ELSEVIER

Journal of Chromatography A, 670 (1994) 39–49

JOURNAL OF CHROMATOGRAPHY A

Chloromethylphenylcarbamate derivatives of cellulose as chiral stationary phases for high-performance liquid chromatography

Bezhan Chankvetadze[☆], Eiji Yashima, Yoshio Okamoto*

Department of Applied Chemistry, Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan

(First received November 9th, 1993; revised manuscript received January 25th, 1994)

Abstract

A new class of eight chloromethylphenylcarbamate derivatives of cellulose was prepared by introducing both an electron-donating methyl group and an electron-withdrawing chloro group on to the phenyl moieties and their chiral recognition abilities were evaluated as chiral stationary phases (CSPs) for high-performance liquid chromatography. The superiority of these derivatives over dichloro- and dimethylphenylcarbamates of cellulose as CSPs was demonstrated for some racemic compounds. The elution order and enantioselectivity were greatly dependent on the positions of the substituents. *Meta-* and *para*-disubstituted derivatives showed higher chiral recognition than *ortho-* and *meta-* or *para*-disubstituted derivatives. The correlation between the chemical shifts of the N–H protons of the carbamate moieties and the enantiomer-resolving abilities of the derivatives is discussed. Some of the derivatives were effective CSPs in both normal- and reversed-phase conditions and could efficiently separate some chiral drug enantiomers.

1. Introduction

In recent years, the separation of enantiomers of various biologically active compounds such as pharmaceuticals, agrochemicals and food additives has become one of the most developing areas of separation science owing to its importance in structure–activity relationship studies, metabolism and chiral pharmacokinetic studies and even in dating some archaeological materials. Direct HPLC enantioseparation, parallel to capillary electrophoresis (CE) and supercritical fluid chromatography (SFC), provides a more promising technique for the analysis of chiral biologically active substances [1–3]. Among many commercially available chiral stationary phases (CSPs) for HPLC enantioseparation, polysaccharide derivative phases are among the most widely used for practical applications. The important advantages of polysaccharides in addition to their availability as natural sources are the ease of substitution and functionalization of the hydroxy groups of the glucose unit and the potential for application in large-scale separations [4,5].

Intensive studies of various polysaccharides, especially those of cellulose derivatives, reveal some correlations between their chiral recognition abilities and electronic and structural properties [4–10]. For benzoate and phenylcarbamate derivatives of cellulose, it has been established that their chiral recognition depends greatly on

* Corresponding author.

☆ Permanent address: Department of Chemistry, Tbilisi State University, Chavchavadze Ave. 1, 380028 Tbilisi, Georgia.

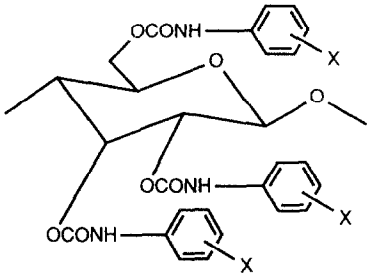
0021-9673/94/$07.00
SSDI 0021-9673(94)00085-N

the type and position of the substituents introduced on to the phenyl group [6,7]. The benzoate derivatives having electron-donating substituents such as methyl groups at *meta* and/or *para* positions showed better chiral resolving abilities than those having electron-withdrawing substituents such as chloro groups [6]. On the other hand, the introduction of either electron-donating or electron-withdrawing substituents tends to improve the optical resolution abilities of phenylcarbamate derivatives CSPs [7,8]. Substitution at a *meta* or *para* position on the phenyl moiety is considered to be more preferable than substitution at an *ortho* position for benzoate and phenylcarbamate derivatives of cellulose to prepare CSPs with a higher chiral recognition ability [6,7].

The tris(3,5-dimethylphenylcarbamate) of cellulose is one of the most powerful CSPs and can be used for both normal- and reversed-phase HPLC enantioseparations [11]. Cellulose tris(3,5-dichlorophenylcarbamate) also shows high chiral recognition, but its practical application is limited because of its exceptionally high solubility in most of chromatographic eluents [7]. In spite of the fact that the above-mentioned and some other polysaccharide derivatives [2,3] are intensively used for the separation of enantiomers of many types of chiral compounds, their chiral recognition mechanism remains obscure.

This work was carried out in order to improve the chiral recognition abilities and enhance the stability of chloro-substituted phenylcarbamates of cellulose by introducing a methyl group as a second substituent. Chloromethylphenylcarbamates of cellulose are versatile systems for studying the relationship between the electronic and structural properties of polysaccharides and their chiral recognition ability [12].

In this study, eight chloromethylphenylcarbamate derivatives of cellulose (Fig. 1) were prepared and their chiral recognition abilities were evaluated as CSPs. The effects of the position of the substituents on the elution order and chiral recognition abilities were studied. Correlations between the chemical shifts of N–H protons of the carbamate moieties of these derivatives and their enantiomer-resolving abilities were examined. The potential of use of these derivatives for reversed-phase HPLC and for the separation of some chiral drug enantiomers was also tested.



1

X=	a: 4-Cl-3-CH_3	e: 2-Cl-4-CH_3	i: 4-CH_3
	b: 3-Cl-4-CH_3	f: 2-Cl-5-CH_3	j: 4-Cl
	c: 3-Cl-2-CH_3	g: 2-Cl-6-CH_3	k: 3,4-$(CH_3)_2$
	d: 5-Cl-2-CH_3	h: 4-Cl-2-CH_3	l: 3,4-Cl_2

Fig. 1. Structures of CSPs.

2. Experimental

2.1. Chemicals

Microcrystalline cellulose (Avicel) was purchased from Merck (Darmstadt, Germany). (3-Aminopropyl)triethoxysilane, 3-chloro-4-methylaniline and 3-chloro-2-methylaniline were of guaranteed reagent grade from Tokyo Kasei (Tokyo, Japan). 2-Chloro-6-methylaniline was obtained from Janssen Chimica (Beerse, Belgium) and triphosgene, pyridine-d_5, 2-chloro-4-methylaniline, 4-chloro-2-methylaniline, 5-chloro-2-methylaniline and 2-chloro-5-methylaniline from Aldrich (Milwaukee, WI, USA). All isocyanates were prepared from the corresponding amines by the conventional method using triphosgene. Wide-pore silica gel (Daiso gel SP-1000, pore size 100 nm, particle size 7 μm) was obtained from Daiso (Osaka, Japan) and was silanized using (3-aminopropyl)triethoxysilane in benzene at 80°C before use. Hexane, 2-propanol and acetonitrile used as components of the eluents were of analytical-reagent grade.

Racemic compounds were obtained from different sources.

2.2. Preparation of tris(chloromethylphenylcarbamate) derivatives of cellulose

Cellulose tris(chloromethylphenylcarbamate) derivatives (**1a–h**) were prepared as described previously [7] by the reaction of cellulose with an excess of corresponding isocyanates in dry pyridine at *ca.* 100°C and isolated as methanol-insoluble fractions. Elemental analyses (Table 1) and IR and ^{1}H NMR spectra showed that hydroxy groups of cellulose were almost completely converted into the carbamate moieties.

2.3. Preparation of stationary phase

Column packing materials were prepared as described previously [7] using macroporous silica gel (Daiso gel SP-1000) and packed into 25 cm × 0.46 cm I.D. stainless-steel tubes by the conventional high-pressure slurry packing technique using a CCP-085 Econo packer pump (Chemco, Osaka, Japan). The plate numbers of the columns were 2000–4000 for benzene with hexane–2-propanol (90:10) at a flow-rate of 0.5 ml/min as the eluent at 20°C. The dead time (t_0) of the columns was determined using 1,3,5-tri-*tert.*-butylbenzene as a non-retained compound.

2.4. Apparatus

All chromatographic experiments were performed on a Jasco Trirotar-II liquid chromatograph equipped with UV (Jasco 875-UV) and polarimetric (Jasco 181-C) detectors. A Model 7125 injector with a 100-μl loop (Rheodyne, Cotati, CA, USA) was used for injection of samples. All column evaluations were carried out at ambient temperature. IR analyses were carried out using a Jasco Fourier transform infrared spectrometer with a Jasco PTL-396 data processor. UV spectra were measured in tetrahydrofuran (THF) solutions using a Jasco Ubest-55 spectrophotometer. Circular dichroism (CD) spectra were measured in THF solutions in a 0.01 cm cell using a Jasco J-720 L spectropolarimeter. ^{1}H NMR spectra were taken in pyridine-d_5 solution at 80°C using a Varian VXR-500 NMR spectrometer operating at 500 MHz. Tetramethylsilane (TMS) was used as the internal standard.

3. Results and discussion

The results of the enantioseparation of fourteen racemic compounds (Fig. 2) on the chloromethylphenylcarbamate derivatives of cellulose are given in Table 2 together with those on 4-methyl-, 4-chloro, 3,4-dimethyl- and 3,4-dichlorophenylcarbamates of cellulose [7]. As can

Table 1
Elemental analysis and N–H chemical shifts of **1a–h**

Compound	C (%)	H (%)	N (%)	Cl (%)	NH–proton, δ (ppm)		
					I	II	III
1a	53.10	4.23	6.22	15.96	10.51	9.88	9.64
1b	53.27	4.24	6.28	15.65	10.57	10.00	9.82
1c	54.15	4.32	6.39	16.13	9.76	9.17	8.85
1d	52.90	4.20	6.31	16.05	9.46	8.85	8.46
1e	54.10	4.22	6.29	16.20	8.95	8.73	8.25
1f	54.06	4.31	6.26	16.09	9.00	8.20	7.96
1g	53.90	4.32	6.36	16.12	9.12	8.90	8.44
1h	54.44	4.39	6.80		9.33	8.80	8.44
Calculated	54.33	3.92	6.34	16.08			

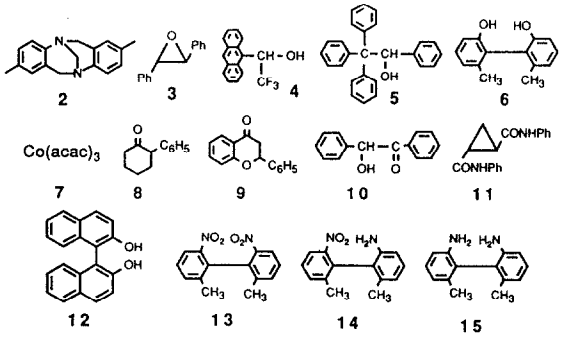


Fig. 2. Structures of racemic compounds.

be seen, more efficient chiral recognition abilities are exhibited by CSPs **1a** and **1b**, which can separate all fourteen racemic compounds with reasonable selectivity, and some racemic compounds were separated better than on dimethyl- (**1k**) and dichloro- (**1l**) phenylcarbamate derivatives of cellulose [7]. It seems noteworthy that these cellulose phenylcarbamate derivatives do not contain a substituent at the *ortho* position on the phenyl moiety. Cellulose tris(3,5-dimethylphenylcarbamate), which is one of the most powerful and widely used columns, could not separate the racemic compound **7**, which was completely separated on **1a** and **1b**. An interesting "synergistic" effect can be observed for some racemic compounds; for example, neither **1k** nor **1l** could separate the compound **5**, which was separated both on **1a** and **1b** with high selectivity ($\alpha = 3.05$ and 1.95, respectively).

The resolving power of the derivatives possessing a substituent at the *ortho* position was relatively low compared with those of **1a** and **1b**. However, these derivatives, particularly **1c** and **1d**, showed characteristic chiral recognition for biphenyl and binaphthyl derivatives (**6** and **12**–**15**). Analogous low chiral recognition was previously found for *ortho*-substituted chloro-, methyl-, dimethyl- and dichlorophenylcarbamates of cellulose [7].

All disubstituted derivatives prepared in this study are scarcely soluble in hexane containing 10–20% of 2-propanol in which cellulose tris(3,5-dichlorophenylcarbamate) is swollen or dissolved.

As can be seen from Table 2, the chiral recognition power of **1l** was almost the same as that of cellulose tris(4-chlorophenylcarbamate) (**1j**) [7], whereas introducing an electron-donating methyl group at the *meta* position to **1j** leads to a substantial increase in the enantiomer resolving ability. However, introduction of methyl group at the *ortho* position (**1h**) lowered the chiral recognition abilities towards most of the racemic compounds used in this study. Introduction of a methyl group at the *meta* position (**1k**) of tris(4-methylphenylcarbamate) (**1i**) of cellulose led to a slight increase in enantioselectivity, but the CSP lost enantioselectivity to the racemic compound **5**, whereas introduction of chloro group at the same *meta* position led to almost the same enantioselectivity without losing enantioselectivity for the racemic compound **5**.

These results clearly demonstrate that the introduction of an electron-donating group at a *meta* or *para* position as a second substituent on the phenyl moiety of cellulose phenylcarbamate derivatives containing one electron-withdrawing substituent at a *meta* or *para* position is much more preferable and *vice versa*. At present it is not clear if this finding can be generalized for all electron-donating and electron-withdrawing groups or whether it may be due to some steric effect.

Some interesting data were obtained from the results of enantioseparation on **1c**, **1d**, **1g** and **1h**, all of which possess a methyl group at the *ortho* position and a chloro group at different positions. The chiral recognition abilities of these derivatives depended on the position of the chloro group and were slightly higher than that of cellulose tris(2-methylphenylcarbamate) [7]. The elution orders of some racemic compounds were reversed depending on the position of the chloro group. In **1h**, the chloro group is far from the carbamate moiety than in other derivatives and the steric hindrance for racemic compounds interacting with the carbamate moiety must be lowest in this instance. Despite this fact, **1c**, **1d** and even **1g** separated most of the racemic compounds **11**–**15**, whereas only **15** could be partially separated on **1h**.

No reverse elution order of any racemic compounds was observed on changing the position of

Table 2
Optical resolution of **2**–**15** on cellulose phenylcarbamate derivatives **1a**–**h**[a]

Compound	**1a**			**1b**			**1c**			**1d**		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
2	1.10(+)	1.13	0.6	0.77(+)	1.25	3.8	0.67(−)	~1		0.80(+)	~1	
3	0.43(+)	3.25	5.0	0.45(+)	2.09	1.6	0.63(+)	~1		0.67(+)	1.30	1.0
4	0.80(−)	1.25	0.9	0.79(−)	1.20	1.3	0.60	1.00		1.53(−)	~1	
5	0.73(+)	3.05	5.0	0.65(+)	1.95	3.8	0.97(+)	1.17	0.7	1.00	1.00	
6	1.80(−)	1.35	1.1	1.45(−)	1.24	0.8	1.47(+)	1.25	0.8	1.90(+)	1.19	
7	5.86(+)[b]	1.44	3.0	2.50(+)[b]	1.67	3.0	3.93(−)[b]	~1		2.87(+)[b]	1.05	
8	1.27(−)	1.26	2.0	1.05(−)	1.19	1.3	1.58(+)	1.08		2.20(−)	~1	
9	1.73(−)	1.06		1.36(−)	1.09	0.8	1.77(+)	~1		1.90(−)	1.08	
10	4.70(−)	1.23	2.7	3.36(−)	1.27	3.5	3.67(+)	1.30	1.3	4.76(+)	1.10	0.9
11	2.13(−)	1.54	1.4	1.10(−)	2.06	3.5	1.50(+)	1.24	1.0	1.30(−)	1.20	
12	4.60(−)	1.54	0.9	3.77(−)	1.22	0.9	3.60(+)	1.50	1.4	4.30(+)	2.28	1.5
13	2.66(−)	1.19	1.5	1.86(−)	1.08	0.8	4.87(+)	1.05		7.66(+)	1.16	0.8
14	2.57(−)	1.56	4.5	1.87(−)	1.82	3.0	3.17(−)	1.24	1.3	4.67(−)	1.33	1.1
15	1.63(−)	1.31	2.0	1.33(−)	1.13		1.37(−)	1.27	0.9	1.33(−)	1.25	0.8
	1e			**1f**			**1g**			**1h**		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
2	1.37(+)	1.09		1.03(+)	~1		1.00(+)	~1		1.08(+)	~1	
3	0.83(+)	1.16	0.7	0.70(+)	1.71	0.8	0.80(+)	~1		0.97(+)	1.07	
4	2.80	1.00		1.67(−)	1.34	0.8	1.50(−)	~1		3.47(−)	~1	
5	2.13	1.00		1.28(+)	1.40		1.07(+)	~1		1.20	1.00	
6	3.67(+)	1.45	0.6	1.87(+)	1.23		2.36(+)	1.20	0.8	3.46(−)	1.11	
7	0.47(+)	~1		0.58(+)	~1		1.33(−)	~1		1.67	1.00	
8	1.10(−)	~1		1.18(−)	~1		0.60(−)	~1		1.43	1.00	
9	2.47(−)	1.04		2.08	1.00		2.40(+)	~1		2.60	1.00	
10	4.08(−)	1.08		3.23	1.00		4.40(+)	1.15	1.3	5.30(−)	1.08	
11	1.67	1.00		0.93	1.00		1.20(+)	1.61	1.1	3.00	1.00	
12	16.33	1.41	1.0	6.07(−)	1.38		7.27	1.56		8.67	1.00	
13	5.40(−)	1.05		4.00(−)	~1		6.80	1.00		8.37	1.00	
14	3.97(−)	1.22	1.0	3.00(−)	1.22		7.40(−)	1.21	1.0	9.40	1.00	
15	1.77(−)	1.26	1.0	1.33(−)	1.30	0.7	6.70(−)	~1		2.63(−)	1.09	
	1i[c]			**1j**[c]			**1k**[c]			**1l**[c]		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
2	0.75(+)	1.48	2.6	0.89(+)	1.16	0.8	0.87(+)	1.49	2.1	0.79(+)	1.47	1.7
3	0.51(+)	1.55	2.4	0.38(+)	1.68	2.3	0.61(+)	1.13	0.6	0.38(+)	1.93	2.9
4	1.54(−)	1.52	3.8	0.48(−)	1.29	1.1	1.76(−)	2.13	4.8	0.33(−)	1.21	
5	1.33(+)	1.37	2.5	0.81(+)	1.95	3.3	1.55(+)	~1		0.48	1.00	
6	2.48(−)	1.30	0.9	0.90(−)	1.20	0.8	1.86(−)	1.87	2.6	1.34(−)	~1	
7	0.90(+)	1.75	3.4	3.16(+)	1.46	2.8	0.57(+)	1.32	1.1	1.21(+)	1.63	2.1
8	1.14(−)	1.20	1.4	1.63(−)	1.16	1.3	0.95(−)	1.20	0.8	1.92(−)	1.31	
9	1.57(+)	1.16	1.5	1.85(+)	1.12	1.1	1.53(−)	1.42	2.6	1.29(+)	1.04	
10	3.00(−)	1.12	1.1	4.00(−)	1.20	2.0	3.24(−)	1.10	0.6	2.77(+)	1.31	1.9
11	1.83(−)	1.35	1.8	1.45(−)	1.44	1.3	1.27(+)	2.39	3.6	0.81(+)	1.15	
12												
13	2.50	1.00		3.95(−)	1.12	1.1				2.18(−)	1.24	1.5
14	2.37(+)	1.34	3.2	2.78(−)	1.30	2.4				2.24(−)	1.20	
15												

[a] The sign in parentheses represents the optical rotation of the first-eluted enantiomer. Eluent, hexane–2-propanol (90:10, v/v); flow-rate, 0.5 ml/min.
[b] Eluent, hexane–2-propanol (98:2, v/v).
[c] Data are taken from ref. 7.

the methyl group in the derivatives bearing a chloro group at position 4 (**1a** and **1h**), whereas the elution order of some racemic compounds was reversed depending on the position of the methyl group in *m*-chlorophenylcarbamates of cellulose (**1b**, **1c** and **1d**). As will be shown below from IR data, intramolecular hydrogen bonding, which probably contributes to maintaining a higher ordered secondary structure of polysaccharide derivatives, is weaker in *ortho*-substituted phenylcarbamate derivatives of cellulose, and probably this is the reason for the dramatic changes in the chiral resolving power and elution order of some racemates. The elution orders of the racemic compounds (**2–15**) were identical on **1a** and **1b**, which may indicate that these two derivatives form the same regular structures, probably owing to strong intramolecular hydrogen bonding.

Comparison of the retention times and enantioselectivities of four biphenyl derivatives (**6**, **13**, **14** and **15**) having a similar structure but different functional groups on the CSPs is of interest. The racemic compounds **13** and **14** possess a nitro group capable of hydrogen bonding with the N–H of the carbamate moiety of cellulose phenylcarbamate derivatives, and they are characterized with longer retention times than the other two derivatives (**6**, **15**). Although a longer retention time does not always lead to a better separation of enantiomers [13], compound **13** with the longest retention time exhibited the lowest enantioselectivity among all the above-mentioned biphenyl derivatives.

Fig. 3 shows the ^{1}H NMR spectra of the NH region of the chloromethylphenylcarbamate derivatives and trisphenylcarbamate (CTPC) of cellulose. The N–H proton chemical shifts of cellulose phenylcarbamate derivatives depended greatly on the position of the substituents, and three resonances corresponding to the N–H protons of carbamate groups at positions 2, 3 and 6 of the glucose units were observed in the N–H regions. The N–H resonance at the lowest field may be assigned to the N–H proton at position 6 [14]. The chemical shifts of the N–H resonances reflect the acidity of N–H protons and shift downfield with increase in acidity of

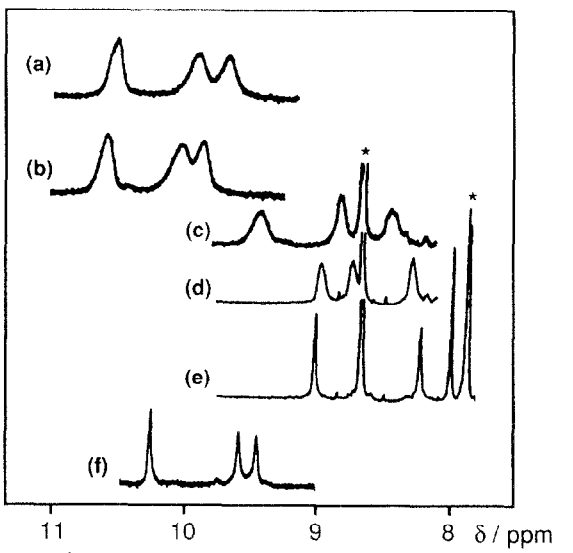


Fig. 3. ^{1}H NMR spectra of cellulose phenylcarbamate derivatives (a) **1a**, (b) **1b**, (c) **1d**, (d) **1e** and (e) **1f** and (f) cellulose trisphenylcarbamate. Pyridine-d_5, 80°C, 500 MHz. Asterisks denote the solvent.

N–H [7]. The N–H protons of **1a** and **1b** resonate slightly downfield of those of CTPC, but upfield of those of cellulose tris(3,5-dichlorophenylcarbamate) [14]. This indicates that the N–H protons of **1a** and **1b** were more acidic than those of CTPC, whereas with *ortho*-substituted derivatives, the N–H resonances dramatically shift upfield, probably owing to steric hindrance of the substituents at the *ortho* position which may disturb the planar structure of the phenylcarbamate residues. The more acidic N–H will interact with appropriate racemic compounds more strongly via hydrogen bonding and if this occurs, the retention times of **13** and **14** on 3,4-disubstituted derivatives will be longer than on *ortho*-substituted derivatives. However, the reverse order was observed (Table 2).

This apparent contradiction can be solved by using the IR spectral data for the above-mentioned phenylcarbamate derivatives of cellulose (Fig. 4). There are at least two N–H peaks in the IR spectra of some derivatives (*e.g.*, **1a**). The peak in the range 3400–3435 cm^{-1} may be assigned to a free N–H residue and that in the range 3330–3350 cm^{-1} to N–H involved in intramolecular hydrogen bonding [7]. Most of

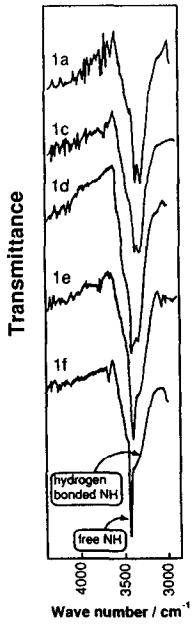


Fig. 4. IR spectra of N–H of chloromethylphenylcarbamate derivatives of cellulose (**1a**, **1c**, **1d**, **1e** and **1f**).

the N–H appears to be free in the case of *ortho*-substituted derivatives, especially 2-chloro-substituted derivatives, and the free N–H will interact strongly with appropriate racemic compounds (in this instance **13** and **14**). This will result in longer retention. However, in 3,4-disubstituted derivatives, the N–H groups are markedly involved in intramolecular hydrogen bonding (Fig. 4) and this part will not contribute to retention if we assume that intramolecularly hydrogen-bonded N–H loses the capability of interacting with appropriate solutes. The racemate **14** possesses both nitro and amino groups and is capable of interacting with both N–H and C=O groups of the carbamate moieties, which may be the reason for the long retention time and high enantioselectivity of this compound. The racemates **6** and **15** will probably interact with the carbonyl fragment of the carbamate moieties which are close to chiral glucose units and this may be the reason for the high enantioselectivity for these compounds.

To demonstrate the importance of hydrogen bonding in chiral recognition on cellulose phenylcarbamate derivatives, separation factors (α) of the racemic compounds **10** and **11** were plotted against N–H chemical shifts of the 6-position of phenylcarbamate derivatives (lowest field resonance) (Fig. 5). As can be seen, reasonable correlations exist between separation factors of the racemic compounds **10** and **11** and N–H chemical shifts. The separation factors generally increased as the N–H chemical shift increased. Other reliable correlations such as this can be found in Table 2 not only for compounds capable of interacting with N–H groups of carbamate moieties (**7–11**, **13**, **14**) but also for other racemic compounds. These results indicate that N–H groups of the carbamate moieties contribute significantly to chiral recognition not only as a local chiral adsorptive site, but also by forming intramolecular hydrogen bonds to maintain a regular structure. The latter will affect both enantioselectivity and column efficiency, in this instance the resolution factor (R_s).

To examine more closely the effect of intramolecular hydrogen bonding on the resolution factor, the R_s value of **15** was divided by the plate numbers (N) of the columns and the resulting R_s/N values were plotted against the N–H chemical shifts of some cellulose phenylcarbamate derivatives (Fig. 6). This plot was constructed in order to exclude the effect of the different efficiencies of the columns, and the racemic compound **15** was chosen because it most probably could not interact with the N–H

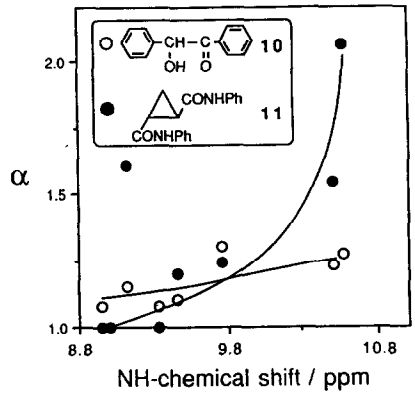


Fig. 5. Dependence of separation factor (α) of benzoin (**10**) and *trans*-cyclopropanedicarboxylic acid dianilide (**11**) on the N–H chemical shift of cellulose phenylcarbamate derivatives (**1a–h**).

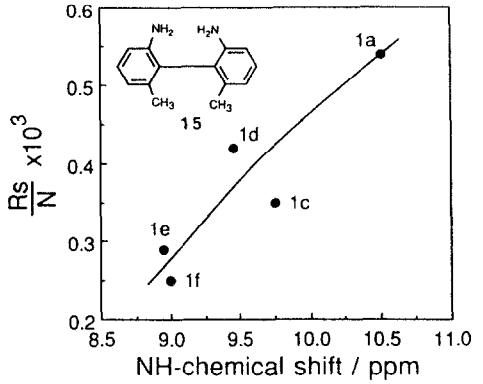


Fig. 6. Dependence of R_s/N for 2,2′-diamino-6,6′-dimethylbiphenyl (**15**) on the N–H chemical shift of cellulose phenylcarbamate derivatives (**1a**, **1c**, **1d**, **1e** and **1f**).

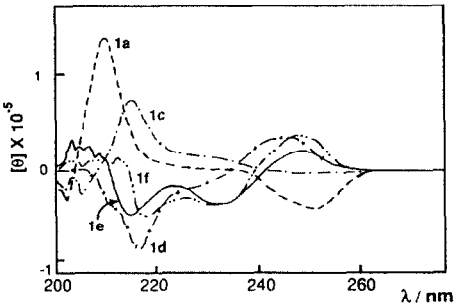


Fig. 7. CD spectra of cellulose phenylcarbamate derivatives (**1a**, **1c**, **1d**, **1e** and **1f**) in THF.

groups of the carbamate moieties as a local chiral adsorptive site and it shows almost the same capacity factor ($k_1' = 1.33–1.77$) and the same separation factor ($\alpha = 1.25–1.31$) on most of the cellulose phenylcarbamate derivatives in this study (**1a**, **1c**, **1d**, **1e** and **1f**). As can be seen from Fig. 6, R_s/N decreases with decrease in N–H chemical shift. This suggests that **1c**, **1d** and especially **1e** and **1f** possess other kinds of adsorbing sites than **1a**, probably owing to their irregular structures and **1a** has a limited number of adsorbing sites owing to its regular structure. IR spectra of the same cellulose phenylcarbamate derivatives (Fig. 4) show that part of the N–H forms intramolecular hydrogen bonds, and the ratio decreased in the same order as that in Fig. 6.

Further evidence for the regular structure of **1a** can be seen in the CD spectra of the above-mentioned cellulose phenylcarbamate derivatives (Fig. 7). The CD spectra showed differences in the patterns, wavelengths of the peak tops and intensities of the peaks, depending on the position of the substituents. The column of **1a** with a high enantiomer resolving ability shows the most intense peak in the region of 210–220 nm (C=O region), which suggests that the structure of **1a** may be highly regular. These results suggest that the position and type of substituents may vary the conformation of the main chain and/or side-chains. This will influence the chiral recognition powers of CSPs.

Hence we can conclude that N–H groups of phenylcarbamate moieties can contribute to chiral recognition by direct interaction with some racemic compounds as a local chiral adsorptive site and also by maintaining a higher ordered secondary structure of CSPs via intramolecular hydrogen bonding, and the latter contribution is more universal and may not depend on the type of a solute and will affect the resolution factor to a considerable extent.

The above-proposed mechanism for the role of intramolecular hydrogen bonding and free N–H groups can explain not only the chiral recognition mechanism of polysaccharide phenylcarbamate derivatives, but also some general physical and chemical properties of these derivatives. For example, the high solubility of cellulose tris(3,5-dichlorophenylcarbamate) may be attributed to a large fraction of free N–H groups with high acidity [7]. Cellulose tris(2-methylphenylcarbamate) shows lyotropic liquid crystallinity, but cellulose tris(2-chlorophenylcarbamate) does not. This may be ascribed to the fact that an *ortho*-substituted chloro group tends to disturb intramolecular hydrogen bonding substantially, whereas a methyl group in the same position does not disturb it, as it can be seen from the IR spectra of these carbamates. Therefore, it is suggested that liquid crystallinity may be closely related to the intramolecular hydrogen bonding abilities of the derivatives.

In the early stages of the development of

cellulose tribenzoate as a CSP, the successful resolution of enantiomers with water-containing eluents was considered to be due to hydrophobic interactions between the CSPs and enantiomers, which plays an important role in chiral recognition [15]. Recently, an attempt was made to improve the chiral recognition abilities of cellulose tris(3,5-dimethylphenylcarbamate) to some hydroperoxides and alcohols by saturation of water in hexane–2-propanol eluent [16] and tris(3,5-dimethylphenylcarbamate) of cellulose was proposed as a successful CSP for the reversed-phase separation of enantiomers [11]. All these data substantially extend the universality of cellulose phenylcarbamate derivatives as CSPs and show that in the absence of interaction of racemic compounds with carbamate fragments via hydrogen bonding in water-containing eluents, alternative chiral sites can contribute to enantioseparation. An example of the enantioseparation of the racemic compound **3** in both normal- and reversed-phase conditions on **1a** is shown in Fig. 8. Better separation was achieved in the former than in the latter instance. Probably the chiral resolving power in reversed-phase systems will be markedly improved by optimizing the separation conditions (buffer system, pH, additives, temperature, etc.), but this is outside the scope of this work. Both normal- (hexane–2-propanol) and reversed-phase (water–acetonitrile, water–ethanol, water–methanol mixtures) enantioseparations were possible on a single column at least for the present separations.

The high chiral recognition abilities exhibited by chloromethylphenylcarbamates of cellulose, especially **1a** and **1b**, were also demonstrated in the separation of enantiomers of some pharmacologically important compounds used in the treatment of various cardio- and cerebrovascular disorders, such as hypertension, angina pectoris and cardiac arrhythmia (Fig. 9). It must be noted that some of these compounds were not separated using cellulose tris(3,5-dimethylphenylcarbamate), which shows high resolving abilities for many racemic compounds including drugs [1,4,7]. Recently, Ching *et al.* [17] reported the separation of acebutolol enantiomers using cellulose tris(3,5-dimethylphenylcarbamate), but the separation factor was low ($\alpha = 1.12$).

4-Aryl-1,4-dihydropyridine calcium antagonists are important peripheral vasodilators and are widely used in the treatment of cerebrocirculatory disorders and hypertension [18]. It has been established that in many instances chiral dihydropyridines such as nitrendipine and nicardipine are superior to the corresponding symmetrically substituted derivatives such as nifedipine [19,20]. Detailed pharmacological studies revealed that enantiomers of chiral dihydropyridines have different, in some instances even opposite, vasodi-

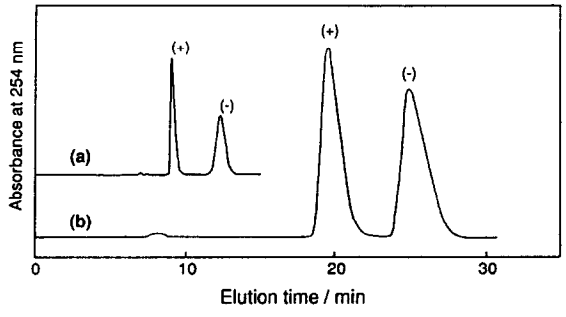


Fig. 8. Separation of enantiomers of *trans*-2,3-diphenyloxirane (**3**) under (a) normal- and (b) reversed-phase conditions on cellulose tris(3-chloro-4-methylphenylcarbamate) (**1b**). Eluent, (a) hexane–2-propanol (90:10), flow-rate 0.5 ml/min; (b) water–acetonitrile (50:50), flow-rate 1.0 ml/min.

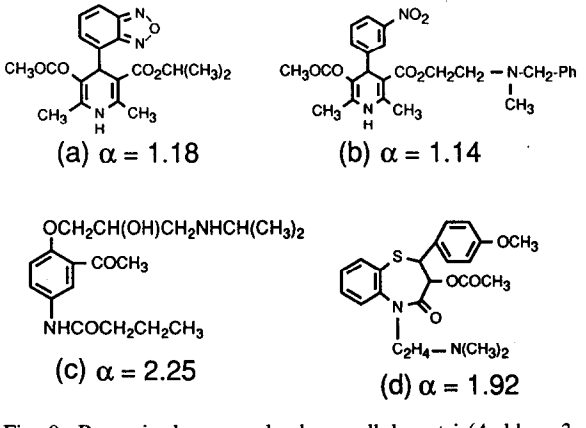


Fig. 9. Racemic drugs resolved on cellulose tris(4-chloro-3-methylphenylcarbamate) (**1a**). (a) Isradipine; (b) nicardipine; (c) acebutolol; (d) *cis*-diltiazem. Eluent, (a–c) hexane–2-propanol (90:10); (d) hexane–2-propanol (80:20).

lating and hypotensive activities and toxicities [21]. In most instances, the 4*S*-enantiomer is more active than the 4*R*-enantiomer [21–23]. The chiral dihydropyridines nitrendipine, nicardipine and isradipine cannot be separated using cellulose tris(3,5-dimethylphenylcarbamate) [4,9]. Enantiomers of these drugs were separated using cellulose tris(4-*tert.*-butylphenylcarbamate) with very long retention times, especially for nicardipine [9].

A chiral thiazepine derivative with the same calcium channel-blocking activity, *cis*-diltiazem, can also be separated using other cellulose [4] and amylose derivatives, but the separation factor on **1a** was much higher, and this will permit the use of this CSP for large-scale separations.

Some other chiral drugs, *e.g.*, the β-blockers propranolol ($k_1' = 0.53$, $\alpha = 2.00$), oxprenolol ($k_1' = 1.18$, $\alpha = 1.14$) and alprenolol ($k_1' = 1.40$, $\alpha = 1.10$) and the antihistaminic carbinoxamine ($k_1' = 7.71$, $\alpha = 1.21$) and doxylamine ($k_1' = 0.93$, $\alpha = 1.85$), can be successfully separated using **1a** and/or **1b**.

4. Conclusions

The chiral recognition abilities of eight chloromethylphenylcarbamate derivatives of cellulose were evaluated as CSPs for HPLC and it was established that 3,4-chloromethylphenylcarbamates of cellulose show higher resolving powers for some racemic compounds than dimethyl- and dichlorophenylcarbamates of cellulose. However, *ortho*-substituted derivatives showed low chiral recognition. Some correlations were established between ^{1}H NMR chemical shifts of the N–H groups of the carbamate moieties and the enantiomer resolving abilities of the above-mentioned cellulose derivatives. It is suggested that the N–H groups of the carbamate moieties will contribute to the chiral recognition as local chiral adsorbing sites and also by maintaining a higher ordered secondary structure of polysaccharide CSPs via intramolecular hydrogen bonding, and the latter is more universal and does not depend on the nature of the solute. The potential of use of these CSPs in reversed-phase conditions and for the separation of some practically important drug enantiomers has also been demonstrated.

5. Acknowledgements

B. Chankvetadze thanks the Japan Cultural Association for financial support during his stay at Nagoya University. The authors are grateful to Professor Emeritus H. Suda of Kanazawa University for providing chiral biphenyl derivatives. This work was partially supported by Grant-in-Aids for Scientific Research Nos. 02555184 and 05559009 from the Ministry of Education, Science and Culture, Japan.

6. References

[1] S. Ahuja, *Chiral Separation by Liquid Chromatography* (*ACS Symposium Series*, No. 471), American Chemical Society, Washington, DC, 1991.
[2] Y. Okamoto and Y. Kaida, *J. Synth. Org. Chem. Jpn.*, 51 (1993) 41.
[3] Y. Okamoto, R. Aburatani, K. Hatano and K. Hatada, *J. Liq. Chromatogr.*, 11 (1988) 2147.
[4] M. Negawa and F. Shoji, *J. Chromatogr.*, 590 (1992) 113.
[5] E. Francotte and R.M. Wolf, *J. Chromatogr.*, 595 (1992) 63.
[6] Y. Okamoto, R. Aburatani and K. Hatada, *J. Chromatogr.*, 389 (1987) 95.
[7] Y. Okamoto, M. Kawashima and K. Hatada, *J. Chromatogr.*, 363 (1986) 173.
[8] Y. Okamoto, M. Kawashima, R. Aburatani, K. Hatada, T. Nishiyama and M. Masuda, *Chem. Lett.*, (1986) 1237.
[9] Y. Okamoto, R. Aburatani, K. Hatada, M. Honda, N. Inotsume and M. Nakano, *J. Chromatogr.*, 513 (1990) 375.
[10] Y. Kaida and Y. Okamoto, *J. Chromatogr.*, 641 (1993) 267.
[11] K. Ikeda, T. Hamasaki, H. Kohno, T. Ogawa, T. Matsumoto and J. Sakai, *Chem. Lett.*, (1989) 1089.
[12] B. Chankbetadze, E. Yashima and Y. Okamoto, *Chem. Lett.*, (1992) 617.
[13] D.T. Witte, J.P. Franke, F.J. Bruggeman, D. Dijksta and R.A. De Zeeuw, *Chirality*, 4 (1992) 389.
[14] Y. Kaida and Y. Okamoto, *Bull. Chem. Soc. Jpn.*, 66 (1993) 2225.

[15] Y. Okamoto, M. Kawashima, K. Yamamoto and K. Hatada, *Chem. Lett.*, (1984) 739.
[16] A. Kunath, E. Hoft and H. Hamann, *J. Chromatogr.*, 588 (1991) 352.
[17] C.B. Ching, B.G. Lim, E.J.D. Lee and S.C. Ng, *Chirality*, 4 (1992) 174.
[18] F. Bossert and W. Vater, *Med. Res. Rev.*, 9 (1989) 291.
[19] L. Dagnino, K.K. Li, M.W. Wolowyk, H. Wynn, C.R. Triggle and E.E. Kraus, *J. Med. Chem.*, 29 (1986) 2525.
[20] M. Iwanami, T. Shibata, M. Fujimoto, R. Kawai, K. Tamazawa, K. Takenata, K. Takahashi and M. Murakami, *Chem. Pharm. Bull.*, 27 (1979) 1426.
[21] S. Goldmann and J. Stoltefuss, *Angew. Chem., Int. Ed. Engl.*, 30 (1991) 1559.
[22] M. Kajino, Y. Wada, Y. Nagai, A. Nagaoka and K. Meguro, *Chem. Pharm. Bull.*, 37 (1989) 2225.
[23] G. Marziniak, A. Delgado, G. Leclerc, J. Velly, N. Decker and J. Schwartz, *J. Med. Chem.*, 32 (1989) 1402.