

Electronic Supplementary Information

Synthesis of highly controlled carbohydrate-polymer based hybrid structures by combining heparin fragments, sialic acid derivatives, and solid phase polymer synthesis

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Materials and Methods

Materials

N-Acetylneuraminic acid (>98%), 3'-Sialyllactose sodium salt (>98%), Methyl- α -D-glucopyranoside (>98%) were purchased from Carbosynth. 2, 2'-(Ethylenedioxy)bis(ethylamine) (98%), Propargyl alcohol (99%), Succinic anhydride (>99%), Triethylsilane (99%), Triisopropylsilane (98%), (+)-Sodium-L-ascorbate (>99.0%), Amberlite® IR 120 H⁺, Amberlite® IR 120 Na⁺, Dowex® 1X4 Cl⁻, Bovine Serum Albumin (BSA) were purchased from Merck (former Sigma Aldrich). Trityl chloride (98%), *p*-Toluic acid (98%), Piperidine (99%), Copper (II) sulfate (98%) were purchased from Acros Organics. *p*-Toluenesulfonic acid monohydrate (98%), Boron trifluoride diethyl etherate (>98%), Acetyl chloride (98%) were purchased from Alfa Aesar. *N*-Bromosuccinimide (99%) was purchased from Merck. Sodium azide (99%) diethyl dithiocarbamate (99%) were purchased from Applichem. PyBOP (Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphat), Fmoc-Lysine(Boc)-OH (>98%) were obtained from Iris Biotech. Silver carbonate (>99%) was obtained from Strem Chemicals. Sodium hypochlorite solution (13%) was obtained from Hoesch. Sodium bromide (>99.5%), (2,2,6,6-tetramethylpiperidinyl) oxyl radical (TEMPO, >99%) were purchased from Fisher scientific (former Fluka). Acetic anhydride (99%), Formic acid (>99%), Magnesium sulfate anhydrous (>99.5% min), Sodium chloride (>99.5%), Sodiumhydrogen carbonate (>99.7%), Disodiumhydrogen phosphate dihydrate (>99%), Potassiumdihydrogen phosphate (>99%), Tris(hydroxymethyl)aminoethan (TRIS, >99.9%), Calcium chloride anhydrous (>96%) were purchased from VWR. Lithium hydroxide monohydrate (>99%) was purchased from Janssen chimica. 9-Fluorenylmethyl chloroformate (Fmoc-Cl, 98%) was purchased from Chempur. *N*, *N*-Diisopropylethylamine (99%) was obtained from Roth. Trifluoroacetic acid (TFA, 99%), 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM, 97%) were purchased from Fluorochem. Tentagel® S RAM (Rink Amide) resin (Capacity 0.25 mmol/g) was purchased from Rapp Polymere. Porcine sodium heparin (MW 15-19 kDa) was purchased from Bioiberica. Heparinase I from *Flavobacterium heparinum* was purchased from Iduron. Fondaparinux®-sodium was purchased as Arixtra® at a concentration of 10 mg / 0.8 mL from the Aspen Pharma Trading Limited as ready-to-use syringes. Peptide synthesis grade *N*, *N*-Dimethylformamide was used for solid phase synthesis. All solvents were of *p.a.* reagent grade. Phosphate buffer pH 6.5 was prepared according Sørensen's buffer: 68.7 vol.% (66.7 mM KH₂PO₄ in Milli-Q) and 31.3 vol.% (66.7 mM Na₂HPO₄*2 H₂O in Milli-Q). Vivaspin® MWCO 2000 and MWCO 3000 units were purchased from Sartorius. Spectra/Por® Float-a-Lyzer® G2 MWCO 0.1 – 0.5 kDa and 0.5 – 1.0 kDa were obtained from Spectrum Labs.

Instrumentation

Nuclear Magnetic Resonance spectroscopy (NMR)

¹H-NMR (300 MHz) spectra were recorded on a Bruker AVANCE III - 300. ¹H-NMR (600 MHz) spectra were recorded on a Bruker AVANCE III - 600. Chemical shifts of all NMR spectra were reported in delta (δ) expressed in parts per million (ppm). For ¹H-NMR the residual, non-deuterated solvent was used as internal standard. The following abbreviations are used to indicate the multiplicities: s, singlet; d, doublet; t triplet; m multiplet.

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR FTIR)

IR spectra were recorded with a Nicolet 6700, attenuated total reflectance Fourier transform infrared spectroscopy (ATR FTIR) spectrometer from Thermo Scientific and spectra analyzed using Omnic software 7.4.

Reversed Phase - High Performance Liquid Chromatography - Mass Spectrometry (RP-HPLC-MS)

Measurements were performed on an Agilent 1260 Infinity instrument coupled to a variable wavelength detector (VWD) (set to 214 nm) and a 6120 Quadrupole LC/MS containing an Electrospray Ionization (ESI) source (operated in positive or negative ionization mode in a *m/z* range of 200 to 2000). As HPLC column a Poroshell 120 EC-C18 (3.0 × 50 mm, 2.5 μ m) RP column from Agilent was used. The mobile phases A and B were H₂O/ACN (95/5) and H₂O/ACN (5/95), respectively. Both mobile phases contained 0.1% of formic acid. Samples were analyzed at a flow rate of 0.4 mL/min using a linear gradient starting with 100% mobile phase A reaching 25, 50 or 75 % mobile phase B within 30 min. The temperature of the column compartment was set to 25 °C. UV and MS spectral analysis was done within the OpenLab ChemStation software for LC/MS from Agilent Technologies.

Strong Anion Exchange - High Performance Liquid Chromatography (SAX-HPLC)

Measurements were performed on an Agilent 1200 instrument coupled to a variable wavelength detector (VWD) (set to 214 nm). As SAX-HPLC column a Zobrax (4.6 × 250 mm, 5.0 μ m) column from Agilent was used. The mobile phases A and B were A: 50 mM NaH₂PO₄, pH 7 in Milli-Q water, B: 50 mM NaH₂PO₄, 800 mM NaCl, pH 7 in Milli-Q water / ACN = 70% : 30%. Samples were analyzed at a flow rate of 1.0 mL/min using the following gradient within 60 min: 0 → 5 min: 95% A, 5% B; 5

→ 40 min: 5 → 100% B; 40 → 60 min: 100% B. The temperature of the column compartment was set to 25 °C. UV spectral analysis was done within the OpenLab ChemStation software for HPLC from Agilent Technologies.

Ultra High Resolution - Mass Spectrometry (UHR-MS)

UHR-MS measurements were performed with a Bruker UHR-QTOF maXis 4G instrument with a direct inlet via syringe pump, an ESI source and a quadrupole followed by a Time Of Flight (QTOF) mass analyzer.

Freeze dryer

The final oligomers were freeze dried with an Alpha 1-4 LD plus instrument from Martin Christ Freeze Dryers GmbH. The main drying method was set to -55 °C and 0.1 mbar.

General Methods

All general methods follow procedures as previously presented for solid phase polymer synthesis¹

Solid phase synthesis protocols

The batch sizes for synthesizing the oligomers using solid phase synthesis varied from 15 µmol to 400 µmol.

Fmoc cleavage

The Fmoc protecting group of the resin as well as from the coupled building blocks or amino acid were cleaved by the addition of a solution of 25% piperidine in DMF. The deprotection was performed twice for 10 min. After that, the resin was washed thoroughly 10 times with DMF.

General coupling protocol

Commercially available Tentagel S RAM (Rink Amide) resin was used as resin for solid phase synthesis. As an example 100 µmol of the resin were swollen in 10 mL of DCM for 20 min and subsequently washed five times with 10 mL of DMF. The Fmoc protecting group of the Tentagel S RAM resin was removed following the Fmoc cleavage protocol. A building block was coupled to the resin using a mixture of 0.5 mmol (5 eq.) of building block and 0.5 mmol PyBOP (5 eq.) dissolved in 4 mL of DMF to which 1 mmol (10 eq.) of DIPEA was added. The mixture was shaken for 30 s under a nitrogen stream for activation and subsequently added to the resin. The resin with the coupling mixture was shaken for 1 h. After that, the resin was washed from excessive reagent 5 times with 10 mL of DMF.

Capping of *N*-terminal primary amine

After successful assembly of the desired number of building blocks on solid phase, the *N*-terminal site was capped with an acetyl group. Therefore, 10 mL acetic anhydride were shaken twice with the resin for 30 min.

General CuAAC protocol

To 100 µmol of the resin loaded with the oligomeric structure, 200 µmol (2 eq.) of protected sialic acid or sialyllactose derivative (**2**, **3**) per azide group, dissolved in 1.5 mL DMF, were added. When propargylated sialic acid (**2**) was used, a flat rate of 100 mg (0.4 mmol) of CuSO₄ and 100 mg (0.5 mmol) of sodium ascorbate were used and dissolved each in 0.75 mL of water and also added to the resin. When propargylated sialyllactose (**3**) was used, a flat rate of 50 mg (0.2 mmol) of CuSO₄ and 50 mg (0.25 mmol) of sodium ascorbate were used and dissolved each in 0.75 mL. This mixture was shaken for 24 h and subsequently washed extensively with water, a 23 mM solution of sodium diethyldithiocarbamate in DMF : H₂O = 1 : 1, DMF and DCM.

Cleavage from solid phase

13 mL of a mixture of 95% TFA, 2.5% of TIPS and 2.5% of DCM were added to the resin and shaken for 1 h. The filtrate was poured into 60 mL cold diethyl ether. The resin was washed with an additional 5 mL of the cleavage mixture which were also added to the cold ether. The resulting precipitate was centrifuged three times and the ether decanted. The crude product was dried over a stream of nitrogen, dissolved in 6 mL of H₂O and lyophilized twice.

General preparative purification protocol of the oligomers

The oligomers were purified by preparative Reversed Phase - High Performance Liquid Chromatography on an Agilent 1260 Infinity instrument coupled to a variable wavelength detector (VWD) (set to 214 nm). As HPLC column a UG80 C18 (20mmI.D.×250 mm, 5 µm) RP column from Shiseido was used. The mobile phases A and B were H₂O and ACN to which 0.1% formic acid were added, respectively. Samples were purified at a flow rate of 20 mL/min using a linear gradient starting with 100% mobile phase A reaching 50% mobile phase B within 15 min. The temperature of the column compartment was room

temperature (18-23° C). UV analysis was done within the OpenLab ChemStation software for LC/MS from Agilent Technologies.

Protective groups deprotection protocol

In order to remove both the acetyl and methyl protective groups of the carbohydrate moieties, 3 mL of a 0.1 M solution of lithium hydroxide monohydrate in a mixture of MeOH : H₂O = 1 : 1 were added to the compound and shaken for 3 h at room temperature (18-23° C) (the pH needs to be checked and has to be at 13 during the deprotection). Subsequently the dissolved and deprotected compound was treated with Amberlite® IR 120 H⁺ ion exchange resin until pH 4-5 was reached. Then the neutralized mixture was dried in *vacuo* at a temperature not higher than 25 °C and finally lyophilized in order to give final deprotected compound.

General ion exchange protocol

Compounds were dissolved in 2 mL of Milli-Q water to which 1 g DOWEX® 1X4 Cl⁻ was added and the mixture was shaken for 10 min. Finally, the resin was filtered off using a 0.45 µm PTFE syringe filter and the product was isolated by lyophilization.

MCPyV capsid production

MCPyV capsids were produced using the established 293 TT cell culture system for virus-like-particle production of papilloma- and polyomaviruses.² Briefly, 293 TT cells cultured in the presence of Hygromycin for SV40 T-antigen expression were transfected with codon-optimized VP1 or VP1- and VP2-coding plasmids^{2b} (pwM and ph2m) and harvested 48 hours post-transfection. Cells were lysed with Triton X-100 and lysates incubated over night at 37 °C for particle maturation. MCPyV particles were purified via salt extraction, followed by CsCl velocity and equilibrium centrifugation steps and ion exchange chromatography (a manuscript containing a detailed description of an optimized purification protocol will be published elsewhere). Integrity of the ca. 50 nm diameter capsid was verified by negative stain transmission electron microscopy (TEM) and dynamic light scattering (DLS).

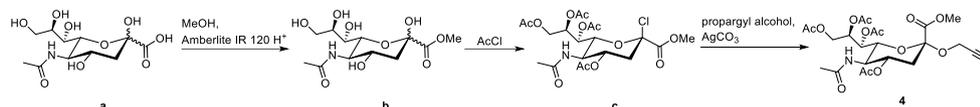
NMR experiments

For STD-NMR, capsids were buffer-exchanged to pure D₂O-buffer (99.5 %, Cortecnet) containing 150 mM NaCl, 1 mM CaCl₂, pH 6.3 using 500 kDa MWCO dialysis devices made from cellulose ester (Spectra Por). The same buffer was used to dissolve compounds **O2** and **O6** to yield 15 mM and 22.5 mM stock solutions, respectively. NMR samples contained 27.1 nM of MCPyV capsids (9.8 µM with respect to the major capsid protein VP1) and 1.5 mM of **O2** or 30.5 nM of MCPyV capsids (11.0 µM of VP1) and 1.1 mM of **O6**, respectively. NMR spectra were recorded at 283 K using 3 mm I.D. MATCH tubes (with 200 µL final sample volume) on a Bruker AVIII-600 spectrometer equipped with a room temperature probe head. For STD-NMR spectra the off- and on-resonance irradiation frequencies were set to -30 ppm and -0.5 ppm, respectively. The irradiation power of the selective pulses was 57 Hz, the saturation time 2 s and the total relaxation delay 3 s. A 50 ms continuous-wave spin-lock pulse at 3.2 kHz was employed and a total number of 1024 scans were recorded. A total of 12 k points was recorded, and spectra were multiplied with a Gaussian window function prior to Fourier transformation using TOPSPIN 3.0 (Bruker).

Experimental Data

Functionalized sialic acid synthesis

Propargyl-functionalized Neu5Ac (**4**) was prepared according to previously published protocols by Šardžik³ and Ebbesen.⁴



Scheme 1: Synthesis sequence of propargylated Neu5Ac.

N-acetyl neuraminic acid methyl ester (b)

30.9 g (100 mmol) of *N*-acetyl neuraminic acid (**a**) were stirred with 6 g of Amberlite® IR120 H⁺ in 400 ml of dry methanol at r.t. until all starting material was consumed. The resin was filtered off and removal of the solvent at reduced pressure afforded methyl ester (**b**) as a white solid in a quantitative yield.

¹H NMR (300 MHz, Deuterium oxide) δ 4.00 – 3.89 (m, 2H, *H*4, 6), 3.85 – 3.76 (m, 1H, *H*5), 3.75 – 3.68 (m, 4H, *-OCH*3, 9'), 3.61 (ddd, ³*J* = 8.9, 6.2, 2.5 Hz, 1H, *H*8), 3.49 (dd, ³*J* = 11.7, 6.2 Hz, 1H, *H*9''), 3.43 (dd, ³*J* = 9.2, 1.2 Hz, 1H, *H*7), 2.19 (dd, ³*J* = 13.1, 4.8 Hz, 1H, *H*3_{eq.}), 1.93 (s, 3H, *Ac*), 1.79 (dd, ³*J* = 13.1, 11.5 Hz, 1H, *H*3_{ax.}).

¹³C NMR (75 MHz, D₂O) δ 174.75, 171.33, 95.27, 70.27, 70.02, 68.11, 66.60, 63.07, 53.41, 51.97, 38.58, 21.99.

MS for C₁₂H₂₁NO₉ (ESI, pos.) *m/z*: [M + Na⁺]⁺ calc.: 346.11; found 346.00, [M + H⁺]⁺ calc.: 324.13; found 324.00, [M - OH]⁻ calc.: 306.11; found 306.20, [M + OH⁻ - H₂O]⁺ calc.: 288.11; found 288.10.

IR (ATR) $\tilde{\nu}_{\max}$: 3322 (br), 2937 (w), 1767 (m), 1633 (m), 1541 (m), 1429 (m), 1371 (m), 1275 (m), 1124 (m), 1028 (s), 894 (m), 575 (s).

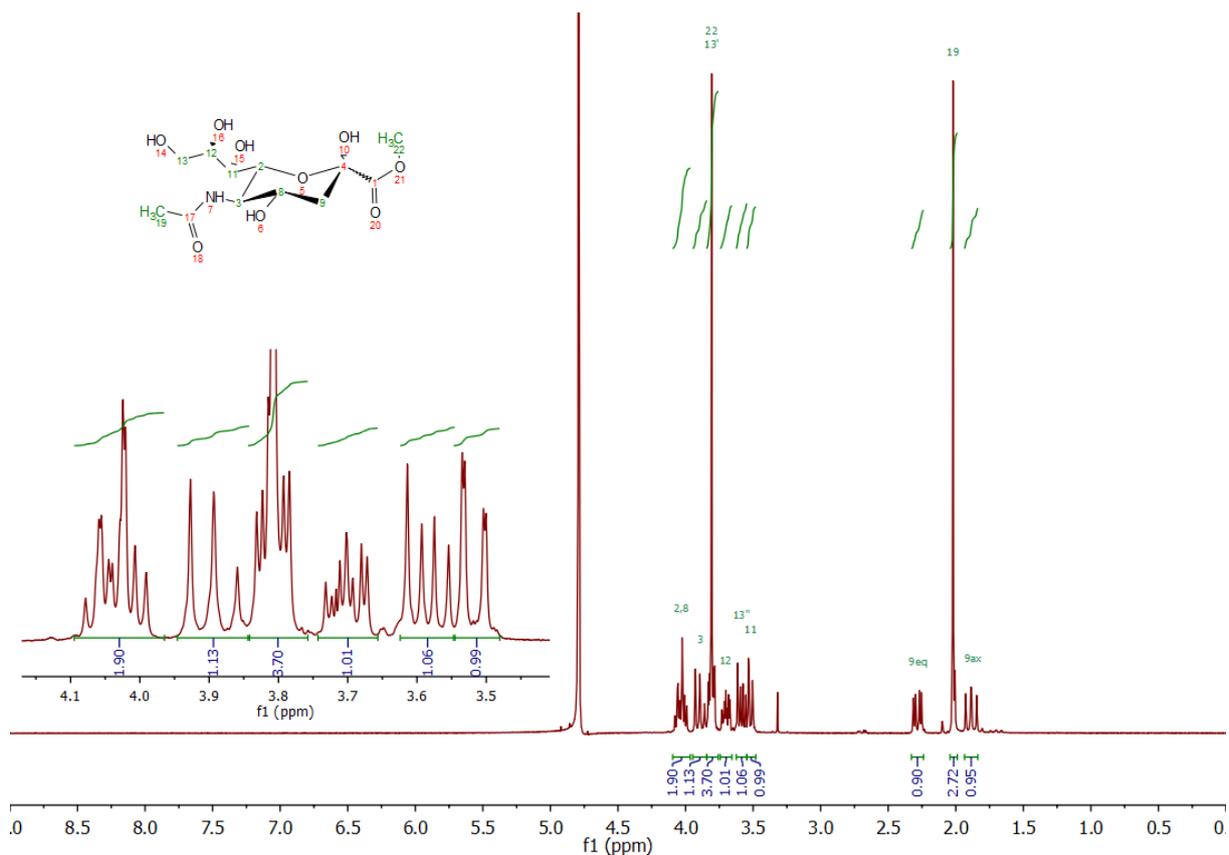


Figure 1: ^1H NMR (300 MHz, Deuterium oxide) of compound **b**.

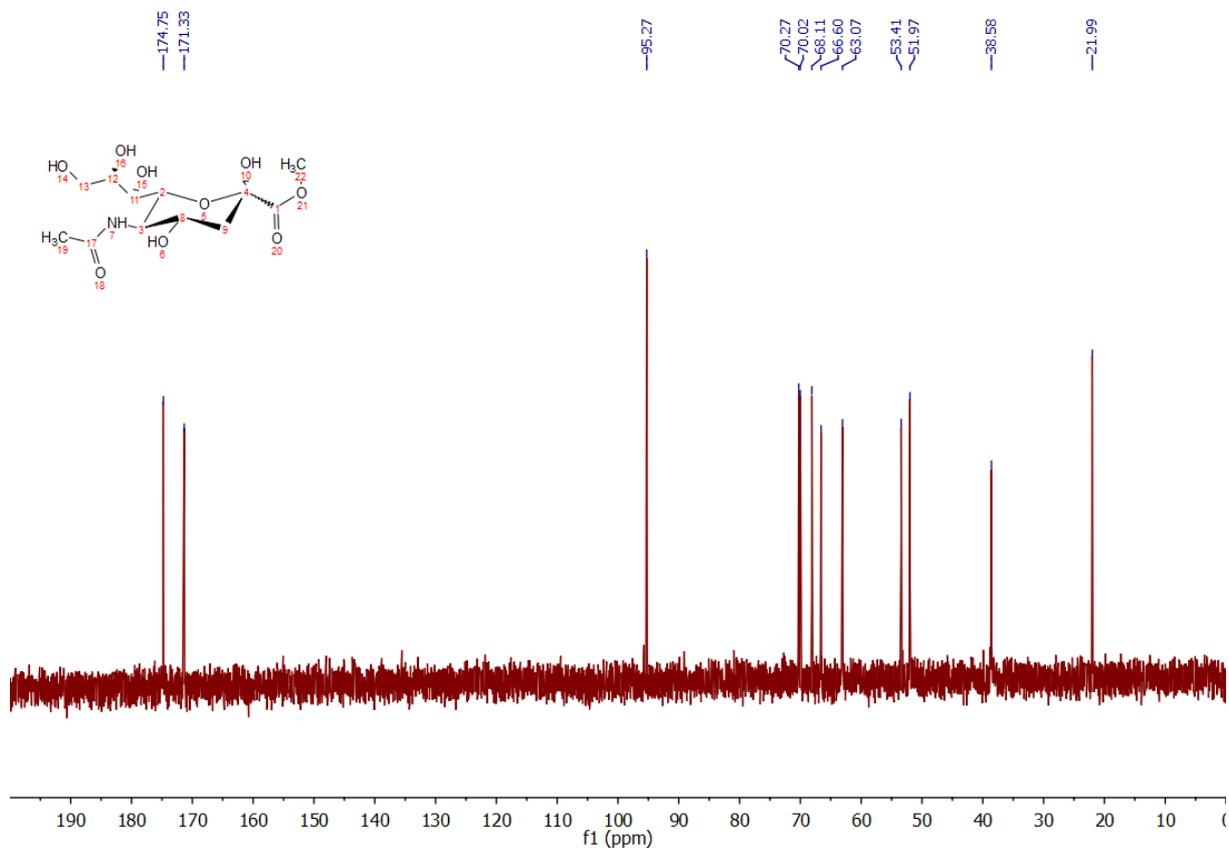


Figure 2: ^{13}C NMR (75 MHz, Deuterium oxide) of compound **b**.

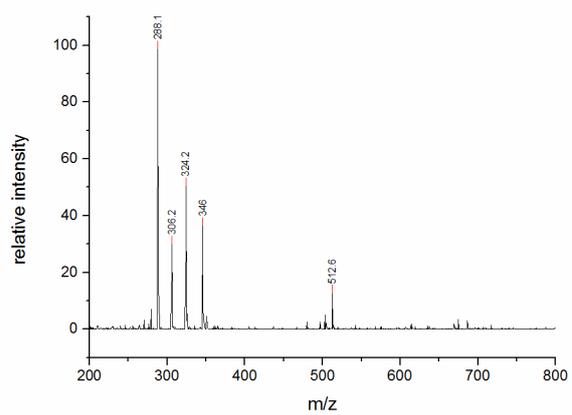


Figure 3: ESI-MS (positive mode) of compound **b**.

Methyl 2-chloro-4,7,8,9-tetra-*O*-acetyl-*N*-acetyl- β -D-neuraminate (**c**)

50 ml of freshly distilled and ice cold (0°C) acetyl chloride were given to 5 g (15.5 mmol) of *N*-acetyl neuraminic acid methyl ester (**b**) in an ice bath under moderate stirring equipped with a gas discharge tube, which leads to a saturated sodium bicarbonate solution. After consumption of the starting material, the remaining acetyl chloride was distilled off at reduced pressure by keeping the temperature not higher than 40 °C to afford the peracetylated and chlorinated intermediate **c** in a quantitative yield. **c** was used without further purification.

^1H NMR (300 MHz, Chloroform-*d*) δ 5.61 (d, $^3J = 10.1$ Hz, 1H, -NH), 5.47 (dd, $^3J = 6.9$, 2.4 Hz, 1H, H7), 5.39 (ddd, $^3J = 11.1$, 4.9 Hz, 1H, H4), 5.17 (ddd, $^3J = 6.8$, 6.0, 2.7 Hz, 1H, H8), 4.43 (dd, $^2J = 12.5$, $^3J = 2.7$ Hz, 1H, H9'), 4.35 (dd, $^3J = 10.8$, 2.4 Hz, 1H, H6), 4.20 (ddd, $^3J = 10.3$ Hz, 1H, H5), 4.06 (dd, $^2J = 12.5$, $^3J = 5.9$ Hz, 1H, H9''), 3.87 (s, 3H, -OCH₃), 2.78 (dd, $^2J = 13.9$, $^3J = 4.8$ Hz, 1H, H3_{eq}.), 2.27 (dd, $^2J = 14.0$, $^3J = 11.2$ Hz, 1H, H3_{ax}.), 2.11 – 1.91 (5s, 5x 3H, 5x Ac).

^{13}C NMR (75 MHz, CDCl₃) δ 171.17, 170.81, 170.73, 170.12, 169.87, 165.75, 96.71, 77.36, 74.00, 70.10, 68.90, 67.01, 62.20, 53.93, 48.90, 40.76, 23.22, 21.06, 20.91, 20.77.

MS for C₂₀H₂₈ClNO₁₂ (ESI, pos.) m/z : [M + H]⁺ calc.: 510.14; found 510.00, [M – Cl + H₂O]⁺ calc.: 492.17; found 492.20, [M – Cl]⁺ calc.: 474.16; found 474.00, [M – Cl – AcOH]⁺ calc.: 414.14; found 414.20.

IR (ATR) $\tilde{\nu}_{\text{max}}$: 2960 (w), 1739 (s), 1658 (m), 1549 (m), 1435 (m), 1370 (m), 1209 (ss), 1031 (s), 599 (m).

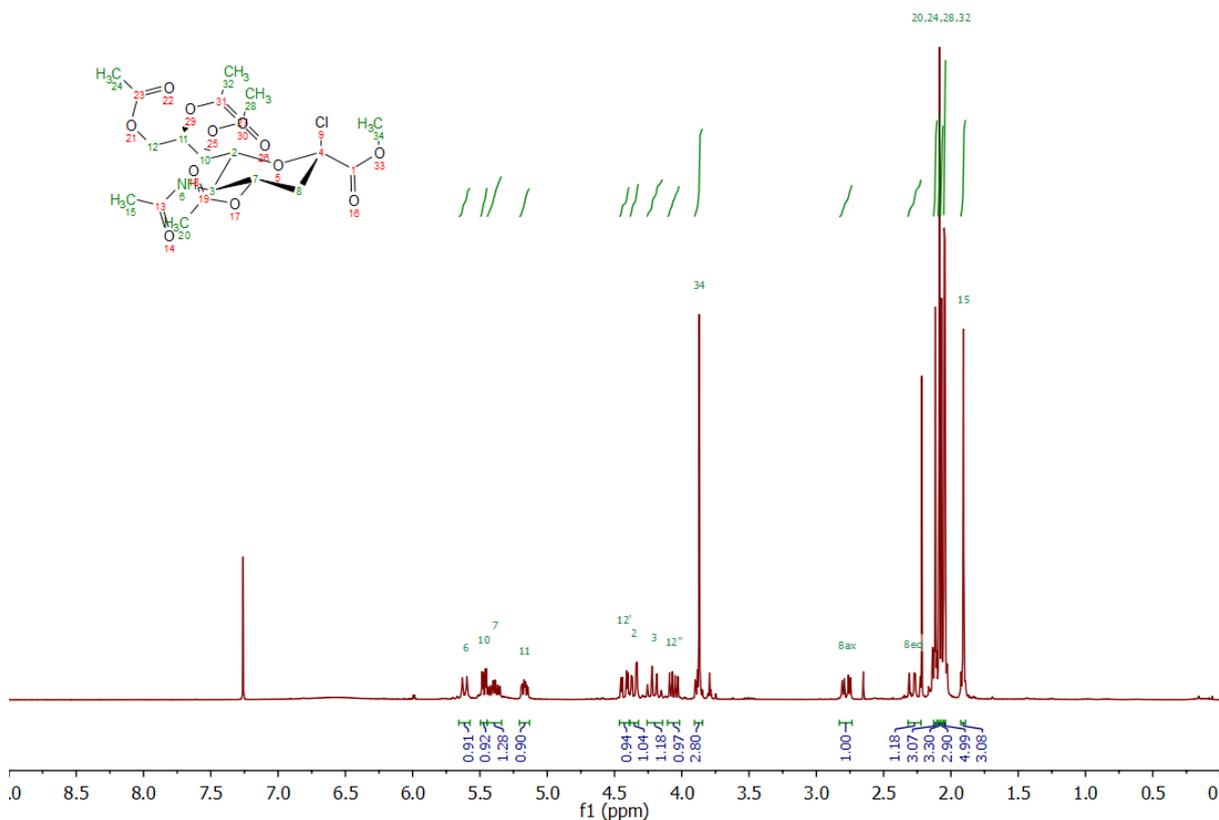


Figure 4: ^1H NMR (300 MHz, Chloroform-*d*) of compound **c**.

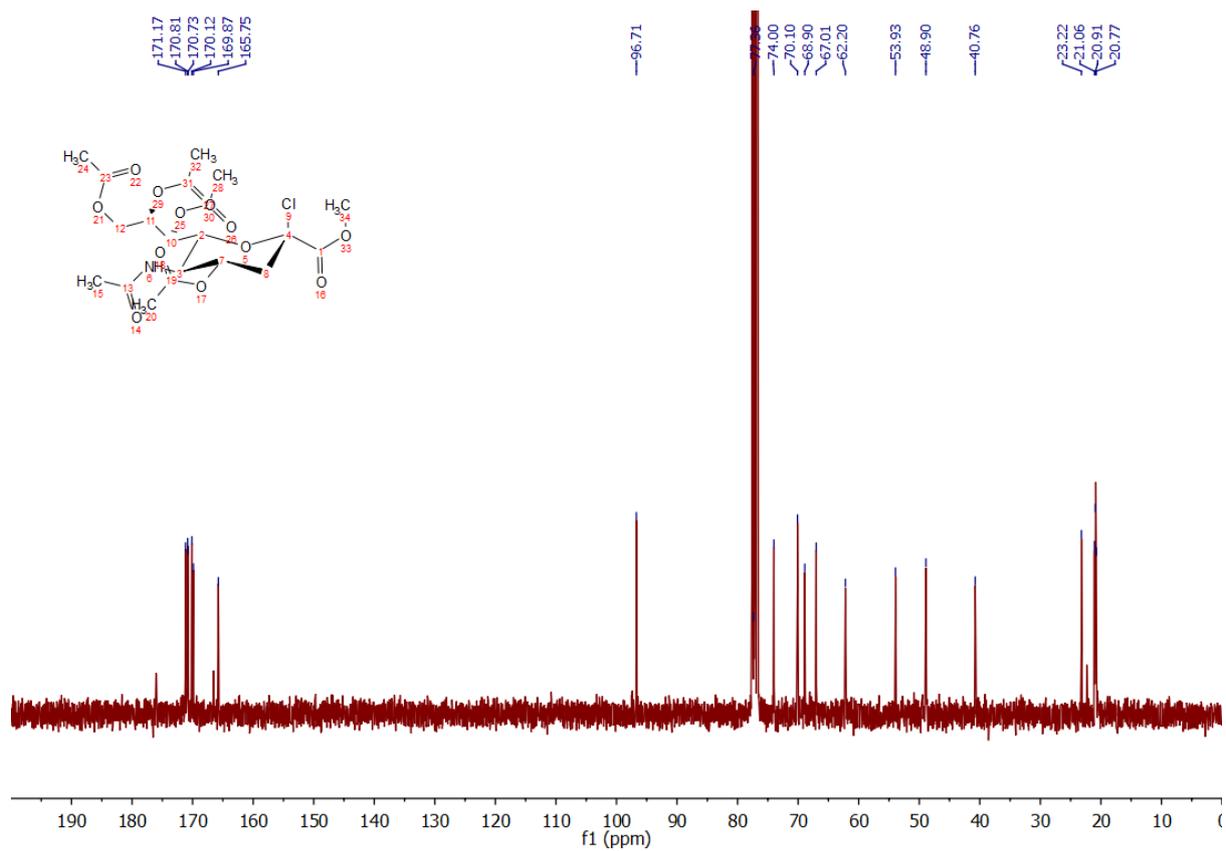


Figure 5: ^{13}C NMR (75 MHz, Chloroform- d) of compound **c**.

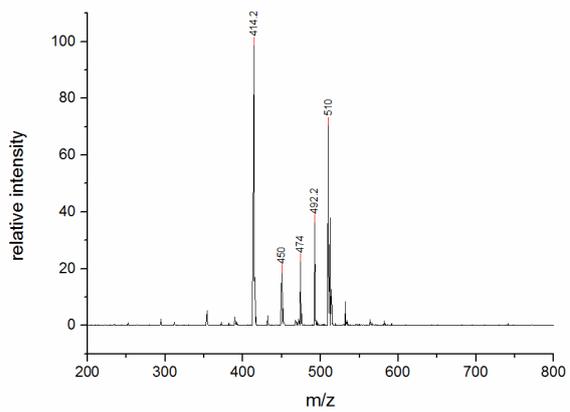


Figure 6: ESI-MS (positive mode) of compound **c**.

Methyl 2-(propargyl)-4,7,8,9-tetra-*O*-acetyl-*N*-acetyl- α -D-neuraminatate (**4**)

7.90 g (15.5 mmol) of **c** were dissolved in 50 ml of freshly distilled propargylic alcohol at r.t., then 2 eq. (8.53 g, 30.9 mmol) of Ag_2CO_3 were added in one portion under rigorous stirring. After 16 h the reaction was stopped by filtering off all solids via vacuum filtration using a pore size 3 frit. The filter cake was washed twice (2x 50 ml) with dichloromethane. The combined organic layers were washed three times with deionized water (3x 100 ml), once with brine (100 ml), dried over MgSO_4 and concentrated under reduced pressure in order to give 3.63 g (6.86 mmol, 45%) of the raw product as a slightly yellow foam ($\alpha : \beta = 5 : 1$, based on ^1H NMR). The raw product was purified via column chromatography (elution gradient EtOAc : nHex = 1 : 1 \rightarrow pure EtOAc. R_f (EtOAc) = 0.5) in order to afford 2.23 g (4.21 mmol, 28%) of a transparent white foam, which consisted mainly of the α -anomer. Recrystallization from EtOH afforded 1.65 g (3.12 mmol, 20%) of the α -form (**4**) as white crystals.

^1H NMR (600 MHz, Chloroform-*d*) δ 5.40 (ddd, $^3J = 8.6, 5.8, 2.8$ Hz, 1H, *H8*), 5.30 (dd, $^3J = 8.6, 1.7$ Hz, 1H, *H9'*), 5.22 – 5.18 (m, 1H, *-NH*), 4.86 (ddd, $^3J = 12.4, 9.6, 4.7$ Hz, 1H, *H4*), 4.39 (dd, $^2J = 15.7, ^4J = 2.5$ Hz, 1H, *propargyl-CH'H*), 4.28 (dd, $^3J = 12.4, 2.8$ Hz, 1H, *H7*), 4.15 (dd, $^2J = 15.7, ^4J = 2.4$ Hz, 1H, *propargyl-CHH''*), 4.10 – 4.02 (m, 3H, *H5, 6, 9''*), 3.80 (s, 3H, *-OCH3*), 2.62 (dd, $^2J = 12.8, ^3J = 4.6$ Hz, 1H, *H3eq.*), 2.43 (t, $^4J = 2.4$ Hz, 1H, *propargyl-CH*), 2.14 – 2.02 (4s, 4x 3H, 4x Ac), 1.97 (dd, $^2J = 12.5$ Hz, $^3J = 12.5$ Hz, 1H, *H3ax.*), 1.87 (s, 3H, Ac).

^{13}C NMR (151 MHz, CDCl_3) δ 171.10, 170.76, 170.33, 170.24, 170.20, 167.91, 98.21, 79.07, 74.62, 72.74, 68.96, 68.39, 67.30, 62.53, 52.98 (2), 49.48, 38.01, 23.31, 21.25, 20.98, 20.95, 20.89.

MS for $\text{C}_{23}\text{H}_{31}\text{NO}_{13}$ (ESI, pos.) m/z : $[\text{M} + \text{Na}]^+$ calc.: 552.17; found 552.20, $[\text{M} + \text{H}]^+$ calc.: 530.19; found 530.20, $[\text{M} - \text{C}_3\text{H}_4\text{O} + \text{H}]^+$ calc.: 474.16; found 474.25, $[\text{M} - \text{C}_3\text{H}_4\text{O} - \text{AcOH} + \text{H}]^+$ calc.: 414.14; found 414.25.

HRMS for $\text{C}_{23}\text{H}_{31}\text{NO}_{13}$ (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calc.: 530.1874; found: 530.1872.

IR (ATR) $\tilde{\nu}_{\text{max}}$: 3318 (w), 3252 (w), 2957 (w), 1748 (s), 1733 (s), 1648 (m), 1547 (m), 1369 (m), 1207 (s), 1037 (s), 620 (m), 602 (m).

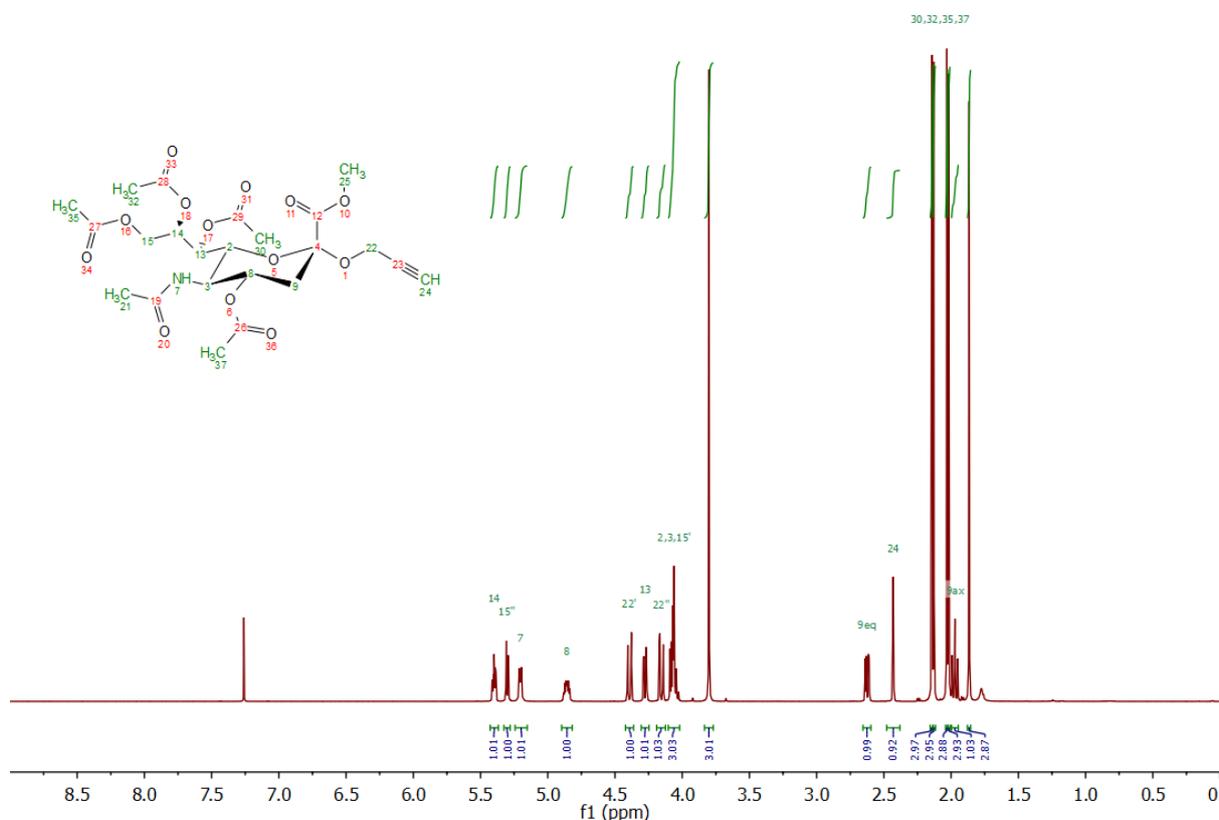


Figure 7: ^1H NMR (600 MHz, Chloroform-*d*) of compound **4**.

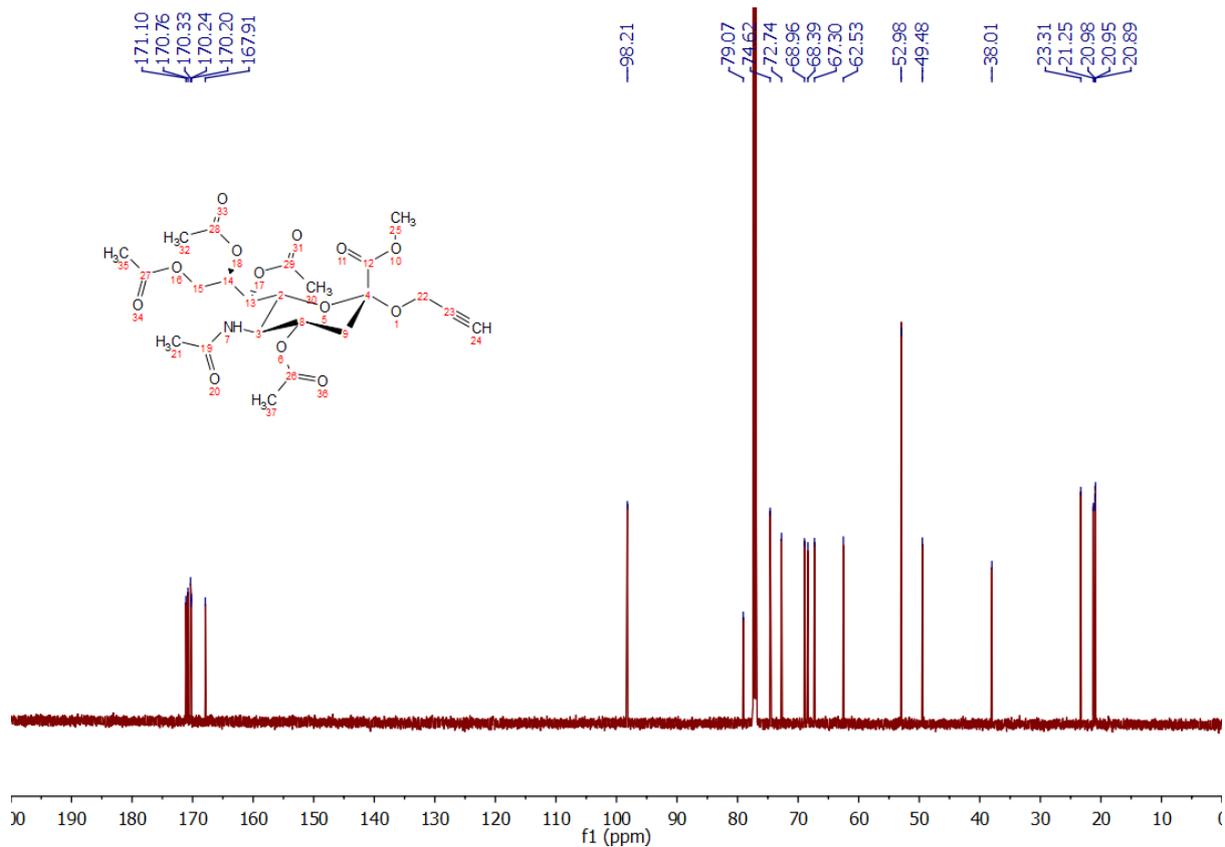


Figure 8: ^{13}C NMR (151 MHz, Chloroform- d) of compound 4.

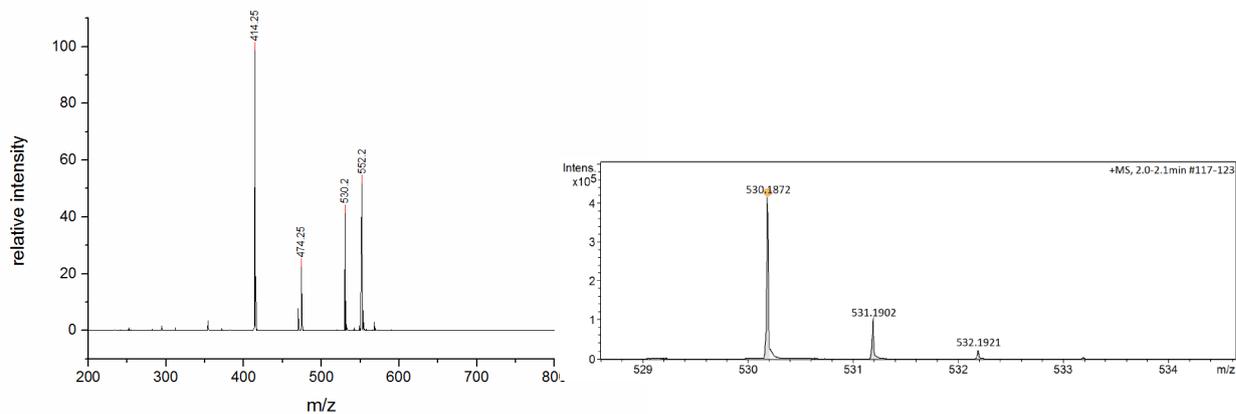
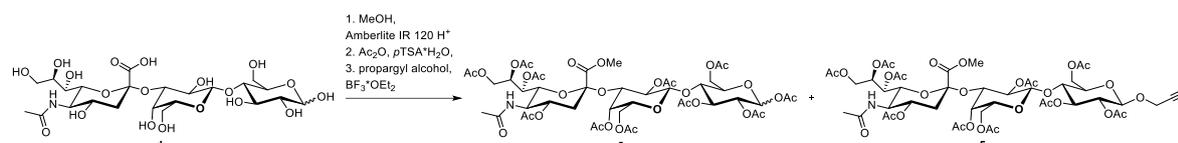


Figure 9: ESI-MS (positive mode), HR-MS (ESI+ Q-TOF, positive mode) of compound 4.

Functionalized 3'-sialyllactose synthesis



Scheme 2: Synthesis sequence of propargylated 3'-sialyllactose.

500 mg (763 μ mol) of 3'-sialyllactose sodium salt (**d**) were suspended in 50 mL of methanol before 0.5 g of Amberlite IR 120 H⁺ were added. After 2 days the esterification was complete (monitored by ESI-MS). Then the resin was filtered off and the solution was dried under reduced pressure. To the raw and non-purified product 50 mL of acetonitrile were added, before 3 eq. per alcohol group (3 x 11 x 763 μ mol = 25 mmol, 2.4 mL) of acetic anhydride and 0.1 eq. per alcohol of *p*-toluenesulfonic acid monohydrate (0.8 mmol, 160 mg) were added while stirring. The reaction was stirred at 40 °C until the ester was completely dissolved and converted. After the solvent was removed under reduced pressure, the product was redissolved in 50 mL of ethyl acetate and 50 mL of water, washed with 50 mL of sat. sodium bicarbonate solution, twice with water (2 x 50 mL) and 50 mL of brine, dried over magnesium sulfate and concentrated to dryness. 855 mg of the raw product were obtained, which were used without further purification. The obtained, peracetylated raw product was dissolved in 50 mL of dichloromethane before 0.5 mL of propargyl alcohol (8.7 mmol, 11.4 eq.) and 0.3 mL of boron trifluoride diethyl etherate (2.4 mmol, 3.1 eq.) were added. After 3 days the reaction was stopped by the addition of 50 mL of water before the product was washed with water, sat. sodium bicarbonate solution, brine (50 mL each), dried over magnesium sulfate and concentrated *in vacuo*. The product was purified via preparative RP-HPLC (20-80% ACN in water, 0.1% formic acid). 123 mg (111 μ mol, 14.6%) of the desired, propargyl functionalized **Methyl 1-(propargyl)-deca-O-acetyl- β -3'-sialyllactoside (5)** was obtained in an α : β ratio of 10% : 90% (based on ¹H NMR) and 130 mg (117 mg, 15.3 %) of the unconverted **Methyl α,β -undeca-O-acetyl-3'-sialyllactosate (e)** was obtained in an α : β ratio of about 70% : 30% (based on ¹H NMR).

Methyl α,β -undeca-O-acetyl-3'-Sialyllactosate (e)

(Methyl (4,7,8,9-tetra-O-acetyl-N-acetyl-neuraminate)yl-(2 \rightarrow 3))-2,4,6-tri-O-acetyl- β -galactopyranosyl-(1 \rightarrow 4) - 1,2,3,6-tetra-O-acetyl- α,β -glucopyranose (e)

¹H NMR (600 MHz, Chloroform-*d*) δ **Glc**: 6.23 (d, ³*J* = 3.6 Hz, 1H, *H*1), 5.00 (dd, ³*J* = 10.3, 3.7 Hz, 1H, *H*2), 5.44 (dd, ³*J* = 10.3, 9.2 Hz, 1H, *H*3), 3.89 (dd, ³*J* = 10.1, 9.2 Hz, 1H, *H*4), 3.85 – 3.83 (m, 1H, *H*5), 4.19 (dd, ²*J* = 12.1, ³*J* = 4.5 Hz, 1H, *H*6'), 4.37 (dd, ²*J* = 12.1, ³*J* = 2.2 Hz, 1H, *H*6''); **Gal**: 4.63 (d, ³*J* = 8.0 Hz, 1H, *H*1), 4.92 (dd, ³*J* = 10.2, 7.9 Hz, 1H, *H*2), 4.50 (dd, ³*J* = 10.2, 3.5 Hz, 1H, *H*3), 4.90 – 4.84 (m, 1H, *H*4), 4.05 – 3.95 (m, 1H, *H*5), 4.05 – 3.95 (m, 2H, *H*6', 6''); **Neu5Ac**: 3.83 (s, 3H, -OCH₃), 1.78 (dd, ²*J* = 13.9 Hz, ³*J* = 11.5, 0.23H, β -*H*3_{ax}), 1.66 (dd, ²*J* = 12.4 Hz, ³*J* = 12.4 Hz, 0.77H, α -*H*3_{ax}), 2.56 (dd, ²*J* = 12.7, ³*J* = 4.6 Hz, 0.68H, α -*H*3_{eq}), 2.44 (dd, ²*J* = 13.7, ³*J* = 5.2 Hz, 0.32H, β -*H*3_{eq}), 4.90 – 4.84 (m, 1H, *H*4), 4.05 – 3.95 (m, 1H, *H*5), 5.17 (d, *J* = 10.2 Hz, 1H, -*NH*), 3.62 (dd, ³*J* = 10.8, 2.8 Hz, 1H, *H*6), 5.39 (dd, ³*J* = 9.4, 2.8 Hz, 1H, *H*7), 5.48 (ddd, ³*J* = 9.4, 4.6, 2.8 Hz, 1H, *H*8), 4.41 (dd, ²*J* = 12.8, ³*J* = 2.8 Hz, 1H, *H*9'), 4.05 – 3.95 (m, 1H, *H*9''), 2.23 – 1.84 (12s, 12x 3H, 12x Ac).

¹³C NMR (151 MHz, Chloroform-*d*) δ 170.98, 170.77, 170.77, 170.54, 170.54, 170.42, 170.35, 170.11, 169.76, 169.71, 169.67, 169.15, 168.05, 101.19, 96.87, 89.17, 75.89, 72.12, 71.50, 70.82, 70.61, 70.09, 70.05, 69.62, 69.42, 67.90, 67.37, 66.88, 62.15, 61.87, 61.68, 53.28, 49.24, 37.51, 23.28, 21.65, 21.11, 21.08, 20.95, 20.91, 20.89, 20.86, 20.84, 20.81, 20.74, 20.64.

RP-HPLC-MS (linear gradient from 0 – 75% eluent B in 30 min at 25 °C): *t*_R = 21.00 min.

MS for C₄₆H₆₃NO₃₀ (ESI, pos.) *m/z*: [M + Na]⁺ calc.: 1132.33; found: 1132.25, [M - C₁₄H₁₉O₁₀ (Glc)]⁺ calc.: 762.25; found: 762.20. HRMS for C₄₆H₆₃NO₃₀ (ESI-TOF) *m/z*: [M + H]⁺ calc.: 1110.3508; found: 1110.3500.

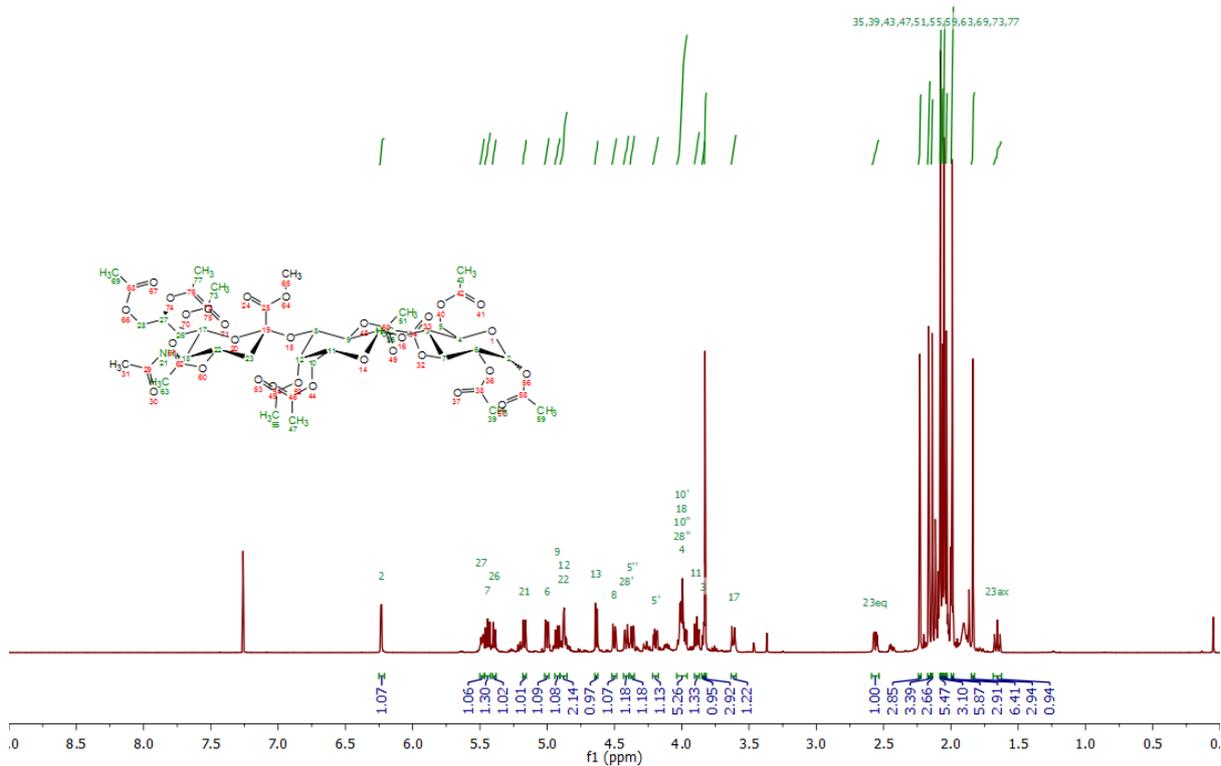


Figure 10: ¹H NMR (600 MHz, Chloroform-d) of compound **e**.

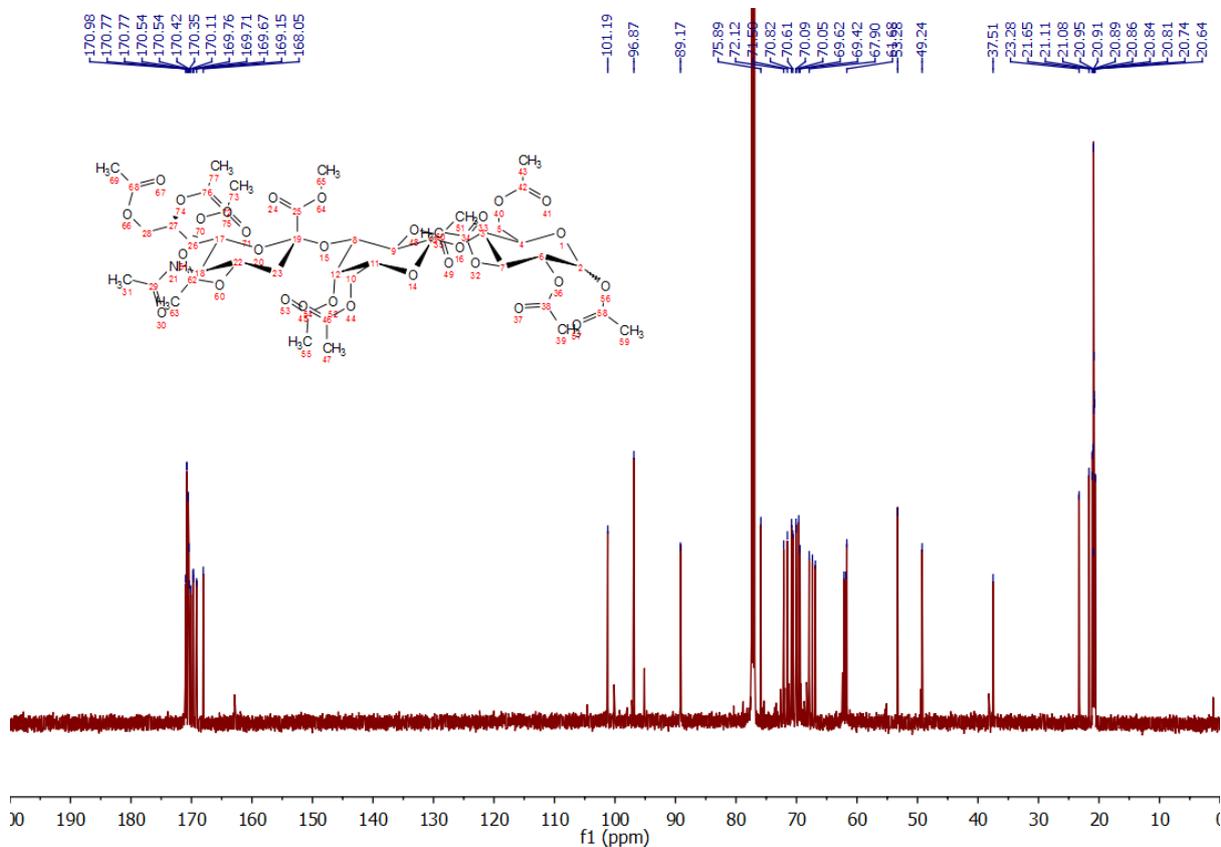


Figure 11: ¹³C NMR (151 MHz, Chloroform-d) of compound **e**.

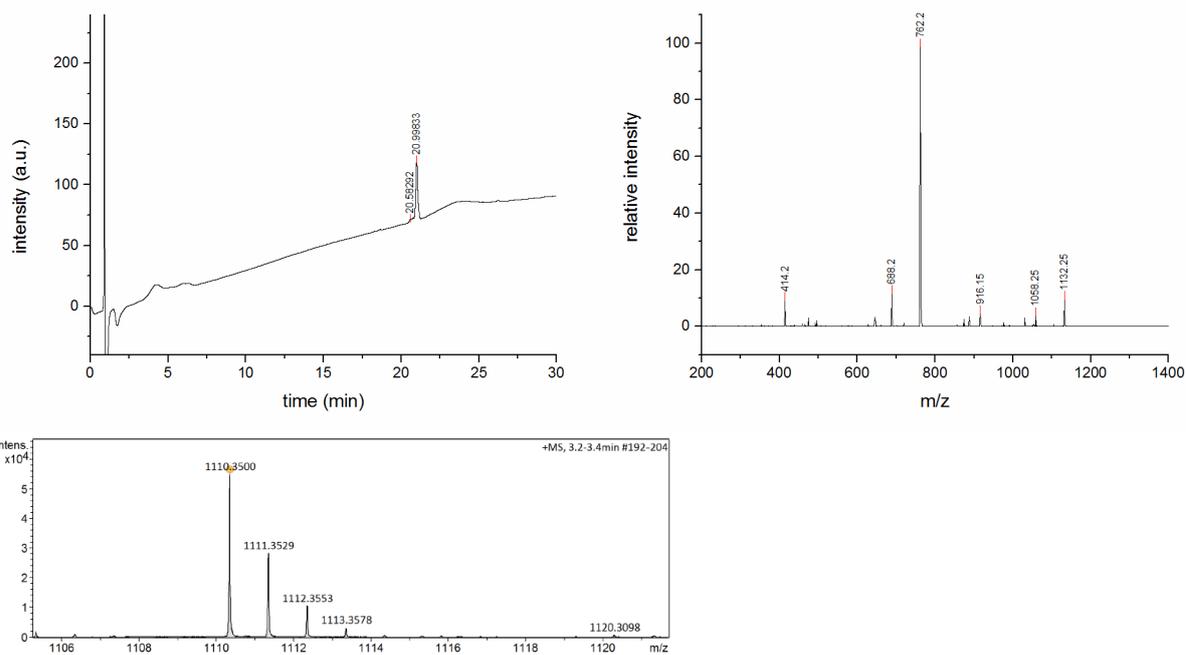


Figure 12: RP-HPLC (linear gradient from 0 - 75% eluent B in 30 min at 25°C), ESI-MS (positive mode), HR-MS (ESI+ Q-TOF, positive mode) of compound e.

Methyl 1-(propargyl)-deca-*O*-acetyl- β -3'Sialyllactoside (5)

(Methyl (4,7,8,9-tetra-*O*-acetyl-*N*-actetyl-neuraminate)yl-(2 \rightarrow 3)-2,4,6-tri-*O*-acetyl- β -galactopyranosyl-(1 \rightarrow 4) -1-(propargyl)-(2,3,6-tri-*O*-acetyl- β -glucopyranoside (5)

^1H NMR (600 MHz, Chloroform-*d*) δ **Propargyl**: 4.33 (d, $^4J = 2.5$ Hz, 2H, *propargyl-CH*₂), 2.45 (t, $^4J = 2.4$ Hz, 0.9H, β -*propargyl-CH*), 2.42 (t, $^4J = 2.4$ Hz, 0.1H, α -*propargyl-CH*); **Glc**: 4.73 (d, $^3J = 7.9$ Hz, 1H, *H1*), 4.94 – 4.89 (m, 1H, *H2*), 5.20 (dd, $^3J = 9.3$, 9.3 Hz, 1H, *H3*), 3.85 – 3.80 (m, 1H, *H4*), 4.04 – 3.95 (m, 1H, *H5*), 4.45 (dd, $^2J = 12.1$, $^3J = 2.1$ Hz, 1H, *H6'*), 4.18 (dd, $^2J = 12.1$, $^3J = 5.3$ Hz, 1H, *H6''*); **Gal**: 4.65 (d, $^3J = 8.0$ Hz, 1H, *H1*), 4.94 – 4.89 (m, 1H, *H2*), 4.50 (dd, $^3J = 10.2$, 3.3 Hz, 1H, *H3*), 4.88 – 4.85 (m, 1H, *H4*), 3.91 – 3.86 (m, 1H, *H5*), 4.04 – 3.95 (m, 4H, *H6'*, *6''*); **Neu5Ac**: 3.83 (s, 3H, *OCH*₃), 1.66 (dd, $^2J = 12.4$ Hz, $^3J = 12.4$ Hz, 1H, *H3ax*), 2.56 (dd, $^2J = 12.6$, $^3J = 4.6$ Hz, 1H, *H3eq*), 4.88 – 4.85 (m, 1H, *H4*), 4.04 – 3.95 (m, 1H, *H5*), 5.15 (d, $^3J = 10.3$ Hz, 1H, *-NH*), 3.66 – 3.60 (m, 1H, *H6*), 5.38 (dd, $^3J = 9.3$, 2.8 Hz, 1H, *H7*), 5.52 (ddd, $^3J = 9.4$, 5.1, 2.8 Hz, 1H, *H8*), 4.40 (dd, $^2J = 12.7$, $^3J = 2.8$ Hz, 1H, *H9'*), 4.04 – 3.95 (m, 1H, *H9''*), 2.23 – 1.84 (11s, 11x 3H, 11x Ac).

^{13}C NMR (151 MHz, Chloroform-*d*) δ 171.00, 170.77, 170.76, 170.61, 170.53, 170.45, 170.37, 169.96, 169.90, 169.75, 169.68, 168.05, 101.06, 98.04, 96.89, 78.29, 76.23, 75.52, 73.35, 72.91, 72.12, 71.52, 71.49, 70.60, 70.02, 69.43, 67.88, 67.42, 66.99, 62.31, 62.24, 61.67, 56.02, 53.28, 49.22, 37.51, 23.29, 21.65, 21.07, 20.96, 20.91, 20.88, 20.82, 20.80, 20.77, 20.75, 20.63.

RP-HPLC-MS (linear gradient from 0 – 75% eluent B in 30 min at 25° C): $t_R = 21.63$ min.

MS for C₄₇H₆₃NO₂₉ (ESI, pos.) m/z : [M + Na⁺]⁺ calc.: 1128.34; found: 1128.15, [M - C₁₄H₁₉O₁₀ (Glc)]⁺ calc.: 762.25; found: 762.20.

HRMS for C₄₇H₆₃NO₂₉ (ESI-TOF) m/z : [M + H⁺]⁺ calc.: 1106.3559; found: 1106.3552.

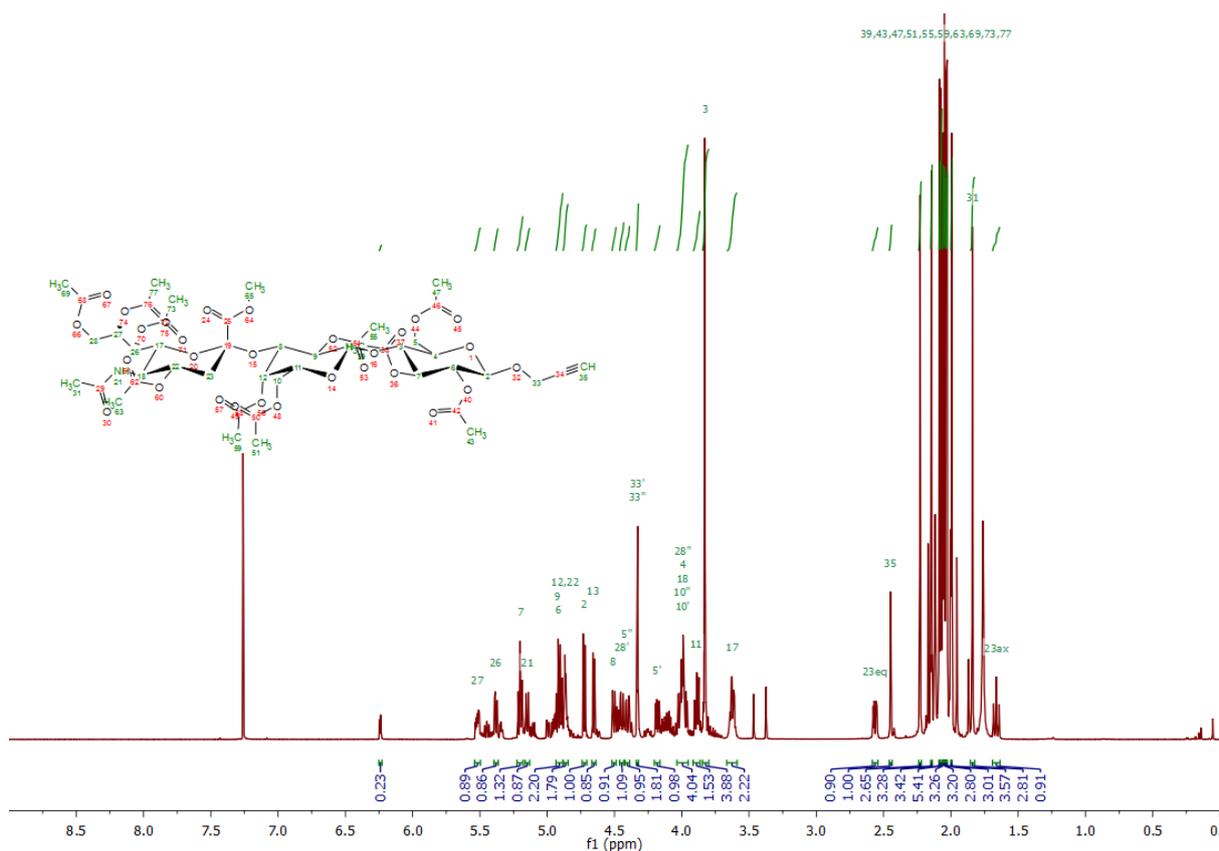


Figure 13: ^1H NMR (600 MHz, Chloroform-*d*) of compound 5.

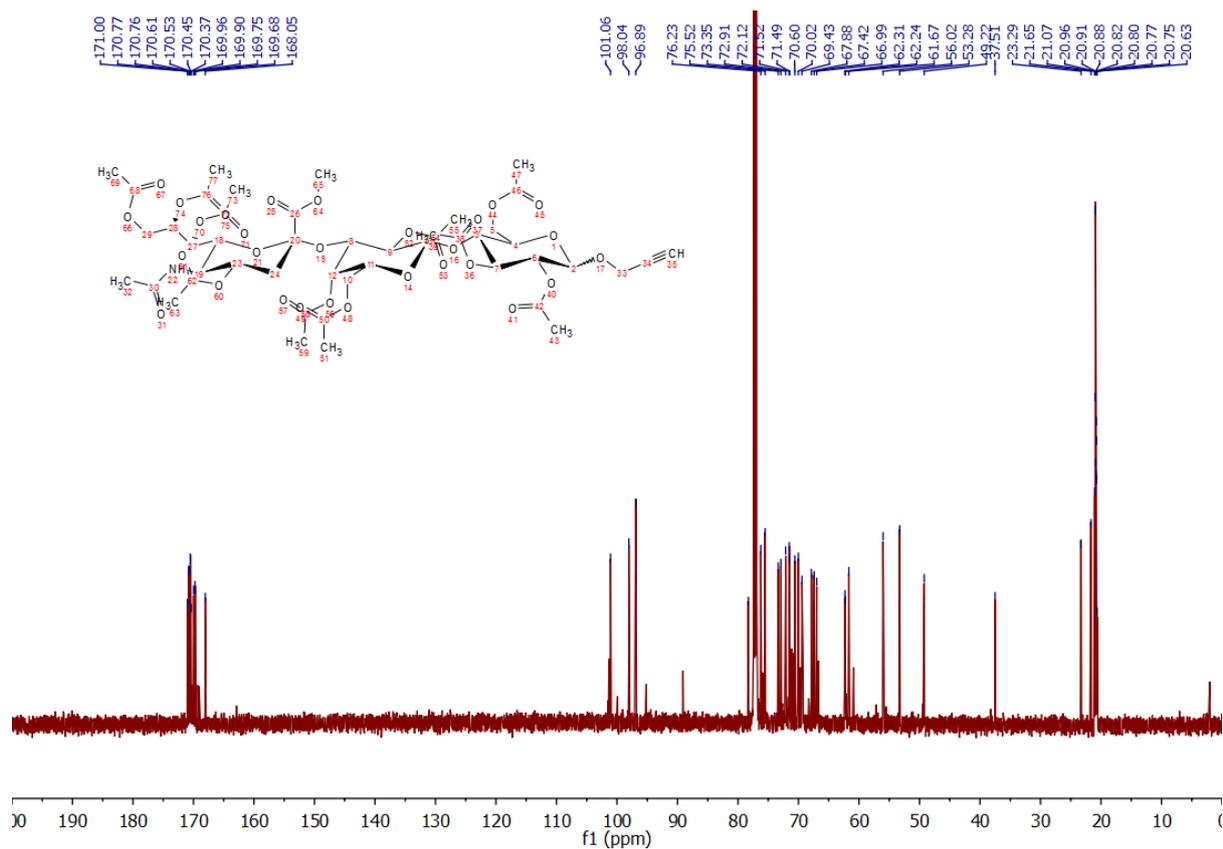


Figure 14: ^{13}C NMR (151 MHz, Chloroform- d) of compound 5.

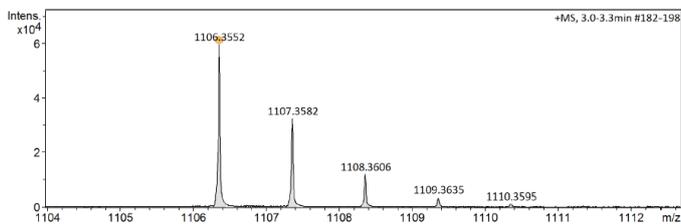
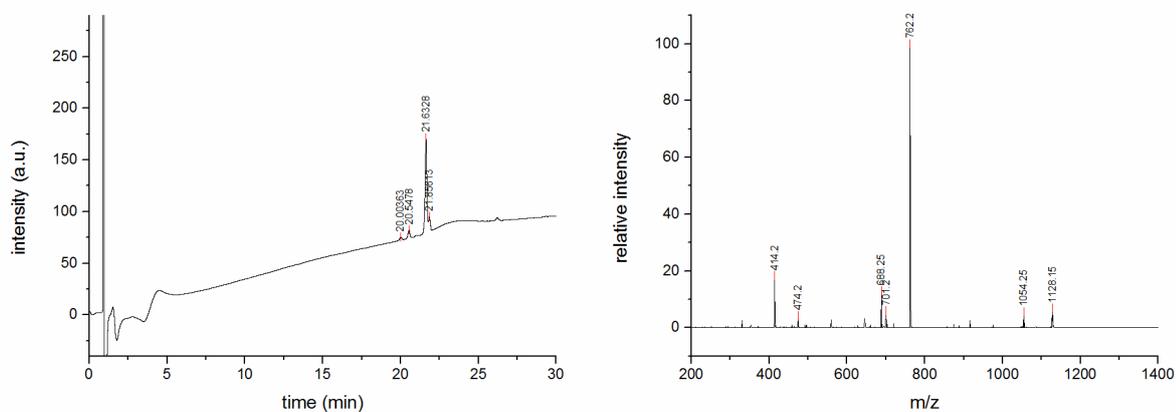
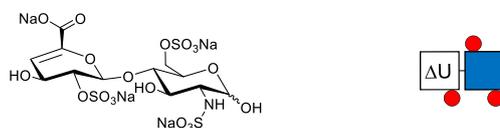


Figure 15: RP-HPLC (linear gradient from 0 - 75% eluent B in 30 min at 25°C), ESI-MS (positive mode), HR-MS (ESI+ Q-TOF, positive mode) of compound 5.

Heparin fragments

Heparin-dp2 (6)



5 g of porcine sodium heparin (MW 15-19 kDa) were dissolved in 50 mM TRIS pH 7.5, 5 mM CaCl₂, 100 mM NaCl, with bovine serum albumin added to 5 mg/mL in a final volume of 40 mL. Heparinase I from *Flavobacterium heparinum* was added to 0.26 I.U. per gram of sodium heparin and the reaction was incubated for several days at 37 °C. The reaction progress was followed at different time points by applying 5-20 μL of the reaction mixture onto analytical gel filtration column (Superdex Peptide 10/300 GL, GE Healthcare) and monitoring the UV absorption at 232 nm. After four days, the cleavage reaction was heat-inactivated and 2 mL of the reaction mixture were filtered at 0.2 μm and loaded onto a self-cast preparative gel filtration column (Bio-Gel P-10 superfine 2.6 cm x 170 cm, Bio-Rad) equilibrated with 50 mM TRIS at pH 7.5, 5 mM CaCl₂, 100 mM NaCl, 0.02 (w/v) % sodium azide. The flow rate was set to 0.2 mL/min, the absorbance at 232 nm was monitored, and 5 mL fractions were collected. Dp2-containing fractions were pooled and freeze-dried. For desalting, the freeze-dried material was dissolved in deionized water, filtered and applied to a HiPrep Desalting 26/10 column (Sephadex G-25 superfine resin, GE Healthcare) using deionized water as running buffer and monitoring the absorbance at 280 nm. Both chromatography steps were conducted at 4 °C. Desalted dp2 was again freeze-dried and stored at -20 °C. Approximately 210 mg (326 μmol, 4.2%, residual sodium chloride) of the Heparin-dp2 fragment were obtained in a purity greater than 99% (SAX-HPLC)

¹H NMR (600 MHz, Deuterium Oxide) δ 5.99 (dd, ³J = 4.6, ⁴J = 1.2 Hz, 1H, ΔUA-H4), 5.54 (dd, ³J = 3.6 Hz, ⁴J = 1.1 Hz, 1H, ΔUA-H1), 5.47 (d, ³J = 3.6 Hz, 1H, GlcN-H1), 4.63 (ddd, ³J = 3.1, ³J = 2.9, ⁴J = 1.3 Hz, 1H, ΔUA-H2), 4.41 – 4.28 (m, 2H, ΔUA-H3, GlcN-H6'), 4.24 (dd, ²J = 11.2, ³J = 2.2 Hz, 1H, GlcN-H6''), 4.18 (ddd, ³J = 10.2, 3.7, 2.0 Hz, 1H, GlcN-H5), 3.89 (dd, ³J = 9.9, 8.6 Hz, GlcN-H4), 3.81 – 3.75 (m, 1H, GlcN-H3), 3.30 (dd, ³J = 10.4, 3.6 Hz, 1H, GlcN-H2). ¹³C NMR (151 MHz, ACN-d₃) δ 168.15, 143.86, 105.55, 96.00, 90.19, 77.67, 74.04, 68.13, 67.07, 65.74, 62.41, 56.81.

SAX – HPLC (isocratic elution in 40 mM Na₂HPO₄, 25 mM KH₂PO₄ in Milli-Q water in 20 min at 25 °C): t_R = 9.93 min. Determined purity: > 99%.

MS for C₁₂H₁₅NNa₄O₁₉S₃ (ESI, neg.) m/z: [M - 2Na⁺ + H⁺]⁻ calc.: 619.93; found 619.90, [M - 3Na⁺ + 2H⁺]⁻ calc.: 597.95; found 597.85, [M - 4Na⁺ + 3H⁺]⁻ calc.: 575.96; found 575.85, [M - 3Na⁺ + 2H⁺ - SO₃]⁻ calc.: 517.99; found 517.90, [M - 4Na⁺ + 3H⁺ - SO₃]⁻ calc.: 496.01; found 496.00. IR

IR (ATR) $\tilde{\nu}_{\max}$: 3185 (s), 2384 (m), 1610 (m), 1402 (m), 1222 (s), 1040 (s), 994 (s), 583 (s).

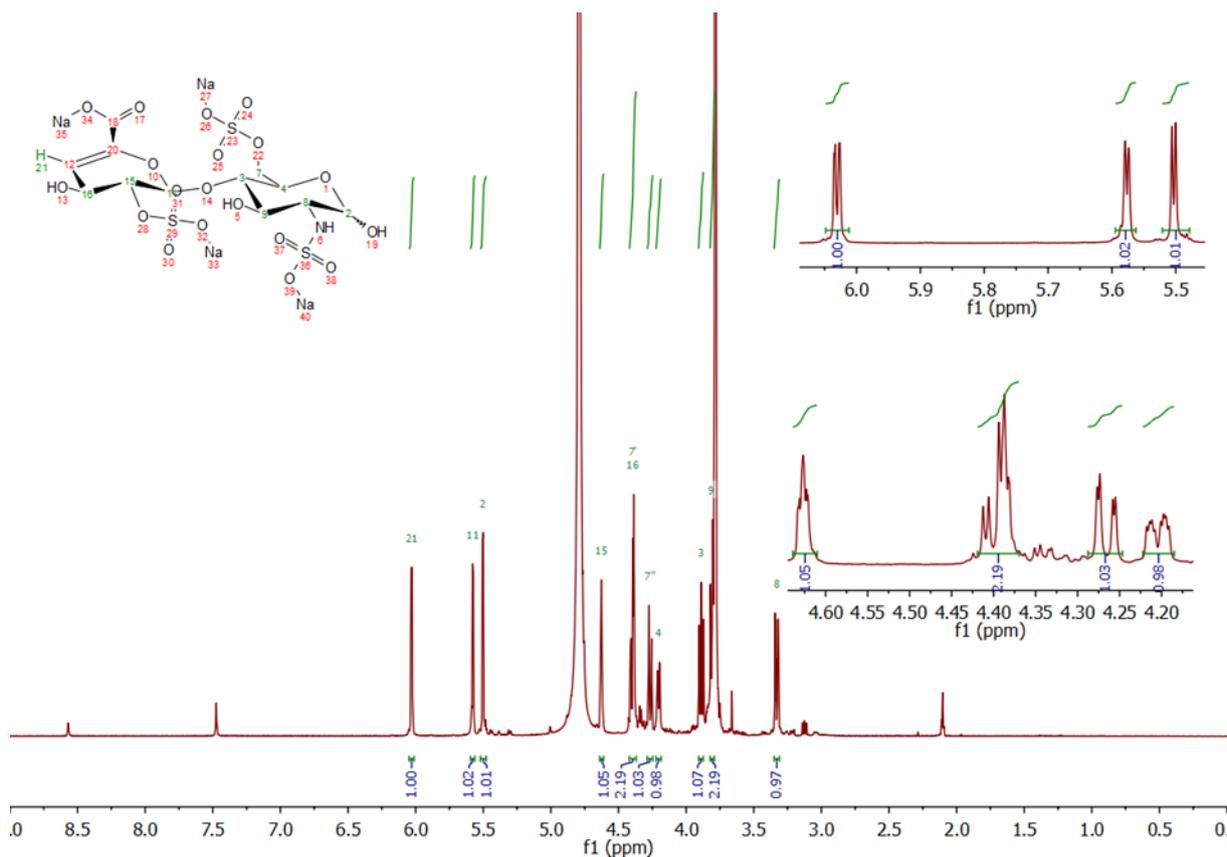


Figure 16: ^1H NMR (600 MHz, Deuterium oxide) of compound **Heparin-dp2**.

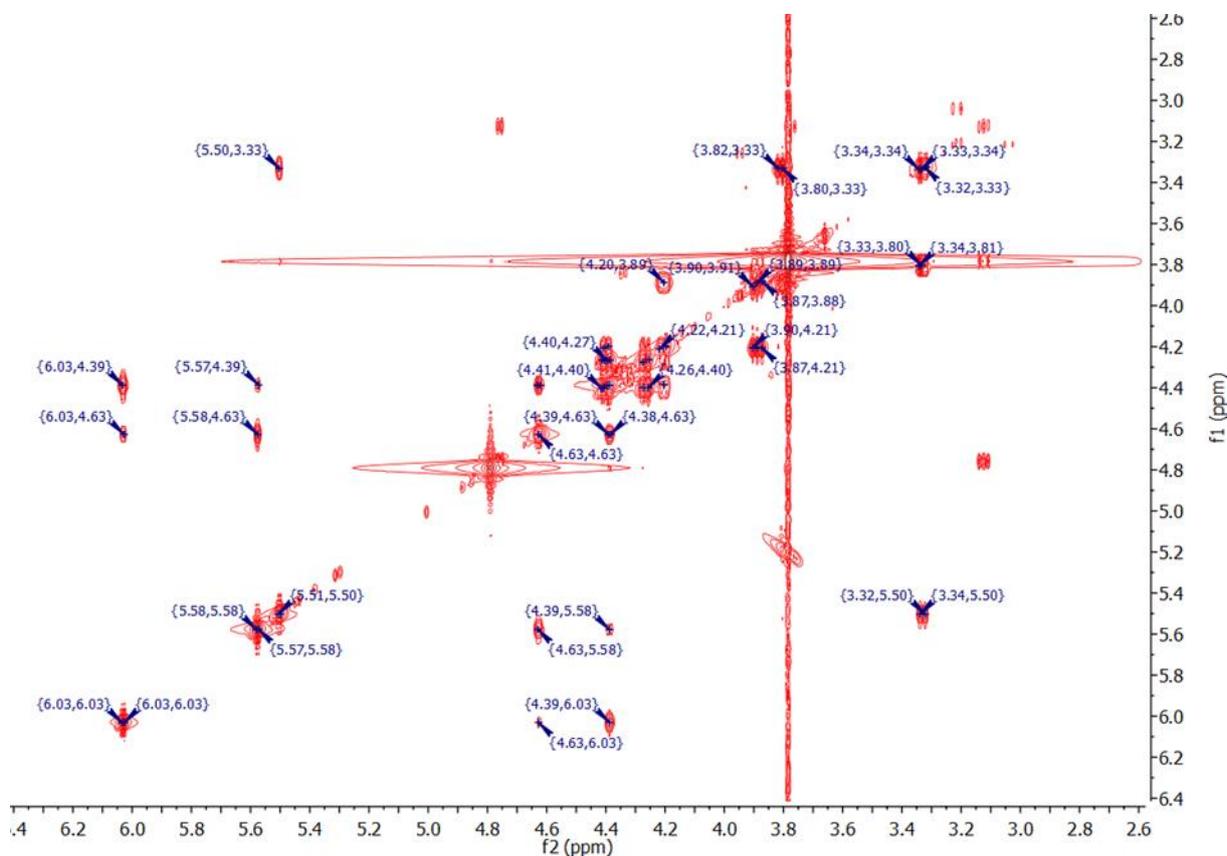


Figure 17: ^1H COSY NMR (600 MHz, Deuterium oxide) of compound **Heparin-dp2**.

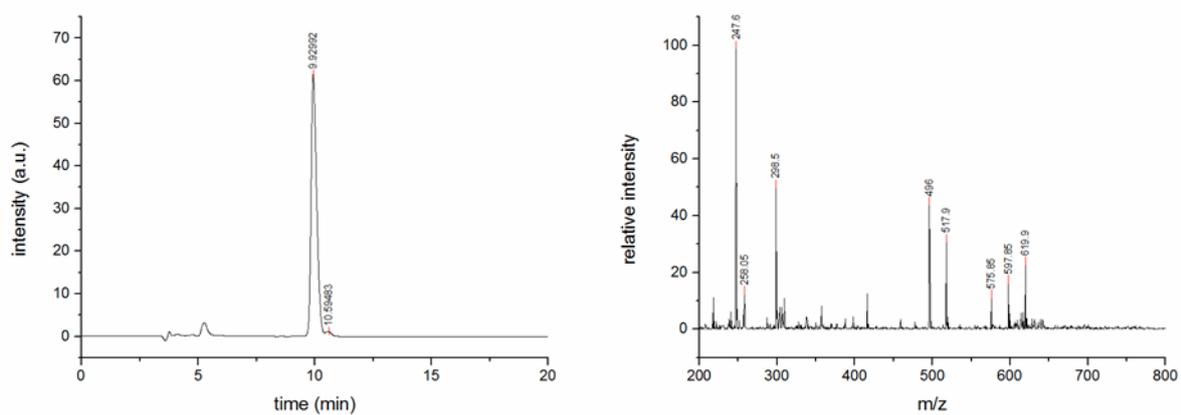
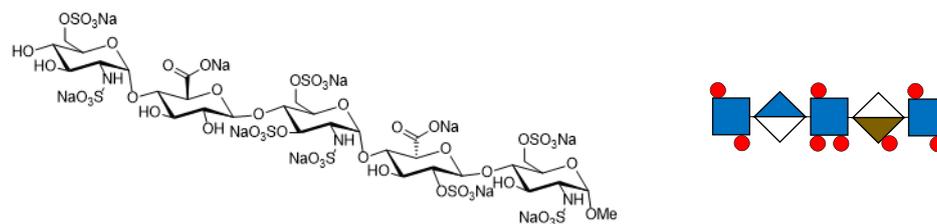


Figure 18: SAX-HPLC (isocratic elution in 40 mM Na_2HPO_4 , 25 mM KH_2PO_4 in Milli-Q water in 20 min at 25° C), ESI-MS (negative mode) of compound **03**.

Fondaparinux[®]-sodium



Fondaparinux-sodium was purchased as Arixtra[®] at a concentration of 10 mg / 0.8 mL from the Aspen Pharma Trading Limited as ready-to-use syringes. The content of 10 syringes (each of them containing 10 mg Fondaparinux-sodium in 0.8 mL of sodium chloride solution) was dialyzed by the use of a Spectra/Por[®] Float-A-Lyzer[®] G2 10 mL unit with a MWCO of 0.1 – 0.5 kDa in order to remove excess of sodium chloride. 98.8 mg (57.1 μmol, 99%) of Fondaparinux were isolated and further analysed.

¹H NMR (600 MHz, Deuterium Oxide) δ 5.67 (d, ³J = 3.8 Hz, 1H, A₅^{nr}-H1), 5.54 (d, ³J = 3.5 Hz, 1H, A₅^{*}-H1), 5.23 (d, ³J = 3.8 Hz, 1H, I₅-H1), 5.06 (d, ³J = 3.6 Hz, 1H, A₅^r-H1), 4.77 – 4.76 (m, 1H, I₅-H5), 4.67 (d, ³J = 7.9 Hz, 1H, G-H1), 4.53 (d, ³J = 10.0 Hz, 1H, A₅^{*}-H3), 4.45 – 4.31 (m, 6H, A₅^{nr}-H6', A₅^{*}-H6', 6'', I₅-H2, A₅^r-H6', 6''), 4.24 – 4.16 (m, 4H, A₅^{nr}-H6'', A₅^{*}-H5, I₅-H3, 4), 4.05 – 3.97 (m, 2H, A₅^{*}-H4, A₅^r-H5), 3.96 – 3.84 (m, 3H, A₅^{nr}-H5, G-H3, 4), 3.81 (m, 2H, G-H5, A₅^r-H4), 3.69 (dd, ³J = 10.6, 9.0 Hz, 1H, A₅^r-H3), 3.69 – 3.58 (m, 2H, A₅^{nr}-H3, 4), 3.49 (dd, ³J = 10.7, 3.4 Hz, 1H, A₅^{*}-H2), 3.46 (s, 4H, G-H2, -OMe), 3.31 (2dd, ³J = 14.0, 10.3, 3.7 Hz, 2H, A₅^{nr}-H2, A₅^r-H2).

MS for C₃₁H₄₃N₃Na₁₀O₄₉S₈ (ESI, neg.) m/z: [M - 2Na⁺]²⁻ calc.: 840.40; found 840.30, [M - 3Na⁺ + H⁺]²⁻ calc.: 829.41; found 829.35, [M - 4Na⁺ + 2H⁺]²⁻ calc.: 818.41; found 818.40, [M - 5Na⁺ + 3H⁺]²⁻ calc.: 807.42; found 807.55, [M - 6Na⁺ + 4H⁺]²⁻ calc.: 796.43; found 796.40, [M - 7Na⁺ + 5H⁺]²⁻ calc.: 785.44; found 785.6.

IR (ATR) $\tilde{\nu}_{\max}$: 3413 (br), 1615 (m), 1419 (m), 1222 (s), 1097 (s), 992 (s), 817 (m), 580 (s).

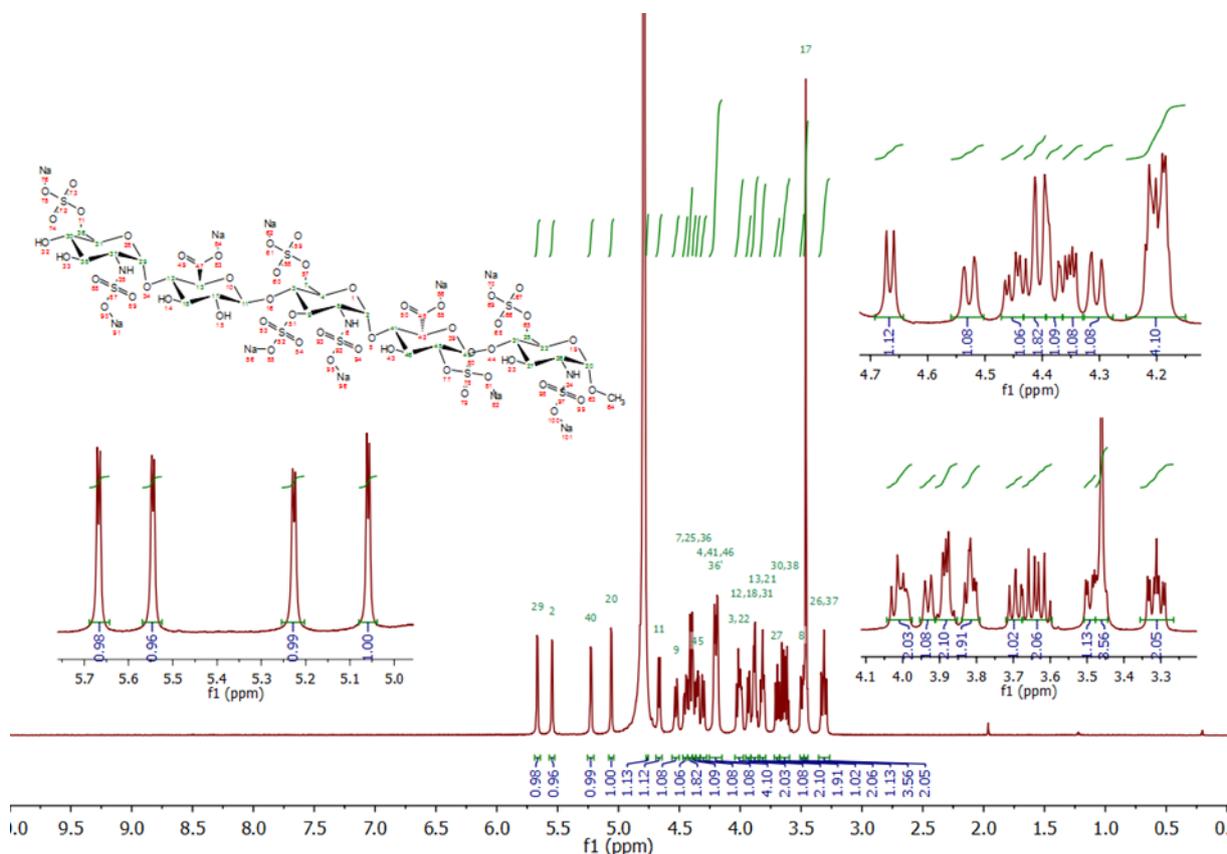


Figure 19: ¹H NMR (600 MHz, Deuterium oxide) of compound **Fondaparinux[®] sodium**.

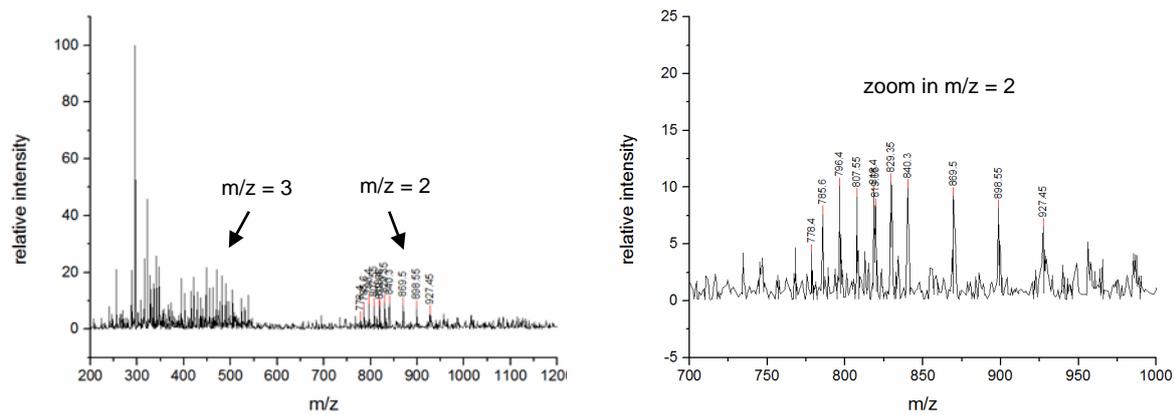
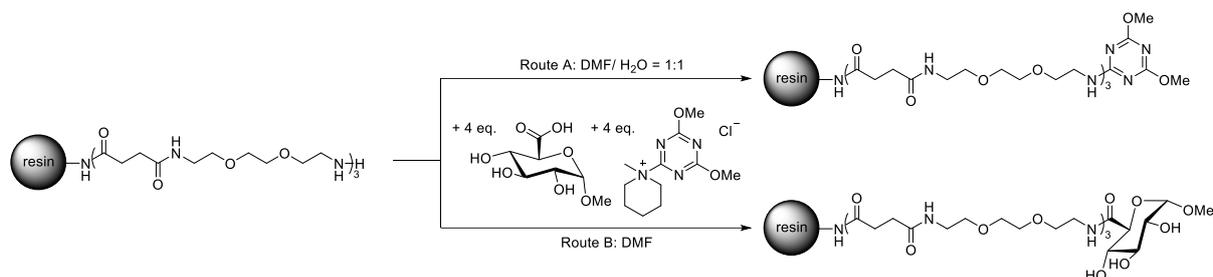


Figure 20: ESI-MS (negative mode) of compound **Fondaparinux®-sodium**. The $m/z = 2$ area was analysed.

Solid phase synthesis approach for coupling of unprotected glucuronic acid



Scheme 3: Synthesis scheme of the DMTMM mediated coupling of unprotected GlcA to a resin-bound oligomer.

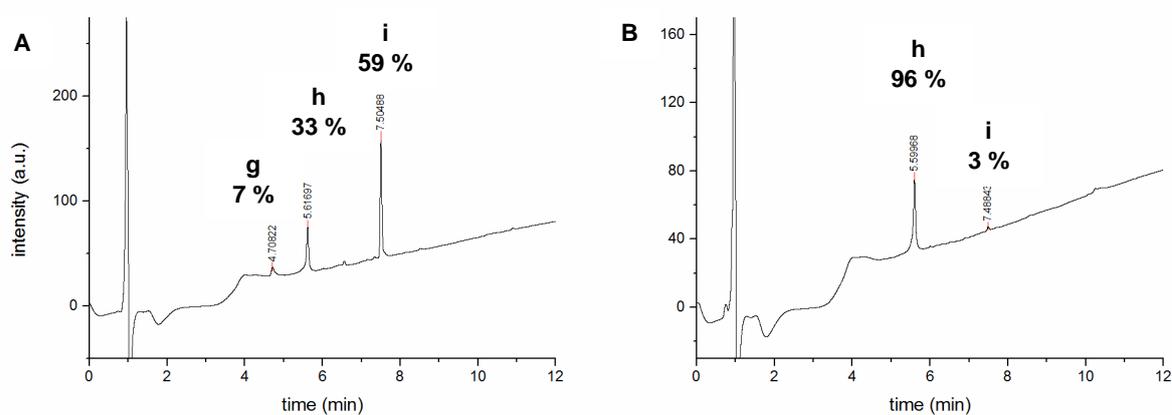
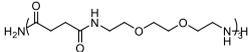
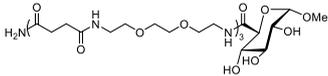
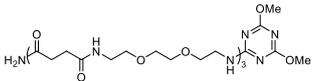


Figure 21: RP-HPLC (linear gradient from 0 – 50% eluent B in 12 min at 25° C) of the investigated Oligomer-GlcA Amide conjugates.

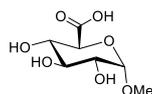
The resin-bound oligomeric sequence (EDS)₃ was synthesized in a 40 μmol batch size according the standard solid phase synthesis protocol using Tentagel® S RAM for test purposes. Microcleavage showed a purity of 98% (data not shown) before further conversion. The test batch was split into two, then methyl-α-D-glucuronic acid (**f**) was coupled using either a 1:1 mixture of DMF and water (**Route A**) or using pure DMF (**Route B**). Four equivalents of the carbohydrate and the coupling agent were used and double coupling was performed. GlcA was preactivated for 60 min with DMTMM in the appropriate solvent before the mixture was given to the resin-bound amine and shaken for another 60 min. Microcleavage was performed and analyzed via RP-HPLC-MS. The use of a 1:1 mixture of DMF and water led primarily to the formation of the amine-triazine product **i**, whereby the choice of pure DMF as solvent led to nearly full conversion to the desired oligoamidoamine-GlcA adduct **h**.

Table 1. Observed products after the conversion of the resin-bound oligomer -(EDS)₃- with methyl- α -D-glucuronic acid (**f**) using a 1:1 mixture of DMF and water (**A**) or pure DMF (**B**)

Product ^{a)} [#]	Structure	Retention time [min]	MW ^{b)} [Da]	Product Composition Route A ^{c)} [%]	Product Composition Route B ^{c)} [%]
g		4.71	calc.: 708.41, found: 708.40.	7	0
h		5.61	calc.: 898.46, found: 898.50.	33	96
i		7.49	calc.: 847.45, found: 847.40.	59	3

^{a)} Products were identified via RP-HPLC-MS; ^{b)} Monoisotopic mass detected as $[M + H^+]^+$; ^{c)} Based on the absorption integrals after microcleavage, test batch was not isolated quantitatively, non-normalized data.

Methyl- α -D-glucuronic acid (f)



Methyl- α -D-glucuronic acid (f) was synthesized according the method described by Adorjan *et al.*⁵

5.00 g (25.75 mmol) of methyl α -D-glucopyranoside, 1.32 g (12.9 mmol) of sodium bromide and 40 mg (0.256 mmol) of TEMPO ((2,2,6,6-tetramethylpiperidinyl) oxyl radical) were dissolved in 250 ml distilled water and cooled to 0°C. Sodium hypochloride solution (60 ml, 13%, 105 mmol) was added dropwise while keeping the pH within 10-11 with sodium hydroxide solution (0.5 M). The reaction mixture was stirred overnight. Excess of sodium hypochloride was deactivated by addition of methanol (50 ml) before the solvent was removed under diminished pressure to half volume. Salts (sodium chloride, sodium bromide), which crystallized by cooling were filtered off. The yellowish supernatant was pipetted off and adjusted to pH 3 with concentrated hydrochloric acid. Hereafter it was precipitated in diethyl ether to remove remaining salts. The solvent was removed under reduced pressure and the product was lyophilized from 60 ml distilled water. 2.65 g (11.55 mmol, 45%) of the product f were obtained as yellow powder.

^1H NMR (600 MHz, Deuterium Oxide) δ 4.89 (d, $^3J = 3.7$ Hz, 1H, H1), 3.96 (d, $^3J = 10.1$ Hz, 1H, H5), 3.75 (dd, $^3J = 9.4, 9.4$, 1H, H3), 3.67 (dd, $^3J = 3.8, 9.8$, 1H, H2), 3.56 (dd, $^3J = 10.1, 9.0$, 1H, H4), 3.49 (s, 3H, -OCH₃) ppm.

^{13}C NMR (151 MHz, D₂O) δ 176.69 (Carbonyl-C), 99.37 (Acetal-C), 72.94, 72.03, 71.99, 71.09, 55.30 (CH₃).

MS for C₇H₁₂O₇ (ESI, pos.) m/z: [2M+Na]⁺: calc.: 439.10; found: 439.00, [M + Na]⁺: calc.: 231.08; found: 231.00.

IR (ATR) $\tilde{\nu}_{\text{max}}$: 3270 (b), 2912 (b), 2862 (w), 1604 (v, s), 1417 (s), 1038 (s).

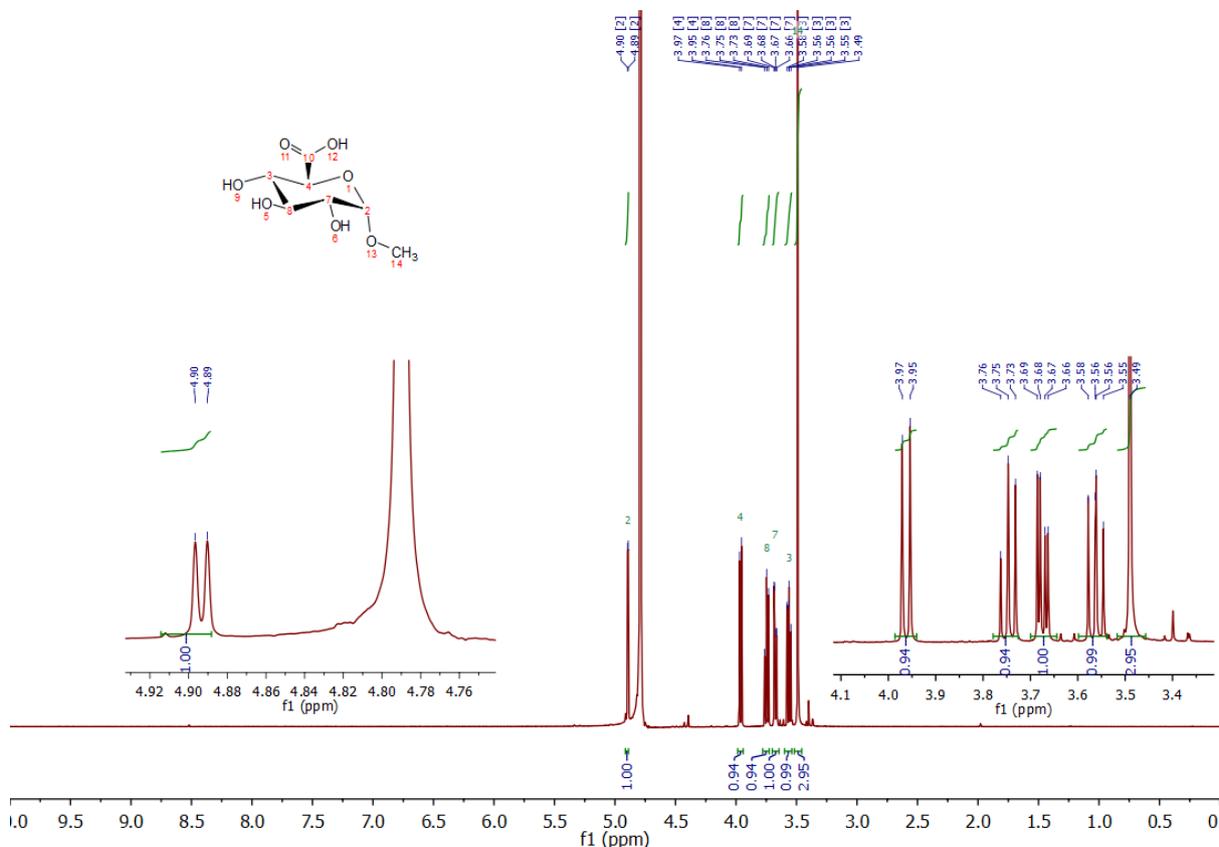


Figure 22: ^1H NMR (600 MHz, Deuterium oxide) of compound f.

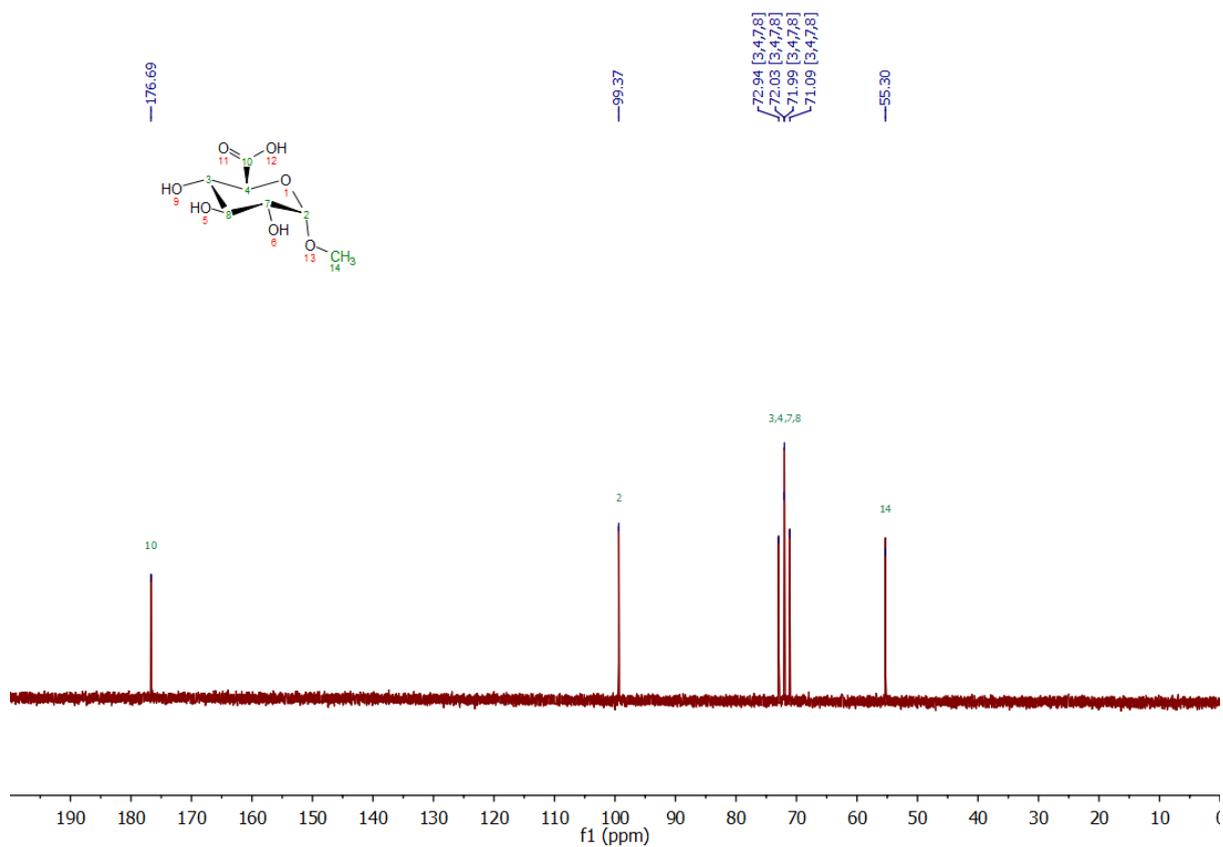


Figure 23: ^{13}C NMR (151 MHz, Deuterium oxide) of compound *f*.

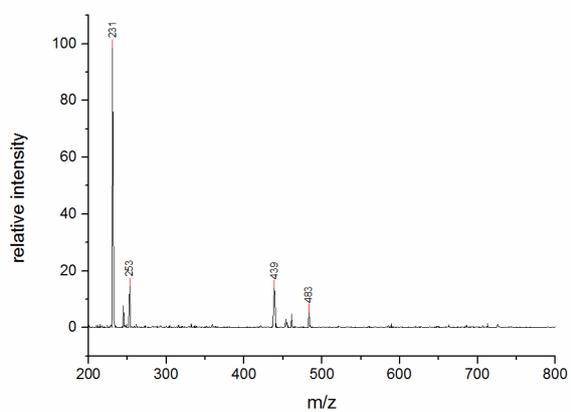
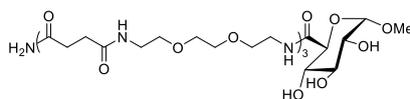


Figure 24: ESI-MS (positive mode) of compound *f*.

(EDS)₃-GlcA amide (h)



(EDS)₃-GlcA amide (h) was synthesized in a 200 μmol scale. Methyl-α-D-glucuronic acid was coupled twice in a four-fold excess with DMTMM as coupling agent to the resin-bound oligomeric sequence -(EDS)₃. The carbohydrate was preactivated for 60 min with DMTMM before the suspension was given to the resin-bound amine and shaken for another 60 min. The compound was purified by ion exchange. For solid phase synthesis and workup protocols see general methods. 72.82 mg (81.1 μmol, 41%) of a white and foamy solid were obtained.

¹H NMR (600 MHz, Deuterium Oxide) δ 4.86 (d, ³J = 3.7 Hz, 1H, *GlcA-H1*), 4.05 (d, ³J = 9.9 Hz, 1H, *GlcA-H5*), 3.69 – 3.67 (m, 15H, *GlcA-H2*; *GlcA-H3*; 3x -O-CH₂-CH₂-O-), 3.63 – 3.60 (m, 12H, 6x O-CH₂), 3.57 (dd, ³J = 9.08, 9.94 Hz, 1H, *GlcA-H4*), 3.47 (m, 2H, CH₂-N-GlcA), 3.42 (s, 3H, *GlcA-OCH₃*), 3.39 (m, 10H, 5x N-CH₂), 2.56 – 2.53 (m, 12H, 6x succinyl CH₂)

RP-HPLC-MS (linear gradient from 0 – 50 % eluent B in 30 min at 25° C): t_R = 9.87 min. Determined purity: 96%.

MS for C₄₇H₆₃NO₂₉ (ESI, pos.) *m/z*: [M + Na]⁺ calc.: 920.44; found: 920.40, [M + H]⁺ calc.: 898.46; found: 898.35, [M + 2H]²⁺ calc.: 449.73; found: 449.80.

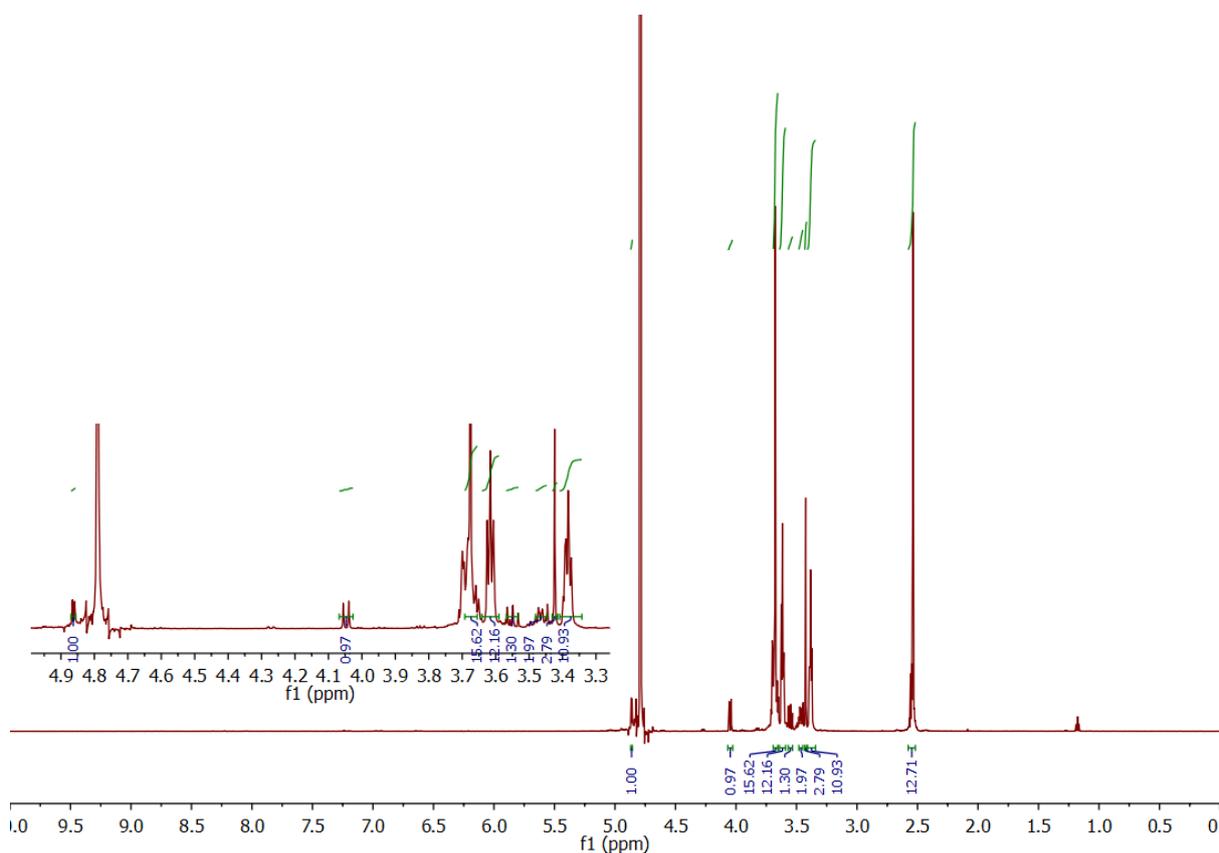


Figure 25: ¹H NMR (600 MHz, Deuterium oxide) of compound **h**.

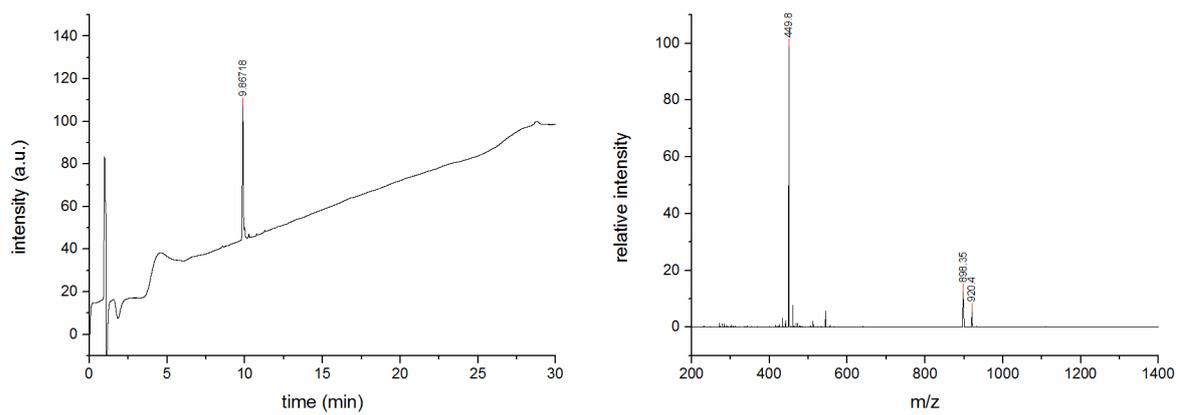


Figure 26: RP-HPLC (linear gradient from 0 - 50% eluent B in 30 min at 25°C), ESI-MS (positive mode) of compound **h**.

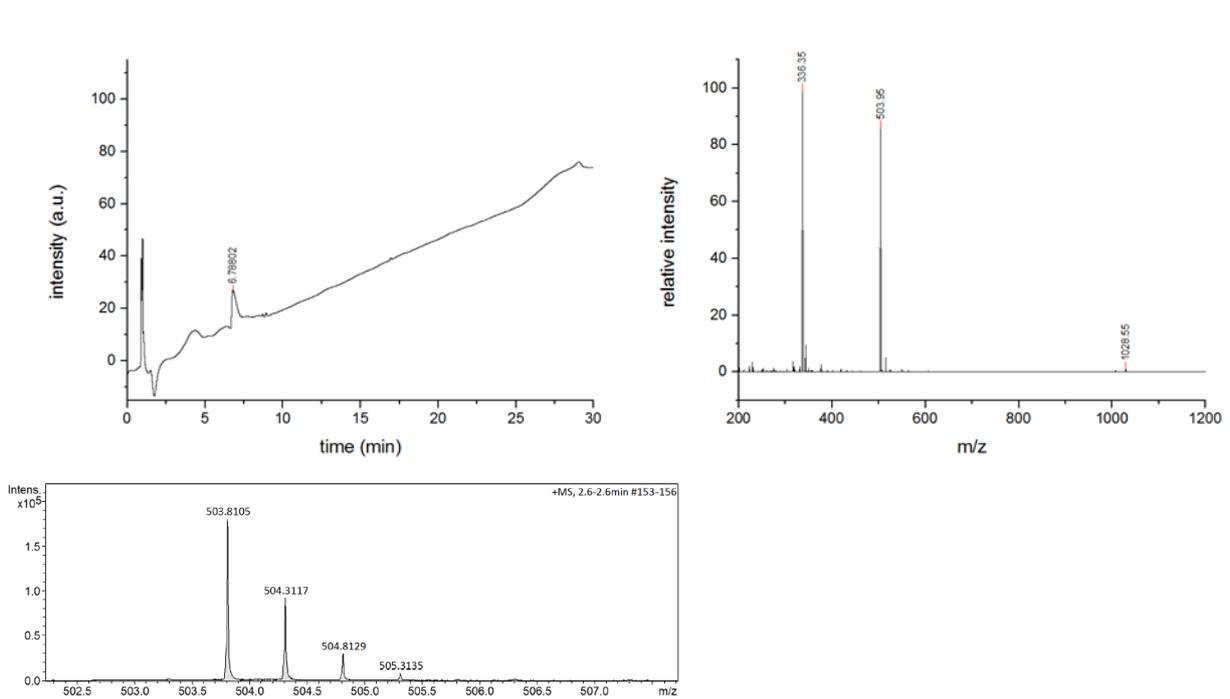
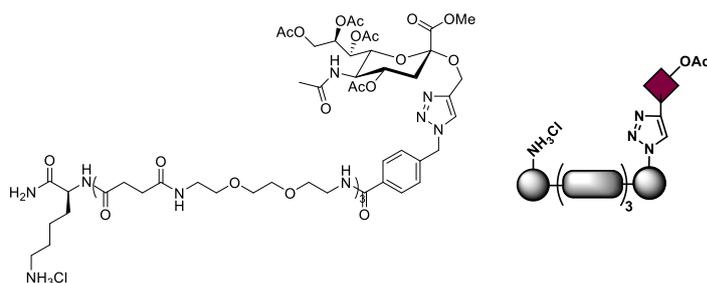


Figure 28: RP-HPLC (linear gradient from 0 - 50% eluent B in 30 min at 25°C), ESI-MS (positive mode), HR-MS (ESI+ Q-TOF, positive mode) of compound **O1**.

N_{ϵ} (HCl)Lys(1)-4-Neu5Ac (protected) (O3)



N_{ϵ} (HCl)Lys(1)-4-Neu5Ac was synthesized in a 100 μmol scale. 38.9 mg (24.9 μmol , 25%) of a white and foamy solid were obtained after purification by preparative HPLC and ion exchange. For solid phase synthesis and workup protocols see general methods.

^1H NMR (600 MHz, Deuterium Oxide) δ 8.01 (s, 1H, *triazole-H*), 7.79 (d, $^3J = 8.3$ Hz, 2H, *aryl-H*), 7.42 (d, $^3J = 8.3$ Hz, 2H, *aryl-H*), 5.73 – 5.64 (m, 2H, *aryl-CH₂-aryl*), 5.38 (dd, $^3J = 8.9, 1.8$ Hz, 1H, *Neu5Ac-H9'*), 5.35 (ddd, $^3J = 8.9, 4.5, 2.5$ Hz, 1H, *Neu5Ac-H8*), 4.92 (ddd, $^3J = 11.8, 10.2, 4.8$ Hz, 1H, *Neu5Ac-H4*), 4.88 (d, $^2J = 13.0$ Hz, 1H, *propargyl-H*), 4.73 (d, $^2J = 12.9$ Hz, 1H, *propargyl-H*), 4.28 – 4.23 (m, 3H, *Lys-CH, Neu5Ac-H6, 9''*), 4.09 (dd, $^3J = 12.7, 4.5$ Hz, 1H, *Neu5Ac-H7*), 3.94 (dd, $^3J = 10.5$ Hz, 1H, *Neu5Ac-H5*), 3.76 – 3.70 (m, 7H, *Neu5Ac-OCH₃, 2x O-CH₂*), 3.68 (s, 6H, 3x *O-CH₂*), 3.66 (s, 4H, 2x *O-CH₂*), 3.63 – 3.57 (m, 12H, 5x *O-CH₂, N-CH₂*), 3.41 – 3.35 (m, 8H, 4x *N-CH₂*), 3.32 (t, $^3J = 5.4$ Hz, 2H, *N-CH₂*), 3.02 (td, $^3J = 7.8, ^2J = 1.7$ Hz, 2H, *Lys-N-CH₂*), 2.73 (dd, $^2J = 13.0, ^3J = 4.8$ Hz, 1H, *Neu5Ac-H3eq.*), 2.63 – 2.45 (m, 12H, 6x *succinyl-CH₂*), 2.20 – 2.04 (4s, 4x 3H, 4x *Neu5Ac-Ac*), 1.97 (dd, $^2J = 13.1, ^3J = 11.7$ Hz, 1H, *Neu5Ac-H3ax.*), 1.93 (s, 3H, *Neu5Ac-Ac*), 1.91 – 1.39 (3m, 6H, 3x *Lys-CH₂*).

RP-HPLC-MS (linear gradient from 0 – 50% eluent B in 30 min at 25° C): $t_R = 16.50$ min. Determined purity: > 99%.

MS for $\text{C}_{67}\text{H}_{106}\text{ClN}_{13}\text{O}_{27}$ (ESI, pos.) m/z : $[\text{M} + 2\text{H}^+ - \text{HCl}]^{2+}$ calc.: 762.87; found: 763.00, $[\text{M} + 3\text{H}^+ - \text{HCl}]^{3+}$ calc.: 508.92; found: 509.00.

HRMS for $\text{C}_{67}\text{H}_{106}\text{ClN}_{13}\text{O}_{27}$ (ESI-TOF) m/z : $[\text{M} + 2\text{H}^+ - \text{HCl}]^{2+}$ calc.: 762.8694; found: 762.8682.

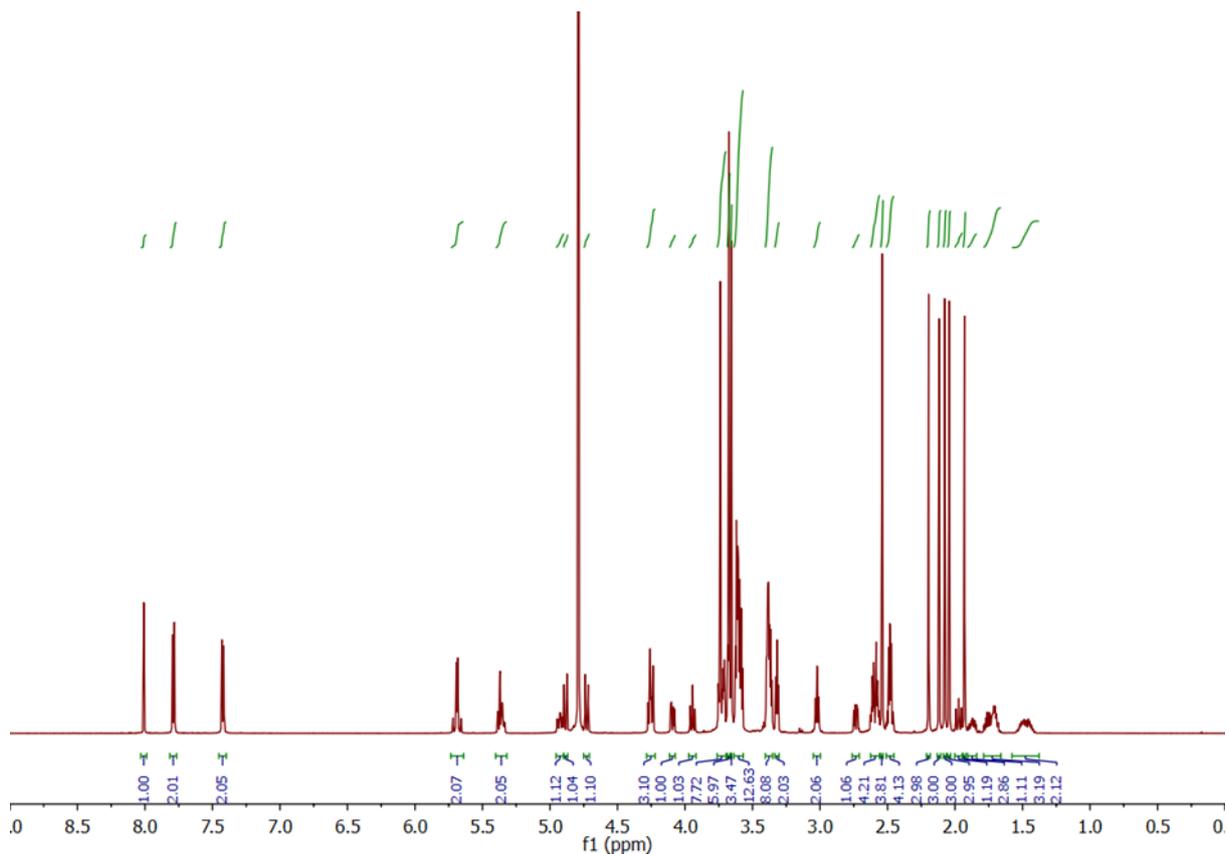


Figure 29: ^1H NMR (600 MHz, Deuterium oxide) of compound **O3**.

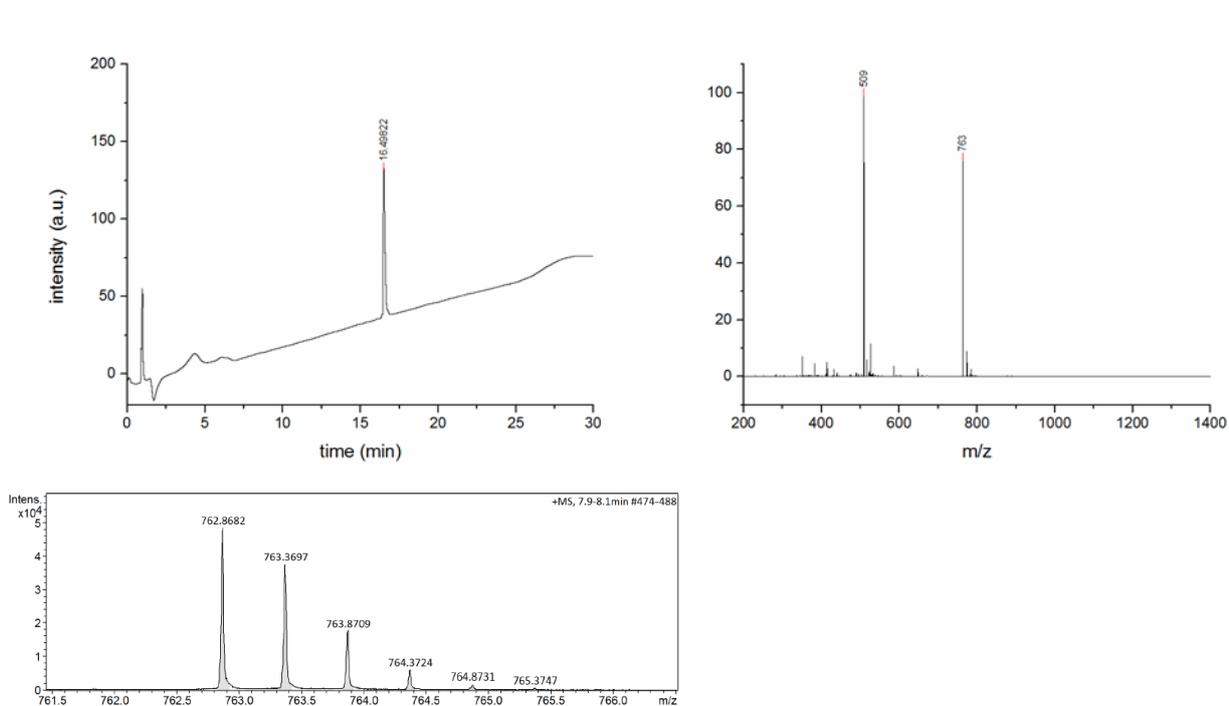
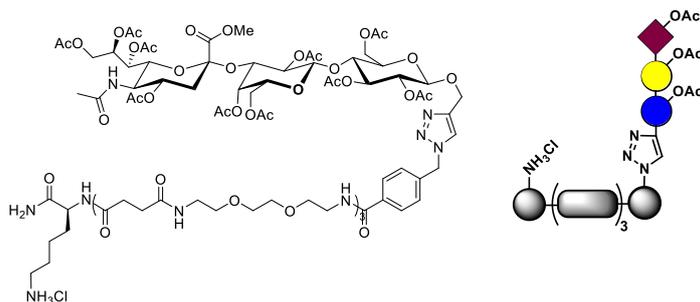


Figure 30: RP-HPLC (linear gradient from 0 - 50% eluent B in 30 min at 25°C), ESI-MS (positive mode), HR-MS (ESI+ Q-TOF, positive mode) of compound **O3**.

N_{ϵ} (HCl)Lys(1)-4-3'Sialyllactose (protected) (O4)



N_{ϵ} (HCl)Lys(1)-4-3'Sialyllactose was synthesized in a 25 μ mol scale. 14.3 mg (6.69 μ mol, 27%) of a white and foamy solid were obtained after purification by preparative HPLC and ion exchange. For solid phase synthesis and workup protocols see general methods.

^1H NMR (600 MHz, Deuterium Oxide) δ 8.11 (s, 1H, *triazole-H*), 7.78 (d, $^3J = 8.2$ Hz, 2H, *aryl-H*), 8.04 (d, $^3J = 8.0$ Hz, 2H, *aryl-H*), 5.70 (s, 2H, *aryl-CH₂-aryl*), 5.52 – 5.44 (m, 2H), 5.42 – 5.22 (m, 1H), 5.19 – 5.08 (m, 1H), 5.01 (d, $^3J = 3.2$ Hz, 1H), 4.96 (ddd, $^3J = 11.9, 10.1, 4.8$ Hz, 1H, *Neu5Ac-H4*), 4.92 (d, $^2J = 13.0$ Hz, 1H, *propargyl-H*), 4.87 – 4.81 (m, 4H), 4.71 (d, $^3J = 8.0$ Hz, 1H, *Gal-H1*), 4.59 (dd, $^3J = 10.2, 3.3$ Hz, 1H, *Gal-H3*), 4.56 – 4.46 (m, 1H), 4.40 (dd, $^2J = 12.9, ^3J = 2.6$ Hz, 1H, *Glc-H6'*), 4.25 (dd, $^2J = 9.2, ^3J = 5.1$ Hz, 1H, *Lys-CH*), 4.22 – 4.09 (m, 4H), 4.06 – 3.99 (m, 1H), 3.96 – 3.91 (m, 1H), 3.90 (s, 3H, *Neu5Ac-OCH₃*), 3.87 (m, 2H), 3.77 – 3.56 (m, 26H, 12x *O-CH₂*, *N-CH₂*), 3.41 – 3.34 (m, 8H, 4x *N-CH₂*), 3.30 (t, $^3J = 5.4$ Hz, 2H, *N-CH₂*), 3.04 – 2.99 (m, 2H, *Lys-N-CH₂*), 2.67 (dd, $^2J = 12.7, ^3J = 4.7$ Hz, 1H, *Neu5Ac-H3eq.*), 2.62 – 2.45 (m, 12H, 6x *succinyl-CH₂*), 2.35 – 1.81 (11s, 11x 3H, 11x Ac), 1.60 (dd, $^2J = 12.3, ^3J = 12.3$ Hz, 1H, *Neu5Ac-H3ax.*), 1.89 – 1.39 (3m, 6H, 3x *Lys-CH₂*).

RP-HPLC-MS (linear gradient from 0 – 50% eluent B in 30 min at 25° C): $t_R = 21.57$ min. Determined purity: 97%.

MS for $\text{C}_{91}\text{H}_{138}\text{ClN}_{13}\text{O}_{43}$ (ESI, pos.) m/z : $[\text{M} + 2\text{H}^+ - \text{HCl}]^{2+}$ calc.: 1050.95; found: 1051.25, $[\text{M} + 3\text{H}^+ - \text{HCl}]^{3+}$ calc.: 700.97; found: 701.25.

HRMS for $\text{C}_{91}\text{H}_{138}\text{ClN}_{13}\text{O}_{43}$ (ESI-TOF) m/z : $[\text{M} + 2\text{H}^+ - \text{HCl}]^{2+}$ calc.: 1050.9539; found: 1050.9531.

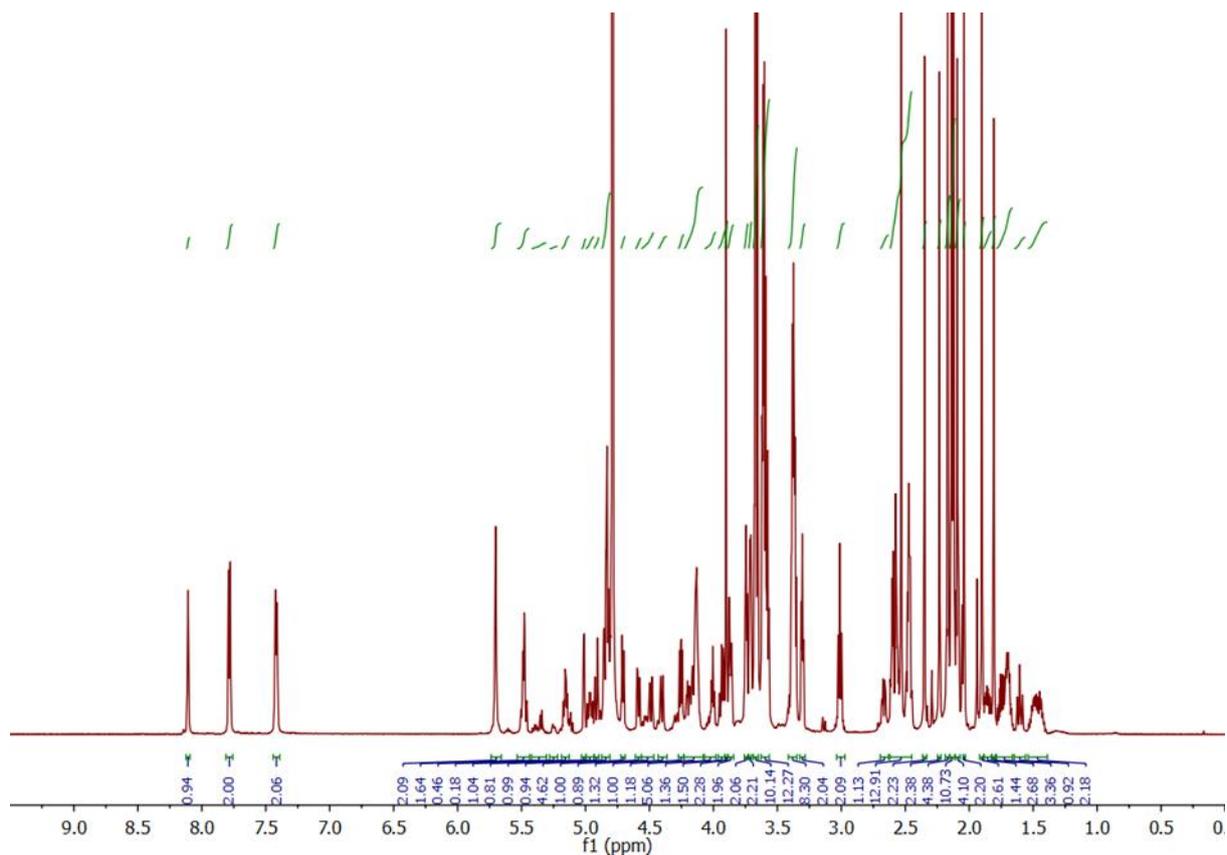


Figure 31: ^1H NMR (600 MHz, Deuterium oxide) of compound **O4**.

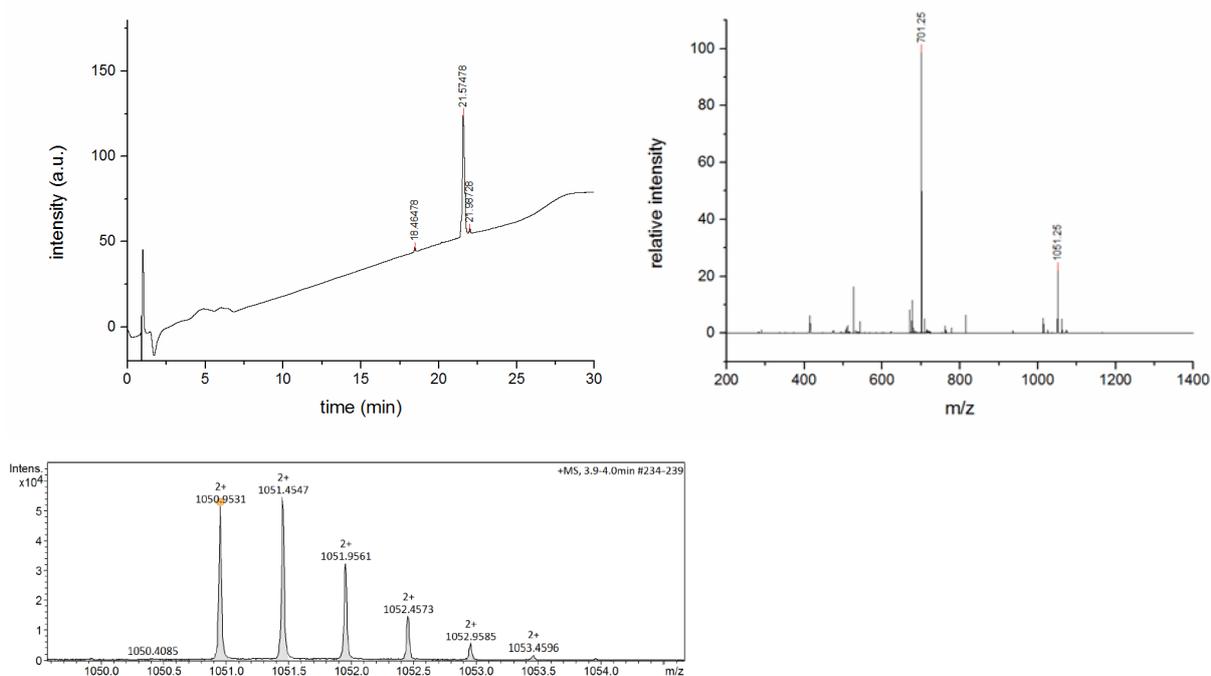
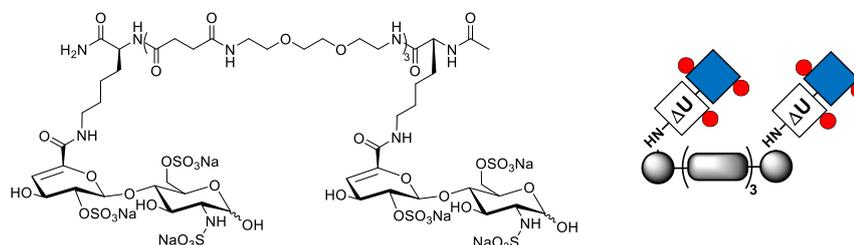


Figure 32: RP-HPLC (linear gradient from 0 - 50% eluent B in 30 min at 25°C), ESI-MS (positive mode), HR-MS (ESI+ Q-TOF, positive mode) of compound **O4**.

Coupling of oligo(amidoamines) and heparin fragments

$N_{\epsilon}(\text{dp}2)\text{Lys}(1,5)\text{-}5$ (O2)



31.9 mg (29.6 μmol) of $N_{\epsilon}(\text{HCl})\text{Lys}(1,5)\text{-}5$, 83.26 mg (125 μmol , 4.2 eq.) of **Heparin-dp2** and 170 mg (614 μmol , 4.9 eq. based on Heparin-dp2) of DMTMM were mixed in a 1 mL glass vial, before 1 mL of a mixture of 0.9 mL dimethylformamide and 0.1 mL phosphate-buffer pH 6.5 were added. The mixture was shaken at room temperature for 24 h, diluted to 5 mL with Milli-Q water and dialyzed using a Vivaspin[®] MWCO 2000 2 ml unit. Separation from mono-substituted product was not quantitative (analysed by HPLC, data not shown) so dialysis was performed again using a MWCO 3000 unit. After ion exchange with Amberlite[®] IR 120 Na^+ and lyophilisation, 13.9 mg (6.16 μmol , 21%) of the desired product were obtained as a white and foamy solid with a purity of 81% (HPLC)-85% (SAX-HPLC). Side products stem from different glucosamine pyranose forms and partial loss and readdition of sulphates during synthesis and workup, either by hydrolysis and condensation or elimination and addition, respectively. Mono-substituted product were removed completely by dialysis.

¹H NMR (600 MHz, Deuterium Oxide) δ 6.00 – 5.94 (m, 2H, 2x $\Delta\text{UA-H}4$), 5.50 – 5.43 (m, 2H, 2x $\Delta\text{UA-H}1$), 5.36 – 5.31 (m, 2H, 2x $\text{GlcN-H}1$), 4.52 – 4.47 (m, 2H, 2x $\Delta\text{UA-H}2$), 4.30 – 3.92 (m, 10H, 2x ($\Delta\text{UA-H}3$, $\text{GlcN-H}5$, 6', 6''), 2x Lys-CH), 3.79 – 3.71 (m, 2H, 2x $\text{GlcN-H}4$), 3.66 – 3.62 (m, 2H, 2x $\text{GlcN-H}3$), 3.59 – 3.56 (m, 12H, 3x $\text{O-CH}_2\text{-CH}_2\text{-O}$), 3.53 – 3.49 (m, 12H, 6x $\text{O-CH}_2\text{-CH}_2\text{-N}$), 3.39 – 3.13 (m, 17H, 6x $\text{O-CH}_2\text{-CH}_2\text{-N}$, 1.5x Lys-N-CH_2), 2.93 – 2.84 (m, 1H, 0.5x Lys-N-CH_2), 2.52 – 2.41 (m, 12H, 6x succinyl-CH_2), 1.96 – 1.90 (m, 3H, backbone-Ac), 1.79 – 1.22 (3m, 12H, 6x Lys-CH_2).

RP-HPLC-MS (linear gradient from 0 – 25% eluent B in 30 min at 25 °C): t_{R} = 10.05 min. Determined purity: 81%.

SAX-HPLC (0 \rightarrow 5 min: 95% A, 5% B; 5 \rightarrow 40 min: 5 \rightarrow 100% B; 40 \rightarrow 60 min: 100% B at 25 °C): t_{R} = 12.75 + 13.21 min. Determined purity: 85%

MS for $\text{C}_{68}\text{H}_{111}\text{N}_{13}\text{Na}_6\text{O}_{51}\text{S}_6$ (ESI, neg.) m/z : $[\text{M} - 6\text{Na}^+ + 4\text{H}^+]^{2-}$ calc.: 1060.75; found: 1060.95, $[\text{M} - 6\text{Na}^+ + 4\text{H}^+ - \text{SO}_3]^{2-}$ calc.: 1020.78; found: 1021.05, $[\text{M} - 6\text{Na}^+ + 4\text{H}^+ - \text{SO}_3 - \text{OH}]^{2-}$ calc.: 1012.28; found: 1011.95, $[\text{M} - 6\text{Na}^+ + 4\text{H}^+ - 2\text{SO}_3]^{2-}$ calc.: 980.80; found: 980.95, $[\text{M} - 6\text{Na}^+ + 3\text{H}^+]^{3-}$ calc.: 706.84; found: 706.95, $[\text{M} - 6\text{Na}^+ + 3\text{H}^+ - \text{SO}_3]^{3-}$ calc.: 680.18; found: 680.40, $[\text{M} - 6\text{Na}^+ + 3\text{H}^+ - 2\text{SO}_3]^{3-}$ calc.: 653.53; found: 653.60, $[\text{M} - 6\text{Na}^+ + 2\text{H}^+]^{4-}$ calc.: 529.87; found: 530.10, $[\text{M} - 6\text{Na}^+ + 2\text{H}^+ - \text{SO}_3]^{4-}$ calc.: 509.89; found: 509.95, $[\text{M} - 6\text{Na}^+ + 2\text{H}^+ - 2\text{SO}_3]^{4-}$ calc.: 489.90; found: 490.00.

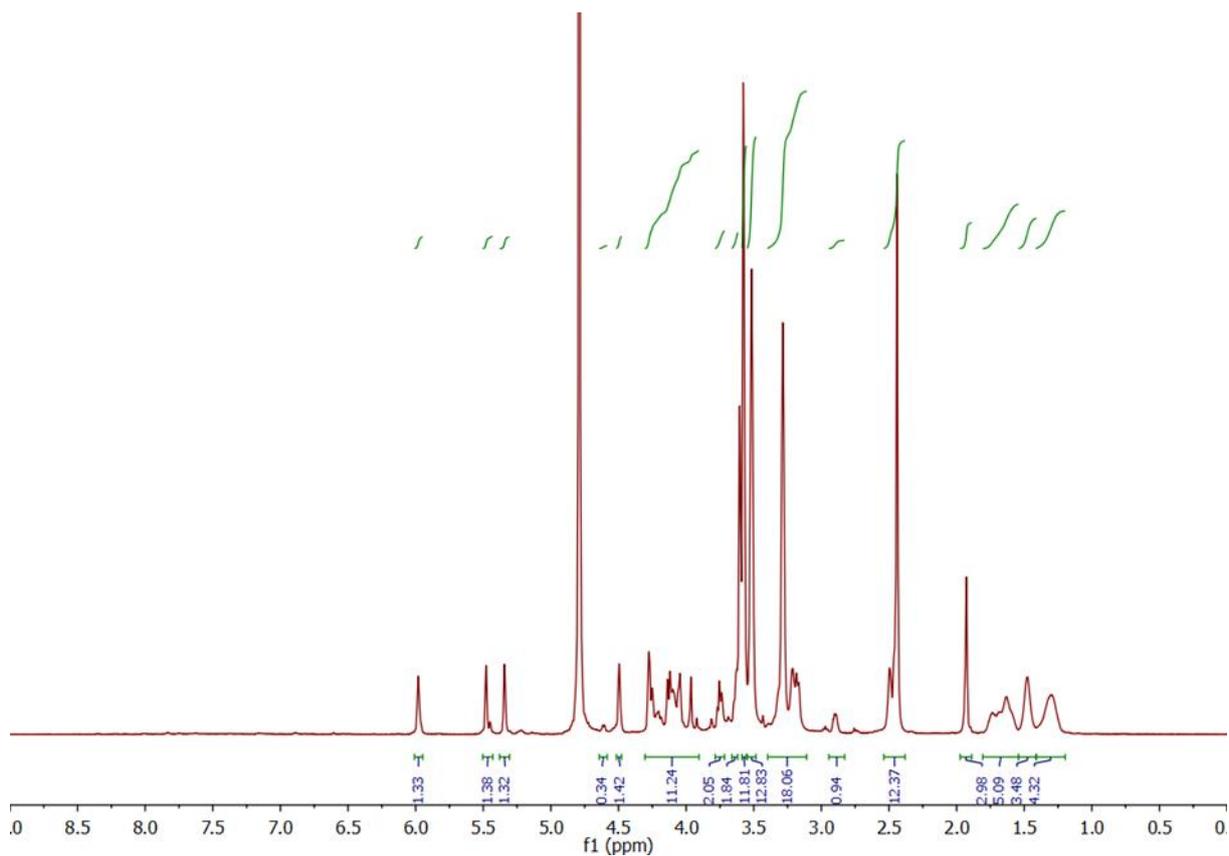


Figure 33: ^1H NMR (600 MHz, Deuterium oxide) of compound **O2**.

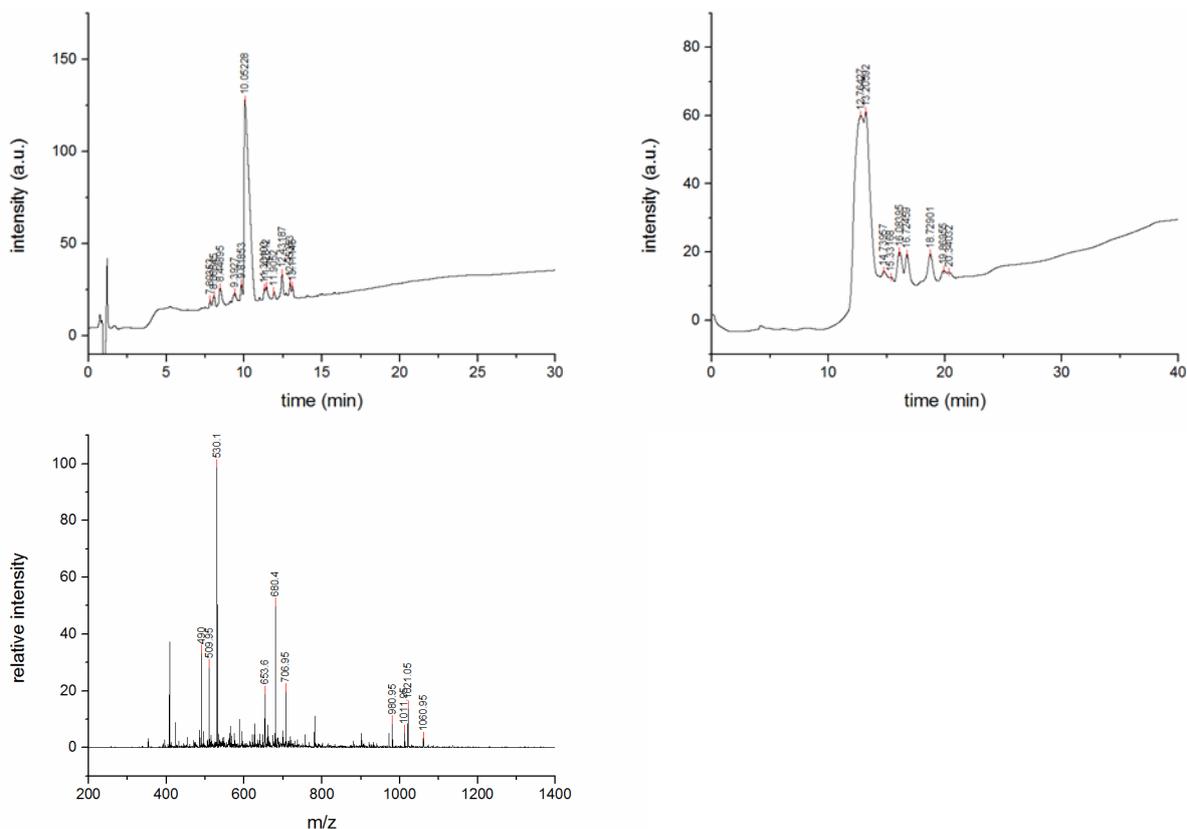
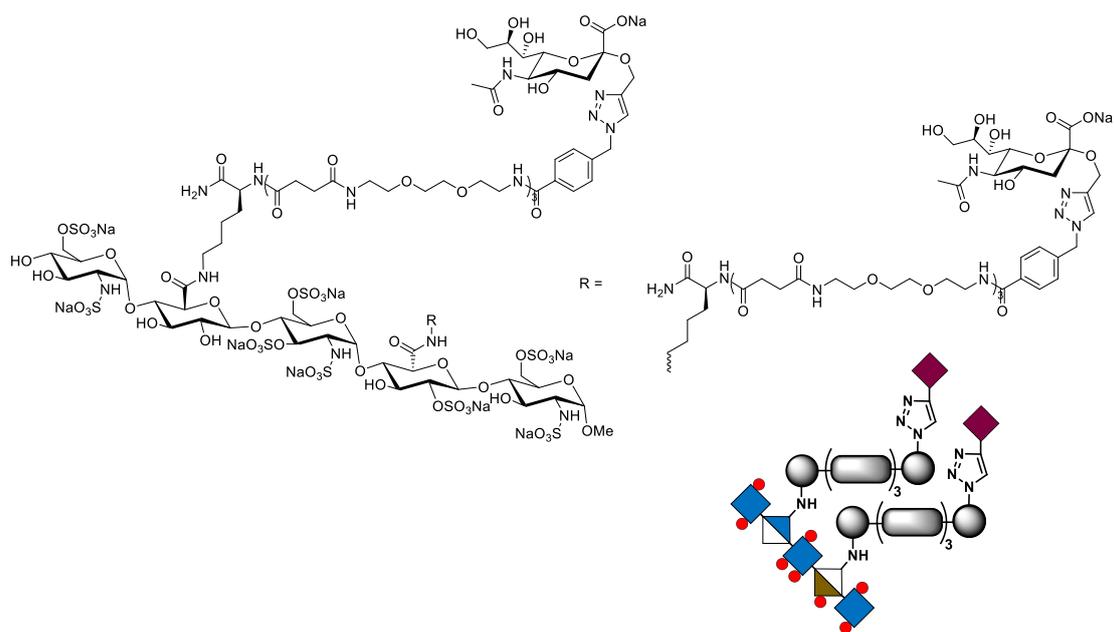


Figure 34: RP-HPLC (linear gradient from 0 – 25% eluent B in 30 min at 25 °C), SAX-HPLC (0 → 5 min: 95% A, 5% B; 5 → 40 min: 5 → 100% B; 40 → 60 min: 100% B at 25 °C), ESI-MS (negative mode) of compound **O2**.

GlcA, IdoA-(N_εLys(1)-4-Neu5Ac)-Fondaparinux (O5) synthesis



5.02 mg **Fondaparinux** (2.91 μmol), 13.6 mg (8.71 μmol , 3 eq.) **N_ε(HCl)Lys(1)-4-Neu5Ac (O3)** and 16.0 mg (57.8 μmol , 20 eq.) DMTMM were combined in a 1 mL glass vial and dissolved in 0.5 ml of a mixture of 0.45 mL dimethylformamide and 0.05 mL phosphate-buffer pH 6.5. The mixture was shaken at room temperature for 24 h, before it was diluted to 5 mL with Milli-Q water and dialyzed using a Vivaspin[®] MWCO 2000 2 ml unit. After lyophilisation the mixture was dissolved in 3 mL of a 0.1 M lithium hydroxide solution (methanol : water = 1 : 1) and shaken for 4 h. The resulting, fully deprotected compound was separated from its protecting groups and excessive lithium hydroxide by dialysis using a Vivaspin[®] MWCO 2000 2 ml unit and finally isolated after ion exchange with Amberlite[®] IR 120 Na⁺ and lyophilisation. 9.4 mg (2.15 μmol , 74%) of the desired product were obtained as a white and foamy solid in a purity of 88% (SAX-HPLC) and 98% (RP-HPLC).

¹H NMR (600 MHz, Deuterium Oxide) δ 8.08 (s, 2H, *triazole-H*), 7.78 (d, ³J = 8.4 Hz, 4H, *aryl-H*), 7.42 (d, ³J = 8.4 Hz, 4H, *aryl-H*), 5.71 (s, 4H, 2x *aryl-CH₂-aryl*), 5.61 (d, ³J = 3.7 Hz, 1H, *A₅^{nr}-H1*), 5.52 (d, ³J = 3.3 Hz, 1H, *A₅^{*}-H1*), 5.23 (d, ³J = 3.7 Hz, 1H, *I₅-H1*), 5.03 (d, ³J = 3.7 Hz, 1H, *A₅^r-H1*), 4.89 (d, ²J = 12.0 Hz, 2H, 2x *propargyl-H*), 4.87 – 4.83 (m, 2H, 2x *Neu5Ac-H4*), 4.75 – 4.71 (m, 1H, *I₅-H5*), 4.62 (d, ²J = 11.8 Hz, 2H, 2x *propargyl-H*), 4.47 – 4.09 (m, 12H, 10x *Fondaparinux-H*, 2x *Lys-CH*), 4.03 – 3.53 (m, 76H, 10x *Fondaparinux-H*, 24x *O-CH₂*, 2x *N-CH₂*, 2x (*Neu5Ac-H4*, 5, 6, 7, 8, 9', 9'')), 3.51 – 3.16 (m, 31H, 3x *A₅-H2*, *G-H2*, *A₅^r-OCH₃*, 2x *Lys-N-CH₂*, 10x *N-CH₂*), 2.75 (dd, ²J = 12.4, ³J = 4.8 Hz, 2H, 2x *Neu5Ac-H3eq.*), 2.65 – 2.44 (m, 24H, 12x *succinyl-CH₂*), 2.04 (s, 6H, 2x *Neu5Ac-Ac*), 1.90 – 1.45 (3m, 12H, 6x *Lys-CH₂*), 1.68 (dd, ²J = 12.2, ³J = 12.2 Hz, 2H, 2x *Neu5Ac-H3ax.*).

RP-HPLC-MS (linear gradient from 0 – 25% eluent B in 30 min at 25° C): t_R = 16.94 min. Determined purity: 98%.

SAX-HPLC (0 → 5 min: 95% A, 5% B; 5 → 40 min: 5 → 100% B; 40 → 60 min: 100% B at 25 °C): t_R = 20.64 min. Determined purity: 88%

MS for C₁₄₇H₂₂₉N₂₉Na₁₀O₉₃S₈ (ESI, neg.) *m/z*: [M – 9Na⁺ + 6H⁺]³⁻ calc.: 1391.41; found: 1391.95, [M – 10Na⁺ + 7H⁺]³⁻ calc.: 1384.08; found: 1384.40, [M – 9Na⁺ + 5H⁺]⁴⁻ calc.: 1043.30; found: 1043.95, [M – 10Na⁺ + 6H⁺]⁴⁻ calc.: 1037.81; found: 1037.95, [M – 10Na⁺ + 6H⁺ - SO₃]⁴⁻ calc.: 1017.82; found: 1018.25, [M – 9Na⁺ + 4H⁺]⁵⁻ calc.: 834.44; found: 834.90, [M – 10Na⁺ + 5H⁺]⁵⁻ calc.: 830.05; found: 830.25, [M – 10Na⁺ + 5H⁺ - SO₃]⁵⁻ calc.: 814.05; found: 814.00, [M – 10Na⁺ + 5H⁺]⁵⁻ calc.: 798.06; found: 798.00.

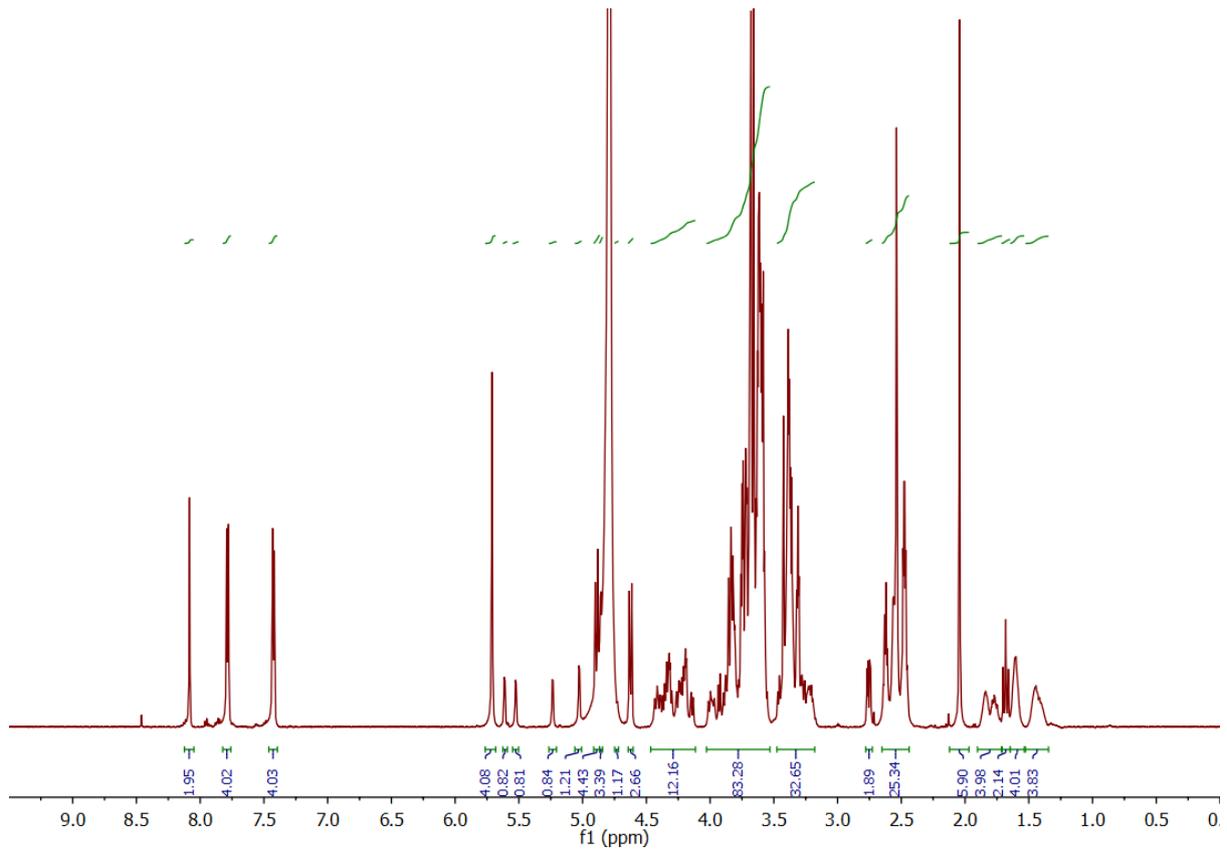


Figure 35: ^1H NMR (600 MHz, Deuterium oxide) of compound **O5**.

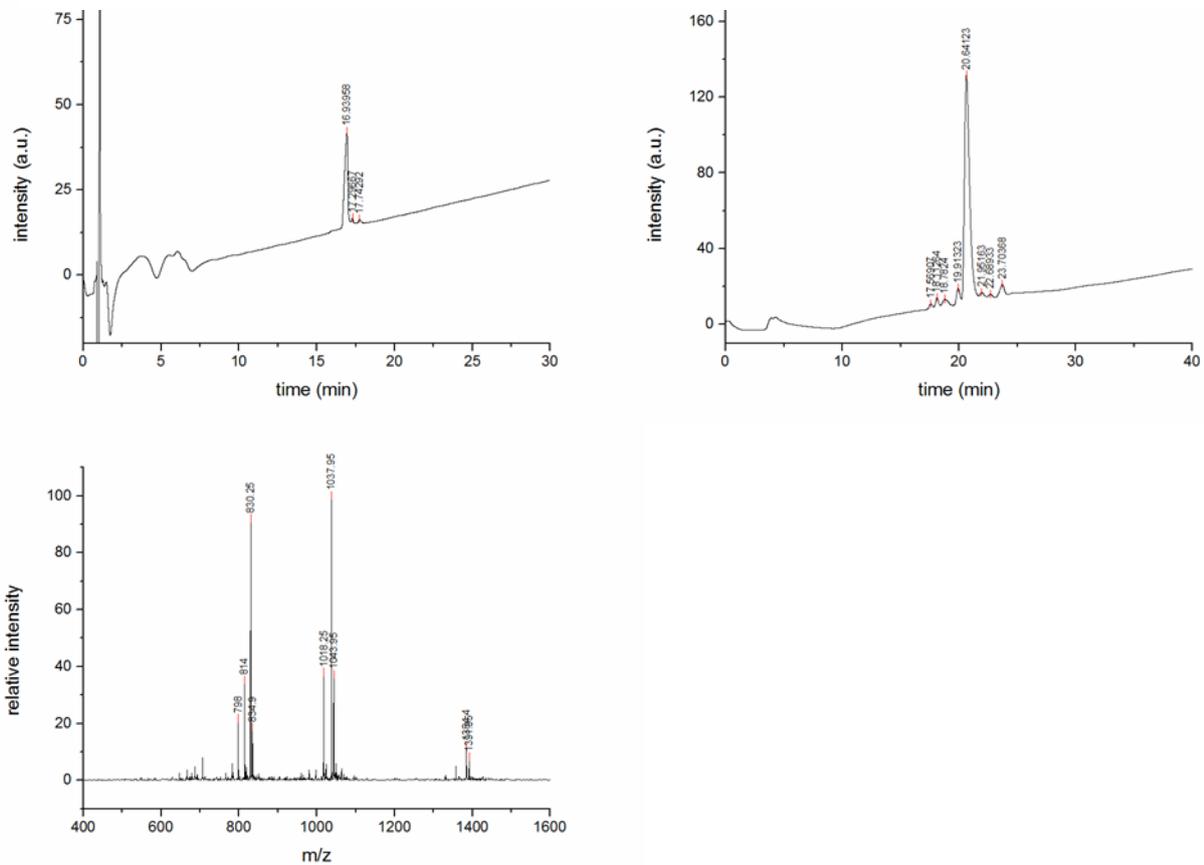
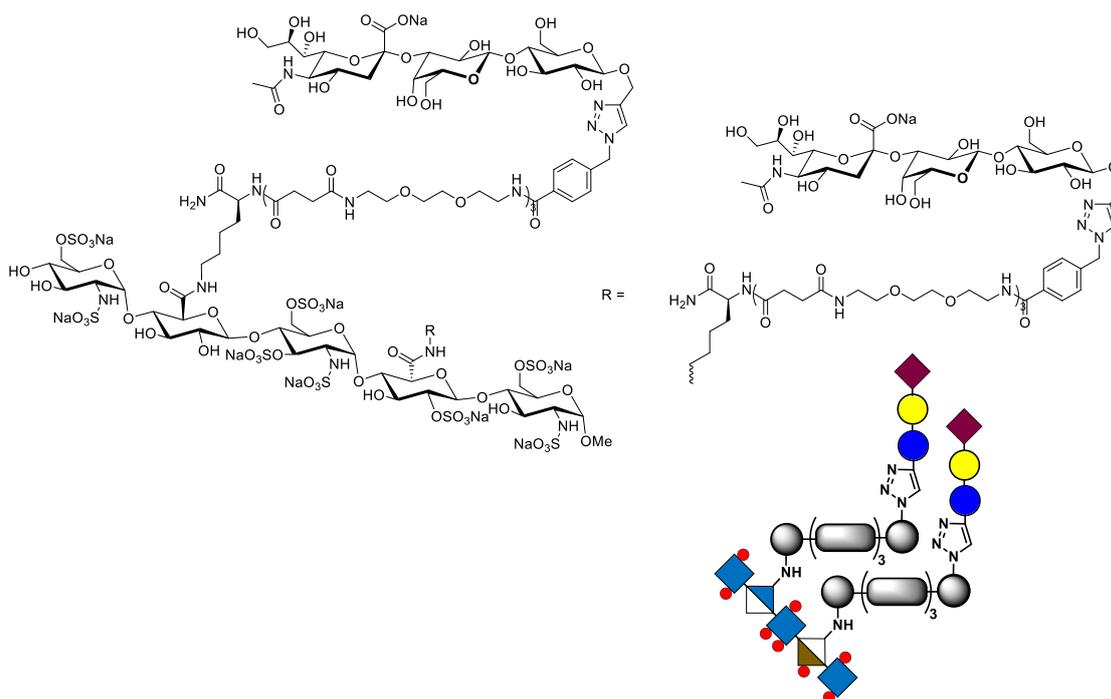


Figure 36: RP-HPLC (linear gradient from 0 – 25% eluent B in 30 min at 25 °C), SAX-HPLC (0 → 5 min: 95% A, 5% B; 5 → 40 min: 5 → 100% B; 40 → 60 min: 100% B at 25 °C), ESI-MS (negative mode) of compound **O5**.

GlcA, IdoA-(N_εLys(1)-4-3'Sialyllactose)-Fondaparinux (O6)



3.09 mg **Fondaparinux** (1.79 μmol), 8.76 mg (4.10 μmol , 2.3 eq.) **N_ε(HCl)Lys(1)-4-3'Sialyllactose (O4)** and 10.8 mg (39.0 μmol , 22 eq.) DMTMM were combined in a 1 mL glass vial and dissolved in 0.5 ml of a mixture of 0.45 mL dimethylformamide and 0.05 mL phosphate-buffer pH 6.5. The mixture was shaken at room temperature for 24 h, before it was diluted to 5 mL with Milli-Q water and dialyzed using a Vivaspin[®] MWCO 2000 2 ml unit. After lyophilisation the mixture was dissolved in 3 mL of a 0.1 M lithium hydroxide solution (methanol : water = 1 : 1) and shaken for 4 h. The resulting, fully deprotected compound was separated from its protecting groups and excessive lithium hydroxide by dialysis using a Vivaspin[®] MWCO 2000 2 ml unit and finally isolated after ion exchange with Amberlite[®] IR 120 Na⁺ and lyophilisation. 7.7 mg (1.53 μmol , 86%) of the desired product were obtained as a white and foamy solid in a purity of 90% $\beta\beta$ (RP-HPLC) and 87% (SAX-HPLC).

¹H NMR (600 MHz, Deuterium Oxide) δ 8.14 (s, 2H, *triazole-H*), 7.79 (d, ³J = 8.3 Hz, 4H, *aryl-H*), 7.44 (d, ³J = 8.2 Hz, 4H, *aryl-H*), 5.72 (s, 4H, 2x *aryl-CH₂-aryl*), 5.61 (d, ³J = 3.7 Hz, 1H, *A₅^{nr}-H1*), 5.53 (d, ³J = 3.4 Hz, 1H, *A₅^{*}-H1*), 5.23 (d, ³J = 3.8 Hz, 1H, *I₅-H1*), 5.02 (d, ³J = 3.7 Hz, 1H, *A₅^r-H1*), 5.00 (d, ²J = 12.7 Hz, 2H, 2x *propargyl-H*), 4.88 (d, ²J = 12.6 Hz, 2H, 2x *propargyl-H*), 4.86 – 4.84 (m, 2H, 2x *Neu5Ac-H4*), 4.62 (d, ²J = 7.8 Hz, 1H, *I₅-H5*), 4.57 (d, ³J = 8.0 Hz, 2H, 2x *Glc-H1*), 4.53 (d, ³J = 7.8 Hz, 2H, 2x *Gal-H1*), 4.46 – 4.09 (m, 12H, 10x *Fondaparinux-H*, 2x *Lys-CH*), 4.03 – 3.53 (m, 100H, 10x *Fondaparinux-H*, 24x *O-CH₂*, 2x *N-CH₂*, 2x (*3'SL-19xH*)), 3.49 – 3.15 (m, 31H, 3x *A₅-H2*, *G-H2*, *A₅^r-OCH₃*, 2x *Lys-N-CH₂*, 10x *N-CH₂*), 2.77 (dd, ²J = 12.4, ³J = 4.6 Hz, 2H, 2x *Neu5Ac-H3eq.*), 2.67 – 2.41 (m, 24H, 12x *succinyl-CH₂*), 2.04 (s, 6H, 2x *Neu5Ac-Ac*), 1.88 – 1.44 (3m, 12H, 6x *Lys-CH₂*), 1.60 (dd, ²J = 12.3, ³J = 12.3 Hz, 2H, 2x *Neu5Ac-H3ax.*).

RP-HPLC-MS (linear gradient from 0 – 25 % eluent B in 30 min at 25 °C): t_R = 16.16 min. Determined purity: 90% $\beta\beta$, 6% $\alpha\beta/\beta\alpha$, < 4% $\alpha\alpha$.

SAX-HPLC (0 → 5 min: 95% A, 5% B; 5 → 40 min: 5 → 100% B; 40 → 60 min: 100% B at 25 °C): t_R = 19.90 min. Determined purity: 87%

MS for C₁₇₁H₂₆₉N₂₉Na₁₀O₁₁₃S₈ (ESI, neg.) m/z: [M – 9Na⁺ + 6H⁺]³⁻ calc.: 1607.48; found: 1608.40, [M – 10Na⁺ + 7H⁺]³⁻ calc.: 1600.15; found: 1600.40, [M – 9Na⁺ + 5H⁺]⁴⁻ calc.: 1205.36; found: 1205.95, [M – 10Na⁺ + 6H⁺]⁴⁻ calc.: 1199.86; found: 1200.30, [M – 10Na⁺ + 6H⁺ - SO₃]⁴⁻ calc.: 1180.00; found: 1179.87, [M – 9Na⁺ + 4H⁺]⁵⁻ calc.: 964.08; found: 964.65, [M – 10Na⁺ + 5H⁺]⁵⁻ calc.: 959.69; found: 959.75, [M – 10Na⁺ + 5H⁺ - SO₃]⁵⁻ calc.: 943.70; found: 943.90.

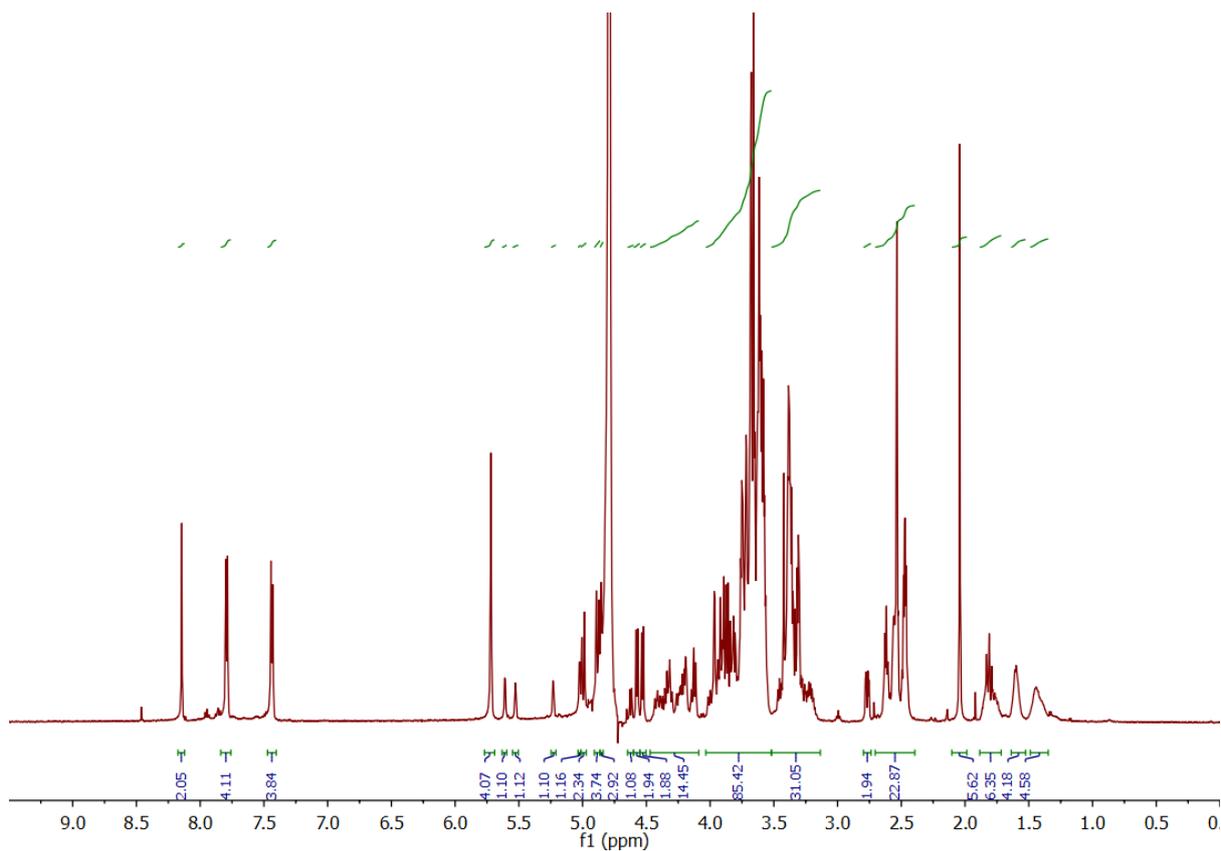


Figure 37: ^1H NMR (600 MHz, Deuterium oxide) of compound **O6**.

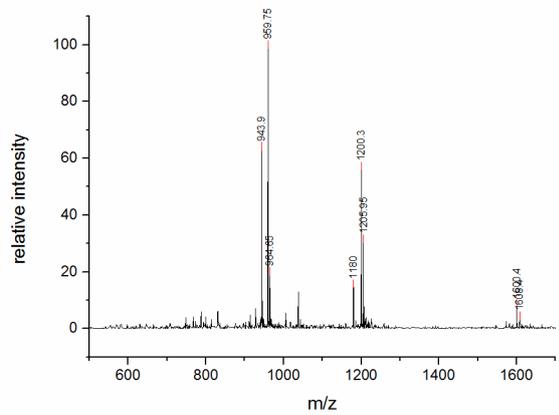
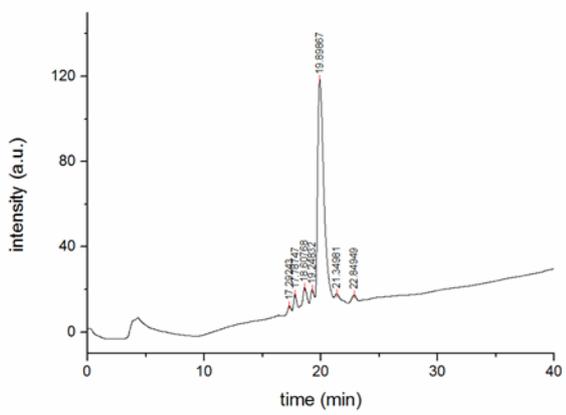
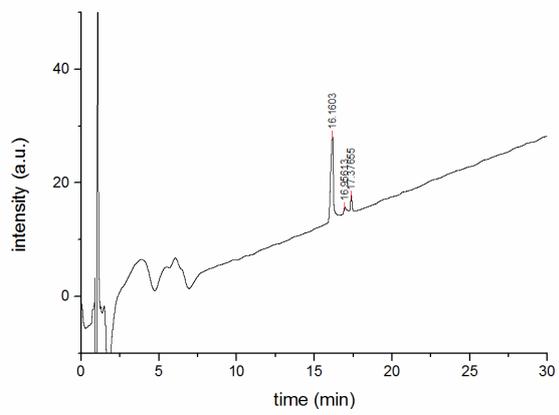


Figure 38: RP-HPLC (linear gradient from 0 – 25% eluent B in 30 min at 25 °C), SAX-HPLC (0 → 5 min: 95% A, 5% B; 5 → 40 min: 5 → 100% B; 40 → 60 min: 100% B at 25 °C), ESI-MS (negative mode) of compound **O6**.

Saturation transfer difference NMR studies

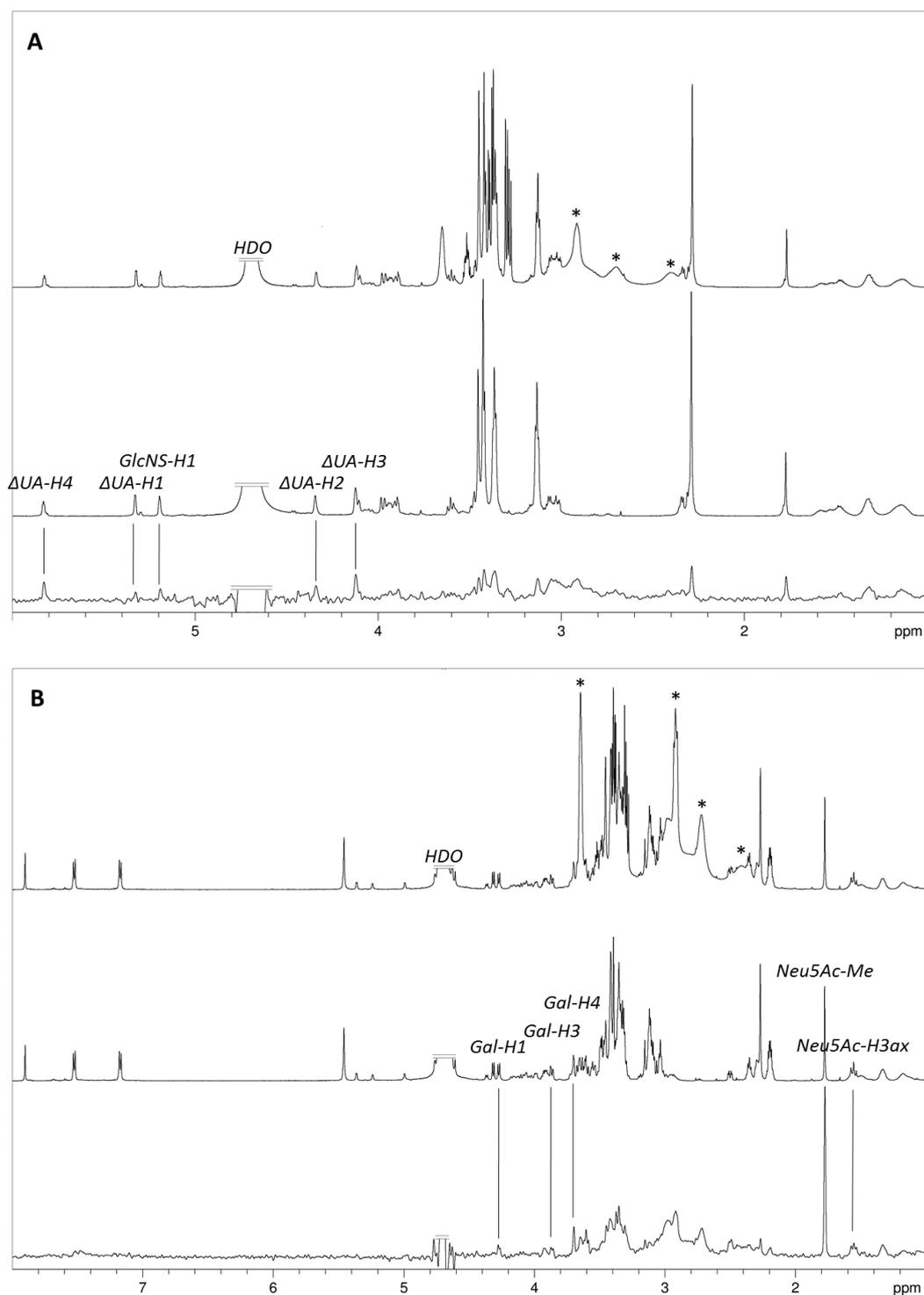


Figure 39: NMR spectra of MCPyV capsids interacting with compounds **O2** and **O6**. **A)** Top: ^1H NMR spectrum of MCPyV capsids with **O2**, 2nd from top: ^1H NMR spectrum of **O2**, bottom: STD-NMR difference spectrum of MCPyV capsids with **O2**. **B)** Top: ^1H NMR spectrum of MCPyV capsids with **O6**, 2nd from top: ^1H NMR spectrum of **O6**, bottom: STD-NMR difference spectrum of MCPyV capsids with **O6**. All spectra were recorded at 283 K in D_2O -buffer containing 150 mM NaCl, 1 mM CaCl_2 , pH 6.3. HDO signals were truncated in each spectrum for the sake of clarity. Unidentified impurities that appear bound to the capsids due to their broad line shape are highlighted with asterisks.

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