# Supporting Information

## Prophylactic antiviral activity of sulfated glycomimetic oligomers and polymers.

Laura Soria-Martinez, Sebastian Bauer, Markus Giesler, Sonja Schelhaas, Jennifer Materlik, Kevin Janus, Patrick Pierzyna, Miriam Becker, Nicole L. Snyder, Laura Hartmann, Mario Schelhaas

### 1. Materials and Methods for Glycomimetic Synthesis and Analysis

1.1 Materials

Acetonitrile (99.9 %, HPLC-grade), Amberlite IR-120, boron triflouride diethyl etherate (for synthesis), concentrated hydrochloric acid (p.a.), diethyl ether (p.a.), 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid, dichloromethane (99.9 %, puriss., p.a.), D-(+)-mannose (99 %), N-hydroxyethylacrylamide, ethyl acetate (99.9 %, puriss., p.a.), palladium on carbon (extent of labeling: 10 wt. % loading, matrix activated carbon support), sodium borohydride, sodium chloride (98 %), tetrahydrofuran (99.9 %, puriss., p.a.) and triisopropylsilane were purchased from Sigma-Aldrich. Chloroform (analytical reagent grade), magnesium sulfate (dried, laboratory reagent grade) and sodium hydrogen carbonate were provided by Fisher Scientific. Azobis(isobutyronitril) (98 %), dichloromethane (p.a.), dimethylformamide (98 %, for peptide synthesis), ethyl acetate (analytical reagent grade) and sulfur trioxide trimethylamine were purchased from ACROS Organics. 2-Bromoethanol (97 %), 2-(2-aminoethoxyethanol) (98 %), ethyl trifluoracetate (99 %), *p*-toluene sulfonic acid (98 %) and sodium methoxide (98 %) were purchased from Alfa Aesar. Ethanolamine, ethylenediamine (99.5 %, for synthesis) and *N*,*N*-diisopropylethylamine (> 99 %) were purchased from Carl Roth. Methanol (p.a.), acetic anhydride (99.7 %) and sodium dithionite were purchased from VWR BDH Prolabo Chemicals. 1,8-Diazabicyclo[5.4.0]undec-7-ene, β-D-Galactose pentaacetate and trichloroacetonitrile were obtained from *fluorochem*. Sodium sulphate (anhydrous) was purchased from Caelo. Triethylamine (99.5 %) was purchased from AppliChem. Formic Acid was purchased from Riedel de haen. Acetic acid (purum, >99 %) was purchased from Honeywell. Anhydrous copper (II) sulfate was purchased from Fluka (coelfen). 2-Cyano-2-propyl dodecyl trithiocarbonate was purchased from abcr. Sodium L(+)-ascorbate was purchased from Panreac AppliChem. Water was purified with a Milli-Q-system by Millipore with a final resistivity of 18 MΩcm.

1.2 Methods

**$^1$H-NMR**

$^1$H-NMR spectra were recorded at room temperature with a Bruker AVANCE III 300 (for 300 MHz) and 600 (for 600 MHz). The chemical shifts were reported relative to solvent peaks (chloroform and water)

as internal standards and reported as δ in parts per million (ppm). Multiplicities were abbreviated as s for singlet, d for doublet, t for triplet and m for multiplet.

**Electrospray Ionization Mass Spectrometry (ESI-MS)**
ESI-MS spectra were recorded with a mass spectrometer type Ion-Trap-API Finningan LCQ Deca.

**Ultra High Resolution Mass Spectrometry (UHR-MS)**
UHR-MS spectra were recorded with a Bruker UHR-QTOF maXis 4G with a direct inlet via syringe pump and an ESI source followed by a quadrupole Time of Flight (QTOF) mass analyser.

**Matrix-Assisted Laser Desorption-Ionization Time of Flight (MALDI-TOF)**
MALDI-TOF spectra were recorded with a MALDI-TOF Ultraflex I provided by Bruker Daltonics. Sinapinic acid matrix was applied in a mixture of acetonitrile and water (ratio of 1:2) was selected.

**Gel Permeation Chromatography (GPC)**
GPC analysis was conducted with an Agilent 1200 series HPLC system and three aqueous GPC columns provided by Polymer Standards Service (PSS). The columns were two Suprema Lux analytical columns (8 mm diameter and 5 µm particle size) and one precolumn (50 mm, 2 x 160 Å of 300 mm and 1000 Å of 300 mm). The eluent was a buffer system consisting of MilliQ water and 30 % acetonitrile with 50 mM $NaH_2PO_4$, 150 mM NaCl and 250 ppm $NaN_3$ with a pH = 7.0 (via addition of 50 mL 3 molar aqueous sodium hydroxide solution) filtered with inline 0.1 µm membrane filter and running at 1 mL per minute. Mullti-angle light scattering is recorded via mimDAWN TREOS and differential refractive index spectra with Optilab rEX both supplied by Wyatt Technologies EU. Data analysis was committed with Astra 5 software and a dn/dc value of 0.156 for each glycopolymer.

**Freeze Dryer**
Lyophilization was performed with an Alpha 1-4 LD instrument provided by Martin Christ Freeze Dryers GmbH. A temperature of –42 °C and a pressure of 0.1 mbar were maintained throughout the freeze-drying process.

**Elemental Analysis**
The ratio of carbon, hydrogen, nitrogen and sulfur were determined using a Vario Micro Cube provided by Analysensysteme GmbH.

**High Pressure Liquid Chromatography (HPLC)**
SAX-HPLC (Strong Anion Exchange) chromatography was performed on an Agilent Technologies 1200 Series LC system using an AT 1200 G1322A degasser, G1311A quarternary pump, G1329A automatic liquid sampler, G1316A thermostatted column compartment and G1314B variable wavelength detector at 214 nm. Buffers were prepared by dissolving 50 mM monosodium phosphate and 800 mM sodium chloride if required, before adjusting to pH 7 with sodium hydroxide. Crude buffer was filtered through GE Healthcare LifeScience Whatman™ 0.2 µm mixed cellulose filter before mixing with HPLC-grade acetonitrile if required. Anion exchange was performed on an Agilent Zorbax SAX column (5 µm, 4.6 x 250 mm with 4.6 x 12.5 mm guard column) at ambient temperature.

RP-HPLC/MS (Reversed Phase-HPLC/Mass Spectroscopy) was performed on an Agilent Technologies 1260 Infinity System using an AT 1260 G4225A degasser, G1312B binary pump, G1329B automatic liquid sampler, G1316C thermostatted column compartment, G1314F variable wavelength detector at 214 nm and an AT 6120 quadropole containing an electrospray ionisation (ESI) source. The mobile phase

consisted of buffer C (water:acetonitrile 95:5, 0.1 % formic acid) and buffer D (water:acetonitrile 5:95, 0.1 % formic acid). HPLC runs were performed on a Poroshell 120 EC-C18 (3.0 × 50 mm, 2.5 µm) RP column from Agilent at a flow rate of 0.4 ml/min 95% buffer A and 5% buffer B (0-5 min), following a linear gradient to 100% buffer B (5-30 min) at 25 °C. ESI-MS for GlcNAc-oligomers and sulfates was performed using 95% buffer A and 5% buffer B without formic acid and a fragmentor voltage of 40-60 V (m/z range of 200 to 2000).

Preparative RP-HPLC was performed by an Agilent 1260 Infinity system with a variable wavelength detector at 214 nm. For oligomer purification a UG80 C18 RP column from Shiseido was used (20 x 250 mm, 5 µm) at a gradient of 10-12.5% acetonitrile in water at room temperature in 15 min. The mobile phase contained 0.1% formic acid.

## 2. Synthesis of GlcNAc-Oligomers

### 2.1 General Protocols

**Synthesis of GlcNAc-oligomers**

Solid phase synthesis (SPS) of unsulfated GlcNAc-oligomers were performed using previously established solid phase polymer synthesis and EDS (**e**thylenedioxy **d**iamine **s**uccinic acid) and TDS (**t**riplebond **d**iamine **s**uccinic acid) building blocks[1].

**Resin handling**

For each reaction, the SPS resin (O1, O3 and O4: Tentagel S® RAM, 0.24 mmol/g, RAPP Polymere; O2: H-Gly-2CT resin, 0,44 mmol/g, Iris Biotech) was filled into BD syringe reactors containing an inlet frit (10 ml for 0.2 mmol batch sizes, 5 ml for 0.05 mmol). Before the first reaction step, the dry resin was swollen in DCM for 20 min (2.5 ml for 0.05 mmol batches, 5 ml for 0.2 mmol batches).

**Fmoc cleavage**

Trailing Fmoc protecting groups from either the resin or attached building blocks were deprotected using 2 x 5 ml 25% piperidine in DMF for 10 and 20 min, respectively using an intermediate washing step with 3 x DMF. After deprotection the resin was additionally washed with 10 x DMF and 3 x DCM. For each washing step 2.5 ml were used for 0.05 mmol batches and 5 ml were used for 0.2 mmol batches.

**Coupling**

For building block coupling (EDS or TDS) a 5-fold excess of building block and a 4.9-fold excess of PyBOP was first dissolved in DMF. Activation was performed by adding a 10-fold excess of diisopropyl ethyl amine (DIPEA) to the reaction solution with shaking for 3-5 min. The activated solution was taken up into the reaction syringe, and the coupling was performed over a period of 1.5 h while continuously shaking. After coupling, the resin was washed with 10 x DMF and 3 x DCM using maximal syringe capacity.

**N-terminal acetylation**

After final Fmoc deprotection, the N-terminus was capped by shaking with acetic anhydride (0.05 mmol batches: 2.5 ml, 0.2 mmol batch: 5 ml) for 30 min. After acetylation the resin was washed with 10 x 5 ml DMF and 3 x 5 ml DCM.

**CuAAC protocol for GlcNAc-$N_3$ (1) ligation**

For sugar ligation 3 eq. of GlcNAc-$N_3$ (**1**) per alkyne group were dissolved in DMF and mixed with 1.3 eq. to sugar Cu(II)$SO_4$ and sodium ascorbate in water.

The click reaction was performed overnight at room temperature, and the remaining copper was removed by extensive washing with cycles of 0.2 M sodium diethyl dithiocarbamate in DMF/water 1:1, DMF:water 1:1, DMF and DCM. Each solution was used to the maximal syringe capacity for each washing step. The washing cycle was continued until both the solution and the resin no longer showed colorization.

***O*-acetyl deprotection**

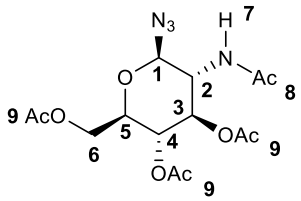
*O*-acetyl groups from each sugar unit were removed by adding 2.5-5 ml NaOMe in methanol (20 mM) for 30 min. Afterwards the resin was washed with 5 x MeOH and 3 x DCM. For each washing step 2.5 ml were used for 0.05 mmol batches and 5 ml were used for 0.2 mmol batches.

**Cleavage from solid support**

For total cleavage from solid support a 95% TFA solution containing 2.5 % TIPS and 2.5 % DCM was added to the resin (1.5 ml for 0.05 mmol batches, 6 ml for 0.2 mmol batch). After 60-70 min, the oligomers were precipitated in 40 ml ice cold diethyl ether. The resin could be washed with DCM and methanol to increase yield. Washing phases were concentrated and then precipitated in ice cold diethyl ether. The precipitate was combined and washed several times with diethyl ether before decanting and drying at high vacuum. Finally, the glycooligomers were lyophilized and purified by preparative HPLC.

## 3. Analytical data of GlcNAc-Oligomers

(*2R,3S,4R,5R,6R*)-5-acetamido-2-(acetoxymethyl)-6-azidotetrahydro-2H-pyran-3,4-diyl diacetate (GlcNAc-$N_3$, **S1**)




Structure **S1** was synthesized by a known protocol from *N*-acetyl glucosamine using acetyl chloride and sodium azide[2,3].

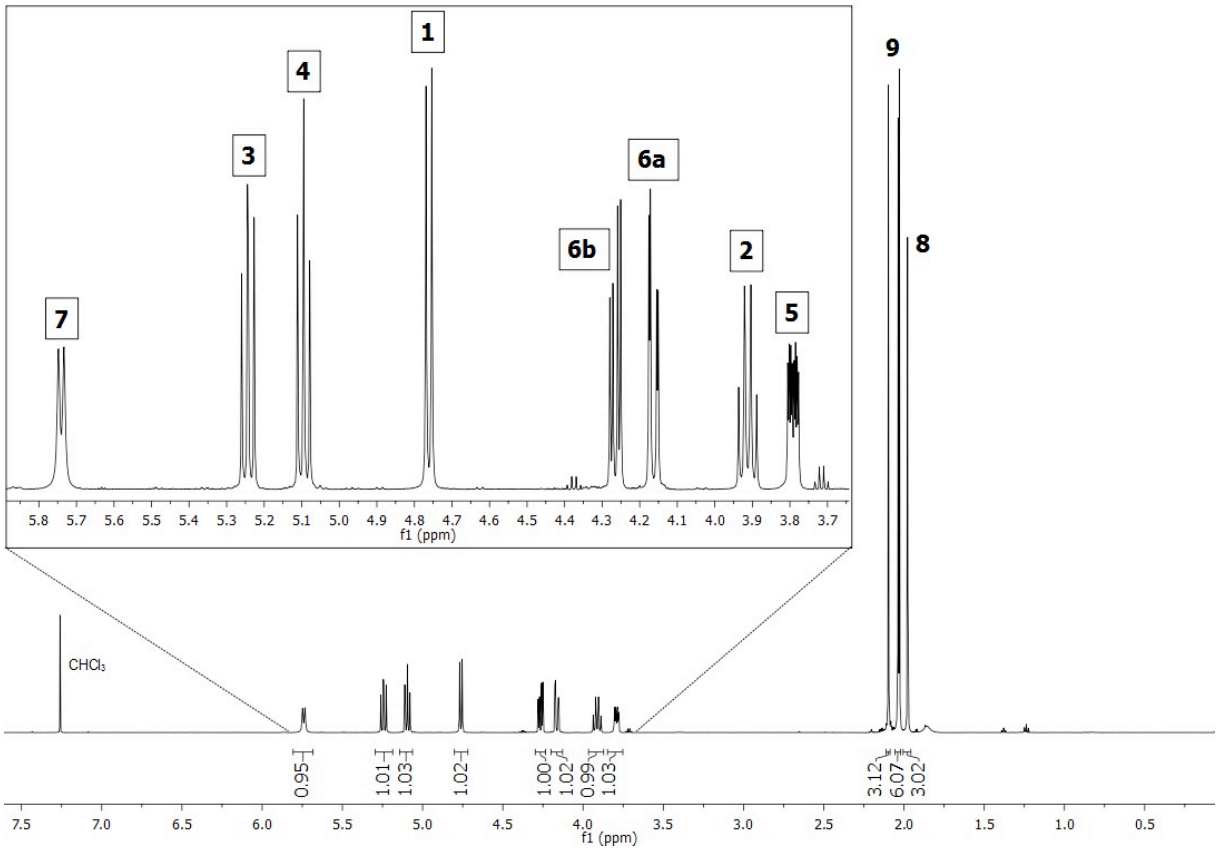



Figure S1: $^1$H-NMR (600 MHz, $CDCl_3$) of compound **S1**.

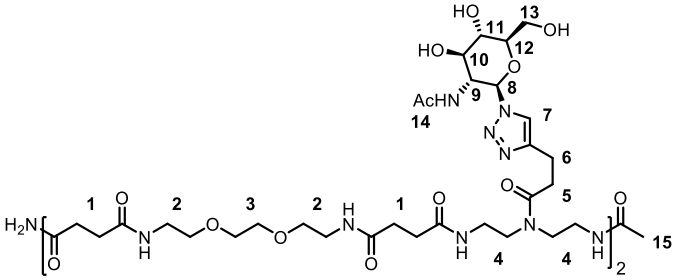
$^{1}$H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.98 (s, 3H, H-8); 2.03 (s, 3H, H-9a); 2.04 (s, 3H, H-9b); 2.10 (s, 3H, H-9c); 3.79 (ddd, 1H, H-5, *J* [Hz]: $H_{5\text{-}6a}$ = 2.3, $H_{5\text{-}6b}$ = 4.9, $H_{4\text{-}5}$ = 10.1); 3.91 (ddd, 1H, H-2, *J* [Hz]: $H_{1\text{-}2}$ = 9.2, $H_{2\text{-}3}$ = 10.6, $H_{2\text{-}7}$ = 8.9); 4.16 (dd, 1H, H-6a, *J* [Hz]: $H_{5\text{-}6a}$ = 2.3, $H_{6a\text{-}6b}$ = -12.4); 4.27 (dd, 1H, H-6b, *J* [Hz]: $H_{5\text{-}6b}$ = 4.9, $H_{6a\text{-}6b}$ = -12.4); 4.76 (d, 1H, H-1, *J* [Hz]: $H_{1\text{-}2}$ = 9.2); 5.09 (dd; 1H; H-4; *J* [Hz]: $H_{3\text{-}4}$ = 9.3, $H_{4\text{-}5}$ = 10.1); 5.24 (dd; 1H; H-3; *J* [Hz]: $H_{2\text{-}3}$ = 10.6, $H_{3\text{-}4}$ = 9.3); 5.74 (d; 1H; H-7; *J* [Hz]: $H_{2\text{-}7}$ = 8.9).

$^{13}$C-NMR (100 MHz, DMSO-$d_6$): δ [ppm] = 170.04, 169.62, 169.52, 169.25, 87.48, 72.77, 72.26, 68.13, 61.66, 52.48.

IR: ν [$cm^{-1}$] = 3362 (w, [N-H]); 2944 (w, [C-H/$CH_2$]); 2879 (w, [C-H/$CH_2$]); 2104 (s, [$N_3$]); 1741 (vs, C=O [OAc]); 1663 (s, C=O [NHAc]); 1518 (s, ν [C-H] + δ [N-H]).

ESI-MS: m/z calc. for $C_{14}H_{21}N_4O_8^+$ $[M+H]^+$: 373.1, found: 373.2.

EDS-(2,4)-GlcNc-(4) (**O1-OH**)




Compound **O1-OH** was lyophilized after removing the solvent from preparative RP-HPLC *in vacuo* and redissolving the resulting solid in 1 ml MilliQ $H_2O$. The final compound was produced in an overall yield of 100 mg (32.4%). Preparative RP-HPLC conditions: 10 - 12.5% MeCN ($H_2O$, 0.1% formic acid) in 10 min.

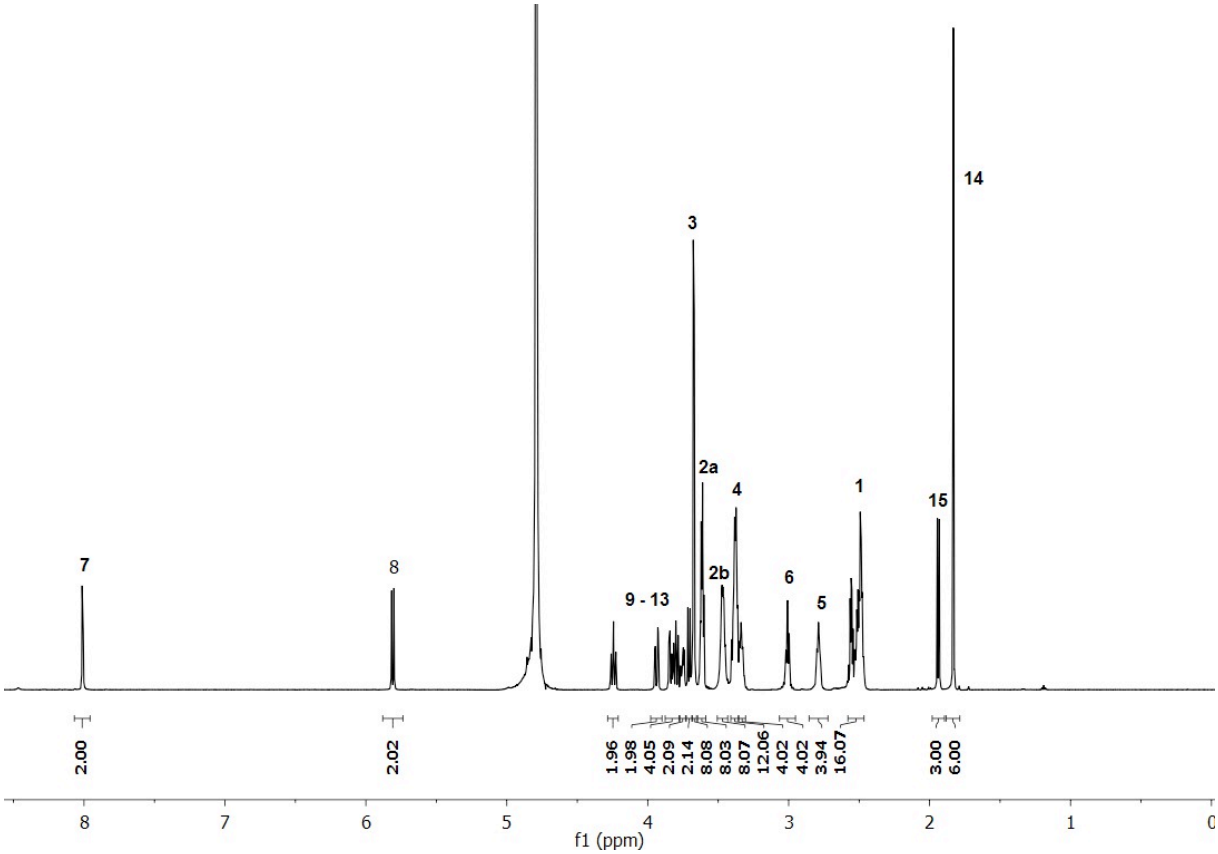
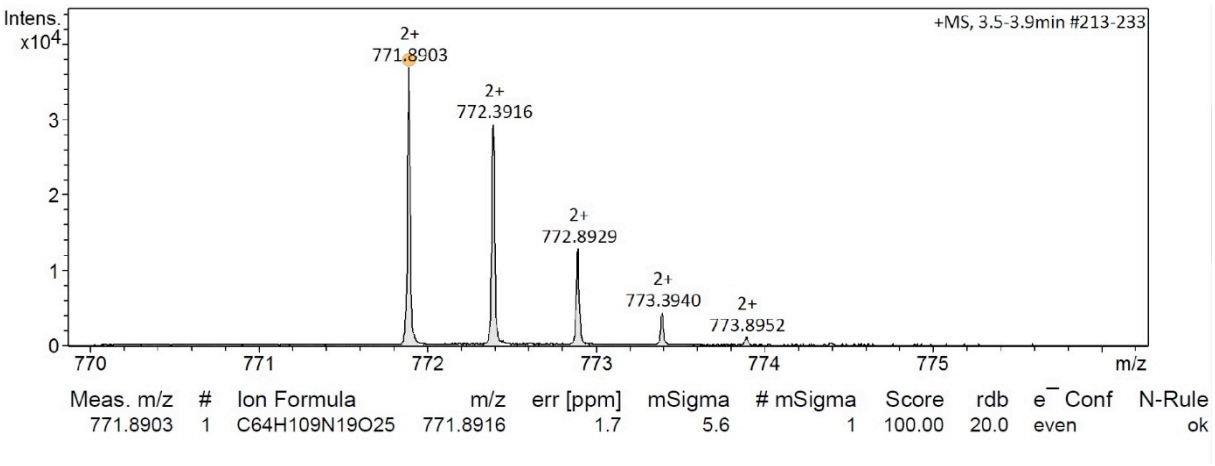



Figure S2: $^{1}$H-NMR (600 MHz, $D_2O$) of compound **O1-OH**.

$^{1}$H-NMR (600 MHz, $D_2O$): δ [ppm] = 1.83 (s, 6H, H-14); 1.93 & 1.95 (2x s, 3H, H-15); 2.45-2.52 (m, 12H, H-1); 2.52-2.59 (m, 4H, H-1 *C-terminus*); 2.79 (m, 4H, H-5); 3.01 (m, 4H, H-6); 3.30-3.35 (m, 4H, H-4 *N-terminus*); 3.35-3.41 (m, 12H, H-4); 3.43-3.52 & 3.59-3.64 (m, 16H, H-2) 3.65-3.69 (m, 8H, H-3); H-9/10/11: 3.70 (m, 2H), 3.80 (dd, $^{3}J_{HH}$ = 8.9 & 10.3 Hz, 2H), & 4.24 (m, 2H); 3.75 (ddd, 2H, H-12, *J* [Hz]: $H_{12\text{-}13a}$ = 1.8, $H_{12\text{-}13b}$ = 5.1, $H_{12\text{-}11}$ = 9.8); 3.84 (dd, 2H, H-13a, *J* [Hz]: $H_{13a\text{-}13b}$ = -12.6, $H_{13a\text{-}12}$ = 5.1); 3.94 (dd, 2H, H-13b, *J* [Hz]: $H_{13a\text{-}13b}$ = -12.6, $H_{13b\text{-}12}$ = 1.8); 5.81 (d, 2H, H-8, *J* = 9.6 Hz); 8.01 (m, 2H, H-7)

Note: Sugar protons 9-11 could not be clearly assigned, because not all *J*-values could be determined.




| Meas. m/z | # | Ion Formula | m/z | err [ppm] | mSigma | # mSigma | Score | rdb | e⁻ Conf | N-Rule |
|---|---|---|---|---|---|---|---|---|---|---|
| 771.8903 | 1 | C64H109N19O25 | 771.8916 | 1.7 | 5.6 | 1 | 100.00 | 20.0 | even | ok |

Figure S3: UHR-MS of compound **O1-OH**.

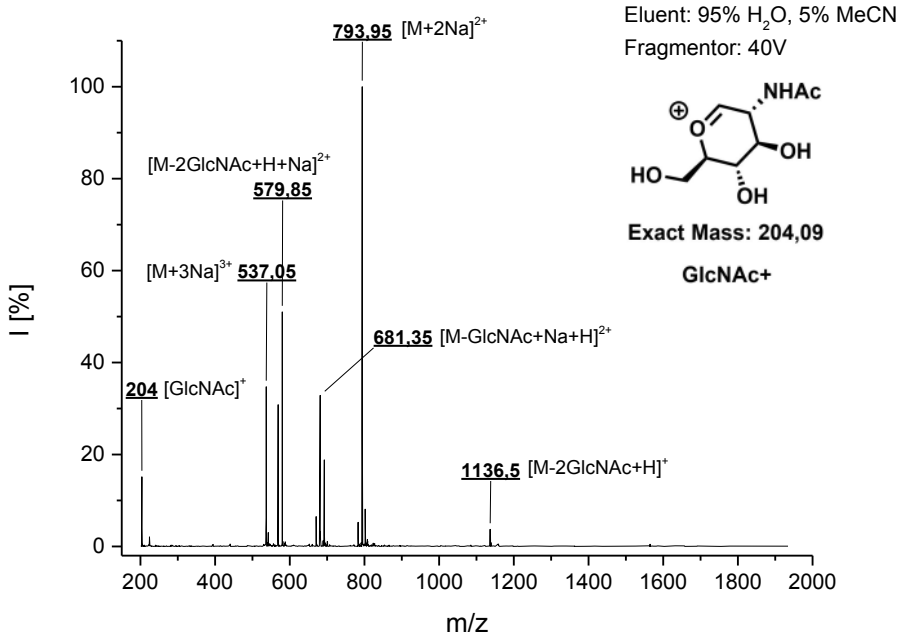



Figure S4: ESI-MS of compound **O1-OH**.

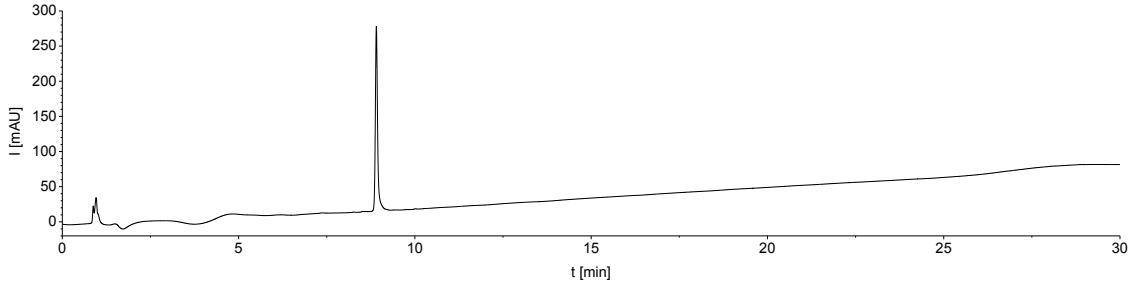



Figure S5: RP-HPLC of **O1-OH**. Method conditions: 95% water, 5% acetonitrile to 50% water, 50% acetonitrile in 30 minutes. 0.1% formic acid. Detection (UV) at 214 nm. Relative purity >95%.

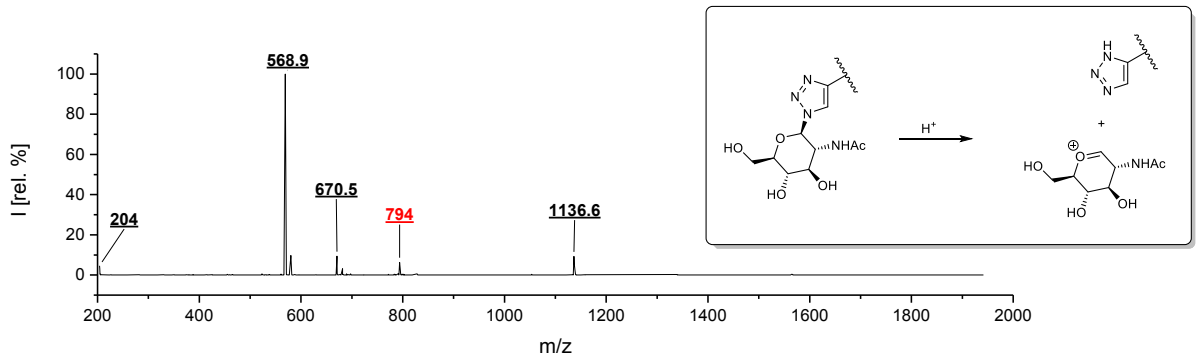
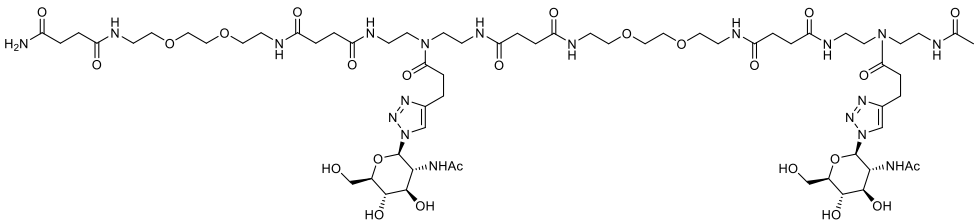
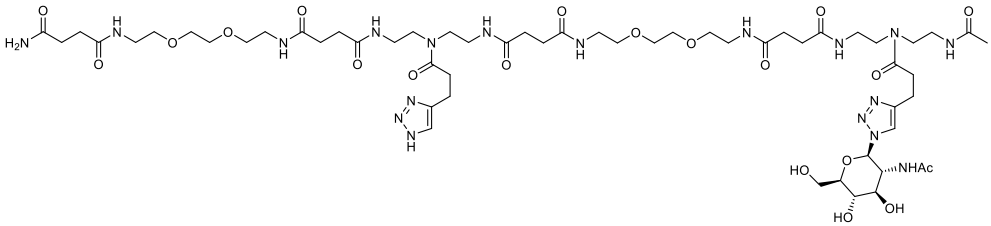
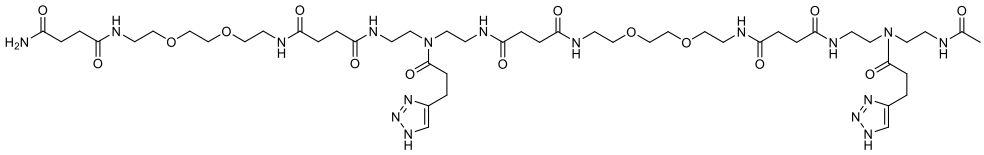
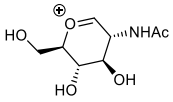
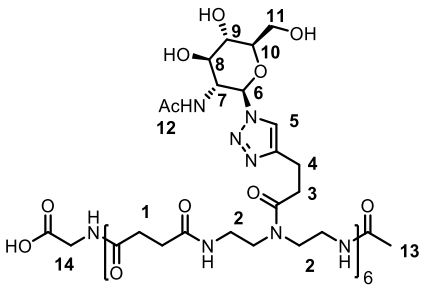



Figure S6: ESI-MS of glycooligomer **O1-OH**. Example of proton induced fragmentation, choosing a 50% MeCN 50% water mixture with 0.1% formic acid as spray eluent. Fragmentor voltage: 100 V.

Table S1: Structural overview of **O1-OH** and fragments from ESI-MS experiment (see Figure S6).

| Structure | m/z (calc.) | m/z (found) |
|---|---|---|
|  | $[M+2Na]^{2+}$ 793.87 | 794.0 |
|  | $[M+2H]^{2+}$ 670.35 | 670.5 |
|  | $[M+H]^{+}$ 1136.62 $[M+2H]^{2+}$ 568.81 | 1136.6 568.9 |
|  | $[M]^{+}$ 204.0 | 204.0 |

Gly-(1-6)-GlcNAc-(6) (**O2-OH**)



Unsulfated **O2-OH** GlcNAc-glycooligomer carrying a glycine linker was synthesized using the protocol described for glycooligomers previously. Briefly, to create the glycine linkage, H-Gly-2CT (Iris Biotech) resin was used. Cleavage from solid support was performed by using 1% TFA for 70 min. Deprotection of the peracetylated OAc GlcNAc moieties was performed after cleavage from solid support using 20 mM NaOMe in methanol followed by Amberlite IR-120 $H^+$ for neutralization. The crude oligomer was purified by preparative HPLC (yield: 22.3 mg, 7%).

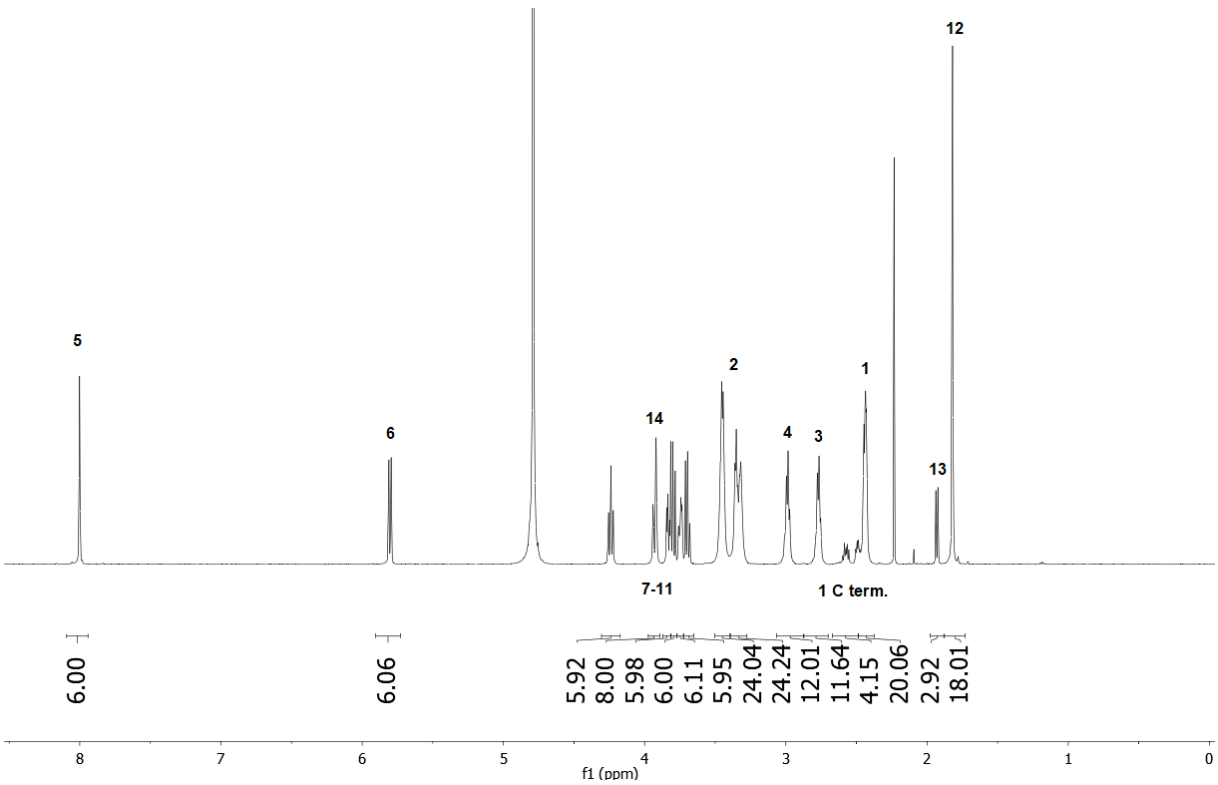
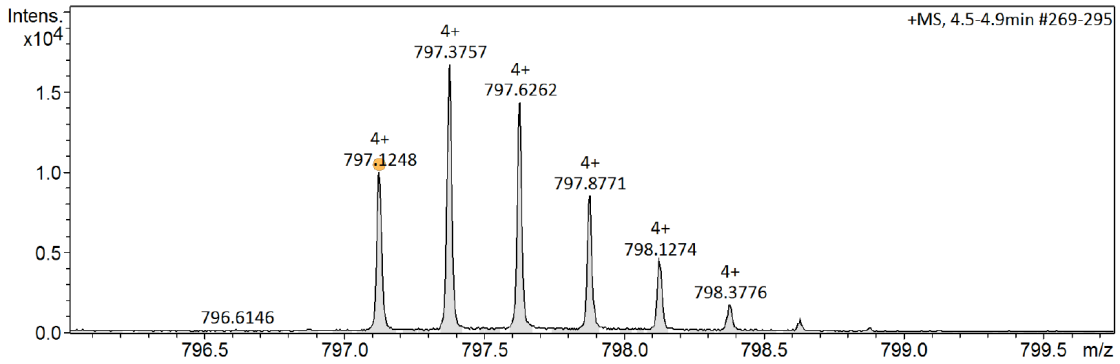



Figure S7: $^1$H-NMR (600 MHz, $D_2O$) of compound **O2-OH**.

$^{1}$H-NMR (600 MHz, $D_2O$): δ [ppm] = 1.82 (s, 18H, H-12); 1.92 & 1.94 (2x s, 3H, H-13); 2.44 (m, 20H, H-1); 2.53-2.54 (m, 4 H, H1 C-terminus); 2.76 (m, 12H, H-3); 2.98 (m, 12H, H-4); 3.25-3.52 (m, 48H, H-2); H-7/8/9: 3.70 (m, 2H), 3.80 (dd, $^{3}J_{HH}$ = 8.9 & 10.0 Hz, 2H), & 4.24 (m, 2H); 3.75 (ddd, 2H, H-10, *J* [Hz]: $H_{10\text{-}11a}$ = 2.2, $H_{10\text{-}11b}$ = 4.8, $H_{9\text{-}10}$ = 9.5); 3.83 (dd, 2H, H-11a, *J* [Hz]: $H_{11a\text{-}11b}$ = -12.5, $H_{11a\text{-}10}$ = 4.8);3.92 (s, 2H, H-14) 3.93 (dd, 2H, H-11b, *J* [Hz]: $H_{11a\text{-}11b}$ = -12.5, $H_{11b\text{-}10}$ = 2.2); 5.80 (d, $^{3}J_{HH}$ = 9.7 Hz, 6H, H-6); 8.00 (s, 6H, H-5).

Note: Sugar protons 7-9 could not be clearly assigned, because not all *J*-values could be determined.




| Meas. m/z | # | Ion Formula | m/z | err [ppm] | mSigma | # mSigma | Score | rdb | e¯ Conf | N-Rule |
|---|---|---|---|---|---|---|---|---|---|---|
| 797.1248 | 1 | C130H209N43O51 | 797.1265 | 2.1 | 13.8 | 1 | 68.00 | 48.0 | even | ok |
| | 2 | C131H209N41O52 | 797.1237 | -1.4 | 13.9 | 2 | 100.00 | 48.0 | even | ok |

Figure S8: UHR-MS of compound **O2-OH** $[M+4H]^{4+}$.

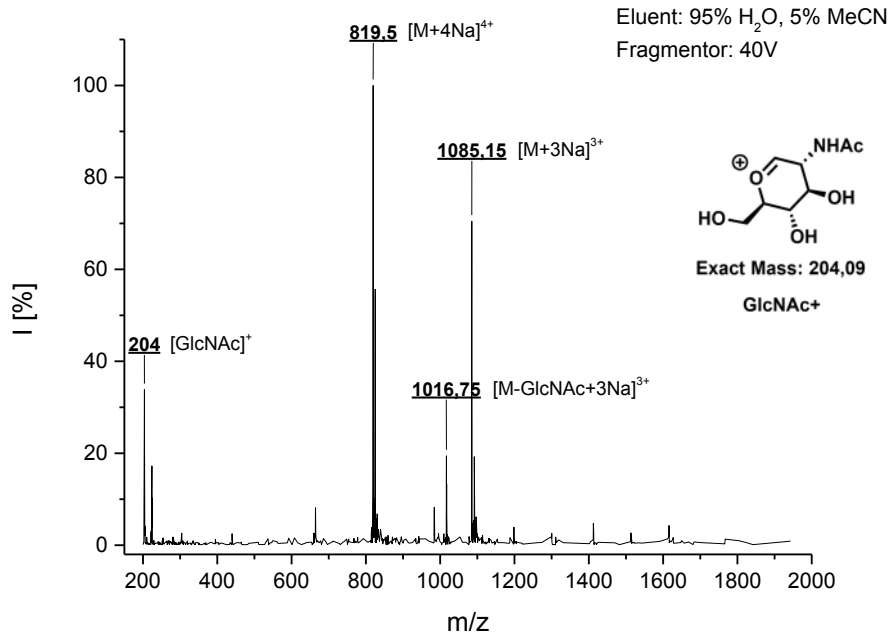



Figure S9: ESI-MS of compound **O2-OH**.

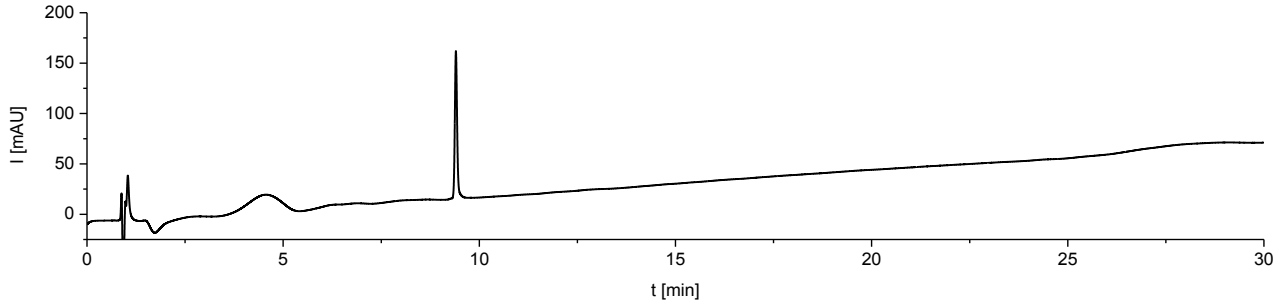
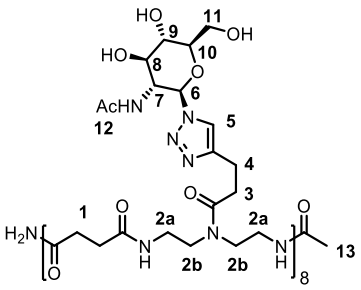



Figure S10: RP-HPLC of **O2-OH**. Method conditions: 95% water, 5% acetonitrile to 50% water, 50% acetonitrile in 30 minutes. 0.1% formic acid. Detection (UV) at 214 nm. Relative purity > 95%.

(1-8)-GlcNAc-(8) (**O3-OH**)



Compound **O3-OH** was lyophilized after removing the solvent from preparative RP-HPLC *in vacuo* and redissolving the resulting solid in 1 ml MilliQ $H_2O$. The final compound was produced in an overall yield of 82 mg (39.5%). Preparative RP-HPLC conditions: 10 - 12.5% MeCN ($H_2O$, 0.1% formic acid) in 10 min.

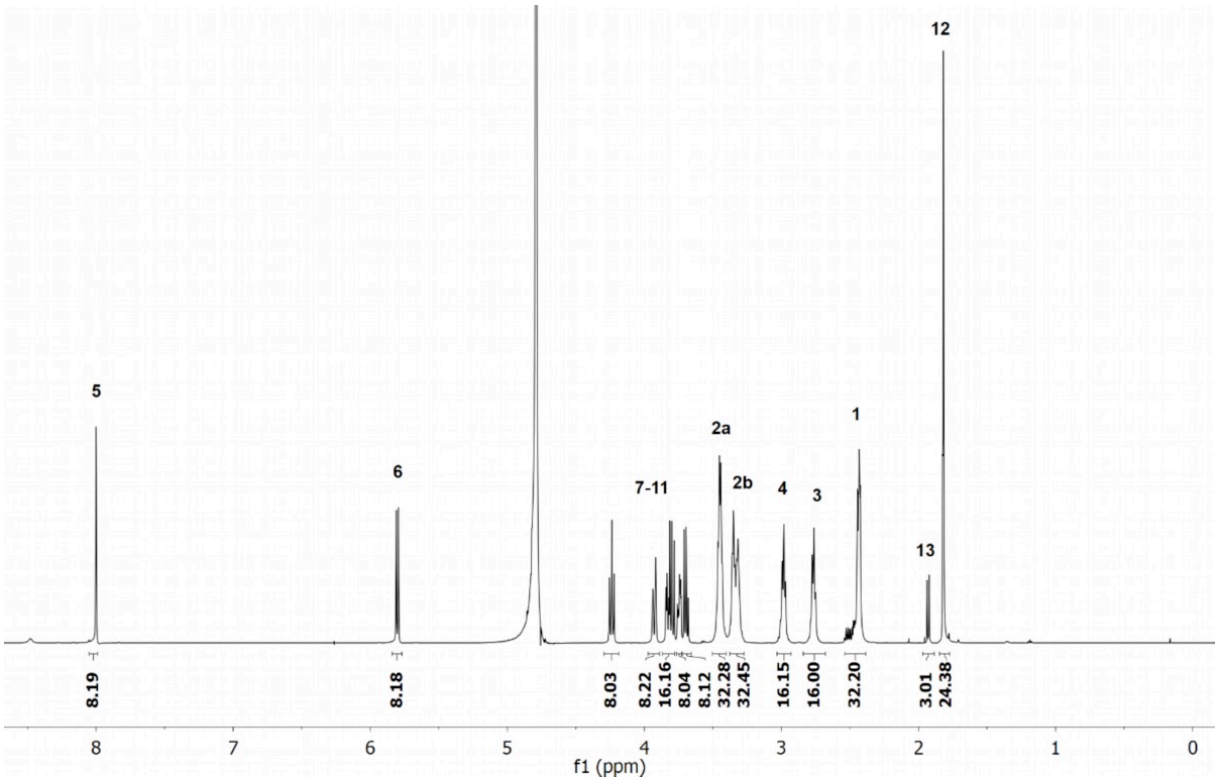



Figure S11: $^1$H-NMR (600 MHz, $D_2O$) of compound **O3-OH**.

$^1$H-NMR (600 MHz, $D_2O$): δ [ppm] = 1.82 (s, 24H, H-12); 1.92 & 1.94 (2x s, 3H, H-13); 2.43 (m, 32H, H-1); 2.76 (m, 16H, H-3); 2.98 (m, 16H, H-4); 3.27-3.40 (m, 32H, H-2b); 3.40-3.52 (m, 32H, H-2a); H-7/8/9: 3.69 (m, 2H), 3.80 (dd, $^3J_{HH}$ = 8.9 & 10.0 Hz, 2H), & 4.24 (m, 2H); 3.74 (ddd, 2H, H-10, *J* [Hz]: $H_{10\text{-}11a}$ = 2.2, $H_{10\text{-}11b}$ = 5.0, $H_{9\text{-}10}$ = 9.6); 3.83 (dd, 2H, H-11a, *J* [Hz]: $H_{11a\text{-}11b}$ = -12.5, $H_{11a\text{-}10}$ = 5.0); 3.92 (s, 2H, H-14) 3.93 (dd, 2H, H-11b, *J* [Hz]: $H_{11a\text{-}11b}$ = -12.5, $H_{11b\text{-}10}$ = 2.2); 5.80 (d, $^3J_{HH}$ = 9.7 Hz, 6H, H-6); 8.00 (s, 8H, H-5).

Note: Sugar protons 7-9 could not be clearly assigned, because not all *J*-values could be determined.

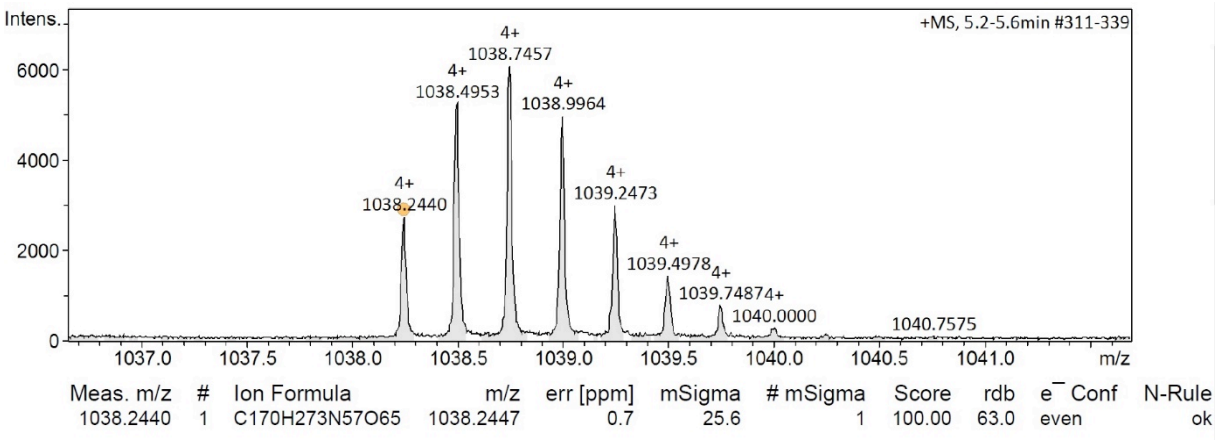



Figure S12: UHR-MS of compound **O3-OH** $[M+4H]^{4+}$.

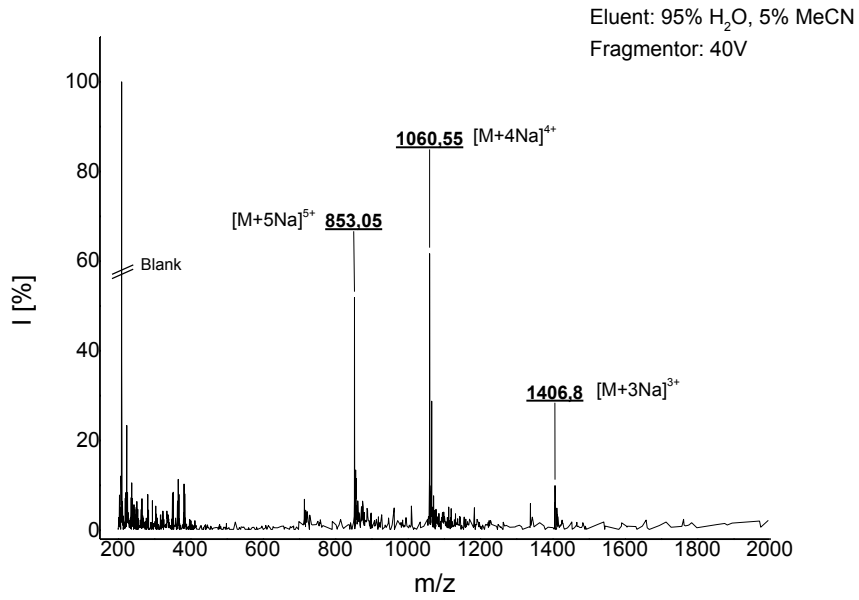



Figure S13: ESI-MS of compound **O3-OH**.

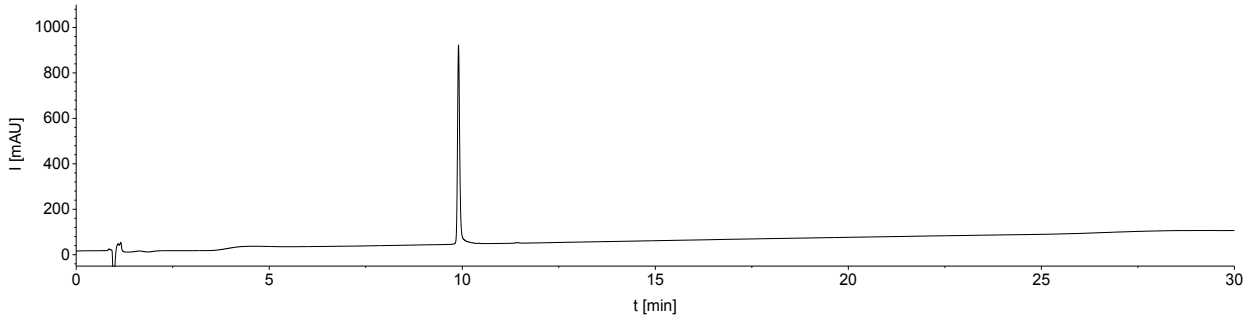
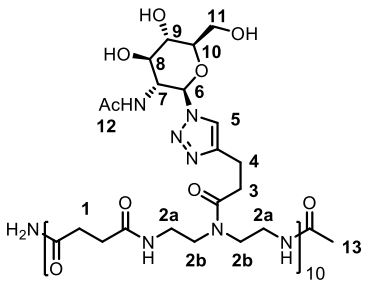



Figure S14: RP-HPLC of **O3-OH**. Method conditions: 95% water, 5% acetonitrile to 50% water, 50% acetonitrile in 30 minutes. 0.1% formic acid. Detection (UV) at 214 nm. Relative purity > 95%.

(1-10)-GlcNAc-(10) (**O4-OH**)



Compound **O4-OH** was lyophilized after removing the solvent from preparative RP-HPLC *in vacuo* and redissolving the resulting solid in 1 ml MilliQ $H_2O$. The final compound was produced in an overall yield of 96 mg (32%). Preparative RP-HPLC conditions: 10 - 12.5% MeCN ($H_2O$, 0.1% formic acid) in 10 min

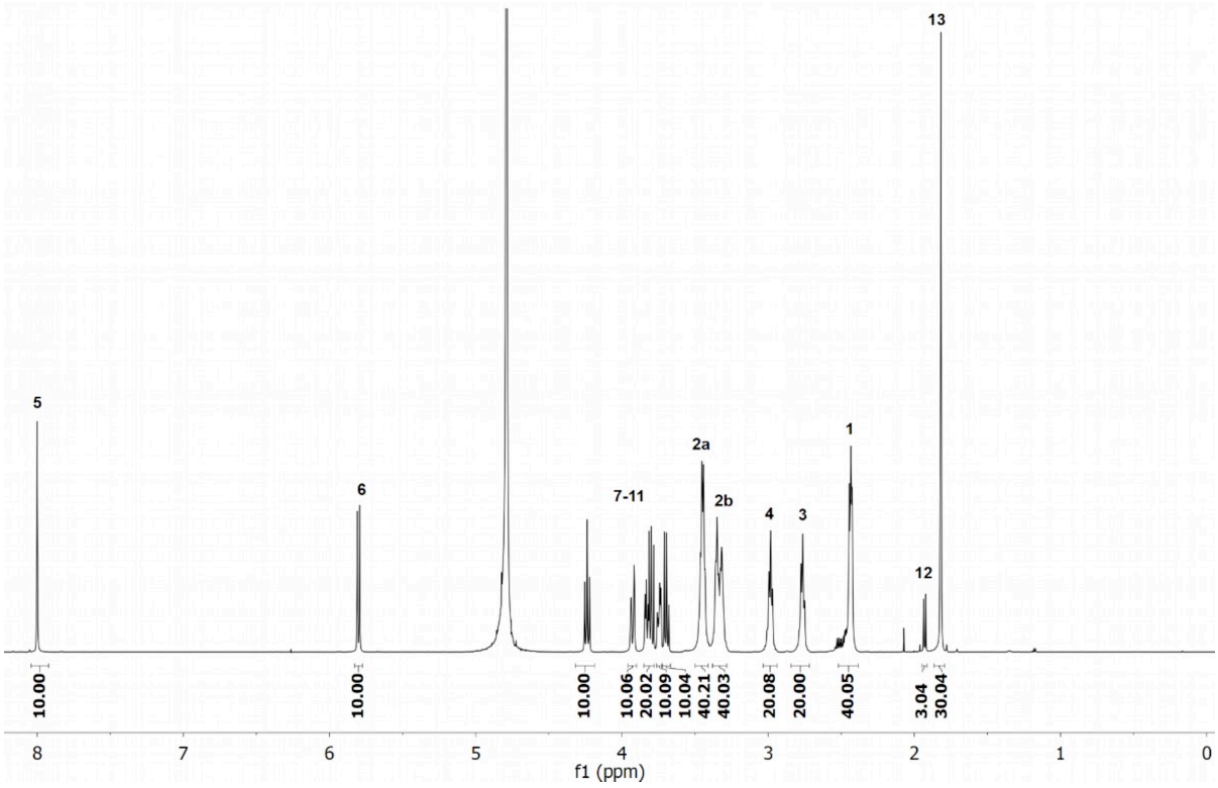
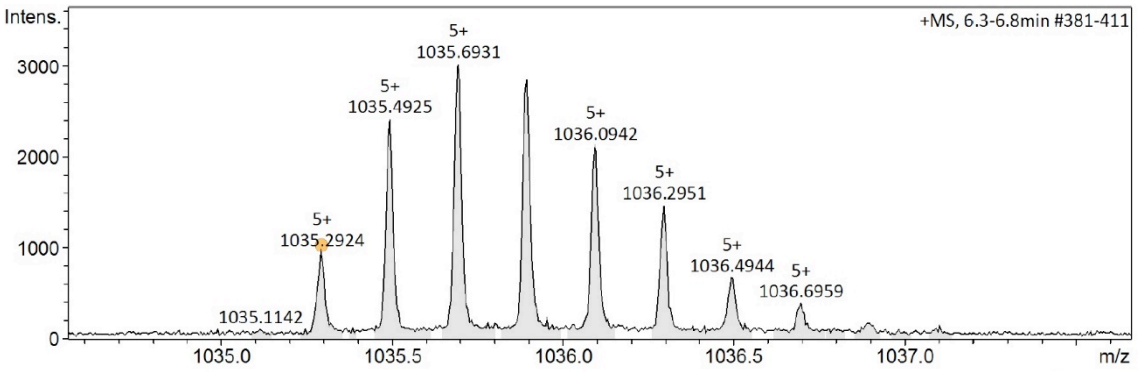



Figure S15: $^1$H-NMR (600 MHz, $D_2O$) of compound **O4-OH**.

$^1$H-NMR (600 MHz, $D_2O$): δ [ppm] = 1.82 (s, 30H, H-12); 1.92 & 1.94 (2x s, 3H, H-13); 2.43 (m, 40H, H-1); 2.76 (m, 20H, H-3); 2.98 (m, 20H, H-4); 3.27-3.40 (m, 40H, H-2b); 3.40-3.52 (m, 40H, H-2a); H-7/8/9: 3.69 (m, 2H), 3.80 (dd, $^3J_{HH}$ = 9.0 & 10.1 Hz, 2H), & 4.24 (m, 2H); 3.74 (ddd, 2H, H-10, *J* [Hz]: $H_{10\text{-}11a}$ = 2.3, $H_{10\text{-}11b}$ = 5.0, $H_{9\text{-}10}$ = 9.7); 3.83 (dd, 2H, H-11a, *J* [Hz]: $H_{11a\text{-}11b}$ = -12.8, $H_{11a\text{-}10}$ = 5.0); 3.92 (s, 2H, H-14) 3.93 (dd, 2H, H-11b, *J* [Hz]: $H_{11a\text{-}11b}$ = -12.8, $H_{11b\text{-}10}$ = 2.3); 5.80 (d, $^3J_{HH}$ = 9.5 Hz, 6H, H-6); 8.00 (s, 8H, H-5).

Note: Sugar protons 7-9 could not be clearly assigned, because not all *J*-values could be determined.




| Meas. m/z | # | Ion Formula | m/z | err [ppm] | mSigma | # mSigma | Score | rdb | e¯ Conf | N-Rule |
|---|---|---|---|---|---|---|---|---|---|---|
| 1035.2924 | 1 | C213H340N69O82 | 1035.2906 | -1.8 | 35.1 | 1 | 42.52 | 78.5 | even | ok |
| | 2 | C212H340N71O81 | 1035.2928 | 0.4 | 35.5 | 2 | 100.00 | 78.5 | even | ok |

Figure S16: UHR-MS of **O4-OH** $[M+5H]^{5+}$.

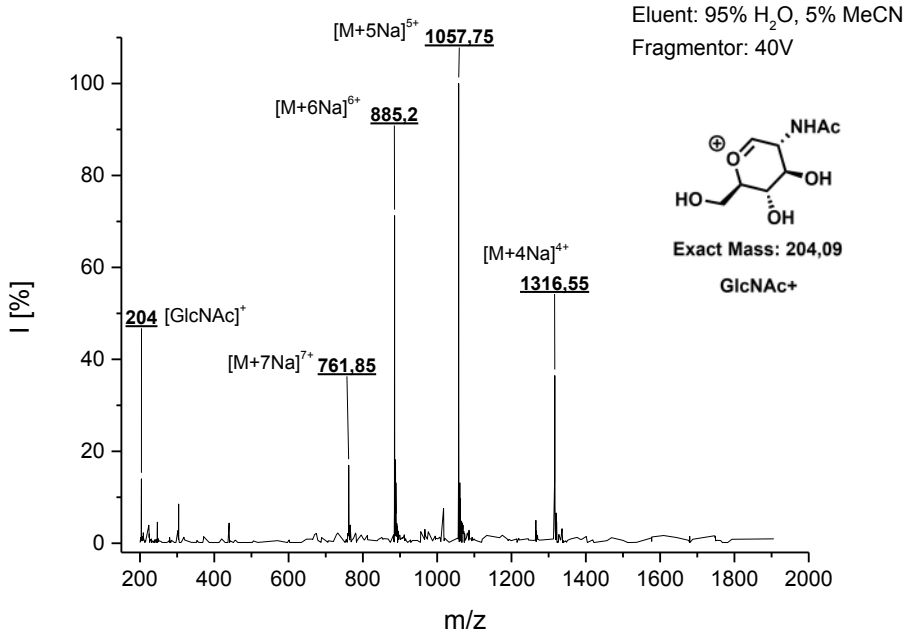



Figure S17: ESI-MS of compound **O4-OH**.

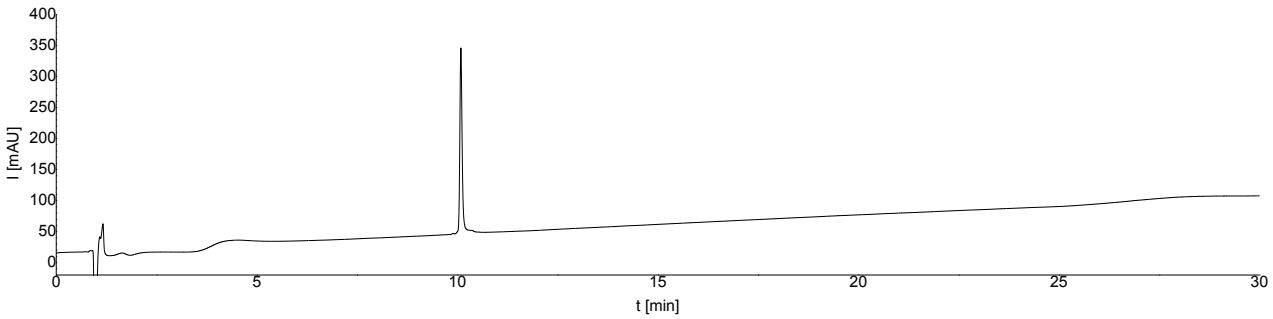



Figure S18: RP-HPLC of **O4-OH**. Method conditions: 95% water, 5% acetonitrile to 50% water, 50% acetonitrile in 30 minutes. 0.1% formic acid. Detection (UV) at 214 nm. Relative purity > 95%.

## 4. Sulfation of GlcNAc-oligomers

For reaction conditions see Table S2. Briefly, the precursor glycooligomer (**O1-OH** – **O4-OH**) and TMA*$SO_3$ were dissolved in dry DMF (8.65 ml/g TMA*$SO_3$) and mixed. After reaction, the mixture was added to a 10% sodium acetate solution in water (10 eq. to TMA*$SO_3$) and concentrated under reduced pressure (60 °C, 15 mbar). The crude product was purified by dialysis against water.


Figure S19: Sulfation of **O2-OH** using dry DMF as solvent and trimethylamine sulfurtrioxide (TMA*$SO_3$) sulfation agent. Sulfation of **O2-OH** was performed using 50 eq. TMA*$SO_3$ at 70°C for 19h.

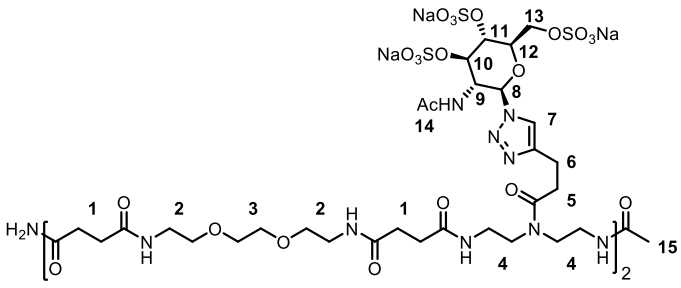
Table S2: Reaction conditions for synthesis of sulfated GlcNAc-glycooligomers.

| Structure | Precursor Glycooligomer | Equivalents[a] TMA*$SO_3$ | Reaction temperature [°C] | Reaction time [h] | d.S.[b] [%] |
|---|---|---|---|---|---|
| O1-1 | O1-OH | 40 | 70 | 18 | 95.0 |
| O1-2 | O1-OH | 40 | 70 | 18 | 98.5 |
| O2 | O2-OH | 50 | 70 | 19 | 98.7 |
| O3 | O3-OH | 40 | 70 | 18 | 89.9 |
| O4 | O4-OH | 40 | 70 | 18 | 85.2 |

(a) Equivalents to OH-groups presented by the respective GlcNAc-glycooligomer. (b) Degree of sulfation was determined by the ration of the anomeric proton signal of fully sulfated GlcNAc (6.02 ppm) to signals of only partially sulfated GlcNAc < 6.02 ppm.

## 5. Analysis of Sulfated Glycooligomers

EDS-(2,4)-GlcNAc(3-6S)-(4) (**O1-1 and O1-2**)



**O1-1**

Compound **O1-1** was obtained from **O1a** (20 mg batch) after dialysis and lyophilization with a yield of 15.1 mg (51.5%).

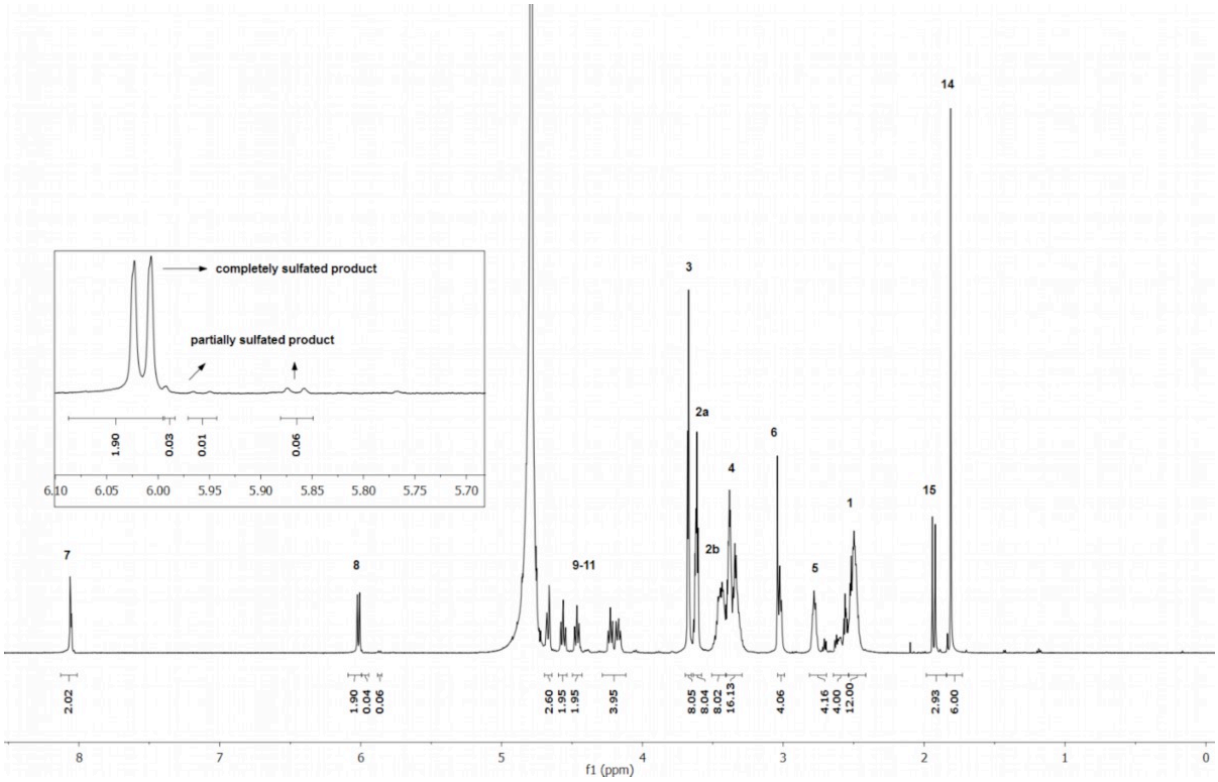



Figure S20: $^{1}$H-NMR (600 MHz, $D_2O$) of compound **O1-1**.

$^{1}$H-NMR (600 MHz, $D_2O$): δ [ppm] = 1.81 (s, 6H, H-14); 1.92 & 1.94 (2x s, 3H, H-15); 2.40-2.53 (m, 12H, H-1); 2.52-2.66 (m, 4H, H-1 *C-terminus*); 2.78 (m, 4H, H-5); 3.03 (m, 4H, H-6); 3.26-3.40 (m, 16H, H-4); 3.40-3.52 (m, 8H, H-2b); 3.61 (m; 8H, H-2a); 3.67 (m, 8H, H-3); H-9-13: 4.17, 4.23, 4.47, 4.56 & 4.67 (5x m, 5x 2H); 6.02 (d, $^{3}J_{HH}$ = 9.8 Hz, 2H, H-8); 8.06 (m, 2H, H-7)

Degree of completely OH-converted product determined by anomeric signal ratio (embedded window): 95.0%.

Note: One of the signals caused by the sugar ring proton is expected to lie beneath the HDO signal at 4.79 ppm (reference signal of $D_2O$).

Proton 8-13 intensity is lowered, due to partially sulfated product (I < 2H).

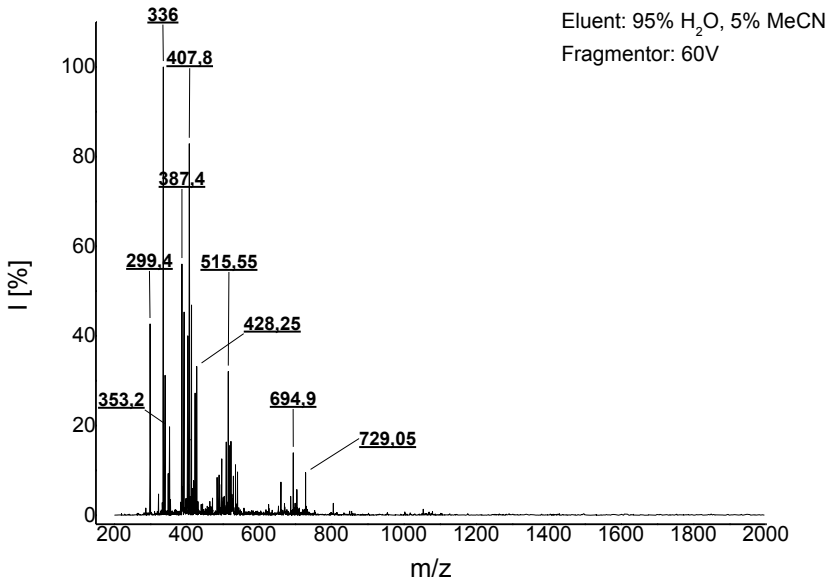



Figure S21: ESI-MS of compound **O1-1**. Negative ion mode.

Table S3: ESI-MS assignment for structure **O1**-**1** (Figure S21). 7x sulfation indicates the existence of an additional $SO_3Na$ attachment.

| 7x sulfated $[C_{64}H_{100}N_{19}O_{46}S_7Na_7]$ | | 6x sulfated $[C_{64}H_{101}N_{19}O_{43}S_6Na_6]$ | | 5x sulfated $[C_{64}H_{102}N_{19}O_{40}S_5Na_5]$ | |
|---|---|---|---|---|---|
| m/z calculated | m/z found | m/z calculated | m/z found | m/z calculated | m/z found |
| $[M-7Na]^{7-}$ = 299.2 | 299.4 | $[M-6Na]^{6-}$ = 335.9 | 336.0 | $[M-5Na]^{5-}$ = 387.3 | 387.4 |
| $[M-6Na]^{6-}$ = 352.9 | 353.2 | $[M-5Na]^{5-}$ = 407.7 | 407.8 | | |
| $[M-5Na]^{5-}$ = 428.1 | 428.3 | $[M-4Na]^{4-}$ = 515.4 | 515.6 | | |
| $[M-4Na]^{4-}$ = 540.8 | 541.1 | $[M-3Na]^{3-}$ = 694.8 | 694.9 | | |
| $[M-3Na]^{3-}$ = 728.8 | 729.0 | | | | |

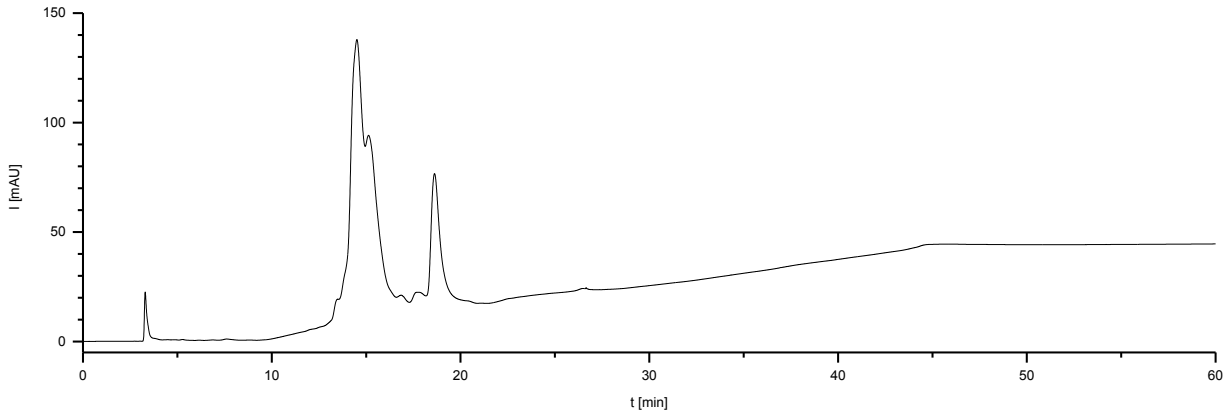



Figure S22: SAX-HPLC of O2-2. Conditions: 0-5 min 40 mM NaCl, 1.5 % MeCN; 5-40 min linear gradient to 800 mM NaCl and 30 % MeCN; 40-60 min 800 mM NaCl and 30 % MeCN to ensure complete elution. Detection at 214 nm.

**O1-2**

Compound **O1-2** was obtained from **O1a** (40.9 mg batch) after dialysis and lyophilization with a yield of 26.3 mg (46.0%).

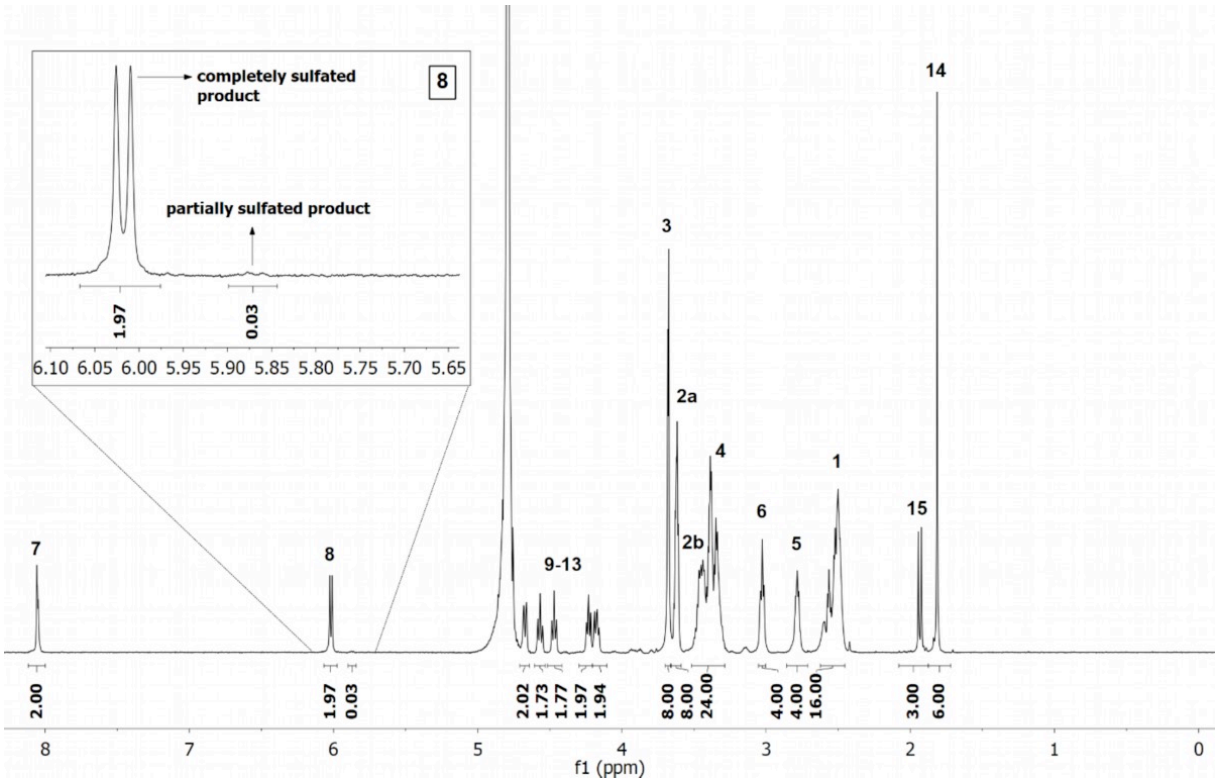



Figure S23: $^{1}$H-NMR (600 MHz, $D_2O$) of compound **O1-2**.

$^{1}$H-NMR (600 MHz, $D_2O$): δ [ppm] = 1.81 (s, 6H, H-14); 1.92 & 1.94 (2x s, 3H, H-15); 2.40-2.53 (m, 12H, H-1); 2.52-2.66 (m, 4H, H-1 *C-terminus*); 2.78 (m, 4H, H-5); 3.03 (m, 4H, H-6); 3.26-3.40 (m, 16H, H-4); 3.40-3.52 (m, 8H, H-2b); 3.61 (m; 8H, H-2a); 3.67 (m, 8H, H-3); H-9-13: 4.17, 4.23, 4.47, 4.56 & 4.67 (5x m, 5x 2H); 6.02 (d, $^{3}J_{HH}$ = 9.8 Hz, 2H, H-8); 8.06 (m, 2H, H-7)

Degree of completely OH-converted product determined by anomeric signal ratio (embedded window): 98.5%.

Note: One of the signals caused by the sugar ring proton is expected to lie beneath the HDO signal at 4.79 ppm (reference signal of $D_2O$).

Proton 8-13 intensity is lowered, due to partially sulfated product (I < 2H).

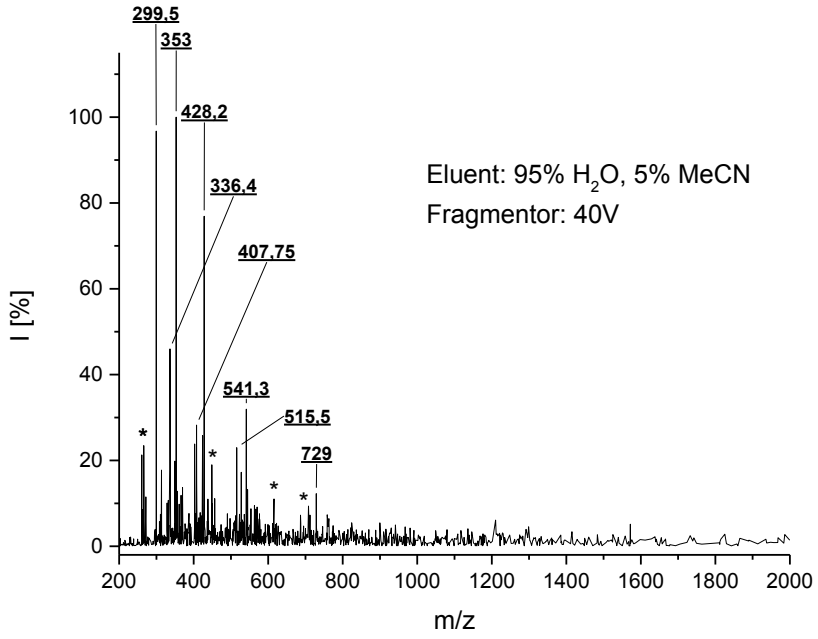



Figure S24: ESI-MS of compound **O1-2**. Negative ion mode.

Table S4: ESI-MS assignment for structure **O1**-**2** (Figure S24). Traces of sulfation below 6x can be found in the weaker m/z signals 200 and 800. Unassigned signals are marked (*). 7x sulfation indicates the existence of an additional $SO_3Na$ attachment.

| 7x sulfated [$C_{64}H_{100}N_{19}O_{46}S_7Na_7$] | | 6x sulfated [$C_{64}H_{101}N_{19}O_{43}S_6Na_6$] | |
|---|---|---|---|
| m/z calculated | m/z found | m/z calculated | m/z found |
| $[M-7Na]^{7-}$ = 299.2 | 299.4 | $[M-6Na]^{6-}$ = 335.9 | 336.2 |
| $[M-6Na]^{6-}$ = 352.9 | 353.0 | $[M-5Na]^{5-}$ = 407.7 | 407.8 |
| $[M-5Na]^{5-}$ = 428.1 | 428.3 | $[M-4Na]^{4-}$ = 515.4 | 515.7 |
| $[M-4Na]^{4-}$ = 540.8 | 541.1 | | |
| $[M-3Na]^{3-}$ = 728.8 | 729.0 | | |

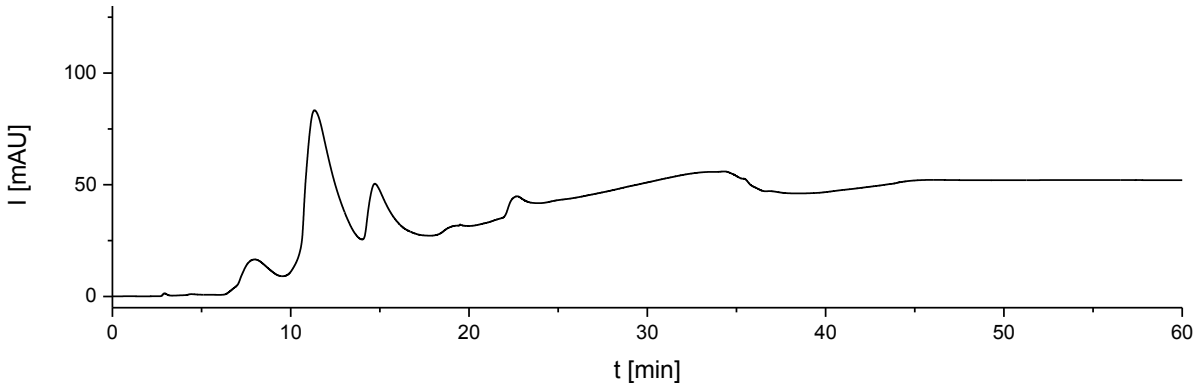
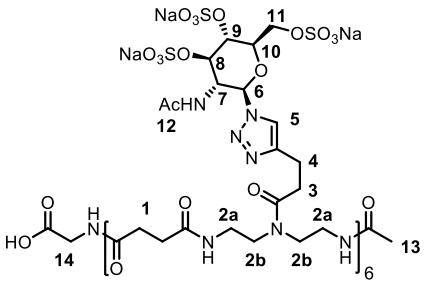



Figure S25: SAX-HPLC of **O1-2**. Conditions: 0-5 min 40 mM NaCl, 1.5 % MeCN; 5-40 min linear gradient to 800 mM NaCl and 30 % MeCN; MeCN; 40-60 min 800 mM NaCl and 30 % MeCN to ensure complete elution. Detection (UV) at 214 nm.

Gly-(1-6)-GlcNAc(3-6S)-(6) (**O2**)



Compound **O2** was obtained from **O2a** (5 mg batch) after dialysis and lyophilization with a quantitative yield of 8.0 mg.

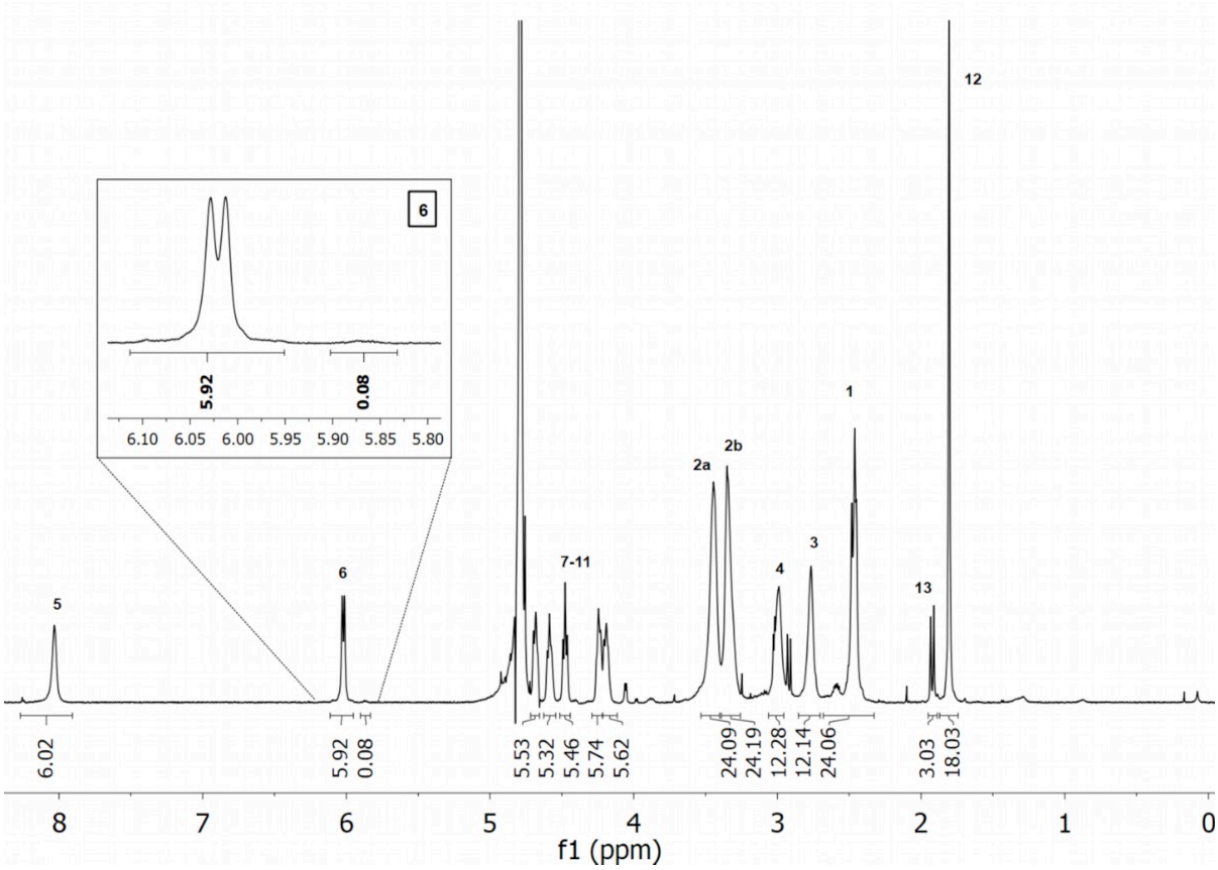



Figure S26: $^{1}$H-NMR (600 MHz, $D_2O$) of compound **O2**.

$^{1}$H-NMR (600 MHz, $D_2O$): δ [ppm] = 1.80 (s, 18H, H-12); 1.91 & 1.93 (2x s, 3H, H-13); 2.46 (m, 20H, H-1); 2.58 (m, 4 H, H1 C-terminus); 2.77 (m, 12H, H-3); 2.99 (m, 12H, H-4); 3.35 & 3.45 (2x br m, 48H, H-2); H-7-11: 4.19, 4.24, 4.48, 4.59, 4.68 (5x m, 5x 6H); 6.02 (d, $^{3}J_{HH}$ = 9.7 Hz, 6H, H-6); 8.03 (br s, 6H, H-5).

Note: Proton 6-11 intensity is lowered, due to partially sulfated product (I < 6H) and because of baseline interference caused by the residual solvent signal at 4.79 ppm (HDO). In addition, one of the H-7-11 signals coincidentally overlaps with signal. The signal for the glycine protons (H-14, I = 2H) could not be assigned.

Degree of completely OH-converted product determined by anomeric signal ratio (embedded window): 98.7%.

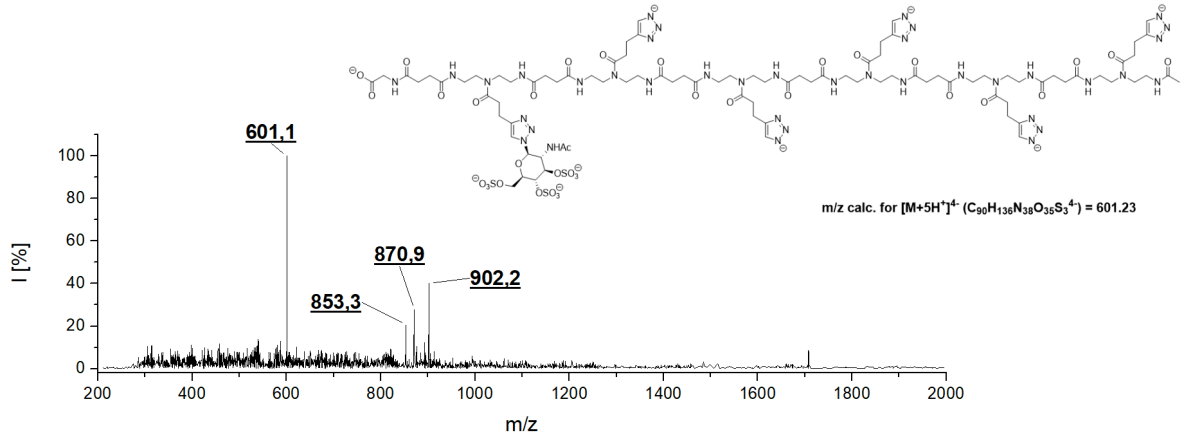



Figure S27: ESI MS of **O2**. Negative ion mode. Note; We were unable to assign the signals at 853.3, 870.9 and 902.2.

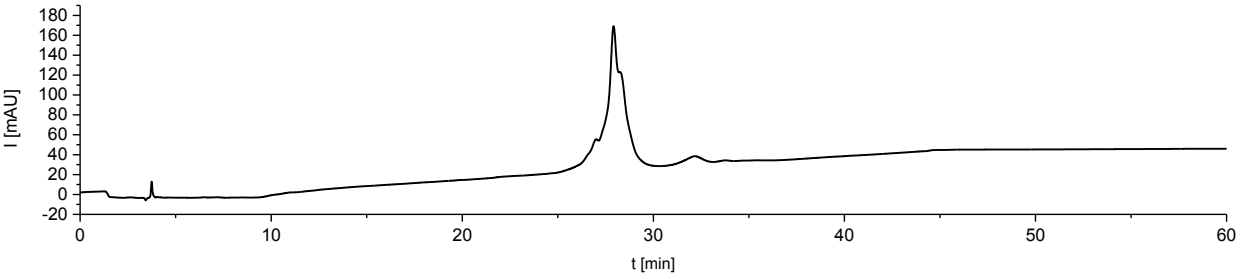
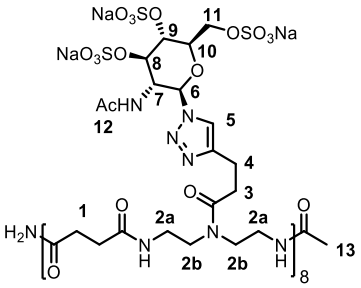



Figure S28: SAX-HPLC of **O2**. Conditions: 0-5 min 40 mM NaCl, 1.5 % MeCN; 5-40 min linear gradient to 800 mM NaCl and 30 % MeCN; 40-60 min 800 mM NaCl and 30 % MeCN to ensure complete elution. Detection (UV) at 214 nm. $t_R$ = 29.1 min.

(1-8)-GlcNAc(3-6S)-(8) (**O3**)




Compound **O3** was obtained from **O3a** (20 mg batch) after dialysis and lyopholization with a yield of 12.8 mg (39.6%).

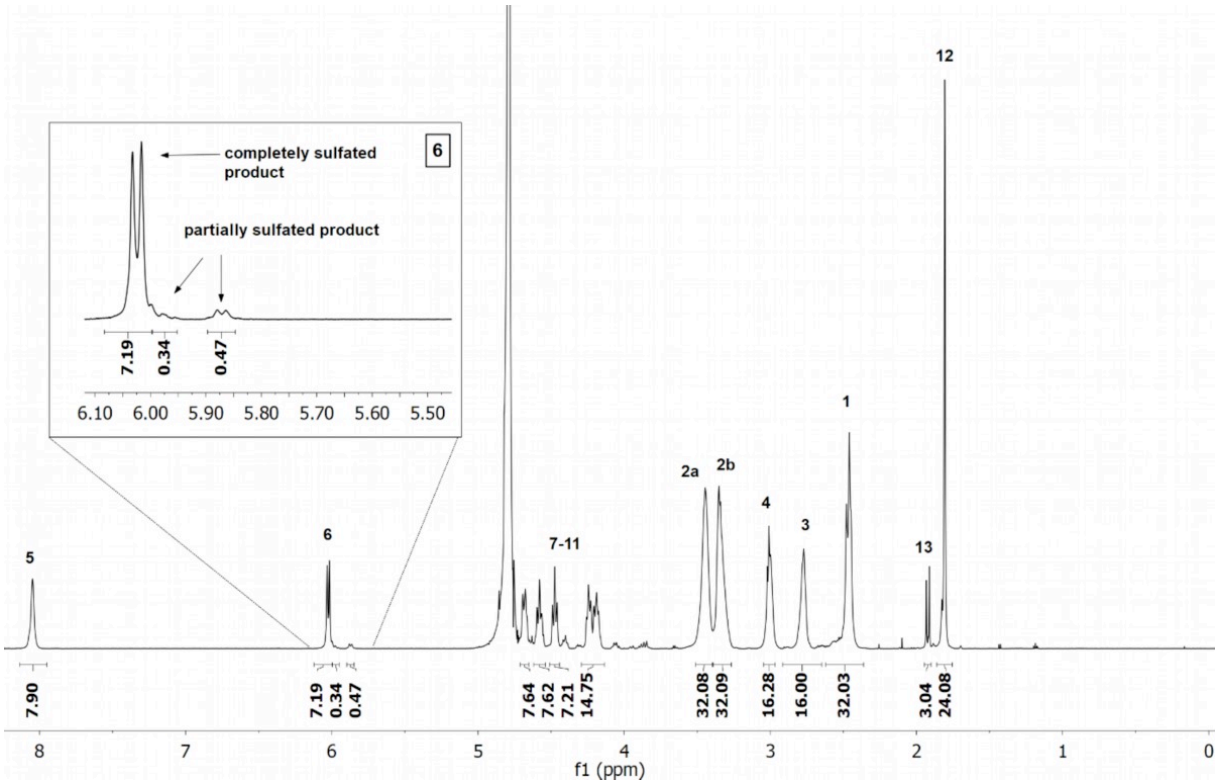



Figure S29: $^{1}$H-NMR (600 MHz, $D_2O$) of compound **O3**.

$^{1}$H-NMR (600 MHz, $D_2O$): δ [ppm] = 1.81 (s, 24H, H-12); 1.91 & 1.93 (2x s, 3H, H-13); 2.46 (m, 32H, H-1); 2.77 (br m, 16H, H-3); 3.01 (br m, 16H, H-4); 3.35 (br m, 32H, H-2b); 3.44 (br m, 32H, H-2a); H-7-11: 4.19, 4.24, 4.47, 4.58 & 4.69 (5x m, 5x 8H); 6.02 (d, $^{3}J_{HH}$ = 9.8 Hz, 8H, H-6); 8.05 (br s, 8H, H-5).

Note: Proton 6-11 intensity is lowered, due to partially sulfated product (I < 8H) and because of baseline interference caused by the residual solvent signal at 4.79 ppm (HDO).

Degree of completely OH-converted product determined by anomeric signal ratio (embedded window): 89.9%

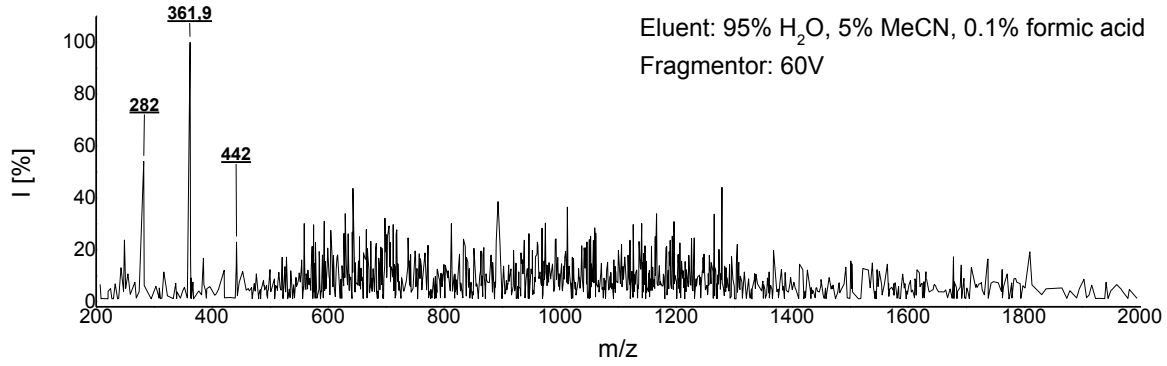
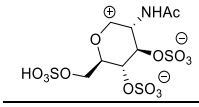
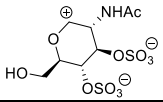
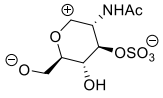
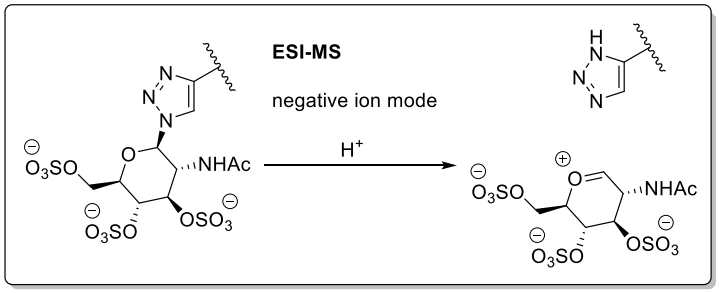



Figure S30: ESI-MS of compound **O3**. Negative ion mode.

Table S5: ESI-MS assignment for structure **O3** (Figure S30).

| Structure | m/z (calc.) | m/z (found) |
|---|---|---|
|  | $[M]^-$ 441.9 | 442.0 |
|  | $[M]^-$ 362.0 | 361.9 |
|  | $[M]^-$ 282.0 | 282.0 |



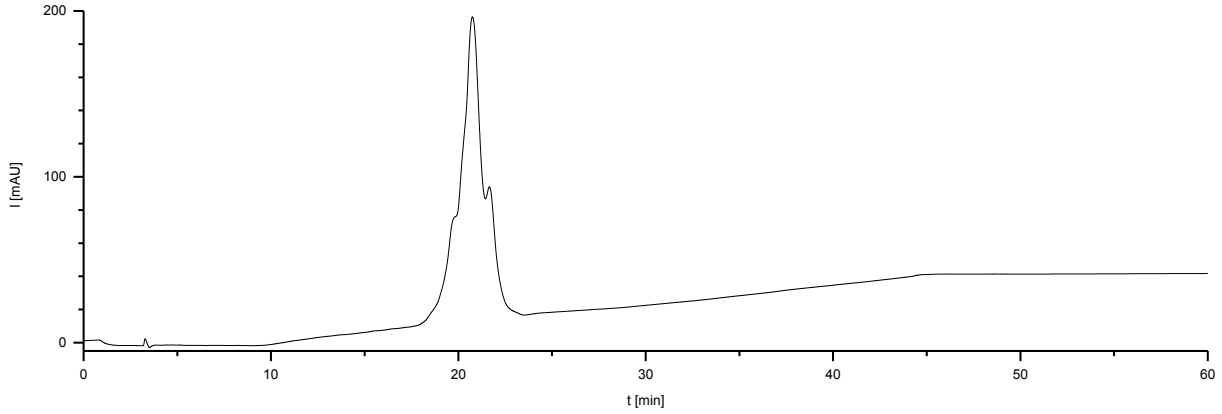
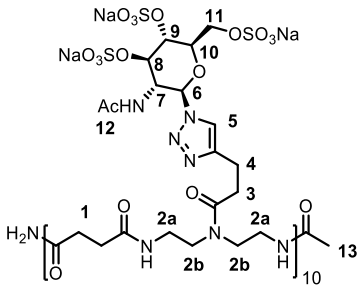



Figure S31: SAX-HPLC of **O3**. Conditions: 0-5 min 40 mM NaCl, 1.5 % MeCN; 5-40 min linear gradient to 800 mM NaCl and 30 % MeCN; 40-60 min 800 mM NaCl and 30 % MeCN to ensure complete elution.

(1-10)-GlcNAc(3-6*S*)-(10) (**O4**)




Compound **O4** was obtained from **O4a** (20 mg batch) after dialysis and lyophilization with a yield of 27.2 mg (93.5%).

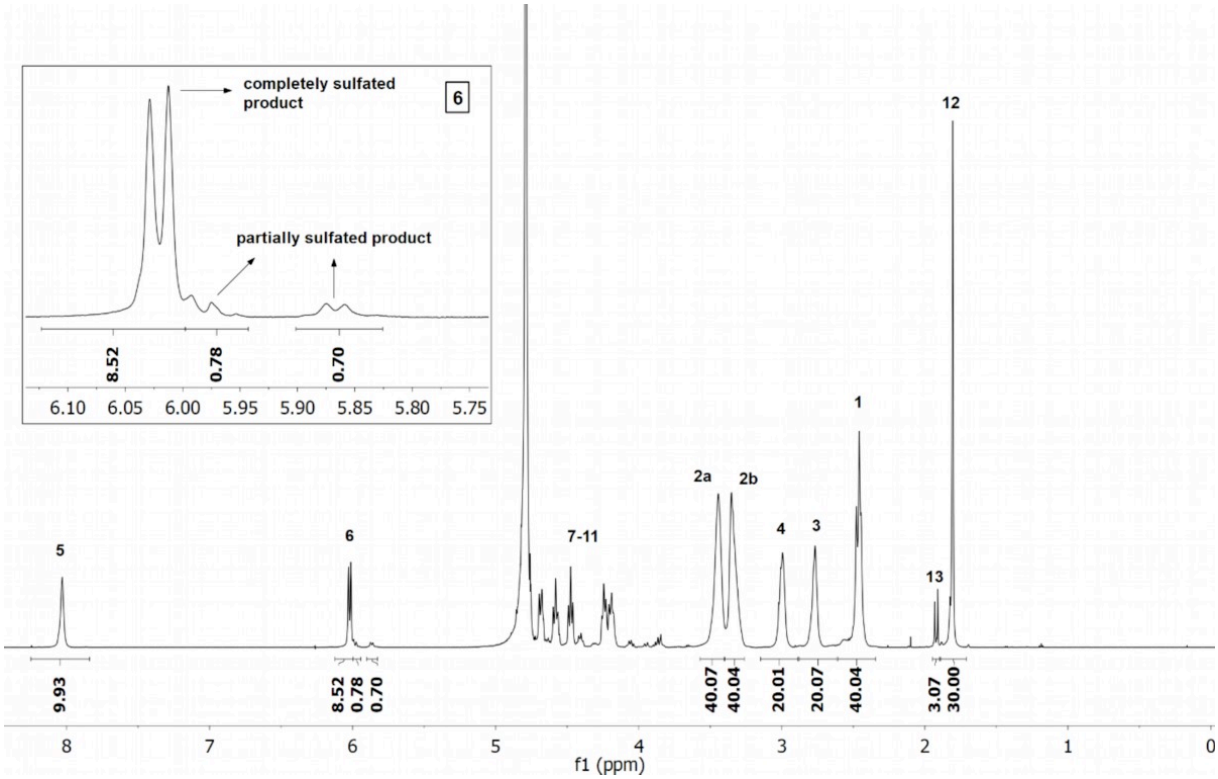



Figure S32: $^{1}$H-NMR (600 MHz, $D_2O$) of compound **O4**.

$^{1}$H-NMR (600 MHz, $D_2O$): δ [ppm] = 1.81 (s, 30H, H-12); 1.91 & 1.93 (2x s, 3H, H-13); 2.46 (m, 40H, H-1); 2.77 (br m, 20H, H-3); 3.01 (br m, 20H, H-4); 3.27-3.40 (br m, 40H, H-2b); 3.40-3.52 (br m, 40H, H-2a); H-7-11: 4.19, 4.24, 4.48, 4.58 & 4.68 (5x m, 5x 10H); 6.02 (d, $^{3}J_{HH}$ = 9.7 Hz, 10H, H-6); 8.03 (br s, 10H, H-5).

Note: Proton 6-11 intensity is lowered, due to partially sulfated product (I < 10H) and because of baseline interference caused by the residual solvent signal at 4.79 ppm (HDO).

Degree of completely OH-converted product determined by anomeric signal ratio (embedded window): 85.2%

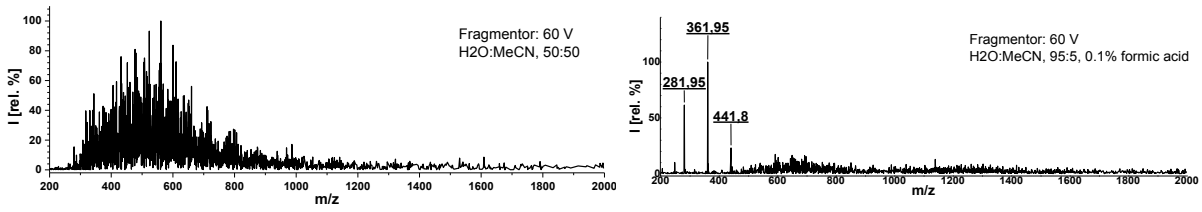
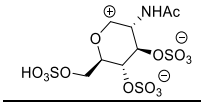
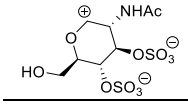
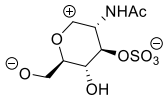
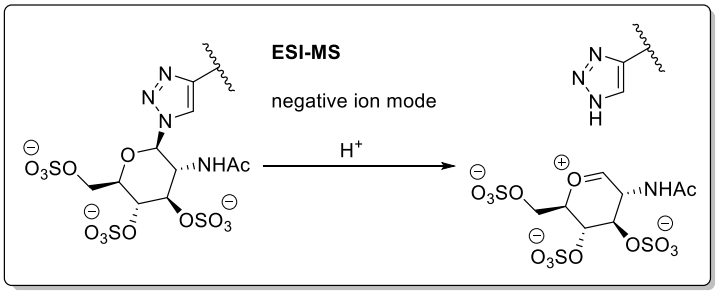



Figure S33: ESI-MS spectra of O4. Comparison of a high fragmenting method (left) to a method yielding sugar fragments (Table S7).

Table S6: ESI-MS assignment for structure **O4** (Figure S33).

| Structure | m/z (calc.) | m/z (found) |
|---|---|---|
|  | $[M]^-$ 441.9 | 442.0 |
|  | $[M]^-$ 362.0 | 361.9 |
|  | $[M]^-$ 282.0 | 282.0 |



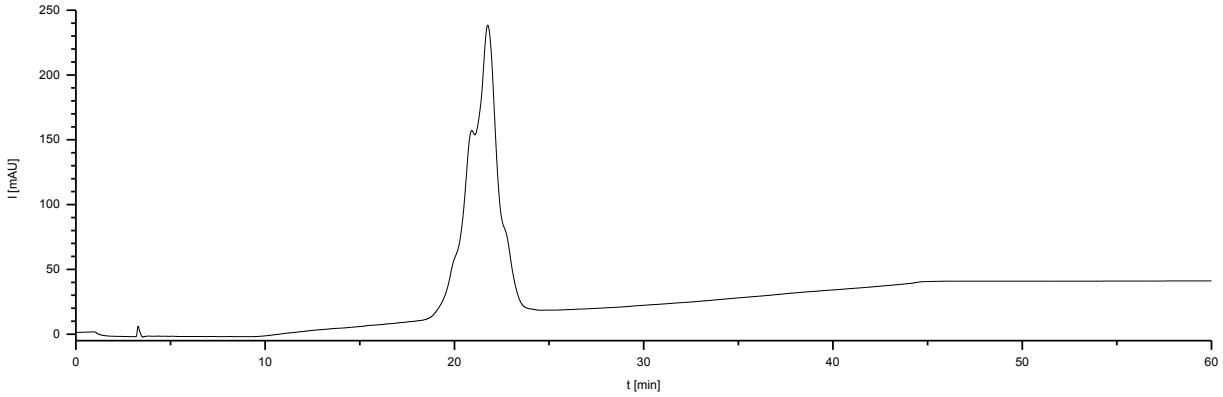
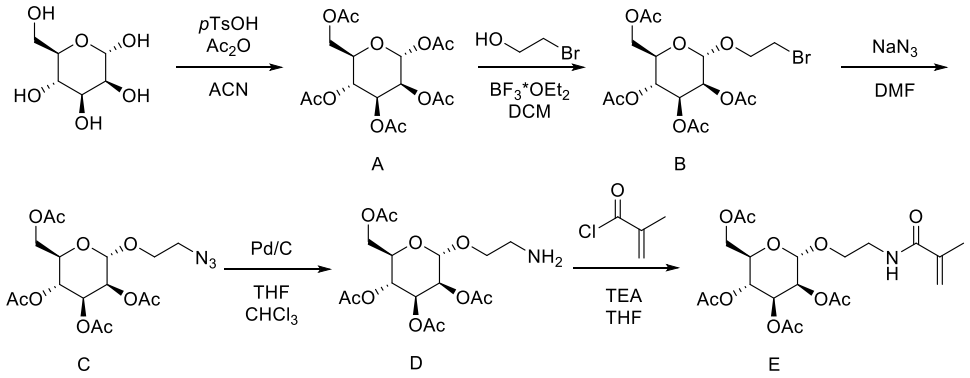




Figure S34: SAX-HPLC of **O4**. Conditions: 0-5 min 40 mM NaCl, 1.5 % MeCN; 5-40 min linear gradient to 800 mM NaCl and 30 % MeCN; 40-60 min 800 mM NaCl and 30 % MeCN to ensure complete elution.

## 6. Synthesis of Glycopolymers

(*N*-[2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)ethyl] methacrylamide




Peracetylation of α-D-mannose was performed adapting a protocol by Watt and Williams[4] and Ponader et al.[1]. In 250 mL acetonitrile α -D-mannose (20 g, 11 mmol) and p-toluene sulfonic acid monohydrate (2.4 g, 13.9 mmol) were dissolved and cooled to 0 °C. After stirring for one hour, acetic anhydride (70 mL, 740 mmol) was added to the white suspension. The reaction mixture turned colorless over 4 hours while reaching room temperature. The solvent was removed under reduced pressure until a brown and highly viscose residue remains in the flask, which was dissolved in 300 mL ethyl acetate. The organic layer was washed with saturated sodium bicarbonate until no further gas was produced. This was followed by a washing procedure including distilled water and brine, followed by drying with magnesium sulfate. Concentration of the organic solvent under reduced pressure yielded **A** as slightly yellow and transparent oil (40.3 g, 103 mmol, 94 % yield).

Synthesis of **B** was performed adapting a protocol by Dahmén et al.[5] and Ponader et al.[1]. In 500 mL round bottom flask, **A** (4.8 g, 109.6 mmol) was dissolved in dichloromethane and stirred for 1 hour while cooling to 0 °C. 2-Bromoethanol (8.5 mL, 119 mmol) was added and the solution was stirred for 30 minutes before addition of boron trifluoride diethyl etherate (65 mL) over one hour. After 3 hours, the ice bath was removed and the mixture was stirred for 72 hours under exclusion of UV-light. To the dark-brown solution, 500 mL of dichloromethane were added and the reaction was cooled to 0 °C for one hour. The organic layer was washed with 2.5 L ice water and then with sodium bicarbonate until no further gas was produced. The organic layer was dried over magnesium sulfate, and the dichloromethane was removed under reduced pressure to produce a grey oil. The crude product was dissolved in ethyl acetate (75 mL) and recrystallized for 72 hours in a freezer at -20 °C. The obtained crystals were washed with cold ethyl acetate and dried under reduced pressure yielding white crystals (17.44 g, 38.4 mmol, 38.6 % yield).

For synthesis of **C**, similar to Lindhorst et al.[6] B (14.95, 32.9 mmol) and sodium azide (8.56 g. 131.6 mmol) were dissolved in 400 mL dimethylformamide and heated to 60 °C. After 72 hours water was added to the solution and the dimethylformamide was removed under reduced pressure. The crude product was dissolved in ethyl acetate and washed with water, saturated sodium bicarbonate and brine.

The organic layer was dried over magnesium sulfate and removed under reduced pressure to give **C** as a colorless oil (12.47 g, 26.45 mmol, 80 % yield).

For synthesis of **D**, adapted from Lindhorst et al.[6] **C** (3.0 g, 7.66 mmol) was dissolved in a mixture of tetrahydrofuran (100 mL) and chloroform (4 mL), and a catalytic amount of palladium on carbon (Pd/C) was added. Afterwards, the apparatus was flushed with hydrogen several times while stirring for 8 hours. The reaction was quenched with 100 mL distilled water. An additional 50 mL chloroform were added to dilute the solution, and the Pd/C was removed via filtration. The water/tetrahydrofuran-layer was washed with another 100 mL chloroform and the solvent was removed under reduced pressure followed by lyophilization to give a yellow-brown solid were obtained (1.92 g, 4.5 mmol, 58 % yield).

Adapted from Yan et al.[7] **D** (1.92 g, 4.17 mmol) was dissolved in tetrahydrofuran (50 mL) and cooled to 0 °C. To this solution was added triethylamine (0.74 mL, 5.3 mmol), followed by the dropwise addition of methacryloyl chloride (0.52 mL, 5.3 mmol) dissolved in 25 mL tetrahydrofuran over 20 minutes with rigorous stirring. The reaction mixture was allowed to reach room temperature overnight. The resulting white precipitate was filtrated off and the solvent was removed under reduced pressure. The crude product was dissolved in ethyl acetate (100 mL) and washed with water until the organic layer turned slightly yellow. Afterwards, the organic layer was washed with saturated sodium bicarbonate solution the evolution of gas ceased, and dried over magnesium sulfate. Removal of the solvent under reduced pressure yielded a grey-brownish and highly viscous product (1.59 g, 3.46 mmol, 83 %, α = 100 %).

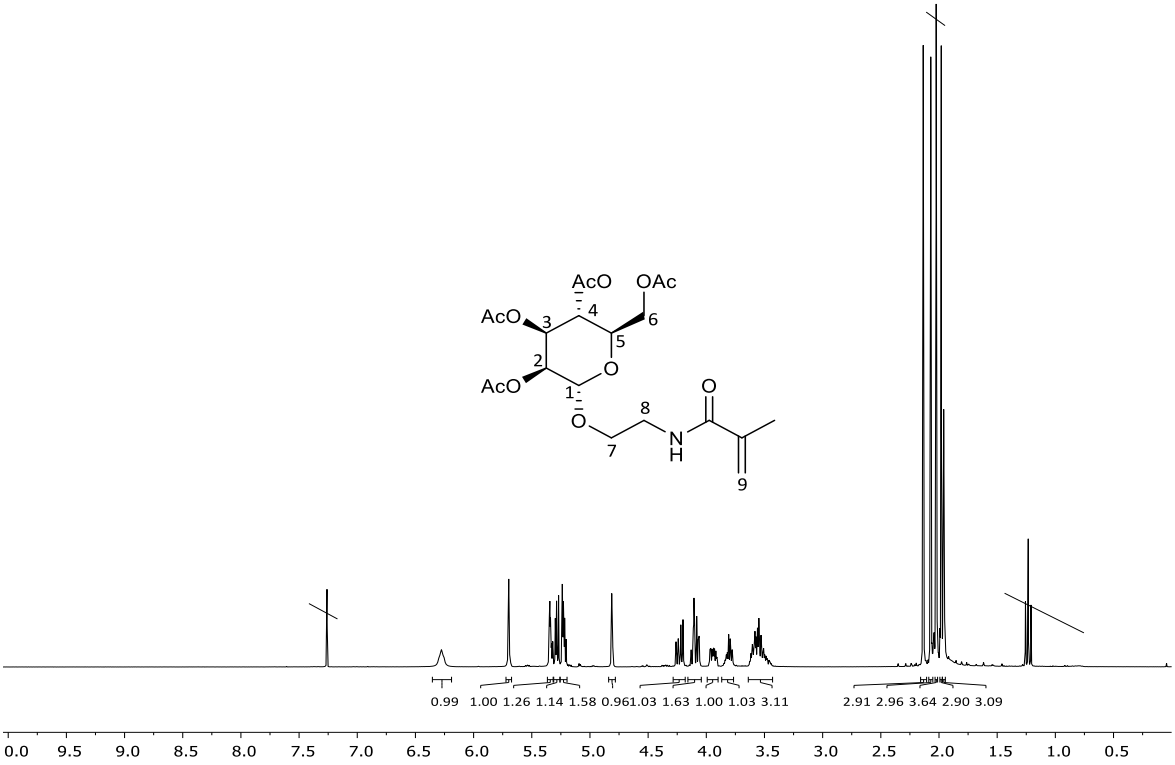



Figure S35: $^{1}$H-NMR (300 MHz, $CDCl_3$) of compound **E**.

$^{1}$H-NMR (300 MHz, $CDCl_3$): δ = 6.36 – 6.26 (m, 1H, H9), 5.73 – 5.66 (m, 1H, H9'), 5.37 – 5.31 (m, 1H, H2), 5.31 – 5.25 (m, 1H, H3), 5.25 – 5.21 (m, 1H, H4), 4.81 (d, $^{3}J_{H1\text{-}H2}$ = 1.6 Hz, 1H, H1), 4.23 (dd, $^{3}J_{H5\text{-}H6}$ = 12.2 Hz, $^{3}J_{H4\text{-}H5}$ = 5.7 Hz, 1H, H5), 4.13 – 4.05 (m, 1H, H6), 4.00 – 3.90 (m, 1H,H6'), 3.85 – 3.76 (m, 1H, H**7**),

3.63 – 3.40 (m, 3H, H7', H8), 2.13 (s, 3H, -$CH_3$), 2.07 (s, 3H, -$CH_3$), 2.02 (s, 3H, -$CH_3$), 1.98 (s, 3H, -$CH_3$), 1,96 (s, 3H, -$CH_3$) ppm.

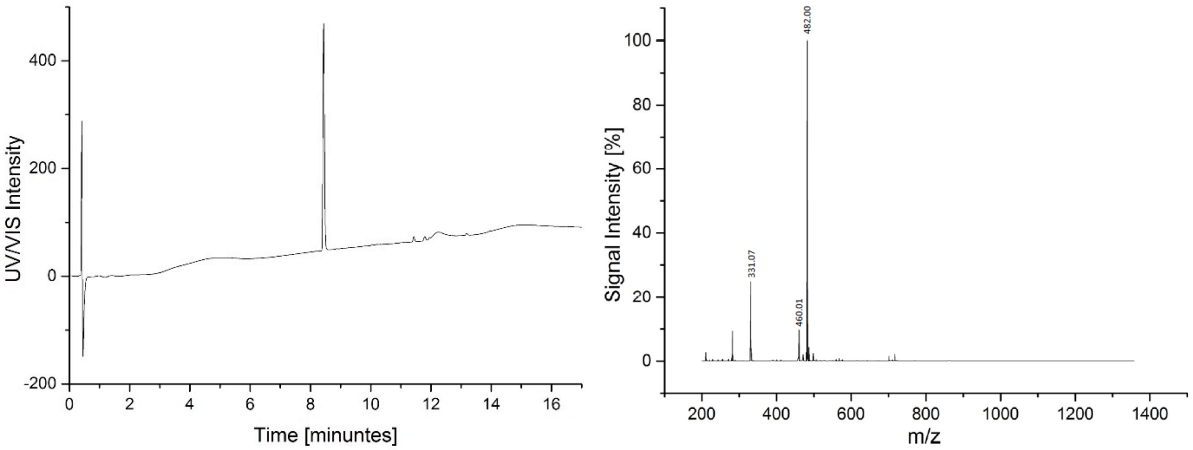
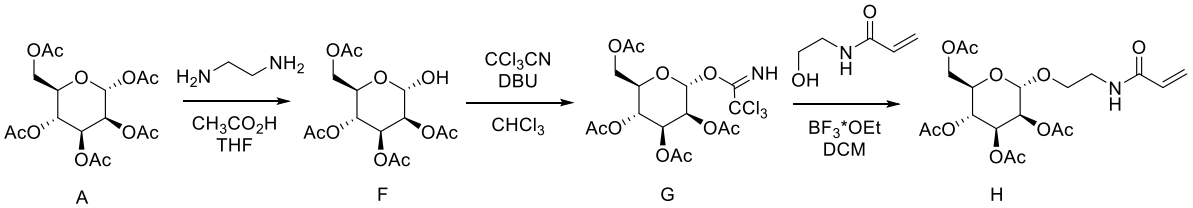



Figure S36**:** LC-MS (positive mode) and direct ESI of compound **E** showing a fragmentation product m/z = 331.07 [calc. 331.10], m/z = 460.01 $[M+H]^+$ [calc. 460.17], m/z = 482.00 $[M+Na]^+$ [calc. 482.17].

## Synthesis of (*N*-[2-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyloxy)ethyl] acrylamide (**H**)




Based on a protocol by Zhang and Kováč[8], anomeric deacetylation of 1,2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose was performed by preparing a solution ethylenediamine (2.2 mL, 34.9 mmol) in tetrahydrofuran (500 mL). While stirring at room temperature, glacial acetic acid (2.2 mL, 40.7 mmol) was added dropwise leading to the formation of a white precipitate. 1,2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranose (11.35 g, 29.1 mmol) was dissolved in tetrahydrofuran (200 mL) and added dropwise to the white suspension. The conversion was monitored via TLC in a 1:1 mixture of *n*-hexane and ethyl acetate ($R_f$ = 0.5) and showed full conversion after 12 hours. Tetrahydrofuran was removed under reduced pressure and the resulting residue was dissolved in water (300 mL). After extracting the water layer with dichloromethane (3 x 300 mL), the organic layer was washed with 1 M hydrochloric acid (3 x 300 mL), distilled water (3 x 300 mL) and saturated sodium bicarbonate solution until the evolution of gas ceased. After drying over sodium sulfate, the solvent was removed under reduced pressure yielding a transparent and highly viscose oil (9.11 g, 24.0 mmol, 89.9 % yield).

Synthesis of **G** was adapted from Fekete et al.[9], and Wu and Sampson[10]. Under nitrogen flow and in presence of molecular sieve (2 Å), **F** (9.3 g, 26.7 mmol) was dissolved in chloroform (250 mL). While stirring at room temperature, DBU (500 µL, 3.3 mmol) was added leading to a yellow solution. After 30 minutes, trichloroacetonitrile (2.8 mL, 28 mmol) was added dropwise causing a change in color from yellow to dark-brown within 3 hours. Conversion was monitored via TLC in ethyl acetate: *n*-hexane (1:1) by observation of a new spot with $R_f$ = 0.8. After 3 hours no further conversion was observed and the solvent was removed under reduced pressure. The desired product was isolated by flash

chromatography (ethyl acetate/*n*-hexane, 3:7). After removing the solvents under reduced pressure, the product was obtained as a light yellow solid ($R_f$ = 0.7) (5.0 g, 10.4 mmol, 40 % yield).

Synthesis of **H** similarly followed the protocol described by Gu et al.[11] and Wu and Sampson[10]. Under inert conditions and argon atmosphere $BF_3$*$Et_2O$ (1.4 mL, 11.6 mmol) was added carefully to a solution of **G** (3.6 g, 7.3 mmol) in dichloromethane (120 mL) and cooled to 0 °C. After stirring for 45 minutes, the solution turned bright-yellow *N*-hydroxyethyl acrylamide (3.6 mL, 31.5 mmol) dissolved in dichloromethane (30 mL) was added dropwise. The mixture was stirred overnight. The solution was washed with ice-cold water (2 x 100 mL) then saturated sodium bicarbonate (2 x 100 mL) until the evolution of gas ceased. The organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude product was purified via flash chromatography in a 2-step procedure. First, products and possible side products were removed with a mixture of ethyl acetate/*n*-hexane of 1:1. In a second step, the product was obtained by flashing with pure ethyl acetate. The desired product has an $R_f$ = 0 in 1:1 ethyl acetate/*n*-hexane, and an $R_f$ = 0.6 in ethyl acetate. After removing the solvent, a colorless, sticky solid was received (1.5 g, 3.5 mmol, 48 %, β = 100 %)

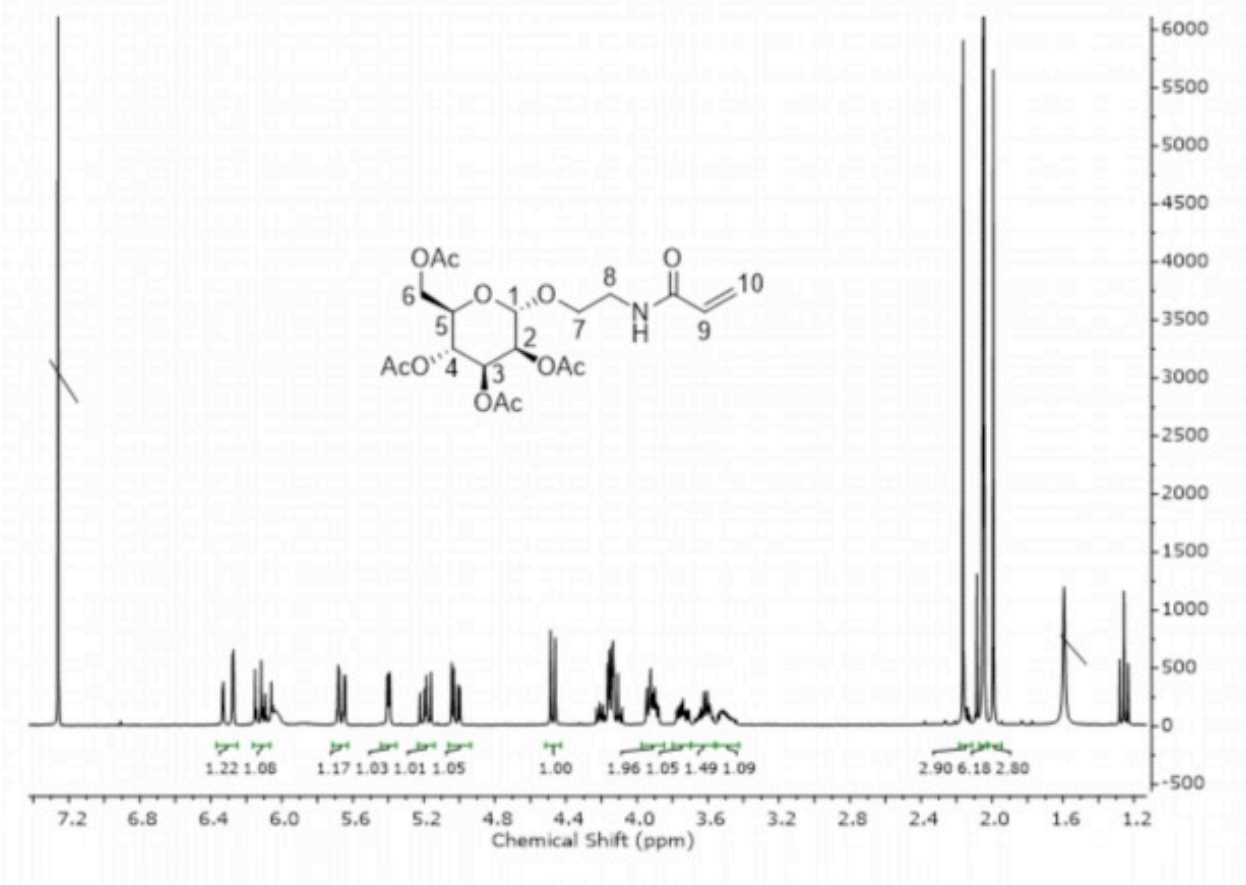



Figure S37: $^1$H-NMR (300 MHz, $CDCl_3$) of compound **H**.

$^1$H-NMR (300 MHz, $CDCl_3$): δ = 6.3 (dd, 2H, H9), 6.17 – 5.98 (m, 2H, -NH-CO-, H10), 5.66 (dd, 1H, H10'), 5.4 (dd, 1H, $^3J_{H4-H5}$ = 1.0 Hz, $^3J_{H4-H3}$ = 3.4 Hz, H4), 5.19 (dd, $^3J_{H1-H2}$ = 7.9 Hz, $^3J_{H2-H1}$=10.5 Hz, 1H, H2), 5.02 (dd, $^3J_{H3-H4}$ = 3.4 Hz, $^3J_{H3-H2}$ =10.5 Hz, 1H, H3)4.47 (m, 2H), 2.16 (s, 3H, -$CH_3$), 2.06 – 2.03 (m, 6H, -$CH_3$), 1.99 (s, 3H, -$CH_3$) ppm.

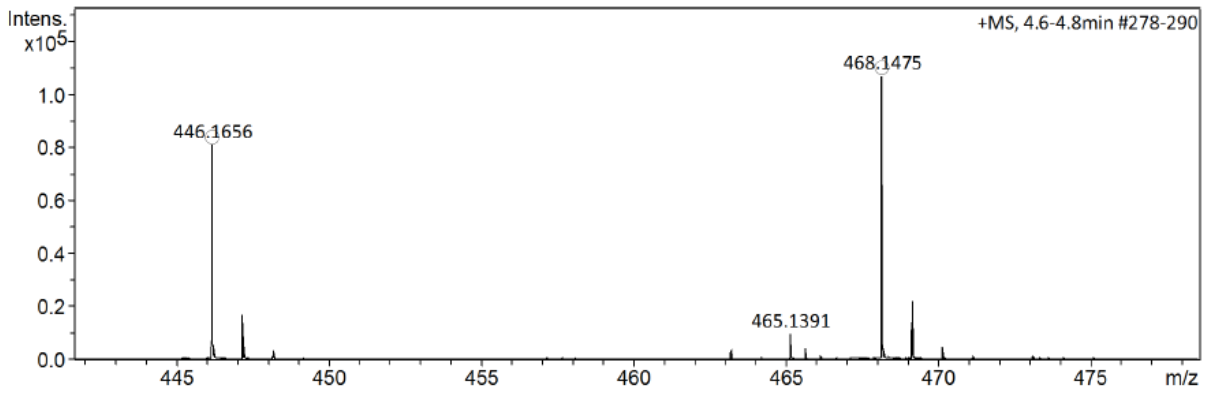


Figure S38: HR-ESI of compound **H**: $[M+H]^+$: 446.1656 – calc. 446.16, $[M+Na]^+$: 468.1475 – calc. 468.16.

## General Protocol for RAFT-Polymerization

The reversible addition-fragmentation chain-transfer (RAFT) polymerization was performed under inert conditions and was based on a general protocol adapted from Toyoshima and Miura[12].

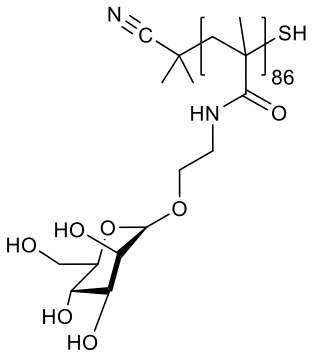
Stock solutions of the different monomers, azobis(isobutyronitrile) (AIBN) and the corresponding chain transfer agent (CTA) were dissolved in dry ethyl acetate to generate a stock solution and were added together into a sealable flask. 3 freeze-pump cycles consisting of 15 minutes flushing with argon followed by 15 minutes of freezing with liquid nitrogen while applying a vacuum of at least $9*10^{-2}$ mbar are completed prior to initiation. Under argon atmosphere, polymerization was initiated at 75 °C while stirring. The polymerization was quenched in liquid nitrogen after 22 hours. Ethyl acetate was removed under reduced pressure and 0.2 M sodium methoxide in methanol was added to the polymerization residue. The reaction mixture was stirred at RT for 2 hours and a white precipitate was obtained. Methanol was removed under reduced pressure and the residue was dissolved in a 20-fold excess of sodium borohydride in water. The final polymer was received after dialysis against distilled water in dialysis tubes with pore sizes from 1000 Da to 2000 Da for three days and subsequent lyophilization.

Table S7: Overview of precursor glycopolymers. Dispersity (D) and molecular number weight ($\overline{M_n}$) are determined via aqueous GPC.

| | **Yield** | **D** | $\overline{M_n}$ [rep. units] |
|---|---|---|---|
| **PM-OH** | 21 mg | 1.25 | 29,200 Da [86] |
| **PG1-OH** | 30 mg | 2.00 | 11,500 Da [40] |
| **PG2-OH** | 37 mg | 1.08 | 13,100 Da [46] |
| **PG-OH** | 25 mg | 1.10 | 23,800 Da [84] |

## 7. Analytical Data of Glycopolymers

Unsulfated Precursor Polymer **PM-OH**




Synthesis of **PM-OH** followed the protocol as described. **E** (177 mg, 385 µmol), 2-cyano-2-propyl dodecyl trithiocarbonat (1.33 mg, 3.85 µmol) and azobis(isobutyronitrile) (AIBN) (0.31 mg, 0.19 µmol) were dissolved in dry ethyl acetate (791 µL). **PM-OH** was obtained as colorless solid were isolated (21 mg, 18.7 %, $M_n$ = 29,200, D = 1.25).

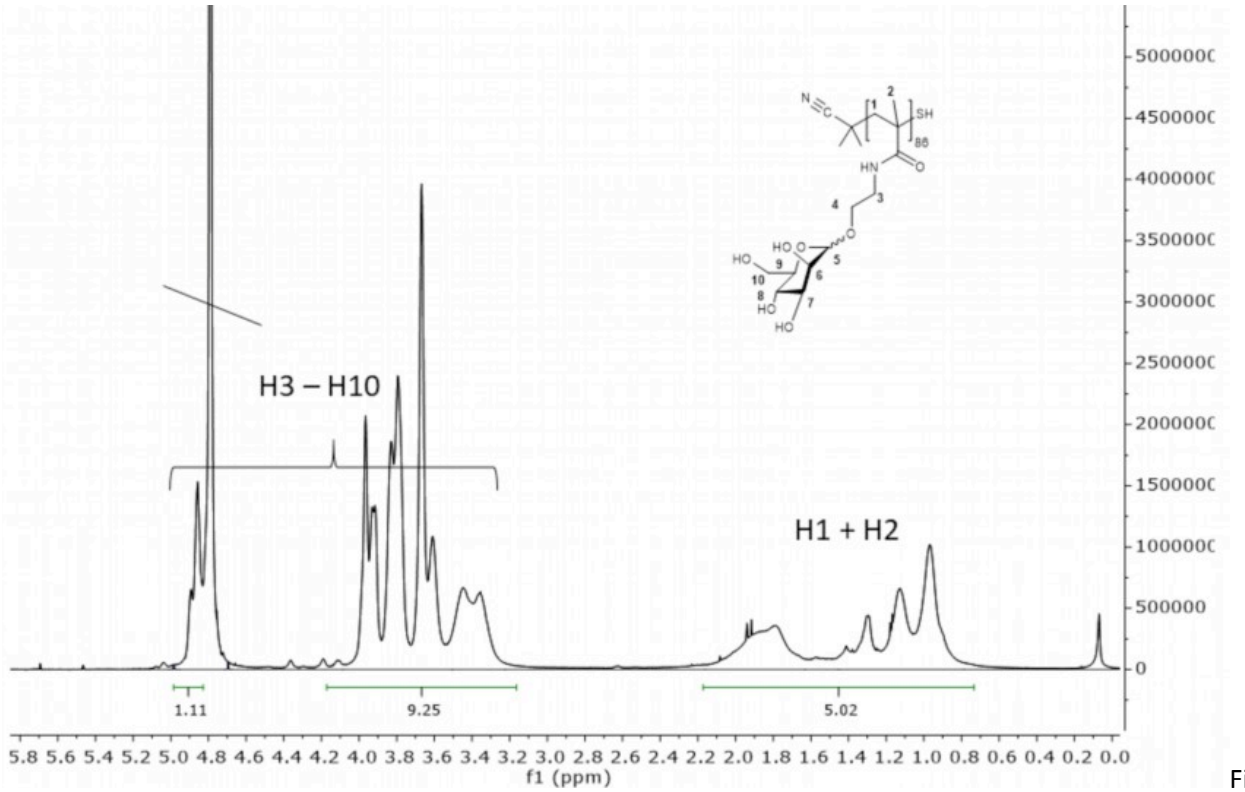



Figure S39: $^1$H-NMR (600 MHz, $D_2O$) of compound **PM-OH**.

$^1$H-NMR (600 MHz, $CDCl_3$): δ = 4.99 – 4.70 (m overlapped with DHO signal, H5, 1H), 4.09 – 3.13 (m, H3,4, 6-10, 10H), 2.17 – 0.79 (broad m, H1, H2, 5H) ppm.

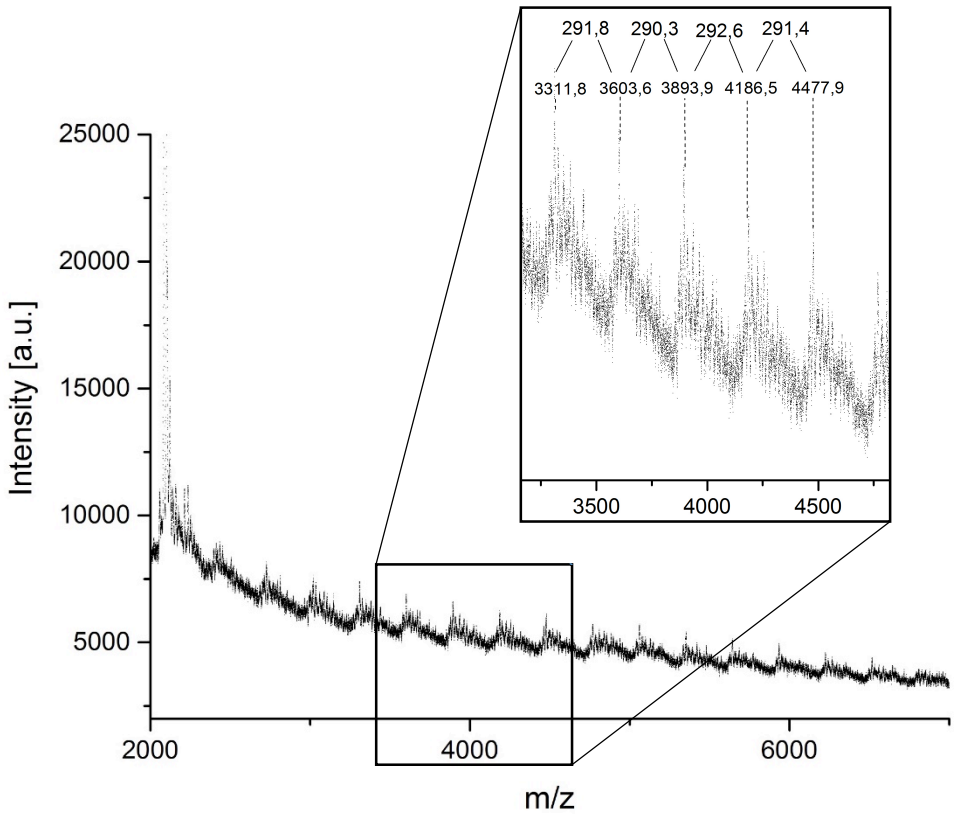



Figure S40: MALDI-TOF MS of **PM-OH**. Note: Glycopolymers proved difficult to analyze by MALDI-TOF analysis likely due to insufficient ionization. Nevertheless, the m/z pattern reveals repeating units for the desired product of 291.52 Da.

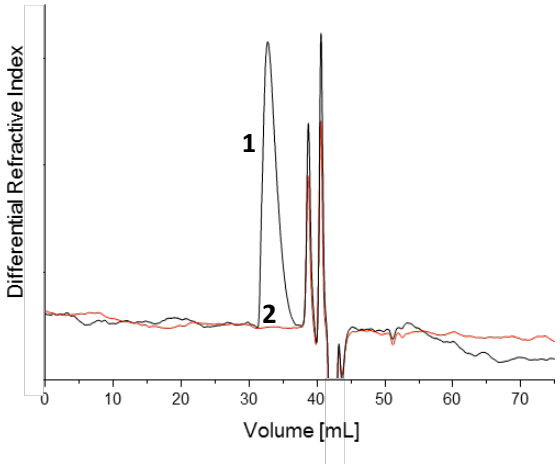
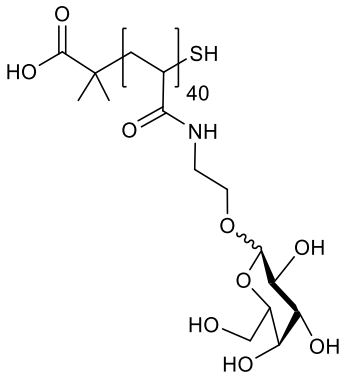



Figure S41: Aqueous GPC of **PM-OH**: $\overline{M}_n$ = 29,200 g/mol, D = 1.25. With 1 = **PM-OH** and 2 = Blank.

Unsulfated Precursor Polymer **PG1-OH**




Synthesis of **PG1-OH** follows the protocol as described previously. **H** (89.28 mg, 200 µmol), 2-cyano-2-propyl dodecyl trithiocarbonat (0.69 mg, 2.0 µmol) and AIBN (0.065 mg, 0.4 µmol]) were dissolved in dry ethyl acetate (566 µL) ethyl acetate. **PG1-OH** was obtained as a white solid (30 mg, 54 %, $M_n$ = 11.500, D = 2.0).

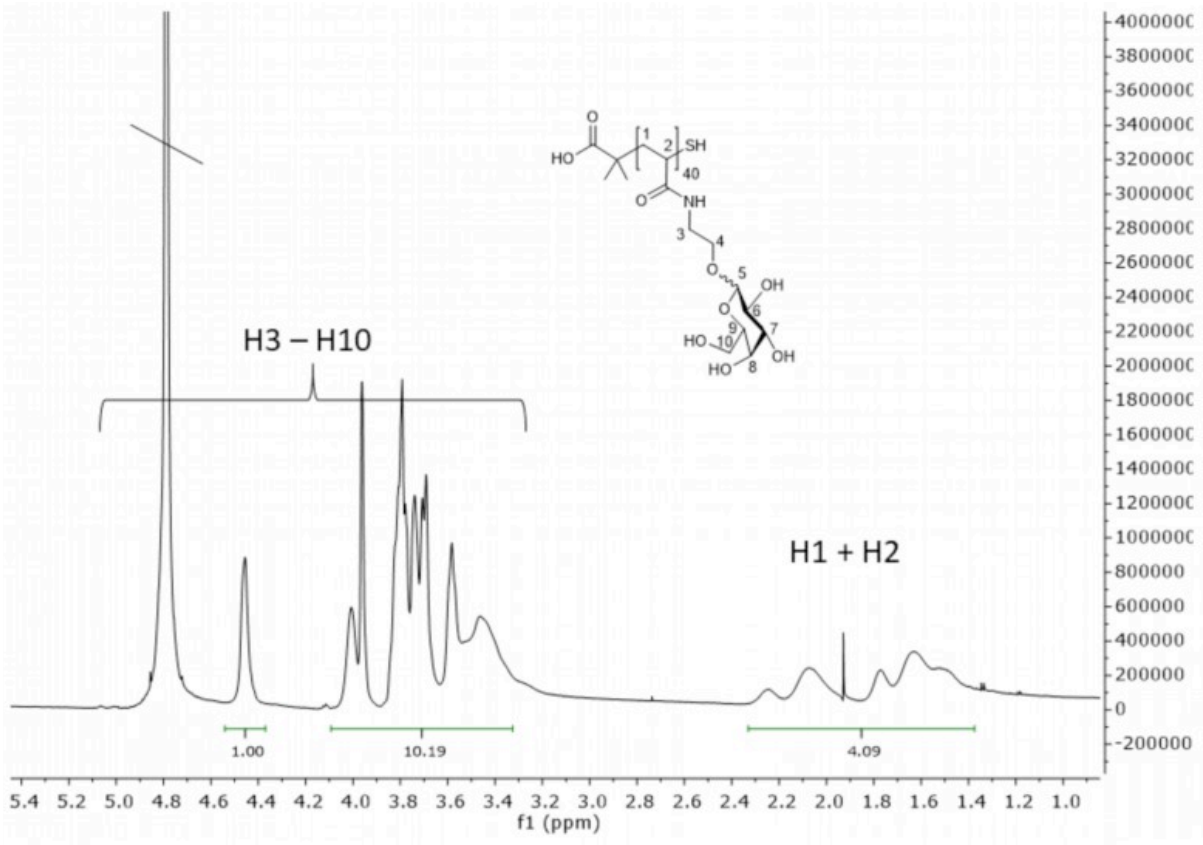



Figure S42: $^1$H-NMR (600 MHz, $D_2O$) of **PG1-OH**.

$^1$H-NMR (600 MHz, $D_2O$): δ = 4.46 (broad s, H5, 1H), 4.09 – 3.32 (m, H3,4-H6-10, 10H), 2.33 – 1,38 (m, H1, H2, 4H) ppm.

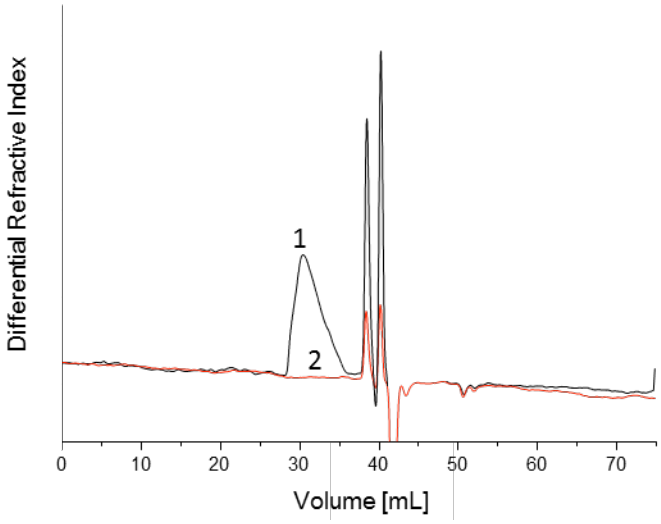
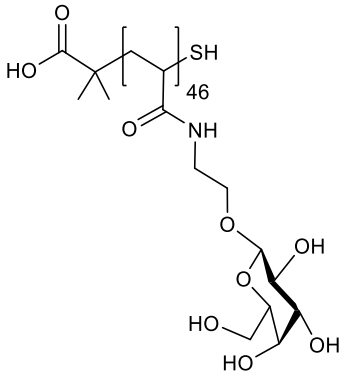



Figure S43: GPC-Diagram of **PG1-OH**: $\overline{M}_n$ = 29,200 g/mol, D =2.0. With 1 = **PG1-OH** and 2 = Blank.

Unsulfated Precursor Polymer **PG2-OH**




Synthesis of **PG2-OH** follows the protocol as described previously. **H** (89.28 mg, 323 µmol), 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (2.9 mg, 8.0 µmol) and AIBN (0.53 mg, 3.2 µmol) were dissolved in dry ethyl acetate (911 µL). The reaction mixture was placed into a sealed microwave tube and degassed within 3 freeze-pump cycles before initiation at 75°C while stirring for 5 hours. **PG2-OH** was obtained as a white polymer (37.2 mg, 41.6 %, $\overline{M}_n$ = 13,100 g/mol, D =1.08).

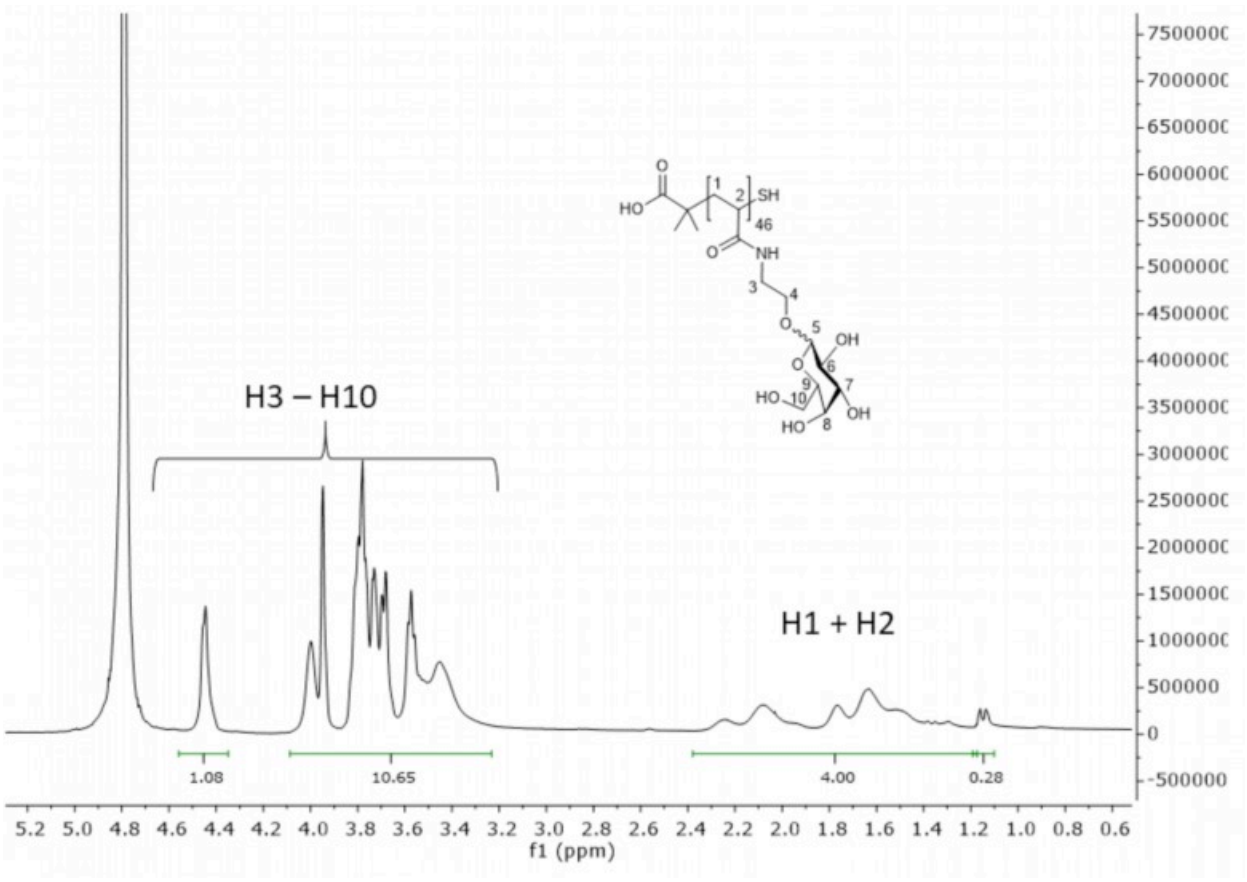



Figure S44: $^{1}$H-NMR (600 MHz, $D_2O$) of **PG2-OH,** 1 + 2 assigned as backbone signals.

$^{1}$H-NMR (600 MHz, $D_2O$): δ = 8.03 (broad s, -COOH), 4.45 (broad s, H5, 1H), 4.09 – 3.16 (m, H3,4,5-8), 2.17 – 1.20 (broad m, H1+2, 4H and 1.17 - 1.13 (2 s, CTA residue, 0.28 H) ppm.

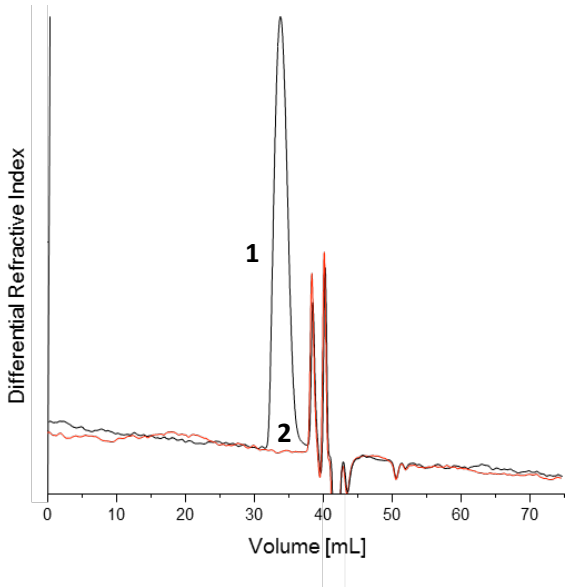



Figure S45: GPC-Diagram of **PG2-OH**: $\overline{M}_n$ = 13,100 g/mol, D =1.08. 1 = **PG2-OH** and 2 = Blank.

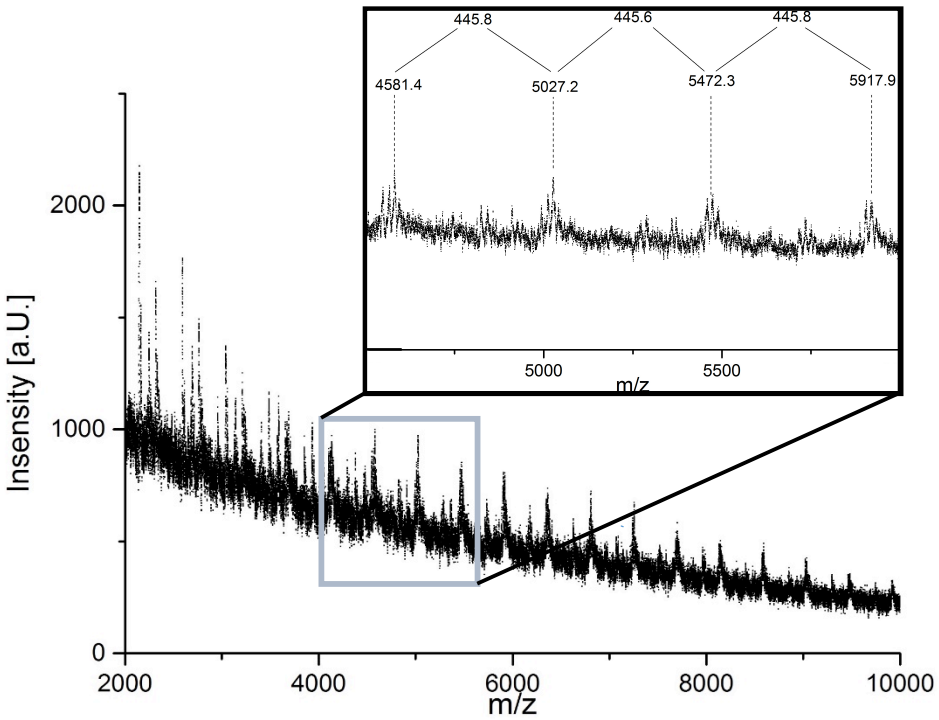
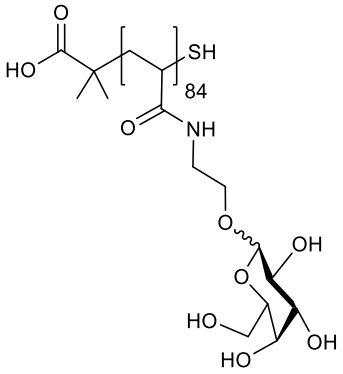



Figure S46: MALDI-TOF of **PG2-OH** before deacetylation. Note: Glycopolymers proved difficult to analyze by MALDI-TOF analysis likely due to insufficient ionization. Nevertheless, the m/z pattern reveals repeating units for the desired product of 445.73 Da.

Unsulfated Precursor Polymer **PG-OH**




Synthesis of **PG-OH** follows the protocol as described previously. **H** (89.01 mg, 200 µmol), 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (0.69 mg, 2.0 µmol) and AIBN (0.65 mg, 0.4 µmol) were dissolved in dry ethyl acetate (564 µL). **PG-OH** was obtained as a white polymer (25 mg, 45.1 %, $\overline{M}_n$ = 23,800 g/mol, D =1.1).

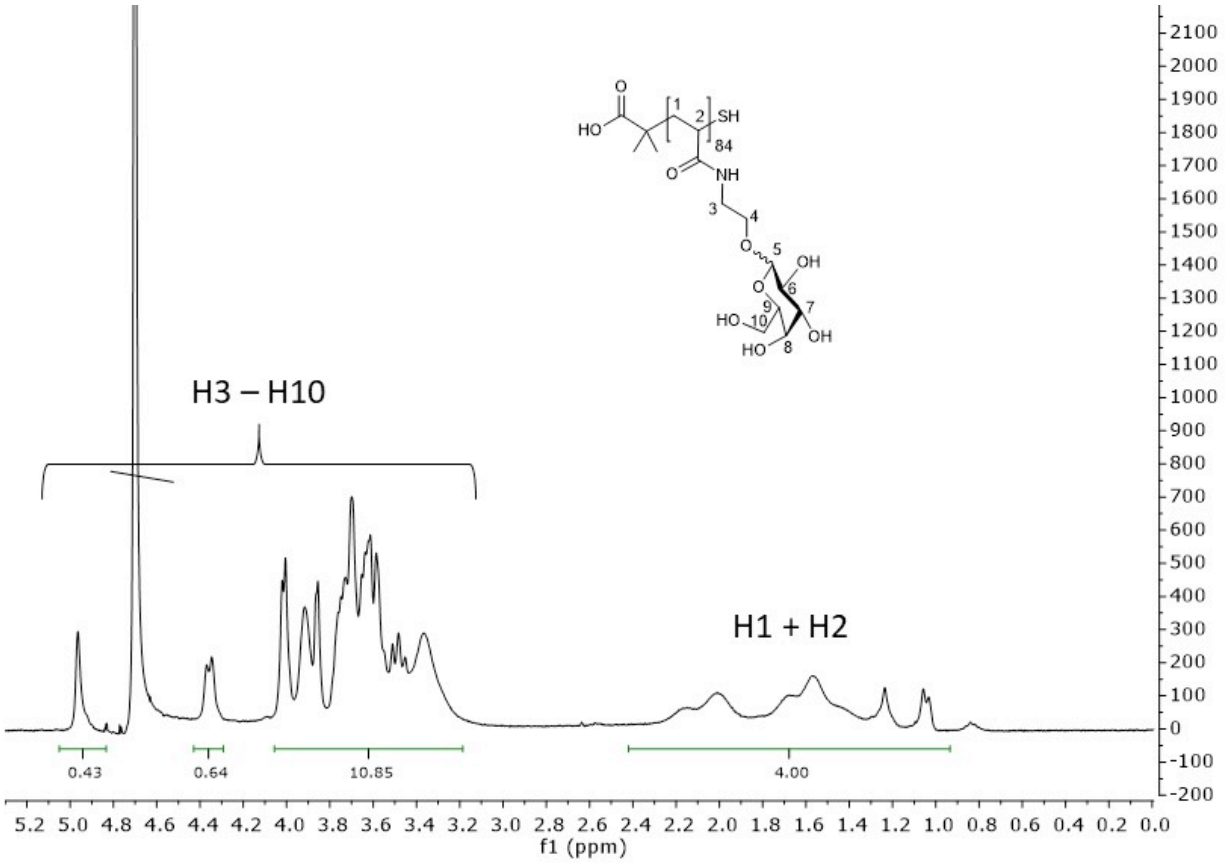
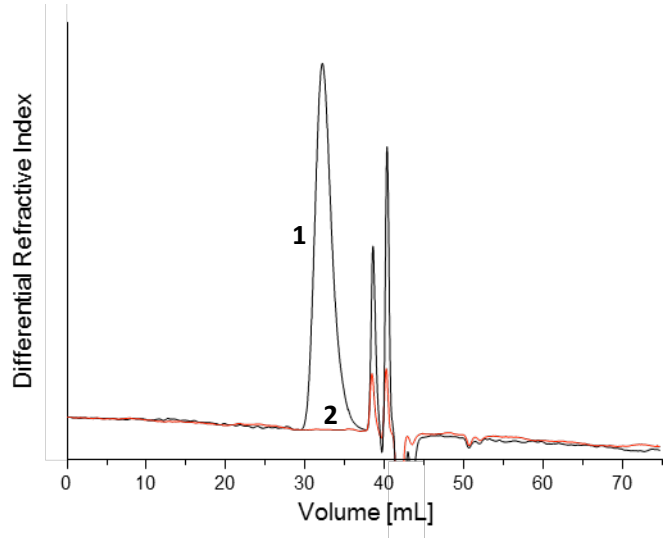



Figure S47: $^{1}$H-NMR (600 MHz, $D_2O$) of compound **PG-OH**.

$^{1}$H-NMR (300 MHz, $D_2O$): δ 5.0 – 4.91 (m, 0.4H,H5α), 4.45 – 4.31 (broad s, H5β, 0.6H), 4.09 – 3.16 (m, H3,4,5-8, 10H), 2.4 – 0.9 (broad m, H1+2, 4H) ppm.




.

Figure S48**:** GPC-Diagram of **PG-OH**: $\overline{M}_n$ = 23,800 g/mol, D =1.1. 1 = **PG-OH** and 2 = Blank.

## 8. Sulfation of glycopolymers

Sulfation of glycopolymers was performed following the protocol described for the sulfation of oligomers **O1** - **O4**, using TMA*$SO_3$ as sulfating agent and NaOAc for quenching. Briefly, glycopolymer and TMA*$SO_3$ (40 eq. per OH-group) were dissolved in dry DMF. The mixture was stirred for 18-19h at 70 °C. After cooling to room temperature, the mixture was added to 10-20% sodium acetate (10-20 eq. to sulfation agent) at 0 °C. The DMF/water mixture was evaporated under reduced pressure (60 °C, 15 mbar) before dialysis (MWCO of 1000-2000 Da) and lyophilization. For detailed reaction conditions see Table S8.

Table S8: Reaction conditions for the sulfation of PM-OH and PG-OH.

| Structure | Weight in unsulfated Glycopolymer[a] [mg] | Weight in TMA*$SO_3$ [mg] | Volume DMF [ml] | Reaction temperature [°C] | Reaction time [h] | Quenching agent[b] | Yield[c] [mg] |
|---|---|---|---|---|---|---|---|
| PM | 5 (PM-OH) | 400 | 3.5 | 70 | 19 | 10% NaOAc (10 eq. to TMA*$SO_3$) | 7.5 (62.5%) |
| PG1 | 14.5 (PG1-OH) | 1386 | 6 | 70 | 18 | 10% NaOAc (10 eq. to TMA*$SO_3$) | 27.3 (76.2%) |
| PG2 | 34 (PG2-OH) | 2727 | 24 | 70 | 18 | 20% NaOAc[d] (20 eq. to TMA*$SO_3$) | 32.6 (38.8%) |

(a) Respective unsulfated glycopolymers are shown in brackets. (b) The crude mixture was added to different volumes of sodium acetate (NaOAc) solution to achieve the respective equivalents. (c) Percentages theoretically derived from a maximum yield of a completely sulfated polymer. (d) As TMA contaminants were found for PM and PG1 (see NMR analysis), PG2 was added to a higher amount of NaOAc-solution. During dialysis, an additional ion exchange was performed using a half saturated NaCl solution to ensure complete removal of contaminants.

## 9. Analysis of Sulfated Glycopolymers

PM

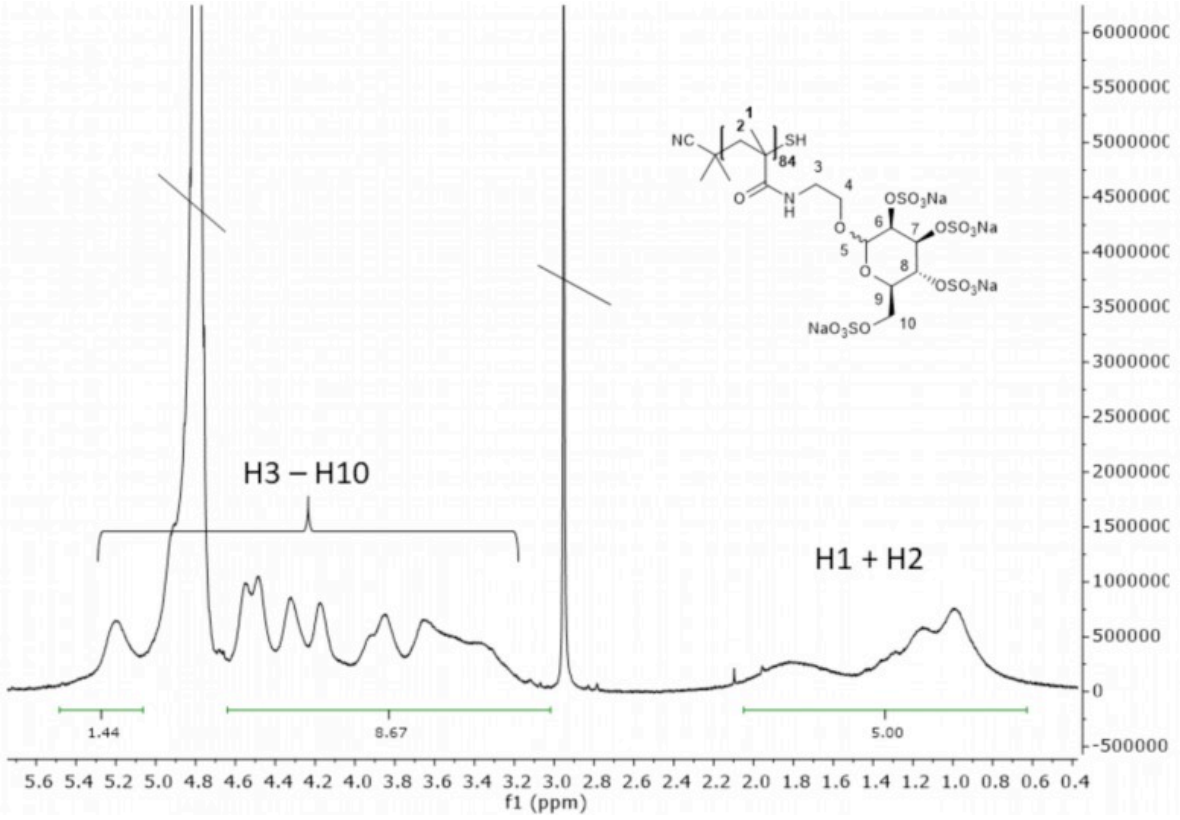



Figure S49: $^{1}$H-NMR (600 MHz, $D_2O$) of **PM**.

$^{1}$H-NMR (600 MHz, $D_2O$): δ = 5.45 – 5.05 (m, H5, 1H, overlapped with DHO); 4.61 – 3.0 (m, H3,4,6-10), 10H) 2.05 – 0.64 (broad m,H1+2, 5H ) ppm.

Note: The singlet at 2.95 ppm is assumed to be trimethylammonium contaminant.

PG1

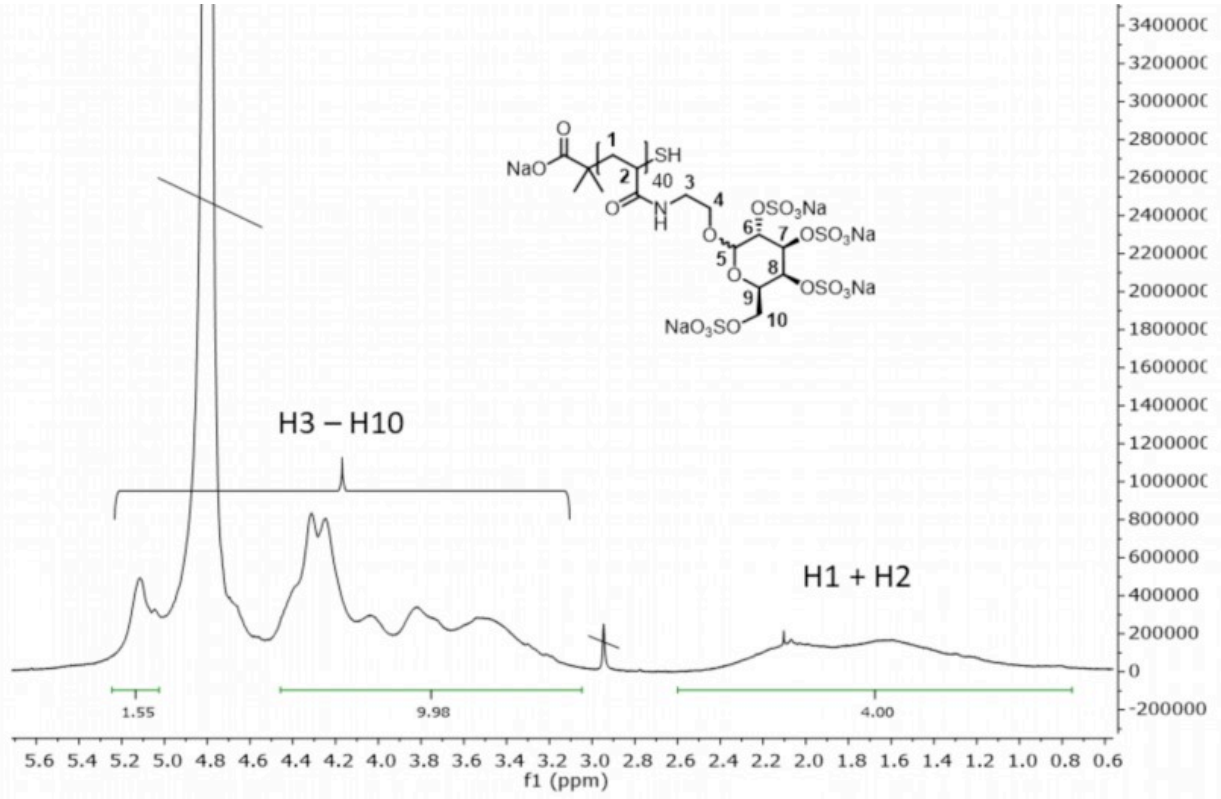



Figure S50: $^{1}$H-NMR (600 MHz, $D_2O$) of **PG1**.

$^{1}$H-NMR (600 MHz, $D_2O$): δ = 5.25 – 5.05 (m, H5, 1H, overlapped with DHO); 4.46 – 3.12 (m, H3,4,6-10), 10H) 2.60 – 0.75 (broad m,H1+2, 4H) ppm.

Note: The singlet at 2.95 ppm is assumed to be trimethylammonium contaminant.

PG2

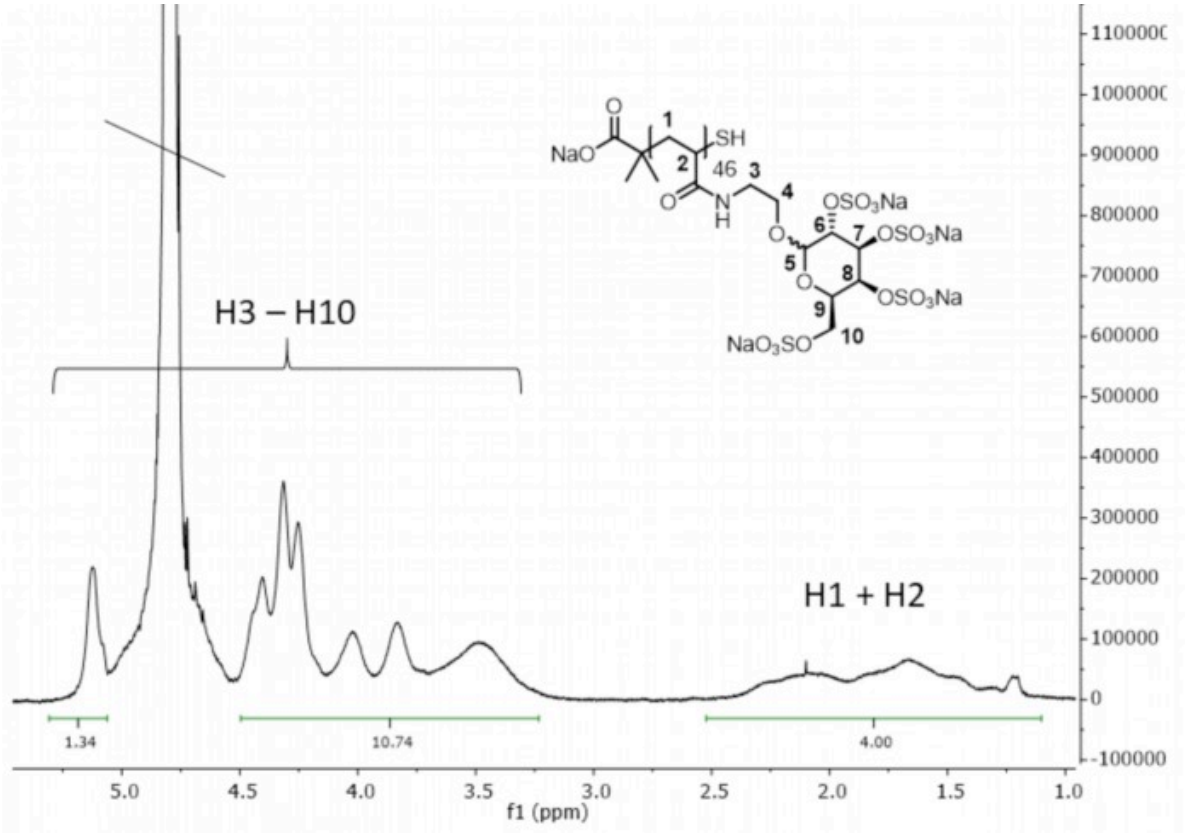



Figure S51: $^{1}$H-NMR (600 MHz, D2O) of **PG2**.

$^{1}$H-NMR (600 MHz, $D_2O$): δ = 5.30 - 5.11 (m, H5, 1H, overlapped with DHO); 4.50 – 3.22 (m, H3,4,6-10), 10H) 2.51 – 1.10 (broad m, H1+2, 4H) ppm.

Table S9: Determination of degree of sulfation of **PG2** by elemental analysis (CHNS analysis).

| **Sulfation[a] (monomer)** | **Unsulfated** | **1x sulf.** | **2x sulf.** | **3x sulf.** | **4x sulf.** | **PG2** |
|---|---|---|---|---|---|---|
| dS [%] | 0 | 25 | 50 | 75 | 100 | **97,6[b]** |
| | Theoretical values for elemental analysis | | | | | Assay result |
| C [%] | 47,7 | 34,8 | 27,5 | 22,7 | 19,3 | 17,2 |
| H [%] | 6,9 | 4,8 | 3,6 | 2,8 | 2,2 | 3,4 |
| N [%] | 5,1 | 3,7 | 2,9 | 2,4 | 2,0 | 1,8 |
| Na [%] | 0,0 | 6,1 | 9,6 | 11,8 | 13,4 | - |
| O [%] | 40,4 | 42,2 | 43,2 | 43,9 | 44,4 | - |
| S [%] | 0,0 | 8,5 | 13,3 | 16,5 | 18,7 | 16,2 |
| S/C ratio | 0 | 0,24 | 0,49 | 0,73 | 0,97 | 0,95 |

(a) Sulfation was derived from the theoretical monomer after sulfation. End groups of the polymer were not considered. The sample was very hygroscopic. Therefore, mass of C/N/S deteriorates, whereas H/O increases. (b) Degree of sulfation determined by S/C ratio correlation function of theoretical values to the elemental analysis results. S/C ratio should not be affected by water.

## 10. Materials and Methods (Biological Assays)

Cell lines, reagents and viruses

Hela cells (from ATCC), HaCaT cells (originated from N. Fusenig, DKFZ, Heidelberg, Germany, kind gift of J.T. Schiller, NIH, Bethesda, USA), 293TT cells (kind gift from J.T.Schiller, NIH, Bethesa, USA.), Vero cells (kind gift from Ari Helenius, ETH, Zürich, Switzerland) and CV-1 cells (kind gift from J. Kartenbeck, DKFZ, Heidelberg, Germany) were kept in Dublecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum. A549 cells (kind gift from C. Buck, NIH, MD, Bethesa, USA) were kept in Roswell Park Memorial Institute Medium (RPMI) with 5% fetal bovine serum. Alexa Fluor 568 succinimidylester was from ThermoFischer Scientific, Atto488N-Phalloidin was from Sigma-Aldrich and RedDot2 was from VWR. Antibodies were from ThermoFischer Scientific (anti-T-antigen SV40, clone PAb108) and BioRad (anti-Nuclear Protein Influenza virus, clone AA5H). Heparin (H4784) and ι-Carrageenan type V (C4014) were from Sigma.

HPV16 and MCPyV pseudoviruses (PsVs) expressing enhanced green fluorescent protein (eGFP) were produced as previously described using the plasmids pClneo-GFP, p16sheLL, and pwM2m, ph2m, phGluc for HPV16 and MCPyV, respectively [13,14]. HPV16 PsVs expressing firefly luciferase as a reporter gene were prepared using plasmids p16sheLL and a plasmid pfLuc consisting of firefly luciferase under control of the CMV promoter. pfLuc was constructed by excising the firefly luciferase gene from pCLucf (Addgene #37328) and by replacing the EGFP within pEGFP-N1 (Clontech). In short, 293TT cells were transfected with the corresponding plasmids, and the harvested PsVs were purified using a linear OptiPrep (25 to 39 %) gradient. Purified SV40 was prepared as previously described[15]. Recombinant HSV-1 (strain 17syn$^{+}$) [16], expressing eGFP under control of the CMV promoter was propagated in Vero cells and purified as previously reported[17]. IAV H1N1 (strain A/Puerto Rico/8/34 (PR8)) stocks were prepared as described previously [18].

Fluorophore-labeled HPV16 PsVs were prepared as described previously [19]. In short, HPV16 PsVs were incubated with Alexa Fluor 568 succinimidylester at a 1:8 molar ratio (L1/dye) for 1h at room temperature. Labeled viruses were separated from free dye by size exclusion chromatography.

Infection assays and cytotoxicity test

To test the inhibitory potential of glycomimetic compounds or natural glycans, viruses were incubated with the compounds at indicated concentrations for 1h at room temperature before adding the inoculum to cells.

For HPV16 or MCPyV infection studies, 4000 HeLa or A549 cells, respectively, were seeded into 96-well optical-bottom-well plates (Greiner) 16 h prior to infection. After incubation of 8ng HPV16 PsV or 85 ng MCPyV PsV with glycomimetics, the inocula were added to cells and incubated at 37℃ for 2h, after which the inoculum was exchanged for fresh growth media. HPV16 and MCPyV-infected cells were fixed with 4% paraformaldehyde (PFA) at 48h and 72h p.i, respectively. Cell nuclei were stained with RedDot2, followed by automated image acquisition using a spinning disc microscope (Zeiss Axio Observer Z1, equipped with a Yokogawa CSU22 spinning disc module; Visitron systems GmbH) with a 20x objective. Infection was scored by automated image analysis using MATLAB-based InfectionCounter as previously described[20]. The infection of each condition was normalized to solvent-treated virus control. As a read-out for cytotoxicity, relative cell counts as a measure of surviving cells treated with glycopolymers, glycooligomers, natural polysaccharides or HPV16 were determined using automated image analysis as described above. Cell counts were normalized to the untreated/uninfected controls (mock).

For HSV-1 infection assays $1.2x10^4$ Vero cells were seeded per well in 96-well optical-bottom-well plates. After incubation of HSV-1 GFP at a multiplicity of infection (MOI) of 0.8 plaque forming units (PFU)/ cell, the inocula were added to cells at 4°C and kept for 1h at 4°C, after which the inoculum was exchanged for fresh growth media and cells were incubated at 37°C. Cells were fixed at 6h p.i. Staining, acquisition and analysis was performed as for HPV16 and MCPyV.

For infection with SV40, 4000 CV-1 cells were seeded as above. After incubation of SV40 with glycomimetics or natural polysaccharides at MOI of 5 PFU/cell, the inocula were added to the cells and exchanged after 2h at 37°C with fresh media. Cells were fixed 24 h p.i. and stained for the expression of SV40 T-antigen as a marker for infection. Scoring for infection occured as before.

For IAV infection, $1x10^4$ A549 cells were seeded, inocula were prepared at MOI 9.5 PFU/cell, incubated with glycomimetics or natural polysaccharides, added to cells and exchanged after 1h at 37°C for fresh medium. Cells were fixed 8h p.i. and stained for IAV nuclear protein (NP) as a read-out for infection. Nuclei staining, acquisition and analysis was conducted as for HPV16 and MCPyV.

IC50 values were calculated using Prism (GraphPad Software, La Jolla California USA, www.graphpad.com) after fitting the data points into a sigmoidal dose-response curve.

HPV16 binding to cells

For binding, 4000 HeLa cells were seeded into 96-well optical-well plates and infected 16 h post seeding. Inocula were prepared as before, but using 1-2.5 ng AF568-labeled HPV16 per well. Cells were fixed 2 h p.i. with 4% PFA and the actin cytoskeleton was stained with Atto488N-phalloidin. Confocal image stacks were acquired as above using a 40x objective. Stacks were converted into maximum intensity projections. The outline of cells was assessed computationally using FIJI and displayed as outline [21].

Mouse vaginal challenge model

All animal procedures were performed in accordance with the German Laws for Animal Protection and were approved by the animal care committee of the local government (North Rhine-Westphalia State Agency for Nature, Environment and Consumer Protection).

Animals were assigned randomly into groups of five mice each. Isoflurane anesthesia (in oxygen) was used to immobilize the animals for pseudovirus application and imaging. Vaginal infection was performed according to Roberts et al.[22]. Briefly, six week old female BalbC mice (Charles River) were injected subcutaneously with 3 mg DepoClinovir (Pfizer, in 100 µl PBS) in the neck five days before challenge with HPV16. 500 ng of HPV16-lucf were preincubated with glycomimetics, carrageenan or heparin (500 µg per mouse) in HEPES buffer. The vaginal epithelium was disrupted with a cytobrush (Cooper Surgical), which was turned clockwise and counterclockwise ten times before infection. Immediately before application of inoculums to the mice, 4 % carboxymethylcellulose was added to increase viscosity to a final concentration of 1.33% (final volume 30µl). Infection was assessed 48h p.i.

by applying 20 µl 20mg/ml luciferin (Synchem) in the vagina and subsequent bioluminescence imaging using an IVIS spectrum (Perkin Elmer). 3 min after application of luciferin, 4 consecutive images were acquired to determine the maximum light emission from luciferase positive cells (exposure time: 2 min, binning: 8; fstop: 1). A circular region of interest of identical size was drawn around the vagina to quantify the signal as photons per second (p/s). The Mann-Whitney Rank Sum Test was used to calculate significant differences (Sigma Plot). Data are displayed as single plots and a line corresponding to the median (GraphPad Software, La Jolla California USA, www.graphpad.com). $P < 0.05$ was considered statistically significant.

Displaying of data and figures

All graphs were generated with GraphPad (GraphPad Software, La Jolla California USA, www.graphpad.com). Microscopy pictures were processed and cell outlines were drawn manually with Fiji[23]. Figures were assembled in Adobe Illustrator CS6.

## 11. References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