Chiral HPLC for efficient resolution of enantiomers

Yoshio Okamoto* and Tomoyuki Ikai

Received 29th July 2008
First published as an Advance Article on the web 29th September 2008
DOI: 10.1039/b808881k

Resolution of racemic compounds is one of the potential ways of obtaining both enantiomers. Among several resolution techniques in the past few decades, direct enantioseparation by high-performance liquid chromatography (HPLC) has significantly advanced, and a large number of chiral stationary phases (CSPs) for HPLC have been developed using both chiral small molecules and polymers with chiral recognition abilities. In this *tutorial review*, after describing the brief history and general view of CSPs, special emphasis will be placed on the studies involving the development and application of polysaccharide-based CSPs in our group.

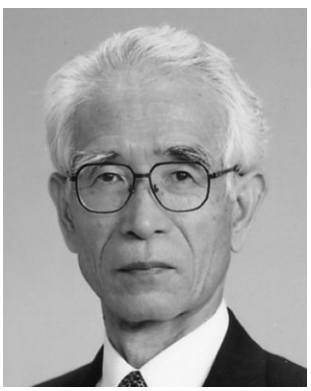
1. Introduction

It is well known that many biologically and pharmacologically interesting compounds are chiral and their physiological properties, such as pharmacodynamics, pharmacokinetics, metabolism, protein binding and toxicity, are often different between enantiomers. In 1858, the first biological enantioselectivity was reported by Louis Pasteur. He found that microorganisms more rapidly consumed (+)-ammonium tartrate than the (−)-isomer. Since then, the role of chirality in biological systems has attracted much attention, and many biological enantioselectivities had already been observed by the early 20th century. These enantioselectivities in organisms had mainly attracted academic interest in pharmacology, medicinal chemistry and biochemistry. However, the drug administration and industry had not made an effort to study the pharmacological effect of each enantiomer in drug candidates mainly because of the difficulty in obtaining and analyzing both pure enantiomers, and most synthetic chiral drugs were used as racemates until the end of the 20th century.

For example, in 1957, a tranquilizer drug (thalidomide) was manufactured and sold as a racemate of *N*-phthalylglutamic acid imide. Unfortunately, the drug was teratogenic and caused serious fetal malformations around the world. This teratogenic effect was attributed to the *S*-(−)-isomer.[1] Although even the administration of the *R*-(+)-isomer could not prevent the tragedy due to the fact that thalidomide is racemized in the body; this disaster brought a profound movement in the pharmaceutical industries. As a result, pharmaceutical companies have been required to systematically investigate the biological activity of individual enantiomers. Today, most of the top selling drugs around the world are administered as single enantiomers having the desired physiological activity.[2]

As in the pharmaceutical field, the analysis of chiral compounds and the preparation of optically active compounds have become more and more important in the fields of agrochemicals, foods, fragrances and functional materials, such as ferroelectric liquid crystals and organic nonlinear optical molecules.

EcoTopia Science Institute, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan. E-mail: okamoto@apchem.nagoya-u.ac.jp; Fax: +81-52-789-3188; Tel: +81-52-789-4600



Yoshio Okamoto

Yoshio Okamoto received his doctorate degree from Osaka University. He was a postdoctoral fellow at the University of Michigan with Professor Overberger. He then moved to Nagoya University as Professor. After retiring in 2004, he became Guest Professor of EcoTopia Science Institute, Nagoya University. He was appointed as Chair Professor of Harbin Engineering University in 2007. He received the Award of Society of Polymer Science, Japan (1982), The Chemical Society of Japan Award for 1999, Chirality Medal (2001), Medal with Purple Ribbon (Japanese Government) (2002), Fujiwara Prize (2005), among others.

Tomoyuki Ikai

Tomoyuki Ikai received his doctorate degrees from the Department of Applied Chemistry, Nagoya University and joined EcoTopia Science Institute, Nagoya University, as Assistant Professor in 2008. In 2008, he spent 5 months with Professor Timothy M. Swager at Massachusetts Institute of Technology as a visiting scientist. His current research concerns the development of chiral stationary phases for chromatography.

In this context, tremendous efforts have been made to develop effective techniques for the preparation of optically active compounds. There are two approaches for obtaining optically active compounds. One is the asymmetric synthesis of a desired enantiomer, and the other is the resolution of a racemic compound into individual enantiomers.

Asymmetric synthesis includes methods using the chiral pool, chiral auxiliaries, asymmetric catalysts and enzymes. Although a large quantity of enantiomers can be economically produced by this approach, only one of the enantiomers is usually obtained from the natural chiral source. This means that if both enantiomers are needed, at least two kinds of chiral source are required for each enantiomer. However, it is sometimes difficult to obtain both of them.

On the other hand, the resolution of a racemate can provide both enantiomers. In 1848, Pasteur discovered that the crystals of racemic sodium ammonium tartrate consisted of two kinds of enantiomorphic shapes.[3] He could manually sort these crystals into each shape using a pair of tweezers and a magnifying glass, and found that these two crystals rotated polarized light in opposite directions. This experiment became the first breakthrough in the field of resolution, and since then the following resolution techniques have been developed: (i) resolution by crystallization including manual sorting of conglomerate, preferential crystallization, crystallization of diastereomers and inclusion in optically active host compound; (ii) kinetic resolution by enzyme; (iii) indirect chromatographic resolution as diastereomers; and (iv) direct chromatographic resolution using chiral stationary phases (CSPs) or chiral mobile phases.

Among these methods, direct resolution using CSPs in high-performance liquid chromatography (HPLC) has grown significantly as a simple and practical method applicable for both analytical and preparative purposes.[4] Recently, the purity (enantiomeric excess) of chiral compounds is mostly estimated by three main methods: NMR spectroscopy, gas chromatography and HPLC with CSPs.[5] Among these, the HPLC method is most frequently used. More than 50% of the determinations have been carried out by chiral HPLC. Thirty years ago when we started the study of enantiomer selective polymerization, the enantiomeric excess could be estimated only by the optical purity measured by a polarimeter with a low sensitivity. This determination method has almost been completely replaced by the above three methods. The recent progress in the HPLC method arises from the development of both efficient CSPs and instruments, such as highly sensitive detectors and the simulated moving bed (SMB) system for preparative separations. More specifically, the introduction of the continuous processes by the SMB system has opened up the possibility for the industrial-scale chromatographic resolution of chiral compounds.[6]

The resolution using CSPs by HPLC can be realized on the basis of the different interaction behavior of the two enantiomers on the CSPs. Fig. 1 shows a typical chromatogram for the HPLC resolution of enantiomers, in which the (−)-isomer elutes first, followed by the (+)-isomer, and complete baseline separation is achieved.[7] The recognition ability of a CSP can be quantitatively evaluated using the following three

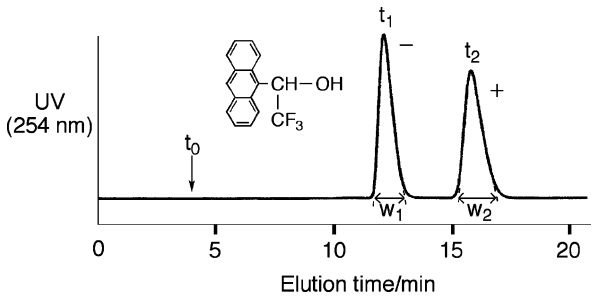


Fig. 1 Resolution of 2,2,2-trifluoro-1-(anthryl)ethanol. CSP: cellulose 3-isopropoxyphenylcarbamate; column: 25 × 0.46 (i.d.) cm; eluent: hexane–2-propanol (90 : 10); flow rate: 0.5 ml min^{-1}. (Reproduced with permission from ref. 7. Copyright 2006, Wiley VCH.)

parameters: retention factor (k), separation factor (α) and resolution factor (Rs), which are defined as follows:

$$k_1 = (t_1 - t_0)/t_0,\ k_2 = (t_2 - t_0)/t_0,$$

$$\alpha = (t_2 - t_0)/(t_1 - t_0) = k_2/k_1$$

$$Rs = 2(t_2 - t_1)/(w_1 + w_2)$$

In these equations, t_1 and t_2 are the retention times of the enantiomers, t_0 is the retention time of a non-retained compound (dead time) and w_1 and w_2 are the peak widths at their bases. For a chromatographic separation, k' shows the degree of interaction with a CSP, α is directly related to the recognition ability of a CSP and Rs is correlated to both the recognition ability of a CSP and the theoretical plate number of a column. The energy difference ($\Delta\Delta G$) in interactions between a CSP and a pair of enantiomers is calculated from the α value ($\Delta\Delta G = -RT \ln \alpha$). The selection of an appropriate CSP, which can produce a sufficient $\Delta\Delta G$, is a key point for successful resolution. Usually, a very small energy difference ($\Delta\Delta G = -0.46$ kJ mol^{-1} corresponding to $\alpha = 1.20$) can result in the baseline resolution of the enantiomers. This is also the reason why a CSP can often resolve a broad range of compounds. Up to now, a tremendous number of CSPs have been developed, and more than one hundred CSPs are commercially available. The chiral selectors used as the CSPs for HPLC can be classified into two types.

The first type of CSP consists of optically active small molecules, which are usually immobilized on a silica gel or organic polymer gel as achiral supports (brush-type CSPs). Since Davankov and Rogozhin initially reported this type of CSP in the early 1970s,[8] a wide range of chiral small molecules have been used as CSPs. The second type of CSP consists of optically active polymers, which are further divided into synthetic and natural polymers.[9] These polymer-type CSPs are usually prepared by coating the polymers on silica gel in order to improve the resolution efficiency and mechanical strength.

In this review, we will discuss the typical chiral small molecules and polymers as CSPs for HPLC. Particularly, special emphasis will be placed on our studies involved in the development and application of polysaccharide-based CSPs, which are the most frequently used for the analysis and preparative separation of chiral compounds.

2. Brush-type CSPs

2.1 Ligand-exchange-type CSPs

In 1971, the first baseline resolution of enantiomers by liquid chromatography was achieved using a ligand-exchange-type CSP,[8] which was developed by Davankov and Rogozhin through immobilizing a cyclic amino acid, L-proline, onto poly(styrene-*co*-divinylbenzene) beads (**1** in Fig. 2). In the early stages of chromatographic chiral separations, the ligand-exchange-type CSPs were often used for the determination of the optical purity of chiral compounds bearing polydentate ligands. However, the frequency of their application has been decreasing, because the ligand-exchange-type CSP can resolve only a quite restricted range of racemates and their operation method is rather complicated compared with the later-developed CSPs.

2.2 Crown ether-based CSPs

Since Cram *et al*. developed the first crown ether-based CSP (**2** in Fig. 3) in 1975,[10] various crown ether-based CSPs have been prepared by introducing appropriate chiral units into the crown ether frameworks. Among them, chiral crown ethers bearing optically active binaphthyl (**3**) and tartaric acid (**4**) units and phenolic pseudo chiral crown ethers (**5**) have been most successfully used as CSPs. These CSPs can efficiently resolve chiral compounds with a primary amino group under reversed phase conditions in the presence of acidic additives. Under these conditions, the oxygen atoms in the crown ether are allowed to interact with the protonated primary amino group of analytes through a multiple hydrogen-bonding interaction. Recently, the applications of crown ether-based CSPs have been extended to the resolution of secondary amino compounds and *N*-acylated amino acids.

2.3 Cyclodextrin-based CSPs

A cyclodextrin-based CSP for the enantioseparation by HPLC was pioneered by Harada *et al*. in 1978.[11] Cyclodextrins are cyclic oligosaccharides with 6, 7 and 8-membered toroidal shapes (Fig. 4). The primary hydroxyl groups at the 6-position of the glucose units are located around the narrower opening of the toroid. The secondary hydroxy groups at the 2- and 3-positions line the opposite wider opening of their cavities. Since the polar hydroxy groups are on the outside of the cyclodextrins, the internal cavity is relatively hydrophobic. The cyclodextrins and their derivatives (**6**) have been used for the chiral mobile phases in capillary electrophoresis and for chiral stationary phases in HPLC. Today, a broad variety of cyclodextrin-based CSPs for HPLC have been commercialized and widely used for the analytical separations of chiral compounds. Due to their relatively low loading capacity, however, they are not very useful for preparative separation.

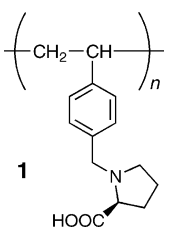


Fig. 2 Structure of ligand-exchange-type CSP.

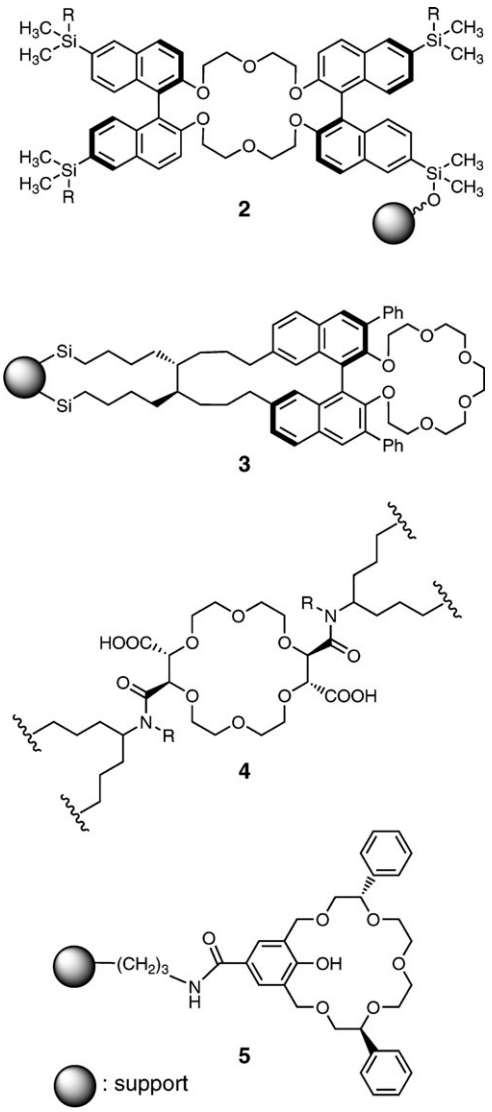


Fig. 3 Structures of crown ether-based CSPs.

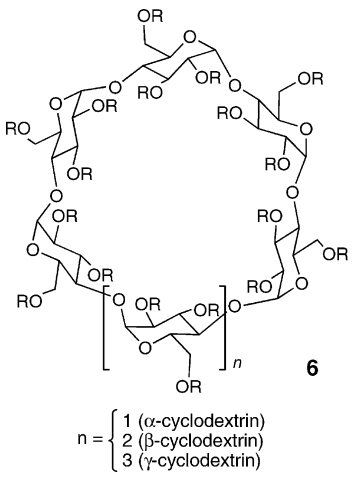


Fig. 4 Structures of cyclodextrin-based CSPs.

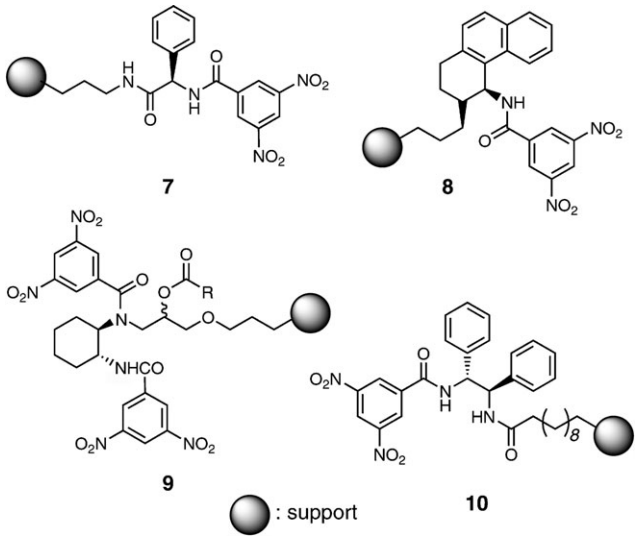


Fig. 5 Structures of donor–acceptor-type CSPs.

Cyclodextrin derivatives are also often used as the CSPs for the gas chromatographic resolution of enantiomers.[12]

2.4 Donor–acceptor-type CSPs

The donor–acceptor-type CSPs were introduced by Pirkle and House in 1979,[13] and this kind of CSP based on 3,5-dinitrobenzoyl-phenylglycine (**7** in Fig. 5) was first commercialized as a CSP for HPLC. Pirkle has also proposed the reciprocity principle for the design of target-specific donor–acceptor-type CSPs. This principle means that the roles of a chiral selector and an analyte are exchangeable, and a well-resolved analyte can be an attractive chiral selector. One of the rationally designed CSPs is the Whelko-01 CSP (**8**), which may be the most effective donor–acceptor type CSP. Additional donor–acceptor-type CSPs based on 1,2-*trans*-diaminocyclohexane (**9**) and 1,2-*trans*-diphenylethylenediamine (**10**) were developed by Gasparrini *et al.*[14] and Uray and Lindner,[15] respectively. These CSPs are also commercially available for the analytical or preparative separation of enantiomers. For the donor–acceptor-type CSPs, the π–π-stacking interaction between the electron-rich and electron-deficient aromatic groups plays a significantly important role in discriminating chiral compounds in addition to hydrogen bonding, dipole–dipole and steric interactions. These donor–acceptor-type CSPs are usually used under normal-phase conditions, but chiral separations under reversed-phase conditions can also be employed.

2.5 Glycopeptide (aglycone)-based CSPs

In 1994, Armstrong *et al*. introduced macrocyclic glycopeptide (aglycone)-based CSPs.[16] Vancomycin (**11**), ristocetin A (**12**), teicoplanin (**13**) and teicoplanin aglycone (**14**) are commercially available as chiral selectors for the glycopeptide (aglycone)-based CSPs (Fig. 6). These phases have been used *via* the immobilization of glycopeptides (aglycone) onto silica gel. The macrocyclic glycopeptides (aglycone) possess several chiral cavities and various kinds of functional groups, such as aromatic, hydroxyl, amino, ester, carboxyl and amido groups. Due to the diversity of functionalities on the macrocyclic glycopeptides (aglycone), these CSPs are able to be used with a variety of mobile phase modes and can resolve a wide range of racemates.

2.6 Ion-exchange-type CSPs

In 1996, Lindner and Lämmerhofer developed the anion-exchange-type CSPs based on *Cinchona* alkaloid derivatives,[17]

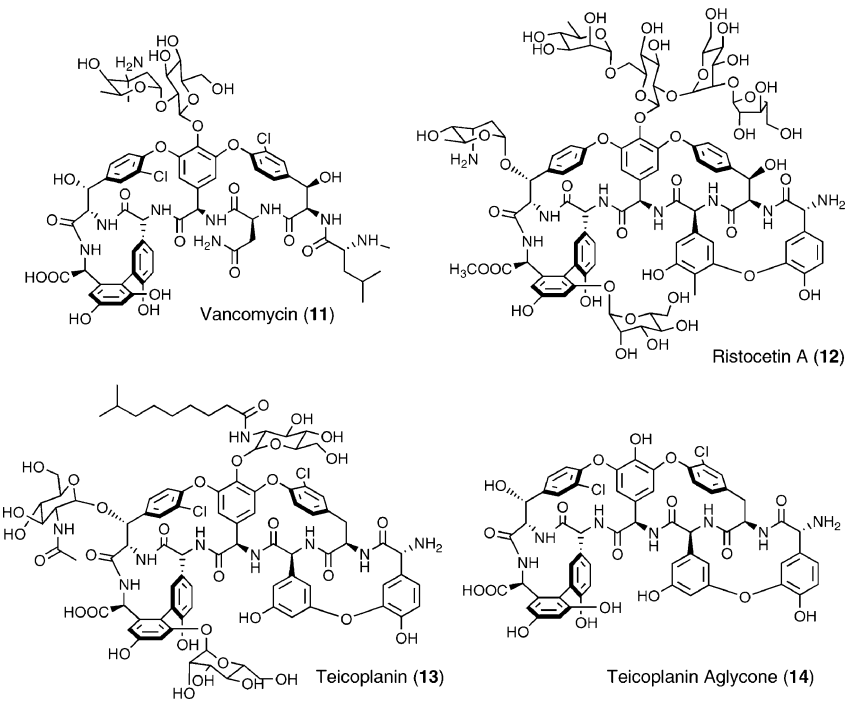


Fig. 6 Structures of glycopeptide (aglycone)-based CSPs.

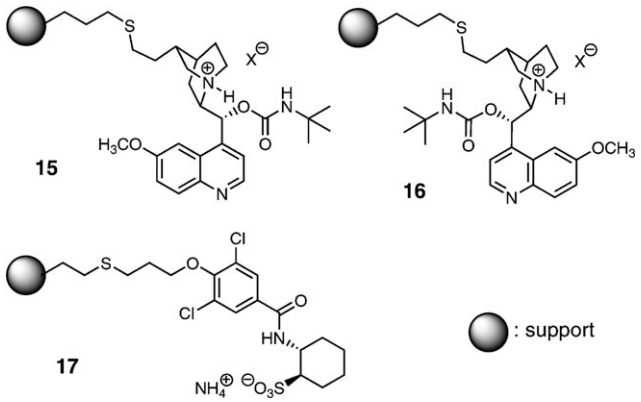


Fig. 7 Structures of ion-exchange-type CSPs.

which have excellent recognition abilities for chiral acidic compounds including important pharmaceuticals and efficient chiral auxiliaries for asymmetric synthesis. 9-*O*-*tert*-Butylcarbamate derivatives of quinine (**15**) and quinidine (**16**) have been commercialized as CSPs under the trade names Chiralpak QN-AX and Chiralpak QD-AX, respectively (Fig. 7). Recently, the cation-exchange-type CSP (**17**) for the resolutions of chiral basic compounds has been developed by the same groups.

3. Synthetic polymer-based CSPs

3.1 Molecular imprinted-type CSPs

Cross-linked polymer gels with enantioselective binding cavities were prepared *via* a molecular imprinting technique by Wulff and Sarhan in 1972.[18] The sizes, shapes and stereochemical natures of these chiral cavities are complementary to an enantiomer used as the template, and therefore, the gel exhibits a higher enantioselective affinity for the template enantiomer than for the other. Until now, numerous molecular imprinted-type CSPs have been reported for the resolution of biologically active compounds, such as amino acids, peptides, hormones and antibiotics.

3.2 Poly(meth)acrylamide-based CSPs

In 1974, Blaschke reported that a polyacrylamide with an optically active side chain (**18** in Fig. 8) showed a chiral recognition for mandelic acid.[19] Since then, several chiral polyacrylamides and polymethacrylamides have been developed as CSPs for HPLC,[20] and many chiral drugs including thalidomide have been resolved using these CSPs on a preparative scale. Typical examples (**19–22**) are shown in Fig. 8. Interestingly, the recognition ability on these polymers is significantly dependent on the synthetic methods. The polymers prepared by the radical polymerization of optically active monomers exhibited a much higher chiral recognition than that prepared by the reaction of poly(acryloyl chloride) with the corresponding chiral amines. The chiral recognition sites in the CSPs must be constructed during the polymerization process. Recently, it was also found that the tacticity of polymethacrylamides influenced their chiral recognition abilities.[21]

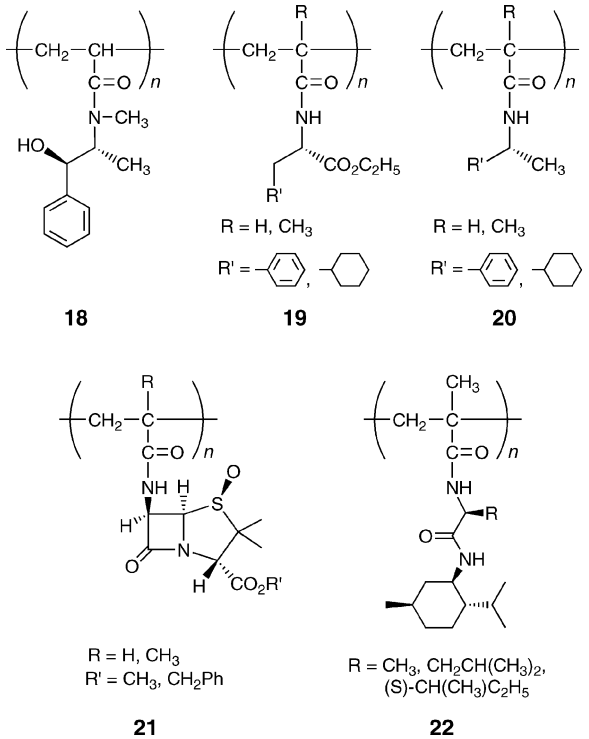


Fig. 8 Structures of poly(meth)acrylamide-based CSPs.

3.3 Polymethacrylate-based CSPs

The polymethacrylates with chiral side chains, such as the (*S*)-1-phenylethyl group, show a very low chiral recognition,[22] but an optically active polymethacrylate with a one-handed helical structure (**23** in Fig. 9) prepared by the helix-sense-selective polymerization with a chiral anionic initiator in 1979[23] showed a high chiral recognition ability, particularly for stereochemically interesting compounds.[24] The chiral recognition on **23** can be attributed to the rigid helical structure accompanying the chiral propeller triphenylmethyl groups. The recognition ability on **23** was evaluated for two different types of CSPs for HPLC; one was prepared with the insoluble **23** ground into small particles and the other by coating the soluble polymer on a macroporous silica gel. These two CSPs showed different chiral recognitions for several racemates, which may be attributed to the different orientation of **23** in the bulk and on the surface of the silica gel. In most cases, methanol or a methanol–water mixture produces better results for enantiomer separations than a nonpolar eluent, such as a hexane–alcohol mixture. This indicates that nonpolar or

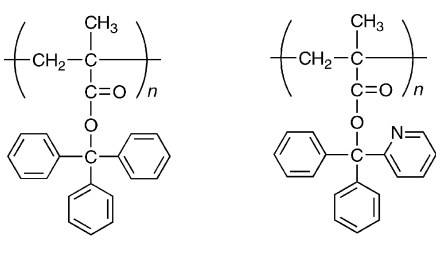


Fig. 9 Structures of polymethacrylate-based CSPs.

hydrophobic interactions between the triphenylmethyl group with a chiral propeller structure and racemates are important for the successful chiral recognition. Therefore, the CSPs can efficiently resolve racemates without any functional groups, which are difficult to be resolved on other CSPs. Besides **23**, the one-handed helical optically active polymer **24** also shows a unique recognition ability.[25]

3.4 Polyamide-based CSPs

Optically active polyamides including poly(α-amino acid)s show a chiral recognition as CSPs for HPLC. Poly(*N*-benzyl-L-glutamine) immobilized onto cross-linked polystyrene beads was used for the resolutions of mandelic acid and hydantoin derivatives.[26]

In 1985, fully synthetic polyamides (**25** in Fig. 10) with chiral recognition abilities were prepared by the polycondensation of chiral dicarboxylic acids with chiral diamines by Saigo *et al.*[27] These polyamide-based CSPs can resolve polar racemates capable of a hydrogen-bonding interaction. The recognition ability of **25** (R = methylene groups) was closely correlated to its crystallizability, which is dependent on the number of methylene groups in the main chain. Polyamides **25** bearing an even number of methylene groups exhibited a higher recognition ability and crystallizability than those bearing an odd number of methylene groups. Some other polyamides (**26**, **27**) have also been prepared as CSPs.

3.5 Tartardiamide-based CSPs

In 1995, Allenmark *et al.* developed conceptually new polymeric-type CSPs,[28] which were prepared *via* the hydrosilylation of C_2-symmetric *N*,*N*′-diallyl-L-tartardiamide derivatives with a multifunctional hydrosilane on an allyl-functionalized silica gel. The CSPs bearing 3,5-dimethylbenzoyl- and 4-*tert*-butylbenzoyl residues are commercially available and have been used for the resolutions of a broad range of pharmaceuticals, including acidic, neutral and basic compounds.

4 Natural polymer-based CSPs

4.1 Protein-based CSPs

Proteins are naturally-occurring polymers composed of α-amino acids as the monomeric unit and possess the ability to discriminate chiral molecules. It is well known that some enzymes, for example hydrolases, exhibit a high enantiomer selection during catalysis. In 1973, Stewart and Doherty first demonstrated that a protein-based CSP based on bovine serum albumin was useful for enantioseparation in liquid chromatography.[29] Allenmark *et al.* conducted extensive research on protein based-CSPs in the 1980s, and the successful enantioseparation of a variety of chiral compounds was achieved. Today, various protein-based CSPs have been commercialized. Typical examples are bovine serum albumin, human serum albumin, α_1-acid glycoprotein, ovomucoid from chicken egg whites, avidin and cellobiohydrolase I. Because the protein-based CSPs can directly resolve biologically active compounds without derivatization under reversed phase conditions, these CSPs play an indispensable role in the bioanalytical monitoring of a chiral drug in the body. However, the protein-based CSPs are not useful for preparative purposes due to the limited number of recognition sites. The stability as CSPs is also a problem, because some proteins readily change their conformation.

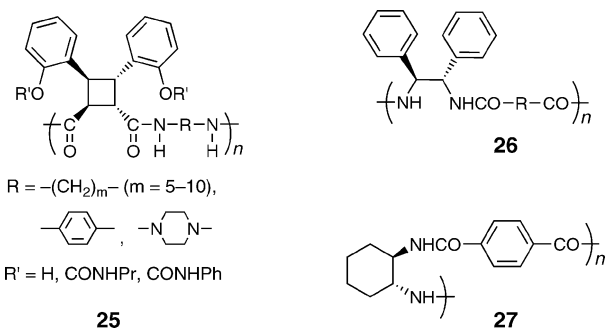


Fig. 10 Structures of polyamide-based CSPs.

4.2.1 Polysaccharide-based CSPs

Fig. 11 shows the proportion of the CSPs for HPLC used for the determination of enantiomeric excess that appeared in the *Journal of the American Chemical Society* in 2005 and 2007.[5] According to these statistics, it can be seen that more than 90% of the determinations by chiral HPLC are performed using the polysaccharide-based CSPs. Furthermore, almost all of the commercial chiral drugs, which are produced by SMB chromatography, seem to be resolved on the polysaccharide-based CSPs.[30] Based on these situations in chiral HPLC, the polysaccharide-based CSPs have been recognized as the most powerful for both analytical and preparative separations.[31]

Polysaccharides, such as cellulose and amylose, are the most abundant on the earth and are optically active. The native polysaccharides themselves can discriminate enantiomers and resolve several racemic compounds by liquid chromatography. However, their recognition abilities are not sufficient to be used practically as CSPs. The first practical CSP derived from polysaccharides was reported by Hesse and Hagel in 1973.[32] They found that the microcrystalline cellulose triacetate **28** (Fig. 12) synthesized under heterogeneous conditions showed a meaningful enantioselectivity, and that the recognition ability is derived from the crystalline structure of cellulose.

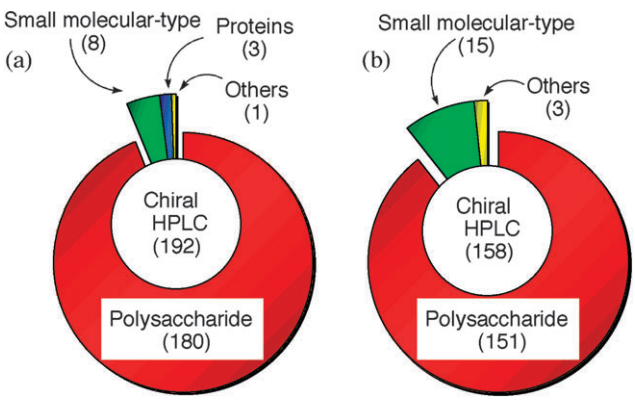


Fig. 11 Distribution of CSPs for HPLC used for the determination of enantiomeric excess that appeared in the *Journal of the American Chemical Society* in 2005 (a) and 2007 (b). The values in parentheses represent the number of the counted papers.

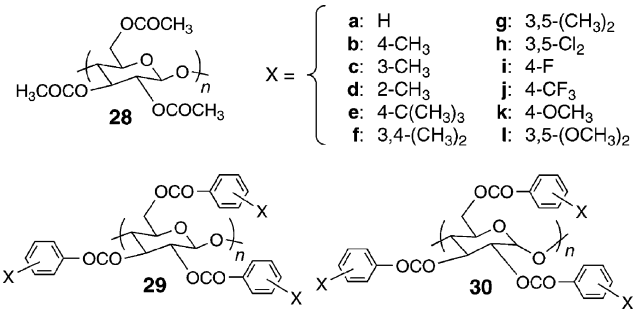


Fig. 12 Structures of ester derivatives of cellulose (**28** and **29**) and amylose (**30**).

Once **28** is dissolved in a solvent, such as dichloromethane, its recognition ability as well as the crystalline structure is completely changed.[33]

Since the 1980s, a large number of cellulose esters have been prepared to evaluate their chiral recognition abilities.[34] Among them, cellulose benzoate and its analogues **29** (Fig. 12) show high chiral recognitions as CSPs for HPLC when they are coated on silica gel. The chiral recognition of the benzoate derivatives is quite dependent on the substituents on the phenyl groups, and especially 4-methylbenzoate **29b** can resolve a broad range of racemates including drugs. Interestingly, the recognition ability of **29b** is dramatically changed by the preparation conditions of the CSPs,[35] such as the coating amount of the derivative and the type and amount of the additives when coated on silica gel. Fig. 13 shows the resolution results of the chrysanthemic acid ethyl ester **31**, which is an intermediate of powerful synthetic pesticides, on the CSPs prepared with or without methyl benzoate as an additive. When a CSP was prepared by coating **29b** in the absence of the additive, the four stereoisomers of **31** were eluted at almost the same time. On the contrary, the (1*R*)-*trans* isomer, which is the most active pesticide form, could be very efficiently separated from the other three isomers on the CSP prepared by coating **29b** on silica gel in the presence of 10 equivalents of methyl benzoate to a glucose unit. Amylose benzoates **30** show a much lower chiral recognition than the cellulose derivatives. This seems to be caused by the lower conformational stability of the amylose derivatives, which allows many conformational isomers to be formed.

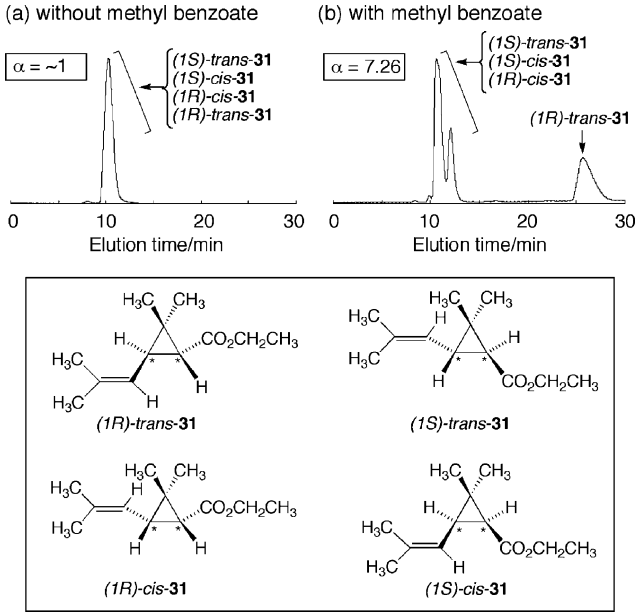


Fig. 13 Chromatograms of chrysanthemic acid ethyl ester (**31**) resolved on CSPs prepared from **29b** without (a) or with (b) methyl benzoate as additive. Asterisks indicate asymmetric carbons. (Reproduced with permission from ref. 35. Copyright 2006, Wiley Periodicals.)

Cellulose phenylcarbamates **32** (Fig. 14) are also useful CSPs for HPLC when coated on silica gel.[36,37] The chiral recognition abilities of these derivatives can be controlled by the nature of the substituents on the phenyl groups. The resolution results of ten racemates **34–43** (Fig. 15) on the nine *para*-substituted phenylcarbamates of cellulose are given in Table 1.[37] The substituents on the phenyl group are displayed in the order of increasing electron-withdrawing power from left to right. The cellulose phenylcarbamates bearing electron-donating substituents, such as alkyl groups, or electron-withdrawing substituents, such as halogens, exhibit higher chiral recognitions than the non-substituted one (**32l**). The substituents seem to influence the polarity of the carbamate group through an inductive effect and change the interaction mode between the cellulose derivatives and the racemates.

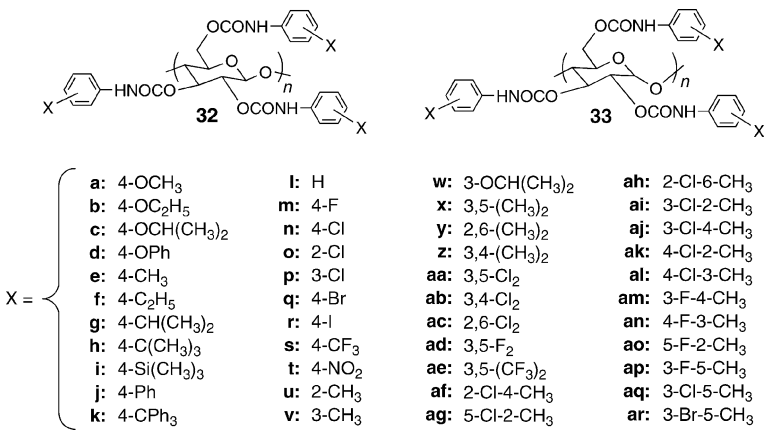


Fig. 14 Structures of phenylcarbamate derivatives of cellulose (**32**) and amylose (**33**).

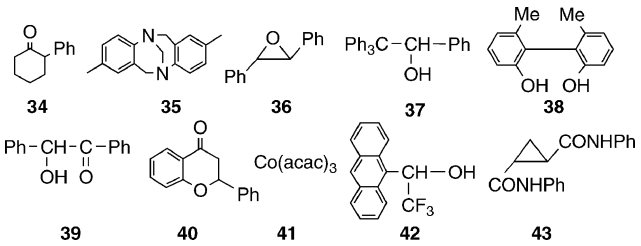


Fig. 15 Structures of racemates.

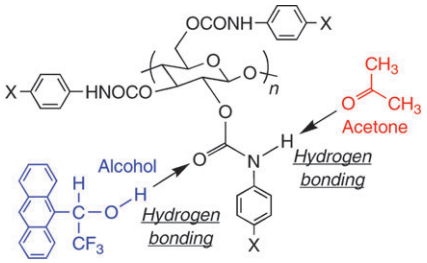


Fig. 16 Possible interaction site of cellulose phenylcarbamates (**32**).

When the electron-donating substituents are introduced on the phenyl groups, the electron density at the carbonyl oxygen of the carbamate groups must be increased, and the retention time of the racemate **42** is increased, because the racemate **42** is mainly adsorbed on the CSPs through hydrogen-bonding interactions with the carbonyl groups (Fig. 16). In contrast, as the electron-withdrawing power of the substituents on the phenyl group becomes stronger, the acidity of the NH proton of the carbamate groups becomes higher. Therefore, acetone is more strongly adsorbed on the CSPs through hydrogen-bonding with the NH groups (Fig. 16). This explanation is supported by the fact that the NH proton resonances of the phenylcarbamates in the ^{1}H NMR spectra shift downfield with an increase in the electron-withdrawing power of the substituents. On the other hand, the derivatives bearing more polar substituents on the phenyl groups, such as methoxy (**32a**) or nitro (**32t**) groups, exhibit low or almost no chiral recognition. These polar groups themselves, which are located far from a chiral glucose unit, may non-enantioselectively interact with the racemates. Therefore, polar substituents should not be introduced on the phenyl group to improve the chiral recognition of cellulose phenylcarbamates.

The chiral recognition on the phenylcarbamate derivatives is also influenced by the position of the substituents.[37] When a methyl group or halogen is introduced at an *ortho* position, the derivatives show a very low recognition ability. Most phenylcarbamate derivatives having a high enantioselectivity form a lyotropic liquid crystalline phase in a highly concentrated solution. However, the *ortho*-substituted derivatives do not show such a liquid crystallinity. This means that the *ortho*-substituted derivatives may not possess a regular higher-order structure, which is extremely important for efficient chiral recognition on polymer-type CSPs.

In contrast to the cellulose derivatives, the 5-chloro-2-methyl- (**33ag**) and 5-fluoro-2-methylphenylcarbamates (**33ao**) of amylose (Fig. 14), which include *ortho* substituents on the phenyl group, show a relatively high chiral recognition.[38] Possible structures of the phenylcarbamates of cellulose and amylose are the left-handed 3/2[39] and 4/3[40] helical chain conformations, respectively. The difference in their higher order structures may be ascribed to the difference in the substituent effect on their chiral recognition.

Among the various phenylcarbamate derivatives, the 3,5-dimethylphenylcarbamates of cellulose (**32x**) and amylose (**33x**) are the most attractive, and can resolve a wide range of racemates. The optimized structures of **32x**[41] and **33x**[42] are shown in Fig. 17. These two 3,5-dimethylphenylcarbamate derivatives are rather complementary in chiral recognition.

The benzoylcarbamates of cellulose (**44**) and amylose (**45**) bearing one more carbonyl group between the carbamate and phenyl groups (Fig. 18), exhibit characteristic chiral recognition abilities, which are different from those of the phenylcarbamates.[43] These derivatives can specifically resolve racemates, such as **38**, **39**, **42** and **43**, capable of hydrogen bonding interactions with the carbonyl groups of the derivatives.

Besides the benzoates and phenylcarbamates, several benzylcarbamates, such as the 1-phenylethyl- (**46a** and **47a**) and 1-phenylpropylcarbamates (**46b** and **47b**) of the polysaccharides, also exhibit high chiral recognition abilities (Fig. 19).[44] Particularly, the amylose (*S*)-1-phenylethylcarbamate (*S*)-**47a** has an

Table 1 Separation factors (α) on the *para*-substituted phenylcarbamates of cellulose[a]

Racemates	OCH$_3$ **32a**	CH$_3$ **32e**	C$_2$H$_5$ **32f**	**32l**	F **32m**	Cl **32n**	Br **32q**	CF$_3$ **32s**	NO$_2$ **32t**
34	1.13 (−)	1.20 (−)	1.19 (−)	1.17 (−)	1.12 (−)	1.16 (−)	1.17 (−)	1.18 (−)	~1 (−)
35	~1 (+)	1.48 (+)	1.11 (+)	1.37 (+)	1.14 (+)	1.16 (+)	1.19 (+)	1.23 (+)	~1 (−)
36	1.34 (+)	1.55 (+)	1.55 (+)	1.46 (+)	1.38 (+)	1.68 (+)	1.70 (+)	1.61 (+)	1.33 (+)
37	1.00	1.37 (+)	1.59 (+)	1.22 (+)	1.64 (+)	1.95 (+)	1.95 (+)	1.48 (+)	1.00
38	1.15 (−)	1.30 (−)	1.33 (−)	1.65 (−)	1.17 (−)	1.20 (−)	1.21 (−)	2.04 (−)	~1 (+)
39	~1 (+)	1.12 (−)	1.14 (−)	~1 (+)	1.14 (−)	1.20 (−)	1.13 (−)	1.10 (−)	1.00
40	~1 (+)	1.16 (+)	1.22 (−)	1.10 (+)	1.13 (+)	1.12 (+)	1.13 (+)	1.14 (+)	1.00
41	~1 (+)	1.75 (+)	1.76 (+)	1.24 (+)	1.53 (+)	1.46 (+)	1.79 (+)	2.06 (+)	~1 (+)
42	1.35 (−)	1.52 (−)	1.57 (−)	1.45 (−)	1.26 (−)	1.29 (−)	1.29 (−)	1.30 (−)	~1 (+)
43	1.00	1.35 (−)	2.12 (−)	1.45 (−)	~1 (−)	1.44 (−)	1.17 (−)	1.22 (−)	~1 (+)

[a] Column: 25 × 0.46 cm (i.d.). Flow rate: 0.5 ml min^{-1}. Eluent: hexane–2-propanol (90 : 10). The signs in parentheses represent the optical rotation of the first-eluted enantiomer.

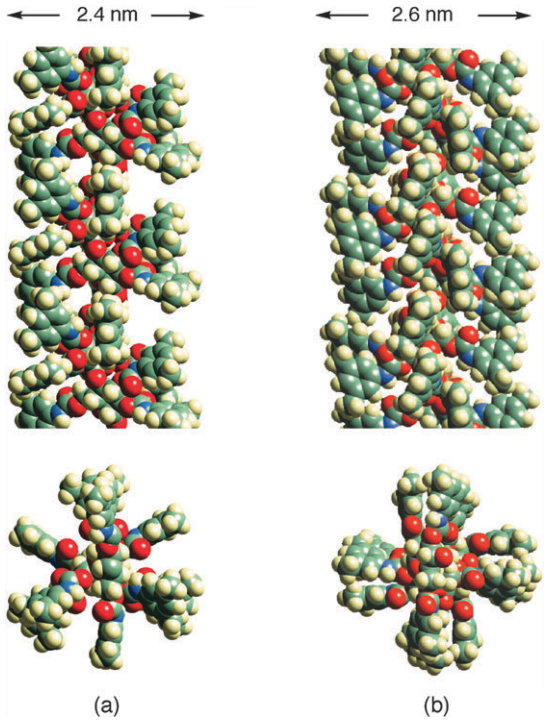


Fig. 17 Optimized structures of 3,5-dimethylphenylcarbamates of cellulose **32x** (a) and amylose **33x** (b). Along (top) and perpendicular (bottom) to the helix axis. (Reproduced with permissions from ref. 41 and ref. 42. Copyright 1999, The Chemical Society of Japan and Copyright 2002, American Chemical Society.)

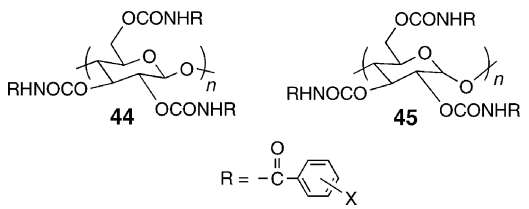


Fig. 18 Structures of benzoylcarbamates of cellulose (**44**) and amylose (**45**).

excellent chiral recognition. Interestingly, the bulkiness of the benzyl moiety significantly influences their chiral recognition. For example, a small group, like benzyl (**46c** and **47c**), and bulky groups, like 2-methyl-1-phenylpropyl (**46d** and **47d**) and diphenylmethyl (**46e** and **47e**), reduce the chiral recognition. This may be because these too-small or too-bulky groups disturb the regular higher-order structure of the derivatives, which is important for efficient chiral recognition.

Although alkylcarbamates, such as the methyl (**48a**) and isopropyl (**48b**) derivatives of cellulose, have a poor chiral recognition ability, some cycloalkylcarbamates, including the cyclohexylcarbamates of cellulose and amylose (**48c** and **49c**), the cyclopentylcarbamate of cellulose (**48d**) and the *exo*-norbornylcarbamate of amylose (**49e**), exhibit high recognitions comparable to the commercial polysaccharide-based CSPs (Fig. 20).[45,46] These cycloalkylcarbamates can also be applied to the CSPs for thin-layer chromatography (TLC) as well as HPLC, because these cycloalkylcarbamates have no

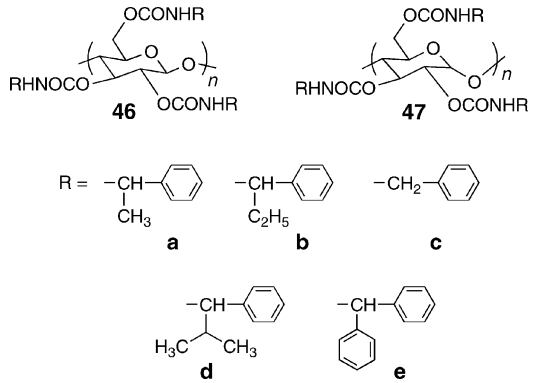


Fig. 19 Structures of benzylcarbamates of cellulose (**46**) and amylose (**47**).

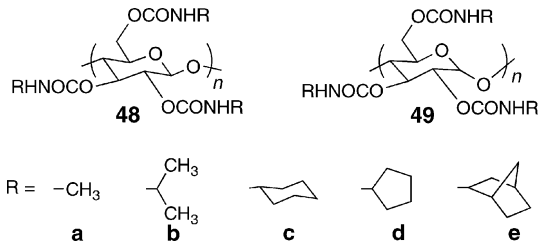


Fig. 20 Structures of cycloalkylcarbamates of cellulose (**48**) and amylose (**49**).

aromatic groups, which disturb simple detection by UV radiation.[45] The resolution results for **42**, **35** and **50** using amylose cyclohexylcarbamate as a CSP for TLC are shown in Fig. 21. The TLC chromatograms were readily detected by UV radiation at 254 nm and showed two spots due to the enantiomers. Because a good correlation was observed between the resolutions by HPLC and TLC, the cycloalkylcarbamate-based CSPs for TLC are expected to be very useful for the rapid setup of the separation conditions for HPLC.

Other phenylcarbamates of the polysaccharides, such as chitin, chitosan, galactosamin, xylan, curdlan, dextran and inulin, have been also prepared and used as the CSPs for HPLC.[47] Their recognition abilities were significantly influenced by the nature of the monomeric units, linkage positions and anomeric configurations. Among them, the 3,5-dimethyl- (**51a**), 3,5-dichloro- (**51b**), 4-chloro- (**51c**) and 4-trifluoromethylphenylcarbamates (**51d**) of chitin[48] and the 3,5-dimethyl- (**52a**), 3,5-dichloro- (**52b**) and 3,4-dichlorophenylcarbamate-ureas (**52e**) of the completely deacetylated chitosan[49] exhibited relatively high recognition abilities (Fig. 22) and could more efficiently resolve some racemates than the corresponding cellulose and amylose derivatives. Especially, some chiral acidic drugs, such as ketoprofen (**53**) and ibuprofen (**54**) (Fig. 23), are efficiently resolved on chitin 3,6-bis(3,5-dichlorophenylcarbamate) (**51b**).

These polysaccharide based CSPs can also be used for supercritical fluid chromatography (SFC). Supercritical fluids possess lower viscosities and higher solute diffusion coefficients than liquids. These properties of supercritical fluids provide the following advantages for chromatographic separations: a high resolution, low pressure drop, rapid column

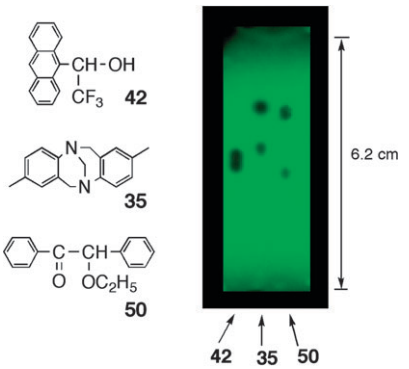


Fig. 21 Enantioseparation of racemates on amylose cyclohexylcarbamate **49c** as CSP for TLC. Eluent, hexane–2-propanol (90 : 10). (Reproduced with permission from ref. 45. Copyright 2000, American Chemical Society.)

equilibration and fast method development. In addition, SFC is particularly important for preparative separations because of a lower solvent consumption and an easier solvent removal compared to HPLC. Since the first chiral separation on the polysaccharide based CSPs in SFC was achieved in 1989,[50] hundreds of successful separations have been demonstrated for a wide variety of racemates. 3,5-Dimethylphenylcarbamates of cellulose (**32x**) and amylose (**33x**) seem to be the most powerful CSPs for chiral separation in SFC as well as HPLC.

4.2.2 Immobilization of polysaccharide derivatives onto silica gel

Although the polysaccharide-based CSPs are very valuable for the efficient resolution of chiral compounds, they have a fatal drawback concerning the solvent selection for mobile phases. Several common organic solvents, such as chloroform, dichloromethane, tetrahydrofuran (THF), ethyl acetate, dioxane, acetone and toluene, cannot be used as mobile phases for these CSPs, because these solvents cause a dissolution or swelling of the polysaccharide derivatives coated on the silica gel and damage the chiral packing materials (CPMs).

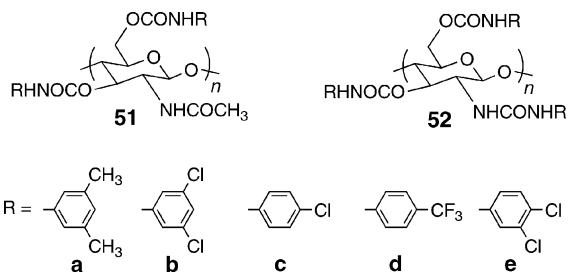


Fig. 22 Structures of phenylcarbamates of chitin (**51**) and phenylcarbamate-ureas of chitosan (**52**).

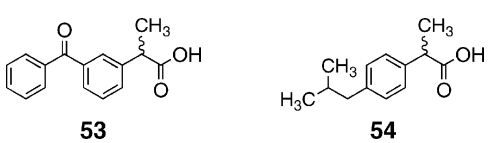


Fig. 23 Structures of ketoprofen (**53**) and ibuprofen (**54**).

Therefore, the above coated-type CPMs based on the polysaccharide derivatives can be used with only restricted kinds of solvents as the mobile phase components. Typical eluents are the hexane–alcohol mixtures for normal-phase chromatography and water–acetonitrile mixtures for reversed-phase chromatography. The expansion of the mobile phase selection opens up the possibility to improve the performance for both the analytical and preparative resolutions. For the analytical resolutions, a refinement of the chiral recognition ability and a reversed elution order of enantiomers might be achieved using the above prohibited solvents.[51] In addition, the selection of a mobile phase with a good sample solubility is absolutely imperative for a high-throughput preparative separation.[52] Therefore, the development of the polysaccharide-based CPMs with a universal solvent compatibility is strongly required.

This requirement has been realized by the immobilization of the polysaccharide derivatives onto silica gel. Over the past two decades, several immobilization methods for the polysaccharide derivatives have been proposed,[53] *i.e.*, the immobilization of the derivatives bearing hydroxy groups with a diisocyanate (a), the chemical bonding of an amylose derivative at an activated chain end (b), the photochemical cross-linking (c), the immobilization of the derivatives bearing vinyl groups with or without a vinyl monomer by radical polymerization (d) and the immobilization of the derivatives bearing alkoxysilyl groups *via* intermolecular polycondensation (e). Among these methods, the immobilization *via* intermolecular polycondensation (method (e))[54] seems to be more valuable because of the simple processing, wide applicability to various polysaccharide derivatives and high immobilization efficiency.

Fig. 24 shows the immobilization *via* the intermolecular polycondensation of triethoxysilyl groups.[54] The 3,5-dimethylphenylcarbamates of cellulose (**55**) and amylose (**56**) bearing 2% and 1% 3-(triethoxysilyl)propyl residues, respectively, were synthesized by the sequential additions of 3,5-dimethylphenyl isocyanate and 3-(triethoxysilyl)propyl isocyanate (Fig. 25). The introduction of the 3-(triethoxysilyl)propyl groups could be identified from the ^{1}H NMR spectra of the obtained polysaccharide derivatives. The derivatives **55** and **56** dissolved in THF and pyridine, respectively, were coated on plain silica gel. The **55** and **56**-coated silica gels were dispersed into a mixture of ethanol, water and trimethylsilyl chloride, and then heated for 10 min at 110 °C. During the reaction, 89% and 86% of the cellulose and amylose derivatives, respectively, could be immobilized through the intermolecular polycondensation of the triethoxysilyl groups. The immobilization efficiency was determined by thermogravimetric (TG) analysis.

The recognition abilities of the immobilized-type CPM-**1** based on the cellulose derivative **55** were evaluated using racemates **34–43**. The resolution results on the CPM-**1** are summarized in Table 2 together with the results on the coated-type CPM-**2**, which is prepared by coating the cellulose derivative **32x** on silica gel. Under the standard chromatographic conditions using an eluent consisting of a hexane–2-propanol mixture, the immobilized-type CPM-**1** showed a chiral recognition similar to the coated-type CPM-**2**. This result suggests that the higher order structure of **55** seems to

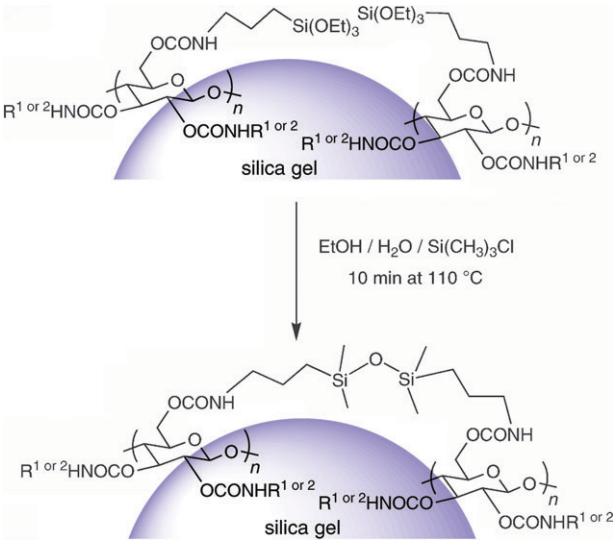


Fig. 24 Scheme of immobilization of polysaccharide derivative *via* intermolecular polycondensation of triethoxysilyl groups.

be very close to that of **32x** coated on the silica gel because of a low degree of cross-linking using only 2% 3-(triethoxysilyl)-propyl group. In addition, eluents containing any proportion of chloroform and THF, which are prohibited for use with the coated-type CPM, can be used for the immobilized-type CPM without reducing its performance. The chiral recognition ability on CPM-**1** was improved for most racemates using eluents containing chloroform and THF. The resolution results on the commercial Chiralpak IB, which is prepared by the immobilization of cellulose 3,5-dimethylphenylcarbamate onto silica gel, are also shown in Table 2. Although the elution orders for all racemates are the same on the two immobilized-type CPMs using a hexane–2-propanol (90 : 10) mixture as the eluent, the chiral recognition abilities on CPM-**1** and Chiralpak IB are slightly different depending on the racemates.

The resolution results on the immobilized-type CPM-**3** based on the amylose derivative **56** are shown in Table 3 together with the data for the coated-type CPM-**4** prepared from the amylose derivative **33x**. As for the cellulose derivatives, most racemates can be better resolved on the immobilized-type CPM-**3** than on the coated-type CPM-**4** by selecting suitable eluents. Compared to the commercial Chiralpak IA containing the immobilized amylose 3,5-dimethylphenylcarbamate, the immobilized-type CPM-**3** exhibited a similar or higher chiral recognition for most racemates (Table 3).

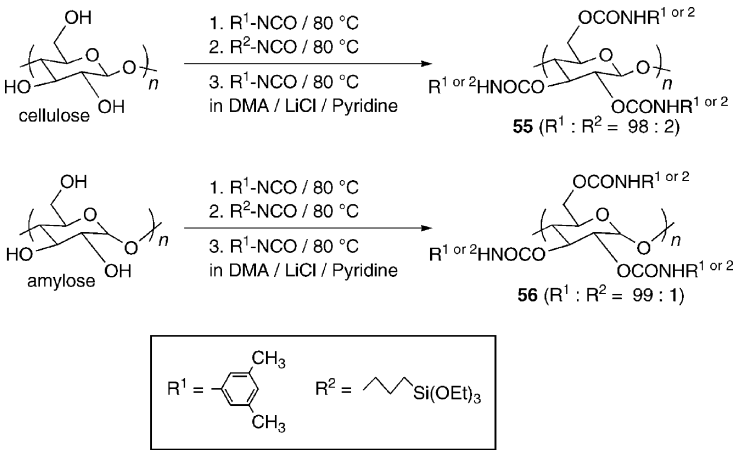


Fig. 25 Synthesis of 3,5-dimethylphenylcarbamates of cellulose (**55**) and amylose (**56**) bearing triethoxysilyl groups.

Table 2 Separation factors (α) on the immobilized-type CPM-**1**, coated-type CPM-**2** and Chiralpak IB[a]

	Immobilized-type CPM-**1**					Coated-type CPM-**2**	Chiralpak IB[b]
Eluents	H–I (90 : 10)	H–C–I (90 : 10 : 1)	H–T–I (90 : 10 : 1)	H–C (70 : 30)	H–T (70 : 30)	H–I (90 : 10)	H–I (90 : 10)
34	1.23 (−)	1.27 (−)	1.19 (−)	1.29 (−)	1.17 (−)	1.17 (−)	1.14 (−)
35	1.53 (+)	1.35 (+)	1.36 (+)	1.06 (+)	1.36 (+)	1.31 (+)	1.22 (+)
36	1.51 (−)	2.12 (−)	1.0	1.93 (−)	~1 (+)	1.96 (−)	1.77 (−)
37	1.15 (+)	1.12 (+)	1.28 (+)	1.16 (+)	~1 (+)	1.12 (+)	1.22 (+)
38	3.52 (−)	1.80 (−)	1.0	1.10 (+)	1.0	2.40 (−)	2.72 (−)
39	1.35 (+)	1.40 (+)	1.35 (+)	—	1.23 (+)	1.50 (+)	1.33 (+)
40	1.21 (−)	1.25 (−)	1.28 (−)	1.09 (−)	1.0	1.34 (−)	1.26 (−)
41	~1 (+)	1.0	1.08 (+)	1.0	1.0	~1 (+)	~1 (+)
42	2.35 (−)	2.93 (−)	2.59 (−)	2.90 (−)	1.56 (−)	2.77 (−)	2.42 (−)
43	1.65 (+)	1.69 (+)	1.60 (+)	—	1.18 (+)	1.99 (+)	1.89 (+)

[a] Column: 25 × 0.20 cm (i.d.). Flow rate: 0.1 ml min^{-1}. Eluent: H: hexane, I: 2-propanol, C: chloroform, T: tetrahydrofuran. The signs in parentheses represent the optical rotation of the first-eluted enantiomer. [b] Column: 25 × 0.46 cm (i.d.). Flow rate: 0.5 ml min^{-1}.

Table 3 Separation factors (α) on the immobilized-type CPM-**3**, coated-type CPM-**4** and Chiralpak IA[a]

	Immobilized-type CPM-**3**					Coated-type CPM-**4**	Chiralpak IA[b]
Eluents	H–I (90 : 10)	H–C–I (90 : 10 : 1)	H–T–I (90 : 10 : 1)	H–C (70 : 30)	H–T (70 : 30)	H–I (90 : 10)	H–I (90 : 10)
34	~1 (−)	1.0	1.0	~1 (+)	1.0	~1 (−)	1.06 (−)
35	1.44 (+)	1.48 (+)	1.67 (+)	~1 (+)	1.24 (+)	1.60 (+)	1.46 (+)
36	2.83 (+)	2.98 (+)	2.83 (+)	2.24 (+)	2.06 (+)	3.33 (+)	2.71 (+)
37	2.11 (+)	1.49 (+)	1.54 (+)	—	1.62 (+)	2.02 (+)	2.07 (+)
38	2.27 (−)	2.26 (−)	2.07 (−)	1.74 (−)	1.36 (−)	2.21 (−)	2.15 (−)
39	1.18 (−)	1.14 (−)	1.13 (+)	1.0	1.48 (+)	1.31 (−)	1.15 (−)
40	1.08 (+)	1.63 (+)	1.31 (+)	1.33 (+)	1.0	~1 (+)	1.17 (+)
41	1.0	1.0	1.0	1.0	1.0	1.0	~1 (−)
42	1.0	1.38 (−)	1.0	1.68 (−)	1.0	1.36 (+)	~1 (+)
43	3.49 (+)	—	—	1.72 (−)	1.0	2.54 (+)	2.06 (+)

[a] Column: 25 × 0.20 cm (i.d.). Flow rate: 0.1 ml min^{-1}. Eluent: H: hexane, I: 2-propanol, C: chloroform, T: tetrahydrofuran. The signs in parentheses represent the optical rotation of the first-eluted enantiomer. [b] Column: 25 × 0.46 cm (i.d.). Flow rate: 0.5 ml min^{-1}.

Today, the commercial immobilized-type CPMs based on polysaccharide derivatives have been widely used for both the analytical and preparative resolutions. For the analytical use, more efficient separations of chiral compounds, which cannot be resolved on the coated-type CPMs, have been attained on the immobilized-type CPMs by selecting a suitable eluent. Meanwhile, the effect of sample solubility in preparative separation of a Ca-sensitizing drug has been investigated using various kinds of solvents.[55] The use of eluents with high solubility, such as dichloromethane and THF, makes it possible to produce the pure enantiomers with high productivity. In the future, the application of the immobilized-type CPMs with a universal solvent compatibility is expected to be more common in all fields of science and technology dealing with chiral compounds.

4.2.3 Polysaccharide-based CPMs for preparative separation

In a large-scale preparative separation, SMB chromatography provides a higher productivity compared to the conventional batch-wise chromatography due to the efficient use of the stationary and mobile phases. The SMB technique is based on a quasi counter-current contact between the stationary and mobile phases mimicking a true moving bed. This is accomplished by the periodic movement of inlet and outlet ports in the same direction to fluid flow. This SMB technique is especially valuable for a binary separation. Therefore, large-scale separations of chiral compounds, which include just binary components consisting of two enantiomers, have been performed using the SMB system since the late 1990s.

The polysaccharide-based CPMs have been frequently used for large-scale separations by SMB chromatography[30] due to their higher loading capacity compared to the other CPMs. However, the current polysaccharide-based CPMs are not the best for large-scale separations, because the CPMs have been prepared by coating or immobilizing the polysaccharide derivatives by *ca.* 20 wt% on silica gel and the major part (80 wt%) of the CPMs is silica gel inactive for chiral recognition. Although the polysaccharide content in the CPMs is requested to be high in order to perform an efficient preparative separation, it cannot be increased by the conventional method without reducing the performance. In order to increase the loading capacity of the CPMs, the bead-type CPMs,[56,57] which are prepared from only polysaccharide derivatives without silica gel, have been prepared and showed a higher loading capacity than the conventional coated-type CPMs. However, the bead-type CPMs suffered from low mechanical resistance against a high pressure due to the absence of rigid inorganic supports. Recently, we developed the organic–inorganic hybrid bead-type CPM for preparative separation, which was prepared from a polysaccharide derivative bearing a small amount of 3-(triethoxysilyl)propyl residues and tetraethyl orthosilicate (TEOS) by a sol–gel reaction.[58]

Fig. 26 shows the preparation scheme and scanning electron microscope (SEM) image of the hybrid bead-type CPM-**5**. The cellulose derivative **55**, TEOS, H_2O and trimethylsilyl chloride were dissolved in a THF and 1-heptanol mixture, and then heated for 9 h at 80 °C. The pretreated solution was then dropwise added to an aqueous solution of sodium lauryl sulfate with mechanical stirring at 1100 rpm. After the sol–gel reaction for 1 h at 80 °C, the hybrid bead was isolated by filtration. The spherical hybrid beads with a mean particle size of less than 20 μm were obtained (Fig. 26). The organic and inorganic contents in the obtained hybrid bead-type CPM-**5** were estimated to be 69 wt% and 31 wt%, respectively, by TG analysis. From the surface analysis of the hybrid bead-type CPM-**5** by energy-dispersive X-ray mapping, it was found that both the organic and inorganic component were well-dispersed at least on a micrometre scale on the surface of the hybrid bead-type CPM-**5**.

The hybrid bead-type CPM-**5** was packed into an HPLC column at a pressure of 400 kg cm^{-2} without any deformation of its spherical shape. This result is different from the previous bead-type CPMs, which are prepared from only polysaccharide derivatives and cannot be applied at high pressure. The inorganic component in the hybrid bead seems to play an important role as a support and confers a sufficient mechanical resistance to the CPM.

In order to demonstrate the potential of the hybrid bead-type CPM-**5** for preparative separation, the loading capacities on the hybrid bead-type CPM-**5** and Chiralpak IB were compared using 2,2,2-trifluoro-1-(anthryl)ethanol (**42**) as a racemate. The results of the preparative separation of **42** on

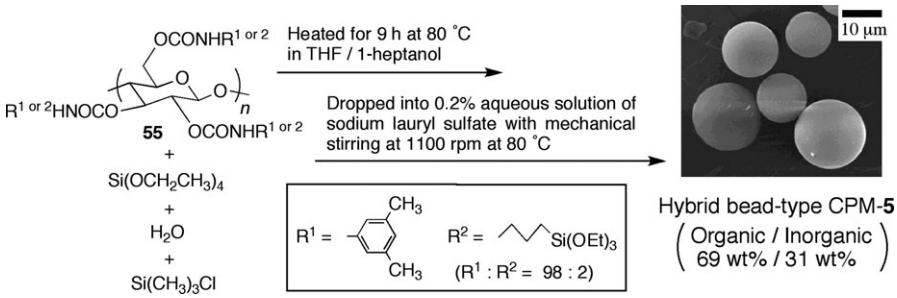


Fig. 26 Scheme of preparation and SEM image of the hybrid bead-type CPM-**5**. (Reproduced with permission from ref. 58. Copyright 2008, Wiley VCH.)

the hybrid bead-type CPM-**5** are shown in Fig. 27. Although the two peaks of the individual enantiomers became closer with an increase in the loading sample from 20 mg to 50 mg, the hybrid bead-type CPM-**5** could readily separate 50 mg of the racemate (Fig. 27a). On the other hand, the Chiralpak IB showed one overlapped peak for the 50 mg loading (Fig. 27b). Under the same flow rate of 1.0 ml min^{-1}, however, the amount of the pure enantiomers obtained per unit time on the hybrid bead-type CPM-**5** did not increase due to the longer retention time compared to the Chiralpak IB. Therefore, the retention time on the hybrid bead-type CPM-**5** was adjusted to that on the Chiralpak IB by changing the flow rate (Fig. 27c). These chromatograms clearly indicate that a higher throughput resolution of a chiral compound can be realized using the hybrid bead-type CPM-**5** *versus* the Chiralpak IB.

4.2.4 Chiral recognition mechanism on polysaccharide derivatives

In contrast to brush type-CSPs, the understanding of the chiral recognition mechanism on polymer-based CSPs is much more difficult, because their chiral recognition usually depends on their higher-order structure. Several approaches have been carried out to clarify the chiral discrimination mechanism of the polysaccharide derivatives.

NMR spectroscopy is one of the most powerful tools for the elucidation of the chiral recognition mechanism at a molecular level. Most carbamate derivatives with high recognition abilities are only soluble in polar organic solvents, such as acetone, THF, pyridine and dimethyl sulfoxide, which strongly interact with the polar carbamate groups of the polysaccharide derivatives. In these polar solvents, the derivatives cannot sufficiently interact with enantiomers for efficient recognition. Therefore, it is difficult to clarify the chiral discrimination mechanism of most carbamate derivatives by NMR spectroscopy.

However, it was found that several carbamate derivatives, such as 4-trimethylsilylphenylcarbamate, 5-fluoro-2-methylphenylcarbamate, 3,5-dichlorophenylcarbamate and cyclohexylcarbamate, are soluble in chloroform and can discriminate enantiomers by NMR spectroscopy as well as by HPLC.

Fig. 28 shows the 400 MHz ^{1}H NMR spectra of (±)-1,1′-bi-2-naphthol (**57**) in the absence (A) and presence (B) of cellulose cyclohexylcarbamate **48c** in $CDCl_3$.[46] The hydroxy and naphthyl (H4) protons of the enantiomers of **57** were apparently separated into two sets of peaks in the presence of **48c**. This clearly indicates that **48c** can recognize the enantiomers even in solution. The hydroxy proton of (−)-**57** was more significantly downfield shifted along with line broadening than that of (+)-**57**. This downfield shift is ascribed to the hydrogen-bonding between the hydroxy group of (−)-**57** and the carbamate group of the cellulose derivative. On the other hand, the naphthyl proton (H4 and H6) resonances exhibited small upfield shifts probably due to the π–π interaction with the carbonyl group of **48c**. The H4 closer to the OH group shows the splitting due to enantiomers, and again (−)-**57** exhibits a larger shift with broadening.

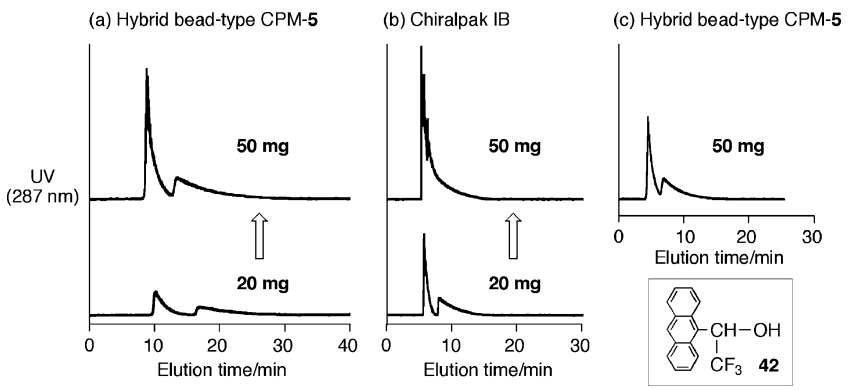


Fig. 27 Preparative separation of *rac*-2,2,2-trifluoro-1-(anthryl)ethanol (**42**) with hexane–2-propanol (90 : 10) as eluent: (a) hybrid bead-type CPM-**5** at flow rate of 1.0 ml min^{-1}; (b) Chiralpak IB at flow rate of 1.0 ml min^{-1}; (c) hybrid bead-type CPM-**5** at flow rate of 2.0 ml min^{-1}. (Reproduced with permission from ref. 58. Copyright 2008, Wiley VCH.)

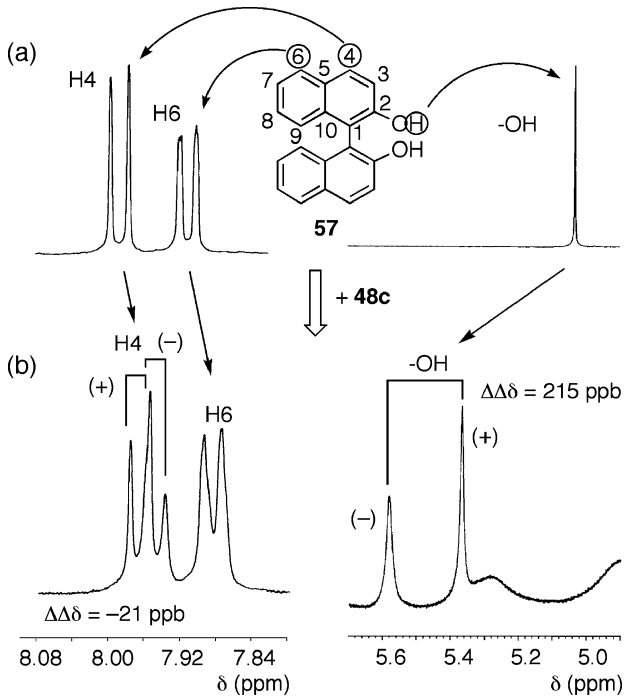


Fig. 28 ^{1}H NMR spectra of selected regions of (±)-**57** in the absence (a) and presence (b) of **48c** in $CDCl_3$. (Reproduced with permission from ref. 46. Copyright 2002, Wiley Liss.)

In the HPLC resolution of (±)-**57** on **48c**, the (+)-isomer is first eluted followed by the (−)-isomer, and baseline separation is achieved (α = 1.14).[46] This means that (−)-**57** interacts more strongly with **48c**. The elution order in HPLC may be related to the larger shifts in the (−)-isomer observed in the ^{1}H NMR.

The results of the chiral discrimination of the other racemates by ^{1}H NMR spectroscopy using **48c** are summarized in Fig. 29.[46] Although the enantiomers **35**, **36** and **40**, which were not resolved or were less retained on **48c** in the HPLC, could not be discriminated by ^{1}H NMR spectroscopy, several proton resonances of enantiomers **37**, **38**, **39** and **42** were separated into two sets of peaks in the presence of **48c**.

The discrimination of enantiomers can also be observed by ^{13}C NMR spectroscopy.[46] Fig. 30 shows that the resonances of

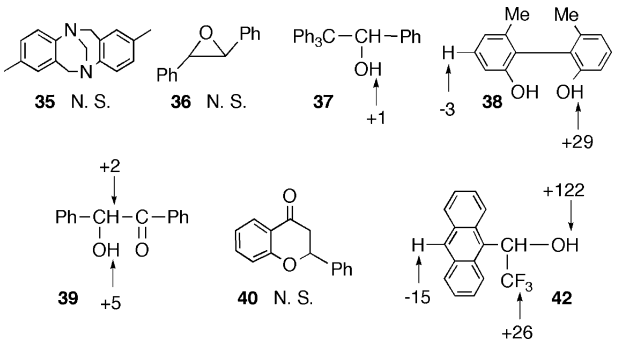


Fig. 29 Compounds enantiomerically recognized by **48c** in ^{1}H and ^{19}F NMR spectroscopy. The protons and fluorine marked with an arrow indicate the recognized ones and figures represent $\Delta\Delta\delta$ (ppb). Negative values indicate upfield shift. N. S.: not separated.

the C1, C2, C4, C7, C8 and C10 carbons of **57** are separated into enantiomers in the presence of **48c** and the (−)-**57** carbon resonances are clearly broadened, as also seen in the ^{1}H NMR spectrum.

More valuable information on the binding geometry and dynamics between the polysaccharides and the enantiomers can be obtained from the spin–lattice relaxation time, ^{1}H NMR titration and intermolecular NOEs.[46,59] Fig. 31 shows the NOESY spectra of **48c**–(−)-**57** (a) and **48c**–(+)-**57** (b) in the region related to the aromatic protons of **57** and the glucose and cyclohexyl methine protons of **48c**.[46] Clear intermolecular NOE cross peaks could be observed between the glucose protons of **48c** and H3, H4 and H9 protons of (−)-**57** (Fig. 31a). On the other hand, the mixture of **48c** and (+)-**57** exhibited no intermolecular NOE cross peaks except for the hydroxy group (Fig. 31b), probably due to a weak interaction. These results indicate that (−)-**57** more strongly binds or interacts with **48c** than (+)-**57**, and the naphthyl protons of (−)-**57** are closely located by the glucose proton of **48c** within less than 5 Å.

A computer simulation, such as molecular mechanics (MM) and molecular dynamics (MD) calculations, is also a useful and effective approach for the qualitative understanding of the chiral recognition on the polysaccharide-based CSPs, for the prediction of the chromatographic behavior and for the design of excellent CSPs.

The interaction energies between the phenylcarbamate (**32l**) or 3,5-dimethylphenylcarbamate (**32x**) of cellulose and *trans*-stilbene oxide (**36**) or benzoin (**39**) were calculated using various force fields and methods.[41] The calculation methods were roughly divided into two types, which apply different methods for enantiomer generation: (1) enantiomers were generated around the NH proton and the carbonyl oxygen of the carbamate group of **32l** and **32x**, which are the most important adsorption sites, and then the calculation of the interaction energy was performed; (2) enantiomers with a particular orientation were randomly generated by the Monte Carlo method on the surface of **32l** and **32x**, and then the calculation of the interaction energy was performed. Although we will not describe the details here, both calculation methods show an agreement with the results obtained by the HPLC resolution of **36** and **39** on **32l** and **32x**.

Recently, in order to clarify the role of eluents in the resolutions on polysaccharide derivatives, Franses *et al.* investigated the interaction between amylose 3,5-dimethylphenylcarbamate (**33x**) and various organic solvents used as eluents.[60] Particularly, the changes in the hydrogen-bonding state, the crystallinity and the side-chain mobility of **33x** upon absorption of organic solvents have been systematically studied by attenuated total reflection infrared spectroscopy (ATR-IR), X-ray diffraction (XRD), ^{13}C cross-polarization/magic-angle spinning (CP/MAS) and MAS solid-state NMR and density functional theory (DFT) modeling. In addition, the differences in the enantioselective adsorption sites among the cellulose 3,5-dimethylphenylcarbamate (**32x**), amylose 3,5-dimethylphenylcarbamate (**33x**) and amylose (*S*)-1-phenylethylcarbamate ((*S*)-**47a**) have also been investigated by the above analytical methods.

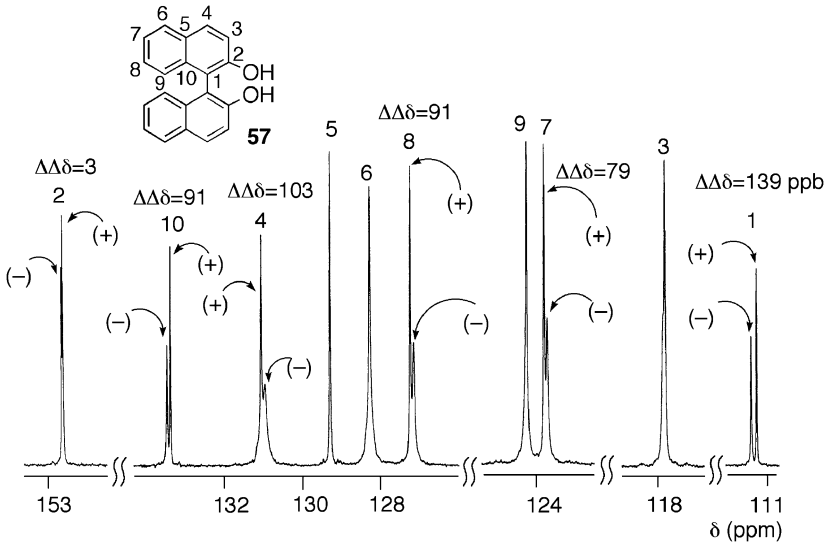


Fig. 30 ^{13}C NMR spectrum of (±)-**57** in the presence of **48c** in $CDCl_3$. (Reproduced with permission from ref. 46. Copyright 2002, Wiley Liss.)

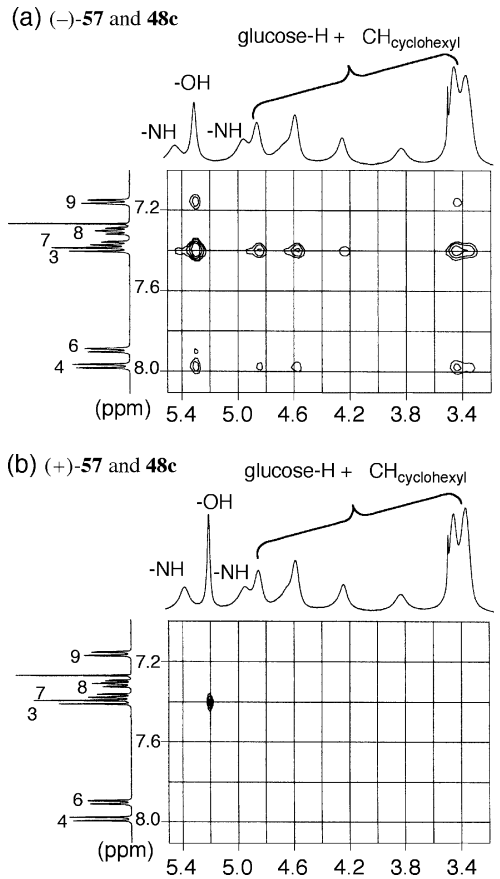


Fig. 31 500 MHz expanded NOESY spectra, at a mixing time of 500 ms, of the mixtures of (−)-**57** and **48c** (a) and (+)-**57** and **48c** (b) in the region between the aromatic protons of **57** and the glucose and cyclohexyl methine protons of **48c** in $CDCl_3$ at 30 °C. (Reproduced with permission from ref. 46. Copyright 2002, Wiley Liss.)

Conclusions

In this review, optically active small molecules and polymers with a chiral recognition ability as the chiral stationary phases for HPLC have been outlined. Today, more than one hundred types of commercially available CSPs have been used for analytical and preparative resolutions around the world. Among them, the polysaccharide-based CSPs have been recognized as the most powerful due to their wide applicability to racemates and high loading capacity. The recent progress in the field of chromatographic resolutions by HPLC, which includes the development of efficient chiral selectors and new types of chiral packing materials and the understandings of chiral recognition mechanisms, will help to produce advances not only in new drugs, but also in various fields of science related to chirality.

Acknowledgements

This work has been supported by Grants from the Ministry of Education, Culture, Sports, Science and Technology and Daicel Chemical Company. We thank all the co-workers who significantly contributed to our studies on chiral HPLC.

References

1 G. Blaschke, H. P. Kraft, K. Fickentscher and F. Köhler, *Arzneim.-Forsch.*, 1979, **29**, 1640.
2 H. Caner, E. Groner, L. Levy and I. Agranat, *Drug Discovery Today*, 2004, **9**, 105.
3 L. Pasteur, *C. R. Acad. Sci.*, 1848, **34**, 535.
4 *Chiral Separation Techniques: A Practical Approach*, ed. G. Subramanian, Wiley-VCH, Weinheim, 3rd edn, 2007.
5 X. M. Chen, C. Yamamoto and Y. Okamoto, *Pure Appl. Chem.*, 2007, **79**, 1561.
6 L. S. Pais, V. G. Mata and A. E. Rodrigues, in *Preparative Enantioselective Chromatography*, ed. G. B. Cox, Blackwell Publishing, Oxford, 2005, ch. 7, pp. 176–204.
7 C. Yamamoto, S. Inagaki and Y. Okamoto, *J. Sep. Sci.*, 2006, **29**, 915.
8 V. A. Davankov and S. V. Rogozhin, *J. Chromatogr.*, 1971, **60**, 280.
9 C. Yamamoto and Y. Okamoto, *Bull. Chem. Soc. Jpn.*, 2004, **77**, 227.
10 G. Dotsevi, Y. Sogah and D. J. Cram, *J. Am. Chem. Soc.*, 1975, **97**, 1259.

11 A. Harada, M. Furue and S. I. Nozakura, *J. Polym. Sci., Polym. Chem. Ed.*, 1978, **16**, 189.
12 L. F. He and T. E. Beesley, *J. Liq. Chromatogr. Relat. Technol.*, 2005, **28**, 1075.
13 W. H. Pirkle and D. W. House, *J. Org. Chem.*, 1979, **44**, 1957.
14 F. Gasparrini, D. Misiti and C. Villani, *J. Chromatogr.*, 1988, **457**, 235.
15 G. Uray and W. Lindner, *Chromatographia*, 1990, **30**, 323.
16 D. W. Armstrong, Y. Tang, S. Chen, Y. Zhou, C. Bagwill and J. R. Chen, *Anal. Chem.*, 1994, **66**, 1473.
17 M. Lämmerhofer and W. Lindner, *J. Chromatogr., A*, 1996, **741**, 33.
18 G. Wulff and A. Sarhan, *Angew. Chem., Int. Ed. Engl.*, 1972, **11**, 341.
19 G. Blaschke, *Chem. Ber.*, 1974, **107**, 237.
20 G. Blaschke, *Angew. Chem., Int. Ed. Engl.*, 1980, **19**, 13.
21 K. Morioka, Y. Suito, Y. Isobe, S. Habaue and Y. Okamoto, *J. Polym. Sci., Part A: Polym. Chem.*, 2003, **41**, 3354.
22 Y. Okamoto and K. Hatada, *J. Liq. Chromatogr.*, 1986, **9**, 369.
23 Y. Okamoto, K. Suzuki, K. Ohta, K. Hatada and H. Yuki, *J. Am. Chem. Soc.*, 1979, **101**, 4763.
24 H. Yuki, Y. Okamoto and I. Okamoto, *J. Am. Chem. Soc.*, 1980, **102**, 6356.
25 Y. Okamoto, H. Mohri and K. Hatada, *Polym. J.*, 1989, **21**, 439.
26 Y. Doi, H. Kiniwa, T. Nishikaji and N. Ogata, *J. Chromatogr.*, 1987, **396**, 395.
27 K. Saigo, Y. Chen, N. Yonezawa, K. Tachibana, T. Kanoe and M. Hasegawa, *Chem. Lett.*, 1985, 1891.
28 S. G. Allenmark, S. Andersson, P. Möller and D. Sanchez, *Chirality*, 1995, **7**, 248.
29 K. K. Stewart and R. F. Doherty, *Proc. Natl. Acad. Sci. U. S. A.*, 1973, **70**, 2850.
30 S. Abel and M. Juza, in *Chiral Separation Techniques: A Practical Approach*, ed. G. Subramanian, Wiley-VCH, Weinheim, Germany, 3rd edn, 2007, ch. 7, pp. 203–273.
31 R. W. Stringham, *Adv. Chromatogr.*, 2006, **44**, 257.
32 G. Hesse and R. Hagel, *Chromatographia*, 1973, **6**, 277.
33 Y. Okamoto, M. Kawashima, K. Yamamoto and K. Hatada, *Chem. Lett.*, 1984, 739.
34 Y. Okamoto, R. Aburatani and K. Hatada, *J. Chromatogr.*, 1987, **389**, 95.
35 C. Yamamoto, K. Yamada, K. Motoya, Y. Kamiya, M. Kamigaito, Y. Okamoto and T. Aratani, *J. Polym. Sci., Part A: Polym. Chem.*, 2006, **44**, 5087.
36 Y. Okamoto, M. Kawashima and K. Hatada, *J. Am. Chem. Soc.*, 1984, **106**, 5357.
37 Y. Okamoto, M. Kawashima and K. Hatada, *J. Chromatogr.*, 1986, **363**, 173.
38 B. Chankvetadze, E. Yashima and Y. Okamoto, *J. Chromatogr., A*, 1995, **694**, 101.
39 H. Steinmeier and P. Zugenmaier, *Carbohydr. Res.*, 1987, **164**, 97.
40 U. Vogt and P. Zugenmaier, *Communication at the European Science Foundation Workshop on Specific Interaction in Polysaccharide Systems*, Uppsada, Sweden, 1983.
41 C. Yamamoto, E. Yashima and Y. Okamoto, *Bull. Chem. Soc. Jpn.*, 1999, **72**, 1815.
42 C. Yamamoto, E. Yashima and Y. Okamoto, *J. Am. Chem. Soc.*, 2002, **124**, 12583.
43 T. Ikai, C. Yamamoto, M. Kamigaito and Y. Okamoto, *Chirality*, 2005, **17**, 299.
44 Y. Kaida and Y. Okamoto, *J. Chromatogr.*, 1993, **641**, 267.
45 T. Kubota, C. Yamamoto and Y. Okamoto, *J. Am. Chem. Soc.*, 2000, **122**, 4056.
46 T. Kubota, C. Yamamoto and Y. Okamoto, *Chirality*, 2002, **14**, 372.
47 Y. Okamoto, J. Noguchi and E. Yashima, *React. Funct. Polym.*, 1998, **37**, 183.
48 C. Yamamoto, T. Hayashi and Y. Okamoto, *J. Chromatogr., A*, 2003, **1021**, 83.
49 C. Yamamoto, M. Fujisawa, M. Kamigaito and Y. Okamoto, *Chirality*, 2008, **20**, 288.
50 P. Macaudiere, M. Caude, R. Rosset and A. Tambue, *J. Chromatogr. Sci.*, 1989, **27**, 383.
51 T. Zhang, C. Kientzy, P. Franco, A. Ohnishi, Y. Kagamihara and H. Kurosawa, *J. Chromatogr., A*, 2005, **1075**, 65.
52 E. Francotte, *J. Chromatogr., A*, 2001, **906**, 379.
53 T. Ikai, C. Yamamoto, M. Kamigaito and Y. Okamoto, *Polym. J.*, 2006, **38**, 91.
54 T. Ikai, C. Yamamoto, M. Kamigaito and Y. Okamoto, *J. Chromatogr., A*, 2007, **1157**, 151.
55 T. Zhang, M. Schaeffer and P. Franco, *J. Chromatogr., A*, 2005, **1083**, 96.
56 E. Francotte and R. W. Wolf, *J. Chromatogr., A*, 1992, **595**, 63.
57 T. Ikai, C. Yamamoto, M. Kamigaito and Y. Okamoto, *J. Sep. Sci.*, 2007, **30**, 971.
58 T. Ikai, C. Yamamoto, M. Kamigaito and Y. Okamoto, *Chem.–Asian J.*, 2008, **3**, 1494.
59 E. Yashima, C. Yamamoto and Y. Okamoto, *J. Am. Chem. Soc.*, 1996, **118**, 4036.
60 R. B. Kasat, Y. Zvinevich, H. W. Hillhouse, K. T. Thomson, N. H. L. Wang and E. I. Franses, *J. Phys. Chem. B*, 2006, **110**, 14114.

