Chiral discrimination of fructo-oligosaccharides toward amino acid derivatives by induced-fitting chiral recognition †

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Among linear permethylated fructo-oligosaccharides containing various monosaccharide moieties at the reducing terminal, **MeSorFru$_3$**, **MeGlc^{6}Fru$_3$**, and **MeFruNys** showed high degrees of enantioselectivity toward binding some amino acid 2-propyl ester salts. In particular **MeFruNys** indicated remarkable chiral discrimination toward [ValOPr^i]$^+$ ($I_R/I_{S\text{-}Dn}$ = 0.14 corresponding to $-\Delta\Delta G_{enan}$ = 1.2 kcal mol^{-1}, *S*-selectivity) and [PheOPr^i]$^+$ ($I_R/I_{S\text{-}Dn}$ = 0.18 corresponding to $-\Delta\Delta G_{enan}$ = 1.0 kcal mol^{-1}, *S*-selectivity). The results of FAB mass, UV–visible spectrometries, thermodynamic parameters, and NMR induced shifts provided evidence for the chiral discrimination of **MeFruNys** toward amino acid ester salts in the "induced-fitting chiral recognition system": the conformation of **MeFruNys** drastically changes from a linear to a pseudo-ring structure with a given cation such as a chiral organic ammonium ion or an alkali metallic ion. This is the first example of chiral discrimination of linear oligosaccharide derivatives toward amino acid derivatives based on the induced-fitting chiral recognition mechanism.

Introduction

Chiral recognition is one of the most fundamental and significant processes in living systems. Many artificial model compounds have been designed and synthesized based on pre-organization. For example, Cram *et al.* comprehensively studied chiral host–guest interaction using chiral crown ether derivatives.[1] The mechanism of chiral recognition in living systems has been gradually made clear.[2] In the case of enzymes such as α-chymotrypsin,[3] haloalkane dehalogenase from *Xanthobacter autotrophicus GJ10*[4] *etc*., it has been recognized that a reversible conformational change of the enzymes is essential for their catalytic activity. The mechanism of enzymatic reaction is clearly explained by "induced-fitting theory".[3] It is a dynamic chiral recognition process in which a drastic conformation change upon complexation induces a new chiral recognition field (site) in the chiral molecule which does not have a sufficient recognization field by itself. Based on the induced-fitting chiral recognition mechanism, new highly functional materials may be designed as improvements on conventional materials based on the usual "Lock–key" concept. For example, the detection of a drastic conformation change would enhance small signals, and application to more highly responsive molecular sensors would be expected.

We paid particular attention to linear fructo-oligosaccharides which consist of β (2→1)-linked fructofuranosides and a reducing terminal monosaccharide moiety (Chart 1). These oligosaccharides contain –O–C–C–O– chain moieties in the backbone as do crown ethers and glymes.‡ Many studies on complexation of oligo- and poly-(ethylene oxide)s with cations have been reported. It has been shown that oligo- and poly-(ethylene oxide)s change their conformation to a pseudo-ring or helical structure to form complexes with cations in gas,[5] solution[6] and crystal states.[7] Therefore, the present linear oligosaccharides are also expected to induce a conformation change by complexation with cations. In order to facilitate observation of the complexation behavior of the oligosaccharides with cations, all of the given oligosaccharides were permethylated in the present study. The permethylated oligosaccharides are soluble in low-polarity organic solvents in which the charge–dipole electrostatic interaction is strengthened by a weak solvent effect.

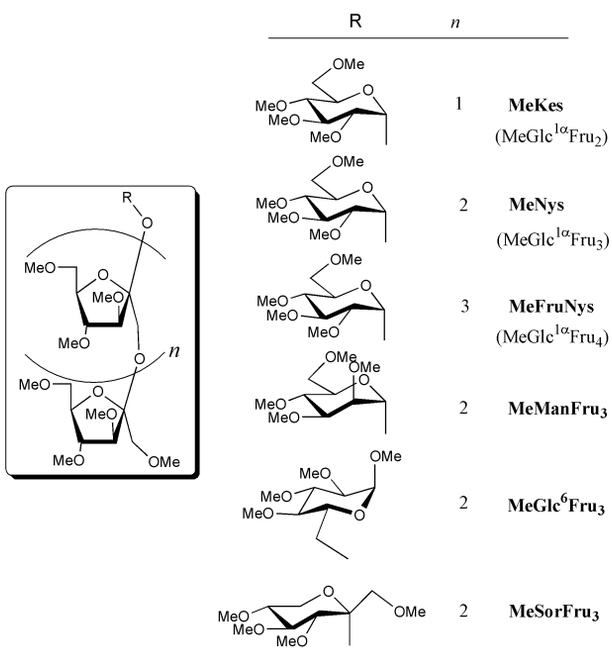

Chart 1 Permethylated fructo-oligosaccharides.

In this paper, we demonstrate the chiral discrimination of the permethylated fructo-oligosaccharides toward amino acid 2-propyl ester salts (Chart 2) and discuss the structures of the

† Experimental details and Fig. S1–S7 are available as supplementary data. For direct electronic access see http://www.rsc.org/suppdata/p2/b0/b007478k/

‡ The IUPAC name for glyme is 1,2-dimethoxyethane.

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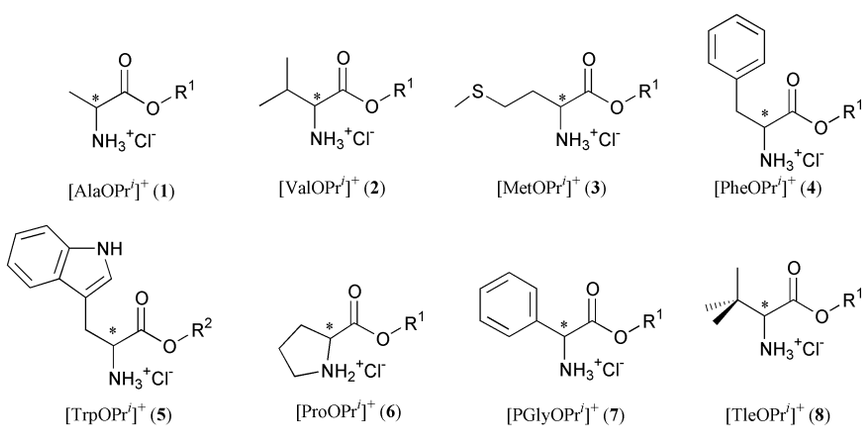
Table 1 Chiral discrimination ability ($I_R/I_{S\text{-}Dn}$ values) of permethylated fructo-oligosaccharides toward amino acid 2-propyl ester salts by FAB mass spectrometry

	Amino acid 2-propyl ester hydrochlorides							
Fructo-oligosaccharides	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
MeKes	1.11	1.19	1.18	1.15	1.18	1.16	0.93	1.23
MeNys	1.16	0.87	1.54	1.08	1.28	1.19	1.56	0.85
MeFruNys	0.45	0.14	0.28	0.18	0.56	1.23	0.26	0.33
MeManFru$_3$	1.09	0.84	1.28	0.98	1.25	1.06	1.20	0.98
MeGlc^{6}Fru$_3$	0.60	0.49	0.79	0.64	0.84	1.16	0.79	0.40
MeSorFru$_3$	0.80	0.51	0.83	0.67	0.77	1.16	0.72	0.40
18C6[a]	0.99	1.00	1.01	1.00	1.00	1.02	1.00	1.04

[a] 18C6 = 18-crown-6.



	R-Enantiomer	*S*-Enantiomer
R^1	$(CH_3)_2CH$-	$(CD_3)_2CD$-
R^2	$(CH_3)_2CH$-	$(CD_3)_2CH$-

Chart 2 Amino acid 2-propyl ester hydrochlorides.

complexes. This is the first observation of the chiral discrimination of linear oligosaccharides on the basis of the induced-fitting chiral recognition mechanism.

Results and discussion

(1) Chiral discrimination

(a) FAB mass spectrometry. The chiral discrimination ability of the given permethylated fructo-oligosaccharides toward amino acid 2-propyl ester hydrochlorides was evaluated by means of the FAB mass spectrometry (MS)–enantiomer-labelled (EL) guest method which had been developed before.[8] In this method, the FAB mass spectra of three component samples in solution (NBA matrix) of permethylated oligosaccharide (S) and a 1 : 1 mixture of *S*-amino acid ester salt labelled with deuterium ($[^2H_n]A_S^+$) and unlabelled *R*-amino acid ester salt (A_R^+) were measured at room temperature, and the two diastereomeric 1 : 1 complex ion peaks differing in molecular weight ($\Delta M_r = n$, *n*: the number of deuteriums) were compared in intensity in order to estimate the chiral discrimination ability (see Experimental section). The relative peak intensity $[I(S + A_R)^+ / I(S + [^2H_n]A_S)^+ = I_R/I_{S\text{-}Dn}]$ values are summarized in Table 1.[9]

The 1 : 1 complex ion peaks of these permethylated oligosaccharides with all the given amino acid ester salts were observed in FAB mass spectra. Chiral discrimination of **MeKes** toward amino acid ester salts (**1**–**6**) was hardly observed ($I_R/I_{S\text{-}Dn} \approx 1.0$). In the case of **MeNys**, the chiral discrimination was very small (toward [MetOPr^i]$^+$, [TrpOPr^i]$^+$, and [PGlyOPr^i]$^+$, $I_R/I_{S\text{-}Dn}$ values = *ca.* 1.5, *R*-selectivity). The trends in the enantioselectivity of **MeNys** and **MeManFru$_3$** were quite similar to each other. That is, the influence of the difference in C^2–OMe configuration in the terminal pyranoside (D-glucose and D-mannose) on the selectivity was mostly negligible. **MeGlc^{6}Fru$_3$** and **MeSorFru$_3$** apparently showed higher chiral discrimination than **MeKes**, **MeNys**, and **MeManFru$_3$**. They both showed $I_R/I_{S\text{-}Dn} \approx 0.5$ (*S*-selectivity) toward [ValOPr^i]$^+$ and [Tle^{t}OPr^i]$^+$. The chiral discrimination of **MeFruNys** was dramatically higher than that of any other studied fructo-oligosaccharides. For [PheOPr^i]$^+$ and [ValOPr^i]$^+$, the $I_R/I_{S\text{-}Dn}$ values were 0.18 and 0.14, respectively (Fig. 1, Table 1). The latter was the highest chiral discrimination obtained in the present series. Comparing the chain length of the given 1-α-D-glucopyranosyl-β-D-fructo-oligosaccharide series, the longer the sugar chain was (*n* = 1–3 in Chart 1), the greater the chiral discrimination ability (**MeKes < MeNys ≪ MeFruNys**). For [ProOPr^i]$^+$ having a rigid and sterically hindered secondary ammonium ion moiety, the 1 : 1 complex ion peaks of the present fructo-oligosaccharides were very small.[10]

(b) Binding ability in solution. The binding ability of **MeFruNys** (or **MeGlc^{6}Fru$_3$**) in solution ($CHCl_3$) was successfully determined by means of UV–visible spectrometry using a known picrate anion (Pic^-) probe for amino acid ester salts (Table 2).[11] By adding **MeFruNys** to an amino acid ester picrate in a chloroform solution at 25 °C, a bathochromic shift ($\Delta\lambda$) of λ_{max} of the picrate ion was observed. This suggests that the ammonium ion in a given amino acid ester salt was bound to the oxygens of the oligosaccharide to form the complex. In general, the cation–picrate anion pair, which forms a tight ion pair in a low-polarity solvent, was separated by complexing

Table 2 Association constants (K_R and K_S) of permethylated fructo-oligosaccharides with amino acid ester picrate in $CHCl_3$ at 298 K and $I_R/I_{S\text{-}Dn}$ values in FAB mass spectrometry

Permethylated oligosaccharide	Amino acid ester picrate	K_R/M^{-1}	K_S/M^{-1}	K_R/K_S	$I_R/I_{S\text{-}Dn}$
MeCF6	$[TrpOPr^i]^+$	1.47×10^3	1.03×10^3	1.4	1.39[a]
MeGlc^{6}Fru$_3$	$[PheOPr^i]^+$	5.37×10^2	7.79×10^2	0.7	0.6
MeFruNys	$[TrpOPr^i]^+$	4.17×10^3	6.61×10^3	0.6	0.6
MeFruNys	$[PheOPr^i]^+$	6.50×10^2	2.05×10^3	0.3	0.2

[a] Ref. 13.

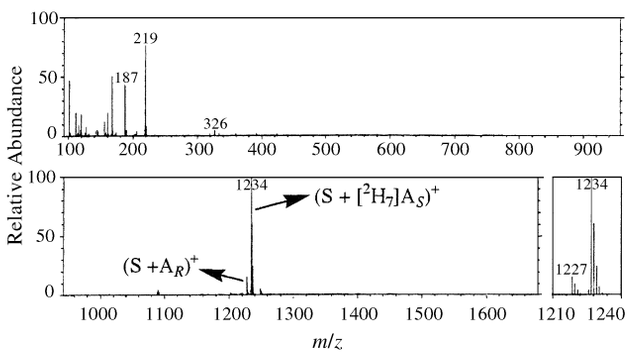


Fig. 1 A typical FAB mass spectrum in the FABMS-EL guest method. S: **MeFruNys**, A: $[ValOPr^i]^+(Cl^-)$. The left peak (*m/z* 1227) is a complex ion $(S + A_R)^+$ and the right (*m/z* 1234) is $(S + [^2H_7]A_S)^+$.

with the host compound. The bathochromic shift was observed because of the more delocalized π-electron system in the picrate anion as the electrostatic cation–anion interaction became weaker owing to the separation (for example, a host–separated ion pair).[12]

Indeed, based on the bathochromic shift behavior, the association constants (K_S and K_R) of **MeFruNys** and **MeGlc^{6}Fru$_3$** with amino acid ester salts could be determined from the UV–visible absorbance changes at given wavelengths. Toward both $[TrpOPr^i]^+$ (Pic^-) and $[PheOPr^i]^+$ (Pic^-), **MeFruNys** showed *S*-selectivity ($K_R/K_S = 0.6$ and 0.3), which is in good agreement with the selectivity by the FABMS-EL guest method ($I_R/I_{S\text{-}Dn} = 0.6$ and 0.2), respectively, in spite of the different solvent (chloroform and NBA matrix). Recently, the chiral discrimination of permethylated cycloinulohexaose (**MeCF6**, Chart 3),

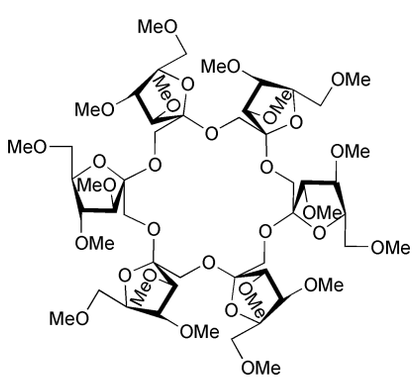


Chart 3 Permethylated cycloinulohexaose (**MeCF6**).

which is a cyclic fructo-oligosaccharide, has been determined by the FABMS-EL guest method.[13] The $I_R/I_{S\text{-}Dn}$ value (1.4) toward $[TrpOPr^i]^+$ was also in good agreement with the K_R/K_S value (1.4).

The thermodynamic parameters of the complexation of **MeFruNys** with $[PheOPr^i]^+$ (Pic^-) were estimated by the K_R and K_S values measured at various temperatures (Table 3). Both of $-\Delta H$ and $-T\Delta S$ in the case of **MeFruNys** with the *S*-enantiomer were larger than those with the *R*-enantiomer. The negative and large enthalpy factor (ΔH) suggests that

Table 3 Thermodynamic parameters[a] of the complexation of **MeFruNys** with $[PheOPr^i]^+$ in $CHCl_3$ at 298 K

Amino acid 2-propyl ester picrate salt	$\Delta H°$/kcal mol^{-1}	$\Delta S°$/cal K^{-1} mol^{-1}	$\Delta G°$/kcal mol^{-1}
R-$[PheOPr^i]^+$	−9.3	−18.1	−3.9
S-$[PheOPr^i]^+$	−11.4	−23.0	−4.6

[a] The parameters were estimated from association constants at various temperatures by the UV titration method. Counter anion = picrate.

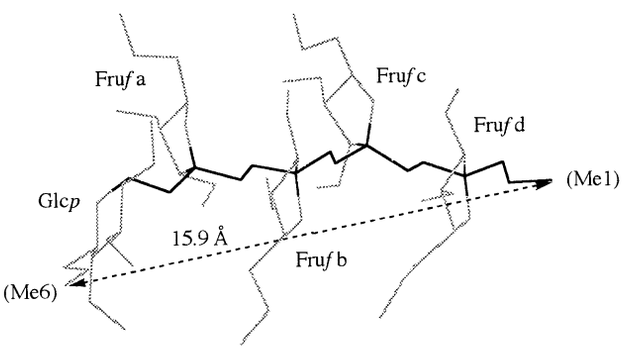


Fig. 2 A typical structure from the molecular dynamics simulation. Black, oxyethylene chain; gray, the pyranose ring and furanose rings.

MeFruNys and the amino acid ester salts were energetically stabilized by complexation. However, the entropy factor ($-T\Delta S$) was large and positive (large entropy loss by the complexation) which greatly cancelled the energetically favorable effect of the enthalpy contribution. This fact suggests that the conformation of the linear and flexible oligosaccharide drastically changed and became fixed upon complexation by the cation ($\Delta H° < 0$, $\Delta S° < 0$).

(2) Structure of a free MeFruNys and a MeFruNys–cation (1 : 1) complex

(a) MD simulation and the self-diffusion coefficient. In order to model the averaged conformation of free **MeFruNys**, molecular dynamics (MD) was simulated using a CFF force field over 50 ps (1 fs per step).[14] The average structure in the MD simulation is shown in Fig. 2. The averaged distance from Me6-Glc*p* to Me1-Fru*f* d of **MeFruNys** was presumed to be about 15.9 Å in the last 30 ps of the equilibrium state. The backbone of **MeFruNys** was a linear structure to which the fructofuranose rings were spirally attached. The self-diffusion coefficient (D) of **MeFruNys** without solvent was estimated to be 9.29×10^{-6} cm^2 s^{-1} atom^{-1}. The D values of **MeFruNys**, and the complex with K^+ or *S*-$[ValOPr^i]^+$ in $CDCl_3$ were evaluated by means of a pulse field gradient technique in NMR spectrometry (Table 4).[15] The evaluated D values were in good agreement with the value determined by MD simulation. The D value of **MeFruNys** was smaller than those of **MeFruNys** complexes because of the increase in molecular weight on association. The magnitude of the D value depends on both

Table 4 The self-diffusion coefficients (D) of **MeFruNys** and the complexes with cations in $CDCl_3$ at 298 K [a]

Guest	Self-diffusion coefficient/10^{-6} cm^2 s^{-1}
None	6.82
$K^+(SCN^-)$	8.96
$[[^2H_7]ValOPr^i]^+(Cl^-)$ [b]	8.63

[a] The concentration of the sample solution was 0.2 M. [b] The self-diffusion coefficient of free $[[^2H_7]ValOPr^i]^+(Cl^-)$ was evaluated as 2.78×10^{-5} cm^2 s^{-1}.

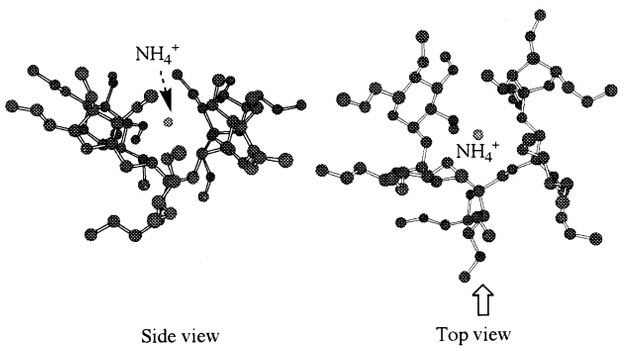


Fig. 3 The estimated structure of a complex of **MeFruNys** with an unsubstituted ammonium ion (NH_4^+) by PM3 calculations. All hydrogens are omitted.

the molecular weight and shape.[16] From the comparison of the above results of the simulation and measurement, it is suggested that free **MeFruNys** has a linear shape.

(b) MO calculations. The 1 : 1 complex ion structure of **MeFruNys** with an unsubstituted ammonium ion (NH_4^+) was modelled by semi-empirical molecular orbital (PM3) calculations (Fig. 3). The ammonium ion was used for determining the position of the cation to simplify the MO calculations rather than using an alkylammonium ion ($R–NH_3^+$) in the amino acid ester salt. One of the structures optimized by the MO calculations was quite similar to the crystalline structure of the complex of **MeCF6** with a barium ion previously determined by X-ray crystallography.[17] In the calculated structure, the cation was surrounded by the –O–C–C–O– chain of **MeFruNys** to construct a quasi-18-crown-6 skeleton. The conformation of the –O–C–C–O– chain ($n = 5$) was a quasi-twist boat type and the arrangement of the –O–C–C–O– torsion angles was $g^+g^+g^+g^+g^+$.[18] The arrangement of the monosaccharide units (Glc*p*-Fru*f* a-Fru*f* b-Fru*f* c-Fru*f* d) was u-u-d-u-u (u = up, d = down) for the pseudo-18-crown-6 ring plane. In the case of the **MeCF6**·Ba^{2+} complex, the –O–C–C–O– torsion angle arrangement was $g^+g^+g^+g^+g^+g^+$ and the fructofuranose ring arrangement was u-u-d-u-u-d. The structure of the **MeFruNys** complex is very close to that of the **MeCF6** complex in which a fructofuranose ring (d) is removed.

(c) UV–visible spectroscopy. In the case of **MeKes** (trisaccharide), bathochromic shift ($\Delta\lambda$) of λ_{max} of alkali metallic and ammonium picrates was hardly observed (Table 5). On the other hand, a bathochromic shift (2–3 nm) was observed with **MeNys** (tetrasaccharide). In the case of **MeFruNys** (pentasaccharide), a bathochromic shift was observed within the range of 2 to 6 nm.[19] These findings were in good agreement with the fact that **MeFruNys** or **MeNys** complexed with an alkali metal ion has a pseudo-ring structure surrounding the cation. The binding ability of **MeFruNys** with K^+ was higher than that of **MeNys** because of the longer –O–C–C–O– chain-unit (larger n in Chart 1) (Table 6). A particular cation size-selectivity of (acyclic) **MeFruNys** could not be observed in the magnitude of the association constants (K) as clearly as the

Table 5 Bathochromic shifts ($\Delta\lambda$/nm) of λ_{max} of alkali metallic and ammonium picrates by adding permethylated fructo-oligosaccharides [a]

Permethylated fructo-oligosaccharides	Alkali metallic picrates				
	Na^+	K^+	Rb^+	Cs^+	NH_4^+
MeKes	0	0	0	0	0
MeNys	3	2	3	3	2
MeFruNys	2 [b]	5 [b]	6 [b]	2 [b]	2 [b]

[a] Solvent: THF–$CHCl_3$ (v/v = 1 : 1), temperature: 298 K. [b] Larger shifts were observed by adding excess permethylated fructo-oligosaccharides, but the isosbestic points disappeared.

selectivity of (cyclic) **MeCF6**. The reason is that the –O–C–C–O– chain moiety in **MeFruNys** is very flexible and a conformation change of the chain moiety can occur to accommodate varying cation size to form a 1 : 1 complex with the cation.

(d) Analogous structure of the complex by a model compound (BzEO5Me). A pentaglyme derivative (**BzEO5Me**) having five units of the –O–C–C–O– moiety was used as a model compound for **MeFruNys** (Chart 4) and the NOE behavior between

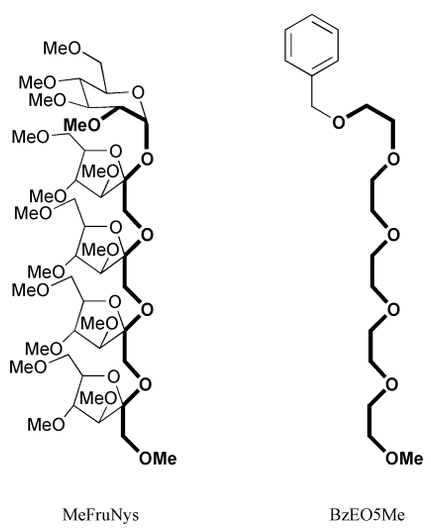


Chart 4 Model compound (**BzEO5Me**) of **MeFruNys**.

protons in the complex of **BzEO5Me** with a cation was examined in $CDCl_3$. In the case of **MeFruNys** complex with the cation, it was not easy to observe the NOE behavior between the protons at both terminals of the pseudo-ring in the complex because the chemical shifts of the methoxy protons were too close. The model compound having a –O–C–C–O– chain, like **MeFruNys**, gives some information regarding the complexation with the cation.

In the case of the potassium ion (counter anion: SCN^-), a small enhancement (NOE: 3%) of the peak area of the methyl proton signal at one terminal was observed by irradiation of the methylene protons in the benzyl group at the other terminal. Adding a potassium ion induced a high-field shift (0.02 ppm) of the methylene protons and a low-field shift (~0.02 ppm) of the other peaks. The high-field shift is ascribed to the closeness of the benzyl group at the other terminal to the methyl group. In the case of *S*-$[[^2H_7]ValOPr^i]^+$ (counter anion: Cl^-), an NOE (*ca.* 8%) between the methylene protons of Val and the terminal methyl protons was observed. The above observations suggest that the complex has a pseudo-ring structure in which the terminal oxygens are located near to each other, and an alkyl group of the amino acid ester closely occupies the ring-open space of the quasi-18-crown-6 ring.

(e) NMR spectroscopy. In comparing the ^{13}C-NMR spectra

Table 6 Logarithm of association constants (K) of permethylated fructo-oligosaccharides with metallic and ammonium ions at 298 K

Permethylated fructo-oligosaccharide	Metallic and ammonium picrate	Solvent	Log K
MeFruNys[a]	Na^+	$CHCl_3$–THF (v/v = 1 : 1)	2.89 ± 0.05
MeFruNys[a]	K^+	$CHCl_3$–THF (v/v = 1 : 1)	3.34 ± 0.05
MeFruNys[a]	Rb^+	$CHCl_3$–THF (v/v = 1 : 1)	3.48 ± 0.05
MeFruNys[a]	Cs^+	$CHCl_3$–THF (v/v = 1 : 1)	4.00 ± 0.05
MeFruNys[a]	NH_4^+	$CHCl_3$–THF (v/v = 1 : 1)	2.94 ± 0.05
MeNys[a]	K^+	$CHCl_3$–THF (v/v = 1 : 1)	1.89 ± 0.06
MeCF6[b]	Na^+	$[^2H_6]$-Acetone	2.2 ± 0.1
MeCF6[b]	K^+	$[^2H_6]$-Acetone	3.8 ± 0.1
MeCF6[b]	Rb^+	$[^2H_6]$-Acetone	3.6 ± 0.1
MeCF6[b]	Cs^+	$[^2H_6]$-Acetone	2.9 ± 0.1

[a] The K values were estimated by the UV titration method. Counter anion: picrate. [b] Ref. 17. The K values were estimated by the ^{1}H-NMR titration method. Counter anion: SCN^-.

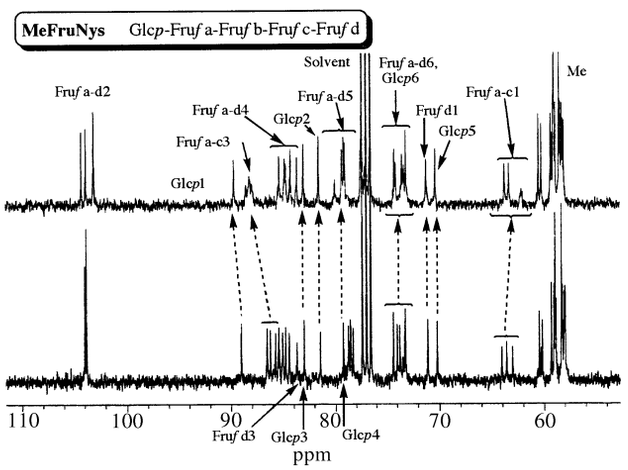


Fig. 4 ^{13}C-NMR spectra of **MeFruNys** (bottom) and the complex with potassium thiocyanate (top) in $CDCl_3$ at 25 °C.

of **MeFruNys** (chain sequence: Glc*p* -Fru*f* a-Fru*f* b-Fru*f* c-Fru*f* d) and its complex with a potassium ion (counter anion: SCN^-), the changes in the chemical shift for Glc*p* and Fru*f* d located at the terminals of the oligosaccharide chain were found to be small. On the other hand, the shift changes of Fru*f* a–c at the central part of the oligosaccharide chain were large (Fig. 4). These specific shifts suggest that the potassium ion is located at a characteristic binding site of **MeFruNys**.

In the ^{1}H-NMR spectra of **[^{2}H$_{51}$]MeFruNys** and its complex with a potassium ion, small shifts in H1 of Glc*p* and H3 of Fru*f* d were observed. The H3 of Fru*f* a–c showed large shift changes (Fig. 5). The H1 and H1′ protons of Fru*f* d at the terminal of the –O–C–C–O– chain moiety directly bound to the cation showed a large low-field and a high-field shift, respectively: thus, the difference of the chemical shifts of vicinal protons increased by complexation with the potassium ion. The motion of the vicinal protons was fixed by the complexation to be clearly distinguished in NMR.

The ^{1}H-NMR shifts of **MeFruNys** induced by adding each enantiomer of $[[^2H_7]ValOPr^i]^+$ were different from each other. The shift found by adding the *S*-enantiomer was larger than that by the *R*-enantiomer (Fig. 6). The difference in the induced shifts seems to relate to the difference of the structure and the concentration of the respective complexes in solution.

From the above results, it is deduced that **MeFruNys** can bind the various cations (alkali metallic ion, ammonium ion, and amino acid ester salts) at the –O–C–C–O– moiety and the structure of the complex has a pseudo-18-crown-6 ring skeleton surrounding the cation.

(3) Proposed chiral discrimination mechanism of MeFruNys

From the above verified results, it is possible to understand the

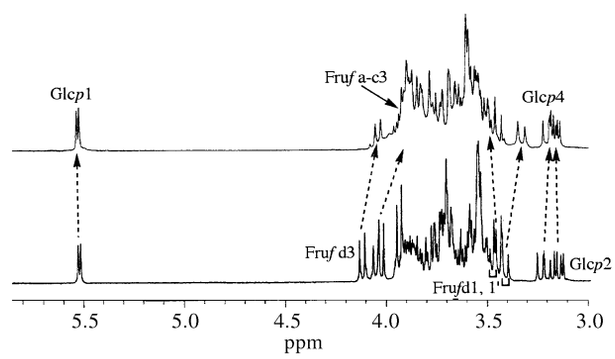


Fig. 5 ^{1}H-NMR spectra of **[^{2}H$_{51}$]MeFruNys** (bottom) and the complex with potassium thiocyanate (top) in $CDCl_3$ at 25 °C.

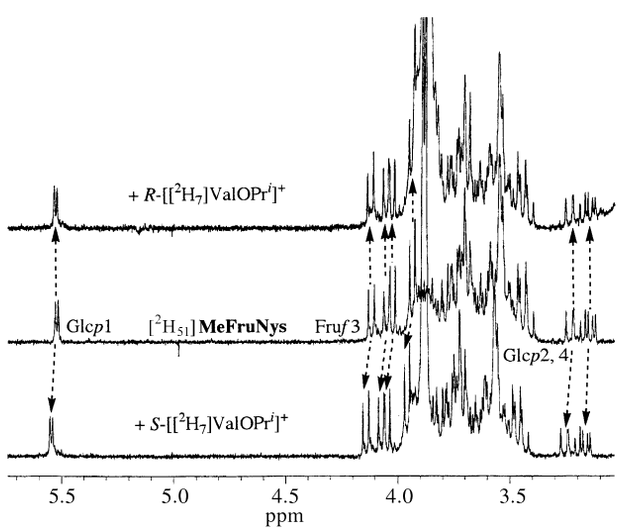
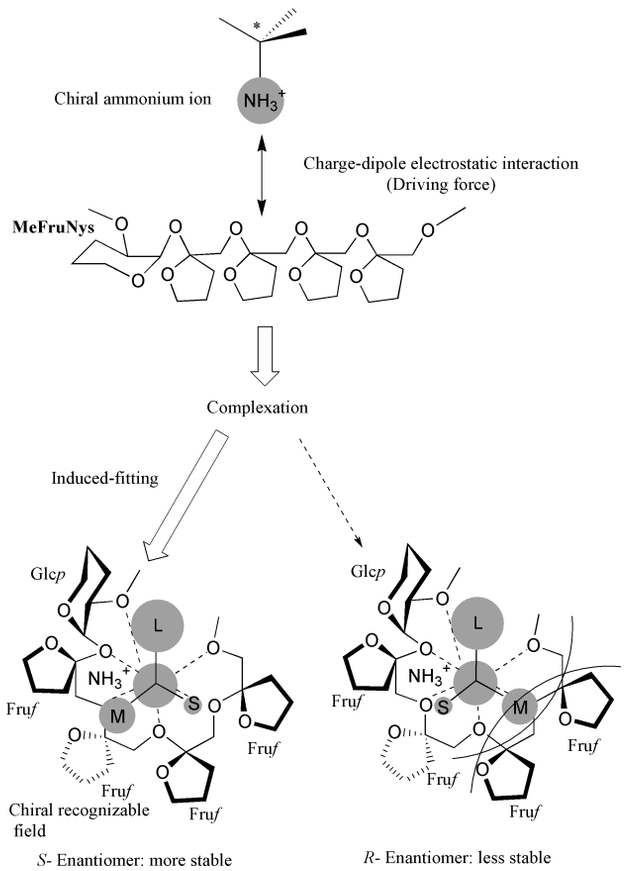


Fig. 6 ^{1}H-NMR spectra of **[^{2}H$_{51}$]MeFruNys** (middle), the complex with *R*-$[[^2H_7]ValOPr^i]^+$ Cl^- (top), and *S*-$[[^2H_7]ValOPr^i]^+$ Cl^- (bottom) in $CDCl_3$ at 25 °C.

chiral discrimination process of **MeFruNys** by the induced-fitting chiral recognition mechanism as follows. **MeFruNys** makes contact with a cation and the structure changes from linear to a pseudo-ring structure to form a 1 : 1 complex such as oligoethylene glycol derivatives.[5–7] In fact, $\Delta S°$ was negative and large in the complexation of **MeFruNys** with the amino acid ester salt ($[PheOPr^i]^+$). The complexation induces a chiral recognition field that consists of six oxygens in the pseudo-crown ether moiety, four fructofuranose rings which contain the sterically effective 3-OMe groups and the terminal glucopyranose ring.

In the case of one enantiomer guest, which forms a better fitting complex with **MeFruNys**, the intermolecular interaction

was understood using the three-point interaction as follows.[20] (i) The ammonium ion moiety of the amino acid ester salt binds the oxyethylene chain *via* charge–dipole electrostatic interactions, hydrogen bonding, *etc*. (as the driving force of the complexation). (ii) The largest substituent group attached to the asymmetric carbon in the amino acid ester salt can be located at the open part (ring-open space) in the pseudo-18-crown-6 ring of **MeFruNys** to avoid the effective repulsion. (iii) The smallest substituent locates itself at the crowded space in the fructofuranose moieties as shown in Scheme 1. In the



Scheme 1 Possible mechanism of chiral discrimination of **MeFruNys** toward the chiral ammonium ion.

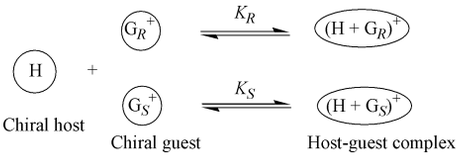
case of the amino acid ester salt with a larger R group such as $[PheOPr^{i}]^{+}$ or $[TrpOPr^{i}]^{+}$, the ordering of the substituents by size is R group > ester group > hydrogen. Therefore, it is expected that **MeFruNys** would form a more stable complex with the *S*-enantiomer of the amino acid ester salts rather than the *R*-enantiomer, in good agreement with the experimental results.

Interestingly, cyclic fructo-oligosaccharides such as **MeCF6** cannot effectively form an induced-fitting complex structure because of the rigid and highly symmetric structure.[17] This must be the reason for the smaller chiral discrimination ability of cyclic **MeCF6**, compared with acyclic **MeFruNys**.[13]

(4) $\Delta\Delta G_{enan}$ values of MeFruNys with various amino acid ester salts

A host–guest complexation equilibrium system containing three components of a chiral host and a pair of enantiomeric (racemic) guests in solution is shown in Scheme 2. If the initial concentrations of the guests ($[G_R^+]_0$ and $[G_S^+]_0$, $[G_R^+]_0 = [G_S^+]_0$) are in large excess compared with the initial concentration of the host ($[H]_0$), the ratio of the association constants (K_R/K_S) in the solution system is represented by eqn. (1).

$$K_R/K_S = [(H + G_R)^+]\,[G_S^+]/\{[(H + G_S)^+]\,[G_R^+]\} \quad (1)$$



Scheme 2 Host–guest complexation in a three component-equilibrium system.

Under the concentration conditions of $[H]_0 \ll [G_R^+]_0$ $(= [G_S^+]_0)$, the concentration ratio of the free guests are approximately equal ($[G_R^+] \approx [G_S^+]_0$). Therefore, the concentration ratio of the diastereomeric host–guest complex ions ($[(H + G_R)^+]/[(H + G_S)^+]$) corresponds to the ratio of the association constants (K_R/K_S) in the equilibrium solution system [eqn. (2)].

$$[(H + G_R)^+]/[(H + G_S)^+] = K_R/K_S \quad (2)$$

We have already reported that $I_R/I_{S\text{-}Dn}$ values at various initial concentrations of host $[H]_0$ in NBA such as chiral crown ethers successfully fit the curve of $[(H + G_R)^+]/[(H + G_S)^+]$ *vs*. $[H]_0$ as shown in eqn. (3).[8a] It is difficult to form complex ions after transfer to the gas phase.

$$I_R/I_{S\text{-}Dn} = [(H + G_R)^+]/[(H + G_S)^+] \quad (3)$$

Indeed, the enantiomeric excess in solution was evaluated from the relative intensity in the FABMS-EL host method.[21] In general, the peak intensity ratio in the FAB mass spectra does not directly reflect the concentration ratio in the matrix.[22] However, as far as the diastereomeric host–guest complex ions are concerned, the concentration ratio is reflected in the peak intensity ratio of a FAB mass spectrum because the transferability factor (efficiency) from the matrix to the gas phase is hardly different in the present diastereomeric complex ions. The $I_R/I_{S\text{-}Dn}$ value was approximately compatible with the K_R/K_S value [eqn. (4)] in the case of $[H]_0 \ll [G_R^+]_0$ $(= [G_S^+]_0)$ from

$$I_R/I_{S\text{-}Dn} \approx K_R/K_S \quad (4)$$

eqn. (2) and (3). Therefore, the relationship is reasonably represented by eqn. (5).

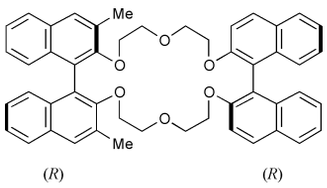
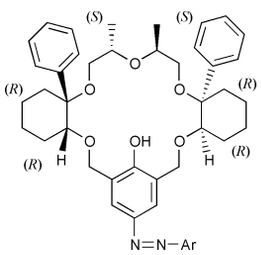
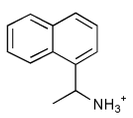
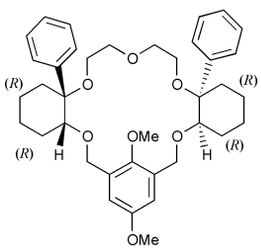
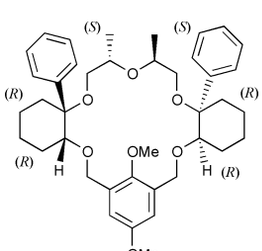
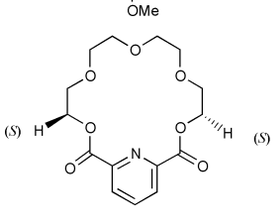
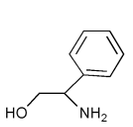
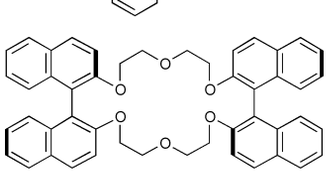
$$\begin{aligned}\Delta\Delta G_{enan} &= \Delta G_{R\text{-enantiomer}} - \Delta G_{S\text{-enantiomer}} \\ &= (-RT\ln K_R) - (RT\ln K_S) \\ &= -RT\ln (K_R/K_S) \\ &\approx -RT\ln (I_R/I_{S\text{-}Dn})\end{aligned} \quad (5)$$

The chiral discrimination ability evaluated by the FABMS method can be simply compared with that found by other means such as NMR, UV, fluorescence spectrometry (FL), extraction methods, *etc.*, using the kcal mol^{-1} unit of $\Delta\Delta G_{enan}$ (chiral difference in the equilibrium free energy change). Typical examples of chiral recognizable compounds are summarized in Table 7,[23] where mostly cyclic crown ether derivatives are involved. The $\Delta\Delta G_{enan}$ values in the 1 : 1 complexation of oligosaccharide derivatives with chiral guests have rarely been reported,[14] although the LC-separation ability of oligosaccharide derivatives is well-known. The acyclic oligosaccharide **MeFruNys** showed nearly the same high degree of chiral discrimination ability as the highly-structured artificial chiral crown ethers and their derivatives which have been utilized as chiral stationary phase (CSP) in LC.[24] The chiral recognition ability of **MeFruNys** is much better than that of permethylated α-cyclodextrin (α-**MeCD**).[23e,f]

Summary

We have demonstrated that **MeFruNys** (a permethylated fructo-oligosaccharide, pentasaccharide) has a remarkably high degree

Table 7 Comparison of the chiral discrimination ability of various hosts toward amino acid derivatives

Chiral host	Chiral guest	$\Delta\Delta G^{\circ}_{enan}$/kcal mol^{-1}	Selectivity	Solvent	Ref.
Still's cyclophane	B[BocNValOMe]	2.5	*S*	$CDCl_3$ [a]	23(*a*)
I 	[PGlyOMe]$^+$	1.9	*R*	$CDCl_3$ [b]	1(*c*)
II 	VII 	1.4	*R*	$CDCl_3$ [c]	23(*b*)
III 	[ValOMe]$^+$	1.2	*R*	$CDCl_3$ [a]	8(*e*)
MeFruNys	(i) [ValOPr^i]$^+$	1.2	*S*	NBA [d]	This work
	(ii) [PheOPr^i]$^+$	1.0	*S*	NBA [d]	This work
IV 	(i) [PheOEt]$^+$	1.0	*R*	NBA [d]	8(*a*)
	(ii) [MetOMe]$^+$	1.0	*R*	NBA [d]	8(*a*)
V 	VIII 	0.9	*R*	(i) Gas phase	23(*c*)
		0.6	*R*	(ii) MeOH	23(*d*)
VI 	[PGlyOMe]$^+$	0.56	*R*	$CDCl_3$ [b]	1(*b*)
MeCF6	[TrpOPr]$^+$	0.2	*R*	(i) NBA [d]	13
		0.2	*R*	(ii) $CDCl_3$ [a]	This work
α-MeCD	(i) [PGlyOPr^i]$^+$	0.04	*S*	NBA [d]	13
	(ii) [FPGlyOH]$^+$ [e]	0.05	*S*	$CDCl_3$ [a]	23(*e*),(*f*)

[a] The NMR titration method. [b] The extraction method. [c] The UV titration method. [d] The FABMS-EL guest method. [e] [FPGlyOH]$^+$ = 4-fluorophenylglycine hydrochloride.

of chiral discrimination toward amino acid ester salts (primary ammonium ions) by the induced-fitting chiral recognition mechanism. It was deduced that acyclic **MeFruNys** became a pseudo-18-crown-6-ring structure surrounding the ammonium ion in the process of complexation with amino acid ester salts, and the chiral discrimination is ascribed to the steric effect of the fructofuranose rings of **MeFruNys** and the substituent of a given amino acid ester salt. This is the complexation-induced selectivity. The chiral discrimination of the saccharide derivatives such as cyclodextrins,[25] amyloses,[26] monosaccharide derivatives,[27] *etc*., was reported and subjected to LC[28] and capillary electrophoresis (CE)[29] *etc*., although their ability for a 1 : 1 complexation was not very high. Until now, however, fructooligosaccharides have not received any attention.[30] They would

be expected to be useful in various applications as chirally recognizable materials in the future.

Experimental

Materials

Permethylated fructo-oligosaccharides: **MeKes**, **MeNys** and **MeFruNys** were prepared by the Hakomori method[31] from commercial samples (Wako) of free inulo-oligosaccharides (hydroxy type) which were optically pure (consisting of D-fructose and D-glucose). Other fructo-oligosaccharides (hydroxy type) were synthesized by the intermolecular transglycosylation of cyclofructan and glycopyranose using an enzyme.[32] **MeManFru$_3$**, **MeGlc^{6}Fru$_3$**, **MeSorFru$_3$** were prepared from the enzyme-synthesized carbohydrates by the Hakomori method. **[$^2H_{51}$]MeFruNys** was permethylated with [$^2H_3$]MeI.

Amino acid 2-propyl ester salts: amino acid 2-propyl ester hydrochlorides were synthesized according to the standard esterification method from commercial D-amino acids and L-amino acids (Sigma, Wako, Tokyo Kasei, and Aldrich) with propan-2-ol or [$^2H_8$]propan-2-ol (99+ atom% D Aldrich). L-[$^2H_8$] Tryptophan 2-propyl ester hydrochloride (***S*-5**) was prepared by esterification with [$^2H_6$]propan-2-ol, ([$^2H_6$]Me_2CHOH, 99.5 atom% D, CDN isotope, Canada) to avoid H–D exchange of the indole moiety. The proportion of deuterium labelled amino acid ester hydrochlorides depends upon that of the alcohol. The deuterium content of the labelled amino acid ester salts was >99%. The above products were dried *in vacuo* at 40 °C before the experiments.

Amino acid 2-propyl ester picrates were prepared by a neutralization reaction of picric acid and amino acid 2-propyl ester in benzene and recrystallized in benzene and ethyl acetate.[11] The products were dried *in vacuo* for 5–10 h at 50 °C and used in the UV–visible titration experiments.

Metallic salts: metallic picrates were prepared by a neutralization reaction of picric acid and metallic hydroxide, and recrystallized in water.[33] After filtration, the products were dried *in vacuo* for 5–10 h at 80 °C. Potassium thiocyanate was used without purification of commercial reagents (Kishida Chemical) after drying under vacuum at 100 °C overnight.

General

UV–visible spectra were recorded with a Hitachi U-3410 UV spectrometer. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded with a JEOL AL-300 spectrometer. TMS was used as the internal standard.

FAB mass spectrometry (MS)–enantiomer labelled (EL) guest method

FAB mass spectra (positive mode) were measured with a JEOL SX-102 mass spectrometer operating at an accelerating voltage of 10 kV with a mass range of *m*/*z* 100–2400. The instrument was equipped with a standard JEOL FAB source and an ion gun. Xenon was used as the atom beam with an emission current of 10 mA and an acceleration of 3 kV. The ion source pressure was typically *ca.* 1–2 × 10^{-5} Torr. The spectra were obtained with a magnet scan rate of 10 s per scan (to *m*/*z* 2400) and the data were processed with a JEOL JMA-DA 6000 data processing system. Calibration was carried out with CsI.

(a) Preparation of sample solutions

A sample solution was prepared by mixing two solutions and a matrix (3-nitrobenzyl alcohol (NBA), Aldrich). FAB mass spectra were measured at room temperature with a deposit of a 1 μL aliquot of the mixed solution which was left overnight to homogenize. The three solutions were as follows: (1) 10 μL of a 1.33 M MeOH solution of a 1 : 1 mixture of unlabelled *R*- and labelled *S*- amino acid ester salts ([A_R^+] = 0.67 M and [[^2H_n]A_S^+] = 0.67 M), (2) 5 μL of a 0.20 M $CHCl_3$ solution of a given permethylated oligosaccharide, and (3) 15 μL of the NBA matrix. The accuracy of the 1 : 1 equivalent concentration of *R*- and *S*- enantiomers was confirmed by the following check: (1) whether the relative intensity ($I_R/I_{S\text{-}Dn}$) values with 18-crown-6 (Tokyo Kasei) which is an achiral host were experimentally obtained as unity (1.00 ± 0.03) or not, (2) whether the relative peak areas were observed as unity in HPLC (column: Daicel CROWNPAK CR; eluent aq. $HClO_4$ pH 1.5) or not (in the case of [PheOPr^i]$^+$).

(b) Relative intensity ($I_R/I_{S\text{-}Dn}$) values

Each relative intensity ($I_R/I_{S\text{-}Dn}$) value in Table 1 was an average of the experimental values of the 10th, 20th, 30th, and 40th scans (n = 4). The observed peak intensity of a permethylated oligosaccharide/*S*-amino acid ester complex ion ($I_{S\text{-}Dn}$) inevitably contains contributions from the amount of (M + 6) or (M + 7) natural abundant isotope derived from the peak intensity of a permethylated oligosaccharide/*R*-amino acid ester complex ion (I_R). However, the theoretical distribution (%) of (M + 6) or (M + 7) was too small to correct. As a typical example, the theoretical contribution from (M + 6) of the complex ion of **MeFruNys** with *R*-[TrpOPr^i]$^+$ (the molecular formula, $C_{61}H_{105}N_2O_{28}$; *m*/*z* 1314) is only 0.1% (JEOL MSroute Data Reduction, Ver. 1.7d, isotope pattern calculation soft). The experimental $I_R/I_{S\text{-}Dn}$ value was 0.56 and that value requires no correction by the natural isotopic distribution.

(c) Effects of the deuterium labelled position on the $I_R/I_{S\text{-}Dn}$ values

Steric effects are an important factor for chiral discrimination. The position labelled with deuterium is a very important point to evaluate the chiral discrimination ability. We confirmed that the labeling of the ester group had little influence on the chiral discrimination, *i.e.*, the $I_R/I_{S\text{-}Dn}$ values by the following experiments. (i) FAB mass spectra of a sample mixture between an oligosaccharide and a 1 : 1 achiral amino acid mixture of labelled glycine 2-[$^2H_7$]propyl ester hydrochloride and unlabelled glycine 2-propyl ester hydrochloride were measured and the I/I_{Dn} values were *ca.* 1.00. (ii) The $I_R/I_{S\text{-}Dn}$ value of **MeFruNys** with a 1 : 1 mixture of labelled *R*-[[$^2H_7$]ValOPr^i]$^+$ and unlabelled *S*-[ValOPr^i]$^+$ was 6.89. The multiplication of the $I_R/I_{S\text{-}Dn}$ value and the $I_S/I_{R\text{-}Dn}$ value was almost unity (0.14 × 6.89 = 0.96).

UV–visible experiments

The bathochromic shift ($\Delta\lambda$) of λ_{max} and the *K* value of the amino acid ester picrate followed by adding permethylated oligosaccharides in distilled $CHCl_3$ were determined as follows. Permethylated fructo-oligosaccharide (solution) or **MeCF6** (solid) was gradually added to a *ca.* 5 × 10^{-5} M $CHCl_3$ solution of a given amino acid ester picrate stirred magnetically in a UV cell until no shift was observed or until the isosbestic relationship disappeared. To maintain the UV cell at an appropriate temperature, water regulated at that temperature in a NESLAB endocal refrigerated circulating bath was allowed to flow into a circulating cell holder. Cell temperature was measured using a thermometer (thermocouple type, TAKARA thermistor instruments Co., D226).

As a typical example, the case of **MeCF6** with *R*-[TrpOPr^i]$^+$ (picrate$^-$) at 25 °C: 25 mL of a 5.39 × 10^{-5} M **MeCF6** $CHCl_3$ solution was prepared in a flask charged with 0.60 mg of *R*-[TrpOPr^i]$^+$ (picrate$^-$) in $CHCl_3$. 3 mL of the solution was charged into a UV cell, and the cell was placed on the cell holder with a reference cell. A **MeCF6** tablet was prepared with a tablet molder (JASCO Tablet size 10 mm) and an oil press (RIKEN POWER Model No, P-1B) with a reducing pressure (a vacuum pump, YAMATO Type, EFOU). A weighed piece of the tablet was added stepwise to the solution of amino acid

ester picrate ([TrpOPr^i]$^+$ (picrate$^-$)) in the UV cell (total 8 pieces). The concentration of **MeCF6** was 0.054, 0.112, 0.158, 0.209, 0.264, 0.375, 0.530 and 0.846 mM, respectively. The ratios of the concentration **[MeCF6]**/[TrpOPr^i]$^+$ were 0.00, 1.01, 2.08, 2.93, 3.89, 4.90, 6.69, 9.84, and 15.7. The *K* values were determined from the spectral changes at various wavelengths using the typical non-linear method (1 : 1 simulation).[34] The calculated *K* values were 1.51×10^3 (376.6), 1.52×10^3 (387.8), 1.44×10^3 (390.1), 1.52×10^3 (401.0), 1.54×10^3 (412.2), and 1.30×10^3 M^{-1} (415.0 nm). The average value was $(1.47 \pm 0.01) \times 10^4$ M^{-1}.

Thermodynamic parameters ($\Delta H°$ and $\Delta S°$) of the complexation of **MeFruNys** with [PheOPr^i]$^+$(Pic$^-$) were calculated from the association constants obtained by the above procedures at various temperatures (3–36 °C).

PM3 Calculations

Calculations were carried out using the PM3 semiempirical molecular orbital procedure as implemented in the MOPAC 7 program package.[35] An Insight II system on a workstation (Silicon Graphics Inc.) was employed. Initial structures were modelled on the basis of the crystalline structures of the **MeCF6**·Ba^{2+} complex[17] and tetraglyme·Hg^{2+} complex.[7]

Molecular dynamics simulation

The models were built using a 3D-Sketcher software module in Cerius^2 ver. 4.2 MatSci system (Molecular Simulation Inc.) on a workstation (Octane, Silicon Graphics Inc.). The structure of the models was optimized using molecular mechanics (consistent force field: CFF ver. 91).[14] The 24 solvent molecules ($CHCl_3$) were located at random. A canonical (NTV) ensemble was used and the temparature was controlled by the Hoover dynamics method. One step of the calculation corresponds to 1 fs. Calculations were repeated over 500 000 steps (500 ps). The properties of averaged molecular length, radius of gyration, and self-diffusion coefficient were evaluated from the last 50 000 steps (50 ps).

NMR spectroscopy

(a) Evaluation of self-diffusion coefficient (*D*). In order to evaluate self-diffusion coefficients, ^{1}H-NMR (600 MHz) spectra were taken with a JEOL JNM-lambda 600 spectrometer equppied with a pulsed field gradient (PFG) probe (NALORAC, NH5XFG). The *D* values of **MeFruNys** and the complexes were evaluated by means of the PFG stimulated echo technique. The sample solutions were charged in a particular symmetric micro tube (Sigemi Standard Joint Co., CMS-005 for JEOL) having the same magnetic susceptibility as $CDCl_3$. The spectra were measured at various PFG power keeping the diffusional interval, PFG pulse width, and temperature (298 K) the same. The concentration of the solutions was 0.2 M. For a typical example, the case of **MeFruNys**: PFG power, 4.4, 8.8, 11.1, 13.3, 15.5, 17.7, and 19.9 G cm^{-1}; diffusional interval, 500 ms; PFG pulse width, 2 ms. The signal of the OMe protons (3.43 ppm) was followed. The relative intensities (I/I_0) for the peak intensity without PFG power were 0.801, 0.470, 0.319, 0.200, 0.118, and 0.0574, respectively. The slope of the linear relationship in the plot ln (I/I_0) *vs.* $(\gamma g \delta)^2(\Delta - \delta/3)$ corresponds to the self-diffusion coefficient; here γ is the gyromagnetic ratio of the proton; *g*, PFG power; δ, PFG pulse width; and Δ, PFG pulse interval. In this case, the *D* value is estimated to be 6.82×10^{-6} cm^2 s^{-1} from the slope of the line ($R^2 = 0.9988$). The *D* value estimated for a different diffusional interval (200 ms) was quite similar.

(b) Induced-shift. The complex solution of an oligosaccharide or **BzEO5Me** with a metallic cation was prepared by means of sonic homogenizer treatment of a large excess of potassium thiocyanate and a given permethylated oligosaccharide in $CDCl_3$. The concentration of the solution was *ca.* 2 mg per 0.6 mL. The metallic content was confirmed by atom absorption experiments, and the formation of 1 : 1 complex was confirmed. All measurements were carried out at 25 °C.

The complex solution of **[^{2}H$_{51}$]MeFruNys** or **BzEO5Me** with [[^{2}H$_7$]ValOPr^i]$^+$ was prepared by adding a $CDCl_3$ solution of [[^{2}H$_7$]ValOPr^i]$^+$ (Cl$^-$) to the host solution. The mole ratio was host–guest = 1 : 10. To ease the observation of shift changes of host proton signals, the amino acid ester salt labelled with deuterium was used.

Acknowledgements

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