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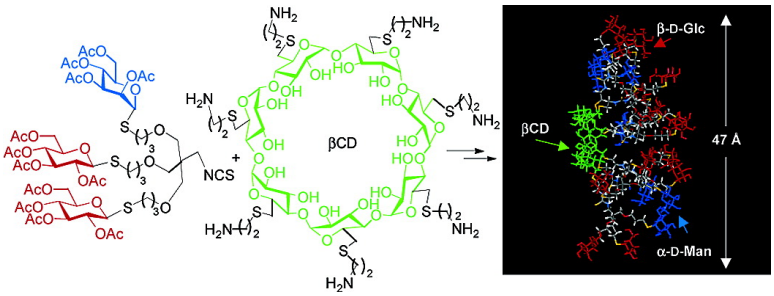

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Probing Secondary Carbohydrate−Protein Interactions with Highly Dense Cyclodextrin-Centered Heteroglycoclusters: The Heterocluster Effect

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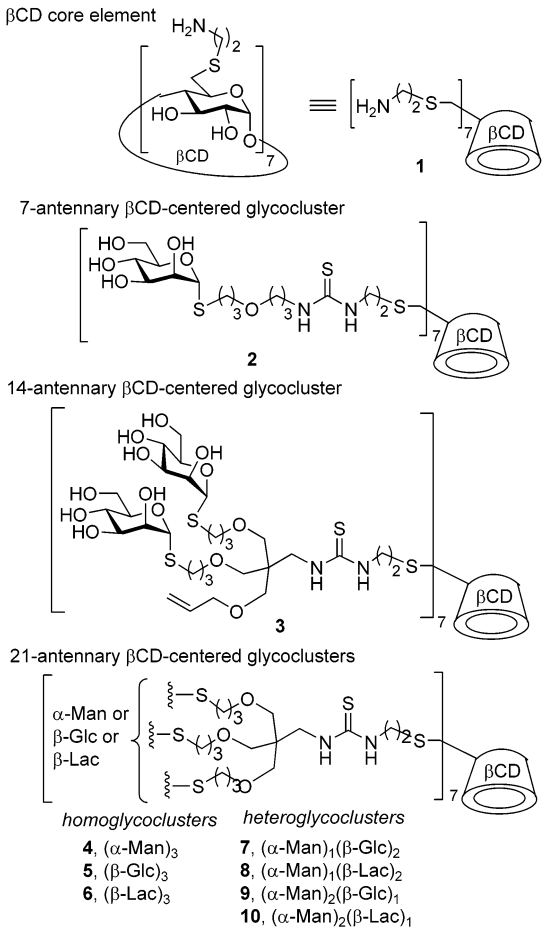
Multivalent interactions between carbohydrate ligands and carbohydrate-binding proteins (lectins) play a crucial role in a large variety of basic cellular recognition processes, which include development, differentiation, morphogenesis, fertilization, the immune response, implantation, cell migration, and cancer metastasis.[1] Synthetic multivalent glycoconjugates with well-defined structures have then considerable potential for the elucidation of the mechanisms involved and as possible inhibitors or effectors. A variety of multivalent oligosaccharide and glycoconjugate mimetics have been synthesized.[2] Typically, these systems incorporate several copies of a single type of sugar on an appropriate scaffold (molecular, dendritic, polymeric). However, in nature, a number of different sugar ligands may be essential for a recognition process. Variations in the expression levels of the sugar epitopes in a heterogeneous environment may also affect the individual binding affinities with a given protein receptor by virtue of secondary interactions.[3] However, only a few procedures that allow the construction of well-defined glycoclusters from different sugar moieties have still been described. Trivalent mixed-type oligosaccharide mimetics have been prepared by using orthogonally protected scaffolds.[4] The generation and deconvolution of dynamic combinatorial libraries of di- and trivalent heteroglycoclusters has also been reported.[5]

Model systems to address issues related to binding events involving heterogeneous cell-surface carbohydrate displays should comply with the need for polyvalency and high density. In this context, we now report on an efficient procedure that allows construction of 21-antennary heteroglycoclusters based on the β-cyclodextrin (βCD) core[6] (Chart 1) and their use as molecular glycocalix surrogates to probe secondary carbohydrate−protein interactions. The synthetic strategy involves the preparation of isothiocyanate-armed triantennary heteroglycodendrons suitable for covalent attachment, through thiourea bridges, to the face-selective functionalized per(C-6)-heptacysteaminyl-βCD derivative **1**, which proved to be a very efficient heptanucleophile for accessing C_7-symmetric hyperbranched structures. The synthesis of the heteroglycodendrons relies on the radical addition of per-O-acetylated 1-thiosugars (**12**−**14**) to the tri-O-allylated pentaerythritol derivative **11**.[7] This reaction affords the anti-Markovnikov adducts in a controlled manner, giving rise to either the mono-, di-, or trivalent

Chart 1. βCD-Centered Homo- and Heteroglycoclusters



derivatives, depending on the reagent relative proportions. The mono- or divalent derivatives were further reacted with another thiosugar to produce the bifunctional ligands. Finally, the glycodendrons were armed for further coupling by transforming the remaining primary hydroxy group into isothiocyanate via an azide intermediate (Scheme 1).

A critical advantage of the above methodology is that it allows sampling compounds with varied, yet perfectly defined densities of the constitutive sugars (D-mannose, D-glucose, or lactose; **4**−**10**) in an overall architecture that favors a highly compact packing by orientational bias (Figure 1a). The 7- and 14-valent mannosyl

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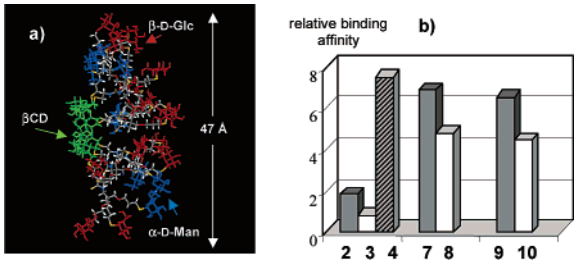
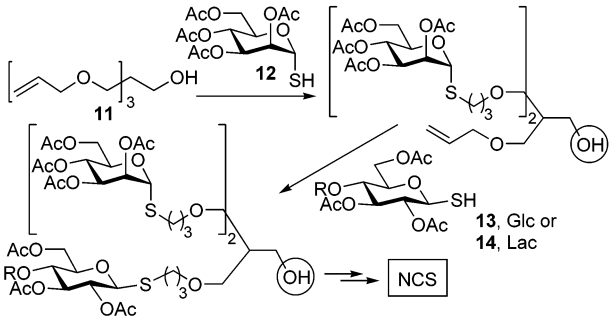


Figure 1. (a) Three-dimensional representation of the βCD-centered heteroglycocluster **7**. (b) Con A binding affinities (ELLA), relative to methyl α-D-mannopyranoside, for compounds **2**−**4** and **7**−**10** in mannose molar basis (IC_{50} ratio/number of mannose units).

Scheme 1. General Strategy for the Synthesis of Isothiocyanate-Armed Heteroglycodendrons



clusters **2** and **3** were also prepared as reference compounds. In this series, we explored the effect on receptor binding of the valency and density of the receptor-binding elements in a homogeneous compared to a heterogeneous environment.

The model lectin that we used for this purpose is the tetrameric plant lectin concanavalin A (Con A) that specifically recognizes α-D-mannopyranosyl (α-Man) epitopes. We considered it of interest to examine the possible interferences of D-glucose (Glc), a rather ubiquitous sugar, and lactose (Lac), a recognition motif for other important lectins, on such a recognition event. The binding affinity was first evaluated by the enzyme-linked lectin assay (ELLA), which provides information on the intrinsic lectin−ligand affinity, devoid of aggregation effects.[1c] The results (Figure 1b) reflected a substantial amplification of the lectin-binding strength for homogeneous α-Man clusters when the monosaccharide units were presented in triads (**4**), in agreement with previous observations,[5,8] while the 21-valent β-Glc and β-Lac conjugates **5** and **6** were not recognized by Con A, as expected from the known specificity of this lectin. Interestingly, the mixed-type α-Man−β-Glc and α-Man−β-Lac heteroglycoclusters (**7**−**10**) exhibited Con A-binding affinities significantly higher than that of the homogeneous conjugates with identical α-Man valency (**2** or **3**), indicative of up to 8-fold higher cluster effects on a mannose molar basis.

It has been speculated that nature uses carbohydrate surface density as an "on−off" switch to regulate biological events by virtue of the cluster effect. Our results suggest that the scenario is probably far more complicated and point to the existence of a *heterocluster effect* which cannot be explained in terms of a difference in effective epitope concentration.[9] The dimensions of the clusters, about 50 Å, probably preclude expanding the distance between two recognition sites in Con A (65 Å), while the presence of the horseradish peroxidase (HRP) label used in ELLA is supposed to prevent cross-linking processes.[1c] Likely, these macromolecules interact with a single binding site per lectin, with proximity/statistical affinity enhancement mechanisms operating.

To get thermodynamic information which could be reliably compared with the ELLA data, isothermal titration microcalorimetry measurements were carried out to evaluate the binding of HRP−Con A to glycoclusters **4**, **9**, and **7** at 25 °C. The results confirmed the 1:1 stoichiometry, in agreement with the absence of precipitation. The obtained $-\Delta G°$ (33.27 ± 1.0, 32.2 ± 3.1, and 31.4 ± 1.3 kJ mol^{-1}), $-\Delta H°$ (152.77 ± 10.5, 71.41 ± 11, and 8.26 ± 2.4 kJ mol^{-1}), and $T\Delta S°$ values (−119.11 ± 10.5, −39.14 ± 11.4, and 24.15 ± 2.4 kJ mol^{-1}, respectively) are indicative of partial enthalpy−entropy compensation, suggesting that a sliding mechanism, promoted by the presence of the secondary epitope, might be at the origin of the observed heterocluster effect. The active role of β-Glc was confirmed by the dramatic decrease in the binding free energy for compound **3** ($-\Delta G° = 21.1 \pm 1.4$ kJ mol^{-1}) as compared with that of **9**, having identical mannose valency.

Regardless of their precise nature, the secondary interactions responsible for the heterocluster effect could have a significant influence on protein binding, resulting in binding selectivity changes at high surface densities.[10] This may be interpreted as a saving mechanism; a relatively low expression level of the putative sugar ligand may be activated by expressing a second, "less costly" epitope. Diversity-oriented methodologies that allow the efficient control of the composition and the geometry of mixed-type glycoclusters may help to understand the basis of these phenomena, which might have implications in the design of synthetic ligands for therapeutically relevant lectins.

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Supporting Information Available: Experimental details for the new compounds, and procedures for Con A affinity measurements (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) No heterocluster effect was observed when comparing triantennary homo- and heteroglycoclusters. In line with results in ref 3, our results support that secondary interactions in the later scenario become significant only at high surface density of the contributing sugars.

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