Measurement of chiral amino acid discrimination by cyclic oligosaccharides: a direct FAB mass spectrometric approach

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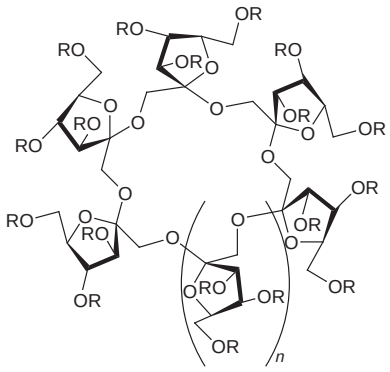
The novel cyclic oligosaccharides, permethylated cyclofructans MECF, 1b and 2b, discriminate enantiomers of chiral amino acid ester hydrochlorides.

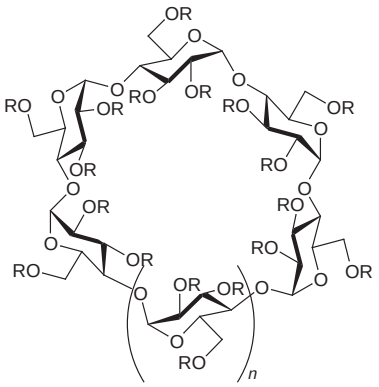
Chiral discrimination of amino acids by cyclodextrins (CD) has been widely investigated in the field of liquid chromatographic and electrophoretic enantioseparations,[1–4] but that by cyclofructans (CF)[5,6] has not as yet been examined. With the application of the FAB mass spectrometry (MS)–enantiomer labelled (EL) guest method,[7–9] one of the rare methods for estimation of the chiral recognition ability of new hosts,[10] we found for the first time that permethylated cyclofructans (MECF) exhibit various degrees of chiral discrimination towards amino acid esters. This is the first successful detection of the chiral amino acid-discriminating ability of cyclic oligosaccharides on a unified scale covering the two series, where various types of complexation mechanism, such as charge-dipole electrostatic, hydrophobic and hydrogen bonding interactions are operative. An oligosaccharide host (H) is complexed with a 1:1 amino acid guest mixture of an unlabelled (*R*)-enantiomer (G_R^+) and a deuterium labelled (*S*)-enantiomer ($G_{S\text{-}Dn}^+$). The enantioselectivity of a given oligosaccharide (host) toward a given racemic amino acid (guest) is quantitatively estimated 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.

The cyclic oligosaccharide hosts **1b**–**5b** were permethylated so that their complex ions were sensitively detected by FAB MS. Amino acid 2-propyl ester hydrochlorides[11] were used as guests so that natural abundance correction[7] was unnecessary. All (*S*)-enantiomers were labelled with deuterium (2-propyl ester: n = 6 or 7). A 1:1 racemic mixture solution of enantiomer guests was prepared by mixing 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 $CHCl_3$ solution were added to 15 μl of the 3-nitrobenzyl alcohol (NBA) matrix. A 1 μl sample of the final mixture was used for obtaining FAB mass spectra. A typical FAB mass spectrum is shown in Fig. 1, and the $I_R/I_{S\text{-}Dn}$ values obtained are summarized in Table 1. MECF6 **1b** and MECF7 **2b** showed higher (*R*)-enantioselectivity for $[Trp\text{-}O\text{-}Pr^i]^+$ ($I_R/I_{S\text{-}Dn}$ = 1.38–1.29; $-\Delta\Delta G_{enan}$ = 150–190 cal mol^{-1}) [$-\Delta\Delta G_{enan} = RT\ln(I_R/I_{S\text{-}Dn})$ was employed for interconversion].[7–9] MECF6 **1b** for $[Tle\text{-}O\text{-}Pr^i]^+$ and MECF7 **2b** for $[Ser\text{-}O\text{-}Pr^i]^+$ or $[Pro\text{-}O\text{-}Pr^i]^+$ provided the next best (*R*)-enantioselectivities. On the other hand, MECF7 **2b** for $[Pgly\text{-}O\text{-}Pr^i]^+$ showed an inverse (*S*)-enantioselectivity ($I_R/I_{S\text{-}Dn}$ = 0.76). β-MECD **4b** and γ-MECD **5b** indicated (*R*)-enantioselectivity for $[Ser\text{-}O\text{-}Pr^i]^+$ and $[Pro\text{-}O\text{-}Pr^i]^+$ ($I_R/I_{S\text{-}Dn}$ = *ca.* 1.15; $-\Delta\Delta G_{enan}$ = *ca.* 80 cal mol^{-1}).

Among many thermodynamic chiral discrimination studies, the chiral discrimination of simple amino acids by CDs on the basis of the host–guest (1:1) intermolecular interactions has rarely been reported until now. In one of the reports, Lincoln determined the relative binding constant (K_R/K_S = 0.92 in water at 295.5 K) of α-MECD **3b** with 4-fluorophenylglycine hydrochloride using the ^{19}F NMR titration method.[12,13] The enantioselectivity of α-MECD **3b** for the corresponding $[Pgly\text{-}O\text{-}Pr^i]^+$ under our FAB MS conditions ($I_R/I_{S\text{-}Dn}$ = 0.94) was in a good agreement with the above K_R/K_S value. It is worthwhile to note that this is further experimental evidence for the parallels between the $I_R/I_{S\text{-}Dn}$ and K_R/K_S values which have been described previously.[7–9] The NOE behavior of α-CD **3a** with Trp using ^1H NMR analysis suggested (*R*)-enantioselectivity,[14]



1a CF6, n = 1, R = H
b MECF6, n = 1, R = Me
2a CF7, n = 2, R = H
b MECF7, n = 2, R = Me



3a α-CD, n = 1, R = H
b α-MECD, n = 1, R = Me
4a β-CD, n = 2, R = H
b β-MECD, n = 2, R = Me
5a γ-CD, n = 3, R = H
b γ-MECD, n 3, R = Me

Guest (counter anion: Cl^-)	Host MECF6 **1b**	MECF7 **2b**	α-MECD **3b**	β-MECD **4b**	γ-MECD **5b**	18-C-6
$[Trp\text{-}O\text{-}Pr^i]^+$	1.38	1.29	1.29	1.23	1.17	0.98
$[Pgly\text{-}O\text{-}Pr^i]^+$	0.99	0.76	0.94	0.91	0.89	0.99
$[Phe\text{-}O\text{-}Pr^i]^+$	1.00	1.01	1.02	1.01	1.00	1.02
$[Tle\text{-}O\text{-}Pr^i]^+$	1.18	1.00	0.95	0.94	0.93	0.97
$[Met\text{-}O\text{-}Pr^i]^+$	1.04	0.95	0.91	0.91	0.92	0.96
$[Ser\text{-}O\text{-}Pr^i]^+$	1.01	1.18	0.95	1.15	0.99	0.96
$[Pro\text{-}O\text{-}Pr^i]^+$	1.08	1.16	1.07	1.07	1.14	0.96
$[Gly\text{-}O\text{-}Pr^i]^+$	1.01	0.99	0.97	0.98		0.99

[a] 18-crown-6 (18-C-6) is the typical achiral host employed. Glycine 2-propyl ester $(Gly\text{-}O\text{-}Pr^i)^+$ is the typical achiral guest employed. Averaged value (n = 4) of 10th, 20th, 30th and 40th scan data. Errors of the $I_R/I_{S\text{-}Dn}$ values are estimated within ±0.04. Tle = *tert*-leucine, Pgly = phenylglycine.

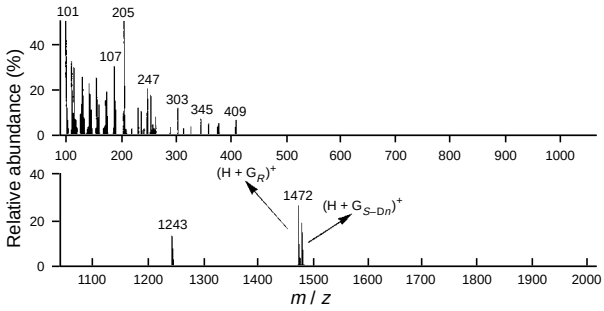


Fig. 1 An example of a FAB mass spectrum from the FABMS/EL guest method. Host: MECF6 **1b**; guest: $[Trp\text{-}O\text{-}Pr^i]^+$. The left peak ($m/z$ 1472) is a complex ion $(H + G_R)^+$ and the right (m/z 1478) is $(H + G_{S\text{-}Dn})^+$.

which was also in line with our results obtained by FAB MS (α-MECD **3b** with $[Trp\text{-}O\text{-}Pr^i]^+$; $I_R/I_{S\text{-}Dn}$ = 1.29).

The crystalline structure of a complex of MECF6 **1b** with Ba^{2+} (counter anion: SCN^-) that was reported previously showed that the cation was coordinated at ten points to the host, four 3-OMe oxygens of the furanose rings and six oxygens in the 18-crown-6 skeleton (Fig. 2).[6] In the case of a complex of MECF6 with amino acid ester hydrochlorides, it is expected that the 3-OMe groups become chiral barriers for the indole or *tert*-butyl groups of the corresponding chiral guest. The difference in the stereochemical complementarity would be origin of the chiral discrimination of MECF6 **1b**.

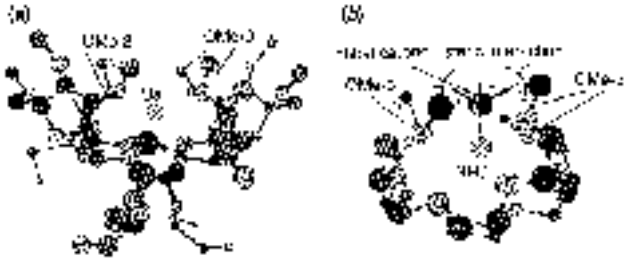


Fig. 2 (*a*) Crystalline complex of **1b** with barium cation (counter anion: SCN^-). Hydrogen atoms are not shown. (*b*) Imaged illustration of a complex of **1b** with an alkylammonium ion. The nitrogen cation of the alkylammonium replaced the barium from (*a*). The α-carbon of the alkylammonium ion was localized at a position of 1.49 Å from the nitrogen, an average C–N distance in amino acids.

We are studying in detail the mechanism of chiral discrimination of the cyclofructans. We hope that cyclofructans and their derivative hosts will be amenable to the further development of new chiral stationary phases and chiral selectors.

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Notes and References

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