Electronic supplementary information (ESI)

The role of phosphopeptides in the mineralisation of silica

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General information

All chemicals and solvents used for the synthesis of peptide building blocks or peptide synthesis were purchased from commercial sources and were not purified further, if not indicated. Unless otherwise noted, all reactions were executed under an argon atmosphere at ambient pressure.

For reaction control by analytical thin layer chromatography TLC plates coated with silica gel 60 F_{254} (*Merck KGaA*) were used and detection was carried out by fluorescence quenching under UV light (λ= 254 nm) or by staining with ninhydrin solution followed by heating to 350 °C. Flash chromatography was performed on silica gel 60 (60 Å, 230-400 mesh) from *Macherey-Nagel*.

The purification of the peptides was achieved by semi-preparative reversed-phase HPLC with a *Thermo Scientific Dionex* UltiMate3000 including a MWD-3000 detector and *a Macherey-Nagel* VP Nucleodur C18 gravity column (125 x 21 mm, 5 µm, 100 Å), using the eluents A: H_2O + 0.1% TFA and B: MeCN + 0.085% TFA. Analytical HPLC was performed on the same system using a *ACE* UltraCore 2.5 SuperC18, 150 x 2.1 µm. Afterwards, the peptides were lyophilised with a *Christ Alpha* 2–4LDplus.

The NMR spectra were recorded on *Bruker* AV 600, AV 500 or HD 500 spectrometers. Chemical shifts (δ) are given in ppm referring to the solvent signal. The coupling constants are ^3J couplings, unless otherwise indicated. All mass spectra were recorded on a *Finnigan* LTQ-FT spectrometer from *Fisher Thermo Scientific*.

Peptide synthesis

Resin loading

2-Chlorotritylchloride resin (1.60 mmol/g) was loaded with Fmoc-Lys(Boc)-OH by adding the protected amino acid (1.50 equiv.) and DIPEA (6.00 equiv.) in DMF (10 mL/g resin) and stirring for 3 h. The resin was washed several times with DMF, MeOH and DCM before it was treated with a mixture of DCM/MeOH/DIPEA (80:15:5) two times for 30 min. Finally, the resin was washed again several times with DMF, MeOH and DCM and dried under vacuum. The loading of the used resin was determined to be 0.62 mmol/g by UV-Vis spectroscopy at 289 nm and 300 nm after cleaving the Fmoc-protecting group with 20% piperidine in DMF for 20 min.

Solid phase peptide synthesis

Peptides were synthesized on a microwave assisted *CEM Liberty Blue* peptide synthesizer. Fmoc-strategy was applied. The 2-chlorotritylchloride resin loaded with Fmoc-Lys(Boc)-OH (scheduled quantity: 0.1 mmol, 1.00 equiv.) was swollen in DMF for 30 min at ambient temperature before it ran through the following cycles of Fmoc-deprotection and amino acid coupling.

- **Fmoc-deprotection:**

  T = 50 °C, $P_{microwave}$ = 30 W, t = 210 s with piperidine (20 w% in DMF, 3.00 mL/deprotection)

- **Amino acid coupling:**

  for amino acids except Fmoc-Arg(Pbf)-OH:

  T = 50 °C, $P_{microwave}$ = 30 W, t = 600 s with Fmoc-protected amino acid (0.2 M in DMF, 5.00 equiv., 2.5 mL/coupling), DIC (0.5 M in DMF, 5.00 equiv., 1.0 mL/coupling) and Oxyma (1.0 M in DMF, 5.00 equiv., 0.5 mL/coupling)

  for Fmoc-Arg(Pbf)-OH:

  1. T = 25 °C, $P_{microwave}$ = 0 W, t = 1500 s
  2. T = 50 °C, $P_{microwave}$ = 35 W, t = 660 s

  with Fmoc-protected amino acid (0.2 M in DMF, 5.00 equiv., 2.5 mL/coupling), DIC (0.5 M in DMF, 5.00 equiv., 1.0 mL/coupling) and Oxyma (1.0 M in DMF, 5.00 equiv., 0.5 mL/coupling).

TBDMS deprotection on the solid phase

The peptide resin (0.10 mmol, 1.00 equiv.) was swollen in THF for 30 min at ambient temperature before it was treated with tetrabutylammonium fluoride (1.0 M in THF, 5.00 equiv./TBDMS group) for 90 min. The resin was washed several times with THF, MeOH, DCM and dried under vacuum.

Phosphorylation on the solid phase

The phosphorylation was carried out under an argon atmosphere with exclusion of moisture. Therefore, the peptide resin (0.10 mmol, 1.00 equiv.) with unprotected serine-hydroxyl groups was placed in a special reaction vessel.

The resin was swollen in dry DMF (2.0 mL/OH group) at rt for 120 min before 1*H*-tetrazol (0.45 M in MeCN, 7.5 equiv./OH group,) and dibenzyl *N,N*-diisopropylphosphoramidite (3.0 equiv./OH group) were added. The resin was treated at rt for 180 min before it was washed several times with dry DMF. The peptide resin was oxidised by adding *tert*-butyl hydroperoxide (5.55 M in decane, 37.7 equiv./OH group,) in dry DMF (2.0 mL/OH group) at rt for 90 min. Finally, the resin was washed again several times with DMF and DCM and dried under vacuum.

Resin cleavage

Resin cleavage was performed with a mixture of TFA/H_2O/TIPS (95:2.5:2.5) for 3 h at ambient temperature. The resin was washed several times with TFA and the combined filtrate was concentrated under reduced pressure. Peptides were precipitated from cold diethyl ether (40 mL), washed two to three times with diethyl ether and lyophilised from water.

Purification

If it was possible, the peptides were purified by semi-preparative reversed-phase HPLC on a *Thermo Scientific Dionex* UltiMate3000 with an MWD-3000 detector with the following conditions.

Column: *Macherey-Nagel* VP Nucleodur C18 gravity (125 x 21 mm, 5 µm, 110 Å)

Gradient: 2-30% B in 30 min (A: water + 0.1% TFA, B: acetonitrile + 0.085% TFA), flow rate: 15.0 mL/min, temperature 28 °C.

2-30% B in 60 min (A: water + 0.1% TFA, B: acetonitrile + 0.085% TFA), flow rate: 15.0 mL/min, temperature 28 °C.

Analytical HPLC

Analytical HPLC was performed on a *Thermo Scientific Dionex* UltiMate3000 with an MWD-3000 detector under the following conditions (if not indicated otherwise).

Column: *ACE* UltraCore 2.5 SuperC18, 150 x 2.1 µm

Gradient: 2-30% B in 10 min (A: water + 0.1% TFA, B: acetonitrile + 0.085% TFA), flow rate: 0.45 mL/min, temperature 28 °C.

Silica precipitation assays

Preparation of silicic acid

A freshly prepared solution of 1 M tetramethoxysilane in 1 mM HCl was incubated at ambient temperature for 45 min and immediately used as a source of silicic acid.

Silica precipitation assay[1]

The polyamine was dissolved in 50 mM sodium acetate buffer (pH 5.5) resulting in a concentration of 10 mM. Each peptide was dissolved in the same buffer resulting in a concentration of 4 mM. Both solutions were mixed, diluted with 50 mM sodium acetate buffer (pH 5.5) to the desired final volume (200 µL) and concentration and incubated for 30 min. Silica precipitation was initiated by the addition of 1 M silicic acid (20 µL), prepared as described above, to the mixture of polyamine and peptide. After exact 10 min at ambient temperature, the silica formation was cancelled by decreasing the pH with 1 M HCl (20 µL). Afterwards, the precipitate was centrifuged (13000 rpm) for 5 min and the silica pellet was washed three times with ultrapure water (400 µL).

β-silicomolybdate assay

The precipitated silica pallet was dissolved in 2 M NaOH (500 µL) for 60 min at ambient temperature and quantified photometrically by a modified β-silicomolybdate method.[2] 1.35 mL HCl (37%) was dissolved in water (40.3 mL) and $[(NH_4)_6Mo_7O_{24} \times 4\ H_2O]$ (774.2 mg) was dissolved in water (9.70 mL). Both solutions were mixed, and the pH was adjusted to 1.12 with 2 M NaOH (molybdate solution). To the sample solution containing dissolved silica (0, 10 or 20 µL) and a corresponding volume of 2 M NaOH (40, 30 or 20 µL), water (160 µL) and molybdate solution (800 µL) were added. The absorbance of the solution was monitored at a wavelength of 370 nm. A silicon atomic absorption standard solution was used to generate calibration curves.

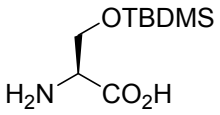
SEM measurements

To ensure an evenly distribution of the silica spheres and prevent aggregation of the spheres, the silica pellet was first suspended in ultrapure water (400 µL) by ultrasonic treatment. Each silica pellet suspension (5 µL) was then dropped onto a surface-modified stainless-steel sample holder and air dried. The surface modification involves two steps, sputter coating the stainless steel with a 6 nm thick gold layer and a subsequent tempering at 300 °C for 10 h. Due to the thermal treatment a dewetting[3] of the gold layer takes places whereby gold nanoparticles are formed. The gold nanoparticles and the partial oxidation of the stainless-steel surface during the thermal treatment create a rough surface on which the silica spheres get stuck and distribute evenly while drying. After the silica pellet suspension is dried, the sample was sputter coated again with a 4 nm thick layer of gold to prevent charging effects and analysed with a scanning electron microscope (JSM-7500F, Jeol, Japan).

Experimental procedures

Building block synthesis

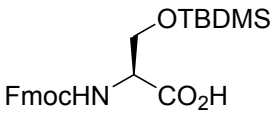
***O*-(*tert*-butyldimethylsilyl)-L-serine (S1)** [4]



S1

L-Serine (2.63 g, 25.0 mmol, 1.0 equiv.) was suspended in dry DMF and cooled to 0 °C. Imidazole (3.41 g, 50.0 mmol, 2.0 equiv.) and TBDMSCl (4.15 g, 27.5 mmol, 1.1 equiv.) were added in one portion, the mixture was allowed to warm to rt and stirred for 18 h. DMF was removed by condensation and the remaining slurry was treated with 80 mL H_2O/pentane (1:1) and stirred for 4 h. The resulting colourless precipitate was filtered off, washed with cold pentane and dried under vacuum to give L-Ser(OTBS)-OH **S1** (3.88 g, 17.7 mmol, 71% yield). ^{1}H-NMR (300 MHz, CD_3OD, 300 K): δ = 4.06 (dd, 1H, ^{2}J=10.9 Hz, ^{3}J=3.8 Hz, β-CH_2) 3.99 (dd, 1H, ^{2}J=10.9 Hz, ^{3}J=6.1 Hz, β-CH_2), 3.61 (dd, 1H, ^{3}J=6.0 Hz, ^{3}J=3.8 Hz, α-CH), 0.93 (s, 9H, TBDMS), 0.13 (s, 6H, TBDMS) ppm; ^{13}C-NMR (75 MHz, CD_3OD, 300 K): δ = 171.7, 63.6, 58.0, 26.4, 19.3, −5.3, −5.4 ppm; HRMS (ESI): calcd. for [$C_9H_{21}NO_3SiH^+$]: 220.1363, found: 220.1369.

***N*-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*O*-(*tert*-butyldimethylsilyl)-L-serine (S2)** [4]

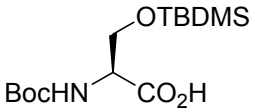


S2

L-Ser(OTBS)-OH **S1** (3.85 g, 17.6 mmol, 1.0 equiv.) was suspended in 5% aqueous solution of Na_2CO_3 (100 mL) and the pH value was adjusted to 8 with 2 M HCl. The mixture was cooled to 0 °C and a solution of FmocOSu (8.89 g, 26.4 mmol, 1.5 equiv.) in acetone (20 mL) was added dropwise. The resulting suspension was allowed to warm to rt and stirred for 18 h. The alkaline aqueous solution was washed with cyclohexane (200 mL) and then acidified to pH 3 with 2 M HCl and extracted with EtOAc (80 mL x 3). The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The obtained crude material was purified by silica gel column chromatography (cyclohexane:EtOAc:AcOH, 3:1:0.01; v/v/v) to afford Fmoc-L-Ser(OTBS)-OH **S2** (5.88 g, 13.3 mmol, 71% yield) as a colourless solid. R_f 0.38 (CH:EE:AcOH, 3:1:0.01, v/v/v); ^{1}H-NMR (300 MHz, $DMSO_{d6}$, 300 K): δ = 12.77 (s, 1H, CO_2H), 7.89 (d, 2H, ^{3}J=7.5 Hz, $Fmoc^{Ar}$), 7.73 (d, 2H, ^{3}J=7.2 Hz, $Fmoc^{Ar}$), 7.42 (t, 2H, ^{3}J=7.1 Hz, $Fmoc^{Ar}$), 7.42 (d, 1H, ^{3}J=8.3 Hz, α-NH), 7.32 (t, 2H, ^{3}J=7.4 Hz, $Fmoc^{Ar}$), 4.33-4.18 (m, 3H, $Fmoc^{Alip}$), 4.18-4.07 (m, 1H, α-CH), 3.90-3.77 (m, 2H, β-CH_2), 0.84 (s, 9H, TBDMS), 0.03 (s, 6H, TBDMS) ppm; ^{13}C-NMR (75 MHz, $DMSO_{d6}$, 300 K): δ = 171.6, 155.9, 143.8, 143.7, 140.7, 127.6, 127.0, 125.2, 120.0, 65.8,

62.7, 56.1, 46.6, 25.7, 17.9, –5.5 ppm; HRMS (ESI): calcd. for [$C_{24}H_{31}NO_5SiNa^+$]: 464.1864, found: 464.1862.

***N*-(*tert*-butoxycarbonyl)-*O*-(*tert*-butyldimethylsilyl)-L-serine (S3)**

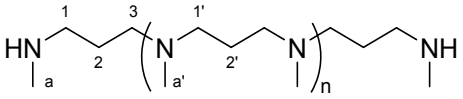


S3

L-Ser(OTBS)-OH **S2** (1.10 g, 5.00 mmol, 1.0 equiv.) was suspended in 5% aqueous solution of Na_2CO_3 (25 mL) and the pH value was adjusted to 8 with 2 M HCl. A solution of Boc_2O (1.31 g, 6.00 mmol, 1.2 equiv.) in acetone (50 mL) was added dropwise. The resulting suspension was stirred at rt for 18 h. The alkaline aqueous mixture was washed with cyclohexane (100 mL) and then acidified to pH 3 with 2 M HCl and extracted with EtOAc (100 mL x 3). The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The obtained crude material was purified by silica gel column chromatography (DCM:AcOH, 1:0.01; v/v) to afford Boc-L-Ser(OTBS)-OH **S3** (1.15 g, 3.61 mmol, 72% yield) as a colourless oil. R_f 0.20 (DCM:AcOH, 1:0.01, v/v); ^{1}H-NMR (300 MHz, $DMSO_{d6}$, 300 K): δ = 12.66 (s, 1H, CO_2H), 6.61 (d, 1H, ^{3}J=8.4 Hz, α-NH) 4.05 (dt, 1H, ^{3}J=8.5 Hz, ^{3}J=5.1 Hz, α-CH), 3.79 (d, 2H, ^{3}J=5.3 Hz, β-CH_2), 1.38 (s, 9H, Boc), 0.84 (s, 9H, TBDMS), 0.02 (s, 6H, TBDMS) ppm; ^{13}C-NMR (75 MHz, $DMSO_{d6}$, 300 K): δ = 171.8, 155.1, 78.1, 62.8, 55.6, 28.1, 25.7, 17.9, –5.5 ppm; HRMS (ESI): calcd. for [$C_{14}H_{29}NO_5SiNa^+$]: 342.1707, found: 342.1705.

LCPA synthesis

LCPA H_3C-NH-[$(CH_2)_3$-N(CH_3)-]$_n$-H (S4) [5]



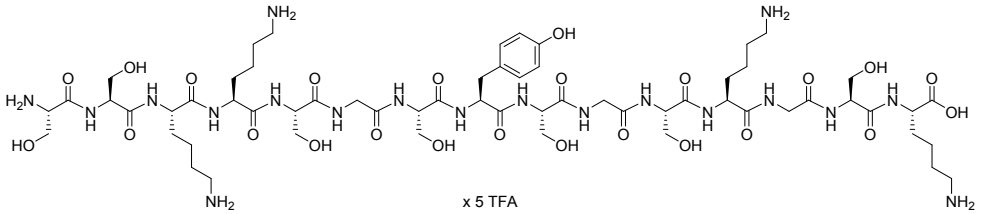
S4

N,*N*-*Bis*[3-(methylamino)propyl]methylamine (4.60 mL, 26.5 mmol, 2.0 equiv.) was dissolved in anhydrous ethanol (1.00 mL) and a solution of 1,3-dibromopropane (1.35 g, 13.3 mmol, 1.0 equiv.) in anhydrous ethanol (4.45 mL) was added dropwise by a syringe pump (25 µL/min) at room temperature. The reaction was performed according to the literature.[5] The LCPA mixture **S4** (1.25 g, 1.54 mmol, 12%) was obtained as a yellow oil. The average chain length was calculated on 12 nitrogens from the integral ratio of terminal and internal protons of the methyl groups. The yield was determined for the average chain length of 12 nitrogen. ^{1}H-NMR (600 MHz, D_2O, 300 K): δ = 2.57 (t, 4H, ^{3}J=7.3 Hz, 1-CH_2), 2.44 (t, 4H, ^{3}J=7.8 Hz, 3-CH_2), 2.40 (t, 36H, ^{3}J=7.8 Hz, 1'-CH_2), 2.34 (s, 6H, a-CH_3), 2.22 (s, 30H, a'-CH_3), 1.70 - 1.65 (m, 22H, 2-,2'-CH_2) ppm. ^{13}C-NMR (75 MHz, D_2O, 300 K): δ = 54.5, 54.3, 48.7, 41.0, 34.4, 25.3, 22.9 ppm. HRMS (ESI): calcd. for [$C_{45}H_{104}N_{12}H^+$]: 813.8580, found: 813.8593.

Peptide synthesis

The following peptides were synthesized under standard coupling condition on a microwave assisted peptide synthesizer (*Liberty Blue*, CEM) on a 0.10 mmol scale with the conditions described above. If it was possible, the peptides were purified by semi-preparative reversed-phase HPLC.

Sil01 **H_2N-S^1-S^2-K^3-K^4-S^5-G^6-S^7-Y^8-S^9-G^{10}-S^{11}-K^{12}-G^{13}-S^{14}-K^{15}-CO_2H (S5)**



S5

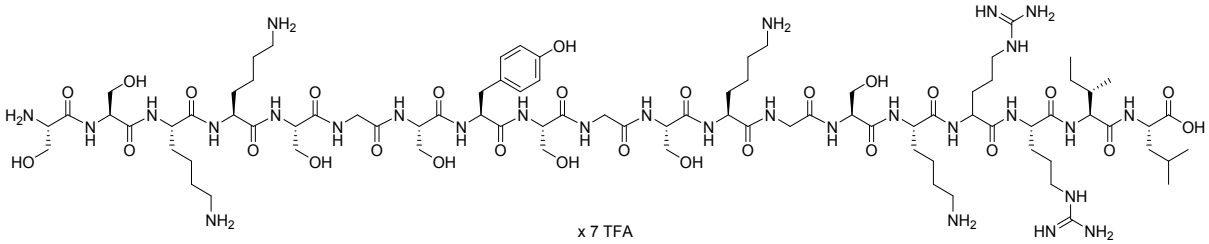
The crude peptide Sil01 **S5** was obtained as a colourless solid (185 mg, 90.5 µmol 91%). The purification of 100 mg of crude peptide by semi-preparative reversed-phase HPLC yields 57 mg pure peptide.

Table 1: ^{1}H-NMR signal assignment for Sil01 **S5** (600 MHz, 300 K, $DMSO_{d6}$).

Amino Acid	δ(α) / ppm	δ(β) / ppm	δ(γ) / ppm	δ(other) / ppm		δ(NH) / ppm
Ser[1]	3.92	3.75, 3.68	-	OH:	5.52	7.68
Ser[2]	4.42	3.64, 3.59	-	OH:	5.15	8.58
Lys[3]	4.25	1.71, 1.53	1.33	δ:	1.52	8.09
				ε:	2.75	
				NH:	7.68	
Lys[4]	4.32 - 4.21	1.71, 1.53	1.33	δ:	1.52	8.17 - 8.00
				ε:	2.75	
				NH:	7.68	
Ser[5]	4.38 - 4.25	3.70 - 3.49	-	OH:	5.11 - 4.98	8.13 - 7.87
Gly[6]	3.86 - 3.68	-	-		-	8.13 - 8.00
Ser[7]	4.38 - 4.25	3.70 - 3.49	-	OH:	5.11 - 4.98	8.13 - 7.87
Tyr[8]	4.48	2.97, 2.70	-	aro.:	7.03, 6.63	8.07
				OH:	9.18	
Ser[9]	4.38 - 4.25	3.70 - 3.49	-	OH:	5.11 - 4.98	8.13 - 7.87
Gly[10]	3.86 - 3.68	-	-		-	8.13 - 8.00
Ser[11]	4.38 - 4.25	3.70 - 3.49	-	OH:	5.11 - 4.98	8.13 - 7.87

Lys[12]	4.32 - 4.21	1.71, 1.53	1.33	δ:	1.52	8.17 - 8.00
				ε:	2.75	
				NH:	7.68	
Gly[13]	3.86 - 3.68	-	-		-	8.13 - 8.00
Ser[14]	4.38 - 4.25	3.70 - 3.49	-	OH:	5.11 - 4.98	8.13 - 7.87
Lys[15]	4.18	1.73, 1.61	1.33	δ:	1.52	8.11
				ε:	2.75	
				NH:	7.68	
				CO_2H:	12.69	

R5 **H_2N-S[1]-S[2]-K[3]-K[4]-S[5]-G[6]-S[7]-Y[8]-S[9]-G[10]-S[11]-K[12]-G[13]-S[14]-K[15]-R[16]-R[17]-I[18]-L[19]-CO_2H (S6)**



S6

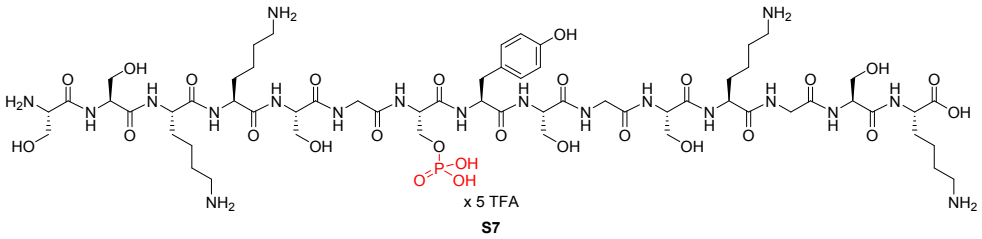
The crude peptide R5 **S6** was obtained as a colourless solid (110 mg, 39.1 µmol 39%). The purification of 50 mg of crude peptide by semi-preparative reversed-phase HPLC yields 18 mg pure peptide.

Table 2: ^{1}H-NMR signal assignment for R5-peptide **S6** (600 MHz, 300 K, $DMSO_{d6}$).

Amino Acid	δ(α) / ppm	δ(β) / ppm	δ(γ) / ppm	δ(other) / ppm		δ(NH) / ppm
Ser[1]	3.92	3.74, 3.67	-	OH:	5.50	7.69
Ser[2]	4.42	3.64, 3.58	-	OH:	5.14	8.57
Lys[3]	4.25	1.71, 1.53	1.33	δ:	1.52	8.09
				ε:	2.75	
				NH:	7.69	
Lys[4]	4.25	1.71, 1.53	1.33	δ:	1.52	8.10
				ε:	2.75	
				NH:	7.69	
Ser[5]	4.39 - 4.24	3.67 - 3.48	-	OH:	5.13 - 4.96	8.14 - 7.87
Gly[6]	3.89 - 3.65	-	-		-	8.14 - 8.00
Ser[7]	4.39 - 4.24	3.67 - 3.48	-	OH:	5.13 - 4.96	8.14 - 7.87

Tyr[8]	4.48	2.97, 2.71	-	aro.:	7.03, 6.63	8.03
				OH:	9.17	
Ser[9]	4.39 - 4.24	3.67 - 3.48	-	OH:	5.13 - 4.96	8.14 - 7.87
Gly[10]	3.89 - 3.65	-	-		-	8.14 - 8.00
Ser[11]	4.39 - 4.24	3.67 - 3.48	-	OH:	5.13 - 4.96	8.14 - 7.87
Lys[12]	4.25	1.71, 1.53	1.33	δ:	1.52	8.10
				ε:	2.75	
				NH:	7.69	
Gly[13]	3.89 - 3.65	-	-		-	8.14 - 8.00
Ser[14]	4.39 - 4.24	3.67 - 3.48	-	OH:	5.13 - 4.96	8.14 - 7.87
Lys[15]	4.25	1.71, 1.53	1.33	δ:	1.52	8.10
				ε:	2.75	
				NH:	7.69	
Arg[16]	4.28	1.72 - 1.44	1.47	δ:	3.08	8.09 – 7.92
				η-NH:	7.55	
Arg[17]	4.28	1.72 - 1.44	1.47	δ:	3.08	8.09 – 7.92
				η-NH:	7.55	
Ile[18]	4.19	1.72	1.43, 1.07	γ:	0.85	7.74
				δ:	0.80	
Leu[19]	4.20	1.52	1.62	δ:	0.88, 0.82	8.16
				CO_2H	12.52	

p1-Sil01 H_2N-S^1-S^2-K^3-K^4-S^5-G^6-pS^7-Y^8-S^9-G^{10}-S^{11}-K^{12}-G^{13}-S^{14}-K^{15}-CO_2H **(S7)**



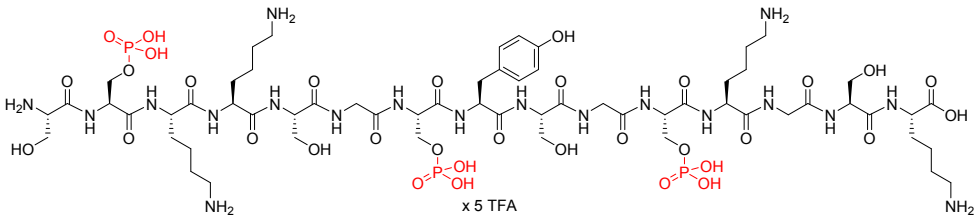
S7

The crude peptide p1-Sil01 **S7** was obtained as a colourless solid (150 mg, 70.6 µmol 71%).

Table 3: ^{1}H-NMR signal assignment for p1-Sil01 **S7** (600 MHz, 300 K, $DMSO_{d6}$).

Amino Acid	δ(α) / ppm	δ(β) / ppm	δ(γ) / ppm	δ(other) / ppm		δ(NH) / ppm
Ser[1]	3.93	3.77, 3.69	-	OH:	5.54	7.71
Ser[2]	4.41	3.74 - 3.53	-	OH:	5.38 - 4.68	8.63
Lys[3]	4.31- 4.21	1.73, 1.57	1.34	δ:	1.53	8.00 - 8.33
				ε:	2.75	
				NH:	7.71	
Lys[4]	4.31- 4.21	1.73, 1.57	1.34	δ:	1.53	8.00 - 8.33
				ε:	2.75	
				NH:	7.71	
Ser[5]	4.39 - 4.27	3.74 - 3.53	-	OH:	5.38 - 4.68	8.33 - 7.82
Gly[6]	3.85 - 3.68	-	-		-	8.00 - 8.33
pSer[7]	4.21	3.93, 3.83	-		-	8.33 - 7.82
Tyr[8]	4.45	2.99, 2.71	-	aro.:	7.03, 6.62	8.10
				OH:	9.17	
Ser[9]	4.39 - 4.27	3.74 - 3.53	-	OH:	5.38 - 4.68	8.33 - 7.82
Gly[10]	3.85 - 3.68	-	-		-	8.00 - 8.33
Ser[11]	4.39 - 4.27	3.74 - 3.53	-	OH:	5.38 - 4.68	8.33 - 7.82
Lys[12]	4.31- 4.21	1.73, 1.57	1.34	δ:	1.53	8.00 - 8.33
				ε:	2.75	
				NH:	7.71	
Gly[13]	3.85 - 3.68	-	-		-	8.00 - 8.33
Ser[14]	4.39 - 4.27	3.74 - 3.53	-	OH:	5.38 - 4.68	8.33 - 7.82
Lys[15]	4.19	1.73, 1.61	1.34	δ:	1.52	8.00 - 8.33
				ε:	2.75	
				NH:	7.68	
				CO_2H:	-	

p3-Sil01 H_2N-S^1-pS^2-K^3-K^4-S^5-G^6-pS^7-Y^8-S^9-G^{10}-pS^{11}-K^{12}-G^{13}-S^{14}-K^{15}-CO_2H (**S8**)



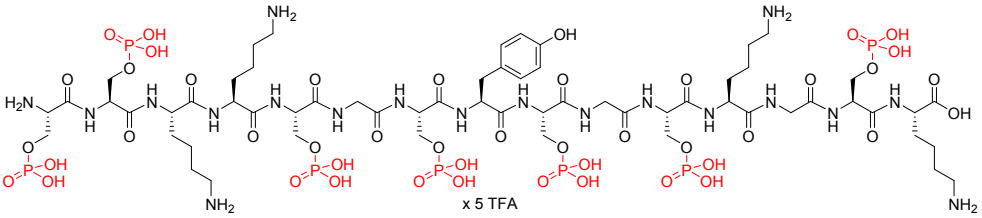
S8

The crude peptide p3-Sil01 **S8** was obtained as a colourless solid (155 mg, 67.8 µmol 68%).

Table 4: ^{1}H-NMR signal assignment for p3-Sil01 **S8** (600 MHz, 300 K, $DMSO_{d6}$).

Amino Acid	δ(α) / ppm	δ(β) / ppm	δ(γ) / ppm	δ(other) / ppm		δ(NH) / ppm
Ser[1]	3.94	3.79, 3.76		-		7.71
pSer[2]	4.28- 4.18	4.12 - 3.90		-		8.44
Lys[3]	4.30- 4.17	1.74, 1.63	1.34	δ:	1.53	8.35 - 7.92
				ε:	2.75	
				NH:	7.71	
Lys[4]	4.30- 4.17	1.74, 1.63	1.34	δ:	1.53	8.35 - 7.92
				ε:	2.75	
				NH:	7.71	
Ser[5]	4.47 - 4.30	3.74 - 3.55	-		-	8.35 - 7.92
Gly[6]	3.84 - 3.70	-	-		-	8.35 - 7.92
pSer[7]	4.28- 4.18	4.12 - 3.90		-		8.35 - 7.92
Tyr[8]	4.51	2.98, 2.75	-	aro.:	7.05, 6.63	8.35 - 7.92
				OH:	9.25	
Ser[9]	4.47 - 4.30	3.74 - 3.55	-		-	8.35 - 7.92
Gly[10]	3.84 - 3.70	-	-		-	8.35 - 7.92
pSer[11]	4.28- 4.18	4.12 - 3.90		-		8.35 - 7.92
Lys[12]	4.30- 4.17	1.74, 1.63	1.34	δ:	1.53	8.35 - 7.92
				ε:	2.75	
				NH:	7.71	
Gly[13]	3.84 - 3.70	-	-		-	8.35 - 7.92
Ser[14]	4.47 - 4.30	3.74 - 3.55	-		-	8.35 - 7.92
Lys[15]	4.30- 4.17	1.74, 1.63	1.34	δ:	1.53	8.35 - 7.92
				ε:	2.75	
				NH:	7.71	
				CO_2H:	-	

p7-Sil01 H_2N-pS^1-pS^2-K^3-K^4-pS^5-G^6-pS^7-Y^8-pS^9-G^{10}-pS^{11}-K^{12}-G^{13}-pS^{14}-K^{15}-CO_2H (S9)



S9

The crude peptide p7-Sil01 **S9** was obtained as a colourless solid (100 mg, 38.4 µmol 38%).

Table 5: ^{1}H-NMR signal assignment for p7-Sil01 **S9** (600 MHz, 300 K, $DMSO_{d6}$ + 5% TFA).

Amino Acid	δ(α) / ppm	δ(β) / ppm	δ(γ) / ppm	δ(other) / ppm		δ(NH) / ppm
pSer^1	4.36	4.19, 4.12	-	-	-	8.44 - 8.07
pSer^2	4.62	4.11 - 4.01	-	-	-	8.93
Lys^3	4.35 - 4.14	1.73, 1.61	1.34	δ:	1.52	8.25 - 8.17
				ε:	2.76	
				NH:	7.66	
Lys^4	4.35 - 4.14	1.73, 1.61	1.34	δ:	1.52	8.25 - 8.17
				ε:	2.76	
				NH:	7.66	
pSer^5	4.59 - 4.50	4.14 - 3.91	-	-	-	8.44 - 8.07
Gly^6	3.88 - 3.72	-	-		-	8.15 - 8.07
pSer^7	4.59 - 4.50	4.14 - 3.91	-	-	-	8.44 - 8.07
Tyr^8	4.50	2.96, 2.71	-	aro.:	7.03, 6.64	8.17
pSer^9	4.59 - 4.50	4.14 - 3.91	-	-	-	8.44 - 8.07
Gly^{10}	3.88 - 3.72	-	-		-	8.15 - 8.07
pSer^{11}	4.59 - 4.50	4.14 - 3.91	-	-	-	8.44 - 8.07
Lys^{12}	4.35 - 4.14	1.73, 1.61	1.34	δ:	1.52	8.25 - 8.17
				ε:	2.76	
				NH:	7.66	
Gly^{13}	3.88 - 3.72	-	-		-	8.15 - 8.07
pSer^{14}	4.59 - 4.50	4.14 - 3.91	-	-	-	8.44 - 8.07
Lys^{15}	4.35 - 4.14	1.73, 1.61	1.34	δ:	1.52	8.25 - 8.17
				ε:	2.76	
				NH:	7.66	
				CO_2H:	-	

Silica precipitation activity

LCPA and peptides

The data points were fitted asymptotically, since a saturation effect was observed at higher concentrations of organic compounds. For the asymptotic fit the data points at which no silica precipitate was detected were excluded to determine the threshold concentration. However, due to the low number of data points of the equimolar mixture of LCPA **S4** and R5 **S6** peptide an asymptotic fit of this data points is not reasonable. The result of the asymptotic fit is shown in Figure 1 and the data of the calculated functions are summarised in Table 6.

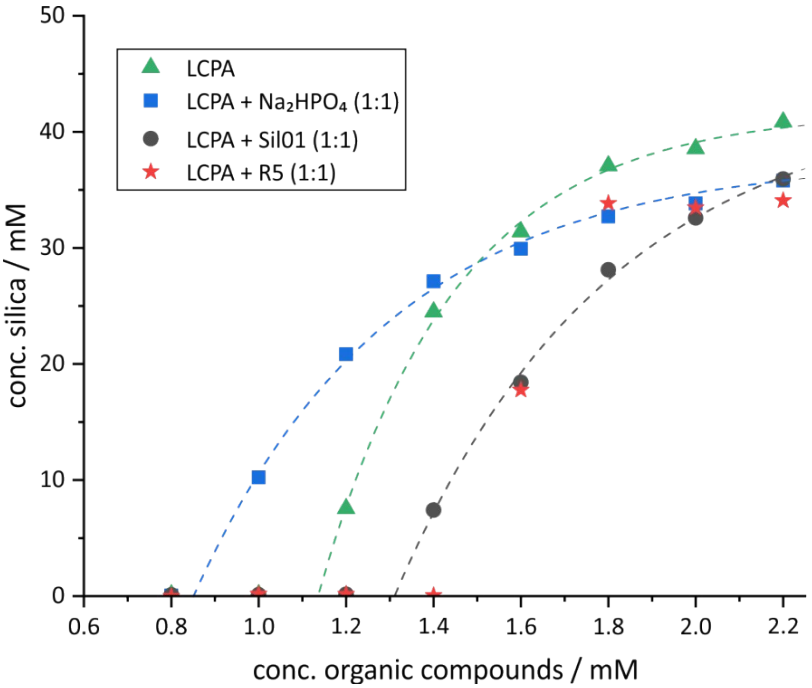


Figure 1: Silica precipitation activity of different mixtures of LCPA **S4** and additives at pH 5.5 in a 50 mM sodium acetate buffer. The silica precipitation took place in a time frame of 10 min and was stopped by the addition of 1.0 M HCl. TMOS served as the source for orthosilicic acid.

Table 6: Data of the asymptotic fit with the function $f(x) = a - bc^x$.

	a	b	c	R^2
LCPA **S4**	41.78	1551.95	0.04	0.9975
LCPA **S4** + Na_2HPO_4 (1:1)	37.70	247.21	0.11	0.9946
LCPA **S4** + Sil01 **S5** (1:1)	43.20	625.47	0.13	0.9972

LCPA and phosphopeptides

The data points were fitted linear, since no saturation effect was observed at higher concentrations of organic compounds. The result of the linear fit is shown in Figure 2 and the data of the calculated functions are summarised in Table 7.

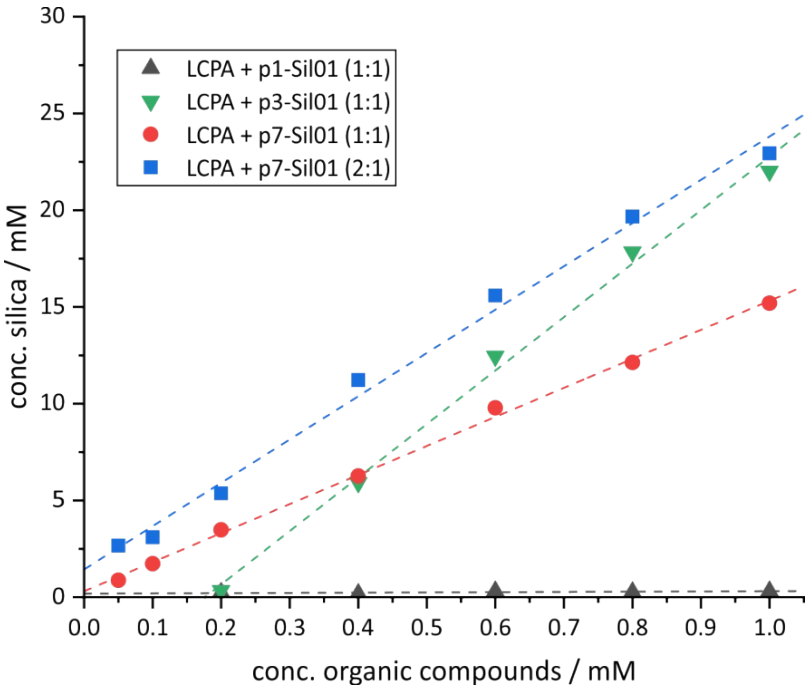


Figure 2: Silica precipitation activity of mixtures of LCPA **S4** and phosphorylated derivatives of Sil01 **S5** at pH 5.5 in a 50 mM sodium acetate buffer. The silica precipitation took place in a time frame of precisely 10 min and was stopped by the addition of 1.0 M HCl. TMOS served as the source for orthosilicic acid.

Table 7: Data of the linear fit with the function $f(x) = a + bx$.

	a	**b**	**R^2**
LCPA **S4** + p1-Sil01 **S7** (1:1)	0.19	0.13	0.4749
LCPA **S4** + p3-Sil01 **S8** (1:1)	–4.86	27.63	0.9946
LCPA **S4** + p7-Sil01 **S9** (1:1)	0.31	15.01	0.9981
LCPA **S4** + p7-Sil01 **S9** (2:1)	1.44	22.37	0.9932

Hydrolysis stability of the serine phosphates

To ensure that the phosphate groups are covalently bound during precipitation, the stability of the hepta-monophosphate p7-Sil01 **S9** against hydrolysis in aqueous buffer systems was tested at different pH values. The hydrolysis was analysed by HSQC spectroscopy. The appearance of serine methylene signals at 3.8 ppm identifies the hydrolysis.

The stability was tested with 2.5 mg p7-Sil01 **S9** in a 100 mM sodium phosphate buffer at pH 3.27, 6.89 and 8.62. The pH values were adjusted with diluted NaOH and H_3PO_4 solutions. p7-Sil01 showed hydrolysis stability at pH 3.27 and 6.89 for more than 4 days and at 8.62 for 1 day without changes in the HSQC spectrum.

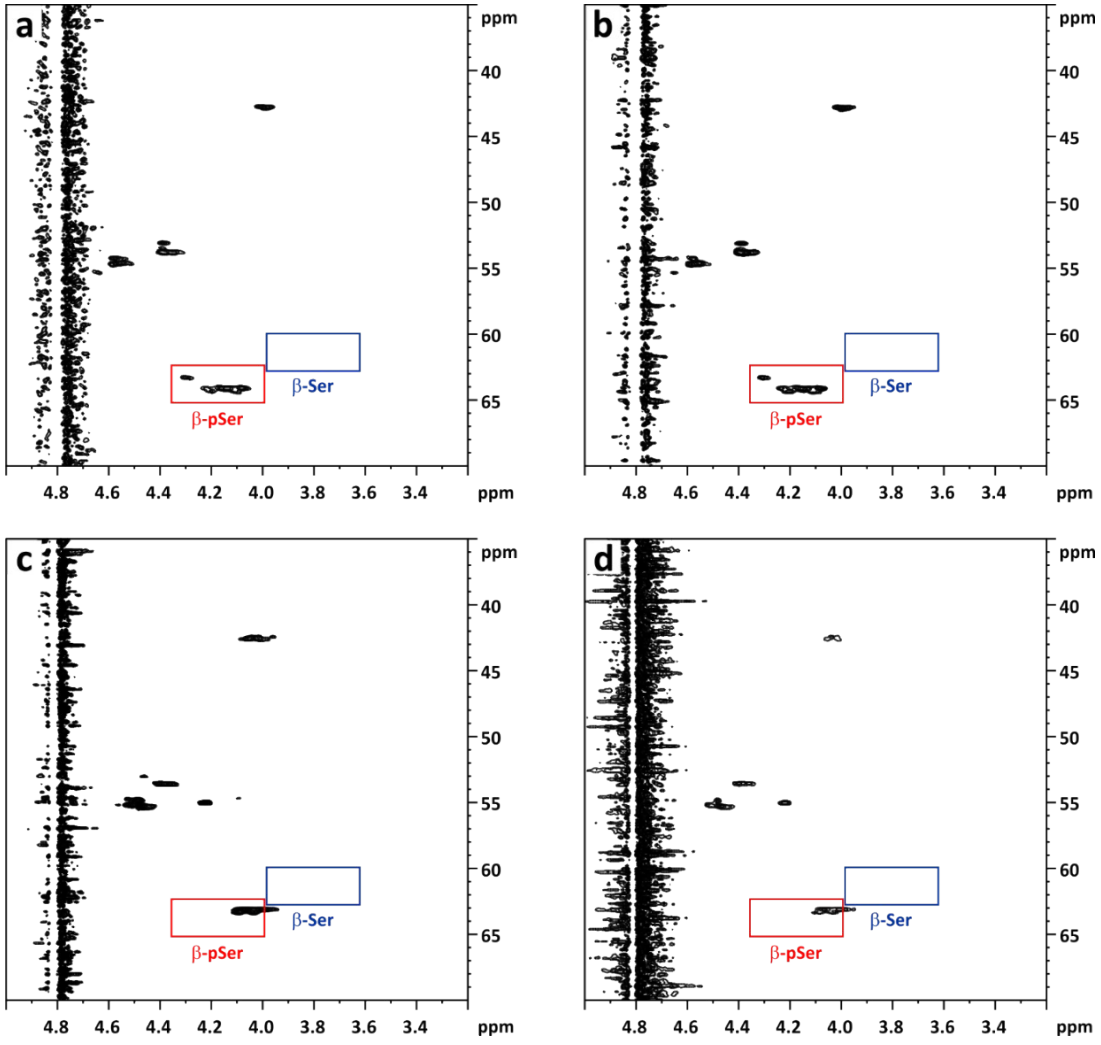


Figure 3: HSQC spectra (600 MHz, 300 K, H_2O/D_2O 9:1) of the synthetic peptide p7-Sil01 **S9**. 100 mM phosphate buffer with pH 3.27 (top) at different times (a) t = 0 h and (b) t = 1 d. 50 mM phosphate buffer with pH 8.62 (bottom) at different times (c) t = 0 h and (d) t = 1 d. The blue boxes highlight the signals from the β-methylene groups of the non-phosphorylated serines. Sufficiently separated are the β-methylene groups of the phosphorylated serines highlighted by red boxes.

HPLC polarity analysis of p7-Sil07

Experiments on the purification of mono-heptaphosphate p7-Sil01 **S9** showed, that it is impossible to purify this peptide by semi-preparative reversed-phase HPLC. Due to the high number of charges, the peptide p7-Sil01 **S9** is too polar to be purified with a C18 column. All attempts to increase the elution time of the peptide by varying the gradients failed. As shown in Figure 4 p7-Sil01 **S9** eluted simultaneously with the injection peak of the analytical HLPC measurement.

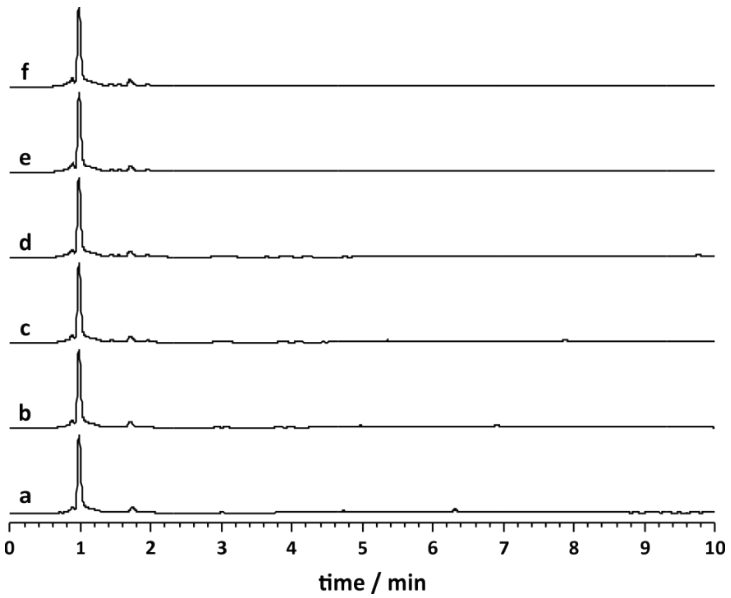


Figure 4: Chromatograms of peptide p7-Sil01 **S9** at different gradients. ACE Ultracore 2.5 SuperC18, 150 mm x 2.1 mm. (a) 2-25% B in 10 min. (b) 2-20% B in 10 min. (c) 2-15% B in 10 min. (d) 2-10% B in 10 min. (e) 2-5% B in 10 min. (f) 2-2% B in 10 min. (A: water + 0.1% TFA, B: acetonitrile + 0.085% TFA), flow rate 0.45 mL/min, temperature 28 °C.

RP-HPLC and ESI-MS analytical data

The following table summarises the analytic data of the RP-HPLC and ESI-MS for the five synthetic peptides.

Table 8: Peptide sequences, HPLC retention times and ESI-MS results.

Peptide	Sequence	rt / min	m/z (calculated)	m/z (ESI-MS)
Sil01	SSKKSGSYSGSKGSK	4.87	737.8784	737.8805
			$[M+2H]^{2+}$	$[M+2H]^{2+}$
R5	SSKKSGSYSGSKGSKRRIL	6.32[a]	1007.0636	1007.0657
			$[M+2H]^{2+}$	$[M+2H]^{2+}$
p1-Sil01	SSKKSGpSYSGSKGSK	4.69	777.8616	777.8627
			$[M+2H]^{2+}$	$[M+2H]^{2+}$
p3-Sil01	SpSKKSGpSYSGpSKGSK	4.26	857.8279	857.8293
			$[M+2H]^{2+}$	$[M+2H]^{2+}$
p7-Sil01	pSpSKKpSGpSYpSGpSKGpSK	0.98	1017.7606	1017.7645
			$[M+2H]^{2+}$	$[M+2H]^{2+}$

[a] 2-50% B in 10 min (A: water + 0.1% TFA, B: acetonitrile + 0.085% TFA), flow rate 0.45 mL/min.

Analytical data

O-(*tert*-butyldimethylsilyl)-L-serine (S1)

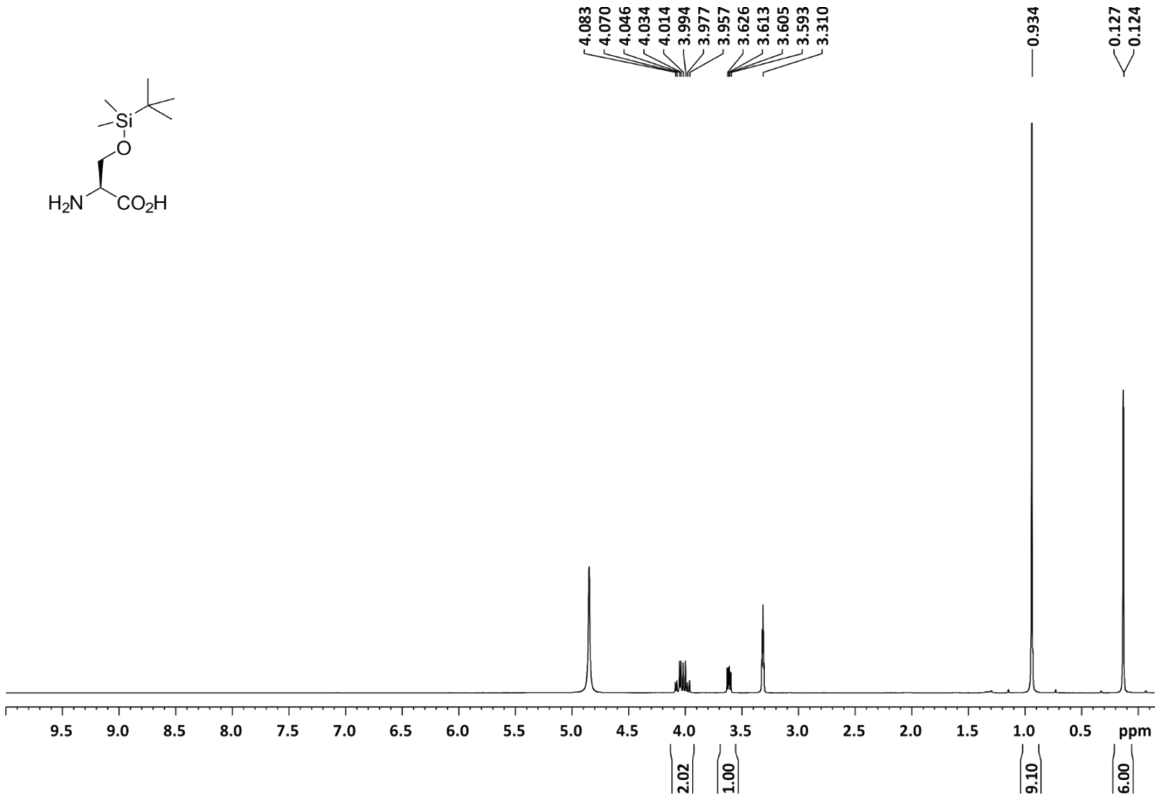


Figure 5: ^{1}H-NMR spectrum of L-Ser(OTBDMS)-OH **S1** (300 MHz, 300 K, CD_3OD).

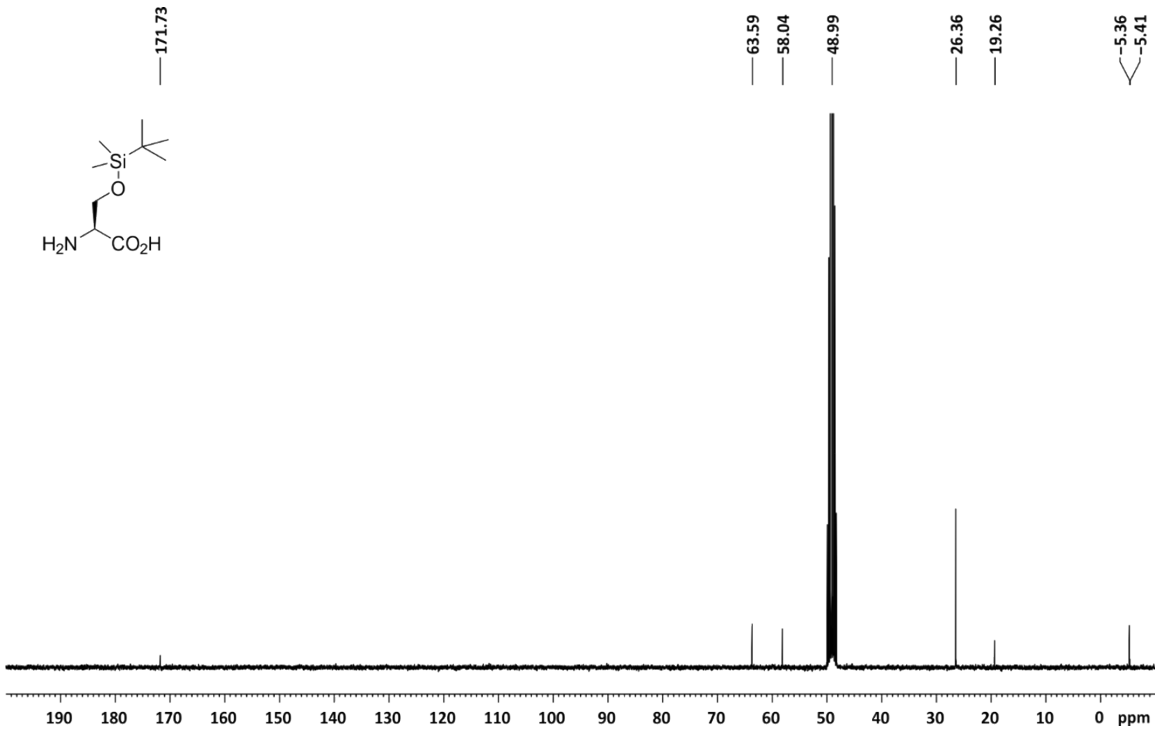


Figure 6: ^{13}C-NMR spectrum of L-Ser(OTBDMS)-OH **S1** (75 MHz, 300 K, CD_3OD).

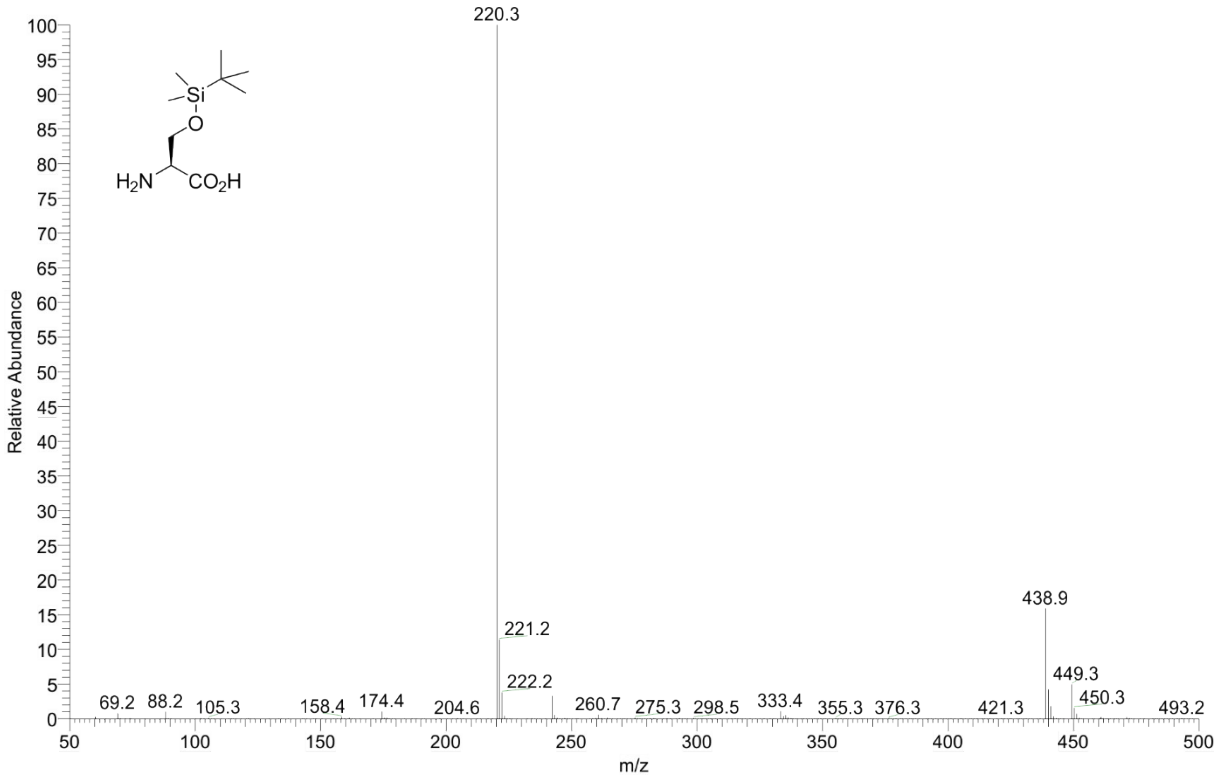


Figure 7: ESI+ mass spectrum with charge pattern m/z of L-Ser(OTBDMS)-OH **S1**.

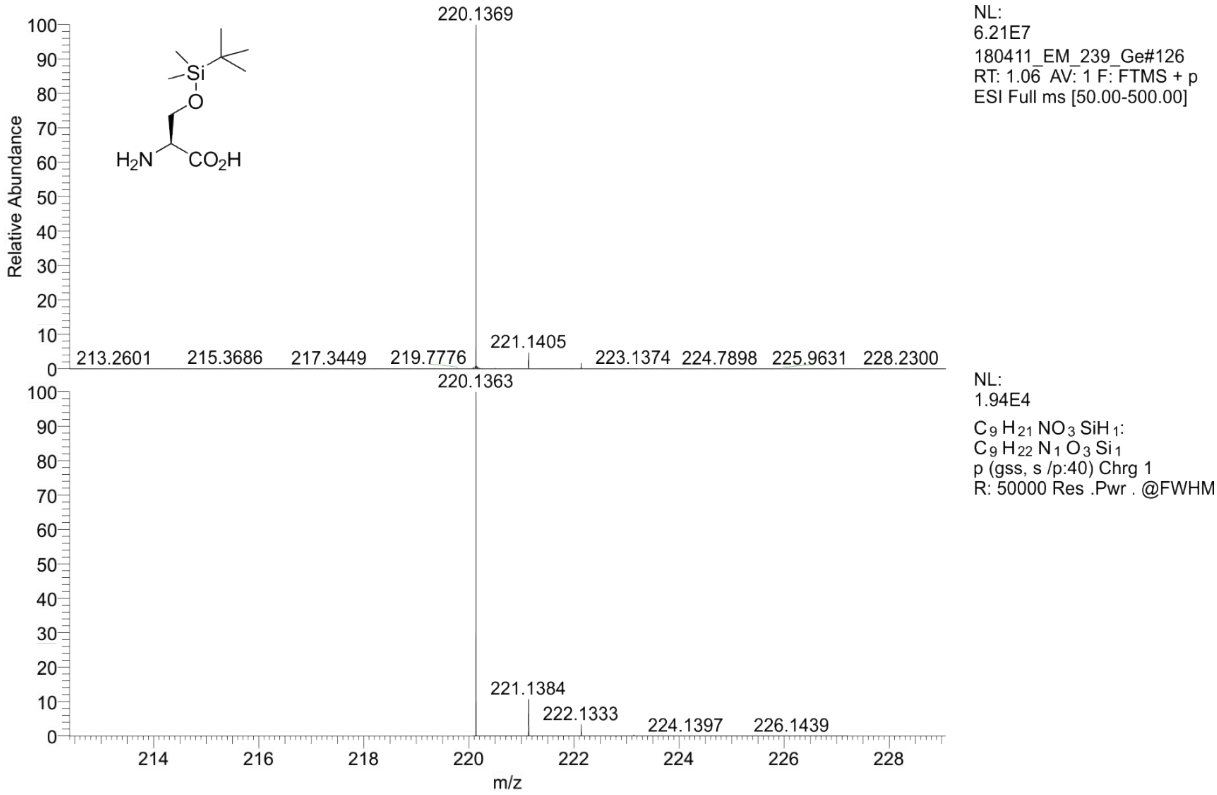


Figure 8: High resolution mass spectrum with isotope pattern of L-Ser(OTBDMS)-OH **S1**.

***N*-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*O*-(*tert*-butyldimethylsilyl)-L-serine (S2)**

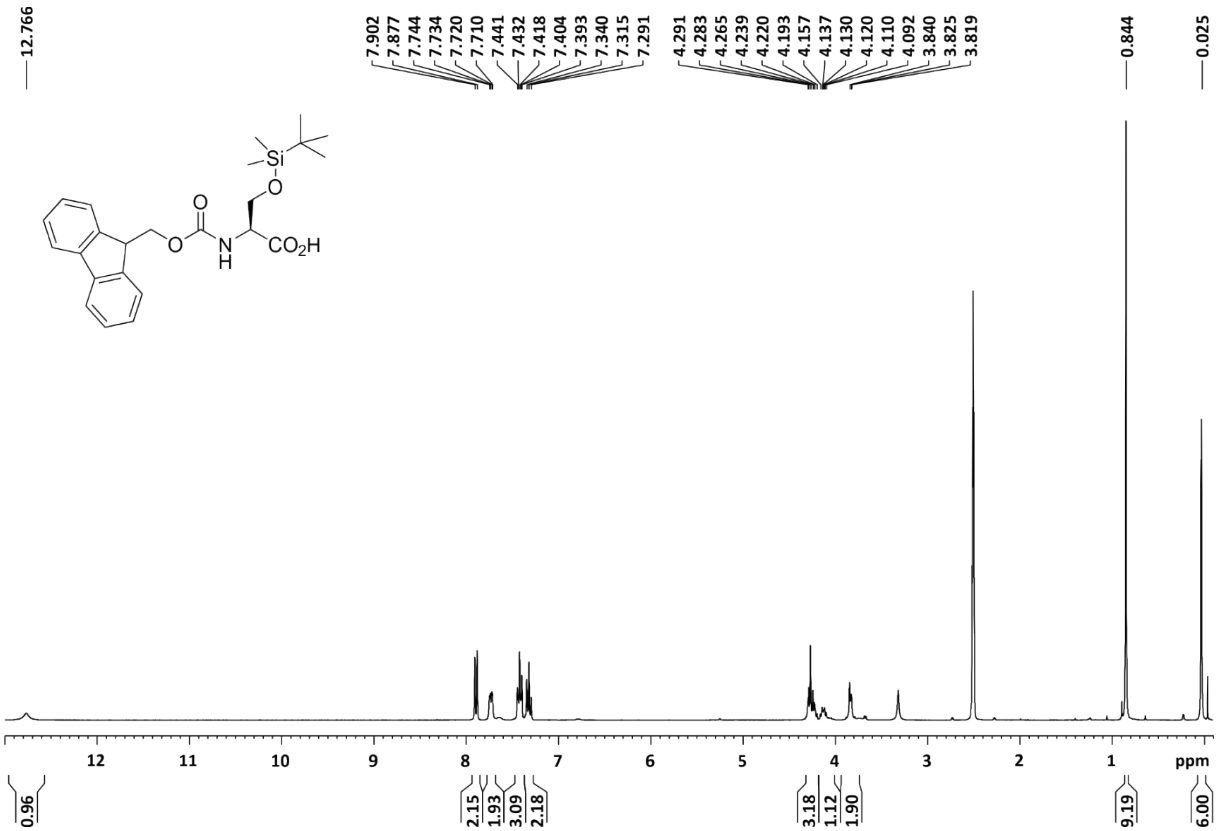


Figure 9: ^{1}H-NMR spectrum of Fmoc-L-Ser(OTBDMS)-OH **S2** (300 MHz, 300 K, $DMSO_{d6}$).

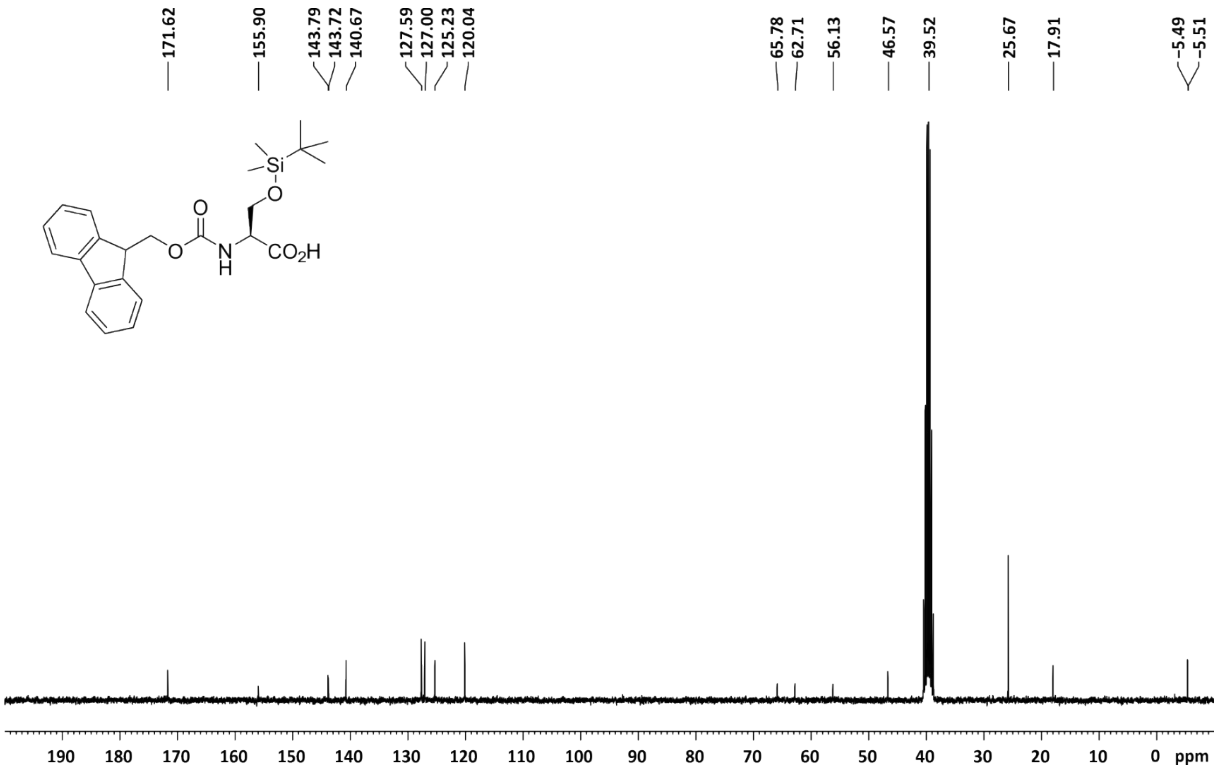


Figure 10: ^{13}C-NMR spectrum of Fmoc-L-Ser(OTBDMS)-OH **S2** (75 MHz, 300 K, $DMSO_{d6}$).

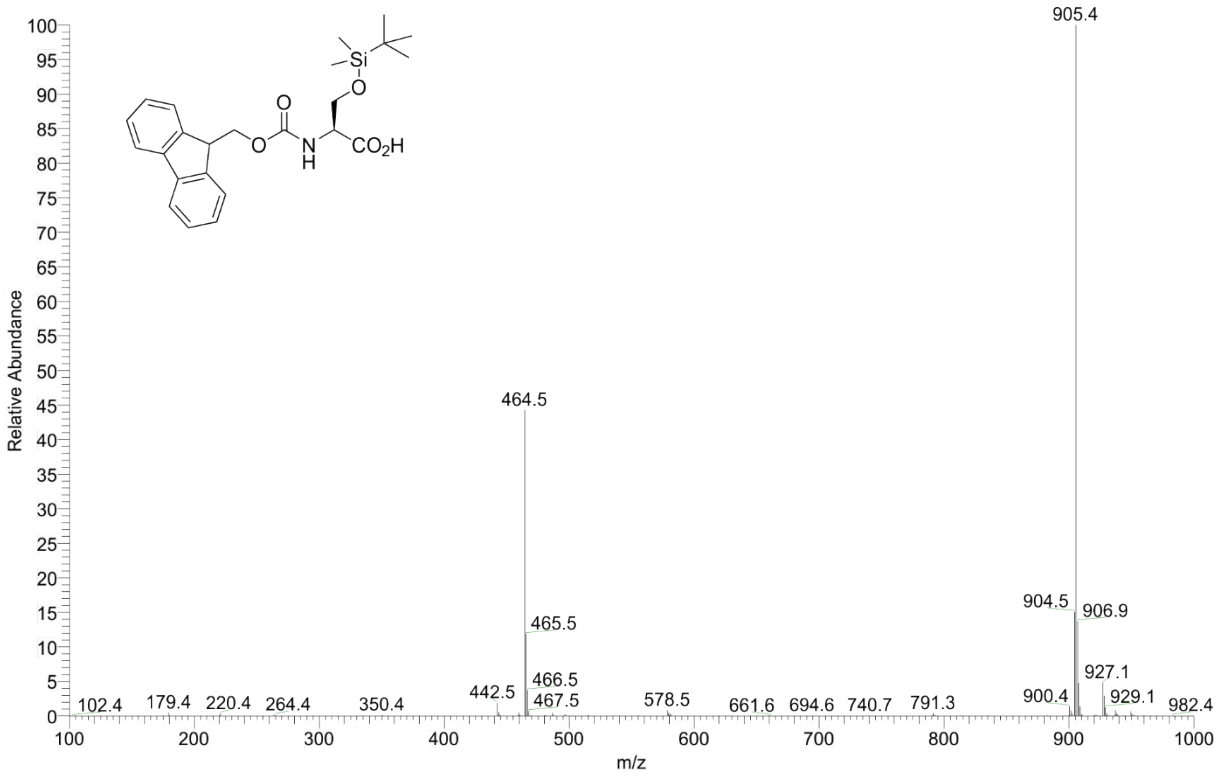


Figure 11: ESI+ mass spectrum with charge pattern m/z of Fmoc-L-Ser(OTBDMS)-OH **S2**.

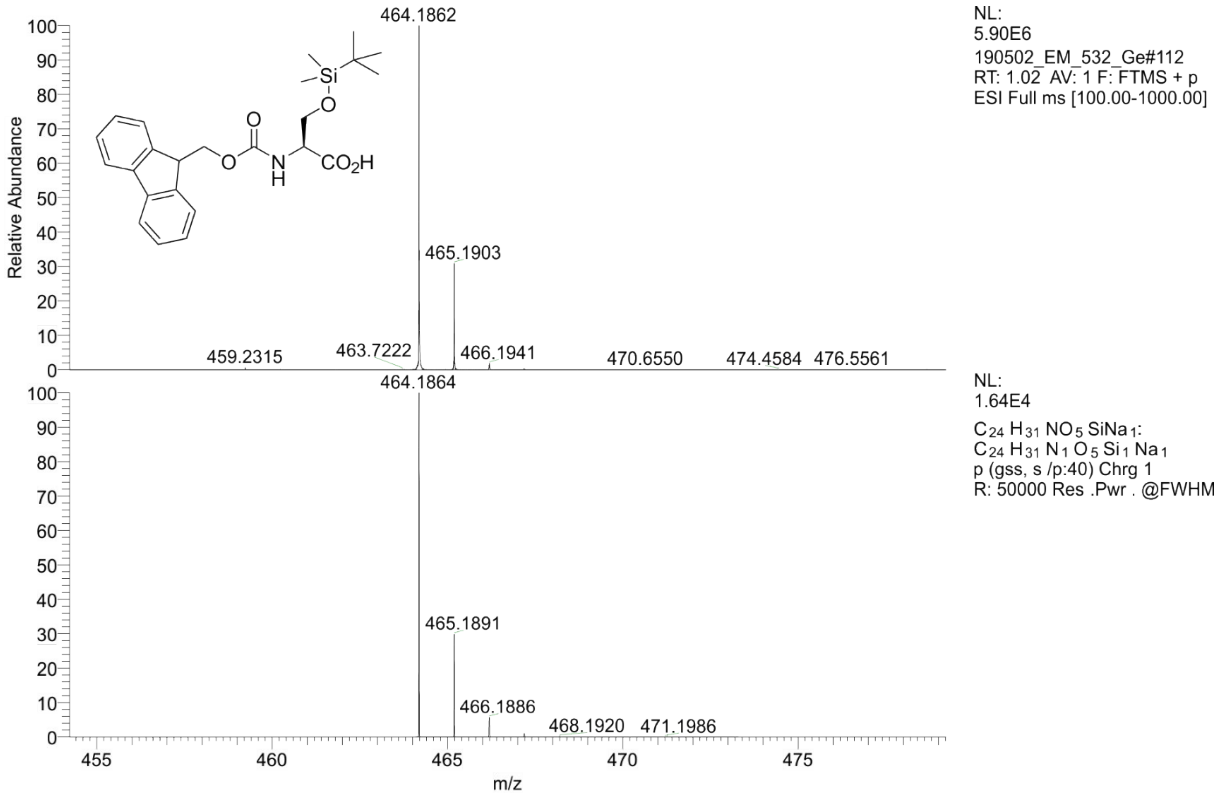


Figure 12: High resolution mass spectrum with isotope pattern of Fmoc-L-Ser(OTBDMS)-OH **S2**.

***N*-(*tert*-butoxycarbonyl)-*O*-(*tert*-butyldimethylsilyl)-L-serine (S3)**

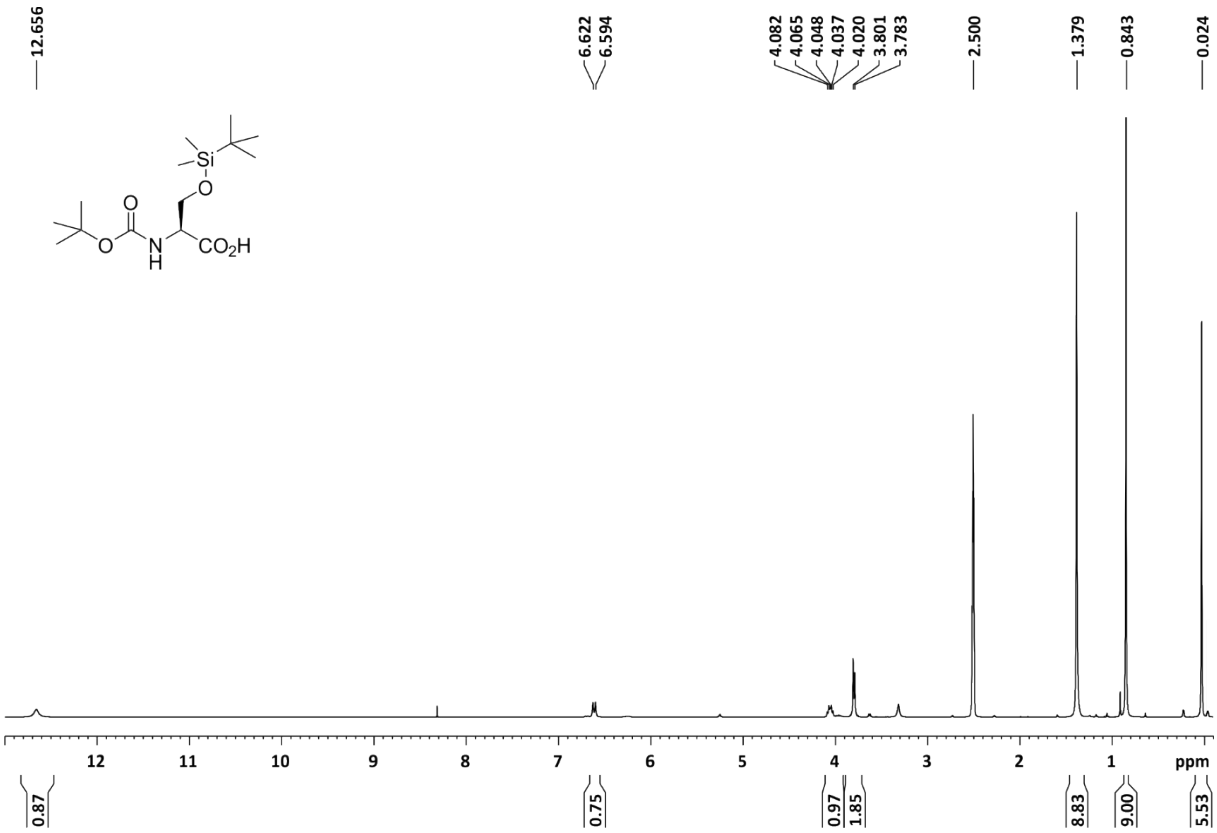


Figure 13: ^{1}H-NMR spectrum of Boc-L-Ser(OTBDMS)-OH **S3** (300 MHz, 300 K, $DMSO_{d6}$).

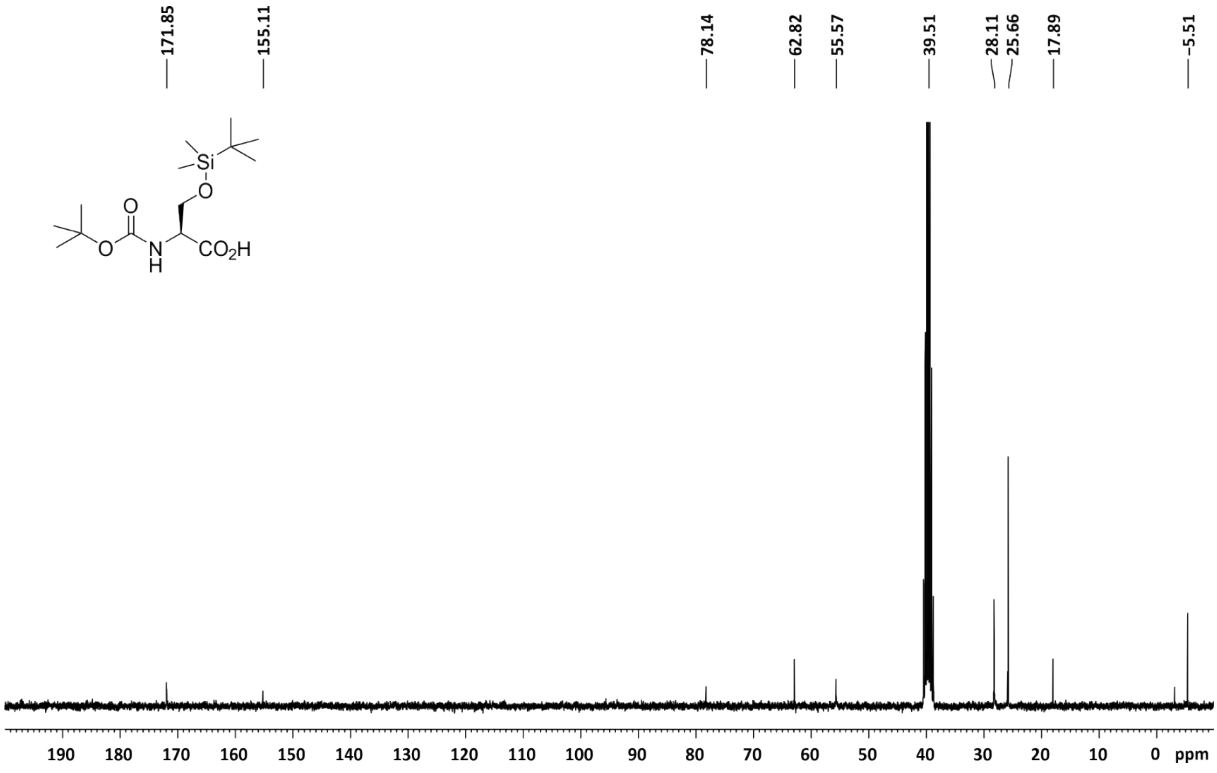


Figure 14: ^{13}C-NMR spectrum of Boc-L-Ser(OTBDMS)-OH **S3** (75 MHz, 300 K, $DMSO_{d6}$).

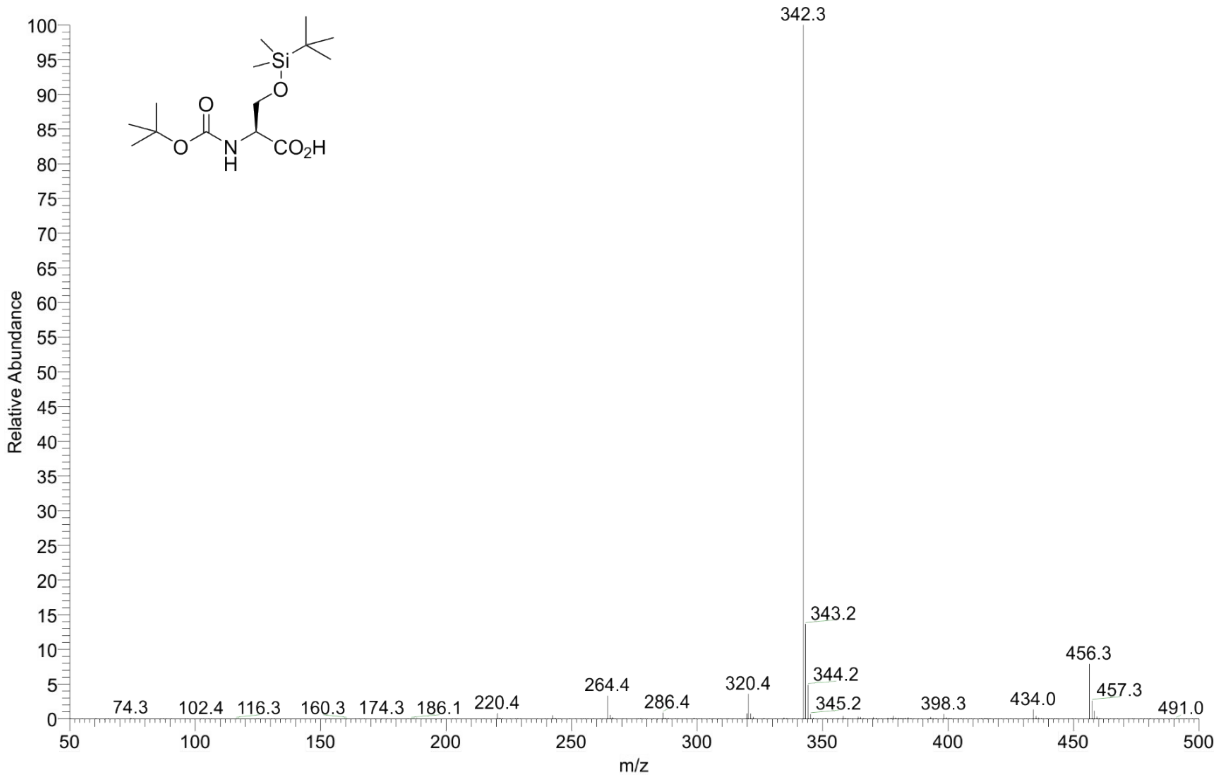


Figure 15: ESI+ mass spectrum with charge pattern m/z of Boc-L-Ser(OTBDMS)-OH **S3**.

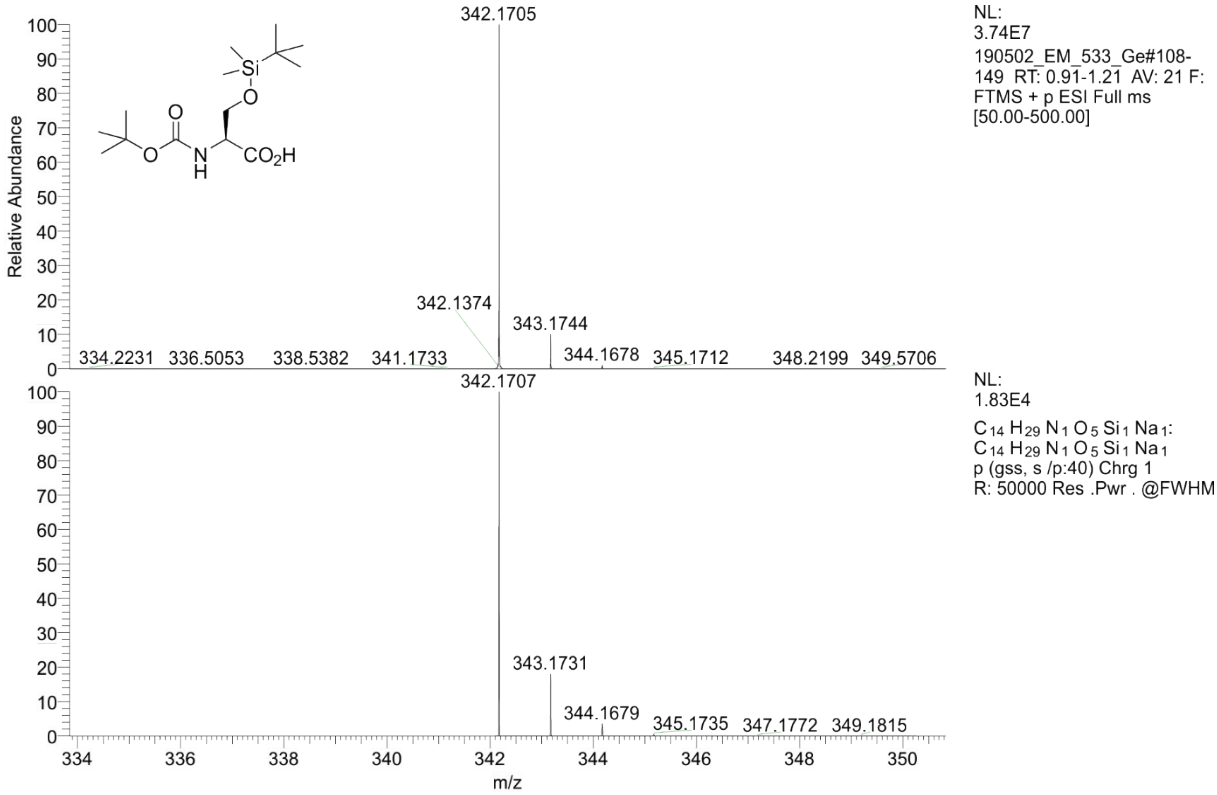


Figure 16: High resolution mass spectrum with isotope pattern of Boc-L-Ser(OTBDMS)-OH **S3**.

LCPA H_3C-NH-[$(CH_2)_3$-N(CH_3)-]$_n$-H (S4)

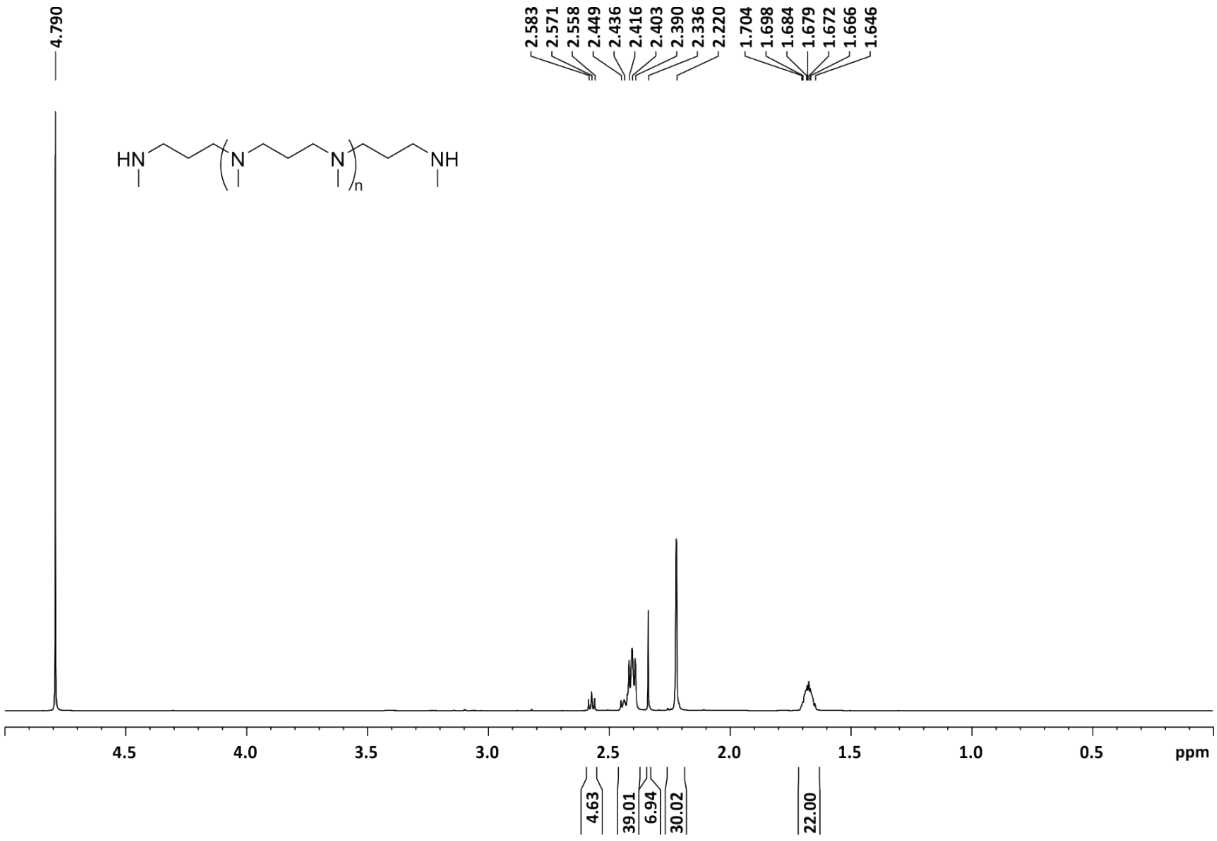


Figure 17: ^{1}H-NMR spectrum of LCPA **S4** (600 MHz, 300 K, D_2O).

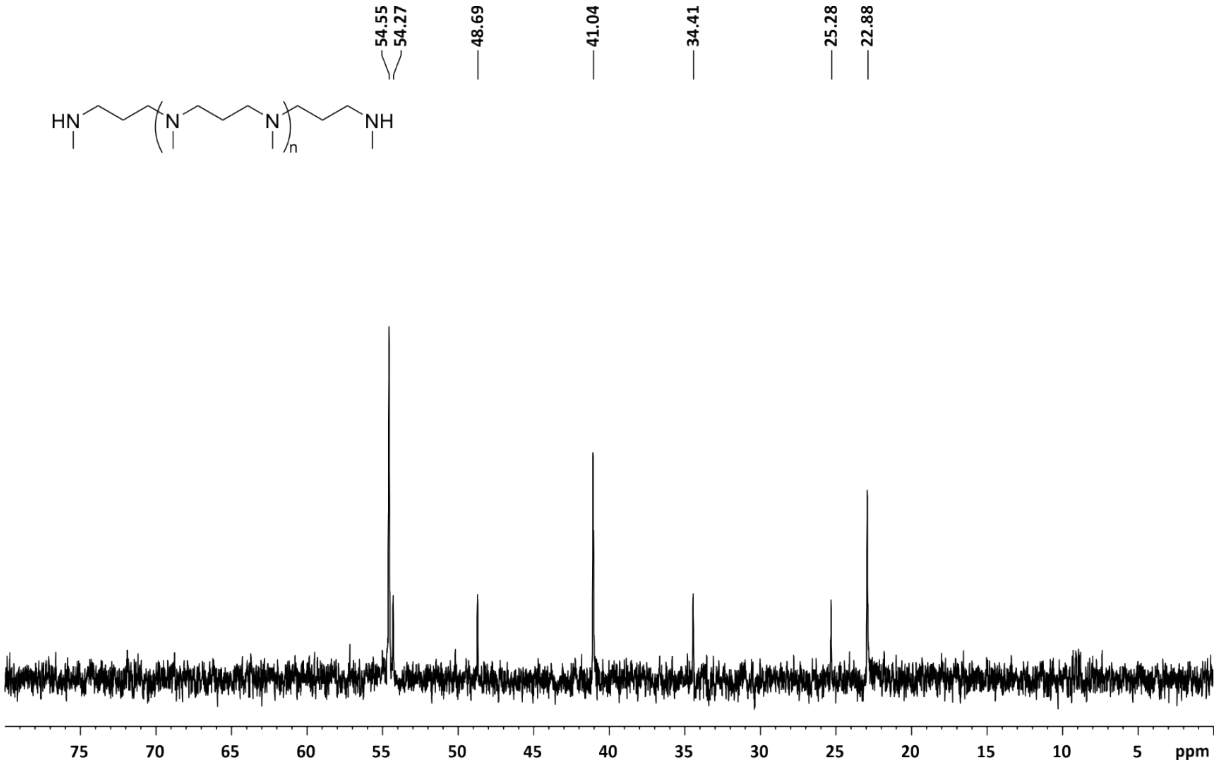


Figure 18: ^{13}C-NMR spectrum of LCPA **S4** (75 MHz, 300 K, D_2O).

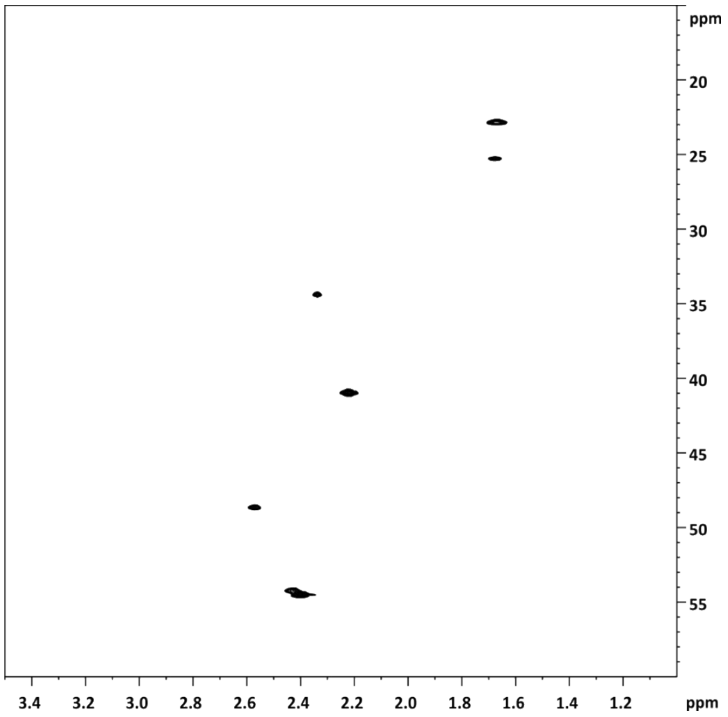


Figure 19: HSQC spectrum of the aliphatic range of LCPA **S4** (600 MHz, 300 K, D_2O).

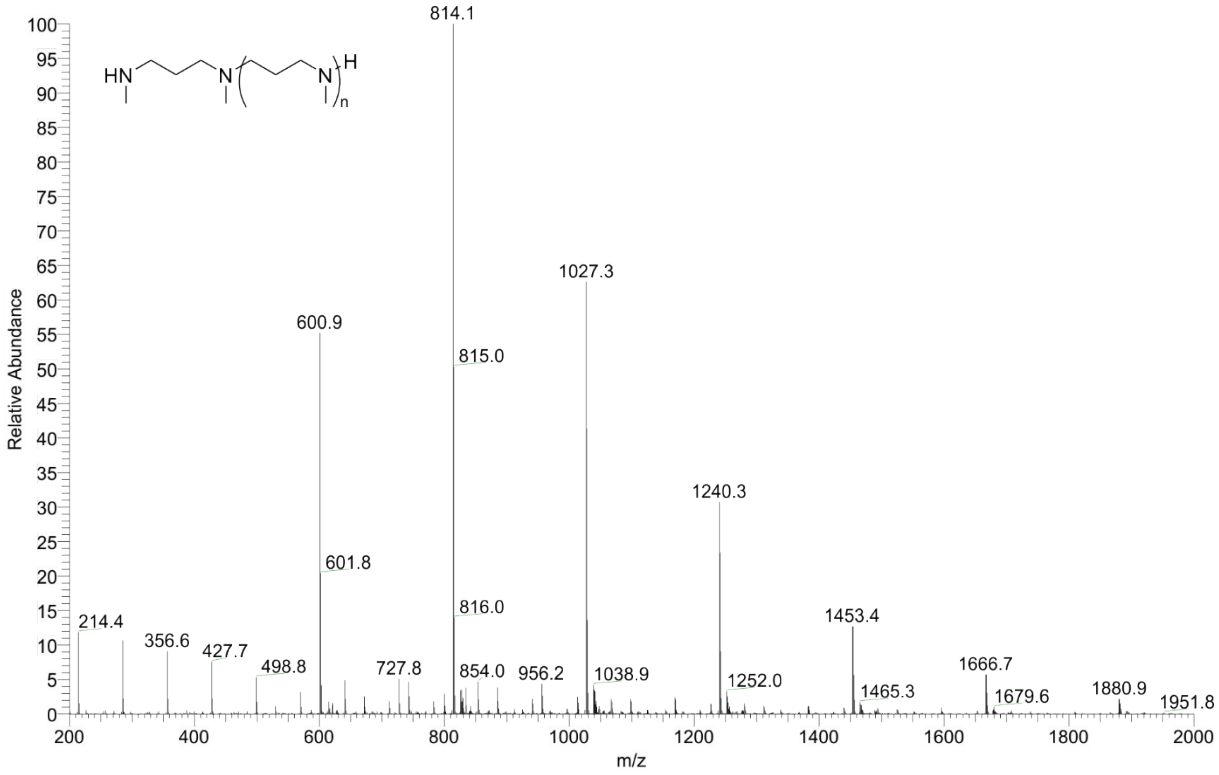


Figure 20: ESI+ mass spectrum with charge pattern m/z of LCPA **S4**. Distribution of chain length of LCPA **S4** mixture describes an approximate Gaussian function with n = 7 (600.9 m/z), n = 10 (814.1 m/z), n = 13 (1027.3 m/z), n = 16 (1240.3 m/z), n = 19 (1453.4 m/z), n = 22 (1666.7 m/z) and n = 25 (1880.9 m/z).

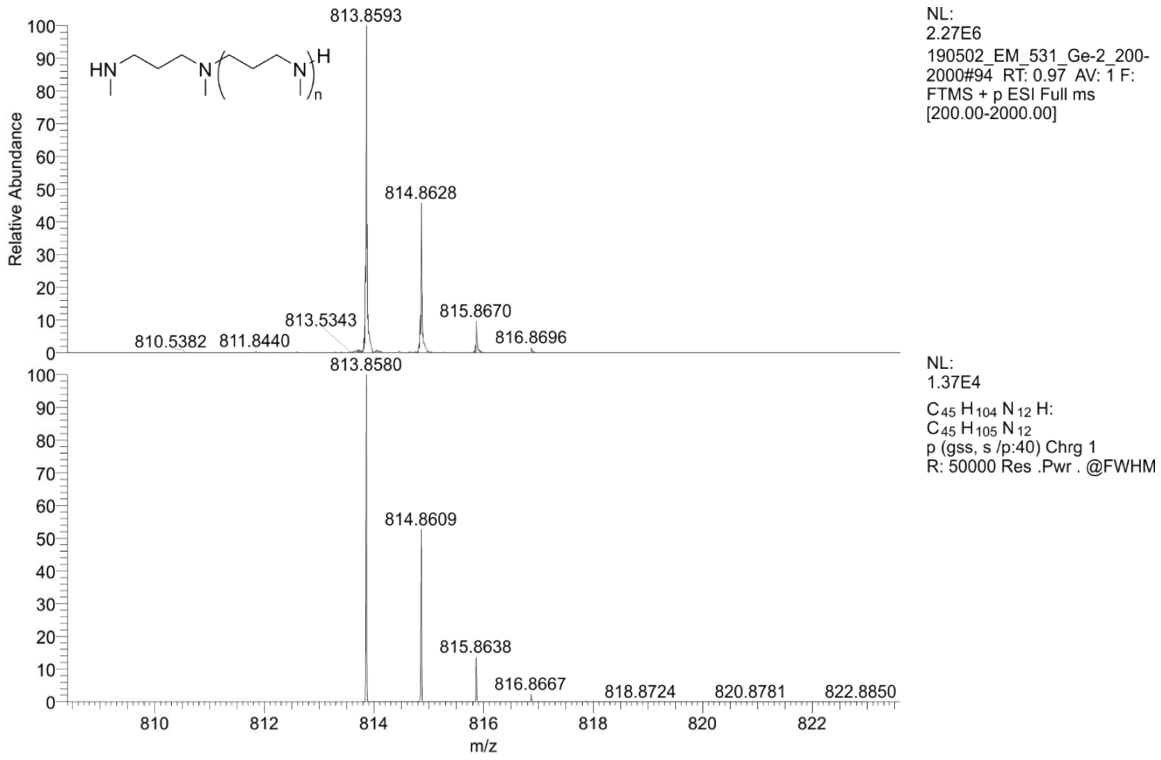


Figure 21: High resolution mass spectrum with isotope pattern of LCPA **S4** with a chain length of 12 nitrogen.

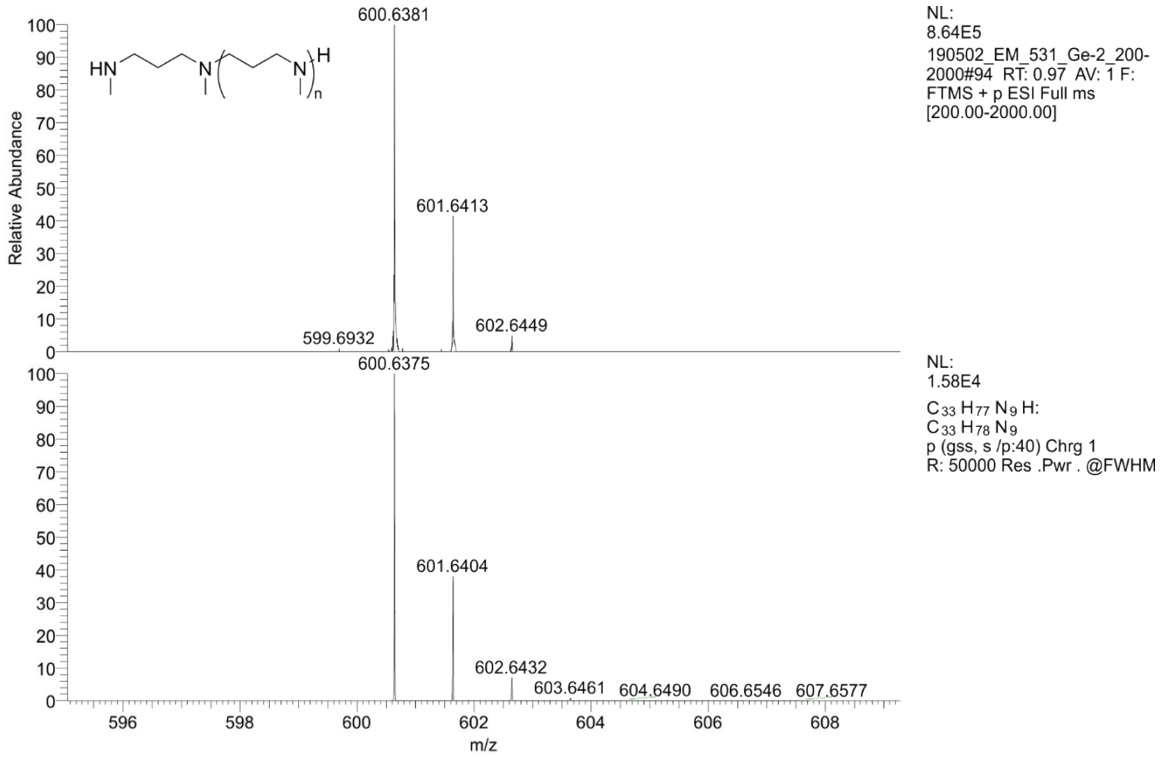


Figure 22: High resolution mass spectrum with isotope pattern of LCPA **S4** with a chain length of 9 nitrogen.

Sil01 H_2N-SSKKSGSYSGSKGSK-CO_2H (S5)

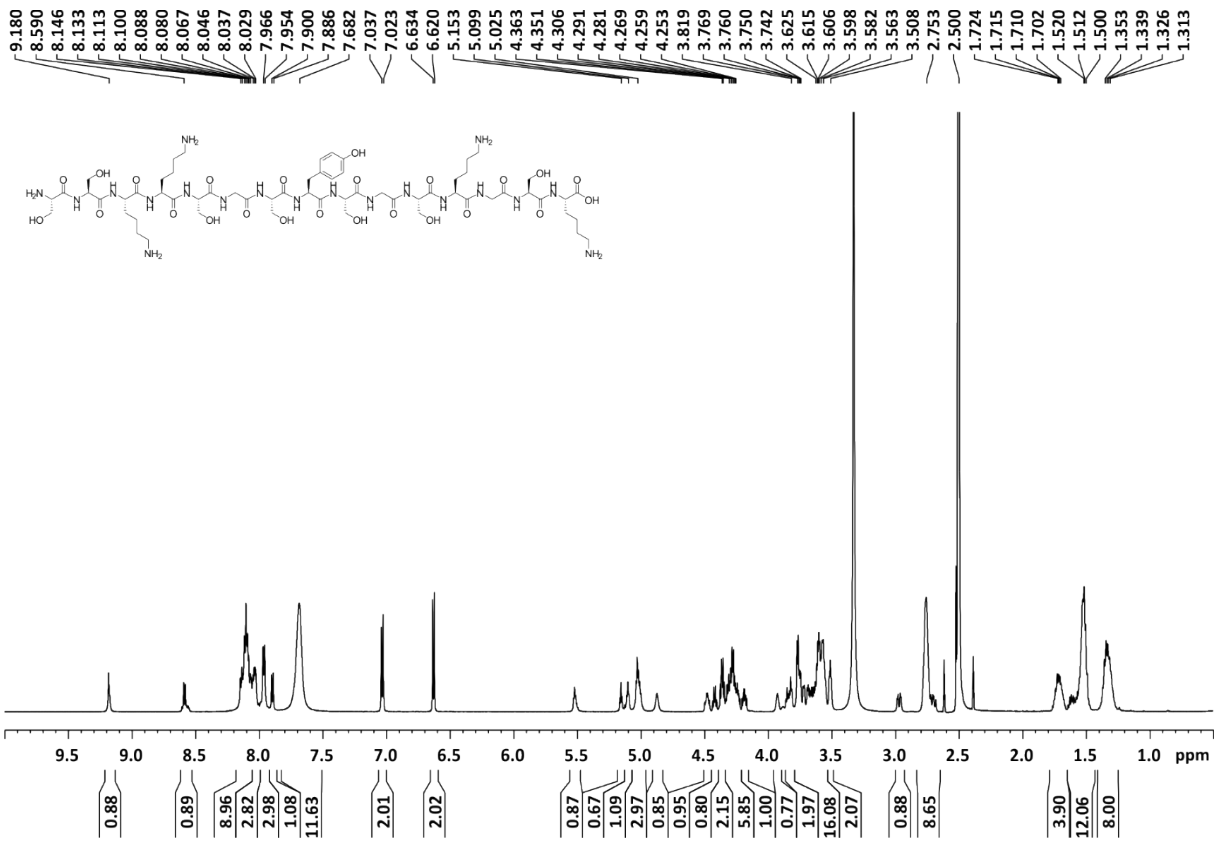


Figure 23: ^{1}H-NMR spectrum of Sil01 **S5** (600 MHz, 300 K, $DMSO_{d6}$).

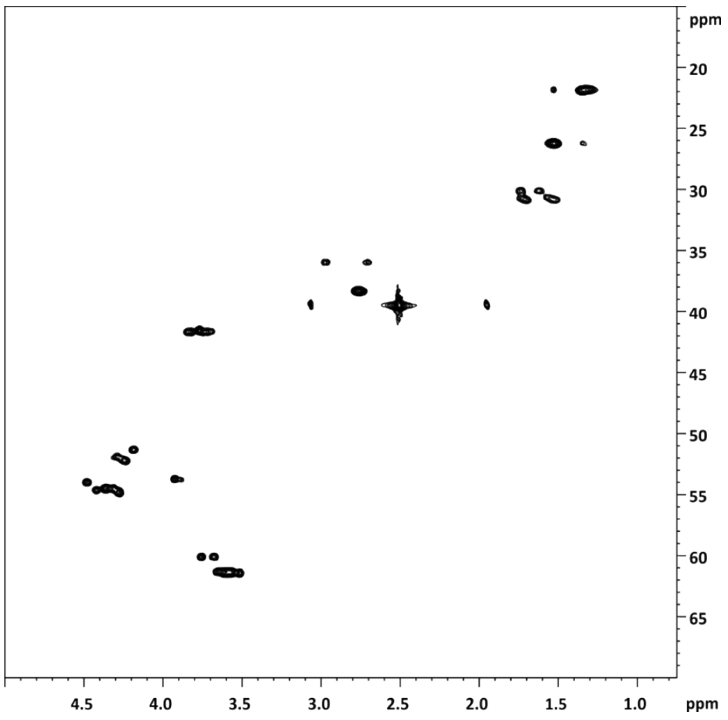


Figure 24: HSQC spectrum of the aliphatic range of Sil01 **S5** (600 MHz, 300 K, $DMSO_{d6}$).

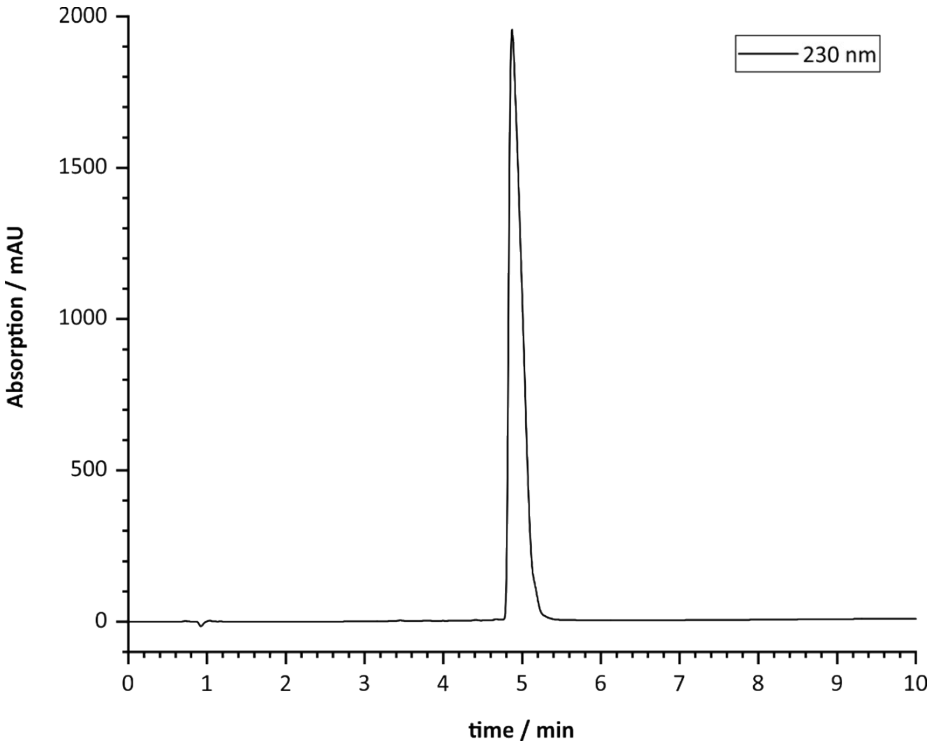


Figure 25: Chromatogram of peptide Sil01 **S5**. ACE Ultracore 2.5 SuperC18, 150 mm x 2.1 mm, 2-30% B in 10 min (A: H_2O + 0.1% TFA, B: CH_3CN + 0.085% TFA), flow rate 0.45 mL/min.

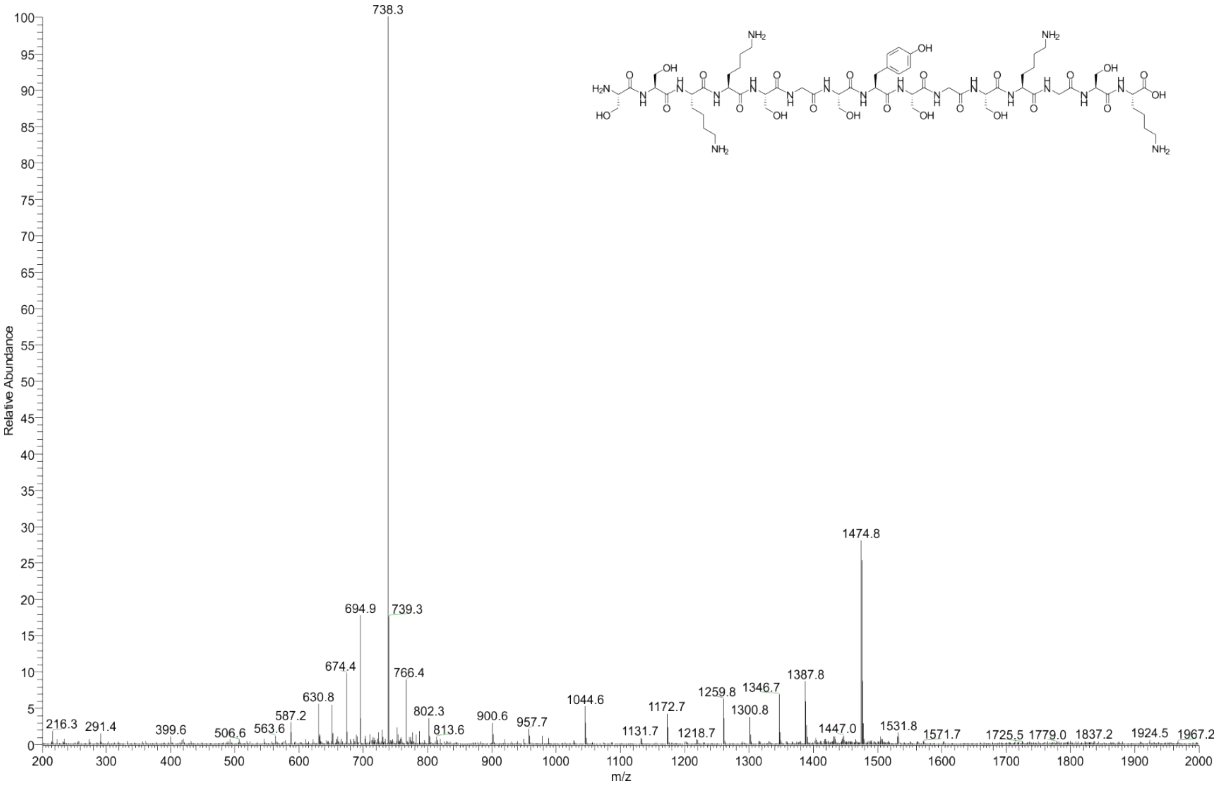


Figure 26: Mass spectrum with charge pattern m/z of peptide Sil01 **S5**.

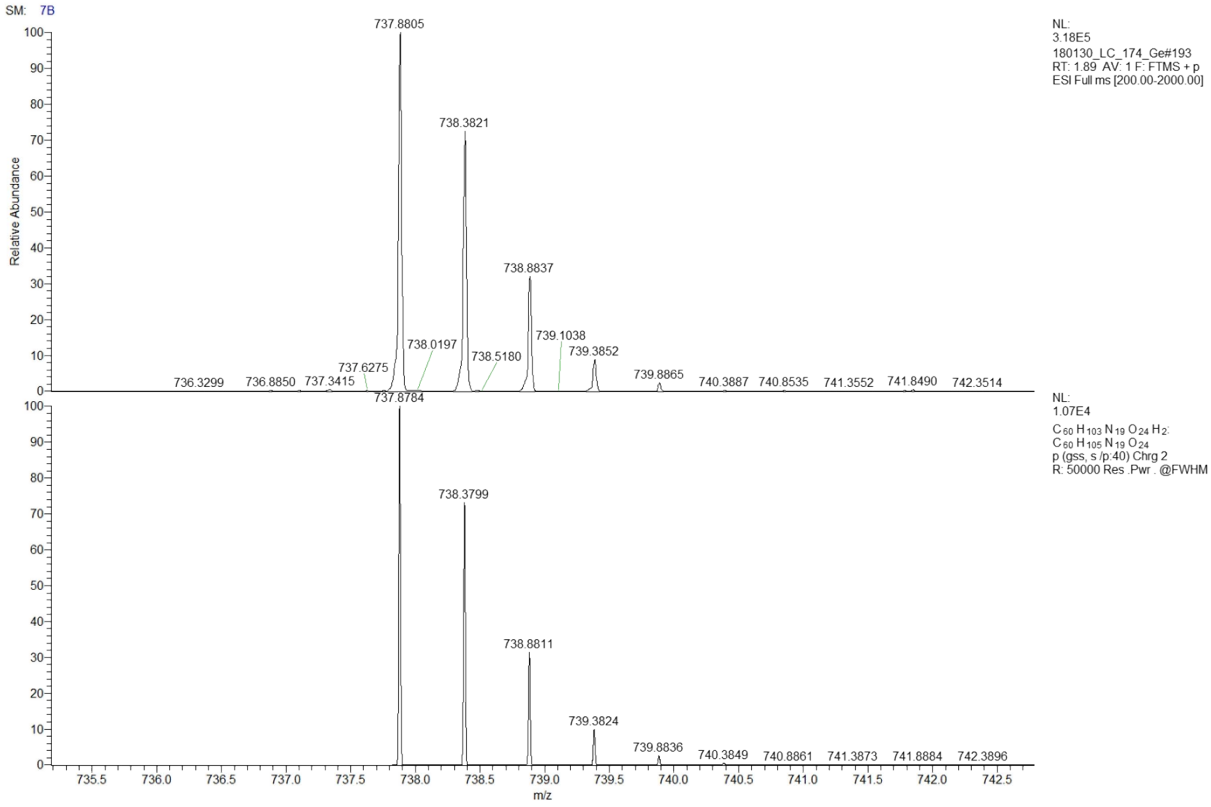


Figure 27: High resolution mass spectrum with isotope pattern of peptide Sil01 **S5**.

R5 peptide H_2N-SSKKSGSYSGSKGSKRRIL-CO_2H (S6)

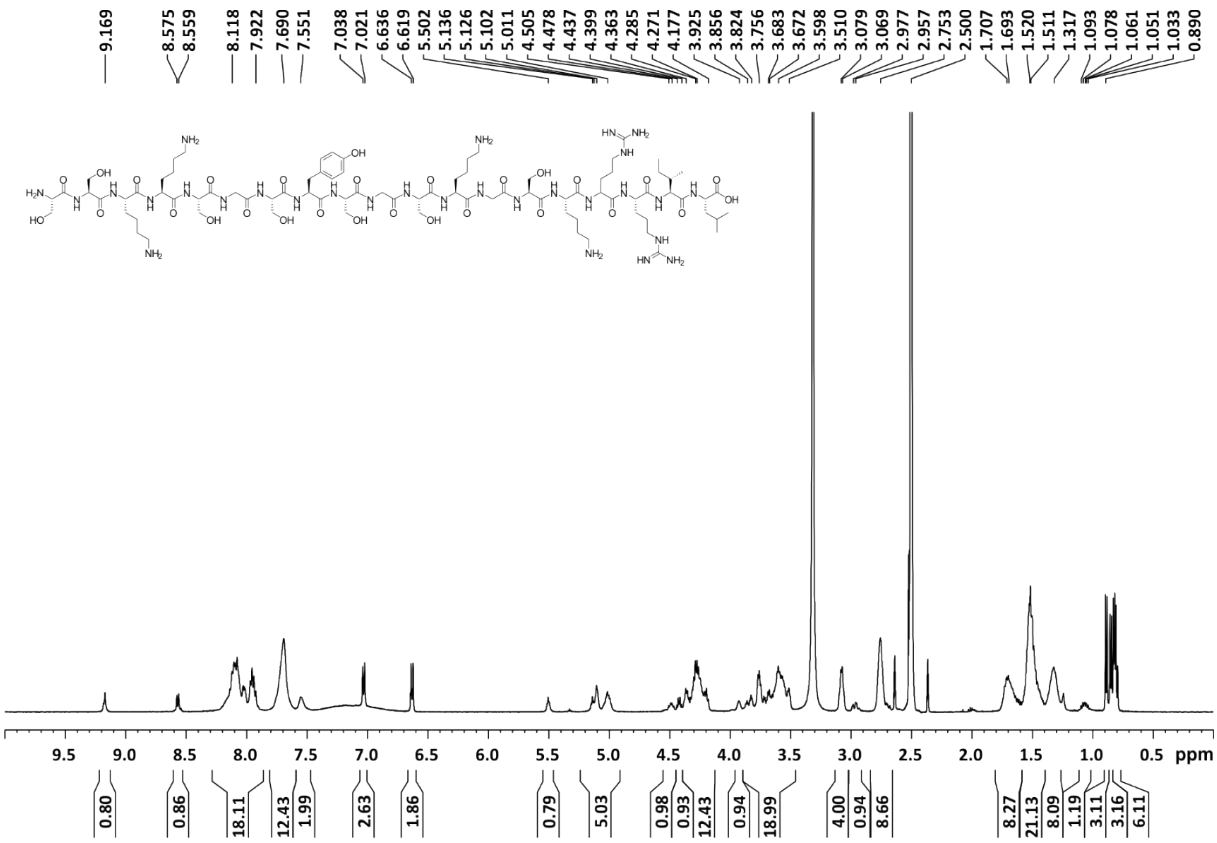


Figure 28: ^{1}H-NMR spectrum of R5 peptide **S6** (600 MHz, 300 K, $DMSO_{d6}$).

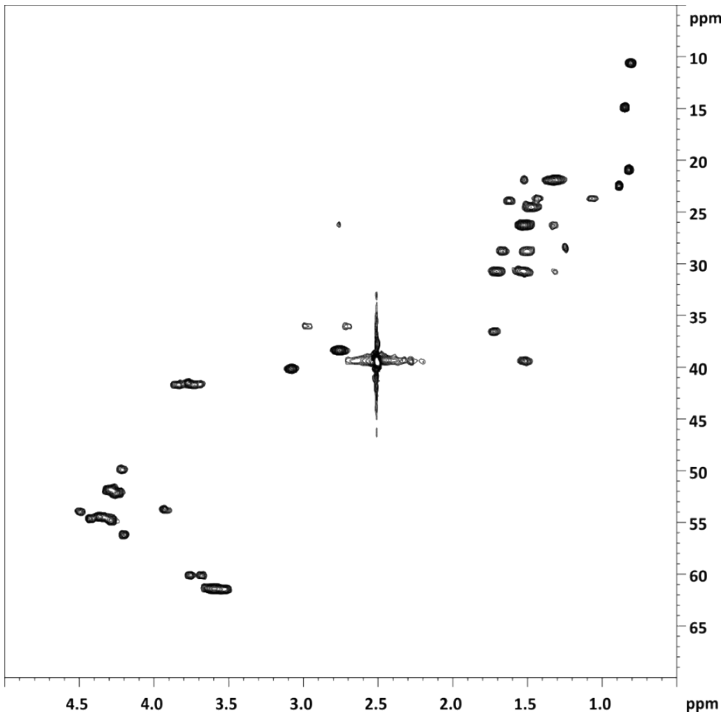


Figure 29: HSQC spectrum of the aliphatic range of R5 peptide **S6** (600 MHz, 300 K, $DMSO_{d6}$).

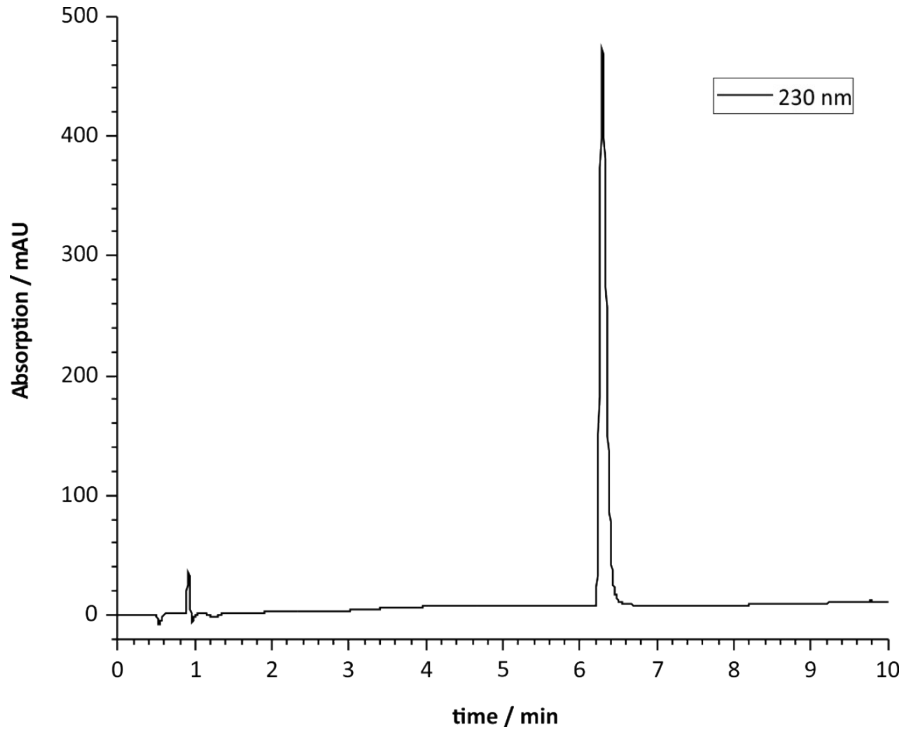


Figure 30: Chromatogram of R5 peptide **S6**. ACE Ultracore 2.5 SuperC18, 150 mm x 2.1 mm, 2-50% B in 10 min (A: H_2O + 0.1% TFA, B: CH_3CN + 0.085% TFA), flow rate 0.45 mL/min.

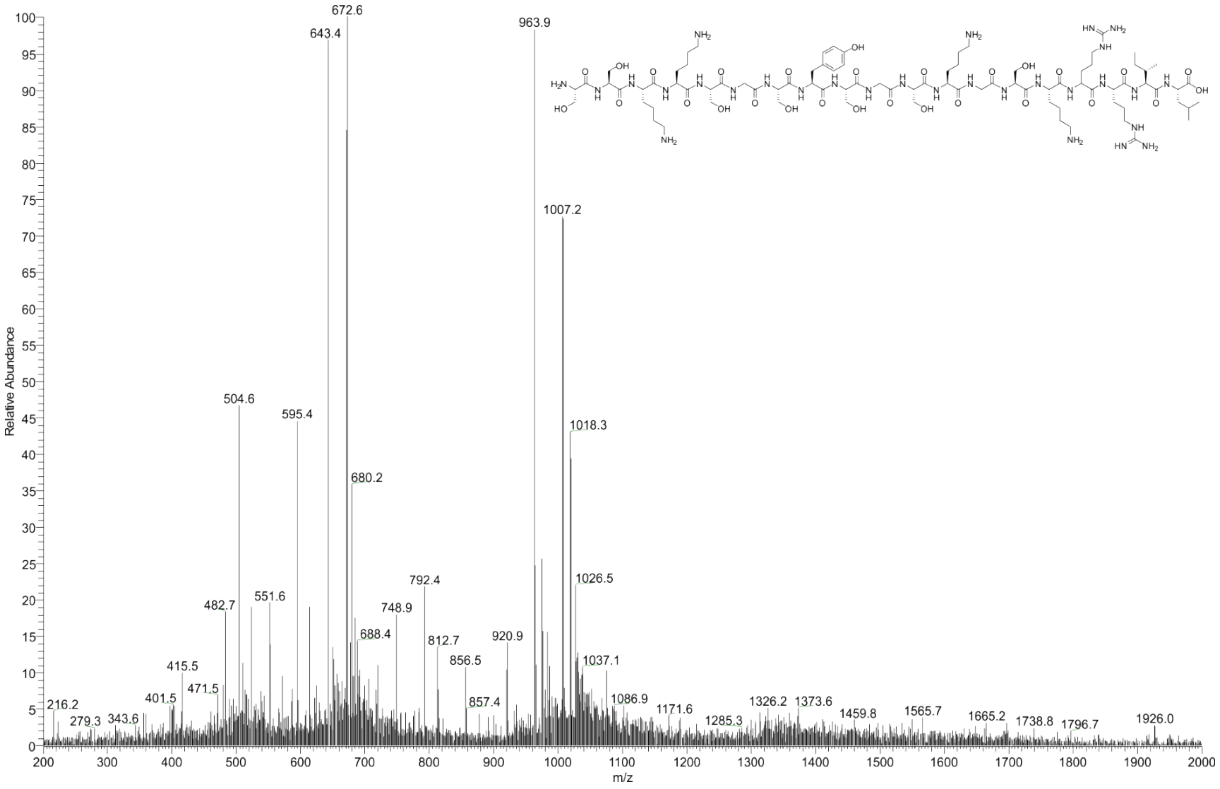


Figure 31: Mass spectrum with charge pattern m/z of R5 peptide **S6**.

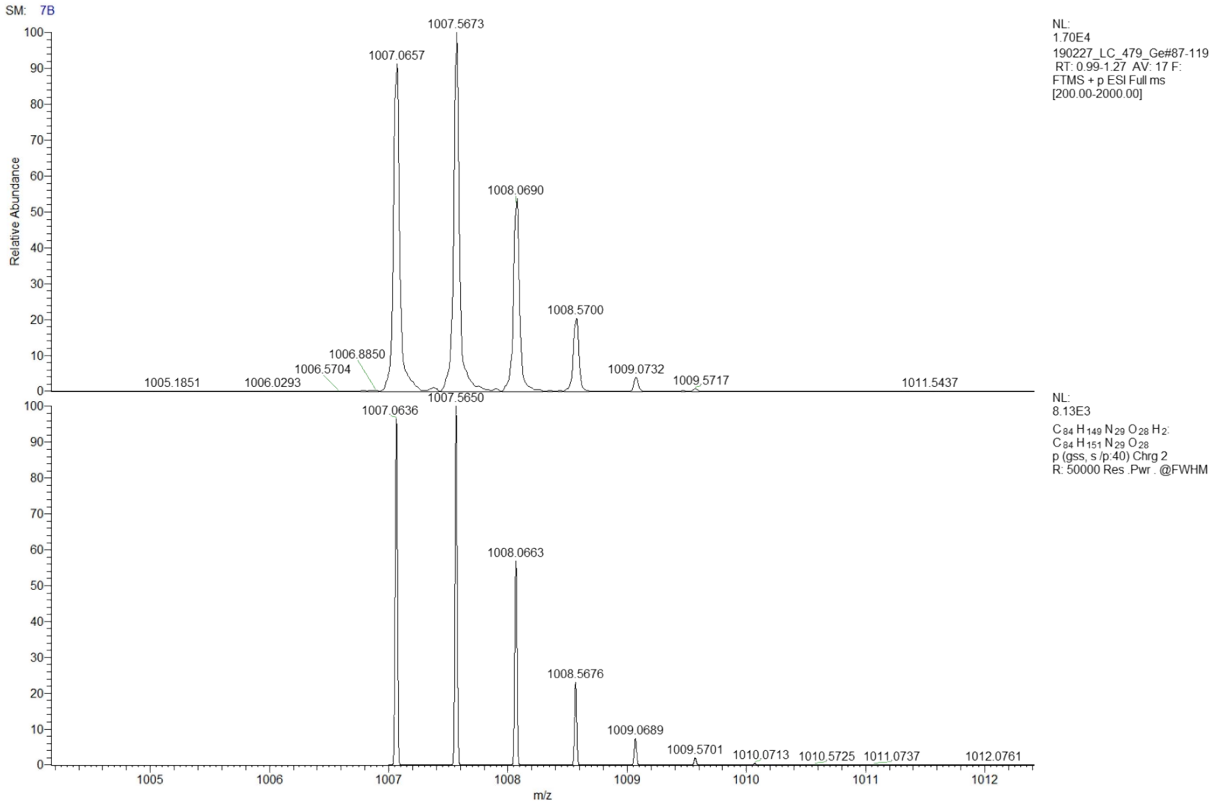


Figure 32: High resolution mass spectrum with isotope pattern of R5 peptide **S6**.

p1-Sil01 H_2N-SSKKSGpSYSGSKGSK-CO_2H (S7)

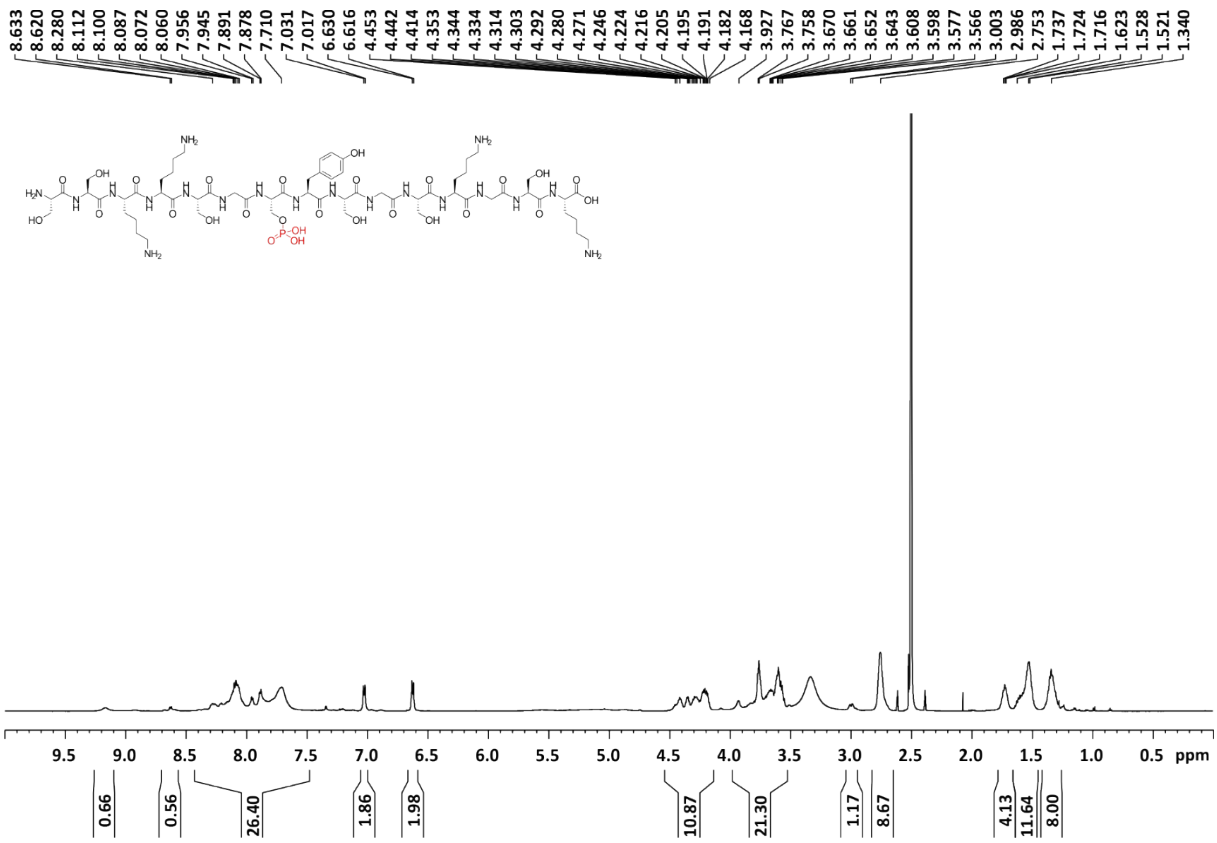


Figure 33: ^{1}H-NMR spectrum of p1-Sil01 **S7** (600 MHz, 300 K, $DMSO_{d6}$).

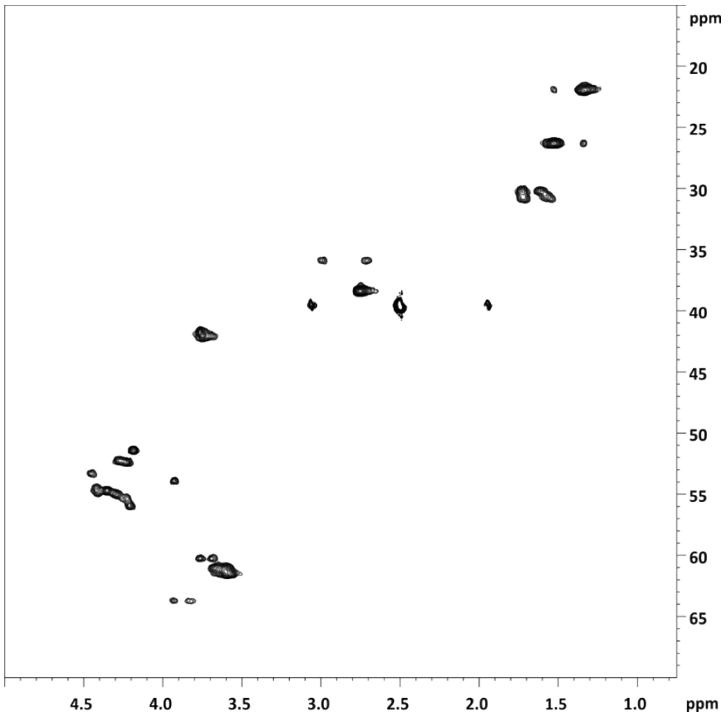


Figure 34: HSQC spectrum of the aliphatic range of p1-Sil01 **S7** (600 MHz, 300 K, $DMSO_{d6}$).

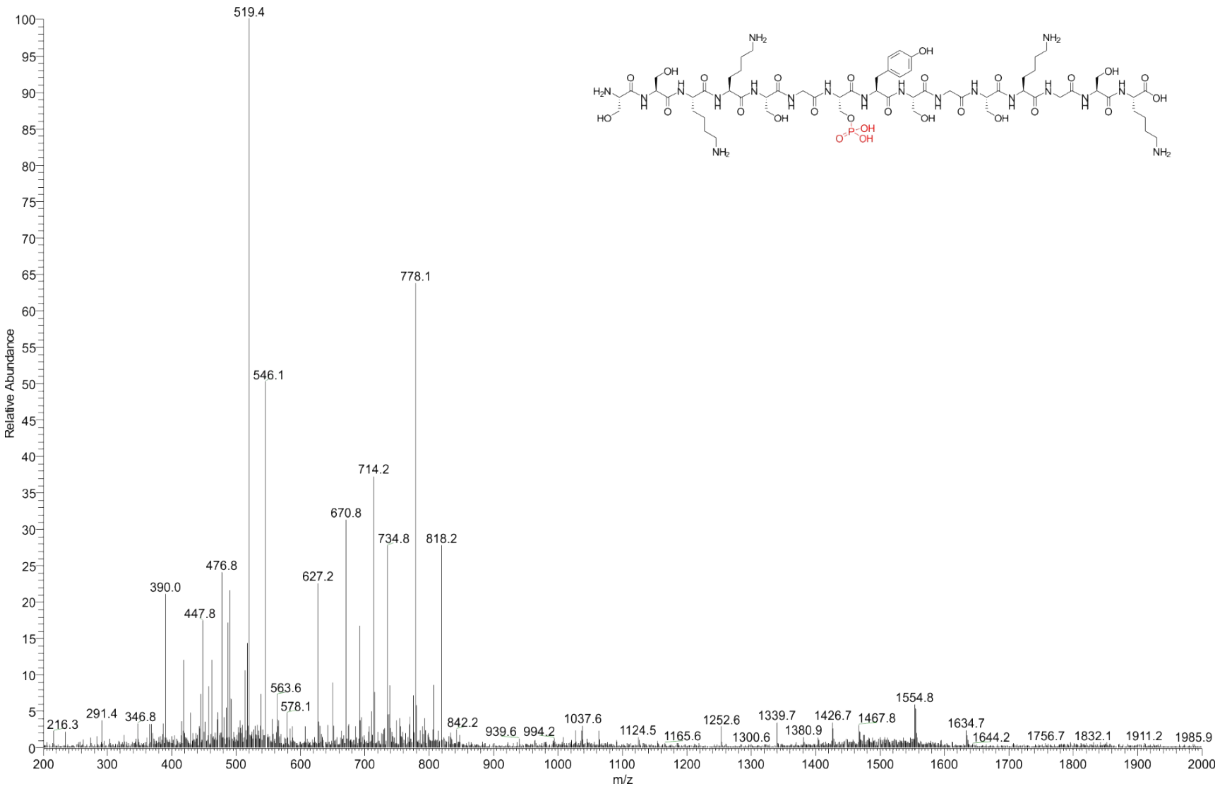


Figure 35: Mass spectrum with charge pattern m/z of peptide p1-Sil01 **S7**.

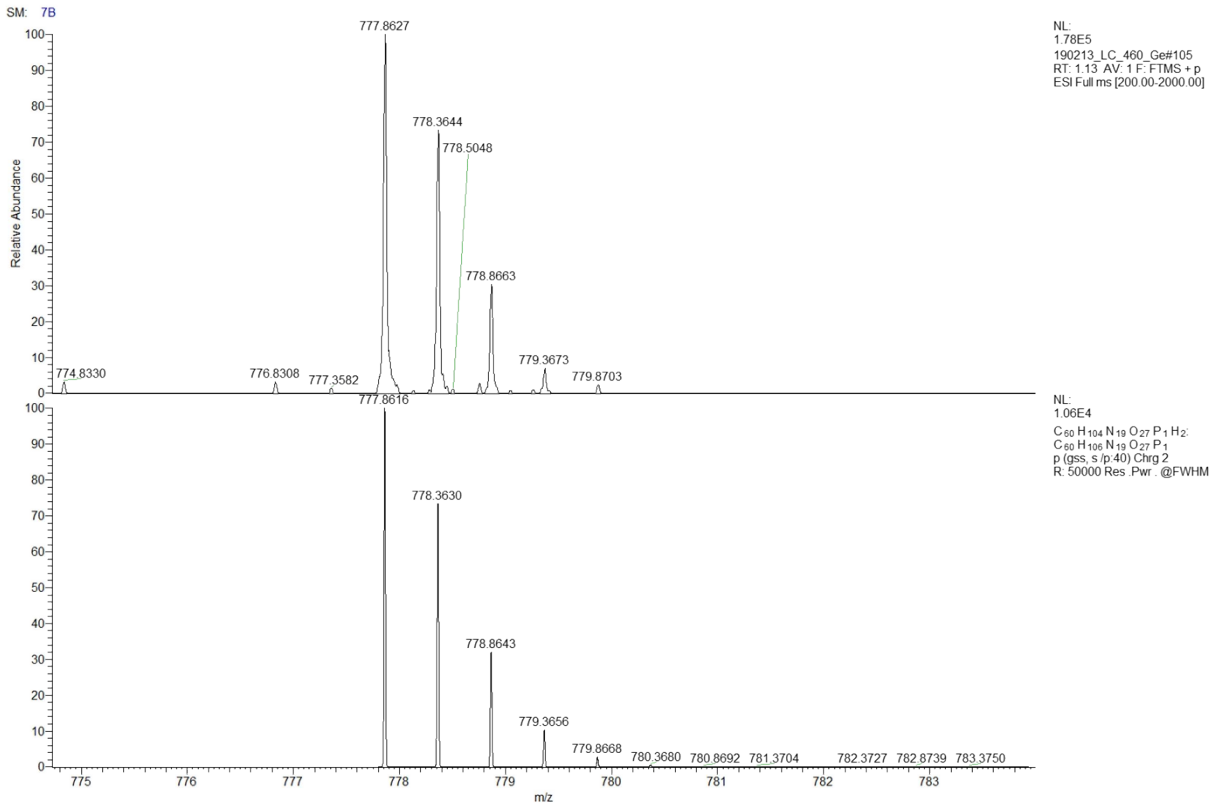


Figure 36: High resolution mass spectrum with isotope pattern of peptide p1-Sil01 **S7**.

p3-Sil01 H_2N-SpSKKSGpSYSGpSKGSK-CO_2H (S8)

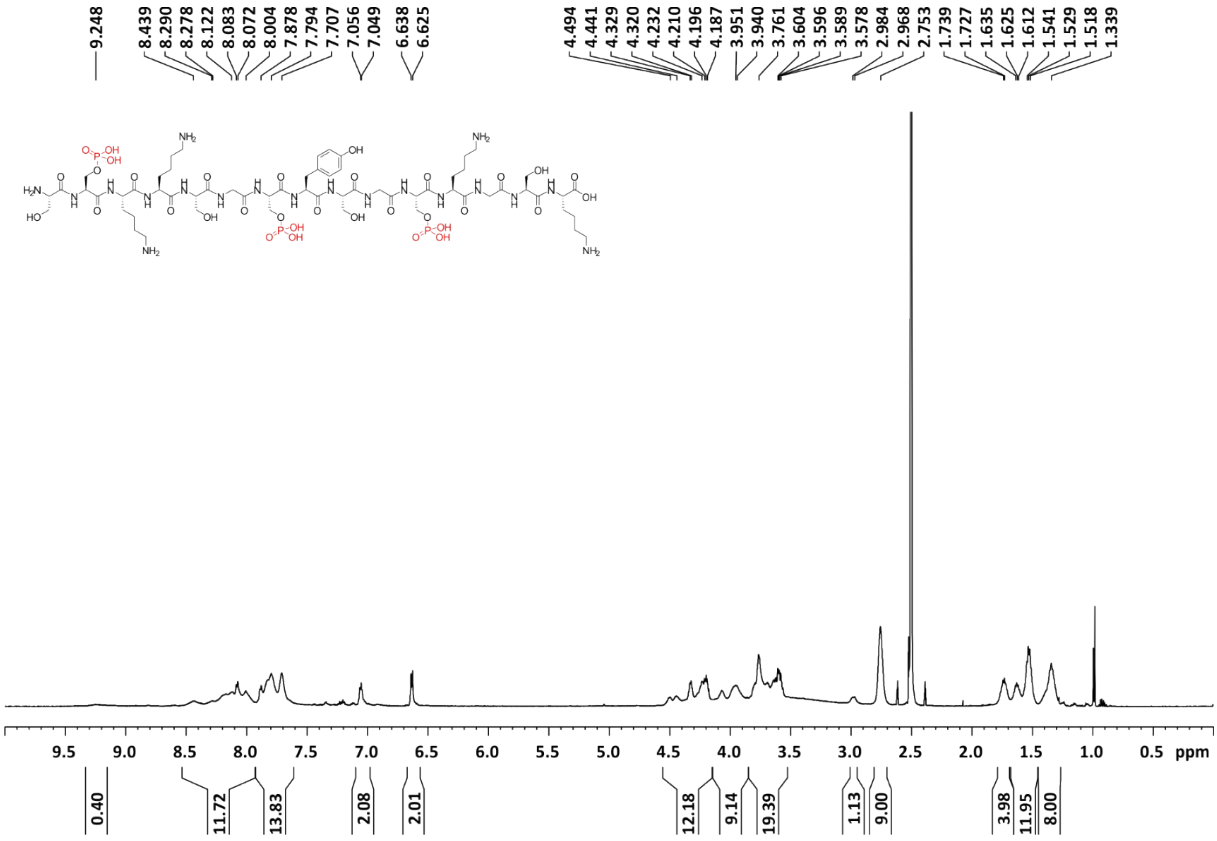


Figure 37: ^{1}H-NMR spectrum of p3-Sil01 **S8** (600 MHz, 300 K, $DMSO_{d6}$). In the range of 3.2 - 4.5 ppm, water is contained as a broad singlet, therefore the integrals appear to be too large.

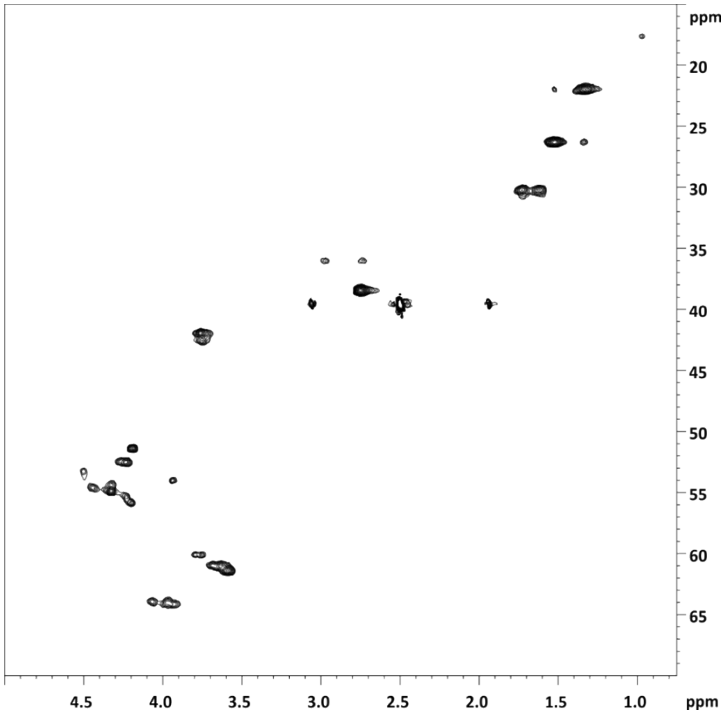


Figure 38: HSQC spectrum of the aliphatic range of p3-Sil01 **S8** (600 MHz, 300 K, $DMSO_{d6}$).

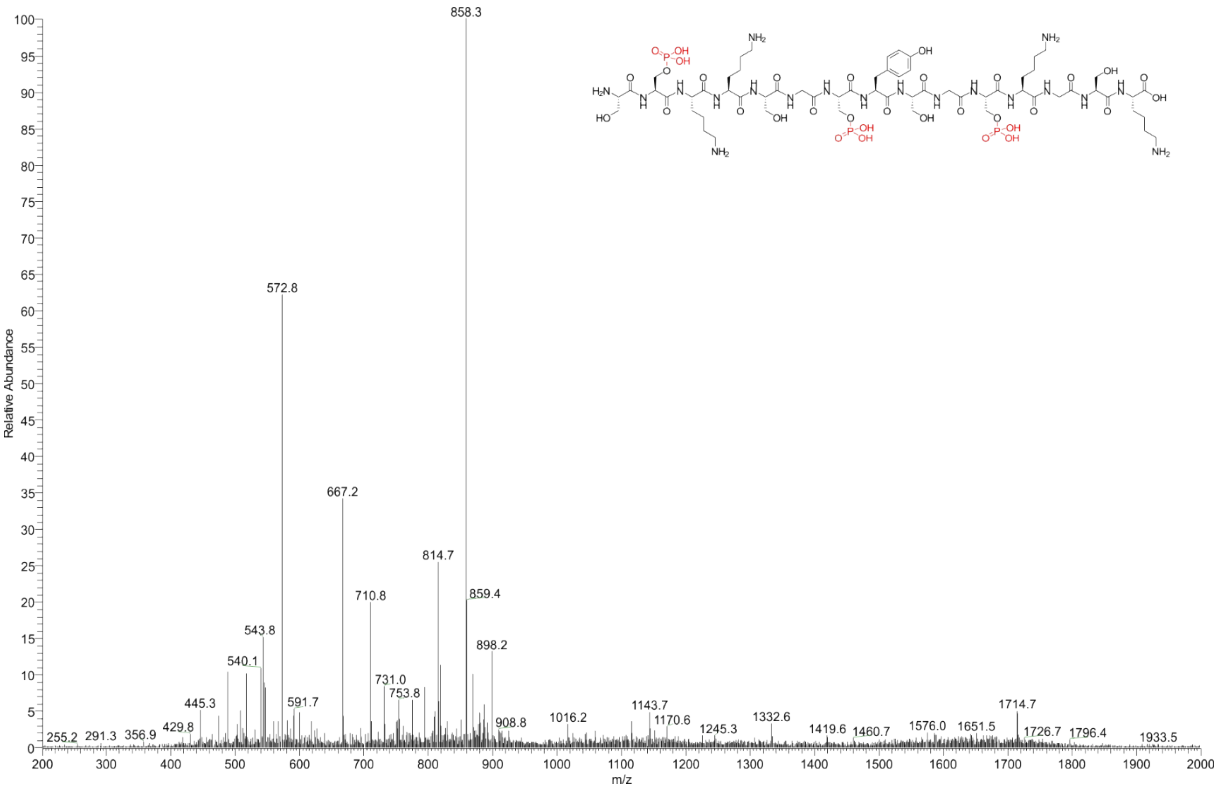


Figure 39: Mass spectrum with charge pattern m/z of peptide p3-Sil01 **S8**.

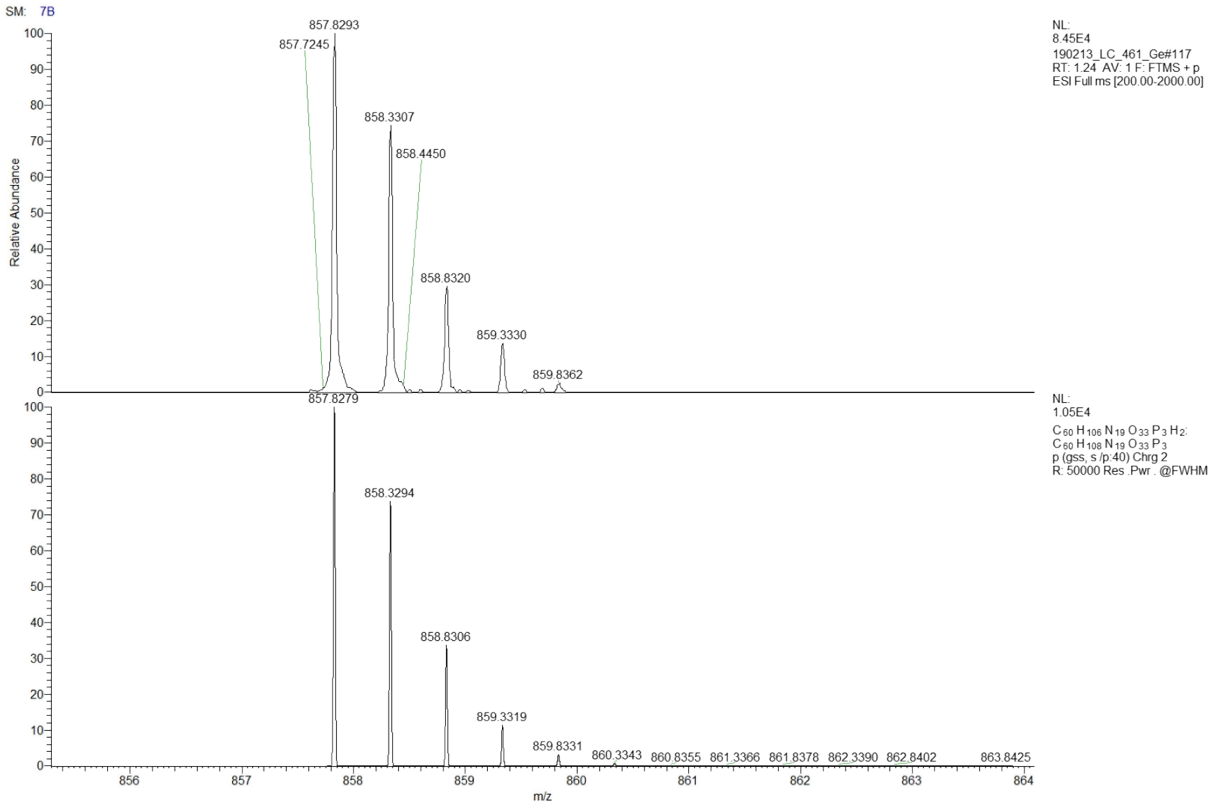


Figure 40: High resolution mass spectrum with isotope pattern of peptide p3-Sil01 **S8**.

p7-Sil01 **H_2N-pSpSKKpSGpSYpSGpSKGpSK-CO_2H (S9)**

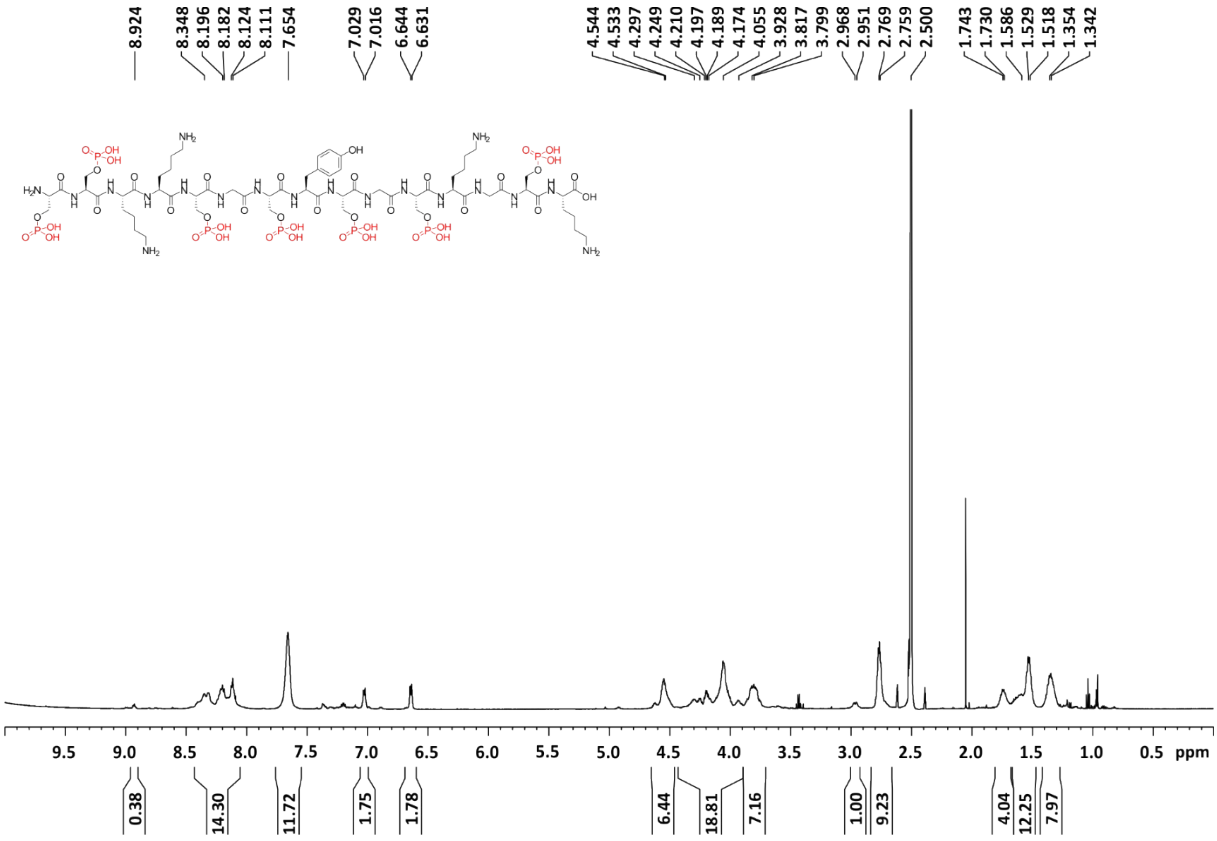


Figure 41: ^{1}H-NMR spectrum of p7-Sil01 **S9** (600 MHz, 300 K, $DMSO_{d6}$ + 5% TFA).

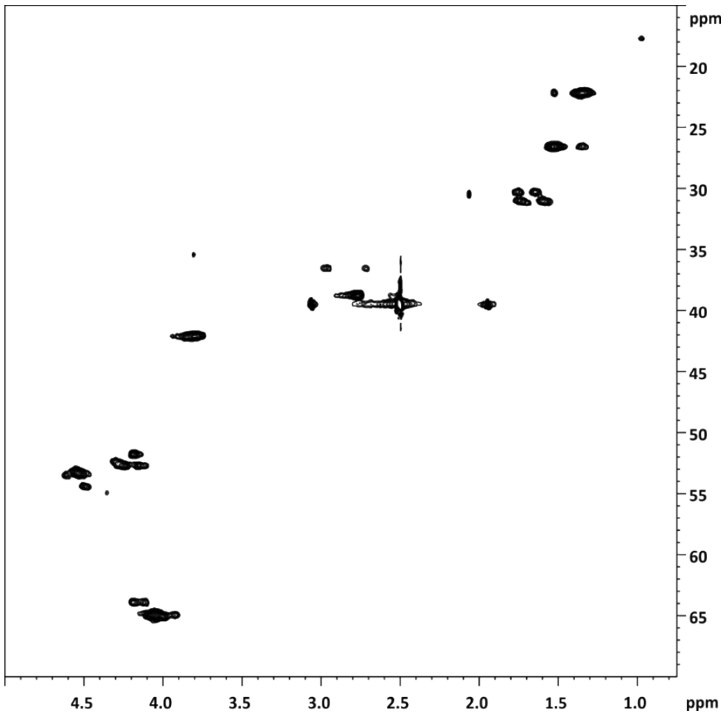


Figure 42: HSQC spectrum of the aliphatic range of p7-Sil01 **S9** (600 MHz, 300 K, $DMSO_{d6}$ + 5% TFA).

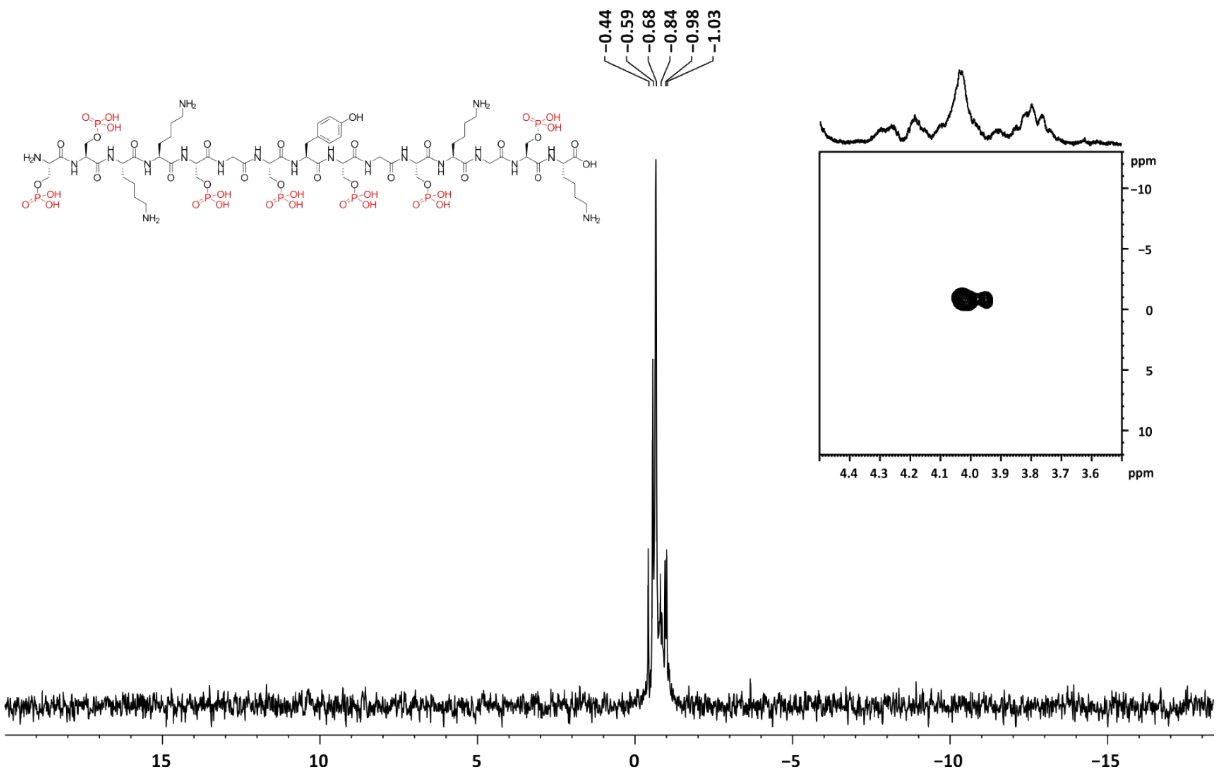


Figure 43: ^{31}P-NMR spectrum of p7-Sil01 **S9** (121 MHz, 300 K, $DMSO_{d6}$ + 5% TFA). Top right HMQC spectrum (correlation between ^{1}H and ^{31}P).

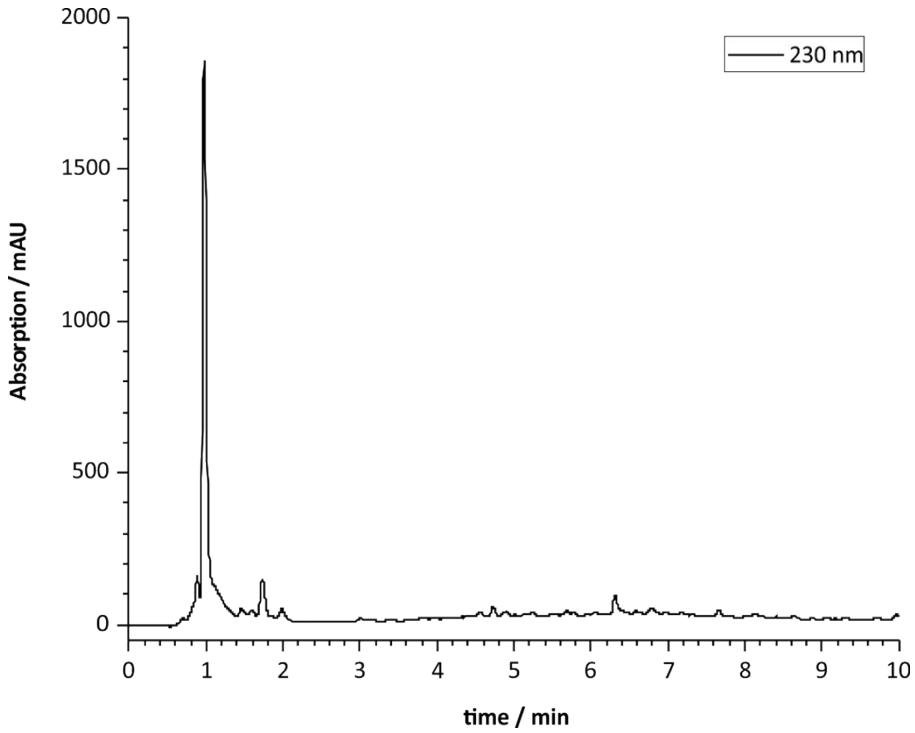


Figure 44: Chromatogram of peptide p7-Sil01 **S9**. ACE Ultracore 2.5 SuperC18, 150 mm x 2.1 mm, 2-30% B in 10 min (A: H_2O + 0.1% TFA, B: CH_3CN + 0.085% TFA), flow rate 0.45 mL/min. It was impossible to separate the p7-Sil01 peptide from the injection peak with our system.

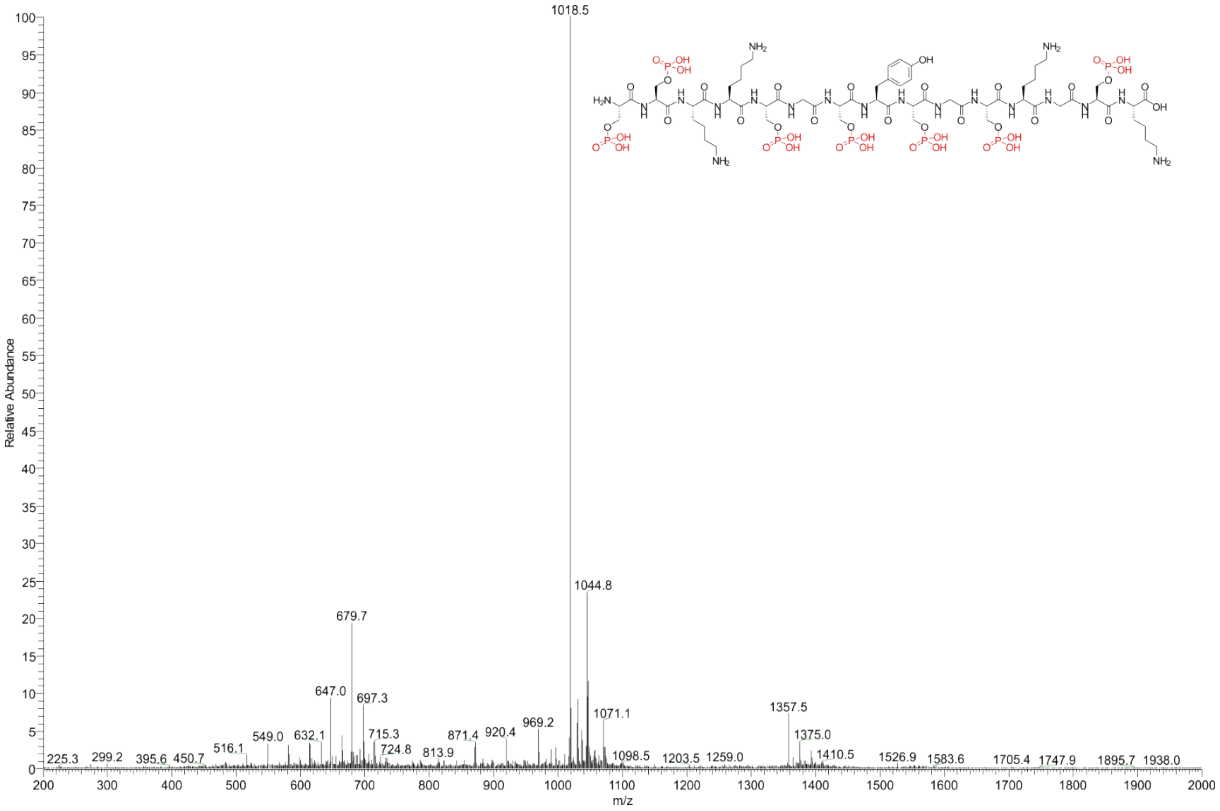


Figure 45: Mass spectrum with charge pattern m/z of peptide p7-Sil01 **S9**.

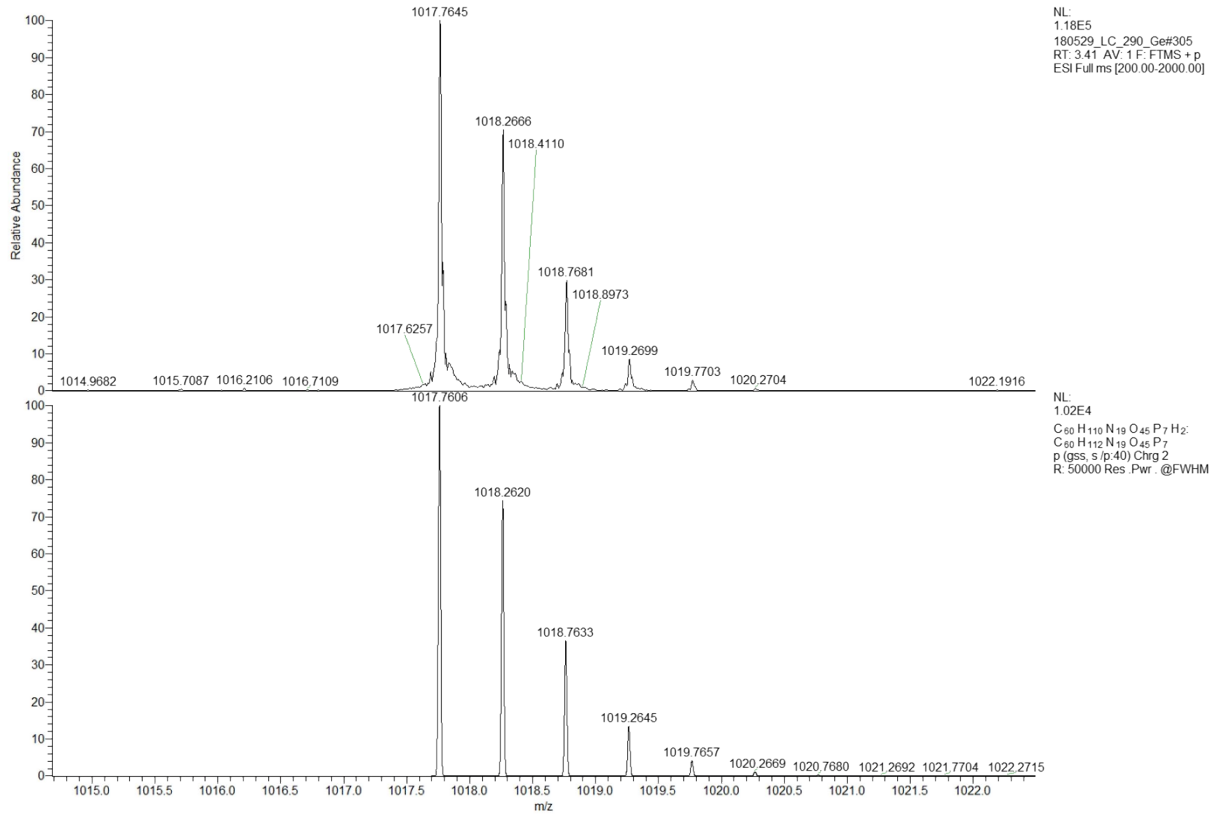


Figure 46: High resolution mass spectrum with isotope pattern of peptide p7-Sil01 **S9**.

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