Supporting information

Table of Contents

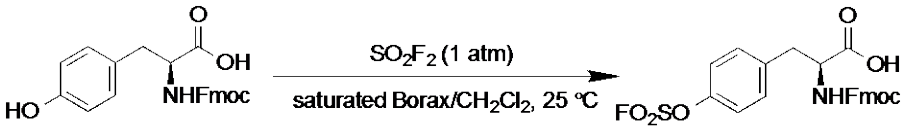
General information

Fmoc-Tyr-OH (97%) was obtained from Combi-Blocks. Borax was procured from MP Biomedicals, LLC. SO_2F_2 was a gift from from Dow AgroScience (99.8%). CH_2Cl_2 (99.9% purity) and dithiothreitol (99%) were obtained from Fisher Scientific. 2-methylpiperidine (99%), cesium carbonate (99.5%), DBU (98%) and ethylene glycol (99.75%) were acquired from Acros Organics. All the reagents and solvents were used directly without further purification. Resin, amino acids and coupling reagents used for peptide synthesis were purchased from Novabiochem Corp. (San Diego, CA, USA). Peptides were synthesized by solid-phase peptide synthesis using a standard Fmoc α-amine protecting group strategy, except for the use of 2-methylpiperidine instead of piperidine for all deprotection steps.

^{1}H NMR spectra were recorded on a Bruker DRX-600 instrument (600 MHz). Chemical shifts quoted in parts per million (ppm) were referenced to 0.0 ppm for tetramethylsilane. The following abbreviations (or combinations thereof) were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, m = multiplet, br = broad. Coupling constants, *J*, were reported in Hertz unit (Hz). ^{13}C NMR spectra were recorded on a Bruker DRX-600 instrument (150 MHz), and were fully decoupled by broad band proton decoupling. ^{19}F NMR spectra were recorded on a Bruker DRX-400 instrument (376 MHz). Chemical shifts were reported in ppm referenced to the internal standard ($CFCl_3$, 0 ppm).

Analytical RP-HPLC was performed using a Waters 600 controller equipped with a Waters 2487 detector. Analytical RP-HPLC was done with a Phenonemex Gemini-NX C18 column (5 μm, 4.6 mm x 150 mm) using a 1.0 mL/min flow rate. For all analytical RP-HPLC chromatograms, baselines were corrected from blank runs. Semi-preparative RP-HPLC was achieved using an Agilent 1260 Infinity system. Semi-preparative HPLC was conducted on a Supelco DiscoveryBIO wide pore C18 column (10 μm, 10 mm x 250 mm) using a 20 mL/min flow rate. High-resolution mass spectra (HRMS) were recorded on an Agilent Mass spectrometer using ESI-TOF (electrospray ionization-time of flight).

Fmoc-Tyr(OSO_2F)-OH



1

To a 2 L single-neck round-bottom flask containing a magnetic stir bar was added Fmoc-Tyr-OH (5.00 g, 12.4 mmol), 150 mL of CH_2Cl_2 and 600 mL of a saturated Borax solution. The mixture was stirred vigorously for 20 minutes. Then the flask was sealed with a Suba-Seal® Septa. The pressure in the flask was reduced using a needle linked to a vacuum pump until the biphasic solution started to degas. Then the SO_2F_2 balloon was attached to the flask and the reaction mixture was stirred vigorously at 25°C for 4.5 h. CH_2Cl_2 was carefully removed using a rotary evaporator. Then 1 M aqueous HCl (150 mL) was slowly added to the reaction mixture while stirring. The mixture was filtered using a filter funnel and the solid was washed with water (80 mL X 3). The solid was dried under vacuum (1 mm Hg) at 50°C for 24 h affording 5.75 g (96% yield, 11.85 mmol) of white solid (mp 133-136 °C). ^{1}H NMR (600 MHz, CD_3OD) δ 7.75-7.65 (m, 2 H), 7.55-7.50 (m, 2 H), 7.35-7.15 (m, 8 H), 4.37 (dd, J_1 = 9.6 Hz, J_2 = 4.8 Hz, 1 H), 4.27-4.15 (m, 2 H), 4.12-4.03 (m, 1 H), 3.23-3.17 (m, 1 H), 2.98-2.90 (m, 1 H); ^{13}C NMR (150 MHz, CD_3OD) δ174.9, 158.5, 150.6, 145.4 (d, J = 6.8 Hz), 142.7, 140.4, 132.7, 129.0, 128.3, 126.4 (d, J = 6.5 Hz), 126.4, 122.0, 121.1, 68.1, 56.7, 48.5, 38.0; ^{19}F NMR (376 Hz, CD_3OD) δ 37.2 (See Fig. S13-S15); HRMS (ESI-TOF) Calculated for $C_{24}H_{20}FNO_7S$ $(M+H)^+$: 486.1017; found: 486.1017 (Fig. S12).

General procedure for the synthesis of fluorosulfated tyrosine (Y(OSO_2F)) peptides

Fmoc-SPPS was performed manually using a plastic syringe with a porous filter or on an Applied Biosystems 443A peptide synthesizer employing Rink amide resin at 200 μmol scale (0.24 mmol/g). The resin was preswollen in CH_2Cl_2 for 1 h before use. Amino acids were coupled using the following reagents and reaction time for preactivation: Fmoc-AA-OH (5 equiv), HCTU (5 equiv), HOBt (5 equiv) and DIPEA (5 equiv)–the reaction period for preactivation was 30 min. The activated amino acid was added to the resin-bound primary amine with stirring or shaking for a coupling period of 30-60 min to generate a new amide bond. Removal of the Fmoc protecting group was achieved using 20% 2-methylpiperidine/DMF (3 x

10 min). The resin was washed 5 X with DMF between each step. After the final Fmoc deprotection step, the resin was washed 5 X with DMF and 5 X with CH_2Cl_2 and dried in air. The crude Y(OSO_2F) peptides were cleaved from the resin and side chain deprotected (except for the OSO_2F protecting group) by stirring with a cleavage cocktail consisting of TFA:TIPS:H_2O (95:2.5:2.5) for 3 h before filtering. The resin was washed 3 X with the cleavage cocktail. The combined filtrates were concentrated carefully by air stream before precipitation utilizing diethyl ether. Precipitated crude peptides were centrifuged at 3000 g at 4°C for 30 min before the removal of ether. 10 mL of water was added to the resulting pellet and the solution was frozen using a dry ice-acetone bath and the peptides were lyophilized. The resulting crude Y(OSO_2F) peptides were analyzed by analytical RP-HPLC. An aliquot of crude peptide **7** and peptide **8** were purified by semi-preparative RP-HPLC and lyophilized to assess the success of synthesizing Y(OSO_2F) peptides. Generally, the peptides were used without further purification for the synthesis of corresponding **sY** peptides to avoid the decrease in yield that results from the additional RP-HPLC purification at the Y(OSO_2F) peptide stage.

General procedure for the deprotection of fluorosulfated tyrosine peptides 7 and S1-S4 to produce the corresponding sulfotyrosine peptides 2-6

Cesium carbonate ($CsCO_3$) powder (10 equiv. for **2**, 12 equiv. for **3**, 15 equiv. for **4**, 18 equiv. for **5** and 20 equiv. for **6**) was dissolved in ethylene glycol (approximately 1 mL of ethylene glycol/ $CsCO_3$ solution was used per 10 mg of crude peptide) by stirring. Equivalents were estimated based on the molecular weight of peptides and the weight of crude peptides. Bicarbonate is not basic enough to trigger the hydrolysis of arylfluorosulfate, thus the equivalents of Cs_2CO_3 used were estimated based on the sum of the Asp, Glu and Tyr and fluorosulfated tyrosine residues. The amount of Cs_2CO_3 utilized needs to exceed the amount needed to deprotonate Asp, Glu and Tyr residues and the Tyr-SO_3H and HF produced in the hydrolysis reaction by at least 5 equivalents. 30-90 mg of crude Y(OSO_2F) peptide powder was then added to the ethylene glycol/ $CsCO_3$ solution. Aliquots of the reaction mixtures were subjected to analytical RP-HPLC analysis until reaction completion (60-120 min). Cs_2CO_3 has good solubility in ethylene glycol and we did not observe any deleterious effects by using up to 30 equiv of Cs_2CO_3. Prolonged reaction times, up to 6 h, did not seem to affect the reaction or the stability of the products either. If the hydrolysis reaction is observed to slow down or stop

before completion, simply add more Cs_2CO_3 powder to accelerate the reaction. The reaction mixture was then purified by semi-preparative RP-HPLC using CH_3CN:20 mM ammonium acetate (20 mM ammonium acetate, pH 9 for **6**) mobile phases. Because it is reported that sulfotyrosine was not stable under acidic conditions, ammonium acetate buffer at near neutral pH was used for HPLC purification of **sY** peptides. Appropriate fractions were collected and lyophilized. The conversion of Y(OSO_2F) peptides to **sY** peptides is excellent, exemplified by the peak shift of the analytical RP-HPLC of purified **7** before and after the reaction (Fig. S1 and S2).

General procedure for the deprotection of fluorosulfated tyrosine peptide 8 to produce the corresponding sulfotyrosine peptide 9

0.5% Dithiothreitol was dissolved in 5% (about 50 equiv. relative to crude **8**) DBU in ethylene glycol (approximately 1 mL per 10 mg of peptide) and stirred for 30 min before adding crude peptide **8**. Although we did not try less equiv. of DBU for the hydrolysis, excess DBU does not seem to affect the reaction or the stability of the product. The reaction mixture was then stirred for 120 min or until the hydrolysis reaction was completed according to RP-HPLC monitoring. The **sY** peptide was then purified by semi-preparative RP-HPLC employing CH_3CN:20 mM ammonium acetate buffer mobile phases. The rate of Y(OSO_2F) peptide **8** hydrolysis to **sY** peptide **9** is excellent, as shown by analytical RP-HPLC monitoring (Fig. S10 and S11).

Reaction between piperidine and arylfluorosulfate

When we used piperidine in the SPPS of peptide **7** we could purify the piperidine addition byproduct for peptide **7** (ESI-MS: calculated for $C_{36}H_{54}N_8O_{15}S$ $(M+H)^+$ 871.4, found 871.2, Fig. S63). To avoid such byproducts, we switched to 2-MP as the base for deprotection in SPPS.

Yield calculation

Peptides purified in ammonium acetate buffer were solubilized and lyophilized at least 5 X until the weight remained constant. Yields of purified Y(OSO_2F) and **sY** peptides were calculated from the adjusted resin loading and based on the assumption that all acidic or basic side chains and N-terminal amines in the peptides were lyophilized as salts with TFA or ammonium acetate. The adjusted resin loading is obtained by multiplying 200 μmol by the percentage of isolated

crude peptides used for further hydrolysis and purification. See Table S1 for details.

Generation and characterization of gaseous ethylene oxide

A mixture of ethylene glycol (12.4 g, 200 mmole) and Cs_2CO_3 (3.2 g, 10 mmole) or DBU (1.5 g, 10 mmole) was prepared in a flask with stirring at room temperature until the solution was homogeneous. The flask was connected by a pipe to another cooled (dry ice and acetone) flask with $CDCl_3$. The whole system was subjected to reduced pressure (vacuum pump) before closing the outlet. Then a balloon was attached to the cooled flask to maintain the pressure of the system. $PhOSO_2F$ (0.88 g, 5 mmole) was added to the mixture of ethylene glycol and the base (Cs_2CO_3 or DBU) with a syringe to perform the ethylene glycolysis of arylfluorosulfate. Large amount of gas was evolved immediately upon the addition of $PhOSO_2F$ with stirring. The generated gaseous material was absorbed in cold $CDCl_3$ in the cooled receiving flask. Characterization of the absorbed gaseous material in $CDCl_3$ by ^1H and ^{13}C NMR confirmed its identity as ethylene oxide (Fig. S6-S8).

Capture of ethylene oxide generated during arylfluorosulfate ethylene glycolysis by a tripeptide

The tripeptide Cys-Gly-Phe-NH_2 (**S5**) was synthesized by SPPS. 0.5 equiv. (relative to peptide **7**) of **S5** was added to the solution of Cs_2CO_3 in ethylene glycol before adding peptide **7** to perform the ethylene glycolysis of arylfluorosulfate based on the above-mentioned protocol. After the reaction the alkylated product tripeptide Cys(S-CH_2-CH_2-OH)-Gly-Phe-NH_2 (**S6**) was purified from the reaction mixture by HPLC and lyophilized for HRMS and NMR characterization.

DADEY(OSO_2F)L-NH_2 (7)

The synthesis was performed on an Applied Biosystems peptide synthesizer using the general SPPS and post cleavage manipulation method outlined above. Crude peptide **7** was analyzed by analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm) and showed a major peak at t_R = 17.6 min (Fig. S16). This crude peptide was used without further purification for the synthesis of **sY** peptide **2**.

Purification of peptide **7** by semi-preparative RP-HPLC (CH_3CN: H_2O mobile phases each with 0.2% TFA, 0% CH_3CN 0-6 min, linear gradient from 0% to 40% CH_3CN over 20 min, t_R = 14.0 min, λ = 210 nm) afforded peptide **7** as a flocculent white powder with a yield of 64%. Analytical RP-HPLC of this material dissolved in ethylene glycol (CH_3CN: 20 mM ammonium acetate buffer mobile phases, 0% CH_3CN 0-5 min, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm) showed a single peak at t_R = 22.8 min (Fig. S1). ^{1}H NMR (600 MHz, DMSO-d_6, aromatic region) δ 8.65 (d, *J* = 7.2 Hz, 1 H), 8.34 (d, *J* = 7.2 Hz, 1 H), 8.14 (s, 3 H), 8.07 (d, *J* = 8.4 Hz, 1 H), 7.96 (d, *J* = 8.4 Hz), 7.83 (d, *J* = 7.8 Hz), 7.47-7.42 (m, 4 H), 7.22 (s, 1 H), 6.99 (s, 1 H) (Fig. S17 and S18), ^{19}F NMR (376 Hz, DMSO-$\mathbf{d}_6$) δ 38.6 (Fig. S57); HRMS (ESI^+): calculated for $C_{31}H_{44}N_7O_{15}SF$ $(M+H)^+$ 806.2673, found 806.2673 (Fig. S19).

DADEsYL-NH_2 (2)

sY peptide **2** was prepared from **7** using the general deprotection procedure employing Cs_2CO_3/ethylene glycol. Purification of peptide **2** by semi-preparative RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, 0% CH_3CN 0-2 min, linear gradient from 0% to 20% CH_3CN over 10 min, t_R = 7.0 min, λ = 210 nm) afforded peptide **2** as a flocculent white powder with a yield of 67%. Purified **2** was analyzed by analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm) and showed a single peak at t_R = 12.2 min (Fig. S20). ^{1}H NMR (600 MHz, D_2O, aromatic region) δ 7.24 (d, *J* = 8.4 Hz, 2 H), 7.18 (d, *J* = 8.4 Hz, 2 H) (Fig. S21 and S22); HRMS (ESI^+): calculated for $C_{31}H_{45}N_7O_{16}S$ $(M+H)^+$ 804.2716, found 804.2717 (Fig. S23).

YEY(OSO_2F)LDYDF-NH_2 (S1)

The synthesis was performed manually using the general SPPS and post cleavage manipulation method. Crude peptide **S1** was analyzed by analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm) and showed a major peak at t_R = 21.1 min (Fig. S24). HRMS (ESI^+): calculated for $C_{55}H_{66}N_9O_{19}SF$ $(M+H)^+$ 1208.4252, found 1208.4251(Fig. S25). This crude peptide was used without further purification for the synthesis of **sY** peptide **3**.

YEsYLDYDF-NH_2 (3)

sY peptide **3** was prepared from **S1** using the general deprotection procedure employing Cs_2CO_3/ethylene glycol. Purification of peptide **3** by semi-preparative RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, 0% CH_3CN 0-2 min, linear gradient from 0% to 20% CH_3CN over 10 min, t_R = 11.5 min, λ = 210 nm) afforded peptide **3** as a flocculent white powder with a yield of 54%. Analysis of this material by analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm) showed a single peak at t_R = 16.7 min (Fig. S26). ^{1}H NMR (600 MHz, D_2O, aromatic region) δ 7.28 (t, *J* = 7.5 Hz, 2 H), 7.23-7.12 (m, 7 H), 6.91 (d, *J* = 5.4 Hz, 2 H), 6.90 (d, *J* = 4.8 Hz, 2 H), 6.71 (d, *J* = 5.4 Hz, 2 H), 6.70 (d, *J* = 5.4 Hz, 2 H) (Fig. S27 and S28); HRMS (ESI^+): calculated for $C_{55}H_{67}N_9O_{20}S$ $(M+H)^+$ 1206.4296, found 1206.4294 (Fig. S29).

Y(OSO_2F)EY(OSO_2F)LDY(OSO_2F)DF-NH_2 (S2)

The synthesis was performed manually using the general SPPS and post cleavage manipulation method. Crude peptide **S2** was analyzed by analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm) showed a major peak at t_R = 26.8 min (Fig. S30). HRMS (ESI^+): calculated for $C_{55}H_{64}N_9O_{23}S_3F_3$ $(M+H)^+$ 1372.3302, found 1372.3302 (Fig. S31). This crude peptide was used without further purification for the synthesis of **sY** peptide **4**.

sYEsYLDsYDF-NH_2 (4)

sY peptide **4** was prepared from **S2** using the general deprotection procedure employing Cs_2CO_3/ethylene glycol. Purification of peptide **4** by semi-preparative RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, 0% CH_3CN 0-6 min, linear gradient from 0% to 15% CH_3CN over 15 min, t_R = 13.4 min, λ = 210 nm) afforded peptide **4** as a flocculent white powder with a yield of 58%. Analysis of this material by analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm) showed a single peak at t_R = 14.9 min (Fig. S32). ^{1}H NMR (600 MHz, D_2O, aromatic region) δ 7.31-7.22 (m, 6 H), 7.20-7.12 (m, 7 H), 7.10 (d, *J* = 8.4 Hz, 2 H), 7.03 (d, *J* = 8.4 Hz, 2 H) (Fig. S33 and S34); HRMS (ESI^+): calculated for $C_{55}H_{67}N_9O_{26}S_3$ $(M+H)^+$ 1366.3432, found 1366.3430 (Fig. S35).

TTPDY(OSO_2F)GHY(OSO_2F)DDKDTLDLNTPVDK-NH_2 (S3)

The synthesis was performed on an Applied Biosystems peptide synthesizer using the general SPPS and post cleavage manipulation method. Crude peptide **S3** was analyzed by analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm) and showed a major peak at t_R = 20.2 min (Fig. S36). HRMS (ESI^+): calculated for $C_{109}H_{162}N_{28}O_{45}S_2F_2$ $(½M+H)^+$ 1343.5402, found 1343.5404 (Fig. S37). This crude peptide was used without further purification for the synthesis of **sY** peptide **5**.

TTPDsYGHsYDDKDTLDLNTPVDK-NH_2 (5)

sY peptide **5** was prepared from **S3** using the general deprotection procedure employing Cs_2CO_3/ethylene glycol. Purification of peptide **5** by semi-preparative RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, 0% CH_3CN 0-6 min, linear gradient from 0% to 30% CH_3CN over 15 min, t_R = 13.0 min, λ = 210 nm) afforded peptide **5** as a flocculent white powder with a yield of 52%. Analytical RP-HPLC of this material (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm) showed a single peak at t_R = 14.4 min (Fig. S38). ^1H NMR (600 MHz, D_2O, aromatic region) δ 8.47 (s, 1 H), 7.24-7.19 (m, 8 H), 7.18-7.14 (m, 4 H), 7.07 (s, 1 H) (Fig. S39 and S40); HRMS (ESI^+): calculated for $C_{109}H_{164}N_{28}O_{47}S_2$ $(½M+H)^+$ 1341.5445, found 1341.5447(Fig. S41).

DADSENSSFY(OSO_2F)Y(OSO_2F)Y(OSO_2F)DY(OSO_2F)LDEVAF-NH_2 (S4)

The synthesis was performed on an Applied Biosystems peptide synthesizer using the general SPPS and post cleavage manipulation method. Crude peptide **S4** was analyzed by analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm) and showed a major peak at t_R = 26.6 min (Fig. S42). HRMS (ESI^+): calculated for $C_{110}H_{138}N_{22}O_{48}S_4F_4$ $(½M+H)^+$ 1370.3999, found 1370.4002 (Fig. S43). This crude peptide was used without further purification for the synthesis of **sY** peptide **6**.

DADSENSSFsYsYsYDsYLDEVAF-NH2 (6)

sY peptide **6** was prepared from **S4** using the general deprotection procedure employing Cs_2CO_3/ethylene glycol. Purification of peptide **6** by semi-preparative RP-HPLC (CH_3CN: 20

mM ammonium acetate buffer mobile phases, pH 9, 5% CH_3CN 0-6 min, linear gradient from 5% to 15% CH_3CN over 20 min, t_R = 19.9 min, λ = 210 nm) afforded peptide **6** as a flocculent white powder with a yield of 36%. Analytical RP-HPLC of this material (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm) showed a single peak at t_R = 15.3 min (Fig. S44). ^1H NMR (600 MHz, D_2O, aromatic region) δ 7.27 (t, *J* = 7.5 Hz, 2 H), 7.23-7.06 (m, 22 H), 7.04-6.98 (m, 2 H) (Fig. S45 and S46). ESI-MS: calculated for $C_{110}H_{142}N_{22}O_{52}S_4$ (⅓M-H)$^-$ 909.3, found 909.4 (Fig. S47). HRMS (ESI$^+$): calculated for $C_{110}H_{142}N_{22}O_{52}S_4$ (½M+H)$^+$ 1366.4086, found 1366.4067 (Fig. S48).

GDY(OSO$_2$F)DSMKEPVFR-NH$_2$ (8)

The synthesis was performed on an Applied Biosystems peptide synthesizer using the general SPPS and post cleavage manipulation method. Crude peptide **8** was analyzed by analytical RP-HPLC (CH_3CN: H_2O mobile phases each with 0.2% TFA, 0% CH_3CN 0-5 min, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm) and showed a major peak at t_R = 25.32 min (Fig. S49). This crude peptide was used without further purification for the synthesis of **sY** peptide **9**. Purification of peptide **8** by semi-preparative RP-HPLC (CH_3CN: H_2O mobile phases each with 0.2% TFA, 0% CH_3CN 0-6 min, linear gradient from 0% to 40% CH_3CN over 20 min, t_R = 19.9 min, λ = 210 nm) afforded peptide **8** as a flocculent white powder with a yield of 30%. Analytical RP-HPLC of this material dissolved in ethylene glycol (CH_3CN: 20 mM ammonium acetate buffer, 0% CH_3CN 0-5 min, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm) showed a single peak at t_R = 24.7 min (Fig. S10). ^1H NMR (600 MHz, D_2O, aromatic region) δ 7.35 (d, *J* = 9.0 Hz, 2 H), 7.32 (d, *J* = 9.0 Hz, 2 H), 7.28 (t, *J* = 7.2 Hz, 2 H), 7.23 (t, *J* = 7.2 Hz, 1 H), 7.19 (d, *J* = 7.8 Hz, 2 H) (Fig. S50 and S51), ^{19}F NMR (376 Hz, H_2O-D_2) δ 37.2 (Fig. S58); HRMS (ESI$^+$): calculated for $C_{61}H_{90}N_{17}O_{22}S_3F$ (M+H)$^+$ 1528.5665, found 1528.5658 (Fig. S52).

GDsYDSMKEPVFR-NH$_2$ (9)

The main peptide product prepared from **8** using the ethylene glycol/Cs_2CO_3 protocol is a byproduct formed by reaction between peptide **9** and the ethylene oxide generated during arylfluorosulfate ethylene glycolysis (HRMS (ESI$^+$): calculated for $C_{63}H_{95}N_{17}O_{24}S_3$ (M+H)$^+$ 1570.5970, found 1570.5965, Figure S9). Thus we switched to a different hydrolysis protocol.

sY peptide **9** was prepared from **8** using the deprotection procedure employing DBU/dithiothreitol/ethylene glycol detailed above. Purification of peptide **9** by semi-preparative RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, 0% CH_3CN 0-6 min, linear gradient from 0% to 20% CH_3CN over 20 min, t_R = 20.6 min, λ = 210 nm) afforded peptide **9** as a flocculent white powder with a yield of 25%. Analytical RP-HPLC of this material (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm) showed a single peak at t_R = 16.1 min (Fig. S53). ^{1}H NMR (600 MHz, D_2O, aromatic region) δ 7.29 (t, *J* = 7.5 Hz, 2 H), 7.26-7.14 (m, 7 H) (Fig. S54 and S55); HRMS (ESI-): calculated for $C_{61}H_{91}N_{17}O_{23}S_3$ (½M-H)$^-$ 761.7745, found 761.7752 (Fig. S56).

Cys-Gly-Phe-NH_2 (S5)

The synthesis was performed on an Applied Biosystems peptide synthesizer using the general SPPS and post-cleavage manipulation method. ^{1}H NMR (600 MHz, D_2O) δ 7.33-7.28 (m, 2 H), 7.26-7.18 (m, 3 H), 4.56-4.51 (m, 1 H), 4.16 (td, J_1 = 5.7 Hz, J_2 = 1.4 Hz, 1 H), 3.92-3.84 (m, 2 H), 3.14-3.06 (m, 1 H), 3.01-2.90 (m, 3 H) (Fig. S60); HRMS (ESI$^+$): calculated for $C_{14}H_{20}N_4O_3S$ (M+H)$^+$ 325.1239, found 325.1230 (Fig. S59).

Cys(S-CH_2-CH_2-OH)-Gly-Phe-NH_2 (S6)

Purification of peptide **S6** from the reaction mixture of peptide **S6** / peptide **7** / ethylene glycol / Cs_2CO_3 by semi-preparative RP-HPLC (CH_3CN: H_2O mobile phases each with 0.2% TFA, 0% CH_3CN 0-6 min, linear gradient from 0% to 20% CH_3CN over 20 min, t_R = 16.9 min, λ = 210 nm) afforded peptide **S6** as a flocculent white powder. ^{1}H NMR (600 MHz, D_2O) δ 7.33-7.28 (m, 2 H), 7.27-7.20 (m, 3 H), 4.57-4.52 (m, 1 H), 4.16-4.12 (m, 1 H), 3.94-3.82 (m, 2 H), 3.70-3.65 (m, 2 H), 3.14-3.02 (m, 2 H), 2.98-2.90 (m, 2 H), 2.71-2.64 (m, 2 H) (Fig. S62); HRMS (ESI$^+$): calculated for $C_{16}H_{24}N_4O_4S$ (M+H)$^+$ 369.1591, found 369.1592 (Fig. S61).

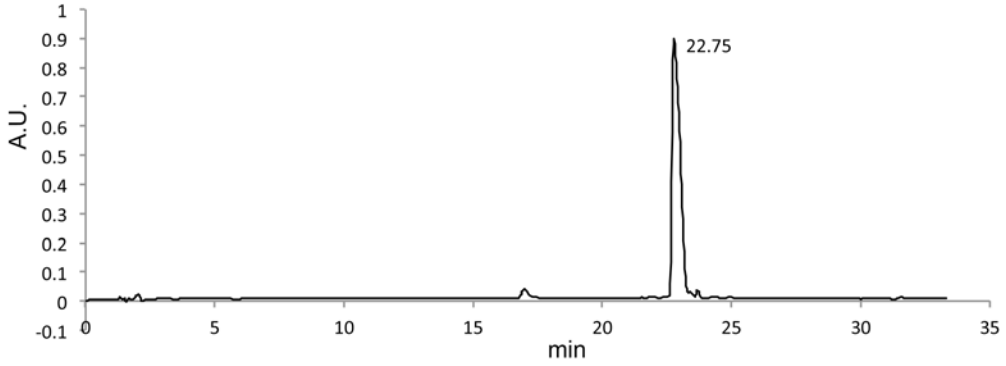


Figure S1. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, 0% CH_3CN 0-5 min, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 22.8 min) chromatogram of purified peptide DADEY(OSO_2F)L-NH_2 **(7)**.

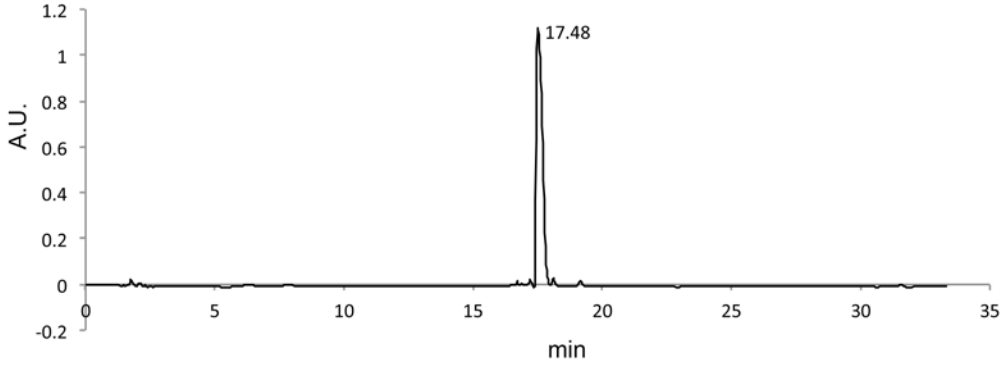


Figure S2. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, 0% CH_3CN 0-5 min, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 17.5 min) chromatogram of the reaction mixture (120 min reaction time) containing the product peptide DADE**sY**L-NH_2 **(2)**.

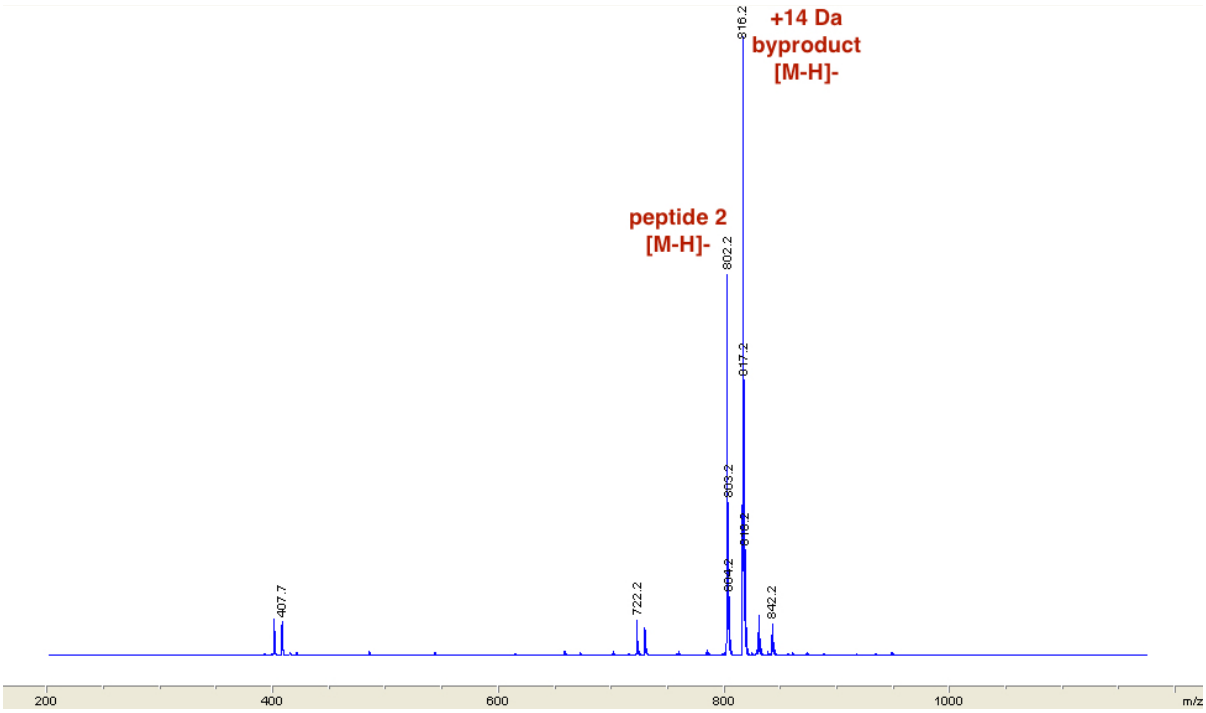


Figure S3. ESI-MS spectrum of the reaction mixture (240 min reaction time) of hydrolyzing / esterifying peptide **7** by Cs_2CO_3 in methanol. A methylated byproduct could be clearly observed.

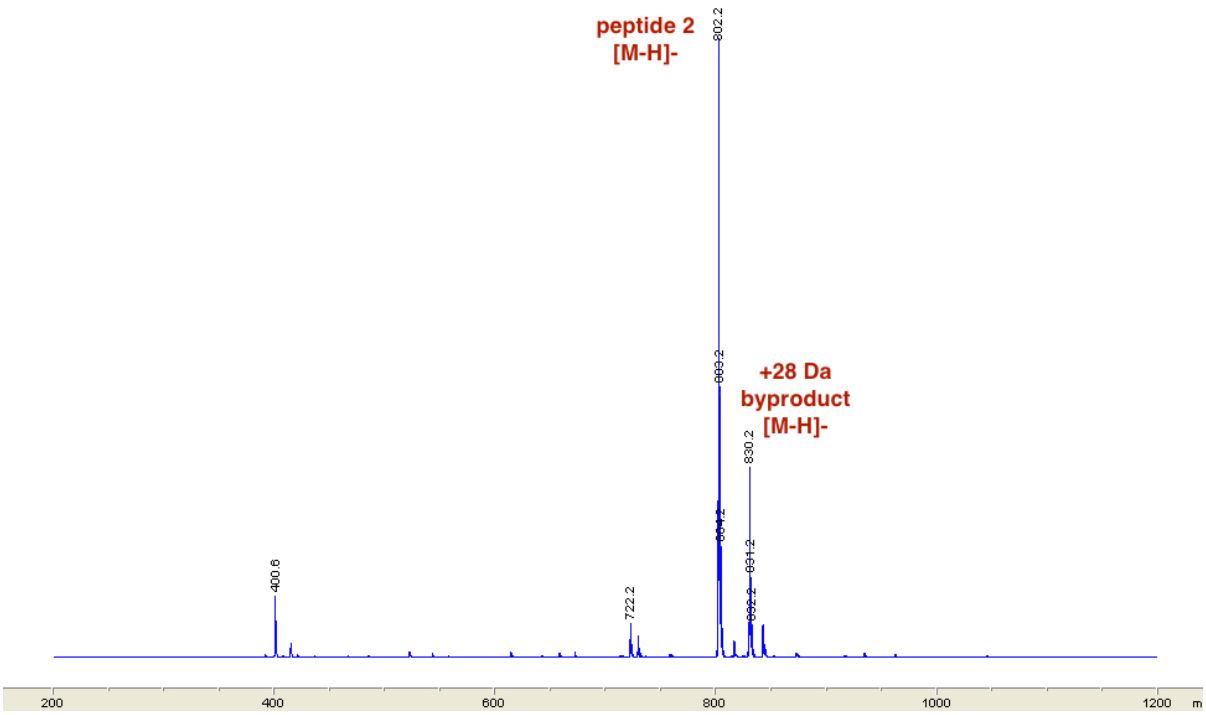


Figure S4. ESI-MS spectrum of the reaction mixture (240 min reaction time) of hydrolyzing / esterifying peptide **7** by Cs_2CO_3 in ethanol. An ethylated byproduct could be clearly observed.

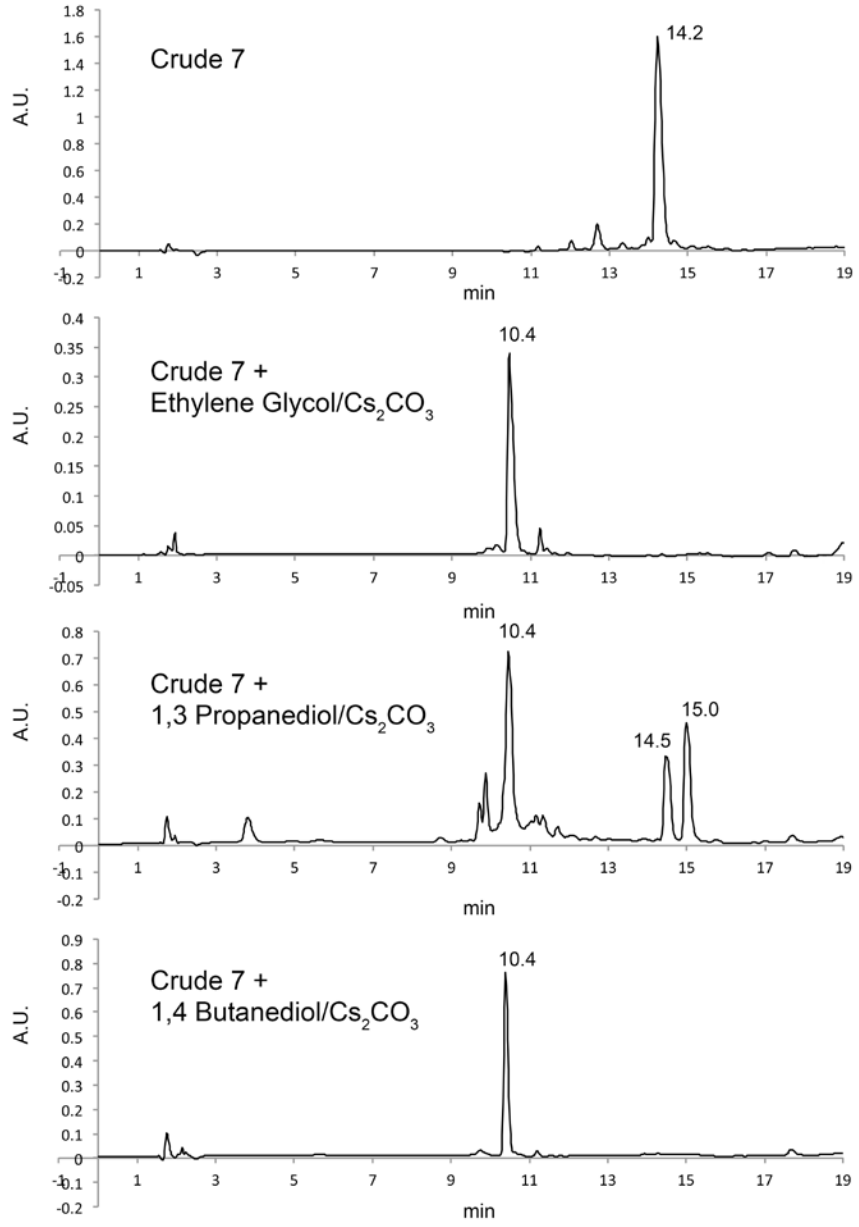


Figure S5. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 100% CH_3CN over 30 min, λ = 210 nm) chromatogram of crude peptide DADEY(OSO_2F)L-NH_2 **(7)** and the reaction mixtures (240 min reaction time) of hydrolysis reactions conducted using different diols. We could not confirm the identities of the isolated byproducts generated with 1,3 propanediol because of the presence of multiple masses in the ESI-MS spectra or because the byproducts do not ionize in the ESI-MS (for t_R = 14.5 and 15 min peaks). The mass for the starting material peptide **7** was not observed in the ESI-MS spectra for t_R = 14.5 and 15 min peaks.

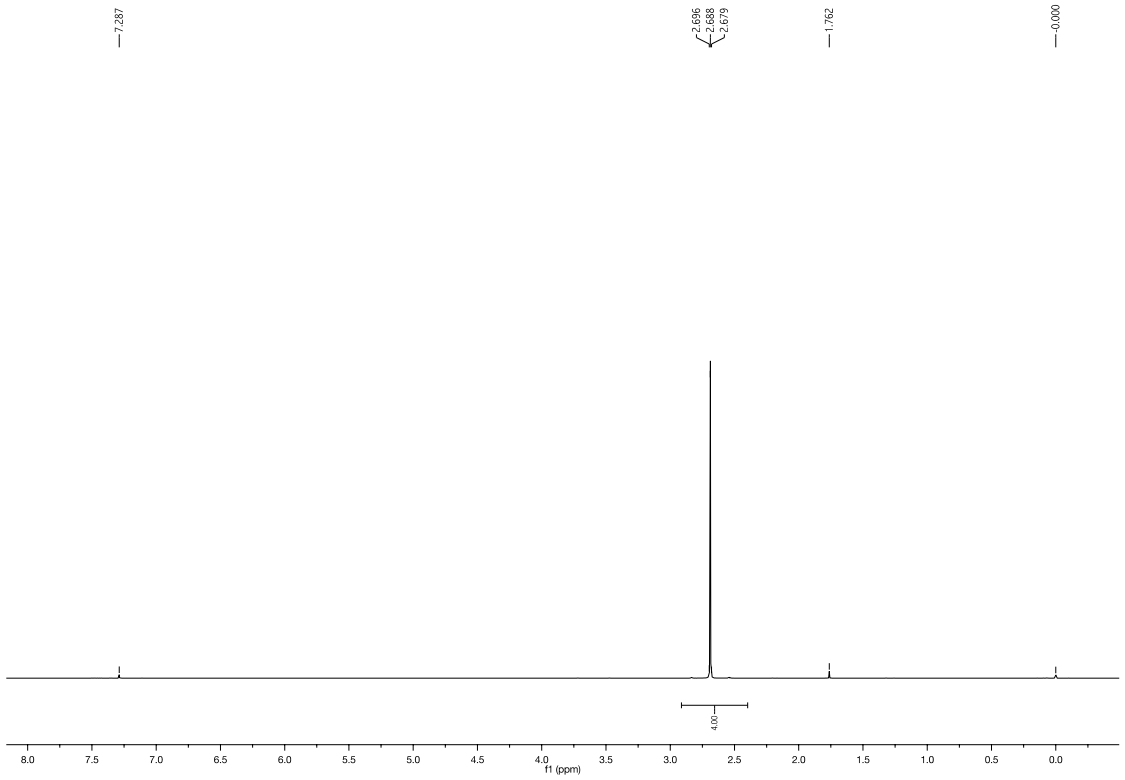


Figure S6. ^{1}H NMR spectrum ($CDCl_3$) for the absorbed ethylene oxide generated by $PhOSO_2F$ ethylene glycolysis employing DBU as the base.

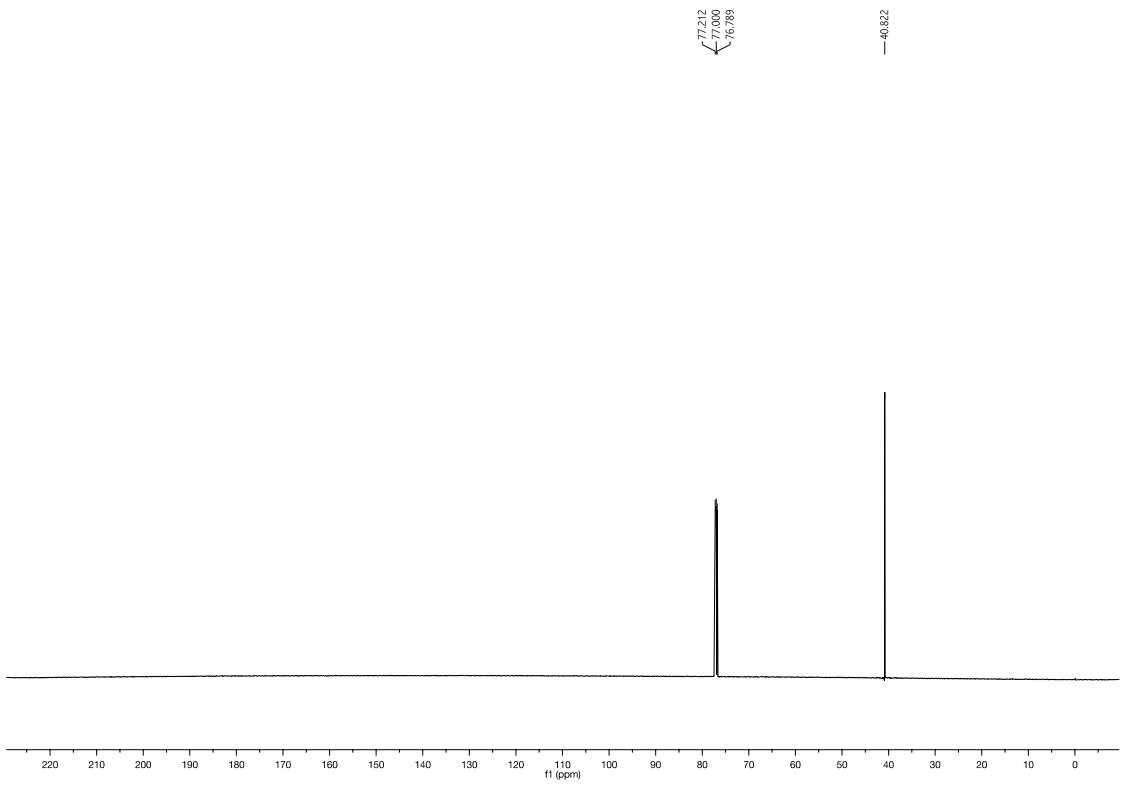


Figure S7. ^{13}C NMR spectrum ($CDCl_3$) for the absorbed ethylene oxide generated by $PhOSO_2F$ ethylene glycolysis with DBU as the base.

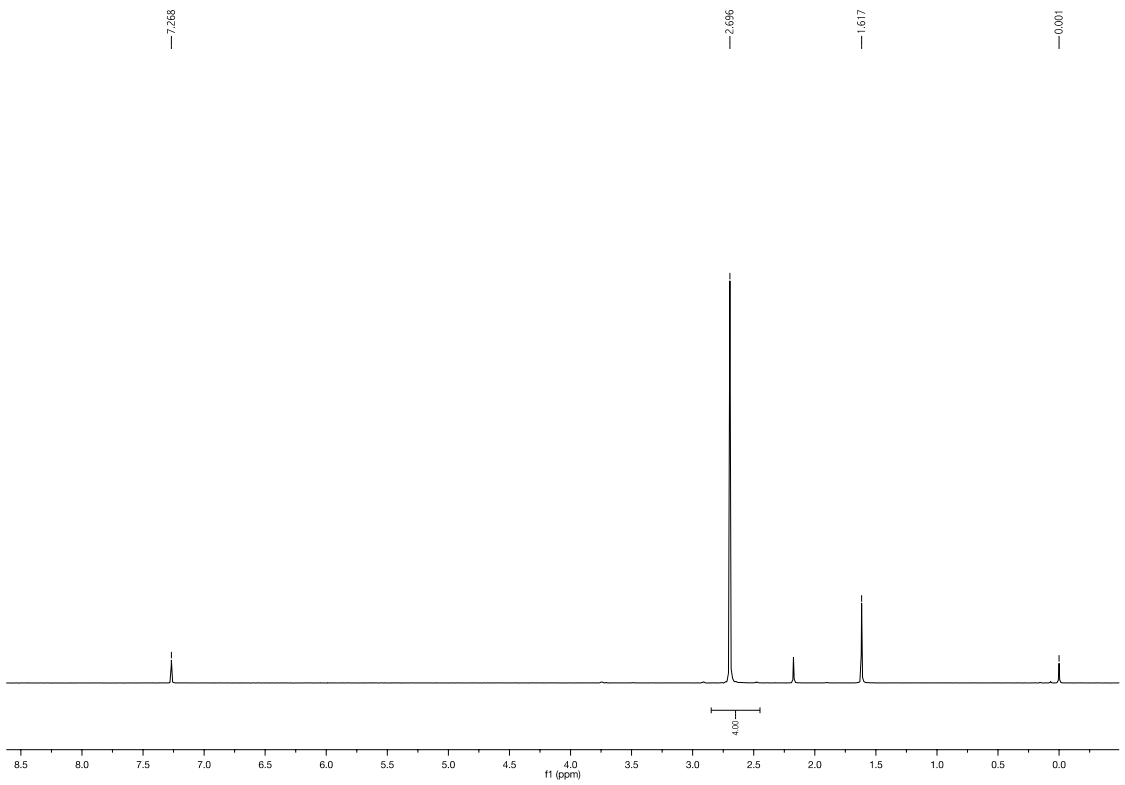


Figure S8. ^{1}H NMR spectrum ($CDCl_3$) for the absorbed ethylene oxide generated by $PhOSO_2F$ ethylene glycolysis with Cs_2CO_3 as the base.

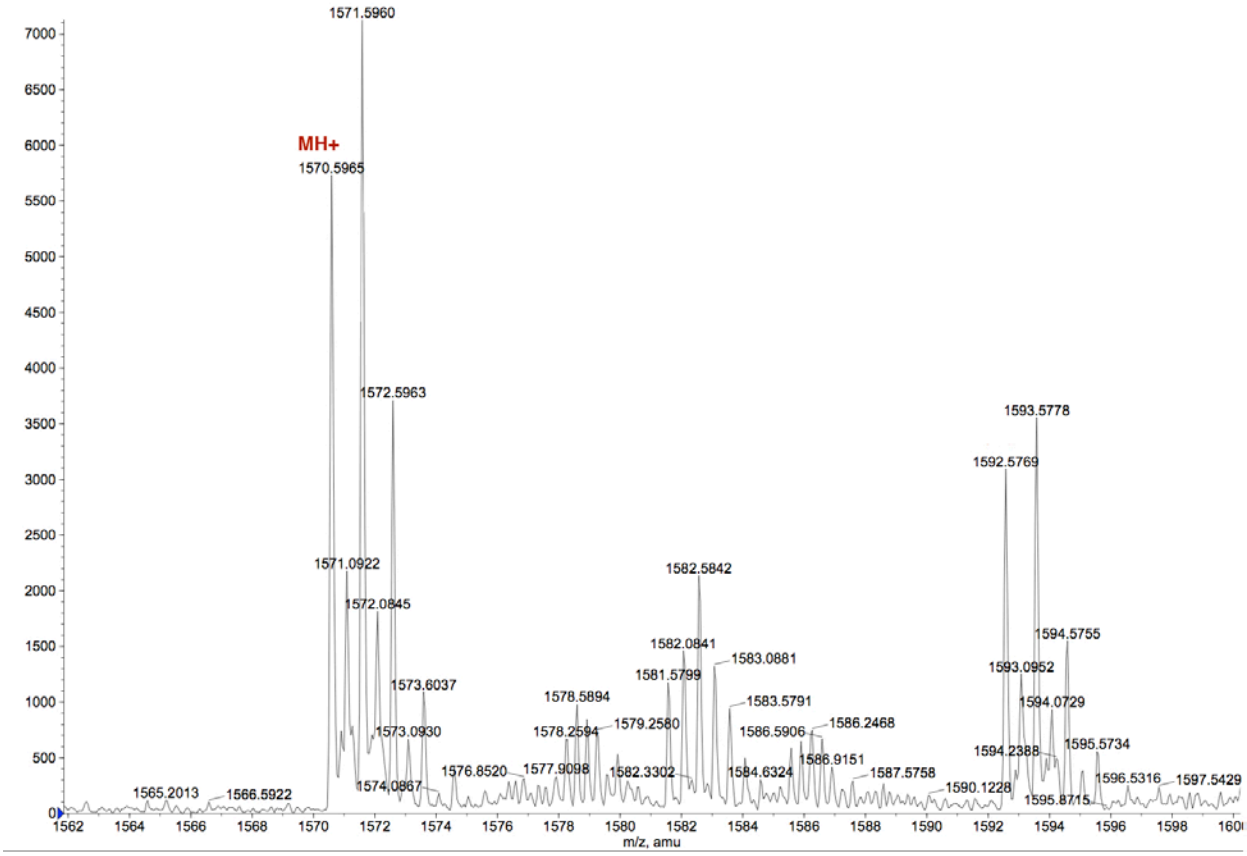


Figure S9. High resolution ESI-TOF spectrum for the purified ethylene oxide-derived thiol alkylated byproduct of peptide **9**.

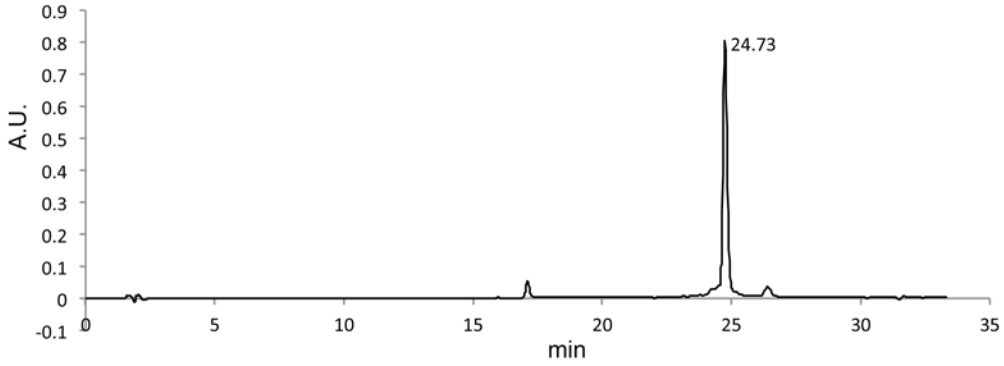


Figure S10. Analytical RP-HPLC (CH_3CN:20 mM ammonium acetate buffer mobile phases, 0% CH_3CN 0-5 min, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 24.7 min) chromatogram of the purified peptide GDY(OSO_2F)DSMKEPCFR-NH_2 **(8)** dissolved in ethylene glycol. Small amount of oxidized peptides (t_R = 26.2 min) disappeared upon dithiothreitol treatment.

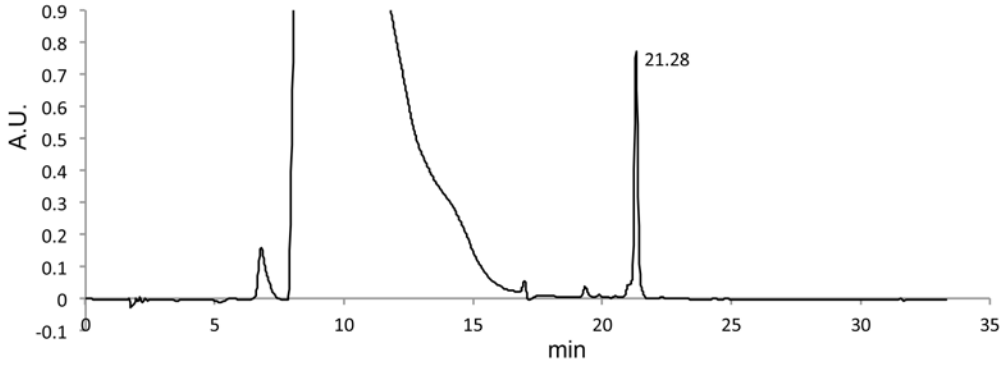


Figure S11. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, 0% CH_3CN 0-5 min, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 21.3 min) chromatogram of the reaction mixture (120 min reaction time) containing GD**sY**DSMKEPCFR-NH_2 **(9)**. DBU and dithiothreitol were eluted within 17 minutes.

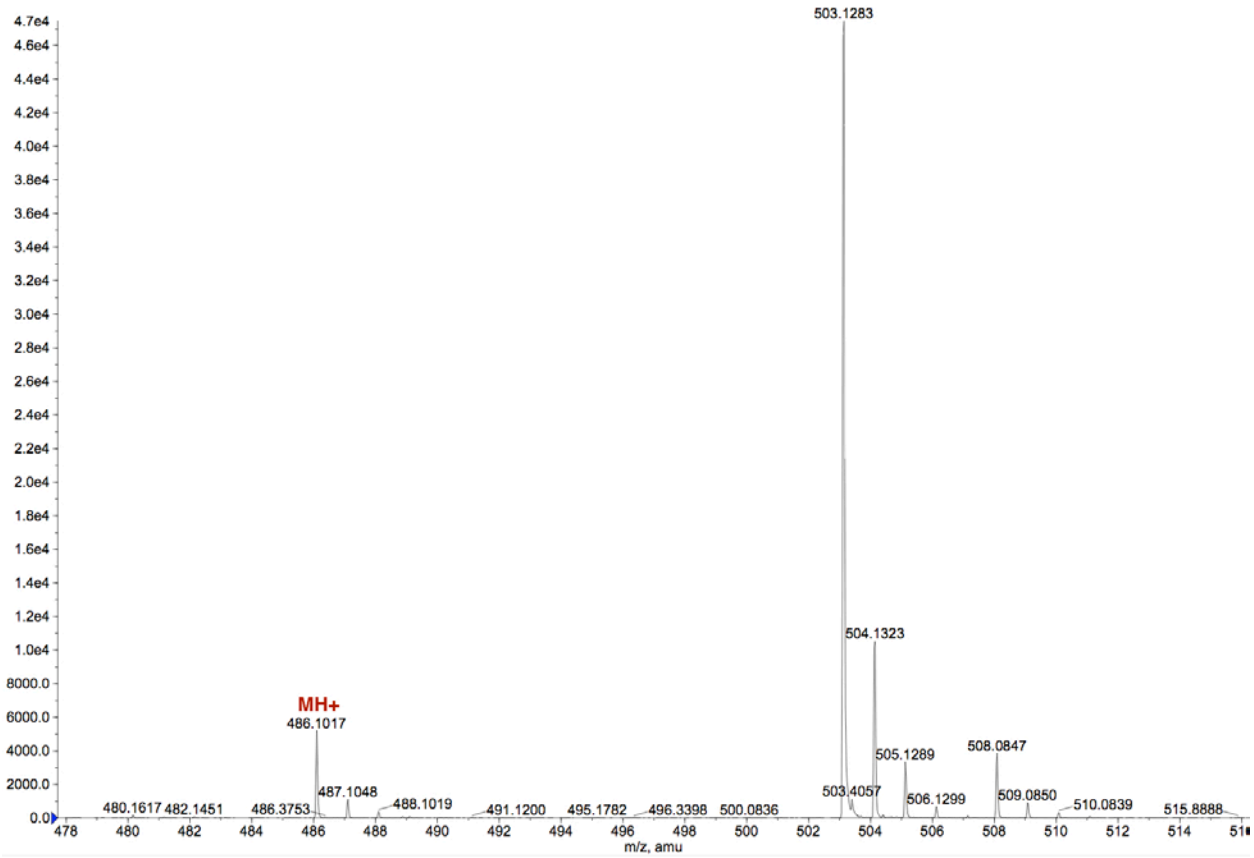


Figure S12. High resolution ESI-TOF spectrum for compound **1**.

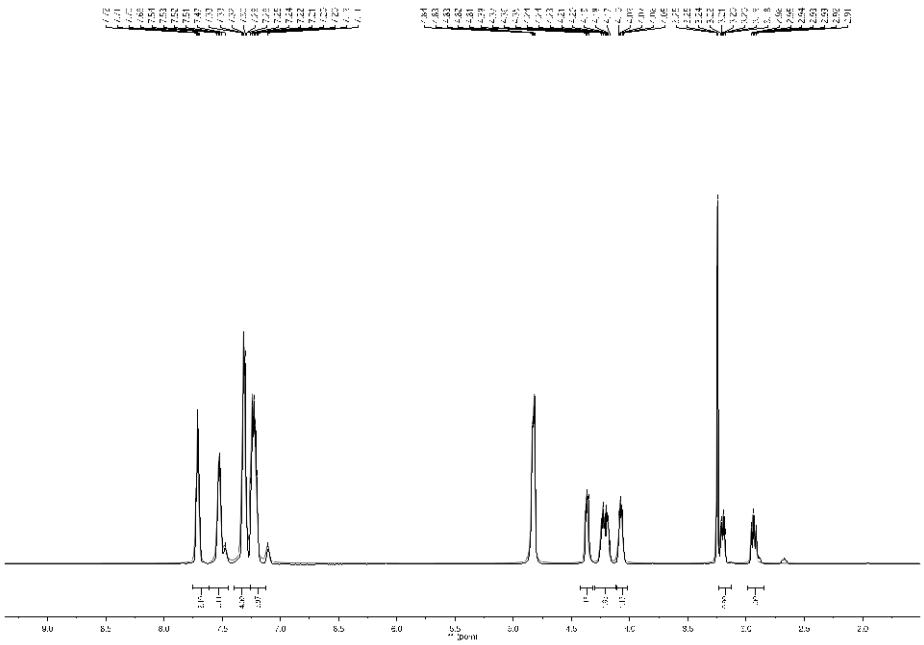


Figure S13. ^{1}H NMR spectrum for compound **1** (CD_3OD).

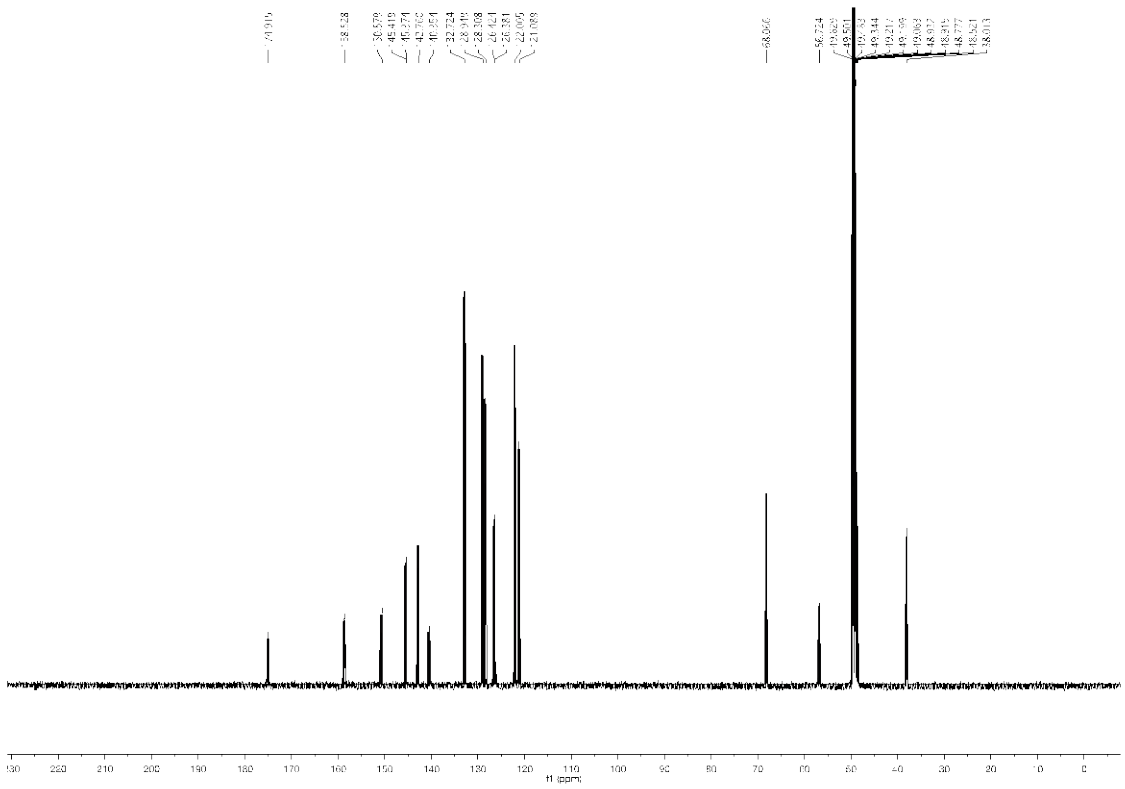


Figure S14. ^{13}C NMR spectrum for compound **1** (CD_3OD).

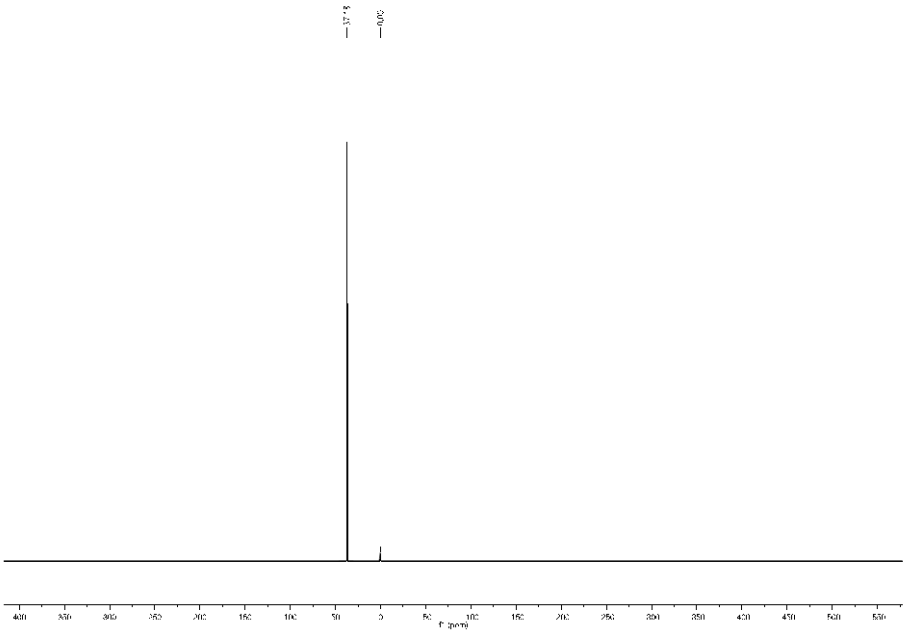


Figure S15. ^{19}F NMR spectrum of compound **1** (CD_3OD).

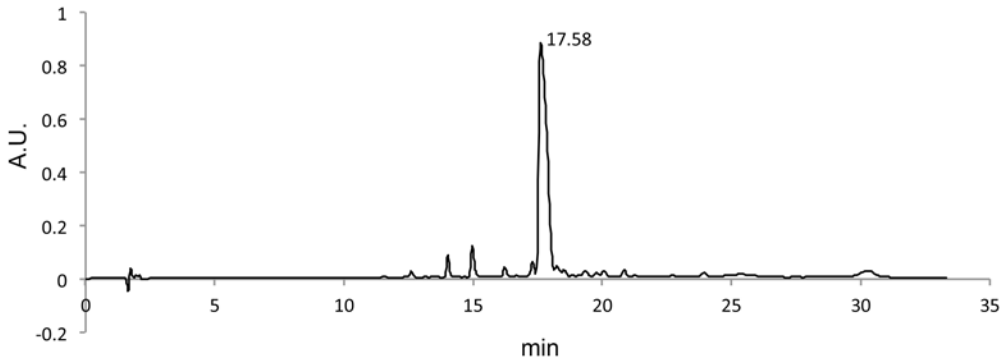


Figure S16. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 17.6 min) chromatogram of crude peptide DADEY(OSO_2F)L-NH_2 **(7)**.

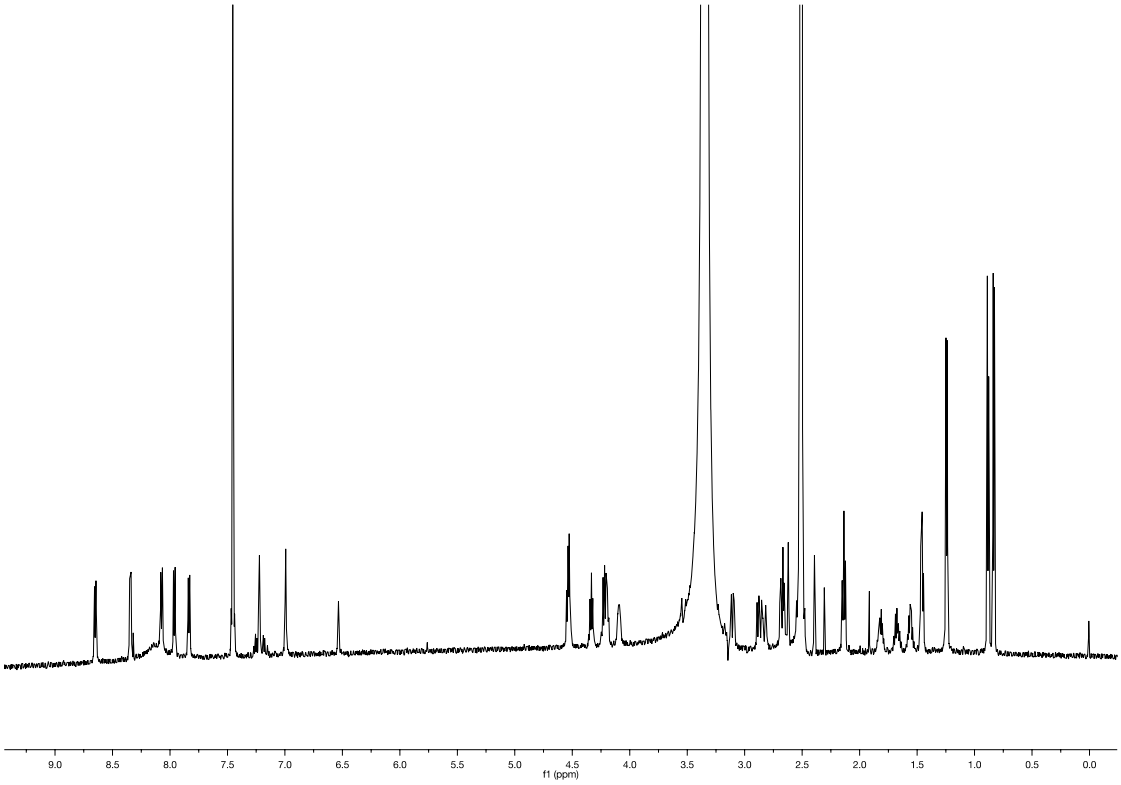


Figure S17. ^{1}H NMR (DMSO-d_6) spectrum of peptide DADEY(OSO_2F)L-NH_2 (**7**).

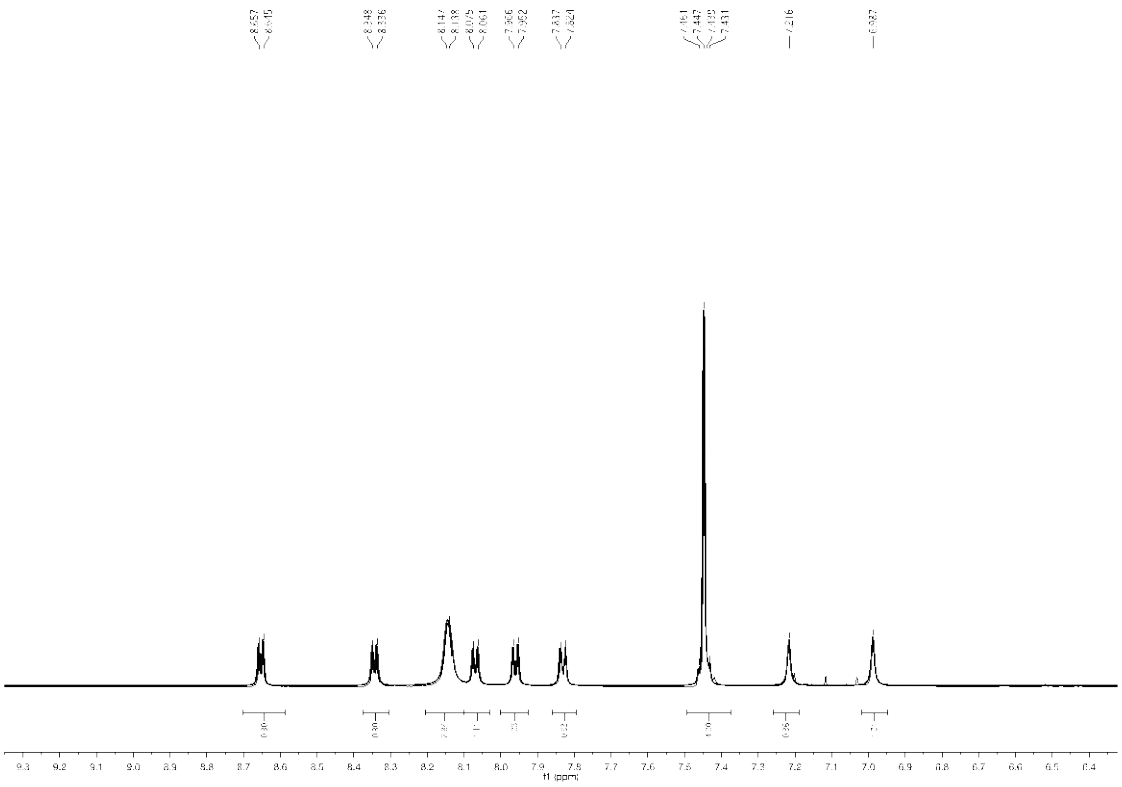


Figure S18. ^{1}H NMR (DMSO-d_6) spectrum of the aromatic region of peptide DADEY(OSO_2F)L-NH_2 (**7**).

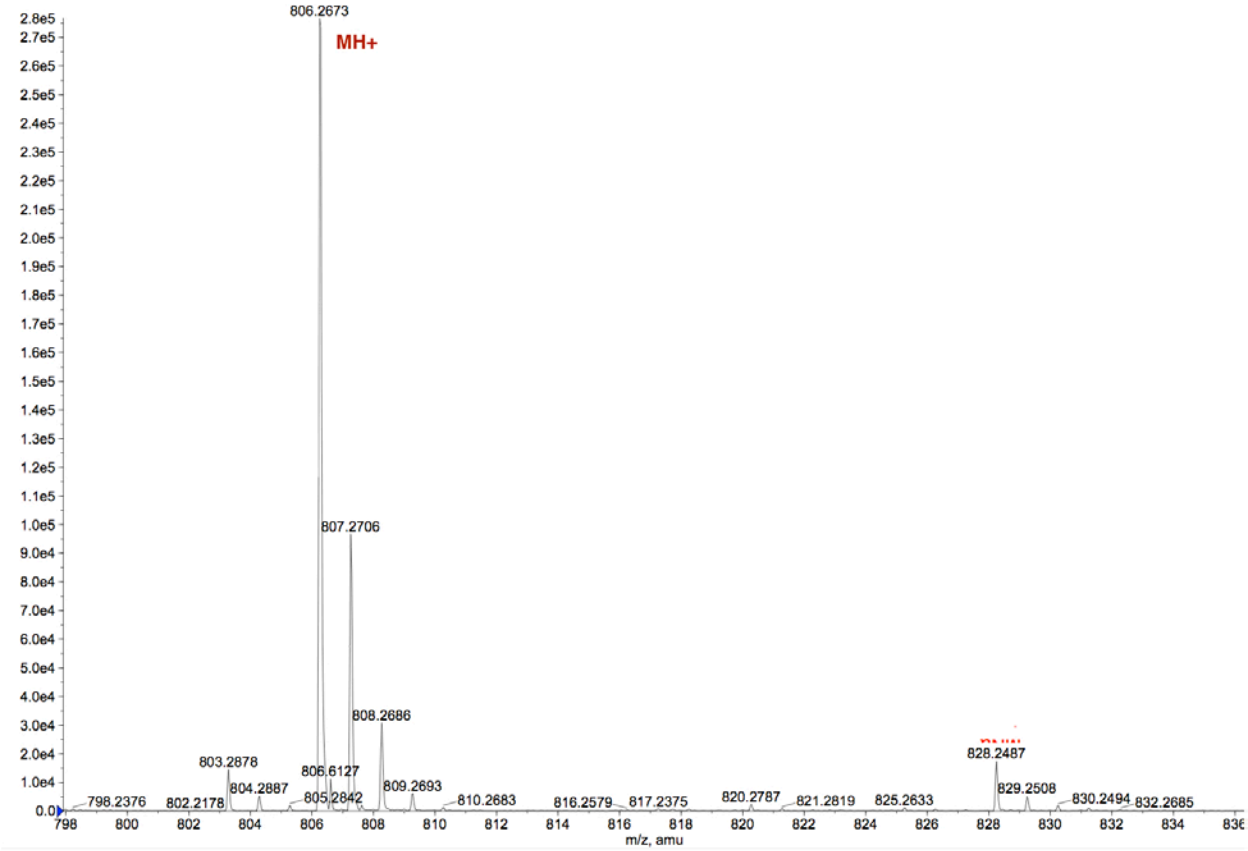


Figure S19. High resolution ESI-TOF spectrum for peptide DADEY(OSO_2F)L-NH_2 (**7**).

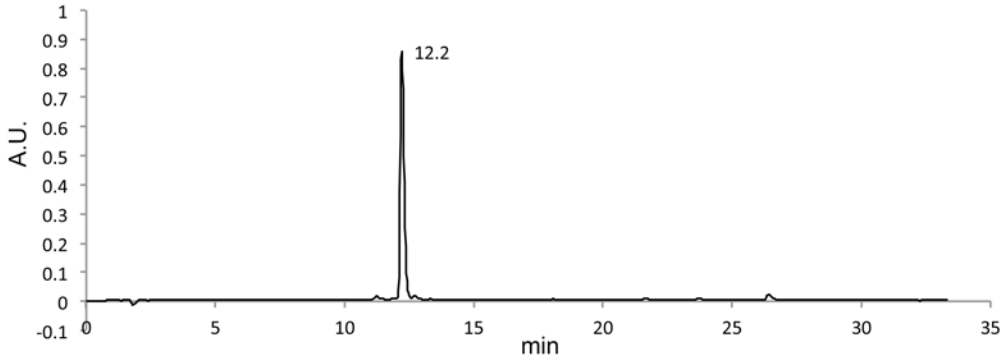


Figure S20. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 12.2 min) chromatogram of purified peptide DADE**sY**L-NH_2 **(2)**.

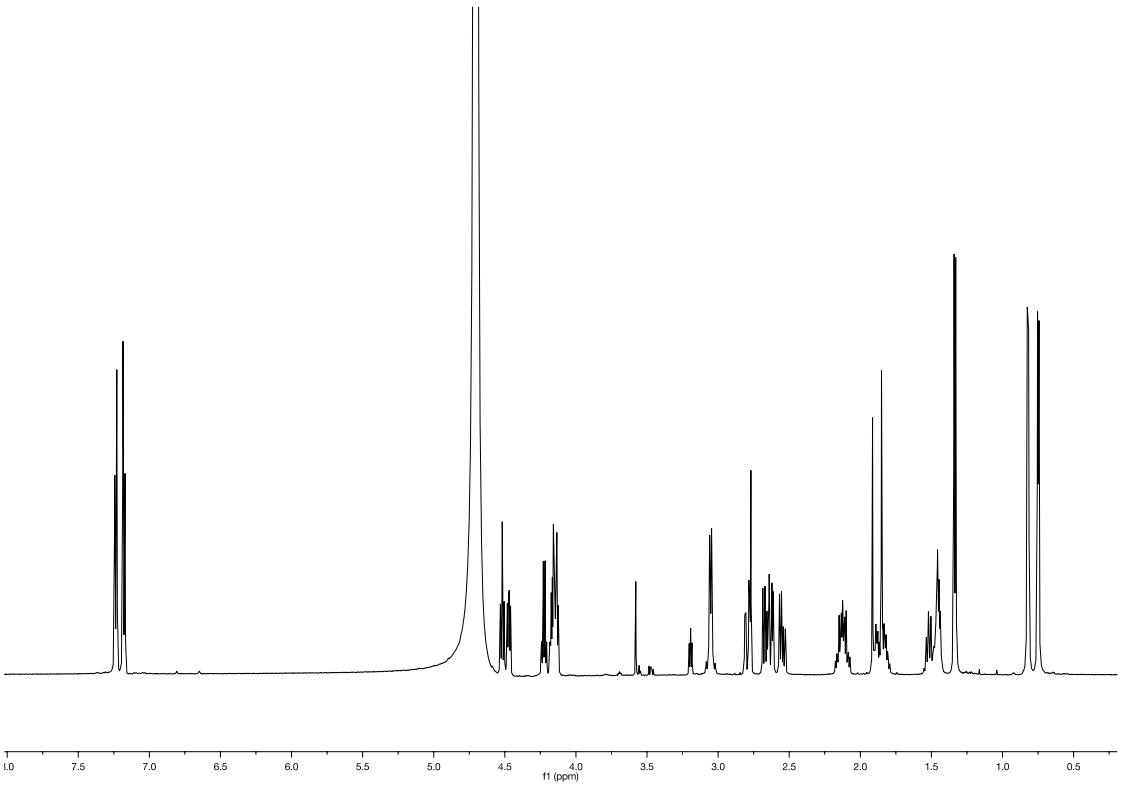


Figure S21. ^{1}H NMR (D_2O) spectrum of peptide DADE**sY**L-NH_2 **(2)**.

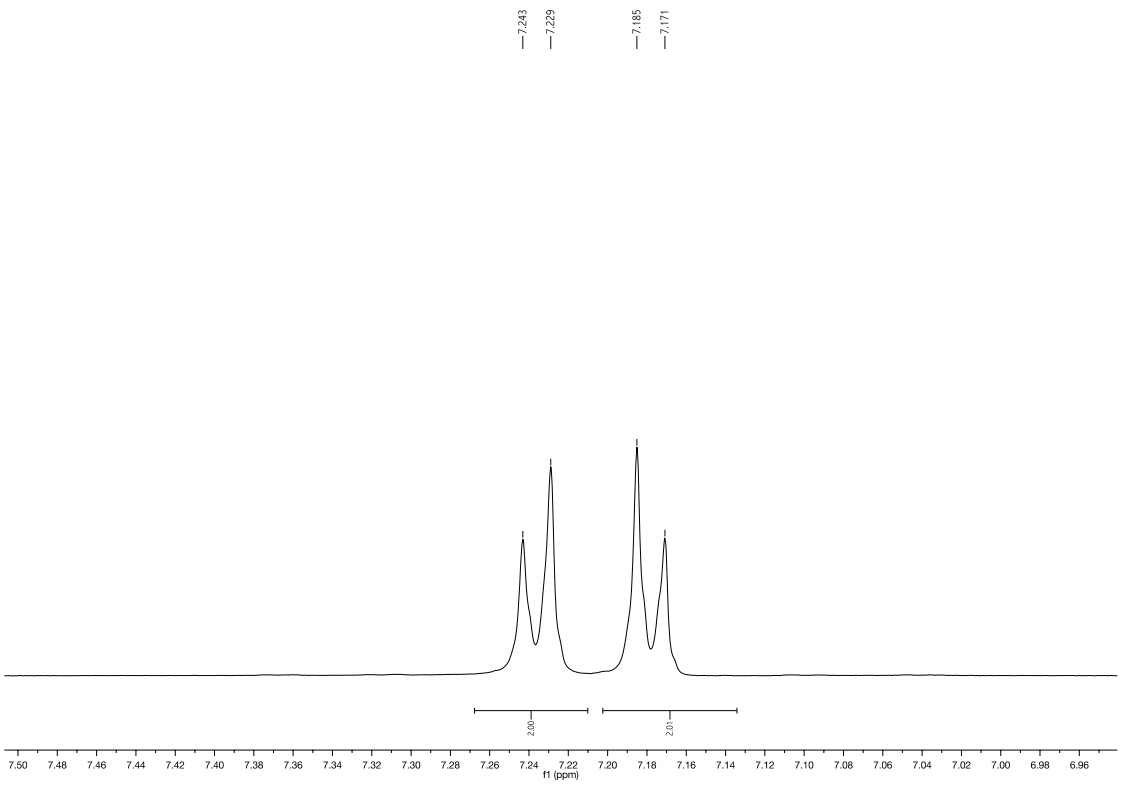


Figure S22. ^{1}H NMR (D_2O) spectrum of the aromatic region of peptide DADE**sY**L-NH_2 **(2)**.

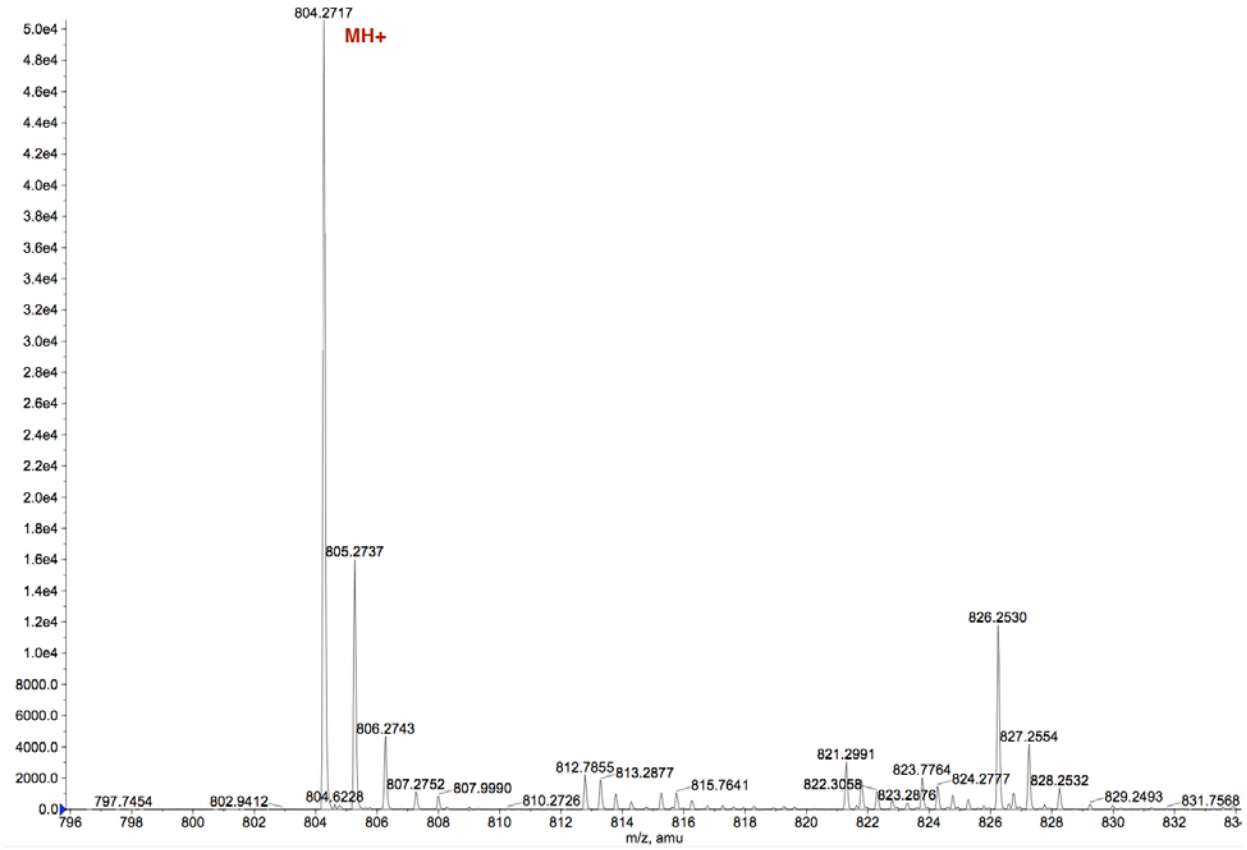


Figure S23. High resolution ESI-TOF spectrum for peptide DADE**sY**L-NH_2 (**2**).

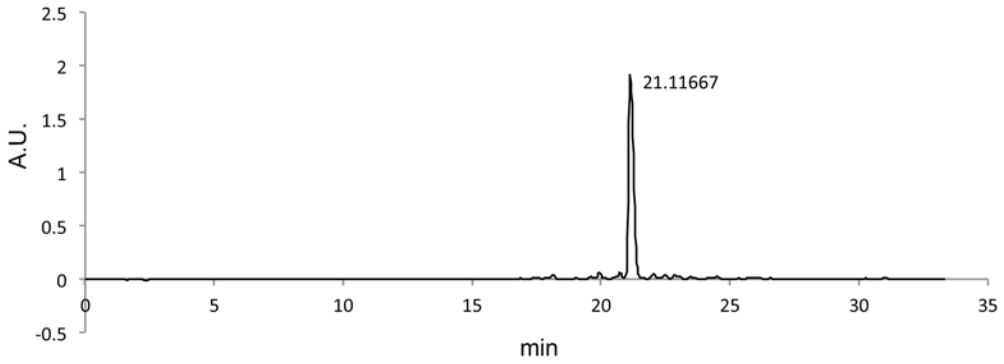


Figure S24. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 21.1 min) chromatogram of crude peptide YEY(OSO_2F)LDYDF-NH_2 **(S1)**.

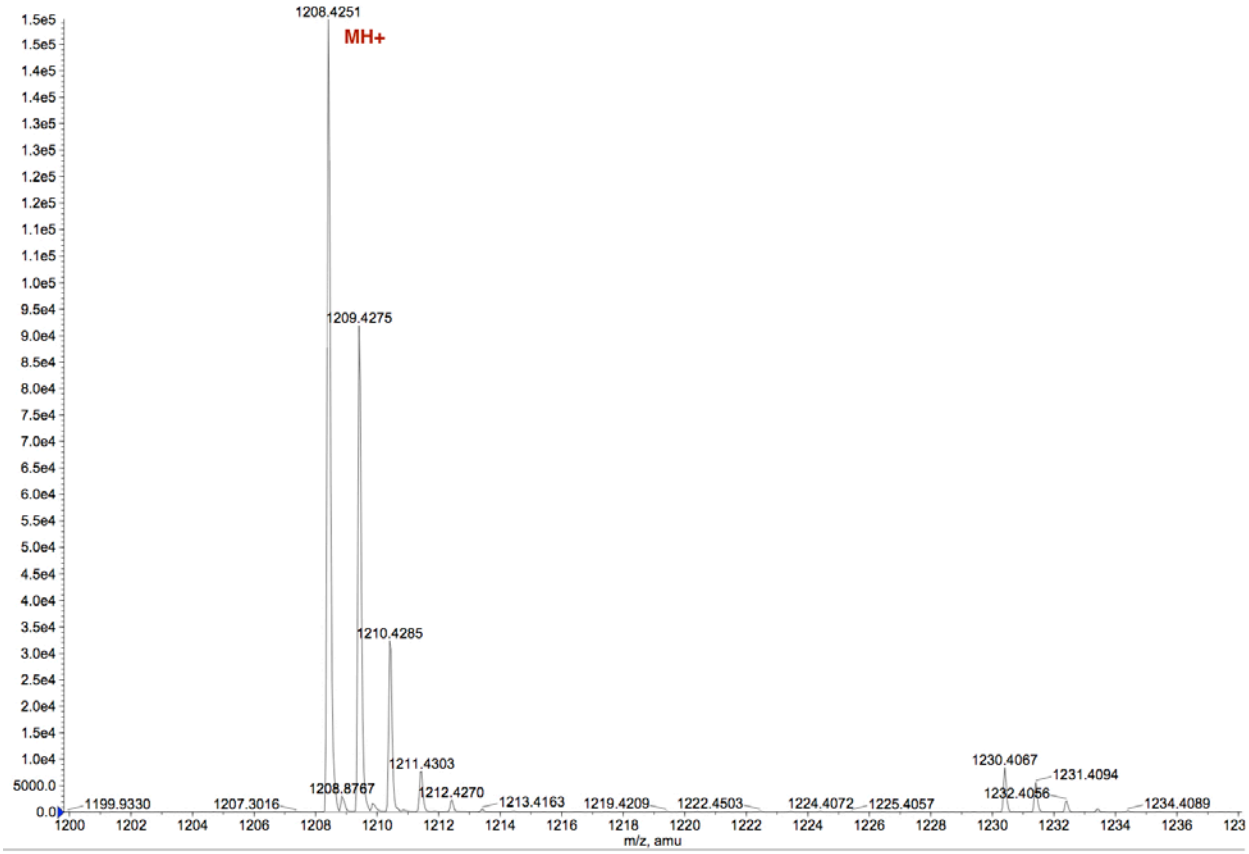


Figure S25. High resolution ESI-TOF spectrum for crude peptide YEY(OSO_2F)LDYDF-NH2 (**S1**).

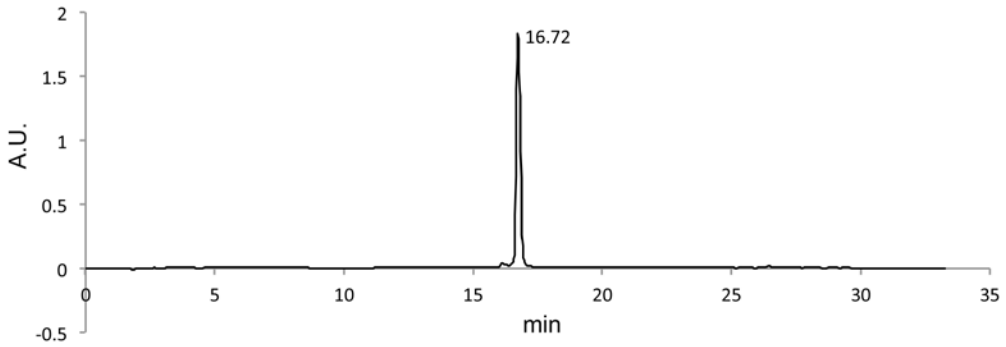


Figure S26. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 16.7 min) chromatogram of purified peptide YE**s**Y**Y**LDYDF-NH_2 **(3)**.

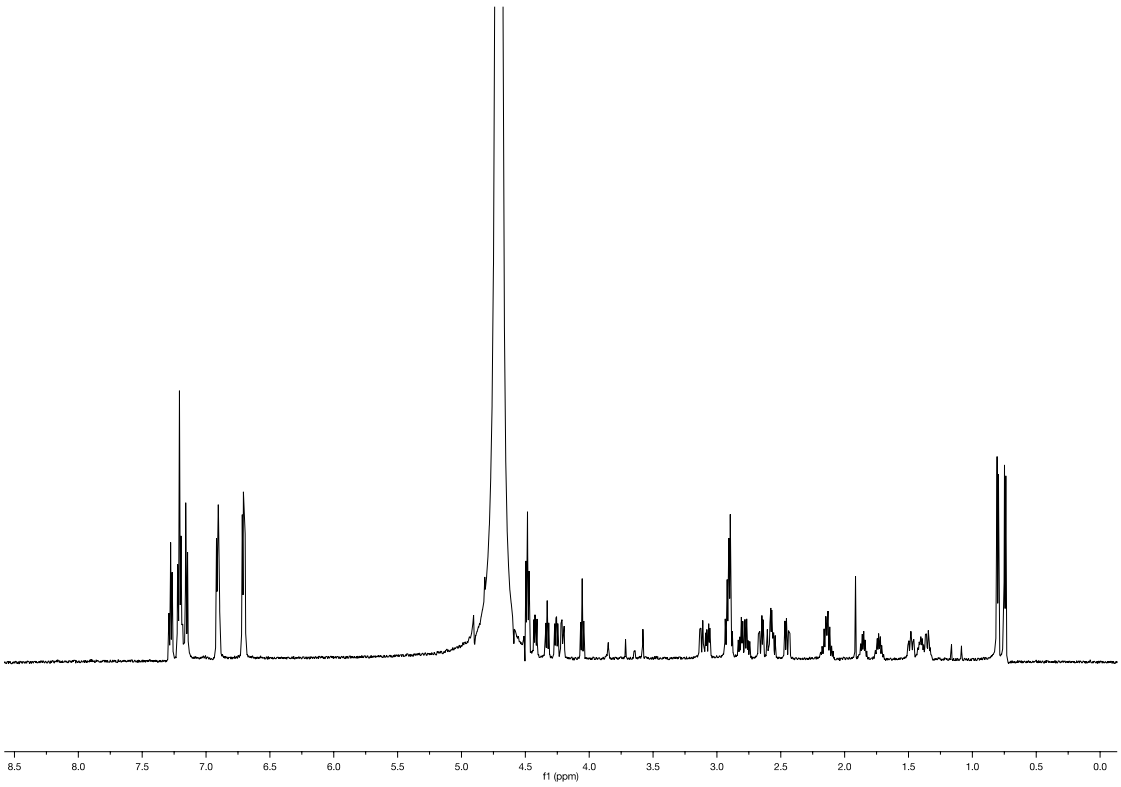


Figure S27. ^{1}H NMR (D_2O) spectrum of peptide YE**sY**LDYDF-NH_2 **(3)**.

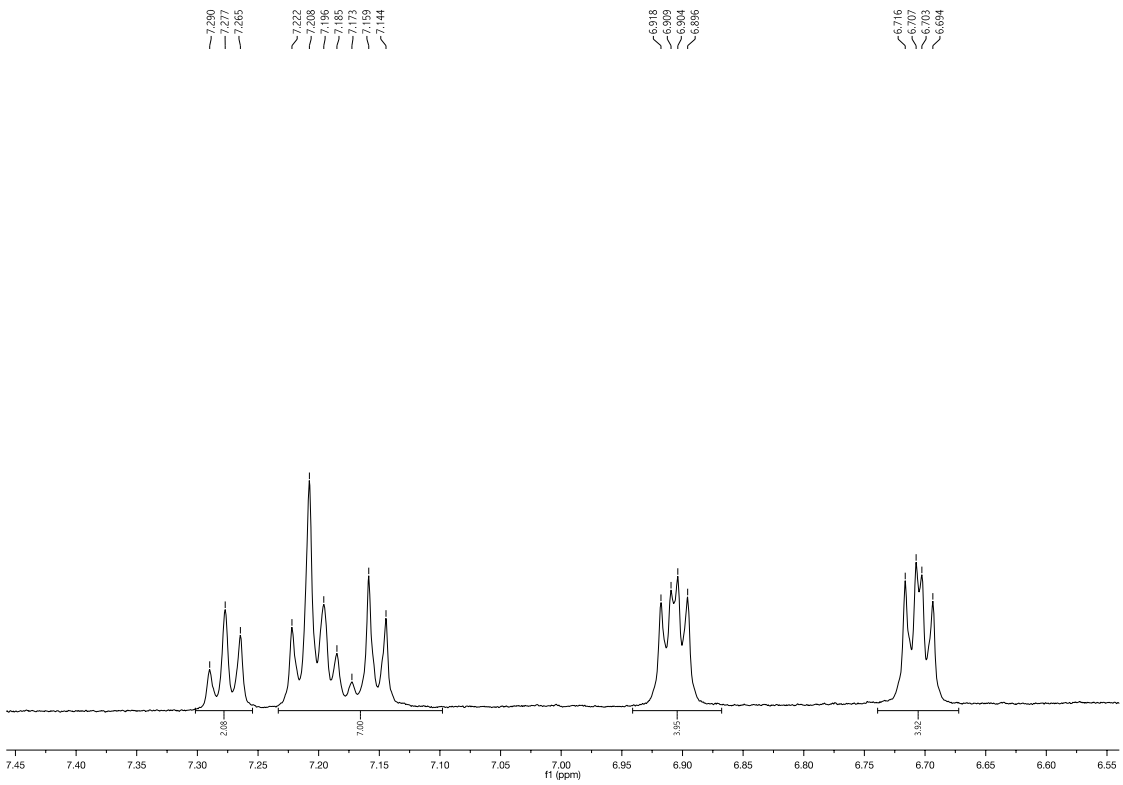


Figure S28. ^{1}H NMR (D_2O) spectrum of the aromatic region of peptide YE**s**Y**Y**LDYDF-NH_2 **(3)**.

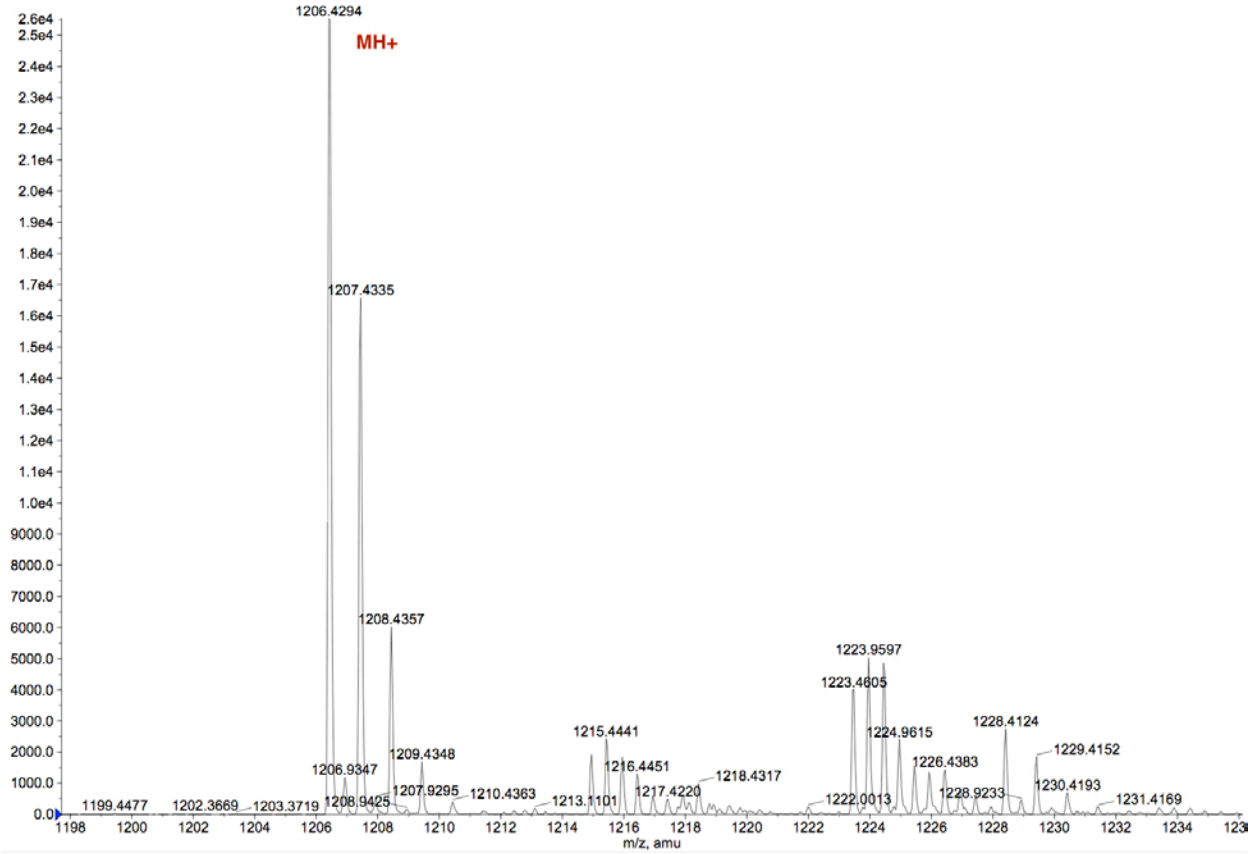


Figure S29. High resolution ESI-TOF spectrum for crude peptide YE**s**Y**L**DYDF-NH2 (**3**).

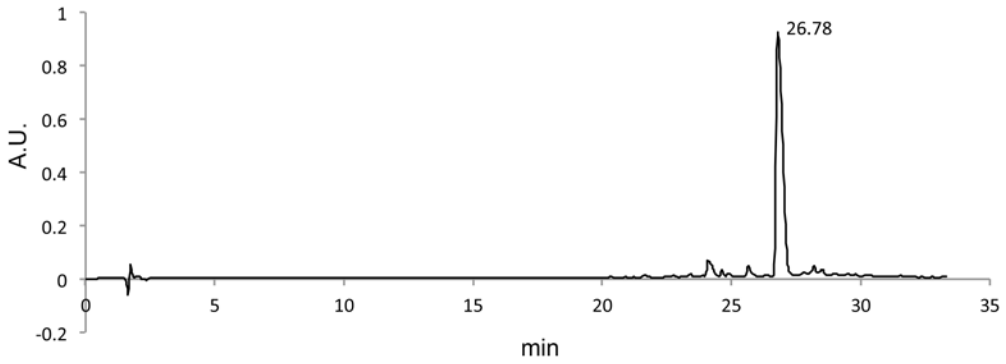


Figure S30. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 26.8 min) chromatogram of crude peptide Y(OSO_2F)EY(OSO_2F)LDY(OSO_2F)DF-NH_2 **(S2)**.

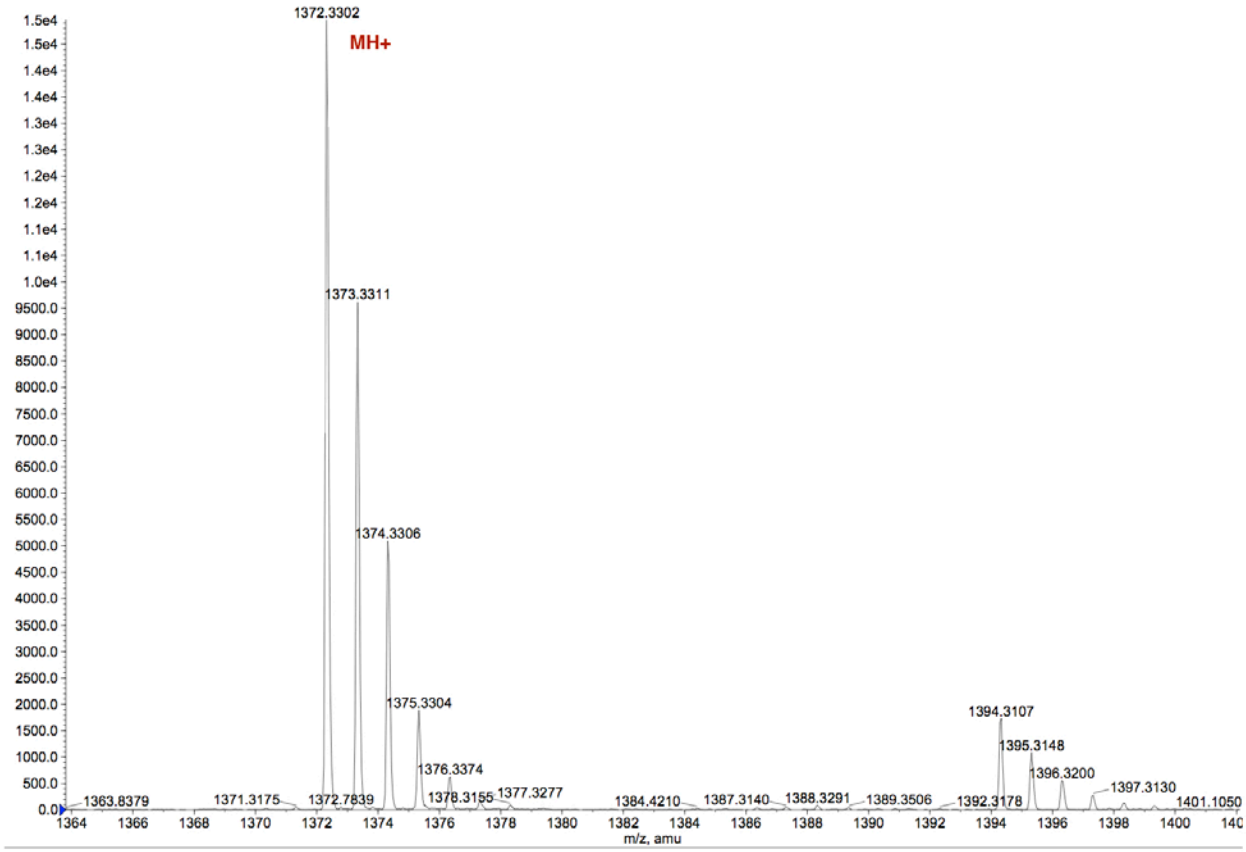


Figure S31. High resolution ESI-TOF spectrum for crude peptide Y(OSO_2F)EY(OSO_2F)LDY(OSO_2F)DF-NH_2 **(S2)**.

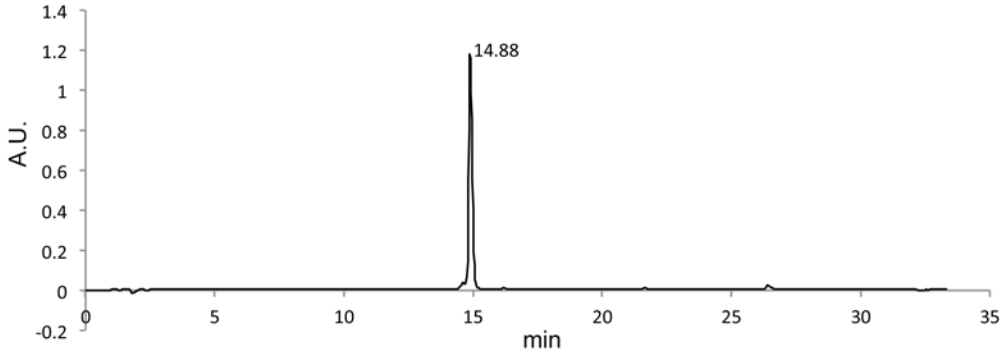


Figure S32. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 14.9 min) chromatogram of purified peptide **sY**E**sY**LD**sY**DF-NH_2 **(4)**.

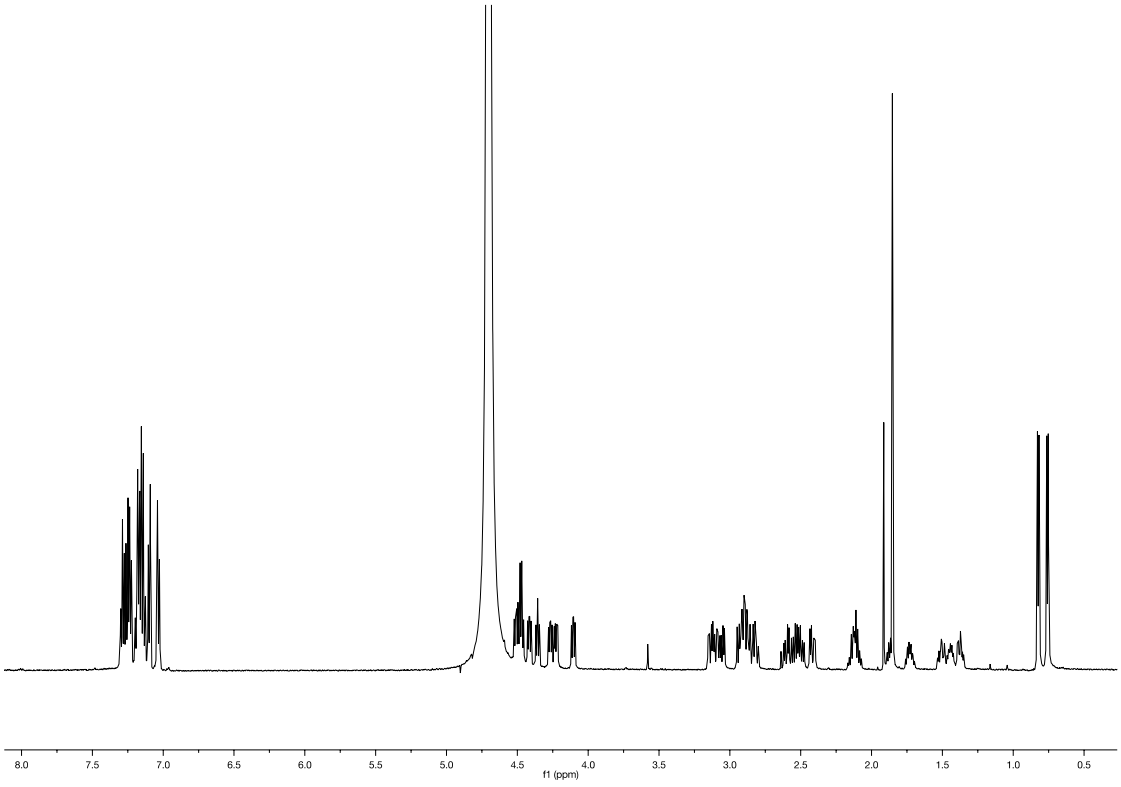


Figure S33. ^{1}H NMR (D_2O) spectrum of peptide **sYEsY**LD**sY**DF-NH_2 **(4)**.

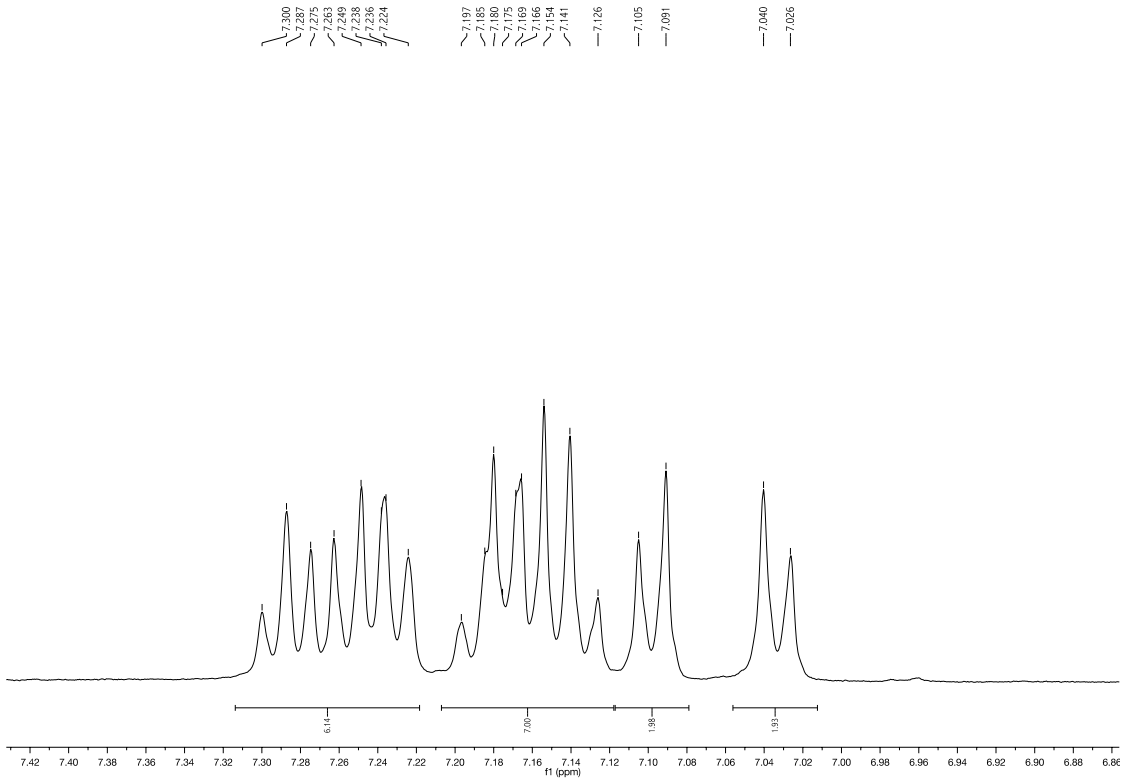


Figure S34. ^{1}H NMR (D_2O) spectrum of the aromatic region of peptide **sY**E**sY**LD**sY**DF-NH_2 **(4)**.

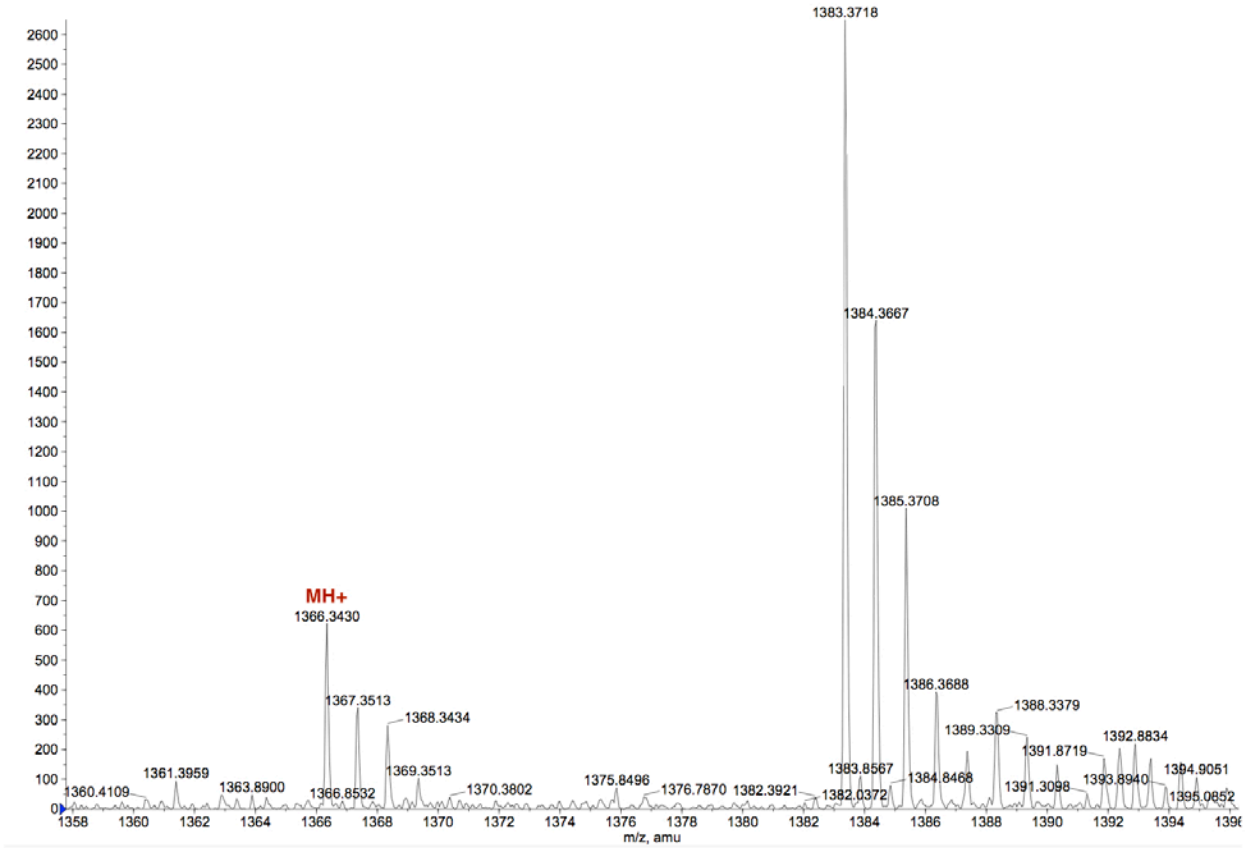


Figure S35. High resolution ESI-TOF spectrum for peptide **sY**E**sY**LD**sY**DF-NH_2 **(4)**.

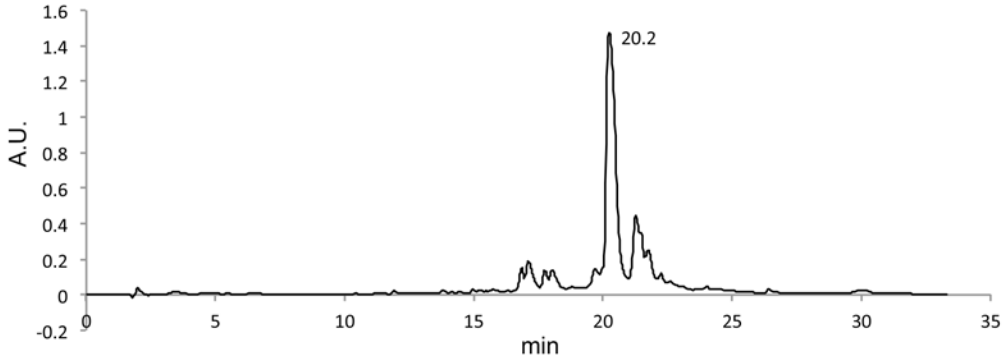


Figure S36. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 20.2 min) chromatogram of crude peptide TTPDY(OSO_2F)GHY(OSO_2F)DDKDTLDLNTPVDK-NH_2 **(S3)**.

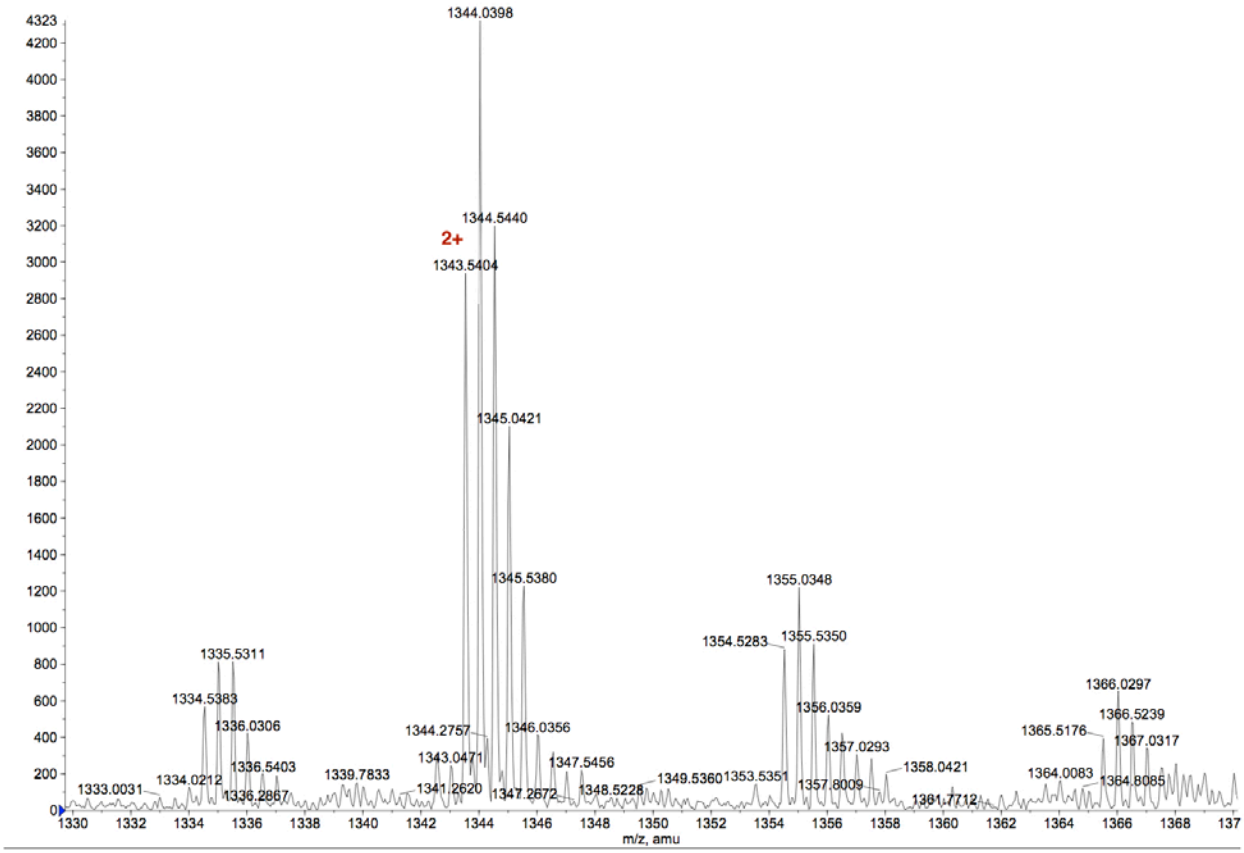


Figure S37. High resolution ESI-TOF spectrum for crude peptide TTPDY(OSO$_2$F)GHY(OSO$_2$F)DDKDTLDLNTPVDK-NH$_2$ **(S3)**.

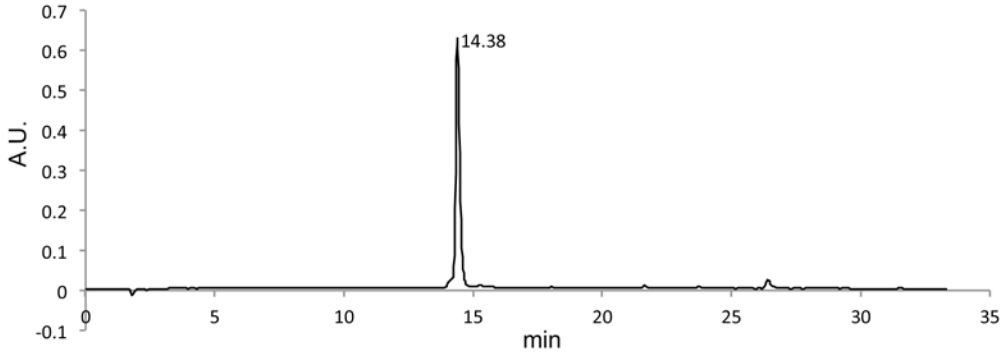


Figure S38. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 14.4 min) chromatogram of purified peptide TTPD**sY**GH**sY**DDKDTLDLNTPVDK-NH_2 **(5)**.

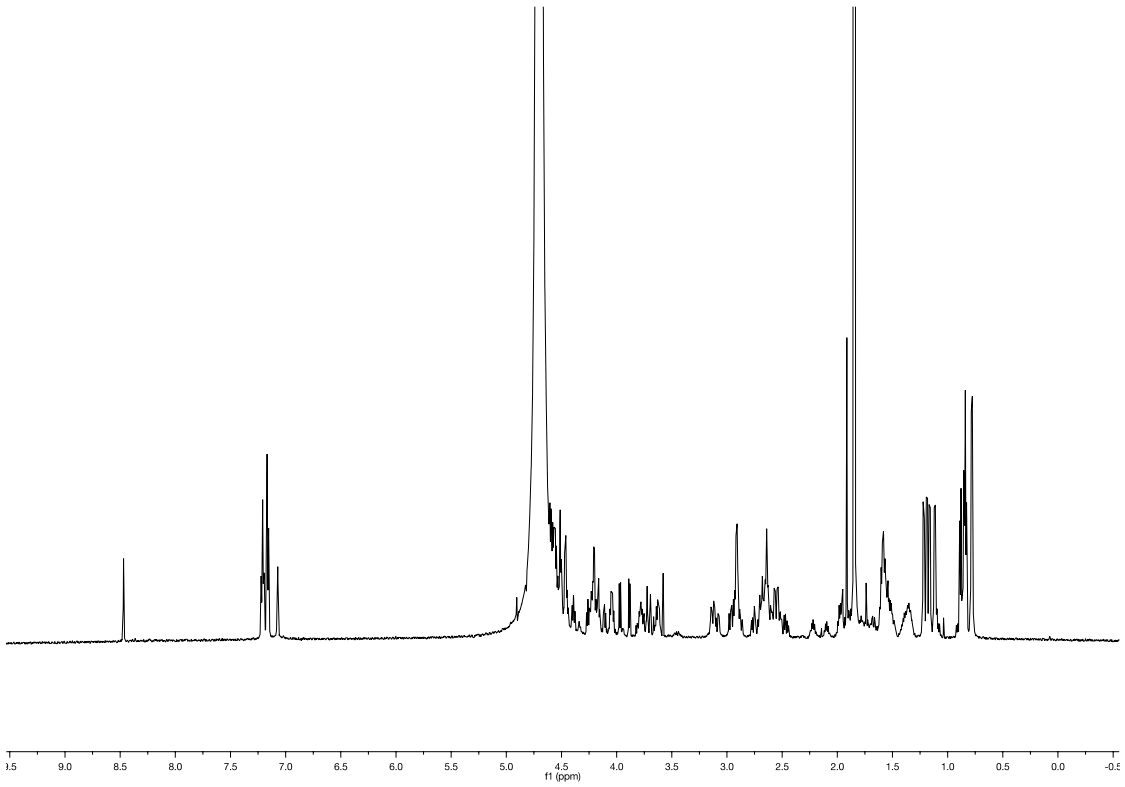


Figure S39. ^{1}H NMR (D_2O) spectrum of peptide TTPD**sY**GH**sY**DDKDTLDLNTPVDK-NH_2 **(5)**.

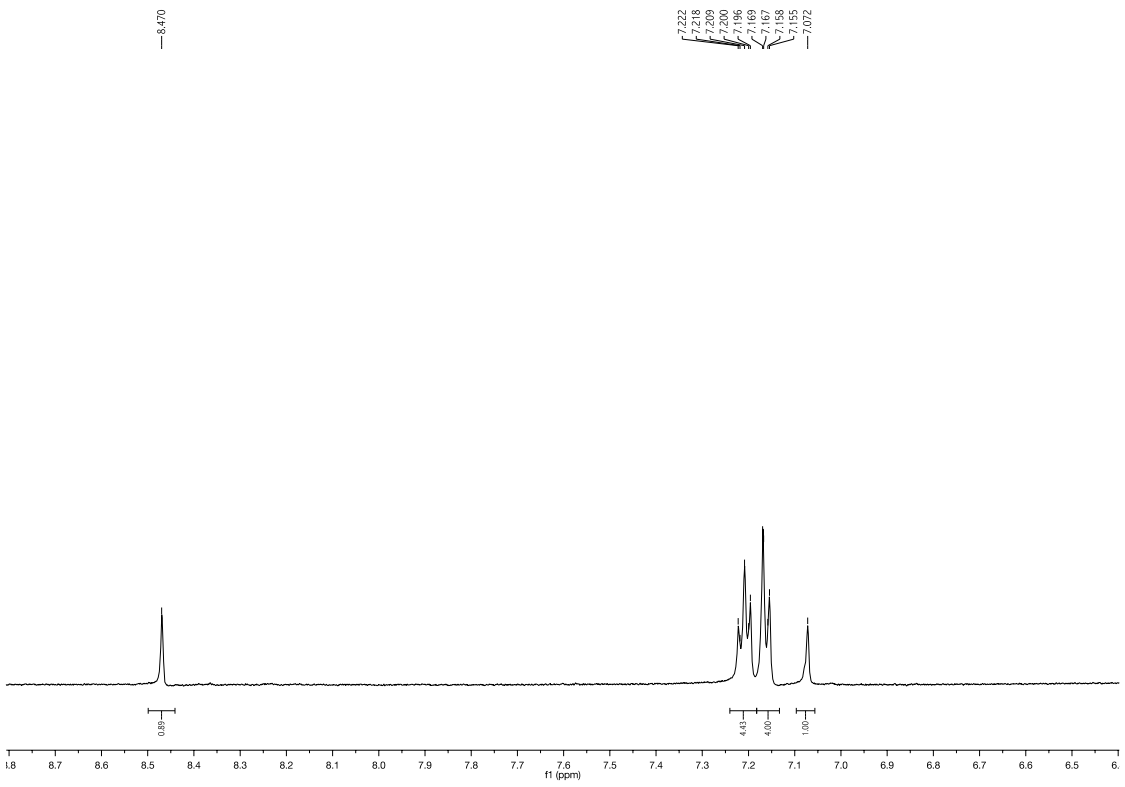


Figure S40. ^{1}H NMR (D_2O) spectrum of the aromatic region of peptide TTPD**sY**GH**sY**DDKDTLDLNTPVDK-NH_2 **(5)**.

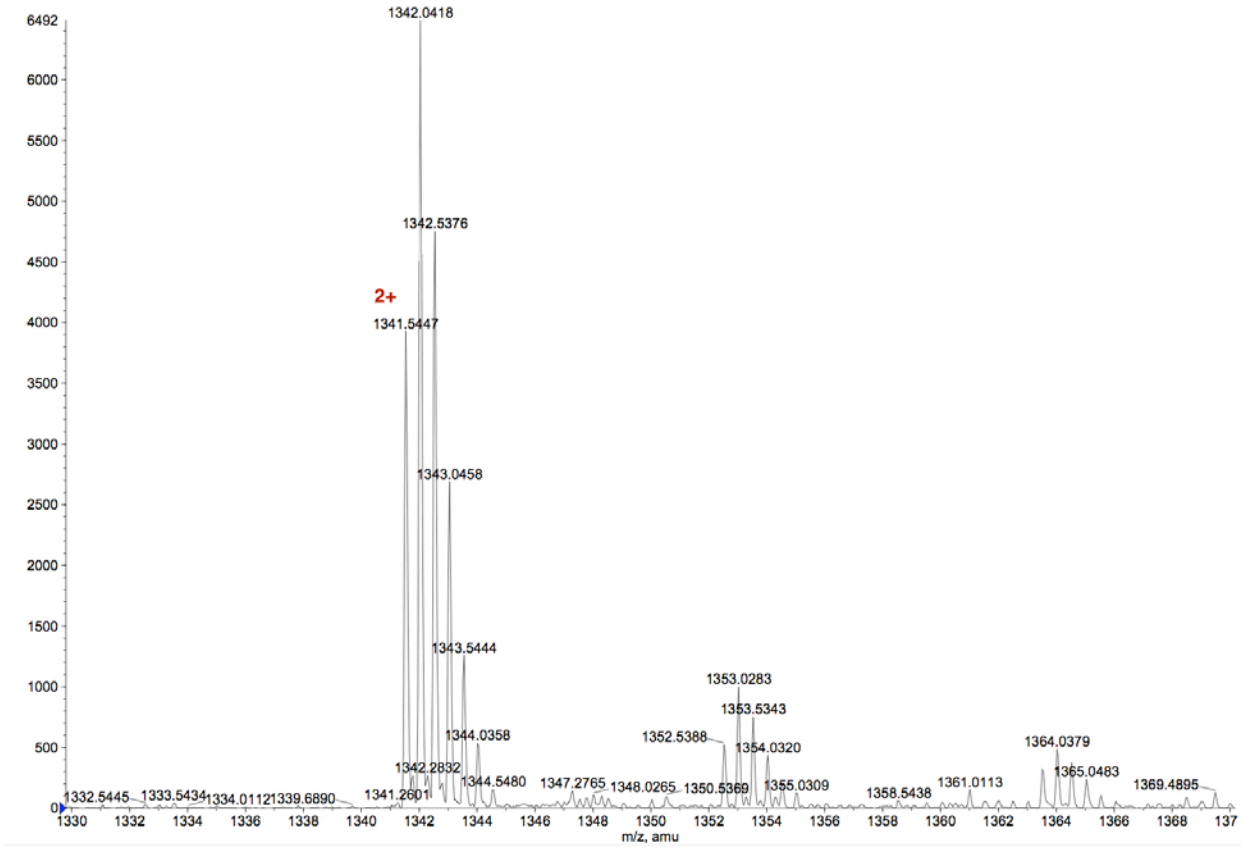


Figure S41. High resolution ESI-TOF spectrum for peptide TTPD**sY**GH**sY**DDKDTLDLNTPVDK-NH_2 **(5)**.

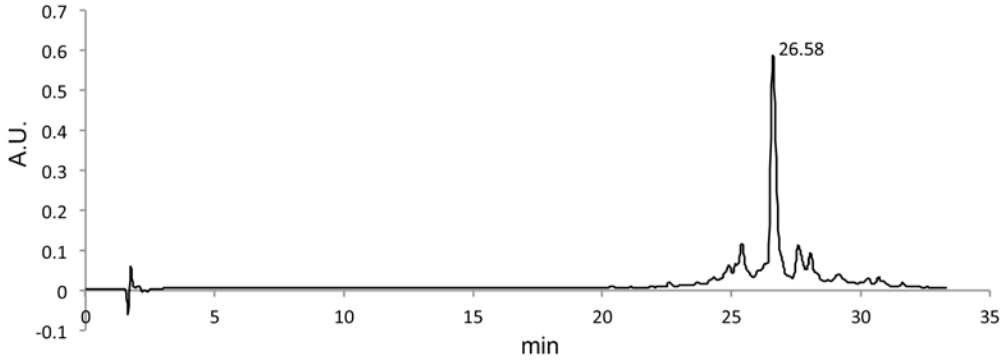


Figure S42. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 26.6 min) chromatogram of crude peptide DADSENSSFY(OSO_2F)Y(OSO_2F)Y(OSO_2F)DY(OSO_2F)LDEVAF-NH_2 **(S4)**.

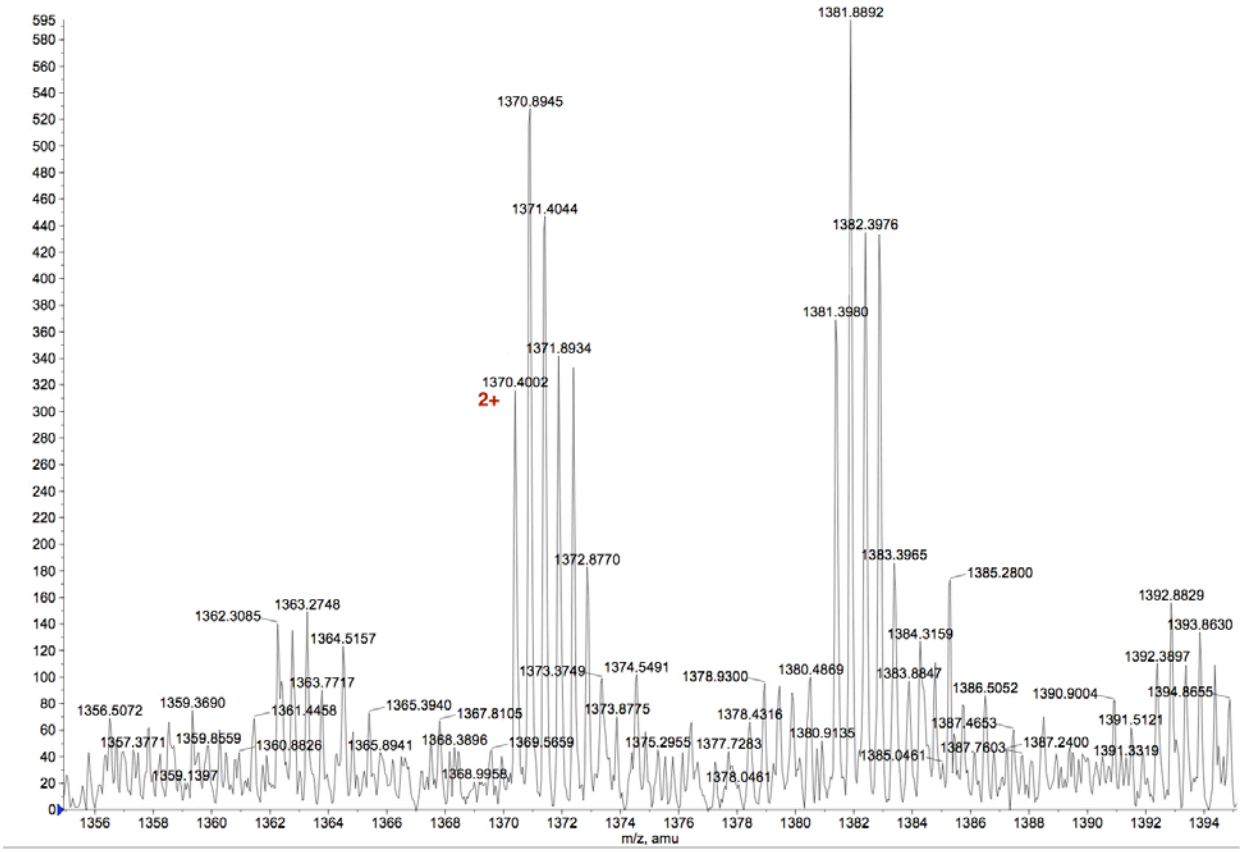


Figure S43. High resolution ESI-TOF spectrum for crude peptide DADSENSSFY(OSO_2F)Y(OSO_2F)Y(OSO_2F)DY(OSO_2F)LDEVAF-NH_2 **(S4)**.

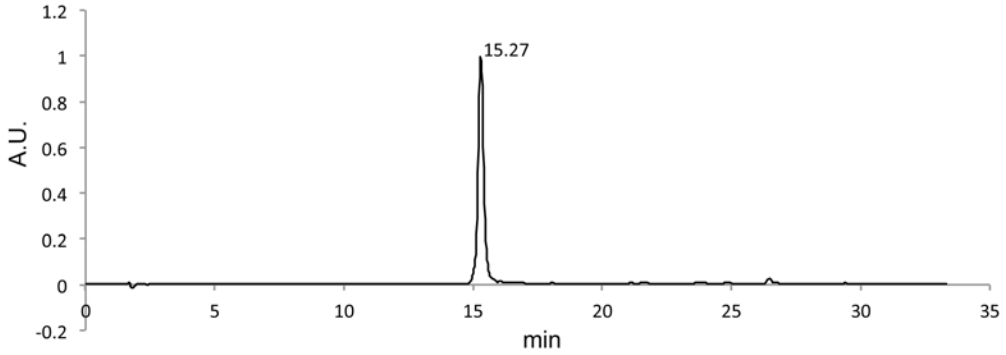


Figure S44. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 15.3 min) chromatogram of purified peptide DADSENSSF**sYsYsY**D**sY**LDEVAF-NH_2 **(6)**.

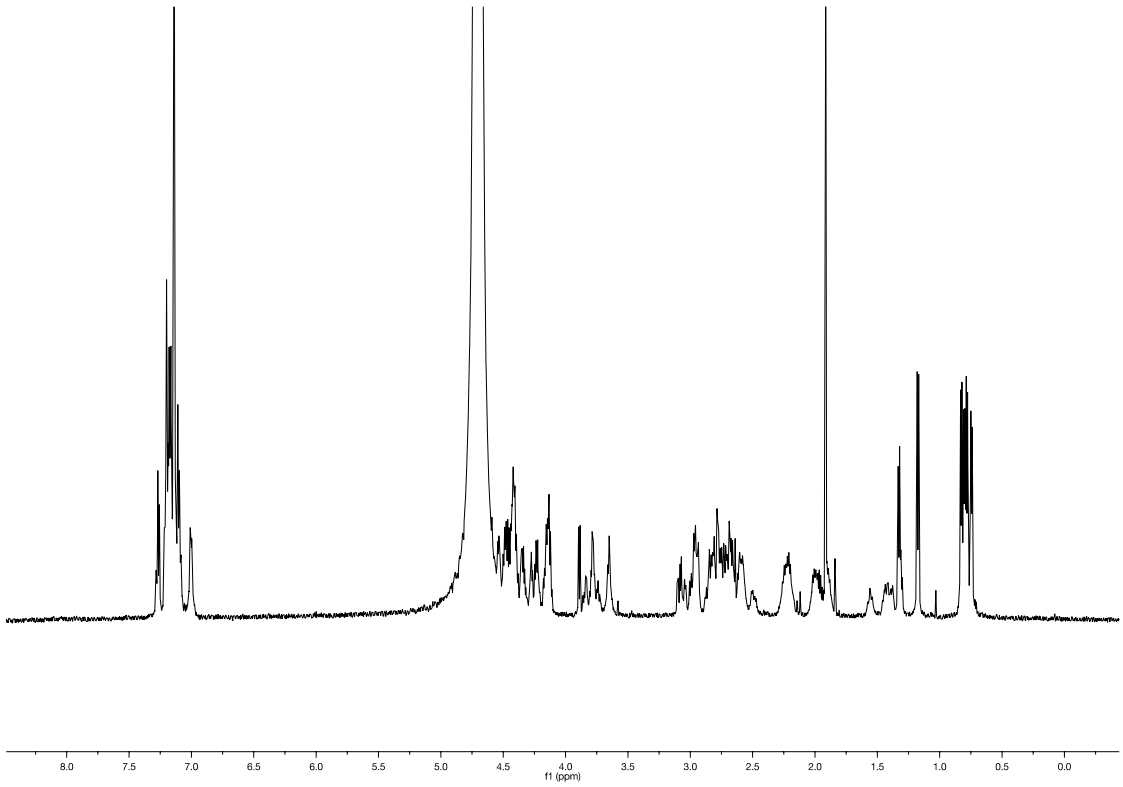


Figure S45. ^{1}H NMR (D_2O) spectrum of peptide DADSENSSF**sYsYsY**D**sY**LDEVAF-NH_2 **(6)**.

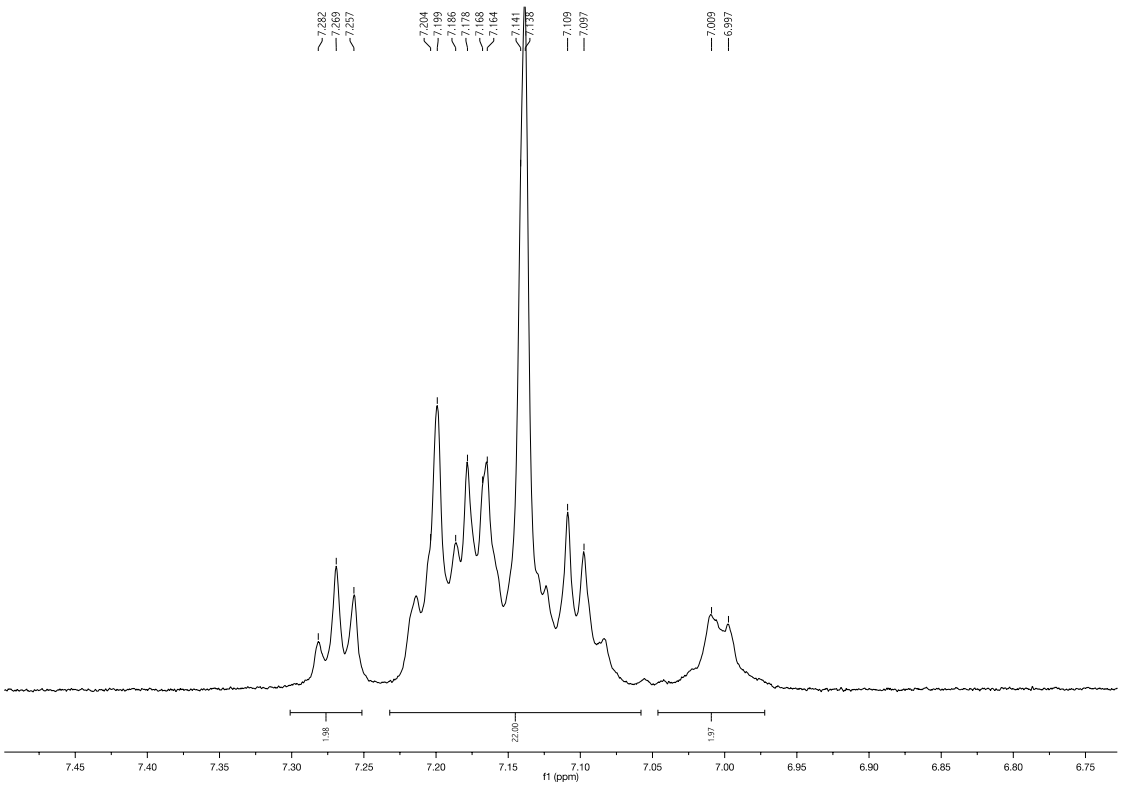


Figure S46. ^{1}H NMR (D_2O) spectrum of the aromatic region of peptide DADSENSSF**sYsYsY**D**sY**LDEVAF-NH_2 **(6)**.

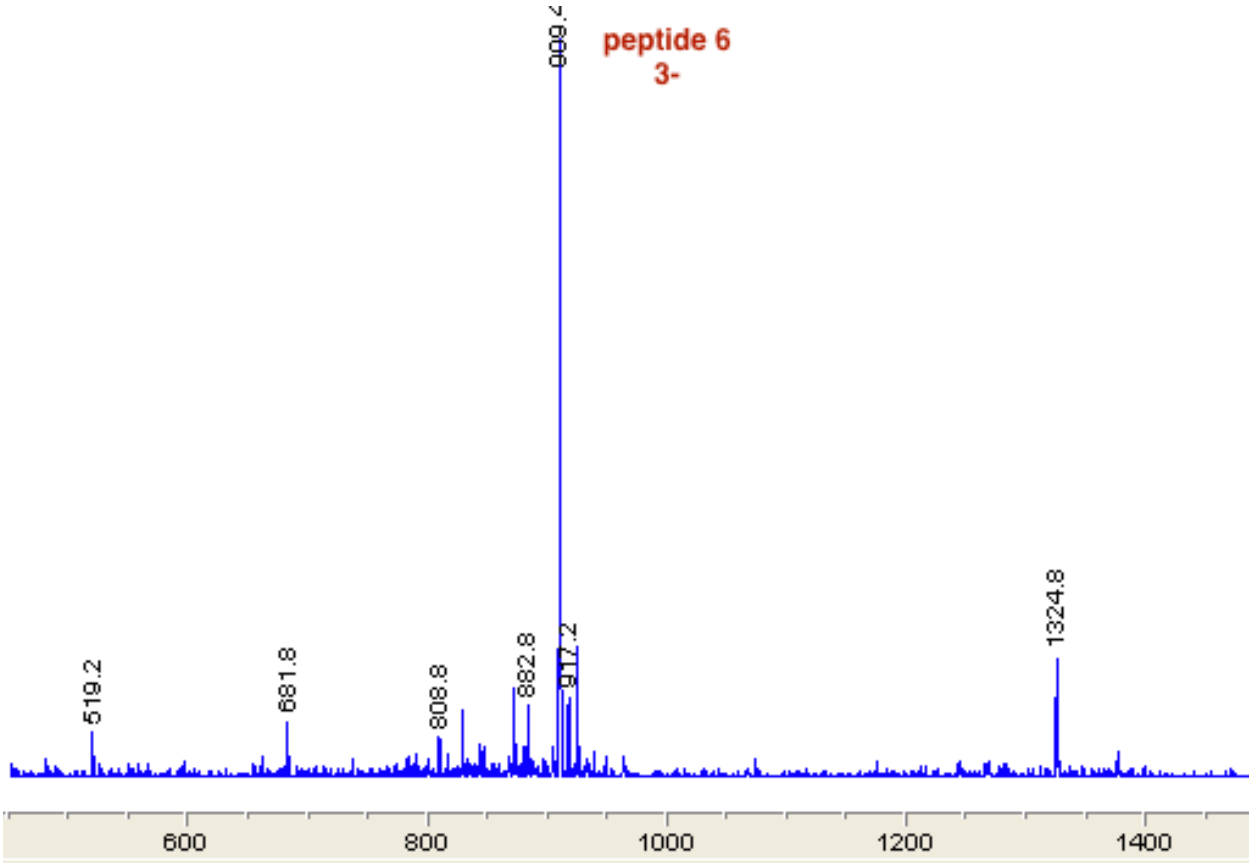


Figure S47. ESI-MS spectrum for peptide DADSENSSF**sYsYsY**D**sY**LDEVAF-NH_2 **(6)**.

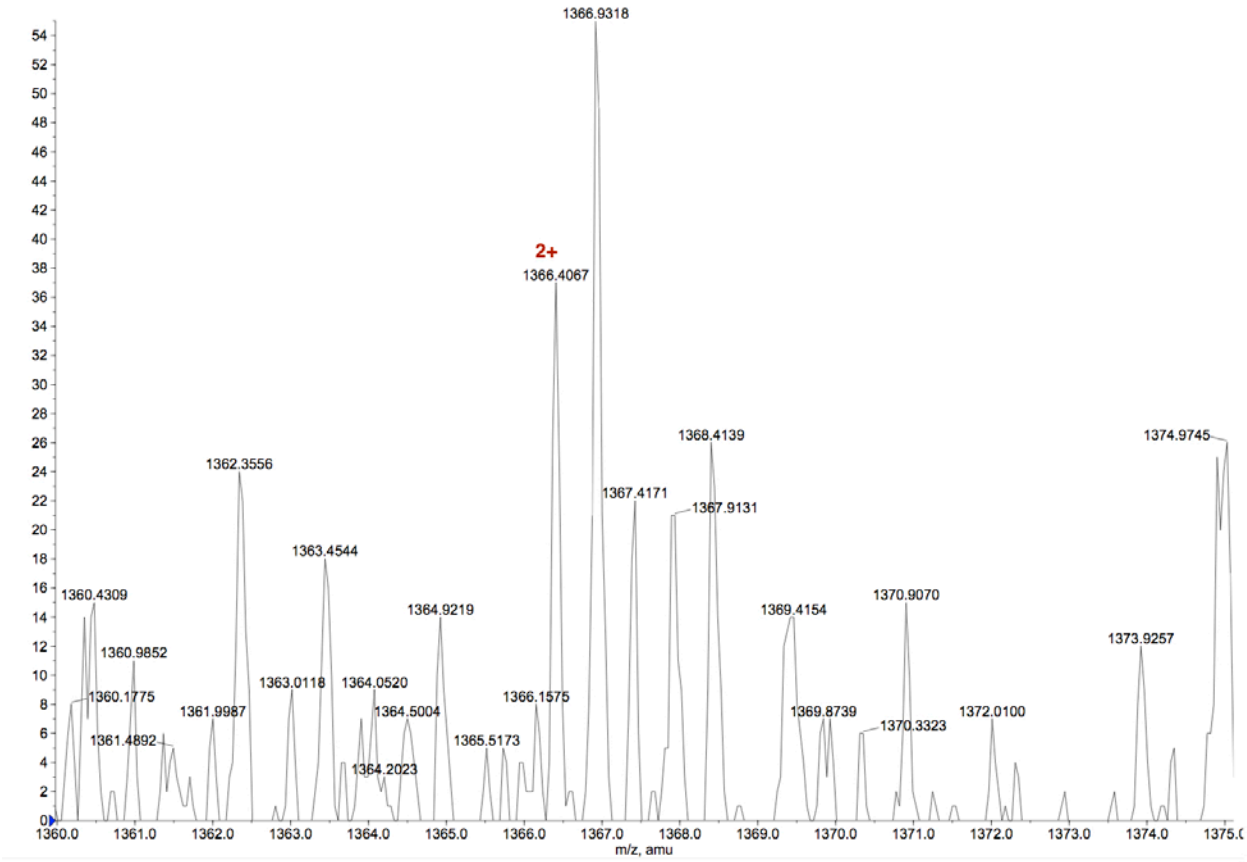


Figure S48. High resolution ESI-TOF spectrum for peptide DADSENSSF**sYsYsY**D**sY**LDEVAF-NH_2 **(6)**.

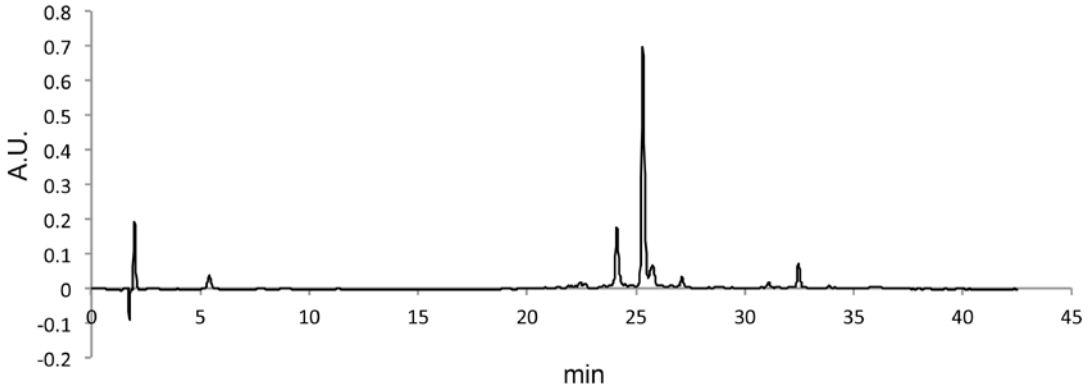


Figure S49. Analytical RP-HPLC (CH_3CN: H_2O mobile phases each with 0.2% TFA, 0% CH_3CN 0-5 min, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 25.32 min) chromatogram of crude peptide GDY(OSO_2F)DSMKEPCFR-NH_2 **(8)**.

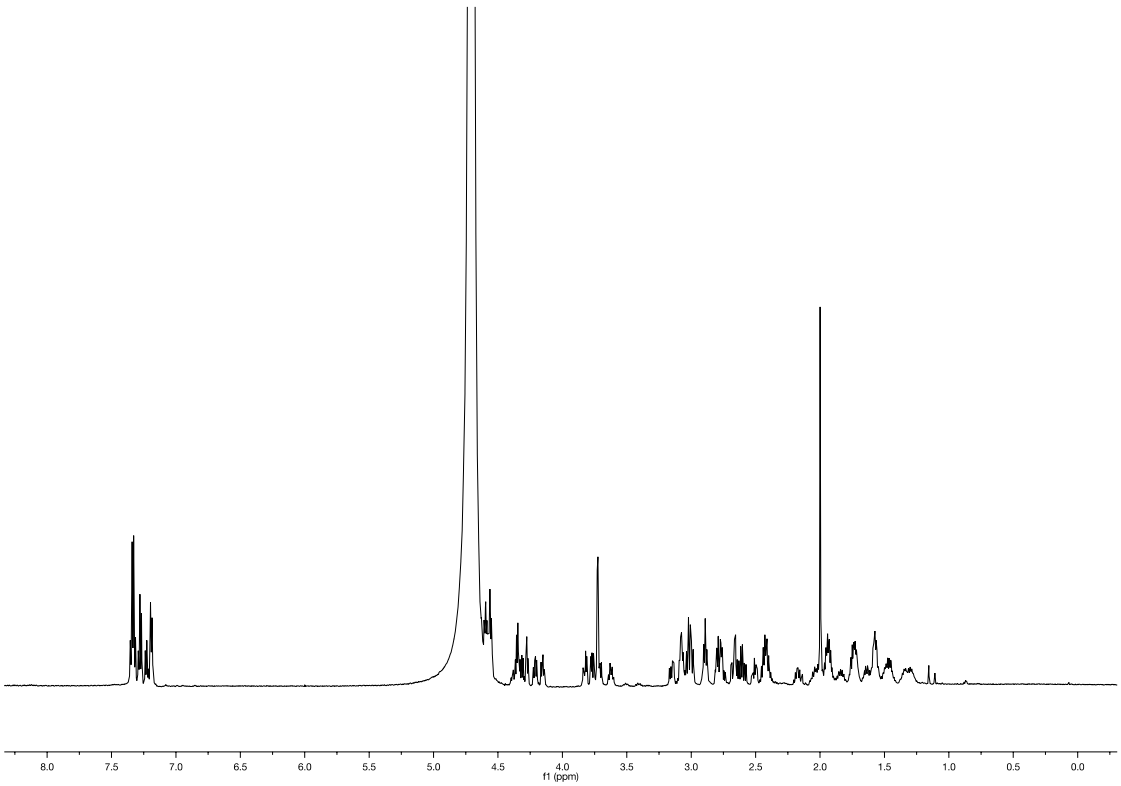


Figure S50. ^{1}H NMR (D_2O) spectrum of peptide GDY(OSO_2F)DSMKEPCFR-NH_2 **(8)**.

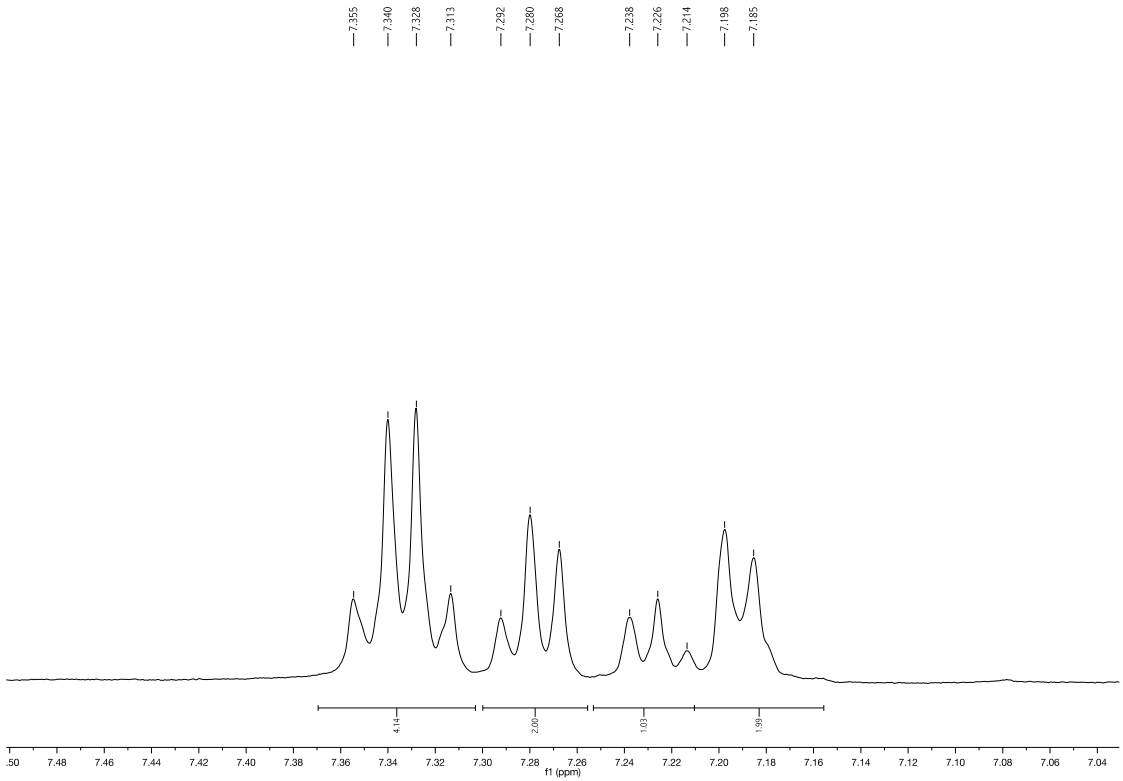


Figure S51. ^{1}H NMR (D_2O) spectrum of the aromatic region of peptide GDY(OSO_2F)DSMKEPCFR-NH_2 **(8)**.

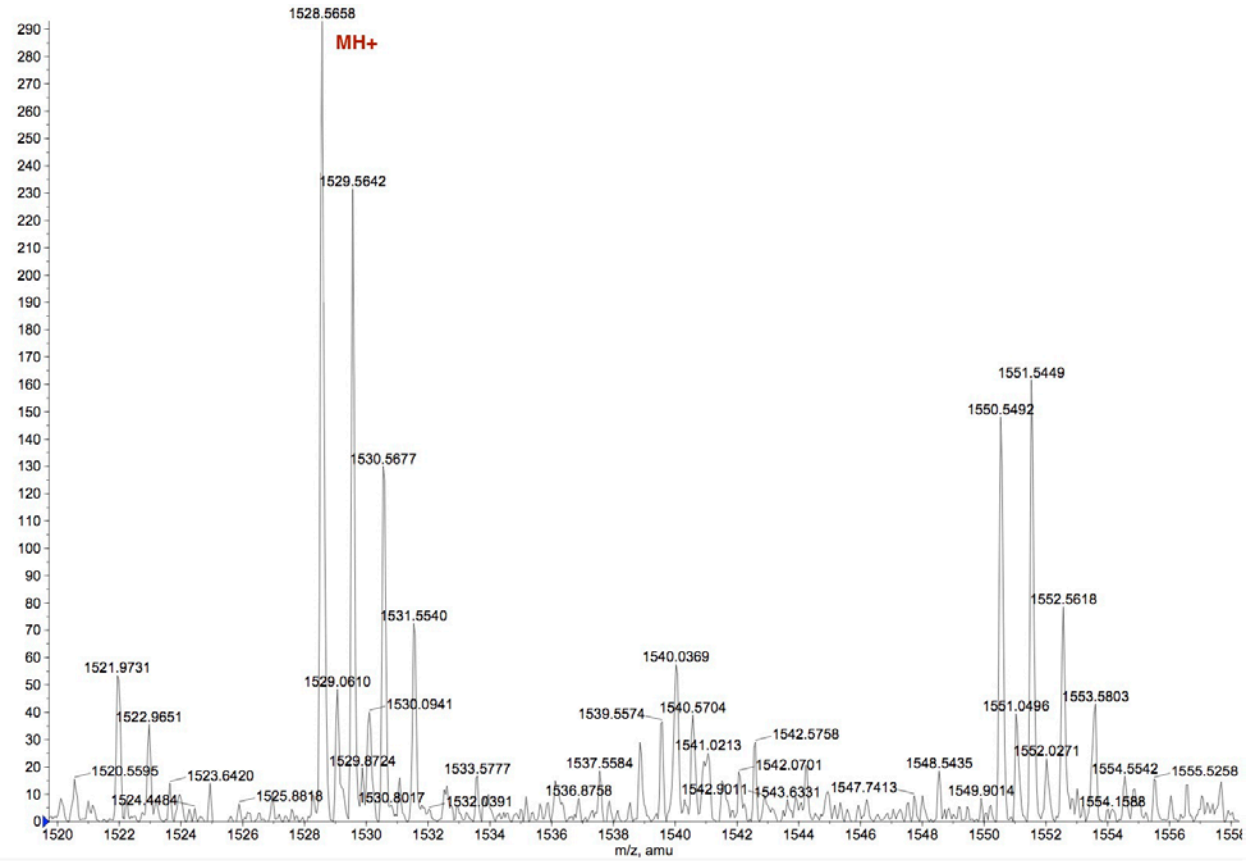


Figure S52. High resolution ESI-TOF spectrum for peptide GDY(OSO_2F)DSMKEPCFR-NH_2 **(8)**.

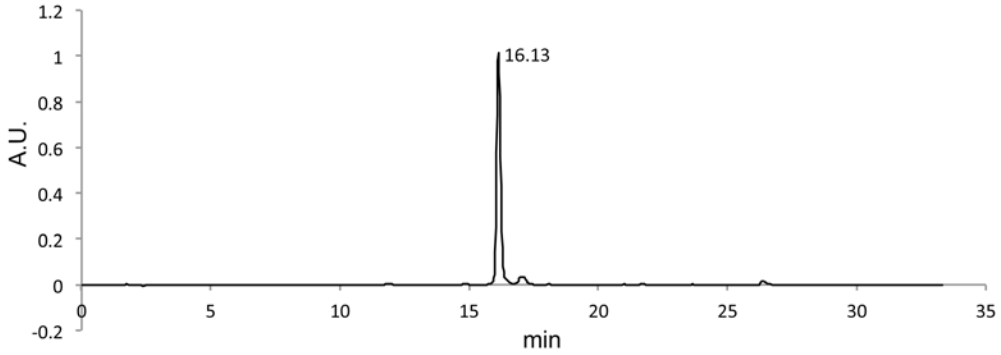


Figure S53. Analytical RP-HPLC (CH_3CN: 20 mM ammonium acetate buffer mobile phases, linear gradient from 0% to 80% CH_3CN over 40 min, λ = 220 nm, t_R = 16.1 min) chromatogram of purified peptide GD**sY**DSMKEPCFR-NH_2 **(9)**.

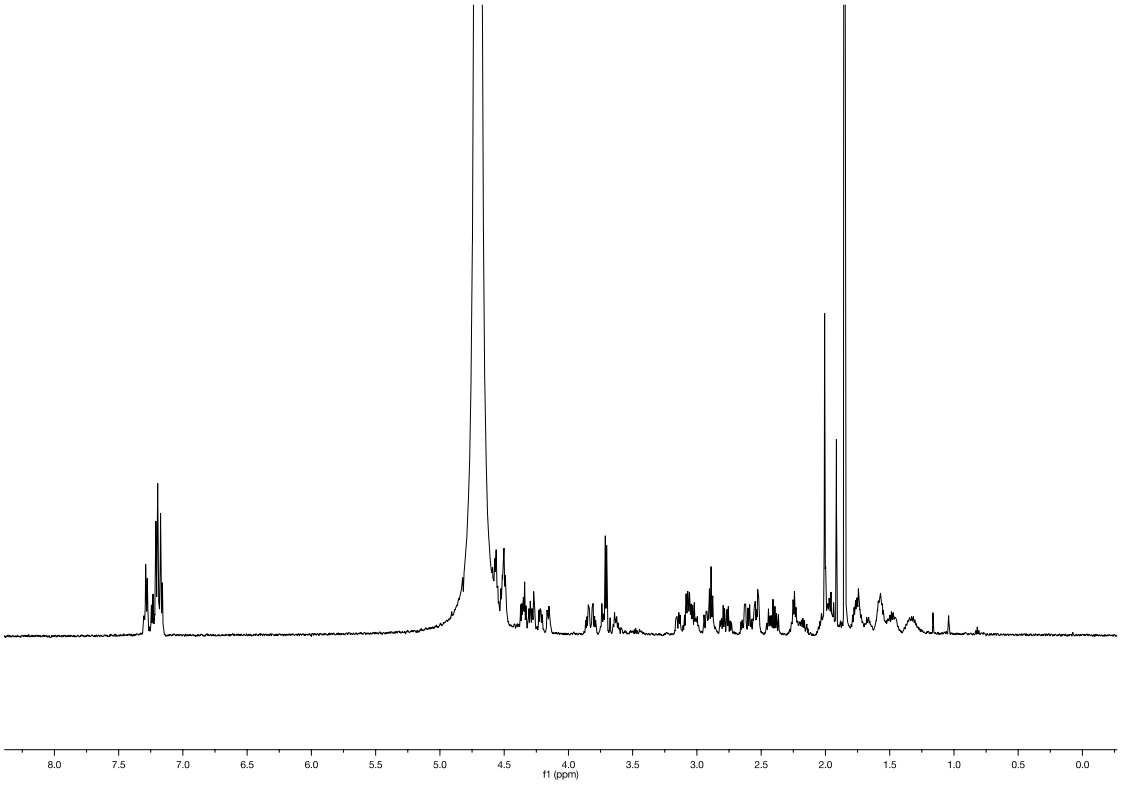


Figure S54. ^{1}H NMR (D_2O) spectrum of peptide GD**sY**DSMKEPCFR-NH_2 **(9)**.

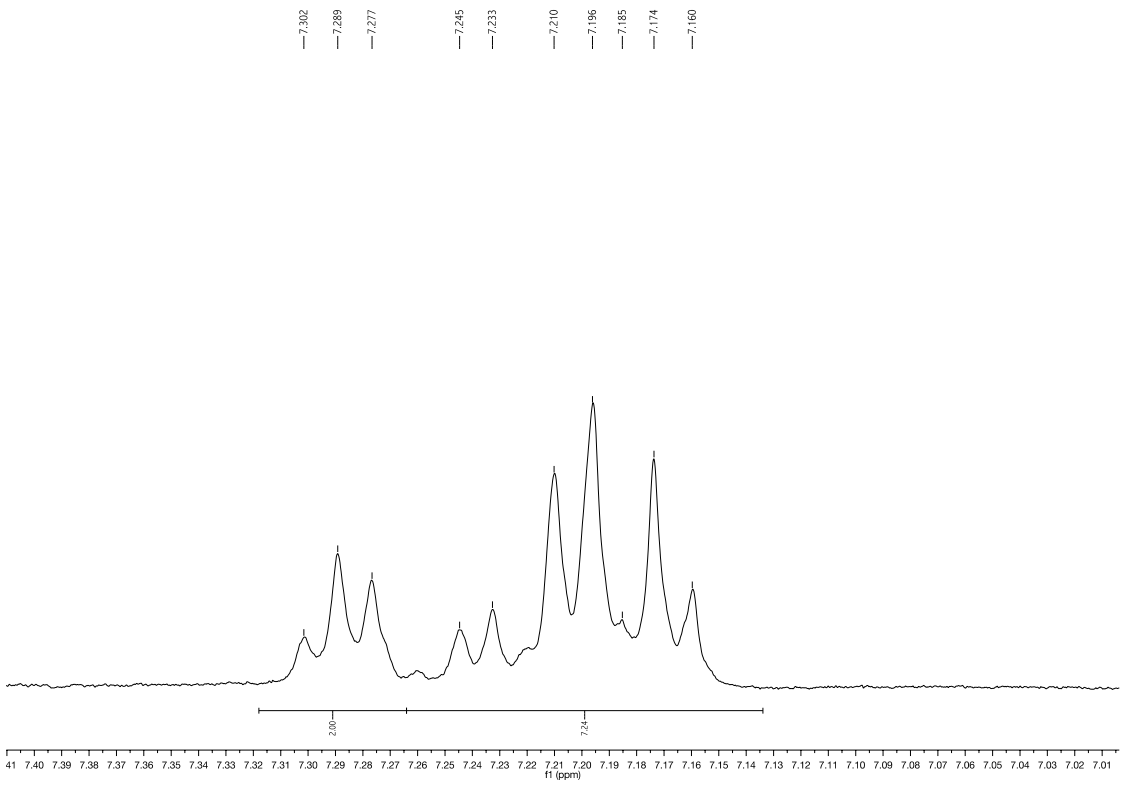


Figure S55. ^{1}H NMR (D_2O) spectrum of the aromatic region of peptide GD**sY**DSMKEPCFR-NH_2 **(9)**.

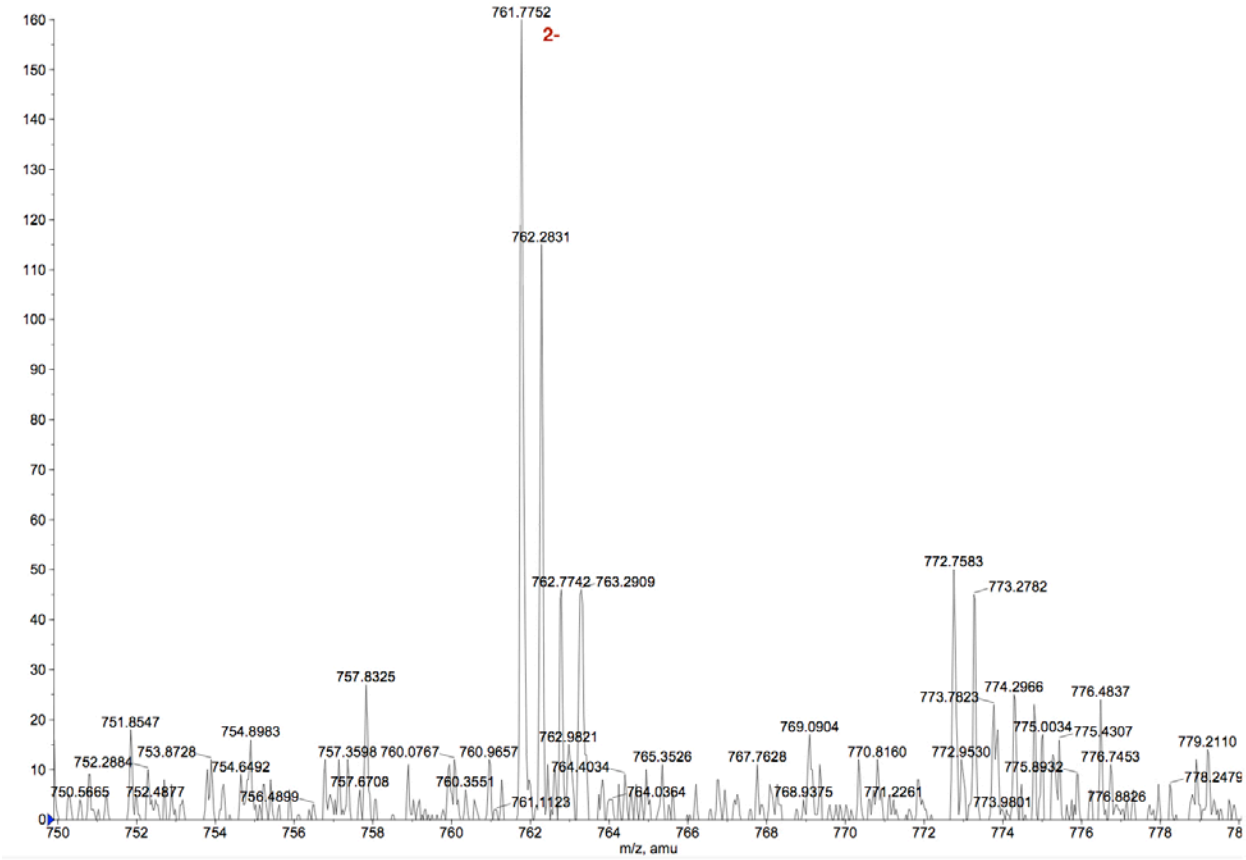


Figure S56. High resolution ESI-TOF spectrum for peptide GD**sY**DSMKEPCFR-NH_2 **(9)**.

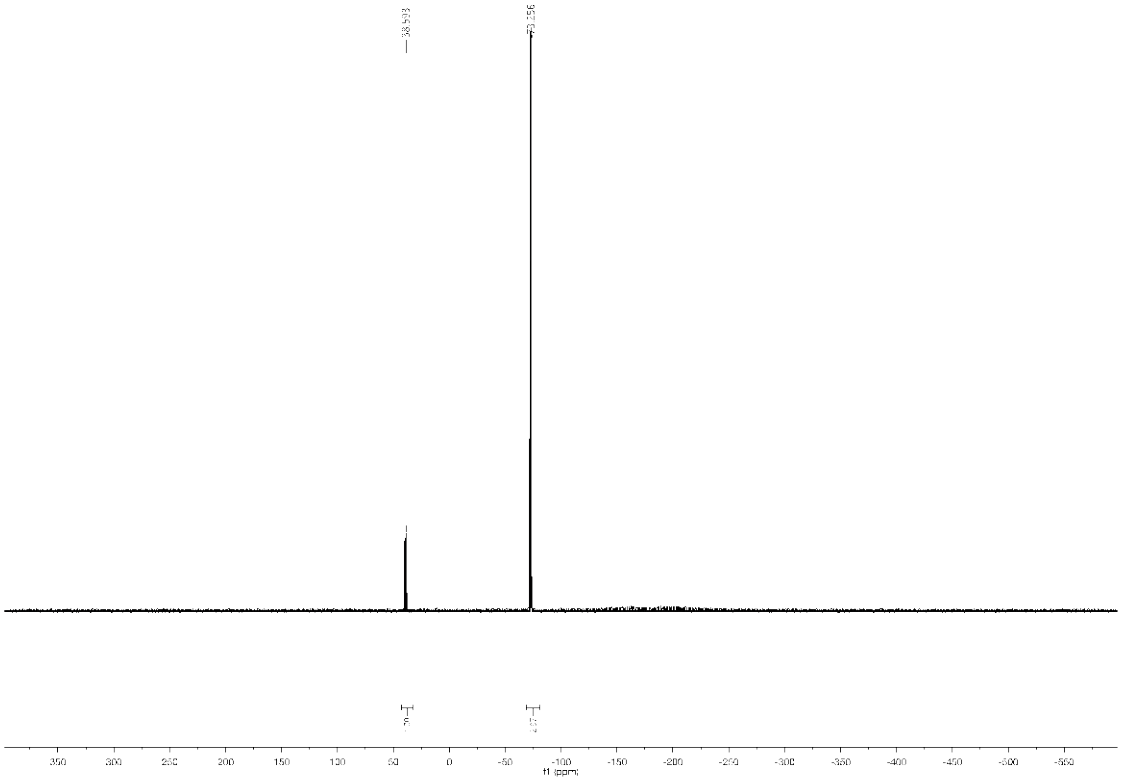


Figure S57. ^{19}F NMR (DMSO-d_6) spectrum of the aromatic region of peptide DADEY(OSO_2F)LNH_2 **(7)**.

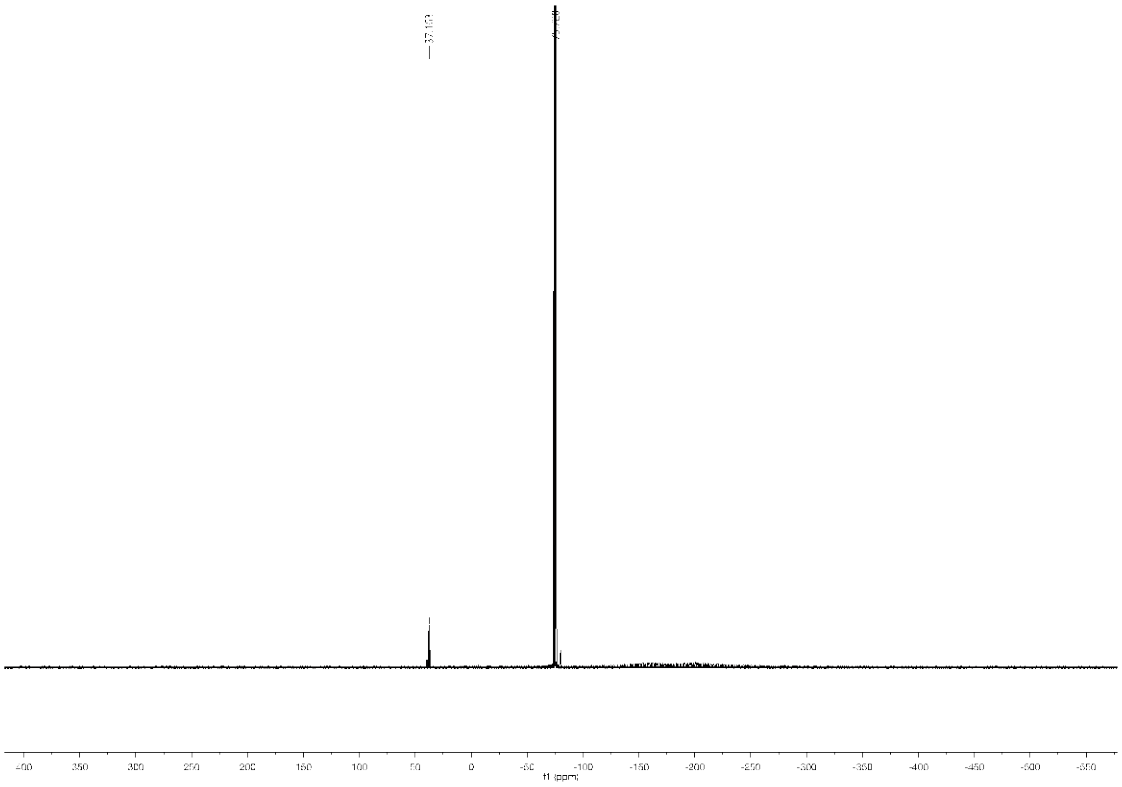


Figure S58. ^{19}F NMR (D_2O) spectrum of the aromatic region of peptide GDY(OSO_2F)DSMKEPCFR-NH_2 **(8)**.

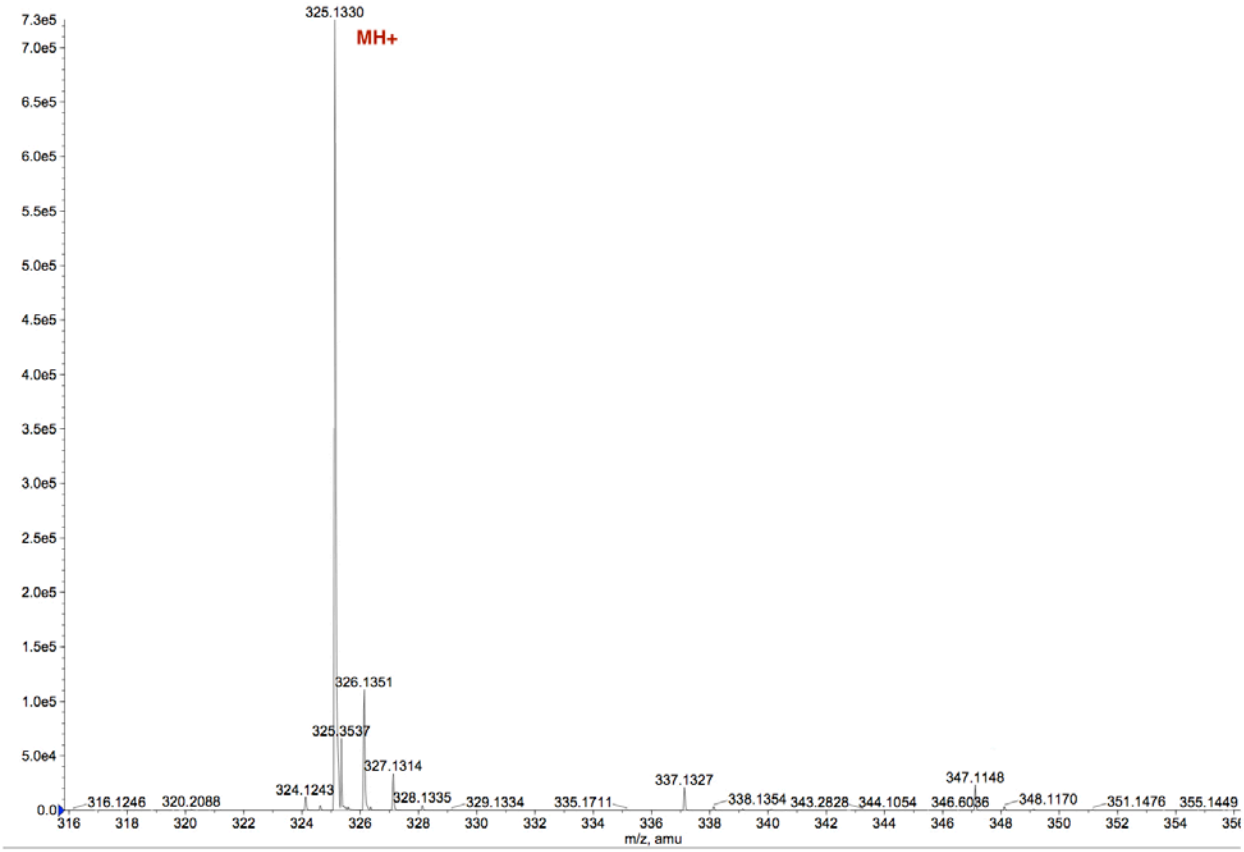


Figure S59. High resolution ESI-TOF spectrum for peptide Cys-Gly-Phe-NH_2 **(S5)**.

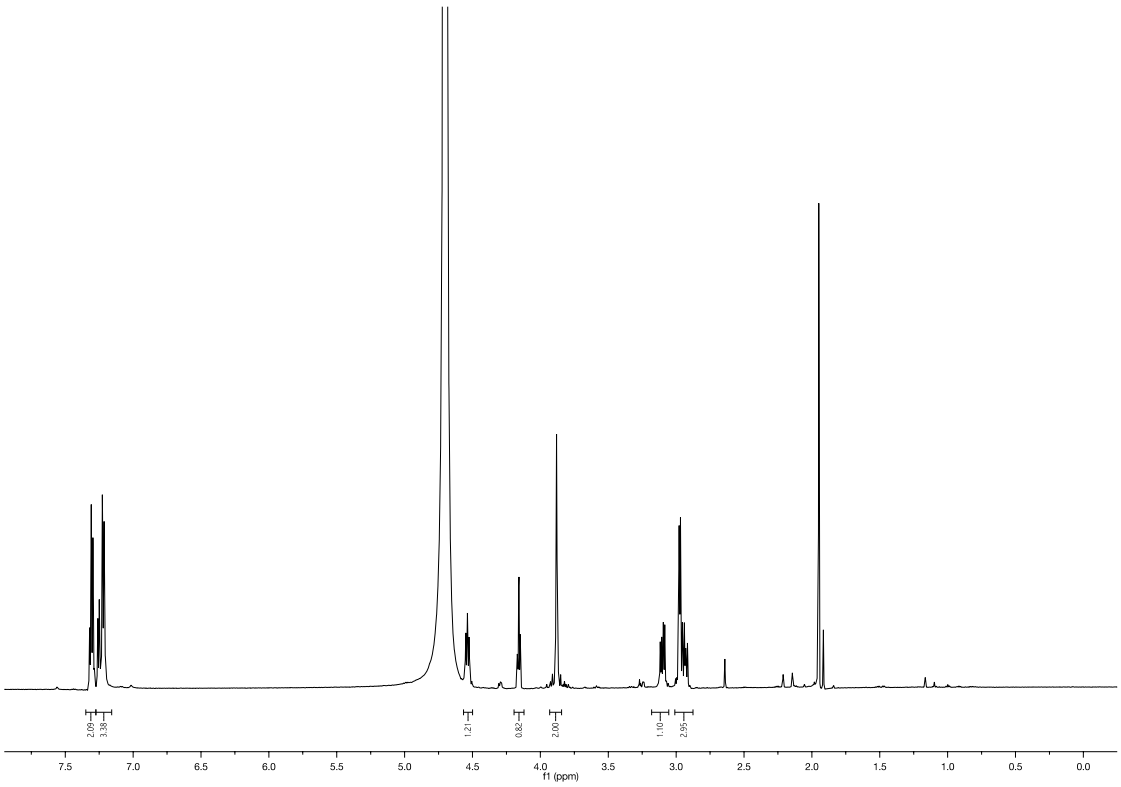


Figure S60. ^{1}H NMR (D_2O) spectrum of peptide Cys-Gly-Phe-NH_2 **(S5)**.

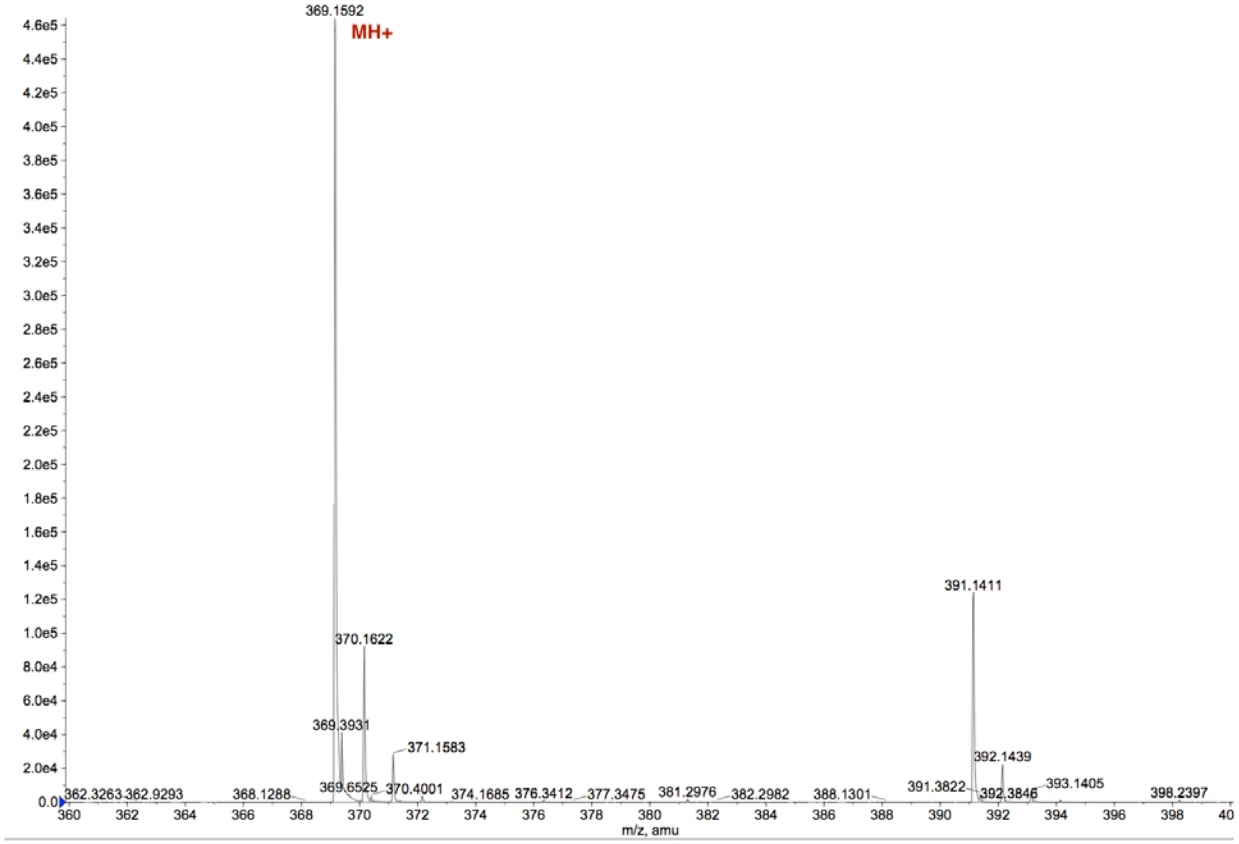


Figure S61. High resolution ESI-TOF spectrum for peptide Cys(S-CH2-CH2-OH)-Gly-Phe-NH_2 **(S6)**.

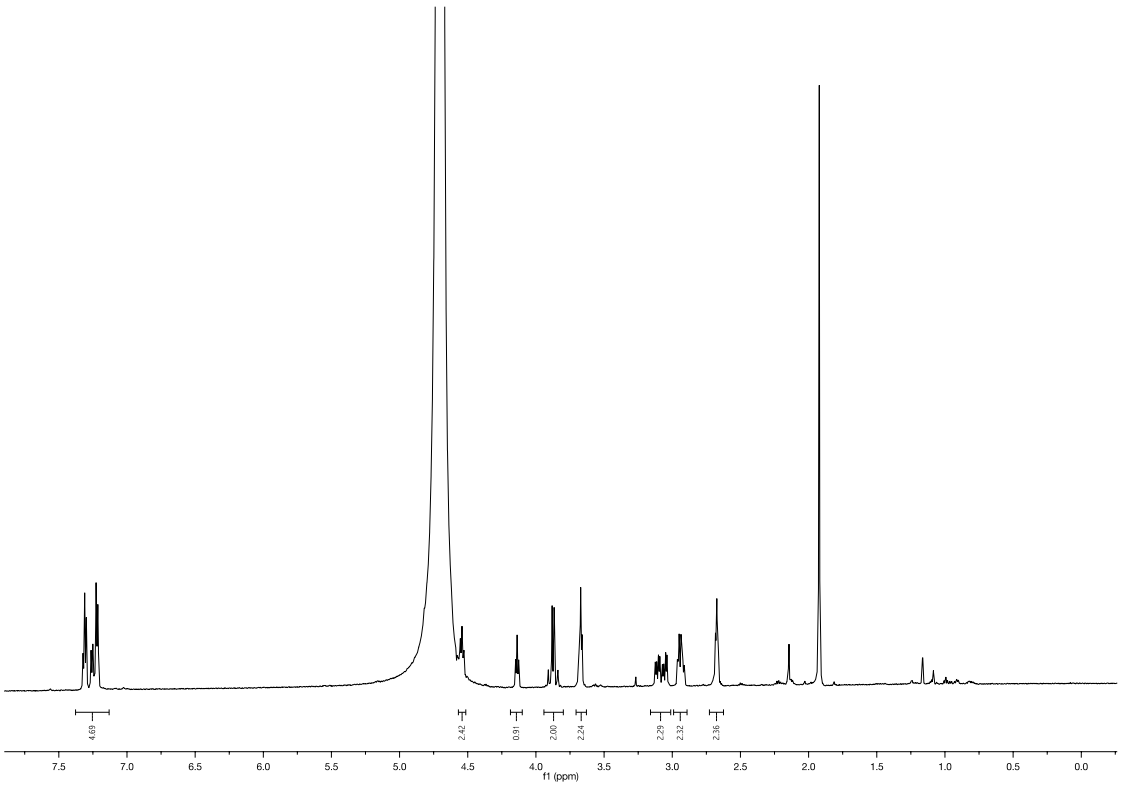


Figure S62. ^{1}H NMR (D_2O) spectrum of peptide Cys(S-CH2-CH2-OH)-Gly-Phe-NH_2 **(S6)**.

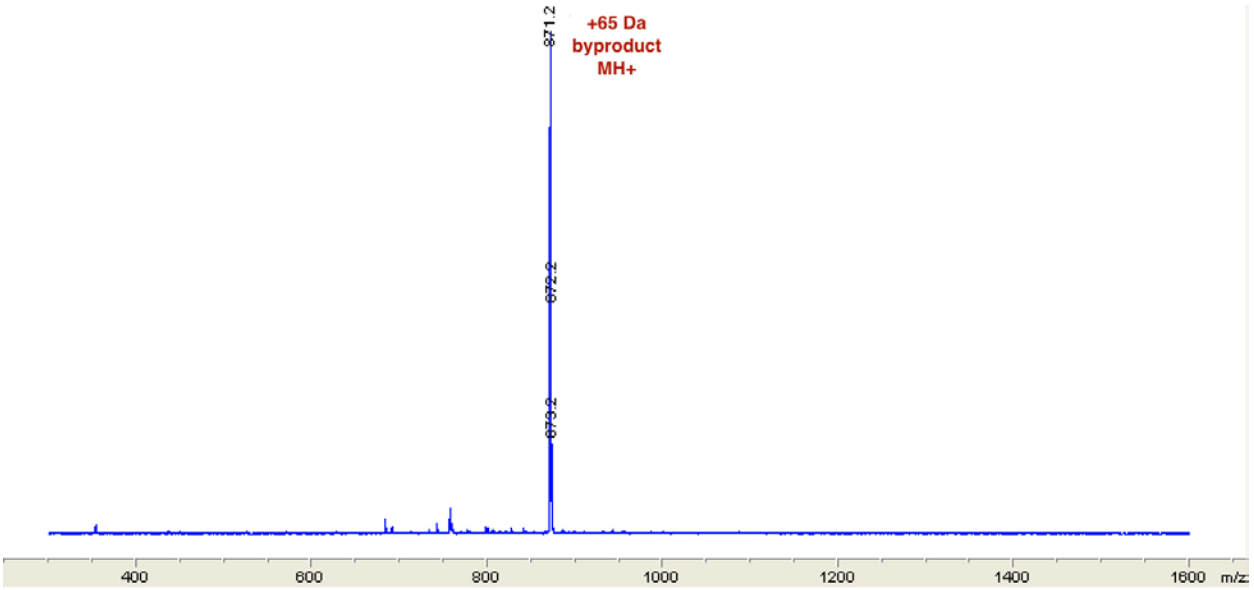


Figure S63. ESI-MS spectrum of the purified piperidine addition byproduct of peptide **7** reacting with piperidine.

Table S1: Peptide synthesis yield summary.

Peptide	Amino Acid Sequence	Recovered Crude Peptide: crude peptide (mg) 0.2 mmol resin loading	RP-HPLC purified peptide yield calculation: input crude peptide weight (mg)	Mw sY peptide + salt (g/mole)	output sY peptide weight (mg)	sY peptide yield
2	DADEsYL-NH_2	152.4	30.5	931	24.80	66.5%
3	YEsYLDYDF-NH_2	226.5	48.8	1334	30.80	53.6%
4	sYEsYLDsYDF-NH_2	236.4	47	1528	35.50	58.4%
5	TTPDsYGHsYDDKDTLDLNTPVDK-NH_2	510.2	87.9	2987	53.70	52.2%
6	DADSENSSFsYsYsYDsYLDEVAF-NH_2	508.3	49.2	2961	20.50	35.8%
9	GDsYDSMKEPCFR-NH_2	356.8	43.7	1714	14.50	34.5%

		crude peptide (mg) 0.2 mmol resin loading	input crude peptide weight (mg)	Mw Y(OSO_2F) peptide + salt (g/mole)	output Y(OSO_2F) peptide weight (mg)	Y(OSO_2F) peptide yield
7	DADEY(OSO_2F)L-NH_2	152.4	20.1	919	15.50	63.9%
8	GDY(OSO_2F)DSMKEPCFR-NH_2	356.8	44.7	1870	18.70	39.9%

Mw = molecular weight