A general sequence independent solid phase method for the site specific synthesis of multiple sulfated-tyrosine containing peptides.

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Abbreviations

Boc: *tert*-butyloxycarbonyl; ^{t}Bu: *tert*-butyl; CH_3CN: acetonitrile; DCE: 1,2-dichloroethane; DCM: dichloromethane; DiPEA: *N*,*N*-diisopropyl-*N*-ethylamine; DMAP: 4-dimetylaminopyridine; Dmbz: 2,6-dimethoxybenzoyl; DMF: dimethylformamide; Fmoc: 9-fluorenylmethoxycarbonyl; HBTU: 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt·H_2O: *N*-hydroxybenzotriazol hydrate; MS (ESI): mass spectrometry (electrospray ionization); MTBE: methyl-*tert*-butyl ether; NMP: *N*-methylpyrrolidone; Rt: retention time; TEA: trietylamine; TFA: trifluoroacetic acid. TIS: triisopropyl silane; Trt: triphenylmethyl or trityl.

General

Peptide grade DCM, NMP, TFA, and HPLC grade solvents were purchased from Biosolve B. V. (Varkenswaard, The Netherlands). DiPEA was obtained from Acros Organics ('s-Hertogenbosch, The Netherlands). Unless stated otherwise, chemicals were obtained from commercial sources and used without further purification.
Zinc dust (<10μm) was activated before use by washing with 2% HCl, washing with ethanol and ether and dried under vacuum according to literature procedure.[1]
Sulfuryl chloride was distilled under atmospheric pressure before use. Pyridine was distilled from CaH_2. TEA was distilled from subsequently ninhydrine and KOH.
Analytical thin layer chromatography (TLC) was performed on Merck pre-coated silica gel 60 F_{254} (0.25mm) plates. Spots were visualized with UV light, ninhydrine, or Cl_2-TDM.[2]
Column chromatography was performed using Silicycle SiliaFlash P60 (40-63 μm). ^{1}H NMR, ^{13}C NMR and two dimensional spectra were obtained on a Varian 300 MHz and 500 MHz spectrometers. Chemical shifts are given in ppm with respect to internal standard TMS for ^{1}H NMR. ^{13}C NMR spectra were recorded using the attached proton test (APT) pulse sequence.
The Fmoc-protected amino acids were purchased from GL Biochem (Shanghai) Ltd. and Fmoc-Tyr(2Cl-Trt)-OH was purchased from Novabiochem (Nottingham, UK).
The side chain protecting groups were chosen as: Boc for lysine, ^{t}Bu for aspartic acid and threonine, Trt for asparagine and histidine. TentaGel™ S RAM resin functionalized with a modified Rink Amide linker, (low crosslinked polystyrene grafted with polyethylene glycol, 0.20-0.27 mmol.g^{-1}, particle size 90 μm) was purchased from Rapp Polymere GmbH, (Germany). ArgoGel™ Wang resin (0.40 mmol/g, particle size 194 μm) was purchased from Argonaut Technologies (Muttenz, Switzerland).

The peptides were synthesized automatically on an Applied Biosystems 433A peptide synthesizer with a UV-monitoring system, which was used to monitor the Fmoc removal step *i.e.* formation of the dibenzofulvene-piperidine adduct absorbing at 301 nm. An additional deprotection cycle and a double coupling of the next amino acid was carried out if the deprotection was slow. The capping solution used was a mixture of 0.5M acetic anhydride , 0.125M DiPEA , and 0.015M HOBt·H_2O in NMP.

Analytical HPLC was performed using an automatic HPLC system (Shimadzu) with an analytical reversed-phase column, an UV detector operating at 214 nm, at a flow rate of 1 mL/min.
A Phenomenex Luna C8 column (100 Å, 5 μm, 250×4.60 mm), a Phenomenex Gemini C18 (110 Å**,** 5 μm, 250 x 4.6 mm), an Alltech Adsorbosphere C8 (90 Å, 5μm, 250 x 4.6 mm) or an Alltech Alltima C8 (100 Å, 5 μm, 250 x 4.6 mm) was used.
Either TFA buffers (buffer A: H_2O:CH_3CN, 95:5, v:v; buffer B: CH_3CN:H_2O:, 60:40, v:v, both containing 0.1% TFA) or NH_4OAc buffers (buffer A: H_2O:CH_3CN, 95:5, v:v; buffer B: CH_3CN:H_2O, 60:40, v:v, both containing 10 mmol of NH_4OAc) were used.
Elution was effected with a linear gradient from 100% A to 100% B over 48 min.

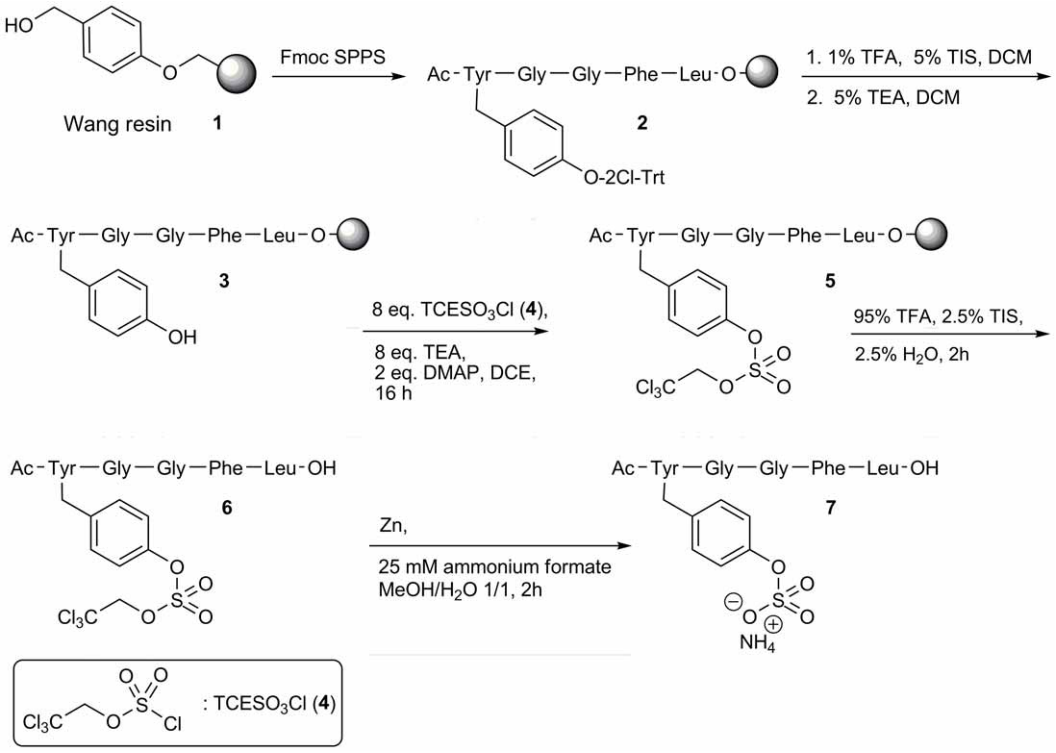
Preparative HPLC was performed using a semiautomatic HPLC system (Applied Biosystems) or a Prep LCMS-QP8000α HPLC system (Shimadzu) with a preparative reversed-phase column, an UV detector operating at 214 nm, at a flow rate of 12.5 mL/min.
A Phenomenex Jupiter C4 (300 Å**,** 10 μm, 250 x 21.2 mm), a Phenomenex Gemini C18 (110 Å**,** 10 μm, 250 x 21.2 mm), an Alltech Adsorbosphere C8 (90 Å, 10 μm, 250 x 22 mm) or an Alltech Alltima C8 (100 Å, 10 μm, 250 x 22mm) was used.
Either TFA buffers (buffer A: H_2O:CH_3CN, 95:5, v:v; buffer B: CH_3CN:H_2O, 60:40, v:v, both containing 0.1% TFA) or NH_4OAc buffers (buffer A: H_2O:CH_3CN, 95:5, v:v; buffer B: CH_3CN:H_2O, 60:40, v:v, both containing 10 mmol of NH_4OAc) were used.
Elution was effected with a linear gradient from 100% A to 100% B over 100 min.

The peptides were characterized using electrospray mass spectrometry (ESI-MS) and was performed on a Thermo Finnigan LCQ DECA XP MAX ion trap mass spectrometer, a Shimadzu LCMS-QP8000 single quadrupole bench-top mass spectrometer or a Waters LCT Time of Flight mass spectrometer (ESI-TOF) (high resolution), all operating in a positive or negative ionization mode.

Synthesis of 2,2,2-trichloroethyl chlorosulfate 4:
2,2,2-trichloroethyl chlorosulfate **4** was synthesized from sulfuryl chloride and 2,2,2-trichloro ethanol, with pyridine as base, as described by Hedayatullah et al. and purified by vacuum distillation in a yield of 82%.[3]

Synthesis of Fmoc-His(Dmbz)-OH

Fmoc-His(Dmbz)-OH was synthesized from Fmoc-His-OH and 2,6-dimethoxy benzoylchloride as described by Zaramella et al. in a yield of 91%.[4]



Supplementary Scheme 1. Solid phase synthesis of sulfated-Leu-enkephalin 7.

Synthesis of sulftated-Leu enkephalin 7:

Fmoc-Leu-OH was attached to the ArgoGel™ Wang resin (0.40 mmol/g) **1** using the procedure of Sieber yielding Fmoc-Leu-ArgoGel Wang resin with a loading of 0.31 mmol/g. [5]

The immobilized peptide **2** was assembled on an automatic ABI 433A Peptide Synthesizer using the ABI FastMoc 0.25 mmol protocols, except that the coupling time was 45 min. instead of 20 min.[6,7] The synthesis was carried out on 0.8061 g Fmoc-Leu preloaded resin. After cleavage of the Fmoc-group by means of a 20% piperidine solution in NMP (3 min. and 7.6 min.), the resin was washed with NMP (5 x). Subsequently, 1 mmol of the appropriate amino acid was dissolved in NMP (2 mL), and HBTU/HOBt (1 mmol, 2.78 mL of 0.36 M) in NMP was added. To this mixture DiPEA (1 mL, 2 M) in NMP was added, and the activated amino acid was then transferred to the reaction vessel. After 45 min, the reaction vessel was drained and the resin was washed with NMP (3 x) followed by an acetylation of any remaining free amino groups with an acetic anhydride capping solution (0.5 M Ac_2O, 0.125 M DiPEA, and 0.015 M HOBt in NMP) for 15 min. The deprotection and coupling reactions were followed by monitoring the dibenzofulvene-piperidine adduct at 301 nm.[7]

The last coupling cycle was followed by removal of the Fmoc-group by a 20% piperidine solution, washing the resin with NMP, and acetylation of the N-terminus by treatment with an acetic anhydride capping solution (0.5 M Ac_2O, 0.125 M DiPEA, and 0.015 M HOBt in NMP) for 15 min. Finally, the resin was washed with NMP (5 x) and DCM (6 x), removed from the reaction vessel, washed with ether, and dried *in vacuo* over P_2O_5.

Next, the selective cleavage of the 2Cl-Trt protecting group from **2** was carried out by treating the resin 10 times 2 min. with a mixture of DCM/TFA/TIS (94/1/5, 20 mL), followed by washing of the resin with 5% TEA in DCM (two times, 20 mL).

The thus obtained resin **3** was swollen in 8 mL of DCE and TEA (280 μL, 2 mmol (8 eq.)) and DMAP (61 mg, 0.5 mmol (2 eq.)) were added and shaken until complete dissolution, then 2,2,2-trichloroethyl chlorosulfate **4** (265 μL, 2 mmol (8 eq.)) was added. After shaking overnight, the resin was washed with DCE (3x), and ether (3x), and dried *in vacuo* over P_2O_5.

The anchored peptide **5** thus obtained was deprotected and cleaved from the solid support by treatment with 25 mL TFA/H_2O/TIS (95:2.5:2.5), for 2 h at room temperature. The mixture was then filtered and the residue washed thoroughly with TFA (2 x 10 mL). The reaction mixture was concentrated *in vacuo* to a volume of approximately 10 mL and the residue was added dropwise to 90 mL MTBE/n-hexane (1/1, v/v) solution. The precipitate was collected by centrifugation (2000 rpm, 10 min.), the supernatant was decanted, and the pellet was resuspended in MTBE/n-hexane (1/1, v/v) (100 mL) and centrifuged again. This was repeated twice. After this, the pellets were dissolved in CH_3CN/water (1/1, v/v) (ca. 60 mL) and lyophilised to give 182 mg of the crude peptide as a white fluffy solid.

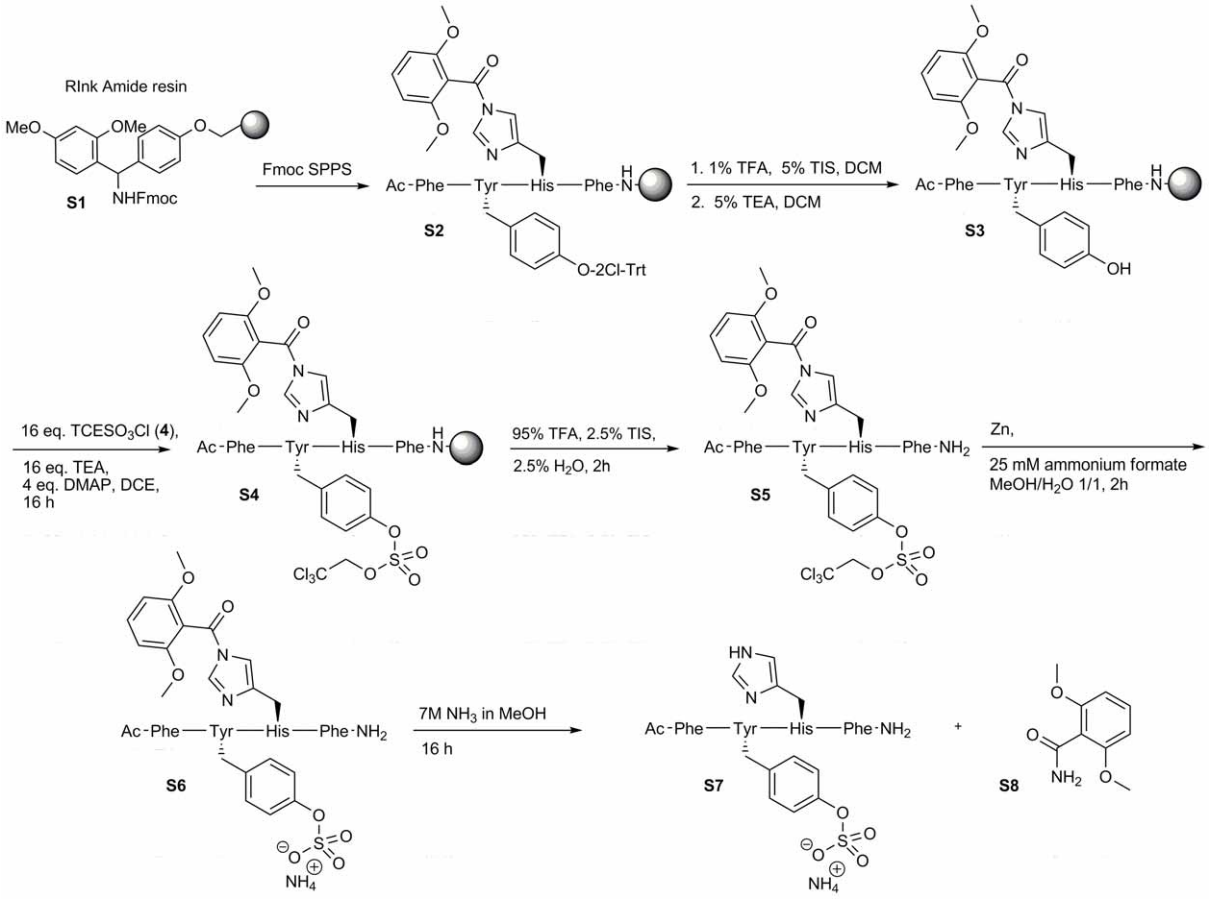
The crude peptide **6** (70 mg) was dissolved in 4 mL buffer A, 4 mL buffer B and 2 mL TFA and purified by prep HPLC (Jupiter C4, TFA buffers) in two runs. Pure fractions were pooled and lyophilized to give 58 mg of pure peptide **6**.

Peptide **6** was characterized by analytical HPLC (Luna C8, TFA buffers, Rt = 48.51 min., Purity >99%) and by ESI-MS (monoisotopic mass $[M-H]^-$ calcd for $C_{32}H_{39}Cl_3N_5O_{11}S$, 806.14; found, 806.45).

After that, 50 mg of peptide **6** was dissolved in 50 mL MeOH and 50 mL 50 mM ammonium formate, 100 mg activated zinc dust was added and the mixture was shaken for 2 h under nitrogen atmosphere. The reaction mixture was filtered, the residue was washed with MeOH and the filtrate concentrated *in vacuo* to remove MeOH, the remaining aqueous solution was lyophilized to yield crude peptide **7**.

The crude peptide **7** (45 mg) was dissolved in 4 mL buffer A and purified by prep HPLC (Jupiter C4, NH_4OAc buffers). Pure fractions were pooled and lyophilized to give 38 mg of pure peptide **7** (63% overall yield based on 0.25 mmol synthesis scale).

Peptide **7** was characterized by analytical HPLC (Luna C8, TFA buffers, Rt = 29.99 min., Purity >99%) and by high resolution ESI-TOF (monoisotopic mass $[M+H]^+$ calcd for $C_{30}H_{40}N_5O_{11}S$, 678.2445; found, 678.2456).



Supplementary Scheme 2. Solid phase synthesis of sulfated testpeptide Ac-FYHF-NH_2.

Synthesis of sulftated Ac-FYHF-NH_2 S7:

Ac-FY(SO_3NH_4)HF-NH_2 was synthesized by manual SPPS on Tentagel™ S RAM resin **S1** (0,24 mmol/g) on a 0.5 mmol scale. Tyrosine was introduced as Fmoc-Tyr(2Cl-Trt)-OH, the histidine was introduced as Fmoc-His(Dmbz)-OH. Mixing was performed by bubbling with nitrogen. After cleavage of the Fmoc-group by means of a 20% piperidine solution in NMP (3 x 8 min., 10 mL), the resin was washed with NMP (3 x 2 min., 10 mL), diethyl ether (2 min., 10 mL) and DCM (3 x 2 min., 10 mL) after which a Kaisertest was performed to confirm the deprotection.[8] Next the resin was washed with NMP (3 x 2 min., 10 mL) and a solution of 2 mmol of the appropriate amino acid, HBTU, HOBT and 4 mmol of DiPEA in 8 mL of NMP was added and mixed for 90 min. After coupling the same washing and testing procedures, as described above, were followed to confirm the coupling. The last coupling cycle was followed by removal of the Fmoc-group by a 20 % piperidine solution, washing the resin with NMP, and acetylation of the N-terminus by treatment with an acetic anhydride capping solution (0.5 M Ac_2O, 0.125 M DiPEA, and 0.015 M HOBt in 10 mL NMP) for 30 min. Finally, resin **S2** was washed with NMP (5 x) and DCM (6 x), removed from the reaction vessel, washed with ether, and dried *in vacuo*.

400 mg of resin **S2** was subjected to the selective deprotection of tyrosine by treating it 10 x 2 min. with 10 mL of a mixture of DCM/TFA/TIS (94/1/5) and washing it twice with 5 % TEA in 10 mL DCM. The thus obtained resin **S3** was swollen in 8 mL of DCE and TEA (222

μL, 1.6 mmol (16 eq.)) and DMAP (49 mg, 0.4 mmol (4 eq.)) were added and shaken until complete dissolution, then 2,2,2-trichloroethyl chlorosulfate **4** (213 μL, 1.6 mmol (16 eq.)) was added. After shaking for 16h, resin **S4** was washed with DCE (3x), and ether (3x), and dried *in vacuo*. Deprotection and cleavage of peptide **S5** from the resin was performed as described earlier.

After lyophilization, peptide **S5** was characterized by analytical HPLC (Adsorbosphere C8, TFA buffers, Rt = 48.73 min., purity >90%) and by ESI-MS (monoisotopic mass $[M+H]^+$ calcd for $C_{46}H_{48}N_7O_{12}S$, 1028.21; found, 1028.10).

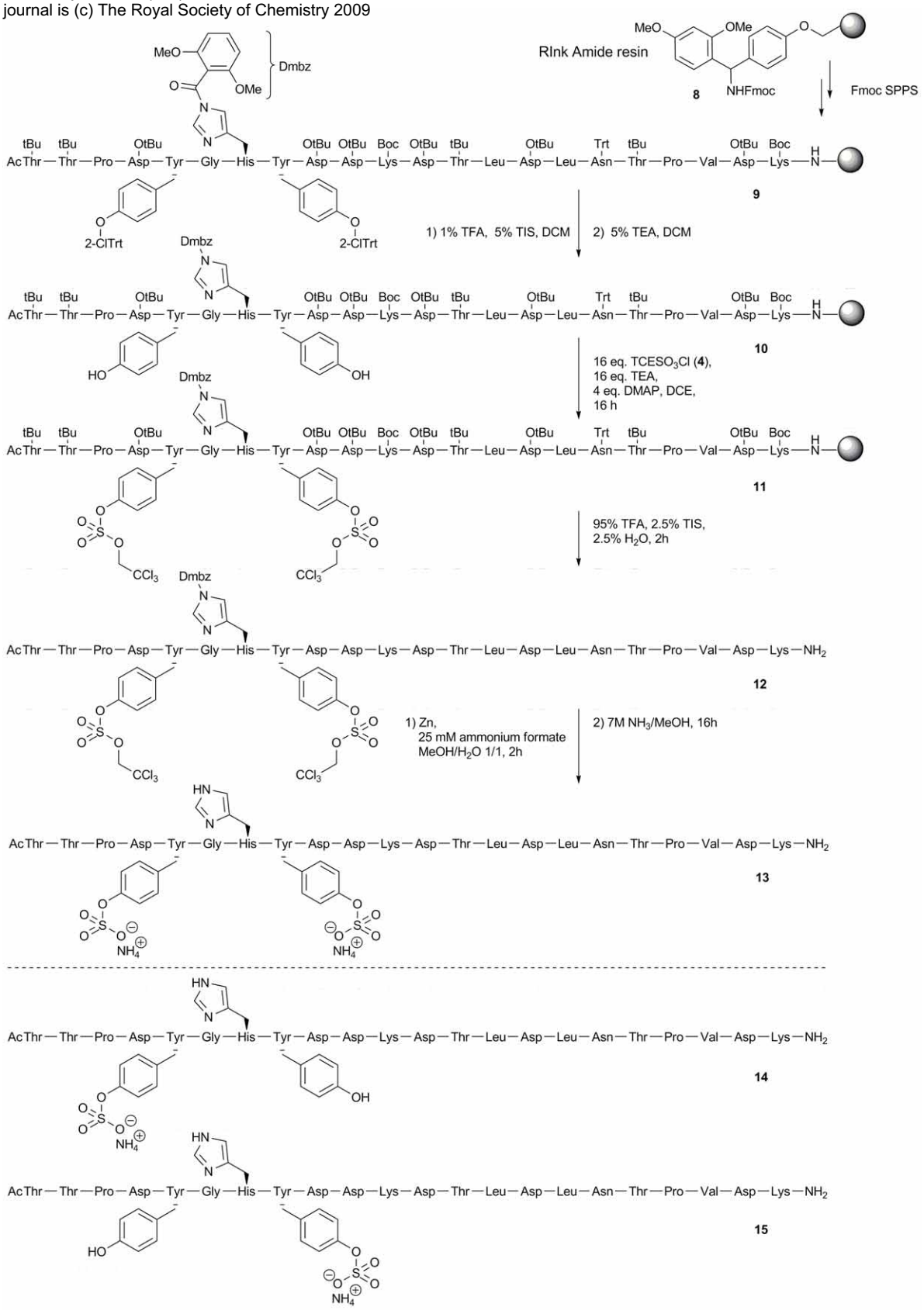
25 mg of peptide **S5** was dissolved in 5 mL MeOH and 5 mL 50 mM ammonium formate, 25 mg activated zinc power was added and the mixture was shaken for 2 h under nitrogen atmosphere. The reaction mixture was filtered, the residue was washed with MeOH/water (1/1, v/v) and the filtrate concentrated in vacuo to remove MeOH and the remaining aqueous solution was lyophilized to give crude peptide **S6**.

The crude peptide **S6** (25 mg) was dissolved in 4 mL buffer A and purified by prep HPLC (Adsorbosphere C8, NH_4OAc buffers). Pure fractions were pooled and lyophilized to give 21 mg of pure peptide **S6**.

Peptide **S6** was characterized by analytical HPLC (Adsorbosphere C8, TFA buffers, Rt = 32.27 min., Purity >95%).

4 mg of peptide **S6** was dissolved in 10 mL 7M NH_3 in MeOH and mixed for 16 hours. After concentration *in vacuo* the peptide was dissolved in 4 mL CH_3CN/water (1/1, v/v) and lyophilised to give 4 mg of the crude peptide **S7** as a white fluffy solid.

Peptide **S7** was characterized by analytical HPLC (Adsorbosphere C8, TFA buffers, Rt = 24.87 min., Purity >75%) and by ESI-MS (monoisotopic mass $[M+H]^+$ calcd for $C_{35}H_{39}N_7O_9S$, 734.25; found, 734.36). The peak at Rt = 10.48 min. was characterized with ESI-MS (monoisotopic mass $[M+H]^+$ calcd for $C_9H_{11}O_3$, 182.07; found 182.05) and appeared to be the removed protecting group 2,6-dimethoxybenzoyl **S8**.



Supplementary Scheme 3. Solid phase synthesis of a disulfated C5a$R_{7\text{-}28}$ mimic 13.

Synthesis of C5a$R_{7\text{-}28}$ diSO3 13:

Peptide **13** was assembled on an automatic ABI 433A Peptide Synthesizer on Tentagel™ S RAM resin (0,25 mmol/g) **8** on a 0.25 mmol scale as described for peptide **7**. Tyrosines 11 and 14 were introduced as Fmoc-Tyr(2Cl-Trt)-OH, histidine 13 was introduced as Fmoc-

His(Dmbz)-OH. The immobilized peptide was assembled on an automatic ABI 433A Peptide Synthesizer as described for peptide **7**.

After the selective cleavage of the 2Cl-Trt protecting groups, as described earlier, the resin was swollen in 8 mL DCE. TEA (554 μL, 4 mmol (16 eq.)) and DMAP (122 mg, 1 mmol (4 eq.)) were added and shaken until complete dissolution, then 2,2,2-trichloroethyl chlorosulfate **4** (533 μL, 4 mmol, (16 eq.)) was added. After shaking overnight, the resin was washed with DCM (3x), and ether (3x), and dried *in vacuo.* Deprotection and cleavage of the peptide from the resin **11**, was performed as described above. After lyophilization crude peptide **12** (680 mg) was obtained as a white fluffy solid. The crude peptide (300 mg) was dissolved in 12 mL buffer A, 12 mL buffer B and 6 mL TFA and purified by prep HPLC (Alltima C8, TFA buffers) in six runs. Pure fractions were pooled and lyophilized to give 85 mg of pure peptide 1**2**. Peptide **12** was characterized by analytical HPLC (Gemini C18, TFA buffers, Rt = 39.58 min., Purity >95%) and by ESI-TOF (monoisotopic mass $[M+2H]^{2+}$ calcd for $C_{124}H_{176}Cl_6N_{28}O_{51}S_2$, 1574.48; found, 1574.78).

Next 80 mg of peptide **12** was dissolved in 40 mL MeOH and 40 mL 50 mM ammonium formate, 160 mg activated zinc dust was added and the mixture was shaken for 2 h under nitrogen atmosphere. Next the reaction mixture was filtered, the residue washed with MeOH/Water (1/1, v/v) and the filtrate concentrated *in vacuo* to remove MeOH and the remaining aqueous solution was lyophilized to obtain the crude peptide. This crude peptide was dissolved in 40 mL 7 M NH_3 in MeOH and was shaken for 16 hours. After concentration *in vacuo* the residue was dissolved in 30 mL CH_3CN/water (1/1, v/v) and lyophilized. The thus obtained peptide was dissolved in 5 mL buffer A and purified by prep HPLC (Gemini C18, NH_4OAc buffers). Pure fractions were pooled and lyophilized to give 13 mg of pure peptide **13**.

Peptide **13** was characterized by analytical HPLC (Gemini C18, TFA buffers, Rt = 21.52 min., Purity >98%) and by ESI-TOF (monoisotopic mass $[M+2H]^{2+}$ calcd for $C_{111}H_{166}N_{28}O_{48}S_2$, 1362.5504; found, 1362.4146).

Synthesis of C5aR$_{7\text{-}28}$ 11SO3 14:

Peptide **14** was assembled on an automatic ABI 433A Peptide Synthesizer on Tentagel® S RAM resin (0,25 mmol/g) **8** on a 0.25 mmol scale as described for peptide **7**. Tyrosine 11 was introduced as Fmoc-Tyr(2Cl-Trt)-OH, histidine 13 was introduced as Fmoc-His(Dmbz)-OH.

After the selective cleavage of the 2Cl-Trt protecting group, as described earlier, the resin was swollen in 8 mL DCE. TEA (285 μL, 2 mmol (8 eq.)) and DMAP (61 mg, 0.5 mmol (2 eq.) were added and shaken until complete dissolution, then 2,2,2-trichloroethyl chlorosulfate **4** (267 μL, 2 mmol, (8 eq.)) was added. After shaking overnight, the resin was washed with DCM (3x), and ether (3x), and dried *in vacuo.* Deprotection and cleavage of the peptide from the resin, was performed as described above. After lyophilization crude peptide (667 mg) was obtained as a white fluffy solid. The crude peptide (450 mg) was dissolved in 27 mL buffer A, 9 mL buffer B and 9 mL TFA and purified by prep HPLC (Alltima C8, TFA buffers) in nine

runs. Pure fractions were pooled and lyophilized to give 150 mg of pure peptide. This peptide was characterized by analytical HPLC (Gemini C18, TFA buffers, Rt = 33.87 min., Purity >98%) and by ESI-TOF (monoisotopic mass $[M+2H]^{2+}$ calcd for $C_{122}H_{175}Cl_3N_{28}O_{48}S$, 1469.55; found, 1469.84).

Next, 145 mg of this protected peptide was dissolved in 50 mL MeOH and 50 mL of 50 mM ammonium formate, 200 mg of activated zinc dust was added and the mixture was shaken for 2h under nitrogen atmosphere. After filtration and subsequent washing of the residue with MeOH/water (1/1, v/v) the filtrate was concentrated *in vacuo* to remove MeOH. The remaining aqueous solution was lyophilized. Next the peptide was dissolved in 40 mL of 7 M NH_3 in MeOH and shaken for 16 hours. After concentration *in vacuo* the residue was dissolved in 40 mL CH_3CN/water (1/1, v/v) and lyophilized. The thus obtained peptide was dissolved in 15 mL buffer A and purified by prep HPLC (Gemini C18, NH_4Oac buffers) in three runs. Pure fractions were pooled and lyophilized to give 83 mg of pure peptide **14**.

Peptide **14** was characterized by analytical HPLC (Gemini C18, TFA buffers, Rt = 21.66 min., Purity >99%)) and by ESI-TOF (monoisotopic mass $[M+2H]^{2+}$ calcd for $C_{111}H_{166}N_{28}O_{45}S$, 1322.565; found, 1322.679).

Synthesis of C5aR$_{7\text{-}28}$ 14SO3 15:

Peptide **15** was assembled on an automatic ABI 433A Peptide Synthesizer on Tentagel™ S RAM resin (0,25 mmol/g) **8** on a 0.25 mmol scale as described for peptide **7.** Tyrosine 14 was introduced as Fmoc-Tyr(2Cl-Trt)-OH, histidine 13 was introduced as Fmoc-His(Dmbz)-OH.

After the selective cleavage of the 2Cl-Trt protecting group, as described earlier, the resin was swollen in 8 mL DCE. TEA (285 µL, 2 mmol (8 eq.)) and DMAP (61 mg, 0.5 mmol (2 eq.) were added and shaken until complete dissolution, then 2,2,2-trichloroethyl chlorosulfate **4** (267 µL, 2 mmol, (8 eq.)) was added. After shaking overnight, the resin was washed with DCM (3x), and ether (3x), and dried *in vacuo.* Deprotection and cleavage of the peptide from the resin, was performed as described above. After lyophilization crude peptide (659 mg) was obtained as a white fluffy solid. The crude peptide (100 mg) was dissolved in 6 mL buffer A, 2 mL buffer B and 2 mL TFA and purified by prep HPLC (Alltima C8, TFA buffers) in two runs. Pure fractions were pooled and lyophilized to give 32 mg of pure peptide. This peptide was characterized by analytical HPLC (Gemini C18, TFA buffers, Rt = 33.28 min., Purity >95%)) and by ESI-TOF (monoisotopic mass $[M+2H]^{2+}$ calcd for $C_{122}H_{175}Cl_3N_{28}O_{48}S$, 1469.55; found, 1469.81).

Next, this protected peptide was dissolved in 20 mL MeOH and 20 mL of 50 mM ammonium formate, 50 mg of activated zinc dust was added and the mixture was shaken for 2h under nitrogen atmosphere. After filtration and subsequent washing of the residue with MeOH/water (1/1, v/v) the filtrate was concentrated *in vacuo* to remove MeOH. The remaining aqueous solution was lyophilized. Next the peptide was dissolved in 20 mL of 7 M NH_3 in MeOH and shaken for 16 hours. After concentration *in vacuo* the residue was dissolved in 20 mL CH_3CN/water (1/1, v/v) and lyophilized. The thus obtained peptide was dissolved in 5 mL

buffer A and purified by prep HPLC (Gemini C18, NH_4Oac buffers). Pure fractions were pooled and lyophilized to give 13 mg of pure peptide **15**.
Peptide **15** was characterized by analytical HPLC (Gemini C18, TFA buffers, Rt = 22.04 min., Purity >95%)) and by ESI-TOF (monoisotopic mass $[M+Zn_2]^{4+}$ calcd for $C_{111}H_{166}N_{28}O_{45}SZn_2$, 692.747; found, 692.728).

Synthesis of C5aR$_{7\text{-}28}$ 16:
The unsulfated version of $C5aR_{7\text{-}28}$ was assembled on an automatic ABI 433A Peptide Synthesizer on Tentagel™ S RAM resin (0,25 mmol/g) on a 0.25 mmol scale as described for peptide **7**. The last coupling cycle was followed by removal of the Fmoc-group by a 20 % piperidine solution, washing the resin with NMP, and acetylation of the N-terminus by treatment with an acetic anhydride capping solution (0.5 M Ac_2O, 0.125 M DiPEA, and 0.015 M HOBt in NMP) for 30 min. Finally, the resin was washed with NMP (5 x) and DCM (6 x), removed from the reaction vessel, washed with ether, and dried *in vacuo*. The anchored peptide was cleaved and deprotected as described for peptide **7**. The crude peptide was purified by prep HPLC (Alltima C8, TFA buffers). Pure fractions were pooled and lyophilized to yield pure $C5aR_{7\text{-}28}$, which was characterized by analytical HPLC (Gemini C18, TFA buffers, Rt = 21.38 min., Purity >99%) and by ESI-TOF (monoisotopic mass $[M+2H]^{2+}$ calcd for $C_{111}H_{166}N_{28}O_{42}$, 1282.5935; found, 1282.5051).

NMR assignments of the peptides 13, 14 and 15. NMR samples of the peptides were prepared in 10% (v/v) D_2O/H_2O sodium phosphate buffers (20 mM, pH 6.2 or 6.5), having peptide concentrations of 1 mM. Sequential ^1H NMR assignments were performed following standard 2D NMR strategies, using a combination of 2D NOESY and 2D TOCSY spectra.[9] All spectra were recorded on a Varian Inova 500 spectrometer at 6 ^oC. Mixing times were set to 60 ms for TOCSY spectra and 400 ms for NOESY spectra. Additional ^{13}C assignments were derived from gradient enhanced ^{13}C-^1H HSQC heteronuclear correlation spectra, using known proton assignments.[10, 11]

Peptide 13

Residue	Cα	Cβ	Cγ	Cδ	Cε	Other
T7	61.93	69.81	21.62			24.51 (Cβ-Acetyl)
T8	59.90	69.87	21.62			
P9	63.33	32.25	27.58	51.22 [b]		
D10	53.85	41.11[b]				
Y11[S]	58.12	38.81		133.20[b]	124.40[b]	
G12	45.33					
H13	55.32	29.03		120.10	136.10	
Y14[S]	57.68	39.06		133.20[b]	124.40[b]	
D15	54.32	41.07[b]				
D16	54.38	41.05[b]				
K17	56.88	32.90	24.71	28.98	42.16 [b]	
D18	54.72	41.11[b]				
T19	62.35	69.70	21.70			
L20	55.55	42.26	26.98[b]	25.07[b], 23.46[b]		
D21	54.29	41.02				
L22	55.58	42.15	26.98[b]	25.07[b], 23.46[b]		
N23	53.49	39.10				
T24	60.39	69.76	21.46			
P25	63.22	32.29	27.58	51.22 [b]		
V26	62.57	32.97	21.13, 20.79			
D27	54.27	41.09[b]				
K28	56.21	32.69	24.87	29.00	42.16 [b]	

Residue	NH	CαH	CβH	CγH	CδH	Other
T7	8.317	4.357	4.173	1.175		2.035 (N-Acetyl)
T8	8.370	4.643	4.184	1.272		
P9		4.315	2.184,1.625	1.971,1.971	3.860,3.691	
D10	8.401	4.538	2.652,2.534			
Y11[S]	8.270	4.537	3.210,3.001		7.285	7.243 (CεH)
G12	8.408	3.804,3.765				
H13	8.198	4.632	3.152,3.036		7.100	8.520 (CεH)
Y14[S]	8.504	4.623	3.186,2.955		7.270	7.236 (CεH)
D15	8.543	4.611	2.716,2.599			
D16	8.379	4.564	2.713,2.713			
K17	8.365	4.268	1.836,1.798	1.435,1.435	1.653,1.594	2.982 (CεH)
D18	8.463	4.649	2.749,2.667			
T19	8.083	4.270	4.252	1.193		
L20	8.263	4.291	1.654,1.571	1.613[b]	0.915,0.852	
D21	8.434	4.576	2.769,2.599			
L22	8.354	4.290	1.644,1.608	1.613[b]	0.925,0.854	
N23	8.529	4.720	2.823,2.764			7.792,6.998 (NδH)
T24	8.071	4.528	4.105	1.246		
P25		4.455	2.303,1.903	2.046,2.008	3.881,3.692	
V26	8.396	4.027	2.036	0.934,0.959		
D27	8.541	4.584	2.729,2.602			
K28	8.496	4.237	1.915,1.754	1.475,1.427	1.673,1.673	2.989 (CεH) 7.753,7.221 (C-NH_2)

Peptide 14

Residue	Cα	Cβ	Cγ	Cδ	Cε	Other
T7	61,92	69,95	21,67			24.45 (Cβ-Acetyl)
T8	59,96	69,74	21,55			
P9	63,28	32,24	27,53	51,22		
D10	53,83	41,22				
Y11^{S}	58,07	38,66		133,13	124,29	
G12	45,29					
H13	55,10	29,10		120,08	136,36	
Y14	58,07	38,98		133,33	118,18	
D15	54,08	41,23				
D16	54,52	40,86				
K17	56,88	32,74	24,67	28,98	42,13	
D18	54,87	40,92				
T19	62,34	69,63	21,71			
L20	55,52	42,26	26,92	24,89 23,61		
D21	54,20	40,88				
L22	55,52	42,14	26,97	25,15 2318		
N23	53,41	39,04				
T24	60,42	69,73	21,46			
P25	63,21	32,27	27,57	51,22		
V26	62,56	32,95	21,09 20,79			
D27	54,20	41,03				
K28	56,19	32,67	24,87	28,98	42,14	

Residue	NH	CαH	CβH	CγH	CδH	Other
T7	8,346	4,353	4,171	1,175		2.035 (N-Acetyl)
T8	8,395	4,639	4,182	1,276		
P9		4,301	2,160 1,605	1,965 1,965	3,868 3,689	
D10	8,434	4,536	2,676 2,562			
Y11^{S}	8,399	4,552	3,216 2,995		7,280	7.225 (CεH)
G12	8,456	3,765 3,765				
H13	8,221	4,649	3,199 3,069		7,183	8.546 (CεH)
Y14	8,455	4,550	3,047 2,886		7,097	6,800 (CεH)
D15	8,509	4,594	2,696 2,574			
D16	8,335	4,531	2,713 2,713			
K17	8,390	4,265	1,840 1,816	1,429 1,429	1,664 1,664	2.990 2,990 (CεH) 7,621 (NξH)
D18	8,439	4,646	2,762 2,674			
T19	8,096	4,269	4,243	1,197		
L20	8,290	4,294	1,657 1,563	1,634	0,911 0,859	
D21	8,454	4,575	2,777 2,597			
L22	8,382	4,288	1,644 1,598	1,602	0,923 0,851	
N23	8,545	4,721	2,829 2,762		7,809 7,022	
T24	8,090	4,522	4,104	1,247		
P25		4,451	2,303 1,896	2,043 1,997	3,884 3,691	
V26	8,427	4,023	2,034	0,930 0,963		
D27	8,568	4,582	2,730 2,633			
K28	8,531	4,233	1,919 1,751	1,474 1,424	1,675 1,675	2.989 (CεH) 7.751,7.226 (C-NH_2)

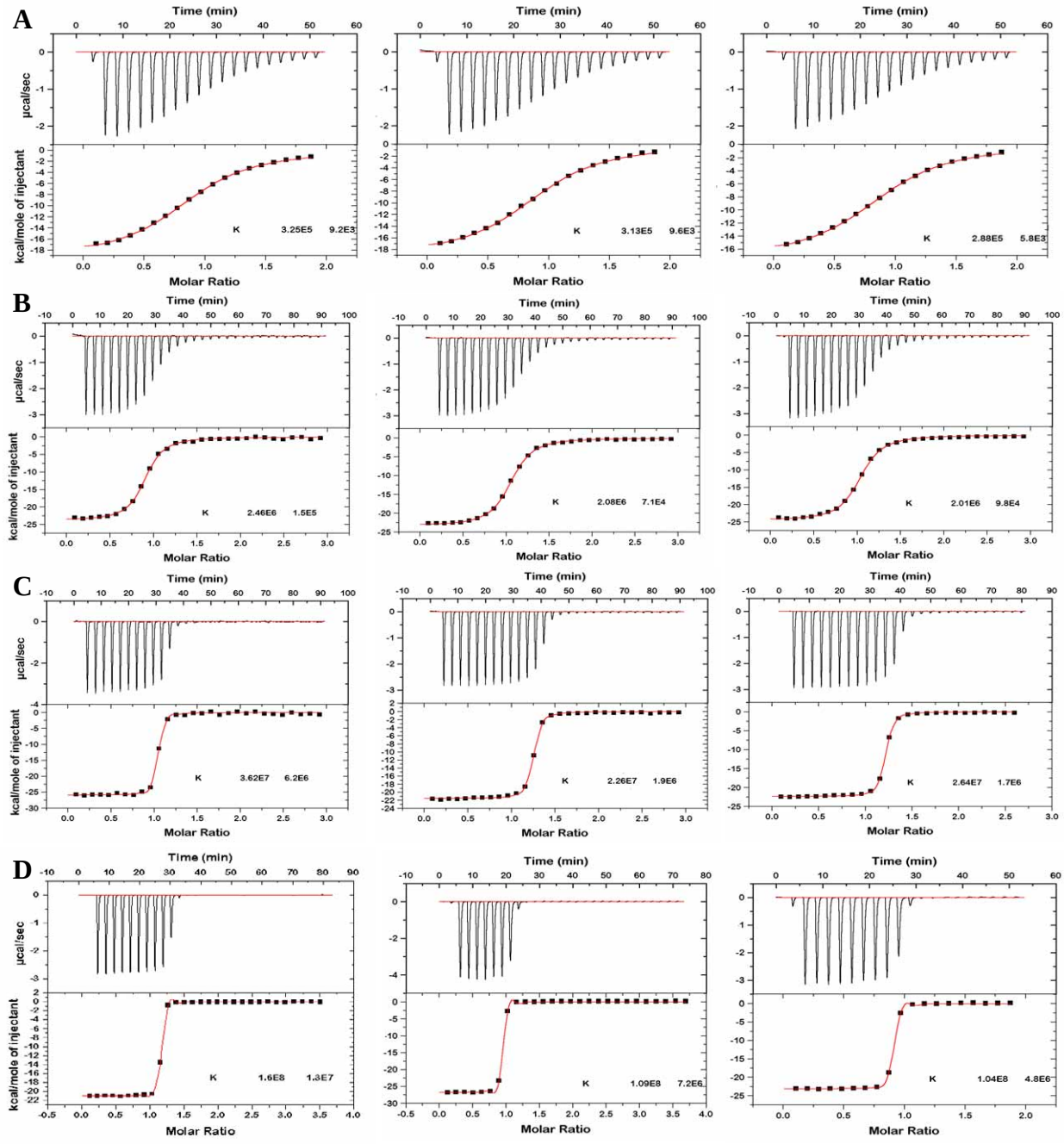
Peptide 15

Residue	Cα	Cβ	Cγ	Cδ	Cε	Other
T7	61,94	69,97	21,68			24.46 (Cβ-Acetyl)
T8	59,91	69,76	21,51			
P9	63,31	32,19	27,49	51,18		
D10	53,80	40,93				
Y11^{S}	58,38	38,54		133,24	118,22	
G12	45,34					
H13	55,36	28,88		120,00	136,30	
Y14^{S}	57,71	39,03		133,24	124,40	
D15	54,28	41,02				
D16	54,36	40,76				
K17	56,85	32,80	24,66	28,98	42,15	
D18	54,76	40,90				
T19	62,34	69,62	21,67			
L20	55,55	42,23	26,94	24,92 23,56		
D21	54,21	40,83				
L22	55,54	42,12	26,99	25,14 23,22		
N23	53,41	39,04				
T24	60,39	69,74	21,46			
P25	63,18	32,28	27,56	51,22		
V26	62,56	32,97	21,09 20,79			
D27	54,26	41,02				
K28	56,20	32,65	24,86	28,97	42,13	

Residue	NH	CαH	CβH	CγH	CδH	Other
T7	8,347	4,350	4,169	1,175		2.035 (N-Acetyl)
T8	8,412	4,637	4,180	1,267		
P9		4,311	2,153 1,602	1,952 1,952	3,864 3,675	
D10	8,458	4,538	2,664 2,534			
Y11	8,174	4,465	3,100 2,933		7,136	6,817(CεH)
G12	8,387	3,768 3,768				
H13	8,178	4,626	3,154 3,030		7,111	8.526 (CεH)
Y14^{S}	8,493	4,625	3,199 2,945		7,273	7,224 (CεH)
D15	8,568	4,616	2,733 2,607			
D16	8,404	4,566	2,725 2,725			
K17	8,400	4,267	1,843 1,184	1,434 1,434	1,661 1,661	2.985 2,985 (CεH) 7,619 (NξH)
D18	8,474	4,653	2,760 2,676			
T19	8,105	4,263	4,242	1,195		
L20	8,290	4,297	1,659 1,566	1,629	0,912 0,855	
D21	8,454	4,575	2,7872,602			
L22	8,387	4,288	1,650 1,599	1,597	0,920 0,852	
N23	8,545	4,721	2,829 2,763		7,812 7,026	
T24	8,094	4,523	4,104	1,248		
P25		4,452	2,307 1,900	2,040 1,998	3,888 3,695	
V26	8,432	4,023	2,033	0,933 0,963		
D27	8,577	4,584	2,726 2,624			
K28	8,540	4,236	1,920 1,755	1,473 1,423	1,671 1,671	2.991 (CεH) 7.755,7.230 (C-NH_2)

ITC measurements.

The ITC measurements have been performed on a MCS Isothermal Titration Calorimeter (Microcal Inc., Northampton, MA). The measuring cell was filled with 1.5 mL of a 30 μM solution of $CHIPS_{31\text{-}121}$ in a 20 mM sodium phosphate buffer at pH 6.5. The syringe was loaded with 250 μL of a 0.5 mM solution of one of the peptides in the same buffer. After each addition of 10 μL of peptide solution, the heat change due to binding of the added peptide is measured. The data were analysed using Microcal Origin software and fitted by non-linear regression analysis.



Supplementary Figure 1: ITC-data fitted with Microcal Origin software. A) $C5aR_{7\text{-}28}$ (**16**); B) $C5aR_{7\text{-}28}$ $11SO_3$ (**14**); C) $C5aR_{7\text{-}28}$ $14SO_3$ (**15**); D) $C5aR_{7\text{-}28}$ $diSO_3$ (**13**).

[1] D. D. Perrin, W. L. F. Armarego, *Purification of laboratory chemicals*, Pergamon Press, Oxford, **1988**, p. 360.
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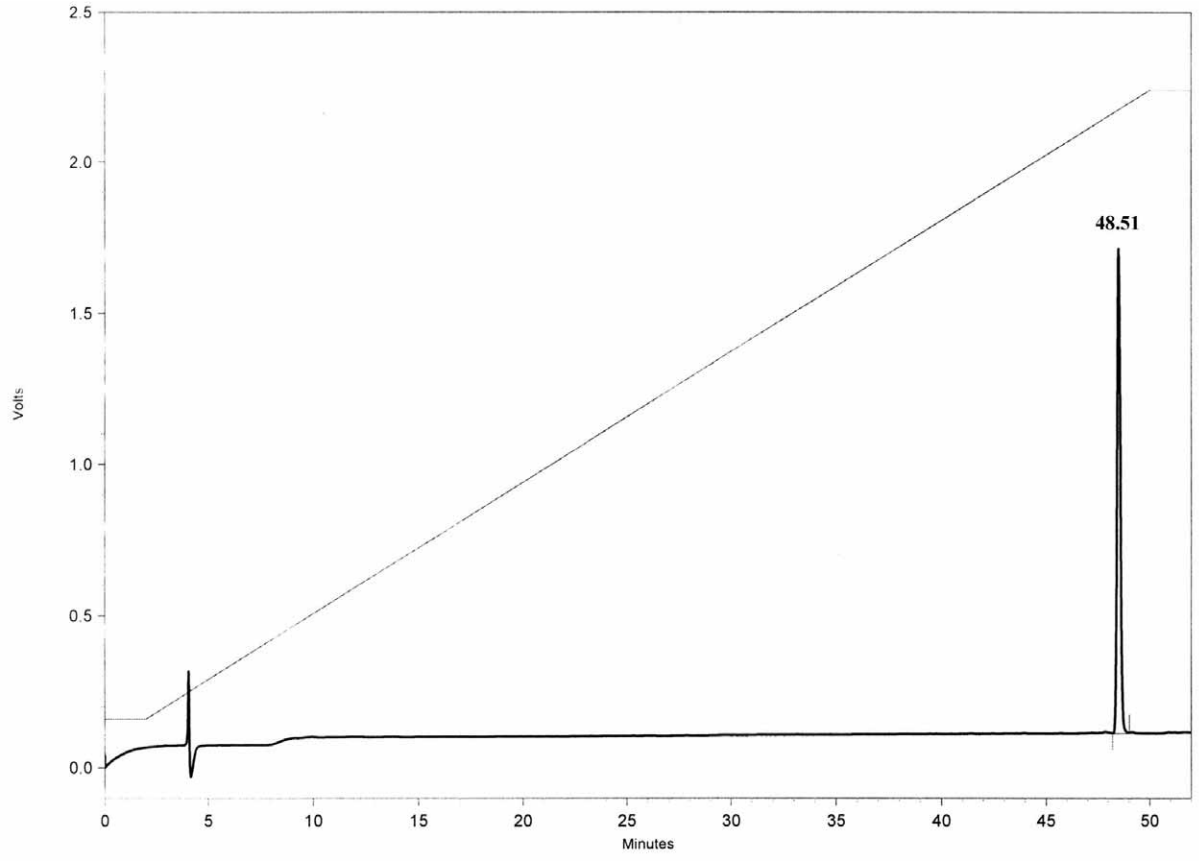
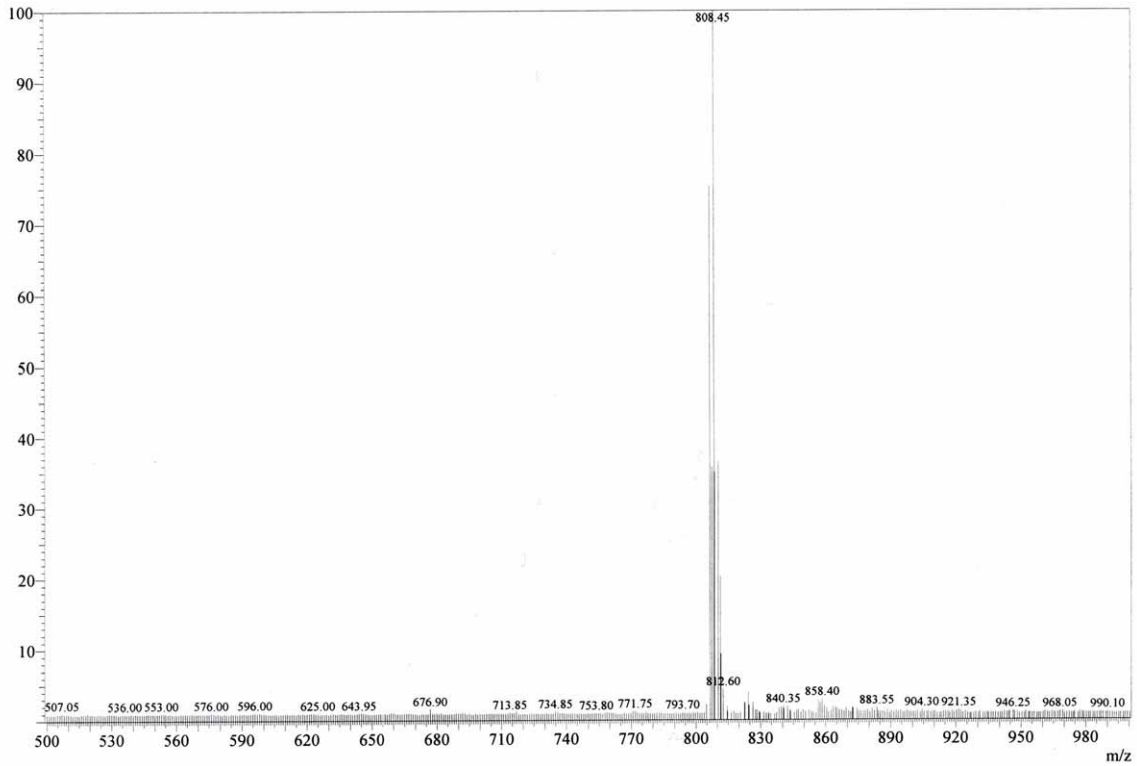
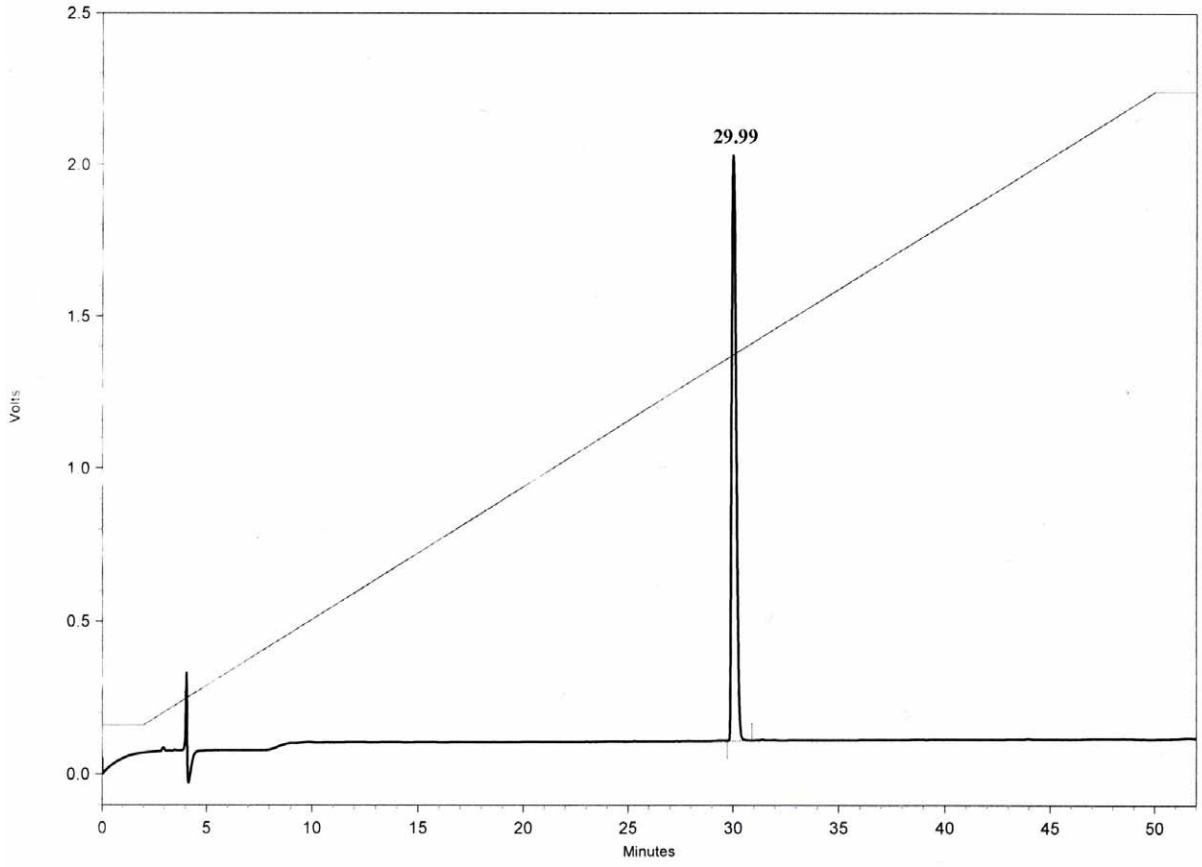
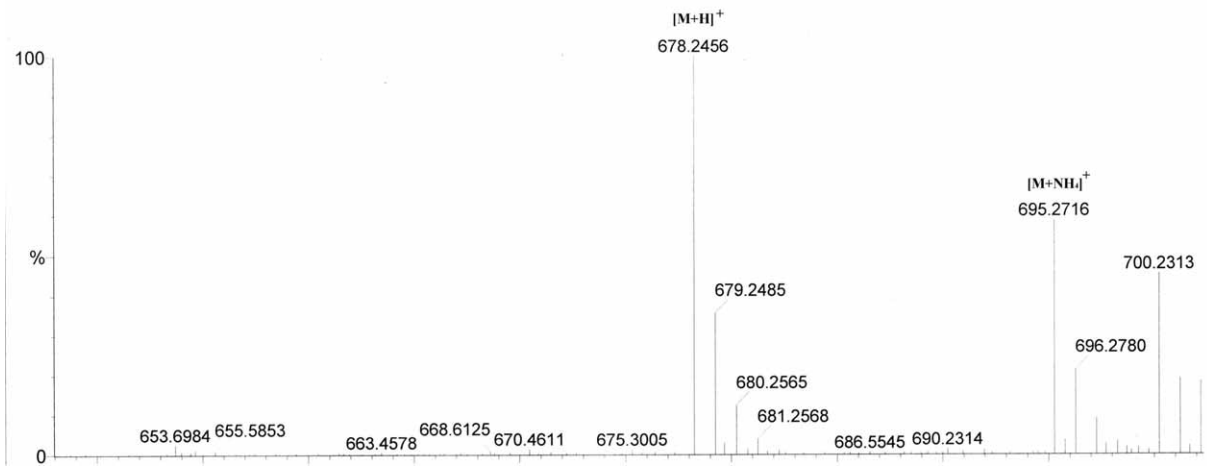
Peptide 6:
Ac-Y(SO_3TCE)GGFL-OH



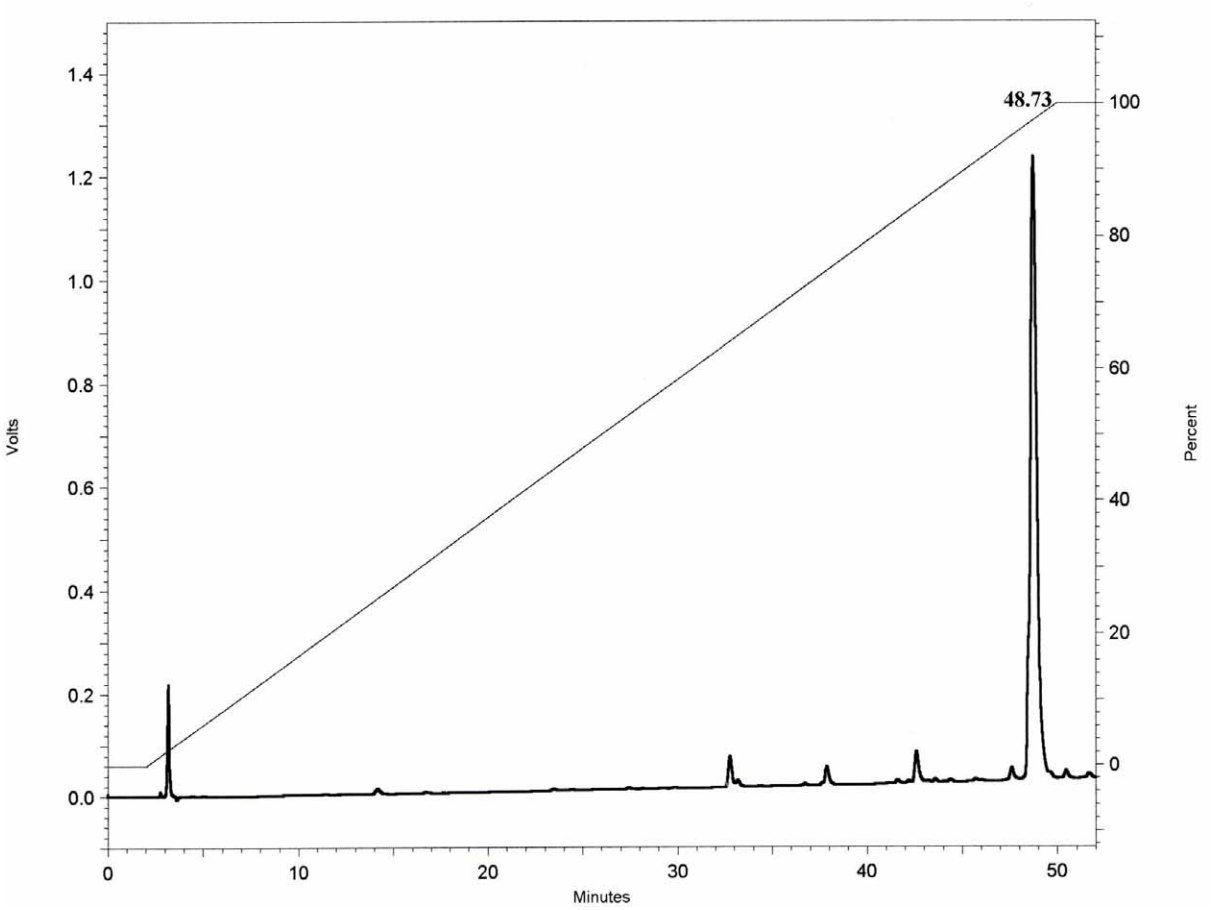


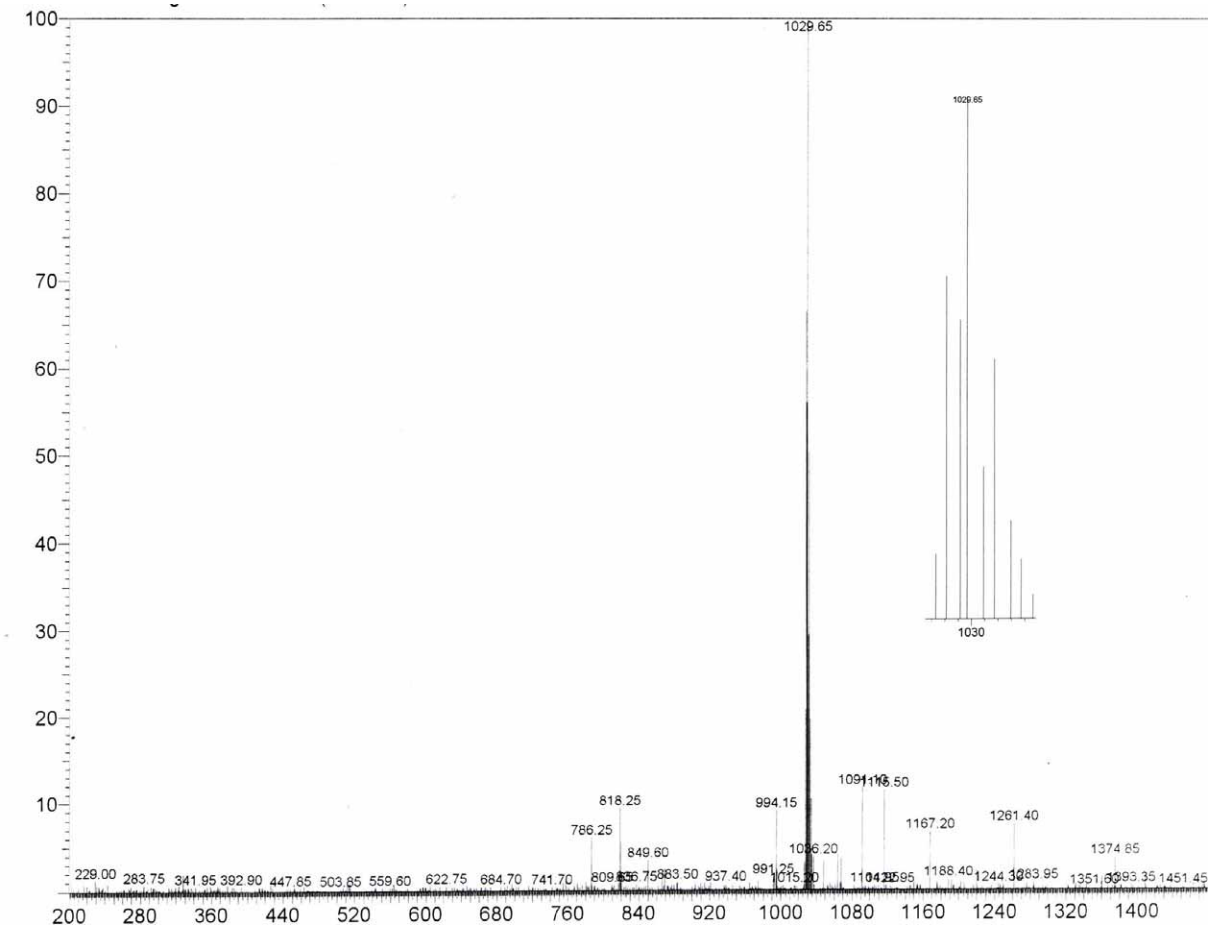
Peptide 7:
Ac-Y(SO_3NH_4)GGFL-OH



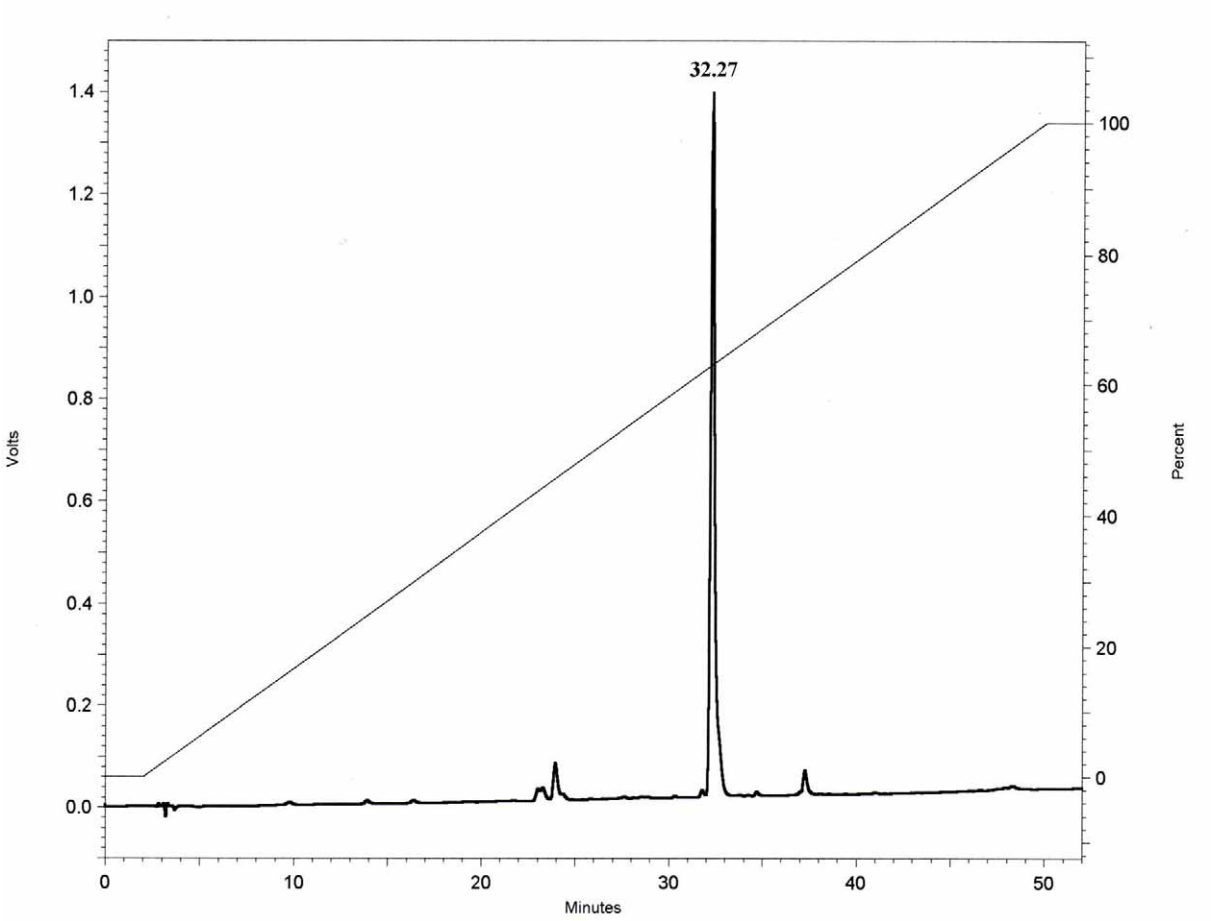


Peptide S5:
Ac-FY(SO_3TCE)H(Dmbz)F-NH_2

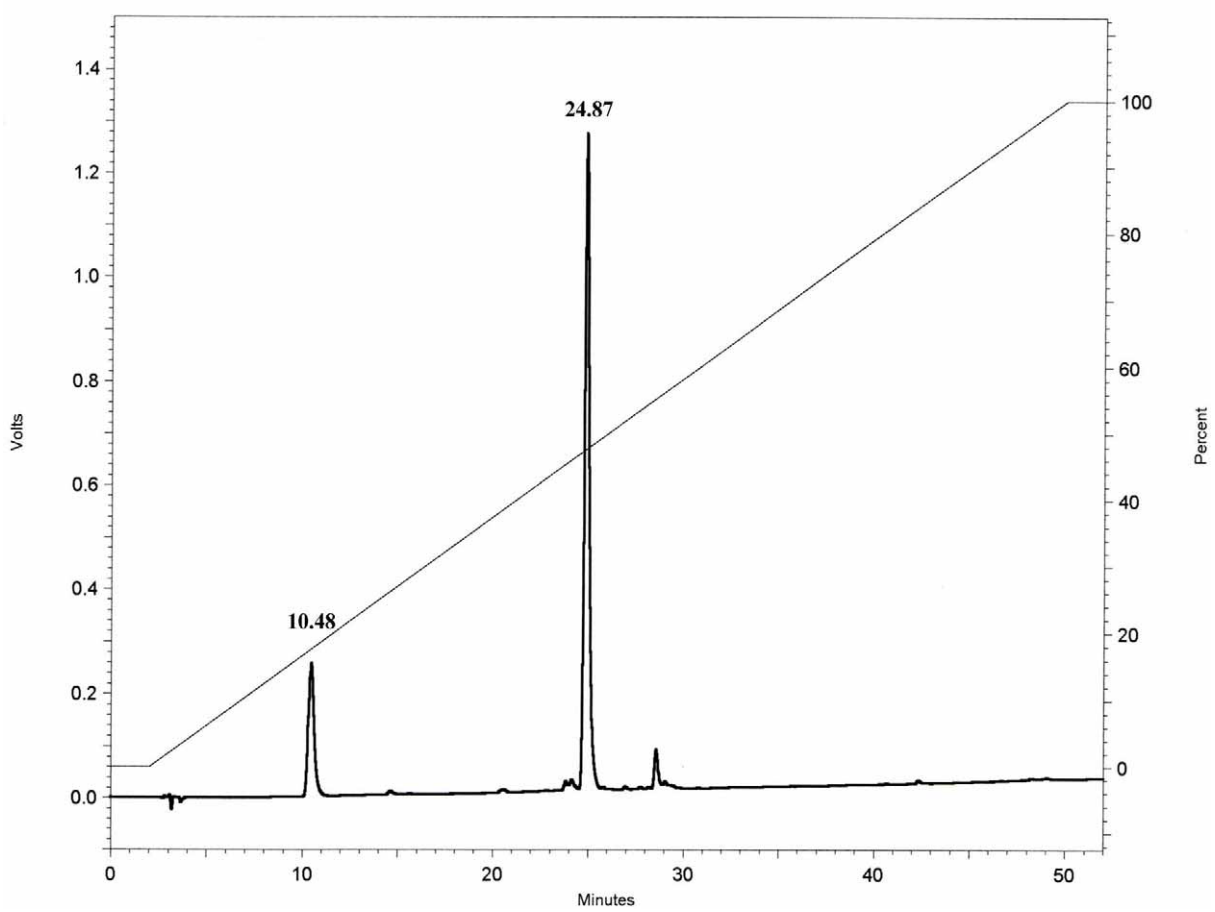


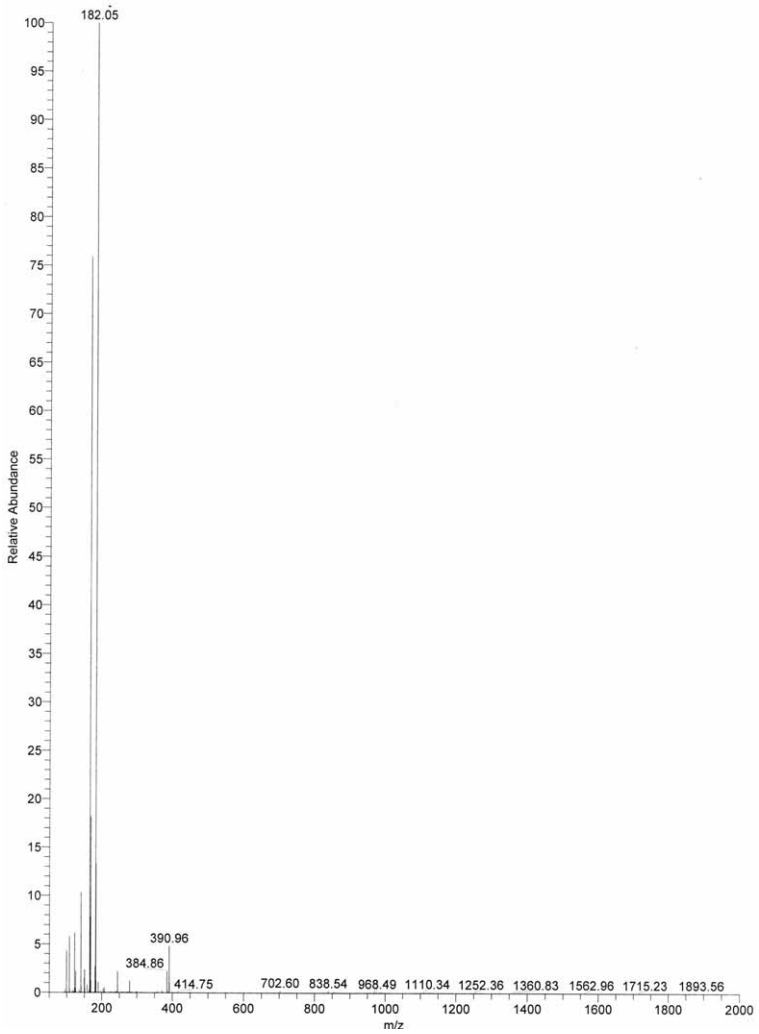


Peptide S6:
Ac-FY(SO_3NH_4)H(Dmbz)F-NH_2

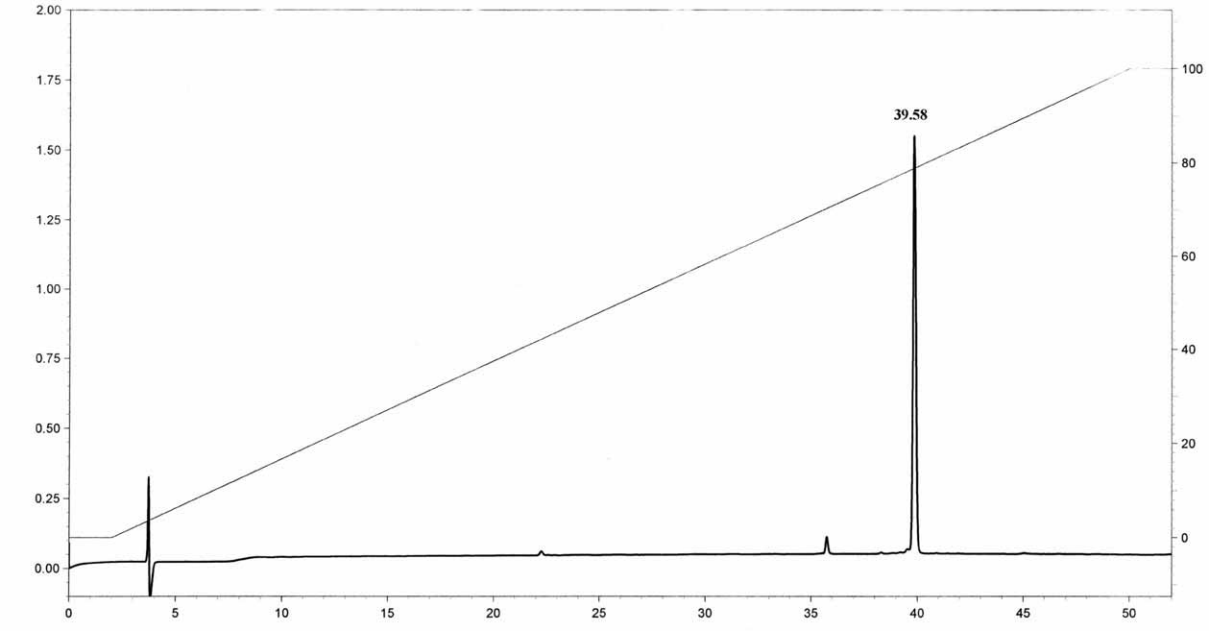


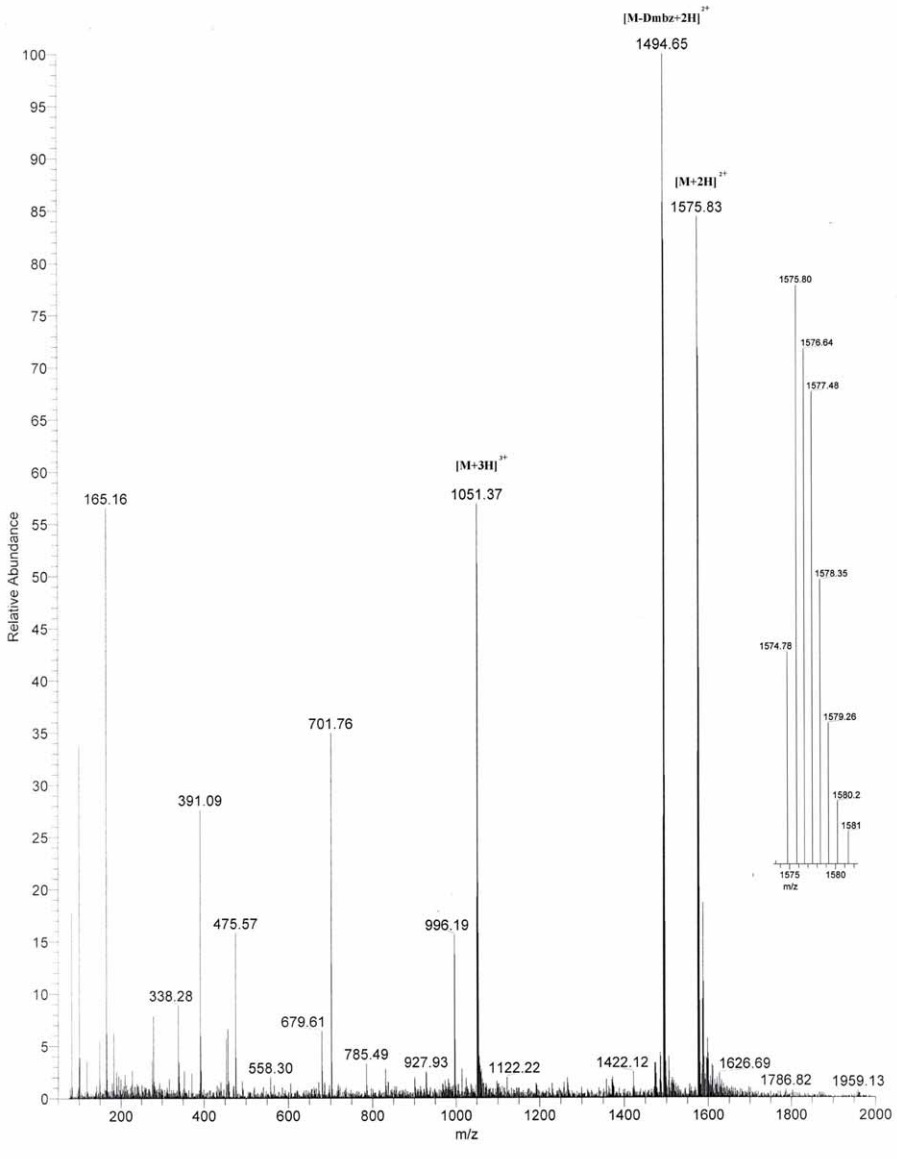
Peptide S7 & Compound S8: Ac-FY(SO_3NH_4)HF-NH_2



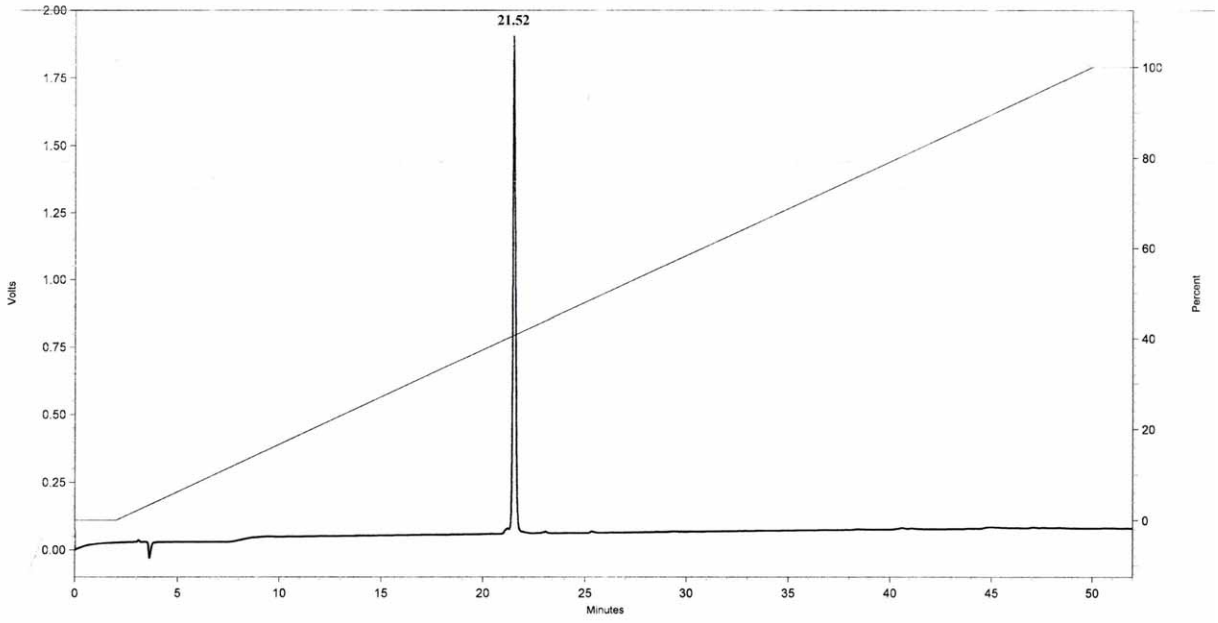

182.05
390.96
384.86
414.75
702.60
838.54
968.49
1110.34
1252.36
1360.83
1562.96
1715.23
1893.56
Relative Abundance
m/z

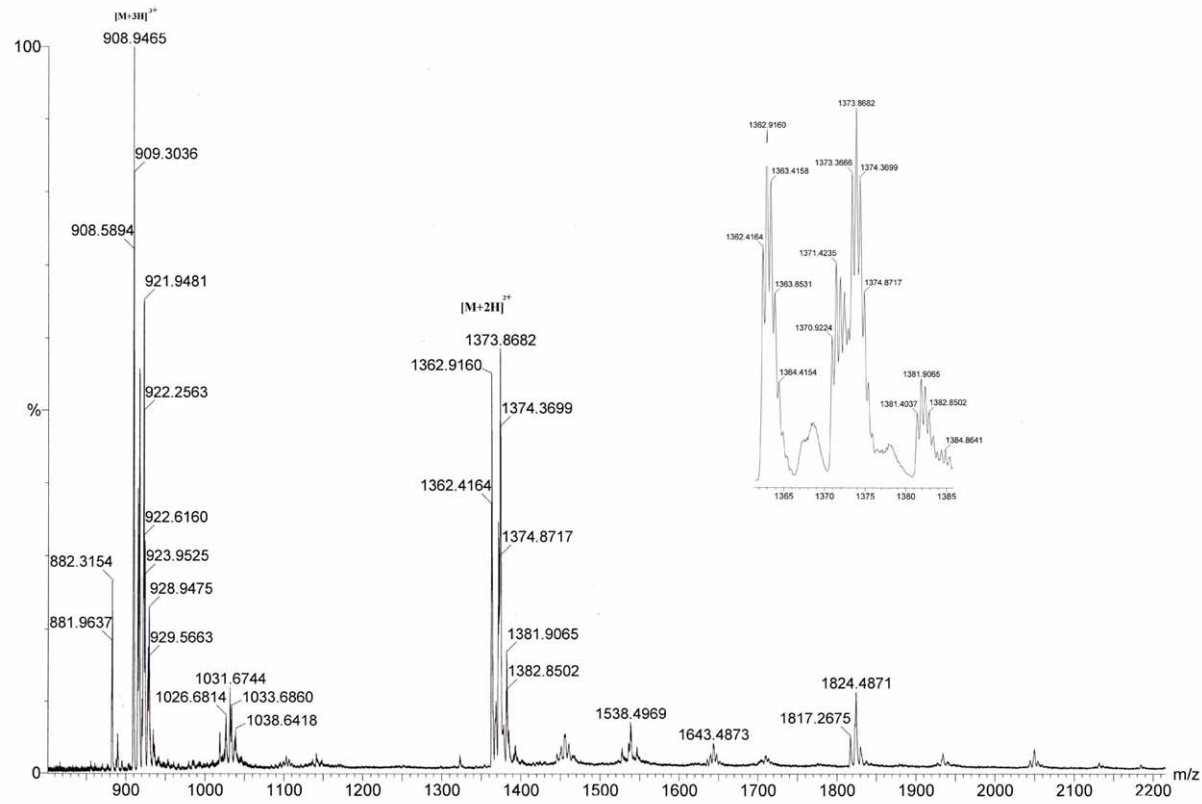
Peptide 12:
Ac-TTPDY(SO_3TCE)GH(Dmbz)Y(SO_3TCE)DDKDTLDLNTPVDK-NH_2



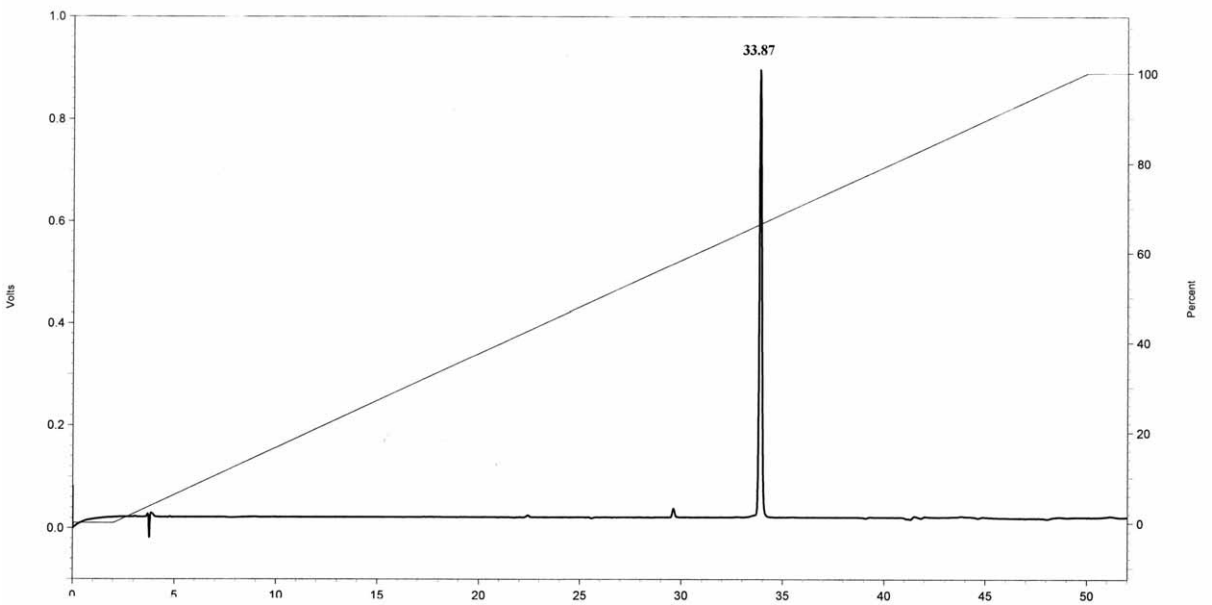


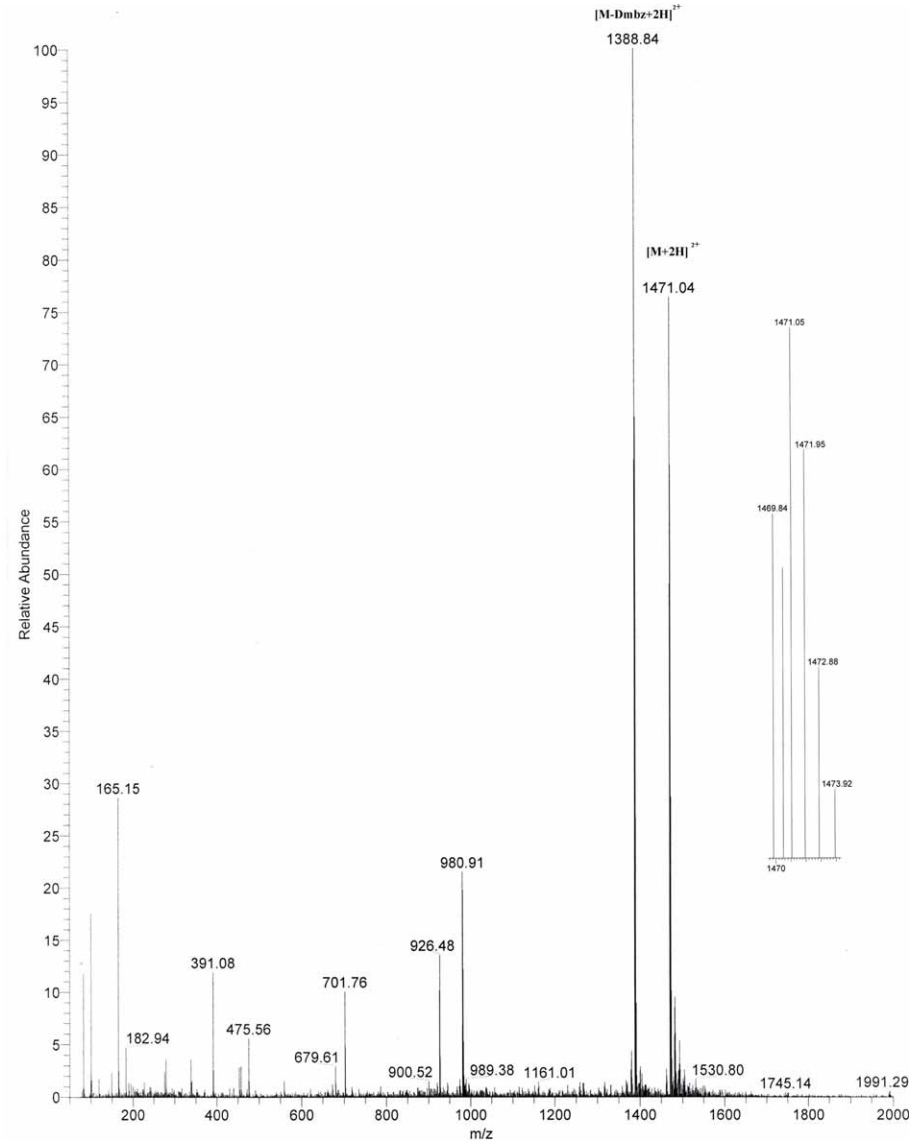
Peptide 13:
Ac-TTPDY(SO_3NH_4)GHY(SO_3NH_4)DDKDTLDLNTPVDK-NH_2



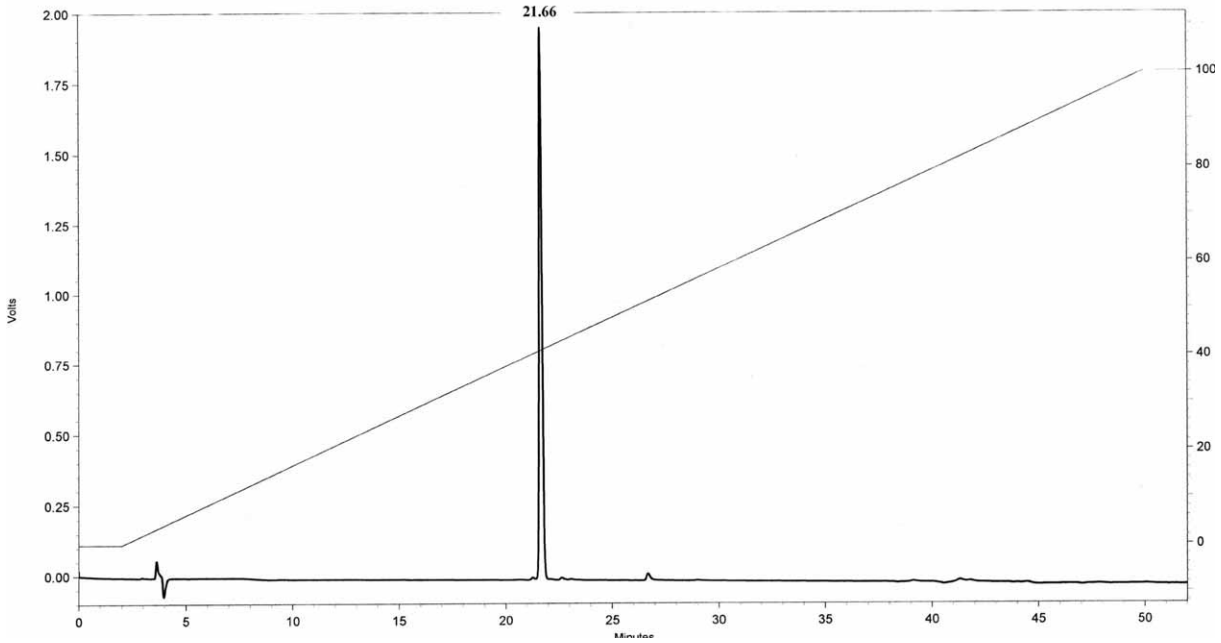


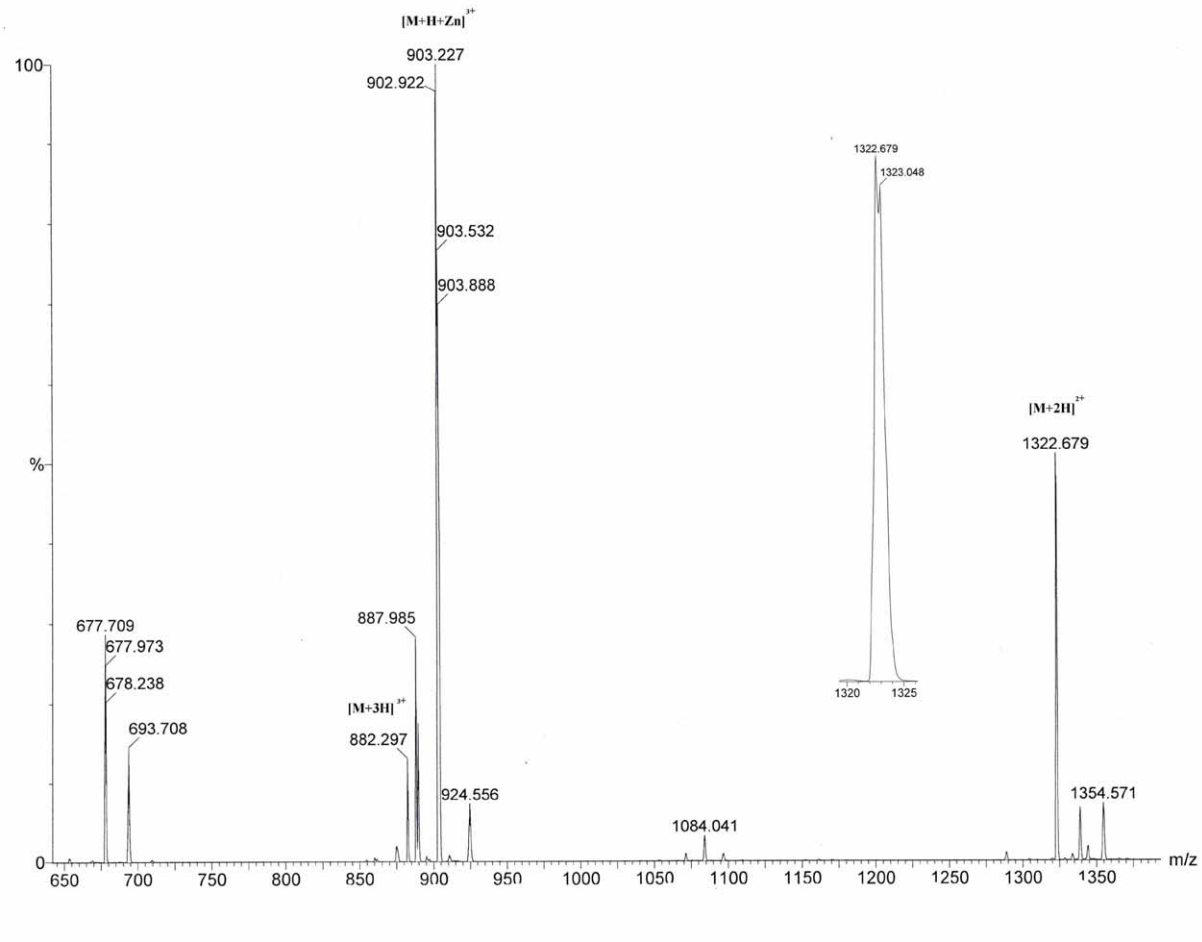
Ac-TTPDY(SO_3TCE)GH(Dmbz)YDDKDTLDLNTPVDK-NH_2



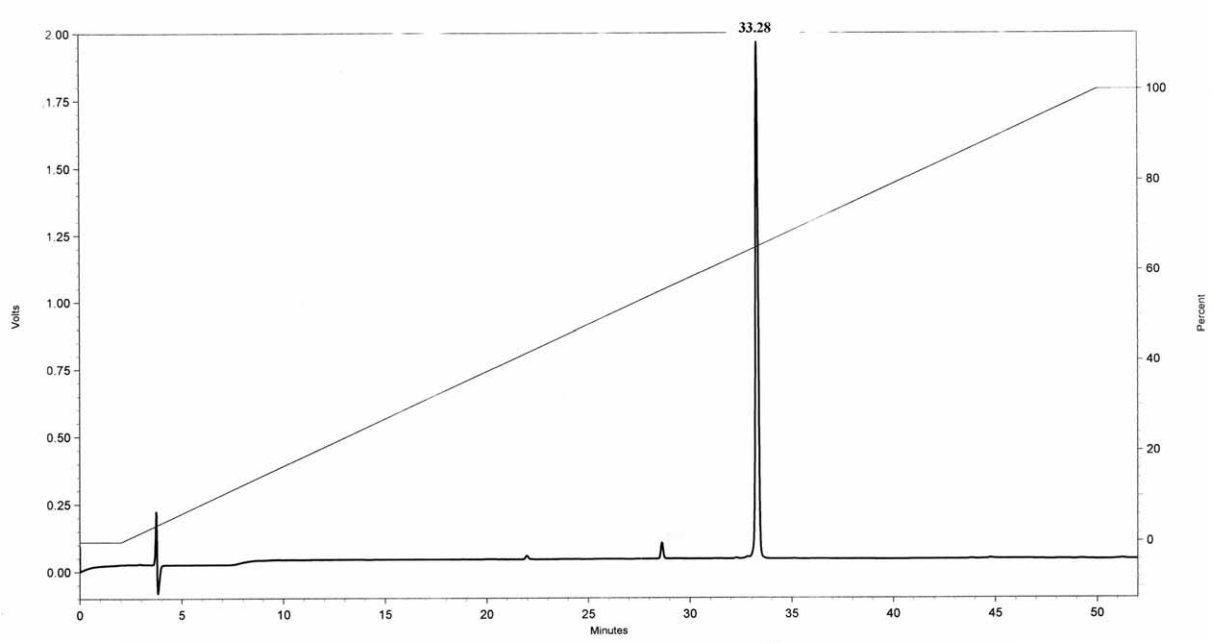


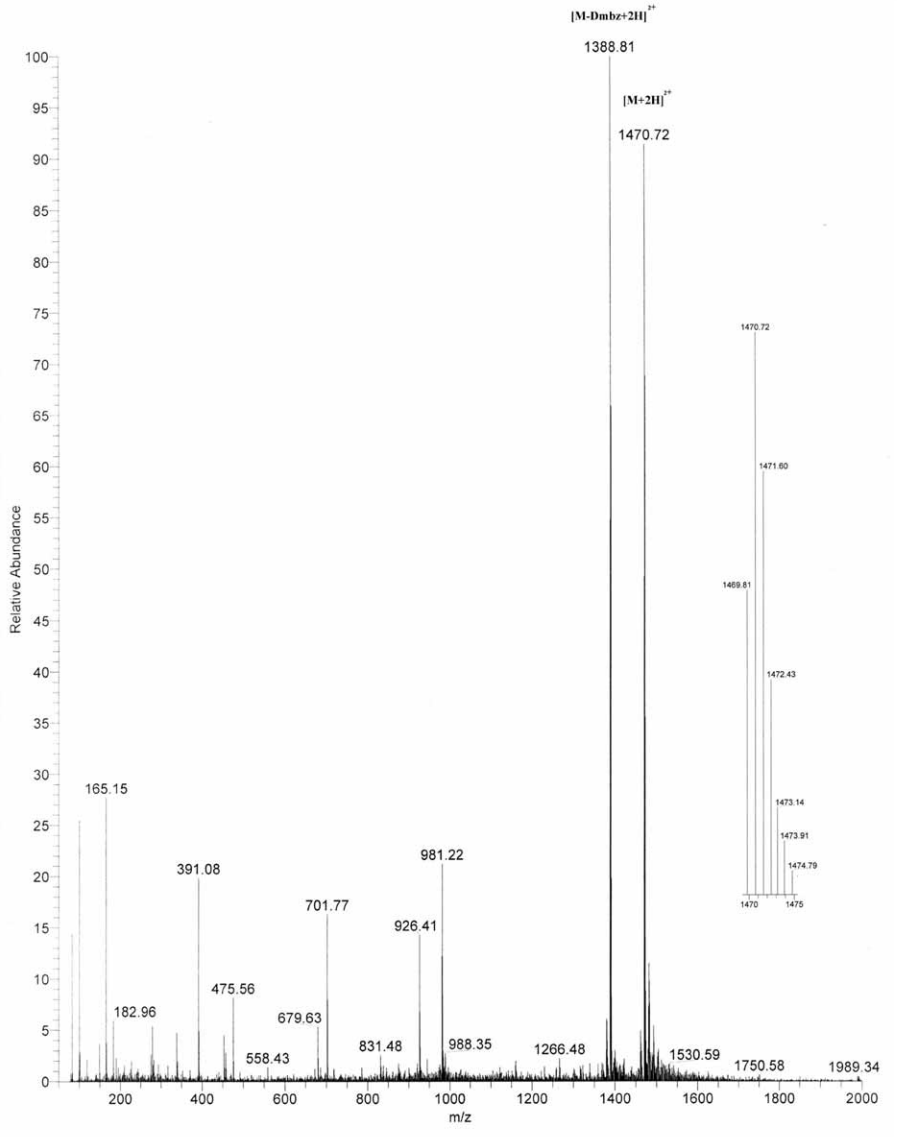
Peptide 14:
Ac-TTPDY(SO_3NH_4)GHYDDKDTLDLNTPVDK-NH_2



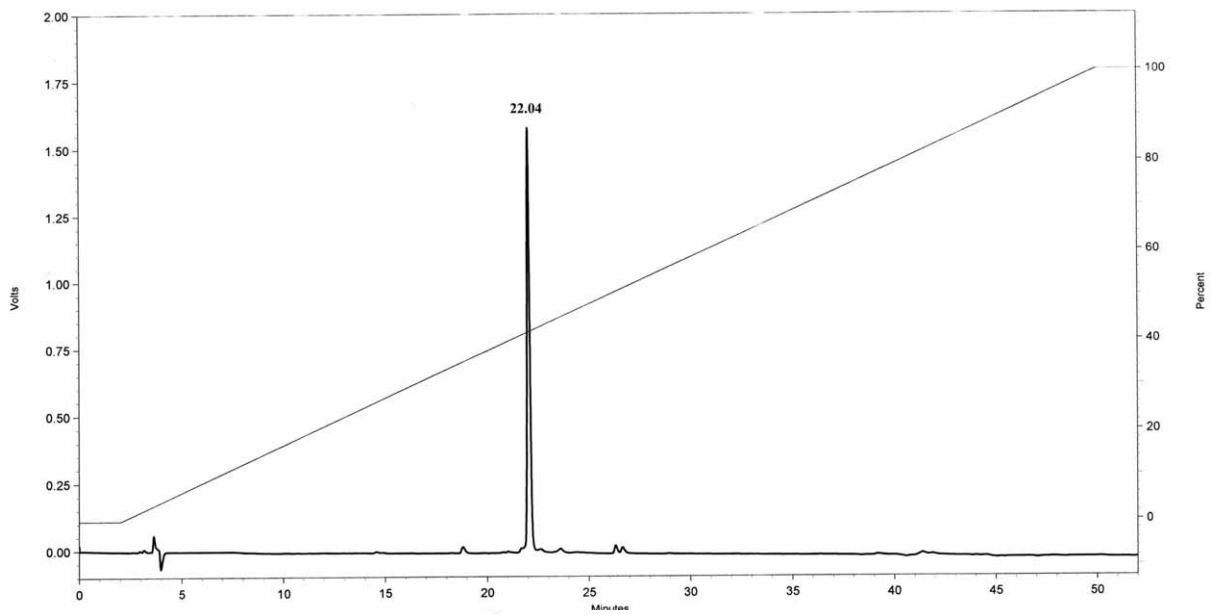


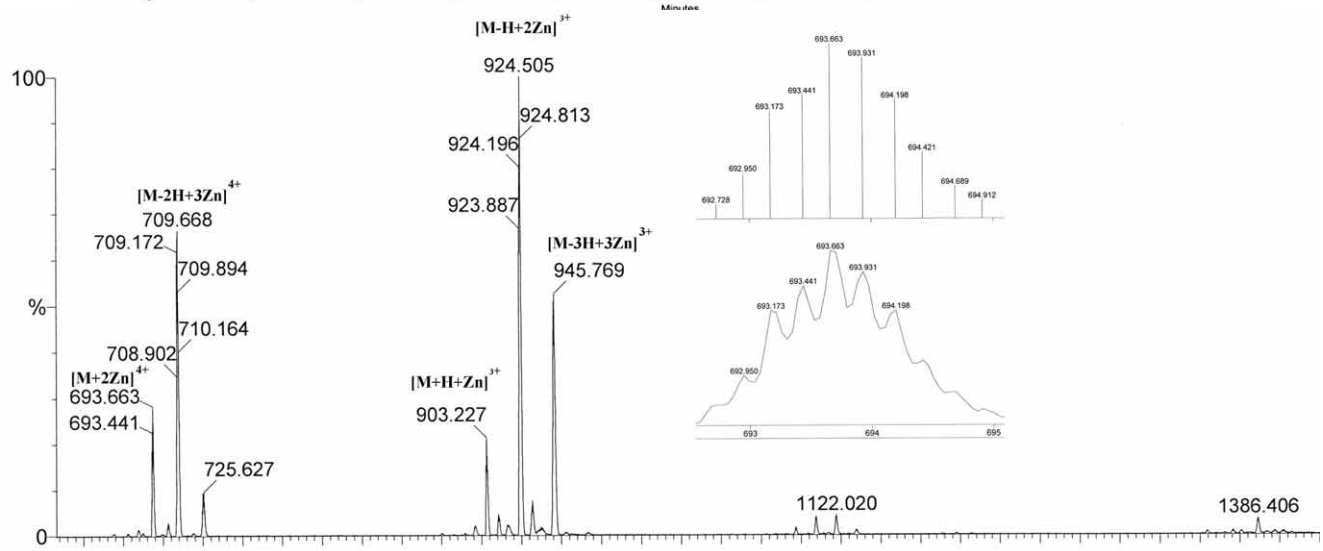
Ac-TTPDYGH(Dmbz)Y(SO_3TCE)DDKDTLDLNTPVDK-NH_2





Peptide 15:
Ac-TTPDYGHY(SO_3NH_4)DDKDTLDLNTPVDK-NH_2





Peptide 16:
Ac-TTPDYGHYDDKDTLDLNTPVDK-NH_2