

Synthesis of Clickable Amphiphilic Polysaccharides as Nanoscopic Assemblies*

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1. General Information

All chemicals and solvents were purchased from Sigma-Aldrich, unless otherwise noted. Anhydrous solvents (dichloromethane, 99.8%, tetrahydrofuran, N,N-dimethylformamide 99.8%) were purchased in capped Sureseal™ bottles and were purified with a Mbraun solvent purification system. Other solvents and reagents were used without further purification. All glassware utilized was flame-dried before use. Glass-backed TLC plates (Silica Gel 60 with a 254 nm fluorescent indicator) were used without further manipulation and stored over desiccant. Silica gel column chromatography was performed using flash silica gel (32-63 μm) and employed a solvent polarity correlated with TLC mobility.

HCT116 cells and HEK293 cells were purchased from American Type Culture Collection (ATCC, <http://www.atcc.org/>). Fetal Bovine Serum (FBS) was purchased from Atlanta Biologicals. McCoy's medium was purchased from Gibco. Dulbecco's modified eagle's medium (DMEM) was

purchased from Lonza. MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] reagent was purchased from Promega.

FT-IR spectra were obtained on a Thermo Scientific Nicolet™ iS™50 Fourier-transform infrared (FT-IR) spectrometer using NaCl plates, with the sample being deposited from CH₂Cl₂ and allowing for evaporation of the solvent before measurement.

¹H-NMR spectra were recorded at 500 MHz on a Varian Inova 500 spectrometer, with tetramethylsilane (TMS) proton signal as the standard. ¹³C-NMR spectra were recorded at 126 MHz on a Varian Inova 500 spectrometer, with TMS carbon signal as the standard.

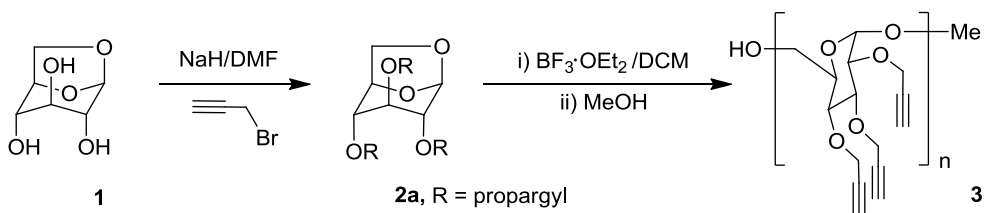
Gel permeation chromatography (GPC) analysis was conducted using a Viscotek GPC system equipped with a TDA270 dual-detector and a column system comprised of one PAS102 and one PAS103 column (Polyanalytik Inc.). The system was equilibrated at 35 °C in THF, which served as the polymer solvent and eluent with a flow rate of 1.0 mL min⁻¹. Polymer solutions were prepared at a known concentration (*ca.* 3 mg mL⁻¹) and an injection volume of 150 μL was used. Data collection and analyses were performed by OmniSEC software system from Malvern Inc.

Mass spectrometry was measured with a Waters LCT Premier™ XE unit.

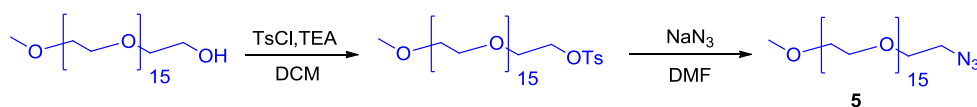
Dynamic light scattering (DLS) measurements were performed by Zetasizer 3000HSA analyzer (Malvern Instruments). Before the analysis, the NP solution was filtered through a 0.45-μm VWR Nylon membrane filter to remove dust particles. Scattered light was collected at a fixed angle of 90°. The calculation of the particle size and size distribution was performed using the installed software from Malvern Instruments.

Transmission Electron Microscope (TEM) measurements were performed using a Philips CM-10 Unit with 60 kV accelerating voltage. Micrographs were collected at 105,000× magnification and calibrated using a 41-nm polyacrylamide bead from NIST. Carbon-coated copper grids were treated with

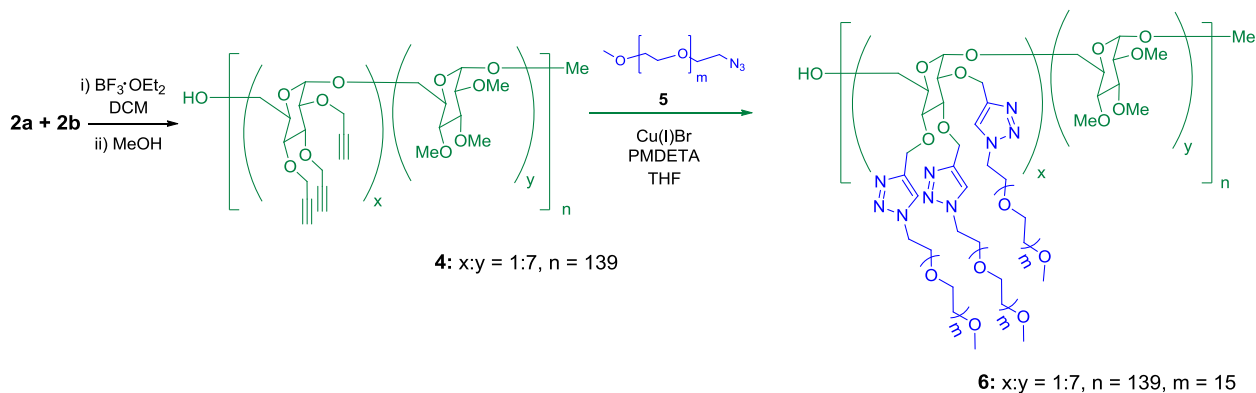
oxygen plasma before deposition of the NP samples. The samples were deposited on the carbon grids for 1 min, and excess samples were wicked away. The samples were allowed to dry under ambient conditions. The number-average particle diameters and standard deviations were generated from the analysis of a minimum of 100 particles.



Scheme 1S. The synthesis of monomer **2a** and its ROPAS to give homo-polysaccharide **3**.



Scheme 2S. The synthesis of azide-functionalized polyethylene glycol (azide-PEG) **5**.



Scheme 3S. The synthesis of co-polymer **4** and the subsequent click conjugation with **5** to give PEG-grafted polysaccharide **6**.

2. Synthesis of 2,3,4-tri-*O*-propargyl-1,6-anhydro- β -D-glucopyranose (**2a**):

To a solution of 1,6-anhydro- β -D-glucose (3.0 g, 18.5 mmol, 1.0 eq.) in DMF (120 mL) was added sodium hydride (NaH) (1.78 g, 74.0 mmol, 4.0 eq.). The reaction was stirred under nitrogen for 20 min. Subsequently, propargyl bromide (CH \equiv C-CH $_2$ -Br) (8.8 g, 74.0 mmol, 4.0 eq.) was added. The solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (hexanes/ethyl acetate = 3:1, R_f = 0.4) to afford the product as yellow gel-like powder (3.8 g, 75%). $[\alpha]_D^{25}$ = -39.9 (c = 1 in CHCl $_3$). $^1\text{H-NMR}$ (500 MHz, CDCl $_3$) δ = 5.48 (s, 1H, β -H1), 4.64 (dd, J = 5.8, 1.3, 1H, H-5), 4.37-4.32 (m, 6H, C \equiv C-CH $_2$), 4.00 (dd, J = 7.3, 1.0, 1H, H-6), 3.82 – 3.77 (m, 1H, H-4), 3.73 (dd, J = 7.3, 5.8, 1H, H-6'), 3.65 – 3.64 (m, 1H, H-3), 3.56 – 3.55 (m, 1H, H-2), 2.50 (ddd, J = 3.9, 3.5, 2.0, 3H, C \equiv C-H). $^{13}\text{C-NMR}$ (126 MHz, CDCl $_3$) δ = 100.37 (β -C1), 79.58-79.50 (C \equiv C-H), 76.06 (C-3), 75.59 (C \equiv C-H), 75.56 (C-4), 75.53 (C \equiv C-H), 75.43 (C-2), 74.13 (C-5), 65.52 (C-6), 57.89-56.85 (C \equiv C-CH $_2$). ESI-MS calc. for C $_{15}$ H $_{16}$ NaO $_5$ [M+Na] $^+$ = 299.27, found: 299.27. IR: 3285(C \equiv C-H stretching), 2965, 2904, 2861(C-H stretching), 2117(C \equiv C stretching), 1148(C-C stretching), 1083(C-O stretching).

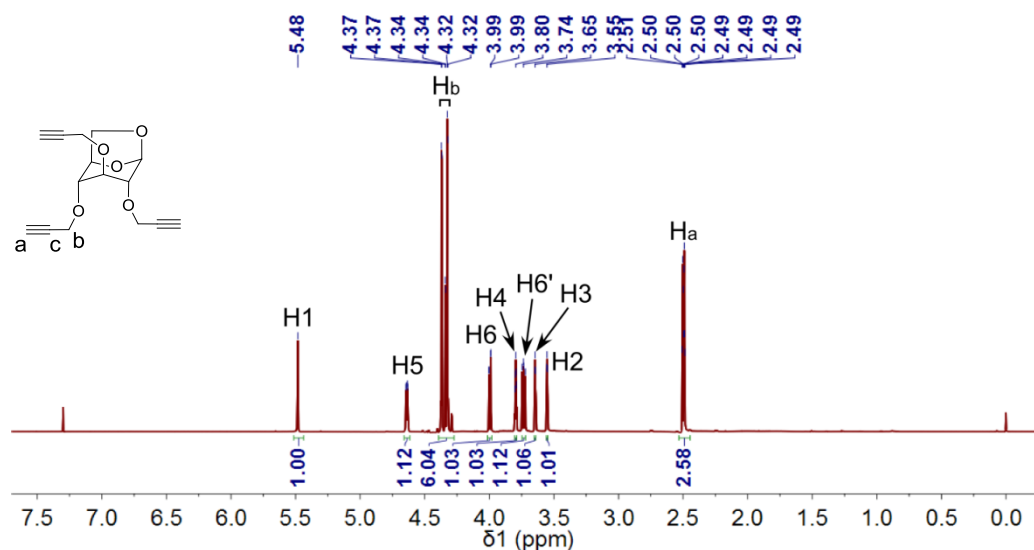


Figure 1S. $^1\text{H-NMR}$ spectrum of monomer **2a**.

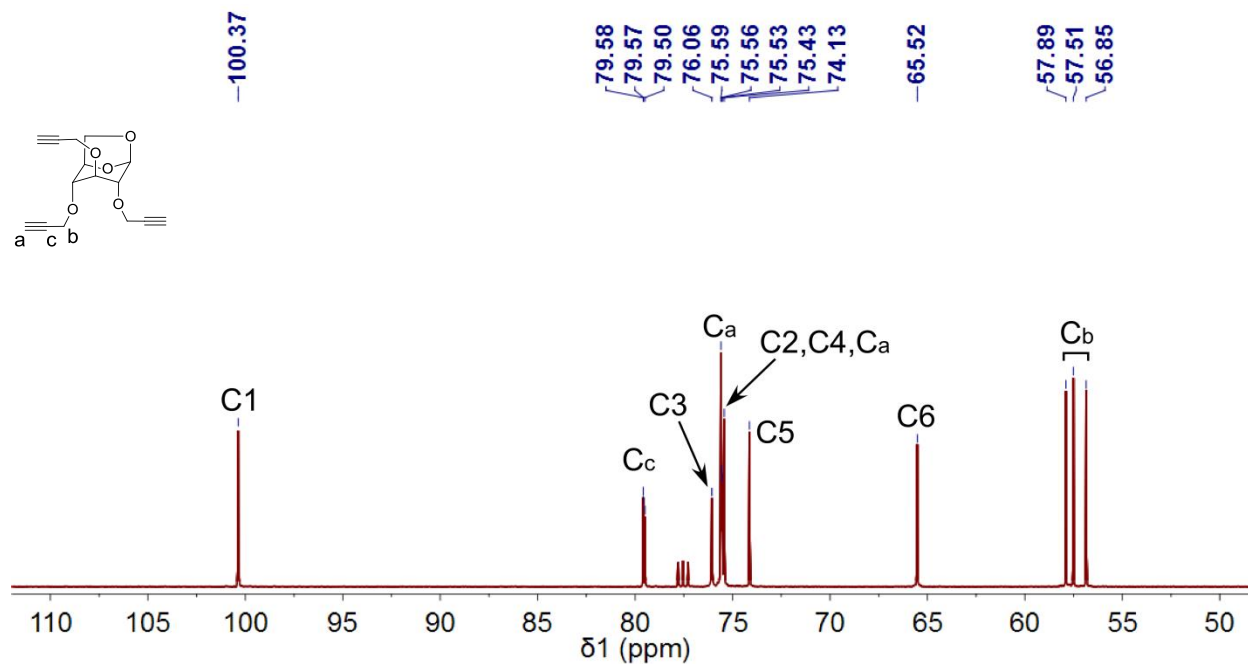


Figure 2S. ^{13}C -NMR spectrum of monomer **2a**.

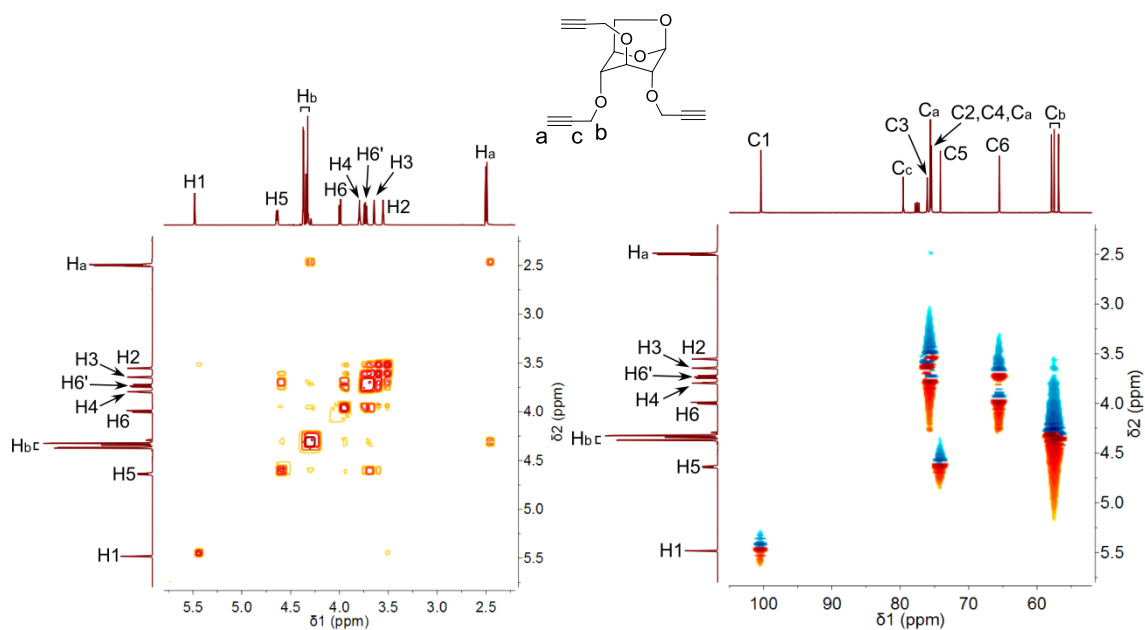


Figure 3S. 2D COSY and HMQC NMR spectra of monomer **2a**.

3. Synthesis of 2,3,4-tri-*O*-methyl-1,6-anhydro- β -D-glucopyranose (**2b**)

To a solution of 1,6-anhydro- β -D-glucose (3.0 g, 18.5 mmol, 1.0 eq.) in DMF (120 mL) was added sodium hydride (NaH) (1.78 g, 74.0 mmol, 4.0 eq.). The reaction was stirred under nitrogen for 20 min. Subsequently, iodomethane (MeI) (10.5 g, 74.0 mmol, 4.0 eq.) was added. The solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (hexanes/ethyl acetate = 8/2, R_f = 0.3) to afford the product as a white powder (2.96 g, 78%). $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ = 5.48 (s, 1H, H-1), 4.64 (dd, J = 5.9, 1.4, 1H, H-5), 3.93 (dd, J = 7.2, 1.1, 1H, H-6), 3.74 (dd, J = 4.4, 3.1, 1H, H-6'), 3.48 (s, 3H, -OMe), 3.47 (s, 3H, -OMe), 3.45 (s, 3H, -OMe), 3.35 – 3.31 (m, 1H, H-4), 3.16 – 3.13 (m, 1H, H-3), 3.13 – 3.09 (m, 1H, H-2). $^{13}\text{C-NMR}$ (126 MHz, CDCl_3) δ = 100.00 (C-1), 79.00 (C-3), 78.44 (C-4), 78.36 (C-2), 73.27 (C-5), 65.32 (C-6), 58.16 (-OMe), 58.02 (-OMe), 57.34 (-OMe).

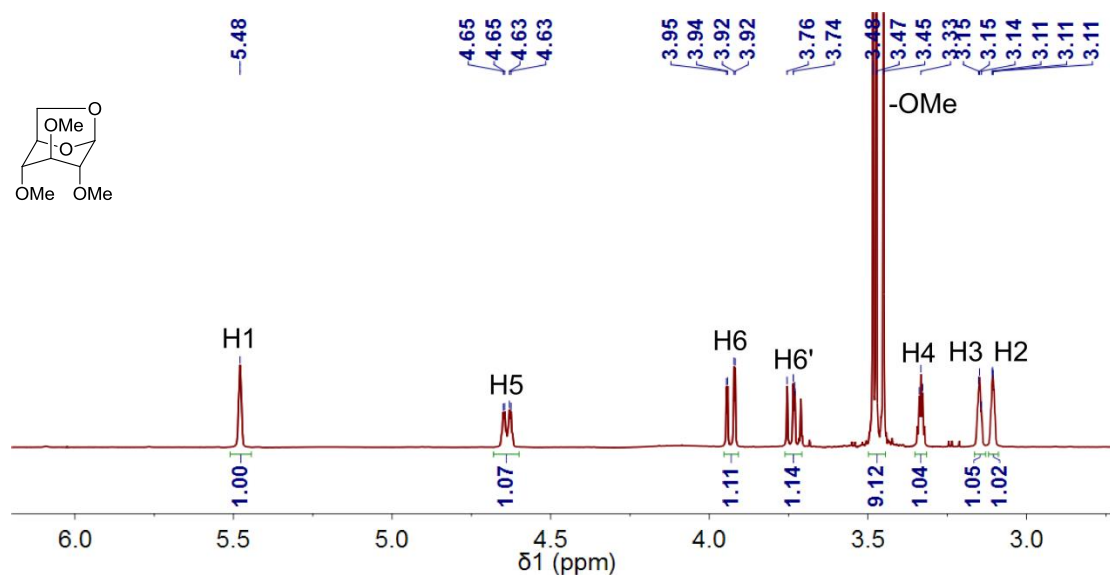


Figure 4S. $^1\text{H-NMR}$ spectrum of monomer **2b**.

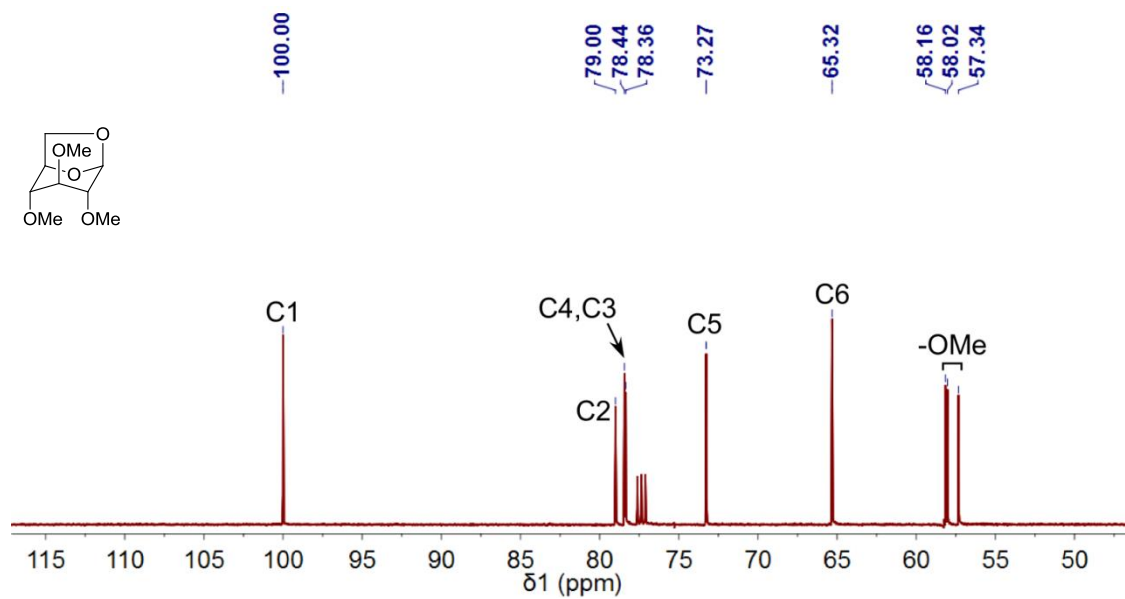


Figure 5S. ^{13}C -NMR spectrum of monomer **2b**.

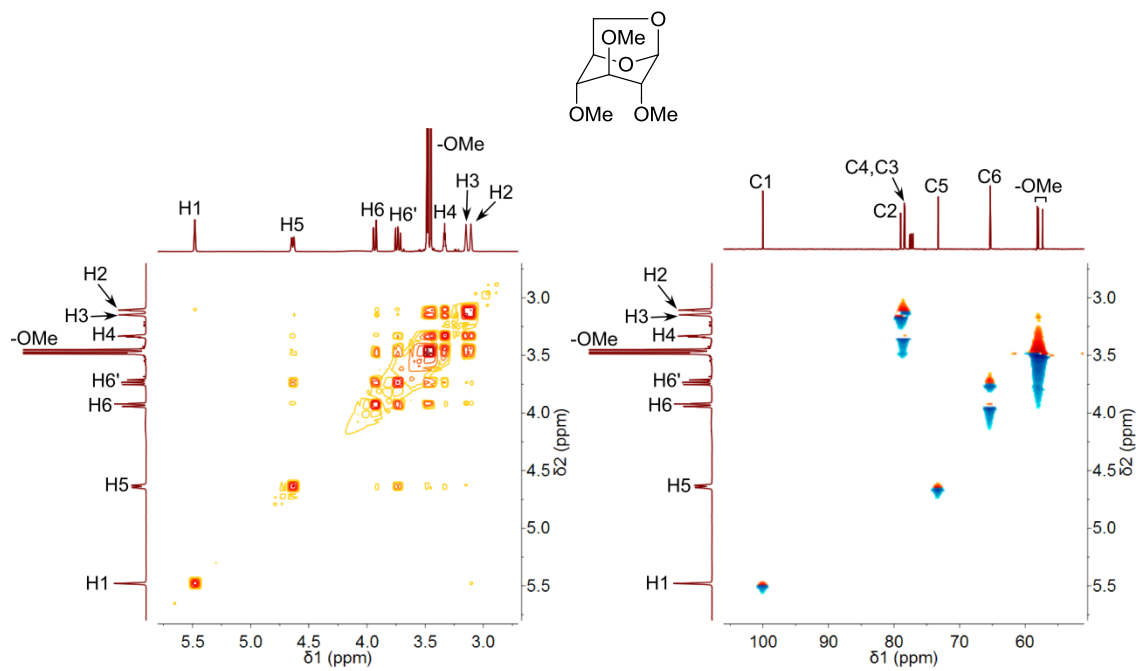


Figure 6S. 2D COSY and HMQC NMR spectra of monomer **2b**.

4. Synthesis of poly(2,3,4-tri-*O*-propargyl- α -D-glucopyranose) (**3**)

To a flame-dried Schlenk flask was added monomer **2a** (0.28 g, 1.0 mmol, 1.0 eq.), which was dried under vacuum for 72 h. Thereafter, anhydrous dichloromethane (3.0 mL) was added *via* cannulation. After three cycles of freeze-pump-thaw, $\text{BF}_3 \cdot \text{OEt}_2$ (7.1 mg, 0.05 mmol, 0.05 eq.) was added *via* cannulation and the reaction mixture was stirred for 4 h. The reaction was quenched by adding MeOH (1 mL) and the product was purified by precipitation in cold MeOH to afford the polymer as a slightly yellow powder (0.121 g, 43%). $M_n^{\text{GPC}} = 36.1$ kDa; PDI = 1.4. $[\alpha]_D^{25} = +176.3$ ($c = 1$ in CHCl_3). $^1\text{H-NMR}$ (500 MHz, CDCl_3) $\delta = 5.03$ (d, 1H, α -H1), 4.53 – 4.40 (m, 4H, $\text{C}\equiv\text{C-CH}_2$), 4.31 (dd, 2H, $\text{C}\equiv\text{C-CH}_2$), 3.84 (t, 2H, H-5 and H-6), 3.77 (d, 2H, H-4 and H-6'), 3.61 – 3.51 (m, 2H, H-2 and H-3), 2.63 – 2.44 (m, 3H, $\text{C}\equiv\text{C-H}$). $^{13}\text{C-NMR}$ (126 MHz, CDCl_3) $\delta = 96.79$ (α -C1), 81.20 (C-5), 80.53-80.10 ($\text{C}\equiv\text{C-H}$), 79.12 (C-2), 76.55(C-3), 75.29-74.54 ($\text{C}\equiv\text{C-H}$), 70.22 (C-4), 66.08 (C-6), 60.42-60.17 ($\text{C}\equiv\text{C-CH}_2$), 57.76 ($\text{C}\equiv\text{C-CH}_2$). IR: 3290 ($\text{C}\equiv\text{C-H}$ stretching), 2931, 2872 (C-H stretching), 2118 ($\text{C}\equiv\text{C}$ stretching), 1161 (C-C stretching), 1084 (C-O stretching).

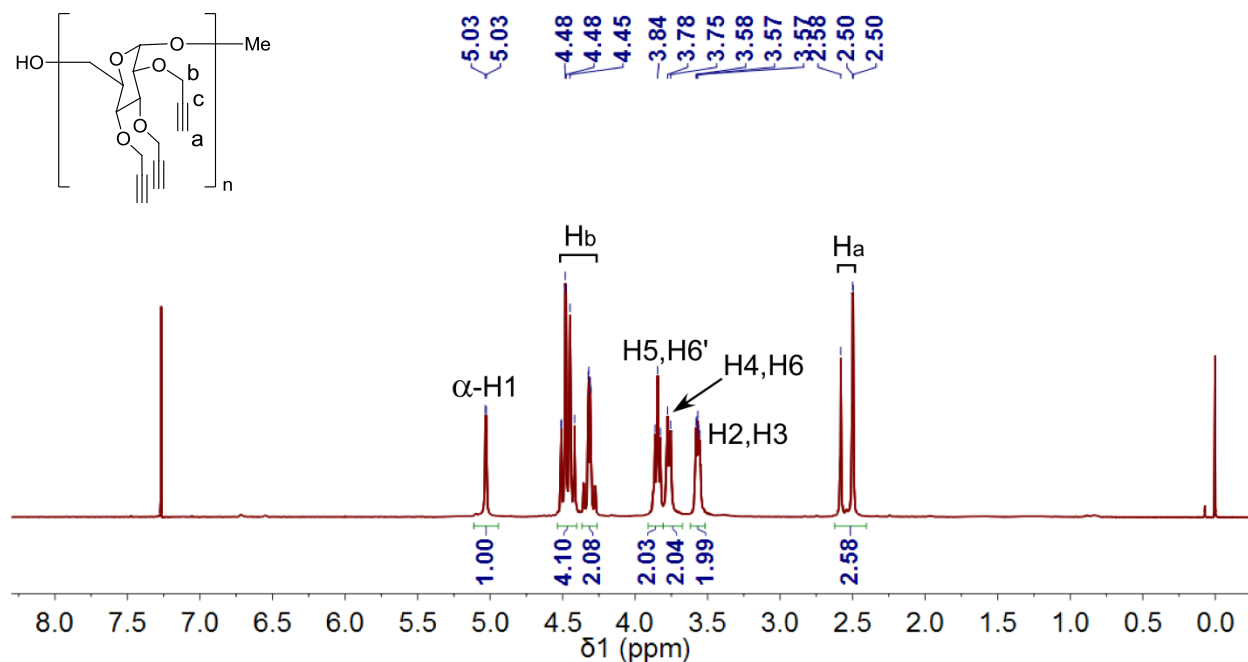


Figure 7S. $^1\text{H-NMR}$ spectrum of polysaccharide **3**.

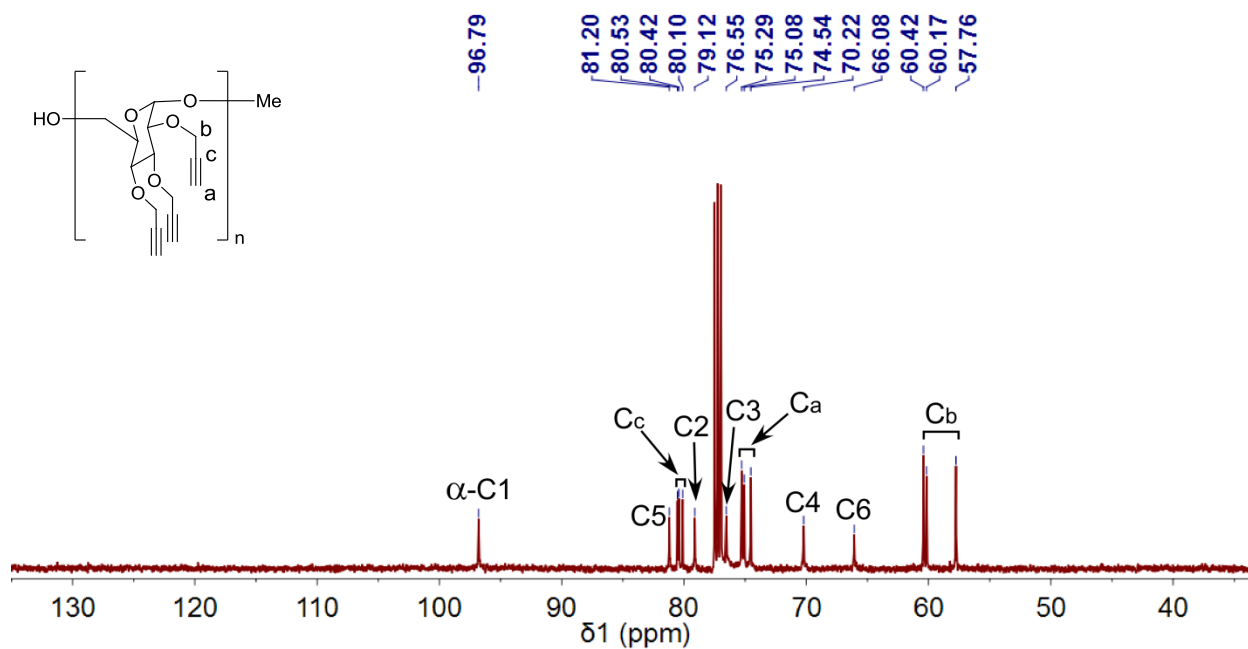


Figure 8S. ^{13}C -NMR spectrum of polysaccharide 3.

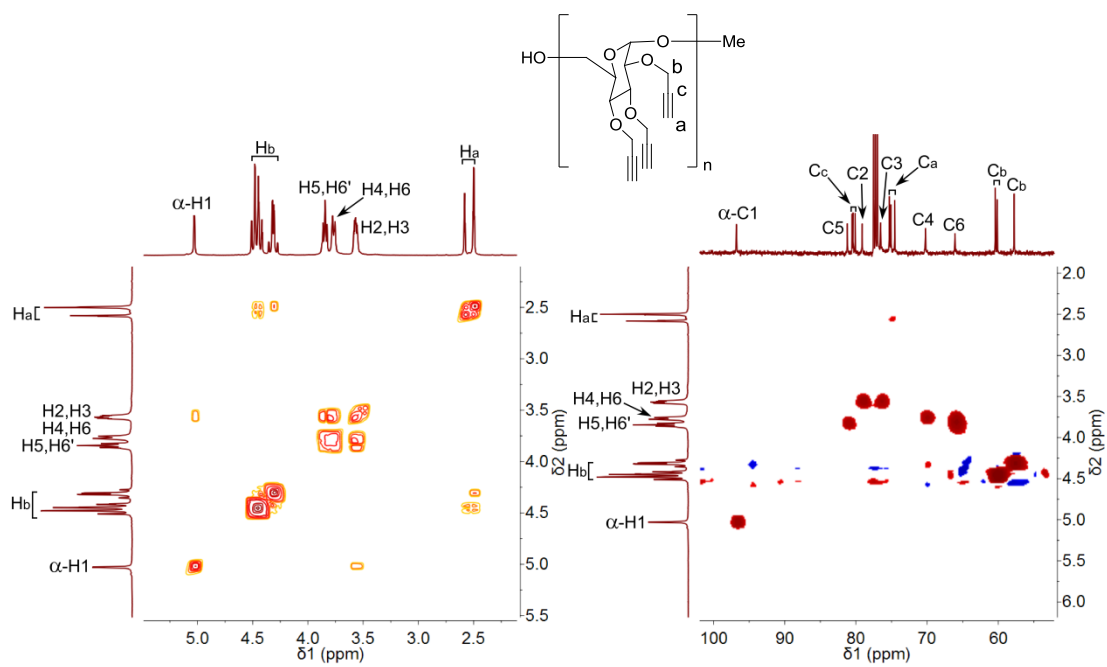


Figure 9S. 2D COSY and HMQC NMR spectra of polysaccharide 3.

5. Synthesis of poly(2,3,4-tri-*O*-propargyl- α -D-glucopyranose)-*co*-(2,3,4-tri-*O*-methyl- α -D-glucose) (**4**)

To a flame-dried Schlenk flask was added monomers **2a** (0.031 g, 0.112 mmol, 1.0 eq.) and **2b** (0.160 g, 0.784 mmol, 7.0 eq.), which were dried under vacuum for 72 h. Therefore, dichloromethane (3.0 mL) was added *via* cannulation. After three cycles of freeze-pump-thaw, $\text{BF}_3 \cdot \text{OEt}_2$ (6.4 mg, 0.045 mmol) was added *via* cannulation and the reaction mixture was stirred at rt for 4 h. The reaction was quenched by adding MeOH (1 mL) and the product was purified by precipitation in cold MeOH to afford the polymer as a slightly yellow powder (0.087 g, 45%). $M_n^{\text{GPC}} = 34.5 \text{ kDa}$; PDI = 1.3. $^1\text{H-NMR}$ (500 MHz, CDCl_3) $\delta = 5.00$ (H-1'), 4.96 (H-1), 4.55-4.24 (C \equiv C-CH $_2$), 3.81-3.12 (sugar protons), 3.60-3.45 (-OMe), 2.58-2.45 (m, 3H, C \equiv C-H). $^{13}\text{C-NMR}$ (126 MHz, CDCl_3) $\delta = 96.81$ (C-1'), 96.57 (C-1), 83.51 (C-3), 81.93 (C-2), 81.35 (C-5'), 80.40-79.99 (C \equiv C-H), 79.63 (C-4), 79.16 (C-2'), 76.69 (C-3'), 75.15-74.46 (C \equiv C-H), 70.39 (C-5), 70.18 (C-4'), 66.12 (C-6), 66.04 (C-6'), 60.92-60.70 (-OMe), 60.41-60.19 (C \equiv C-CH $_2$), 58.48 (-OMe), 57.85 (C \equiv C-CH $_2$). IR: 3256 (C \equiv C-H stretching), 2984, 2932, 2872 (C-H stretching), 2115 (C \equiv C stretching), 1158 (C-C stretching), 1101 (C-O stretching).

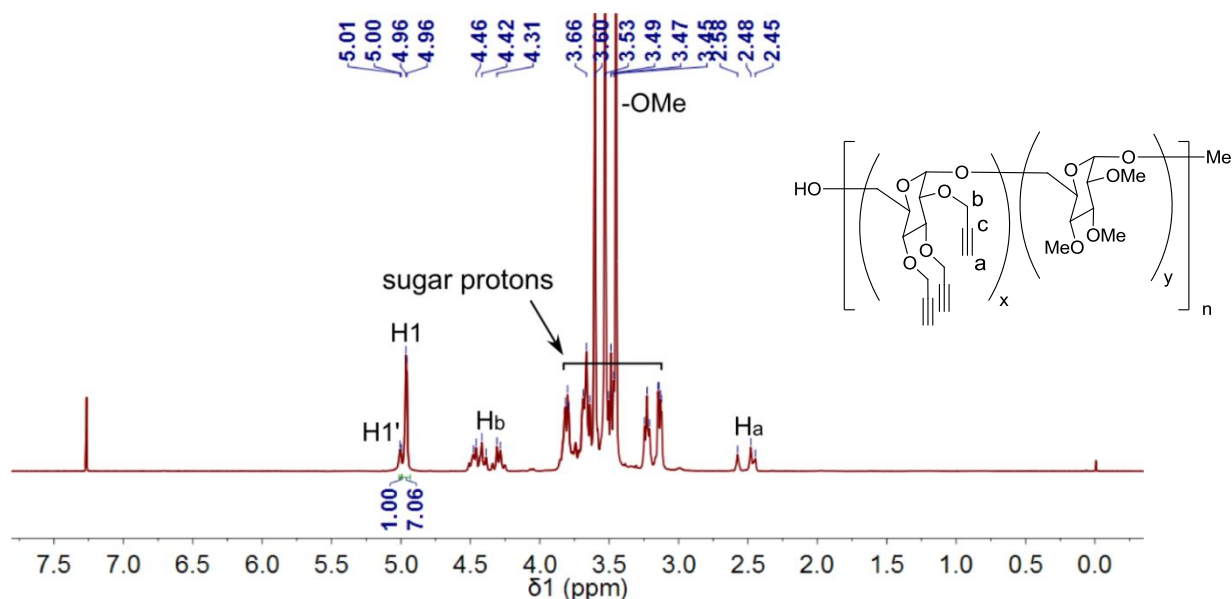


Figure 10S. $^1\text{H-NMR}$ spectrum of co-polymer **4**.

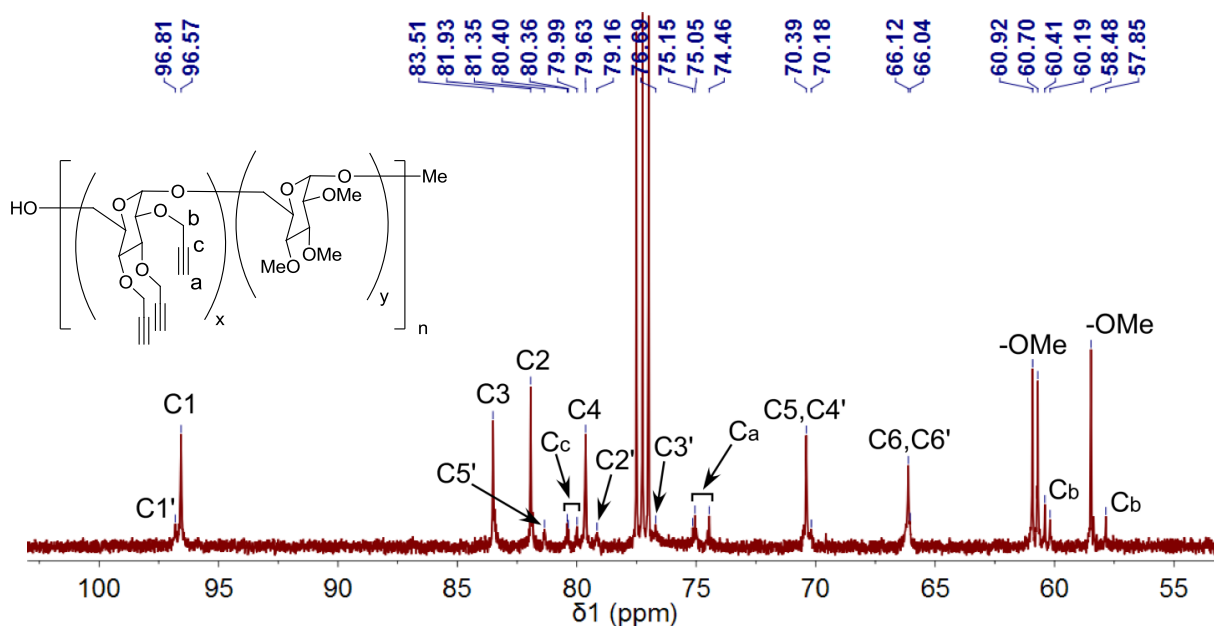


Figure 11S. ^{13}C -NMR spectrum of co-polymer **4**.

6. Synthesis of poly(2,3,4-tri-*O*-propargyl- α -D-glucopyranose)-*co*-(2,3,4-tri-*O*-methyl- α -D-glucose) with different monomer ratios

A: (**2a:2b** = 1:2): The synthesis was performed in a same way as that of co-polymer **4** with a monomer ratio: **2a:2b** = 1:2. $M_n^{\text{GPC}} = 32$ kDa; PDI = 1.4. ^1H -NMR (500 MHz, CDCl_3) $\delta = 5.03$ (H-1'), 4.98 (H-1), 4.48 – 4.33 ($\text{C}\equiv\text{C}-\text{CH}_2$), 3.84 – 3.17 (sugar protons), 3.62-3.50 (-OMe), 2.59 – 2.50 (m, 3H, $\text{C}\equiv\text{C}-\text{H}$). ^{13}C -NMR (126 MHz, CDCl_3) $\delta = 96.59$ (C-1'), 96.36 (C-1), 83.27 (C-3), 81.71 (C-2), 81.12 (C-5'), 80.18-79.77 ($\text{C}\equiv\text{C}-\text{H}$), 79.43 (C-4), 78.93 (C-2'), 76.27 (C-3'), 74.80-74.20 ($\text{C}\equiv\text{C}-\text{H}$), 70.17 (C-5 and C-4'), 65.91 (C-6 and C-6'), 60.66-60.43 (-OMe), 60.15-60.91 ($\text{C}\equiv\text{C}-\text{CH}_2$), 58.20 (-OMe), 57.61 ($\text{C}\equiv\text{C}-\text{CH}_2$).

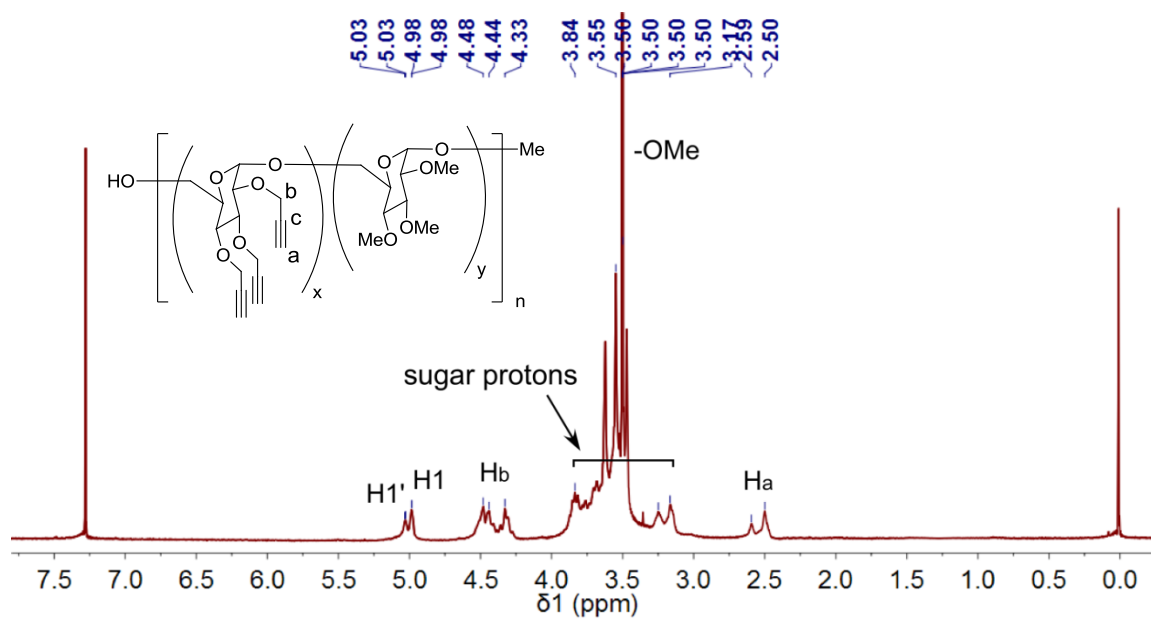


Figure 12S. $^1\text{H-NMR}$ spectrum of co-polymer ($2\mathbf{a}:2\mathbf{b} = 1:2$).

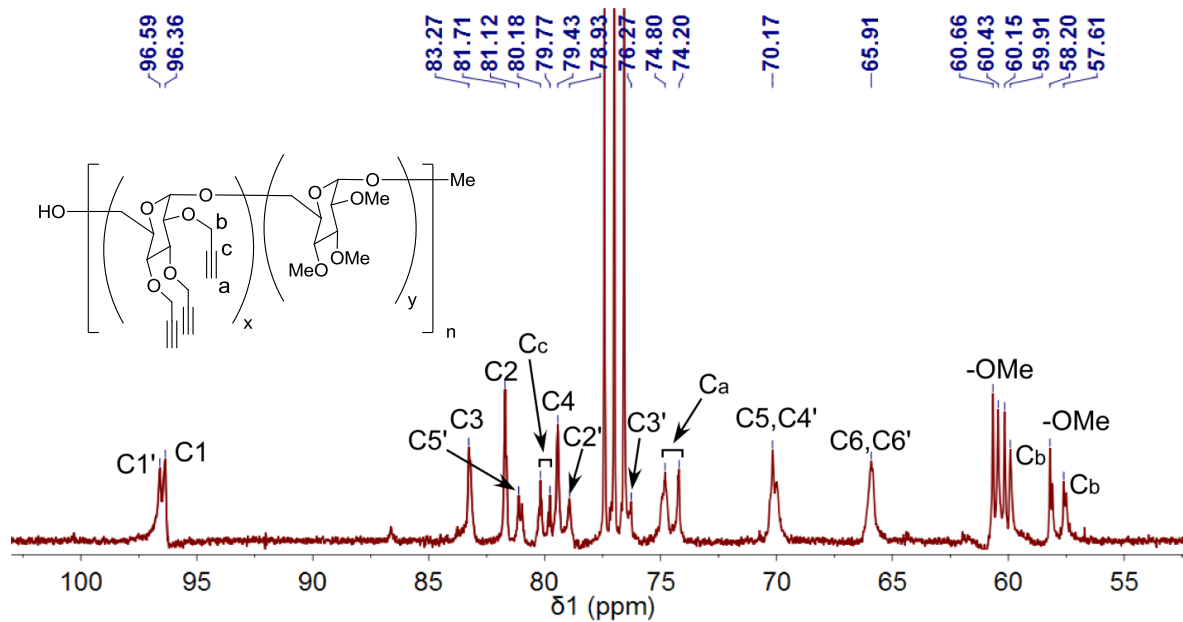


Figure 13S. $^{13}\text{C-NMR}$ spectrum of co-polymer ($2\mathbf{a}:2\mathbf{b} = 1:2$).

B: (2a:2b = 1:15): The synthesis was performed in a same way as that of co-polymer **4** with monomer ratio: **2a:2b** = 1:15. $M_n^{\text{GPC}} = 31 \text{ kDa}$; PDI = 1.4. $^1\text{H-NMR}$ (500 MHz, CDCl_3) $\delta = 5.02\text{-}4.98$ (H-1' and H-1), 4.44 – 4.31 (C \equiv C-CH $_2$), 3.84 – 3.17 (sugar protons), 3.63-3.48 (-OMe), 2.60 – 2.47 (m, 3H, C \equiv C-H). $^{13}\text{C-NMR}$ (126 MHz, CDCl_3) $\delta = 96.27$ (C-1' and C-1), 83.23 (C-3), 81.67 (C-2), 81.07 (C-5'), 80.11-80.06 (C \equiv C-H), 79.37 (C-4), 78.88 (C-2'), 76.36 (C-3'), 75.78-74.19 (C \equiv C-H), 70.11 (C-5 and C-4'), 65.85 (C-6 and C-6'), 60.59-60.37 (-OMe), 60.10-59.88 (C \equiv C-CH $_2$), 58.18 (-OMe), 57.56 (C \equiv C-CH $_2$).

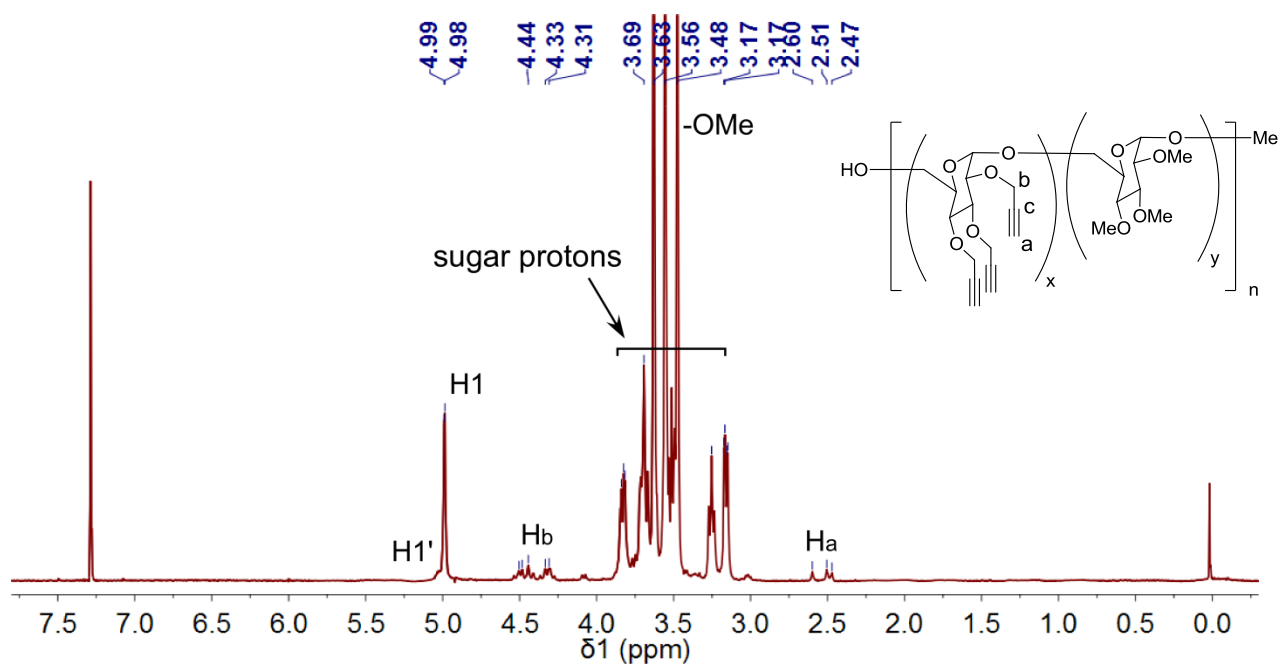


Figure 14S. $^1\text{H-NMR}$ spectrum of co-polymer (**2a:2b** = 1:15).

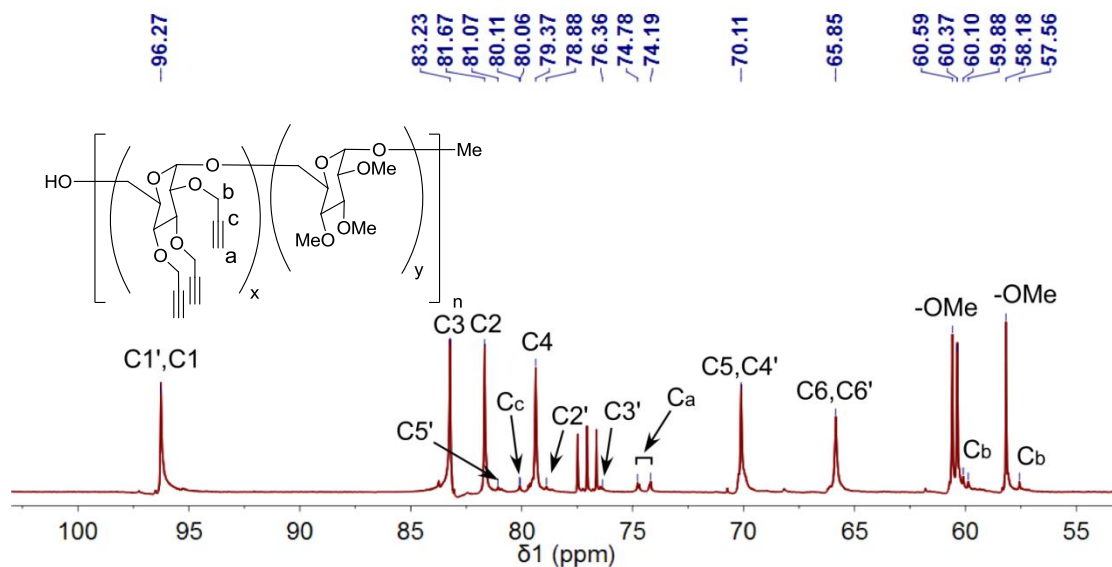


Figure 15S. ^{13}C -NMR spectrum of co-polymer (**2a:2b** = 1:15).

7. Synthesis of azido-methoxypolyethylene glycol (**5**)

To a solution of methoxypolyethylene glycol (mPEG) ($M_n = 750$) (7.5 g, 10.0 mmol, 1.0 eq.) in DCM (200 mL) was added *p*-toluenesulfonyl chloride (2.28 g, 12.0 mmol, 1.2 eq.) and triethylamine (1.0 g, 10.0 mmol, 1.0 eq.). The reaction was stirring at rt for 24 h. The reaction mixture was extracted with dichloromethane (2×300 mL) and water. The organic layer was combined, washed with brine, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was added into a solution of DMF (100mL) with sodium azide (0.98 g, 15.0 mmol, 1.5 eq.). The reaction mixture was stirred at 65 °C for 24 h. The solvent was removed under reduced pressure and the reaction mixture was suspended in ethyl acetate, any insoluble salt was filtered by Busch funnel. The ethyl acetate solution was collected and the solvent was removed under reduced pressure to give the product as a slightly yellow powder (6.9 g, 89%). ^1H -NMR (500 MHz, CDCl_3) δ = 3.58-3.56 (m, 56H, $-\text{OCH}_2\text{CH}_2-$), 3.49-3.43 (m, 2H, $-\text{CH}_2\text{N}_3$), 3.30 (s, 3H, $-\text{CH}_3$). ^{13}C -NMR (126 MHz, CDCl_3) δ = 72.07-70.19 ($-\text{OCH}_2\text{CH}_2-$), 59.18 ($-\text{CH}_3$), 50.84 ($-\text{CH}_2\text{N}_3$). IR: 2104 ($\text{N}\equiv\text{N}$ stretching), 2863 (C-H stretching), 1113 (C-O stretching).

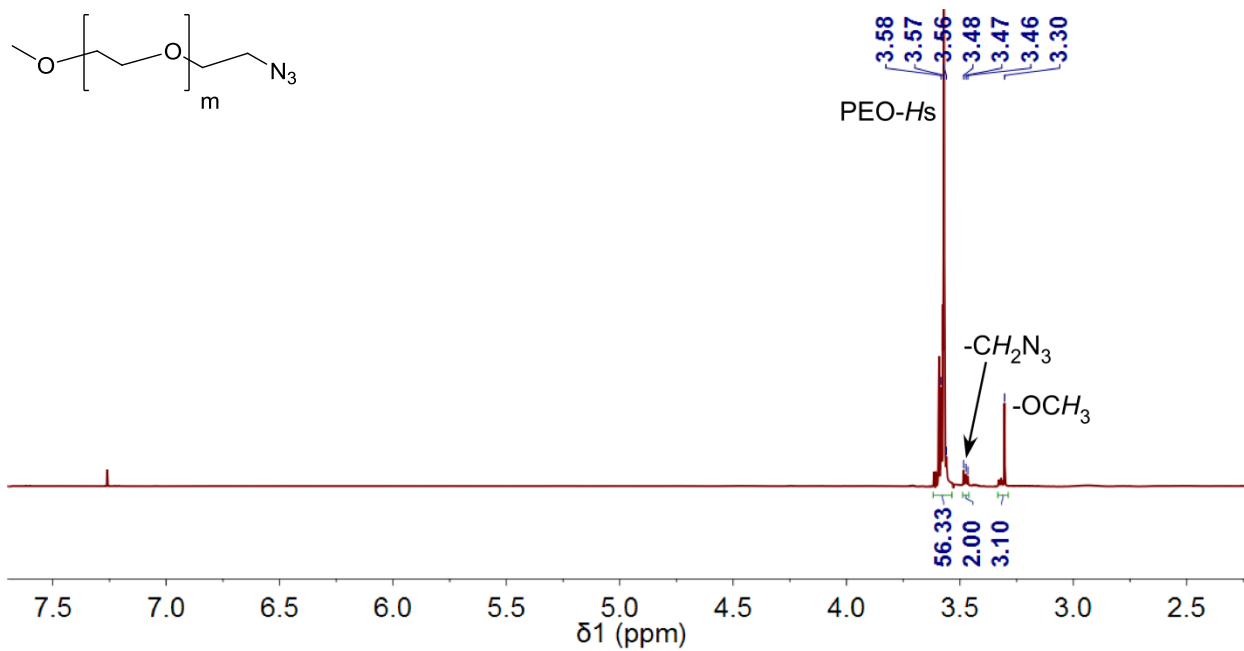


Figure 16S. $^1\text{H-NMR}$ spectrum of azido-methoxypolyethylene glycol **5**.

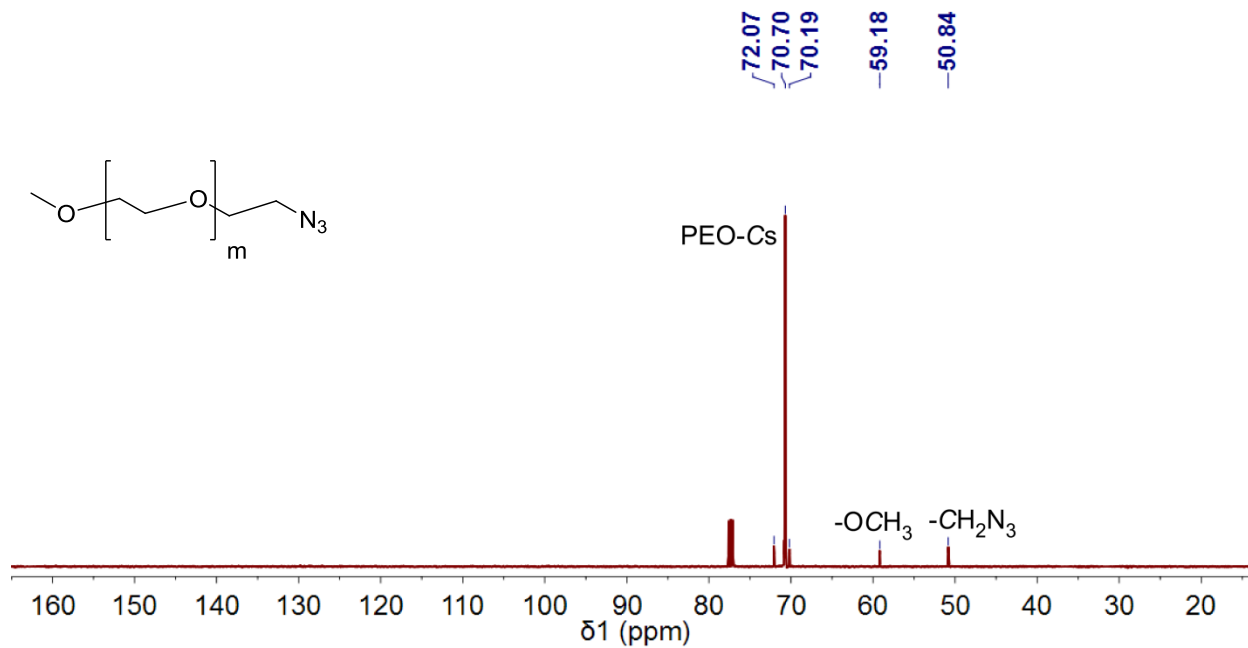


Figure 17S. $^{13}\text{C-NMR}$ spectrum of azido-methoxypolyethylene glycol **5**.

8. Synthesis of PEG-grafted polysaccharide **6**

To a flame-dried 10-mL Schlenk flask was added co-polymer **4** (0.069 g, $M_n^{\text{GPC}} = 36.1$ kDa, 0.088 mmol alkynes) and **5** (0.20 g, 0.27 mmol). Anhydrous THF (3.0 mL) was added. After three cycles of freeze-pump-thaw, Cu(I)Br (6.7 mg, 0.047 mmol) and N,N,N',N',N''-pentamethyldiethylenetriamine (10.4 mg, 0.06 mmol) were added. The reaction was stirred at rt for 8 h. The reaction mixture was loaded into a short column of neutral alumina to remove the copper catalyst. The reaction mixture was precipitated in diethyl ether (3×100 ml) to afford the product as a white powder (0.23 g, 86%). $M_n^{\text{GPC}} = 54$ kDa; PDI = 1.3. $^1\text{H-NMR}$ (500 MHz, CDCl_3) $\delta = 4.97$ (H-1), 4.52 ($\text{C}\equiv\text{C-CH}_2$), 3.90-3.81, 3.66-3.61 (PEO-Hs), 3.54-3.38 (-OMe), 3.25-3.14 (sugar protons). $^{13}\text{C-NMR}$ (126 MHz, CDCl_3) $\delta = 124.76$ -124.39 (triazole-Cs), 96.60 (C-1), 83.52 (C-3), 81.94 (C-2), 79.66 (C-4), 72.17, 70.80 (PEO-Cs), 70.41, 69.67 (C-5), 66.15 (C-6), 60.93 (-OMe), 60.71 (-OMe), 59.29 (-OMe PEG), 58.47 (-OMe), 50.27 (triazole- CH_2 -). IR: 2996, 2986, 2874 (C-H stretching), 1185 (C-C stretching), 1082 (C-O stretching).

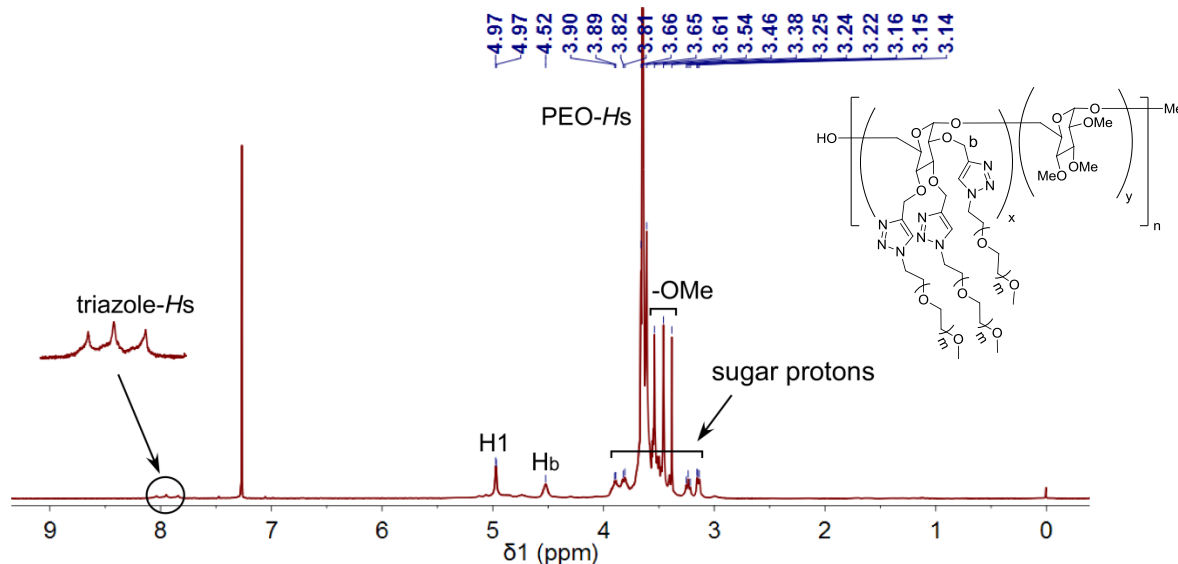


Figure 18S. $^1\text{H-NMR}$ spectrum of PEG-grafted polysaccharide **6**.

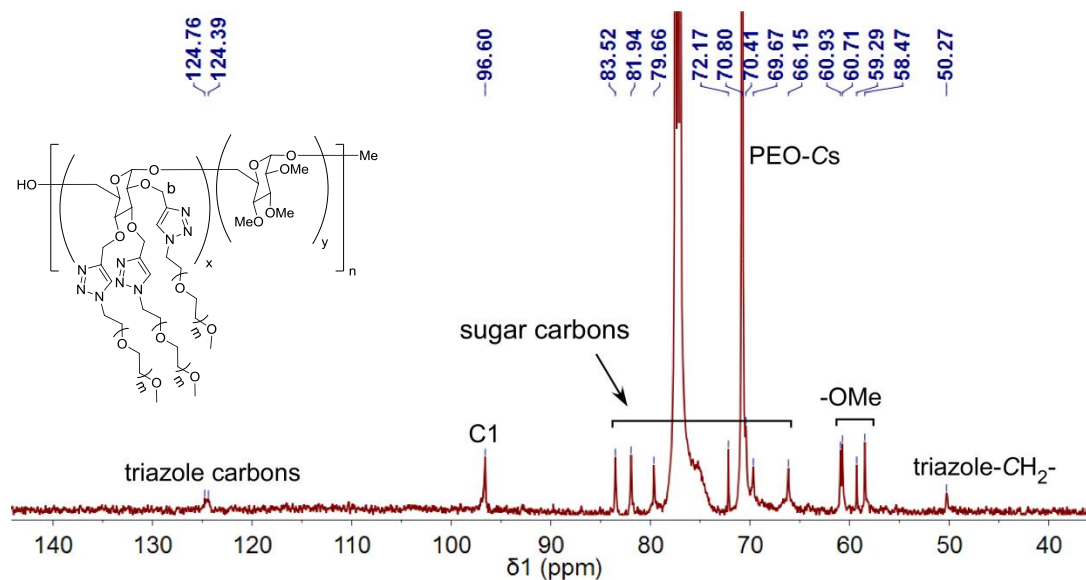


Figure 19S. ^{13}C -NMR spectrum of PEG-grafted polysaccharide **6**.

9. Synthesis of NPs

PEG-grafted polysaccharide **6** (10 mg) was dissolved in N,N-dimethylformamide (DMF, 10 mL) to make a solution with a concentration of 1 mg mL^{-1} . The solution was stirred at rt for 2 h. Nanopure water (10 mL) was added dropwise over a time period of 1 h. The solution was stirred for another 4 h and then dialyzed against nanopure water for 72 h to afford a nanoparticle solution. Nanoparticle concentration: 0.30 mg mL^{-1} ; TEM sizes: $94 \pm 15 \text{ nm}$; Hydrodynamic light scattering (DLS) sizes: D_h (intensity) = 226 ± 35 ; D_h (volume) = $245 \pm 66 \text{ nm}$.

10. Cell Cytotoxicity Assay on HCT116 and HEK293 cells.

The media formulation used for HCT116 was McCoy's 5A medium + 10% Fetal Bovine Serum (FBS), and the media formulation used for HEK293 cells was Dulbecco's modified eagle's medium (DMEM) + 10% FBS. All experiments were performed at 37°C .

HCT116 and HEK293 cells were seeded on 96-well plates at a density of 5000 cells per well for *ca.* 24 h. A full media replacement was then conducted which contained various concentrations of nanoparticles (Figure 20S). The treatments were performed for 2 h. Subsequently, another full media replacement was conducted and the cells were incubated for 72 h. To perform the MTS assay, the existing media was replaced with 120 μL of MTS reagents, which contained 100 μL of the cell's respective media + 20 μL of CellTiter 96 Aqueous One Solution (Promega) in each well for 2.5 h at 37 $^{\circ}\text{C}$. The absorbance at 490 nm was obtained using a PerkinElmer Wallac Victor3 1420 Multilabel Counter. Cell viability was normalized to untreated control cells.

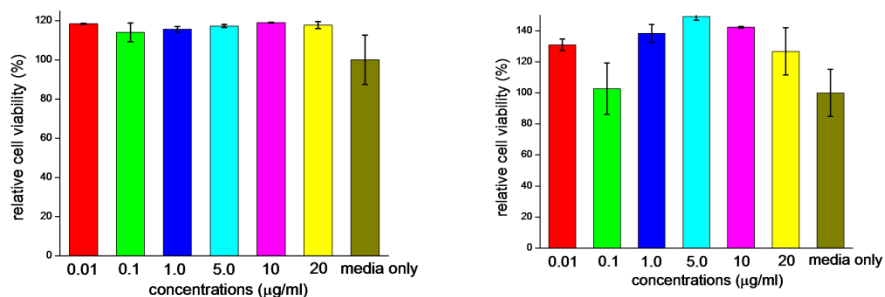


Figure 20S: The cell viability studies using HCT116 colon cancer cells (left) and HEK293 cells (right).