JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

pubs.acs.org/JACS

Modular Synthesis of Amphiphilic Janus Glycodendrimers and Their Self-Assembly into Glycodendrimersomes and Other Complex Architectures with Bioactivity to Biomedically Relevant Lectins

Virgil Percec,*[,†] Pawaret Leowanawat,[†] Hao-Jan Sun,[†,∥] Oleg Kulikov,[†] Christopher D. Nusbaum,[†] Tam M. Tran,[†] Annabelle Bertin,[†] Daniela A. Wilson,[†] Mihai Peterca,[†] Shaodong Zhang,[†] Neha P. Kamat,[§] Kevin Vargo,[#] Diana Moock,[#] Eric D. Johnston,[§] Daniel A. Hammer,[§,#] Darrin J. Pochan,[▽] Yingchao Chen,[▽] Yoann M. Chabre,[‡] Tze C. Shiao,[‡] Milan Bergeron-Brlek,[‡] Sabine André,[⊥] René Roy,[‡] Hans-J. Gabius,[⊥] and Paul A. Heiney[∥]

[†]Roy & Diana Vagelos Laboratories, Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6323, United States

[∥]Department of Physics and Astronomy, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6396, United States

[§]Department of Bioengineering and Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, Pennsylvania 19104, United States

[#]Department of Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6323, United States

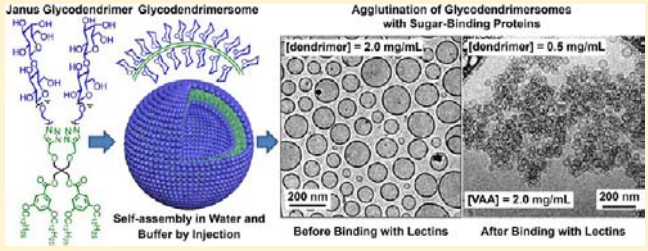
[▽]Department of Materials Science & Engineering, University of Delaware, Newark, Delaware 19716, United States

[‡]Department of Chemistry, Université du Québec à Montréal, P.O. Box 8888, Succ. Centre-Ville, Montréal, Québec, Canada, H3C 3P8,

[⊥]Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Ludwig-Maximilians-University, Veterinärstrasse 13, 80539 Munich, Germany

Supporting Information

ABSTRACT: The modular synthesis of 7 libraries containing 51 self-assembling amphiphilic Janus dendrimers with the monosaccharides D-mannose and D-galactose and the disaccharide D-lactose in their hydrophilic part is reported. These unprecedented sugar-containing dendrimers are named amphiphilic Janus glycodendrimers. Their self-assembly by simple injection of THF or ethanol solution into water or buffer and by hydration was analyzed by a combination of methods including dynamic light scattering, confocal microscopy, cryogenic transmission electron microscopy, Fourier transform analysis, and micropipet-aspiration experiments to assess mechanical properties. These libraries revealed a diversity of *hard* and *soft* assemblies, including unilamellar spherical, polygonal, and tubular vesicles denoted glycodendrimersomes, aggregates of Janus glycodendrimers and rodlike micelles named glycodendrimer aggregates and glycodendrimermicelles, cubosomes denoted glycodendrimercubosomes, and solid lamellae. These assemblies are stable over time in water and in buffer, exhibit narrow molecular-weight distribution, and display dimensions that are programmable by the concentration of the solution from which they are injected. This study elaborated the molecular principles leading to single-type soft glycodendrimersomes assembled from amphiphilic Janus glycodendrimers. The multivalency of glycodendrimersomes with different sizes and their ligand bioactivity were demonstrated by selective agglutination with a diversity of sugar-binding protein receptors such as the plant lectins concanavalin A and the highly toxic mistletoe *Viscum album* L. agglutinin, the bacterial lectin PA-IL from *Pseudomonas aeruginosa*, and, of special biomedical relevance, human adhesion/growth-regulatory galectin-3 and galectin-4. These results demonstrated the candidacy of glycodendrimersomes as new mimics of biological membranes with programmable glycan ligand presentations, as supramolecular lectin blockers, vaccines, and targeted delivery devices.



INTRODUCTION

Glycans of glycoconjugates adorn cell surfaces with recognition elements for sugar-binding proteins (lectins) to mediate functions such as cell−cell recognition and adhesion, initiation of signaling, delivery, and routing, also relevant for immune recognition.[1] Multivalent glycan displays are essential to overcome the weak interactions between individual sugars and proteins to generate ligand selectivity to sugar-binding protein receptors. In addition to the structure of the glycans, the spatial mode of

Received: April 3, 2013
Published: May 21, 2013

   dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

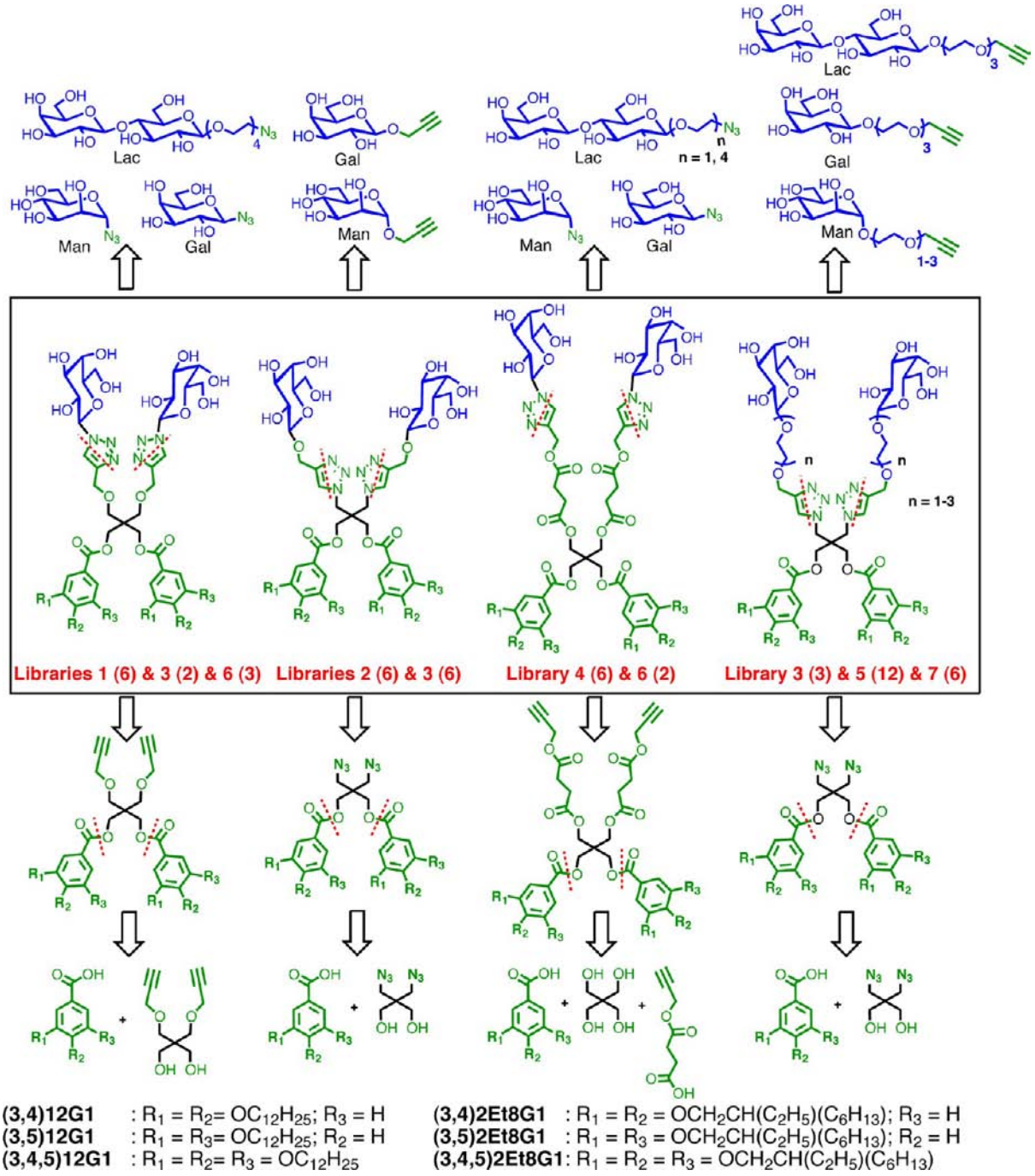
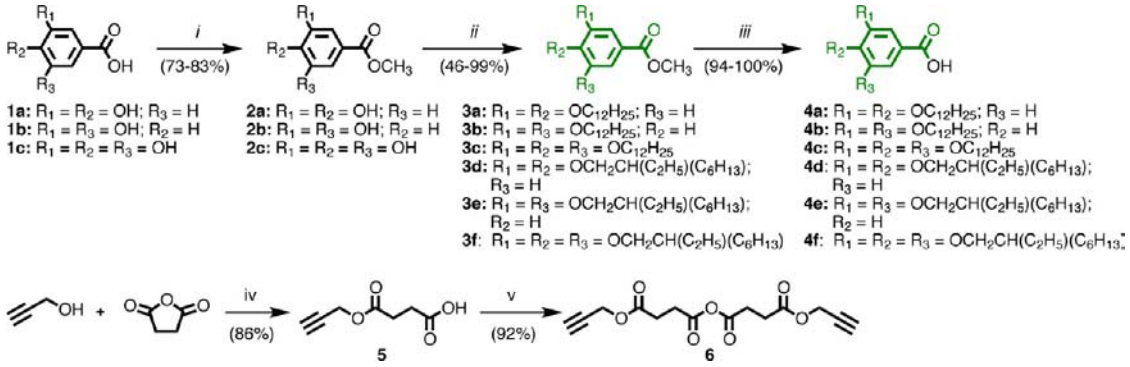


Figure 1. Modular approaches to the synthesis of seven libraries of amphiphilic Janus glycodendrimers. The numbers in between parentheses represent the number of Janus dendrimers in the corresponding library.

presentation, its dynamics, and adaptability play a salient role in turning glycan presence into ligand or counter-receptor functionality. The goal of understanding the route to specific recognition and control of cell physiology explains the chemical efforts toward tailoring diverse multivalent glycoconjugate displays accomplished via multivalent scaffolds. Mimics of natural glycoconjugates (glycoproteins and glycolipids) include glycopeptides,[2] glycopolymers,[3] glycodendrimers,[4−6] glycoliposomes and synthetic glycolipids,[5c] cyclic clusters such as cyclophanes,[6] and glycodynamers.[7] Their availability facilitates experimental approaches to understand the functioning of complex assemblies such as microdomains in membranes and can also form the basis for potential medical applications such as blocking undesired lectin binding in inflammation/tumor progression or in infection, or stimulating the immune response by vaccination.[4] Glycopolymers, glycodynamers, and glycodendrimers provide some accessible mimics for the biodisplay of glycans, but all require complex multistep synthesis to generate multivalency and are often built on toxic scaffolds. Vesicles presenting carbohydrates are more similar to biological cell membranes but lack precise structure because they are prepared by coassembly of several components by hydration followed by multiple fractionation via extrusion.[8] This results in a random distribution of carbohydrates over the surface of the vesicle. Therefore, a simple route to hard and soft vesicles that are fully programmable in regards to ligand density and type is in demand.

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

Scheme 1. Synthesis of the First-Generation Hydrophobic Dendritic Acids 4a–f and of the Alkyne Anhydride 6[a]



[a]Reagents and conditions: (i) H_2SO_4 (cat.), MeOH, reflux; (ii) 1-bromododecane or 2-ethylhexyl bromide, K_2CO_3, DMF (80 °C); (iii) KOH, EtOH, (reflux); (iv) DMAP (cat.) CH_2Cl_2 (25 °C); (v) DCC, CH_2Cl_2 (0–25 °C).

Additions to the existing panel of glycoconjugates that mimic biological membranes will broaden their range of applications.

Recently, we reported a new class of amphiphiles called Janus dendrimers that self-assemble by simple injection of their ethanol solution in water into narrow size distribution and stable-in-time vesicles called dendrimersomes.[9a] Moving toward applicability, we report here a simple strategy to an accelerated modular synthesis of the first examples of amphiphilic Janus glycodendrimers containing two identical carbohydrates in their hydrophilic part. They self-assemble by simple injection of their solution made in water-miscible solvents such as THF and ethanol into water and buffer and by hydration,[8] in unilamellar hard and soft spherical, polygonal, and tubular vesicles[9a,b,10] denoted glycodendrimersomes, aggregates of rodlike micelles[9a,11] named glycodendrimermicelles, aggregates of Janus glycodendrimers named glycodendrimer aggregates or, for short, dendrimer aggregates, cubosomes[9a,12] denoted glycodendrimercubosomes, and hard lamellae. By analogy with dendrimersomes and other complex architectures reported to self-assemble from simple amphiphilic Janus dendrimers,[9] all supramolecular assemblies generated in water from Janus glycodendrimers are obtained with a predictable size that amplifies the multivalency of presentation of their sugars from 2 to *n*, display narrow molecular mass distribution that in the case of vesicles and liposomes is considered to be monodisperse,[12k] and are stable over time. Structurally, they provide models for biological cell membranes with the typical glycan presence on their surface. These assemblies are of general interest as platforms for glycan ligand presentation because they offer a simple supramolecular approach to simulate the naturally multivalent display of carbohydrates.[1,3,4] They also may be engineered into devices used in the lectin-mediated delivery of drugs, genes, imaging agents, and pharmaproteins used as therapeutics to block lectins and to act as vaccines targeting lectins on dendritic cells.[1,3,4,9a,10–12] The spherical nature of the supramolecular assemblies produced from self-assembling amphiphilic Janus glycodendrimers provides an additional advantage to covalent glycodendrimers[4] as mimics, because their synthesis is simple and their water cavity can be exploited. Glycodendrimersomes offer an additional advantage to glycodendrimers,[4] by providing mimics of the biological membranes rather than only modeling their surface. Therefore, they are expected not only to be delivery devices but also to serve the same functions as covalent glycodendrimers.[4] To prove bioactivity in binding to lectins and thereby in principle access the potential applications mentioned above, we demonstrate selective agglutination of glycodendrimersomes of different sizes with the plant lectin concanavalin A (Con A), the toxic mistletoe lectin *Viscum album* agglutinin (VAA), a potential biohazard akin to ricin, the bacterial lectin PA-IL from *Pseudomonas aeruginosa,* and two human lectin members of the galectin family, galectin-3, Gal-3, and galectin-4, Gal-4, potently acting in adhesion, growth regulation, and glycan routing.[13]

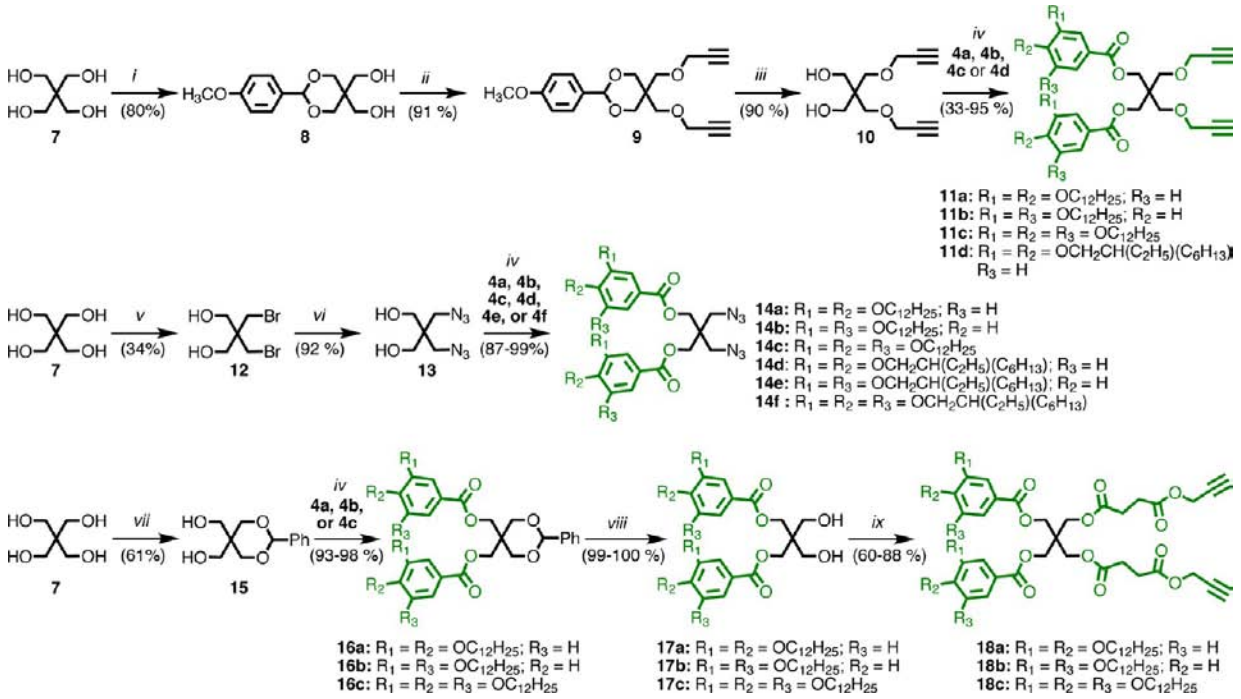
RESULTS AND DISCUSSION

Selection of Modular Synthetic Strategies and Primary Structures of Amphiphilic Janus Glycodendrimers. Four accelerated modular strategies were elaborated for the synthesis of seven libraries of amphiphilic Janus glycodendrimers (Figure 1).

Libraries 1 and 2 shown in the first two left side columns from Figure 1 are constitutional isomers and were expected to provide the shortest synthetic routes to self-assembling amphiphilic Janus glycodendrimers. The modular synthetic methodologies for these two libraries involve the synthesis of twin-hydrophobic dendrons containing *n*-alkyl groups and are functionalized with alkyne or azide attached directly to the focal point of the Janus dendrimer precursor. In the second step of this modular methodology, the unprotected monosaccharides D-mannose and D-galactose, containing alkyne and azide aglyconic groups as models for any other carbohydrate headgroups, were rapidly combined with the complementary twin-hydrophobic dendrons via copper-catalyzed click chemistry. Discrimination between alkyne vs azide at each of the two dendrimer frangments as the preferred mode of synthesis together with assembly was the driving force behind these experiments. The modular strategy employed in the synthesis of library 3 consisting of a mixture of constitutional isomers is similar to that used for libraries 1 and 2 except that, to increase solubility, the *n*-alkyl groups of the twin-hydrophobic dendrons were replaced with branched alkyls. A third modular approach was used to construct libraries 4 and 6 (third column from the left side of Figure 1). Library 4 contains a succinic ester spacer between the twin-hydrophobic dendrons and their alkynes used in the click chemistry. This spacer was incorporated to enhance solubility and flexibility, and as shown later, an important factor in recognition studies. This library contains D-mannose, D-galactose, and D-lactose in the hydrophilic part of the Janus dendrimers. The fourth modular approach (libraries 3, 5, and 7) incorporates hydrophilic oligooxyethylene spacers between the carbohydrate and the twin-hydrophobic dendrimers. These spacers were expected to increase hydrophilicity, flexibility, and solubility.

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055–9077

Scheme 2. Synthesis of the Twin-Hydrophobic Dendrons Functionalized with Alkyne and Azide Groups at Their Apex[a]



[a]Reagents and conditions: (i) *p*-Anisaldehyde, HCl, water (25 °C); (ii) propargyl bromide, NaH, DMF (0−25 °C); (iii) CH_3CO_2H−water (50 °C); (iv) DCC, DPTS, CH_2Cl_2 (25 °C); (v) HBr, H_2SO_4−AcOH (reflux); (vi) NaN_3, DMSO (110 °C); (vii) benzaldehyde, HCl, water, (viii) H_2, Pd/C, MeOH−CH_2Cl_2 (25 °C); (ix) **6**, DMAP, pyridine, CH_2Cl_2 (25 °C).

These modular strategies evolved during the analysis of the structure and properties of the supramolecular assemblies generated from each individual library of amphiphilic Janus glycodendrimers and were directed toward the construction of soft single-type glycodendrimersomes containing D-mannose, D-galactose, and D-lactose in the hydrophilic part, which are glycan ligands for the five lectin receptors selected for binding experiments. Details of these design strategies and their selection will be discussed in each subsection dedicated to the synthesis and analysis of individual libraries. This study required the investigation of no less than seven libraries containing 51 compounds demonstrating the challenges encountered when taking the concept of simple amphiphilic Janus dendrimers that self-assemble into dendrimersomes[9a,b] to the more complex amphiphilic Janus glycodendrimers that self-assemble into glycodendrimersomes.

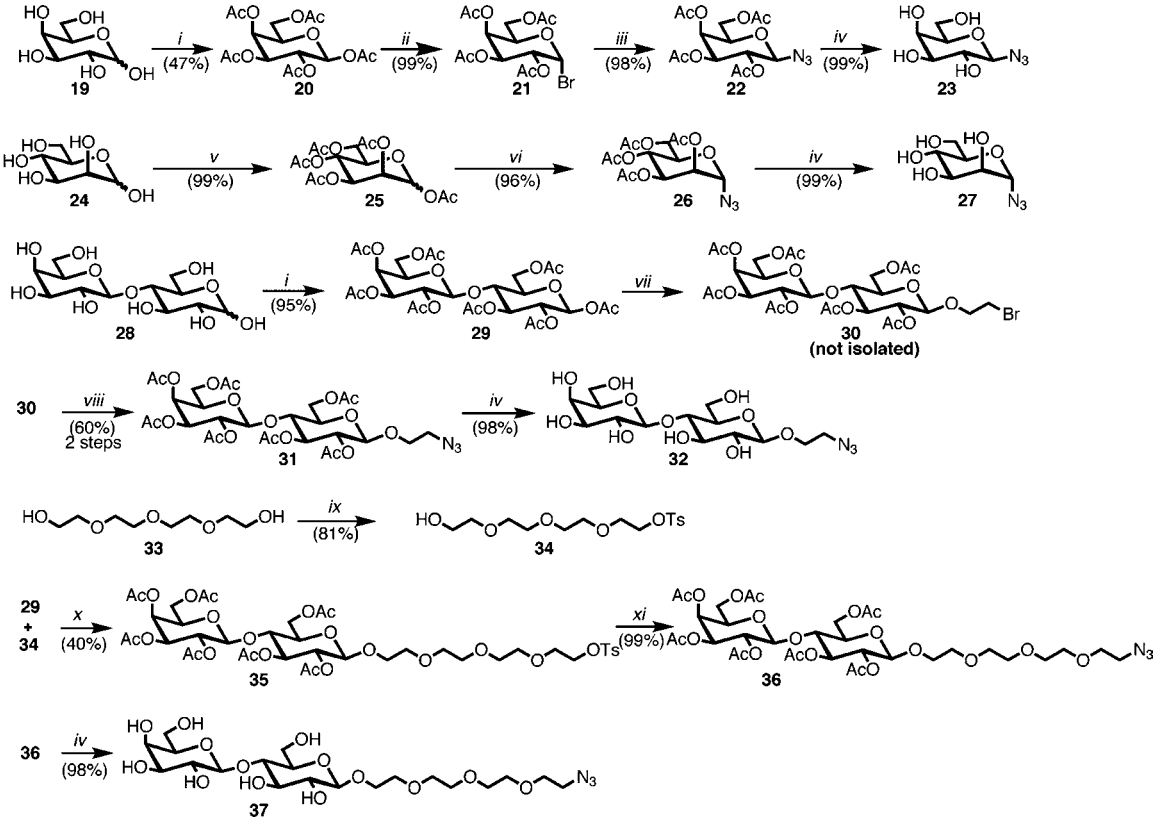
Synthesis of Twin-Hydrophobic Dendrons Functionalized with Alkyne and Azide Groups. The first-generation hydrophobic dendritic acids **4a**−**d** were synthesized from natural phenolic acids[14] as reported previously[9a,15] (Scheme 1). Compounds **4e**,**f** are new derivatives synthesized by the same procedures as those used for the previously reported compounds.[9a] Esterification of protocatechuic acid (**1a**),[14a−g] α-resorcylic acid (**1b**),[14] and gallic acid (**1c**)[14a,c−g] in refluxing MeOH with H_2SO_4 as catalyst yielded methyl 3,4-dihydroxybenzoate (**2a**), methyl 3,5-dihydroxybenzoate (**2b**), and methyl 3,4,5-trihydroxybenzoate (**2c**) in 73−83% yield. Etherification of **2a**−**c** with 1-bromododecane or 2-ethylhexyl bromide gave first-generation hydrophobic esters **3a**−**f** containing linear and branched alkyl groups on the periphery in 46−99% yield. The hydrophobic first-generation dendritic acids **4a**−**f** were obtained by the hydrolysis of the corresponding esters **3a**−**f** with KOH in refluxing ethanol (94−100% yield).

The modular methodology elaborated for the preparation of libraries of amphiphilic Janus glycodendrimers involves three independent steps. The first one consists of the synthesis of three libraries of twin-hydrophobic dendrons functionalized with alkyne directly attached to their apex, via a succinic ester at their apex, or with azide directly attached to the apex (Scheme 2). In the first step of this synthesis, pentaerythritol **7** was monoprotected with *p*-anisaldehyde under acidic conditions to generate the methoxybenzylidene acetal **8** in 80% yield.[16] Compound **8** was etherified with propargyl bromide to produce **9** in 91% yield after column chromatography.[17] Deprotection of **9** with HOAc in water yielded **10** (90% yield).[17b] Esterification of **10** with **4a**−**d** in the presence of DCC/DPTS in CH_2Cl_2 produced **11a**−**d** in 33−95% yield after column chromatography. Bromination of **7** with HBr and H_2SO_4−AcOH at reflux generated **12** in 34% yield which upon reaction with NaN_3 in DMSO at 110 °C for 16 h produced **13** in 92% yield. Esterification of **13** with **4a**−**f** with DCC/DPTS in CH_2Cl_2 at 25 °C generated **14a**−**f** in 87−99% yield. The second library of hydrophobic twin-dendrons functionalized with alkynes on their periphery contains a succinic ester spacer that connects the alkyne to the pentaerythritol branching point (Scheme 2, bottom). In this case, pentaerythritol **7** was monoprotected as benzylidene acetal **15**[18] (61% yield), which after esterification with **4a**−**c** produced **16a**−**c** in 93−98% yield. Deprotection of the benzylidene acetal by hydrogenolysis using Pd/C and H_2 generated **17a**−**c** in 99−100% yield. The esterification of **17a**−**c** with the acetylene anhydride **6**,[19] that was prepared by the esterification of propargyl alcohol with succinic anhydride followed by dehydration with DCC (bottom of Scheme 1), produced **18a**−**c** in 60−88% yield.

Synthesis of the Glycosyl Azides of D-Galactose, D-Mannose, and D-Lactose. The stereoselective synthesis of

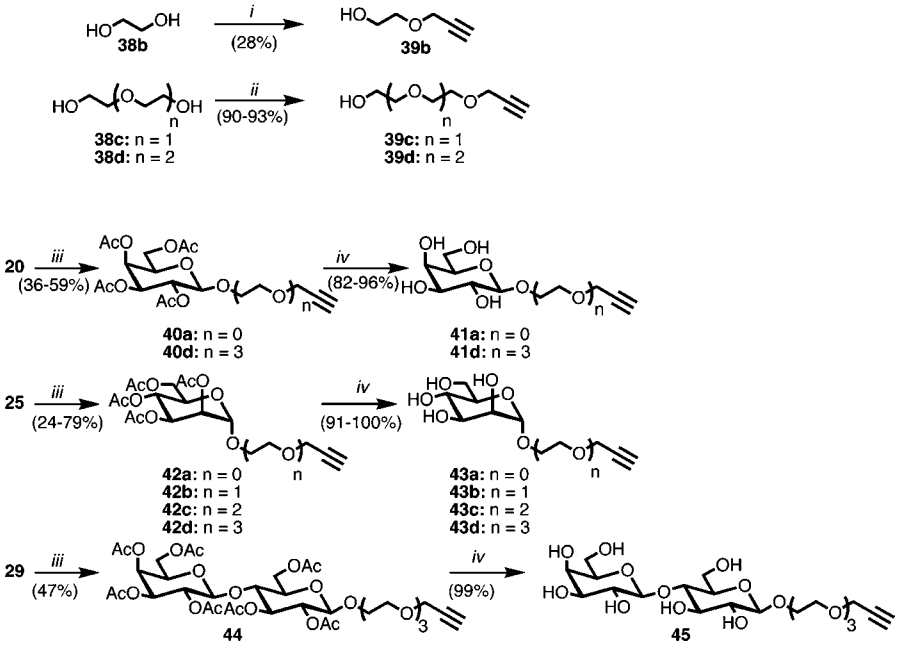
dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

Scheme 3. Stereoselective Synthesis of the Glycosyl Azides 23, 27, 32, and 37[a]



[a]Reagents and conditions: (i) AcONa, Ac_2O, (reflux); (ii) 33% HBr/AcOH (25 °C); (iii) NaN_3, DMSO (25 °C); (iv) 1 M MeONa in MeOH, MeOH (25 °C); (v) I_2, Ac_2O, (0−25 °C); (vi) $TMSiN_3$, $SnCl_4$, CH_2Cl_2 (25 °C); (vii) 2-bromoethanol, $BF_3{\cdot}Et_2O$, CH_2Cl_2 (0 to 25 °C); (viii) NaN_3, DMF (80 °C); (ix) TsCl, pyridine, CH_2Cl_2 (25 °C); (x) $BF_3{\cdot}Et_2O$, CH_2Cl_2 (0 to 25 °C); (xi) NaN_3, NaI, DMF (70 °C).

Scheme 4. Synthesis of the Propargylated Glycosides 41a,d, 43a−d, and 45[a]

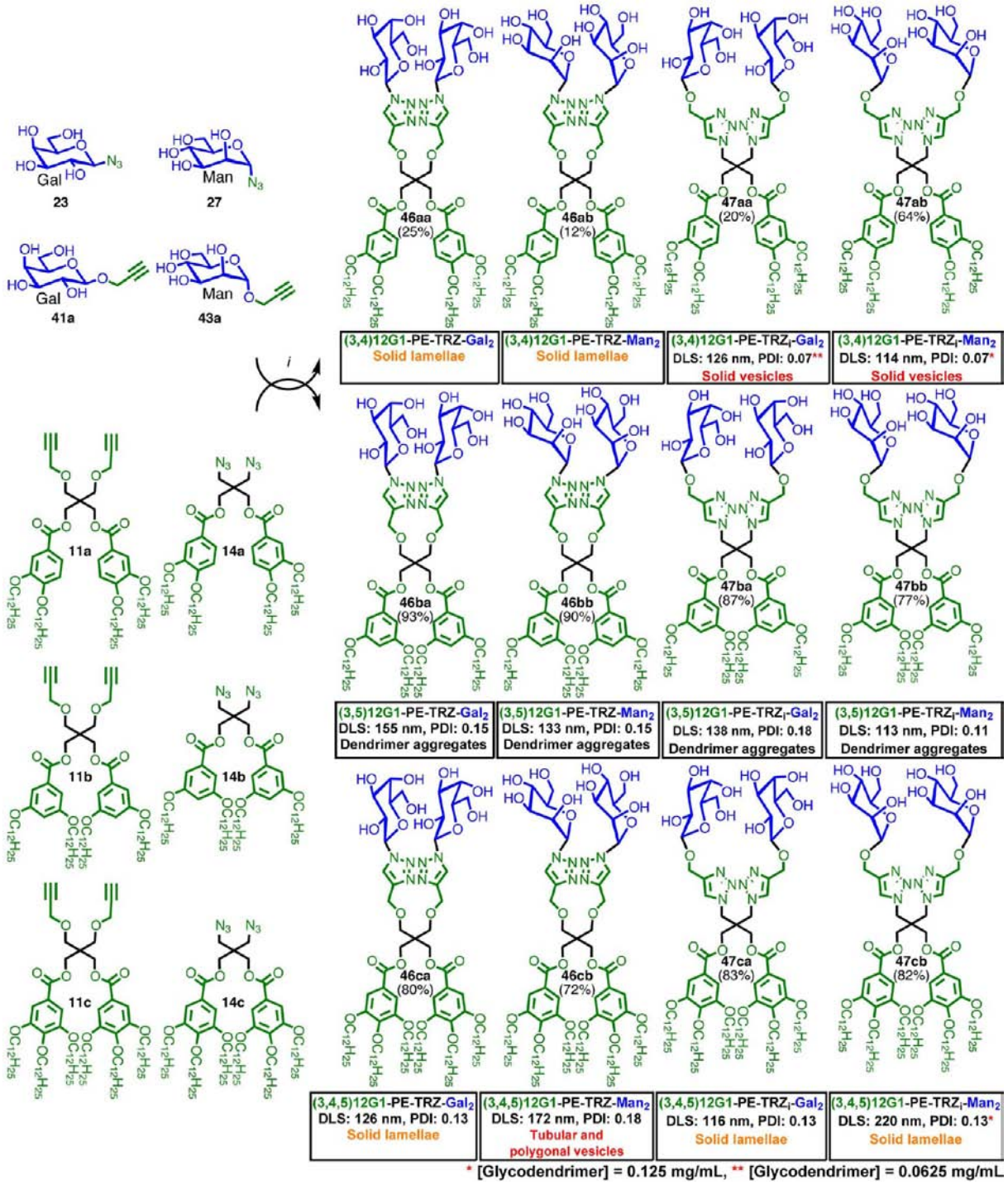


[a]Reagents and conditions: (i) NaH, propargyl bromide, toluene (25 °C); (ii) propargyl bromide, ^{t}BuOK, THF (25 °C); (iii) **39b−d**, or propargyl alcohol, $BF_3{\cdot}Et_2O$, CH_2Cl_2 or CH_3CN (0−25 °C); (iv) 1 M MeONa in MeOH (25 °C).

the glycosyl azides of D-galactose,[20,21] D-mannose,[20,21] and D-lactose[22] is outlined in Scheme 3. Acetylation of **19** with Ac_2O at reflux in the presence of AcONa gave the β-anomer **20** (47% yield)[23] that was transformed into the α-anomer **21** with HBr/AcOH.[24]

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

Scheme 5. Modular Synthesis of Constitutional Isomeric Libraries 1 (46aa to 46cb) and 2 (47aa to 47cb) Containing 12 Amphiphilic Janus Glycodendrimers with D-Mannose and D-Galactose and the Summary of Their Self-Assembly by Injection of Their THF Solution into Water[a]



[a]Reagents and conditions: (i) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, THF/water (25 °C).

Compound **21** was treated without isolation with NaN_3 in DMSO to produce the β-D-glycosyl azide **22** in 98% yield after column chromatography.[25] Standard Zemplén de-O-acetylation of **22** with MeONa in MeOH generated **23** (99%).[20] Acetylation of **24** with Ac_2O catalyzed by I_2 produced the mixture of α- and β-anomers **25** in 99% yield.[26] The α-azide **26** was obtained by reacting **25** with $TMSN_3$ in the presence of $SnCl_4$ in CH_2Cl_2 in 96% yield after column chromatography.[21] Reaction of **26** with MeONa in MeOH at 25 °C produced **27** (99% yield).[21] Compound **28** was acetylated under the same conditions as for **19** to give the β-anomer **29** (95% yield)[23] which was reacted with 2-bromoethanol in the presence of $BF_3.OEt_2$ in CH_2Cl_2 at 25 °C to give **30**. Compound **30** was treated without purification with NaN_3 to give **31** (60% for two steps). Standard Zemplén de-O-acetylation of **31** with MeONa in MeOH at 25 °C produced **32** (98%).[27] Intermediate **29** was also reacted with tetra(ethylene glycol)

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

monotosylate **34**. Compound **34** was prepared from tetra(ethylene glycol), **33**, and TsCl in the presence of pyridine as base.[28]

Lactosyl acetate **29** was reacted with **34** in the presence of $BF_3{\cdot}OEt_2$ in CH_2Cl_2 at 0−25 °C and subsequently with NaN_3 in the presence of NaI in DMF at 70 °C to generate **36** in 99% yield. Final lactosyl azide **37** was obtained in 98% yield by the reaction of **36** with MeONa in MeOH.[27]

Synthesis of the Propargylated Glycosides of D-Galactose, D-Mannose, and D-Lactose. Compounds **39b−d** were synthesized in 28%, 90%, and 93% yield (after purification by column chromatography) by the monoetherification of ethylene glycol, **38b**, di(ethylene glycol), **38c**, and tri(ethylene glycol), **38d**, with propargyl bromide following literature procedures (Scheme 4).[29,30] Glycosylation of **20**, **25**, and **29** with propargylated alcohols, **39b−d** was performed in either CH_2Cl_2 or CH_3CN and was catalyzed with $BF_3{\cdot}Et_2O$ at 0 °C to yield **40a,d** (36−59% yield), **42a−d**, and **44** in 26−79% yield after purification by column chromatography.[30,31] Standard Zemplén de-O-acetylation of **40a,d**, **42a−d**, and **44** with MeONa in MeOH at 25 °C produced **41a,d**, **43a−d**, and **45** in 82−100% yield.[30,31]

Accelerated Modular Synthesis of Two Constitutional Isomeric Libraries Containing 12 Amphiphilic Janus Glycodendrimers Presenting D-Mannose and D-Galactose. The first of the two constitutional isomeric libraries of amphiphilic Janus glycodendrimers was synthesized by an accelerated modular synthesis via click chemistry-mediated assembly[32] of the twin-hydrophobic dendrons containing alkynes directly attached to their apex, **11a−c** from Schemes 2, with the glycosyl azides **23**, **27** of D-galactose and D-mannose from Scheme 3. This process generated the six amphiphilic Janus glycodendrimers **46aa,ab,ba,bb,ca,cb** from the left two columns of Scheme 5. The second constitutional isomeric library was produced by the click chemistry of the twin-hydrophobic dendrons containing the azide groups at their apex **14a−c** from Scheme 2, with the glycosyl alkynes **41a**, **43a** from Scheme 4 to generate the six amphiphilic Janus glycodendrimers **47aa,ab,ba,bb,ca,cb** from the right two columns of Scheme 5. These libraries were designed to select the most suitable methodologies and products.

Self-Assembly of Janus Glycodendrimers by Injection of Their THF or Ethanol Solution into Water and Analysis by DLS, Cryo-TEM, and Micropipet-Aspiration Experiments. Hard and Soft Assemblies. The simplest method for the self-assembly of amphiphilic molecules into vesicles and liposomes involves the injection of their solution in a water-miscible solvent such as ethanol or THF into water or buffer.[8] This methodology has been shown to be efficient for the self-assembly of amphiphilic Janus dendrimers into monodisperse vesicles called dendrimersomes.[9a,b] The resulting assemblies were first analyzed by dynamic light scattering (DLS) for size, polydispersity (PDI), and stability in time. Assemblies stable in time were subsequently analyzed by cryogenic-transmission electron microscopy (cryo-TEM) to determine their structure. Sharp edges in various supramolecular assemblies indicate structures generated from crystalline or glassy unilamellar membranes that will be called hard assemblies, while continuous surfaces indicate fluid or soft assemblies. Micropipet aspiration experiments[9a] carried out on giant vesicles obtained by hydration are complementary to cryo-TEM experiments in the discrimination between hard and soft glycodendrimersomes. This combination of methods will be used to discuss the results summarized in Scheme 5 for the constitutional isomeric libraries 1 and 2. Compounds **46aa,ab** display limited solubility in THF. All other Janus glycodendrimers from Scheme 5 are soluble in THF but not in ethanol. Assemblies of compounds **46aa,ab** produced by the injection of 100 μL of their solution containing 1.25 mg/mL in THF into 2 mL of Millipore water generated a final concentration of 0.0625 mg/mL that is stable for less than 1 h. To overcome the limited solubility of **46aa,ab** in THF, various modifications of their primary structure were investigated. They include the replacement of *n*-alkanes from their hydrophobic twin-dendrons with branched alkanes and incorporation of various hydrophilic and hydrophobic spacers in different parts of the Janus glycodendrimer. These modifications will be discussed in the next sections. Injection of an aliquot of THF solutions (100 μL of 10 mg/mL) of all Janus glycodendrimers from Scheme 5 except **47aa** (100 μL of 1.25 mg/mL), **47ab,cb** (100 μL both of 2.5 mg/mL), into Millipore water (2 mL) followed by 5 s of vortex mixing induces self-assembly. The size distribution values of all assemblies from Scheme 5 are narrow and within the values that are considered monodisperse for vesicles.[9a,12k,33] Traditionally, monodisperse vesicles are prepared by hydration followed by complex and multiple extrusion procedures.[12k,34]

The dimensions of all assemblies from Scheme 5 range from 114 to 126 nm and are suitable for drug delivery and other applications.[12k,35] These Janus dendrimers self-assemble into solid lamellae (Figure 2a,c), solid vesicles named solid or hard glycodendrimersomes (Figure 2b), glycodendrimer aggregates (Figure SF7c,d,g, Supporting Information), and tubular and polygonal dendrimersomers (Figure 2c,d).

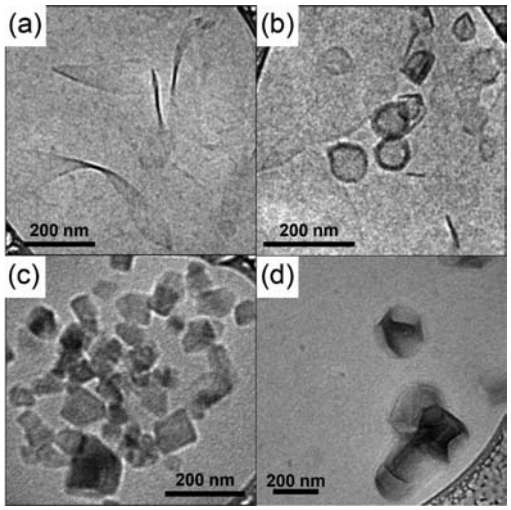
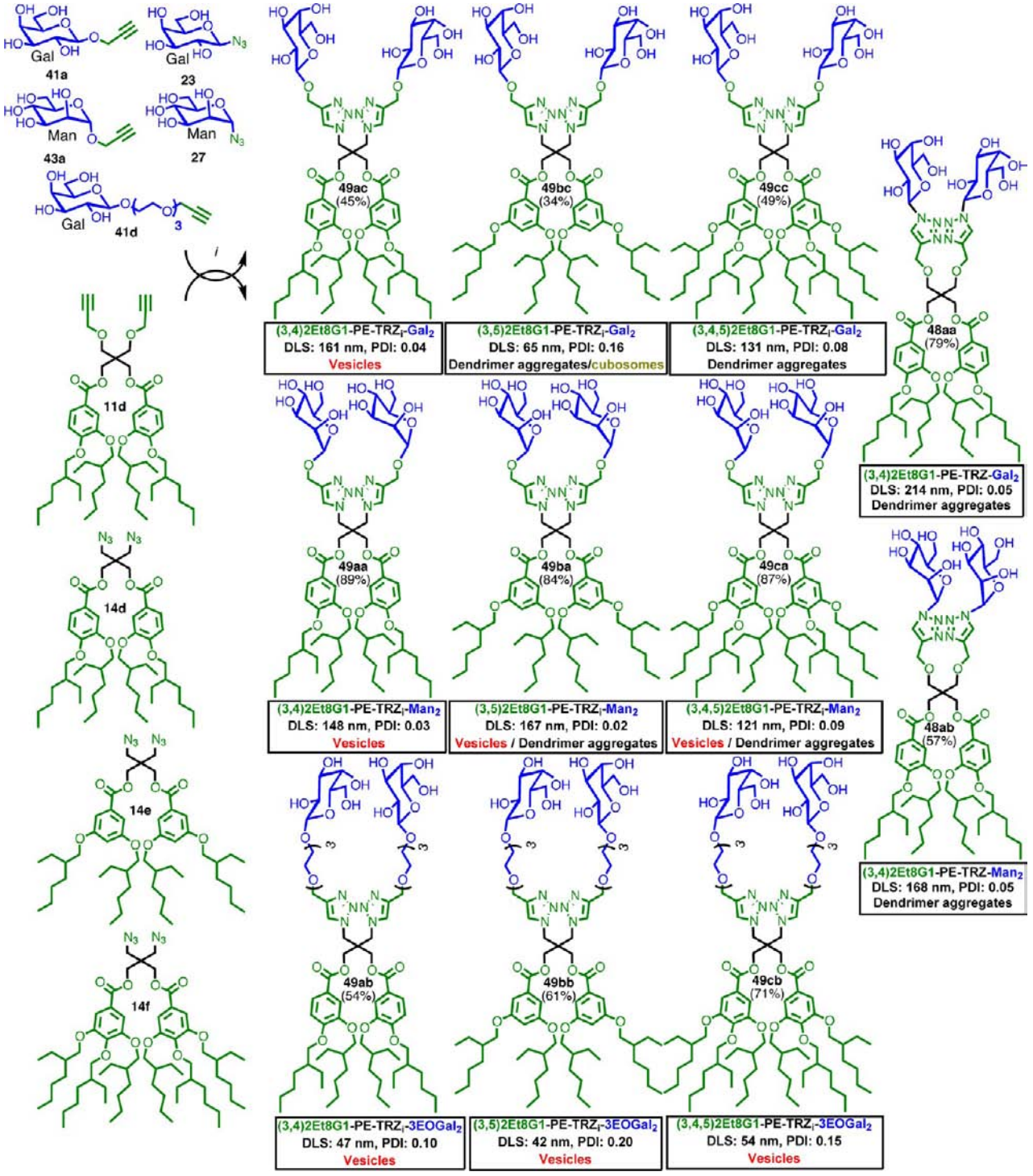


Figure 2. Selected cryo-TEM images of solid lamellae assembled from (a) (3,4)12G1-PE-TRZ-Gal_2 **46aa**, (b) solid glycodendrimersomes assembled from (3,4)12G1-PE-TRZ_i-Man_2 **47ab**, (c) solid lamellae assembled from (3,4,5)12G1-PE-TRZ-Gal_2 **46ca**, and (d) polygonal glycodendrimersomes assembled from (3,4,5)12G1-PE-TRZ-Gal_2 **46cb**.

Compounds **46aa,ab** self-assemble into sheetlike solid lamellae (Figure 2a,c). These 2D sheetlike as well as other solid morphologies imply that their membrane is either crystalline or amorphous below its glass transition temperature. Both prevent the closing process required to form a 3D vesicle. A hard vesicle is a 3D particle constructed from a unilamellar bilayered membrane in a way similar to that for regular fluid and soft vesicles (Figure 2b). The sharp edges/corners of the structure indicate that its membrane is rigid. The glycodendrimer aggregates are not traditional micelles because micelles should have dimensions

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

Scheme 6. Modular Synthesis of Library 3 (48aa to 49bb) Containing 11 Amphiphilic Janus Glycodendrimers with Branched Hydrophobic Alkyl Groups and D-Mannose and D-Galactose in Their Hydrophilic Part and the Summary of Their Self-Assembly by Injection of Their THF Solution into Water[a]



[a]Reagents and conditions: (i) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF/water (25 °C).

of about two molecular lengths of the Janus dendrimer, which are smaller than sizes observed (Figure SF7c,d,g). The glycodendrimer aggregates are hard droplets consisting of a Janus glycodendrimer-rich phase suspended in water which are similar to a hard oil droplet (Figure SF7c,d,g). The phase separation of hydrophilic and hydrophobic segments happens only on the particle surface but not inside where it is disordered due to slow kinetics. Compound **46cb** generated a mixture of morphologies indicating that the rates of formation of each morphology are similar (Figure 2d, Figure SF7h), and/or that they have similar stabilities. It should be noted that at concentrations smaller than 0.5 mg/mL, the nanotubular morphology is dominant (Figure SF7h), while at concentrations higher than 0.5 mg/mL, the polygonal glycodendrimersome that has a shape similar to an

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

icosahedron is dominant (Figure 2d). A quantitative and comparative analysis of solid and soft assemblies by a combination of cryo-TEM and micropipet-aspiration experiments will be presented in a different section. Cryo-TEM images of structures assembled from **46ab,ba,bb,cb**, and **47aa,ba,ca,cb** of libraries 1 and 2 are in Figure SF7.

Modular Synthesis and Analysis of Library 3 Containing 11 Amphiphilic Janus Glycodendrimers with Branched Alkyl Groups in Their Hydrophobic Part and D-Mannose or D-Galactose. The low solubility of Janus glycodendrimers from libraries 1 and 2 (Scheme 5) prompted us to explore pathways to prevent membrane crystallization and enhance solubility and flexibility to generate soft assemblies.

Three twin-hydrophobic dendrons containing branched alkyl groups in their hydrophobic part and alkyne (**11d**) or azide (**14d−f**) groups were combined with the carbohydrates **23** and **27** containing azide groups, with the carbohydrates **41a** and **43a** containing the alkyne directly attached, and with **41d** that has the alkyne attached to the carbohydrate via a tri(ethylene glycol) spacer. This provided eleven Janus glycodendrimers (Scheme 6). All these Janus glycodendrimers are soluble in THF (10 mg/mL to 40 mg/mL) and after injection produced concentrations in water from 0.5 mg/mL up to 2 mg/mL. The injection in water of their THF solutions generated assemblies with polydispersity ranging from 0.03 to 0.20. Five of these Janus glycodendrimers, **49ac,aa,ab,bb,cb**, produced only soft glycodendrimersomes with size ranging from 42 to 167 nm. Compounds **49ba,ca** assemble into mixtures of soft glycodendrimersomes and dendrimer aggregates, **48aa,ab,cc** into dendrimer aggregates, while **49bc** into mixtures of dendrimer aggregates and cubosomes. The analysis of the structure of cubosomes will be presented in a later section. These experiments demonstrated a remarkable improvement in solubility, polydispersity, and ability to generate single-component glycodendrimersomes. Selected examples of cryo-TEM experiments are shown in Figure 3. Compounds with

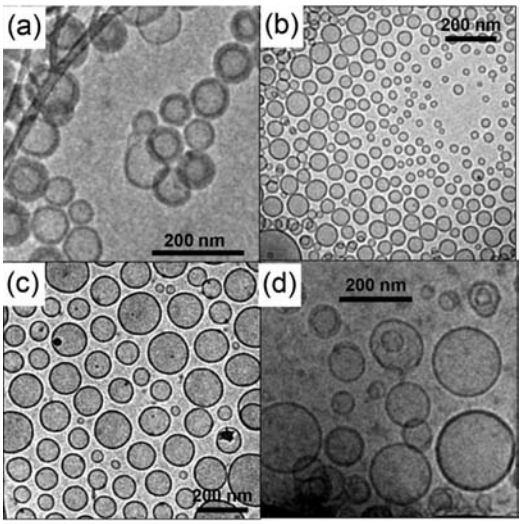


Figure 3. Selected cryo-TEM images of glycodendrimersomes assembled from (a) (3,4)2Et8G1-PE-TRZ$_i$-Man$_2$ **49aa**, (b) (3,4)2Et8G1-PE-TRZ$_i$-3EOGal$_2$ **49ab**, (c) (3,5)2Et8G1-PE-TRZ$_i$-3EOGal$_2$ **49bb**, and (d) (3,4,5)2Et8G1-PE-TRZ$_i$-3EOGal$_2$ **49cb**.

branched alkyl chains in the hydrophobic side and a long tri(ethylene glycol) spacer in the hydrophilic side (Figure 3b,c,d) induce the formation of high-quality glycodendrimersomes. Interestingly, for the branched compound without hydrophilic spacer (Figure 3a), the resulting glycodendrimersomes possess walls with two distinct membrane thicknesses. The thick wall is three times larger than that of the regular thin wall which is about the thickness of two Janus dendrimer lengths (~7 nm). The inner wall of the thick vesicles has darker color than the rest of the wall, indicating a more ordered and denser packing of molecules forming the vesicle wall, while the lighter color signifies a disordered aggregate of compound-rich phase surrounding the vesicle. This appearance suggests that the absence of the hydrophilic spacer does not favor the assembly of unilamellar membranes but instead induces the formation of kinetically trapped intermediates such as thick-wall vesicles that cannot be stabilized into unilamellar vesicles.

The efficiency of soft vesicle formation is also dependent on the number and position of the alkyl chains from the hydrophobic side (Scheme 6, Figure 3). Cryo-TEM images of structures assembled from **49ca,ba,ab,aa,ac,bc** of library 3 are in Figure SF8, Supporting Information.

Modular Synthesis and Analysis of Library 4 Containing Six Janus Glycodendrimersomes with a Succinic Ester Spacer in the Hydrophobic Part and D-Mannose, D-Galactose, or D-Lactose. A second approach to enhance solubility and flexibility involved the incorporation of a succinic ester spacer in between the twin-hydrophobic dendron and its alkyne groups, while maintaining linear *n*-alkyl groups on the periphery of the dendron (Scheme 7). The twin-hydrophobic dendrons **18a−c** were coupled with carbohydrates containing the azide group attached directly (**23**, **27**), to generate the six Janus glycodendrimers from Scheme 7. These molecules show acceptable solubility in THF to result after injection in a concentration of 0.25 to 0.5 mg/mL in water. The polydispersity of their assemblies is narrow (0.14 to 0.21).

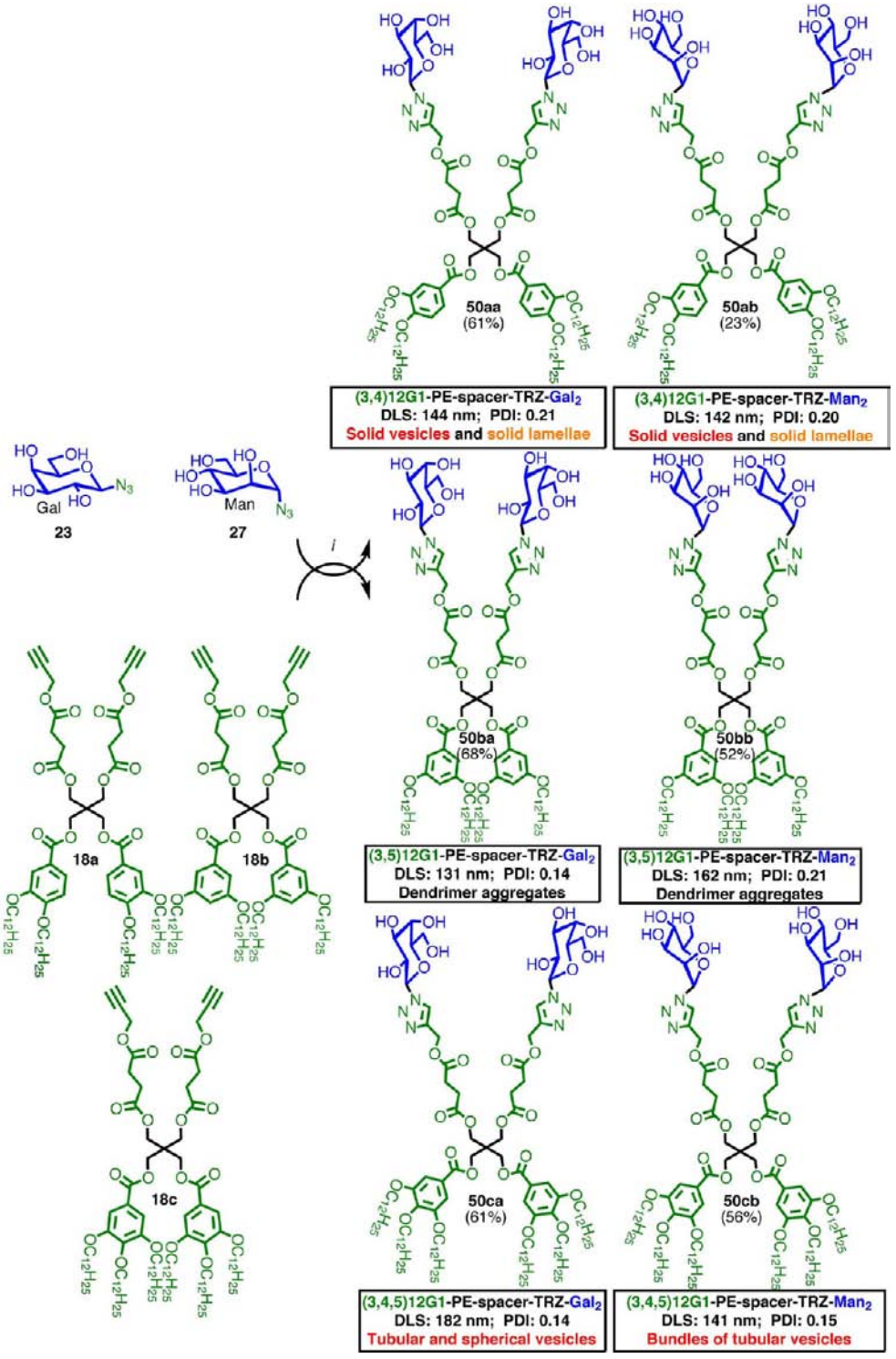
The dimensions of assemblies resulted from these Janus dendrimers range from 131 to 182 nm. However, their 3,4-disubstituted dendrons (**50aa,ab**) generate solid lamellae and vesicles, while the 3,5-disubstituted compounds (**50ba,bb**) produce dendrimer aggregates. Only the 3,4,5-trisubstituted molecules (**50ca,cb**) lead to interesting assemblies consisting of mixtures of tubular and soft spherical glycodendrimersomes and also bundles of tubular dendrimersomes. Figure 4 illustrates examples of these assemblies. Solid vesicles displaying an asymmetric shape with sharp edges are shown in Figure 4a,b. The curved feature in the TEM images is the projection of curved solid lamellae with an edge-on orientation. They are membranes that are too stiff to form a 3D hard vesicle by closing the broken solid vesicle.

The nanotubular structures from Figure 4c aggregate and produce bundles at high concentration. At concentrations lower than 0.25 mg/mL, single tubes are observed. The hexagonal arrangement of rings observed in Figure 4c is the projection of tubular bundles with their long axis perpendicular to the film. This image indicates that the nanotubes possess uniform diameter and length. In addition, their diameter to length aspect ratio appears to be constant. Cryo-TEM images of structures assembled from compounds **50bb,ba** are in Figure SF9a and SF9b, Supporting Information.

Modular Synthesis and Analysis of Library 5 Containing 12 Janus Glycodendrimers with Linear Hydrophobic *n*-Alkyl Groups and D-Mannose or D-Galactose Connected by Mono-, Di- and Tri(ethylene glycol) Spacers. Libraries 1, 2 (Scheme 1), and 4 (Scheme 7) contained linear *n*-alkyl groups in their hydrophobic part. To increase solubility and flexibility, a succinic ester spacer was incorporated between

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

Scheme 7. Modular Synthesis of Library 4 (50aa to 50cb) Containing 6 Amphiphilic Janus Glycodendrimers with Hydrophobic Linear *n*-Alkyl Groups, a Succinic Ester Spacer, D-Mannose, D-Galactose, or D-Lactose in their Hydrophilic Part and the Summary of Their Self-Assembly by the Injection of Their THF Solution into Water[a]



[a]Reagents and conditions: (i) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF/water (25 °C).

the hydrophobic and hydrophilic fragments of the Janus dendrimers from library 4. Libraries 1, 2, and 4 generated mostly hard assemblies and tubular vesicles. The replacement of the linear *n*-alkyl groups with a branched hydrophobic segment together with a tri(ethylene glycol) spacer produced only vesicles (see **49ab,bb,cb** from library 3 of Scheme 6). Therefore, we decided to investigate the role of ethylene glycol spacers in the case of Janus dendrimers containing linear *n*-alkyl groups in their hydrophobic part. Subsequently, we combined **14a−c** with **43b,c**, **41d**, and **43d** containing ethylene glycol, di(ethylene glycol), and tri(ethylene glycol) spacers between D-mannose or D-galactose and the alkyne group to generate the library 5

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

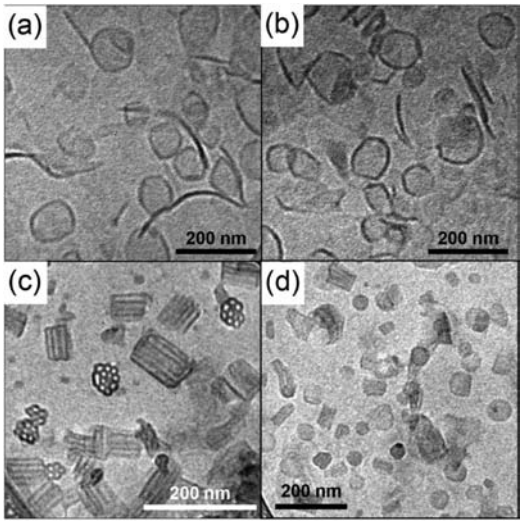


Figure 4. Selected cryo-TEM images of solid lamellae and solid glycodendrimersomes assembled from (a) (3,4)12G1-PE-spacer-TRZ-Man_2 **50ab** and (b) (3,4)12G1-PE-spacer-TRZ-Gal_2 **50aa**; tubular glycodendrimersome bundles assembled from (c) (3,4,5)12G1-PE-spacer-TRZ-Man_2 **50cb**; mixture of tubular and spherical glycodendrimersomes assembled from (d) (3,4,5)12G1-PE-spacer-TRZ-Gal_2 **50ca**.

(Scheme 8) containing 12 Janus glycodendrimers. All Janus glycodendrimers from Scheme 8 are soluble both in THF (5 to 10 mg/mL) and in ethanol. After injection, their concentration in water ranges from 0.25 to 0.5 mg/mL. The polydispersity of these assemblies ranges from 0.05 to 0.26 and their size from 43 to 222 nm. Cryo-TEM images of structures assembled from **51aa**,**ab**,**ac**,**ad**,**cc**,**cd** are in Figure SF10, Supporting Information. The 3,4-disubstituted hydrophobic segments of the Janus dendrimer (top row in Scheme 8) lead to solid lamellae for **51aa** and solid vesicles for **51ab**,**ac**,**ad**. The replacement of the 3,4-disubstituted hydrophobic part with a 3,5-disubstituted (middle row in Scheme 8) produced soft vesicles for **51ba**,**bb**,**bc** and cubosomes for **51bd**. The transition from 3,5-disubstitution to 3,4,5-trisubstitution (bottom row in Scheme 8) changed the assemblies in water from soft to hard generating solid lamellae for **51ca**,**cb**,**cc**,**cd**. Selected examples of soft and hard assemblies from library 5 are in Figure 5. The dependence of the structure assembled in water on the 3,4-, 3,5-, and 3,4,5-substitution patterns is in line with the molecular mechanism of unilamellar structure formation reported in a previous publication for simple dendrimersomes.[9b]

Modular Synthesis of Library 6 Containing Five Janus Glycodendrimers with and without Succinic Ester Spacer and D-Lactose Connected via Mono- and Tetra(ethylene glycol) Spacers. The main goal of these investigations is to delineate the molecular principles producing single-type soft glycodendrimersomes and other complex glycoarchitectures with D-mannose, D-galactose, and D-lactose on their periphery. The experiments reported in Schemes 6 and 8 provided molecular instructions for the design of soft glycodendrimersomes presenting D-mannose and D-galactose. Scheme 9 demonstrates the development of the molecular principles that yield glycodendrimersomes with D-lactose as sugar headgroup. On the basis of the experience accumulated with the other libraries, 3,4-, 3,5-, and 3,4,5- di- and trisubstituted hydrophobic patterns with and without succinic acid ester and alkyne, **11a**−**c**, and **18c**, were selected and combined with D-lactose-containing ethylene glycol and tetra(ethylene glycol) spacers connected to the azide group, **32** and **37**, to generate the five Janus glycodendrimers from Scheme 9. All these Janus glycodendrimers are soluble in THF (5 to 40 mg/mL), and their assemblies are stable over time in water in the range of concentrations from 0.25 to 2.0 mg/mL. The polydispersity of these assemblies ranges from 0.15 to 0.29, while their size varies from 46 to 406 nm. With the exception of **52ad**, the only 3,4-disubstituted compound which forms a mixture of soft vesicles and bundles of rodlike micelles, all other Janus glycodendrimers generate soft glycodendrimersomes. It is interesting that **52ad** forms rodlike micelles aligned side by side. This complex arrangement leads to a long ribbonlike structure (Figure 6b). Additional selected cryo-TEM pictures of these assemblies are in Figure 6 and of glycodendrimersomes assembled from **52cd** in Figure SF9c.

Modular Synthesis of Library 7 Containing Six Janus Glycodendrimersomes with Branched and Linear Hydrophobic Alkyl Groups and D-Lactose Connected via a Tri(ethylene glycol) Spacer. The diversity of glycodendrimersomes with D-lactose was increased by combining linear and branched alkyl groups in the hydrophobic part of the twin-dendrons containing azides (**14a**−**f**) with D-lactose containing the alkyne group attached via a tri(ethylene glycol) spacer (**45**) (Scheme 10). This strategy provided six new Janus glycodendrimers. Library 7 generated a remarkable series of results. All Janus glycodendrimersomes from Scheme 9 are soluble in THF, and their assemblies are stable over time in water in concentrations higher than 0.5 and up to 2.0 mg/mL. Even at these high concentrations in water these assemblies display size distributions ranging from 0.17 to 0.23 and sizes from 97 to 393 nm. Janus glycodendrimer **53aa** assembles in a combination of soft glycodendrimersomes and rodlike micelles, while **53ca** produces solid vesicles and lamellae. Compounds **53ba**,**da**,**ea**,**fa** self-assemble into soft glycodendrimersomes. Representative cryo-TEM are shown in Figure 7.

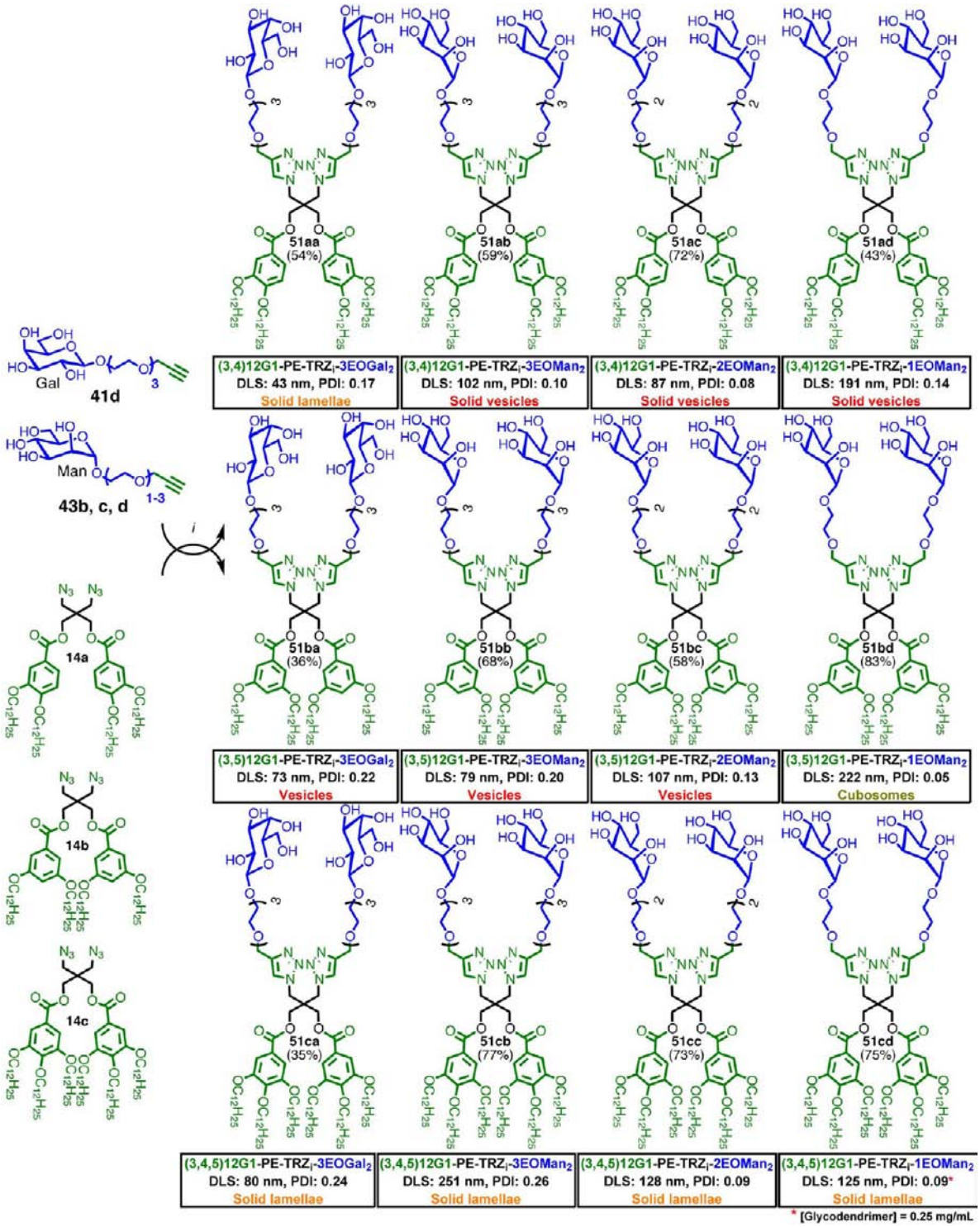
The morphology of rodlike micelles assembled from **53aa** (Figure 7a) is similar to that obtained from **52ad** (Figure 6b). It was observed that self-assembly shifted toward a higher concentration of soft vesicles for the case of **53aa** compared with **52ad**. This indicates that vesicle formation is favored by a shorter hydrophilic spacer (tri-, **53aa**, vs tetra(ethylene glycol), **52ad**).

The small sheetlike features from Figure 7c are a suspension of solid lamellae coexisting with a high concentration of soft glycodendrimersomes

Discrimination between Hard and Soft Glycodendrimersomes and Determination of Their Structure and Physical Properties. A combination of techniques including confocal microscopy on giant glycodendrimersomes containing hydrophobic dyes in their membrane, micropipet-aspiration experiments on giant glycodendrimersomes, and cryo-TEM before and after annealing above the melting or glass transition temperature of the assembly was used to discriminate between soft and hard glycodendrimersomes. Figure 8 shows examples of micropipet-aspiration experiments performed on soft (**51bc**, **52dd** in Figure 8a) and hard (**50aa**, **51ad** in Figure 8a) giant glycodendrimersomes prepared by hydration. The solid membranes made from **50aa**, **51ad** cannot be aspirated, and, therefore, the vesicle from **50aa** buckles in response to the applied suction pressure. On the other hand, the mechanical properties of fluid dendrimersomes can be determined through the micropipet-aspiration in which a controlled pressure applied to the vesicle results in a uniform tension along the membrane. Two such examples generated from **51bc**, **52dd** are in Figure 8a. Figure 8b illustrates the comparison of the elastic moduli of

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

Scheme 8. Modular Synthesis of Library 5 (51aa to 51 cd) Containing 12 Amphiphilic Janus Glycodendrimers with Linear *n*-Alkyl Groups, D-Mannose, or D-Galactose Connected via Mono-, Di-, and Tri(ethylene glycol) Spacers and Summary of Self-Assembly by Injection of THF or EtOH Solution into Water[a]



[a]Reagents and conditions: (i) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF/water (25 °C).

glycodendrimersomes obtained from **52dd**, **51bc**,**bb** with that of polymersomes made from the diblock copolymer PEO_{30}-b-PBD_{46} and of liposomes made from the lipid HSPC.[36] In response to the increase in membrane tension, the fluid vesicle stretches and exhibits a lateral expansion, resulting in an increase in area. The slope of the area change in response to the membrane tension is the elastic modulus of the membrane. The linear change in membrane area in response to the applied membrane tension indicates that the glycodendrimersomes obtained from **52dd**, **51bc**,**bb** are generated from a fluid unilamellar membrane (Figure 8c). Vesicles of **52dd** were able to stretch to critical area strains greater than 20%, while vesicles of **51bc** were

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

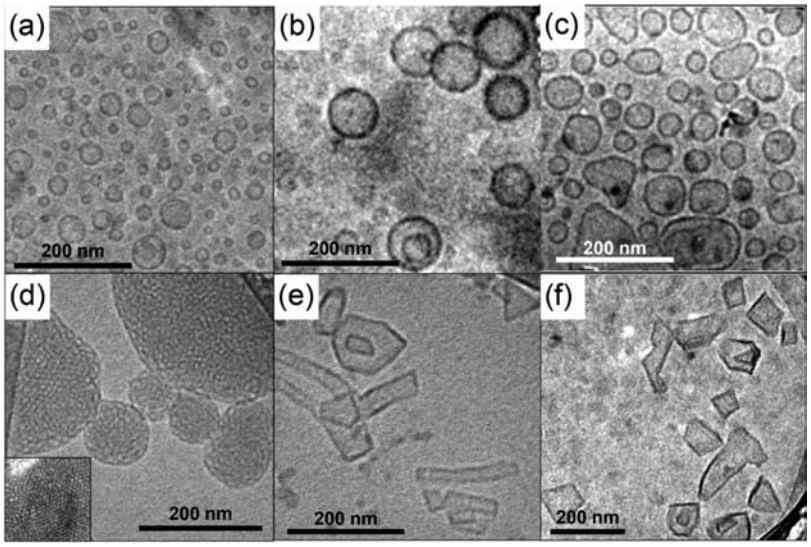


Figure 5. Selected cryo-TEM images of glycodendrimersomes assembled from (a) (3,5)12G1-PE-TRZ$_i$-3EOGal$_2$ **51ba**, (b) (3,5)12G1-PE-TRZ$_i$-3EOMan$_2$ **51bb**, (c) (3,5)12G1-PE-TRZ$_i$-2EOMan$_2$ **51bc**; glycodendrimercubosomes assembled from (d) (3,5)12G1-PE-TRZ$_i$-1EOMan$_2$ **51bd**; solid lamellae assembled from (e) (3,4,5)12G1-PE-TRZ$_i$-3EOGal$_2$ **51ca**, and (f) (3,4,5)12G1-PE-TRZ$_i$-3EOMan$_2$ **51cb**.

only able to extend to 5−10% critical area strains. These results show that the membrane of **52dd** has an elastic modulus of 49.6 ± 15 dyn/cm (average result obtained with 11 vesicles) and is more elastic than that of **51bc**, with an elastic modulus of 163 ± 30 dyn/cm (average result obtained with 13 vesicles). Figure 8d indicates that unlike polymersomes of PEO_{30}-b-PBD_{46}, vesicles of **52dd** display hysteresis. The filled circles are based on measurements during initial vesicle stretching. Open circles are measurements taken during vesicle relaxation. Figure 8e,f presents confocal microscopy images of giant soft glycodendrimersomes formed by **51bc** and **52dd**, respectively. A microphotograph of giant solid glycodendrimersome of **51ad** is in Figure 8g. The giant glycodendrimersomes (size larger than 10 μm) were prepared by the film hydration method.[9a] Soft vesicles (Figure 8e,f) exhibit a uniform spherical shape, while hard vesicles (Figure 8g) display a less regular structure, thus confirming the results of the cryo-TEM and micropipet-aspiration experiments.

The thickness of the membrane forming the vesicle wall was measured from the cryo-TEM images.[37] The membrane thickness is approximately equal to the length of two Janus glycodendrimers (6.8 ± 0.5 to 7.5 ± 0.5 nm) that construct the unilamellar bilayer except for the thick vesicles obtained from **49aa**,**ba** (Table ST1, Figure SF23, Supporting Information).

The annealing effect of the solid lamellae was also studied. Figure 9 shows the cryo-TEM images of the nanostructure formed by **51cb** before and after annealing. The sample was prepared by injection at room temperature with a concentration of 0.5 mg/mL in water.

A solid lamellae constructed from the stiff membrane formed by **51cb** is in Figure 9a. After annealing at 60 °C, above the glass transition temperature, for 30 min, a change from solid lamellae to spherical vesicles was observed (Figure 9b). This result indicates that the solid lamellae with sharp edges in the as-prepared solution represents a kinetically trapped morphology constrained by the stiff membrane. Upon annealing above the glass transition temperature, the membrane becomes flexible and closes up to form a soft spherical vesicle. The wall may become solid again or change back to a solid lamellae when the temperature is brought back to room temperature. So far, the

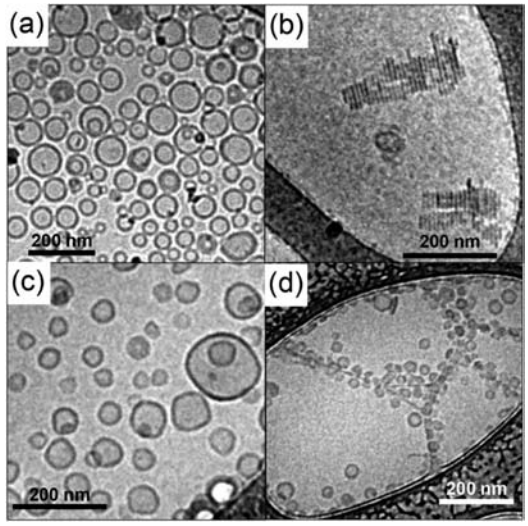


Figure 6. Selected cryo-TEM images of (a) glycodendrimersomes assembled from (3,5)12G1-PE-TRZ-4EOLac$_2$ **52bd**, (b) rodlike glycodendrimermicelle bundles assembled from (3,4)12G1-PE-TRZ-4EOLac$_2$ **52ad**, (c) glycodendrimersomes assembled from (3,4,5)12G1-PE-spacer-TRZ-4EOLac$_2$ **52dd**, and (d) glycodendrimersomes assembled from (3,4,5)12G1-PE-spacer-TRZ-1EOLac$_2$ **52dc**.

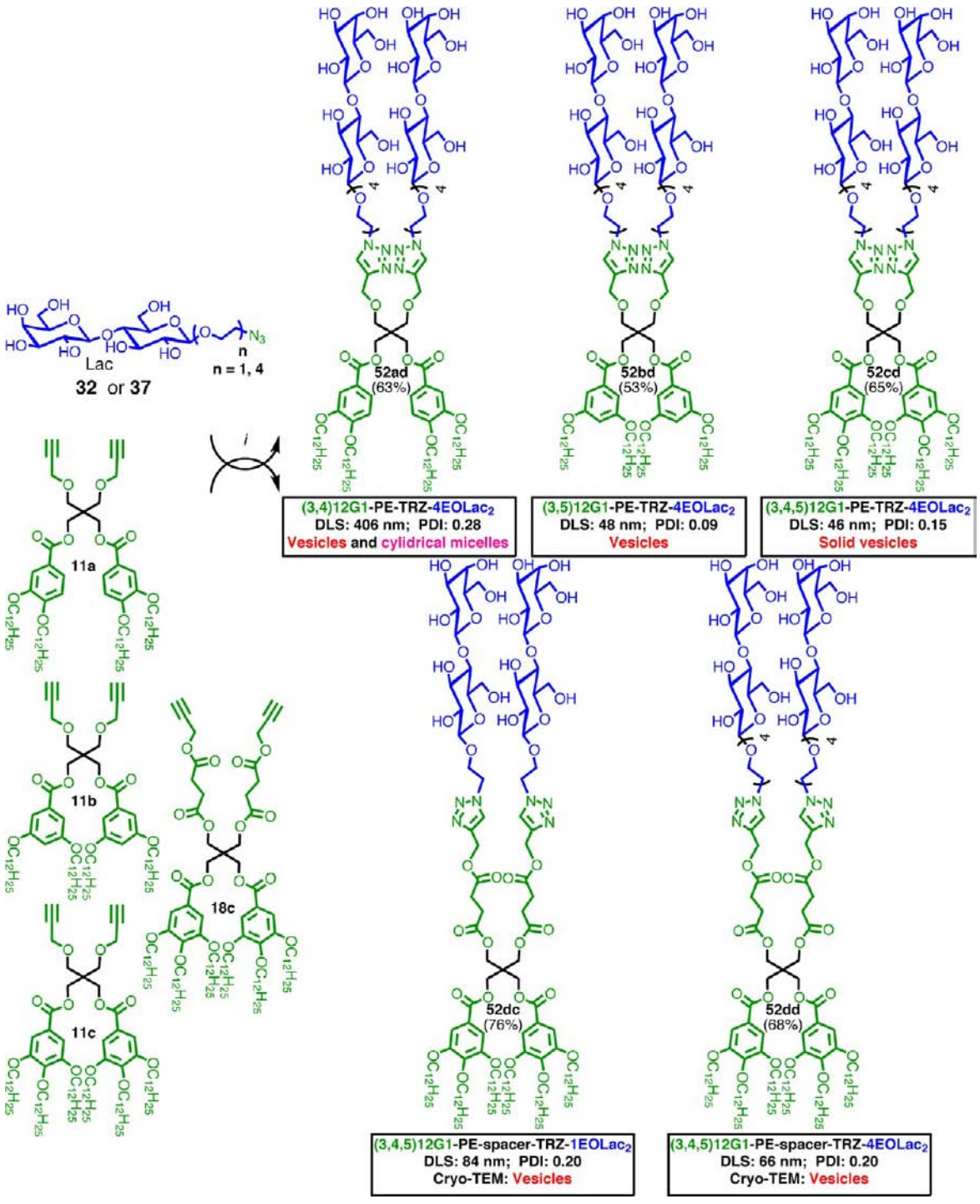
vesicles investigated remain smooth and spherical in shape as in the case shown in Figure 9b. These experiments reveal that glycodendrimersomes are the thermodynamic product of self-assembly in water. Additional cryo-TEM images of solid glycodendrimersomes assembled from **51cb** obtained after annealing are presented in Figure SF11, Supporting Information.

To our knowledge, the transition from tubular and solid lamellae to spherical vesicles upon annealing was observed only once in the case of a stereocomplex of amphiphilic peptides.[2b] The experiments reported here indicate that this morphological change could be general for solid aggregates generated from hard amphiphilic assemblies in water.

Assembly of Glycodendrimersomes with Different Dimensions by the Injection Method. Vesicles with different diameters and narrow polydispersity are desirable to relate

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

Scheme 9. Modular Synthesis of Library 6 (52ad to 52dd) Containing 5 Amphiphilic Janus Glycodendrimers with Linear *n*-Alkyl Groups and D-Lactose and Summary of Self-Assembly by Injection of THF Solution into Water[a]



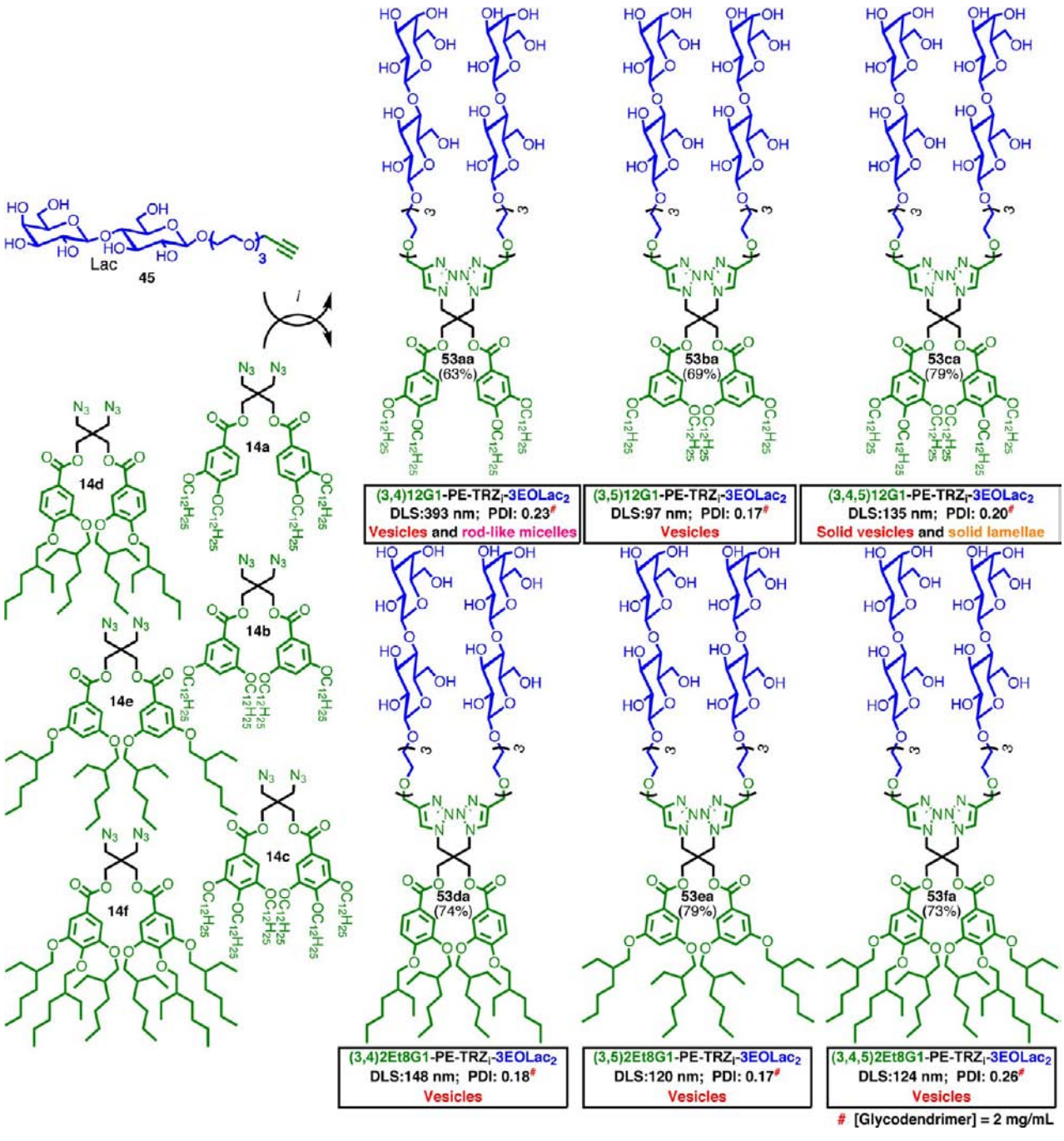
[a]Reagents and conditions: (i) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF/water (25 °C).

structure to activity in bioassays important for use as delivery nanocontainers, vaccines, lectin blockers, and targeted delivery devices and for molecular recognition experiments with programmable glycan ligand presentations of biological membranes. They are also required for the determination of their structure and physical properties.

Therefore, the preparation of glycodendrimersomes with different dimensions by the injection method was investigated. Figure 10 shows the concentration−size relation experiments on the assembly of glycodendrimersomes from **49bb** by injection in water to a final concentration in water from 0.125 mg/mL to 10 mg/mL. The DLS results (Figures 10a) demonstrated the increase of vesicle size with increasing the final concentration in water. At 10 mg/mL, the solution is opaque due to the large particle size and high particle density but does not exhibit any sign of precipitation. At a concentration above 10 mg/mL, vesicles with an average size on the order of a micrometer were obtained. These results demonstrated that it is possible to

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

Scheme 10. Modular Synthesis of Library 7 (53aa to 53fa) Containing 6 Amphiphilic Janus Glycodendrimers with Linear and Branched Alkyl Groups and D-Lactose and Their Self-Assembly by Injection of Their THF Solution into Water[a]



[a]Reagents and conditions: (i) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF/water (25 °C).

generate giant glycodendrimersomes by simple injection. These giant vesicles are stable in water and buffer even at concentrations higher than 10 mg/mL. Figure 10b–f shows the corresponding cryo-TEM images at the indicated concentration. The relationship between size and concentration observed by DLS is supported by cryo-TEM. Cryo-TEM images at concentrations higher than 4 mg/mL reveal multiple giant vesicles squeezing themselves in the copper grids because of large size and high particle density. These vesicles freely deform when touching each other, indicating the high flexibility of their soft vesicle wall. Other glycodendrimersomes exhibit a similar dependence between size and concentration. Cryo-TEM images of **49ab**, **52bd,dd**, **51ba**, and **49aa** over a range of concentrations are in Figures SF12–SF16, Supporting Information.

Structure of Glycodendrimercubosomes by the Analysis of the Fourier Transform of Their Cryo-TEM. Cubosomes are 3D bicontinuous liquid crystal particles with cubic lattice symmetry assembled from amphiphilic molecules displaying a large specific surface area. Figure 11 shows the cryo-TEM images of cubosomes formed by **51bd** at a concentration of 1.0 mg/mL. The unique structure of cubosomes and their ability to simultaneously incorporate water- and oil-soluble compounds led to an active area of research for controlled-release applications.[38]

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055–9077

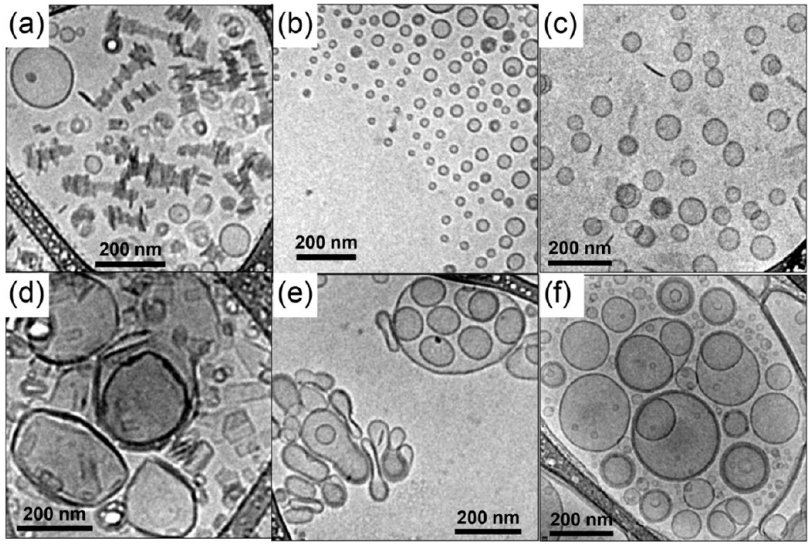


Figure 7. Selected cryo-TEM images of glycodendrimersomes and rodlike glycodendrimermicelles assembled from (a) (3,4)12G1-PE-TRZ$_i$-3EOLac$_2$ **53aa**, (b) glycodendrimersomes assembled from (3,5)12G1-PE-TRZ$_i$-3EOLac$_2$ **53ba**, (c) glycodendrimersomes and solid lamellae assembled from (3,4)2Et8G1-PE-TRZ$_i$-3EOLac$_2$ **53da**, (d) solid and polygonal glycodendrimersomes assembled from (3,4,5)12G1-PE-TRZ$_i$-3EOLac$_2$ **53ca**, (e) glycodendrimersomes assembled from (3,5)2Et8G1-PE-TRZ$_i$-3EOLac$_2$ **53ea**, and (f) glycodendrimersomes assembled from (3,4,5)2Et8G1-PE-TRZ$_i$-3EOLac$_2$ **53fa**.

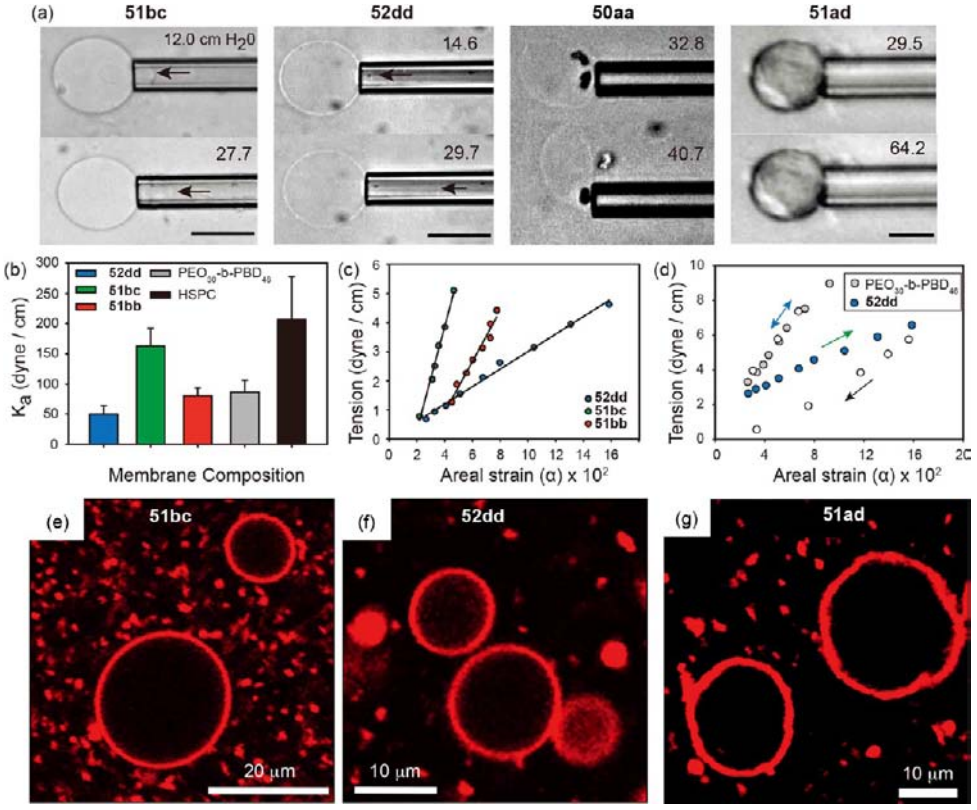


Figure 8. (a) Micropipet aspiration of soft glycodendrimersomes from (3,5)12G1-PE-TRZ$_i$-2EOMan$_2$ **51bc** and (3,4,5)12G1-PE-spacer-TRZ-4EOLac$_2$ **52dd**, and solid glycodendrimersome from (3,4)12G1-PE-spacer-TRZ-Gal$_2$ **50aa** and (3,4)12G1-PE-TRZ$_i$-1EOMan$_2$ **51ad**; scale bar is 25 μm. (b) Comparison of elastic moduli of glycodendrimersomes from **52dd**, **51bc**, and **51bb** with polymersomes of PEO$_{30}$-b-PBD$_{46}$ and liposomes from lipid HSPC. (c) Plot of tension vs areal strain for glycodendrimersomes from **52dd**, **51bc**, and **51bb**. (d) Comparison of tension vs areal strain plot for polymersome PEO$_{30}$-b-PBD$_{46}$ and glycodendrimersome **52dd**. In d, filled circles are measurements during initial vesicle stressing, and open circles are measurements during vesicle relaxation. Confocal microscopy images of (e, f) giant soft glycodendrimersomes and (g) giant hard glycodendrimersomes containing the hydrophobic Nile red dye.

Their structures can be elucidated by the analysis of the Fourier transform of their cryo-TEM images.[39] Figure 11a,b shows cryo-TEM of cubosomes at different orientations. The texture of the particle results from the projection of the

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

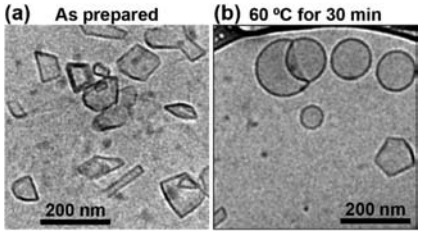


Figure 9. (a) Cryo-TEM image of (3,4,5)12G1-PE-TRZ$_i$-3EOMan$_2$ **51cb** obtained from an as-prepared solution at 0.5 mg/mL. (b) Cryo-TEM image of the same solution after annealing at 60 °C for 30 min. The transition from solid lamellae to vesicles is observed.

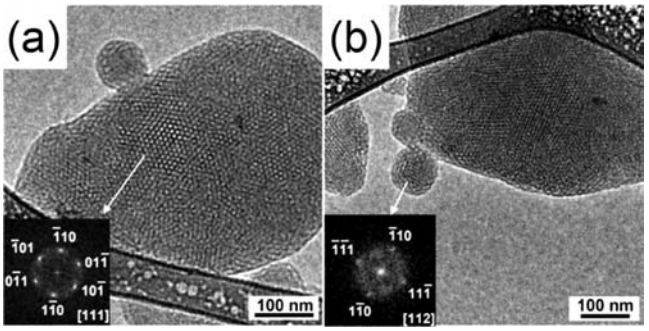


Figure 11. Cryo-TEM images of (3,5)12G1-PE-TRZ$_i$-1EOMan$_2$ **51bd** glycodendrimercubosome with $Pn\bar{3}m$ symmetry at 1.0 mg/mL. The inset in panel a shows the Fourier transform of a particle with a hexagonal arrangement of reflections corresponding to {110} crystallographic planes, indicating orientation along the [111] direction. The Fourier transform in panel b shows the {111} reflections, indicating orientation along the [112] direction, which has about a 20° tilt with respect to panel a.

bicontinuous structure of the cubosome. The inset in Figure 11a shows the Fourier transform of the indicated area. The hexagonal arranged bright spots are the {110} crystallographic plane reflections that suggest that the cubosome is oriented along the [111] direction. In the inset of Figure 11b, the square pattern resulted from particles oriented along the [112] direction that shows the reflections of {111} plane. On the basis of these observations, the cubosome of **51bd** exhibits a $Pn\bar{3}m$ cubic symmetry. The particle in Figure 11b is tilted about 20° with respect to that in Figure 11a.

Molecular Design Principles for Janus Glycodendrimers Assembling into Glycodendrimersomes with Narrow Size Distribution and Stable over Time in Buffer. Figure 12 outlines the structures of the 16 Janus glycodendrimers that self-assemble by injection in water and/or in buffer in single-type soft glycodendrimersomes. These soft glycodendrimersomes exhibit narrow molar mass distribution and are stable over time. Their dimensions, polydispersity, and stability in water (marked blue and yellow) and PBS and HEPES buffers (marked yellow) are indicated in Figure 12. The dimensions of glycodendrimersomes assembled in water and PBS are similar but smaller than those assembled in HEPES. Ten of these Janus glycodendrimersomes, highlighted in yellow, assemble in glycodendrimersomes that are also stable in buffer. Six of them contain a tri(ethylene glycol) or tetra(ethylene glycol) spacer in the hydrophilic part and a branched or linear 3,5-disubstituted pattern in the hydrophobic part. Three of them contain the same oligooxyethylenic spacer length as that of the previous six molecules but have a 3,4,5-branched or linear hydrophobic pattern in the hydrophobic part. Only one among these ten has a 3,4-disubstituted branched hydrophobic pattern with the same hydrophilic spacer length. Based on these observations, the following molecular design principles were established for the structure of Janus glycodendrimers that are expected to produce soft spherical glycodendrimersomes stable in buffer. Any mono- or disaccharide attached via a tri(ethylene glycol) or tetra(ethylene glycol) spacer with a 3,5-disubstituted linear or branched hydrophobic pattern will most probably self-assemble into uniform single-type soft glycodendrimersomes with favorable mechanical properties, narrow molar mass distribution, and stability over time in buffer. This conclusion supports previous predictions concerning the dimensions, stability, and mechanical properties, elaborated for simple amphiphilic Janus dendrimers and dendrimersomes.[9b] An additional requirement for the assembly of

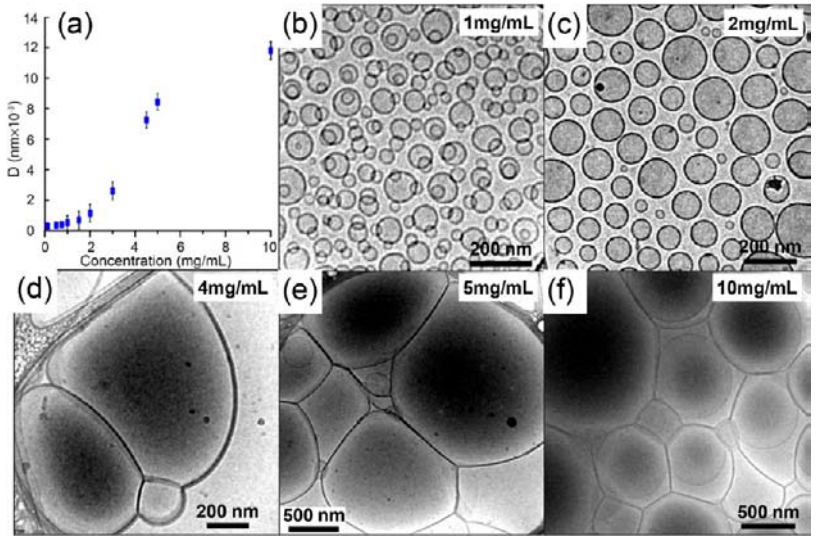


Figure 10. (a) Plot of glycodendrimersome diameter vs concentration. The average vesicle size was obtained from DLS measurements. Cryo-TEM images of glycodendrimersomes assembled from (3,5)2Et8G1-PE-TRZ$_i$-3EOGal$_2$ **49bb** at concentrations of (b) 1 mg/mL, (c) 2 mg/mL, (d) 4 mg/mL, (e) 5 mg/mL, and (f) 10 mg/mL.

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

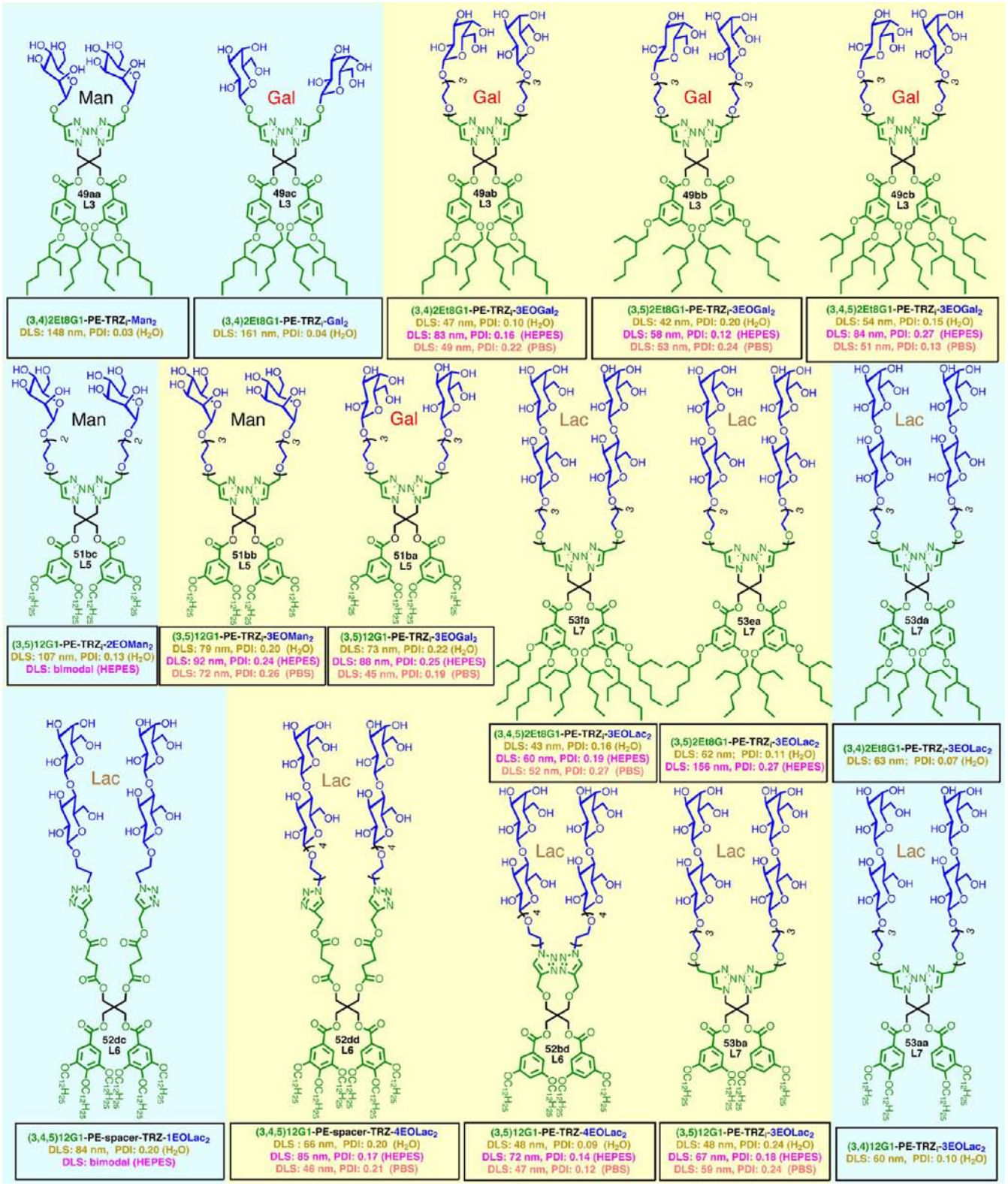


Figure 12. Structures of amphiphilic Janus glycodendrimers and their single-type soft glycodendrimersomes with narrow polydispersity and good stability in buffer (marked in yellow) and in water (marked in blue and in yellow) as assembled by injection. The library number is indicated under the molecule code.

soft glycodendrimersomes is a glass transition or melting temperature after hydration lower than room temperature. Janus glycodendrimers with a 3,4- and 3,4,5-substitution pattern tend to crystallize while those with a 3,5-substituted pattern are amorphous with low glass transitions when containing tri- and tetra(ethylene glycol) fragments. This trend explains the results from Figure 12.

Agglutination of Glycodendrimersomes with Plant, Bacterial, and Human Lectins. Glycodendrimersomes are of interest to enable specific surface contacts of their glycan display

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

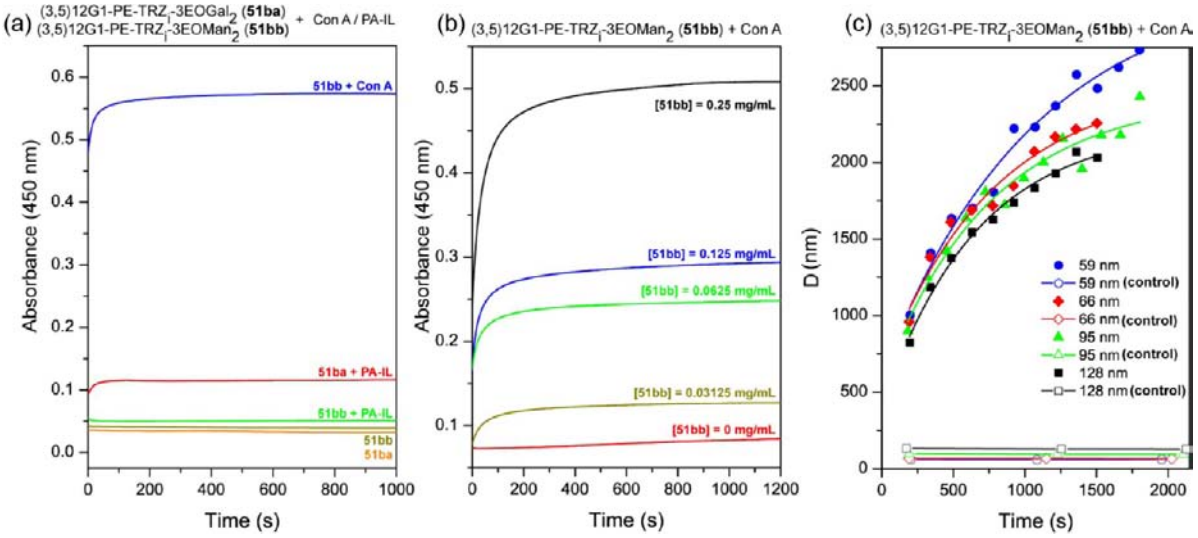


Figure 13. (a) Agglutination of dendrimersomes of (3,5)12G1-PE-TRZ$_i$-3EOGal$_2$ (**51ba**) and (3,5)12G1-PE-TRZ$_i$-3EOMan$_2$ (**51bb**) in the presence of Con A and PA-IL. [**51ba**] = 0.5 mg/mL (900 μL), [**51bb**] = 0.5 mg/mL (600 μL), [Con A] = 0.3 mg/mL (100 μL), [PA-IL] = 0.0625 mg/mL (100 μL) in HEPES buffer (1.0 mM $MnCl_2$ and 1.0 mM $CaCl_2$). (b) Agglutination of dendrimersomes of (3,5)12G1-PE-TRZ$_i$-3EOMan$_2$ (**51bb**) at different concentrations (0 to 0.25 mg/mL, 900 μL) in the presence of Con A (0.125 mg/mL, 100 μL) in 10 mM HEPES (1.0 mM $MnCl_2$ and 1.0 $CaCl_2$). (c) Agglutination of dendrimersomes of **51bb** with different sizes in the presence of Con A recorded by DLS. [**51bb**] = 0.0625 mg/mL (400 μL), [Con A] = 0.5 mg/mL (100 μL) in HEPES buffer (1.0 mM $MnCl_2$ and 1.0 mM $CaCl_2$).

with receptors in lectin-targeted delivery of a cargo or as pharmaceuticals when for example they block the lectin-dependent docking of viruses onto cells.[1−4,40] An essential prerequisite for considering bioapplications is to ascertain the ligand bioactivity of the sugar head groups in molecular recognition experiments with lectin receptors. Agglutination of glycodendrimersomes by lectins demonstrates an assumed amplification of the multivalency of the carbohydrates at the transition from the Janus glycodendrimer to glycodendrimersome from 2 to *n*. To reveal general reactivity, we present initial experiments on agglutination of glycodendrimersomes by various types of plants (the plant lectin concanavalin A, Con A, that binds to D-mannose,[41b] and the European mistletoe lectin *Viscum album* L. agglutin, VAA,[41c−f] that reacts with β-galactosides), bacterial (the β-galactoside-binding lectin PA-IL from *Pseudomonas aeruginosa*, a bacterium affecting cystic fibrosis patients and immunocompromized individuals, that binds to D-galactose[41a]) and human galectine-3, Gal-3, and galectine-4, Gal-4, sharing binding to D-lactose.[41g−i] Qualitative agglutination experiments were monitored in 10 mM HEPES buffer by a combination of DLS, UV−vis, and cryo-TEM experiments. DLS experiments sense the agglutination process by a size increase in time that is observed upon mixing the appropriate glycodendrimersomes with the corresponding lectin. This correlation of mutually fitting specificities excludes carbohydrate-independent mechanisms.

A change in absorbance was invariably detected, revealing bioactivity of the sugar head groups. Representative UV−vis experiments over a time course of up to 33 min are shown in Figure 13a for the agglutination of **51ba** with PA-IL and of **51bb** with Con A. Control experiments for **51ba** and **51bb** in the absence of the lectin are shown in the bottom of Figure 13a. A control experiment for sugar specificity, for **51bb** with PA-IL, is shown in the bottom of Figure 13a. The increase in absorbance with time reflects the agglutination process. A concentration dependence of the agglutination of Con A with **51bb** together with a control experiment are shown in Figure 13b. A constant

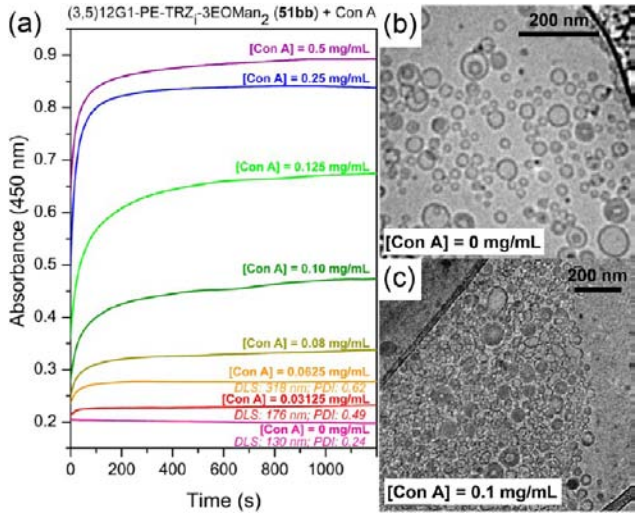


Figure 14. (a) Agglutination of soft dendrimersomes of (3,5)12G1-PE-TRZ$_i$-3EOMan$_2$ (**51bb**) in the presence of different concentrations of Con A. [**51bb**] = 0.5 mg/mL (900 μL), [Con A] = 0 − 0.5 mg/mL (100 μL) in 10 mM HEPES buffer (1.0 mM $MnCl_2$ and 1.0 mM $CaCl_2$) (a). Corresponding Cryo-TEM images at indicated Con A concentration. The agglutination effect can be clearly visualized (b, c).

concentration of Con A was used in combination with different concentrations of **51bb**. Finally, Figure 13c shows the agglutination of glycodendrimersomes of different diameters assembled from **51bb** with Con A. Control experiments for each diameter are also presented. In the range of diameters investigated, the smallest glycodendrimersome provides the fastest agglutination, probably due to the highest surface/volume ratio.

These results demonstrate the importance of the injection method for the simple and rapid preparation of soft glycodendrimersomes with different dimensions, mechanical properties, dynamics, adaptability, and degree of multivalency. In the next experiment, concentration of Con A was varied while concentration of **51bb** was maintained constant. The agglutination

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

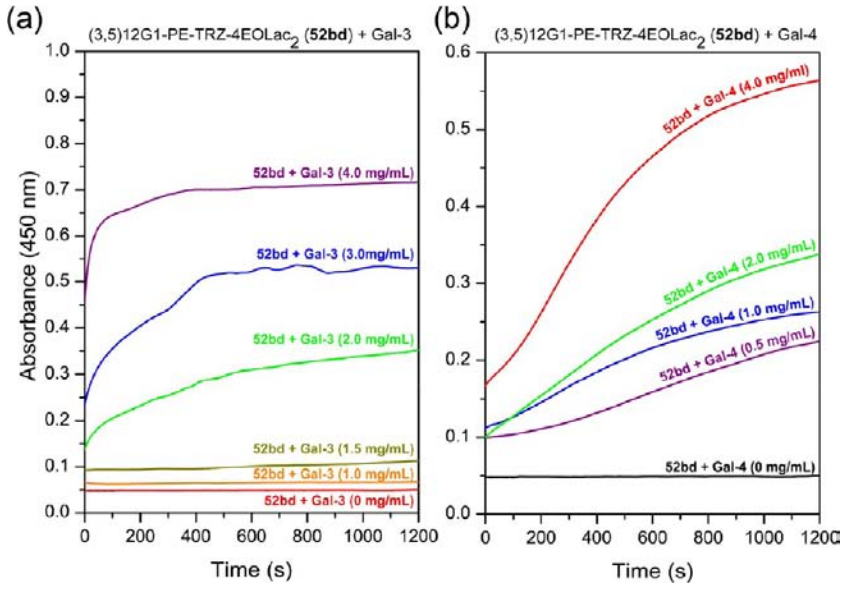


Figure 15. Agglutination of dendrimersomes of (3,5)12G1-PE-TRZ-4EOLac$_2$ (**52bd**) in the presence of different concentration of Gal-3 (a) and Gal-4 (b). [**52bd**] = 1.0 mg/mL (900 μL), [Gal-3] = 0−4.0 mg/mL (100 μL), [Gal-4] = 0−4.0 mg/mL (100 μL) in 10 mM HEPES buffer (1.0 mM $MnCl_2$ and 1.0 mM $CaCl_2$).

monitored by UV−vis is shown in Figure 14a. Figure 14b,c describes the agglutination of **51bb** with Con A monitored by cryo-TEM. Additional results on agglutination monitored by cryo-TEM and UV−vis are in Figures SF17, SF19−SF22, and SF24−SF26, Supporting Information. Having ascertained bioactivity with the plant leguminous and bacterial lectins, we next proceeded to test two human lectins. While sharing specificity to lactose, Gal-3 and Gal-4 have a different molecular design. Gal-3 has a collagenase-sensitive N-terminal tail relevant for aggregation when interacting with multivalent ligands, while Gal-4 presents two carbohydrate recognition domains connected by a 42-amino acid linker peptide.[42]

It was thus of interest to ascertain bioactivity to human lectins as well as characterize the resulting profiles of a monomeric galectin capable to form aggregates with a bivalent protein of this family. Figure 15a shows the agglutination of **52bd** with Gal-3 (cryo-TEM images in Figure SF19), while Figure 15b displays the course of agglutination of **52bd** with Gal-4 (cryo-TEM images in Figure SF20). In both cases, the concentration of **52bd** was maintained constant, while the concentrations of Gal-3 and Gal-4 were varied. Control experiments supporting lectin dependence of the agglutination experiments are also shown in Figure 15a,b. Evidently, the obtained courses are different, yet reaching similar plateau values. The nature of the lectin thus influences the way agglutination proceeds. In this context, it should be noted that the mode of counter-receptor cross-linking on the cell surface will determine the strength of the adhesive contacts (in *trans*, between "cells") and the response by initiating signaling (in *cis*, on a "cell" surface), directing attention to further work along this line. Interestingly, the plant toxin followed a saturation kinetics for Gal-3. The agglutination profiles of **52bd** with different concentrations of VAA investigated by UV−vis are shown in Figure SF18a, Supporting Information, while the similar experiment monitored by cryo-TEM is in Figure SF18b−e. Compound **51ba** that contains D-galactose was also agglutinated by VAA (Figures SF21 and SF25) and PA-IL (Figure SF22), as expected based on the specificity of lectins. These results on courses of agglutination reveal that the glycodendrimersomes react differently to lectins, topology and adaptability of the soft vesicles with favorable mechanical properties playing a role and thus making them valuable models for further studies to relate lectin structure and glycan display on the course of agglutination. To our knowledge, this study is the most comprehensive when considering the diversity of lectins used.[1e]

■ CONCLUSIONS

Seven libraries containing 51 self-assembling amphiphilic Janus glycodendrimers with three types of biogenic carbohydrates in their hydrophilic part have been synthesized by a simple and efficient accelerated modular strategy. These Janus glycodendrimers self-assemble by simple injection of their solution in a water-miscible solvent into water and buffer. The resulting supramolecular structures were analyzed by a combination of methods to determine their structure and to delineate the molecular principles leading to narrow size distribution and stability over time in water and in buffer for single-type soft and hard assemblies, including unilamellar spherical, polygonal, and tubular glycodendrimersomes, Janus glycodendrimer aggregates, glycodendrimercubosomes, and solid lamellae. Sixteen of these amphiphilic Janus glycodendrimers result in soft glycodendrimersomes with dimensions programmable via the concentration of the solution from which they are injected. Ten of them, **49ab**, **49bb**, **49cb** (library 3), **51ba**, **51bb** (library 5), **52dd**, **52bd** (library 6), and **53ba**, **53ea**, **53fa** (library 7) containing the same carbohydrates form glycodendrimersomes that are also stable in buffer. Binding studies of these glycodendrimersomes performed by agglutination experiments with plant, bacterial, and human lectin receptors of biomedical interest demonstrated bioactivity of the multivalent ligand display of their sugar headgroups, supporting potential applications. Of note, the apparent differences in the courses of agglutination by human Gal-3 and Gal-4 revealed a topological dimension, beyond the cross-linking of surface-presented D-lactose in both cases. Therefore, these assemblies are models of biological membranes to delineate structure−activity correlations at the level of multivalent, soft, dynamic, and adaptable surface recognition, also enabling

dx.doi.org/10.1021/ja403323y | J. Am. Chem. Soc. 2013, 135, 9055−9077

exploration of utility for targeting and lectin blocking. Mapping agglutination courses with native and structurally engineered proteins, also in the inhibitory setting mimicking physiologically relevant systems of functional competition,[43] will be crucial to test this concept. These experiments demonstrated that glycodendrimersomes provide a new, simple, and efficient mimic of cell membranes, broadening the toolbox of glycopolymers, glycodynamers, glycopeptides, liposomes/nanoparticles, and glycodendrimers. This new type of programmable surface ligand display with interactions in *cis* and in *trans* extends the application of surface-based carbohydrate microarrays that are used in high-throughput detection and specificity analysis of proteins[44] to dynamic measurements in solution, at the same level of specificities but with more complex functions. Mixing compounds with different headgroups even enables the route toward cell-membrane-like complexity, offering flexibility and accessibility changes for detailed bioexploration in solution, not amendable by planar arrays on surface, with read-out for *cis*- and *trans*-interactions and versatile physicochemical analysis possible on structural properties, as documented by cryo-TEM, even after exposure to lectins. The library approach used to discover and predict the primary structures[9,45] that are the platform for glycodendrimersomes is thus taken from principles of complex supramolecular systems,[9,45] in the realm of glycobiology, encouraging both studies on fundamental structure–activity correlations and on applications. As the initial discovery of different agglutination courses attests, work toward selective sugar receptors blocking/targeting, for example in bacterial/viral attachment, lectin blocking in inflammation/tumor progression, or delivery to antigen-processing dendritic cells in vaccination, will be based on these new tools for multivalent display even possibly beyond carbohydrates as ligands.

■ ASSOCIATED CONTENT

Supporting Information

Experimental synthetic procedures with complete structural and self-assembly analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

Percec@sas.upenn.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support by the National Science Foundation (grants DMR-1066116 and DMR-1120901) and by the P. Roy Vagelos Chair at the University of Pennsylvania is gratefully acknowledged. R.R. is grateful to NSERC for financial support and for a CRC in Therapeutic Chemistry. P.L. thanks the Royal Thai Government for a graduate fellowship and H.J.G. for EC funding (GlycoHIT grant agreement 260600 and GLYCOPHARM PITN-GA-2012-317297).

■ REFERENCES

(1) (a) Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357–2364. (b) Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Curr. Opin. Chem. Biol.* **2000**, *4*, 696–703. (c) Roy, R. *Curr. Opin. Struct. Biol.* **1996**, *6*, 692–702. (d) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2755–2794. (e) Gabius, H.-J., Ed. *The Sugar Code. Fundamentals of Glycosciences*; Wiley-VCH: Weinheim, 2009. (f) Gabius, H.-J.; André, S.; Jiménez-Barbero, J.; Romero, A.; Solís, D. *Trends Biochem. Sci.* **2011**, *36*, 298–313. (g) Lepenies, B.; Yin, J.; Seeberger, P. H. *Curr. Opin. Chem. Biol.* **2010**, *14*, 404–411. (h) Seeberger, P. H.; Werz, D. B. *Nature* **2007**, *446*, 1046–1051.

(2) (a) Kramer, J. R.; Deming, T. J. *J. Am. Chem. Soc.* **2012**, *134*, 4112–4115. (b) Ueda, M.; Makino, A.; Imai, T.; Sugiyama, J.; Kimura, S. *Chem. Commun.* **2011**, *47*, 3204–3206. (c) André, S.; Renoudet, O.; Bossu, I.; Dumy, P.; Gabius, H.-J. *J. Pept. Sci.* **2011**, *17*, 427–437. (d) Roy, R.; Zanini, D.; Meunier, S. J.; Romanoska, A. *Chem. Commun.* **1993**, 1869–1872.

(3) (a) Ladmiral, V.; Melia, E.; Haddleton, D. M. *Eur. Polym. J.* **2004**, *40*, 431–449. (b) Kanai, M.; Mortell, K. H.; Kiessling, L. L. *J. Am. Chem. Soc.* **1997**, *119*, 9931–9932. (c) Miura, Y. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 5031–5036. (d) Spain, S. G.; Gibson, M. I.; Cameron, N. R. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 2059–2072. (e) Godula, K.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2010**, *132*, 9963–9965. (f) Voit, B.; Appelhans, D. *Macromol. Chem. Phys.* **2010**, *211*, 727–735. (g) Schatz, C.; Lecommandoux, S. *Macromol. Rapid Commun.* **2010**, *31*, 1664–1684. (h) Roy, R. *Trends Glycosci. Glycotechnol.* **1996**, *8*, 79–99. (i) Coullerez, G.; Seeberger, P. H.; Textor, M. *Macromol. Biosci.* **2006**, *6*, 634–647. (j) Spain, S. G.; Cameron, N. R. *Polym. Chem.* **2011**, *2*, 60–68. (k) Gou, Y.; Geng, J.; Richards, S.-J.; Burns, J.; Remzi Becer, C.; Haddleton, D. M. *J. Polym. Sci., Part A: Polym. Chem.* **2013**, *51*, 2588–2597. (l) Spaltenstein, A.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 686–687. (m) André, S.; Ortega, P. J. C.; Perez, M. A.; Roy, R.; Gabius, H.-J. *Glycobiology* **1999**, *9*, 1253–1261. (n) Roy, R.; Tropper, F. D. *Chem. Commun.* **1988**, 1058–1060. (o) Bovin, N. V.; Gabius, H.-J. *Chem. Soc. Rev.* **1995**, *24*, 413–421.

(4) (a) Chabre, Y. M.; Roy, R. *Curr. Top. Med. Chem.* **2008**, *8*, 1237–1285. (b) Touaibia, M.; Roy, R. *Mini-Rev. Med. Chem.* **2007**, *7*, 1270–1283. (c) Imberty, A.; Chabre, Y. M.; Roy, R. *Chem.—Eur. J.* **2008**, *14*, 7490–7499. (d) Sirois, S.; Touaibia, M.; Chou, K.-C.; Roy, R. *Curr. Med. Chem.* **2007**, *14*, 3232–3242. (e) Chabre, Y. M.; Roy, R. *Adv. Carbohydr. Chem. Biochem.* **2010**, *63*, 165–393. (f) Höebel, S.; Loos, A.; Appelhans, D.; Schwarz, S.; Seidel, J.; Voit, B.; Aigner, A. *J. Controlled Release* **2011**, *149*, 146–158. (g) Lo Conte, M.; Robb, M. J.; Hed, Y.; Marra, A.; Malkoch, M.; Hawker, C. J.; Dondoni, A. *J. Polym. Sci., Part A: Polym. Chem.* **2011**, *49*, 4468–4475. (h) Kikkeri, R.; Grünstein, D.; Seeberger, P. H. *J. Am. Chem. Soc.* **2010**, *132*, 10230–10232. (i) Kikkeri, R.; Liu, X.; Adibekian, A.; Tsai, Y.-H.; Seeberger, P. H. *Chem. Commun.* **2010**, *46*, 2197–2199. (j) Zanini, D.; Roy, R. *J. Am. Chem. Soc.* **1997**, *119*, 2088–2095. (k) de Paz, J. L.; Noti, C.; Böhm, F.; Werner, S.; Seeberger, P. H. *Chem. Biol.* **2007**, *14*, 879–887. (l) Hecht, M.-L.; Stallforth, P.; Silva, D. V.; Adibekian, A.; Seeberger, P. H. *Curr. Opin. Chem. Biol.* **2009**, *13*, 354–359. (m) Ribeiro-Viana, R.; García-Vallejo, J. J.; Collado, D.; Pérez-Inestrosa, E.; Bloem, K.; van Kooyk, Y.; Rojo, J. *Biomacromolecules* **2012**, *13*, 3209–3219. (n) Chabre, Y. M.; Roy, R. *Chem. Soc. Rev.* **2013**, *42*, 4657–4708.

(5) (a) Kikkeri, R.; Liu, X.; Adibekian, A.; Tsai, Y.-H.; Seeberger, P. H. *Chem. Commun.* **2010**, *46*, 2197–2199. (b) Laurino, P.; Kikkeri, R.; Azzouz, N.; Seeberger, P. H. *Nano Lett.* **2011**, *11*, 73–78. (c) Jayaraman, N.; Maiti, K.; Naresh, K. *Chem. Soc. Rev.* **2013**, *42*, 4640–4656. (d) Hecht, M.-L.; Stallforth, P.; Silva, D. V.; Adibekian, A.; Seeberger, P. H. *Curr. Opin. Chem. Biol.* **2009**, *13*, 354–359. (e) Röckendorf, N.; Lindhorst, T. K. *Top. Curr. Chem.* **2001**, *217*, 201–238. (f) Ribeiro-Viana, R.; García-Vallejo, J. J.; Collado, D.; Pérez-Inestrosa, E.; Bloem, K.; van Kooyk, Y.; Rojo, J. *Biomacromolecules* **2012**, *13*, 3209–3219.

(6) (a) Kalovidouris, S. A.; Turnbull, W. B.; Stoddart, J. F. *Can. J. Chem.* **2002**, *80*, 983–991. (b) Nelson, A.; Stoddart, J. F. *Carbohydr. Res.* **2004**, *339*, 2069–2075. (c) Barbera, J.; Garces, A. C.; Jayaraman, N.; Omenat, A.; Serrano, J. L.; Stoddart, J. F. *Adv. Mater.* **2001**, *13*, 175–180. (d) Ashton, P. R.; Boyd, S. E.; Brown, C. L.; Jayaraman, N.; Nepogodiev, S. A.; Stoddart, J. F. *Chem.—Eur. J.* **1996**, *2*, 1115–1128. (e) Ballardini, R.; Colonna, B.; Gandolfi, M. T.; Kalovidouris, S. A.; Orzel, L.; Raymo, F. M.; Stoddart, J. F. *Eur. J. Org. Chem.* **2003**, 288–294. (f) Turnbull, W. B.; Kalovidouris, S. A.; Stoddart, J. F. *Chem.—Eur. J.* **2002**, *8*, 2988–3000. (g) Roy, R.; Kim, J. M. *Angew. Chem., Int. Ed.* **1999**, *38*, 369–372. (h) Leyden, R.; Velasco-Torrijos, T.; André, S.; Gouin, S.; Gabius, H.-J.; Murphy, P. V. *J. Org. Chem.* **2009**, *74*, 9010–9026. (i) André, S.; Grandjean, C.; Gautier, F. M.; Bernardi, S.; Sansone, F.; Gabius, H.-J.;

Ungaro, R. *Chem. Commun.* **2011**, *47*, 6126−6128. (j) André, S.; Jarikote, D. V.; Yan, D.; Vincenz, L.; Wang, G. N.; Kaltner, H.; Murphy, P. V.; Gabius, H.-J. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 313−318.
(7) (a) Ruff, Y.; Buhler, E.; Candau, S.-J.; Kesselman, E.; Talmon, Y.; Lehn, J.-M. *J. Am. Chem. Soc.* **2010**, *132*, 2573−2584. (b) Ruff, Y.; Lehn, J.-M. *Angew. Chem., Int. Ed.* **2008**, *47*, 3556−3559. (c) Ruff, Y.; Lehn, J.-M. *Biopolymers* **2008**, *89*, 486−496.
(8) (a) Wagner, A.; Vorauer-Uhl, K.; Katinger, H. *Eur. J. Pharm. Biopharm.* **2002**, *54*, 213−219. (b) Batzri, S.; Korn, E. D. *Biochim. Biophys. Acta* **1973**, *298*, 1015−1019. (c) Kremer, J. M. H.; Esker, M. W. J.; Pathmamanoharan, C.; Wiersena, P. H. *Biochemistry* **1977**, *16*, 3932−3935. (d) Bridson, R. H.; Santos, R. C. D.; Al-Duri, B.; McAllister, S. M.; Robertson, J.; Alpar, H. O. *J. Pharm. Pharmacol.* **2006**, *58*, 775−785. (e) Szoka, F.; Papahadjopoulos, D. *Annu. Rev. Biophys. Bioeng.* **1980**, *9*, 467−508. (f) Monnard, P. A.; Deamer, D. W. *Methods in Enzymology*; Duzgunes, N., Ed.; Academic Press: New York, 2003; pp 133−151.
(9) (a) Percec, V.; Wilson, D. A.; Leowanawat, P.; Wilson, C. J.; Hughes, A. D.; Kaucher, M. S.; Hammer, D. A.; Levine, D. H.; Kim, A. J.; Bates, F. S.; Davis, K. P.; Lodge, T. P.; Klein, M. L.; De, V. R. H.; Aqad, E.; Rosen, B. M.; Argintaru, A. O.; Sienkowska, M. J.; Rissanen, K.; Nummelin, S.; Ropponen, J. *Science* **2010**, *328*, 1009−1014. (b) Peterca, M.; Percec, V.; Leowanawat, P.; Bertin, A. *J. Am. Chem. Soc.* **2011**, *133*, 20507−20520. (c) Peterca, M.; Imam, M. R.; Leowanawat, P.; Rosen, B. M.; Wilson, D. A.; Wilson, C. J.; Zeng, X.; Ungar, G.; Heiney, P. A.; Percec, V. *J. Am. Chem. Soc.* **2010**, *132*, 11288−11305.
(10) (a) Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 113−158. (b) Thomas, J. L.; Tirrell, D. A. *Acc. Chem. Res.* **1992**, *25*, 336−342. (c) Lasic, D. D.; Papahadjopoulos, D. *Science* **1995**, *267*, 1275−1276. (d) Discher, B. M.; Won, Y. Y.; Ege, D. S.; Lee, J. C. M.; Bates, F. S.; Discher, D. E.; Hammer, D. A. *Science* **1999**, *284*, 1143−1146. (e) Barenholz, Y. *Curr. Opin. Colloid Interface Sci.* **2001**, *6*, 66−77. (f) Guo, X.; Szoka, F. C. *Acc. Chem. Res.* **2003**, *36*, 335−341. (g) Allen, T. M.; Cullis, P. R. *Science* **2004**, *303*, 1818−1822. (h) Haluska, C. K.; Riske, K. A.; Marchi-Artzner, V.; Lehn, J.-M.; Lipowsky, R.; Dimova, R. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15841−15846. (i) Kita-Tokarczyk, K.; Meier, W. *Chimia* **2008**, *62*, 820−825. (j) van Dongen, S. F. M.; de Hoog, H.-P. M.; Peters, R. J. R. W.; Nallani, M.; Nolte, R. J. M.; van Hest, J. C. M. *Chem. Rev.* **2009**, *109*, 6212−6274. (k) Farokhzad, O. C.; Langer, R. *ACS Nano* **2009**, *3*, 16−20. (l) Antonietti, M.; Förster, S. *Adv. Mater.* **2003**, *15*, 1323−1333. (m) Schulz, M.; Glatte, D.; Meister, A.; Scholtysek, P.; Kerth, A.; Blume, A.; Bacia, K.; Binder, W. H. *Soft Matter* **2011**, *7*, 8100−8110.
(11) (a) Kataoka, K.; Harada, A.; Nagasaki, Y. *Adv. Drug Delivery Rev.* **2001**, *47*, 113−131. (b) Miyata, K.; Christie, R. J.; Kataoka, K. *React. Funct. Polym.* **2011**, *71*, 227−234. (c) Francis, M. F.; Cristea, M.; Winnik, F. M. *Pure Appl. Chem.* **2004**, *76*, 1321−1335. (d) Haag, R.; Kratz, F. *Angew. Chem., Int. Ed.* **2006**, *45*, 1198−1215. (e) Marguet, M.; Edembe, L.; Lecommandoux, S. *Angew. Chem., Int. Ed.* **2012**, *51*, 1173−1176.
(12) (a) Spicer, P. T. *Curr. Opin. Colloid Interface Sci.* **2005**, *10*, 274−279. (b) Larsson, K. *J. Phys. Chem.* **1989**, *93*, 7304−7314. (c) Prashar, D.; Sharma, D. *Asian J. Res. Pharm. Sci.* **2011**, *1*, 59−62. (d) Nguyen, T.-H.; Hanley, T.; Porter, C. J. H.; Larson, I.; Boyd, B. J. *J. Pharm. Pharmacol.* **2010**, *62*, 856−865. (e) Boyd, B. J. *Int. J. Pharm.* **2003**, *260*, 239−247. (f) Almgren, M.; Edwards, K.; Karlsson, G. *Colloids Surf., A* **2000**, *174*, 3−21. (g) Hyde, S. T.; Andersson, S.; Ericsson, B.; Larsson, K. *Zeik. Kristallogr.* **1984**, *168*, 213−219. (h) Unverzagt, C.; André, S.; Seifert, J.; Kojima, S.; Fink, C.; Srikrishna, G.; Freeze, H.; Kayser, K.; Gabius, H.-J. *J. Med. Chem.* **2002**, *45*, 478−491. (i) Rizwan, S. B.; Boyd, B. J.; Rades, T.; Hook, S. *Exp. Opin. Drug Delivery* **2010**, *7*, 1133−1144. (j) André, S.; Kozár, T.; Kojima, S.; Unverzagt, C.; Gabius, H.-J. *Biol. Chem.* **2009**, *390*, 557−565. (k) Zhu, T. F.; Szostak, J. W. *PLoS One* **2009**, *4*, e5009−e5009.
(13) (a) Gabius, H.-J.; Walzel, H.; Joshi, S. S.; Kruip, J.; Kojima, S.; Gerke, V.; Kratzin, H.; Gabius, S. *Anticancer Res.* **1992**, *12*, 669−676. (b) Gabius, H.-J.; Darro, F.; Remmelink, M.; André, S.; Kopitz, J.; Danguy, A.; Gabius, S.; Salmon, I.; Kiss, R. *Cancer Invest.* **2001**, *19*, 114−126. (c) Sanchez-Ruderisch, H.; Fischer, C.; Detjen, K. M.; Welzel, M.; Wimmel, A.; Manning, J. C.; André, S.; Gabius, H.-J. *FEBS J.* **2010**, *277*, 3552−3563. (d) Amano, M.; Eriksson, H.; Manning, J. C.; Detjen, K. M.; André, S.; Nishimura, S.-I.; Lehtiö, J.; Gabius, H.-J. *FEBS J.* **2012**, *279*, 4062−4080. (e) Kaltner, H.; Gabius, H.-J. *Histol. Histopathol.* **2012**, *27*, 397−416. (f) Ledeen, R. W.; Wu, G.; André, S.; Bleich, D.; Huet, G.; Kaltner, H.; Kopitz, J.; Gabius, H.-J. *Ann. N.Y. Acad. Sci.* **2012**, *1253*, 206−221. (g) Smetana, K., Jr.; André, S.; Kaltner, H.; Kopitz, J.; Gabius, H.-J. *Expert Opin. Ther. Targets* **2013**, *17*, 379−392.
(14) (a) Herrmann, K. *Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 315−347. (b) Cuvelier, M. E.; Richard, H.; Berset, C. *Biosci., Biotechnol., Biochem.* **1992**, *56*, 324−325. (c) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. *Free Radical Biol. Med.* **1996**, *20*, 933−956. (d) Natella, F.; Nardini, M.; Di Felice, M.; Scaccini, C. *J. Agric. Food Chem.* **1999**, *47*, 1453−1459. (e) Fukumoto, L. R.; Mazza, G. *J. Agric. Food Chem.* **2000**, *48*, 3597−3604. (f) Baderschneider, B.; Winterhalter, P. *J. Agric. Food Chem.* **2001**, *49*, 2788−2798. (g) Kawabata, J.; Okamoto, Y.; Kodama, A.; Makimoto, T.; Kasai, T. *J. Agric. Food Chem.* **2002**, *50*, 5468−5471.
(15) (a) Percec, V.; Ahn, C. H.; Bera, T. K.; Ungar, G.; Yeardley, D. J. P. *Chem.—Eur. J.* **1999**, *5*, 1070−1083. (b) Ropponen, J.; Nummelin, S.; Rissanen, K. *Org. Lett.* **2004**, *6*, 2495−2497.
(16) (a) Chen, W.; Meng, F.; Li, F.; Ji, S.-J.; Zhong, Z. *Biomacromolecules* **2009**, *10*, 1727−1735. (b) Jiang, Z.-X.; Yu, Y. B. *J. Org. Chem.* **2007**, *72*, 1464−1467.
(17) (a) Al-Mughaid, H.; Grindley, T. B. *J. Org. Chem.* **2006**, *71*, 1390−1398. (b) Ortega-Muñoz, M.; Perez-Balderas, F.; Morales-Sanfrutos, J.; Hernandez-Mateo, F.; Isac-Garcia, J.; Santoyo-Gonzalez, F. *Eur. J. Org. Chem.* **2009**, 2454−2473.
(18) Issidorides, C. H.; Gulen, R. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. *IV*, pp 679−680.
(19) Antoni, P.; Hed, Y.; Nordberg, A.; Nyström, D.; von Holst, H.; Hult, A.; Malkoch, M. *Angew. Chem., Int. Ed.* **2009**, *48*, 2126−2130.
(20) Vicente, V.; Martin, J.; Jimenez-Barbero, J.; Chiara, J. L.; Vicent, C. *Chem.—Eur. J.* **2004**, *10*, 4240−4251.
(21) Geng, J.; Lindqvist, J.; Mantovani, G.; Chen, G.; Dayers, C. T.; Clarkson, G. J.; Haddleton, D. M. *QSAR Comb. Sci.* **2007**, *26*, 1220−1228.
(22) (a) Cai, L.; Ban, L.; Guan, W.; Mrksich, M.; Wang, P. G. *Carbohydr. Res.* **2011**, *346*, 1576−1580. (b) Kale, R. R.; Mukundan, H.; Price, D. N.; Harris, J. F.; Lewallen, D. M.; Swanson, B. I.; Schmidt, J. G.; Iyer, S. S. *J. Am. Chem. Soc.* **2008**, *130*, 8169−8171.
(23) Wolfrom, M. L.; Thompsom, A. *Methods Carbohydr. Chem.* **1963**, *2*, 211.
(24) Mitchell, A. A.; Pratt, M. R.; Hruby, V. J.; Polt, R. *J. Org. Chem.* **2001**, *66*, 2327−2342.
(25) Kumar, R.; Maulik, P. R.; Misra, A. K. *Glycoconjugate J.* **2008**, *25*, 595−602.
(26) Ravindranathan Kartha, K. P.; Field, R. A. *Tetrahedron* **1997**, *53*, 11753−11766.
(27) Hasegawa, T.; Fujisawa, T.; Numata, M.; Matsumoto, T.; Umeda, M.; Karinaga, R.; Mizu, M.; Koumoto, K.; Kimura, T.; Okumura, S.; Sakurai, K.; Shinkai, S. *Org. Biomol. Chem.* **2004**, *2*, 3091−3098.
(28) Zhang, L.; Sun, L.; Cui, Z.; Gottlieb, R. L.; Zhang, B. *Bioconjugate Chem.* **2001**, *12*, 939−948.
(29) Nieman, J. A.; Nair, S. K.; Heasley, S. E.; Schultz, B. L.; Zerth, H. M.; Nugent, R. A.; Chen, K.; Stephanski, K. J.; Hopkins, T. A.; Knechtel, M. L.; Oien, N. L.; Wieber, J. L.; Wathen, M. W. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3039−3042.
(30) Michel, O.; Ravoo, B. J. *Langmuir* **2008**, *24*, 12116−12118.
(31) Poláková, M.; Belánová, M.; Mikušová, K. n.; Lattová, E.; Perreault, H. L. N. *Bioconjugate Chem.* **2011**, *22*, 289−298.
(32) (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004−2021. (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596−2599. (c) Johnson, J. A.; Finn, M. G.; Koberstein, J. T.; Turro, N. J. *Macromol. Rapid Commun.* **2008**, *29*, 1052−1072. (d) Iha, R. K.; Wooley, K. L.; Nyström, A. M.; Burke, D. J.; Kade, M. J.; Hawker, C. J. *Chem. Rev.* **2009**, *109*, 5620−5686. (e) Fernandez-Megia, E.; Correa, J.; Riguera, R. *Biomacromolecules* **2006**, *7*, 3104−3111. (f) Fernandez-Megia, E.; Correa, J.; Rodríguez-Meizoso, I.; Riguera, R. *Macromolecules* **2006**,

39, 2113−2120. (g) Binder, W. H.; Sachsenhofer, R. *Macromol. Rapid Commun.* **2008**, *29*, 952−981.
(33) Joannic, R.; Auvray, L.; Lasic, D. D. *Phys. Rev. Lett.* **1997**, *78*, 3402−3405.
(34) Hope, M. J.; Bally, M. B.; Webb, G.; Cullis, P. R. *Biochim. Biophys. Acta* **1985**, *812*, 55−65.
(35) (a) Nagayasu, A.; Uchiyama, K.; Kiwada, H. *Adv. Drug Delivery Rev.* **1999**, *40*, 75−87. (b) Davis, M. E.; Chen, Z.; Shin, D. M. *Nat. Rev. Drug Discovery* **2008**, *7*, 771−782.
(36) (a) Kamat, N. P.; Robbins, G. P.; Rawson, J.; Therien, M. J.; Dmochowski, I. J.; Hammer, D. A. *Adv. Funct. Mater.* **2010**, *20*, 2588−2596. (b) Cheng, Z.; Elias, D. R.; Kamat, N. P.; Johnston, E. D.; Poloukhtine, A.; Popik, V.; Hammer, D. A.; Tsourkas, A. *Bioconjugate Chem.* **2011**, *22*, 2021−2029.
(37) Davis, K. P.; Lodge, T. P.; Bates, F. S. *Macromolecules* **2008**, *41*, 8289−8291.
(38) (a) Anderson, D. M.; Wennerstroem, H. *J. Phys. Chem.* **1990**, *94*, 8683. (b) Scriven, L. E. *Nature* **1976**, *263*, 123.
(39) (a) Sagalowicz, L.; Michel, M.; Adrian, M.; Frossard, P.; Rouvet, M.; Watzke, H. J.; Yaghmur, A.; de Campo, L.; Glatter, O.; Leser, M. E. *J. Microsc.* **2006**, *221*, 110. (b) de Campo, L.; Yaghmur, A.; Sagalowicz, L.; Leser, M. E.; Watzke, H.; Glatter, O. *Langmuir* **2004**, *20*, 5254.
(40) (a) Shiao, T. C.; Roy, R. *New J. Chem.* **2012**, *36*, 324−339. (b) Durka, M.; Buffet, K.; Iehl, J.; Holler, M.; Nierengarten, J.-F.; Taganna, J.; Bouckaert, J.; Vincent, S. P. *Chem. Commun.* **2011**, *47*, 1321−1323. (c) Durka, M.; Buffet, K.; Iehl, J.; Holler, M.; Nierengarten, J.-F.; Vincent, S. P. *Chem.—Eur. J.* **2012**, *18*, 641−651. (d) Cecioni, S.; Oerthel, V.; Iehl, J.; Holler, M.; Goyard, D.; Praly, J.-P.; Imberty, A.; Nierengarten, J.-F.; Vidal, S. *Chem.—Eur. J.* **2011**, *17*, 3252−3261. (e) Nierengarten, J.-F.; Iehl, J.; Oerthel, V.; Holler, M.; Illescas, B. M.; Munoz, A.; Martin, N.; Rojo, J.; Sanchez-Navarro, M.; Cecioni, S.; Vidal, S.; Buffet, K.; Durka, M.; Vincent, S. P. *Chem. Commun.* **2010**, *46*, 3860−3862. (f) Compain, P.; Decroocq, C.; Iehl, J.; Holler, M.; Hazelard, D.; Mena Barragan, T.; Ortiz Mellet, C.; Nierengarten, J.-F. *Angew. Chem., Int. Ed.* **2010**, *49*, 5753−5756. (g) Voskuhl, J.; Stuart, M. C. A.; Ravoo, B. J. *Chem.—Eur. J.* **2010**, *16*, 2790−2796. (h) Vico, R. V.; Voskuhl, J.; Ravoo, B. J. *Langmuir* **2011**, *27*, 1391−1397. (i) Kauscher, U.; Ravoo, B. J. *Beilstein J. Org. Chem.* **2012**, *8*, 1543−1551. (j) Kingery-Wood, J. E.; Williams, K. W.; Sigal, G. B.; Whitesides, G. M. *J. Am. Chem. Soc.* **1992**, *114*, 7303−7305. (k) Bernardes, G. J. L.; Kikkeri, R.; Maglinao, M.; Laurino, P.; Collot, M.; Hong, S. Y.; Lepenies, B.; Seeberger, P. H. *Org. Biomol. Chem.* **2010**, *8*, 4987−4996.
(41) (a) Imberty, A.; Wimmerova, M.; Mitchell, E. P.; Gilboa-Garber, N. *Microbes Infect.* **2004**, *6*, 221−228. (b) Goldstein, I. J.; Poretz, R. D. In *The Lectins Properties, Functions and Applications in Biology and Medicine*; Goldstein, I. J.; Liener, I. E.; Sharon, N., Eds.; Academic, San Diego, 1986, pp 233−247. (c) Lee, R. T.; Gabius, H.-J.; Lee, Y. C. *J. Biol. Chem.* **1992**, *267*, 23722−23727. (d) Galanina, O. E.; Kaltner, H.; Khraltsova, L. S.; Bovin, N. V.; Gabius, H.-J. *J. Mol. Recogn.* **1997**, *10*, 139−147. (e) Jiménez, M.; André, S.; Barillari, C.; Romero, A.; Rognan, D.; Gabius, H.-J.; Solís, D. *FEBS Lett.* **2008**, *582*, 2309−2312. (f) Giguère, D.; André, S.; Bonin, M. A.; Bellefleur, M. A.; Provencal, A.; Cloutier, P.; Pucci, B.; Roy, R.; Gabius, H. J. *Bioorg. Med. Chem.* **2011**, *19*, 3280−3287. (g) Wu, A. M.; Wu, J. H.; Liu, J.-H.; Singh, T.; André, S.; Kaltner, H.; Gabius, H.-J. *Biochimie* **2004**, *86*, 317−236. (h) Krzeminski, M.; Singh, T.; André, S.; Lensch, M.; Wu, A. M.; Bonvin, A. M. J. J.; Gabius, H.-J. *Biochim. Biophys. Acta* **2011**, *1810*, 150−161. (i) Vokhmyanina, O. A.; Rapoport, E. M.; André, S.; Severov, V. V.; Ryzhov, I.; Pazynina, G. V.; Korchagina, E.; Gabius, H.-J.; Bovin, N. V. *Glycobiology* **2012**, *22*, 1207−1217.
(42) (a) André, S.; Liu, B.; Gabius, H.-J.; Roy, R. *Org. Biomol. Chem.* **2003**, *1*, 3909−3916. (b) Ahmad, N.; Gabius, H.-J.; André, S.; Kaltner, H.; Sabesan, S.; Roy, R.; Liu, B.; Macaluso, F.; Brewer, C. F. *J. Biol. Chem.* **2004**, *279*, 10841−10847. (c) Kopitz, J.; Bergmann, M.; Gabius, H.-J. *IUBMB Life* **2010**, *62*, 624−628. (d) Kopitz, J.; Ballikaya, S.; Andre, S.; Gabius, H. J. *Neurochem. Res.* **2012**, *37*, 1267−1276. (e) Stancic, M.; Slijepcevic, D.; Nomden, A.; Vos, M. J.; de Jonge, J. C.; Sikkema, A. H.; Gabius, H. J.; Hoekstra, D.; Baron, W. *Glia* **2012**, *60*, 919−935.
(43) Kopitz, J.; von Reitzenstein, C.; André, S.; Kaltner, H.; Uhl, J.; Ehemann, V.; Cantz, M.; Gabius, H.-J. *J. Biol. Chem.* **2001**, *276*, 35917−3563.
(44) (a) Love, K. R.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3583−3586. (b) Ko, K.-S.; Jaipuri, F. A.; Pohl, N. L. *J. Am. Chem. Soc.* **2005**, *127*, 13162−13163. (c) de Paz, J. L.; Noti, C.; Seeberger, P. H. *J. Am. Chem. Soc.* **2006**, *128*, 2766−2767. (d) Tully, S. E.; Rawat, M.; Hsieh-Wilson, L. C. *J. Am. Chem. Soc.* **2006**, *128*, 7740−7741.
(45) (a) Percec, V.; Cho, W. D.; Ungar, G.; Yeardley, D. J. P. *J. Am. Chem. Soc.* **2001**, *123*, 1302−1315. (b) Percec, V.; Mitchell, C. M.; Cho, W. D.; Uchida, S.; Glodde, M.; Ungar, G.; Zeng, X. B.; Liu, Y. S.; Balagurusamy, V. S. K.; Heiney, P. A. *J. Am. Chem. Soc.* **2004**, *126*, 6078−6094. (c) Percec, V.; Peterca, M.; Sienkowska, M. J.; Ilies, M. A.; Aqad, E.; Smidrkal, J.; Heiney, P. A. *J. Am. Chem. Soc.* **2006**, *128*, 3324−3334. (d) Percec, V.; Won, B. C.; Peterca, M.; Heiney, P. A. *J. Am. Chem. Soc.* **2007**, *129*, 11265−11278. (e) Rosen, B. M.; Wilson, D. A.; Wilson, C. J.; Peterca, M.; Won, B. C.; Huang, C. H.; Lipski, L. R.; Zeng, X. B.; Ungar, G.; Heiney, P. A.; Percec, V. *J. Am. Chem. Soc.* **2009**, *131*, 17500−17521. (f) Rosen, B. M.; Wilson, C. J.; Wilson, D. A.; Peterca, M.; Imam, M. R.; Percec, V. *Chem. Rev.* **2009**, *109*, 6275−6540. (g) Menjoge, A. R.; Kannan, R. M.; Tomalia, D. A. *Drug Discovery Today* **2010**, *15*, 171−185. (h) Tomalia, D. A. *Soft Matter* **2010**, *6*, 456−474. (i) Damasceno, P. F.; Engel, M.; Glotzer, S. C. *Science* **2012**, *337*, 453−457.