Supporting Information for:

“Synthetic Enantiopure Carbohydrate Polymers that are Highly Soluble in Water and Noncytotoxic”

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GENERAL METHODS AND INSTRUMENTATION

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian 500 MHz spectrometer. FTIR spectra were obtained by pressing a small amount of dry sample onto a horizontal attenuated total reflectance (ATR) adapter on a Nicolet FT-IR. Specific rotations were determined using a Rudolph Autopol II polarimeter operating at 589 nm in a 50 mm pathlength cell. Polymer molecular weights for protected polymers (**P1'**, **P2'**) were determined by gel permeation chromatography (GPC) versus polystyrene standards using THF as the eluent at a flow rate of 1.0 mL/min with a Styragel column (HR4E, 7.8 x 300 mm) in series with a refractive index detector. Polymer molecular weights for deprotected polymers (**P1**, **P2**) were determined by GPC versus dextran standards using 0.10 M $NaNO_2$, 0.010 M phosphate buffer at pH 7.4 as the eluent at a flow rate of 0.5 mL/min with a PL aquagel-OH 30 (7.5 x 300 mm) and a Shodex Ohpak (KB-804, 8.0 x 300 mm) in series with a refractive index detector. Unless otherwise mentioned, all chemicals were purchased from Sigma-Aldrich, Alfa-Aesar, or Acros and used as received. Monomer **1**[1] and initiator **2**[2] were synthesized as previously reported.

General procedure for synthesis of polymer 1 series

In an oven-dried flask, lactam **1** (0.500 g, 1.09 mmol) and 4-nitrobenzoyl chloride (4.0 mol% for DP_{th} = 25, 2.0 mol% for DP_{th} = 50, 1.0 mol% for DP_{th} = 100) were dissolved in 10 mL of distilled tetrahydrofuran (without BHT) which had been dried over molecular sieves. Because small quantities of 4-nitrobenzoyl chloride were required, a stock solution of 4-nitrobenzoyl chloride in tetrahydrofuran was prepared immediately before use and the appropriate volume was added to the reaction flask. The reaction flask was cooled to 0°C in an ice bath. Next, the appropriate volume of a 0.5 M solution of LiHMDS in THF (10 mol% for DP_{th} = 25, 5.0 mol% for DP_{th} = 50, 2.5 mol% for DP_{th} = 100) was added and the solution was stirred for 0.5 hr, at which time the reaction was allowed to warm to room temperature. After 0.5 hr at room temperature, complete consumption of **1** was confirmed by thin-layer chromatography with 1:1 ethyl acetate and hexane as the eluent. To quench the reaction, a drop of saturated NH_4Cl aqueous solution was added. The THF was removed and the resulting solid was redissolved in diethyl ether (25 mL) and washed with 1 M HCl, sat'd $NaHCO_3$, and brine. After drying over sodium sulfate, the product was isolated by evaporation of solvent. The product was dissolved in minimal dichloromethane and precipitated by adding dropwise into a flask of stirred, cold pentane (50 mL). The resulting solid was collected by filtration, redissolved in dichloromethane, and precipitated in cold methanol and dried. After drying under high vacuum, a white powder was isolated with quantitative or close to quantitative mass recovery. Spectral data is listed for **P1'$_{25}$**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (br s, 1H), 7.15 (br m, 15H), 5.85 (br s, 1H), 4.75-3.1 (br m, 12H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.1, 138.8, 138.4, 128.3, 127.8, 127.5, 74.1, 73.2, 72.1, 71.7, 68.5, 46.7. IR (ATR): 3342 br (NH), 1675 (amide I), 1521 br (amide II), 1064 cm^{-1}.

General procedure for synthesis of polymer 2 series

In an oven-dried flask, lactam **1** (0.500 g, 1.09 mmol) and initiator **2** (4.0 mol% for DP_{th} = 25, 2.0 mol% for DP_{th} = 50, 1.0 mol% for DP_{th} = 100) were dissolved in 10 mL of distilled tetrahydrofuran (without BHT) which had been dried over molecular sieves. Because small quantities of initiator **2** were required, a stock solution in tetrahydrofuran was prepared immediately before use and the appropriate volume was added to the reaction flask. The reaction flask was cooled to 0 °C in an ice bath. Next, the appropriate volume of a 0.5 M solution of LiHMDS in THF (10 mol% for DP_{th} = 25, 5.0 mol% for DP_{th} = 50, 2.5 mol% for DP_{th} = 100) was added and the solution was stirred for 0.5 hr, at which time the reaction was allowed to warm to room temperature. After 0.5 hr at room temperature, complete consumption of **1** was confirmed by thin-layer chromatography with 1:1 ethyl acetate and hexane as the eluent. To quench the reaction, a drop of saturated NH_4Cl aqueous solution was added. The THF was removed and the resulting solid was redissolved in diethyl ether (25 mL) and washed with 1 M HCl, sat'd $NaHCO_3$, and brine. After drying over sodium sulfate, the product was isolated by evaporation of solvent. The product was dissolved in minimal dichloromethane and precipitated by adding dropwise into a flask of stirred, cold pentane (50 mL). The resulting solid was collected by filtration, redissolved in dichloromethane, and precipitated in cold methanol and dried. After drying under high vacuum, a white powder was isolated with quantitative or close to quantitative mass recovery.

General procedure for debenzylation of polymers

Polymer **1** or **2** (0.200 - 0.400 g depending on sample) was dissolved in 5.0 mL of tetrahydrofuran. Next, 1.5 equiv. of LiHMDS (from 1.0 M stock solution in tetrahydrofuran) was added to this solution and the solution was stirred for 5 minutes at room temperature. The solution was then added dropwise into a rapidly stirred solution of sodium in anhydrous liquid ammonia (50 mL) at −78 °C under nitrogen. Sodium was washed in hexane and cut into small pieces before addition. The solution's deep blue color was maintained by adding additional sodium. After 1 hour at −78 °C, sat'd ammonium chloride was added until the blue color disappeared. After evaporation of the ammonia at room temperature, the resulting aqueous layer was washed with diethyl ether twice. The aqueous solution was dialyzed for 2 days with 3 water changes. Polymers $\mathbf{P1}_{25}$ and $\mathbf{P2}_{25}$ were dialyzed using 500 Da MWCO tubing. Polymers $\mathbf{P1}_{50}$ and $\mathbf{P2}_{50}$ were dialyzed using 1 kDa MWCO tubing, and $\mathbf{P1}_{100}$ and $\mathbf{P2}_{100}$ were dialyzed using 3.5 kDa MWCO tubing. After lyophilization, the resulting white solid was suspended in methanol and collected by filtration as a white solid. Yields over both steps, polymerization and deprotection, ranged from 66 – 94% and are reported in the main text. Spectral data is listed for $\mathbf{P1}_{25}$: ^{1}H NMR (500 MHz, D_2O) δ 5.78 (d, *J* = 5.2 Hz, 1H), 4.25 (d, *J* = 11.4 Hz, 1H), 3.95 – 3.89 (s, 1H), 3.66 (m, 3H), 3.20 (dd, *J* = 11.4, 5.3 Hz, 1H); ^{13}C NMR (126 MHz, D_2O) δ 171.9, 75.4, 72.7, 67.9, 66.0, 61.7, 46.8. IR (ATR): 3550-3200 br, 1670 (amide I), 1515 br (amide II), 1131, 1053, 975, 789 cm^{-1}.

Cell culture and *in vitro* cell viability assay

Chinese hamster ovary (CHO) cells were cultured in F-12K media supplemented with 10% fetal bovine serum (FBS) and 1% L-glutamine-penicillin-streptomycin (GPS). Liver hepatocellular carcinoma (HepG2) cells were cultured in DMEM media supplemented with 10% FBS and 1% GPS. *Homo sapiens* cervix adenocarcinoma cells (HeLa) were cultured in a complete DMEM medium with 10% fetal bovine serum, 1% GPS, and 0.1 mM MEM non-essential amino acid (Sigma). All cell lines were purchased from ATCC (Manassas, VA) and maintained at 37°C in 5% CO_2 with humidity. When cells reach 80% to 90% confluency, they were split at a 1:4 ratio using a standardized trypsin-based detachment protocol for all experiments.

To determine cytotoxicity when cells were in the presence of $\mathbf{P2}_{25}$ or $\mathbf{P2}_{100}$, an *in vitro* cell viability assay was performed using a standard MTS proliferation assay protocol (CellTiter 96® Aqueous One, Promega, Madison, WI). Cells were seeded at 25,000 cells/well in 96-well plates and incubated overnight at 37°C. The media was removed and replaced with culture media containing 2 mg/mL $\mathbf{P2}_{25}$ or $\mathbf{P2}_{100}$. Cells were incubated for another 24 or 48 hours before measuring cell viability with an MTS reagent. Absorbance was recorded at 492 nm with a multi-plate reader and cell viability was calculated in relation to control cells in media.

Rhodamine-labeling of $\mathbf{P2}_{25}$ and $\mathbf{P2}_{100}$.

A 5(6)-carboxy-X-rhodamine N-succinimidyl ester was used to label $\mathbf{P2}_{25}$ and $\mathbf{P2}_{100}$ in order to monitor polymer uptake in HepG2 cells. A solution containing 10 mg/mL $\mathbf{P2}_{25}$ or $\mathbf{P2}_{100}$ dissolved in distilled water with 0.10 M $NaHCO_3$ was prepared. A second solution was prepared containing 2 mg/mL 5(6)-Carboxy-X-Rhodamine N-succinimidyl ester dissolved in DMSO. A portion of the DMSO solution (100 µL) was added to 400 µL of the aqueous solution. After 30 minutes at room temperature, the reaction mixture was dialyzed overnight in distilled water using a 1 kDa MW cut-off dialysis membrane with three water changes. The sample was isolated by lyophilization and rhodamine labeling of the polymer was confirmed using polyacrylamide gel electrophoresis.

Confocal microscopy for uptake studies

HepG2 cells were plated at a density of 500,000 cells/well in a 6-well plate containing a 1.5 cm (18 x 18 mm) glass coverslip in each well and were incubated overnight at 37°C. The media was removed and replaced with fresh culture media containing 25 µg/mL rhodamine-labeled $\mathbf{P2}_{25}$ or $\mathbf{P2}_{100}$ followed by a 24-hour incubation. To prepare for confocal microscopy imaging, the coverslips were washed twice with cold PBS and cells were treated with 4% formaldehyde for 20 minutes in 37°C. Subsequently, cells were washed twice with PBS containing magnesium and calcium. Cells were incubated for 8 minutes with 3 µg/mL Hoechst trihydrochloride trihydrate solution in PBS and 100 µg/mL of a concanavalin A 488 conjugate in PBS to stain the nucleus and the membrane, respectively. Cells were washed twice with cold PBS, followed by another two washes with PBS containing calcium and magnesium. The coverslips were carefully removed from the wells and mounted on glass slides

with a Prolong Gold Anti-Fade® reagent. Samples were imaged using a Zeiss LSM 510 Meta Live Cell confocal microscope under a 405 nm laser (blue, nucleus), a 488 nm laser (green, cell membrane), and a 543 nm laser (red, rhodamine conjugates).

Quantitative analysis of *rh*-P2$_{25}$ and *rh*-P2$_{100}$ uptake, 4°C endocytosis inhibition, and asialofetuin inhibition in HepG2 Cells monitored by flow cytometry

HepG2 cells were seeded at 500,000 cells/well in a 6-well plate and incubated overnight at 37°C with 5% CO_2. The culture media was removed and replaced with fresh culture media (2.0 mL) containing 25 μg/mL *rh*-**P2$_{25}$** or *rh*-**P2$_{100}$**. The cells were incubated for 24 hours before washing twice with PBS and harvesting with a standard trypsin-detachment protocol. Cell suspensions were centrifuged at 250*g* for 5 minutes and were resuspended twice in cold FACS buffer (PBS (1x) with 1% BSA and 1% NaN_3). Flow cytometry was performed on an LSRII instrument with a 561 nm laser line. Uptake was compared to a rhodamine-labeled dextran standard, *rh*-**Dex10k**. 10,000 events were recorded per sample and data analysis was performed with FlowJo® software.

To further probe the mechanism of *rh*-**P2$_{25}$** uptake, cells were plated in the same manner described above, and subseqently incubated at 4°C in media for 1 hour followed by incubation in media (2.0 mL) containing 25 μg/mL *rh*-**P2$_{25}$** for 4 hours at 4°C. For asialofetuin (AF) inhibition, cells were incubated in media (2.0 mL) containing 25 μg/mL *rh*-**P2$_{25}$** with the addition of AF at 1.0 mg/mL or 4.0 mg/mL for 24 hours at 37°C with 5% CO_2. Both 4°C incubation and AF inhibition were quantified with flow cytometry, as describe above.

References

1. Kałuża, Z.; Abramski, W.; Bełżecki, C.; Grodner, J.; Mostowicz, D.; Urbański, R.; Chmielewski, M. “Cycloaddition of chlorosulfonyl isocyanate to sugar vinyl ethers.” *Synlett* **1994**, *07*, 539-541.

2. Manoharan, M.; Rajeev, K. G. “Oligonucleotides comprising a non-phosphate backbone linkage.” US Patent 287260, 2006.

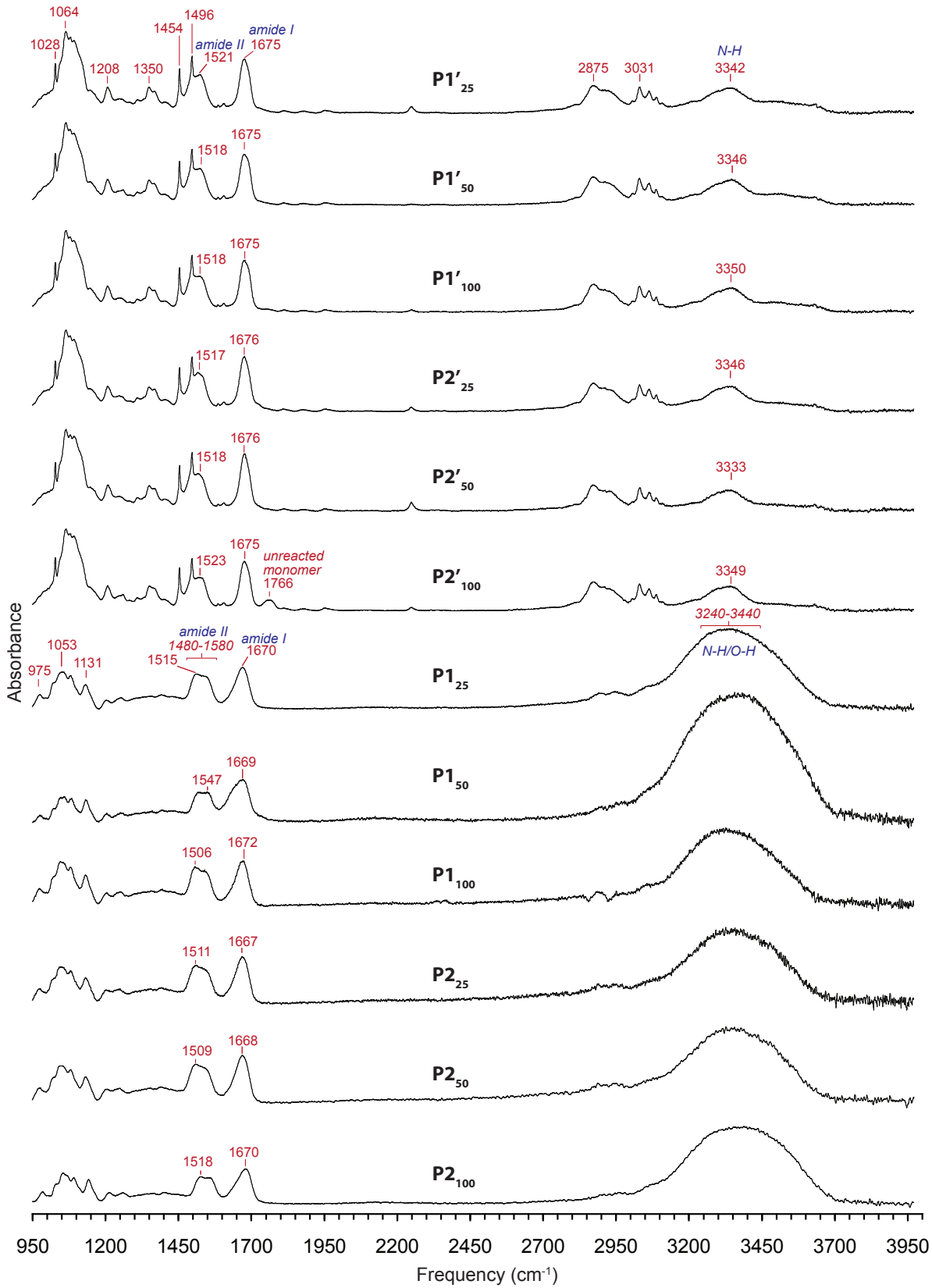


Figure S1. Infrared spectra of protected (**P1'**, **P2'**) and deprotected (**P1**, **P2**) polymers. Major bands are labelled in red with the energy (cm^{-1}) for $\mathbf{P1'_{25}}$ and $\mathbf{P1_{25}}$. In subsequent spectra, only those bands which vary are identified. Spectra for **P1'** and **P2'** were collected by drop-casting a film from a concentrated chloroform solution onto an ATR crystal. Spectra for **P1** and **P2** were obtained by pressing a dry sample of the polymer onto an ATR crystal.

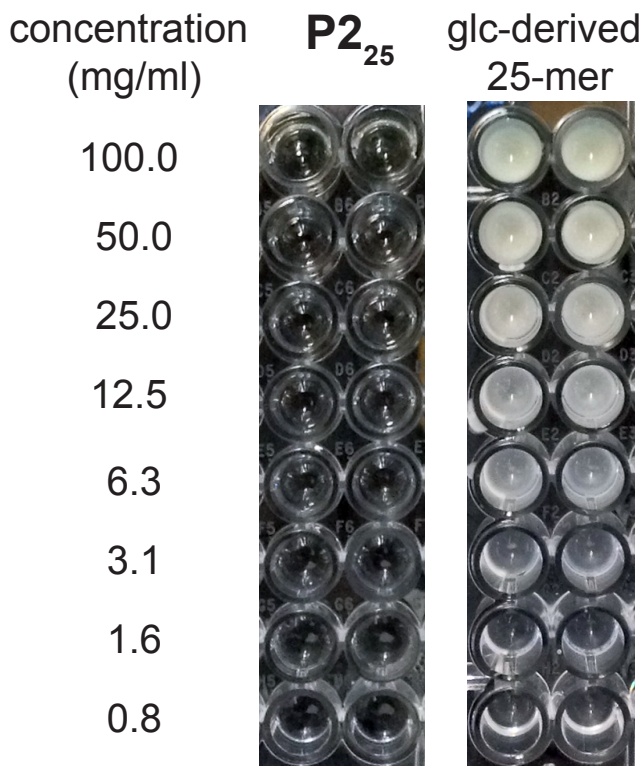


Figure S2. The image shows solutions of **P2**$_{25}$ (left) and a glc-derived 25-mer (right) in distilled water at various concentrations in a 96-well plate after incubation at 37 °C for 48 hours.

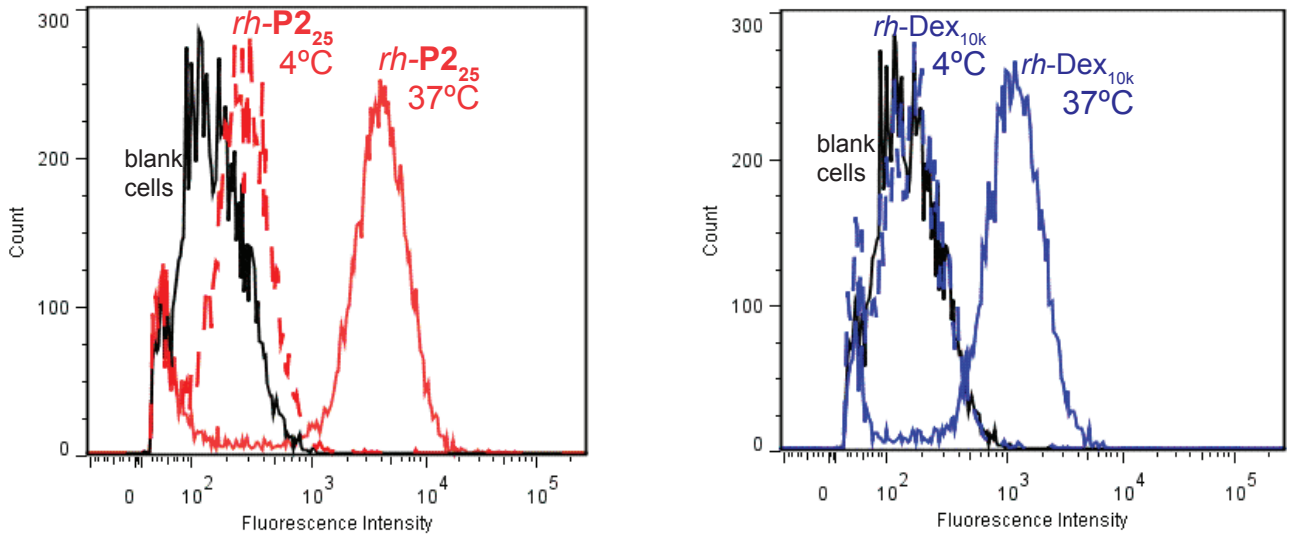
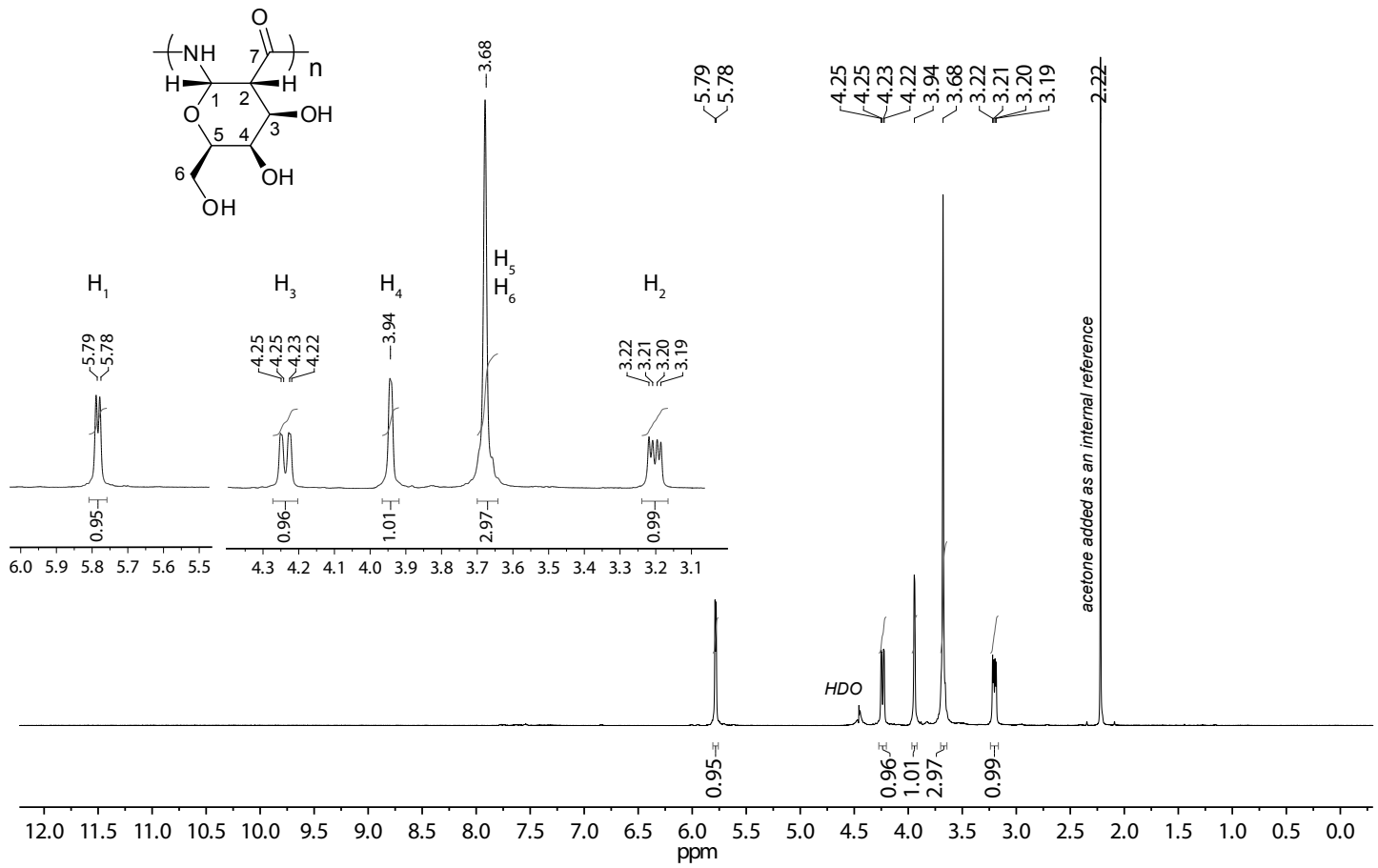
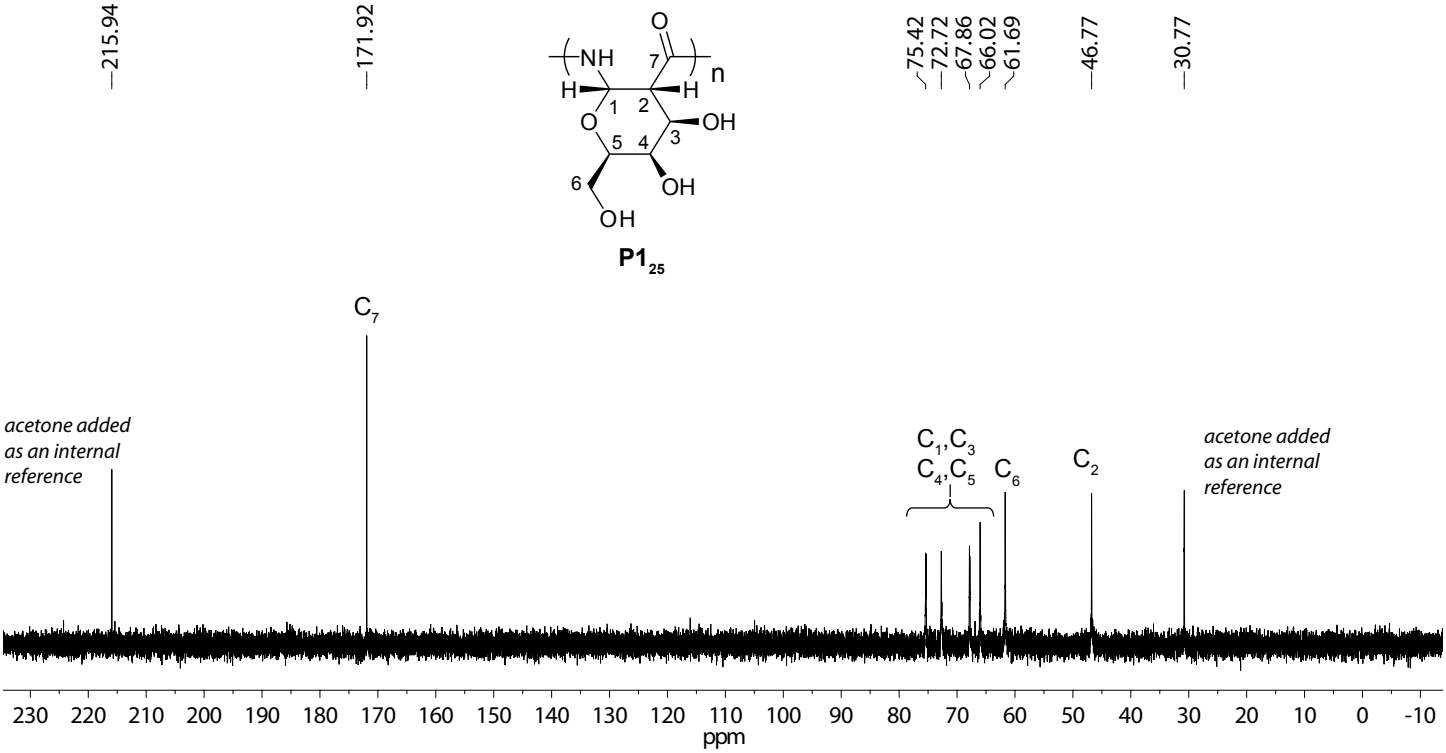


Figure S3. The inhibition of cell uptake at 4 °C is shown for *rh*-**P2**$_{25}$ (left) and *rh*-Dex$_{10k}$ (right) after a 4 hour incubation. The large decreases in uptake observed for both polymers confirms that uptake occurs mostly by endocytosis rather than passive diffusion across the cell membrane.

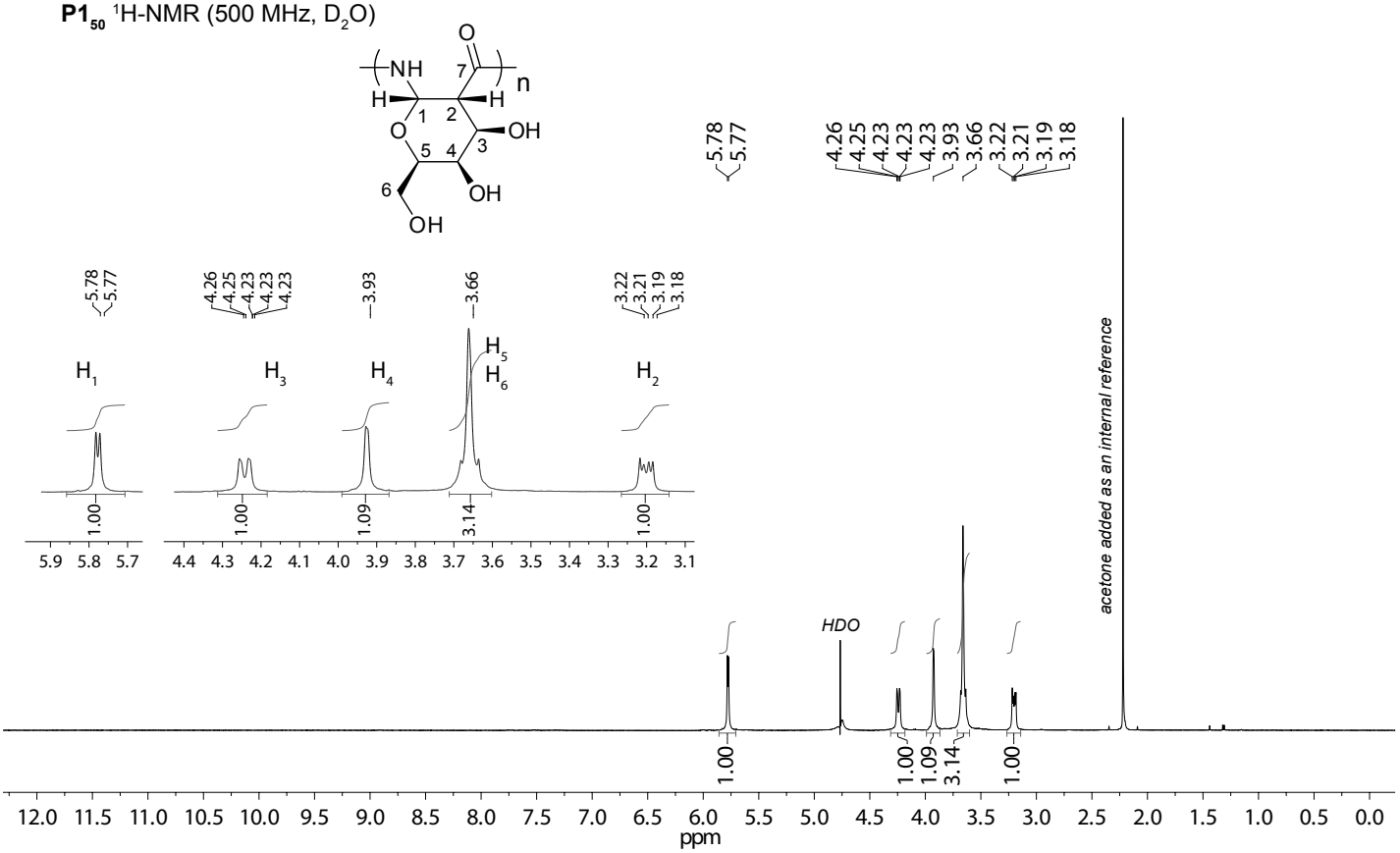
P1$_{25}$ ^{1}H-NMR (500 MHz, D_2O)



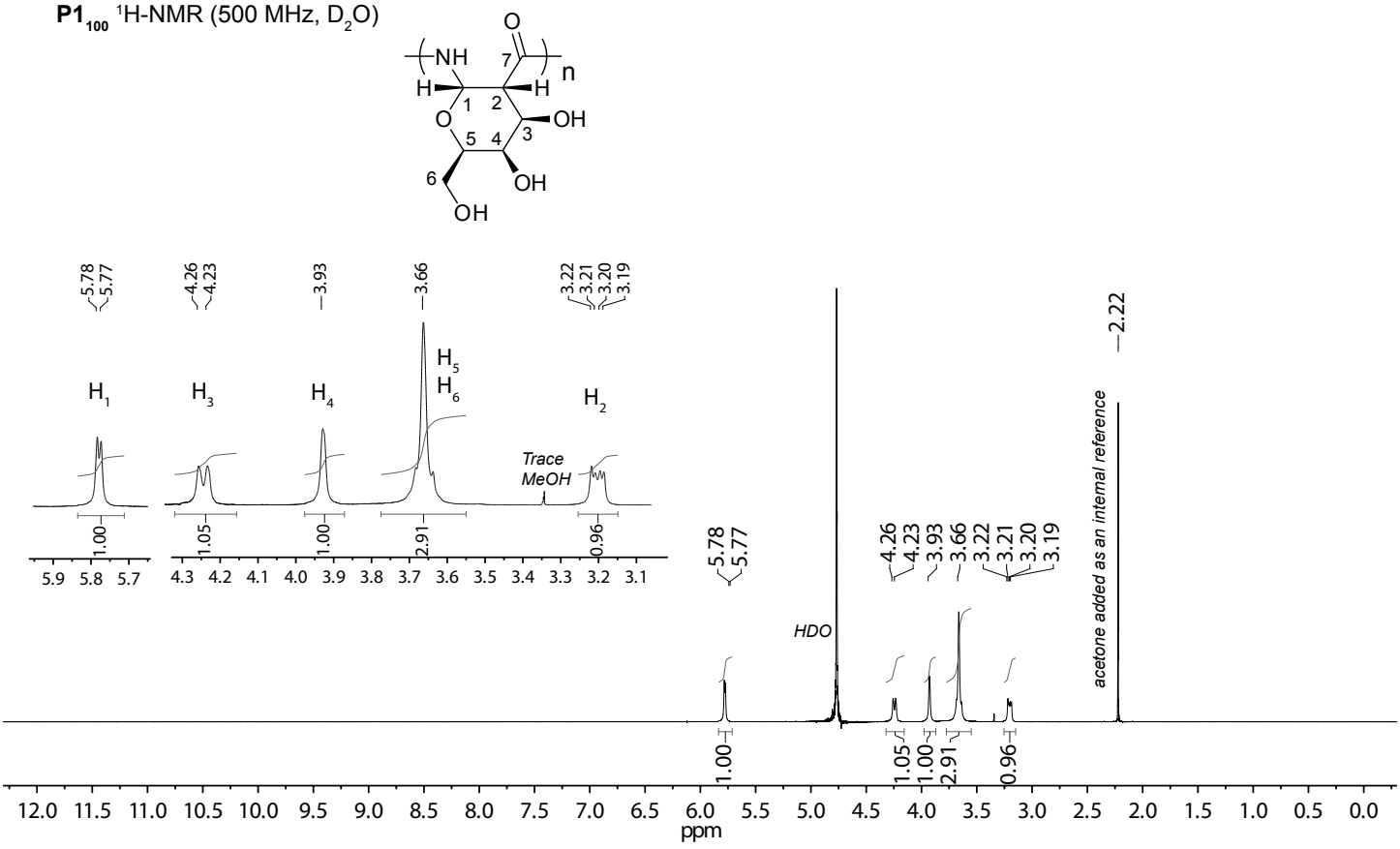
P1$_{25}$ (126 MHz, D_2O)



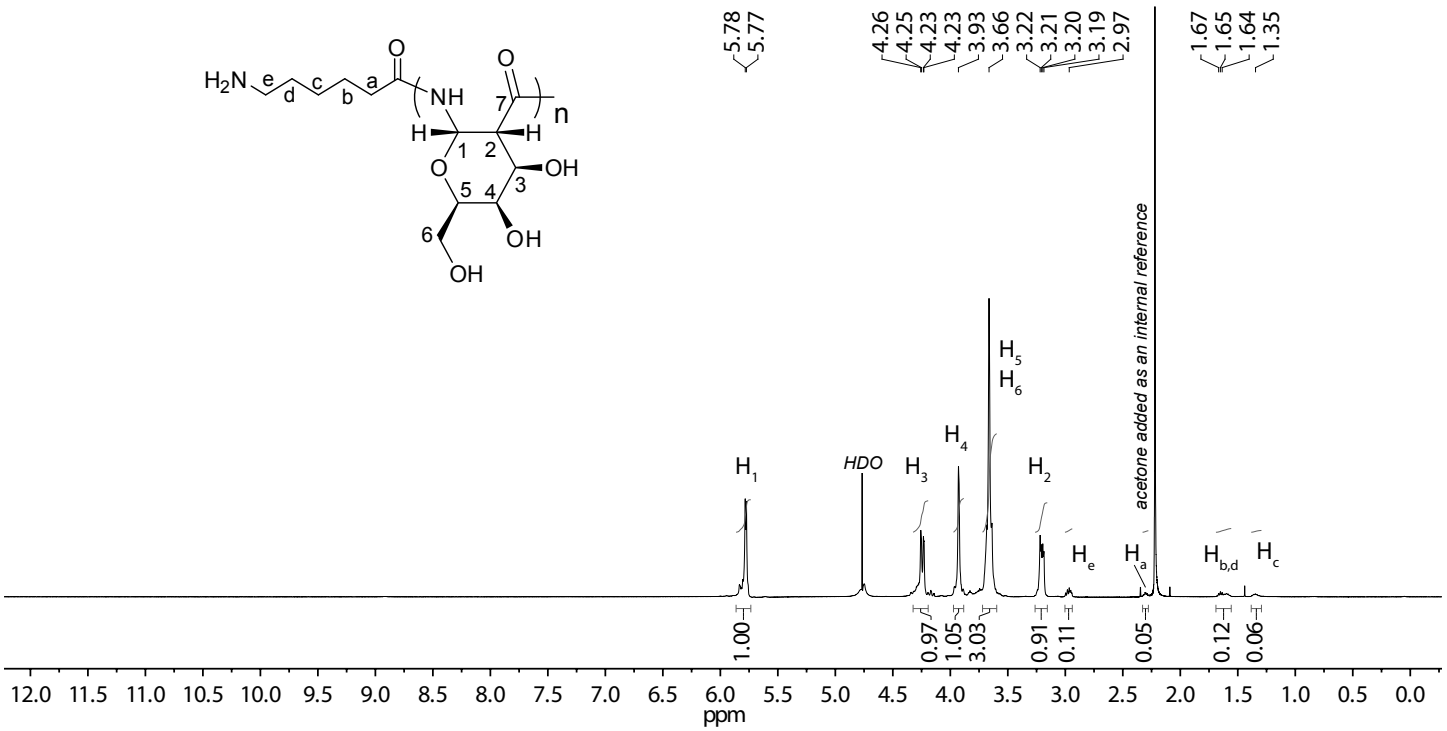
P1$_{50}$ ^{1}H-NMR (500 MHz, D_2O)



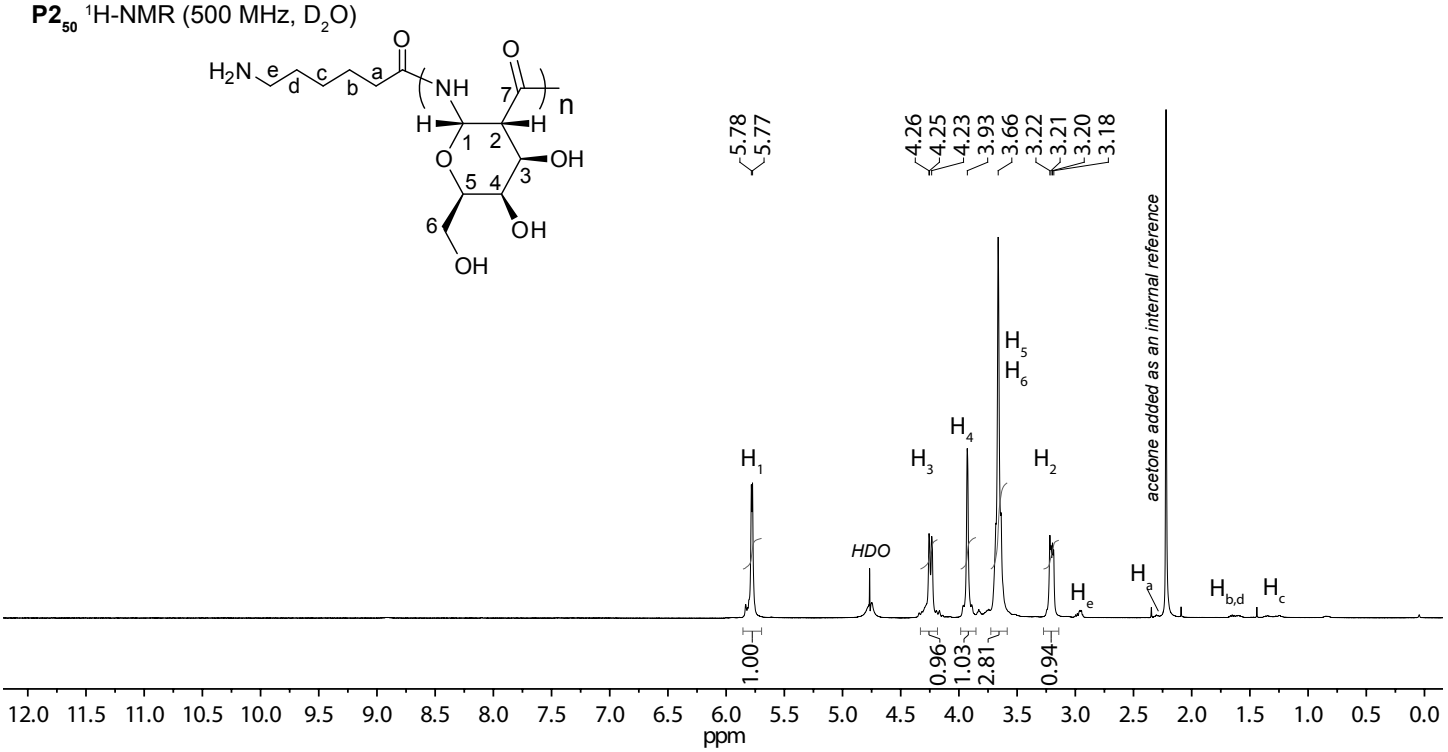
P1$_{100}$ ^{1}H-NMR (500 MHz, D_2O)



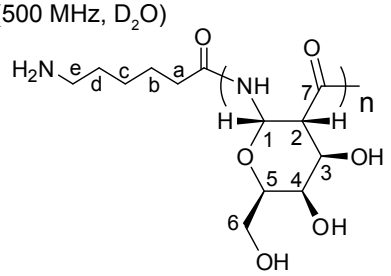
P2$_{25}$ ^{1}H-NMR (500 MHz, D_2O)



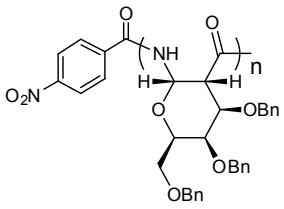
P2$_{50}$ ^{1}H-NMR (500 MHz, D_2O)



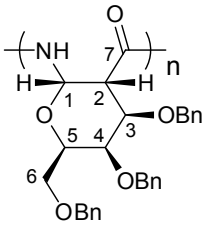
P2$_{100}$ ^{1}H-NMR (500 MHz, D_2O)



P1'$_{25}$ ^{1}H-NMR (500 MHz, $CDCl_3$)

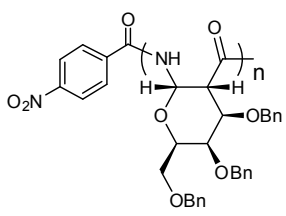


P1'$_{25}$ (126 MHz, $CDCl_3$)

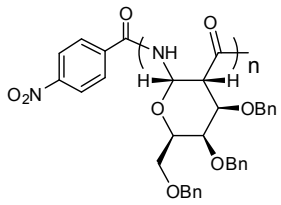


P1$_{25}$

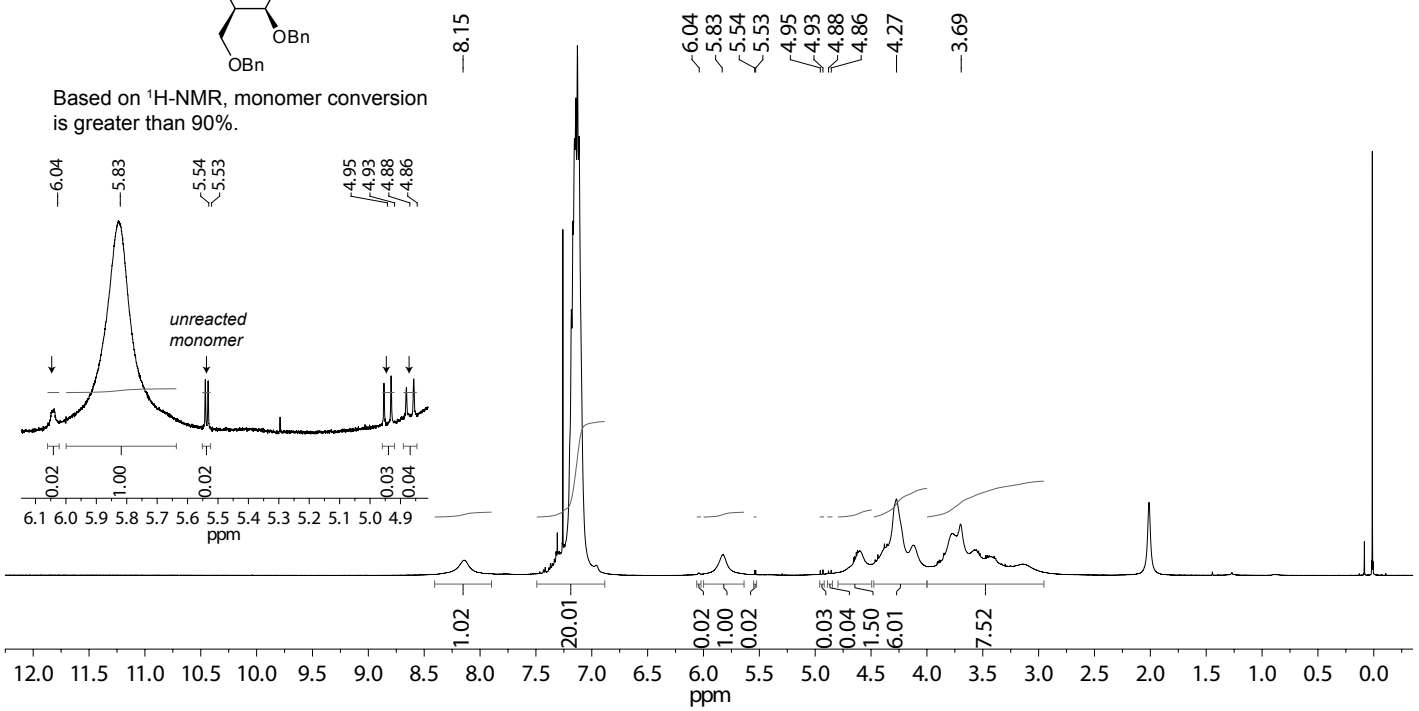
P1'$_{50}$ ^{1}H-NMR (500 MHz, $CDCl_3$)



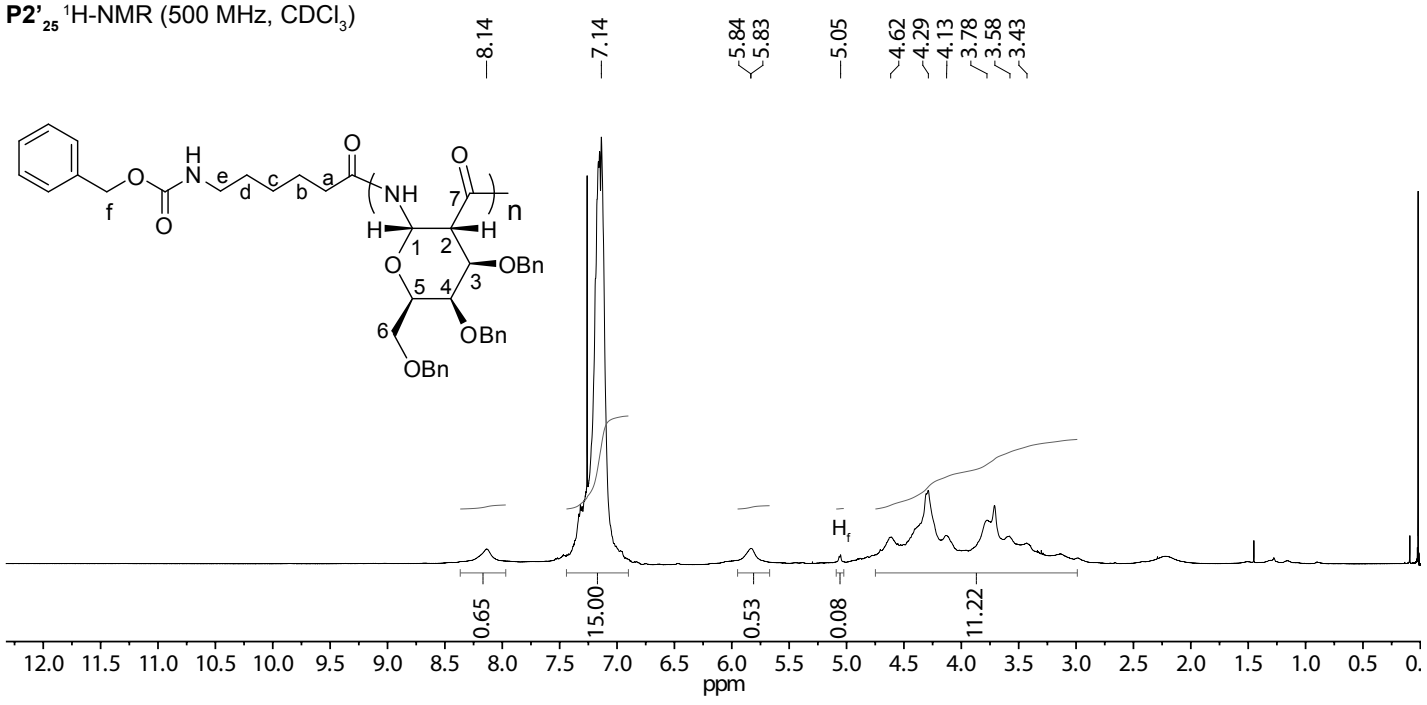
P1'$_{100}$ ^{1}H-NMR (500 MHz, $CDCl_3$)



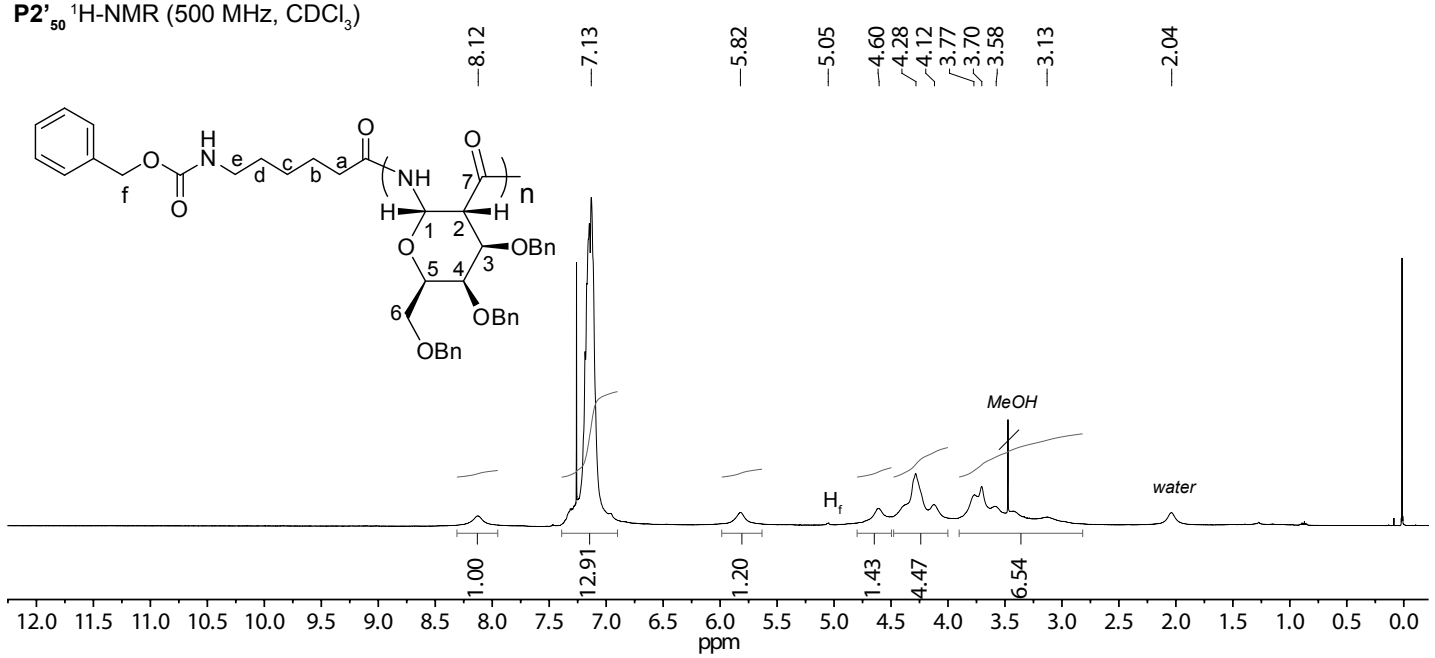
Based on ^{1}H-NMR, monomer conversion is greater than 90%.



P2'$_{25}$ ^{1}H-NMR (500 MHz, $CDCl_3$)



P2'$_{50}$ ^{1}H-NMR (500 MHz, $CDCl_3$)



P2'$_{100}$ ^{1}H-NMR (500 MHz, $CDCl_3$)

Based on ^{1}H-NMR, monomer conversion is approximately 83%, with 17% monomer remaining.