Supporting Information

End-functionalised glycopolymers as glycosaminoglycan mimetics inhibit HeLa cell proliferation

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1. Materials and characterization.

1.1 Materials

Sodium azide (99.5%), cis-5-norbornene-exo-2,3dicarboxylic anhydride (98%, NDA), ethyl vinyl ether (97%, EVE), 3-amiqnopropyne (97%), 6-Chlorohexanol (95%), maleic acid (99%), N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (98%, EDC), N-hydroxysuccinimide (98%, NHS), N,N’-Dicyclohexylcarbodiimide (99%, DCC), 4-Dimethylaminopyridine (99%, DMAP), 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT, 97%), N-methylmorpholine (NMM, 99.5%), palladium hydroxide on carbon (20% Pd(OH)$_2$), benzaldehyde dimethyl acetal (98%), (+/-)-10-camphorsulfonic acid (99%), sodium methylate (97%), 2,2,2-trichloroethyn chloroformate (99%, Troc-Cl), triethylamine(99.5%), sulfur trioxide pyridine complex (SO$_3$·Py, 97%), Amberlite® IR-120 cation exchange resin (H$^+$ form) were purchased from Aladdin (Shanghai, China) and used without further purification. 6-maleimidohexanooic acid (98%), nucleic acid dye DAPI, and 3-(4, 5-dimethyl-2-thiazoly)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) and Grubbs-type catalysts (G 3rd) were purchased from Sigma Aldrich (St. Louis, MO, USA). SephadexTM LH-20 was purchased from GE healthcare. Sulfur trioxide triethylamine complex (96%) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Other chemical reagents were purchased from Energy Chemical and used directly. Minimum essential medium (MEM) was obtained from Gibco (Rockville, MD, USA). Fetal bovine serum (FBS) and trypsin were obtained from Gibco-Invitrogen (Grand Island, NY, USA). The primary antibodies p-ERK, ERK, p-AKT, AKT, p-Pi3K, Pi3K, p-mTOR, mTOR, and Actin were obtained from CST (Boston, MA, USA). The secondary antibodies were obtained from HuaBio (Hangzhou, China). Heparanase assay toolbox were purchased from Cisbio Assay (France) and heparinase ((human recombinant heparanase 7570-GH) purchased from R&D systems (Minneapolis, MN, USA).
1.2 Characterization

Nuclear magnetic resonance (NMR) spectra were recorded on an Agilent DD2 spectrometer (500 MHz). The chemical shifts of all the NMR spectra were reported in delta (δ) units and expressed as parts per million (ppm). The NMR spectra were referenced using CD$_3$OD (¹H NMR δ = 3.31 ppm, ¹³C NMR δ = 49.00 ppm), and D$_2$O (¹H NMR δ = 4.79 ppm). Zeta potential and spherical nanomicelle diameters analysis of GP1-6 were performed by dynamic light scattering measurement using the zetasizer Nanoparticle Analyser (Nano ZS 90; Malvern Instruments, Ltd., Malvern, United Kingdom). Transmission electron microscope (TEM) images of samples were obtained by a JEOL JSM 5410 transmission electron microscope (Japan) and statistics by Nano Measurer. Absorbance and fluorescence intensity measurements were performed by microplate reader Tecan SparkControl™ V2.3.5. Confocal laser scanning microscope (CLSM) images were taken by a LSM 700 confocal scanning microscope (A1+A1R+, Nikon, Japan).
2. Synthetic routes of the galactosamine building blocks with diverse sulfation patterns.

Scheme S1. Synthetic routes of the diverse sulfated monosaccharide (1, 2, 3, 4, 5, 6).
Reagents and conditions: (a) 0.6 M NaOH in water/1,4-dioxane (1:1), 80 °C, 1 h. (b) Et$_3$N, Ac$_2$O, CH$_3$OH, rt, 1 h. (c) SO$_3$·Et$_3$N, DMF, 50 °C, 10 h. (d) Pd/C, H$_2$, CH$_3$OH/H$_2$O(1:1), rt, 2 h. (e) CH$_3$ONa, CH$_3$OH, rt, 1 h. (f) α,α-Dimethoxytoluene, CSA, CH$_3$CN, rt, 3 h. (g) SO$_3$·Et$_3$N, Et$_3$N, Py, rt, 6 h. (h) Ac$_2$O, Py, rt, 12 h. (i) 70% CH$_3$COOH in H$_2$O, 60 °C, 2 h. (j) CH$_3$ONa, CH$_3$OH, -5 °C, 6 h.
3. Synthetic routes of the distinct glycopolymers with diverse sulfation patterns.

Scheme S2. Synthetic routes of sulfated glycopolymers (GP1, GP2, GP3, GP4, GP5, GP6).
4. Synthetic procedure of compounds.

4-Methylphenyl 3,4,6-tri-O-acetyl-2-deoxy-1-thio-2-(2',2',2'-trichloroethoxycarbonylamino)-β-D-galactopyranoside (17)

To a solution of D-glucosamine hydrochloride (2 g, 9.28 mmol) in 40 mL H₂O, sodium bicarbonate (1.95 g, 23.19 mmol, 2.5 eq.) was added. The mixture was cooled to 0 ºC and TrocCl (1.92 mL, 13.92 mmol, 1.5 eq.) was added. The mixture was stirred at room temperature for 5 h after which TLC indicated full conversion. The solution was concentration under reduced pressure. The residue was acetylated by adding pyridine (20 mL) and Ac₂O (10 mL) and stirred at room temperature for 2 h after which TLC indicated full conversion. The mixture was concentrated and the residue was cooled to 0 ºC. The residue was re-dissolved in 25 mL CH₂Cl₂. BF₃·Et₂O (0.99 mL, 6.8 mmol, 1.5 eq.) and TolSH (1.13 g, 9.05 mmol, 2 eq.) were added and the solution was warmed up to room temperature. The mixture was stirred at room temperature overnight after which TLC indicated full conversion and quenched by adding sodium bicarbonate aqueous. The mixture was then extracted with CH₂Cl₂. the combined organic layers were dried over Na₂SO₄, concentrated and purified by column chromatography to afford 17 as a white solid (3.02 g, 70%). ¹H NMR (500 MHz, CDCl₃): δ 7.48 - 7.37 (m, 2H), 7.26 (d, J = 3.0 Hz, 1H), 7.09 (t, J = 19.9 Hz, 2H), 5.37 (t, J = 8.3 Hz, 1H), 5.19 (dd, J = 10.7, 2.4 Hz, 1H), 5.10 (d, J = 9.0 Hz, 1H), 4.83 (d, J = 10.3 Hz, 1H), 4.79 (d, J = 12.0 Hz, 1H), 4.72 (d, J = 12.0 Hz, 1H), 4.18 (dd, J = 11.3, 7.0 Hz, 1H), 4.11 (dd, J = 11.4, 6.2 Hz, 1H), 3.94 - 3.84 (m, 2H), 2.34 (s, 3H), 2.12 (s, 3H), 2.04 (d, J = 4.9 Hz, 3H), 1.97 (d, J = 3.7 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 170.40, 170.14, 170.21, 153.93, 138.52, 133.22, 129.70, 128.58, 87.54, 74.50, 74.43, 70.90, 66.91, 61.67, 51.35, 29.69, 21.16, 20.67, 20.64, 20.60.

6-Azidohexan-1-ol (18)

6-Chlorohexanol (2.5 mL, 18.74 mmol), sodium azide (3.65 g, 56.2 mmol) and sodium hydroxide (0.038 g, 0.9 mmol) were dissolved in 200 mL H₂O and stirred at room temperature for 3 days after which TLC indicated full conversion. The mixture was then extracted with CH₂Cl₂ till the product was completely
transferred from aqueous layer to organic layer. The organic layer was dried (Na$_2$SO$_4$) and evaporated under reduced pressure giving the colourless oil (2.47 g, 92%). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 3.64 (t, J = 6.5 Hz, 2H), 3.27 (t, J = 6.9 Hz, 2H), 1.73 – 1.51 (m, 4H), 1.50 – 1.32 (m, 4H). $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 62.71, 51.38, 32.53, 28.80, 26.52, 25.33.

6-Azidoxy 3,4,6-tri-O-acetyl-2-deoxy-2-(2',2',2'-trichloroethoxycarbonylamino)-β-D-galactopyranoside (7)

To a solution of compound 17 (500 mg, 1.08 mmol) and 18 (308.82 mg, 2.16 mmol) in 12 mL anhyd DCM with argon protection was added 4 Å molecule sieve. The mixture was stirred at room temperature for 0.5 h and then cooled to -20 ºC, NIS (392.75 mg, 2.16 mmol, 2 eq.) and TMSOTf (47.4 µL, 0.32 mmol, 0.3 eq.) were added. The mixture was stirred for 0.5 h after which TLC indicated full conversion and quenched with Et$_3$N. The mixture was filtered, evaporated and purified by column chromatography to afford 7 as light yellow syrup (574.9 mg, 88%). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 5.37 (d, J = 2.6 Hz, 1H), 5.23 (d, J = 9.9 Hz, 1H), 5.11 (d, J = 6.2 Hz, 1H), 4.80 - 4.56 (m, 3H), 4.14 (ddd, J = 26.8, 11.2, 6.8 Hz, 2H), 3.93 - 3.85 (m, 2H), 3.77 (d, J = 8.9 Hz, 1H), 3.48 (dd, J = 16.2, 6.9 Hz, 1H), 3.25 (t, J = 6.8 Hz, 2H), 2.13 (d, J = 9.9 Hz, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.58 (s, 4H), 1.36 (s, 4H). $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 170.40, 170.32, 170.20, 154.02, 101.09, 95.48, 77.21, 74.40, 70.59, 70.11, 69.63, 66.69, 61.33, 53.00, 51.31, 29.27, 28.70, 26.39, 25.41, 20.67, 20.60.

6-Azidoxy 2-deoxy-2-amino-β-D-galactopyranoside (19)

To a solution of compound 7 (200 mg) in MeOH was added MeONa to adjust the pH to 10. The mixture was stirred at room temperature for 30 min then neutralized with Dowex 50-X8 resin (H$^+$ form), filtered, and concentrated. The residue was re-dissolved in 2 mL 1,4-dioxane and 0.6 M NaOH (2 mL) was added. The solution was refluxed at 80 ºC for 1 h after which TLC indicated full conversion. The mixture was neutralized with 1 M HCl and concentrated to afford compound 19 for further use without purification.

6-Azidoxy 2-deoxy-2-acetamido-β-D-galactopyranoside (20)
Compound 19 was dissolved in 1.5 mL CH$_3$OH, Et$_3$N (20 μL) and Ac$_2$O (20 μL) were added to the solution and stirred at room temperature for 1 h after which TLC indicated full conversion. The mixture was concentrated to afford compound 20 (32 mg, 95%). $^1$H NMR (500 MHz, CD$_3$OD): δ 4.37 (d, J = 8.4 Hz, 1H), 3.95 - 3.86 (m, 2H), 3.85 (d, J = 3.0 Hz, 1H): 3.75 (h, J = 4.5 Hz, 2H), 3.60 (dd, J = 10.7, 3.1 Hz, 1H), 3.52 - 3.46 (m, 2H), 3.29 (t, J = 6.9 Hz, 2H), 1.99 (s, 3H), 1.59 (dd, J = 12.6, 5.9 Hz, 4H), 1.39 (dt, J = 14.0, 7.0 Hz, 4H).

$^{13}$C NMR (126 MHz, CD$_3$OD): δ 101.73, 75.20, 71.85, 68.95, 68.25, 68.14, 61.08, 52.87, 51.00, 29.28, 29.14, 28.50, 26.13, 25.27, 21.69.

6-Aminohexyl 2-deoxy-2-acetamido-β-D-galactopyranoside (1)

To a solution of compound 20 (30 mg, 0.086 mmol) in 1:1 CH$_3$OH/H$_2$O was added Pd(OH)$_2$/C. The mixture was filled with H$_2$ and stirred at room temperature for 2 h after which TLC indicated full conversion. Pd(OH)$_2$/C was separated by filtration through a Celite pad and the filtrate was concentrated to give the desired compound 1 as white solid (20.53 mg, 74%). $^1$H NMR (500 MHz, D$_2$O): δ 4.32 (d, J = 8.5 Hz, 1H), 3.81 (d, J = 3.2 Hz, 1H), 3.80 - 3.72 (m, 2H), 3.71 - 3.61 (m, 2H), 3.59 (dd, J = 10.7, 3.0 Hz, 1H), 3.56 - 3.52 (m, 1H), 3.50 - 3.43 (m, 1H), 2.93 - 2.84 (m, 2H), 1.91 (s, 3H), 1.79 (s, 1H), 1.54 (d, J = 7.4 Hz, 4H), 1.49 - 1.40 (m, 4H).

$^{13}$C NMR (126 MHz, D$_2$O): δ 101.37, 75.00, 70.93, 70.15, 67.69, 60.87, 52.36, 47.33, 28.26, 25.30, 24.55, 22.14.

6-Azidohexyl 2-deoxy-2-acetamido-3,4,6-tri-O-sulfo-β-D-galactopyranoside (21)

To a solution of compound 20 (40 mg, 0.12 mmol) in DMF (1 mL) was added SO$_3$•Et$_3$N (500 mg, 3.6 mmol, 10 eq. per OH). The reaction mixture was stirred at 50 °C for 10 h. Upon confirmation of the completely conversion by TLC, the mixture was purified by Sephadex LH-20 gel filtration (MeOH) to give the desired product 21 as a syrup (57.6 mg, 85%). $^1$H NMR (500 MHz, CD$_3$OD): δ 4.96 (d, J = 2.8 Hz, 1H), 4.43 (d, J = 8.4 Hz, 2H), 4.35 (dd, J = 11.2, 2.9 Hz, 1H), 4.27 (dd, J = 11.8, 8.3 Hz, 1H), 4.11 (dd, J = 11.1, 8.4 Hz, 1H), 3.94 (dd, J = 8.2, 2.8 Hz, 1H), 3.88 (dt, J = 9.8, 6.2 Hz, 1H), 3.51 (dt, J = 9.8, 6.5 Hz, 1H), 3.29 (d, J = 6.9 Hz, 2H), 1.95 (s, 4H), 1.58 (td, J = 13.0, 11.6, 7.2 Hz, 3H), 1.38 (p,
J = 4.5, 4.1 Hz, 4H). $^{13}$C NMR (126 MHz, CD$_3$OD): $\delta$ 174.39, 100.82, 75.24, 74.05, 72.14, 70.48, 67.80, 67.75, 50.94, 28.42, 27.96, 25.57, 24.62, 22.25.

6-Aminohexyl 2-deoxy-2-acetamido-3,4,6-tri-O-sulfo-β-D-galactopyranoside (5)

As the same procedure for the compound 1 preparation, the compound 21 (32 mg, 0.054 mmol) was hydrogenated to afford 5 as a white solid (21.4 mg, 70%). $^1$H NMR (500 MHz, D$_2$O): $\delta$ 4.84 (s, 1H), 4.59 (d, $J$ = 8.5 Hz, 1H), 4.39 (dd, $J$ = 11.0, 2.5 Hz, 1H), 4.15 - 4.08 (m, 1H), 4.00 (d, $J$ = 6.0 Hz, 1H), 3.92 - 3.85 (m, 1H), 3.80 (d, $J$ = 5.4 Hz, 1H), 3.61 - 3.54 (m, 1H), 2.90 (dd, $J$ = 15.7, 7.8 Hz, 2H), 1.91 (s, 3H), 1.61 - 1.43 (m, 4H), 1.25 (d, $J$ = 30.1 Hz, 4H). $^{13}$C NMR (126 MHz, CD$_3$OD): $\delta$ 174.57, 101.76, 75.19, 73.97, 72.09, 70.61, 67.88, 50.94, 39.35, 28.21, 26.48, 24.98, 24.39, 22.18.

6-Azidohexyl 2-deoxy-2-N-sulfo-β-D-galactopyranoside (22)

To a solution of compound 19 (45 mg, 0.15 mmol) in 1 mL pyridine was added 0.1 mL Et$_3$N and SO$_3$·Et$_3$N (35 mg, 1.5 eq.). The mixture was stirred at room temperature for 6 h. Upon confirmation of the completely conversion by TLC, the mixture was purified by Sephadex LH-20 gel filtration (MeOH) to give the desired product 22 as a syrup (46.6 mg, 82%). $^1$H NMR (500 MHz, CD$_3$OD): $\delta$ 4.38 (d, $J$ = 8.2 Hz, 1H), 3.90 (dt, $J$ = 9.3, 6.6 Hz, 1H), 3.85 (d, $J$ = 3.0 Hz, 1H), 3.78 (d, $J$ = 3.1 Hz, 1H), 3.76 (d, $J$ = 5.1 Hz, 2H), 3.57 (dt, $J$ = 9.4, 6.6 Hz, 1H), 3.50 (t, $J$ = 6.1 Hz, 1H), 3.35 (m, 1H), 2.90 (t, $J$ = 6.5 Hz, 2H), 1.67 - 1.56 (m, 4H), 1.49 - 1.39 (m, 4H). $^{13}$C NMR (126 MHz, CD$_3$OD): $\delta$ 101.58, 75.21, 73.69, 68.93, 67.85, 61.14, 56.80, 51.02, 29.20, 28.46, 26.21, 25.26.

6-Aminohepxyl 2-deoxy-2-N-sulfo-β-D-galactopyranoside (3)

As the same procedure for the compound 1 preparation, the compound 22 (20 mg, 0.052 mmol) was hydrogenated to afford 3 as a white solid (14.36 mg, 77%). $^1$H NMR (500 MHz, D$_2$O): $\delta$ 4.40 - 4.36 (m, 1H), 3.83 (d, $J$ = 3.3 Hz, 2H), 3.68-3.57 (m, 3H), 3.54 – 3.42 (m, 2H), 3.06 (d, 1H), 2.90 ($J$ = 7.21, 2H), 1.61 – 1.48 (m, 4H), 1.38 – 1.24 (m,
4H). $^{13}$C NMR (126 MHz, D$_2$O): $\delta$ 101.65, 74.81, 72.15, 70.13, 67.76, 60.96, 56.60, 39.30, 28.32, 26.50, 25.10, 24.49.

6-Azidoethyl 2-deoxy-2-sulfo-3,4,6-tri-sulfo-$\beta$-D-galactopyranoside (23)

As the same procedure for the compound 21 preparation, the compound 19 (28 mg, 0.092 mmol) was sulfated to afford 23 as syrup (41.4 mg, 72%). $^1$H NMR (500 MHz, CD$_3$OD): $\delta$, 4.94 (d, $J = 2.2$ Hz, 1H), 4.62 (d, $J = 8.2$ Hz, 1H), 4.51 - 4.45 (m, 1H), 4.41 (dd, $J = 15.2$, 6.2 Hz, 2H), 4.27 (dd, $J = 11.7$, 8.1 Hz, 1H), 4.05 - 4.00 (m, 1H), 3.95 (dd, $J = 15.4$, 6.4 Hz, 1H), 3.28 (m, 2H), 3.59 (m, 1H), 1.61 (d, $J = 5.5$ Hz, 4H), 1.41 (s, 4H). $^{13}$C NMR (126 MHz, D$_2$O): $\delta$ 99.87, 74.59, 73.73, 73.09, 69.38, 67.71, 59.48, 50.97, 29.13, 28.46, 26.11, 25.27.

6-Aminohexyl 2-deoxy-2-sulfo-3,4,6-tri-sulfo-$\beta$-D-galactopyranoside (6)

As the same procedure for the compound 1 preparation, the compound 23 (22 mg, 0.035 mmol) was hydrogenated to afford 6 as a white solid (16.9 mg, 80%). $^1$H NMR (500 MHz, D$_2$O): $\delta$ $^1$H NMR (500 MHz, Deuterium Oxide) $\delta$ 4.86 (t, $J = 6.0$ Hz, 1H), 4.56 (d, $J = 12.1$ Hz, 1H), 4.51 - 4.41 (m, 1H), 4.22 (d, $J = 12.0$ Hz, 1H), 4.10 (dt, $J = 24.2$, 8.2 Hz, 2H), 3.92 - 3.77 (m, 1H), 3.74 - 3.57 (m, 1H), 3.57 – 3.48 (m, 1H), 2.89 (t, $J = 7.5$ Hz, 2H), 1.54 (d, $J = 20.9$ Hz, 4H), 1.37 – 1.24 (m, 4H).

6-Azidoethyl 4,6-O-benzylidene-2-deoxy-2-(2',2',2'-trichloroethoxycarbonylamino)-$\beta$-D-galactopyranoside (8)

To a solution of compound 7 (200 mg, 0.33 mmol) in MeOH was added MeONa to adjust the pH to 10. The mixture was stirred at room temperature for 30 min then neutralized with Dowex 50-X8 resin (H⁺ form), filtered, and concentrated. The residue was re-dissolved in 7 mL CH$_3$CN, a catalytic amount of (+/-)-10-camphorsulfonic acid (8 mg, 0.035 mmol) and benzaldehyde dimethyl acetal (102.7 μL, 0.68 mmol) were added. The mixture was stirred at room temperature for 4 h until the TLC showed full conversion, and 500 μL Et$_3$N was added to quench the reaction. CH$_3$CN was evaporated and the residue was purified to afford 8 as a white solid (167 mg, 89%). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.54 - 7.46 (m, 2H), 7.39 - 7.32 (m, 3H), 5.56 (s, 1H), 5.27 (s, 1H), 4.72 (t, $J = 11.4$ Hz, 2H), 4.57 (d, $J =$
$6.1 \text{ Hz, 1H}$, $4.32 \text{ (d, } J = 12.4 \text{ Hz, 1H)}$, $4.19 \text{ (d, } J = 2.8 \text{ Hz, 1H)}$, $4.07 \text{ (d, } J = 12.4 \text{ Hz, 1H)}$, $4.02 \text{ - 3.88 (m, 2H)}$, $3.64 \text{ (s, 1H)}$, $3.51 - 3.40 \text{ (m, 2H)}$, $3.25 \text{ (t, } J = 6.8 \text{ Hz, 2H)}$, $2.84 \text{ (s, 1H)}$, $1.58 \text{ (s, 4H)}$, $1.35 \text{ (d, } J = 22.9 \text{ Hz, 4H)}$. $^{13}C \text{ NMR (126 MHz, CDCl}_3\text{): }$ δ 154.63, 137.38, 129.27, 128.38, 126.40, 101.35, 100.38, 77.22, 75.03, 74.55, 70.18, 69.32, 69.14, 66.56, 55.83, 51.34, 30.93, 29.28, 28.72, 26.42, 25.48.

6-Azidohexyl 3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-acetamido-β-D-galactopyranoside (24)

To a solution of compound 8 (130 mg, 0.23 mmol) in 4 mL 1,4-dioxane was added 0.6 M NaOH (4 mL). The solution was refluxed at 80 °C for 1 h after which TLC indicated full conversion. The mixture was neutralized with 1 M HCl and concentrated. The residue was dissolved in pyridine (4 mL) and Ac$_2$O (2 mL) and stirred at room temperature overnight. The solution was concentrated and purified to afford compound 24 (100 mg, 92%). $^1H \text{ NMR (500 MHz, CDCl}_3\text{): }$ δ 7.57 – 7.45 (m, 2H), 7.36 (p, $J = 5.5$, 4.4 Hz, 3H), 5.54 (d, $J = 12.9$ Hz, 2H), 5.42 (dd, $J = 11.3$, 3.6 Hz, 1H), 4.92 (d, $J = 8.3$ Hz, 1H), 4.36 (d, $J = 3.6$ Hz, 1H), 4.32 (d, $J = 12.0$ Hz, 1H), 4.09 – 4.03 (m, 1H), 3.92 (ddd, $J = 11.5$, 6.3, 2.9 Hz, 2H), 3.54 (s, 1H), 3.50 (dd, $J = 9.4$, 6.6 Hz, 1H), 3.25 (t, $J = 6.9$ Hz, 2H), 2.08 (s, 3H), 1.95 (s, 3H), 1.59 (t, $J = 6.7$ Hz, 4H), 1.38 (d, $J = 7.5$ Hz, 4H). $^{13}C \text{ NMR (126 MHz, CDCl}_3\text{): }$ δ 170.87, 170.28, 137.65, 128.97, 128.13, 126.31, 100.85, 100.00, 73.21, 70.46, 69.20, 69.03, 66.32, 52.06, 51.37, 45.76, 28.77, 26.43, 25.50, 23.56, 20.96.

6-Azidohexyl 3-O-acetyl-4,6-di-O-sulfo-2-deoxy-2-acetamido-β-D-galactopyranoside (25)

Compound 24 (60 mg, 0.13 mmol) was heated to 60 °C and 70% (v/v) CH$_3$COOH (3 mL) was added. The mixture was stirred for 2 h after which TLC indicated full conversion. The mixture was concentrated and the residue was dissolved in DMF (1 mL). SO$_3$·Et$_3$N (500 mg, 3.6 mmol, 10 eq. per OH) was added and the reaction mixture was stirred at 50 °C for 10 h. Upon confirmation of the completely conversion by TLC, the mixture was purified by Sephadex LH-20 gel filtration (MeOH) to give the desired product 25 as a syrup (57.7 mg, 85%). $^1H \text{ NMR (500 MHz, CD}_3\text{OD): }$ δ 4.90 (d, $J = 3.2$ Hz, 1H), 4.77 (d, $J = 2.7$ Hz, 1H), 4.47 (d, $J = 8.4$ Hz,
6-Aminohexyl 4,6-di-O-sulfo-2-deoxy-2-acetamido-β-D-galactopyranoside(4)

To a solution of 25 (28 mg, 0.051 mmol) in CH₃OH (2 mL), MeONa was added to adjust the pH to 9. The mixture was stirred at -5 ºC for 30 min then neutralized with Dowex 50-X8 resin (H⁺ form), filtered, and concentrated. The residue was dissolved in 1:1 CH₃OH/H₂O and Pd(OH)₂/C was added. The mixture was filled with H₂ and stirred at room temperature for 2 h after which TLC indicated full conversion. Pd(OH)₂/C was separated by filtration through a Celitepad and the filtrate was concentrated to give the desired compound 4 as white solid (29 mg, 58%). ¹H NMR (500 MHz, D₂O): δ 4.61 (s, 1H), 4.41 (t, J = 4.2 Hz, 1H), 4.21 (dd, J = 11.5, 3.3 Hz, 1H), 4.13 – 4.07 (m, 1H), 3.95 (dd, J = 8.8, 3.2 Hz, 1H), 3.79 (dd, J = 10.4, 5.6 Hz, 3H), 3.55(m, 1H), 2.94 – 2.84 (m, 2H), 2.00 – 1.87 (m, 3H), 1.61 – 1.43 (m, 4H), 1.27 (dd, J = 9.3, 4.7 Hz, 4H). ¹³C NMR (126 MHz, CD₃OD): δ 172.20, 101.58, 75.55, 72.67, 70.95, 69.05, 67.71, 53.47, 51.01, 29.11, 28.50, 26.12, 25.24, 21.62.

6-Azidohexyl 4,6-O-benzylidene-2-deoxy-2-acetamido-β-D-galactopyranoside(26)

To a solution of compound 8 (130 mg, 0.23 mmol) in 4 mL 1,4-dioxane was added 0.6 M NaOH (4 mL). The solution was refluxed at 80 ºC for 1 h after which TLC indicated full conversion. The mixture was neutralized with 1 M HCl and concentrated. The residue was dissolved in CH₃OH (2 mL), Et₃N (20 μL) and Ac₂O (20 μL) were added and stirred at room temperature for 1 h. The solution was concentrated and purified to afford 26 as a syrup (85 mg, 85%). ¹H NMR (500 MHz, CDCl₃): δ 7.50 (dd, J = 7.2, 2.1 Hz, 2H), 7.39 - 7.31 (m, 3H), 5.43 (s, 1H), 4.57 (d, J = 8.3 Hz, 1H), 4.19 (d, J = 12.4 Hz, 1H), 4.00 (d, J = 3.0 Hz, 1H), 3.97 - 3.85 (m, 3H), 3.83 - 3.75 (m, 1H), 3.50 - 3.43 (m, 1H), 3.34 (s, 1H), 3.25 (t, J = 6.9 Hz, 2H), 2.00(s, 3H), 1.64 -1.52 (m, 4H), 1.42 - 1.30 (m, 4H). ¹³C NMR (126 MHz, CDCl₃): δ 171.82, 137.85, 129.10, 128.21, 126.51, 101.13, 100.55, 75.21, 70.40, 69.14,
6-Azidohexyl 4,6-O-benzylidene-2-deoxy-2-acetamido-3-O-sulfo-β-D-galactopyranoside (27)
As the same procedure for the preparation of compound 21, the compound 26 (28 mg, 0.092 mmol) was sulfated to give the desired product 27 as a syrup (24 mg, 61%). 1H NMR (500 MHz, CD3OD): δ 7.54 (d, J = 7.8 Hz, 2H), 7.34 (d, J = 6.0 Hz, 3H), 5.63 (s, 1H), 4.60 (d, J = 8.3 Hz, 2H), 4.48 (dd, J = 11.1, 3.3 Hz, 1H), 4.24 – 4.09 (m, 3H), 3.95 – 3.85 (m, 1H), 3.58 (s, 1H), 3.52 (d, J = 9.6 Hz, 1H), 3.28 (t, J = 6.9 Hz, 2H), 3.14 (q, J = 7.0 Hz, 1H), 1.96 (s, 3H), 1.59 (s, 4H), 1.40 (s, 4H). 13C NMR (126 MHz, CD3OD): δ 172.30, 138.27, 128.36, 127.47, 126.23, 101.52, 100.82, 75.30, 73.84, 69.02, 68.84, 66.44, 50.97, 50.48, 46.42, 29.06, 28.49, 26.08, 25.21, 21.74.

6-Aminohexyl 2-deoxy-2-acetamido-3-O-sulfo-β-D-galactopyranoside (2)
As the same procedure for the compound 1 preparation, the compound 27 (24 mg, 0.047 mmol) was hydrogenated to afford 2 as a syrup (13.45 mg, 72%). 1H NMR (500 MHz, CD3OD): δ 4.44 (d, J = 8.4 Hz, 1H), 4.23 (s, 1H), 4.09 (s, 1H), 3.85 (s, 1H), 3.76 (s, 1H), 3.60 (d, J = 33.4 Hz, 3H), 3.48 (s, 1H), 2.83 (s, 2H), 1.87 (s, 3H), 1.46 (d, J = 41.4 Hz, 4H), 1.22 (s, 4H). 13C NMR (126 MHz, CD3OD): δ 172.25, 101.86, 77.82, 74.83, 69.09, 66.35, 60.94, 50.57, 39.38, 28.81, 26.97, 25.63, 25.21, 21.89.

6-(exo-N-norbornene-2,3-dicarboximide)hexanoic acid (11)
Cis-5-norbornene-exo-2,3-dicarboxylic anhydride (2.0 g, 12.20 mmol) was dissolved in 60 mL of anhydrous toluene purged with argon gas. Then 6-Aminocaproic acid (1.9 g, 14.64 mmol) and Et3N (1.5 g, 14.64 mmol) were added and the flask was fitted with a Dean-Stark trap and a condenser. The reaction mixture was refluxed in oil bath at 120 °C for 15 h after which TLC indicated full conversion. The solvent was evaporated under reduced pressure. The product was purified by column chromatography to give a white solid (3.2 g, 95%). The spectrum for the desired product was the same as reported previously. 6-(exo-N-norbornene-2,3-dicarboximide)hexanoic acid N-succinimide ester (12)
To a solution of compound 11 (2.00 g, 9.04 mmol) in 30 mL CH2Cl2, EDC
(1.935 g, 12.66 mmol, 1.4 eq.) and NHS (1.162 g, 12.7 mmol, 1.4 eq.) were added and stirred at room temperature for 4 h. The mixture was concentrated and purified to afford 12 as white solid (2.76 g, 96%). ¹H NMR (500 MHz, CDCl₃): δ 6.24 (s, 2H), 3.49 - 3.35 (m, 2H), 3.21 (s, 2H), 2.78 (s, 4H), 2.63 (s, 2H), 2.55 (t, J = 7.4 Hz, 2H), 1.79 - 1.64 (m, 2H), 1.55 (dt, J = 15.1, 7.6 Hz, 2H), 1.45 (t, J = 11.6 Hz, 1H), 1.37 (dt, J = 15.3, 7.8 Hz, 2H), 1.16 (d, J = 9.8 Hz, 1H). ¹³C NMR (126 MHz, CDCl₃): δ 178.00, 169.17, 168.33, 137.76, 47.74, 45.09, 42.72, 38.20, 30.66, 27.20, 25.96, 25.55, 24.02.

Cis-butenedioic-bis(6-azidohexyl) ester (15)

To a solution of maleic acid (300 mg, 2.58 mmol) in 10 mL CH₂Cl₂ was added compound 18 (740 mg, 5.17 mmol, 2 eq.). The mixture was cooled to 0 ºC and DCC (640 mg, 3.11 mmol, 1.2 eq.) and DMAP (95 mg, 0.78 mmol, 0.3 eq.) were added. The mixture was stirred at room temperature and white precipitation was occurred. After TLC indicated full conversion, the mixture was concentrated under reduced pressure. The residue was re-dissolved in minimal amount of CH₂Cl₂, filtrated to remove white precipitation, and CH₂Cl₂ was evaporated. The residue was purified by silica gel column to afford compound 15 as white solid (540 mg, 57%). ¹H NMR (500 MHz, CDCl₃): δ 6.24 (s, 2H), 4.19 (t, J = 6.6 Hz, 4H), 3.27 (t, J = 6.9 Hz, 4H), 1.71 - 1.66 (m, 4H), 1.63 - 1.59 (m, 4H), 1.43 - 1.38 (m, 8H). ¹³C NMR (126 MHz, CDCl₃): δ 165.27, 129.77, 65.13, 51.30, 28.71, 28.30, 26.33, 25.45.

6-(N-Maleimido)-N-(2-propynyl)hexanamide (14)

To a solute of 6-maleimidohexanoic acid (300 mg, 1.42 mmol) in 8 mL CH₃OH was added propargylamine (182 µL, 3.30 mmol, 2.3 eq.) and DMTMM (786 mg, 2.84 mmol, 2 eq.). The mixture was stirred at 40 ºC for 3 h after which TLC indicated full conversion. The mixture was concentrated under reduced pressure and purified to give compound 14 as white solid (257.4 mg, 73%). ¹H NMR (500 MHz, CDCl₃): δ 6.68 (s, 2H), 5.71 (s, 1H), 4.03 (dd, J = 5.0, 2.3 Hz, 2H), 3.50 (t, J = 7.2 Hz, 2H), 2.22 (t, J = 2.0 Hz, 1H), 2.18 (t, J = 7.5 Hz, 2H), 1.72 - 1.64 (m, 2H), 1.59 (dd, J = 14.9, 7.5 Hz, 2H), 1.36 - 1.27 (m, 2H). ¹³C NMR (126 MHz, CDCl₃): δ 172.26, 170.83, 134.05, 79.58, 71.53, 37.54, 36.08,

**Cis-butenedioic-bis[6-(4-(N-maleimido)-hexanamide)methyl-1H-1,2,3-triazol-4-yl]hexyl] ester (16)**

Compound **15** (36 mg, 0.1 mmol) and compound **14** (48.78 mg, 0.2 mmol, 2 eq.) were dissolved in 2 mL THF and 1.5 mL H₂O was added. To this was added freshly prepared stock solutions of CuSO₄·5H₂O (200 μL, 25 mg/mL, 0.2 eq.) and Na-ascorbate (212 μL, 99.055 mg/mL, 1.2 eq.). The mixture was stirred at 40 ºC for 1 h after which TLC indicated full conversion. The mixture was concentrated and washed with CH₂Cl₂. The organic layer was dried (Na₂SO₄) and evaporated under reduced pressure. The residue was purified by silica gel column to give compound **16** as white solid (35 mg, 47 %). ¹H NMR (500 MHz, CDCl₃): δ 7.57 (s, 1H), 6.68 (s, 4H), 6.23 (s, 2H), 5.30 (s, 1H), 4.50 (d, J = 5.6 Hz, 4H), 4.33 (t, J = 7.2 Hz, 4H), 4.15 (t, J = 6.5 Hz, 4H), 3.49 (t, J = 7.2 Hz, 6H), 2.18 (t, J = 7.5 Hz, 4H), 1.94 - 1.86 (m, 4H), 1.65 (dd, J = 13.3, 7.1 Hz, 11H), 1.45 - 1.30 (m, 11H). ¹³C NMR (126 MHz, CDCl₃): δ 172.88, 170.85, 165.29, 134.04, 129.79, 122.27, 64.98, 54.27, 50.18, 37.60, 36.16, 34.87, 30.06, 28.17, 26.29, 26.02, 25.24, 24.95.

**General procedure for the preparation of polymer containing NHS ester (13)**

A solution of **12** (30 mg, 1 eq.) in 1 mL CH₂Cl₂ was stirred at -78 ºC for 30 min with argon protection. A stock solution of the Grubbs 3rd catalyst was freshly prepared at 14.2 mg•mL⁻¹ in CH₂Cl₂. Following the addition of desirable amounts of the catalyst solution, the mixture was stirred vigorously at -78 ºC for 20 min and then stirred at room temperature protected from light. The reaction time for the complete consumption of the monomer was monitored by thin layer chromatography (TLC). Following the end of the reaction, excess ethyl vinyl ether was added to quench the reaction, after which the mixture was stirred for 30 min. Following stirring, the excess Et₂O (×5 Volume) was added and light brown precipitate was formed. The suspension was centrifuged and then the ether was decanted. Repeat the process.
of centrifuging and decanting to afford the final polynorbornyl NHS ester as a grey solid. The polymer was stored at -20 °C and characterized by ¹H-NMR.

**Terminal maleimidation of NHS-containing polymer chain (13-TA)**

In a typical polymerization experiment, a solution of 12 (70 mg, 0.19 mmol) in 2 mL CH₂Cl₂ was stirred at -78 °C for 30 min with argon protection. A stock solution of the Grubbs 3rd catalyst was freshly prepared at 14.2 mg•mL⁻¹ in DCE. Following the addition of 100 μL catalyst solution, the mixture was stirred vigorously at -78 °C for 20 min and then stirred at room temperature protected from light. Upon TLC indicated full conversion, excess compound 16 was added to quench the reaction, after which the mixture was stirred for 30 min. Following stirring, the excess Et₂O (×5 Volume) was added and light brown precipitate was formed. The suspension was centrifuged and then the ether was decanted. Repeat the process of centrifuging and decanting to afford the final polynorbornyl NHS ester as a grey solid. The polymer was stored at -20 °C and characterized by ¹H-NMR.

**General procedure for post-modification of NHS-containing polymer with sugar units**

Before the condensation of NHS-containing polymer with sugar units, NHS-containing polymer (1 eq., based on monomer) was dissolved in 1.2 mL DMF to prepared solution A, and terminal amine functionalized galactosamine (1.2 eq.) was dissolved in 200 μL deionized water to prepare solution B. Afterward, solution A and Et₃N (5 eq.) were added to solution B quickly. The mixture was stirred vigorously under room temperature for 5 h after which TLC indicated full conversion. The mixture was dialyzed against deionized water for 3 days using cellulose membranes with a molecular weight cut off of 3.5 kDa and lyophilized to obtain the purified glycopolymer and maleimide containing glycopolymer as a white powder. The glycopolymers were stored at -20 °C and characterized by ¹H-NMR.

**General procedure for coupling glycopolymer with Cy3-NH₂**

Glycopolymer with NHS activated esters and Cy3-NH₂ (2 eq., based on glycopolymer) was dissolved in 1.2 mL PBS buffer (0.1 M, pH = 8.5). The mixture was stirred vigorously under room temperature for 5 h after which TLC indicated successful coupling. The mixture was
dialyzed against deionized water for 3 days using cellulose membranes with a molecular weight cut off of 3.5 kDa and lyophilized to obtain the purified glycopolymer with Cy3 a pink powder. The glycopolymers were stored at -20 ºC.

General procedure for the end-capping of glycopolymers with iRGD cyclic peptide (GP-iRGD)

The maleimide containing glycopolymer (1 eq., based on maleimide) was dissolved in HEPES buffer and iRGD cyclic peptide (1.5 eq.) was added. The mixture was stirred gently at room temperature for 24 h. The mixture was dialyzed against deionized water for 3 days using cellulose membranes with a molecular weight cutoff of 3.5 kDa to remove inorganic salt and excess iRGD cyclic peptide. The dialysate was lyophilized to obtain iRGD cyclic peptide capped glycopolymer as a white powder. The glycopolymers were stored at -20 ºC.
5. NMR spectra of compounds.

**Figure S1-1.** $^1$H NMR spectrum of compound 17

**Figure S1-2.** $^{13}$C NMR spectrum of compound 17
Figure S2-1. $^1$H NMR spectrum of compound 18

Figure S2-2. $^{13}$C NMR spectrum of compound 18
Figure S3-1. $^1$H NMR spectrum of compound 7.

Figure S3-2. $^{13}$C NMR spectrum of compound 7.
Figure S4-1. $^1$H NMR spectrum of compound 20

Figure S4-2. $^{13}$C NMR spectrum of compound 20.
Figure S5-1. $^1$H NMR spectrum of compound 1

Figure S5-2. $^{13}$C NMR spectrum of compound 1.
Figure S6-1. $^1$H NMR spectrum of compound 21.

Figure S6-2. $^{13}$C NMR spectrum of compound 21.
Figure S7-1. $^1$H NMR spectrum of compound 5.

Figure S7-2. $^{13}$C NMR spectrum of compound 5.
Figure S8-1. $^1$H NMR spectrum of compound 22.

Figure S8-2. $^{13}$C NMR spectrum of compound 22.
Figure S9-1. $^1$H NMR spectrum of compound 3.

Figure S9-2. $^{13}$C NMR spectrum of compound 3.
Figure S 10-1. $^1$H NMR spectrum of compound 23.

Figure S10-2. $^{13}$C NMR spectrum of compound 23.
Figure S11-1. $^1$H NMR spectrum of compound 6.

Figure S12-1. $^1$H NMR spectrum of compound 8.
Figure S12. $^{13}$C NMR spectrum of compound 8.

Figure S13. $^1$H NMR spectrum of compound 24.
**Figure S13-2.** $^{13}$C NMR spectrum of compound 24.

**Figure S14-1.** $^1$H NMR spectrum of compound 25.
Figure S14-2. $^{13}$C NMR spectrum of compound 25.

Figure S15-1. $^1$H NMR spectrum of compound 4.
Figure S15-2. $^{13}$C NMR spectrum of compound 4.

Figure S16-1. $^1$H NMR spectrum of compound 26.
Figure S16-2. $^{13}$C NMR spectrum of compound 26.

Figure S17-1. $^1$H NMR spectrum of compound 27.
Figure S17-2. $^{13}$C NMR spectrum of compound 27.

Figure S18-1. $^1$H NMR spectrum of compound 2.
Figure S18-2. $^{13}$C NMR spectrum of compound 2.

Figure S19-1. $^1$H NMR spectrum of compound 12.
Figure S19-2. $^{13}$C NMR spectrum of compound 12.

Figure S20-1. $^1$H NMR spectrum of compound 15.
Figure S20-2. $^{13}$C NMR spectrum of compound 15.

Figure S21-1. $^1$H NMR spectrum of compound 14.
Figure S21-2. $^{13}$C NMR spectra of compound 14.

Figure S22-1. $^1$H NMR spectra of compound 16.
Figure S22-2. $^{13}$C NMR spectra of compound 16.

Figure S23. $^1$H NMR spectra of compound 13.
Figure S24. $^1$H NMR spectra of compound 13-TA.
6. NMR spectra for glycopolymers with specific sulfation pattern.

Figure S25. $^1$H NMR spectra of compound GP1.

Figure S26. $^1$H NMR spectra of compound GP2.
Figure S27. $^1$H NMR spectra of compound GP3.

Figure S28. $^1$H NMR spectra of compound GP4.
Figure S29. $^1$H NMR spectra of compound GP5.

Figure S30. $^1$H NMR spectra of compound GP6.
7. Design of polymer backbone with different length.

Table S1. Properties of the synthesized polymer backbone.

<table>
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<th>Catalyst</th>
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<th>(M_n) (NMR)</th>
<th>yield</th>
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<td>100</td>
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<td>1 %</td>
<td>37.44 kDa</td>
<td>95%</td>
</tr>
</tbody>
</table>

Figure S31. \(^1\)H NMR spectra of polymer backbone. Degree of polymerization (DP) were determined by \(^1\)H NMR integrations of polymer olefin signals (5.3-5.9 ppm) to phenyl signal (7.5 ppm).
8. Flow cytometric analysis and confocal images of glycopeptide mimetic with different time.

Figure S32. GP5-iRGD localizes to the cytoplasm of HeLa. (A) Changes in fluorescence intensity of GP5-iRGD with different time. (B) Confocal images of HeLa cells incubated with GP5-iRGD (20 μg•mL⁻¹) for 2 h and 4 h.
9. NMR spectra for terminating agent

**Figure S33.** $^1$H NMR spectra of compound 14, 15, 16, 13-TA.

10. Reference