Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Optimization of Ring-Opening Metathesis Polymerization (ROMP) under Physiologically Relevant Conditions

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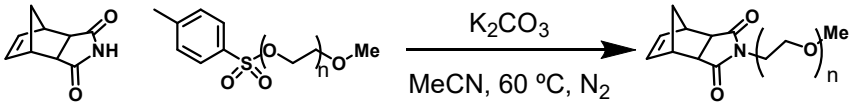
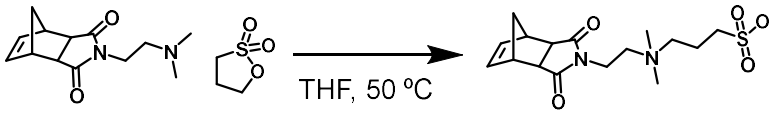
General Considerations. *Materials*: All reagents and solvents were used as obtained from commercial sources. [1,3-Bis(2,4,6-trimethylphenyl)-4-[(4-ethyl-4-methylpiperazin-1-ium-1-yl)methyl]imidazolidin-2-ylidene]-(2-i-propoxybenzylidene)dichlororuthenium(II) chloride (**AquaMet)** was purchased from Strem Chemicals. Compounds **S1**[1], **PEG-OTs**[2] **NB-NMe$_2$**[3] **oNB-PEG**[2], **oNB-NMe$_2$**[4] **oNB-Sulfo**[5] and **NB-OMe**[1] were synthesized as previously reported.

Instrumentation: ^{1}H and ^{13}C NMR spectra were recorded on a Varian 600 MHz spectrometer. Chemical shifts are reported in delta (δ) units, expressed in parts per million (ppm) downfield from tetramethylsilane using the residual protio-solvent as an internal standard ($CDCl_3$, ^{1}H: 7.26 ppm and ^{13}C: 77.16 ppm; DMSO, ^{1}H: 2.50 ppm and ^{13}C: 39.52; D_2O, ^{1}H: 4.79 ppm). Molecular mass data was obtained using a Micromass Quattro Ultima Triple Quadrupole mass spectrometer. Gel Permeation Chromatography (GPC) setup consists of: a Shimadzu pump, 2 in-line Phenomenex Phenogel 5 µm size-exclusion columns (10^3 and 10^4 Å) within a Shimadzu column oven, and a Shimadzu RID-10A differential refractive index detector. The mobile phase consisted of THF at a 1 mL/min flow rate at 30 °C. The sample injection volume was 25 µL. Peaks were calibrated against a set of polystyrene standards. A BioTek Synergy HT plate reader was used for catalyst decomposition studies. DLS was conducted with a Malvern Panalytical Zetasizer Nano ZS.



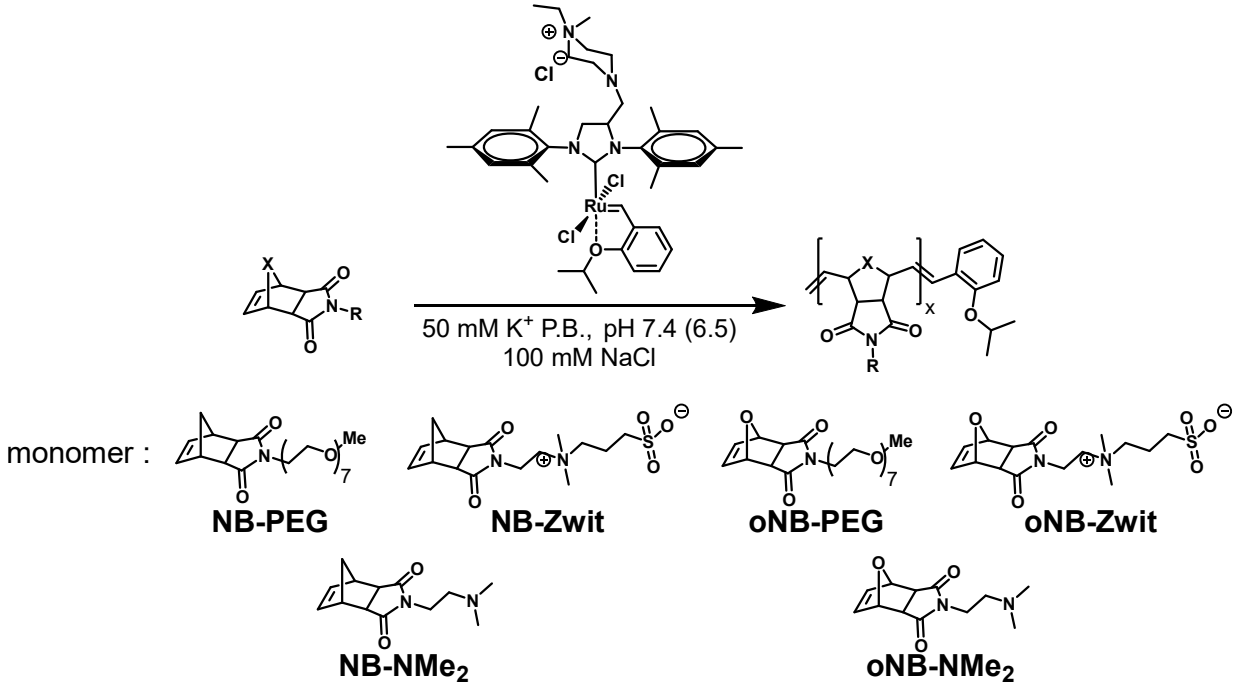
S1 **PEG-OTs** **NB-PEG**

Synthesis of **NB-PEG**: Into a round bottom flask was added stir bar, **S1** (1.22 g, 7.48 mmol, 1.2 eq.) and K_2CO_3 (2.24 g, 16.21 mmol, 2.6 eq.). A solution of **PEG-OTs** (3.15 g, 6.23 mmol, 1.0 eq.) in 35 mL acetonitrile was then added to the flask and the reaction vessel was sealed. The flask was then lowered into a pre heated oil bath and stirred for 18 hours. The reaction mixture was allowed to cool and the solid precipitate was removed via vacuum filtration. The filtrate was then concentrated under reduced pressure and the crude residue was purified by column chromatography using a gradient from EtOAc to 5% MeOH/EtOAc. This yielded a colorless viscous oil (1.7 g, 55 % yield). ^{1}H-NMR spectra were consistent with the previous synthesis of this molecule.[1] ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.28 (s, 2H), 3.73 – 3.46 (m, 24H), 3.38 (s, 3H), 3.26 (s, 2H), 2.68 (s, 2H), 1.48 (d, J = 9.8 Hz, 1H), 1.36 (d, J = 9.8 Hz, 1H).



NB-NMe$_2$ **NB-Sulfo**

Synthesis of **NB-Sulfo**: Into a scintillation vial was added stir bar, **NB-NMe$_2$** (196 mg, 0.84 mmol, 1.0 eq.) and THF (2 mL). 1,3-propane sultone (101 mg, 0.84 mmol, 1.0 eq.) was then added to the vial and the reaction vessel was sealed. The vial was then lowered into a pre heated oil bath and stirred for 18 hours. A white precipitate precipitated out of solution. The reaction mixture was allowed to cool and the solid precipitate was isolated via vacuum filtration. The solids were washed 2x with 10 mL diethyl ether and allowed to dry under vacuum. Product was isolated as a white solid (126 mg, 42 % yield). ^{1}H NMR (600 MHz, D_2O) δ 6.39 (s, 2H), 4.08 – 3.95 (m, 2H), 3.68 – 3.46 (m, 4H), 3.28 (s, 2H), 3.24 (s, 6H), 3.03 (m, 2H), 2.93 (s, 2H), 2.35 – 2.21 (m, 2H), 1.56 (d, J = 10.2 Hz, 1H), 1.23 (d, J = 10.2 Hz, 1H). LRMS: exact mass [M] calcd for: $C_{16}H_{23}N_2O_5S$ 356.14; $[M+H]^+$, found 357.07.



General Polymerization Procedure for Conditions Optimization and Monomer Scope

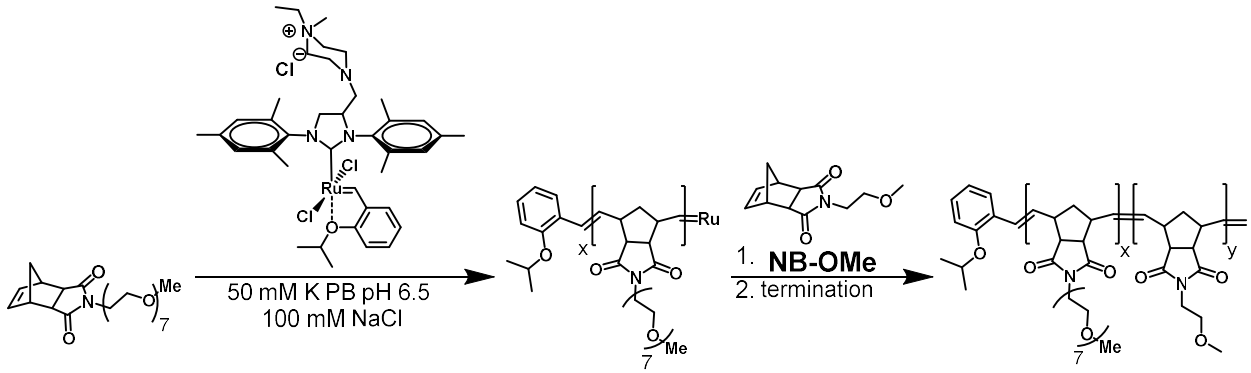
In an Eppendorf tube, **NB-PEG** (15.5 mg, 0.031 mmol, 50.0 eq.) was dissolved in 155 μL of 100 mM potassium phosphate buffer, pH 7.4. To the same Eppendorf tube, 77.5 μL of 400 mM NaCl and 52.5 μL of deionized water were added. In a separate Eppendorf tube, **AquaMet** (0.5 mg, 0.00062 mmol, 1.0 eq.) was dissolved in deionized water to yield a 20 mg/mL stock solution. The **AquaMet** solution was vortexed vigorously until all solids dissolved into solution. **AquaMet** stock (25 μL) was added to the original Eppendorf tube

containing the monomer solution, yielding a 50 mM K^+ P.B., pH 7.4 with 100 mM NaCl reaction media that is 0.10 M in monomer. The tube was immediately placed onto a shaker at room temperature for 1 hour. 1 μL of diethylene glycol monovinyl ether was added to the tube after one hour to terminate polymerization. The tube was left on the shaker for 10 additional minutes. The sample was dried at reduced pressure and the resulting residue was dissolved in appropriate solvent for analysis by ^{1}H-NMR and GPC.

Table S1. Monomer scope for ROMP under physiological conditions with GPC data

Entry[a,b]	Substrate	pH	% Conversion	M_n (kDa)[c]	Đ
1	**NB-PEG**	7.4	93	26.8	1.86
2	**NB-PEG**	6.5	>99	40.0	1.87
3	**NB-Sulfo**[d]	7.4	83	N/A	N/A
4	**NB-Sulfo**[d]	6.5	>99	N/A	N/A
5	**NB-NMe_2**	7.4	0	N/A	N/A
6	**NB-NMe_2**	7.4	0	N/A	N/A
7	**oNB-PEG**	7.4	35	1.4	3.81
8	**oNB-PEG**	6.5	64	16.3	1.26
9	**oNB-Sulfo**[d]	7.4	10	N/A	N/A
10	**oNB-Sulfo**[d]	6.5	22	N/A	N/A
11	**oNB-NMe_2**	7.4	0	N/A	N/A
12	**oNB-NMe_2**	6.5	0	N/A	N/A

[a] Polymerizations were conducted at room temperature open to air for one hour. [b] [**monomer**] = 0.1 M, [**1**] : [**monomer**] = 1 : 50. [c] The molecular weights of polymers were determined by SEC-GPC in THF using polystyrene standards. [d] Unable to obtain molecular weight data as polymers are insoluble with our current GPC setup.



General Polymerization Procedure for Synthesis of Block Copolymers and ROMPISA

In an Eppendorf tube, **NB-PEG** (15.5 mg, 0.031 mmol, 20.0 eq.) was dissolved in 155 μL of 100 mM potassium phosphate buffer, pH 6.5. To the same Eppendorf tube, 77.5 μL of 400 mM NaCl and 15 μL of deionized water were added. In a separate Eppendorf tube, **AquaMet** (1.25 mg, 0.0015 mmol, 1.0 eq.) was dissolved in deionized water to yield a 20 mg/mL stock solution. The **AquaMet** solution was vortexed vigorously until all solids dissolved into solution. **AquaMet** stock (62.5 μL) was added to the original Eppendorf tube containing the monomer solution. The tube was immediately placed onto a shaker at room temperature for 25 minutes. An aliquot of this reaction mixture was then added to a Eppendorf tube containing **NB-OMe** (6.9 mg, 0.031 mmol, 20.0 eq relative to initial catalyst loading) in 20 μL of 50 mM potassium phosphate buffer, pH 6.5 100 mM NaCl which was allowed to react for an additional 60 minutes. To the remaining volume of the initial reaction mixture was added 1 μL of diethylene glycol monovinyl ether. After 60 minutes, the diblock polymerization was then terminated with 1 μL of diethylene glycol monovinyl ether. The tube was left on the shaker for 10 additional minutes. An aliquot of the crude reaction mixture was then diluted 100-fold in PBS, filtered via 0.45 μm cellulose acetate syringe filter and analyzed by DLS (D_h = 105 nm). Both reaction mixtures were dried at reduced pressure and the resulting residues were dissolved in appropriate solvent for analysis by ^{1}H-NMR and GPC. By ^{1}H-NMR, full monomer conversion for both **NB-PEG** and **NB-OMe** was observed. By GPC, first block: $M_{n,exptl}$ = 11.1 kDa, $M_{n,theor}$ = 9.9 kDa, Đ = 1.67; diblock: $M_{n,exptl}$ = 25.4 kDa, $M_{n,theor}$ = 18.7 kDa, Đ = 1.56.

General Procedure for Preparation of **AquaMet** Solutions for UV-Vis Spectroscopy

UV-Vis spectral decomposition: 133 μL of **AquaMet** stock solution (0.6 mg/mL in diH_2O) was added to a cuvette containing 500 μL 100 mM potassium phosphate buffer, 250 μL 400 mM NaCl, and 117 μL diH_2O. Final solution concentrations: 50 mM K^+ P.B., 100 mM NaCl; [**AquaMet**] = 100 μM. Spectral changes were recorded at periodic time points.

*Kinetic plots for **AquaMet** decomposition*: In a 96 well plate, 10 μL of **AquaMet** stock solution (2.4 mg/mL in diH_2O) was added to each well. 200 μL of aqueous media with desired pH and NaCl concentration was then added to corresponding well. Kinetic analysis for each buffer composition was conducted in triplicate. The 96 well plate was

then placed into a BioTek Synergy HT plate reader and the peak at 376 nm was monitored every 5 minutes over 200 minutes. Temperature was maintained constant at 22 ºC throughout the duration of the experiment.

General Procedure for Monitoring ROMP in buffered D_2O by ^1H-NMR

All stock solutions were made using D_2O. In an Eppendorf tube, added **NB-PEG** (24.7 mg, 0.05 mmol, 50.0 eq.), 250 µL 100 mM potassium phosphate buffer pH 7.4, 50 µL 1 M NaCl and 100 µL D_2O. This solution was then transferred to an NMR tube. 100 µL of **AquaMet** stock solution (8 mg/mL, 0.8 mg, 0.001 mmol, 1.0 eq.) was then quickly added. ^1H-NMR scans were recorded every 120 seconds.

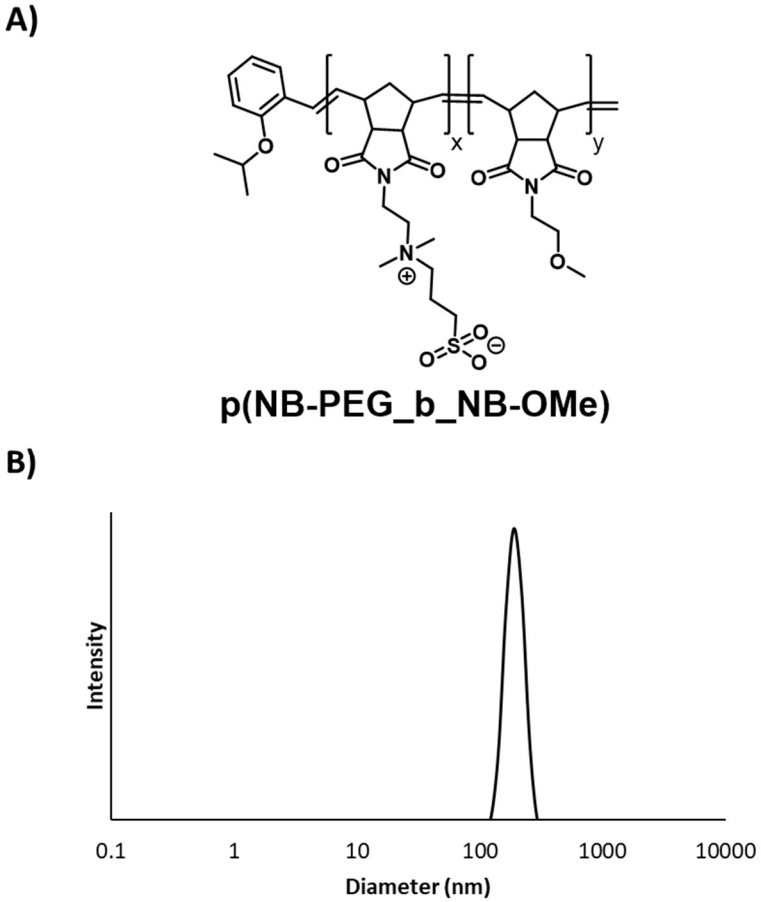


Figure S1. A) Structure of **p**(**NB-Sulfo_b_NB-OMe**). B) DLS of **p**(**NB-Sulfo_b_NB-OMe**). D_h = 194 nm.

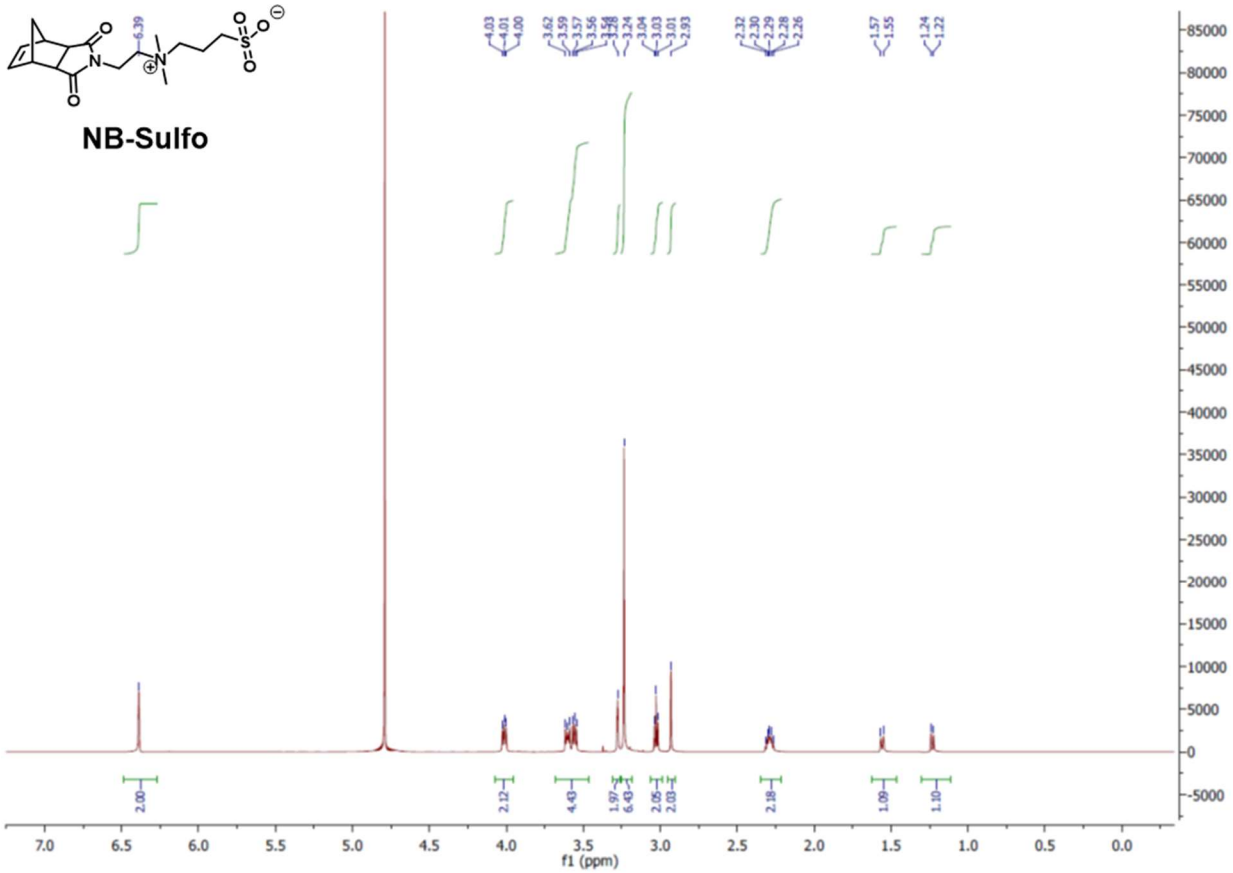


Figure S2. ^{1}H NMR of **NB-Sulfo**

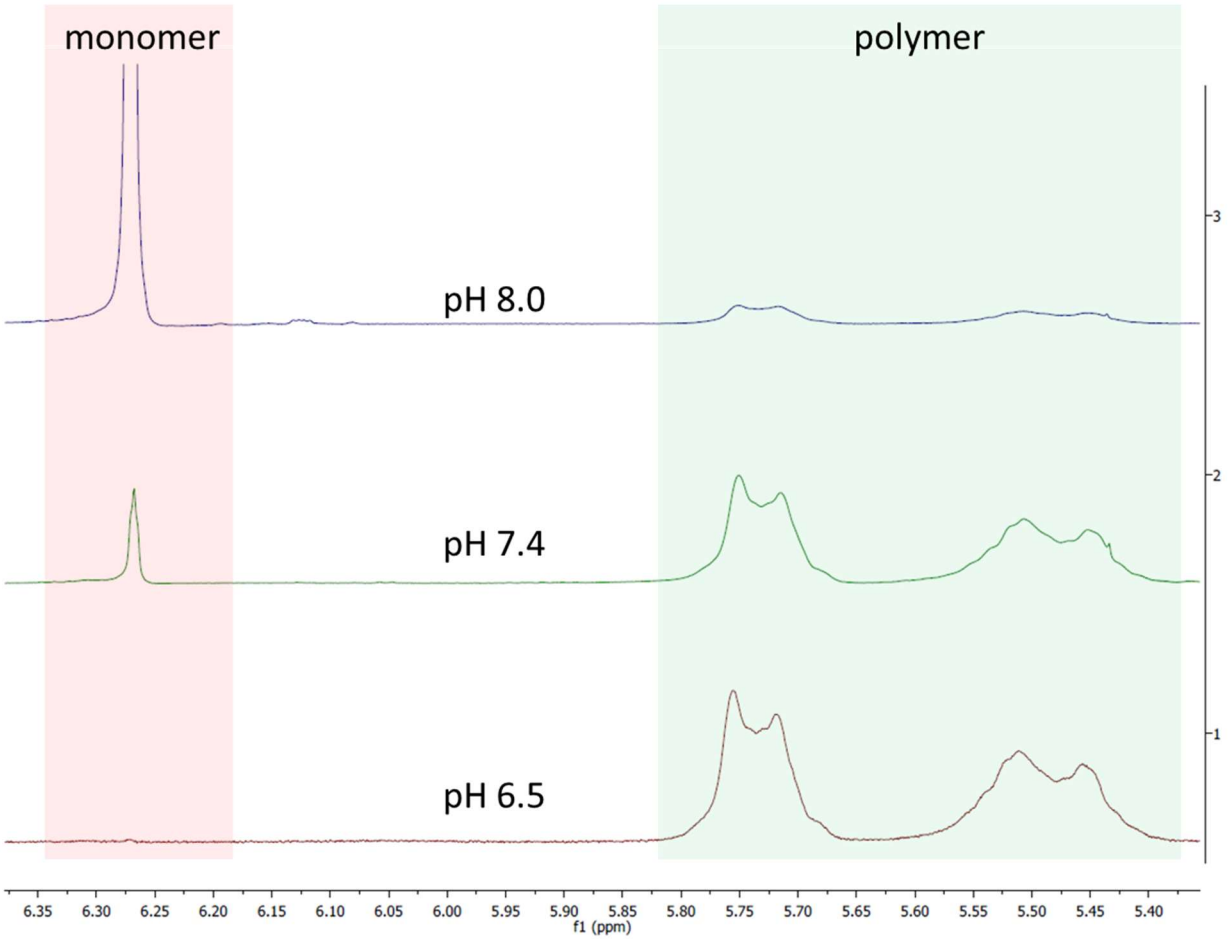


Figure S3. Crude polymerization mixtures of **NB-PEG** (0.1 M initial concentration) in 50 mM K^+ P.B, 100 mM NaCl at various pH. All polymerizations were terminated after 60 minutes.

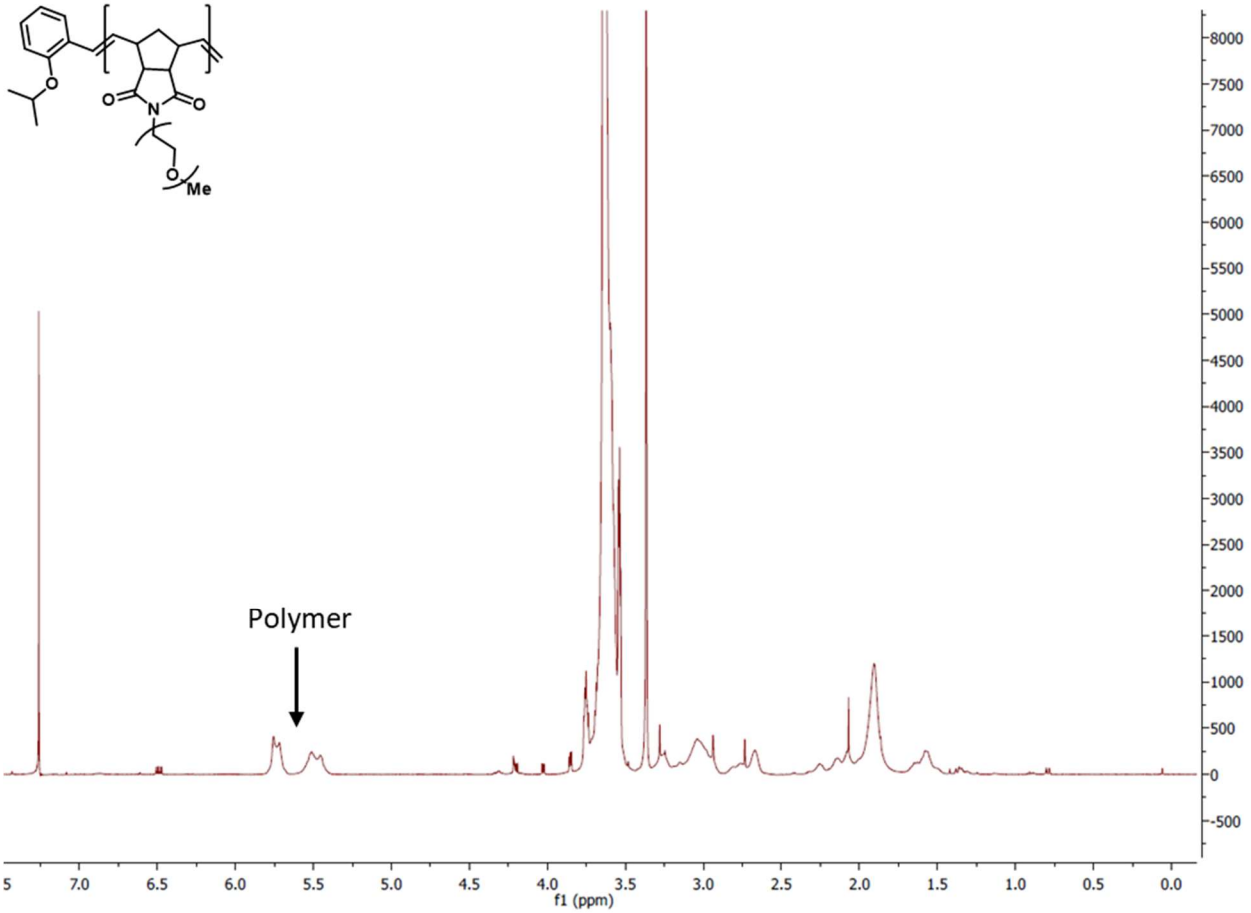


Figure S4. ^{1}H-NMR in $CDCl_3$ of crude polymerization mixture of **NB-PEG** in 50 mM K^+ P.B, pH 6.5 100 mM NaCl. Only the olefinic monomer and polymer peaks have been annotated. Peak at ~6.5 ppm corresponds to diethylene glycol monovinyl ether.

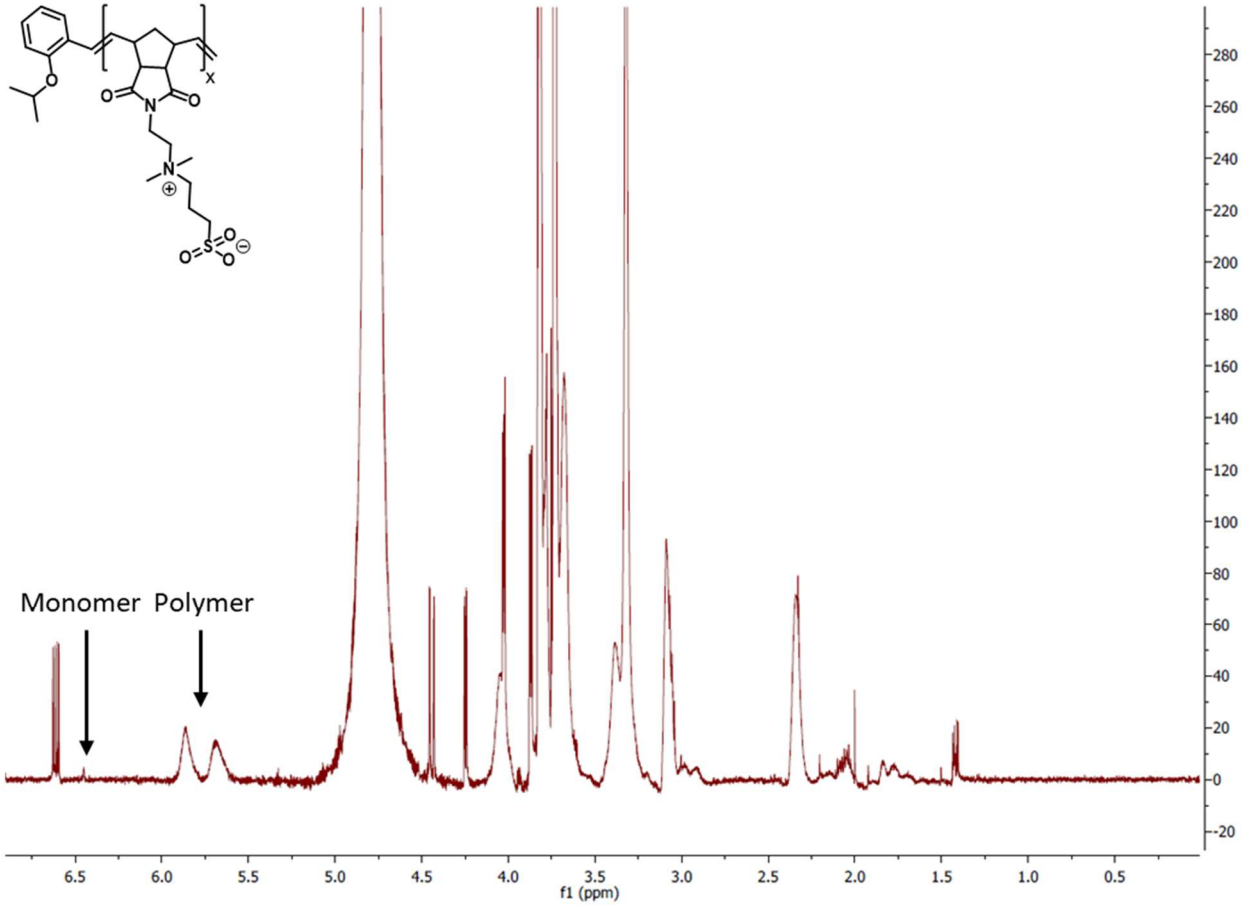


Figure S5. ^{1}H-NMR in D_2O of crude polymerization mixture of **NB-Sulfo** in 50 mM K^+ P.B, pH 6.5 100 mM NaCl. Only the olefinic monomer and polymer peaks have been annotated. Peak at ~6.6 ppm corresponds to diethylene glycol monovinyl ether.

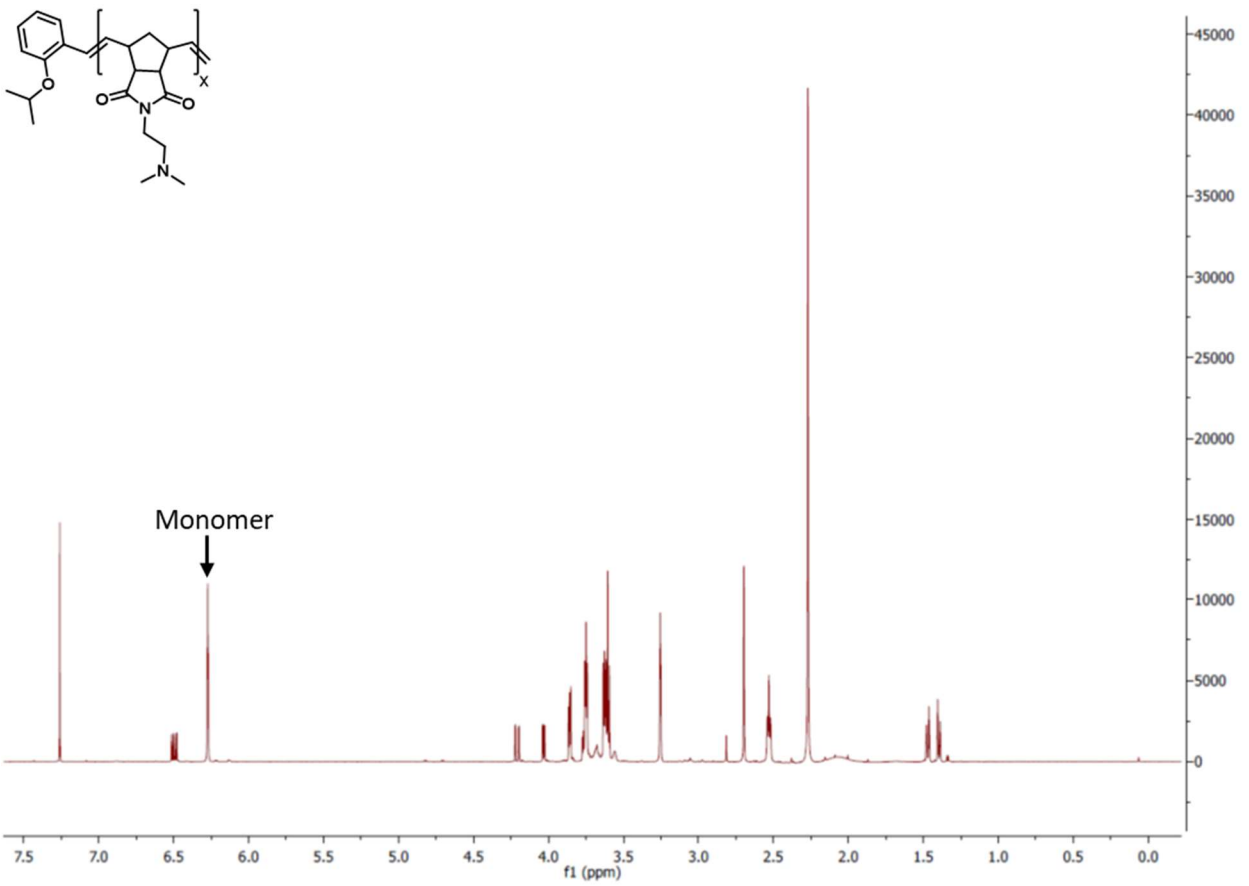


Figure S6. ^{1}H-NMR in $CDCl_3$ of crude polymerization mixture of **NB-NMe$_2$** in 50 mM K^+ P.B, pH 6.5 100 mM NaCl. Only the olefinic monomer and polymer peaks have been annotated. Peak at ~6.5 ppm corresponds to diethylene glycol monovinyl ether.

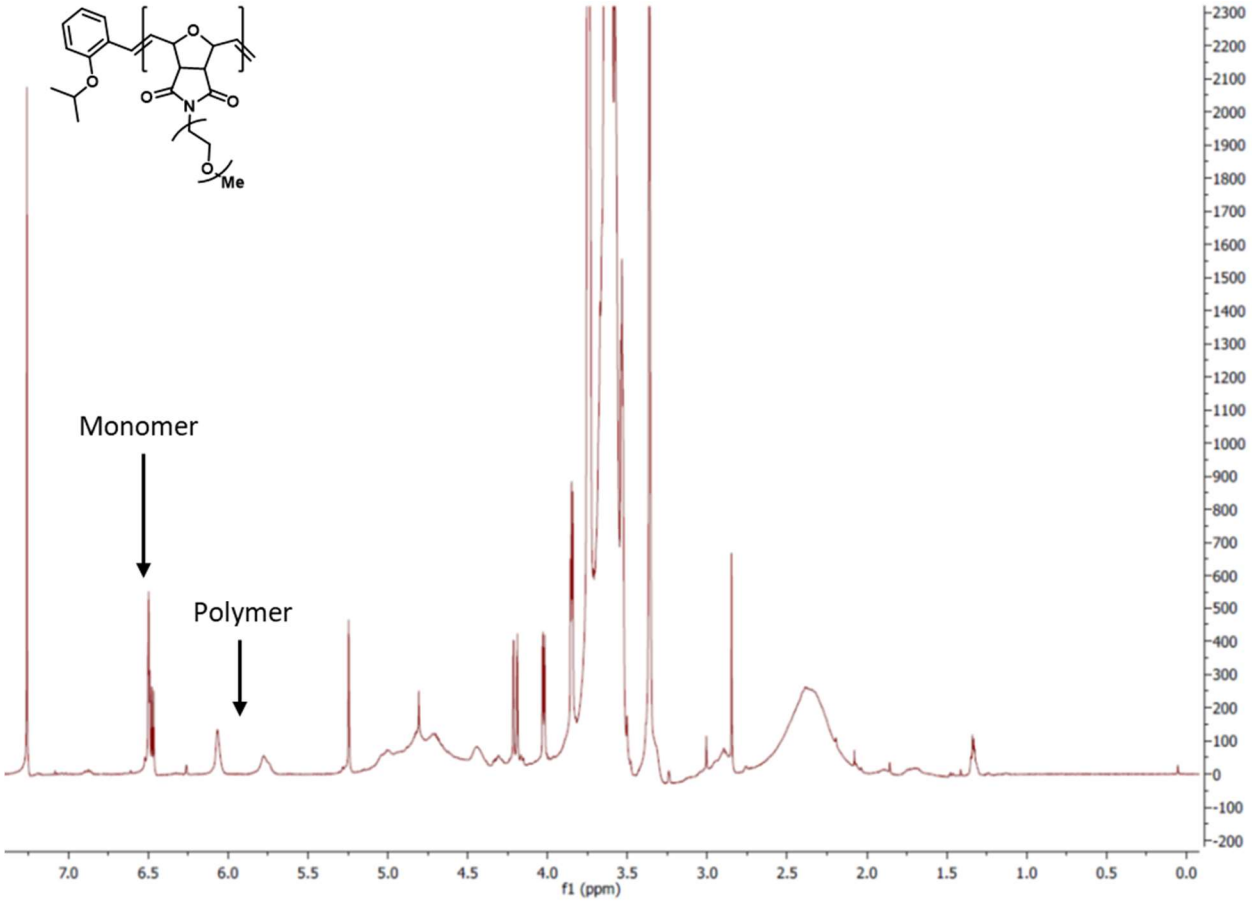


Figure S7. ^{1}H-NMR in $CDCl_3$ of crude polymerization mixture of **oNB-PEG** in 50 mM K^+ P.B, pH 6.5 100 mM NaCl. Only the olefinic monomer and polymer peaks have been annotated. Peak at ~6.5 ppm corresponds to diethylene glycol monovinyl ether.

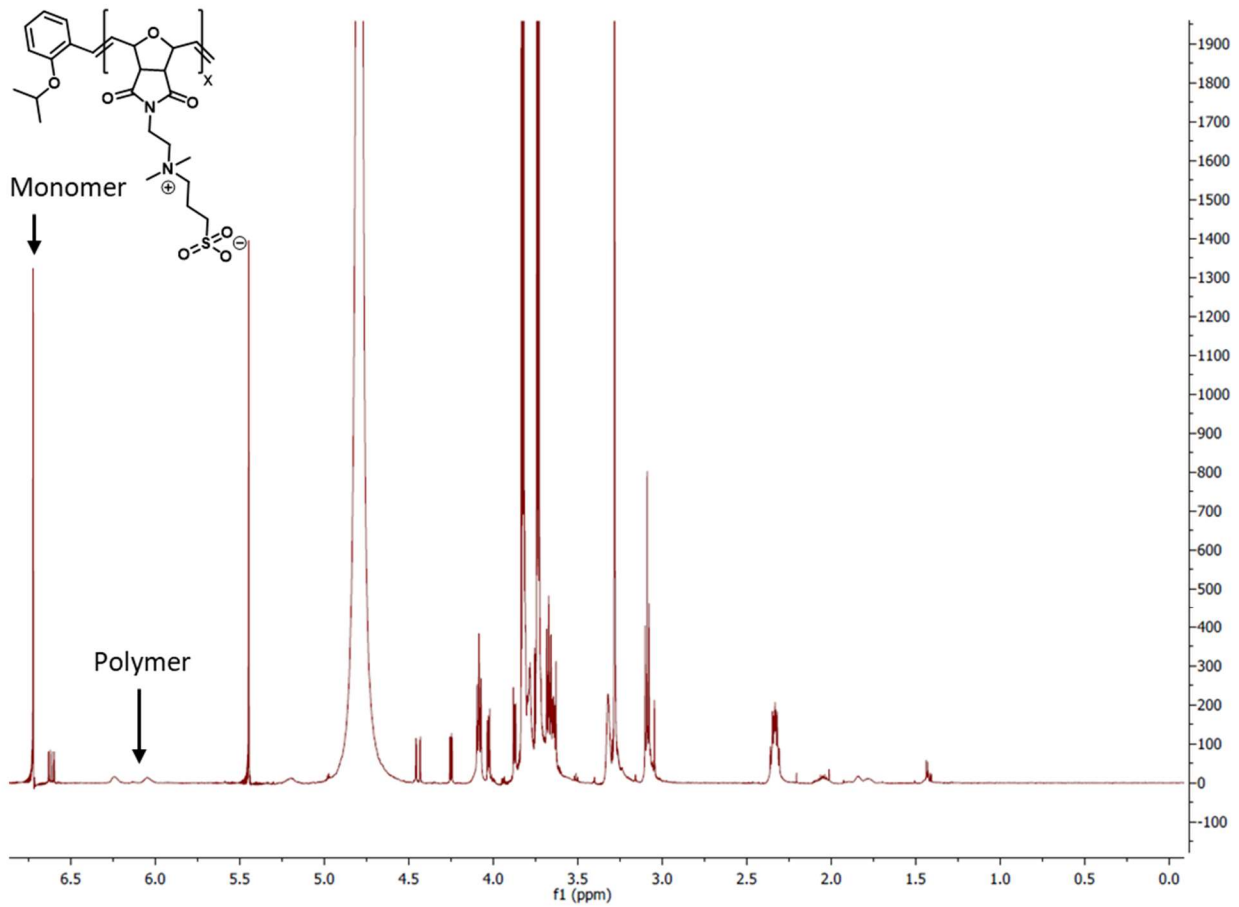


Figure S8. ^{1}H-NMR in D_2O of crude polymerization mixture of **oNB-Sulfo** in 50 mM K^+ P.B, pH 6.5 100 mM NaCl. Only the olefinic monomer and polymer peaks have been annotated. Peak at ~6.6 ppm corresponds to diethylene glycol monovinyl ether.

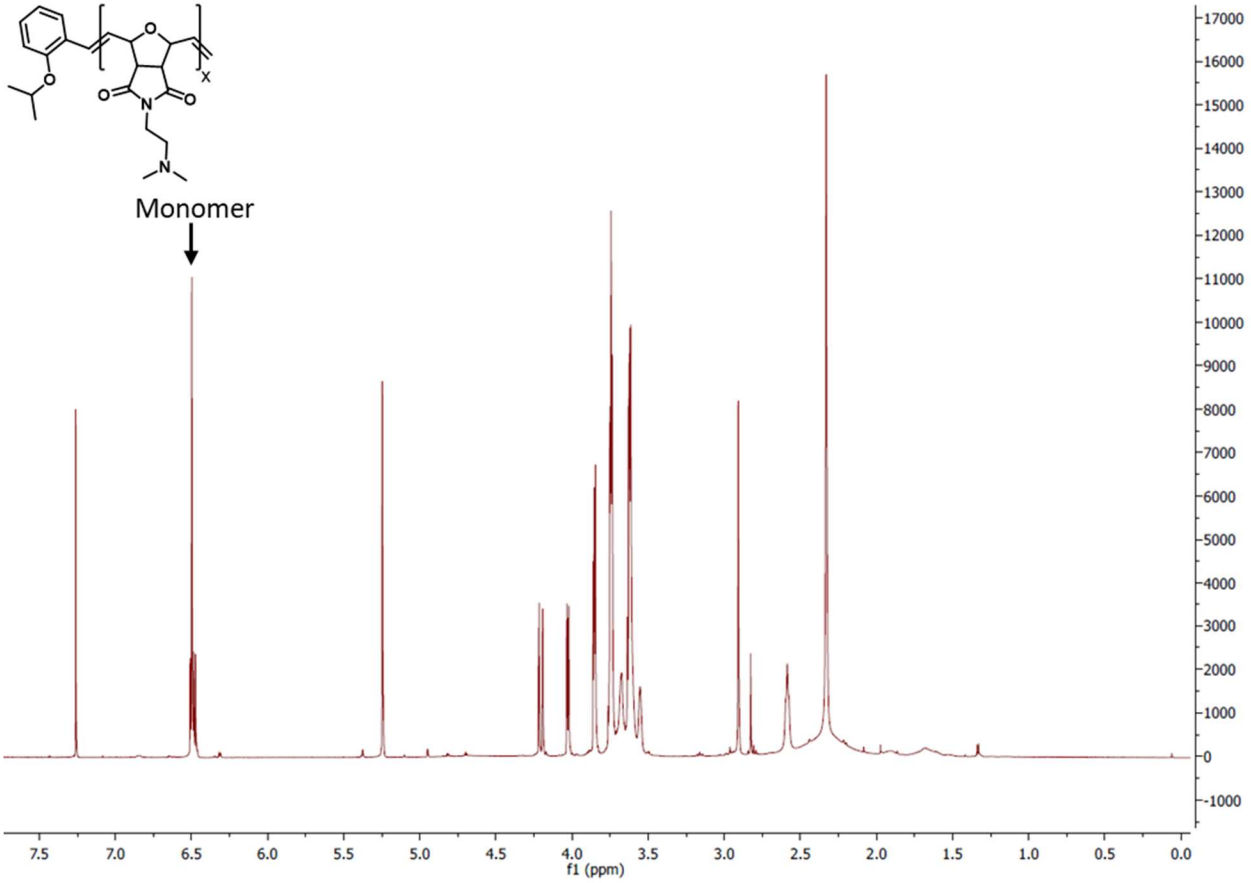


Figure S9. ^{1}H-NMR in $CDCl_3$ of crude polymerization mixture of **oNB-NMe$_2$** in 50 mM K^+ P.B, pH 6.5 100 mM NaCl. Only the olefinic monomer and polymer peaks have been annotated. Peak at ~6.5 ppm corresponds to diethylene glycol monovinyl ether.

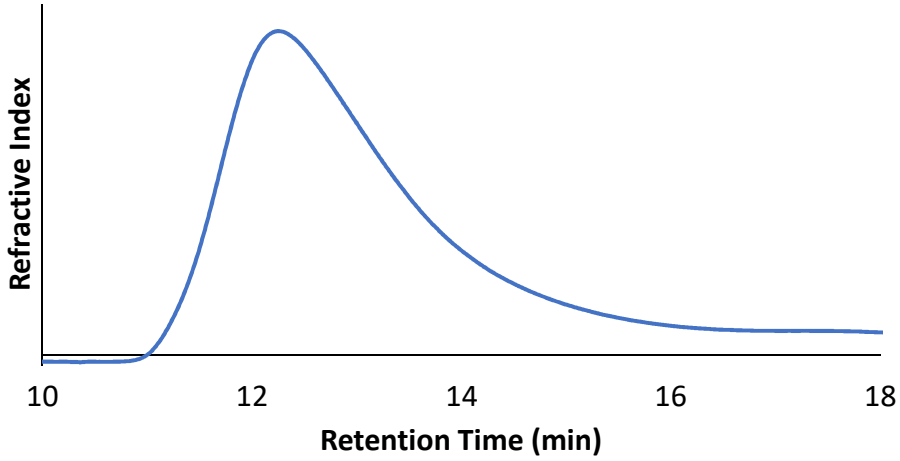


Figure S10. SEC-GPC trace of poly(**NB-PEG**) polymerized in pure water.

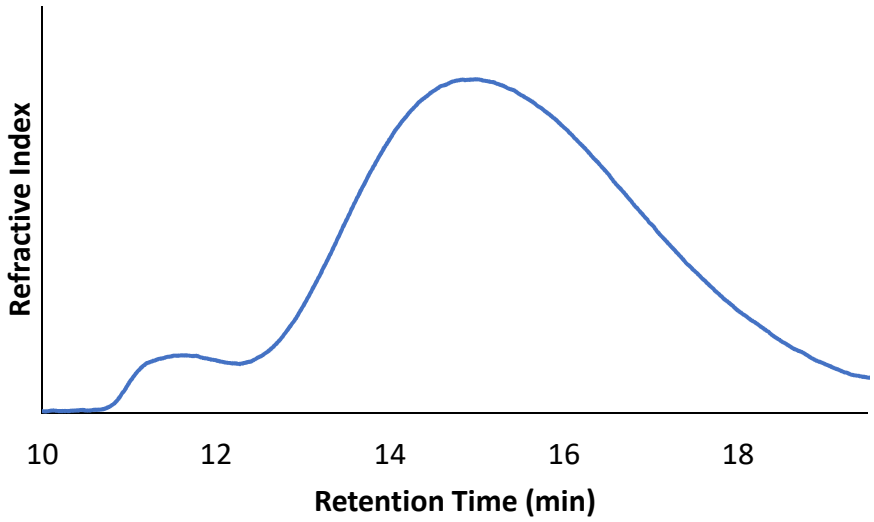


Figure S11. SEC-GPC trace of poly(**NB-PEG**) polymerized in 50 mM K^+ P.B. pH 7.4 and 10 mM NaCl.

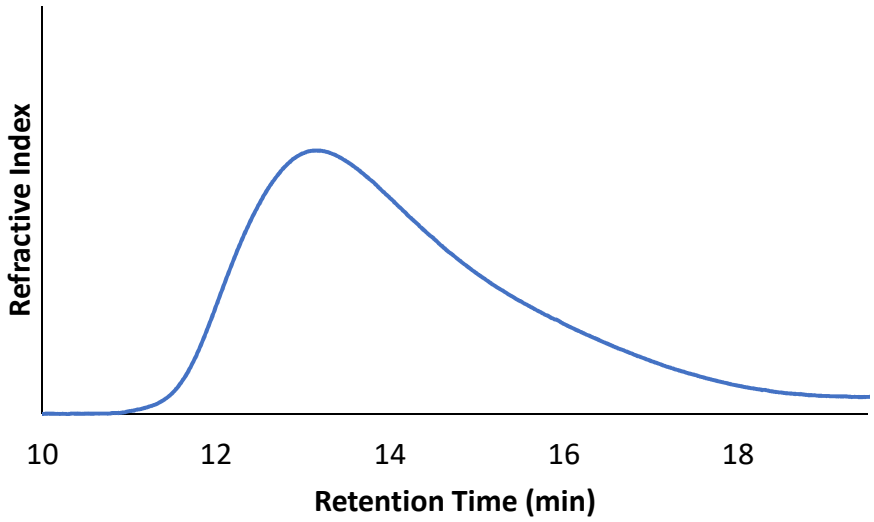


Figure S12. SEC-GPC trace of poly(**NB-PEG**) polymerized in 50 mM K^+ P.B. pH 7.4 and 50 mM NaCl.

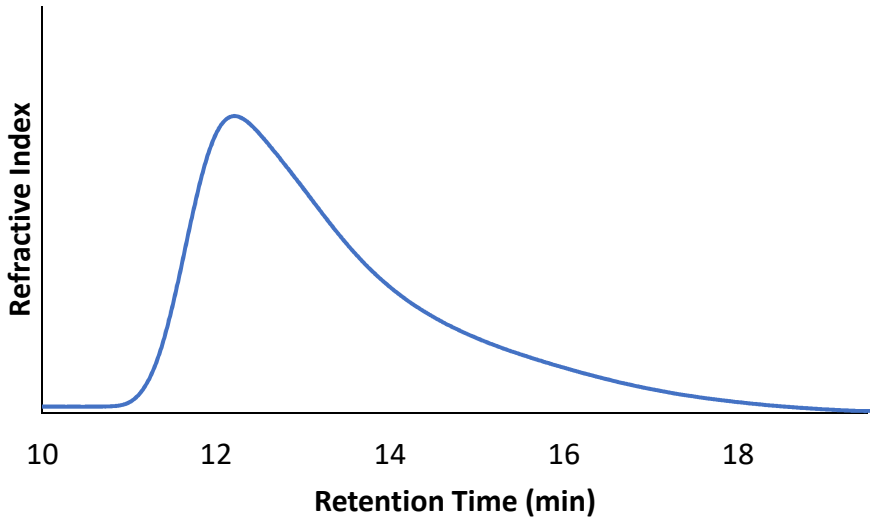


Figure S13. SEC-GPC trace of poly(**NB-PEG**) polymerized in 50 mM K^+ P.B. pH 7.4 and 100 mM NaCl.

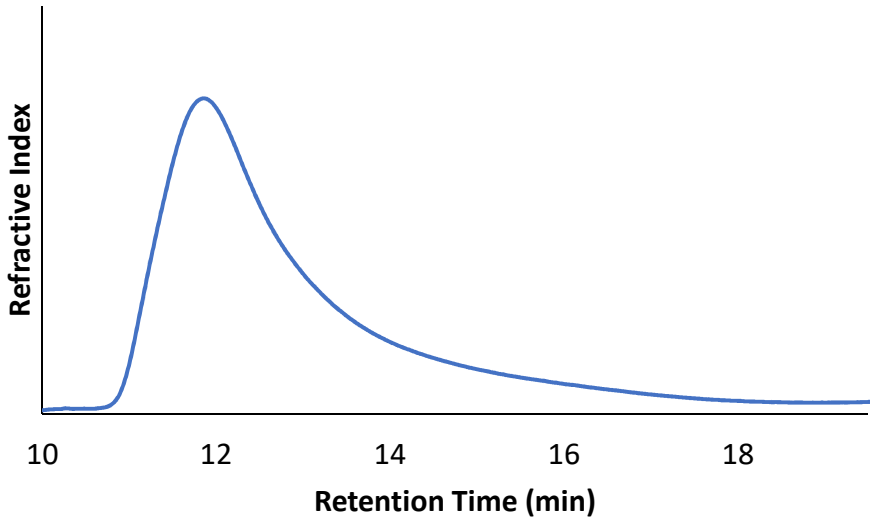


Figure S14. SEC-GPC trace of poly(**NB-PEG**) polymerized in 50 mM K^+ P.B. pH 6.5 and 100 mM NaCl.

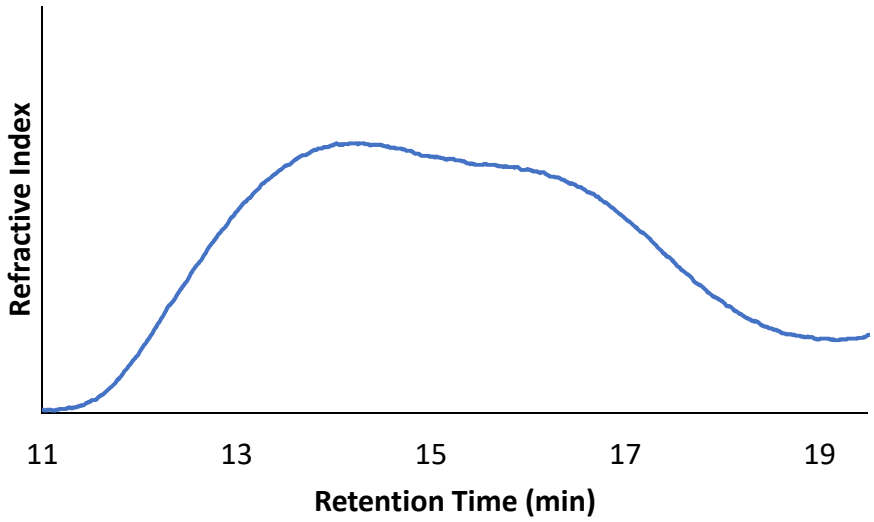


Figure S15. SEC-GPC trace of poly(**NB-PEG**) polymerized in 50 mM K^+ P.B. pH 8.0 and 100 mM NaCl.

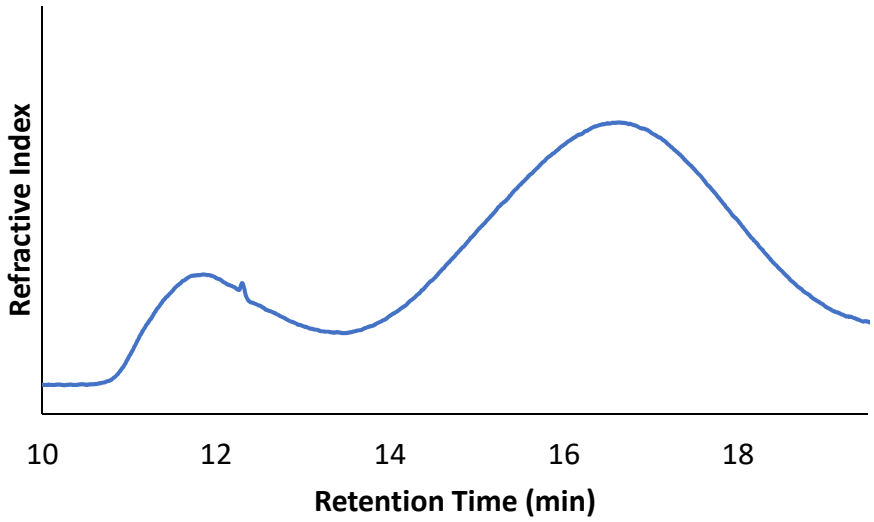


Figure S16. SEC-GPC trace of poly(**NB-PEG**) polymerized in 50 mM MOPS pH 7.4.

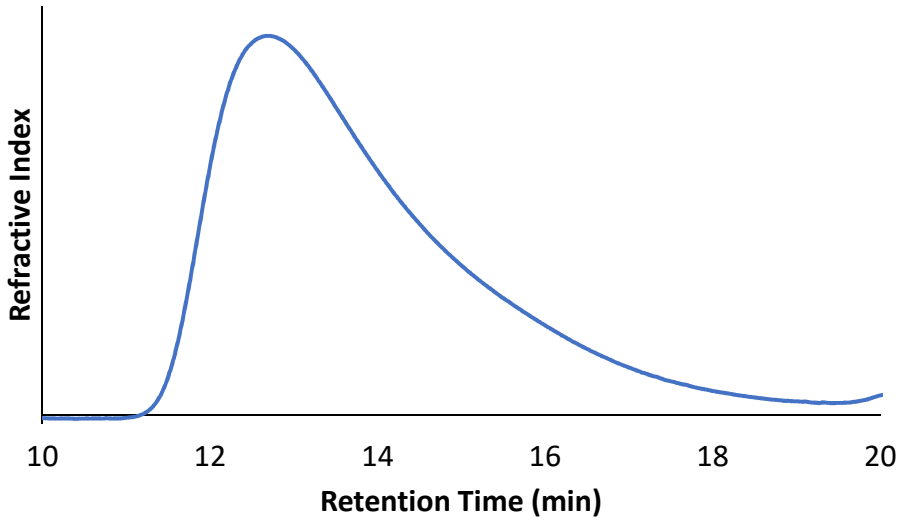


Figure S17. SEC-GPC trace of poly(**NB-PEG**) polymerized in 50 mM MOPS pH 7.4 and 50 mM $MgCl_2$.

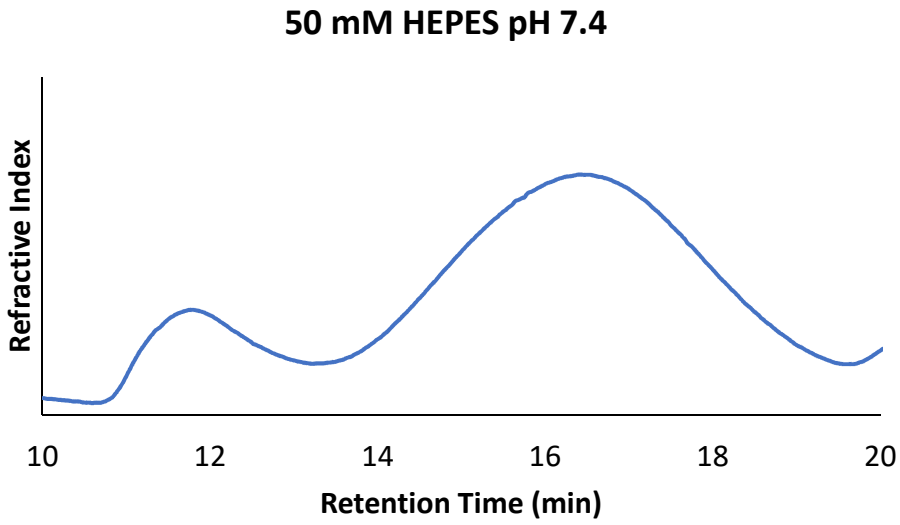


Figure S18. SEC-GPC trace of poly(**NB-PEG**) polymerized in 50 mM HEPES pH 7.4.

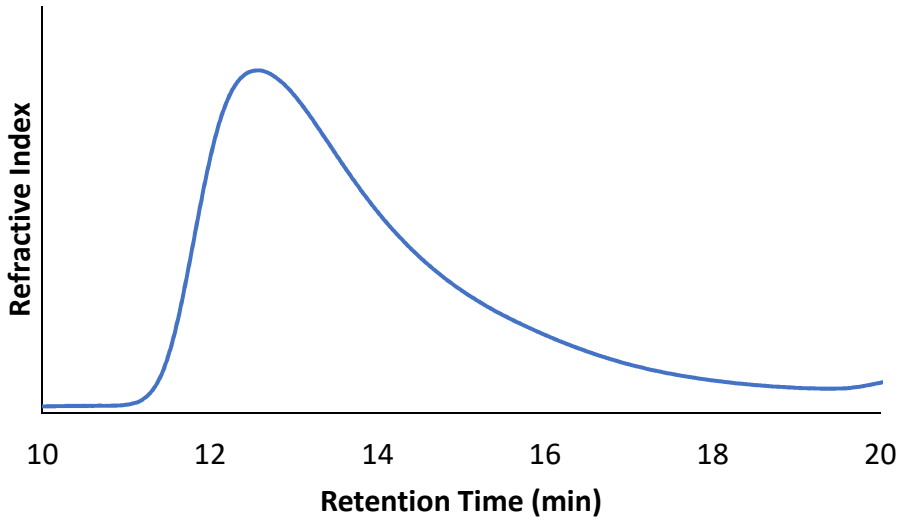


Figure S19. SEC-GPC trace of poly(**NB-PEG**) polymerized in 50 mM HEPES pH 7.4 and 50 mM $MgCl_2$.

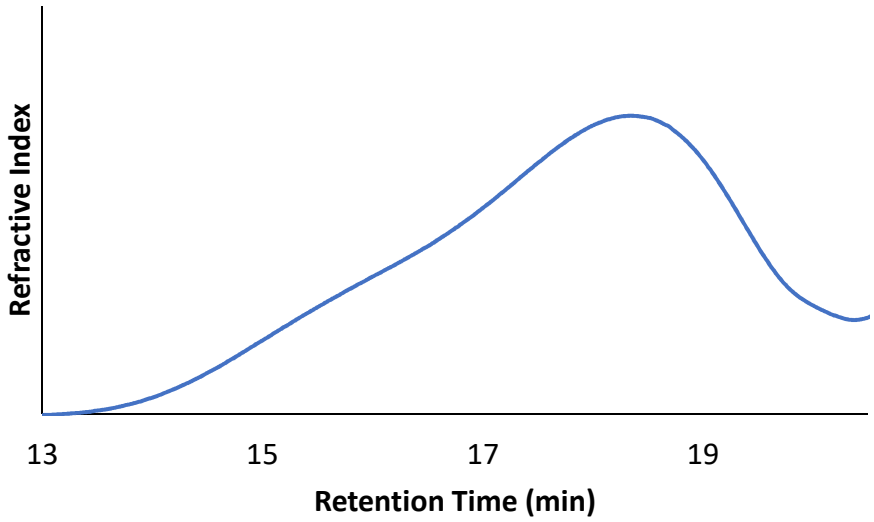


Figure S20. SEC-GPC trace of poly(**oNB-PEG**) polymerized in 50 mM K^+ P.B. pH 7.4 and 100 mM NaCl.

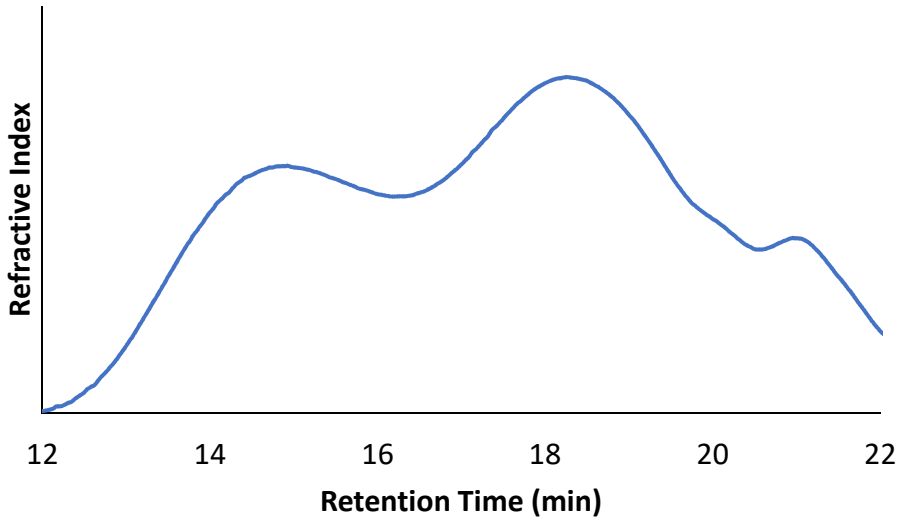


Figure S21. SEC-GPC trace of poly(**oNB-PEG**) polymerized in 50 mM K^+ P.B. pH 6.5 and 100 mM NaCl.