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Preparation and Host-Guest Interactions of Novel Macrocyclic Sugar Clusters Having Mono- and Oligosaccharides

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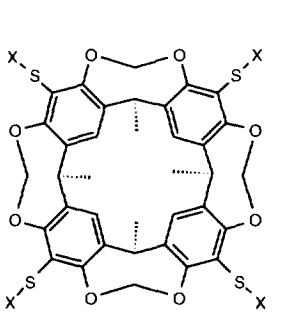
Abstract: Novel macrocyclic sugar clusters, which are constructed with a macrocyclic skeleton and four carbohydrate branches composed of glucose, maltose, or maltotriose derivatives individually, were prepared. Host–guest interactions between the hosts and a fluorescent guest, 8-anilinonaphthalene-1-sulphonate, were examined in aqueous media. The sugar clusters having maltose, or maltotriose residues strongly bind concanavaline A, but never peanut lectin.

Naturally occurring cell-surface oligosaccharides, presenting as a cluster, are known to play important roles in specific cellular-recognition in various biological processes.[1] In order to perform a functional simulation of cell-surface oligosaccharides, we have previously prepared macrocyclic sugar clusters having eight polar side chains with terminal glucose and galactose residues and examined their capability as a host in aqueous media.[2] We now designed macrocyclic sugar clusters, which are constructed with a macrocyclic skeleton and four carbohydrate moieties composed of glucose, maltose, or maltotriose derivatives individually (**1a**, **1b**, and **1c**, respectively). In this communication, we report on the preparation and guest-binding behavior of the sugar clusters as a host, with emphasis on microenvironmental polarity of the guest-binding sites provided by hosts **1a-c**.



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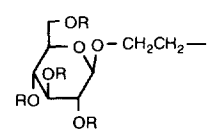

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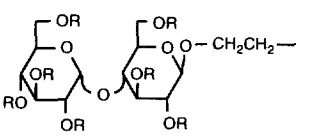

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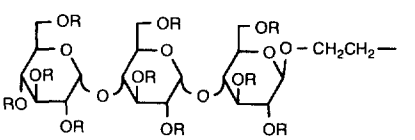

1c R = H; **2c** R = Ac

3 H–

Sugar clusters **1a-c** were synthesized by following the reaction sequences in Scheme 1. A sugar cluster having glucose residues **1a** was prepared by condensation of macrocyclic tetrathiol compound (**3**)[3] and 2-bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**4a**).[4] Successful coupling of **3** and **4a** was carried out in the presence of Cs_2CO_3 in DMF at room temperature to give the precursors of sugar cluster

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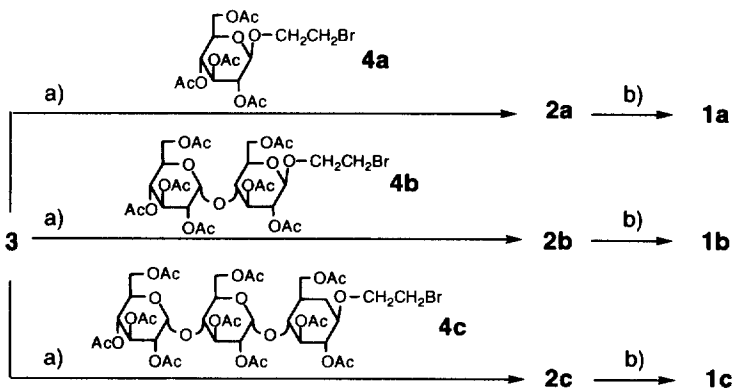
bearing glucose residues (**2a**, 63%) after purification by column chromatography on silica gel. Upon deacetylation with MeONa/MeOH, compound **2a** was converted into unprotected sugar cluster derivative **1a**. The use of 2-bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-β-D-glucopyranoside (**4b**)[5] and 2-bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-α-D-glucopyranosyl]-β-D-glucopyranoside (**4c**)[5] in place of **4a** afforded the corresponding precursors of sugar cluster bearing maltose and maltotriose residues (**2b** and **2c**, respectively). Compounds **2b** and **2c** were converted to **1b** and **1c**, respectively, after the method applied to the preparation of **1a**. All the novel products were purified by column chromatography and identified by ^{1}H NMR, TOF-MS, and IR spectroscopy as well as by elemental analyses.[6]



Scheme 1. Reagents : a) Cs_2CO_3; b) MeONa, MeOH.

The guest-binding behavior of hosts **1a-c** toward fluorescent probe, 8-anilinonaphtalene-1-sulphonate (ANS) whose emission is extremely sensitive to change in microenvironmental polarity experienced by the molecule, was examined by fluorescence spectroscopy in aqueous HEPES buffer (0.05 M, pH 7.2, μ 0.1 with NaCl) at 25 °C. Upon addition of the hosts to the HEPES buffer containing ANS (1.0 μM), a fluorescence intensity originating from the guest increased along with a concomitant blue shift of the fluorescence maximum, reflecting formation of the corresponding host-guest complexes (Fig. 1). Moreover, the extent of change in fluorescence intensity upon addition of the hosts under otherwise identical conditions is in the following sequential order with respect to the hosts: **1c** > **1b** > **1a**. Stoichiometry for the complexes formed with the hosts and ANS was investigated by the Job's continuous variation method. The results revealed that the present hosts underwent complex formation with the guest in a 1:1 molar ratio of host to guest. The binding constants (K) of **1a-c** toward ANS were evaluated on the basis of the Benesi-Hildebrand relationship for a 1:1 host-guest interaction in a manner as described previously.[7] The calculated K values are summarized in Table 1 together with the microenvironmental polarity parameter, E_T^N, which was evaluated from the fluorescence maximum in a manner similar to that reported previously.[7] As is obvious from the data in Table 1, the K values and the microenvironmental polarity of the guest-binding sites provided by hosts **1a-c** were subject to change by the size, shape and hydrophobic character of the three-dimensional internal cavity of the hosts. The E_T^N value for ANS in host **1c** (E_T^N = 0.53; approximately equal to the value for 2-propanol) is smaller than those for ANS upon complexation with **1b** and **1a**. Therefore, the guest-binding sites of **1c** are well shielded from bulk aqueous phase to become largely hydrophobic relative to those of **1b** and **1a**.

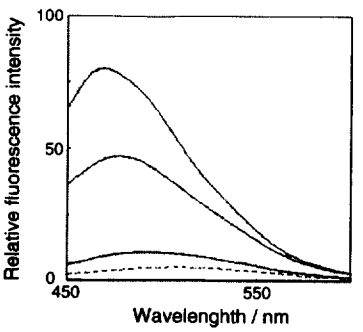


Fig.1 Fluorescence spectra of ANS in the presence of **1a** (a), **1b** (b), and **1c** (c) in aqueous HEPES buffer. Concentrations in M: ANS, 1.0 x 10^{-6}; hosts, 8.0 x 10^{-5}. The dotted line refers to background spectrum originating from ANS alone.

Table 1
Binding constants (K / M^{-1}), microenvironmental polarity parameters (E_T^N) for ANS molecule incorporated into sugar clusters **1a-c** in aqueous HEPES buffer (0.05 M, pH 7.2, μ 0.1 with NaCl) at 25 °C.

Host [a]	K / M^{-1}	E_T^N [b]
1a	900	0.85
1b	1600	0.75
1c	2600	0.53

a) Concentrations in M: ANS, 1.0 x 10^{-6}; hosts, 2.0 x 10^{-5} – 1.0 x 10^{-4}.

b) E_T^N values assigned to water and tetramethylsilane (TMS) are 1.00 and 0.00 as the most polar and apolar solvents, respectively.

Concanavaline A (Con A)[8] is an α-D-glucoside-binding lectin which is composed of four subunits, each having a carbohydrate-binding site. The recognition abilities of sugar clusters **1a-c** toward Con A were investigated by the precipitation method[9] in aqueous media in a manner as described previously.[10] Fig.2 shows that a solution of Con A (7.5 μM) becomes turbid with **1b** having four terminal α-D-glucosyl residues (50 μM), reflecting formation of a cross-linked aggregate; deaggregation occurs upon addition of a large excess of glucose (50 mM) as a competitive inhibitor. Peanut lectin (PNA), a galactose binding lectin, never interacts with **1b.** The specific binding of **1c** with Con A was also confirmed by the identical method, in a manner similar to those exercised by **1b** with the identical lectins. On the other hand, compound **1a** having four β-D-glucosyl residues, shows less binding affinity for Con A in aqueous media.

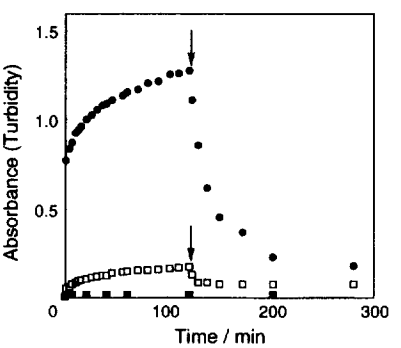


Fig. 2 Time courses of the turbidity change at 25℃ for a solution of **1b** (50 μM) and Con A (7.5 μM) (●), **1a** (50 μM) and Con A (7.5 μM) (□), and **1b** (50 μM) and PNA (7.5 μM) (■) in HEPES buffer (0.01M, pH 7.2, [NaCl] = 0.5M, [$MnCl_2$] = 0.1M, and [$CaCl_2$] = 0.1M). Arrows indicate addition of glucose (50 mM).

In conclusion, the present sugar clusters having mono- and oligosaccharides are capable of acting as host toward guest molecules in aqueous media. The differences in internal cavities among the hosts (i.e., size,

shape, and hydrophobic character of the binding sites) were reflected in the binding affinities toward the guest and the microenvironmental effects of the guest-binding sites of the hosts.

References and Notes

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[5] These compounds were prepared in a manner similar to that applied to the synthesis of **4a**. The configurations at the anomeric positions in **4a-c** were confirmed from the ^{1}H NMR spectra: $J_{1,2}$ 7.6–7.8 Hz.

[6] **1a**: mp 219–223 °C; ^{1}H NMR [500MHz, CD_3OD, 303 K] δ = 1.81 (d, J = 7.5 Hz, 12H, $CHCH_3$), 3.03 (m, 8H, OCH_2CH_2S), 3.14 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 9.0 Hz, 4H, H-2), 3.25 (m, 4H, H-5), 3.26 (m, 4H, H-4), 3.36 (dd, 4H, H-3), 3.61 [m, 4H, OCH_2CH_2S (nonequivalent)], 3.67 (dd, $J_{5,6}$ = 5.0, J_{gem} = 12 Hz, 4H, H-6), 3.84 (dd, $J_{5,6}$ = 5.0, J_{gem} = 12 Hz, 4H, H-6), 3.89 [m, 4H, OCH_2CH_2S (nonequivalent)], 4.27 (d, $J_{1,2}$ = 8.0 Hz, 4H, H-1), 4.30 (d, J = 7.5 Hz, 4H, inner OCH_2O), 5.00 (q, J = 7.5 Hz, 4H, $CHCH_3$), 5.92 (d, J = 7.5 Hz, 4H, outer OCH_2O), 7.45 (s, 4H, Ar-H); IR (KBr) 3400 (OH) cm^{-1}; Found: C, 52.46; H, 5.75%. Calcd for $C_{68}H_{88}O_{32}S_4 \cdot 1/2H_2O$: C, 52.53; H, 5.77%. TOF-MS *m/z* 1552 [M^+ + Li]; calcd M for $C_{68}H_{88}O_{32}S_4$, 1545. **1b**: mp 209–214 °C; ^{1}H NMR [500MHz, CD_3OD–D_2O (9:1 v/v), 303 K] δ = 1.81 (d, J = 7.0 Hz, 12H, $CHCH_3$), 3.01 (m, 8H, OCH_2CH_2S), 3.23 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 8.0 Hz, 4H, H-2), 3.33 (m, 4H, H-4'), 3.40 (m, 4H, H-5), 3.51 (m, 4H, H-2'), 3.56 (dd, $J_{3,4}$ = 9.0, $J_{4,5}$ = 9.0 Hz, 4H, H-4), 3.64 (m, 8H, H-3', OCH_2CH_2S (nonequivalent)]. 3.67 (m, 4H, H-3), 3.69 (m, 4H, H-5'), 3.78–3.86 (m, 16H, H-6, H-6'), 3.90 [m, 4H, OCH_2CH_2S (nonequivalent)], 4.32 (d, $J_{1,2}$ = 8.0 Hz, 4H, H-1), 4.28 (d, J = 7.5 Hz, 4H, inner OCH_2O), 4.95 (q, J = 7.0 Hz, 4H, $CHCH_3$), 5.22 (d, $J_{1',2'}$ = 3.5 Hz, 4H, H-1'), 5.96 (d, J = 7.5 Hz, 4H, outer OCH_2O), 7.45 (s, 4H, Ar- H); IR (KBr) 3400 (OH) cm^{-1}; Found: C, 49.98; H, 6.08%. Calcd for $C_{92}H_{128}O_{52}S_4 \cdot 1H_2O$: C, 49.95; H, 5.92%. TOF-MS *m/z* 2217 [M^+ + Na]; calcd M for $C_{92}H_{128}O_{52}S_4$, 2194. **1c**: mp 228–230 °C; ^{1}H NMR [500MHz, CD_3OD–D_2O (9:1 v/v), 323 K] selected data δ = 1.78 (m, 12H, $CHCH_3$), 4.24 (m, 4H, inner OCH_2O), 4.90 (m, 4H, $CHCH_3$), 5.33, 5.26 (m, 4H, H-1', H-1''), 6.06 (m, 4H, outer OCH_2O), 7.48 (s, 4H, Ar- H); IR (KBr) 3400 (OH) cm^{-1}; Found: C, 48.08; H, 6.00%. Calcd for $C_{116}H_{168}O_{72}S_4 \cdot 3H_2O$: C, 48.10; H, 6.05%. TOF-MS *m/z* 2864 [M^+ + Na]; calcd M for $C_{116}H_{168}O_{72}S_4$, 2841. Satisfactory elemental analyses and ^{1}H NMR spectra were also obtained for **2a-c**.

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