Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Direct access of site-specifically phosphorylated-lysine peptides from solid-support

Supporting Information

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List of abbreviations

NMR	nuclear magnetic resonance
MHz	Megahertz
FTIR	fourier transform infrared spectroscopy
HPLC	high performance liquid chromatography
UV	ultraviolet
RP	reversed-phase
FLD	fluorescence detection
MeCN	acetonitrile
TFA	trifluoroacetic acid
UPLC	ultra performance liquid chromatography
Fmoc	fluorenylmethyloxycarbonyl
HBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOBt	1-hydroxybenzotriazole
LC	liquid chromatography
MS	mass spectrometry
ESI	electrospray ionization
TOF	time of flight
LTQ	linear triple quadrupole
ETD	electron transfer dissociation
Et_3N	triethylamine
THF	tetrahydrofuran
$CDCl_3$	chloroform
NaOH	sodium hydroxide
HRMS	high resolution mass spectrometry
HCl	hydrochloric acid
HMBC	heteronuclear multiple bond correlation
DIPEA	*N*,*N*-diisopropylethylamine
HATU	1-[bis(dimethylamino)-methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HMBA	hydroxymethylbenzoic acid
TG	tentagel
DMF	dimethylformamide
TIS	triisopropylsilane
DCM	dichloromethane
D_2O	deuterium oxide

1 General Information

Materials: All reagents, starting materials, amino acids, and solvents were purchased from commercial suppliers and used without further purification if not further mentioned.

NMR: ^{1}H, ^{13}C, and ^{31}P-NMR spectra were recorded on a Jeol ECX-400 400 MHz spectrometer, Bruker Ultrashield 300 MHz spectrometer or Bruker Ultrashield AV 600 MHz at ambient temperature. The chemical shifts are reported in ppm relatively to the residual solvent peak.

IR: The spectrum was characterized by a **Bruker Tensor 27 FTIR** spectrometer in the range of 4000-400 cm^{-1}.

Analytical HPLC: HPLC-UV traces were obtained using a Waters 600S controller system, a Waters 616 pump, a Waters 717plus autosampler, a Waters 2489 UV/Visible detector connected to a 3100 mass detector using a Kromasil C18 5µm, 250 x 4.6 mm RP-HPLC column with a flow rate of 1.0 mL/min. The following solvent and gradient was applied for all peptides if not further mentioned: (A = H_2O + 0.1% TFA, B = MeCN + 0.1% TFA) 5% B 0-5 min, 5-100% 5-36 min, 100% B 36-42 min. HPLC-UV chromatograms were recorded at 220 nm. The data was processed by Empower Pro software.

Analytical UPLC: UPLC-UV traces were obtained using a Waters H-class instrument, equipped with a Quaternary Solvent Manager, a Waters autosampler, a Waters TUV detector connected to a 3100 mass detector using a Acquity UPLC-BEH C18 1.7 µM 2.1x50mm RP column with a flow rate of 0.6 mL/min. The following solvent and gradient was applied for all peptides if not further mentioned: (A = H_2O + 0.1% TFA, B = MeCN + 0.1% TFA) 1% B 0-5 min, 1-99% 5-15 min, 99% B 15-17 min. UPLC-UV chromatograms were recorded at 220 nm.

Solid-phase peptide synthesis: Peptides were synthesized with an ABI 433A Peptide Synthesizer (Applied Biosystems) via standard Fmoc-based conditions (Fast-Fmoc protocol with HOBt/HBTU conditions) on a Novasyn TG HMBA resin with a loading of 0.22 mmol/g.

Semi-preparative HPLC purification: Phosphoramidate ester peptides were purified on a JASCO LC-2000 system using a reversed phase Kromasil C18 column (25 x 250 mm) at a flow rate of 16 mL/min using a TFA gradient (0% B 0-5 min; 0-100% B 5-55 min; 100% B 55-60 min) in water/acetonitrile system (A = H_2O + 0.1% TFA, B = MeCN + 0.1% TFA). Phosphorlylated lysine peptide was purified on a Shimadzu HPLC system using a reversed phase Nucleodur C18 HTec column (10 x 250 mm) at a flow rate of 3 mL/min using an acetonitrile/water gradient (0% B 0-5 min; 0-40% B 5-55 min; 40-100% B 55-85 min) in alkaline aqueous buffer (pH 8.2) as a mobile phase (A = 10 mM Ammonium Acetate in H_2O, B = MeCN + 10 % of sol. A).

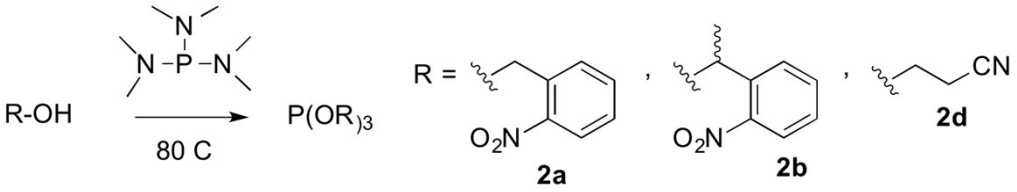
High-resolution mass spectra (HRMS): Characterization of phosphites and peptides were done with an Agilent 6210 ToF LC/MS system.

ETD Tandem MS analysis: For LC-MS analysis, peptides were dissolved in 6 μL water (7.1 μM) and analyzed by a reversed-phase capillary liquid chromatography system (Dionex Ultimate 3000 NCS-3500RS Nano, Thermo Scientific) connected to an LTQ Orbitrap XL mass spectrometer (Thermo Scientific) equipped with an ETD module (Thermo Scientific). LC separations were performed on a capillary column (Acclaim PepMap C18, 2 μm 100 Å, 250 mm × 75 μm i.d., Dionex) at an eluent flow rate of 300 nl/min using a gradient of 2–23% B in 20 min. Mobile phase A contained 0.1% formic acid in water, and mobile phase B contained 0.1% formic acid in acetonitrile. Mass spectra were acquired in a data-dependent mode with one MS survey scan (with a resolution of 30,000) in the Orbitrap and ETD scans of the phosphorylated peptide precursor ions in the Orbitrap mass analyzer at a resolution of 7,500. Triple charged peptide ions were fragmented using an include list. Activation time for ETD was 70 ms. Before ETD experiments, the ETD source was tuned to optimal signal of fluoranthene anions. MS spectra and ETD fragment ion spectra of phosphopeptides were manually verified and compared with the theoretical fragment ions phosphorylated lysine peptides considering all possible phosphorylation sites.

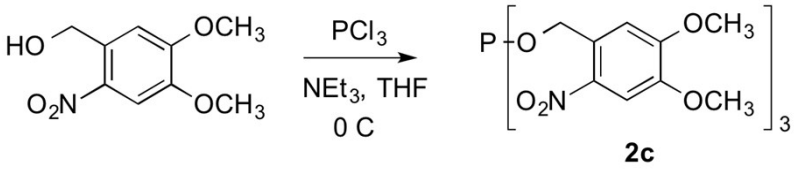
2 Synthesis of phosphites

2-nitrobenzyl alcohol and 4,5-dimethoxy-2-nitrobenzyl alcohol were purschased from Sigma Aldrich. Phosphite **2a** was synthesized by the condensation of hexamethyltriphosphorous triamide and the alcohol.[1] Phosphites **2b** and **2d** were synthesized as previously described.[2] Phosphite **2c** was synthesized as described in Scheme S2. Acros silica gel (60 A, 0.035-0.070 mm) was used as stationary phase for normal phase chromatography.

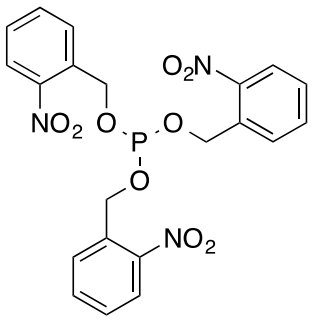
Scheme S1. Synthesis of phosphites **2a, 2b and 2d**



Scheme S2. Synthesis of phosphites **2c**



2.1 Synthesis of tris(2-nitrobenzyl) phosphite (2a)

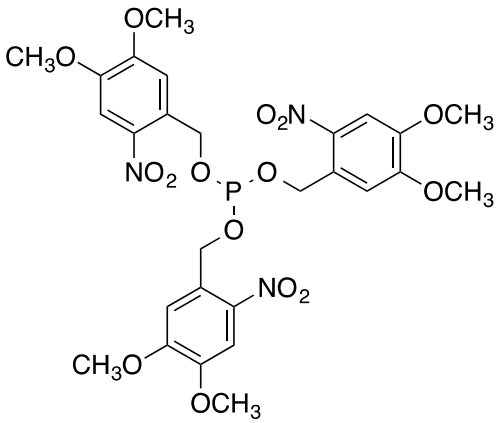


Chemical Formula: $C_{21}H_{18}N_3O_9P$
Exact Mass: 487.0781
Molecular Weight: 487.3608

Hexamethylphosphorous triamide (1.0 mL, 0.89 g, 5.4 mmol, 1eq) was added to 2-nitrobenzyl alcohol (2.5 g, 16.3 mmol, 3 eq) at 80 °C. The mixture was stirred under argon stream at 100 °C for 3 h. Compound **2a** (1.38 g, 2.83 mmol, 53%) was obtained as a orange powder after column chromatography (1% Et_3N, hexane/ethyl acetate: 7/3).

^{1}H NMR (400 MHz, $CDCl_3$-d1) δ = 8.11-8.09 (d, *J* = 8.3 Hz, 3H), 7.80-7.78 (d, *J* = 7.4 Hz, 3H), 7.67-7.63 (t, *J* = 7.7 Hz, 3H), 7.47-7.43 (t, *J* = 7.7 Hz, 3H), 5.38-5.36 (d, *J* = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, CDCl3-d1) δ = 146.6, 134.4 (d), 133.9, 128.4, 128.2, 124.8, 61.59 (d). ^{31}P NMR (161 MHz, $CDCl_3$-d1) δ = 140.34. HRMS for $C_{21}H_{18}N_3NaO_9P$ $[M+Na]^+$ calcd: 510.0673, found: 510.0673. FT-IR: νmax (neat, cm-1): 2359 (s), 2342 (s), 1518 (s), 1447 (w), 1435 (w), 1195 (w), 1014 (m), 986 (s), 859 (m), 843 (m), 816 (s), 788 (s), 722 (s), 668 (m). R_f (hexane/ethyl acetate: 4/1) = 0.3.

2.2 Synthesis of tris(4,5-dimethoxy-2-nitrobenzyl) phosphite (2c)



Chemical Formula: $C_{27}H_{30}N_3O_{15}P$
Exact Mass: 667.1415
Molecular Weight: 667.5168

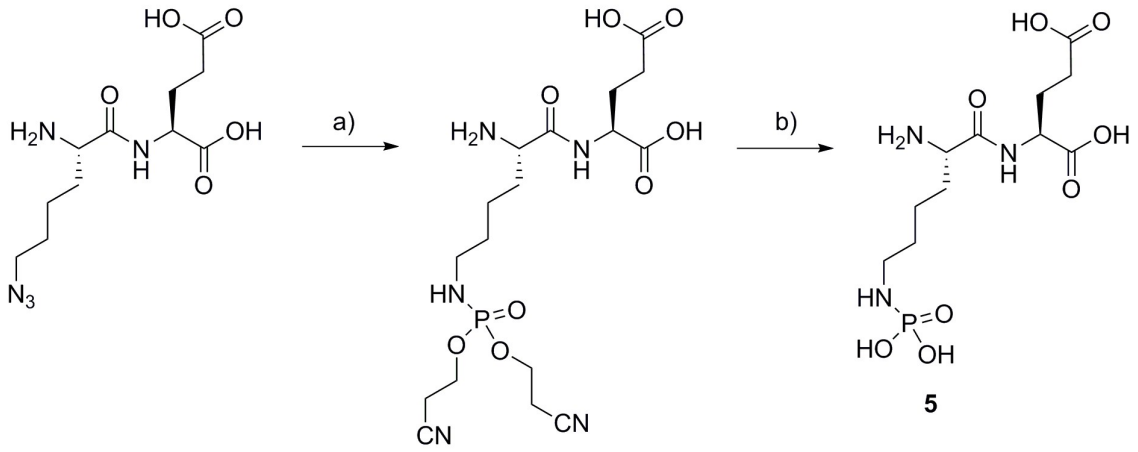
Phosphorus trichloride (0.11 mL, 0.17 g, 1.25 mmol, 1eq) was dissolved in dry THF (7 ml). The reaction was cooled to 0 ºC and Et_3N was added slowly (0.70 mL, 5.0 mmol, 4 eq). Then, a solution of (4,5-dimethoxy-2-nitrophenyl)methanol (0.8 g, 3.75 mmol, 3eq) in THF (12 mL) was added dropwise for 20 min. The reaction was stirred for 2h at 0 ºC and it was allowed to come to room temperature and stir overnight. Compound **2c** (0.38 g, 1.5 mmol, 45%) was obtained as a yellow powder after column chromatography (1% Et_3N, toluene/ethyl acetate: 7/3).

^{1}H NMR (400 MHz, $CDCl_3$-d1) δ = 7.68 (s, 3H), 7.25 (s, 3H), 5.42-5.40 (d, *J* = 7.0 Hz, 6H), 3.94 (s, 18H). ^{13}C NMR (100 MHz, MeCN-d3) δ = 154.1, 148.0, 139.0, 129.9 (d), 109.4, 108.0, 61.9 (d), 56.6 (d). ^{31}P NMR (161 MHz, $CDCl_3$-d1) δ = 139.18. HRMS for $C_{27}H_{30}N_3O_{15}P$ $[M+Na]^+$ calcd: 690.1307, found: 690.1307. FT-IR: νmax (neat, cm-1): 2361 (m), 2341 (w), 1580 (w), 1525 (s), 1505 (m), 1326 (m), 1273 (s), 1218 (s), 1065 (s), 979 (s), 866 (w), 797 (m). R_f (toluene/ethyl acetate: 1/1) = 0.7.

3 Stability of the P(=O)-N bond under neutral and basic conditions

Procedure for the synthesis of substrate 5: Phosphorylated dipeptide **5** was synthesized as previously described.[2] After the identification of the product **5** by 1H, 31P HMBC-NMR, the free phosphoramidate dipeptide **5** was used without further purification to check the stability of the P-N under slightly and harsh basic conditions.

Scheme S3. Synthesis of phosphorylated lysine dipeptide **5**.



Reagents and Conditions: a) 5 eq of phosphite **2d** in DMF, 24 h, 45 ºC. Subsequent addition was needed. b) 250 mM NaOH , 20 min and then 250 mM HCl

Determination of the phosphorylation decay by NMR: Aliquots of dipeptide **5** (0.4 mM) were dissolved in ammonium acetate salt (10 mM) at pH 8.2 and pH 10.3. For the pH 13.2 measurement, the aliquots were dissolved in 220 mM NaOH. To monitor the stability, the ε-methylene protons that coupled to ^{31}P nuclei were integrated versus the ε-methylene protons of lysine. Experiments were run for 16h.

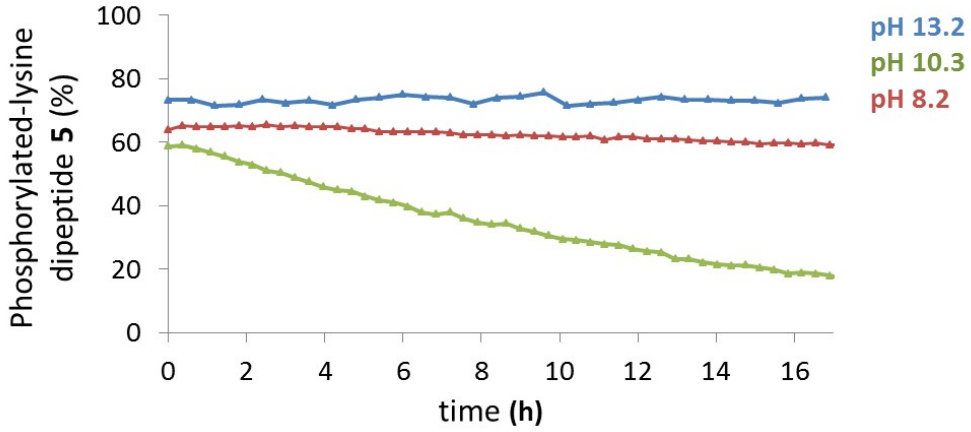


Figure S1. Stability of phosphoramidate bond under slightly and harsh basic conditions monitored by 1H-NMR

^{1}H-NMR

10 mM NH_4Ac pH 8.2

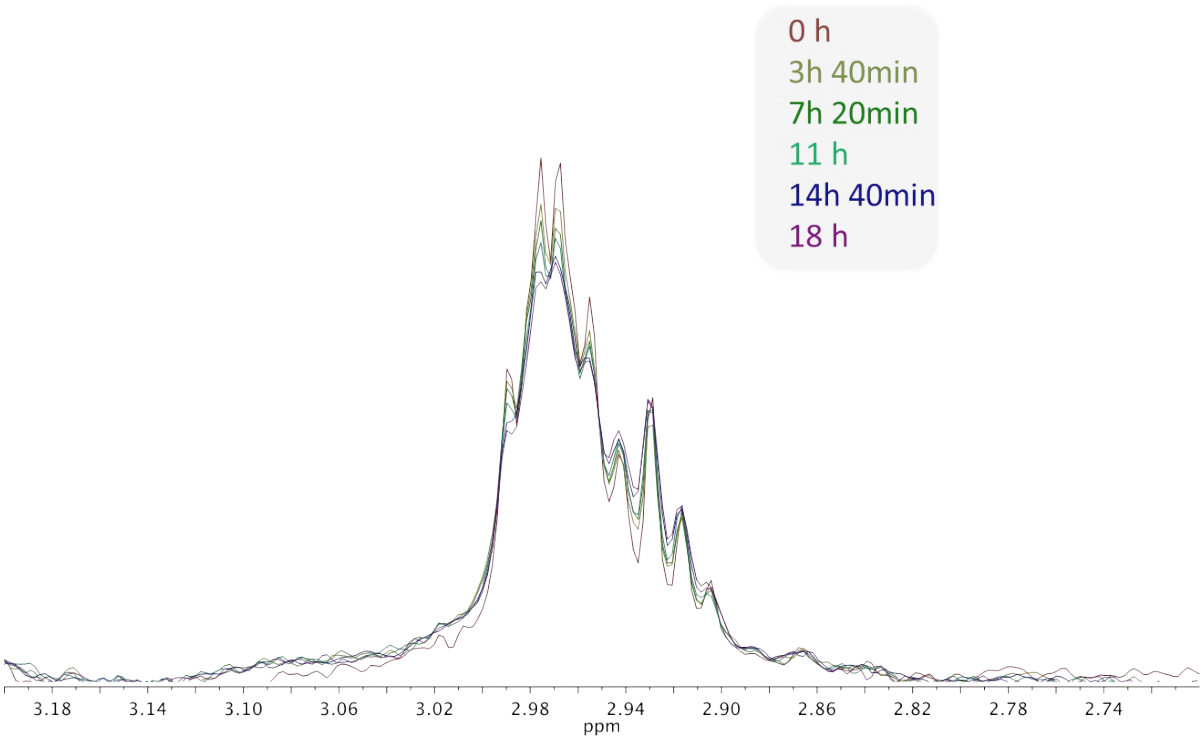


Figure S2. ^{1}H-NMR showing the signal of ε-CH_2 for phospholysine (3-2.95 ppm) and lysine (2.95-2.90 ppm). No changes in signal intensity can be observed over a time of 16 hours.

^{1}H-NMR

10 mM NH_4Ac pH 13.2

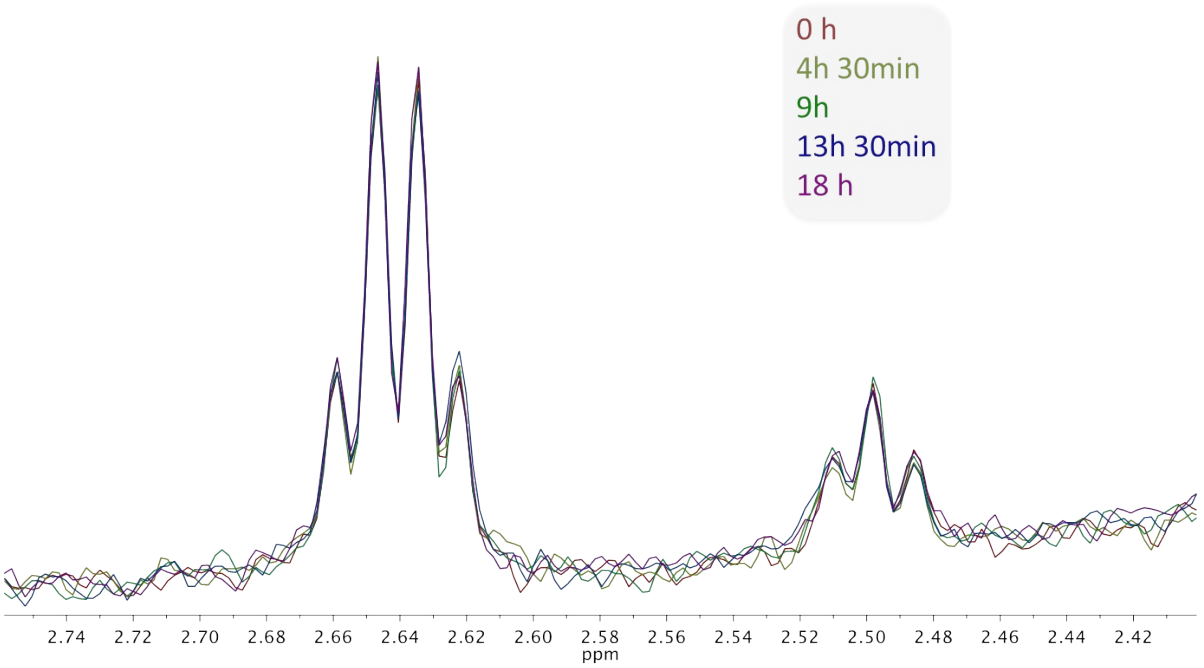


Figure S3. ^{1}H-NMR showing the signals of ε-CH_2 for phospholysine (2.67-2.62ppm) and lysine (2.52-2.48 ppm). No changes in signal intensity can be observed over a time of 16 hours.

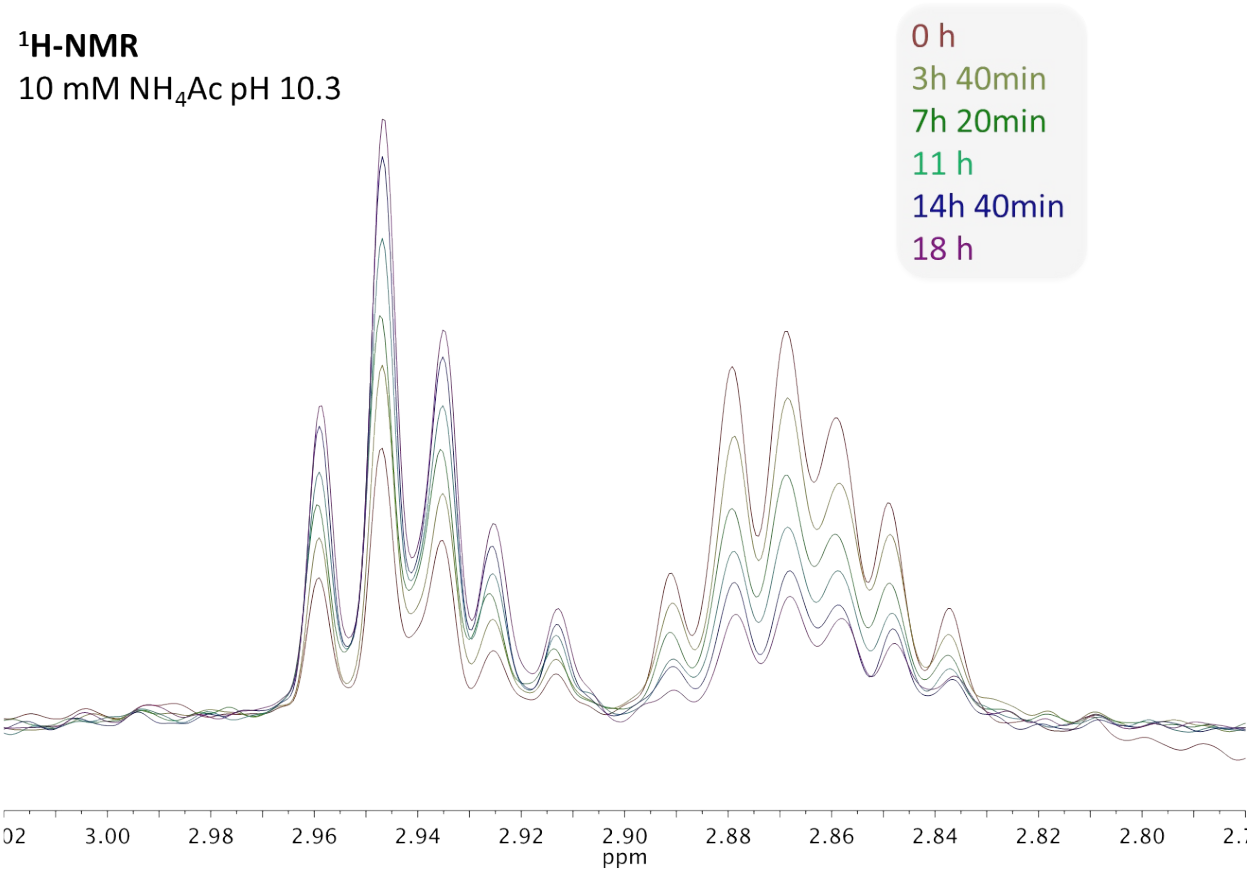


Figure S4. ^{1}H-NMR showing the signals of ε-CH_2 for phospholysine (2.90-2.83 ppm) and lysine (2.96-2.91 ppm). Under these conditions a decay of phospholysine can be observed via the rise of the lysine signals and the decay of the phospholysine signal.

4 Solid-phase synthesis of *o*-nitrobenzyl protected phosphoramidates peptides

4.1 Azidolysine peptide synthesis on the solid support

General procedure: A small amount of beads of Novasyn TG HMBA resin peptide **1a** (30 mg, 0.005 mmol) were incubated with TFA cocktail (2 mL 95% TFA, 5% TIS) for 2h. The beads were washed with 10% DIPEA in DCM, DCM and DMF. The peptide beads were cleaved off the resin with 250 mM NaOH in dioxane at 0 ºC for 20 min. After neutralization with HCl, the peptide was lyophilized. The peptide was analyzed by HPLC-UV. Identity of the product was confirmed using MS.

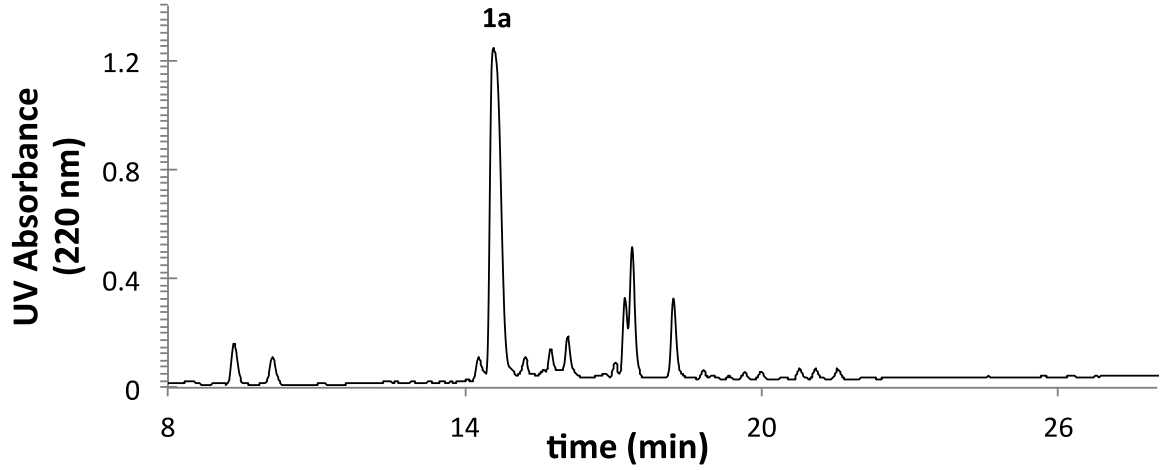
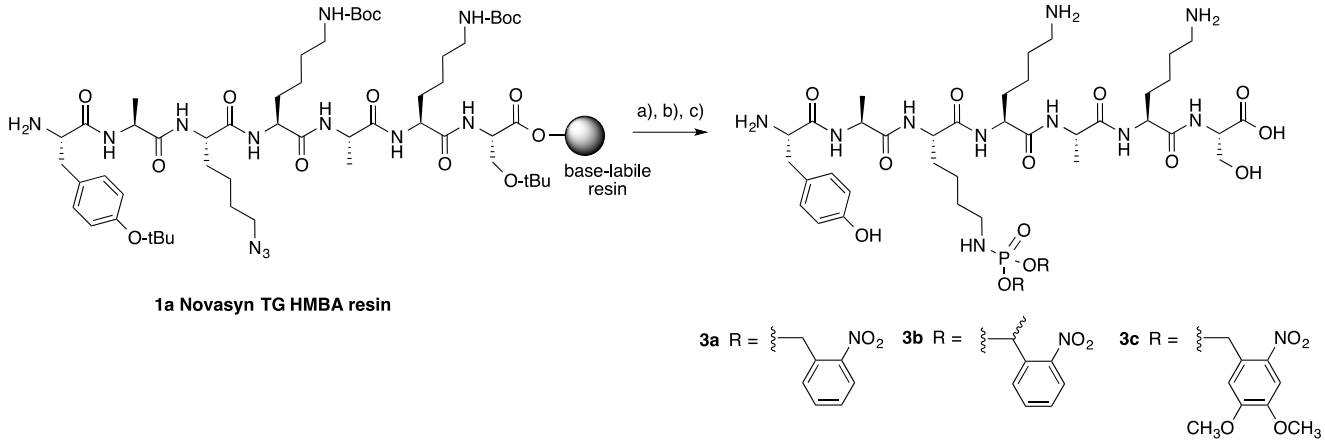


Figure S6. HPLC-UV trace of the crude peptide after NaOH cleavage

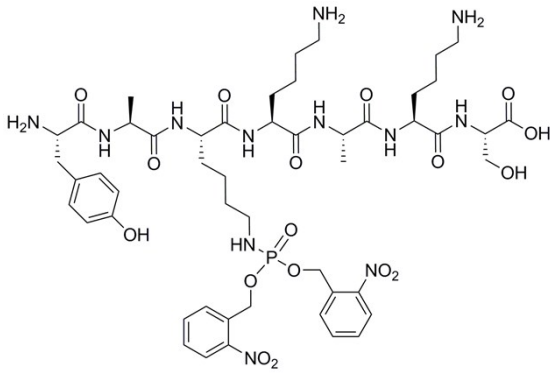
4.2 Staudinger-Phosphite reaction on the solid support

Scheme S4. Staudinger-Phosphite reaction on solid support



Reagents and conditions: a) 2.5 mL 95% TFA, 5% TIS for 2h. b) phosphite **2a**, **2b** or **2c** (2 x 5 eq) in DMF, 24 h - 48 h, 45 ºC c) 1.0 mL of 1M NaOH in dioxane (4 mL) for 20 min at 0 ºC.

N^6-(bis(2-nitrobenzyl)-phosphoramidate lysine peptide **3a**



3a

Chemical Formula: $C_{50}H_{73}N_{12}O_{17}P$
Exact Mass: 1144.4954
Molecular Weight: 1145.1748

Novasyn TG HMBA resin containing ε-azido lysine peptide **1a** (150 mg, 0.025 mmol) was swelled in DMF (400 µL) and a solution of phosphite **2a** (60.9 mg, 0.125 mmol, 5eq) in DMF (100 µL) was added. The reaction mixture was incubated at 45 ºC for 24 h. Then, a fresh solution of phosphite **2a** (60.9 mg, 0.125 mmol, 5eq) in DMF (100 µL) was added and the mixture incubated for another 24 h. Excess phosphite was washed out and the resin was dried in DCM. Peptide was cleaved off the resin with 250 mM NaOH in dioxane at 0 ºC for 20 min. After neutralization with HCl, purification via semi-preparative HPLC yielded peptide **3a** (0.65 mg, 0.57 µmol) in 2% yield; HRMS: m/z: 573.2552 $[M+2H]^{2+}$ (calcd. m/z: 573.2550). See appendix for spectra.

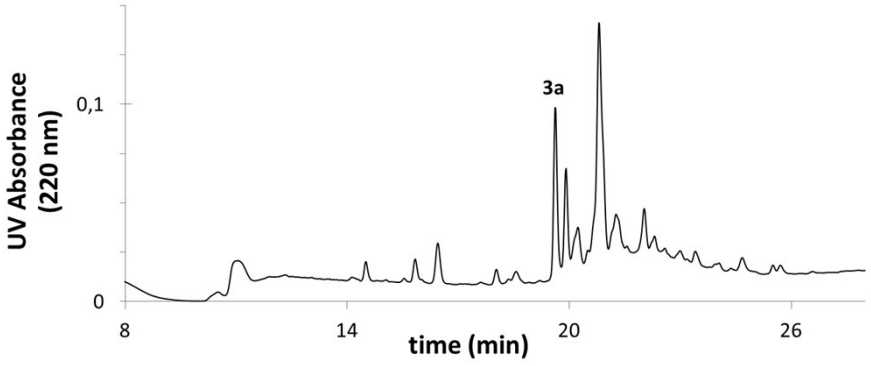


Figure S7. HPLC-UV trace of the crude reaction after NaOH cleavage

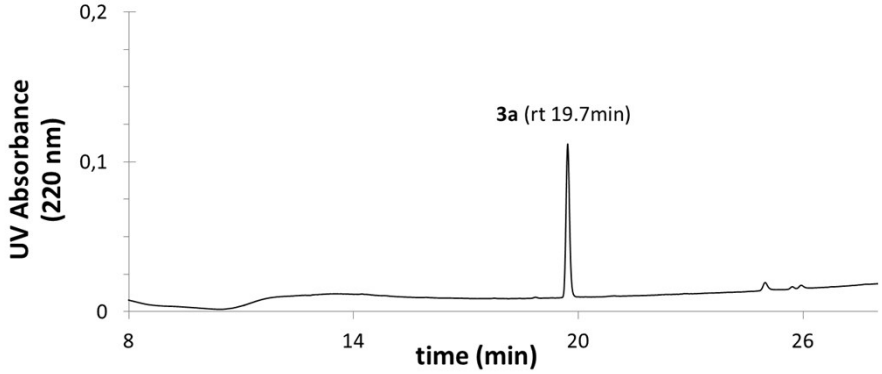
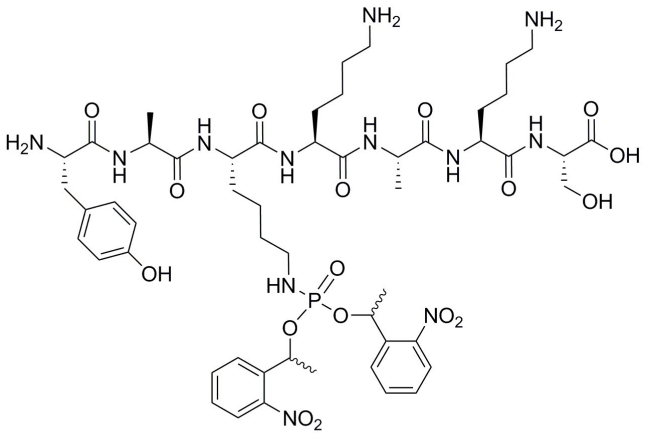


Figure S8. HPLC-UV pure trace of phosphoramidate **3a**

N^6-(bis(1-(2-nitrophenyl)ethoxy))-phosphoramidate lysine peptide **3b**



3b

Chemical Formula: $C_{52}H_{77}N_{12}O_{17}P$
Exact Mass: 1172,52673
Molecular Weight: 1173,22876

Novasyn TG HMBA resin containing ε-azido lysine peptide **1a** (150 mg, 0.025 mmol) was swelled in DMF (400 µL) and a solution of phosphite **2b** (66.1 mg, 0.125 mmol, 5eq) in DMF (100 µL) was added. The reaction mixture was incubated at 45 ºC for 24 h. Then, a fresh solution of phosphite **2b** (66.1 mg, 0.125 mmol, 5eq) in DMF (100 µL) was added and the mixture incubated for another 24 h. Excess phosphite was washed out and the resin was dried in DCM. Peptide was cleaved off the resin with 250 mM NaOH in dioxane at 0 ºC for 20 min. After neutralization with HCl, purification via semi-preparative HPLC yielded peptide **3b** (2.1 mg, 1.8 µmol) in 7% yield; HRMS: m/z: 587.2719 $[M+2H]^{2+}$ (calcd. m/z: 587.2706). ^{31}P-NMR (243 MHz, D_2O at pH 7.5) δ = 9.30, 9.28. 9.27 and 8.28. See appendix for spectra' s.

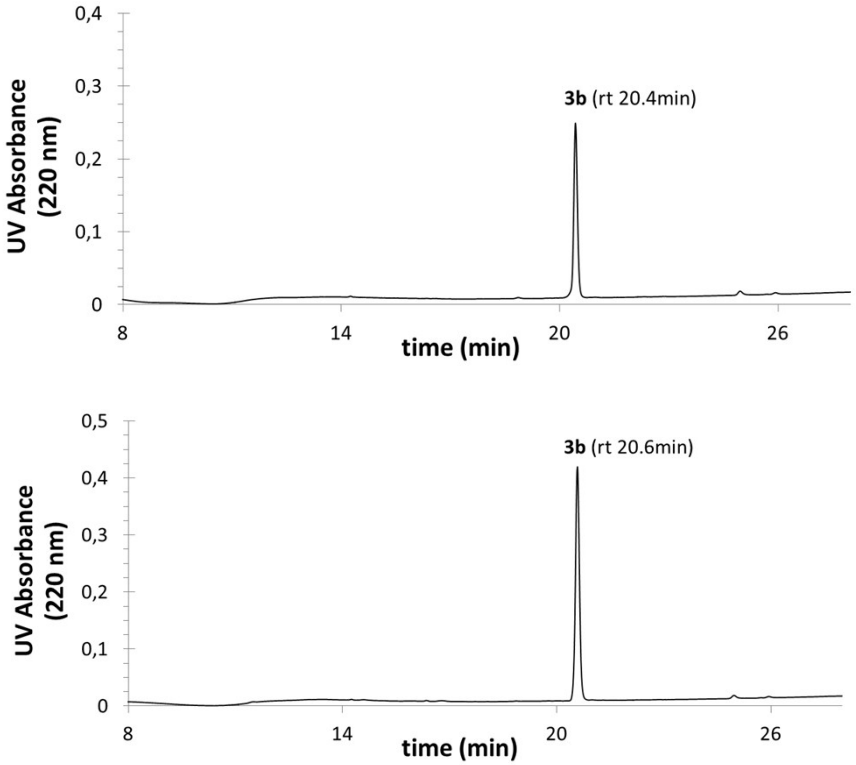
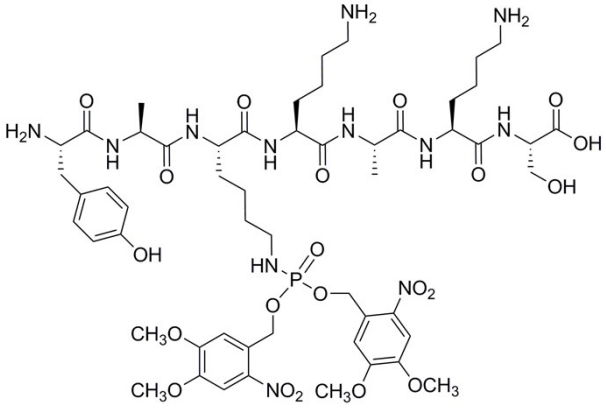


Figure S9. HPLC-UV pure traces of both diastereoisomers formed of phosphoramidate **3b**

N^6-(bis((4,5-dimethoxy-2-nitrobenzyl)) phosphoramidate lysine peptide **3c**



3c

Chemical Formula: $C_{54}H_{81}N_{12}O_{21}P$
Exact Mass: 1264.5377
Molecular Weight: 1265.2788

Novasyn TG HMBA resin containing ε-azido lysine peptide **1a** (42 mg, 7 μmol) was swelled in DMF (200 μL) and a solution of phosphite **2c** (23.3 mg, 70 μmol, 5eq) in DMF (100 μL) was added. The reaction mixture was incubated at 45 ºC for 24 h. Then, a fresh solution of phosphite **2c** (23.3 mg, 70 μmol, 5eq) in DMF (100 μL) was added and the mixture incubated for another 24 h. Excess phosphite was washed out and the resin was dried in DCM. Peptide was cleaved off the resin with 250 mM NaOH in dioxane at 0 ºC for 20 min. After neutralization with HCl, purification via semi-preparative HPLC yielded peptide **3c** (0.25 mg, 0.2 μmol) in 3% yield; HRMS: m/z: 633.2762 $[M+2H]^{2+}$ (calcd. m/z: 633.2761). See appendix for spectra.

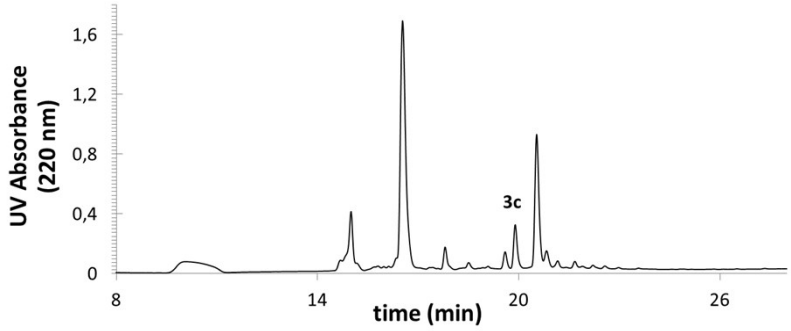


Figure S10. HPLC-UV trace of the crude reaction after NaOH cleavage

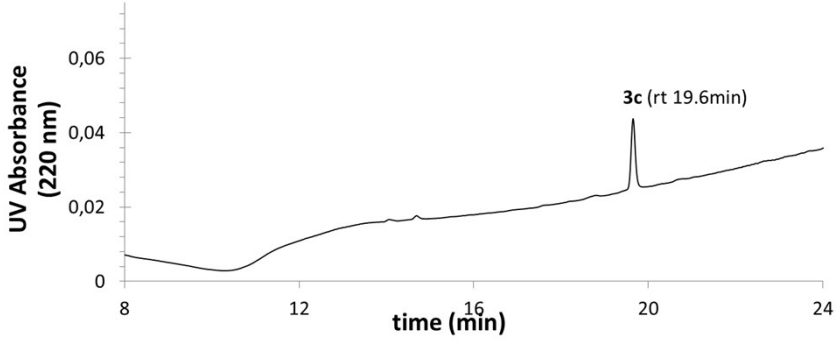
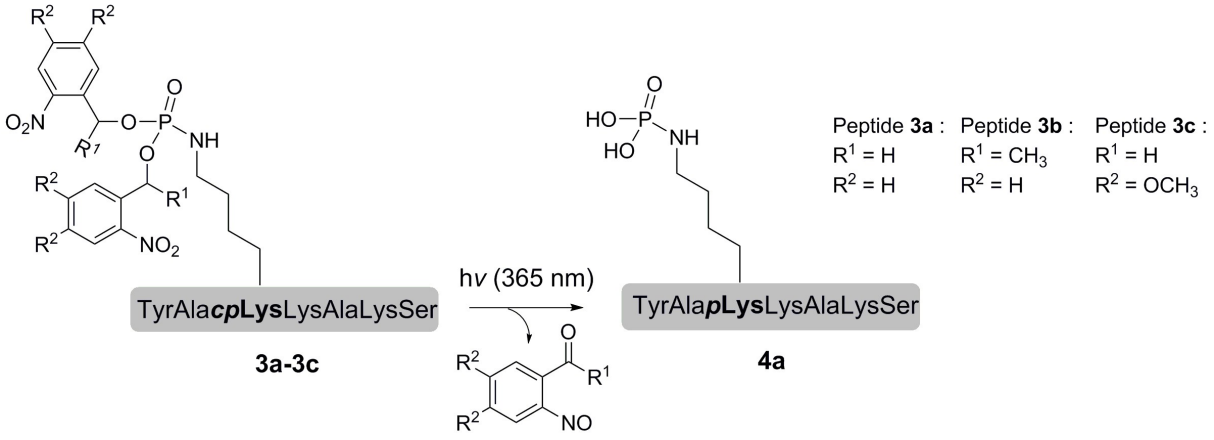


Figure S11. HPLC-UV pure trace of phosphoramidate **3c**

5 UV-irradiation of *o*-nitrobenzyl protected phosphormidate peptides 3a, 3b and 3c.

General procedure: A phosphate (20 mM, pH 7.3) buffered solution (100 µL) of peptide **3a**, **3b** (292 µM) or **3c** (146 µM) was irradiated at 355 nm with laser (Spectron Laser Systems, settings: 10 hz, 10 ns and 50 mJ per pulse) for 15 sec and 30 sec on ice. Inosine (500 µM) was used as internal standard. The samples were injected into the HPLC-UV and the identification of the peaks was confirmed by MS.



Scheme S5. Uncaging of peptides **3a**, **3b** and **3c** upon irradiation with UV-light at 355 nm. ***cpLys*** = *caged phosphoramidate Lysine peptide*

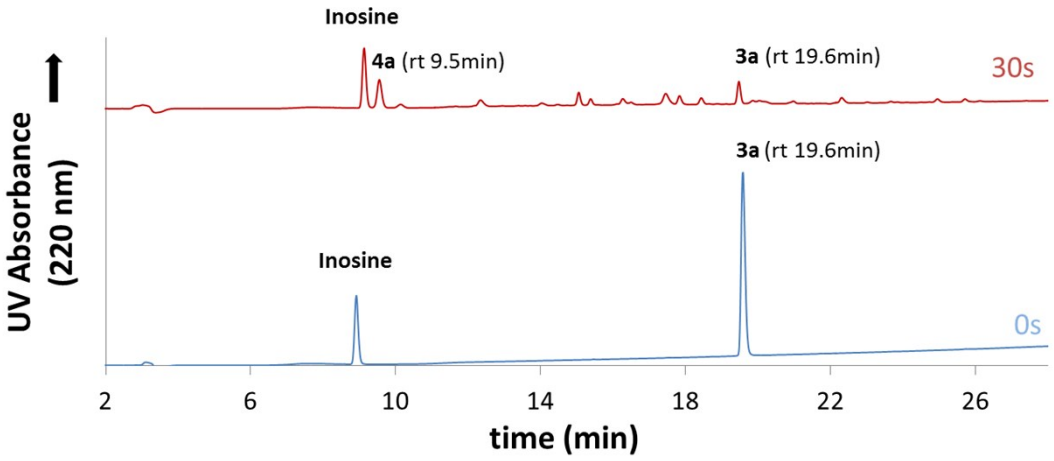


Figure S12. HPLC-UV traces of **3a** (NB) uncaging

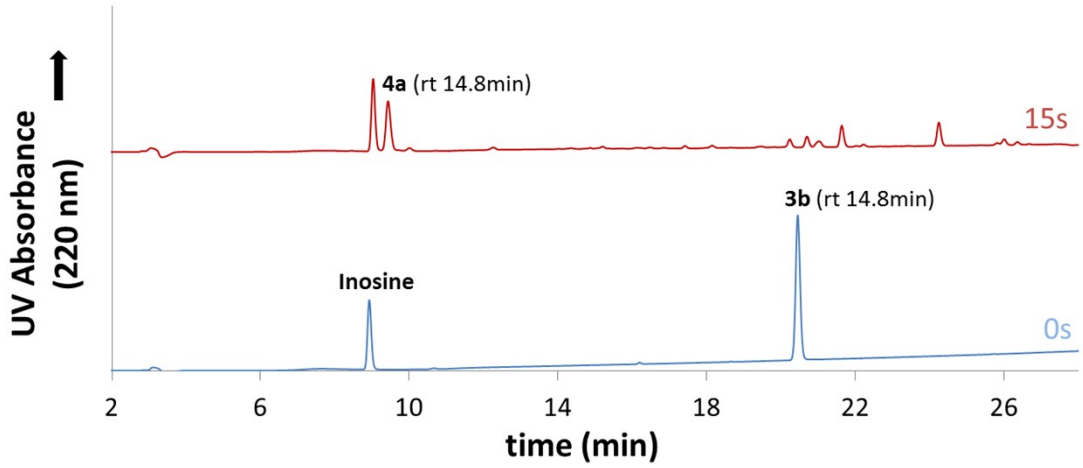


Figure S13. HPLC-UV traces of **3b** (NB) uncaging

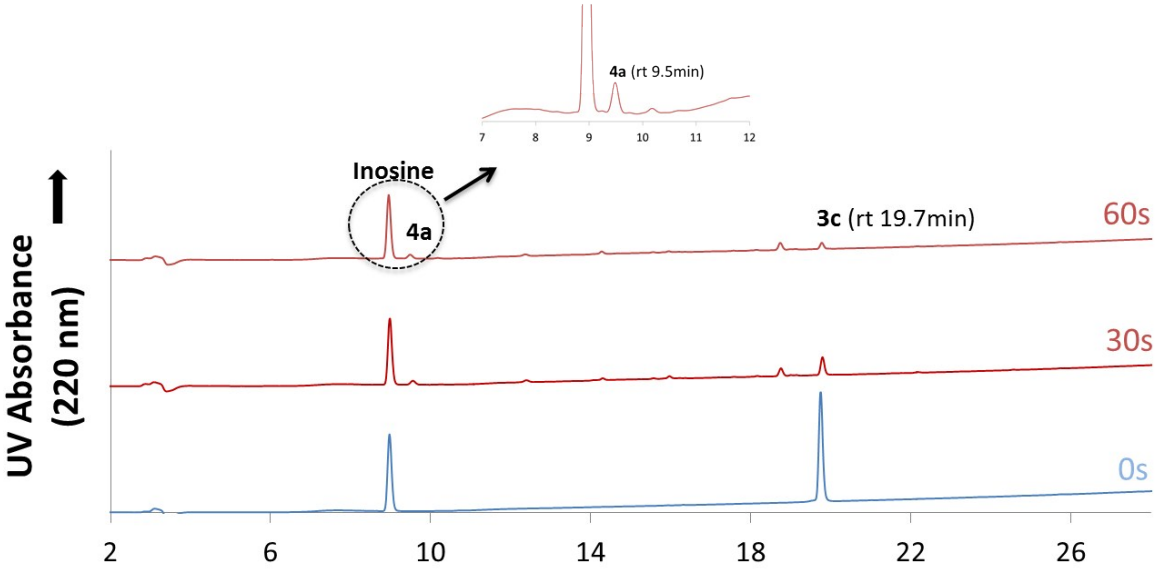


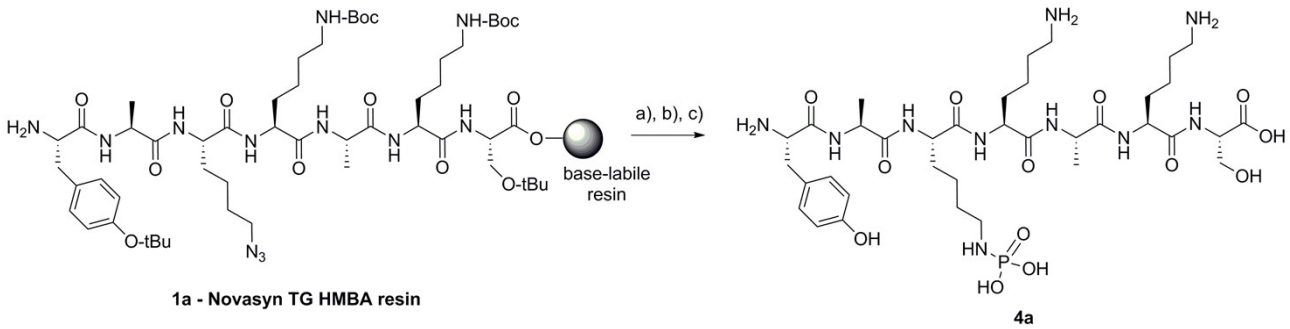
Figure S14. HPLC-UV traces of **3c** (DMNB) uncaging

6 Synthesis of phosphorylated lysine peptides using a base-cleavable phosphite

6.1 Synthesis of phospholysine peptide **4a**

Procedure: Novasyn TG HMBA resin containing ε-azido lysine peptide **1a** (50 mg, 8 µmol) was swelled in DMF (100 µL) and a solution of phosphite **2d** (9.6 mg, 40 µmol, 5eq) in DMF (50 µL) was added. The reaction mixture was incubated at 45 ºC for 24 h. Subsequent addition of phosphite was performed every 12 h. The reaction mixture was incubated at 45 ºC for 48 h. The reaction mixture was incubated at 45 ºC for 48 h. Excess phosphite was washed out and the resin was dried in DCM. Peptide was cleaved off the resin with 250 mM NaOH in dioxane at 0 ºC for 20 min. After neutralization with HCl, the identity of the peptide was confirmed by HPLC-UV-MS. HRMS: m/z: 438.2226 $[M+2H]^{2+}$ (calcd. m/z: 438.2229). See *chapter 7* for spectra.

Scheme S6. Staudinger-Phosphite reaction on solid support



Reagents and conditions: a) 2.5 mL 95% TFA, 5% TIS for 2h. b) phosphite **2d** (4 x 5 eq) in DMF, 24 h - 48 h, 45 ºC c) 1.0 mL of 1M NaOH in dioxane (4 mL) for 20 min at 0 ºC.

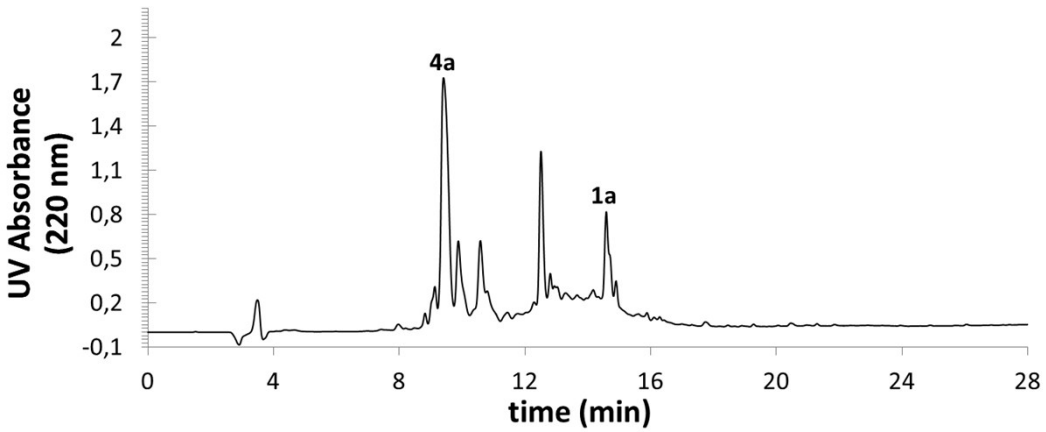
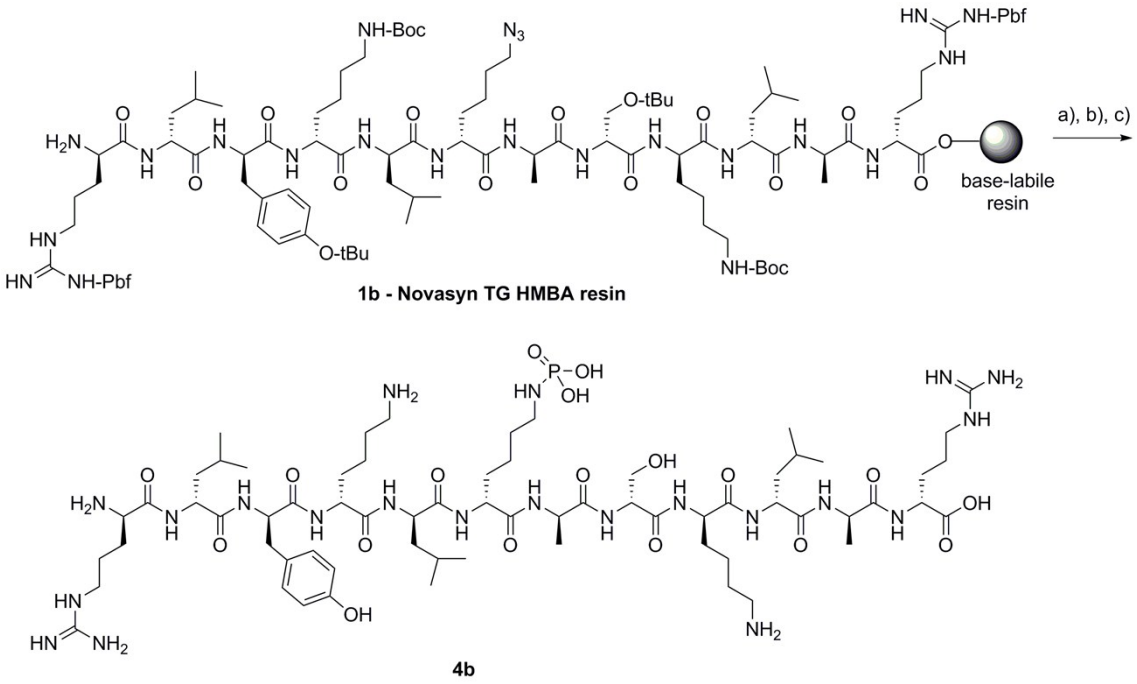


Figure S15. HPLC-UV trace of pLys peptide **4a** after 48h incubation

6.2 Synthesis of phospholysine peptide **4b**

Procedure: Novasyn TG HMBA resin containing ε-azido lysine peptide **1b** (150 mg, 0.025 mmol) was swelled in DMF (700 µL) and a solution of phosphite **2d** (120.5 mg, 0.5 mmol, 10eq) in DMF (100 µL) was added. Subsequent addition of phosphite was performed every 6 h. The reaction mixture was incubated at 45 ºC for 48 h. Excess phosphite was washed out and the resin was dried in DCM. Peptide was cleaved off the resin with 250 mM NaOH in dioxane at 0 ºC for 20 min. After neutralization with HCl, purification via semi-preparative HPLC yielded peptide **4b** (1.2 mg, 0.79 µmol) in 3% yield; HRMS: m/z: 509.6352 $[M+2H]^{2+}$ (calcd. m/z: 509.6359). See *chapter 7* for spectra.

Scheme S7. Staudinger-Phosphite reaction on solid support



Reagents and conditions: a) 2.5 mL 95% TFA, 5% TIS for 2h. b) phosphite **2d** (4 x 5 eq) in DMF, 48 h, 45 ºC c) 1.0 mL of 1M NaOH in dioxane (4 mL) for 20 min at 0 ºC.

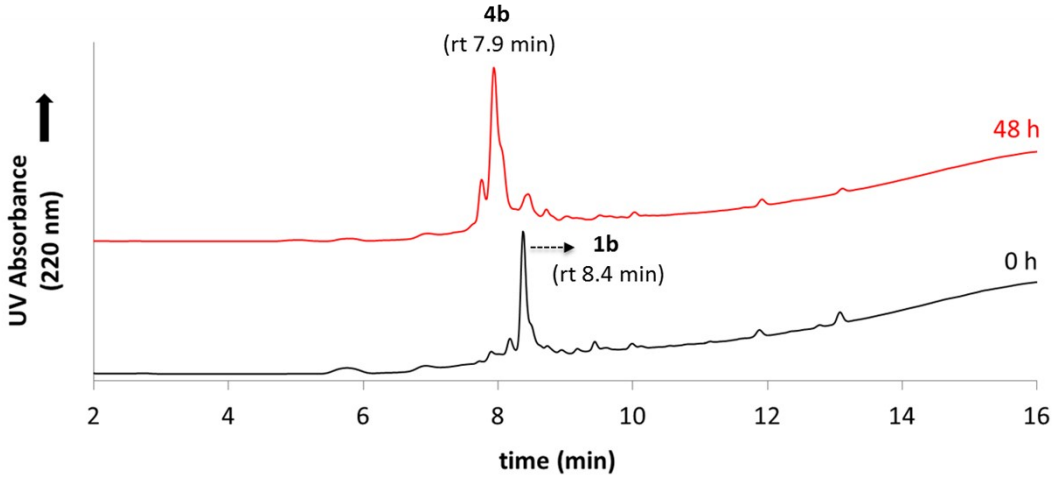


Figure S16. HPLC-UV traces of azido peptide **1b** (at the bottom) at 0h and of phospholysine peptide **4b** after 48h incubation and NaoH cleavage(on the top)

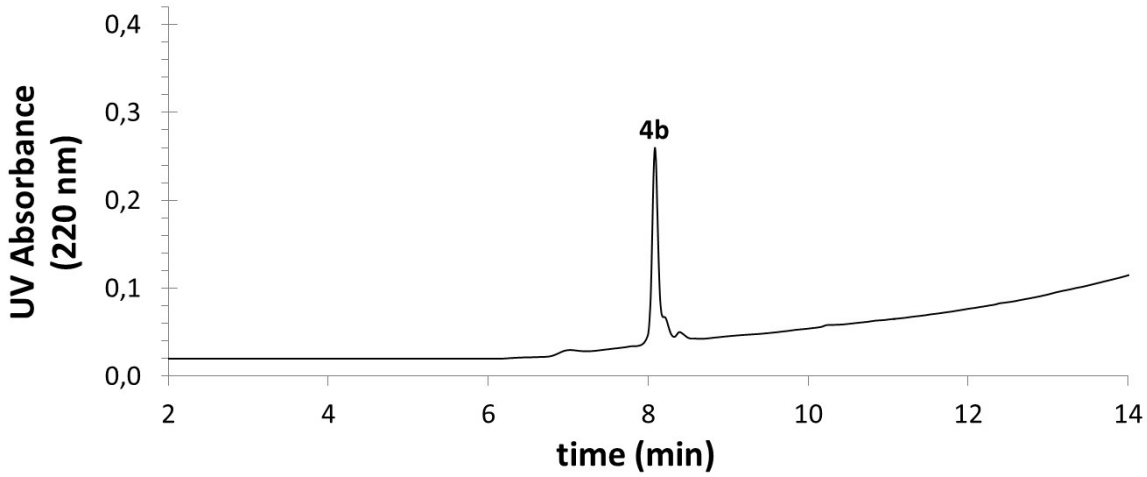


Figure S17. HPLC-UV trace of phospholysine peptide **4b** after semi-prepartive HPLC.

7 Characterization of the phosphorylated site by ETD MS

7.1 Analysis by ETD MS of phospholysine **4a**

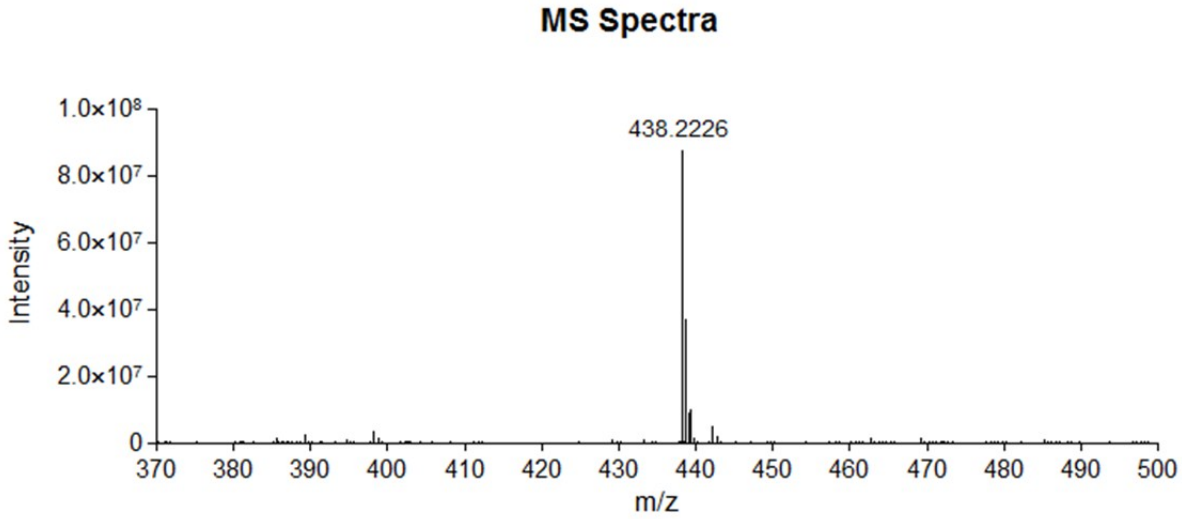


Figure S18. MS Spectra of phospholysine peptide **4a**

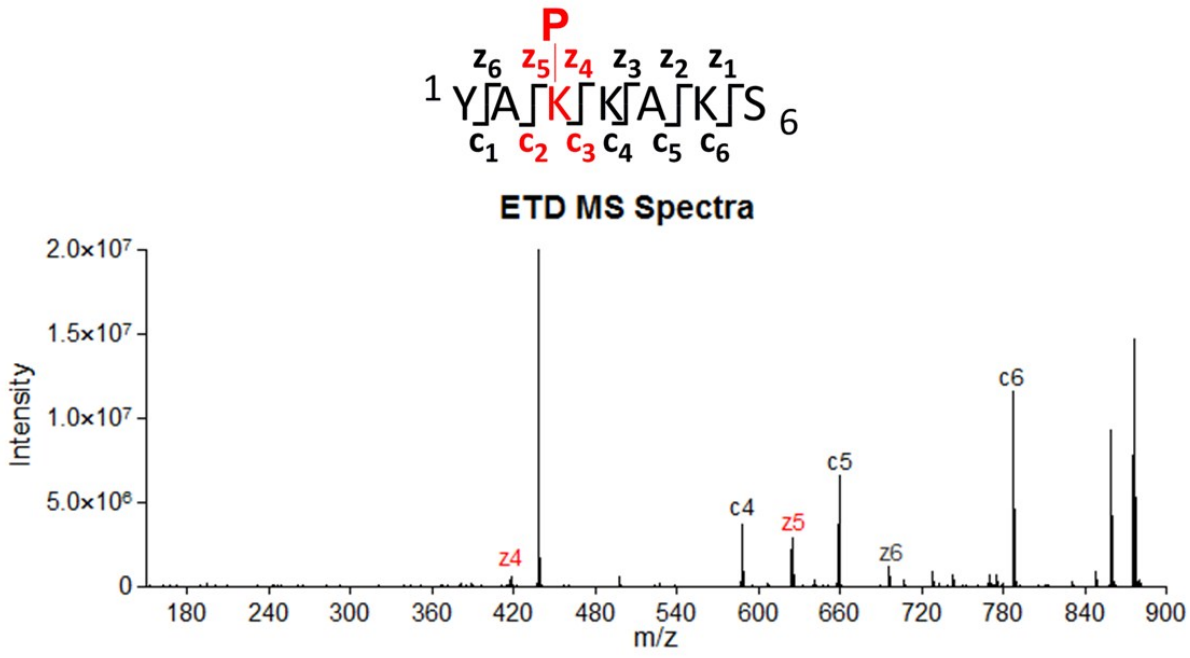


Figure S19. ETD MS Spectra of the 2+ charge state precursor ion of phospholysine peptide **4a**.

7.2 Analysis by ETD MS of phospholysine **4b**

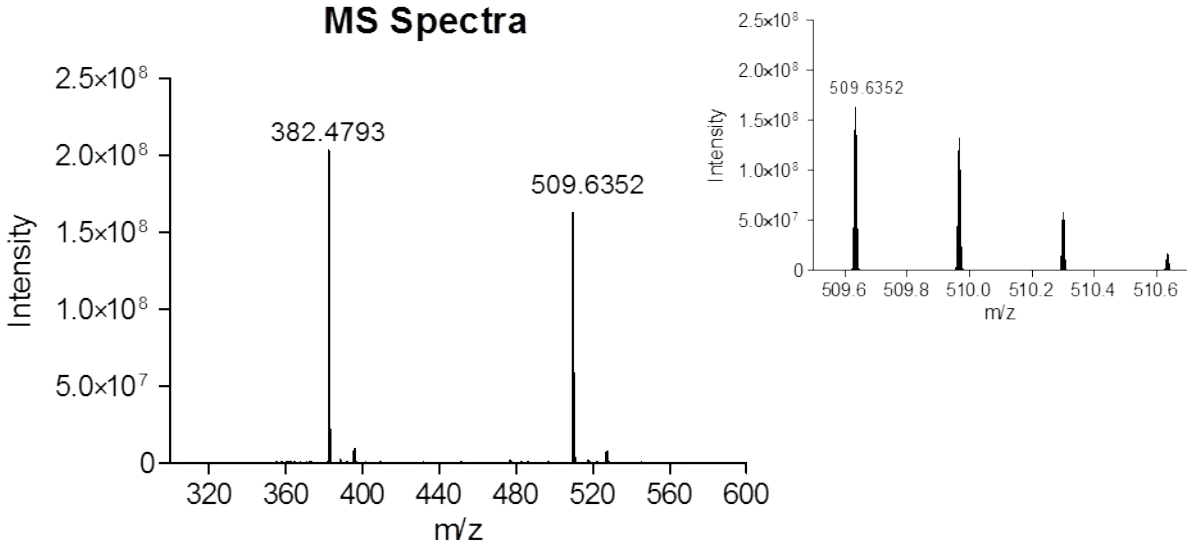


Figure S20. MS Spectra of phospholysine peptide **4b**

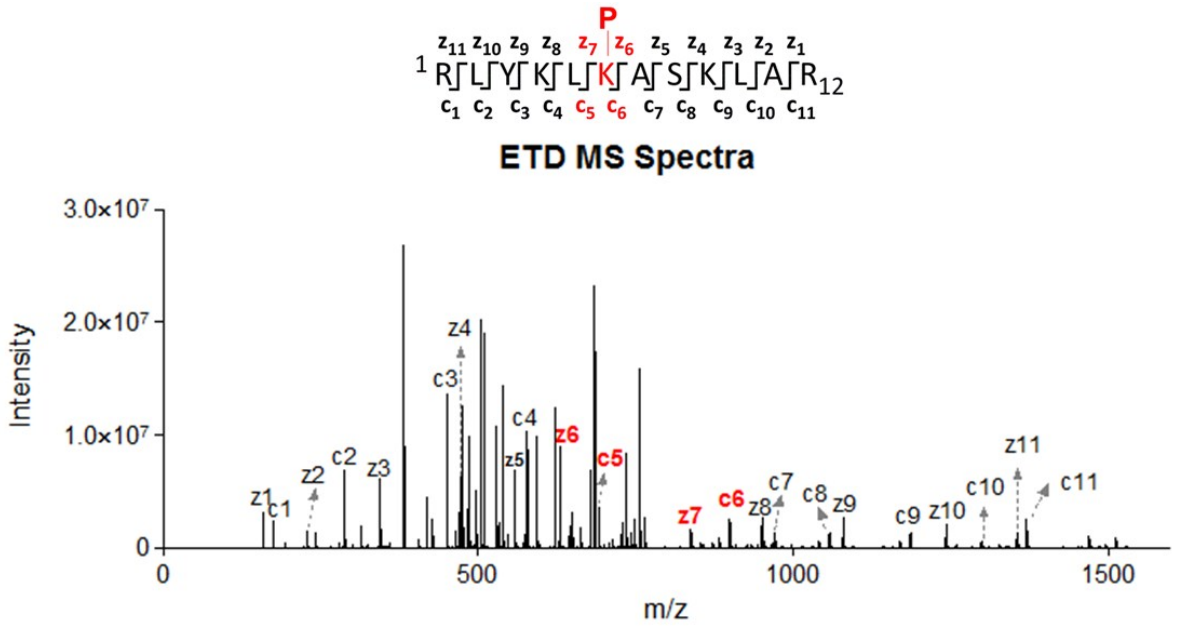


Figure S21. ETD MS Spectra of the 4+ charge state precursor ion of phospholysine peptide **4b**.

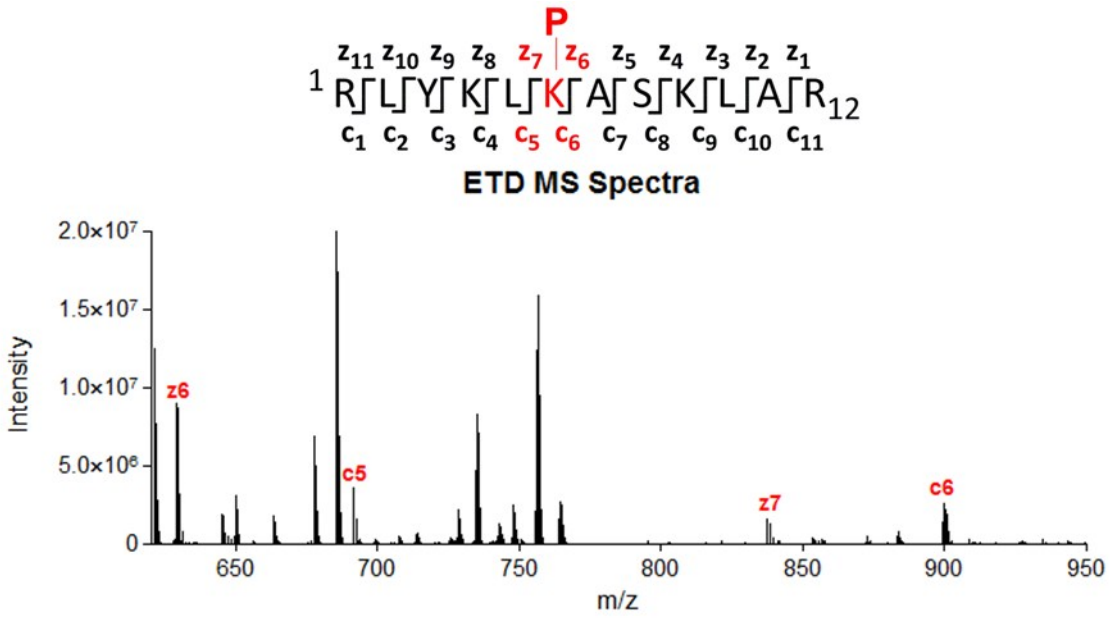


Figure S22. ETD MS Spectra of the 4+ charge state precursor ion of phospholysine peptide **4b**. (m/z range 620-920).

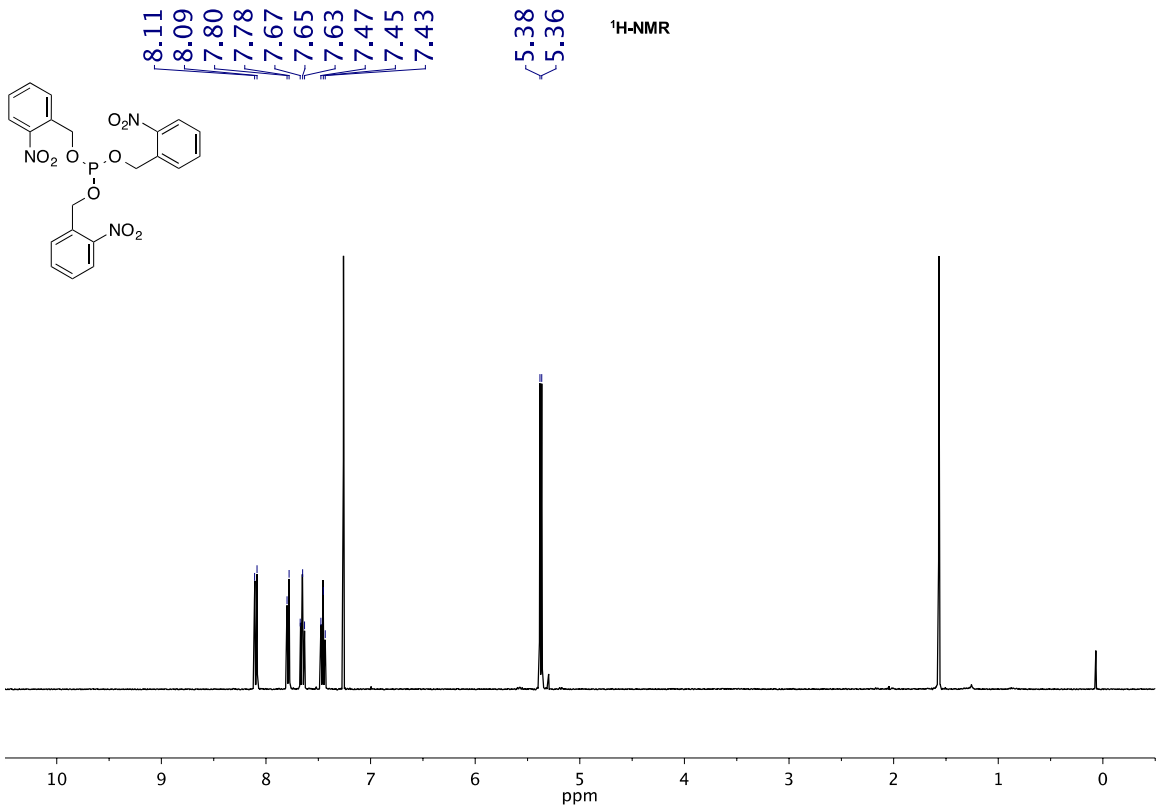
8 Literature

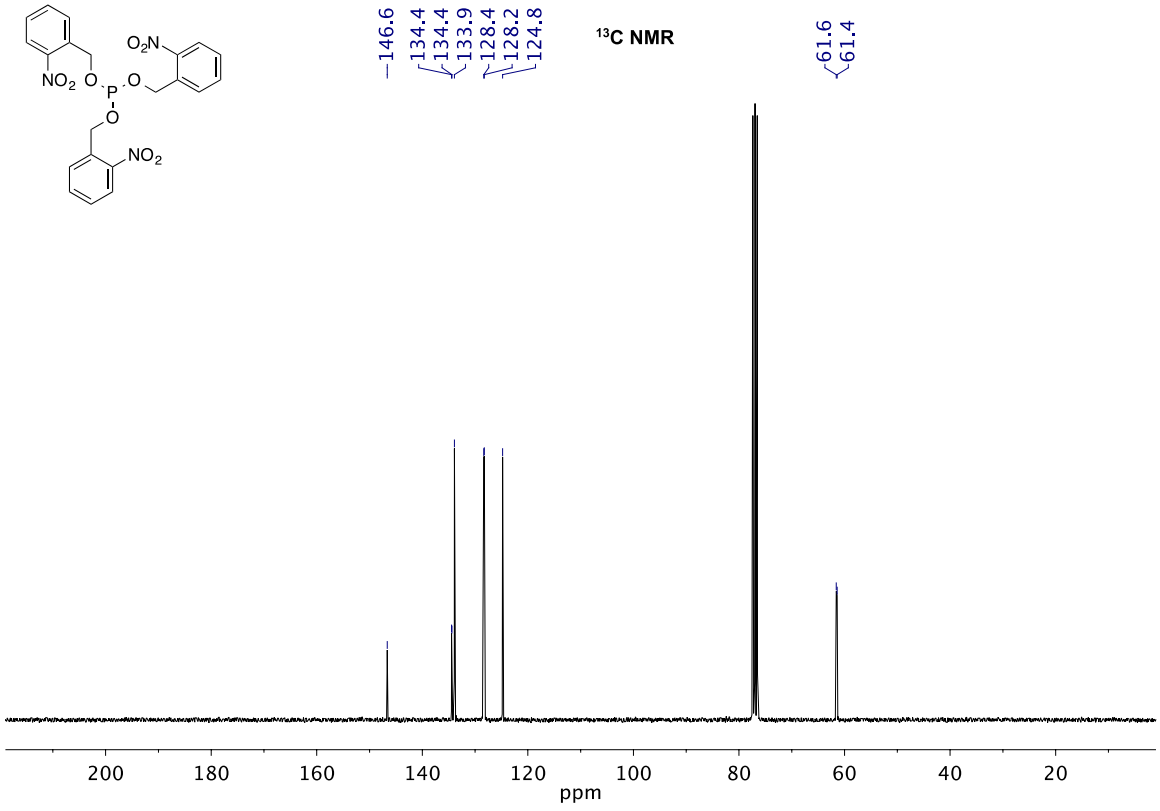
[1] Skopek, K.; Gladysz, J. A. *J. Organomet. Chem*. **2008**, 693, 857-866

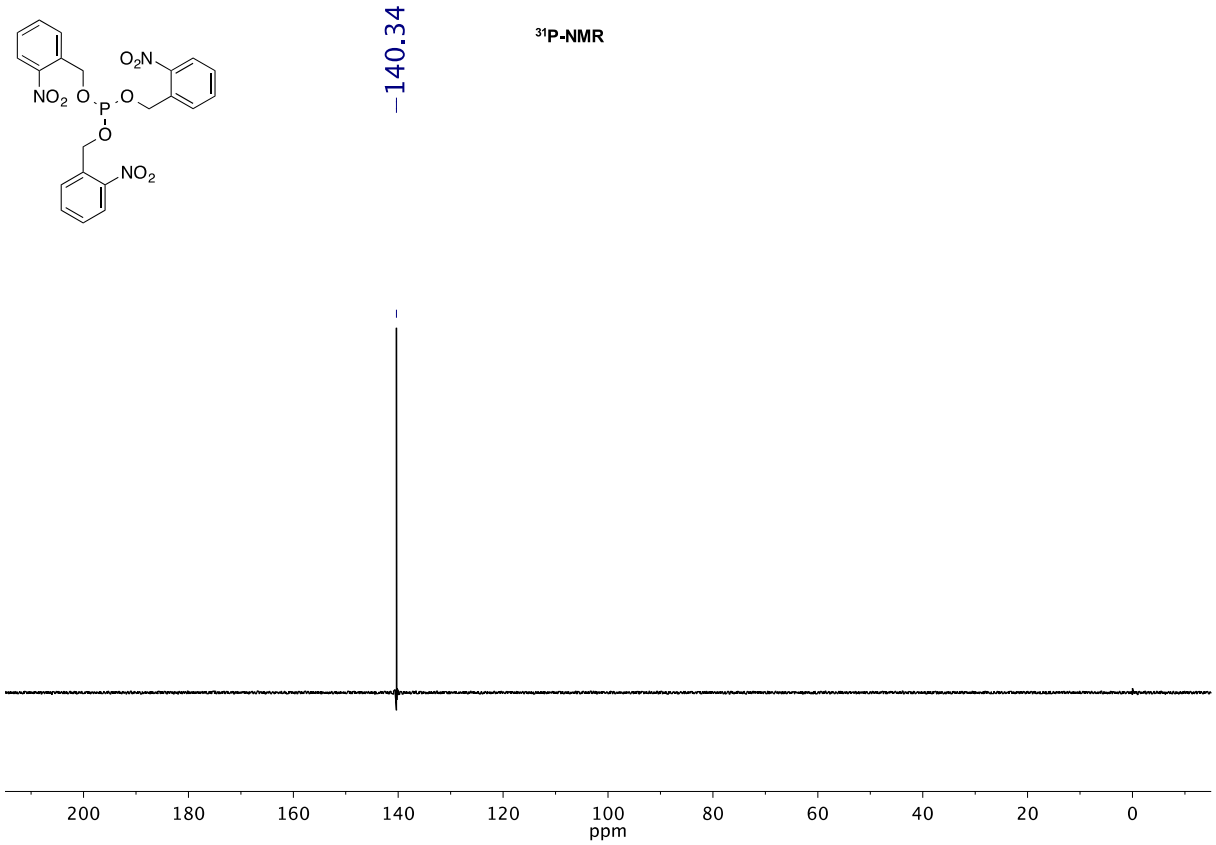
[2] Bertran-Vicente, J.; Serwa, R. A.; Schümann, M.; Schmieder, P.; Krause, E.; Hackenberger, C. P. R. *J. Am. Chem. Soc.* **2014,** 136(39)*,* 13622-13628.

9 Appendix

tris(2-nitrobenzyl) phosphite (**2a**)







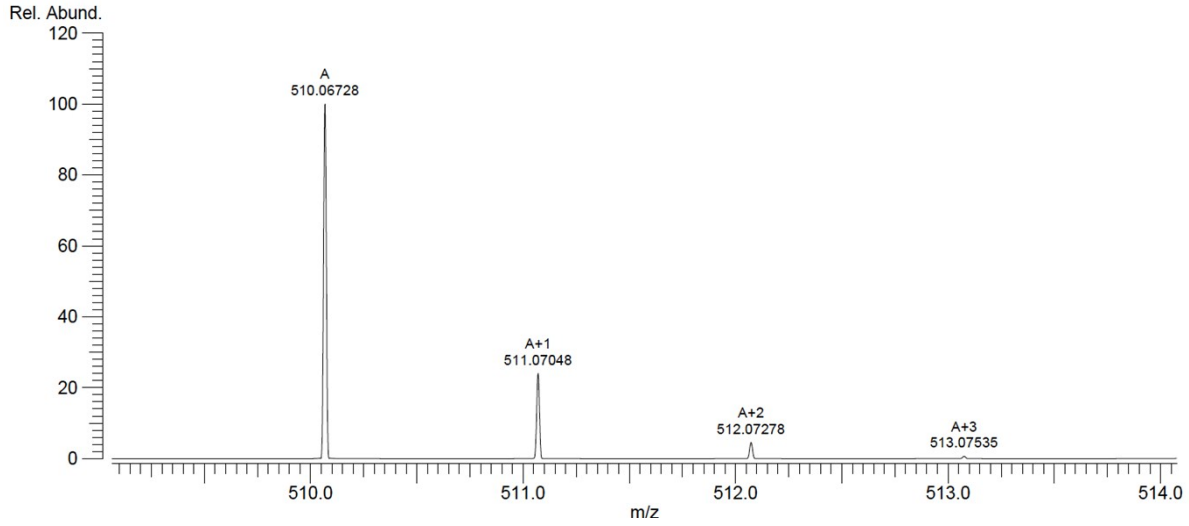
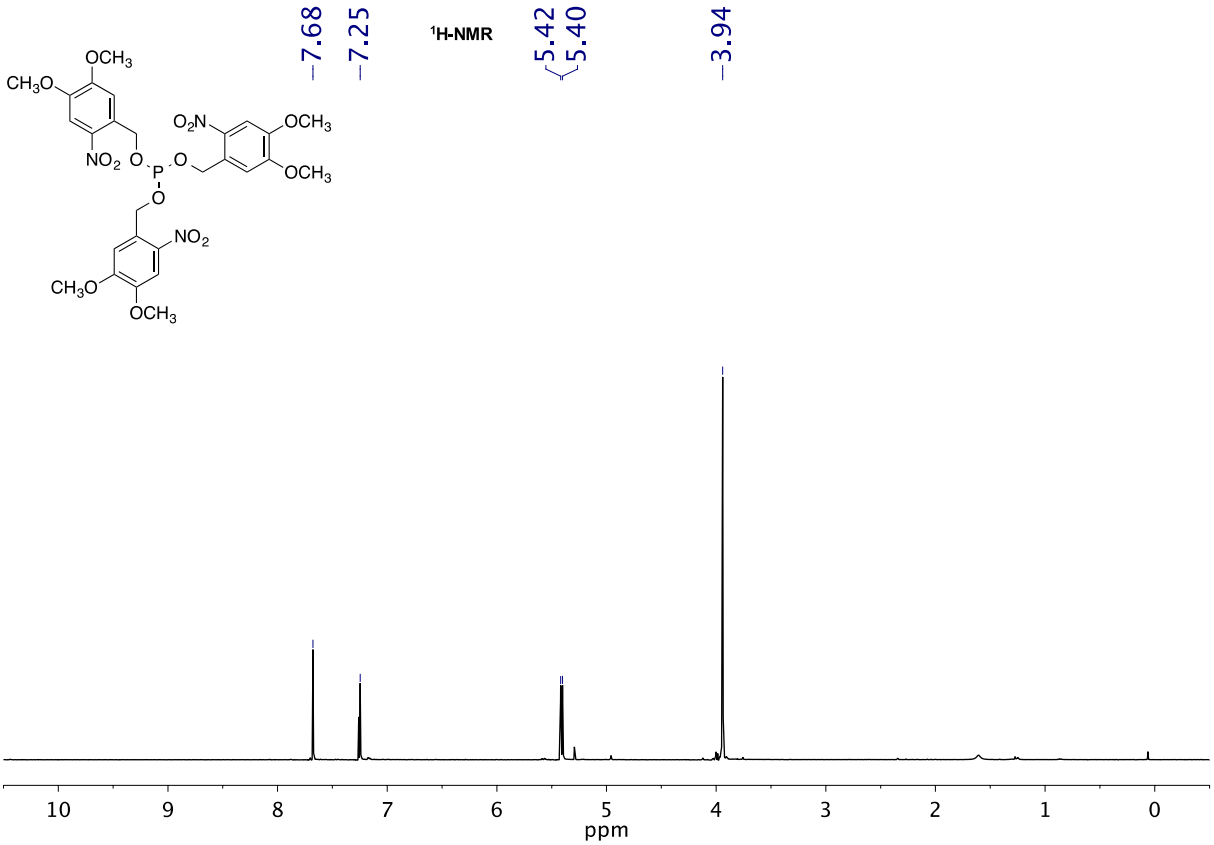
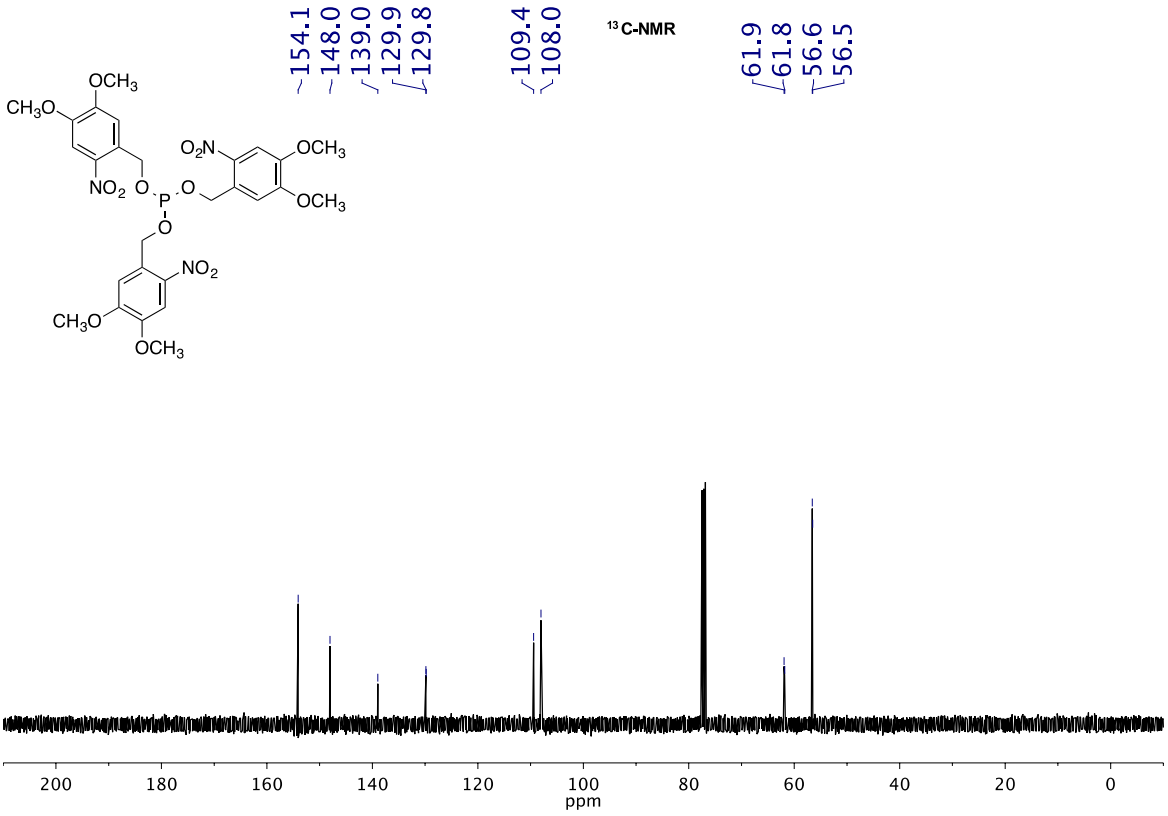
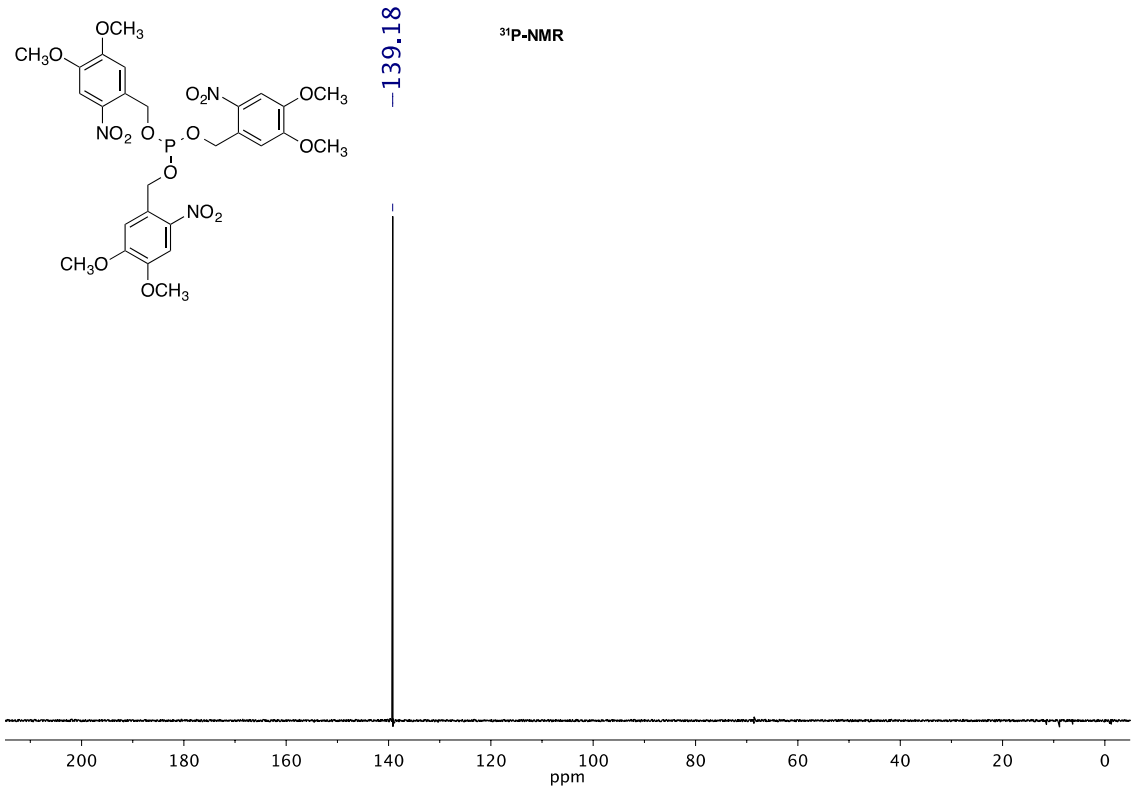


Figure S23. ^{1}H, ^{31}P-NMR and HR-MS of phosphite **2a**

tris(4,5-dimethoxy-2-nitrobenzyl) phosphite (**2c**)







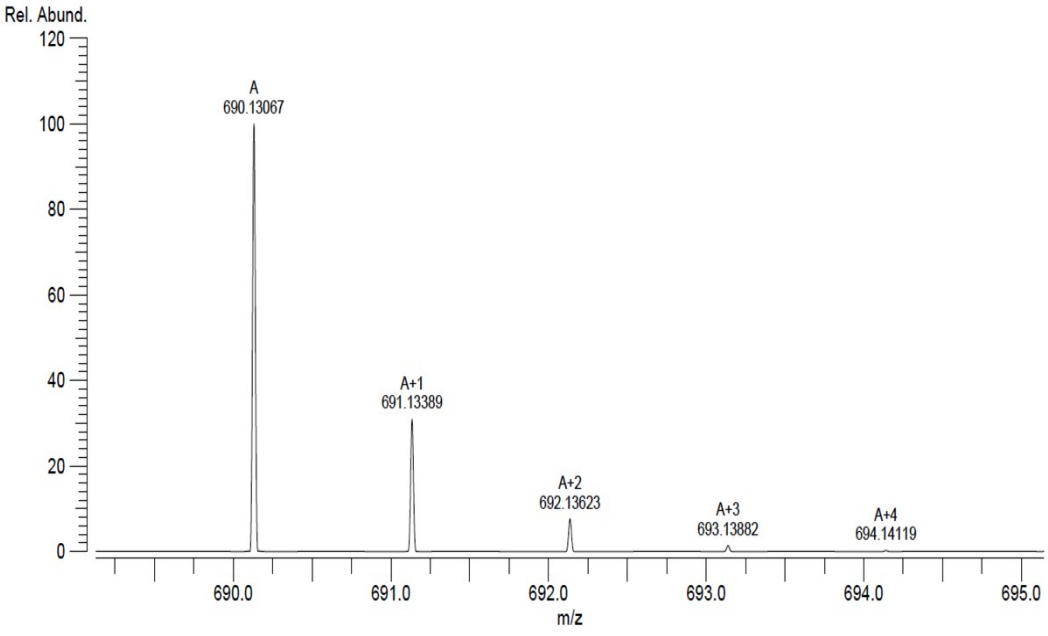


Figure S24. ^{1}H, ^{13}C, ^{31}P-NMR and HR-MS of phosphite **2c**

N^6-(bis(2-nitrobenzyl)-phosphoramidate lysine peptide **3a**

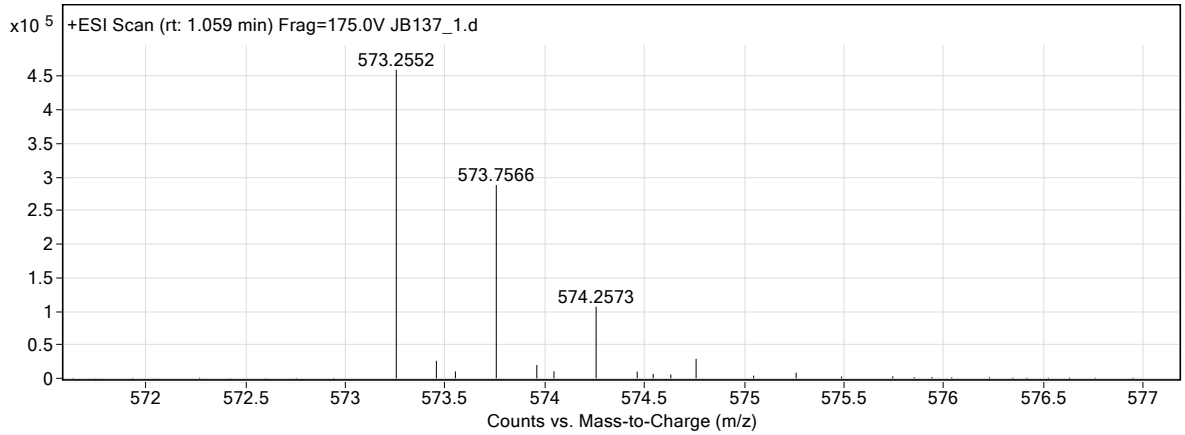
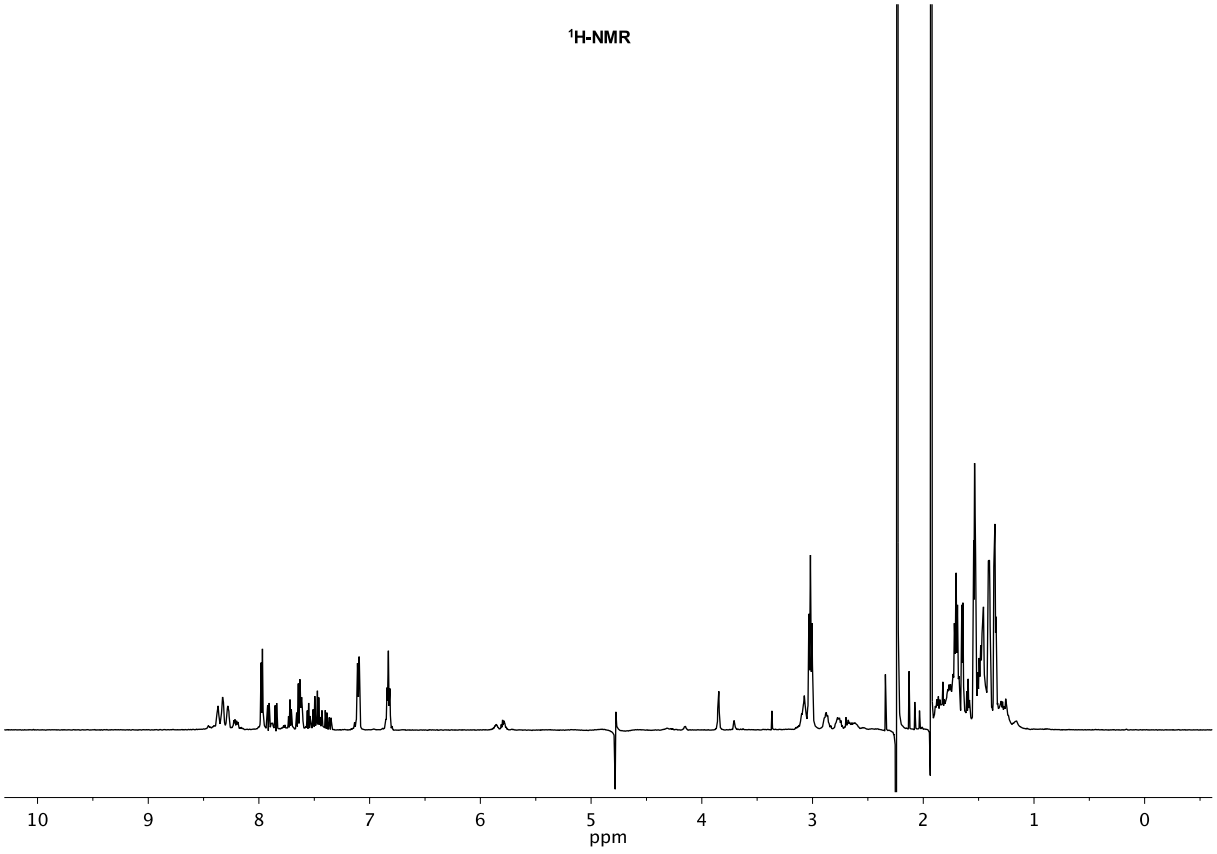
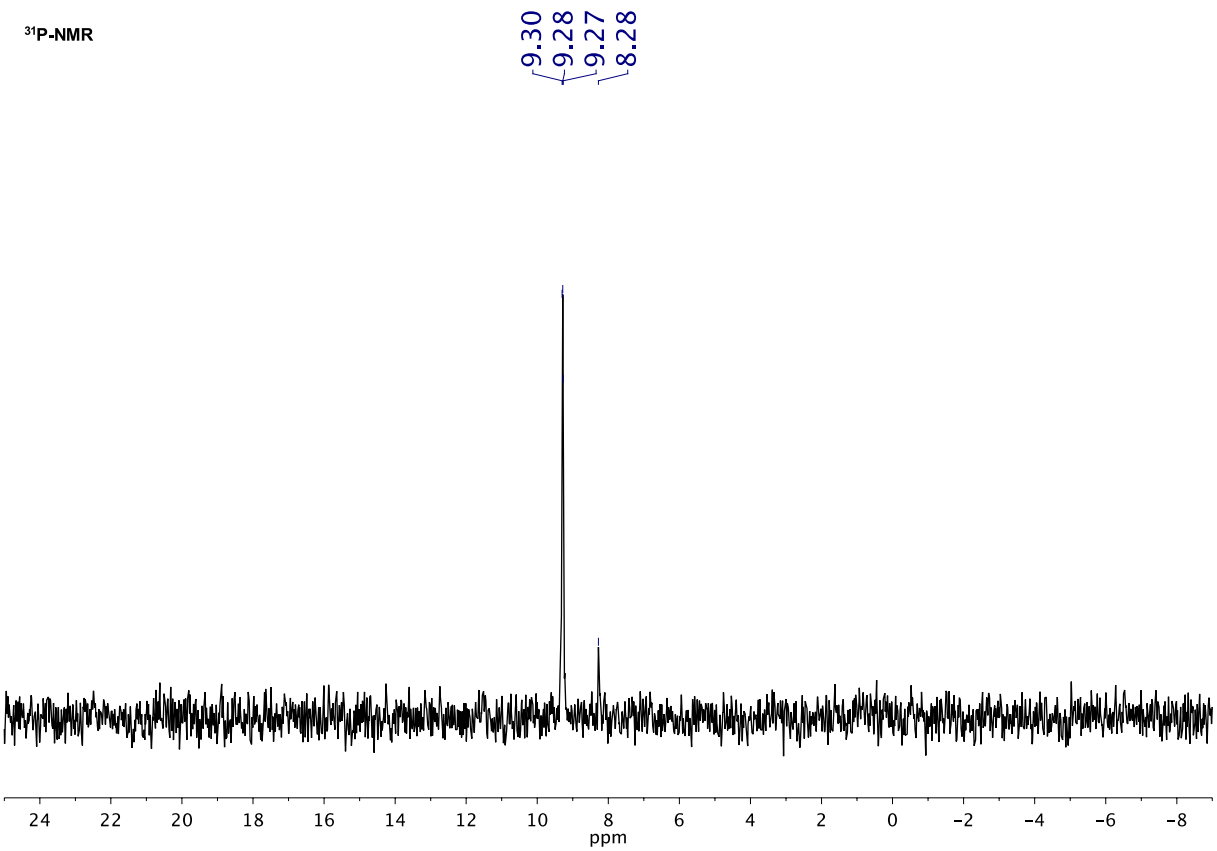
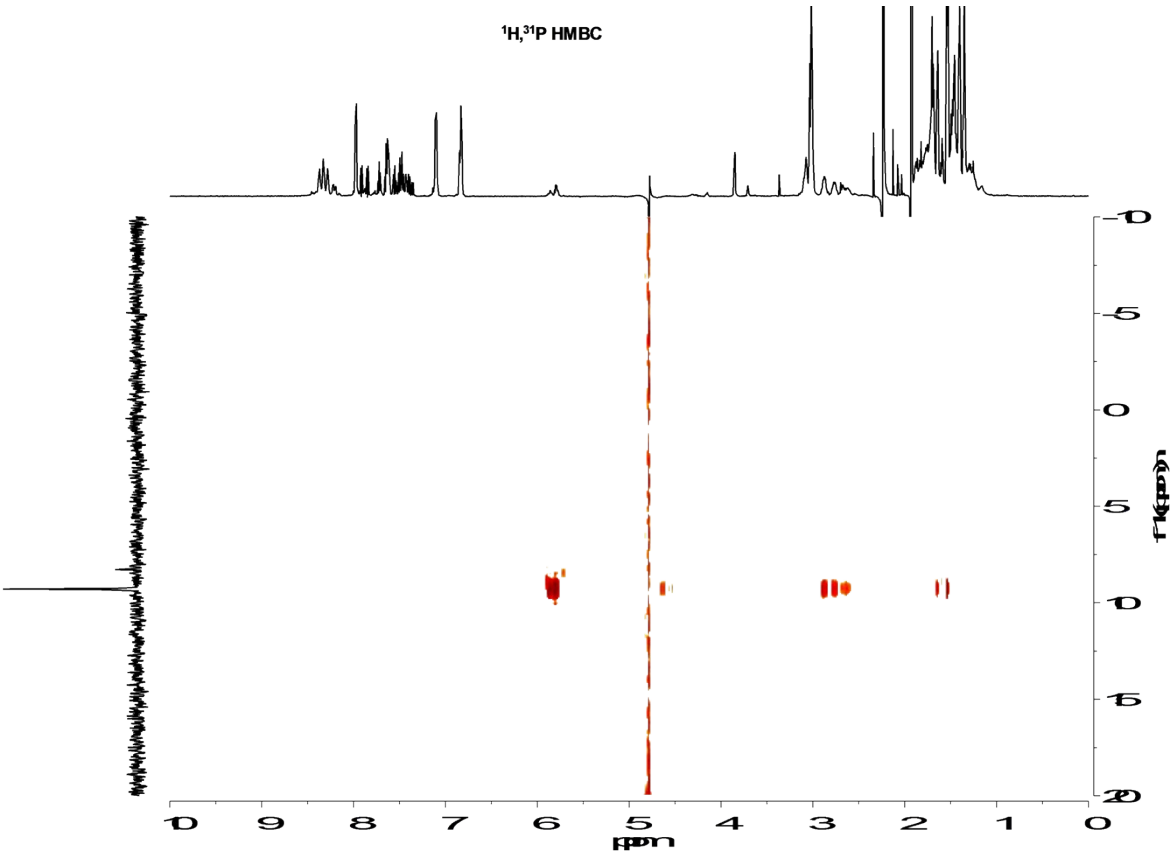


Figure S25. HR-MS of phosphoramidate **3a**

N^6-(bis(1-(2-nitrophenyl)ethoxy))-phosphoramidate lysine peptide **3b**




31P-NMR
9.30
9.28
9.27
8.28
24 22 20 18 16 14 12 10 8 6 4 2 0 −2 −4 −6 −8
ppm


1H,31P HMBC
10 9 8 7 6 5 4 3 2 1 0
ppm

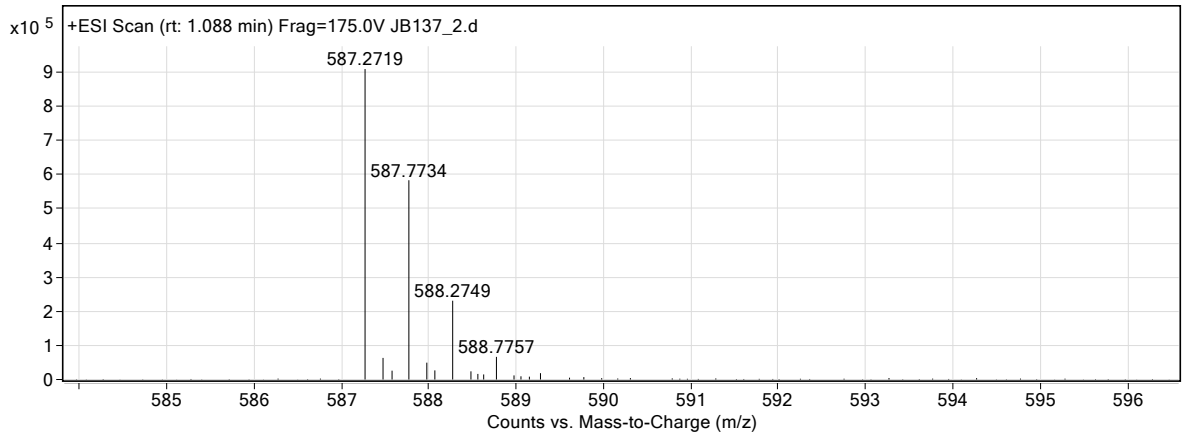


Figure S26. ^{1}H, ^{31}P, $^{1}H,^{31}P$-HMBC NMR and HR-MS of phosphoramdiate **3b**

<u>*N*^{6}-(bis((4,5-dimethoxy-2-nitrobenzyl)) phosphoramidate lysine peptide **3c**</u>

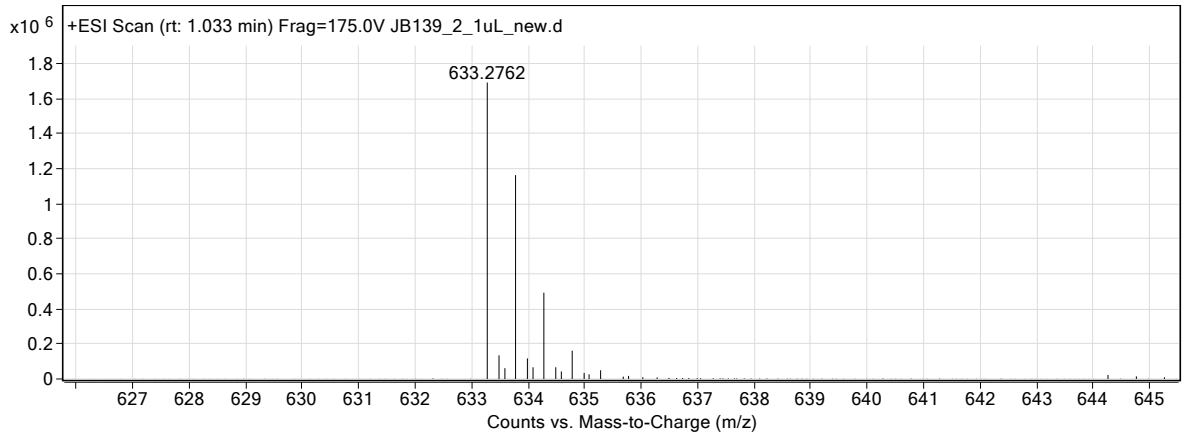


Figure S27. HR-MS of phosphoramidate **3c**