Evaluation of Chiral Amino Acid Discrimination by a Permethylated Cyclic Tetrasaccharide, *cyclo*-{→6)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→}, Using FAB Mass Spectrometry

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The novel cyclic oligosaccharide, permethylated tetrasaccharide (CTS), determinates the enantiomers of chiral amino acid isopropyl ester hydrochlorides.

Chiral recognition is one of the fundamental and important processes in living systems, and chirality and the related technology are one of the most significant subjects in pharmacy, biochemistry, organic chemistry, etc.[1,2] It is well known that some kinds of cyclic oligosaccharide derivatives such as cyclodextrin (CD)[3] and cyclofructan (CF)[4] show chiral discrimination toward chiral molecules and/or ions, and the CD derivatives have been applied as the chiral stationary phase in gas and liquid chromatography.[5,6] However, chiral discrimination of cyclic tetrasaccharide (CTS) (Chart 1),[7] which is a cyclic oligosaccharides, has not as yet been examined. As CTS has a polyether ring moiety $(–O–C–C–O–C–O–C–C–C–)_2$ in the molecular center, CTS is expected to associate with cation guests via charge–dipole electrostatic interactions. As is well known, chiral crown ether derivatives, which are cyclic polyethers, have been reported as having high enantioselectivity toward primary chiral ammonium ions, and are applied to chiral separations.[8] With the application of the FAB mass spectrometry (MS)-enantiomer labeled (EL) guest method,[9,10] which is one of the methods for estimation of the chiral recognition ability of new hosts,[11] we found for the first time that permethylated CTS (MeCTS) exhibits various degrees of chiral discrimination toward ammonium ions of amino acid isopropyl esters. A MeCTS host (H) is complexed with a 1:1 amino acid guest mixture of an unlabeled R enantiomer (G_R^+) and a deuterium-labeled S enantiomer ($G_{S\text{-}Dn}^+$, n: number of deuterium atoms). The enantioselectivity of MeCTS is quantitatively evaluated from the relative peak intensity value $[I(H + G_R)^+/I(H + G_{S\text{-}Dn})^+ = I_R/I_{S\text{-}Dn}]$ of the two host–guest diastereomeric complex ion peaks in the FAB mass spectrum.

CTS was permethylated[12] so that its complex ion was sensitively detected by FABMS. Amino acid isopropyl ester hydrochlorides were used as guests so that natural abundance correction was unnecessary. All S enantiomer guests were labeled with deuterium (isopropyl ester: $n = 6$ or 7). A 1:1 racemic mixture solution of enantiomer guests was prepared by mixing together an equal amount of a 0.67 M MeOH solution of each enantiomer. A 10 μL aliquot of the guest solution and a 5 μL aliquot of a 0.20 M host MeOH solution were added to 15 μL of the a 3-nitirobenzyl alcohol (NBA) matrix. A 1 μL sample of the final mixture was used for obtaining the FAB mass spectra. A typical mass spectrum is shown in Figure 1, and the $I_R/I_{S\text{-}Dn}$ values obtained are summarized in Table 1 with the data of Me–α-CF. MeCTS showed larger chiral discrimination ability than Me–α-CF except for the case of Trp–O–*i*-Pr^+. MeCTS showed S selectivity toward the primary ammonium ions of the given guests. The enantionselectivity for Val–O–*i*-Pr^+ and Pgly–O–*i*-Pr^+ is large among the primary ammonium ions. For secondary ammonium ion Pro–O–*i*-Pr^+ MeCTS showed R selectivity clearly. Val–O–*i*-Pr^+ and Pgly–O–*i*-Pr^+ have a secondary carbon atom neighboring on the each stereo center. But, Pro–O–*i*-Pr^+ is also very constrained around the stereo center. It is suggested that when MeCTS binds to chiral ammonium ions to make complexes, the steric interactions between the host and guests occur near the stereo center of the guests.

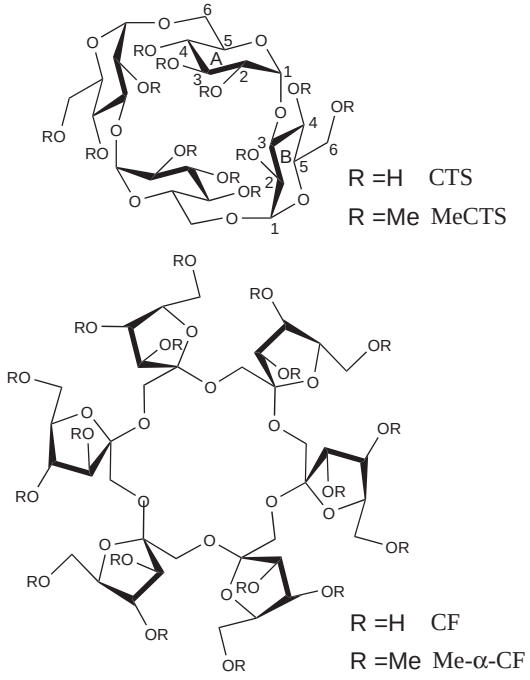

Chart 1. Cyclic tetrasaccharide (CTS) and α-cyclofructan (α-CF) derivatives.

In NMR studies, the spectral changes of MeCTS by resulting from addition of ammonium salts of amino acid esters were too small to estimate the association constants ($K <$

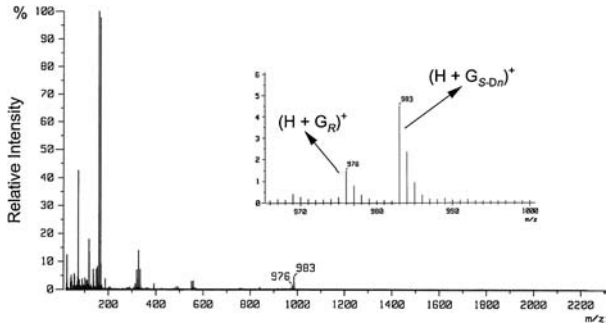

Figure 1. Example of mass spectra of complex ions of MeCTS with Val–O–*i*-Pr^+ in the FABMS/EL-guest method. NBA was used as the matrix. Molecular weights of H, $G_R{}^+$, and $G_{S\text{-}Dn}{}^+$ are 816, 160, and 167, respectively.

Table 1. The relative peak intensity ($I_R/I_{S\text{-}Dn}$ values) of the complex ions $(H + G_R)^+$ and $(H + G_{S\text{-}Dn})^+$ of MeCTS or Me–α-CF (H) with ammonium ions of amino acid isopropyl esters ($G_R{}^+$ and $G_{S\text{-}Dn}{}^+$) in FAB mass spectrometry[a]

Amino acid isopropyl ester	Permethylated cyclic oligosaccharide	
	MeCTS	Me–α-CF
Ala–O–*i*-Pr^+	0.90	0.94
Val–O–*i*-Pr^+	0.33	1.28
Pro–O–*i*-Pr^+	3.72	1.08
Met–O–*i*-Pr^+	0.54	1.04
Phe–O–*i*-Pr^+	0.68	1.00
Trp–O–*i*-Pr^+	0.81	1.38
Pgly–O–*i*-Pr^+	0.48	0.99

[a]The counter anions of the chiral ammonium ion guests were chloride (Cl^-). The matrix of FABMS was 3-nitrobenzyl alcohol (NBA). The accuracy of the $I_R/I_{S\text{-}Dn}$ values were 1.00 ± 0.04. Pgly = phenylglycine (2-amino-2-phenylacetic acid).

$1\,M^{-1}$). The specific shifts of MeCTS proton signals were induced by adding ammonium thiocyanate in acetone-d_6 at 298 K ($\log K = 1.0 \pm 0.1$). The limiting downfield shifts are summarized in Table 2. The protons of H5A, H6A, H1B, H2B, and Me2B showed the relatively large shifts, which suggest that ammonium ions locate close to the protons and bind to the oxygen atoms neighboring the protons. Thus, the oxygen atoms of O5A, O6A (or O1B), and O2B are binding points of ammonium ions. When potassium thiocyanate was used as a guest, the proton signals responded in the same manner. The structure of the complex between MeCTS and the potassium ions calculated and optimized by MM (force field: CVFF) based on the crystalline structure of CTS[13] supports the proposed structure on the basis of the limiting shifts.

The ammonium ion moiety fixes at the oxygen atoms as shown in Figure 2. As MeCTS adopts a C_2-symmetry, the space around the stereo center divides into two parts. The three substituents of the α-carbon of the guest locate in different steric environments. Therefore, each enantiomer of the guest was distinguished effectively. In the case of Me–α-CF complex, as each substituent locates in similar environments because of C_3 (C_6)-symmetry, the chiral discrimination ability toward chiral amino acid esters becomes smaller than that of MeCTS.[14]

We are studying in detail the enantioselective complexation of CTS derivatives. CTS and the its derivatives can be applied in to chiral separation technology in the future.

Table 2. ^{1}H NMR induced downfield shifts (ppm) of the peaks of MeCTS by complexation with $NH_4{}^+$ (SCN^-) in acetone-d_6 at 298 K

Proton	Limiting shift/ppm	Proton	Limiting shift/ppm
H1A	0.05	H1B	0.17
H2A	0.06	H2B	0.19
H3A	—[a]	H3B	−0.02[b]
H4A	0.04	H4B	—[a]
H5A	−0.18[b]	H5B	0.08
H6A	0.22	H6B	—[a]
H6′A′	0.07	H6′B′	—[a]
Me2A	0.02	Me2B	0.17
Me3A	0.01	Me4B	0.00
Me4A	0.03	Me6B	0.02

[a]The shifts did were not estimated exactly because of the overlaps in other peaks. The limiting shift of each peak was calculated from the association constant and the induced shift under certain host and guest concentration conditions. [b]Negative values show upfield shifts.

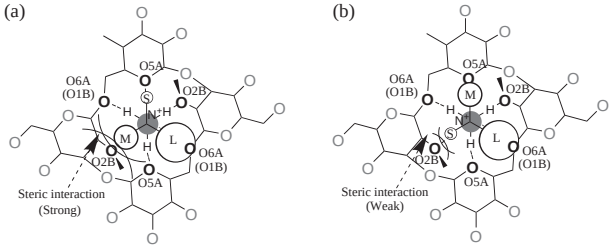

Figure 2. Illustrations of complexes of MeCTS with chiral organic ammonium ions. (a) Complex of CTS with one enantiomer of guest; (b) with another one.

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