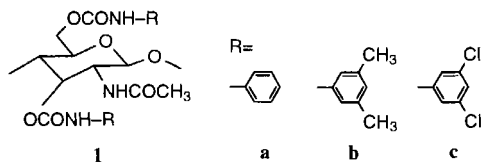
Enantioseparation by Using Chitin Phenylcarbamates as Chiral Stationary Phases for High-Performance Liquid Chromatography

Chiyo Yamamoto, Tomoko Hayashi, Yoshio Okamoto,* and Shiro Kobayashi†
Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-8603
†Department of Materials Chemistry, Graduate School of Engineering, Kyoto University, Kyoto 606-8501

(Received September 10, 1999; CL-990778)

Three chitin derivatives, 3,6-bis(phenylcarbamate), 3,6-bis(3,5-dimethylphenylcarbamate), and 3,6-bis(3,5-dichlorophenylcarbamate), were prepared and their chiral recognition abilities as chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC) were evaluated. Among the three, the 3,5-dimethyl- and 3,5-dichlorophenylcarbamates showed a relatively high chiral recognition ability. Especially, some chiral acidic drugs such as ibuprofen and ketoprofen were efficiently resolved on the 3,5-dichlorophenylcarbamate.

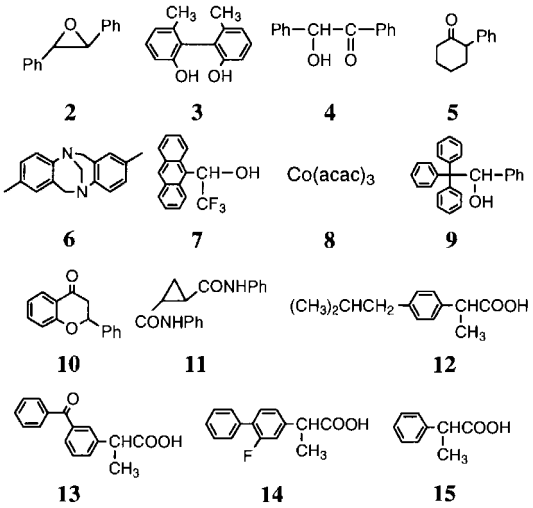
The phenylcarbamate derivatives of polysaccharides, particularly cellulose and amylose, show high chiral recognition when used as chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC).[1-5] Among the many derivatives, the 3,5-dimethylphenylcarbamates of cellulose[6] and amylose[7] are very useful CSPs and can resolve a wide range of racemates. The phenylcarbamate derivatives of other polysaccharides such as chitosan, garactosamine, xylan, curdran, dextran, and inulin were also examined as CSPs.[8,9] However, these derivatives, in most cases, exhibit lower separation for many racemates than the 3,5-dimethylphenylcarbamates of cellulose and amylose. Chitin is also a very readily available polysaccharide. However, until recently, this polysaccharide had not been used for the preparation of CSPs probably because of the difficult derivatization due to poor solubility. Cass and co-workers recently reported the synthesis and chiral recognition as CSPs of the phenylcarbamate and 3,5-dimethylphenylcarbamate of chitin.[10] However, these carbamates had very poor chiral recognition ability and could resolve only a few compounds. Judging from their experimental data, the phenyl- and 3,5-dimethylphenylcarbamate derivatives that they used as CSPs must have possessed 20-30% of unsubstituted hydroxy groups, and this may be the main reason for the very low chiral recognition of the carbamates.



1 **a** **b** **c**

In the present work, we prepared the completely carbamoylated phenylcarbamate derivatives and found that these derivatives exhibit a higher chiral recognition as CSPs for HPLC. As the derivatives, 3,6-bis(phenylcarbamate) (**1a**), 3,6-bis(3,5-dimethylphenylcarbamate) (**1b**), and 3,6-bis(3,5-dichlorophenylcarbamate) (**1c**) were examined.

Chitin from shrimp shells was obtained from Sigma. Phenyl isocyanate (Wako, Japan), 3,5-dichlorophenyl isocyanate (Tokyo Kasei, Japan) and 3,5-dimethylphenyl isocyanate (Daicel, Japan) were used as obtained. Porous spherical silica gel (Daiso gel SP-1000, pore size; 100 nm, particle size; 7 μm) was silanized with (3-aminopropyl)triethoxysilane in benzene in the presence of a catalytic amount of dry pyridine at 90 °C. Racemates (**2-15**) were commercially available or prepared by the usual method.



2 **3** **4** **5** **6** **7** **8** **9** **10** **11** **12** **13** **14** **15**

Chitin was dissolved in dry *N*,*N*-dimethylacetamide containing lithium chloride at 80 °C for 24 h, and then allowed to react with an excess of a corresponding phenyl isocyanate in dry pyridine. The chitin derivatives were isolated as the methanol-insoluble fraction (yield 83-88%). The ^{1}H-NMR data and elemental analysis (Table 1) of the derivatives showed that the hydroxy groups of chitin were almost quantitatively converted into the carbamate moieties.[11]

Table 1. Elemental analysis of chitin derivatives

CSPs	Calculated / %			Found / %		
	C	H	N	C	H	N
1a	59.9	5.3	9.5	59.9	5.5	9.5
1b	62.8	6.3	8.5	62.8	6.5	8.6
1c	45.6	3.3	7.3	45.6	3.4	7.3

Packing materials were prepared using macroporous silica gel as previously described[12] and were packed into a stainless-steel tube (25 × 0.46 cm i.d.) by a conventional high-pressure slurry packing technique using a model CCP-085 Econo packer pump (Chemco, Japan). The plate numbers of the columns were 4600-6700 for benzene with a mixture of hexane–2-propanol (90:10) as the eluent at a flow rate of 0.5 ml/min. The dead time (t_0) of the columns was estimated using 1,3,5-tri-*tert*-butylbenzene as a nonretained compound.

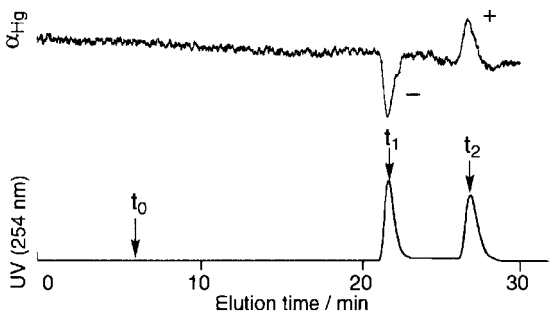


Figure 1. Resolution of benzoin (**4**) on chitin bis(3,5-dichlorophenylcarbamate) (**1c**)

The agreement between the calculated and found values of the elemental analysis listed in Table 1 indicates that two hydroxy groups of a glucosamine residue were completely converted into phenyl or 3,5-dimethylphenylcarbamate groups. On the other hand, in the case of the chitin carbamate derivatives reported in the literature,[10] the conversions into the phenyl and 3,5-dimethylphenylcarbamate groups seem to be about 80 and 70%, respectively, based on the elemental analysis data. These different results may be attributed to the difference in the dissolving abilities of the solvents used in the synthesis.

The HPLC resolution on the CSPs (**1a-c**) was examined for racemates **2-15**. Figure 1 shows a chromatogram of the resolution of benzoin (**4**) on a column packed with chitin bis(3,5-dichlorophenylcarbamate) (**1c**). The enantiomers eluted at retention times of t_1 and t_2 showing complete separation. Capacity factors, k_1' [=$(t_1-t_0)/t_0$] and k_2' [=$(t_2-t_0)/t_0$] were 2.34 and 3.12, respectively, and the separation factor α [=k_2'/k_1'], which reflects the chiral recognition ability of a CSP, was estimated to be 1.33.

Table 2. Resolution of racemates on chitin carbamates[a]

Racemates	1a		1b		1c	
	k_1'	α	k_1'	α	k_1'	α
2	0.30 (–)	~1	0.21 (+)	~1	0.20 (–)	~1
3	2.29 (–)	1.24	1.41 (–)	1.30	1.30 (–)	1.17
4	3.31 (+)	1.04	1.93 (–)	1.10	2.34 (–)	1.33
5	1.19 (–)	~1	0.73 (+)	~1	1.28 (+)	1.39
6	0.32 (+)	~1	0.29 (+)	1.14	0.34 (–)	~1
7	1.01 (–)	1.17	0.97 (–)	1.25	0.29	~1
8	1.05 (+)	1.18	0.82 (+)	1.06	0.49 (–)	1.12
9	1.02 (+)	~1	0.59 (+)	1.17	0.23 (+)	~1
10	1.27 (+)	1.20	0.96 (–)	1.54	2.71 (+)	1.34
11	1.57 (–)	~1	0.70 (–)	1.14	0.34 (+)	~1

[a]Eluent, hexane–2-propanol (90/10, v/v); flow rate, 0.5 ml min^{-1}. The signs in parentheses represent the opitical rotation of the first-eluted enantiomer.

Table 3. Resolution of 2-arylpropionic acids on chitin carbamates[a]

Racemates	1a		1b		1c	
	k_1'	α	k_1'	α	k_1'	α
12	0.79 (–)	~ 1	0.67 (+)	~ 1	0.54 (+)	1.11
13	9.69 (+)[b]	1.23	3.81 (+)	1.21	8.29 (+)[b]	1.72
14	1.73 (–)	1.06	1.33 (–)	1.08	0.29 (+)[c]	1.10
15	1.74 (+)	1.17	1.25 (+)	1.41	0.87 (+)	1.39

[a]The signs in parentheses represent the optical rotation of the first-eluted enantiomer. Flow rate: 0.5 ml min^{-1}. Eluent: hexane–2-propanol–CF_3COOH (95/5/1, v/v/v). [b]Flow rate: 1.0 ml min^{-1}. [c]Eluent: hexane–2-propanol–CF_3COOH (90/10/1, v/v/v).

As shown in Table 2, among the chitin derivatives used in this study, 3,5-dimethyl- and 3,5-dichlorophenylcarbamates showed relatively high chiral recognition abilities. The chitin derivatives better resolved some racemates, for instance, compound **5** on **1c**, compound **8** on **1a**, and compound **10** on **1b**, with larger α values than on the 3,5-dimethylphenylcarbamates of cellulose and amylose.

Table 3 shows the results of the resolutions of four 2-arylpropionic acids. Here, a small amount of trifluoroacetic acid was added to the eluent.[13] The acids **12-15** were better resolved on the chitin carbamates, especially on 3,5-dichlorophenylcarbamate, than on the 3,5-dimethylphenylcarbamates of cellulose and amylose. The α value (1.72) observed for **13** on **1c** may be the largest one for the normal phase HPLC enantioseparation on the polysaccharide derivatives prepared so far. Therefore, **1c** appears to be useful for the resolution of various 2-arylpropionic acids.

In the previous study by Cass, *et al.*,[10] the resolution of racemates **4**, **6**, **7**, **10**, and **13** by **1a** and **1b** were also examined under the analogous conditions shown in Table 2. However, only two racemates **7** (α=1.1) and **10** (α=1.4) were resolved on **1a** and on **1b**, respectively. These α values were smaller than those observed on the corresponding derivatives prepared in this study, and other compounds were not resolved. The improved resolution abilities of our CSPs may be ascribed to the almost total substitution of hydroxy groups with the carbamate residues. It has also been found that a high degree of substitution of the hydroxy groups of cellulose with carbamate residues is important to prepare CSPs with high enantioselectivity.[14]

In this work, the chiral recognition abilities as CSPs for HPLC of three chitin derivatives, in which the hydroxy groups were almost quantitatively converted to the carbamate moieties, were evaluated. High chiral recognition abilities were observed for the 3,5-dimethyl- and 3,5-dichlorophenylcarbamates. Some chiral 2-arylpropionic acids were efficiently resolved on the 3,5-dichlorophenylcarbamate.

References and Notes

1 Y. Okamoto and E. Yashima, *Angew. Chem. Int. Ed.*, **37**, 1020 (1998).
2 E. Yashima, C. Yamamoto, and Y. Okamoto, *Synlett*, **1998**, 344.
3 E. Yashima and Y. Okamoto, *Bull. Chem. Soc. Jpn.*, **68**, 3289 (1995).
4 Y. Okamoto and Y. Kaida, *J. Chromatogr. A*, **666**, 403 (1994).
5 J. Dingenen, "A Practical Approach to Chiral Separations by Liquid Chromatography," ed by G. Subramanian, VCH Publishers, Weinheim (1994), p.115.
6 Commercial name: Chiralcel OD (Daicel).
7 Commercial name: Chiralpak AD (Daicel).
8 Y. Okamoto, M. Kawashima, and K. Hatada, *J. Am. Chem. Soc.*, **106**, 5357 (1984).
9 Y. Okamoto, J. Noguchi, and E. Yashima, *Reactive & Functional Polymer*, **37**, 183 (1998).
10 Q. B. Cass, A. L. Bassi, and S. A. Matlin, *Chirality*, **8**, 131 (1996).
11 The presence of a small amount (ca. 10%) of free amino groups has been pointed out for chitin. This may react with isocyanate to form urea bond. However, this can only slightly (0.3% for carbon content) affect the results of elemental analysis.
12 Y. Okamoto, M. Kawashima, and K. Hatada, *J. Chromatogr.*, **363**, 173 (1986).
13 Y. Okamoto, R. Aburatani, Y. Kaida, K. Hatada, N. Inotsume, and M. Nakano, *Chirality*, **1**, 239 (1989).
14 Y. Okamoto, unpublished data.