Supporting information

A Chemical Probe for Protein Crotonylation

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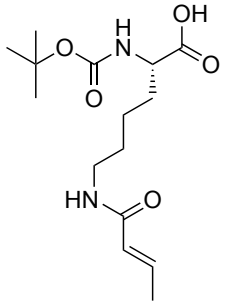
General Methods.

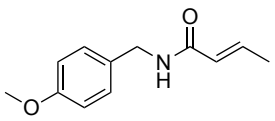
All commonly used chemicals were purchased from Sigma Aldrich (Milwaukee, WI) or Fisher Scientific (Pittsburgh, PA) and used without further purification. Slide-A-Lyzer dialysis cassettes and mini-dialysis devices where purchased from ThermoFisher Scientific (Waltham, MA). Size exclusion chromatography was performed on an AKTA FPLC system (GE Healthcare Life Sciences) equipped with a Superdex 200 Increase 10/300 GL column. Analytical reversed-phase HPLC (RP-HPLC) was performed on an Agilent 1200 series instrument with a C18 column (Vydac, 5 μm, 4.6 x 150 mm, 1 mL/min) using 0.1% trifluoroacetic acid (TFA) in water (HPLC solvent A), and 90% acetonitrile, 0.1% TFA in water (HPLC solvent B) as the mobile phases. Reversed-phase preparative scale purifications were performed on an Agilent 1200 series instrument using a semi-preparative C18 column (Vydac, 12 μm, 10 x 250 mm, flow 4 mL/min), on a Waters preparative HPLC system (equipped with a Water 2535 Binary Gradient Module and a Waters 2489 UV detector) using a C18 preparative column (Vydac, 15-20 μm, 20 x 250 mm, flow 15 mL/min) and on a Biotage Isolera using a C18 column (Biotage SNAP Ultra column KP-C18-HS). Flash chromatography was performed on a Biotage Isolera using a silica column (Biotage SNAP Ultra, HP-Sphere 25μm). LS-MS analysis was performed on an Agilent 6120 Quadrupole LC/MS system with a C18 column (Agilent Zorbax, 3.5 μm, 4.6 x 100 mm) using 0.1% formic acid (FA) in water and 90% acetonitrile, 0.1% FA in water. ESI-MS analysis was performed on a MicrOTOF-Q II ESI-Qq-TOF mass spectrometer (Bruker Daltonics, Billerica, MA). Gel and western blot images were acquired with an ImageQuant LAS 4000 instrument (GE Healthcare, Little Chalfont, United Kingdom) or with an Odyssey Infrared Imager (Li-Cor, Lincoln, NE). ^{1}H NMR spectra were recorded on a Bruker UltraShield Plus 500 MHz instrument and are internally referenced to residual solvent signals as noted, $CDCl_3$: 7.26 ppm, CD_3OD: 3.31 ppm, $(CD_3)_2SO$: 2.50 ppm). Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, br = broad signal, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets) and coupling constant (Hz). ^{13}C NMR spectra were recorded on a Bruker UltraShield Plus 500 MHz and are internally referenced to residual solvent signals as noted: $CDCl_3$: 53.84 ppm, CD_3OD: 49.00 ppm, $(CD_3)_2SO$: 39.52 ppm. Data for ^{13}C NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet), and J_{C-P} coupling constant (Hz) (when applicable). When applicable, doublets in the ^{13}C NMR spectra are denoted as d and singlets as s. ^{31}P NMR spectra were recorded on a Bruker UltraShield Plus 500 MHz. Data for ^{31}P NMR are reported in chemical shift (δ ppm).

Synthesis

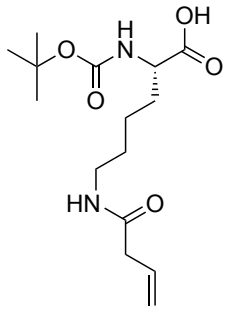


N^{α}-Boc,N^{ε}-crotonyl lysine (2a): 0.5 g (2.0 mmol) Boc-lysine was dissolved in 50 mL DMF. To this was added 0.46 g (3.5 mmol, 0.44 mL, 1.5eq.) crotonic anhydride and 1.7 mL (10 mmol) *N*,*N*-Diisopropylethylamine (DIPEA). The reaction was stirred at room temperature overnight. The solvent was removed in vacuo and the product, **2a**, purified by column chromatography (reversed-phase chromatography using a 30 g C18 column (Biotage), linear gradient 0-70% MeCN in 25 CV, H_2O / MeCN + 0.1% TFA), yielding a transparent oil (0.25 g, 0.8 mmol, 42%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 – 6.73 (m, 1H), 5.89 – 5.77 (m, 1H), 4.36 – 4.10 (m, 1H), 3.42 – 3.25 (m, 2H), 1.91 – 1.82 (m, 3H), 1.80 – 1.29 (m, 15H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.4, 167.2, 156.2, 141.1, 124.6, 80.4,

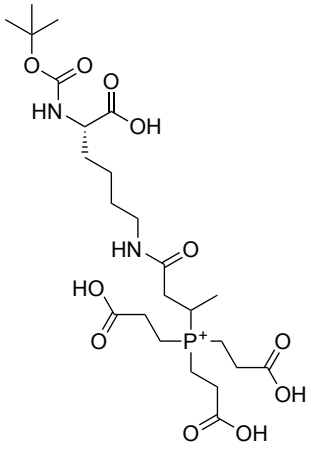
53.2, 39.4, 32.0, 28.9, 28.5, 22.5, 18.0. Exact mass (HRMS) calc. for $C_{10}H_{19}N_2O_3^+$(M - CO_2 - C_4H_8 + H)$^+$ 215.1390, found 215.1387. Note only the mass corresponding to neutral loss of the Boc group from **2a** was detected in positive mode ESI.



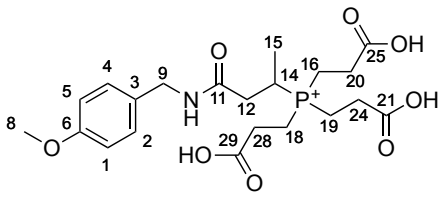
(*E*)-*N*-(4-methoxybenzyl)but-2-enamide (2b): 2.0 g (14.6 mmol, 1.9 mL) of 4-methoxybenzylamine was dissolved in 50 mL MeCN. To this was added 2.7 g (17.5 mmol, 2.2 mL, 1.2 eq.) of crotonic anhydride and 12.3 g (146 mmol, 10 eq.) $NaHCO_3$. The reaction was stirred at room temperature overnight. Excess of $NaHCO_3$ was removed by filtration and the solvent was removed in vacuo yielding a pale yellow solid. The solid was dissolved in ethyl acetate and washed with sat. bicarbonate and brine, and subsequently dried on magnesium sulfate. Product was purified by silica column chromatography (ethyl acetate/hexane, linear gradient 0-100% EtOAc in 12 CV) yielding a white solid (0.8 g, 3.8 mmol, 26%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 – 7.18 (m, 2H), 6.95 – 6.80 (m, 3H), 5.83 – 5.74 (m, 1H), 5.64 (br, 1H), 4.43 (d, *J* = 5.6 Hz, 2H), 3.79 (s, 3H), 1.85 (dd, *J* = 6.9, 1.7 Hz, 3H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.8, 159.2, 140.5, 130.5, 129.4, 125.0, 114.2, 55.5, 43.2, 17.9. Exact mass (HRMS) calc. for $C_{12}H_{16}NO_2^+$ (M+H)$^+$ 206.1176, found 206.1206.



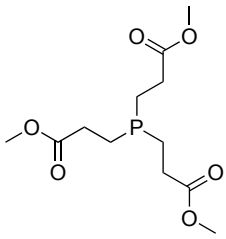
N^{α}-Boc,N^{ε}-3-butenoyl lysine (2d): 1.7 g (20.3 mmol, 1.7 mL, 5 eq.) of butenoic acid was dissolved in 50 mL DCM. To this solution was added 1.7 g (8.2 mmol, 2 eq.) of *N,N′*-Dicyclohexylcarbodiimide. The reaction was stirred at room temperature for 1 hour. The urea by-product was removed by filtration and the solvent was removed in vacuo to afford the crude anhydride. This was dissolved in a minimal amount of DMF and added dropwise to a solution of Boc-Lys-OH (1.0 g, 4.1 mmol, 1 eq.) dissolved in 100 mL DMF. The reaction was stirred at room temperature for 2 hours. The solvent was removed in vacuo and the suspension was dissolved in ethyl acetate and subsequently washed with 0.1 M HCl and brine. The organic layer was dried over magnesium sulfate and the solvent was removed in vacuo. The desired product, **2d**, was purified by reversed-phase chromatography using a 30 g C18 column (Biotage, gradient 0-70%MeCN in 25 CV, H_2O / MeCN + 0.1% TFA), yielding a transparent oil (2.9 mmol, 0.9 g, 70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.00 – 5.85 (m, 1H), 5.32 – 5.14 (m, 2H), 4.40 – 4.03 (m, 1H), 3.36 – 3.18 (m, 2H), 3.05 (d, *J* = 7.1, 1.3 Hz, 2H), 1.97 – 1.26 (m, 15H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.0, 172.1, 156.1, 130.9, 120.7, 80.6, 53.2, 41.4, 39.5, 31.9, 28.9, 28.5, 22.4. Exact mass (HRMS) calc. for $C_{10}H_{19}N_2O_3^+$(M - CO_2 - C_4H_8 + H)$^+$ 215.1390, found 215.1373. Note only the mass corresponding to neutral loss of the Boc group from **2d** was detected in positive mode ESI.



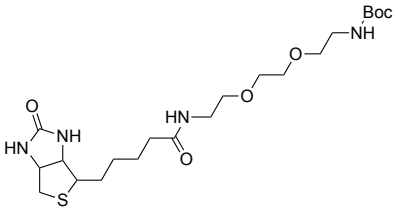
N^{α}-Boc,N^{ε}-crotonyl lysine TCEP adduct (3a): 20 mg (64 μmol) of **2a** and 182 mg (640 μmol, 10 eq.) tris(2-carboxyethyl)phosphine (TCEP) was dissolved in a total volume of 4 mL 50 mM Tris, pH 8 (pH adjusted after addition of TCEP with 5N NaOH). The reaction was stirred at room temperature overnight. Product was purified by semi-preparative RP-HPLC C18 column (Waters), gradient 0-50% MeCN in 60 min (flow 3.5 mL/min, H_2O / MeCN + 0.1% TFA). ^{1}H NMR (500 MHz, MeOD) δ 4.13 – 4.01 (m, 1H), 3.27 – 3.14 (m, 2H), 3.05 (s, 1H), 2.89 – 2.42 (m, 14H), 1.91 – 1.77 (m, 1H), 1.74 – 1.61 (m, 1H), 1.61 – 1.50 (m, 2H), 1.48 – 1.41 (m, 11H), 1.34 (dd, $^3J_{H\text{-}P}$ = 17.2, $J_{H\text{-}H}$ = 7.2 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.3, 172.42 (d, $J_{C\text{-}P}$ = 15.7 Hz), 168.5 (d, $J_{C\text{-}P}$ = 13.0 Hz), 155.6, 78.0, 53.5, 38.6, 35.0, 30.5, 28.6, 28.2, 25.9 (d, $J_{C\text{-}P}$ = 2.9 Hz), 23.1, 22.8 (d, $J_{C\text{-}P}$ = 48.5 Hz), 12.8, 12.7 (d, $J_{C\text{-}P}$ = 48.9 Hz); ^{31}P NMR (203 MHz, MeOD) δ 41.16. Exact mass (HRMS) calc. for $C_{24}H_{42}N_2O_{11}P^+$ $(M)^+$ 565.2521, found 565.2514



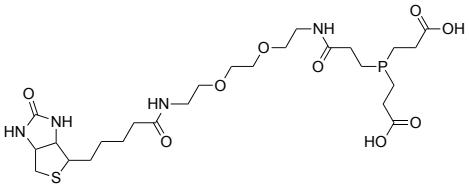
(*E*)-*N*-(4-methoxybenzyl)but-2-enamide TCEP adduct (3b): 100 mg (0.48 mmol) of **2b** was dissolved in 4 mL of DMF. To this was added a solution of 698 mg (2.4 mmol, 5eq.) TCEP in a total volume of 4 mL 50 mM Tris, pH 8, 6M GuHCl. The reaction was stirred at room temperature overnight. The product was purified by column chromatography (reversed-phase chromatography using a 30 g C18 column (Biotage), gradient 0-70% in 25 CV, H_2O / MeCN + 0.1% TFA) yielding 56 mg (0. 22 mmol, 45%) of a white solid. ^{1}H NMR (500 MHz, MeOD) δ 7.23 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 4.32 (s, 2H), 3.77 (s, 3H), 3.13 – 3.00 (m, 1H), 2.90 – 2.48 (m, 14H), 1.33 (dd, $^3J_{H\text{-}P}$ = 17.2 Hz, $J_{H\text{-}H}$ = 7.2 Hz, 3H); ^{13}C NMR (126 MHz, MeOD) δ 174.0 (d, $^3J_{C21C25C29\text{-}P}$ = 12.9 Hz), 170.8 (d, $^3J_{C11\text{-}P}$ = 11.1 Hz), 160.6 (s, C6), 131.6 (s, C3), 130.2 (s, C1C5), 115.0 (s, C2C4), 55.7 (s, C8), 44.0 (s, C9), 36.6 (d, $^2J_{C12\text{-}P}$ = 1.9 Hz), 26.9 (d, $^2J_{C20C24C28\text{-}P}$ = 3.6 Hz), 25.2 (d, $^1J_{C14\text{-}}$ = 49.0 Hz), 14.9 (d, $^1J_{C16C18C19\text{-}P}$ = 49.7 Hz), 13.6 (d, $^2J_{C15\text{-}P}$ = 2.6 Hz);^{31}P NMR (203 MHz, MeOD) δ 41.2. Exact mass (HRMS) calc. for $C_{21}H_{31}NO_8P^+$ $(M)^+$ 456.1781, found 456.1763



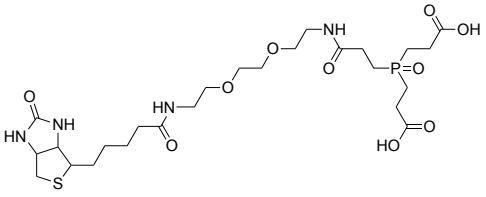
Trimethyl ester analog of TCEP (1c): Compound was previously reported and synthesized accordingly.[1] 250 mg (0.88 mmol) of TCEP was dissolved in 10 mL of degassed MeOH containing 250 mg amberlyst (Amberlyst 15 hydrogen form, Sigma-Aldrich). The reaction was stirred at room temperature for 3 hours under an inert atmosphere. The amberlyst was removed by filtration and the solvent removed in vacuo. Product was purified by column chromatography (reversed-phase chromatography using a 30 g C18 column (Biotage), gradient 0-70% MeCN in 25 CV, H_2O / MeCN + 0.1% TFA) yielding 96 mg (37%) of a transparent oil. ^{1}H NMR (501 MHz, $CDCl_3$) δ 3.74 (s, 9H), 2.83 – 2.65 (m, 6H), 2.37 – 2.13 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.5 (d, $^3J_{C\text{-}P}$ = 7.6 Hz), 52.4, 29.2 (d, $^2J_{C\text{-}P}$ = 3.8 Hz), 19.0 (d, $^1J_{C\text{-}P}$ = 23.6 Hz). ^{31}P NMR (203 MHz, D_2O) δ -26.8.



BocNH-PEG2-biotin (5): 100 mg of biotin (0.41 mmol) and 78.5 mg (0.5 mmol, 1.2 eq.) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) were dissolved in 5 mL of 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) pH 6. To this was added 152 mg (0.61 mmol, 145 µL, 1.5 eq.) of *N*-Boc-2,2-(ethylenedioxy)diethylamine. The pH was adjusted using 6 M HCl and the reaction was stirred at room temperature for 2 hours. Product was purified by column chromatography (reversed-phase chromatography using a 30 g C18 column (Biotage), gradient 0-70% MeCN in 25 CV, H_2O / MeCN + 0.1% TFA), yielding 175 mg of a white solid (0.37 mmol, 90%). ^{1}H NMR (500 MHz, MeOD) δ 4.52 – 4.45 (m, 1H), 4.30 (dd, *J* = 7.9, 4.4 Hz, 1H), 3.62 (s, 4H), 3.58 – 3.49 (m, 4H), 3.37 (t, 2H), 3.25 – 3.18 (m, 3H), 2.93 (dd, *J* = 12.8, 5.0 Hz, 1H), 2.71 (d, *J* = 12.7 Hz, 1H), 2.22 (t, *J* = 7.3 Hz, 2H), 1.81 – 1.53 (m, 4H), 1.50 – 1.38 (m, 11H); ^{13}C NMR (126 MHz, MeOD) δ 174.8, 164.7, 157.0, 78.7, 69.9, 69.9 (obscured peak), 69.7, 69.2, 61.9, 60.2, 55.6, 39.8, 39.6, 38.9, 35.3, 28.4, 28.1, 27.4, 25.4. Exact mass (HRMS) calc. for $C_{21}H_{39}N_4O_6S^+(M+H)^+$ 475.2585, found 475.2571



Probe 4: Compound **5** (175 mg, 0.36 mmol) was Boc deprotected in a mixture of DCM/TFA (50:50, 10 mL) for 1 hour at room temperature. Solvents were removed by lyophilization yielding a white solid (0.35 mmol, 151 mg, 98%). The free amino product was used without further purification. Biotin-PEG2-amine (66 mg, 176 µmol), EDC (176 µmol 33.7 mg, 1 eq.) and 47.9 mg (352 µmol, 2 eq.) 1-hydroxy-7-azabenzotriazole (HOAt) were dissolved in 1.5 mL DMF (degassed) under an inert atmosphere. This solution was added to TCEP (528 µmol, 150 mg, 3 eq.) dissolved in 1.5 mL degassed DMF containing 148 µL (704 µmol, 4 eq.) DIPEA under an inert atmosphere. The reaction was stirred at room temperature for 30 min. Product was purified by RP-HPLC using a semi-preparative C18 column (gradient 0-50% MeCN in 60 min at a flow rate of 3.5 mL/min, H_2O / MeCN + 0.1% TFA) yielding a white solid (77 µmol, 44 mg, 41%). ^{1}H NMR (500 MHz, MeOD) δ 4.55 – 4.43 (m, 1H), 4.31 (dd, *J* = 7.9, 4.5 Hz, 1H), 3.62 (s, 4H), 3.56 (t, *J* = 5.6 Hz, 2H), 3.55 (t, *J* = 5.7 Hz, 2H), 3.43 – 3.33 (m, 4H), 3.25 – 3.18 (m, 1H), 3.00 – 2.67 (m, 8H), 2.58 – 2.40 (m, 6H), 2.22 (t, *J* = 7.4 Hz, 2H), 1.81 – 1.53 (m, 4H), 1.51 – 1.38 (m, 2H); ^{13}C NMR (126 MHz, MeOD) δ 176.2, 175.7 (d, $^3J_{C\text{-}P}$ = 7.0 Hz), 173.4 (d, $^3J_{C\text{-}P}$ = 4.5 Hz), 166.1, 71.3, 71.3, 70.6, 70.5, 63.4, 61.6, 57.0, 41.1, 40.6, 40.3, 36.8, 29.8 (d, $^2J_{C\text{-}P}$ = 6.3 Hz), 29.8, 29.5, 29.1 (d, $^2J_{C\text{-}P}$ = 4.5 Hz), 26.9, 17.6 (d, $^1J_{C\text{-}P}$ = 51.4 Hz), 17.5 (d, $^1J_{C\text{-}P}$ = 52.3 Hz); ^{31}P NMR (203 MHz, MeOD) δ -26.03. Exact mass (HRMS) calc. for $C_{25}H_{44}N_4O_9PS^+(M+H)^+$ 607.2561, found 607.2558. RP-HPLC t_r = 11.3 min (gradient 0-73% MeCN in 30 min, H_2O / MeCN + 0.1% TFA, Figure S15).

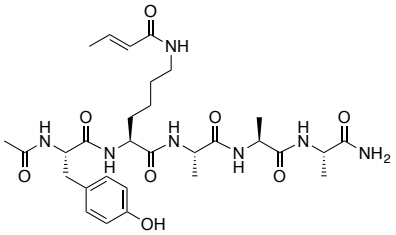


Oxidized Probe 4: 6.5 mg of **4** was dissolved in 500 µL of 10 mM MES buffer pH and cooled in an ice bath. 16 µL of a 3% H_2O_2 solution was added (final concentration 0.1% v/v) and incubated for 5 min. The oxidized product was immediately purified by RP-HPLC on a semi-preparative C18 column (gradient 0-50% MeCN in 60 min, flow rate = 3.5 mL/min) yielding a white solid. Exact mass (HRMS) calc. for

$C_{25}H_{44}N_4O_{10}PS^+(M+H)^+$ 623.2510, found 623.2484. RP-HPLC (LC-MS) t_r = 16.5 min (gradient 0-73% MeCN in 30 min, H_2O / MeCN + 0.1% FA, Figure S15).

General Procedures for Peptide Synthesis. Peptides were synthesized on either a Trityl-OH Chemmatrix resin for C-terminal hydrazides or on a Rink amide-Chemmatrix resin for C-terminal carboxamides. Peptides were either synthesized using manual addition of the reagents (using a stream of dry N_2 to agitate the reaction mixture) or on a Liberty Peptide Synthesizer equipped with a Discovery microwave module (CEM, Matthews, NC). Lysine crotonyl residues where introduced via the incorporation of Fmoc-Lys(Alloc)-OH, and subsequent alloc deprotection and functionalized with the crotonyl group as described below. For C-terminal acyl hydrazides, the procedure of Fang et al.[2] was used with slight modifications. In a representative synthesis, 595 mg of Trityl-OH resin (0.25 mmol) was placed in a polypropylene reaction vessel and swelled in DMF for 20 minutes. A solution of 1% of $SOCl_2$ in DCM (10 mL) was added to the resin and the reaction vessel was shaken overnight. An additional 100 μL of $SOCl_2$ was added and allowed to react for 2 hours. The resin was washed with DCM, 2% DIPEA in DMF and with DMF. Fmoc-hydrazine (254 mg, 4 eq.) in 5 mL DMF, and 100 μL DIPEA (5 eq.) was added to the resin and allowed to react for 1 hour. The resin was subsequently washed three times with DMF. Conventional Fmoc solid phase synthesis was then used to assemble the peptide chain using the methods outlined below. For manual peptide synthesis, a typical procedure is given here. The Fmoc group was deprotected with 3 mL of 20% piperidine in DMF and performed twice (one deprotection for 30 secs followed by an additional deprotection for 15 min). Between each deprotection step, as well as all subsequent synthetic steps, flow washes were used (3 x 5 sec with DMF). Coupling was performed using 5 eq. of monomer, 4.9 eq. of 0.5 M HBTU/HOBt and 10 eq. of DIPEA with 2 min pre-activation. Lysine crotonyl functionalization was performed as follows. The alloc group was deprotected with tetrakis(triphenylphosphine)Pd^0 (0.1 eq. per alloc group) and 1,3 dimethylbarbituric acid (5 eq. per alloc group) in DCM for 2 x 20 min. The resin was washed with DCM, DMF, 5% DIPEA in DMF and DMF. Crotonylation was performed with crotonic anhydride (10 eq. per crotonyl group) and DIPEA (5 eq. per crotonyl group) in DMF for 15 min. The procedure was repeated once more to ensure full acylation. Cleavages were performed with 95% TFA, 2.5% TIS and 2.5% H_2O. The peptide was then precipitated with diethyl ether, dissolved in water with 0.1% TFA and analyzed via RP-HPLC. Semi-preparative or preparative RP-HPLC purification was then used to isolate the peptide of interest.

Synthesis of model peptide 2c. Model peptide **2c** was synthesized on a Rink Amide-Chemmatrix using manual synthesis. The crude product was purified via preparative RP-HPLC. Analytical data: RP-HPLC gradient 0-73% MeCN, t_r = 17.6 min (Figure S14). Expected mass: 631.3 Da, found $(M+H)^+$ = 632.3 Da.



Synthesis of crotonylated histone peptides H3K18Cr(1-28) and H4pKCr$_5$(1-37). Standard Fmoc SPPS chemistry using Trityl-OH Chemmatrix resin, as outlined above, was applied to generate C-terminal peptide hydrazides corresponding to H3(1-28)K18Cr and H4(1-37)K5/8/12/16/20Cr with N-terminal acetylation. Analytical data: RP-HPLC gradient 0-73%

MeCN, H3K18Cr t_r= 28.2 min. Expected mass: 3120.8 Da, found 3120.9 Da; RP-HPLC gradient 0-73%B, H4pKCr t_r= 17.6 min Expected mass: 4333.1 Da, found 4333.3 Da.

Preparation of recombinant histones. Unmodified recombinant human histones (H2A, uniprot ID: Q6FI13; H2B, uniprot ID: O60814; H3C96A, C110A, uniprot ID: P68431; H4, uniprot ID:P62805) were produced in and purified from *E. coli* according to published protocols.[3]

Expression of p300 in Sf9 cells. Full-length p300 was expressed in Sf9 cells and purified according to published procedures.[4]

Semisynthesis of modified histones, H4pAc H3K18Cr and H4pCr. Histone H4pAc was prepared by semisynthesis as described previously.[5] Analogously, histones H3K18Cr and H4pCr were prepared, with minor modifications, using the peptides described above. Notably, the H3K18Cr and H4pCr proteins were not subjected to radical desulfurization (this is incompatible with the crotonyl group) and were thus used as cysteine mutants (H3K18CrA29C and H4pCr$_5$A38C). Analytical data: RP-HPLC gradient 0-73% MeCN, histone H3K18Cr t_r= 28.3 min. Expected mass: 15350.0 Da, found 15350.9 Da. RP-HPLC gradient 0-73% MeCN, H4pCr t_r= 25.5 min. Expected mass: 11650.8 Da, found 11650.9 Da. See Figure S16 for analytical data.

Histone octamer formation. Octamers of wild-type (WT), H3K18Cr, H4pCr and H4pAc were prepared according to published protocols.[6] Octamers were analyzed by SDS PAGE (Figure S17) and stored at -20°C.

Chromatin assembly. Mononucleosomes were prepared according to published protocols.[6] Briefly, histone octamers and recombinant 601 DNA in 10 mM TrisHCl, pH 7.5 at 4°C, 2 M KCl, 0.1 mM EDTA, where placed in a Slide-A-Lyzer mini dialysis device (3.5 kDa cutoff) and dialyzed into 200 mL 10 mM TrisHCl, pH 7.5, 1.4 M KCl, 0.1 mM EDTA, 1 mM DTT (start buffer), at 4°C for 1 hour. 300 mL of 10 mM TrisHCl, pH 7.5, 10 mM KCl, 0.1 mM EDTA, 1 mM DTT (end buffer) was then added over the course of 6 hours. Subsequently, two rounds of dialysis with 200 mL end buffer (2 hours each) was performed. Samples were recovered, centrifuged (max speed table centrifuge, 4°C, 5 min) and the supernatant isolated. Quantification of mononucleosomes was performed by their UV absorbance at 260 nm and analyzed by native gel electrophoresis (5x TBE gel) (see Figure S17). Molar ratios of DNA and octamer where empirically optimized for each case. Nucleosome arrays were prepared as previously described.[7] 12-mer arrays were analyzed by native gel electrophoresis (1% agarose / 2% polyacrylamide APAGE gels) (see Figure S17).

General procedure for TCEP/phosphine addition to model compound 2a and model peptide 2c. Either **2a** or **2c** were dissolved in 200 μL of buffer containing 50 mM TrisHCl, pH 8, 6M GuHCl to a final concentration of 2 mM. To this was added the phosphine from a stock solution of 1M in the same buffer to the appropriate concentration. The reaction mixture was incubated at 37°C. For analysis, a 10 μL aliquot was taken and diluted with HPLC solvent A to 50 μL and used for HPLC or LC-MS analysis. The pH screen was performed using a series of buffers (100 mM acetate, 50 mM TrisHCl, 50 mM BisTris, pH 4-10, 6 M GuHCl).

Analysis of crotonylated nucleosomes using chemical probe 4. Mononucleosomes (200 nM) were reacted with probe **4** (4 mM) in 50 mM TrisHCl, pH 8 (total volume 20 μL) for 16 hours at

37 °C. SDS loading buffer was then added, followed by boiling (5 min), and the sample was analyzed by SDS PAGE (15% TrisHCl gel, 170V, 65 min). Semi-dry transfer was performed on Immun-Blot PVDF membranes (Biorad, 20V for 20 min at 4ºC). These were then blocked (1 hour, 5% milk in TBST) and probed with Streptavidin-800 (5000x dilution of Licor IRDye® 800CW Streptavidin in TBST + 0.1% SDS) for 1 h at room temperature.

Proteomic analysis of H3K18Cr mononucleosomes reacted with probe 4.
Mononucleosomes containing H3K18Cr (400 nM) were incubated with 4 mM probe **4** for 16 hours at 37 °C. Samples were desalted using C8-stage tips, reduced with 5 mM DTT at 60 °C for 10 min, and alkylated with 15 mM chloroacetamide for 30 min in the dark. Samples were digested with 1 µg ArgC (Sigma) overnight at 37 °C, and then incubated with an additional 0.25 µg ArgC for 3 hours. Samples were lyophilized, resuspended in 0.1% formic acid, and analyzed using an Easy-nLC-Orbitrap-Fusion-Lumos system (Thermo Scientific, USA). Samples were loaded onto a 45 cm nano-capillary column (75 um ID) packed with 1.9 µm C18-AQ (Dr. Maisch, Germany). The mass spectrometer was operated in data-dependent mode with full survey scans (*m/z* 400–1500) in the Orbitrap followed by up to 20 MS/MS scans with CID fragmentation in the ion trap. A dynamic exclusion list was used to exclude peptides previously sequenced within the last 60 sec. Raw files were searched using Byonic, MS-Amanda and Sequest HT algorithms within the Proteome Discoverer 2.1 suite (Thermo Scientific, USA). A custom modification of +674.2750 Da on Lys residues was also defined as a dynamic modification (the crotonyl group conjugated to probe **4**, denoted K* in main text and Figure S18). ArgC digestions were allowed 2 missed cleavages. Files were searched against a custom database of human histone proteins supplemented with common contaminants. Scaffold (version Scaffold_4.7.5, Proteome Software Inc., Portland, OR) was used to validate peptide (95% FDR, Scaffold Local algorithm) and protein (99%, Protein Prophet algorithm) identifications. See fully annotated spectra Figure S18.

Detection of H3K18Cr mononucleosomes in a *E.coli* lysate. *E. coli* lysates supplemented with mononucleosomes containing H3K18Cr (50-400 nM) were incubated with probe **4** (4 mM) for 16 hours before being analyzed by western-blot as described above. For pull-down experiments *E. coli* lysates supplemented with H3K18Cr mononucleosomes (400 nM) were reacted with probe **4** (4 mM) in 10 mM TrisHCl, pH 8, 10 mM KCl for 16 hours at 37 ºC. Excess probe was then removed by dialysis (2 x 0.5L 50 mM TrisHCl, pH 8 for 2 hours at 4 ºC) and the resulting solution incubated with 50 µL of pre-washed magnetic Streptavidin beads (New England Biolabs) for 1 hour at room temperature. Following washing, the beads were eluted by boiling in 2X SDS buffer for 10 min. The eluent was analyzed by western-blot as described before.

Cell culture and protein extraction. HEK293T cells were cultured in DMEM, supplemented with 10% FBS, at 37 °C under 5% CO_2. Cells where treated with or without 20 mM sodium crotonate (prepared by dissolving crotonic acid in water and titrating with sodium hydroxide to pH 7.4) at 80% confluency (~$8*10^6$ cells) and harvested 24 hours later. Cells where washed with ice cold PBS and flash frozen. Histones where extracted using the EpiQuik Total Histone Extraction Kit (Epigentek, OP-0006-100). Nuclear extract was prepared as follows: Cells were resuspended in 1 mL LB1 buffer (50 mM HEPES, pH 7.5, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.25% Triton X-100, protease inhibitors), incubated on ice for 10 min and centrifuged for 5 min (400g at 4 °C). The supernatant was removed, and the pellet was resuspended in 500 µL of LB2 buffer (10 mM TrisHCl, pH 8, 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, protease inhibitors) and

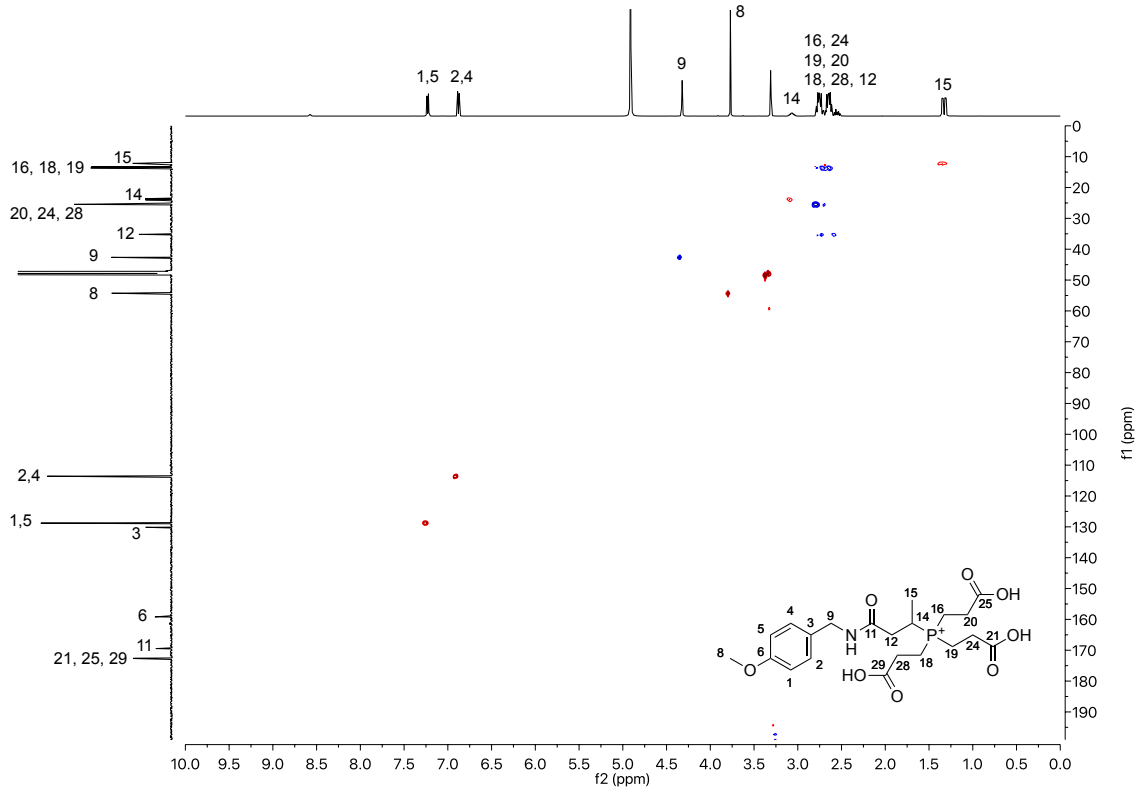
incubated on ice for 10 min. After centrifugation (1300g, 5 min at 4 °C) the supernatant was removed, and the pellet was resuspended in 75 μL LB3 buffer (10 mM TrisHCl, pH 8, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% Na-deoxycholate, 0.5% N-lauroylsarcosine, protease inhibitors). Resuspension was aided by sonicating the sample for 5 secs (30% amplitude). Triton X-100 was added (1% final) and the sample was boiled for 5 min. The denatured supernatant was isolated by centrifugation (max speed, 5 min, room temperature) in preparation for the reaction with probe **4**.

Detection of crotonylation in cellular proteins. Histones or nuclear extracts extracted from HEK293T cells (± sodium crotonate) were incubated with probe **4** (4 mM) for 16 hours at 37 °C. SDS loading buffer was then added and the sample boiled for 5 min. Samples were then analyzed by western-blot as described above (see Analysis of crotonylated nucleosomes using chemical probe **4**).

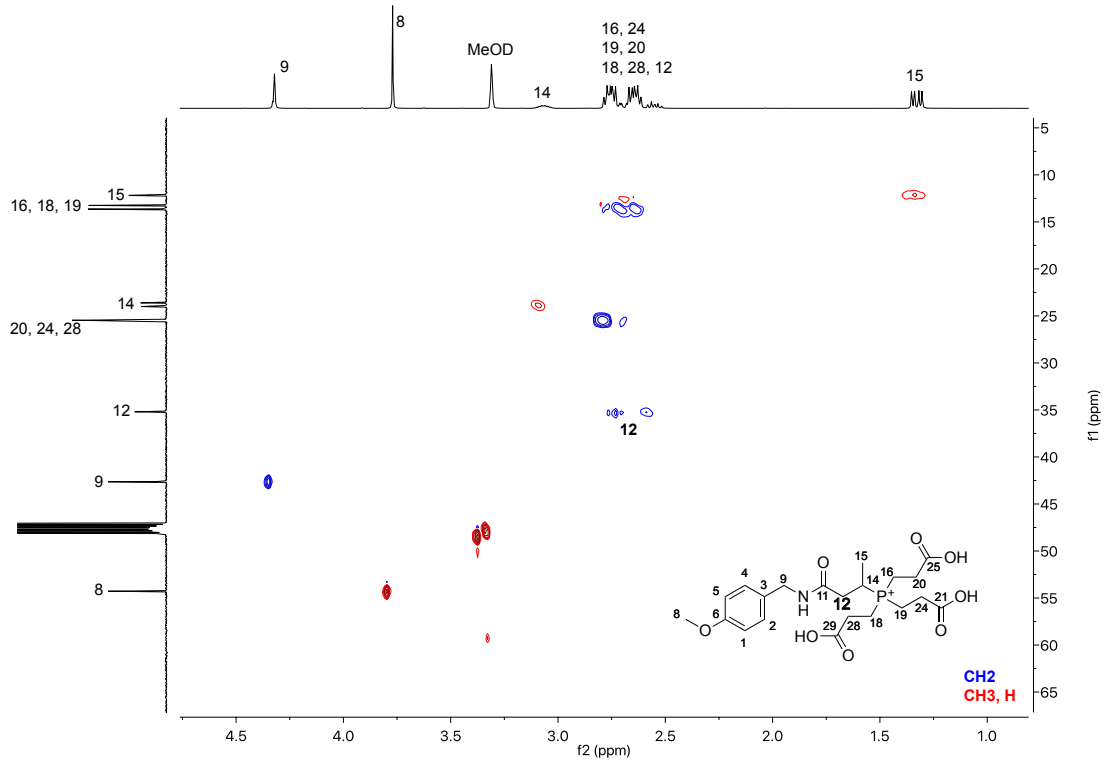
p300 mediated crotonylation/acetylation of nucleosomal arrays. Reconstituted nucleosome arrays (200 nM per 601 site), Cr-CoA/Ac-CoA (100 μM) and purified p300 (50 ng) were incubated in 60 μL reaction buffer (50 mM TrisHCl, pH 8, 10% glycerol, 0.1 mM EDTA) at 30 °C for 1 hour. Following dialysis against 50 mM TrisHCl, pH 8, 8 M urea (2 x 0.5L) for 2 hours at room temperature, the reaction mixture was divided into 3 parts for western blot analysis employing either the indicated antibodies or probe **4.** Analysis by probe **4** was as described above.

Inhibition of antibody recognition by TCEP. WT 12-mer arrays (200 nM per 601 site) were crotonylated using p300, as described above. After the p300 reaction, excess Cr-CoA was removed by dialysis (2x0.5L 50 mM TrisHCl, pH 8 for 2 hours at 4 °C) and the samples were incubated with 200 mM TCEP for 16 hours at room temperature. Excess of TCEP was removed by dialysis and the samples were analyzed by western blot as described above.

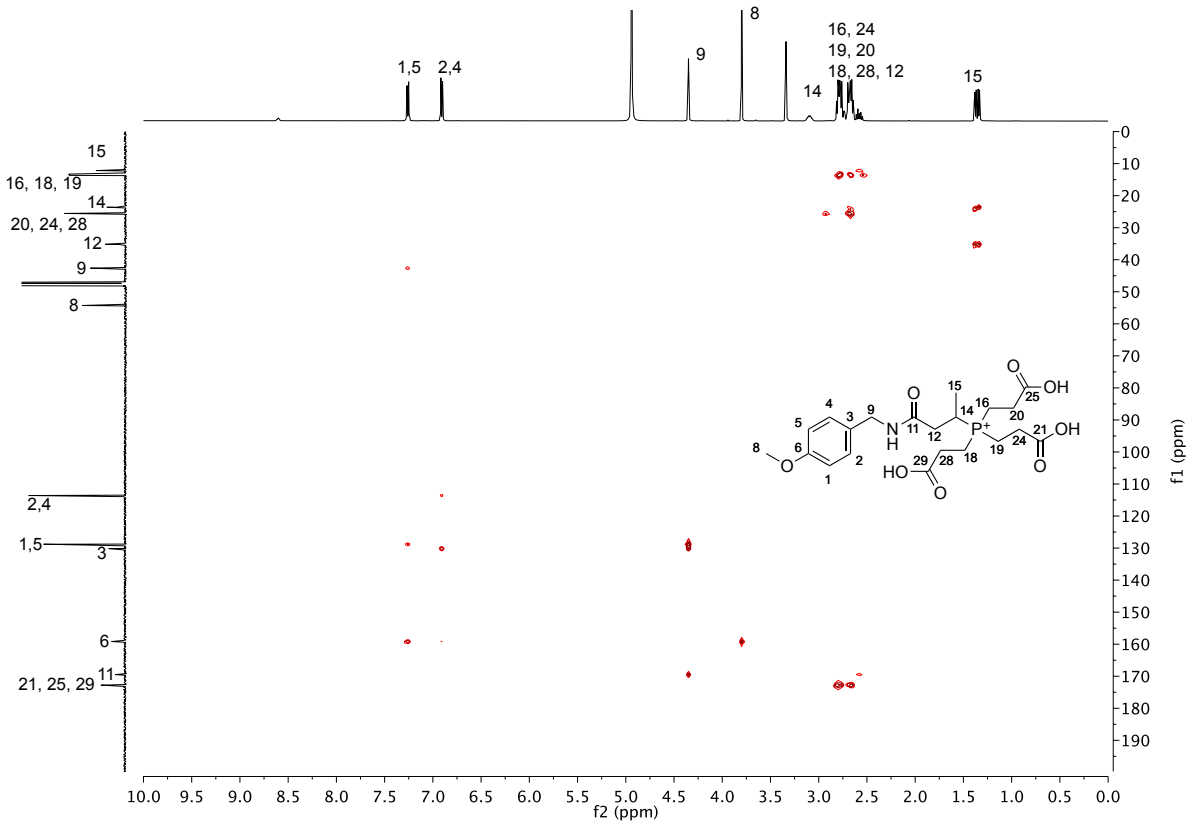
a) HSQC (MeOD, 500 MHz) **3b**



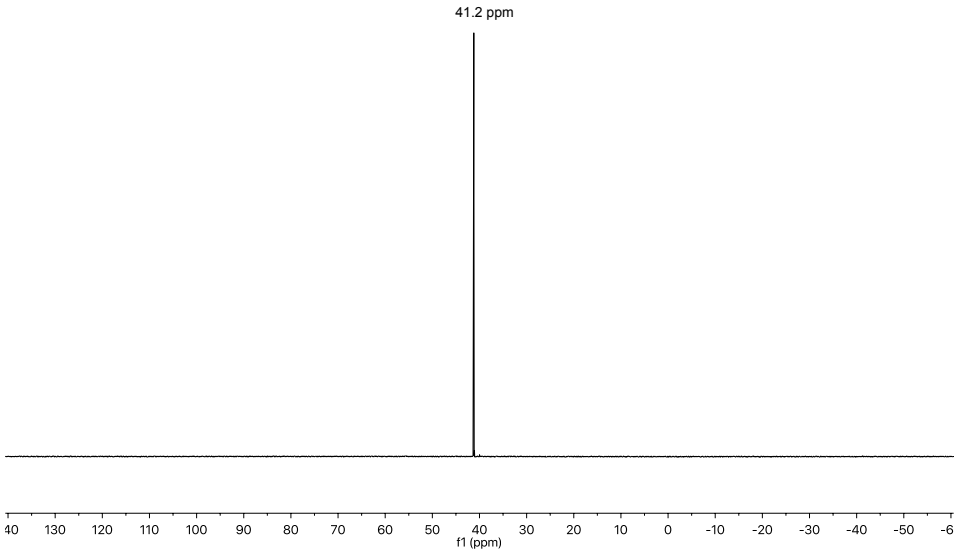
Zoom in from above spectra:



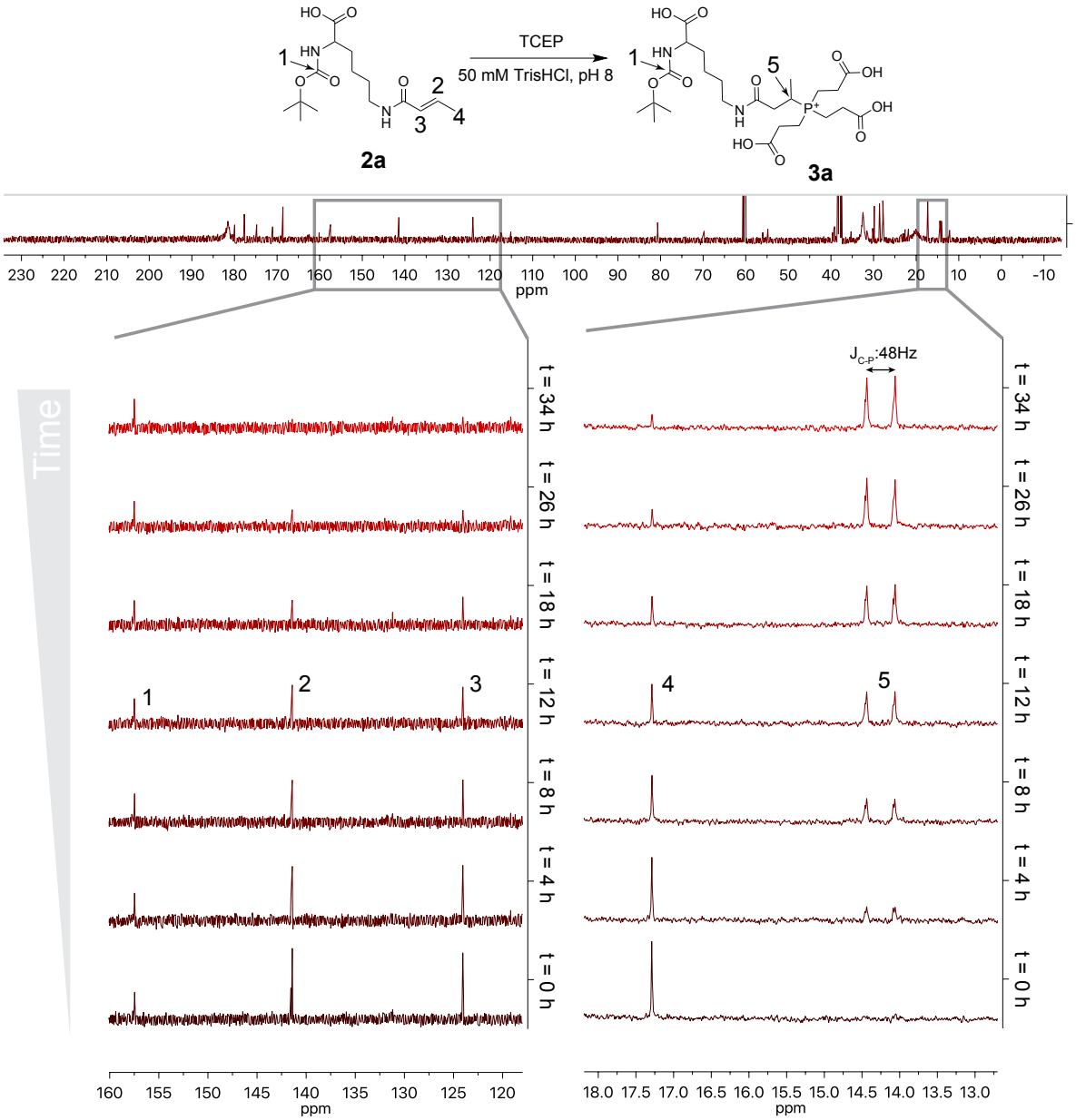
b) HMBC (MeOD, 500 MHz) **3b**



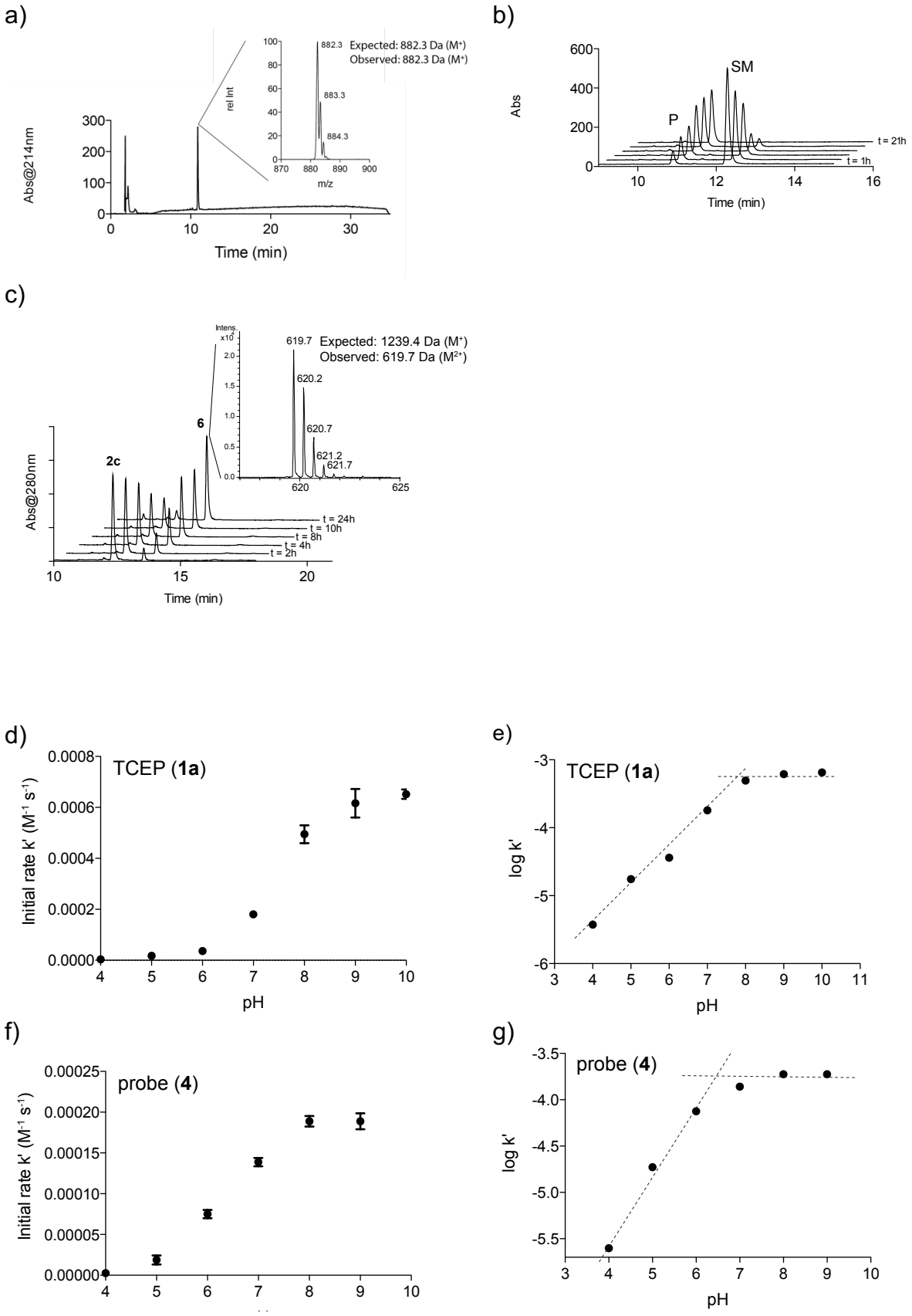
c) ^{31}P-NMR (MeOD, 203 MHz) **3b**



d)



Supplementary Figure S1: NMR analysis of model compound **3b** formed from the reaction of TCEP and model compound **2b**. Peak assignment relied on the HSQC and HMBC spectra. a) HSQC spectra of **3b**. Note, the generation of a methylene (carbon 12) signal indicates the loss of the olefin moiety in **2b** upon reaction with TCEP. b) HMBC spectra of **3b**. c) ^{31}P-NMR spectra of **3b**. The chemical shift of 41.2 ppm is consistent with a β-phosphonium species.[8] d) Time course experiment of **2a** (40 mM) with **1a** (160 mM) in aqueous buffer (50 mM TrisHCl, pH 8) at room temperature. Note, the disappearance of the ^{13}C signals of the olefin (denoted in the spectra as 2 and 3) and the appearance of the resulting CH_2 signal (denoted in the spectra as 5).

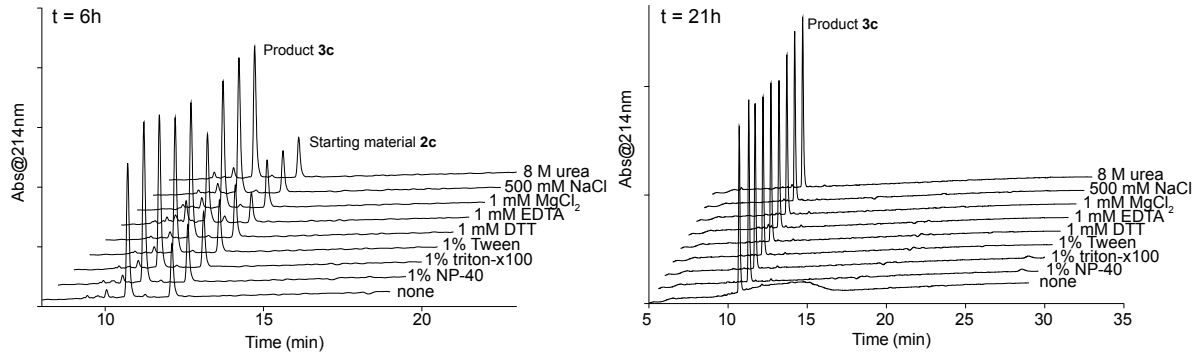

a)
Abs@214nm
Time (min)
rel Int
m/z
882.3
883.3
884.3
Expected: 882.3 Da (M+)
Observed: 882.3 Da (M+)
b)
Abs
SM
P
t = 21h
t = 1h
Time (min)
c)
Abs@280nm
Intens.
x10
2c
6
619.7
620.2
620.7
621.2
621.7
Expected: 1239.4 Da (M+)
Observed: 619.7 Da (M2+)
t = 24h
t = 10h
t = 8h
t = 4h
t = 2h
Time (min)
d)
TCEP (1a)
Initial rate k' (M-1 s-1)
pH
e)
TCEP (1a)
log k'
pH
f)
probe (4)
Initial rate k' (M-1 s-1)
pH
g)
probe (4)
log k'
pH

Supplementary Figure S2: pH screen of the reaction between TCEP (**1a**) or probe (**4**) with model compound **2c**. a) Analytical C18 RP-HPLC chromatogram (0-73% MeCN from 5-35 min, H_2O / MeCN (0.1% TFA) of the reaction mixture between **1a** with **2c** after 21h. The starting materials are fully converted to the addition product **3c**. In-set shows ESI-MS analysis of the product. Reaction conditions: 200 mM TCEP, 2 mM **2c**, in 50 mM TrisHCl, 6 M GuHCl, pH 8, 37 ºC. b) Time course of the reaction in panel a. Starting material (**2c**) (SM) and product (P) (**3c**) are indicated in the RP-HPLC stack. c) Analytical C18 reversed-phase HPLC chromatogram (0-73% MeCN from 5-35min, H_2O / MeCN (0.1% TFA) of the reaction mixture between probe **4** with **2c**. Starting material (**2c**) (SM) and product (P) (**6**) are indicated in the RP-HPLC stack. In-set shows ESI-MS analysis of the product **6**. Reaction conditions: 200 mM **4**, 2 mM **2c**, in 50 mM TrisHCl, 6 M GuHCl, pH 8, 37 ºC. d) Initial rates of the reaction (denoted k') of TCEP (**1a**) with **2c** are plotted against the pH. Reaction conditions: 200 mM TCEP (**1a**), 2 mM **2c**, in 100 mM Acetate, 50 mM TrisHCl or 50 mM BisTris, pH 4-10, at 37 °C. e) Data from d was re-plotted as a log of the k' versus pH to show a pH-rate profile. f) Initial rates of the reaction (denoted k') of probe **4** with **2c** are plotted against the pH. Reaction conditions: 200 mM TCEP (**1a**), 2 mM **2c**, in 100 mM Acetate, 50 mM TrisHCl or 50 mM BisTris, pH 4-10, at 37 ºC. e) Data from d was re-plotted as a log of the k' versus pH to show a pH-rate profile.

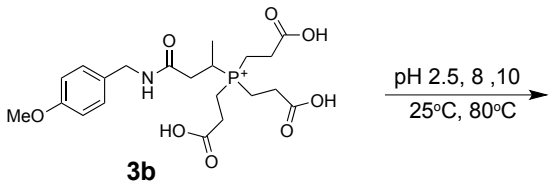
a)

Additive	Conversion (t=21 h)
None	full
NP-40 (end 1%)	full
Triton-x100 (end 1%)	full
Tween 20 (end 1%)	full
DTT (end 1 mM)	full
EDTA (1 mM)	full
$MgCl_2$ (1 mM)	full
NaCl (end 500 mM)	full
Urea (end 8M)	full

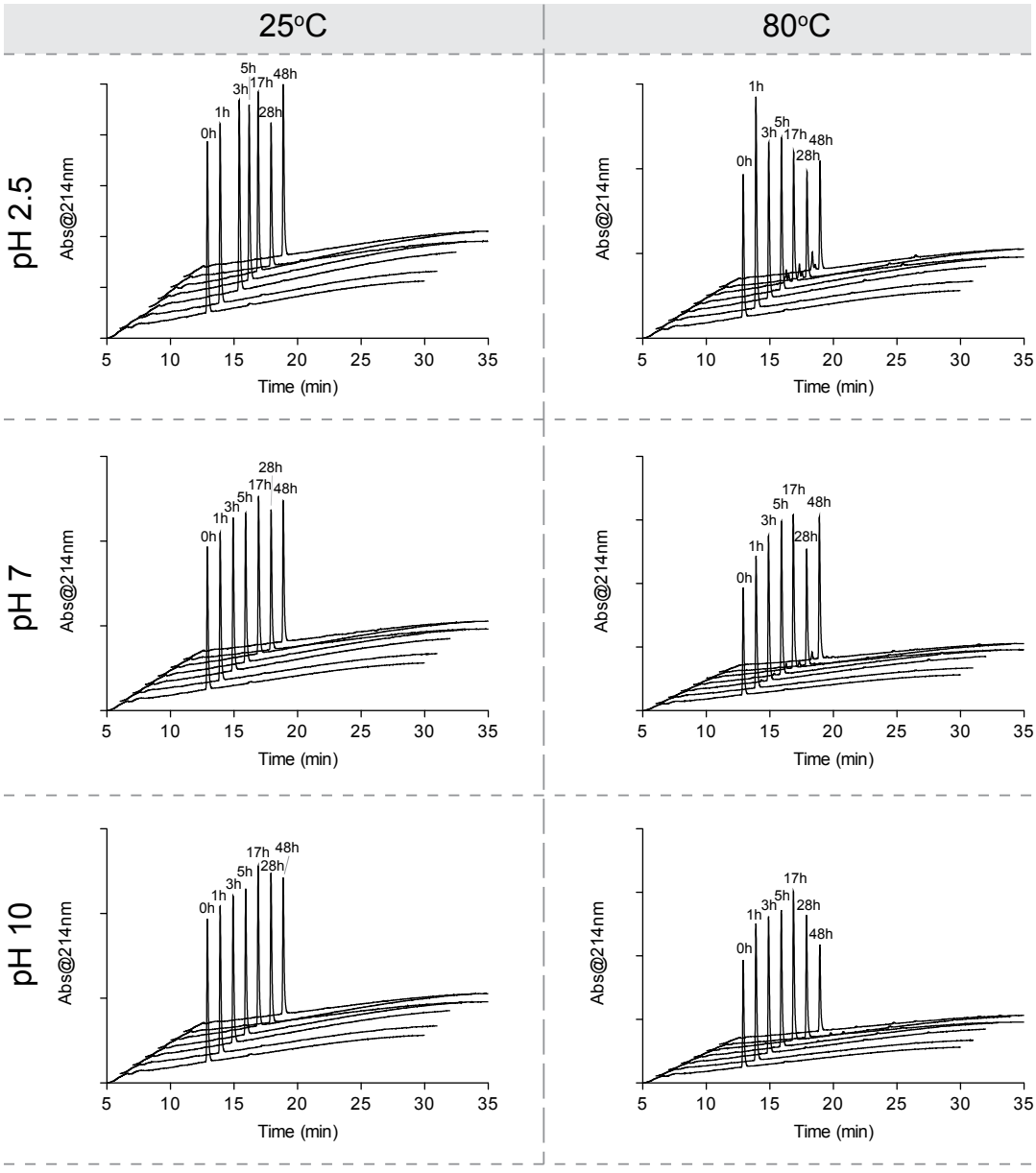
b)



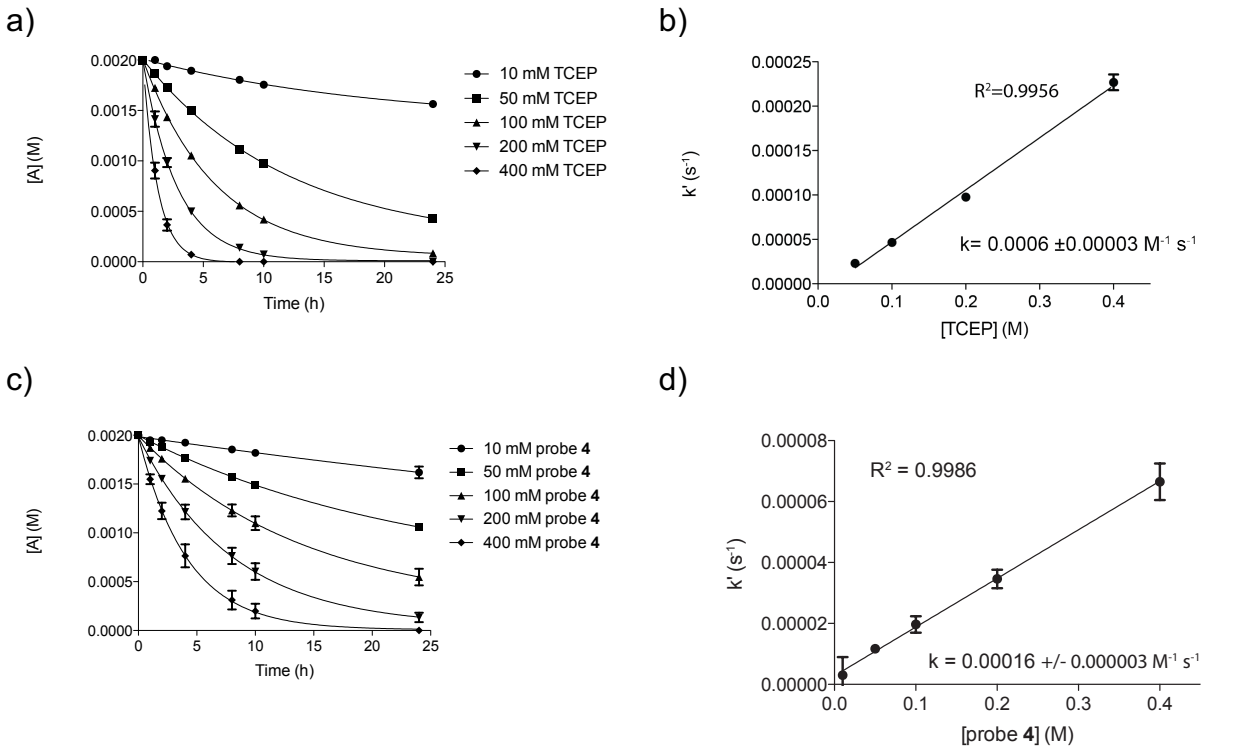
c)


MeO
N
H
O
P+
OH
HO
O
pH 2.5, 8 ,10
25°C, 80°C

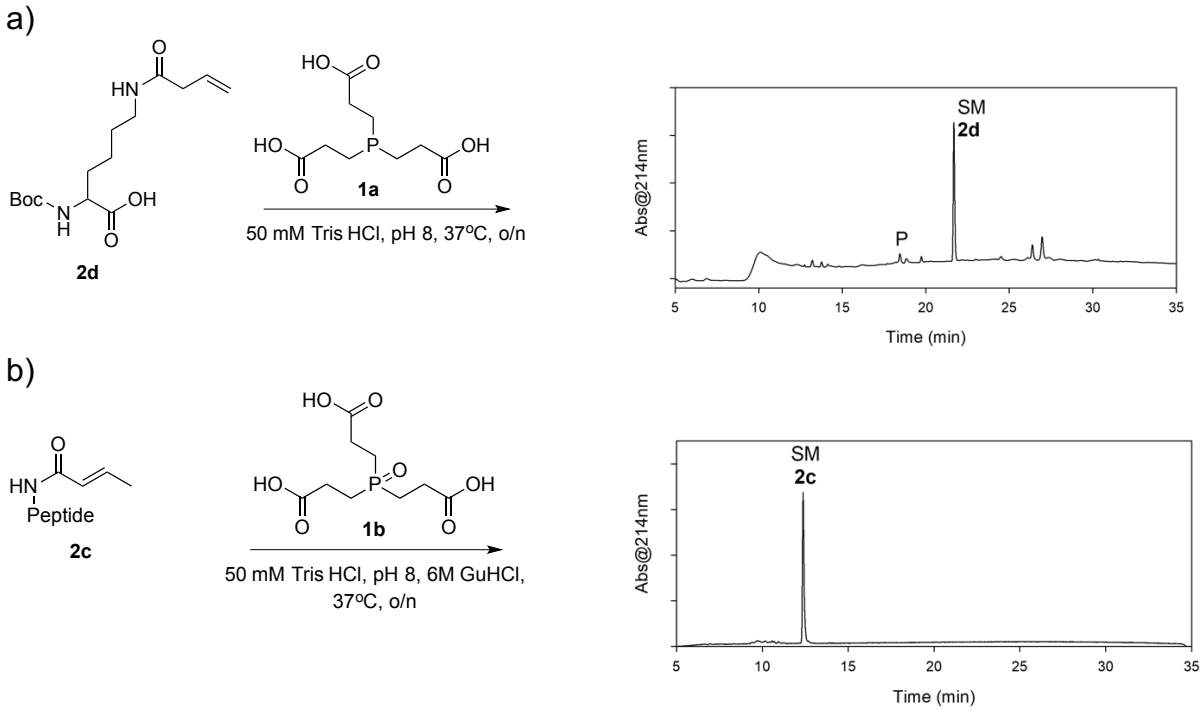
3b


25°C
80°C
pH 2.5
pH 7
pH 10
Abs@214nm
Time (min)
0h
1h
3h
5h
17h
28h
48h

Supplementary Figure S3: Scope of the reaction between TCEP and the crotonyl compound (**2b**/**2c**). a) Table showing effect of additives commonly used in protein biochemistry on the reaction between **2c** with **1a**. Reaction conditions are as stated in panel a Figure S2. Full conversion was achieved in all cases after 21 hours. b) RP-HPLC-traces of the reaction between **2c** with **1a** (after 6 hours, left panel; after 21 hours, right panel) with additives listed in panel a. c) Evaluation of the stability of the phosphonium species **3b** in aqueous buffer. Compound **3b** (2 mM in 100 mM acetate, 50 mM TrisHCl or 50 mM BisTris) was subjected to a range of conditions (pH: 2.5, 8, and 10; temperature: 25°C and 80°C) and analyzed by RP-HPLC (0-73% MeCN from 5-35min, H_2O / MeCN (0.1% TFA)) at several time points (t = 0 h to t = 48h).

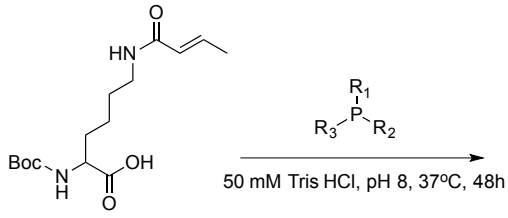


Supplementary Figure S4: Kinetic analysis of the reaction. a) Kinetic traces of the reaction of TCEP **1a** (concentration range 10-400 mM) with **2c** (2 mM) in 50 mM TrisHCl, 6 M GuHCl, pH 8, 37 ºC. Conversions were calculated using the RP-HPLC areas of the starting material (SM) and the product (P) peaks from traces using 280 nm detection (assuming similar extinction coefficients of SM and P). Concentration of **2c** ([A]) at a given time point was fit to a single exponential decay generating rate constants (k'). Experiments were performed in triplicate. b) Calculation of the second order rate constant. Pseudo-first order approximation analysis was used to calculate the second order rate constant, in which [TCEP] >> [**2c**]. Rate constants (k') determined in a) were plotted against [TCEP] and the resulting slope represents the second order rate constant of the reaction as 6 (± 0.3) x 10^{-4} M^{-1} s^{-1}. c) Kinetic traces of the reaction of probe **4** (concentration range 10-400 mM) with **3c** (2 mM) in 50 mM TrisHCl, 6 M GuHCl, pH 8, 37 ºC. Calculation of the second order rate constant as per panel b. k = 1.6 (± 0.3) x 10^{-4} M^{-1} s^{-1}.

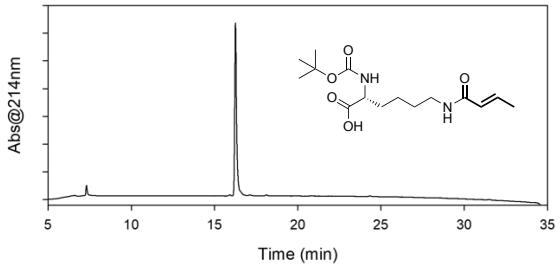


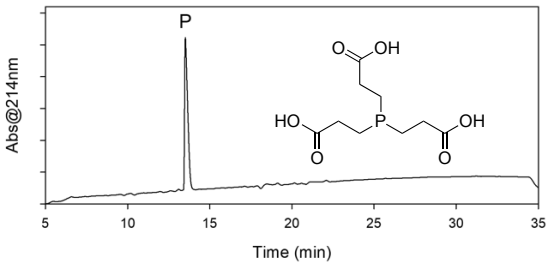
Supplementary Figure S5: a) Reaction of compound **2d**, containing the 3-butenoyl acyl group, with TCEP (**1a**). b) Reaction of the model peptide (**2c**) with the oxidized version of TCEP (**1b**). Reaction conditions: 2 mM of **2c**,**d** and 200 mM of **1a**,**b**, in 50 mM TrisHCl, pH 8, at 37 ºC for 16 hours. Reactions were analyzed on a LC-MS. SM = starting material, P = product.

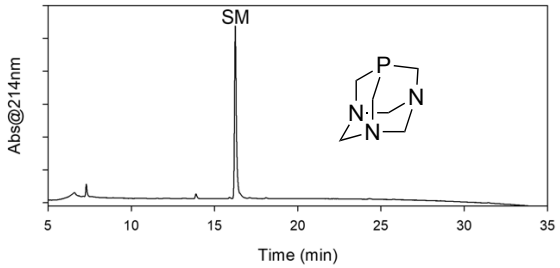
a)

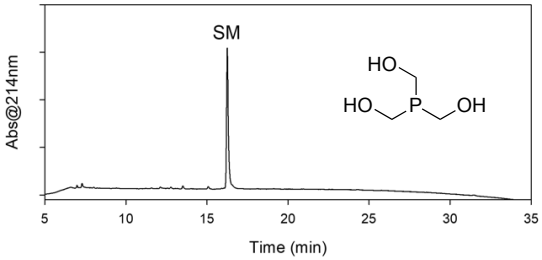

HN
Boc
N
H
OH
O
R1
R3
P
R2
50 mM Tris HCl, pH 8, 37°C, 48h

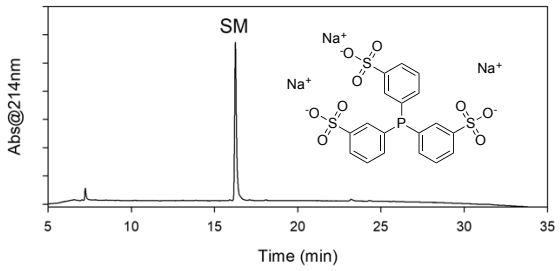
2a

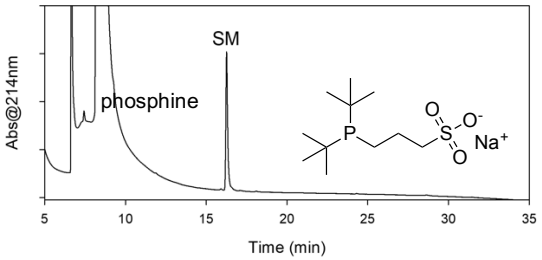

Abs@214nm
Time (min)

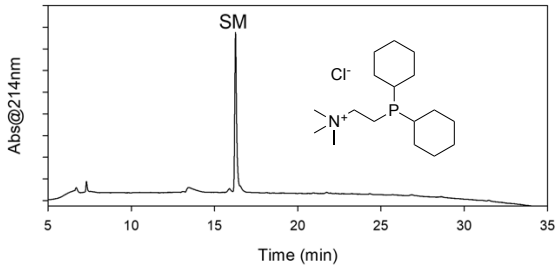

P
Abs@214nm
Time (min)


SM
Abs@214nm
Time (min)

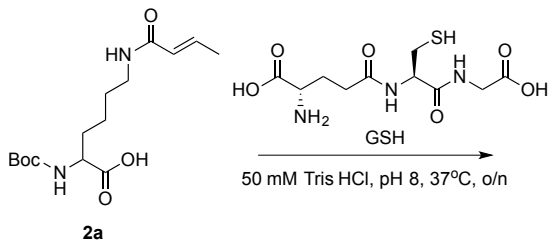

SM
Abs@214nm
Time (min)

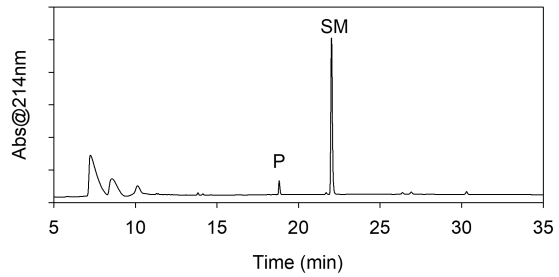

SM
Abs@214nm
Time (min)


SM
phosphine
Abs@214nm
Time (min)

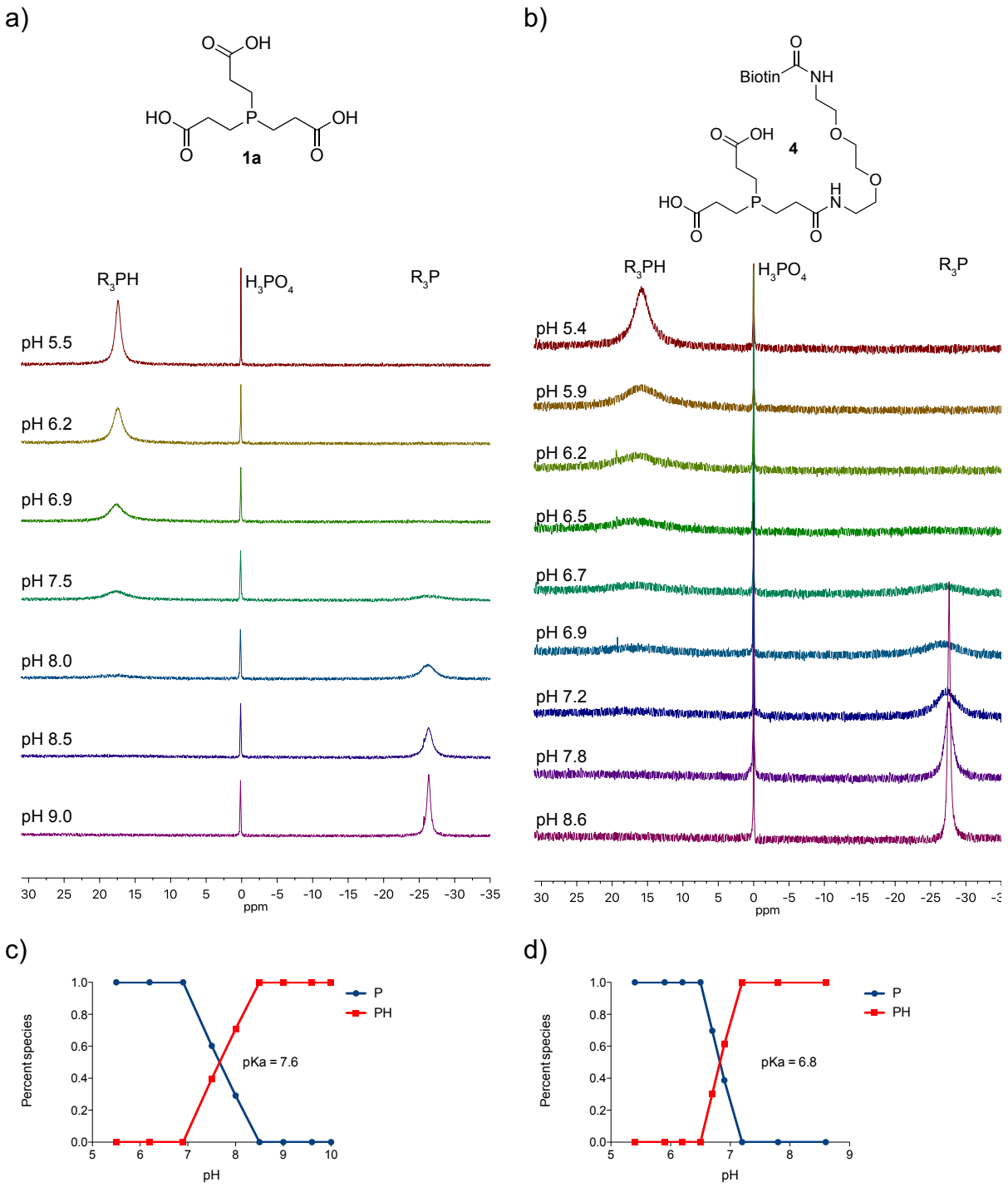

SM
Abs@214nm
Time (min)

b)





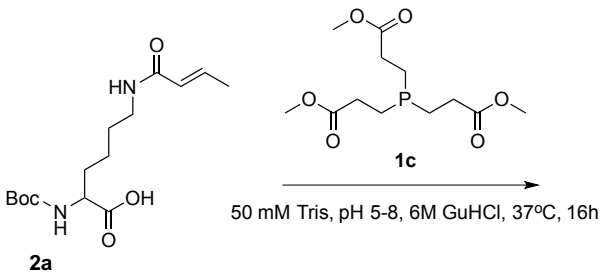
Supplementary Figure S6: a) Reactivity of a series of structurally diverse water-soluble phosphines with model compound **2a**. Reaction conditions: 2 mM **2a** reacted with 100 mM phosphine in 50 mM TrisHCl, 6M GuHCl, pH 8, at 37°C for 48 hours. Reaction mixtures were analyzed by RP-HPLC and ESI-MS. SM = starting material (RP-HPLC trace, top left). P = product. The TCEP reaction served as the positive control (RP-HPLC trace, top right). b) Reaction of **2a** with glutathione (GSH). Reaction conditions: 2 mM **2a** reacted with 200 mM red-GSH in 50 mM TrisHCl, 6M GuHCl, pH 8, at 37°C for 16 hours. Reaction mixture was analyzed by RP-PHLC and ESI-MS and only trace amounts of reaction product were observed.



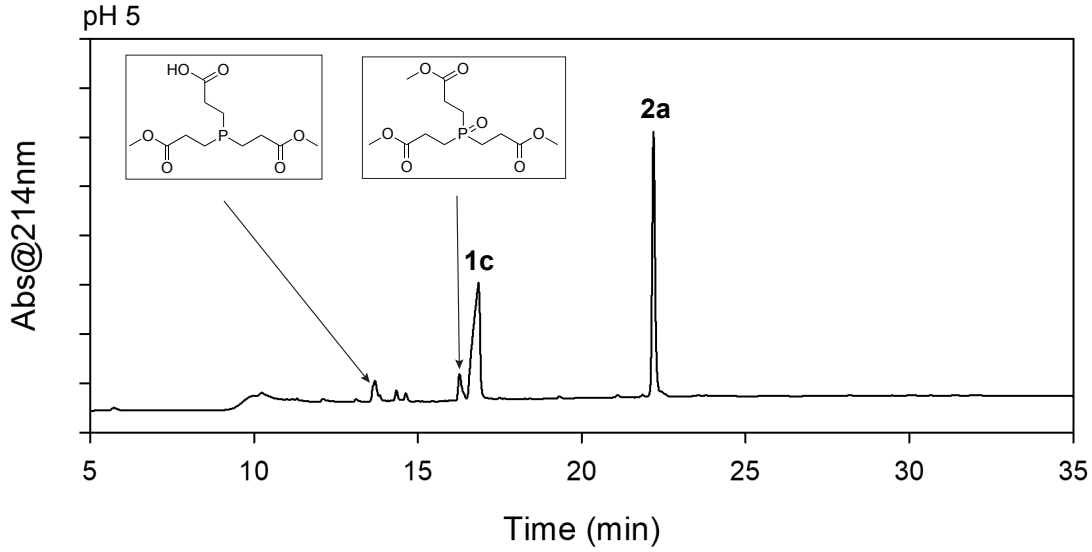
Supplementary Figure S7: pKa determination for TCEP and probe **4**. pH titration was performed according to a literature procedure.[9] a) ^{31}P-NMR spectra of TCEP (50 mM) at different pH. An insert with D_2O with $H_3PO_4^-$ was used for locking and served as external standard. Protonated (17.4 ppm) and deprotonated (-26.4 ppm) P-species are denoted in the spectra and correspond to literature values.[9] b) ^{31}P-NMR spectra of probe **4** (25 mM) at different pH. c-d) Ratios of peak integrals where determined (integrals where corrected using the external signal) and plotted against pH. pKa was determined using the Henderson-Hasselbalch equation: $pKa = pH + \log\frac{A^-}{AH}$ in

which A^- is represented by the integral of the R_3P species and AH by the integral of the R_3PH species. The pKa of TCEP was determined to be 7.6 and probe **4** of 6.8. These values are similar as reported in the literature[9,1] for TCEP (7.6) and monomethyl ester analogue of TCEP (6.6), respectively.

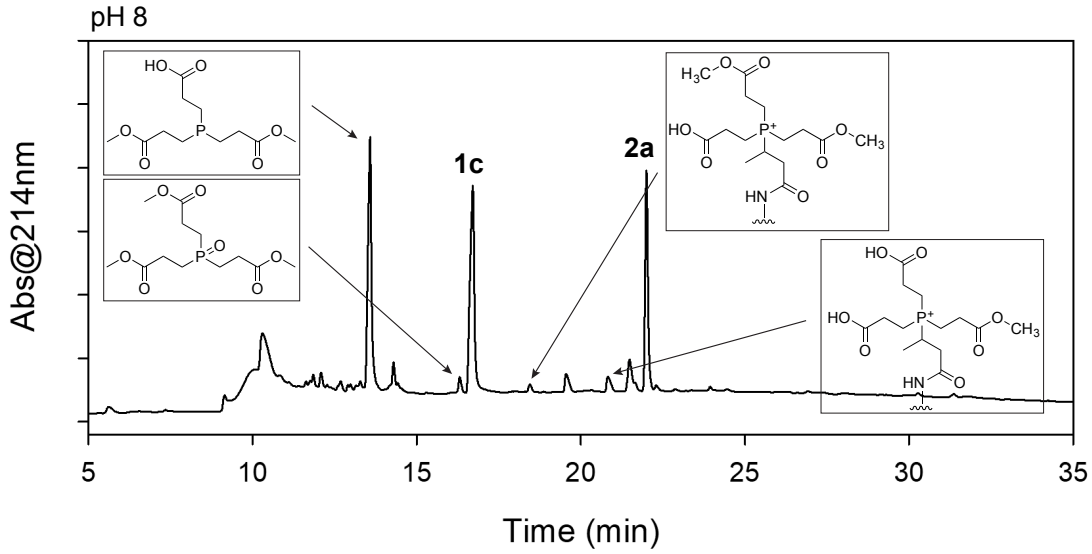
a)


2a
1c
50 mM Tris, pH 5-8, 6M GuHCl, 37ºC, 16h

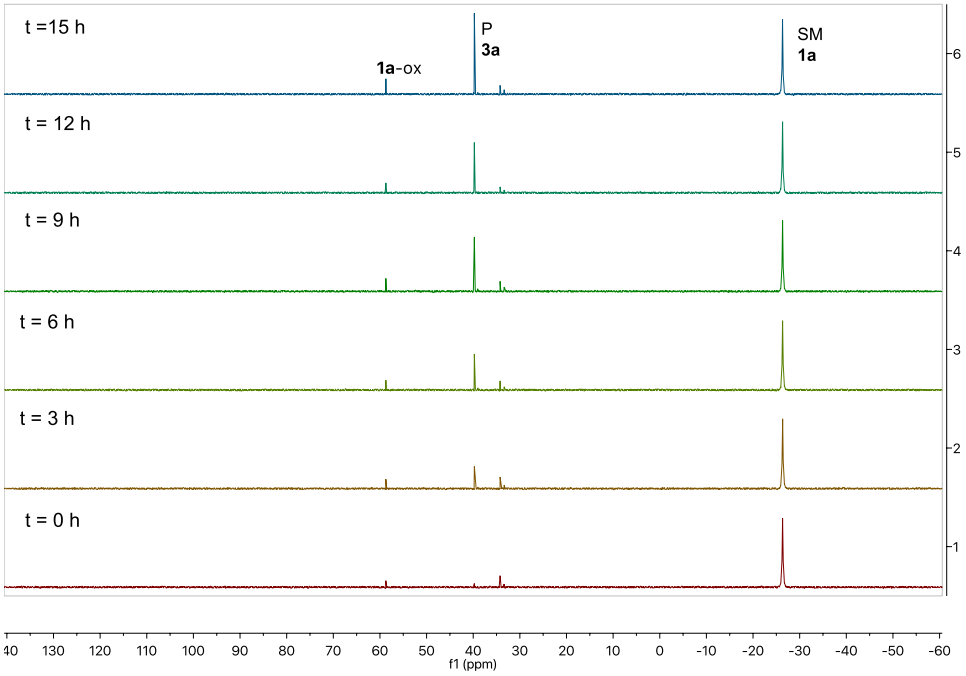
b)


pH 5
Abs@214nm
2a
1c
Time (min)

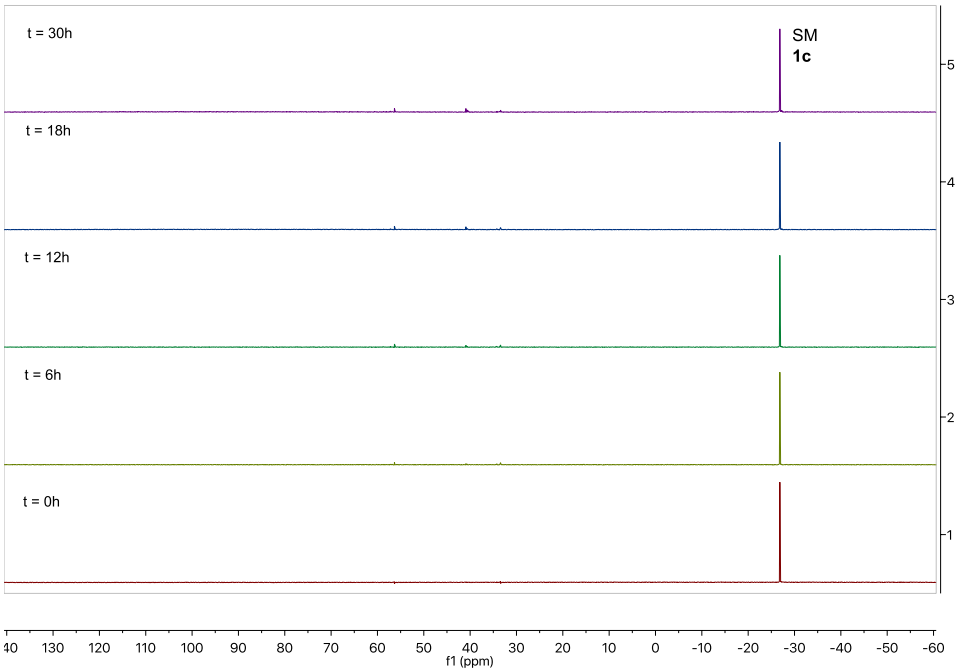
c)


pH 8
Abs@214nm
1c
2a
Time (min)

d)

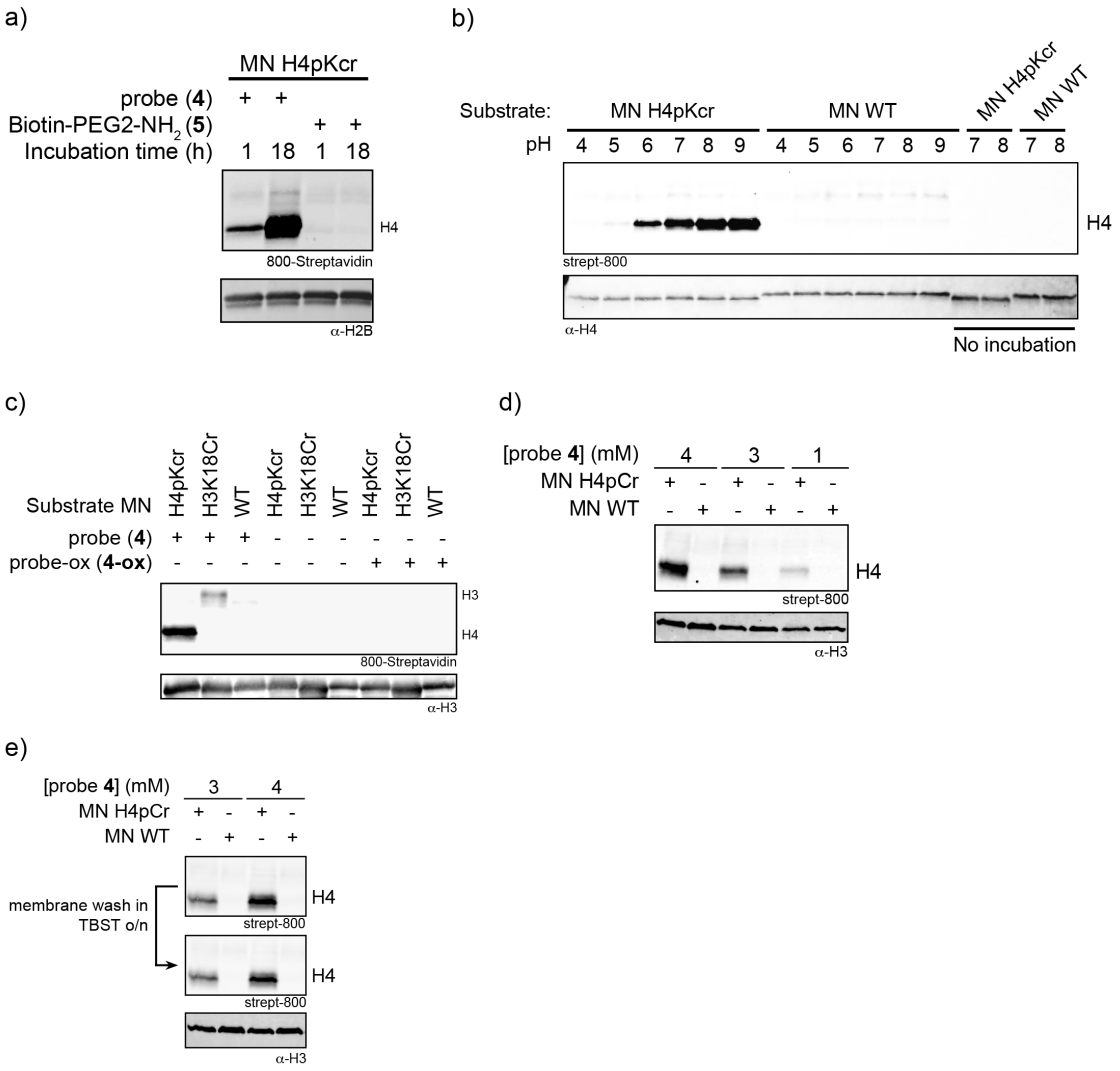


e)

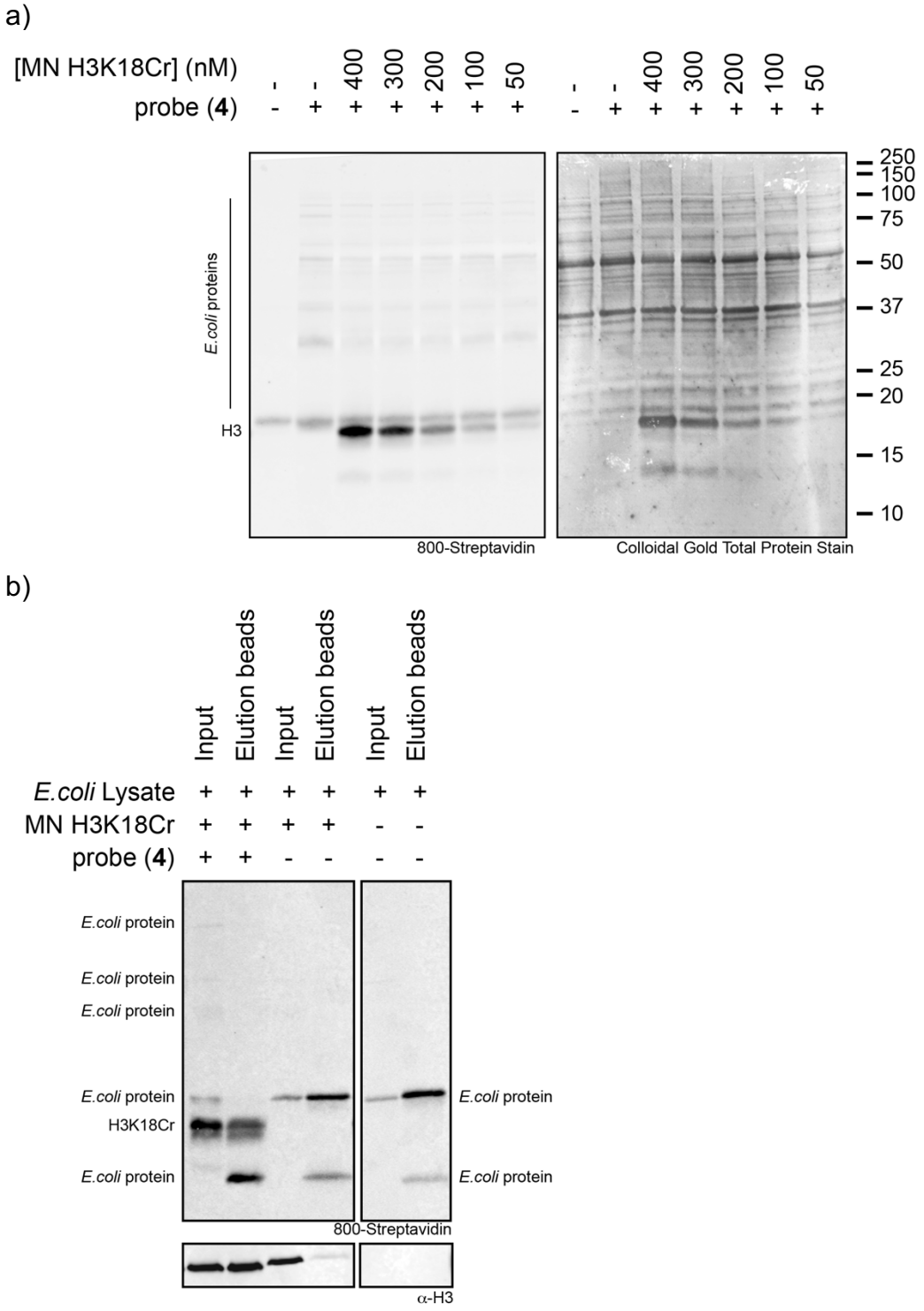


Supplementary Figure S8: Reaction of **1c** with model compound **2a**. a) Reaction scheme. b-c) RP-HPLC chromatograms of the reaction mixture at pH 5 and pH 8 after 16 hours, respectively. Indicated peaks were characterized by ESI-MS. No reaction between **1c** and **2a** was observed. By contrast, hydrolysis products of **1c** (i.e. mono- and bis-methyl esters) were found to react with **2a**. d-e) As an alternative approach for studying the reaction of **2a** with **1c**, ^{31}P-NMR based experiments were utilized. The product formation of either the reaction with **1c** or **1a** with **2a** was

followed by ^{31}P-NMR over time. Conditions: 10 mM of **2a** and 19.2 mM (1.6 eq.) of **1a**,**c** in 50 mM TrisHCl, pH 8, 6M GuHCl, at room temperature. An insert with D_2O was used for the ^{31}P-NMR signal lock.

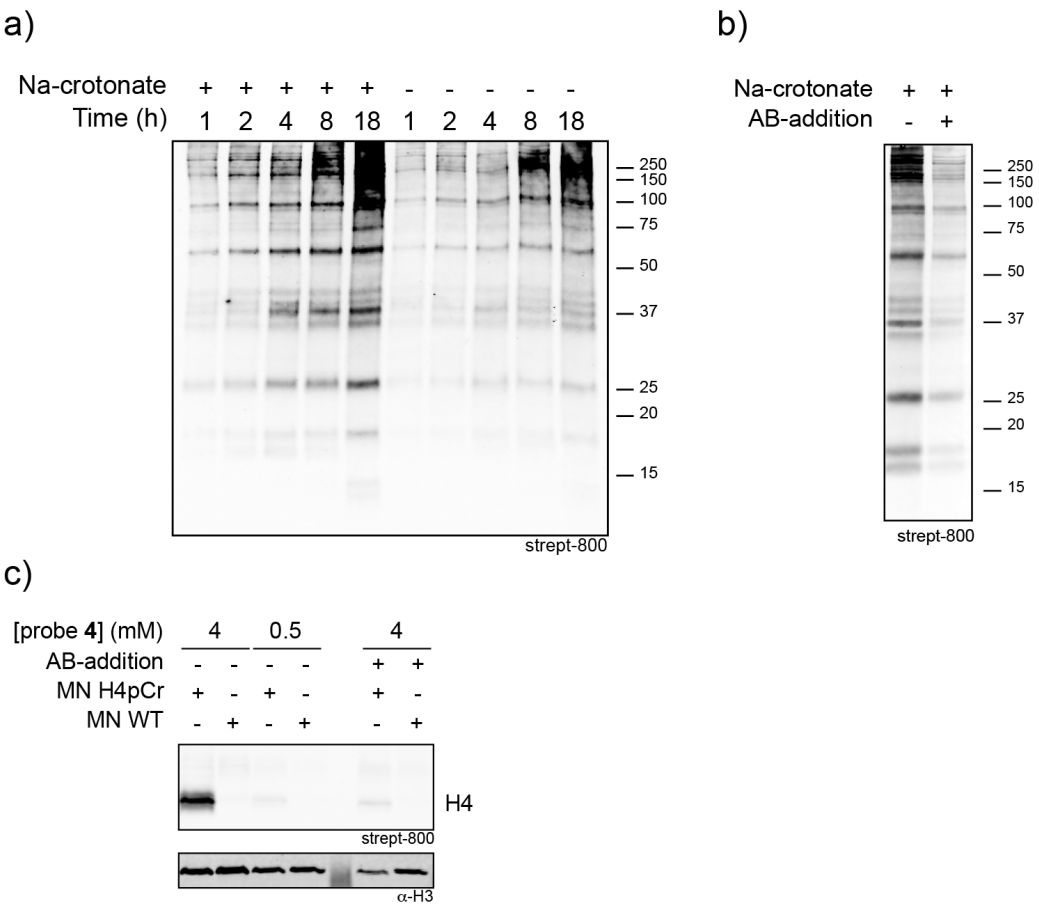


Supplementary Figure S9: Detection of crotonylation in mono-nucleosomes (MNs) with chemical probe **4**. a) Time course experiment utilizing probe **4** (4 mM) with MNs bearing pre-installed H4pCr marks (200 nM). The MNs were incubated with the probe for either 1h or 18h, at 37°C, before being analyzed by western-blot employing a biotin detection system (dye-conjugated streptavidin). An α-H2B antibody was used as a loading control. The pre-installed crotonylation mark is detected by the probe within an hour. No detection was seen using the precursor of the probe (**5**), lacking the TCEP warhead. b) Western blot analysis of a pH screen of the reaction of probe **4** (4 mM) with H4pCr MNs (200 nM). Detection as described in a). An α-H4 antibody was used as a loading control (see Figure S2). c) Detection of MNs containing H4pCr, H4K18Cr versus wild-type (WT) by probe **4** after 16 h incubation. Detection as described in panel a, with the use of an α-H3 antibody as a loading control. No detection of crotonylated MNs was observed using the oxidized version the probe (**4-ox**). d) Detection of MN H4pCr with different probe **4** concentrations after 16 h incubation. e) Stability of the Western blot signal of the probe towards MN H4pCr was evaluated. Extensive washing of the membrane in TBST did not lead to a significant decrease of the Western blot signal.

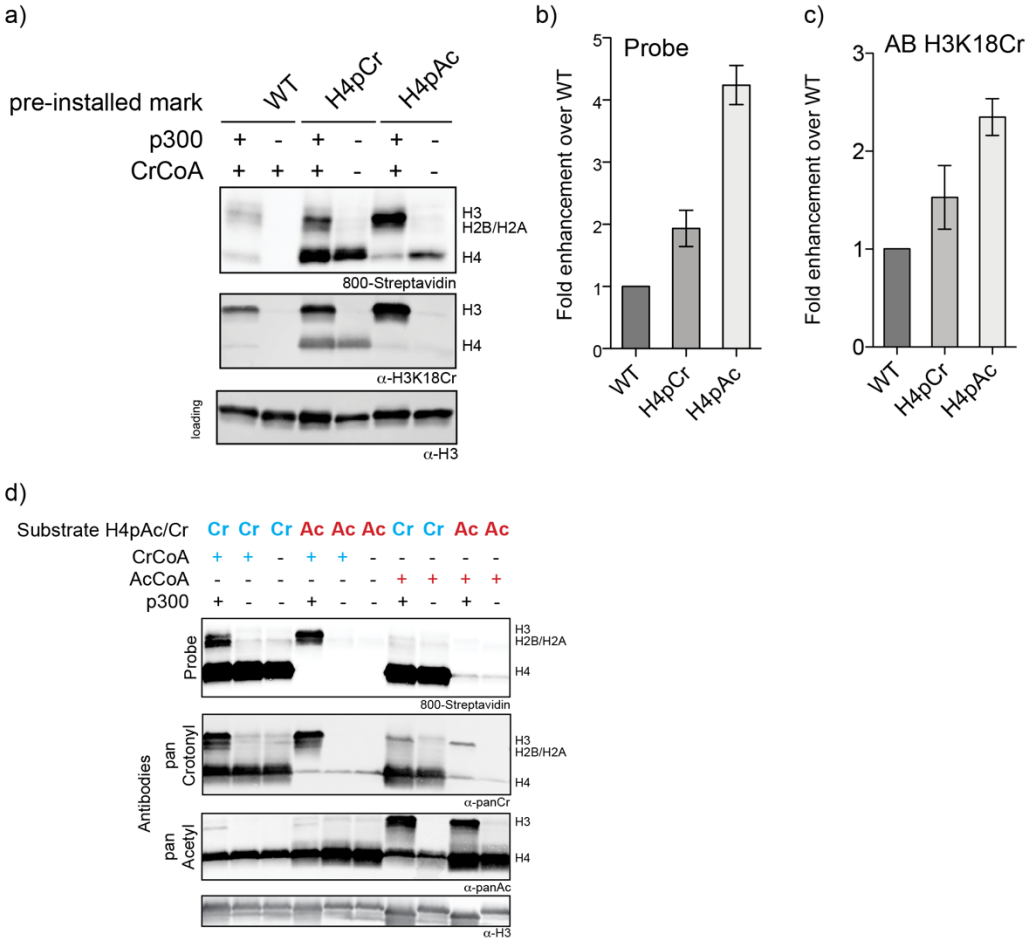


Supplementary Figure S10. a) Analysis of the detection limit of probe **4** when used in a complex protein mixture. MNs containing H3K18Cr were added to an *E.coli* lysate at different concentrations (50-400 nM) and incubated with the probe **4** (4 mM) for 16 hours (in 50 mM TrisHCl, pH 8, 150 mM NaCl), before being analyzed by western-blot employing a biotin detection system (dye-conjugated streptavidin). A colloidal gold protein stained membrane was used as a loading control. b) Affinity capture of MNs containing H3K18Cr conjugated with probe

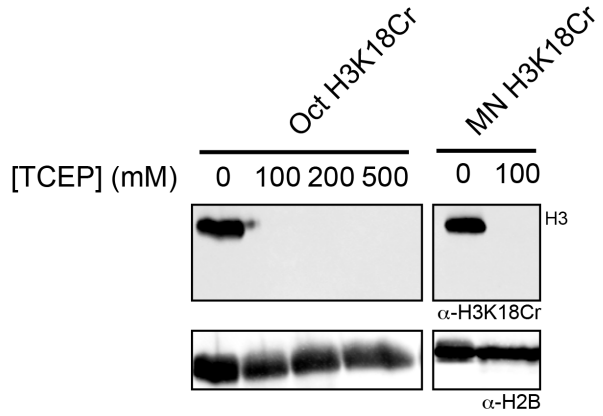
4 from a complex protein mixture. H3K18Cr MNs (400 nM) were added to an *E.coli* lysate and incubated with probe **4** (4 mM) for 16 hours. Excess probe was removed by dialysis and magnetic streptavidin beads were used to capture crotonylated proteins. Proteins were eluted from the beads by boiling in SDS sample buffer. Analysis by western-blot as described in a), an α-H3 antibody was used as a loading control.



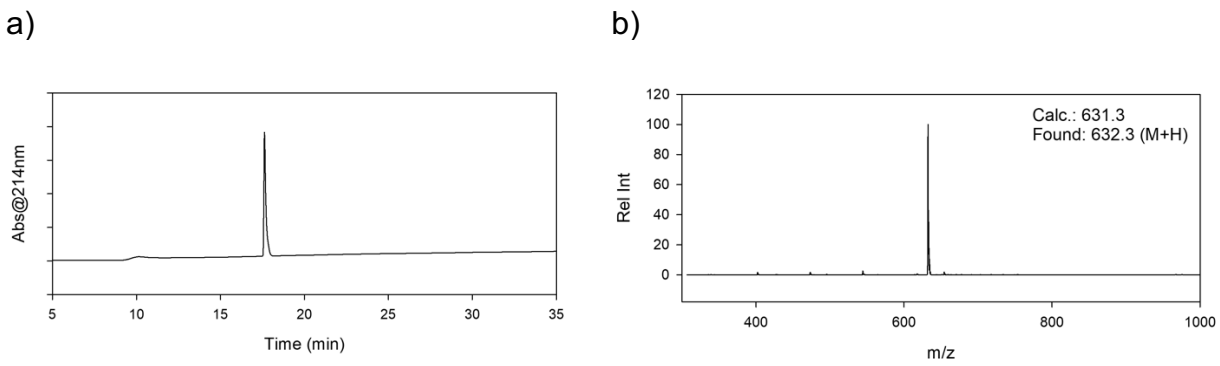
Supplementary Figure S11. Analysis of protein crotonylation. a) Western blot analysis of nuclear extract from HEK293 cells, either grown in the absence or presence of sodium crotonate. The denatured supernatant was boiled in the presence Triton X-100 and isolated by centrifugation (max speed, 5 min, room temperature) in preparation for the reaction with probe **4** for the indicated times. b) Blocking of probe detection was accomplished by co-incubating the probe with the pan specific crotonyl antibody (PTM biolabs, PTM-501, 20.000x dilution) for 18 hr. c) Same as in panel b, except recombinant mononucleosomes (MNs) containing H4pCr were used.



Supplementary Figure S12: Western blot analysis of p300 mediated crotonylation of 12-mer nucleosomal arrays bearing either no-, H4pCr- or H4pAc marks. a) As in Figure 3 (main text). For comparison, reactions were also analyzed with anti-H3K18Cr antibodies as indicated. b-c) Densitometry analysis of western blots of crotonylation levels of histones H3, H2B and H2A (signal of histone H4 is omitted since the mark is pre-installed in the substrates H4pCr and H4pAc) detected by probe **4** (b) and by anti-H3K18Cr (c). Fold enhancement is plotted over p300 activity on WT nucleosomal arrays (n = 3). d) Western blot analysis of p300 mediated crotonylation/acetylation of 12-mer nucleosomal arrays bearing either no-, H4pCr- or H4pAc marks. Detection as described in a).

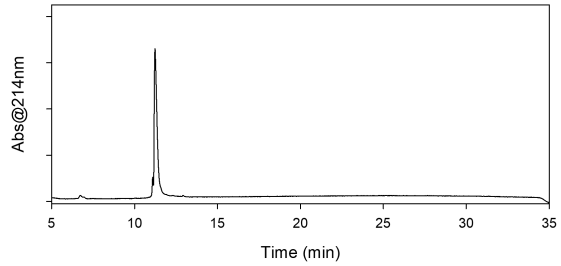


Supplementary Figure S13. Effect of TCEP treatment on antibody recognition of H3K18Cr histone octamers (Oct) or MNs (200 nM) bearing the H3K18Cr mark. Samples were incubated with TCEP (0-500 mM) for 16 hours at 37°C, before being analyzed by western-blot employing anti-H3K18Cr antibody. An α-H2B antibody was used as a loading control.

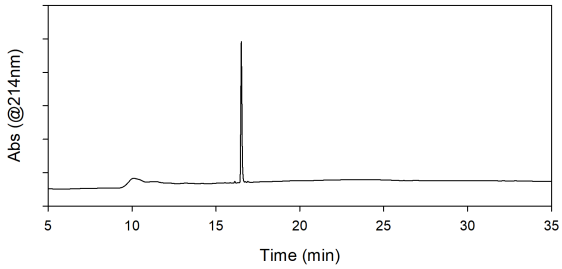


Supplementary Figure S14: Characterization of model peptide **2c**. a) Analytical C18 RP-HPLC of purified peptide **2c** (0-73% MeCN from 5-35min). b) ESI-MS of **2c.**

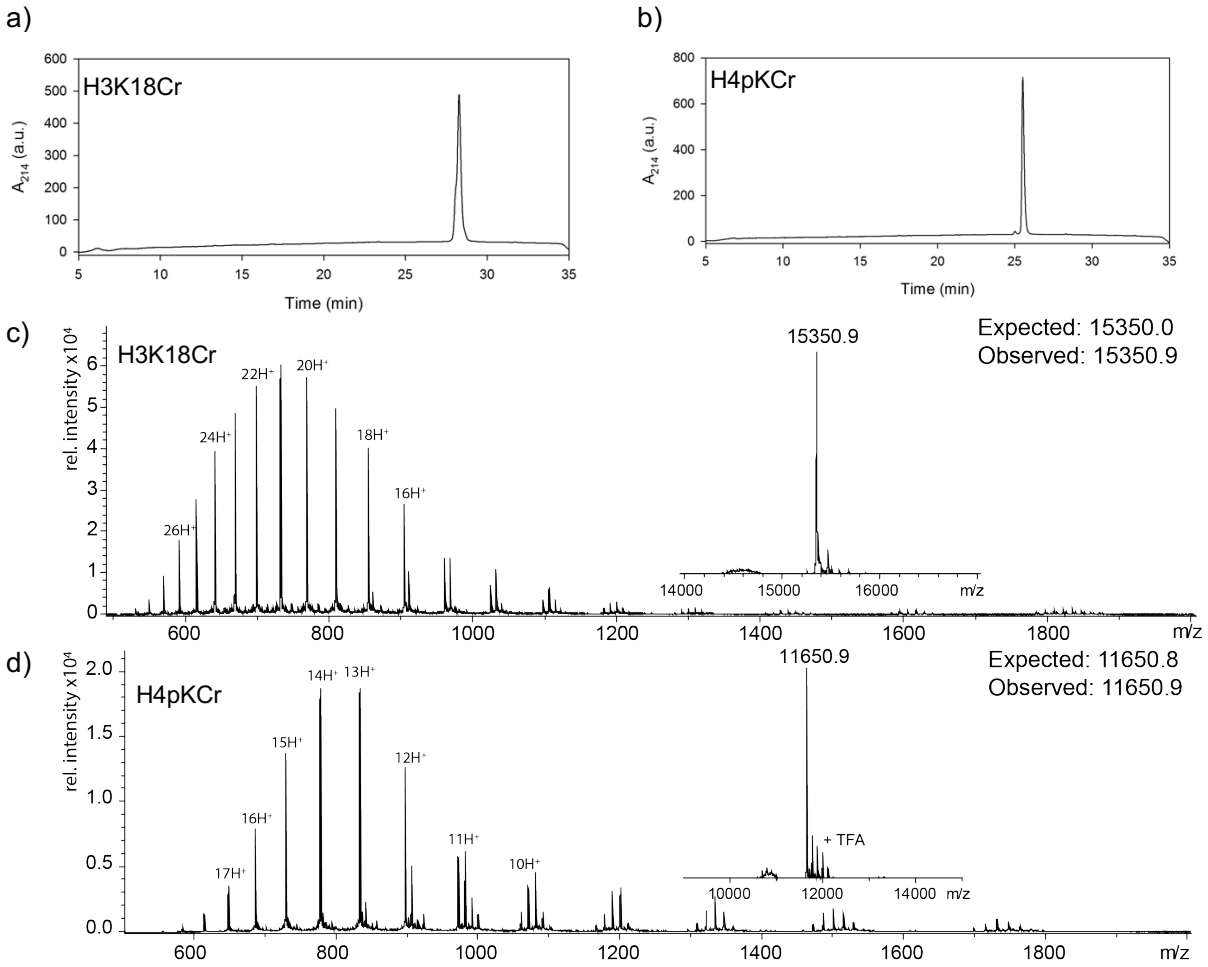
a)
Probe **4**



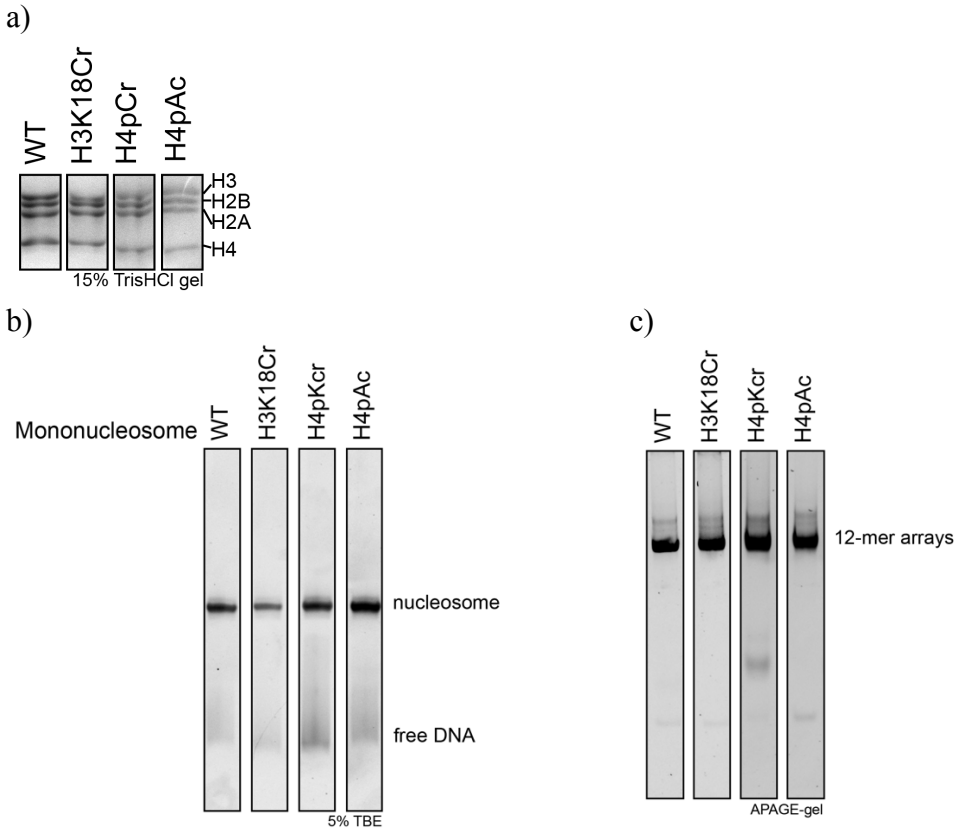
b)
Probe **4-ox**



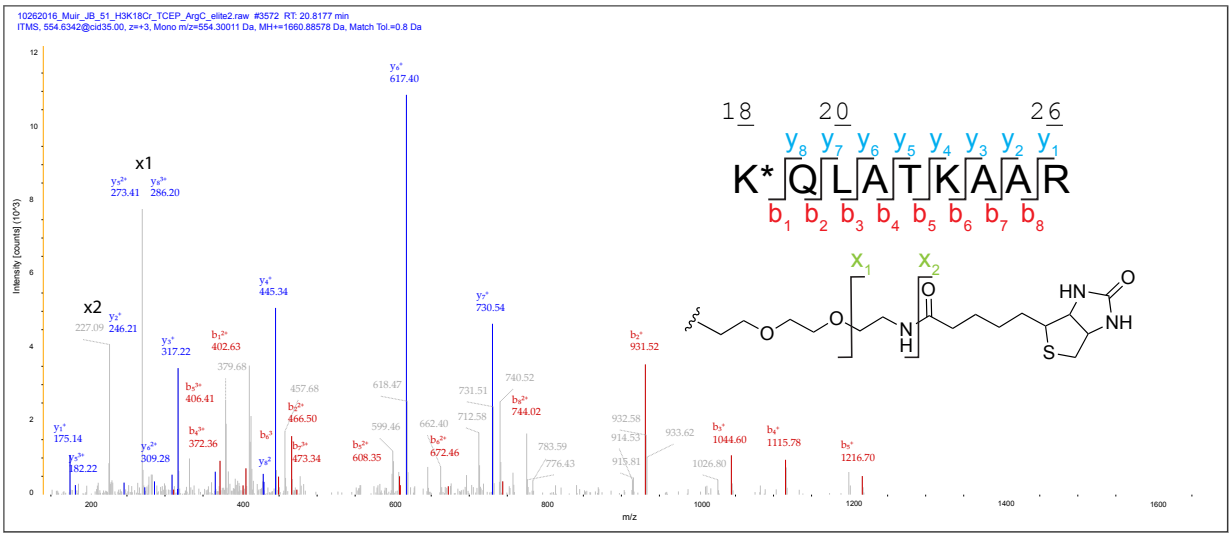
Supplementary Figure S15: a) HPLC traces of probe **4** (0-73% MeCN from 5-35min, H_2O / MeCN (0.1% TFA). b) HPLC trace (LC-MS) of probe **4-ox** (0-73% MeCN from 5-35min, H_2O / MeCN (0.1% FA).



Supplementary Figure S16: Characterization of semi-synthetic histones. a-b) Analytical C18 RP-HPLC chromatograms of purified H3K18Cr and H4pKCr histones, respectively (0-73% MeCN from 5-35min). c-d) ESI-MS of purified H3K18Cr and H4pKCr histones, respectively (deconvoluted masses are depicted in the inset).

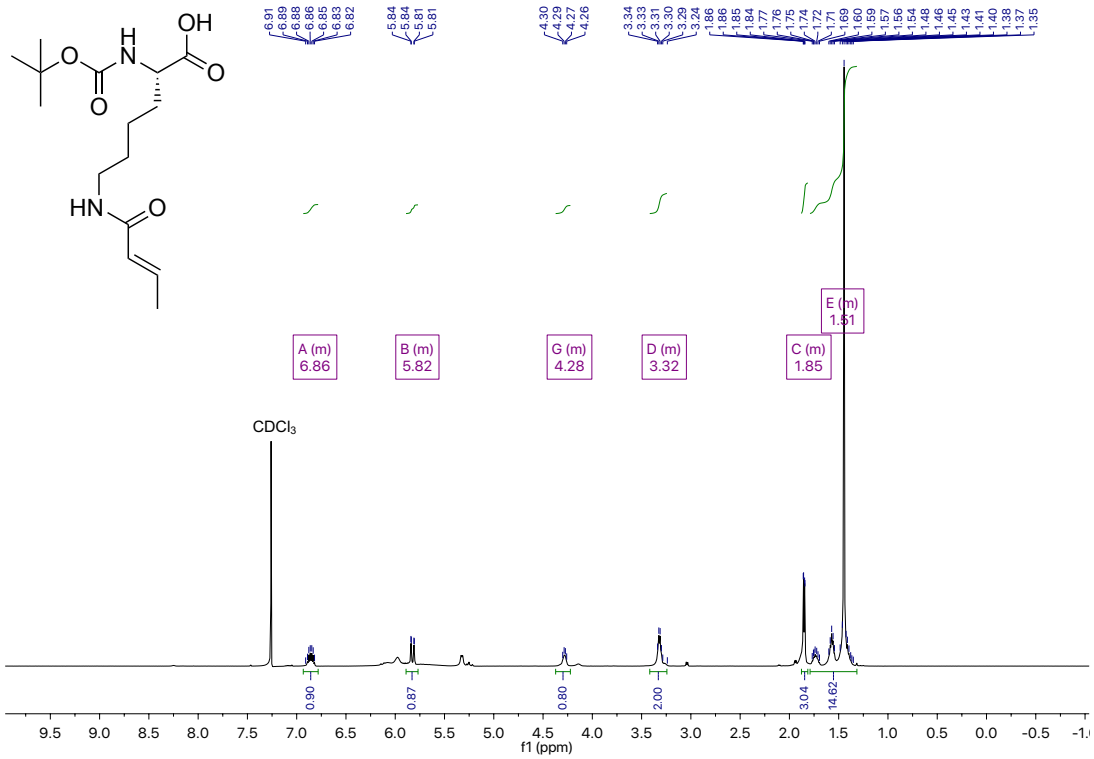


Supplementary Figure S17: a) SDS-PAGE analysis of WT, H3K18Cr, H4pCr and H4pAc containing histone octamers. Samples were analyzed on a 15% TrisHCl gel (stained with coomassie blue). b) Native gel analysis of WT, H3K18Cr, H4pKCr, and H4pAc mononucleosomes (5% TBE gel, stained with SYBR™ Gold). c) APAGE analysis of WT, H3K18Cr, H4pKCr and H4pAc 12-mer arrays (1% agarose / 2% polyacrylamide APAGE gels, stained with SYBR™ Gold).

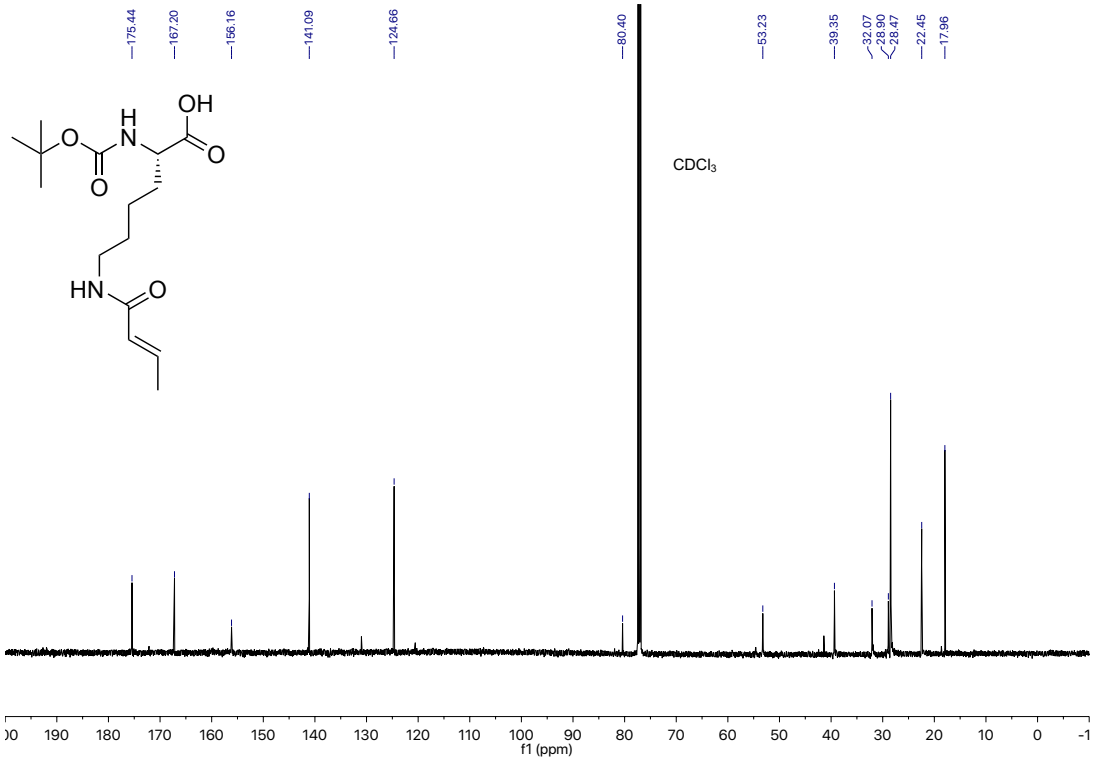


Supplementary Figure S18: Fully Annotated spectra in Figure 2 (main text).

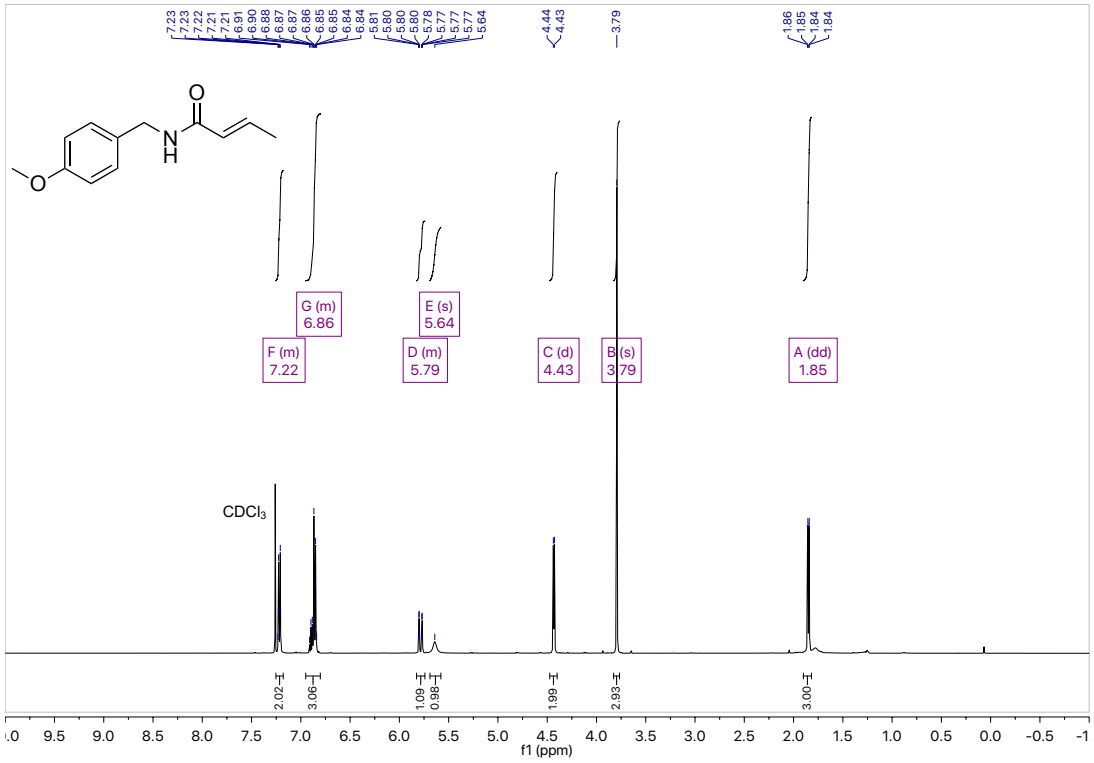
^{1}H-NMR ($CDCl_3$, 500 MHz) **2a**



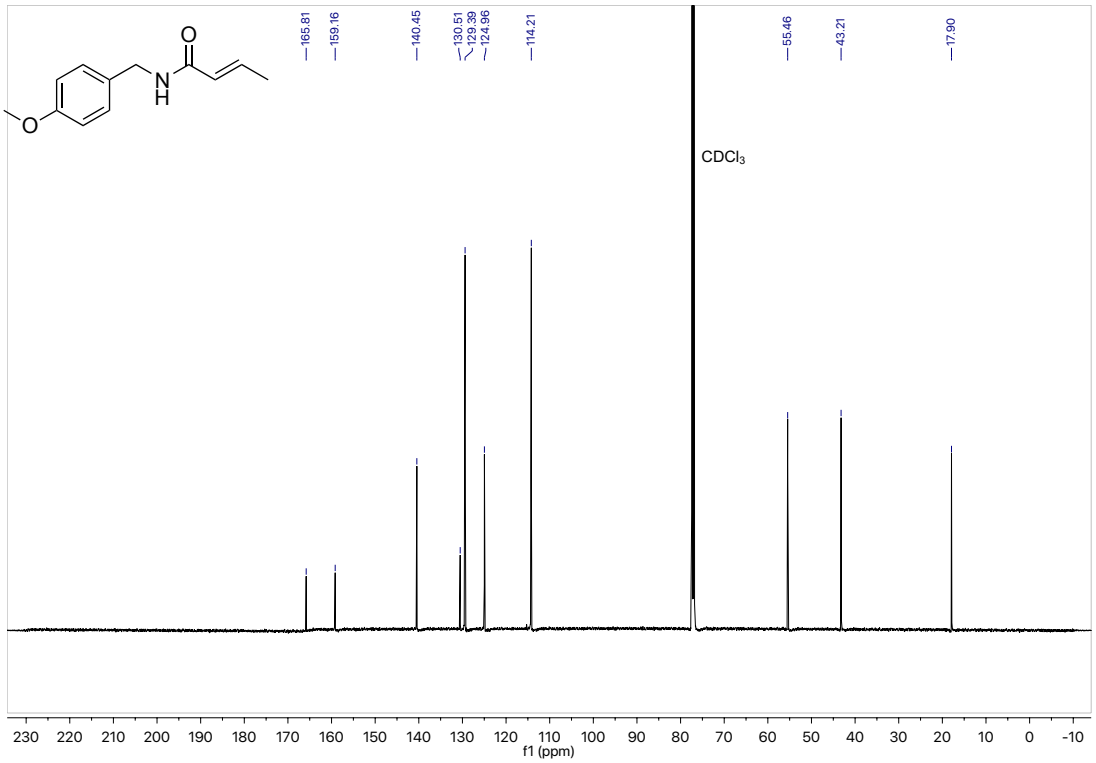
^{13}C-NMR ($CDCl_3$, 500 MHz) **2a**



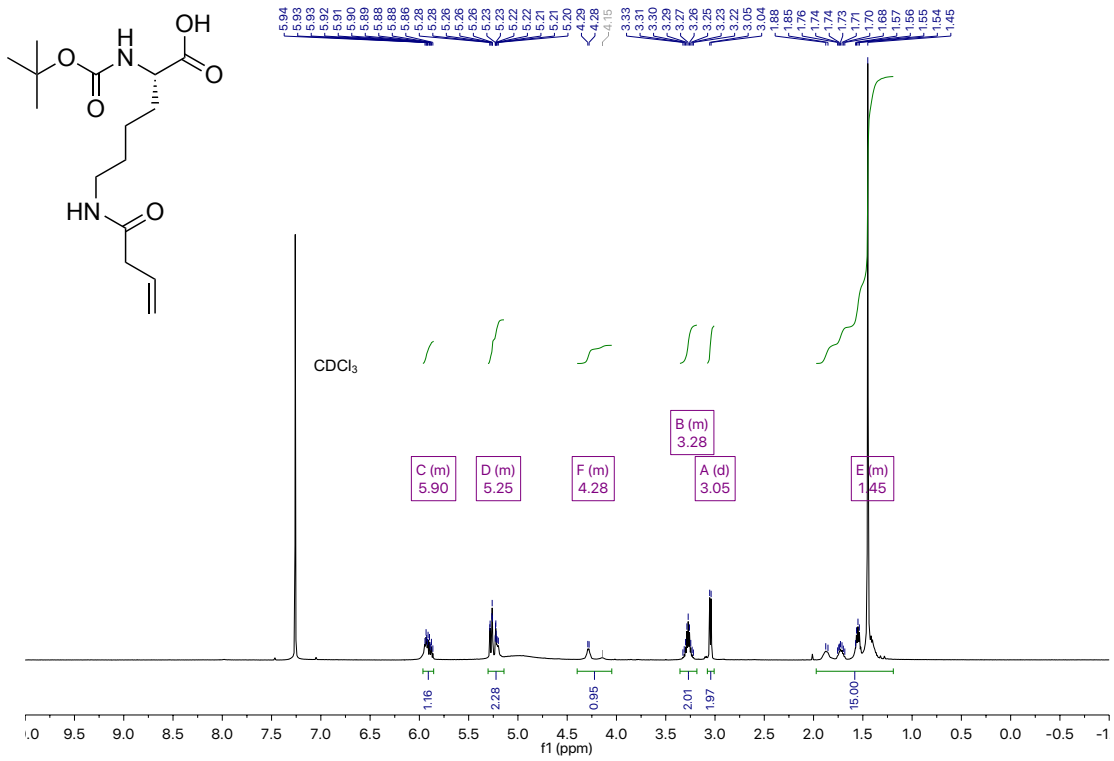
^{1}H-NMR ($CDCl_3$, 500 MHz) **2b**



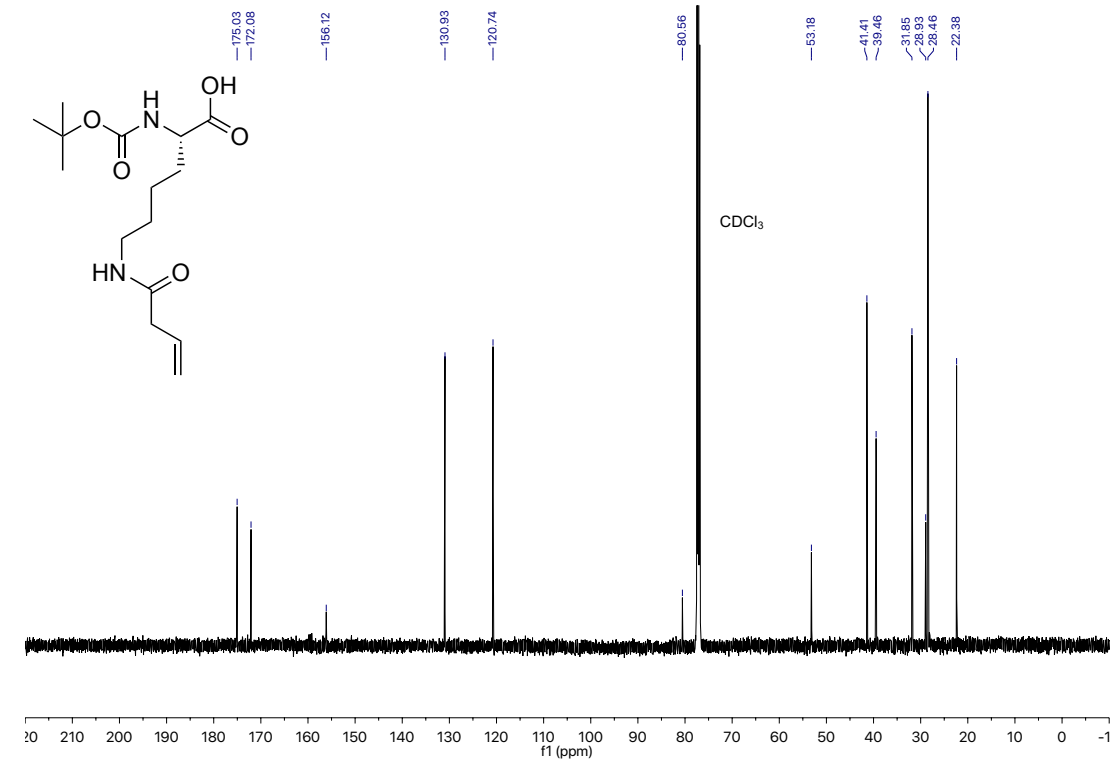
^{13}C-NMR ($CDCl_3$, 500 MHz) **2b**



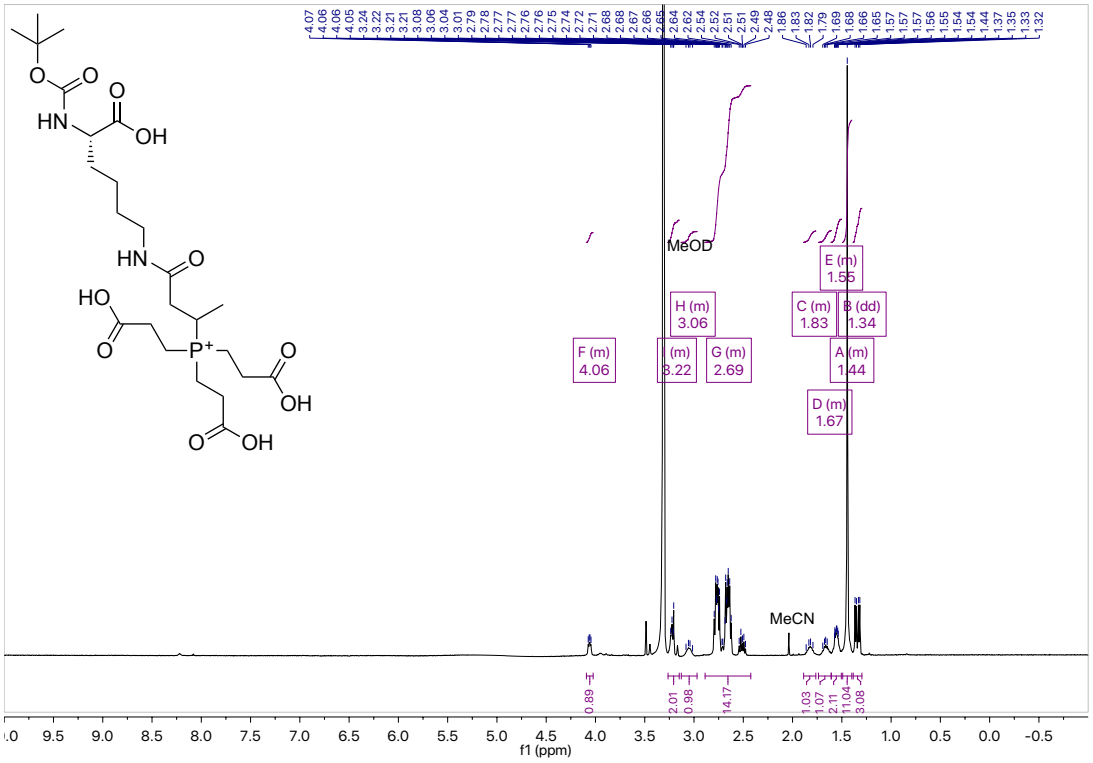
^{1}H-NMR ($CDCl_3$, 500 MHz) **2d**



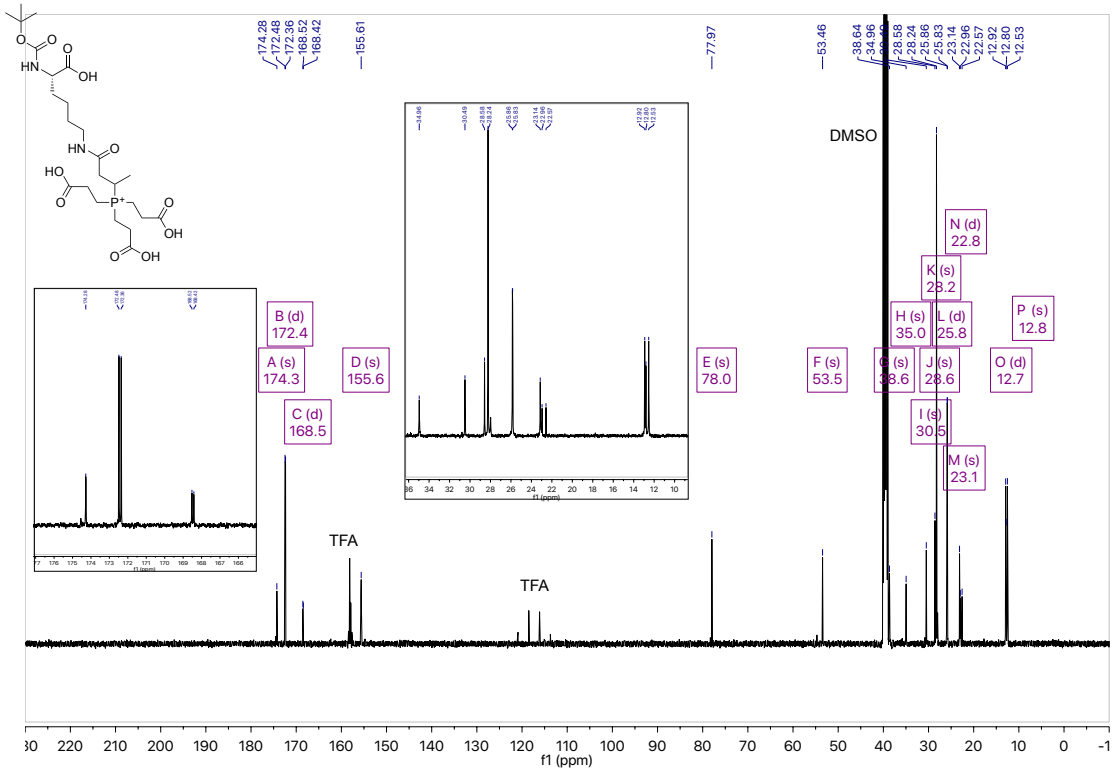
^{13}C-NMR ($CDCl_3$, 500 MHz) **2d**



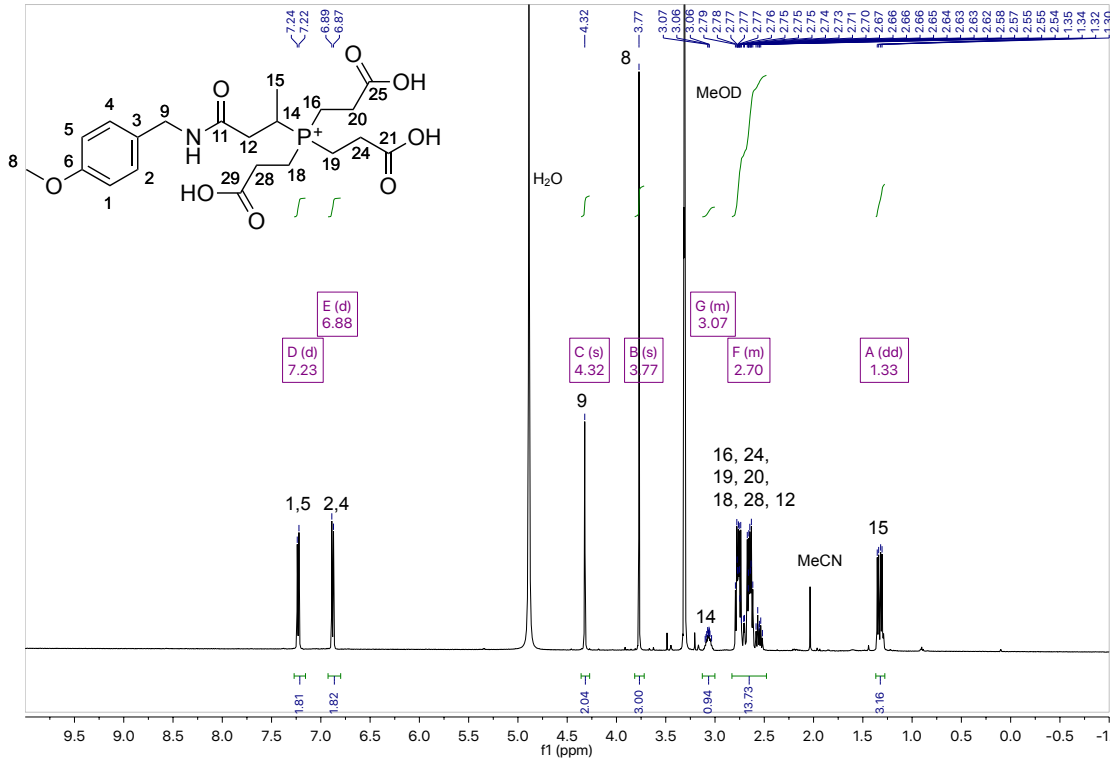
^{1}H-NMR ($CDCl_3$, 500 MHz) **3a**



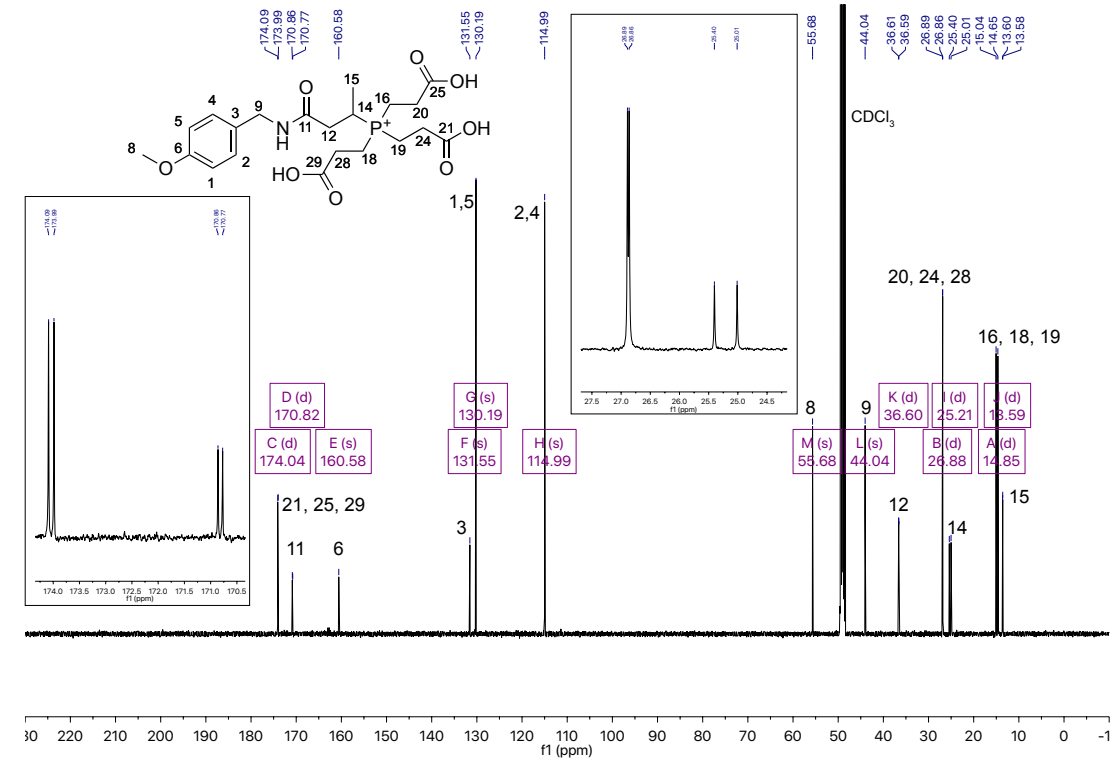
^{13}C-NMR ($CDCl_3$, 500 MHz) **3a**



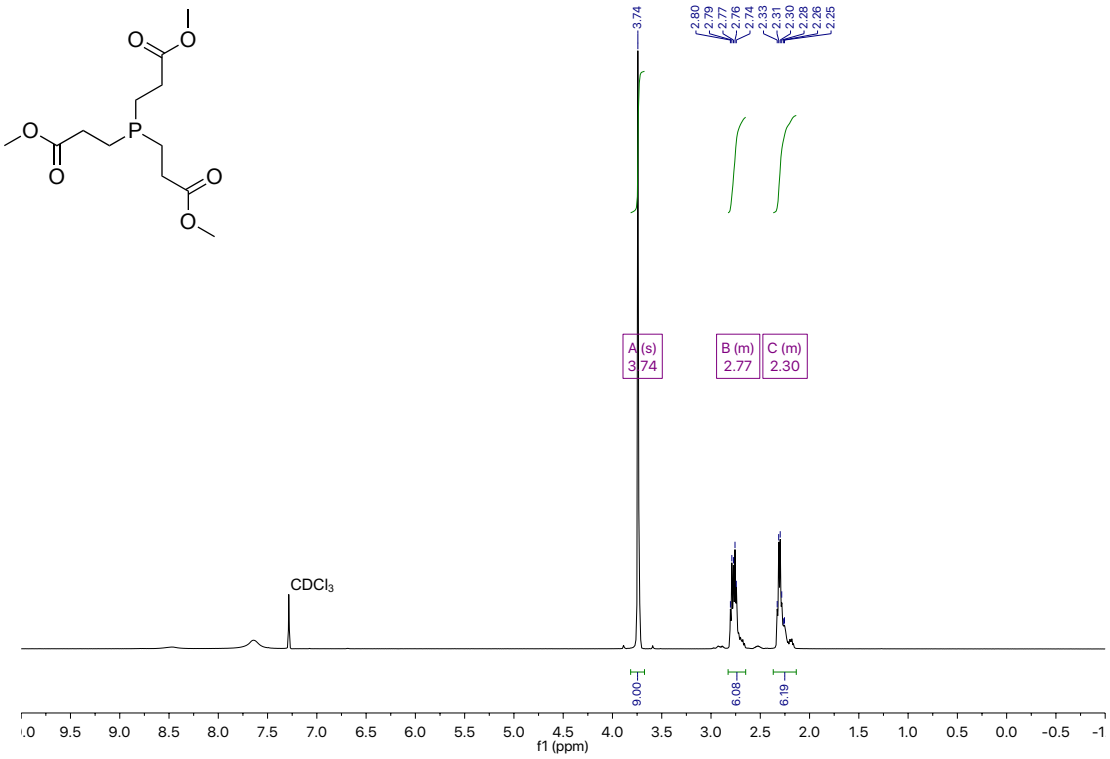
^{1}H-NMR ($CDCl_3$, 500 MHz) **3b**



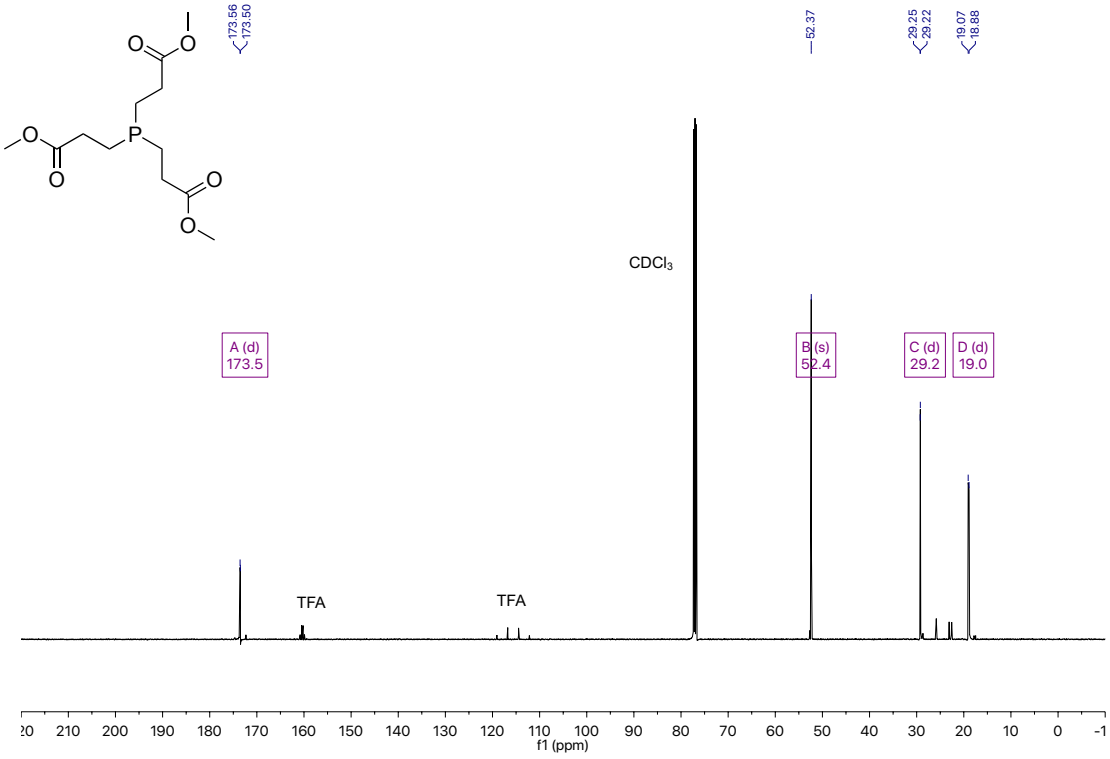
^{13}C-NMR ($CDCl_3$, 500 MHz) **3b**



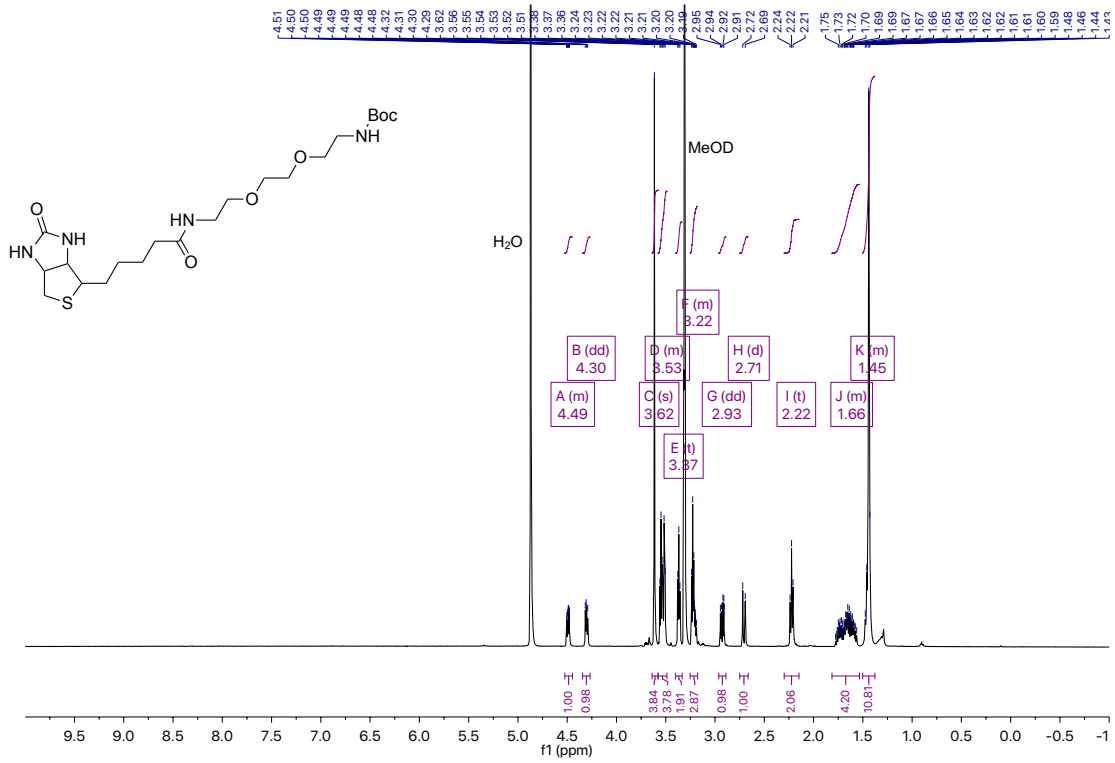
^{1}H-NMR ($CDCl_3$, 500 MHz) **1c**



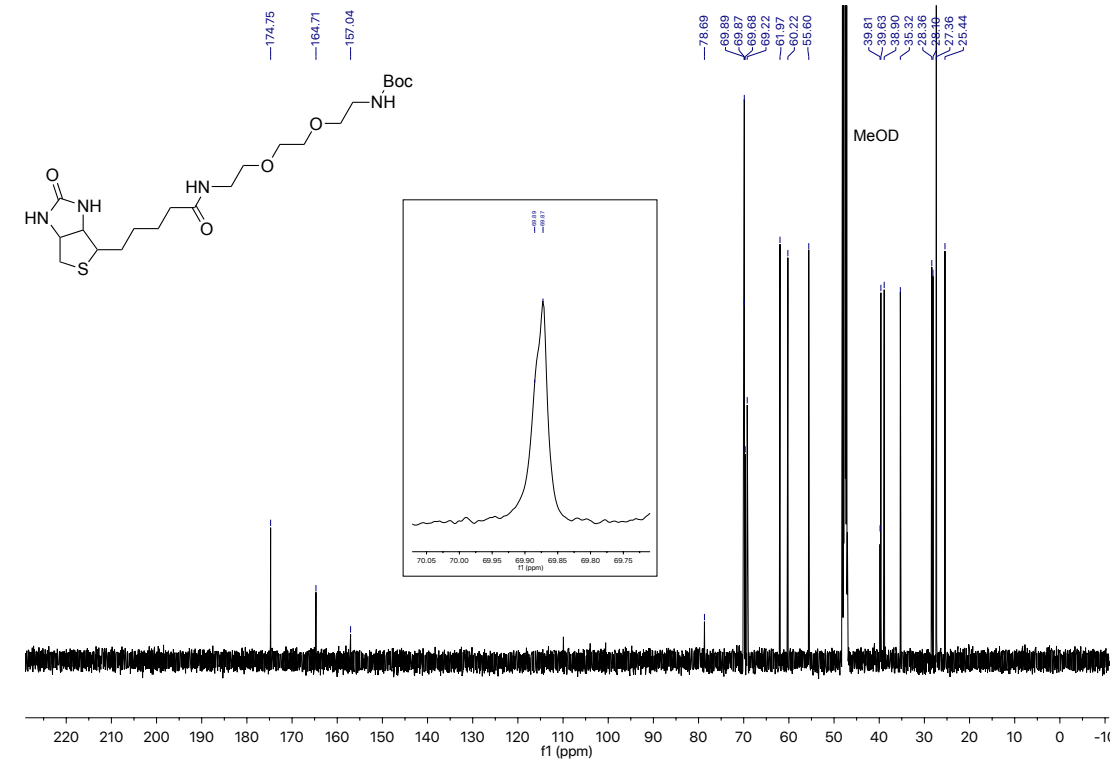
^{13}C-NMR ($CDCl_3$, 500 MHz) **1c**



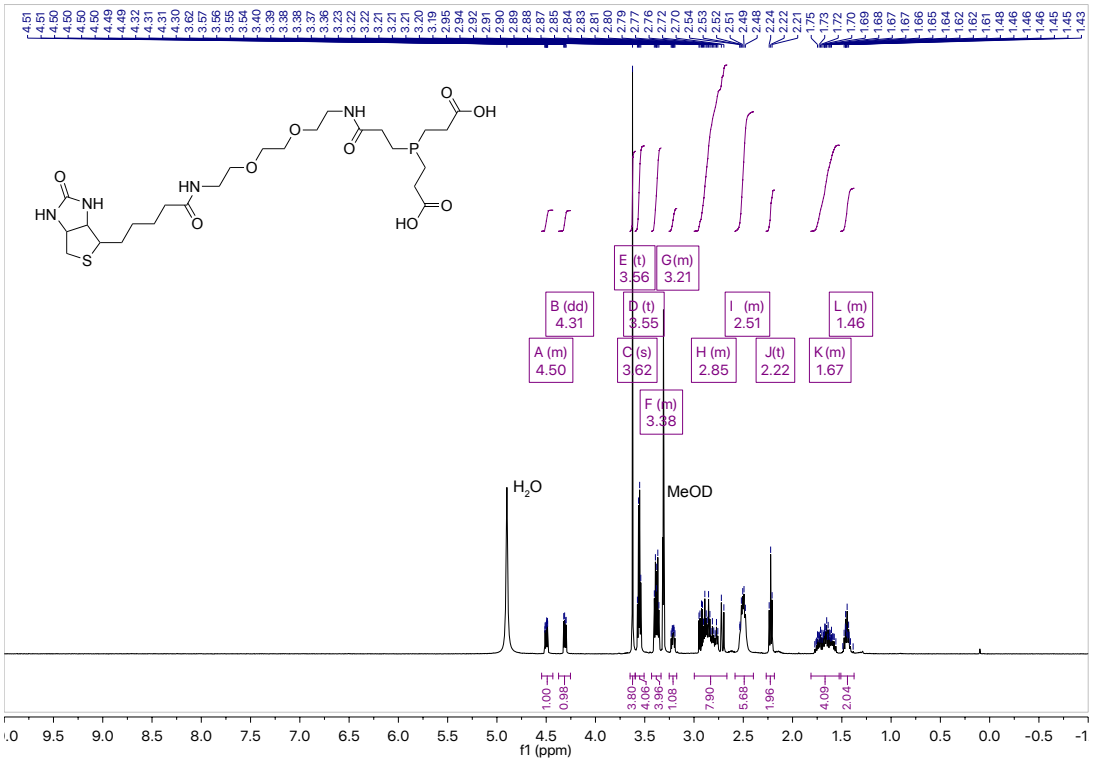
^{1}H-NMR (MeOD, 500 MHz) **5**



^{13}C-NMR (MeOD, 500 MHz) **5**



^{1}H-NMR (MeOD, 500 MHz) **4**



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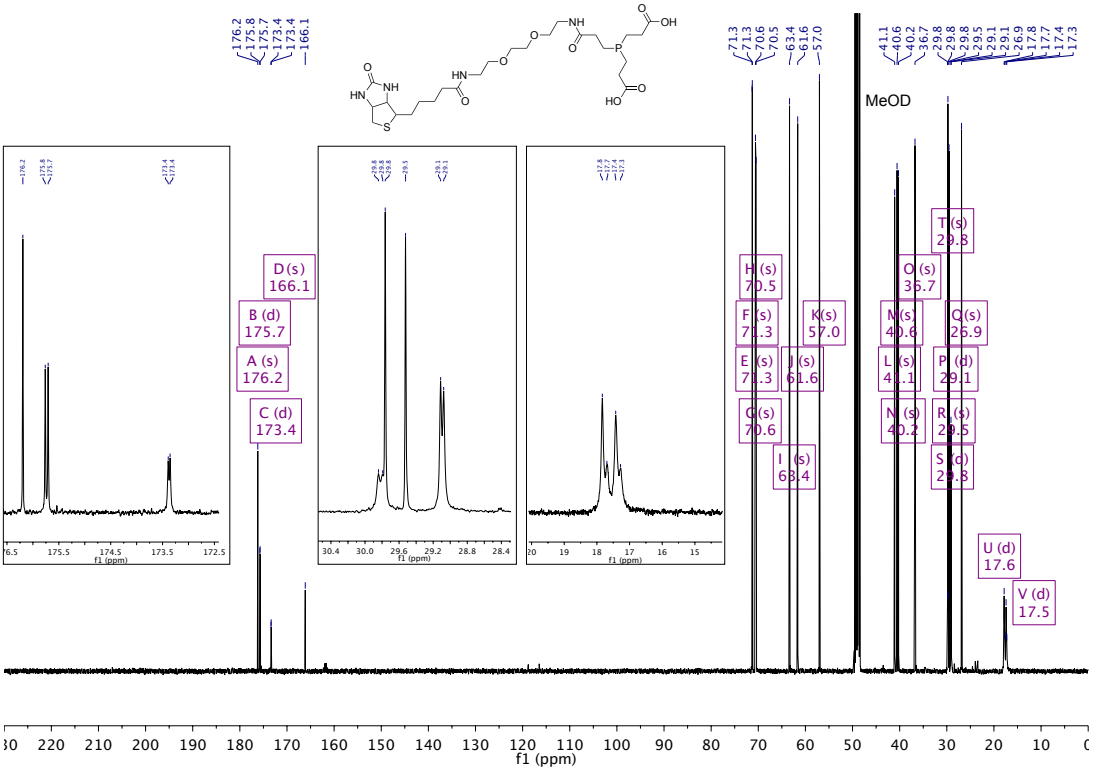

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