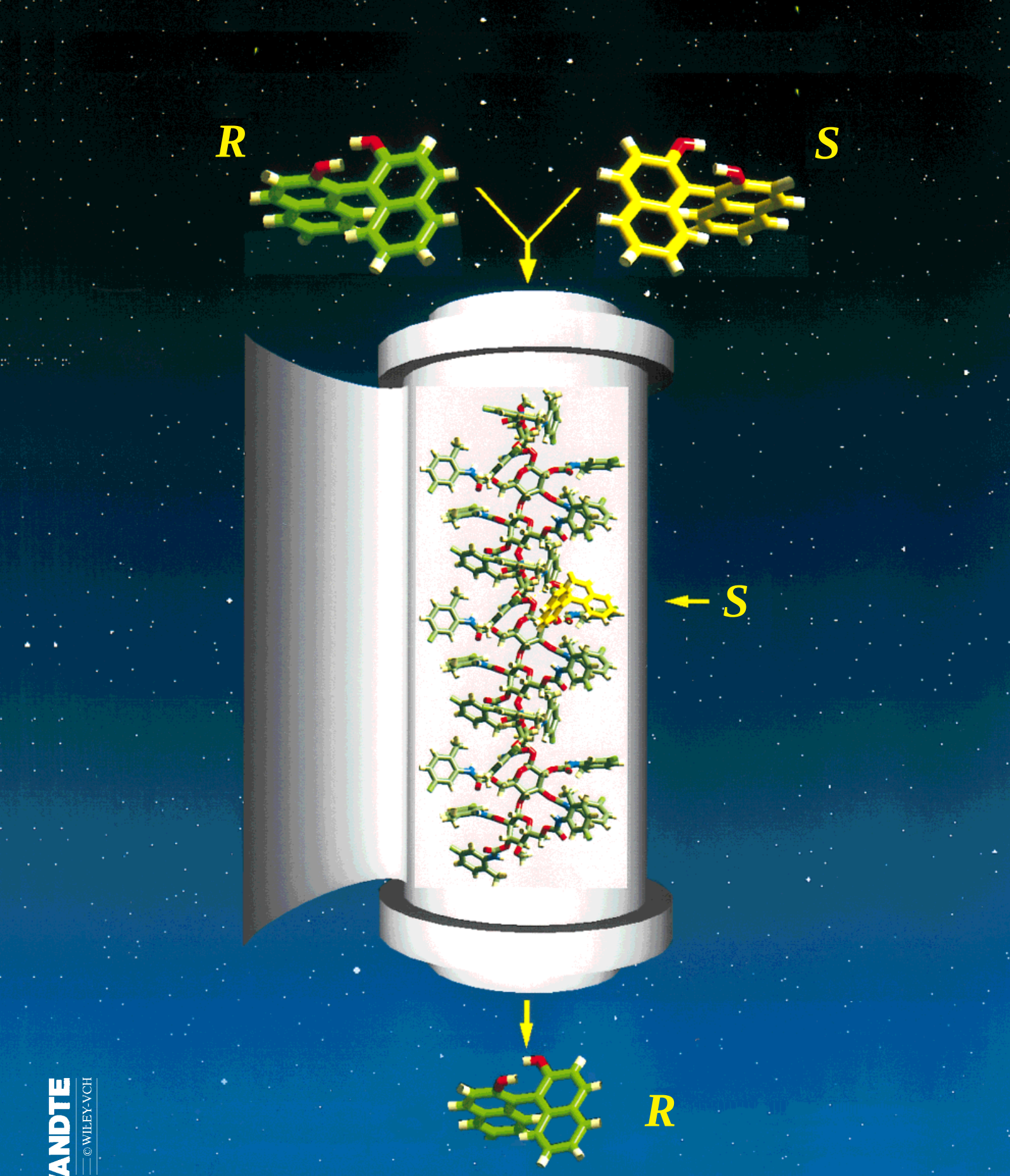

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Insight into the separation of enantiomers by HPLC on polysaccharide derivatives

Polysaccharide Derivatives for Chromatographic Separation of Enantiomers

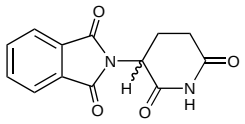
Yoshio Okamoto* and Eiji Yashima

In living systems, chirality is a critical factor. However, this fact had frequently been ignored because enantiomer separations were very difficult and laborious, and most synthetic chiral drugs were therefore commercialized as racemates. In the past two decades, this situation has significantly changed because chromatographic enantioseparations, particularly by high-performance liquid chromatography (HPLC), have been markedly improved. This resolution procedure has already been recognized in many fields as one of the most useful methods for the analysis and separation of enantiomers. Now, most racemates appear to be resolved by liquid chromatography with commercially available chiral stationary phases. Among them, polysaccharide-based phases such as cellulose esters as well as phenylcarbamates of cellulose and amylose are some of the most popular ones. These can be used to resolve a wide range of racemates including aliphatic and aromatic compounds with or without functional groups and many drugs from an analytical to a preparative scale. These polysaccharide phases offer a great chance to resolve enantiomers of interest. Although the mechanism for discrimination between enantiomers on polysaccharide phases has not yet been satisfactorily elucidated, some interesting approaches have been performed by chromatographic, computational, and spectroscopic studies. Mechanistic studies at a molecular level are of great importance and interest from the viewpoint of molecular recognition, which will provide a chance to develop a more efficient polysaccharide-based chiral stationary phase. This review focuses on the direct separation of enantiomers on polysaccharide derivatives after providing a brief historical background.

Keywords: chirality · enantiomeric resolution · liquid chromatography · molecular recognition · polysaccharides

1. Introduction

In 1980 G. Blaschke reported the first comprehensive review of chromatographic enantioseparation entitled "Chromatographic Resolution of Racemates" in this journal.[1] At that time there was no chiral stationary phase (CSP) for high-performance liquid chromatography (HPLC) commercially available, and liquid chromatography (LC) was mostly used to resolve enantiomers for preparative, but not for analytical, purposes. The chirality of drugs was frequently neglected, and most chiral drugs were used as racemates. Nevertheless, Blaschke et al. succeeded in resolving many drugs using optically active polyacrylamide gels on a preparative scale and evaluated the different pharmacological behaviors between the enantiomers.[1] For the first time, they resolved (±)-thalidomide (**1**), which had been used as a hypnotic drug and sedative under the name Contergan and induced a tragedy owing to its potent teratogenicity. They reported that the teratogenic action may be caused only by the *S* isomer, whereas the *R* isomer may not show any teratogenic behavior, even at high doses.[2] Although this conclusion remains controversial, because each enantiomer racemizes at physiological pH or after injection into rabbits,[3] this is one of the key studies through which people recognized the importance of evaluating the difference in the pharmacological behaviors of both enantiomers of a drug.



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As Blaschke predicted in 1980, chromatographic enantioseparations, and in particular resolution by HPLC, have significantly advanced in the past two decades, and are now generally recognized in many fields of science dealing with chiral compounds as one of the most powerful methods available for obtaining both pure enantiomers and for determining enantiomer composition.[4] The determination of enantiomeric excess (*ee*) or optical purity was a laborious task before 1980, and there existed only three practical methods: polarimetric, NMR spectroscopic, and chromatographic (by HPLC or GC).[5] The latter requires prior conversion of the enantiomers into diastereomeric derivatives. These methods could be used to estimate the enantiomeric excess for a limited number

[*] Prof. Y. Okamoto, Dr. E. Yashima
Department of Applied Chemistry, Graduate School of Engineering
Nagoya University, Chikusa-ku, Nagoya 464-8603 (Japan)
Fax: (+81) 52-789-3188
E-mail: okamoto@apchem.nagoya-u.ac.jp

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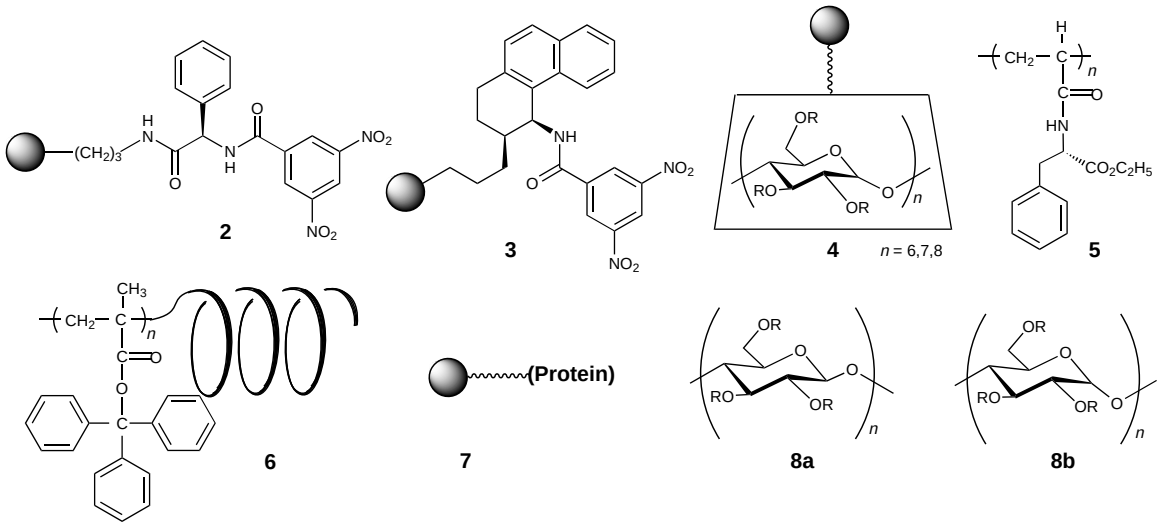


Figure 1. Typical chiral stationary phases for HPLC. The gray spheres represent silica gel.

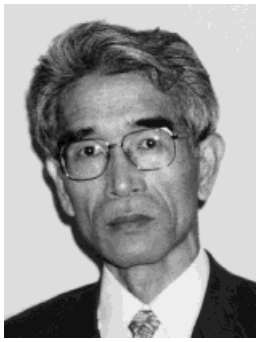
of chiral compounds with a relatively low accuracy. However, most racemates now appear to be resolved by HPLC.[6]

Particularly in the pharmaceutical industry, enantiomer separations by HPLC have become essential for the research and development of chiral drugs,[3b, 7] since enantiomers of chiral drugs often exhibit marked differences in their biological activity in living systems.[8] This forces regulatory agencies to issue the guidelines for the development of chiral drugs, which requires the submission of data on pharmacokinetics as well as physiological, toxicological, and metabolic activities of the drug enantiomers.[9] As a result of these trends, marketing of chiral drugs as a single enantiomer is increasing.[3b, 10] The annual sales of enantiomerically pure drugs reached 45 billion dollars in 1994 and will certainly increase in the future.[3b, 10] The recent significant development of instruments coupled with sensitive detectors and high-resolution columns packed with efficient CSPs have made a major contribution to the advancement of HPLC as an effective technique for the separation of enantiomers.

The design and preparation of a CSP capable of effectively recognizing chirality is the key point in developing this chromatographic enantioseparation technique. Therefore, many CSPs have been prepared for HPLC, and more than 100 CSPs have been introduced on the market since 1981.

There are basically two types of CSPs (Figure 1): One type consists of small chiral molecules (e.g. **2**–**4**) which are usually immobilized on a support silica gel ("brush-" or "Pirkle-type" CSPs), and the other is derived from an optically active polymer (e.g. **5**–**8**) which can be used as a porous gel or with silica gel. A large number of brush-type CSPs have been prepared[4, 11] since Pirkle developed the first commercially available CSP **2** in 1981.[12] Typical examples of the latter CSPs are polyacrylamides (**5**) developed by Blaschke,[1, 13] one-handed helical polymethacrylates (**6**),[14] proteins (**7**),[4b] and polysaccharide derivatives (**8**),[15] which have been reviewed in detail elsewhere. Besides the CSPs described above, the cross-linked polymer gels with chiral cavities generated with a chiral molecule as a template by the molecular-imprinting

Yoshio Okamoto was born in Osaka, Japan, in 1941. He received both his B.A. (1964) and Ph.D. (1969) from Osaka University. He joined the Faculty of Engineering at Osaka University as an assistant in 1969, and then 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, molecular recognition on stereoregular polymers, and enantioseparation by HPLC.



Y. Okamoto

E. Yashima

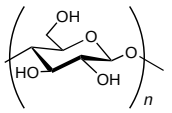
Eiji Yashima was born in Kyoto, Japan, in 1958. He received his Ph.D. from Osaka University. After spending five years at Kagoshima University, he joined Okamoto's group at Nagoya University in 1991 and was appointed Associate Professor in 1995 and Professor in 1998. His current research concerns synthesis of helical polymers with optical activity, enantioseparation by HPLC, and computational studies in the recognition of chirality.

technique are also interesting materials as CSPs and biomimetic sensors.[16]

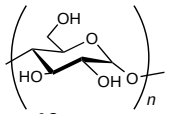
HPLC is also recognized as a valuable technique for the isolation and purification of chiral drugs and natural products on a large scale,[17] and competes with other synthetic and separation methods such as asymmetric synthesis and kinetic resolution with biocatalysts.[10b] This review focuses on the direct separation of enantiomers on polysaccharide-based CSPs, which appear to be among the most popular CSPs. The wide applicability and usefulness of the polysaccharide-based CSPs in the analysis and preparation of enantiomers are demonstrated by a comprehensive survey of the literature.[6, 15] In particular, cellulose triacetate, tribenzoates, and phenylcarbamate derivatives of cellulose and amylose show high abilities to recognize chirality. The mechanism for discrimination between enantiomers on polysaccharide phases has not yet been satisfactorily elucidated, but some interesting approaches to understanding the mechanism have been performed by chromatographic elucidation and computational and spectroscopic studies. These approaches should provide a guide for the selection of a suitable column and the development of a novel polysaccharide-based CSP. Therefore, the significance of the mechanistic studies on the polysaccharide-based CSPs are comprehensively reviewed here.

2. Historical Background of the Use of Polysaccharides and Their Derivatives in Enantiomer Separation

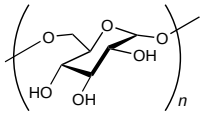
The earliest chromatographic resolutions of enantiomers were carried out with natural chiral adsorbents such as wool and polysaccharides. Polysaccharides are among the most important and abundant natural biopolymers with optical activity, and usually function as a structural unit or an energy source in living systems. The typical polysaccharides cellulose (**9**), starch (amylose) (**10**), and dextran (**11**) have a regular repeating unit of D-glucose throughout the polymers, and were used as chiral adsorbents for chromatographic enantiomer separation owing to their easy availability.



9: Cellulose

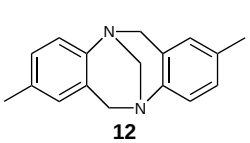


10: Amylose



11: Dextran

Resolution by enantioselective adsorption of racemic dyes containing phenylalanine or mandelic acid residues was attained for the first time on wool in the early 1920s.[18] The first chromatographic resolution was reported by Henderson and Rule in 1938; they partially resolved racemic *p*-phenylenebis(iminocamphor) into its enantiomers on a column packed with the disaccharide lactose; however, the maximum optical purity of the fractions was only about 6%.[19] Later Prelog and Wieland more efficiently resolved Tröger's base (**12**) with the same lactose column.[20]



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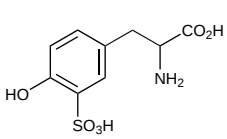

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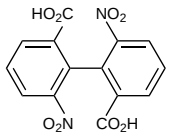

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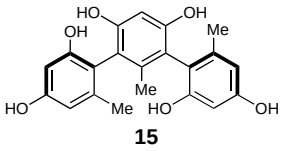

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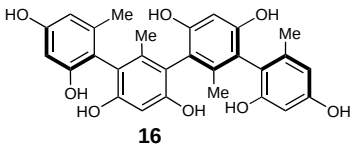

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The first utilization of the chirality of polysaccharides appears to be demonstrated by Kotake et al.[21] They separated several racemic amino acid derivatives, for instance **13**, into two spots of enantiomers by cellulose paper chromatography. Dalgliesh[22a] extended this work and proposed the three-point rule.[22] The rule that three simultaneous interactions are necessary for the discrimination of enantiomers in bimolecular systems has quite often been quoted for explaining the mechanism for discrimination between enantiomers, even nowadays. Furthermore, cellulose thin-layer chromatography[23] and column liquid chromatography with cellulose and potato starch[24] were developed and used for the resolution of racemates, usually amino acids.

Most of the early attempts at column chromatography were conducted with a low efficiency. Nevertheless, Musso demonstrated the usefulness of a native polysaccharide as a chiral adsorbent and succeeded in the complete resolution of atropisomeric biphenyl derivatives **14**–**16** on potato starch.[25] Later Yuasa found that thoroughly purified native cellulose with a high degree of crystallinity [crystal form I; microcrystalline cellulose (Avicel)] can completely resolve amino acids such as tryptophan by liquid chromatography.[26] Carboxymethylcellulose and Sephadex ion exchangers composed of a three-dimensional network of dextran cross-linked by epichlorohydrin effectively resolved many chiral metal complexes.[27] In 1993 Wulff et al. prepared amylose immobilized on silica gel through enzymatic polymerization and found that the CSP resolved racemic menthol by HPLC.[28] These materials do not seem to be practically useful CSPs because of their relatively low resolving abilities.

However, many water-soluble polysaccharides, including heparin and amylose, have recently been found to be effective chiral additives for high-performance capillary electrophoresis (HPCE).[29] Although the enantioselectivity of the polysaccharides is not very high, the extremely high column efficiency of HPCE makes them capable of completely resolving many enantiomers including chiral drugs. This has been reviewed elsewhere.[29, 30]

3. Enantiomer Separation by HPLC

Figure 2 shows a chromatogram for the resolution of Tröger's base (**12**, 0.06 mg) on amylose tris(3,5-dimethylphenylcarbamate) (ADMPC,[43] **78**). The enantiomers elute at t_1 and t_2 with complete separation. Capacity factors k'_1 and k'_2

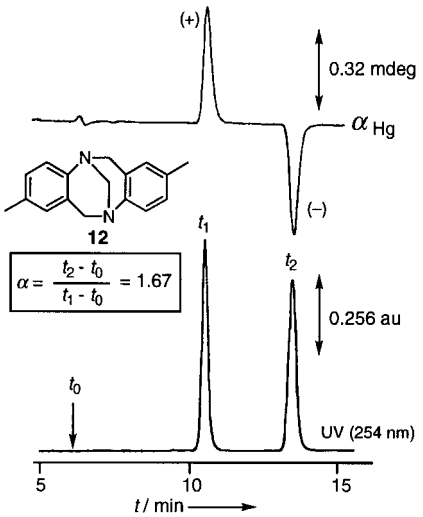


Figure 2. Chromatographic resolution of the enantiomers of **12** on ADMPC (**78**, Chiralpak AD). Column: 25 × 0.46 cm (inner diameter); eluent: hexane/2-propanol (9/1); flow rate: 0.5 mL min^{-1}. t = elution time, mdeg = 1 × 10^{-3}°, au = absorbance units.

and separation factor α are determined as shown in the figure. From the α value the difference in Gibbs energy ($\Delta\Delta G^{\neq}$) at a given temperature can be calculated by using Equation (1). For the base-line separation of enantiomers, a separation factor of about 1.2 is needed, which corresponds to a difference in Gibbs energy of only 0.11 kcal mol^{-1} between the CSP and the two enantiomers. In chromatographic enantioseparation, in addition to UV or a refractive index (RI) detector, a polarimetric or a circular dichroism (CD) detector,[31] which are sensitive to only chirality, can also be used. This is a great advantage of HPLC over other analytical methods such as gas chromatography and NMR spectroscopy.

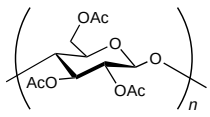
$$\Delta\Delta G^{\neq} = -RT\ln\alpha \quad (1)$$

3.1. Polysaccharide Esters as Chiral Stationary Phases

As mentioned above, native polysaccharides themselves are not practically useful CSPs in HPLC owing to their low enantioselectivities and mechanical properties. However, some modified polysaccharides show high chromatographic properties in the resolution of a variety of racemates.

3.1.1. Cellulose Triacetate as Chiral Stationary Phases

Cellulose triacetate was prepared by Hesse and Hagel in 1973 and is the first practical CSP derived from polysaccharides.[32] They found that this derivative, which was prepared by heterogeneous acetylation of native microcrystalline cellulose (Avicel) in benzene, possesses interesting properties for resolving chiral compounds by LC, although the resolving power of a partially acetylated cellulose is poor.[33] The triacetate is believed to preserve a structure closely related to that of native cellulose (form I) and has been called "microcrystalline cellulose triacetate" (CTA-I, **17**). CTA-I has been employed with ethanol/water as the eluent for the resolution of various racemates, especially stereochemically interesting, nonpolar compounds and aromatic pharmaceuticals.[1, 13, 15a] The high loading capacity of CTA-I makes it one of the most popular CSPs for a large-scale, medium-pressure separation by LC.[17] Some stereochemically interesting compounds completely resolved on CTA-I are shown in Figure 3.[13, 32b, 34]



17: CTA-I

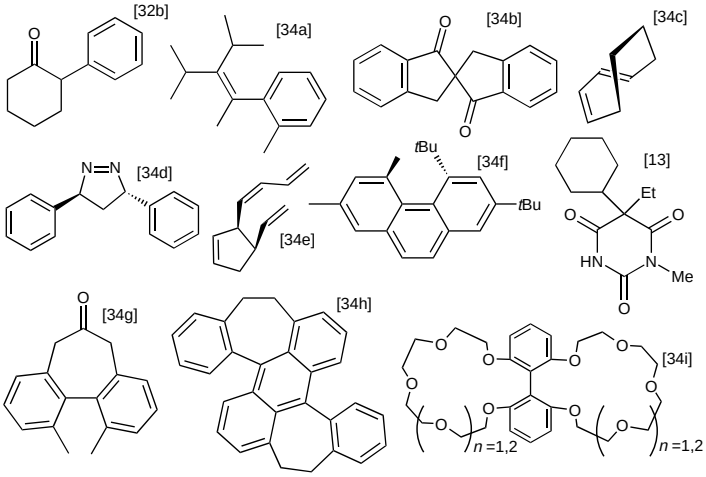
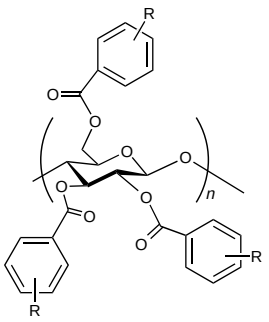


Figure 3. Compounds whose enantiomers were resolved on CTA-I (**17**) with large α values, along with literature references.

Hesse and Hagel pointed out that the microcrystallinity of CTA-I is essential for recognizing chirality. The resolving ability is substantially reduced and, in some cases, there is a reversal of the order in which enantiomers are eluted once CTA-I is dissolved in a solvent.[32] However, when CTA-I was coated on silica gel for HPLC from a solution, the CTA afforded another useful CSP.[35] As expected, the ability to recognize chirality was completely different from that of CTA-I. For instance, enantiomers of Tröger's base (**12**) eluted in reversed order on the two triacetate columns.[35a] The new CSP has greater advantages than CTA-I in column efficiency and durability. These results have aroused wide interest in the use of derivatized polysaccharides such as benzoates and phenylcarbamates as CSPs for HPLC, and most polysaccharide-based CSPs so far developed have been prepared by coating on silica gel.

3.1.2. Cellulose Benzoates as Chiral Stationary Phases

In Scheme 1 are shown the structures of cellulose tribenzoates prepared by our group, some of which are also useful CSPs when coated on silica gel. The effect of alkyl, halogen, trifluoromethyl, and methoxy substituents on the phenyl groups of cellulose tribenzoate (CTB, **24**, Chiralcel OB) was systematically studied.[36] An inductive effect of the substituents was observed to affect enantioselectivity: The benzoate derivatives having electron-donating substituents, such as a methyl group, showed better recognition of chirality than those having electron-withdrawing substituents, such as a



R
18: 4-CH_3O
19: 3,5-$(CH_3O)_2$
20: 4-$(CH_3)_3C$
21: 4-CH_3
22: 3-CH_3
23: 2-CH_3
24: H
25: 4-F
26: 4-CF_3
27: 3,5-$(CH_3)_2$
28: 3,5-Cl_2

Scheme 1. Structures of cellulose tribenzoate derivatives.

halogen. However, the strongest electron donor, the methoxy group, was not suitable because of the high polarity of the substituent itself.

Among the benzoates, cellulose tris(4-methylbenzoate) (**21**, Chiralcel OJ) exhibits a high resolving power for various racemates, including drugs, and has become a practically useful CSP. Some stereochemically interesting compounds resolved on **21** are shown in Figure 4.[37] Nonaromatic compounds are also resolved well on CTB (Figure 5).[15a,c, 38] Mannschreck et al.[39] and Francotte et al.[40] independently prepared spherical beads of cellulose tribenzoate derivatives and found them to be useful CSPs, particularly for preparative purposes because of their high loading capacity. Among these cellulose esters, triacetate, tribenzoate, tris(4-methylbenzoate), and tricinnamate have been on the market as CSPs. Tribenzoates of amylose show a low ability to recognize chirality.[36]

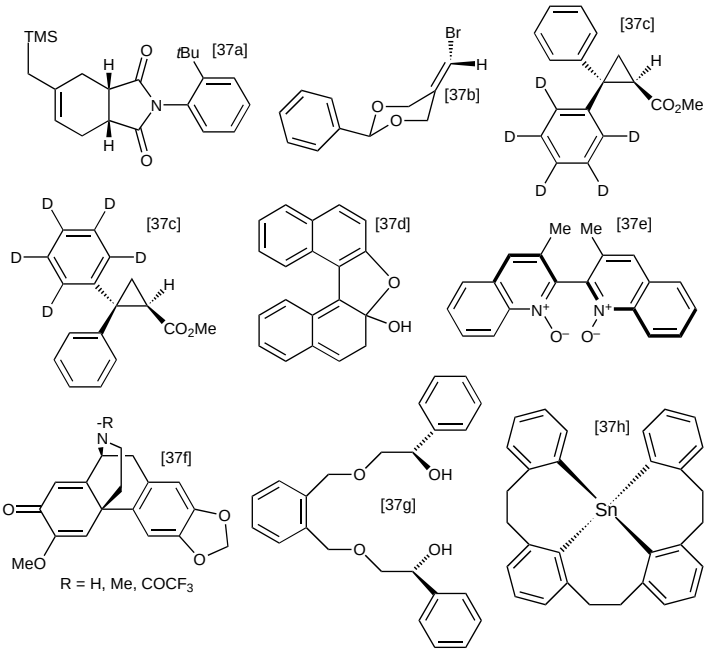


Figure 4. Compounds whose enantiomers were resolved on cellulose tris(4-methylbenzoate) (**21**, Chiralcel OJ), along with literature references.

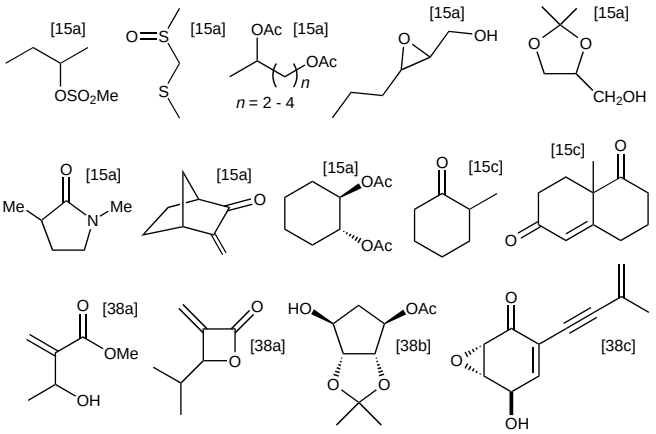
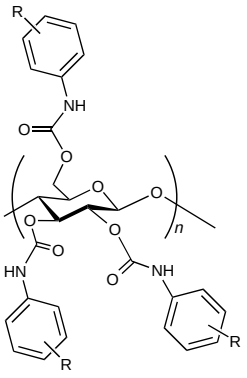


Figure 5. Nonaromatic compounds whose enantiomers were resolved on CTB (**24**, Chiralcel OB), along with literature references.

3.2. Phenylcarbamates of Cellulose and Amylose as Chiral Stationary Phases

We prepared a series of cellulose tris(phenylcarbamate) derivatives (Scheme 2) by the reaction of microcrystalline cellulose (Avicel) with substituted phenyl isocyanates.[15g, 41]



R	
29: 4-CH_3O	**49**: 3,5-$(CH_3)_2$
30: 4-C_2H_5O	**50**: 2,6-$(CH_3)_2$
31: 4-$(CH_3)_2CHO$	**51**: 3,4,5-$(CH_3)_3$
32: 4-$(CH_3)_2CHCH_2O$	**52**: 3,5-Cl_2
33: 4-$(CH_3)_3Si$	**53**: 3,4-Cl_2
34: 4-CH_3	**54**: 2,6-Cl_2
35: 4-CH_3CH_2	**55**: 3,5-F_2
36: 4-$(CH_3)_2CH$	**56**: 3,5-$(CF_3)_2$
37: 4-$(CH_3)_3C$	**57**: 2-Cl-4-CH_3
38: 3-CH_3	**58**: 2-Cl-5-CH_3
39: 2-CH_3	**59**: 2-Cl-6-CH_3
40: H	**60**: 3-Cl-2-CH_3
41: 4-F	**61**: 3-Cl-4-CH_3
42: 4-Cl	**62**: 4-Cl-2-CH_3
43: 2-Cl	**63**: 4-Cl-3-CH_3
44: 3-Cl	**64**: 3-F-4-CH_3
45: 4-Br	**65**: 5-F-2-CH_3
46: 4-CF_3	**66**: 3-F-5-CH_3
47: 4-NO_2	**67**: 3-Cl-5-CH_3
48: 3,4-$(CH_3)_2$	**68**: 3-Br-5-CH_3

Scheme 2. Structures of cellulose trisphenylcarbamate derivatives.

They are among the most thoroughly investigated polysaccharide-based CSPs with respect to chromatographic enantioseparation and the mechanism for discrimination between enantiomers. They can resolve a wide range of racemates having various functional groups, although their resolving abilities depend greatly on the substituents on the phenyl groups.

The results for the resolution of ten racemates (**12**, **69**–**77**; acac = acetylacetonate) on *para*- or *meta*-substituted derivatives are presented in Table 1. Most of the CSPs listed in Table 1 can separate all of the racemates, depending on the substituents. Generally, substitution with an electron-donating methyl group or an electron-withdrawing halogen at the *meta* and/or *para* position improves the resolving ability for

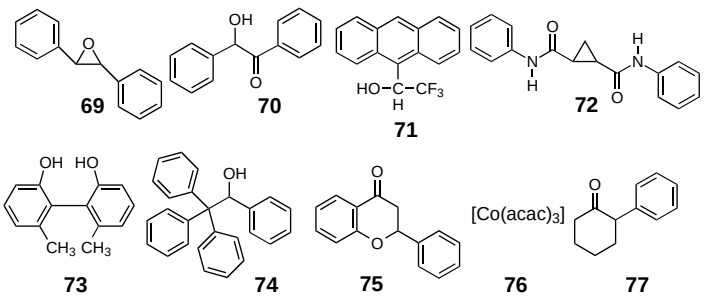
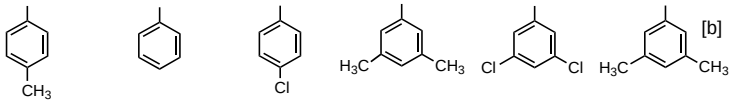


Table 1. Separation factors (α) in the resolution of racemates of **12** and **69**–**77** on phenylcarbamate derivatives of cellulose and amylose.[a]

CSP:



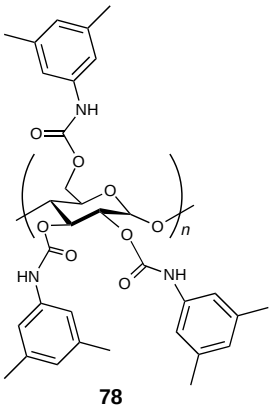
	34	**40**	**42**	**49**	**52**	**78**
Racemate						
12	1.48 (+)	1.37 (+)	1.16 (+)	1.32 (+)	1.65 (+)	1.67 (+)
69	1.55 (+)	1.46 (+)	1.68 (+)	1.68 (−)	1.84 (+)	3.04 (+)
70	1.12 (−)	≈1 (+)	1.20 (−)	1.58 (+)	1.21 (−)	1.21 (−)
71	1.52 (−)	1.45 (−)	1.29 (−)	2.59 (−)	1.38 (−)	1.15 (+)
72	1.35 (−)	1.45 (−)	1.44 (−)	3.17 (−)	1.41 (+)	2.01 (+)
73	1.30 (−)	1.65 (−)	1.20 (−)	1.83 (−)	1.11 (+)	2.11 (−)
74	1.37 (+)	1.22 (+)	1.95 (+)	1.34 (+)	1.29 (+)	1.98 (+)
75	1.16 (+)	1.10 (+)	1.12 (+)	1.41 (−)	1.20 (−)	1.12 (+)
76	1.75 (+)	1.24 (+)	1.46 (+)	≈1 (+)	1.82 (+)	≈1 (−)
77	1.20 (−)	1.17 (−)	1.16 (−)	1.15 (−)	1.26 (−)	≈1 (−)

[a] Column: 25 × 0.46 cm (inner diameter); eluent: hexane/2-propanol (90/10); flow rate: 0.5 mL min^{-1}. The sign in parentheses indicates the optical rotation of the first enantiomer to be eluted. [b] Amylose tris(3,5-dimethylphenylcarbamate) (**78**).

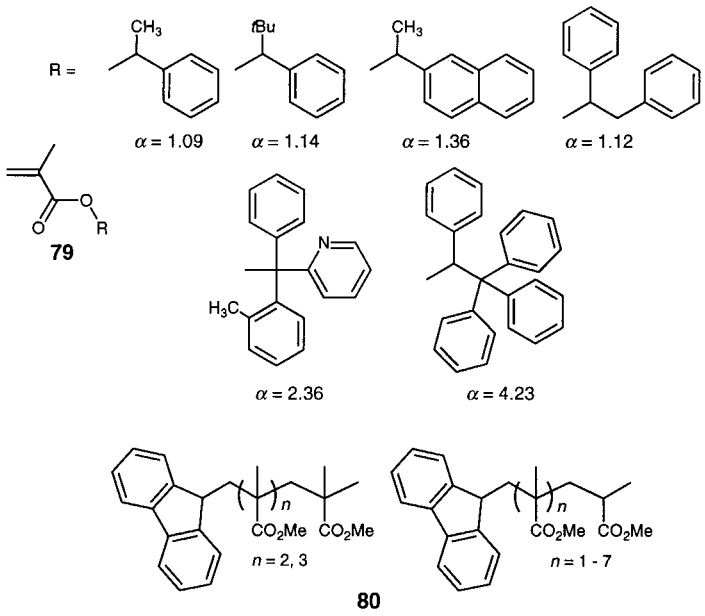
many racemates, but *ortho*-substituted derivatives such as **39**, **43**, and **54** do not recognize chirality well. The derivatives with heteroatom substituents, such as the alkoxy group in **29** and **30** or the nitro group **47**, also show poor abilities to recognize chirality.[41] Phenylcarbamates bearing both electron-donating methyl and electron-withdrawing chloro or fluoro groups on the phenyl moieties exhibit a high resolving ability for many racemates; cellulose phenylcarbamates **61**, **64**, and **67** recognize chirality particularly well.[42]

Similarly, the ability of amylose phenylcarbamates to recognize chirality was improved by introducing methyl or chloro groups on the phenyl moieties.[43] However, in contrast to the cellulose derivatives, tris(4-methoxyphenylcarbamate) and tris(5-chloro-2-methylphenylcarbamate) of amylose show a relatively high recognition of chirality.[44] This may be ascribed to the differences in their higher order structures. Possible structures are left-handed 3/2 and 4/1 helical chain conformations for tris(phenylcarbamates) of cellulose (CTPC)[45] and amylose (ATPC),[46] respectively. These different higher order structures seem to be responsible for the different influence of the substituents on the resolving power of the cellulose and amylose derivatives.

Among the tris(phenylcarbamate) derivatives of cellulose and amylose prepared so far, 3,5-disubstituted derivatives such as 3,5-dimethylphenylcarbamates (CDMPC, **49**, Chiralcel OD) and 3,5-dichlorophenylcarbamates (**52**) of cellulose and tris(3,5-dimethylphenylcarbamate) of amylose (ADMPC, **78**, Chiralpak AD) show quite excellent enantioselectivity for a variety of racemates (Table 1).[41, 43] The CSP **52** has a unique ability to recognize

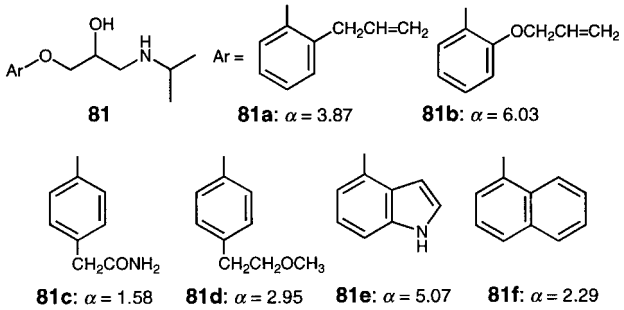


chirality and can resolve bulky methacrylates **79**[41] and stereoisomers of oligo(methyl methacrylate)s **80**.[14c,e] This markedly contributes to the elucidation of the mechanism of helix-sense selective polymerization of triarylmethyl methacrylates leading to an optically active, one-handed helical polymethacrylate (CSP **6** in Figure 1). However, owing to the high solubility in an eluent with a high proportion of 2-propanol, application is rather limited. This defect was overcome by chemical bonding of the CSP to 3-aminopropyl-silanized silica gel with a diisocyanate as a spacer.[47]



CDMPC resolves a variety of chiral compounds, including aromatic hydrocarbons, axial and planar dissymmetric compounds, metal-containing compounds, chiral sulfur or phos-

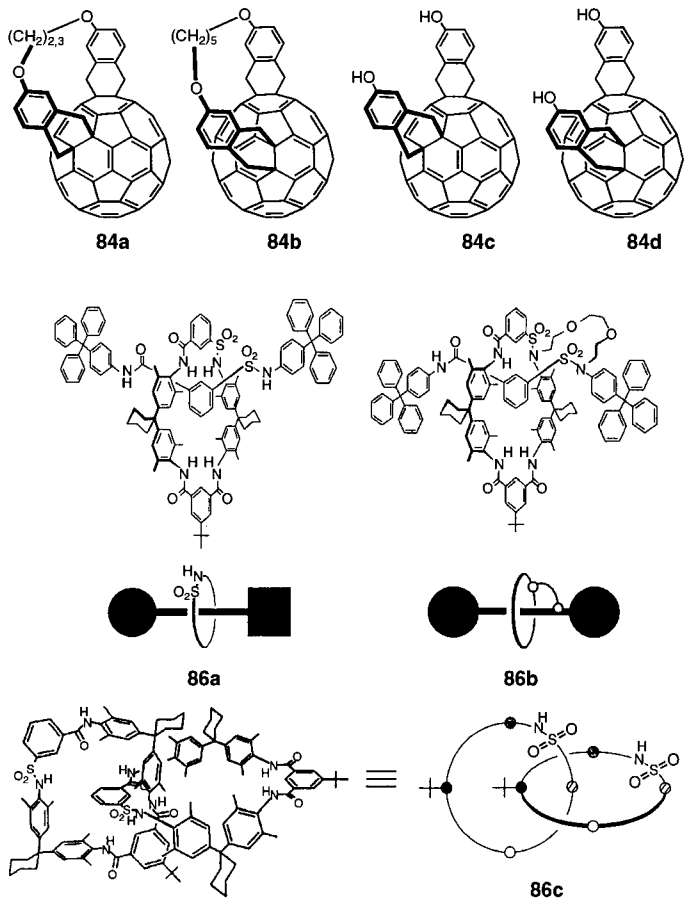
phorus compounds, cyano or carbonyl compounds, amines, carboxylic acids, alcohols, amino acid derivatives, ethers, and many drugs[6, 15] including β-adrenergic blocking agents (β-blockers) such as **81**. In the resolution of β-blockers on



CDMPC, the (*R*)-(+)-isomers usually eluted first followed by the (*S*)-(−)-isomers, and there was complete separation.[48]

Some stereochemically interesting compounds and important chiral building blocks recently resolved on CDMPC are shown in Figure 6.[49, 50] Some of them cannot be separated by other methods and were resolved for the first time by HPLC. Figure 7 displays the chromatograms for the resolution of several racemates on CDMPC. Abscisic acid (**82**),[51] the metallocene derivative **83**,[52] the chiral C_{60} derivatives **84**,[53] the aromatic hydrocarbons **85**,[54] and the topologically interesting rotaxanes **86a, b** and catenane **86c**[55] were completely separated into their enantiomers.

The ability of ADMPC to recognize chirality is as effective as that of CDMPC. Some enantiomers are eluted in the reverse order on the two CSPs (Table 1), suggesting that these two are complementary in recognizing chirality. Enantiomers not resolved on CDMPC may be resolved on ADMPC, and



vice versa.[15g,h] Several chiral compounds recently resolved on ADMPC are shown in Figure 8.[56]

It is very important to select a suitable eluent to achieve efficient resolution of the enantiomers.[15] A mixture of

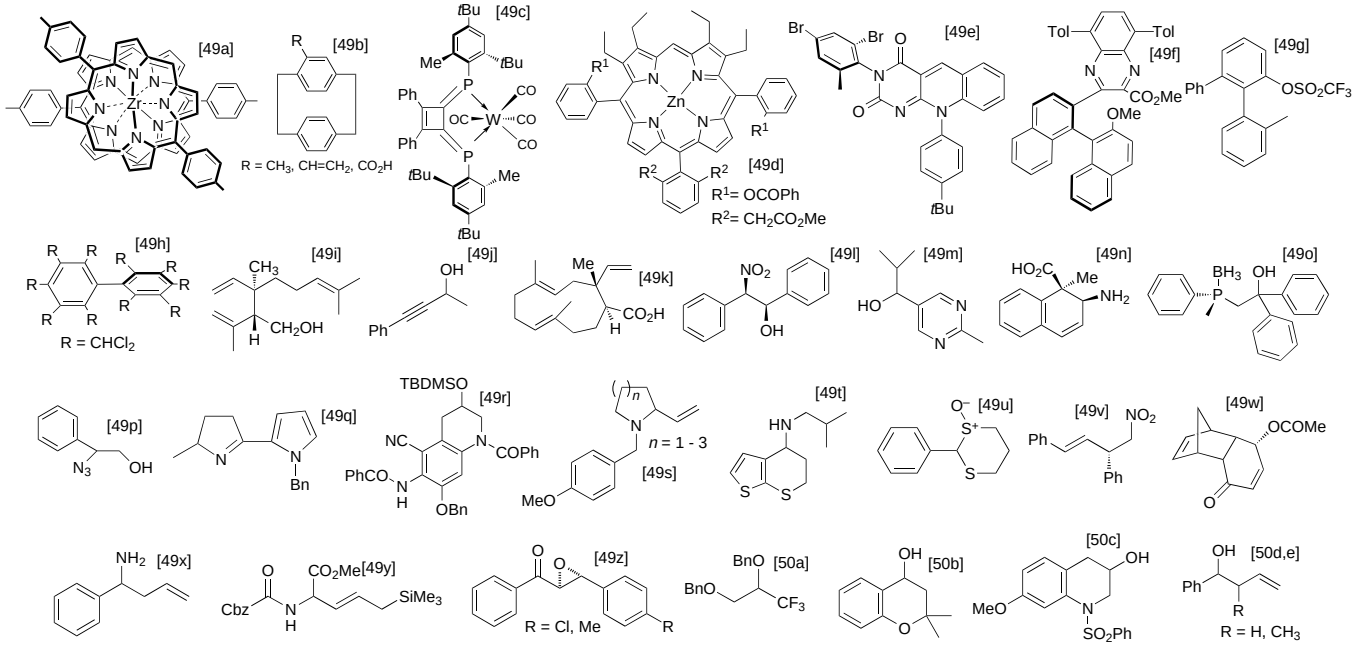


Figure 6. Compounds whose enantiomers were resolved on CDMPC (**49**, Chiralcel OD), along with literature references. Tol = tolyl, Bn = benzyl, TBDMS = *tert*-butyldimethylsilyl.

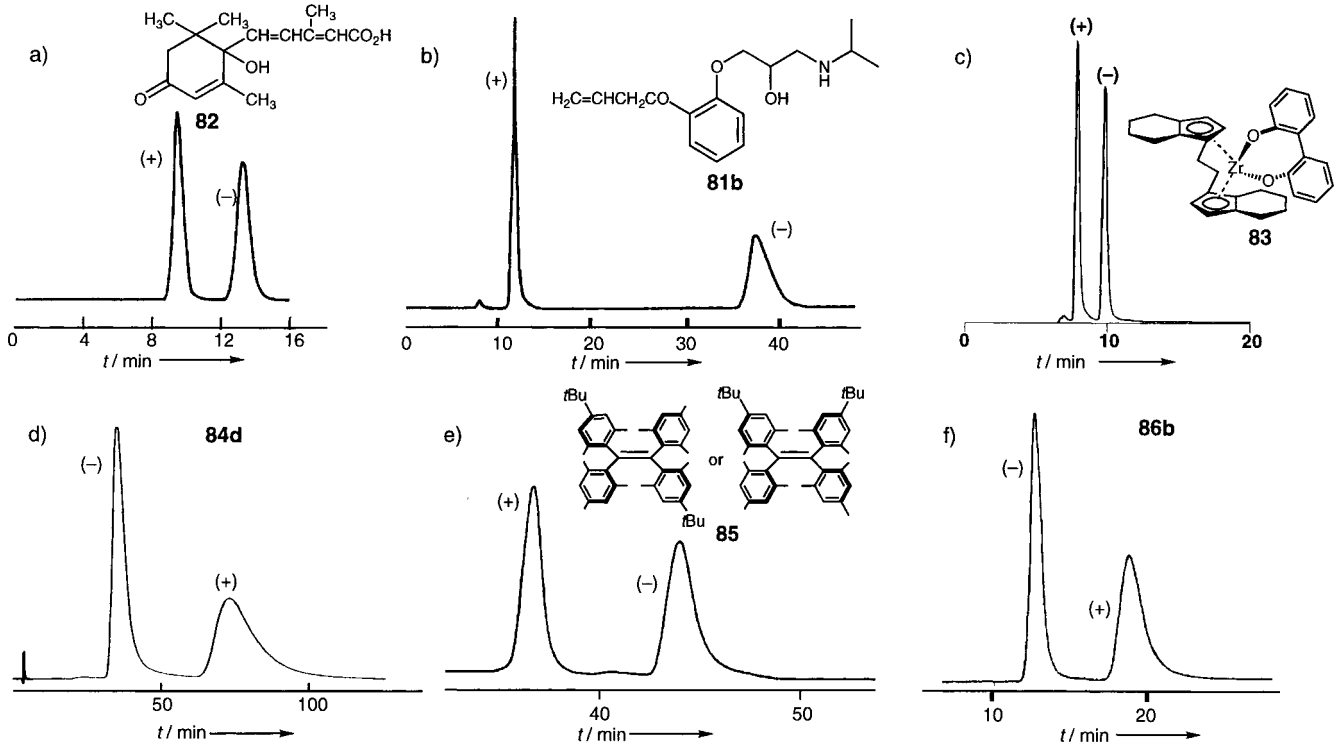


Figure 7. Chromatograms for the resolution of a) abscisic acid (**82**),[51] b) oxprenolol (**81b**),[48] c) the *ansa*-zirconocene derivative **83**,[52] d) the chiral C_{60} derivative **84d**,[53] e) the tetraarylethene derivative **85**,[54] and f) the chiral rotaxane **86b**[55] on CDMPC (**49**). Column: 25 × 0.46 cm (inner diameter); eluent: a) hexane/2-propanol/trifluoroacetic acid (80/20/0.1), b) hexane/2-propanol/diethylamine (80/20/0.1), c) hexane/ethanol (9/1), d) hexane/2-propanol (7/3), e) isooctane, f) hexane/ethanol (85/15); flow rate: 0.5 mL min^{-1} (a–c, f), 1.0 mL min^{-1} (d, e). t = elution time.

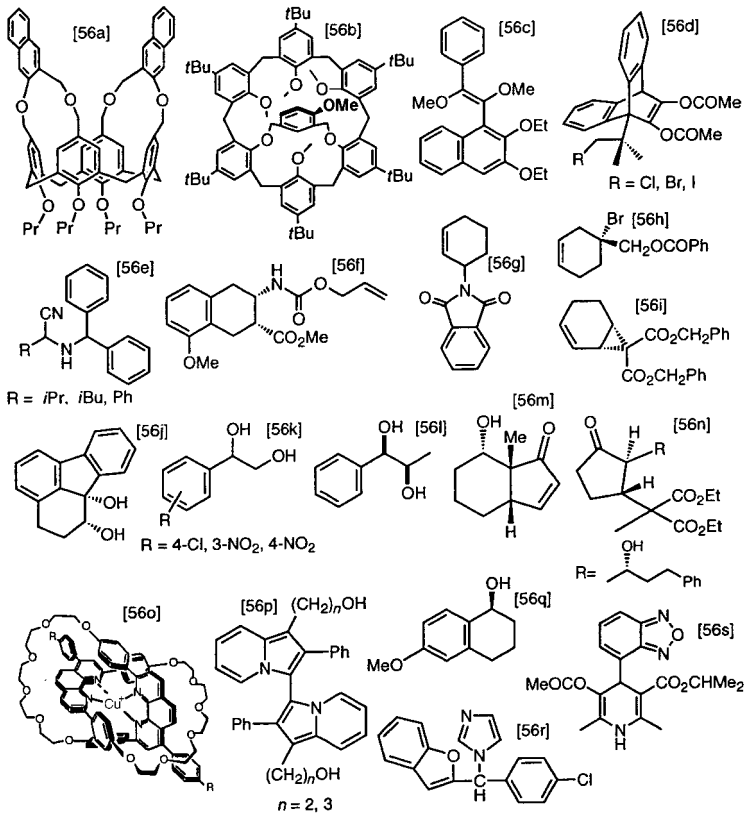


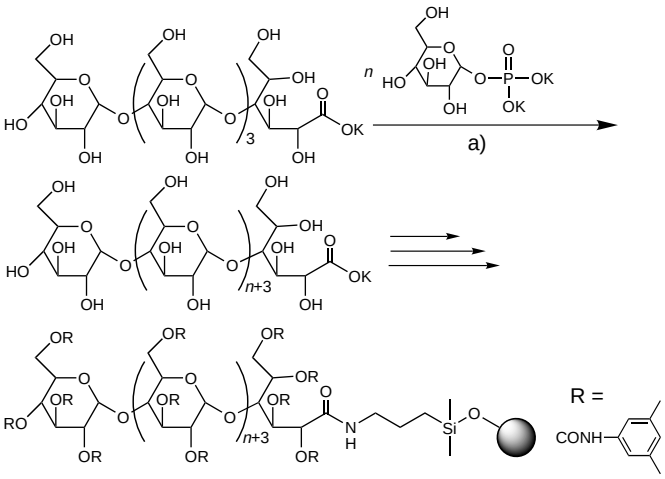
Figure 8. Compounds whose enantiomers were resolved on ADMPC (**78**, Chiralpak AD), along with literature references.

hexane and 2-propanol or ethanol is often used for the separation of enantiomers on polysaccharide-based CSPs. The structures of the alcohol can influence the enantioselectivity.[57] When an analyte has an acidic group (e.g. **82**), addition of a small amount of strong acid (e.g. CF_3COOH) gives better separation (Figure 7a).[58] For basic amino compounds, addition of a small amount of diethylamine or isopropylamine is recommended for reducing tailing of the peaks (Figure 7b).[48] Aqueous eluents are also particularly useful for investigating the pharmacokinetics and pharmacodynamics of chiral drugs in living systems.[59] Supercritical-fluid liquid chromatography (SFC) with carbon dioxide and alcohols as a mobile phase can also be applied to the polysaccharide phases.[60] However, other solvents such as chloroform and THF cannot be used as the main mobile phase because the polysaccharide-based CSPs are soluble or swollen in the solvents.

To overcome this defect, CDMPC and ADMPC were regioselectively bonded to silica gel at the 2-, 3-, and 6-positions of the glucose units with a diisocyanate spacer.[47] These CSPs were able to discriminate between enantiomers better than nonregioselectively bonded CSPs, although their abilities to recognize chirality were slightly lower than those of the coated-type CSPs. The above-mentioned CSPs may be chemically bonded to silica gel through several hydroxyl groups of the polysaccharides, which may

cause an alternation of higher order structures of the polysaccharides and thus decrease the ability to recognize chirality. Similar chemically bonded CDMPC phases were also prepared through radical polymerization of the allyl groups of silica gels and a CDMPC derivative bearing vinyl groups.[61]

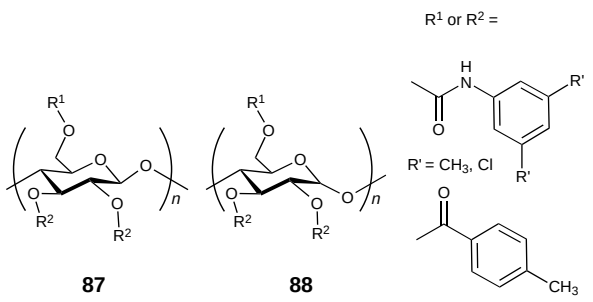
Recently, ADMPC was successfully bonded chemically to silica gel only at the reducing terminal residue of amylose (Scheme 3).[62a] Amylose with the desired chain length was



Scheme 3. Synthesis of ADMPC bound on silica gel through enzymatic polymerization. a) Potato phosphorylase. The gray sphere represents silica gel.

readily prepared by the polymerization of the dipotassium salt of α-D-glucose 1-phosphate with functionalized maltooligosaccharides in the presence of a phosphorylase isolated from potato.[28, 62b] The amylose was successfully bonded to silica gel at the reducing terminal residue, and then allowed to react with 3,5-dimethylphenyl isocyanate to afford CSPs with excellent resolving abilities—comparable to that of the coated-type CSP—and high durabilities with respect to solvents such as THF and chloroform. Some racemates were more efficiently resolved on the new bonded-type CSP than the coated-type CSP using chloroform as a component of the mobile phase.[62a]

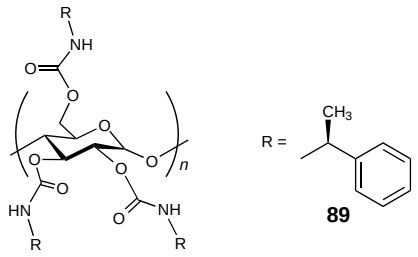
Regioselectively carbamoylated and/or benzoylated celluloses **87** and amyloses **88** were prepared, and their abilities to recognize chirality were evaluated.[63] Some racemates are



more efficiently resolved on the CSPs. Porous spherical silica gels with a mean particle size of 7–10 μm and a mean pore diameter of 100–400 nm are often used as a support. However, the surface and type of support also influence the enantioselectivity of the polysaccharide-based CSPs.[64]

3.3. Aralkylcarbamates of Polysaccharides as Chiral Stationary Phases

Alkylcarbamates, such as methyl- and cyclohexylcarbamates of cellulose, are poor in recognizing chirality. Although tris(*tert*-butylcarbamate) of cellulose can completely resolve some racemates, it cannot resolve as many racemates as the phenylcarbamates of polysaccharides. However, several tris-(aralkylcarbamate)s of cellulose and amylose exhibited characteristic resolving abilities that are different from those of the phenylcarbamates of polysaccharides.[65] In particular, 1-phenylethylcarbamates of polysaccharides exhibit a high ability to recognize chirality, although benzylcarbamate and other more bulky aralkylcarbamates are not as effective. The resolving abilities of 1-phenylethylcarbamates depend on the chirality of the aralkyl group; the (*S*)-1-phenylethylcarbamate of amylose (**89**, Chiralpak AS) can recognize chirality. Some



of the racemates shown in Figure 9,[65-67] including the chiral telluroxide **90**,[66] are better resolved on the CSP than other phenylcarbamates of polysaccharides including CDMPC and ADMPC.

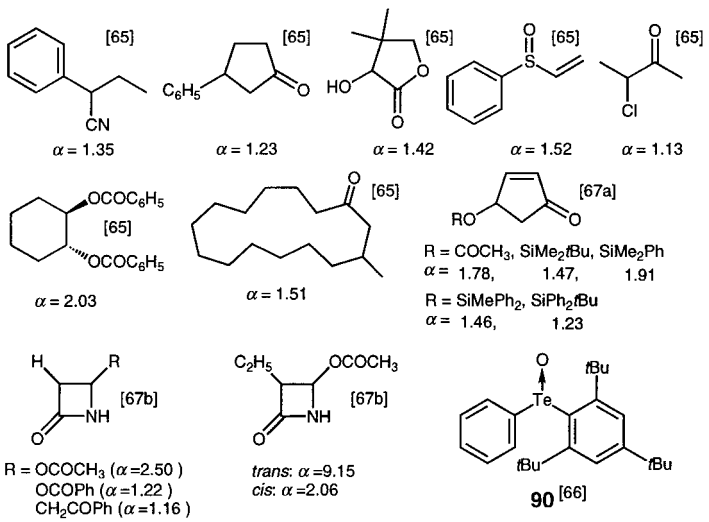


Figure 9. Compounds whose enantiomers were better resolved on amylose tris((*S*)-1-phenylethylcarbamate) (**89**, Chiralpak AS) than other polysaccharide-based CSPs, along with literature references.

4. Mechanism for Discrimination between Enantiomers on Polysaccharide Derivatives

An understanding of the recognition of chirality at a molecular level is of great interest and importance in many fields of chemistry and biology.[68] Furthermore, recognition of chirality plays an essential role in the fields of enantioseparation. In the past few years, several approaches to clarifying the mechanism for the recognition of chirality on CSPs for liquid chromatographies have been attempted by means of chromatography, NMR spectroscopy,[69] X-ray analysis, and computational methods.[70] The CSPs that have been most intensively studied in this respect are cyclodextrin-based CSPs and Pirkle-type CSPs;[4, 69, 70] rational models for interactions between the CSPs and enantiomers have been proposed.

In contrast, very few mechanistic studies on chiral discrimination at a molecular level have been performed for polymeric CSPs.[71] Chiral polymers usually have a number of different binding sites with different affinities to enantiomers, and the determination of their exact structures in the solid state or in solution is laborious. This makes it difficult to evaluate a precise mechanism for the recognition of chirality on the polymeric CSPs.

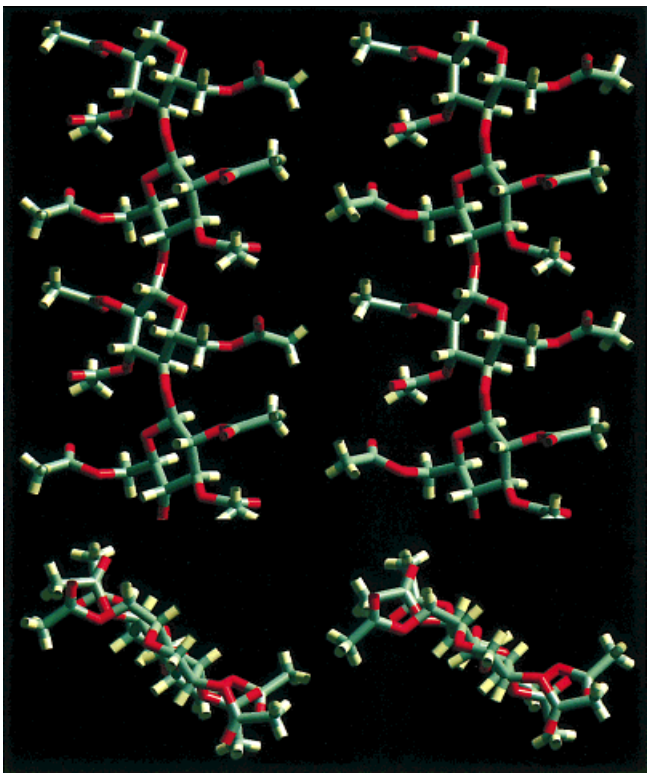


Figure 10. Calculated structure of the laminate CTA-I matrix on the basis of an X-ray structure analysis. The coordinates of each atom are taken from ref. [72c]. View along the chain axis (top) and perpendicular to the chain axis (bottom).

4.1. Cellulose Esters

Although CTA-I has been used as a CSP for a long time, the mechanism for the recognition of chiral has not been satisfactorily elucidated. This is probably because of difficulty in determining the complex structure of CTA-I, which seems to be characterized by the presence of many adsorption sites for interactions, despite structural investigations by means of X-ray crystallography,[15a, 45a, 72] calculations,[73] and solid-state ^{13}C NMR spectrscopy.[74] Hesse and Hagel,[32] and later Francotte et al.,[72a] proposed an inclusion mechanism by which enantiomers may be adsorbed in the chiral cavities consisting of the laminate CTA-I matrix (Figure 10).[72c] The inclusion of molecules in the cavity may be governed not by the attractive interaction through functional groups, but mainly by the shape of the molecules.[75] The inclusion mechanism seems to explain the chiral discrimination of CTA-I, since many fully aromatic or nonaromatic hydrocarbons without any functional groups (Figure 3) can be resolved on this CSP.

Other theoretical studies[76] and the X-ray analysis of a model compound[77] also support the inclusion mechanism. Heterogeneous acetylation may provide a supramolecular structure of CTA-I in which multiple interaction sites with specific surface and cavities inside the matrix may be responsible for the high ability to recognize chirality for a wide range of enantiomers.[75] The existence of such a supramolecular structure of CTA was also proposed based on NMR investigations in solution.[78] However, such an NMR spectrum may not be useful for elucidating the mechanism for discrimination between enantiomers on CTA-I, because the microcrystallinity in the solid state plays a dominant role for the enantioselectivity. A detailed solid-state NMR study may be necessary.

Cellulose triacetate coated on silica gel from a solution may consist of CTA-II and amorphous regions; the enantioselectivity is sensitive to the coating solvent.[15a, 72b] The main chiral adsorption sites of CTB derivatives are considered to be the polar carbonyl groups of esters, which can interact with the racemates through hydrogen bonding and dipole–dipole interactions for discrimination between enantiomers.[36] Wainer et al. proposed a similar mechanism, an attractive binding with steric fit involving hydrogen bonding and dipole–dipole interactions rather than inclusion, on the basis of the separation properties of a series of structurally related chiral aromatic amides and alcohols on the CTB phases.[79]

However, the ability to recognize chirality is highly dependent on the conditions for the preparation of the CSPs, and particularly on the solvent used to dissolve CTB derivatives in coating process, as observed for CTA. This means that other factors, such as the morphology of CTB derivatives, may be closely related to the enantioselectivity.[36] Francotte and Zhang pointed out the importance of the supramolecular structure of CTB derivatives for recognizing chirality.[40c] Grinberg et al. recently investigated the thermodynamic profiles for the resolution of a chiral diol on cellulose tris(4-methylbenzoate) (**21**).[80] They found an interesting temperature-dependent enantioseparation behavior: At low temperature the enantioselectivity is driven by entropy due to inclusion, whereas at high temperature it is governed by

enthalpy mainly due to an attractive interaction through hydrogen bonding.

Based on the X-ray structural analysis, Steinmeier and Zugenmaier proposed a left-handed 3/2-helical structure for CTB regardless of the preparation conditions (Figure 11).[45a] Modeling of CTB was also reported by Francotte et al.[40a] and

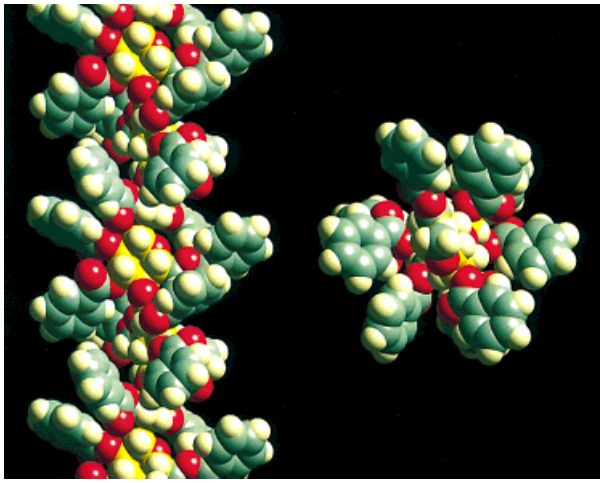


Figure 11. Optimized structure of CTB (**24**) based on the structure determined by X-ray analysis.[45a] The energy minimization was carried out with the Dreiding force field in Cerius^2 (MSI). The glucose carbon atoms of CTB are shown in yellow for clarity. View along the helix axis (left) and perpendicular to the helix axis (right).

Grinberg et al.,[80] and their proposed main-chain structures are rather similar to that of CTA-I. Oguni et al. investigated the mechanism for discrimination between enantiomers on the CTB derivative **21** by means of ^{13}C NMR spectroscopy in solution.[71a,c] Several signals of 1-phenylethanol were split into the signals of enantiomers in the presence of **21**. This is interesting for elucidating the mechanism for discrimination between enantiomers, and further experiments may provide additional information.

4.2. Phenylcarbamates of Cellulose and Amylose

Most of the cellulose tris(phenylcarbamate) derivatives form a lyotropic liquid crystalline phase in a highly concentrated solution (Figure 12)[41, 81] and show high crystallinity under a polarizing microscope when they are cast from a solution. This indicates that the phenylcarbamates on the silica surface are presumably arranged in a regular fashion. Such an ordered structure seems to be very important for efficient recognition of chirality by CSPs derived from polymers. A few cellulose phenylcarbamate derivatives and alkylcarbamates which do not form such a liquid crystalline phase showed a poor ability to recognize chirality. The mechanism for the recognition of chirality by phenylcarbamates of cellulose and amylose has been proposed on the basis of chromatographic, computational, and spectroscopic studies.

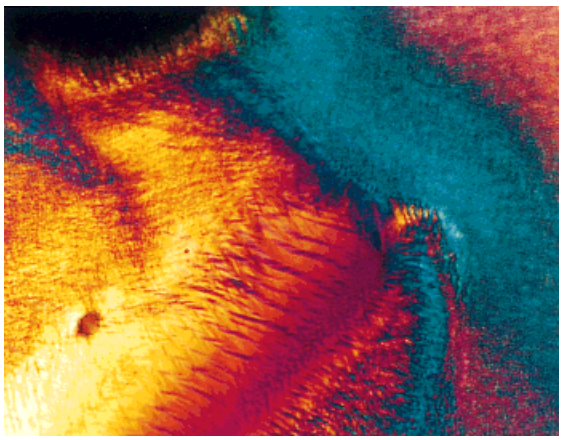


Figure 12. A photomicrograph of a lyotropic liquid crystalline phase of cellulose tris(phenylcarbamate) in a highly concentrated solution under polarized light.

4.2.1. Chromatographic Studies

As mentioned above, the abilities of phenylcarbamates of polysaccharides to recognize chirality are greatly affected by the substituents on the phenyl groups. To understand the effect quantitatively, the retention times of acetone and the first isomer of alcohol **71** eluted on the 4-substituted CSPs are plotted against the Hammett parameter σ of the substituents (Figure 13).[41] The retention times of acetone tend to increase

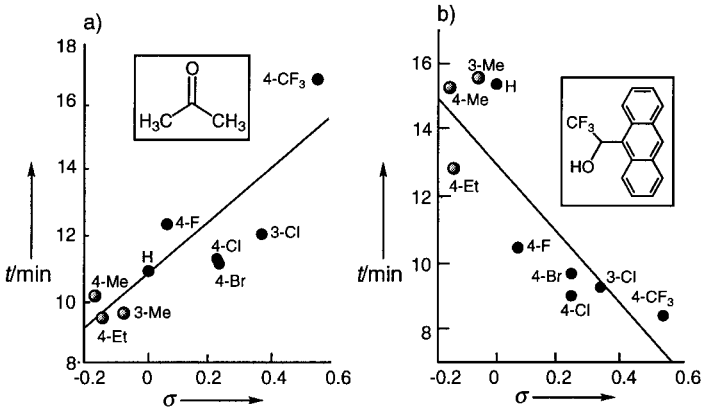


Figure 13. Plots of retention times t of a) acetone and b) the first enantiomer of **71** to elute on cellulose tris(phenylcarbamate) derivatives against the Hammett parameter σ of the substituents.

as the electron-withdrawing power of the substituents increases, whereas those of the first isomer of **71** eluted decrease. These results indicate that the main chiral adsorption sites are probably the polar carbamate groups, which are able to interact with a racemate through hydrogen bonding to the NH and C=O groups; in the case of the latter, dipole–dipole interactions also play a role (Figure 14). The nature of the substituents (X) on the phenyl groups influences the polarity of the carbamate residues, which must change the resolving ability. Therefore, acetone is more strongly adsorbed on the CSPs with the more acidic NH protons through a hydrogen-bonding interaction.

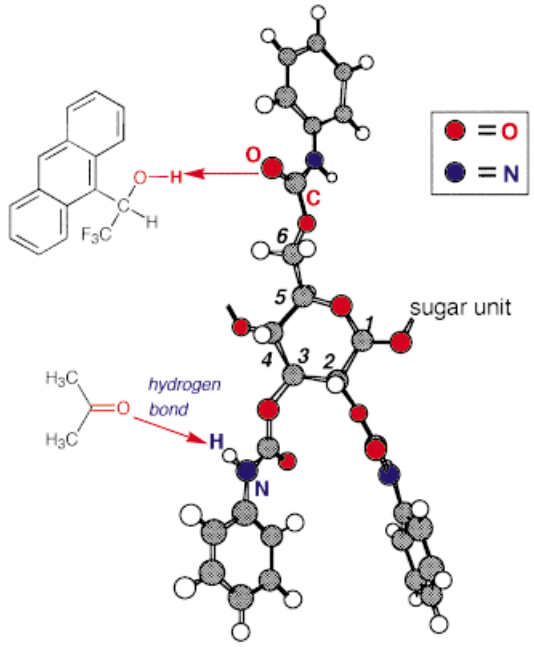


Figure 14. Possible interaction sites of cellulose tris(phenylcarbamate) derivatives.

On the other hand, when X is an electron-donating group (e.g. methyl) the electron density at the carbonyl oxygen atom of the carbamates is expected to increase. Therefore, alcohols are more strongly adsorbed on the CSPs through a hydrogen-bonding interaction. When X is a polar group (e.g. nitro or methoxy) the racemates may interact with X, which will result in a lowering of the resolving ability (see Section 3.2) since X is far away from a chiral glucose residue. Therefore, substitutions with bulky alkoxy groups such as isopropoxy or isobutoxy groups (**31** and **32**, Scheme 2) improve the resolving ability by reducing the interactions at the ether oxygen atom.[44a]

Although the phenylcarbamate derivatives of polysaccharides have become popular CSPs, the mechanism for discrimination between enantiomers at a molecular level has remained obscure. A determination of the exact structures of the phenylcarbamate derivatives is necessary to reveal the mechanism. Figure 15 shows a stable structure of CTPC that was optimized by molecular-mechanics calculations starting from the proposed X-ray crystal structure of CTPC.[45] CTPC has a left-handed 3/2-helical conformation, and the glucose residues are regularly arranged along the helical axis. A chiral helical groove with polar carbamate groups exists parallel to the main chain. The polar carbamate groups are preferably located inside, and hydrophobic aromatic groups are placed outside the polymer chain so that polar enantiomers may predominantly interact with the carbamate residues in the groove through hydrogen-bond formation. This interaction seems to be important for efficient chiral discrimination, especially in normal-phase HPLC.

In addition to these polar interactions, $\pi-\pi$ interactions between phenyl groups of a CTPC derivative and aromatic groups of a solute may play a role in the recognition of chirality, because several nonpolar aromatic compounds have also been resolved.[49b] Consequently, the interaction of the carbamate groups of CSPs with racemates seems to be most important for effective recognition of chirality. This speculation is supported by NMR studies.

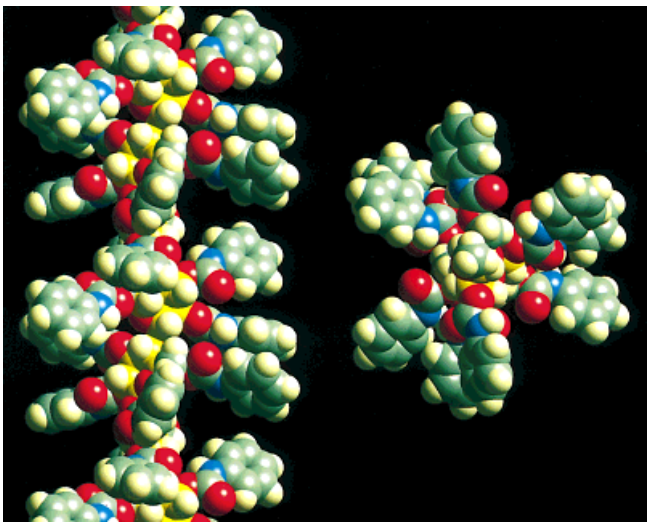


Figure 15. Optimized structure of CTPC based on the structure determined by X-ray analysis.[45] The glucose carbon atoms of CTPC are shown in yellow for clarity. View along the helix axis (left) and perpendicular to the helix axis (right).

4.2.2. NMR Studies

CTPC seems to maintain its helical structure even in solution.[82] However, most phenylcarbamates of the polysaccharides with resolving ability as high as CSPs are soluble only in polar solvents such as acetone, pyridine, and THF. In these polar solvents the derivatives show poor recognition of chirality because the solvents preferentially interact with the polar carbamate residues. Therefore, it was difficult to elucidate the mechanism for discrimination between enantiomers with NMR spectroscopy in these solvents. However, we recently found that several phenylcarbamate derivatives—for instance, tris(4-trimethylsilylphenylcarbamate) (**33**)[83] and tris(5-fluoro-2-methylphenylcarbamate) (**65**)[42b] of cellulose (Scheme 2)—are soluble in chloroform, and discriminate between enantiomers in ^{1}H and ^{13}C NMR spectroscopies as well as HPLC. This permitted us, for the first time, to investigate the chiral interaction occurring in solution by NMR spectroscopy. The phenylcarbamate derivatives resolved many racemates by HPLC, and the chromatographic results can be directly correlated to those from spectroscopy.

Figure 16 shows the ^{1}H NMR spectra of (±)-*trans*-stilbene oxide (**69**) in the absence (a) and presence of **33** (b). The signal for the methine proton of **69** is split into two singlets in the presence of **33**,[83] and only the signal of the (−) isomer is shifted downfield. This clearly indicates that **33** can discriminate between the enantiomers even in solution. In the chromatographic separation of (±)-**69** on the CSP **33**, the (+) isomer was eluted first followed by the (−) isomer, and complete base-line separation was achieved ($\alpha = 1.55$). This order of elution correlates well with the downfield shift of the (−) isomer observed in the ^{1}H NMR spectrum.

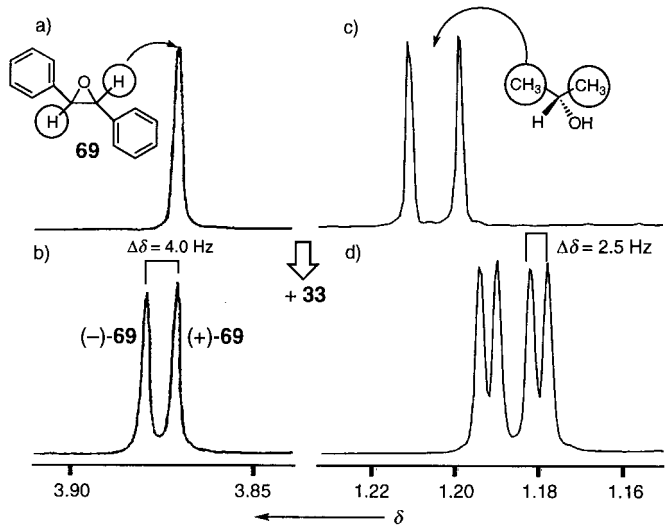


Figure 16. ^{1}H NMR spectra of *trans*-stilbene oxide (**69**, 5 mg) in a) the absence and b) the presence of **33** (40 mg) in $CDCl_3$ (1.0 mL) at 22 °C. The peaks were assigned with enantiomerically pure (+)- and (−)-**69**. c), d) The changes in the methyl proton resonances of 2-propanol (3 μL) added to **69** (5 mg) and **69** (5 mg) + **33** (20 mg) are also shown.

As previously mentioned, the most important adsorption site for discrimination between enantiomers on phenylcarbamate derivatives may be the polar carbamate residue (Figure 14). For **69**, the oxygen atom of the oxirane ring may interact with the NH proton of the carbamate residue through hydrogen bonding. Therefore, addition of a hydrogen acceptor, such as acetone, does not result in splitting of the methine proton signal.[83] A similar change in the ^{1}H NMR spectrum of **69** was also induced upon addition of 2-propanol. Interestingly, the signals for the methyl groups of 2-propanol were shifted upfield and split into a pair of doublets (Figure 16d), indicating that the two methyl groups were magnetically nonequivalent in the presence of **33**. The chirality of **33** seems to force 2-propanol to bind in a diastereotopic environment, and allows the recognition of enantiotopic methyl groups.[83, 84]

The ^{1}H NMR signals of other racemic mixtures, including Tröger's base (**12**) and benzoin (**70**), were also separated into two sets of peaks in the presence of **33**. Moreover, in the case of secondary alcohols such as 2-heptanol and 2-octanol, the methyl protons at the end of the longer chain and very remote from the stereogenic center were enantiomerically discriminated in the presence of **33** (Figure 17), whereas the methine and another methyl protons were not recognized. This

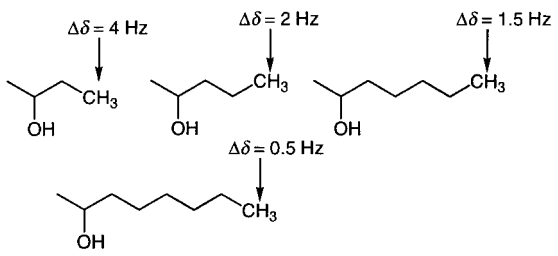
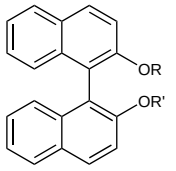


Figure 17. Secondary alcohols whose enantiomers can be distinguished by **33**. The protons marked with an arrow are those that are recognized with ^{1}H NMR spectroscopy.

suggests that the methyl protons of the alkyl chains may be specifically located near chiral **33**, so that the signals for the methyl protons split into signals for both enantiomers: Compound **33** can function as a chiral shift reagent.

The phenylcarbamate derivative **65** can discriminat between enantiomers of 2,2′-dihydroxy-1,1′-binaphthyl (**91a**) and 2,2′-dihydroxy-6,6′-dimethyl-1,1′-biphenyl (**73**) very well in HPLC.[85] Figure 18 shows a chromatogram for the resolution of **91a** on **65** with a large separation factor (α = 4.23) and complete base-line separation; the *R* isomer is eluted first followed by the *S* isomer. Analogue **73** is also resolved completely with a large α value (3.22). As in the separation of **91a**, the *S* isomer is retained longer.



91a: R = R' = H
91b: R = H, R' = CH_3
91c: R = R' = CH_3

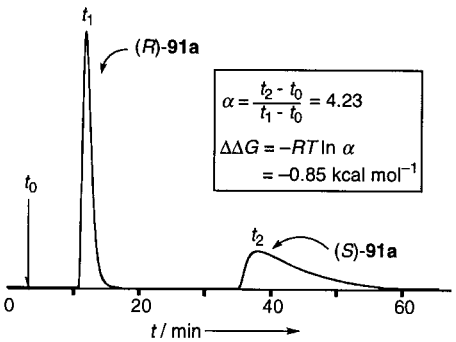
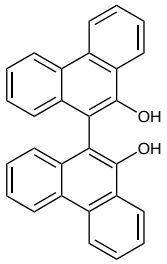


Figure 18. Chromatogram of the enantioseparation of (*RS*)-1,1′-bi-2-naphthol (**91a**) on CSP **65** with hexane/2-propanol (9/1) as the eluent at 20 °C. Column: 25 × 0.46 cm (inner diameter); flow rate: 1.0 mL min^{-1}.

The polymer **65** also discriminates between the enantiomers of **91a** and **73**, as shown by ^{1}H and ^{13}C NMR spectroscopy.[85] Figure 19 shows the ^{1}H NMR (a, b) and ^{13}C NMR spectra (c) of (*RS*)-**91a** in the absence (a) and presence of **65** (b, c) in $CDCl_3$. Each signal for the hydroxyl and naphthyl protons H4 and H6 of **91a** is distinctly split into two signals for the enantiomers. The signals for the OH protons of (*S*)-**91a** are more downfield-shifted with line broadening than the corresponding signals for (*R*)-**91a**, whereas the signals for the H4 and H6 protons of (*S*)-**91a** are shifted upfield with broadening. This indicates that (*S*)-**91a** interacts more strongly with **65**. The downfield shift of the OH resonances is ascribed to hydrogen-bond effects, and the upfield shifts for the aromatic protons are probably due to the π-stacking or shielding effect of a neighboring aromatic ring of **65**. There are similar splittings of signals for **73** into those for its enantiomers in the presence of **65**, and the resonances for the OH proton of (*S*)-**73** are more downfield-shifted than the corresponding signals for (*R*)-**73**. Mono- and di-O-methylated derivatives **91b, c** and bulky **92** are hardly separated in HPLC and cannot be distinguished in the ^{1}H NMR spectrum. Hydrogen bonding through the hydroxy group



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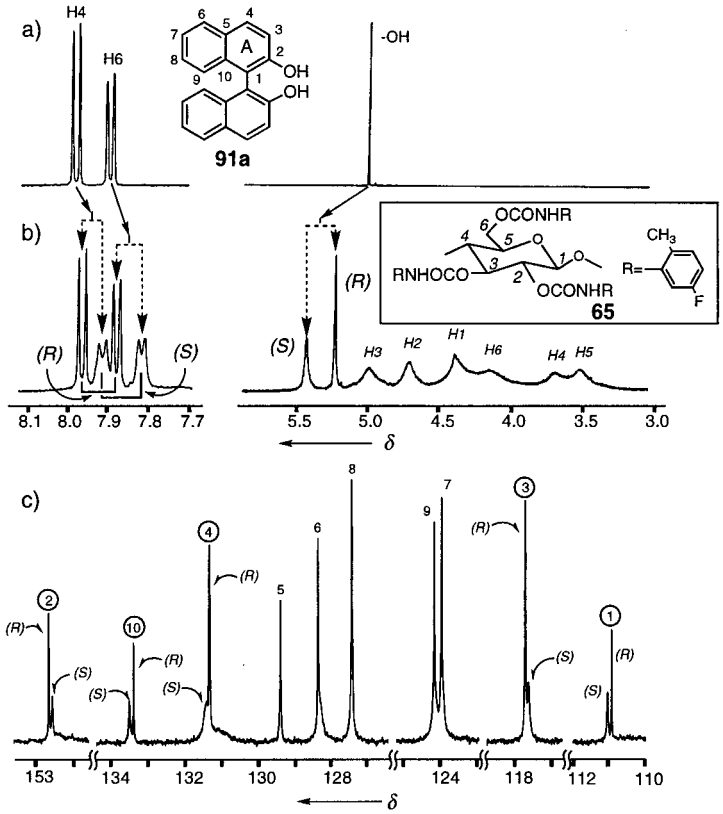


Figure 19. Selected region of the a), b) ^{1}H and c) ^{13}C NMR spectra of (*RS*)-**91a** in the absence (a) and presence (b, c) of **65** in $CDCl_3$ at 23 °C.

of **91a** seems to be the main force for its retention and resolution.

The discrimination of enantiomers in the presence of **65** by ^{13}C NMR spectroscopy is also possible for **91a** and **73**.[85] The signals for carbon atoms C1 – C4 and C10 of **91a** are separated into the signals for the enantiomers, and the resonances for (*S*)-**91a** are markedly broadened. The signals exhibiting large splittings belong to carbon atoms of ring A (Figure 19), indicating that ring A may be favorably located close to the chiral glucose residue. In other words, with ring A (*S*)-**91a** may insert into the chiral groove of **65** to form the hydrogen bond. Measurements of the relaxation time of the complexes also support this speculation.[85] The relaxation times (T_1) of all carbon atoms of (*R*)- and (*S*)-**91a** are shorter in the presence of **65** than those of free **91a**, and those T_1 values for (*S*)-**91a** are always shorter than the corresponding values for (*R*)-**91a** (Table 2). A remarkable reduction in T_1 is observed for carbon atoms C1 – C5 and C10 attached to ring A. These results indicate that the mobility of ring A is restricted by an intermolecular hydrogen bond between the hydroxl groups

Table 2. Spin-lattice relaxation times T_1 [s] of **91a** in the presence of **65** in $CDCl_3$ at 19 °C.

C atom	T_1 (*S*)	T_1 (*R*)	C atom	T_1 (*S*)	T_1 (*R*)
1	7.47	8.82	6	0.97	1.02
2	4.20	5.31	7	0.90	0.93
3	0.90	1.07	8	0.86	1.01
4	0.82	0.99	9	0.92	1.00
5	3.07	4.03	10	2.98	4.15

at C2 and the carbonyl group of the carbamate residues of **65**.

^{1}H NMR titrations of **65** with (*S*)- and (*R*)-**91a** were performed and a Job plot of the continuous changes in chemical shifts for the complex **65** · (*S*)-**91a** was prepared to investigate the binding sites of **65** and the stoichiometry of the complexation. The H2 proton resonance of a glucose unit of **65** is dramatically affected by addition of (*S*)-**91a** and shifts upfield upon binding, whereas the other glucose proton resonances move only slightly. The significant upfield shift of the H2 proton resonance indicates that a naphthyl ring of (*S*)-**91a** may be closely located above the H2 proton. In the Job plot the maximal complex formation occurs at about 0.5 mol fraction of the glucose residue of **65**. This indicates that each glucose unit of **65** may have the same binding affinity to (*S*)-**91a**, which may be attributed to the regular structure of **65** even in a solution.[85]

The most valuable information for the geometry of the complex between **65** and (*S*)-**91a** was obtained by 2D NOESY spectroscopy.[85] Figure 20 shows the NOESY spectrum of **65** · (*S*)-**91a**. Several cross-peaks for clear intermolecular

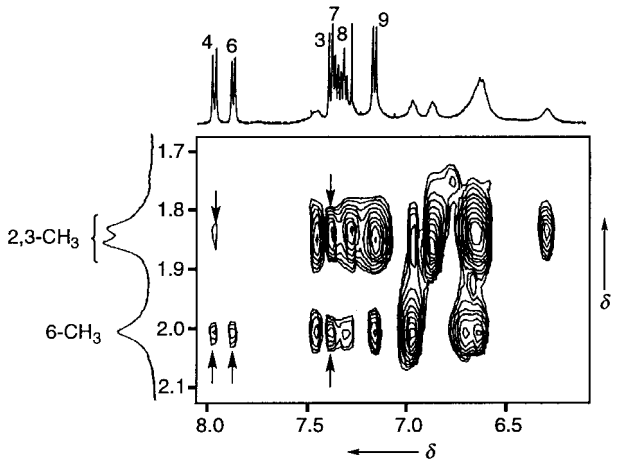


Figure 20. NOESY spectrum ($CDCl_3$, 23 °C) of a mixture of (*S*)-**91a** and **65** (molar ratio 1:2) at a mixing time of 300 ms. F_2 axis: region of the arene protons of **65** and **91a**; F_1 axis: region of the methyl protons on the phenyl moiety of **65**. See text for further details.

NOEs between the aromatic H4, H6, and H7 protons of (*S*)-**91a** and the methyl protons of **65** are represented by arrows. On the other hand, a NOESY spectrum of a mixture of (*R*)-**91a** and **65** is very similar to the spectrum of free **65**, and no intermolecular NOEs between **65** and (*R*)-**91a** are observed. These data indicate that (*S*)-**91a** binds or interacts with **65** more strongly than (*R*)-**91a**, and that the naphthyl protons of (*S*)-**91a** are located at a distance of less than 5 Å to the methyl protons of **65**. These observations are in accord with the results of HPLC and 1D NMR experiments.

The enantioselectivities (α) and the thermodynamic parameters $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$ for the more stable complex **65** · (*S*)-**91a** and the difference in Gibbs energy ($\Delta\Delta G^{\ddagger}$) in the chiral discrimination process can be separately determined by ^{1}H NMR titrations in solution and by HPLC. On the basis of the HPLC and NMR data combined with the structural data

of cellulose trisphenylcarbamate (CTPC) determined by X-ray analysis, a model structure for the **65** · (*S*)-**91a** complex can be proposed (Figure 21).[85] According to this model (*S*)-**91a** is in a chiral groove of **65** and directed toward the H2 proton of the glucose through intermolecular hydrogen bonds between the OH protons and the carbonyl oxygen atoms of **65** (dashed line). This model satisfactorily explains all the NMR data, including the intermolecular NOE data and the results of the titrations. Moreover, the difficulty of 10,10′-dihydroxy-9,9′-biphenanthryl (**92**) to discriminate between enantiomers is reasonably explained: The bulky phenanthryl groups of **92** can prevent the formation of hydrogen bonds. These results should provide useful information both for understanding the mechanism for discrimination between enantiomers on other polysaccharide-based CSPs and for designing even better CSPs.

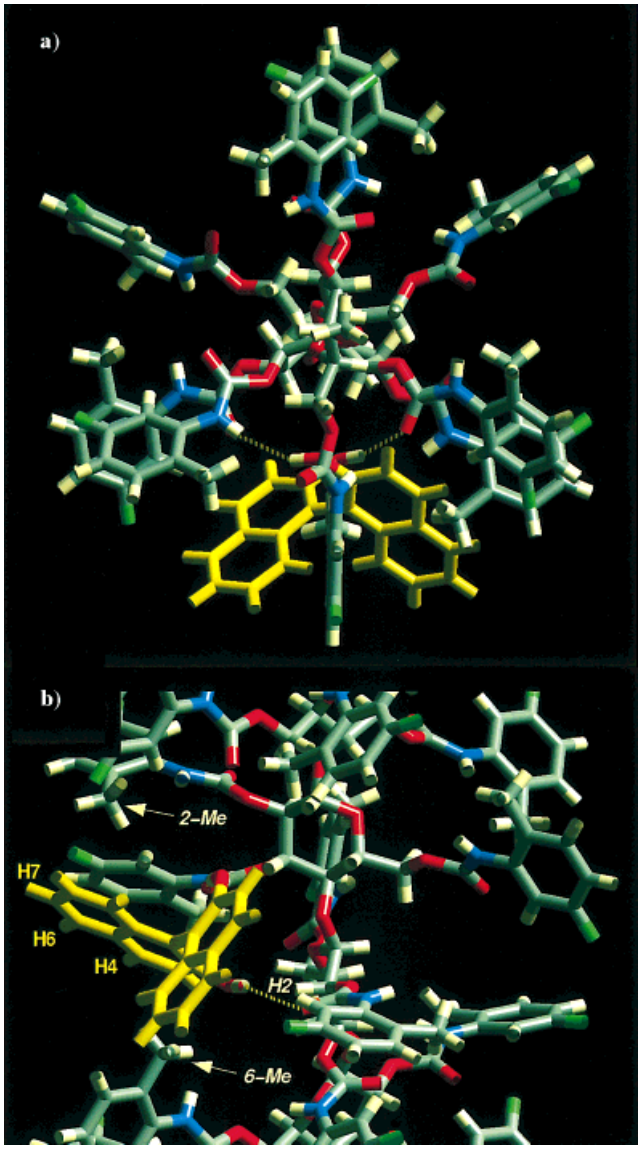


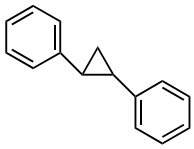
Figure 21. Calculated sturcture of the complex **65** · (*S*)-**91a**. The (*S*)-**91a** portion is shown in yellow, and dashed lines correspond to hydrogen bonds. a) View perpendicular to the helix axis; b) expanded region of the same structural model as viewed along the helix axis. Reproduced with permission from ref. [85].

Commercially available amylose-based CSPs **78** and **89** were recently found to be soluble in chloroform, and can discriminate between enantiomers for many compunds in NMR spectroscopy as well as HPLC.[86] Further NMR studies, especially NOESY experiments coupled with a computer modeling, will certainly serve to propose a rationale for discrimination between enantiomers at a molecular level.

4.2.3. Computational Studies

A computer simulation involving molecular-mechanics (MM) and molecular-dynamics (MD) calculations may be a useful and effective approach for elucidating the mechanism for the recognition of chirality on other $CDCl_3$-insoluble phenylcarbamate derivatives of polysaccharides, and for predicting the order of elution for the enantiomers. Lipkowitz et al. have been extensively studying the mechanism for the recognition of chirality from theoretical viewpoints with the aid of chromatography on CSPs made from optically active small molecules.[70] The interaction energies between the CSPs and the enantiomers were calculated by MM, MD, and quantum-mechanical calculations, and the mechanisms for the recognition of chirality have been proposed on the basis of the above calculations.

We recently carried out MM calculations on the interaction energies between CTPC[87] or CDMPC[88] and *trans*-stilbene oxide (**69**) or *trans*-1,2-diphenylcyclopropane (**93**) to gain insight into the mechanism for the recognition of chirality on $CDCl_3$-insoluble phenylcarbamate derivatives. CTPC is a good candidate for such a study, because its structure was postulated on the basis of an X-ray analysis[45] and it shows a high ability to recognize chirality for HPLC as a CSP as well as a CDMPC. In a chromatographic enantioseparation, **69** was completely resolved on CTPC ($\alpha = 1.46$) and CDMPC ($\alpha = 1.68$). However, the order of elution is reversed: The *R*,*R* isomer eluted first followed by the *S*,*S* isomer on CTPC, whereas the *S*,*S* isomer eluted first on CDMPC. Racemic **93** was not separated on CTPC ($\alpha \approx 1$).



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The calculations were performed by two methods:

1) A molecule of the enantiomer was placed in space and rotated at specified angles around each NH proton in the 2-, 3-, and 6-positions of the carbamoyl group of CTPC or CDMPC, which may be considered to be the most important adsorption site for **69** on the basis of chromatographic resolution and NMR studies. The interaction energy was then calculated at each point of a grid on the CTPC or CDMPC molecule with all possible combinations of the rotation angles of the enantiomer. The calculation results were evaluated with the lowest interaction energy and the distribution of the interaction energy.

2) A molecule of the enantiomer with a particular orientation was randomly generated with the Monte Carlo meth-

od[89a] on the surface of a CTPC or CDMPC molecule defined by a particular van der Waals radius, which was obtained with the technique of "blowing up" the atomic radii.[89b] MM calculations between the molecules were then performed step by step. The results of these calculations were evaluated with the averaged interaction energy.

In both calculations an octamer or nanomer of CTPC and CDMPC molecules, whose structure was 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.

Although they will not be described in detail here, the results of both calculations were in good agreement with the observed chromatographic resolution on CTPC and CDMPC.[87, 88] The calculations suggested that the lowest or averaged interaction energy between CTPC and (*S*,*S*)-**69** is lower than that between CTPC and (*R*,*R*)-**69**, whereas a reversed enantiomer preference was observed for CDMPC and **69**. This indicates that (*S*,*S*)-**69** may interact more closely with CTPC than (*R*,*R*)-**69**, which in turn interacts more strongly with CDMPC.

Figure 22 provides a graphical view of the interaction between CTPC and (*S*,*S*)-**69**. Compound (*S*,*S*)-**69** is bound in a chiral groove, and each phenyl group may interact with the phenyl groups of CTPC through $\pi-\pi$ interactions; the ether oxygen atom of (*S*,*S*)-**69** is located near the NH proton of CTPC and forms a hydrogen bond. Although the same calculation was performed for **93** and CTPC, almost no different interaction energy was observed for the enantiomers.

The reason why CDMPC shows the opposite enantioselectivity toward **69** is not clear at present. The optimized structure of CDMPC (Figure 23) shows a similar left-handed 3/2 helix as for CTPC, but the aromatic rings are arranged differently. This may be responsible for the reversed enantioselectivity of CTPC and CDMPC. CTPC and CDMPC also behave differently in dilute solutions: Norisue et al. reported that CDMPC exhibits pronounced optical anisotropy, in contrast to other cellulose derivatives including CTPC, which may be related to the characteristics of CDMPC.[90]

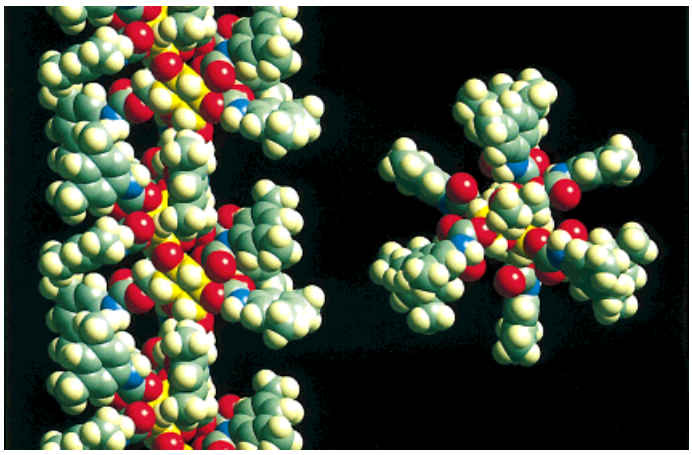


Figure 23. Optimized structure of CDMPC. The glucose carbon atoms of CDMPC are shown in yellow for clarity. View along the helix axis (left) and perpendicular to the helix axis (right).

In the above calculations, interactions between a single chain of the polysaccharides and an enantiomer are taken into consideration, since polar racemates may interact preferentially with polar carbamate residues inside the polymer chain. However, besides these polar interactions, the $\pi-\pi$ interaction between the phenyl group of a CTPC derivative and the aromatic groups of a solute may play a role in the recognition of chirality, because several nonpolar aromatic compounds have also been resolved.[15, 49b] Especially under reverse-phase conditions with aqueous eluents, certain kinds of hydrophobic chiral cavities between the CTPC chains (Figure 24) must play a dominant role for effective recognition of chirality. Further computational studies of this system will be useful.

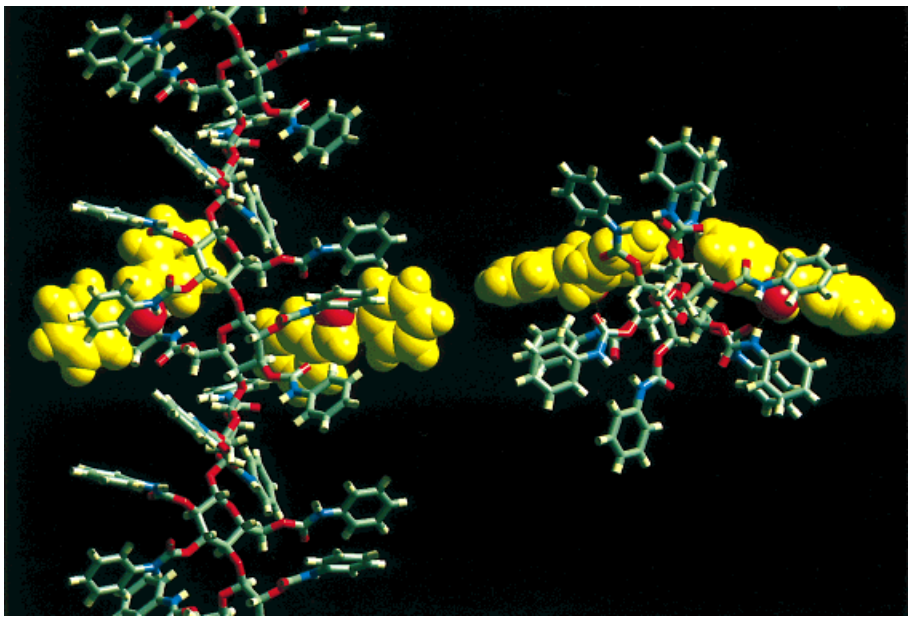


Figure 22. Calculated structure of complex CTPC·(*S*,*S*)-**69** (yellow) formed through hydrogen bonds. View along the helix axis (left) and perpendicular to the helix axis (right).

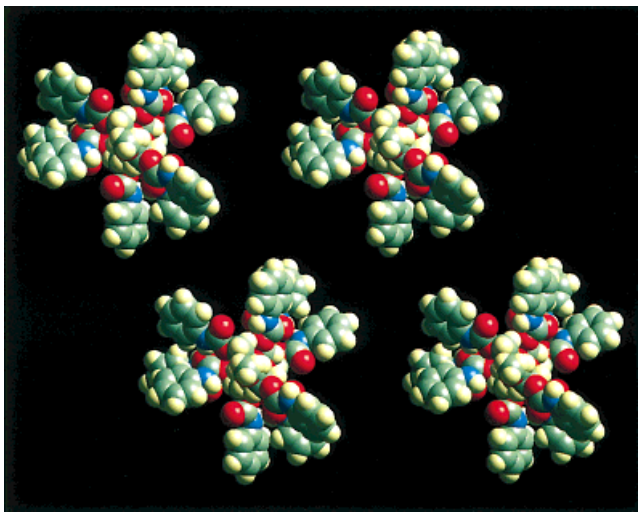
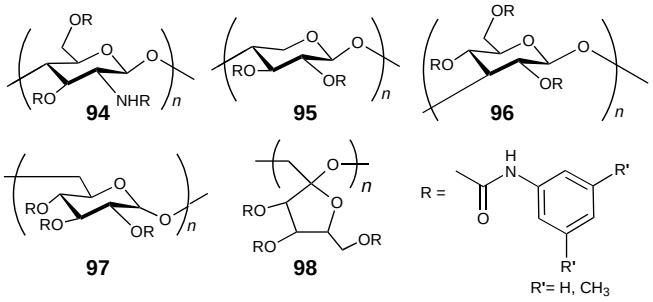


Figure 24. Calculated structures of CTPC aggregates as viewed perpendicular to the helix axis.

5. Other Phenylcarbamates of Polysaccharides, Oligosaccharides, and Cyclodextrins

Phenylcarbamates of other polysaccharides such as chitosan (**94**), xylan (**95**), curdlan (**96**), dextran (**97**), and inulin (**98**) were also prepared and used as CSPs.[91] The abilities to



recognize chirality depended markedly on the nature of the monosaccharide units and position and type of the linkage. Among the polysaccharide derivatives, the 3,5-dimethylphenylcarbamates of chitosan and xylan show a relatively high ability to recognize chirality, although the 3,5-dimethylphenylcarbamates of cellulose and amylose often exhibit better resolving power. However, some racemates were better resolved on the phenylcarbamates than on the corresponding cellulose and amylose derivatives. For instance, $[Cr(acac)_3]$ and $[Co(acac)_3]$ (**76**) were more efficiently resolved on **95b** ($R' = CH_3$; Figure 25). The ability of a CSP derived from phenylcarbamoylated chitin to recognize chirality is very low.[91c] Phenylcarbamates of amylopectin, one of the constituents of starch which is made up of highly branched chains of glucose units, exhibit an enantioselectivity that is rather similar to that of the corresponding amylose derivative ADMPC.[91d]

3,5-Dimethylphenylcarbamates of cellooligosaccharides (**99**) and 3,5-dimethylphenyl-, 4-chloro-, and 4-bromophenylcarbamates of maltooligosaccharides (**100**) were prepared, and their abilities to recognize chirality were compared with

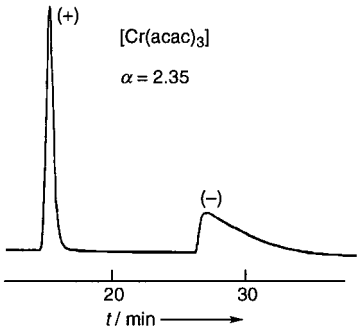
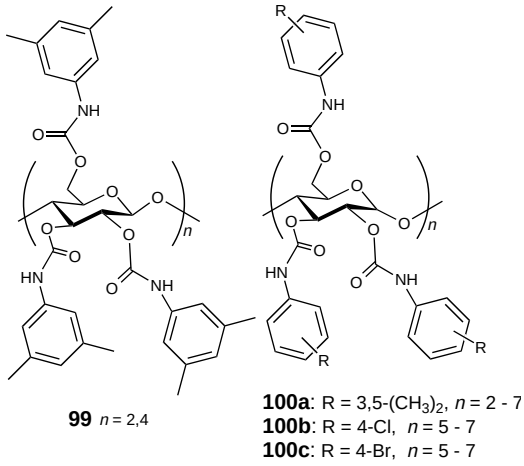


Figure 25. Separation of (±)-chromium(III) tris(acetylacetonate) on bis(3,5-dimethylphenylcarbamate) of xylan (**95**, $R = CH_3$) with hexane/2-propanol (9/1) as the eluent. Column: 25 × 0.46 cm (inner diameter); flow rate: 0.5 mL min^{-1}.

those of the corresponding polysaccharide derivatives to obtain information on the influence of the higher order structures of the polysaccharide derivatives.[92a,b] The ability of



99 to recognize chirality is lower than that of the cellulose derivative CDMPC, whereas the resolving ability of **100a** is not so different from that of the corresponding amylose derivatives when $n = 4-7$. This indicates that the cellooligosaccharides have a different structure from that of CDMPC, whereas the maltooligosaccharides have an ordered structure similar to those of the amylose derivatives when n is greater than 4. These explanations were supported by conformational studies of the oligomers by CD spectroscopy.[92a,b] Chemically bonded type CSPs derived from benzoylglucosamine and 1-(1-naphthyl)ethylcarbamoylated maltooligosaccharides were prepared, and their resolving abilities were evaluated; some racemates were completely resolved on the CSPs.[92c,d]

MM and MD calculations of the 4-chloro- and 4-bromophenylcarbamates of maltohexaose, models of the amylose derivatives, were performed, and their conformations were examined to propose a model structure for the corresponding amylose phenylcarbamates.[92b] The initial structures were constructed from the crystal structure data of amylose, which has a left-handed 6/1-helical structure.[93a] The conformations of the 4-chloro- and 4-bromophenylcarbamates of maltohexaose were transformed from a left-handed 6/1 helix to almost left-handed 5/1 and 4/1 helices, respectively, after MD

simulations.[92b] Figure 26 shows the structures of native amylose (a) and a model of the amylose trisphenylcarbamate (b) derived from the calculated structure of 4-bromophenylcarbamate of maltohexaose. The helical pitch of the amylose phenylcarbamate is similar to that of amylose tris(phenylcarbamate) proposed by Zugenmaier et al.[46] Although the

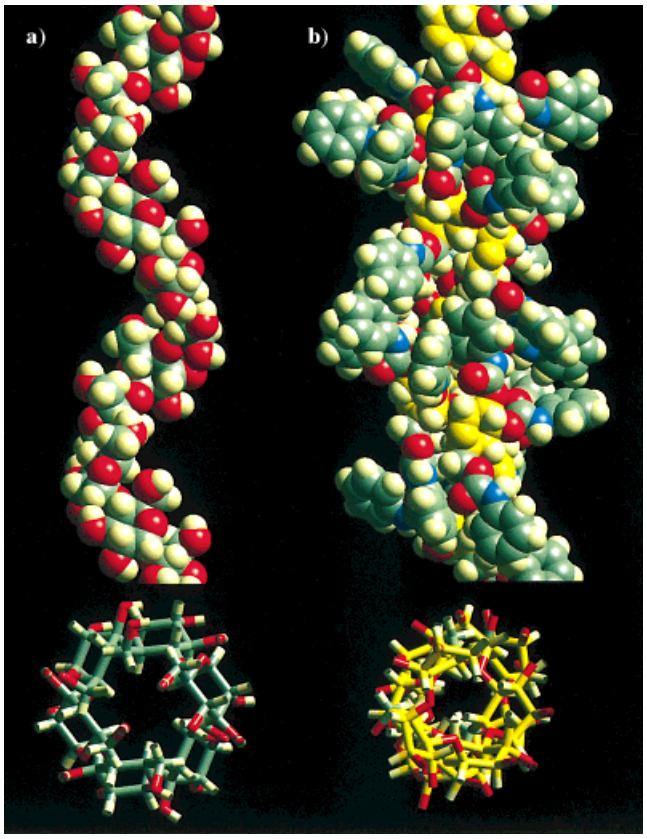
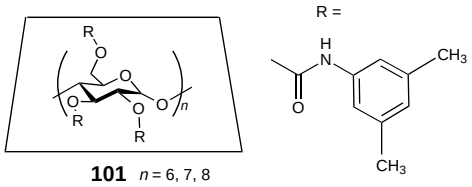


Figure 26. a) Crystal structure of native amylose. b) A possible structure of ATPC. The structure of ATPC was constructed on the basis of the optimized structure of 4-bromophenylcarbamates of maltohexaose.[92b] The glucose carbon atoms of ATPC are shown in yellow. View along the helix axis (top) and perpendicular to the helix axis (bottom); all side groups and protons are omitted for clarity. The initial atomic coordinates of native amylose were taken from the crystal structure data [93a] in the Cambridge Structural Database 3D Graphics Search System.[93b]

reason for the difference in the structures of the oligomers depending on the kind of the halogen atom is not clear, the present results indicate that the amylose phenylcarbamate derivatives may not have a structure similar to that of native amylose (a left-handed 6/1 helix), but rather contain a tight, left-handed 4/1 or 5/1 helix. Wainer et al. also proposed a model of ADMPC on the basis of computer simulations; the helix seems to be larger than a 4/1 or 5/1 helix.[94]

Cyclic oligosaccharides, cyclodextrins, consist of the same α-D-glucose unit as amylose and can separate many racemates by an inclusion mechanism in GC,[95] HPCE,[29, 30] and HPLC.[11a] The CSPs consisting of cyclodextrins bonded to silica gel are commercially available. On the other hand, the abilities of 3,5-dimethylphenylcarbamates of cyclodextrins **101** to recognize chirality are quite different from those of native cyclodextrins and ADMPC. This indicates that the



101 $n = 6, 7, 8$

amylose derivative must possess a higher order structure that is different from that of the cyclodextrin derivatives.[92a, 96]

Figure 27 shows the structures of β-cyclodextrin[97] (a) and its 3,5-dimethylphenylcarbamate (b): Both rims of the derivatized cyclodextrin are covered with 3,5-dimethylphenylcarbamate residues, and, therefore, the enantioselectivity of the derivatized phase may be governed by the interaction with the polar carbamate residues and may be independent of the inclusion known for native β-cyclodextrin. These phenylcarbamates of linear and cyclic oligomers are soluble in $CDCl_3$ and exhibit chiral discrimination in NMR spectroscopy as well as HPLC;[83b] the results of NMR spectroscopy, in particular for the cellooligosaccharides, may be useful for understanding the mechanism for the recognition of chirality by CDMPC, which is not soluble in chloroform.

6. Miscellaneous Applications

The polysaccharide derivatives have a great merit for the easy preparation of a film (membrane), which can be used as a new device in the enantiomer-separation system.[98] The CDMPC membrane prepared by coating a solution of CDMPC in THF on a Teflon membrane filter as a support showed a high ability for enantioselective adsorption; for instance, oxprenolol (**81b**) enriched in the *S* isomer up to 60% *ee* was obtained by a single adsorption–desorption procedure. This system can be applied to the enantioselective permeation of oxprenolol in an organic media.[99] Oxprenolol obtained through the membrane was enriched in the *S* isomer up to 50% *ee* in the initial stage, and enrichment in the *R* isomer for a source phase (23% *ee*) was achieved.

CDMPC can also be utilized as a "chiral belt" for the continuous, rapid, and preparative resolution of **81b** with a motor.[100] Figure 28 illustrates an apparatus used for the resolution. A CDMPC belt (111 cm long) was fitted with the apparatus and rotated at a constant speed (66 cm h^{-1}) by a motor. A) The belt entered a solution of racemic **81b** (100 mg 100 mL^{-1}) for enantioselective adsorption, B) hexane/2-propanol (95/5) for enantioselective desorption, C) hexane/2-propanol (7/3) for desorption, and finally D) hexane for rinsing. The source phase A gradually became rich in the *R* isomer, and the enatiomeric excess reached 28% after 8 h when 65 mg of **81b** was transported from the source phase. The receiving phase C became rich in the *S* isomer with up to 68% *ee* at the initial stage (after 2 h). The present method can be scaled up without any difficulty, and may be used for a large-scale separation.

The separation of enantiomers through chiral membranes, such as liquid membranes with a chiral mobile carrier[101] or solid chiral polymer membranes,[102] is attractive for the

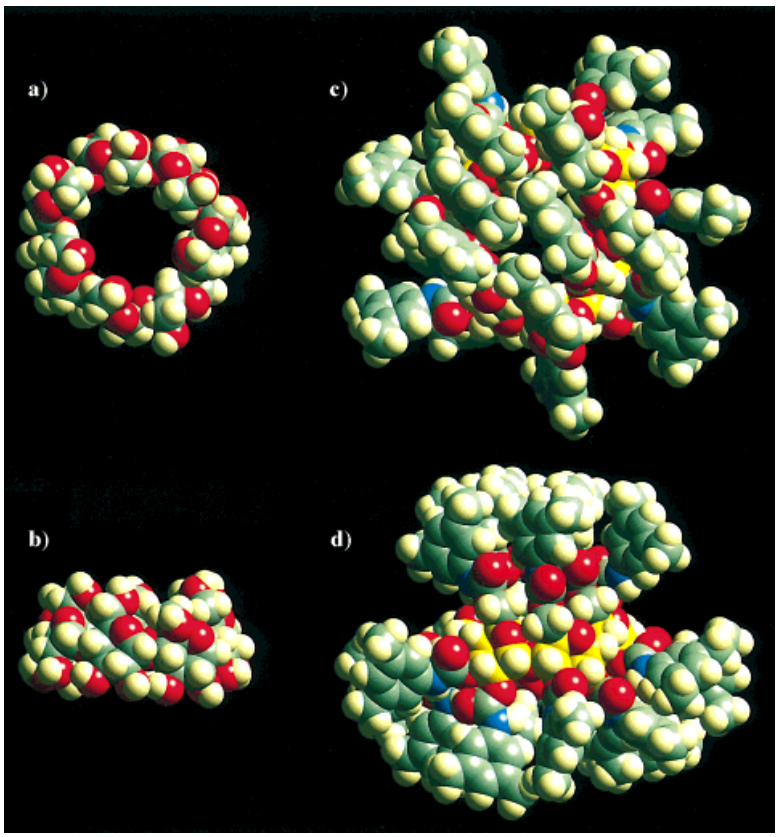


Figure 27. Plan (a, c) and side views (b, d) of the crystal structure of β-cyclodextrin (a, b) and the possible structure of 3,5-dimethylphenylcarbamoylated β-cyclodextrin (c, d) optimized by MM and MD calculations. The glucose carbon atoms of phenylcarbamoylated β-cyclodextrin are shown in yellow for clarity. The initial atomic coordinates of β-cyclodextrin were taken from the crystal structure data[97] in the Cambridge Structural Database 3D Graphics Search System.[93b]

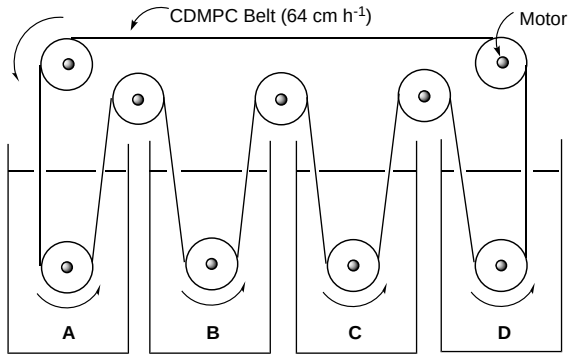
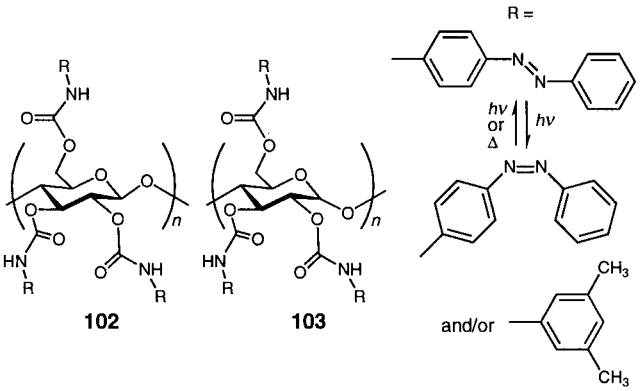


Figure 28. An apparatus used in the resolution of (±)-oxprenolol (**81b**) with a chiral belt. See text for further details.



further development of separation technology in this field. However, their efficiency and enantioselectivity are, at present, not high enough for practical use.

The polysaccharide derivative membranes bearing a photoresponsive 4-phenylazophenyl residue (**102** and **103**) can sensitively respond to light and/or heat and exhibit interesting chiral properties in enantioselective adsorption.[103a] The ability to recognize chirality is influenced by *trans*–*cis* isomerization of the pendant azobenzene residues. The *trans* membranes showed higher enantioselectivity than the *cis* membranes, and the selectivity could be reversibly controlled in an on–off fashion by photoisomerization of the pendant azobenzene moieties. When the azo group of the membrane **102** was *trans*-oriented, **81b** with 43 % *ee* was adsorbed, and when it was *cis*-oriented, **81b** with 38 % *ee* was adsorbed (Figure 29). Thus, the photocontrol of the recognition of chirality was realized for the first time with the photoresponsive chiral polymer membranes. Interestingly, recognition of chirality by a CSP consisting of **102** depends on the

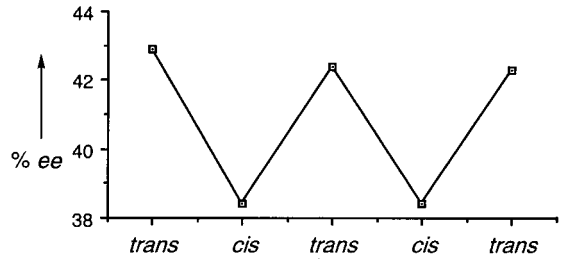


Figure 29. Change in the enantiomeric excesses (*ee*) of **81b** adsorbed on the **102** membrane during the *trans*–*cis* isomerization (irradiation time 4 h for *trans* → *cis*, 2 h for *cis* → *trans*). The portions of *trans* and *cis* were 100 and 80 %, respectively.

proportion of *trans* or *cis* orientations of the azobenzene residues.[103b]

Polysaccharides and their derivatives were also used as chiral catalysts[104] and chiral templates[105] in asymmetric synthesis and asymmetric polymerization, respectively. Bredig et al. used diethylaminocellulose as the first polymeric chiral catalyst for an asymmetric cyanohydrin reaction in 1932.[104a] Relatively high optical yields of up to 77% were obtained in the hydrogenation of 2-phenyl-1-butene with a complex made up of rhodium and 2,3-*O*-bis(diphenylphosphanyl)-6-*O*-triphenylmethylcellulose.[104b] Similar rhodium catalysts derived from D-glucose show very high enantioselectivities of up to 99% *ee* in asymmetric hydrogenations.[104c]

7. Summary and Outlook

We have prepared many polysaccharide derivatives and have evaluated them as CSPs for HPLC; some of them are commercially available.[106] A wide range of racemates including aliphatic and aromatic compounds with or without functional groups and many drugs have been resolved on the CSPs. Among the CSPs prepared so far, 3,5-dimethylphenylcarbamates of cellulose (Chiralcel OD), amylose (Chiralpak AD), and cellulose tris(4-methylbenzoate) (Chiralcel OJ) are excellent for recognizing chirality. This allowed the successful resolution of more than 80% of the racemates tested.[15g,h] These CSPs can be used not only for analytical purposes, but also for the preparative-scale separation of enantiomers.

The utility of the polysaccharide-based CSPs for the large-scale preparative separation of enantiomers has been realized. Francotte has reviewed the preparative-scale chromatographic resolution of enantiomers, and concludes that even in the preparative resolutions about 70% of the CSPs used for this purpose are derived from polysaccharide derivatives.[17c] In particular, the recently developed simulated moving-bed (SMB) chromatography has a great potential for the industrial-scale preparation of pure enantiomers, and has been utilized.[3b, 7b, 17c, 107] SMB chromatography is a continuous solid–liquid countercurrent process. It allows the continuous introduction of the feed and thus permits large amounts of eluent to be saved.

The elucidation of a mechanism for discrimination between enantiomers on the CSPs at a molecular level appears to be essential for further developments in this exciting area. Elucidation of the exact mechanism for the discrimination of chirality on the CSPs must serve to predict the order in which enantiomers are eluted and to develop a superior polysaccharide-based CSP.

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[106] Coated-type, cellulose-based CSPs are available from Daicel: triacetate (**17**, Chiralcel OA), tribenzoate (**24**, Chiralcel OB), tris(4-methylbenzoate) (**21**, Chiralcel OJ), tricinnamate (Chiralcel OK), trisphenylcarbamate (**40**, Chiralcel OC), tris(4-methylphenylcarbamate) (**34**, Chiralcel OG), tris(4-chlorophenylcarbamate) (**42**, Chiralcel OF), tris(3,5-dimethylphenylcarbamate) (**49**, Chiralcel OD). Microcrystalline CTA-I (**17**) is also available from Daicel (Chiralcel CA-I), Merck (Cellulose triacetate), and Macherey-Nagel (Cellulose Ce-AC-40XF). Coated-type, amylose-based CSPs are commercialized from Daicel: tris(3,5-dimethylphenylcarbamate) (**78**, Chiralpak AD), tris((*S*)-1-phenylethylcarbamate) (**89**, Chiralpak AS).

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