Supporting Information

Selective and Reversible Photochemical Derivatization of Cysteine Residues in Peptides and Proteins

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Table of contents:

General Information. All organic solvents were dried and freshly distilled before use. Flash chromatography was performed using 40-63 μm silica gel. All NMR spectra were recorded on 400 MHz instruments in $CDCl_3$ and referenced to TMS unless otherwise noted. Solutions were prepared using HPLC grade water, and acetonitrile. Photolyses were carried out using mini-Rayonet photochemical reactor equipped with 8 fluorescent UV lamps (4W, 300 or 350) or a hand-held lamp equipped with 2 fluorescent UV lamps (4W, 300 or 350).

Materials: 1-iodo-2-(2-(2-methoxyethoxy)ethoxy)ethane,[1] 2-(2-(Vinyloxy)ethoxy)ethyl tosylate,1 6-(hydroxymethyl)naphthalene-1,7-diol (NQMP **1b**),[2] 8-TEG-3-(hydroxymethyl)naphthalen-2-ol (NQMP-TEG **2c**),1 8-(Biotin-TEG)-3-(hydroxymethyl)naphthalen-2-ol (NQMP **1d**),1 and 1-α-(3-Azidopropyl)-2,3,4,6-tetra-*O*-acetyl-D-mannopyranoside (**S2**),[3] were prepared following the previously reported procedures. Fmoc-L-amino acid derivatives and resins were purchased from NovaBioChem and Applied Biosystems; peptide synthesis grade DMF was obtained from EM Science and *N*-methylpyrrolidone (NMP) from Applied Biosystems; Bovine Serum Albumin (BSA) was acquired from Thermo Scientific.

2-(2-(vinyloxy)ethoxy)ethyl iodide (**S1**): NaI (1.5g, 10 mmol) was added to a solution of 2-(2-(vinyloxy)ethoxy)ethyl tosylate (1.43 g, 5 mmol) in acetone (20 mL) and reaction mixture was refluxed for 8h. Solvent was removed under reduced pressure, the residue was dissolved in dichloromethane, washed with brine, dried over anhydrous magnesium sulfate, and the solvent was removed in vacuum. The product was purified by chromatography (60% EtOAc in hexanes) to yield 0.99 g (82%) of **S1** as a yellowish oil. ^{1}H NMR: 6.40 (dd, *J* = 14.3, 6.8 Hz, 1H), 4.10 (dd, *J* = 14.3, 2.0 Hz, 1H), 3.94 (dd, *J* = 6.8, 2.0 Hz, 1H), 3.81 – 3.73 (m, 2H), 3.73 – 3.63 (m, 4H), 3.18 (t, *J* = 7.0 Hz, 2H). ^{13}C NMR: 151.95, 87.18, 72.37, 69.49, 67.54, 2.91. FW calc. (C6H11IO2): 241.9804, EI-HRMS: 241.9810.

Nonapeptides Ac-Cys-Lys-Tyr-Trp-Gly-Arg-Gly-Asp-Ser-NH$_2$ (4), ***Cys-Lys-Tyr-Trp-Gly-Arg-Gly-His-Ser-NH$_2$ (4-His)***, ***Ac-Met-Lys-Tyr-Trp-Gly-Arg-Gly-Asp-Ser-NH$_2$ (6)***, *and* ***Met-Lys-Tyr-Trp-Gly-Arg-Gly-His-Ser-NH$_2$ (6-His)*** were synthesized following a standard Fmoc chemistry protocol on Rink amide AM resin (0.25 mmol). Peptides preparation was accomplished on a Applied Biosystems ABI 433A peptide synthesizer equipped with UV-detector using N^{α}-Fmoc-protected amino acids and 2-(1H-bezotriazole-1-yl)-oxy-1,1,3,3-tetramethyl hexafluorophosphate (HBTU)/1-Hydroxybenzotriazole (HOBt) as the activating reagents. The resin was treated with cleavage solution of 95:2.5:2.5 TFA/water/TIS (5 mL) for 1 h. The resin was filtered, washed with TFA (5 mL), and the filtrate was then concentrated in

vacuum to approximately 1/3 of its original volume. The peptide was precipitated by using diethyl ether (0°C, 100 mL) and recovered by centrifugation at 3000 rpm for 15 min. The crude peptide was purified by RP-HPLC on a semi-preparative C-18 column using a linear gradient solvent (0-95% CH_3CN in H_2O, 0.1% TFA in 40 min; flow: 1 mL/min) and the appropriate fractions were lyophilized to afford target peptide. Peptide **4**: FW Calcd. for $C_{48}H_{70}N_{15}O_{14}S^+$ (M + H^+) 1112.4942, MALDI HRMS found 1112.6621.

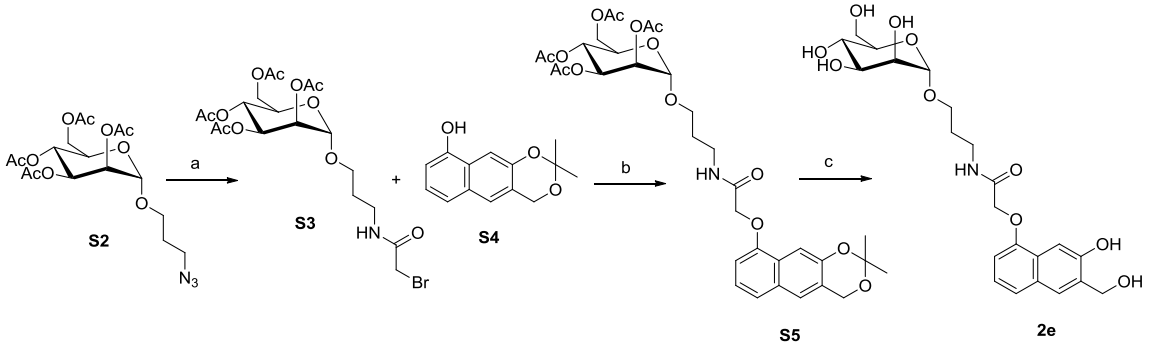
Peptide **4-His**: FW Calcd. $C_{48}H_{70}N_{17}O_{11}S^+$ for (M + H^+) 1092.5161. MALDI HRMS found 1092.5121.

Peptide **6**: FW Calcd for $C_{50}H_{74}N_{15}O_{14}S^+$ (M + H^+) 1140.5255. MALDI HRMS found 1140.5098.

Peptide **6-His**: FW Calcd for $C_{50}H_{74}N_{17}O_{11}S^+$ (M + H^+) 1120.5469. MALDI HRMS found 1120.6261.

Peptide 4 dimer [Ac-Cystine-Lys-Tyr-Trp-Gly-Arg-Gly-Asp-Ser-NH_2]$_2$ (**7**): Peptide **4** (40 mg) was dissolved in 2:8 DMSO/H_2O (4 ml) and stirred at r.t. for 2 days. The dimer was purified by RP-HPLC on a semi-preparative C-18 column using a linear solvent gradient (0-95% CH_3CN in H_2O, 0.1% TFA in 40 min; flow: 1 mL/min). Appropriate fractions were lyophilized to afford the target peptide **7**. F.W. Calcd. for $C_{96}H_{137}N_{30}O_{28}S_2^+$ (M + H^+): 2221.9655; MALDI HRMS found: m/z 2221.8560.

Preparation of **NQMP-*D*-mannopyranoside** conjugate **1e**.



Reagents and conditions: (a) i) H_2, Pd/C, MeOH; ii) $BrCH_2COCl$, Pyridine, DCM; (b) **S4**, NaOH, DMF; (c) i) 7N NH_3 in MeOH; ii) 2:8 H_2O/AcOH

1-α-(3-Bromoacetyl-propyl)-2,3,4,6-tetra-*O*-acetyl-D-mannopyranoside (**S3**). Palladium hydroxide (20 wt% Pd on carbon, 0.11 g) was added to a solution of 1-α-(3-azidopropyl)-2,3,4,6-tetra-*O*-acetyl-D-mannopyranoside (**S2**, 0.431 g. 1.0 mmol) in MeOH (40 ml). The mixture was stirred under H_2 for 2 h. Solids were removed by filtration; the solvent was removed under

reduced pressure. The crude material was dissolved in dry CH_2Cl_2 and bromoacetyl chloride (0.21 ml, 2.0 mmol), Et_3N (0.558 ml, 4.0 mmol) were added. The reaction mixture was stirred at r.t. for 2 h and solvent was removed under reduced pressure. The crude product was purified by flash chromatography (1:2 to 1:0 EtOAc/CH_2Cl_2) to give **S3** (0.280 g, 53%) as brownish oil. ^{1}H NMR (300 MHz): 6.81 (br, 1H, N*H*), 5.26-5.12 (m, 3H), 4.73 (s, 1H, H-1), 4.19 (dd, 1H, *J* =5.3, 12.3 Hz, H-5), 4.00 (m, 1H, H-5´), 3.89 (m, 1H), 3.79 (s, 2H, BrC*H*$_2$), 3.72 (m, 1H, NHC*H*$_2$), 3.44 (m, 1H, NHC*H*$_2$), 3.32 (m, 2H, OC*H*$_2$CH$_2$), 2.06 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.79 (m, 2H, OCH$_2$C*H*$_2$). ^{13}C NMR (75 MHz): 170.74, 170.12, 169.79, 166.05, 97.80, 69.56, 69.15, 68.67, 66.65, 66.28, 62.66, 38.08, 29.22, 28.92, 20.96, 20.86, 20.80, 20.77. FW Calcd for $C_{19}H_{28}BrNNaO_{11}^+$ (M + Na^+): 548.0738; MALDI HRMS found: m/z 548.0668.

Protected 1-α-(3-(NQMP-acetyl)propyl)-2,3,4,6-tetra-*O*-acetyl-D-mannopyranoside (**S5**). NaOH (12.0 mg, 0.3 mmol) was added to a solution of **S4** (69.1 mg, 0.3 mmol) in methanol (10 ml). After 10 min at r.t. solvent was removed under reduced pressure and the resulting salt re-dissolved in DMF (20 ml). A solution of bromide **S3** (131.6 mg, 0.25 mmol) in DMF (10 ml) was added to the reaction mixture and it was stirred at r.t. for 4 h. The solvent was removed under reduced pressure. And the residue purified by flash chromatography (1:0 to 4:1 CH_2Cl_2/EtOAct) to give **S5** (160 mg, 95%) as yellowish oil. ^{1}H NMR (300 MHz): 7.51 (s, 1H), 7.41 (s, 1H), 7.33 (d, 1H, *J* = 8.5 Hz), 7.16 (t, 1H, *J* = 7.9 Hz), 6.79 (t, 1H, *J* = 6.1 Hz, N*H*), 6.69 (d, 1H, *J* = 7.3 Hz), 5.28-5.17 (m, 3H), 5.01 (s, 2H), 4.75 (s, 1H, H-1), 4.61 (s, 2H), 4.24 (dd, 1H, *J* =5.3, 12.3 Hz, H-5), 4.05 (m, 1H, H-5´), 3.95 (m, 1H), 3.72 (m, 1H), 3.44 (m, 3H), 2.10 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.92 (s, 3H, Ac), 1.86 (t, 2H, *J* = 6.3 Hz, OCH$_2$C*H*$_2$), 1.56 (s, 6H). ^{13}C NMR (75 MHz): 170.79, 170.15, 169.98, 169.91, 168.66, 152.18, 150.03, 129.71, 125.83, 123.73, 122.08, 121.48, 106.44, 105.83, 100.19, 97.96, 69.74, 69.25, 68.70, 68.04, 66.44, 66.25, 62.78, 61.26, 36.55, 29.74, 25.17, 25.12, 21.05, 20.91, 20.87, 20.83. FW Calcd for $C_{33}H_{41}NNaO_{14}^+$ (M + Na^+): 698.2419; MALDI HRMS: m/z 698.2216.

1-α-(3-(NQM-acetyl)-propyl)-D-mannopyranoside (**S6**). **S5** was dissolved in 7N metanolic ammonia (20 ml) at 0°C and stirred at r.t. for 12 hours. The solvent was removed under reduced pressure, residue re-dissolved in 2:8 H_2O/HOAc (20 ml), and stirred at r.t. for 2 hours. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on Iatrobeads (10-20% MeOH in CH_2Cl_2) to give **S6** (61 mg, 65%) as colorless syrup. ^{1}H NMR (300 MHz, CD_3OD): 7.75 (s, 1H), 7.54 (s, 1H), 7.38 (d, 1H, *J* = 8.2 Hz), 7.15 (t, 1H, *J* = 7.9 Hz), 6.76 (d, 1H, *J* = 7.3 Hz), 4.80 (s, 2H), 4.70 (d, 1H, *J* =1.5 Hz, H-1), 4.65 (s, 2H), 3.86-3.48 (m, 6H), 3.46-3.28 (m, 4H), 1.82 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD): 170.18,

153.45, 152.39, 131.03, 129.77, 126.29, 126.19, 122.50, 121.33, 105.76, 103.13, 100.52, 73.54, 71.47, 70.95, 67.79, 67.50, 64.96, 61.78, 60.06, 36.40, 29.16. FW Calcd for $C_{22}H_{29}NNaO_{10}^{+}$ (M + Na^{+}) 490.1684; MALDI HRMS: m/z 490.1181

BSA-Vinyl Ether conjugate (**9c**). **S1** (1.92 mg, 8μmol) was added to a solution of BSA (53 mg, ca 0.8μmol) in 3mL of 0.1N phosphate buffer (pH=8) + 0.5 mL of acetonitrile and gently shaked using mechanical shaker for 12 h at r.t. Ellman's test shows complete conversion of free thiol group in BSA. Aqueous layer was washed with ethyl acetate and was freeze-dried to produce **9b** (63 mg – includes some phosphate salt). The modified BSA **9b** was further purified by multiple spin filtration.

BSA-TEG-Me conjugate (**9b**). Was obtained using similarly procedure but 1-iodo-2-(2-(2-methoxyethoxy)ethoxy)ethane instead of **S1**.

Determination of BSA sulfhydryl contents with Ellman's reagent.

0.01M solution of Ellman's reagent (5,5′-dithio-bis(2-nitrobenzoic acid)) was prepared by dissolving 4 mg of the reagent in 1 mL of 0.1M Tris buffer (pH =8.2) containing 1 mM EDTA. 250 μL of ca. 10 μM solution of BSA was added to a UV cell containing 2.5 mL of buffer and 150 μL of Ellman's reagent solution. After 15 min of incubation, the absorbance at 412 nm has been recorded against a control sample without a protein. Similar measurements were conducted using methanol as co-solvent.

Source of BSA	SH / BSA (mol/mol)	Methanol
Boehringer Gmbh	0.66, 0.60, 0.48	0%
	0.72	50%
Pierce	0.85	0%
Sigma	0.49	0%
	0.64, 0.64	25%
	0.55, 0.56	50%

It should be mentioned that Ellman's test is not a very reliable method for quantitative determination of free thiols in proteins. First of all, the cleavage of DTNB (5,5′-dithio-bis(2-nitrobenzoic acid)) shows complex kinetics, which depends on the pH of the solution, substrate concentration, and presence of co-solvents.[4] Secondly, two different extinction coefficients are reported for TNB (5-thio-2-nitrobenzoic acid) dianion.[5,6] Finally, Ellman's test is known to

underestimate SH contents in proteins.[4b,7] Therefore, accurate determination of thiol concentration requires calibration against an authentic sample, which is not possible for BSA. In fact, Ellman's test of BSA usually produces values in the range of 50 to 80%[8] but sometimes as low as 25%[9] of free SH groups. It has been reported that in papain reaction of the active cysteine with Ellman's reagent is reversible and position of the equilibrium depends on the pH of the solution.[10] Since Cys34 residue in BSA also possesses unusually high acidity (pKa ~ 5),[11] similar non-stoichiometric reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DNTB) is possible.

Procedures

Photochemical Labeling of Peptides 4, **6**, and **7**. 0.1 mM PBS solutions of peptides **4**, **6**, and **7** were irradiated with 300 or 350 nm fluorescent lamps in the presence of variable amounts of NQMP-TEG (**2c**, 0.1 – 0.4 mM). Composition of the photolysates was analyzed using HPLC (Figure S1). Peptide **4**-NQMP-TEG conjugate (**5**) was isolated using semi-prep C-18 HPLC column and characterized by ESI-HRMS (Figure S2). The concentration of labeled peptide **5** was determined by UV spectroscopy using characteristic 330 nm band of NQMP chromophore. This solution was then used in calibration of HPLC instruments.

Control Experiments: 0.1 mM PBS solutions of NQMP-TEG (**2c**) and peptide **4** were irradiated separately for 12 min using both 300 and 350 nm fluorescent lamps. No decomposition or losses of materials were detected by HPLC.

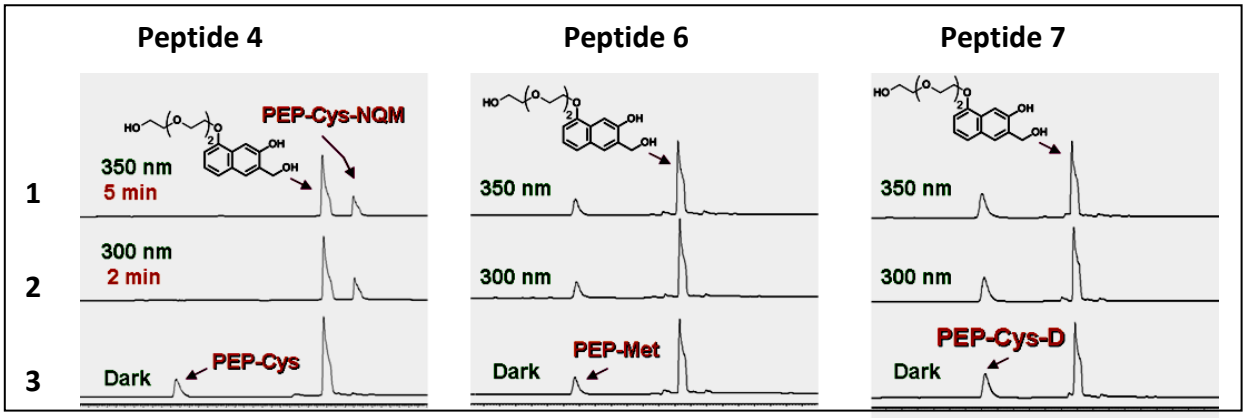
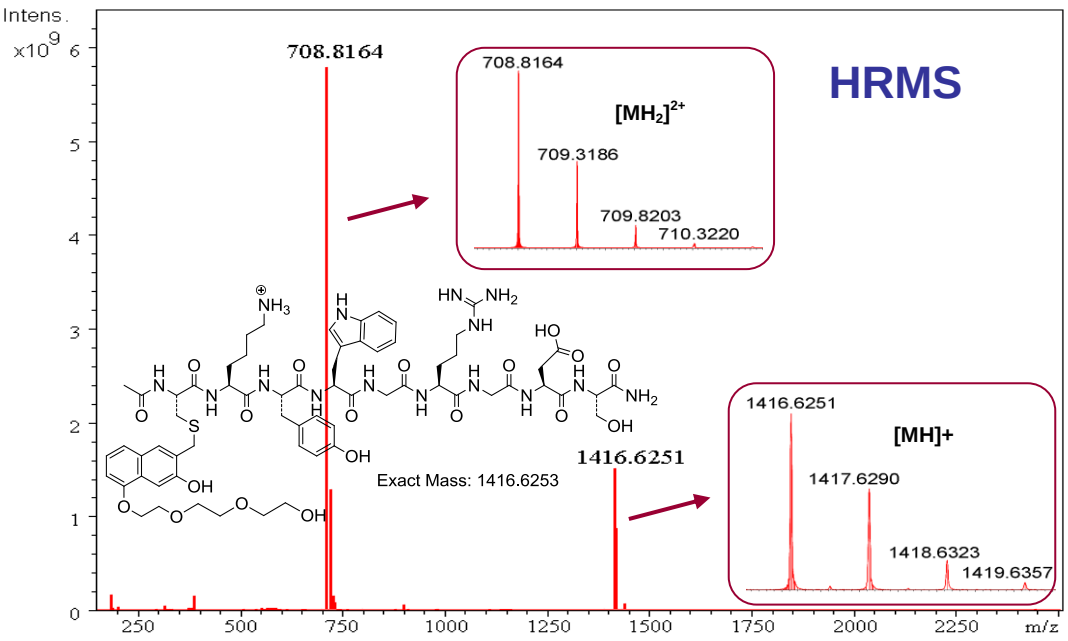


Figure S1. HPLC analysis of peptides **4**, **6**, and **7** (0.1 mM in PBS) photo-labeling with NQMP-TEG (**2c**, 0.4 mM): Lane 1 shows HPLC traces after 5 min irradiation of with 350 nm light; Lane 3: 2 min irradiation at 300 nm; Lane 3: 30 minutes incubation with now irradiation (dark control).



MS/MS:

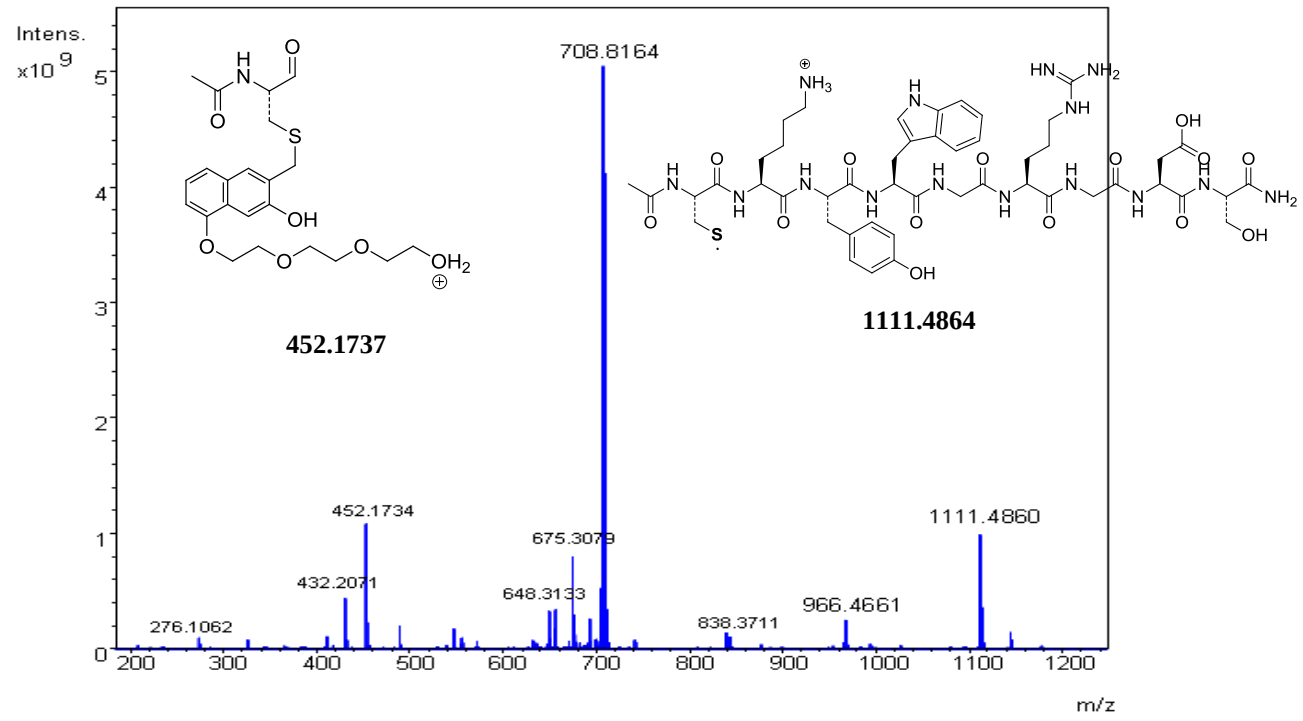


Figure S2. High Resolution ESI MS and MS/MS spectra of peptide-NQMP-TEG conjugate **5**.

Spectrophotometric measurements of BSA (9b) concentration.

9b quantification was conducted by measuring absorbance of at 278 nm. This wavelength corresponds to the maxima of a strong BSA band (ε= 49,200; Fig.S3, green spectrum).

258 nm and 331 nm wavelengths were employed for separate quantification of NQMP and BSA chromophores in protein **10a** solutions. At 258 nm NQMP chromophore has low extinction coefficient (ε= 5,600 Figure S2, red spectrum), while BSA shows much stronger absorbance (ε= 28,900). NQMP has a weak band with maximum at 331 nm (ε= 5,200), where BSA has virtually no absorbance (ε= 180, Figure S3).

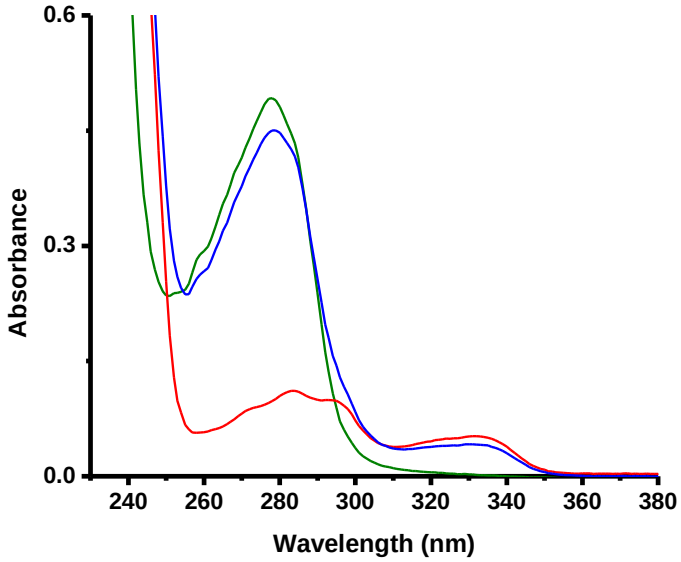


Figure S3. UV spectra of PBS solutions of BSA (**9a**, 10 μM, green line); NQMP-biotin (**1d**, 10 μM, red line); BSA-NQMP-Biotin (**10a**, 7.4 μM, blue line).

Quantification of the total protein.

a) Bradford protein assay: different concentrations of protein solutions were mixed with Coomassie Blue solution (Bio-Rad) The absorption of the resulting solution at 584 nm was measured in a multiwell microplate using BMG Labtech POLARstar Optima reader. The amount of protein is analyzed by comparing the absorption to a protein standard curve.
b) Alternatively, total protein concentration in some samples was determined by bicinchoninic acid assay (BCA Protein Assay Reagent Kit; Pierce, USA) following the manufacturer's instruction.

Biotin Quantification.

The amount of biotin in samples of BSA-biotin conjugates **10d** and **11d** was quantified by using Fluorescence Biotin Quantitation Kit (Thermo Scientific). Briefly, the fluorescence intensity of the dye of avidin significantly increases when the weakly interacting quencher HABA (4'-hydroxyazobenzene-2-carboxylic acid) is displaced by the biotin. The premix fluorescence dye labeled avidin with HABA (DyLight Reporter) is added to the protein solution containing conjugated biotin. Because of its higher affinity for avidin, biotin displaces the HABA, allowing the avidin to fluoresce. The fluorescence intensity was measured on a BMG Labtech POLARstar Optima reader. The amount of biotin is measured in a microplate by comparing the fluorescence to a biotin standard curve.

Western Blot Analysis. The photo-derivatized and control BSA samples (25 μg of protein per lane) were resolved on a 4−20% SDS-PAGE gel (Bio-Rad) and transferred to a nitrocellulose membrane. Next the membrane was blocked in blocking buffer (nonfat dry milk (5%; Bio-Rad) in PBST (PBS containing 0.1% Tween-20 and 0.1% Triton X-100)) for 2 h at RT. The blocked membrane was then incubated for 1 h at r.t. with an antibiotin antibody conjugated to horseradish peroxidase (HRP) (1:100000; Jackson ImmunoResearch Lab, Inc.) in blocking buffer and washed with PBST (4 × 10 min). Final detection of HRP activity was performed using ECL Plus chemiluminescent substrate (Amersham), exposure to film (Kodak) and development using a digital X-ray imaging machine (Kodak). Coomassie Brilliant blue staining was used to confirm total protein loading.

General Procedures for Photochemical Biotinylation of Proteins.

Solutions of BSA **9a**, its derivatives **9b**,**c** (3.6 mg in 5 mL of PBS buffer; 11 μm), and NQMP-biotin (**1d**, 100 μM) were irradiated with 350 nm fluorescent lamp for 2 min. The excess of reagent was removed by spin filtration or using sephadex-G25 PD-10 column. Ellman's test conducted of the resulting fractions was negative. Measurements of total protein concentration along with quantification of biotin produce biotin to BSA ratios around unity for spin-filter purification and ca. 1.4 for Sephadex chromatographic separation of the protein (single pass, Table S1). Our experiments with NQMP-DNS labeled BSA shows that complete removal of non-specifically bound reagent requires three passes through a Sephadex column.

Table S1. Quantification of Bovine Serum Albumin Photo-labeling with **1d**

Substrate	Protein purification	Protein quantification Method	Biotin/BSA ratio
10d	Spin filter	Bradford	0.99±0.03[a)]
10d	Spin filter	Spectrophotometric	1.00±0.03[b)]
10d	SEC	Bicinchoninic acid	1.38±0.18[a)]
10d	SEC	Spectrophotometric	1.39±0.07[b)]
11d	Spin filter	Bradford	1.05±0.06[a)]
11d	Spin filter	Spectrophotometric	1.00±0.04[b)]

[a)] Total biotin was determined using FluoReporter® Biotin Quantitation Assay Kit by Themo Scientific. [b)] Total biotin was assumed to be equal concentration of NQMP chromophore, which was measured spectrophotometrically.

Photochemical labeling of BSA with Dansyl fluorophore. 500 μL of 68 μM solution of BSA in PBS and 250 μL of 1 mM solution NQMP-Dansyl conjugate (NQMP-DNS, **1f**, Scheme 1) in PBS with 16% of DMSO were added to 1.75 mL of PBS (pH=7.1). Three sets of the solutions were prepared; first two were incubated in the dark for 30 and 90 min. The third was irradiated in mini-Rayonet photoreactor equipped with 350 nm fluorescent tubes for 6 min. The low molecular weight components were removed from photolysate and controls by passing it through a Sephadex-G25 PD-10 column three times. The excess of the solvents was removed by spin-filtration and the solutions were reconstituted to 3.5 mL for fluorescence measurements. The MALDI-TOF spectra of the product **10f** indicate mass increase from of 499 ± 120 Da from native BSA **9a** (Figure S4). This mass is consistent with mono-derivatization of BSA with NQMP-DNS (MW = 423).

The Dansyl and BSA contents were determined using spectroscopic method to produce Dansyl to BSA ratio was 1.77±0.30 after a single pass through a PD-10 column**.** The control dark experiment produces Dansyl to BSA ratios 0.41±0.07 after 30 min and 0.43±0.15 after 90 min of incubation. This non-specific binding is not surprising since dansyl-amide is known to have significant affinity to BSA binding site I.[12] Only after the third separation, the Dansyl to BSA ratio drops below 5% in control experiments. It also important to note that non-specific dansyl labeling does not survive SDS-PAGE (*vide infra*).

Fluorescent imaging of the gel: PBS solutions containing 12 μM of BSA and 100 μM of NQMP-DNS were irradiated with 350 nm lamps or incubated in the dark for 30 and 90 min (*vide supra*). The unconjugated NQMP-DNS was removed by passing the samples through a PD-10 column and fractions collected were further filtered and concentrated using a 10K MWCO Nanosep® centrifugal devices (Life Sciences). The BSA samples (1.2 uM) were added to native sample buffer and resolved using a 4-20 % Tris-HCl gel (Bio-Rad) without boiling. The gel was imaged using a ChemiDoc MP imaging system (Bio-Rad). The fluorescence readout has been performed using 302 nm excitation and 548-630 nm filter (Amber or Ethidium bromide filter) in the recording channel. Coomassie staining of the gel was done to confirm total protein loading.

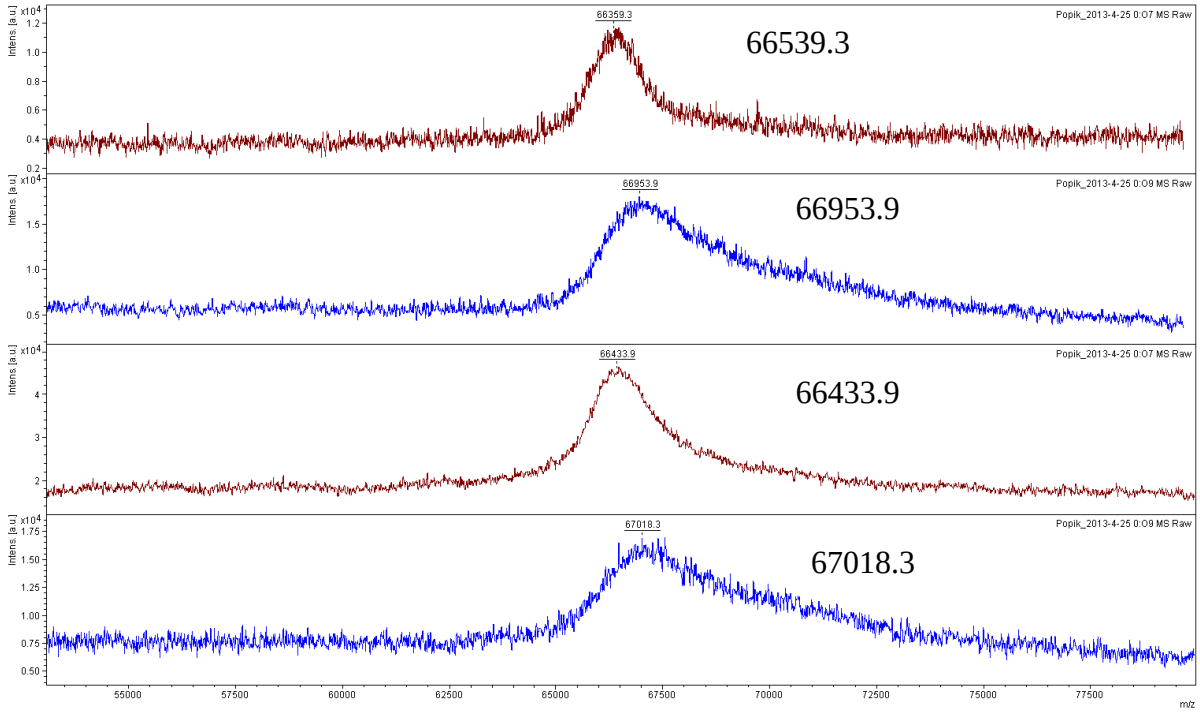


Fig. S4. MALDI-TOF spectrum of a control (brick-red) and NQMP-DNS labeled BSA (blue)

Derivatization of BSA with Mannose. Solutions of BSA (**9a**, 11 μM in PBS buffer), and NQMP-mannose (**1e**, 100 μM) were irradiated with 350 nm fluorescent lamp for 2 min. The excess of reagent was removed by spin filtration.

Quantification of conjugated mannose in the sample of BSA-mannose conjugate **10e** was performed on DIONEX ICS-3000 HPAEC chromatograph using deionized water and 200 mM NaOH as an eluent. Sample preparation: 1-2 mg of mannose-conjugated BSA sample and standard D-(+)-mannose were treated with 2 M TFA in water (250 μL) for 4 h at 100 °C. Sample

and standard were spin dried, re-dissolved in water (500 µL) and filtered. Sample concentration was then determined based on the calibration curves of mannose standards.

Photochemical Removal of the Biotin Tag from BSA derivative 10d. A solution of BSA-biotin conjugate **10d** (2 µM PBS buffer) was irradiated with 350 nm fluorescent lamp for 2 min. Protein was isolated by spin filtration and submitted to the Western blot analysis (Fig. 2B).

Photo-triggered catch and release of BSA using NeutrAvidin Agarose gel: 200 µL of PBS solution containing 0.01mM of BSA and 0.1 mM of NQMP-biotin conjugate (**1d**) were irradiated for 5 min using 350 nm fluorescent lamps. 70 µL of high capacity NeutrAvidin Agarose resin (Thermo Scientific) was mixed in using vortex and the mixture was incubated in dark for 30 min. The suspension was then centrifuged and the supernatant was removed. No BSA can be detected in the supernatant solution by assay method. The derivatized Agarose gel was washed fresh PBS solution (3 x 200 µL). The washes contain no detectable amounts of NQMP-biotin conjugate **1d**. NeutrAvidin Agarose gel was re-suspended in 200 µL of PBS buffer and irradiated for 5 min using 350 nm fluorescent lamps. The gel was separated from the aqueous phase by centrifugation and washed with PBS (3 x 250 µL). The combined supernatant was analyzed for released BSA by spectrophotometric and Bradford assay methods.

The procedure was repeated with two modifications:

A) Second irradiation was conducted in 400 µL of PBS

B) Second irradiation was conducted in 200 µL of PBS but in the presence of 0.1 mM of ethyl vinyl ether.

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[1] S. Arumugam and V. V. Popik. *J. Am. Chem. Soc.*, **2011***, 133*,15730.

[2] S. Arumugam and V. V. Popik. *J. Am. Chem. Soc.,* **2011**, *133*, 5573.

[3] W. Hayes, H. M. I. Osborn, S. D. Osborne, R. A. Rastalib, and B. Romagnoli. *Tetrahedron* **2003**, *59*, 7983.

[4] (a) J. Sedlak and R. H. Linsday. *Anal. Biochem.,* **1968**, *25*, 192; (b) C. K. Riener, G. Kada, and H. J. Gruber. *Anal. Bioanal. Chem.,* **2002**, *373*, 266.

[5] G. L. Ellman. *Arch. Biochem. Biophys.*, **1959**, *82*, 70.

[6] P. W. Riddles, R. L. Blakeley, and B. Zerner. *Anal. Biochem.,* **1979**, *94*, 75.

[7] H. Faulstich, P. Tews, and D. Heintz. *Anal. Biochem.* **1993**, *206*, 357; S. K. Wright and R. E. Viola. *Anal. Biochem.,* **1998**, *265*, 8.

[8] J. Janatova, J.K. Fuller, and M. J. Hunter. *J. Biol. Chem.* **1968**, *243*, 3612; S.-L. Chen and K.-H. Kim, *Arc. Biochem. Biophys.,* **1985**, *239*, 163; T. Yasuhara and K. Nokihara, *Anal. Chem.,* **1998**, *70*, 3505.

[9] S. Chesne, P. Rondeau, S. Armenta, and E. Bourdon, *Biochim.,* **2006**, *88*,1467; E. Katchalski, G. S. Benjamin, and V. Gross. *J. Am. Chem.,* **1957**, *79*, 4096.

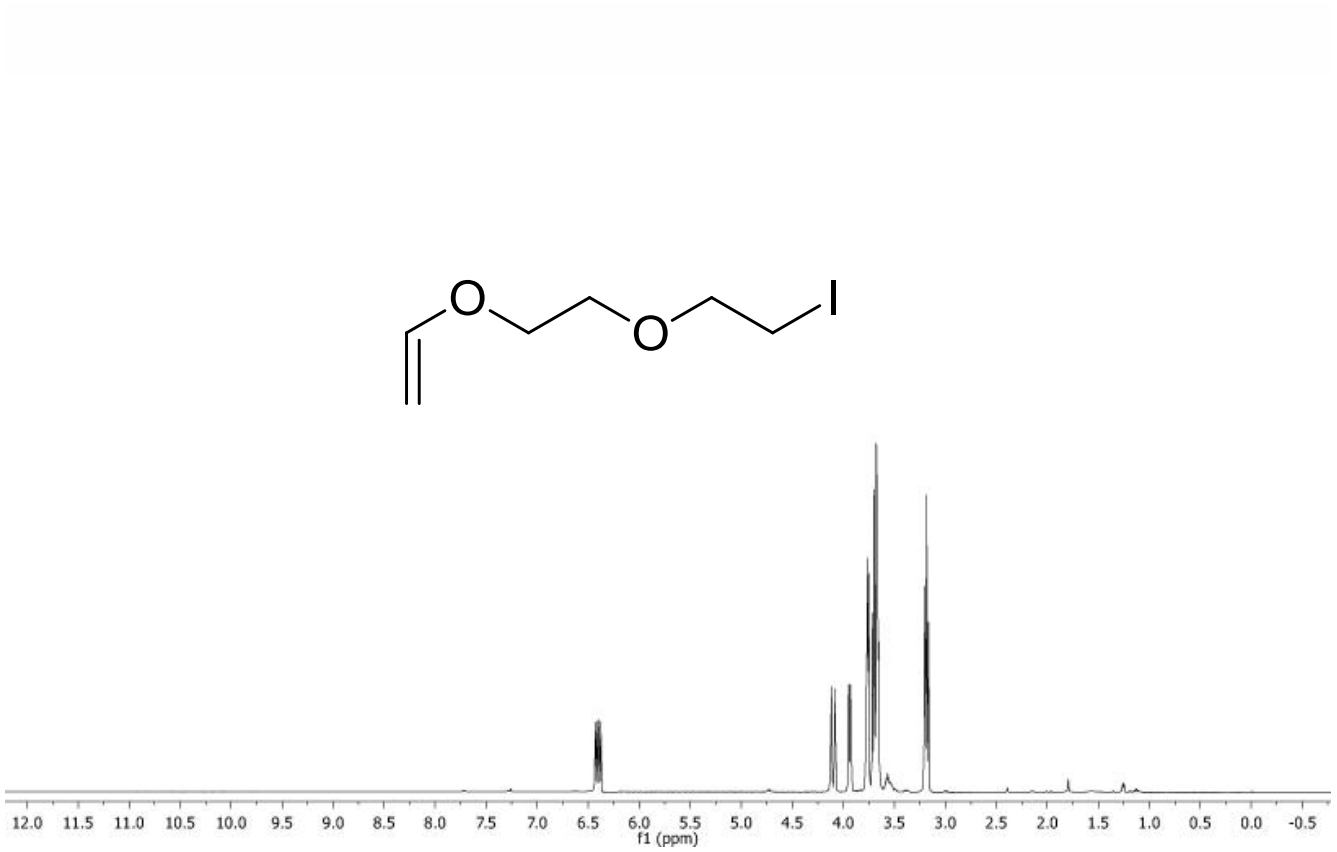
[10] K. Brocklehurst and G. Little *Biochem. J.*, **1973**, *133*, 67.

[11] S. D. Lewis, D. C. Misra, and J. A. Shafer, *Biochemistry*, **1980**, *19*, 6129.

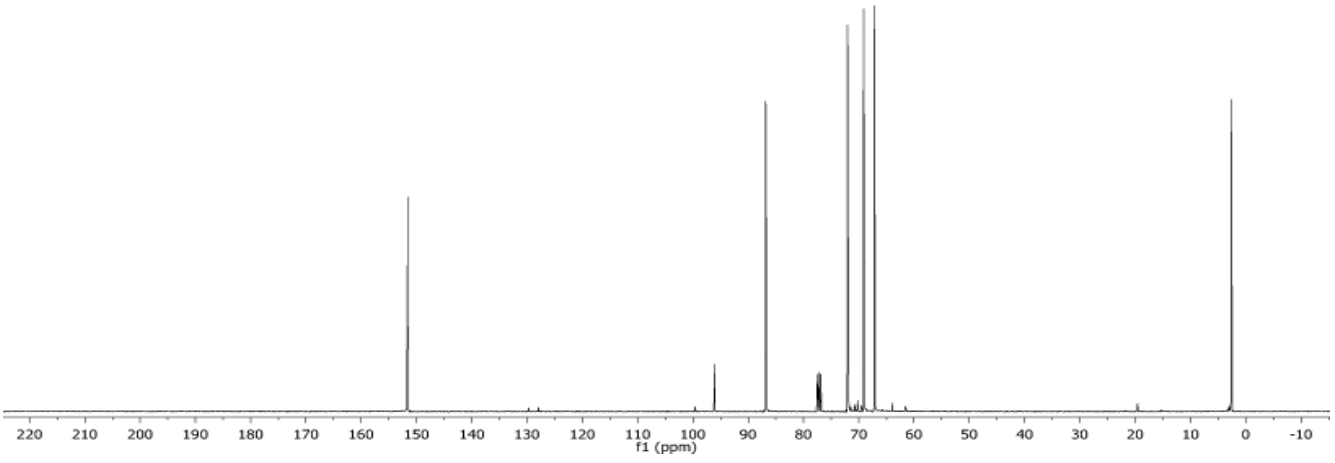
[12] V. S. Jisha, K. T. Arun, M. Hariharan, and D. Ramaiah. *J. Am. Chem. Soc.,* **2006**, *128*, 6024.

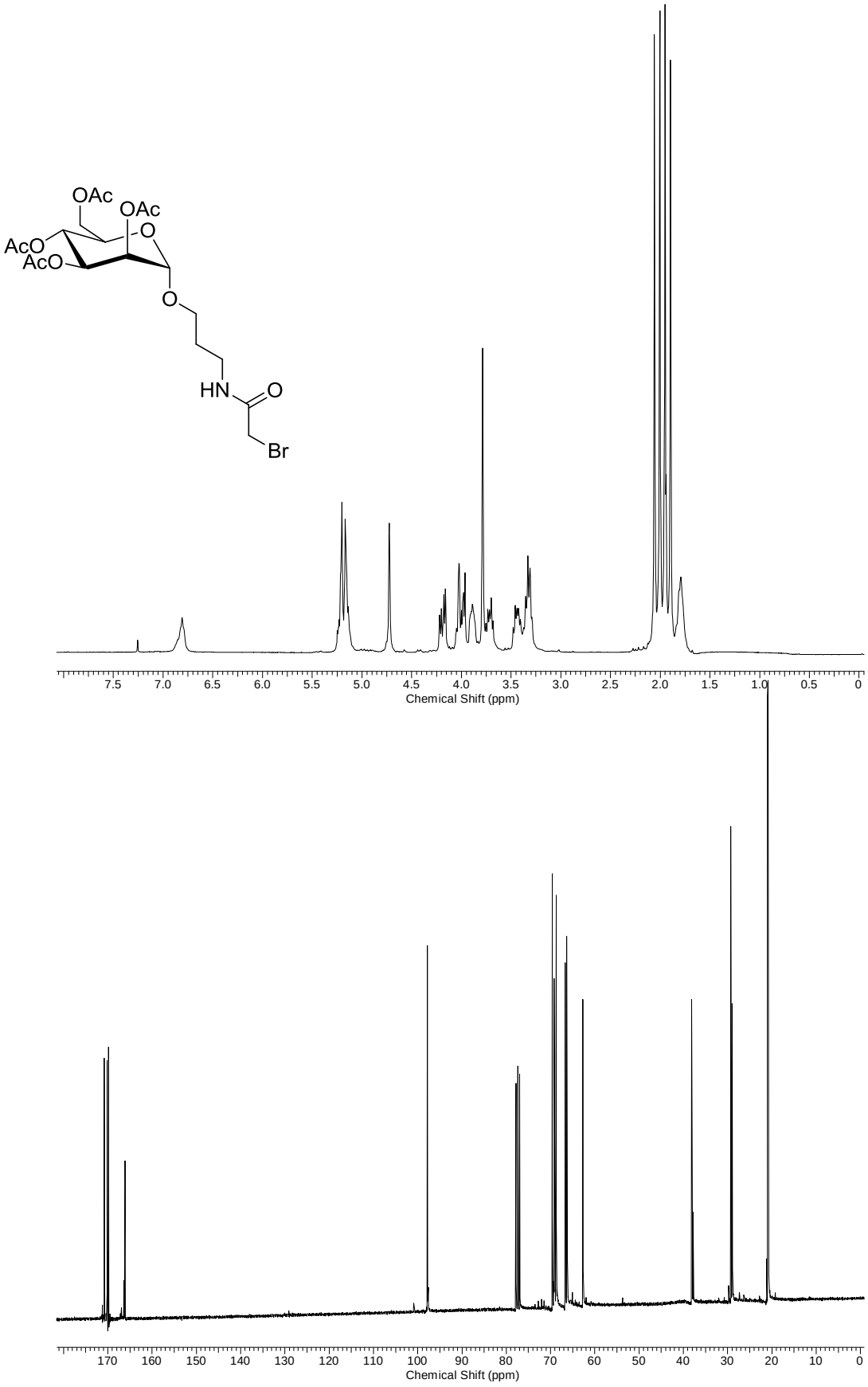
^{1}H and ^{13}C Spectra

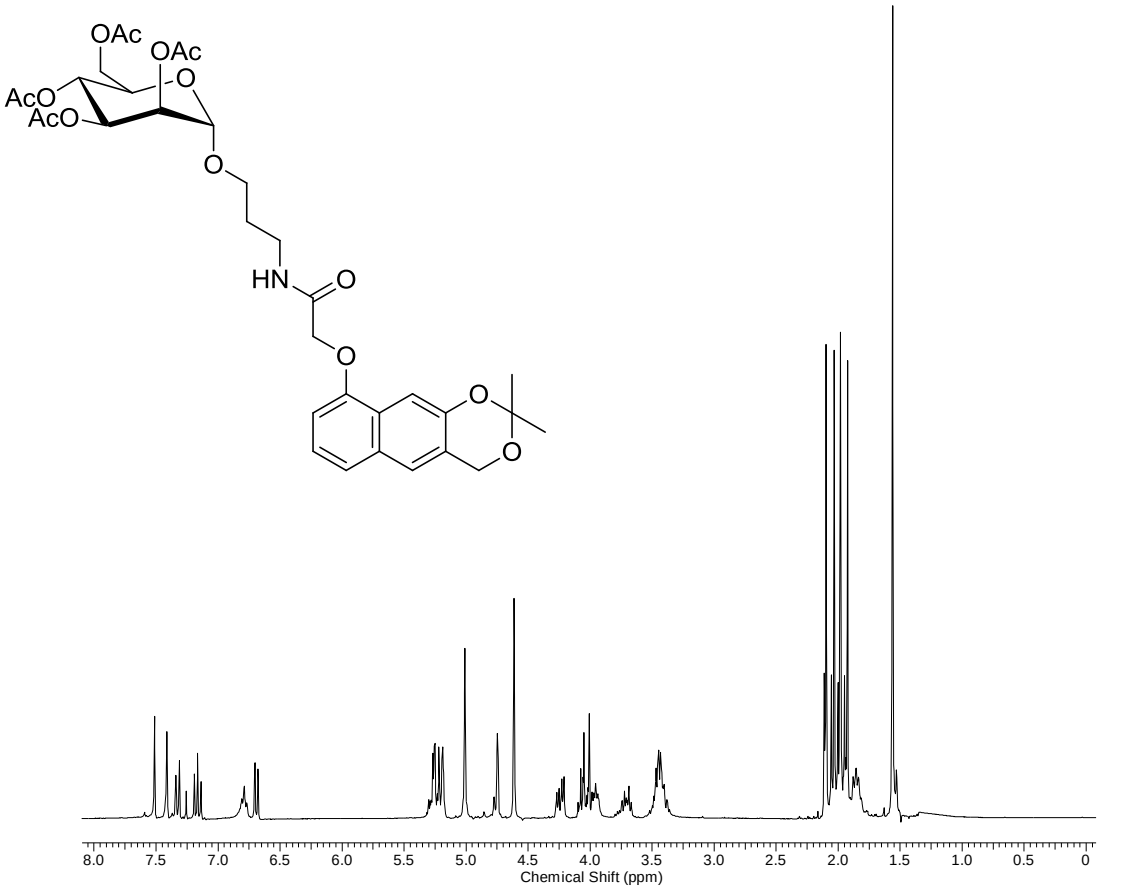
as-DEg-Ovinyl-I_Proton_20081219_01

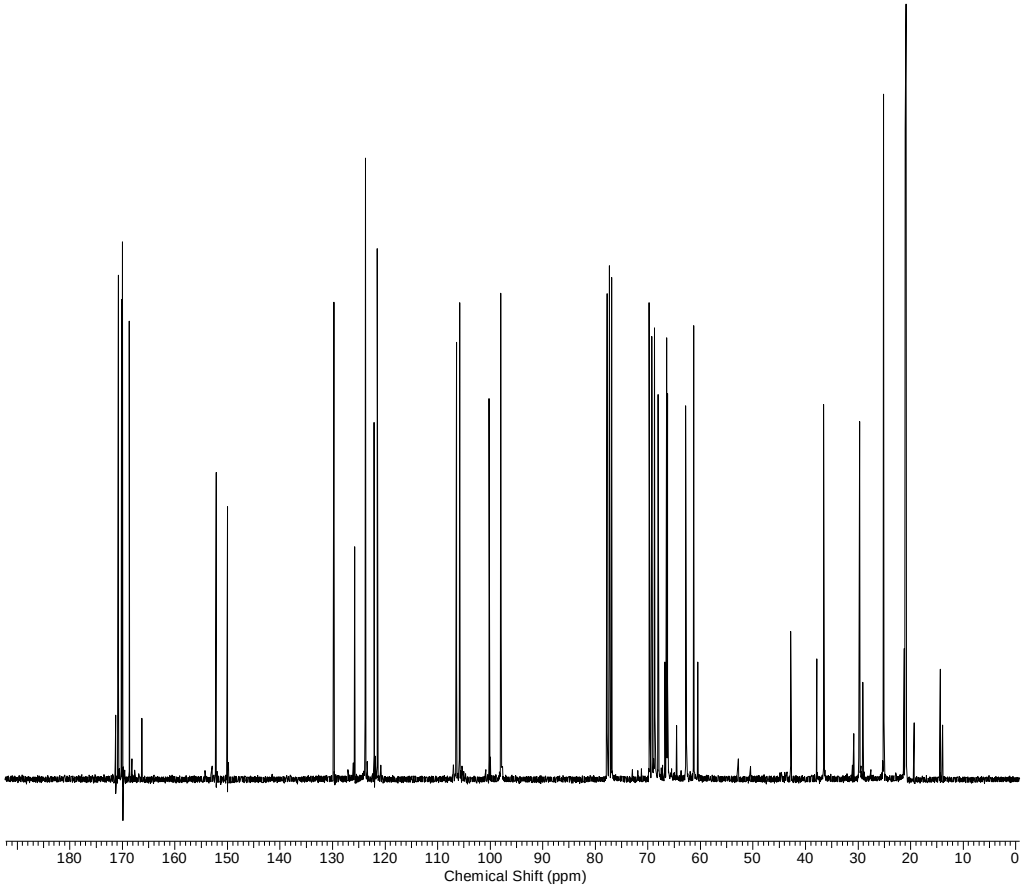


as-DEg-Ovinyl-I_Carbon_20081219_01




OAc
OAc
O
AcO
AcO
O
HN
O
Br
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
Chemical Shift (ppm)


OAc
OAc
AcO
AcO
O
O
HN
O
O
O
O
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)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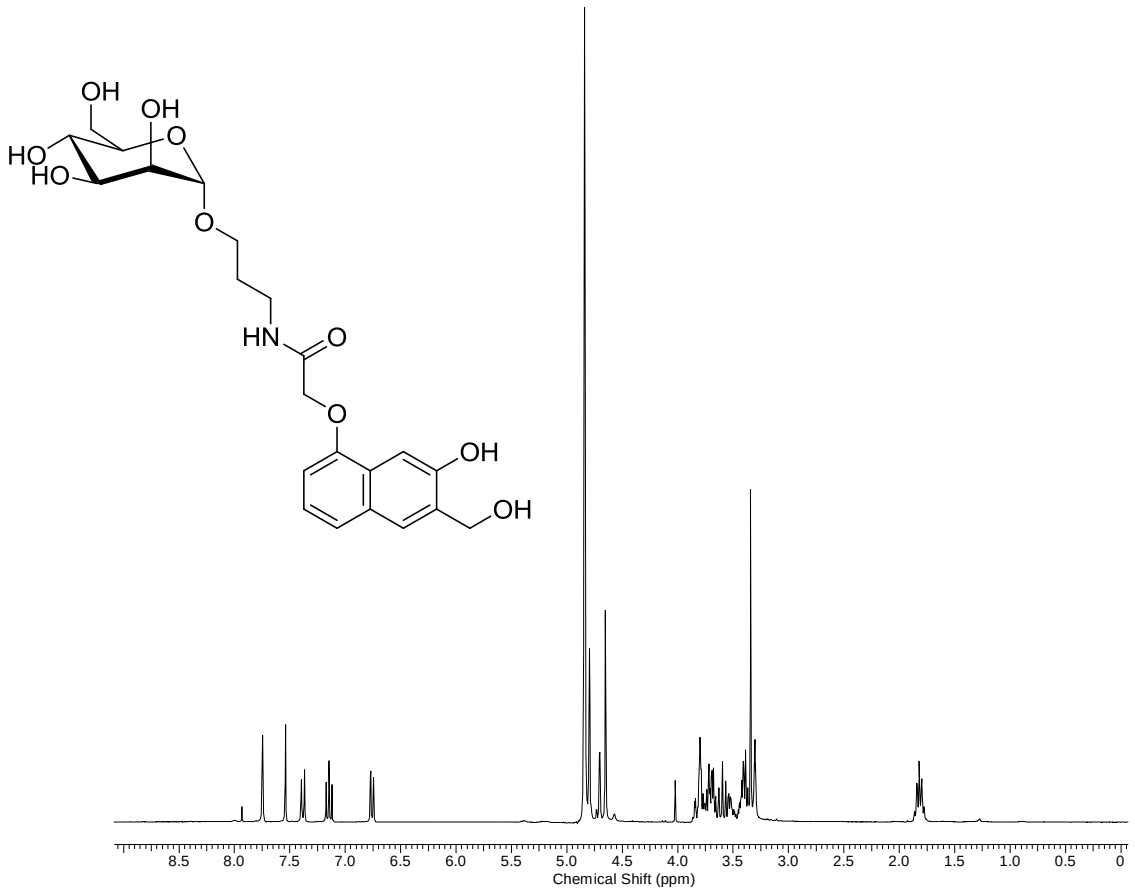
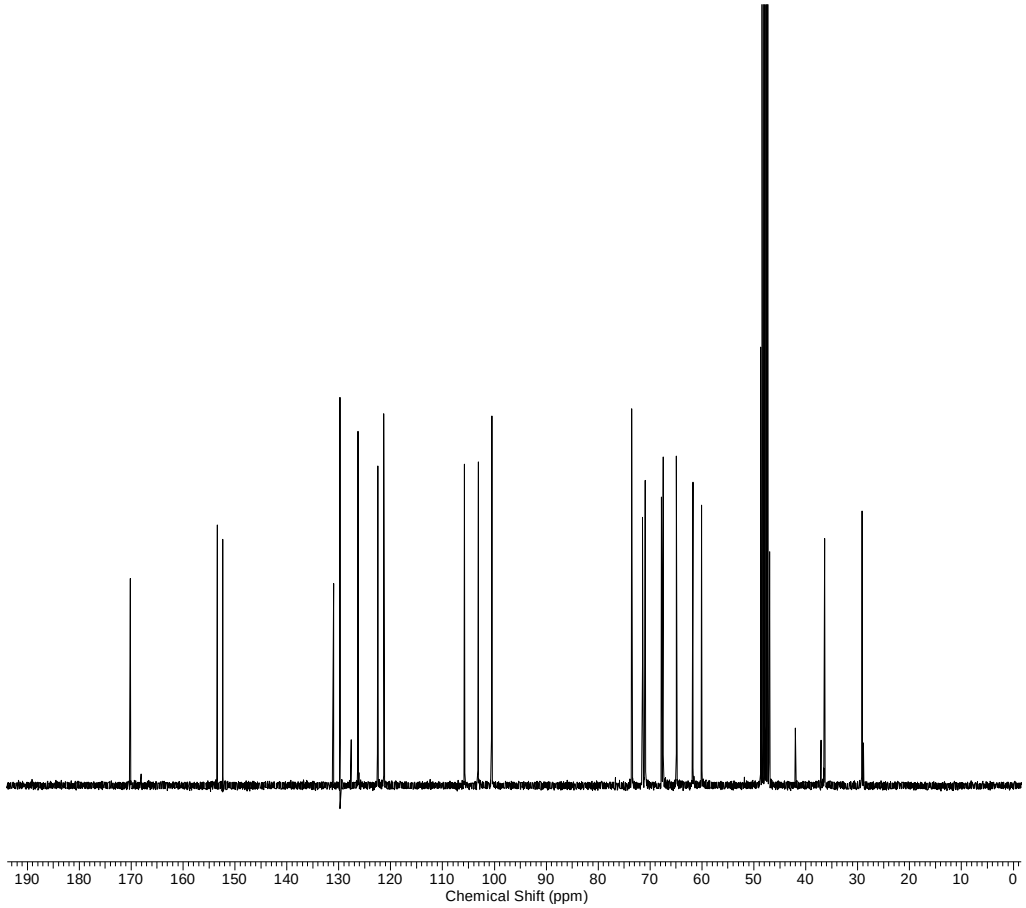
Chemical Shift (ppm)


OH
OH
O
HO
HO
O
HN
O
O
OH
OH
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)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Chemical Shift (ppm)