# 3-Fluoro-, 3-chloro- and 3-bromo-5-methylphenylcarbamates of cellulose and amylose as chiral stationary phases for high-performance liquid chromatographic enantioseparation

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## Abstract

3-Fluoro-, 3-chloro- and 3-bromo-5-methylphenylcarbamates of cellulose and amylose were prepared and their chiral recognition abilities as chiral stationary phases for high-performance liquid chromatography (HPLC) were evaluated and compared with those of the 3,5-difluoro-, 3,5-dichloro- and 3,5-dimethylphenylcarbamates of cellulose and amylose. The introduction of both an electron-donating methyl group and an electron-withdrawing halogen group onto the phenyl moieties markedly modified the polarities of the carbamate residues. Among the cellulose derivatives, cellulose tris(3-fluoro-5-methylphenylcarbamate) showed an excellent chiral recognition ability and resolved some racemates better than the corresponding 3,5-difluoro- and 3,5-dimethylphenylcarbamates of cellulose. The amylose derivatives exhibited a characteristic chiral recognition depending on the substituents. The effects of substituents on chiral discrimination is discussed on the basis of enantioseparation results in HPLC, IR, $^1$H NMR and circular dichroism (CD) data. Some chiral drugs were better resolved on cellulose tris(3-chloro-5-methylphenylcarbamate) than cellulose tris(3,5-dimethylphenylcarbamate).    © 1997 Elsevier Science B.V.

## 1. Introduction

Phenylcarbamate derivatives of polysaccharides such as cellulose and amylose show high chiral recognition abilities for a variety of racemates including many drugs as chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC) [1–8]. Chiral recognition abilities of the polysaccharide derivatives depend greatly on the

species and position of substituents introduced on the phenyl groups of the carbamate derivatives. The introduction of electron-donating or electron-withdrawing substituents at the 3- or 4-position tends to improve the optical resolution abilities of CSPs [9]. Thus, 3,5-dimethylphenylcarbamates of cellulose and amylose on market belong to some of the most widely used CSPs for HPLC enantioseparation [1–9]. Tris(3,5-dichlorophenylcarbamate) of cellulose also shows high chiral recognition, but its practical application is limited because of high solubility in
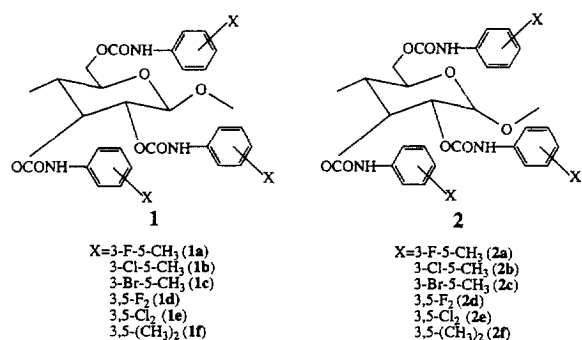
*Corresponding author.

X=3-F-5-CH₃ (1a)    X=3-F-5-CH₃ (2a)
  3-Cl-5-CH₃ (1b)      3-Cl-5-CH₃ (2b)
  3-Br-5-CH₃ (1c)      3-Br-5-CH₃ (2c)
  3,5-F₂ (1d)          3,5-F₂ (2d)
  3,5-Cl₂ (1e)         3,5-Cl₂ (2e)
  3,5-(CH₃)₂ (1f)      3,5-(CH₃)₂ (2f)

Fig. 1. Structure of CSPs.

most chromatographic eluents [9]. Mechanical mix-
ing of tris(3,5-dimethylphenylcarbamate) and
tris(3,5-dichlorophenylcarbamate) of cellulose may
not lead to the substantial improvement of chiral
resolving ability [10].

Recently, phenylcarbamate derivatives having
both an electron-donating methyl group and an
electron-withdrawing chloro or fluoro group on the
phenyl moieties were found to exhibit high enantio-
separation for many racemates [11–13]; particularly,
3-chloro-4-methyl-, 4-chloro-3-methyl- and 3-fluoro-
4-methylphenylcarbamates of cellulose and amylose
tris(5-chloro-2-methylphenylcarbamate) showed very
high chiral recognition ability comparable to those of
3,5-dimethylphenylcarbamates of cellulose and amy-
lose, whereas the corresponding 3,5-disubstituted
phenylcarbamate derivatives, such as 3-chloro-5-
methylphenylcarbamates of cellulose and amylose,
have not yet been prepared. Here we prepared 3-
fluoro- (a), 3-chloro- (b) and 3-bromo-5-
methylphenylcarbamates (c) of cellulose (1) and
amylose (2) (Fig. 1) and their chiral recognition

abilities as CSPs were evaluated and compared with
those of the corresponding 3,5-difluoro- (d) [14],
3,5-dichloro- (e) and 3,5-dimethylphenylcarbamates
(f) of cellulose and amylose.

## 2. Experimental

### 2.1. Chemicals

Microcrystalline cellulose (Avicel) was purchased
from Merck (Darmstadt, Germany). Amylose with
degree of polymerization (DP ca. 70) was a gift from
Nakano Vinegar (Handa, Japan). (3-Aminopropyl)-
triethoxysilane, 4-fluoro-2-methylaniline and 4-
bromo-2-methylaniline were of guaranteed reagent
grade from Tokyo Kasei (Tokyo, Japan). 2-Methyl-
4-nitroaniline was from Aldrich (Milwaukee, WI,
USA). Wide pore silica gel (Daiso gel SP-1000, pore
size, 100 nm; particle size, 7 μm) was kindly
supplied from Daiso (Osaka, Japan) and was silan-
ized using (3-aminopropyl)triethoxysilane in ben-
zene in the presence of a catalytic amount of dry
pyridine at 80°C before use. Hexane and 2-propanol
used as components of eluents were of reagent grade.
Racemates (3–12 in Fig. 2) were commercially
available (Aldrich) or prepared by the usual method
[15].

All isocyanates were prepared with standard meth-
ods starting from 4-fluoro-2-methylaniline, 2-methyl-
4-nitroaniline and 4-bromo-2-methylaniline, respec-
tively [16]. Detailed procedures for the syntheses of
three isocyanates are described in Fig. 3. 3-Fluoro-5-
methylphenyl isocyanate; b.p. 68°C (13 mmHg; 1
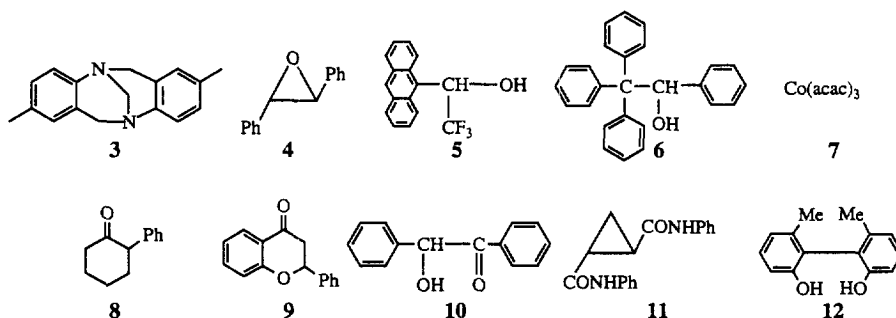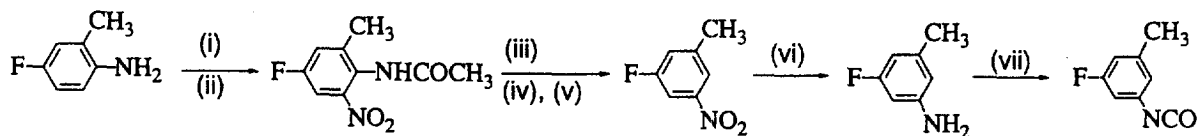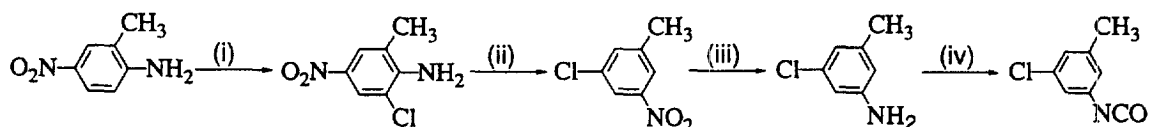mmHg=133.322 Pa). [IR (neat)]: 2270 (ν_{NCO}); ¹H



Fig. 2. Structure of racemates.

(a) Synthsis of 3-fluloro-5-methylphenyl isocyanate



Reagent and conditions: (i) Ac₂O in pyridine, 94%; (ii) HNO₃-H₂SO₄ in CH₃CO₂H, quant.; (iii) H₂SO₄/H₂O (10/1), 39%; (iv) NaNO₂-H₂SO₄, quant.;(v) Cu₂O-H₂SO₄ in dioxane, 69%; (vi) SnCl₂ in CH₃OH, HCl, 65%; (vii) HCl, triphosgene in toluene, 27%.

(b) Synthsis of 3-chloro-5-methylphenyl isocyanate



Reagent and conditions: (i) KClO₄, quant.; (ii) NaNO₂-H₂SO₄, in EtOH, quant.; (III) SnCl₂ in 95% EtOH, HCl, 73%; (iv) HCl, triphosgene in toluene, 43%.

(c) Synthsis of 3-bromo-5-methylphenyl isocyanate
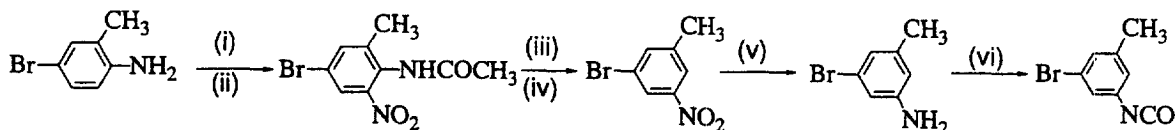


Reagent and conditions: (i) Ac₂O in pyridine, 94%; (ii) HNO₃-H₂SO₄ in CHCl₃, 72%.; (iii) H₂SO₄/H₂O (10/1), 94%; (iv) NaNO₂-H₂SO₄, in EtOH, 87%; (v) SnCl₂ in CH₃OH, HCl, 84%; (vi) HCl, triphosgene in toluene, 33%.

Fig. 3. Schemes for the syntheses of 3-fluoro-5-methylphenyl isocyanate (a), 3-chloro-5-methylphenyl isocyanate (b) and 3-bromo-5-methylphenyl isocyanate (c).

NMR ($C^2HCl_3$): $\delta$ 2.32 (s, $CH_3$, 3H), 6.60–6.74 (m, aromatic, 3H). 3-Chloro-5-methylphenyl isocyanate; b.p. 50°C (2 mmHg). [IR (neat)]: 2264 ($\nu_{NCO}$); $^1H$ NMR ($C^2HCl_3$): $\delta$ 2.30 (s, $CH_3$, 3H), 6.79, 6.89, 6.99 (s, aromatic, 3H). 3-Bromo-5-methylphenyl isocyanate; b.p. 70°C (2 mmHg). [IR (neat)]: 2268 ($\nu_{NCO}$); $^1H$ NMR ($C^2HCl_3$): $\delta$ 2.30 (s, $CH_3$, 3H), 6.84, 7.06, 7.16 (s, aromatic, 3H).

## 2.2. Preparation of trisphenylcarbamate derivatives of cellulose and amylose

Phenylcarbamate derivatives of cellulose and amylose were prepared by the reaction of cellulose or amylose with an excess of the corresponding phenyl isocyanate derivatives in dry pyridine at ca. 80°C [9,17]. The phenylcarbamates obtained were isolated as a methanol-insoluble fraction (yield 83–98%). $^1H$

NMR spectra and elemental analyses showed that hydroxy groups of cellulose and amylose were almost quantitatively converted into the carbamate moieties. 1a: 83% yield; [IR (KBr)]: 3320, 3430 ($\nu_{NH}$), 1738 ($\nu_{C=O}$); $^1H$ NMR ([$^2H$_5]pyridine): $\delta$ 1.90, 2.03, 2.20 (s, $CH_3$, 9H), 3.48, 3.80, 4.42, 4.63, 5.01, 5.40 (br, glucose protons, 7H), 6.16–7.38 (br, aromatic, 9H), 9.60, 9.79, 10.47 (br, NH, 3H). Anal. calcd. for $(C_{30}H_{28}O_8N_3F_3)_n$: C, 58.53; H, 4.58; N, 6.82%. Found: C, 58.49; H, 4.54; N, 6.92%. 1b: 98% yield; [IR (KBr)]: 3322, 3390 ($\nu_{NH}$), 1738 ($\nu_{C=O}$); $^1H$ NMR([$^2H$_5]pyridine): $\delta$ 1.87, 2.01, 2.20 (s, $CH_3$, 9H), 3.46, 3.74, 4.38, 4.59, 4.96, 5.35 (br, glucose protons, 7H), 6.38–8.15 (br, aromatic, 9H), 9.50, 9.78, 10.42 (br, NH, 3H). Anal. calcd. for $(C_{30}H_{28}O_8N_3Cl_3)_n$: C, 54.18; H, 4.24; N, 6.32%. Found: C, 54.09; H, 4.30; N, 6.55%. 1c: 92% yield; [IR (KBr)]: 3300, 3402 ($\nu_{NH}$), 1738 ($\nu_{C=O}$); $^1H$

NMR([$^2$H_5]pyridine): $\delta$ 1.90, 2.02, 2.23 (s, CH$_3$, 9H), 3.53, 3.78, 4.40, 4.63, 4.98, 5.40 (br, glucose protons, 7H), 6.58–7.76 (br, aromatic, 9H), 9.53, 9.80, 10.44 (br, NH, 3H). Anal. calcd. for (C$_{30}$H$_{28}$O$_8$N$_3$Br$_3$)$_n$: C, 45.13; H, 3.53; N, 5.26%. Found: C, 45.10; H, 3.60; N, 5.28%. **2a**: 92% yield; [IR (KBr)]: 3300, 3434 ($\nu_{NH}$), 1748 ($\nu_{C=O}$); $^1$H NMR([$^2$H_5]pyridine): $\delta$ 1.87, 1.99, 2.38 (s, CH$_3$, 9H), 4.59, 4.89, 5.02, 5.32, 5.50, 5.74 (br, glucose protons, 7H), 6.21–7.66 (br, aromatic, 9H), 9.57, 9.75, 10.43 (br, NH, 3H). Anal. calcd. for (C$_{30}$H$_{28}$O$_8$N$_3$F$_3$)$_n$: C, 58.53; H, 4.58; N, 6.82%. Found: C, 58.47; H, 4.60; N, 6.99%. **2b**: 95% yield; [IR (KBr)]: 3290, 3406 ($\nu_{NH}$), 1738 ($\nu_{C=O}$); $^1$H NMR([$^2$H_5]pyridine): $\delta$ 1.84, 1.98, 2.37 (s, CH$_3$, 9H), 4.20, 4.34, 4.62, 5.07, 5.42, 5.71 (br, glucose protons, 7H), 6.44–8.10 (br, aromatic, 9H), 9.50, 9.64, 10.36 (br, NH, 3H). Anal. calcd. for (C$_{30}$H$_{28}$O$_8$N$_3$Cl$_3$)$_n$: C, 54.18; H, 4.24; N, 6.32%. Found: C, 54.09; H, 4.33; N, 6.34%. **2c**: 94% yield; [IR (KBr)]: 3306, 3400 ($\nu_{NH}$), 1738 ($\nu_{C=O}$); $^1$H NMR([$^2$H_5]pyridine): $\delta$ 1.88, 2.01, 2.40 (s, CH$_3$, 9H), 4.59, 4.70, 5.09, 5.29, 5.52, 5.75 (br, glucose protons, 7H), 6.65–8.05 (br, aromatic, 9H), 9.68, 10.42 (br, NH, 3H). Anal. calcd. for (C$_{30}$H$_{28}$O$_8$N$_3$Br$_3$)$_n$: C, 45.13; H, 3.53; N, 5.26%. Found: C, 45.10; H, 3.64; N, 5.37%.

## 2.3. Preparation of stationary phase

A column packing material was prepared as described previously [9,17] using macroporous silica gel and packed into 25 cm×0.46 cm I.D. stainless-steel tubes by conventional high-pressure slurry packing technique using a model CCP-085 Econo packer pump (Chemco, Osaka, Japan). The plate numbers of columns were 3300–8500 for benzene with hexane–2-propanol (90:10, v/v), 0.5 ml/min, as an eluent at 20°C. The dead time ($t_0$) of the columns was estimated using 1,3,5-tri-*tert*.-butylbenzene as a non-retained compound.

## 2.4. Apparatus

All chromatographic experiments were performed on a JASCO BIP-I liquid chromatograph equipped

with a UV (JASCO-875-UV) and a polarimetric (JASCO 181-C) detectors. A Model 7125 injector containing 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 FT-IR 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 0.01 cm cell using a JASCO J-720 L spectropolarimeter. $^1$H NMR spectra were taken in [$^2$H_5]pyridine solution at 80°C using a Varian VXR-500 or Varian Gemini 400 NMR spectrometer operating at 500 or 400 MHz, respectively. Tetramethylsilane was used as the internal standard.

## 3. Results and discussion

Fig. 4 shows a chromatogram of the resolution of (±)-*trans*-stilbene oxide (**4**) on a column packed with cellulose tris(3-fluoro-5-methylphenylcarbamate) (**1a**). The enantiomers eluted at retention times
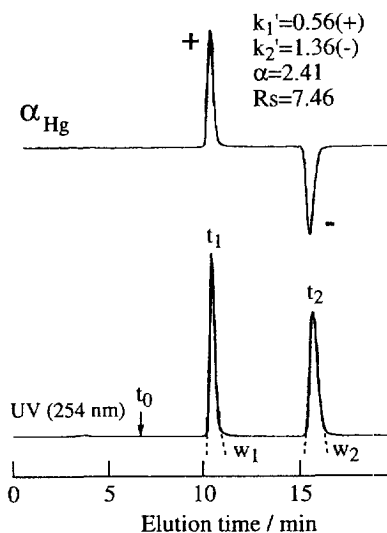


Fig. 4. Separation of enantiomers of (±)-*trans*-stilbene oxide (**4**) on cellulose tris(3-fluoro-5-methylphenylcarbamate) (**1a**). Chromatographic conditions are shown in Table 1.

Table 1
Resolution of **3–12** on phenylcarbamate derivatives of cellulose

| Racemate | 1a: 3-F-5-CH$_3$ | | | 1b: 3-Cl-5-CH$_3$ | | | 1c: 3-Br-5-CH$_3$ | | | 1d: 3,5-F$_2$[a] | | | 1e: 3,5-Cl$_2$[a] | | | 1f: 3,5-(CH$_3$)$_2$[a] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $k_1'$ | $\alpha$ | $R_s$ | $k_1'$ | $\alpha$ | $R_s$ | $k_1'$ | $\alpha$ | $R_s$ | $k_1'$ | $\alpha$ | $R_s$ | $k_1'$ | $\alpha$ | $R_s$ | $k_1'$ | $\alpha$ | $R_s$ |
| 3 | 0.77(+) | 1.58 | 4.68 | 0.74(+) | 1.95 | 5.22 | 0.65(+) | 1.80 | 6.00 | 0.88(+) | 1.73 | 3.25 | 0.87(+) | 1.65 | 3.89 | 0.97(+) | 1.31 | 1.92 |
| 4 | 0.56(+) | 2.41 | 7.46 | 0.54(+) | 2.05 | 5.13 | 0.45(+) | 1.88 | 5.20 | 0.51(+) | 1.81 | 3.06 | 0.56(+) | 1.84 | 4.20 | 0.74(-) | 1.68 | 3.22 |
| 5 | 0.76 | 1.42 | 2.94 | 0.69(-) | 1.43 | 1.90 | 0.59 | 1.36 | 2.64 | 0.34(-) | 1.27 | | 0.28(-) | 1.38 | 0.87 | 2.13(-) | 2.59 | 6.40 |
| 6 | 0.71(+) | 1.55 | 3.43 | 0.69(+) | 1.17 | 0.97 | 0.68(+) | 1.04 | | 0.44(+) | ca.1 | | 0.40(+) | 1.29 | 0.84 | 1.37(+) | 1.34 | 1.87 |
| 7 | 0.88(+) | 1.49 | 3.39 | 0.57(+) | 1.39 | 1.32 | 0.46(+) | 1.26 | 1.46 | 1.72(+) | 2.36 | 6.94 | 0.76(+) | 1.82 | 4.06 | 0.42(+) | ca.1 | |
| 8 | 1.68(+) | 1.36 | 4.47 | 1.90(-) | 1.33 | 3.33 | 1.50(-) | 1.29 | 3.49 | 3.62(-) | 1.21 | 1.90 | 2.65(-) | 1.26 | 1.95 | 1.17(-) | 1.15 | 0.90 |
| 9 | 1.72(-) | 1.11 | 1.65 | 1.43(-) | 1.22 | 2.18 | 1.11(-) | 1.19 | 2.21 | 2.01(-) | 1.18 | 1.64 | 1.55(-) | 1.20 | 1.48 | 1.47(-) | 1.41 | 3.08 |
| 10 | 4.18(-) | 1.44 | 6.84 | 3.04(-) | 1.11 | 1.41 | 2.31(-) | 1.09 | 1.46 | 3.77(-) | ca.1 | | 3.08(-) | 1.21 | 1.91 | 2.43(+) | 1.58 | 4.38 |
| 11 | 1.08 | 1.11 | 0.80 | 0.81(+) | 1.22 | 1.26 | 0.66(+) | 1.22 | 1.12 | 0.68(+) | 1.63 | 2.46 | 0.59(+) | 1.41 | 1.47 | 0.83(+) | 3.17 | 6.17 |
| 12 | 2.05(-) | 1.71 | 3.26 | 3.28(-) | 1.33 | 1.97 | 1.76(-) | 1.15 | 1.28 | 1.70(+) | 1.05 | | 1.62(+) | 1.11 | 0.75 | 2.36(-) | 1.83 | 4.39 |

Conditions: column, 25×0.46 cm I.D.; eluent, hexane–2-propanol (90:10); flow rate, 0.5 ml/min. The sign of optical rotation of the first-eluting isomer is shown in the parentheses of $k_1'$.

[a] Data are cited from Ref. [14].

Table 2
Resolution of **3–12** on phenylcarbamate derivatives of amylose

| Racemate | 2a: 3-F-5-CH$_3$ | | | 2b: 3-Cl-5-CH$_3$ | | | 2c: 3-Br-5-CH$_3$ | | | 2d: 3,5-F$_2$[a] | | | 2e: 3,5-Cl$_2$[a] | | | 2f: 3,5-(CH$_3$)$_2$[a] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $k_1'$ | $\alpha$ | $R_s$ | $k_1'$ | $\alpha$ | $R_s$ | $k_1'$ | $\alpha$ | $R_s$ | $k_1'$ | $\alpha$ | $R_s$ | $k_1'$ | $\alpha$ | $R_s$ | $k_1'$ | $\alpha$ | $R_s$ |
| 3 | 0.90(+) | 1.90 | 8.30 | 0.94(+) | 2.20 | 7.80 | 0.67(+) | 2.29 | 7.81 | 0.78(+) | 1.13 | | 0.84(+) | 1.34 | 2.27 | 0.53(+) | 1.58 | 2.30 |
| 4 | 0.71(+) | 1.89 | 7.42 | 0.71(+) | 2.10 | 6.75 | 0.44(+) | 2.23 | 6.03 | 0.32(+) | 1.30 | 0.70 | 0.50(+) | 1.32 | 1.69 | 0.42(+) | 3.04 | 6.67 |
| 5 | 0.78 | 1.11 | | 0.97(-) | 1.29 | 1.81 | 0.68 | 1.30 | 1.47 | 0.39 | 1.00 | | 0.37 | 1.00 | | 1.30(+) | 1.15 | 0.75 |
| 6 | 1.50(+) | 3.79 | 15.29 | 1.84(+) | 4.70 | 17.60 | 1.24(+) | 4.00 | 13.24 | 0.48(+) | 1.64 | 1.83 | 0.88(+) | 2.25 | 6.05 | 2.65(+) | 1.98 | 5.48 |
| 7 | 0.56(+) | 1.07 | | 0.42(+) | ca.1 | | 0.31(+) | ca.1 | | 2.58(-) | 1.08 | | 0.61(+) | ca.1 | | 0.25(+) | ca.1 | |
| 8 | 1.39(+) | ca.1 | | 1.36(+) | ca.1 | | 0.95(+) | ca.1 | | 2.19(-) | ca.1 | | 1.26(-) | ca.1 | | 0.61(-) | ca.1 | |
| 9 | 2.11(+) | ca.1 | | 1.89(+) | 1.06 | 0.68 | 1.33(+) | 1.07 | 1.08 | 1.40(+) | 1.50 | 2.36 | 1.62(+) | 1.10 | 1.02 | 0.93(+) | 1.12 | 0.77 |
| 10 | 6.23(-) | 1.27 | 4.73 | 6.30(-) | 1.11 | 2.15 | 4.28(-) | 1.07 | 1.08 | 3.53(-) | ca.1 | | 6.08(+) | ca.1 | | 3.14(-) | 1.21 | 2.07 |
| 11 | 1.72(+) | 4.36 | 11.68 | 1.05(+) | 1.71 | 2.52 | 0.90(+) | 3.13 | | 0.83(+) | 1.51 | 1.76 | 0.59(-) | 1.11 | | 3.25(+) | 2.01 | 3.59 |
| 12 | 1.90(-) | 1.36 | 1.87 | 2.94(-) | 1.12 | | 2.12(-) | 1.16 | 0.65 | 2.03 | 1.09 | | 1.43(+) | ca.1 | | 2.46(-) | 2.11 | 6.38 |

Conditions are shown in Table 1.

[a] Data are cited from Ref. [14].

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 0.56 and 1.36, and separation factor $\alpha$ [$=k_2'/k_1'$] and resolution factor $R_s$ [$=2(t_2-t_1)/(w_1+w_2)$] were estimated to be 2.41 and 7.46, respectively.

The results of the enantioseparation of ten racemates, Tröger base (3), 4, 1-(9-anthryl)-2,2,2-trifluoroethanol (5), 1,2,2,2-tetraphenylethanol (6), cobalt(III) tris(acetylacetonate) (7), 2-phenylcyclohexanone (8), flavanone (9), benzoin (10), trans-cyclopropanedicarboxylic acid dianilide (11) and 2,2'-dihydroxy-6,6'-dimethylbiphenyl (12) on the 3-halogen-5-methylphenylcarbamates of cellulose (1a–1c) and amylose (2a–2c) are give in Table 1 and Table 2, respectively. For comparison, the enantioseparation results on 3,5-difluoro- (1d and 2d), 3,5-dichloro- (1e and 2e) and 3,5-dimethylphenylcarbamates (1f and 2f) of cellulose and amylose [9,14] are also shown in Table 1 and Table 2, respectively. Among three 3-halogen-5-methylphenylcarbamates of cellulose, 1a with a fluoro substituent at the 3-position showed higher resolving power than 1b and 1c with a chloro and bromo group at the 3-position, respectively, and can resolve all ten racemates with reasonable enantioselectivity. For instance, compounds 4, 6, 7, 8, 10 and 12 were resolved on 1a with larger $\alpha$ values than those on 1b and 1c. It seems worth mentioning that some racemates (3, 4 and 8) were separated better on 1a–1c than on 3,5-dimethylphenylcarbamate of cellulose (1f) which is one of the most powerful and popular columns. Moreover, 1f could not separate the racemic compound 7, which was separated completely on 1a–1c. Similarly, 6 and 10 which were not resolved on 1d were completely resolved on 1a. These results suggest that the 3-halogen-5-methylphenylcarbamates may be a unique CSP possessing both chiral recognition abilities of the corresponding 3,5-dihalogen-substituted phenylcarbamates and 3,5-dimethylphenylcarbamate. The resolving power of the cellulose derivatives tends to decrease in the order 1a>1b>1c, although most racemates were completely or partially resolved even on 1c. No reversed elution order was observed for enantiomers on three 3-halogen-5-methylphenylcarbamates of cellulose (1a–1c) and amylose (2a–2c).

Comparison of the enantioselectivity and the elution order of racemates on the polysaccharide derivatives listed in Table 1 gave useful information regarding the effect of simultaneous introduction of both electron-donating and electron-withdrawing substituents onto the phenyl moieties on the chiral recognition ability. The differences in the chiral recognition abilities of the cellulose derivatives (1a–1f) were observed depending on the racemates. For example, in the resolution of 4, the (+) enantiomer eluted first on 1a–1e, but the (−) enantiomer eluted first on 1f. 1a–1c showed a similar enantioselectivity to those of 1d and 1e, indicating that a similar chiral discrimination seems to proceed on 1a–1e for 4. On the contrary, 1a–1c showed a similar chiral discrimination to that of 1f in the resolution of 12; the (+) enantiomer eluted first on 1a–1c and 1f, while the (−) enantiomer eluted first on 1d and 1e. These differences in chiral discrimination may be explained as follows.

The most important adsorbing site for chiral recognition on the phenylcarbamate derivatives of polysaccharides is considered to be the polar carbamate residues, which can interact with a racemate via hydrogen bonding on the NH and C=O groups and the dipole–dipole interaction on the C=O (Fig. 5) [1–5,9,11–15]. The importance of hydrogen bonding for chiral discrimination has recently been supported by $^1$H NMR studies using $C^2HCl_3$-soluble phenylcarbamate derivatives of polysaccharides [17–19]. It was also reported [9] that the introduction of either electron-donating or electron-withdrawing substituents at the 3- or 4-position onto the phenyl
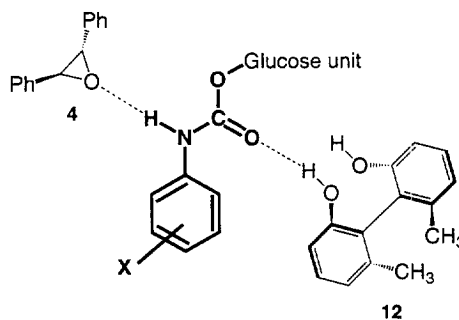


Fig. 5. Schematic interactions of 4 and 12 with the phenylcarbamoyl residue.

moieties tends to improve optical resolution abilities of phenylcarbamate derivatives of cellulose. For instance, cellulose tris(3,5-dimethylphenylcarbamate) (**1f**), having electron-donating substituents can effectively resolve polar compounds such as alcohols which can interact with the carbonyl groups of the carbamate moieties, and the derivatives having electron-withdrawing substituents, for instance, cellulose tris(3,5-dichlorophenylcarbamate) (**1e**), possess higher resolving power for the compounds bearing a carbonyl and/or ether oxygen that can be adsorbed on the N–H groups of the carbamate moieties. The characteristic enantioseparation of many racemates on the cellulose phenylcarbamate derivatives has been proving this explanation. Intramolecular hydrogen bonds between adjacent carbamate moieties of neighboring glucose units also contribute to maintain a higher-order structure of the phenylcarbamate derivatives. Such a higher-order structure must be important for effective chiral recognition of these derivatives [12]. Besides these polar interactions, the π–π interaction between the phenyl group of

phenylcarbamate derivatives and the aromatic groups of a solute may play some role for chiral recognition, because several nonpolar aromatic compounds have also been resolved [20]. In order to investigate this, $^1$H NMR, IR and CD spectra of the carbamates were measured (Fig. 6 and Fig. 7).

Fig. 6A shows $^1$H NMR spectra of the N–H region of the phenylcarbamate derivatives of cellulose (**1b**, **1e**, and **1f**). The N–H proton chemical shifts of the derivatives were greatly dependent on the substituents, and two or three resonances corresponding to the N–H protons of the carbamate groups at 2, 3 and 6 positions of glucose units were observed. The N–H resonance at the lowest field may be assigned to the N–H proton at the 6 position [21]. The chemical shifts of the N–H resonances reflect the acidity of N–H protons and shift downfield with an increase in acidity of N–H [9]. The NH protons of **1b** resonate downfield of those of **1f**, but upfield of those of **1e**. There are almost no differences in the chemical shifts of the NH proton resonances between **1a**–**1c** irrespective of the
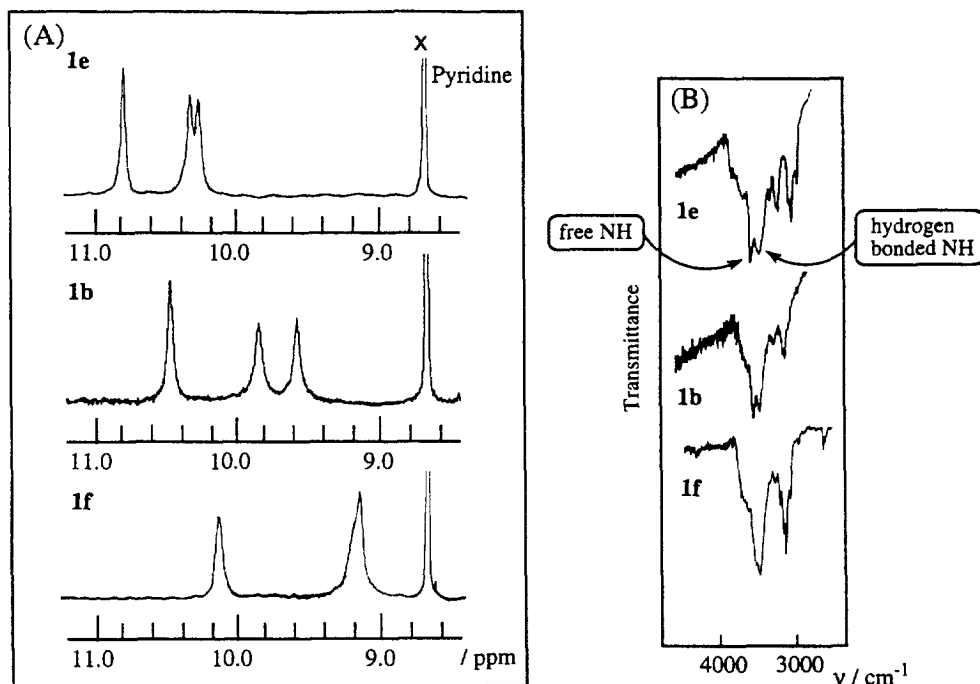


Fig. 6. $^1$H NMR ([$^2$H_5]pyridine, 80°C, 500 MHz) (A) and IR (KBr) (B) spectra of cellulose trisphenylcarbamate derivatives (**1b**, **1e** and **1f**).
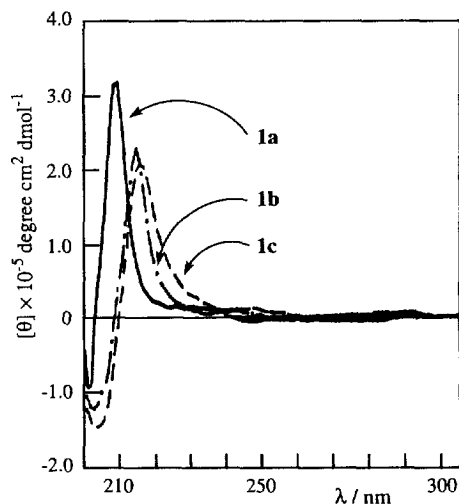
Fig. 7. CD spectra of cellulose trisphenylcarbamate derivatives (1a, 1b and 1c) in THF.

halogen atoms on the phenyl groups. These results indicate that the NH protons of 1a–1c are more acidic than 1f, but less acidic than 1e. The same changes may be induced on the carbonyl sites; the electron density of the carbonyl groups of 1a–1c may be enhanced compared with those of 1d and 1e.

The racemate of 4 probably interacts with the carbamate residues through the hydrogen bond, as shown in Fig. 5. The ether oxygen may interact more strongly with acidic NH group of the phenylcarbamate residue of 1a–1e than that of the 3,5-dimethylphenylcarbamate residue (1f). Thus, the cellulose derivatives 1a–1e are expected to show a similar chiral discrimination toward 4. On the other hand, the most important site of 12 for the interaction with the CSPs seems to be the hydroxy group. Therefore, 12 may interact mainly with the carbonyl group of the phenylcarbamate residue as shown in Fig. 5. Therefore, 1a–1c will show a similar chiral discrimination to that of 1f in the resolution of 12.

The N–H groups of phenylcarbamate moieties can contribute to chiral recognition by direct interaction with some racemates as a chiral site and also by maintaining 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 solute [12]. Fig. 6B shows IR spectra of the N–H region of the phenylcarbamates

of cellulose. Two peaks are observed around 3500 cm$^{-1}$; the peak in the lower wave number is assigned to the N–H group involved in intramolecular hydrogen bond between the carbamate residues of neighboring glucose units and the peak in the higher wave number to a free N–H bond [9,12]. High fraction of the latter band indirectly means more ordered secondary structure of the derivatives. Both free N–H and intramolecularly hydrogen bonded N–H were observed in the IR spectra of the derivatives. A large fraction of the N–H appears to be free in the case of 1e, while the N–H groups are markedly involved in intramolecular hydrogen-bonding in 1f. 1b shows almost the same intensities of these two peaks in its IR spectrum, indicating that introduction of the methyl substituent at the 5 position increases the ratio of hydrogen bonded N–H groups, which means the formation of more ordered rigid structure of the derivative compared with those of 1d and 1e. The derivative 1b may have an intermediate character between 1e and 1f. Other derivatives 1a and 1c also showed similar IR spectra. However, some racemates can be resolved better on 1a–1c rather than 1d–1f as mentioned before. This must be the result of the balance of the polarity of the carbamate residue and intramolecular hydrogen bond ability of 1a–1c.

Another piece of evidence for the regular structure of 1a–1c can be seen in their CD spectra (Fig. 7). The CD spectra showed similar patterns, but different intensity of the peaks depending on the substituents. The derivative 1a with high 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. This may influence the chiral recognition power of the CSP.

All 3-halogen-5-methylphenylcarbamates of cellulose (1a–1c) and amylose (2a–2c) prepared in this study are scarcely soluble in hexane with 0–20% of 2-propanol mixture and have a longer life time as a CSP over cellulose tris(3,5-dichlorophenylcarbamate) coated on silica gel.

In the amylose derivatives, similar substituent effects on the enantioselectivity was also observed, although the effect was less pronounced compared with that on cellulose derivatives.

The new amylose derivatives showed relatively high chiral recognition ability comparable to that of

amylose tris(3,5-dimethylphenylcarbamate) (**2f**). The enantioselectivity of the derivatives was dependent on the racemates. For instance, the racemates **11** and **6** were separated better on **2a** and **2b** than on **2d–2f** with very high $\alpha$ values (4.36 and 4.70, respectively). The derivatives **2a–2c** also showed an intermediate character between **2d** or **2e** and **2f** in their IR and $^1$H NMR spectra. The CD spectra of the amylose derivatives showed a different pattern of CD depending on the substituents. These amylose derivatives may have different ordered structures, which

may result in characteristic high chiral recognition ability of each derivative.

The high chiral recognition abilities exhibited by the new polysaccharide phases were also proven in the separation of enantiomers of some pharmacologically important compounds such as nimodipin, articain, camazepam and acebutolol (Fig. 8). It must be denoted that under the same chromatographic conditions these compounds were not separated on tris(3,5-dimethylphenylcarbamate) of cellulose (**1f**) showing high resolving abilities for many racemates,
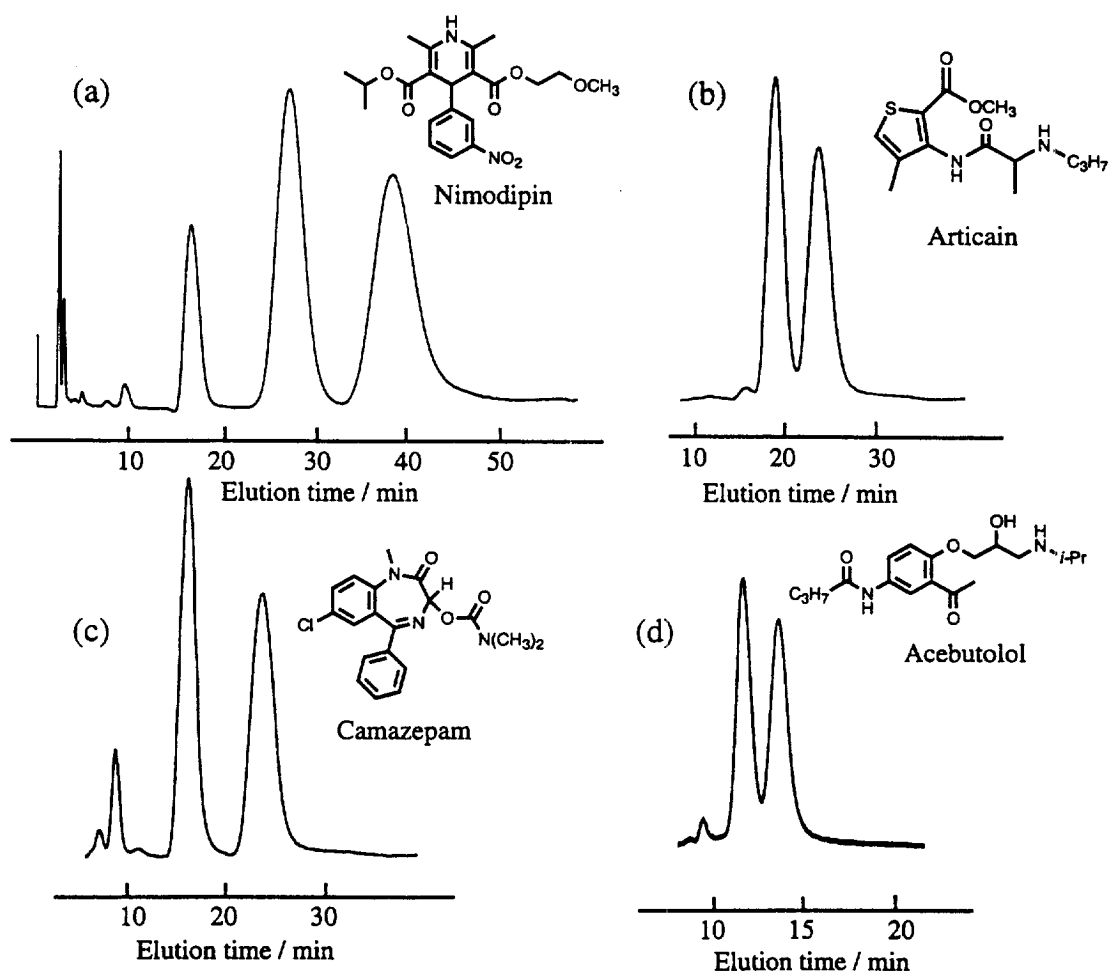


Fig. 8. Separation of enantiomers of nimodipin (a), articain (b), camazepam (c) and acebutolol (d) on cellulose tris(3-chloro-5-methylphenylcarbamate) (**1b**). Eluent: hexane–2-propanol (93:7) (a), hexane–2-propanol (85:15) (b), and hexane–ethanol (85:15) (c and d). Flow-rate: 1.0 ml/min.
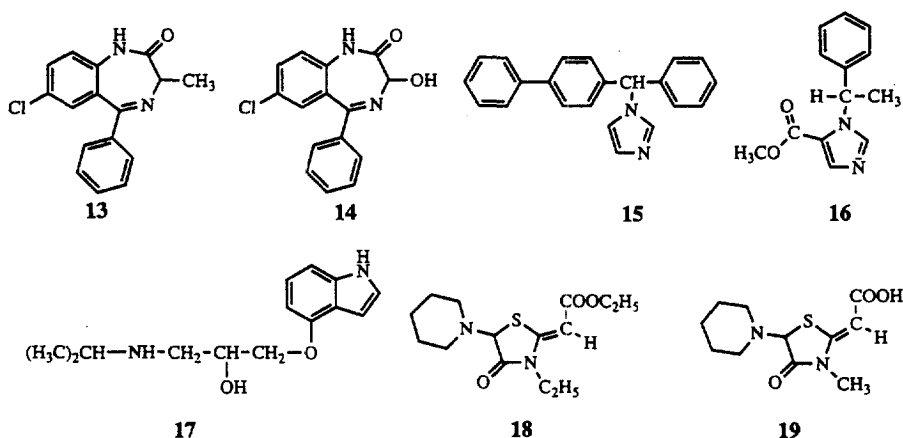
Fig. 9. Structure of racemates (13–19).

including drugs [5,9,22–24]. Moreover, the CSP **1b** completely resolved other drugs (**13–19**) (Fig. 9) better than **1f** (Table 3).

tives. Potential of use of new CSPs for separation of some practically important drug enantiomers was also demonstrated.

## 4. Conclusion

Chiral recognition abilities of six new 3-halogen-substituted-5-methylphenylcarbamates of cellulose and amylose as CSPs for HPLC were evaluated and compared with those of the corresponding 3,5-difluoro-, 3,5-dichloro- and 3,5-dimethylphenylcarbamates. Some correlations were established between the polarity of the carbamate moieties and enantiomer resolving abilities of the above mentioned deriva-

## Acknowledgements

Table 3
Resolution of **13–19** on **1b** and **1f**

| Racemate | **1b**: 3-Cl-5-CH$_3$ | | | **1f**: 3,5-(CH$_3$)$_2$ | | |
|---|---|---|---|---|---|---|
| | $k'_1$ | $\alpha$ | $R_s$ | $k'_1$ | $\alpha$ | $R_s$ |
| 3-Methylbenzodiarepine(**13**)[a] | 2.0 | 1.47 | 1.5 | 1.46 | 1.22 | 0.8 |
| Oxazepam(**14**)[b] | 2.0 | 1.28 | 1.0 | 5.8 | 1.37 | 1.7 |
| Bifonazol(**15**)[b] | 10.2 | 1.40 | 1.5 | | | |
| Metomidate(**16**)[b] | 5.8 | 1.20 | 1.0 | 1.31 | 1.16 | 0.7 |
| Pindolol(**17**)[c] | 3.2 | 1.50 | 1.6 | 3.17[d] | 5.07[d] | >3[d] |
| Piprozolin(**18**)[c] | 2.1 | 2.70 | 3.7 | 5.04 | 1.31 | 2.0 |
| Etozolin(**19**)[c] | 3.1 | 2.90 | 3.7 | 1.6 | 2.45 | 4.0 |

[a] Eluent, hexane–2-propanol (90:10); flow-rate, 1.0 ml/min.
[b] Eluent, hexane–2-propanol (85:15); flow-rate, 1.0 ml/min.
[c] Eluent, hexane–2-ethanol (85:15); flow-rate, 1.0 ml/min.
[d] Eluent, hexane–2-propanol–HNEt$_2$ (80:20:0.1); flow-rate, 0.5 ml/min.

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