Enzyme-Polymer Conjugates with Photocleavable Linkers for Control Over Protein Activity

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Supporting Information – Table of Contents

Material and Methods.

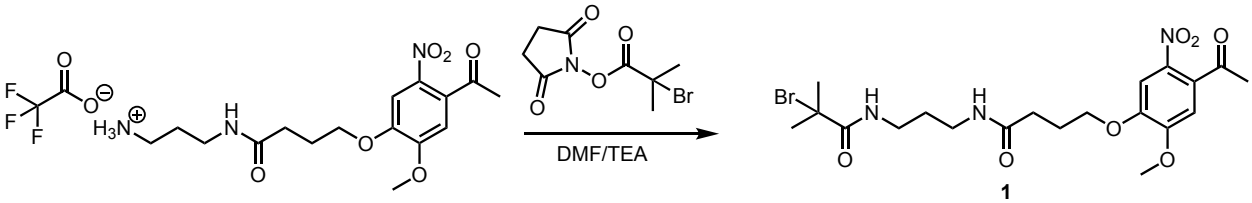
Acetovanillone was purchased from Oakwood Chemicals. O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) and N,N'-disuccinimidyl carbonate (DSC) were purchased from Chem-Impex International. Tin(II) 2-ethylhexanoate, fluorescamine, and tris(2-dimethylaminoethyl)amine, were purchased from Alfa Aesar. All other chemicals were purchased from Sigma-Aldrich and Fisher Scientific and were used without purification unless otherwise noted. For PEGA, 100 ppm BHT and 100 ppm MEHQ inhibitors were removed by passing through a plug of basic alumina. Copper bromide (Cu(I)Br) was purified by stirring in glacial acetic acid for 16 hours, filtering and rinsing with ethanol and diethyl ether twice and storing in a desiccator. Lysozyme from chicken egg white, Catalog No. J60701.06, Lot: V041520 was purchased from Sigma Aldrich. Lyophilized cells from Micrococcus Lysodeikticus ATCC No. 4698 were purchased from Sigma Aldrich. For photocleavage experiments, a 4 watt, 365 nm lamp was used (brand: UVP).

Analytical Techniques.

Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker AV 400 MHz and Bruker AV 500 MHz spectrometer. Proton NMR spectra were acquired with a relaxation delay for 2 s for small molecules. Abbreviations are s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet. Mass spectrometry for small molecules and lysozyme macroinitiators was obtained on an Agilent Q-TOF 6530 Liquid Chromatography/Mass Spectrometry (LC/MS) using a 10-95% acetonitrile gradient over 15 minutes. Mass spectrometry for lysozyme conjugates was obtained on a Bruker Ultraflex Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF)using sinapinic acid as the matrix. The matrix solution was prepared with a final concentration

of 10 mg/mL sinapinic acid in 50/50 acetonitrile/water with 0.1% TFA. For sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS PAGE), BioRad Any kDa Mini-PROTEAN-TGX™ gels were used. SDS-PAGE protein standards were obtained from Bio-Rad (Precision Plus Protein Prestained Standards). Small molecule purification was done *via* flash chromatography and conducted on a Biotage Isolera One auto-column system. Size exclusion chromatography (SEC) measurements in N,N-dimethylformamide (DMF) were performed on an Infinity 1260 II high-performance liquid chromatography (HPLC) system from Agilent equipped with a diode array detector DAD from Wyatt technology. The system also included a multiangle light scattering detector MALS and differential refractive index detector dRI from Wyatt technology. Polymers were separated on two PLgel Mixed-D gel columns PL1110-6504 (300 x 7.5 mm) (exclusion limits from 200 to 400 000 Da) at a flow rate of 0.6 mL min^{-1}. Column temperatures were held at 40°C in DMF with LiBr (0.1 M). Molar masses were calculated from PMMA standards. Conjugates were purified and analyzed by Fast Protein Liquid Chromatography on a Bio-Rad BioLogic DuoFlow chromatography system equipped with a HiTrap SP HP column employing a method of 0 to 1 M NaCl in 10 mM PB, pH 6.0 (Buffer A: 10 mM PB, pH 6.0; Buffer B: 10 mM PB, pH 6.0 + 1 M NaCl; 0.4 mL/min; 1 x 0.10 mL injections; 2 CV 0% Buffer B, then 8 CV 0- 100% Buffer B, then 2 CV 100% Buffer B). Plate reader assays for protein quantification by BSA standard assay, fluorescamine, and lysozyme activity assays were run on a Molecular Devices Spectramax iD3 instrument and data analysis was conducted on SoftMax Pro 7.1 software. Infrared spectroscopy (IR) data was collected using a Perkin Elmer University ATR Sampling Accessor Spectrum One FT-IR Spectrometer with 80 scans.

Photocleavable Initiator Synthesis.

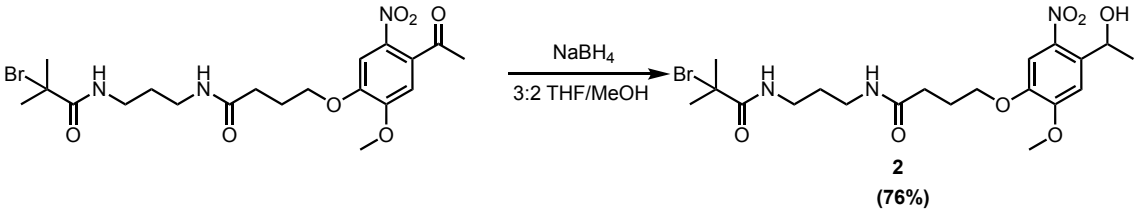


Synthesis of 1.

This reagent was prepared as previously reported.[1] 4-(4-Acetyl-2-methoxy-5-nitrophenoxy)-N-(3-aminopropyl)butanamide (1.16 g, 1 Eq, 3.28 mmol) was dissolved in 10 mL of dry DMF. Anhydrous triethylamine (TEA) (352 mg, 458 µL, 1 Eq, 3.28 mmol) and 2,5-dioxopyrrolidin-1-yl 2-bromo-2-methylpropanoate (1.73 g, 2 Eq, 6.57 mmol) were then added. Reaction proceeded for 16 hours at 25 °C. After reaction, the crude mixture was then precipitated into dry diethyl ether three times. 1.18 g (72% yield) product was collected as a yellow wax.

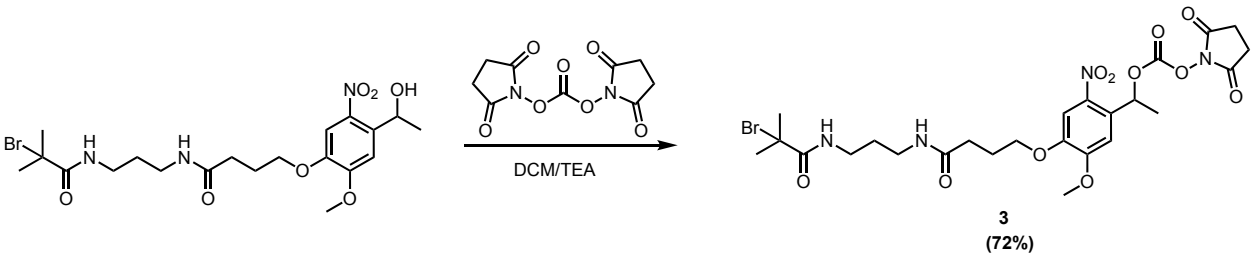
^{1}H NMR (500 MHz, chloroform-*d*) δ 7.60 (s, 1H), 7.23 (s, 1H), 6.74 (s, 1H), 6.34 (s, 1H), 4.15 (t, *J* = 6.2 Hz, 2H), 3.95 (s, 3H), 3.29 (m, *J* = 6.2, 1.7Hz, 4H), 2.46 (s, 2H), 2.26 – 2.16 (m, 2H), 1.94 (d, *J* = 1.8 Hz, 6H), 1.66 (m, *J* = 7.7, 5.5, 4.0, 2H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 200.11, 172.97, 172.87, 154.23, 148.86, 138.39, 132.81, 108.74, 107.07, 77.24, 62.00, 56.62, 53.45, 36.71, 35.85, 32.69, 32.34, 30.84, 30.41, 24.10. IR: ν = 2155, 1967, 1964, 1706, 1645, 1517, 1363, 1278,

1205, 1036, 649 cm^{-1}. ESI-MS calculated for $C_{20}H_{28}BrN_3O_7$ $[M+H]^+$ Expected: 502.11 Observed: 502.13 ^1H and ^{13}C NMR results are similar to what was previously published.[1]



Synthesis of 2.
4-(4-Acetyl-2-methoxy-5-nitrophenoxy)-N-(3-(2-bromo-2-methylpropanamido)propyl)-butanamide (809 mg, 1 Eq, 1.61 mmol) was dissolved in 10 mL of 3:2 tetrahydrofuran/methanol (THF/MeOH) with a stir bar in a glass scintillation vial. Sodium borohydride (243 mg, 4 Eq, 6.44 mmol) was then added in portions, resulting in the evolution of H_2. Reaction was allowed to proceed at 25 °C for 3 h. Saturated NH_4Cl was then added to quench the reaction, and the organic layer was collected. The aqueous layer was then extracted three times with ethyl acetate. The organic layers were combined and dried with $MgSO_4$ to yield and concentrated. 620 mg (76%) of product was collected as a yellow wax.

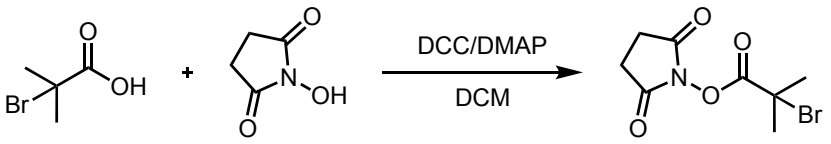
^1H NMR (500 MHz, chloroform-*d*) δ 7.55 (s, 1H), 7.30 (s, 1H), 7.22 (d, *J* = 7.2 Hz, 1H), 6.32 (t, *J* = 6.4 Hz, 1H), 5.54 (q, *J* = 6.3 Hz, 1H), 4.10 (t, *J* = 6.2 Hz, 2H), 3.97 (s, 3H), 3.34 – 3.21 (m, 4H), 2.56 (s, 1H), 2.44 (t, *J* = 7.2 Hz, 2H), 2.27 – 2.15 (m, 2H), 1.94 (s, 6H), 1.70 – 1.59 (m, 2H), 1.54 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 172.82, 172.80, 154.01, 146.86, 139.59, 137.15, 109.15, 108.77, 68.59, 65.71, 62.00, 56.37, 36.71, 35.92, 32.38, 32.34, 29.71, 24.92. IR: ν = 2659, 2330, 1629, 1507, 1262, 1093, 731 cm^{-1}. ESI-MS calculated for $C_{20}H_{30}BrN_3O_7$ $[M+H]^+$ Expected: 504.13 Observed: 504.14



Synthesis of 3.
N-(3-(2-Bromo-2-methylpropanamido)propyl)-4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanamide (732 mg, 1 Eq, 1.45mmol) was dissolved in 20 mL dry dichloromethane (DCM). N.N'-disuccinimidyl carbonate (1.86 g, 5 Eq, 7.26 mmol) and anhydrous TEA (0.147 g, 202 µL, 1 Eq, 1.45 mmol) were then added to the flask. Reaction was allowed to stir at 25 °C for 16 h. Afterwards, reaction mixture was quenched and washed three times with $NaHCO_3$ solution, dried over $MgSO_4$, and concentrated. Product was purified by gradient column chromatography (90% DCM, 10% MeOH). 685 mg (73%) of product was collected as an orange-yellow wax.

^1H NMR (500 MHz, chloroform-*d*) δ 7.63 (s, 1H), 7.07 (s, 1H), 6.49 (q, *J* = 6.3 Hz, 1H), 6.27 (s, 1H), 4.11 (q, *J* = 7.2 Hz, 2H), 4.04 (s, 3H), 3.28 (m, *J* = 12.2, 8.2, 6.9, 4H), 2.80 (s, 4H), 2.45 (t, *J*

= 7.2 Hz, 2H), 2.21 (p, *J* = 6.8 Hz, 2H), 1.95 (s, 6H), 1.65 (p, *J* = 6.1 Hz, 2H), 1.59 (m, 3H). ^{13}C NMR (126 MHz, chloroform-*d*)) δ 172.77, 172.72, 171.16, 168.46, 154.50, 150.58, 147.66, 139.27, 131.21, 109.17, 107.30, 68.58, 62.06, 60.41, 56.53, 36.64, 32.88, 32.36, 29.68, 25.43, 24.86, 21.07. IR: ν = 2658, 2330, 1697, 1628, 1508, 1197, 1070, 636 cm^{-1}.



(77%)

Synthesis of NHS-bromoisobutyrate initiator .

This procedure was adapted from Zhang, A. et al.[2] To a 250 mL round bottom flask were added 2-bromo-2-methylpropanoic acid (10 g, 1 Eq, 60 mmol), N-hydroxysuccinimide (NHS) (10 g, 1.5 Eq, 90 mmol), N,N'-dicydclohexylcarbodiimide (DCC) (19 g, 1.5 Eq, 90 mmol), and 4-dimethylaminopyridine (DMAP) (1.5 g, 0.2 Eq, 12 mmol). These reagents were suspended in 60 mL of DCM. Reaction was allowed to stir for 16 hours at 25 ℃. The crude reaction was then filtered and purified by gradient column chromatography (85% DCM, 15% MeOH). 12.2 g (77%) of product was collected as a white solid.

^{1}H NMR (500 MHz, chloroform-*d*) δ 2.86 (s, 2H), 2.08 (d, *J* = 1.2 Hz, 3H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 168.53, 167.49, 51.14, 30.73, 25.64. IR: ν = 2661, 2331, 1078 cm^{-1}. ^{1}H and ^{13}C NMR results are similar to what was previously published.[2]

General Procedure for the AGET ATRP of PEGA with 3.

To a 10 mL Schlenk flask was added Me_6TREN (33 µL, 0.12 mmol, 4 Equiv.), which was diluted with 0.1 mL of solvent. This solution was allowed to deoxygenate via sparging with argon for 15 minutes. To a second 10 mL Schlenk flask was added $PEGA_{Mn = 480}$ (0.68 mL, 1.5 mmol, 50 Equiv.), **3** (20 mg, 0.031 mmol, 1 Equiv.) and DMF as an internal standard. The monomer-initiator solution was diluted with 0.1 mL of solvent and 10 µL of dimethylsulfoxide (DMSO) to fully dissolve the initiator. The solution was also deoxygenated via sparging with argon for 30 minutes. Meanwhile, $CuBr_2$ (6.9 mg, 0.031 mmol, 1 Equiv.) was added to the first Schlenk flask under a positive argon flow. Upon addition of $CuBr_2$, the catalyst complex solution became blue. Sparging with argon was continued for an additional 15 minutes. A stock solution of ascorbic acid dissolved in solvent was prepared and deoxygenated via sparging for 30 minutes. After 30 minutes, the monomer-initiator solution was first transferred via an argon-flushed syringe into the catalyst complex solution. Then, ascorbic acid (3.3 mg, 0.05 mL, 0.019 mmol, 0.6 Equiv.) was fed slowly into the reaction solution via an argon-flushed syringe. Upon complete addition of the ascorbic acid, the reaction mixture turned olive green in color. Reaction was allowed to stir for 15 hours at 30 ℃ under a closed argon atmosphere. Reaction was quenched by exposing solution to air and diluting with THF. To purify polymer, the crude solution was passed through a neutral alumina oxide column and precipitated into cold diethyl ether. Wash with cold diethyl ether was carried out three times. After drying under high vacuum, the final polymer was then characterized by ^{1}H NMR and DMF SEC analysis (with PMMA standards).

Polymer Entry 3 (Table 1):
^{1}H NMR (400 MHz, D_2O) δ 7.57 (s, 1H), 7.29 (s, 1H), 4.14 (s, 280H), 3.26 (s, 385H), 2.29 (s, 166H), 1.73 (m, 253H).
M_n (kDa): 41.5 (± 3.60%)
$Đ$: 1.33

This procedure was followed for other entries, but no characterization was obtained for those entries due to unsuccessful monomer conversion.

General Procedure for the SARA ATRP of PEGA with 3.

To a 20 mL Schlenk flask was added Me_6TREN (87 µL, 0.34 mmol, 3 Equiv.), which was diluted with 3 mL of solvent. This solution was allowed to deoxygenate via sparging with argon for 15 minutes. To a second Schlenk flask was added PEGA (1.3 g, 11 mmol, 100 Equiv.), initiator (30 mg, 0.11 mmol, 1 Equiv.) and DMF as an internal standard. The monomer-initiator solution was diluted with 1 mL of isopropanol (IPA) to dissolve the monomer and 100 µL of DMSO to fully dissolve the initiator. After 15 minutes, copper nanopowder (4.3 mg, 0.068 mmol, 0.6 Equiv.) was added to the first Schlenk flask under a positive argon flow, and then finally $CuBr_2$ (10 mg, 0.045 mmol, 0.4 Equiv.) was added. Upon addition of $CuBr_2$, the catalyst complex solution became blue in color with black specs of copper nanopowder at the bottom. The catalyst complex solution was allowed to deoxygenate for another 15 minutes *via* sparging with argon. Finally, to initiate the polymerization, the monomer-initiator solution was transferred via an argon-flushed syringe into the catalyst complex solution. Reaction was allowed to stir for 1 hour at 25 °C under a closed argon atmosphere. Reaction was quenched by exposing the solution to air and diluting with THF. To purify polymer, the crude solution was passed through a neutral alumina oxide column and precipitated into cold diethyl ether. Washing with cold diethyl ether was carried out three times. After drying under high vacuum, the final polymer was then characterized by ^{1}H NMR and DMF SEC analysis (with PMMA standards).

Polymer Entry 5 (Table 1):
^{1}H NMR (400 MHz, D_2O) δ 7.55 (s, 1H), 7.27 (s, 1H), 4.11 (s, 143H), 3.24 (s, 218H), 2.24 (s, 86H), 1.70 (m, 162H).
M_n (kDa): 31.3 (± 4.90%)
$Đ$: 1.15

Polymer Entry 6 (Table 1):
^{1}H NMR (400 MHz, D_2O) δ 7.57 (s, 1H), 4.13 (s, 565H), 3.26 (d, J = 2.7 Hz, 1029H), 2.30 (m, 344H), 1.73 (m, 552H).
M_n (kDa): 90.4 (± 3.60%)
$Đ$: 1.29

General Procedure for Hydrolysis Studies of Photocleavable Initiator:

A sample of the photocleavable initiator was dissolved in 10% (v/v) deuterated acetonitrile (acetonitrile-*d*) and diluted with either pH 6 PBS buffer or pH 7.4 deuterated water. ^{1}H NMR analysis was used to quantify retainment of the NHS end-group in comparison to the hydrolyzed product. The following time points were taken: 0, 1.6, 2.9, 3.8, 5.4, 6.9, 27.3, and 30.2 hours. Percent retainment was determined based on the ratio of photocleavable initiator to hydrolyzed product at the first ^{1}H NMR measurement of t = 0.

General Procedure for Conjugation of 90 kDa Photocleavable Polymer to Lysozyme:

A 1 mg/mL solution of lysozyme was prepared in pH 9, 50 mM sodium carbonate buffer. After the protein concentration was determined via a BCA assay, the 90 kDa photocleavable polymer (Table 1 Entry 6) (2.9 μmol, 4 equiv with respect to lysine residues) dissolved in dimethyl sulfoxide (DMSO) was added to the protein solution (final DMSO concentration of 5%). The conjugation reaction was allowed to proceed for 5 h at 25 °C.

Procedure for the Preparation of Lysozyme Macroinitiator:

A stock solution of 10.53 mg/mL lysozyme was prepared in pH 8 carbonate buffer. 1 mg of lysozyme (95 μL) was diluted up to 200 μL with pH 8 carbonate buffer. Then, 50 equivalents of initiator (dissolved in 10 μL of DMSO) was added to the 1 mg solution of lysozyme. Conjugation was allowed to proceed for 16 hours at 25 °C. Afterwards, lysozyme samples (modified and unmodified) were purified via the use of 3 kDa molecular weight cut off (MWCO) centriprep filters. Three washes with MilliQ H_2O for 15 minutes, 13.2 rpm were carried out during this purification process. Finally, purified samples were rediluted to a total volume of 1 mL using pH 8 carbonate buffer. % Modification was determined by a fluorescamine assay.

Procedure for the Polymerization From Lysozyme Macroinitiator:

To a 10 mL Schlenk flask is added Me_6TREN (7.4 mg, 8.7 μL, 1.26 Eq, 0.032 mmol), copper (I) bromide (4.6 mg, 0.98 μL, 1.26 Eq, 0.032 mmol), and 0.1 mL of pH 8 phosphate buffer. This was allowed to stir at 0 °C using an ice bath. Upon addition of the copper bromide, the appearance of the blue deactivating Cu^{2+} species in solution was observed. Meanwhile, to another 10 mL Schlenk flask was added PEGA (3.13 g, 3.13 mL, 250 Eq, 6.5 mmol), lysozyme macroinitiator (200 μg, 5.0 x 10^{-3} Eq, 0.0140 μL), ethyl 2-bromo-2-methyl-propanoate (5.0 mg, 3.8 μL, 1 Eq, 26 μmol) as sacrificial initiator, and DMF (5.6 mg, 6.0 μL, 3 Eq, 77 μmol) which was used as an internal standard). Deoxygenation via sparging with argon was undertaken for 15 minutes. To initiate the polymerization, the monomer and initiator solution was transferred by a syringe argon-flushed needle to the copper catalyst complex flask. Polymerization was allowed to proceed for 1 hour at 25 °C.

Procedure for the Characterization of Polymer by 'Graft-From':

This experiment was carried out as we previously reported.[3] Briefly, protein-polymer conjugate was buffer exchanged by centriprep ultracentrifugation (MWCO 3kDa) to digestion buffer (10 mM Tris HCl, 2 mM $CaCl_2$, pH 7.4). 100 µL of this sample was then added to 100 µL Proteinase K at 2 mg/mL in digestion buffer. The solution was incubated in a 50 °C water bath for 24 hours. Peptide fragments were removed by centriprep ultracentrifugation (MWCO 3 kDa) before analysis by SEC.

^{1}H NMR (400 MHz, D_2O) δ 7.57 (s, 1H), 7.31 (s, 1H), 4.15 (s, 3506H), 3.78 – 3.37 (m, 60714H), 3.28 (s, 5744H), 2.28 (s, 1646H), 1.75 (m, 3252H).
M_n (kDa): 117.1 kDa
$Đ$: 1.16

General Procedure for Photocleavage of Protein-Polymer Conjugates:

Samples, diluted up to 100 µL of pH 8 phosphate buffer, were irradiated with UV light (365 nm) for 1 hour at 4 °C to allow for photocleavage of the polymer from the protein. Afterwards, samples were immediately used for activity assay or further characterization. It has been reported that cleavage of *ortho*-nitrobenzyl groups is 80% complete within 10 minutes at 3.5 mW/cm^2 at 365 nm.[4]

General Procedure for Activity Assay of Protein-Polymer Conjugates/Lys:

Samples were added to a transparent 96-well plate. All samples were mixed with 200 µL of a suspension of lyophilized Micrococcus Lysodeikticus cells (0.15 mg/mL in 50 mM potassium phosphate buffer, pH 6.24). Absorbance measurements were taken at 450 nm for 30 minutes over 30 second intervals. The change in absorbance over 120 minutes was determined and used to determine protein activity.

Control Experiment on Native Lysozyme Activity Upon Exposure to UV Irradiation:

5 µg of native lysozyme was diluted up to 100 µL of MilliQ H_2O in a 96 well-plate. These samples were allowed to be exposed to UV irradiation for a given amount of time. The following exposure time points were carried out: 0 minutes, 15 minutes, 30 minutes, 60 minutes, 90 minutes, and 120 minutes. Each time point was done in triplicate. After the final exposure time point of 120 minutes all samples were mixed with 200 µL of a suspension of lyophilized *Micrococcus Lysodeikticus* cells (0.15 mg/mL in 50 mM potassium phosphate buffer, pH 6.24). Absorbance measurements were taken at 450 nm for 120 minutes over 30 second intervals. The change in absorbance over 120 minutes was determined and used to determine protein activity.

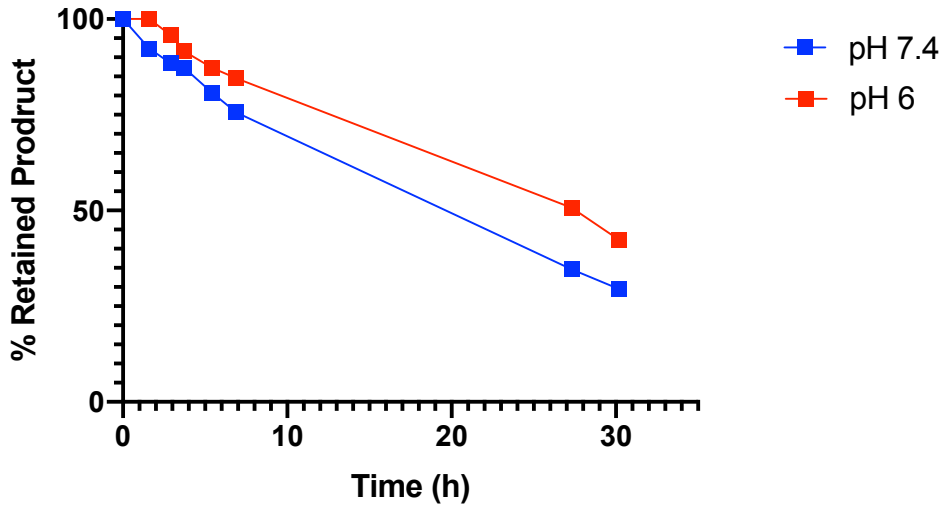


Figure S1. Hydrolysis studies of photocleavable initiator

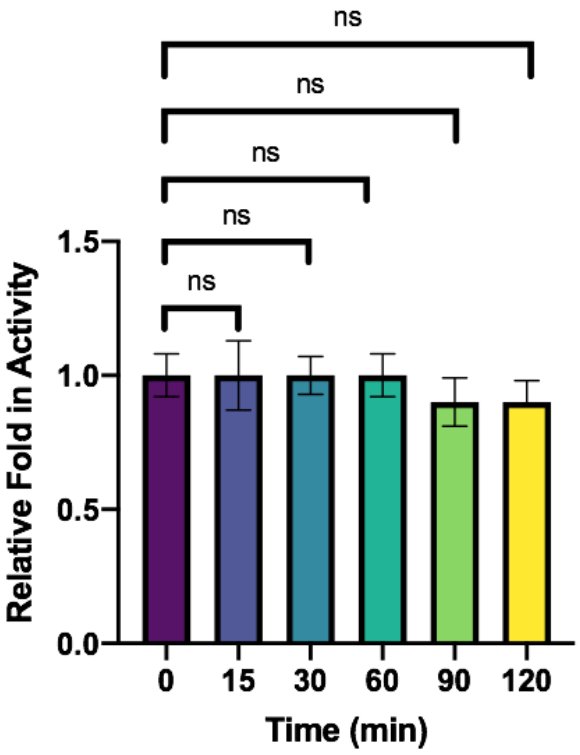


Figure S2. Impact of native lysozyme activity upon exposure to UV irradiation. Each measurement was conducted in triplicate and statistical significance was determined *via* a one-way ANOVA with multiple comparisons (ns = $p \geq 0.05$ compared against the sample at t = 0 min).

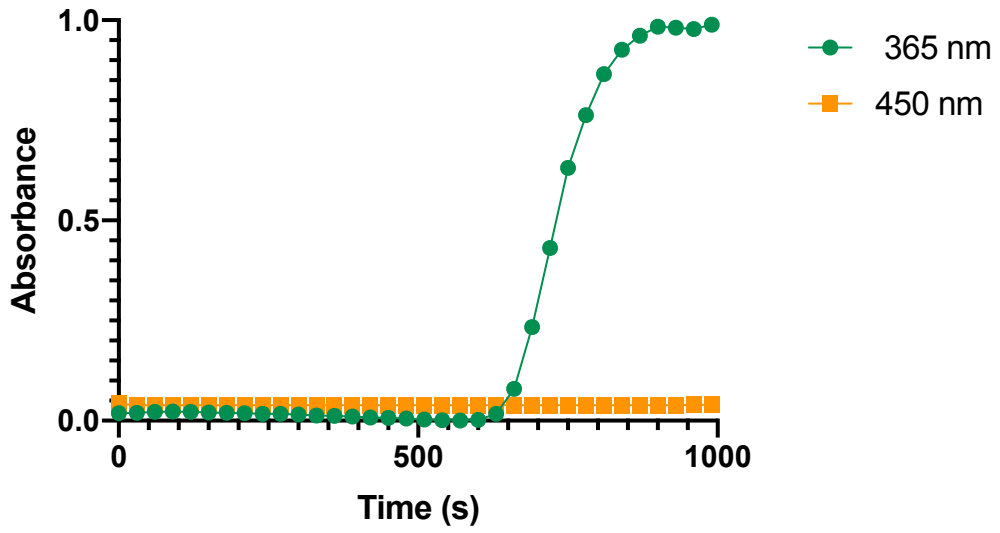


Figure S3. Absorbance measurements of conjugate after exposure to different wavelengths over time. Measurements were taken at 365 nm and 450 nm, respectively, over the course of 16 minutes at 30 second intervals. The result shows that 450 nm utilized in the activity assay does not change protein absorbance, while 365 does, due to cleavage of the polymer.

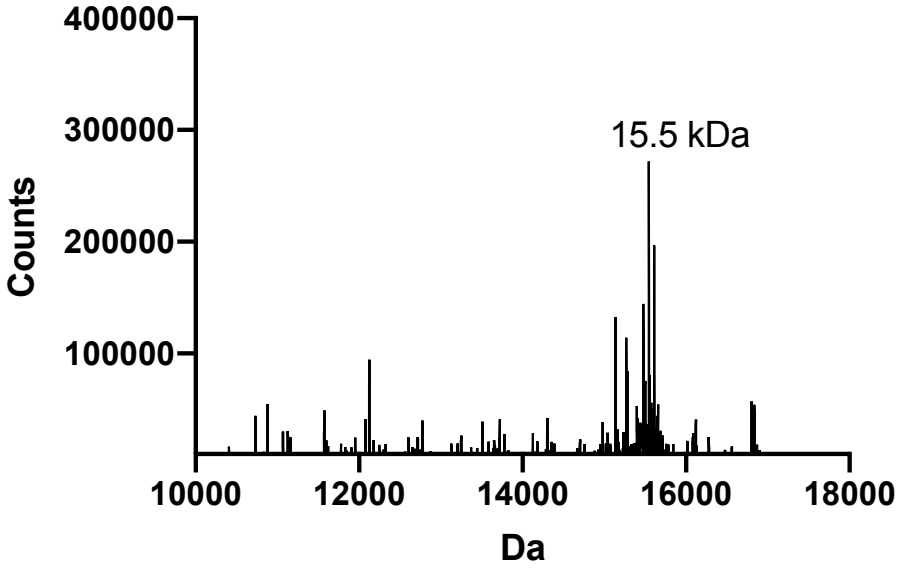


Figure S4. LCMS spectrum of non-cleavable lysozyme macroinitiator.

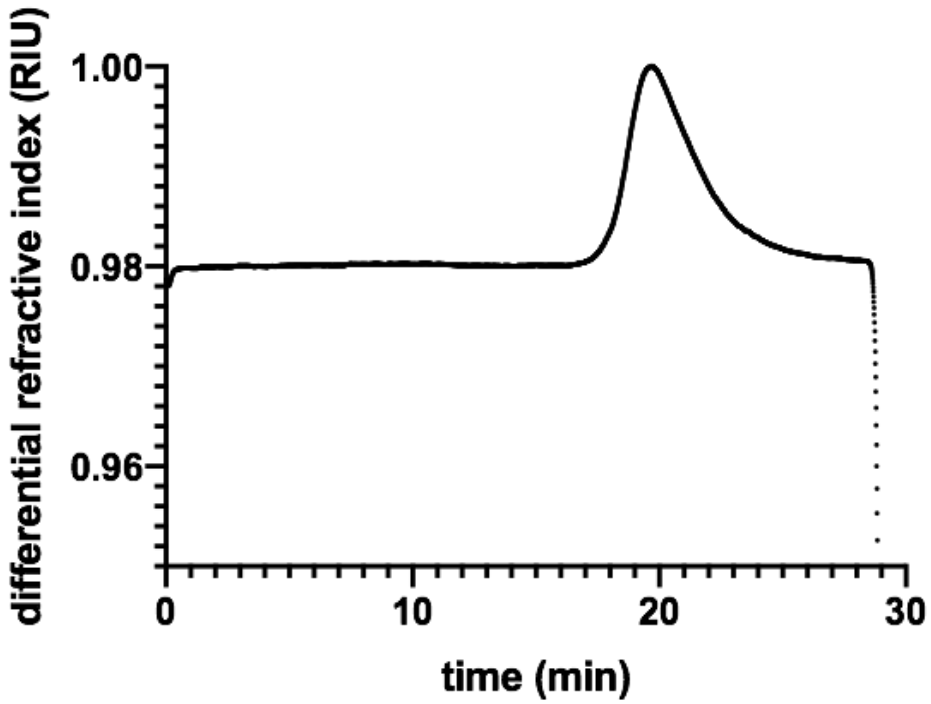


Figure S5. DMF SEC Trace of Polymer from *grafting-from*.

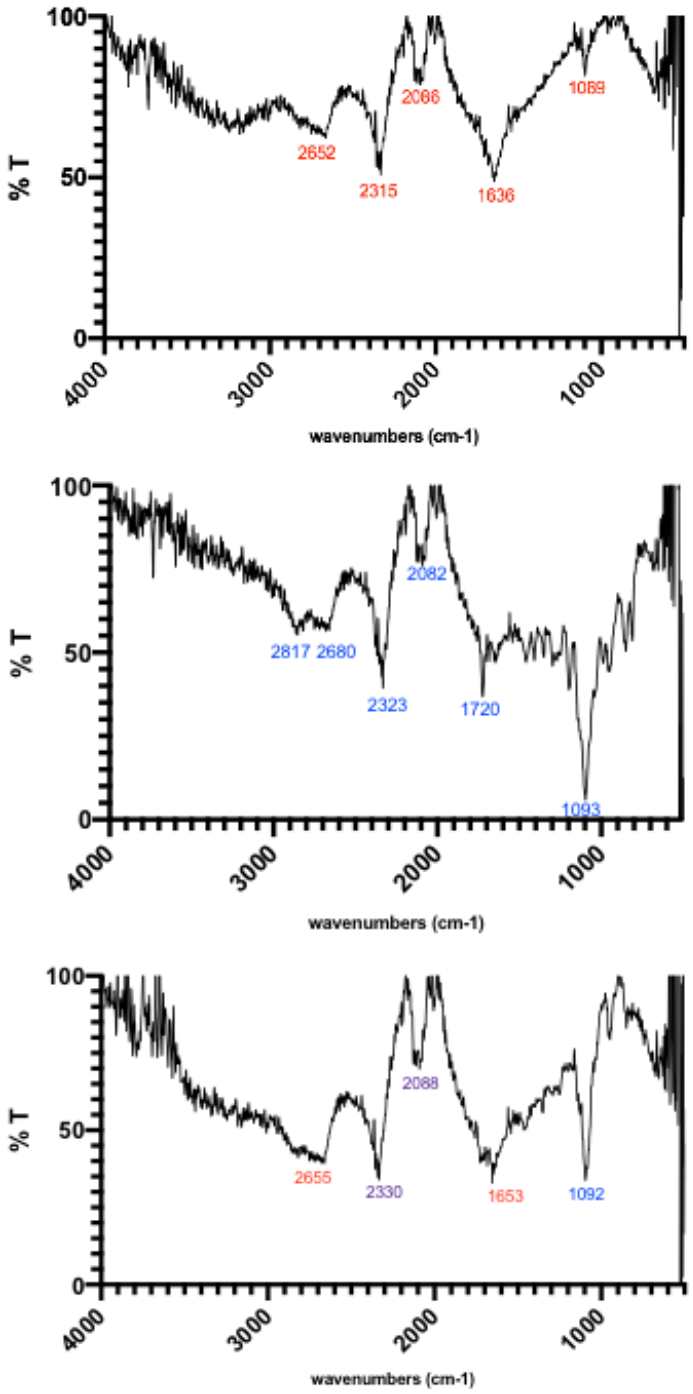


Figure S6. IR analysis of *grafting-from* conjugate. Lysozyme macroinitiator (top), PEGA monomer (middle), lysozyme polymer conjugate (bottom).

Other Polymer SEC Traces

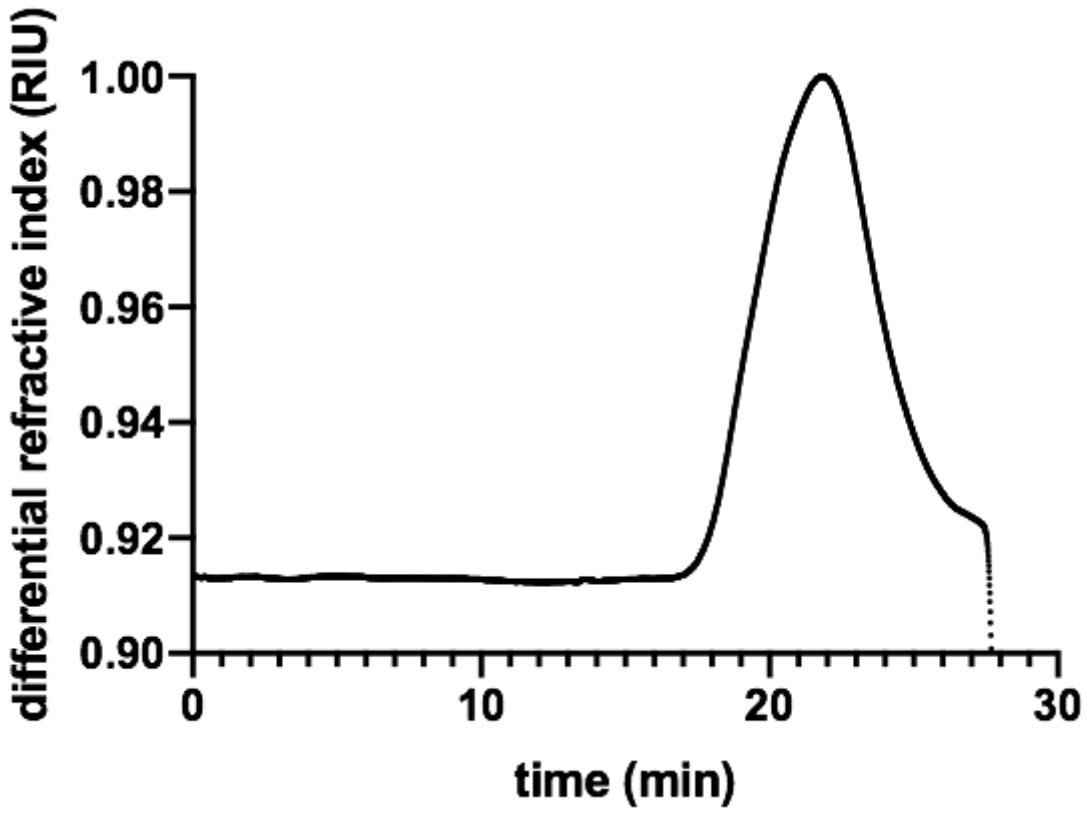


Figure S7. DMF SEC trace of polymer Entry 3 (Table 1).

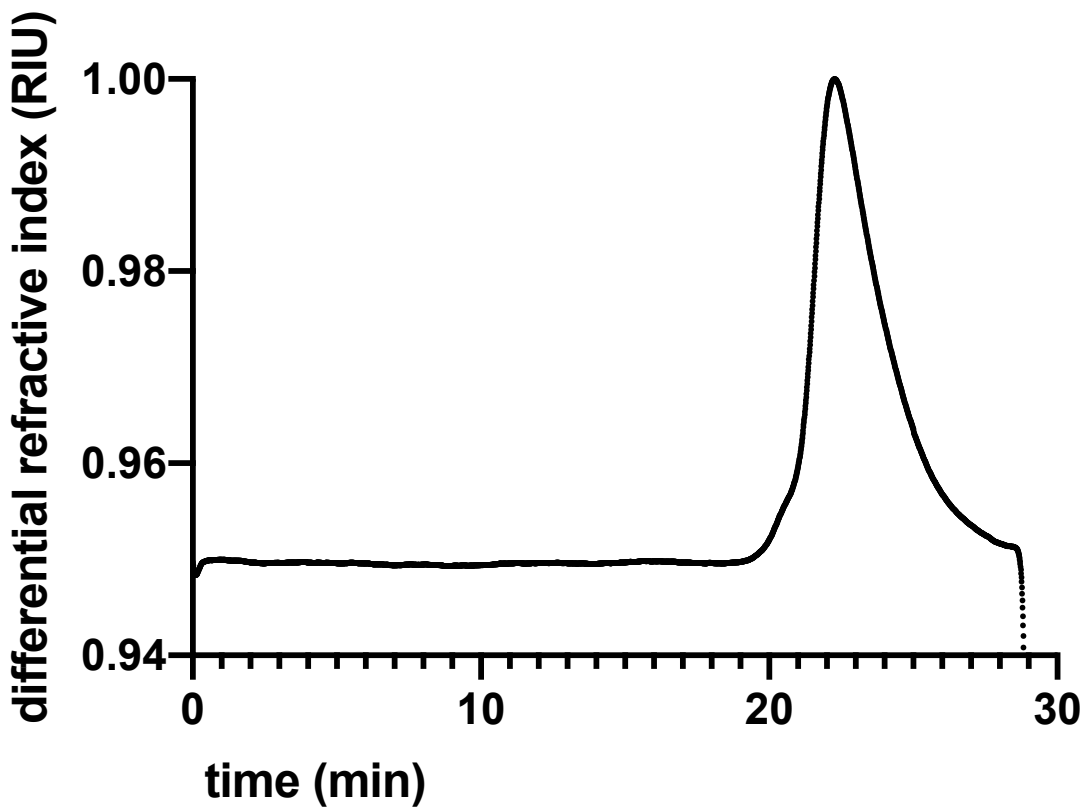


Figure S8. DMF SEC trace of polymer Entry 5 (Table 1).

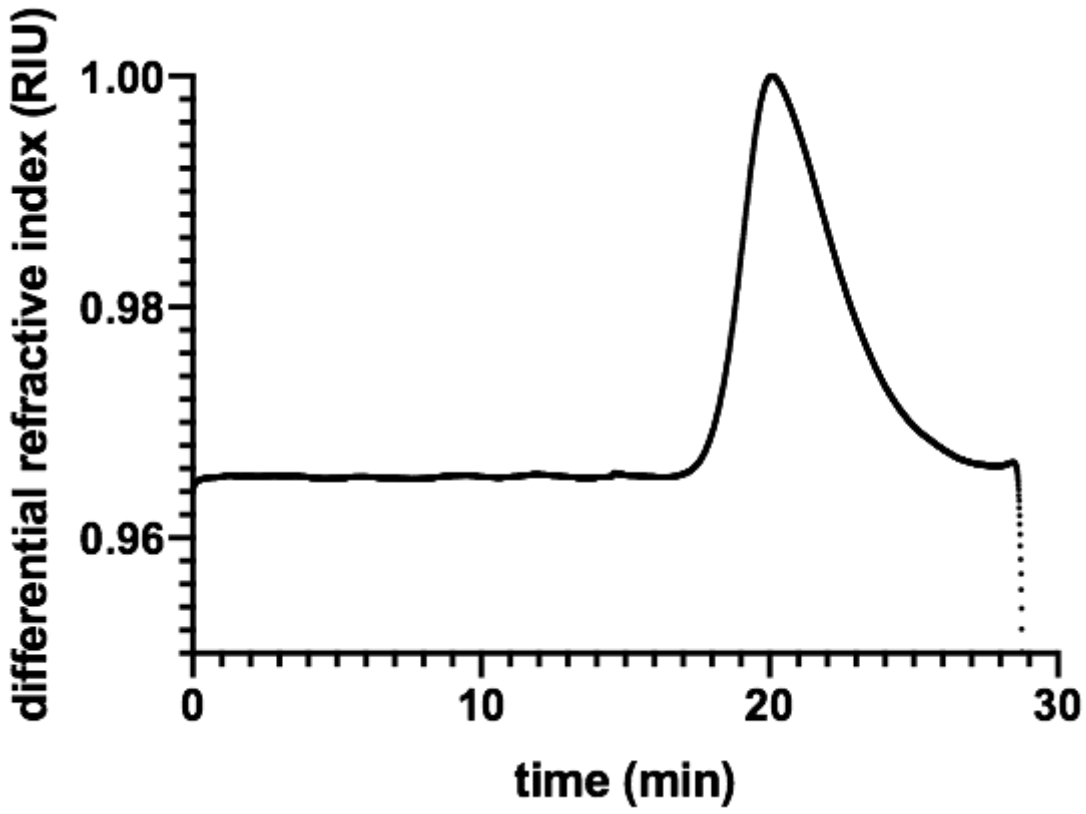


Figure S9. DMF SEC trace of polymer Entry 6 (Table 1).

^{1}H NMR and ^{13}C NMR Spectrum for Photocleavable Initiator Synthesis

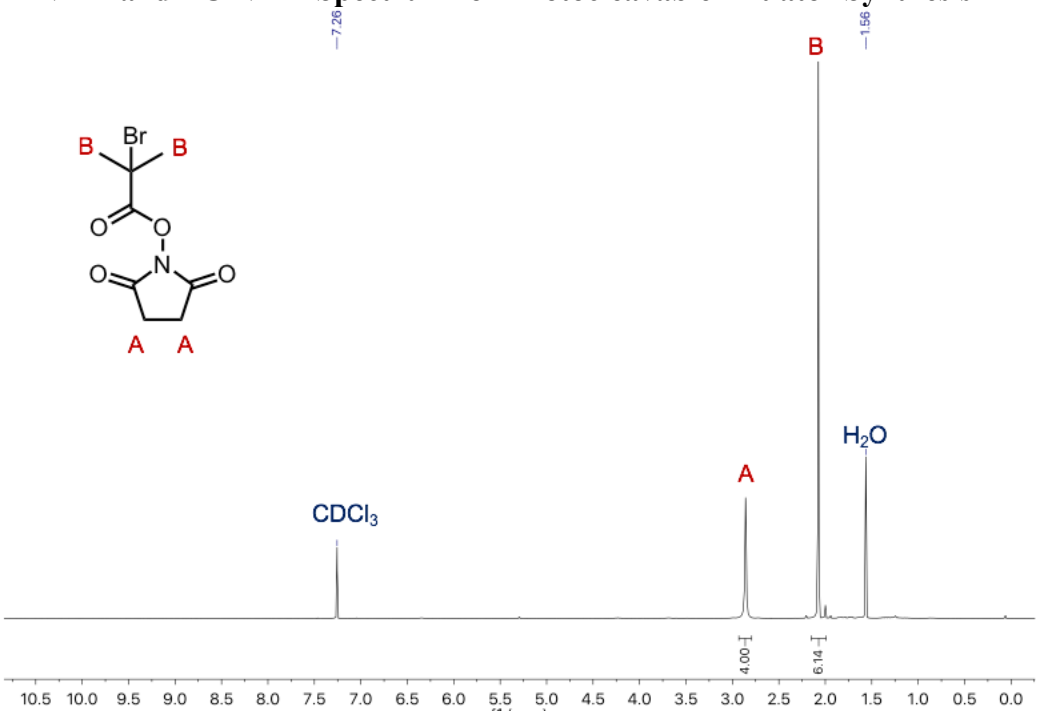


Figure S10. ^{1}H NMR spectrum of NHS-isobromobutyrate in $CDCl_3$.

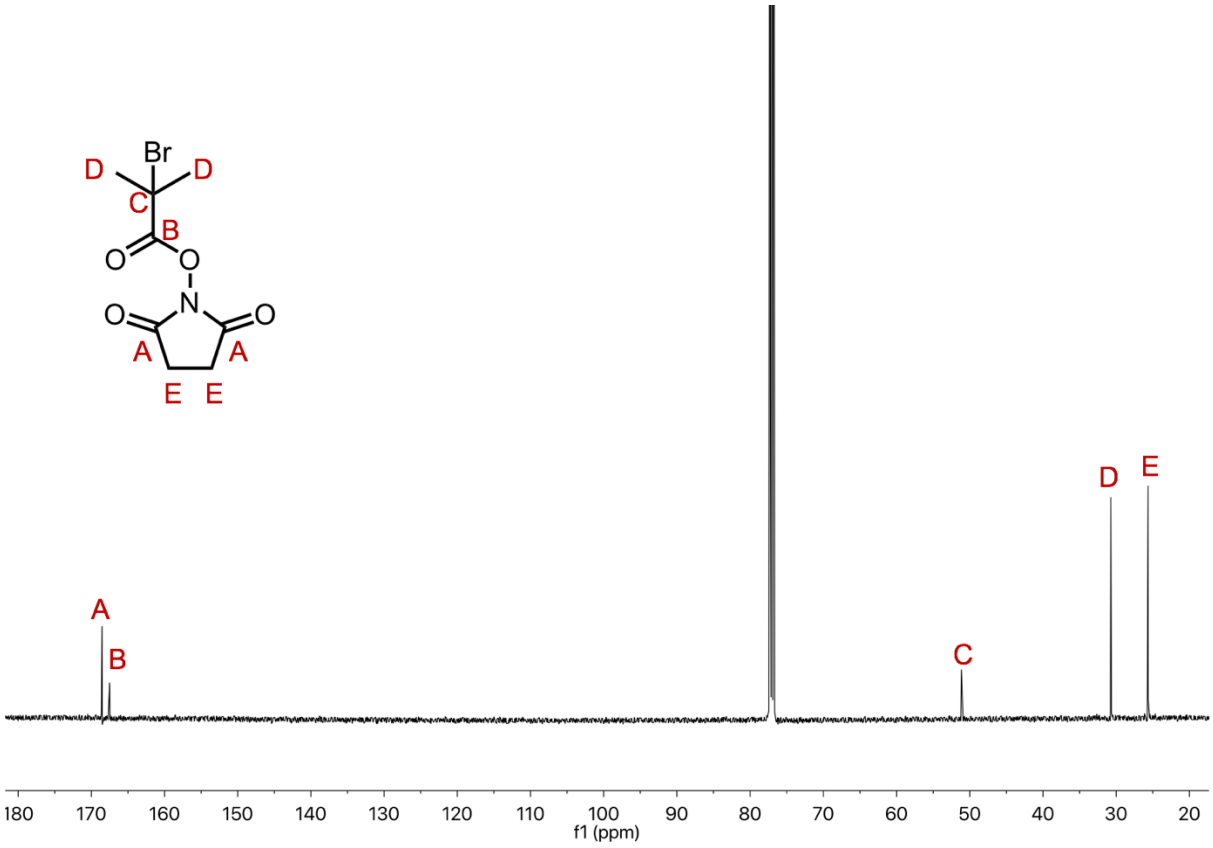


Figure S11. ^{13}C NMR spectrum of NHS-isobromobutyrate in $CDCl_3$.

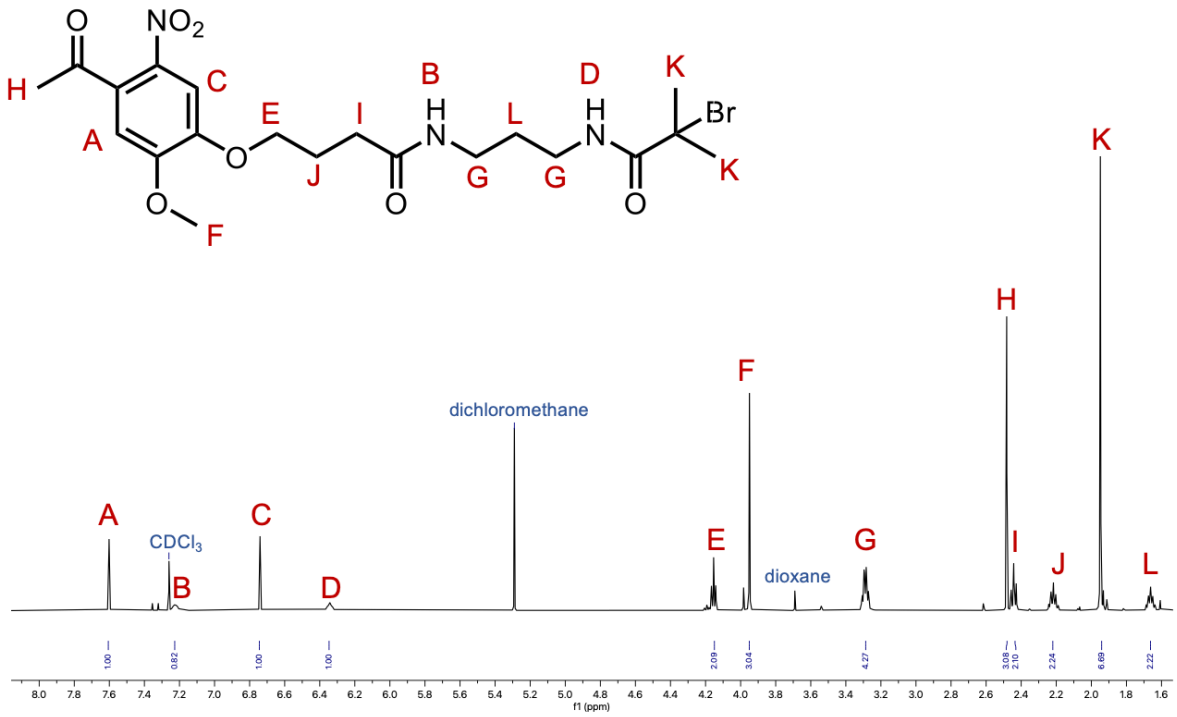


Figure S12. ^{1}H NMR spectrum of **1** in $CDCl_3$.

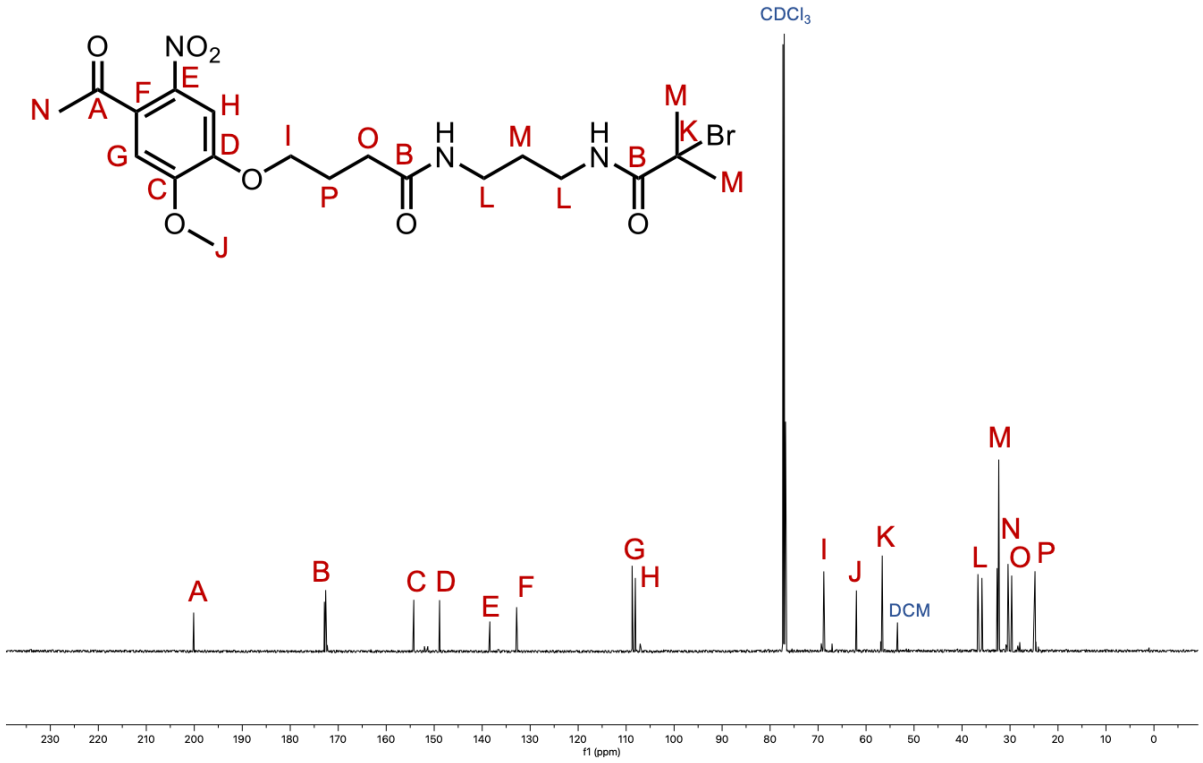


Figure S13. ^{13}C NMR spectrum of **1** in $CDCl_3$.

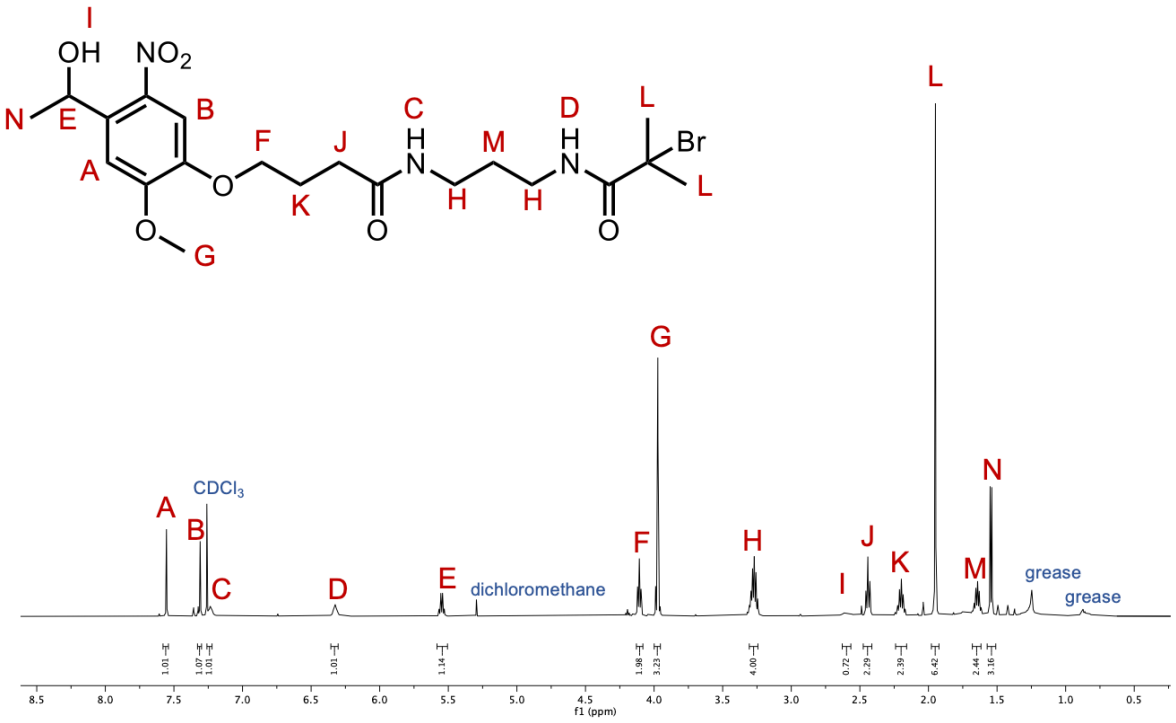


Figure S14. ^{1}H NMR spectrum of **2** in $CDCl_3$.

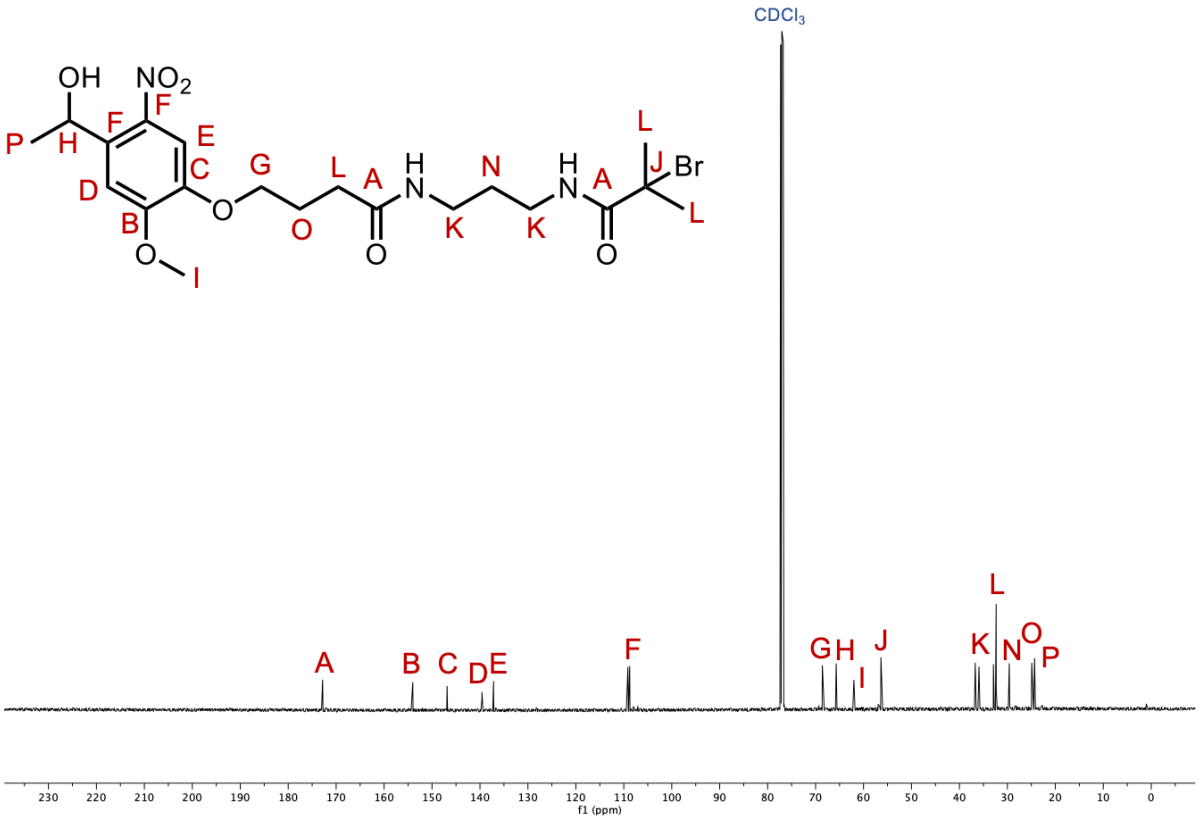


Figure S15. ^{13}C NMR spectrum of **2** in $CDCl_3$.

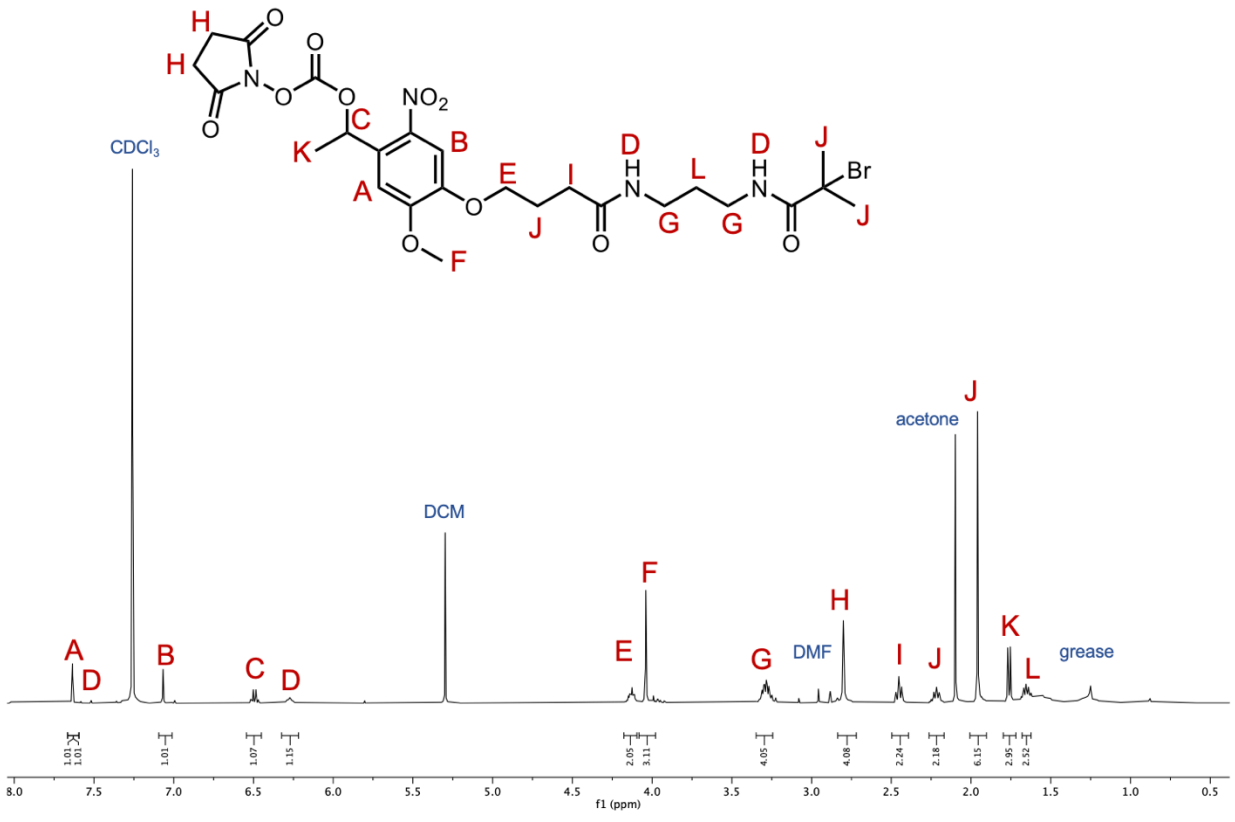


Figure S16. ^{1}H NMR spectrum of **3** in $CDCl_3$.

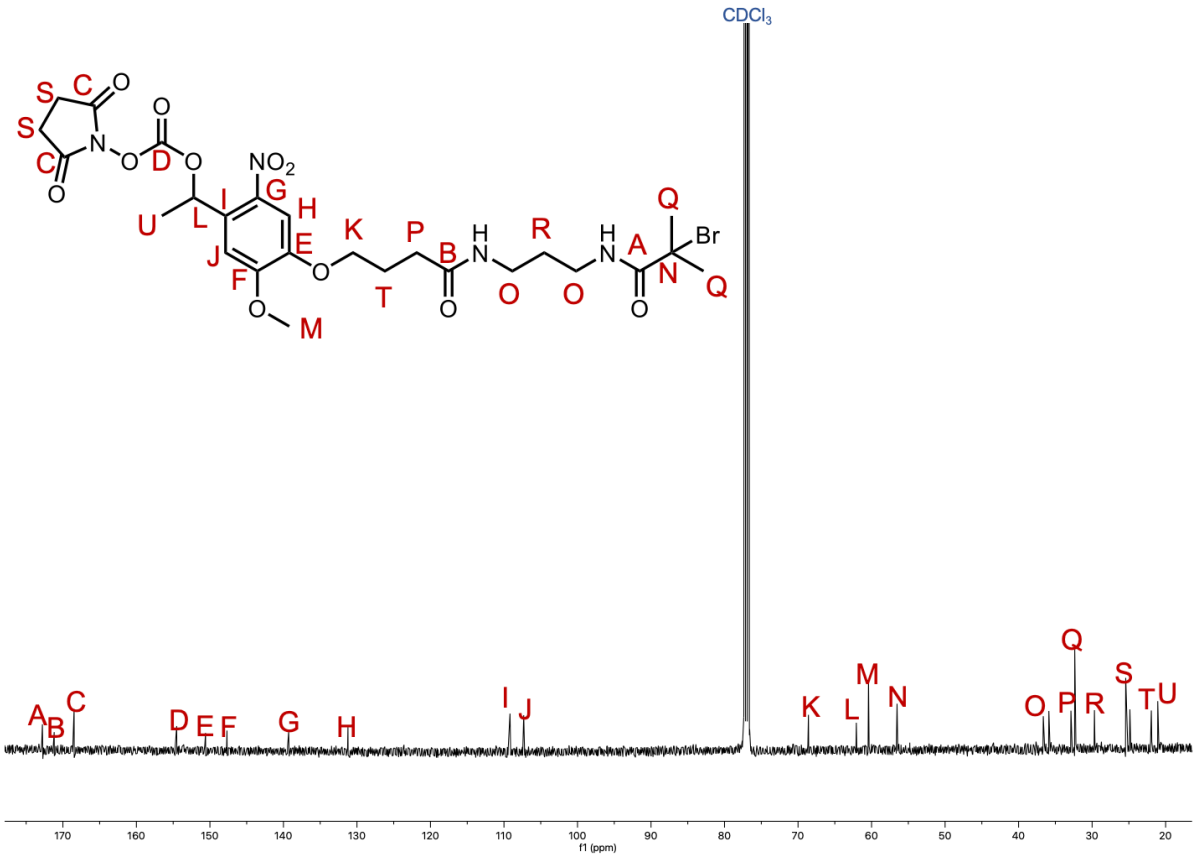


Figure S17. ^{13}C NMR spectrum of **3** in $CDCl_3$.

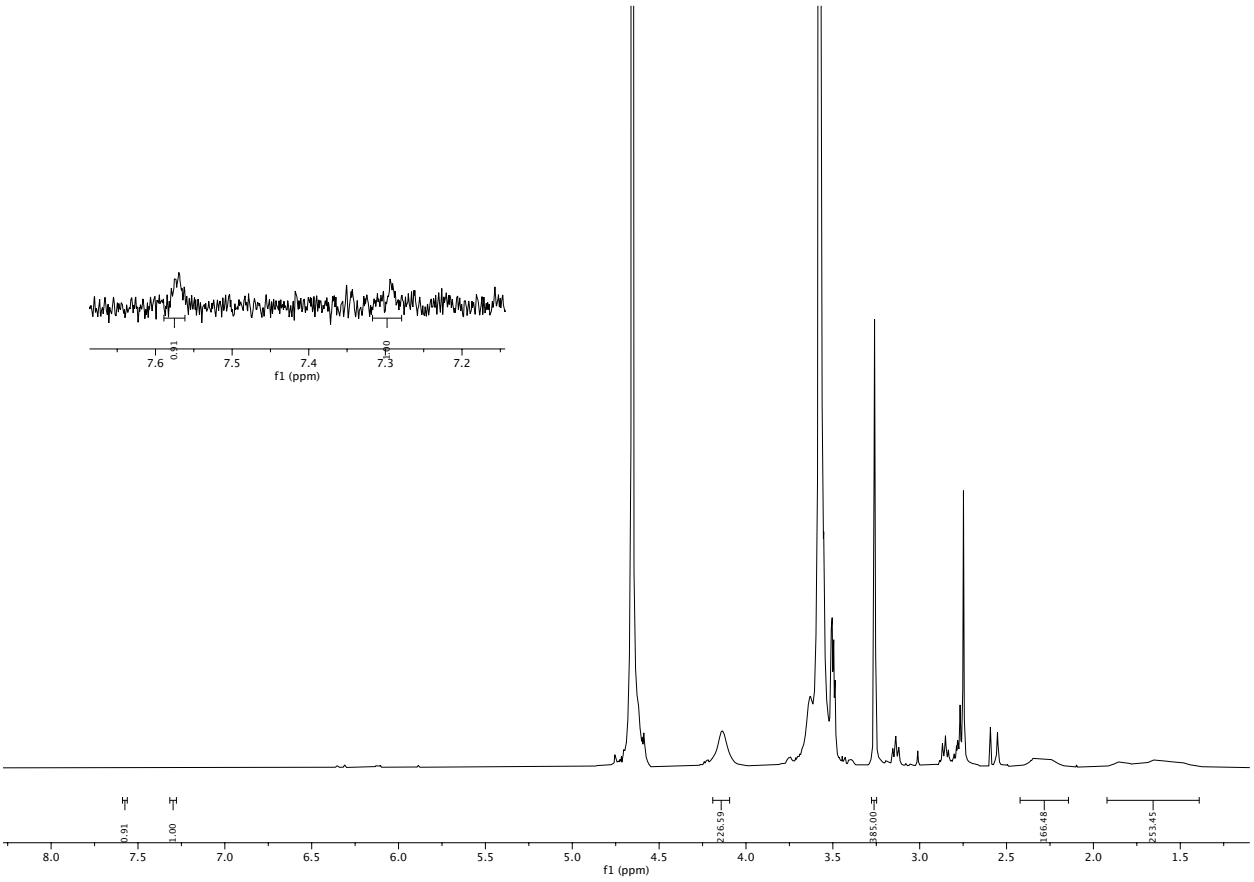


Figure S18. ^{1}H NMR spectrum of Entry 3 Table 1 in D_2O.

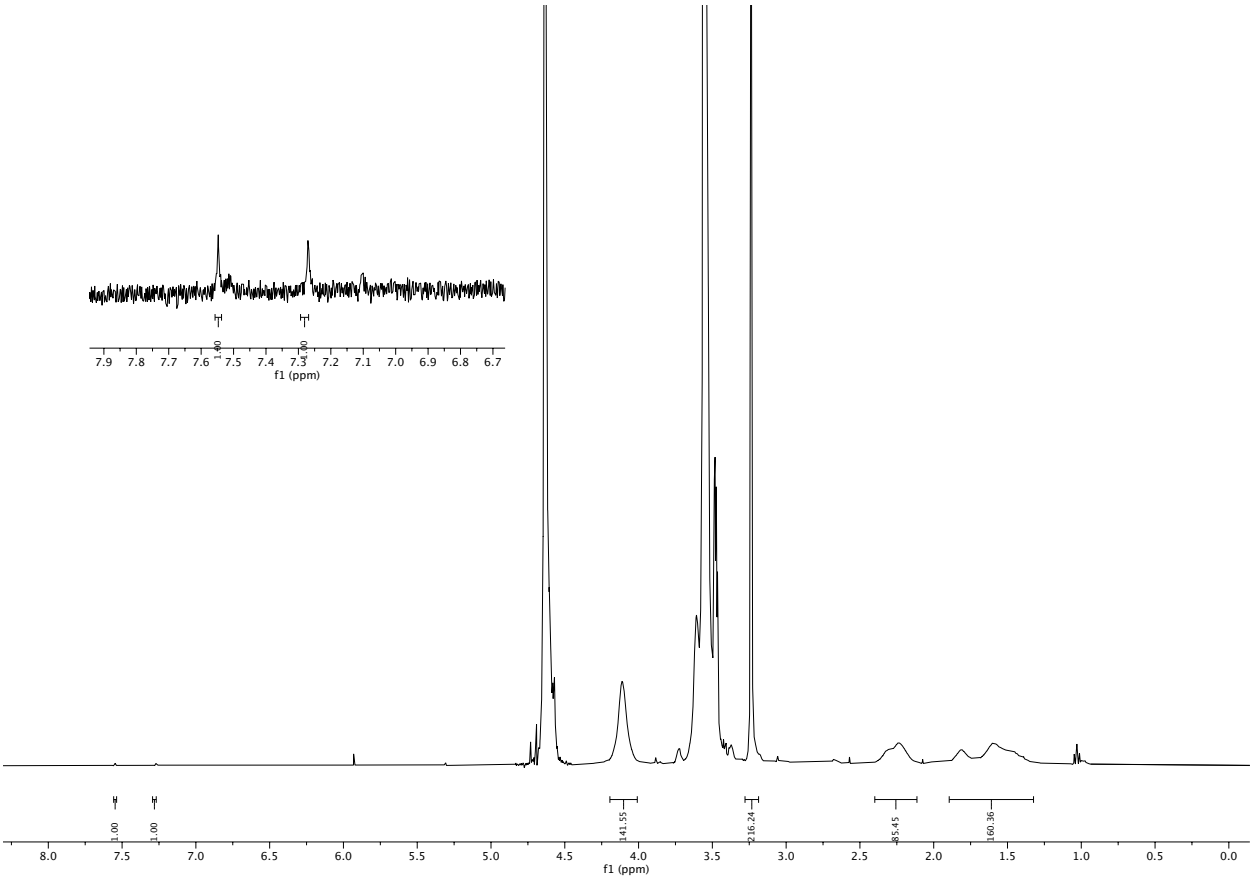


Figure S19. ^{1}H NMR Spectrum of Entry 5 Table 1 in D_2O.

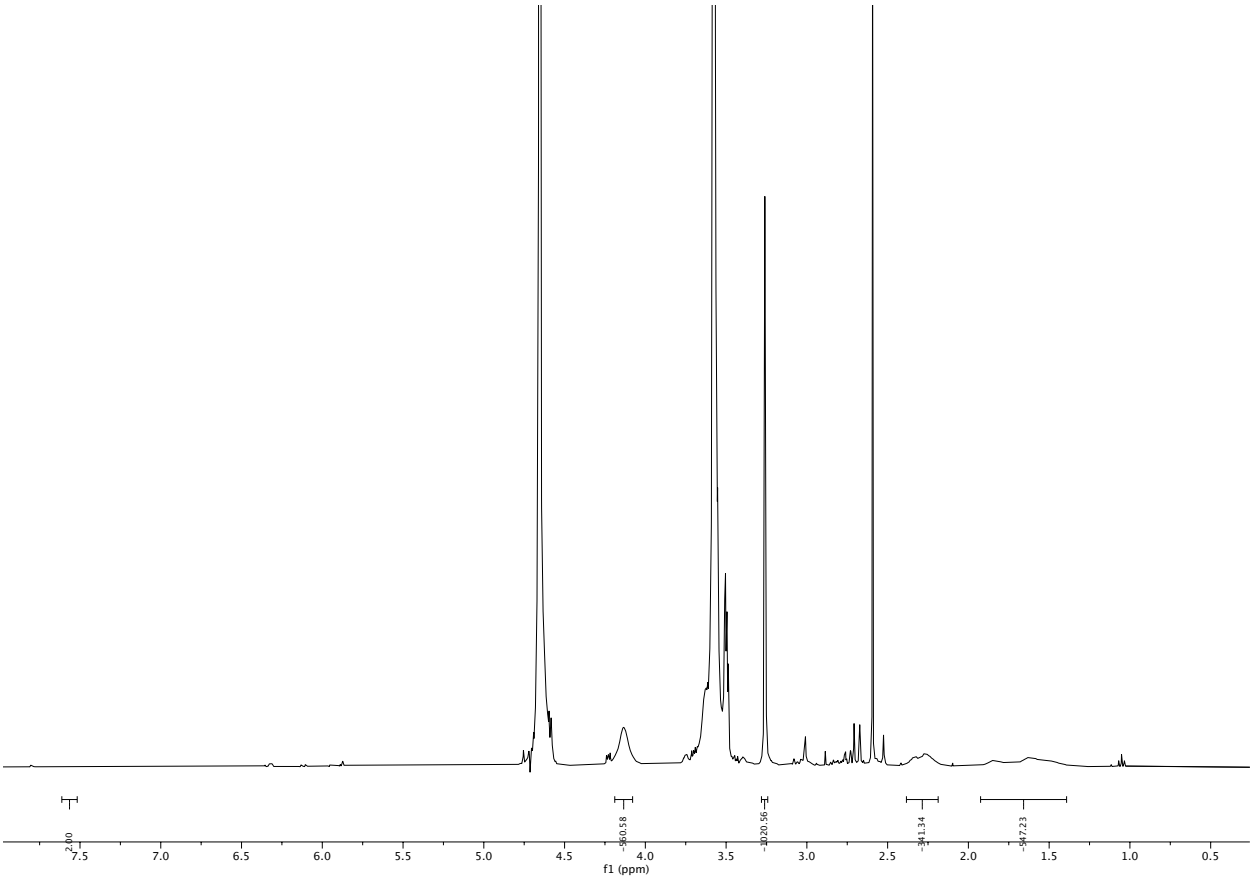


Figure S20. ^{1}H NMR Spectrum of Entry 6 Table 1 in D_2O.

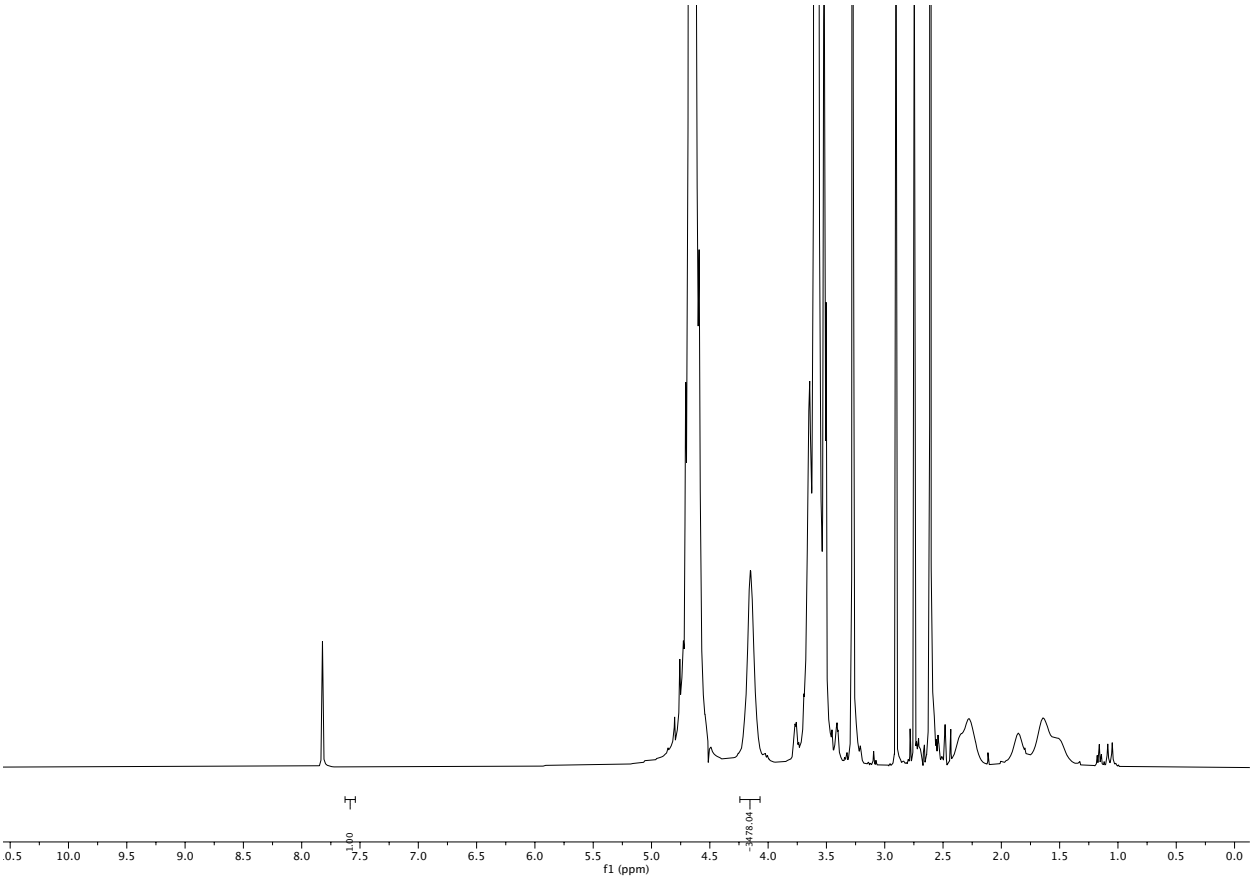


Figure S21. ^{1}H NMR Spectrum of *grafting-from* polymer after protein digestion in D_2O.

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