Chiral Discrimination by 4-Chloro- and 4-Bromophenylcarbamates of Maltooligosaccharides in HPLC, NMR, and CD

Eiji Yashima, Pennapa Sahavattanapong, Chiyo Yamamoto, and Yoshio Okamoto*

Department of Applied Chemistry, School of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-01

(Received March 31, 1997)

4-Chloro- and 4-bromophenylcarbamates of maltooligosaccharides ($n=5$, 6, and 7) were prepared and their chiral recognition abilities as a chiral stationary phase for HPLC were evaluated. The maltooligosaccharides carbamates exhibited a characteristic chiral recognition depending on the racemates and resolved several racemates in HPLC. The enantioselectivity as well as elution order of enantiomers on the maltooligosaccharide derivatives were similar to those on the corresponding amylose phenylcarbamte derivatives. The maltooligosaccharides also showed chiral discrimination for some racemates in NMR; the proton resonances of racemates, such as *trans*-stilbene oxide, benzoin, and 1,2,2,2-tetraphenylethanol were split into a pair of peaks due to the enantiomers. A good correlation between the NMR results and the chromatographic elution order was observed for most racemates. An enantiomeric guest, bilirubin, was also chirally discriminated by the maltooligosaccharides in chloroform to show an induced CD. The structures of the maltohexaose derivatives were examined by molecular mechanics and molecular dynamics simulations.

Chromatographic enantioseparations by high-performance liquid chromatography (HPLC) have developed considerably in the past decade and have become a highly useful method for both analytical and preparative separations of enantiomers. Since chiral stationary phases (CSPs) are key materials for this separation, many CSPs have been prepared.[1—5] We found that phenylcarbamate derivatives of cellulose and amylose are very effective CSPs for enantioseparation of a wide range of racemates, including many drugs.[6—8] The chiral recognition abilities of the derivatives depend greatly on the substituents introduced on the phenyl moieties. The introduction of an electron-donating or electron-withdrawing substituent, for instance, a methyl or chloro group, at the *meta*- and/or *para*-position of the phenyl group improves the chiral recognition ability.[9]

Although the mechanism of chiral discrimination on the polysaccharide derivatives has not been satisfactorily elucidated at a molecular level, some interesting approaches to understanding the mechanism have been carried out by chromatographic elucidation,[8] computational methods,[10] and spectroscopic methods.[11,12] Determination of the exact structures of the phenylcarbamate derivatives should be necessary in order to solve the mechanism. Zugenmaier et al. reported a left-handed 3/2 helical structure for cellulose trisphenylcarbamate by means of X-ray analysis.[13] They also proposed a left-handed 4/1 helical structure for amylose trisphenylcarbamate.[14]

The present work was carried out in order to obtain information regarding the structure of the phenylcarbamate derivatives of amylose. For this purpose, a series of 4-chloro- (**1a—1c**) and 4-bromophenylcarbamates (**2a—2c**) of maltooligosaccharides ($n=5$, 6, and 7) (Chart 1) were prepared, since the corresponding amylose tris(4-chloro- or 4-bromophenylcarbamate)s were recently found to show high chiral recognition as CSPs for HPLC.[15] In addition, a heavy atom introduction at the *para*-position on the phenyl group of the maltooligosaccharides would be preferable for X-ray analysis if they crystallize. In this study the chiral recognition abilities of the 4-chloro- and 4-bromophenylcarbamates of maltooligosaccharides were examined as CSPs for HPLC. In the course of this study, we have found that these maltooligosaccharides were soluble in chloroform and exhibited chiral discrimination in NMR and circular dichroism (CD). These findings may be useful to investigate the chiral recognition mechanism of the phenylcarbamate derivatives of amylose using the oligomers as a model compound. Molecular mechanics and molecular dynamics calculations of the maltooligosaccharides were also carried out for the structure investigation of the derivatives.

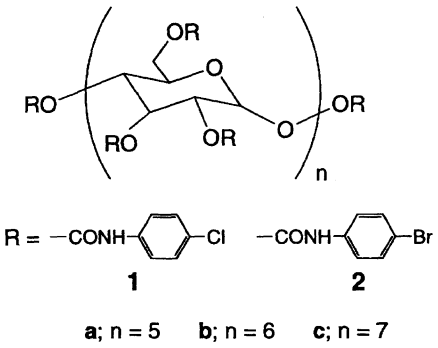


Chart 1.

Experimental

Materials. Maltooligosaccharides ($n=5$, 6, 7) were kindly supplied from Nihon Shokuhin Kako (Yaizu, Shizuoka). All solvents

used in the preparation of CSPs were of analytical reagent grade, they were carefully dried, and distilled before use. Solvents used in the chromatographic experiments were of HPLC grade. $CDCl_3$ (99.8 atom %D, Nacalai tesque) was dried over molecular sieves 4A (Nacalai tesque) and stored under nitrogen. Racemates (Chart 2) were commercially available or prepared by the usual method.[16] (*S*)-(−)-1,1′-Bi-2-naphthol ($[\alpha]_D^{25}$ −32°, *c* 1.5 g dL^{-1}, THF) and 4-chloro- and 4-bromophenyl isocyanates were obtained from Tokyo Kasei. (*S*)-(+)-Benzoin was from Aldrich. (−)- and (+)-Isomers of *trans*-stilbene oxide (**3**) and 1,2,2,2-tetraphenylethanol (**8**) were obtained by chromatographic enantioseparation of the racemates using cellulose tris(3,5-dimethylphenylcarbamate) as a CSP.[8,9] Bilirubin was purchased from Tokyo Kasei and used without further purification. The purity of bilirubin was checked by ^{1}H NMR.[17,18] We also measured a CD spectrum of bilirubin in CH_2Cl_2 in the presence of quinidine and found that the magnitude of the Cotton effects was almost the same as the reported data.[19] These results clearly indicate that the bilirubin used in this study consisted of almost pure IX isomer. Chloroform used for the CD measurements of bilirubin was distilled in vacuum over sodium hydride and stored under nitrogen. Tris(4-chlorophenylcarbamate) and tris(4-bromophenylcarbamate) of amylose were prepared according to the previously reported method.[15]

Preparation of 4-Chloro- and 4-Bromophenylcarbamates of Maltooligosaccharides. 4-Chloro- and 4-bromophenylcarbamates of maltooligosaccharides were prepared by the reaction with an excess of the corresponding phenyl isocyanates in dry pyridine at ca. 80 °C.[11,15] A typical procedure for the preparation of 4-chlorophenylcarbamate of maltopentaose (**1a**) is described below. Maltopentaose (1.0 g) which had been dried in vacuo at room temperature for ca. 2 h was allowed to react with an excess of 4-chlorophenyl isocyanate (8.0 ml, 63 mmol) in dry pyridine (20 ml) at ca. 80 °C for 20 h. A crude product isolated as methanol/H_2O (6/1 (v/v)) insoluble fraction contained a urea derivative as a by-product from the IR spectrum. Purification was then carried out by reprecipitation of the product from chloroform to hexane. The obtained product was collected by centrifugation, washed with hexane, and dried in vacuo at 60 °C for 3 h (75% yield).

4-Chlorophenylcarbamate of Maltopentaose (1a): IR (KBr) 3332, 3400 (ν_{NH}), 1721 cm^{-1} ($\nu_{C=O}$); ^{1}H NMR (pyridine-d_5) δ = 4.0—6.0 (br, glucose protons, 7H), 6.9—7.8 (br, aromatic, 12H), 9.6—11.0 (br, NH, 3H). Found: C, 52.03; H, 3.26; N, 7.08%. Calcd for $(C_{149}H_{120}O_{43}N_{17}Cl_{17})_n$: C, 52.03; H, 3.52; N, 6.93%.

4-Chlorophenylcarbamate of Maltohexaose (1b): 39% yield. IR (KBr) 3300, 3406 (ν_{NH}), 1742 cm^{-1} ($\nu_{C=O}$); ^{1}H NMR (pyridine-d_5) δ = 4.0—6.1 (br, glucose protons, 7H), 6.8—7.8 (br, aromatic, 12H), 9.5—11.0 (br, NH, 3H). Found: C, 52.05; H, 3.36; N, 6.93%. Calcd for $(C_{176}H_{142}O_{51}N_{20}Cl_{20})_n$: C, 52.04; H, 3.52; N, 6.90%.

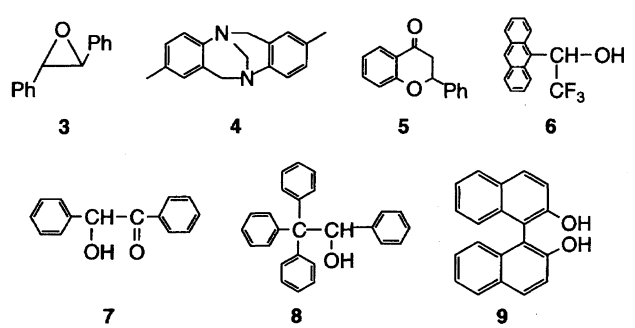


Chart 2.

4-Chlorophenylcarbamate of Maltoheptaose (1c): 43% yield. IR (KBr) 3320, 3408 (ν_{NH}), 1721 cm^{-1} ($\nu_{C=O}$); ^{1}H NMR (pyridine-d_5) δ = 4.0—6.1 (br, glucose protons, 7H), 6.8—7.8 (br, aromatic, 12H), 9.6—11.0 (br, NH, 3H). Found: C, 52.05; H, 3.34; N, 6.85%. Calcd for $(C_{203}H_{164}O_{59}N_{23}Cl_{23})_n$: C, 52.04; H, 3.53; N, 6.88%.

4-Bromophenylcarbamate of Maltopentaose (2a): 21% yield. IR (KBr) 3308, 3400 (ν_{NH}), 1740 cm^{-1} ($\nu_{C=O}$); ^{1}H NMR (pyridine-d_5) δ = 3.8—6.0 (br, glucose protons, 7H), 7.0—7.8 (br, aromatic, 12H), 9.6—10.7 (br, NH, 3H). Found: C, 42.65; H, 2.86; N, 5.65%. Calcd for $(C_{149}H_{120}O_{43}N_{17}Br_{17})_n$: C, 42.66; H, 2.88; N, 5.68%.

4-Bromophenylcarbamate of Maltohexaose (2b): 30% yield. IR (KBr) 3332, 3400 (ν_{NH}), 1725 cm^{-1} ($\nu_{C=O}$); ^{1}H NMR (pyridine-d_5) δ = 3.8—6.0 (br, glucose protons, 7H), 7.0—7.8 (br, aromatic, 12H), 9.4—10.8 (br, NH, 3H). Found: C, 42.69; H, 2.87; N, 5.75%. Calcd for $(C_{176}H_{142}O_{51}N_{20}Br_{20})_n$: C, 42.69; H, 2.89; N, 5.66%.

4-Bromophenylcarbamate of Maltoheptaose (2c): 39% yield. IR (KBr) 3306, 3400 (ν_{NH}), 1734 cm^{-1} ($\nu_{C=O}$); ^{1}H NMR (pyridine-d_5) δ = 3.8—6.0 (br, glucose protons, 7H), 7.0—7.8 (br, aromatic, 12H), 9.4—10.7 (br, NH, 3H). Found: C, 42.78; H, 2.85; N, 5.74%. Calcd for $(C_{203}H_{164}O_{59}N_{23}Br_{23})_n$: C, 42.72; H, 2.90; N, 5.65%.

Preparation of Stationary Phase. A column packing material was prepared as described previously[11,15] 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). The plate numbers of columns were 3000—8000 for benzene with hexane/2-propanol (99/1 (v/v)) as an eluent at a flow rate of 0.5 ml min^{-1}. The dead time (t_0) of the columns was estimated using 1,3,5-tri-*t*-butylbenzene as a non-retained compound.

Instruments. All chromatographic experiments were performed on a JASCO 980-PU liquid chromatograph equipped with a UV (JASCO-970-UV) and a polarimetric (JASCO OR-990) detector. 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 THF solutions using a JASCO Ubest-55 spectrophotometer. CD spectra of the maltooligosaccharides and bilirubin were measured in THF (ca. 0.5 mg ml^{-1}) and chloroform (0.175 mg ml^{-1}), respectively, in a 0.05 cm quartz cell using a JASCO J-720 L spectropolarimeter. ^{1}H NMR spectra were taken in pyridine-d_5 solution at 80 °C using a Varian VXR-500 NMR spectrometer operating at 500 MHz. TMS was used as the internal standard.

Computational Studies. Molecular modeling, molecular mechanics, and molecular dynamics calculations were performed with the Dreiding force field (version 2.21)[20] as implemented in Cerius^2 software (version 1.6.2, Molecular Simulations Inc., Burlington, MA, USA) running on an Indigo^2–Extreme graphics workstation (Silicon Graphics). Charges on atoms were calculated using QEq[20] in Cerius^2 and total charges of molecules were zero. First, the coordinates of a repeating unit of amylose were taken from the crystal structure data of amylose[21] in the Cambridge Structural Database 3D Graphics Search System.[22] Each hydroxy group at the 2, 3, and 6 positions of glucose units was then replaced by 4-chloro- or 4-bromophenylcarbamoyloxy residues in a similar way to the reported method.[11] Next, the monomeric units were connected to give hexamers of **1b** and **2b** having the same left-handed 6/1 helix to native amylose, respectively. The phenylcarbamoyl groups were then

added at the 1- and 4-hydroxy groups of both ends of the hexamers. The energy minimization was accomplished first by Conjugate Gradient 200 (CG 200) and then by Fletcher Powell (FP) until the root mean square (rms) value became less than 0.01 kcal mol^{-1} Å^{-1}, respectively. Molecular dynamics simulations were run for 300 ps at 500 K with a step size of 1 fs under constant NVT conditions using the Hoover algorithm; the energy minimization was finally accomplished by CG 200, followed by FP in a similar way.

Results and Discussion

Chromatographic Enantioseparation. Figure 1 shows a chromatogram of the resolution of racemic flavanone (**5**) on a column packed with 4-bromophenylcarbamate of maltohexaose (**2b**) coated on silica gel. The enantiomers eluted at retention times of t_1 and t_2 and were completely separated. Capacity factors, k'_1 [$=(t_1-t_0)/t_0$] and k'_2 [$=(t_2-t_0)/t_0$], were 2.97 and 3.91, respectively. The separation factor α [$=k'_2/k'_1$] reflecting the chiral recognition ability of a CSP was estimated to be 1.32. The results of enantioseparation of six racemates, *trans*-stilbene oxide (**3**), Tröger base (**4**), **5**, 1-(9-anthryl)-2,2,2-trifluoroethanol (**6**), benzoin (**7**), and 1,2,2,2-tetraphenylethanol (**8**), on **1a**—**c** and **2a**—**c** are given in Tables 1 and 2, respectively. For comparison, resolution results on the corresponding amylose derivatives, tris(4-chlorophenylcarbamate) (Cl-AM) and tris(4-bromophenylcarbamate) (Br-AM) of amylose are also shown in Tables 1 and 2. Because the maltooligosaccharide derivatives were soluble in polar solvents, a nonpolar eluent such as a hexane/2-propanol (99/1 (v/v)) was used as the eluent, while hexane/2-propanol (90/10 (v/v)) was employed for the resolution on Cl-AM and Br-AM.

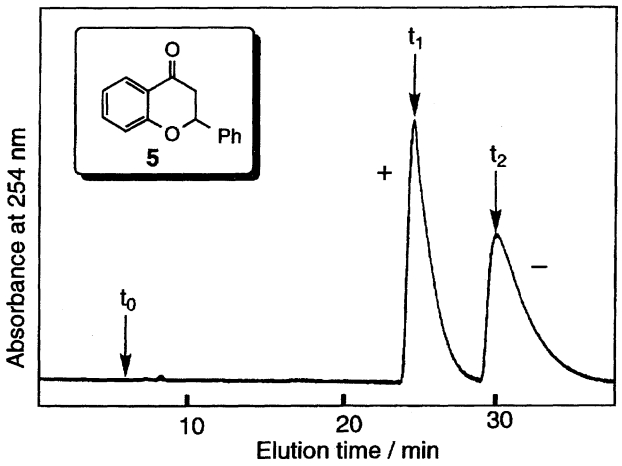


Fig. 1. Resolution of racemic flavanone (**5**) on a column packed with 4-bromophenylcarbamate of maltohexaose (**2b**). Chromatographic conditions are shown in Table 1.

As shown in Tables 1 and 2, clear correlation was not observed between the number (n) of glucose units and the separation factor (α). Although, the amylose derivatives showed the best resolving power for most of racemates, especially for **3** and **8**, the maltooligosaccharides exhibited a characteristic chiral recognition depending on the racemates. For instance, compounds **6** and **7** were better resolved on **1a** with larger α values than those on **1b** and **1c**, while **3** and **4** were more effectively resolved on **1b** and **1c**, respectively.

Table 1. Enantioseparation Results on *p*-Chlorophenylcarbamates of Maltooligosaccharides (**1a**—**1c**) and Amylose (Cl-AM)

	1a[a]		**1b**[a]		**1c**[a]		Cl-AM[b]	
	k'_1 [c]	α	k'_1 [c]	α	k'_1 [c]	α	k'_1 [c]	α
3	0.50 (+)	ca.1	0.26 (+)	1.27	0.53 (+)	1.23	0.53 (+)	3.02
4	2.68 (+)	ca.1	1.29 (+)	1.09	2.33 (+)	1.13	0.79 (+)	1.37
5	3.01 (+)	1.31	1.79 (+)	1.31	3.39 (+)	1.26	0.88 (+)	1.37
6	3.91	1.33	2.82	1.21	14.30	1.04	0.67	1.17
7	9.48 (+)	1.42	6.38 (+)	1.39	4.86 (+)	1.34	5.14 (−)	1.40
8	1.33 (+)	1.36	1.32 (+)	1.51	2.24 (+)	1.38	1.12 (+)	8.29

a) Eluent, hexane/2-propanol (99/1); flow rate, 0.5 ml min^{-1}. b) Eluent, hexane/2-propanol (90/10), flow rate, 0.5 ml min^{-1}. c) The sign in parentheses represents optical rotation of the first-eluted isomer.

Table 2. Enantioseparation Results on *p*-Bromophenylcarbamates of Maltooligosaccharides (**2a**—**2c**) and Amylose (Br-AM)

	2a[a]		**2b**[a]		**2c**[a]		Br-AM[b]	
	k'_1 [c]	α	k'_1 [c]	α	k'_1 [c]	α	k'_1 [c]	α
3	0.45 (+)	ca.1	0.47 (+)	1.36	0.49 (+)	1.24	0.33 (+)	2.54
4	1.68 (+)	ca.1	3.02 (+)	ca.1	2.61 (+)	ca.1	0.79 (+)	1.16
5	2.78 (+)	1.21	2.97 (+)	1.32	3.37 (+)	1.26	1.06 (+)	1.05
6	4.91	ca.1	4.70	1.14	4.15	1.00	0.42	1.00
7	3.35 (+)	1.93	9.77 (+)	1.37	10.67 (+)	1.26	2.69 (−)	1.08
8	0.76 (+)	1.23	2.58 (+)	1.60	1.68 (+)	1.38	0.80 (+)	2.86

a) Eluent, hexane/2-propanol (99/1); flow rate, 0.5 ml min^{-1}. b) Eluent, hexane/2-propanol (90/10), flow rate, 0.5 ml min^{-1}. c) The sign in parentheses represents optical rotation of the first-eluted isomer.

Similarly, **5** and **7** were better resolved on **2b** and **2a**, respectively, among **2a**—**2c** CSPs. It seems worth mentioning that some racemates (**5**—**7**) which were not or only partially resolved on Br-AM were almost completely resolved on either **2a** or **2b**. The elution order of enantiomers was the same on **1a**—**1c** and Cl-AM and on **2a**—**2c** and Br-AM except for that for **7**. Similar differences in the chromatographic enantioseparation were also observed between 3,5-dimethylphenylcarbamates of maltooligosaccharides (n=2—7) and amylose.[23]

Figure 2 showed the CD spectra of the maltooligosaccharide derivatives together with those of the corresponding amylose derivatives in THF. The CD spectra of the maltooligosaccharides showed similar patterns with almost the same intensities of the peaks irrespective of the number of glucose units, whereas those of the amylose derivatives exhibited more intense peaks in the same region. These results suggest that the maltooligosaccharides and the amylose derivatives may have a similar conformation, in which the amylose derivatives may have a more rigid structure. This may be the reason for the chiral recognition abilities of the amylose derivatives being higher than those of the maltooligosaccharides.

Another reason for differences in chiral discrimination between the phenylcarbamate derivatives of the maltooligosaccharides and amylose may be the influence of two terminal glucose units of the maltooligosaccharides, which each have four carbamate residues at the end glucose units. Since the most important adsorbing site for chiral recognition on the phenylcarbamate derivatives of polysaccharides has been 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,[6—9,24] such terminal carbamate residues may show different chiral discrimination compared with those derived from the main chain glucose units.[23] Consequently, these conformational and structural differences appear to influence the chiral recognition abilities of the phenylcarbamate derivatives of the oligo- and polysaccharides.

Chiral Discrimination in NMR. Since the maltooligosaccharides prepared in this study are soluble in chloroform, the interaction between enantiomers and the oligomers occurring in solution can be investigated using NMR spectroscopy. The corresponding amylose derivatives are not soluble in chloroform.

Figure 3(A) shows the 500 MHz ^{1}H NMR spectra of (±)-**8** (5 mg, 0.014 mmol) in the absence (a) and presence (b) of **1a** (30 mg, 0.048 mmol of glucose unit) in $CDCl_3$ (0.9 ml). The methine and OH proton signals of **8** appearing as two doublets centered at δ = 6.317 and 2.231, respectively, were enantiomerically separated into two pairs of doublets in the presence of **1a**. This clearly indicates that **1a** can recognize the enantiomers even in solution. On the basis of the measurement with enantiomerically pure (+)- and (−)-**8**, it was found that the methine and OH protons of the (−)-**8** were more largely shifted to downfield than the (+)-**8** protons. This indicates that the OH groups of **8** may interact with **1a** through hydrogen bonding on the C=O groups. In the chromatographic enantioseparation of (±)-**8** on the CSP **1a**, the (+)-isomer eluted first, followed by the (−)-isomer; complete baseline separations were achieved, indicating that the (−)-isomer adsorbs more strongly on **1a**. This elution

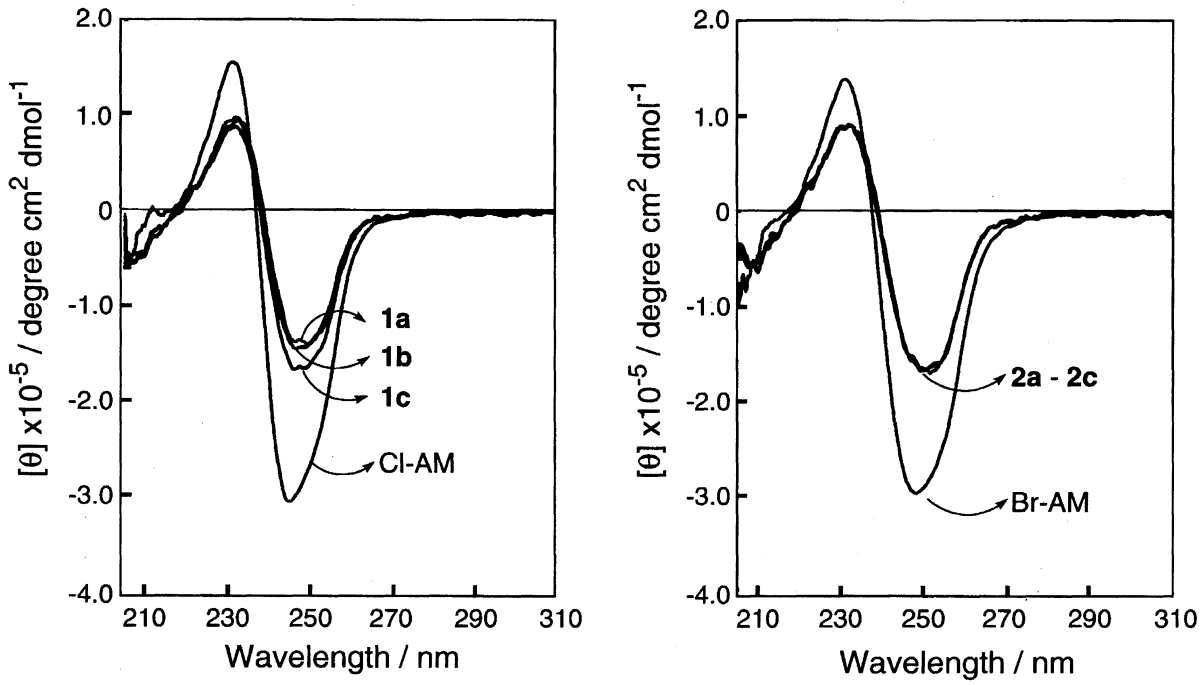


Fig. 2. CD spectra of the maltooligosaccharide derivatives and the corresponding amylose derivatives in THF at ambient temperature. **1a**—**1c**; 7.4—7.5×10^{-4} M of glucose units, **2a**—**2c**; 6.0—6.2×10^{-4} M of glucose units, Cl-AM; 1.6×10^{-3} M of glucose units, Br-AM; 1.3×10^{-3} M of glucose units (1 M = 1 mol dm^{-3}).

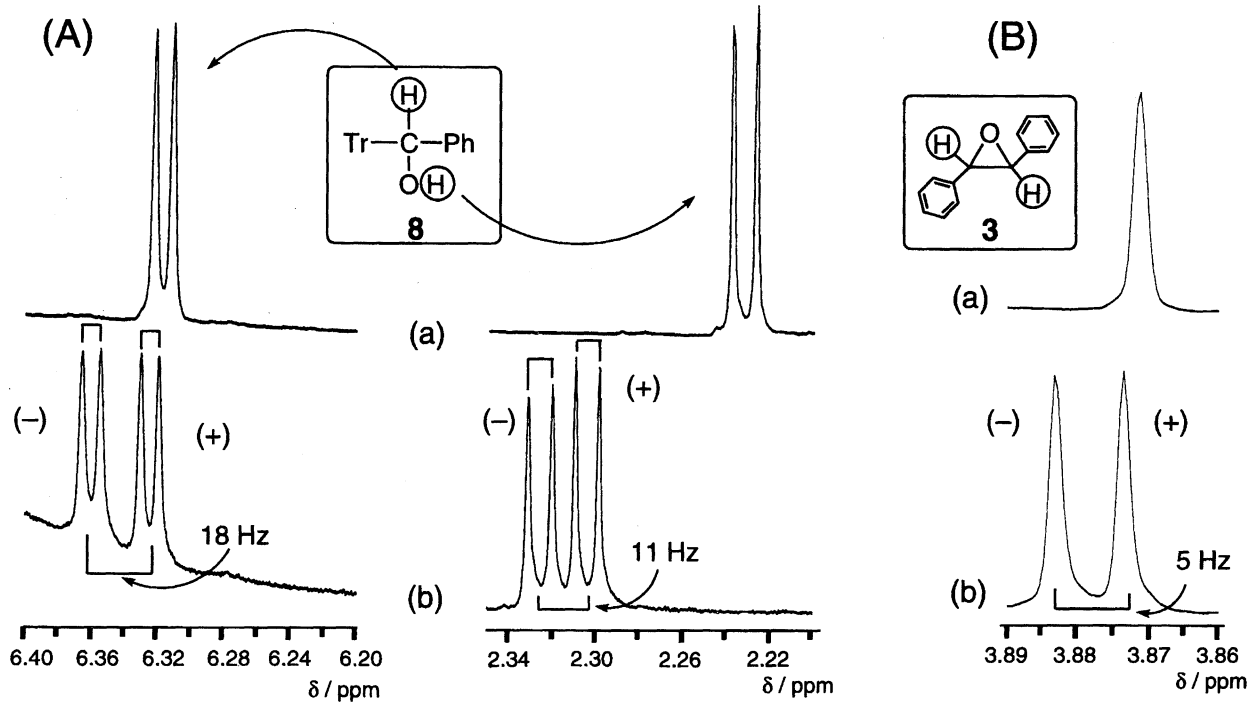


Fig. 3. ^{1}H NMR spectra of the methine and OH proton resonances of **8** (5 mg) (A) and the methine proton resonance of **3** (5 mg) (B) in the absence (a) and presence of **1a** (30 mg) and **2a** (30 mg) (b), respectively, in $CDCl_3$ (0.9 ml)) at ambient temperature.

order may be associated with the shift of the (−)-isomer of **8** observed in the ^{1}N NMR. Similarly, **2a** recognized the enantiomers of **3** in $CDCl_3$ (Fig. 3(B)).

Other phenylcarbamate derivatives of the maltooligosaccharides also showed chiral discrimination for some racemates such as **3**, **7**, **8**, and 1,1′-bi-2-naphthol (**9**) (Tables 3 and 4) in $CDCl_3$. A similar good correlation between the NMR results and the chromatographic elution order on the maltooligosaccharides phases was also observed for the enantiomers of **3** and **8**. However, in the case of **7**, $\Delta\delta$ values of the first-eluted enantiomer in HPLC were larger than those of the second-eluted enantiomer. One possible reason may be solvent effects. The chromatographic enantioseparation was carried out using a mixture of hexane/2-propanol, whereas $CDCl_3$ was used for the ^{1}H NMR experiments.

Chiral Discrimination of Bilirubin. The maltooligosaccharide derivative **1a** also discriminated an enantiomeric guest, such as (4*Z*, 15*Z*)-bilirubin IXa (BR) in chloroform and showed an induced bisignate CD[19,25–27]; $\Delta\varepsilon$ at a shorter wavelength ($\Delta\varepsilon_1$ at ca. 420 nm) is negative and that at a longer wavelength ($\Delta\varepsilon_2$ at ca. 470 nm) is positive (Fig. 4). No absorption of the maltooligosaccharide is observed in this region. Other 4-chloro-substituted maltooligosaccharide derivatives exhibited similar induced CDs, but 4-bromo-substituted derivatives showed rather weak induced CDs; $\Delta\varepsilon_1 = -0.2$ and $\Delta\varepsilon_2 = +0.4$ for **2a**, $\Delta\varepsilon_1 = -0.1$ and $\Delta\varepsilon_2 = +0.3$ for **2b**. Based on exciton-coupling theory,[19,25] BR bound to the derivatives is considered to have preferentially an (*R*)-helix conformation. Recently, *para*-halogenated cellulose trisphenylcarbamate (CTPC) derivatives were also found through CD spectroscopic study to be able to selectively bind one of the BR enantiomers in solution and in the film.[28] On the contrary to the maltooligosaccharides, the BR enantiomer having an (*S*)-helix conformation was bound preferentially to the CTPC derivatives. The reason for differences in chiral discrimination of BR between the phenylcarbamate derivatives of cellulose and maltooligosaccharides is not clear at present time, although the difference in their ordered structures may be important.

Computational Studies. As previously mentioned, the 4-chloro- and 4-bromophenylcarbamates of maltooligosaccharides may have a similar conformation to those of the corresponding amylose derivatives, on the basis of the chromatographic and CD studies. Therefore, molecular mechanics and molecular dynamics calculations of the maltohexaose derivatives, **1b** and **2b**, as a model of the amylose derivatives were performed using the Dreiding force field[20] and the conformations of the **1b** and **2b** were examined. The initial structures of **1b** and **2b** were constructed from the crystal structure data of amylose having a left-handed 6/1 helical structure.[21] The energy minimization and molecular dynamics calculations were then performed to the **1b** and **2b**. Figure 5 shows the structures of **1b** and **2b** after molecular dynamics simulations. The conformations of the **1b** and **2b** were transformed from a left-handed 6/1 helix to almost left-handed 5/1 and 4/1 helices, respectively, after 300 ps molecular dynamics simulations at 500 K. The same helical conformations were maintained during the molecular dynamics simulations even at 300 K. The structure of **2b** was very similar to that of amylose trisphenylcarbamate proposed by Zugenmaier.[14] Although the reason for the difference in the structures between **1b** and **2b** depending on the kind of the halogen atom is not clear, the present results indicate that the corresponding amylose phenylcarbamate derivatives

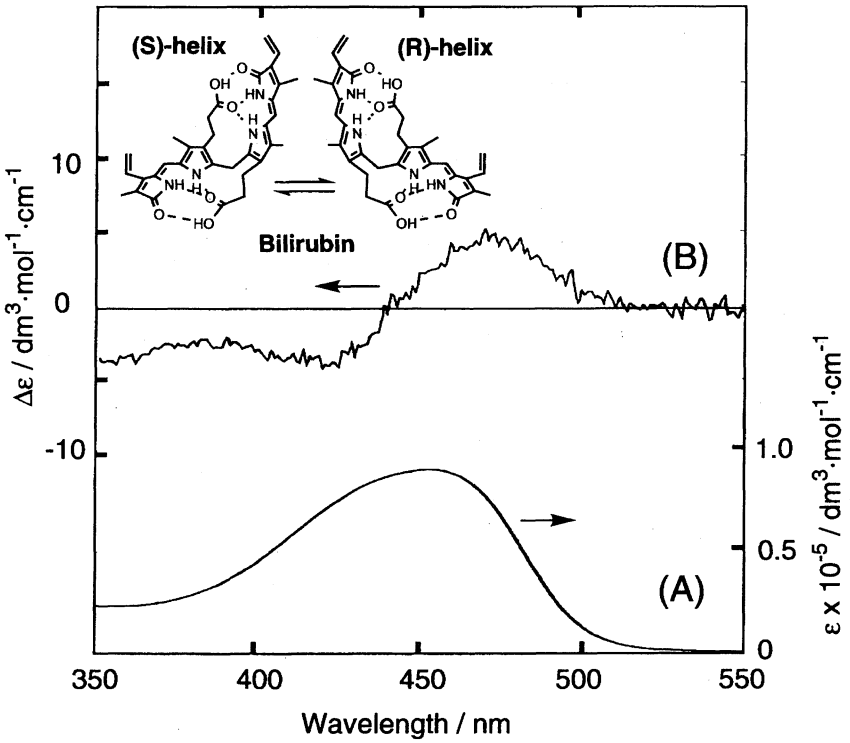


Fig. 4. UV-visible (A) and CD (B) spectra of bilirubin (3×10^{-4} M) in chloroform in the presence of **1a** (3×10^{-2} M of glucose units) at ambient temperature.

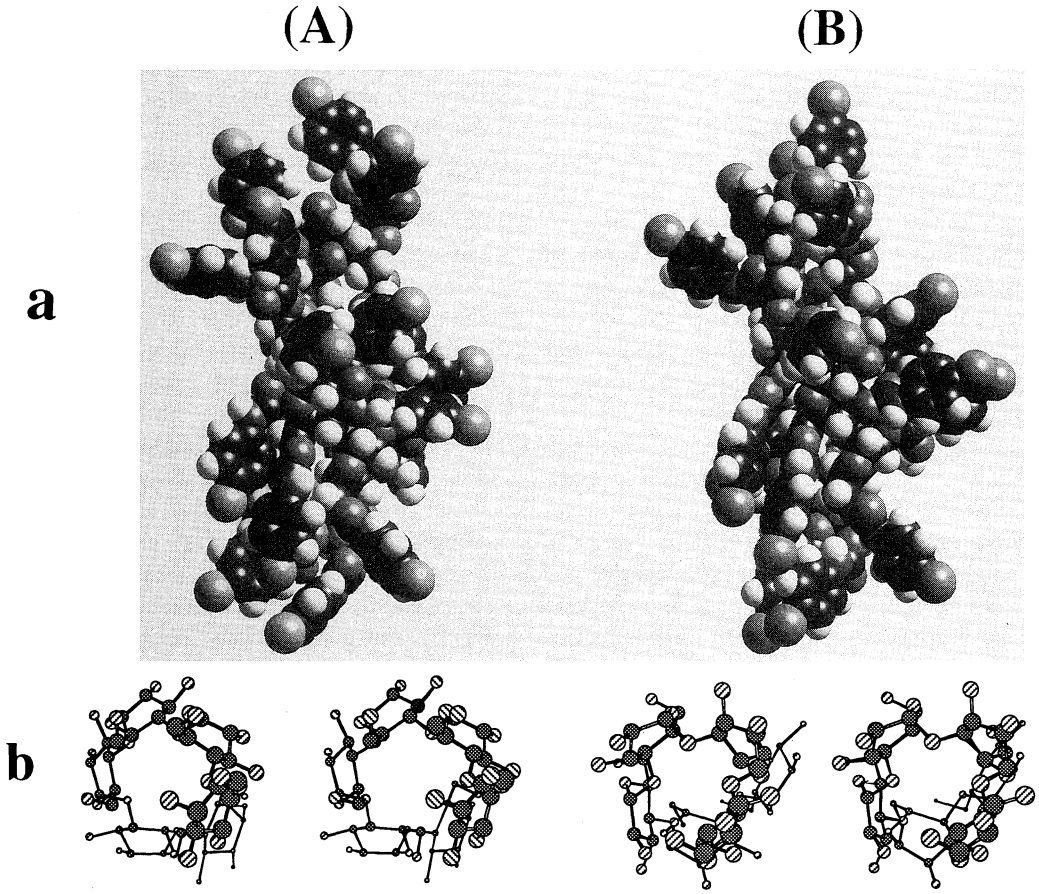
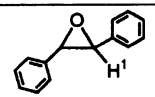
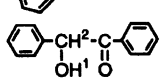
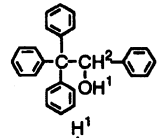
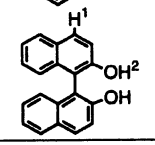


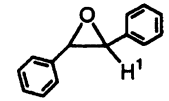
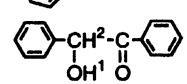
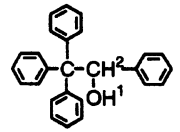
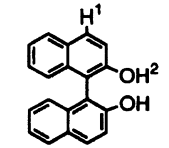
Fig. 5. Computer-generated depiction of the structures of **1b** (A) and **2b** (B) after molecular dynamics simulations. (a) Along the helix axis. (b) Perpendicular to the chain axis (stereoviews): All side groups and protons are omitted for clarity.

Table 3. Chiral Discrimination of **3**, **7**—**9** by **1a**—**1c** in ^{1}H NMR[a)]

	Racemates	1a				1b				1c			
		α[b)]	Proton	$\Delta\delta$[c)] [Hz]	$\Delta\Delta\delta$[d)] [Hz]	α[b)]	Proton	$\Delta\delta$[c)] [Hz]	$\Delta\Delta\delta$[d)] [Hz]	α[b)]	Proton	$\Delta\delta$[c)] [Hz]	$\Delta\Delta\delta$[d)] [Hz]
3		ca.1 (+)	H^1	−1(+), 4(−)	5	1.27 (+)	H^1	−1(+), 5(−)	6	1.23 (+)	H^1	−1.5(+), 1.5(−)	3
7		1.42 (+)	H^1 H^2	48(−), 51.5(+) 11(−), 13.5(+)	3.5 2.5	1.39 (+)	H^1	38.5(−), 44(+)	5.5	1.34 (+)	H^1	31.5(−), 35(+)	3.5
8		1.36 (+)	H^1 H^2	36.5(+), 47.5(−) 4(+), 22(−)	11 18	1.51 (+)	H^1 H^2	40(+), 48.5(−) 3.5(+), 21.5(−)	8.5 18	1.28 (+)	H^1 H^2	37.5(+), 44(−) 1(+), 17(−)	6.5 16
9			H^1 H^2	−8(−), −12(+) 101(−), 118(+)	4 17		H^1 H^2	−9(−), −13.5(+) 110(−), 130(+)	4.5 20		H^1 H^2	−10(−), −14(+) 104.5(−), 126.5(+)	4 22

a) Racemate, 5 mg; **1a**—**1c**, 30 mg; $CDCl_3$, 0.9 ml. b) Separation factor in chiral HPLC (see Table 1). The sign in parentheses represents optical rotation of the first-eluted isomer. c) $\Delta\delta$ ($= \delta - \delta_f$) indicates the induced shift of enantiomers in the presence of **1a**—**1c**. δ and δ_f are the chemical shifts of paticular proton of a racemate in the presence and the absence of **1a**—**1c**, respectively. d) $\Delta\Delta\delta$ ($= |\Delta\delta_S - \Delta\delta_R|$) indicates the difference in the chemical shift between enantiomers.

Table 4. Chiral Discrimination of **3**, **7**—**9** by **2a**—**2c** in ^{1}H NMR[a)]

	Racemates	2a				2b				2c			
		α[b)]	Proton	$\Delta\delta$ [Hz]	$\Delta\Delta\delta$ [Hz]	α[b)]	Proton	$\Delta\delta$ [Hz]	$\Delta\Delta\delta$ [Hz]	α[b)]	Proton	$\Delta\delta$ [Hz]	$\Delta\Delta\delta$ [Hz]
3		ca.1 (+)	H^1	1(+), 6(−)	5	1.36 (+)	H^1	1.5(+), 6.5(−)	5	1.24 (+)	H^1	1(+), 6.5(−)	5.5
7		1.93 (+)	H^1 H^2	42(−), 47(+) 9(−), 17(+)	5 8	1.37 (+)	H^1 H^2	33(−), 37.5(+) 8(−), 11(+)	4.5 3	1.26 (+)	H^1	28(−), 32(+)	4
8		1.23 (+)	H^1 H^2	28(+), 37(−) −2(+), 13.5(−)	9 15.5	1.60 (+)	H^1 H^2	29.5(+), 37.5(−) −3(+), 14(−)	8 17	1.38 (+)	H^1 H^2	25.5(+), 31(−) −3.5(+), 9(−)	5.5 12.5
9			H^1 H^2	−5.0(−), −11.5(+) 93(−), 116.5(+)	6.5 23.5		H^1 H^2	−6.5(−), −11(+) 92.5(−), 112(+)	4.5 19.5		H^1 H^2	−7.5(−), −12(+) 81.5(−), 108(+)	4.5 26.5

a) Racemate, 5 mg; **2a**—**2c**, 30 mg; $CDCl_3$, 0.9 ml. b) Separation factor in chiral HPLC (see Table 2). The sign in parentheses represents optical rotation of the first-eluted isomer.

may not have a structure similar to native amylose which has a left-handed 6/1 helix. The carbamates appear to have a rather tight, left-handed 4/1 or 5/1 helix.

A direct elucidation of the structures of the maltooligosaccharide derivatives may be possible by means of X-ray analysis if the derivatives give crystals suitable for X-ray analysis. Attempts to obtain crystals of the maltooligosaccharides are now in progress.

This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, and by the Proposal-Based Advanced Industrial Technology R&D Program from NEDO, Japan.

References

1) "A Practical Approach to Chiral Separations by Liquid Chromatography," ed by G. Subramanian, VCH, New York (1994).
2) Y. Okamoto, *Chemtech*, **1987**, 176.
3) W. H. Pirkle and T. C. Pochapsky, *Chem. Rev.*, **89**, 347 (1989).
4) D. R. Taylor and K. Maher, *J. Chromatogr. Sci.*, **30**, 67 (1992).
5) S. G. Allenmark, "Chromatographic Enantioseparation: Methods and Application," Wiely, New York (1988).
6) Y. Okamoto, Y. Kaida, R. Aburatani, and K. Hatada, "Chiral Separations by Liquid Chromatography," ed by S. Ahuja, ACS Symposium Series 471, Washington D. C. (1991), Chap. 5.
7) Y. Okamoto and Y. Kaida, *J. Chromatogr., Sect. A*, **666**, 403 (1994).
8) E. Yashima and Y. Okamoto, *Bull. Chem. Soc. Jpn.*, **68**, 3289 (1995).
9) Y. Okamoto, M. Kawashima, and K. Hatada, *J. Chromatogr.*, **363**, 173 (1986).
10) E. Yashima, M. Yamada, Y. Kaida, and Y. Okamoto, *J. Chromatogr., Sect. A*, **694**, 347 (1995).
11) E. Yashima, C. Yamamoto, and Y. Okamoto, *J. Am. Chem. Soc.*, **118**, 4036 (1996).
12) E. Yashima, M. Yamada, C. Yamamoto, M. Nakashima, and Y. Okamoto, *Enantiomer*, **2** (1997), in press.
13) U. Vogt and P. Zugenmaier, *Ber. Bunsenges. Phys. Chem.*, **89**, 1217 (1985).
14) U. Vogt and P. Zugenmaier, "Communication at the European Science Foundation Workshop on Specific Interaction in Polysaccharide Systems," Uppsada, Sweden (1983).
15) E. Yashima, E. Kasashima, and Y. Okamoto, *Chirality*, **9**, 63 (1997).
16) Y. Kaida and Y. Okamoto, *Bull. Chem. Soc. Jpn.*, **65**, 2286 (1992).
17) D. A. Lightner and J.-S. Ma, *Spect. Lett.*, **17**, 317 (1984).
18) X. X. Zhu, G. R. Brown, and L. E. St-Pierre, *Can. J. Spectrosc.*, **32**, 49 (1987).
19) D. A. Lightner, J. K. Gawronski, and W. M. D. Wijekoon, *J. Am. Chem. Soc.*, **109**, 6354 (1987).
20) S. L. Mayo, B. D. Olafson, and W. A. Goddard, III, *J. Phys. Chem.*, **94**, 8897 (1990); A. K. Rappe and W. A. Goddard, III, *J. Phys. Chem.*, **95**, 3358 (1991).
21) A. Sarko and A. Biloski, *Carbohydr. Res.*, **79**, 11 (1980).
22) F. H. Allen and O. Kennard, *Chem. Design Automation News*, **8**, 31 (1993).
23) R. Aburatani, Y. Okamoto, and K. Hatada, *Bull. Chem. Soc. Jpn.*, **63**, 3606 (1990).
24) B. Chankvetadze, E. Yashima, and Y. Okamoto, *J. Chromatogr., Sect. A*, **694**, 101 (1995).
25) D. A. Lightner, J. K. Gawronski, and K. Gawronska, *J. Am. Chem. Soc.*, **107**, 2456 (1985).
26) K. Kano, K. Yoshiyasu, and S. Hashimoto, *Chem. Lett.*, **1990**, 21.
27) Y. Murakami, O. Hayashida, and Y. Nagai, *J. Am. Chem. Soc.*, **116**, 2611 (1994).
28) E. Yashima, H. Fukaya, P. Sahavattanapong, and Y. Okamoto, *Enantiomer*, **1**, 193 (1996).