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Review

Polysaccharide-based chiral stationary phases for high-performance liquid chromatographic enantioseparation

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Abstract

Recent developments of polysaccharide-based chiral stationary phases (CSPs) for the direct separation of enantiomers in high-performance liquid chromatography (HPLC) are mainly reviewed together with the results on mechanistic studies by means of chromatography, NMR and mass spectroscopies, and computational methods. Miscellaneous applications of polysaccharide derivatives to the newly developed, chiral dynamic high-performance liquid chromatography (DHPLC) for obtaining a nonracemic compound are also described.

Keywords: Reviews; Chiral stationary phases, LC; Enantiomer separation; Polysaccharides

Contents

1. Introduction

In the past two decades, chromatographic enantioseparations, particularly direct separation of enantiomers by high-performance liquid chromatography (HPLC), have advanced markedly and this resolution procedure has become one of the most useful methods in many fields dealing with drugs, natural products, agrochemicals, etc., not only for determining their optical purity, but also for obtaining optical isomers on a large scale [1–4]. Particularly, in the

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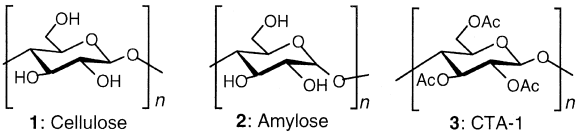
pharmaceutical industry, chiral HPLC has already been recognized to be essential for the research and development of chiral drugs [5–7]: detailed investigation of the pharmacokinetics, physiological, toxicological, and metabolic activities of both enantiomers is necessary before use. These trends have resulted in an increasing number of drugs marketed as single-isomer forms in the pharmaceutical industry; annual sales of single-isomer chiral drugs reached U.S.$96.4 billion in 1998 and will increase in the future [7]. Moreover, the recently developed simulated moving-bed (SMB) chromatography, allows large-scale, preparative separations of enantiomers on a ton scale which may accelerate the production of single-isomer chiral drugs [8–12].

The design and development of a chiral stationary phase (CSP) capable of effective chiral recognition of a wide range of enantiomers is the key point of the chiral HPLC technique. A number of CSPs for HPLC have been prepared and more than 100 CSPs have been commercialized. The CSPs usually consist of either small chiral molecules or chiral polymers immobilized on a support such as silica gel. The polymers can also be used as porous gel. Since details of the HPLC enantioseparation on these different types of CSPs have been described elsewhere [1–6], and chromatographic enantioseparation on polysaccharide-based CSPs as well as the effects of chromatographic conditions such as mobile phases and temperature on chiral recognition using CSPs have been thoroughly reviewed previously [13–18], this review mainly focuses on the recent development of the resolution of enantiomers on polysaccharide benzoate and phenylcarbamate derivatives as CSPs, which appear to be among the most used CSPs in organic, bioorganic, and pharmaceutical chemistry [8,13–18]. The mechanism of chiral discrimination on polysaccharide-based CSPs and their miscellaneous applications are also reviewed briefly in this article.

2. Polysaccharide esters and carbamates as CSPs

Polysaccharides such as cellulose (**1**) and amylose (**2**) are among the most abundant optically active biopolymers with perfectly defined structures and can resolve enantiomers including amino acid derivatives and atropisomeric biphenyl derivatives, although their chiral recognition ability is not high [19–21]. The first practically useful CSP derived from polysaccharides was prepared by Hesse and Hagel in 1973 [22], that is microcrystalline cellulose triacetate (**3**, CTA-I).

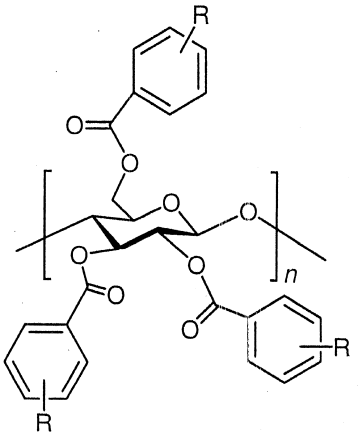


This CSP shows interesting chiral resolving properties in liquid chromatography and many aromatic and aliphatic enantiomers have been resolved on CTA-I [23,24], probably through an inclusion mechanism in the chiral cavities of the CTA-I matrix [22,25]. CTA-I has a merit, that is its high loading capacity, which makes it one of the most popular CSPs for large-scale, medium-pressure, liquid chromatographic separation of enantiomers [23,24].

Okamoto et al. previously succeeded in making a synthetic helical vinylpolymer with a predominantly one-handed screw-sense by asymmetric polymerization of triphenylmethyl methacrylate with chiral catalysts [26,27]. They later discovered that the resulting optically active poly(triphenylmethyl methacrylate) ((+)-PTrMA)) exhibits a high chiral recognition ability for various racemic compounds with aromatic groups [28], especially when (+)-PTrMA was adsorbed on macroporous silica gel [29,30]. With this procedure, a practically useful CSP with higher resistance to compression could easily be prepared. (+)-PTrMA was the first commercialized CSP derived from a synthetic chiral polymer (Chiralpak OT(+) from Daicel). Since then, most chiral polymer-based CSPs have been prepared by coating them on silica gel.

Okamoto et al. applied this procedure to the preparation of a coated-type CSP derived from CTA-I. This new CTA afforded another useful CSP [31,32] and the chiral recognition ability was completely different from that of CTA-I. These findings aroused great interest in the preparation of derivatized polysaccharides, including their benzoates and phenylcarbamates, as CSPs by coating them on silica gel.

The structures of the tribenzoates (**4**) and phenylcarbamates (**5**) of cellulose prepared by

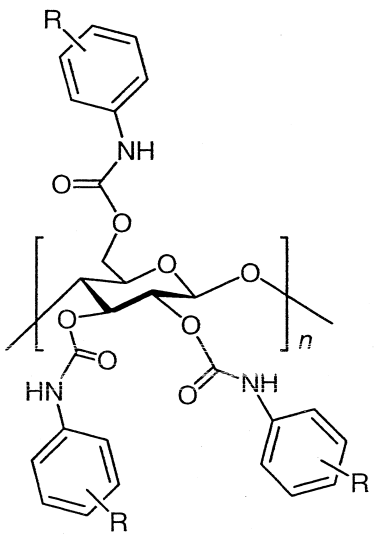


4: R =
a: 4-CH_3O
b: 3,5-$(CH_3O)_2$
c: 4-$(CH_3)_3C$
d: 4-CH_3
e: 3-CH_3
f: 2-CH_3
g: H
h: 4-F
i: 4-CF_3
j: 3,5-$(CH_3)_2$
k: 3,5-Cl_2

Scheme 1. Structures of cellulose tribenzoates.

Okamoto et al. are shown in Schemes 1 and 2, respectively [16–18,33,34]. These CSPs resolved a wide range of racemates having various functional groups, depending greatly on the substituents on the phenyl groups. An inductive effect of the substituents on enantioselectivity was observed, but the derivatives with heteroatom substituents, such as methoxyl and nitro groups, showed low chiral recognition because of the high polarity of the substituent itself [33,34]. Among the benzoates, cellulose tris(4-methylbenzoate) (**4d**, Chiralcel OJ (**OJ**)) exhibites very high chiral recognition for various racemic compounds, including drugs, and appears to be a practically useful CSP. Some racemic compounds recently resolved on **OJ** [35–45] and cellulose tribenzoate (**4g**, Chiralcel OB (**OB**)) [46–48] are shown in Fig. 1.

Mannschreck prepared microcrystalline cellulose tribenzoate powders [49] and Francotte prepared spherical beads of cellulose tribenzoate derivatives [50,51]. They directly used these bulk cellulose benzoates as CSPs for a preparative purpose because of their high loading capacity. Francotte pointed out the importance of the supramolecular structure for chiral recognition on the basis of resolution results of many racemates on the CSPs derived from the spherical beads [50,51]. Wainer and co-workers proposed the attractive binding-steric fit formulation mechanism for the retention of enantiomers on the basis of the separation of a series of enantiomers [52] which involved hydrogen bonding and dipole–dipole interaction rather than inclusion. Oguni et al. found that **OJ** also recognized the enantiomers of 1-phenylethanol in solution by means of ^{13}C-NMR spectroscopy as well as in HPLC [53,54]; several carbon resonances of 1-phenylethanol were split into the enantiomers in the presence of **OJ**. This NMR



5: R =		
a: 4-CH_3O	**q**: 4-Br	**ag**: 2-Cl-5-CH_3
b: 4-C_2H_5O	**r**: 4-I	**ah**: 2-Cl-6-CH_3
c: 4-$(CH_3)_2CHO$	**s**: 4-CF_3	**ai**: 3-Cl-2-CH_3
d: 4-$(CH_3)_2CHCH_2O$	**t**: 4-NO_2	**aj**: 3-Cl-4-CH_3
e: 4-$(CH_3)_3Si$	**u**: 2,5-$(CH_3)_2$	**ak**: 4-Cl-2-CH_3
f: 4-CH_3	**v**: 3,4-$(CH_3)_2$	**al**: 4-Cl-3-CH_3
g: 4-CH_3CH_2	**w**: 3,5-$(CH_3)_2$	**am**: 5-Cl-2-CH_3
h: 4-$(CH_3)_2CH$	**x**: 3,5-$(CH_3)_2$-4-CH_3O	**an**: 3-F-4-CH_3
i: 4-$(CH_3)_3C$	**y**: 2,6-$(CH_3)_2$	**ao**: 5-F-2-CH_3
j: 3-CH_3	**z**: 3,4,5-$(CH_3)_3$	**ap**: 4-F-3-CH_3
k: 2-CH_3	**aa**: 3,5-Cl_2	**aq**: 3-F-5-CH_3
l: H	**ab**: 3,4-Cl_2	**ar**: 3-Cl-5-CH_3
m: 4-F	**ac**: 2,6-Cl_2	**as**: 3-Br-5-CH_3
n: 4-Cl	**ad**: 3,5-F_2	**at**: 4-Ph-N=N
o: 2-Cl	**ae**: 3,5-$(CF_3)_2$	
p: 3-Cl	**af**: 2-Cl-4-CH_3	

Scheme 2. Structures of cellulose trisphenylcarbamates.

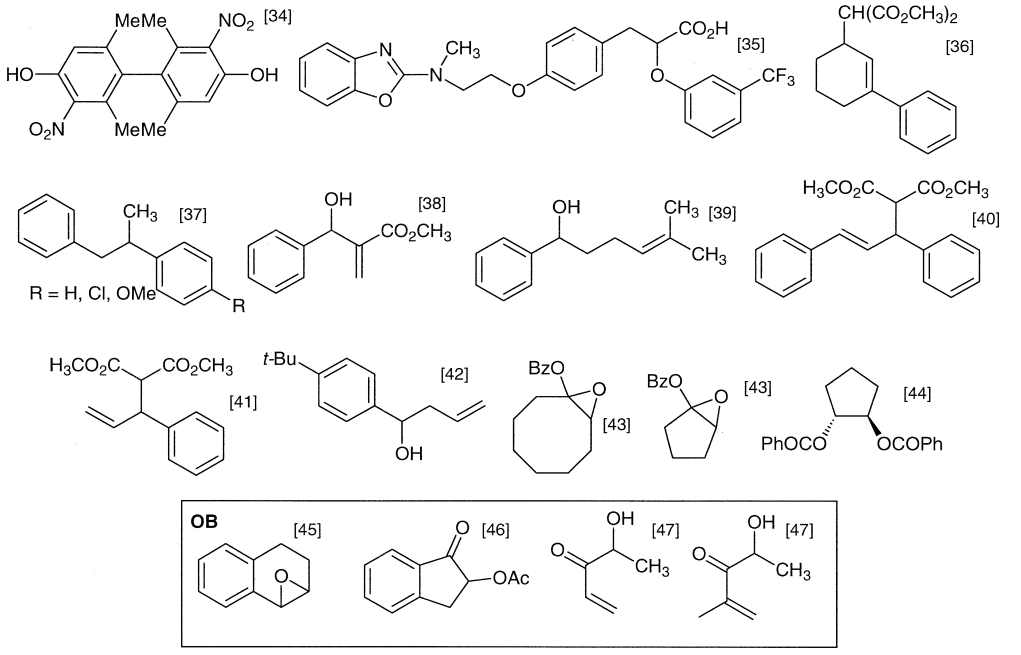
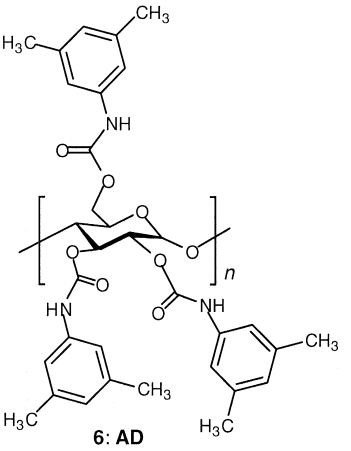


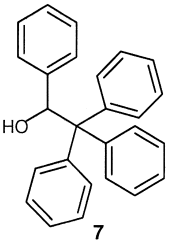
Fig. 1. Compounds resolved on Chiralcel OB or Chiralcel OJ. The numbers next to the structures represent literature references.

method appears to be useful for elucidating the chiral discrimination mechanism at a molecular level.

Okamoto's group prepared a series of cellulose trisphenylcarbamate derivatives (Scheme 2) and investigated intensively the effects of substituents on the enantioseparation and proposed a mechanism of chiral discrimination based on chromatographic, computational, and spectroscopic methods [16,18]. Among the prepared tris(phenylcarbamate) derivatives of cellulose, tris(3,5-dimethylphenylcarbamate) of cellulose (**5w**, Chiralcel OD (**OD**)) [34] shows particularly interesting and excellent resolving ability for a variety of racemic compounds, including many stereochemically interesting organic compounds and chiral drugs [13–18]. Similarly, amylose tris(3,5-dimethylphenylcarbamate) (**6**, Chiralpak AD (**AD**)) exhibits quite excellent enantioselectivity for a variety of racemates [55]. Some racemic compounds recently resolved on **OD** and **AD** are shown in Fig. [56–72] and Fig. 3 [73–89], respectively, and Fig. 4 [90–93] and Fig. 5 [94–97] show typical chromatograms of the resolution of stereochemically interesting organic compounds and chiral drugs on phenylcarbamates of polysaccharides. The universal usefulness of these CSPs is also described in recent review articles [16–18]. Other phenylcarbamates of cellulose and amylose show a characteristic enantioselectivity depending on the enantiomers. For instance, amylose tris(4-chlorophenylcarbamate) resolved 1,2,2,2-tetraphenylethanol (**7**) with a separation factor (α) of 8.29 [98].



6: **AD**



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Recently, a series of phenylcarbamate derivatives having both an electron-donating methyl group and an electron-withdrawing halogen group on the phenyl moiety were prepared to evaluate their chiral

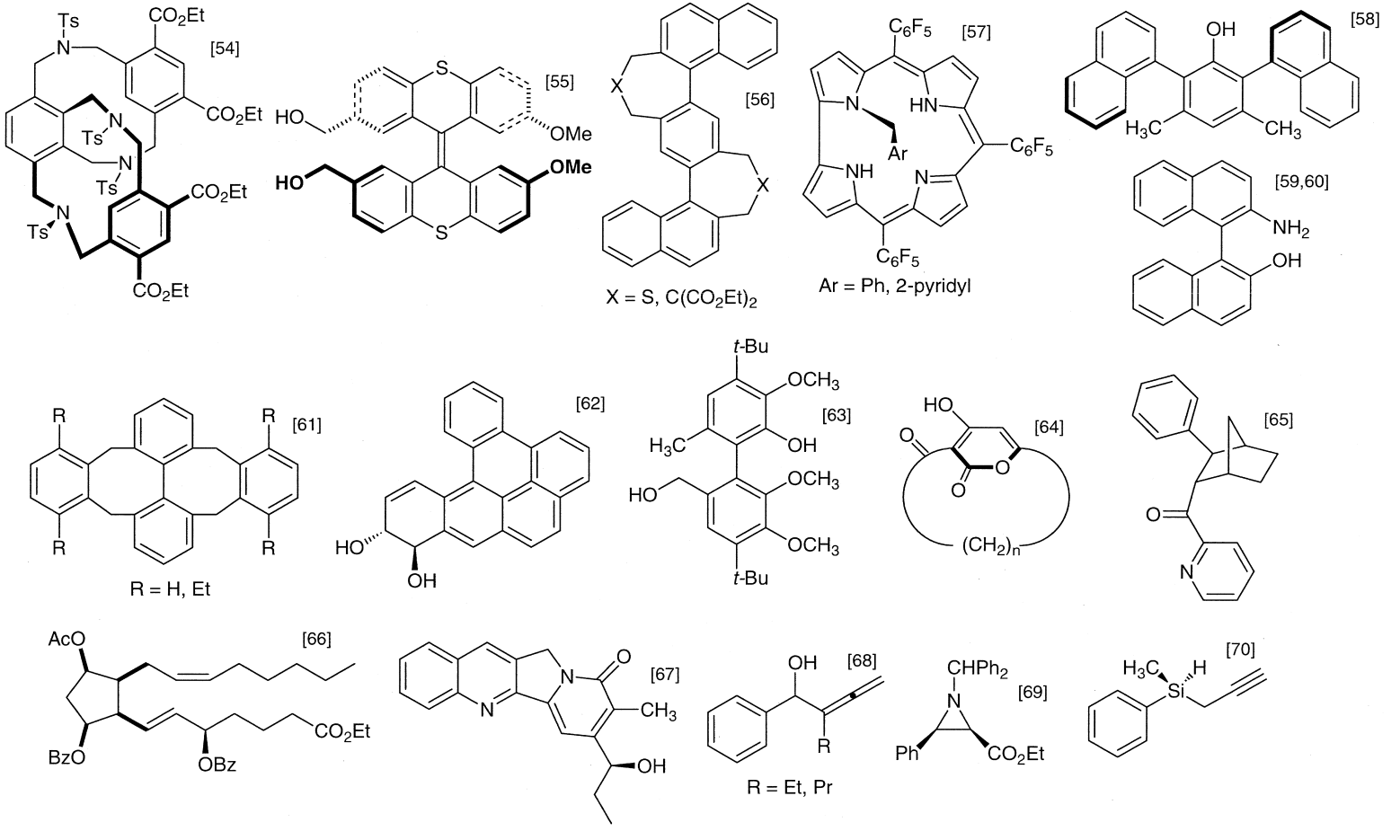


Fig. 2. Compounds resolved on Chiralcel OD. The numbers next to the structures represent literature references.

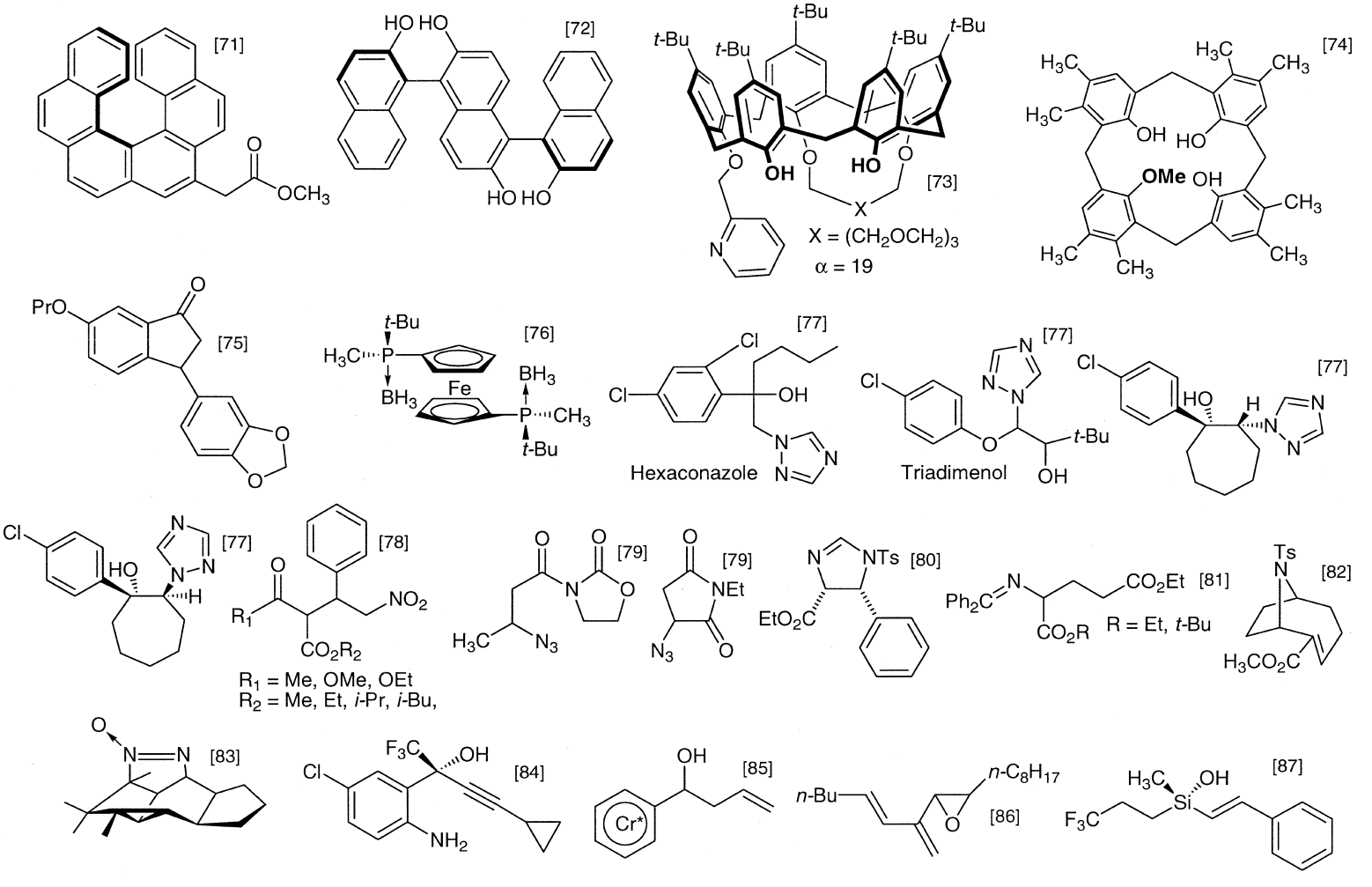


Fig. 3. Compounds resolved on Chiralpak AD. The numbers next to the structures represent literature references.

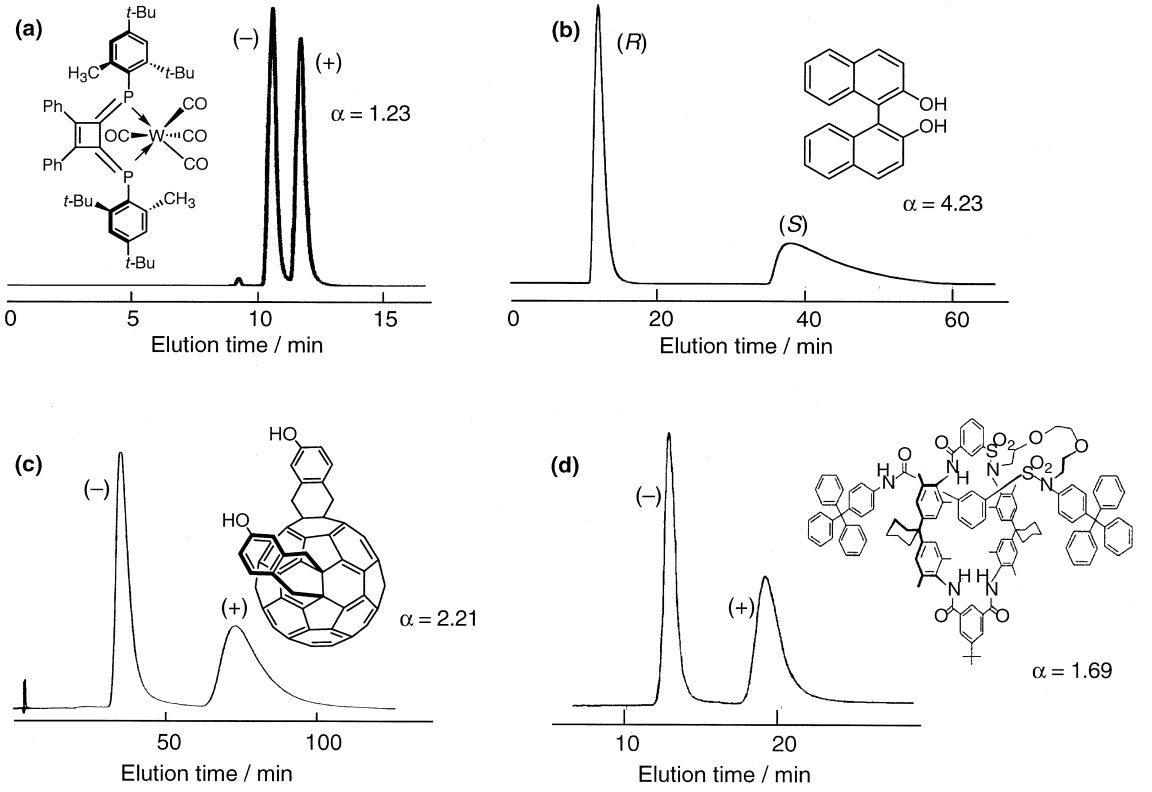
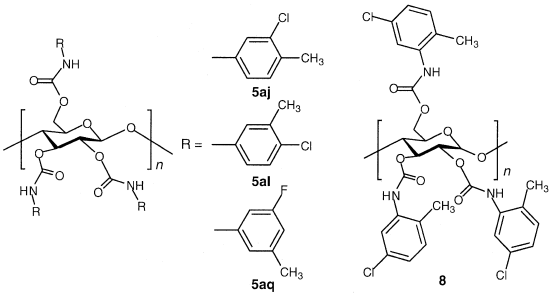


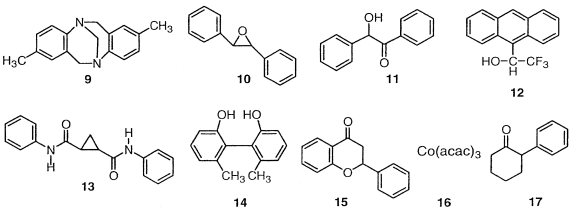
Fig. 4. Chromatograms of the separation of a metal-containing compound, 3,4-bis(2,4-di-*tert*.-butyl-6-methylphenylphosphinidene)-1,2-diphenylcyclobutene·$W(CO)_4$ (a) [90], 1,1′-bi-2-naphthol (b) [91], a chiral C_{60} derivative (c) [92], and a chiral rotaxane (d) [93] on cellulose tris((3,5-dimethylphenylcarbamate) (**OD**) (a,c,d) and cellulose tris(5-fluoro-2-methylphenylcarbamate) (**5ao**) (b). Column, 25×0.46 cm (I.D.); eluent, hexane (a), hexane–2-propanol (90:10 (b), 70:30 (c)), and hexane–ethanol (85:15) (d); flow-rate, 0.5 ml/min (a,d), 1.0 ml/min (b,c). Chromatograms (a) and (d) are reproduced, with permission, from Ref. [90] (Copyright 1997, Chemical Society of Japan) and from Ref. [93] (Copyright 1997, American Chemical Society), respectively.

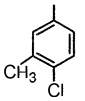
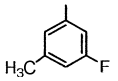
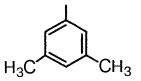
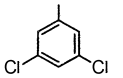
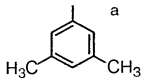
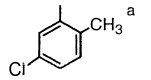
recognition abilities [99–102]. Among them, the 3-chloro-4-methyl- (**5aj**) [99], 4-chloro-3-methyl- (**5al**) [99], and 3-fluoro-5-methylphenylcarbamates (**5aq**) [102] of cellulose and 5-chloro-2-methylphenylcarbamates (**8**) of amylose [100] were found to be practically useful CSPs and showed high chiral recognition ability.



The resolution results for 10 racemates (**7**, **9**–**17**) on these CSPs using separation factors (α) are shown in Table 1 together with those on **OD** and **AD**. Some racemates were resolved better on these mixed-type CSPs than **OD** and **AD**. The introduction of both an electron-donating group and an electron-withdrawing group on the phenyl moieties may modify the polarities of the carbamate residues. Some chiral drugs (or intermediates) (**18**–**23**) were better resolved on the mixed CSPs [102–104]. Generally, substitution at the *meta*- and/or *para*-position improved the resolution ability for cellulose phenylcarbamates, but *ortho*-substitution decreased the chiral recognition ability. However, in contrast, the *ortho*-substituted amylose phenylcarbamates such as **8** showed relatively high chiral recognition [100]. This may be ascribed to the differences in their

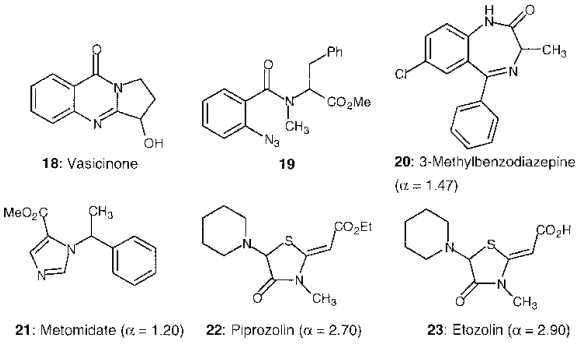
Table 1
Separation factors (α) in the resolution of racemates (**7**, **9**–**17**) on phenylcarbamate derivatives of cellulose and amylose[a]



Racemate	CSP					
	 **5al**	 **5aq**	 **5w (OD)**	 **5aa**	 **6**: **AD**	 **8**
7	3.05 (+)	1.55 (+)	1.34 (+)	1.29 (+)	1.98 (+)	1.67 (+)
9	1.13 (+)	1.58 (+)	1.32 (+)	1.65 (+)	1.67 (+)	1.13 (−)
10	3.25 (+)	2.41 (+)	1.68 (−)	1.84 (+)	3.04 (+)	1.70 (+)
11	1.23 (−)	1.44 (−)	1.58 (+)	1.21 (−)	1.21 (−)	1.16 (+)
12	1.25 (−)	1.42	2.59 (−)	1.38 (−)	1.15 (+)	1.14 (−)
13	1.54 (−)	1.11	3.17 (−)	1.41 (+)	2.01 (+)	3.09 (+)
14	1.35 (−)	1.71 (−)	1.83 (−)	1.11 (+)	2.11 (−)	1.28 (+)
15	1.06 (−)	1.11 (−)	1.41 (−)	1.20 (−)	1.12 (+)	1.18 (+)
16	1.44 (+)[c]	1.49 (+)	ca. 1 (+)	1.82 (+)	ca. 1 (−)	1.18 (+)
17	1.26 (−)	1.36 (−)	1.15 (−)	1.26 (−)	ca. 1 (−)	1.24 (−)

[a] Conditions: column, 25×0.46 cm I.D.; eluant, hexane–2-propanol (90:10); flow-rate, 0.5 ml/min. The sign of optical rotation of the first-eluted isomer is shown in parentheses.
[b] Phenylcarbamates of amylose.
[c] Eluant, hexane–2-propanol (98:2).

higher-order structures. Left-handed 3/2 and 4/1 helical chain conformations were postulated for tris(phenylcarbamates) of cellulose (**5l**, CTPC) [105] and amylose (ATPC) [106], respectively. These different higher-order structures must be responsible for the different influence of the substituents on the resolving power of the cellulose and amylose phenylcarbamates.



OD and **AD** were regioselectively bonded to silica gel at the 2, 3 and 6 positions of glucose units with 4,4′-diphenylmethylene as a spacer to improve durability (Fig. 6) [107]. A variety of eluents can be used in chiral HPLC using these bonded-type CSPs. However, these CSPs show slightly lower chiral recognition than the coated-type CSPs, because reaction with the diisocyanate may occur through some hydroxyl groups of the polysaccharides, which will cause an alternation of higher-order structures of the polysaccharides. Similar chemically bonded-type polysaccharide-based phases have also been prepared through radical polymerization of allyl groups of silica gels and a polysaccharide derivative bearing vinyl groups [108].

Chemically bonding to silica gel at either end of the polysaccharide chains may be ideal. For **AD**, this ideal chemical bonding to silica gel succeeded [109]: amylose with the desired chain length was prepared

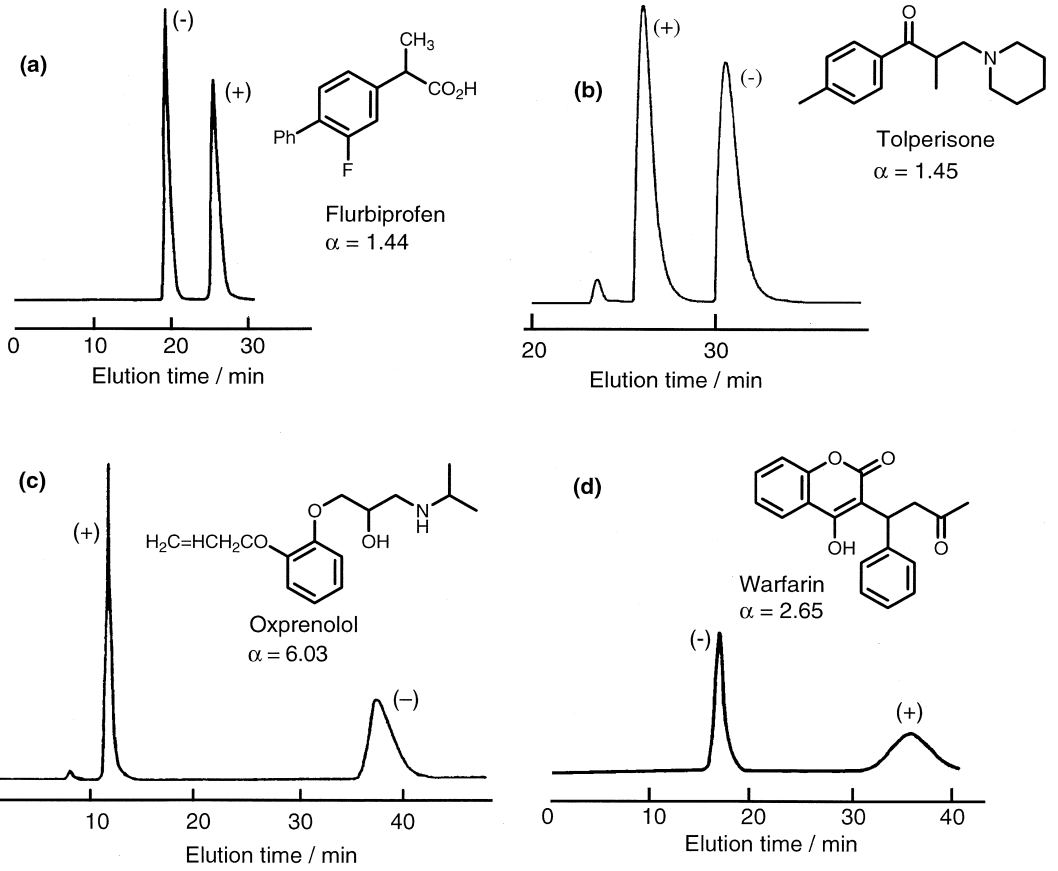


Fig. 5. Chromatograms of the separation of flurbiprofen (a) [94], tolperisone (b) [95], oxprenolol (c) [96] and warfarin (d) [97] on amylose tris(3,5-dimethylphenylcarbamate) (**AD**) (a), xylan bis(3,5-dichlorophenylcarbamate) (b), and cellulose tris(3,5-dimethylphenylcarbamate) (**OD**) (c,d). Column, 25×0.46 cm (I.D.); eluent, hexane–2-propanol–trifluoroacetic acid (95:5:1) (a), hexane–2-propanol (90:10) (b), hexane–2-propanol–Et_2NH (80:20:0.1) (c), and hexane–2-propanol–HCO_2H (80:20:0.1) (c); flow-rate, 0.5 ml/min. Chromatograms (a), (b), and (c) are reproduced, with permission, from Ref. [94] (Copyright 1989, Wiley–VCH), Ref. [95] (Copyright 1998, Elsevier), and Ref. [96] (Copyright 1986, Chemical Society of Japan), respectively.

by the polymerization of α-D-glucose 1-phosphate dipotassium salt with functionalized maltooligosaccharides using a phosphorylase from potato. The amylose was then bonded to silica gel at the reducing terminal residue, and was allowed to react with 3,5-dimethylphenyl isocyanate to afford a CSP with an excellent resolving ability comparable to that of the coated-type **AD**. The bonded-type **AD** shows high durability against many organic solvents, including tetrahydrofuran (THF) and chloroform. Francotte recently developed an alternative method to improve the durability of polysaccharide-based CSPs using a cross-linking technique (Scheme 3) [7].

Although alkylcarbamates, such as methyl- and cyclohexylcarbamates of cellulose, did not afford a useful CSP, some of the tris(aralkylcarbamate) of cellulose and amylose, particularly tris((*S*)-1-phenylethylcarbamate) of amylose (**24**, Chiralpak AS (**AS**)), exhibit high chiral recognition [110,111]. This CSP resolved racemates such as β-lactam antibiotics better than other polysaccharides carbamates including **OD** and **AD**.

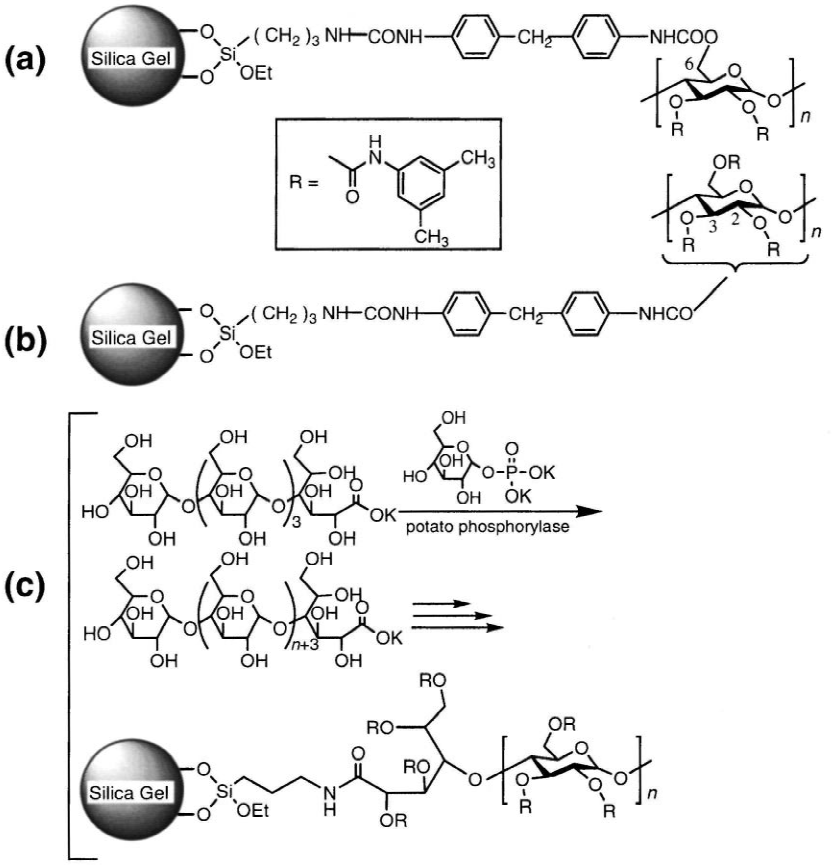
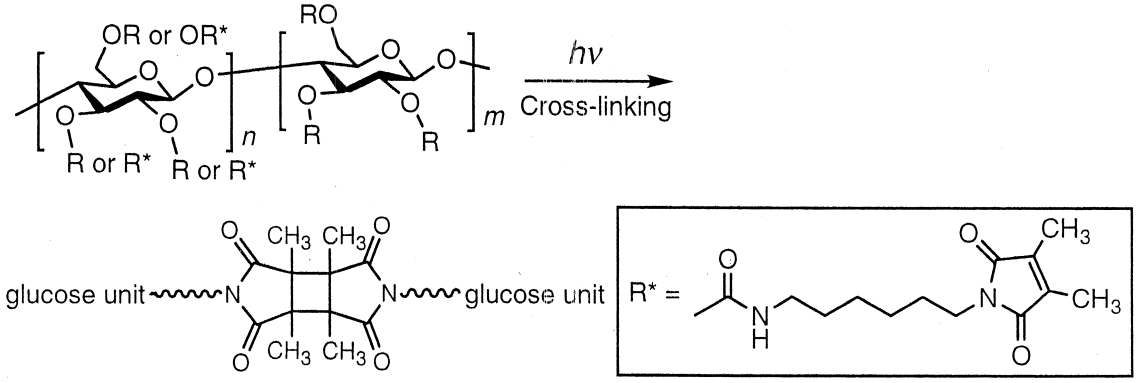
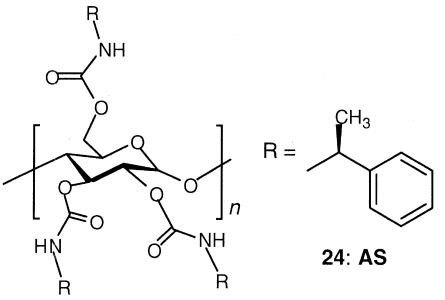


Fig. 6. Structures of chemically bonded-type ADMPC phases. The synthetic scheme of ADMPC bound on silica gel through enzymatic polymerization is also shown in (c).

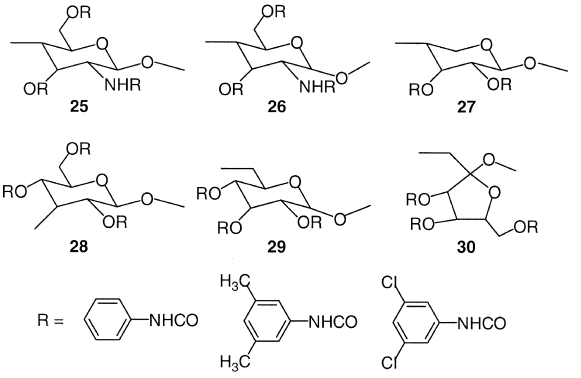


Scheme 3. Synthesis of polysaccharide-based CSPs through cross-linking [7].



24: **AS**

Trisphenylcarbamates, tris(3,5-dichloro- or 3,5-dimethylphenylcarbamate) of other polysaccharides such as chitosan (**25**), galactosamine (**26**), xylan (**27**), curdlan (**28**), dextran (**29**), and inulin (**30**) were also prepared and their chiral recognition abilities evaluated [95]. The chiral recognition abilities depended markedly on the nature of the monosaccharide units, the linkage position, and the linkage type. Among the polysaccharide derivatives, the 3,5-dimethylphenylcarbamates of chitosan and xylan and the 3,5-dichlorophenylcarbamate of galactosamine showed relatively high chiral recognition ability, although the 3,5-dimethylphenylcarbamates of cellulose and amylose often exhibit better resolving power. However, some racemates were better resolved on the phenylcarbamates than the corresponding cellulose and amylose derivatives.



3. Mechanism of chiral recognition on phenylcarbamates of polysaccharides

The chiral recognition mechanism of polysaccharide-based CSPs has been investigated extensively, usually based on chromatographic methods. This approach may give a lot of information, particularly thermodynamic parameters for the interactions between solutes and the CSPs during the resolution [3]. However, in order to understand chiral recognition at a molecular level, spectroscopic approaches including NMR combined with computer modeling seem to be necessary. Phenylcarbamates of cellulose are one of the most intensively investigated CSPs on the basis of chromatographic, computational, and spectroscopic methods and detailed results have already been reviewed elsewhere [18,91]; therefore, here, the author briefly summarizes the results.

As described above, the chiral recognition abilities of phenylcarbamates of polysaccharides are greatly influenced by the substituents on the phenyl moieties, since the substituents modify the polarity of the carbamate groups. This suggests that the most important adsorbing sites for chiral discrimination on phenylcarbamate derivatives may be the polar carbamate groups.

Fig. 7 shows the possible structure of CTPC obtained by molecular mechanics calculation based on the proposed structure of CTPC by X-ray analysis [105] and possible interaction sites of CTPC. CTPC possesses a left-handed three-fold (3/2) helix and glucose residues are regularly arranged along the helical axis. A chiral helical groove with polar carbamate residues exists along the main chain. The polar carbamate groups are nicely located inside, and hydrophobic aromatic groups outside, the polymer chain and, therefore, enantiomers coming from outside the groove can effectively interact with the polar carbamate groups via hydrogen bonding with the NH and C=O groups and dipole–dipole interaction on the C=O [34]. Therefore, the nature of the substituents on the phenyl groups affects the polarity of the carbamate residues, which leads to different chiral resolving powers.

Besides these polar interactions, $\pi-\pi$ interactions between the phenyl group of a CSP and an aromatic group of the solute may play some role in chiral recognition, particularly in reversed-phase mode HPLC separation.

3.1. NMR studies

CTPC appears to maintain its helical structure even in solution [112]. However, most phenylcarba-

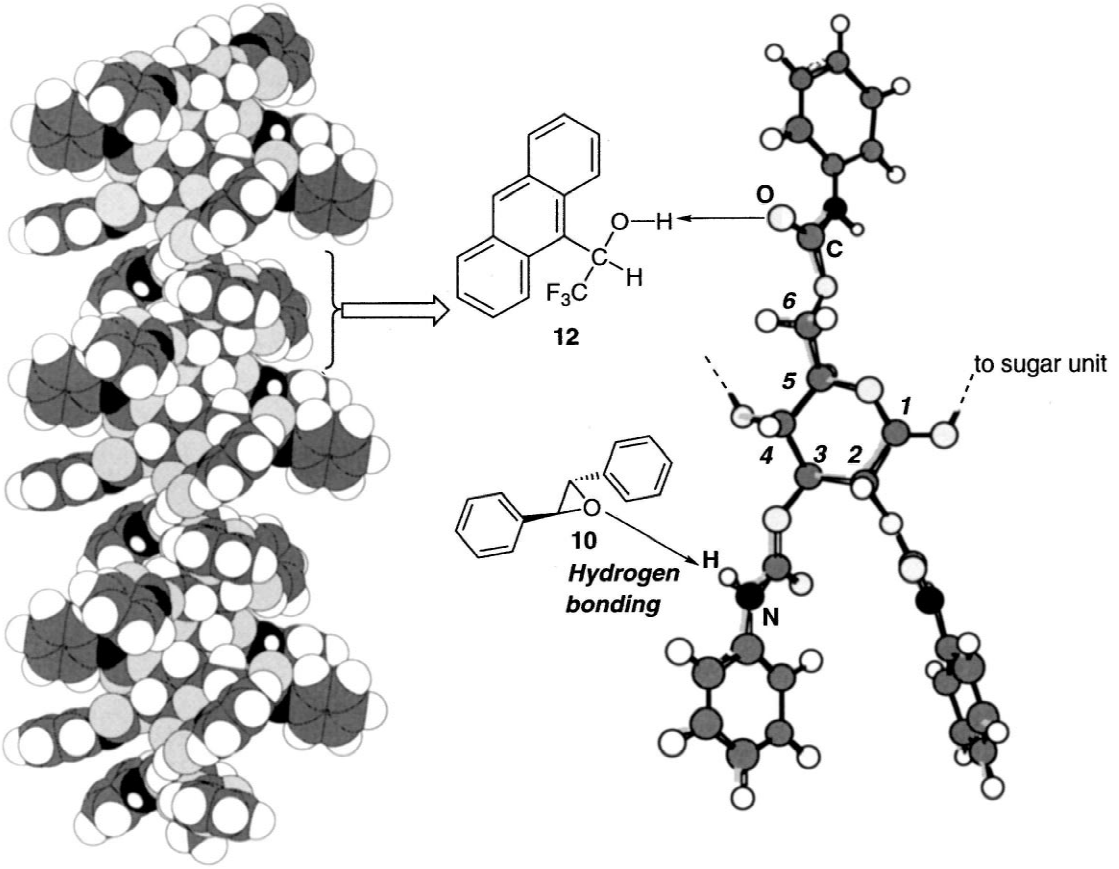


Fig. 7. Optimized structure (left) and possible interaction sites (right) of CTPC derivatives.

mate derivatives with high chiral resolving ability as CSPs are soluble only in polar solvents such as pyridine and THF. In such polar solvents, chiral discrimination of enantiomers by NMR cannot be detected because of the strong interaction of the solvents with the polar carbamate residues. However, some phenylcarbamate derivatives, for instance tris(4-trimethylsilylphenylcarbamate) (**5e**) [113] and tris(5-fluoro-2-methylphenylcarbamate) (**5ao**) [101], of cellulose were found to be soluble in chloroform and showed chiral discrimination for many racemic compounds in ^{1}H- and ^{13}C-NMR spectroscopies as well as in HPLC [114].

Fig. 8 shows the 500 MHz ^{1}H-NMR spectra of (±)-*trans*-stilbene oxide (**10**) in the presence and absence of **5e**. The methine proton of **10** was separated into two singlets in the presence of **5e** and only the methine proton of the (−)-isomer shifted downfield [113]. This indicates that **5e** can discriminate the enantiomers even in solution and may interact strongly with the (−)-isomer. In the chromatographic separation of racemic **10** on the CSP, the (+)-isomer eluted first, followed by the (−)-isomer and complete baseline separation was attained. This elution order is well correlated with the downfield shift of the (−)-isomer observed in ^{1}H-NMR. **5e** could discriminate other various enantiomers in solution and the structures of racemates enantiomerically recognized by **5e** are shown in Fig. 8, indicating that **5e** can work as a chiral shift reagent [114].

5ao is also soluble in chloroform and can discriminate the enantiomers of atropisomeric 1,1′-bi-2-naphthol (**31**) and 2,2′-dihydroxy-6,6′-dimethylbiphenyl in ^{1}H- and ^{13}C-NMR with large chemical shift differences of the enantiomers as well

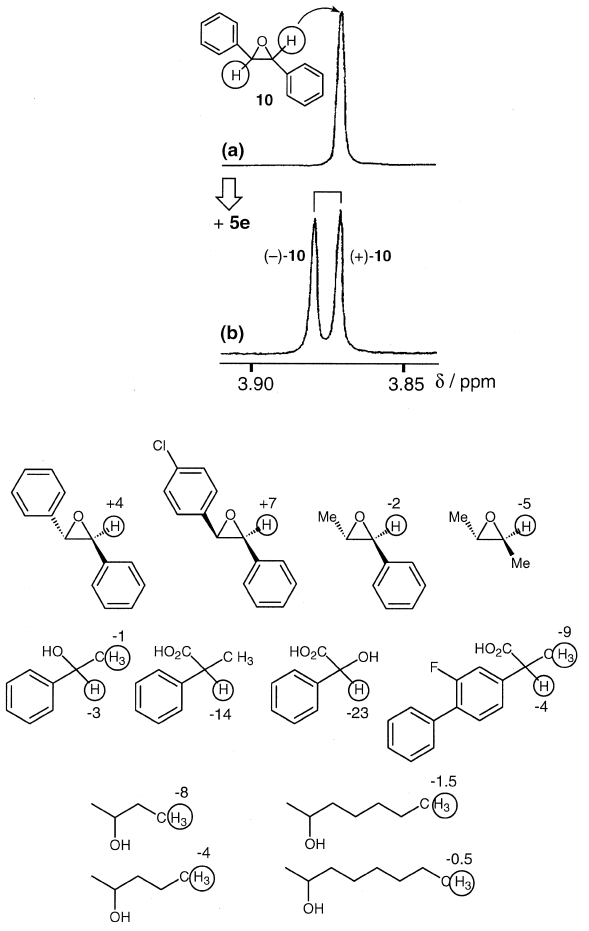
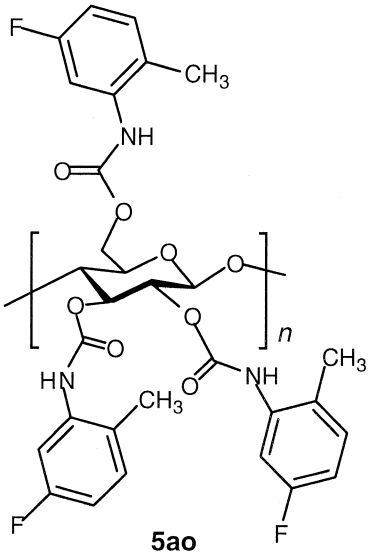


Fig. 8. ^{1}H-NMR spectra of the methine proton resonance region of *trans*-stilbene oxide (5 mg, **10**) in the absence (a) and presence (b) of cellulose tris(4-trimethylsilylphenylcarbamate) (40 mg, **5e**) in $CDCl_3$ (1.0 ml) at 22°C. Peak assignment was carried out with enantiomerically pure (+)- and (−)-**10**. Compounds enantiomerically recognized by **5e** in ^{1}H-NMR are also shown; figures represent ΔΔδ (ppb) and a negative value indicates an upfield shift [114].

as in HPLC with large separation factors ($\alpha > 3$) (for the chromatographic resolution result of **31** on CSP **5ao**, see Fig. 4b). Complete baseline separation of **31** and the biphenyl derivative was achieved with the elution order of enantiomers: the (*R*)-isomers eluted first followed by the (*S*)-isomers. In the ^{1}H- and ^{13}C-NMR spectra of **31** in the presence of **5ao**, the hydroxy and some aromatic protons and carbon resonances of **31** and the biphenyl derivative are clearly separated into a pair of peaks due to the nonequivalence of complexation-induced chemical shifts for the enantiomers. The binding geometry and dynamics between **5ao** and the enantiomers of **31** were investigated on the basis of spin-lattice relaxation time, ^{1}H-NMR titrations, and intermolecular NOEs in the presence of **5ao**. Based on these results, combined with molecular modeling, it was found that (*S*)-**31** site-selectively binds to the phenylcarbamoylated cellulose derivative **5ao** through multiple interactions, including intermolecular hydrogen bonding, π–π and/or CH–π interactions, to afford a 1:1 complex [91]. These results should provide useful information, both for understanding the chiral discrimination mechanism of the other polysaccharide-based CSPs and for designing more effective CSPs.



3.2. Mass spectrometric studies

The stronger binding of (*S*)-**31** than (*R*)-**31** to **5ao** was also detected through a mass spectrometric study [115,116]. The relative peak intensity (RPI) of complexes of a chiral host with deuterated and nondeuterated guests has recently been used for detecting chiral discrimination events in chiral host–guest chemistry [117].

A mixture of (*RS*)-**31** and **5ao** was ionized by electron ionization (EI) operated by temperature programming at 32°C/min from 25 to 400°C. Reconstructed ion current (RIC) profiles of (*R*)- and (*S*)-**31**

showed different shapes, indicating the different adsorption and/or desorption of **31** from the chiral adsorbent **5ao**. Direct chiral discrimination in EI-MS was confirmed using partially deuterated **31** at the 3 and 3′ positions. A mixture of (*S*)-**31** and (*R*)-**31**-d_2 or (*S*)-**31**-d_2 and (*R*)-**31** in $CHCl_3$ containing **5ao** was directly inserted into the ion source to measure EI mass spectra.

The mass spectra at scan numbers 30–35, 80–85, and 140–145 in RIC profiles of (*S*)-**31**-d_2 (M_r 288) and (*R*)-**31** (M_r 286) with **5ao** are shown in Fig. 9a, b, and c, where the differences in the ratios of *m*/*z* 288 to *m*/*z* 286 are clearly detected. A plot of the ratio of *m*/*z* 288 to 286 (the mean value of six scans) versus the scan number is also shown in Fig. 9d. At the beginning of the scan, the relative peak intensity of *m*/*z* 286 was larger than that of *m*/*z* 288, which increased as the sample temperature was raised, while the former (*m*/*z* 286) decreased. These results indicate that the molecule of M_r 288 [(*S*)-**31**-d_2] vaporizes more slowly at higher temperature than the molecule of M_r 286 [(*R*)-**31**]. When a mixture of (*S*)-**31** and (*R*)-**31**-d_2 was used instead, the relative intensity of *m*/*z* 286 to 288 (the reciprocal of the above ratio in Fig. 9d) showed the same tendency.

When optically inactive polystyrene was used as adsorbent, no difference in the relative peak intensity at *m*/*z* 288 to 286 was detected. Moreover, in the resolution of (*RS*)-**31** and (*RS*)-**31**-d_2 on the CSP, no isotope effect was observed. These results clearly indicate that the difference in EI mass spectra is due to the difference in desorption between the enantiomers from the chiral adsorbent **5ao**. This method can be used to discriminate the chirality of other enantiomers of small molecules, if they show peaks in their EI mass spectra in the presence of chiral polymers. Similar chiral recognition was also detected by negative ion fast-atom bombardment mass spectrometry (FAB-MS) [116].

3.3. Computational studies

Computer simulations involving molecular mechanics (MM) and molecular dynamics (MD) are also

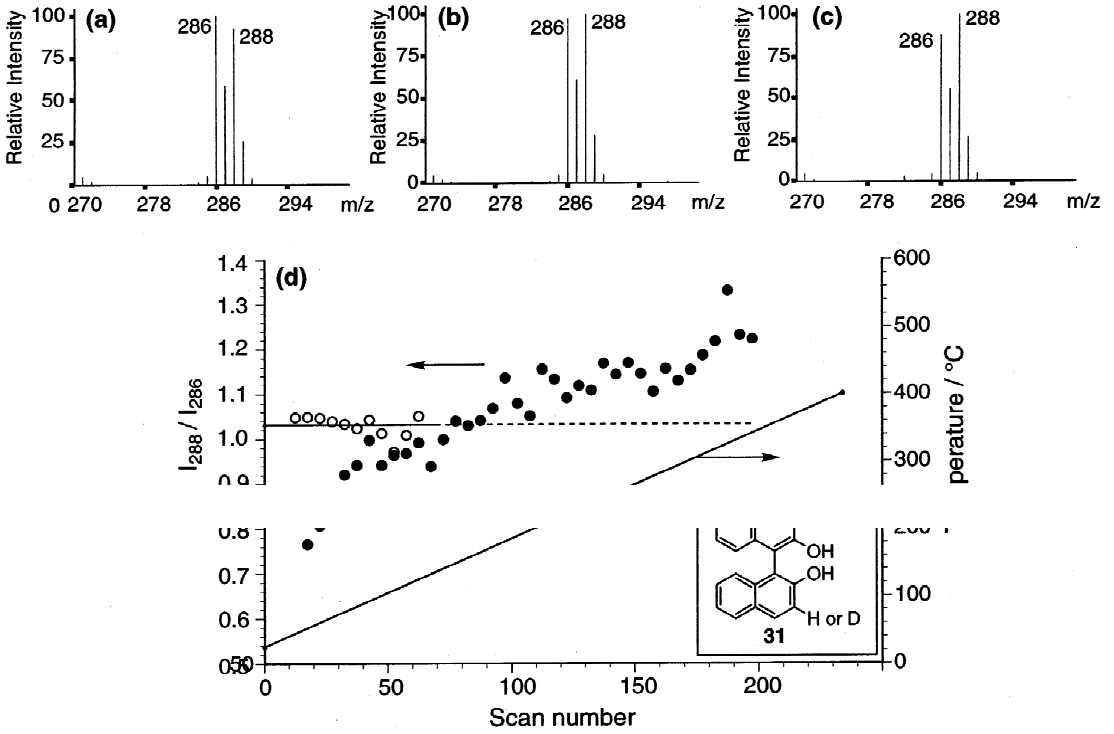


Fig. 9. Mass spectra of a mixture of (*S*)-**31**-d_2 (M_r 288) and (*R*)-**31** (M_r 286) in the presence of **5ao** at scan numbers 30–55 (a), 80–85 (b), and 140–145 (c) in RIC profiles and ratio of the peak intensity versus scan number with (○) and without (●) **5ao** (d).

applied to calculate the interaction energies between the CSPs and enantiomers. Especially, Lipkowitz et al. have been extensively studying the mechanism of chiral recognition from a theoretical viewpoint [118,119]. For $CDCl_3$-soluble phenylcarbamate derivatives of polysaccharides, one can use the above NMR techniques. However, most phenylcarbamates of polysaccharides with high resolving ability are soluble only in polar solvents. For such $CDCl_3$-insoluble phenylcarbamates, computer simulation involving MM and MD calculations will be a useful and effective approach for elucidating the chiral recognition mechanism and for predicting the elution order of enantiomers.

Calculations of the interaction energies between CTPC or **OD** and *trans*-stilbene oxide (**10**) or benzoin (**11**) were recently performed by various methods using different force fields [120]. The calculations were roughly divided into two methods. (1) Enantiomers were generated and tumbled around each NH proton and the C=O oxygen of the 2, 3, and 6 positions of the carbamate group of CTPC or **OD** at specified angles which are considered to be the most important adsorption sites, and the interaction energy was then calculated at each point of a grid on the CTPC or **OD** molecule using all possible combinations of the rotation angles of the enantiomer. (2) Enantiomers with a particular orientation were randomly generated by the Monte Carlo method on the surface of CTPC and **OD** defined by the particular van der Waals radius using the reported technique of 'blowing up' the atomic radii [121], and then the interaction energy was calculated. The calculation results were evaluated with the averaged interaction energy. In both calculations, an octamer or nanomer of CTPC and **OD** molecules constructed based on the X-ray data of CTPC, followed by optimization with MM and MD calculations, was used. Enantiomers were generated on the middle part of the polymers so as to avoid the influence of the end groups of the polymers. Detailed calculation results are not described here, but the results of both calculations were in good agreement with the results for the chromatographic resolution of **10** and **11** by CTPC and **OD** [120].

Fig. 10 shows the computer graphics of the interaction between CTPC and the (*S,S*)-(−)-**10** with the lowest hydrogen bond energy. The (*S,S*)-(−)-**10** is bound in a chiral groove, and each phenyl group may interact with the phenyl groups of CTPC

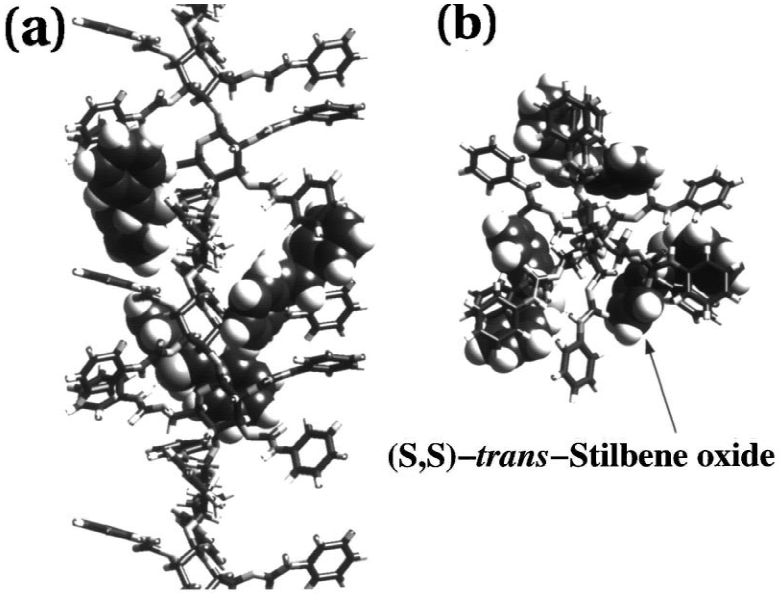


Fig. 10. Calculated structure of the CTPC–(*S,S*)-**10** complex formed through hydrogen bonds. View along the helix axis (a) and perpendicular to the helix axis (b). (Reproduced, with permission, from Ref. [120]. Copyright 1999, Chemical Society of Japan.)

through π–π interactions; the ether oxygen atom of (*S*,*S*)-(−)-**10** is located near the NH proton of CTPC and can form a hydrogen bond. (*S*,*S*)-(−)-**10** apparently enters CTPC to form a hydrogen bond with the NH proton of the carbamoyl group.

These methods are useful for a qualitative understanding of the chiral recognition mechanism of cellulose phenylcarbamates, although the use of MD calculations will be needed to simulate the dynamic behavior of the interactions occurring in chromatography. This approach is not restricted to the study of chiral recognition and is applicable to a variety of host–guest interaction systems.

4. Miscellaneous applications

4.1. Dynamic high-performance liquid chromatography

Chromatographic enantioseparation on CSPs can be used for obtaining both optically pure enantiomers in 50% yield separately. However, when the newly developed, chiral dynamic high-performance liquid chromatography (DHPLC) is applied to stereolabile enantiomers at high temperatures, nonracemic compounds can be obtained from a racemic mixture [122]. DHPLC is a powerful tool for investigating dynamic processes of interconverting enantiomers [123–128] and kinetic data and enantiomerization barriers for stereolabile compounds can be obtained from a series of temperature-dependent plateaus and peak shapes by chiral DHPLC (Fig. 11a) [123–128]. The use of CSPs for the equilibrium of interconverting enantiomers opens the possibility for combining separation techniques and equilibrium shift in one step; in principle, one enantiomerically pure enantiomer may be obtained from a racemate in 100% yield.

Fig. 11b shows a typical HPLC chromatogram for the resolution of a racemic spiro compound (**32**) on **OD**. The spiro compound enantiomerizes thermally and photochemically through [1,6]-ring-opening of the C–O bond next to the spiro center and consecutive ring closure. Compound **32** is stable enough at 20°C and can be completely resolved without any enantiomerization while, at higher temperatures (50°C), the enantiomerization processes lead to plateau-like elution profiles (Fig. 11c).

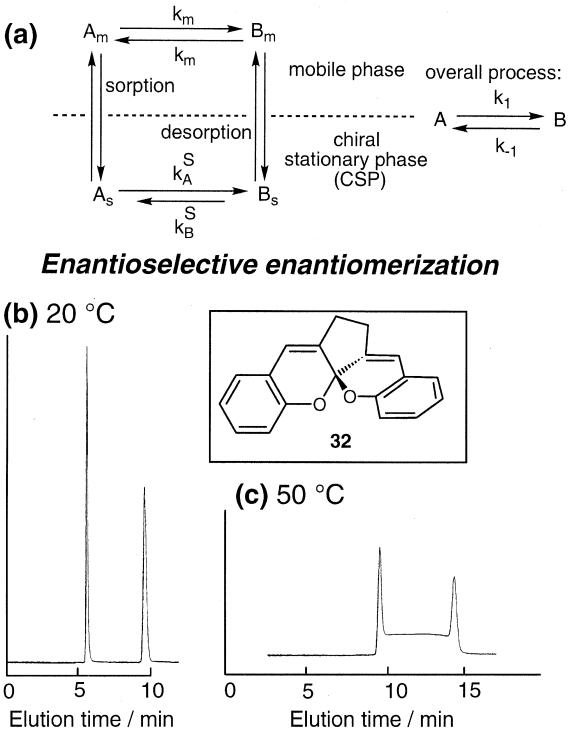


Fig. 11. Processes occurring during DHPLC (a), where A and B represent the first- and second-eluted enantiomer and the indices m and s indicate the enantiomer in the mobile phase and a sorbed enantiomer. Chromatograms for the resolution of spiro compound **32** on **OD** with hexane–2-propanol (90:10) as eluent at 20 (b) and 50°C (c) are also shown; flow-rate, 1.0 (b) and 0.25 ml/min (c). (Reproduced, with permission, from Ref. [122]. Copyright 1998, Wiley–VCH.)

In the heterogeneous CSP–mobile phase system, the adsorbed, longer-retaining enantiomer (B) is enriched, whereas the eluent may contain a racemic mixture under equilibrium conditions. Accordingly, only a racemic mixture is expected on elution under the usual conditions (constant temperature and flow). However, when the time interval after elution of the first peak (A) until elution of the second (B) can be kept as short as possible, enantioselective enantiomerization may be possible. Experiments were carried out as follows. After injection of a racemic solution of **32** into the HPLC system, the mobile-phase flow (1.5 ml/min) was stopped after 2 min. After an appropriate time (1–6 h) for equilibration, the mobile-phase flow was restarted, which gave chromatograms showing an excess of the second-

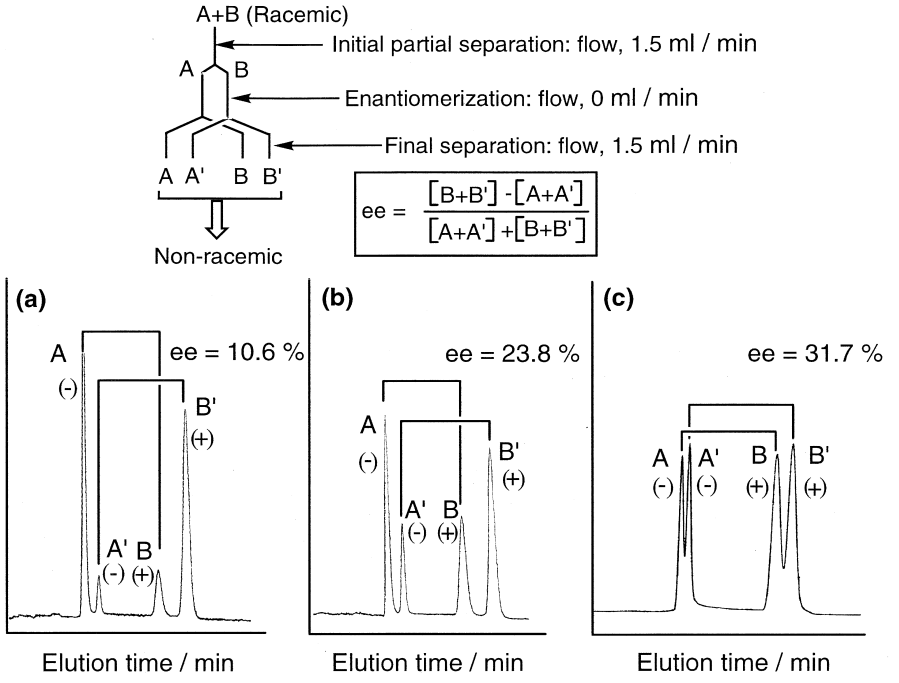


Fig. 12. DHPLC chromatograms of compound **32** on **OD** with hexane–2-propanol (99:1) as eluent at 40°C. Equilibration time: 16 min (a), 57 min (b) and 6 h (c). (Reproduced, with permission, from Ref. [122]. Copyright 1998, Wiley–VCH.)

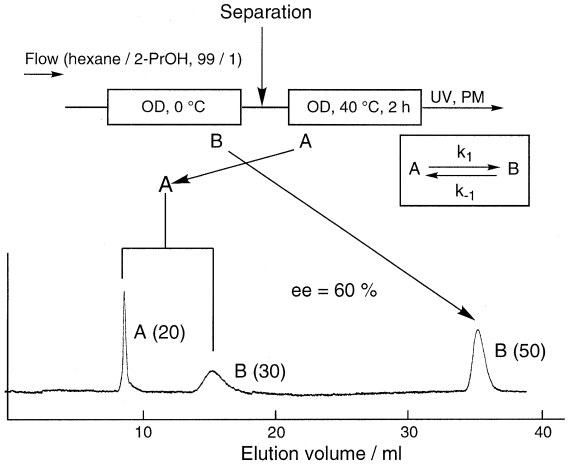


Fig. 13. Two-column system for enantioselective enantiomerization of one enantiomer of **32** during chiral DHPLC. The chromatogram for spiro compound **32** in enantioselective enantiomerization on the CSPs is also shown. (Reproduced, with permission, from Ref. [122]. Copyright 1998, Wiley–VCH.)

eluted enantiomer. The chromatograms usually show two peaks for the enantiomers, but in the case of high α values ($\alpha = 2.29$), four peaks appeared (Fig. 12). Peaks A and A′ as well as B and B′ were derived from enantiomers already separated during the initial separation and nonracemic mixtures up to 32%ee were obtained by chiral DHPLC.

To increase the enantiomeric excess of **32** by chiral DHPLC, the following two CSPs systems were employed (Fig. 13). The basic idea is that enantiomerization occurs on the second CSP at relatively high temperature, but thermal interconversion might be suppressed on the first CSP at low temperature. After the first-eluting enantiomer A had left the first column at 0°C and had been transported to the second at 40°C, the flow was stopped for enantioselective enantiomerization of A (2 h). Three peaks could be detected in the chromatogram, as expected (Fig. 13); the first and second peaks were due to A (ca. 20 mol%) and B (ca. 30 mol%), respectively, and peak B was derived from A on the second CSP by enantioselective enantiomerization. The last peak is noninterconverted B (50 mol%).

Finally, nonracemic **32** of 60%ee was obtained by this simple stop-and-flow technique combined with an effective CSP. The equilibrium between A and B was not completely reached after 2 h and if equilibrium had been completely attained, the enantiomeric excess may be even higher (68%ee). More cycles of enantioselective equilibration of one enantiomer will lead to an enantiomerically pure compound.

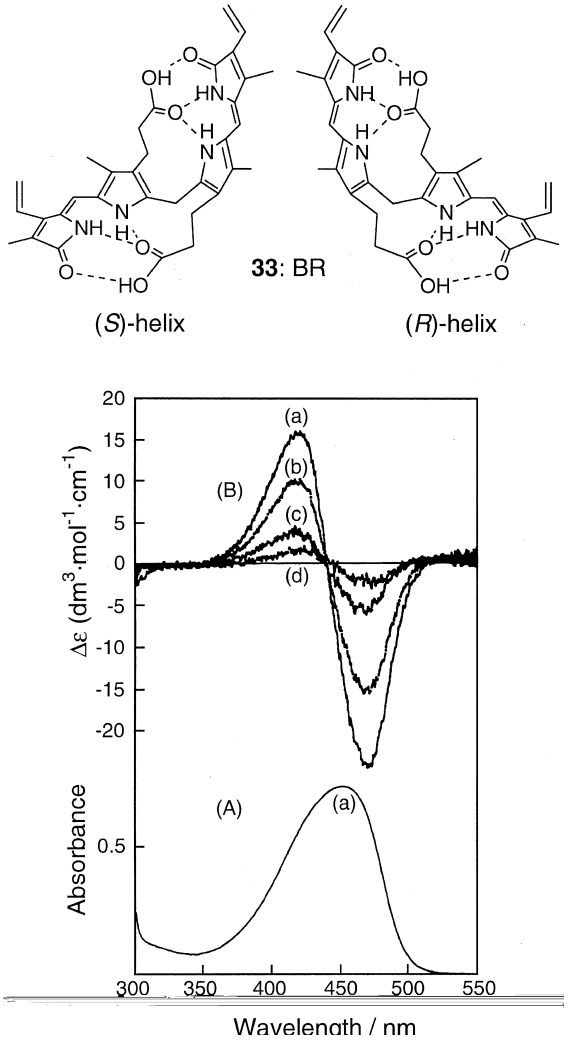


Fig. 14. Two enantiomeric conformations of bilirubin and UV–visible (A) and CD (B) spectra of bilirubin ($3\cdot10^{-4}$ *M*) in chloroform–ethanol in the presence of CTPC derivatives ($3\cdot10^{-2}$ *M* glucose units); **5q** (a), **5p** (b), **5f** (c), and **5ad** (d). (Reproduced, with permission, from Ref. [129]. Copyright 1996, Gordon and Breach.)

Chiral DHPLC may be useful in order to transform an interconvertible racemic mixture into an excess of one enantiomer and to separate the enantiomers at the same time. Particularly, where only one enantiomer is of interest, chiral DHPLC may be a new approach to obtain enantiomerically pure compounds in 100% yield.

4.2. Induced circular dichroism of bilirubin

As mentioned above, some cellulose trisphenylcarbamate derivatives are soluble in chloroform and exhibit chiral discrimination for a variety of enantiomers in NMR as well as in HPLC. It was found that these phenylcarbamate derivatives could induce the chirality of enantiomeric guests such as (4*Z*,15*Z*)-bilirubin IXα (**33**, BR). This was confirmed based on its asymmetric conformation in chloroform and in a film by means of CD spectroscopy [129]. BR is not optically active, but has two enantiomeric helical conformations maintained by six intramolecular hydrogen bonds between two carboxylic acid moieties and two pyrromethenone NH protons (Fig. 14). These (*R*)- and (*S*)-helical conformers are in dynamic equilibrium in an achiral solution [130], but some optically active compounds can enantioselectively bind to BR to induce circular dichroism (CD) spectra in solution [131–133].

A significant induced CD was observed in the UV–visible region for BR in the presence of some CTPC derivatives in chloroform. Fig. 14 shows typical CD spectra of BR ($3\cdot10^{-4}$ *M*) in $CHCl_3$ in the presence of CTPC derivatives (**5f**, **5p**, **5q**, and **5ad** in Scheme 2; $3\cdot10^{-2}$ glucose unit *M*). The intensity of the induced CD for the chiral bichromophoric BR was significantly influenced by the kind and position of substituents on the phenyl group of CTPC. Especially, *para*-halogenated CTPC derivatives induced the most intense CD. The exciton-coupling theory predicts that an enantiomer of BR having an (*S*)-helix conformation is bound preferentially to CTPC derivatives. The intermolecular hydrogen bond between the phenylcarbamoyl moieties of the CTPC derivatives and BR is presumed to be the main binding force for the asymmetric transformation of BR, because addition of a small amount of 2-propanol to a chloroform solution of BR

containing CTPC derivatives markedly diminishes the CD signal. In the film state, CTPC–BR complexes also exhibit an induced CD identical in pattern and sign to those in solution, and the intensity is greater than that observed in chloroform for the same molar ratio. The CTPC film incorporated one of the enantiomeric BR in excess and the film may be used as a device for a chiroptical switch.

5. Conclusion

Polysaccharide-based CSPs can resolve a wide range of racemic compounds including many chiral drugs. Some benzoates and phenylcarbamates of cellulose and amylose as well as CTA are commercially available. The utility of polysaccharide-based CSPs for the preparative scale separation of enantiomers has already been recognized [12–18]. Particularly, SMB chromatography has great potential for the industrial scale preparation of pure enantiomers and has already been employed [8–12]. SMB chromatography is a continuous solid–liquid countercurrent process. It allows continuous feeding of the racemic mixture and collection of each enantiomer, and thus large amounts of eluent can be saved.

Elucidation of the chiral discrimination mechanism on CSPs at a molecular level appears to be essential for further developments in this exciting area. Understanding of chiral recognition at a molecular level is of particular importance for further developments of more effective CSPs, and some approaches to the clarification of the chiral recognition mechanism on polysaccharide-based CSPs for HPLC have been attempted by means of chromatography, NMR, and computational methods. The results will serve to develop more effective polysaccharide-based CSPs.

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