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Expeditive Synthesis of Glycodendrimer Scaffolds Based on Versatile TRIS and Mannoside Derivatives

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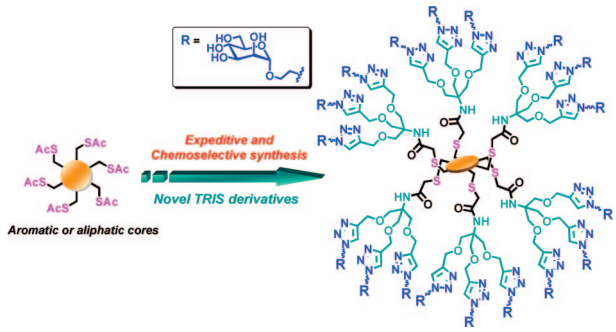

A new family of glycodendrimer scaffolds containing 12 and 18 peripheral α-D-mannopyranosidic units has been synthesized by Cu(I)-catalyzed [1,3]-dipolar cycloadditions using sulfurated dendritic scaffolds bearing alkyne functionalities and novel TRIS derivatives.

Multiple carbohydrate−protein interactions are responsible, at the molecular level, for several critical biological events such as cellular adhesion and recognition, physiological function regulation, and pathogenic infections. In spite of the weakness of these fundamental interactions in terms of affinity and selectivity on a per-saccharide basis, these attractive forces are dramatically 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".[1] In the case of pathogenic infections, a critical step in host-tissue colonization and biofilm formation is achieved through bacterial adhesion commonly mediated by carbohydrate-binding lectins expressed on or shed from bacterial surfaces. One striking example is found in type 1 fimbriae from *Escherichia coli* that are the most common type of adhesive appendages in several enterobacteria and mediate mannose-specific adhesion of uroepithelial cells via the 30 kDa lectin-like subunit FimH.[2] X-ray crystal-structure studies have recently revealed that the lectin domain of uropathogenic *E. coli* FimH possesses a carbohydrate recognition domain (CRD) at its tip, which can accommodate one α-D-mannopyrannoside residue.[3]

For our ongoing research program aimed at the study of mannose protein binding with the synthesis and biological evaluation of multivalent glycomimetic inhibitors against bacterial adhesion,[4,5] original dendritic architectures are needed. Hence, the straightforward preparation of a new family of mannosylated dendrimers is proposed in order to pursue the systematic study concerning the modulation of critical structural parameters toward high specificity. The proposed strategy is based on the synthesis of sulfurated and polypropargylated versatile dendritic cores onto which mannoside and other sugar moieties will be efficiently attached by a Cu(I)-catalyzed azide-alkyne [1,3]-dipolar cycloaddition reaction (CuAAC),[6] in high yields and with complete regioselectivity. The construction of these dendritic cores emanates from the use of thioacetylated derivatives of pentaerythritol and tetra- or hexasubstituted benzene engaged in nucleophilic substitution or 1,4-conjugate addition to afford adequately functionalized novel TRIS (tris(hydroxymethyl)aminomethane) derivatives. Although pentaerythritol has recently been successfully used for the construction of highly branched mannopyranoside structures which presented nanomolar affinities to *E. coli*,[7] tetrakis- and hexakis(bromomethyl)benzene represent less common compounds for the synthesis of glycodendrimers. Since the synthesis of "arborols" proposed by Newkome et al. in 1985,[8] the widespread use of TRIS and its AB_3-monomer derivatives afforded highly functionalized structures, taking advantage of its geometry for a rapid dendritic growth. This scaffold has been used for the accelerated synthesis of dense carbohydrate-containing dendrimers[9] and more recently for the efficient syntheses of glycodendrons presented as potential carbohydrate vaccine candidates and antiviral agents.[10] Consequently, the present work deals with original multivalent dendritic architectures that

(1) (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*, 2755–2794.

(2) Hung, C.-S.; Bouckaert, J.; Hung, D.; Pinkner, J.; Widberg, C.; DeFusco, A.; Auguste, C. G.; Strouse, R.; Langerman, S.; Waksman, G.; Hultgren, S. J. *Mol. Microbiol.* **2002**, *44*, 903–915.

(3) Bouckaert, J.; Berglund, J.; Schembri, M.; De Genst, E.; Cools, L.; Wuhrer, M.; Hung, C. S.; Pinkner, J.; Slattergard, R.; Zavialov, A.; Choudhary, D.; Langerman, S.; Hultgren, S. J.; Wyns, L.; Klemm, P.; Oscarson, S.; Knight, S. D.; De Greve, H. *Mol. Microbiol.* **2005**, *55*, 441–455.

(4) Nagahori, N.; Lee, R. T.; Nishimura, S. I.; Pagé, D.; Roy, R.; Lee, Y. C. *ChemBioChem* **2002**, *3*, 836–844. Roy, R. *Trends Glycosci. Glycotechnol.* **2003**, *15*, 291–310.

(5) For specific applications of mannosylated dendrimers: (a) Touaibia, M.; Roy, R. *Mini-Rev. Med. Chem.* **2007**, *7*, 1270–1283. (b) Touaibia, M.; Roy, R. Carbohydrate-Protein and Carbohydrate-Carbohydrate Interactions. In *Comprehensive Glycoscience*; Kamerling, J. P. Ed.; Elsevier: Amsterdam, 2007; Vol. 3, pp 821−870.

(6) (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) Antoni, P.; Nyström, D.; Hawker, C. J.; Hult, A.; Malkoch, M. *Chem. Commun.* **2007**, 2249–2251. (d) Dedola, S.; Nepogodiev, S. A.; Field, R. A. *Org. Biomol. Chem.* **2007**, *5*, 1006–1017.

(7) (a) Touaibia, M.; Shiao, T. C.; Papadopoulos, A.; Vaucher, J.; Wang, Q.; Benhamioud, K.; Roy, R. *Chem. Commun.* **2007**, 380–382. (b) Touaibia, M.; Wellens, A.; Shiao, T. C.; Wang, Q.; Sirois, S.; Bouckaert, J.; Roy, R. *ChemMedChem* **2007**, *2*, 1190–1201.

(8) Newkome, G. R.; Yao, Z.-Q.; Baker, G. R.; Gupta, V. K. *J. Org. Chem.* **1985**, *50*, 2003–2004.

(9) Ashton, P. R.; Boyd, S. E.; Brown, C. L.; Jayaraman, N.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **1997**, *36*, 732–735.

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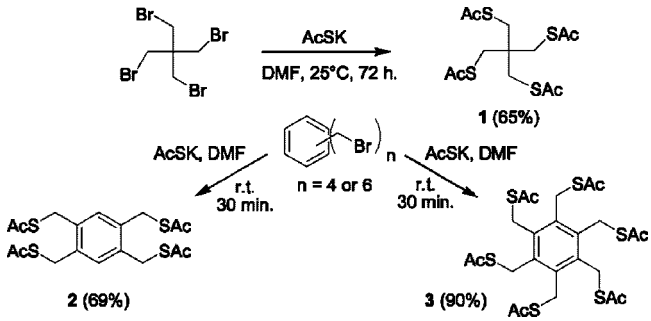
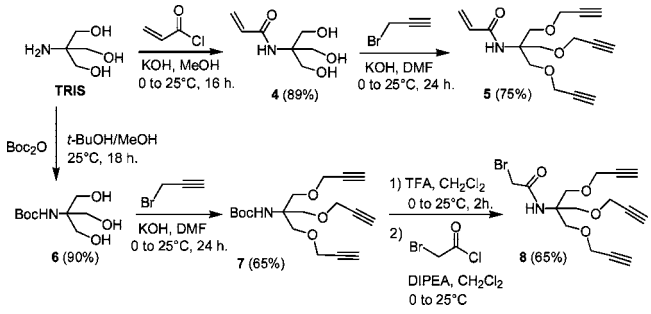
SCHEME 1. Synthesis of Thioacetylated Cores



SCHEME 2. Synthesis of Propargylated TRIS Derivatives

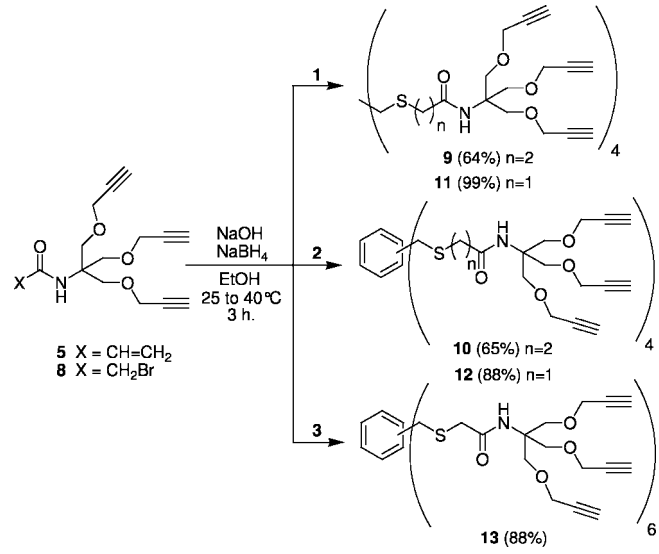


represent ideal candidates for our ongoing systematic study to design efficient but specific inhibitors of viral or bacterial infections.

The synthesis of thioacetylated aliphatic or aromatic cores, from commercially available pentaerythritol tetrabromide and 1,2,4,5-tetrakis- or hexakis(bromomethyl)benzene, respectively, constitutes the initial step of the dendritic growth. The use of potassium thioacetate in DMF at room temperature allowed the desired bromide displacement to afford the known pentaerythritol tetrathioacetate[11] (**1**) and the original aromatic derivatives **2** and **3** in an efficient way and with reduced reaction times (Scheme 1).

Coupling reactions between these sulfurated cores and TRIS moieties containing complementary functions acting as electrophiles have been investigated. As shown in Scheme 2, two closely related strategies have been employed to introduce suitable functions on TRIS, leading to the desired new tripropargylated compounds **5** and **8** in a concise and efficient way. *N*-Acryloyltris[(propargyloxy)methyl]aminomethane (**5**) has been synthesized in a two-step orthogonal sequence (thus avoiding protection/deprotection reactions), from the initial selective acryloylation of TRIS in methanolic potassium hydroxide media (**4**, 89%), according to the procedure described by Pucci et al.,[12] followed by propargylation under basic conditions (KOH, DMF) in 75% yield. The synthetic way to obtain 2-bromoacetamidotris[(propargyloxy)methyl]aminomethane (**8**) was based on the almost quantitative synthesis of the *N*-Boc TRIS derivative **6**.[13] Propargylation under the above conditions afforded *N*-(*tert*-butyloxycarbonyl)tris[(propargyloxy)methyl]aminomethane (**7**)

SCHEME 3. Synthesis of Polypropargylated Dendritic Cores



in 65% yield. The quantitative removal of the Boc protecting group with TFA at room temperature and the use of the corresponding salt without further purification in a coupling reaction with bromoacetyl chloride in the presence of DIPEA afforded the desired compound **8** (65%).

As mentioned before, the critical step of our dendritic growth resides in the coupling reaction between thioacetylated cores and TRIS derivatives **5** and **8**. Conditions were optimized to afford satisfactory thioalkylation and were established for a 1,4-conjugate addition reaction between pentaerythritol tetraacetate (**1**) and the acryloylated derivative **5** (see Table 1 in the Supporting Information for optimization conditions).

The first attempt, concerning the use of basic conditions classically encountered for deacetylation reactions (NEt_3, MeOH) for in situ formation of thiolate intermediates, failed. We next turned our attention to reductive conditions ($NaBH_4$), following Braga's procedure, described as an efficient sulfur addition reaction from thiocetate compounds on alkyl halides.[14] In this case, the desired dodecapropargylated compound **9** was obtained in rather low yield (29%), in spite of solvent variation. Finally, our efforts were successfully directed toward the use of combined basic and reductive conditions proposed by Fall et al.[15] (NaOH, $NaBH_4$ in EtOH) for efficient in situ alkylation in a reduced reaction time (64%). Surprisingly, yields were not improved with prolonged reaction time and no desired compound was observed with the use of THF as solvent. The optimized conditions described above were used for the efficient synthesis of the aromatic dodecapropargylated compound **10** (65%) (Scheme 3). Unfortunately, attempts to obtain an octadecapropargylated derivative from the hexathioacetate core **3** failed.

Interestingly, we found that, under the same experimental conditions, in situ reduction of cores **1** and **2** and even **3** followed by an S_N2 reaction with bromide derivative **8** afforded the corresponding dodeca- and octadecapropargylated dendritic scaffolds **11**−**13**, respectively, in excellent yields (Scheme 3). The almost quantitative yields obtained for the S_N2 reaction in

(10) Wang, S.-K.; Liang, P.-H.; Astronomo, R. D.; Hsu, T.-L.; Hsieh, S.-L.; Burton, D. R.; Wong, C.-H. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 3690–3695.

(11) Wan, Y.; Mitkin, O.; Barnhurst, L.; Kurchan, A.; Kutateladze, A. *Org. Lett.* **2000**, *2*, 3817–3819.

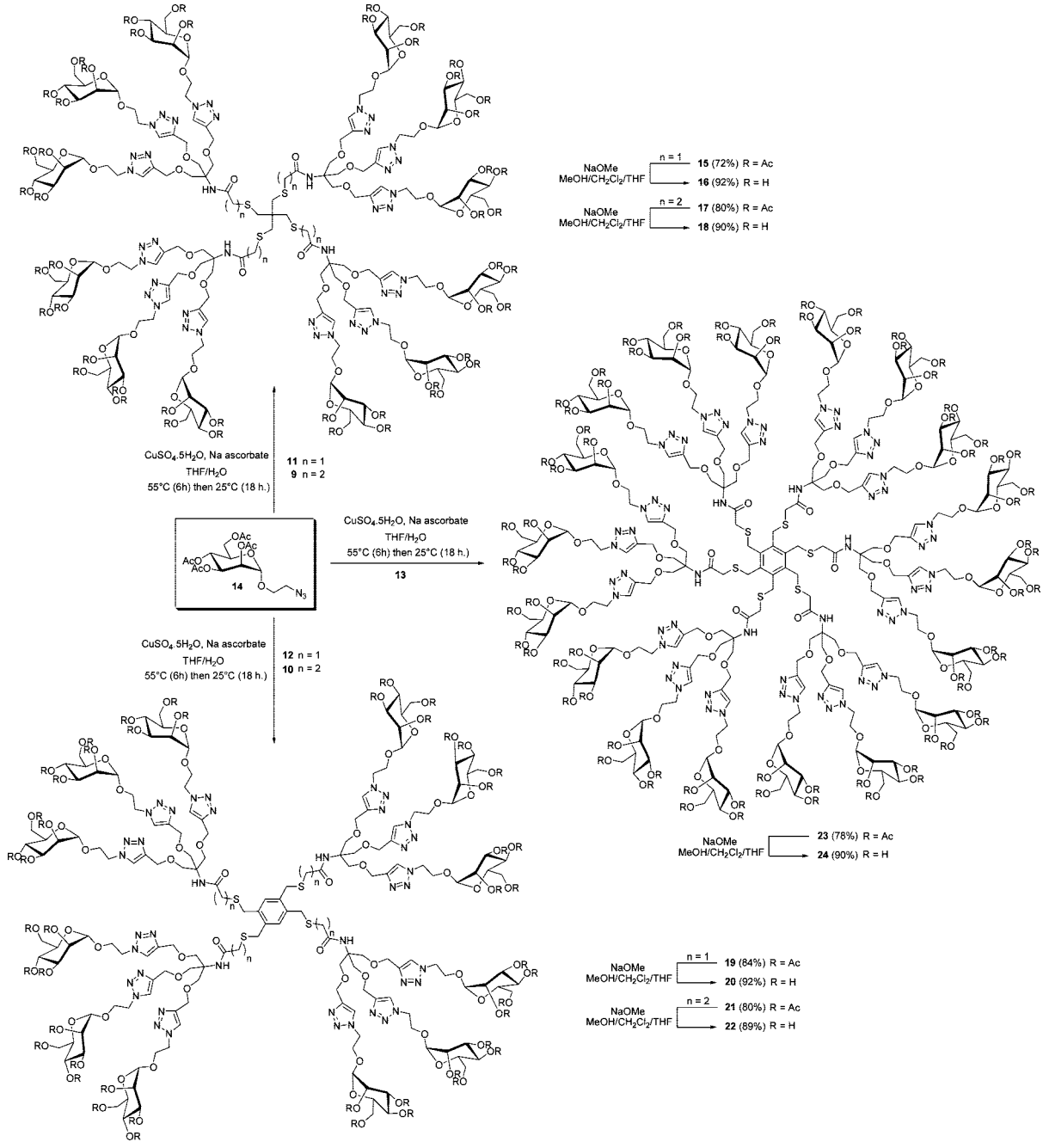
(12) Pucci, B.; Maurizis, J. C.; Pavia, A. A. *Eur. Polym. J.* **1991**, *27*, 1101–1106.

(13) Segura, M.; Sansone, F.; Casnati, A.; Ungaro, R. *Synthesis* **2001**, *14*, 2105–2112.

(14) Braga, A. L.; Appelt, H. R.; Schneider, P. H.; Silveira, C. C.; Wessjohann, L. A. *Tetrahedron: Asymmetry* **1999**, *10*, 1733–1738.

(15) Fall, Y.; Diouf, O.; Gómez, G.; Bolaño, T. *Tetrahedron Lett.* **2001**, *44*, 6069–6072.

SCHEME 4. Synthesis of Mannosylated Dendrimers



comparison to those observed for 1,4-conjugate addition might be explained by the absence of possible retro-Michael reactions. Furthermore, faster relative kinetics for the nucleophilic substitution may compete efficiently against the likelihood of thiol oxidation to disulfide, particularly for reactions involving the hexasubstituted derivative **3**. The symmetry of these novel stable polypropargylated scaffolds described above, associated with the presence of the characteristic ^{1}H and ^{13}C NMR, IR, and MS signals, facilitated their unequivocal characterization. Thus, their treatment with the known 2-azidoethyl-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside[16] (**14**) using Cu(I)-catalyzed click reactions provided four G(0) dendrimers in good to excellent yields containing 12 acetylated D-mannopyrannoside residues (**15**, **17**, **19**, and **21**) and one exhibiting 18 peripheral protected glycans (**23**) (Scheme 4). Generally, the conditions under which the Cu(I) catalyst was generated in situ afforded better yields than those using Cu(I) species (CuI) directly. In all cases, analysis of the ^{1}H NMR spectra of the mannoside dendrimers revealed calculated integrations for the triazole protons (δ 7.78 ppm) respective to the anomeric protons (δ 4.82 ppm), and complete disappearance of the acetylenic signals (δ 2.49 ppm), thus confirming together with HRMS completion of the multiple one-pot cycloadditions. De-*O*-acetylation under slightly modified Zemplén conditions (NaOMe, MeOH/CH_2Cl_2/THF) furnished the desired dodeca- and octadecavalent glycodendrimers **16**, **18**, **20**, **22**, and **24**, respectively, in excellent yields.

(16) (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.; GarciaCastro, R.; Bernad, A.; Rojo, J. *Bioconjugate Chem.* **2003**, *14*, 817–823.

The functionalized dendrimer **24**, presenting six adjacent thioacetyl groups around a central benzene core, may provide an interesting architecture and carbohydrate orientation on its own, given the possibility of the side chains to be situated alternately above and below the plane of the central benzene residue.[17] Hence, the global relaxed peripheral sugar density and specific 2-fold tripodal orientation of the carbohydrate moieties may constitute another fundamental parameter to exploit in the study of various protein binding processes.

In conclusion, we have developed an expeditive and systematic route toward the synthesis of a series of dodeca- to octadecamannopyranoside G(0) dendrimers by using triazole chemistry and building from the corresponding stable polypropargylated scaffolds. Two of these latter species (**9** and **10**) were obtained according to an efficient orthogonal and chemoselective sequence, and all were synthesized with simple and generally high yielding reactions. Work is in progress on the synthesis of the corresponding higher generation dendritic architectures possessing optimized valencies and topologies. Indeed, studies have shown that a plateau of inhibitory potency was reached as a function of dendrimer scaffolding generation, above which the binding interactions begin to diminish. The surface congestion induced inaccessibility of individual sugars toward their protein receptors.[18] Moreover, it has been observed that the structures and valency represented critical factors in the optimization of individual binding interactions.[19] In order to confirm those important observations with our structures, biological studies are currently under investigation to estimate their therapeutic potential, notably for the inhibition of adhesion of *E. coli* toward urothelial infections and other mannoside binding proteins such as DC-SIGN.[5]

Experimental Section

Octadecapropargylated Derivative 13. To a solution of the hexa(thioacetylated) core **3** (20.0 mg, 0.033 mmol, 1.0 equiv) in dry EtOH (2.0 mL) were added at room temperature finely ground NaOH (15.8 mg, 0.395 mmol, 12.0 equiv) and $NaBH_4$ (15.0 mg, 0.395 mmol, 12.0 equiv) under a nitrogen atmosphere. After 5 min, 2-bromoacetamido[tris(propargyloxy)methyl]aminomethane (**8**; 91.6 mg, 0.257 mmol, 7.8 equiv) was added to the solution, which was then warmed to 40 °C for 3 h. The solvent was removed under reduced pressure, and the resulting crude material was chromatographed on a silica gel column.

Data for **13**: eluent for column chromatography CH_2Cl_2/EtOAc, 80:20 to 75:25 (v/v); yield 88%; colorless oil; $R_f = 0.53$ (CH_2Cl_2/EtOAc 70:30 (v/v)); ν_{max}(NaCl)/cm^{-1} 3310 s (br), 3005 s, 2910 s (br), 2113 s, 1673 s, 1652 s, 1540 m, 1472 m, 1250 s, 1146 s, 796 s (br); δ_H (300 MHz; $CDCl_3$) 2.49 (t, 18H, *J* 2.4, C≡C*H*), 3.37 (s, 12H, SCH_2CO), 3.86 (s, 36H, CH_2O), 4.15 (s, 12H, $C_{ar}CH_2$S), 4.16 (d, 24H, $J = 2.4$ Hz, OCH_2C≡CH), 6.70 (br s, 6H, N*H*); δ_C (75.5 MHz; $CDCl_3$) 31.6, 37.7, 58.7, 59.5, 68.4, 75.1, 79.7, 136.1, 168.8; *m*/*z* (TOF$^+$ HRMS) for $C_{102}H_{120}N_6O_{24}S_6$ 1003.341 19 [M + 2H]$^{2+}$, found 1003.340 69.

Sample Procedure for the Preparation of Dendrimer 23. To a solution of **13** (20 mg, 0.010 mmol, 1.0 equiv) in a 1:1 (v/v) mixture of water and tetrahydrofuran (3.0 mL) were added 2′-azidoethyl-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**14**; 93.6 mg, 0.224 mmol, 22.5 equiv), $CuSO_4 \cdot 5H_2O$ (15.0 mg, 0.060 mmol, 6.0 equiv) and sodium ascorbate (11.8 mg, 0.060 mmol, 6.0 equiv). While it was stirred, the mixture was first heated to 55 °C for 6 h and left at room temperature for an additional 18 h. Ethyl acetate (15 mL) was added, and the solution was washed with saturated aqueous NH_4Cl (2 × 10 mL), water (10 mL), and brine (5 mL). Organics were collected, dried over Na_2SO_4, and concentrated to dryness in vacuo.

Data for **23**: eluent for column chromatography CH_2Cl_2/MeOH, 100:0 to 90:10 (v/v)); yield 78%; white solid; $R_f = 0.45$ (CH_2Cl_2/MeOH 93:7 (v/v)); mp 87−90 °C; δ_H (300 MHz; $CDCl_3$) 1.95, 2.01, 2.07, 2.11 (4 × s, 216H, COCH_3), 3.37 (s, 12H, SCH_2CO), 3.67 (br s, 18H, H_5), 3.81 (s, 36H, C_qCH_2O), 3.90 (m, 36H, OCH_2CH$_2$), 4.05 (s, 12H, $C_{ar}CH_2$S), 4.06 (m, 18H, H_{6b}), 4.18 (m, 18H, H_{6a}), 4.56 (br s, 36H, CH═CCH_2), 4.57 (m, 36H, OCH$_2$CH_2N), 4.82 (br s, 18H, H_1), 5.18−5.30 (m, 54H, H_2, H_3, H_4), 7.08 (br s, 6H, N*H*), 7.80 (br s, 18H, C*H*═C); δ_C (75.5 MHz; $CDCl_3$) 20.6, 20.7, 20.7, 20.8, 31.3, 37.2, 49.4, 62.1, 64.6, 65.6, 66.3, 68.7, 69.2, 97.4, 124.0, 136.3, 144.8, 169.6, 169.8, 169.9, 170.5, 170.7; *m*/*z* (TOF$^+$ HRMS) for $C_{390}H_{534}N_{60}O_{204}S_6$ 2379.296 78 [M + 4H]$^{4+}$, found 2379.301 30.

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Supporting Information Available: Text and figures giving experimental details for the synthesis of compounds **1**−**13** and **15**−**24** and ^{1}H and ^{13}C NMR spectra of compounds **2**, **3**, and **5**−**24**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Hardy, A. D. U.; MacNicol, D. D.; Swanson, S.; Wilson, D. R. *Tetrahedron Lett.* **1978**, *38*, 3579–3582. (b) D'Aleo, A.; Williams, R. M.; Osswald, F.; Edamana, P.; Hahn, U.; van Heyst, J.; Tichelaar, F. D.; Vögtle, F.; De Cola, L. *Adv. Funct. Mater.* **2004**, *14*, 1167–1177.

(18) Roy, R. *Top. Curr. Chem.* **1997**, *187*, 241–274.

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