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3,5-Dimethylphenylcarbamates of cellulose and amylose regioselectively bonded to silica gel as chiral stationary phases for high-performance liquid chromatography

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Abstract

3,5-Dimethylphenylcarbamates of amylose (ADMPC) (**1**) and cellulose (CDMPC) (**2**) chemically bonded to 3-aminopropylsilica gel were prepared with 4,4′-diphenylmethane diisocyanate as a spacer and their optical resolution abilities were evaluated as chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC). To investigate the influence of the position of the glucose unit in immobilization on chiral recognition, the polysaccharide derivatives were regioselectively bonded to the silica surface. ADMPC regioselectively bonded at the 6-position to silica gel possesses a higher optical resolving power than that bonded at the 2- or 3-position. The chiral recognition ability of the former CSP was almost comparable to that of ADMPC coated on silica gel. For CDMPC, the position of glucose in immobilization on silica gel hardly affected the chiral recognition. The enantioselectivities of these CSPs were also influenced by the amount of the diisocyanate used for immobilization. These chemically bonded-type CSPs were able to be used with eluents such as $CHCl_3$ in which the polysaccharide derivatives are soluble or swollen. A few racemates which were not or poorly separated on the coated-type CSP were more efficiently resolved on the chemically bonded-type CSP using $CHCl_3$ as a component of the mobile phase.

1. Introduction

Cellulose and amylose are the most accessible optically active polymers. These polysaccharides are known to show a chiral recognition which is not high enough for practical use. However, their benzoate [1,2] and phenylcarbamate derivatives [3–9] exhibit a high chiral recognition ability and afford practically useful chiral stationary phases (CSPs) for HPLC. We previously reported that twenty substituted trisphenylcarbamate derivatives of cellulose exhibited characteristic optical resolving powers as CSPs for HPLC when they were coated on silica gel [4]. Among them, tris(3,5-dimethylphenylcarbamate) (CDMPC) (**2**) and tris(3,5-dichlorophenylcarbamate) were the best derivatives with respect to chiral recognition. Of the derivatives of amylose, the tris(3,5-dimethylphenylcarbamate) (ADMPC) (**1**) was again the most efficient CSP for many racemates [5].

These CSPs have been prepared by coating the polysaccharide phenylcarbamate derivatives on silica gel and the tris(3,5-dimethylphenylcarbam-

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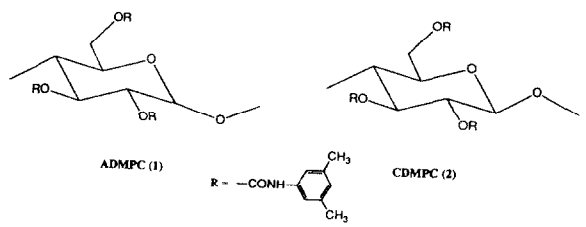


ate) phases possess a high durability when a hexane–2-propanol mixture is used as the eluent, whereas for the cellulose tris(3,5-dichlorophenylcarbamate) phase an eluent containing more than 10% of 2-propanol can not be used because of the high solubility. Some solvents such as tetrahydrofuran (THF) and $CHCl_3$, in which the polysaccharide derivatives themselves are dissolved or swollen, are unable to be used as eluents. To improve these defects, the derivatives were chemically bonded to 3-aminopropylsilanized silica gel with diisocyanates as a spacer non-regioselectively through the hydroxy groups at the 2-, 3- or 6-position of the glucose units [10]. However, the chiral recognition ability was greatly changed by immobilization, usually showing a lower resolving ability than those of coated-type CSPs.

In this study, we prepared chemically bonded-type CSPs regioselectively linked to aminopropyl-functionalized silica gel with a diisocyanate, and their chiral recognition abilities were evaluated.

2. Experimental

2.1. Chemicals

Racemic solutes were obtained from different sources [11]. Amylose of guaranteed reagent grade was purchased from Nacalai Tesque (Kyoto, Japan) (M_r 16 000) or was a gift from Nakano Vinegar (Handa, Japan) (M_r 11 000). Cellulose (Avicel) was obtained from Merck (Darmstadt, Germany). Triphenylmethyl chloride, 4,4′-diphenylmethane diisocyanate and 3-aminopropyltriethoxysilane were of guaranteed reagent grade from Tokyo Kasei (Tokyo, Japan). 3,5-Dimethylphenyl isocyanate was prepared from 3,5-dimethylaniline and triphosgene by a conventional method. Porous spherical silica gel with a mean particle size of 7 μm and a mean pore diameter of 100 nm was kindly supplied by Daiso Chemical (Osaka, Japan). All solvents used in the preparation of CSPs were of analytical reagent grade, carefully dried and distilled before use. Solvents used in the chromatographic experiments were of HPLC grade.

2.2. Preparation of CSPs

The CSPs were prepared by the reaction of polysaccharide derivatives and 3-aminopropyl-functionalized silica gel with 4,4′-diphenylmethane diisocyanate. Polysaccharides were regioselectively bonded to silica gel on their glucose units. Fig. 1 shows the immobilization of ADMPC to macroporous silica gel at the 6-position of the glucose unit. Macroporous silica gel was treated with an excess of the silanizing reagent 3-aminopropyltriethoxysilane in dry benzene.

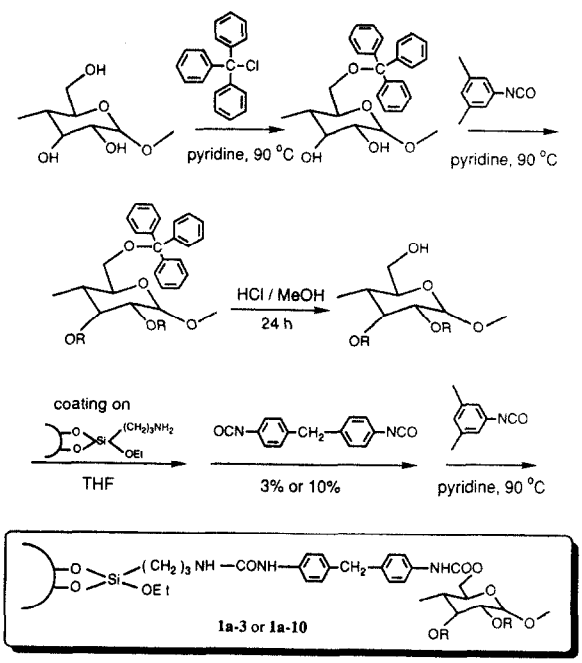


Fig. 1. Regioselective bonding of ADMPC at the 6-position.

Amylose (3.00 g) was allowed to react in pyridine (60 ml) at 80–90°C for 24 h with a large excess triphenylmethyl chloride (10.32 g), which can react only with the primary hydroxy group at the 6-position to form a trityl ether. After confirming the formation of the trityl ether by IR spectrometry, an excess of 3,5-dimethylphenyl isocyanate (9.55 g) was added to form carbamate residues with the hydroxy groups at the 2- and 3-positions. The 2,3-bis(3,5-dimethylphenylcarbamoyl)-6-O-trityl amylose obtained was suspended in a large excess of methanol containing a small amount of hydrochloric acid so as to remove the trityl group at room temperature. The amylose 2,3-bis(3,5-dimethylphenylcarbamate) (0.79 g) thus obtained was dissolved in THF, and the solution was coated on 3-aminopropylsilica gel (3.30 g) as described previously [4]. After THF had been removed *in vacuo*, the amylose derivative on silica gel was dispersed in a mixture of dry toluene (10 ml) containing 4,4′-diphenylmethane diisocyanate (3 or 10 mol-% based on the 6-position hydroxy groups of amylose) and dry pyridine (2 ml), and then the mixture was heated at 90–100°C. After 5 h, an excess of 3,5-dimethylphenyl isocyanate was added and allowed to react with the remaining hydroxy groups at the 6-position at 90–100°C for 24 h. The CSP (**1a–3** or **1a–10**) thus obtained was collected by filtration and washed with THF to remove free ADMPC derivative. The content of ADMPC derivative bonded to the silica surface was estimated by elemental analysis.

The ADMPC derivative (**1b–3** or **1b–10**) immobilized regioselectively via secondary hydroxy groups at the 2- and 3-positions of the glucose unit was also prepared according to Fig. 2 in order to investigate the influence of the positions of chemical bonding on enantioselectivity. For comparison, ADMPC non-regioselectively bonded to silica gel at the 2-, 3- and 6-positions (**1c–3**) was also synthesized directly from amylose on silica gel according to the previous report [10] for cellulose.

The CDMPC derivatives regioselectively bonded to the silica surface (**2a** and **2b**) were analogously prepared by the procedures for the CSPs of amylose derivatives described above.

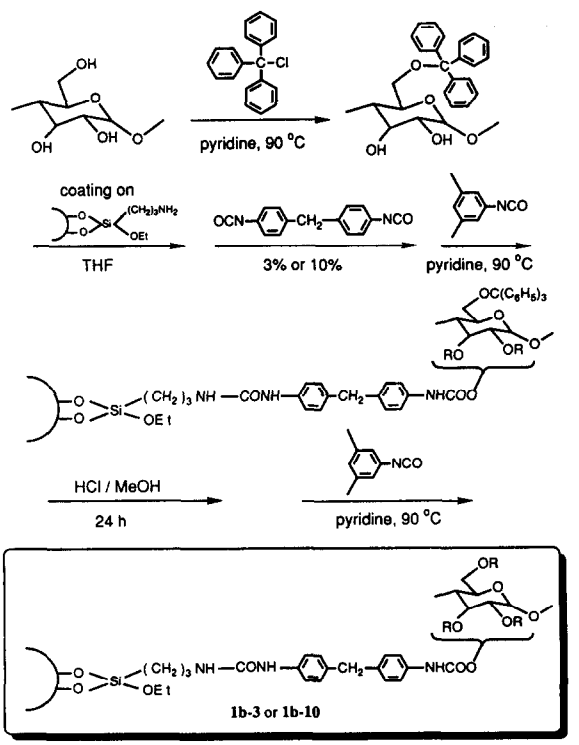


Fig. 2. Regioselective bonding of ADMPC at the 2- and 3-positions.

Regioselectivity in immobilization of amylose and cellulose derivatives is presumed to be more than 90% and 70%, respectively, judging from the results of the regioselective carbamoylation of amylose and cellulose with 3,5-dimethylphenyl and 3,5-dichlorophenyl isocyanates reported previously [12]. A regioselectivity of 90% for the amylose derivative means that, for instance, with **1a**, a chemical bond between amylose and silica gel is formed to the extent of 90% at the 6-position and 10% at the 2- or 3-position.

2.3. Apparatus and chromatography

The carbamoylated amylose and cellulose bonded to silica gel were packed into stainless-steel columns (250 × 4.6 mm I.D.) by the conventional high-pressure slurry-packing procedure [4]. Chromatographic experiments were performed on a Jasco BIP-I chromatograph equipped with a Jasco Model 875 UV detector

(254 nm), a Jasco DIP-181C polarimetric detector (mercury lamp, no filter, flow cell 50 × 3 mm I.D.) and a Jasco RC-228 recorder at room temperature. Separation was carried out with hexane–2-propanol (90:10) at a flow-rate of 0.5 ml/min. A racemate solution (1–10 μl) was injected into the chromatographic system with a Rheodyne Model 7125 injector (20-μl loop). The dead time (t_0) was determined with 1,3,5-tri-*tert*.-butylbenzene as a non-retained compound. Most columns exhibited theoretical plate numbers of 3000–5000 against benzene.

IR spectra were taken on a Jasco IR-810 spectrophotometer as KBr pellets. ^{1}H NMR spectra were measured with a Varian VXR500 spectrometer (500 MHz) using TMS as an internal standard.

3. Results and discussion

3.1. Preparation of CSPs

Fig. 3 shows the Fourier transform (FT) IR spectrum of ADMPC (**1a–3** in Fig. 1) immobilized at the 6-position on the silica surface with diisocyanate corresponding to 3 mol-% of the primary hydroxy group. Two peaks due to the carbamoyl and phenyl groups were observed at around 1720 and 1620 cm^{-1}, respectively. This suggests that ADMPC was immobilized on the silica surface. The existence of the polysaccharide derivatives on the silica surface was also confirmed for other packing materials. The contents of ADMPC and CDMPC on the packing materials were estimated to be 7–23 mass-% by elemental analyses (Table 1). In most instances, the amount of the immobilized polysaccharide derivatives was greater in the reaction with 10% than in the reaction with 3% diisocyanate. When dichlorodiphenylsilanized silica gel was employed in place of 3-aminopropylsilica gel, only a small amount of the cellulose derivative was fixed on the silica surface, probably through cross-linking between cellulose chains [10]. This suggests that the immobilization of polysaccharides can be attributed mainly to bond formation between the amino group of 3-aminopropylsilanized silica gel and the hydroxy groups of the polysaccharides by the diisocyanate. Therefore, the methods illustrated in Figs. 1 and 2 seem suitable for the regioselective immobilization of the polysaccharide derivatives.

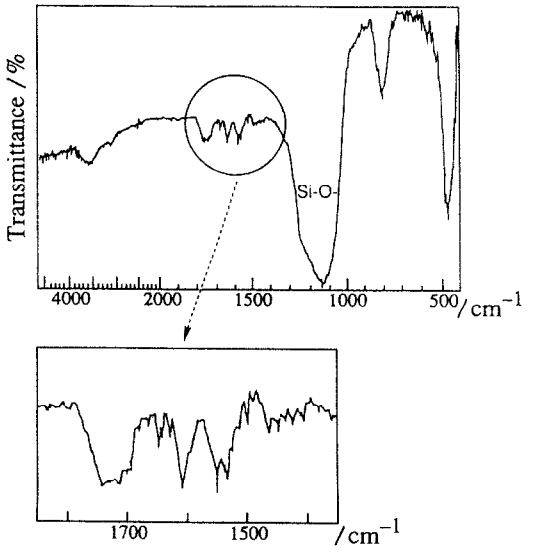


Fig. 3. FT-IR spectrum of ADMPC chemically bonded to silica gel at the 6-position (**1a–3**).

3.2. Optical resolution on amylose CSPs

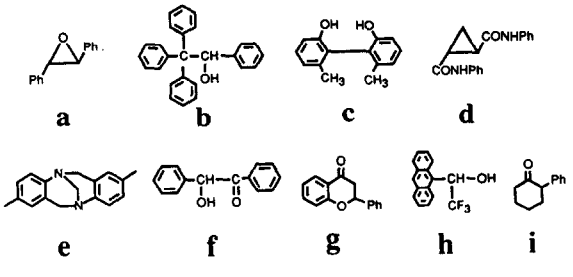
Fig. 4 shows a chromatogram of the resolution of racemic 2,2′-dihydroxy-6,6′-dimethylbiphenyl (**c**) on a column packed with ADMPC (**1a–3**) bonded at the 6-position of glucose with 3 mol-% diisocyanate. The enantiomers eluted at retention times of t_1 and t_2 showing complete separation. The capacity factors (k_1' and k_2'), which were evaluated as $(t_1 - t_0)/t_0$ and $(t_2 - t_0)/t_0$, were 0.67 and 1.40, respectively. The separation factor $[\alpha = k_2'/k_1' = (t_2 - t_0)/(t_1 - t_0)]$ and the resolution factor $[R_s = 2(t_2 - t_1)/(W_1 + W_2)]$ were determined to be 2.10 and 4.42, respectively.

In Table 2 are summarized the results for the resolution of racemic compounds **a–i** on the five ADMPC phases which were fixed with different amounts of diisocyanate at different positions on the glucose unit. The results with the coated ADMPC phase, which was prepared by physical

Table 1
Preparation of CSPs

No.	Polysaccharide	Positions	Diisocyanate[a] (mol-%)	Elemental analyses			Amount of polysaccharide[b] (mass-%)
				C (%)	H (%)	N (%)	
1a–3	ADMPC	6	3.0	5.33	0.53	0.64	7.7
1a–10		6	10.0	7.17	0.71	0.84	11.0
1b–3		2,3	3.0	5.89	0.62	0.68	8.8
1b–10		2,3	10.0	12.22	1.10	1.30	21.5
1c–3		2,3,6	3.0	12.87	1.27	1.39	23.0
2a–3	CDMPC	6	3.0	7.70	0.77	0.79	12.2
2a–10		6	10.0	10.51	0.71	1.03	17.7
2b–3		2,3	3.0	6.94	0.33	0.89	10.6
2b–10		2,3	10.0	12.15	0.70	1.35	21.3
2c–3		2,3,6	3.0	11.71	0.68	1.05	20.3
2c–10		2,3,6	10.0	11.92	0.45	1.27	20.8
Silica gel[c]	–	–	–	0.63	0.15	0.07	–

[a] Diisocyanate used for the preparation of CSPs based on hydroxy groups of polysaccharide.
[b] The amount of polysaccharide derivatives on silica gel calculated from C (%) of CSPs.
[c] 3-Aminopropylsilica gel.



adsorption of ADMPC on silica gel, are also shown for comparison [7]. The results indicate that the chiral discrimination ability depends on both the position of the glucose unit immobilized and the amount of diisocyanate used. The chiral recognition ability tended to decrease as the degree of chemical bonding between the amylose derivatives and the silica surface increased, as reported previously [10]. However, it should be noted that the ADMPC phases (**1a**) immobilized at the 6-position show a higher resolving power than the other CSPs (**1b** and **1c**) immobilized irregularly at the 2- and 3-positions or at the 2-, 3- and 6-positions. In particular, **1a–3**, showed a high chiral recognition comparable to that of the coated-type phase.

The most important adsorbing site of

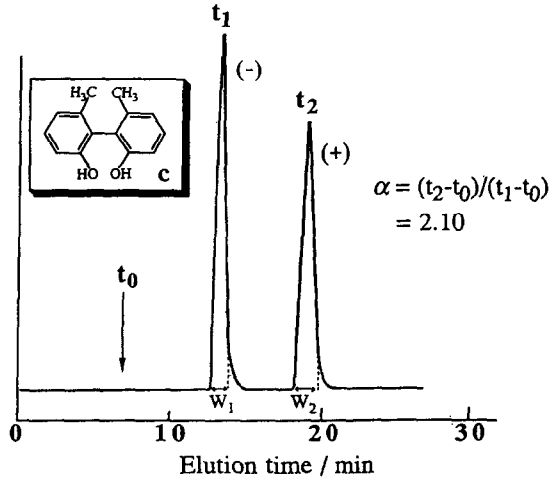


Fig. 4. Optical resolution of 2,2′-dihydroxy-6,6′-dimethylbiphenyl on **1a–3**. Eluent, hexane–2-propanol (90:10); flow-rate, 0.5 ml/min.

phenylcarbamate derivatives of polysaccharide for chiral recognition has been considered to be the carbamate residues [4]. The racemates probably interact with the carbamate residues through hydrogen bonding, as shown in Fig. 5. However, the enantioselectivities of these CSPs depended greatly on the kind of racemate. For example, in the resolution of **d** and **e**, these

Table 2
Optical resolution of racemates (**a–i**) on ADMPC-fixed phases (**1a-3–1c-3**)

Racemate	**1a-3**			**1a-10**			**1b-3**			**1b-10**			**1c-3**			Coated-type[a]		
	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
a	0.14 (+)	2.53	2.27	0.20 (+)	2.44	2.68	0.14 (+)	2.08	2.00	0.42 (+)	1.55	1.88	0.44 (+)	1.99	4.22	0.42 (+)	3.04	6.67
b	0.70 (+)	1.94	4.12	1.05 (+)	1.85	3.71	0.60 (+)	1.75	2.93	1.52 (+)	1.58	3.14	1.79 (+)	1.75	5.52	2.65 (+)	1.98	5.48
c	0.67 (−)	2.10	4.42	0.97 (−)	1.98	3.60	0.75 (−)	1.63	2.29	1.96 (−)	1.48	2.40	1.88 (−)	1.89	4.47	2.46 (−)	2.11	6.38
d	0.84 (+)	1.75	1.82	1.35 (+)	1.68	1.64	0.87	1.0		2.45	1.0		3.59 (+)	1.60	1.31	3.25 (+)	2.01	3.59
e	0.20 (+)	1.37	0.62	0.31 (+)	1.38	0.84	0.69 (+)	*ca.* 1		10.24	1.0		0.99 (+)	*ca.* 1		0.53 (+)	1.58	2.30
f	1.04 (−)	1.09	0.61	1.45 (−)	1.05	0.32	1.14 (+)	1.09		3.21 (+)	1.14	1.47	3.62	1.0		3.14 (−)	1.21	2.07
g	0.34 (+)	*ca.* 1		0.49 (+)	1.10	0.61	0.46 (+)	*ca.* 1		1.30 (+)	1.11		1.30 (+)	1.16	1.19	0.93 (+)	1.12	0.77
h	0.45	1.0		0.58	1.0		0.49	*ca.* 1		1.27	1.13		1.29	1.07		1.30 (+)	1.15	0.75
i	0.65	1.0		0.80	1.0		0.30 (−)	*ca.* 1		0.85 (−)	*ca.* 1		2.04	1.0		0.61 (−)	*ca.* 1	

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

Table 3
Optical resolution of racemates (**a–i**) on CDMPC-fixed phases (**2a-3–2c-10**)

Racemate	**2a-3**			**2a-10**			**2b-3**			**2b-10**			**2c-3**			**2c-10**		Coated-type[a]	
	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	k_1'	α
a	0.32 (−)	1.30	1.64	0.46 (−)	1.24	1.10	0.23 (−)	1.46	1.67	0.50 (−)	*ca.* 1		0.32 (+)	*ca.* 1	2.20	0.06 (+)	*ca.* 1	0.74 (−)	1.68
b	0.79 (−)	1.11		1.12 (−)	1.25	1.32	0.52 (+)	1.11		1.16 (+)	*ca.* 1		0.64 (+)	1.14		0.16	1.0	1.37 (+)	1.34
c	0.64 (−)	3.47	5.55	0.93 (−)	2.74	4.48	0.62 (−)	2.76	4.34	1.10 (−)	4.54	3.14	0.70 (−)	2.63		0.17 (−)	*ca.* 1	2.36 (−)	1.83
d	0.50 (+)	1.40	2.06	0.74 (+)	1.34	1.47	0.46 (+)	1.46	1.60	0.96	1.0		0.79	1.0	1.09	0.19	1.0	0.83 (+)	3.17
e	0.45 (+)	1.40	2.30	0.52 (+)	1.47	2.08	0.41 (+)	1.32		1.03 (+)	1.49		1.27	1.0		0.60	1.0	0.97 (+)	1.32
f	1.17 (+)	1.31	3.56	1.58 (+)	1.28	2.97	0.93 (+)	1.29	1.62	2.09 (+)	1.19		1.51 (+)	1.16	2.65	0.44	1.0	2.43 (+)	1.58
g	0.70 (+)	1.19	2.20	1.00 (−)	1.17	1.48	0.51 (−)	1.21	1.37	1.20 (−)	*ca.* 1		0.82 (−)	*ca.* 1	0.84	0.20	1.0	1.47 (−)	1.41
h	0.77 (−)	2.33	10.8	1.10 (−)	2.32	8.93	0.68 (−)	2.12	3.16	1.25 (−)	1.87	1.80	0.92 (−)	1.63		0.30	1.0	2.13 (−)	2.59
i	0.55 (−)	1.22	1.33	0.74 (−)	1.22	1.25	0.41 (−)	1.19		0.85 (−)	1.28	0.91	0.60 (−)	1.22		0.16 (−)	*ca.* 1	1.17 (−)	1.15

Eluent, hexane–2-propanol (90:10, v/v); flow-rate, 0.5 ml/min. The signs in parentheses represent the optical rotation of the first-eluted enantiomer.
[a] Data taken from ref. 4.

racemates were sufficiently separated on 6-position-fixed phases (**1a–3** and **1a–10**), but almost no chiral discrimination was attained on the 2- and 3-positions-fixed phases, **1b–3** and **1b–10**. This indicates that the enantiomers of **d** and **e** may be discriminated mainly on the carbamate groups at the 2- and 3-positions. This speculation was also supported by the results of the enantioseparation of a variety of racemates using regioselective carbamoylated amylose with 3,5-dimethylphenyl and 3,5-dichlorophenyl isocyanates [12]. Among the carbamate groups, those at the 2- and 3-positions seem to be responsible for efficient chiral recognition in the amylose derivatives; that is, the regular arrangement of carbamate groups at the 2- and 3-positions may be important for a high enantioseparation [12]. Moreover, the formation of a covalent bond through the primary hydroxy groups at the 6-position may be suitable for keeping the sterically preferred conformation of the main chain of amylose.

In the resolution of **f**, the (−)-isomer eluted first on **1a–3** and **1a–10**, whereas the (+)-isomer eluted first on **1b–3** and **1b–10**. This indicates that for **f** the mechanism of chiral recognition is different between **1a** and **1b**. The enantiomers of **f** may be discriminated mainly on the carbamate residues at the 2- or 3-position of **1a** and at the 6-position of **1b**. The former carbamate residues probably interact more strongly with the (+)-isomer of **f** and the later carbamate with the (−)-isomer.

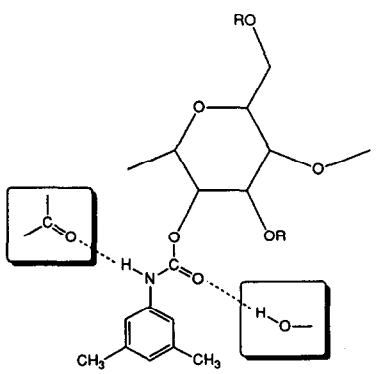


Fig. 5. Schematic interaction of the racemic compounds with the phenylcarbamoyl residue.

3.3. Optical resolution on cellulose CSPs

The results of the optical resolution of **a–i** on the six cellulose derivatives are listed in Table 3. The regioselectively fixed CSPs (**2a** and **2b**) showed obviously a higher enantioselectivity than the non-regioselectively fixed CSPs (**2c**), analogously to the case of the CSPs based on ADMPC. The lower degree of chemical bonding between cellulose and silica gel seems to be better. Although the 6-position-fixed phase had a higher optical resolving power with ADMPC-fixed CSPs, in the cellulose derivatives a significant difference in chiral recognition between the 6-position-bonded phase (**2a–3**) and the 2- and 3-positions-bonded phase (**2b–3**) was not observed. These results suggest that the mechanism of enantioseparation may be similar between on **2a–3** and **2b–3**; racemates are likely to interact simultaneously with multiple 3,5-dimethylphenylcarbamate residues at the 2- and 3-positions and 6-position of neighbouring glucose units [12]. In the case of the amylose derivatives, the carbamate groups at the 2- and 3-positions are often more important for the optical resolution than that at the 6-position, as mentioned above. The difference observed between the cellulose and amylose derivatives can be explained as follows.

The possible structures of the phenylcarbamates of cellulose [13] and amylose [14] have been reported on the basis of X-ray analysis by Vogt and Zugenmaier. The cellulose derivative possesses the conformation of a left-handed threefold (3/2) helix. Racemates can simultaneously interact with all the carbamate residues in a complicated manner, because the residues at the 2- and 3-positions of a glucose unit and the 6-position of neighbouring glucose units are located close to each other. In the cellulose derivatives, the carbamate residue at the 6-position may be as important for chiral recognition as those at the 2- and 3-positions, and therefore the CDMPC-bonded phases show little influence on chiral recognition with respect to the posi-

tions of chemical bonding. On the other hand, the conformation of the amylose derivative is proposed to be a left-handed fourfold (4/1) helix [14]. The racemates cannot interact simultaneously with the phenylcarbamate residues at the 2- and 3-positions of a glucose unit and the 6-position of the neighbouring glucose units, since these are remote from each other. In the amylose derivatives, the carbamate residues at the 2- and 3-positions seem to play a more important role than that isolated at the 6-position. Hence the 6-position-bonded ADMPC phases may show a high optical resolution ability without decreasing the chiral recognition ability of the carbamate residues at the 2- and 3-positions.

3.4. Separation of racemates under different chromatographic conditions

As mentioned above, the chemically bonded-type CSPs can be used with solvents such as $CHCl_3$ and THF which swell or dissolve ADMPC. Enantioseparation of some racemates using these eluents was studied preliminarily on column **1a–3** (Fig. 6). 2-Phenylcyclohexanone (**i**), which could not be separated on either the **1a–3** or the ADMPC-coated-type column under normal-phase conditions [hexane–2-propanol (90:10)], was almost completely separated into enantiomers when a small amount of $CHCl_3$ (5%) was added to the mobile phase. Similarly, flavanone (**g**) was completely resolved on the **1a–3** column using hexane–$CHCl_3$–2-propanol as the eluent. The enantioselectivity ($\alpha = 1.60$) of **g** on **1a–3** [eluent hexane–$CHCl_3$–2-propanol (95:5:1)] was much greater than that on the ADMPC-coated phase ($\alpha = 1.12$) under normal-phase conditions. A similar result was obtained in the separation of **h** on **1a–3** by use of the eluent hexane–$CHCl_3$–2-propanol (95:5:1) ($\alpha = 1.34$); the separation factor of **h** on the coated-type phase [eluent hexane–2-propanol (90:10)] was 1.15. The presence of a much smaller amount of 2-propanol may improve the chiral discrimination ability of the ADMPC phase, on which the hydrogen bonding between the CSP and racemates may play an important role. This

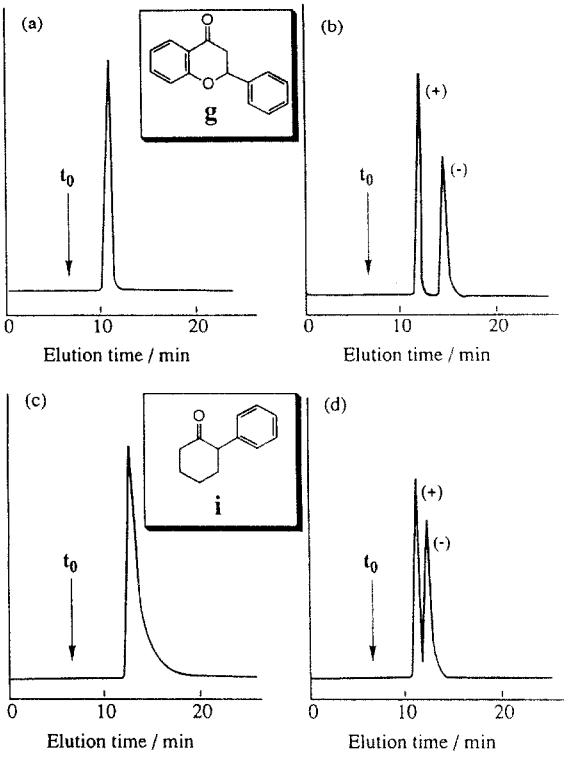


Fig. 6. Effect of eluent on optical resolution of (a and b) **g** and (c and d) **i**. Eluent: (a and c) hexane–2-propanol (90:10); (b) hexane–$CHCl_3$–2-propanol (95:5:1); (d) hexane–$CHCl_3$ (95:5).

may also be ascribed to some conformational change of ADMPC because ADMPC is swollen in $CHCl_3$. The selection of the composition of the eluent seems very important for achieving efficient optical resolution. Clearly, more studies must be done to investigate the influence of solvents on the chiral recognition and elucidate the separation mechanism.

4. Conclusions

ADMPC and CDMPC could be regioselectively immobilized on 3-aminopropylsilica gel with diisocyanate. The chiral recognition abilities of the amylose and cellulose derivatives bonded non-regioselectively at the 2-, 3- and 6-positions were also evaluated. In the amylose derivatives, the optical resolving ability of the 6-position-

bonded phase with a smaller extent of chemical bonding was higher than that of the 2- and 3-positions-bonded phase, but it was slightly lower than that of ADMPC-coated phase. On the other hand, for the cellulose derivatives, a clear dependence of enantioseparation on the position of the glucose unit for immobilization could not be observed. With both derivatives, regioselectively bonded phases had a superior chiral discrimination to non-regioselectively bonded phases. The optical resolving power of these CSPs was also influenced by the degree of immobilization by diisocyanate in the preparation of CSPs. Since these chemically bonded phases were not damaged by polar solvents such as $CHCl_3$, a more efficient separation of racemates was attained by the use of an eluent containing a small amount of $CHCl_3$, which could not be applied with the coated CSPs.

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