Supplementary Material (ESI) for Chemical Communications

Phosphonamidate peptide synthesis by Staudinger reactions of silylated phosphinic acids and esters

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1 General information

Materials: All reagents, amino acids, starting materials and solvents were purchased from commercial suppliers and used without further purification if not further mentioned. Phenyl phosphinic acid (**11a**) was purchased from ACROS ORGANICS and Ethyl phenylphosphinate (**7b**) from Aldrich. Dry solvents (CH_3CN, CH_2Cl_2, DMF) were purchased from ACROS ORGANICS.

Peptide Synthesis: Peptides were synthesized using standard amide coupling conditions HBTU/HOBt utilizing TG HMBA resin (Novabiochem). The first amino acid was attached by MSNT/Melm activation method and cleavage from the resin was performed with ice-cold NaOH (1M)/dioxane (1:3) for 15 min. Coupling and cleavage procedures are described by Novabiochem.

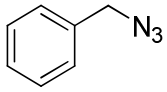
Methodes: LC-UV and LC-MS spectra were recorded on an Agilent 6210 TOF LC/MS system, Agilent Technologies, Santa Clara, CA, USA. Spray voltage was set to 4 kV. Drying gas flow rate was set to 25 psi. Separation of the sample was performed on a Luna 5u C18(2) 100 A column (5 µm, 4.6×150 mm) at a flow rate of 0.5 mL/min. The following solvent (A =1% AcOH in H_2O, B =1% AcOH in MeCN) gradient was applied: 0% B 0-3 min; 0-100% B 3-14 min; 100% B 14-18 min. Preparative HPLC purification for peptides was performed on a JASCO LC-2000 Plus system using a Kromasil RP18 column (25×250 mm) at a flow rate of 16 mL/min. The following solvent (A =1% AcOH in H_2O, B =1% AcOH in MeCN) gradient was applied: 0% B 0-3 min; 0-30% B 3-8 min; 30-100% B 8-35 min; 100% B 35-43 min; 100-20% B 43-45 min.

Flash chromatography was performed on silica gel (Acros Silicagel 60 A, 0.035-0.070 mm). TLC was performed on aluminium-backed silica plates (60 F254, 0.2 mm) which were developed using potassium permanganate as visualising agent. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at Jeol ECX/400.

2 Synthesis of organic compounds

2.1 Synthesis of azido compounds

2.1.1 Synthesis of benzyl azide (9b)



Sodium azide (715 mg, 11.0 mmol) was dissolved in 200 mL DMSO and benzyl bromide (1.71 g, 1.19 mL, 10 mmol) was added to the solution. The reaction mixture was stirred for at least 2 h at room temperature. 200 mL H_2O were added and after cooling to room temperature, the aqueous solution was extracted with diethyl ether (3 x 100 mL). The combined organic layers were washed with water (2x 100 mL) and brine (100 mL). After drying over $MgSO_4$ the ether was removed under reduced pressure to yield the pure azide **9b** in a yield of 90% as colourless oil.[1]

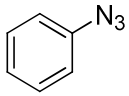
^{1}H-NMR (250 MHz, $CDCl_3$): δ (ppm) = 7.56 – 7.28 (m, 5H, Ph), 4.36 (s, 2H, CH_2).

2.1.2 Synthesis of phenylic azides 9a and 9c-e

Phenylic azides **9** were synthesized following a procedure by Hecht et al.[2]

General procedure A: The aniline derivative (13 mmol) was suspended in 80 mL hydrochloric acid (17%) at room temperature and then ethanol was added until a clear solution was obtained. The solution was cooled to 0 °C and $NaNO_2$ (1.5 eq.) was added in small portions. After stirring at 0 °C for 15-30 min. NaN_3 (1.5 eq.) was slowly added and the mixture was stirred for additional 2 h at room temperature. The reaction mixture was extracted with diethyl ether (3x 80 mL) and the combined organic fractions were washed with saturated $NaHCO_3$-solution (3x 50 mL) and with brine (50 mL). After drying over $MgSO_4$ the ether was removed under reduced pressure and the desired azides **9** were obtained without further purification.

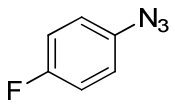
2.1.2.1 Phenyl azide (9a)



Phenyl azide (**9a**) was obtained according to **General procedure A** as a brown liquid in a yield of 90%.

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.86 (dd, *J* = 7.6, 0.7 Hz, 2H, CH), 7.04 (td, *J* = 7.6, 0.7 Hz, 1H, CH), 7.27 (t, *J* = 7.6 Hz, 2H, CH). [3]

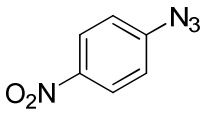
2.1.2.2 1-Azido-4-fluorobenzene (9c)



1-Azido-4-fluorobenzene (**9c**) was obtained according to **General procedure A** as a yellow liquid in a yield of 40%.

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.95-7.05 (m, 4H).[4]

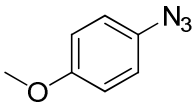
2.1.2.3 1-Azido-4-nitrobenzene (9d)



1-Azido-4-bromobenzene (**9d**) was obtained according to **General procedure A** as a yellow liquid in a yield of 96%.

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.23 (d, *J* = 9.0 Hz, 2H, CH), 7.13 (d, *J* = 9.0 Hz, 2H, CH).[2]

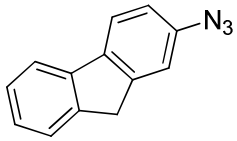
2.1.2.4 1-Azido-4-methoxybenzene (9e)



1-Azido-4-methoxybenzene (**9e**) was obtained according to **General procedure A** as a brown liquid quantitatively.

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.95 – 6.90 (m, 2H, CH), 6.88 – 6.83 (m, 2H, CH), 3.76 (s, 3H, OMe,).[2]

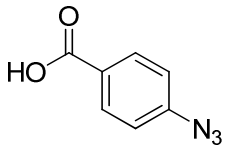
2.1.2.5 2-Azido-9H-fluorene (9g)



1-Azido-4-bromobenzene (**9g**) was obtained according to **General procedure A** as a yellow solid in a yield of 67%.

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.73 (d, *J* = 8.1 Hz, 2H, CH,), 7.53 (d, *J* = 7.4 Hz, 1H, CH), 7.37 (t, *J* = 7.4 Hz, 1H, CH), 7.29 (td, *J* = 7.4, 1.1 Hz, 1H, CH), 7.21 (d, *J* = 1.6 Hz, 1H, CH), 7.04 (dd, *J* = 8.2, 2.2 Hz, 1H, CH), 3.88 (s, 1H, CH_2).[4]

2.1.3 4-Azido benzoic acid (9f)

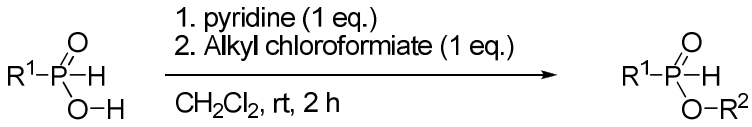


4-Aminobenzoic acid (**9f**) (2.00 g, 15.0 mmol) was dissolved in 10 mL water and concentrated sulphuric acid (98%, 3 mL) and additional water (3 mL) were added. The suspension was cooled to 0°C and a solution of $NaNO_2$ (1.06 g, 15.8 mmol) in of water (3 mL) was added under constant stirring. Sodium azide (1.20 g 18.5 mmol) was added to the brown solution. After additional 10-15 min. of stirring at 0°C the precipitate was filtered and washed several times with water. 4-Azidobenzoic acid was dissolved in ethyl acetate and dried over $MgSO_4$ to yield the product in a yield of 75%.[5]

^{1}H-NMR (250 MHz, CD_3OD): δ (ppm) = 8.17 – 7.89 (m, 2H, CH), 7.24 – 6.90 (m, 2H, CH), 4.84 (s, 1H, OH).

2.2 Synthesis of phosphinic esters 7 and acids 11

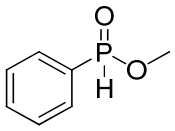
2.2.1 Method A: Hewitt reaction



General procedure B: The phosphinic acid was dissolved in 80 mL dichloromethane and one equivalent formiate was added to the solution followed by one equivalent of pyridine. When the

evanescent had stopped, the reaction mixture was refluxed for 15 min. and cooled to room temperature. The reaction mixture was poured into hydrochloric acid (0.1 M, 30 mL) and the organic layer was washed with water (50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the pure ester was obtained without further purification. [6]

2.2.1.1 Methyl phenylphosphinate (7a)

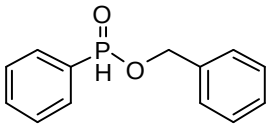


Methyl phenylphosphinate (**7a**) was obtained according to **General procedure B** as a pale liquid in a yield of 82%.

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.68-7.91 (m, 2H, CH), 7.41-7.69 (m, 3H, CH), 7.52 (d, *J* = 566.12 Hz, 1H, P-H); 3.81 (d, *J* = 12 Hz, CH_3).

HRMS (ESI-TOF): Exact mass calculated for $C_7H_{10}O_2P^+$ $[M+H]^+$: 157.0413, found: 157.0411 $[M+H]^+$.

2.2.1.2 Benzyl phenylphosphinate (7c)

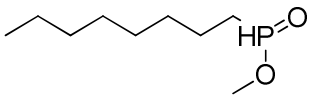


Benzyl phenylphosphinate (**7c**) was obtained according to **General procedure B** as a yellow liquid in a yield of 91%.

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.78 (m, 2H, CH), 7.65 (d, *J* = 566.5 Hz, 1H, P-H), 7.64 – 7.56 (m, 1H, CH), 7.55 – 7.46 (m, 2H, CH), 7.41 – 7.28 (m, 5H, CH), 5.16 (dd, *J* = 11.7, 8.3 Hz, 1H, CH_2), 5.07 (dd, *J* = 11.8, 9.4 Hz, 1H, CH_2);

HRMS (ESI-TOF): Exact mass calculated for $C_{13}H_{13}NaO_2P^+$ $[M+Na]^+$: 255.0545, found: 255.0532 $[M+Na]^+$.

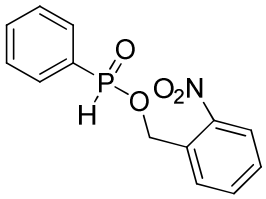
2.2.1.3 Methyl octylphosphinate (7d)



Methyl octylphosphinate (**7d**) was obtained according to **General procedure B** as a yellow liquid in a yield of 75%.

^{1}H-NMR (400 MHz, CD_3Cl): δ (ppm) = 3.69 (d, *J* = 11.7 Hz, 3H, CH_3), 1.74 – 1.59 (m, 2H, CH_2), 1.55 – 1.41 (m, 2H, CH_2), 1.35 – 1.12 (m, 10H, CH_2), 0.78 (t, *J* = 6.7 Hz, 3H, CH_3); ^{13}C-NMR (101 MHz, CD_3Cl): δ (ppm) = 52.8 (d, *J* = 7.1 Hz), 31.7 (d, *J* = 3.0 Hz), 30.5 (d, *J* = 4.2 Hz), 30.3 (d, *J* = 3.9 Hz), 29.0 (d, *J* = 6.2 Hz), 28.9 (d, *J* = 4.9 Hz), 22.6 (d, *J* = 1.4 Hz), 20.7 (d, *J* = 2.8 Hz), 20.6 (d, *J* = 3.1 Hz), 14.0; ^{31}P-NMR (162 MHz, CD_3Cl) δ = 41.63 (pt).[7]

2.2.2 Synthesis of 2-Nitrobenzyl phenylphosphinate (7e)

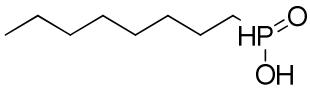


Phenyl phosphinic acid **11a** (994 mg, 7.00 mmol) and *o*-nitrobenzyl alcohol (1.07 g, 7.00 mmol) were dissolved in anhydrous CH_2Cl_2 (120 mL) and EDC·HCl (2.17 g, 14.0 mmol) was added. The reaction mixture was stirred at room temperature for 16 h, washed with saturated KH_2PO_4 (2 times 100 mL), saturated $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The product **7e** was obtained in a yield of 70% containing a small amount of alcohol.

^{1}H-NMR (400 MHz, CD_3Cl): δ (ppm) = 8.07 (dd, *J* = 8.2, 1.0 Hz, 1H, CH), 7.76 (m, 3H, CH), 7.70 (d, *J* = 570.8 Hz, 1H, P-H), 7.67 – 7.55 (m, 2H, CH), 7.53 – 7.41 (m, 3H, CH), 5.54 (dd, *J* = 14.7, 8.2 Hz, 1H, CH_2), 5.43 (dd, *J* = 14.6, 7.7 Hz, 1H, CH_2); ^{13}C-NMR (101 MHz, CD_3Cl): δ (ppm) = 146.9, 134.3, 133.7 (d, *J* = 3.0 Hz), 132.4 (d, *J* = 7.8 Hz), 130.9 (d, *J* = 12.2 Hz), 129.2, 129.0, 128.9, 128.9 (d, *J* = 132.2 Hz), 127.9, 125.2, 64.30 (d, *J* = 4.9 Hz).

HRMS (ESI-TOF): Exact mass calculated for $C_{13}H_{14}N_2O_4P^+$ $[M+H]^+$: 278.0577, found: 278.0554 $[M+H]^+$.

2.2.3 Synthesis of Octyl phoshinic acid (11b)

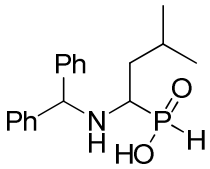


$NaH_2PO_2 \cdot H_2O$ (530 mg, 5.00 mmol) and 1-hexene (168 mg, 251 µL, 2.00 mmol) were dissolved in MeOH (10 mL) and Et_3B (1 M THF, 2 mL, 2 mmol) was added at room temperature. After 2 h the solvent was removed under reduced pressure and redissolved in ethylacetate. The organic solution was extracted with saturated $KHSO_4$-solution. The aqueous phase was extracted with ethyl acetate (2x) and the combined organic layers were dried over $MgSO_4$. The organic solvent was removed and the phosphinic acid **11b** was obtained in a yield of 80% without further purification.[8]

^{1}H-NMR (400 MHz, CD_3Cl): δ (ppm) = 10.81 (bs, 1H, OH), 7.10 (d, *J* = 540 Hz, 1H, P-H), 1.1-1.80 (m, 14H, CH_3), 0.88 (t, *J* = 6 Hz, 3H, CH_3).

2.2.4 Synthesis of Leucine and Alanin phosphinic acid derivatives

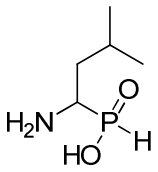
2.2.4.1 1-(Benzhydrylamino)-3-methylbutylphosphinic acid



α-Methyl butyraldehyde (2.49 mL, 22.7 mmol) was added to a refluxing solution of diphenylmethylamine hydrochloride (5.00 g, 22.7 mol) and 50% aqueous hypophosphorous acid (2.37 mL, 22.7 mol) in water (25 mL). The reaction mixture was heated for 3 h and the product was filtered and washed with water and acetone. The product was obtained in a yield of 85% as a white solid and used without further purification.[9]

^{1}H-NMR (400 MHz, CD_3CN/D_2O 1:1): δ (ppm) = 7.14 (d, 1H, *J*=570 Hz, P-H), 7.51-7.40 (m, 10H, Ar), 5.81 (s, 1H, Ar_2CH), 3.14-3.01 (m, 1H, CH), 1.78-1.53 (m, 3H, CH_2, CH), 0.66 (m, 6H, CH_3).

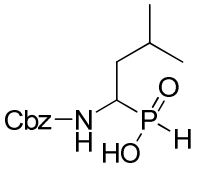
2.2.4.2 1-amino-3-methylbutylphosphinic acid



To diphenylmethylaminophosphonous acid 48% hydrobromic acid *(5* times by weight) was added and the mixture was heated to 100 °C for 2 h. The reaction mixture was evaporated to dryness and the residue was taken up in water. The aqueous solution was washed several times with ether and the water was again removed under reduced pressure. The residue was dissolved in ethanol and propylene oxide was added until precipitation started. After standing for 24h the product was filtered off and the phosphinic acid was obtained in a yield of 67%.

^{1}H-NMR (400 MHz, D_2O): δ (ppm) = 6.85 (d, 1H, *J*= 533 Hz, P-H), 3.15-3.01 (m, 1H, PCHN), 1.68-1.38 (m, 3H, CH_2,CH), 0.88-0.81 (m, 6H, CH_3).

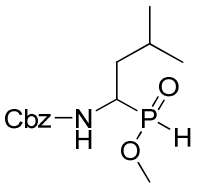
2.2.4.3 1-(benzyloxycarbonylamino)-3-methylbutylphosphinic acid (11c)



To a solution of the amino phosphinic acid (7.43 mmol, 1.12 g) in 1 eq. of NaOH-solution (2M), 1 eq. of benzyl chloroformate and 1 eq. of NaOH-solution (2M) were added at 0°C. The reaction mixture was stirred for 2 h at 0°C and the extracted with diethylether. The aqueous solution was acidified with hydrochloric acid (2M) and extracted with ethyl acetate (3x). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The pure compound **11c** was obtained as a white solid in a yield of 70%.

^{1}H-NMR (400 MHz, DMSO): δ (ppm) = 8.09 (bs, 1H, OH), 7.30–7.22 (m, 5H, CH), 6.77 (d, *J* = 526.8 Hz, 1H, PH), 5.05 (s, 2H, CH_2), 3.69–3.57 (m, 1H, CH), 1.71–1.59 (m, 1H, CH), 1.54–1.30 (m, 3H, CH, CH_2), 0.86 (d, *J* = 6.5 Hz, 3H, CH_3), 0.81 (d,*J* = 6.4 Hz, 3H, CH_3).

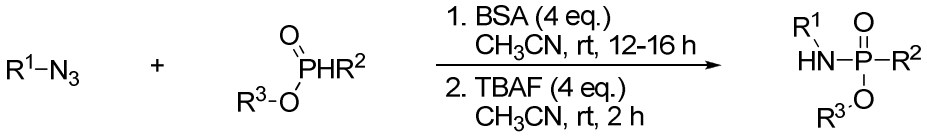
2.2.4.4 Benzyl 1-(methoxyhydrophosphoryl)-3-methylbutylcarbamate (7f)



The phosphinic acid **11c** (15 mmol) was suspended in trimethyl phosphite (20 mmol) and heated at 60 °C for 1-2 h. The phosphite was distilled off and the residue was triturated with a mixture of pentane and diethylether and allowed to stand at -10°C for several days until a white solid was obtained. After filtration the phosphinate **7f** was dried in the high vacuum and could be used without further purification in a yield of 53%.[10]

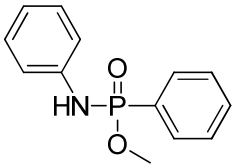
^{1}H-NMR (400 MHz, DMSO): δ (ppm) = 0.82-0.990 (m, 6H, CH_3), 1.34-1.35 (m, 2H, CH_2), 1.55-1.57 (m, 1H, CH), 1.62-1.67 (m, 2H, CH_2), 3.65 (d, *J* = 11.3 Hz, 3H, CH_3), 3.68 (d, *J* = 11.4 Hz, 3H, CH_3), 3.72-3.90 (m, 1H, CH), 5.10 (m, 2H, CH_2), 7.21-7.40 (m, 5H, Ph).

2.3 Synthesis of Phosphonamidates 2



General Procedure C: The azide (2 mmol) was dissolved in 3 mL acetonitrile and one equivalent phosphinate **7** was added followed by BSA (4 eq.). The reaction mixture was stirred at room temperature over night. Then TBAF (2 eq.) was added and the solution was stirred for additional 30 min. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate. The organic solution was extracted with water to remove acetamide. The organic layer was concentrated under reduced pressure and purified by flash chromatography (2:1→4:1, ethyl acetate: cyclohexane).

2.3.1 Methyl *N,P*-diphenylphosphonamidate (2a)

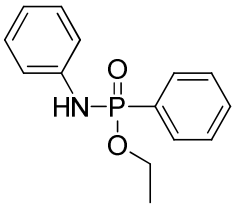


Methyl *N,P*-diphenylphosphonamidate (**2a**) was obtained as a pale solid following **General Procedure C** in a yield of 91%.

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.85 (ddd, *J* = 13.6, 8.1, 1.1 Hz, 2H, CH), 7.49 (tt, *J* = 2.7, 1.3 Hz, 1H, CH), 7.44 – 7.37 (m, 2H, CH), 7.14 (q, *J* = 7.7 Hz, 2H, CH), 6.97 – 6.92 (m, 2H, CH), 6.87 (t, *J* = 7.4 Hz, 1H, CH), 3.85 (d, *J* = 11.3 Hz, 3H, CH_3); ^{13}C-NMR (101 MHz, $CDCl_3$): δ (ppm) = 140.4, 132.3, 131.5 (d, *J* = 10.1 Hz), 129.8 (d, *J* = 178.1 Hz), 129.3, 128.7 (d, *J* = 14.8 Hz), 121.5, 117.5 (d, *J* = 6.2 Hz), 51.2 (d, *J* = 6.0 Hz); ^{31}P-NMR (400 MHz, $CDCl_3$): δ (ppm) = 19.85.

HRMS (ESI-TOF): Exact mass calculated for $C_{13}H_{14}N_2O_4P^+$ $[M+H]^+$: 248.0846, found: 248.0835 $[M+H]^+$.

2.3.2 Synthesis of Ethyl *N,P*-dipenyl phosphonamidate (2c)



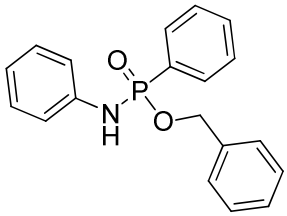
Phenyl azide (**9a**) (357 mg, 3.00 mmol) was dissolved in CH_2Cl_2 (5 mL) and commercially available ethyl phenylphosphinate (**7b**) (481 µL, 3.00 mmol) was added followed by TMSCl (1.15 mL, 9.00 mmol) and Et_3N (1.25 mL, 9.00 mmol). The reaction mixture was stirred over night at room temperature and was quenched with TBAF (1M THF, 9 mL). The solvent was evaporated and the residue purified by column chromatography (2:1, EA: CH). The product was obtained as a pale solid in a yield of 82%.

^{1}H-NMR (400 MHz, CD_3OD): δ (ppm) = 7.86 – 7.78 (m, 2H, CH), 7.58 – 7.51 (m, 1H, CH), 7.50 – 7.43 (m, 2H, CH), 7.13 (t, *J* = 7.8 Hz, 2H, CH), 7.01 (dd, *J* = 8.5, 0.9 Hz, 2H, CH), 6.89 – 6.82 (m, 1H, CH), 4.24 – 4.09 (m, 2H, CH_2), 1.34 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C-NMR (101 MHz, CD_3OD): δ (ppm) = 141.9 (d, *J* = 1.7 Hz), 133.6 (d, *J* = 3.0 Hz), 132.6 (d, *J* = 10.3 Hz), 131.3 (d, *J* = 174.8 Hz), 130.1, 129.7 (d, *J* = 14.7 Hz), 122.6, 119.0 (d, *J* = 6.9 Hz), 62.62 (d, *J* = 5.9 Hz);^{31}P-NMR (162 MHz, CD_3OD) δ (ppm) = 19.51.

HRMS (ESI-TOF): Exact mass calculated for $C_{13}H_{14}N_2O_4P^+$ $[M+H]^+$: 262.0991, found: 262.0989 $[M+H]^+$.

2.3.3 Benzyl *N,P*-diphenylphosphonamidate (2d)

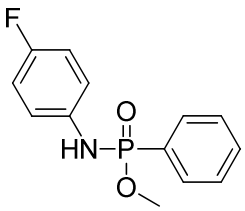
Benzyl *N*,*P*-diphenylphosphonamidate (**2d**) was obtained as a yellow solid following **general procedure C** in a yield of 80% as a pale solid.



^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.91 – 7.81 (m, 2H), 7.51 (td, *J* = 7.4, 1.4 Hz, 1H), 7.36 (t, *J* = 23.2 Hz, 2H), 7.13 (t, *J* = 7.9 Hz, 2H), 6.90 (dd, *J* = 16.0, 7.6 Hz, 3H), 5.26 (dd, *J* = 11.6, 7.5 Hz, 1H, CH_2), 5.09 (dd, *J* = 11.6, 6.8 Hz, 1H, CH_2); ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) = 140.1, 136.2, 132.5, 131.6 (d, *J* = 10.1 Hz), 130.9, 129.4, 128.8, 128.7, 128.5, 128.3, 121.7, 117.8 (d, *J* = 6.1 Hz), 66.8 (d, *J* = 81.3 Hz); ^{31}P-NMR (400 MHz, $CDCl_3$): δ (ppm) = 18.53.

HRMS (ESI-TOF): Exact mass calculated for $C_{19}H_{19}NO_2P^+$ $[M+H]^+$: 324.1148, Found: 324.1164 $[M+H]^+$.

2.3.4 Methyl *N*-4-fluorophenyl-*P*-phenylphosphonamidate (2e)

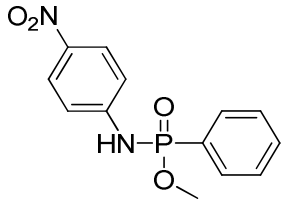


Methyl *N*-4-fluorophenyl-*P*-phenylphosphonamidate (**2e**) was obtained as a white solid following **General Procedure C** in a yield of 61%.

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.85 – 7.77 (m, 2H, CH), 7.53 – 7.46 (m, 1H, CH), 7.44 – 7.37 (m, 2H, CH), 7.05 (s, 2H, CH), 6.92 – 6.78 (m, 2H, CH), 3.84 (d, *J* = 11.3 Hz, 3H, CH_3); ^{13}C-NMR (101 MHz, $CDCl_3$): δ (ppm) = 158.2 (d, *J* = 240.0 Hz), 136.4 (d, *J* = 2.6 Hz), 132.6 (d, *J* = 3.0 Hz), 131.6 (d, *J* = 10.2 Hz), 129.7 (d, *J* = 177.6 Hz), 128.8 (d, *J* = 14.8 Hz), 119.2 (dd, *J* = 7.5, 6.7 Hz), 116.1 (d, *J* = 22.5 Hz), 51.4 (d, *J* = 6.4 Hz); ^{31}P-NMR (400 MHz, $CDCl_3$): δ (ppm) = 20.04.

HRMS (ESI-TOF): Exact mass calculated for $C_{13}H_{14}N_2O_4P^+$ $[M+H]^+$: 266.0741, Found: 266.0735 $[M+H]^+$.

2.3.5 Methyl *N*-4-nitrophenyl-*P*-phenylphosphonamidate (2f)

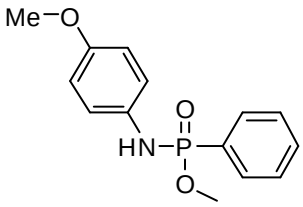


Methyl *N*-4-nitrophenyl-*P*-phenylphosphonamidate (**2f**) was obtained as a white solid following **General Procedure C** in a yield of 72%.

^{1}H-NMR (400 MHz, CD_3OD): δ (ppm) = 8.09 – 8.00 (m, 2H), 7.89 – 7.82 (m, 2H), 7.61 (td, J = 7.7, 2.6 Hz, 1H), 7.52 (td, *J* = 8.0, 4.3 Hz, 2H), 7.21 – 7.14 (m, 2H), 3.85 (d, *J* = 11.6 Hz, 3H); ^{13}C-NMR (101 MHz, CD_3OD): δ (ppm) = 148.83, 142.98, 134.22 (d, *J* = 3.0 Hz), 132.70 (d, *J* = 10.5 Hz), 129.99 (d, *J* = 14.9 Hz), 129.63 (d, *J* = 176.0 Hz), 126.26, 118.23 (d, *J* = 7.2 Hz), 52.78 (d, *J* = 6.0 Hz); ^{31}P-NMR (162 MHz, $CDCl_3$) δ (ppm) = 20.00.

HRMS (ESI-TOF): Exact mass calculated for $C_{13}H_{14}N_2O_4P^+$ $[M+H]^+$: 293.0686, Found: 293.0682 $[M+H]^+$.

2.3.6 Methyl *N*-4-methoxyphenyl-*P*-phenylphosphonamidate (2g)

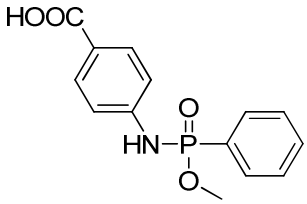


Methyl *N*-4-methoxyphenyl-*P*-phenylphosphonamidate (**2g**) was obtained as an orange solid following **General Procedure C** in a yield of 76%.

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.88 – 7.76 (m, 2H, CH), 7.48 (td, *J* = 7.2, 1.0 Hz, 1H, CH), 7.36-7.50 (m, 2H, CH), 6.92 – 6.85 (m, 2H, CH), 6.79 (bs, 1H, NH), 6.73 – 6.64 (m, 2H, CH), 3.83 (d, *J* = 11.6 Hz, 3H, CH_3), 3.68 (s, 1H, CH_3); ^{13}C-NMR (101 MHz, $CDCl_3$): δ (ppm) = 155.0, 133.7, 132.5 (d, *J* = 3.1 Hz), 131.8 (d, *J* = 10.1 Hz), 130.3 (d, *J* = 177.2 Hz), 128.9 (d, *J* = 14.8 Hz), 119.7 (d, *J* = 6.3 Hz), 115.0, 55.8, 51.5 (d, *J* = 6.3 Hz); ^{31}P-NMR (162 MHz, $CDCl_3$) δ (ppm) = 20.22.

HRMS (ESI-TOF): Exact mass calculated for $C_{14}H_{16}N_2O_3P^+$ $[M+H]^+$: 291.0893, found: 291.0938 $[M+H]^+$.

2.3.7 4-(Methoxy(phenyl)phosphorylamino)benzoic acid (2h)

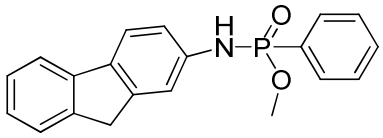


The compound **2h** was obtained according to the **General Procedure C** described. To protect the acid functionality BSA was added before the methyl phenyl phosphinate and HF·pyridine was added instead of TBAF until the product precipitates. The phosphonamidate was filtrated and dried under high vacuum. The pure product **2h** was obtained as a white solid in a yield of 82%.

^{1}H-NMR (400 MHz, CD_3OD): δ (ppm) = 12.51 (s, 1H, OH), 8.68 (d, *J* = 7.8 Hz, 2H, CH), 7.80 – 7.70 (m, 2H, CH), 7.62 – 7.47 (m, 3H, CH), 7.12 (d, *J* = 8.7 Hz, 2H, CH), 3.71 (d, *J* = 11.3 Hz, 3H); ^{13}C-NMR (101 MHz, CD_3OD): δ (ppm) = 167.6, 146.4, 132.9, 131.7 (d, *J* = 10.2 Hz), 131.3, 131.4 – 131.1 (m), 130.7 (d, *J* = 171.8 Hz), 129.3 (d, *J* = 14.3 Hz), 123.2, 117.1 (d, *J* = 7.0 Hz), 51.9 (d, *J* = 5.8 Hz). ^{31}P-NMR (162 MHz, CD_3OD) δ (ppm) = 18.14.

HRMS (ESI-TOF): Exact mass calculated for $C_{14}H_{15}NO_4P^+$ $[M+H]^+$: 292.0733, found: 292.0738 $[M+H]^+$.

2.3.8 Methyl *N*-9H-fluoren-2-yl-P-phenylphosphonamidate (2i)

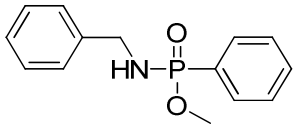


Methyl *N-9H*-fluoren-2-yl-P-phenylphosphonamidate (**2i**) was obtained as a yellow solid following **General Procedure C** in a yield of 90%.

^{1}H-NMR (400 MHz, CD_3Cl): δ (ppm) = 7.69 – 7.61 (m, 2H, CH), 7.40 (d, *J* = 7.5 Hz, 1H, CH), 7.34 (d, *J* = 8.2 Hz, 1H, CH), 7.31 (dd, *J* = 7.4, 1.4 Hz, 1H, CH), 7.27 – 7.20 (m, 3H, CH), 7.07 (t, *J* = 7.4 Hz, 1H, CH), 7.01 – 6.95 (m, 2H, CH), 6.79 (dd, *J* = 8.2, 2.1 Hz, 1H, CH), 3.64 (d, *J* = 11.4 Hz, 3H, CH_3), 3.53 (s, 2H, CH_2); ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) = 144.6, 142.4, 141.2 (d, *J* = 1.1 Hz), 138.9 (d, *J* = 1.2 Hz), 135.6, 132.4 (d, *J* = 3.1 Hz), 131.3 (d, *J* = 10.4 Hz), 129.9, 129.0 (d, *J* =176.4 Hz), 128.5 (d, *J* = 14.9 Hz), 126.5, 125.6, 124.6, 120.2, 118.8, 116.5 (d, *J* = 6.8 Hz), 114.5 (d, *J* = 6.7 Hz), 51.3 (d, *J* = 6.2 Hz), 36.58. ^{31}P-NMR (400 MHz, $CDCl_3$): δ (ppm) = 20.04.

HRMS (ESI-TOF): Exact mass calculated for $C_{20}H_{19}NO_2P$+ [M+H]+: 336.1148, found: 336.1146 $[M+H]^+$.

2.3.9 Methyl N-benzyl-P-phenylphosphonamidate (2b)

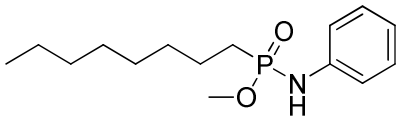


Methyl *N*-benzyl-*P*-phenylphosphonamidate (**2b**) was obtained as a white solid following **General Procedure C** in a yield of 54%.

^{1}H-NMR (400 MHz, CD_3Cl): δ (ppm) = 7.83 – 7.75 (m, 2H, CH), 7.53 – 7.46 (m, 1H, CH), 7.45 – 7.38 (m, 2H, CH), 7.30 – 7.17 (m, 5H, Ph), 4.11 – 3.97 (m, 2H, CH_2), 3.68 (d, *J* = 11.1 Hz, 3H, CH_3), 3.47 (s, 1H, NH); ^{13}C-NMR (400 MHz, CD_3Cl): δ (ppm) = 139.7 (d, *J* = 6.3 Hz), 132.0 (d, *J* = 3.0 Hz), 131.5 (d, *J* = 9.8 Hz), 130.4 (d, *J* = 173.6 Hz), 128.6, 128.4, 127.5, 127.3, 51.3 (d, *J* = 5.8 Hz), 44.9; ^{31}P-NMR (400 MHz, CD_3Cl): δ (ppm) = 24.72;

HRMS (ESI-TOF): Exact mass calculated for $C_{14}H_{17}NO_2P^+$ $[M+H]^+$: 262.0991, found: 262.0989 $[M+H]^+$.

2.3.10 Synthesis of Methyl P-octyl-N-phenylphosphonamidate (2j)



The compound (**2j**) was synthesized according to **General Procedure C** and obtained in yield of 30%.

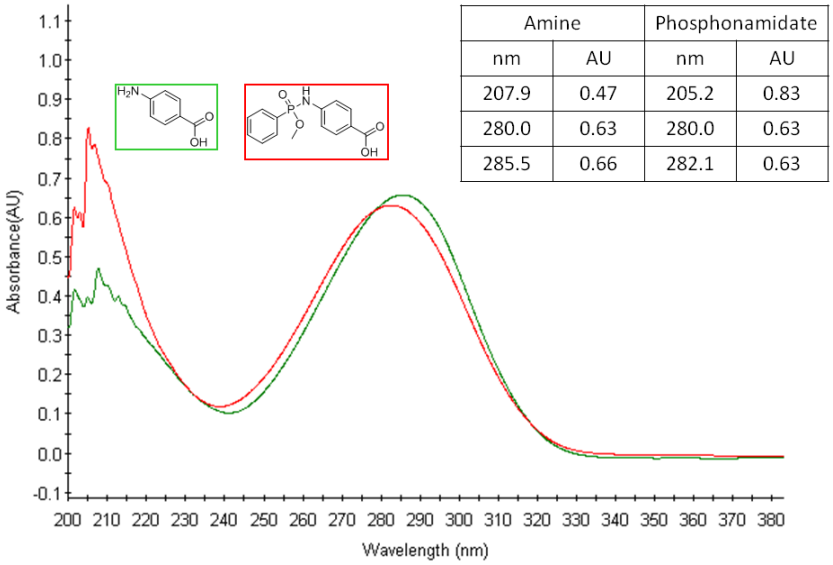
^{1}H-NMR (400 MHz, CD_3Cl): δ (ppm) = 7.23 (t, *J* = 7.9 Hz, 2H, CH), 6.98 (d, *J* = 8.2 Hz, 1H, CH), 6.92 (dd, *J* = 9.2, 5.6 Hz, 1H, CH), 6.73 (d, *J* = 9.0 Hz, 1H, CH), 3.70 (d, *J* = 11.2 Hz, 3H, CH_3), 1.98 – 1.78 (m, 2H), 1.70 – 1.49 (m, 2H), 1.35 – 1.25 (m, 4H), 1.25 – 1.11 (m, 4H, CH_2), 0.81 (t, J = 9.3 Hz, 3H, CH_3); ^{13}C-NMR (101 MHz, CD_3Cl): δ (ppm) = 140.67 (s), 129.60 (s), 121.36 (s), 117.17 (d, J = 6.1 Hz), 50.33 (d, J = 7.3 Hz), 31.86 (s), 30.61 (d, J = 16.9 Hz), 29.30 – 28.78 (m), 26.22 (s), 24.94 (s), 22.69 (s), 21.92 (d, J = 4.3 Hz), 14.17 (s); ^{31}P-NMR (162 MHz, CD_3Cl) δ (ppm) = 33.81.

HRMS (ESI-TOF): Exact mass calculated for $C_{14}H_{17}NO_2P^+$ $[M+H]^+$: 284.1774, found: 284.1801 $[M+H]^+$.

3 Synthesis of Phosphonamidate-Peptides (2k-o, 3a-c)

3.1 Comparison of the UV spectra of 4-Amino benzoic acid and 4-(Methoxy(phenyl)phosphorylamino)benzoic acid (2h)

Comparison of the UV spectra of the phosphonamidate (0.05 mM in MeOH) and the corresponding amine (0.05 mM in MeOH) shows no difference in the absorbance at 280 nm. Because of this similarity, the UV trace at 280 nm was used for the determination of the ratio between the desired phosphonamidate peptide and the amino peptide resulting from the hydrolysis of the P-N-bond.

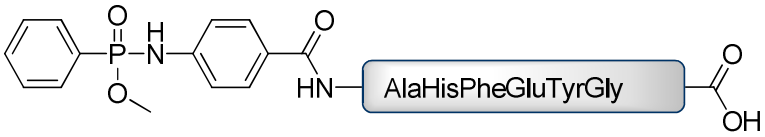


Amine		Phosphonamidate	
nm	AU	nm	AU
207.9	0.47	205.2	0.83
280.0	0.63	280.0	0.63
285.5	0.66	282.1	0.63

3.2 Preparation of Phosphonamidate-Peptides

General Procedure: The resin bound peptide was deprotected with 95% TFA (2.5% H_2O, 2.5% TIS) for 2 h. After washing of the resin with DMF and CH_2Cl_2 the resin was dried under high vacuum and flushed with argon. After swelling in 3 mL CH_2Cl_2 the phosphinic acid derivative and BSA were added. The reaction was allowed to proceed for 20 h at r.t. and under slight agitation. The resin was washed with CH_2Cl_2 and the ice-cold cleavage cocktail (1 M NaOH/ dioxane, 1:3, 1 mL) was added. After 15 min. The filtrate was neutralized with 1 M HCl and the conversions were determined by LC-MS, in which the UV trace was recorded at 280 nm.

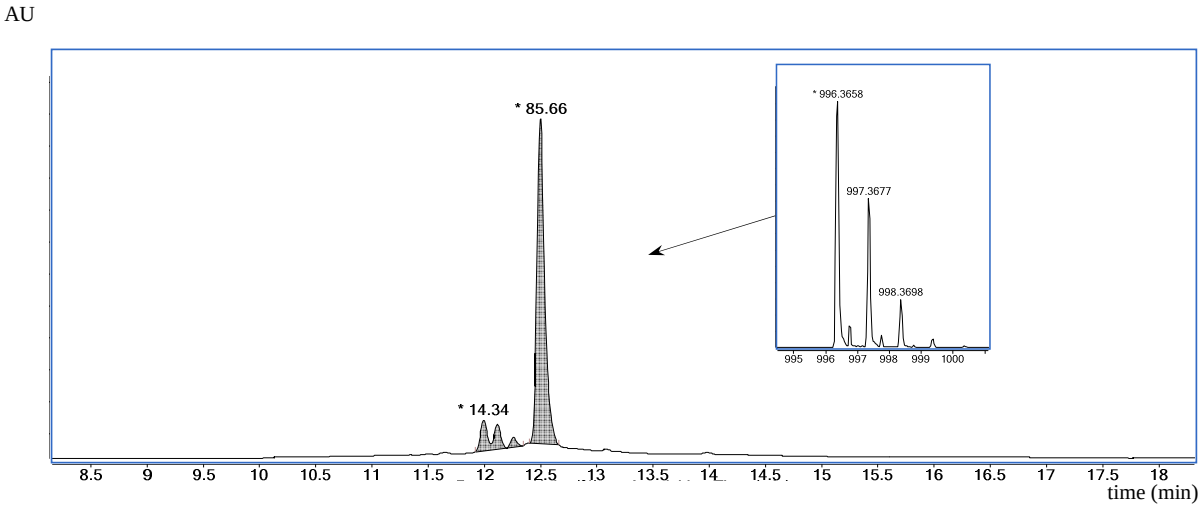
3.2.1 Synthesis of peptide 2k



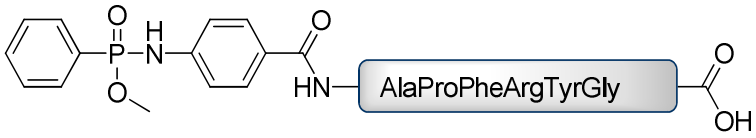
Compound **2k** was obtained from azido peptide **10a** (0.01 mmol) and Methyl phenylphosphinate (**7a**) (10 eq.) with BSA (40 eq.) according to the general procedure. An LC-MS analysis shows a conversion of 86%.

HRMS (ESI-TOF): *m/z* =996.3658 $[M+H]^+$ (calcd.: *m/z* =996.3651).

LC-MS (280 nm):

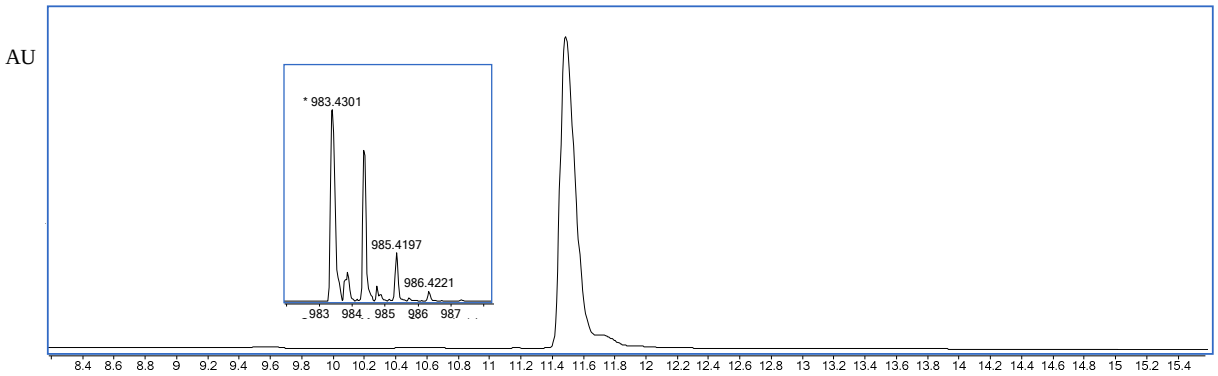


3.2.2 Synthesis of peptide 2l



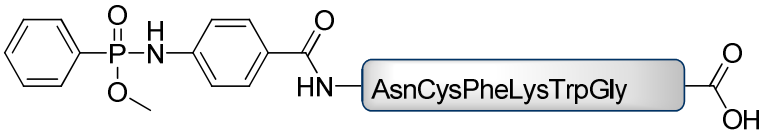
Compound **2l** was obtained from azido peptide **10b** (0.01 mmol) and Methyl phenylphosphinate (**7a**) (50 eq.) with BSA (200 eq.) according to the general procedure. The product was purified by HPLC and the pure phosphonamidate peptide **2l** was obtained in an overall isolated yield of 35%.

LC-MS (280 nm):



HRMS (ESI-TOF): *m/z* =983.4276 $[M+H]^+$ (calcd.: *m/z* =983.4175).

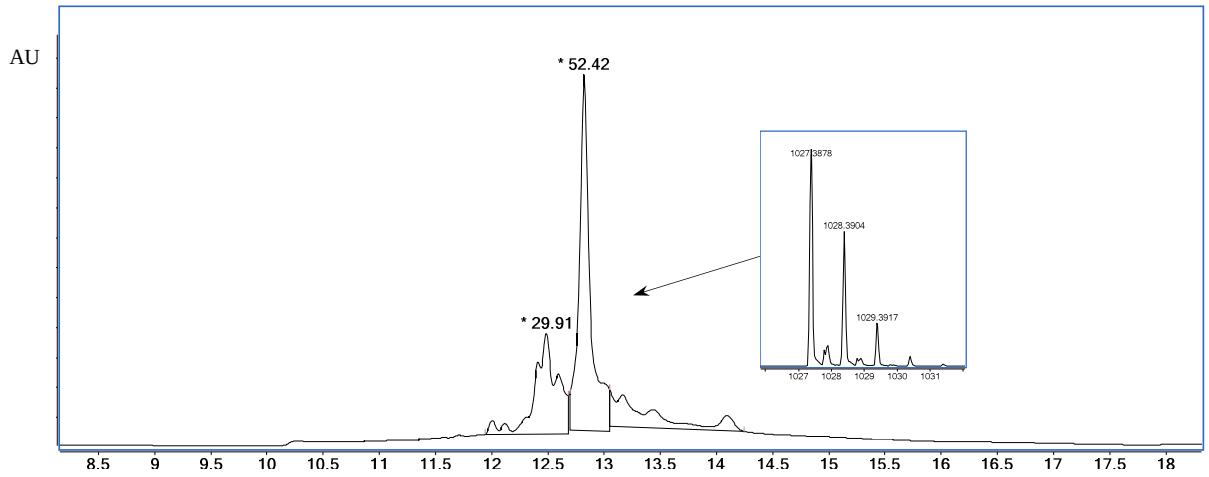
3.2.3 Synthesis of peptide 2m



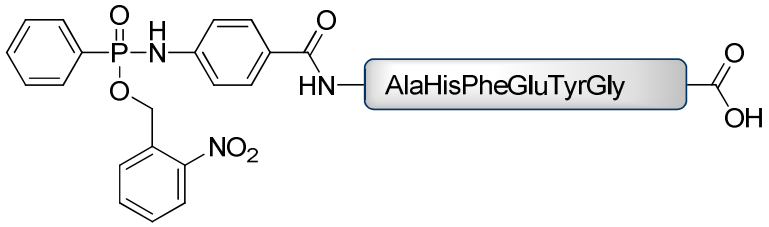
Phosphonamidate peptide **2m** was obtained from azido peptide **10c** (0.01 mmol) and Methyl phenylphosphinate (**7a**) (10 eq.) with BSA (40 eq.) according to the general procedure. LC-MS analysis of the crude product revealed a conversion of 52%.

HRMS (ESI-TOF): *m/z* =1027.3880 $[M+H]^+$ (calcd.: *m/z* =1027.3896).

LC-MS (280 nm):



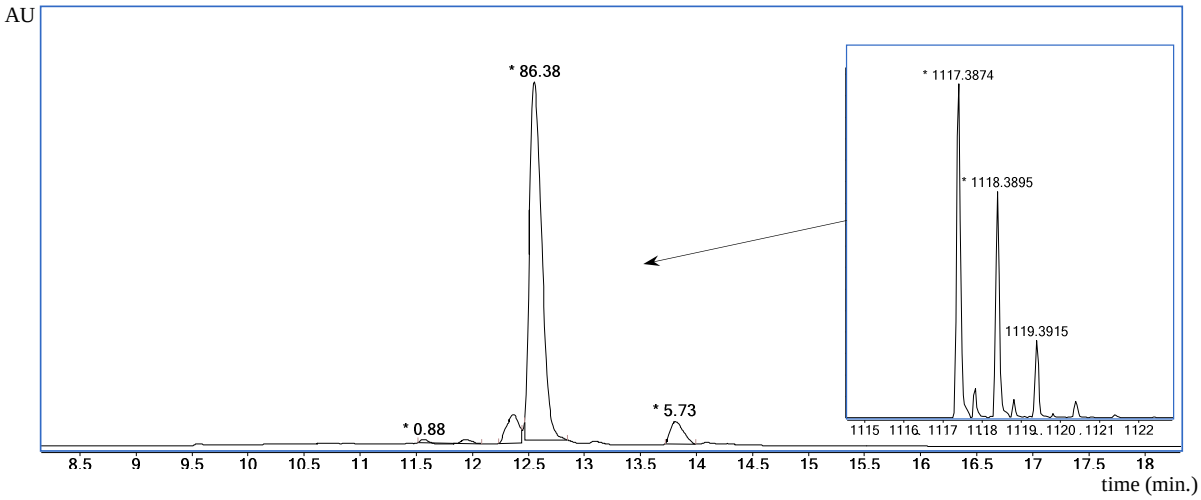
3.2.4 Synthesis of peptide 2n



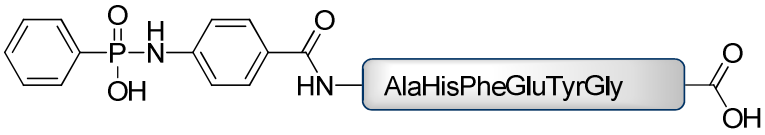
Compound **2n** was obtained from azido peptide **10a** (7.1 µmol) and 2-Nitrobenzyl phenylphosphinate (**7e**) (100 eq.) with BSA (400 eq.) according to the general procedure. LC-MS analysis of the crude product **2n** showed a conversion of 86%.

HRMS (ESI-TOF): m/z =1117.3882 [M+H]+ (calcd.: m/z =1117.3815).

LC-MS (280 nm):



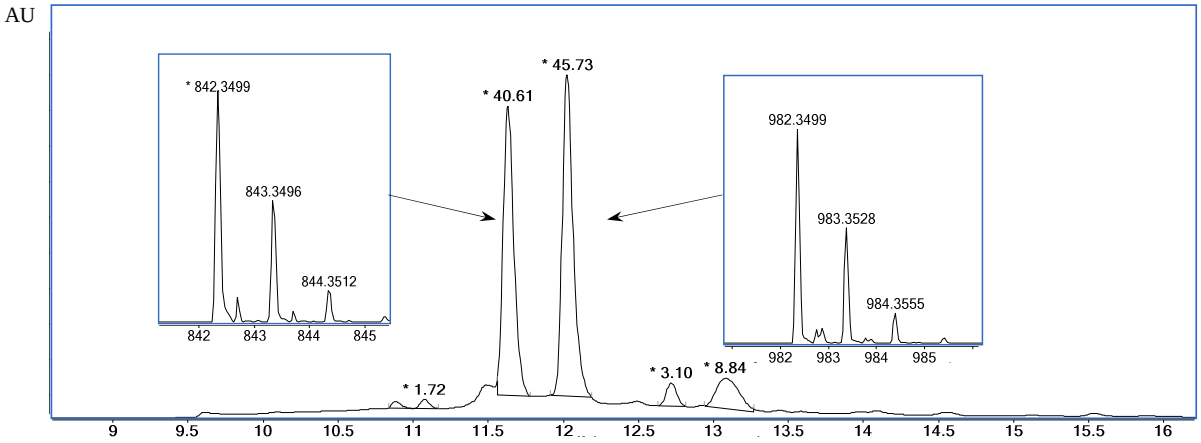
3.2.5 UV-cleavage of the o-Nitro-benzylester



The crude o-nitro benzylphosphonamidate peptide (**2n**) solution obtained after cleavage of the peptide from the resin was irradiated with a UV lamp for 10 min. and analyzed again by LC-MS. In addition to the desired phosphonamidate **3a** (46%) the amino peptide (41%) can be detected due to P-N-bond cleavage.

HRMS (ESI-TOF): *m/z* =982.3499 $[M+H]^+$ (calcd.: *m/z* =982.3495).

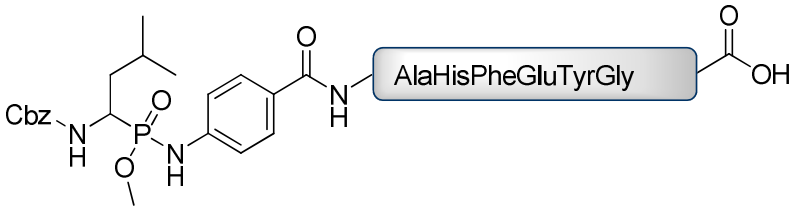
LC-MS (280 nm):



Amino peptide:

HRMS (ESI-TOF): *m/z* =842.3499 $[M+H]^+$ (calcd.: *m/z* =842.3468).

3.2.6 Synthesis of peptide 2o

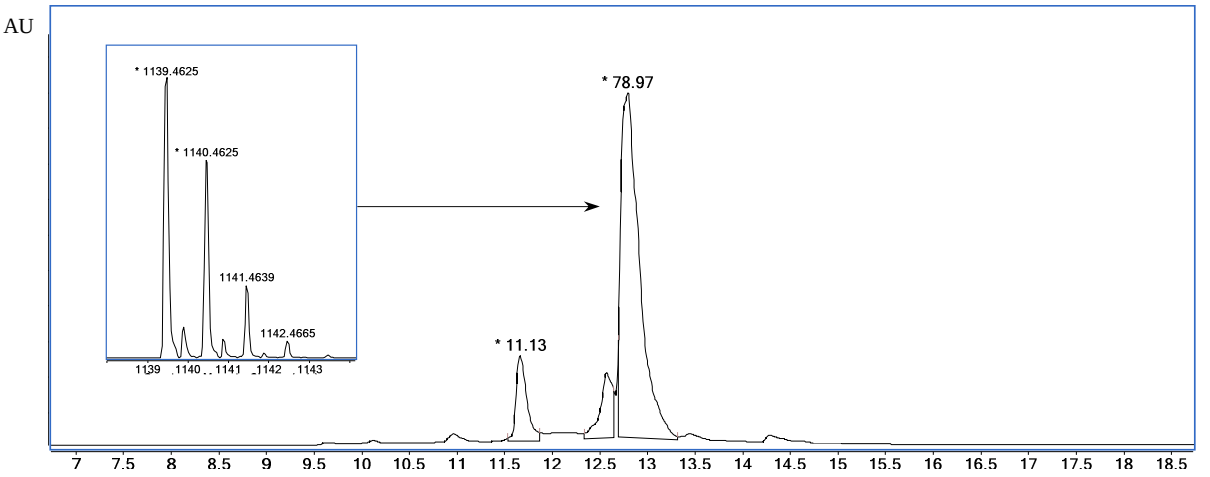


Phosphonamidate peptide **2o** was obtained from azido peptide **10a** (2.5 µmol) and phosphinate **2f** (60 eq.) with BSA (180 eq.) according to the general procedure. An LC-MS spectrum of the crude product **2o** is shown below. Integration of the UV-trace at 280 nm shows about 11% of the amino peptide and 79% of the desired phosphonamidate **2o**.

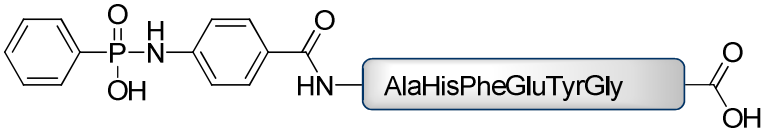
Separation of the sample was performed on a Luna 5u C18(2) 100 A column (5 µm, 4.6×150 mm) at a flow rate of 0.6 mL/min. The following solvent (A =1% NH_4Ac in H_2O, B = MeCN) gradient was applied: 0% B 0-3 min; 0-100% B 3-14 min; 100% B 14-18 min.

HRMS (ESI-TOF): m/z = 1139.4625 [M+H]+ (calcd.: m/z = 1139.4598).

LC-MS (280 nm):



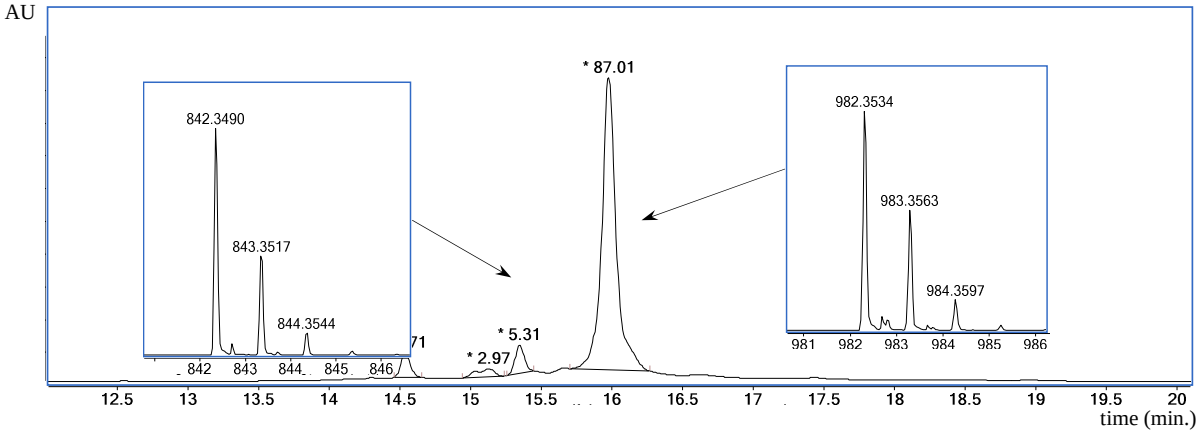
3.2.7 Synthesis of peptide 3a



Phosphonamidate peptide **3a** was obtained from azido peptide **10a** (2.5 µmol) and phenyl phosphinic acid **11a** (50 eq.) with BSA (300 eq.) according to the general procedure. An LC-MS spectrum of the crude product showed about 5% of the amino peptide and 87% of the desired phosphonamidate.

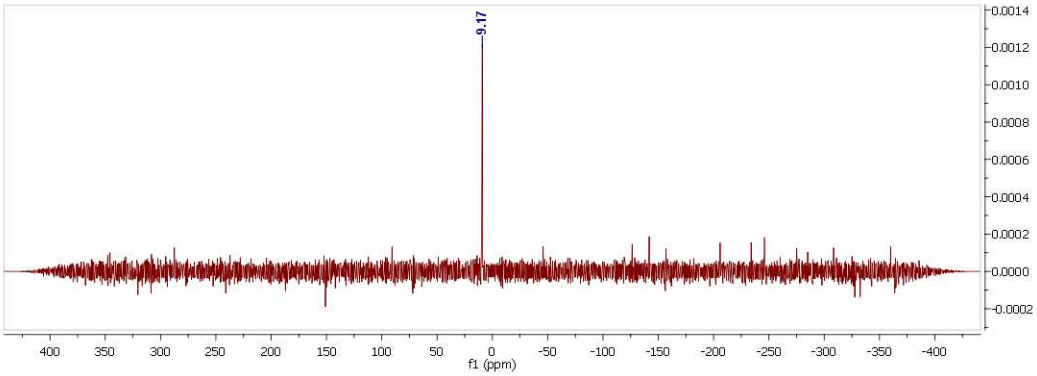
HRMS (ESI-TOF): *m/z* =982.3517 $[M+H]^+$ (calcd.: *m/z* =982.3495).

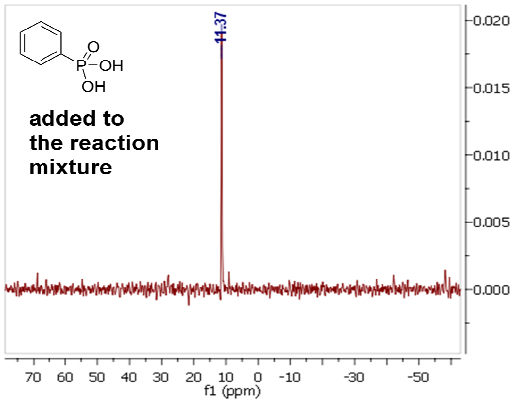
LC-MS (280 nm):



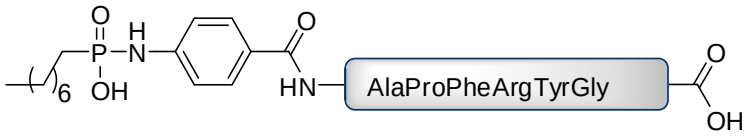
HRMS (ESI-TOF): *m/z* =982.3517 [M+H]+ (calcd.: *m/z* =982.3495).

Measurement of a ^{31}P-NMR of **3a** showed just one phosphorous species at 9.17 ppm.





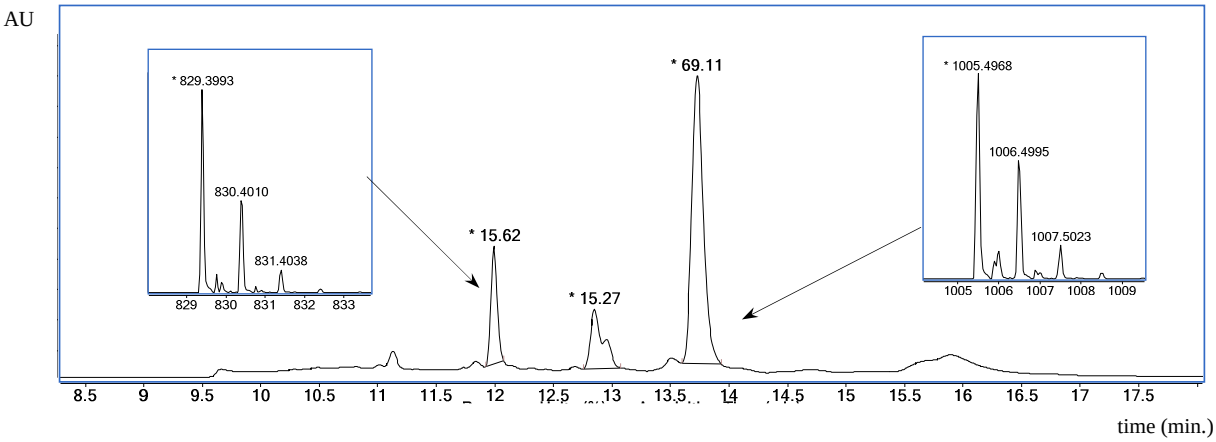
3.2.8 Synthesis of peptide 3b



Phosphonamidate peptide **3b** was obtained from azido peptide **10b** (0.0025 mmol) and Octyl phosphinic acid (**11b**) (100 eq.) with BSA (300 eq.) according to the general procedure. An LC-MS spectrum of the crude product **3b** showed about 16% of the amino peptide and 69% of the desired phosphonamidate.

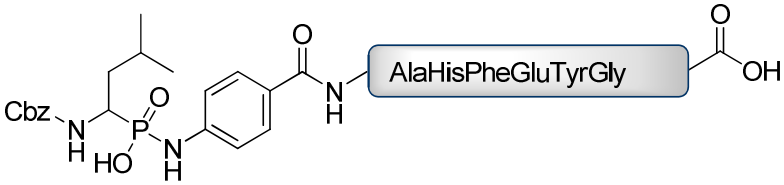
HRMS (ESI-TOF): *m/z* =1005.4979 $[M+H]^+$ (calcd.: *m/z* =1005.4958).

LC-MS (280 nm):



HRMS (ESI-TOF): *m/z* =829.3993 $[M+H]^+$ (calcd.: *m/z* =829.3991).

3.2.9 Synthesis of phosphonamidate peptide 3c

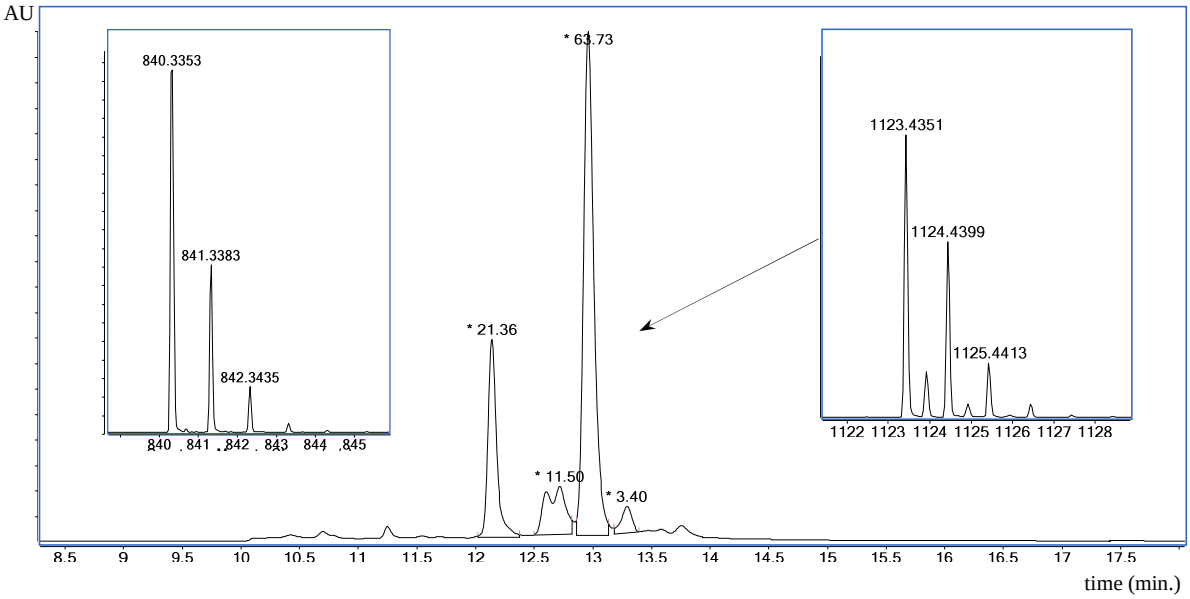


Phosphonamidate peptide **3c** was obtained from azido peptide **10a** (1.8 µmol) and 1-(Benzyloxycarbonylamino)-3-methylbutylphosphinic acid (**11c**) (80 eq.) with BSA (240 eq.) according to the general procedure. An LC-MS spectrum of the crude product **3c** is shown below. Integration of the UV-trace at 280 nm shows about 11% of the amino peptide and 64% of the desired phosphonamidate **3c**.

Separation of the sample was performed on a Luna 5u C18(2) 100 A column (5 µm, 4.6×150 mm) at a flow rate of 0.6 mL/min. The following solvent (A =1% NH_4Ac in H_2O, B = MeCN) gradient was applied: 0% B 0-3 min; 0-100% B 3-17 min; 100% B 17-19 min.

HRMS (ESI-TOF): m/z =1123.4351 $[M-H]^-$ (calcd.: m/z =1123.4296).

LC-MS spectrum (280 nm):



HRMS (ESI-TOF): *m/z* =840.3353 $[M-H]^-$(calcd.: *m/z* =840.3322).

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