Journal of Chromatography A, 666 (1994) 403–419
Elsevier Science B.V., Amsterdam

CHROM. 25 545

Review

Resolution by high-performance liquid chromatography using polysaccharide carbamates and benzoates as chiral stationary phases

Yoshio Okamoto* and Yuriko Kaida

Department of Applied Chemistry, Faculty of Engineering, Nagoya University Furo-cho, Chikusa-ku, Nagoya 464-01 (Japan)

ABSTRACT

Polysaccharides such as cellulose and amylose are the most accessible optically active polymers and can be readily modified to triesters and triscarbamates. In this review, triscarbamates of the polysaccharides as chiral stationary phases (CSPs) for HPLC are extensively discussed. Cellulose benzoates such as 3- and 4-methylbenzoates are also briefly reviewed. Chiral recognition of phenylcarbamate derivatives of cellulose depends on the substituents introduced on the phenyl groups, and 3,5-dimethylphenylcarbamate afforded the most useful CSP, which can separate a wide range of racemates. Amylose tris(3,5-dimethylphenylcarbamate) also shows high chiral recognition. Chiral recognition of 3,5-dimethylphenylcarbamates of oligosaccharides is also discussed. Amylose tris[(*S*)-1-phenylethyl-carbamate] is a useful derivative that can efficiently separate many enantiomers of carbonyl compounds.

CONTENTS

1. INTRODUCTION

Recently, optically active compounds have been attracting much attention in various fields dealing with drugs, agrochemicals, ferroelectric liquid crystals, etc., and therefore their preparation and analysis have been becoming increasingly important. In the past 10 years, optical resolution by high-performance liquid chromatography (HPLC) has progressed rapidly, and has become a practical and useful method not only for determining optical purity but also for obtaining optical isomers. The development of

* Corresponding author.

0021-9673/94/$26.00
SSDI 0021-9673(93)E0987-6

chiral stationary phases (CSPs) is a key point of this method; many CSPs for HPLC have been developed, and nearly 100 are commercially available [1]. CSPs for HPLC have usually been prepared with either chiral small molecules or polymers with optical resolving power (see, *e.g.*, ref. 2). The CSPs derived from small molecules are prepared by chemically bonding the chiral small molecules to a support, usually silica gel, and their chiral recognition can in some instances be predictable from that of the chiral small molecules themselves. On the other hand, chiral recognition of polymeric CSPs often depends on the higher order structure of the chiral polymers, and therefore it is difficult to predict their chiral recognition only from the character of a monomer unit. Polymeric CSPs are of interest and attractive because an unexpected high chiral recognition ability may appear owing to the higher order structure of polymers. In this review, the optical resolution of various racemates on the derivatives of polysaccharides, cellulose and amylose, is discussed. Many of them are commercially available (Table 1).

An example of chromatographic optical resolution is shown in Fig. 1. The capacity factors $[k'_1 = (t_1 - t_0)/t_0, k'_2 = (t_2 - t_0)/t_0]$ are 0.75 and 1.11, respectively. The separation factor ($\alpha = k'_2/k'_1$), representing the chiral recognition ability of a CSP, is 1.48, and the resolution factor $[R_s = 2(t_2 - t_1)/(W_1 + W_2)]$ is 2.16. In most separations with polysaccharide phases, complete separation of enantiomers is attained if α is larger than 1.2. When $\alpha = 1.2$, the energy difference, $\Delta\Delta G$ ($= RT \ln \alpha$), between the interaction of a CSP with a pair of enantiomers is only 0.11 kcal/mol (1 kcal = 4.184 kJ). In other words, complete resolution can be attained even with a very small energy difference in enantiomer discrimination. The degree of chiral discrimination in one

TABLE 1

COMMERCIALLY AVAILABLE CHIRAL COLUMNS

Polysaccharide derivative	Trade name	Distributor
Microcrystalline cellulose triacetate	Chiralcel CA-1	Daicel
	Cellulose triacetate	Merck
	Cellulose Cel-AC-40XF	Macherey–Nagel
Cellulose triacetate (coated on silica gel)	Chiralcel OA	Daicel
Cellulose tribenzoate (coated on silica gel)	Chiralcel OB	Daicel
Cellulose trisphenylcarbamate (coated on silica gel)	Chiralcel OC	Daicel
Cellulose tris(3,5-dimethylphenylcarbamate) (coated on silica gel)	Chiralcel OD	Daicel
	Chiralcel OD-R	Daicel
Cellulose tris(4-chlorophenylcarbamate) (coated on silica gel)	Chiralcel OF	Daicel
Cellulose tris(4-methylphenylcarbamate) (coated on silica gel)	Chiralcel OG	Daicel
Cellulose tris(4-methylbenzoate) (coated on silica gel)	Chiralcel OJ	Daicel
Cellulose tricinnamate (coated on silica gel)	Chiralcel OK	Daicel
Amylose tris(3,5-dimethylphenylcarbamate) (coated on silica gel)	Chiralpak AD	Daicel
Amylose tris[(*S*)-1-phenylethylcarbamate] (coated on silica gel)	Chiralpak AS	Daicel

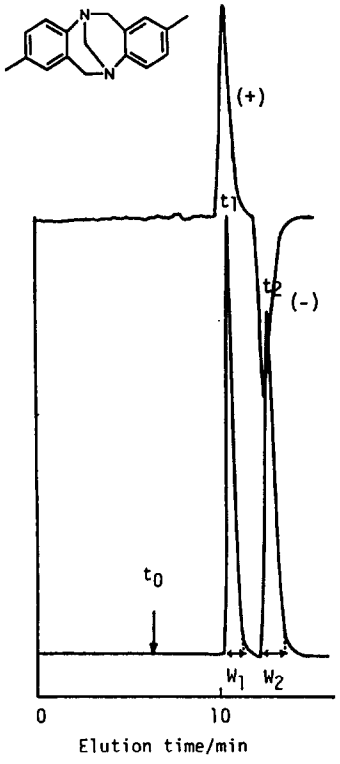


Fig. 1. Resolution of Tröger base on cellulose tris(4-methylbenzoate).

process of adsorption and desorption is small. Therefore, to attain efficient optical resolution on a polymeric CSP, it may be preferable for the polymer to have a regular structure, which will result in efficient accumulation of the discrimination process.

2. CELLULOSE ESTERS

Cellulose and amylose are the most accessible naturally occurring optically active polymers. These polysaccharides themselves show chiral recognition [3,4], but do not afford practical CSPs. However, their derivatization brings about practically useful CSPs with high chiral recognition that can separate a wide range of racemic compounds into enantiomers.

In 1973, Hesse and Hagel [5] found that microcrystalline cellulose triacetate (MCT) (Fig. 2, **1**) prepared under heterogeneous reaction conditions showed a high chiral recognition abili-

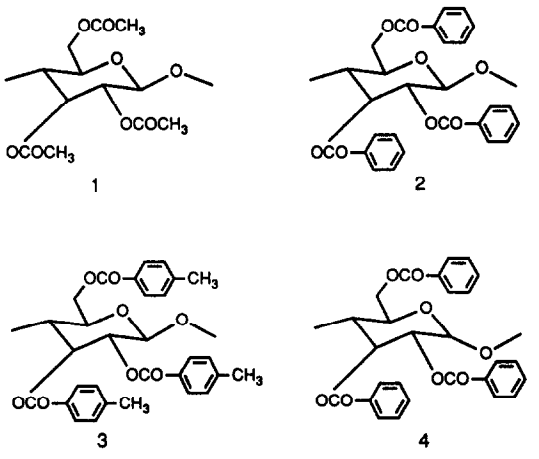


Fig. 2. Cellulose triacetate (**1**), tribenzoate (**2**) and tris(4-methylbenzoate) (**3**) and amylose tribenzoate (**4**).

ty. This chiral recognition ability originates from the crystal structure of native cellulose. When MCT was dissolved in a solvent and adsorbed on silica gel, the chiral recognition of the resulting triacetate was different from that of MCT, and the elution orders of optical isomers of several racemates such as Tröger base were reversed [6,7]. This change in chiral recognition is ascribed to the different conformations of cellulose triacetates [8]. These results clearly indicate that the optical resolving ability of polymeric CSPs depends greatly on their higher order structure. Compounds having aromatic groups are particularly well resolved [9,10]. Racemic compounds may be incorporated into the chiral cavity consisting of MCT chains for chiral recognition.

Among various kinds of cellulose esters, cellulose tribenzoate (Fig. 2, **2**) and its derivatives show high chiral recognition abilities when they are adsorbed on macroporous silica gel [6,7,11]. The optical resolving abilities of the benzoate derivatives are dependent on the substituents on the phenyl groups. The most important adsorbing site of the derivatives may be the carbonyl group, and its polarity may be changed by the substituents on the phenyl groups. The derivative may interact with racemic compounds having carbonyl groups through dipole–dipole interactions and with compounds having hydroxy or amino groups through hydrogen bonding [12,13]. The benzoates having an electron-donating sub-

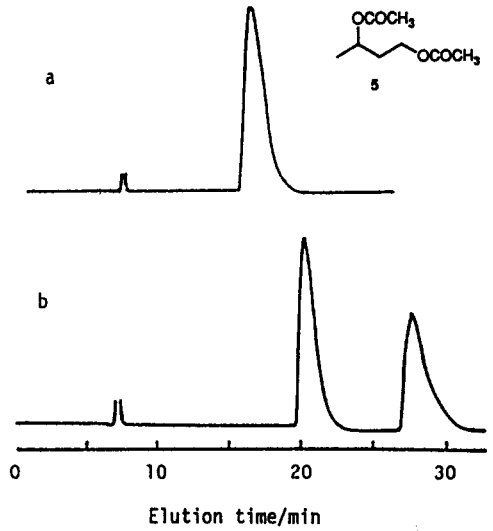


Fig. 3. Optical resolution on 1,3-diacetoxybutane (5) on cellulose tribenzoate (2) coated on silica gel (a) with CH_2Cl_2 and (b) with CH_2Cl_2–nitrobenzene (10:1). Eluent hexane–2-propanol (90:10); flow-rate, 0.5 ml/min.

stituent such as a methyl group showed a higher chiral recognition ability than those having an electron-withdrawing substituent such as a chloro group. The enhancement of electron density on the carbonyl oxygen may be important. Thus, the 3- or 4-methylbenzoate of cellulose can resolve various kinds of racemates. The chiral recognition ability of these derivatives also depends on the process of preparation, which influences the higher order structure of CSPs. Fig. 3 shows the chromatograms for the optical resolution of 1,3-diacetoxybutane (5) on 2 [8]. The CSP which was prepared by coating 2 on silica gel from a dichloromethane solution cannot resolve 5 (Fig. 3a), whereas that from a dichloromethane–nitrobenzene (10:1) solution can separate 5 into optical isomers (Fig. 3b).

Fig. 4 shows the compounds resolved on cellulose tris(4-methylbenzoate) (3, Chiralcel OJ) [14–24]. Many drugs have been resolved.

Recently, Francotte and co-workers [25] re-

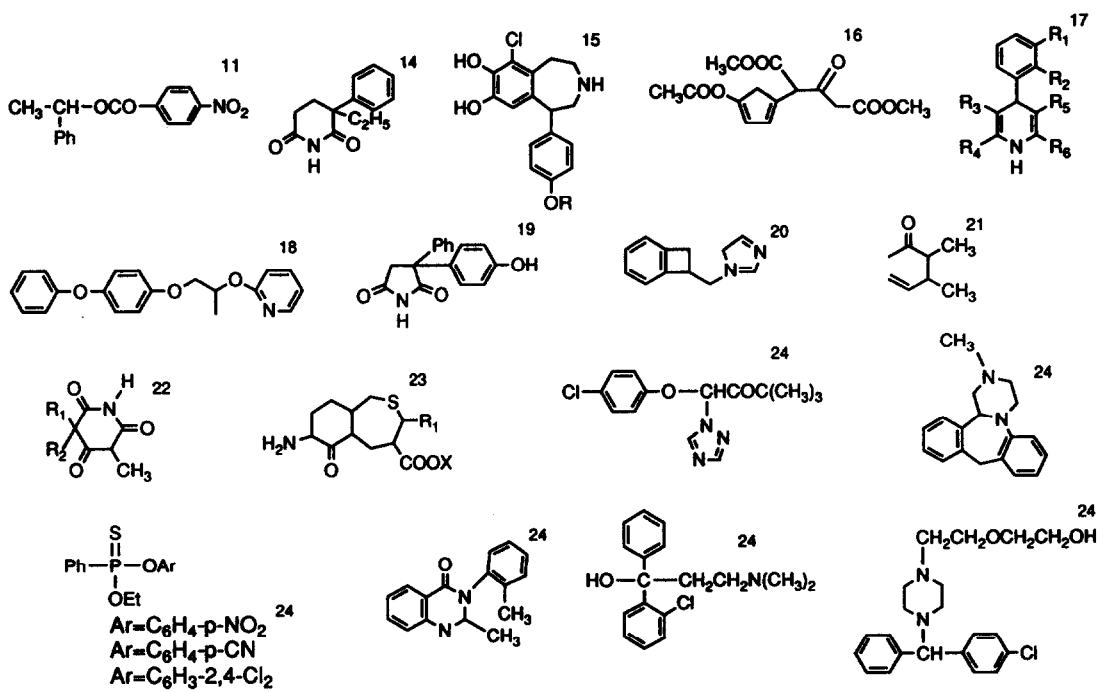
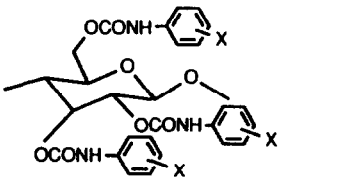


Fig. 4. Compounds resolved on cellulose tris(4-methylbenzoate) (3, Chiralcel OJ). The numbers next to the structures refer to literature references.



Cellulose phenylcarbamates

X =

6: 4-CH_3O 17: 3-CH_3

7: 4-$C(CH_3)_3$ 18: 2-CH_3

8: 4-CH_3 19: 3,4-$(CH_3)_2$

9: 4-C_2H_5 20: 3,5-$(CH_3)_2$

10: 4-$CH(CH_3)_2$ 21: 2,6-$(CH_3)_2$

11 : H 22: 3,4,5-$(CH_3)_3$

12: 4-Br 23: 3,5-Cl_2

13: 4-Cl 24: 3,5-F_2

14: 4-F 25: 3,5-$(CF_3)_2$

15: 4-CF_3 26: 4- $-N=N-$

16: 4-NO_2

Fig. 5. Phenylcarbamate derivatives of cellulose.

ported that the polymer gels of cellulose tris-(methylbenzoate)s prepared without using silica gel are useful CSPs for HPLC. The gel may be suitable for preparative separation. Amylose benzoates (**4**) showed low chiral recognition [26].

3. CELLULOSE PHENYLCARBAMATES

Cellulose tris(phenylcarbamate) (CTPC) and its derivatives (Fig. 5, **6–26**) are easily prepared by the reaction of cellulose with corresponding phenyl isocyanate derivatives, and give useful CSPs for HPLC when coated on silica gel [27,28].

The optical resolving ability of the phenylcarbamates also depends on the substituents on the phenyl groups [27]. Table 2 gives the separation factors (α) for the optical resolution of racemates (Fig. 6, **27–36**) on eleven 4-substituted phenylcarbamates of cellulose (**6–16**). The derivatives having electron-donating substituents such as alkyl groups, especially a *tert.*-butyl group (**7**) [28], or electron-withdrawing substituents such as halogens show a higher chiral recognition ability than the non-substituted phenylcarbamate **11**, although the derivatives having methoxy or nitro groups show a low chiral recognition ability. The most important adsorbing site of CTPC for chiral recognition may be the carbamate residues near chiral glucose units, and the polarity of the residue must be influenced by the substituents on phenyl groups. If an electron-donating substituent such as methyl is introduced on the phenyl, the electron density of carbonyl will be increased, and if an electron-withdrawing group such as chloro is introduced, the acidity of the NH group will become higher. These inductive effects of substituents can be confirmed by ^1H NMR spectroscopy. The NH proton resonances of the phenylcarbamate derivatives having electron-withdrawing substituents shift downfield com-

TABLE 2

SEPARATION FACTORS (α) ON CELLULOSE PHENYLCARBAMATES

Eluent, hexane–2-propanol (90:10); flow-rate, 0.5 ml/min; temperature, 25°C.

Compound	6	7	8	9	10	11	12	13	14	15	16
27	1(+)	1.74(+)	1.48(+)	1.11(+)	1.17(+)	1.37(+)	1.19(+)	1.16(+)	1.14(+)	1.23(+)	1(−)
28	1.35(−)	1.75(−)	1.52(−)	1.57(−)	1.59(−)	1.45(−)	1.29(−)	1.29(−)	1.26(−)	1.30(−)	1(+)
29	1.34(+)	1.27(+)	1.55(+)	1.55(+)	1.43(+)	1.46(+)	1.70(+)	1.68(+)	1.38(+)	1.61(+)	1.33(+)
30	1.00	2.24(−)	1.35(−)	2.12(−)	2.14(−)	1.45(−)	1.17(−)	1.44(−)	1(−)	1.22(−)	1(+)
31	1.15(−)	1.50(−)	1.30(−)	1.33(−)	1.39(−)	1.65(−)	1.21(−)	1.20(−)	1.17(−)	2.04(−)	1(+)
32	1.00	1.36(+)	1.37(+)	1.59(+)	1.47(+)	1.22(+)	1.95(+)	1.95(+)	1.64(+)	1.48(+)	1.00
33	1(+)	1.45(+)	1.16(+)	1.22(+)	1.23(+)	1.10(+)	1.13(+)	1.12(+)	1.13(+)	1.14(+)	1.00
34	1(+)	2.50(+)	1.75(+)	1.76(+)	2.46(+)	1.24(+)	1.79(+)	1.46(+)	1.53(+)	2.06(+)	1(+)
35	1.13(−)	1.22(−)	1.20(−)	1.19(−)	1.15(−)	1.17(−)	1.17(−)	1.16(−)	1.12(−)	1.18(−)	1(−)
36	*ca.* 1(+)	1.07(−)	1.12(−)	1.14(−)	1.17(−)	*ca.* 1(−)	1.13(−)	1.20(−)	1.14(−)	1.10(−)	1.00

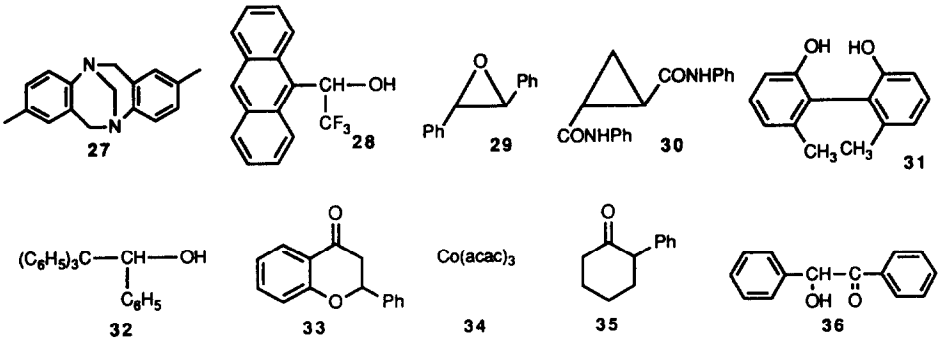


Fig. 6. Racemic compounds used for evaluating optical resolving ability.

pared with those of the derivatives having electron-donating substituents. The elution time of acetone becomes longer from left to right in the order given in Table 2. Acetone probably interacts with the NH protons of the carbamate residues mainly through hydrogen bonding, as shown in Fig. 7. Racemates with a polar group probably interact with the carbamate residue mainly through hydrogen bonding for efficient chiral recognition. For instance, a racemic compound having a hydroxy group may interact with the carbonyl groups of carbamate residues, and that having a carbonyl group with the NH groups of carbamate residues. On the other hand, the derivatives bearing methoxy or nitro groups on the phenyl groups show low chiral recognition

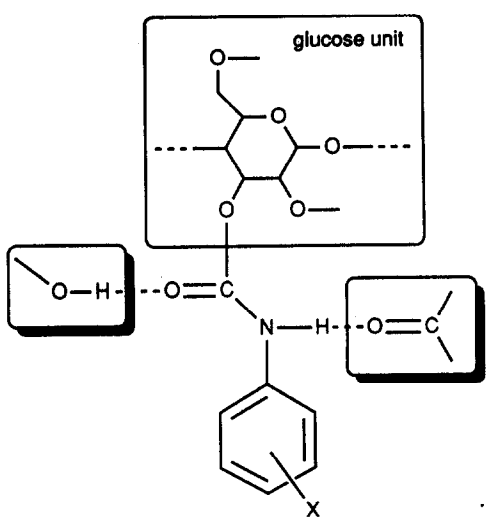


Fig. 7. Schematic interaction of racemates with a carbamate residue.

abilities. This seems to be ascribable to the fact that racemates must interact with these substituents far from the main chains of chiral polysaccharides. Such an interaction cannot result in effective chiral discrimination and will reduce the chiral recognition ability of the CSP. Accordingly, introduction of a bulky alkoxy group such as an isopropoxy group on the phenyl group can improve the optical resolving ability because the interaction between racemates and ether oxygen is prevented owing to steric hindrance [29]. The interaction on the carbamate residues appears to be most important to induce efficient chiral discrimination.

The optical resolution of racemates on various methyl-substituted phenyl carbamates of cellulose (**8**, **17–22**) has been examined (Table 3). The derivatives bearing methyl groups at the 3- or 4-position of phenyl groups show high chiral recognition. Concentrated solutions of these derivatives form lyotropic liquid crystal phases [27,30]. This indicates that these derivatives probably possess the regular higher order structure on the silica surface. On the other hand, the derivatives bearing a methyl group at the 2-position on the phenyl group show low chiral recognition abilities and clear liquid crystallinity was not observed for 2,6-dimethylphenylcarbamate. The steric hindrance of methyl groups at the 2-position may prevent the phenylcarbamate from adopting a regular higher order structure. In the cellulose phenylcarbamate system, the regular higher order structure may be preferable to attain high chiral recognition, probably because the chiral sites existing in the regular structure are more suitable to show high chiral

TABLE 3

SEPARATION FACTORS (α) ON CELLULOSE METHYL-SUBSTITUTED PHENYLCARBAMATES

Eluent, hexane–2-propanol (90:10); flow-rate, 0.5 ml/min; temperature, 25°C.

Compound	8	17	18	19	20	21	22
27	1.48(+)	1.45(+)	1(+)	1.49(+)	1.32(+)	1(+)	1.48(+)
28	1.52(−)	1.56(−)	1.10(−)	2.13(−)	2.59(−)	1.17(+)	1.77(−)
29	1.55(+)	1.28(+)	1.35(+)	1.13(+)	1.68(−)	1(−)	1.36(−)
30	1.35(−)	1(−)	1(−)	2.39(+)	3.17(+)	1.36(+)	1.22(+)
31	1.30(−)	2.63(−)	1(+)	1.87(−)	1.83(−)	1.34(−)	2.09(−)
32	1.37(+)	1.45(+)	1(+)	1(+)	1.34(+)	1(−)	1(+)
33	1.16(+)	1.14(+)	1(−)	1.42(−)	1.41(−)	1(−)	1.21(−)
34	1.75(+)	1.29(+)	1(+)	1.32(+)	1(+)	1(−)	1(+)
35	1.20(−)	1.17(−)	1(−)	1.20(−)	1.15(−)	1(+)	1.08(−)
36	1.12(−)	*ca.* 1(−)	1.10(+)	1.31(+)	1.58(+)	*ca.* 1(+)	1.18(+)

recognition than the sites in other structures. Therefore, the chiral recognition ability appears to increase with increasing regularity. Of course, a polymer with a regular higher order structure does not always lead to higher chiral recognition than a polymer with an irregular structure, because some of chiral sites existing in the irregular polymer may show higher chiral recognition than the sites in the regular polymer. However, in this case, an efficient separation may be difficult to achieve.

Among these cellulose phenylcarbamates, cellulose tris(3,5-dimethylphenylcarbamate) (**20**, Chiralcel OD) shows a particularly high optical resolving ability, affording a practically useful CSP. The CSP is stable in an eluent system consisting of a mixture of hexane and 2-propanol. The authors attempted resolution of 510 racemates on **20**; 229 were completely resolved and 86 partially resolved, that is, about 62% of the racemates were resolved on **20**. The CTPC derivatives can be applied to the separation of a wide range of racemates (Fig.8) [31–118].

Although a hexane–2-propanol mixture has been mainly used as the eluent for these cellulose columns, a water–acetonitrile mixture containing perchloric acid can also be used as an eluent for the optical resolution of several drugs such as β-blockers on **20** [119]. The $\pi-\pi$ interaction between racemates and the phenyl groups of CSPs may also play an important role in the separation under reversed-phase conditions. Such a possibility has also been discussed even in normal-phase separations [33].

Table 4 shows the chiral recognition abilities of various 3,5-disubstituted phenylcarbamates of cellulose (**20**, **23–25**) [120]. Large differences were observed in the k_1' and α values between dimethyl (**20**) and dichloro (**23**) or difluoro (**24**) derivatives. The dichlorophenylcarbamate derivative (**23**) shows high chiral recognition, but does not afford a practical CSP owing to its high solubility in solvents. To improve this defect, **23** was chemically bonded to silica gel [121]. However, the chiral recognition ability became lower when 10% of hydroxy groups of cellulose were chemically bonded to silica gel. The ditrifluoromethyl derivative (**25**) showed poor chiral recognition, and the elution times of most of the racemates on **25** were shorter than those on other derivatives. Ditrifluoromethyl groups seem to prevent the racemates from interacting with the carbamate residues of **25**.

The exact structures of these phenylcarbamates of cellulose must be determined in order to reveal the mechanism of chiral recognition at the molecular level. Fig. 9 shows the possible structure of cellulose tris(phenylcarbamate) (**11**), reported by Vogt and Zugenmaier [122] on the basis of X-ray analysis. The cellulose derivative possesses a conformation of a left-handed threefold (3/2) helix. A chiral helical groove or ditch with polar carbamate residues exits along the main chain. If a racemate can enter this groove,

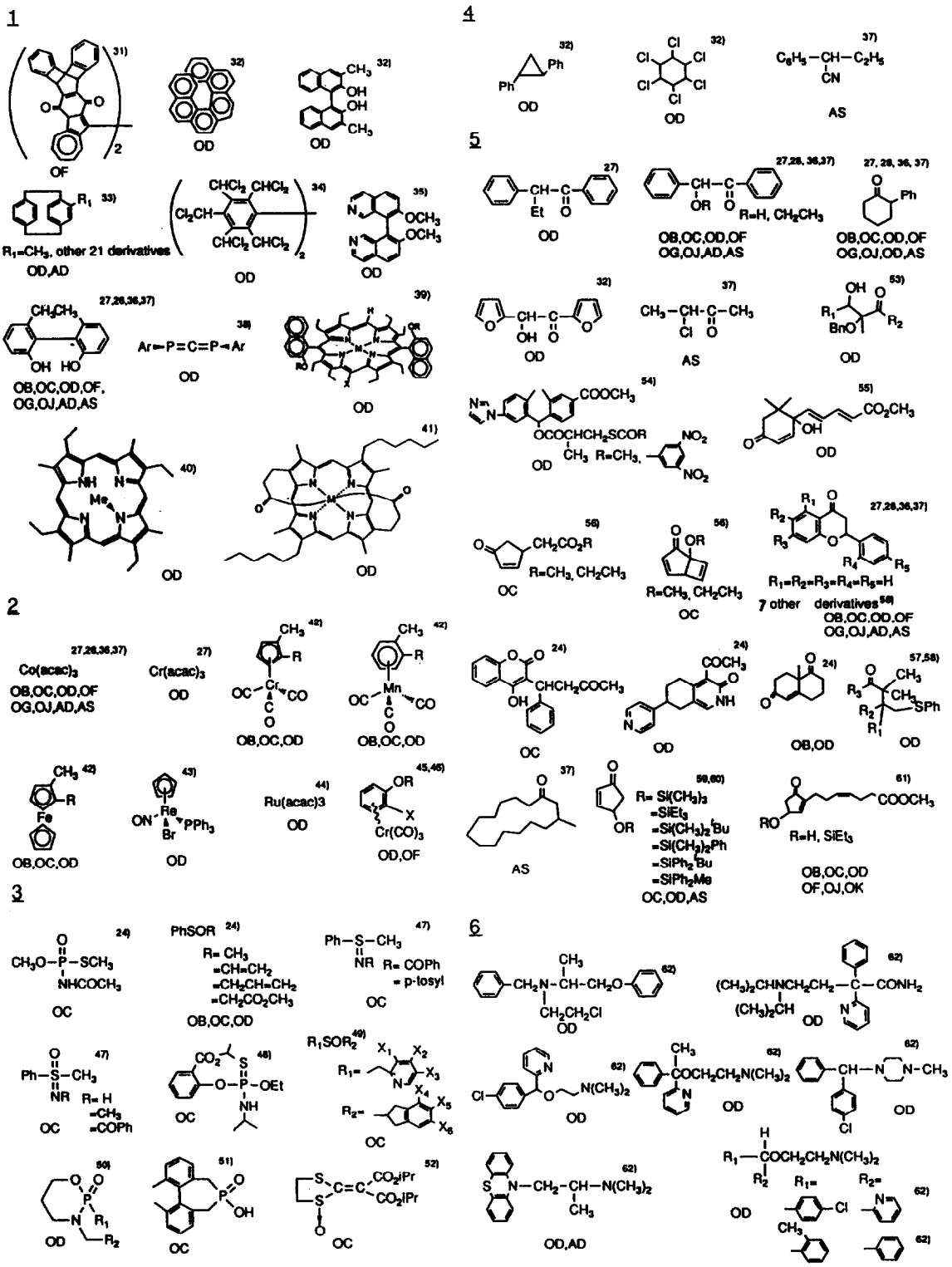
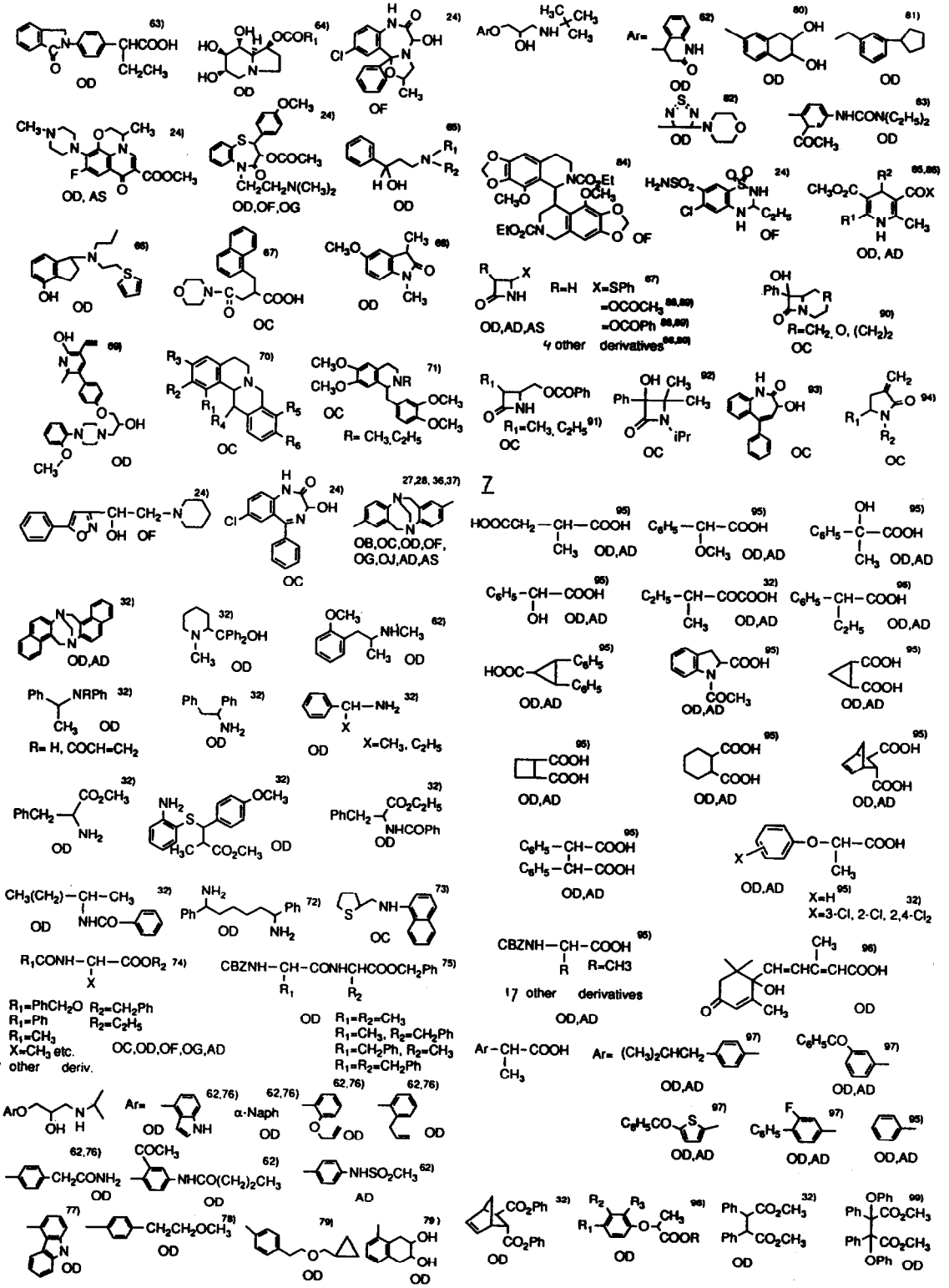

1
OF
OD
OD
R1=CH3, other 21 derivatives
OD,AD
OD
OD
OB,OC,OD,OF,
OG,OJ,AD,AS
OD
OD
OD
OD
4
OD
OD
AS
5
OD
OB,OC,OD,OF
OG,OJ,AD,AS
OB,OC,OD,OF
OG,OJ,OD,AS
OD
AS
OD
OD
OD
OC
OC
OD
7 other derivatives
OB,OC,OD,OF
OG,OJ,AD,AS
OC
OD
OB,OD
OD
AS
OC,OD,AS
OB,OC,OD
OF,OJ,OK
2
Co(acac)3
OB,OC,OD,OF
OG,OJ,AD,AS
Cr(acac)3
OD
OB,OC,OD
OB,OC,OD
OB,OC,OD
OD
Ru(acac)3
OD
OD,OF
3
OC
OB,OC,OD
OC
OC
OC
OC
OD
OC
OC
6
OD
OD
OD
OD
OD
OD,AD

Fig. 8.



(Continued on p. 412)

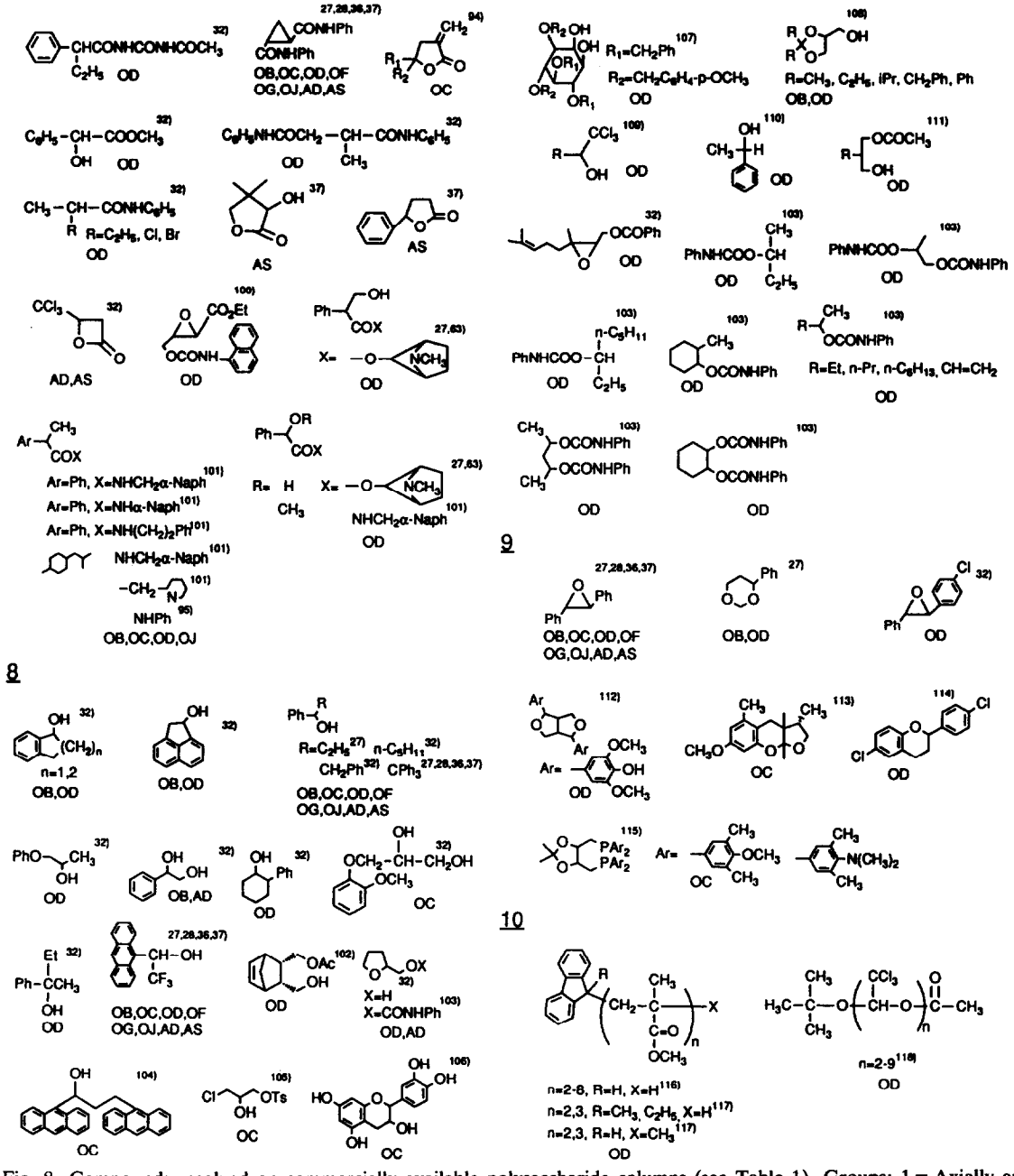


Fig. 8. Compounds resolved on commercially available polysaccharide columns (see Table 1). Groups: 1 = Axially and planar disymmetric compounds; 2 = metal-containing compounds; 3 = compounds possessing chiral sulphur or phosphorous atom; 4 = hydrocarbon, halide and cyano compounds; 5 = ketones; 6 = amines and derivatives; 7 = acids and derivatives; 8 = alcohols and derivatives; 9 = ethers; 10 = oligomers.

the racemate may be efficiently discriminated into enantiomers. To attain efficient separation, the helical structure must be highly regular. Electron-donating groups such as methyl may contribute to stabilizing the above helical conformation through intramolecular hydrogen bonding between the carbamate residues and the $\pi-\pi$ stacking of phenyl groups.

TABLE 4

OPTICAL RESOLUTION ON 3,5-DISUBSTITUTED CELLULOSE PHENYLCARBAMATES

Eluents, hexane–2-propanol (90:10); flow-rate, 0.5 ml/min; temperature, 25°C.

Compound	20		23		24		25	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α
27	0.97(+)	1.32	0.87(+)	1.65	0.88(+)	1.73	0.31(+)	1
28	2.13(−)	2.59	0.28(−)	1.38	0.34(−)	1.27	0.65(−)	1.42
29	0.74(−)	1.68	0.56(+)	1.84	0.51(+)	1.81	0.25(+)	1.54
30	0.83(+)	2.17	0.59(+)	1.41	0.68(+)	1.63	0.20(−)	1
31	2.36(−)	1.83	1.62(+)	1.11	1.70(+)	1.05	0.79(+)	1
32	1.37(+)	1.34	0.40(+)	1.29	0.44(+)	1	0.09(+)	1
33	1.47(−)	1.41	1.55(−)	1.20	2.01(−)	1.18	0.51(−)	1
34	0.42(+)	1	0.76(+)	1.82	1.72(+)	2.36	0.34(+)	1.47
35	1.17(−)	1.15	2.65(−)	1.26	3.62(−)	1.21	1.66(−)	1.09
36	2.43(+)	1.58	3.08(−)	1.21	3.77(−)	*ca.* 1	1.60(−)	1.28

Cellulose tris(4-azophenylcarbamate) (**26**) can change its azo group structure between *cis* and *trans* by irradiation with light [123]. The chiral recognition of **26** depends on the structure of azo groups.

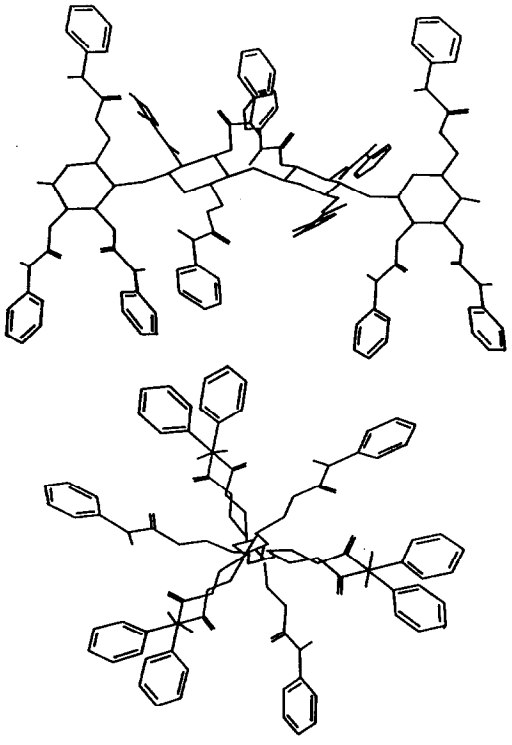
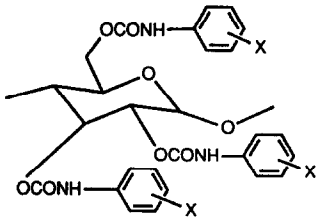


Fig. 9. Structure of cellulose trisphenylcarbamate (**11**) [122]. Top, along the chain axis; bottom, perpendicular to the chain axis.

4. AMYLOSE PHENYLCARBAMATE DERIVATIVES

Amylose phenylcarbamates (Fig. 10, **37–39**) have also been used as CSPs [36]. In Table 5, the results of optical resolution on **37–39** are summarized. The tris(3,5-dimethylphenylcarbamate) derivative **37** (Chiralpak AD) exhibits the highest chiral recognition for most racemates. The optical resolving abilities of the amylose derivatives are different from those of the corre-



Amylose phenylcarbamates

X =

37: 3,5-$(CH_3)_2$

38: H **39**: 3,5-Cl_2

Fig. 10. Phenylcarbamate derivatives of amylose.

TABLE 5

OPTICAL RESOLUTION ON AMYLOSE PHENYLCARBAMATES

Eluent, hexane–2-propanol (90:10); flow-rate, 0.5 ml/min; temperature, 25°C.

Compound	37			38			39		
	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
27	0.53(+)	1.58	2.30	0.77(+)	1.28	1.10	0.84(+)	1.34	2.27
28	1.30(+)	1.15	0.75	0.61	1.00		0.37	1.00	
29	0.42(+)	3.04	6.67	0.39(+)	1.46	1.29	0.50(+)	1.32	1.69
30	3.25(+)	2.01	3.59	1.83(+)	1.52	1.70	0.59(−)	1.11	
31	2.46(−)	2.11	6.38	1.15(−)	1.53	1.74	1.10(+)	*ca.* 1	
32	2.65(+)	1.98	5.48	1.42(+)	1.86	3.11	0.88(+)	2.25	6.05
33	0.93(+)	1.12	0.77	2.21(+)	1.51	2.27	1.62(+)	1.10	1.02
34	0.25(−)	*ca.* 1		1.80(−)	1.28	0.87	0.63(+)	*ca.* 1	
35	0.61(−)	*ca.* 1		1.19(−)	*ca.* 1		1.26(−)	*ca.* 1	
36	3.14(−)	1.21	1.23	3.72(+)	*ca.* 1		6.08(+)	*ca.* 1	

sponding cellulose derivatives, and both are complementary to each other. The most important adsorbing site for chiral recognition must also be carbamate residues. The structure of amylose tris(phenylcarbamate) (**38**) has been reported to be a left-handed fourfold (4/1) helix on the basis of X-ray analysis [124]. The difference in chiral recognition of cellulose and amylose derivatives must be due to the different configuration of the glucose residue and higher order structure.

The optical resolution of 338 racemic compounds on **37** has been attempted by our group. Among them, 112 racemates have been completely resolved and 102 racemates partially resolved. Consequently, in the optical resolution of 510 racemates by the two 3,5-dimethylphenylcarbamates **20** and **37**, 186 racemates have been resolved only on **20**, 85 only on **37** and 129 on both. In total, 400 (78%) of the racemates have been resolved at least on one of the two CSPs. Some of the racemic compounds efficiently separated on **37** (Chiralpak AD) are shown in Fig. 8.

5. PHENYLCARBAMATES OF OTHER POLYSACCHARIDES

Phenylcarbamates of other polysaccharides (Fig. 11) such as xylan (**40**), curdlan (**41**), chitosan (**42**), dextran (**43**) and inulin (**44**), were

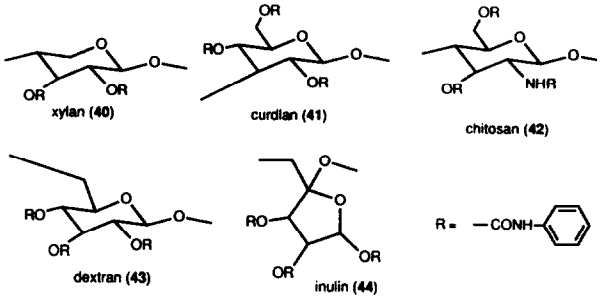


Fig. 11. Phenylcarbamates of polysaccharides.

also synthesized and their chiral recognition abilities were evaluated [27]. Among these derivative, xylan derivative showed a high chiral recognition ability. Xylan tris(3,5-dichlorophenylcarbamate) separated a calcium ion antagonist, nicaldipine [85].

6. PHENYLCARBAMATES OF OLIGOSACCHARIDES

The optical resolving abilities of 3,5-dimethylphenylcarbamates (Fig. 12) of linear oligomers of D-glucose, cellooligosaccharides (**45**, $n = 2$, 4) and maltooligosaccharides (**46**, $n = 2$–7) and cyclic oligomers, α-, β- and γ-cyclodextrin (**47**, $n = 6$–8), were also investigated to elucidate the influence of higher order structure of the polysaccharide derivatives on chiral recognition [125]. Chiral recognition by the cellooligosac-

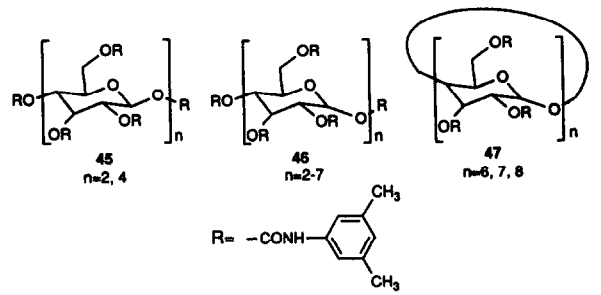


Fig. 12. 3,5-Dimethylphenylcarbamates of oligosaccharides.

charide derivatives was lower than that on the cellulose derivative **20**. On the other hand, the maltooligosaccharide derivatives with $n = 4–7$ showed a chiral recognition ability similar to that of the amylose derivative **37**. These results are ascribable to the fact that the structures of the cellooligosaccharide derivatives are different from that of the cellulose derivative and those of the maltooligosaccharide derivatives are similar to that of the amylose derivative. These structural difference and similarity were presumed from the circular dichroism (CD) spectral data of these derivatives. Each of the cyclodextrin derivatives showed a characteristic chiral recognition different from those of other linear oligomers.

Many cyclodextrin-based CSPs have been reported and are commercially available [126,127]. Recently, we found that the CSPs consisting of 3,5-dimethylphenylcarbamoylated β-cyclodextrin showed different chiral recognition depending on whether β-cyclodextrin is chemically bonded to silica gel through their secondary hydroxy groups at the 2- and 3-position or primary hydroxy groups at the 6-position [128]. The derivative bonded to silica at the 2- or 3-position showed higher optical resolving power.

7. ARALKYLCARBAMATES OF CELLULOSE AND AMYLOSE

Alkylcarbamates of cellulose, such as methyl- and isopropylcarbamates, show low chiral recognition, but some aralkylcarbamates of cellulose and amylose possess high chiral recognition abilities [37,129]. As aralkylcarbamates, benzyl- (**48**), 1-phenylethyl- (**49**), 1-phenylpropyl- (**50**),

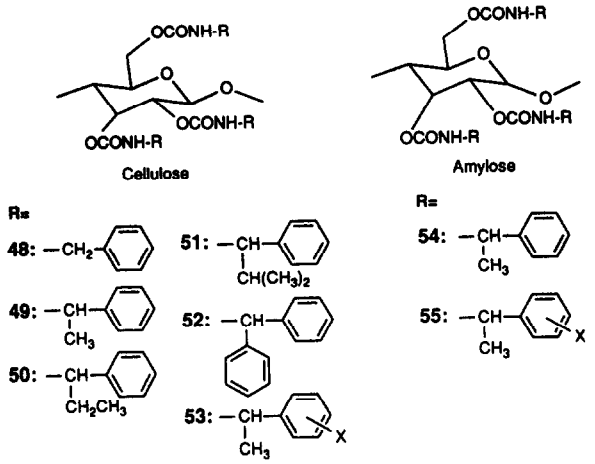


Fig. 13. Aralkylcarbamates of cellulose (**48–52**) and amylose (**54**, **55**).

2-methyl-1-phenylpropyl- (**51**) and 1,1-diphenylmethylcarbamates (**52**) (Fig. 13) have been prepared to be used as CSPs. In the cellulose derivatives, **49** and **50** showed higher chiral recognition abilities than other derivatives. Similar results were obtained with respect to amylose aralkylcarbamates. These results indicate that only 1-phenylethyl and 1-phenylpropyl groups may be suitable for keeping the regular higher order structure of the polysaccharide derivatives, which is preferable to attaining efficient chiral discrimination.

The influence of the chirality of the 1-phenylethyl group on the chiral discrimination of **49** and **54** was also studied [37]. For the cellulose derivatives, (*R*)-**49** showed a higher chiral recognition ability than (*R*,*S*)- and (*S*)-**49** derivatives. Of the amylose derivatives, (*S*)-**54** was superior to other derivatives. Their ^{1}H NMR and CD spectra indicate that the conformation of these derivatives may depend on the chirality of the 1-phenylethyl group. The fact that (*R*)- and (*RS*)-**49** form lyotropic liquid crystalline phases in tetrahydrofuran but (*S*)-**49** does not also suggests that the higher order structures of these derivatives may be different, depending on the chirality. This difference in the structure may cause the different chiral recognition abilities.

In these aralkylcarbamates, the amylose derivative (*S*)-**54** (Chiralpak AS) often shows the highest optical resolving ability for a variety of

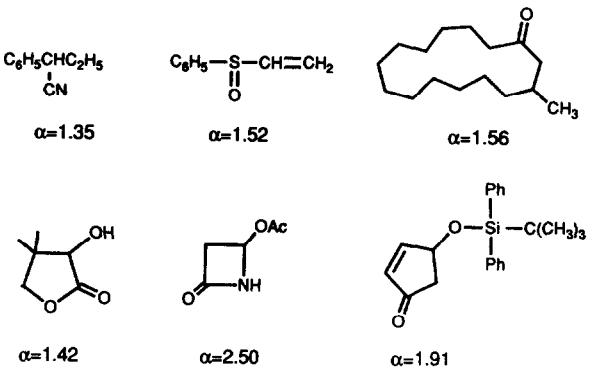


Fig. 14. Compounds efficiently resolved on amylose tris[(*S*)-1-phenylethylcarbamate] [(*S*)-**54**, Chiralpak AS].

compounds, and many carbonyl compounds which cannot be sufficiently resolved on the phenylcarbamate derivatives of polysaccharides have been resolved on this phase. Some examples of compounds that are well resolved are shown in Fig. 14. These include a β-lactam and a 4-hydroxy-2-cyclopentenone derivative.

The optical resolving abilities of the derivatives of 1-phenylethylcarbamates of cellulose (**53**) and amylose (**55**) having a methyl or a chloro group on their phenyl groups were also evaluated [129]. The chiral recognition depended on the nature and position of the substituents on the phenyl groups.

8. CONCLUSIONS

Among the CSPs discussed, commercially available phases are summarized in Table 1 and racemic compounds which can be resolved mainly on the carbamate phases are summarized in Fig. 8. A very wide range of racemic compounds including axially and planar asymmetric compounds, metal-containing compounds, compounds possessing chiral sulphur or phosphorus atoms, hydrocarbons, halides, cyano compounds, ketones, amines and their derivatives, acids and their derivatives, alcohols and their derivatives, ethers and oligomers can be separated into enantiomers. Selection of a chiral column capable of resolving a given compound is often a time-consuming, troublesome problem. In the above polysaccharide systems, this may not be the case, because some of the CSPs can resolve many compounds. We usually first test the separation using Chiralcel OD, followed by Chiralcel OJ or Chiralpak AD. With these three columns, about 80% of the compounds have been resolved. If the separation on these three is unsuccessful, then we test Chiralpak AS, Chiralcel OB and other cellulose-based phases.

Recently Vandenbosch *et al.* [130] reported that cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel-OD) is useful for separating racemic drugs as well as the protein phases [130]. A systematic preparative separation has been developed [131]. Accordingly, optical resolution with polysaccharide phases will become more important not only for analytical separations of racemates but also for preparative separations to obtain optically active compounds. Resolution using a membrane of cellulose tris(3,5-dimethylphenylcarbamate) has also been examined [132].

To develop further excellent polysaccharide-based CSPs, clarification of the chiral discrimination mechanism on the foregoing derivatives and the establishment of a regioselective modification method for polysaccharides appear to be necessary.

9. ACKNOWLEDGEMENT

Part of our research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1 S. Ahuja, in S. Ahuja (Editor), *Chiral Separations by Liquid Chromatography* (*ACS Symposium Series*, No. 471) American Chemical Society, Washington, DC, 1991, p. 1.
2 S.G. Allenmark, *Chromatographic Enantioseparation*, Ellis Horwood, Chichester, 1988; A.M. Krstulovic (Editor), *Chiral Separation by HPLC*, Ellis Horwood, Chichester, 1989.
3 S. Yuasa, A. Shimada, K. Kameyama, M. Yasui and K. Adzuma, *J. Chromatogr. Sci.*, 18 (1980) 311; T. Fukuhara, M. Isoyama, A. Shimada, M. Itoh and S. Yuasa, *J. Chromatogr.*, 387 (1987) 562.
4 G. Gubitz, W. Jellenz, D. Schomleber, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 31.

5 G. Hesse and R. Hagel, *Chromatographia*, 6 (1973) 227; G. Hesse and R. Hagel, *Liebigs Ann. Chem.*, (1976) 916.
6 A. Ichida, T. Shibata, I. Okamoto, Y. Yuki, H. Namikoshi and Y. Toga, *Chromatographia*, 19 (1984) 280; T. Shibata, I. Okamoto and K. Ishii, *J. Liq. Chromatogr.*, 9 (1986) 313.
7 Y. Okamoto, M. Kawashima, K. Yamamoto and K. Hatada, *Chem. Lett.*, (1984) 739.
8 T. Shibata, T. Sei, H. Nishimura and K. Deguchi, *Chromatographia*, 24 (1986) 552.
9 A. Mannschreck, H. Koller and R. Wernicke, *Kontakte*, 1 (1985) 40.
10 A. Hussenius, R. Isaksson and O. Matsson, *J. Chromatogr.*, 405 (1987) 155.
11 Y. Okamoto, R. Aburatani and K. Hatada, *J. Chromatogr.*, 389 (1987) 95.
12 I.W. Wainer and M.C. Alembik, *J. Chromatogr.*, 358 (1986) 85; I.W. Wainer, M.C. Alembik and E. Smith, *J. Chromatogr.*, 388 (1987) 65.
13 I.W. Wainer, R.M. Stiffin and T. Shibata, *J. Chromatogr.*, 411 (1987) 139.
14 Y. Aboul-Enein and M.R. Islam, *J. Chromatogr. Sci.*, 28 (1990) 307.
15 P. Camilleri, C.A. Dyke, S.J. Paknoham and L.A. Senior, *J. Chromatogr.*, 498 (1990) 414.
16 M. Mori, S. Nukui and M. Shibasaki, *Chem. Lett.*, (1991) 1797.
17 P.A. Soons, M.C.M. Roosemalen and D.D. Breimer, *J. Chromatogr.*, 528 (1990) 343.
18 M. Okamoto and H. Nakazawa, *J. Chromatogr.*, 568 (1991) 147.
19 S. Eto, H. Noda and A. Noda, *J. Chromatogr.*, 568 (1991) 157.
20 F.A. Maris, P.J.M. Vervoort and H. Hindriks, *J. Chromatogr.*, 547 (1991) 45.
21 E.J. Corey and D.-H. Lee, *J. Am. Chem. Soc.*, 113 (1991) 4026.
22 M. Murata and K. Achiwa, *Tetrahedron Lett.*, 32 (1991) 6763.
23 U. Nagai and V. Pavone, *Heterocycles*, 28 (1989) 589.
24 *Technical Report*, Daicel Chemical Industry, Tokyo, 1993.
25 E. Francotte and R.M. Wolf, *Chirality*, 3 (1991) 43; E. Francotte, R.W. Lang and T. Winkler, *Chirality*, 3 (1991) 177.
26 Y. Okamoto, T. Fukumoto and R. Aburatani, unpublished results.
27 Y. Okamoto, M. Kawashima and K. Hatada, *J. Am. Chem. Soc.*, 106 (1984) 5357; Y. Okamoto, M. Kawashima and K. Hatada, *J. Chromatogr.*, 363 (1986) 173.
28 Y. Okamoto, K. Hatano, R. Aburatani and K. Hatada, *Chem. Lett.*, (1989) 715.
29 Y. Ohashi, Y. Kaida, E. Yashima and Y. Okamoto, *Polym. Prepr.*, Jpn., 41 (1992) 701.
30 U. Vogt and P. Zugenmaier, *Makromol. Chem. Rapid. Commun.*, 4 (1983) 759.
31 J. Daub, L. Jakob, J. Salbeck and Y. Okamoto, *Chimica*, 39 (1985) 393.
32 Y. Okamoto, upublished results; Y. Okamoto and Y. Kaida, *J. Synth. Org. Chem. Jpn.*, 51 (1993) 41.
33 H. Hopf, W. Grahn, D.G. Barret, A. Gerdes, J. Hilmer, J. Huckler, Y. Okamoto and Y. Kaida, *Chem. Ber.*, 123 (1990) 841.
34 S.E. Biali, B. Kahr, Y. Okamoto, R. Aburatani and K. Mislow, *J. Am. Chem. Soc.*, 110 (1988) 1917.
35 K. Yamamoto, K. Watanabe, H. Chikamatsu, Y. Okamoto and Y. Yoshida, *J. Chem. Soc., Chem. Commun.*, (1987) 807.
36 Y. Okamoto, R. Aburatni, T. Fukumoto and K. Hatada, *Chem. Lett.*, (1987) 1857.
37 Y. Okamoto, Y. Kaida, H. Hayashida and K. Hatada, *Chem. Lett.*, (1990) 902.
38 M. Yoshifuji, K. Toyota, Y. Okamoto and T. Asakura, *Tetrahedron Lett.*, 31 (1990) 2311.
39 H. Ogoshi, K. Saita, K. Sakurai, T. Watanabe, H. Toi, Y. Aoyama and Y. Okamoto, *Tetrahedron Lett.*, 27 (1986) 6365; Y. Aoyama, K. Saita, H. Toi, H. Ogoshi and Y. Okamoto, *Tetrahedron Lett.*, 28 (1987) 4853.
40 H. Kubo, T. Aida, S. Inoue and Y. Okamoto, *J. Chem. Soc., Chem. Commun.*, (1988) 1015.
41 K. Konishi, K. Oda, K. Nishida, T. Aida and S. Inoue, *J. Am. Chem. Soc.*, 114 (1992) 1313.
42 Y. Yamazaki, N. Morohashi and K. Hosono, *J. Chromatogr.*, 542 (1991) 129.
43 J.A. Ransder, C.M. Garner and J.A. Gladysz, *Organometallics*, 10 (1991) 1631.
44 H. Kobayashi, H. Matsuzawa, Y. Kaizu and A. Ichida, *Inorg. Chem.*, 26 (1987) 4318.
45 Y. Kaida, Y. Okamoto, J.-C. Chambron, D.K. Mitchell and J.-P. Sauvage, *Tetrahedron Lett.*, 34 (1993) 1019.
46 K. Nakamura, K. Ishihara, A. Ohno, M. Uemura, H. Nishimura and Y. Hayashi, *Tetrahedron Lett.*, 31 (1990) 3603.
47 I.W. Wainer, M.C. Alembic and C.R. Johnson, *J. Chromatogr.*, 361 (1986) 374.
48 M. Ueji and C. Tomizawa, *J. Pestic. Sci.*, 11 (1986) 447.
49 R. Erlandsson, R. Isaksson, R. Lorentzon and P. Lindberg, *J. Chromatogr.*, 532 (1990) 305.
50 R. Masurel and I.W. Wainer, *J. Chromatogr.*, 490 (1989) 133.
51 S. Kanoh, N. Tamura, M. Motoi and H. Suda, *Bull. Chem. Soc. Jpn.*, 60 (1987) 2308.
52 M. Uchida, M. Sumida and M. Hirano, *J. Pestic. Sci.*, 11 (1986) 573.
53 S. Kobayashi, I. Shiina, J. Izumi and T. Mukaiyama, *Chem. Lett.*, (1992) 373.
54 M. Tsurata, N. Shiotsuki, T. Matsumoto and T. Oe, *Yakugaku Zasshi*, 109 (1989) 26.
55 I. Railton, *J. Chromatogr.*, 346 (1986) 321.
56 F. Toda and K. Tanaka, *J. Chem. Soc., Chem. Commun.*, (1986) 1426.
57 A. Toshimitsu, C. Hirosawa and S. Tanimoto, *Chem. Lett.*, (1992) 239.
58 M. Krause and R. Galensa, *J. Chromatogr.*, 514 (1990) 147.

59 Y. Okamoto, R. Aburatani, M. Kawashima, M. Hatada and N. Okamura, *Chem. Lett.*, (1986) 1767.
60 Y. Kaida and Y. Okamoto, *Chem. Lett.*, (1992) 85.
61 L. Miller and C. Weyker, *J. Chromatogr.*, 484 (1989) 337; 511 (1990) 97.
62 Y. Okamoto, R. Aburatani, K. Hatano and K. Hatada, *J. Liq. Chromatogr.*, 11 (1988) 2147.
63 G. Perrone and M. Farina, *J. Chromatogr.*, 520 (1990) 373.
64 A.L. Margolin, *CHEMTECH*, (1991) 160.
65 D.T. Witte, P.J. Swart, J.-P. Hianke and R.A. de Boer, *Chirality*, 4 (1992) 62.
66 D.T. Witte, J. Bosman, R.A. de Zeeuw, J.H. de Boer and D.A. Doornbos, *J. Chromatogr.*, 558 (1991) 333.
67 K. Inoguchi, T. Morimoto and K. Achiwa, *J. Organomet. Chem.*, 370 (1989) 9.
68 T.B.K. Lee and G.K. Wong, *J. Chromatogr.*, 523 (1990) 317.
69 M. Rudolph, *J. Chromatogr.*, 535 (1990) 161; M. Rudolf, P. Volk and G. Schniedel, *J. Chromatogr.*, 535 (1990) 263.
70 K. Tagahara, J. Koyama, T. Okatani and Y. Suzuta, *Chem. Pharm. Bull.*, 34 (1986) 5166.
71 R. Noyori, M. Ohta, Y. Hisao, M. Kitamura, T. Ohta and H. Takaya, *J. Am. Chem. Soc.*, 108 (1986) 7117.
72 K. Yamamoto, H. Yumioka, Y. Okamoto and H. Chikamatsu, *J. Chem. Soc., Chem. Commun.*, (1987) 168.
73 T. Mukaiyama, H. Asanuma, I. Hachiya and T. Harada, *Chem. Lett.*, (1991) 1209.
74 Y. Okamoto, Y. Kaida, R. Aburatani and K. Hatada, *J. Chromatogr.*, 477 (1989) 367.
75 S.-H. Wu, S.-L. Lin, S.-Y. Lai and T.-H. Chou, *J. Chromatogr.*, 514 (1990) 325.
76 Y. Okamoto, M. Kawashima, R. Aburatani, K. Hatada, T. Nishiyama and M. Masuda, *Chem. Lett.*, (1986) 1237.
77 Th. Hollenhorst and G. Blaschke, *J. Chromatogr.*, 585 (1991) 329.
78 M.S. Ching, M.S. Lennard, A. Gregory, G.T. Tucker, D.R. Rutledger and C. Garrick, *J. Chromatogr.*, 497 (1989) 313.
79 C.R. Lee, J.-P. Porziemsky, M.-C. Aubert and A.M. Krstulovic, *J. Chromatogr.*, 539 (1991) 55.
80 A.M. Krstulovic, M.H. Fouchet, J.T. Burke, G. Gillet and A. Durand, *J. Chromatogr.*, 452 (1988) 477.
81 H.Y. Aboul-Erein and M.R. Islam, *Chirality*, 1 (1990) 301.
82 H.Y. Aboul-Erein and M.R. Islam, *J. Chromatogr.*, 511 (1990) 109.
83 C. Harttmann, O. Krauss, H. Spahn and E. Mutschler, *J. Chromatogr.*, 496 (1989) 387.
84 Y. Okamoto, D. Dirnberger, T. Burgemeister, G. Dannhardt and W. Wiegrebe, *Arch. Pharm.* (Weinheim, Ger.), 319 (1986) 1122.
85 Y. Okamoto, R. Aburatani, K. Hatada, M. Honda, N. Inotsume and M. Nakano, *J. Chromatogr.*, 513 (1990) 375.
86 H. Ebiike, Y. Terao and K. Achiwa, *Tetrahedron Lett.*, 32 (1991) 5805.
87 T. Kaneko, Y. Okamoto and K. Hatada, *J. Chem. Soc., Chem. Commun.*, (1987) 1511.
88 Y. Okamoto, Y. Senoh, H. Nakane and K. Hatada, *Chirality*, 1 (1989) 216.
89 Y. Kaida and Y. Okamoto, *Chirality*, 4 (1992) 122.
90 F. Toda, K. Tanaka and M. Yagi, *Tetrahedron*, 43 (1987) 1495.
91 T. Yamada, H. Suzuki and T. Mukaiyama, *Chem. Lett.*, (1987) 295.
92 F. Toda, M. Yagi and S. Soda, *J. Chem. Soc., Chem. Commun.*, (1987) 1413.
93 X.-L. Lu and S.K. Yang, *J. Chromatogr.*, 535 (1990) 229.
94 M. Lienne, M. Caude, R. Rosset and A. Tambuté, *J. Chromatogr.*, 448 (1988) 55.
95 Y. Okamoto, R. Aburatani, Y. Kaida and K. Hatada, *Chem. Lett.*, (1988) 1125.
96 Y. Okamoto, R. Aburatani and K. Hatada, *J. Chromatogr.*, 448 (1988) 454.
97 Y. Okamoto, R. Aburatani, Y. Kaida, K. Hatada, N. Inotsume and M. Nakano, *Chirality*, 1 (1989) 239.
98 S.-H. Wu, S.-Y. Lai, S.-L. Lin, F.-Y. Chu and K.-T. Wang, *Chirality*, 3 (1991) 67.
99 P. Pappas, B.C. Pappas, Y. Okamoto and H. Sakamoto, *J. Org. Chem.*, 54 (1988) 4404.
100 J.H. Kennedy and L.O. Weigel, *Chirality*, 4 (1992) 132.
101 D.M. McDaniel and B.G. Snider, *J. Chromatogr.*, 404 (1987) 123.
102 M. Murata, S. Ikoma and K. Achiwa, *Chem. Pharm. Bull.*, 38 (1990) 2329.
103 Y. Okamoto, Z.-K. Cao, R. Aburatani and K. Hatada, *Bull. Chem. Soc. Jpn.*, 60 (1987) 3999.
104 S. Tazuke, Y. Miyamoto, T. Ikeda and K. Tachibana, *Chem. Lett.*, (1986) 953.
105 S. Hamaguchi, T. Ohashi and K. Watanabe, *Agric. Biol. Chem.*, 50 (1986) 375.
106 T. Ozawa, Tsukuba University, personal communication.
107 S. Ozaki, M. Kohno, H. Nakahira, M. Bunya and Y. Watanabe, *Chem. Lett.*, (1988) 77.
108 Y. Terao, K. Tsuji, M. Murata, A. Achiwa, T. Nishino, N. Watanabe and K. Sato, *Chem. Pharm. Bull.*, 37 (1989) 1653.
109 E.J. Corey and J.O. Link, *J. Am. Chem. Soc.*, 114 (1992) 1906.
110 A. Kunath, E. Hoft, H.-J. Hamann and J. Wagner, *J. Chromatogr.*, 558 (1991) 177.
111 K. Tsuji, Y. Terao and K. Achiwa, *Tetrahedron Lett.*, 30 (1989) 6189.
112 M. Tanahashi, M. Karina and T. Higuchi, in *Proceedings of the Fourth International Symposium on Wood and Pulping Chemistry*, Vol. 2, 1987, p. 343.
113 A. Ichihara, M. Nonaka, S. Sakamura, R. Sato and A. Tajimi, *Chem. Lett.*, (1988) 27.
114 M.G. Quaglla, N. Desideri, E. Bossi, I. Sestili and C. Conti, *Chirality*, 4 (1992) 65.
115 K. Yoshikawa, K. Inoguchi, T. Morimoto and K. Achiwa, *Heterocycles*, 31 (1990) 1413.

116 T. Nakano, Y. Okamoto and K. Hatada, *J. Am. Chem. Soc.*, 114 (1992) 1318.
117 T. Nakano, Y. Okamoto and K. Hatada, unpublished results.
118 K. Ute, K. Oka, Y. Okamoto, K. Hatada, F. Xi and O. Vogl, *Polym. J.*, 12 (1991) 1419.
119 K. Ikeda, T. Hamasaki, H. Kohno, T. Ogawa, T. Matsumoto and J. Sakai, *Chem. Lett.*, (1989) 1089; A. Ishikawa and T. Shibata, *J. Liq. Chromatogr.*, 16 (1993) 859.
120 Y. Okamoto, R. Aburatani and K. Hatada, *Bull. Chem. Soc. Jpn.*, 63 (1990) 955.
121 Y. Okamoto, R. Aburatani, S. Miura and K. Hatada, *J. Liq. Chromatogr.*, 10 (1987) 1613.
122 U. Vogt and P. Zugenmaier, *Ber. Bunsenges. Phys. Chem.*, 89 (1985) 1217.
123 Y. Okamoto, H. Sakamoto and K. Hatada, *Chem. Lett.*, (1986) 983.
124 U. Vogt and P. Zugenmaier, presented at the *European Science Foundation Workshop on Scientific Interaction in Polysaccharide Systems, Uppsala, 1983.*
125 R. Aburatani, Y. Okamoto and K. Hatada, *Bull. Chem. Soc. Jpn.*, 63 (1990) 3606.
126 D.W. Armstrong, C.D. Chang and S.-H. Lee, *J. Chromatogr.*, 539 (1991) 83.
127 D.W. Armstrong, A.M. Stalcup, M.L. Hilton, J.D. Duncun, J.R. Faulkner, Jr. and S.-C. Chang, *Anal. Chem.*, 62 (1990) 1601; D.W. Armstrong, A.M. Stalcup and C.-D. Chang, *J. Chromatogr.*, 540 (1991) 83.
128 T. Hargitai, Y. Kaida and Y. Okamoto, *J. Chromatogr.*, 628 (1993) 11; T. Hargitai and Y. Okamoto, *J. Liq. Chromatogr.*, 16 (1993) 848.
129 Y. Kaida and Y. Okamoto, *J. Chromatogr.*, 641 (1993) 267.
130 C. Vandenbosch, D. Massart and W. Lindner, *J. Pharm. Biomed. Anal.*, 10 (1992) 895.
131 I. Okamoto, A. Ichida, T. Shibata, K. Mori and Y. Okamoto, *Nippon Kagaku Kaishi*, (1992) 133; M. Negawa and F. Shoji, *J. Chromatogr.*, 590 (1992) 113.
132 E. Yashima, J. Noguchi and Y. Okamoto, *Chem. Lett.*, (1992) 1959.