

Accounts

Optically Active Polymers for Chiral Separation

Chiyo Yamamoto and Yoshio Okamoto*

Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-8603

Received September 18, 2003; E-mail: okamoto@apchem.nagoya-u.ac.jp

One of the most practically important functions of optically active polymers is chiral recognition. In the past two decades, various optically active polymers have been utilized as the chiral stationary phases (CSPs) for high-performance liquid chromatography, which is today the most powerful and practical method not only for analyzing enantiomers but also for obtaining them in a pure form. In this account, the emphasis lies on the polymethacrylate and polysaccharide derivatives that we have developed. One-handed helical poly(triphenylmethyl methacrylate) has been prepared by asymmetric anionic polymerization and shows high chiral recognition, especially for the compounds which have no functional groups. Polysaccharide derivatives, particularly cellulose and amylose phenylcarbamates, can resolve a wide range of racemates, and their abilities are significantly influenced by the substituents introduced on the phenyl moieties. Cellulose and amylose cycloalkylcarbamates and chitin phenylcarbamates also show high chiral recognition; the former can be utilized as the CSPs for thin-layer chromatography because of the absence of UV absorption. The immobilization of the polysaccharide derivatives onto a silica support via chemical bonding improves their durability for various solvents and also their resolving ability for some compounds. Several phenylcarbamates are soluble in chloroform, which allowed one to study their chiral recognition mechanism at a molecular level, particularly by NMR spectroscopy. A computational method can also be applied for mechanistic studies.

Recently, optically active polymers have attracted a great deal of attention. One of the reasons for this is the chiral nature of living systems. Most naturally occurring polymers, such as proteins, nucleic acids, and polysaccharides, are chiral and optically active; they often possess a specific conformation associated with their chirality. Some of the natural polymers have been utilized for enantiomeric differentiation in vitro as chiral catalysts, chiral hosts, and chiral adsorbents. Various optically active polymers have been designed and synthesized to mimic these functions.[1,2] Most of the polymers have been prepared by one of three typical methods: the polymerization of optically active monomers, the modification of optically active polymers, and the asymmetric polymerization of prochiral monomers.

One of the most remarkable applications of optically active polymers is the chiral separation of racemic compounds by high-performance liquid chromatography (HPLC) using the polymers as chiral stationary phases (CSPs).[3–11] The determination of optical purity or enantiomeric excess (ee) had been a hard task until 1980, since only two practical methods, using a polarimeter and using an NMR instrument, existed. In the 1980s, the chiral separation by gas chromatography (GC) and HPLC using CSPs had significantly advanced, and today the ee of most compounds is determined mainly by three methods: NMR, GC, and HPLC.[12] In particular, the direct separation of enantiomers by HPLC has become the most powerful method not only for analyzing enantiomers but also for obtaining them in a pure form. The preparation of a CSP capable of effective chiral recognition is the key step in this method. Therefore, many CSPs for HPLC have been prepared, and about 100 of them have been commercialized. The CSPs for HPLC are classified into two types: one consists of optically active small molecules,[8,13,14] and the other is based on optically active polymers. The small molecules are usually bonded onto silica gel. On the other hand, the polymers can be used as the CSPs by coating on silica gel or by cross-linking as a gel. The chiral recognition by small molecule CSPs depends mainly on that of the small molecules themselves, but polymeric CSPs often show chiral recognition that depends on the higher-order structure of the polymers, which makes understanding the chiral recognition mechanism difficult. Today, the most widely used CSPs are the derivatives of polysaccharides, probably because of the very broad applicability of these CSPs.[12,15–19] In this account, we will discuss various optically active polymers as the CSPs for HPLC. Special emphasis is placed on the polysaccharide derivatives recently developed in our group.

1. Optically Active Polymers with Chiral Recognition Ability

Figure 1 shows some typical CSPs prepared from optically active polymers (**1–16**); CSPs **1–13** are totally synthetic polymers including vinyl polymers (**1–5**), polyamides (**6–10**), polyurethanes (**11**), polyacetylene (**12**), and a polysaccharide analogue (**13**). CSPs **14–16** are based on natural polymers, proteins

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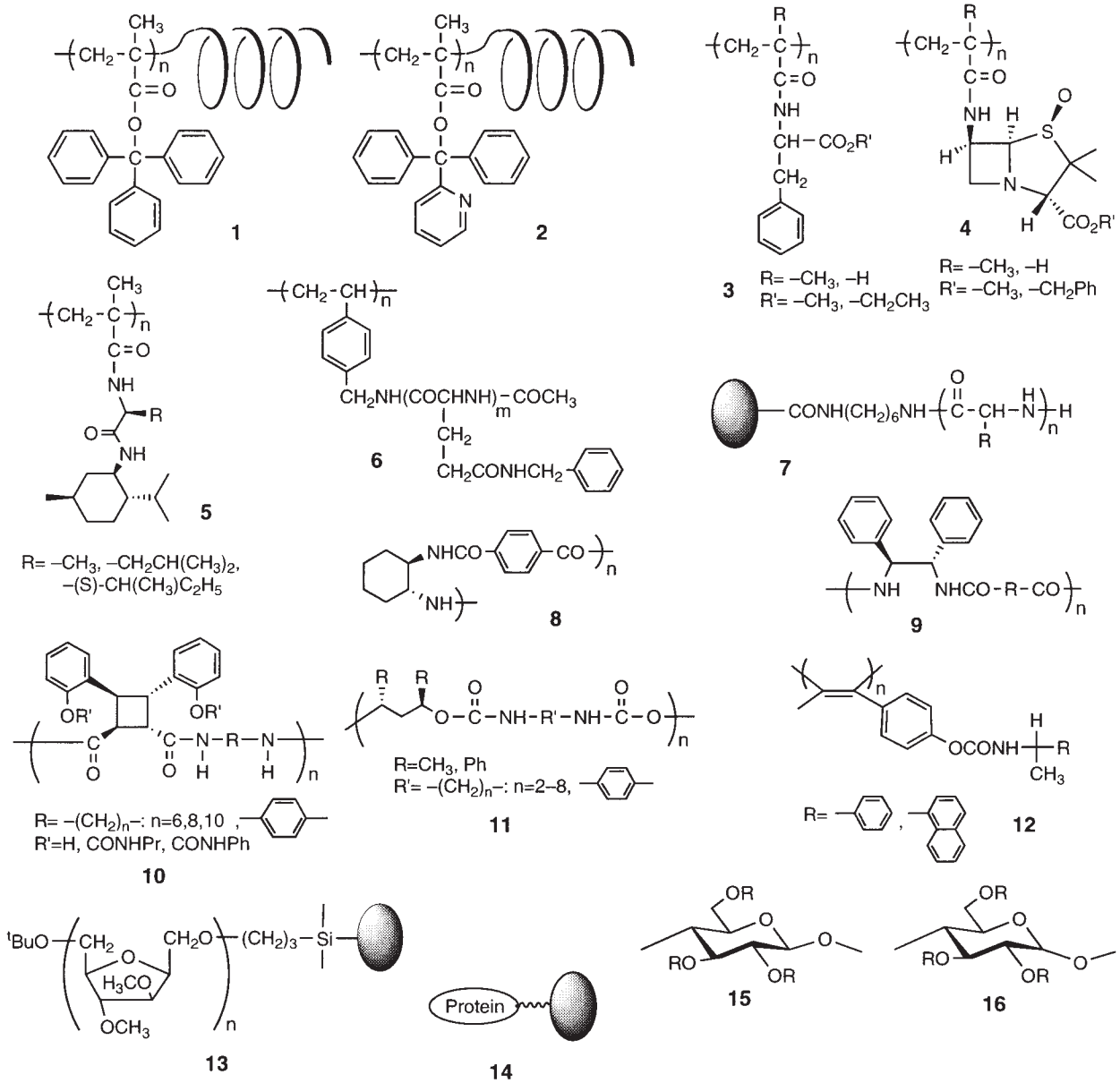


Fig. 1. Chiral polymer stationary phases for HPLC.

(**14**) and polysaccharides (**15**, **16**).

The chiral recognition ability of a CSP can be quantitatively evaluated from a chromatogram of enantiomer resolution. Figure 2 shows the chromatogram for the HPLC resolution of Tröger base on cellulose tris(3,5-difluorophenylcarbamate). The (+)-isomer elutes first, followed by the (−)-isomer, and complete baseline separation is achieved. The results of the separation can be evaluated by three parameters: —capacity factors (k'), separation factor (α), and resolution factor (Rs)—defined as follows:

$$\begin{aligned} &k_1' = (t_1 - t_0)/t_0, \quad k_2' = (t_2 - t_0)/t_0, \\ &\alpha = (t_2 - t_0)/(t_1 - t_0) = k_2'/k_1', \\ &Rs = 2(t_2 - t_1)/(w_1 + w_2), \end{aligned} \tag{1}$$

where t_1 and t_2 are the retention times of the enantiomers, t_0 is the dead time (retention time of a nonretained compound), and w_1 and w_2 are the bandwidths of the peaks. In a chromatographic separation, α is directly related to the chiral recognition ability of a CSP, and Rs is correlated to both the chiral recognition ability of a CSP and the column efficiency (theoretical plate number). The energy difference ($\Delta\Delta G$) for the interactions between a CSP and a pair of enantiomers is estimated from the α value using the equation, $\Delta\Delta G = -RT \ln \alpha$. A separation factor of $\alpha = 1.20$ corresponds to $\Delta\Delta G = -0.11$ kcal/mol, which is usually sufficient for the baseline separation of enantiomers. A small energy difference in the chiral discrimination process can realize the complete separation of enantiomers.

2. Synthetic Polymers

2.1 Vinyl Polymers. 2.1.1 Polymethacrylate: Triphenylmethyl methacrylate (TrMA) is a unique monomer that gives an almost completely isotactic polymer by anionic polymerization in both nonpolar and polar solvents. Even free-radical polymerization provides an isotactic-rich poly(TrMA) (**1**).[20] The isotac-

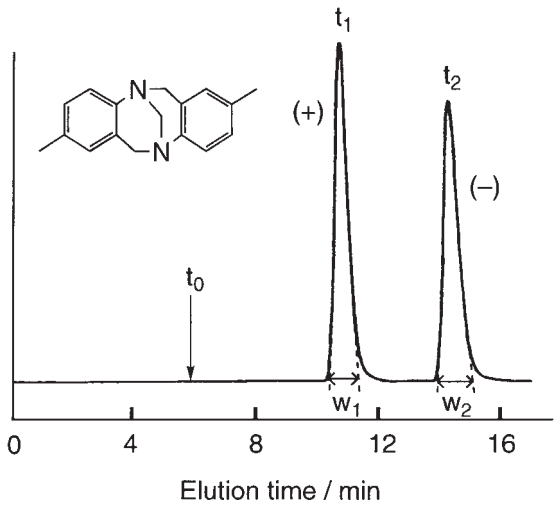


Fig. 2. Optical resolution of Tröger base on cellulose tris(3,5-difluorophenylcarbamate) column: 25 × 0.46 (i.d.) cm; eluent: hexane/2-propanol (90/10); flow rate: 0.5 mL/min.

tic specificity of the TrMA polymerization is attributed to the helix formation of the main chain, which is constructed through the self-organization of the bulky side groups and is stabilized by steric repulsion of the groups. Therefore, when the bulky triphenylmethyl groups are removed from the polymer by hydrolysis of the ester linkage, the helical structure is lost.

The conventional anionic or radical polymerization of TrMA gives a mixture of equal amounts of enantiomeric right- and left-handed helices. In 1979, the first synthesis of one-handed helical **1** was achieved by asymmetric anionic polymerization using a complex of *n*-BuLi with (−)-sparteine ((−)-Sp) (Fig. 3).[21] This was the first example of an optically active vinyl polymer, the chirality of which is caused mainly by helicity. Since then, many chiral ligands have been examined to control the helicity, and some of them can lead to almost a 100% one-handed helix. For example, the complexes of 9-fluorenyllithium (FlLi) with (−)-Sp, (+)- and (−)-2,3-dimethoxy-1,4-bis(dimethylamino)butane (DDB), and (+)-(1-pyrrolidinylmethyl)-pyrrolidine (PMP) (Fig. 4), produce an almost perfectly isotactic one-handed helical **1** through the polymerization in toluene at a low temperature.[22–24] The solvent used for the polymerization significantly affects the helix-sense-selectivity. When the polar solvent THF was used for the polymerization, the helix-sense-selectivity was significantly reduced. The polymer with a degree of polymerization (DP) above 100 is insoluble in solvents, but the polymer with a relatively low DP is soluble in THF.

The optically active **1** shows a high optical activity and intense circular dichroism (CD) due both to the helicity of the main chain and to the triphenylmethyl group with a chiral propeller structure. The chiral recognition ability of **1** was evaluated for two different types of the CSPs for HPLC; one was prepared with the insoluble **1** ground into small particles[25] and the other by coating the soluble polymer on a macroporous silica gel.[26] Among the many polymer-type CSPs in Fig. 1, CSP **1**, especially the coated-type, shows a particularly high chiral recognition ability for various kinds of racemates. The chiral recognition ability of insoluble **1** with a high molecular weight (DP = 220) was estimated using a column packed with small ground polymer particles.[25] Although various racemates were resolved on this column, it was difficult to prepare an efficient packing material due to its brittleness. This defect was overcome by coating the soluble **1** of DP = ca. 50 on macroporous silica gel.[26] The **1**-coated silica gel showed higher resistance to compression and a longer lifetime than the CSP based on the insoluble **1**. The insoluble and soluble **1**-based CSPs showed different chiral recognition for several racemates, which may be attributed to the different orientation of **1** in bulk and on the surface of silica gel.[27]

So far, more than 200 racemic compounds, including hydrocarbons, esters, amides, halides, and phosphoric compounds, have been resolved on the coated-type **1**; some of them are shown in Fig. 5. The CSP is particularly useful for the resolution of stereochemically interesting compounds that are diffi-

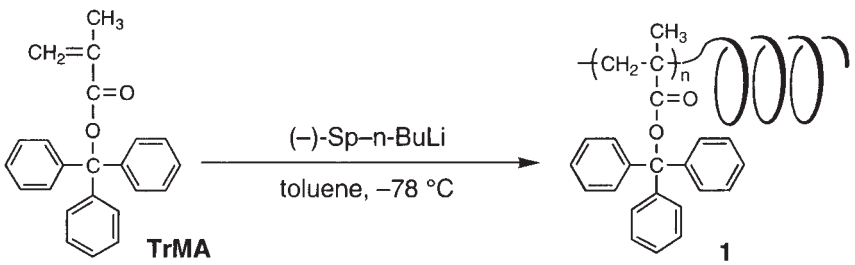


Fig. 3. Asymmetric polymerization of TrMA.

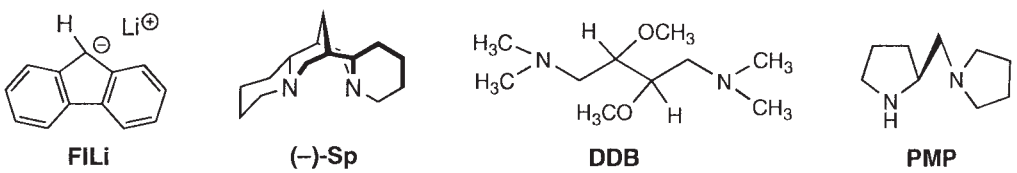


Fig. 4. Structures of 9-fluorenyllithium and chiral ligands.

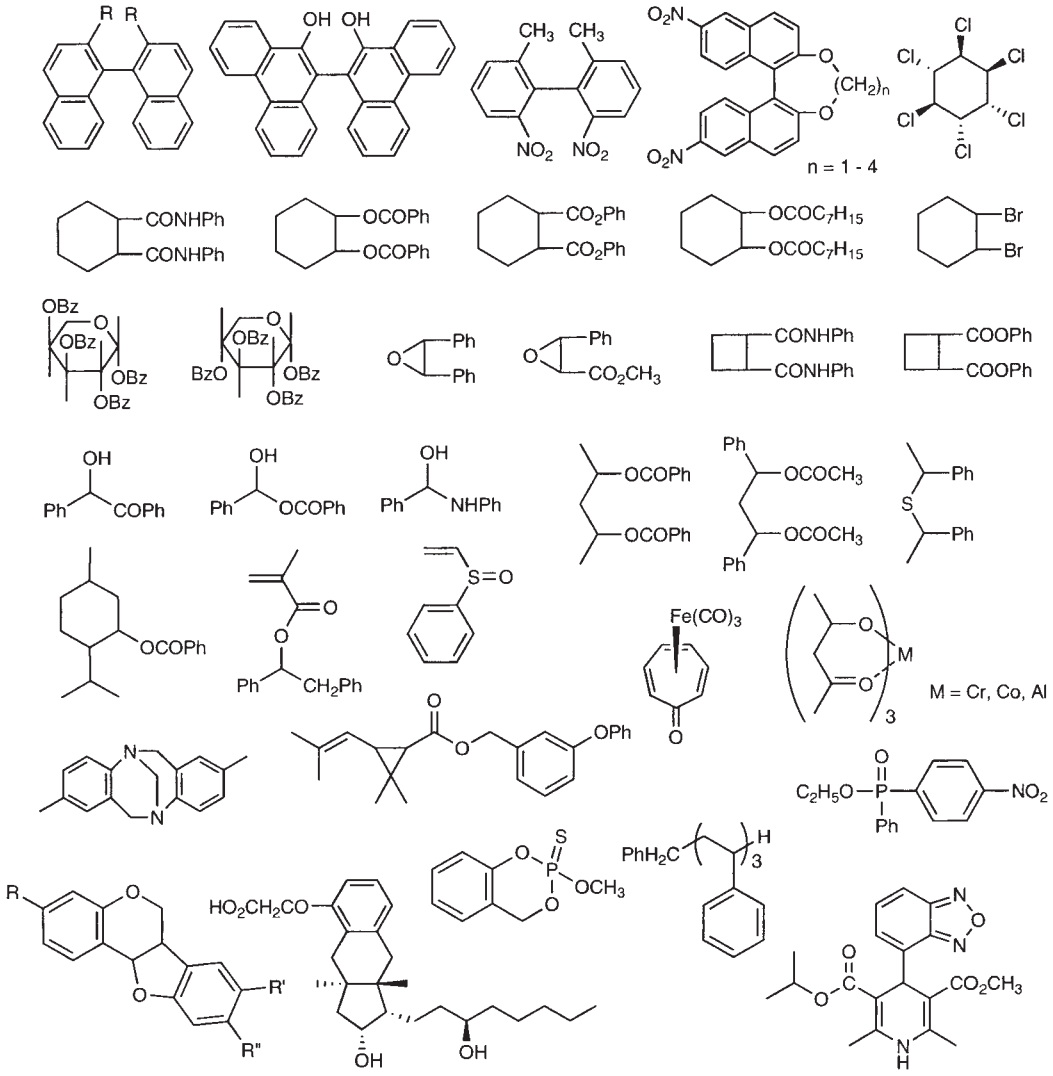


Fig. 5. Compounds resolved on optically active **1**.

cult to be resolved by other methods due to the lack of functionalities (Fig. 6).[25,28–45]

The eluents usable for the coated-type **1** are limited; the solvents, such as chloroform, THF, and aromatic hydrocarbon, which dissolve the polymer, cannot be used. In most cases, methanol or a methanol–water mixture gives better results for enantiomer separations than a nonpolar eluent such as a hexane–alcohol mixture. This indicates that nonpolar or hydrophobic interactions between **1** and a nonpolar group of an analyte are important for effective chiral recognition. The most important chiral recognition site of **1** seems to be the triphenylmethyl group with a chiral propeller structure.[27]

The chemically-bonded **1** CSP has also been prepared.[46] The chemical bonding of **1** with silica gel was attained by two methods: (a) the reaction of a block copolymer of TrMA and 3-trimethoxysilylpropyl methacrylate with silica gel or (b) the reaction between **1** having a terminal group, Ph–NH–CH_2CH_2–N(Ph)–, with the silica gel pretreated with (3-aminopropyl)triethoxysilane using toluene-2,4-diyldiisocyanate (Fig. 7). The obtained chemically-bonded CSP showed chiral recognition similar to that of the coated-type CSP when methanol was used as eluent. Using this chemically-bonded CSP, racemic helical polymers, for instance, an equimolar mixture of right- and left-handed **1** was separated into optical isomers.[46] Besides the (±)-**1**, some racemic polymethacrylates with a helical structure were resolved on this CSP using chloroform as an eluent.[47,48]

Although methanol is a good eluent for the resolution on **1**, the triphenylmethyl groups of **1** are slowly solvolyzed in this solvent. Therefore, when an alcoholic eluent is used, it is preferable to use the column at low temperatures. In addition, the column must be stored under a non-alcoholic condition. In order to counter this defect, an electron-withdrawing pyridyl group was introduced in place of a phenyl group in TrMA. Diphenyl-2-pyridylmethyl methacrylate (D2PyMA) also afforded an one-handed helical optically active polymer poly(D2PyMA) (**2**) by the helix-sense-selective polymerization with a chiral anionic initiator.[49–51] Such control of one-handedness is much more difficult compared with the polymerization of TrMA, because the side-chain pyridyl group interacts with the counter cation of the growing end and prevents the coordination of a chiral ligand with a counter cation. Most chiral ligands did not work effectively and only PMP gave a completely single-handed helical **2** with a narrow molecular weight distribu-

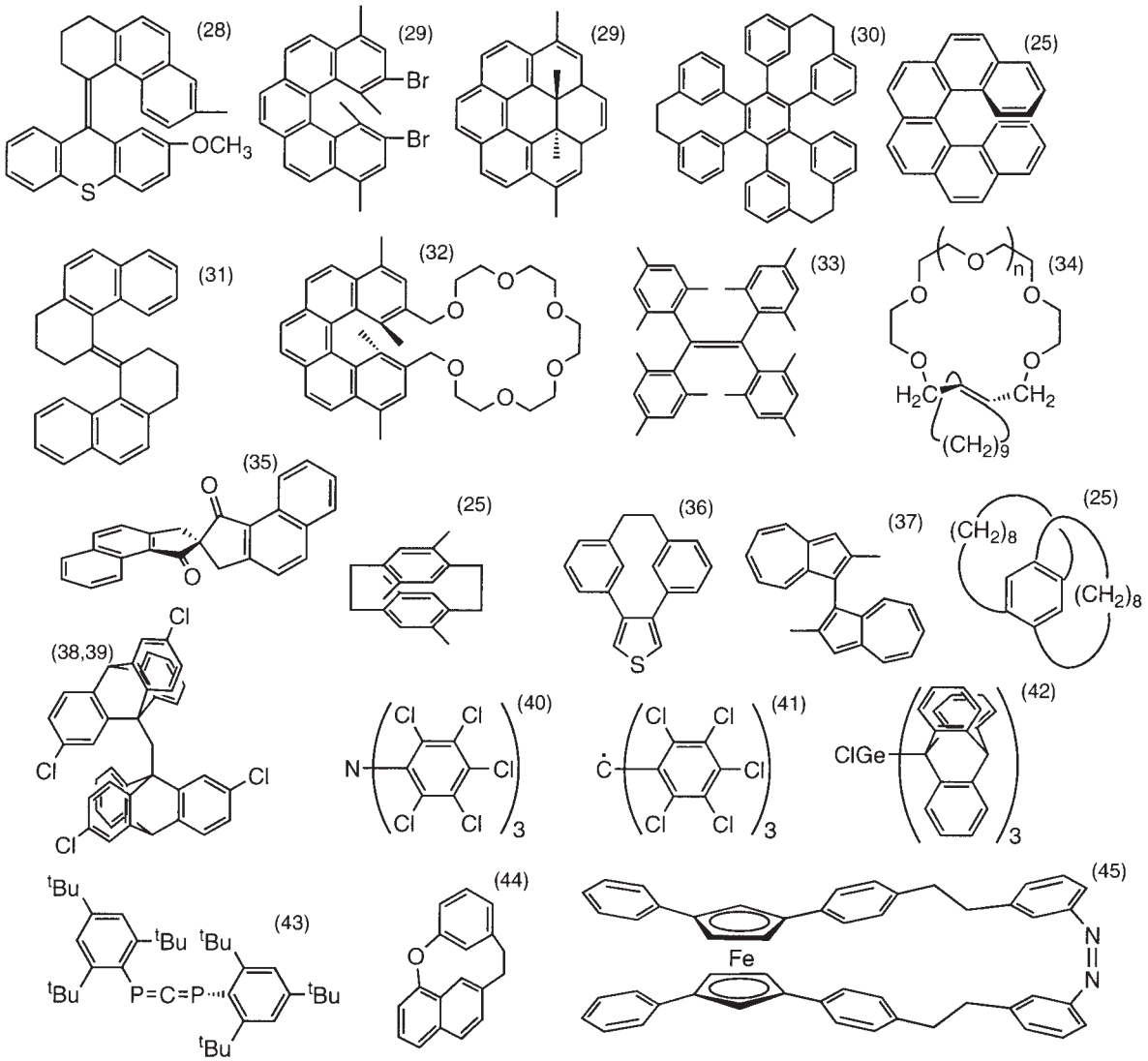


Fig. 6. Stereochemically interesting compounds resolved on optically active **1**.

tion.[50,51] The stability of the optically active **2**-coated silica gel against the solvolysis by methanol is 16-fold higher than that of **1**-coated silica gel.[52] The chiral recognition by **2** is basically similar to that by **1**; many racemic compounds have also been resolved by HPLC using **2**.[52] However, for the separation of polar racemates such as alcohols, the interaction through a hydrogen bond between the pyridyl group of **2** and the racemates also plays some important role along with the hydrophobic or nonpolar interactions.

Although the one-handed helical **1** shows high chiral recognition abilities towards various types of compounds, most optically active polymethacrylates bearing a simple chiral side chain such as the (*S*)-1-phenylethyl group show almost no chiral recognition ability.[27] This suggests that the chiral recognition by **1** is a rare case and can be attributed to the rigid helical structure accompanying the chiral propeller triphenylmethyl group. Figure 8 shows examples of the optically active polymethacrylates with chiral recognition ability. The polymethacrylates bearing a chiral urea moiety were synthesized by the radical polymerization of the chiral monomers prepared from 2-methacryloyloxyethyl isocyanate and the corresponding amine.[53,54] The polymers resolved some racemates. When (*R*)-(+)-1-(1-naphthyl)ethylamine was bonded to silica gel, the packing material showed chiral recognition different from that of the poly(methacrylate) with the same chiral amine residue (**17**), indicating that the higher-ordered structure of the polymer may influence the chiral recognition. The optically active polymethacrylates having binaphthol[55] or (+)-5-oxobornyl moieties[56] in the side chain (**18**, **19**) were also synthesized by radical polymerization. Both the polymers coated on silica gel separated several racemates. The influence of the stereoregularity on resolution has not yet been reported. The chiral recognition by **18** and **19** may simply arise from the binaphthyl and oxobornyl groups, respectively.

2.1.2 Polyacrylamide and Polymethacrylamide: In 1980, Blaschke and co-workers reported the chiral separation of drug enantiomers by optically active polyacrylamides and polymethacrylamides.[57–59] At that time, no commercially available CSPs existed for HPLC, and most chiral synthetic drugs were used as racemates. Many chiral drugs including thalidomide were resolved on the polymers, and the difference in pharmacological behavior between the enantiomers was evaluated.[60]

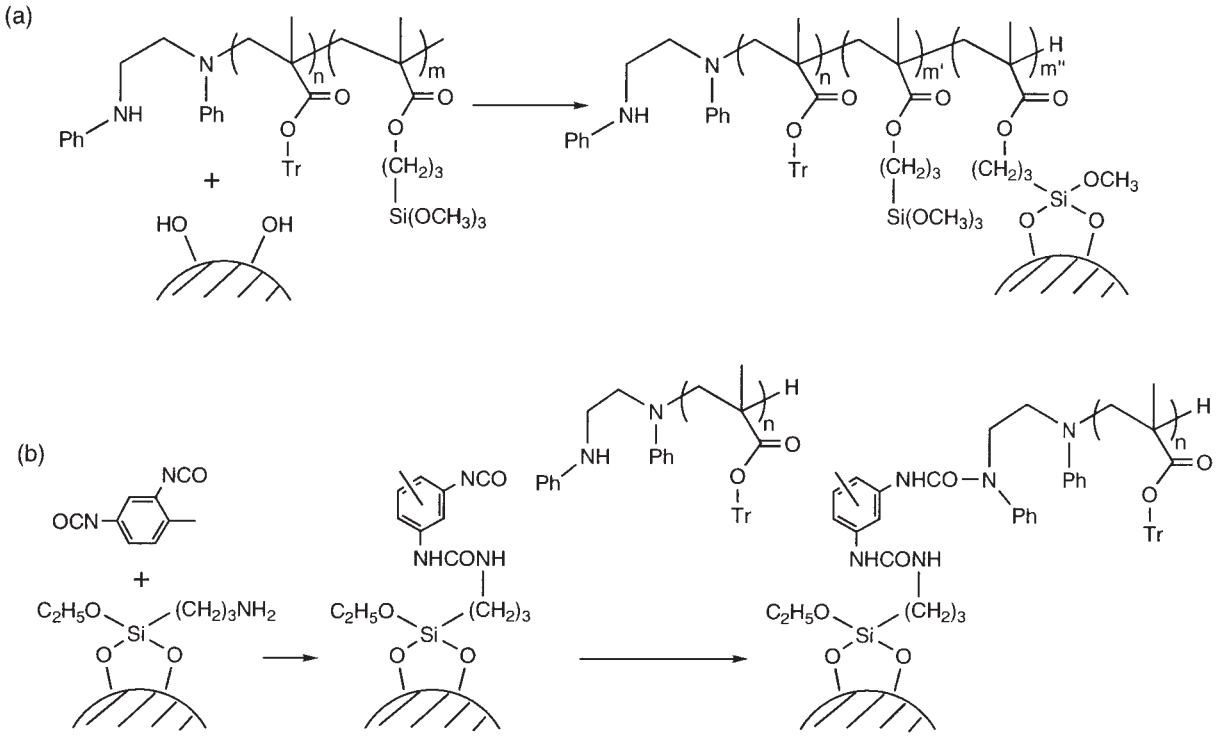


Fig. 7. Preparation of chemically bonded CSP **1**.

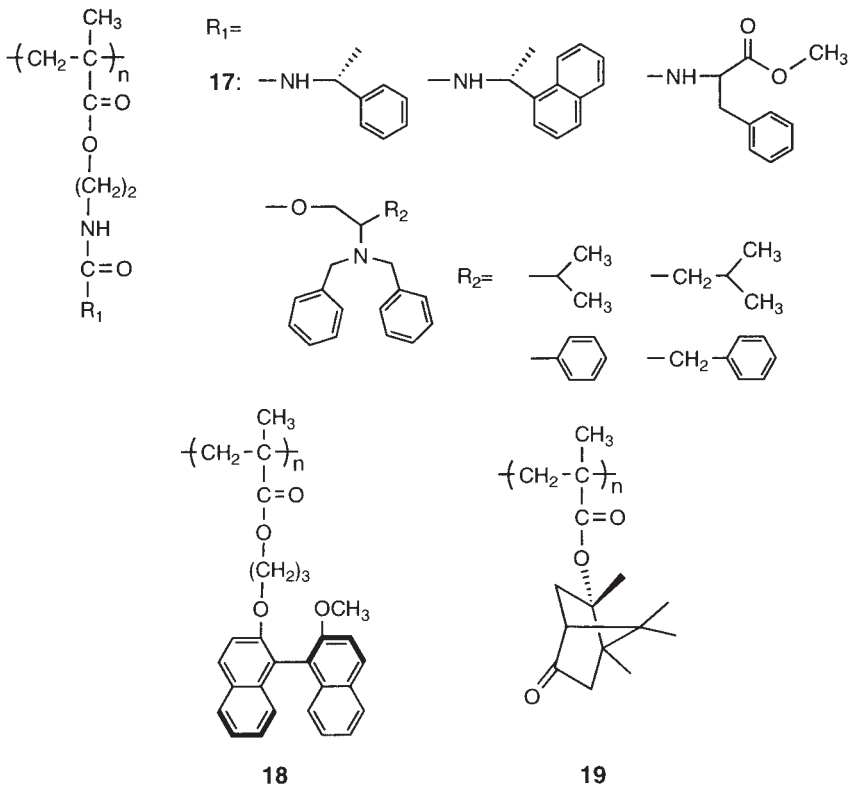
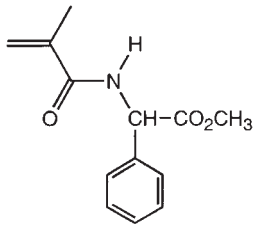


Fig. 8. Structures of the optically active polymethacrylates with chiral recognition ability.

This is one of the key studies, which informed us about the importance of clarification of the pharmacological behavior of both enantiomers. They also reported that the chiral recognition ability of the polymers is influenced by synthetic methods; that is, only the polymers prepared by the radical polymerization of optically active monomers exhibited high chiral recognition, while the same polymer prepared by the reaction of poly(acryloyl chloride) with the corresponding chiral amine showed a low



20

Fig. 9. Structure of *N*-(α-methoxycarbonylbenzyl)methacrylamide (**20**).

chiral recognition.[57] The chiral recognition sites with a high ability must be constructed through the polymerization process.

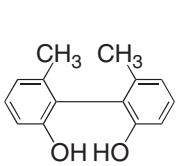
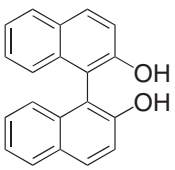
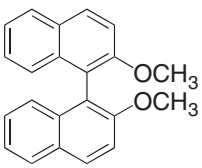
N-Monosubstituted (meth)acrylamides possess an amide hydrogen and therefore cannot be polymerized by an anionic method. Only the radical method, which generally produces an atactic or syndiotactic-rich polymer, can be applied. The chiral separation power of an optically active vinyl polymer is considered to depend on the tacticity, which influences the polymer conformation and higher-order structure. Recently, we found that Lewis acids such as ytterbium trifluoromethanesulfonate ($Yb(OTf)_3$) and yttrium trifluoromethanesulfonate ($Y(OTf)_3$) significantly influence the stereochemistry of the radical polymerization of meth(acrylamide)s.[61–65] Based on this result, we prepared the optically active poly(*N*-((*R*)-α-methoxycarbonylbenzyl)methacrylamides) (poly((*R*)-**20**)) (Fig. 9) that are rich in isotactic and syndiotactic sequences and we evaluated their chiral recognition abilities as the CSPs for HPLC.[66] The syndiotactic poly((*R*)-**20**)) (mm/mr/rr = ~0/13/87) was obtained in the absence of the Lewis acids using n-Bu_3B–O_2 as an initiator at a low temperature in THF, while the isotactic poly((*R*)-**20**) (mm/mr/rr = 87/13/~0) was prepared in the presence of $Yb(OTf)_3$. These two tactic polymers and the polymer prepared by the conventional radical method (initiator: AIBN, temperature: 60 °C, and solvent: THF) (mm/mr/rr = 6/29/65) were evaluated as CSPs (Table 1). From this study, it was found that the chiral recognition abilities of the polymethacrylamides are affected by the stereoregularity. Higher α values for the racemates having hydroxy groups, such as 2,2′-dihydroxy-6,6′-dimethylbiphenyl and 1,1′-bi-2-naphthol, were observed for the highest syndiotactic poly-**20**, and the α values decreased as the syndiotacticity decreased. The capacity factor was more significantly affected by the tacticity. This is because the isotactic poly-**20** may preferentially form intramolecular hydrogen bonds, which prevent the polymer from effectively interacting with the racemates. In the chiral recognition of racemates having a hydroxy group by poly-**20**, the hydrogen bonding interaction plays an important role, and therefore the di-*O*-methylated derivative of 1,1′-bi-2-naphthol was not resolved.

Some other polyacrylamides and polymethacrylamides (**4**, **5**) have also been prepared for use as CSPs.[67,68] Polymethacrylamide (**4**; R = CH_3, R′ = CH_2Ph) possessing a penicillin sulfoxide skeleton resolved various aromatic racemates. The stationary phase possessing only the monomer unit of **4** showed a lower resolving ability. This indicates that the alignment of the pendant chiral groups along the polymer backbone may be important for chiral recognition. The CSPs consisting of **5** were prepared by two methods: one by covalently bonding **5** having a vinyl group to silica gel through radical polymerization for analytical purposes and the other by bead polymerization in the presence of a cross-linking reagent for preparative purposes. The obtained CSPs showed a high recognition for several drugs.

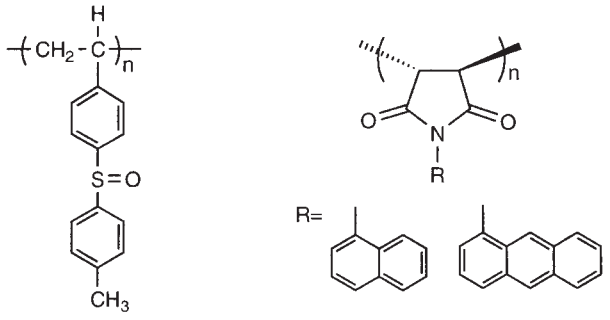
An optically active polystyrene derivative (**21**) (Fig. 10) with a chiral sulfoxide moiety can resolve several aromatic alcohols and amines.[69] Poly(*N*-1-naphthylmaleimide) and poly(*N*-1-anthrylmaleimide) (**22**) (Fig. 10) obtained by asymmetric anionic polymerization with chiral organometal complexes showed chiral recognition for several racemates, including 1,1′-bi-2-naphthol. The chiral recognition in solution by **22** (R = 1-anthryl) was also detected by ^1H NMR spectroscopy.[70] This polymer has a predominant (*R*,*R*) or (*S*,*S*) configuration of the main chain produced by a trans-opening of the maleimide monomer. The polymer probably has a helical structure arising from the main chain asymmetry.[71,72]

2.2 Polyamides. Several optically active polyamides including poly(α-amino acid)s show chiral recognition as the CSPs for HPLC. Cross-linked porous polystyrene beads incorporating poly(*N*-benzyl-L-glutamine) (**6**) were prepared via poly(γ-methyl-L-glutamate) and used for the resolution of man-

Table 1. Enantioseparation of Racemates on Poly((*R*)-**20**)[a)]

						
Tacticity mm/mr/rr	k_1'	α	k_1'	α	k_1'	α
~0/13/87	1.62 (+)	1.48	2.51 (–)	1.14	2.01 (–)	~1
6/29/65	1.22 (+)	1.31	2.32 (–)	~1	1.64 (–)	~1
87/13/~0	0.66 (+)	~1	2.26 (–)	~1	0.67 (–)	~1

a) Flow rate: 0.1 mL/min, column: 2.0 (i.d.) × 250 mm, eluent: hexane–2-propanol (70:30). The signs in parentheses show the optical rotation of the first-eluted enantiomer.



21 **22**

Fig. 10. Structures of polystyrene derivative (**21**) and poly(N-substituted maleimide)s (**22**) with chiral recognition ability.

delic acid and hydantoin derivatives.[73,74] The main chain of the immobilized poly(*N*-benzyl-L-glutamine) assumes an α-helical structure, which seems to be responsible for the chiral recognition. A dimer ($m = 2$) **6** showed no chiral recognition. However, the polymers with $m = 14$ or 36 achieved the baseline separation of a hydantoin derivative, but the polymer with $m =$ ca. 250 showed lower recognition. These results suggest that the vicinities of the helical chain end may recognize enantiomers. Some amino acid derivatives were resolved on poly(L-leucine) or poly(L-phenylalanine) chemically bonded to poly(methyl acrylate) mocroporous beads (**7**) or on the spheres consisting of the polypeptide alone.[75]

Polyamides **8**,[76] **9**,[77] and **10**[78–80] prepared from chiral diamines or chiral dicarboxylic acids by polycondensation can separate polar racemates, which interact with the CSP through hydrogen bonding. The chiral recognition ability of **10** (R′ = methylene groups) was affected by the crystallizability of **10**, which depended on the number of methylene groups in the main chain. An odd–even effect was observed; the polyamide **10** having an even number of methylene groups shows higher recognition ability than those having an odd number of methylene groups.

2.3 Other Synthetic Polymers. Optically active polyurethanes (**11**) have been prepared by the polyaddition of chiral diols to various diisocyanates.[81] Their chiral recognition depends on the diisocyanate residues. Polyurethanes derived from 1,3-diphenylpropanediol and aliphatic diisocyanates exhibited high chiral recognition for 1,1′-bi-2-naphthol derivatives, while those from aromatic diisocyanates showed poor recognition. Wide angle X-ray diffraction studies showed that the chiral recognition abilities depended on the crystallinity.

Various polyacetylene derivatives have been synthesized to develop new functional polymers such as conducting materials, nonlinear optical materials, and gas-permeable membranes. The polymers possess a repetition of double and single bonds, and therefore, at least four possible structures exist with respect to these bonds: cis-transoid, cis-cisoid, trans-transoid, and trans-cisoid. The stereoregularity of the polyphenylacetylene can be estimated by ^{1}H NMR spectroscopy, in which the chemical shift and line shape of the main chain's olefinic protons and aromatic protons are sensitive to the structural isomers. Phenylacetylene derivatives usually produce stereoregular polymers with an almost completely cis-transoidal structure by use of a rhodium catalyst.[82] Polyphenylacetylene derivatives (**12**) with optically active substituents at the para position have been prepared using $[RhCl(nbd)]_2$ (nbd: norbornadiene) to examine the chiral recognition ability as CSPs.[83,84] The polymers show intense CD peaks due to the main chain conjugated structure, indicating that the polymers possess a one-handed helical conformation. The **12**-coated silica gel resolved several enantiomers including Tröger's base and spiropyran derivatives. In order to investigate the effect of stereoregularity on chiral recognition ability, we prepared **12** (R = Ph) via a different route. At first, 4-(*tert*-butyldimethylsiloxy)phenylacetylene was polymerized in toluene using a tungsten catalyst WCl_6/Ph_4Sn, followed by desilylation with tetrabutylammonium fluoride. The resultant poly(4-hydroxyphenylacetylene) was then allowed to react with (*R*)-(+)-1-phenylethyl isocyanate to give **12** (R = Ph). The obtained stereoirregular polymer showed poor chiral recognition. This result clearly indicates that the one-handed helical conformation induced by a stereoregular main chain with chiral side groups is essential for effective chiral recognition. The chemically bonded-type CSP of **12** (R = naphthyl) was also prepared by polymerization with a rhodium catalyst in the presence of silica gel having a phenylacetylene residue on its surface (Fig. 11). The obtained CSP exhibited chiral recognition rather similar to that of the coated type-CSP and can be used with polar solvents such as THF and chloroform which dissolve the polymer.

Several optically active polyacetylene derivatives have also been prepared to be used as the chiral solid membranes to separate enantiomers.[85–87]

Glycosylated poly(phenyl isocyanide)s (**23**) (Fig. 12) bearing the chiralities of both the saccharide side chain (α-/β-glucose and α-/β-galactose) and the helical polyisocyanide main chain were prepared. The chiral recognition abilities of their 3,5-dimethylphenylcarbamate derivatives were compared with those of poly(*N*-phenylacrylamide) derivative (**24**) having a flexible backbone.[88] The CD spectrum of the rigid helical polymers indicated that the polymers have a regular structure due to the chirality of the α- and β-anomeric center of the sugar moieties. These polymers showed chiral recognition for some racemates, depending on the stereostructure of the pendant sugars. Among them, the α-galactose-carrying poly(phenyl isocyanide) derivative (**24**(Gal-α)) showed the highest chiral recognition ability. However, their CD intensity was smaller than those expected for the complete right- or left-handed helices, indicating that these poly(phenyl isocyanide)s are a mixture of the right- and left-handed helical polymers or sequences. This suggests that, if the one-handed helical poly(phenyl isocyanide)s could be prepared, their chiral separation abilities would be significantly enhanced.

Polysaccharide-analogue polyether **13** prepared by anionic cyclopolymerization was chemically bonded to silica gel.[89] The CSP resolved several α-amino acids, such as tryptophan and phenylglycine. The chiral recognition ability of an analogue of **13** has been evaluated.[90]

Many cross-linked polymer gels possessing chiral cavities have been prepared by a molecular imprinting technique using a monomer or template molecule with an optically active template moiety.[91–93] Figure 13 shows the template monomers hav-

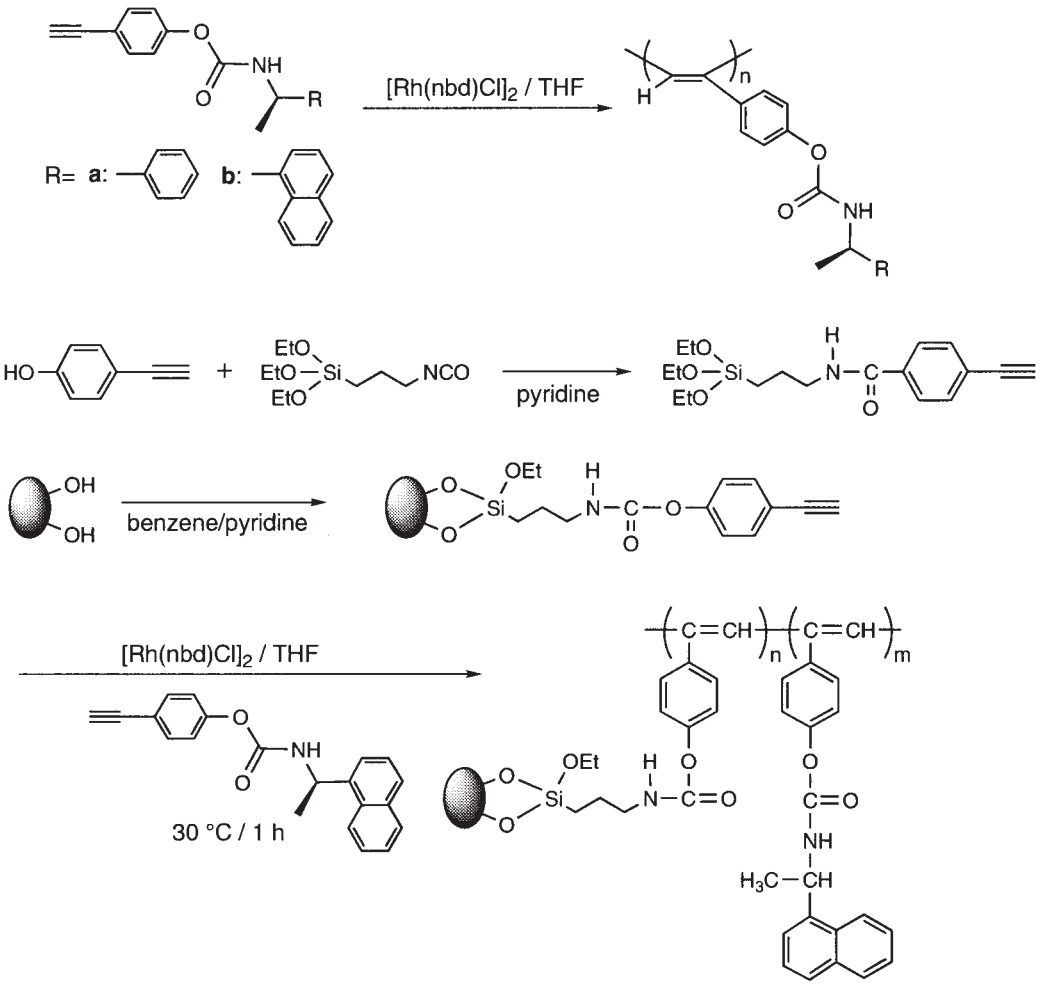


Fig. 11. Immobilization of optically active polyacetylene derivatives onto silica gel.

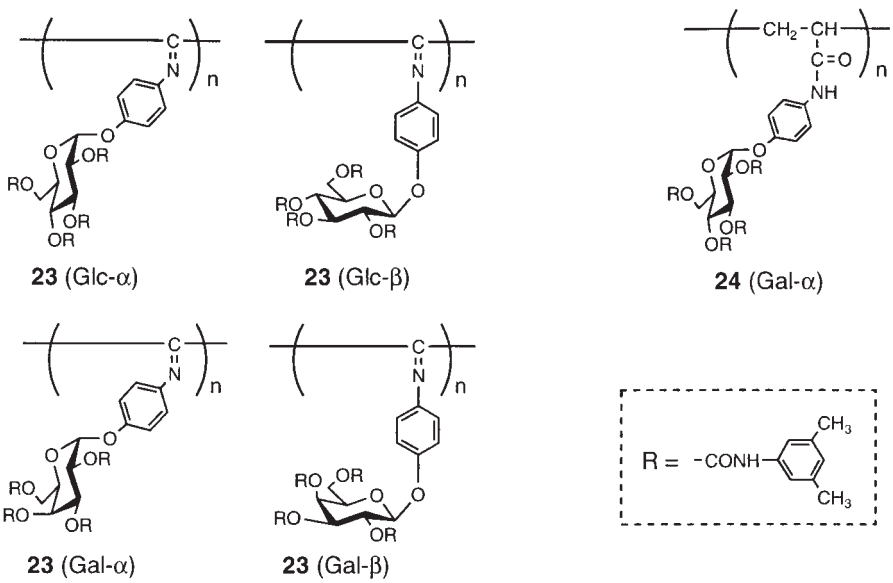


Fig. 12. Rigid helical poly(phenyl isocyanide)s carrying phenylcarbamoylated sugars (**23**), together with the corresponding flexible analogue poly(*N*-phenylacrylamide) polymer (**24**).

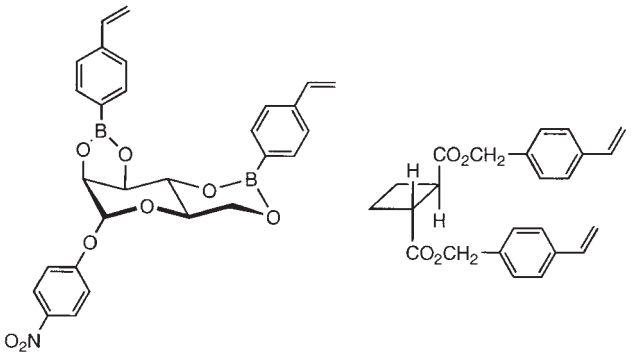


Fig. 13. Structures of chiral template monomers.

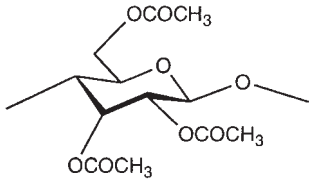
ing a removable chiral moiety. After polymerization, a chiral gel can be obtained by removing the template moieties. The gel showed a higher affinity for the templated enantiomer. Molecular imprinting using racemic templates is also known.[94]

3. Natural Polymers and Their Derivatives

3.1 Proteins. Proteins are naturally-occurring polymers composed of α-amino acids. Many kinds of proteins exist in nature, and most of them have a chiral recognition ability to some extent. However, structural proteins such as wool, silk, and human hair usually show very low chiral recognition. So far, various proteins have been evaluated as the CSPs for HPLC, and the following proteins have been commercialized: albumins such as bovine serum albumin[95] and human serum albumin,[96] glycoproteins such as α_1-acid glycoprotein,[97] ovomucoid from chicken egg whites,[98] avidin,[99] and enzymes such as cellobiohydrolase I[100] and pepsin.[101]

The protein-based CSPs can often directly resolve chiral drugs without derivatization under reversed phase conditions using aqueous mobile phases. This is valuable to investigate the metabolism of a drug in a body using blood and urine. On the other hand, these phases also have several disadvantages, such as low loading capacity and low stability as CSPs. The retention and enantioselectivity for racemates on the protein-based CSPs are significantly affected by chromatographic conditions, such as the eluent, column temperature, and pH, which may vary protein conformation. The protein-based CSPs are useful for analytical purposes but not for preparative separation, because of the very low loading capacity due to the limited number of recognition sites. Various attempts have been examined to improve the performance of the protein-based CSPs. The stability of the CSPs is enhanced through crosslinking of the proteins.[102] The protein fragment or protein domain, which plays the central role for chiral recognition, was used to increase the loading capacity of the CSPs and to understand the chiral recognition site of the CSPs.[103–105] In the future, the preparation of more suitable protein-based CSPs for a target molecule might be realized by genetic technology.

3.2 DNA. Nucleic acid, DNA, and RNA aptamers have been recently used for affinity chromatography. The aptamer can be prepared by chemical synthesis in a short time with good reproducibility and accuracy. In addition, its sequence is easily changed and modified at precise locations to modulate the binding selectivity. Very recently, a DNA aptamer specifically designed for a target D-peptide (arginine-vasopressin) was prepared and immobilized on a streptavidin chromatographic support.[106] On the obtained CSPs, the D-peptide is strongly retained, while the L-peptide elutes in the dead volume under the optimal binding condition. Furthermore, the aptamer column is stable in a phosphate buffer containing $MgCl_2$. Therefore, DNA aptamers may have a great potential as new target-specific chiral selectors for HPLC.



25

Fig. 14. Structure of cellulose triacetate (**25**).

3.3 Polysaccharides. Polysaccharides such as cellulose and amylose are some of the most readily available optically active polymers. Although the polysaccharides have stereoregular sequences consisting of D-glucose, their chiral recognition abilities are not high enough to be used as CSPs. However, they can be readily converted to esters and carbamates through the reaction with suitable reagents. The derivatized polysaccharide-based CSPs show much better chromatographic and enantioselective properties than the native polysaccharides themselves. At present, more than ten polysaccharide-based CSPs are commercially available and these are extensively used for both analytical and preparative separation of enantiomers because of their versatility and high loadability.

3.3.1 Cellulose Esters: The first practical CSP derived from polysaccharides is the microcrystalline cellulose triacetate (**25**) (Fig. 14) found by Hesse and Hagel in 1973.[107,108] They prepared it by the heterogeneous acetylation of native microcrystalline cellulose (Avicel) in benzene, and therefore, it possesses a structure closely related to that of native cellulose (CTA-I). Interestingly, the obtained CTA-I exhibited completely different chiral recognition abilities before and after dissolving it in a solvent.[109,110] For example, the elution order of Tröger base enantiomers on the CTA-I was opposite to that on the CSP prepared by coating a solution of CTA-I on silica gel.[110] This is ascribed to different higher-order structures or different supramolecular structures of the two **25**s. CTA-I has been used for the resolution of a wide range of racemates, especially nonpolar aromatic compounds and aromatic pharmaceuticals, using an ethanol–water mixture as the eluent.[57,59,111] Stereochemically interesting compounds completely separated on the CTA-I are shown in Fig. 15.[112–120]

Cellulose tribenzoates are readily obtained by reacting cellulose with the corresponding benzoyl chlorides (Fig. 16).[110,121] The benzoate derivatives having electron-donating substituents, such as a methyl group, showed better chiral recognition ability than those having electron-withdrawing substituents such as a halogen. Among the benzoates, cellulose tris(4-methylbenzoate) (**26b**) showed a high chiral recognition for various racemates. These CSPs have been used by coating the benzoates dissolved in a solvent such as methylene dichloride onto silica gel. The chiral recognition of **27a** was significantly influenced by the solvents in which **27a** was dissolved for coat-

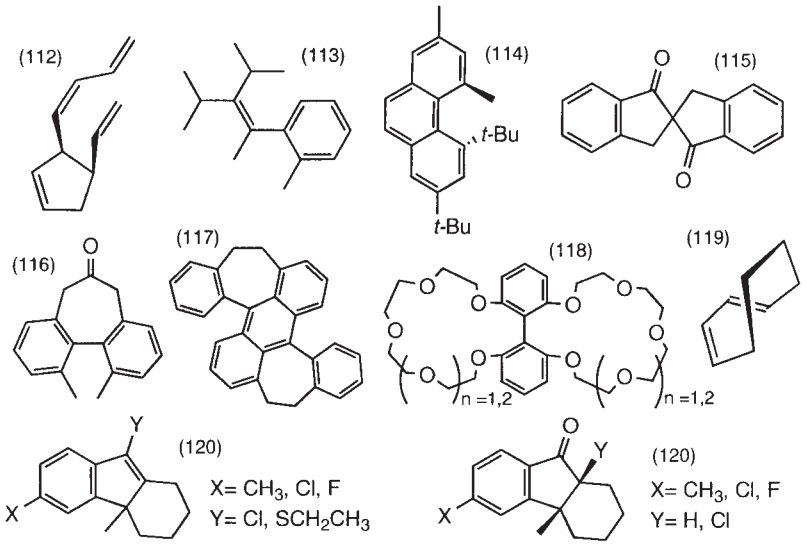


Fig. 15. Compounds resolved on microcrystalline cellulose triacetate (CTA I).

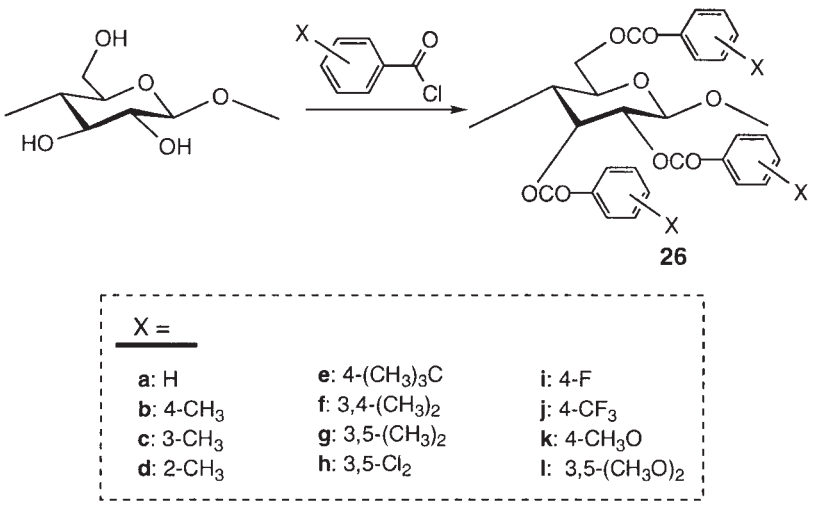


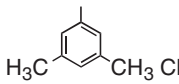
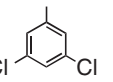
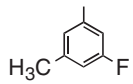
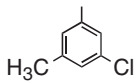
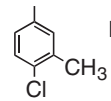
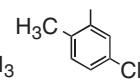
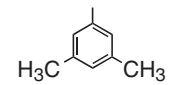
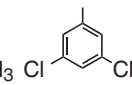
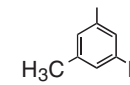
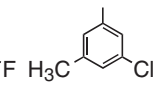
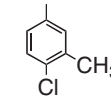
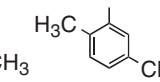
Fig. 16. Preparation and structures of cellulose tribenzoates (**22**).

ing.[111,121,122] The higher-order structure of **27a** coated on silica gel must depend on the coating solvents. Tribenzoates of amylose show lower chiral recognition than the cellulose tribenzoates.[121]

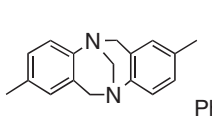
3.3.2 Cellulose and Amylose Phenylcarbamates: Cellulose and amylose are easily converted to various trisphenylcarbamate derivatives (**27**, **28**) by reacting them with the corresponding phenyl isocyanates (Fig. 17). These derivatives are also coated on silica gel to be used as the CSPs. The effect of the substituents on chiral recognition has been systematically investigated.[123–125] Their chiral recognition abilities are significantly influenced by the substituents on the phenyl group. Generally, the introduction of an electron-donating methyl group or an electron-withdrawing halogen at the *m*- and/or *p*-position of the phenyl ring improves the chiral recognition ability for many racemates. In particular, tris(3,5-dimethylphenylcarbamate) derivatives of both cellulose and amylose (**27x**, **28n**) exhibit excellent resolving ability for a variety of racemates. The columns of **27x** and **28n** are commercially available and have been most widely utilized for enantioseparation in many fields. Examples resolved on **27x** and **28n** are shown in Figs. 18[126–140] and 19,[140–154] respectively. On **27x** and **28n**, the enantiomers of many racemic compounds are eluted in the reverse order, suggesting that these two CSPs are complementary in recognizing chirality. Many enantiomers unresolved on **27x** can be resolved on **28n**, and vice versa. In the resolution of about 500 racemates in our group, about 80% of the racemates were separated into enantiomers on at least one of the two columns.

Interestingly, the phenylcarbamate derivatives having both an electron-donating group and an electron-withdrawing group on the phenyl moieties possess a high chiral recognition abilities for many racemates.[155–158] The results of the enantioseparation of 10 racemates on the cellulose and amylose phenylcarbamates having methyl and/or halogen on the phenyl group are summarized in Table 2. For cellulose phenylcarbamates, the substitution at the ortho-position decreased the chiral recognition ability. On the other hand, the ortho-substituted amylose phenylcarbamates such as **28t** show relatively high chiral recognition. These results may be ascribed to their different higher-order structures. Zugenmaier et al. proposed left-handed 3/

Table 2. Separation Factors (α) in the Resolution of **a**–**j** on Cellulose and Amylose Phenylcarbamates[a)]

	Cellulose derivatives						Amylose derivatives					
												
Racemate	**27x**	**27aa**	**27ap**	**27aq**	**27al**	**27ag**	**28n**	**28p**	**28x**	**28y**	**28s**	**28t**
a	1.31 (+)	1.65 (+)	1.58 (+)	1.95 (+)	1.13 (+)	ca. 1 (+)	1.58 (+)	1.34 (+)	1.90 (+)	2.20 (+)	1.43 (+)	1.13 (–)
b	1.68 (–)	1.84 (+)	2.41 (+)	2.05 (+)	3.25 (+)	1.30 (+)	3.04 (+)	1.32 (+)	1.89 (+)	2.10 (+)	1.66 (+)	1.70 (+)
c	1.58 (+)	1.21 (–)	1.44 (–)	1.11 (–)	1.23 (–)	1.10 (+)	1.21 (–)	ca. 1 (+)	1.27 (–)	1.11 (–)	1.05 (–)	1.16 (+)
d	1.83 (–)	1.11 (+)	1.71 (–)	1.33 (–)	1.35 (–)	1.19 (+)	2.11 (–)	ca. 1 (+)	1.36 (–)	1.12 (–)	1.32 (–)	1.28 (+)
e	1.15 (–)	1.26 (–)	1.36 (–)	1.33 (–)	1.26 (–)	ca. 1 (–)	ca. 1 (–)	ca. 1(–)	ca. 1 (+)	ca. 1 (+)	ca. 1 (–)	1.24 (–)
f	2.59 (–)	1.38 (–)	1.42	1.43 (–)	1.25 (–)	ca. 1 (–)	1.15 (+)	1.00	1.11	1.29 (–)	1.00	1.14 (–)
g	ca. 1 (+)	1.82 (+)	1.49 (+)	1.39 (+)	1.44 (+)[b)]	1.05 (+)[b)]	ca. 1 (+)	ca. 1 (+)	1.07 (+)	ca. 1 (+)	ca. 1 (–)	1.18 (+)
h	1.34 (+)	1.29 (+)	1.55 (+)	1.17 (+)	3.05 (+)	1.00	1.98 (+)	2.25 (+)	3.79 (+)	4.70 (+)	1.88 (+)	1.67 (+)
i	1.41 (–)	1.20 (–)	1.11 (–)	1.22 (–)	1.06 (–)	1.08 (–)	1.12 (+)	1.10 (+)	ca. 1 (+)	1.06 (+)	1.11 (+)	1.18 (+)
j	3.17 (+)	1.41 (+)	1.11	1.22 (+)	1.54 (–)	1.20 (–)	2.01 (+)	1.11 (–)	4.36 (+)	1.71 (+)	1.34 (+)	3.09 (+)

a) The sign in parentheses represents the optical rotation of the first-eluted enantiomer. Eluent, hexane–2-propanol (90:10); flow rate, 0.5 mL/min. b) Eluent, hexane–2-propanol (98:2).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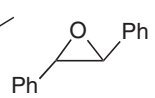
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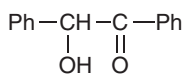
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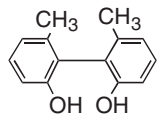
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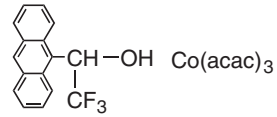
e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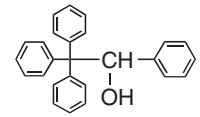
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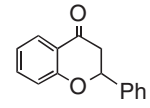
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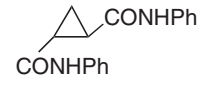
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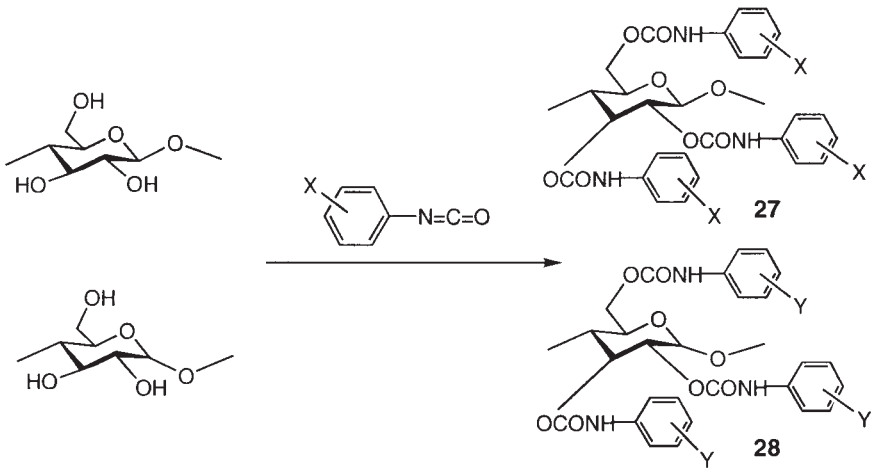


X =

a: H	**l:** 4-Ph_3C	**w:** 3,4-$(CH_3)_2$	**ah:** 2-Cl-6-CH_3
b: 4-CH_3	**m:** 4-F	**x:** 3,5-$(CH_3)_2$	**ai:** 3-Cl-2-CH_3
c: 4-C_2H_5	**n:** 4-Cl	**y:** 2,6-$(CH_3)_2$	**aj:** 3-Cl-4-CH_3
d: 4-$(CH_3)_2CH$	**o:** 2-Cl	**z:** 3,4,5-$(CH_3)_3$	**ak:** 4-Cl-2-CH_3
e: 4-$(CH_3)_3C$	**p:** 3-Cl	**aa:** 3,5-Cl_2	**al:** 4-Cl-3-CH_3
f: 4-CH_3O	**q:** 4-Br	**ab:** 3,4-Cl_2	**am:** 3-F-4-CH_3
g: 4-C_2H_5O	**r:** 4-I	**ac:** 2,6-Cl_2	**an:** 4-F-3-CH_3
h: 4-$(CH_3)_2CHO$	**s:** 4-CF_3	**ad:** 3,5-F_2	**ao:** 5-F-2-CH_3
i: 4-PhO	**t:** 4-NO_2	**ae:** 3,5-$(CF_3)_2$	**ap:** 3-F-5-CH_3
j: 4-$(CH_3)_3Si$	**u:** 2-CH_3	**af:** 2-Cl-4-CH_3	**aq:** 3-Cl-5-CH_3
k: 4-Ph	**v:** 3-CH_3	**ag:** 5-Cl-2-CH_3	**ar:** 3-Br-5-CH_3

Y =

a: H	**h:** 4-Ph	**o:** 3,4,5-$(CH_3)_3$	**u:** 3-F-4-CH_3
b: 4-CH_3	**i:** 4-F	**p:** 3,5-Cl_2	**v:** 4-F-3-CH_3
c: 4-$(CH_3)_2CH$	**j:** 4-Cl	**q:** 3,4-Cl_2	**w:** 5-F-2-CH_3
d: 4-$(CH_3)_3C$	**k:** 4-Br	**r:** 3,5-F_2	**x:** 3-F-5-CH_3
e: 4-CH_3O	**l:** 4-I	**s:** 4-Cl-3-CH_3	**y:** 3-Cl-5-CH_3
f: 4-PhO	**m:** 3,4-$(CH_3)_2$	**t:** 5-Cl-2-CH_3	**z:** 3-Br-5-CH_3
g: 4-$(CH_3)_3Si$	**n:** 3,5-$(CH_3)_2$		

Fig. 17. Preparation and structures of trisphenylcarbamates of cellulose (**27**) and amylose (**28**).

$2^{159,160}$ and $4/3^{161}$ helical chain conformations for tris(phenylcarbamate)s of cellulose and amylose, respectively. This structural difference seems to be responsible for the different influence of the substituents on the chiral recognition.

Cellulose tris(3,5-dichlorophenylcarbamate) (**27aa**) shows a unique chiral recognition ability. Although the separation factors in hexane–2-propanol (90/10) listed in Table 2 are high, the CSP is slowly damaged because **27aa** is soluble in hexane containing 10–20% 2-propanol. This higher solubility of **27aa** limits its application as the CSP. This defect is overcome by chemically bonding **27aa** to silica gel.[162] On the other hand, **27aa** is insoluble in polar solvents such as alcohols, acetonitrile, and water. Recently, **27aa** was found to show high chiral recognition using alcohol as an eluent.[163–165] For example, a very high enantioseparation factor, exceeding 110, was obtained in the enantioseparation of 2-(benzylsulfinyl)benzamide on **27aa** with 2-propanol as an eluent (Fig. 20).[163] This α value may be the highest among the enantioseparations on the polysaccharide derivatives by HPLC.

3.3.3 Cellulose and Amylose Alkylcarbamates: Alkylcarbamates, such as methyl- and isopropylcarbamates, show very low chiral recognition as CSPs. This is attributed to the lack of a regular higher-order structure, which may be important for these derivatives to show high chiral recognition. Most of the cellulose tris(phenylcarbamate) derivatives form a lyotropic liquid crystalline phase in a high concentration and show high crystallinity under a polarizing microscope when they are cast from solution,[124,166] while the above alkylcarbamates do not form a lyotropic liquid crystalline phase. These alkyl carbamates coated on the silica surface from a solution probably do not have an ordered structure. Bulky cycloalkyl groups were introduced in order to prepare alkylcarbamate derivatives hav-

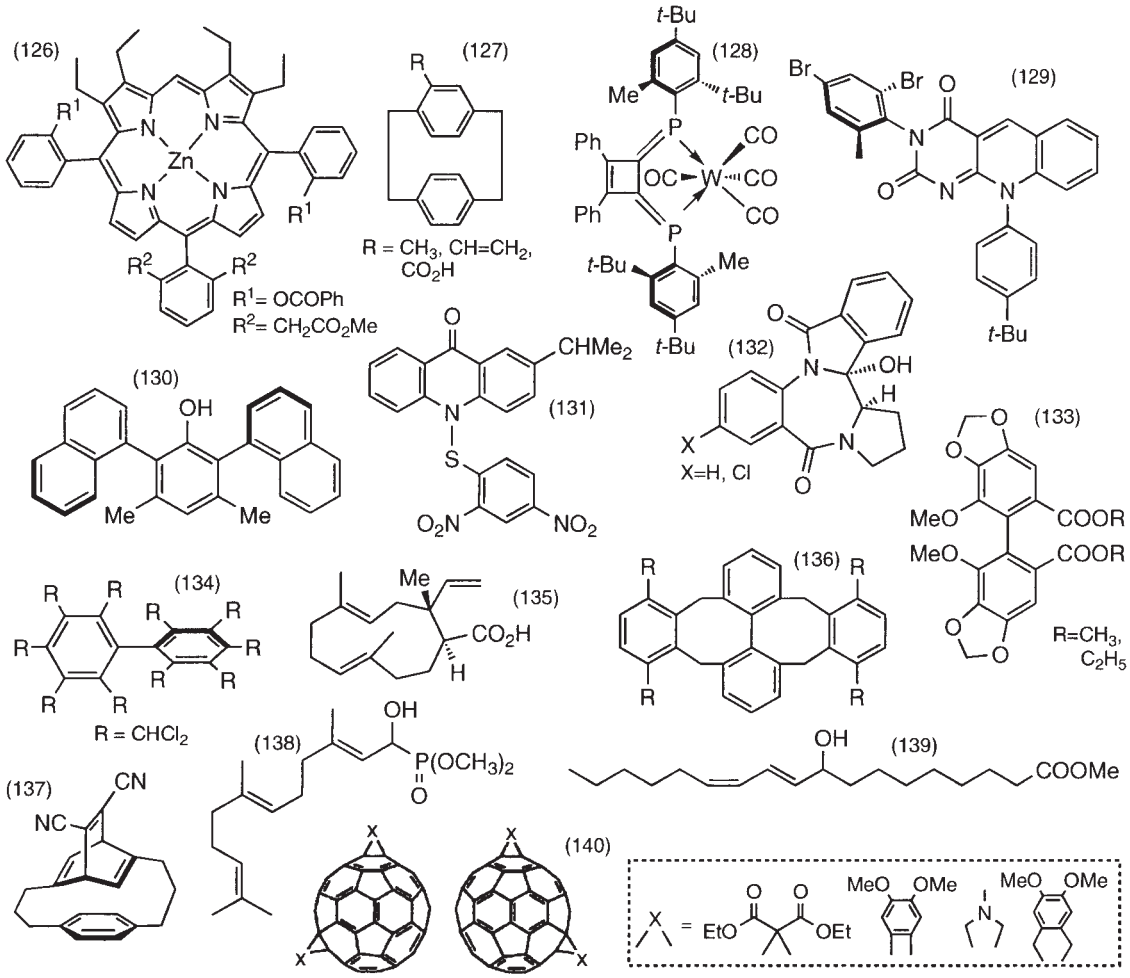


Fig. 18. Compounds resolved on cellulose tris(3,5-dimethylphenylcarbamate) (**27x**).

ing a regular higher-order structure. Then cyclohexyl- and norbornylcarbamates (**29**, **30**) (Fig. 21) of cellulose and amylose were found to exhibit high chiral recognition for a wide range of racemates.[167,168] Their chiral recognition abilities are comparable to those of 3,5-dimethylphenylcarbamate derivatives **27x** and **28n**. These cycloalkylcarbamates of cellulose exhibit high crystallinity when cast from a solution.

The phenylcarbamates of the polysaccharides are difficult to use as the CSPs for thin layer chromatography (TLC) due to the difficulty in detection by UV irradiation. However, these cycloalkylcarbamates can be used as the CSPs for TLC because of the absence of UV absorption above 220 nm.[167] The TLC plate was prepared by coating a slurry containing **30a**-coated silica gel and a fluorescent indicator on a standard glass. The resolution of Tröger base, 1-(9-anthryl)-2,2,2-trifluoroethanol, and benzoin ethyl ester was performed using hexane–2-propanol (90/10) as a mobile phase. The TLC chromatogram was readily detected by UV radiation at 254 nm and showed the resolution of racemates into each enantiomer (Fig. 22). The TLC results can be compared with those obtained in HPLC with the same CSP. Although the α values in HPLC are slightly larger than those in TLC, a good correlation is observed between these α values, which enables rapid set up of the condition for the HPLC resolution. The cycloalkylcarbamates can be very useful CSPs for TLC as well as for HPLC resolution.

3.3.4 Cellulose and Amylose Benzylcarbamates: As mentioned above, methyl- and isopropylcarbamates exhibit low chiral recognition, probably due to the insufficient steric requirements to form a regular higher-order structure. Therefore, several tris(benzylcarbamate) derivatives of cellulose and amylose as shown in Fig. 23 were evaluated as CSPs. Among the five derivatives, 1-phenylethylcarbamates (**31b**, **32b**) and 1-phenylpropylcarbamates (**31c**, **32c**) show high chiral recognition,[169,170] although that of the remaining three is very low. In the CD spectra of the cast films of these aralkylcarbamates, intense peaks were observed only for **31b**, **31c**, **32b**, and **32c**, indicating that **31b**, **31c**, **32b**, and **32c** probably possess more regular higher-order structures than the other derivatives. The chiral recognition abilities of the 1-phenylethylcarbamate derivatives depend greatly on the chirality of the 1-phenylethyl group. For the cellulose derivatives, (*R*)- and (*RS*)-**31b** show higher resolving abilities than (*S*)-**31b**. On the other hand, amylose 1-phenylethylcarbamates **32b** often show higher chiral recognition abilities than the cellulose derivatives, and (*S*)-**32b** and (*RS*)-**32b**, particularly the former, exhibit higher abilities than (*R*)-**32b** in contrast to the cellulose derivatives. Not only the glucose unit but also the chirality of the 1-phenylethyl group plays an important role in the chiral recognition. (*S*)-**32b** shows high chiral recognition especially for β-lactams[171] and 3-hydroxy-2-cyclopentanone derivatives.[172]

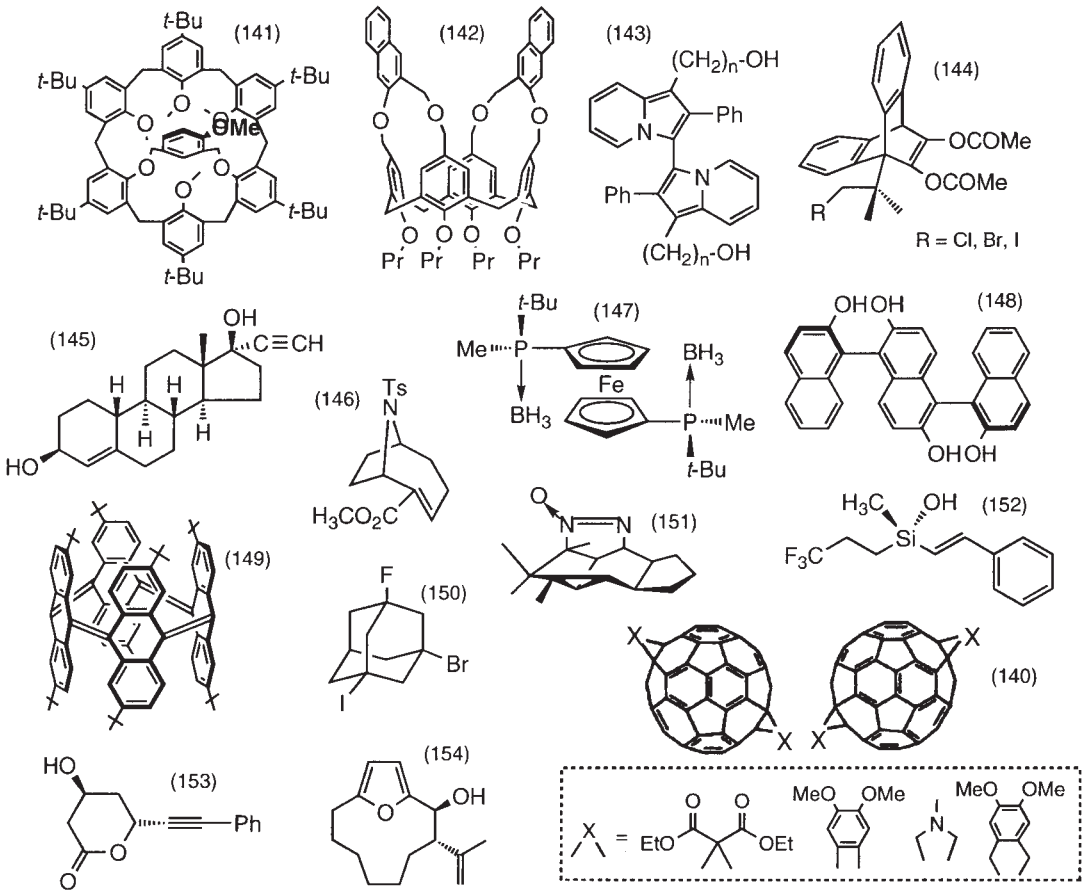


Fig. 19. Compounds resolved on amylose tris(3,5-dimethylphenylcarbamate) (**28n**).

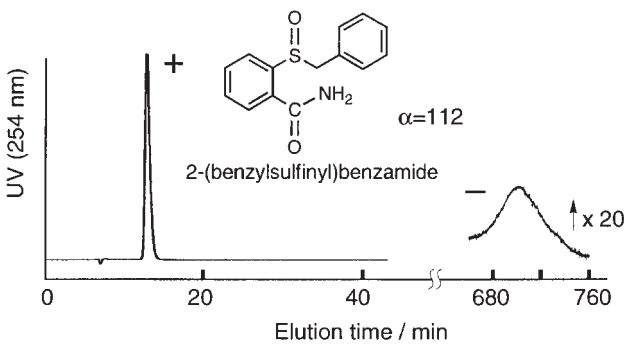


Fig. 20. Enantioseparation of 2-(benzylsulfinyl)benzamide on **27aa** using 2-propanol as an eluent. Column: 25 × 0.46 (i.d.) cm; flow rate: 0.5 mL/min.

3.3.5 Other Phenylcarbamates of Polysaccharides: 3,5-Dimethyl- and 3,5-dichlorophenylcarbamates of other polysaccharides[173] including chitin (**33**),[174] chitosan (**34**), galactosamine (**35**), curdlan (**36**), dextran (**37**), xylan (**38**), and inulin (**39**) were prepared (Fig. 24) and their chiral recognition abilities as the CSPs for HPLC were evaluated. The enantioselectivity and the elution order of enantiomers differ among these polysaccharides depending on the sugar units, linkage position, and linkage type. Among these phenylcarbamate derivatives, 3,5-dimethylphenylcarbamates of chitin (**33a**), chitosan (**34a**), and xylan (**38a**) and 3,5-dichlorophenylcarbamates of chitin (**33b**) and galactosamine (**35b**) showed relatively high chiral recognition abilities; indeed, some racemates were better resolved on these phenylcarbamates than on **27x** and **28n**. Other carbamates also performed high resolution for a few specific racemates and resolved them better than the cellulose and amylose derivatives.

As already mentioned, several pairs of enantiomers are elut-

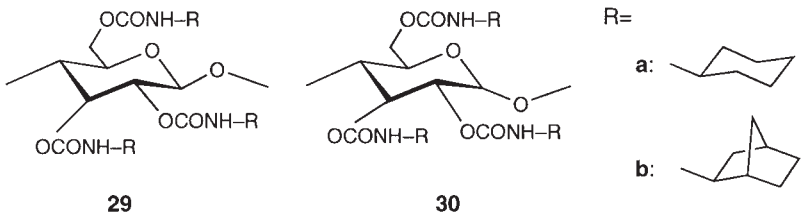
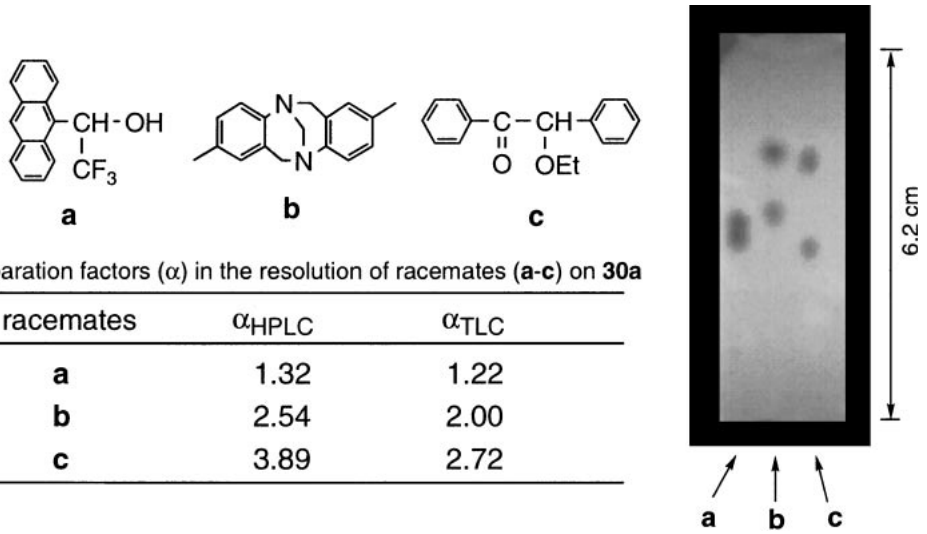


Fig. 21. Structures of cycloalkylcarbamates of cellulose (**29**) and amylose (**30**).



Separation factors (α) in the resolution of racemates (**a**-**c**) on **30a**

racemates	α_{HPLC}	α_{TLC}
a	1.32	1.22
b	2.54	2.00
c	3.89	2.72

Fig. 22. Enantioseparation of racemates (**a**–**c**) on amylose triscyclohexylcarbamate (**30a**) as CSPs for HPLC and TLC. (Reprinted from Ref. 167. Copyright 2000, American Chemical Society.)

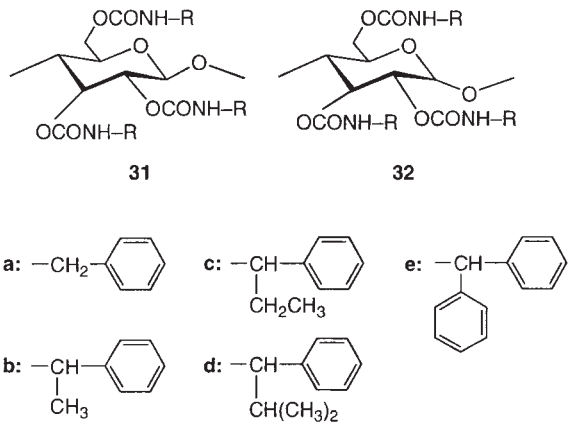


Fig. 23. Structures of benzylcarbamates of cellulose (**31**) and amylose (**32**).

ed in reversed order on the 3,5-dimethylphenylcarbamates of cellulose (**27x**) and amylose (**28n**). Cellulose and amylose are different only in the linkage type, α-linkage and β-linkage. Similar reversed enantioselectivities were also observed for a few racemates between **34a** β-(1 → 4) and **35a** α-(1 → 4) consisting of D-glucosamine units, and for some between **34b** and **35b**.[173]

Table 3 shows the results of the resolution of acidic drugs such as ketoprofen and ibuprofen using hexane–2-propanol containing trifluoroacetic acid as eluents. These acids were better resolved on chitin carbamates, particularly on **33b**, than on **27x** and **28n**.[174]

3.3.6 Immobilization of Cellulose and Amylose Phenylcarbamates: The polysaccharide-based chiral stationary phases are usually prepared by coating the polysaccharide derivatives on macroporous silica gel. Therefore, a solvent such as THF, chloroform, and ethyl acetate, in which polysaccharide

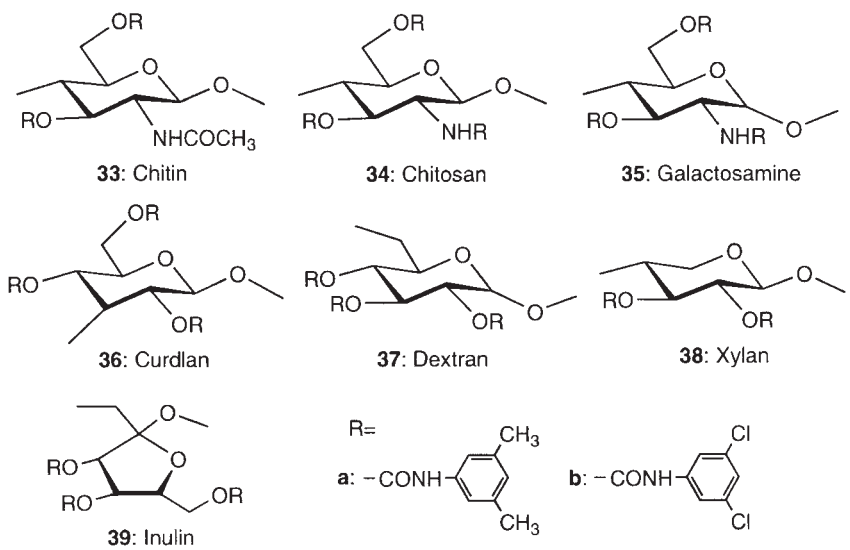
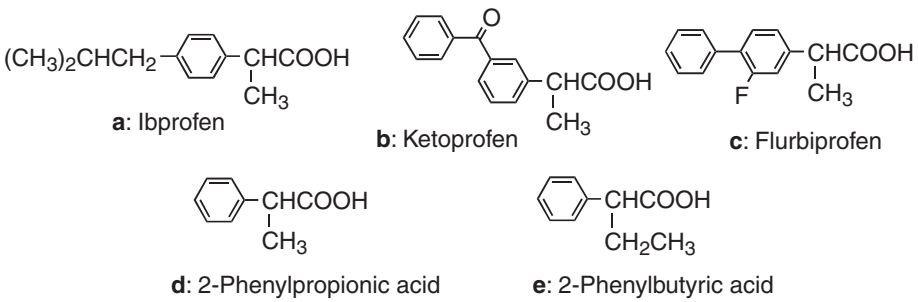


Fig. 24. Structures of trisphenylcarbamates of various polysaccharides (**33**–**39**).

Table 3. Resolution of Acids (**a**–**c**) on the Phenylcarbamate Derivatives of Chitin (**33**), Cellulose (**27x**), and Amylose (**28n**)[a)]

	Chitin			Cellulose	Amylose
Acid		**33a**	**33b**	**27x**	**28n**
a	~1 (–)	~1 (+)	1.11 (+)	1.06 (–)[b)]	~1 (+)[b)]
b	1.23 (+)[c)]	1.21 (+)	1.72 (+)[c)]	1.03 (–)	1.18 (–)[d)]
c	1.06 (–)	1.08 (–)	1.10 (+)[e)]	1.00	1.44 (–)
d	1.17 (+)	1.41 (+)	1.39 (+)	1.19 (–)[b)]	1.14 (–)[e)]
e	1.22 (+)[e)]	1.48 (+)	1.48 (+)	1.39 (–)	1.13 (–)

a) The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Flow rate: 0.5 mL/min. Eluent: hexane–2-propanol–CF_3COOH (95:5:1). b) Eluent: hexane–2-propanol–CF_3COOH (98:2:1). c) Flow rate: 1.0 mL/min. d) Eluent: hexane–2-propanol–CF_3COOH (80:20:1). e) Eluent: hexane–2-propanol–CF_3COOH (90:10:1).



derivatives are dissolved or swollen, cannot be used as the main component of an eluent because the CSPs coated on silica gel are damaged. Several chemically bonded-type CSPs have been prepared to overcome this defect.

3,5-Dimethylphenylcarbamates of cellulose and amylose were randomly and regioselectively chemically bonded to 3-aminopropylsilanized silica gel at the 2-, 3-, and 6-positions of the glucose units using 4,4′-diphenylmethane diisocyanate (Fig. 25a).[162,175] For the cellulose phenylcarbamate **27x**, the position of glucose in the immobilization on silica gel hardly affected the chiral recognition, but the amylose phenylcarbamate **28n** regioselectively bonded at the 6-position to silica gel showed a higher chiral recognition than that bonded at the 2- or 3-position. Although the obtained chemically bonded-type CSPs can be used with a variety of eluents, some deterioration of chiral recognition in comparison to the corresponding coated-type CSPs was observed, probably due to alteration of the regular higher-order structures of the polysaccharides. The structure regularity may be reduced.

In order to reproduce the chiral recognition ability of the coated-type CSP precisely for a bonded-type CSP, chemically bonding to silica gel only at the chain end of the polysaccharide may be ideal. As shown in Fig. 25b, **28n** was chemically bonded to silica gel only at the reducing terminal residue of amylose, which has a desired chain length and a narrow molecular weight distribution produced by the enzymatic polymerization of α-D-glucose 1-phosphate dipotassium salt using phosphorylase isolated from potato.[176] This bonded-type CSP of **28n** showed excellent resolving ability, similar to that of the coated-type CSP, and can be used with organic solvents including THF and chloroform.

Other immobilization methods have been reported, such as the photochemical cross-linking of **27x**[177,178] and the polymerization of 3,5-dimethylphenylcarbamates of cellulose, amylose, and chitosan having a 10-undecenoylcarboxylate group.[179–181]

Recently, cellulose phenylcarbamate derivatives having a styryl or methacryloyl group at the 6-position of the glucose unit were synthesized. They were immobilized onto silica gel via radical copolymerization with vinyl monomers, such as styrene, isoprene, *t*-butyl acrylate, or *t*-butyl methacrylate, under various conditions (Fig. 25c).[182–184] As the content of the vinyl group introduced on the cellulose derivatives was reduced, the immobilization became more difficult, although the obtained phase exhibited higher chiral recognition. In order to attain more efficient immobilization of the cellulose derivative with a vinyl group by using a lower amount of a vinyl monomer, a vinyl group was also introduced onto the silica surface. The immobilized CSPs showed basically a similar chiral recognition to that of the coated-type CSPs and could be stably used with the eluent containing 10% chloroform; some racemates were better resolved with this eluent. This immobilization technique can also be applied not only to cellulose derivatives but also to other polysaccharide derivatives.

On the chemically bonded-type CSPs, various solvents can be used as the eluents. This expands the number of separable racemates that are not resolved by a coated-type CSPs. For ex-

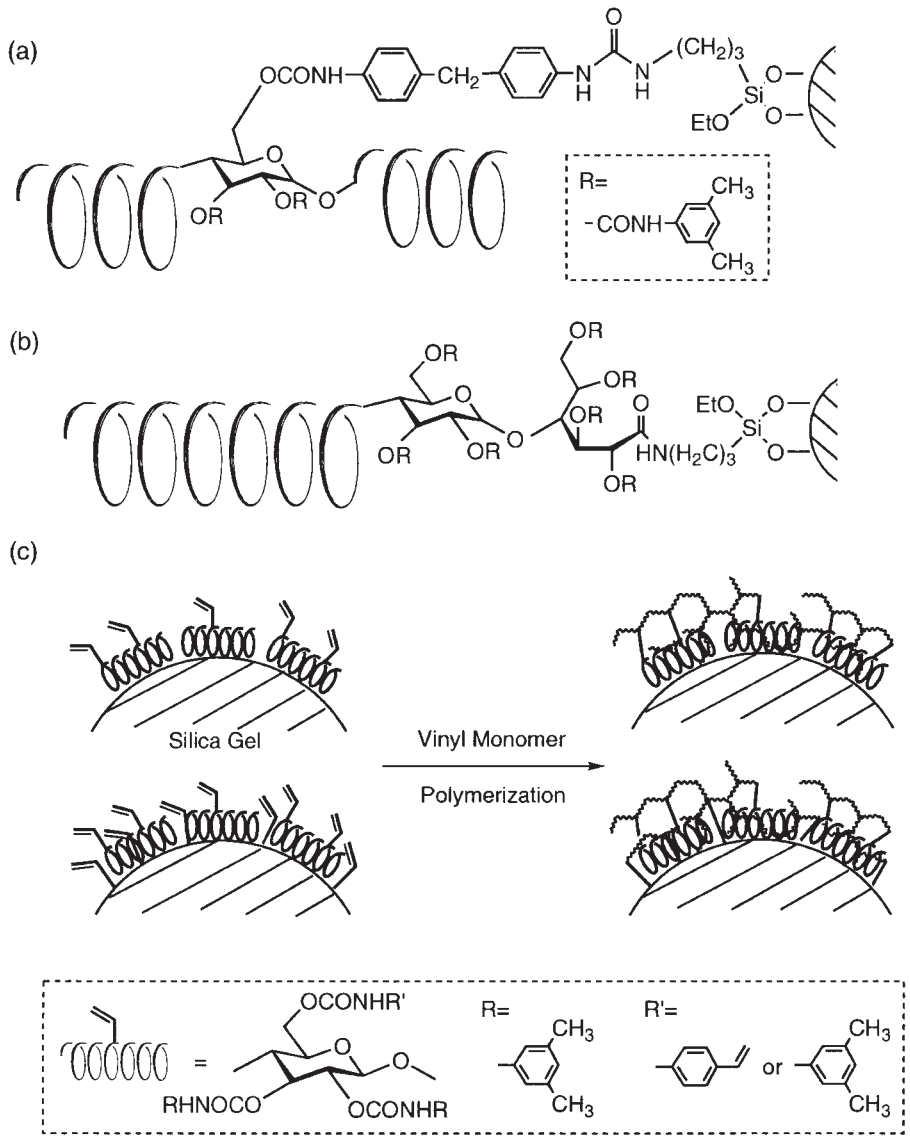


Fig. 25. Polysaccharide derivatives immobilized CSPs.

ample, on the bonded-type CSP of **28n**, topologically interesting catenanes and molecular knots were successfully resolved using a hexane/chloroform/2-propanol mixture (Fig. 26).[185–187] The first direct HPLC resolution of the smallest chiral fullerene C_{76} was also achieved on this CSP using a hexane/chloroform mixture (80:20) as the eluent.[188] In this case, to obtain high enantiomeric purity recycling was necessary because of the low degree of resolution.

3.3.7 Monolithic CSP Using Polysaccharide Derivatives: Recently, a very fast enantioseparation of 1-(9-anthryl)-2,2,2-trifluoroethanol was attained using **27x** coated on monolithic silica materials. The analysis time is below 30 s, which may be the shortest time among the baseline HPLC enantioseparations (Fig. 27).[189] Macroporous silica gel is usually used for the polysaccharide-based CSPs as a support material. On this material, when the linear flow-rate of a mobile phase is increased to reduce the analysis time, a significant pressure drop and peak broadening due to mass transfer are often observed. On the other hand, monolithic silica materials can reduce these problems, and therefore expeditious analysis and more efficient preparative separation will be realized. A monolithic CSP has been prepared by filling a commercially available monolithic silica column (50 × 4.6 mm) with an acetone solution of **27x**, followed by slow evaporation of the solvent at room temperature. After this procedure was repeated twice, 12 wt % **27x** was coated on the monolithic silica material. Using the obtained monolithic CSP, one could increase the linear flow rate to 20 mL/min, and the enantiomers eluted at 7.2 s and 18.5 s with a complete separation. The enantioseparation within a minute enables a high throughput in ee determination.

3.4 Mechanism of Chiral Recognition on Polysaccharide Phenylcarbamates. In the past decade, many attempts to clarify the mechanism of chiral recognition on CSPs for liquid chromatography have been made by means of chromatography, NMR spectroscopy,[190–193] X-ray analysis, and computational methods.[194–197] The most successful studies were performed with small molecule CSPs, while only a few mechanistic studies on chiral discrimination at a molecular level have been reported on polymeric CSPs. A number of different interaction sites with a different affinity for enantiomers exist on chiral pol-

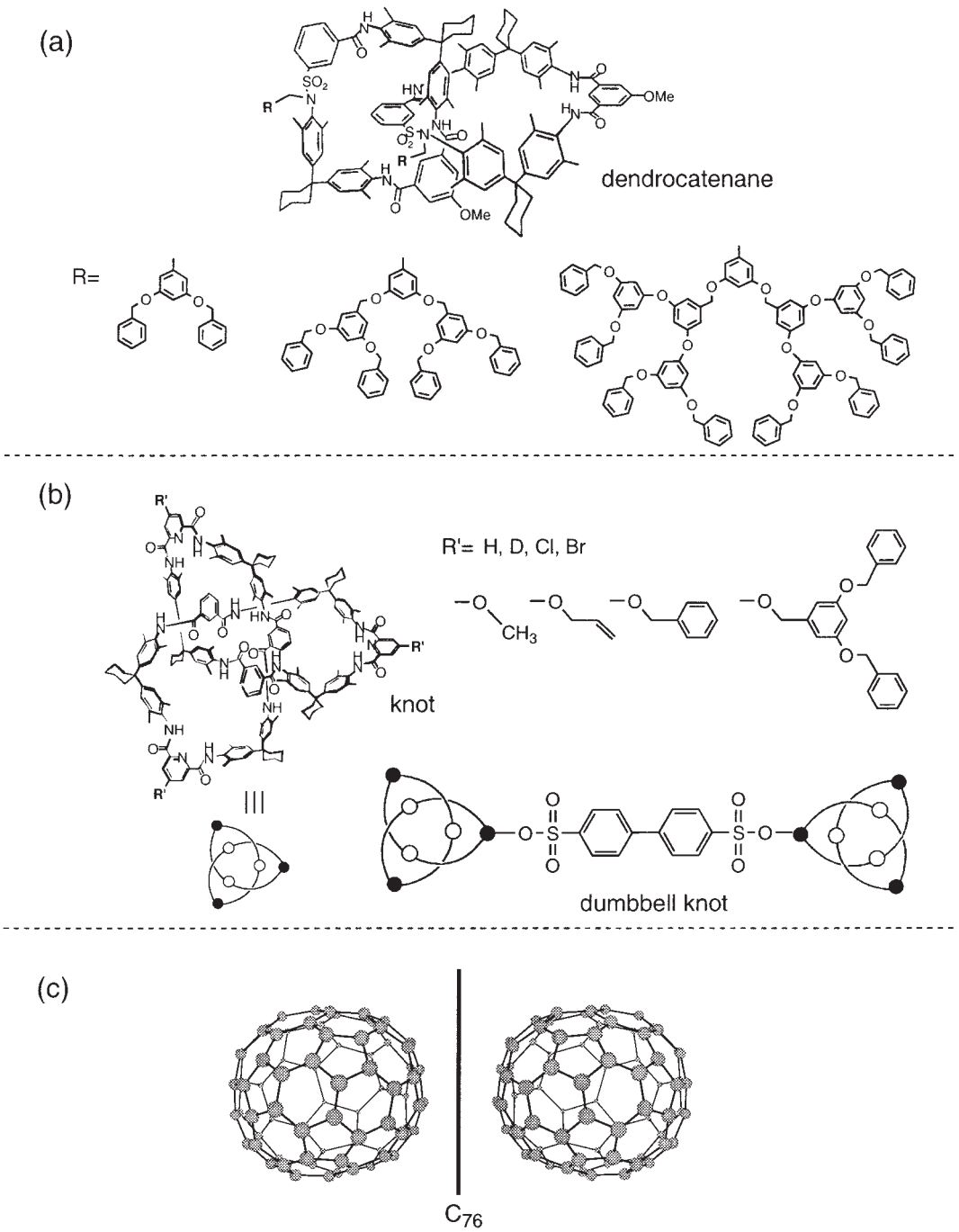


Fig. 26. Structures of dendrocatenanes (a), molecular knot (b), and C_{76} (c) resolved on chemically bonded-**28n** CSP.

ymers, and the determination of their precise structures both in the solid state and in solution is not easy. This makes understanding the chiral recognition mechanism of polymeric CSPs difficult.

The chiral recognition mechanism of the polysaccharide-based CSPs had been investigated, mostly based on chromatographic methods, and had not yet been satisfactorily elucidated at a molecular level. Recently, spectroscopic[168,198–201] and computational approaches[202,203] have been carried out on the polysaccharide-based CSPs.

3.4.1 Chromatographic Studies: In order to obtain superior CSPs, more than two hundred polysaccharide derivatives have been prepared. Particularly, phenylcarbamate derivatives of cellulose and amylose having various substituents on the phenyl moieties were systematically examined. As mentioned above, the chiral recognition abilities of the phenylcarbamates of polysaccharides are greatly influenced by the substituents on the phenyl moieties. To evaluate the effect of the substituents on the interaction between CSPs and solutes, the retention time of acetone was measured on the CSPs of 3- and 4-substituted phenylcarbamates of cellulose.[124] Results show that acetone tends to interact more strongly with the CSPs having electron-withdrawing substituents than with CSPs having electron-donating substituents. Figure 28 shows the schematic in-

teraction of the carbamate residues and solutes. Probably, acetone is mainly adsorbed on the stationary phases via hydrogen-bonding with an NH proton, and this interaction is expected to be stronger when the proton is more acidic, due to the electron-withdrawing power of the substituents. On the other hand, alcohols capable of hydrogen bonding with the C=O of the carbamate group tend to interact more strongly with the CSPs having electron-donating substituents. These results indicate that the main chiral interaction sites are probably the polar carbamate groups, which can interact with a racemate via hydrogen bonding on NH and C=O groups and a dipole–dipole interaction on C=O.

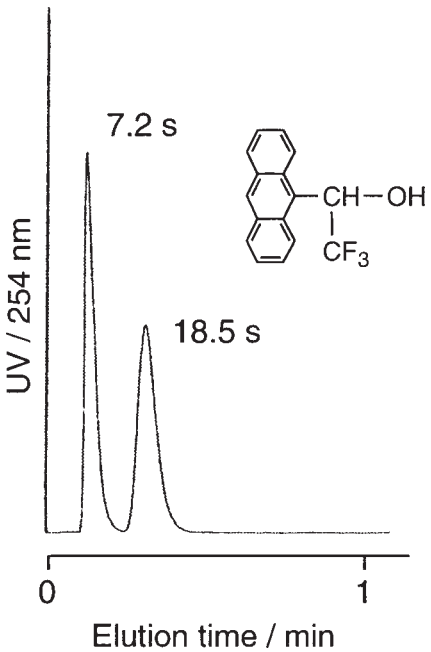


Fig. 27. Enantioseparation of 2,2,2-trifluoro-1-(9-anthryl)-ethanol using **27x** in situ coated 12% (w/w) on monolithic silica material as chiral stationary phase. Column: 4.6 × 50 mm; mobile phase: hexane/2-propanol 90/10 (v/v); flow rate: 20 mL/min.

To elucidate the chiral recognition mechanism of the polysaccharide phenylcarbamates at a molecular level, one needs their precise structures. Figure 29 shows the stable structures of **27a** and **27x**[203] optimized by molecular-mechanics calcula-

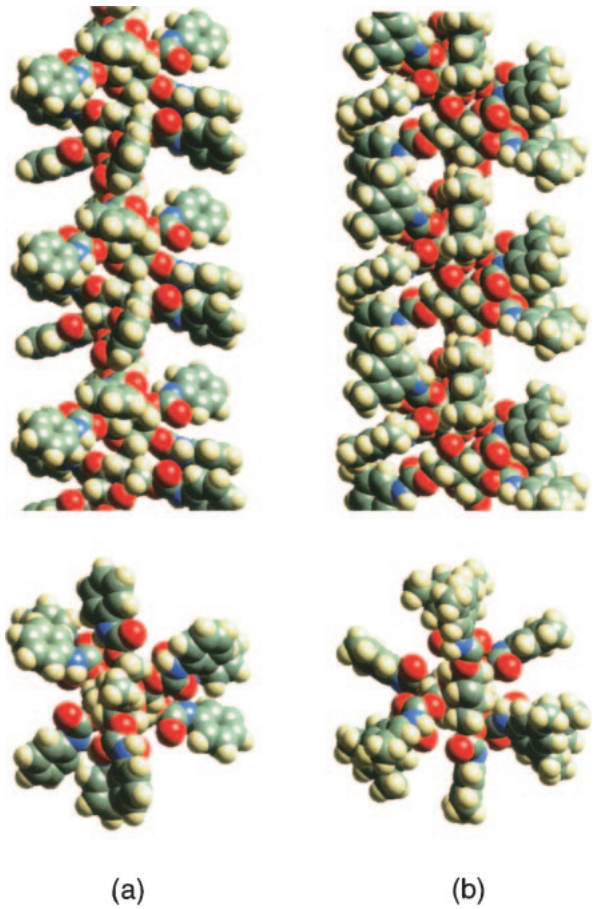


Fig. 29. Optimized structures of phenylcarbamate **27a** (a) and 3,5-dimethylphenylcarbamate **27x** (b) derivatives of cellulose. View along the helix axis (top) and perpendicular to the helix axis (bottom).

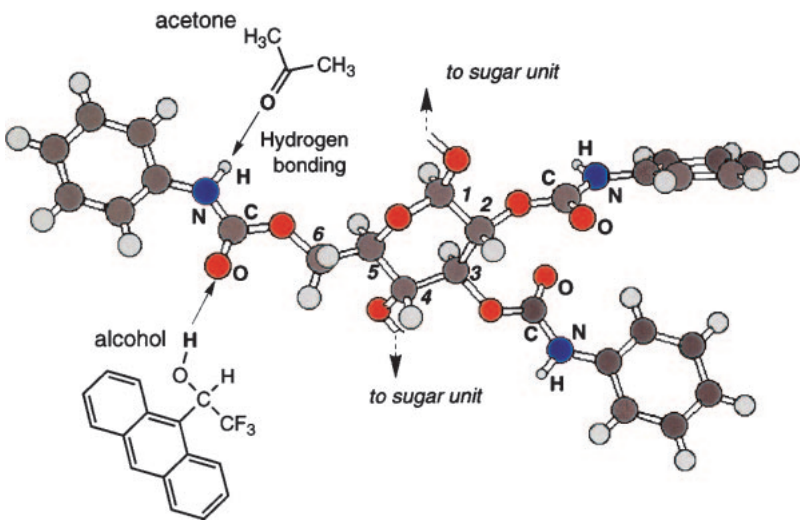


Fig. 28. Possible interaction sites of cellulose trisphenylcarbamates.

tions using the three-dimensional periodic boundary condition on the basis of the X-ray crystal structure of **27a** proposed by Zugenmaier.[159,160] These optimized structures have similar left-handed 3/2 helical conformations, and the glucose residues are regularly arranged along the helical axis. A chiral helical groove surrounded with polar carbamate groups exists along the main chain. The polar carbamate groups are preferably located inside, while hydrophobic aromatic groups are located outside the polymer chain. Therefore, polar enantiomers can predominantly interact with the carbamate residues in the groove through hydrogen-bond formation. When a polar group such as methoxy or nitro exists on the phenyl group, the chiral recognition ability of the CSP decreases due to the interaction between the polar substituent far from the chiral glucose unit and a racemate. Therefore, when the substituent is a bulkier alkoxy group, such as isopropoxy or isobutoxy, the bulky substituent can prevent racemates from interacting with the ether oxygen of the alkoxy group. This actually improved the chiral recognition ability.[204]

The phenylcarbamates of polysaccharides can also separate several nonpolar aromatic compounds,[127] which cannot interact with the carbamate group through a polar interaction. This means that, besides the polar interactions, a nonpolar interaction, such as a π–π interaction between the phenyl groups of phenylcarbamates and an aromatic group of a racemate, may play some role in chiral recognition, particularly under reversed-phase mode HPLC conditions.

From the pictures for **27a** and **27x**, the main-chain structures appear similar, but the conformation of the side groups is not the same. Because **27x** has two methyl groups on a phenyl group, some steric repulsion between the side chains may occur. This structural difference may be responsible for the different enantioselectivity of **27a** and **27x** toward some racemates. The substituents on the phenyl group affect not only the polarity of the carbamate residues but also the structures of phenylcarbamates.

On the other hand, the structures of amylose phenylcarbamate derivatives have not yet been determined by X-ray studies. Wenslow and Wang investigated the structure of **28n** by solid-state NMR; they pointed out that **28n** forms a helical structure with less than six folds in the solid state.[205] Recently, we found that **28n** with a low degree of polymerization (DP) is soluble in chloroform. We investigated the structure of **28n** in solution by NMR using the 2D NOESY technique coupled with computer modeling.[201] Figure 30 shows the optimized structure of **28n** with a left-handed 4/3 helix as the most probable one. For its construction, the information on the interproton distance of the glucose protons, that is H1–H4′, was obtained by measuring the peak volumes of the cross and diagonal peaks at different mixing times in the NOESY spectra. Combined with the energy profile of the dimer model of **28n**, two dihedral angles in the glycoside bond were determined, and the polymer model was then constructed under three-dimensional periodic boundary conditions. Similar to the cellulose derivatives, the polar carbamate groups are located inside the polymer chain and the aromatic groups are outside.

Although **27x** and **28n** have the same side chains, these two are quite different in their helical structures and conformations of the side chains. The former has a left-handed 3/2 helix and the latter a left-handed 4/3 helix.

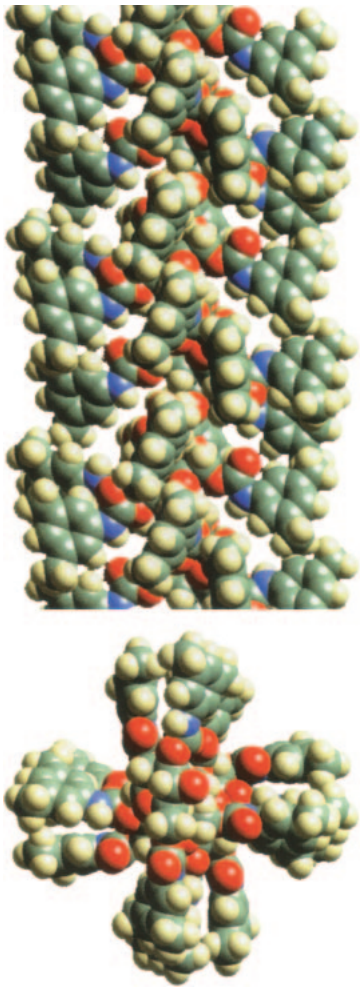


Fig. 30. Optimized structure of amylose tris(3,5-dimethylphenylcarbamate) (**28n**). View along the helix axis (top) and perpendicular to the helix axis (bottom). (Reprinted from Ref. 201. Copyright 2002, American Chemical Society.)

The influence of the degree of polymerization on the higher-order structures of polysaccharide phenylcarbamates is still not clear. This was investigated by a chromatographic approach and circular dichroism (CD) spectroscopy using cellooligosaccharide and maltooligosaccharide derivatives.[206] The high chiral recognition abilities of cellulose and amylose must be due to the helical higher-order structure. Such an ordered structure seems to begin at a rather low degree of polymerization for the amylose derivatives, but may begin at a higher degree for the cellulose derivatives. On the other hand, 3,5-dimethylphenylcarbamates of cyclodextrins showed quite different chiral recognition and CD spectra from those of **28n** and the maltooligosaccharide derivatives.[206] The cyclodextrin derivatives must have conformations different from those of the linear maltooligosaccharide derivatives.

3.4.2 NMR Studies: NMR spectroscopy is one of the most powerful tools for revealing chiral recognition mechanisms at a molecular level; NMR results show that cellulose trisphenylcarbamate maintains a helical structure even in solution.[207]

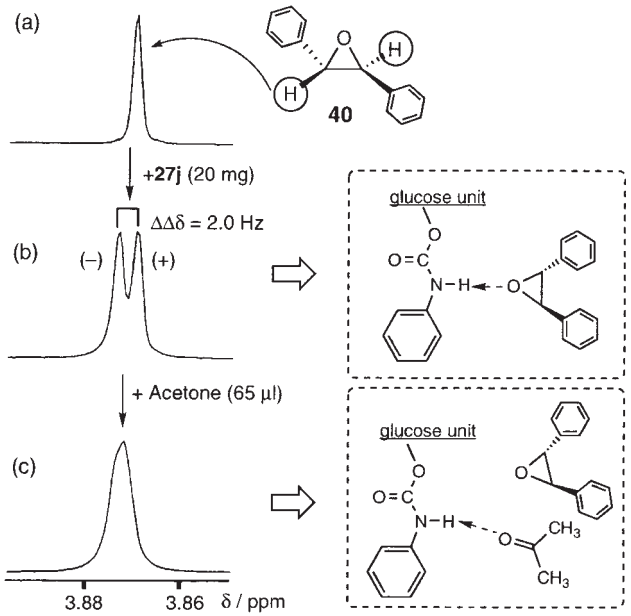


Fig. 31. ^{1}H NMR spectra of **40** in the presence of **27j** in $CDCl_3$ (1.0 mL) at 22 °C. **27j**: 0 (a), and 20 mg (b and c), acetone: 0 (a and b), and 65 μL (c).

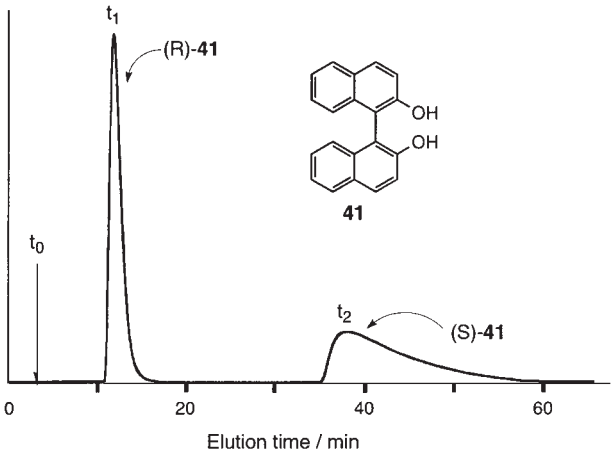


Fig. 32. Chromatogram of the enantioseparation of **41** on **27ao** with hexane/2-propanol (90/10) as eluent. (Reprinted from Ref. 200. Copyright 1996, American Chemical Society.)

However, most phenylcarbamate derivatives of polysaccharides with a high chiral recognition ability are soluble only in polar solvents, such as acetone, pyridine, and THF, which strongly interact with the polar carbamate residues. In these solvents, the chiral discrimination of enantiomers is hardly detected by NMR. Therefore, it was difficult to elucidate the chiral discrimination mechanism of most phenylcarbamate derivatives by NMR spectroscopy.

However, in the past ten years, several phenylcarbamates of cellulose and amylose, for instance, tris(4-trimethylsilylphenylcarbamate)s (**27j**, **28g**),[198,199] tris(5-fluoro-2-methylphenylcarbamate)s (**27ao**, **28w**),[157,200] and cyclohexylcarbamate,[168] are known to be soluble in chloroform, in which the chiral recognition for many racemates was detected by ^{1}H and ^{13}C NMR as well as in HPLC. These findings enabled us to investigate the interactions occurring in solution using NMR spectroscopy.

The high chiral recognition ability of **27j** is comparable to that of **27x**. Figure 31 shows the ^{1}H NMR spectra of (±)-*trans*-stilbene oxide (**40**) in the absence (a) and presence (b) of **27j**. The singlet signal for the methine proton of **40** at δ 3.871 is enantiomerically separated into two singlet peaks in the presence of **27j**, and only the (−)-isomer is shifted downfield. The chemical shift difference $\Delta\Delta\delta$ of the peaks increased with an increase in the amount of **27j** and with a decrease in temperature. This clearly indicates that **27j** can recognize the enantiomers even in solution. In the chromatographic enantioseparation of (±)-**40** on CSP **27j**, the (+)-isomer eluted first followed by the (−)-isomer, and complete baseline separation was achieved ($\alpha = 1.55$). This elution order is associated with the downfield shift of the (−)-isomer observed in the ^{1}H NMR.[198,199] For **40**, the oxygen atom of the oxirane ring may interact with the NH proton of the carbamate residue through hydrogen-bonding. The addition of acetone capable of hydrogen bonding with the NH proton prevents the interaction between **27j** and **40**, and the splitting of the methine proton signal of **40** disappears (Fig. 31c). Many other racemates are also enantiomerically recognized by **27j** in $CDCl_3$.

Cellulose tris(3,5-dichlorophenylcarbamate) having a high solubility as already mentioned above is dissolved in chloroform in the presence of a small amount of 2-propanol or an acid and also exhibits chiral recognition for some racemates.[199]

In HPLC, cellulose tris(5-fluoro-2-methylphenylcarbamate) (**27ao**) discriminates the enantiomers of 1,1′-bi-2-naphthol (**41**) with a large separation factor ($\alpha = 4.23$) (Fig. 32).[200] In ^{1}H and ^{13}C NMR spectroscopies, **27ao** also effectively discriminates the **41** enantiomers.[200] Figure 33 shows the 500 MHz ^{1}H NMR spectra of (*RS*)-**41** in the absence (a) and presence (b) of **27ao** in $CDCl_3$. Each of the hydroxy and naphthyl protons (H4 and H6) of **41** is clearly separated into two peaks due to the enantiomers in the presence of **27ao** and the former shifts downfield by hydrogen-bonding and the latter upfield by π-stacking or a shielding effect due to a neighboring aromatic ring of **27ao**. The signals due to (*S*)-**41** are more significantly shifted, accompanied by line broadening. This result indicates that (*S*)-**41** interacts more strongly with **27ao** in accordance with the chromatographic elution order of the enantiomers. Similar splittings due to **41** enantiomers are also observed in ^{13}C NMR spectroscopy.[200] Interestingly, only the carbons near the hydroxy groups are separated into enantiomers. This indicates that these carbons may be favorably located close to the chiral glucose residue to form hydrogen-bonding.

Information on the binding site of **27ao** and on the stoichiometry of the complexation was obtained by ^{1}H NMR titrations of **27ao** with (*S*)- and (*R*)-**41** and the Job plot for the **27ao**–(*S*)-**41** complex.[200] The details of binding geometry and dynamics between **27ao** and the enantiomers of **41** were investigated on the spin–lattice relaxation time, ^{1}H NMR titration, and intermolecular NOEs in the presence of **27ao**.[200] Figure 34 shows the NOESY spectrum of **27ao**–(*S*)-**41** in the regions between the aromatic protons and the methyl protons on the phenyl group of **27ao**, and the column slices taken through the tops of the three methyl peaks on the phenyl groups of **27ao**. A few clear intermolecular NOE cross peaks noted by arrows in the figure can be observed, indicating that the naphthyl protons of (*S*)-

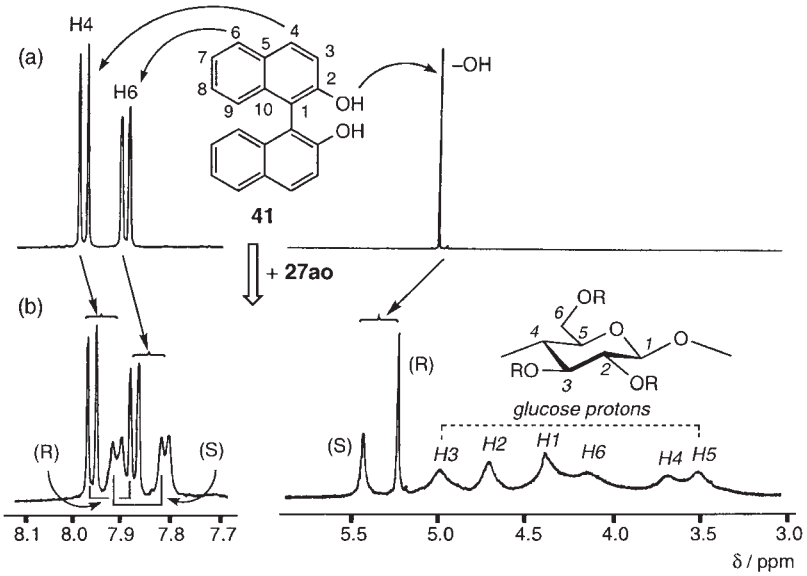


Fig. 33. ^{1}H NMR spectra of selected region of (*RS*)-**41** in the absence (a) and presence (b) of **27ao** in $CDCl_3$ at 23 °C. (Reprinted from Ref. 200. Copyright 1996, American Chemical Society.)

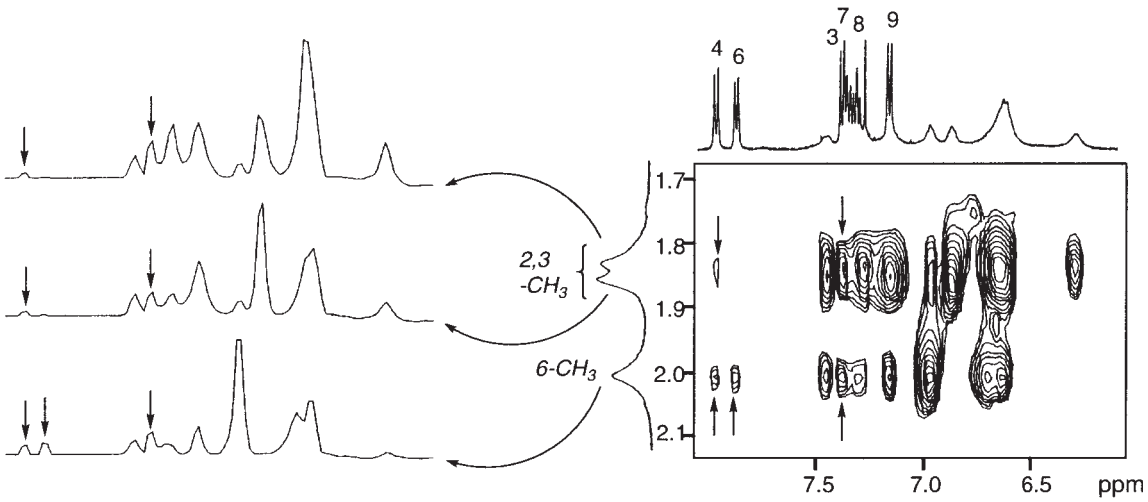


Fig. 34. Expanded NOESY spectra (right hand side) at a mixing time of 300 ms of the mixtures of (*S*)-**41** and **27ao** (molar ratio, 1:2) in the region between the aromatic protons (**41** and **27ao**) and methyl protons on the phenyl moiety of **27ao** in $CDCl_3$ at 30 °C. On the left hand side is shown the column slices taken through the tops of the three methyl peaks on the phenyl groups of **27ao**. (Reprinted from Ref. 200. Copyright 1996, American Chemical Society.)

41 are closely located to the methyl protons of **27ao** within less than 5 Å. On the other hand, no intermolecular NOE cross peaks were detected in the NOESY spectrum of **27ao**–(*R*)-**41**, probably due to a weak interaction. These observations agreed with the results obtained in the chiral HPLC and 1D NMR experiments. Using the HPLC and NMR data combined with the structural data for cellulose trisphenylcarbamate determined by X-ray analysis, a model has been proposed for the **27ao**–(*S*)-**41** complex as shown in Fig. 35, where two hydroxy protons of (*S*)-**41** form hydrogen bonds with the carbonyl oxygens of the carbamate groups of **27ao**. This interaction model satisfactorily explains all the NMR data including the intermolecular NOE and titration results.

Similar to the chiral HPLC experiment, the enantioselectivity (α) in the NMR experiment can also be calculated by the ^{1}H NMR titrations. The obtained value was 10.6, which is 2.5 times that estimated by the chiral HPLC using **27ao** as the CSP.[200] This difference may be ascribed to the use of different solvents in the HPLC and NMR measurements. It is difficult to use the same solvent in HPLC and NMR, because chloroform dissolves the **27ao** coated on silica gel.

As previously mentioned, we recently found that **28n** prepared from low-molecular-weight amylose (DP = ca. 100) obtained by enzymatic polymerization is soluble in chloroform and exhibits chiral discrimination toward many enantiomers in NMR as well as in HPLC.[201] In this case, the direct comparison of the NMR and HPLC data could be performed using chloroform and the bonded-type CSP, in which **28n** was chemically bonded to silica gel only at the chain end. Good agreement between the NMR and HPLC data was observed in the chiral

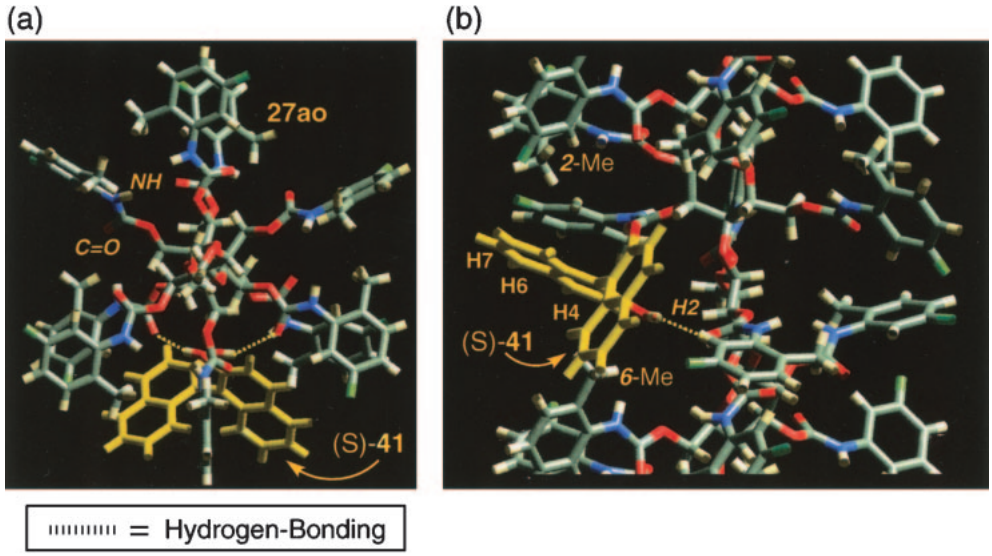


Fig. 35. Calculated structure of the complex **27ao**–(*S*)-**41**. View perpendicular to the helix axis (a), and expanded region of the same structure model as viewed along the helix axis (b). (Reprinted from Ref. 200. Copyright 1996, American Chemical Society.)

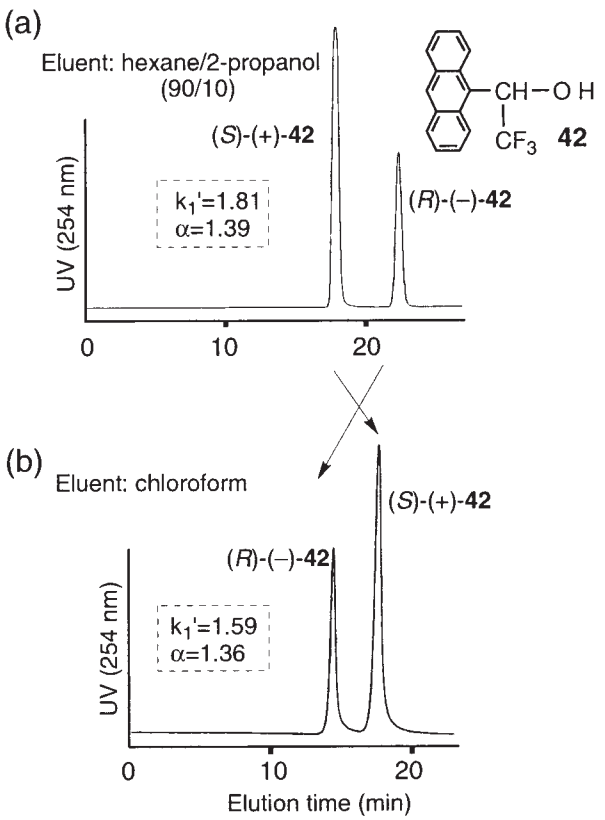


Fig. 36. Chromatograms of the enantioseparation of 1-(9-anthryl)-2,2,2-trifluoroethanol (**42**) on coated-type **28n** with hexane/2-propanol (90/10) as the eluent (a) and chemically bonded-type **28n** with chloroform as the eluent (b) at 25 °C. Column, 25 × 0.46 (i.d.) cm; flow rate, 0.5 mL/min. The (*S*)-**42**/(*R*)-**42** ratio is 2/1.

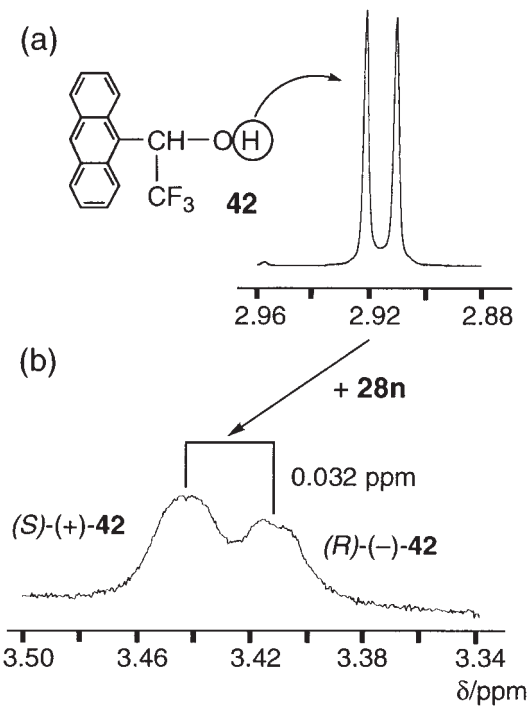


Fig. 37. ^{1}H NMR spectra of the OH proton of **42** in the absence (a) and presence (b) of **28n** in $CDCl_3$ at 23 °C.

recognition. Figure 36 shows the resolution of 1-(9-anthryl)-2,2,2-trifluoroethanol (**42**) on **28n** with hexane–2-propanol and chloroform as the eluents. In hexane–2-propanol (9/1), (*S*)-(+)-**42** eluted first, followed by (*R*)-(−)-**42**, whereas in chloroform (*R*)-(−)-**42** eluted first. The HPLC results with chloroform are consistent with the NMR results in Fig. 37, where the OH proton of (*S*)-(+)-**42** is more significantly shifted downfield than that of the corresponding (*R*)-(−)-**42**. The binding geometry between **28n** and (*S*)-(+)-**42** was investigated by ^{1}H NMR titration, and a rational model of the complex has been proposed (Fig. 38).

3.4.3 Computational Studies: As explained above, NMR is a powerful tool to investigate the chiral recognition mechanism of CSPs, but most phenylcarbamates of the polysaccharides with a high resolving ability are soluble only in polar sol-

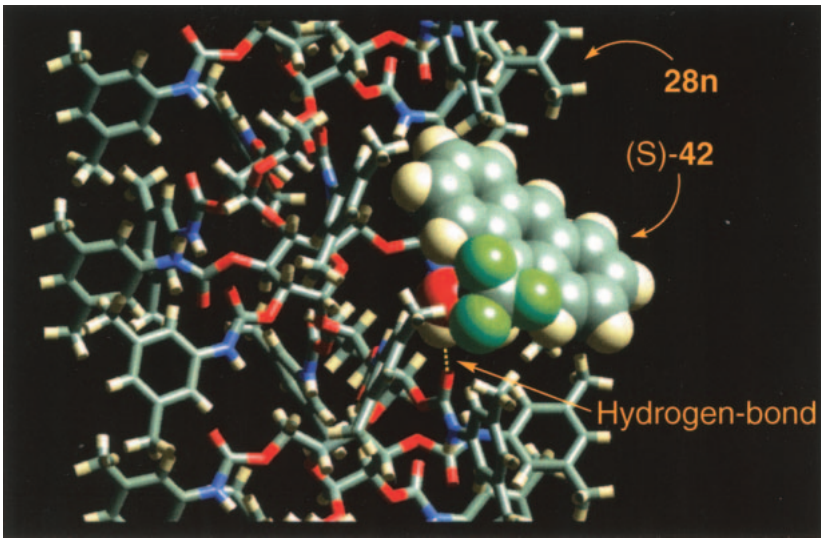


Fig. 38. Calculated structure of the complex **28n**–(*S*)-**42**. The dashed line corresponds to hydrogen bond.

vents, such as pyridine, THF, and DMF. In such cases, the interaction between the solvent and the phenylcarbamates is too strong to detect the interaction between the phenylcarbamates and a racemate. For these $CDCl_3$-insoluble phenylcarbamate derivatives, a computer simulation involving molecular-mechanics (MM) and molecular dynamics (MD) calculations may be a useful and effective approach for elucidating the mechanism of the chiral recognition, which might allow prediction of the elution order of enantiomers.

To study the chiral recognition mechanism for small molecule CSPs, various successful attempts have been carried out from theoretical viewpoints especially by Lipkowitz et al.[194,195] The interaction energies between the CSPs and enantiomers were calculated by MM, MD, and quantum mechanics calculations, and the chiral recognition mechanisms have been proposed on the basis of the calculation results.

To explain the chiral recognition mechanism of the $CDCl_3$-insoluble phenylcarbamate derivatives, we performed interaction energy calculations between cellulose tris(phenylcarbamate) (**27a**) or tris(3,5-dimethylphenylcarbamate) (**27x**) and *trans*-stilbene oxide (**40**) or benzoin by various methods using different force fields.[203] For the interaction energy calculation, the exact structures of the phenylcarbamate derivatives and enantiomers are required. Because **27a** exhibits a high chiral recognition as the CSP and its structure has been determined on the basis of the X-ray data, it is a suitable CSP for the calculation. In a chromatographic enantioseparation, **40** is completely resolved on both CSPs [**27a** ($\alpha = 1.46$), **27x** ($\alpha = 1.68$)], with the reversed elution order of enantiomers; the (*R*,*R*)-isomer elutes first on **27a**, and the (*S*,*S*)-isomer on **27x**. On the other hand, benzoin is completely separated on **27x** ($\alpha = 1.58$) but is not separated on **27a**.

The methods used for calculating the interaction energy are roughly divided into the following two types, which differ in enantiomer generation methods.

In one method, the center of a cubic sampling box ($r = 4$ Å) with mesh ($r' = 0.5$ Å) is placed on the NH proton and C=O oxygen of the carbamate residue of **27a** and **27x**, and then the enantiomers are generated at each grid point and rotated at 15° intervals for *x*, *y*, and *z* axes, individually. The interaction energy calculation is carried out at each NH proton and C=O oxygen at the 2-, 3-, and 6-positions of the glucose units. The calculation results are evaluated by the lowest interaction energy and the distribution of the interaction energy (Fig. 39).

In another method, the enantiomers with a particular orientation are randomly generated by the Monte Carlo method on the surface of **27a** and **27x** defined by the particular van der Waals radius using the reported technique of "blowing up" the atomic radii.[208] The MM calculations between the molecules are then performed step by step. The results of these calculations are evaluated with the averaged interaction energy.

In the calculations, nonamers of **27a** and **27x** were used, and the enantiomers were generated around their middle sections to avoid the influence of the end groups. The obtained results of both calculations were in good agreement with the chromatographic resolution data for **40** and benzoin.[203] The lowest or averaged interaction energy between **27a** and (*S*,*S*)-**40** obtained in the calculation was lower than that between **27a** and (*R*,*R*)-**40**, whereas an opposite enantiomer preference was observed for **27x** and **40**. Although the same calculation was performed for the benzoin–**27a** system, almost no difference in the interaction energies was observed for the enantiomers.

The clear interaction energy differences between the enantiomers was recognized only when the enantiomers were generated inside **27a** and **27x**, indicating that the polar carbamate residues of the cellulose carbamates may be the most important adsorbing and chiral recognition site for polar racemates. Figure 40 shows the calculated structure of the **27a**–(*S*,*S*)-**40** complex having the lowest interaction energy obtained by the second method employing the Monte Carlo technique.[203] The (*S*,*S*)-**40** is bound in the chiral groove to form a hydrogen bond between the ether oxygen of **40** and the NH proton of the carbamate group of **27a**, and each phenyl group appears to interact with the phenyl groups of **27a** through π–π interactions.

In the above calculations, the interaction energies are calculated between a single polysaccharide chain and an enantiomer, because polar racemates can preferentially come into the chiral groove of the polymer chain with the polar carbamate residues.

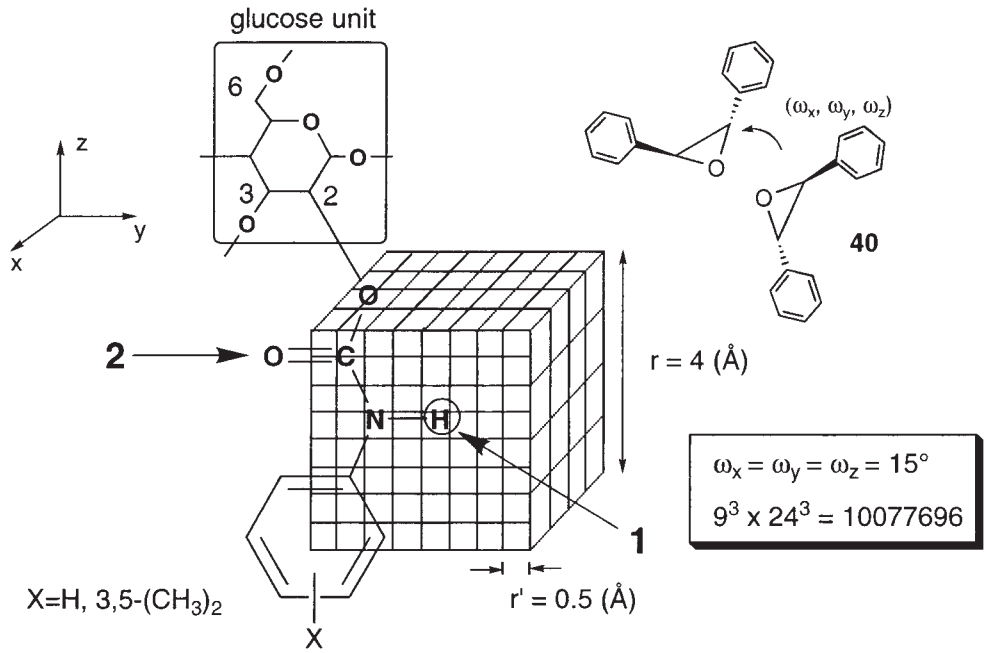


Fig. 39. Method of calculation of interaction energy between **27a** and enantiomers of **40** or benzoin.

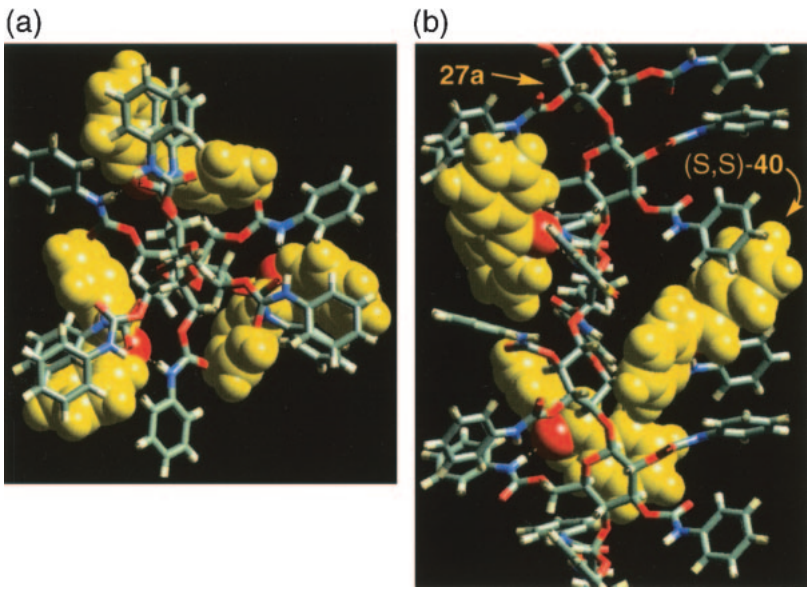


Fig. 40. Calculated structure of the **27a**–(*S*,*S*)-**40** complex formed through hydrogen bondings. View perpendicular to the helix axis (a) and along the helix axis (b).

However, besides these polar interactions, hydrophobic interactions such as π–π interaction between the phenyl group of the phenylcarbamate derivatives and the aromatic groups of a enantiomer may contribute to the chiral recognition, especially under reversed-phase conditions. In this case, the hydrophobic chiral space created by the phenyl moieties of the phenylcarbamates may also play an important role in chiral recognition. In addition, solvent molecules must be taken into consideration. Further computational studies are required for a more accurate prediction of chromatographic enantioseparation. The methods described above are useful for a qualitative understanding of the chiral recognition mechanism of the polysaccharide derivatives.

4. Conclusions

In this account, optically active polymers having a chiral recognition ability as the chiral stationary phases for HPLC have been reviewed. Most polymers with a high chiral recognition power possess regular higher-order structures, which seem to be the most important factor in efficient chiral recognition by the polymeric CSPs. Although it is not easy to control the higher-order structure of fully synthetic polymers, several natural polymers with a stereo-controlled structure and a high functionality exist. Polysaccharides such as cellulose and amylose are the most readily available optically active polymers with stereoregular sequences. The chiral recognition abilities of native

polysaccharides themselves are not remarkable, but they can be readily converted to the esters and carbamates with high chiral recognition abilities. At present, more than ten polysaccharide-based CSPs have been on the market and are being extensively used for both analytical and preparative separation of enantiomers.

For the preparative separation, simulated moving bed (SMB) chromatography, which is a powerful method for the separation of a two-component mixture, was successfully introduced in the early 1990s.[209] This has already been employed for the preparative separation of pharmaceutical compounds.

On the other hand, miniaturized separation techniques such as capillary liquid chromatography (CLC), capillary electrophoresis (CE), and capillary electrochromatography (CEC) have also been investigated.[210] These techniques offer advantages such as low consumption of the stationary and mobile phases, low cost, and fewer environmental problems.

The chiral recognition mechanism of the polysaccharide derivatives has been solved to some extent by means of chromatography, NMR, and computational methods. Understanding of the mechanism on CSPs at a molecular level will help in further development of more effective CSPs.

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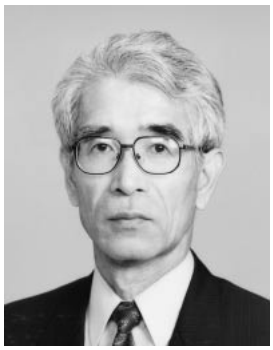

Yoshio Okamoto was born in Osaka in 1941. He received his bachelor's (1964), master's (1966), and doctoral (1969) degrees from Osaka University, Faculty of Science. He joined Osaka University, Faculty of Engineering Science as an assistant in 1969, and spent two years (1970–1972) at the University of 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 a Professor. His research interests include stereocontrol in polymerization reaction, asymmetric polymerization, optically active polymers, and enantioseparation by HPLC. He has received The Award of the Society of Polymer Science, Japan (1982), The Chemical Society of Japan Award for Technical Development (1991), The Award of The Chemical Society of Japan (1999), Chirality Medal (2001), and Medal with Purple Ribbon (2002), among others.

Chiyo Yamamoto was born in Aichi in 1970. She received her B.S. degree in 1994, M.S. degree in 1996, and Ph. D degree in 1999 from Nagoya University under the supervision of Professors Y. Okamoto and E. Yashima. After being a postdoctoral fellow of JSPS for four months, she accepted a position as research assistant in 1999 at Nagoya University, where she currently studies with Y. Okamoto. In 2000 she spent five months at University of California, Berkeley as a postdoctoral fellow with Prof. J. M. J. Fréchet. Her current research concerns the development of chiral stationary phases for chromatography and the studies on chiral recognition mechanism.