Supporting Information

Protein ROMP: Aqueous Graft-*from* Ring-Opening Metathesis Polymerization

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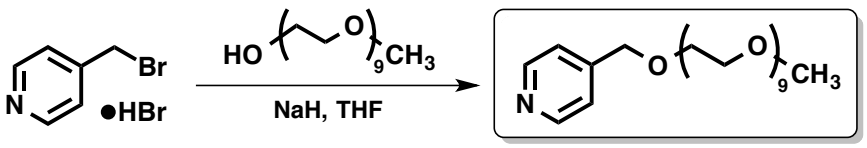
Materials. Methylene chloride (reagent grade), methylene chloride (extra dry), and deuterated chloroform were purchased from Acros Organics. Methanol, tetrahydrofuran (dried over molecular sieves), ethyl acetate, pentane, and hexane were purchased from Fisher Scientific. Dimethyl sulfoxide was purchased from AMRESCO. 4-bromomethyl pyridine hydrobromide, *cis*-5-Norbornene-*exo*-2,3-dicarboxylic anhydride, 5-Norbornene-*exo*-2-carboxylic acid, and Grubbs' 2nd generation catalyst were purchased from Sigma-Aldrich. *Poly*(ethylene glycol) monomethyl ether ($M_n \simeq 350$), and sodium hydride (60% oil dispersion) were purchased from Alfa-Aesar. Diethylene glycol monovinyl ether was purchased from TCI. 4-dimethylaminopyridine was purchased from AnaSpec, Inc. N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride was purchased from Chem-Impex International. Egg white lysozyme was purchased from bioWORLD. All reagents were used directly, without further purification.

Instrumentation. ^{1}H- and ^{13}C-NMR spectra were obtained using either a 300 MHz Varian Gemini spectrometer or a 600 MHz Varian Inova NMR spectrometer. All NMR spectra were analyzed against residual solvent peaks. Matrix-assisted laser desorption-ionization (MALDI) and nanostructure-assisted laser desorption-ionization (NALDI) spectra were obtained with a Bruker Autoflex III MALDI-TOF-TOF mass spectrometer equipped with a 200 Hz Smartbeam II laser system. For all MALDI experiments, samples were analyzed as a 1:1 solution with dihydroxybenzoic acid (DHB, Acros Organics) matrix using a Bruker ground steel target plate in the range of *m/z* = 10 – 20 kDa. For all NALDI experiments, neat samples without matrix were analyzed using a Bruker NALDI nanostructured target plate accessory in the range of *m/z* = 100 – 1000 Da. Electrospray ionization mass spectrometry (ESI-MS) was performed using a Thermo Scientific LCQ DECA ion trap mass spectrometer equipped with a 4 kV electrospray source. UV-VIS measurements were acquired using a Shimadzu BioSpec-nano UV-VIS spectrophotometer. Gel permeation chromatography (GPC) was performed on a Shimadzu Prominence GPC instrument equipped with a Shimadzu RID10A differential refractometer detector. Stationary phase was two Phenomenex 10E3A size exclusion columns in sequence maintained at 40 ^{o}C. GPC mobile phase was anhydrous THF at a flow rate of 1.0 mL/min. Size exclusion chromatography (SEC) was performed using a GE Healthcare AKTA-FPLC 900 chromatography system equipped with a Superdex 75 10/300 GL size exclusion column. For all FPLC experiments, the mobile phase was 50 mM phosphate buffer (pH 7.4) at a flow rate of 0.4 mL/min. SDS polyacrylamide gel electrophoresis (PAGE) was performed on Novex NuPAGE 4-12% bis-tris protein gels (1.0mm x 12 well) (35 minutes, 200 V, 10X SDS-PAGE running buffer, pH 8.3). Gels were stained with Coomassie SimplyBlue SafeStain (Life Technologies). Barium iodide gel staining was performed according to literature[1] by incubation with a 5% w/w solution of barium chloride (Fisher) followed by a 0.1 M solution of iodine (Fluka).



Scheme S1. *Preparation of PEG-substituted pyridine ligand (PPL).*

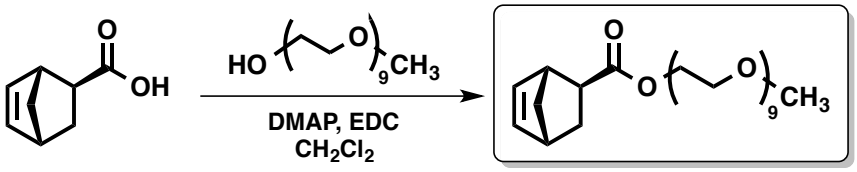
para-*poly*(ethylene glycol)-substituted pyridine ligand (PPL). The novel ligand was prepared based on standard Williamson ether synthesis protocols.[2] Poly(ethylene glycol) monomethyl ether (M_n ~ 350, 2.1 g, 6.0 mmol) was added dropwise to a slurry of sodium hydride (432 mg, 6.0 mmol) in 15 mL dry THF and stirred for 30 minutes under nitrogen atmosphere at 25 ^oC. 4-bromomethyl pyridine hydrobromide (2.28 g, 9.0 mmol) was then added, followed immediately by another portion of sodium hydride (432 mg, 6.0 mmol). The slurry was allowed to stir vigorously under nitrogen atmosphere for 12 hours at 25 ^oC. During this time, a color change from pale-yellow to dark maroon was observed. The heterogeneous mixture was then filtered via fine fritted funnel, and the filtrate concentrated under reduced pressure. The clear orange oil was then subjected to silica gel chromatography using a 5% MeOH:CH_2Cl_2 mobile phase which afforded pure product as a clear colorless oil (640 mg, 24%). The low product yield may be attributed to the known propensity of bromomethyl pyridine to undergo self-condensation under these conditions.

^1H NMR (300.1 MHz, $CDCl_3$, 17 ^oC, ppm, **Figure S1**): δ = 8.56 (2H, d, *J* = 6.0 Hz) 7.26 (2H, d, J = 6.0 Hz) 4.58 (2H, s) 3.64 (PEG methylene, m) 2.98 (3H, s).

^{13}C NMR (600 MHz, $CDCl_3$ 25 ^oC, ppm, **Figure S2**) δ = 149.8, 121.9, 77.4, 77.2, 77.0, 72.0, 71.6, 70.7, 70.3, 59.2.

MS (NALDI-TOF, **Figure S12**) m/z: [M] Calculated for $C_{19}H_{33}NO_7$ (n = 5) 343.46; Found 343.945 (17%), Calculated for $C_{21}H_{37}NO_8$ (*n* = 6) 387.45; Found 387.990 (46%), Calculated for $C_{23}H_{41}NO_9$ (*n* = 7) 431.57; Found 432.048 (79%), Calculated for $C_{25}H_{45}NO_{10}$ (*n* = 8) 475.54; Found 476.093 (100%), Calculated for $C_{27}H_{49}NO_{11}$ (*n* = 9) 519.67; Found 520.164 (97%), Calculated for $C_{29}H_{53}NO_{12}$ (*n* = 10) 563.69; Found 564.216 (76%), Calculated for $C_{31}H_{57}NO_{13}$ (*n* = 11) 607.77; Found 608.250 (48%), Calculated for $C_{33}H_{61}NO_{14}$ (*n* = 11) 651.74; Found 652.289 (25%).

For all subsequent stoichiometric calculations using this product, an M_n of 476 Da was assumed as given by the highest-abundance peak shown by NALDI-MS.



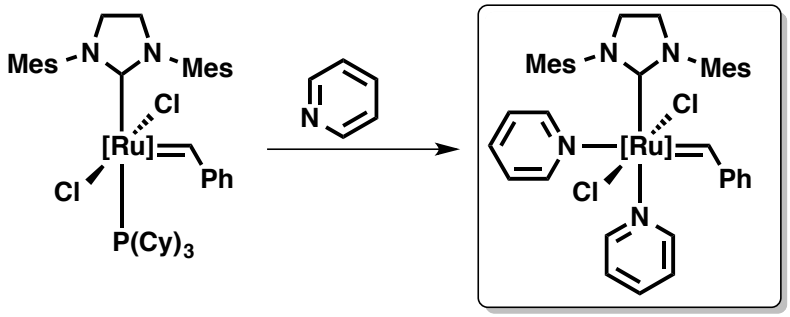
Scheme S2. *Preparation of PEG monoester norbornene monomer (**4**).*

***exo-poly*(ethylene glycol) monoester norbornene (4).** The monomer was prepared based on a previously-reported procedure.[3] 5-Norbornene-*exo*-2-carboxylic acid (1.38 g, 10 mmol), poly(ethylene glycol) monomethyl ether (3.50 g, 10 mmol), and DMAP (122.17 mg, 1.0 mmol) were dissolved in 25 mL dry CH_2Cl_2. EDC (1.92 g, 10 mmol) was then added and the mixture was refluxed for 12 hours under nitrogen atmosphere at 38 ^oC. The mixture was then concentrated under reduced pressure to a white sticky oil which was taken up into ethyl acetate (100 mL) and water (50 mL). The organic layer was washed with two aliquots of saturated sodium bicarbonate solution (100 mL) and two aliquots of brine (100 mL). The organic layers were combined, concentrated under reduced pressure, and the clear oily residue was subjected to silica gel chromatography using a gradient of 1% - 6% MeOH:CH_2Cl_2 in increments of 100 mL to yield pure product as a clear pale-yellow oil that was soluble in both CH_2Cl_2 and water (3.4 g, 82%).

^1H NMR (300.1 MHz, $CDCl_3$, 17 ^oC, ppm, **Figure S3**): δ = 6.13 (2H, m) 4.24 (2H, PEG methylene, br t, *J* = 9.0 Hz) 3.64 (PEG methylene, m) 3.37 (3H PEG methyl, s) 3.04 (1H, br s) 2.91 (1H, br s) 2.26 (1H, m) 1.94 (1H, m) 1.53 (1H, d, *J* = 6.0 Hz) 1.36 (2H, m).

MS (NALDI-TOF, **Figure S13**) *m/z*: [M] Calculated for $C_{15}H_{24}O_5$ (*n* = 3) 284.35; Found 284.762 (10%), Calculated for $C_{28}H_{24}O_6$ (*n* = 4) 328.40; Found 328.848 (37%), Calculated for $C_{19}H_{32}O_7$ (*n* = 5) 372.46; Found 372.914 (76%), Calculated for $C_{21}H_{36}O_8$ (*n* = 6) 416.51; Found 416.968 (100%), Calculated for $C_{23}H_{40}O_9$ (*n* = 7) 460.56; Found 461.023 (95%), Calculated for $C_{25}H_{44}O_{10}$ (*n* = 8) 504.62; Found 505.093 (73%), Calculated for $C_{27}H_{48}O_{11}$ (*n* = 9) 548.67; Found 549.128 (44%), Calculated for $C_{29}H_{52}O_{12}$ (*n* = 10) 592.72; Found 593.175 (20%).

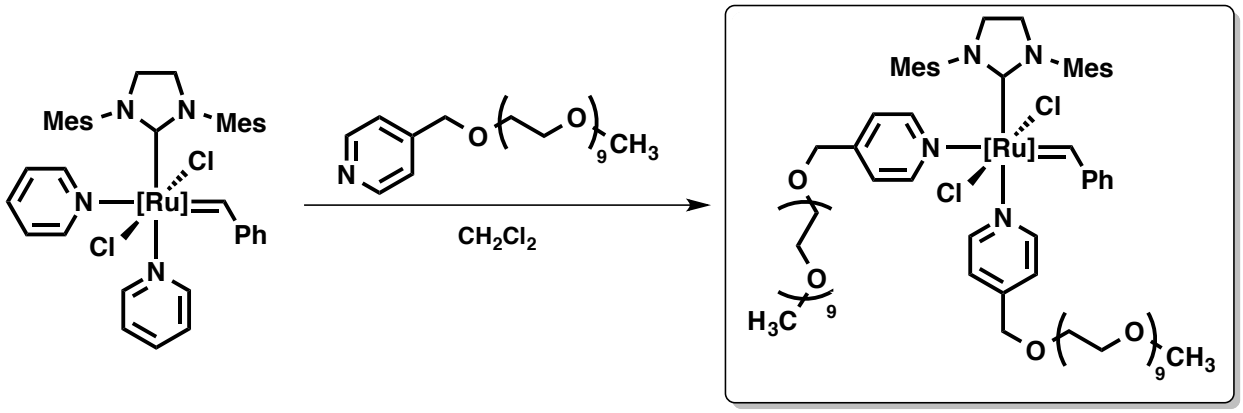
For all subsequent stoichiometric calculations using this product, an M_n of 417 Da was assumed as given by the highest-intensity peak shown by NALDI-MS.



Scheme S3. *Preparation of Grubbs' 3rd generation catalyst.*

Grubbs' 3rd generation catalyst. Grubbs' 2nd generation catalyst was converted to the 3rd generation catalyst according to a previously-reported procedure.[4] To a 20 mL scintillation vial equipped with a magnetic stir bar was added Grubbs' 2nd generation catalyst, [(H_2IMes)(PCy_3)($Cl)_2$Ru=CHPh] (100 mg, 0.12 mmol). The red powder was dissolved directly in 1.0 mL pyridine, and allowed to stir under nitrogen atmosphere for 5 minutes at 25 ^oC until all red color disappeared to yield a clear, dark green solution. 10 mL of cold pentane was then added and the solution was allowed to stir for 5 more minutes. The green precipitate that formed was isolated via fine fritted funnel, and washed with 20 mL of cold pentane. The green powder was then collected, dried under reduced pressure, and stored as pure Grubbs' 3rd generation catalyst of the form [(H_2IMes)($py)_2$($Cl)_2$Ru=CHPh], (66 mg, 76%).

^{1}H NMR (600 MHz, $CDCl_3$, 25 ^oC, ppm, **Figure S4A**): δ = 19.20 (alkylidene, 1H, s) 8.66 (s) 7.86 (s) 7.66 (d, *J* = 12 Hz) 7.45 (t, J = 12 Hz) 7.21 (s) 7.05 (t, *J* = 18 Hz) 6.98 (s) 6.90 (s) 6.74 (s) 4.15 (br d, *J* = 78 Hz) 2.63 (s) 2.29 (s).



Scheme S4. *Preparation of water-soluble Grubbs' catalyst (**2**).*

Water-soluble Grubbs' catalyst (2). The catalyst was prepared based on a modified version of a previously-reported protocol.[5] A 25 mL round-bottom flask equipped with a 1.0 cm magnetic stir bar was charged with *para-poly*(ethylene glycol)-substituted pyridine (60 mg, 120 μmol) dissolved in 1.0 mL dry CH_2Cl_2 and stirred for 5 min. Grubbs' 3rd generation catalyst (30 mg, 40 μmol) dissolved in 1.0 mL dry CH_2Cl_2 and was then added dropwise via syringe, the flask was purged with nitrogen, and wrapped in aluminum foil to keep out light. Step 1: The solution was allowed to stir at 25 °C for 30 minutes in the dark. Step 2: Nitrogen inlet and outlet lines were then attached to the flask and the CH_2Cl_2 was allowed to slowly evaporate under a weak nitrogen stream over another 30 minutes. Step 3: The contents were then concentrated further under high vacuum for 5 minutes to yield a green-yellow oily residue. The residue was then re-dissolved in 1.0 mL dry CH_2Cl_2, and steps 1 – 3 were repeated 5 more times. After the last cycle, the dark green oily residue was re-suspended in 1.0 mL phosphate-buffered saline (pH 6.5), sonicated in cold water for 3 minutes to ensure adequate dissolution, and subjected to centrifugation at 15k rpm for 1 minute to remove insoluble particulates. The clear dark green supernatant was removed via pipette and used directly for aqueous metathesis chemistry.

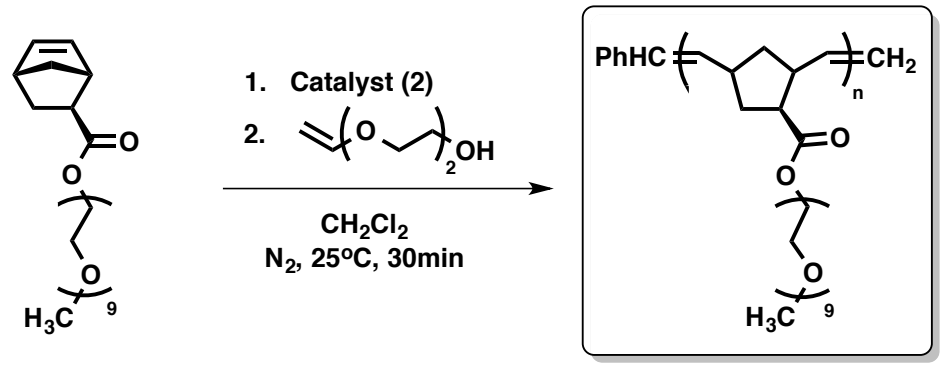
^{1}H NMR (600 MHz, $CDCl_3$, 25 °C, ppm, **Figure S4B**): δ = 19.16 (alkylidene, 1H, s) 8.57 (s) 7.78 (s) 7.63 (d, *J* = 6.0 Hz) 7.43 (t, *J* = 12 Hz) 7.26 (s) 7.22 (s) 7.03 (t, *J* = 12 Hz) 6.95 (s) 6.86 (s) 6.71 (s) 4.56 (s) 4.39 (s) 4.13 (br d, *J* = 78 Hz) 7.26 (s) 3.63 (PEG methylene, m) 3.35 (PEG methyl, s) 2.61 (s) 2.40 (s) 2.27 (s) 1.39 (s) 1.23 (s) 1.09 (s).

^{1}H NMR (600 MHz, D_2O, 25 ^{o}C, ppm, **Figure S5**): δ = 18.99 (alkylidene, 1H, s) 18.32 (alkylidene, 1H, s) 18.03 (alkylidene, 1H, s) 8.51 (s) 7.95 (s) 7.72 (s) 7.46 (s) 7.27 (s) 7.21 (s) 7.00 (s) 6.54 (s) 4.67 (s) 3.73 (PEG methylene, m), 3.37 (PEG methyl, s) 2.58 (s) 2.36 (s) 2.25 (s) 1.98 (s) 1.64 (s).

^{13}C NMR (600 MHz, $CDCl_3$, 25 ^{o}C, ppm, **Figure S6**): δ = 218.33, 151.85, 151.73, 149,94, 147.63, 130.39, 129.79, 129.49, 127.87, 121.80, 121.46, 72.10, 71.64, 70.77, 70.68, 70.39, 59.18, 33.52, 23.65, 21.20.

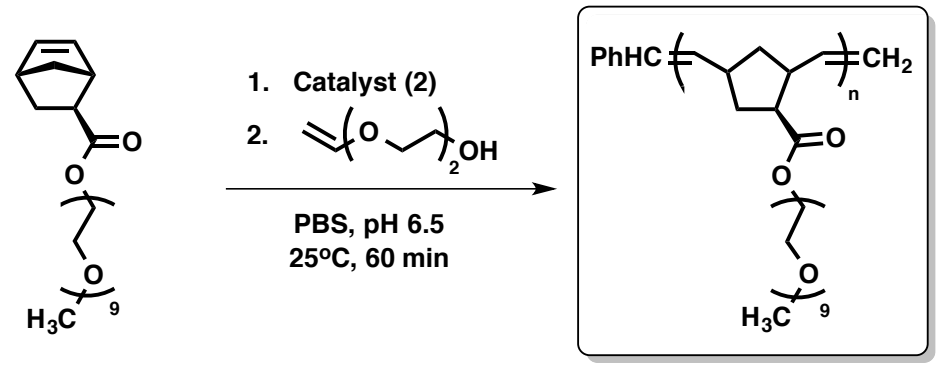
Polymerization kinetics. 1.0 mg monomer was dissolved in 900 µL D_2O and the solution was added to an NMR tube. The NMR tube was purged with nitrogen and capped. An initial NMR spectrum was collected for use as time = 0. The NMR tube was then ejected and a solution of catalyst (**2**) in 100 µL D_2O was quickly added through the cap via syringe. The cap was then wrapped in parafilm and NMR spectra were immediately collected once per minute for 60 minutes at 25 ^{o}C. Monomer conversion and rate of initiation was determined by monitoring relative integration of olefin proton signals from both monomer and polymer over time.

Stability studies. An NMR tube was charged with 20 mg catalyst (**1**) dissolved in 500 µL D_2O, purged with nitrogen and capped with a propylene cap secured with parafilm. The sample was analyzed via ^{1}H NMR every 30 minutes for 10 hours at 25 ^{o}C. The stability of the catalyst was determined by monitoring the number, relative integration, and chemical shift of peaks observed in the alkylidene region of the spectrum between 16.5 and 19.5 ppm.



Scheme S5. *ROMP of (**4**) in organic conditions*

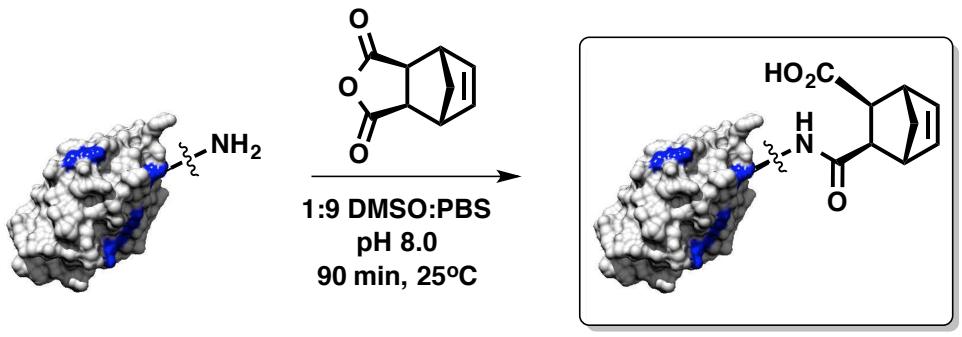
ROMP of (4) with catalyst (2) in CH_2Cl_2. A 2-dram vial, equipped with a magnetic stir bar was charged with *exo-poly*(ethylene glycol) monoester norbornene monomer (**4**) (50 mg, 87.5 µmol) dissolved in 0.5 mL dry CH_2Cl_2 . The vial was capped with a rubber septum, and purged with nitrogen. 115 µL of 31 mg/mL modified Grubbs' catalyst (**2**) in dry CH_2Cl_2 (3.56 mg, 4.89 µmol, M:C = 20) was then added quickly via syringe and the vial capped and purged with nitrogen again. The solution was allowed to stir in the dark for 30 minutes and then quenched with diethylene glycol monovinyl ether (50 µL, 386 µmol). The solution was then concentrated and precipitated into cold hexane. The hexane was decanted and the residue collected and dried under high vacuum to yield clear brown oil that was soluble in CH_2Cl_2 and water (45 mg, 90%). Complete conversion of monomer to polymer was confirmed via ^{1}H NMR of crude product. ^{1}H NMR of purified product is shown in **Figure S7**.



Scheme S6. *ROMP of (**4**) in aqueous conditions.*

ROMP of (4) with catalyst (2) in phosphate buffer. A 2-dram vial equipped with a magnetic stir bar was charged with *exo-poly*(ethylene glycol) monoester norbornene monomer (**4**) (55.8 mg, 97.7 µmol) dissolved in 0.5 mL 10 mM phosphate buffer (pH 6.5). 115 µL of 31 mg/mL modified Grubbs' catalyst (**2**) in 10 mM phosphate buffer (pH 6.5, 3.56 mg, 4.89 µmol, M:C = 20) was then added quickly via syringe and the vial capped. The solution was allowed to stir in the dark for 60 minutes and then quenched with diethylene glycol monovinyl ether (50 µL, 386 µmol). The solution was then concentrated under high vacuum, redissolved in 200 µL CH_2Cl_2 and precipitated into cold hexane. The hexane was decanted and the residue collected and dried under high vacuum to yield clear brown oil that was soluble in CH_2Cl_2 and water (48 mg, 86%). Complete conversion of monomer to polymer was confirmed via ^{1}H NMR of crude product. ^{1}H NMR of purified product is shown in **Figure S8.** M_n = 62,600; PDI = 1.13 (**Figure S9**).

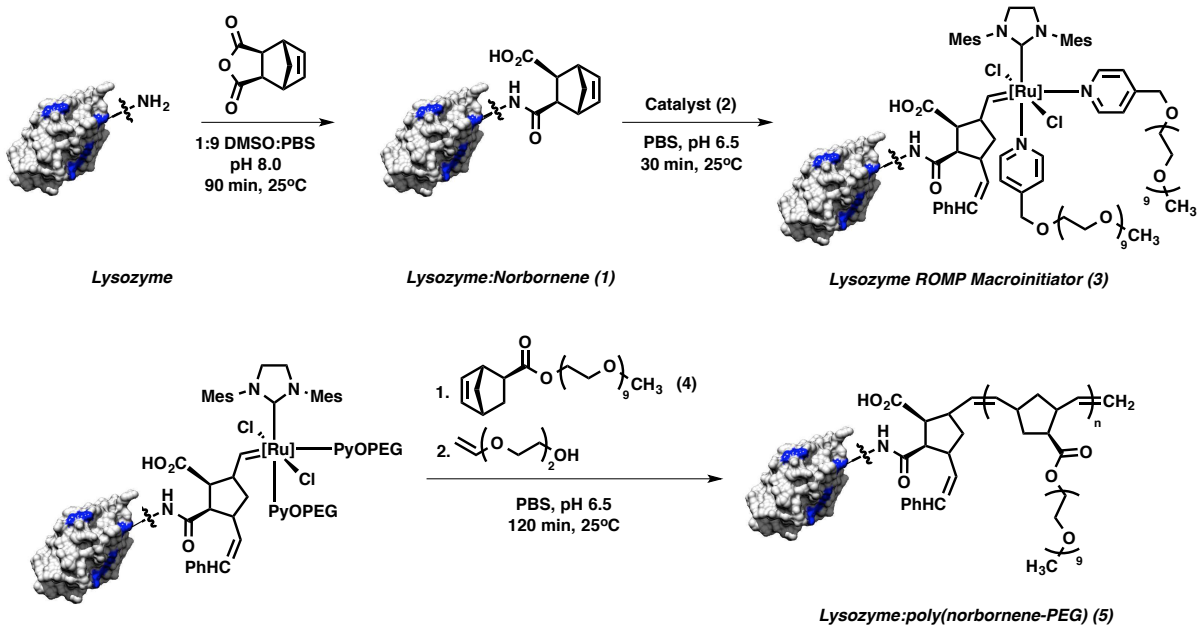
Verification of livingness in phosphate buffer. A 2-dram vial equipped with a magnetic stir bar was charged with *exo-poly*(ethylene glycol) monoester norbornene monomer (**4**) (100 mg, 0.22 mmol) dissolved in 0.5 mL 10 mM phosphate buffer (pH 6.5). Modified Grubbs' catalyst (**2**) in 10 mM phosphate buffer (pH 6.5, 7.34 mg, 10 µmol, M:C = 22) was then added quickly via syringe and the vial capped. The solution was allowed to stir in the dark for 20 minutes. Another identical aliquot of *exo-poly*(ethylene glycol) monoester norbornene monomer (**4**) was then added and the solution was allowed to stir in the dark for another 20 minutes. s100 µL samples were removed before and after the second addition of monomer. Complete conversion of monomer to polymer in both steps was confirmed via ^{1}H NMR (**Figure S10**) and an increase in molecular weight between steps was verified via GPC (**Figure S11**).



Scheme S7. *Preparation of lysozyme:norbornene conjugate (**1**).*

Lysozyme:norbornene conjugate (1). To a 20 mL scintillation vial equipped with a magnetic stir bar was added egg white lysozyme (10 mg, 0.7 μmol), dissolved in 9.0 mL 10 mM phosphate buffer (pH 6.5), and stirred at 25 ^{o}C for 10 minutes until no solid particulates were visible. *cis*-5-Norbornene-exo-2,3-dicarboxilic anhydride (8.6 mg, 52.5 μmol) was dissolved in 1.0 mL DMSO, and slowly added to the lysozyme solution dropwise over 45 minutes. pH of the solution was constantly monitored using litmus paper and adjusted to approximately pH 8.0 using 1.0 M NaOH. After addition was completed, the solution was stirred for an additional 60 minutes. Free norbornene was removed via centrifugal spin filtration through a molecular weight cut-off filter (3.5 kDa, 10k rpm, 90 minutes, 3x). The resulting supernatant was diluted to and stored at a concentration of 1.0 mg/mL as verified by UV-VIS spectroscopy at a wavelength of 280 nm (ε_{280} = 38,940 cm^{-1} M^{-1}). Number of lysine residue modifications was verified via MALDI-TOF-MS (**Figure S14**) and percent purity was verified via FPLC to be >95% based on the relative integration of norbornene signals at 220 nm at ~14.0 and ~17.0 mL (**Figure S15**).

MS (MALDI-TOF, **Figure S14**) m/z: [M] Calculated for (n = 3) 14,808; Found 14,805 (24%), Calculated for (*n* = 4) 14,972; Found 14,968 (60%), Calculated for (*n* = 5) 15,136; Found 15,135 (100%), Calculated for (*n* = 6) 15,300; Found 15,299 (95%), Calculated for (*n* = 7) 15,465; Found 15,549 (56%).



Scheme S8. *Preparation of macroinitiator (**3**) and subsequent protein:polymer conjugate (**5**).*

Preparation of protein:polymer conjugates (5). Norbornene-functionalized lysozyme (3.0 mg, 0.2 μmol) in 1.0 mL phosphate buffer (pH 6.5) was added to a 20 mL scintillation vial equipped with a magnetic stir bar. 2.0 mL of the prepared aqueous solution of modified Grubb's catalyst (**2**) is quickly added (7.3 mg, 10 μmol). The solution was allowed to stir in the dark at 25 ^{o}C for 30 minutes. The dark green crude macroinitiator complex solution was purified via centrifugal spin filtration through a molecular weight cut-off filter (10 kDa, 8k rpm, 10 minutes, 6x). The macroinitiator was considered pure when the filtrate became clear and no free catalyst was seen via gel electrophoresis. After filtration, equal volumes of the macroinitiator solution (**3**) were added to five 1.5 mL Eppendorf tubes, each containing 0, 50, 100, 200, and 400 molar equivalents per protein of *exo-poly*(ethylene glycol) monoester norbornene monomer (**4**) dissolved in 100 μL of phosphate buffer (pH 6.5). The Eppendorf tubes were then agitated via orbital shaker for 120 minutes. Diethylene glycol monovinyl ether (5 μL, 38.6 μmol) was then added to each tube, the tubes were vortexed and allowed to shake for 60 more minutes. The crude protein:polymer conjugate solutions (**Figure S16**) were then purified via centrifugal spin filtration through a molecular weight cut-off filter (10 kDa, 8k rpm, 10 minutes, 6x). FPLC analysis showed isolated peaks at low elution volumes indicating the presence of purified protein:polymer conjugate (**5**) (**Figure S17**).

Figure S1. ^{1}H NMR spectrum of PEG-substituted pyridine ligand (PPL).

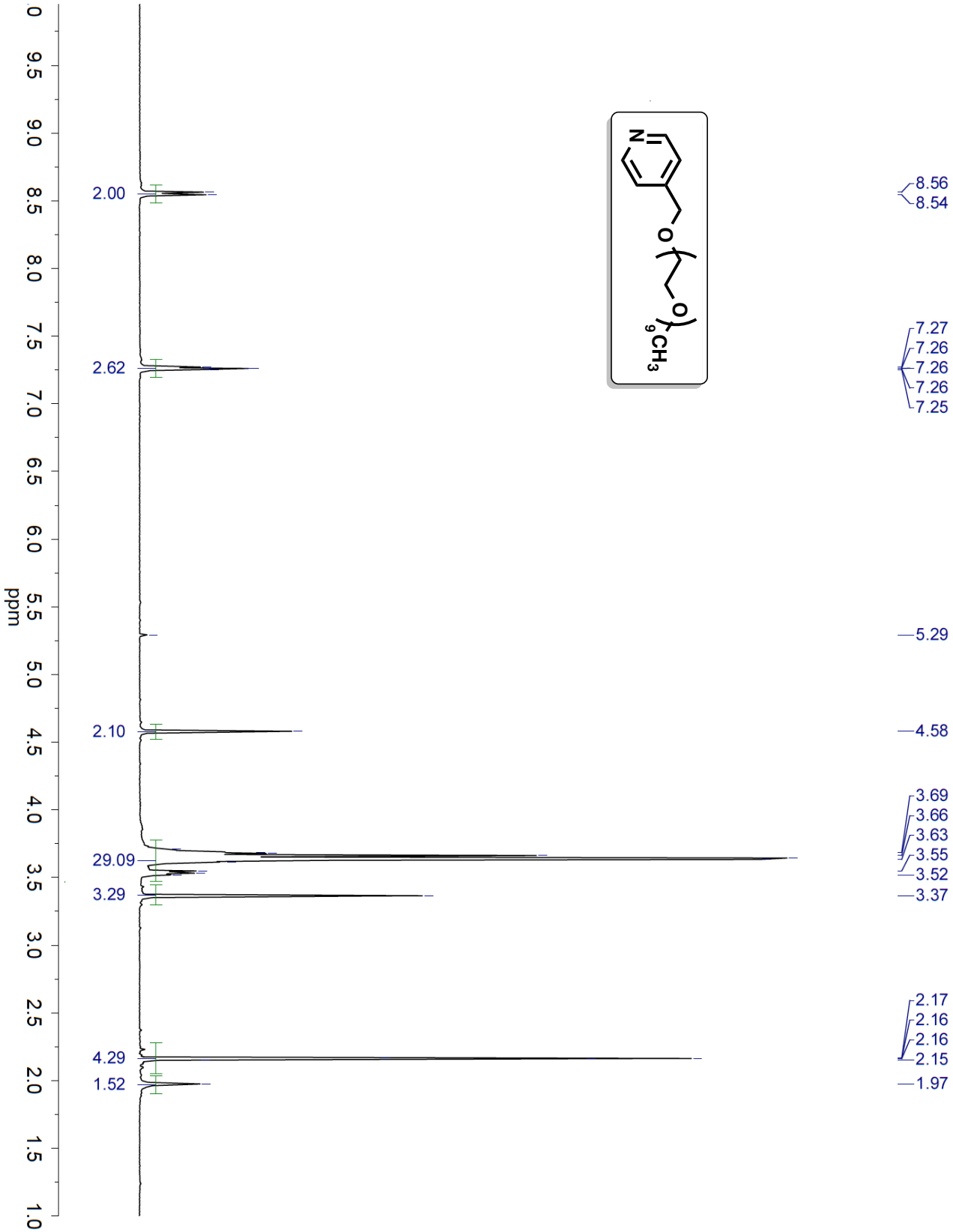


Figure S2. ^{13}C NMR spectrum of PEG-substituted pyridine ligand (PPL).

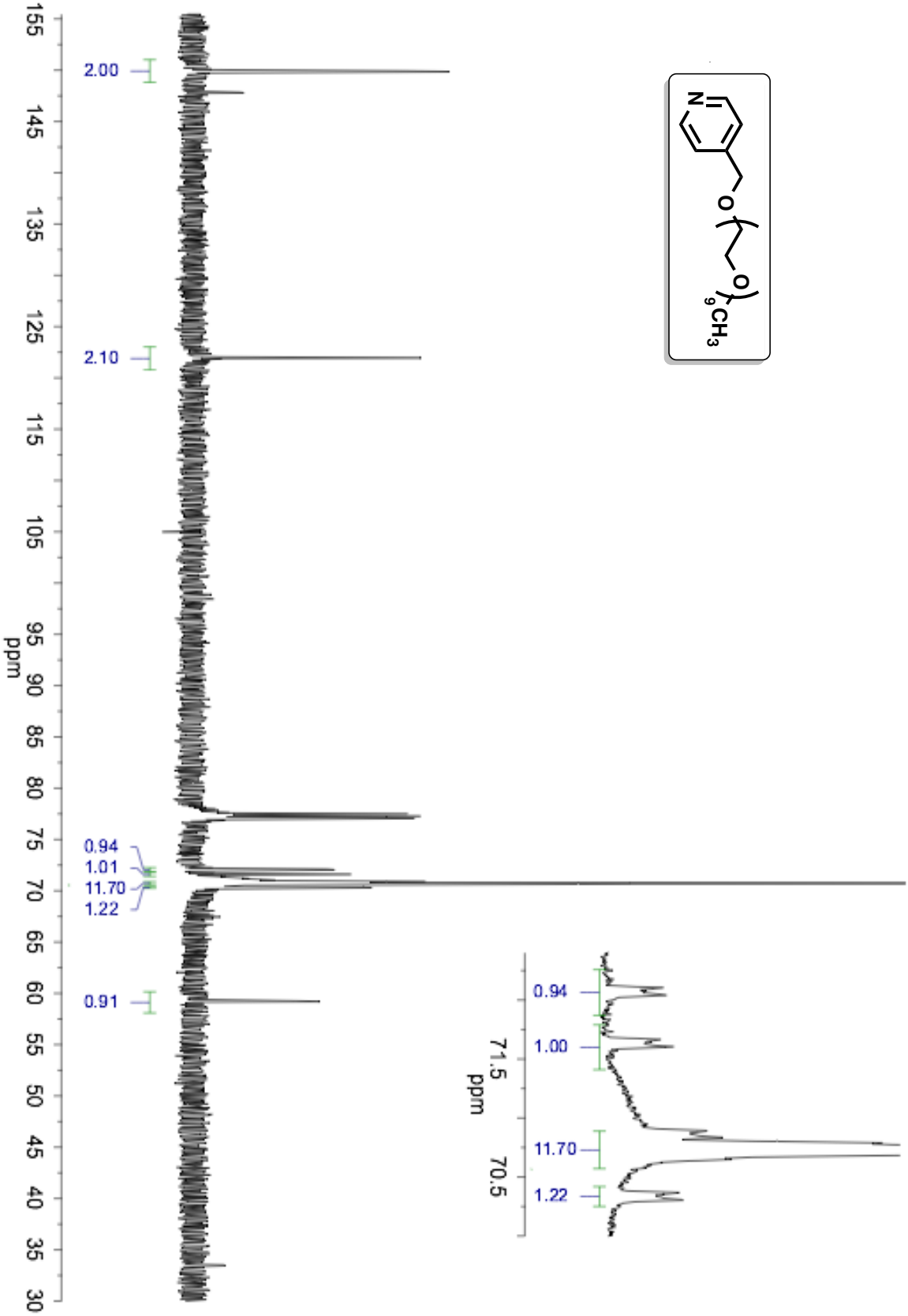


Figure S3. NMR spectrum of PEG-monoester norbornene monomer (**4**).

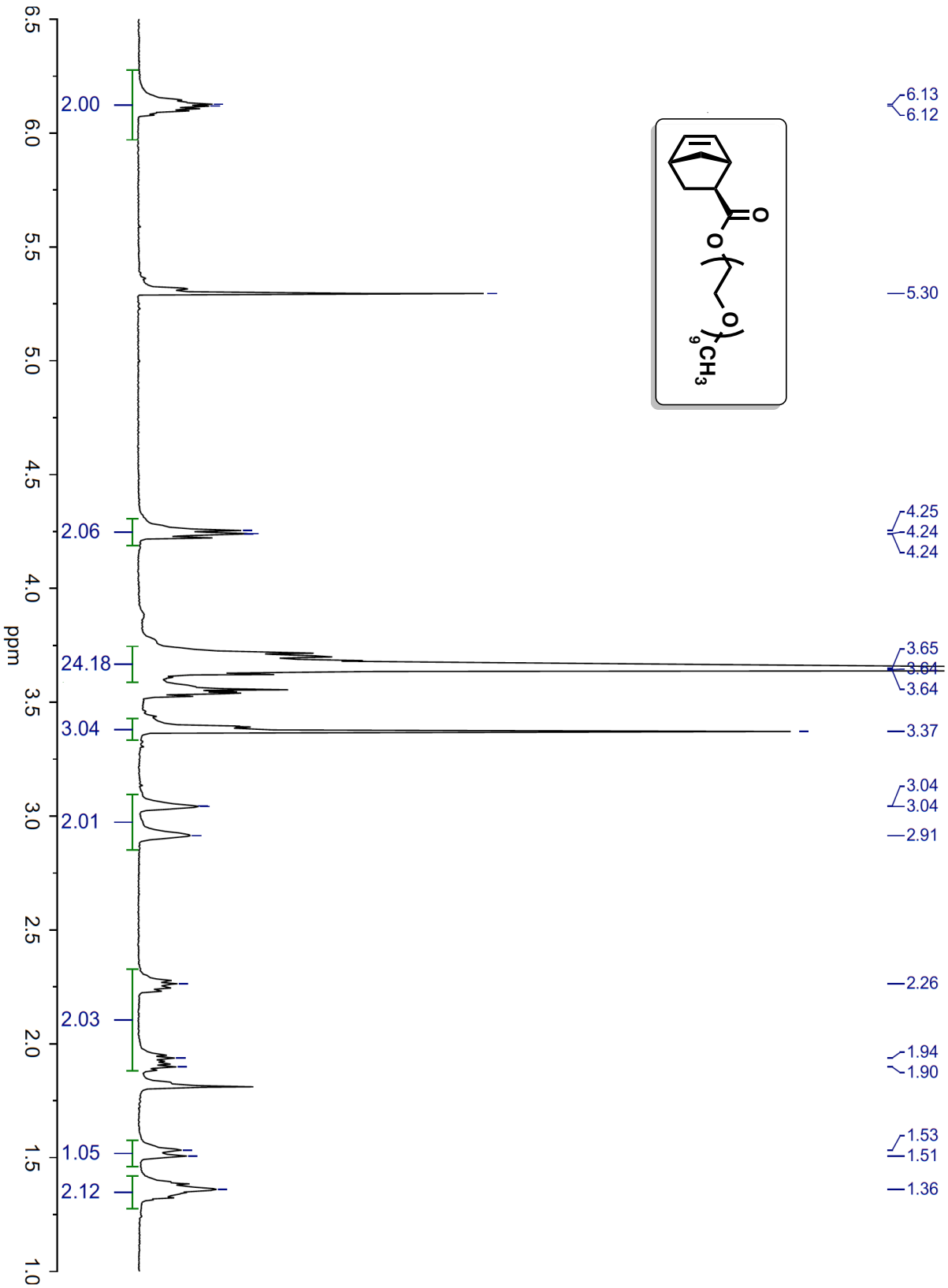


Figure S4. ^{1}H NMR spectra of regular Grubbs' 3rd generation catalyst (**A**) and modified, water-soluble Grubbs' 3rd generation catalyst (**2**) (**B**) in $CDCl_3$.

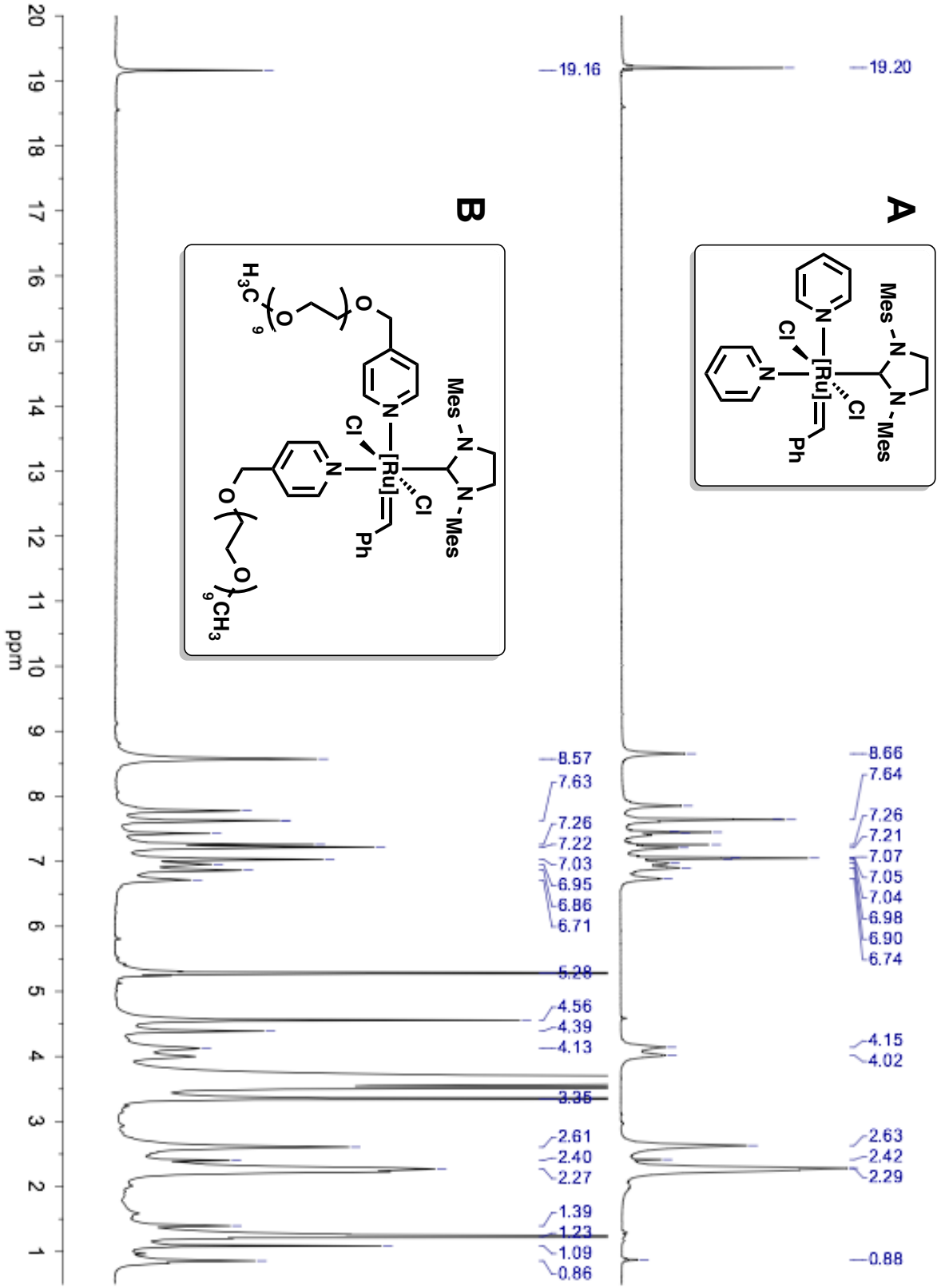


Figure S5. ^{1}H NMR spectrum of modified, water-soluble Grubbs' 3rd generation catalysts in D_2O.

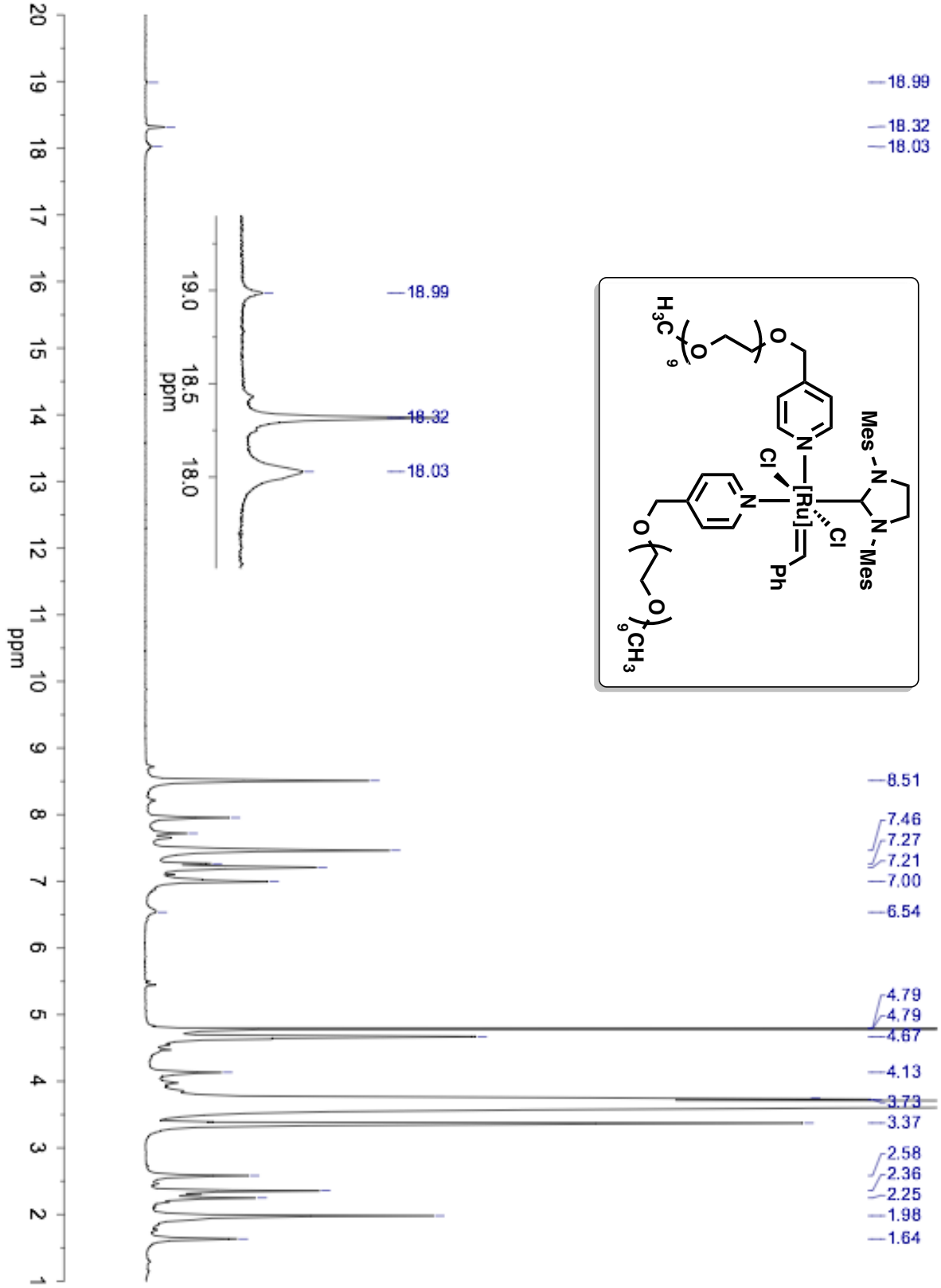


Figure S6. ^{13}C NMR spectrum of modified, water-soluble Grubbs' 3rd generation catalysts in D_2O.

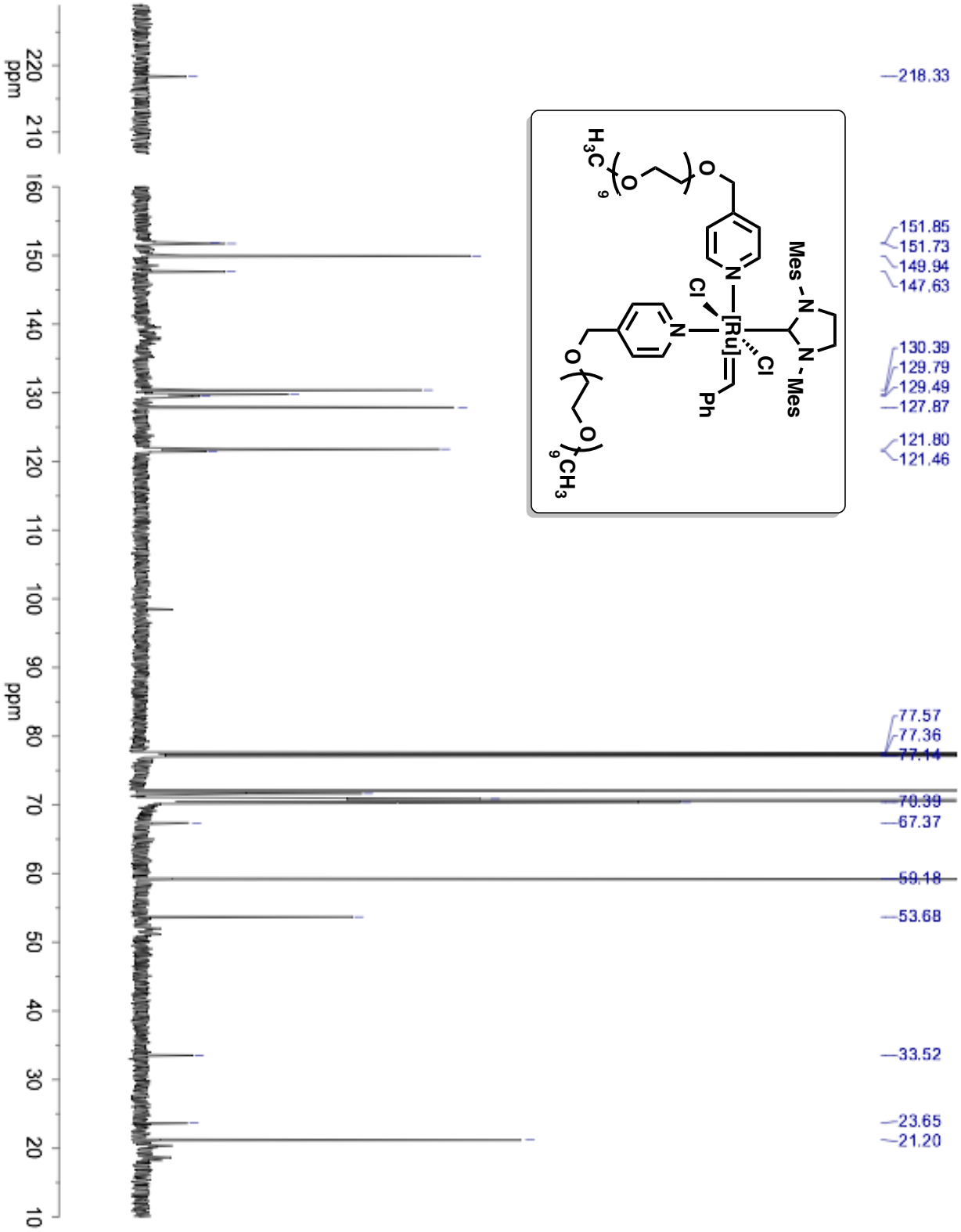


Figure S7. ^{1}H NMR spectrum of poly(norbornene-PEG) via ROMP of (**4**) with catalyst (**2**) in organic conditions. 600 MHz, $CDCl_3$, 25 ^{o}C. Alkylidene region shown to the side indicating complete removal of catalyst.

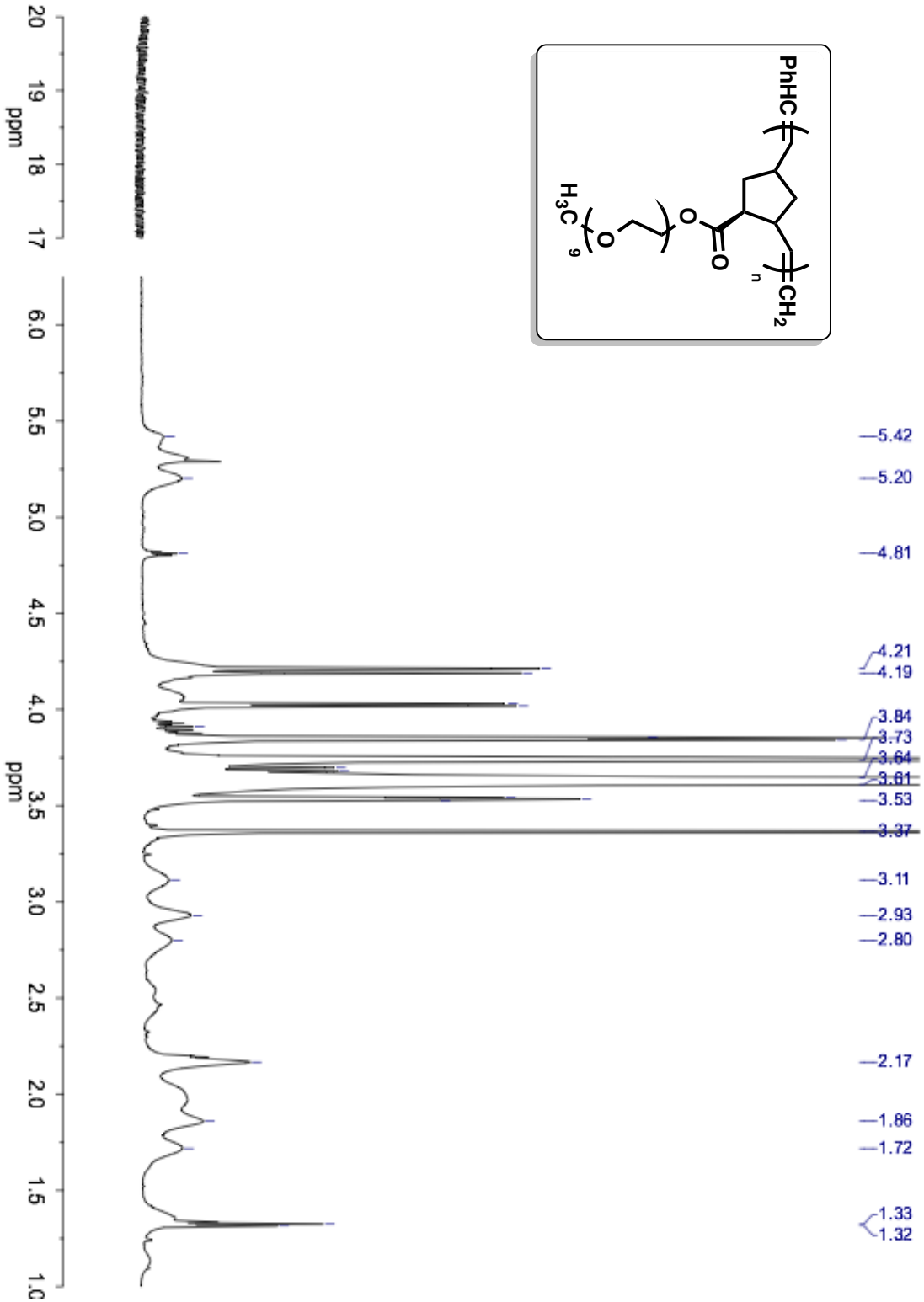


Figure S8. ^{1}H NMR spectrum of poly(norbornene-PEG) via ROMP of (**4**) with catalyst (**2**) in phosphate-buffer at pH 6.5. 300 MHz, $CDCl_3$, 25 ^{o}C.

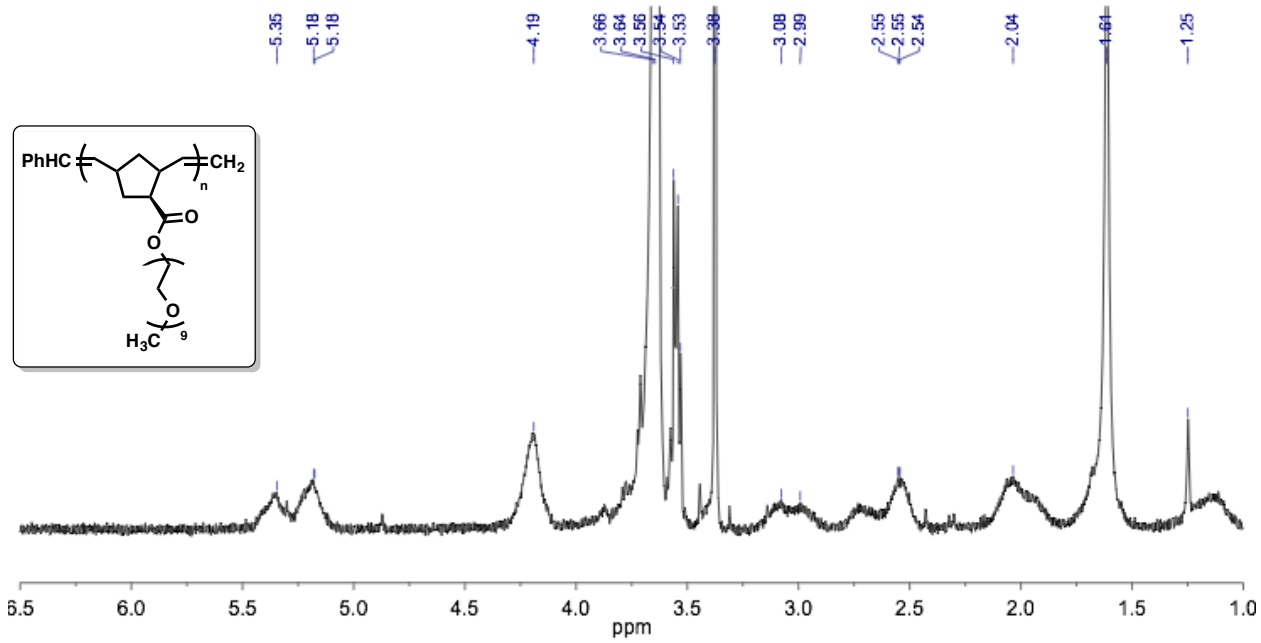


Figure S9. THF GPC of poly(norbornene-PEG) via ROMP of (**4**) with catalyst (**2**) in phosphate-buffer at pH 6.5. M_n = 62,600; PDI = 1.13.

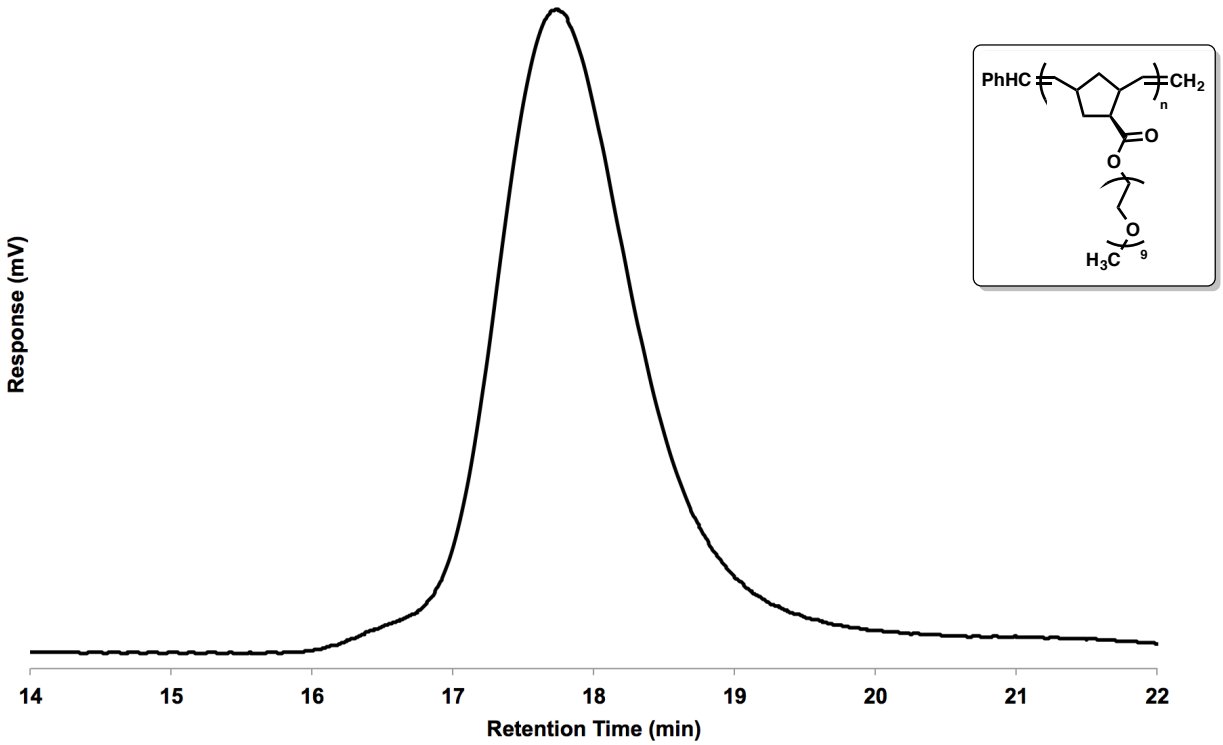


Figure S10. ^{1}H NMR spectra of (**A**): *exo-poly*(ethylene glycol) monoester norbornene monomer (**4**). (**B**): poly(norbornene-PEG) after one addition of momoner (**4**). (**C**) poly(norbornene-PEG) after second addition of monomer (**4**). Disappearance of monomer peaks shown with arrow.

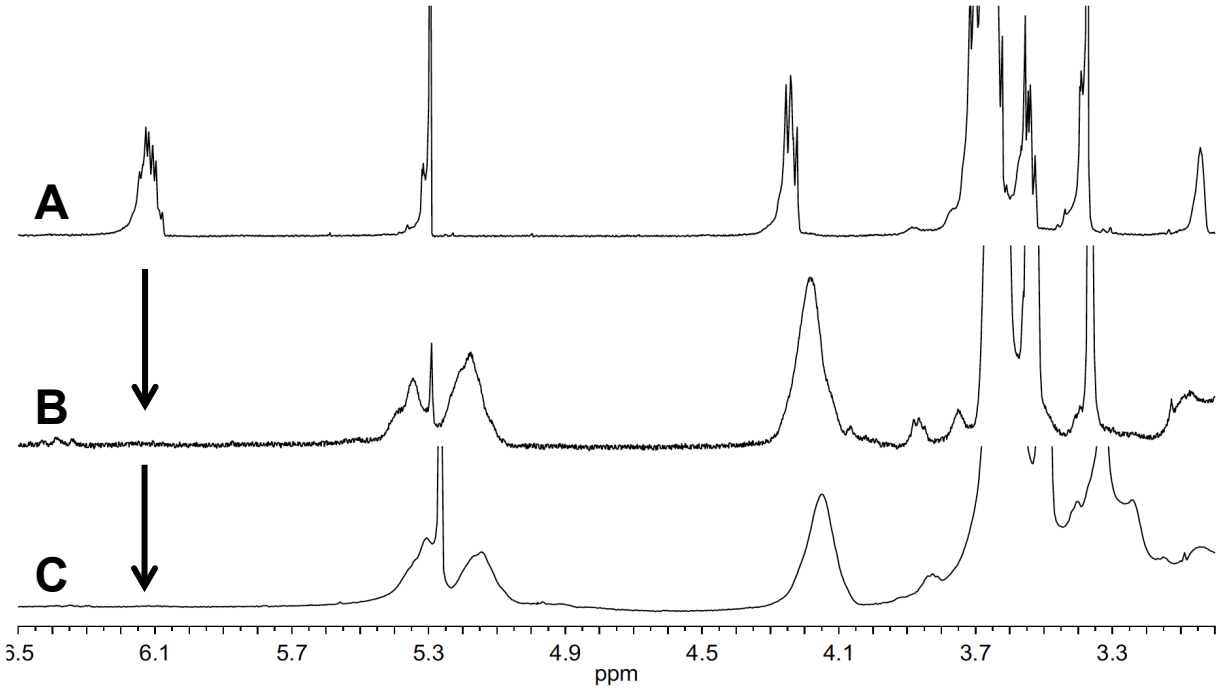


Figure S11. THF GPC of poly(norbornene-PEG) after one addition of momoner (**4, Solid Line**) and poly(norbornene-PEG) after second addition of monomer (**4, Dashed Line**).

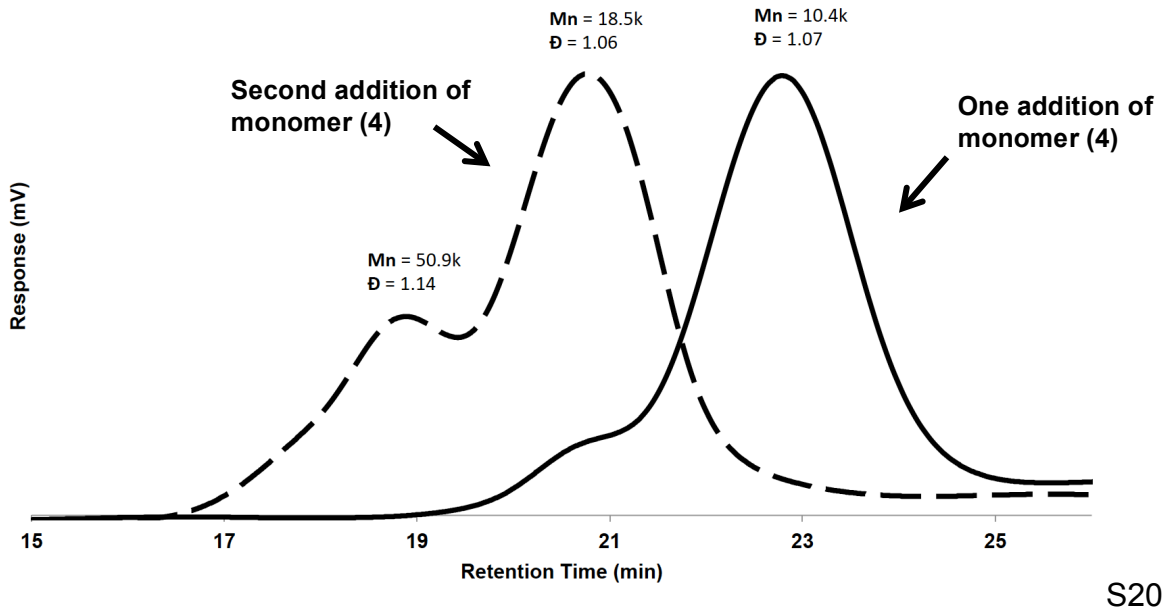


Figure S12. NALDI spectrum of PEG-substituted pyridine ligand (PPL).

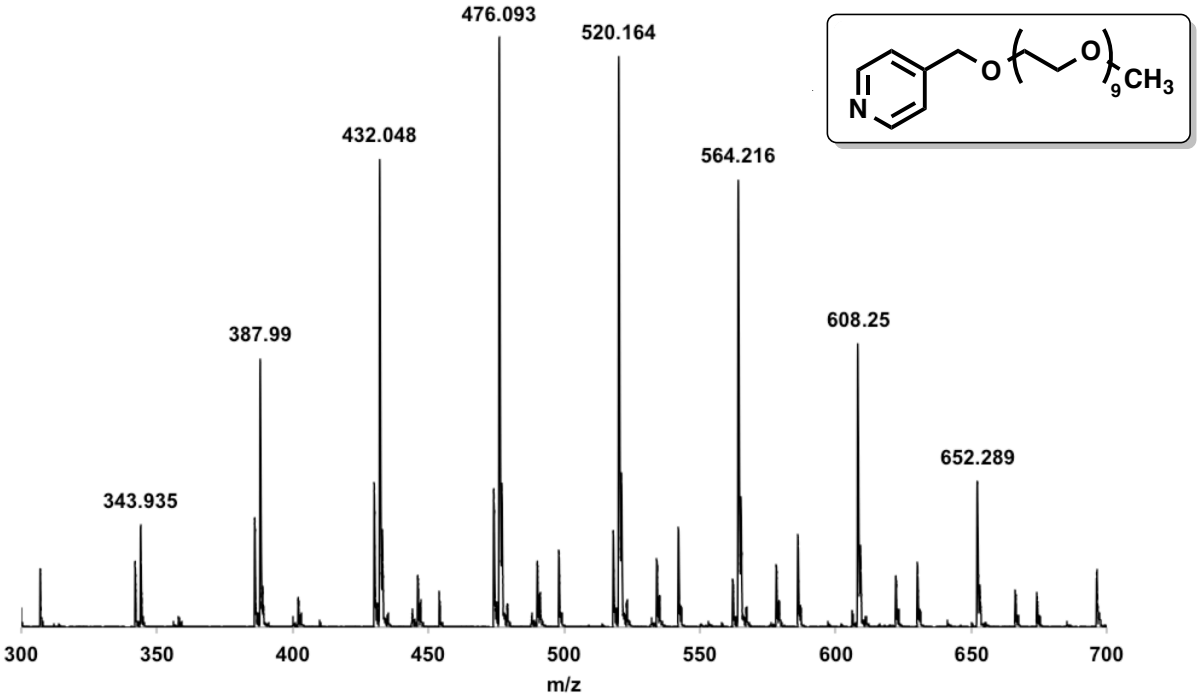


Figure S13. NALDI spectrum of PEG-monoester norbornene monomer (**4**).

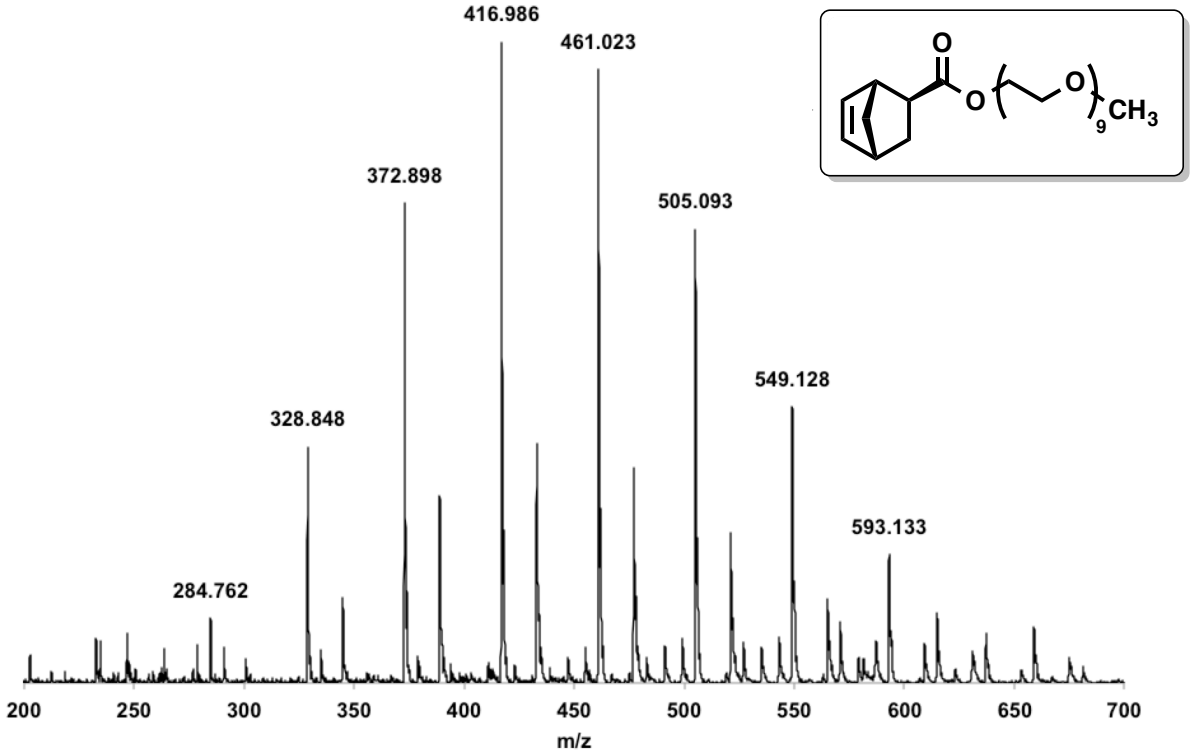
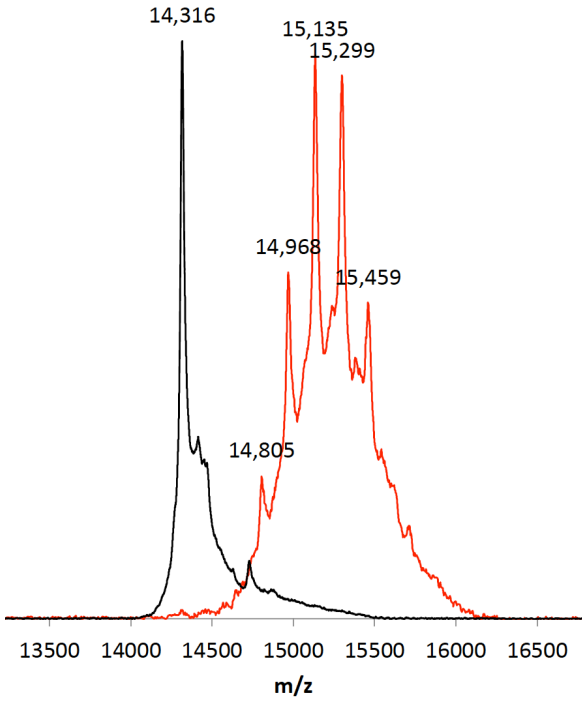


Figure S14. MALDI spectrum of lysozyme:norbornene conjugate product. (**Black** = Lysozyme wild-type, **Red** = Lysozyme:norbornene conjugate).



Functionalized Lysine Residues	Calculated (Da)	Found (Da, %)
3	14,808	14,805 (24%)
4	14,972	14,968 (60%)
5	15,136	15,135 (100%)
6	15,300	15,299 (95%)
7	15,465	15,459 (56%)

Figure S15. FPLC of lysozyme:norbornene conjugate at 220 nm. Norbornene attached to lysozyme surface and free norbornene impurities in solution are shown.

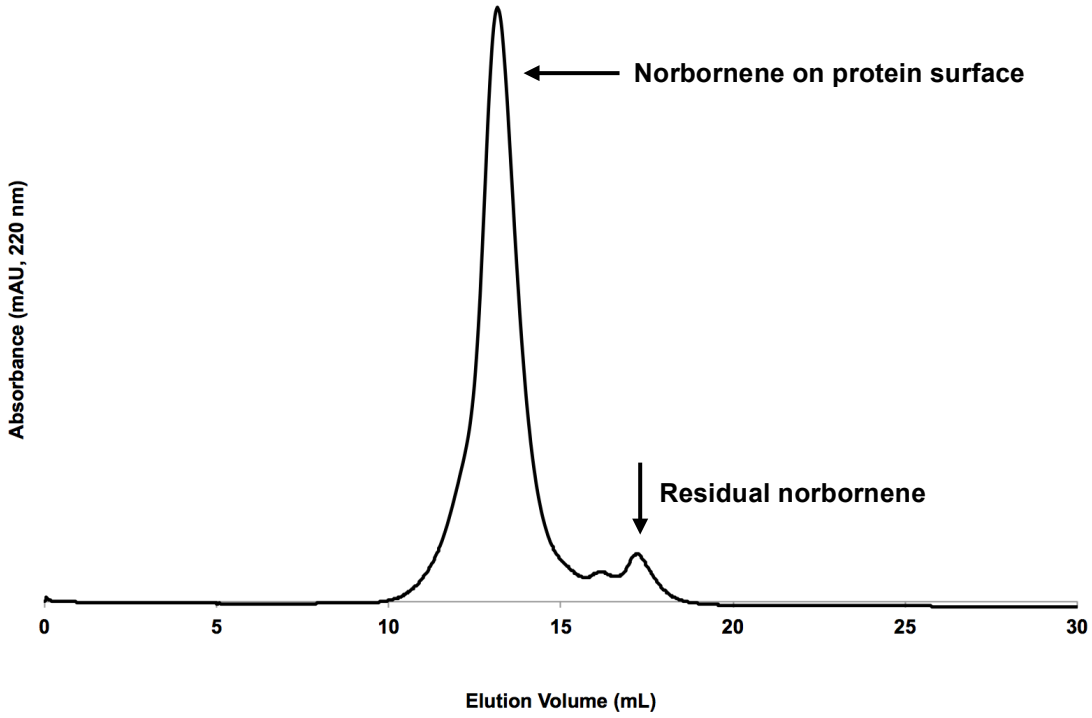


Figure S16. FPLC of unpurified protein:polymer conjugate (**5**) derived from ROMP of (**3**) with 400 equivalents of monomer (**4**). Absorbance measured at 280 nm.

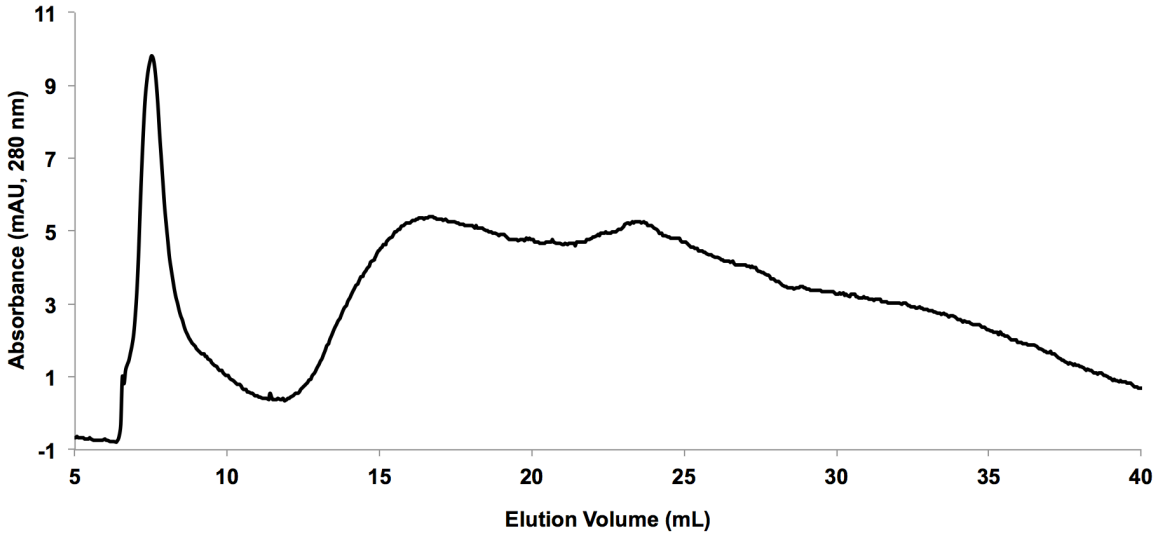


Figure S17. FPLC of lysozyme species at various stages of modification following purification. Absorbance measured at 280 nm.

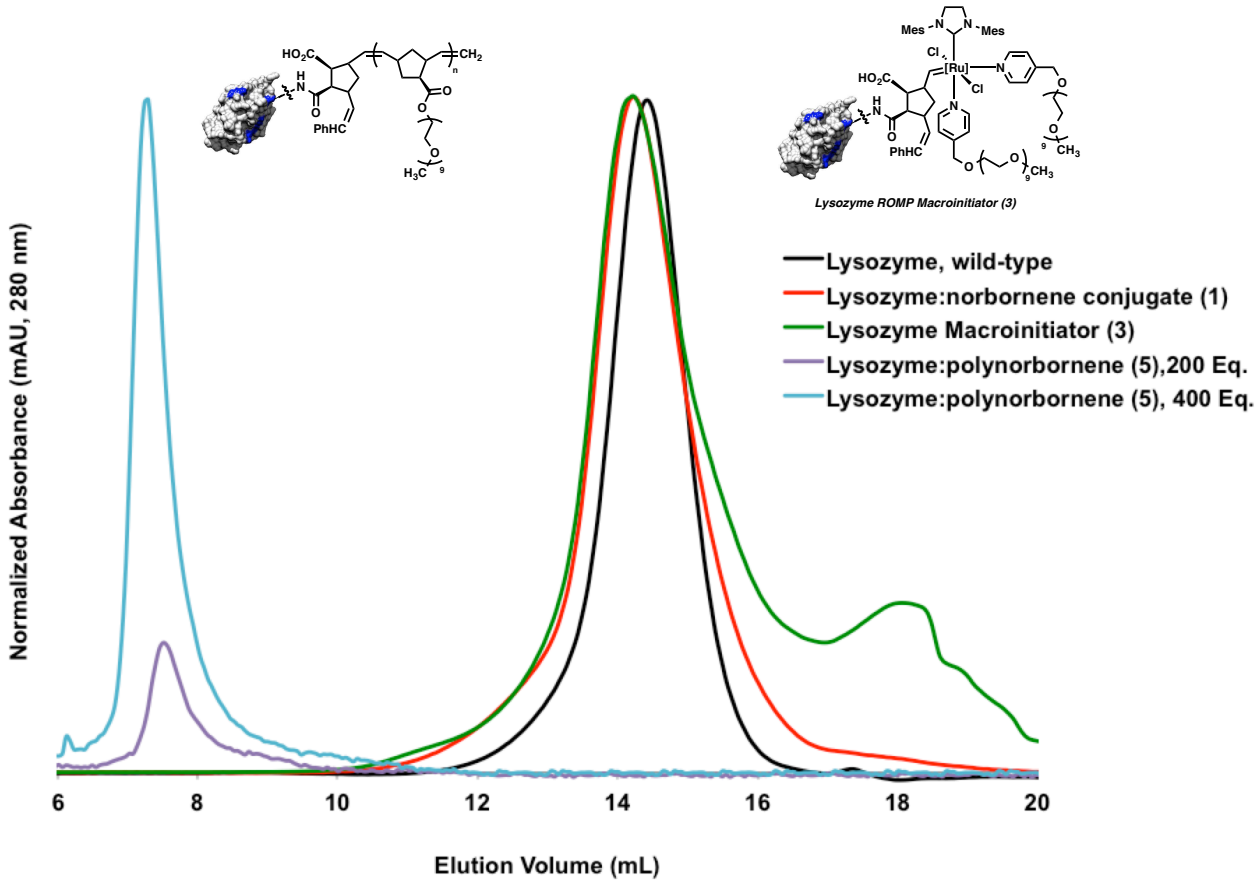


Figure S18. PAGE gels, stained with coomassie showing comparison in pattern and color between protein-polymer conjugate and free poly(norbornene). L = protein ladder, A = lysozyme, B = lysozyme:norbornene (**1**), C = monomer (**4**), D = *poly*(norbornene-PEG), M_n = ~40 kDa , E-G represent protein:polymer conjugates derived from ROMP of (**3**) with 100, 200, and 400 equivalents of monomer (**4**).

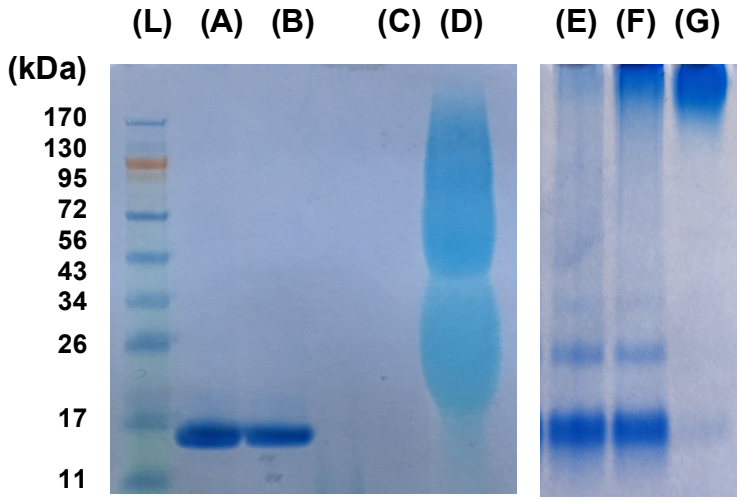
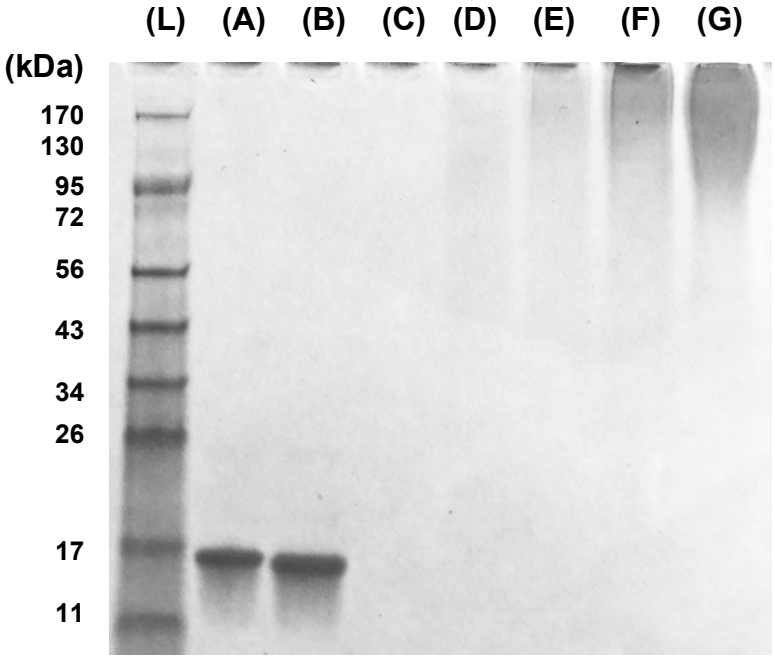


Figure S19. PAGE gels, stained with coomassie showing purified protein:polymer conjugates (5) after 12 hours at at 25 ^{o}C. L = protein ladder, A = lysozyme, B = lysozyme:norbornene (**1**), C-G represent protein:polymer conjugates derived from ROMP of (3) with 0, 50, 100, 200, and 400 equivalents of monomer (**4**).



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