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Accounts

Chiral Discrimination on Polysaccharides Derivatives

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Chromatographic enantioseparations, particularly resolution by high-performance liquid chromatography (HPLC), have advanced considerably in the past decade, and have become a practically useful method not only for determining their optical purity, but also for obtaining optical isomers. The preparation of a chiral stationary phase (CSP) capable of effective chiral recognition is the key to this separation technique. We have found that polysaccharide derivatives, particularly cellulose esters and phenylcarbamate derivatives of cellulose and amylose, are very effective CSPs, and can resolve a wide range of racemates, including drugs. A variety of polysaccharides derivatives involving tribenzoates and tris(phenylcarbamates) of cellulose and amylose were prepared and their chiral recognition abilities were evaluated as CSPs in HPLC. The chiral recognition and resolving ability of the derivatives depend greatly on the substituents introduced on the phenyl moieties. The introduction of electron-donating or electron-withdrawing substituents improved the chiral recognition ability. Among the many derivatives of polysaccharides, 3,5-dimethylphenylcarbamates of cellulose and amylose and cellulose tris(4-methylbenzoate) show high chiral recognition, which has allowed the resolution of more than 80% of the racemates tested. These CSPs can be used for both analytical and preparative separations of enantiomers. Some significant mechanistic aspects are also briefly reviewed.

Since optically active compounds have recently aroused wide interest in many fields related to pharmaceuticals, natural products, agrochemicals, and ferroelectric liquid crystals, their preparation and analysis are of increasing importance. Since living systems consist of chiral proteins, nucleic acids, and polysaccharides, living organisms often show quite different physiological behaviors toward one of a pair of enantiomers, especially drugs.$^{1)}$ Therefore, a detailed investigation of the pharmacokinetics, physiological, toxicological, and metabolic activities of both enantiomers of drugs have become necessary for developing chiral drugs; an increasing number of drugs have been marketed as single-isomer forms in the pharmaceutical industry.

Asymmetric synthesis is a fascinating method for obtaining optically active compounds, and the optical resolution of racemates is another way to obtain pure isomers. During the past decade, chromatographic enantioseparations, particularly resolution by high-performance liquid chromatography (HPLC), have advanced considerably and have become practically useful methods for determining optical purity and for obtaining optical isomers. Particularly, in the pharmaceutical industry, chiral HPLC has become essential for the research and development of chiral drugs.$^{2)}$ The preparation of a chiral stationary phase (CSP) capable of effective chiral recognition is the key to this separation technique. Therefore, many CSPs for HPLC have been prepared,$^{3-8)}$ and about 110 CSPs have been commercialized. Large-scale, preparative HPLC systems have already been put on the market as a process for the isolation and purification of chiral drugs and natural products.

There are basically two types of CSPs. One consists of a chiral selector consisting of a chiral small molecule that is usually bound to an achiral support, such as silica gel; the second is based on a chiral polymer which can be used as a porous gel or can be bound to silica gel. The former CSPs have been reviewed.$^{5-9)}$ The latter CSPs include polyacrylamides,$^{10)}$ one-handed helical polymethacrylates,$^{11,12)}$ polyamides,$^{13)}$ proteins,$^{14-16)}$ and polysaccharide derivatives. Here, we review the resolution of enantiomers on CSPs consisting of polysaccharide derivatives, particularly cellulose esters and phenylcarbamate derivatives of cellulose and amylose, which appear to be one of the most useful CSPs.$^{17-20)}$

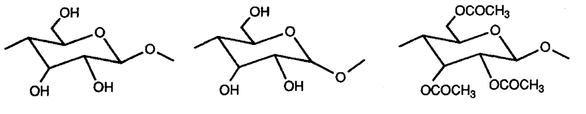
Although the mechanism of chiral discrimination on polysaccharide phases has not yet been satisfactorily elucidated, some interesting approaches to understanding the mechanism have been made using chromatographic, computational, and spectroscopic methods. Mechanistic studies are of importance and interest from the viewpoint of molecular recognition, which will lead to the development of a novel polysaccharide-based CSP. Some significant mechanistic aspects are also briefly reviewed.

Polysaccharide Esters

Polysaccharides, such as cellulose (**1**) and amylose (**2**), are among the most abundant naturally occurring polymers with optical activity. They can be used as a CSP, although their chiral resolving ability is not very high. Kotake et al.[21)] reported on the first enantioseparation of aromatic amino acids by paper chromatography. Later, Dalgliesh extended the work and proposed the three-point rule,[22)] which has often been quoted for explaining the chiral discrimination mechanism.

Although thoroughly purified native cellulose with a high degree of crystallinity (crystal form I; microcrystalline cellulose) possesses an ability of complete resolution of amino acids by liquid chromatography,[23)] the material may not afford a practically useful CSP because of insufficient resolving ability and difficulty in handling. On the other hand, cellulose is easily converted to a variety of derivatives, such as triesters and tricarbamates, by reactions on active hydroxyl groups with corresponding reagents. The resulting derivatized polysaccharide phases offer great merit regarding the chromatographic and enantioselective properties, compared with native cellulose. Presently, more than ten CSPs consisting of polysaccharide derivatives have appeared on the market and have been extensively used for both analytical and preparative separation of enantiomers.[17—20,24)]

The first widely used CSP derived from cellulose is the microcrystalline cellulose triacetate (**3**, CTA-I), which is prepared by the heterogeneous acetylation of native microcrystalline cellulose in benzene (Chart 1). CTA-I is believed to preserve a structure closely related to native cellulose.[25)] The CSP shows interesting chiral resolving properties in liquid chromatography, although the chiral resolution power of partially acetylated cellulose is poor.[26)] Hesse and Hagel have pointed out that the microcrystallinity of CTA-I is essential for chiral recognition, since the resolving ability is substantially reduced, and reversal of the elution order occurs in some cases once the triacetate is dissolved in a solvent.[25)] CTA-I has been employed for the resolution of various racemates, especially aromatic compounds, using an ethanol-water mixture as the eluent on both the analytical and preparative scale.[27—29)] The high loading capacity of CTA-I makes it one of the most usable stationary phases for the large-scale separation of pharmaceuticals.[27)]



1: Cellulose **2: Amylose** **3: Cellulose triacetate**

Chart 1.

The mechanism for chiral recognition on CTA-I has not yet been satisfactorily elucidated, probably because of a difficulty in determining the complex structure of CTA-I; however, an inclusion mechanism has been postulated by which aromatic compounds may be adsorbed in chiral cavities inside the CTA-I matrix.[25,30,31)]

When CTA-I is coated on macroporous silica gel from a solution, it affords another CSP (Chiralcel OA).[32,33)] As expected, its chiral recognition ability is completely different from that of CTA-I. The new CSP is of greater advantage than CTA-I in column efficiency, durability, and choice of eluents as the mobile phase. An X-ray crystallographic analysis of the material has suggested that it is amorphous rather than a crystal,[34)] indicating that microcrystallinity is not essential for chiral recognition. Chromatograms of the resolution of Tröger base (**4**) on the two CSPs are shown in Fig. 1.[32)] Enantiomers of **4** were eluted in reversed order on two triacetate columns. This may be ascribed to the different higher-order structures, or a different supramolecular structure of the two triacetates. Therefore, the chiral recognition of CSP is greatly dependent on the conditions of the preparation of CSP; for instance, the coating solvent

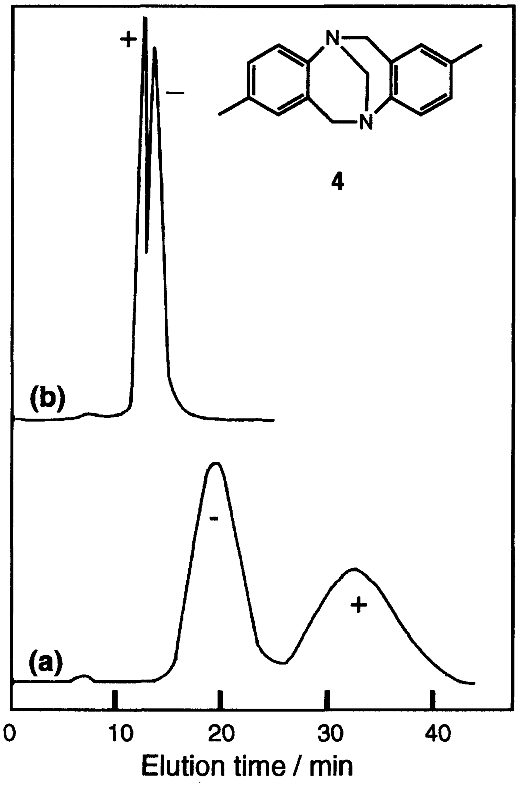


Fig. 1. Optical resolution of Tröger base (**4**) on microcrystalline cellulose triacetate (**3**, CTA-I) (a) and **3** coated on silica gel (b). Column, 25×0.46 cm (i.d.); eluent, ethanol–H_2O (7/3); flow rate, 0.5 ml min^{-1}.

and molecular weight of cellulose.[34]

These results provided a breakthrough in this area, and have aroused wide interest in the use of other derivatized polysaccharides for chiral HPLC. Figure 2 shows the structures of cellulose tribenzoates prepared by our group.[35] The influence of substituents on the phenyl groups of cellulose tribenzoate (CTB, **11**, Chiralcel OB) was systematically studied.[35] As substituents, alkyl, halogen, trifluoromethyl, methoxyl, and nitro groups were employed. Figure 3 shows a chromatogram of the resolution of **4** on cellulose tris(4-methylbenzoate) (**8**, Chiralcel OJ). The enantiomers eluted at t_1 and t_2 show complete separation. The capacity factors (k'_1) and k'_2), separation factor (α), and resolution factor (R_s) were determined as shown in the figure. Table 1 shows the results of the resolution of four racemates (**4**, **17**—**19**) on CTB derivatives **8**, **11**, and **12**.[35] The resolution ability depended greatly on the substituents. Benzoate derivatives having electron-donating substituents, such as methyl group, showed a better chiral recognition ability than did those having electron-withdrawing substituents, such as halogen groups. Among the benzoates, cellulose tris(4-methylbenzoate) (**8**) exhibited a high chiral recognition for various racemates, including drugs, and appears to be a practically useful CSP. Some stereochemically interesting compounds and chiral drugs resolved on **8** are shown in Fig. 4.[35—54] Several non-aromatic compounds (Fig. 5) are also resolved on CTB (**11**).[55]

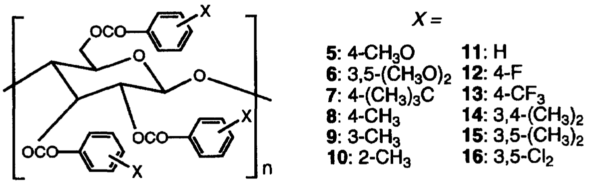


Fig. 2. Structures of cellulose tribenzoate derivatives.

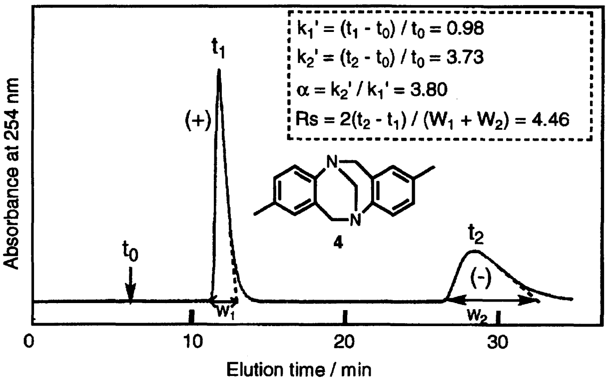
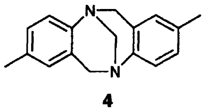
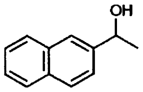
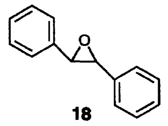
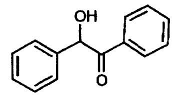


Fig. 3. Chromatographic resolution of **4** on cellulose tris(4-methylbenzoate). Column, 25×0.46 cm (i.d.); eluent, hexane–2-propanol (9/1); flow rate, 0.5 ml min^{-1}. (Reprinted from Ref. 35 Copyright 1987, Elsevier Science B.V.).

Mannschreck et al.,[56] and later Francotte et al.,[57—59] prepared spherical beads of cellulose tribenzoate and its derivatives with a methyl-substituent on the phenyl group, and found that these are useful CSPs, particularly for preparative purposes, because of their high loading capacity. The CTB-derivative beads exhibit enantioselectivity comparable to that of the corresponding coated-type CTB derivatives on silica gel.

The main chiral adsorbing sites of CTB derivatives are considered to be the polar carbonyl groups of esters, which can interact with racemates through hydrogen bonding and a dipole–dipole interaction for chiral discrimination.[35] Wainer and co-workers have proposed a similar mechanism[60] based on the chromatographic enantioseparation of a series of chiral aromatic amides[61] and alcohols[62] on the CTB phase. The chiral recognition ability of the CTB phases was greatly dependent on the conditions of the preparation of CSP,[35] particularly on the solvent used to dissolve CTB derivatives in the coating process, as observed in the case of cellulose triacetate. Therefore, other factors, such as the morphology of CTB derivatives, must be related to the enantioseparation.[35,57] Steinmerier and Zugenmaier proposed a left-handed 3/2 helical structure for CTB, independent of the preparation conditions.[63] Similar results were also given by Francotte et al. by means of an X-ray analysis.[57] Recently, Oguni et al. investigated the chiral discrimination mechanism of cellulose tris(4-methylbenzoate) by means of ^{13}C NMR spectroscopy.[64] Several carbon resonances of 1-phenylethanol were split into a pair due to enantiomers in the presence of the polymer. This approach may be useful to elucidate the chiral discrimination mechanism at the molecular level.

Table 1. Separation Factors (α) in the Resolution of **4** and **17**—**19** on Cellulose Tribenzoates[a)]

Racemates CSP	 **4**	 **17**	 **18**	 **19**
8: 4-CH_3	3.80(+)	1.23(−)	1.17(+)	1.23(−)
11: H	1.00	1.15(−)	1.31(+)	1.08(+)
12: 4-F	ca. 1(+)	1.09(−)	1.21(+)	1.16(−)

a) Column, 25×0.46 cm (i.d.); eluent, hexane–2-propanol (90/10); flow rate, 0.5 ml min^{-1}. The sign in parentheses shows optical rotation of the first-eluted isomer.

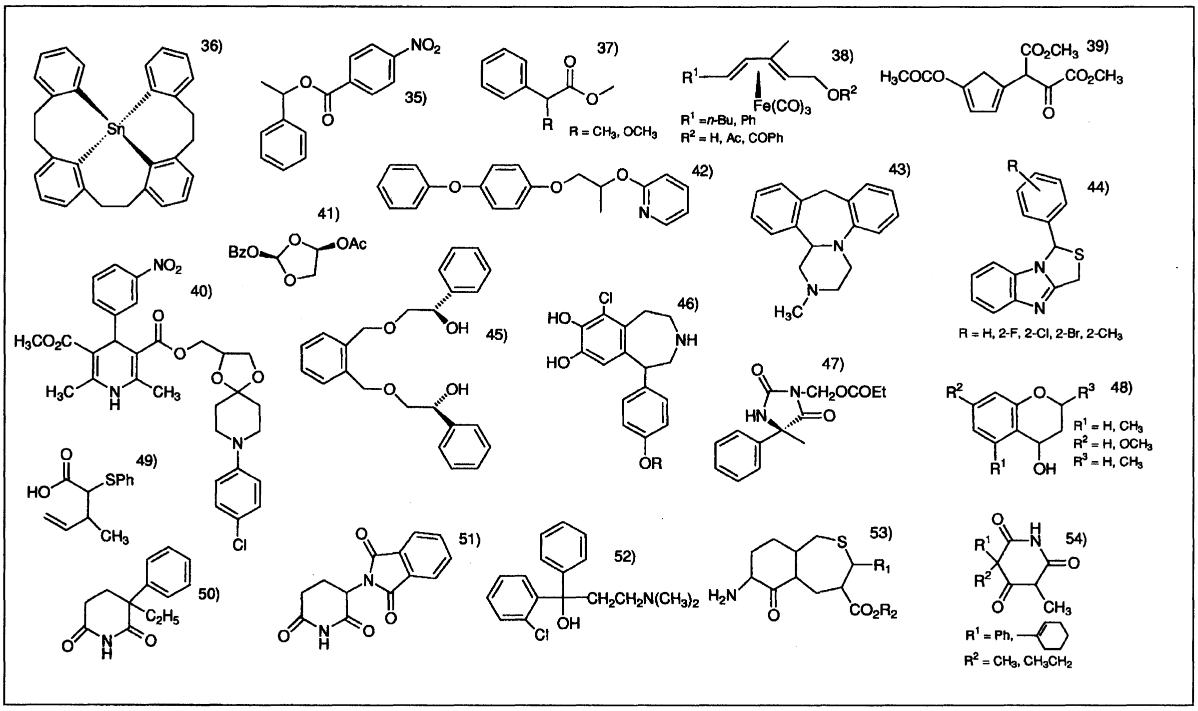


Fig. 4. Compounds resolved on cellulose tris(4-methylbenzoate) (Chiralcel OJ). The numbers next to the structures represent references.

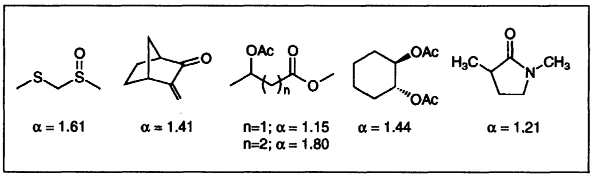
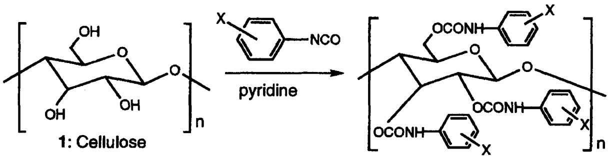


Fig. 5. Non-aromatic compounds resolved on cellulose tribenzoate (Chiralcel OB).

Among these cellulose triesters, triacetate, tribenzoate, tris(4-methylbenzoate), and tricinnamate have been commercialized as CSPs.

Tribenzoates of amylose showed low chiral recognition.[35)]



X =

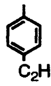
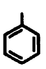
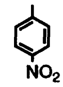
20: 4-CH_3O
21: 4-C_2H_5O
22: 4-$(CH_3)_2CHO$
23: 4-$(CH_3)_2CHCH_2O$
24: 4-$(CH_3)_3Si$
25: 4-CH_3
26: 4-CH_3CH_2
27: 4-$(CH_3)_2CH$
28: 4-$(CH_3)_3C$
29: 3-CH_3
30: 2-CH_3
31: H
32: 4-F
33: 4-Cl
34: 2-Cl
35: 3-Cl
36: 4-Br
37: 4-CF_3
38: 4-NO_2
39: 3,4-$(CH_3)_2$
40: 3,5-$(CH_3)_2$
41: 2,6-$(CH_3)_2$
42: 3,4,5-$(CH_3)_3$
43: 3,5-Cl_2
44: 3,4-Cl_2
45: 2,6-Cl_2
46: 3,5-F_2
47: 3,5-$(CF_3)_2$
48: 2-Cl-4-CH_3
49: 2-Cl-5-CH_3
50: 2-Cl-6-CH_3
51: 3-Cl-2-CH_3
52: 3-Cl-4-CH_3
53: 4-Cl-2-CH_3
54: 4-Cl-3-CH_3
55: 3-F-4-CH_3
56: 5-F-2-CH_3

Fig. 6. Preparation and structures of cellulose tris(phenylcarbamate) derivatives.

Phenylcarbamates of Cellulose and Amylose

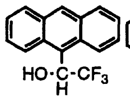
Cellulose tris(phenylcarbamate) derivatives are readily prepared by the reaction of microcrystalline cellulose with substituted phenyl isocyanates (Fig. 6), and have been the most deeply investigated polysaccharide phases with respect to the enantioseparation and the mechanism of chiral discrimination.[17—20)] These can resolve a wide range of racemates having various functional groups when coated on silica gel. The chiral recognition abilities of a series of cellulose phenylcarbamate derivatives have been evaluated,[19,65)] and the chiral recognition mechanism has been proposed on the basis of chromatographic, computational, and spectroscopic methods. Their chiral recognition ability was greatly influenced by the substituents on the phenyl groups.[65)] The separation factors (α) for ten racemates (**4**, **18**, **19**, **57**—**63**) on 4-substituted derivatives are shown in Table 2, where the substituents on the phenyl groups are arranged from the left to right column in the order of increasing electron-withdrawing powers of the substituents. Most CSPs can separate all of the racemates, except for a few cases, although the α values depend greatly on the 4-substituents. The derivatives with heteroatom substituents, such as the methoxyl and nitro groups, show poor chiral recognition.[65)] Generally, the introduction of an electron-donating methyl group or an electron-withdrawing halogen at the 3- and/or 4-position improved the resolution ability for many racemates, but 2-substituted derivatives showed low chiral recognition. Recently, phenylcarbamate derivatives having both an electron-donating methyl group and an electron-withdrawing chloro or fluoro group on the phenyl moieties were found to exhibit high enantiosep-

Table 2. Separation Factors (α) in the Resolution on 4-Substituted Phenylcarbamate Derivatives of Cellulose[a)]

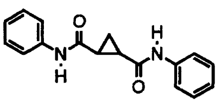
CSP Racemates[b)]	 OCH$_3$	 CH$_3$	 C$_2$H$_5$		 F	 Cl	 Br	 CF$_3$	 NO$_2$
4	ca. 1(+)	1.48(+)	1.11(+)	1.37(+)	1.14(+)	1.16(+)	1.19(+)	1.23(+)	ca. 1(−)
18	1.34(+)	1.55(+)	1.55(+)	1.46(+)	1.38(+)	1.68(+)	1.70(+)	1.61(+)	1.33(+)
19	ca. 1(+)	1.12(−)	1.14(−)	ca. 1(+)	1.14(−)	1.20(−)	1.13(−)	1.10(−)	1.00
57	1.35(−)	1.52(−)	1.57(−)	1.45(−)	1.26(−)	1.29(−)	1.29(−)	1.30(−)	ca. 1(+)
58	1.00	1.35(−)	2.12(−)	1.45(−)	ca. 1(−)	1.44(−)	1.17(−)	1.22(−)	ca. 1(+)
59	1.15(−)	1.30(−)	1.33(−)	1.65(−)	1.17(−)	1.20(−)	1.21(−)	2.04(−)	ca. 1(+)
60	1.00	1.37(+)	1.59(+)	1.22(+)	1.64(+)	1.95(+)	1.95(+)	1.48(+)	1.00
61	ca. 1(+)	1.16(+)	1.22(−)	1.10(+)	1.13(+)	1.12(+)	1.13(+)	1.14(+)	1.00
62	ca. 1(+)	1.75(+)	1.76(+)	1.24(+)	1.53(+)	1.46(+)	1.79(+)	2.06(+)	ca. 1(+)
63	1.13(−)	1.20(−)	1.19(−)	1.17(−)	1.12(−)	1.16(−)	1.17(−)	1.18(−)	ca. 1(−)

a) Column, 25×0.46 cm (i.d.); eluent, hexane–2-propanol (90/10); flow rate, 0.5 ml min^{-1}. The sign in parentheses shows optical rotation of the first-eluted isomer.

b)



57



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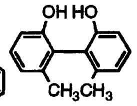

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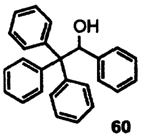

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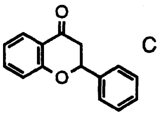
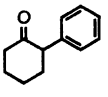


61

Co(acac)$_3$

62



63

aration for many racemates; particularly, 3-chloro-4-methyl-, 4-chloro-3-methyl-, and 3-fluoro-4-methylphenylcarbamates of cellulose (**52**, **54**, and **55**) showed a high chiral recognition ability.[66—68)]

The retention times of acetone on the 4-substituted CSPs tend to increase as the electron-withdrawing power of the substituents increases (Fig. 7(a)).[65)] On the other hand, the retention times of the first eluted isomer of alcohol (**57**) decrease roughly as the electron-withdrawing power of the substituents increases (Fig. 7(b)).[65)] These results indicate that the main chiral adsorbing sites are probably the polar carbamate groups, as shown in Fig. 8, and that the groups can interact with a racemate via hydrogen bonding on NH and C=O groups and the dipole–dipole interaction on C=O. The acidity of the NH proton increases along with an increase in the electron-withdrawing power of the substituents (X) on the phenyl groups. Therefore, acetone is more strongly adsorbed on the CSPs with the more acidic NH protons via a hydrogen-bonding interaction. This observation is associated with the fact that the NH resonances shift downfield as the electron-withdrawing power of the substituents (X) on the phenyl groups increases.[65)]

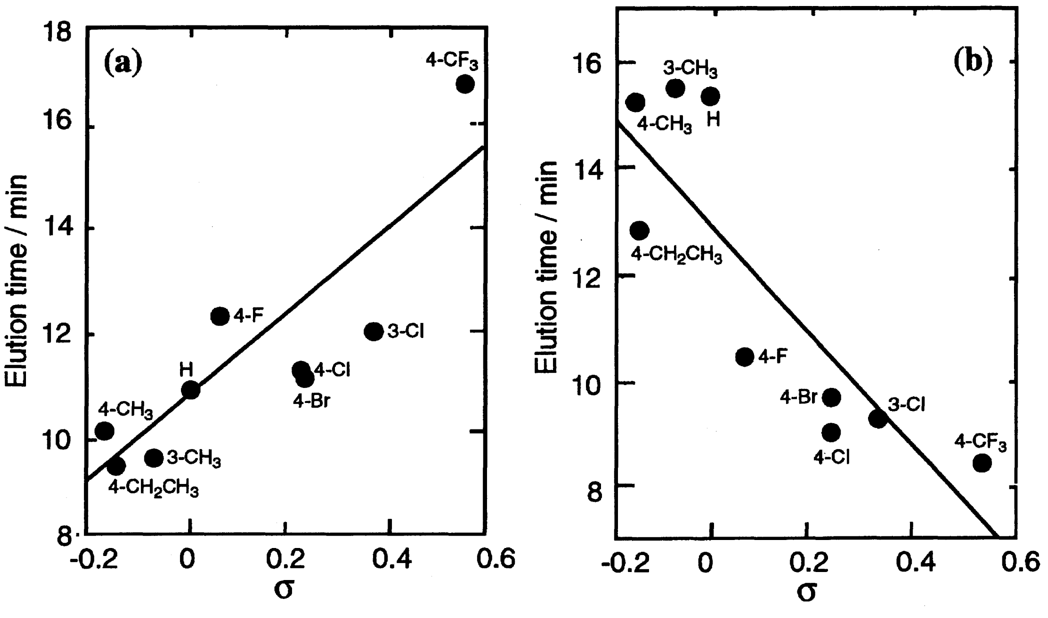


Fig. 7. Plots of retention times of acetone (a) and the first-eluting enantiomer of compound **57** on cellulose tris(phenylcarbamate) derivatives against the Hammett's σ values. (Reprinted from Ref. 65 Copyright 1986, Elsevier Science B.V.).

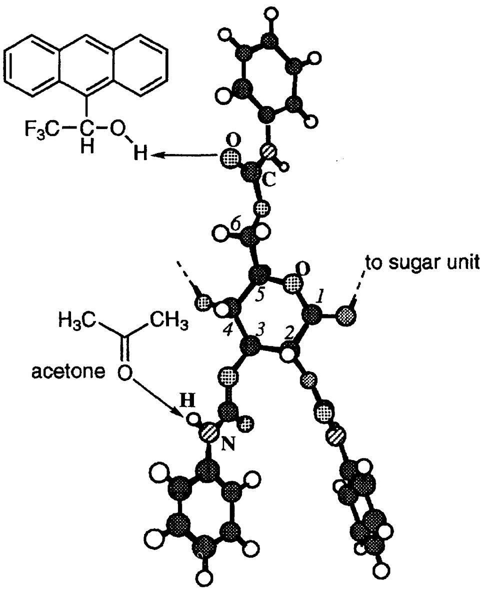
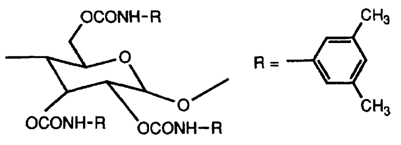


Fig. 8. Possible interaction sites of cellulose tris(phenylcarbamate) derivatives.

On the other hand, when X is the electron-donating one, such as a methyl group, the electron density of the carbonyl oxygen of the carbamates is expected to increase. Therefore, alcohols are more strongly adsorbed on the CSPs via a hydrogen-bonding interaction. When X, itself, is a polar group, such as a nitro or methoxyl group, racemates can interact with X substituents. Since X is far from a chiral glucose residue, such an interaction on X should reduce the optical resolving ability, as can be seen in Table 2. Therefore, bulky alkoxyl substituents, such as isopropoxyl (**22**) and isobutoxyl (**23** in Fig. 6), improve the resolving ability by reducing the interactions on the ether oxygen.[69] The interaction of the carbamate groups of CSPs with racemates seems to play the most important role for effective chiral recognition. This is described later in more detail.

Similarly, the chiral resolving ability of amylose derivatives was improved by introducing methyl or chloro groups on the phenyl moieties.[70,71] However, in contrast to the cellulose derivatives, tris(4-methoxyphenylcarbamate)[69] and tris(5-chloro-2-methylphenylcarbamate)[72] of amylose showed high chiral recognition. Zugenmaier et al. proposed left-handed 3/2 and 4/1 helical structures for cellulose tris(phenylcarbamate) (**31**)[63,73] and amylose tris(phenylcarbamate),[74] respectively. These different higher-order structures might be responsible for the different influence of the substituents on the resolving ability of the cellulose and amylose phenylcarbamate derivatives.

Among the tris(phenylcarbamate) derivatives of cellulose and amylose so far prepared, 3,5-disubstituted derivatives, such as 3,5-dimethyl- (**40**) (CDMPC, Chiralcel OD) and 3,5-dichlorophenylcarbamates (**43**) of cellulose and tris(3,5-dimethylphenylcarbamate) of amylose (**64**) (ADMPC, Chiralpak AD) (Chart 2), show particularly interesting and excellent resolving ability for a variety of racemates.[65,70] The results of the resolution of ten racemates (**4**, **18**, **19**, **57**—**63**) on these CSPs are shown in Table 3. The CSP **43** shows a unique chiral recognition; for example, the CSP can resolve bulky esters[65] and stereoisomers of oligo(methyl methacrylate)s,[75] which markedly contributed to our understanding of the stereochemistry of the asymmetric polymerization of triarylmethyl methacrylates. However, this CSP has a defect due to a high solubility in the eluent. One can not use an eluent containing a high 2-propanol content. This defect was overcome by chemically bonding the CSP to 3-aminopropylsilanized



64: ADMPC (Chiralpak AD)

Chart 2.

Table 3. Resolution on 3,5-Disubstituted Phenylcarbamates of Cellulose and Amylose[a)]

Racemate	**40**		**43**[b)]		**64**	
	k_1'[c)]	α	k_1'[c)]	α	k_1'[c)]	α
4	0.97(+)	1.32	0.87(+)	1.65	0.53(+)	1.58
18	0.74(−)	1.68	0.56(+)	1.84	0.42(+)	3.04
19	2.43(+)	1.58	3.08(−)	1.21	3.14(−)	2.01
57	2.13(−)	2.59	0.28(−)	1.38	1.30(+)	1.15
58	0.83(+)	3.17	0.59(+)	1.41	3.25(+)	2.01
59	2.36(−)	1.83	1.62(+)	1.11	2.46(−)	2.11
60	1.37(+)	1.34	0.40(+)	1.29	2.65(+)	1.98
61	1.47(−)	1.41	1.55(−)	1.20	0.93(+)	1.12
62	0.42(+)	ca. 1	0.76(+)	1.82	0.25(−)	ca. 1
63	1.17(−)	1.15	2.65(−)	1.26	0.61(−)	ca. 1

a) Column, 25×0.46 cm (i.d.); eluent, hexane–2-propanol (90/10); flow rate, 0.5 ml min^{-1}. b) Eluent, hexane–2-propanol (95/5). c) The sign in parentheses shows optical rotation of the first-eluted isomer.

silica gel with a diisocyanate as a spacer. The detailed method and results on the chemical bonding are described later.

CDMPC resolves a variety of racemates, including aromatic hydrocarbons, amines, carboxylic acid,[76] alcohols, amino acid derivatives,[77] and many drugs,[78] including β-adrenergic blocking agents (β-blockers).[79] Some stereochemically interesting compounds and drugs recently resolved on CSP are shown in Fig. 9[80—106] and Fig. 10,[78,79,107—128] respectively. In the chromatographic resolution of many β-blockers on CDMPC, (R)-(+)-isomers eluted first, followed by (S)-(−)-isomers, which showed complete separation.[78,79] Figure 11[78,79,113,115] shows the resolution chromatograms of several racemic drugs on CDMPC and ADMPC.

Although CDMPC exhibits a high resolving ability for many compounds, several drugs are separated better on other phenylcarbamate derivatives than on CDMPC.[78]

We examined the resolution of 510 racemates on CDMPC; 229 of them were almost resolved and 86 were partially resolved with two overlapping peaks.[19] This means that about 62% of 510 racemates were resolved on CSP. The tris(3,5-dimethylphenylcarbamate) may be one of the most powerful CSPs. We also tested the enantioseparation of 384 racemates on an ADMPC column; 107 of them were completely resolved and 102 were partially resolved, showing two peaks. Consequently, when two tris(3,5-dimethylphenylcarbamate)s of cellulose and amylose were used for 510 racemates, 186 racemates were separated only on CDMPC, 85 only on ADMPC, and 129 on both columns. This means that 400 racemates, about 78% of 510 racemates, were separated into enantiomers at least on either of the two columns. Some enantiomers exhibit a reversed elution order on the two CSPs (Table 3), suggesting that these two are complementary in chiral recognition for many racemates. Enantiomers not resolved on CDMPC may be resolved on ADMPC, and vice versa.[19] Some drugs and chiral compounds efficiently resolved on ADMPC are shown in Fig. 12.[78,115,121,129—142]

As an eluent, a hexane-2-propanol mixture is often used for the separation of enantiomers. Other alcohols, including methanol and ethanol, can also be used, and the structure of alcohols influences the enantioseparation.[24] When an analyte has a basic amino group, the addition of a small amount of an amine, such as diethylamine or isopropylamine, is recommended for reducing tailing of the peaks.[79] For acidic compounds, the addition of a small amount of a strong acid, such as CF_3COOH, often induces better separation.[76] Aqueous eluents are also particularly usable to investigate the pharmacokinetics and pharmacodynamics of chiral drugs in living systems.[143,144] Supercritical fluid liquid chromatography using carbon diox-

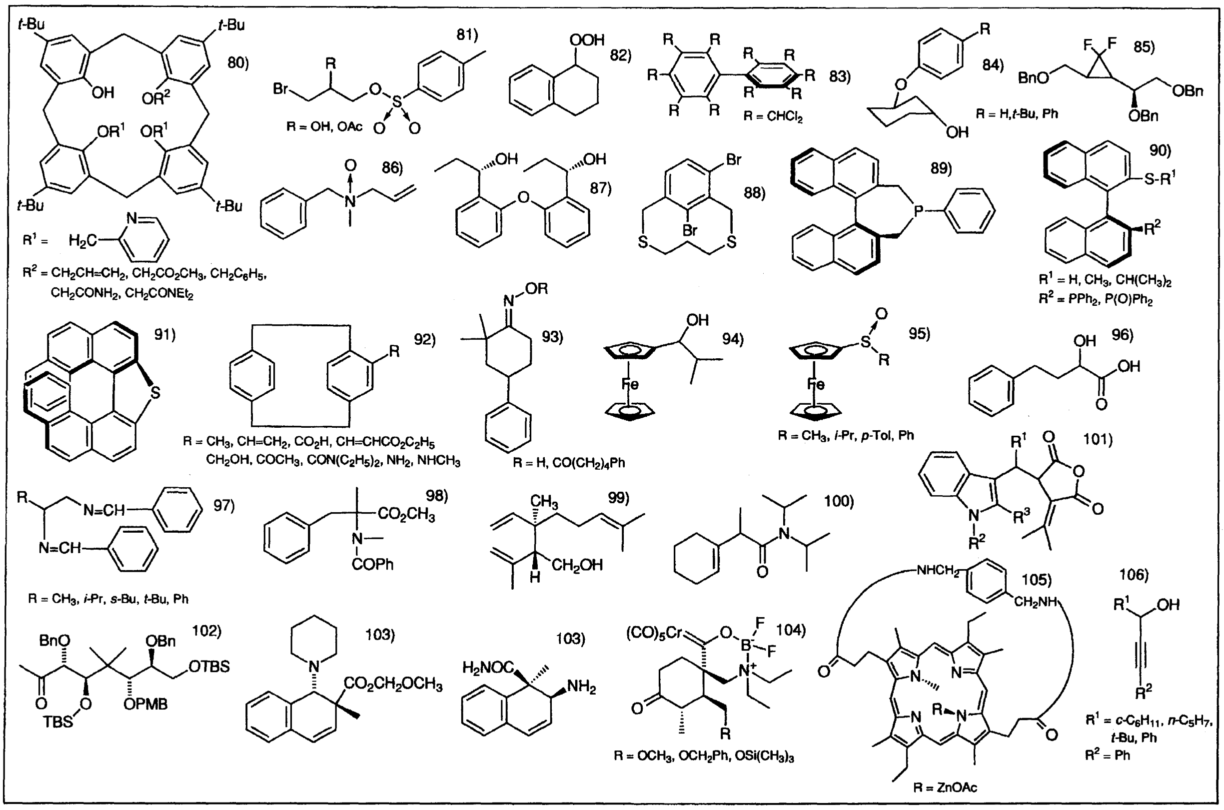


Fig. 9. Compounds resolved on cellulose tris(3,5-dimethylphenylcarbamate) (**CDMPC**, Chiralcel OD). The numbers next to the structures represent references.

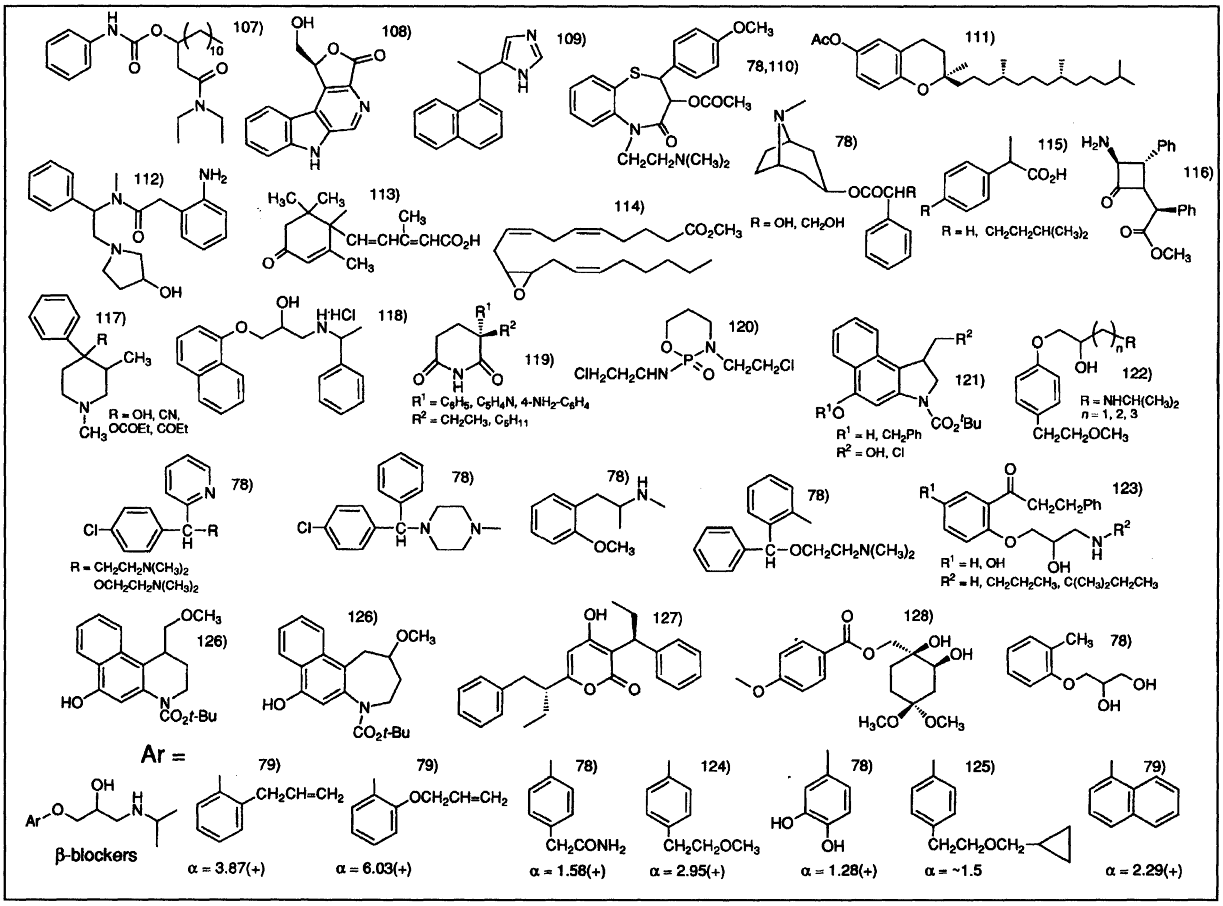


Fig. 10. Drugs resolved on cellulose tris(3,5-dimethylphenylcarbamate) (**CDMPC**, Chiralcel OD). The numbers next to the structures represent references.

ide and alcohols as a mobile phase can also be applied to the polysaccharide phases.[145] However, other solvents, such as chloroform and tetrahydrofuran, in which the polysaccharides are dissolved or swollen, are unable to be used as the main mobile phases. To improve this defect, the phenylcarbamate derivatives of cellulose were chemically bonded to silica gel using diisocyanate as a spacer.[146] However, the chiral recognition ability was reduced compared to that of coated-type CSPs when 10% of the hydroxyl groups of cellulose were chemically bonded to amino-functionalized silica gel with diisocyanate non-regioselectively.

Recently, CDMPC and ADMPC regioselectively bonded to silica gel were prepared with 4,4′-diisocyanatodiphenylmethane as a spacer (Fig. 13).[147] ADMPC regioselectively bonded at the 6-position to silica gel (Fig. 13(a)), by using a small amount of diisocyanate, showed a higher resolving ability than bonded at the 2- or 3-position (Fig. 13(b)). On the other hand, for CDMPC, a clear dependence of enantioseparation on the position of the glucose unit for immobilization could not be observed. In both CDMPC and ADMPC, the regioselectively bonded phases showed a superior chiral discrimination to non-regioselectively bonded phases. Since these chemically bonded phases are not damaged by polar solvents, such as chloroform, some racemates were more efficiently resolved on the chemically bonded-type CSP using chloroform as a component of the mobile phase. The above-mentioned CSPs may be chemically bonded to silica gel through plural hydroxyl groups of polysaccharides, which cause an alternation of higher-order structure of the polysaccharides, and these decrease the chiral recognition ability.

Very recently, 3,5-dimethylphenylcarbamate of amylose was successfully chemically bonded to silica gel only at the reducing terminal residue of amylose (Fig. 13(c)).[148] Amylose having the desired chain length was readily prepared by the polymerization of α-D-glucose 1-phosphate dipotassium salt with functionalized maltooligosaccharides using a phosphorylase isolated from potato. The amylose was successfully bonded to silica gel at the reducing terminal residue, and was allowed to react with 3,5-dimethylphenyl isocyanate to afford CSPs with excellent resolving ability and high durability against solvents, such as THF and chloroform.[149]

Mechanism of Chiral Discrimination on Phenylcarbamates of Polysaccharides

Most cellulose tris(phenylcarbamate) derivatives

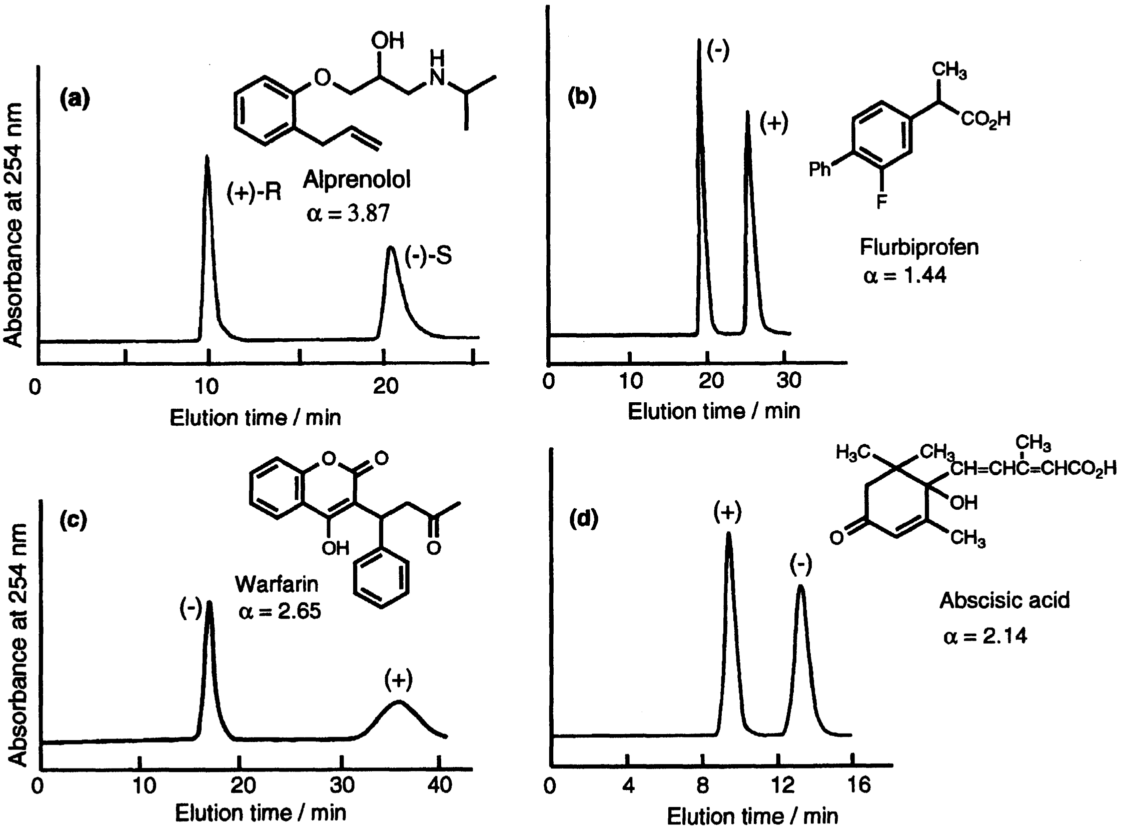


Fig. 11. Chromatograms of resolution of alprenolol (a),[79] flurbiprofen (b),[115] warfarin (c),[78] and abscisic acid (d)[113] on cellulose tris(3,5-dimethylphenylcarbamate) (a, c, and d) and amylose tris(3,5-dimethylphenylcarbamate) (b). Column, 25×0.46 cm (i.d.); eluent, hexane–2-propanol (9/1) (a), hexane–2-propanol–trifluoroacetic acid (95/5/1) (b), hexane–2-propanol–HCO_2H (80/20/0.1) (c), hexane–2-propanol–trifluoroacetic acid (80/20/1) (d); flow rate, 0.5 ml min^{-1}.

form a lyotropic liquid crystalline phase in a highly concentrated solution,[65,150] and show a high crystallinity under a polarizing microscope when they are cast from a solution. This means that the carbamate derivatives coated on silica gel from a solution also have an ordered structure in which phenylcarbamate groups are regularly arranged. Such an ordered structure seems to be very important for efficient chiral recognition on CSPs derived from polymers. A few cellulose phenylcarbamate derivatives and alkylcarbamates do not form such a liquid crystalline phase, and show low chiral recognition.[65]

The most important adsorbing sites for chiral discrimination on phenylcarbamate derivatives are probably the polar carbamate groups, as shown in Fig. 8; these groups are capable of interacting with a racemate via hydrogen bonding with NH and C=O groups and the dipole–dipole interaction on C=O.[19,65] Hence, the nature of the substituents on the phenyl groups affects the polarity of the carbamate residues, which must change the chiral resolving ability.

Although the phenylcarbamate derivatives of polysaccharides have become popular, the chiral discrimination mechanism at a molecular level is not clear. A determination of the exact structures of the phenylcarbamate derivatives should be made in order to reveal the mechanism. Figure 14 shows the stable structure optimized by a molecular mechanics calculation using Dreiding force field[151] based on the proposed structure for cellulose tris(phenylcarbamate) (CTPC) by an X-ray analysis.[63,73] CTPC has a left-handed 3/2 helical conformation, and the glucose residues are regularly arranged along the helical axis. A chiral helical groove, or ditch, with polar carbamate residues exists along the main chain. The polar carbamate groups are favorably located inside, and hydrophobic aromatic groups are placed outside the polymer chain so that polar enantiomers may insert in the groove to interact with the carbamate residues via hydrogen-bonding formation. This interaction seems to be important for efficient chiral discrimination.

Besides these polar interactions, the π–π interaction between the phenyl group of a CTPC derivative and the aromatic groups of a solute may play some role for chiral recognition, because several nonpolar aromatic compounds have also been resolved.[92]

CTPC appears to maintain its helical structure even in solution.[152] However, most of the phenylcarbamate derivatives with high chiral resolving ability as CSPs are soluble only in polar solvents, such as pyridine and

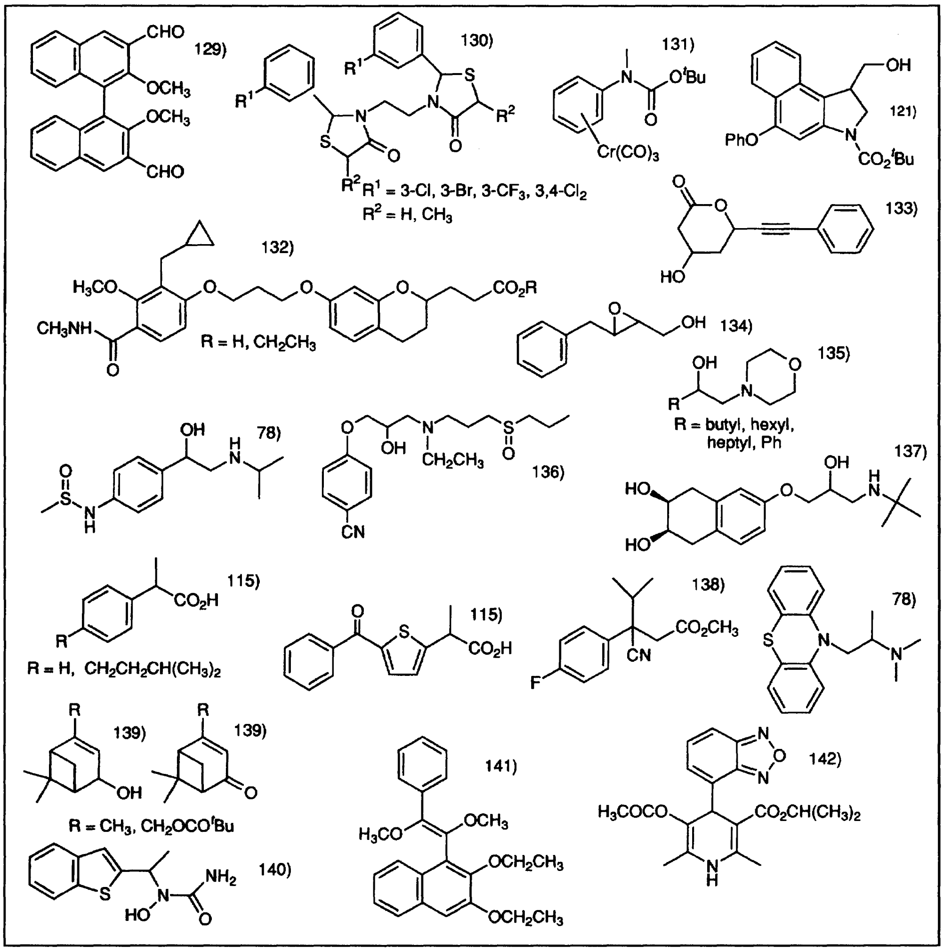


Fig. 12. Compounds resolved on amylose tris(3,5-dimethylphenylcarbamate) (**ADMPC**, Chiralpak AD). The numbers next to the structures represent references.

THF. In such polar solvents, the chiral discrimination of enantiomers by NMR is hardly detected due to a strong interaction of the solvents with the polar carbamate residues. Therefore, it was difficult to elucidate the chiral discrimination mechanism by NMR. Recently, we have found that several new phenylcarbamate derivatives, for example tris(4-trimethylsilylphenylcarbamate) (**24**)[153] and tris(5-fluoro-2-methylphenylcarbamate) (**56**)[68] of cellulose (Chart 3), are soluble in chloroform, and show chiral discrimination in ^{1}H and ^{13}C NMR spectroscopies as well as in HPLC. This, for the first time, permits us to investigate the chiral interaction occurring in solution by NMR. The phenylcarbamate derivatives resolved many racemates by HPLC. The chromatographic results can be directly correlated to the spectroscopic ones.

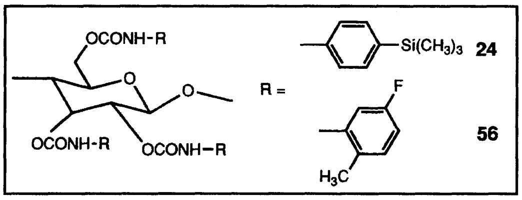


Chart 3.

Figure 15 shows the 500 MHz ^{1}H NMR spectra of (±)-*trans*-stilbene oxide (**18**) in the presence and absence of **24**. The methine proton of **18** was separated into two singlet resonances in the presence of **24**.[153] This clearly indicates that **24** can discriminate the enantiomers even in solution. The measurement using enantiomerically pure (+)- and (−)-**18** indicated that only the methine proton of (−)-isomer shifted downfield, whereas that of the (+)-isomer scarcely shifted. In the chromatographic enantioseparation of (±)-**18** on the CSP **24**, the (+)-isomer eluted first, followed by the (−)-isomer, and complete base-line separation was attained ($\alpha = 1.55$). The (−)-isomer must be adsorbed more strongly on **24**. This elution order is well related with the downfield shift of the (−)-isomer observed in the ^{1}H NMR.

As previously described, the most important adsorbing site for chiral discrimination on phenylcarbamate derivatives is the carbamate residue, which can interact with enantiomers via hydrogen bonding, as shown in Fig. 8. For **18**, the cyclic ether oxygen may interact with the NH proton of the carbamate residue via hydrogen bonding. Therefore, the addition of a hydrogen acceptor, such as acetone, resulted in no splitting

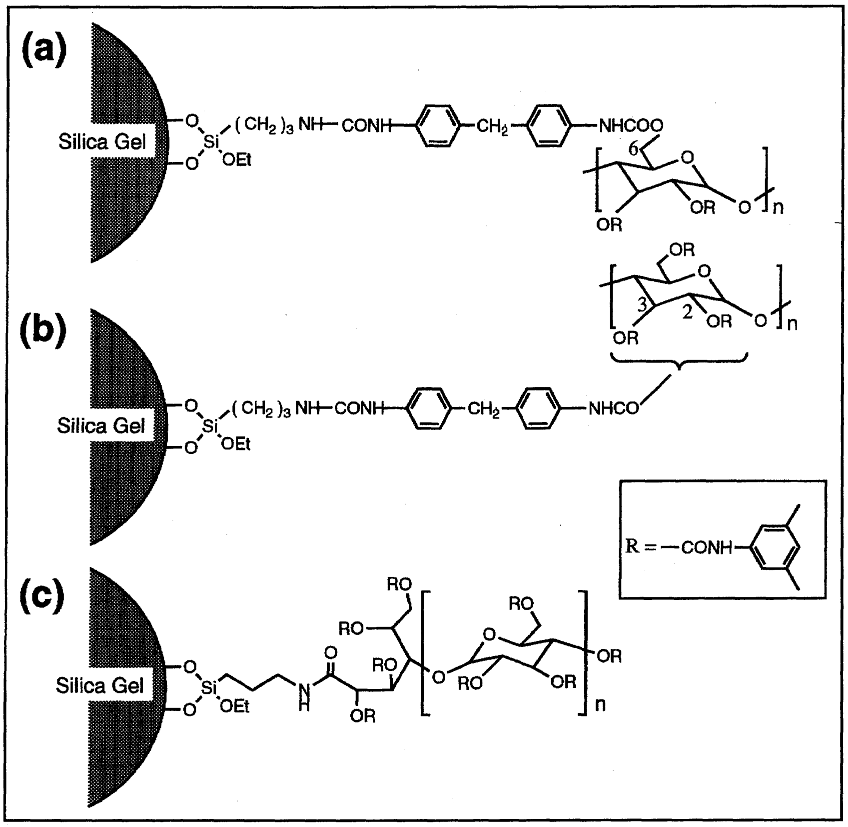


Fig. 13. Structures of **ADMPC** chemically bonded to silica gel at the 6- (a), 2,3-position (b) of glucose units, and at the reducing terminal residue of amylose (c).

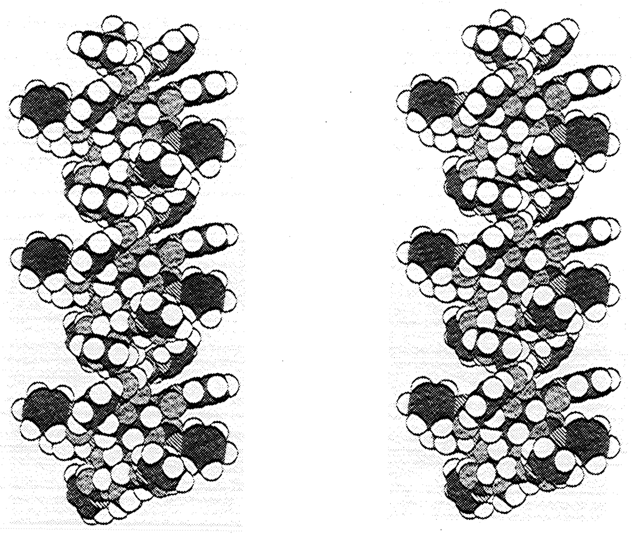


Fig. 14. Stereoviews of optimized structure of cellulose tris(phenylcarbamate) (**CTPC**). The energy minimization was carried out using Dreiding force field[151] under three dimensional periodic boundary conditions by Cerius^2 (Molecular Simulations Incorp.).

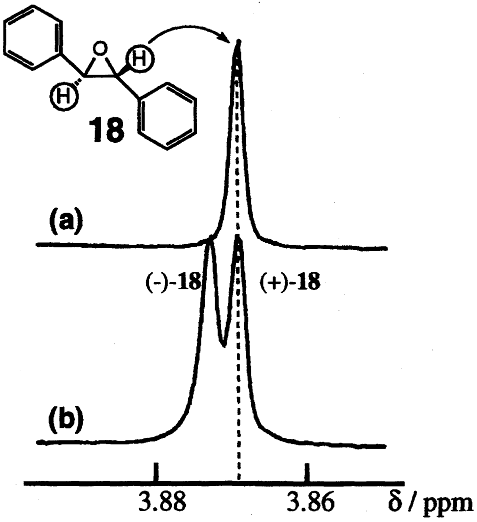


Fig. 15. 500 M Hz ^{1}H NMR spectra of *trans*-stilbene oxide (5 mg, **18**) in the absence (a) and presence (b) of cellulose tris(4-trimethylsilylphenylcarbamate) (20 mg, **24**) in $CDCl_3$ (1.0 ml) at 22 °C (TMS).

of the methine proton.[153] An analogous change in the ^{1}H NMR of the methine proton of **18** was also observed by the addition of 2-propanol in place of acetone. In this case, interestingly, the methyl groups of 2-propanol split into a pair of doublets, indicating that the two methyl groups were magnetically non-equivalent in the presence of **24**.[153] The ^{1}H NMR signals of other enantiomers, including Tröger base (**4**), benzoin (**19**), 2,2′-dihydroxy-1,1′-binaphthyl, and several *s*-alcohols, were also separated into two sets of peaks in the presence of **24** in $CDCl_3$ (Fig. 16).[154] The present results suggest that the phenylcarbamate derivatives of the polysaccharides

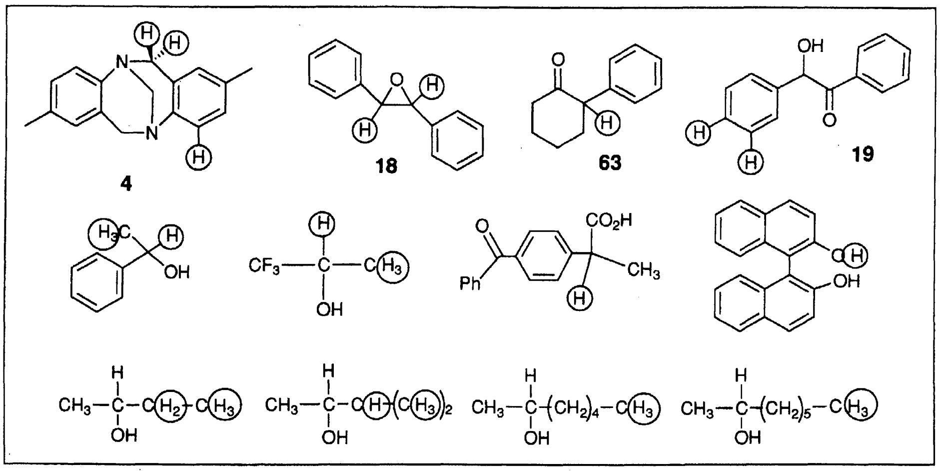


Fig. 16. Compounds enantiomerically discriminated by cellulose tris(4-trimethylsilylphenylcarbamate) (**24**) in ^{1}H NMR. The recognized protons are marked by circles.

may be useful as a chiral shift reagent. The phenylcarbamate derivative **56** also showed chiral discrimination for some racemates in CDCl$_3$; especially, enantiomers of 2,2′-dihydroxy-1,1′-binaphthyl and 2,2′-dihydroxy-6,6′-dimethyl-1,1′-biphenyl were distinctly discriminated by **56** in ^{1}H and ^{13}C NMR as well as in HPLC. The interaction between **56** and the alcohols was extensively investigated at the molecular level by using the 2D-NOESY technique.[155]

On the other hand, a computer simulation may be a useful and effective approach for elucidating the chiral recognition mechanism on other CDCl$_3$-insoluble phenylcarbamate derivatives of polysaccharides, and for predicting the elution order of enantiomers.

Very recently, we carried out force-field calculations of the interaction energies between cellulose tris(phenylcarbamate) (CTPC) (**31**; Fig. 14) and *trans*-stilbene oxide (**18**) or *trans*-1,2-diphenylcyclopropane (**65**) (Chart 4) using Quanta/Charmm and Molecular Interaction programs (Molecular Simulations Inc.) in order to gain insight into the chiral recognition mechanism of phenylcarbamate derivatives.[156] CTPC is a suitable phenylcarbamate derivative, because its structure has been postulated on the basis of an X-ray analysis, and shows high chiral recognition as a CSP for HPLC, as previously described. In a chromatographic enantioseparation, **18** was completely resolved (α=1.46) on CTPC, and the (+)-(R,R) isomer eluted first followed by the (−)-(S,S) isomer; **65** was not separated on the same column (α=ca. 1).

An enantiomer molecule of **18** was placed and tumbled around the NH proton of the carbamate group at specified angles. The interaction energy was then calculated at each point of a grid on the CTPC molecule optimized with Charmm force field, using all possible combinations of the rotation angles of the enantiomer. The calculation results suggested that the lowest interaction energy between CTPC and (−)-(S,S)-**18** is −22.16 kcal mol^{-1}, whereas that of (+)-(R,R)-**18** is −19.73 kcal mol^{-1}, indicating that (−)-(S,S)-**18** may more closely interact with CTPC than (+)-(R,R)-**18**. Figure 17 shows computer graphics of the interaction between CTPC and (−)-(S,S)-**18** with the lowest energy; (S,S)-**18** is bound in a chiral groove and each phenyl group may interact with the phenyl groups of CTPC via a π–π interaction, and the ether oxygen of

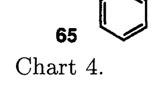

65

Chart 4.

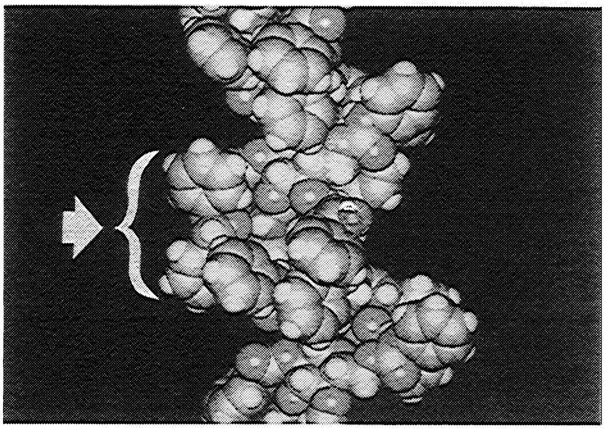


Fig. 17. Computational graphics of the interaction between cellulose tris(phenylcarbamate) (CTPC) and (S,S)-*trans*-stilbene oxide (**18**) (arrowed) exhibiting the lowest interaction energy. The energy minimization was carried out using Charmm force field in Quanta (Molecular Simulations Incorp.). (Reprinted from Ref. 156. Copyright 1995, Elsevier Science B.V.).

(*S*,*S*)-**18** is located near to the NH proton of CTPC. The distance between them is 2.5 Å, which is short enough to form hydrogen bonding, while that for (*R*,*R*)-**18** is 3.2 Å.[156] Although the same calculation was performed for **65**, almost no different interaction energy was observed for the enantiomers of **65**. These calculations agreed with the observed chromatographic resolution on CTPC. Computational studies will be useful and appreciable to the fields of chiral discrimination.[157]

Other Phenylcarbamates of Polysaccharides

Phenylcarbamates of other polysaccharides such as chitosan (**66**), xylan (**67**), curdlan (**68**), dextran (**69**), and inulin (**70**) were also prepared and used as CSPs (Chart 5). Some of the carbamates showed unique and interesting resolution properties. However, these can not resolve as many racemates as can the cellulose tris(phenylcarbamates).[158]

Although alkylcarbamates, such as methylcarbamate and cyclohexylcarbamate of cellulose, showed poor chiral recognition, some of the tris(aralkylcarbamate) of cellulose and amylose (Fig. 18) showed characteristic resolving abilities for many racemates different from those of the phenylcarbamates of the polysaccharides.[159] Among the aralkylcarbamates shown in Fig. 18, only 1-phenylethyl- and 1-phenylpropylcarbamates of the polysaccharides exhibit high chiral recognition, and benzylcarbamate and other more bulky carbamates show lower resolving ability. The bulkiness of the former two aralkyl groups may be suitable for the carbamates to have regular higher-order structures, because only these two show a lyotropic liquid crystalline phase. The chiral recognition abilities of 1-phenylethylcarbamate of cellulose and amylose depend on the chirality of the carbamate group. Particularly, (*S*)-1-phenylethylcarbamate of amylose (Chiralpak AS) showed high chiral recognition, and some of the racemates shown in Fig. 19[52,159—165] are better resolved on the CSP than other polysaccharides carbamates, including phenylcarbamate derivatives.

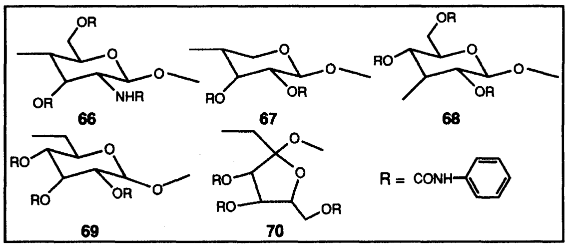


Chart 5.

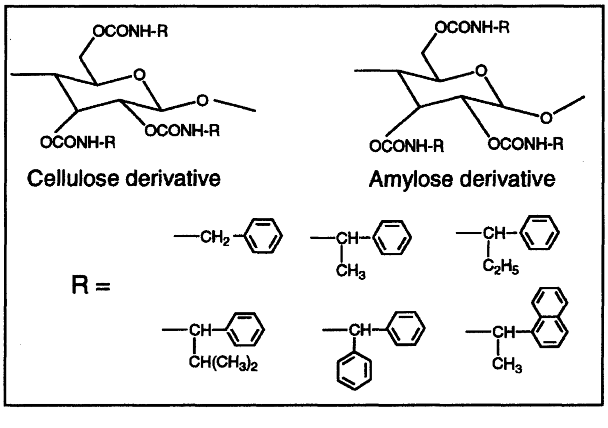


Fig. 18. Structures of aralkylcarbamates of cellulose and amylose.

Phenylcarbamates of Oligosaccharides

3,5-Dimethylphenylcarbamates of oligosaccharides, cellooligosaccharide (**71**), maltooligosaccharide (**72**), and cyclodextrins (**73**) were prepared (Fig. 20), and their chiral recognition abilities were compared with those of the corresponding polysaccharide derivatives in order to obtain information about the influence of the higher-order structure of the polysaccharide derivatives.[166]

The results of the optical resolution of several racemates on these oligosaccharides derivatives are summarized in Table 4.[166] The chiral recognition abilities of 3,5-dimethylphenylcarbamates of cellooligosaccharides ($n=2$, 4) were lower than that of the cellulose derivative (CDMPC), while in the linear maltooligosaccharides ($n=2$—6) the resolving abilities of 4—6 mer were similar to each other and were not so different from the ability of amylose tris(3,5-dimethylphenylcarbamate) (ADMPC). These results clearly indicate that cellooligosaccharide derivatives have a different structure from that of CDMPC, whereas the maltooligosaccharides have a similar ordered structure to that of ADMPC when *n* is more than 4. These explanations were supported through conformational studies of the oligomers by CD spectroscopy.[166] The intensities of the CD spectra of the cellooligosaccharide derivatives were smaller than that of CDMPC (Fig. 21(a)), while the CD patterns of the maltooligosaccharides were quite similar, except for 2 mer (Fig. 21(b)). Consequently, the cellooligosaccharides may have less-ordered structures and the maltooligosaccharides ($n>3$) may have highly ordered structures similar to ADMPC; these must be the reason for the low and high resolving abilities of those oligomers, respectively.

β-Cyclodextrin, cyclic oligomers with the same glucose unit as amylose, can separate racemates by inclusion[167] and the CSPs consisting of cyclodextrins bonded to silica gel have been commercialized. On the other hand, 3,5-dimethylphenylcarbamates of cyclodextrins showed quite different chiral recognition from that of ADMPC, indicating that the amylose derivative must possess a higher-order structure that is different from those of the cyclodextrin derivatives.[166]

Two different types of 3,5-dimethylphenylcarbamate of β-cyclodextrin immobilized on silica gel have been prepared: One has immobilization probably through the 6-position,[168] and the other through the 2- and/or

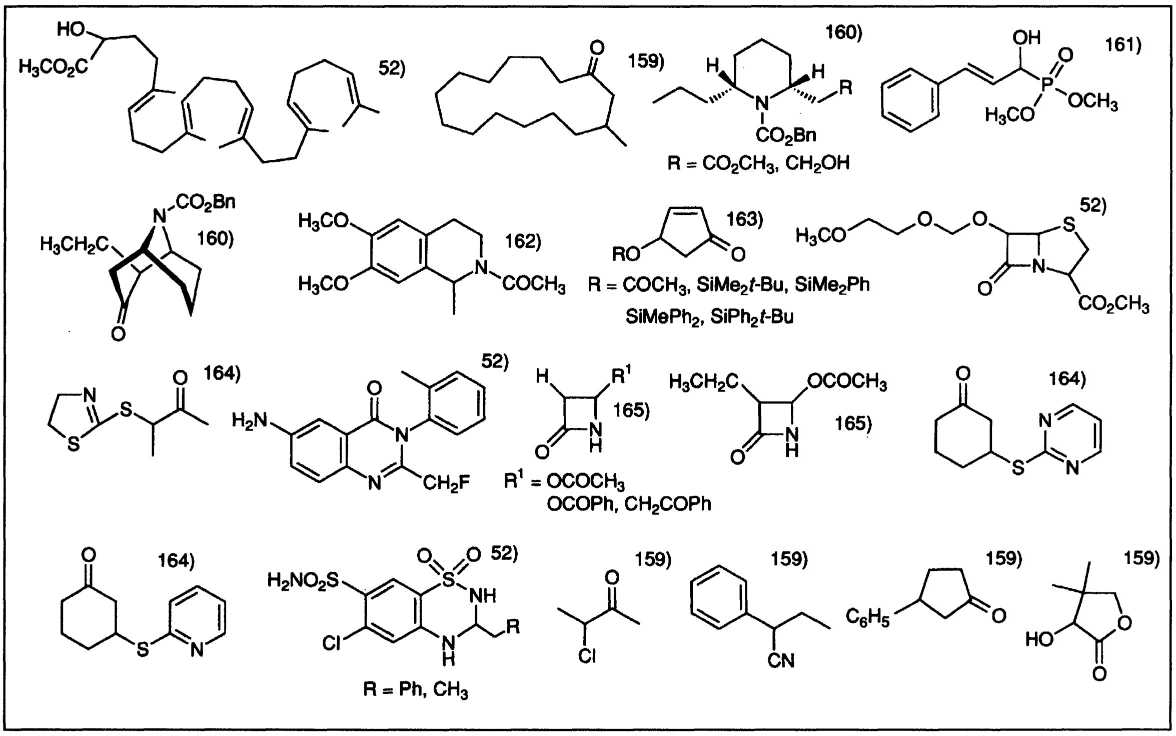


Fig. 19. Compounds resolved on amylose tris((*S*)-1-phenylethylcarbamate) (Chiralpak AS). The numbers next to the structures represent references.

Table 4. Separation Factors (α) in the Resolution on 3,5-Dimethylphenylcarbamates of Oligosaccharides[a)]

CSP Racemates[b)]	**71a**[b)] (*n*=2)	**71b**[c)] (*n*=4)	**72a** (*n*=2)	**72b**[c)] (*n*=3)	**72c** (*n*=4)	**72d** (*n*=5)	**72e** (*n*=6)	**73a** (*n*=6)	**73b** (*n*=7)	**73c** (*n*=8)
4	1.38(+)	ca. 1(+)	ca. 1(−)	1.00	1.34(+)	1.12(+)	1.11(+)	ca. 1(−)	1.15(−)	1.52(−)
18	ca. 1(−)	1.21(+)	ca. 1(+)	1.28(+)	1.11(+)	1.11(+)	ca. 1(+)	1.36(+)	1.37(+)	1.16(−)
57	1.00	Not eluted	ca. 1(+)		1.54(−)	1.59(−)	1.18(−)	Not eluted	1.00	1.10(−)
61	1.37(+)	1.00	1.00	1.31(+)	1.60(+)	1.21(+)	1.36(+)			
63	ca. 1(−)	1.13(−)	ca. 1	1.00	1.06(−)	1.14(−)	1.06(−)	1.30(−)	1.22(−)	1.05(+)

a) Column, 25×0.46 cm (i.d.); eluent, hexane–2-propanol (99/1); flow rate, 0.5 ml min^{-1}. The sign in parentheses shows optical rotation of the first-eluted isomer. b) Eluent, hexane–2-propanol (98/2). c) Eluent, hexane.

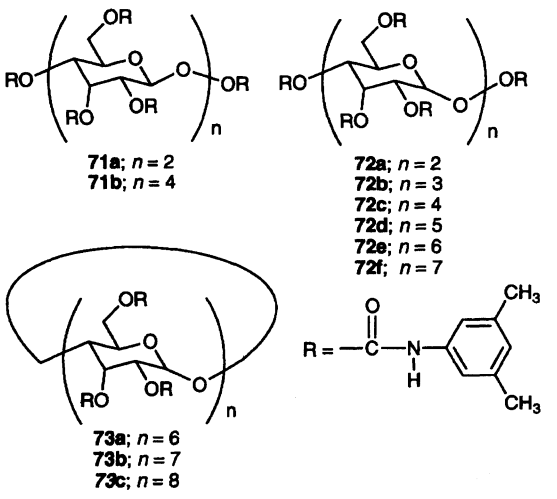


Fig. 20. Structures of 3,5-dimethylphenylcarbamates of cellooligosaccharides (**71**), maltooligosaccharides (**72**), and cyclodextrins (**73**).

3-position[169)] of the glucose units. The chiral recognition abilities of both CSPs are rather different, and the latter seems to show a higher chiral recognition ability.[170)]

Resolution by Polysaccharide Derivative Membranes

The present polysaccharide derivatives are of great advantage for the easy preparation of a film (membrane), in which one of the enantiomers can be preferentially adsorbed by enantioselective adsorption. The CDMPC membrane prepared by coating a THF solution of CDMPC on a Teflon® membrane filter as a support showed a high chiral recognition ability; for instance, oxprenolol (**74**; β-blocker) rich in (*S*)-(−)-isomer up to 60%ee was obtained by a single adsorption–desorption procedure.[171)] This system can be applicable to the enantioselective permeation of oxprenolol in an organic media.[172)]

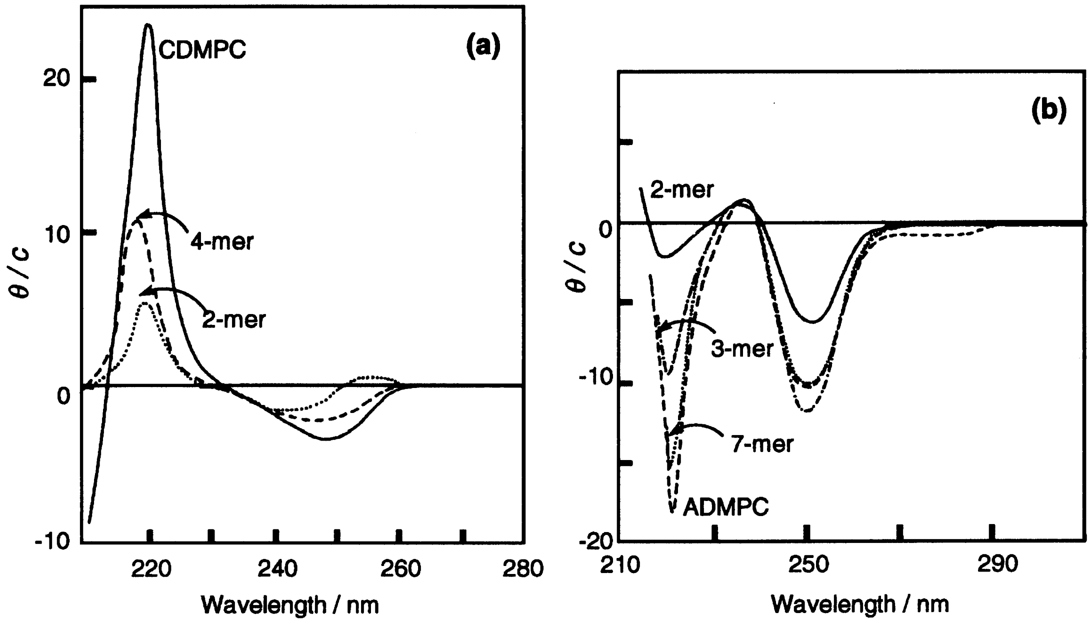


Fig. 21. CD spectra of 3,5-dimethylphenylcarbamates of cellooligosaccharides (**71**) and cellulose (**CDMPC**) (a), and those of maltooligosaccharides (**72**) and amylose (**ADMPC**) (b) in THF. The concentration was ca. 1 mg ml^{-1}.

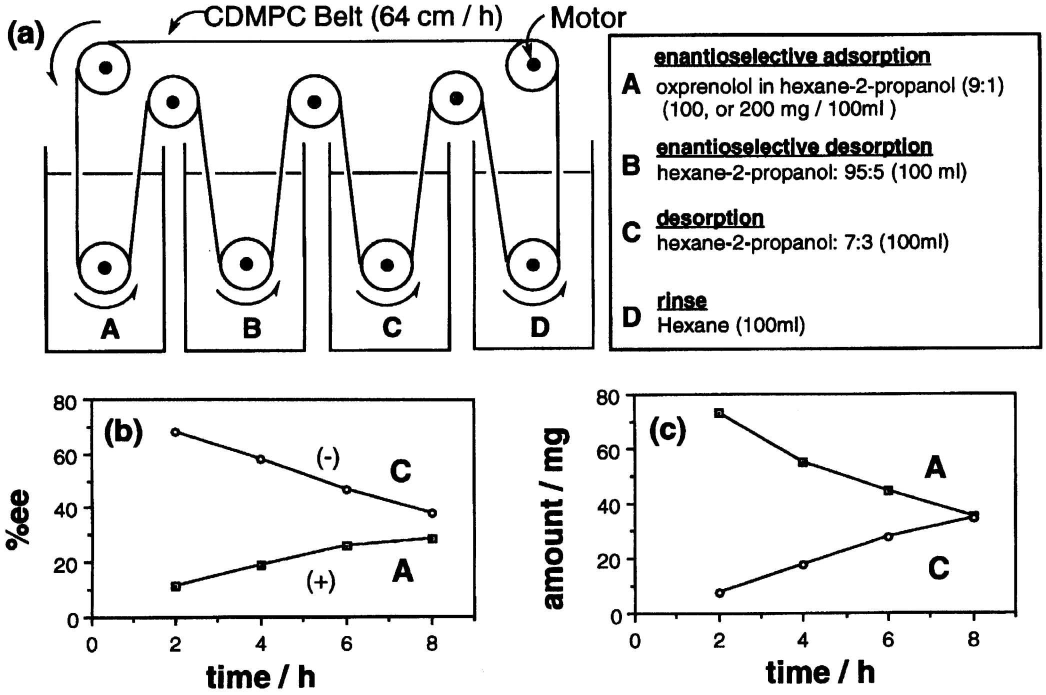
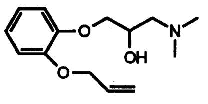
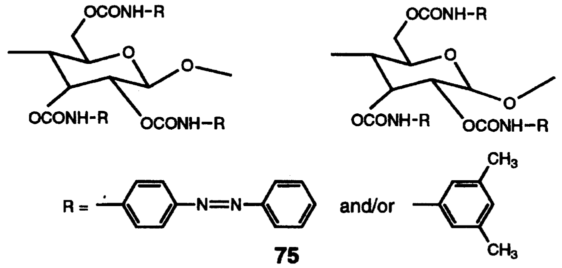


Fig. 22. An apparatus used in the resolution of (±)-oxprenolol (**74**) by *chiral belt* (a) and change in ee (b) and the amount (c) of **74** in the source (A) and receiving (C) phases; rotating rate, 64 cm h^{-1}; concentration of **74** in the source phase (A), 100 mg/100 ml.

Moreover, CDMPC can be utilized as a *chiral belt* for the continuous and preparative enantioseparation of **74** using a motor.[173)] An apparatus used for the resolution is illustrated in Fig. 22(a). The CDMPC belt (111 cm length) was fitted with the apparatus and rotated at a constant speed of 66 cm h^{-1} by a motor. The belt goes into a racemic solution of **74** (A) for enantioselective-adsorption, next into an enantioselective-desorption solvent (B), a desorption solvent (C), and then a rinse solvent (D). The source phase (A) gradually became rich in (*R*)-isomer, and reached up to 28%ee after 8 h when about 65 mg of **74** in the source phase was transported, while **74** in the receiving phase (C) was rich in (*S*)-isomer which shows more effective activity as a β-adrenergic blocking agent than (*R*)-isomer, up to 68%ee at the initial stage (after 2 h). A high level of



74



75

Chart 6.

enantioselectivity (67%ee at the initial stage) and high-speed transport of **74** by a factor of 1.5 were achieved, even when the concentration of **74** in the source phase (A) doubled (200 mg/100 ml).[173] The present method can be scaled up without any difficulty, and may be used for a large-scale separation.

The polysaccharide derivative membranes bearing a 4-phenylazophenyl residue (**75**) (Chart 6) responded sensitively to light, and/or heat and exhibited interesting features in enantioselective adsorption. The *trans* membranes showed higher enantioselectivity than did the *cis* membranes, and the selectivity could be reversibly controlled in an on-off fashion by photoisomerization of the pendant azobenzene moieties.[174]

Conclusion

A wide range of racemates, including aliphatic and aromatic compounds with or without functional groups and many pharmaceuticals, can be resolved on the CSPs derived from polysaccharide derivative CSPs. The utility of the polysaccharide CSPs for large-scale preparative separation of enantiomers is now generally realized. A regular higher-order structure of the polysaccharide derivatives must be responsible for effective chiral recognition. For further developments in this area, the elucidation of a chiral discrimination mechanism on the CSPs at a molecular level appears to be essential. Some interesting results with respect to the mechanism through a spectroscopic analysis and calculations have been accomplished. Elucidation of the exact chiral discrimination mechanism on the CSPs must serve to predict the elution order of enantiomers and to develop a more excellent polysaccharide-based CSP.

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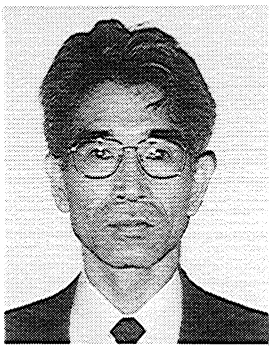
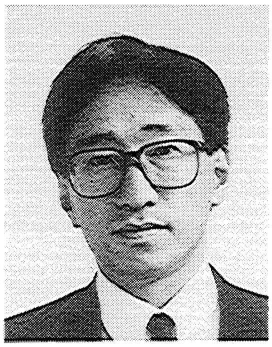


Yoshio Okamoto was born in Osaka in 1941. He received both his B.A. (1964) and Ph. D degrees (1969) from Osaka University, Faculty of Science. He joined Osaka University, Faculty of Engineering Science as an assistant in 1969, and spent two years (1970—1972) at the University of Michigan as a postdoctoral fellow with Professor C. G. Overberger. In 1983 he was promoted to Associate Professor, and in 1990 he moved to Nagoya University as Professor. His research interests include stereocontrol in polymerization, asymmetric polymerization, optically active polymers, and enantioseparation by HPLC. He received the Awards of the Society of Polymer Science, Japan in 1982 and the Chemical Society of Japan Award for Technical Development in 1991.



Eiji Yashima was born in Kyoto in 1958. He studied polymer chemistry at Osaka University, where he received his Ph. D on the asymmetric polymerization of methacrylates under the supervision of Professors K. Hatada and Y. Okamoto (Osaka University). After spending five years at Kagoshima University as Assistant Professor, where he worked with Professors N. Miyauchi and M. Akashi on the synthesis of functional polymers, he joined Okamoto's group at Nagoya University in 1991 and was appointed as Associate Professor in 1995. His current research concerns helical polymer synthesis with optical activity, enantioseparation by HPLC, and computational studies in chiral recognition.