A Click Approach to Unprotected Glycodendrimers†

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ABSTRACT: Click chemistry in combination with ultrafiltration has allowed the quick, efficient, and reliable multivalent conjugation of unprotected alkyne-derived carbohydrates to three generations of azido-terminated gallic acid−triethylene glycol dendrimers under aqueous conditions. The reported procedure allows the atom economical incorporation of up to 27 unprotected fucose, mannose, and lactose residues, in reproducible high yields (up to 92%), requiring only catalytic amounts of Cu. The completion of the conjugation process was clearly established in all cases by both ^{1}H NMR and MALDI-TOF MS.

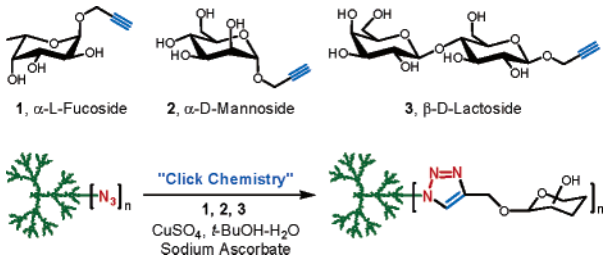
A plethora of biological processes such as fertilization, pathogen invasion, toxin and hormone mediation, and cell−cell interactions rely on carbohydrate−receptor interactions.[1] Such a level of selectivity attained by Nature is, however, in marked contrast to the low affinity and specificity shown by monosaccharidic ligands. To circumvent this shortcoming, Nature has profited from multivalency. Thus, both receptors and carbohydrates are displayed in a multivalent fashion, enabling their simultaneous interaction in a global process characterized by higher affinity and specificity than the monovalent interactions.[2]

This cooperative effect, generally referred to as "cluster effect",[3] has opened up the possibility of inhibiting or promoting carbohydrate−receptor interactions with synthetic multivalent glycoconjugates.[4] Among them, glycodendrimers are especially attractive due to their monodisperse nature and the possibility of controlling their size and the number of carbohydrates at the periphery.[5] Several strategies have been reported to anchor carbohydrates to dendrimers. However, the introduction of unprotected carbohydrates, an interesting approach avoiding tedious final deprotection steps, and leading to more efficient couplings for steric reasons, has been underdeveloped in our opinion. Thus, although active esters and isothiocyanates have been reported for that purpose, both suffer from lengthy activation steps of the coupling partners.[6]

With the purpose of synthesizing several generations of dendritic glycoconjugates for the study of some biological processes, we decided to explore new pathways for their preparation from unprotected carbohydrates. The desired strategy should not only proceed under aqueous conditions, and render activation steps unnecessary, but also fulfill atom economy requirements. With this in mind, we turn our attention to the recently described Cu(I)-catalyzed azide−alkyne [3 + 2] cycloaddition,[7] the most efficient among the click reactions,[8] as it proceeds in high yields, under aqueous conditions, and with complete regioselectivity.

In our approach, we resolved to locate azide and alkyne functional groups on the dendritic and the carbohydrate partners,

Scheme 1



respectively. Azide-terminated dendrimers were preferred to those incorporating terminal alkynes because of the potential bias of the latter to Cu(II)-catalyzed intradendritic oxidative coupling.[9] Also, better results have been reported by Fokin, Sharpless, and Finn in the click decoration of azide-functionalized cowpea mosaic virus.[10] In addition, the required *O*-alkynyl carbohydrates are easily accessible.

Herein, we describe the efficient and quick decoration of azido-terminated dendrimers with alkyne-derived unprotected carbohydrates under aqueous conditions by means of the use of click chemistry. The resulting glycodendrimers are isolated in high yields, after purification of the reaction mixture by ultrafiltration (Scheme 1).[11]

Experimental Section

General Methods. CH_2Cl_2, Et_3N, CH_3CN, and DMF were distilled from CaH_2. Column chromatography was performed with 70−230 and 230−400 mesh silica gel. Thin-layer chromatography (TLC) was done on silica 60/F-254 aluminum-backed plates (E. Merck). Ultrafiltration was performed on Amicon stirred cells with Amicon YM1 membranes. IR spectra were recorded with a Bruker IFS-66v spectrometer. NMR spectra were recorded with Varian Inova 400 MHz, Bruker DPX 250 MHz, and Varian Inova 750 MHz spectrometers in D_2O, CD_3OD, or $CDCl_3$. Chemical shifts are reported in ppm (δ units) downfield from internal tetramethylsilane ($CDCl_3$) or the HOD signal (D_2O and CD_3OD). (+)-D-Mannose, (−)-L-fucose, and (+)-D-lactose were purchase from Fluka, (+)-sodium L-ascorbate was from Sigma, and $CuSO_4$ was obtained from Prolabo.

MALDI-TOF MS. MALDI-TOF MS were carried out on a Bruker Autoflex or on a 4700 Applied Biosystems operating in linear mode for masses higher than 6000−8000 Da and in reflected mode for lower masses. 2-(4-Hydroxyphenylazo)benzoic acid

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(HABA) was used as matrix and NaCl as a cationizing agent. Samples were dissolved in MeOH−H_2O (1:1) at a concentration 5 × 10^{-4} M. HABA was dissolved in dioxane at a concentration 0.05 M. Sample (20 μL) and matrix (80 μL) solutions were mixed, and then 1 μL of 0.02 M NaCl was added. Finally, 1 μL of the resulting mixture was placed on the MALDI plate. In the case of **[G3]-Man** and **[G3]-Lac**, samples were dissolved in H_2O (1 mg/mL), and 2,5-dihydroxybenzoic acid (DHB) dissolved in MeOH−H_2O (1:1) was employed as matrix.

2-(2-(2-Azidoethoxy)ethoxy)ethyl-4-*p*-toluenesulfonate (6). NaN_3 (1.137 g, 17.5 mmol) was added to a solution of chlorohydrin **5** (1.27 mL, 8.75 mmol) in DMF (35.0 mL). The reaction was stirred at 100 °C for 12 h under Ar. After cooling at room temperature, the solvent was evaporated to give a residue that was suspended in CH_2Cl_2 and then filtered through Celite. The filtrate was evaporated to afford 2-[2-(2-azidoethoxy)ethoxy]ethanol (1.58 g, 100%) as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 3.77−3.61 (m, 10H), 3.41 (t, J = 5.0 Hz, 2H). ^{13}C NMR (63 MHz, $CDCl_3$) δ: 71.4, 69.4, 69.1, 68.8, 60.1, 49.7. Anal. Calcd for $C_9H_{13}N_3O_3$: C, 47.36; H, 5.76; N, 12.75. Found: C, 47.13; H, 5.91; N, 12.80.

2-[2-(2-Azidoethoxy)ethoxy]ethanol (1.54 g, 8.79 mmol) was dissolved in CH_2Cl_2 (8.80 mL). Pyridine (1.4 mL, 17.60 mmol) was added, and the mixture was cooled to 0 °C. Then, TsCl (2.51 g, 13.21 mmol) was added in portions over a 20 min period, and the resulting solution was allowed to warm to room temperature. After 12 h of stirring, the reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with aqueous HCl (15 mL, 3%) and brine (15 mL). The organic layer was dried (Na_2SO_4) and concentrated to give a crude product that was purified by silica gel chromatography (EtOAc/hexanes, 1:2.5) to afford **6** (2.58 g, 89%) as a colorless oil. NMR spectral data of **6** matched well those previously reported.[12] ^{1}H NMR (250 MHz, $CDCl_3$, Me_4Si) δ: 7.79 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.5 Hz, 2H), 4.20 (t, J = 4.7 Hz, 2H), 3.71−3.57 (m, 8H), 3.36 (t, J = 5.0 Hz, 2H), 2.44 (s, 3H).

3,4,5-Tri-(2-(2-(2-azidoethoxy)ethoxy)ethyl)benzoic Acid.[12] A mixture of **6** (7.01 g, 21.28 mmol), methyl gallate (0.98 g, 5.32 mmol), and K_2CO_3 (7.35 g, 53.20 mmol) in DMF (53 mL) was heated at 80 °C for 18 h. Then, it was allowed to reach room temperature (rt) and was partitioned between EtOAc (300 mL) and H_2O (300 mL). The aqueous phase was extracted with EtOAc (2 × 200 mL). The combined organic phase was washed with brine (200 mL) and then was dried (Na_2SO_4) and concentrated to give a crude product that was purified by silica gel chromatography (EtOAc/hexanes, 2:1) to afford methyl 3,4,5-tri-(2-(2-(2-azidoethoxy)ethoxy)ethyl)benzoate (3.03 g, 87%) as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$, Me_4Si) δ: 7.28 (s, 2H), 4.23−4.16 (m, 6H), 3.88−3.64 (m, 27H), 3.37 (t, J = 5.0 Hz, 6H).

Aqueous KOH (9.1 mL, 1 M) was added to a solution of methyl 3,4,5-tri-(2-(2-(2-azidoethoxy)ethoxy)ethyl)benzoate (3.02 g, 18.42 mmol) in EtOH (107 mL). The resulting solution was heated at reflux for 2 h and then was allowed to reach rt. Neutralization with Amberlite IR-120, followed by filtration and evaporation, afforded pure acid **4** (2.93 g, 100%) as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$, Me_4Si) δ: 10.50 (br s, 1H), 7.26 (s, 2H), 4.23−4.19 (m, 6H), 3.91−3.64 (m, 24H), 3.38 (t, J = 4.9 Hz, 6H). Anal. Calcd for $C_{25}H_{39}N_9O_{11}$: C, 46.80; H, 6.13; N, 19.65. Found: C, 46.65; H, 6.22; N, 19.73.

[G1]-N_3. EDC·HCl (168 mg, 0.87 mmol) was added to a stirred solution of acid **4** (280 mg, 0.44 mmol) and 1-propylamine (0.073 mL, 0.87 mmol) in CH_2Cl_2 (4 mL). Stirring was continued under Ar for 14 h. Then, CH_2Cl_2 (15 mL) was added, and the reaction mixture was washed with brine (3 × 10 mL). The organic layer was dried (Na_2SO_4) and concentrated to give a crude product that was purified by silica gel chromatography (3% hexane in EtOAc) to afford **[G1]-N_3** (252 mg, 85%) as a colorless oil. IR (KBr): 2929, 2873, 2106, 1651, 1639, 1240 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ: 7.04 (s, 2H), 6.16 (br s, 1H), 4.23−4.19 (m, 6H), 3.88−3.66 (m, 24H), 3.42−3.37 (m, 8H), 1.67−1.60 (m, 2H), 0.98 (t, J = 7.4 Hz, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ: 166.9, 152.4, 141.3, 130.1, 107.0, 72.3, 70.72, 70.68, 70.66, 70.6, 70.5, 70.0, 69.7, 69.1, 50.6, 41.8, 22.9, 11.4. MALDI-TOF MS (HABA, reflected mode, *m*/*z*): 705.3. Calcd for $[M + Na]^+$, $C_{28}H_{46}N_{10}O_{10}Na$: 705.7. Anal. Calcd for $C_{28}H_{46}N_{10}O_{10}$: C, 46.26; H, 6.79; N, 20.52. Found: C, 46.29; H, 7.15; N, 20.32.

[G2]-N_3. Pd/C (22 mg, 20%) was added to a solution of **[G1-N_3]** (110 mg, 0.116 mmol) in MeOH (2 mL). The resulting mixture was stirred under H_2 (1 atm) for 24 h. Then, the catalyst was removed by filtration through Celite, and the filtrate was concentrated to give a crude product that was analyzed by ^{1}H NMR (complete disappearance of the CH_2N_3 signal). To a solution of the above residue and acid **4** (356 mg, 0.556 mmol) in CH_2Cl_2 (1.70 mL), HOBt·*n*H_2O (85 mg, 0.556 mmol), and EDC·HCl (107 mg, 0.556 mmol) were added. Stirring was continued for 22 h under Ar. Then, CH_2Cl_2 (15 mL) was added, and the reaction mixture was washed with brine (3 × 10 mL). The organic layer was dried (Na_2SO_4) and concentrated to give a crude product that was purified by silica gel chromatography (10% MeOH in EtOAc) to afford **[G2]-N_3** (292 mg, 84%) as a colorless oil. IR (KBr): 2928, 2872, 2106, 1652, 1121 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ: 7.12 (s, 2H), 7.09 (s, 4H), 7.04 (s, 2H), 4.19−4.10 (m, 24H), 3.86−3.59 (m, 102H), 3.39−3.36 (m, 20H), 1.65−1.60 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ: 167.0, 166.9, 152.2, 152.1, 140.9, 130.2, 129.5, 106.8, 106.6, 72.2, 70.6, 70.5, 70.4, 70.3, 70.0, 69.8, 69.7, 69.6, 68.7, 50.5, 41.8, 39.8, 22.7, 11.4. MALDI-TOF MS (HABA, reflected mode, *m*/*z*): 2497.9. Calcd for $[M + Na]^+$, $C_{103}H_{163}N_{31}O_{40}Na$: 2498.6. Anal. Calcd for $C_{103}H_{163}N_{31}O_{40}$: C, 49.97; H, 6.64; N, 17.54. Found: C, 49.57; H, 6.73; N, 17.87.

[G3]-N_3. Pd/C (22 mg, 20%) was added to a solution of **[G2-N_3]** (65 mg, 0.026 mmol) and aqueous HCl (0.131 mL, 0.2 M) in MeOH (0.70 mL). The resulting mixture was stirred under H_2 (1 atm) for 24 h. Then, the catalyst was removed by filtration through Celite, and the filtrate was concentrated to give a crude product that was analyzed by ^{1}H NMR (complete disappearance of the CH_2N_3 signal). To a solution of the above residue and acid **4** (283 mg, 0.441 mmol) in CH_3CN (0.75 mL), Et_3N (0.61 mL, 0.441 mmol), HOBt·*n*H_2O (60 mg, 0.441 mmol), and EDC·HCl (85 mg, 0.441 mmol) were added. Stirring was continued for 28 h under Ar. Then, CH_2Cl_2 (10 mL) was added, and the reaction mixture was washed with brine (3 × 8 mL). The organic layer was dried (Na_2SO_4) and concentrated to give a crude product that was purified by silica gel chromatography (10% MeOH in EtOAc) to afford **[G3]-N_3** (165 mg, 80%) as a colorless oil. IR (KBr): 2926, 2872, 2105, 1652, 1119 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ: 7.12−7.10 (m, 26H), 4.17−4.14 (m, 78H), 3.84−3.60 (m, 336H), 3.40−3.35 (m, 56H), 1.68−1.55 (m, 2H), 0.91 (t, J = 7.6 Hz, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ: 166.9, 152.2, 152.0, 141.0, 129.4, 106.8, 106.7, 72.2, 70.7, 70.6, 70.5, 70,4, 70.3, 70.2, 70.0, 69.9, 69.6, 69.5, 69.4, 68.7, 50.5, 50.4, 41.7, 39.8, 29.5, 22.7, 11.3. MALDI-TOF MS (HABA, reflected mode, *m*/*z*): 7878.0. Calcd for $[M + Na]^+$, $C_{328}H_{514}N_{94}O_{130}Na$: Calcd 7877.1. Anal. Calcd for $C_{328}H_{514}N_{94}O_{130}$: C, 50.16; H, 6.60; N, 16.76. Found: C, 50.05; H, 6.85; N, 16.43.

2-Propynyl α-L-Fucopyranoside (1). To a stirred solution of protected fucoside **7**[13] (142 mg, 0.313 mmol) in CH_2Cl_2 (1 mL) under Ar, TMSI (0.045 mL, 0.313 mmol) was added. The resulting solution was stirred at room temperature for 20 min, and then it was added to a stirred solution of propargyl alcohol (0.037 mL, 0.626 mmol) and 2,6-di-*tert*-butylpyridine (0.070 mL, 0.313 mmol) in CH_2Cl_2 (2 mL). Stirring was continued for 5 h at room temperature, and then MeOH (3 mL) was added. After 20 min of stirring, the reaction mixture was evaporated and purified by column chromatography (CH_2Cl_2/MeOH 10%) to give pure **1** (62 mg, 97%) as a white solid; mp 142−143 °C. ^{1}H NMR (250 MHz, D_2O) δ: 4.85 (d, J = 3.2 Hz, 1H), 4.12 (d, J = 2.3 Hz, 2H), 3.92 (q, J = 6.3 Hz, 1H), 3.64−3.62 (m, 3H), 2.71 (t, J = 2.3 Hz, 1H), 1.02 (d, J = 6.6 Hz, 3H). ^{13}C NMR (63 MHz, D_2O) δ: 97.5, 79.0, 75.7, 71.6, 69.3, 67.6, 67.0, 55.0, 15.1. Anal. Calcd for $C_9H_{14}O_5$·$0.7H_2O$: C, 50.30; H, 7.22. Found: C, 50.13; H, 6.88.

2-Propynyl α-D-Mannopyranoside (2). To a solution of trichloroacetimidate **8**[14] (719 mg, 1.495 mmol) in CH_2Cl_2 (15 mL) under Ar, powdered 4 Å ms and propargyl alcohol (0.172 mL, 2.918

mmol) were added. The resulting mixture was cooled to 0 °C, and a solution of $BF_3{\cdot}OEt_2$ (0.020 mL, 0.160 mmol) in CH_2Cl_2 (1.6 mL) was added dropwise for 10 min. The stirring was continued at room temperature overnight. Then, 3 drops of Et_3N were added, and the reaction was filtered through Celita. The filtrate was evaporated to give a crude product that was recrystallized from EtOAc/hexane, affording pure **9** (507 mg, 90%) as colorless crystals. NMR spectral data of **9** matched well those previously reported.[15]

Aqueous KOH (2.1 mL, 1 M) was added to a solution of **9** (100 mg, 0.259 mmol) in EtOH (6.5 mL). The resulting solution was stirred at room temperature for 1 h 30 min and then was neutralized with Amberlite IR-120, filtered, and evaporated, affording a residue that was purified by column chromatography (CH_2Cl_2/MeOH 20%) to give pure **2** (55 mg, 97%) as an oil. ^{1}H NMR (250 MHz, CD_3-OD) δ: 4.99 (d, J = 1.3 Hz, 1H), 4.30 (d, J = 2.4 Hz, 2H), 3.87 (dd, J = 11.8 Hz, J = 2.2 Hz, 1H), 3.82 (dd, J = 3.1 Hz, J = 1.7 Hz, 1H), 3.75−3.63 (m, 3H), 3.56−3.52 (m, 1H), 2.88 (t, J = 2.4 Hz, 1H). ^{13}C NMR (63 MHz, CD_3OD) δ: 100.3, 80.5, 76.5, 75.6, 73.0, 72.5, 70.0, 63.3, 55.3. Anal. Calcd for $C_9H_{14}O_6$ H_2O: C, 45.74; H, 6.83. Found: C, 45.75; H, 6.85.

2-Propynyl β-D-Lactopyranoside (3). Acetyl groups in lactoside **10**[16] (2.90 g, 4.30 mmol) were hydrolyzed under the same conditions as in mannoside **9** to give, after column chromatography (CH_2Cl_2/MeOH 20%), pure **3** (2.57 g, 93%) as a white foam. ^{1}H NMR (250 MHz, D_2O) δ: 4.73 (d, J = 8.0 Hz, 1H), 4.53−4.49 (m, 3H), 4.07−3.56 (m, 11H), 3.40 (dt, J = 2.3 Hz, J = 7.4 Hz, 1H), 2.98 (t, J = 2.3 Hz, 1H). ^{13}C NMR (63 MHz, D_2O) δ: 103.6, 101.0, 79.4, 78.9, 77.0, 76.0, 75.5, 75.0, 73.3, 73.2, 71.6, 69.2, 61.7, 60.6, 57.3. Anal. Calcd for $C_{15}H_{24}O_{11}{\cdot}2H_2O$: C, 43.25; H, 6.73. Found: C, 42.90; H, 6.35.

General Procedure for the Multivalent Glycosidation of [Gn]-N_3. Dendrimers **[Gn]-N_3** were dissolved in *t*-BuOH−H_2O (1:1) to give a 0.1 M final concentration of terminal azides. Then, pyranosides **1**, **2**, or **3** (200 mol % per terminal N_3) and freshly prepared aqueous solutions of $CuSO_4$ (1 mol % per N_3) and sodium ascorbate (5 mol % per N_3) were added. The resulting solution was stirred at room temperature for 24−72 h and then readily purified by ultrafiltration (G2 and G3 glycodendrimers, Amicon YM1, acetone−H_2O 1:1, 3 × 30 mL). In the case of G1 glycodendrimers, the reaction mixture was concentrated and purified by column chromatography (CH_2Cl_2/MeOH/H_2O, 3:3:0.2).

[G1]-Fuc. From **[G1]-N_3** (38 mg, 0.056 mmol), **1** (68 mg, 0.336 mmol), 0.1 M sodium ascorbate (84 μL), and 0.1 M $CuSO_4$ (17 μL) dissolved in *t*-BuOH (0.65 mL)−H_2O (0.50 mL), and after 24 h of reaction time following the general procedure described above, **[G1]-Fuc** (56 mg, 79%) was obtained as a white foam. ^{1}H NMR (750 MHz, D_2O) δ: 8.05 (s, 1H), 8.04 (s, 2H), 7.14 (s, 2H), 4.94 (d, J = 3.6 Hz, 1H), 4.92 (d, J = 3.3 Hz, 2H), 4.69−4.57 (m, 12H), 4.23−4.19 (m, 4H), 4.16−4.12 (m, 2H), 3.96−3.57 (m, 36H), 3.34 (t, J = 7.2 Hz, 2H), 1.64−1.56 (m, 2H), 1.10−1.06 (m, 9H), 0.93 (t, J = 7.4 Hz, 3H). ^{13}C NMR (63 MHz, D_2O) δ: 169.8, 152.6, 140.3, 130.4, 126.0, 107.0, 99.1, 99.0, 72.4, 70.3, 70.2, 70.1, 69.8, 69.3, 69.1, 68.5, 67.3, 61.1, 50.6, 42.4, 22.7, 15.8, 11.4. MALDI-TOF MS (HABA, reflected mode, *m*/*z*): 1311.7. Calcd for [M + Na]$^+$, $C_{55}H_{88}N_{10}O_{25}Na$: 1312.3. Anal. Calcd for $C_{55}H_{88}N_{10}O_{25}{\cdot}2H_2O$: C, 48.97; H, 6.82; N, 10.38. Found: C, 48.69; H, 6.98; N, 9.98.

[G1]-Man. From **[G1]-N_3** (34 mg, 0.050 mmol), **2** (67 mg, 0.307 mmol), 0.02 M sodium ascorbate (375 μL), and 0.02 M $CuSO_4$ (75 μL) dissolved in *t*-BuOH (0.60 mL)−H_2O (0.35 mL), and after 24 h of reaction time following the general procedure described above, **[G1]-Man** (50 mg, 75%) was obtained as an oil. ^{1}H NMR (750 MHz, D_2O) δ: 8.06 (s, 1H), 8.04 (s, 2H), 7.11 (s, 2H), 4.87 (br s, 1H), 4.86 (br s, 2H), 4.72−4.53 (m, 12H), 4.20−4.19 (m, 4H), 4.13−4.12 (m, 2H), 3.92 (t, J = 4.9 Hz, 4H), 3.89 (t, J = 4.9 Hz, 2H), 3.85−3.83 (m, 3H), 3.83−3.81 (m, 3H), 3.80 (dd, J = 2.1 Hz, J = 5.0 Hz, 1H), 3.78 (dd, J = 5.0 Hz, J = 2.0 Hz, 2H), 3.74−3.55 (m, 27H), 3.33 (t, J = 7.1 Hz, 2H), 1.63−1.58 (m, 2H), 0.93 (t, J = 7.4 Hz, 3H). ^{13}C NMR (63 MHz, D_2O) δ: 166.9, 152.6, 144.2, 130.4, 126.6, 107.0, 103.6, 102.0, 78.9, 76.0, 75.4, 75.0, 73.3, 73.2, 71.6, 70.3, 69.3, 69.2, 62.5, 61.6, 60.7, 50.6, 42.5, 22.7, 11.4. MALDI-TOF MS (HABA, reflected mode, *m*/*z*): 1359.9 Calcd for [M + Na]$^+$, $C_{55}H_{88}N_{10}O_{28}Na$: 1360.3. Anal. Calcd for $C_{55}H_{88}N_{10}O_{28}{\cdot}H_2O$: C, 47.91; H, 6.53; N, 10.16. Found: C, 48.09; H, 6.82; N, 9.93.

[G1]-Lac. From **[G1]-N_3** (25 mg, 0.037 mmol), **3** (84 mg, 0.221 mmol), 0.1 M sodium ascorbate (56 μL), and 0.1 M $CuSO_4$ (11 μL) dissolved in *t*-BuOH (0.50 mL)−H_2O (0.40 mL), and after 24 h of reaction time following the general procedure described above, **[G1]-Lac** (50 mg, 75%) was obtained as an oil. ^{1}H NMR (750 MHz, D_2O) δ: 8.06 (s, 1H), 8.05 (s, 2H), 7.14 (s, 2H), 4.89 (d, J = 12.5 Hz, 1H), 4.85 (d, J = 11.8 Hz, 2H), 4.73 (d, J = 11.8 Hz, 1H), 4.70 (d, J = 11.8 Hz, 2H), 4.62−4.58 (m, 6H), 4.47−4.41 (m, 6H), 4.20 (br s, 4H), 4.12 (br s, 2H), 4.00−3.50 (m, 58H), 3.34 (t, J = 7.1 Hz, 2H), 3.29 (t, J = 8.4 Hz, 2H), 1.64−1.59 (m, 2H), 0.94 (t, J = 7.1 Hz, 3H). ^{13}C NMR (63 MHz, D_2O) δ: 169.7, 152.6, 144.2, 144.1, 144.0, 140.3, 130.3, 126.5, 126.3, 106.9, 103.6, 102.0, 79.0, 78.9, 76.0, 75.4, 75.0, 73.3, 73.2, 71.6, 70.3, 70.0, 69.8, 69.3, 69.2, 62.5, 61.6, 60.7, 50.6, 42.5, 22.7, 11.4. MALDI-TOF MS (HABA, reflected mode, *m*/*z*): 1847.4 Calcd for [M + Na]$^+$, $C_{73}H_{118}N_{10}O_{43}Na$: 1846.7. Anal. Calcd for $C_{73}H_{118}N_{10}O_{43}{\cdot}4H_2O$: C, 46.21; H, 6.65; N, 7.18. Found: C, 45.90; H, 6.93; N, 6.91.

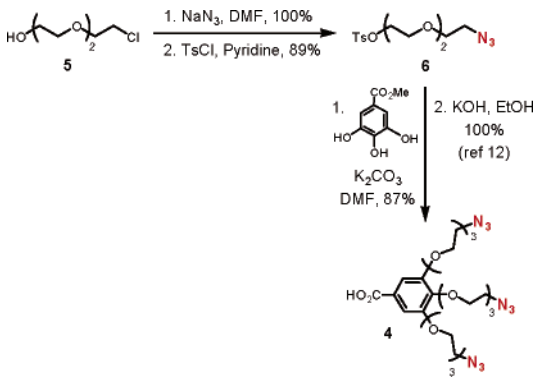
[G2]-Fuc. From **[G2]-N_3** (63 mg, 0.025 mmol), **1** (93 mg, 0.460 mmol), 0.1 M sodium ascorbate (113 μL), and 0.1 M $CuSO_4$ (23 μL) dissolved in *t*-BuOH (1.00 mL)−H_2O (0.80 mL), and after 36 h of reaction time following the general procedure described above, **[G2]-Fuc** (93 mg, 85%) was obtained as a white foam. ^{1}H NMR (750 MHz, D_2O) δ: 8.04 (s, 3H), 8.02 (s, 6H), 7.09 (s, 2H), 7.08 (s, 4H), 6.97 (s, 2H), 4.93−4.91 (m, 9H), 4.68−4.55 (m, 42H), 4.06−3.53 (m, 156H), 3.26 (t, J = 6.9 Hz, 2H), 1.56−1.52 (m, 2H), 1.06−1.05 (m, 27H), 0.88 (t, J = 7.3 Hz, 3H). ^{13}C NMR (63 MHz, D_2O) δ: 170.0, 152.1, 144.3, 140.9, 139.9, 139.8, 129.4, 125.6, 106.4, 98.8, 72.0, 70.0, 69.8, 69.3, 69.0, 68.2, 67.0, 50.2, 42.4, 40.0, 22.5, 15.5, 11.1. MALDI-TOF MS (HABA, reflected mode, *m*/*z*): 4317.6. Calcd for [M + Na]$^+$, $C_{184}H_{289}N_{31}O_{85}Na$: 4318.4. Anal. Calcd for $C_{184}H_{289}N_{31}O_{85}{\cdot}8H_2O$: C, 49.76; H, 6.87; N, 9.78. Found: C, 49.48; H, 6.50; N, 9.93.

[G2]-Man. From **[G2]-N_3** (56 mg, 0.023 mmol), **2** (89 mg, 0.408 mmol), 0.1 M sodium ascorbate (105 μL), and 0.1 M $CuSO_4$ (20 μL) dissolved in *t*-BuOH (1.00 mL)−H_2O (0.80 mL), and after 36 h of reaction time following the general procedure described above, **[G2]-Man** (92 mg, 92%) was obtained as an oil. ^{1}H NMR (750 MHz, D_2O) δ: 8.07 (s, 3H), 8.04 (s, 6H), 7.10 (s, 2H), 7.08 (s, 4H), 6.98 (s, 2H), 4.86−4.85 (m, 9H), 4.79−4.69 (m, 42H), 4.11−3.54 (m, 174H), 3.23 (t, J = 6.6 Hz, 2H), 1,54−1.47 (m, 2H), 0.85 (t, J = 7.3 Hz, 3H). ^{13}C NMR (63 MHz, D_2O) δ: 169.5, 152.4, 152.3, 144.1, 129.8, 126.1, 106.8, 100.0, 73.6, 72.7, 71.2, 71.1, 70.6, 70.4, 70.3, 70.2, 70.1, 69.7, 69.5, 69.3, 69.2, 68.9, 67.3, 67.1, 61.4, 61.3, 60.2, 50.6, 42.2, 40.4, 22.9, 11.5. MALDI−TOF MS (HABA, reflected mode, *m*/*z*): 4466.4 Calcd for [M + Na]$^+$, $C_{184}H_{289}N_{31}O_{94}Na$: 4462.4. Anal. Calcd for $C_{184}H_{289}N_{31}O_{94}{\cdot}3H_2O$: C, 50.47; H, 6.57; N, 9.66. Found: C, 50.12; H, 6.93; N, 9.45.

[G2]-Lac. From **[G2]-N_3** (60 mg, 0.024 mmol), **3** (166 mg, 0.436 mmol), 0.1 M sodium ascorbate (108 μL), and 0.1 M $CuSO_4$ (22 μL) dissolved in *t*-BuOH (1.00 mL)−H_2O (0.80 mL), and after 36 h of reaction time following the general procedure described above, **[G2]-Lac** (120 mg, 86%) was obtained as a white foam. ^{1}H NMR (750 MHz, D_2O) δ: 8.08 (br s, 3H), 8.06 (s, 6H), 7.10 (s, 2H), 7.09 (s, 4H), 6.98 (s, 2H), 4.97−4.86 (m, 9H), 4.78−4.73 (m, 9H), 4.58−4.42 (m, 42 H), 4.09−3.53 (m, 219 H), 3.33−3.31 (m, 9H), 3.28 (t, J = 7.1 Hz, 2H), 1.61−1.55 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H). ^{13}C NMR (63 MHz, D_2O) δ: 169.5, 169.4, 152.5, 144.3, 144.2, 140.4, 129.8, 126.6, 126.5, 126.4, 126.3, 103.7, 102.1, 79.1, 76.1, 75.5, 73.4, 73.2, 72.8, 71.6, 70.8, 70.6, 70.4, 70.3, 70.2, 69.9, 69.7, 69.6, 69.4, 69.2, 69.0, 62.6, 61.7, 60.8, 50.7, 42.5, 40.5, 22.9, 11.6. MALDI-TOF MS (HABA, linear mode, *m*/*z*): 5920.3. Calcd for [M + Na]$^+$, $C_{238}H_{379}N_{31}O_{139}Na$: 5921.7. Anal. Calcd for $C_{238}H_{379}N_{31}O_{139}{\cdot}8H_2O$: C, 47.26; H, 6.53; N, 7.18. Found: C, 47.51; H, 6.32; N, 6.83.

[G3]-Fuc. From **[G3]-N_3** (60 mg, 7.64 μmol), **1** (83 mg, 0.410 mmol), 0.1 M sodium ascorbate (103 μL), and 0.1 M $CuSO_4$ (21

Scheme 2



μL) dissolved in *t*-BuOH (1.00 mL)-H_2O (0.80 mL), and after 72 h of reaction time following the general procedure described above, **[G3]-Fuc** (91 mg, 91%) was obtained as a white foam. ^{1}H NMR (750 MHz, D_2O) δ: 8.06−8.00 (m, 27H), 7.10 (s, 6H), 7.08 (s, 12H), 7.03 (s, 2H), 7.00 (s, 4H), 6.98 (s, 2H), 4.93−4.91 (m, 27H), 4.68−4.54 (m, 132H), 4.12−3.44 (m, 498H), 3.21−3.18 (m, 2H), 1.51−1.48 (m, 2H), 1.12−1.01 (m, 81H), 0.83 (t, J = 7.2 Hz, 3H). ^{13}C NMR (63 MHz, D_2O) δ: 169.1, 152.5, 152.4, 144.8, 144.6, 140.3, 129.8, 125.9, 106.8, 99.2, 72.8, 72.4, 70.7, 70.6, 70.3, 70.2, 69.7, 69.4, 69.0, 68.6, 67.3, 63.1, 61.2, 50.6, 40.4, 22.9, 15.9, 11.6. MALDI-TOF MS (HABA, linear mode, *m*/*z*): 13333.7. Calcd for $[M + Na]^+$, $C_{571}H_{892}N_{94}O_{265}Na$: 13336.7. Anal. Calcd for $C_{571}H_{892}N_{94}O_{265}\cdot 8H_2O$: C, 50.90; H, 6.75; N, 9.78. Found: C, 51.27; H, 7.12; N, 9.45.

[G3]-Man. From **[G3]-N_3** (34 mg, 4.33 μmol), **2** (50 mg, 0.229 mmol), 0.1 M sodium ascorbate (58 μL), and 0.1 M $CuSO_4$ (12 μL) dissolved in *t*-BuOH (0.70 mL)-H_2O (0.60 mL), and after 72 h of reaction time following the general procedure described above, **[G3]-Man** (54 mg, 91%) was obtained as a white foam. ^{1}H NMR (750 MHz, D_2O) δ: 8.10−8.03 (m, 27H), 7.10 (s, 6H), 7.08 (s, 12H), 7.03 (s, 2H), 7.00 (s, 4H), 6.98 (s, 2H), 4.92−4.86 (m, 27H), 4.76−4.52 (m, 132H), 4.18−3.31 (m, 552H), 3.25−3.19 (m, 2H), 1.54−1.48 (m, 2H), 0.86−0.80 (m, 3H). MALDI-TOF MS (DHB, linear mode, *m*/*z*): 13693.0. Calcd for $[M + H]^+$, $C_{571}H_{893}N_{94}O_{292}$: 13746.7. Anal. Calcd for $C_{571}H_{892}N_{94}O_{292}\cdot 10H_2O$: C, 49.20; H, 6.55; N, 9.45. Found: C, 49.37; H, 6.23; N, 9.72.

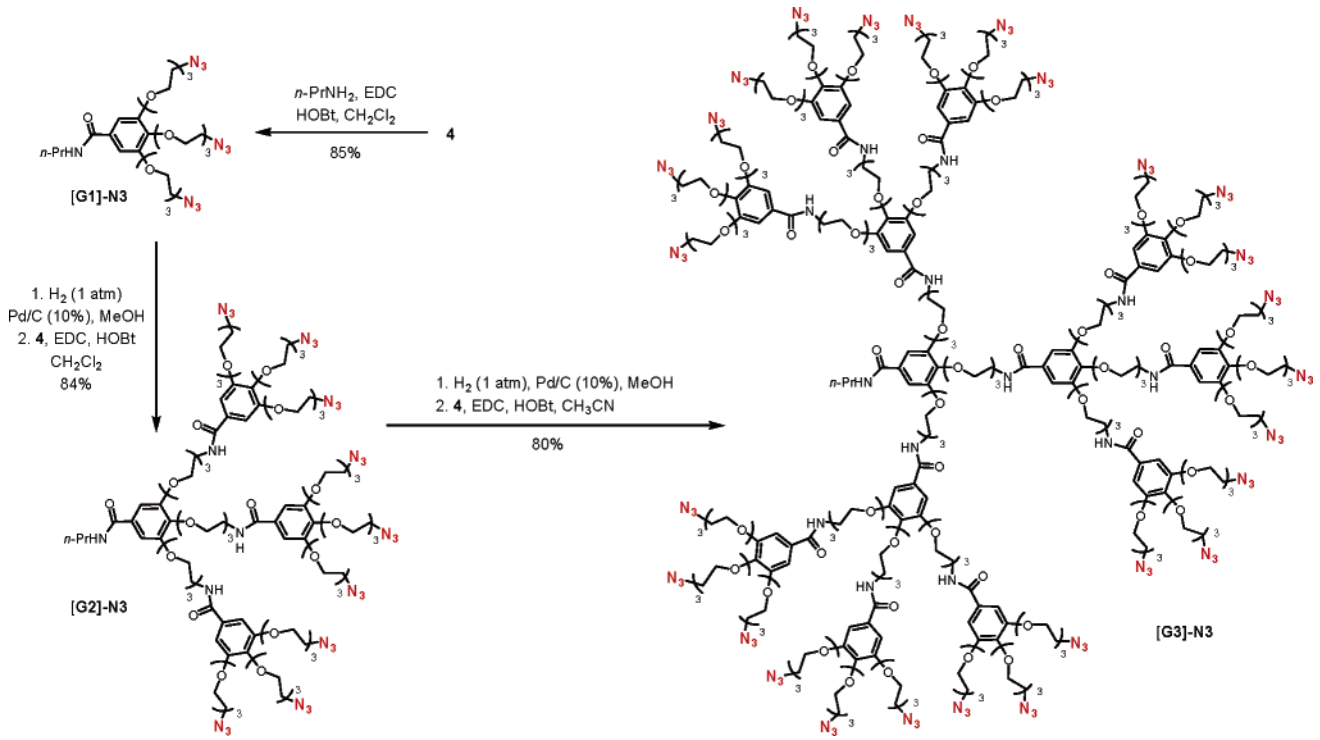
[G3]-Lac. From **[G3]-N_3** (36 mg, 4.58 μmol), **3** (90 mg, 0.237 mmol), 0.1 M sodium ascorbate (62 μL), and 0.1 M $CuSO_4$ (12 μL) dissolved in *t*-BuOH (0.70 mL)−H_2O (0.60 mL), and after 72 h of reaction time following the general procedure described above, **[G3]-Lac** (74 mg, 90%) was obtained as a white foam. ^{1}H NMR (750 MHz, D_2O) δ: 8.11−8.05 (m, 27H), 7.13−6.95 (m, 26H), 4.97−4.84 (m, 27H), 4.80−4.67 (m, 27H), 4.65−4.38 (m, 132H), 4.20−3.42 (m, 687H), 3.37−3.27 (m, 27H), 3.26−3.19 (m, 2H), 1.56−1.46 (m, 2H), 0.87−0.81 (m, 3H). ^{13}C NMR (63 MHz, D_2O) δ: 169.5, 152.8, 145.2, 144.9, 140.7, 130.1, 129.6, 129.2, 128.9, 128.6, 128.3, 128.1, 127.6, 127.0, 126.8, 107.2, 104.0, 102.4, 79.4, 76.3, 75.8, 75.4, 74, 6, 73.2, 71.9, 71.6, 71.0, 70.9, 70.7, 70.0, 69.6, 69.4, 62.4, 62.0, 61.1, 51.0, 43.3, 40.7, 23.4, 11.6. MALDI-TOF MS (DHB, linear mode, *m*/*z*): 18116.9. Calcd for $[M + H]^+$, $C_{733}H_{1163}N_{94}O_{427}$: 18124.4; Anal. Calcd for $C_{733}H_{1162}N_{94}O_{427}\cdot 15H_2O$: C, 47.82; H, 6.48; N, 7.15. Found: C, 47.57; H, 6.11; N, 6.80.

Results and Discussion

With the aim of synthesizing several generations of azide-terminated dendrimers, we turned our attention to a dendritic core **4** comprising gallic acid and triethylene glycol, due to its solubility in H_2O and easy structural modification by changing the number and length of spacer units. Acid **4** has been previously reported,[12] although through an inefficient process (23%, four steps from triethylene glycol), what has hampered the spread of this dendritic family so far. Therefore, before accomplishing the glycodendrimer synthesis, we started looking for a more efficient synthesis of acid **4** from commercially available chlorohydrin **5** (Scheme 2). Indeed, treatment of **5** with NaN_3 in DMF at 100 °C and tosylation of the resulting triethylene glycolazide with TsCl in pyridine led to tosylate **6** in an excellent 89% overall yield. Then, coupling of **6** with gallic acid methyl ester and hydrolysis afforded acid **4** in 87% overall yield.[12]

With a reliable synthesis of acid **4** in hand, three generations of azide-terminated dendrimers, carrying a hydrolytically stable amide bond at the focal point, were divergently synthesized. Thus, dendrimer of generation one **[G1]-N_3** was readily obtained

Scheme 3



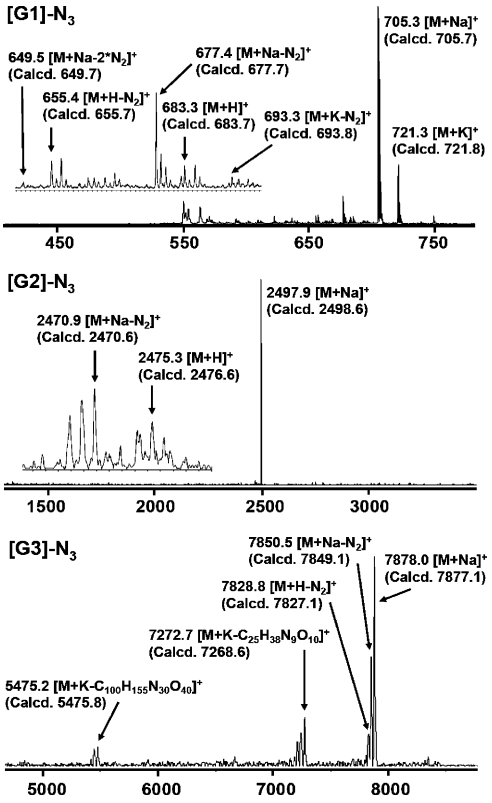


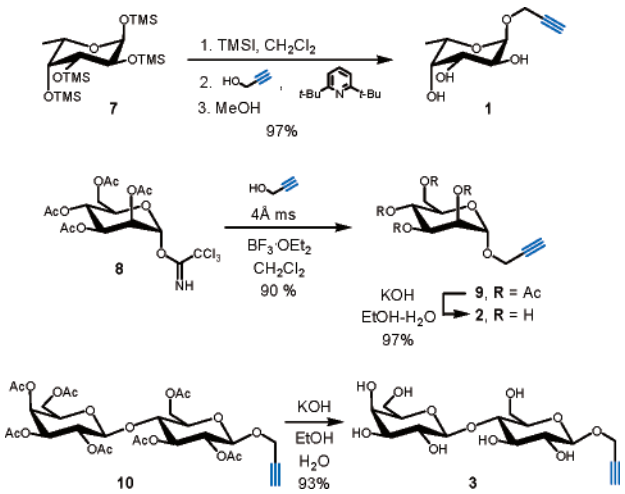
Figure 1. MALDI-TOF MS of **[G1]-N_3**, **[G2]-N_3**, and **[G3]-N_3**.

from acid **4** and *n*-$PrNH_2$ through an amide linkage [EDC, HOBt, CH_2Cl_2] in 85% yield. Hydrogenation of the terminal azides in **[G1]-N_3** [H_2 (1 atm), Pd/C (10%), MeOH] and treatment of the resulting dendritic amine with 3.5 equiv of acid **4** (EDC, HOBt, CH_2Cl_2) led to the dendrimer of generation two **[G2]-N_3** in 84% yield. By repetition of the same sequence hydrogenation-amide formation with acid **4** (18 equiv), **[G3]-N_3** was obtained in a very good 80% yield (Scheme 3). **[Gn]-N_3** dendrimers were characterized by NMR, MALDI−TOF MS, and elemental analysis. The presence of azide groups was confirmed by the appearance of intense signals at 2106−2108 cm^{-1} in the IR spectra of these species.

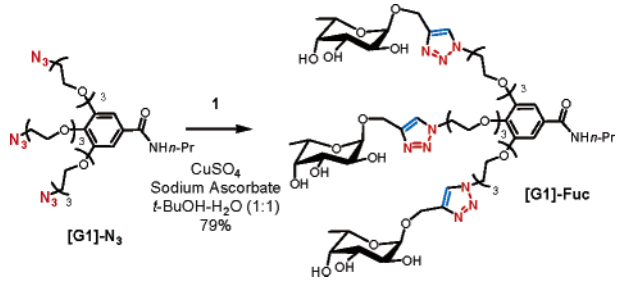
The generation growth in the synthesis of these dendrimers and the completion of the substitution were easily followed by ^1H NMR thanks to the characteristic signals of the methylene protons adjacent to the azide (3.35−3.40 ppm) and amino groups (2.84−2.91 ppm). Especially interesting was the effect of generation on the shift and shape of the aromatic signals in the ^1H NMR spectrum. Thus, while a singlet at 7.04 ppm appeared in the ^1H NMR spectrum of **[G1]-N_3**, and three singlets at 7.04, 7.09, and 7.12 ppm (in a ratio 1:2:1) for **[G2]-N_3**, only a broad signal became visible in the same region of the **[G3]-N_3** spectrum.

MALDI-TOF MS of **[Gn]-N_3** allowed us to clearly confirm beyond doubt the completion of the substitution and the purity of the products. Spectra were best obtained with 2-(4-hydroxyphenylazo)benzoic acid (HABA) as matrix and NaCl as cationizing agent (Figure 1).[17] In all cases the molecular weight of the peaks with highest intensity matched well those for the calculated sodium adducts $[M + Na]^+$. Lower intensity peaks agreed well with species derived from protonation or potassium cationization and from several losses of N_2 from azide groups which occurred during the MALDI experiment.[18] Also, in the spectrum of **[G3]-N_3**, two lower intensity peaks at *m*/*z* 7272.7 and 5475.2 are present. They have been ascribed to fragmentation of amide linkages due to the increasingly higher laser power required in the MALDI experiments, of the heavier dendrimers. The peak at 5475.2 has been attributed to $[M + K-C_{100}H_{155}N_{30}O_{40}]^+$ (calcd 5475.8) and results from the fragmentation at an inner amide bond, while the peak at 7272.7 is attributed to $[M + K-C_{25}H_{38}N_9O_{10}]^+$ (calcd 7268.6) and arises from the MALDI fragmentation at an outer amide bond.

Scheme 4



Scheme 5



Thus, in this way pure and well-characterized azido-terminated dendrimers amenable to surface decoration by means of click chemistry have been prepared. To test the viability of the Cu(I)-catalyzed azide−alkyne [3 + 2] cycloaddition for the ready synthesis of unprotected glycodendrimers from **[G1]-N_3**, **[G2]-N_3**, and **[G3]-N_3**, three unprotected alkyne glycosides **1**−**3**, derived respectively from α-L-fucose, α-D-mannose, and *β*-D-lactose, were prepared (Scheme 4), and their coupling under click conditions with the above azide dendrimers was studied.

Fucoside **1** was obtained from the readily available trimethylsilylated fucose **7**,[13] by treatment with TMSI, and then with propargyl alcohol. Subsequent deprotection of the TMS groups with MeOH led to **1** in an excellent 97% yield. The mannose derivative **2** was obtained in very good yield (87%) from mannose trichloroacetimidate **8**,[14] by treatment with propargyl alcohol (BF_3·OEt_2, CH_2Cl_2), followed by hydrolysis of the acetate groups. Similarly, hydrolysis of the acetate groups of **10**[16] led to the alkyne derived lactose **3** in an excellent 93% yield (Scheme 4).

Indeed, when **[G1]-N_3** was treated with fucoside **1** in the presence of $CuSO_4$ (1 mol % per terminal azide) and sodium ascorbate (5 mol % per terminal azide) in *t*-BuOH−H_2O (1:1, 26 h, rt), pure **[G1]-Fuc** was obtained in 79% yield, after purification by silicagel chromatography (Scheme 5). The complete incorporation of three fucose residues in **[G1]-Fuc** was clearly established by ^1H NMR thanks to the disappearance of the signal (3.40−3.35 ppm) characteristic of the methylene

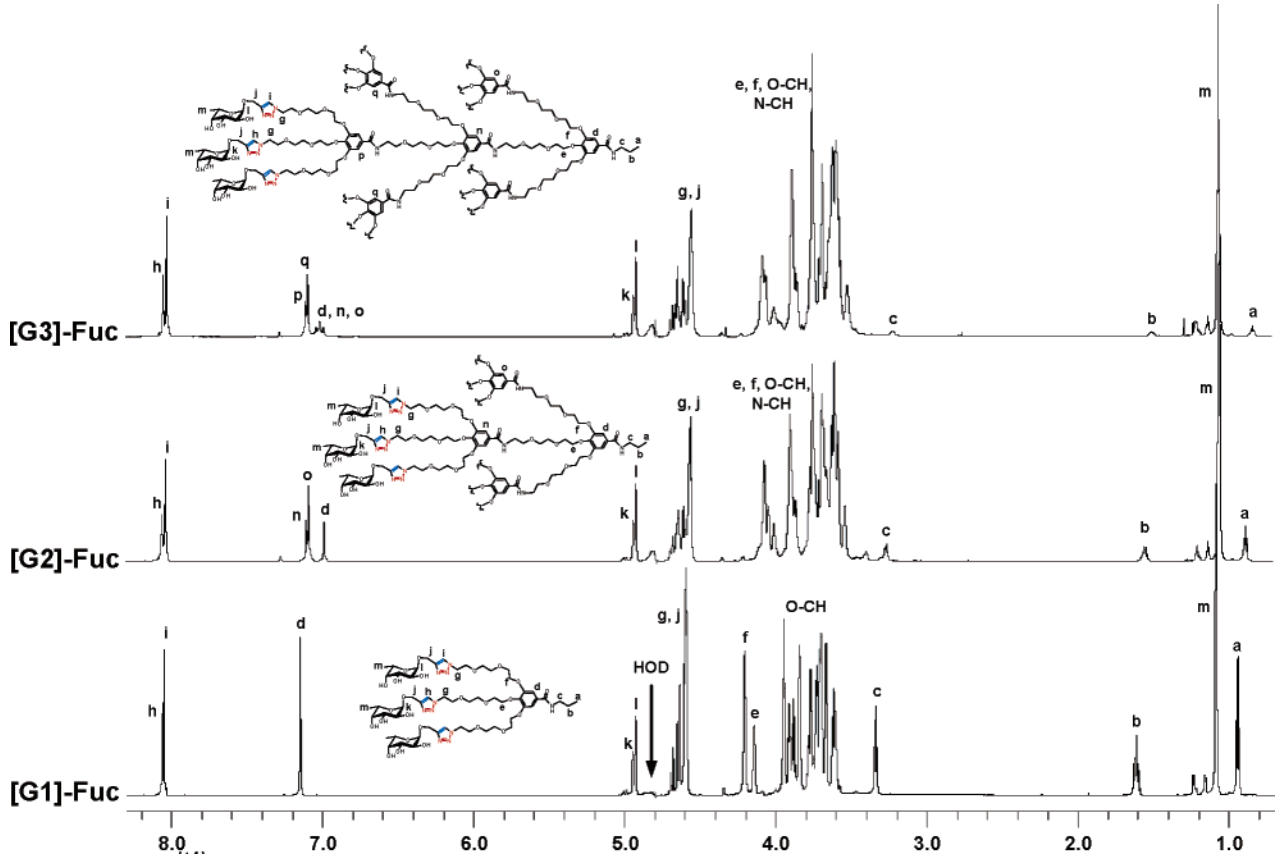


Figure 2. ^{1}H NMR spectra of **[G1]-Fuc**, **[G2]-Fuc**, and **[G3]-Fuc** (750 MHz, D_2O).

protons adjacent to the azide groups. Also, the presence of two singlets at 8.05 and 8.04 ppm (1:2 ratio), corresponding to the triazol protons, and of two doublets at 4.94 (J = 3.6 Hz) and 4.92 (J = 3.3 Hz) (1:2 ratio), due to the anomeric protons, indicates the presence of three fucose residues (Figure 2).

The completion of the multivalent glycosidation and the purity of **[G1]-Fuc** was also confirmed by the MALDI-TOF MS, showing the presence of a single peak at m/z = 1311.7 matching well the calculated value for the $[M + Na]^+$ adduct at m/z = 1312.3 (Figure 3).

Similarly, when mannoside **2** and lactoside **3** were coupled to **[G1]-N$_3$**, the resulting multivalent glycosides were obtained in good yields: **[G1]-Man**, 75%; and **[G1]-Lac**, 75% (Scheme 6). Both **[G1]-Man** and **[G1]-Lac** showed NMR and MALDI-TOF features similar to those of the fucose counterpart. The completion of the conjugation process was established, as before, using those techniques (Table 1 and Supporting Information).

When G2 and G3 dendrimers were treated with glycosides **1**, **2**, and **3** under similar conditions as above, pure glycoconjugates incorporating 9 and 27 residues of fucose, mannose, and lactose, respectively, for **[G2]-N$_3$** and **[G3]-N$_3$** were obtained in excellent yields (85−92%, Scheme 6). Purification of these highly polar species, with up to 189 hydroxyl groups in the case of **[G3]-Lac**, was better realized by means of ultrafiltration through Amicon YM1 membranes, a technique that in our hands quickly delivered pure glycodendrimers of G2 and G3 in higher yields and considerably less time than standard chromatography led to the G1 counterparts.

The analysis of the ^{1}H NMR spectra of glycodendrimers of G2 and G3 showed, for all of them, complete disappearance of the signal at 3.40−3.35 ppm that corresponds to the methylene protons adjacent to the azide groups, confirming the completion of the multivalent glycoconjugation (Figure 2). The presence

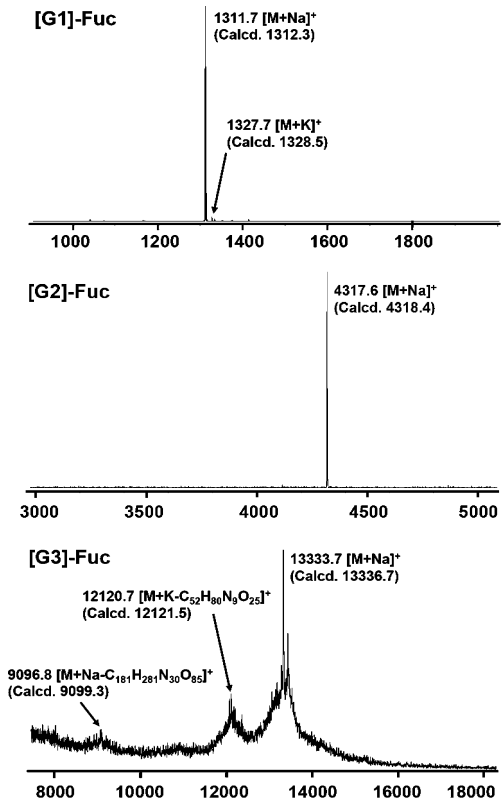


Figure 3. MALDI-TOF MS of **[G1]-Fuc**, **[G2]-Fuc**, and **[G3]-Fuc**.

of two sets of signals for the triazol and anomeric protons, in the same 1:2 ratio previously seen for the G1 glycodendrimers, revealed the chemical resemblance of all the glycosides at the external C-3 and C-5 gallic acid positions (the same applies

Scheme 6

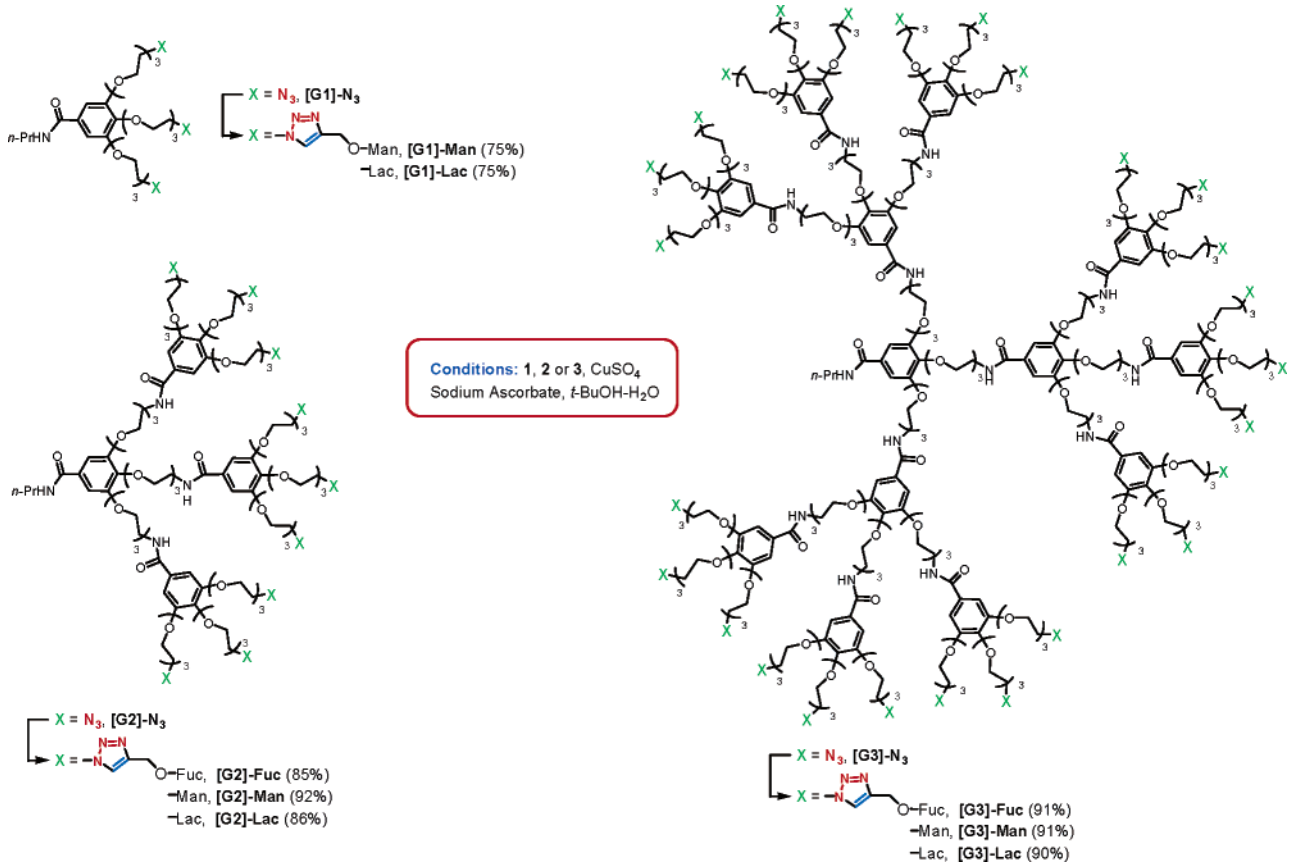


Table 1. Molecular Weights of Glycodendrimers Measured by MALDI-TOF MS

dendrimer		MW[a]	dendrimer		MW[a]
[G1]-Fuc	1311.7	[1312.3][b]	**[G2]-Lac**	5920.3	[5921.7][b]
[G1]-Man	1359.9	[1360.3][b]	**[G3]-Fuc**	13333.7	[13336.7][b]
[G1]-Lac	1847.4	[1846.7][b]	**[G3]-Man**	13693.0	[13746.7][c]
[G2]-Fuc	4317.6	[4318.4][b]	**[G3]-Lac**	18116.9	[18124.4][c]
[G2]-Man	4466.4	[4462.4][b]			

[a] Numbers in brackets are calculated molecular weights. [b] $[M + Na]^+$. [c] $[M + H]^+$.

for all the glycosides at the C-4 positions) independently of the dendrimer generation.

The completion of the substitution and the purity of the resulting glycosylated products were again confirmed by MALDI-TOF MS (Table 1). Thus, very clean MALDI-TOF spectra were obtained for the G2 glycodendrimers (Figure 3 and Supporting Information), with fine peaks centered at *m*/*z* values matching those for the calculated sodium or potassium adducts. In the case of the lactose derivative, minute fragmentation at the labile anomeric positions can also be detected.

As generation increases, dendrimers fly with more difficulty in MALDI-TOF MS, and higher laser power must be applied. Consequently, the quality of the baseline is degraded, and broader peaks, as well as some fragmentation, appear in the MALDI-TOF spectra of the G3 glycodendrimers (Figure 3 and Supporting Information). Thus, two intense and broad peaks at *m*/*z* between 12 000 and 14 000 are present in the MALDI-TOF spectrum of **[G3]-Fuc** (Figure 3). The most intense one is at *m*/*z* 13 333.7 and corresponds to the expected sodium adduct (calcd for $[M + Na]^+$: 13 336.7), while the less intense peak at 12 120.7 and another one at 9096.8 correspond to fragmentations during the MALDI experiment. These have been attributed to cleavages at outer (calcd for $[M + K - C_{52}H_{80}N_9O_{25}]^+$: 12121.5) and inner amides (calcd for $[M + Na - C_{181}H_{281}N_{30}O_{85}]^+$: 9099.3), as previously seen for **[G3]-N_3**. The MALDI-TOF spectra of the mannose and lactose G3 counterparts are shown in the Supporting Information and present similar features to those described for the other glycodendrimers. Overall, our results indicate that characterization of high-molecular-weight dendrimers of this type by MALDI-TOF exclusively presents some problems due to cleavage of the macromolecule by the laser bean producing fragments that could be erroneously interpreted as the result of incomplete reaction. ^{1}H NMR monitoring of that transformation can be used to ensure the integrity of the dendrimers and the completeness of the reaction.

Conclusion

Click chemistry in combination with ultrafiltration has proved to be an invaluable tool for the quick, efficient, and reliable conjugation of unprotected alkyne-derived carbohydrates to dendritic systems incorporating terminal azides, as opposed to the lengthier process of employing protected glycosides or introducing the click functionality on each generation of previously synthesized dendrimers.

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Supporting Information Available: MALDI-TOF spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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