Supporting information for

Antiviral Agents from Multivalent Presentation of Sialyl Oligosaccharides on Brush Polymers

Shengchang Tang,[†] Wendy B. Puryear,[‡,§] Brian M. Seifried,[†] Xuehui Dong,[†] Jonathan A. Runstadler,[‡, §] Katharina Ribbeck,[‡] and Bradley D. Olsen[*,†]

Department of Chemical Engineering,[†] Department of Biological Engineering,[‡] and Division of Comparative Medicine,[§] 77 Massachusetts Avenue, Cambridge, MA 02139, USA

*To whom correspondence should be addressed: B.D.O. (Email: bdolsen@mit.edu)

Table of Contents

S1. Supplementary Figures Cited in the Main Text

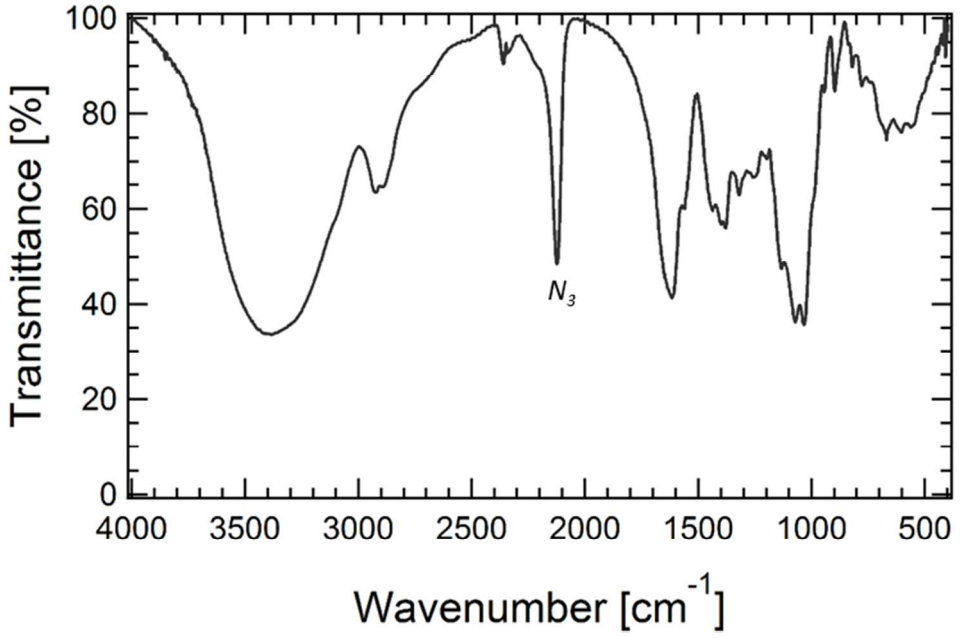


Figure S1. FTIR spectrum of 6'-sialyllactose azide. The peak at 2123 cm^{-1} corresponds to the antisymmetric stretch of azide.

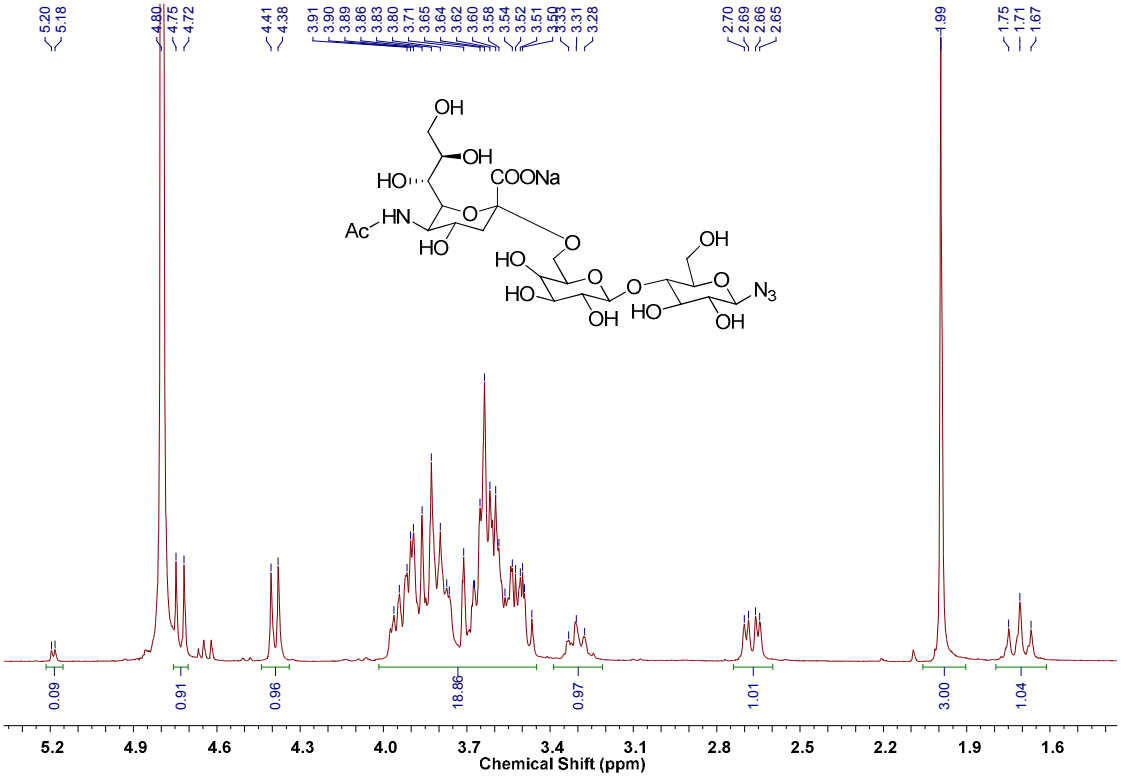


Figure S2. ^{1}H NMR spectrum of 6'-Sialyllactose azide in D_2O (300 MHz).

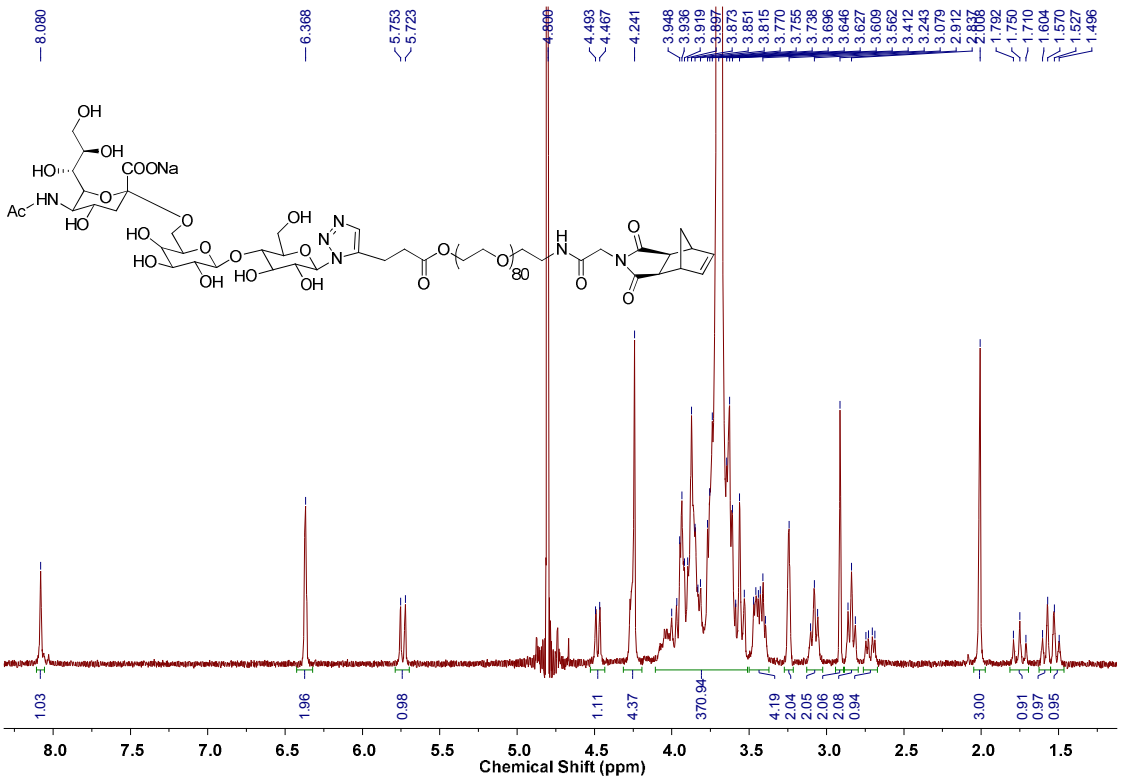


Figure S3. ^{1}H NMR spectrum of NB-PEG-SA in D_2O (300 MHz).

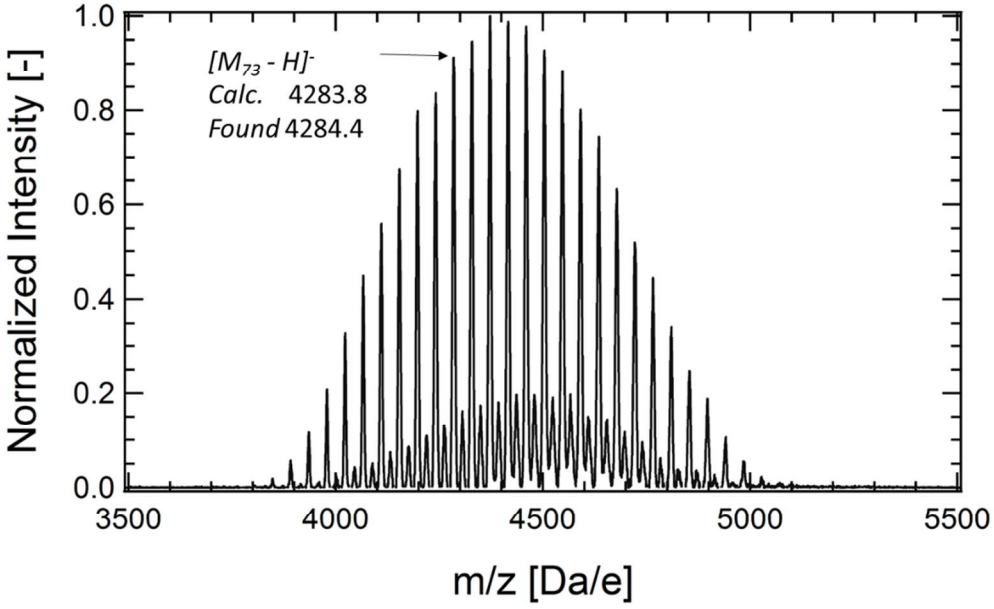


Figure S4. MALDI-TOF MS of NB-PEG-SA (negative linear mode).

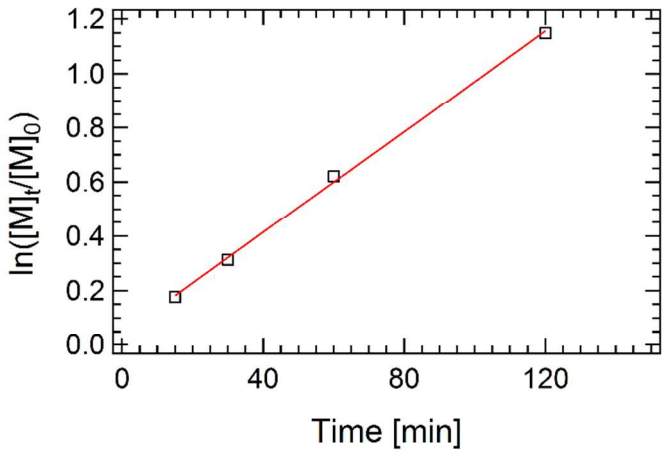


Figure S5. Representative kinetics in ROMP showing linear chain growth. Polymerization condition: [MM] = 0.025 M, target DP = 50. The propagation rate constant was determined from the slope, which gives k_p = 0.0093 min^{-1}.

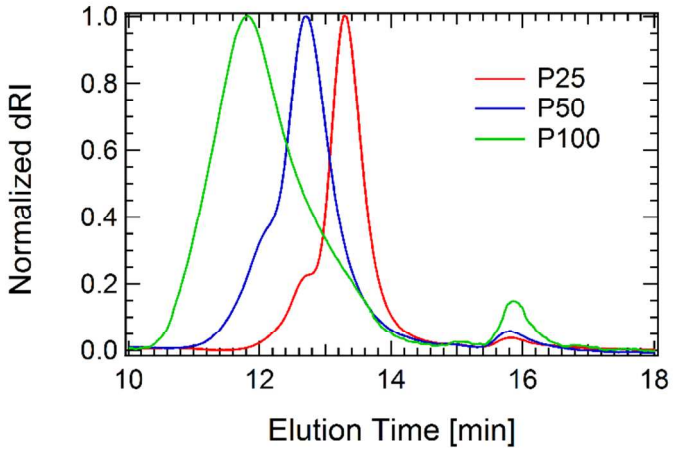


Figure S6. GPC traces of brush homopolymers P25, P50 and P100 (crude polymerization mixture). The peaks appearing at ca. 16 min were from residual MMs.

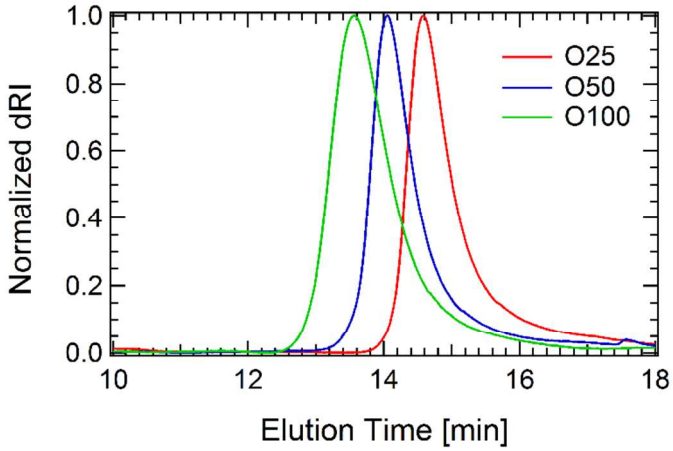


Figure S7. GPC traces of brush polymers O25, O50 and O100 (crude polymerization mixture).

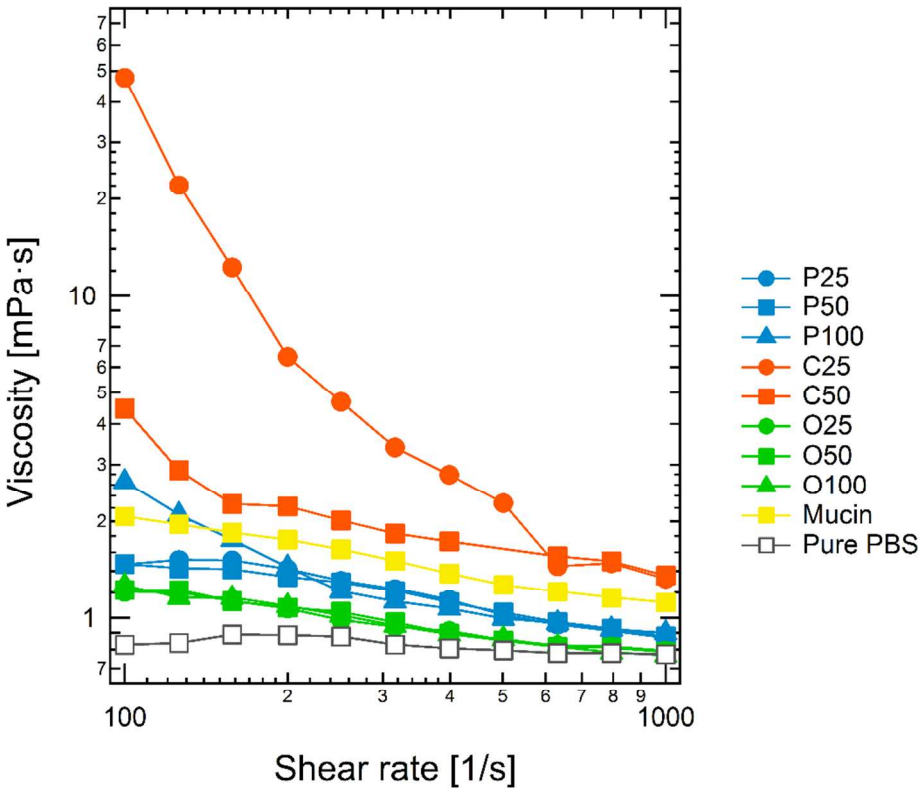


Figure S8. Viscosity measurement of brush polymer solutions at 37 °C. The sialic acid concentration in all solutions was 0.5 mM; therefore, C25 and C50 polymers are at higher concentration and therefore have higher viscosities due to the lower density of sialic acid on these brushes.

S2. Materials

Hydroxyl poly(ethylene glycol) amine, HCl salt (M_n = 3500 kg/mol, $Đ$ = 1.03) was purchased from JenKem Technology USA. Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) was purchased from Click Chemistry Tools. 6'-sialyllactose sodium salt was purchased from Carbosynth Limited. Mucin from the bovine submaxillary gland was purchased from Millipore (Cat# 499643, Lot# D00160765). While there are always concerns that the purification procedures for mucins may change their form from the native structure, the purification procedure for submaxillary gland mucins has been demonstrated to preserve both the high-molecular-weight structure and the sialic acid content of the original molecules.[1, 2]

Compounds *cis*-5-norbornene-*exo*-2,3-dicarboxylic anhydride[3], *N*-(glycine)-*cis*-5-norbornene-*exo*-dicarboximide[4], catalyst G3 $(H_2IMes)(pyr)_2(Cl)_2Ru{=}CHPh$[5], were prepared according to published procedures.

All other chemical reagents were purchased from commercial sources (Sigma-Aldrich and VWR) and used as received unless otherwise noted.

S3. Instrumentation and Characterization

NMR spectra were recorded on a Mercury 300 MHz spectrometer or an INOVA 500 MHz spectrometer. The residual undeuterated solvent peaks were used for references. The following abbreviations are used

to denote the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Coupling constants *J* are reported in Hertz (Hz).

Gel permeation chromatography (GPC) measurements were performed on an Agilent 1260 LC system with two columns (ResiPore, 300 × 7.5 mm, Agilent Technologies, CA) in series at 70 °C and a flow rate of 1 mL/min, where DMF with 0.02 M LiBr was used as the mobile phase. The molecular weights were determined using a Wyatt miniDAWN TREOS multi-angle light scattering detector and a Wyatt Optilab T-rEX differential refractive index detector.

Matrix-assisted laser desorption/ionization time-of- flight (MALDI-TOF) mass spectra were obtained on a Bruker Microflex instrument equipped with a 337 nm nitrogen laser in Koch Institute at MIT. α-Cyano-4-hydroxycinnamic acid (CHCA) was used as matrix. Low resolution mass spectra (LRMS) of small molecules were obtained from liquid chromatrography-mass spectrometry (LC-MS) analysis using an Agilent 6130 quadrupole mass spectrometer.

High performance liquid chromatography (HPLC) purification was performed on an Agilent 1260 system with a Zorbax 300SB-C18 PrepHT column. The mobile phase was a mixture of 0.1% acetic acid in MilliQ water and HPLC-grade acetonitrile.

Fourier transform infrared (FTIR) measurements were performed on a Thermo Nexus 870 spectrometer and results were processed using OMNIC software.

Ultraviolet–visible (UV-vis) absorbance scans were obtained on a Tecan Infinite 200 Pro microplate reader at 1 nm resolution.

Viscosity measurements were performed on an Anton Paar MCR 702 rheometer operated at the single-drive mode with a cone-and-plate geometry (50 mm diameter, 0.5° cone angle and 50 μm truncation gap). The temperature was controlled by a Peltier plate and was kept constant at 37 °C. Water evaporation was minimized by adding deionized water on top of the cone. Shear viscosities were determined in the shear rate range of 100 – 1000 1/s.

Fluorescence microscopy images were acquired at 10× magnification at the A488 channel on an Olympus IX-81 inverted fluorescence microscope. The exposure time was fixed at 250 ms for consistency.

S4. General Procedures for Ring Opening Metathesis Polymerization (ROMP)

All polymerizations were performed in degassed DMF (HPLC grade) under nitrogen atmosphere in a glovebox. A stock solution of G3 catalyst was freshly prepared at a concentration of 4 mg/mL. In a typical experiment, a 2 mL vial equipped with a small stir bar was charged with 100 μL of MM solution. Under vigorous stirring, the desired amount of catalyst solution was added to the vial to initiate polymerization. The final concentration of MMs was adjusted from 0.01 to 0.05 M such that the gelation was retarded during polymerization to enhance kinetic control. The reaction time was varied from 2 to 24 h to maximize the conversion of MMs, and the reaction was terminated by addition of 1 – 2 drops of cold ethyl vinyl ether. The solution was then diluted and a small aliquot was taken for GPC analysis.

Polymers were diluted with 14 mL of MilliQ water and transferred to a 15 mL centrifugal filter (50 kDa MWCO for entries 1-5 in Table 1, and 10 kDa MWCO for entries 6-8 in Table 1). The solution was concentrated to less than 1 mL by spinning at 4,000 RPM at room temperature. The remaining solution was again diluted with 14 mL of water. After the process was repeated 5 – 10 times to ensure complete removal of unreacted MMs, the remaining solution was lyophilized to afford pure brush polymers for subsequent studies.

S5. Hemagglutination Inhibition Assay

In a typical experiment, 25 μL of virus suspension containing 4 hemagglutinin units (HAU) was added to the first column of a 96-well V-bottom plate and was serially diluted to the columns after. Then 25 μL of polymer solution ([SA] = 0.5 mM) in Dulbecco's phosphate buffered saline (DPBS without Ca2+ and Mg2+, Lonza BioWhittaker™) was added to each well. After the mixture was incubated at room temperature for 30 min, 50 μL of 0.5% chicken erythrocytes was added to each well. The entire plate was gently tapped to ensure good mixing of the components, and the results were reported after 1 h incubation at room temperature. Incomplete hemagglutination could be identified by a teardrop pattern when the well plate was tilted. The titer was determined from the maximum dilution that still showed complete hemagglutination inhibition.

S6. In Vitro Infection Assay

The in vitro infection assays were performed using a procedure slightly modified from a previous report.3 MDCK-SIAT1-CMV-PB1 cells were cultured in D10 medium to reach 80 – 100% confluency, trypsinized, resuspended and diluted to 300,000 cells/mL. In the infection experiments, 30,000 cells were seeded in each well of a 96-well flat bottom plate (tissue-culture treated) and were allowed to adhere to the plate at 37 °C overnight (16 – 18 h). Medium was then removed and replaced by 150 μL of infection growth medium (IGM). After incubation at 37 °C for 3 h, the medium was removed and replaced by 50 μL of polymer solution at various concentrations in DPBS. After incubation at 37 °C for 2 h, 5 μL of engineered A/WSN/1933 (H1N1) virus with PB1-GFP reporter genes (1×10^7 virus particles/mL) was gently added to each well. For the negative control, 5 μL of DPBS was added. Solutions were removed after another 2 h incubation step, and cells were washed with 150 μL DPBS three times. After 150 μL of IGM was added to each well, cells were cultured at 37 °C for 18 h to allow expression of GFP. The number of GFP positive cells was counted with an Accuri C6 flow cytometer. Results were analyzed using the FlowJo software and 0.5% GFP-positive cells was set for the uninfected control.

Media used in the studies was prepared according to the recipes shown below. Solutions were filtered through a 0.22 μm PES filter and stored at 4 °C.

(1) D10 medium: DMEM (HyCloneTM, #SH30243.01) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 ug/ml streptomycin.

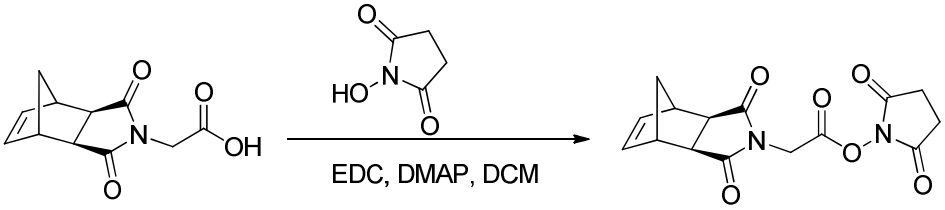
(2) IGM: OptiMEM (InvitrogenTM, #31985-088) supplemented with 0.01% FBS (heat-inactivated), 0.3% BSA (InvitrogenTM, #15260-037), 100 U/ml penicillin and 100 μg/ml streptomycin, and 100 μg/ml calcium chloride.

S7. Acidic Ninhydrin Assay

To determine SA content in the commerically available mucin sample, a published procedure[6] was adopted with slight modification. Acid ninhydrin reagent was prepared by stirring 1 g of ninhydrin in 24 mL of glacial acetic acid until the ninhydrin was completely dissolved. Then, 16 mL of concentrated HCl (ca. 36%) was slowly added to the mixture. In a typical assay setup, mucin solutions, acid ninhydrin reagent and glacial acetic acid were mixed at a 1:1:1 volume ratio. The mixture was boiled for exactly 10 min on a heat block and was cooled down immediately in a chilled water bath. Subsequently, 100 μL of the reaction mixture was added to a Greiner UV-Star® 96 well plate (flat bottom), and an absorbance scan was taken from 350 nm to 700 nm. The absorbance at 470 nm was used to quantify the sialic acid content. Experiments were performed in triplicates. The sialic acid content was determined to be ca. 10 wt% in mucins.

S8. Synthetic Procedures for Macromonomers

Synthesis of NB-NHS

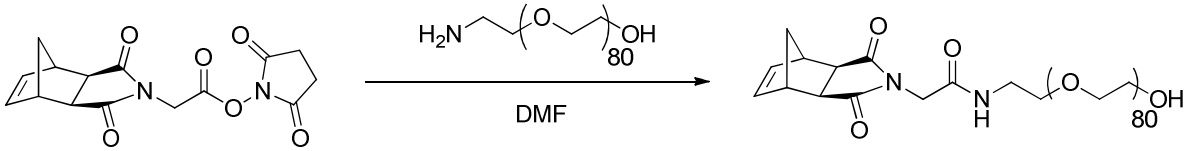


To a 300 mL round bottom flask was added *N*-(glycine)-*cis*-*5*-norbornene-*exo*-dicarboximide (500 mg, 2.26 mmol), *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl, 650 mg, 3.39 mmol), and 4-(dimethylamino)pyridine (DMAP, 41.4 mg, 0.34 mmol). Dichloromethane (100 mL) was then added to dissolve the solids. After 15 minutes, *N*-hydroxy succinimide (390 mg, 3.39 mmol) was added, and the reaction was stirred at room temperature overnight. Solvent was removed under vacuum, and the residue was purified by silica gel flash column chromatography using 50 – 80% EtOAc in hexanes to give white solids (319.4 mg, 44% yields, R_f = 0.2, 50% EtOAc in hexanes).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.27 (s, 2H), 4.53 (s, 2H), 3.28 (s, 2H), 2.81 (s, 4H), 2.75 (s, 2H), 1.53-1.44 (m, 2H).

LRMS (ESI) m/z calculated for $C_{15}H_{15}N_2O_6$ $[M + H]^+$ 319.1, found 319.0.

Synthesis of NB-PEG-OH



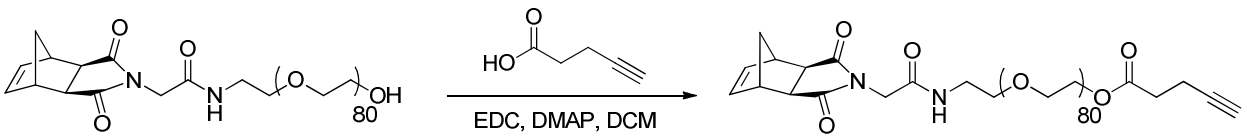
Hydroxyl PEG amine (1 g) was dissolved in 100 mL of 1 M sodium carbonate solution and stirred for 30 min to liberate the free amine. The aqueous solution was extracted by dichloromethane (3 × 50 mL). The combined organic phase was dried over Na_2SO_4, and the solvent was removed under vacuum to afford 0.96 g white solid (yield 96%).

Purified hydroxyl PEG amine was dissolved in anhydrous N,N-dimethylformamide (DMF, 30 mL), and NB-NHS ester (109.4 mg, 0.34 mmol) was added in one portion. After reaction overnight, solvent was removed under vacuum, and the residue was redissolved in a small amount of dichloromethane (ca. 10 mL) and precipitated in cold diethylether. The precipitation was performed three times to give a white solid (0.98 g, yield 93%).

^{1}H NMR (500 MHz, D_2O): 6.35 (t, 2H, J = 2.0 Hz), 4.22 (s, 2H), 3.82 (t, 2H, J = 4.5 Hz), 3.73 – 3.58 (m, 384H), 3.53 (m, 2H, J = 4.5 Hz), 3.39 (t, 2H, J = 5.0 Hz), 3.23 (s, 2H, J = 1.5 Hz), 2.89 (s, 2H), 1.57 (d, 1H, J = 10.0 Hz), 1.50 (dt, 1H, J = 1.5 Hz and 10.0 Hz).

The structure was also confirmed by MALDI-TOF MS (Figure S16).

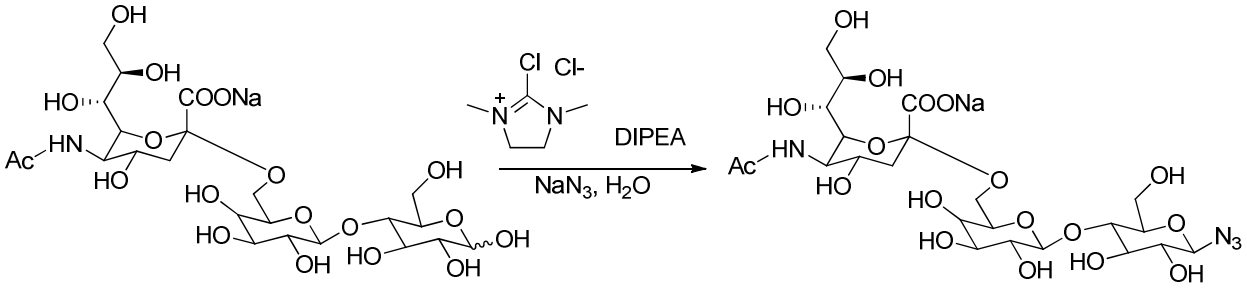
Synthesis of NB-PEG-alkyne



NB-PEG-OH (300 mg, 81 μmol), pentynoic acid (11.9 mg, 122 μmol), DMAP (2.0 mg, 16 μmol) were dissolved in 10 mL of dichloromethane. EDC·HCl (23.3 mg, 122 μmol) was then added to the solution, and the reaction was stirred at room temperature overnight. After solvent was evaporated under vacuum, the crude product was dissolved in a small amount of dichloromethane and precipitated in cold diethylether. The precipitation was performed three times to give a white solid (290 mg, yield 94%).

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.76 (s, 1H), 6.26 (s, 2H), 4.26 (t, 2H, J = 5.0 Hz), 4.15 (s, 2H), 3.78 (t, 2H, J = 5.0 Hz), 3.72 – 3.54 (m, 384H), 3.49 (t, 2H, J = 5.0 Hz), 3.45 (m, 2H), 3.30 (s, 2H), 2.72 (s, 2H), 2.58 (m, 2H), 2.50 (m, 2H), 1.98 (t, 1H, J = 2.5 Hz), 1.87 (d, 1H, J = 10.0 Hz), 1.51 (d, 1H, J = 10.0 Hz).

Synthesis of 6'-sialyllactose azide

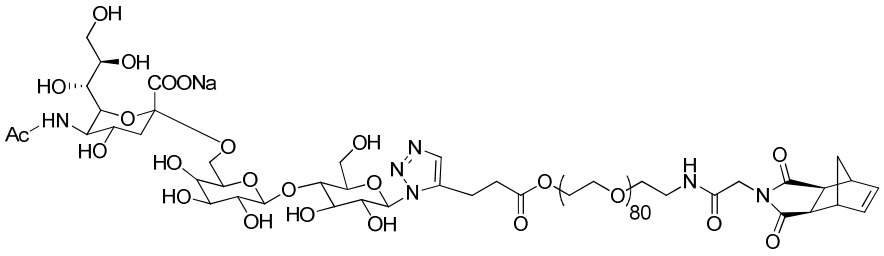


The synthetic procedure was slightly modified from the published literature.[7-9] First, *N*,*N*-diisopropylethylamine (DIPEA, 1.33 mL, 7.63 mmol) was added to a clear solution containing 6'-sialyllactose sodium salt (500 mg, 0.76 mmol) and sodium azide (496 mg, 7.63 mmol) in 1.57 mL of water. After 2-chloro-1,3-dimethylimidazolinium (DMC, 387 mg, 2.29 mmol) was added, the two phase mixture was stirred vigorously at 0 °C for 1 h. The solvent was then evaporated under vacuum, and DMF was added to dissolve the residual solid. Undissolved sodium azide was filtered, and the filtrated was concentrated under vacuum. The residual was dissolved in 50 mL of water and extracted with DCM (50 mL × 2). The aqueous phase was loaded onto a short ion-exchange column packed with Amberlite IR-120 resin (hydrogen form, previously activated with 1 M NaOH aqueous solution), and eluted with MilliQ water. The solution was neutralized to pH 6.5, concentrated under vacuum, dialyzed against water (MWCO 500 Da), and lyophilized to give a white powder (413 mg, yield 80%).

^{1}H NMR (300 MHz, D_2O): δ 5.19 (d, 0.09 H, J = 3.6 Hz), 4.74 (d, 0.91H, J = 8.7 Hz), 4.39 (d, 1H, J = 7.8 Hz), 4.00-3.43 (m, 18H), 3.31 (t, 1H, J = 8.7 Hz), 2.67 (dd, 1H, J = 4.5 Hz and 12.3 Hz), 1.99 (s, 3H), 1.71 (t, 1H, J = 12.3 Hz). The spectrum is similar to reference[9], and 90% of the product is β anomer.

LRMS (ESI) m/z calculated for $C_{23}H_{37}N_4O_8$ $[M - Na]^-$ 657.2, found 657.2.

Synthesis of NB-PEG-SA

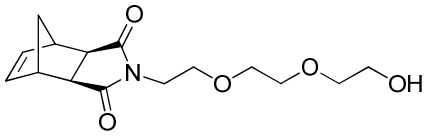


TBTA stock solution was prepared in DMF at a concentration of 88.7 mg/mL, and $CuSO_4$ stock solution was prepared in MilliQ water at a concentration of 26.7 mg/mL. Then, the Cu(II)-TBTA complex was prepared by mixing 50 µL TBTA solution and 50 µL $CuSO_4$ stock solution. The mixture was vortexed to ensure homogeneous mixing. Separately, NB-PEG-alkyne (290 mg, 76 µmol) and 6-SA-Lac-N_3 (56.8 mg, 84 µmol) was dissolved in 4 mL of DMF/water mixture (1:1 v/v). Then 100 µL of Cu(II)-TBTA complex (0.1 equiv. to azide) was added *via* a syringe, and the resulting solution was degassed by sparging with nitrogen gas. The reaction was initiated by injecting 100 µL of freshly prepared sodium ascorbate solution (66.2 mg/mL in water, 0.4 equiv. to azide), and the color of mixture immediately turned light yellow. The reaction was stirred at room temperature overnight to maximize conversion. The reaction mixture was filtered through a 0.45 µm PTFE filter and purified by preparatory HPLC using a gradient of 10-60% MeCN in water with 0.1% acetic acid over 30 min. The combined pure fractions were neutralized to pH 6.5, concentrated under vacuum, dialyzed against MilliQ water (MWCO 3500 Da) and finally freeze-dried to give a white powder (247 mg, yield 72%).

^{1}H NMR (300 MHz, D_2O): 8.08 (s, 1H), 6.37 (s, 2H), 5.74 (d, 1H, J = 9.0 Hz), 4.48 (d, 1H, J = 7.8 Hz), 4.24 (m, 2H), 4.10 – 3.51 (m, 371H), 3.44 (m, 4H), 3.24 (s, 2H), 3.08 (t, 2H, J = 6.9 Hz), 2.91 (s, 2H), 2.84 (t, 2H, J = 6.9 Hz), 2.72 (dd, 1H, J = 4.8 Hz and 12.3 Hz), 2.01 (s, 3H), 1.75 (t, 1H, J = 12.3 Hz), 1.59 (d, 1H, J = 9.9 Hz), 1.51 (d, 1H, J = 9.9 Hz).

The structure was also confirmed by MALDI-TOF MS (Figure S4).

Synthesis of NB-OEG3-OH

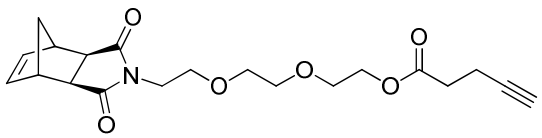


A round bottom flask was charged with *cis*-5-norbornene-*exo*-2,3-dicarboxylic anhydride (0.95 g, 5.80 mmol), 2-(2-(2-aminoethoxy)ethoxy)ethanol (0.95 g, 6.28 mmol), triethylamine (80 µL, 0.58 mmol) and toluene (20 mL). After the reaction was refluxed at 140 °C overnight with a Dean-Stark apparatus, the reaction was cooled to room temperature, and the solvent was removed under vacuum. The residue was dissolved in 30 mL of DCM, washed with 0.1 M HCl (a.q.), and washed with brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to give a slightly yellow viscous oil (1.63 g, yield 95%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.28 (t, 2H, J = 1.8 Hz), 3.76 – 3.50 (m, 12H), 3.30 – 3.23 (m, 2H), 2.69 (d, 2H, J = 1.4 Hz), 1.66 (br), 1.49 (d, 1H, J = 9.9 Hz), 1.35 (d, 1H, J = 9.9 Hz).

LRMS (ESI) m/z calculated for $C_{20}H_{26}NO_6$ $[M + H]^+$ 296.1, found 296.1.

Synthesis of NB-OEG3-AK

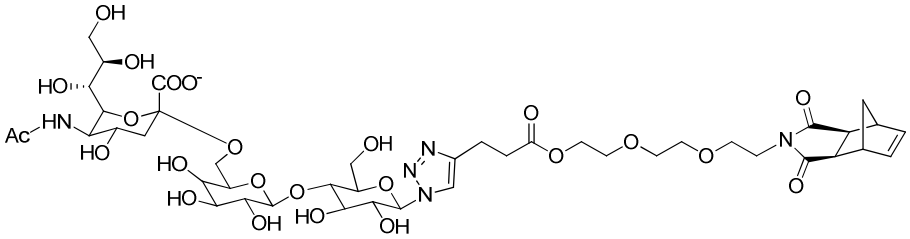


NB-OEG3-OH (738 mg, 2.5 mmol), pentynoic acid (250 mg, 2.5 mmol), and DMAP (100 mg, 0.82 mmol) were dissolved in 15 mL of dichloromethane. EDC·HCl (580 mg, 3.0 mmol) was then added to the solution, and the reaction was stirred at room temperature overnight. After solvent was evaporated under vacuum, the reaction mixture was redissolved in 20 mL DCM, washed with water and purified over silica gel column chromatography using EtOAc/hexanes (3:1, v/v) to afford a slightly yellow viscous oil (577 mg, yield 62%, R_f = 0.58).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.27 (t, 2H, J = 1.8 Hz), 4.30 – 4.14 (m, 2H), 3.73 – 3.61 (m, 6H), 3.60-3.54 (m, 4H), 3.27 – 3.24 (m, 2H), 3.31 – 3.20 (m, 2H), 2.67 (d, 2H, J = 1.2 Hz), 2.61 – 2.53 (m, 2H), 2.53 – 2.45 (m, 2H), 1.97 (t, 1H, J = 2.6 Hz), 1.47 (d, 1H, J = 9.9 Hz), 1.35 (d, 1H, J = 9.9 Hz).

LRMS (ESI) m/z calculated for $C_{20}H_{26}NO_6$ $[M + H]^+$ 376.2, found 376.2.

Synthesis of NB-OEG3-SA



The synthesis was performed using a similar method as the compound NB-PEG-SA, and the reaction was monitored by LC-MS to ensure > 90% conversion. The reaction mixture was purified by preparatory HPLC using a gradient of 5-60% MeCN in water with 0.1% acetic acid over 20 min. The combined pure fractions were neutralized to pH 6.5, mixed with 60 mg of metal scavenger (Ethylenediaminetriacetic acid acetamide, polymer-bound, #656844, Aldrich, 20 equiv. relative to the original amount of $CuSO_4$ in reactant mixture) and stirred overnight to remove residual Cu^{2+}. The beads were filtered, and the filtrate was dialyzed against MilliQ water (MWCO 500 Da) and finally freeze-dried to give a white powder (yield 60%).

^{1}H NMR (300 MHz, D_2O): 8.06 (s, 1H), 6.31 (s, 2H), 5.73 (d, 1H, J = 9.3 Hz), 4.47 (d, 1H, J = 7.8 Hz), 4.21 (s, 2H), 4.11 – 3.47 (m, 31H), 3.17 (s, 2H), 3.06 (t, 2H, J = 6.9 Hz), 2.87 – 2.76 (m, 4H), 2.70 (dd, 1H, J = 4.5 Hz and 12.0 Hz), 2.00 (s, 3H), 1.75 (t, 1H, J = 12.0 Hz), 1.45 (d, 1H, J = 9.9 Hz), 1.27 (d, 1H, J = 9.9 Hz).

LRMS (ESI) m/z calculated for $C_{43}H_{62}N_5O_{24}$ $[M - Na]^-$ 1032.4, found 1032.4.

S9. Additional Supporting Figures

A. GPC

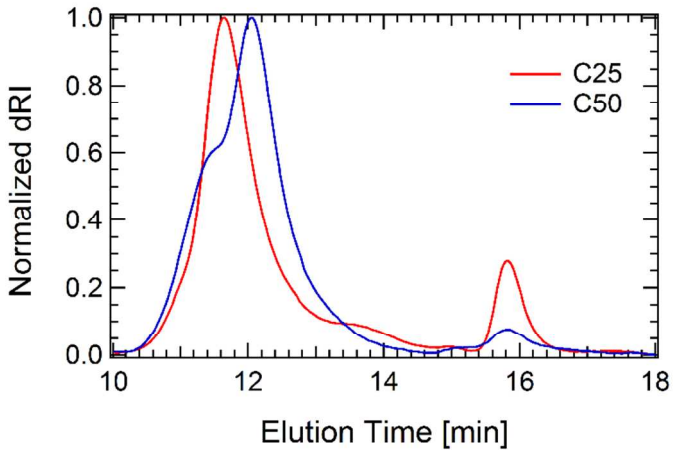


Figure S9. GPC traces of brush copolymers C25 and C50 (crude polymerization mixture). Relative poor control over the molecular weight distribution is attributed to rapid gelation during polymerization.

B. NMR spectra

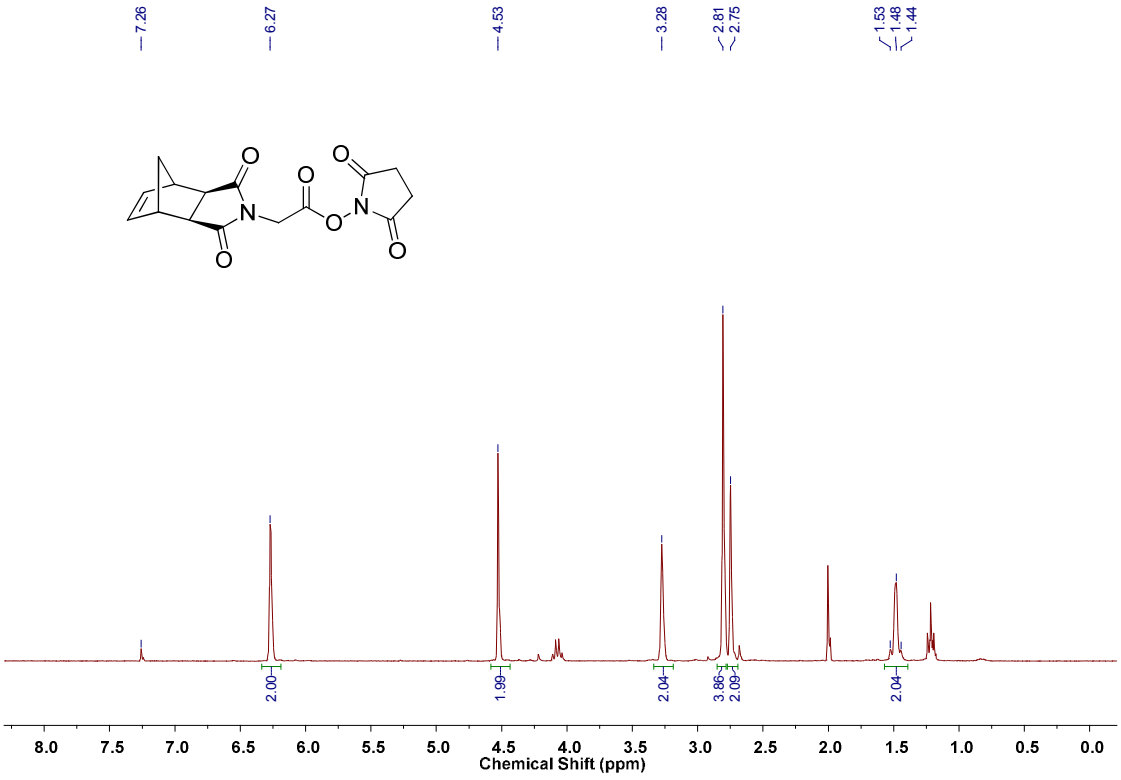


Figure S10. ^{1}H NMR spectrum of NB-NHS in $CDCl_3$ (300 MHz).

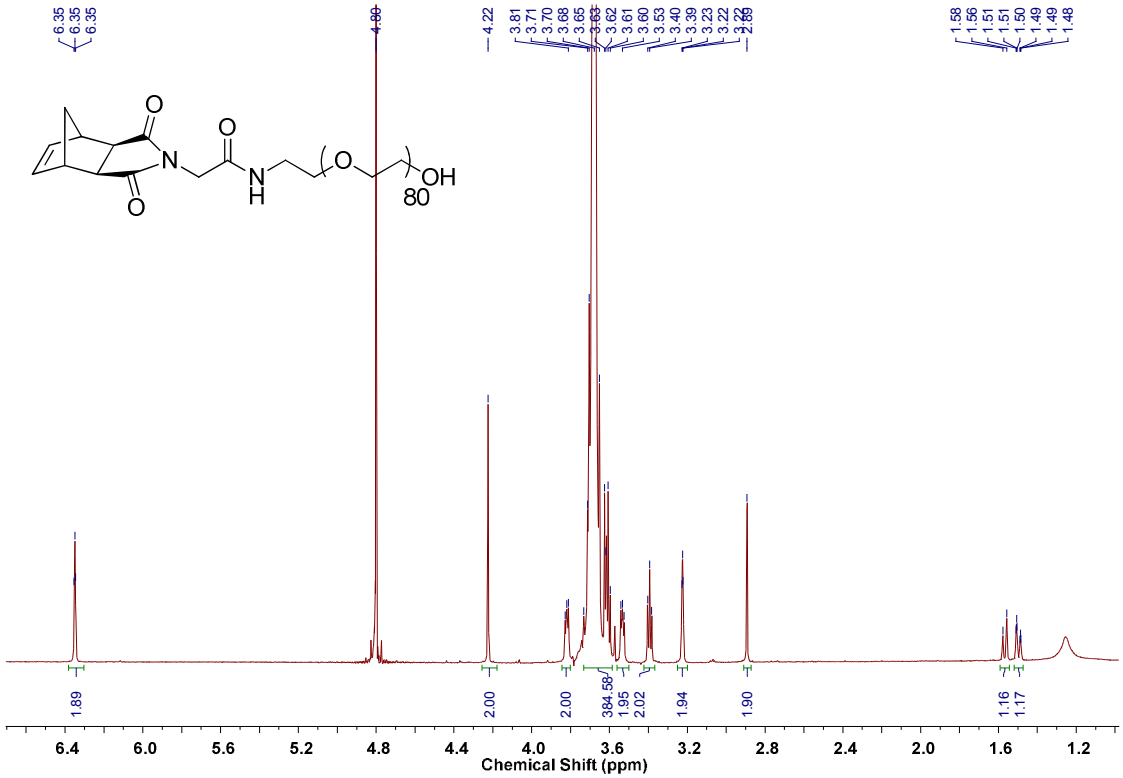


Figure S11. ^{1}H NMR spectrum of NB-PEG-OH in D_2O (500 MHz).

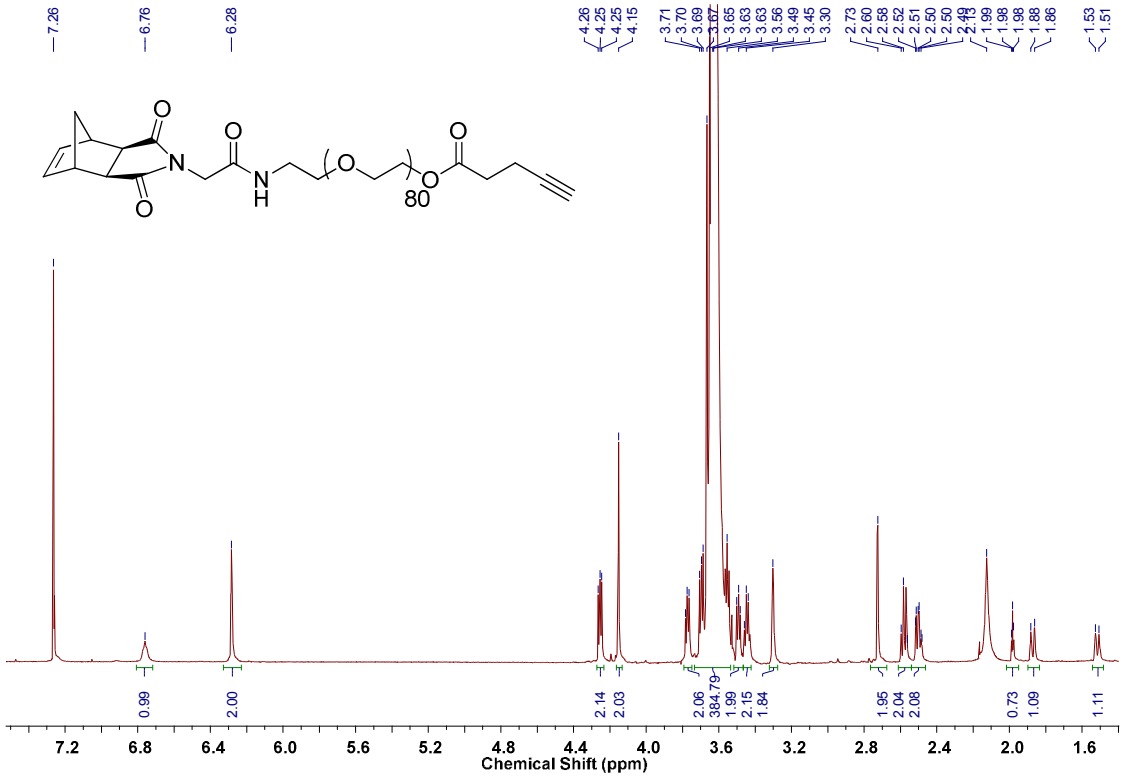


Figure S12. ^{1}H NMR (300 MHz) spectrum of NB-PEG-AK in $CDCl_3$.

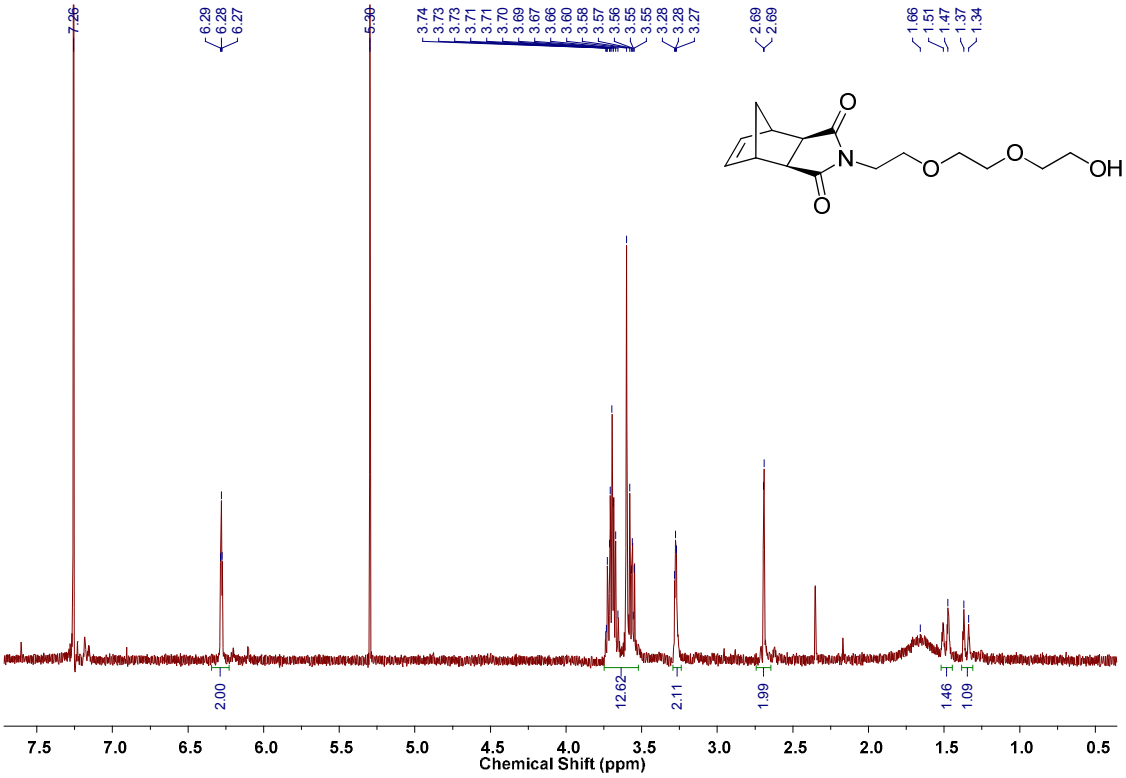


Figure S13. ^{1}H NMR spectrum of NB-OEG3-OH in $CDCl_3$ (300 MHz).

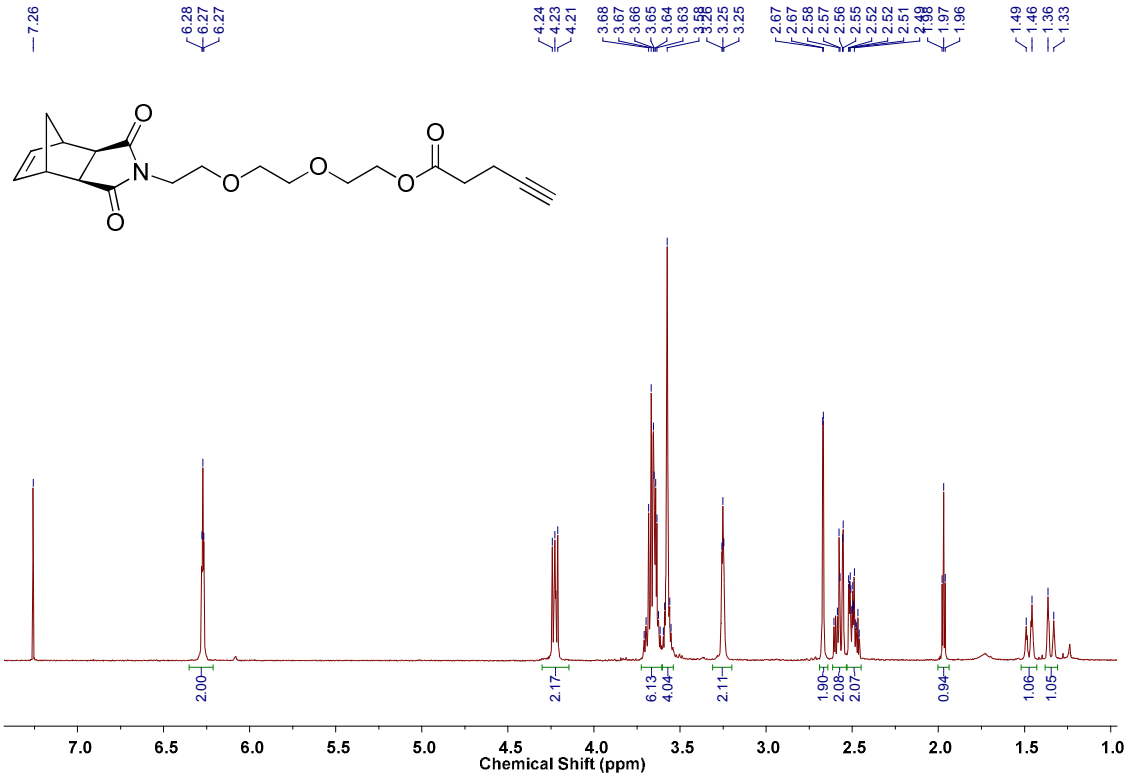


Figure S14. ^{1}H NMR (300 MHz) spectrum of NB-OEG3-AK in $CDCl_3$.

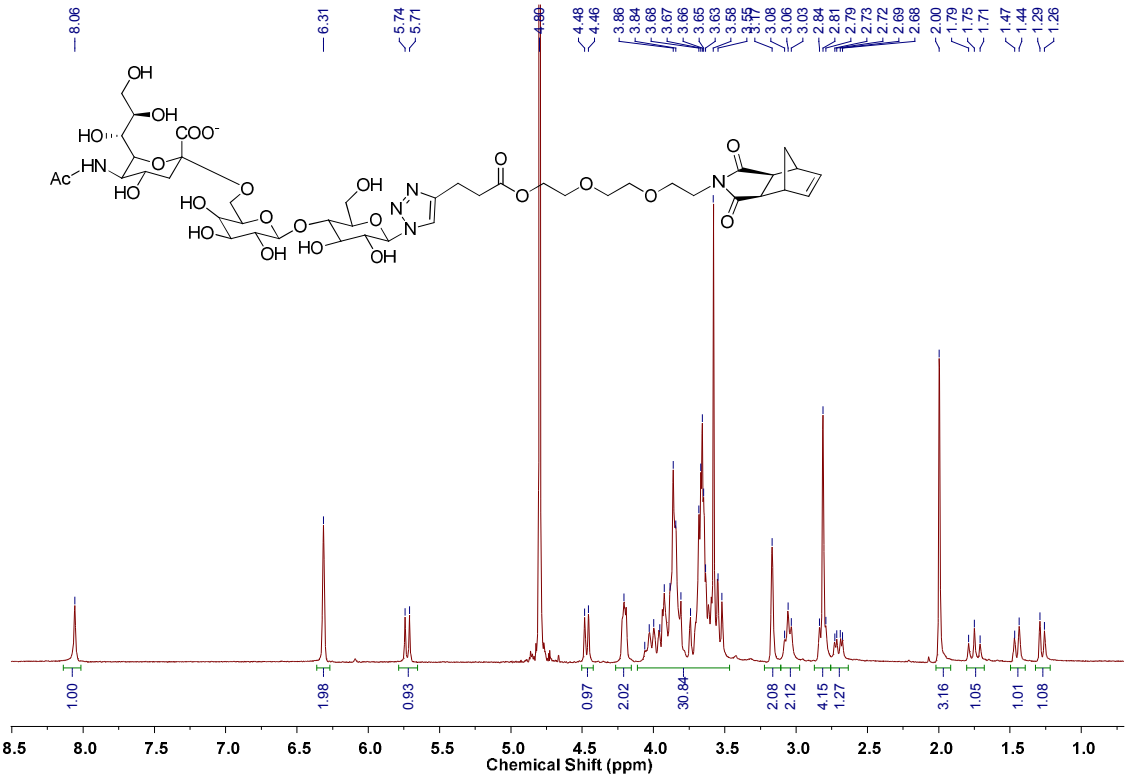


Figure S15. ^{1}H NMR spectrum of NB-OEG3-SA in D_2O (300 MHz).

C. MALDI-TOF MS

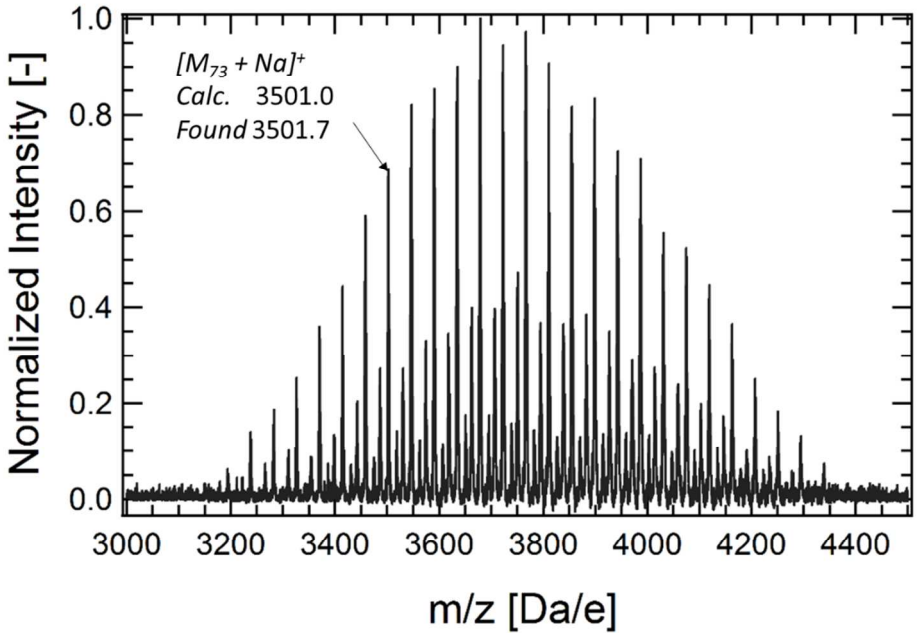


Figure S16. MALDI-TOF MS of NB-PEG-OH (positive reflector mode).

S10. References:

1. Hill, H. D.; Reynolds, J. A.; Hill, R. L., *Journal of Biological Chemistry* **1977,** *252* (11), 3791-8.
2. Tettamanti, G.; Pigman, W., *Archives of Biochemistry and Biophysics* **1968,** *124*, 41-50.
3. Matson, J. B.; Grubbs, R. H., *Journal of the American Chemical Society* **2008,** *130* (21), 6731-6733.
4. Conrad, R. M.; Grubbs, R. H., *Angewandte Chemie International Edition* **2009,** *48* (44), 8328-8330.
5. Love, J. A.; Morgan, J. P.; Trnka, T. M.; Grubbs, R. H., *Angewandte Chemie International Edition* **2002,** *41* (21), 4035-4037.
6. Yao, K.; Ubuka, T.; Masuoka, N.; Kinuta, M.; Ikeda, T., *Analytical Biochemistry* **1989,** *179* (2), 332-335.
7. Tanaka, T.; Nagai, H.; Noguchi, M.; Kobayashi, A.; Shoda, S.-i., *Chemical Communications* **2009,** (23), 3378-3379.
8. Vinson, N.; Gou, Y.; Becer, C. R.; Haddleton, D. M.; Gibson, M. I., *Polymer Chemistry* **2011,** *2* (1), 107-113.
9. Tanaka, T.; Ishitani, H.; Miura, Y.; Oishi, K.; Takahashi, T.; Suzuki, T.; Shoda, S.-i.; Kimura, Y., *ACS Macro Letters* **2014,** *3* (10), 1074-1078.