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Structure Control of Polysaccharide Derivatives for Efficient Separation of Enantiomers by Chromatography

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Contents

1. Introduction 6077
2. Landmarks in the Development of Polysaccharide-Based CSPs 6080
3. Esters of Cellulose and Amylose 6081
4. Cellulose Phenylcarbamates 6081
5. Amylose Phenylcarbamates 6083
6. Other Polysaccharide Carbamates 6084
7. Regioselectively Substituted Polysaccharide Derivatives 6085
8. Compounds Resolved by Polysaccharide Derivatives 6086
9. Chiral Recognition Mechanism on Polysaccharide Derivatives 6086
10. Separation by Chiral SFC 6089
11. Immobilization of Polysaccharide Derivatives onto Chromatographic Supports 6090
12. Chiral Separations on Monolithic Silica Columns Containing Polysaccharide Derivatives 6094
13. Preparative Separation Using Polysaccharide-Based Chiral Packing Materials 6096
14. Conclusions 6097
15. Acknowledgments 6097
16. Note Added after ASAP Publication 6098
17. References 6098

1. Introduction

Chirality is a ubiquitous feature in living systems. Today, it is widely recognized that many biologically interesting compounds, such as drugs, agrochemicals, food additives, and fragrances, are chiral and their physiological properties usually rely on their chirality due to the extremely high chiral discrimination ability of enzymes and receptors.[1−6] Particularly, the different pharmacological effects between enantiomers are ultimately an important concern in the pharmaceutical field in which only one enantiomer of a chiral drug often exhibits the desirable therapeutic activity, while the other shows an antagonistic function, side effects, or even toxic effects.[7−14]

During the late 1950s and early 1960s, a racemate of *N*-phthalylglutamic acid imide, which is a sedative and hypnotic drug known as thalidomide, was administrated to pregnant women and caused the birth of approximately 10 000 babies with malformations. Later, Blaschke pointed out that this teratogenic effect was attributed to the *S*-(−)-isomer.[15] Even though the administration of the pure *R*-(+)-isomer could not halt the disaster because thalidomide is unstable in the body and easily undergoes racemization,[16−18] this tragedy undoubtedly brought a profound movement in the drug administration and industries. In 1992, the U.S. Food and Drug Administration issued a specific guideline for the production of new chiral drugs,[19] which demanded a systematic investigation of the biological behavior of their individual enantiomers and significantly encouraged the development of single enantiomer drugs.[20−22] Today, most of the best-selling drugs around the world are administered as single enantiomers with the desired therapeutic activity,[23] and the annual sales of single enantiomer drugs are expected to exceed 200 billion dollars in 2008. Furthermore, the preparation of single enantiomers has also become important in the fields of functional materials, such as ferroelectric liquid crystals and organic nonlinear optical molecules.[24−26]

Based on this historical background, substantial efforts have been undertaken to develop practical techniques for the preparation of enantiomers with a high enantiomeric excess (ee). In general, two approaches are utilized for this objective, asymmetric synthesis and chiral separation. Asymmetric synthesis using chiral sources, such as chiral pools, chiral auxiliaries, asymmetric catalysts, and enzymes, has significantly progressed over the last few decades.[27−41] Although the large-scale preparation of enantiomers can be economically attained using this approach, the products do not always show a high ee, and therefore, a further purification step may be inevitable. Additionally, nature produces only one of the enantiomers as a chiral source in most cases. This means that if both enantiomers of the target compounds are required, at least two kinds of chiral sources are necessary for each enantiomer. However, it is sometimes difficult to obtain both of them.

On the other hand, the chiral separation approach can easily provide both enantiomers with a high ee. Since Pasteur first isolated two enantiomers of sodium ammonium tartrate using a magnifying glass and a pair of tweezers in 1848,[42,43] the innovation of chiral separation techniques has attracted great interest. Basically, chiral separations[44] are carried out by (i) crystallization,[45−47] (ii) enzymatic kinetic resolution,[48−50] and (iii) chromatographic separation.[51−53]

Specifically, direct chiral separation using chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC) has significantly evolved during the past few decades and is recognized as the most popular and reliable tool for both the analysis of enantiomer compositions and the preparation of pure enantiomers.[51−64] Chiral separations can

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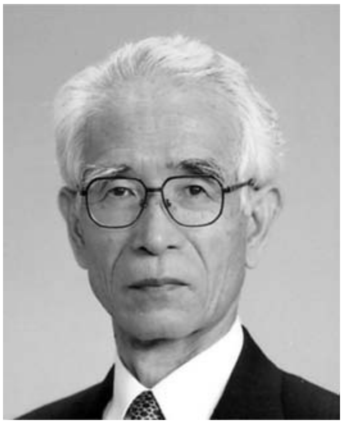


Yoshio Okamoto received his bachelor (1964), master (1966), and doctrate (1969) degrees from Osaka University, Faculty of Science. He joined Osaka University, Faculty of Engineering Science, as Assistant Professor in 1969, and spent two years (1970−1972) at the University of Michigan as a postdoctoral fellow with Professor C. G. Overberger. In 1983, he was promoted to Associate Professor, and in 1990, he moved to Nagoya University as Professor. In 2004, he retired from Nagoya University, and from then until March 2009, he had been Guest Professor of EcoTopia Science Institute, Nagoya University. He has also been appointed as the Chair Professor of Harbin Engineering University in China since 2007. His research interests include stereocontrol in polymerization reaction, asymmetric polymerization, optically active polymers and enantioseparation by HPLC. He received The Awards of the Society of Polymer Science, Japan (1982), The Chemical Society of Japan Award for Technical Development for 1991, The Chemical Society of Japan Award for 1999, Molecular Chirality Award (1999), Chirality Medal (2001), Medal with Purple Ribbon (Japanese Government) (2002), Fujiwara Prize (2005), and Thomson Scientific Research Front Award 2007 (2007), among others.

be achieved using chiral mobile phases for the HPLC.[65,66] One of the advantages of this method is that the association constant between enantiomers and chiral selectors can be estimated by changing the concentration of chiral selectors in mobile phases.[67,68] However, this approach is not mainstream due to the disadvantages of the inconvenience of preparative purpose. Besides HPLC, supercritical fluid chromatography (SFC) has received increasing attention and has played a dominant role in the progress of chromatographic chiral separation due to its high resolution, low organic solvent consumption, short analysis time, and high productivity.[69−77] In addition, capillary electrophoresis, capillary electrochromatography, and microchip techniques have also been developed for small-scale separations and used for the microanalysis of chiral compounds.[78−86] Furthermore, the recent incremental growth of simulated moving bed (SMB) chromatography has opened up the possibility for industrial-scale chromatographic separations of chiral compounds, which is one of the final goals of the pharmaceutical industry.[87−92]

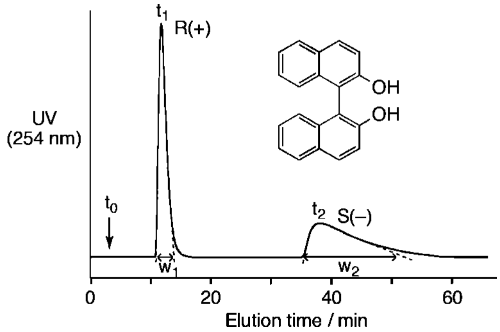


Figure 1. Chiral separation of *rac*-1,1′-bi-2-naphthol: CSP = cellulose 5-fluoro-2-methylphenylcarbamate; column, 25 cm × 0.46 cm (i.d.); eluent = hexane/2-propanol (90/10); flow rate = 1.0 mL/min. Reprinted with permission from ref 93. Copyright 1996 American Chemical Society.

Chiral separation using CSPs by HPLC is based on the occurrence of labile diastereomeric complexes with different energies on the CSPs. The interactions utilized for the chiral separations are generally hydrogen bonding, $\pi-\pi$ interactions, dipole−dipole interactions, steric hindrance, hydrophobic interactions, and electrostatic interactions. Figure 1 shows a typical chromatogram for the HPLC separation of enantiomers, in which the (+)-isomer elutes first, followed by the (−)-isomer, and baseline separation is accomplished.[93] The signs of the optical rotation in this manuscript are based on those obtained at 589 nm in hexane/2-propanol (90/10). The chiral recognition ability of a CSP can be quantitatively estimated using the following three factors: retention factor (k), separation factor (α), and resolution factor (R_s), which are defined as follows:

$$k_1 = (t_1 - t_0)/t_0 \tag{1}$$

$$k_2 = (t_2 - t_0)/t_0 \tag{2}$$

$$\alpha = (t_2 - t_0)/(t_1 - t_0) = k_2/k_1 \tag{3}$$

$$R_s = 2(t_2 - t_1)/(w_1 + w_2) \tag{4}$$

In these formulas, t_0, t_1, and t_2 are the retention times of a nonretained compound, first-eluted enantiomer, and second-eluted enantiomer, respectively, and w_1 and w_2 are the peak widths at the bases. For the chromatographic separations, k represents the degree of interaction between a CSP and a corresponding enantiomer, α is directly correlated to the recognition ability of a CSP, and R_s shows both the recognition ability of a CSP and the theoretical plate number of a column. The energy difference ($\Delta\Delta G$) in the interactions between a CSP and an individual enantiomer can be evaluated from the α value ($\Delta\Delta G = -RT \ln \alpha$). The selection of a suitable CSP, which produces a sufficient $\Delta\Delta G$, is essential for successful separations. Usually, the

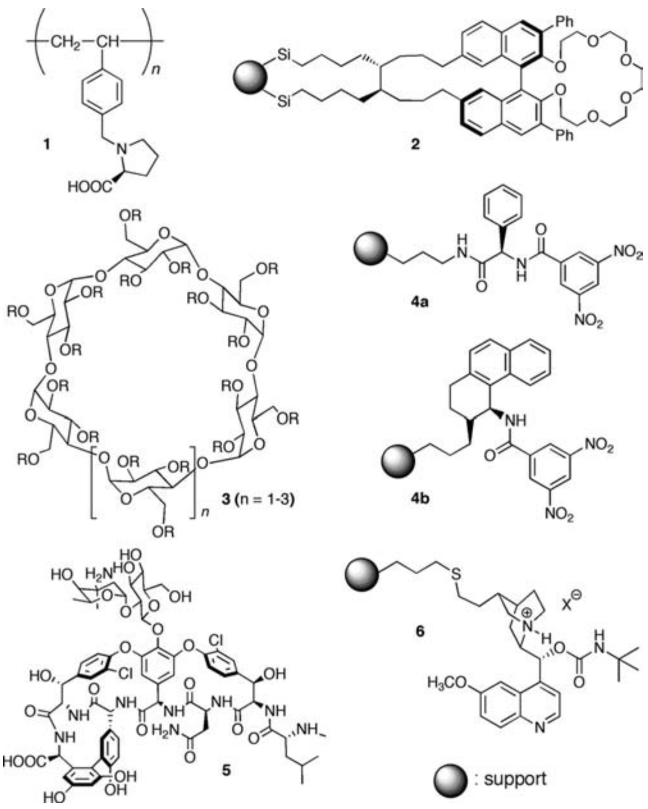


Figure 2. Structures of molecular-type CSPs.

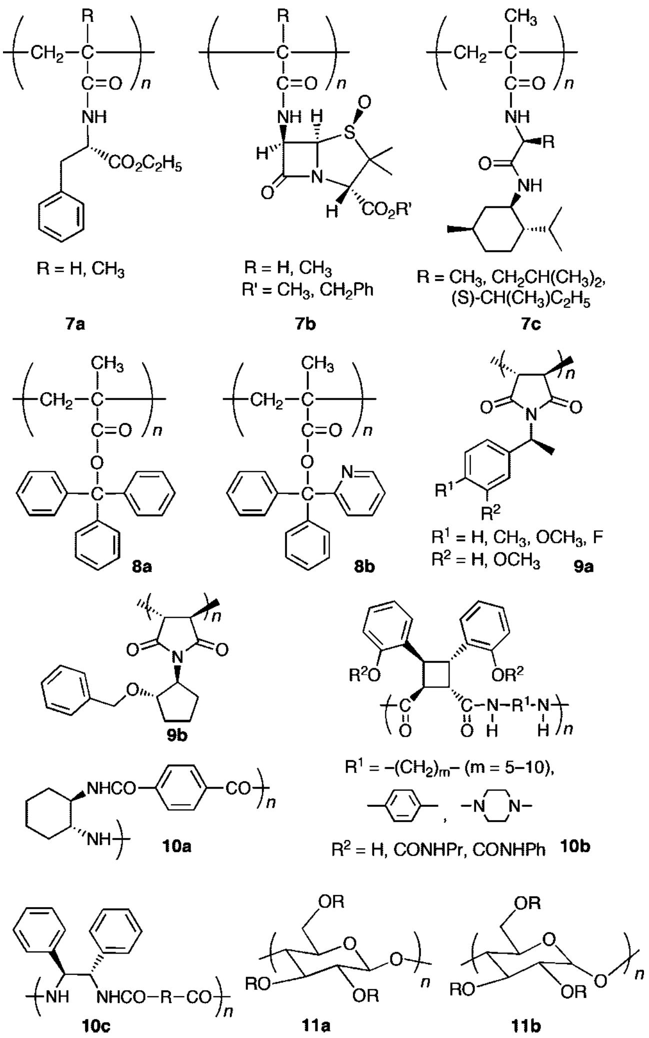


Figure 3. Structures of polymer-type CSPs.

baseline separation of the enantiomers can be achieved at a very small energy difference ($\Delta\Delta G = -0.46$ kJ/mol corresponding to $\alpha = 1.20$). This is one of the reasons chromatographic separation using a CSP can be used for a wide range of chiral compounds.

Up to now, the development of powerful CSPs with high chiral recognition abilities, wide applicability, and high loading capacity for preparative separation has attracted much attention, and the number of commercially available CSPs exceeds 100. These CSPs for HPLC can be prepared using both chiral molecules and polymers with chiral recognition abilities.

Since Davankov et al. accomplished the first baseline separation of enantiomers using molecular-type CSPs in 1971,[94] a wide range of chiral compounds, which include an amino acid (**1**),[94−96] a crown ether (**2**),[97−99] a cyclodextrin (**3**),[100−104] π-basic or π-acidic aromatic compounds (**4**),[105−113] a macrocyclic glycopeptide (**5**),[114−116] and a cinchona alkaloid (**6**),[117−119] have been used as CSPs (Figure 2). This type of CSP is usually immobilized on silica gel or organic polymer gel as achiral supports (brush-type CSPs). The polymer-type CSPs are further divided into synthetic and natural chiral polymers.[54,120] Typical examples of the synthetic polymers are a molecularly imprinted polymer gel,[121,122] poly(meth)acrylamide (**7**),[123−130] polymethacrylate (**8**),[131−135] poly(maleimide) (**9**),[136−138] and polyamide (**10**),[139−144] and those of the natural polymers include a polysaccharide derivative (**11**)[145−151] and a protein[152−157] (Figure 3). Except for cross-linked polymer gels, these polymers are usually coated on silica gel in order to improve their mechanical strength and resolution efficiency.

In the 1970s, the purity (ee) of chiral compounds was usually estimated by the measurement of their optical activity with a polarimeter having a low sensitivity. In this method, one had to compare the observed optical activity with that of the pure enantiomer, which was usually difficult to prove. Therefore, it sometimes occurred that though a compound was regarded to be enantiomerically pure, later it was not the case, and the optical purity exceeded 100%. This determination method has almost been completely replaced during the last 30 years, and recently, three main methods consisting of NMR, gas chromatography, and HPLC with CSPs have been mainly utilized. Among them, the HPLC method is the most popular, and more than 50% of the determinations have been carried out by chiral HPLC.[158] Figure 4 shows the proportion of CSPs used for the ee determination by chiral HPLC that appeared in the *Journal of the American Chemical Society* in 2005 and 2007. These circle graphs show that approximately 90% of the ee determinations are carried out using the polysaccharide-based CSPs. In addition, the polysaccharide-based CSPs have often been used for the large-scale purification of many commercial chiral drugs in SMB chromatography.[159] From the viewpoint of these recent trends in chiral HPLC, the polysaccharide-based CSPs have been recognized as the most powerful ones for both analytical and preparative separations.[145−151]

In this review, we will present the development and application of polysaccharide-based CSPs for the efficient separation of enantiomers. First, the development of the polysaccharide-based CSPs with a high recognition ability through their structure control is comprehensively described after a brief history of advances in the polysaccharide-based CSPs. The chiral recognition mechanism of the polysaccharide-based CSPs is then thoroughly discussed. Finally, the

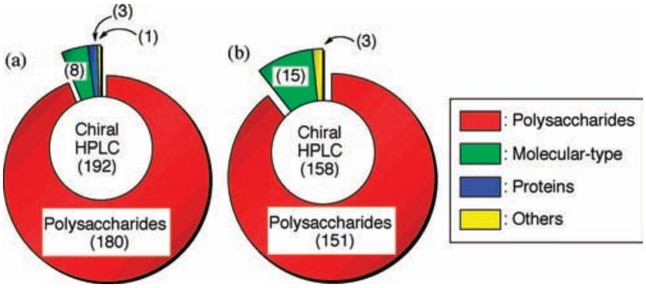


Figure 4. Distribution of CSPs for HPLC used for the determination of enantiomeric excess reported in the *Journal of the American Chemical Society* in 2005 (a) and 2007 (b). The values in parentheses represent the number of the counted papers. Reprinted with permission from refs 64 and 158. Copyright 2008 The Royal Society of Chemistry and 2007 International Union of Pure and Applied Chemistry.

Table 1. Landmarks in the Development of Polysaccharide-Based CSPs and Others for HPLC[a]

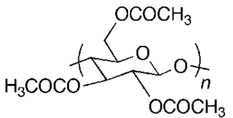
year	landmarks
1938	**chromatographic resolution by lactose** (Henderson, Rule)
1944	**resolution of Tröger base by lactose** (Prelog et al.)
1951	**resolution by paper chromatography using cellulose** (Kotake et al.)
1952	three-point rule (Dalgliesh)
1966	resolution by gas chromatography (Gil-Av et al.)
1971	ligand exchange chromatography (Davankov et al.)
1972	molecular imprinted method (Wulff, Sarhan)
1973	**microcrystalline cellulose triacetate** (Hesse, Hagel)
	protein-based CSPs (Stewart, Doherty)
1974	polyacrylamide-based CSPs (Blaschke)
1975	crown ether-based CSPs (Cram et al.)
1978	**resolution by potato starch** (Musso)
	cyclodextrin-based CSPs (Harada et al.)
1979	pirkle-type CSP (Pirkle, House)
	helical poly(triphenylmethyl methacrylate) (Okamoto et al.)
1984	**cellulose tribenzoate and trisphenylcarbamate** (Daicel, Okamoto)
	commercialization of cellulose-based CSPs (Daicel)
	immobilized CSP based on cyclodextrin (Armstrong)
1986	**cellulose tris(3,5-dimethylphenylcarbamate)** (Okamoto et al.)
1987	**amylose tris(3,5-dimethylphenylcarbamate)** (Okamoto et al.)
	immobilized CSP based on polysaccharide phenylcarbamate (Okamoto et al.)
1994	macrocyclic glycopeptide-based CSPs (Armstrong et al.)
1995	tartardiamide-based CSPs (Allenmark et al.)
1996	cinchona alkaloid-based CSPs (Lindner, Lämmerhofer)
2004	**commercialization of immobilized CSP based on polysaccharide phenylcarbamate** (Daicel)

[a] Boldface lines are related to the development polysaccharide-based CSPs.

current applications of polysaccharide-based CSPs, such as a chiral separation in SFC, immobilization of polysaccharide derivatives, use of the monolithic silica column, and preparative separation, are summarized.

2. Landmarks in the Development of Polysaccharide-Based CSPs

During the early studies of the chromatographic resolution of enantiomers, some polysaccharides and oligosaccharides were used as chiral adsorbents. Significant landmarks in the studies and development using polysaccharides and their derivatives are summarized in Table 1, together with the development of other phases for liquid chromatography. In 1938, Henderson and Rule reported the partial chromatographic resolution of *p*-phenylene bis(imino camphar) using a disaccharide lactose,[160] and in 1944, Prelog succeeded in the partial resolution of Tröger base using the same sugar.[161] Kotake resolved amino acid derivatives by paper chromatography in 1951.[162,163] Musso succeeded in the complete resolution of biphenyl derivatives using potato starch.[164] Wulff also demonstrated the effectiveness of amylose in the resolution of menthol.[165] More recently, microcrystalline cellulose has also been used as a CSP to completely resolve amino acids by liquid chromatography.[166,167] However, the performances of these saccharides except for the cyclic oligomers, that is, the cyclodextrins,[100−104] are not adequate enough to be used as CSPs for HPLC. Although the CSPs based on cyclodextrins have been commercialized, these are not popular as shown in Figure 4.



12 (CTA-I)

Figure 5. Structure of cellulose triacetate **12** (**CTA-I**).

More practically useful materials are obtainable through modification of these saccharides, particularly cellulose and amylose. In 1973, the first practical CSP derived from polysaccharides was reported by Hesse and Hagel.[168,169] They found that the microcrystalline cellulose triacetate, CTA-I (**12** in Figure 5), which was synthesized by heterogeneous acetylation of the native microcrystalline cellulose, showed a meaningful chiral recognition ability in liquid chromatography. The crystalline structure of CTA-I is believed to preserve that of the native cellulose (form I)[169,170] and the recognition ability seems to be derived from its crystalline structure. CTA-I has been employed for the chiral separations of a variety of compounds, such as nonpolar or less polar compounds and aromatic pharmaceuticals, using an ethanol and water mixture as the eluent.[124,125,171] Due to its high loading capacity, CTA-I is still used today for the large-scale chiral separation in medium-pressure liquid chromatography.[172−176] Interestingly, once CTA-I is dissolved in a solvent and coated on silica gel, its recognition ability is completely changed from that of CTA-I.[168,169,171,177,178] For example, the opposite elution order of the enantiomers of Tröger base was observed using the two kinds of cellulose triacetate-based CSPs.[177] The difference in the recognition ability seems to be derived from the change in its crystalline structure. In addition, the latter CSP prepared by coating has significant advantages, such as higher resolution and higher mechanical strength, when compared with CTA-I.[177,178] Based on these early investigations, the study of polysaccharide-based CSPs for HPLC has blossomed since the 1980s.

In 1984, the tribenzoate[177,178] and trisphenylcarbamate[179] of cellulose were reported by our group and Daicel Chemical Industries, and four kinds of polysaccharide-based CSPs, including these two derivatives, were commercialized from Daicel. Cellulose tris(3,5-dimethylphenylcarbamate) (commercial names, Chiralcel OD, Kromasil CelluCoat, RegisCell, Eurocel 01, and Lux Cellulose-1) and amylose tris(3,5-dimethylphenylcarbamate) (commercial names, Chiralpak AD, Kromasil AmyCoat, RegisPack, and Europak 01), which are the most frequently used CSPs, were developed in 1986[180] and 1987,[181] respectively. In 1987, the immobilization of polysaccharide phenylcarbamates onto silica gel was attempted in order to increase its durability against solvent,[182] and the first immobilized CSP based on amylose tris(3,5-

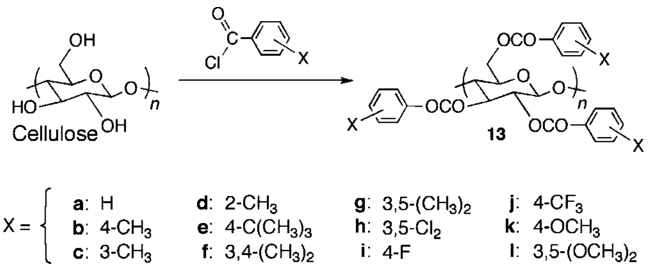


Figure 6. Synthesis and structure of cellulose benzoates (**13**).

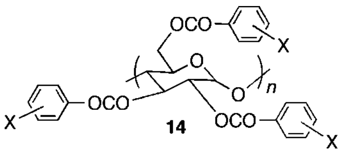


Figure 7. Structure of amylose benzoates (**14**).

dimethylphenylcarbamate), which has a universal solvent applicability, was launched under the trade name Chiralpak IA from Daicel Chemical Industries in 2004.[183] With these polysaccharide-based CSPs, today, nearly 90% of chiral compounds can be successfully separated.[146,183]

3. Esters of Cellulose and Amylose

Since Hesse and Hagel developed a practical CSP based on the microcrystalline cellulose triacetate CTA-I in 1973,[168] various kinds of cellulose esters have been prepared to evaluate their recognition abilities as CSPs for HPLC. Among them, the cellulose benzoates **13** (Figure 6) show high recognition abilities when used as both a microcrystalline form[184] and a coated form on silica gel.[177,178,185] The recognition ability of the benzoate derivatives significantly depends on the nature of the substituents on the phenyl groups. The effect of the substituents, which include alkyl, halogen, trifluoromethyl, and methoxy groups, has been systematically studied.[185] The benzoate derivatives with electron-donating substituents, such as an alkyl group, have a tendency to show a higher recognition ability than those with electron-withdrawing substituents, such as a halogen and trifluoromethyl. The most likely reason for this observation is that the electron density of the carbonyl groups of the cellulose derivatives is significantly influenced through an inductive effect of the substituents on the phenyl groups. However, the electron-donating methoxy group does not work to increase the recognition ability due to the high polarity of the substituent itself. Among these cellulose benzoates, 4-methylbenzoate **13b** exhibits an especially high chiral recognition ability and has been used for the resolutions of a broad range of chiral compounds including drugs.[145,146,186−194] On the other hand, the amylose benzoates (**14** in Figure 7) show much lower recognition abilities than the cellulose derivatives **13**.[195] This may result from the lower conformational stability of the amylose derivatives, which causes many conformational isomers to be formed.

Recently, we found that the recognition ability of cellulose 4-methylbenzoate **13b** can be controlled using additives when the derivative is coated on silica gel.[196] Figure 8 shows the chromatograms of the chrysanthemic acid ethyl ester (**15** in Figure 9), which is the intermediate of effective synthetic pesticides, on the CSPs prepared with or without methyl benzoate (**16**) as an additive. When a CSP was prepared by coating **13b** on silica gel in the absence of the additive, all of the stereoisomers of **15** were eluted at almost the same time. In contrast, the (1*R*)-*trans* isomer, which shows the strongest pesticidal activity, could be very efficiently separated from the other three isomers (α = 7.26) on the CSP prepared in the presence of 10 equiv of **16** to a glucose unit. The additive **16** is expected to interact with the benzoate moieties of **13b** via dipole−dipole and $\pi-\pi$ interactions, which probably affect the orientation of the benzoate moieties and also the higher-order structure of the cellulose 4-methylbenzoate **13b**. A schematic representation of a possible structural change in **13b** after imprinting the additives is shown in Figure 10. This computer simulation result suggests that the orientation of the side chains of **13b** may be changed through the interaction of **13b** with additives **16**. If the **16**-imprinted structure of **13b** is stable, it would be maintained even after the removal of the imprinted molecule **16**.

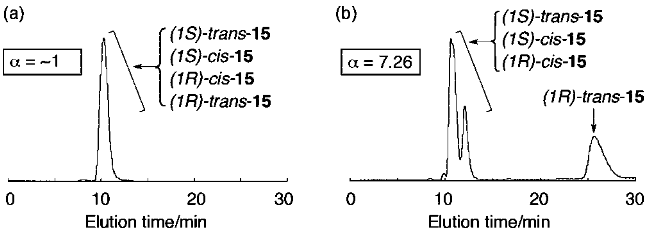


Figure 8. Chromatograms of chrysanthemic acid ethyl ester (**15**) resolved on CPMs prepared from **13b** in the (a) absence and (b) presence of methyl benzoate as additive. Reprinted with permission from ref 196. Copyright 2006 Wiley Periodicals.

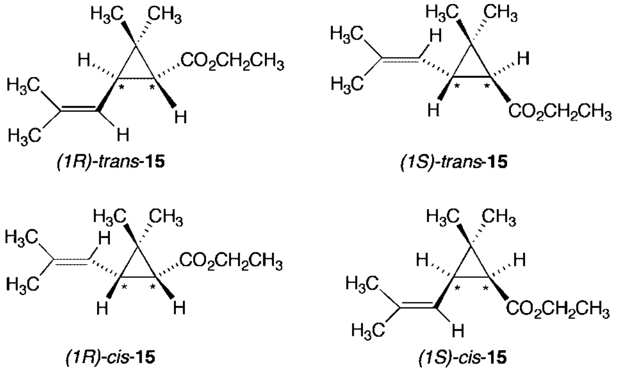


Figure 9. Structures of chrysanthemic acid ethyl ester (**15**). Asterisks indicate asymmetric carbons.

4. Cellulose Phenylcarbamates

Cellulose phenylcarbamate derivatives **17** (Figure 11) are also useful chiral selectors for HPLC when coated on silica gel.[179,180] The recognition abilities of these derivatives can significantly change depending on the substituents on the phenyl groups. The resolution results of ten racemates, **19**−**28** (Figure 12), on the nine *para*-substituted phenylcarbamates of cellulose are given in Table 2.[180] The substituents on the phenyl group are arranged in the order of their increasing electron-donating powers from left to right. Compared with the nonsubstituted cellulose derivative (**17g**), the phenylcarbamates bearing electron-withdrawing substituents, such as halogens, or electron-donating substituents, such as alkyl groups, exhibit better chiral recognitions. These substituents appear to perturb the polarity of the carbamate group via an inductive effect and affect the interaction mode between the cellulose derivatives and the chiral analytes.

When the electron-withdrawing substituents are introduced on the phenyl groups, the acidity of the NH proton of the carbamate groups becomes high. Therefore, the retention time

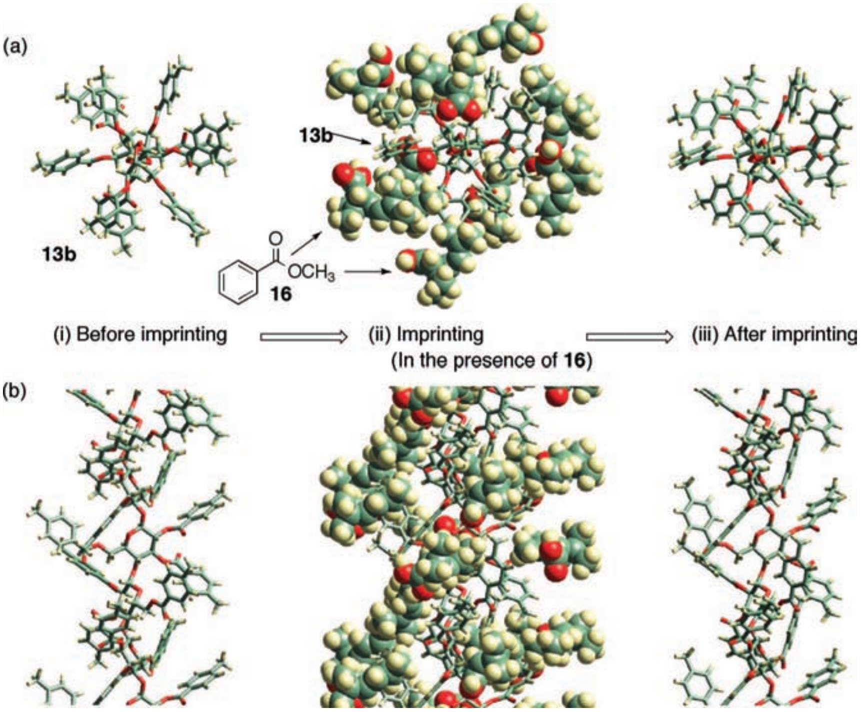
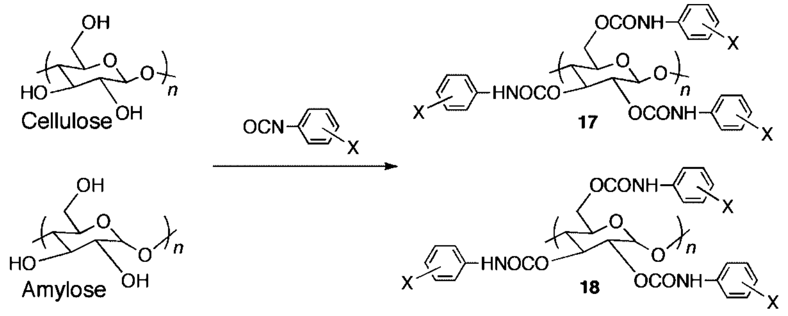


Figure 10. Schematic representation of the structural change of **13b** through the interaction between **13b** and **16**. View (a) perpendicular to the helix axis and (b) along the helix axis. Compounds **13b** and **16** are represented by cylinders and VDW spheres, respectively. Reprinted with permission from ref 196. Copyright 2006 Wiley Periodicals.



X =

a: 4-NO_2
b: 4-CF_3
c: 4-I
d: 4-Br
e: 4-Cl
f: 4-F
g: H
h: 4-CPh_3
i: 4-Ph
j: 4-$Si(CH_3)_3$
k: 4-$C(CH_3)_3$
l: 4-$CH(CH_3)_2$
m: 4-C_2H_5
n: 4-CH_3
o: 4-OPh
p: 4-$OCH(CH_3)_2$
q: 4-OC_2H_5
r: 4-OCH_3
s: 2-Cl
t: 2-CH_3
u: 2-OCH_3
v: 3-Cl
w: 3-CH_3
x: 3-OCH_3
y: 3-OC_2H_5
z: 3-$OCH(CH_3)_2$
aa: 3,5-$(OCH_3)_2$
ab: 3,5-$(CH_3)_2$
ac: 2,6-$(CH_3)_2$
ad: 3,4-$(CH_3)_2$
ae: 3,5-Cl_2
af: 3,4-Cl_2
ag: 2,6-Cl_2
ah: 3,5-F_2
ai: 3,5-$(CF_3)_2$
aj: 2-Cl-4-CH_3
ak: 5-Cl-2-CH_3
al: 2-Cl-6-CH_3
am: 3-Cl-2-CH_3
an: 3-Cl-4-CH_3
ao: 4-Cl-2-CH_3
ap: 4-Cl-3-CH_3
aq: 5-F-2-CH_3
ar: 3-F-4-CH_3
as: 4-F-3-CH_3
at: 3-F-5-CH_3
au: 3-Cl-5-CH_3
av: 3-Br-5-CH_3

Figure 11. Synthesis and structures of phenylcarbamate derivatives of cellulose (**17**) and amylose (**18**).

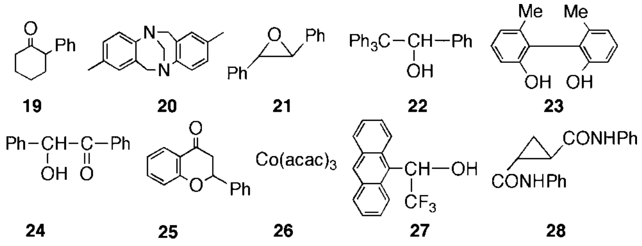
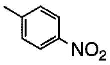
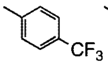
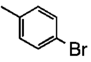
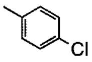
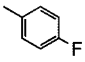
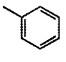
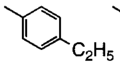
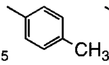
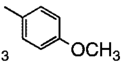


Figure 12. Structures of racemates **19**−**28**.

of acetone on the CSPs with the electron-withdrawing substituents is increased, because acetone is mainly adsorbed on the derivatives through a hydrogen-bonding interaction with the NH groups. In contrast, as the electron-donating power of the substituents on the phenyl group becomes stronger, the electron density at the carbonyl oxygen of the carbamate groups must be higher, and the racemate **27** is more strongly adsorbed on the derivatives through hydrogen bonding with the carbonyl groups. Meanwhile, the derivatives bearing fairly polar substituents on the phenyl groups, such as the nitro (**17a**) or methoxy (**17r**) groups, exhibit a low or almost no recognition ability. Because the nitro or methoxy groups are located far from a chiral glucose unit, these polar groups themselves are expected to cause a nonenantioselec-

Table 2. Separation Factors (α) on the *para*-Substituted Phenylcarbamates of Cellulose[a]

Racemates	 **17a**	 **17b**	 **17d**	 **17e**	 **17f**	 **17g**	 **17m**	 **17n**	 **17r**
19	~1 (–)	1.18 (–)	1.17 (–)	1.16 (–)	1.12 (–)	1.17 (–)	1.19 (–)	1.20 (–)	1.13 (–)
20	~1 (–)	1.23 (+)	1.19 (+)	1.16 (+)	1.14 (+)	1.37 (+)	1.11 (+)	1.48 (+)	~1 (+)
21	1.33 (+)	1.61 (+)	1.70 (+)	1.68 (+)	1.38 (+)	1.46 (+)	1.55 (+)	1.55 (+)	1.34 (+)
22	1.00	1.48 (+)	1.95 (+)	1.95 (+)	1.64 (+)	1.22 (+)	1.59 (+)	1.37 (+)	1.00
23	~1 (+)	2.04 (–)	1.21 (–)	1.20 (–)	1.17 (–)	1.65 (–)	1.33 (–)	1.30 (–)	1.15 (–)
24	1.00	1.10 (–)	1.13 (–)	1.20 (–)	1.14 (–)	~1 (+)	1.14 (–)	1.12 (–)	~1 (+)
25	1.00	1.14 (+)	1.13 (+)	1.12 (+)	1.13 (+)	1.10 (+)	1.22 (–)	1.16 (+)	~1 (+)
26	~1 (+)	2.06 (+)	1.79 (+)	1.46 (+)	1.53 (+)	1.24 (+)	1.76 (+)	1.75 (+)	~1 (+)
27	~1 (+)	1.30 (–)	1.29 (–)	1.29 (–)	1.26 (–)	1.45 (–)	1.57 (–)	1.52 (–)	1.35 (–)
28	~1 (+)	1.22 (–)	1.17 (–)	1.44 (–)	~1 (–)	1.45 (–)	2.12 (–)	1.35 (–)	1.00

[a] Column = 25 cm × 0.46 cm (i.d.); flow rate = 0.5 mL/min; eluent = hexane−2-propanol (90:10). The signs in parentheses represent the optical rotation of the first-eluted enantiomer.

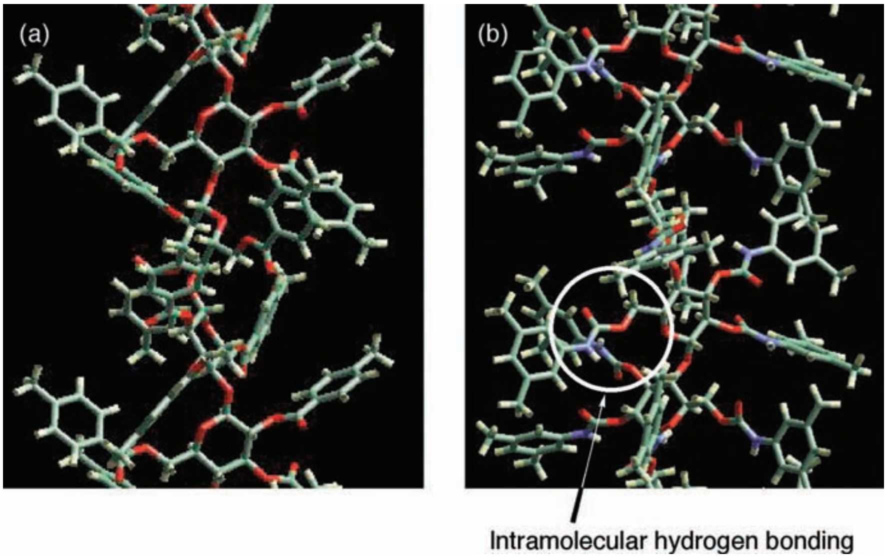


Figure 13. Structures of (a) 4-methylbenzoate **13b** and (b) 3,5-dimethylphenylcarbamate **17ab** of cellulose. Reprinted with permission from ref 196. Copyright 2006 Wiley Periodicals.

tive interaction with the chiral analytes. Consequently, polar substituents should not be introduced on the phenyl group for enhancement of the recognition abilities of cellulose phenylcarbamates.

The chiral recognition on the phenylcarbamate derivatives is also affected by the position of the substituents.[180,197] As opposed to the *para*-substituted phenylcarbamate derivatives, most of the *ortho*-substituted derivatives show rather low recognition abilities. The phenylcarbamate derivatives possessing a high recognition ability usually provide a lyotropic liquid crystallinity in a highly concentrated solution.[180] However, the *ortho*-substituted derivatives do not form such a liquid crystalline phase, indicating that the *ortho*-substituted derivatives may not have a regular higher-order structure, which is extremely important for efficient chiral recognition on polymer-type CSPs.[54,120]

In contrast to the benzoate derivatives, the chiral recognition of cellulose phenylcarbamates is only slightly influenced by the conditions used for the preparation of the packing material. This difference seems to be caused by the different conformational stabilities between the cellulose benzoates and cellulose phenylcarbamates.[196] The structures of cellulose 4-methylbenzoate **13b**[198] and cellulose 3,5-dimethylphenylcarbamate **17ab**,[199,200] which have been calculated on the basis of the left-handed 3/2-helical structure of the cellulose benzoate **13a** and cellulose phenylcarbamate **17g**, respectively, determined by X-ray structural analysis, are shown in Figure 13. For the phenylcarbamates, intramolecular hydrogen bonding exists between the adjacent carbamate moieties of the neighboring glucose units, and this interaction may contribute to maintaining their rigid regular structure. The existence of hydrogen bonding between the adjacent carbamate moieties was confirmed by the measurement of the IR spectrum in the NH region of the cellulose phenylcarbamate, which shows two peaks assigned to the free NH and hydrogen-bonded NH groups.

5. Amylose Phenylcarbamates

For amylose phenylcarbamates **18** (Figure 11), the introduction of methyl or chloro groups on the phenyl groups produces a positive effect on their recognition ability.[181,201,202] The amylose derivative **18x**, which has a methoxy group on the *meta*-position, also exhibits a rather high recognition ability. Compared with the derivative **18x**, the chiral recognition ability of amylose 3,5-dimethoxyphenylcarbamate **18aa** is significantly decreased,[203] although the derivative **18aa** is useful for resolutions of several chiral drugs.[204,205] The additional methoxy group introduced on another *meta*-position may prevent the racemates from interacting with the carbamate group, which is considered to be the most important adsorbing site for chiral recognition. In contrast

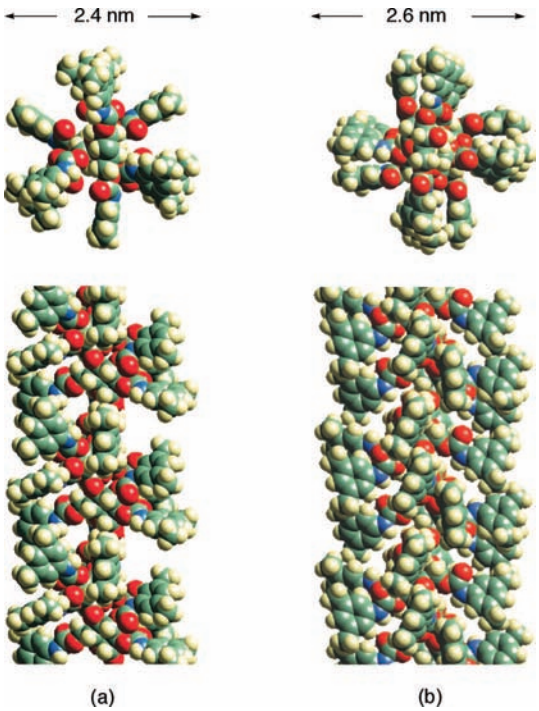


Figure 14. Optimized structures of 3,5-dimethylphenylcarbamates of (a) cellulose (**17ab**) and (b) amylose (**18ab**), perpendicular to (top) and along (bottom) the helix axis. Reprinted with permission from refs 200 and 209. Copyright 1999 The Chemical Society of Japan and 2002 American Chemical Society.

to the cellulose derivatives, however, amylose derivatives with substituents at the *ortho*-position, such as the 5-fluoro-2-methyl- (**18aq**) and 5-chloro-2-methylphenylcarbamates (**18ak**), exhibit a relatively high recognition ability,[201,202] and particularly, the **18ak**-based CSP has been commercially available from Phenomenex as Lux Amylose-2 and from Daicel as Chiralpak AY-H. The phenylcarbamates of cellulose and amylose are reported to have the left-handed 3/2[198,199] and 4/3[206] helical conformations, respectively. The difference in their helical structures may result in the difference in the substituent effect on their chiral recognition.

The effect of column temperature on the chiral separation on amylose derivatives was carefully investigated by Wang et al.[207,208] Once the amylose derivative **18ab** is heated to 50 °C in a polar solvent, including ethanol, methanol, and water, its recognition ability is changed. This phenomenon may suggest that the derivative **18ab** shows a thermally induced irreversible conformational change, which results in the alternation of the separation mechanism. The change of the recognition ability of **18ab** depends on the polar component of the mobile phase, and such an irreversible change could not be observed in a solvent containing 2-propanol.

Among the various phenylcarbamate derivatives already developed, the 3,5-dimethylphenylcarbamates of cellulose (**17ab**) and amylose (**18ab**) are recognized as the most powerful and attractive CSPs for HPLC. These two chiral selectors are rather complementary in chiral recognition and can resolve a wide range of chiral compounds. The optimized structures of **17ab**[200] and **18ab**[209] are shown in Figure 14.

6. Other Polysaccharide Carbamates

Several benzylcarbamate derivatives of cellulose and amylose showed characteristic recognition abilities, and particularly, the 1-phenylethyl- (**29a** and **30a** in Figure 15) and 1-phenylpropylcarbamates (**29b** and **30b**) exhibit a high recognition,[210,211] which are different from those of the phenylcarbamate derivatives. Interestingly, the chirality of the benzyl groups significantly influences their recognition abilities. For example, the amylose (*S*)-1-phenylethylcarbamate, (*S*)-**30a**, has a better chiral recognition ability compared with (*R*)-1-phenylethylcarbamate, (*R*)-**30a**, and (*R*,*S*)-1-phenylethylcarbamate (*R*,*S*)-**30a**. An NMR study of these derivatives indicated that the chirality of the 1-phenylethyl group seems to influence the conformation of the glucose units. In contrast, the benzyl-, 2-methyl-1-phenylpropyl-, and diphenylmethylcarbamate derivatives of cellulose and amylose showed low or almost no recognition ability. This may be because these too-small or too-bulky benzyl moieties prevent the derivatives from forming the regular higher-order structure, which plays an important role in the efficient chiral recognition.

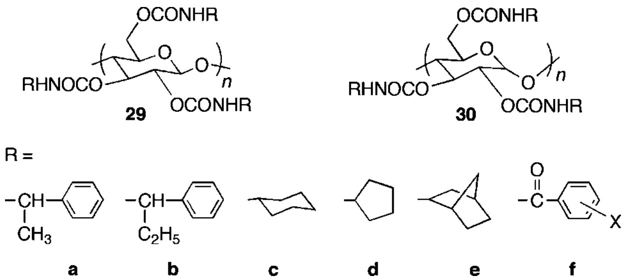


Figure 15. Structures of benzylcarbamates (**a** and **b**), cycloalkylcarbamates (**c**−**e**), and benzoylcarbamates (**f**) of cellulose (**29**) and amylose (**30**).

For the same reason, the alkylcarbamate derivatives, including the methyl- and isopropylcarbamates of cellulose, also have a very low recognition ability. However, several cycloalkylcarbamates, such as the cyclohexylcarbamate, cyclopentylcarbamate, and *exo*-norbornylcarbamate of cellulose and amylose (**29c**−**e** and **30c**−**e** in Figure 15), exhibit an excellent recognition ability comparable to the commercial polysaccharide-based CSPs.[212,213] Because these cycloalkylcarbamates do not contain aromatic groups, they poorly absorb UV light. Therefore, these cycloalkylcarbamate-based CSPs can also be utilized for resolutions by thin-layer chromatography (TLC) using a UV radiation detection.[212] The chiral separations of **27**, **20**, and **31** by TLC have been performed using amylose cyclohexylcarbamate as a CSP (Figure 16). These compounds were readily detected by UV radiation at 254 nm, and the TLC chromatograms clearly showed two spots corresponding to the enantiomers. Since the separations by TLC were strongly correlated with those by HPLC, the cycloalkylcarbamate-based CSPs for TLC are expected to allow a rapid setup of the separation conditions for HPLC.

The benzoylcarbamates of cellulose (**29f**) and amylose (**30f**), which have an additional carbonyl group between the carbamate and phenyl groups, have also been prepared as CSPs for HPLC.[214] These benzoylcarbamate derivatives exhibit a specific recognition ability for chiral compounds bearing a hydrogen bonding donor, and racemates such as **23**, **24**, **27**, and **28**, capable of hydrogen bonding with the carbonyl groups, could be efficiently separated on these CSPs.

Besides the cellulose and amylose derivatives, various carbamate derivatives of polysaccharides, such as chitin, chitosan, galactosamin, xylan, dextran, curdlan, and inulin,

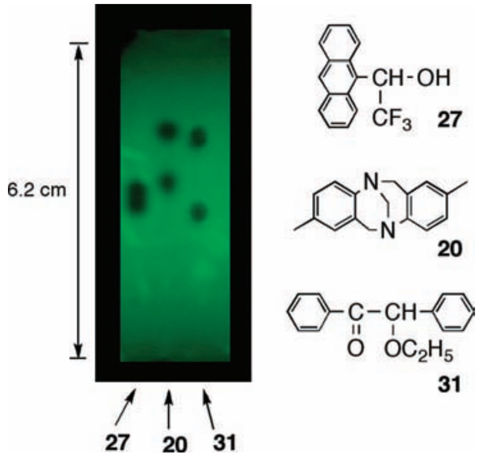


Figure 16. Chiral separations of racemates on amylose cyclohexylcarbamate **30c** as CSP for TLC: eluent, hexane−2-propanol (90:10). Reprinted with permission from ref 212. Copyright 2000 American Chemical Society.

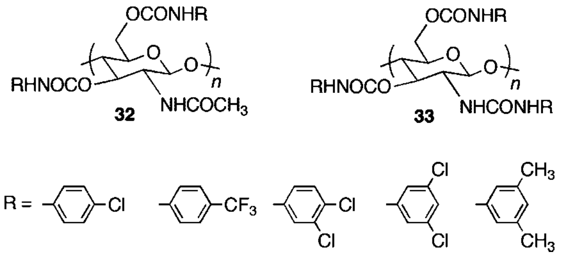


Figure 17. Structures of phenylcarbamates of chitin (**32**) and phenylcarbamate ureas of chitosan (**33**).

have been prepared and applied as CSPs for HPLC.[179,215−219] Their recognition abilities significantly depend on the linkage positions, anomeric configurations, and nature of the monomeric units. Among them, the chitin (**32**)[220,221] and chitosan (**33**)[222] derivatives as shown in Figure 17 exhibited a relatively high recognition ability for a wide range of chiral compounds including drugs. For example, ketoprofen and ibuprofen could be more effectively separated on these derivatives than on the phenylcarbamates of cellulose and amylose.[220]

7. Regioselectively Substituted Polysaccharide Derivatives

Various kinds of cellulose and amylose derivatives have already been developed as CSPs for HPLC, and most of them have the same substituent at the 2-, 3-, and 6-positions of the glucose unit as already discussed. Meanwhile, several cellulose and amylose derivatives with different substituents at the 2-, 3-, and 6-positions have also been prepared in order to expand the range of applications as CSPs.[223]

In 1993, the regioselectively substituted derivatives of cellulose (**34**) and amylose (**35**), which have different carbamate groups at the 2,3-positions and at the 6-position, were first prepared via protection of the primary hydroxy group at the 6-position (Figure 18).[224] Since then, polysaccharide derivatives bearing different carbamate groups or benzoate groups at the 2,3-positions and at the 6-position have been synthesized on the basis of the above synthetic procedure and used as CSPs for HPLC.[223,225] The chromatographic behavior of the regioselectively substituted derivatives is quite different from the corresponding derivatives with the same substituents at the 2-, 3-, and 6-positions, and some racemates can be more efficiently separated on these regioselectively substituted derivatives. Although it is difficult to provide a comprehensive interpretation about the influence of substituents at the 2,3-positions and at the 6-position, amylose derivatives have a tendency to be more influenced by the substituents at the 2- and 3-positions than that at the 6-position.[226] On the other hand, the substituents at the 2-, 3-, and 6-positions of the cellulose derivatives seem to complicatedly influence the chiral recognition, and their abilities significantly depend on the kind of racemates. The difference in the chromatographic results between the cellulose and amylose derivatives may be ascribed to the difference in their higher order structures.[198−200,206,209]

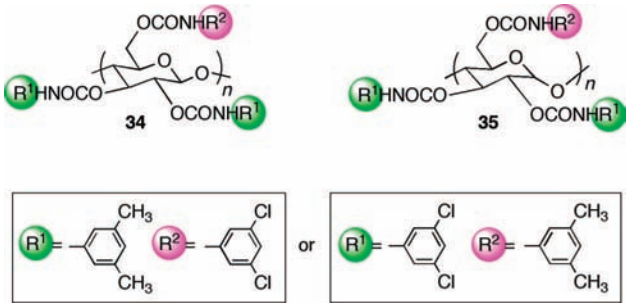


Figure 18. Structures of cellulose (**34**) and amylose (**35**) derivatives bearing different carbamate groups at the 2-, 3-positions and at the 6-position of a glucose unit.

The regioselective introduction of different substituents at the 2- and 3-positions has been considered to be difficult, because both of the hydroxy groups at the 2- and 3-positions are secondary and show a similar reactivity. However, Dicke recently reported that an amylose derivative bearing a benzoate group only at the 2-position could be synthesized via a reaction with vinyl benzoate in dimethyl sulfoxide.[227] By using this method, our group has successfully synthesized amylose derivatives (**36** and **37**) bearing different substituents on each of the 2-, 3-, and 6-positions (Figure 19).[228] The structures of these derivatives have been confirmed by ^{1}H and ^{13}C NMR and elemental analysis.

The recognition abilities on **36** and **37** have been evaluated for HPLC, and the resolution results are summarized in Table 3. For comparison, the results on a commercially available Chiralpak AD, which consists of the amylose 3,5-dimethylphenylcarbamate (**18ab**) as the chiral selector, are also shown. Amylose derivatives **36** and **37** exhibited similar or better recognition abilities when compared with Chiralpak AD, and racemates **19**, **25**, and **26**, which cannot be efficiently separated on Chiralpak AD, were separated on both **36** and **37**. The separation factors on **36** and **37** bearing reversed substituents at the 3- and 6-positions are quite different. Specifically, for the resolution of **19**, **25**, and **27**, the elution order of each enantiomer was reversed. These results indicate that the electronic effect of the methyl and chloro groups on the phenyl groups may significantly affect the recognition ability of the CSPs.

Polysaccharide derivatives bearing different substituents at all three hydroxy groups are expected to be quite effective for understanding the chiral discrimination mechanism on the polysaccharide-based CSPs at a molecular level. Further systematic preparations of regioselectively substituted derivatives seem to be attractive.

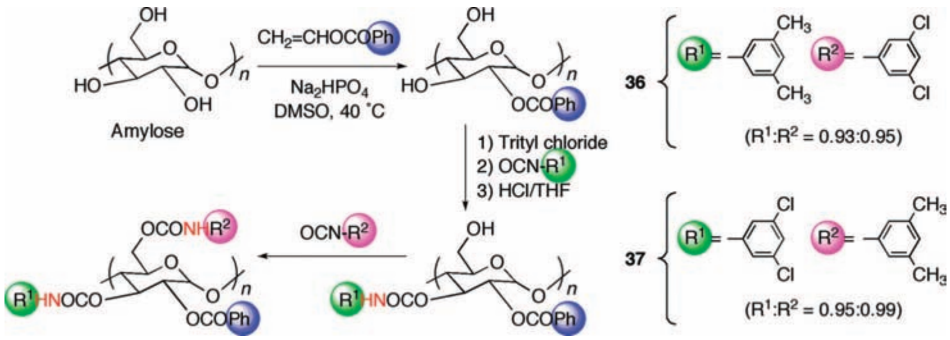


Figure 19. Synthesis of amylose derivatives (**36** and **37**) bearing different substituents at the 2-, 3-, and 6-positions of a glucose unit.

Table 3. Separation Factors (α) on the Amylose Derivatives[a]

	36	**37**	Chiralpak AD[b]
19	1.15 (−)	1.24 (+)	1.02 (−)
20	1.85 (+)	2.31 (+)	1.70 (+)
21	1.30 (−)	~1 (−)	2.81 (+)
22	1.07 (+)	1.22 (+)	2.24 (+)
23	1.11 (−)	1.12 (−)	2.22 (−)
24	1.73 (−)	1.97 (−)	1.31 (−)
25	1.17 (−)	1.13 (+)	1.04 (+)
26	1.75 (−)	2.46 (−)	~1 (−)
27	1.18 (−)	1.23 (+)	1.39 (+)
28	3.21 (+)	3.71 (+)	1.59 (+)

[a] Column = 25 cm × 0.20 cm (i.d.); flow rate = 0.1 mL/min; eluent = hexane−2-propanol (90:10). The signs in parentheses represent the optical rotation of the first-eluted enantiomers. [b] Column = 25 cm × 0.46 m (i.d.); flow rate = 0.5 mL/min.

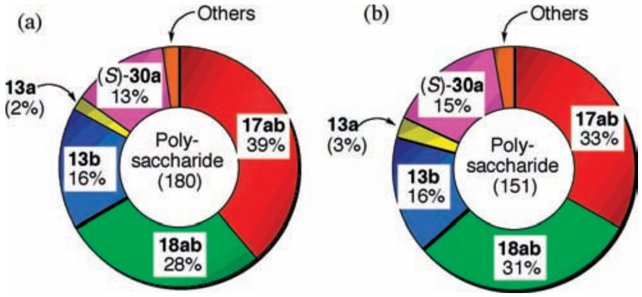


Figure 20. Proportion of polysaccharide-based CSPs used in chiral HPLC for the determination of enantiomeric excess reported in the *Journal of the American Chemical Society* in 2005 (a) and 2007 (b). The values in parentheses represent the number of the counted papers. Reprinted with permission from ref 158. Copyright 2007 International Union of Pure and Applied Chemistry.

8. Compounds Resolved by Polysaccharide Derivatives

As already described, some of the above polysaccharide-based CSPs have been commercialized since the 1980s and utilized for the separations of a wide range of chiral compounds with or without functional groups. Figure 20 shows the proportion of the polysaccharide-based CSPs used in chiral HPLC in order to estimate the ee of products that appeared in the *Journal of the American Chemical Society* in 2005 and 2007.[158] According to these statistics, the 3,5-dimethylphenylcarbamates of cellulose, **17ab** (Chiralcel OD), and amylose, **18ab** (Chiralpak AD), seem to be the most popular ones, followed by cellulose 4-methylbenzoate, **13b** (Chiralcel OJ), and amylose (*S*)-1-phenylethylcarbamate, (*S*)-**30a** (Chiralpak AS). Figure 21 shows the recent examples of chiral compounds resolved by the polysaccharide-based CSPs, which include alcohols,[229] amines,[186,230] carboxylic acids,[231] ethers,[232] carbonyl[233] and cyano[234] compounds, aromatic hydrocarbons,[187,235] nonaromatic compounds,[236] metal-containing compounds,[237−239] chiral sulfur[240] or phosphorus[241,242] compounds, and axially,[243−245] planar,[246] and topologically[247−249] asymmetric compounds. For more examples of the available chiral compounds, see the reviews[54,145−151] and catalogs.[250,251]

9. Chiral Recognition Mechanism on Polysaccharide Derivatives

In the past two decades, many attempts to clarify the chiral recognition mechanism on CSPs for liquid chromatography have been carried out by NMR spectroscopy,[252−255] X-ray analysis,[256,257] and computational methods.[258−268] The most successful study was made for the molecular-type CSPs, while only a few studies on the chiral recognition mechanism at a molecular level have been reported for the polymer-type CSPs.[269−271] This is because a variety of interaction sites with different affinities for enantiomers exist on chiral polymers and the determination of their precise structures both in the solid and in solution states is not easy. Until now, several approaches have been performed to elucidate the chiral recognition mechanism of the polysaccharide derivatives.

NMR spectroscopy is well-known to be one of the most effective tools for the elucidation of the chiral recognition mechanism at a molecular level. Most carbamate derivatives with high recognition abilities are only soluble in polar organic solvents, such as acetone, dimethyl sulfoxide, tetrahydrofuran (THF), pyridine, etc. In these solvents, the derivatives cannot sufficiently interact with enantiomers for efficient recognition due to the strong interaction between the solvent molecules and the polysaccharide derivatives. Therefore, it is difficult to clarify the chiral discrimination mechanism on polysaccharide derivatives by NMR spectroscopy.

In the past 15 years, however, it was found that several carbamate derivatives, such as 4-trimethylsilylphenyl-,[272−274] 5-fluoro-2-methylphenyl-,[93] 3,5-dichlorophenyl-,[273] and cyclohexylcarbamate,[213] are soluble in chloroform and can discriminate enantiomers by NMR spectroscopy as well as by HPLC. Figure 22 shows the 500 MHz ^{1}H NMR spectra of *rac*-*trans*-stilbene oxide (**21**) in the absence (a) and presence (b) of cellulose 4-trimethylsilylphenylcarbamate **17j** in $CDCl_3$.[272−274] The methine proton resonance of the enantiomer **21** was significantly separated into two sets of singlet peaks in the presence of **17j**, and only the resonance of the (*S*,*S*)-isomer is downfield shifted. The chemical shift difference ($\Delta\Delta\delta$) of these two peaks increased with an increase in the amount of **17j** and with a decrease in temperature. This clearly indicates that **17j** can recognize the enantiomers even in solution. Because the most important adsorption site for chiral recognition on the phenylcarbamate derivatives may be the polar carbamate residues, the oxygen

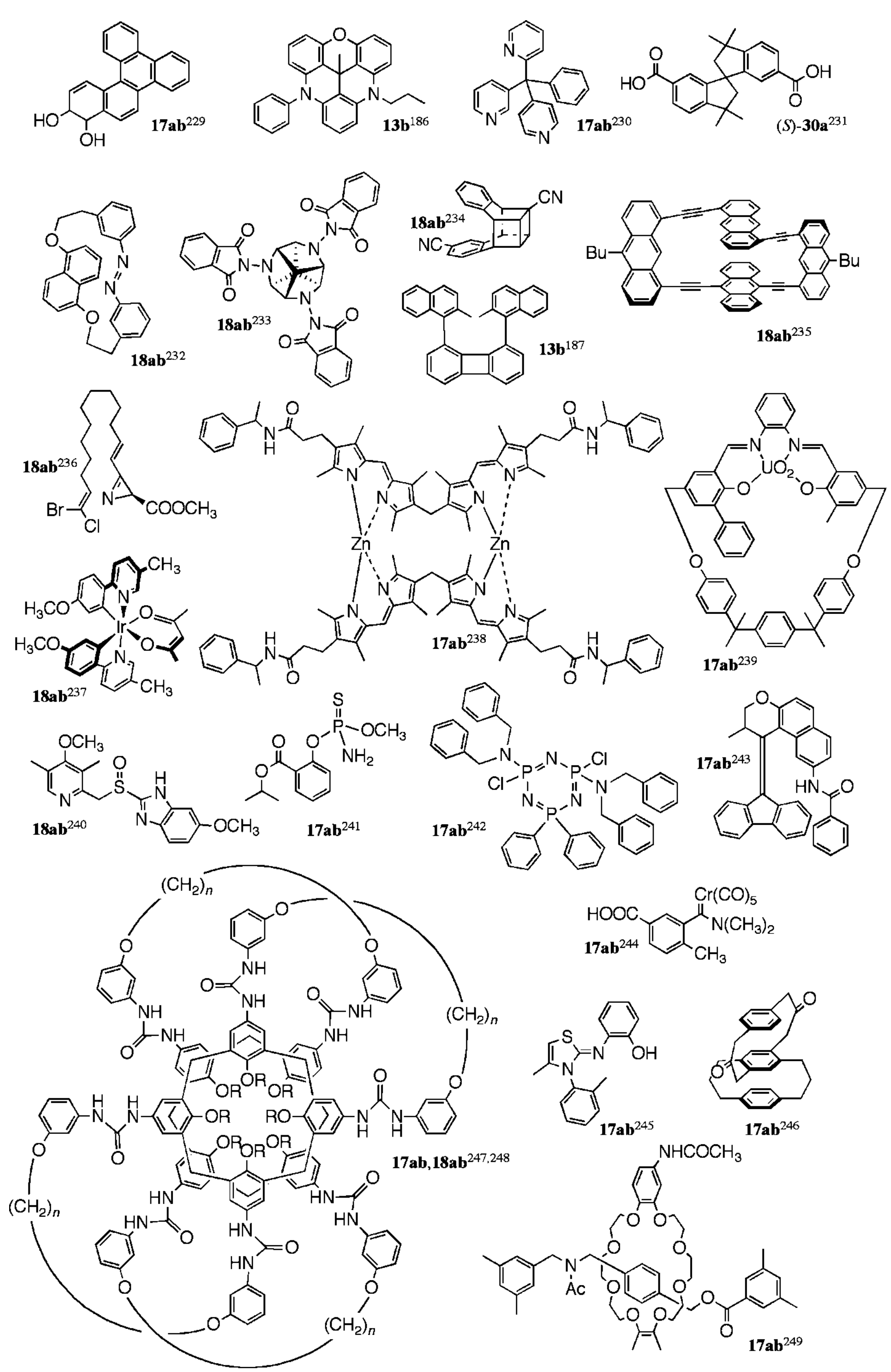


Figure 21. Structures of chiral compounds resolved on the polysaccharide-based CSPs.

atom of the oxirane ring in **21** may interact with the NH proton of the carbamate group through hydrogen bonding. Therefore, the addition of acetone, which is a hydrogen bonding acceptor and prevents the interaction between **21** and **17j** through hydrogen bonding with the NH proton, causes the splitting of the methine proton resonance to disappear. Many other racemates can be also recognized on **17j** in $CDCl_3$.[273] In the case of secondary alcohols, such as 2-heptanol and 2-octanol, the methyl protons at the end of the longer chain, which are situated the farthest from the asymmetric carbon, were enantiomerically recognized in the presence of **17j** (Figure 23),[273] while the methylene and other methyl protons were not discriminated. These results suggest that the methyl protons of the long alkyl chains may be specifically located deep in the chiral **17j**.

The cellulose derivative **17aq** also discriminates the enantiomers of **38** by ^1H and ^{13}C NMR spectroscopy.[93] Figure 24 shows the ^1H NMR spectra of *rac*-**38** in the absence (a) and presence (b) of **17aq** in $CDCl_3$. Each signal for the hydroxyl and naphthyl (H4 and H6) protons of **38** is apparently split into two signals due to the enantiomers. The signals for the hydroxy protons of (*S*)-**38** are more downfield shifted with line broadening than that for (*R*)-**38**, while the signals for the H4 and H6 protons of (*S*)-**38** are upfield shifted with broadening. This means that (*S*)-**38** has a stronger interaction with **17aq**. The downfield shift of the hydroxy protons is probably due to hydrogen bonding between the hydroxy group of (*S*)-**38** and the carbamate group of the cellulose derivative, and the upfield shifts of the aromatic protons may be ascribed to the π-stacking or

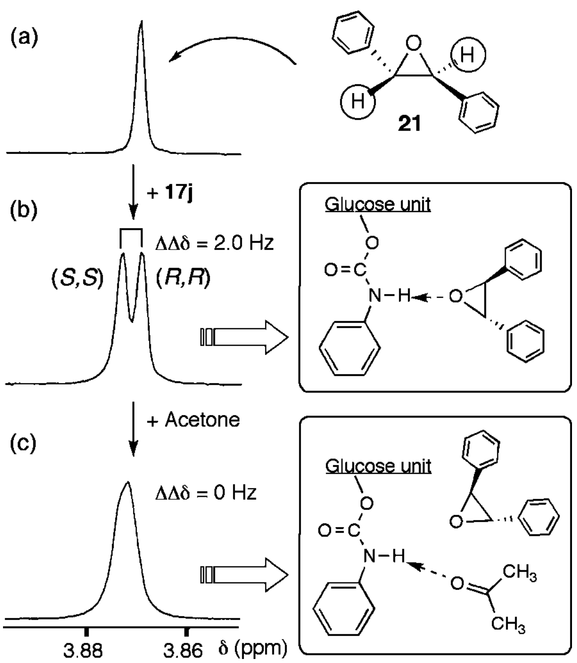


Figure 22. ^{1}H NMR spectra of *rac*-**21** in the presence of **17j** in CDCl$_3$ (1.0 mL): **17j**, (a) 0 and (b, c) 20 mg; acetone, (a, b) 0 and (c) 65 μL. Reprinted with permission from ref 272. Copyright 1994 The Chemical Society of Japan.

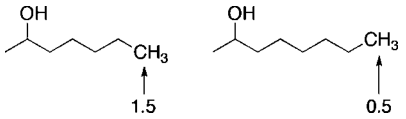


Figure 23. Secondary alcohols enantiomerically recognized by **17j** in ^{1}H NMR. The protons marked with an arrow indicate the recognized ones, and figures represent ΔΔδ (Hz).

shielding effect of a neighboring aromatic ring of **17aq**. During the HPLC separation of *rac*-**38** on **17aq**, the (*R*)-isomer is first eluted followed by the (*S*)-isomer, and baseline separation is then achieved ($\alpha = 4.23$) (Figure 1). This elution order in HPLC agrees with the large shifts in the (*S*)-isomer observed in the ^{1}H NMR spectrum.

Figure 25 shows the discrimination of the enantiomers of **38** by ^{13}C NMR spectroscopy.[93] The resonances of the C1−C4 and C10 carbons of **38**, which are located near the hydroxy groups, are separated into enantiomers in the presence of **17aq**, and the carbon resonances of (*S*)-**38** clearly become broader than those of (*R*)-**38**, as well as the observation in the ^{1}H NMR spectrum. This indicates that ring A of (*S*)-**38** may be favorably located in the chiral groove of **17aq**. Measurements of the relaxation time also support this speculation. Furthermore, ^{1}H NMR titrations of **17aq** with (*S*)- and (*R*)-**38** and a Job plot of the continuous changes in chemical shifts for the complex **17aq**−(*S*)-**38** were performed in order to investigate the binding sites of **17aq** and the stoichiometry of the complexation.[93] The H2 proton resonance of a glucose unit is dramatically upfield shifted by the addition of (*S*)-**38**, while the other glucose proton resonances only slightly move. This upfield shift of the H2 proton resonance indicates that the H2 proton may be located above a naphthyl ring of (*S*)-**38**. The Job plot shows that the maximum complex formation occurs at around 0.5 mol fraction of the glucose unit of **17aq**. This means that each glucose unit of **17aq** may have the same binding affinity for (*S*)-**38** probably due to the regular structure of **17aq** even in solution. More valuable information on the binding geometry and dynamics between the polysaccharides and the enantiomers can be obtained from the intermolecular NOEs. Figure 26 shows the NOESY spectra of **17aq**−(*S*)-**38** (a) and **17aq**−(*R*)-**38** (b) in the region related to the methyl protons on the phenyl group of **17aq** and the aromatic protons.[93] Clear intermolecular NOE cross peaks denoted by the arrows could be observed between the methyl proton of **17aq** and the aromatic protons H4, H6, and H7 of (*S*)-**38** (Figure 26a). On the other hand, the mixture of **17aq** and (*R*)-**38** exhibited no intermolecular NOE cross peaks (Figure 26b), probably due to a weak interaction. These results indicate that (*S*)-**38** more strongly binds or interacts with **17aq** than (*R*)-**38**, and the naphthyl protons of (*S*)-**38** are closely located to the glucose proton of **17aq** within less than 5 Å. These observations correspond to the results of the HPLC and 1D NMR experiments. Based on the HPLC and NMR data combined with the structural data for the cellulose phenylcarbamate (**17g**) determined by X-ray analysis, a computational structure has been proposed for the **17aq**−(*S*)-**38** complex (Figure 27).[93] This calculation model shows that two hydroxy protons of (*S*)-**38** interact with the carbonyl oxygens of the carbamate groups of **17aq** through hydrogen bonding.

The chiral recognition mechanism of molecular-type CSPs, such as cyclodextrins, cinchona alkaloids, and Pirkle-type CSPs, have been extensively investigated from theoretical viewpoints especially by Lipkowitz et al.[258,259] The interaction energies between the CSPs and enantiomers were calculated by molecular-mechanics (MM), molecular dynamics (MD), and quantum-mechanical calculations, and the recognition mechanisms of the chiral small molecules have been proposed. These computer simulations are also a powerful approach for the qualitative understanding of the chiral recognition on the polysaccharide-based CSPs. Several attempts have already been carried out for predicting the chromatographic behavior on the polysaccharide-based CSPs.

The interaction energy calculations between the phenylcarbamate (**17g**) or 3,5-dimethylphenylcarbamate (**17ab**) of cellulose and *trans*-stilbene oxide (**21**) or benzoin (**24**) were performed using various force fields.[200,275] During a chromatographic resolution, **21** can be completely separated on both **17g** ($\alpha = 1.46$) and **17ab** ($\alpha = 1.68$). However, their elution orders are opposite; the (*R*,*R*)-isomer is first eluted on **17g** and the (*S*,*S*)-isomer on **17ab**. Meanwhile, **17ab** can efficiently separate benzoin ($\alpha = 1.58$), but **17g** cannot ($\alpha = \sim 1$).

The applied calculations were roughly divided into the following two methods, which are different based on the enantiomer generation methods: (1) Enantiomers were generated around the carbonyl oxygen and the NH proton of the carbamate group of **17g** and **17ab** and rotated at 15° intervals for the *x*, *y*, and *z* axes, individually. The interaction energy calculation is performed for each carbonyl oxygen and NH proton at the 2-, 3-, and 6-positions of the glucose units with all possible combinations of the rotation angles of the enantiomers. The calculation results are evaluated with the lowest interaction energy and the distribution of the interaction energy. (2) Enantiomers with a particular orientation were randomly generated by the Monte Carlo method on the surface of **17g** and **17ab**, and then the interaction energy was estimated step-by-step through the MM calculation between the molecules.[276] In both calculations, the nonamers of **17g** and **17ab** were utilized as chiral selectors, and the

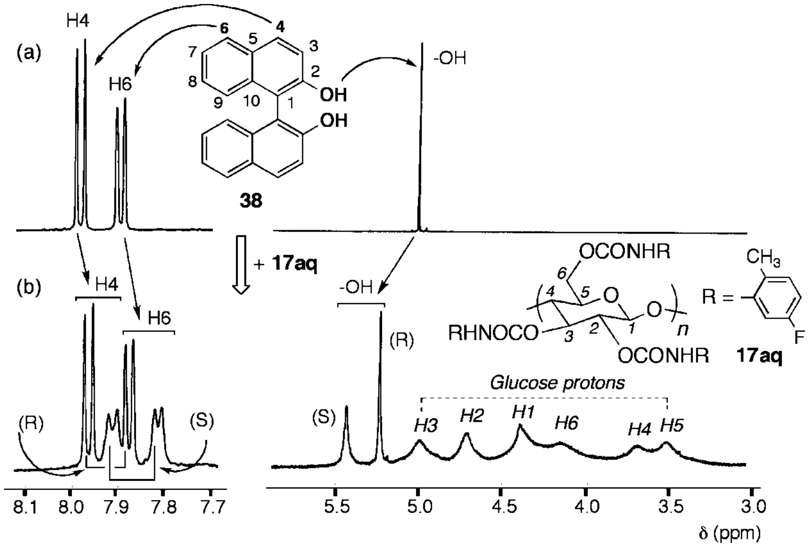


Figure 24. ^{1}H NMR spectra of a selected region of *rac*-**38** in the absence (a) and presence (b) of **17aq** in $CDCl_3$. Reprinted with permission from ref 93. Copyright 1996 American Chemical Society.

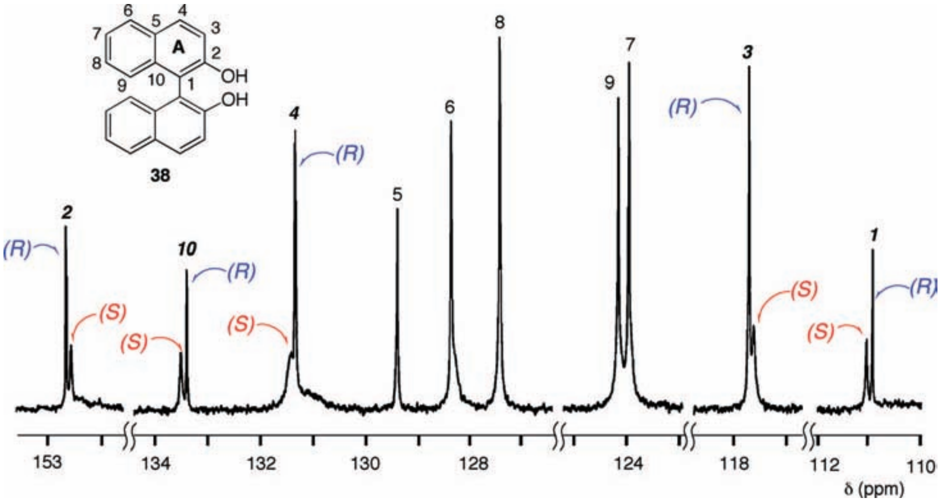


Figure 25. ^{13}C NMR spectrum of *rac*-**38** in the presence of **17aq** in $CDCl_3$. Reprinted with permission from ref 93. Copyright 1996 American Chemical Society.

enantiomers were generated around the middle part of their structures in order to avoid the influence of the end groups.

Both calculation results well agreed with the chromatographic resolution data. The lowest or averaged interaction energy between **17g** and (*S*,*S*)-**21** was lower than that between **17g** and (*R*,*R*)-**21**, while an opposite enantiomer preference was observed for the **17ab**−**21** system. In the case of benzoin (**24**), almost no difference in the interaction energies with **17g** was observed for the enantiomers.

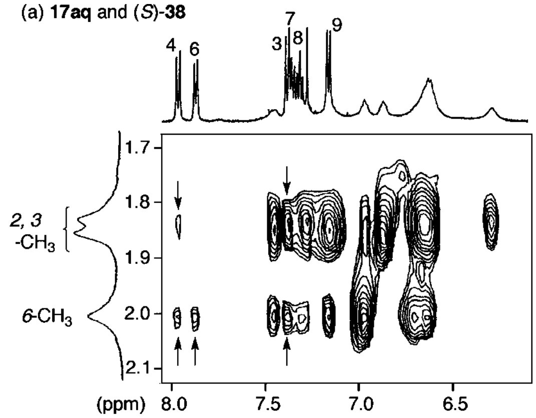
Figure 28 shows a graphical view of the interaction mode between **17g** and (*S*,*S*)-**21**,[200] which has the lowest interaction energy obtained by the second calculation method using the Monte Carlo technique. (*S*,*S*)-**21** is bound in a chiral groove of **17g** through hydrogen bonding between the ether oxygen atom of (*S*,*S*)-**21** and the NH proton of the carbamate group of **17g**. In addition, each phenyl group of **21** may interact with the phenyl groups of **17g** through $\pi-\pi$ interactions. Although the actual reason for the opposite enantioselectivity of **17g** and **17ab** toward **21** is unknown, the different arrangement of aromatic groups in **17g** and **17ab** is expected to be responsible for the reversed enantioselectivity.

Recently, using attenuated total reflection infrared spectroscopy (ATR-IR), density functional theory (DFT) modeling, X-ray diffraction (XRD), and ^{13}C cross-polarization/magic-angle spinning (CP/MAS) and MAS solid-state NMR, Franses et al. investigated the difference in the enantioselective adsorption sites among the cellulose 3,5-dimethylphenylcarbamate (**17ab**), amylose 3,5-dimethylphenylcarbamate (**18ab**), and amylose (*S*)-1-phenylethylcarbamate ((*S*)-**30a**).[277] They concluded that the carbamate groups in these polymers show quite different interaction energies in their hydrogen bonding and that the carbonyl, NH, and phenyl groups of these polymers are expected to be oriented in different ways. Additionally, in order to clarify the role of eluents during the chiral discrimination on polysaccharide derivatives, the interaction between **18ab** and various organic solvents used as eluents was also investigated.[278,279] Particularly, the changes in the hydrogen bonding state, the crystallinity, and the side-chain mobility of **18ab** upon the absorption of organic solvents have been systematically investigated by the above analytical method.

10. Separation by Chiral SFC

The polysaccharide-based CSPs can also be used for SFC.[280−284] Supercritical fluids possess lower viscosities and

(a) **17aq** and (*S*)-**38**



(b) **17aq** and (*R*)-**38**

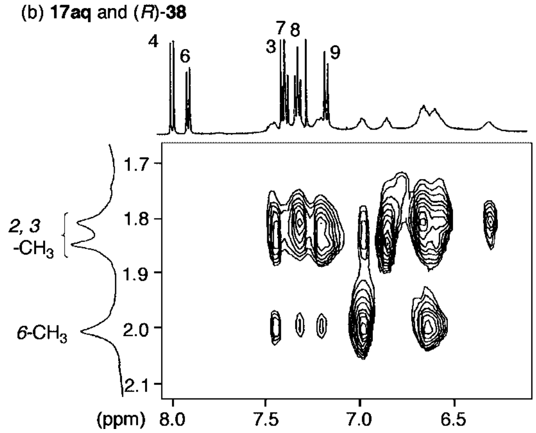


Figure 26. Expanded 500 MHz NOESY spectra at a mixing time of 300 ms of the mixtures of (a) **17aq** and (*S*)-**38** and (b) **17aq** and (*R*)-**38** in the region between the aromatic protons (**17aq** and **38**) and the methyl protons on the phenyl groups of **17aq** in $CDCl_3$ at 30 °C. Reprinted with permission from ref 93. Copyright 1996 American Chemical Society.

higher solute diffusion coefficient than liquids. These features of supercritical fluids provide the following advantages as mobile phases for chromatographic separations: a rapid column equilibration, fast method development, low pressure drop, and high resolutions.[285] The characteristics of supercritical fluids, such as density, solubility, and eluting strength, can be changed by manipulating the back-pressure and temperature. This means that the chromatographic behavior including retention times, separation efficiencies, and resolutions can be easily controlled even without changing eluents.

Supercritical carbon dioxide with modest critical parameters ($T_c = 31.3$ °C, $P_c = 7.39$ MPa) is most widely used as the main component of eluents for SFC because it is relatively inexpensive, nontoxic, and nonflammable. In addition, carbon dioxide can be produced by condensation from the atmosphere or recovery from industrial exhaust gas. Therefore, carbon dioxide is more environmentally friendly than petroleum-based solvents typically used for HPLC. Because pure carbon dioxide has a weak eluting strength, the addition of polar organic solvents, such as alcohols and acetonitrile, is usually necessary to increase the eluting strength of mobile phases and decrease the retention time.[286]

Since the first chiral separation on the polysaccharide-based CSPs in SFC was demonstrated in 1988,[280] hundreds of successful separations have been attained for a wide variety of racemates. The separation results have been summarized in several reviews[69−77] and books,[284] and recently, Heyden et al. reviewed an extensive study on chiral separations in SFC.[72] The 3,5-dimethylphenylcarbamates of cellulose (**17ab**) and amylose (**18ab**) seem to be the most powerful CSPs for SFC as well as for HPLC. In the future, the chiral separations in SFC will be more common particularly in the field of large-scale preparative separations from the viewpoint of a reduced solvent consumption and easier solvent removal than those for HPLC.

11. Immobilization of Polysaccharide Derivatives onto Chromatographic Supports

Although the polysaccharide derivatives exhibit a powerful recognition ability as chiral selectors for chiral packing materials (CPMs), they have a serious drawback of the narrow range of mobile phase selections. The polysaccharide-based CPMs have been conventionally prepared by physically coating the polysaccharide derivatives on a chromatographic support without chemical bonding.[177,180] Therefore, the common organic solvents, such as THF, chloroform, dichloromethane, toluene, ethyl acetate, and acetone, cannot be used as eluents, because these solvents damage the CPMs through dissolution or swelling of the polysaccharide derivatives coated on the support. Due to their dissolution or swelling properties, the coated-type CPMs based on polysaccharide derivatives can only be used with rather restricted types of solvents as the eluent components. Typical eluents are the alkane−alcohol mixtures and water−acetonitrile mixtures for normal- and reversed-phase chromatography, respectively. Because the selection of a suitable eluent makes it possible to improve the enantioselectivities, resolutions, solubility and stability of samples, and productivity for pure enantiomers,[55,183,287−291] the development of the polysaccharide-based CPMs with a universal solvent compatibility is strongly desired.

The drawback of mobile phase selections can be solved by immobilizing the polysaccharide derivatives on the chromatographic supports. Several immobilization methods of the polysaccharide derivatives have been developed over the past two decades.[158,292−297] The first immobilized CPMs based on the polysaccharide derivatives were prepared by our group in 1987.[182] The immobilization scheme is presented in Figure 29. The cellulose derivatives were immobilized on the 3-aminopropyl-functionalized silica gel using a diisocyanate as the cross-linker, which was expected to react with the hydroxy groups on cellulose and the amino groups on the silica gel, and also between the hydroxy groups of different cellulose chains. Furthermore, regioselective immobilization was investigated using the polysaccharide derivatives bearing hydroxy groups at the 2- and 3-positions or only at the 6-position of the glucose unit.[226] The regioselectively immobilized CPMs apparently exhibit a higher recognition ability than the nonregioselectively immobilized CPM. Zou et al. prepared the immobilized CPMs derived from other polysaccharide derivatives using bisfunctional reagents.[298−302] Fréchet and Svec et al. reported a similar immobilization method, in which the polysaccharide derivative has been immobilized on organic polymer beads as a support instead of silica gel.[303]

However, these immobilized CPMs showed low chiral recognition, particularly when a large amount of cross-linker was used to achieve a high immobilization efficiency. The high number of chemical linkages between the polysaccharide derivatives and silica gel may destroy the regular higher order structure of the polysaccharide derivatives, which is prerequisite for a high recognition

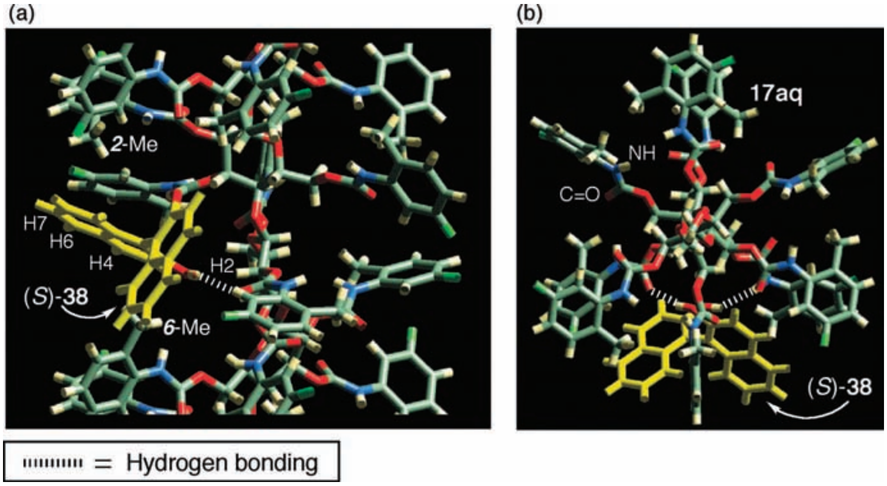


Figure 27. Calculated structure of the complex **17aq**−(*S*)-**38**. View (a) along and (b) perpendicular to the helix axis. Reprinted with permission from ref 93. Copyright 1996 American Chemical Society.

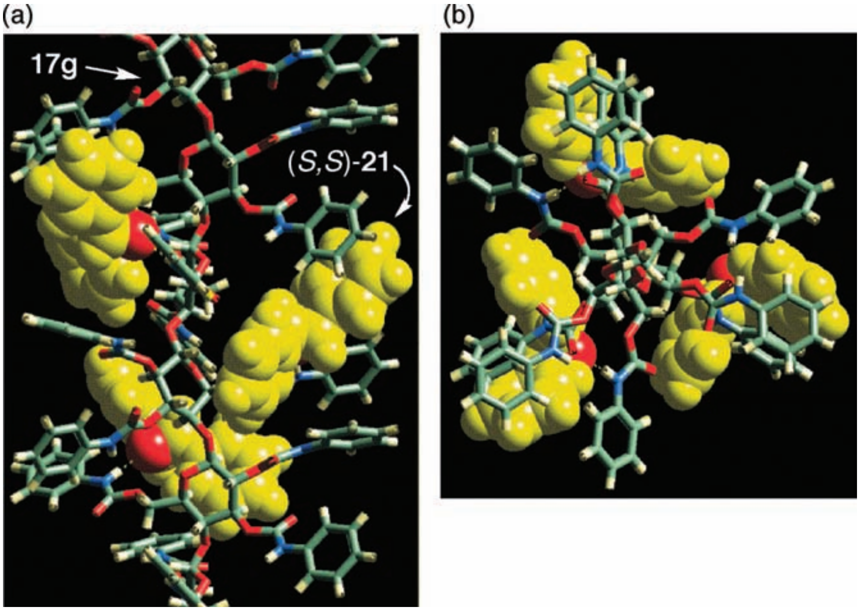


Figure 28. Calculated structure of the complex **17g**−(*S*,*S*)-**21** formed through hydrogen bondings. View (a) along and (b) perpendicular to the helix axis. Reprinted with permission from ref 200. Copyright 1999 The Chemical Society of Japan.

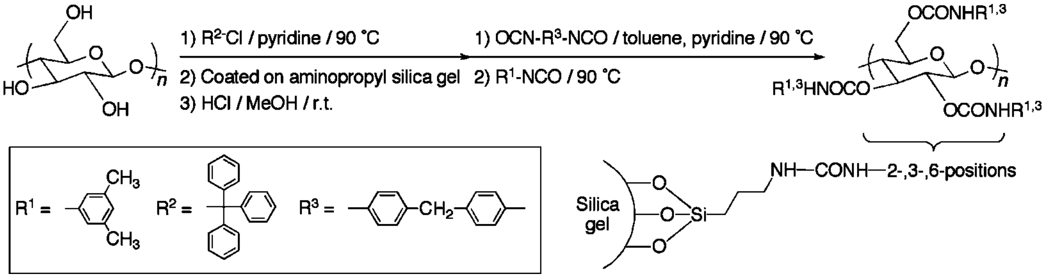


Figure 29. Immobilization of polysaccharide derivatives using diisocyanate.

ability. To prevent the disturbance of the regular higher order structure of the polysaccharide derivatives, amylose was chemically bonded to silica gel only at the reducing terminal end.[304,305] The immobilization scheme is presented in Figure 30. This method consists of three steps.[304] First, amylose was prepared by the enzymatic polymerization of α-D-glucose 1-phosphate dipotassium using a potato phosphorylase as the catalyst. The obtained amylose, which has the desired chain length and narrow molecular weight distribution, is then bonded to the silica gel only at the reducing terminus. Finally, in order to convert the remaining hydroxyl groups of the amylose to carbamate groups, the amylose-bonded silica gel is treated with a large excess of 3,5-dimethylphenyl isocyanate. This immobilized CPM exhibited a comparable recognition ability to the conventional coated-type CPM and can resolve several topologically interesting compounds[306−313] and the chiral C_{76} fullerene[314] using eluents containing chloroform (Figure 31). However, this method can only be used for amylose derivatives, and the preparation process is quite complicated.

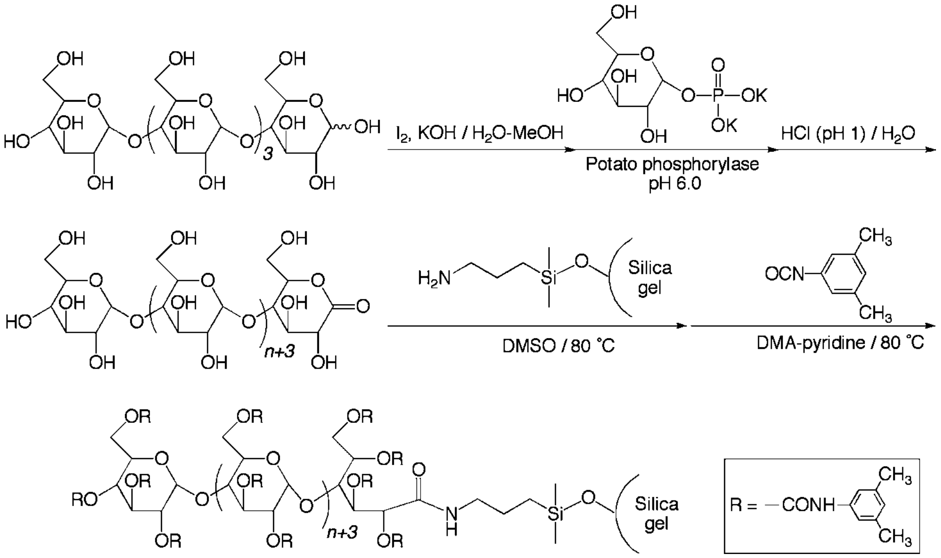


Figure 30. Immobilization of the amylose derivative at an activated chain end.

The immobilization of cellulose 4-vinylbenzoate onto a modified silica gel via radical polymerization was reported by Kimata et al.[315,316] In this method, cellulose 4-vinylbenzoate was coated on the acryloyl-functionalized silica gel, and then, the coated silica gel dispersed in a solvent was heated in the presence of a radical initiator, such as benzoyl peroxide (Figure 32a). Although the obtained immobilized CPM could be used with the eluents containing dichloromethane or THF, it showed a lower enantioselectivity compared with the conventional coated CPM. Because the cellulose derivative contains a large number of styryl groups along the polymer chain, its regular higher order structure is expected to be disturbed during the immobilization process. Oliveros et al. described the analogous immobilization of polysaccharide phenylcarbamates bearing 10-undecenoyl groups as the vinyl group (Figure 32b).[297,317−321] However, this method also provides the immobilized CPMs with a slightly low recognition ability.

The immobilization efficiency of the polysaccharide derivatives bearing vinyl groups is expected to be increased by the addition of a vinyl monomer to the immobilization system, and an efficient immobilization might result in a high recognition ability. By using this strategy, our group has investigated the immobilization of the polysaccharide derivative bearing a vinyl group onto silica gel through radical copolymerization with a vinyl monomer (Figure 32c).[322−327] As a result of exploring the optimum immobilization conditions by changing the type and amount of the vinyl group introduced to the derivatives or the vinyl monomers, the immobilized CPMs exhibit a recognition ability close to that of the coated one.

Francotte et al. reported the photochemical immobilization of polysaccharide derivatives.[328−331] In this method, the polysaccharide derivatives could be immobilized onto silica gel by exposure to UV irradiation, even though the polysaccharide derivatives did not contain any photopolymerizable groups (Figure 33). The immobilization mechanism has not yet been clarified, but the cross-linking reaction between the polysaccharide derivatives is expected to occur during the UV irradiation. The 3,4- and 3,5-dichlorophenylcarbamates and 3-trifluoromethyl-4-chlorophenylcarbamate of cellulose have been immobilized onto silica gel through this method, and their recognition abilities have been evaluated.[331] These immobilized CPMs can separate a wide range of racemates with choice of a suitable eluent.

Cellulose derivatives bearing hydroxy and azido groups have also been immobilized onto γ-glycidoxypropyl-functionalized silica gel (G-silica gel)[332] and 3-aminopropyl-functionalized silica gel (A-silica gel),[333] respectively (Figure 34).

Recently, we developed an efficient immobilization method by the intermolecular polycondensation of 1−2% triethoxysilyl groups introduced to the polysaccharide derivatives (Figure 35).[334,335] During this immobilization, the higher order structure of the polysaccharide derivatives may not be disordered, because the derivatives can be efficiently immobilized onto silica gel using a small amount of triethoxysilyl groups and there is a low degree of cross-linking. Therefore, the immobilized CPMs showed a high recognition ability as do the conventional coated-type CPMs. Compared with the other immobilization methods discussed above, this immobilization method seems to be more valuable with respect to the simplicity of the processing, immobilization efficiency, chiral recognition, and applicability to various polysaccharide derivatives.

Today, several immobilized CPMs with a universal solvent compatibility are commercially available under the trade names Chiralpak IA,[183] Chiralpak IB,[287] and Chiralpak IC[288] (Daicel), which contain the polysaccharide derivatives **18ab**, **17ab**, and **17ae** as chiral selectors, respectively. These immobilized CPMs have been used for both analytical and preparative separations and provided a better performance than the corresponding coated CPMs with selection of a suitable eluent.

For analytical HPLC, more efficient separations of chiral compounds (Figure 36) that cannot be resolved or can be resolved to a small degree on the coated CPMs have been attained on the immobilized CPMs using the prohibited solvents.[183,287,336−341] This improvement of the recognition ability may partly be due to the change in the conformation of the polysaccharide derivatives. The immobilized CPMs are also useful for separation of chiral compounds with a low solubility in a conventional eluent. For example, compound **39** is difficult to analyze using the conventional coated CPM due to the low solubility of **39** in a hexane/alcohol mixture. However, as shown in Figure 37, **39** can

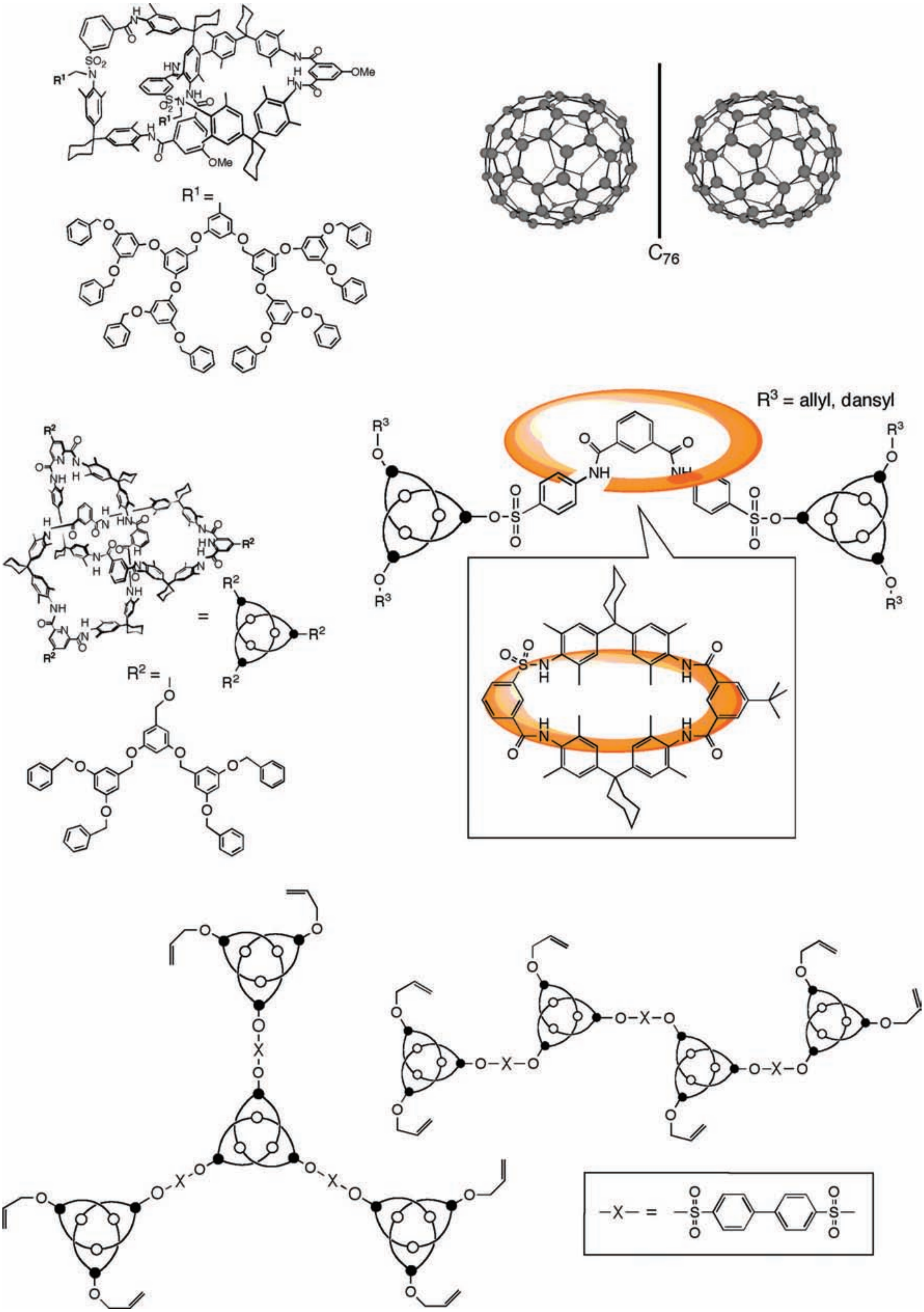


Figure 31. Structures of topologically interesting compounds and chiral fullerene C_{76}.

be efficiently resolved on Chiralpak IA using a mixture of hexane/$CHCl_3$/CH_2Cl_2 as the eluent,[342] which cannot be used for the coated type. The immobilized CPMs possess the advantage that direct monitoring for asymmetric synthesis occurring in the prohibited solvent, such as chloroform, dichloromethane, and toluene can be performed without further purification and removal of the prohibited solvents. Actually, Chiralpak IA and Chiralpak IB have been used for direct reaction monitoring for asymmetric synthesis in prohibited solvents.[343−346] For a large-scale separation, the selection of a solvent with a good sample solubility is extremely important,[55,290,291] although the compromise among the enantioselectivity, resolution and retention time should also be considered. The effect of sample solubility on the

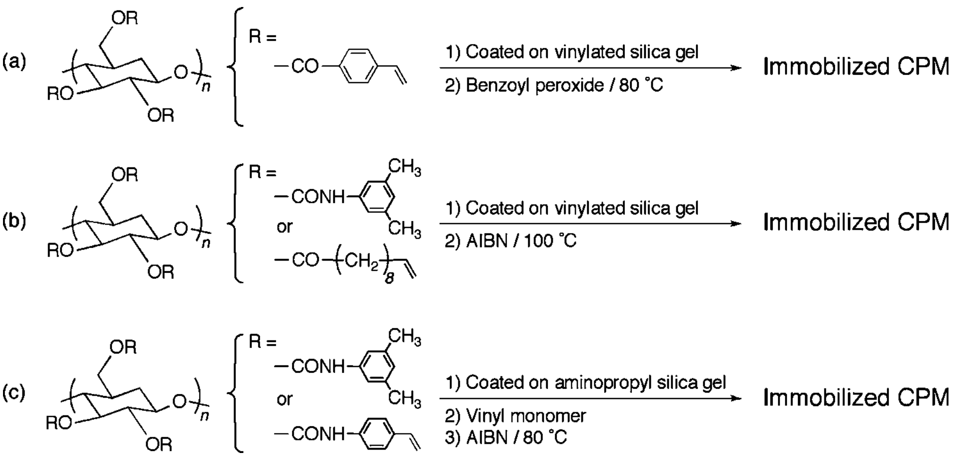


Figure 32. Immobilization of polysaccharide derivatives bearing vinyl groups via radical polymerization.

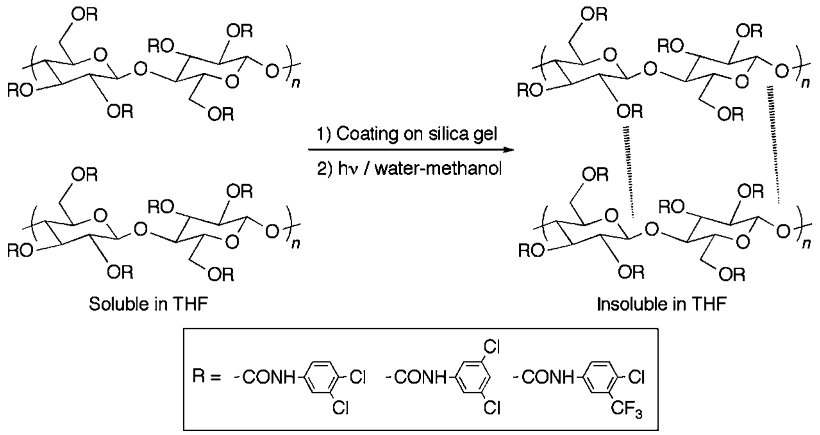


Figure 33. Immobilization of the polysaccharide derivatives via photochemical cross-linking.

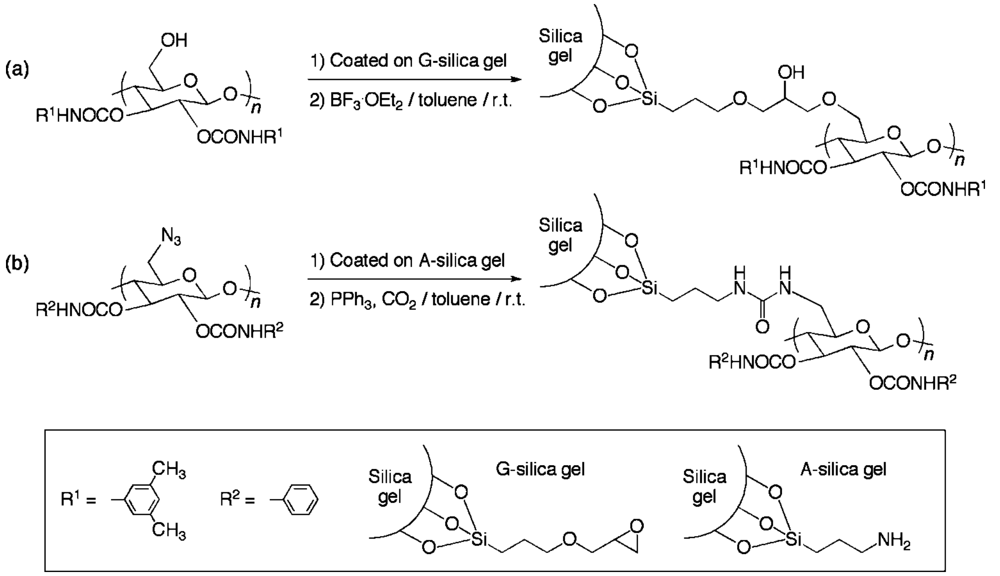


Figure 34. Immobilization of polysaccharide derivatives bearing hydroxy groups and azido groups onto G-silica gel (a) and A-silica gel (b), respectively.

preparative separation of a Ca-sensitizing drug (**40** in Figure 38) has been significantly investigated using Chiralpak IA.[347] The use of eluents with a high solubility, such as dichloromethane and THF, makes it possible to produce pure enantiomers with a high productivity.

12. Chiral Separations on Monolithic Silica Columns Containing Polysaccharide Derivatives

Rapid chromatographic analysis is becoming more prevalent in the fields of combinatorial chemistry, genomics, and

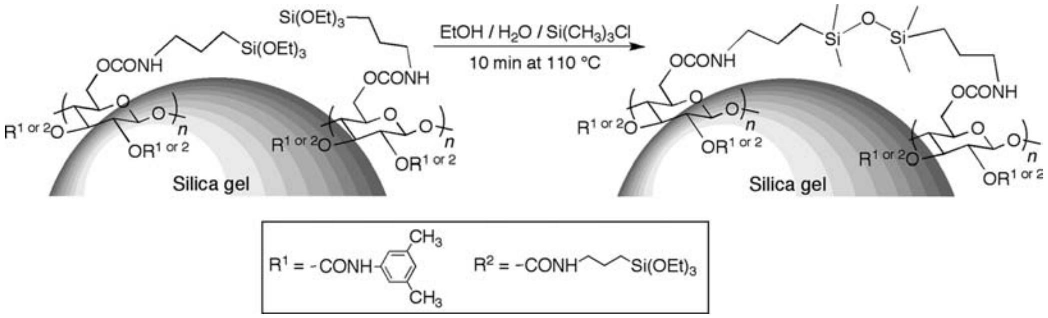


Figure 35. Immobilization of polysaccharide derivatives via intermolecular polycondensation of triethoxysilyl groups.

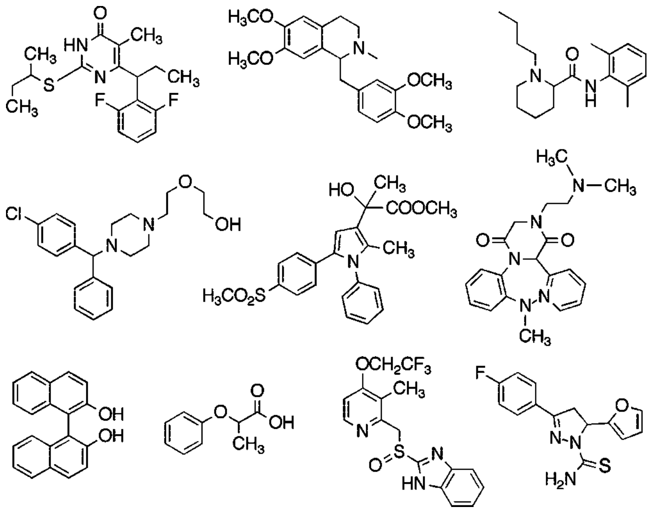


Figure 36. Examples of chiral compounds efficiently resolved on Chiralpak IA and Chiralpak IB with prohibited eluents.

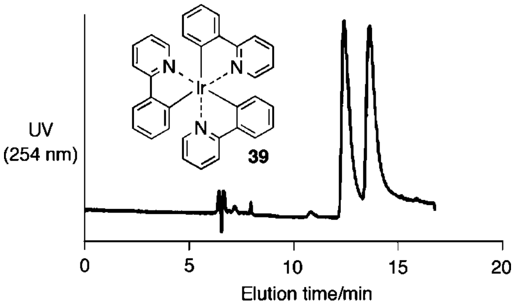


Figure 37. HPLC chromatogram of **39** on Chiralpak IA. Eluent = hexane/$CHCl_3$/CH_2Cl_2 (75:20:5); flow rate = 0.5 mL/min. Reprinted with permission from ref 342. Copyright 2007 Wiley-VCH.

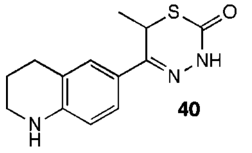


Figure 38. Structure of the Ca-sensitizing drug **40**.

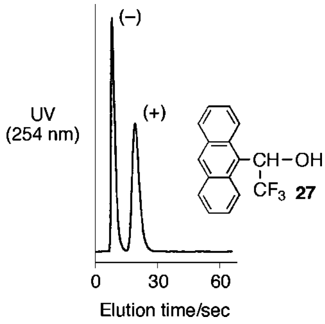


Figure 39. Chiral separation of *rac*-2,2,2-trifluoro-1-(9-anthryl)-ethanol (**27**) using **17ab** *in situ* coated at 12% (w/w) on monolithic silica gel as chiral stationary phase. Column = 50 mm × 4.6 mm (i.d.). Eluent = hexane/2-propanol, 90/10. Flow rate = 20 mL/min. Reprinted with permission from ref 354. Copyright 2003 The Chemical Society of Japan.

proteomics, in which a large number of analytes are generated. The analytical run time is dependent on the linear flow rate of the mobile phase as well as the retention characteristics of the analytes, column length, and dynamic characteristics of the stationary phases.

Macroporous spherical silica gels with a mean particle size of 3−10 μm are usually used as the supporting material for the polysaccharide-based CPMs. In this case, when the flow rate is increased to shorten the analysis time, a substantial pressure drop and peak broadening due to mass transfer resistances are often observed. On the other hand, monolithic silica gels, which are made of a single piece of porous silica gel, have attracted considerable attention as alternative supports for a rapid chromatographic analysis.[348−353] The pressure drop through monolithic silica gel supports is significantly lower and the column efficiency on the linear flow rate of the mobile phase is much flatter compared with the spherical silica supports.

Recently, several attempts have been made to combine the high recognition ability of polysaccharide derivatives with the favorable kinetic properties of monolithic silica gel supports.[332,354−359] In 2003, the first monolithic CPM based on polysaccharide derivatives was prepared by filling a commercially available monolithic silica column (50 mm × 4.6 mm (i.d.)) with an acetone solution of cellulose 3,5-dimethylphenylcarbamate (**17ab**), followed by slowly drying the solvent at ambient temperature and pressure.[332,354] After *in situ* coating, the cellulose derivative was loaded at ca. 12 wt % on the monolithic silica gel. The obtained monolithic CPM can be effectively employed even at the very high flow rate of 20 mL/min, and the baseline separation of the racemate (**27**) can be accomplished within the analysis time below 30 s (Figure 39). The rapid enantioseparation within a minute opens up the new possibility for high-throughput analysis by chiral HPLC.

Miniaturization of chiral separation techniques has become one of the major trends in analytical chemistry during the past few years.[360,361] Because miniaturized techniques are environmentally friendly and cost-effective, they are suitable for the screening of large compound libraries. Downscaling of the commonly used HPLC columns (4.0−4.6 mm (i.d.)) to the capillary format (0.1−0.5 mm (i.d.)) seems to be a promising approach for parallel and multidimensional analysis. Therefore, enantioseparations using capillary electro-

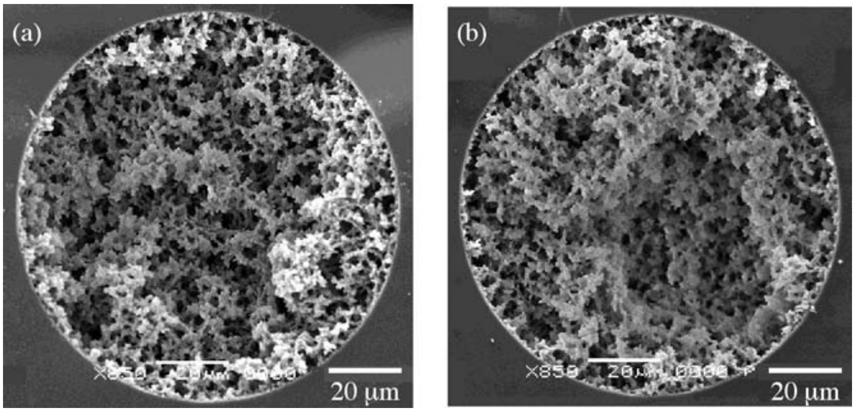


Figure 40. Scanning electron microscope images of monolithic silica capillary before (a) and after (b) modification with polysaccharide derivative. Reprinted with permission from ref 359. Copyright 2006 Wiley-VCH.

phoresis, capillary liquid chromatography (CLC), capillary electrochromatography (CEC),[78−86] and laboratory-on-chip[362−369] have been intensively studied during the past few years. Until now, various types of capillary columns including wall-coated open tubular capillaries, packed fused silica capillaries, and monolithic capillaries have been utilized as tools for miniaturized chiral separations.[370−379] In the wall-coated open tubular capillaries, the inside wall of the capillaries was modified with the appropriate chiral selectors. Their recognition abilities were not very high because of the low content of chiral selectors. For the packed fused silica capillaries, the traditional CPMs for HPLC can be utilized without additional modification. However, it suffered from bubble formation during the frit fabrication. The frit is considered to be a main source of fragility, low reproducibility, and peak dispersion of the capillary columns. On the other hand, the monolithic silica capillary columns have the significant advantages of no need for frits for maintaining the CPMs, in addition to the low back-pressure and a flatter dependence of the column efficiency on the linear flow rate.

Monolithic silica capillary columns based on 3,5-dimethylphenylcarbamate of cellulose (**17ab**) and amylose (**18ab**) have been prepared by *in situ* coating.[355−357] The amount of chiral selectors can be increased by repeating the coating process. The obtained monolithic capillaries were used for enantioseparations in CLC or CEC, and several chiral compounds were efficiently resolved in a short time of less than 1 min. These results indicate that enantioseparation on the monolithic capillary column containing the polysaccharide derivatives appears to be fairly useful for the rapid microanalysis of chiral compounds. As well as a silica-based monolithic capillary, the organic polymer-based monolithic capillary column modified with cellulose 3,5-dimethylphenylcarbamate (**17ab**) can be used for enantioseparation in CEC as reported by Zou et al.[358]

The immobilization of polysaccharide derivatives onto monolithic silica gel in capillary columns has also been investigated.[359] Scanning electron microscope (SEM) images of the monolithic capillaries were taken before and after modification with the polysaccharide derivative (Figure 40). From the SEM observations, it was found that the modification does not cause any significant change in the morphology of the monolithic packing material. The obtained CPMs with covalent attachment of the polysaccharide derivatives have a universal solvent compatibility as described in the previous section.

13. Preparative Separation Using Polysaccharide-Based Chiral Packing Materials

In preparative separations, SMB chromatography provides a higher productivity compared with the conventional batch-wise chromatography because of the efficient utilization of the stationary and mobile phases.[87−92,380−387] SMB chromatography is based on a quasi-countercurrent contact between the stationary and mobile phases mimicking a true moving bed. This is realized by the periodic movement of the inlet and outlet ports in the same direction as the mobile phase flow. In order to efficiently operate SMB chromatography, many parameters, such as the diameter and length of the column, total column number, eluent, feed, raffinate, extract and recycle fluid flow, and switch time interval, should be correctly regulated. Therefore, the process design of SMB chromatography is usually optimized by computer simulations. SMB chromatography is particularly effective for the separation of binary components. Since the late 1990s, therefore, large-scale separations of chiral compounds, which include just two enantiomers, have been performed using SMB chromatography by the pharmaceutical industry.[87−92]

As well as analytical separations, the polysaccharide-based CPMs have been frequently used for large-scale preparative separations by SMB chromatography because of their higher loading capacity compared with the other CPMs.[55] However, the current polysaccharide-based CPMs are not optimal for preparative separations, because the CPMs have been prepared by coating or immobilizing the polysaccharide derivatives at only ca. 20 wt % on silica gel and the major part (80 wt %) of the CPMs consist of silica gel inactive for the enantioseparation. In order to improve the productivity for preparative separations, an increase in the polysaccharide content of the CPMs is highly required. However, it is quiet difficult to increase the content via conventional methods without reducing the performance.

In the past, the bead-type CPMs, which consist of only polysaccharide derivatives without silica gel support, have been developed to increase the productivity for preparative separations.[388−391] Although these bead-type CPMs showed a higher loading capacity than the conventional coated-type CPMs, the bead-type CPMs suffered from their low mechanical strength due to the absence of rigid inorganic supports.

Recently, we developed an organic−inorganic hybrid bead-type CPM for preparative separation,[392] which has a sufficient mechanical strength applicable for the practical use

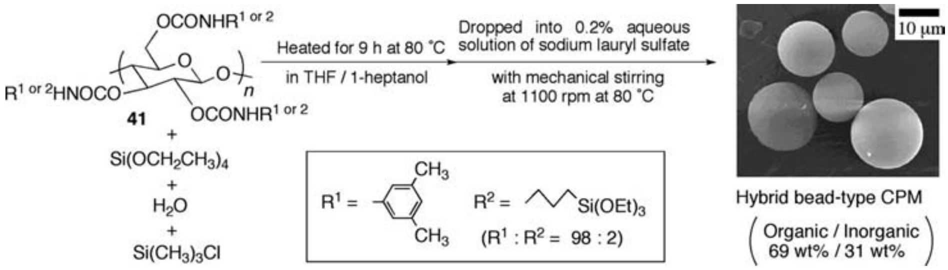


Figure 41. Scheme of preparation and SEM image of the hybrid bead-type CPM. Reprinted with permission from ref 392. Copyright 2008 Wiley-VCH.

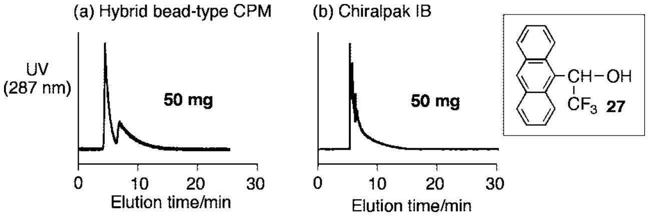


Figure 42. Preparative separation of *rac*-2,2,2-trifluoro-1-(anthryl)ethanol (**27**) with hexane/2-propanol (90/10) as eluent: (a) hybrid bead-type CPM at flow rate of 2.0 mL/min; (b) Chiralpak IB at flow rate of 1.0 mL/min. Reprinted with permission from ref 392. Copyright 2008 Wiley-VCH.

of HPLC. Figure 41 shows the preparation scheme of the hybrid bead-type CPM via a sol−gel reaction between a polysaccharide derivative bearing a small amount of 3-(triethoxysilyl)propyl residues, **41**, and tetraethyl orthosilicate (TEOS). The hybrid beads have a spherical shape with a mean particle size of less than 20 μm (Figure 41). The organic and inorganic contents in the hybrid bead-type CPM were estimated to be 69 wt % and 31 wt %, respectively, and were well-dispersed on the surface of the hybrid bead-type CPM at least on a micrometer level.

The hybrid beads were packed into an HPLC column at a pressure of 40 MPa without any transformation of their spherical shape. This is different from the results of previous bead-type CPMs consisting of only an organic component, which cannot be applied at high pressure. These results indicate that the 31% inorganic component in the hybrid bead seems to play an adequate role as a support and provides a sufficient mechanical strength to the CPM.

In order to verify the potential ability of the hybrid bead-type CPM for preparative separation, the loading capacities on the hybrid bead-type CPM and the commercially available Chiralpak IB, which are packed using the same size columns, were compared. The chromatograms of the preparative separation of 2,2,2-trifluoro-1-(anthryl)ethanol, **27**, on the hybrid bead-type CPM and Chiralpak IB are shown in Figure 42, in which the retention time on the hybrid bead-type CPM was adjusted to that on the Chiralpak IB by controlling the flow rate. The hybrid bead-type CPM could readily separate 50 mg of **27** (Figure 42a), while the Chiralpak IB showed one overlapped peak for the 50 mg loading (Figure 42b). These chromatograms clearly indicated that a high-throughput resolution of a chiral compound can be realized using the hybrid bead-type CPM.

In the future, combination of the hybrid bead-type CPM and the SMB technique is expected to provide a significant benefit to the production of optically pure compounds.

14. Conclusions

In this review, the development and applications of polysaccharide derivatives for the efficient chromatographic separation of enantiomers have been outlined. The chiral recognition abilities of native polysaccharides themselves are not sufficient for use as chiral selectors for CPMs, but their abilities can be readily improved by structure control including primary, secondary, and tertiary structures of the polysaccharides. At present, more than ten polysaccharide-based CPMs are already commercially available and frequently used around the world due to their wide applicability to racemates and high loading capacity. The chiral recognition mechanism of the polysaccharide derivatives has been clarified to some extent by NMR and IR spectroscopy, X-ray analysis, and computational methods. Understanding of the chiral discrimination mechanism at a molecular level will help with the prediction of the elution orders of enantiomers and the development of more efficient CPMs. As well as for HPLC, the polysaccharide-based CPMs have also been used for SFC, which offer several advantages, such as a rapid column equilibration, fast method development, low pressure drop, and high resolutions. In the future, chiral separations by SFC will be more common particularly in the field of large-scale preparative separations from the viewpoint of a low solvent consumption and easy solvent removal and recycling. Furthermore, the immobilized-type CPMs based on polysaccharide derivatives with a universal solvent compatibility have opened up the possibility to improve the performance of both analytical and preparative separations of chiral compounds. Recently, monolithic silica gel supports have attracted significant attention as a supporting material for a rapid chromatographic analysis, because the pressure drop through monolithic silica gel supports is significantly lower and the column efficiency on the linear flow rate of the mobile phase is much flatter compared with the spherical silica supports. For preparative separations, SMB chromatography, which is a powerful method for the separation of binary components, has been successfully performed particularly in the pharmaceutical industry. We expect that the applications of the polysaccharide-based CPMs will make a meaningful contribution to further growth in all fields of science related to chirality, such as drugs, agrochemicals, food additives, fragrances, and functional materials. On the other hand, the development of a preparation method of the CSPs that can efficiently resolve target chiral compounds is also highly desired. This may even change the strategy used for the synthesis of chiral compounds.

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16. Note Added after ASAP Publication

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17. References

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