection wavelength was 254 nm. The void volume in normal phase conditions was determined using tri-tert-butylbenzene.



### Cellulose Derivative and Chiral Stationary Phases

The chiral selector and the stationary phases have been prepared as it is indicated on Figure 1[9]. The elemental analysis for the cellulose derivative was C 67.37, H 7.62, N 3.78%. This analysis corresponds to a cellulose derivative having 1 10-undecenoyl group and 1.6 3,5-dimethylphenylaminocarbonyl groups for each glucose unity. The same result was obtained from the $H^1$-NMR data.

## RESULTS AND DISCUSSION

The cellulose derivative was fixed on allyl silica gel (**A**), "end capped" silica gel (**B**), non-treated silica gel (**C**), alumina (**D**) and graphite (**E**) in a

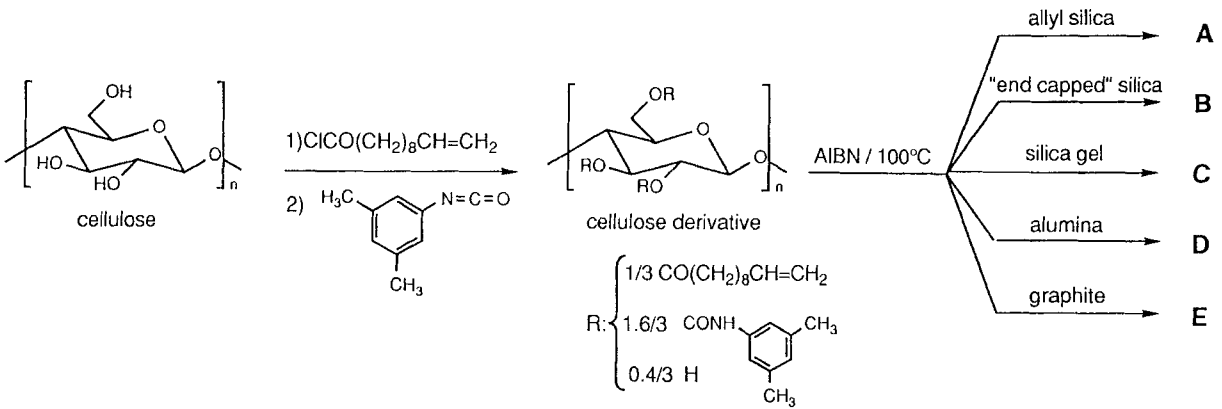


**FIGURE 1**
Preparation of Chiral Stationary Phases

radical reaction by means of the C-C double bonds on the undecenoyl group. The fixation process took place either by polymerization of the cellulose derivative with groups able to react on the matrix surface (allyl, in CSP **A**), or by reticulation of the 10-undecenoyl groups of the cellulose derivative itself on the matrix surface. The fixation resulted in the insolubilization of the cellulose derivative, which was soluble in chloroform before the treatment but was not soluble, even in boiling chloroform, after the reaction.

The elemental analyses of the resulting CSPs, all of which were obtained under the same experimental conditions, are given in Table 1. The nitrogen percentages, and therefore the amount of chiral selector fixed by stationary phase unit mass is almost the same for all supports and independent of the matrix.

In Tables 2 to 4, several chromatographic results obtained with these CSPs are presented. As a result of the fixation process, these CSPs were eluted with heptane/chloroform mixtures (Table 2). Moreover, the same column can be used on reverse and normal phase conditions, and changed from one to the other with the only precaution of using perfectly miscible solvents . However, all CSPs showed better enantioselectivity in normal phase conditions than in reversed phase conditions. CSPs **B** (end capped silica gel as a matrix) and **E** (graphite as a matrix) showed high retention times against several racemic compounds in normal phase conditions. However, the selectivity did not improve when they were used in reverse phase (ACN/water, ACN/$NaClO_4$, ...). Generally, in the resolution of racemic compounds **1** to **9**, the enantioselectivity was slightly



**TABLE 1**
Elemental Analyses of the Chiral Supports

| CSP | %C | %H | %N |
|---|---|---|---|
| **A** | 15.58 | 1.80 | 0.94 |
| **B** | 12.65 | 2.14 | 0.71 |
| **C** | 12.84 | 2.02 | 0.90 |
| **D** | 12.44 | 1.83 | 0.84 |
| **E** | 93.19 | 1.36 | 0.85 |

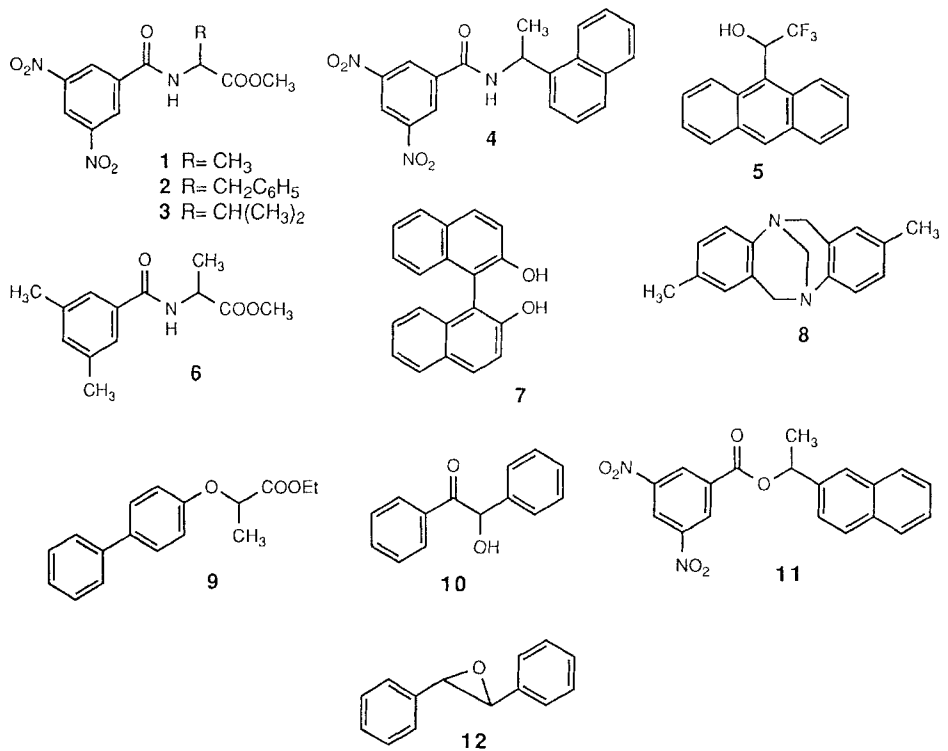


**FIGURE 2**
Chemical Structures of Racemic Test Compounds

**TABLE 2**
Chromatographic Results Obtained with Chiral Stationary Phases **B**, **C**, **D** and **E**.

| RACEMIC COMPOUNDS | B | | C | | D | | E | | mobile phase Heptane/$Cl_3CH$ |
|---|---|---|---|---|---|---|---|---|---|
| | $k'_1$ | $\alpha$ | $k'_1$ | $\alpha$ | $k'_1$ | $\alpha$ | $k'_1$ | $\alpha$ | |
| **1** | 1.81 | 1.15 | 5.64 | 1.05 | 3.45 | 1.00 | 4.04 | 1.14 | 25:75 |
| **2** | 0.98 | 1.12 | 2.24 | 1.00 | 1.85 | 1.00 | 3.52 | 1.00 | 25:75 |
| **3** | 0.76 | 1.20 | 1.79 | 1.09 | 1.33 | 1.00 | 1.83 | 1.18 | 25:75 |
| **4** | 3.88 | 1.27 | >15* | - | 7.53 | 1.19 | >15* | - | 50:50 |
| **5** | 1.77 | 1.99 | >15* | - | 5.52 | 1.76 | >15* | - | 50:50 |
| **6** | 1.31 | 1.00 | 2.91 | 1.00 | 2.12 | 1.12 | 1.14 | 1.00 | 75:25 |
| **7** | 0.97 | 1.18 | 3.69 | 1.05 | >15* | - | 1.44 | 1.21 | 50:50 |
| **8** | 2.94 | 1.00 | 2.53 | 1.00 | 2.76# | 1.17 | 1.19# | 1.18 | 75:25 |
| **9** | 0.72 | 1.17 | 1.06 | 1.13 | 1.31 | 1.25 | 1.22 | 1.11 | 90:10 |

$k'_1$, Capacity factor for the first eluted enantiomer; $\alpha$, selectivity factor. Column: 15 cm x 0.46; flow rate: 1ml/min.
* When 100% chloroform was used as mobile fase. # Mobile phase: 90:10 Heptane/$Cl_3CH$.

**TABLE 3**
Chromatographic Results Obtained with Chiral Stationary Phase **A**

| RACEMIC COMPOUNDS | Heptane/Chloroform | | | Heptane/2-Propanol | | | Acetonitrile/Water | |
|---|---|---|---|---|---|---|---|---|
| | k'1 | α | Rs | k'1 | α | Rs | k'1 | α |
| 1 | 2.27[a] | 1.23 | 1.33 | 12.68[a] | 1.12 | 1.00 | 5.98[a] | 1.00 |
| 2 | 1.35[a] | 1.22 | 1.06 | 8.91[b] | 1.20 | 1.48 | 4.78[b] | 1.00 |
| 3 | 1.00[a] | 1.26 | 1.12 | 4.61[b] | 1.00 | - | 12.19[a] | 1.00 |
| 4 | 1.47[a] | 1.23 | 1.19 | 6.12[b] | 1.28 | 1.88 | 8.94[b] | 1.08 |
| 5 | 1.30[a] | 2.05 | 4.61 | 0.87[b] | 1.73 | 2.97 | 5.08[b] | 1.12 |
| 6 | 2.01[b] | 1.10 | - | 2.42[a] | 1.24 | 1.74 | 4.29[a] | 1.09 |
| 7 | 1.54[c] | 1.24 | 1.49 | 2.96[a] | 1.16 | 1.21 | 13.58[a] | 1.00 |
| 8 | 4.12[b] | 1.00 | - | 1.10[b] | 1.12 | - | 12.77[a] | 1.04 |
| 9 | 1.09[d] | 1.18 | - | 0.65[a] | 1.25 | 1.20 | 6.18[b] | 1.00 |
| 10 | 5.54[d] | 1.32 | 1.23 | 2.03[a] | 1.22 | 1.70 | 5.58[a] | 1.08 |
| 11 | 7.55[d] | 1.09 | - | 5.42[a] | 1.07 | - | 17.89[b] | 1.00 |
| 12 | 1.00[d] | 1.75 | 2.06 | 0.64[a] | 1.27 | 1.30 | 6.63[b] | 1.00 |
| | a) 25:75 Heptane/$Cl_3CH$<br>b) 75:25 Heptane/$Cl_3CH$<br>c) 50:50 Heptane/$Cl_3CH$<br>d) 90:10 Heptane/$Cl_3CH$ | | | a) 90:10 Heptane/2-PrOH<br>b) 80:20 Heptane/2-PrOH | | | a) 40:60 ACN/$H_2O$<br>b) 60:40 ACN/$H_2O$ | |

$k'_1$, Capacity factor for the first eluted enantiomer; α, selectivity factor; Rs, resolution. Column: 15 cm x 0.46; flow rate: 1ml/min.

**TABLE 4**
Chromatographic Results Obtained with Chiral Stationary Phase **A**

| RACEMIC COMPOUNDS | Heptane/2-Propanol | | | Acetonitrile/Water | | | Acetonitrile/Phosphate buffer | |
|---|---|---|---|---|---|---|---|---|
| | k'1 | α | Rs | k'1 | α | Rs | k'1 | α |
| TEMAZEPAM | 12.88[a] | 1.13 | - | 5.92[a] | 1.17 | 1.60 | | |
| LORMETAZEPAM | 16.89[a] | 1.00 | - | 7.12[a] | 1.27 | 2.48 | | |
| OXAZEPAM | 9.90[a] | 1.27 | 1.76 | 3.88[a] | 1.25 | 2.19 | | |
| LORAZEPAM | 9.84[a] | 1.64 | 3.48 | 3.76[a] | 1.28 | 2.43 | | |
| WARFARIN | 4.85[a] | 1.81 | 3.03 | 2.97[a] | 1.17 | - | | |
| VERAPAMIL | 2.21[b] | 1.00 | - | 5.38[b] | 1.03 | - | 7.28[a] | 1.00 |
| BENDROFLUMETIAZIDE | 8.34[b] | 1.17 | - | | | | 4.24[a] | 1.00 |
| METOPROLOL | 1.61[b] | 1.24 | 1.22 | 4.91[c] | 1.00 | - | 0.90[a] | 1.13 |
| ATENOLOL | 9.78[b] | 1.14 | - | 0.74[c] | 1.00 | - | 0.11[a] | 1.00 |
| PROPANOLOL | 1.91[b] | 1.18 | - | 2.39[b] | 1.04 | - | 2.99[a] | 1.00 |
| NADOLOL | 3.43[b] | 1.36 | 1.25 | 1.21[c] | 1.00 | - | 0.50[b] | 1.00 |
| PINDOLOL | 5.51[b] | 2.84 | 5.35 | 6.83[c] | 1.36 | 1.30 | 2.60[b] | 1.23 |
| NAPROXEN | 4.48[c] | 1.16 | 1.31 | | | | 5.39[b] | 1.00 |
| IBUPROFEN | 1.19[c] | 1.09 | - | | | | 12.09[b] | 1.00 |
| | a) 90:10 Hept./2-PrOH<br>b) 80:20:0.1 Hept./2-PrOH/DEA<br>c) 98:2:0.1 Hept./2-PrOH/TFA | | | a) 40:60 ACN/$H_2O$<br>b) 40:60 ACN/$NaClO_4$ 0.1M<br>c) 20:80 ACN/$NaClO_4$ 0.1M<br>b,c) flow rate: 0.5 ml/min | | | a) 40:60 ACN/Phosphate buffer 0.03M pH: 5.8<br>b) 20:80 ACN/Phosphate buffer 0.03M pH: 5.8 | |

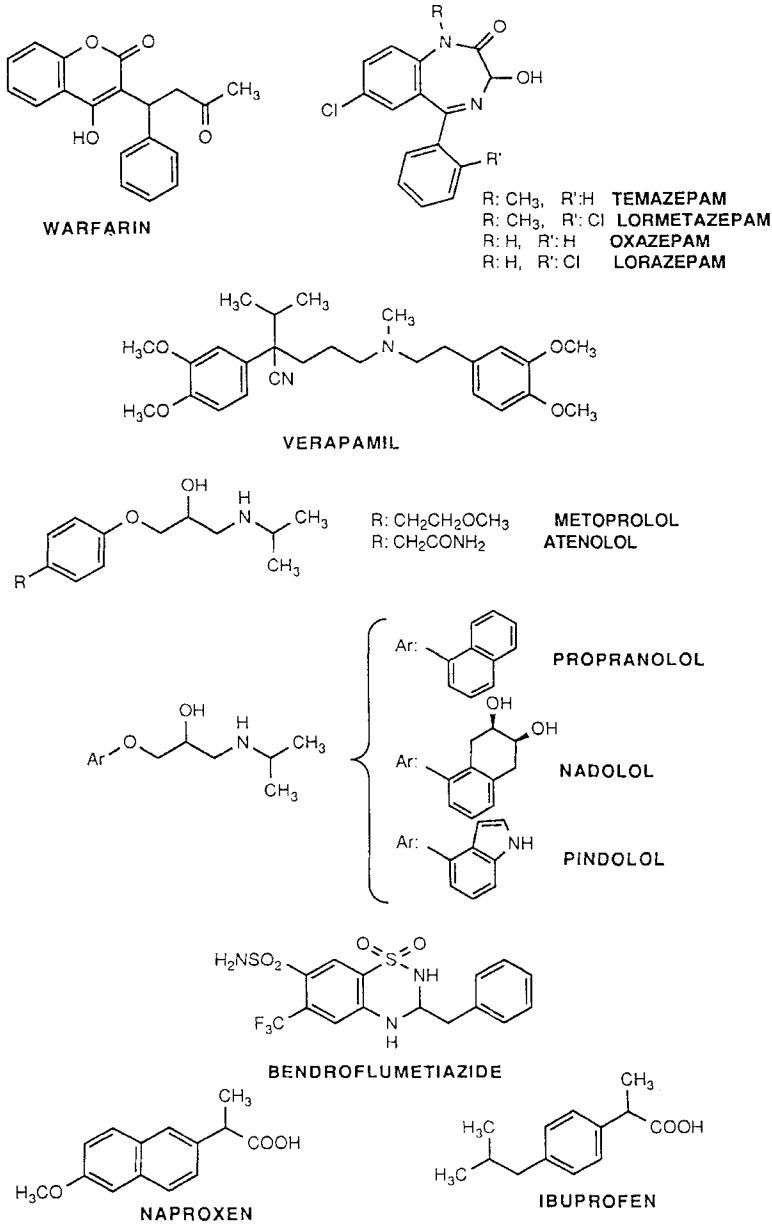


**FIGURE 3**
Chemical Structures of Racemic Drugs (Table 4)

better when heptane/chloroform was used as mobile phase rather than heptane/2-propanol The best results were obtained with CSP **A** (Table 3).

In Table 4, the resolution of several racemic drugs including benzodiazepines, aminoalcohols and arylpropionic acids, under normal (heptane/2-propanol) and reverse phase conditions (ACN/water, ACN/$NaClO_4$, ACN/phosphate buffer), is presented.

## CONCLUSION

The method described here for the preparation of polysaccharide chiral stationary phases allows the fixation of the chiral selector, a 3,5-dimethylphenyl/10-undecenoyl mixed derivative of cellulose, on all kinds of chromatographic matrices. The resulting CSPs are resistant to usual HPLC solvents and conditions.

Although several variables have not yet been optimized, such as the nature of the starting polysaccharide derivative, the ratio of reagents or the order of introduction of these to the polysaccharide (these will be the object of forthcoming papers), the results presented here are promising.



## ACKNOWLEDGEMENTS

Financial support from the Comisión Interministerial de Ciencia y Tecnologia and from the Generalitat de Catalunya (Project n. QFN91-4201) of Spain is acknowledged.

P. L. Present address: Instituto de Ciencia de Materiales de Aragón, Facultad de Ciencias, Universidad de Zaragoza-CSIC, 50009-Zaragoza (Spain).

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Received: November 11, 1994
Accepted: November 29, 1994