Tris(cyclohexylcarbamate)s of Cellulose and Amylose as Potential Chiral Stationary Phases for High-Performance Liquid Chromatography and Thin-Layer Chromatography

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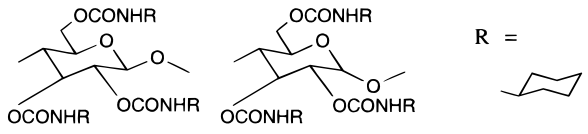
Abstract: Cyclohexylcarbamates of cellulose and amylose were prepared and their resolving abilities for enantiomers were evaluated as chiral stationary phases (CSPs) for high-performance liquid chromatography. The CSPs showed high resolving abilities, which are comparable to those of popular CSPs, tris(3,5-dimethylphenylcarbamate)s of cellulose and amylose. The cycloalkylcarbamates could also be used as CSPs for thin-layer chromatography because of the absence of a phenyl group, which causes the difficulty of detection by UV radiation. In addition, these two derivatives were soluble in chloroform and exhibited chiral discrimination to some chiral compounds in ^{1}H NMR spectroscopy as well as in HPLC.

Introduction

Cellulose and amylose are the most accessible optically active polymers on the earth. These polysaccharides are known to exhibit a high chiral recognition as phenylcarbamate derivatives[1] and afford very useful chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC).[2] The chiral recognition mechanism of the derivatives has also been studied by NMR and computer simulation.[3] However, because the phenylcarbamate derivatives strongly absorb ultraviolet (UV) light, which is conveniently used for detection in thin-layer chromatography (TLC), these are not eligible for practical CSPs in TLC as well as most other useful CSPs in HPLC.[4] On the other hand, alkylcarbamate derivatives such as methyl and isopropyl derivatives of the polysaccharides exhibit much lower chiral recognition as CSPs for HPLC,[1b,2a] although they poorly absorb UV light.

In this study, we found that tris(cyclohexylcarbamate)s of cellulose (**1**) and amylose (**2**) showed high chiral recognition abilities and can be used as the CSPs in both HPLC and TLC. Chiral recognition of **1** was also observed by NMR spectroscopy.



1 **2**

Experimental Section

Chemicals. Cellulose (Avicel, DP ~ 200) and fluorescent indicator F_{254} for thin-layer chromatography were purchased from Merck. Amylose (DP ~ 200) was a gift from Ajinoki. (3-Aminopropyl)-triethoxysilane was of guaranteed reagent grade from Tokyo Kasei. *N*,*N*-Dimethylacetamide and cyclohexyl isocyanate were obtained from Kishida and LiCl from Wako. Wide-pore silica gel (Daiso gel SP-1000, pore size 100 nm, particle size 7 μm) was kindly supplied by Daiso and was silanized using (3-aminopropyl)triethoxysilane in benzene at 80 °C before use. All solvents used in the preparation of CSPs were of

(1) (a) Okamoto, Y.; Kawashima, M.; Hatada, K. *J. Am. Chem. Soc.* **1984**, *106*, 5357. (b) Okamoto, Y.; Kawashima, M.; Hatada, K. *J. Chromatogr.* **1986**, *363*, 173. (c) Okamoto, Y.; Aburatani, R.; Fukumoto, T.; Hatada, K. *Chem. Lett.* **1987**, 1857. (d) Okamoto, Y.; Aburatani, R.; Hatano, K.; Hatada, K. *J. Liq. Chromatogr.* **1988**, *11*, 2147. (e) Oguni, K.; Oda, H.; Ichida, A. *J. Chromatogr. A* **1995**, *694*, 91. (f) Allenmark, S. G. *Chromatographic Enantioseparation*; Ellis Horwood: Chichester, 1988. (g) Francotte, E. *J. Chromatogr. A* **1992**, *576*, 1. (h) Francotte, E. *J. Chromatogr. A* **1994**, *666*, 565.

(2) For reviews: (a) Okamoto, Y.; Yashima, E. *Angew. Chem., Int. Ed.* **1998**, *37*, 1020. (b) Yashima, E.; Yamamoto, C.; Okamoto, Y. *Synlett* **1998**, 344. (c) Yashima, E.; Okamoto, Y. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 3289. (d) Okamoto, Y.; Kaida, Y. *J. Chromatogr. A* **1994**, *666*, 403. (e) Ishikawa, A.; Shibata, T. *J. Liq. Chromatogr.* **1993**, *16*, 859.

(3) (a) Yashima, E.; Yamamoto, C.; Okamoto, Y. *J. Am. Chem. Soc.* **1996**, *118*, 4036. (b) Yashima, E.; Yamada, M.; Okamoto, Y. *Chem. Lett.* **1994**, 579. (c) Yashima, E.; Yamada, M.; Yamamoto, C.; Nakashima, M.; Okamoto, Y. *Enantiomer* **1997**, *2*, 225. (d) Yashima, E.; Yamada, M.; Kaida, Y.; Okamoto, Y. *J. Chromatogr. A* **1995**, *694*, 347. (e) Lipkowitz, K. B. In *A Practical Approach to Chiral Separations by Liquid Chromatography*; Subramanian, G., Ed.; VCH: New York, 1994; Chapter 2. (f) Lipkowitz, K. B.; Anderson, A. G. In *Computational Approaches in Supramolecular Chemistry*; Wipff, G., Ed.; Kluwer: Dordrecht, 1994. (g) Lipkowitz, K. B. *J. Chromatogr. A* **1995**, *694*, 15. (h) Topiol, S.; Sabio, M.; Moroz, J.; Caldwell, W. B. *J. Am. Chem. Soc.* **1990**, *110*, 8367. (i) Lipkowitz, K. B.; Pearl, G.; Coner, B.; Peterson, M. A. *J. Am. Chem. Soc.* **1997**, *119*, 600. (j) Yamamoto, C.; Yamada, M.; Yashima, E.; Okamoto, Y. *Polym. Prepr. Jpn.* **1995**, *44*, 3029. (k) Yamamoto, C.; Yashima, E.; Okamoto, Y. *Bull. Chem. Soc. Jpn*, **1999**, *72*, 1815.

(4) (a) Pirkle, W. H.; Finn, J. M.; Schreiner, J. L.; Hamper, B. C. *J. Am. Chem. Soc.* **1981**, *103*, 3964. (b) Welch, C. J. *Adv. Chromatogr.* **1995**, *35*, 171. (c) Shinbo, T.; Yamaguchi, T.; Nishimura, K.; Sugiura, M. *J. Chromatogr. A* **1987**, *405*, 145. (d) Chang, S. C.; Reid, G. L., III; Chang, C, D.; Armstrong, D. W. *Trends Anal. Chem.* **1993**, *12*, 144. (e) Blaschke, G. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 13. (f) Blaschke, G. *J. Liq. Chromatogr.* **1986**, *9*, 341. (g) Okamoto, Y.; Okamoto, I.; Yuki, H.; Murata, S.; Noyori, R.; Takaya, H. *J. Am. Chem. Soc.* **1981**, *103*, 6971. (h) Okamoto, Y.; Hatada, K. *J. Liq. Chromatogr.* **1986**, *9*, 369. (i) Okamoto, Y.; Nakano, T. *Chem. Rev.* **1994**, *94*, 349. (j) Hermansson, J.; Eriksson, M. *J. Liq. Chromatogr.* **1986**, *9*, 621. (k) Allenmark, S. G. *J. Liq. Chromatogr.* **1986**, *9*, 425. (l) Miwa, T.; Miyakawa, T. *J. Chromatogr.* **1987**, *408*, 316. (m) Erlandsson, P.; Marle, I.; Hansson, L.; Isaksson, R.; Pettersson, C.; Pettersson, G. *J. Am. Chem. Soc.* **1990**, *112*, 4573. (n) Wainer, I. W.; Jadaud, P.; Schombaum, G. R.; Kadadker, S. V.; Henry, M. P. *Chromatographia* **1988**, *25*, 903. (o) Noctor, T.; Felix, G.; Wainer, I. W. *Chromatographia* **1991**, *31*, 55. (p) Haginaka, J.; Murashima, T.; Seyama, C. *J. Chromatogr. A* **1994**, *666*, 203.

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analytical reagent grade, carefully dried, and distilled before use. Solvents used in chromatographic experiments were of HPLC grade. $CDCl_3$ (99.8 atom % D, Nacalai) was dried over molecular sieves 4A (Nacalai) and stored under nitrogen. Racemates (**3**−**13**) were commercially available or were prepared by the usual method.[5]

Synthesis of Cellulose Tris(cyclohexylcarbamate) (1). Cellulose tris(cyclohexylcarbamate) (**1**) was prepared by the reaction[1b] of cellulose (1.0 g) dissolved in an *N*,*N*-dimethylacetamide (15 mL)−LiCl (1.5 g) mixture at 100 °C for 24 h and an excess of cyclohexyl isocyanate (4.0 g, 32 mmol) and pyridine (20 mL) were added to the polysaccharide solution. The reaction was continued for 36 h at 100 °C. The resulting carbamate derivative was isolated as a methanol-insoluble fraction. The degree of substitution of the hydroxy groups was determined to be nearly three for **1** by elemental analysis and ^{1}H NMR spectroscopy. IR (KBr) 3449, 3336 (ν_{NH}), 1719 ($\nu_{C=O}$); ^{1}H NMR ($CDCl_3$, 60 °C, TMS) δ 1.16−1.28, 1.60−1.86 (br, cyclohexyl protons, 30H), 3.36, 3.89, 4.26, 4.53, 4.82 (br, cyclohexyl protons and glucose protons, 10H), 4.92, 5.18, 5.60 (br, NH, 3H). Anal. Calcd for $(C_{27}H_{43}O_8N_3)_n$: C, 60.32; H, 8.06; N, 7.82. Found: C, 59.48; H, 8.57; N, 7.76.

Synthesis of Amylose Tris(cyclohexylcarbamate) (2). Amylose (1.0 g) was first dissolved in an *N*,*N*-dimethylacetamide (10 mL)−LiCl (1.0 g) mixture at 80 °C for 24 h and then an excess of cyclohexyl isocyanate (3.3 g, 26 mmol) and pyridine (20 mL) were added to the polysaccharide solution. The reaction was continued for 24 h at 80 °C. The resulting carbamate derivative was isolated as a methanol-insoluble fraction. The degree of substitution of the hydroxy groups was determined to be nearly three for **2** by elemental analysis and ^{1}H NMR spectroscopy. IR (KBr) 3444, 3319 (ν_{NH}), 1718 ($\nu_{C=O}$); ^{1}H NMR ($CDCl_3$, 60 °C, TMS) δ 1.27, 1.60−1.96 (br, cyclohexyl protons, 30H), 3.40, 4.12, 4.24, 4.40, 4.80, 5.10, 5.20, 7.15 (br, cyclohexyl protons, glucose protons and NH, 13H). Anal. Calcd for $(C_{27}H_{43}O_8N_3)_n$: C, 60.32; H, 8.06; N, 7.82. Found: C, 59.49; H, 8.39; N, 7.66.

Preparation of the Stationary Phase. Packing materials were prepared by coating the carbamate derivatives on macroporous silica gel (Daiso Gel, particle size 7 μm, pore size 100 nm). Then these were packed in a stainless steel tube (25 cm × 0.46 cm (i.d.)) by slurry methods. The plate numbers of columns **1** and **2** were 6100 and 5700, respectively, for benzene using a hexane−2-propanol (90:10) mixture as the eluent at a flow rate of 0.5 mL min^{-1}.[5] 1,3,5-Tri-*tert*-butylbenzene was used as a nonretained compound to estimate dead time (t_0).[6]

Preparation of TLC Plates. 2 (0.75 g) was dissolved in 10 mL of tetrahydrofuran (THF). The solution was added to the above silanized macroporous silica gel (3 g), and wetted silica gel was dried under vacuum. This coating process was repeated with the remaining carbamate solution. The silica gel (1.5 g) and fluorescent indicator (0.1 g) were mixed in methanol (ca. 3 mL). The slurry was carefully poured on a standard slide glass (76 × 26 mm), and then spread to form a uniform layer (0.3 mm). The plate was dried in an oven for 30 min at 110 °C. The layer weight was found to be 0.13 g after drying.

Apparatus. Chromatographic experiments were performed on a JASCO 980-PU chromatograph equipped with a UV (JASCO 970-UV) and a polarimetric (JASCO OR-990) detector at room temperature. A solution of racemate (1−25 μL) was injected into the chromatographic system with a Rheodyne Model 7125 injector. IR spectra were measured on a JASCO FT/IR-620 spectrometer as a KBr pellet. ^{1}H NMR spectra were measured with a Varian Gemini-2000 spectrometer (400 MHz) using TMS as an internal standard.

Results and Discussion

HPLC Resolution on Tris(cyclohexylcarbamate)s of Cellulose (1) and Amylose (2). The results of resolution of eleven racemates (**3**−**13**) on the cellulose derivative (**1**) and amylose derivative (**2**) are given in Table 1, and Figure 1 shows the chromatogram of resolution of racemic benzoin ethyl ether (**13**) on a column packed with **2**. 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 1.00 and 3.89, respectively. Separation factor α [$=k_2'/k_1'$] and resolution factor Rs

(5) Kaida, Y.; Okamoto, Y. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 2286.

Table 1. Resolution of Racemates (**3**−**13**) on Tris(cyclohexylcarbamate)s of Cellulose (**1**) and Amylose (**2**)[a]

	1			**2**		
	k_1'	α	Rs	k_1'	α	Rs
3	1.11 (−)	1.60	3.82	1.02 (−)	1.39	2.78
4	0.26 (+)	1.19		0.44 (+)	∼1	
5	2.33 (−)	1.52	4.90	1.42 (−)	1.32	2.15
6	0.28 (+)	∼1		0.46 (+)	2.54	6.43
7	6.65 (+)	1.47	4.56	3.43 (−)	1.36	1.58
8	0.14	1.0		0.13	1.0	
9	0.41 (−)	1.22		0.71 (+)	1.70	4.77
10	0.63 (+)	∼1		0.89 (+)	1.48	3.60
11	4.44 (+)	2.43	5.31	0.84 (+)	1.17	1.13
12	1.98 (−)	1.18	2.10	4.33 (−)	1.12	1.73
13	0.22	1.0		1.00 (−)	3.89	16.27

[a] Eluent, hexane−2-propanol (90:10); flow rate, 0.5 mL min^{-1}. The signs in parentheses represent the optical rotation of the first-eluted enantiomer.

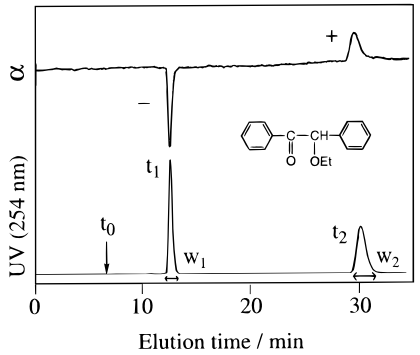
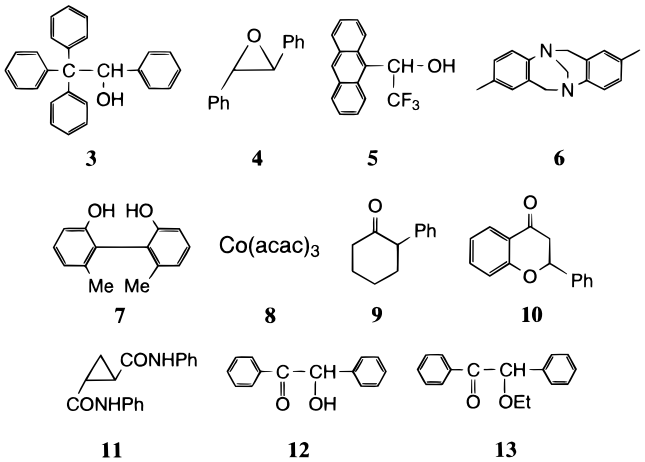


Figure 1. Separation of enantiomers of benzoin ethyl ether (**13**) on **2**. Chromatographic conditions are shown in Table 1.

$[=2(t_2 - t_1)/(w_1 + w_2)]$ were found to be 3.89 and 16.27, respectively. Dead time (t_0) was estimated to be 6.3 min with 1,3,5-tri-*tert*-butylbenzene using a hexane−2-propanol (90/10, v/v) mixture as an eluent at a flow rate of 0.5 mL min^{-1} (12 kg cm^{-2}).[6] This value was similar to the t_0 values (6.0 min) for the columns packed with phenylcarbamate-based packing materials prepared under conditions similar to those described here.[1b] This agreement suggests that one may basically evaluate the chiral recognition abilities of cyclohexylcarbamates and phenylcarbamates of polysaccharides by comparing the α-values for these CSPs.



Both **1** and **2** showed high chiral recognition abilities. For instance, the chiral recognition ability of **1** was higher for

(6) Koller, H.; Rimböck, K.-H.; Mannschreck, A. *J. Chromatogr.* **1983**, *282*, 89.

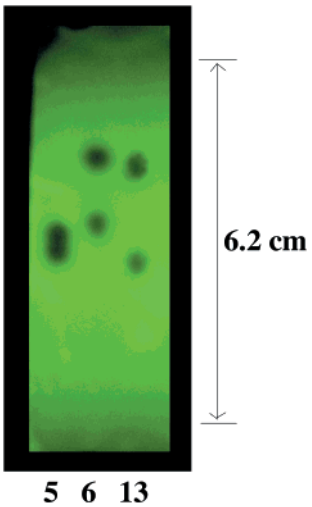


Figure 2. TLC chromatogram showing the separation of **5**, **6**, and **13**. Eluent, hexane−2-propanol (90:10). Spots were detected using a 254 nm UV hand lamp.

racemic compounds **3** and **9** than the very popular CSP, cellulose tris(3,5-dimethylphenylcarbamate),[1b,2a−d,7] and **2** more efficiently resolved racemates **5**, **6**, **9**, **10**, and **13** than the amylose tris-(3,5-dimethylphenylcarbamate).[1c,2a−d,8] Therefore, racemates **6**, **9**, **10**, and **13** were better resolved on **2** than both the tris(3,5-dimethylphenylcarbamate)s. Besides the racemates shown in Figure 1, several racemates such as *trans*-cyclobutane dicarboxylic acid dianilide ($\alpha = 3.52$) and 1-(1,2,3,4-tetrahydronaphthyl) benzoate ($\alpha = 2.00$) on **1** and *trans*-4-chlorostilbene oxide ($\alpha = 2.53$) and hydrobenzoin ($\alpha = 1.91$) on **2** were resolved also efficiently with a large α value. These results suggest that the CSPs **1** and **2** will be very useful as well as the tris(3,5-dimethylphenylcarbamate)s. The elution order of the enantiomers of the two racemates **7** and **9** were reversed on **1** and **2**. It has been shown that the tris(3,5-dimethylphenylcarbamate)s of cellulose and amylose exhibit complementary separation for many racemates, and the enantiomers often elute in a reversed order.[2] Analogous phenomena appear to be realized on **1** and **2**. The CSPs **1** and **2** were quite stable for at least 80 h under the chromatographic condition with a hexane−2-propanol (90:10) mixture as the eluent.

TLC Resolution on Tris(cycloalkylcarbamate) of Amylose (2). When cellulose tris(3,5-dimethylphenylcarbamate) was used as a CSP for TLC, the detection of sample spots by irradiation of UV light which is usually applied in TLC was possible only for compound **5** containing a fluorescent anthryl residue. The detection of other compounds with a phenyl group was difficult because of strong UV absorption of the CSPs with phenyl groups. So far, successful TLC resolution on the CSPs containing a phenyl group has been reported only for the racemates with a fluorescent residue.[9] However, because **2** has no phenyl group, the sample spots in TLC are expected to be detectable by UV irradiation.

The solutes, **5**, **6**, and **13**, were dissolved in a hexane−2-propanol (90:10) mixture at a concentration of approximately 0.3 mg mL^{-1} and applied as spots 1 cm above the bottom edge of the TLC plate (76 × 26 mm) using glass capillaries and developed using a mobile phase (hexane−2-propanol (90:10)) at room temperature. The results of TLC resolution of **5**, **6**, and **13** are shown in Figure 2. The chromatograms were readily detected by UV radiation at 254 nm, showing two spots of enantiomers. These spots were identified by using each pure enantiomer. The TLC results are evaluated by the ratios (R_{f1} and R_{f2}) of the developments for enantiomer and solvent and were obtained as $R_{f1} = 0.47$ and $R_{f2} = 0.52$ for **5**, 0.55 and 0.71 for **6**, and 0.45 and 0.69 for **13**.

(7) Commercial name: Chiralcel OD (Daicel).
(8) Commercial name: Chiralpak AD (Daicel).
(9) (a) Bhushan, R.; Parshad, V. *J. Chromatogr. A* **1996**, *736*, 235. (b) Armstrong, D. W.; Zhou, Y. *J. Liq. Chromatogr.* **1994**, *17*, 1695.

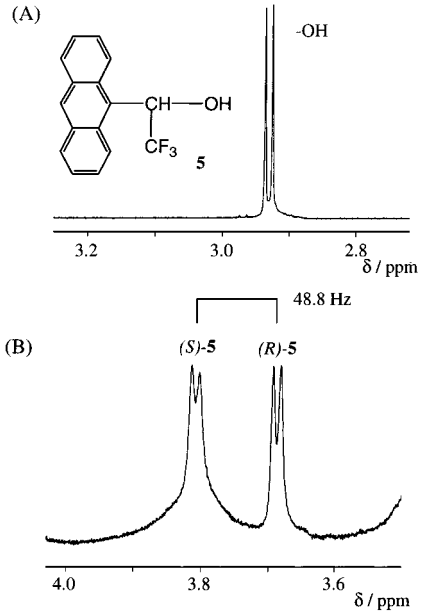


Figure 3. ^{1}H NMR spectra of a selected region *(RS)*-**5** (18.1 mM) in the absence (A) and presence (B) of **1** (37.2 mM glucose units) in $CDCl_3$.

The R_{f1} and R_{f2} values can be correlated with the capacity factors (k_1' and k_2') and separation factor (α) in HPLC by eqs 1−3:

$$k_1'_{TLC} = \frac{h_0 - h_1}{h_1} = \frac{1}{R_{f1}} - 1 \quad (1)$$

$$k_2'_{TLC} = \frac{h_0 - h_2}{h_2} = \frac{1}{R_{f2}} - 1 \quad (2)$$

$$\alpha_{TLC} = \frac{k_2'_{TLC}}{k_1'_{TLC}} = \frac{R_{f1}(1 - R_{f2})}{R_{f2}(1 - R_{f1})} \quad (3)$$

where h_0 is the length of solvent development, and h_1 and h_2 are the lengths of enantiomer development. Thus, a more developed enantiomer with a large R_f value should be less retained to give a small k' value. From the R_{f1} and R_{f2} values, the α_{TLC} values for **5**, **6**, and **13** are estimated to be 1.22, 2.00, and 2.72, respectively, which are qualitatively in agreement with the corresponding α values 1.32, 2.54, and 3.89 in HPLC shown in Table 1. The α values in HPLC are larger than those in TLC. This result indicates that the HPLC resolution is more efficient than the TLC resolution. This must be due at least partly to the fact that the HPLC column was packed at a very high pressure which can increase the relative volume of the CSP compared with a mobile phase. The TLC plate has been prepared under an atmosphere. In HPLC, the separation factor (α) of ca. 1.20 is usually sufficient for baseline separation. On the other hand, our TLC chiral plate showed two spots due to the enantiomers of **5** ($\alpha = 1.34$). These results suggest that the cycloalkylcarbamates of polysaccharides are expected to be very useful CSPs for TLC, which will enable rapid setup of the conditions for the HPLC resolution. When **2** was coated on a commercial silica

gel TLC plate (Merck (6.5 cm × 2.0 cm)), the resolution of **6** was not efficiently attained, showing lower separation, $R_{f1} = 0.33$ and $R_{f2} = 0.43$. This is ascribed to the difference in the silica gel. The silica gel used for the commercial TLC plate has a much larger surface area compared with the silica gel used in this work.

Chiral Discrimination by Tris(cyclohexylcarbamate) of Cellulose (1) in NMR. As shown in Table 1 in the resolution of **5** on **1** by HPLC, the enantiomers were completely resolved with the separation factor ($\alpha = 1.52$) and eluted as a second peak indicating that (*S*)-(+)-**5** interacts more strongly with the stationary phase **1**. Figure 3 shows the 400 MHz ^{1}H NMR spectra of (*RS*)-**5** in the absence (A) and presence (B) of **1** in $CDCl_3$. The hydroxy proton (2.931 ppm, $J = 4.4$ Hz) of the enantiomers of **5** was significantly separated into two peaks (3.686 and 3.808 ppm, $\Delta\Delta\delta$=48.8 Hz, $J = 4.4$ Hz) in the presence of **1**. Some anthryl protons and fluorine were also split with relatively small chemical shift differences. This clearly indicates that **1** can recognize the enantiomers even in solution. A similar result has been observed in a cellulose trisphenylcarbamate system.[3a] On the basis of the measurement with enantiomerically pure (*S*)- and (*R*)-**5**, it became clear that the hydroxy protons ((*S*)-**5**−OH) were more largely shifted downfield than the corresponding ((*R*)-**5**-OH), indicating that ((*S*)-**5**-OH) interacts more strongly with **1**. The downfield shift of the OH resonances is ascribed to hydrogen bond formation. The larger chemical shift movement of the ((*S*)-**5**-OH) resonances than ((*R*)-**5**-OH) observed in the ^{1}H NMR is associated with the chromatographic elution order of the enantiomer. Because of the absence of aromatic protons of the alkylcarbamate derivatives, the chemical shift differences ($\Delta\Delta\delta$) between the enantiomers in the aromatic region can be easily detected. This suggests that **1** and **2** may also be useful as chiral shift reagents.

Conclusion. We found that cellulose and amylose tris(cyclohexylcarbamate)s showed high chiral recognition in HPLC and can be used as the CSPs in TLC and chiral shift reagents in ^{1}H NMR. Other alkylcarbamates, particularly cycloalkylcarbamates, are also expected to show high chiral recognition in both HPLC and TLC.

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