# Cellulose 3,5-Dimethylphenylcarbamate Immobilized onto Silica Gel via Copolymerization with a Vinyl Monomer and Its Chiral Recognition Ability as a Chiral Stationary Phase for HPLC

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A new cellulose phenylcarbamate derivative having a vinyl group at the 6-position was synthesized and immobilized onto silica gel via radical copolymerization with styrene. The immobilization was efficiently attained using a small amount of styrene. The chiral recognition abilities of the immobilized chiral stationary phase (CSP) were similar to those of the CSP coated on silica gel.

Phenylcarbamate derivatives of cellulose and amylose are known to show a high chiral recognition as chiral stationary phases (CSPs) in high-performance liquid chromatography (HPLC).[1,2] Particularly, cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC, **1**) (Figure 1) has been widely used to separate a broad range of racemates. The CSPs are usually

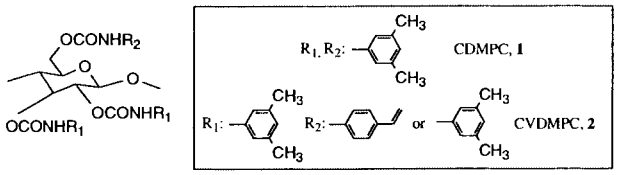


**Figure 1**. Structures of CSPs for HPLC.

prepared by coating the polysaccharide derivatives on macroporous silica gel;[1b] therefore, the solvents such as tetrahydrofuran (THF) and chloroform, which dissolve or swell the polysaccharides, cannot be added to the mobile phase. To overcome this defect, we prepared the CSPs in which the polysaccharide phenylcarbamates were randomly[3] and regioselectively[4] chemically bonded to 3-aminopropyl silica gel with diisocyanates through the hydroxy groups of the polysaccharides. However, these bonded CSPs showed a slightly lower chiral recognition ability, particularly at a high chemical bond content, than the corresponding coated CSPs. We also prepared the amylose tris(3,5-dimethylphenylcarbamate) CSP chemically bonded to silica gel only at the reducing terminal residue of amylose by the enzymatic polymerization.[5] This bonded CSP showed a resolving power similar to that of the coated CSP and can be used with solvents such as THF and chloroform. However, this technique cannot be applied to the cellulose derivatives. Kimata and co-workers prepared a bonded CSP via the polymerization of cellulose *p*-vinylbenzoate on acrylamide-immobilized silica gel.[6] This bonded CSP seems to show a slightly lower chiral selectivity than the corresponding coated CSP, and this technique has not yet been applied to other polysaccharide derivatives. Oliveros and Minguillón prepared the immobilized CSPs via the polymerization of cellulose, amylose, and chitosan derivatives having both 10-undecenoylcarboxylate and 3,5-dimethylphenylcarbamate groups on the allyl-silica gel.[7] They observed relatively high enantioselectivities with the cellulose and chitosan derivatives. However, the immobilization degree of the polysaccharide derivatives on silica gel was not high probably due to the low reactivity of the 10-undecenoyl group, and the amylose derivatives CSPs showed a large deterioration in enantioseparation ability. Francotte and co-workers prepared the immobilized CSPs via photochemical cross-linking of polysaccharide derivatives having no photopolymerizable functional groups.[8] Although this technique may be simple, the details of the process are not clear.

In the present study, in order to develop an efficient immobilization method without changing the enantioseparation ability, we prepared a cellulose 3,5-dimethylphenylcarbamate derivative bearing about a 30% *p*-vinylphenylcarbamate moiety at the 6-position of a glucose ring (CVDMPC, **2**), and found that it was efficiently immobilized on silica gel via radical copolymerization with styrene.

The cellulose derivative **2** bearing a vinyl group was synthesized as shown in Figure 2.[9] The ratio of the substituents at the 6 position was determined to be about 7:3 ((3,5-dimethylphenylcarbamate):(4-vinylphenylcarbamate)) by $^1$H NMR spectroscopy.

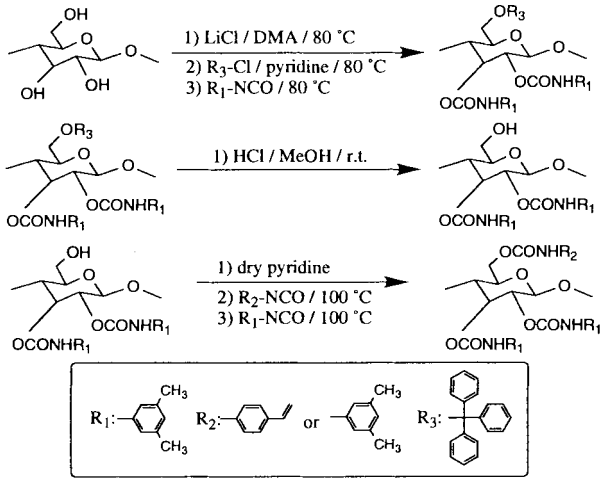


**Figure 2**. Schemes of the synthesis of **2**.

The **2**-coated silica was prepared with a THF solution of **2** (0.75 g/10 mL) and 3-aminopropyl macroporous silica gel (Daisogel, particle size 7 μm, pore size 100 nm) according to previous methods.[1b] A hexane solution of styrene and azobisisobutyronitrile (2 mol% against the total amount of vinyl groups) was added to the **2**-coated silica, and then the cellulose

derivative was immobilized by the copolymerization with styrene at 60 °C for 20 h. The **2**-immobilized silica was washed with THF and then dried. The amount of the immobilized **2** was estimated by $^1$H NMR analysis of the THF wash solution using an internal standard.

The **2**-bonded silica gel was packed in a stainless-steel tube (25 cm × 0.20 cm or 0.46 cm (i.d.)). The plate numbers of the columns were about 2000 (0.20 cm (i.d.)) or 6000 (0.46 cm (i.d.)) for benzene using hexane–2-propanol (90:10) as the eluent at a flow rate of 0.1 or 0.5 mL/min. Tri-*t*-butylbenzene was used as the non-retained compound to estimate the dead time ($t_0$).[10]

During the immobilization process, most of the coated cellulose derivative **2** was fixed on the silica surface when the amount of styrene ranged from 10 to 50 wt% against **2** (Table 1). However, when the styrene was reduced to 5 wt%, the amount of **2** immobilized on the silica became 86%. Without styrene, only 50% of **2** was immobilized on the surface under the same conditions. These results indicate that styrene can significantly contribute to the efficient immobilization of the cellulose derivative on silica gel.

**Table 1.** Separation factors (α) on cellulose phenylcarbamates[a]

| Styrene / **2** | 50 wt% | 30 wt% | 10 wt% | 5 wt% | Coated type | |
|---|---|---|---|---|---|---|
| Immobilized **2** | >99% | >99% | 99% | 86% | **1** | **2** |
| **3** | 1.53(+) | 1.60(+) | 1.68(+) | 1.45(+) | 1.32(+) | 1.34(+) |
| **4** | 1.23(+) | 1.17(+) | ca. 1(+) | 1.33(–) | 1.68(–) | 1.51(–) |
| **5** | 1.14(+) | 1.16(+) | 1.18(+) | 1.34(+) | 1.58(+) | 1.35(+) |
| **6** | ca. 1(+) | ca. 1(+) | 1.12(+) | ca. 1(+) | 1.34(+) | ca. 1(+) |
| **7** | 1.31(–) | 1.31(–) | 1.32(–) | 1.22(–) | 1.15(–) | 1.19(–) |
| **8** | 1.73(–) | 1.81(–) | 1.96(–) | 2.18(–) | 2.57(–) | 2.27(–) |
| **9** | ca. 1(+) | ca. 1(+) | 1.32(+) | 1.17(+) | ca. 1(+) | 1.13(+) |
| **10** | ca. 1(–) | 1.08(–) | 1.13(–) | 1.22(–) | 1.41(–) | 1.28(–) |
| **11** | ca. 1(+) | ca. 1(+) | ca. 1(+) | 1.42(+) | 2.17(+) | 1.67(+) |
| **12** | 2.63(–) | 2.76(–) | 3.20(–) | 2.57(–) | 1.83(–) | 2.16(–) |

[a]Capacity factor with optical rotation (in parentheses) of the first-eluted enantiomer ($k_1'$) = ($t_1$-$t_0$)/$t_0$ and separation factor (α) = $k_2'/k_1'$, where $t_1$ and $t_2$ are retention time of the first-eluted enantiomer and second eluted enantiomer, respectively. Eluent; hexane–2-propanol (90:10).

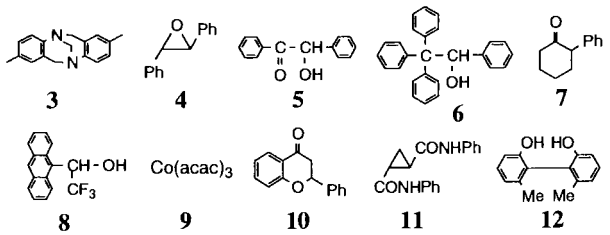


**Figure 3**. Structures of racemates.

The resolution results of ten racemates (**3**–**12**) (Figure 3) on the immobilized CSPs are given in Table 1 together with the data for the coated **1** and **2**. The coated CSP **2** shows a slightly higher chiral selectivity for some racemates (**3**, **7**, **9**, **12**), but a slightly lower one for the other racemates than the coated CSP **1**. The elution order of the racemates was always consistent. As the content of styrene to **2** decreased from 50 to 10 wt%, the value of the capacity factor, $k_1'$, increased. For example, the $k_1'$ values of racemate **3** were 0.63, 0.75, and 0.88 on the CSPs containing 50, 30, and 10 wt% styrene, respectively. The styrene residues existing near the cellulose derivatives may disturb the interaction between the enantiomers and cellulose derivatives. When the ratio of styrene to **2** was reduced to 5 wt%, the $k_1'$ value also decreased due to the decrease in the amount of immobilized **2**. The $k_1'$ value was 0.64 for racemate **3** when the styrene content was 5 wt%. The chiral recognition abilities of **2** were basically maintained after the polymerization process, when the styrene content was low. As the styrene content increases, the polysaccharide derivative will be more tightly immobilized and may change its conformation which is closely related with chiral recognition. The immobilized CSPs were stable for the eluent containing 10% chloroform, and some racemates were better resolved with chloroform.

A cellulose 3,5-dimethylphenylcarbamate derivative having a styryl group was efficiently immobilized via the radical copolymerization with styrene. The resulting CSPs showed a high enantioseparation ability similar to that of the popular **1**-coated CSP (Chiralcel OD) and could be used with chloroform as the eluent. This immobilization technique can be applied to other polysaccharide derivatives regardless of the type of supports. We are now exploring the optimum conditions for the immobilization of the cellulose derivatives on the silica surface by changing several factors, such as the type and content of a vinyl monomer for copolymerization and the type and amount of the vinyl group introduced to the cellulose derivatives. The introduction of a vinyl group on the silica surface is also expected to improve the immobilization efficiency by a radical process.

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