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REVIEW ARTICLES

Immobilized Polysaccharide-Based Chiral Stationary Phases for HPLC

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ABSTRACT: Polysaccharide derivatives, such as phenylcarbamates and benzoates of cellulose and amylose, are known to show high chiral recognition abilities for many racemates when used as chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC). This type of CSPs has usually been prepared by coating the polysaccharide derivatives onto a macroporous silica gel without a chemical bond. Therefore, rather limited numbers of solvents can be used as eluents, and solvents, such as chloroform and tetrahydrofuran (THF), which swell or dissolve the polysaccharide derivatives cannot be used. The selection of a suitable eluent is very important for both analytical and preparative separations. In order to enhance the versatility of the polysaccharide derivative-based CSPs, the derivatives have to be immobilized. Here we review the immobilization methods of the polysaccharide derivatives mainly onto silica gel. [DOI 10.1295/polymj.38.91]

KEY WORDS Immobilization / Polysaccharide / Resolution / High Performance Liquid Chromatography / HPLC / Chiral Stationary Phase / CSP /

Optically active compounds have been attracting much attention in many fields of science, and the demand for single enantiomers in the pharmaceutical industry has also been increasing. However, the separation or resolution of enantiomers is often laborious because most physical and chemical properties of enantiomers are identical. In the early 1970s, the first baseline separation of enantiomers by liquid chromatography was reported by Davankov,[1] and in 1981, the chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC) developed by Pirkle[2] were for the first time commercialized. In the 1980s, the instrumentation for HPLC had remarkably advanced, and many efficient CSPs for HPLC had also been developed.[3–9] Today, most chiral compounds appear to be resolved by HPLC using CSPs.[10]

The CSPs have been prepared with optically active small molecules[6,8,11] or polymers,[3–7,12–15] which are usually supported on silica gel. Among a large number of CSPs so far developed, phenylcarbamates of cellulose and amylose (Figure 1) exhibit broad applicability to a wide range of compounds.[10,16–21] Some of the polysaccharide-based CSPs for HPLC have been commercially available. However, these polysaccharide-based CSPs can be used with a limited number of solvents, because some organic solvents, such as THF, chloroform, toluene, ethyl acetate, and acetone, dissolve or swell the polysaccharide derivatives and destroy their packed columns. Due to this solubility of the polysaccharide derivatives, the coated CSPs have usually been used with eluents consisting of alkanes/alcohols mixtures or aqueous solvents containing alcohols or acetonitrile. These limitations in the mobile phase selection are sometimes a serious problem for efficient analytical and preparative resolution of enantiomers.[10,22] For a large scale preparative separation, good solubility of the sample is essential for high productivity.[22–25] Therefore, versatility in the solvent selection is highly desired.

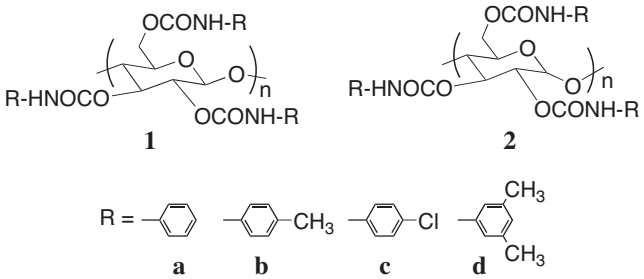

Figure 1. Structures of phenylcarbamate derivatives of cellulose (**1**) and amylose (**2**).

This problem can be solved through immobilization of the polysaccharide derivatives onto chromatographic supports. Once CSPs are immobilized on a support, a number of solvents can be used as the mobile phases. The selection of a suitable eluent will improve the

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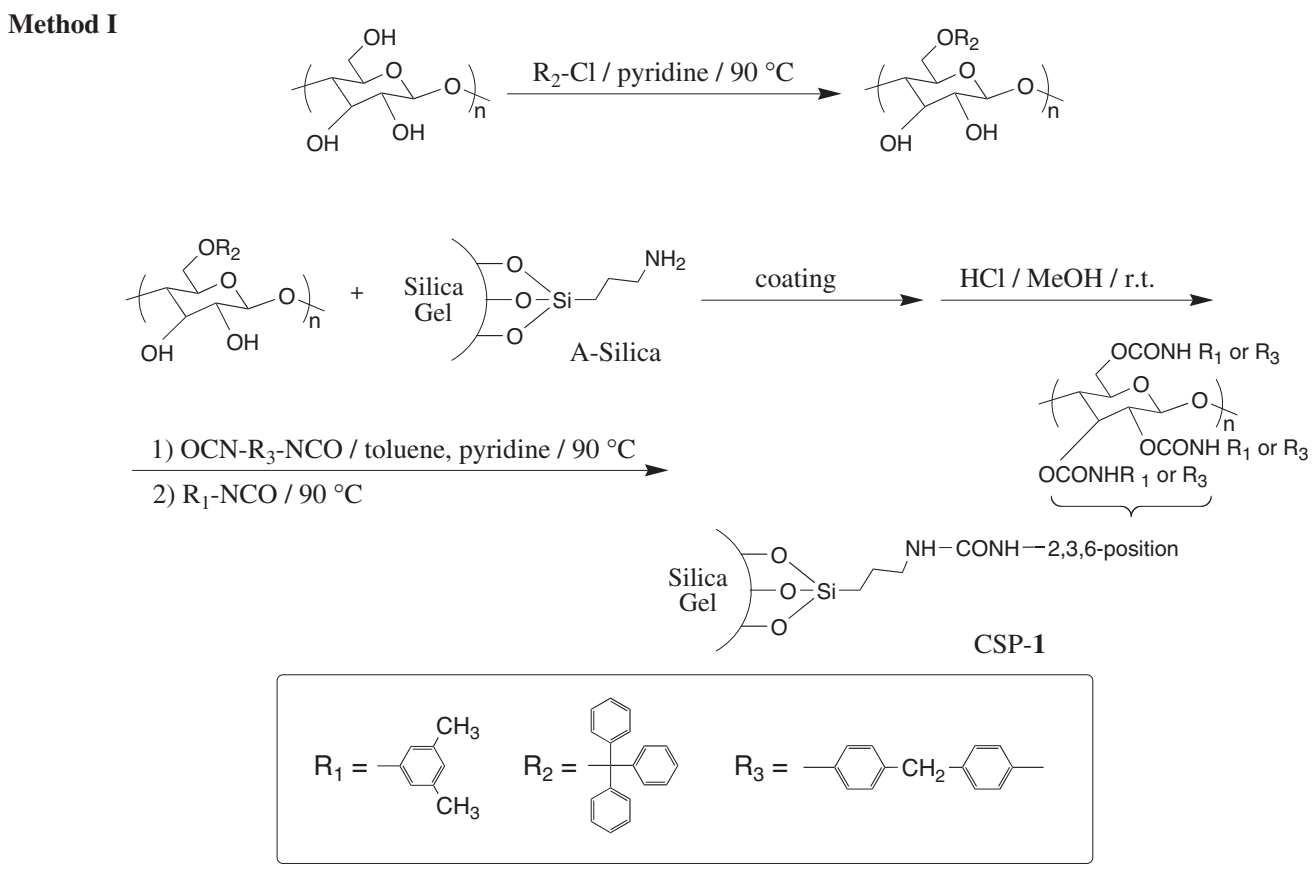

Figure 2. Non-regioselective immobilization of the cellulose derivative at the 2-, 3-, and 6-positions.

resolution and may provide higher enantioselectivity. Additionally, in the case of immobilized CSPs, even the solutions obtained from various synthetic media can be directly injected. For these reasons, several immobilized polysaccharide-based CSPs have been prepared in the past decade. In this review, we will summarize various immobilization methods of polysaccharide derivatives on silica gel supports.

IMMOBILIZATION OF POLYSACCHARIDE DERIVATIVES USING DIISOCYANATE

The first immobilization of polysaccharide derivatives on silica gel was investigated by our group in 1987.[26] The reaction was conducted between polysaccharide derivatives and 3-aminopropyl functionalized-silica gel (A-silica) using a diisocyanate as a cross-linker, which was expected to react with the free amino groups on the silica surface and the hydroxyl groups on the polysaccharide, and also between the hydroxy groups of different polysaccharide chains.

Figure 2 shows the preparation scheme (Method I) of the immobilized phase (CSP-**1**), in which diisocyanate (OCN-R_3-NCO) was expected to connect between the hydroxy groups at the 2-, 3-, and 6-positions of the glucose unit and A-silica. Firstly, 6-*O*-trityl-cellulose dissolved in chloroform was coated onto the A-silica, and the coated material was then treated with methanol containing a small amount of hydrochloric acid to regenerate free cellulose. The cellulose on the A-silica was allowed to react with a certain amount of 4,4′-diphenylmethane diisocyanate, and finally the remaining unreacted hydroxyl groups of the cellulose were derivatized to the carbamate with an excess of 3,5-dimethylphenyl isocyanate.

The regioselectively immobilized CSPs-**2** and -**3** were also prepared according to Methods II and III (Figures 3 and 4), respectively,[27] in which a cellulose derivative was immobilized onto the A-silica through the hydroxy groups at the 2- and 3-positions or only the 6-position, respectively. 4,4′-Diphenylmethane diisocyanate was allowed to react with the A-silica coated with either 6-*O*-trityl-cellulose (Method II) or 2,3-bis(3,5-dimethylphenylcarbamoyl) cellulose (Method III). An excess of 3,5-dimethylphenyl isocyanate was then added to react with the remaining hydroxy groups of cellulose. The immobilized amylose derivative phases, CSPs-**5**, **6**, and **7**, were similarly prepared according to Methods I, II, and III, respectively.[27]

These immobilized CSPs have been evaluated using a hexane–2-propanol mixture as an eluent, and their enantioselectivities for racemic compounds (**3**–**12** in Figure 5) are compared to those of the corresponding coated-type CSPs-**4** and **8**,[28,29] which have

Method II

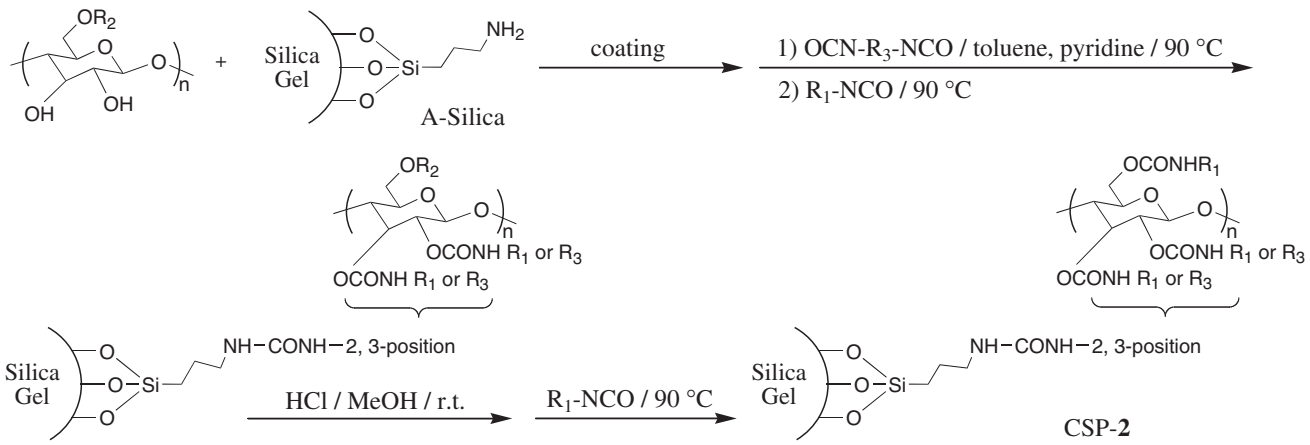

Figure 3. Regioselective immobilization of the cellulose derivative at the 2- and 3-positions.

Method III

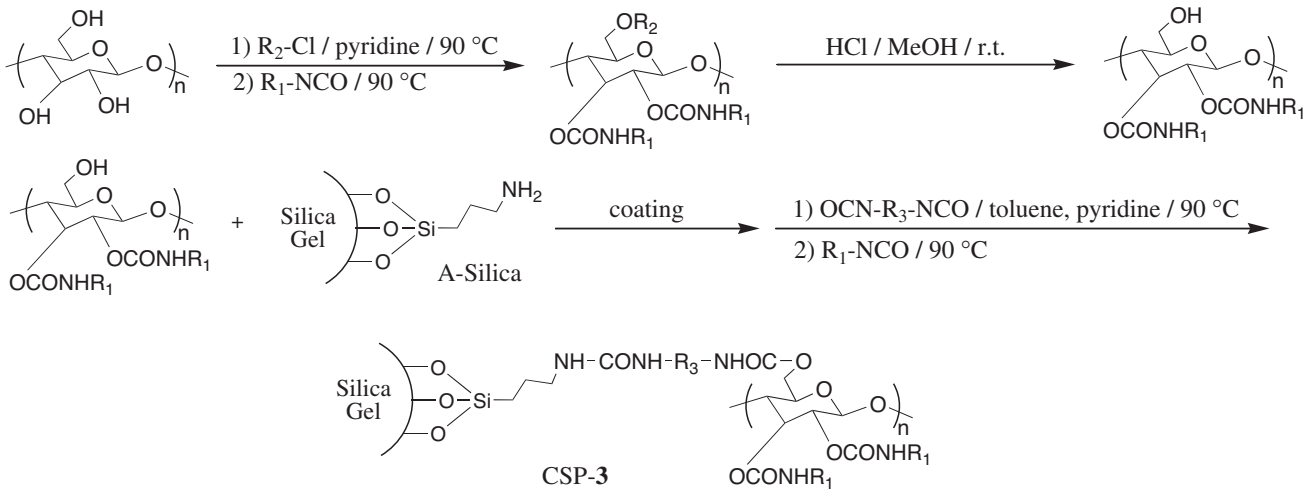

Figure 4. Regioselective immobilization of the cellulose derivative at the 6-position.

been prepared by physical adsorption of the polysaccharide derivatives, **1d** and **2d**, on silica gel, respectively (Tables I and II). For both the cellulose and amylose derivatives, some decreases in the chiral recognition ability have been observed for the immobilized CSPs prepared by the three methods compared with that of the coated-type CSPs. This decrease in enantioselectivity may be caused by the alteration and disturbance of the higher order structure of the polysaccharide derivatives through the chemical bonding to the silica gel. The chiral recognition ability seems to be dependent on the amount of the diisocyanate used for immobilization. Thus, the enantioselectivity decreases as the degree of chemical bonding between the polysaccharide derivatives and the silica increases.

For the cellulose-based phases, the regioselectively immobilized CSPs-**2** and -**3** obviously show a higher enantioselectivity than the non-regioselectively immobilized CSP-**1** (Table I). However, a clear difference in enantioselectivity is not observed between the CSPs immobilized at the 2- and 3-positions (Method II) and at the 6-position (Method III). These results suggest that the higher order structure is not significantly changed by the position used for the immobilization.

On the other hand, for the amylose derivatives, the chiral recognition ability of CSP-**7** immobilized at the 6-position is higher than that of CSP-**6** immobilized at the 2- and 3-positions, although both the regioselectively immobilized CSPs show a superior chiral discrimination to non-regioselectively-bonded CSP-**5** (Table II). For example, racemates **3** and **11** are sufficiently separated on CSP-**7**, but almost no chiral discrimination is observed on CSP-**6**. This indicates that **3** and **11** may be discriminated mainly based on the

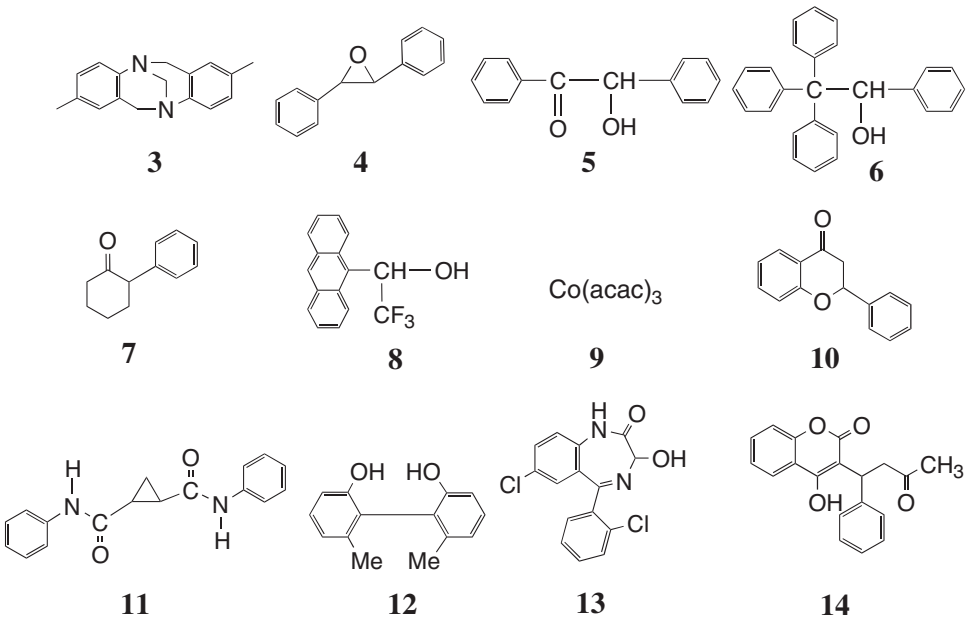

Figure 5. Structures of the racemates.

Table I. Separation factors on cellulose derivative-immobilized CSPs[a]

CSP	Cellulose derivative			
	CSP-**1** (Method I)	CSP-**2** (Method II)	CSP-**3** (Method III)	CSP-**4** (coated **1d**)
Racemates				
3	1.0	1.32 (+)	1.40 (+)	1.32 (+)
4	*ca.* 1 (+)	1.46 (−)	1.30 (−)	1.68 (−)
5	1.16 (+)	1.29 (+)	1.31 (+)	1.58 (+)
6	1.14 (+)	1.11 (+)	1.11 (−)	1.34 (+)
7	1.22 (−)	1.19 (−)	1.22 (−)	1.15 (−)
8	1.63 (−)	2.12 (−)	2.33 (−)	2.59 (−)
10	*ca.* 1 (−)	1.21 (−)	1.19 (+)	1.41 (−)
11	1.0	1.46 (+)	1.40 (+)	3.17 (+)
12	2.63 (−)	2.76 (−)	3.47 (−)	1.83 (−)

[a]The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Eluent; hexane–2-propanol (90:10). Flow-rate; 0.5 mL/min.

Table II. Separation factors on amylose derivative-immobilized CSPs[a]

CSP	Amylose derivative			
	CSP-**5** (Method I)	CSP-**6** (Method II)	CSP-**7** (Method III)	CSP-**8** (coated **2d**)
Racemates				
3	*ca.* 1 (+)	*ca.* 1 (+)	1.37 (+)	1.58 (+)
4	1.99 (+)	2.08 (+)	2.53 (+)	3.04 (+)
5	1.0	1.09 (+)	1.09 (−)	1.21 (−)
6	1.75 (+)	1.75 (+)	1.94 (+)	1.98 (+)
7	1.0	*ca.* 1 (−)	1.0	*ca.* 1 (−)
8	1.07	*ca.* 1	1.0	1.15 (+)
10	1.16 (+)	*ca.* 1 (+)	*ca.* 1 (+)	1.12 (+)
11	1.60 (+)	1.0	1.75 (+)	2.01 (+)
12	1.89 (−)	1.63 (−)	2.10 (−)	2.11 (−)

[a]The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Eluent; hexane–2-propanol (90:10). Flow-rate; 0.5 mL/min.

carbamate residues at the 2- or 3-position of amylose.

The different behavior between the cellulose and amylose derivatives may be explained as follows. The cellulose phenylcarbamates are known to have a 3/2 left-handed helical conformation[30] and form a lyotropic liquid crystalline phase at a high concentration in solution.[31] This suggests that the cellulose derivatives have a rather stiff chain. This conformation may be less influenced by the immobilization shown in Figures 3 and 4. On the other hand, the corresponding amylose derivatives do not form a lyotropic liquid crystalline phase, indicating that the polymer chains may be more flexible, and its conformation may be more sensitively changed depending on the immobilization position.

Because the immobilized CSPs are totally insoluble in any solvents, eluents containing THF and $CHCl_3$, which dissolve or swell the polysaccharide derivatives, can be used. A more efficient separation of some racemates, which are not separated with hexane–2-propanol, can be attained with an eluent containing a small amount of $CHCl_3$. This improvement may partly be ascribed to the change in the conformation of the polysaccharide derivatives.

Recently, Zou *et al.* prepared immobilized CSPs derived from other polysaccharide derivatives by a similar method using a bisfunctional reagent.[32–36] In addition to HPLC, the obtained CSPs have been applied to nano-liquid chromatography and capillary electrochromatography.[35,36] However, these CSPs

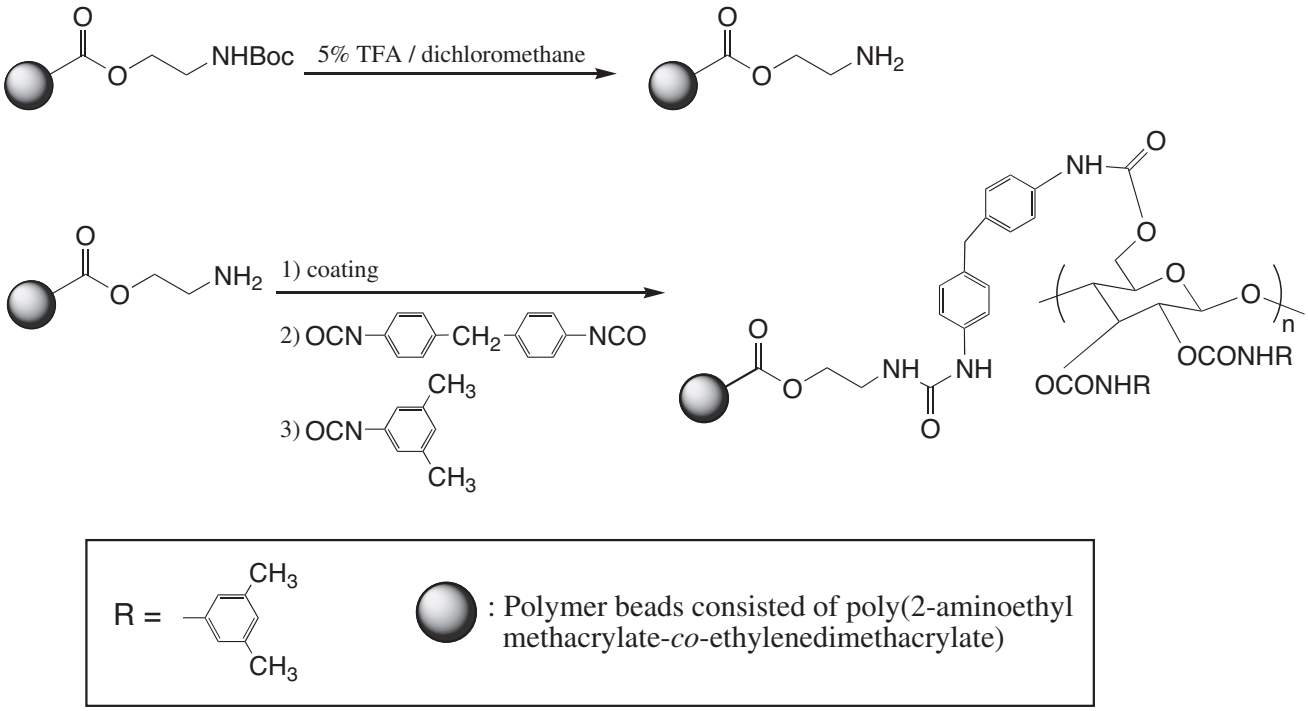

Figure 6. Scheme of immobilization of cellulose derivatives on polymer beads.

show rather low enantioselectivity probably due to the above-mentioned reason.

For the brush type CSPs,[37,38] the replacement of the silica gel with polymer beads results in a significant improvement in chiral recognition. This result indicates that numerous residual silanol groups located on the surface of the silica gel may generate non-specific interactions and lead to a decrease in chiral recognition. Considering these results, in 2003, Fréchet and Svec *et al.* prepared polysaccharide-immobilized CSPs using polymer beads as a support.[39] In these CSPs, the polysaccharide derivatives have been bonded to the internal surface of the poly(2-aminoethyl methacrylate-*co*-ethylenedimethacrylate) beads instead of the silica gel in order to eliminate the influence of the silanol groups. The immobilization mechanism is similar to that of Method III; a diisocyanate reacted with the free amino group on the polymer beads and the hydroxyl groups on the polysaccharide derivatives (Figure 6). Unlike the brush type, these CSPs did not show any significant enhancement in chiral recognition compared with the silica-immobilized CSPs using the same selector and an identical amount of diisocyanate. This result may be associated with the fact that complete coverage of the surface of both the silica and polymer supports has been achieved by using polysaccharides as chiral selectors, and therefore, the support itself has no influence on the enantioselectivity of the CSPs, because the racemates cannot access the native surface of the support.

IMMOBILIZATION OF POLYSACCHARIDE DERIVATIVES HAVING VINYL GROUPS BY POLYMERIZATION

The immobilization of cellulose tris(*p*-vinylbenzoate) on a modified silica by radical copolymerization was reported by Kimata *et al.* in 1993.[40,41] In this method, cellulose tris(*p*-vinylbenzoate) was coated on the A-silica modified with acryloyl chloride. The coated CSP suspended in a solvent was then heated in the presence of benzoyl peroxide as a radical initiator. After washing, about 95% of cellulose tris(*p*-vinylbenzoate) was confirmed to be immobilized on the silica gel. Although the immobilized CSP could be used with eluents containing dichloromethane or tetrahydrofuran, it showed a slightly lower enantioselectivity than the coated CSP, which was prepared without the polymerization process. Because the cellulose derivative must contain a large number of styryl groups on its polymer chain, the derivative may be very tightly immobilized on the silica. Therefore, the regular higher order structure of the cellulose derivative would be destroyed resulting in a lower enantioselectivity.

Oliveros *et al.* reported an analogous immobilization of polysaccharide derivatives containing partly polymerizable groups.[42,43] They prepared a polysaccharide derivative bearing both 3,5-dimethylphenylcarbamate and 10-undecenoyl groups as the polymerizable group.

This cellulose derivative has been prepared by the

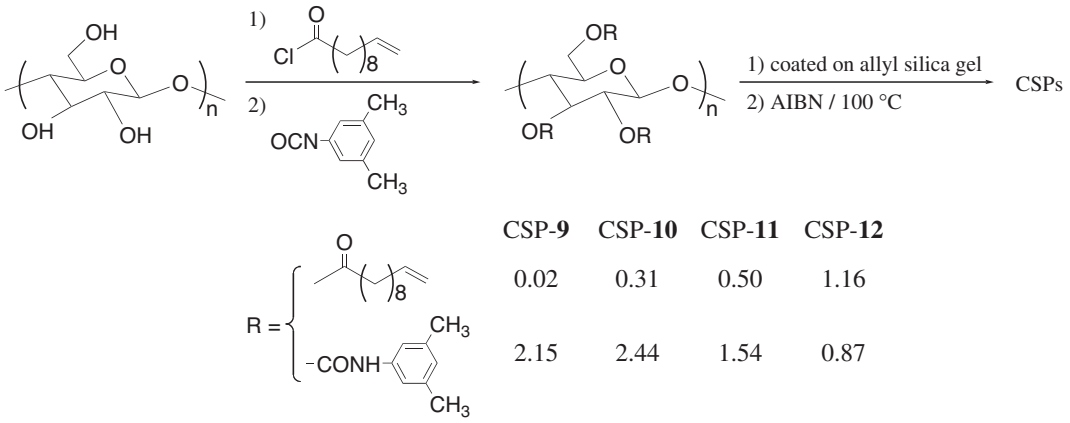

Figure 7. Scheme of immobilization of 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose with different content of 10-undecenoyl groups on allylsilica gel.

Table III. Separation factors on CSPs-**9**–**12** with heptane–2-propanol as mobile phase[a]

CSP	CSP-**9**	CSP-**10**	CSP-**11**	CSP-**12**	Heptane–2-Propanol
Racemates					
3	1.0	1.21	1.10	1.0	98:2
4	1.69	1.49	1.0	1.0	98:2
5	1.37	1.33	1.16	1.0	90:10
8	2.22	2.20	1.70	1.23	98:2
13	1.43	1.56	1.85	1.32	90:10
14	2.17	2.17	1.73	1.63	90:10

[a]Flow-rate 1 mL/min.

Table IV. Separation factors on CSPs-**9**–**12** with heptane–chloroform as mobile phase[a]

CSP	CSP-**9**	CSP-**10**	CSP-**11**	CSP-**12**	Heptane–chloroform
Racemates					
4	2.37	2.03	1.47	1.0	95:5
5	1.15	1.15	1.05	1.0	80:20
8	2.42	2.29	1.86	1.36	50:50
13	1.0	1.18	1.13	1.04	50:50
14	2.40	2.15	1.70	1.66	50:50

[a]Flow-rate 1 mL/min.

successive addition of 10-undecenoyl chloride and 3,5-dimethylphenyl isocyanate. After coating on a support, the derivative was heated in the presence of α,α'-azobisisobutyronitrile (AIBN) for immobilization. The immobilization can take place through two processes; one is the reaction of the 10-undecenoyl groups on the cellulose with allyl groups introduced on silica gel, and the other is the reticulation of 10-undecenoyl groups themselves. Both processes induce a decrease in the chiral recognition ability due to the disturbance of the structure of the polysaccharide derivative. In order to examine this influence of the immobilization on the enantioselectivity, four cellulose derivatives with different contents of 10-undecenoyl groups (from 0.02 to 1.16 groups per glucose unit) were synthesized and immobilized onto allylsilica gel (Figure 7).[44] CSP-**9** had a lower content of the cellulose derivative on silica because it was prepared from the derivative with the lowest content of vinyl groups.

These CSPs were evaluated with heptane/2-propanol and heptane/chloroform mixtures as the eluents, and the results are summarized in Tables III and IV, respectively. In both systems with heptane–2-propanol and heptane–chloroform, the chiral recognition abilities of CSPs-**11** and **12** with a higher degree of immobilization are lower than those of CSPs-**9** and **10** with a lower degree of immobilization. The derivative (CSP-**10**) with approximately 0.3 10-undecenoyl groups per glucose unit seems to be suitable for effective immobilization and high chiral recognition.

In this immobilization, two kinds of substituents, the 10-undecenoyl group and the 3,5-dimethylphenylcarbamate group, were introduced on cellulose. The former was for immobilization of the polysaccharide on the chromatographic support and the latter for high chiral discrimination. However, introduction of the different substituents on cellulose may deform the regular higher order structure of the cellulose derivative. In order to maintain the structural regularity in the cellulose derivative, particularly the higher order helical structure, two kinds of structurally similar substituents were introduced on cellulose (**16** in Figure 8).[45] The cellulose derivative **16** having polymerizable alkenoxybenzoyl groups instead of the previous 10-undecenoyl groups was synthesized for immobilizing on allylsilica gel.

When the chromatographic behavior of the CSPs derived from **15** and **16** were compared, better chiral recognition ability was observed for the CSP derived from **16**. However, this CSP had a lower cellulose

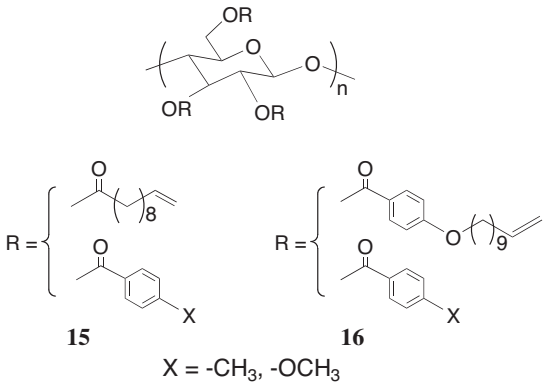

Figure 8. Structures of the cellulose benzoates with different kind of vinyl groups (**15** and **16**).

content than the CSP derived from **15** due to the low reactivity of the alkenoxybenzoyl groups compared to that of the 10-undecenoyl groups.

In order to improve the immobilization efficiency, the alkenoxybenzoyl group content was increased, and this increase did not induce a decrease in the chiral recognition ability, contrary to the results using the cellulose derivative **15** with a higher content of 10-undecenoyl groups. These results indicate that the higher order structure of these cellulose derivatives bearing the alkenoxybenzoyl group may be maintained during the immobilization process. Therefore, the introduction of a 4-substituted aromatic ring is considered to be important for maintaining the structural regularity of the cellulose derivative.

This immobilization method has also been successfully applied to amylose and chitosan derivatives.[46,47]

IMMOBILIZATION OF POLYSACCHARIDE DERIVATIVES *VIA* COPOLYMERIZATION WITH A VINYL MONOMER

The above immobilization methods have at least one of the following disadvantages: lower chiral recognition, low immobilization efficiency, and limitation of applicable polysaccharides.

The low immobilization efficiency of the polysaccharide derivatives with vinyl groups is expected to be improved through the copolymerization of a vinyl monomer added into the immobilization system, and efficient immobilization might be attained without changing the chiral recognition ability. In this strategy, a cellulose derivative bearing a vinyl group at the 6-position of a glucose unit was immobilized onto silica gel *via* radical copolymerization with a vinyl monomer (Figure 9).[48–51]

The cellulose derivative bearing a regioselective vinyl group was synthesized by protecting the hydroxy group at the 6-position, as shown in Figure 10.[48,50] The ratio of the substituents (3,5-dimethylphenylcarbamate and 4-vinylphenylcarbamate) at the 6-position of the derivative was about 7:3. The obtained cellulose derivative was coated onto the A-silica (see Figure 2), and styrene and AIBN were then added to the coated silica gel. The immobilization of the cellulose derivative was attained by heating the mixture at 60 °C for 20 h, and the mixture was fully washed with THF. The amount of the immobilized derivative was estimated by ^{1}H NMR analysis of the THF washing solution. The immobilization results on the A-silica using 0, 5, 10, 30, and 50 wt % styrene relative to the cellulose derivative, and the chiral recognition abilities of the obtained immobilized CSPs are shown in Table V. Most of the coated cellulose derivatives have been immobilized on silica gel using 10–50 wt % styrene. However, when the styrene content was reduced to 0 or 5 wt %, the amount of the immobilized cellulose derivative was decreased to 50 or 86%, respectively. These results indicate that styrene can significantly contribute to the efficient immobilization of the cellulose derivative on silica gel. Chiral recognition was highest for CSP-**16** immobilized with 5 wt % styrene for some racemates, and a further increase in styrene caused a decrease in the α value. This is because the higher order structure of the cellulose derivative may be changed during the immobilization process, especially at high styrene contents. The styrene

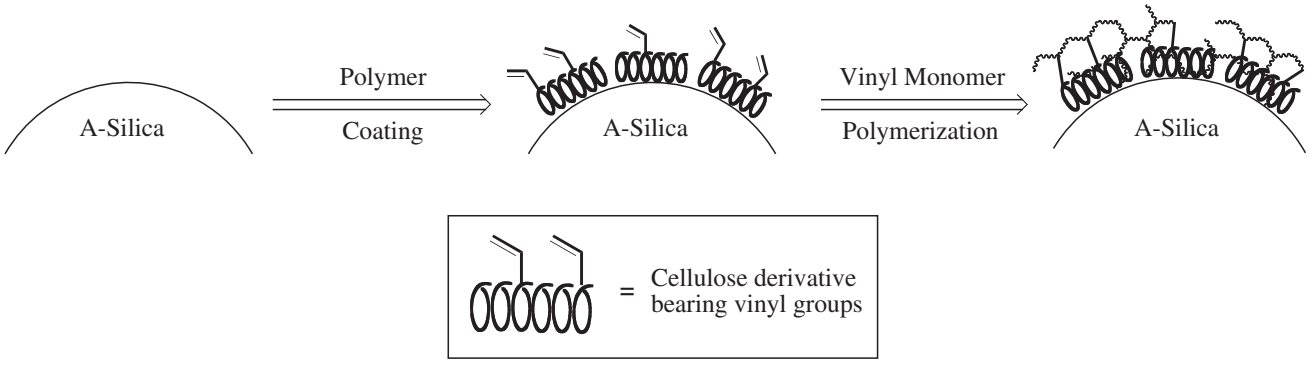

Figure 9. Scheme of immobilization of cellulose derivatives bearing vinyl groups with vinyl monomers.

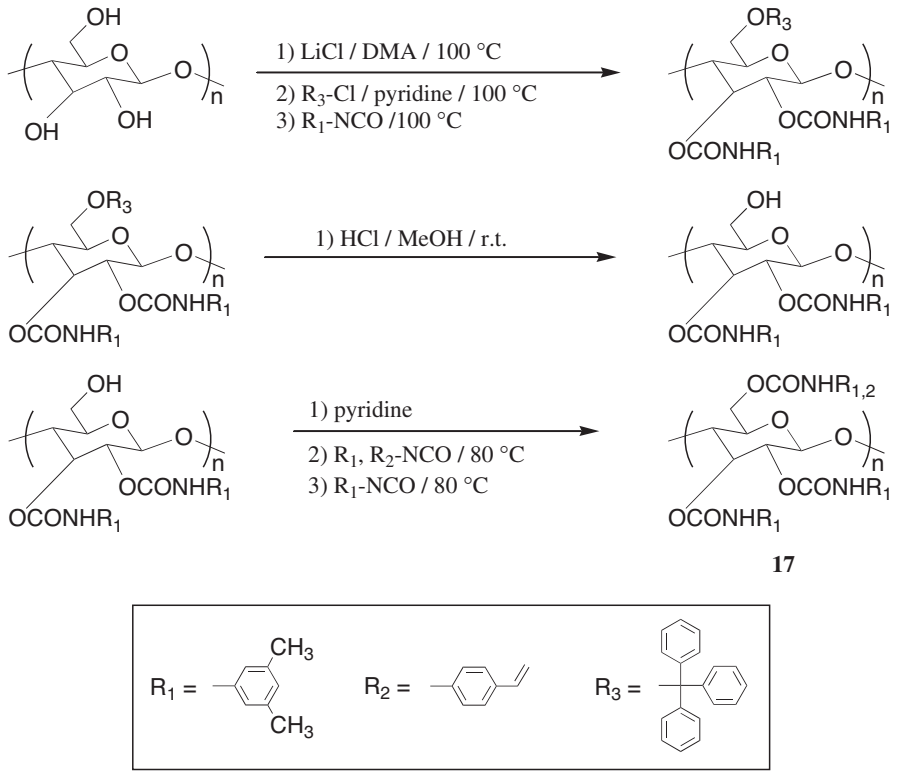

Figure 10. Scheme of the synthesis of cellulose derivatives (**17**).

Table V. Effect of styrene content on immobilization and separation factors on **17**[a]

CSP	CSP-**13**	CSP-**14**	CSP-**15**	CSP-**16**	CSP-**17**
Styrene/Cellulose deriv.	50 wt %	30 wt %	10 wt %	5 wt %	0 wt %
Immobilized cellulose deriv.	> 99%	> 99%	99%	86%	50%
Racemates					
3	1.53 (+)	1.60 (+)	1.68 (+)	1.45 (+)	1.45 (+)
4	1.23 (+)	1.17 (+)	*ca.* 1 (+)	1.33 (−)	*ca.* 1 (−)
5	1.14 (+)	1.16 (+)	1.18 (+)	1.34 (+)	1.25 (+)
6	*ca.* 1 (+)	*ca.* 1 (+)	1.12 (+)	*ca.* 1 (+)	*ca.* 1 (+)
7	1.31 (−)	1.31 (−)	1.32 (−)	1.22 (−)	1.26 (−)
8	1.73 (−)	1.81 (−)	1.96 (−)	2.18 (−)	1.92 (−)
9	*ca.* 1 (+)	*ca.* 1 (+)	1.32 (+)	1.17 (+)	*ca.* 1 (+)
10	*ca.* 1 (−)	1.08 (−)	1.13 (−)	1.22 (−)	1.23 (−)
11	*ca.* 1 (+)	*ca.* 1 (+)	*ca.* 1 (+)	1.42 (+)	1.40 (+)
12	2.63 (−)	2.76 (−)	3.20 (−)	2.57 (−)	2.74 (−)

[a]The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Eluent; hexane–2-propanol (90:10). Flow rate; 0.1 mL/min. Silica gel; A-Silica. [Vinyl group]/[AIBN] = 50. Solvent for polymerization; hexane.

units in CSP may generate non-selective interaction sites for the enantiomers and lead to a decrease in chiral recognition. These results suggest that an optimized amount of styrene has to be used for both efficient immobilization and chiral recognition. About 10 wt % vinyl monomer relative to the cellulose derivative seems to be suitable for these purposes.

The influence of vinyl monomer on the immobilization of the polysaccharide derivatives is shown in Table VI,[50] where 10 wt % of styrene, isoprene, *t*-butyl acrylate (*t*BuA), and *t*-butyl methacrylate (*t*BuMA) relative to the cellulose derivative has been used for immobilization. A high immobilization efficiency can be attained with all the vinyl monomers and most cellulose derivatives have been successfully immobilized. However, the enantioselectivities are slightly different for these immobilized CSPs, indicating that the vinyl monomers influence the chiral recognition to some extent.

As explained before, styrene seems to disturb the enantioselectivity through non-stereoselective interaction between the cellulose derivative and the enan-

Table VI. Effect of the kind of vinyl monomer on immobilization and separation factors on **17**[a]

CSP	CSP-**15**	CSP-**18**	CSP-**19**	CSP-**20**
Vinyl monomer	Styrene	Isoprene	tBuA	tBuMA
Immobilized cellulose deriv.	99%	99%	90%	90%
Racemates				
3	1.68 (+)	1.77 (+)	1.57 (+)	1.53 (+)
4	*ca.* 1 (+)	1.13 (−)	1.29 (−)	1.29 (−)
5	1.18 (+)	1.17 (+)	1.29 (+)	1.28 (+)
6	1.12 (+)	1.15 (+)	*ca.* 1 (+)	*ca.* 1 (+)
7	1.32 (−)	1.30 (−)	1.25 (−)	1.25 (−)
8	1.96 (−)	1.84 (−)	2.16 (−)	2.12 (−)
9	1.32 (+)	1.27 (+)	1.21 (+)	1.20 (+)
10	1.13 (−)	1.11 (−)	1.21 (−)	1.20 (−)
11	*ca.* 1 (+)	1.00	1.45 (+)	1.42 (+)
12	3.20 (−)	1.69 (−)	2.84 (−)	2.84 (−)

[a]The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Eluent; hexane–2-propanol (90:10). Flow rate; 0.1 mL/min. Silica gel; A-Silica. Vinyl monomer; 10 wt %. [Vinyl group]/[AIBN] = 50. Solvent for polymerization; hexane.

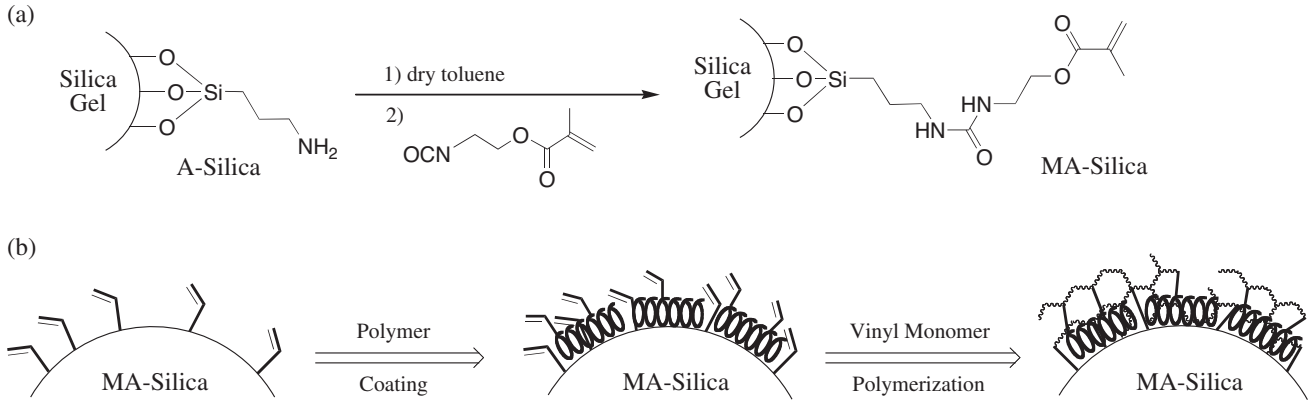

Figure 11. Scheme of the introduction of a vinyl group on silica surface (a) and the immobilization of the cellulose derivative (b).

tiomers. Therefore, the styrene content should desirably be as small as possible. To attain effective immobilization with a small amount of styrene, MA-silica bearing a vinyl group on the silica surface has been prepared by the reaction of the A-silica with 2-methacryloyloxyethyl isocyanate (Figure 11).[49,50] The immobilization results on the MA-silica and A-silica with 0 and 5 wt % styrene and the chiral recognition abilities are summarized in Table VII. When 5 wt % styrene is used in the immobilization process, the amount of the cellulose derivative immobilized on the MA-silica and A-silica are 97 and 86%, respectively. The immobilization efficiency has been improved using the MA-silica. However, the chiral recognition ability of CSP-**21** prepared with the MA-silica is slightly lower than that of CSP-**16** on the A-silica. This decrease in the α values may mainly be attributed to a non-enantioselective interaction between the polar urea bond on the MA-silica and the enantiomers.

Cellulose derivative **18** bearing a methacryloyloxy group has also been prepared (Figure 12) and immobilized using 10 wt % styrene on the A-Silica (Table VIII).[50,51] The derivative has also been quantitatively immobilized on the A-silica, and the chiral recognition ability of the obtained CSP-**23** is similar to that of CSP-**15** prepared using the cellulose derivative **17** with a *p*-vinylphenyl group. 2-Methacryloyloxyethyl isocyanate used for CSP-**23** is commercially available and stable. In contrast, 4-vinylphenyl isocyanate used for CSP-**15** is not commercially available and is not easy to handle due to its high polymerizability. Therefore, 2-methacryloyloxyethyl isocyanate seems to be more useful for preparing the cellulose derivatives.

When **18** is immobilized on the A-silica *via* copolymerization with 10 wt % 2,3-dimethylbutadiene in place of styrene, the chiral recognition ability of immobilized CSP-**24** is close to that of coated-type CSP-**25**, although the immobilization efficiency is slightly low.[51] This result may be attributed to the fact that the incorporated 2,3-dimethylbutadiene residue is inert to the racemates and interacts less with them due

Table VII. Effect of introduction of vinyl group to silica gel on immobilization and separation factors on **17**[a]

CSP	CSP-**21**	CSP-**22**	CSP-**16**	CSP-**17**
Silica surface	MA-Silica	MA-Silica	A-Silica	A-Silica
Styrene/Cellulose deriv.	5 wt %	0 wt %	5 wt %	0 wt %
Immobilized cellulose deriv.	97%	70%	86%	50%
Racemates				
3	1.73 (+)	1.66 (+)	1.45 (+)	1.45 (+)
4	1.00	1.13 (−)	1.33 (−)	*ca.* 1 (−)
5	1.19 (+)	1.24 (+)	1.34 (+)	1.25 (+)
6	1.09 (+)	1.07 (+)	*ca.* 1 (+)	*ca.* 1 (+)
7	1.32 (−)	1.29 (−)	1.22 (−)	1.26 (−)
8	1.90 (−)	1.96 (−)	2.18 (−)	1.92 (−)
9	1.23 (+)	1.18 (+)	1.17 (+)	*ca.* 1 (+)
10	1.14 (−)	1.17 (−)	1.22 (−)	1.23 (−)
11	1.12 (+)	1.31 (+)	1.42 (+)	1.40 (+)
12	4.27 (−)	3.34 (−)	2.57 (−)	2.74 (−)

[a]The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Eluent; hexane–2-propanol (90:10). Flow rate; 0.1 mL/min. [Vinyl group]/[AIBN] = 50. Solvent for polymerization; hexane.

Table VIII. Effect of the kind of vinyl monomer on immobilization and separation factors on **18**[a]

CSP	CSP-**23**		CSP-**24**		CSP-**25**
Vinyl monomer	Styrene		2,3-DMBD		Coated **18**
Immobilized cellulose deriv.	99%		88%		
Eluent	**A**	**B**	**A**	**B**	**A**
Racemates					
3	1.90 (+)	1.68 (+)	1.71 (+)	1.63 (+)	1.75 (+)
4	*ca.* 1 (−)	*ca.* 1 (−)	1.20 (−)	1.51 (−)	1.48 (−)
5	1.22 (+)	1.20 (+)	1.27 (+)	1.30 (+)	1.29 (+)
6	1.07 (+)	1.20 (+)	1.15 (+)	1.21 (+)	1.15 (+)
7	1.37 (−)	1.36 (−)	1.30 (−)	1.34 (−)	1.30 (−)
8	1.95 (−)	2.50 (−)[b]	2.16 (−)	2.93 (−)	2.32 (−)
9	1.16 (+)	*ca.* 1 (+)	1.20 (+)	1.0	1.16 (+)
10	1.10 (−)	1.12 (−)	1.18 (−)	1.23 (−)	1.21 (−)
11	*ca.* 1 (+)	Not eluted	*ca.* 1 (+)	Not eluted	*ca.* 1 (+)
12	4.13 (−)	1.80 (−)[b]	3.80 (−)	1.97 (−)	3.15 (−)

[a]The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Eluent; A: hexane–2-propanol (90:10), B: hexane–chloroform–2-propanol (90:10:1). Flow rate; 0.1 mL/min. Silica gel; A-Silica. The content of the vinyl monomer; 10 wt %. [Vinyl group]/[AIBN] = 50. Solvent for polymerization = hexane. [b]Flow rate; 0.2 mL/min.

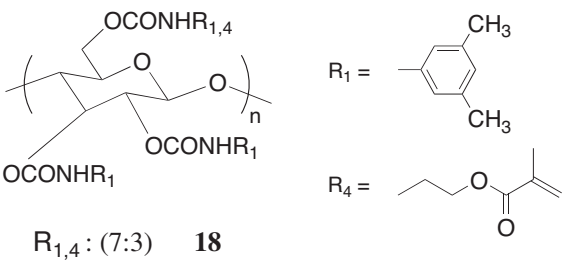

Figure 12. Structure of the cellulose derivative bearing a methacryloyloxy groups.

to the absence of the phenyl group, and the higher order structure of the cellulose derivative is not significantly changed through the immobilization process because of the lower glass-transition temperature of the poly(2,3-dimethylbutadiene) compared to that of polystyrene. Even after washing the immobilized column with 100% chloroform for about 80 h, no polymer was washed out from CSP-**24**, and k'_1 and α values on CSP-**24** for the 10 racemates were hardly changed. Six racemates, **4**–**8** and **10**, were better resolved on CSP-**24** with the eluent containing 10% chloroform than in the resolution without using chloroform.

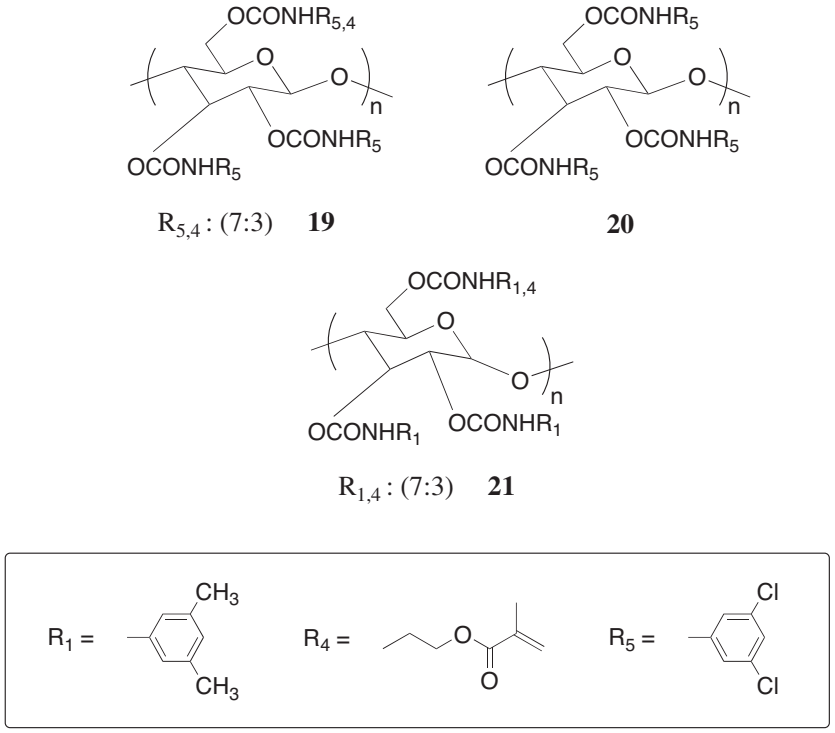

Figure 13. Structures of polysaccharide derivatives for CSPs.

Table IX. Immobilization and separation factors on CSPs immobilized and coated cellulose and amylose derivatives[a]

CSP	CSP-**26**		CSP-**27**	CSP-**28**		CSP-**8**
Polysaccharide deriv.	**19**		**20**	**21**		**2d**
Immobilized polysaccharide deriv.	94%		coated-type	99%		coated-type
Eluent	**A**	**B**	**A**	**A**	**B**	**A**
Racemates						
3	1.34 (+)	1.21 (+)	1.65 (+)	1.34 (+)	1.31 (+)	1.58 (+)
4	1.59 (+)	1.44 (+)	1.84 (+)	1.84 (+)	1.86 (+)	3.04 (+)
5	1.09 (+)	1.12 (+)	1.21 (−)	*ca.* 1 (−)	*ca.* 1 (−)	1.21 (−)
6	*ca.* 1 (+)	1.16 (+)	1.29 (+)	1.76 (+)	1.48 (+)	1.98 (+)
7	1.30 (−)	1.30 (−)	1.26 (−)	*ca.* 1 (−)	*ca.* 1 (−)	*ca.* 1 (−)
8	1.32 (−)	1.71 (−)	1.38 (−)	1.12 (−)	1.33 (−)	1.15 (−)
9	1.52 (+)	1.34 (+)	1.82 (+)	1.0	1.0	1.0
10	1.15 (−)	1.10 (−)	1.20 (−)	1.22 (+)	1.34 (+)	1.12 (+)
11	1.11 (−)	Not eluted	1.41 (+)	1.10 (+)	Not eluted	2.01 (+)
12	1.29 (−)	1.13 (−)	1.11 (+)	1.95 (−)	1.79 (−)	2.11 (−)

[a]The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Eluent; A: hexane–2-propanol (90:10), B: hexane–chloroform–2-propanol (90:10:1). Flow rate; 0.1 mL/min. Silica gel; A-Silica. Vinyl monomer = 2,3-dimethylbutadiene (10 wt %). [Vinyl group]/[AIBN] = 30. Solvent for polymerization = toluene.

This immobilization method has been applied to other cellulose derivative (**19**) and amylose derivative (**21**) (Figure 13).[51] The immobilization onto the A-silica with 2,3-dimethylbutadiene as a vinyl monomer and the chiral recognition ability on the immobilized- and coated-type CSPs are shown in Table IX. Although **20** coated on CSP-**27** gradually disappeared from the A-silica surface due to the dissolution in a hexane–2-propanol mixture,[28] this solvent can be utilized for CSP-**26** as the eluent. When 10 wt % 2,3-dimethylbutadiene was used for the immobilization, 94% of **19** was immobilized on the silica gel, and **21** was almost completely immobilized. Both immobilized CSPs can maintain their high chiral recognition ability and can be used with an eluent containing 10% chloroform. These results indicate that, by this immobilization strategy, the cellulose and amylose phenylcarbamate derivatives are efficiently immobilized on silica gel almost without losing their high chiral recognition abilities.

The immobilization of the cellulose derivatives bearing vinyl groups non-regioselectively at the 2-,

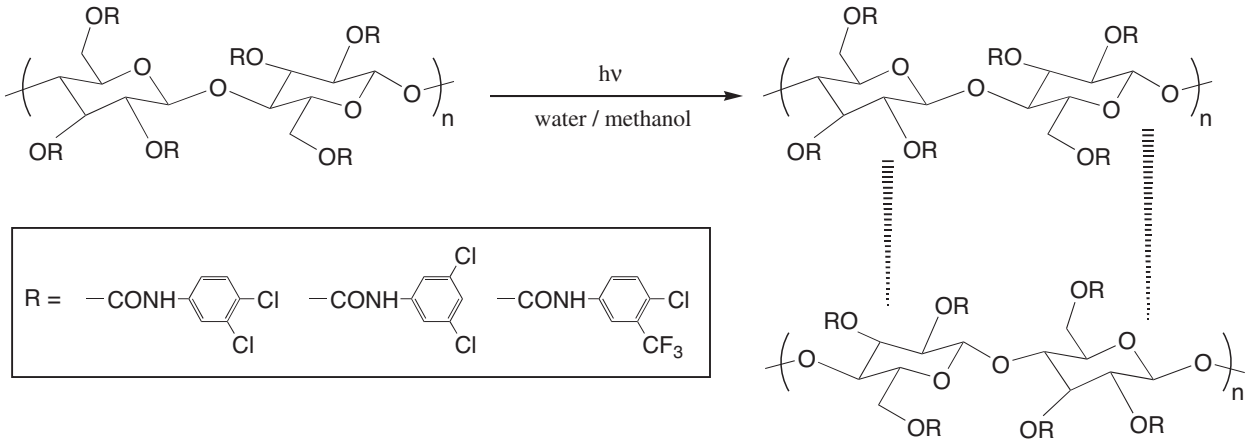

Figure 14. Scheme of photochemical immobilization of polysaccharide derivatives.

3-, and 6-positions of the glucose unit is of course possible.[50] These derivatives can be easily prepared by a one-pot process. The cellulose derivatives with the randomly introduced vinyl groups show an efficiency for immobilization similar to those with a regioselective vinyl group, although the former shows a slightly lower chiral recognition ability than the latter. Therefore, this synthetic method is valuable for the facile preparation and immobilization of cellulose derivatives having a vinyl group on silica gel.

OTHER METHODS

Francotte *et al.* described the thermal and photochemical immobilization of polysaccharide derivatives.[22,52–55] Recently, three halogenated phenylcarbamate derivatives of cellulose have been immobilized onto silica gel by photochemical immobilization and their chiral recognition abilities have been evaluated.[55]

3,4- and 3,5-dichlorophenylcarbamates, and 3-trifluoromethyl-4-chlorophenylcarbamate of cellulose are obtained by the reaction of cellulose with the corresponding halogenophenyl isocyanates. So far, these derivatives had not been used as CSPs due to their high solubility. In order to obtain immobilized CSPs from these derivatives, the derivatives coated on silica gel were irradiated using an immersing UV-mercury lamp in a mixture of water-methanol (Figure 14). Although the photopolymerizable functional groups were not introduced on these cellulose derivatives, the derivatives appeared to be immobilized on silica. The obtained CSPs can be used with eluents such as hexane/2-propanol and heptane/chloroform. The mechanism of immobilization has not yet been elucidated, but cross-linking between the cellulose derivatives may occur during irradiation. Three photochemically immobilized CSPs show relatively high enantioselectivities for a wide range of racemates by selecting the eluents.

A polysaccharide-analogue has been immobilized on silica gel and used as a CSP for the resolution of racemic amino acid derivatives.[56–58] This CSP was prepared by a two-step reaction as shown in Figure 15. A polysaccharide-analogue polyether with a (trimethoxy)silyl end-group was prepared by anionic polymerization, and the resulting polymer was then immobilized onto silica gel by the reaction of (trimethoxy)silyl groups with silanol groups on the silica surface.[56]

The chiral recognition abilities for various amino acid derivatives on the obtained CSPs have been evaluated using aq. $NaClO_4$ (pH 2)/CH_3CN as an eluent. Regardless of the kind of alkoxy substituent in the polymers, the D-isomers are firstly eluted in most cases and the racemates bearing a bulky substituent on the chiral carbon, such as phenylglycine and 1-(1-naphthyl)ethylamine, can be easily resolved. The ethoxy group is more suitable for high enantioselectivity than the methoxy group.[57] The chiral recognition ability seems to depend on the 3,4-di-*O*-substituted alkyl groups in the polymers.

IMMOBILIZATION OF AN AMYLOSE DERIVATIVE AT A CHAIN END

As mentioned above, the polysaccharide derivatives can be immobilized onto silica gel by the method using a diisocyanate and polymerization. However, these immobilized CSPs showed a somewhat lower enantioselectivity than the corresponding coated-type CSPs, particularly when a larger amount of a cross-linker or a vinyl monomer was used. These multisite chemical linkages may disturb the regular higher order structure of the polysaccharide derivatives, which is important for high enantioselectivity.

To overcome this defect mainly due to the disturbance of the regular structure of the polysaccharide derivatives, amylose was chemically bonded to silica

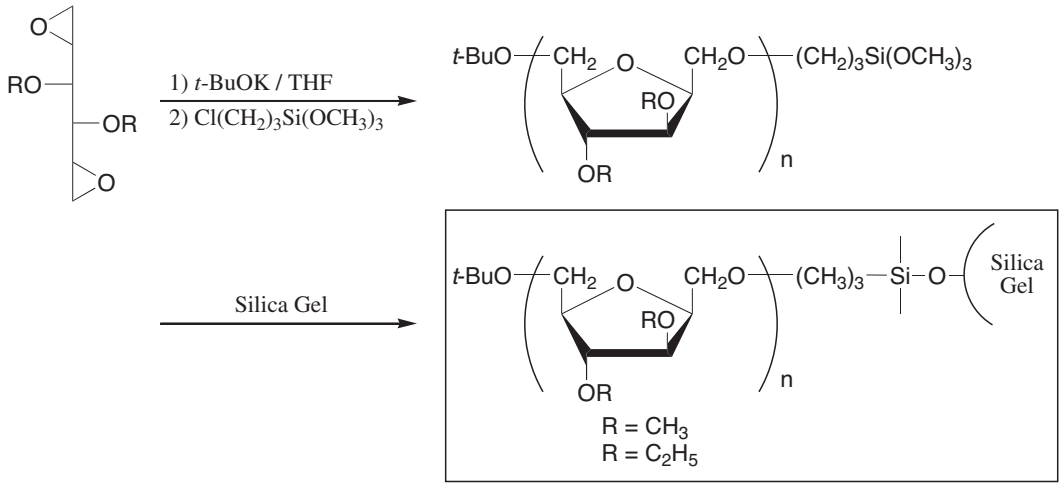

Figure 15. Scheme of immobilization of (1→6)-2,5-anhydro-D-glucitol derivatives on silica gel.

Method I

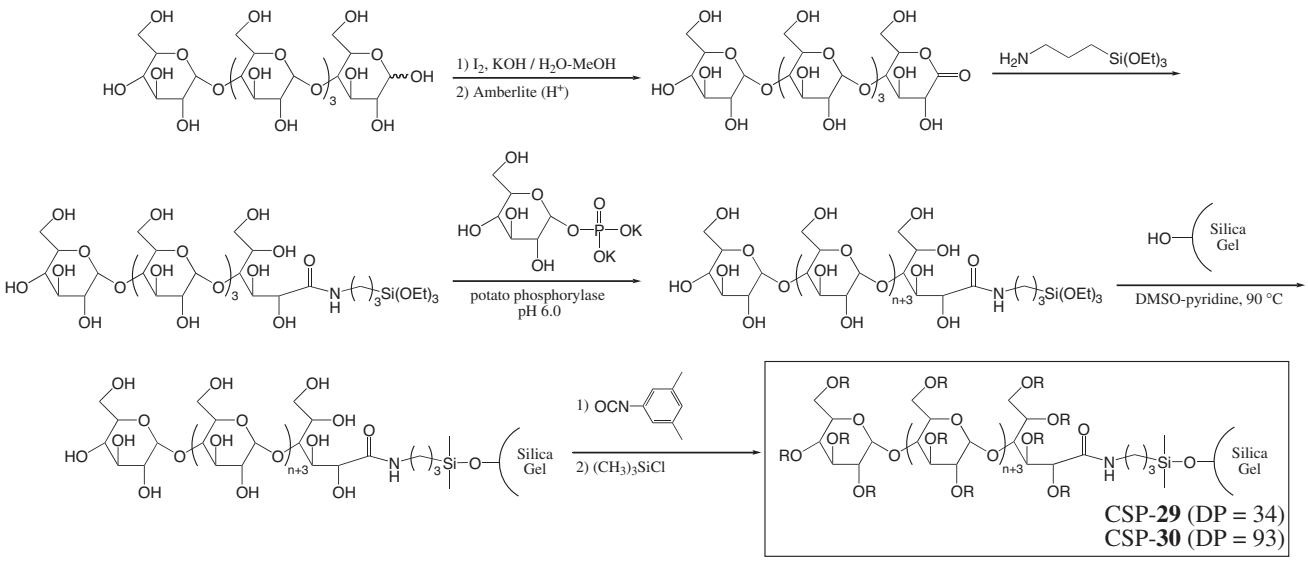

Figure 16. Scheme of immobilization of amylose derivative to silica gel only at the reducing terminal residue (Method I).

gel only at a reducing terminal residue.[59,60] This immobilization method consists of two steps;[59] firstly, amylose was prepared by the enzymatic polymerization of α-D-glucose 1-phosphate dipotassium catalyzed by a potato phosphorylase using two kinds of primers derived from maltopentaose.[61–63] The amylose chains, which have a desired chain length and a narrow molecular mass distribution, are then bonded to silica gel to be used as CSPs. Two kinds of preparation methods, Methods I and II, are described in Figures 16 and 17, respectively. In Method I, maltopentaose is lactonized for reaction with 3-aminopropyltriethoxysilane. Amylose chains are then extended by enzymatic polymerization, and the resulting amylose bearing a triethoxysilyl group at the terminal residue is allowed to react with silica gel for immobilization. In Method II, maltopentaose is first oxidized to be converted into a potassium gluconate at a reducing terminal residue, and the enzymatic polymerization is then performed. After the lactonization, the amylose is immobilized onto the A-silica. Finally, both the amylose-conjugated silica gels are treated with a large excess of 3,5-dimethylphenyl isocyanate to derivatize the remaining hydroxyl groups of amylose to the carbamates.

These methods have two big advantages; one is that the immobilization onto silica gel only at the reducing terminal residue of amylose can be achieved, and the other is that amylose with a desired chain length and a narrow molecular mass distribution can be easily prepared. In particular, the former feature is important for maintaining the regular higher order structure of the amylose derivative.

Four immobilized CSPs having different degrees of

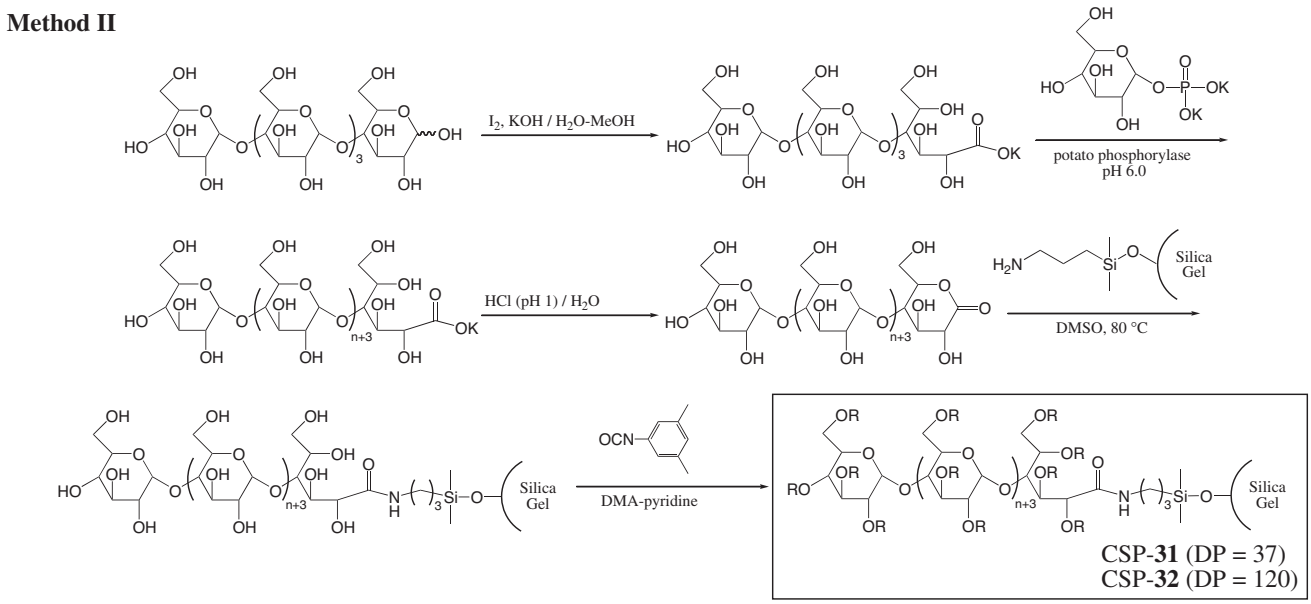

Figure 17. Scheme of immobilization of amylose derivative to silica gel only at the reducing terminal residue (Method II).

Table X. Separation factors on CSPs immobilized to silica gel only at the reducing terminal residue of amylose[a]

CSP	CSP-**29** (Method I, DP = 34)	CSP-**31** (Method II, DP = 37)	CSP-**30** (Method I, DP = 93)	CSP-**32** (Method II, DP = 120)	CSP-**8** (coated **2d**)
Racemates					
3	1.19 (+)	1.44 (+)	1.10 (+)	1.56 (+)	1.58 (+)
4	1.85 (+)	1.96 (+)	2.03 (+)	2.56 (+)	3.04 (+)
5	*ca.* 1 (−)	*ca.* 1 (−)	1.08 (−)	1.07 (−)	1.21 (−)
6	1.76 (+)	2.01 (+)	1.81 (+)	2.18 (+)	1.98 (+)
7	*ca.* 1 (−)	*ca.* 1 (−)	*ca.* 1 (−)	*ca.* 1 (−)	*ca.* 1 (+)
9	*ca.* 1 (−)	*ca.* 1 (+)	*ca.* 1 (−)	*ca.* 1 (+)	*ca.* 1 (−)
10	1.08 (+)	1.23 (+)	1.10 (+)	1.33 (+)	1.12 (+)
11	1.24 (+)	1.33 (+)	1.44 (+)	2.15 (+)	2.01 (+)
12	1.61 (−)	1.50 (−)	1.83 (−)	1.68 (−)	2.11 (−)

[a]The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Eluent; hexane–2-propanol (90:10). Flow-rate; 0.5 mL/min.

polymerization (DP) of amylose have been prepared by Methods I and II, and their chiral recognition abilities are summarized in Table X. The results indicate that the chiral discrimination ability depends on both the DP of amylose and the preparation method. The chiral recognition ability increases with an increase in the DP of amylose, and the CSPs prepared by Method II are superior to those by Method I if the DPs are similar. Methods I and II differ only in the surface treatment processes. The silica surface in Method I has been end-capped with trimethylsilyl chloride, while that in Method II has been end-capped with 3,5-dimethylphenyl isocyanate.

Among the four immobilized CSPs, CSP-**32** with the largest DP shows the highest enantioselectivity. Additionally, the chiral recognition ability of CSP-**32** is comparable to that of coated-type CSP-**8**, and some racemates are even better resolved on CSP-**32**. Because a higher order structure of the amylose derivative may be maintained during the immobilization process due to the chemical bonding to silica gel, the chiral recognition of CSP-**32** seems to be as high as that of the coated-type CSP-**8**. These immobilized CSPs have a greater durability when exposed to various polar solvents and can be used with an eluent consisting of only THF without any damage.

ENANTIOSEPARATIONS WITH ELUENTS CONTAINING $CHCL_3$

Because the immobilized-type CSPs can be used with eluents containing $CHCl_3$ and THF, some racemates which are not resolved by a coated-type CSPs can be separated into enantiomers.[64–76] For example, topologically interesting catenanes,[67] molecular knots,[71,72] and knotaxane,[73] which is rotaxane with knots as stoppers, have been completely resolved on CSP-**32** using a hexane/chloroform/2-propanol mix-

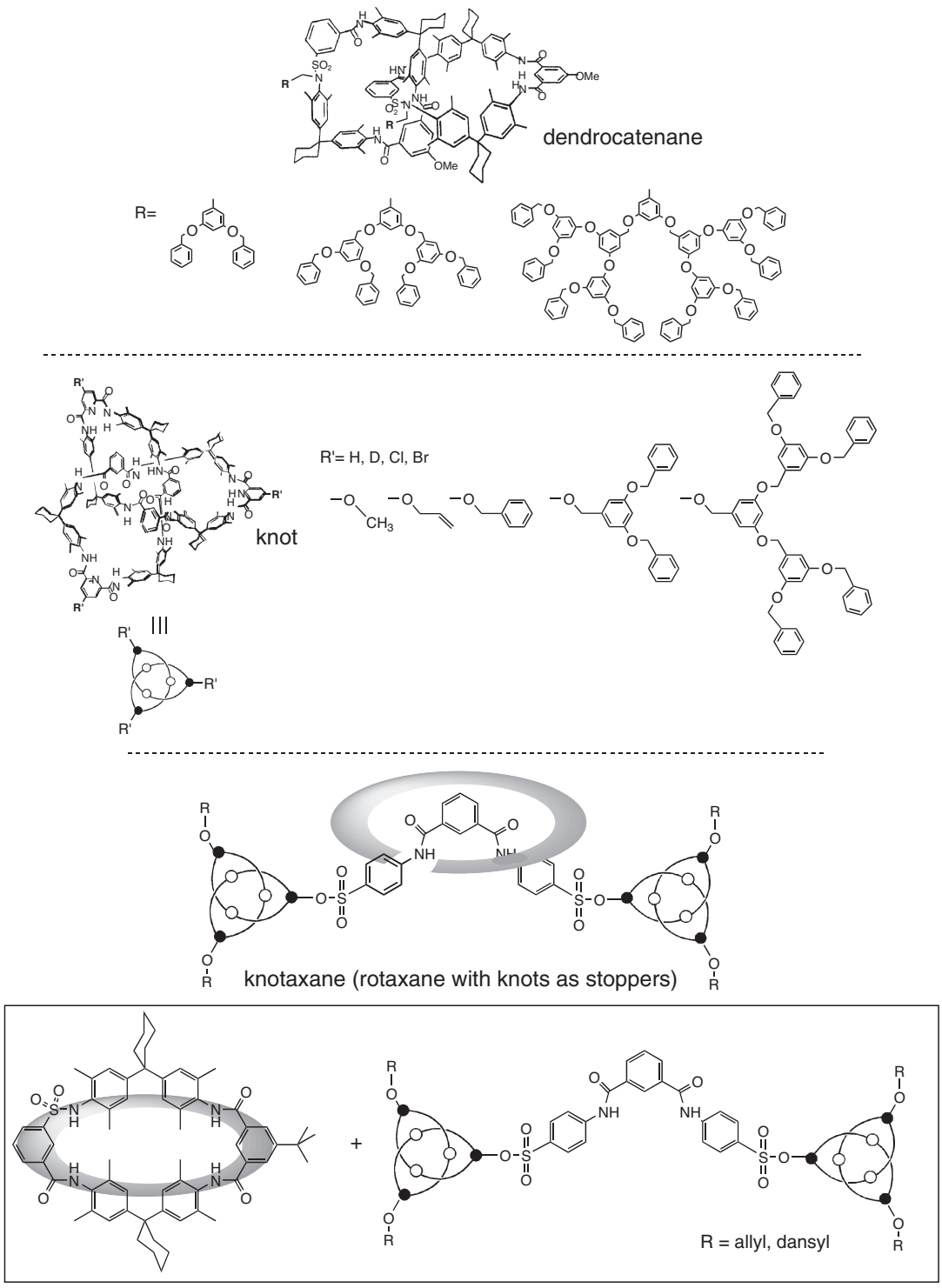

Figure 18. Structures of catenanes, knots, and knotaxane.

ture (Figure 18). In addition, the linear, branched, and cyclic knotane oligomers can be sufficiently resolved with an eluent containing chloroform (Figure 19).[74,75] This CSP achieved the first direct HPLC enantioseparation of the chiral fullerene C_{76} using a hexane/chloroform mixture (80:20) as the eluent.[76] In this case, to increase the enantiomeric purity, recycling has been applied because of the low degree of resolution.

CONCLUSIONS

Several methods for preparing immobilized polysaccharide-based CSPs have been reviewed. The immobilization of polysaccharide derivatives provides the following advantages: 1) expansion of the kind of solvents which can be used as the eluent and the injection solvent; 2) improvement of the chiral recog-

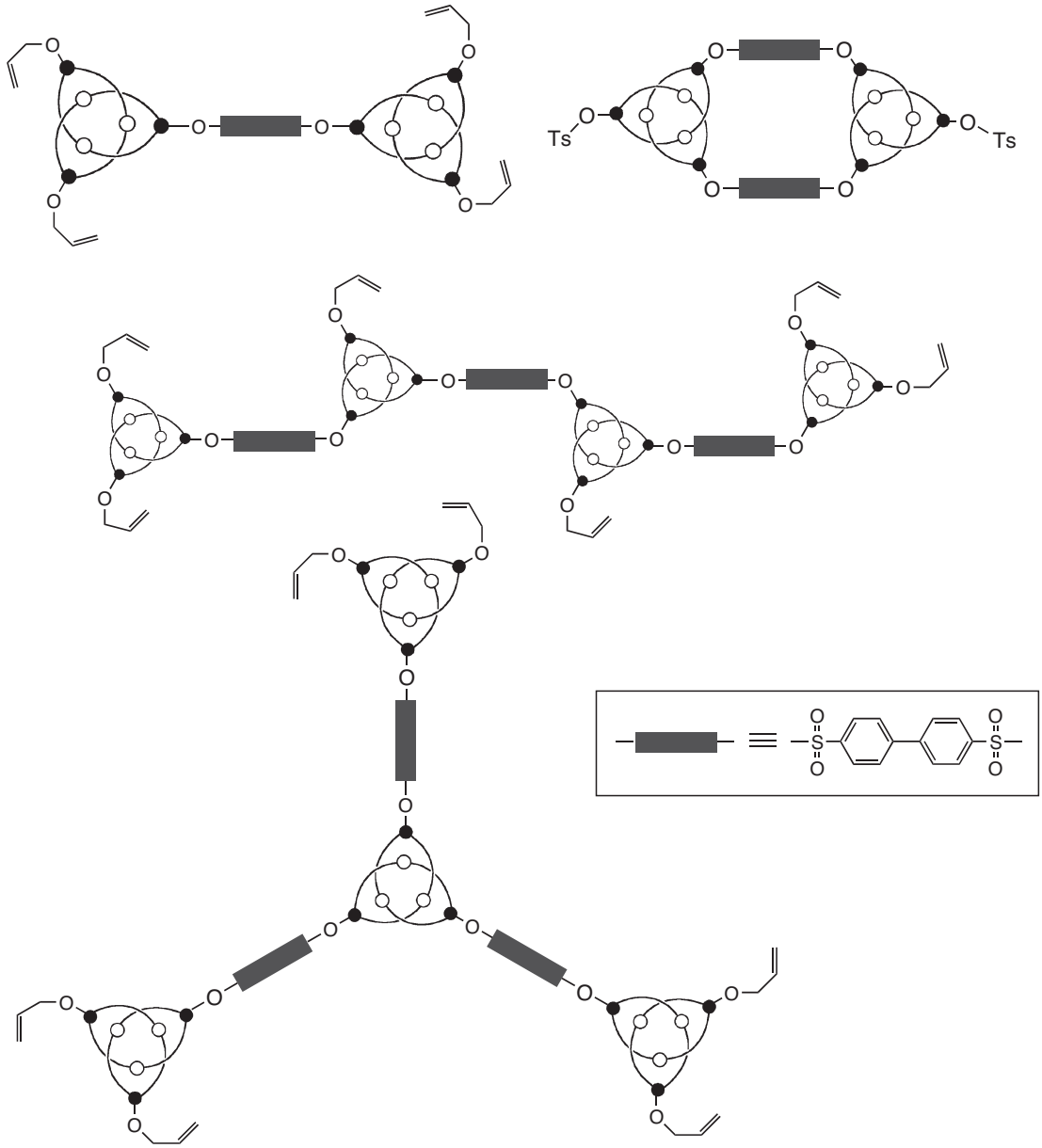

Figure 19. Structures of molecular knots with linear, branched, and cyclic architectures.

nition ability in the analytical separation; 3) increase in the loading capacity in the preparative separation; and 4) long life of the column. We expect that the immobilized CSPs will become more popular and will replace the coated-type CSPs.

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