

Mimicking Biological Membranes with Programmable Glycan Ligands Self-Assembled from Amphiphilic Janus Glycodendrimers**

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Abstract: An accelerated modular synthesis produced 18 amphiphilic Janus glycodendrimers with three different topologies formed from either two or one carbohydrate head groups or a mixed constellation with a noncarbohydrate hydrophilic arm. By simple injection of their THF solutions into water or buffer, all of the Janus compounds self-assembled into uniform, stable, and soft unilamellar vesicles, denoted glycodendrimersomes. The mixed constellation topology glycodendrimersomes were demonstrated to be most efficient in binding plant, bacterial, and human lectins. This evidence with biomedically relevant receptors offers a promising perspective for the application of such glycodendrimersomes in targeted drug delivery, vaccines, and other areas of nanomedicine.

Multivalent displays of glycan ligands from the surface of biological membranes are emulated by glycopolymers^[1] and glycodendrimers^[2] with carbohydrates in each repeat unit or at the chain ends. They are efficient for recognition of the carbohydrate-binding proteins, lectins. Owing to their lack of an encapsulating cavity and an imprecise spatial ligand presentation, these multivalent glycoconjugates only mimic the functionality of the surface of biological membranes in

a primitive way. Vesicles, liposomes, and polymersomes are endowed with an internal cavity,^[3] thus making them candidates for mimicking the supramolecular multivalency of biological membranes.^[4] A few examples of carbohydrate-containing vesicles with a random distribution of glycan on their surfaces were generated by complex and time-consuming co-assembly.^[4a,5] Recently, a simple method for the assembly of amphiphilic Janus dendrimers to produce monodisperse, stable over time, and impermeable dendrimersomes with predictable dimensions and properties was reported.^[6] Seven libraries, containing 51 amphiphilic Janus glycodendrimers (Figure 1 a), were screened to discover ten that form

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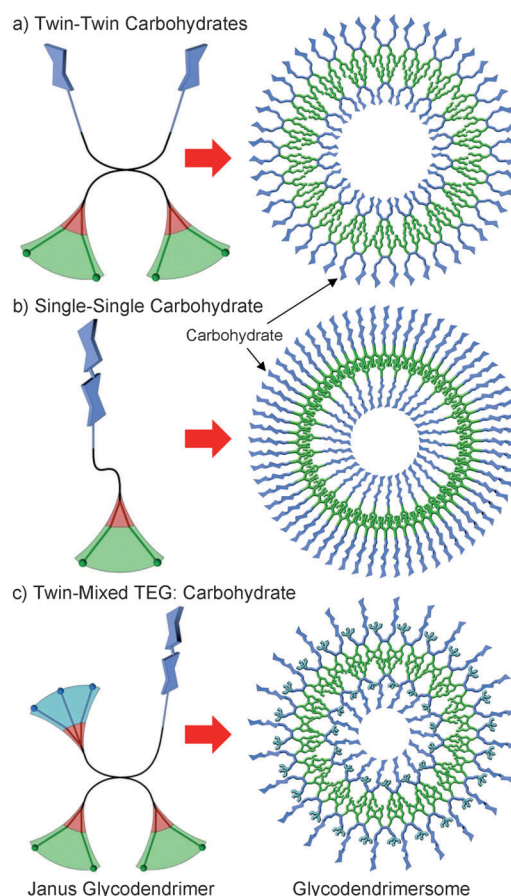


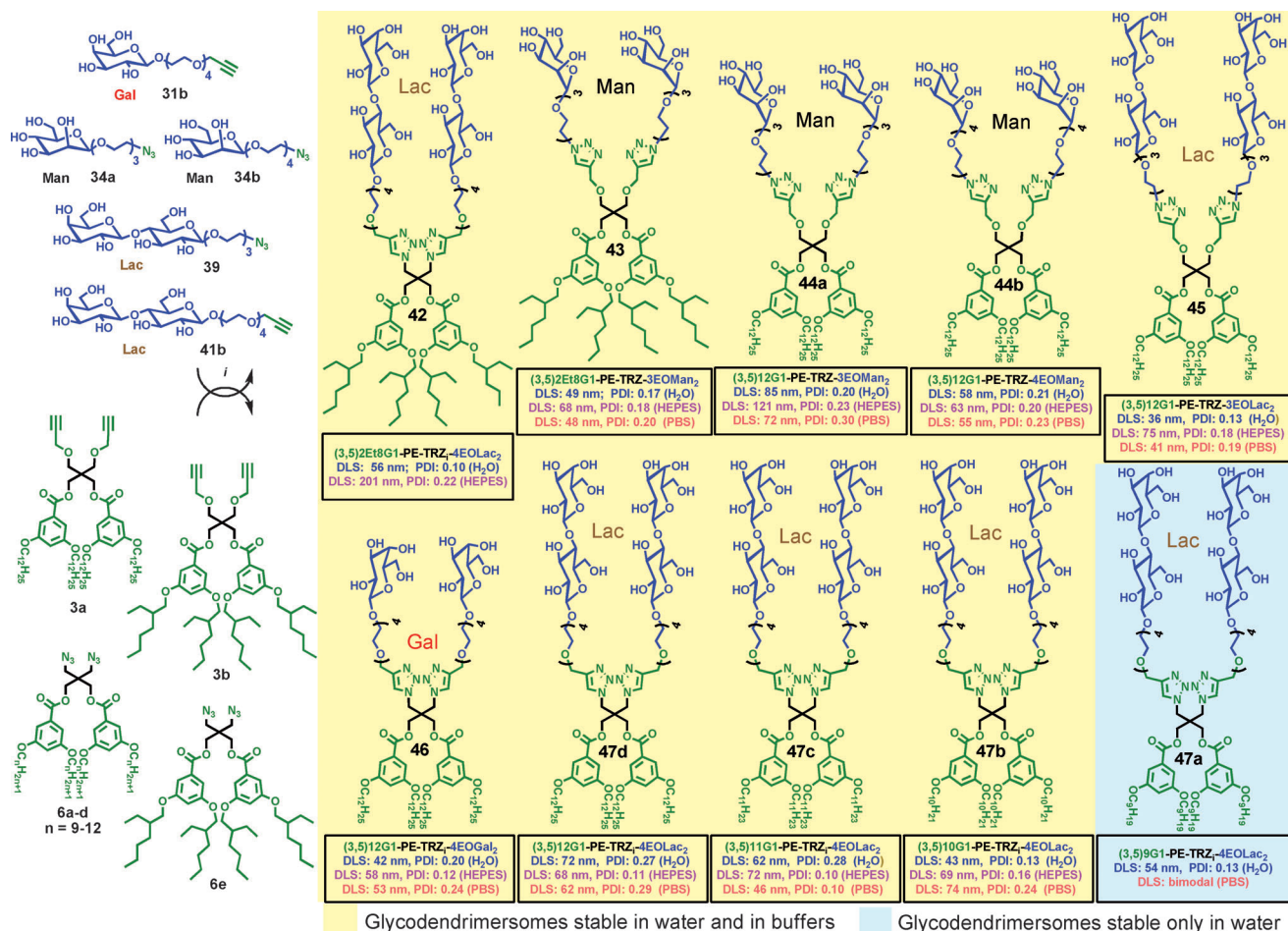
Figure 1. Three different topologies of amphiphilic Janus glycodendrimers and the structure of the corresponding multivalent glycodendrimersomes: a) twin-twin carbohydrates, b) single-single carbohydrate and c) twin-mixed TEG:carbohydrate. Color code: hydrophilic blue, hydrophobic green, aromatic red.

soft unilamellar multivalent glycodendrimersomes that are stable in water and in buffers.^[7] This enormous synthetic effort prompted us to investigate the capability to predict the assembly of soft unilamellar glycodendrimersomes from the simplest primary structures of twin-twin carbohydrate,^[7] single-single carbohydrate, and twin-mixed TEG:carbohydrate amphiphilic Janus glycodendrimers (Figure 1). This prediction was accessed by exploring a previously discovered twin-twin carbohydrate frame^[7] together with methodologies employed for amphiphilic Janus dendrimers.^[6a,c] Bioactivity was tested by agglutination assays performed with a mannoside-specific plant lectin (concanavalin A, ConA),^[2d] a galactoside-specific bacterial lectin (PA-IL),^[8] and the human galectin-7 (hGal-7).^[9]

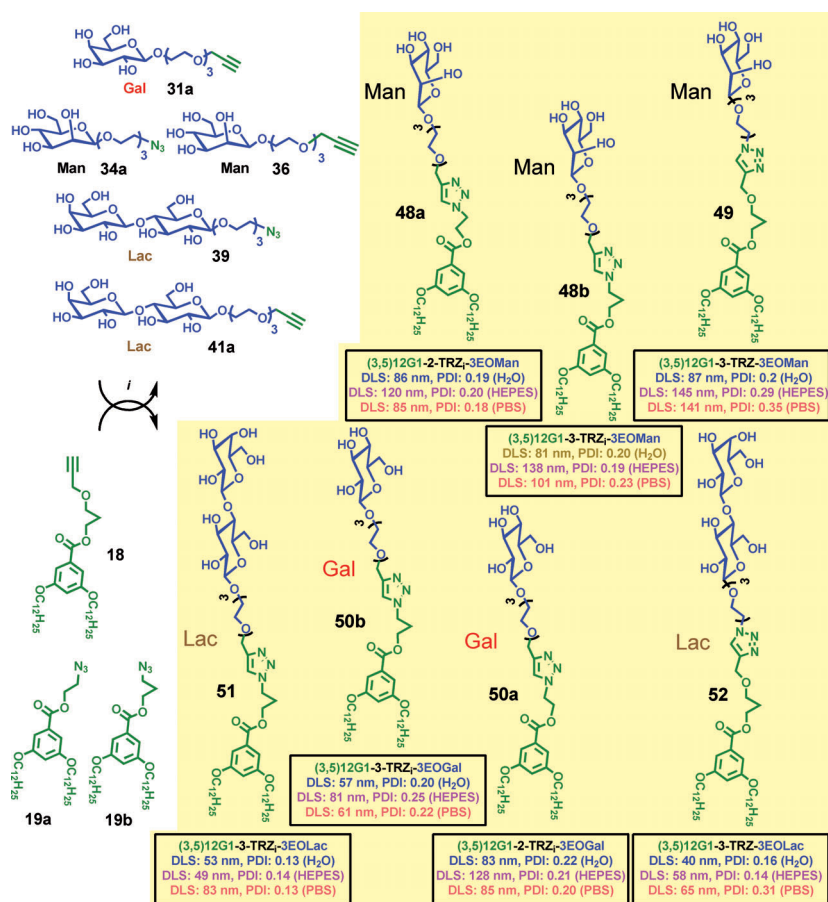
Three libraries containing 18 Janus compounds with different topologies were synthesized through an accelerated modular strategy^[7] (see the Supporting Information). These libraries include ten twin-twin carbohydrate (Scheme 1), seven single-single carbohydrate (Scheme 2), and three twin-mixed TEG:carbohydrate (Scheme 3) amphiphilic Janus glycodendrimers. In this study, we were concerned with the simplest possible first generation dendrons, denoted

minidendrons,^[10a] as models or maquettes for the discovery of novel architectural motifs that may also be accessible from higher generations during self-assembly in water. The role of minidendrons^[2g,10a,b] and Janus minidendrimers is analogous to that of simple peptides used to understand the molecular engineering involved in the assembly of more complex proteins, or of maquettes used by sculptors and architects to appreciate various aspects of full-size objects.^[10a,c] The twin-twin topology was inspired by a previous report.^[7] The single-single^[6c] topology was generated by splitting the most successful twin-twin structures into single-single carbohydrate (Scheme 1 and 2). The twin-mixed TEG:carbohydrate were designed by reassembling a single-single amphiphilic Janus dendrimer discovered recently^[6c] with a single-single carbohydrate amphiphilic Janus glycodendrimer from Scheme 2.

Library 1 contains ten twin-twin carbohydrate Janus glycodendrimers presenting three different carbohydrates; D-mannose (Man), D-galactose (Gal), and D-lactose (Lac); conjugated to the hydrophobic parts of minidendrons (Scheme 1). Regardless of the type of carbohydrate and the pattern of the alkyl groups from the hydrophobic part, all of the Janus molecules self-assembled into monodisperse^[4f] and



Scheme 1. Modular synthesis of library 1, which consists of ten constitutional isomeric twin-twin carbohydrate compounds with D-Man, D-Gal, and D-Lac as headgroups. Reagents and conditions: CuSO₄·5 H₂O, sodium ascorbate, THF/water, 23 °C. The diameter (in nm) and polydispersity (PDI) of the glycodendrimersomes were measured by DLS in water and in HEPES and PBS (0.5 mg mL⁻¹). THF = tetrahydrofuran, HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid, PBS = phosphate buffered saline.



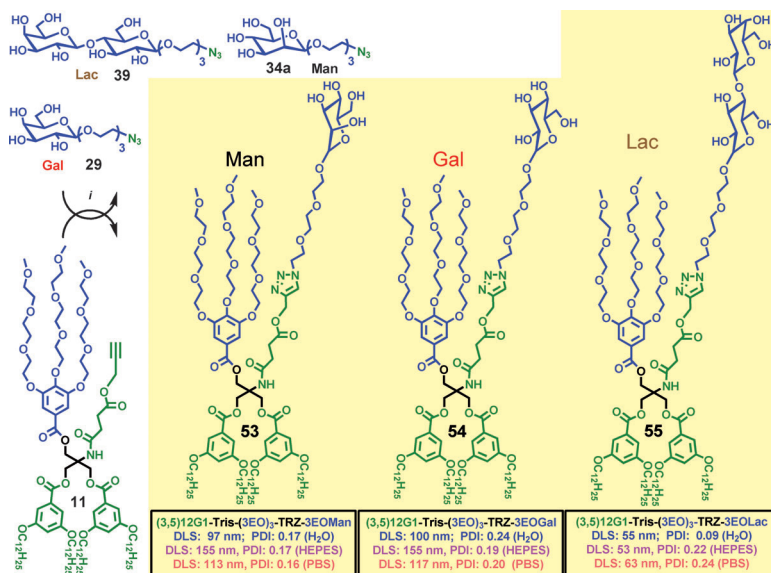
Scheme 2. Modular synthesis of library 2, which consists of seven constitutional single-single carbohydrate compounds with D-Man, D-Gal, and D-Lac as headgroups. Reagents and conditions: $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, sodium ascorbate, THF/water, 23 °C. The diameter (in nm) and polydispersity (PDI) of the glycodendrimer vesicles were measured by DLS in water and in HEPES and PBS (0.5 mg mL^{-1}).

soft unilamellar glycodendrimer vesicles (Scheme 1 and Figure SF1 in the Supporting Information). This demonstrates the predictability of the primary structures and their assembly in water by small variations of a previously discovered chemical frame.^[7] Glycodendrimers **47b–d**, which have more than nine carbon atoms in linear alkyl chains, and compounds **42** and **43**, which have branched hexyl groups, self-assembled into stable vesicles in HEPES and PBS (Scheme 1). Vesicles formed by **47a**, which contains only nine carbon atoms in the alkyl chains (highlighted in blue in Scheme 1), showed bimodal size distribution in PBS and precipitated from HEPES.

A key feature of bioregulation on a cell surface is the modulation of ligand presentation to make it relevant for cell adhesion and signaling.^[11] Even though the glycan ligands of glycodendrimer vesicles formed with twin-twin carbohydrates were demonstrated to be multivalent,^[7] it is fundamental to understand whether the excessively high number of carbohydrates from the hydrophilic part of these vesicles is required for

binding. Two approaches were elaborated to answer this question. The first involved seven single-single carbohydrate from Library 2 with a single hydrophobic dendron and a single carbohydrate headgroup, which reduce their mass by half compared to their twin-twin analogues (Figure 1b and Scheme 2). The second involved three compounds from Library 3 with two identical hydrophobic dendrons, one carbohydrate headgroup, and one hydrophilic dendron containing three triethylene glycol (TEG) monomethyl ether groups, denoted twin-mixed TEG:carbohydrate. In order to reduce the steric hindrance of binding to the target,^[4d] the carbohydrates were appended to the distal end of a long stretching chain so that the targeting ligands are extended beyond the vesicle surface (Figure 1c and Scheme 3). All of the single-single carbohydrate and twin-mixed TEG:carbohydrate self-assembled into uniform vesicles with submicron size in water, PBS, and HEPES (Figure 2 and Figure SF2,3). These glycodendrimer vesicles were used to test bioactivity.

To do so, the known capacity of di- and tetrameric lectins to form bridges between cells (agglutination) was exploited and three types of lectins were selected based on 1) their different sugar specificities and involvement of Ca^{2+} , and 2) their biofunctionality, deliberately including a virulence factor and a potent endogenous (human)



Scheme 3. Modular synthesis of library 3, which consists of three twin-mixed TEG:carbohydrate compounds with D-Man, D-Gal, and D-Lac as headgroups. Reagents and conditions: $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, sodium ascorbate, THF/water, 23 °C. The diameter (in nm) and polydispersity (PDI) of the glycodendrimer vesicles were measured by DLS in water and in HEPES and PBS (0.5 mg mL^{-1}).

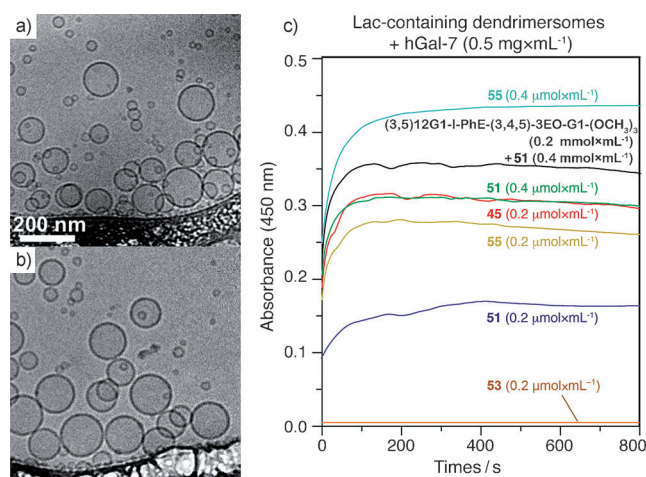


Figure 2. Representative cryo-TEM images of glycodendrimerosomes self-assembled by injection of THF solutions of a) **45** (0.5 mg mL^{-1}) in PBS or b) **50a** (0.5 mg mL^{-1}) in HEPES. c) Agglutination assay with Lac-containing glycodendrimerosomes (in $\mu\text{mol mL}^{-1}$) with different topologies in the presence of hGal-7 (0.5 mg mL^{-1}) in PBS buffer.

effector.^[2d,8,12] The absorbance profiles of agglutination assays with ConA (Figure SF4,5), PA-IL (Figure SF6), and hGal-7 (Figure 2) in HEPES or PBS were recorded by UV/Vis spectroscopy. The availability of glycodendrimerosomes with different sugar headgroups afforded rigorous specificity controls, as illustrated by the lack of reactivity of hGal-7 with Man-presenting glycodendrimerosome **53** (Figure 2c). After adding hGal-7 to the solution of Lac-presenting vesicles, the absorbance increased steadily until a plateau was reached (Figure 2c). Similar results were obtained from assays of ConA with vesicles self-assembled by D-Man-containing dendrimers (Figure SF5) and PA-IL with vesicles derived from D-Gal-containing dendrimers (Figure SF6). These results support the hypothesis of maintained selectivity and bioactivity.

In order to study the effect of the topology of these glycodendrimerosomes on the bioactivity of the corresponding glycodendrimerosomes, it is reasonable to keep the molar concentration of the ligand identical in each solution. The final concentration of twin-mixed molecule **55** and single-single **51** was adjusted to $0.4 \mu\text{mol mL}^{-1}$ to give $0.4 \mu\text{mol mL}^{-1}$ of D-lactose. The concentration of twin-twin **45** had to be set to $0.2 \mu\text{mol mL}^{-1}$. Interestingly, the agglutination curve of **51** at $0.4 \mu\text{mol mL}^{-1}$ overlapped with that of **45** at $0.2 \mu\text{mol mL}^{-1}$, which may indicate that the hydrodynamic volume of single-single glycodendrimerosomes on the vesicle surface is half that of their twin-twin counterparts. On the other hand, twin-mixed **55** at $0.4 \mu\text{mol mL}^{-1}$ showed the highest absorbance in the series, which indicates that a diluted density of glycan ligand favors the contact between the carbohydrate and protein because it leads to reduced steric hindrance.^[4d] The steric hindrance can also be reduced in the vesicles co-assembled with **51** ($0.4 \mu\text{mol mL}^{-1}$) and the nonsugar dendrimer (3,5)12G1-I-PhE-(3,4,5)-3EO-G1-(OCH₃)₃,^[6c] for which the density of sugar on the vesicle surface was diluted, thus leading to higher binding efficiency than with self-assembly by **51** ($0.4 \mu\text{mol mL}^{-1}$). Similar to carbohydrate-containing

vesicles co-assembled from glycolipids and phospholipids,^[5d] carbohydrate ligands are likely randomly distributed on the surface of vesicles formed from the physical mixture of **51** ($0.4 \mu\text{mol mL}^{-1}$) and (3,5)12G1-I-PhE-(3,4,5)-3EO-G1-(OCH₃)₃,^[6c] while the ligand distribution can be chemically controlled by twin-mixed **55**. The advantage of this type of chemical control of ligand distribution becomes evident when considering the highest bioactivity exhibited by the vesicles derived from twin-mixed topology (Figure 1c) in binding plant (Figure SF5), bacterial (Figure SF6), and human (Figure 2c) lectins.

In conclusion, three libraries containing 18 amphiphilic Janus glycodendrimerosomes with D-mannose, D-galactose, and D-lactose in the hydrophilic part have been synthesized through an accelerated modular strategy. These compounds exhibit three different topologies (Figure 1): ten are twin-twin carbohydrate amphiphilic Janus glycodendrimerosomes constructed with two identical hydrophobic dendrons and two identical carbohydrate headgroups (Library 1), seven are single-single carbohydrate glycodendrimerosomes built with one hydrophobic dendron and one carbohydrate (Library 2), and three are twin-mixed TEG:carbohydrate glycodendrimerosomes endowed with two identical hydrophobic dendrons, one TEG monomethyl ether containing hydrophilic dendron, and one carbohydrate (Library 3). Monodisperse and stable soft unilamellar vesicles, denoted glycodendrimerosomes, were prepared by simple injection of THF solutions of the glycodendrimerosomes into water or buffer. Notably, all 18 of the Janus compounds self-assemble into soft unilamellar multivalent glycodendrimerosomes, a result that highlights the extremely high reliability of the prediction strategy based on the primary structure of the Janus glycodendrimerosomes.^[7] By performing agglutination assays with the biomedically relevant plant lectin ConA, bacterial lectin PA-IL, and human galectin-7, specific and potent bioactivity for the glycodendrimerosomes was demonstrated, which proves the spatial arrangement of multivalent glycan display of glycodendrimerosomes. With the multivalent glycan ligands extending out of the vesicle surface, the glycodendrimerosomes formed by twin-mixed amphiphilic Janus glycodendrimerosomes (Figure 1c) showed the highest binding affinity for lectins and proved to have the optimal glycan ligand display. We demonstrated that the most efficient supramolecular glycan multivalency is determined by a combination of numbers and topology rather than only numbers. This novel biological membrane mimic is expected to be of interest for targeted drug delivery, vaccines,^[11c] and various fundamental and technological areas of nanomedicine.^[13]

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