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Chemical immobilization of azido cellulose phenylcarbamate onto silica gel via Staudinger reaction and its application as a chiral stationary phase for HPLC

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Abstract—Azido cellulose phenylcarbamate (AzCPC) was synthesized regioselectively and chemically immobilized onto aminized silica gel to afford urea-bonded chiral stationary phases (CSPs). The obtained CSPs showed good enantioselectivity in HPLC towards various racemates using normal phase eluants (hexane/2-propanol, hexane/chloroform or hexane/2-propanol/chloroform).

Cellulose derivatives play an important role in enantioseparations as chiral stationary phases (CSPs) for chiral resolutions.[1–5] Traditionally, cellulose based CSPs are prepared via physical coating of their derivatives onto macroporous silica gel.[6,7] However these CSPs are generally applicable for normal phase separations with limited choices of eluants (such as alkane–alcohol mixtures). Consequently, the development of chemically bonded cellulose based CSPs were aimed at overcoming these limitations and have gained more attention in recent years.

Chemically bonded CSPs (also known as chemically immobilized CSPs) usually comprise three components: (a) the substrate (often functionalized silica gel); (b) the chiral selector which distinguishes two enantiomers from each other; and (c) the linkage which chemically bonds the substrate and the chiral selector.

Several methodologies for chemical immobilization of cellulose based CSPs have been developed. Kimata et al. reported the synthesis of cellulose tris(*p*-vinylbenzoate) and its chemical bonding onto arylamide-immobilized silica gel via polymerization.[8] Minguillón et al. reported the bonding of 3,5-dimethylphenylcarbamates of cellulose, amylose and chitosan possessing a 10-undecenoyl group onto silica gel by heterogeneous coupling of the double bonds and reticulation.[9–12] Okamoto and co-workers have reported the immobilization of 3,5-dimethylphenylcarbamates of the polysaccharides bearing vinyl groups through copolymerization with other vinyl monomers.[13–19] All the above methods involved a pre-coating step and a chemical bonding step. In the pre-coating step, the polysaccharide derivatives were coated onto the surface of modified silica gel by solvent evaporation. In the bonding step, the polysaccharide derivatives were chemically bonded by reactions between functional groups on the polysaccharide derivatives and the silica gel surface. Thus, these immobilization approaches are regarded as the 'bonding-with-pre-coating' approach (or method I).

Enomoto reported an alternative method for the preparation of amylose CSPs via enzymatic polymerization of α-D-glucose 1-phosphate dipotassium to obtain an amylose derivative, which was then chemically bonded onto silica gel.[20] In this approach, the amylose derivative was not pre-coated onto silica gel prior to the chemical immobilization step. Thus this method is regarded as the 'bonding-without-pre-coating' approach (or method II).

To date, investigations on the effects of different synthetic approaches (method I and method II) for chemically bonded cellulose based CSP remain scarce. Herein, we report the first novel synthetic approach

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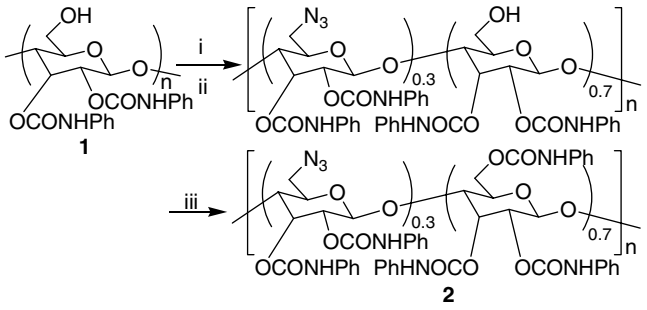

Figure 1. Synthesis of cellulose 6-deoxy-6-azide/phenylcarbamate **2**. (i) DMF, I_2, PPh_3, imidazole, 80 °C; (ii) NaN_3, 80 °C; (iii) Ph–NCO, pyridine, 80 °C. The amount of azido group was determined by ^1H NMR analysis of cellulose 6-deoxy-6-diisopropylurea/phenylcarbamate, which was synthesized by Staudinger reaction between **2** and diisopropylamine.

for azido-functionalized cellulose, which is used as a key intermediate towards the preparation of urea-bonded cellulose based CSPs (Figs. 1 and 2). In addition, we also attempt to explain the effects of different synthetic approaches (method I and method II) for urea-bonded cellulose based CSPs through structural characterization and their application as CSPs in HPLC.

Azido-functionalized cellulose derivative **2** (Fig. 1) was prepared from cellulose derivative **1** via a modified approach to that described by us earlier[21] for the synthesis of azido-functionalized cyclodextrin. The synthesis of **1** has been reported by Okamoto et al.[22] The hydroxyl groups of **1** were partially converted to an azido group, then the remaining 6-hydroxyls were converted to phenylcarbamates, yielding cellulose 6-deoxy-6-azide/phenylcarbamate **2**.[23]

In this study, two CSPs were prepared via Staudinger reaction of **2** with aminized silica gel. By comparison of the two CSPs from method I and method II, the influence of the pre-coating step was investigated. Silica gel (Kromasil, particle size 5 μm, pore size 100 Å) was aminized by reaction with 3-aminopropyltriethoxysilane in dry toluene.[7] CSP **AzCPC-II** was prepared by direct chemical immobilization of **2** onto aminized silica gel using THF as the solvent (method II).[24] CSP **AzCPC-I** was synthesized following method I: **2** was pre-coated onto the aminized silica gel in THF and immobilized in toluene.[25] The amount of immobilized **2** was determined by elemental analyses.

Elemental analyses showed that CSP **AzCPC-I** contained 15.58% C, 2.03% H and 3.24% N, whereas CSP **AzCPC-II** contained 10.43% C, 1.69% H and 2.15% N. From the elemental analyses data, the amounts of immobilized cellulose derivative **2** on CSP **AzCPC-I** and CSP **AzCPC-II** were 18.22 g and 8.10 g per 100 g CSP, respectively. This indicates that the pre-coating step has significantly improved the immobilization efficiency[9–12] from 40% to 91%. This is in agreement with the stronger IR peak of CSP **AzCPC-I** at 1735 cm^{-1} attributed to stretching of the carbonyl group of **2**.

Both CSPs were packed individually into stainless-steel columns (Phenomenex, 250 mm * 0.46 mm i.d.) by the slurry method. The plate numbers per meter were about 20,000 (CSP **AzCPC-I**) and 18,000 (CSP **AzCPC-II**) measured for biphenyl in hexane/2-propanol (90:10) at a flow rate of 0.5 mL/min. 1,3,5-Tri-*tert*-butylbenzene was used to estimate the dead time (t_0).[26] The enantioseparation results of eleven racemates (**3**–**13**) (Fig. 3) on CSP **AzCPC-I** and CSP **AzCPC-II** are shown in Table 1. Capacity factors: $k'_1 = (t_1 - t_0)/t_0$ and $k'_2 = (t_2 - t_0)/t_0$ where t_1 and t_2 are the retention times of the first and second eluted enantiomers. Selectivity: $\alpha = k'_2/k'_1$ and the resolution: $Rs = 2(t_2 - t_1)/(W_1 + W_2)$ where W_1 and W_2 are the baseline width of the first and second eluted peaks.

For racemates **3**–**9**, CSP **AzCPC-I** showed both a higher selectivity and a higher resolution than CSP **AzCPC-II**. The higher enantioselectivity was due to the larger amount of bonded chiral selector **2**. Among the four flavanone derivatives **10**–**13**, **11** was separated when 10% (mobile phase A) or 5% (mobile phase B) 2-propanol in hexane was used. Although **10** and **12** were not

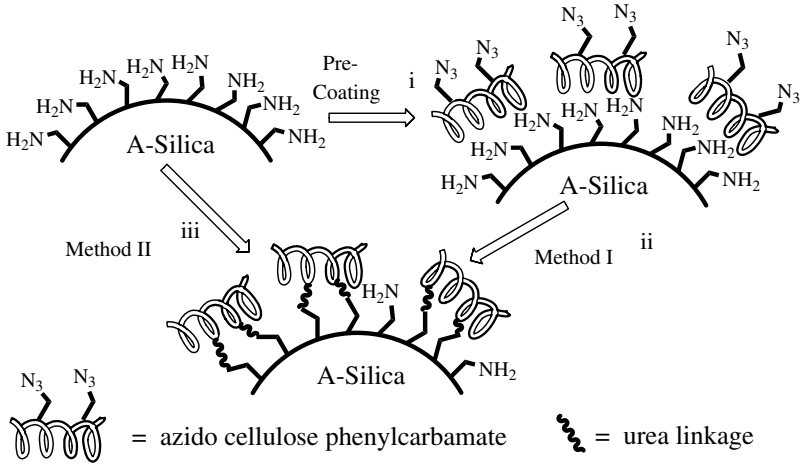

Figure 2. Synthesis of CSP **AzCPC-I** and CSP **AzCPC-II**. (i) Aminized silica gel, compound **2**, THF; (ii) pre-coated silica gel, toluene, PPh_3, CO_2, rt; (iii) aminized silica gel, compound **2**, THF, PPh_3, CO_2, rt.

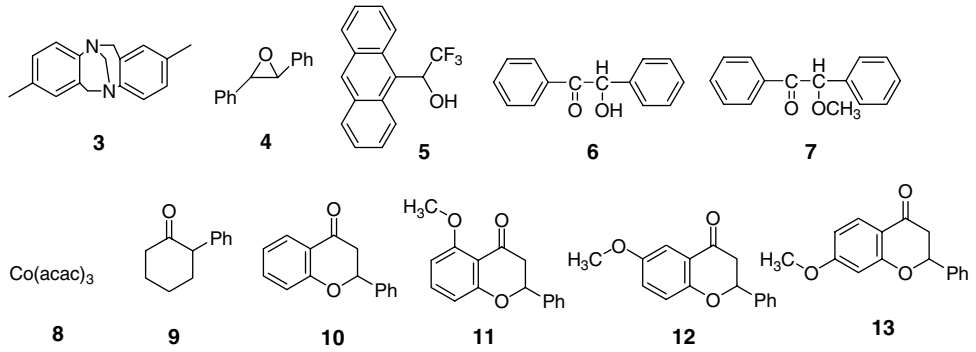

Figure 3. Structures of racemates.

Table 1. HPLC enantioseparation results for CSP **AzCPC-I** and CSP **AzCPC-II** using mobile phase A: hexane/2-propanol = 90/10; B: hexane/2-propanol = 95/5; flow rate = 0.5 mL/min; UV detection at 254 nm

Entry	CSP **AzCPC-I**			CSP **AzCPC-II**		
	k_1'	α	Rs	k_1'	α	Rs
Mobile phase A						
3	1.25	1.09	0.70	0.60	1.00	/
4	0.66	1.27	1.66	0.29	1.21	0.66
5	5.38	1.21	1.48	4.11	1.15	1.33
6	6.19	1.09	0.97	3.29	1.06	0.73
7	1.71	1.19	1.54	0.87	1.16	1.14
8	3.61	1.10	0.73	1.37	1.07	0.63
9	1.98	1.09	0.89	0.79	1.05	0.51
10	2.26	1.00	/	0.60	1.00	/
11	9.19	1.15	0.93	3.49	1.12	1.05
12	2.98	1.00	/	0.95	1.00	/
13	4.62	1.00	/	1.44	1.00	/
Mobile phase B						
3	1.38	1.08	0.69	0.69	1.06	0.55
4	0.66	1.30	1.72	0.28	1.28	0.81
5	5.98	1.22	2.38	8.77	1.17	2.13
6	8.56	1.09	0.94	4.70	1.06	0.80
7	2.15	1.19	1.63	1.03	1.18	1.54
8	5.79	1.11	0.78	2.32	1.08	0.71
9	2.35	1.09	0.91	0.97	1.04	0.42
10	6.46[a]	1.05[a]	0.63[a]	2.25[a]	1.04[a]	0.62[a]
11	19.71	1.16	1.00	4.95	1.12	1.17
12	8.42[a]	1.05[a]	0.56[a]	2.40[a]	1.05[a]	0.65[a]
13	5.98	1.00	/	2.55	1.00	/

/: No separation.
[a] Hexane/2-propanol = 99/1.

Table 2. HPLC enantioseparation results for CSP **AzCPC-I**; mobile phase A: hexane/2-propanol = 90/10, B: hexane/2-propanol/chloroform = 90/5/5, C: hexane/2-propanol/chloroform = 90/1/9 and D: hexane/chloroform = 90/10; flow rate = 0.5 mL/min; UV detection at 254 nm

Entry	Mobile phase A			Mobile phase B		
	K_1'	α	Rs	k_1'	α	Rs
3	1.25	1.09	0.70	1.08	1.00	/
4	0.66	1.27	1.66	0.52	1.31	1.79
5	5.38	1.21	1.48	6.61	1.25	1.68
6	6.19	1.09	0.97	5.59	1.07	0.92
7	1.71	1.19	1.54	1.37	1.20	1.62
8	3.61	1.10	0.73	1.33	1.07	0.58
9	1.98	1.09	0.89	1.50	1.08	0.79
10	2.26	1.00	/	1.71	1.00	/
11	9.19	1.15	0.93	7.37	1.19	1.31
12	2.98	1.00	/	2.19	1.00	/
13	4.62	1.00	/	3.33	1.00	/
	Mobile phase C			Mobile phase D		
3	1.41	1.00	/	4.69	1.00	/
4	0.49	1.25	2.00	1.63	1.47	3.97
5	34.04	1.34	3.28	[a]	[a]	[a]
6	7.78	1.08	1.01	22.46	1.09	0.98
7	1.86	1.27	2.22	3.77	1.00	/
8	1.45	1.00	/	3.14	1.00	/
9	1.93	1.08	0.76	3.58	1.00	/
10	2.28	1.00	/	3.97	1.00	/
11	11.06	1.28	2.31	44.79	1.24	1.43
12	2.94	1.05	0.58	3.38	1.00	/
13	4.90	1.00	/	16.48	1.00	/

/: No separation.
[a] Analyte does not elute.

separated, they were resolved using a mobile phase containing a smaller amount (1%) of 2-propanol. However, using mobile phase A, CSP **AzCPC-I** showed a slightly lower resolution (0.93) for **11** although its selectivity (1.15) was higher than that for CSP **AzCPC-II** (1.12). This lower resolution was probably due to greater peak broadening as the capacity factor k_1' of **11** on CSP **AzCPC-I** (9.19) was much larger than that on CSP **AzCPC-II** (3.49).

The immobilized CSPs were compatible with chloroform and improved results were obtained for several analytes (Table 2). The addition of chloroform to the mobile phase led to enantioseparation of racemate **12**, which was not resolved using hexane/2-propanol (90:10). An increase the amount of chloroform in the mobile phase also increased the resolution of some of the racemates (**4**, **5**, **7**, **11**). For example, the resolution of **4** increased from 1.66 (mobile phase A, Fig. 4A) to 3.97 (mobile phase D, Fig. 4B).

In summary, a novel method for the preparation of urea-bonded cellulose CSP has been reported. The Staudinger reaction described involves the reaction of an azido-functionalized cellulose derivative with amino-functionalized silica gel in the presence of carbon dioxide and triphenylphosphine. The pre-coating of the azido-functionalized cellulose derivative onto aminized

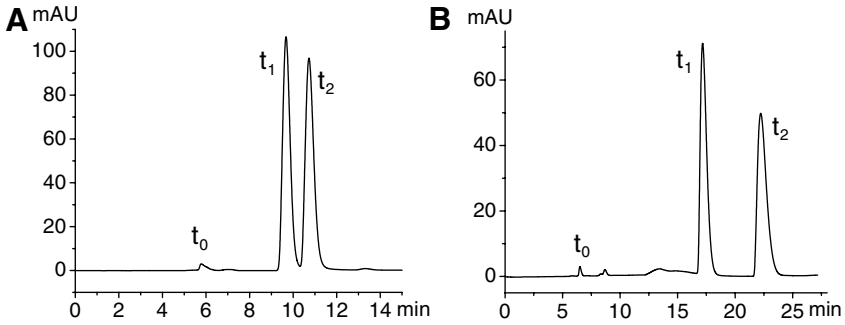

Figure 4. Chromatograms for the resolution of **4** on CSP **AzCPC-I**.

silica gel prior to chemical immobilization was found to enhance significantly the immobilization efficiency, which also led to better enantioselectivity of the resulting CSP. The obtained CSPs showed high enantioseparation ability towards various racemates. Enantioseparation using chloroform in the mobile phase led to better separation than traditional hexane/2-propanol mobile phases for some of the racemates studied. Further investigation towards optimized immobilization conditions and enantioseparation results will be reported elsewhere.

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23. *Synthesis of cellulose 6-deoxy-6-azide/phenylcarbamate* **2**: 8.6 g of **1** (21 mmol) was reacted with iodine (12.7 g, 50 mmol), triphenylphosphine (13.1 g, 50 mmol) and imidazole (6.8 g, 100 mmol) in DMF (120 mL) at 80 °C. After 24 h, an excess of sodium azide was added and the reaction was stirred for another 24 h. The remaining hydroxyl groups at the 6-position were treated with excess phenyl isocyanate in pyridine to yield **2** (8.0 g, 75%): IR (cm^{-1}): 3392 and 3315 (N–H, s), 3060 (arom C–H, m), 2955 (sp^3 C–H, m), 2108 (N_3, s), 1737 (C=O, s); ^1H NMR (300 MHz, acetone-d_6) δ: 8.62–8.32 (NH), 7.39–6.75 (arom H), 5.01–3.66 (anhydrous glucose H).
24. *Preparation of CSP* **AzCPC-II**: Aminized silica gel (4.0 g) was suspended in dry THF (100 mL), and dry CO_2 was bubbled through the slurry. Cellulose derivative **2** (1.0 g) and triphenylphosphine (1.0 g) were dissolved in THF (100 mL) and added to the reaction mixture, which was stirred at room temperature overnight. The resulting CSP **AzCPC-II** was filtered and subjected to Soxhlet extraction using THF overnight to remove unreacted **2**, after which it was dried under vacuum to afford the desired CSP (yield 3.8 g, 76%). Elemental analysis: C, 10.43; H, 1.69; N, 2.15.
25. *Preparation of CSP* **AzCPC-I**: A pre-coating step was performed. Cellulose derivative **2** (1.0 g) was dissolved in THF (20 mL) and coated onto aminized silica (4.2 g). The pre-coated aminized silica gel was dried under vacuum and further chemically bonded under the same conditions described for CSP **AzCPC-II** except that the solvent was toluene (60 mL) in which **2** was insoluble. The CSP was subjected to the same extraction and drying process to afford the desired CSP (yield 4.0 g, 77%). Elemental analysis: C, 15.58; H, 2.03; N, 3.24.
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