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# Hexaphenylbenzene as a Rigid Template for the Straightforward Syntheses of "Star-Shaped" Glycodendrimers

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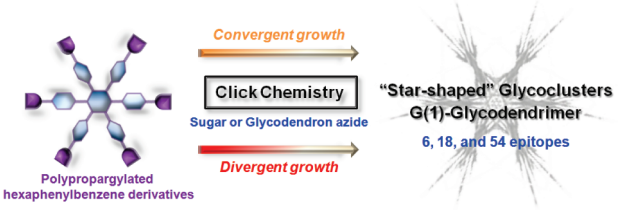


Original glycodendrimers emanating from propargylated hexaphenylbenzene cores and containing up to 54 peripheral sugar ligands have been synthesized by Cu(I)-catalyzed [1,3]-dipolar cycloadditions using both convergent and divergent approaches.

Several important biological events such as cellular adhesion and recognition, regulation of physiological functions, and pathogenic infections are mediated, at the molecular level, by multiple carbohydrate−protein interactions.[1] More specifically, bacterial adhesion processes are commonly achieved through carbohydrate-binding lectins expressed on or shed from bacterial surfaces that can also be involved in host-tissue colonization and biofilm formation. In spite of the weaknesses of these key binding interactions in terms of avidity and selectivity on a per-saccharide basis, these attractive forces are dramaticaly and naturally reinforced by the presence of multiple copies of both the ligands and the receptors that are engaged in a phenomena known as the "glycoside cluster or dendritic effect".[2]

Biological applications of synthetic poly-glycosylated derivatives span from specific screening and targeting of lectins toward antiinfective strategies to their use in photodynamic therapy or as vaccines, adjuvants, immunotherapy, and anti-angiogenic agents.[3] As part of our ongoing research program aimed at the understanding of initial steps involved in infectious phenomenon via saccharide-lectin recognition processes, the elaboration and biological evaluation of multivalent glycomimetic inhibitors are needed.[3−5] Hence, the straightforward preparation of a new family of multivalent glycosylated architectures built around a more rigid hexaphenylbenzene scaffold is proposed. This additional core will help pursuing our systematic investigations regarding the roles played by the subtle but critical modulations of structural parameters responsible for high avidity, with specific and tailored presentation of peripheral recognition moieties.[3] By virtue of their flexible scaffolds and linkers, previous glycodendrimers showed negative cooperativity upon binding to lectins as shown by their increasingly contributing entropic lost.[6] Hence, by designing rigidified scaffolds with less flexible conformational and translational mobilities, we aimed at counterbalancing the negative effects observed by flexible scaffolds such as PAMAM, PPI, and polyesters.[3] Noteworthy is the fact that water-soluble dendrimers emanating from aromatic shape-persistent and rigid scaffolds can provide interesting properties including host−guest molecular recognition,[7] and induce autoassembly mechanisms responsible for the amplification of the inhibition.[8] Furthermore, the inherent rigidity of the distorted but symmetrical inner aromatic system, as observed from X-ray crystals of hexakis-(4-hydroxyphenyl)benzene **1**,[9] can afford unique homogeneous epitopes' spatial orientation. More specifically, although few examples of glycoclusters organized around a radial hexaphenylbenzene core have already been described, synthetic methodologies remain limited to the [2 + 2 + 2] dicobalt octacarbonyl-catalyzed cyclotrimerizations.[10] Those strategies strictly require preliminary preparations of diglycosylated aromatic alkynes. Consequently, the cumbersome syntheses of these precursors provide rather limited access to "molecular asterisks" of higher paucivalency.

In light of these considerations, we report herein the design of original polypropargylated scaffolds obtained via a

(1) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637–674.
(2) (a) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321–327. (b) Roy, R. *Curr. Opin. Struct. Biol.* **1996**, *6*, 692–702. (c) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754–2794. (d) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555–578.
(3) Chabre, Y. M.; Roy, R. *Adv. Carbohydr. Chem. Biochem.* **2010**, *63*, 165–393.
(4) (a) Nagahori, N.; Lee, R. T.; Nishimura, S. I.; Pagé, D.; Roy, R.; Lee, Y. C. *ChemBioChem* **2002**, *3*, 836–844. (b) Roy, R. *Trends Glycosci. Glycotechnol.* **2003**, *15*, 291–310. (c) Touaibia, M.; Roy, R. Carbohydrate-Protein and Carbohydrate-Carbohydrate Interactions. In *Comprehensive Glycoscience*; Kamerling, J.-P., Ed.; Elsevier: Boston, MA, 2007; Vol. 3, pp 821−870.
(5) For reviews on glycoclusters and glycodendrimers: (a) Chabre, Y. M.; Roy, R. *Curr. Top. Med. Chem.* **2008**, *8*, 1237–1285. (b) Imberty, A.; Chabre, Y. M.; Roy, R. *Chem.—Eur. J.* **2008**, *14*, 7490–7499. (c) Li, Y.; Cheng, Y.; Xu, T. *Curr. Drug Discovery Technol.* **2007**, *4*, 246–254.
(6) Dam, T. K.; Roy, R.; Pagé, D.; Brewer, C. F. *Biochemistry* **2002**, *41*, 1359–1363.
(7) Sakamoto, J.; Müllen, K. *Org. Lett.* **2004**, *6*, 4277–4280.
(8) Sleiman, M.; Varrot, A.; Raimundo, J.-M.; Gingras, M.; Goekjian, P. G. *Chem. Commun.* **2008**, 6507–6509.
(9) Kobayashi, K.; Shirasaka, T.; Sato, A.; Horn, E.; Furukawa, N. *Angew. Chem., Int. Ed.* **1999**, *38*, 3483–3486.
(10) (a) Roy, R.; Corazon Trono, M.; Giguère, D. *ACS Symp. Ser.* **2005**, *896*, 137–150. (b) Dominique, R.; Liu, B.; Das, S. K.; Roy, R. *Synthesis* **2000**, *6*, 862–868. (c) Kaufman, R. J.; Sidhu, R. S. *J. Org. Chem.* **1982**, *47*, 4941–4947.


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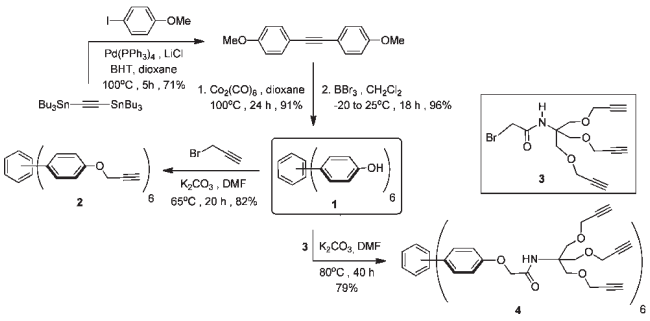
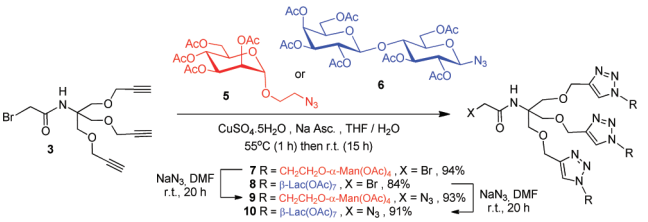
**SCHEME 1. Synthesis of Polypropargylated Hexaphenylbenzene Derivatives 2 and 4**




postfunctionalization strategy. The corresponding multivalent glycoarchitectures rose from both divergent and convergent accelerated dendritic growth using click chemistry. In practice, the synthetic sequence was initiated by a double Stille coupling involving bis(tributylstannyl)acetylene and *p*-iodoanisole in the presence of lithium chloride, tetrakis-(triphenylphosphine)palladium (0), and 2,6-di-*tert*-butyl-4-methylphenol in dioxane at reflux, to give the corresponding symmetrical diarylalkyne in 71% yield (Scheme 1).[11]

$[Co_2(CO)_8]$-catalyzed cyclotrimerization of the resulting bis(*p*-methoxyphenyl)acetylene and subsequent methyl ether deprotection with $BBr_3$ provided efficient access to key hexahydroxylated precursor **1** in 87% yield over two steps.[9] Direct propargylation with propargyl bromide and $K_2CO_3$ in DMF at 65 °C afforded the novel hexafunctionalized analogue **2** in a satisfactory 82% yield. Multiplication of peripheral propargyl functions was further addressed with the use of orthogonal $AB_3$ synthon 2-bromoacetamido-tris[(propargyloxy)methyl]-aminomethane **3**, obtained according to a four-steps sequence from TRIS (tris(hydroxymethyl)aminomethane) as previously described.[12] The application of similar experimental conditions led to the unprecedented octadecapropargylated scaffold **4** without significant diminution of substitutions' efficiency (Scheme 1). Completion of the reaction was supported by analysis of NMR spectra, revealing a unique signal pattern for extended inner aromatic core consisting of a characteristic pair of doublets at $\delta$ = 6.43 and 6.67 ppm in $^1$H NMR and five distinct signals at $\delta$ = 113.1, 132.2, 134.2, 140.0, and 154.4 ppm in $^{13}$C NMR. Furthermore, expected correspondence between observed integrations for aromatic and acetylenic signals (24 protons vs 18 protons), together with HRMS analysis confirmed the desired structure. Having key-scaffolds **2** (6-mer) and **4** (18-mer) in hands, we next turned our attention toward the preparation of higher multivalent architectures containing from 6 to 54 surface carbohydrate ligands. The introduction of the modified mannopyranoside or lactopyranoside termini was efficiently achieved using Cu(I)-catalyzed azide−alkyne [1,3]-dipolar cycloaddition reaction (CuAAC).[13] To this end, known saccharide derivatives,

**SCHEME 2. Structures of Glycosyl Azides 5 and 6 and Synthesis of Dendronized Derivatives 9 and 10**




2-azidoethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside **5**[14] and lactosyl azide **6**[15] containing the necessary complementary function, were prepared (Scheme 2).

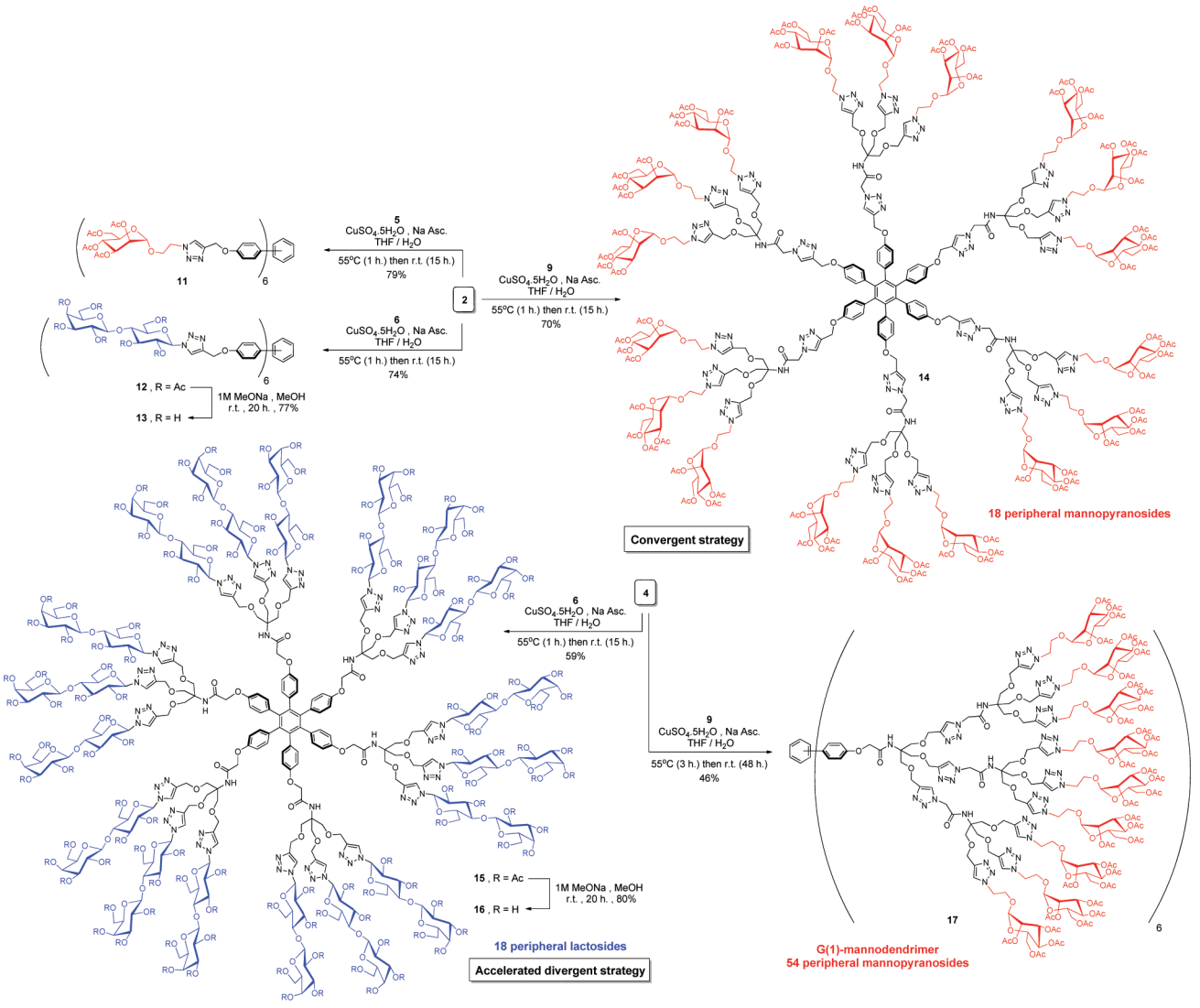
To access higher multivalent functionalities, dendronizations of sugar azides were achieved from tripropargylated synthon **3** on which were "clicked" sugar azides **5** or **6** to afford brominated analogues **7** and **8** in 94 and 84% yield, respectively. For the necessary convergent strategy, subsequent transformation of bromides **7** and **8** into azide derivatives was conveniently carried out in the presence of $NaN_3$ in DMF, yielding desired mannosylated and lactosylated dendrons **9** and **10**, respectively, in more than 91% yields, both exhibiting an active focal azido function (Scheme 2).

Coupling of monovalent glycosyl azides **5** and **6** on *O*-propargylated hexaphenylbenzene core **2** with standard CuAAc conditions in the presence of substoichiometric amount of $CuSO_4$ and sodium ascorbate in a homogeneous THF/water mixture afforded fully substituted star-shaped hexavalent derivatives **11** and **12** in 79 and 74% yield, respectively (Scheme 3). De-O-acetylation of lactosylated analogue **12** under usual Zemplén conditions (NaOMe, MeOH) furnished fully water-soluble "glycoasterisk" **13** in a satisfactory yield of 77%. Furthermore, a convergent approach involving treatment of the analogous mannosylated dendron **9** under similar experimental conditions efficiently furnished the extended octadecavalent architecture **14** in a 70% yield.

An accelerated divergent strategy was subsequently applied from "hypercore"[16] **4** and acetylated lactosyl azide **6**. Using standard CuAAC conditions resulted in the octadecalactosylated cluster **15** (59%) containing 18 triazole groups radially distributed in one layer and a different inner space compared to the mannosylated analogue **14** described above. This last structural parameter might be important in respect to the study of the dual rigidity/compaction's influence responsible for specific topographical epitope orientation. Interestingly, although clicked moieties consisted in different saccharides and valencies, comparable global yields were obtained for both octadecavalent structures, regardless of the multiple one pot reactions' number (70% yield for **14**, 6 coupling reactions vs 58% yield for **15**, 18 coupling reactions). In fact, both strategies indifferently afforded excellent partial yields superior or equal to 95%. Transesterification was then carried out under basic conditions (1 M MeONa in

(11) Cummins, C. H. *Tetrahedron Lett*. **1994**, *35*, 857–860.
(12) Chabre, Y. M.; Contino-Pépin, C.; Placide, V.; Shiao, T. C.; Roy, R. *J. Org. Chem*. **2008**, *73*, 5602–5605.
(13) (a) Wu, P.; Malkoch, M.; Hunt, J. N.; Vestberg, R.; Kaltgrad, E.; Finn, M. G.; Folkin, V. V.; Sharpless, K. B.; Hawker, C. J. *Chem. Commun*. **2005**, 5775–5777. (b) Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc*. **2005**, *127*, 210–216. (c) Dedola, S.; Nepogodiev, S. A.; Field, R. A. *Org. Biomol. Chem*. **2007**, *5*, 1006–1017. (d) Meldal, M.; Tornoe, C. W. *Chem. Rev*. **2008**, *108*, 2952–3015. (e) Aragão-Leoneti, V.; Campo, V. L.; Gomes, A. S.; Field, R. A.; Carvalho, I. *Tetrahedron* **2010**, *66*, 9475–9492.
(14) (a) Chernyak, A. Y.; Sharma, G. V.; Kononov, L. O.; Krishna, P. R.; Levinsky, A. B.; Kochetchov, N. K.; Rao, A. V. R. *Carbohydr. Res*. **1992**, *223*, 303–309. (b) Arce, E.; Nieto, P. M.; Diaz, V.; Garcia Castro, R.; Bernad, A.; Rojo, J. *Bioconjugate Chem*. **2003**, *14*, 817–823.
(15) Tropper, F. D.; Andersson, F. O.; Braun, S.; Roy, R. *Synthesis* **1992**, 618–620.
(16) Wooley, K. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc*. **1991**, *113*, 4252–4261.

**SCHEME 3. Elaboration of Hexavalent (11, 12, and 13) and Octadecavalent (14, 15, and 16) Glycosylated Architectures Together with Glycodendrimer 17 Containing 54 Mannopyranoside Termini**




MeOH) to afford fully hydroxylated lactocluster **16** in a satisfactory yield (80%). Application of the corresponding accelerated methodology involving coupling of dendritic core **4** and mannosylated dendron **9** furnished G(1)-mannodendrimer **17** (48%) exhibiting 54 protected mannoside termini and containing two distinct TRIS layers in internal and middle sections of the dendritic platform. As observed previously, classical click chemistry conditions, using only an additional short initial heating period, gave access to hyperfunctionalized and packed structure in 48% yield, corresponding to an excellent 96% yield per individual coupling reactions and corroborating the reliability of the accelerated dendritic growths developed in this study.

In all cases, analysis of the $^{1}$H and $^{13}$C NMR spectra of the multivalent glycosylated derivatives revealed expected integrations for the triazole protons respective to the internal aromatic and outer anomeric protons, complete disappearance of the acetylenic signals ($\delta$ 3.47 ppm for **2**, and $\delta$ 2.40 ppm for **4**, in $^{1}$H NMR), thus, confirming together with mass spectrometry and infrared spectroscopy unequivocal completion of the multiple one-pot click reactions (Table 1).

**TABLE 1. High- and Low-Resolution ESI$^{+}$-MS Experiments for Multivalent Glycosylated Compounds 11−17**

| entry | compound (valency) | calculated mass ($m/z$) | experimental mass |
|---|---|---|---|
| 1 | **11** (6) | 1681.57138 ($[M + 2H]^{2+}$) | 1681.56904[a] |
| 2 | **12** (6) | 1609.49995 ($[M + 3H]^{3+}$) | 1609.49710[a] |
| 3 | **13** (6) | 1531.52443 ($[M + 2H]^{2+}$) | 1531.52785[a] |
| 4 | **14** (18) | 2057.32 ($[M + 5H]^{5+}$) | 2057.27[b] |
| 5 | **15** (18) | 3548.68 ($[M + 4H]^{4+}$) | 3548.74[b] |
| 6 | **16** (18) | 1778.82873 ($[M + 5H]^{5+}$) | 1778.82137[a] |
| 7 | **17** (54) | 30551.26 ($[M + H]^{+}$) | 30550.70[b] |

[a]High-resolution mass spectrometry experiment with theoretical mass calculated from exact mass. [b]Low-resolution mass spectrometry experiment with theoretical mass calculated from molecular weight.

In conclusion, we have developed an expeditive and systematic route toward the synthesis of original glycoclusters and G(1)-glycodendrimers built from circular and aromatic polypropargylated scaffolds. All the multivalent glycoconjugates were obtained with simple and generally high yielding reactions including nucleophilic substitutions to generate synthons and CuAAC reactions to covalently

attached saccharidic moieties. To this end, two straightforward synthetic pathways have been addressed and generated high valency derivatives containing up to 54 peripheral epitopes in satisfactory yields. Interestingly, the hydrophobic character of the inner aromatic core has also been counterbalanced by the hydroxylated sugar derivatives, affording fully water-soluble derivatives. This critical property combined with the specific spatial presentation of the termini radiating from these "star-shaped" structures have actually encouraged the evaluation of their binding properties on relevant lectins. Thus, promising preliminary data for lactosylated dendrimers **13** and **16** already highlighted their strong inhibitory potencies against galectin-3 binding to mucins with a marked multivalent effect and low micromolar values for octadecavalent derivative **16**. In addition, results compared well with previously studied lactosylated PAMAM dendrimers[17] and detailed experimental procedures will be reported in due course.

## Experimental Section

**General Procedure for Functionalization of 1.** To a solution of **1** (1.0 equiv, $C$ = [0.04 M]) and $K_2CO_3$ (7.2 equiv) in dry DMF was added corresponding halide (9.0 equiv) over a 10 min period and under a nitrogen atmosphere. The mixture was stirred and warmed at 65−80 °C for additional 20−40 h. The resulting yellowish residue was diluted with ethyl acetate (10 mL) and washed with two 20 mL portions of water, 10 mL of brine, and dried over $Na_2SO_4$. Filtration and evaporation of the solvent afforded crude products which were subsequently purified either by precipitation (for **2**) or by silica gel column chromatography (for **4**).

**Data for Hexapropargylated Derivative 2.** Obtained from precipitation, with a minimum amount of ethyl acetate and then cold hexanes, as an off-white solid (89.0 mg, 0.141 mmol) with a 82% yield. Mp > 250 °C (decomposition); $^1$H NMR (600 MHz, DMSO-$d_6$, $\delta$ ppm): 6.75 (d, $J$ = 8.7 Hz, C$H_{ar}$, 12H), 6.47 (d, $J$ = 8.7 Hz, C$H_{ar}$, 12H), 4.54 (d, $J$ = 1.9 Hz, OC$H_2$C≡CH, 12H), 3.44 (t, $J$ = 1.9 Hz, OCH$_2$C≡C$H$, 6H); $^{13}$C NMR (150 MHz, DMSO-$d_6$, $\delta$ ppm): 154.5 ($C_{q\text{-}ar}$O), 139.9 ($C_{ar}$ central), 133.5 ($C_q$=CH), 131.8 ($C_{ar\text{-}central}C_q$=$C$H), 112.9 ($OC_{q\text{-}ar}$=$C$H), 79.2 (OCH$_2C$≡CH), 77.9 (OCH$_2$C≡$C$H), 55.2 (O$C$H$_2$C≡CH); $m/z$ (ESI$^+$ HRMS) for $C_{60}H_{42}O_6$ = 859.30542 [M + H]$^+$, found 859.30267; 881.28736 [M + Na]$^+$, found 881.28463.

**Data for Octadecapropargylated Derivative 4.** Obtained from purification by silica gel chromatography (eluent: $CH_2Cl_2$/MeOH 98:2) (57.0 mg, 0.0250 mmol) in a 79% yield, as a yellowish oil. $R_f$ = 0.41 $CH_2Cl_2$/MeOH (95:5); $^1$H NMR (600 MHz, $CDCl_3$, $\delta$ ppm): 6.81 (br s, 6H, N$H$), 6.67 (d, $J$ = 8.7 Hz, C$H_{ar}$, 12H), 6.44 (d, $J$ = 8.7 Hz, C$H_{ar}$, 12H), 4.19−4.09 (m, OC$H_2$C=O, $C_q$C$H_2$O, 48H), 3.81 (br s, OC$H_2$C≡CH, 36H), 2.40 (br s, OCH$_2$C≡C$H$, 18H); $^{13}$C NMR (150 MHz, $CDCl_3$, $\delta$ ppm): 167.7 ($C$ONH), 154.4 ($C_{q\text{-}ar}$O), 140.0 ($C_{ar\text{-}central}$), 134.2 ($C_q$=CH), 132.2 ($C_{ar\text{-}central}C_q$=$C$H), 113.1 ($OC_{q\text{-}ar}$=$C$H), 79.4 (OCH$_2C$≡CH), 74.8 (OCH$_2$C≡$C$H), 68.2 ($C_qCH_2$O), 66.8 ($C_q$), 59.0 (O$C$H$_2$C=O), 58.5 (O$C$H$_2$C≡CH); $m/z$ (ESI$^+$ HRMS) for $C_{132}H_{132}N_6O_{30}$ = 761.30687 [M + 3H]$^{3+}$, found 761.30919; 1141.45667 [M + 2H]$^{2+}$, found 1141.45772; 1163.43861 [M + 2Na]$^{2+}$, found 1163.43813.

**General Procedure for Zemplén Reaction.** Acetylated cluster or dendrimer was dissolved in dry MeOH and a solution of sodium methoxide (1 M in MeOH, 5 $\mu$L per 30 min period) was added until slow precipitation of the product. Additional 50 $\mu$L of the sodium methoxide solution was injected and the mixture was stirred overnight. Ten milliliters of MeOH was then added and the mixture was transferred into a centrifuge tube (15 mL). After a first centrifugation, solvent was removed and the remaining white solid was subsequently resuspended with 15 mL of MeOH ancentrifugeded (4 times). Water was finally added for entire solubilization and the solution was neutralized by addition of ion-exchange resin (Amberlite IR 120 H$^+$) until pH 6−7. After filtration, water was removed *in vacuo* with rotary evaporator. The residue was then lyophilized to yield the fully deprotected and hydrosoluble glycocluster or glycodendrimer.

**Data for Derivative 16.** $^1$H NMR (600 MHz, $D_2O$, $\delta$ ppm): 7.96 (s, $H_{triazole}$, 18H), 6.48 (br s, C$H_{ar}$, 12H), 6.15 (br s, C$H_{ar}$, 12H), 5.56 ($d_{app}$, $H_{1gal}$, 18H), 4.37−4.30 (m, $H_{1glu}$, OC$H_2C_{triazole}$, 54H), 4.08 (br s, OC$H_2$CO, 12H), 3.89 ($t_{app}$, $H_{2glu}$, 18H), 3.83−3.41 (m, $C_qCH_2O$, $H_{2gal}$, $H_{3gal}$, $H_{3glu}$, $H_{4gal}$, $H_{4glu}$, $H_{5gal}$, $H_{5glu}$, $H_{6gal}$, $H_{6glu}$, 234H); $^{13}$C NMR (150 MHz, $D_2O$, $\delta$ ppm): 169.8 ($C$ONH), 154.8 ($C_{q\text{-}ar}$O), 143.9 ($C_{triazole}$), 139.7 ($C_{ar\text{-}central}$), 133.9 ($C_q$=CH), 131.9 ($C_{ar\text{-}central}C_q$=$C$H), 123.8 ($CH_{triazole}$), 112.4 ($OC_{q\text{-}ar}$=$C$H), 102.5 ($C_{1gal}$), 86.9 ($C_{1glu}$), 77.3 ($C_{4glu}$), 77.0 ($C_{3glu}$), 75.0 ($C_{5glu}$), 74.1 ($C_{2glu}$), 72.1 ($C_{3gal}$), 71.7 ($C_{5gal}$), 70.5 ($C_{2gal}$), 68.2 ($C_{4gal}$), 67.8 ($C_qCH_2O$), 65.8 (O$C$H$_2$CO), 63.2 (O$C$H$_2C_{triazole}$), 60.6 ($C_{6gal}$), 59.3 ($C_{6glu}$) 59.1 ($C_q$); $m/z$ (ESI$^+$ HRMS) for $C_{348}H_{510}N_{60}O_{210}$ = 1778.82873 [M + 5H]$^{5+}$, found 1778.82137.

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**Supporting Information Available:** Experimental details for the synthesis of compounds **1**−**4**, **7**−**17**. List of NMR spectra ($^1$H, $^{13}$C, COSY), FT−IR spectra, and MS experiments for compounds **1**, **2**, **4**, **7**−**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

(17) André, S.; Cejas Ortega, P. J.; Alamino Perez, M.; Roy, R.; Gabius., H.-J. *Glycobiology* **1999**, *9*, 1253–1261.