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High-performance liquid chromatographic enantioseparation using chitin carbamate derivatives as chiral stationary phases

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Abstract

Chitin carbamate derivatives including 4-substituted and 3,5-disubstituted phenylcarbamates, 1-phenylethylcarbamates, and cycloalkylcarbamates were synthesized and coated on macroporous silica gel to evaluate their chiral recognition abilities as chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC). Among the derivatives, the 3,5-dimethylphenyl, 4-chlorophenyl, and 4-trifluoromethylphenylcarbamates showed relatively high-chiral recognition abilities when a hexane–2-propanol mixture was used as the eluent. The CSPs based on the chitin 3,5-dimethylphenyl and 3,5-dichlorophenylcarbamates could be stably used in the presence of chloroform and ethyl acetate as a component of the eluents, and a few racemates were more sufficiently resolved by the addition of a small amount of chloroform in the mobile phase. Some racemates were more efficiently resolved under the reversed phase condition.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Chitin carbamate; Phenyl carbamates; 1-Phenylethylcarbamates; Cycloalkylcarbamates

1. Introduction

Biologically active chiral compounds often exhibit quite different physiological behaviors between enantiomers. Therefore, the pharmacokinetic and physiological activities of both enantiomers must be investigated before use. Chromatographic enantioseparation by high-performance liquid chromatography (HPLC) using a chiral stationary phase (CSP) has advanced in the past two decades and has become a practically useful method not only for determining their optical purity but also for obtaining optical isomers. The development of effective CSPs is the key for this method, and many CSPs consisting of a small optically active molecule and an optically active polymer have been prepared [1–3]. In the latter polymer-based CSPs, most polymers with high-chiral recognition possess a regular higher-order structure, which seems to be the important factor for efficient chiral recognition. Polysaccharides such as cellulose and amylose are the most readily available stereoregular polymers. These polysaccharides can be converted into various derivatives on hydroxy groups. So far, a large number of phenylcarbamate derivatives of cellulose and amylose have been prepared [4–6] and it was found that the introduction of alkyl or halogen groups at the *m*- or *p*-position on the phenyl groups significantly influence the chiral recognition abilities [6]. Among the many derivatives, the 3,5-dimethylphenylcarbamates of cellulose and amylose exhibit high-chiral recognition and have been widely used to resolve a broad range of racemates [6–9]. The chiral recognition by arylalkylcarbamates has also been evaluated and 1-phenylethylcarbamates were found to show high-chiral recognition abilities depending on the chirality of the arylalkyl group [10,11]. More recently, cycloalkylcarbamates, such as cyclohexyl and norbornylcarbamates, of cellulose and amylose were found to be useful CSPs for thin-layer chromatography (TLC) as well as for HPLC [12,13].

Phenylcarbamates of other polysaccharides including xylan, dextran, chitosan, curdlan, galactosamine, and inulin were also prepared, and their chiral recognition abilities as CSPs for HPLC were quite dependent on the nature of the monosaccharide units and linkage position and type. Some of their 3,5-dimethylphenyl and 3,5-dichlorophenylcarbamates showed a relatively high-chiral recognition [14,15].

Chitin is also one of the most abundant natural optically active polymers consisting of *N*-acetyl-D-glucosamine units

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linked by β-(1 → 4), and its derivatives had not been used as CSPs for HPLC probably due to the difficulty of the derivatization owing to its low solubility in solvents. In 1996, Cass et al. [16] prepared chitin phenylcarbamates and evaluated their abilities as CSPs for HPLC. However, these derivatives showed a low-chiral recognition, which may be attributed to the low substitution of the hydroxy group of chitin to the carbamate group based on the elemental analysis data.

In our previous study [17], we prepared three chitin phenylcarbamates, 3,6-bis(phenylcarbamate), 3,6-bis(3,5-dimethylphenylcarbamate), and 3,6-bis(3,5-dichlorophenylcarbamate), and their chiral recognition abilities were evaluated. Among them, the 3,5-dimethylphenyl and 3,5-dichlorophenylcarbamates exhibited relatively high-chiral recognition abilities, especially for some chiral drugs such as ibuprofen and ketoprofen.

In this study, various chitin phenylcarbamates having alkyl and halogen groups at the *m*- or *p*-positions of the phenyl moiety, arylalkylcarbamates, and cycloalkylcarbamates were prepared and their chiral resolution abilities were evaluated as CSPs for HPLC. Because of the poor solubility of the chitin derivatives, the eluents containing chloroform and ethyl acetate, which cannot be used as eluents for other polysaccharide-type CSPs, could be examined as eluents. Chiral separation on the chitin phenylcarbamates under reversed phase conditions was also investigated.

2. Experimental

2.1. Chemicals and reagents

Chitin from shrimp shells was purchased from Sigma. Phenyl isocyanate and lithium chloride were obtained from Wako. The 3,5-dimethylphenyl and 4-tert-butylphenyl isocyanates were kindly supplied by Daicel. The 3,5-dichlorophenyl, 4-chlorophenyl, 4-bromophenyl, and (*S*)- and (*R*)-methylbenzyl isocyanates, 4-bromo-2-methylaniline, and (3-aminopropyl)triethoxysilane were from Tokyo Kasei. The 4-methylphenyl, 4-isopropylphenyl, 4-fluorophenyl, 4-iodophenyl, 3,5-bis(trifluoromethyl)phenyl, and cyclopentyl isocyanates, and (±)-exo-2-aminonorbornane were purchased from Aldrich. The 4-trifluoromethylphenyl isocyanate was obtained from AZmax. Cyclohexyl isocyanate was from Kishida. The porous spherical silica gel (Daiso gel SP-1000) with a mean particle size of 7 μm and a mean pore diameter of 100 nm was kindly supplied from Daiso Chemical. Pyridine and *N*,*N*-dimethylacetamide were from Kanto Chemical as anhydrous reagents. The racemates were commercially available or were prepared by the usual method [18].

2.2. Synthesis of chitin phenylcarbamates

The 3-bromo-5-methylphenyl [9] and (±)-exo-2-norbornyl isocyanates [13] were prepared from 4-bromo-2-methylaniline and (±)-exo-2-aminonorbornane, respectively. Chitin carbamates (Fig. 1) were prepared by the reaction of chitin (1.0 g) with the corresponding isocyanates. Chitin was dissolved in an *N*,*N*-dimethylacetamide (15 ml)–LiCl (1.5 g) mixture at 80 °C for 24 h, and then an excess of an isocyanate (1.3 eq) and pyridine (5 ml) were added to the chitin solution. The reaction was continued for 24 h at 80 °C. The resulting chitin phenylcarbamate derivative was isolated as the methanol-insoluble fraction. The ^{1}H NMR data and elemental analysis (Table 1) of the derivatives indicated that the hydroxy groups of chitin were almost quantitatively converted into the carbamate moieties.

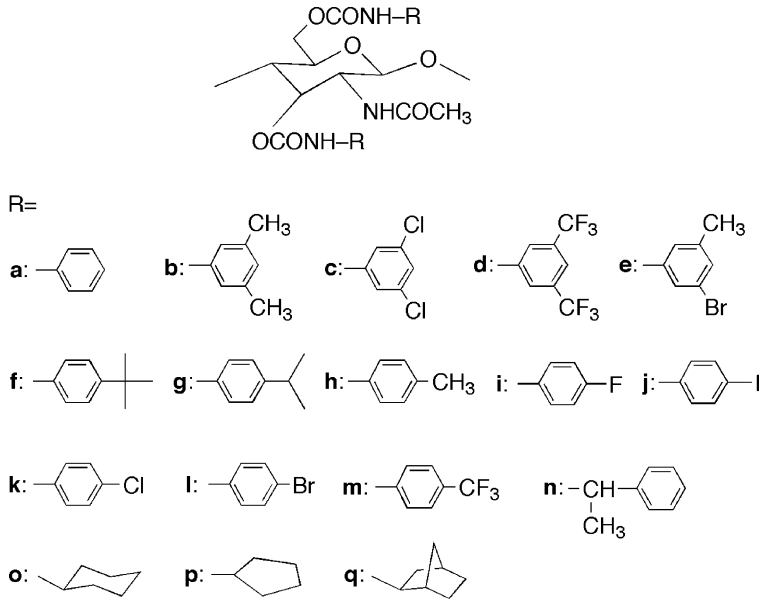

Fig. 1. Structures of chitin carbamates.

Table 1
Elemental analysis of chitin carbamates

CSPs	Calculated (%)			Found (%)		
	C	H	N	C	H	N
1a	62.8	6.3	8.5	62.8	6.5	8.6
1b	59.9	5.3	9.5	59.9	5.5	9.5
1c	45.6	3.3	7.3	45.6	3.4	7.3
1d	43.8	2.7	5.9	43.8	2.7	5.9
1e	46.0	6.7	4.0	46.0	6.8	4.3
1f	65.1	7.1	7.6	64.9	7.4	7.7
1g	64.0	6.7	8.0	63.0	6.9	8.2
1h	61.4	5.8	9.0	61.5	6.0	9.0
1i	55.4	4.4	8.8	53.9	4.8	8.5
1j	38.1	3.1	6.1	38.0	3.3	6.0
1k	51.8	4.2	8.2	51.1	4.3	8.1
1l	44.1	3.5	7.0	44.1	3.6	6.9
1m	49.9	3.7	7.3	49.9	3.8	7.4
1n-(*S*)	62.3	6.3	8.5	62.0	6.6	8.6
1n-(*R*)	62.3	6.3	8.5	59.4	6.7	8.2
1o	58.3	7.8	9.3	58.3	7.8	9.1
1p	56.5	7.3	9.9	56.3	7.6	10.1
1q	60.4	7.4	8.8	52.4	7.2	8.1
Chitin	47.3	6.5	6.9	47.3	6.7	7.0

Estimated based on a repeated *N*-acetylglucosamine unit.

2.3. Preparation of stationary phase

The packing materials were prepared by coating the carbamate derivatives on silanized macroporous silica gel as previously described [6]. As a coating solvent, tetrahydrofuran (THF) was used for only 3,5-dichlorophenylcarbamate and 3,5-bis(trifluoromethylphenylcarbamate) because of the low solubility of the chitin phenylcarbamates. Other chitin derivatives were dissolved in DMSO–THF or pyridine–THF (2:1 to 8:1, v/v). The packing materials were packed into a stainless-steel tube (25 cm × 0.46 cm i.d.) by a conventional high-pressure slurry packing technique using a model CCP-085 Econo packer pump (Chemco). The plate numbers of the columns were 4000–8000 for benzene with hexane–2-propanol (90:10) as the eluent at a flow rate of 0.5 ml/min. 1,3,5-Tri-*tert*-butylbenzene and acetone were used as the non-retained compound for estimating the dead time (t_0) under normal and reversed phase conditions, respectively.

2.4. Apparatus

The chromatographic experiments were performed on a Jasco PU 980 Intelligent HPLC pump equipped with UV (JASCO 970-UV) and polarimetric (JASCO OR-990) detectors at room temperature. A solution (1–10 μl) of a racemate was injected into the chromatographic system with a Rheodyne Model 7125 injector. The ^{1}H NMR spectra were taken in pyridine-d_5 or DMSO-d_6 at 80 °C using a Varian Gemini-2000 NMR spectrometer (400 MHz).

3. Results and discussion

In order to obtain the carbamate derivatives with a high enantioselectivity, it may be important to completely synthesize the disubstituted derivatives. The polysaccharide carbamate derivatives having a low degree of substitution

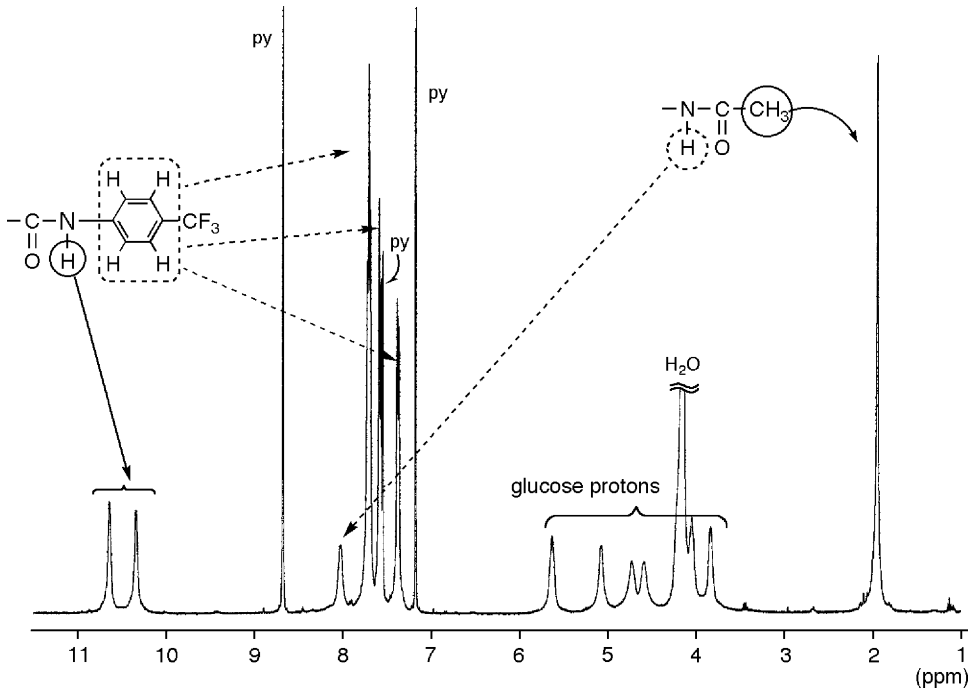

Fig. 2. ^{1}H NMR spectrum of chitin bis(4-trifluoromethylphenylcarbamate) (**1m**) (pyridine-d_5, 80 °C, 400 MHz).

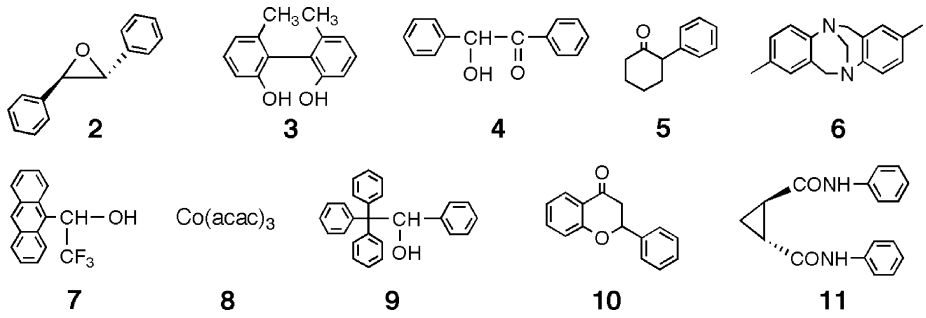

Fig. 3. Structures of racemates.

of the hydroxy groups with carbamate residues probably show poor chiral resolution abilities due to their irregular structure. However, the complete derivatization of chitin would be difficult due to its poor solubility. For the complete derivatization of chitin to carbamate derivatives, chitin was dissolved in *N*,*N*-dimethylacetamide–lithium chloride and then allowed to react with the excess isocyanates in the presence of pyridine. The results of the elemental analysis are summarized in Table 1 which indicates that most carbamates, except for **1i**, **1n**-(*R*), and **1q**, are completely derivatized. Fig. 2 shows the ^{1}H NMR spectrum of chitin bis(4-trifluoromethylphenylcarbamate) (**1m**) as an example for a quantitatively derivatized chitin bisphenylcarbamates.

The HPLC resolution on the CSPs **1a**–**p** was examined for ten racemates, *trans*-stilbene oxide (**2**), 2,2′-dihydroxy-6,6′-dimethylbiphenyl (**3**), benzoin (**4**), 2-phenylcyclohexanone (**5**), Tröger base (**6**), 1-(9-anthryl)-2,2,2-trifluoroethanol (**7**), cobalt(III) tris(acetylacetonate) (**8**), 1,2,2,2-tetraphenylethanol (**9**), flavanone (**10**), and *trans*-cyclopropanedicarboxylic acid dianilide (**11**) (Fig. 3). Fig. 4 shows a chromatogram of the resolution of **10** on the column packed with chitin bis(4-chlorophenylcarbamate) (**1k**). The enantiomers eluted at the retention times of t_1 (=20.3 min) and t_2 (=27.9 min) showing complete separation. The capacity factors, $k_1'(=(t_1 - t_0)/t_0)$ and $k_2'(=(t_2 - t_0)/t_0)$, were 2.10 and 3.26, respectively, and the separation factor $\alpha(=k_2'/k_1')$ was found to be 1.55.

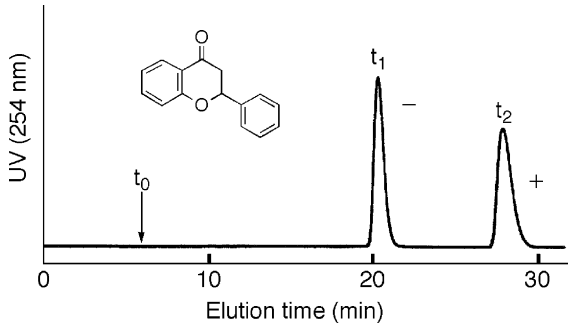

Fig. 4. Resolution of flavanone (**10**) on chitin bis(4-chlorophenylcarbamate) (**1k**).

3.1. 3,5-Disubstituted phenylcarbamates of chitin

In a previous study, the chitin phenyl (**1a**), 3,5-dimethylphenyl (**1b**), and 3,5-dichlorophenylcarbamates (**1c**) were prepared and the high-chiral recognition by **1b** and **1c** has been observed [17]. In the present study, the additional two derivatives, 3,5-di(trifluoromethyl)phenylcarbamate (**1d**) and 3-bromo-5-methylphenylcarbamate (**1e**), were synthesized and their chiral recognition abilities were compared with those of **1a**–**c**. Table 2 shows the capacity factors for the first-eluted enantiomer (k_1') and separation factors (α) for 10 racemates **2–11** on the 3,5-disubstituted phenylcarbamates of chitin. The chiral recognition abilities are significantly influenced by the substituents on the phenyl groups, and the introduction of the substituents onto a phenyl group improves the chiral recognition. The racemates except for **2** can be separated on at least one of the 3,5-disubstituted phenylcarbamates. Among the 3,5-disubstituted phenylcarbamates, **1b** exhibited the highest chiral recognition ability. **1c**, **1d**, and **1e** also showed a relatively high-chiral recognition for some racemates. **1b** and **1c**, which has the substituents with the opposite electronic properties, i.e., electron-donating and electron-withdrawing, are complementary in chirality recognition to some extent. Enantiomers not resolved on **1b** were resolved on **1c**, and vice versa. **1d** also has two electron-withdrawing trifluoromethyl groups and exhibits a similarity to **1c** in chiral recognition ability. The chiral recognition ability of **1e** bearing both an electron-donating methyl group and an electron-withdrawing bromine is not clearly related to those of **1b** and **1c**.

3.2. 4-Substituted phenylcarbamates of chitin

Eight 4-substituted phenylcarbamates of chitin (**1f**–**m**) were prepared and their chiral recognition abilities as CSPs for HPLC were compared for the ten racemates (**2**–**11**) as shown in Table 3, where the substituents are arranged in the decreasing order of electron-donating power from left to right. Only chitin bis(4-bromophenylcarbamate) (**1l**) was not dissolved in any solvents, and therefore, its chiral separation ability was not evaluated as a coated-type CSP.

Table 2
Resolution of racemates on chitin 3,5-disubstituted phenylcarbamate

Racemate	**1a**		**1b** ($(CH_3)_2$)		**1c** (Cl_2)		**1d** ($(CF_3)_2$)		**1e** ((Br, CH_3))	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
2	0.30 (−)	∼1	0.21(+)	∼1	0.20 (−)	∼1	0.15 (−)	∼1	0.24 (+)	∼1
3	2.29 (−)	1.24	1.41 (−)	1.30	1.30(−)	1.17	0.63 (−)	1.25	2.01 (−)	1.28
4	3.31 (+)	1.04	1.93(−)	1.10	2.34(−)	1.33	1.69(+)	1.12	1.86 (−)	1.19
5	1.19 (−)	∼1	0.73(+)	∼1	1.28(+)	1.39	1.10(+)	1.07	1.01(−)	1.12
6	0.32 (+)	∼1	0.29(+)	1.14	0.34(−)	∼1	0.14(−)	∼1	0.33(−)	∼1
7	1.01(−)	1.17	0.97 (−)	1.25	0.29	∼1	0.15 (−)	1.26	0.62(−)	1.15
8	1.05 (+)	1.18	0.82 (+)	1.06	0.49 (−)	1.12	0.28 (+)	∼1	0.41 (+)	1.13
9	1.02 (+)	∼1	0.59 (+)	1.17	0.23 (+)	∼1	0.10 (−)	∼1	0.47 (+)	1.22
10	1.27 **(−)**	1.20	0.96(−)	1.54	2.71 (−)	1.34	0.56(−)	1.13	1.00 (−)	1.39
11	1.57 (−)	∼1	0.70(−)	1.14	0.34(+)	∼1	0.32(+)	1.34	0.78 (+)	∼1

Flow rate: 0.5 ml/min. The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Eluent: hexane–2-propanol (90:10, v/v).

Among these 4-substituted phenylcarbamate derivatives, the 4-methylphenyl (**1h**), 4-chlorophenyl (**1k**), and 4-trifluoromethylphenylcarbamates (**1m**) exhibited relatively high-chiral recognition abilities. Regarding to the 4-alkylphenylcarbamates, 4-methylphenylcarbamate (**1h**) exhibited the highest chiral recognition ability. Bulkier alkyl groups, *tert*-butyl (**1f**) and isopropyl groups (**1g**), reduced the k'_1 values and the chiral recognition ability for most racemates.

The most important adsorbing site for the chiral recognition on the phenylcarbamate derivatives of cellulose and amylose under normal phase conditions has been considered to be the polar carbamate residue, which can interact with enantiomers mainly through intermolecular hydrogen bonding on the NH or C=O groups [6,19,20]. The adsorbing powers of these sites may be strongly influenced by the nature of the substituents on the phenyl group [6] For the chitin phenylcarbamates, the most important adsorbing sites are considered to be the polar carbamate and acetamide residues, and the polarities of these sites must be influenced by the substituents on the phenyl group. For example, racemates **5**, **8**, and **10**, which have carbonyl and ether oxygens, tend to interact more strongly with the CSPs **1i–m** having electron-withdrawing substituents than the CSPs **1f–h** having electron-donating substituents. This is because the acidity of the NH proton of carbamate group increases along with an increase in the electron-withdrawing power of the substituents on the phenyl group. On the other hand, alcohols

Table 3
Resolution of racemates on chitin 4-substituted phenylcarbamate

Racemate	**1f** (*t*-Bu)		**1g** (*i*-Pr)		**1h** (CH_3)		**1a** (H)	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α
2	0.18 (+)	∼1	0.20 (+)	∼1	0.28 (+)	∼1	0.30 (−)	∼1
3	1.75 (−)	1.21	2.06 (−)	1.11	2.66 (−)	1.35	2.29 (−)	1.24
4	1.22 (−)	∼1	1.10 (−)	1.05	2.21 (+)	1.10	3.31 (+)	1.04
5	0.62 (−)	1.06	0.52 (−)	1.10	0.79 (−)	∼1	1.19 (−)	∼1
6	0.21	1.00	0.21 (+)	∼1	0.34 (−)	∼1	0.32 (+)	∼1
7	0.81	∼1	0.79	∼1	1.34 (−)	1.24	1.01 (−)	1.17
8	0.22 (+)	∼1	0.23 (+)	∼1	0.46 (+)	1.33	1.05 (+)	1.18
9	0.44 (−)	∼1	0.40 (+)	∼1	0.77 (+)	1.13	1.02 (+)	∼1
10	1.20 (−)	1.73	0.85 (−)	1.68	0.89 (−)	1.35	1.27 (−)	1.20
11	0.42 (−)	1.38	0.39 (−)	1.36	1.03 (+)	∼1	1.57 (−)	−1
	1i (F)		**1j** (I)		**1k** (Cl)		**1m** (CF_3)	
2	0.24 (−)	∼1	0.18 (+)	∼1	0.27 (−)	1.15	0.18	1.00
3	1.43 (−)	1.29	1.39 (−)	1.13	2.24 (−)	1.24	1.58 (−)	1.20
4	2.43 (+)	1.02	2.23 (+)	∼1	2.65 (+)	1.09	1.90 (+)	1.19
5	1.04 (−)	∼1	1.00 (+)	∼1	1.47 (+)	1.03	1.22 (+)	1.07
6	0.32 (−)	∼1	0.41 (−)	∼1	0.35 (−)	1.14	0.22 (−)	1.23
7	0.72 (−)	1.08	0.41	∼1	0.47	∼1	0.35	∼1
8	0.72 (+)	1.24	0.78 (+)	∼1	0.83 (+)	1.22	0.54 (+)	1.13
9	0.45 (+)	1.18	0.43 (+)	∼1	0.47 (+)	1.28	0.18 (+)	1.22
10	1.22 (−)	1.39	1.35 (−)	1.53	2.10 (−)	1.55	2.26 (−)	1.62
11	1.13 (+)	∼1	0.64 (+)	∼1	0.71 (+)	1.13	0.39 (+)	1.44

Flow rate: 0.5 ml/min. The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Eluent: hexane–2-propanol (90:10, v/v).

Table 4
Resolution of racemates on chitin 1-phenylethylcarbamates and cycloalkylcarbamates

Racemate	**1n**-(*S*)		**1n**-(*R*)		**1o**		**1p**	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α
2	0.26 (+)	~1	0.08 (+)	~1	0.09 (+)	~1	0.10 (+)	~1
3	3.12 (+)	~1	2.55 (−)	1.20	2.87 (−)	1.06	2.36 (−)	1.03
4	1.92 (−)	1.14	0.65 (+)	~1	1.21 (+)	~1	1.10 (+)	~1
5	0.50 (−)	1.16	0.13 (−)	~1	0.21 (−)	1.29	0.21 (−)	1.24
6	0.26 (−)	~1	0.05 (+)	~1	0.15 (−)	~1	0.12 (+)	~1
7	1.85 (−)	1.44	0.94 (−)	~1	1.73	~1	1.72	~1
8	0.33 (+)	~1	0.14	1.00	0.10 (+)	~1	0.10	1.00
9	0.91 (+)	~1	0.28 (−)	1.25	0.57 (−)	~1	0.51 (+)	~1
10	0.58 (−)	~1	0.13	1.00	0.24 (−)	~1	0.24 (−)	~1
11	1.37 (−)	~1	0.58 (+)	1.29	0.84 (+)	~1	0.93 (+)	~1

Flow rate: 0.5 ml/min. The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Eluent: hexane–2-propanol (90:10, v/v).

3, **7** and **9** having hydrogen capable of hydrogen bonding with C=O of carbamate group tend to interact more strongly with the CSPs **1i**–**m** than the CSPs **1f**–**h**, indicating that the electron-donating substituents increase the electron density of the carbonyl oxygen of the carbamates.

3.3. Arylalkyl- and cycloalkylcarbamates of chitin

The results of the HPLC resolution on chitin arylalkylcarbamates having chiral side groups, (*S*)- and (*R*)-1-phenylethylcarbamates (**1n**-(*S*) and **1n**-(*R*)), and cycloalkylcarbamates (**1o**, **p**) are summarized in Table 4. In our previous study on the cellulose and amylose 1-phenylethylcarbamates [10,11], the chiral recognition abilities depended on the chirality of the 1-phenylethyl groups, and the (*S*)-1-phenylethylcarbamate of amylose showed a high-chiral recognition ability. On the other hand, for the cellulose derivatives, the (*R*)-1-phenylethylcarbamate showed a higher chiral recognition ability than the (*S*)-isomer.

The elemental analysis data (Table 1) suggest that **1n**-(*R*) may possess 20% of the unsubstituted hydroxy groups different from **1n**-(*S*). Therefore, we tried to enhance the substitution degree through the reaction of **1n**-(*R*) with excess isocyanate. However, no further reaction proceeded. Both the **1n** derivatives exhibited low-chiral recognition abilities, but half of the racemates were resolved on either of the derivatives.

For cycloalkylcarbamates, norbornylcarbamate (**1q**) appears to be partially substituted based on the result of the elemental analysis. The obtained derivative was not soluble in any solvent, and therefore, the CSP of **1q** could not be prepared. The cyclopentyl, cyclohexyl, and norbornylcarbamates of cellulose and amylose exhibit a high-chiral recognition as well as the 3,5-dimethylphenylcarbamates of cellulose and amylose, which are very popular CSPs for HPLC. These cycloalkylcarbamates may also be used as the CSPs for thin-layer chromatography due to the absence of a phenyl group [12]. However, the chitin cycloalkylcarbamates, **1o**

Table 5
Effect of eluents on resolution of racemates on **1b**[a]

Racemate	Hexane–2-propanol (90:10)		Hexane–$CHCl_3$–2-propanol (90:10:1)		Hexane–$CHCl_3$ (90:10)		Hexane–AcOEt-2-propanol (90:10:1)	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α
2	0.27 (+)	~1	0.25 (+)	1.12	0.62 (+)	1.40	0.24 (+)	1.33
3	1.27 (−)	1.29	4.92 (−)[b]	1.36	32.6 (−)[b]	1.21	3.70 (−)	1.32
4	1.98 (−)	1.12	3.01 (−)[b]	1.13	14.2 (−)[b]	~1	2.73 (−)	1.15
5	0.82 (+)	~1	0.79 (+)	1.06	3.07 (+)[b]	~1	0.98 (+)	~1
6	0.30 (+)	1.13	0.35 (+)	1.31	1.84 (+)	1.62	0.61 (+)	~1
7	1.11 (−)	1.19	5.76 (−)[b]	1.13	38.8 (−)[b]	1.12	2.10[b]	~1
8	1.08 (+)	~1	0.38 (+)	1.21	2.57 (+)[b]	1.27	3.85 (+)	1.19
9	0.62 (+)	1.18	0.99 (+)[b]	1.29	3.42 (+)[b]	~1	0.82 (+)	1.37
10	1.01 (−)	1.54	1.02 (−)	1.75	3.46 (−)[b]	2.04	1.04 (−)	1.63
11	0.73 (−)	1.11	–[c]	–[c]	–[c]	–[c]	15.3 (−)[b]	1.24

[a] Flow rate: 0.5 ml min^{-1}. The signs in parentheses represent the optical rotation of the first-eluted enantiorner.
[b] Flow rate: 1.0 ml min^{-1}.
[c] Not eluted.

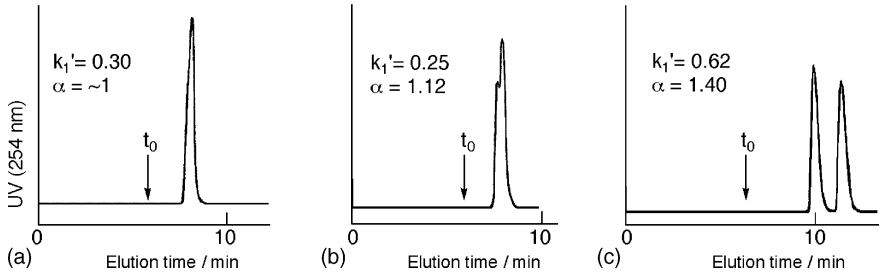

Fig. 5. Chromatograms of the resolution of trans-stilbene oxide (**2**) on **1c** with (a) hexane–2-propanol (90:10), (b) hexane–$CHCl_3$–2-propanol (90:10:1), and (c) hexane–$CHCl_3$ (90:10) as eluent.

Table 6
Effect of eluents on resolution of racemates on **1c**[a]

Racemate	Hexane–2-propanol (90:10)		Hexane–$CHCl_3$–2-propanol (90:10:1)		Hexane–AcOEt–2-propanol (90:10:1)	
	k'_1	α	k'_1	α	k'_1	α
2	0.20 (−)	~1	0.20 (−)	~1	0.12 (+)	~1
3	1.30 (−)	1.17	7.15 (−)[b]	1.08	3.70 (−)	1.13
4	2.34 (−)	1.33	3.70 (−)[b]	1.38	1.32 (−)	1.08
5	1.28 (+)	1.39	1.36 (+)	1.24	0.74 (+)	1.30
6	0.34 (−)	~1	0.40 (+)	1.15	0.34 (−)	1.68
7	0.29	~1	1.85 (−)[b]	1.10	0.58[b]	~1
8	0.49 (−)	1.12	0.15 (+)	1.33	1.05 (+)	1.86
9	0.23 (+)	~1	0.57 (−)	1.19	0.24 (+)	~1
10	2.71 (−)	1.34	2.57 (−)	1.25	0.57 (−)	1.18
11	0.34 (+)	~1	–[c]	–[c]	5.45 (+)[b]	1.24

[a] Flow rate: 0.5 ml/min. The signs in parentheses represent the optical rotation of the first-eluted enantiorner.
[b] Flow rate: 1.0 ml/min.
[c] Not eluted.

and **1p**, showed a low-chiral recognition and could separate only the two racemates **3** and **5**.

3.4. Effect of eluent composition on enantioseparation

The coated-type chiral packing materials of the cellulose and amylose derivatives cannot be used with solvents such as THF and chloroform, which dissolve or swell the polysaccharide derivatives. Therefore, in order to improve this defect, several methods for the immobilization of the cellulose and amylose derivatives onto silica gel have been examined [21–32]. On the other hand, the solubilities of the chitin phenylcarbamates prepared in this study are very low, indicating that a wide range of solvents can be utilized as the eluents without immobilizing the chitin derivatives on silica gel.

Table 7
Resolution of racemates on **1b** and **1c** under normal and reversed phase conditions[a]

Racemates	**1b**				**1c**			
	Hexane–2-propanol[b] (90:10)		MeOH–H_2O[c] (75:25)		Hexane–2 propanol[b] (90:10)		MeOH–H_2O[c] (75:25)	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α
2	0.27 (+)	~1	1.92 (+)	1.22	0.20 (−)	~1	1.60 (+)	1.35
3	1.27 (−)	1.29	0.30 (+)	~1	1.30 (−)	1.17	0.16 (+)	~1
4	1.98 (−)	1.12	0.70 (−)	1.16	2.34 (−)	1.33	0.68 (−)	1.18
5	0.82 (+)	~1	0.93 (+)	1.19	1.28 (+)	1.39	2.34 (+)	1.43
6	0.30 (+)	1.13	1.28 (−)	1.05	0.34 (−)	~1	1.69 (−)	1.27
7	1.11 (−)	1.19	1.25	~1	0.29	~1	0.30	~1
8	1.08 (+)	~1	0.27 (+)	1.11	0.49 (−)	1.12	0.31 (+)	1.42
9	0.62 (+)	1.18	3.74 (+)	1.19	0.23 (+)	~1	1.26 (+)	~1
10	1.01 (−)	1.54	2.84 (−)	1.45	2.71 (−)	1.34	3.00 (−)	1.11
11	0.73 (−)	1.11	0.31 (+)	~1	0.34 (+)	~1	0.48 (−)	~1

[a] The signs in parentheses represent the optical rotation of the first-eluted enantiomer.
[b] Flow rate: 0.5 ml/min.
[c] Flow rate: 0.4 ml/min.

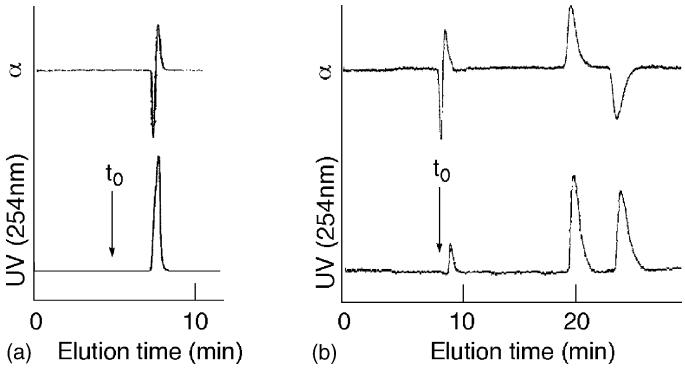

Fig. 6. Chromatograms of the resolution of *trans*-stilbene oxide (**2**) on **1c** with (a) hexane–2-propanol (90:10) and (b) methanol–H_2O (75:25) as eluent. The flow rate was (a) 0.5 ml/min and (b) 0.4 ml/min.

The enantioseparation of the racemates using chloroform and ethyl acetate as an eluent component was studied on **1b** (Table 5). The racemates except for **7** were more efficiently resolved by using hexane–$CHCl_3$–2-propanol (90:10:1) as the eluent rather than hexane–2-propanol (90:10).

Fig. 5 shows the chromatograms of the resolution of **2** on **1b** using hexane–2-propanol (90:10), hexane–$CHCl_3$–2-propanol (90:10:1), and hexane–$CHCl_3$ (90:10) as eluents. Racemate **2** was not separated on **1b** with a hexane–2-propanol (90:10) mixture; however, partial (α = 1.12) and complete resolutions (α = 1.40) were attained by reducing the 2-propanol content and simultaneously adding chloroform. 2-Propanol may prevent the interaction between the enantiomers and the carbamate moiety of the phenylcarbamate derivatives, and therefore, the reduction of the amount of 2-propanol in the eluent increased the capacity factor, k'_1, especially for the racemates having hydrogen capable of hydrogen bonding. Racemate **11** was not eluted with the eluents containing chloroform.

The separation factors for seven racemates (**2**–**4**, **8**–**11**) with hexane–ethyl acetate–2-propanol (90:10:1) were higher than those with hexane–2-propanol (90:10). CSP **1b** was stable for the eluent containing 10% chloroform or ethyl acetate.

The resolution on CSP **1c** using the eluents containing $CHCl_3$ and ethyl acetate was also examined (Table 6). The effectiveness of the eluents for the resolution depends on the racemates; the highest α values were obtained for **3**, **5**, and **10** with hexane–2-propanol, **4**, **7**, and **9** with hexane–$CHCl_3$–2-propanol, and **6**, **8**, and **11** with hexane–ethyl acetate–2-propanol.

The chitin phenylcarbamates (**1b**, **c**) also exhibited high-chiral recognition abilities under a reversed phase condition with methanol–H_2O (75:25) (Table 7). Under the normal phase condition with a hexane–alcohol mixture, enantiomers can interact with the CSPs through a polar interaction, for instance, hydrogen-bonding and dipole–dipole interactions. Besides these polar interactions, the π–π interaction between the phenyl group of the CSP and the aromatic groups of the enantiomers may play some role in the chiral recognition, because several nonpolar aromatic compounds have also been resolved. On the other hand, this nonpolar interaction may play a major role under the reversed phase condition. Comparing the results under the reversed phase condition with those under the normal phase condition, the resolved racemates seem to be different from each other; the racemates having the hydrogen capable of hydrogen bonding (**3**, **7**, **9**, and **11**) seems to be resolved under the normal phase, while hydrophobic racemates (**2**, **5**, **9**, and **10**) are more efficiently retained and resolved under the reversed phase condition. Fig. 6 shows the chromatograms of **2** on **1c**. Racemate **2**, which could not be separated on **1c** with hexane–2-propanol as the eluent, was completely separated into enantiomers using methanol–H_2O (75:25).

4. Conclusions

New chitin carbamate derivatives were synthesized and used as the CSPs in HPLC. The chiral recognition abilities were significantly influenced by the substituents on the phenyl groups, and among the derivatives, the 3,5-dimethylphenyl, 4-chlorophenyl, and 4-trifluoromethylphenylcarbamates were found to show high-chiral recognitions. The solubilities of the chitin derivatives were very low, and therefore, the range of the solvents that can be used as eluents was expanded. In the addition of chloroform or ethyl acetate to the eluents, chitin derivatives **1b** and **1c** could be safely used as CSPs and showed higher chiral recognition abilities than those observed with hexane–2-propanol. Chitin bisphenylcarbamates have a polar acetamide residue at 2-position of glucose unit differing from cellulose and amylose trisphenylcarbamates, and the acetamide residue may play same important role for recognition enantiomers similar to phenylcarbamate residue. This might be a reason why chitin phenylcarbamates exhibit the high-chiral recognition ability comparable to cellulose and amylose phenylcarbamates.

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