A Macrocyclic Sialic Acid Cluster as a Host, as an Adsorbate, and as a Ligand for Lectin and Virus

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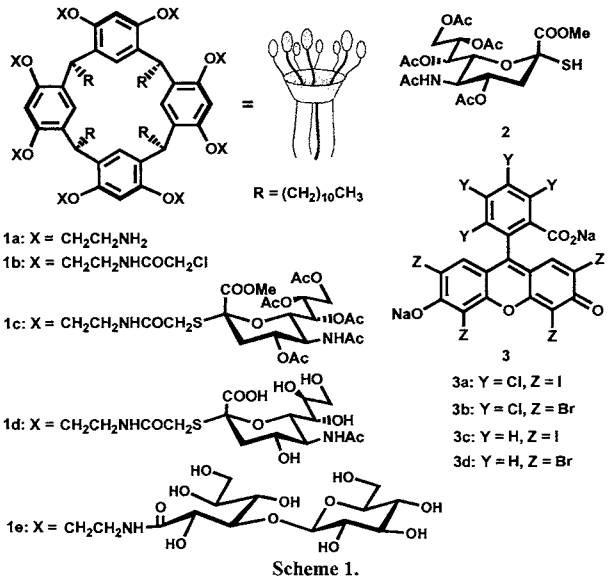
A calix[4]resorcarene-based macrocyclic octa(sialic acid) derivative **1d** was prepared. It forms complexes with such guests as rose bengal in water. It forms a closely packed monolayer on an SPR sensor chip; a sialo-targeting lectin is readily adsorbed on the saccharide residues exposed to bulk water. Compound **1d** also inhibits the hemagglutination of human erythrocytes and also cytopathic effect of MDCK (Madine-Darby canine kidney) cells mediated by human influenza A viruses at concentrations of 5-50 μM.

Sialyl oligosaccharides play important roles in many cellular events such as differentiation and multiplication, viral and bacterial infection, adhesion, and cancer-related phenomena. A variety of multiantennal sialic (*N*-acetylneuramic) acid derivatives (polymers,[1] dendrimers,[2] surfactant aggregates,[3] etc.) have so far been prepared and their interactions with biological receptor sites such as influenza viruses were studied in light of the so-called saccharide cluster effects.[4] We have recently introduced a macrocyclic version of saccharide clusters.[5] They are composed of a bowl-shaped macrocyclic framework, four long alkyl chains on one side of the macrocycle, and clustering eight saccharide moieties on the other (Scheme 1). An ultimate goal of this project is to provide saccharide clusters with a multifunctionality. We report here on the diverse interaction modes of the sialic acid derivative as a host, as an adsorbate, and as a ligand for sialo-targeting lectins and viruses.

An octa(sialic acid) derivative of calix[4]resorcarene (**1d**, R = undecyl; molecular weight = 4373) was prepared by coupling the corresponding octa(chloroacetamide) **1b**, obtained from octaamine **1a**,[5a] with protected glycosyl thiol **2**[6] and subsequent deprotection of the resulting octa(thioglycoside) **1c** (Scheme 1).[7] Compound **1d** is highly water-soluble. There is no evidence for its aggregation at ≤1 mM as far as surface tension and dynamic light scattering are concerned. The complexing ability of host **1d** was investigated with rose bengal (**3a**), a well-known singlet oxygen generator, as an anionic guest at pH = 7.8. The isosbestic spectral change (Figure 1),



Scheme 1.

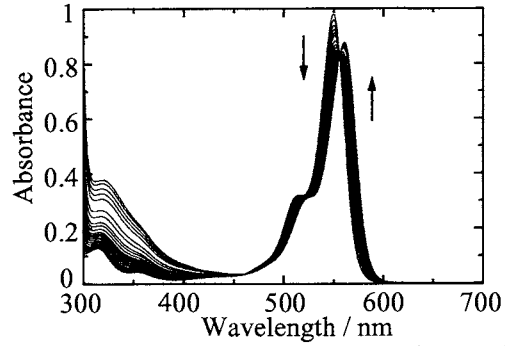


Figure 1. Electronic spectra for a series of solutions of guest **3a** (1.0×10^{-5} M) and varying amounts of host **1d** (0 - 5.0×10^{-4} M) in water at pH 7.8 (25 mM Tris-HCl buffer) at 25 °C.

coupled with continuous-variation Job analysis (not shown), indicates a 1:1 complexation with $K = 2.1 \times 10^4$ M^{-1} being evaluated from least-squares curve fitting. The affinities of other members of the halo-substituted fluorescein family sharply decrease in the order of phloxine B (**3b**, $K = 1740$ M^{-1}) > erythrosine B (**3c**, 800) > eosin Y (**3d**, <50).

Adsorption of a substance may be directly detected by surface plasmon resonance (SPR).[8] Compound **1d** having four alkyl ($C_{11}H_{23}$) tails is readily immobilized from an aqueous solution on a hydrophobized (covered with long-chain alkanethiol) sensor chip (gold) of SPR (BIAcore), resulting in an increase in resonance unit of ΔRU = 1280 at saturation. If the generally accepted relationship[9] is applicable, the observed ΔRU corresponds to a packing density of 0.18 molecules/nm^2 or inversely an occupation area of 5.7 nm^2/molecule for adsorbed **1d**. The occupation area is very close to an estimated cross-sectional area of the saccharide cluster part (diameter, 2-2.5 nm) of compound **1d** in a folded conformation. These results leave little doubt that adsorbate **1d** forms a closely packed monolayer with its long alkyl chains embedded in the hydrophobic forest on the solid surface. The clustering sialic acid residues exposed to bulk water in turn serve as a target of Japanese horseshoe crab lectin from *tachypleus tridentatas* (TTA,[10] from Seikagaku Kogyo), which binds to acetamino sugars, sialic acid in

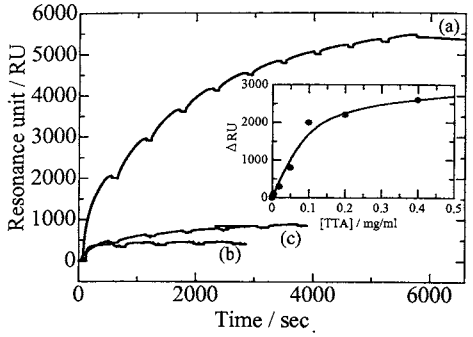


Figure 2. Sensorgrams for the adsorption of lectin, on the hydrophobized sensor chip coated with compound **1d** or **1e**: TTA vs **1d** (a), Concanavalin A vs **1d** (b), and TTA vs **1e** (c). A 40-μl portion of a lectin solution (0.1 mg/ml) was repeatedly added after adsorption/desorption was complete for the previous injection. Running buffer is 50 mM Tris-HCl (pH = 7.0) with [NaCl] = 150 mM and [$CaCl_2$] = 40 mM. Inset: variation in ΔRU with [TTA] for the first injection in (a).

particular. The observed value of ΔRU = 5310 (Figure 2, a) is much larger than that (ΔRU = 410) for a mannose/glucose-specific lectin Concanavalin A (b) or that (ΔRU = 860) for TTA with a glucose-functionalized surface coated with compound **1e**[5b,8c] (c). The strong **1d**-TTA interaction may be evidenced by the poor inhibitory effect of sialic acid, which in a great excess (50 mg/ml) inhibits the adsorption of TTA (0.1 mg/ml) by only ~50%.

It is well known that sialyl oligosaccharides serve as ligands for influenza viruses. The interactions of artificial sialylated substances with the viruses are conveniently evaluated by their inhibitory effects on the virus-mediated hemagglutination[11] and also by their capability of neutralizing the cytopathic effects of the viruses on the MDCK (Madine-Darby canine kidney) cells.[12] The present sialic acid cluster **1d** inhibits hemagglutination of human erythrocytes at the concentration of 44 μg/ml (10 μM), 22 μg/ml (5 μM), or 44 μg/ml (10 μM) for the A/PR/8/34 (H1N1), A/Memphis/1/71 (H3N2), or A/Aichi/2/68 (H3N2) strain of influenza A virus, respectively.[13] Cluster **1d** also shows a moderate neutralization activity with IC_{50} (concentration required for 50% neutralization) = 200 μg/ml (46 μM), 100 μg/ml (23 μM), and 200 μg/ml (46 μM) for the three types of strains.[14]

To summarize, compound **1d** is multifunctional. The aromatic cavity incorporates a guest molecule, the alkyl chains provide a site of adsorption on a hydrophobic surface, and the saccharide cluster part interacts with sialo-targeting lectins and viruses. Different functions may be uniquely combined. We are particularly concerned about the use of this type of macrocycles as specific transporters of included guests as either drugs or probes to the biological receptor sites. Design of high-density sialo-functionalized materials is also a subject of intense attention.

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Virus hemagglutination and neutralization assay was carried out in University of Shizuoka. Correspondence on this point should be addressed to this author.

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7 **1a** → **1b** ($(ClCH_2CO)_2O$ in CH_2Cl_2 at rt under Ar for 12 h; 91%), **1b** → **1c** (in DMSO-Et_3N (99:1) at rt under Ar for 12 h; 88%), **1c** → **1d** (NaOMe in MeOH-DMSO (2:1) at rt for 8 h, followed by ion exchange (Dowex 50W X-8(H^+)) and dialysis (Spectra/Por membrane MWCO 1000); 95%). **1c**: ^{1}H NMR (selected signals) (DMSO-d_6, 400 MHz) δ 0.81 (t, 12H, CH_2CH_3), 1.65 (s, 24H, $NCOCH_3$), 1.91, 1.93, 1.99, and 2.06 (each s, each 24H, $OCOCH_3$), 3.72 (s, 24H, OCH_3); Found: C, 54.21; H, 6.64; N, 3.84%. Calcd for $C_{264}H_{384}N_{16}O_{112}S_8$: C, 54.38; H, 6.64; N, 3.84%. **1d**: ^{1}H NMR (selected signals) (DMSO-d_6, 400 MHz) δ 0.83 (t, 12H, CH_2CH_3), 1.88 (s, 24H, $NCOCH_3$); MALDI-TOF MS (2,5-dihydroxybenzoic acid + NaCl) 4423.52 ([M - H + 2Na]$^+$, 4424.77); Found: C, 50.88; H, 7.07; N, 4.78%. Calcd for $C_{192}H_{304}N_{16}O_{80}S_8 \cdot 8H_2O$: C, 51.05; H, 7.14; N, 4.96%.

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13 A solution of **1d** (1 mg/ml) was serially diluted two-fold with phosphate buffered saline (PBS) containing 0.01% gelatin in 96-well microtiter plates. To each plate was added a suspension of virus (2^4 HA unit) and 0.5% human erythrocytes and hemagglutination was inspected visually. See reference 11 for the detailed procedure.

14 Monolayers of MDCK cells maintained in Eagle's minimum essential medium (EMEM) containing 5% of fetal calf serum were inoculated with 100 μl of $TCID_{50}$ (50% tissue-culture infectious dose) of virus in the presence of **1d** (0.01-2000 μg/ml). After removal of the inoculum, the monolayers were washed with EMEM. The activities of lactate dehydrogenase (LDH) released from virus-infected MDCK cells were assayed by colorimetry. See reference 12 for the detailed procedure.