Electronic Supplementary Material (ESI) for Chemical Communications

N-Terminal α-Amino Group Modification of Peptides by an Oxime Formation/Exchange Reaction Sequence

Karen Ka-Yan Kung, Kong-Fan Wong, King-Chi Leung and Man-Kin Wong*

State Key Laboratory of Chirosciences and Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Hong Kong, China

mankin.wong@polyu.edu.hk

Supporting Information

General Procedure

All reagents were commercially available and used without further purification. Milli-Q® water used as reaction solvent in peptide modification and LC-MS was deionised using a Milli-Q® Gradient A10 system (Millipore, Billerica, USA). Flash column chromatography was performed using silica gel 60 (230–400 mesh, ASTM) with n-hexane/EtOAc or CH_3OH/CH_2Cl_2 as eluent. ^1H and ^{13}C NMR spectra were recorded on Bruker DPX-400, DPX-600, Varian Unity Inova 400 NB and 500 NB spectrometers. The chemical shifts are expressed in ppm and coupling constants are given in Hz. Data for ^1H NMR are recorded as follows: chemical shift (δ, ppm), muplicity (s, singlet; br s, broad singlet; d, doublet; dd, double doublet; t, triplet; td, triplet of doublets; m, multiplet), coupling constant (Hz), integration. Data for ^{13}C NMR are reported in terms of chemical shift (δ, ppm). Low resolution mass spectra (MS) and high resolution mass spectra (HR-MS) were obtained on Waters Micromass Q-Tof 2™ with positive ESI in terms of mass to charge ratio (m/z).

ESI–MS Analysis of Peptide Modification

The mass spectrometer was performed over a m/z range of 100-2000 on Waters Micromass Q-Tof 2™ with positive ESI, and the raw spectra were deconvoluted by the MassLynx 4.1 Transform Program (Waters, Manchester, UK). Desolvation and source temperatures were 150 °C and 80 °C respectively. Operating conditions optimized for the detection of reaction mixture were the following: capillary voltage 3 kV, sample cone voltage 30 V, extraction voltage 4 V and collision cell voltage 10 eV.

LC–MS Analysis of Peptide Modification

Mass spectrometry analysis was performed using the ESI source of Q-Tof 2™ (Waters-Micromass, Manchester, UK) in the positive ion mode. The CapLC® system (Waters, Manchester, UK) was equipped with a Poroshell 300SB-C18 column (1.0 mm ID × 75 mm , 5μm) with ZORBAX

Poroshell guard column (1.0 mm ID × 17 mm, 5 µm) (Agilent-Technologies Inc., Wilmington, USA). Mobile-phase A was made of 0.5% formic acid in Milli-Q® water. Mobile-phase B was made of 0.5% formic acid in CH_3CN. 2 µl of sample was injected with a flow rate of 40 µl/min at 25 ^oC. The initial conditions for separation were 3% B for 3 min, followed by a linear gradient to 70% B by 30 min, 3% B by 31 min then to 3% B by 45 min. The mass spectrometer was performed over a m/z range of 200–2000, and the raw spectra were deconvoluted by the MassLynx 4.1 Transform Program (Waters, Manchester, UK). Desolvation and source temperatures were 150 °C and 80 °C respectively. Operating conditions optimized for the detection of reaction mixture were the following: capillary voltage 3 kV, sample cone voltage 30 V, extraction voltage 4 V and collision cell voltage 10 eV.

MALDI-TOF-MS Analysis of Peptide Modification for PEG-modified Peptide 6b

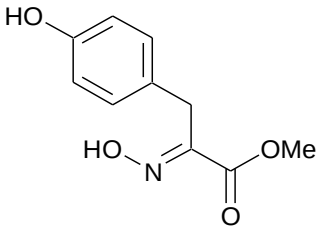
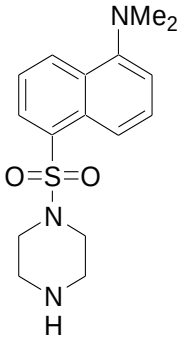
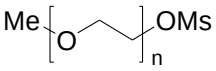
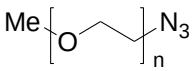
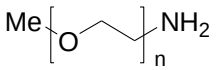
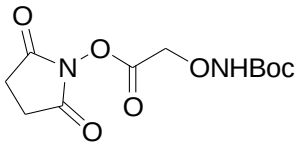
An aliquot of 10 µl of sample was mixed with 10 µl of 10 mg/mL CHCA matrix in CH_3CN/H_2O (1:1) with 0.1% TFA. An aliquot of 1 µl of the sample-matrix mixture was spotted onto the stainless steel target plate and air-dried. The target plate was then mounted onto a MALDI Micro-MX Time-of-Flight mass spectrometer (Waters, Milford, MA) for analysis. The laser of the MALDI source was a 337 nm pulse laser (Model 337Si-63, Spectra Physics, Mountain View, CA) operating at a pulse frequency of 10 Hz. The mass spectrometer was operated in positive and reflectron mode. The flight tube and reflectron voltage of the TOF mass analyzer were set at +12 kV and −5.2 kV, respectively. The extraction voltage and extraction delay were set at 2 kV and 500 ns respectively. The mass spectrometry analysis was performed over a m/z range of 500–2000.

Calculation of Peptide Conversion

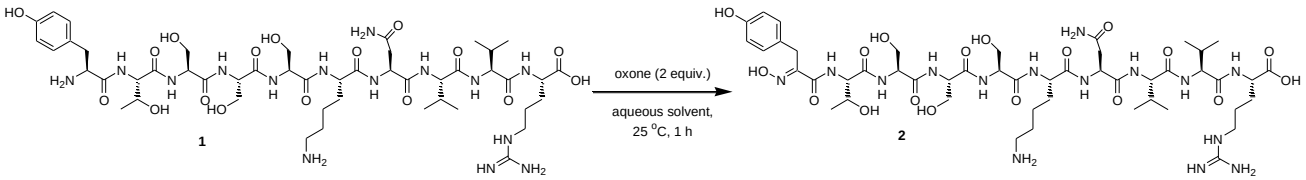
The crude reaction mixture of unmodified peptides (peptide) and modified peptides (product) was subjected to LC-MS or ESI-MS analysis. After data processing by MassLynx 4.1 Transform Program, peptide conversion at different time intervals was determined by measuring the relative peak intensities of peptide and product in the mass spectrum as follows:

$$Peptide\ Conversion\ (\%) = \left(1 - \frac{Relative\ Intensity\ of\ Peptide}{Relative\ Intensities\ of\ Peptide\ and\ Product}\right) \times 100\%$$

Literature References

 **4**	N. Kotoku, H. Tsujita, A. Hiramatsu, C. Mori, N. Koizumi and M. Kobayashi, *Tetrahedron*, 2005, **61**, 7211–7218.
 *N*-Dansylpiperazine	V. Sashuk, D. Schoeps, and H. Plenio, *Chem. Commun.*, 2009, **7**, 770–772.
 **5bb**	(a) B. C. Mei, K. Susumu, I. L. Medintz and H. Mattoussi, *Nat. Protoc.*, 2009, **4**, 412–423; (b) K. Susumu, B. C. Mei and H. Mattoussi, *Nat. Protoc.*, 2009, **4**, 424–436.
 **5bc**	(a) B. C. Mei, K. Susumu, I. L. Medintz and H. Mattoussi, *Nat. Protoc.*, 2009, **4**, 412–423; (b) K. Susumu, B. C. Mei and H. Mattoussi, *Nat. Protoc.*, 2009, **4**, 424–436.
 **5bd**	(a) B. C. Mei, K. Susumu, I. L. Medintz and H. Mattoussi, *Nat. Protoc.*, 2009, **4**, 412–423; (b) K. Susumu, B. C. Mei and H. Mattoussi, *Nat. Protoc.*, 2009, **4**, 424–436.
 **7**	S. Deroo, E. Defrancq, C. Moucheron, A. Kirsch-De Mesmaeker and P. Dumy, *Tetrahedron Lett.*, 2003, **44**, 8379–8382.

Procedure for Oxidative *N*-Terminal Modification of Peptide 1



A mixture of peptide **1**, YTSSSKNVVR, (10 μL of 1 mM in H_2O), oxone (2 μL of 10 mM in H_2O, 2 equiv.), and $NaHCO_3$ (5 μL of 30 mM in H_2O, 1.5 mM) in H_2O (83 μL) was stirred at 25 °C for 1 h. The resulting mixture (10 μL) was diluted with 90 μL H_2O and 1% formic acid in CH_3CN (100 μL). The diluted mixture was analyzed by MS analysis for determination of the peptide conversion. Site-specific modified peptide **2** was determined by ESI-MS/MS analysis (Figure S1).

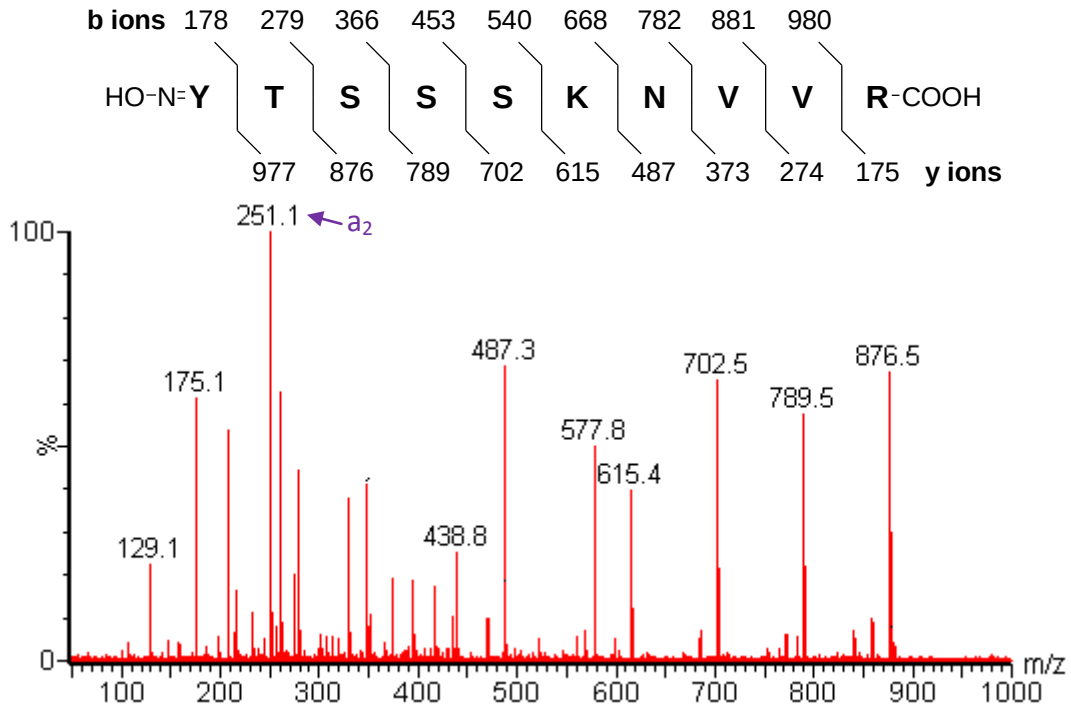


Figure S1. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide **2** (ESI source, doubly charged ion of m/z = 577.8).

Time Courses for Oxidative *N*-Terminal Modification of Peptide 1

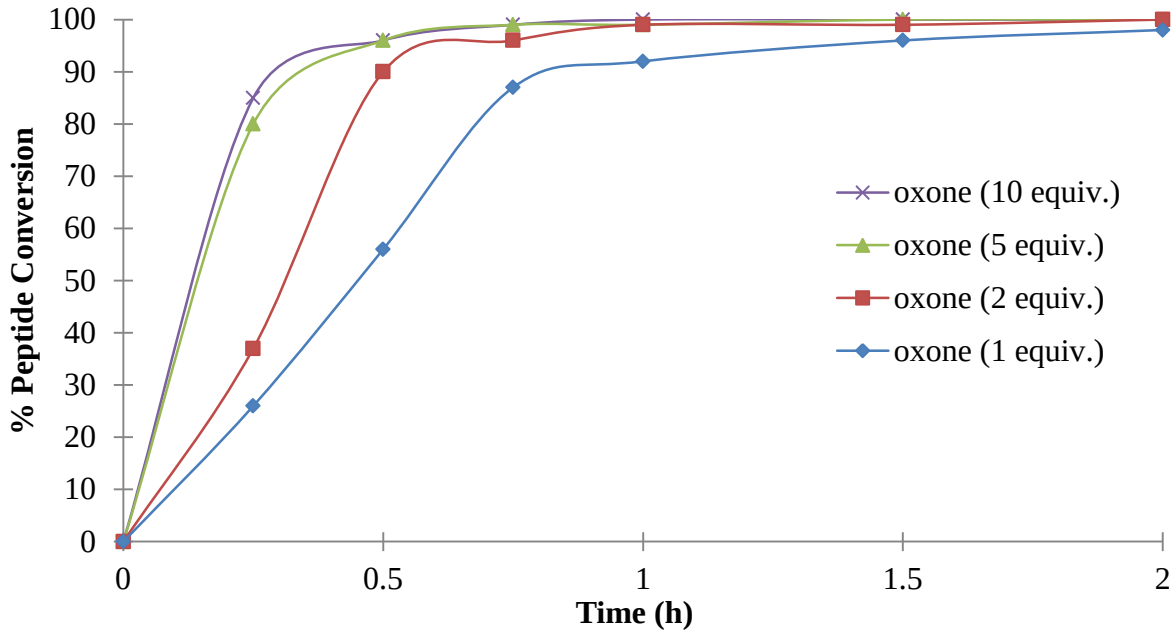


Figure S2. Time course of *N*-terminal α-amino group modification of peptide **1** (100 μM) with various concentrations of oxone in $NaHCO_3$ solution (1.5 mM, pH 8.3) at 25 ^{o}C.

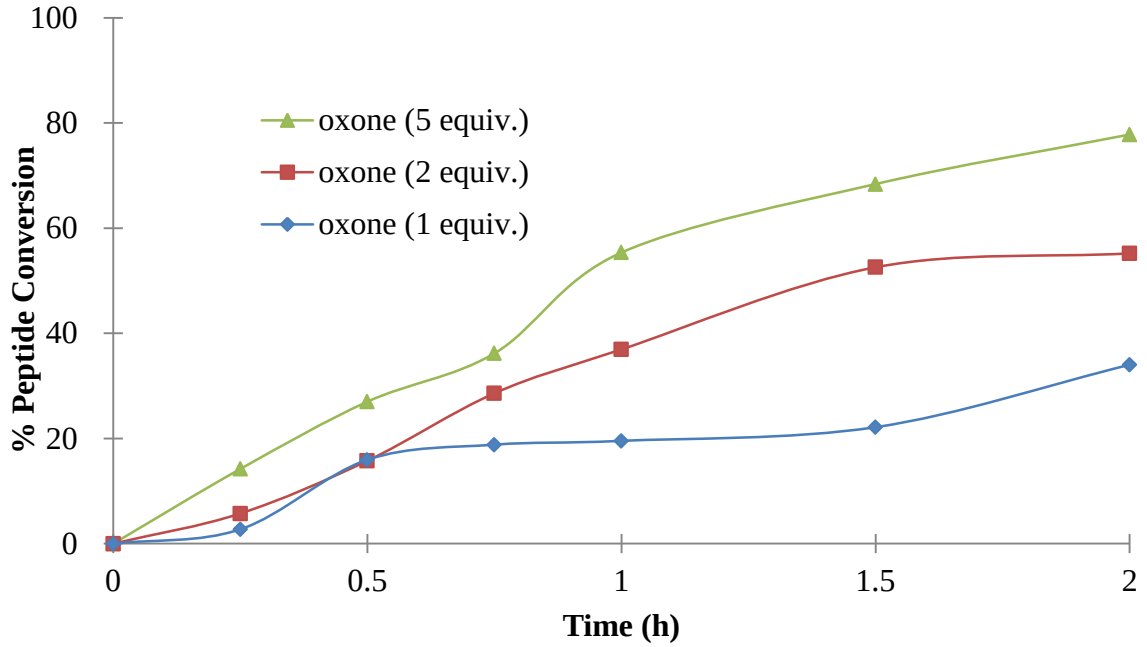
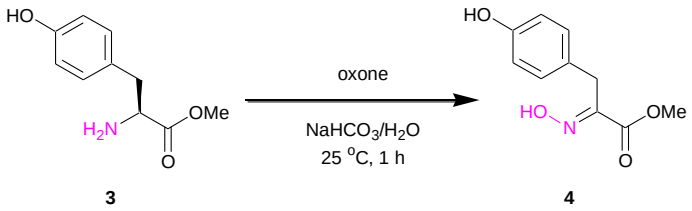


Figure S3. Time course of *N*-terminal α-amino group modification of peptide **1** (100 μM) with various concentrations of oxone in $NaHCO_3$ solution (1.5 mM, pH 8.3) at 4 ^{o}C.

Model Study of *N*-Terminal Modification of L-Tyrosine Methyl Ester 3



3 **4**

To a round-bottom flask containing L-tyrosine methyl ester **3** (20 mg, 0.1 mmol) and $NaHCO_3$ (130 mg, 1.5 mmol) in H_2O (10 mL) was added oxone (124 mg, 0.2 mmol). After stirring at 25 ^oC for 1 h, the reaction mixture was extracted with CH_2Cl_2 (15 mL × 3). The combined layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (30–40% EtOAc/n-hexane) to provide a product **4** in 91% conversion and 67% isolated yield as a white solid; 67% yield, white powder; analytical TLC (silica gel 60) (80% EtOAc/n-hexane), R_f = 0.69; ^1H NMR (400 MHz, CD_3OD) δ 7.07 (d, *J* = 8.5 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 2H), 3.82 (s, 2H), 3.76 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 164.81, 155.78, 151.11, 129.79, 127.29, 115.00, 51.50, 29.08; ESI-MS *m/z* 210 $[M+H]^+$.

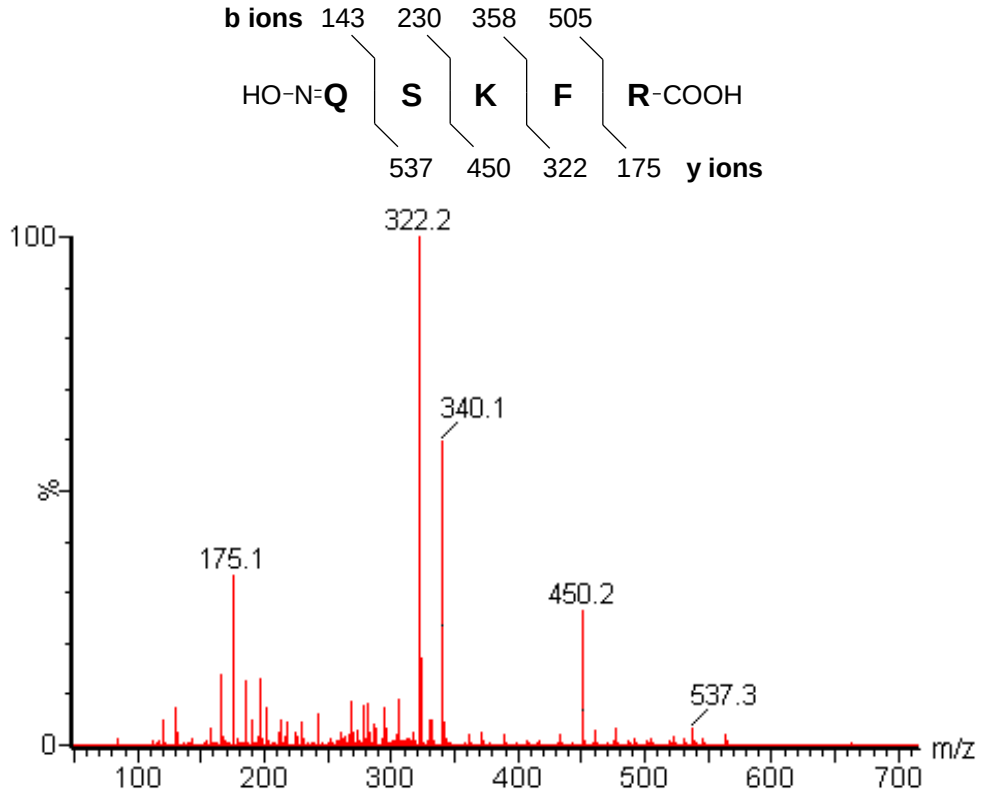


Figure S4. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide QSKFR (Table 2, entry 1) (ESI source, doubly charged ion of m/z = 340.1).

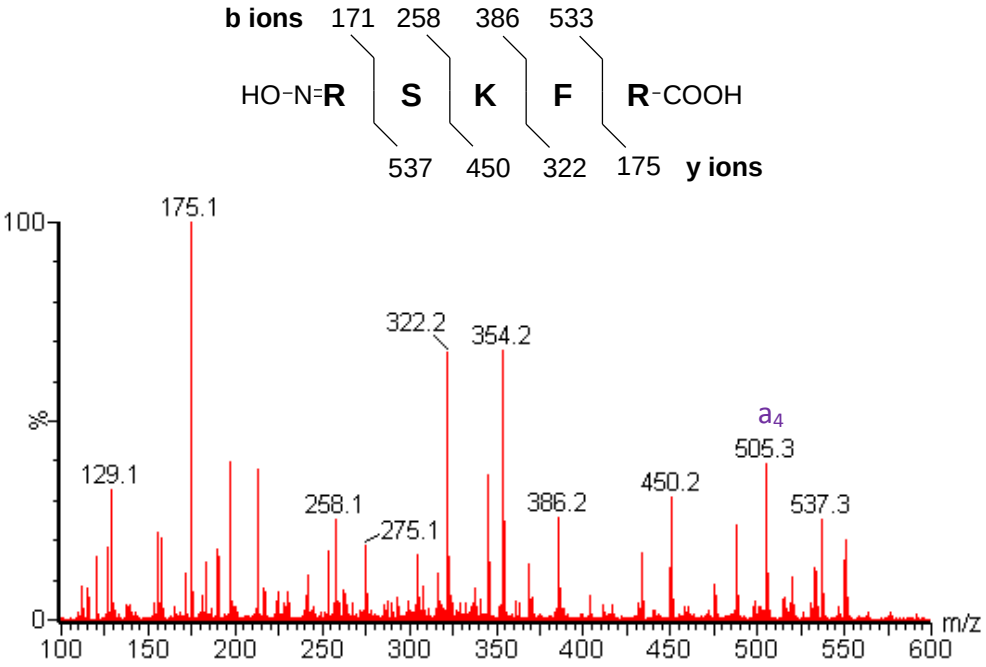


Figure S5. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide RSKFR (Table 2, entry 2) (ESI source, doubly charged ion of m/z = 354.2).

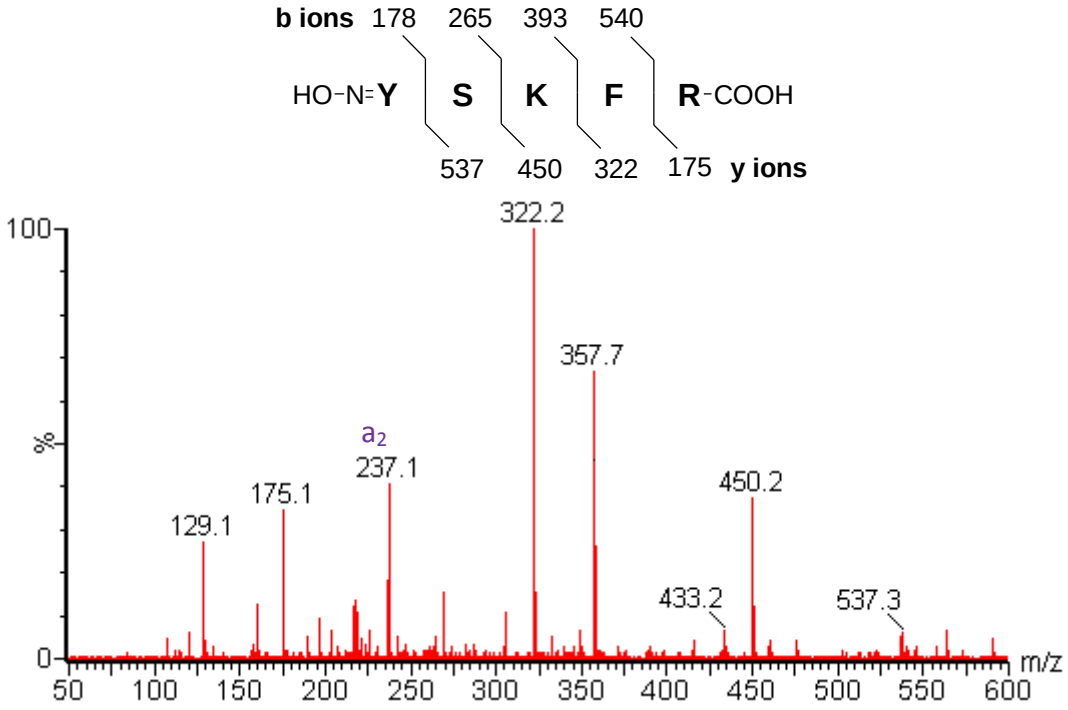


Figure S6. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide YSKFR (Table 2, entry 3) (ESI source, doubly charged ion of m/z = 357.7).

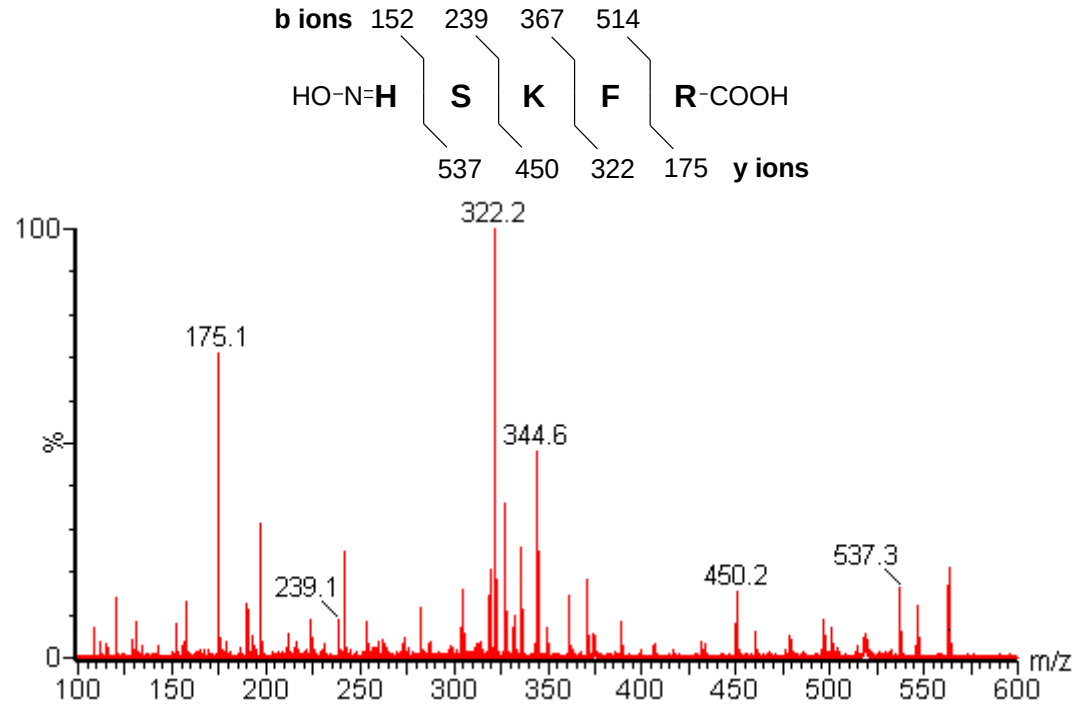


Figure S7. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide HSKFR (Table 2, entry 4) (ESI source, doubly charged ion of m/z = 344.6).

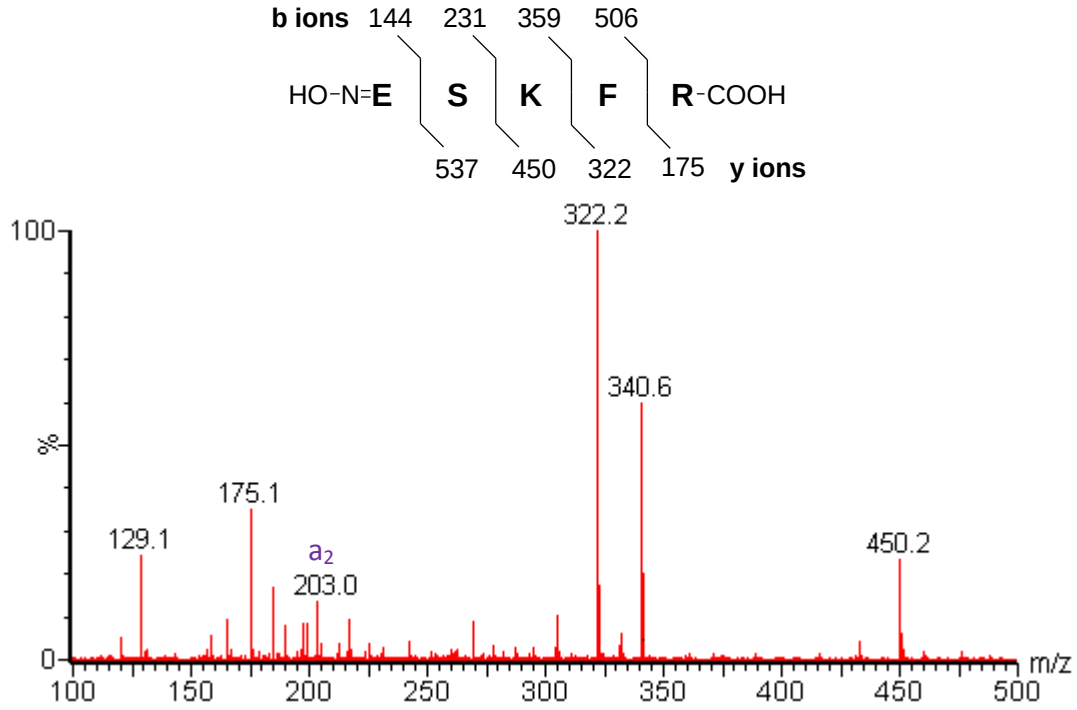


Figure S8. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide ESKFR (Table 2, entry 5) (ESI source, doubly charged ion of m/z = 340.6).

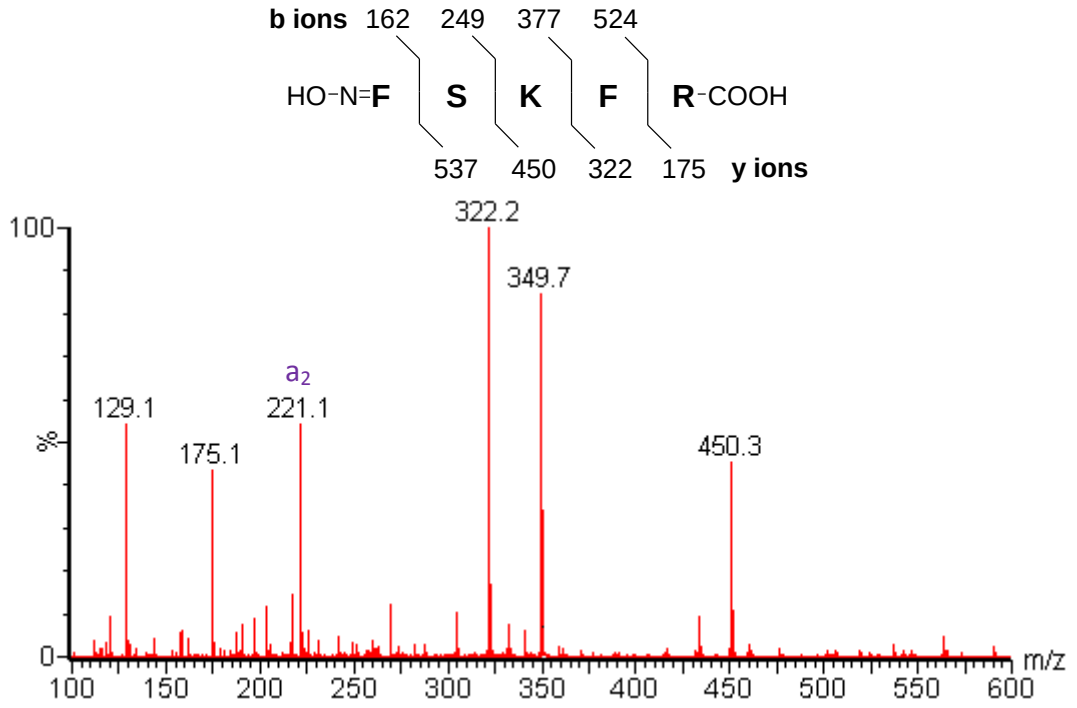


Figure S9. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide FSKFR (Table 2, entry 6) (ESI source, doubly charged ion of m/z = 349.7).

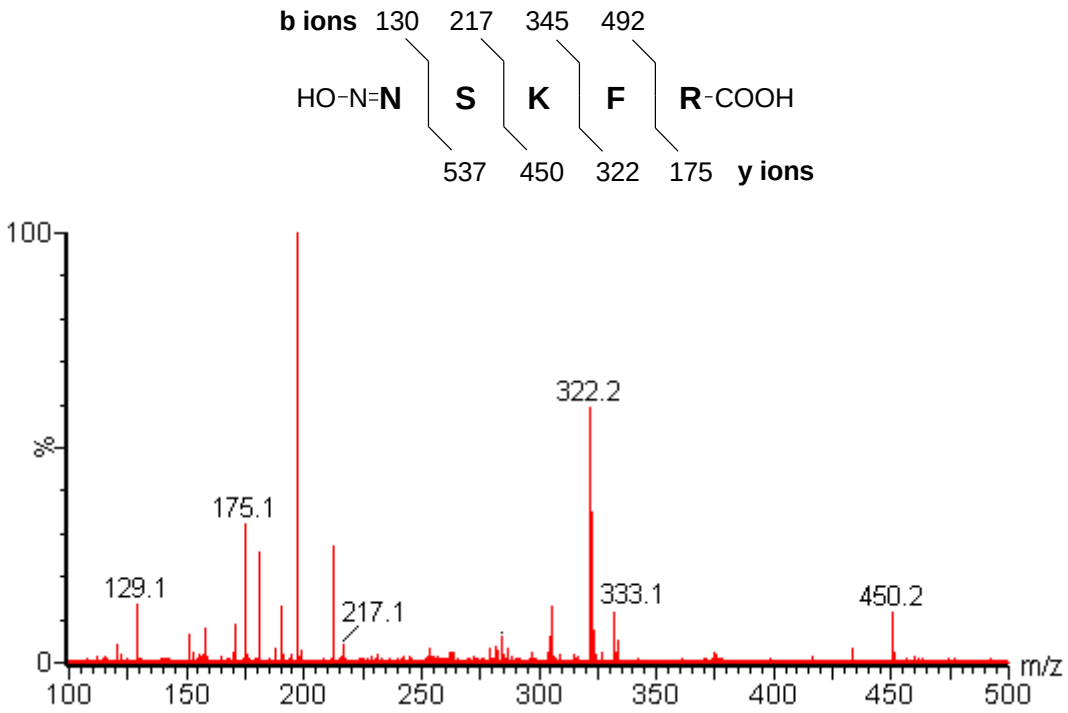


Figure S10. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide NSKFR (Table 2, entry 7) (ESI source, doubly charged ion of m/z = 333.1).

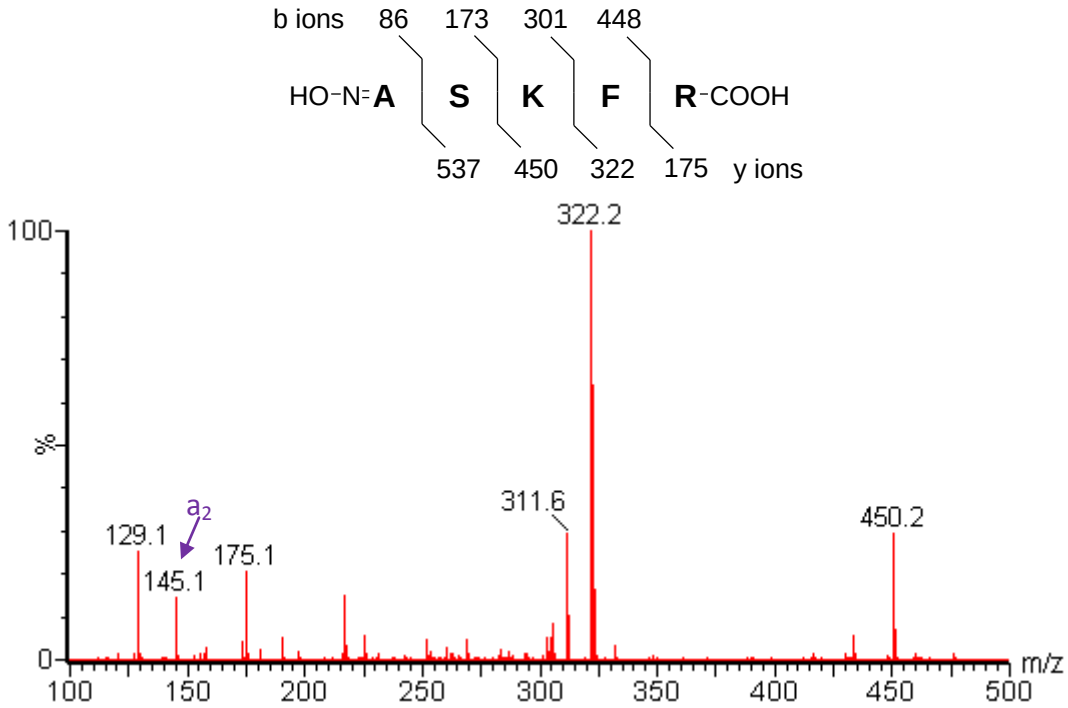


Figure S11. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide ASKFR (Table 2, entry 8) (ESI source, doubly charged ion of m/z = 311.6).

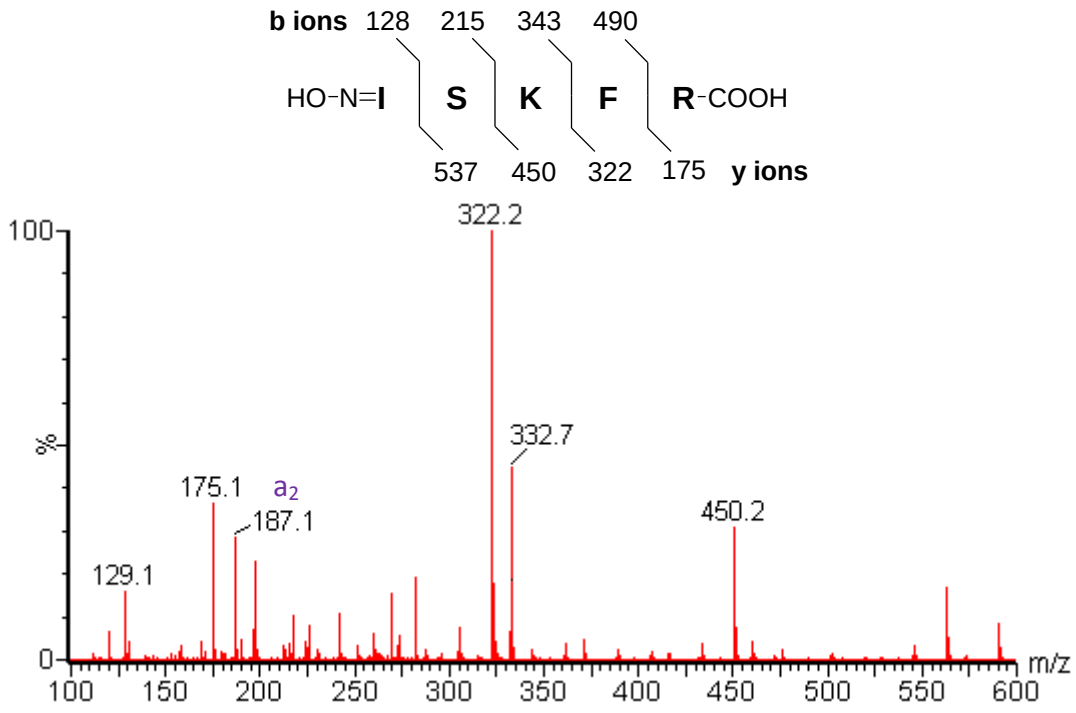


Figure S12. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide ISKFR (Table 2, entry 9) (ESI source, doubly charged ion of m/z = 332.7).

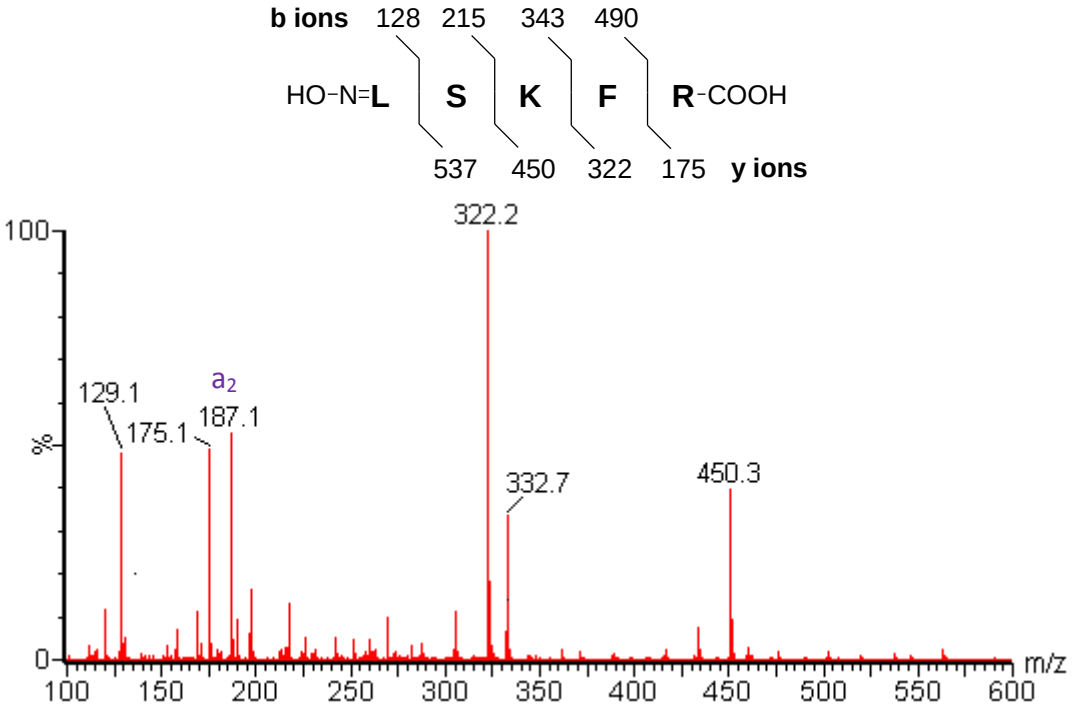


Figure S13. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide LSKFR (Table 2, entry 10) (ESI source, doubly charged ion of m/z = 332.7).

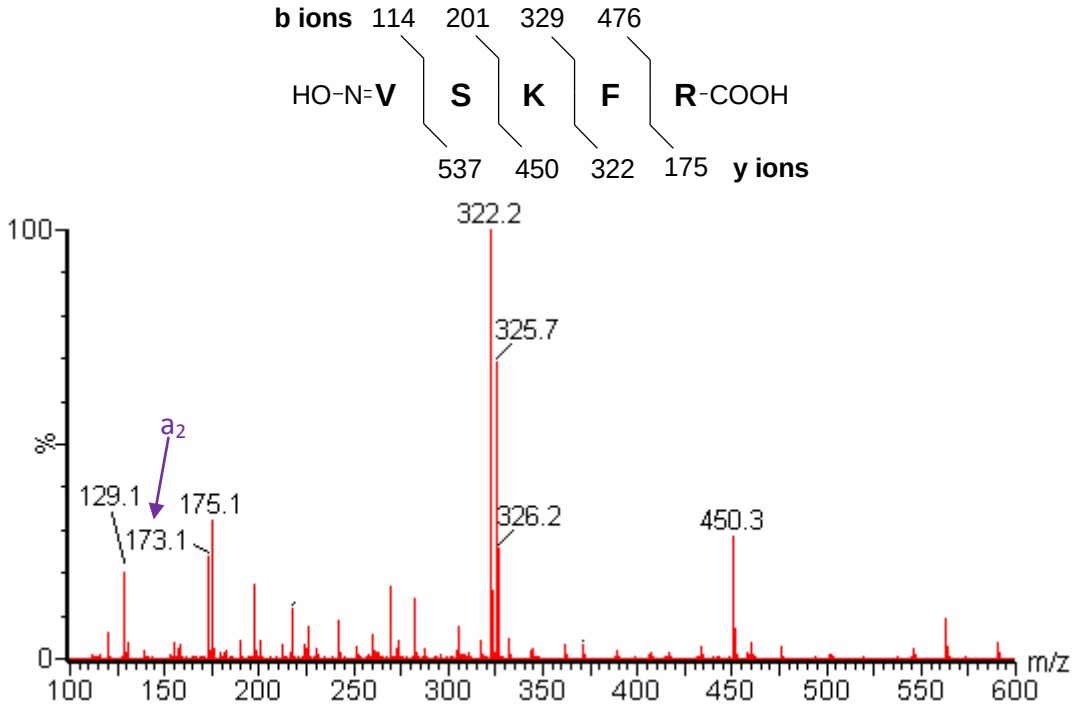


Figure S14. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide VSKFR (Table 2, entry 11) (ESI source, doubly charged ion of m/z = 325.7).

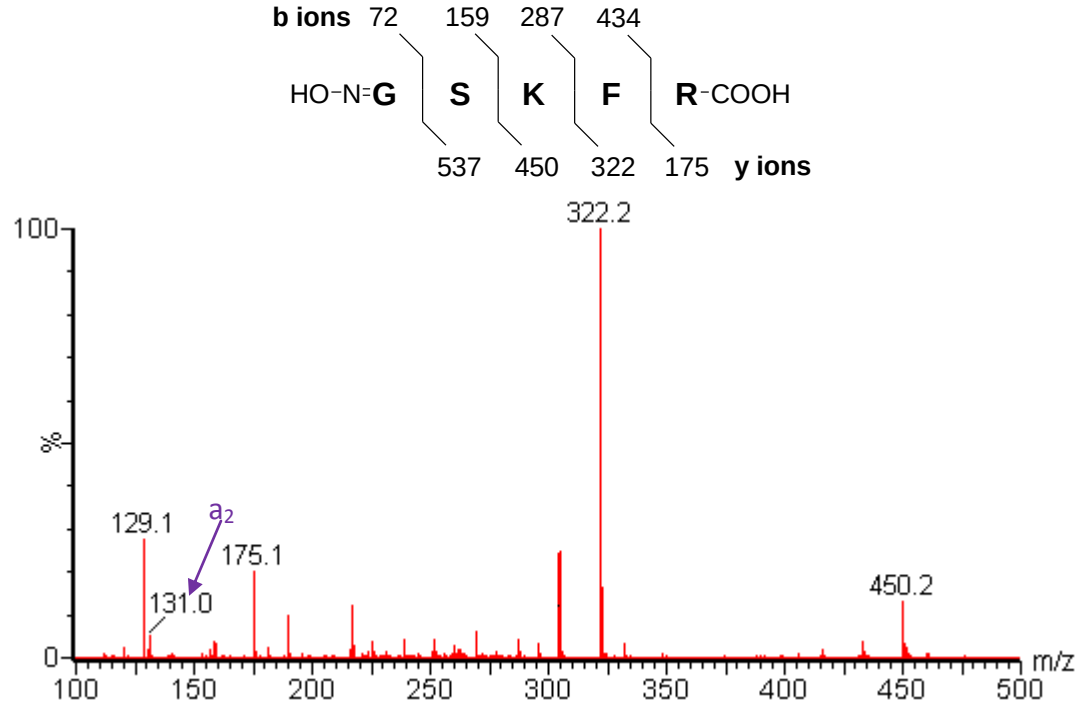


Figure S15. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide GSKFR (Table 2, entry 12) (ESI source, doubly charged ion of m/z = 304.6).

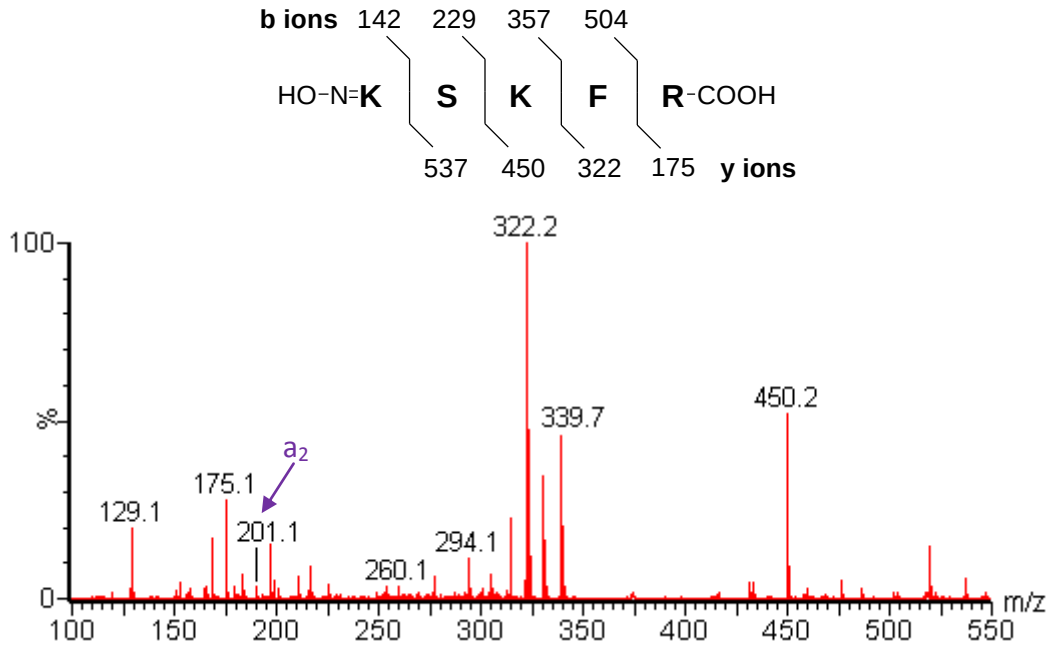


Figure S16. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide KSKFR (Table 2, entry 13) (ESI source, doubly charged ion of m/z = 339.7).

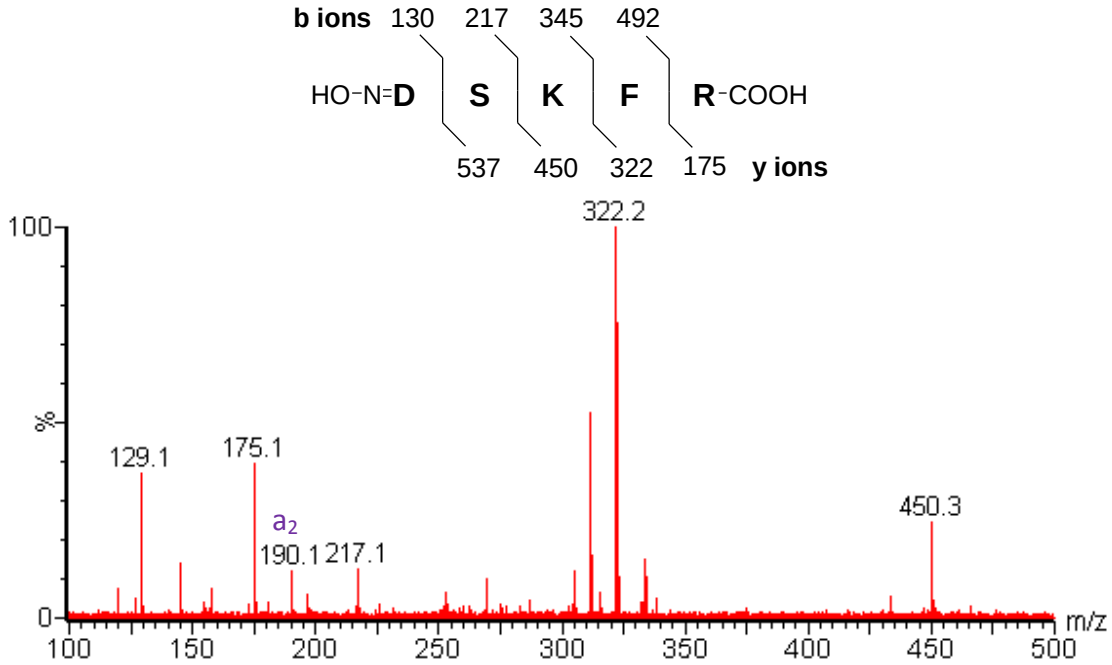


Figure S17. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide DSKFR (Table 2, entry 14) (ESI source, doubly charged ion of m/z = 333.6).

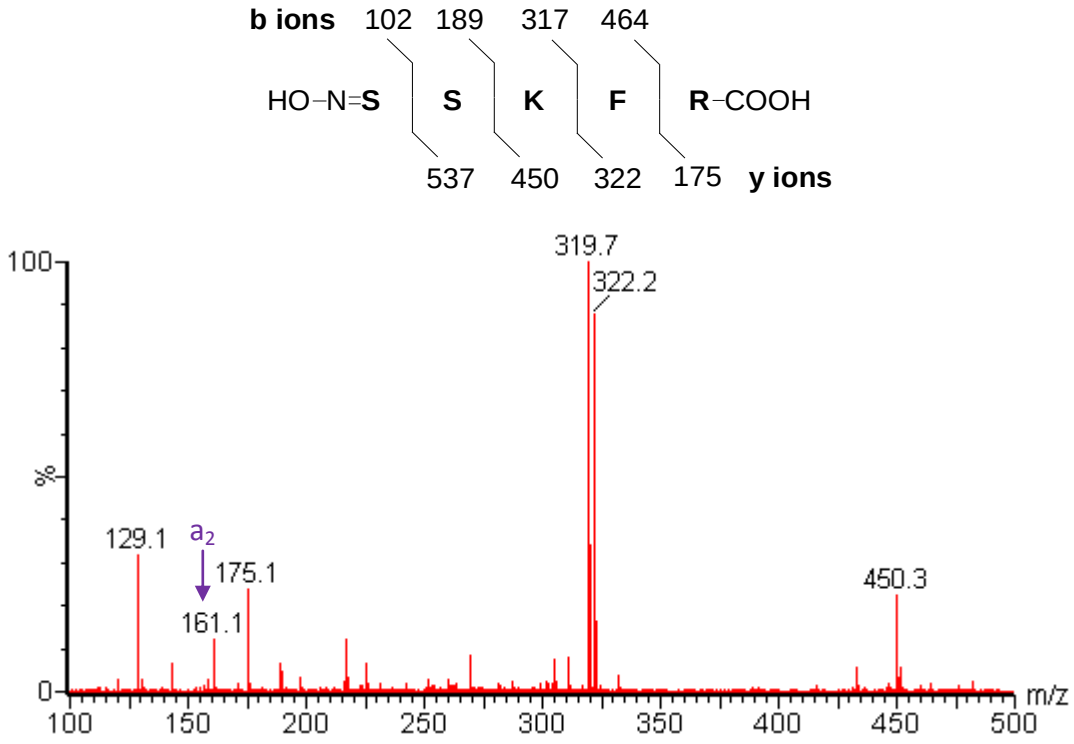


Figure S18. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide SSKFR (Table 2, entry 15) (ESI source, doubly charged ion of m/z = 319.7).

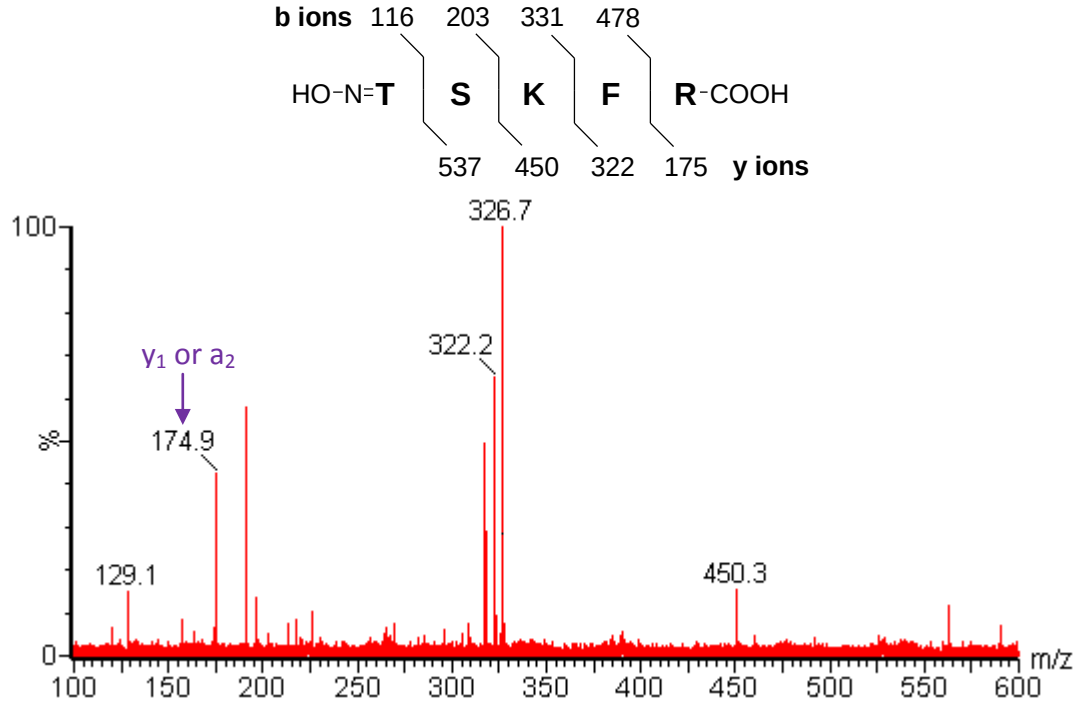


Figure S19. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide TSKFR (Table 2, entry 16) (ESI source, doubly charged ion of m/z = 326.7).

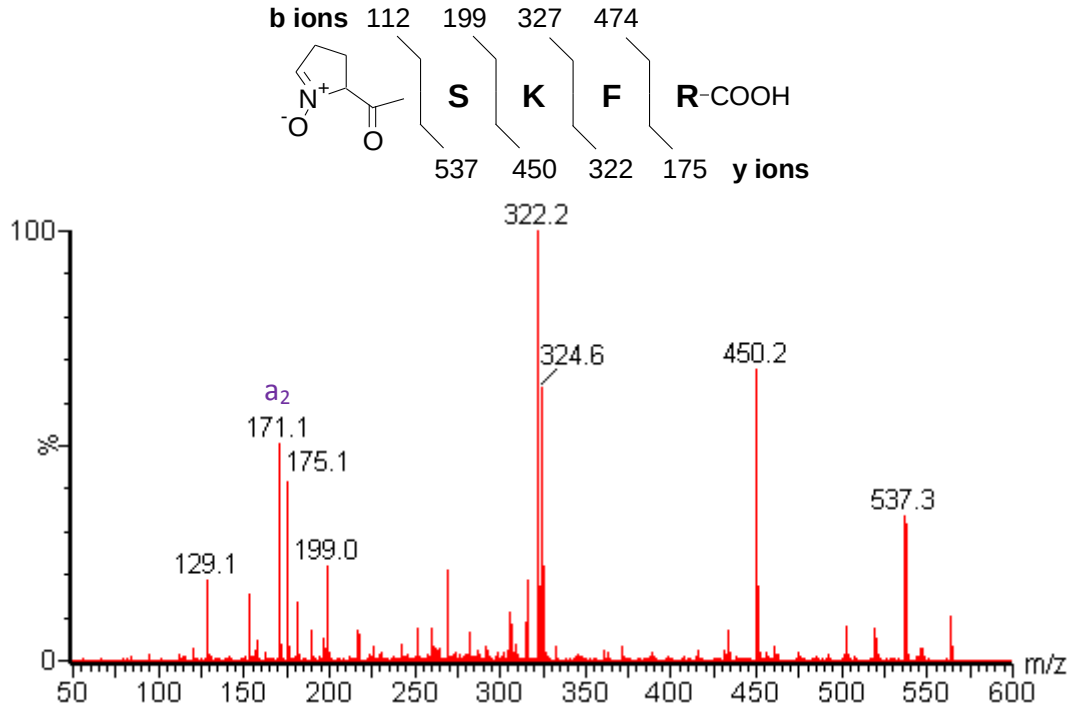


Figure S20. Q-TOF MS/MS spectrum of *N*-terminal modified peptide PSKFR (Table 2, entry 17) (ESI source, doubly charged ion of m/z = 324.6).

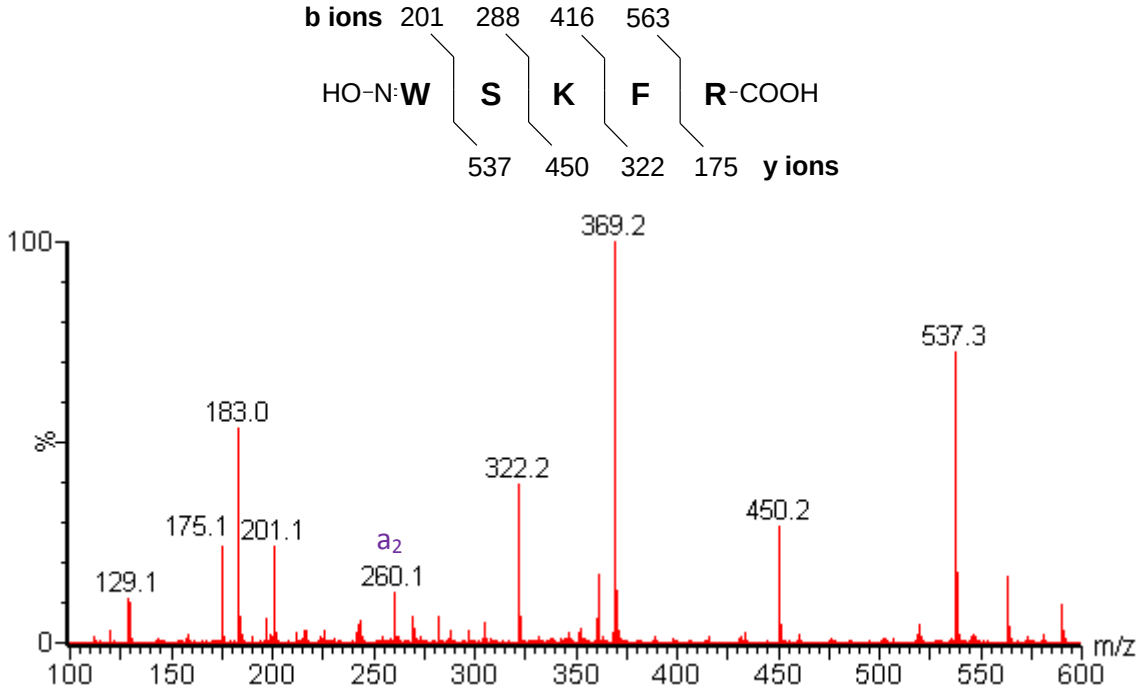


Figure S21. Q-TOF MS/MS spectrum of *N*-terminal oxime-modified peptide WSKFR (Table 2, entries 18–20) (ESI source, doubly charged ion of m/z = 369.2).

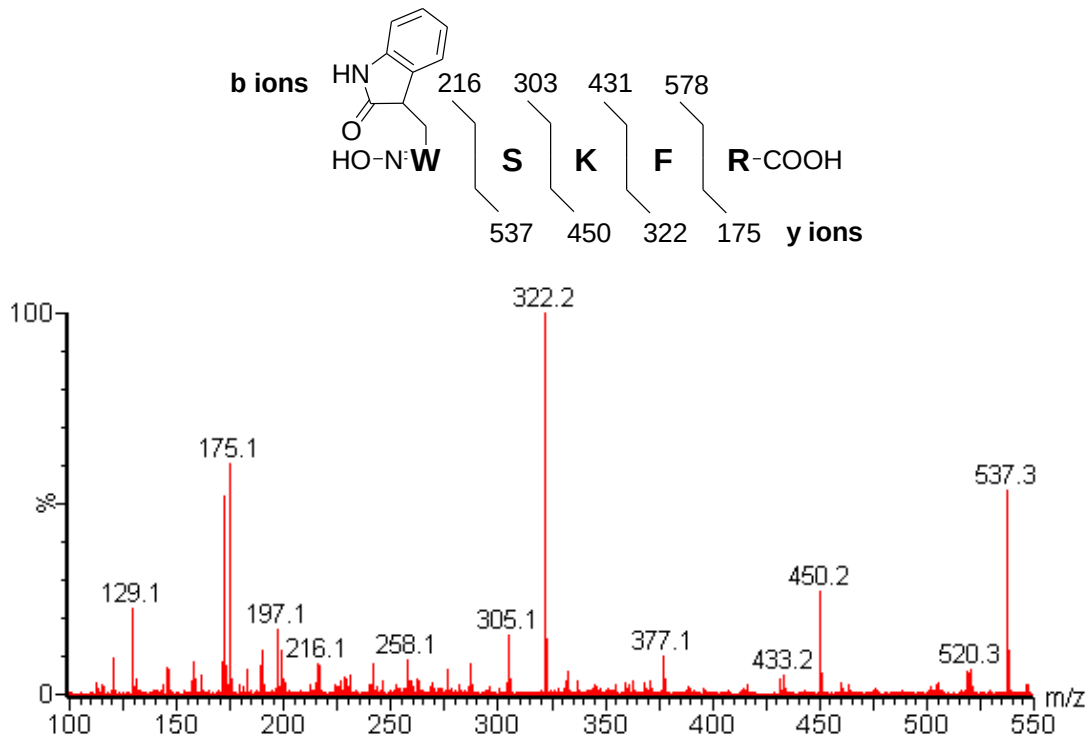


Figure S22. Q-TOF MS/MS spectrum of *N*-terminal modified peptide WSKFR (Table 2, entries 18–20) (ESI source, doubly charged ion of m/z = 377.1).

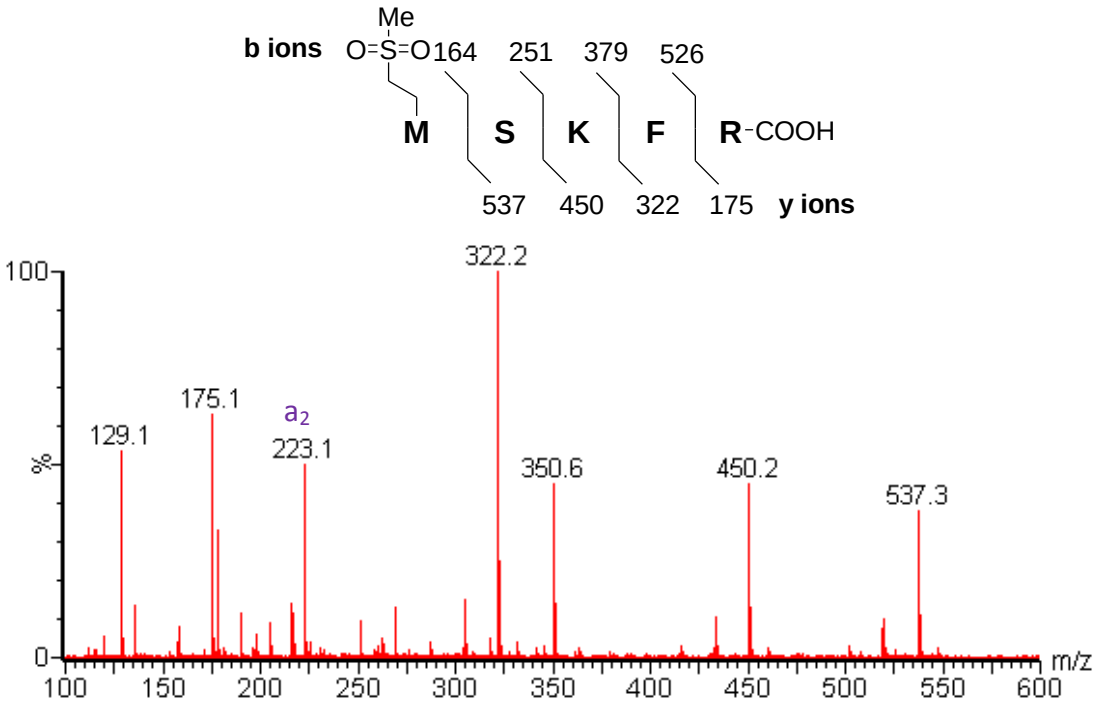


Figure S23. Q-TOF MS/MS spectrum of *N*-terminal modified peptide MSKFR (Table 2, entry 21) (ESI source, doubly charged ion of m/z = 350.6).

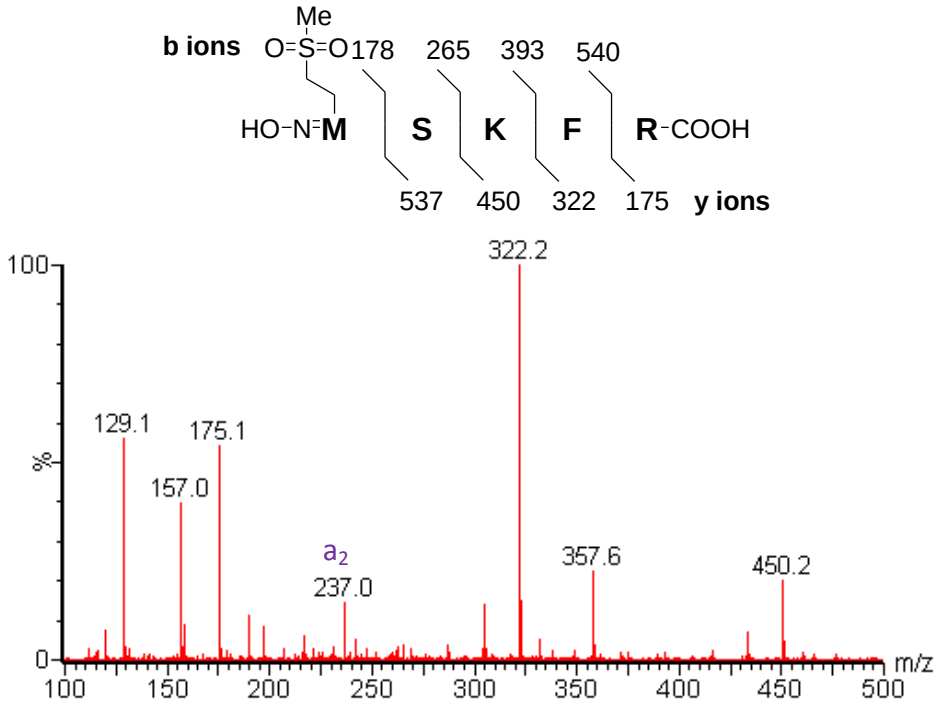


Figure S24. Q-TOF MS/MS spectrum of *N*-terminal modified peptide MSKFR (Table 2, entry 21) (ESI source, doubly charged ion of m/z = 357.6).

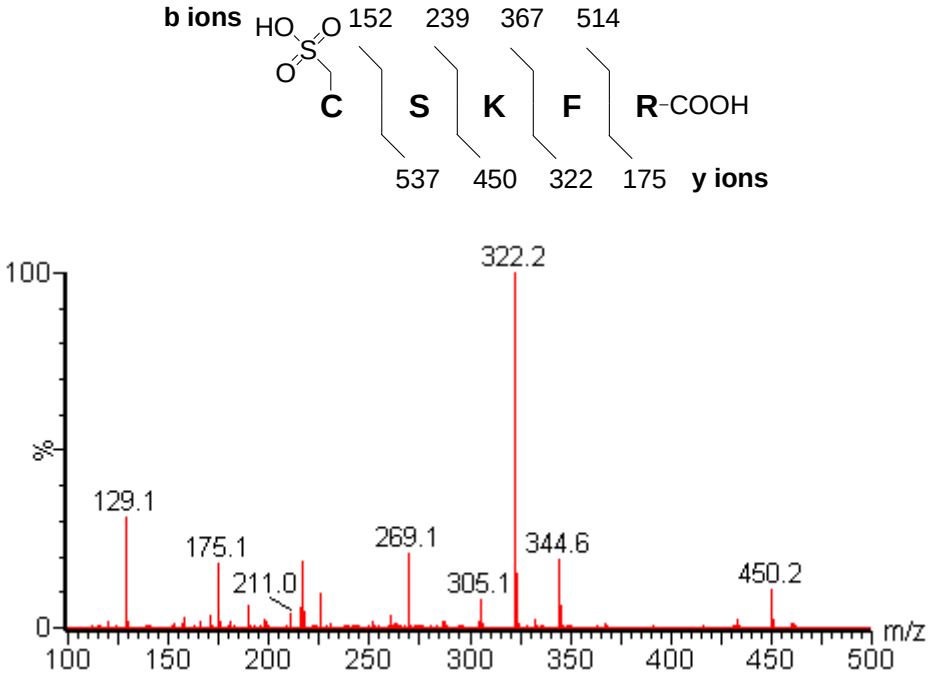


Figure S25. Q-TOF MS/MS spectrum of *N*-terminal modified peptide CSKFR (Table 2, entries 22–23) (ESI source, doubly charged ion of m/z = 344.6).

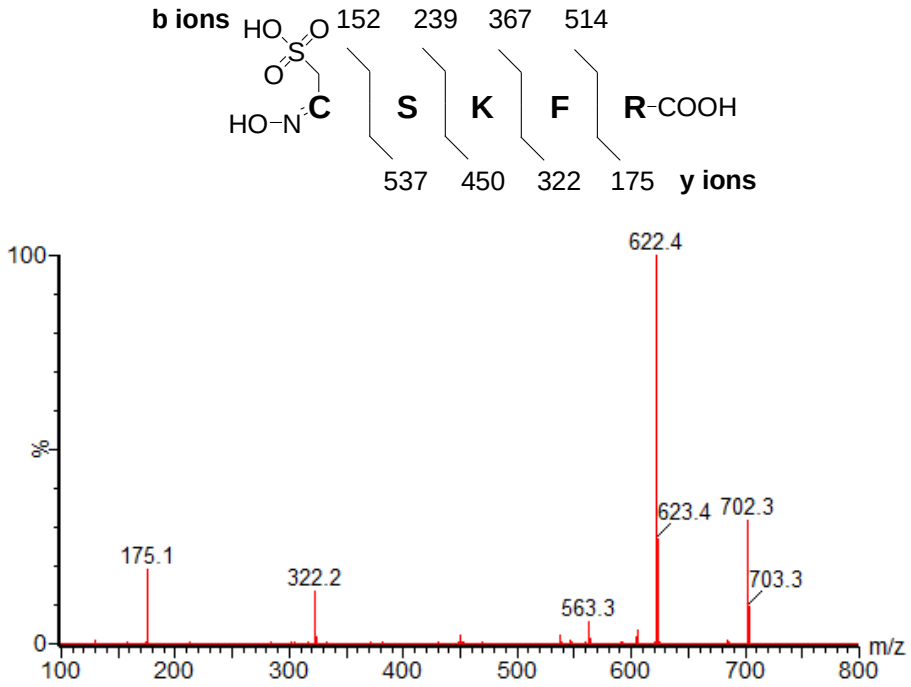
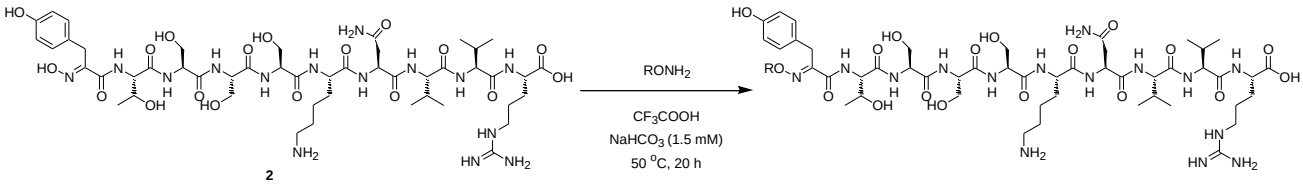


Figure S26. Q-TOF MS/MS spectrum of *N*-terminal modified peptide CSKFR (Table 2, entries 22–23) (ESI source, singly charged ion of m/z = 702.3).

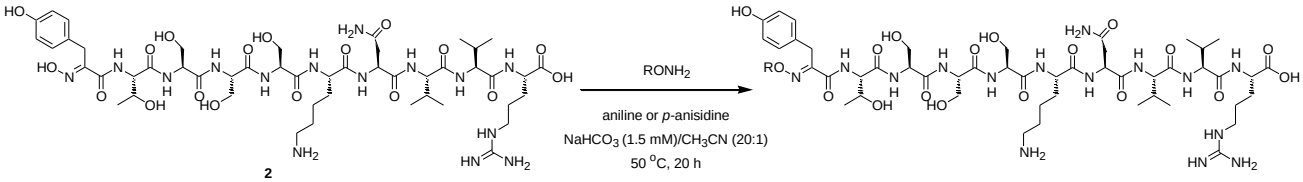
Procedure for Transoximation of Oxime-modified YTSSSKNVVR using Trifluoroacetic Acid



2

A mixture of oxime-modified YTSSSKNVVR, **2** (50 μL of crude reaction mixture), 10 mM methylhydroxylamine or *O*-benzylhydroxylamine in H_2O (2 μL, 1000 equiv.), trifluoroacetic acid (5 μL, 0.65 equiv.) in H_2O (43 μL) was stirred at 50 ^oC for 20 h. The resulting mixture was added with 1% formic acid in CH_3CN (100 μL). The diluted mixture was analyzed by ESI-MS for determination of the oxime-modified peptide conversion (Figures S27–28).

Procedure for Transoximation of Oxime-modified YTSSSKNVVR using Aniline or *p*-Anisidine



2

A mixture of oxime-modified YTSSSKNVVR, **2** (50 μL of crude reaction mixture), 10 mM methylhydroxylamine or *O*-benzylhydroxylamine in H_2O (2 μL, 1000 equiv.), 200 mM aniline or *p*-anisidine in CH_3CN (5 μL, 100 equiv.) in H_2O (43 μL) was stirred at 50 ^oC for 20 h. The resulting mixture was added with 1% formic acid in CH_3CN (100 μL). The diluted mixture was analyzed by ESI-MS/MS for determination of the oxime-modified peptide conversion (Figures S27–28).

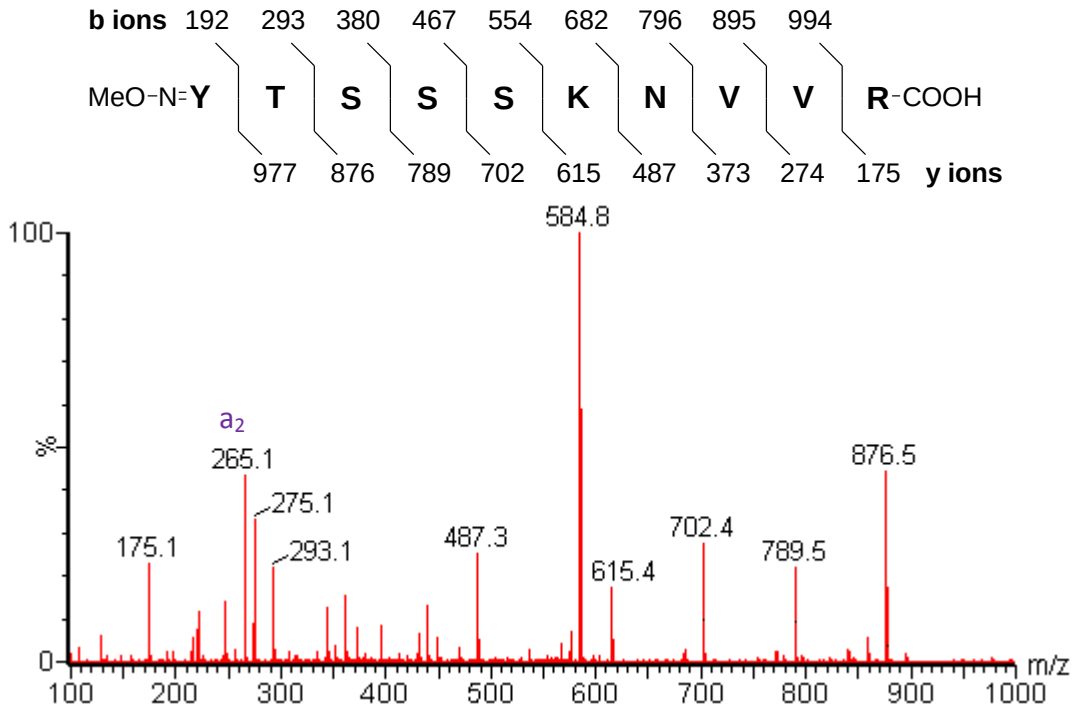


Figure S27. Q-TOF MS/MS spectrum of *N*-terminal methoxylamine-functionalized peptide YTSSSKNVVR (ESI source, doubly charged ion of m/z = 584.8).

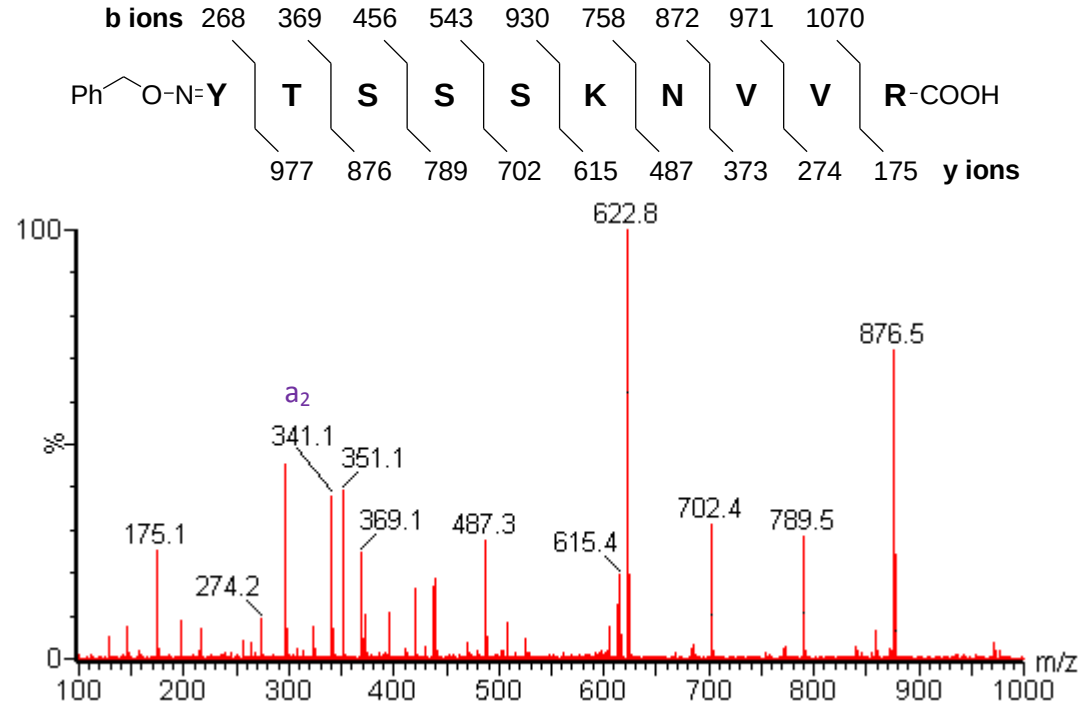
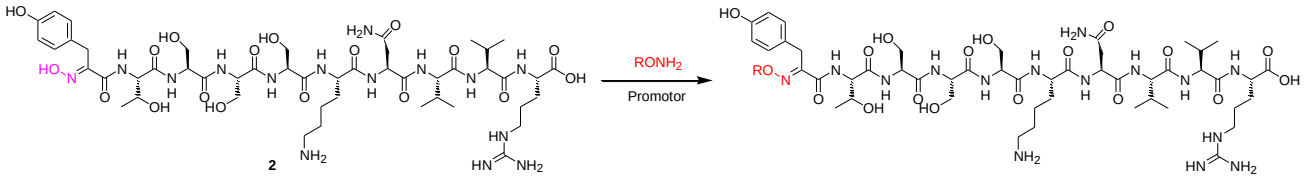


Figure S28. Q-TOF MS/MS spectrum of *N*-terminal *O*-benzylhydroxylamine-functionalized peptide YTSSSKNVVR (ESI source, doubly charged ion of m/z = 622.8).

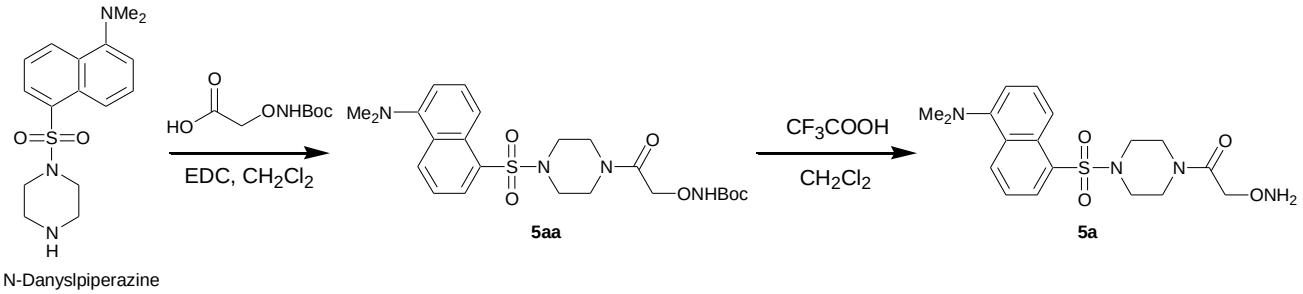
Table S1. Screening conditions for transoximation of oxime-modified YTSSSKNVVR.



Entry	Promotor	Promotor (equiv.)	$RONH_2$	$RONH_2$ (equiv.)	Temperature (^oC)	Conversion of peptide 2 (%)[a]
1	CF_3COOH	0.65	$PhCH_2ONH_2$	20	50	98
2	CF_3COOH	0.65	$PhCH_2ONH_2$	50	50	97
3[b]	CF_3COOH	0.65	$PhCH_2ONH_2$	50	50	33
4	CF_3COOH	0.13	$PhCH_2ONH_2$	50	50	46
5	aniline	100	$MeONH_2$	1000	25	3
6	aniline	100	$MeONH_2$	1000	40	80
7	aniline	100	$PhCH_2ONH_2$	1000	25	2
8	aniline	100	$PhCH_2ONH_2$	1000	40	82
9	aniline	100	$PhCH_2ONH_2$	1000	50	96
10[b]	aniline	100	$PhCH_2ONH_2$	1000	50	25
11	aniline	100	$PhCH_2ONH_2$	100	50	23
12	aniline	100	$PhCH_2ONH_2$	500	50	62
13	aniline	200	$PhCH_2ONH_2$	200	50	6
14	aniline	200	$PhCH_2ONH_2$	500	50	29
15	aniline	200	$PhCH_2ONH_2$	1000	50	55
16	aniline	500	$PhCH_2ONH_2$	200	50	3
17	aniline	500	$PhCH_2ONH_2$	1000	50	52
18	aniline	1000	$PhCH_2ONH_2$	200	50	0
19	aniline	1000	$PhCH_2ONH_2$	500	50	25
20	aniline	1000	$PhCH_2ONH_2$	1000	50	46
21	*p*-anisidine	100	$PhCH_2ONH_2$	100	50	10
22	*p*-anisidine	100	$PhCH_2ONH_2$	500	50	37
23	*p*-anisidine	100	$PhCH_2ONH_2$	1000	50	93
24[b]	*p*-anisidine	100	$PhCH_2ONH_2$	1000	50	21

[a] Determined by ESI-MS. [b] 4 h.

General Scheme for Synthesis of Dansyl-functionalized Hydroxylamine 5a

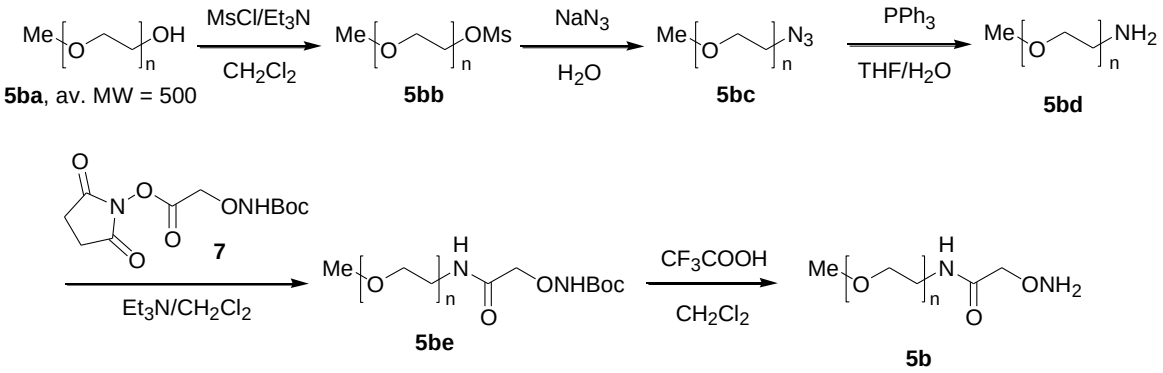


Compound 5aa: a solution of *N*-dansylpiperazine (35 mg, 110 μmol), (Boc-aminooxy)acetic acid (19 mg, 0.13 mmol) and *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC, 26 mg, 0.17 mmol) in 10 mL CH_2Cl_2 was stirred at room temperature under nitrogen atmosphere for overnight. The reaction mixture was washed by water (50 mL) and then dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel using 5% CH_3OH/CH_2Cl_2 as eluent to give **5a**; yield: 49 mg (100 μmol, 91%); pale green powder; analytical TLC (silica gel 60) (10% CH_3OH/CH_2Cl_2), R_f = 0.64; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, *J* = 8.5 Hz, 1H), 8.35 (d, *J* = 8.5 Hz, 1H), 8.20 (dd, *J* = 7.5 Hz, 1H), 7.85 (s, -NH), 7.55 (dd, *J* = 8.0 Hz, 2H), 7.19 (d, *J* = 8.5 Hz, 1H), 4.57 (s, 2H), 3.42–3.45 (m, 2H), 3.20–3.22 (m, 4H), 2.89 (s, 5H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.71, 156.22, 152.11, 132.41, 131.30, 130.99, 130.47, 130.31, 128.46, 123.32, 119.45, 115.57, 82.19, 73.50, 73.46, 65.99, 45.68, 45.57, 45.43, 44.48, 41.42, 28.31, 15.41; ESI-MS m/z 493 $[M+H]^+$; HRMS (ESI) for $C_{23}H_{33}N_4O_6S$, calcd. 493.2121, found 493.2115.

Compound 5a: a solution of **5aa** (55g, 0.10 mmol) and trifluoroacetic acid (TFA, 2 mL) in 4 mL CH_2Cl_2 was stirred at room temperature for 30 min. The reaction mixture was concentrated under reduced pressure and triturated by CH_2Cl_2 twice (10 mL × 2). The residue was purified by flash column chromatography on silica gel using 10% CH_3OH/CH_2Cl_2 as eluent to give **5a**; yield: 35 mg (90 μmol, 90%), pale green powder; analytical TLC (silica gel 60) (10% CH_3OH/CH_2Cl_2), R_f = 0.54; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, *J* = 8.5 Hz, 1H), 8.39 (d, *J* = 8.5 Hz, 1H), 8.20 (dd, *J* = 7.5 Hz, 1H), 7.53 (dd, *J* = 8.0 Hz, 2H), 7.20 (d, *J* = 8.0 Hz, 1H), 4.57 (d, *J* = 8.5 Hz, 2H), 3.63–3.66 (m, 2H),

3.53–3.56 (m, 2H), 3.15–3.18 (m, 4H), 2.89 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.68, 156.74, 151.93, 131.12, 130.92, 128.33, 123.29, 119.56, 115.52, 71.86, 45.87, 45.53, 44.82, 41.48, 21.69, 15.74; ESI-MS m/z 393 $[M+H]^+$; HRMS (ESI) for $C_{18}H_{25}N_4O_4S$, calcd. 393.1597, found 393.1609.

General Scheme for Synthesis of PEG-functionalized Hydroxylamine 5b



Compound 5bb: poly(ethylene glycol) methyl ether 500 **5ba** (10 g, 20 mmol) was dissolved in dry CH_2Cl_2 (20 mL) and triethylamine (10 mL, 72 mmol) under nitrogen atmosphere. Methanesulfonyl chloride (5.5 mL, 71 mmol) was added at 0 ^{o}C. After the addition, the ice bath was removed. The reaction mixture was allowed to warm up to room temperature slowly and stirred for 4 h. H_2O (100 mL) was then added to quench the reaction. The crude reaction mixture was washed with 1 M HCl solution (20 mL), saturated $NaHCO_3$ solution (20 mL), and brine (20 mL), and the organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure to obtain a brown liquid as mesylate **5bb**; yield: 9.6 g (19.2 mmol, 95%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.38 (t, J = 4.5 Hz, 2H), 3.77 (t, J = 4.5 Hz, 2H), 3.63–3.68 (m, 36H, PEG), 3.54–3.56 (m, 2H), 3.38 (s, 3H), 3.09 (s, 3H).

Compound 5bc: mesylate **5bb** (6.3 g, 11 mmol) and sodium azide (1.8 g, 27.2 mmol) were dissolved in H_2O (12 mL) under nitrogen atmosphere. The mixture was refluxed for 18 h. After the reaction, the resulting mixture was extracted with CH_2Cl_2 (50 mL × 3). The combined organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure to afford azide **5bc** as a pale brown liquid; yield: 4.8 g (9 mmol, 82 %); ^{1}H

NMR (400 MHz, $CDCl_3$): δ = 3.64–3.68 (m, 38H, PEG), 3.55 (t, J = 2.4 Hz, 2H), 3.40 (d, J = 2.4 Hz, 2H), 3.38 (s, 3H).

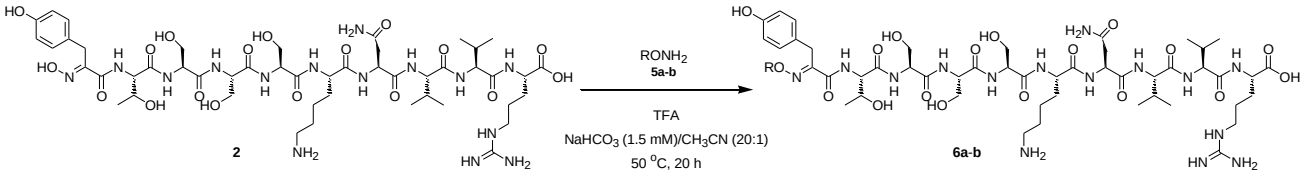
Compound 5bd: a mixture of azide **5bc** (2.63 g, 5 mmol) and triphenylphosphine (1.59 g, 6 mmol) was dissolved in tetrahydrofuran (15 mL) under nitrogen atmosphere. The mixture was stirred for 3 h, followed by addition of H_2O (0.21 mL) and allowed to stir for 18 h. After the reaction, 1 M HCl solution (15 mL) was added to the crude reaction mixture and washed with diethyl ether (20 mL × 3). The aqueous layer was cooled to 0 ^oC, neutralized by careful addition of KOH (2 g, 36 mmol), and extracted with CH_2Cl_2 (20 mL × 3). The combined organic layer was washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure to give amine **5bd** as a pale yellow liquid; yield: 2.1 g (4.25 mmol, 85 %); ^1H NMR (500 MHz, $CDCl_3$): δ = 3.64–3.66 (m, 40H, PEG), 3.54–3.56 (m, 2H), 3.52 (t, J = 5.2 Hz, 2H), 3.38 (s, 3H), 2.81 (t, J = 5.2 Hz, 2H).

Compound 5be: amine **5bd** (0.25 g, 0.5 mmol), (Boc-aminooxy)acetic acid *N*-hydroxysuccinimide ester **7** (0.17 g, 0.6 mmol), triethylamine (0.11 mL, 0.75 mmol) were dissolved in dry CH_2Cl_2 (5 mL) under nitrogen atmosphere. The mixture was stirred at room temperature for 24 h. After addition of H_2O (5 mL), the resulting mixture was extracted with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure to obtain *N*-Boc aminooxy compound **5be** as a pale yellow liquid; yield: 0.32 g (0.48 mmol, 95 %); ^1H NMR (500 MHz, $CDCl_3$): δ = 8.07 (br s, 1H), 4.31 (s, 2H), 3.63–3.68 (m, 34H, PEG), 3.59 (t, J = 5.5 Hz, 2H), 3.55 (t, J = 5.5 Hz, 2H), 3.47–3.51 (m, 2H), 3.38 (s, 3H), 1.48 (s, 9H).

Compound 5b: to a solution of *N*-Boc aminooxy compound **5be** (0.1 g, 0.15 mmol) in CH_2Cl_2 (1 mL) in an ice bath was slowly added trifluoroacetic acid (1 mL) in dropwise. The mixture was then warmed to room temperature and stirred for 30 min. After the reaction, the solvent was evaporated to nearly dryness under reduced pressure and diluted with H_2O (5 mL). The aqueous solution in an ice bath was neutralized by slow addition of solid $NaHCO_3$ until no CO_2 evolution was observed. The

solution was then extracted with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure to give aminooxy compound **5b** as a pale yellow liquid; yield: 71.5 mg (0.13 mmol, 83 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.16 (br s, 1H), 4.15 (s, 2H), 3.64–3.67 (m, 35H, PEG), 3.60 (t, J = 5.0 Hz, 2H), 3.55 (t, J = 5.0 Hz, 2H), 3.50–3.53 (m, 2H), 3.38 (3H, s).

Procedure for Transoximation of Oxime-modified YTSSSKNVVR with Functionalized Hydroxylamines using Trifluoroacetic Acid



A mixture of oxime-modified YTSSSKNVVR, **2** (50 μL of crude reaction mixture), 10 mM aminooxy-compound **5a** or **5b** in CH_3CN (2 μL, 1000 equiv.), trifluoroacetic acid (5 μL, 0.65 equiv.) and H_2O (43 μL) was stirred at 50 ^oC for 20 h. The resulting mixture was added with 1% formic acid in CH_3CN (100 μL). The diluted mixture was analyzed by LC-MS or MALDI-TOF for determination of the *N*-terminal functionalized peptides conversions (Figures S29–30).

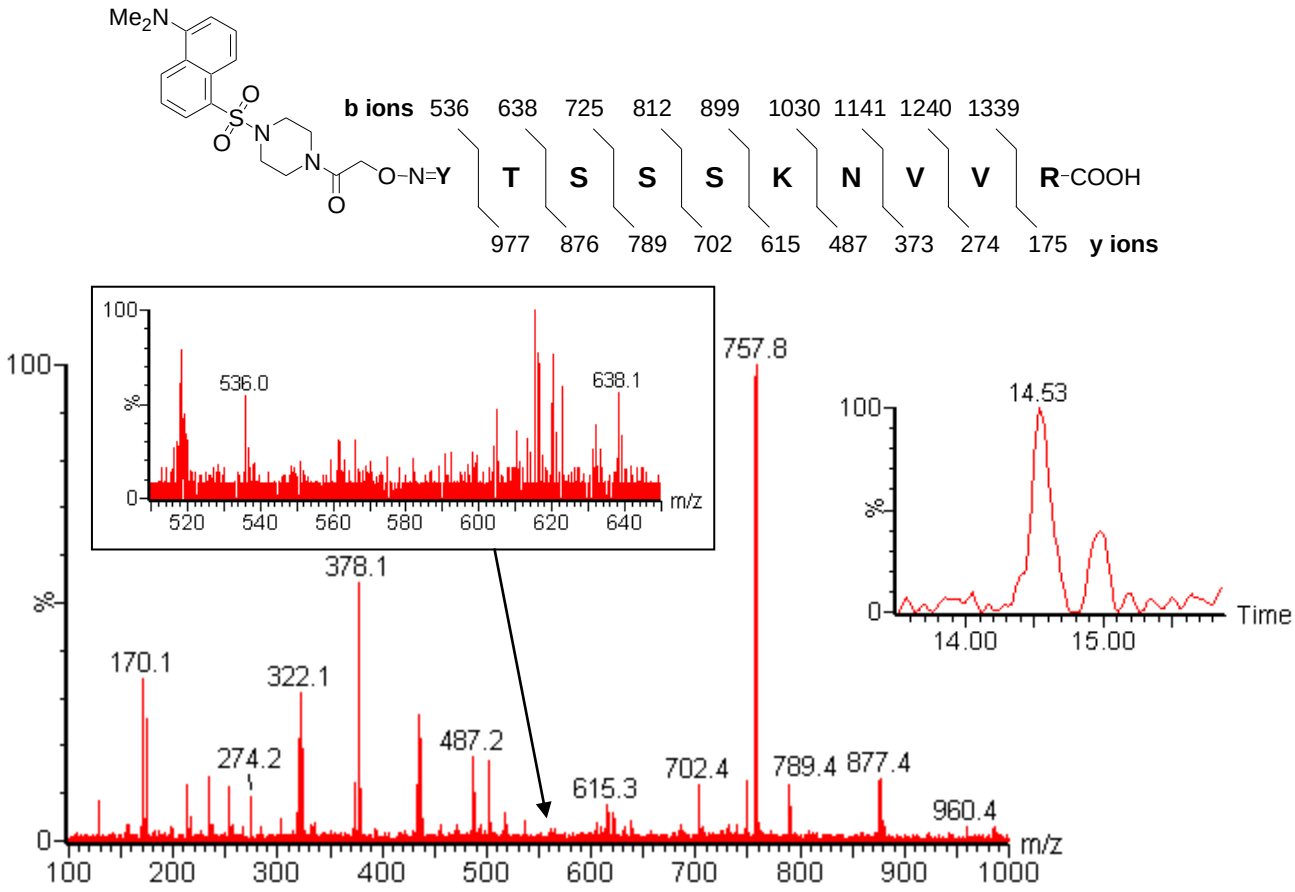


Figure S29. Q-TOF MS/MS spectrum of *N*-terminal dansyl-functionalized peptide YTSSSKNVVR, **6a**, (ESI source, doubly charged ion of m/z = 757.8) and the XIC chromatogram of **6a** at t = 14.53 min (inset).

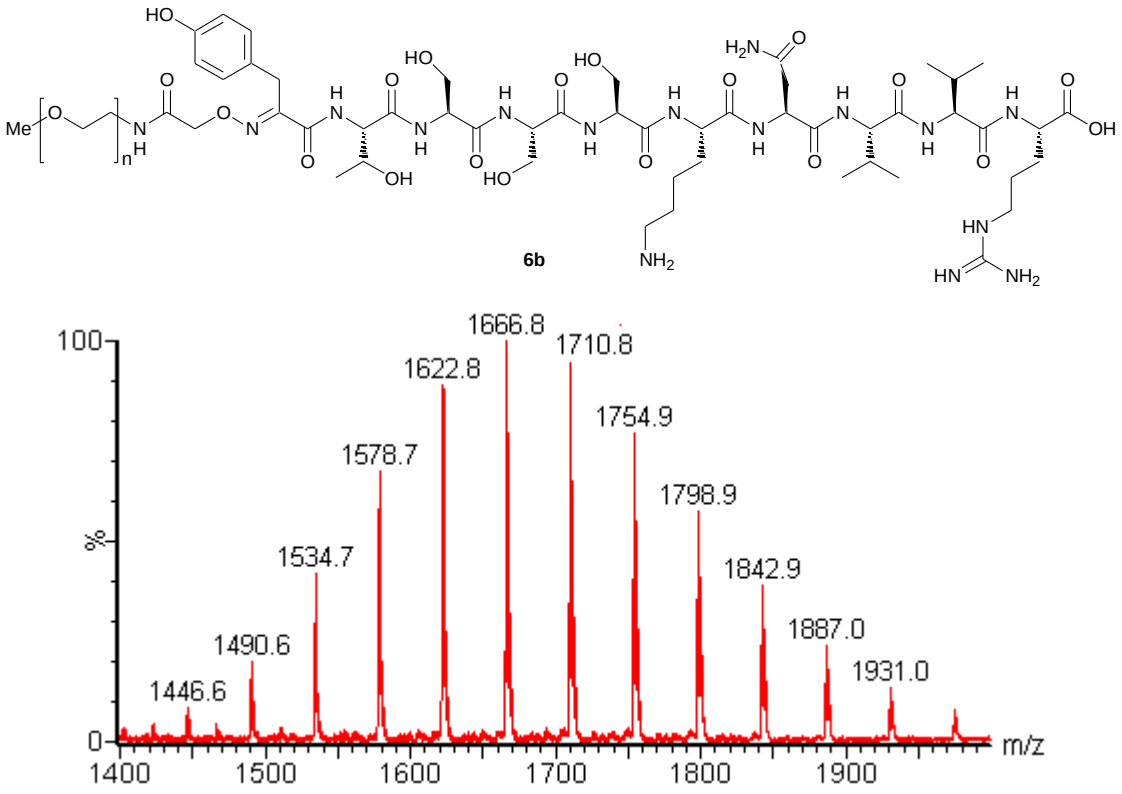
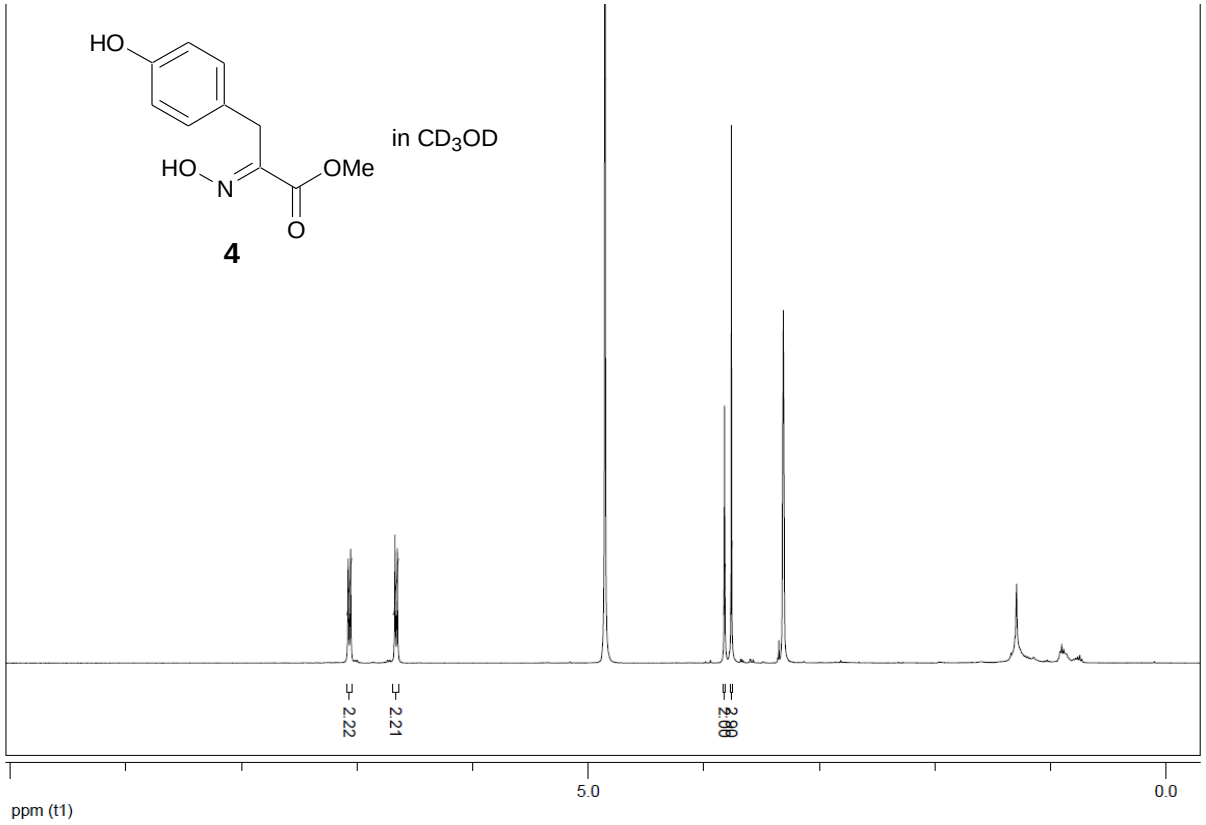
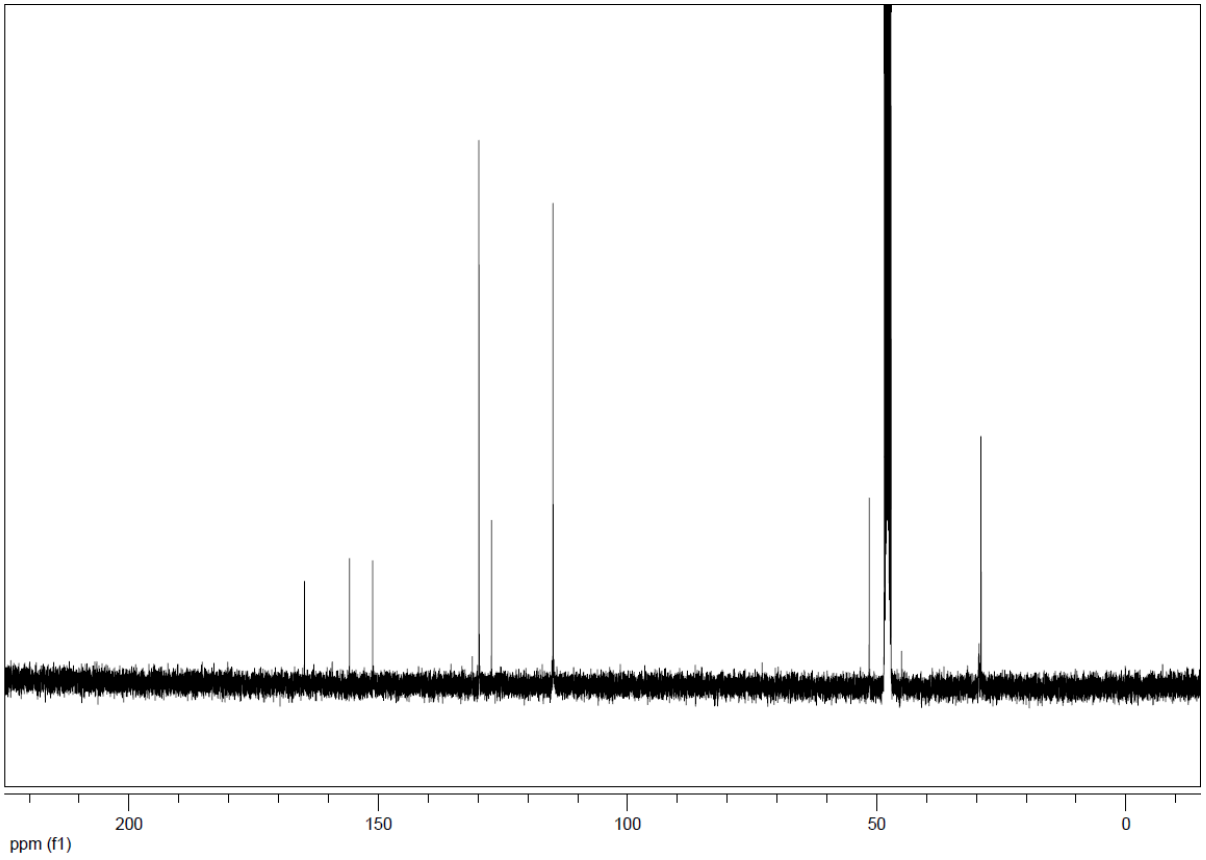


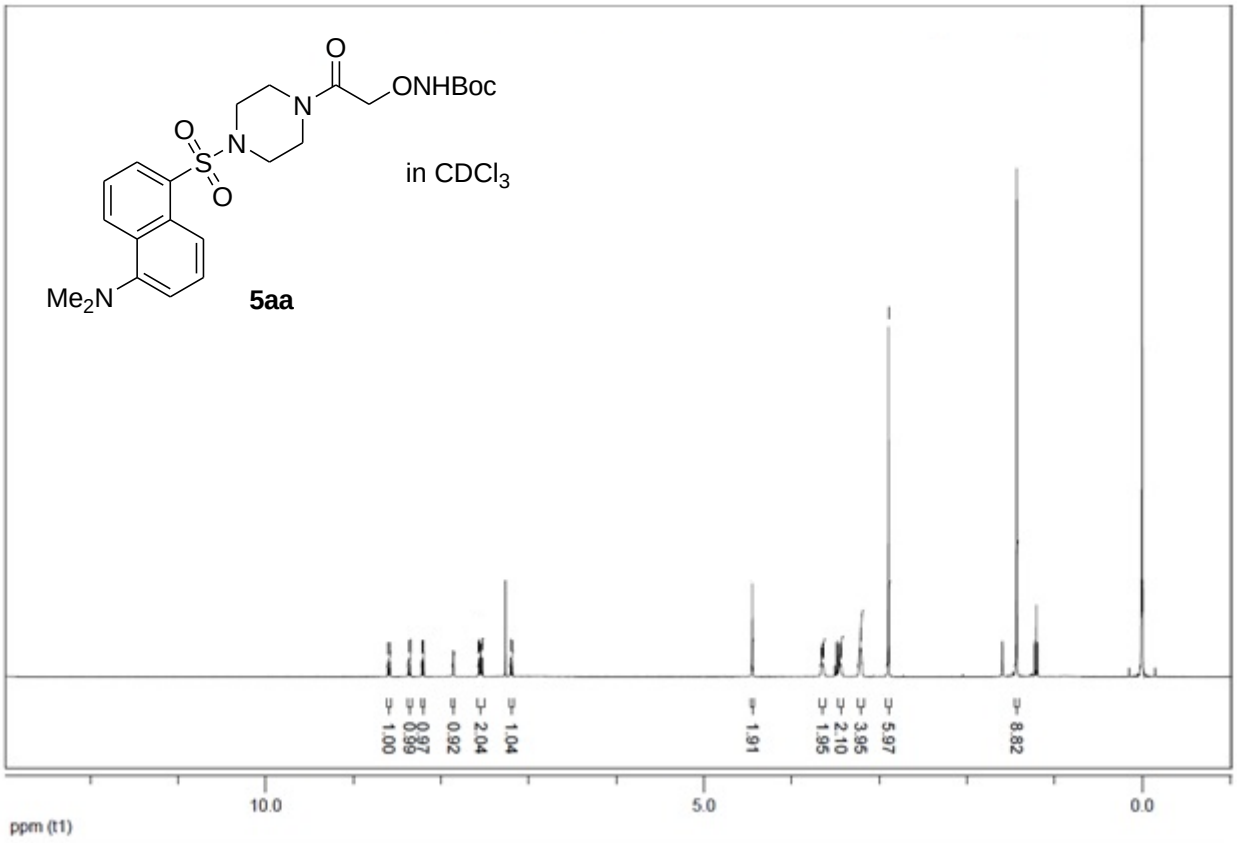
Figure S30. MALDI-TOF spectrum of *N*-terminal PEG-functionalized peptide YTSSSKNVVR, **6b** (ESI source, singly charged ions of m/z = 1446.6–1931.0).

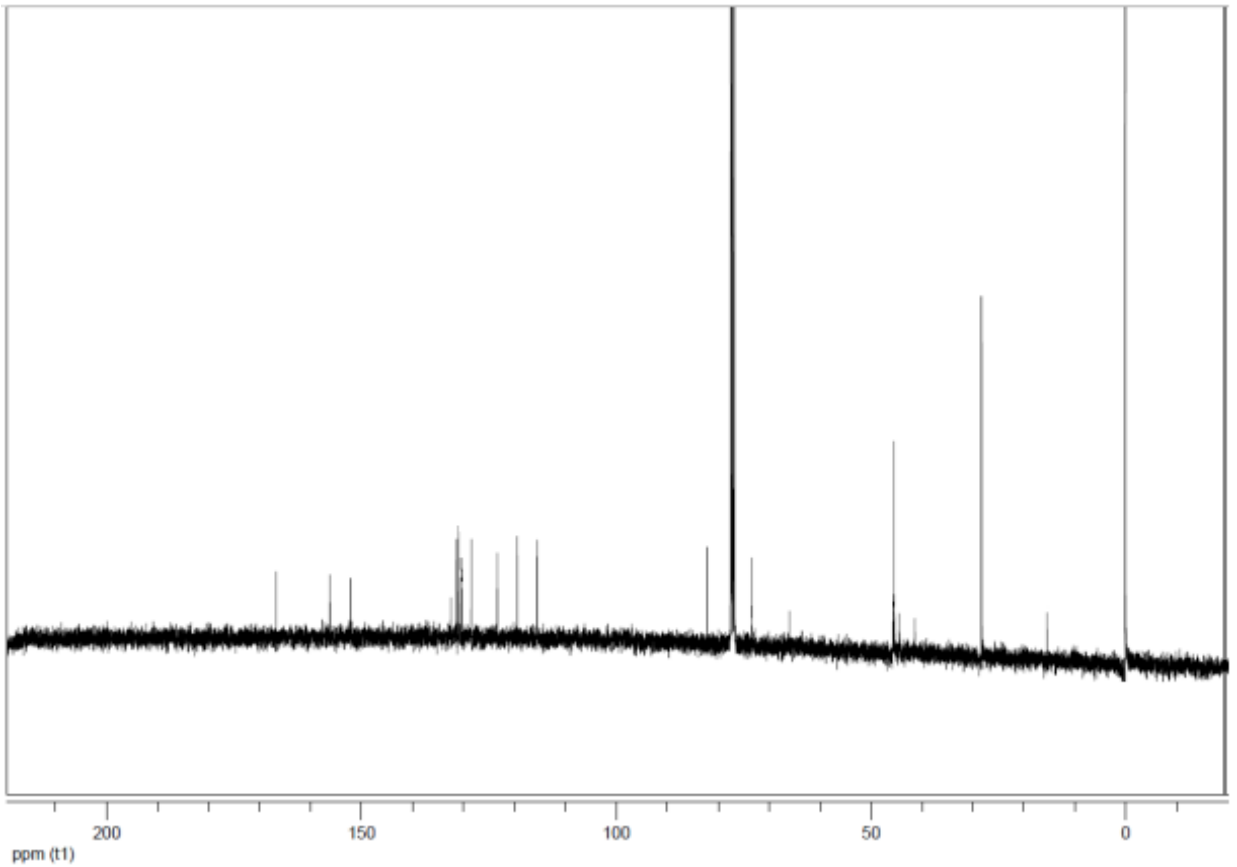
NMR Spectra of 4



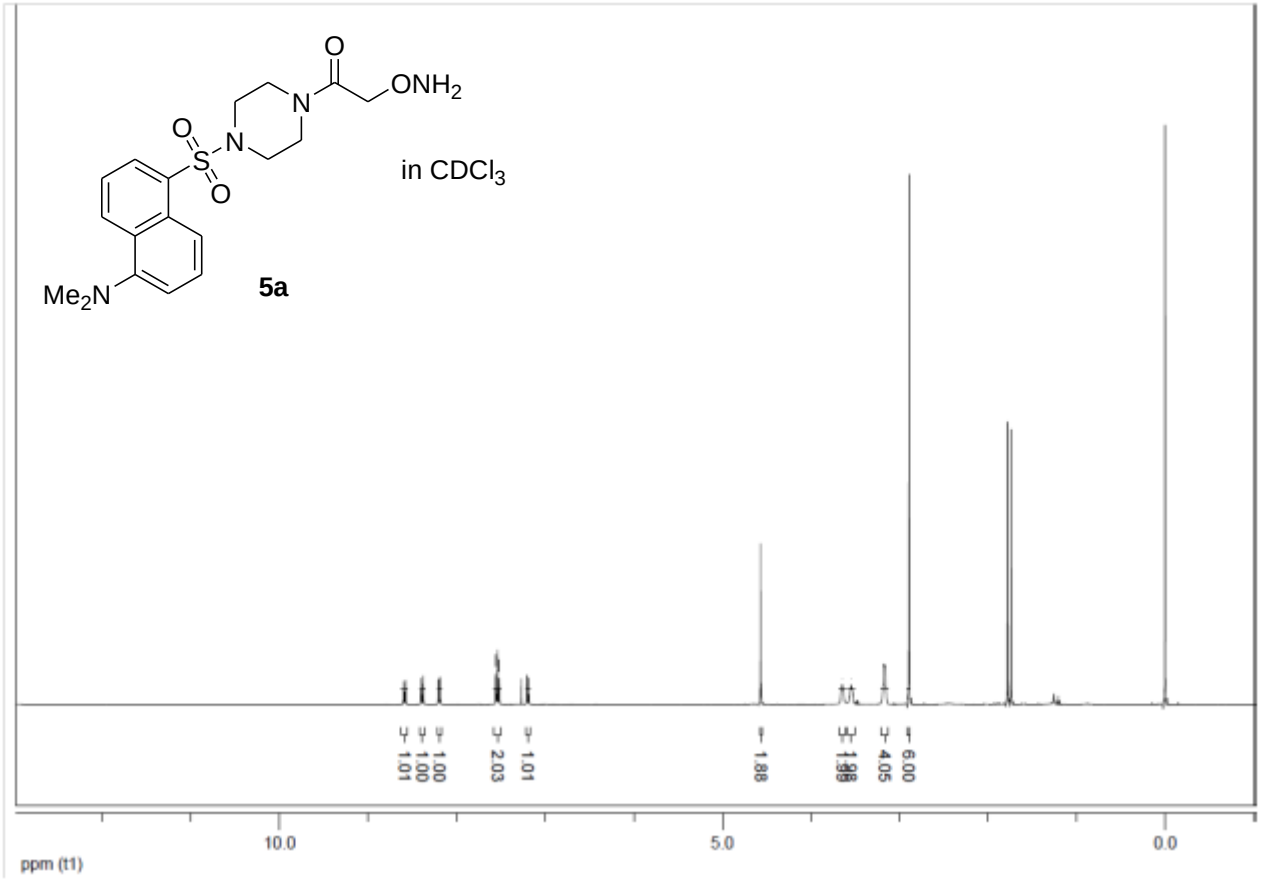


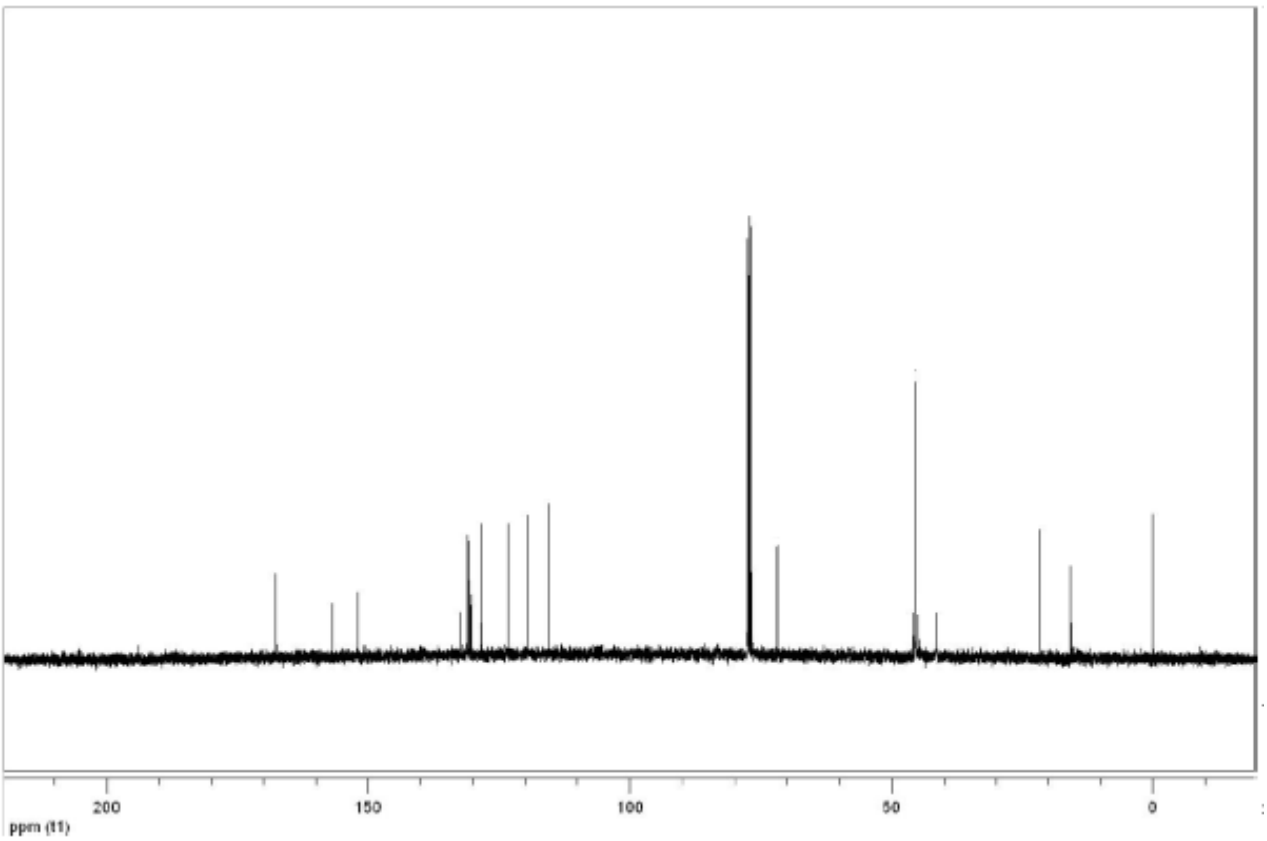
NMR Spectra of 5aa



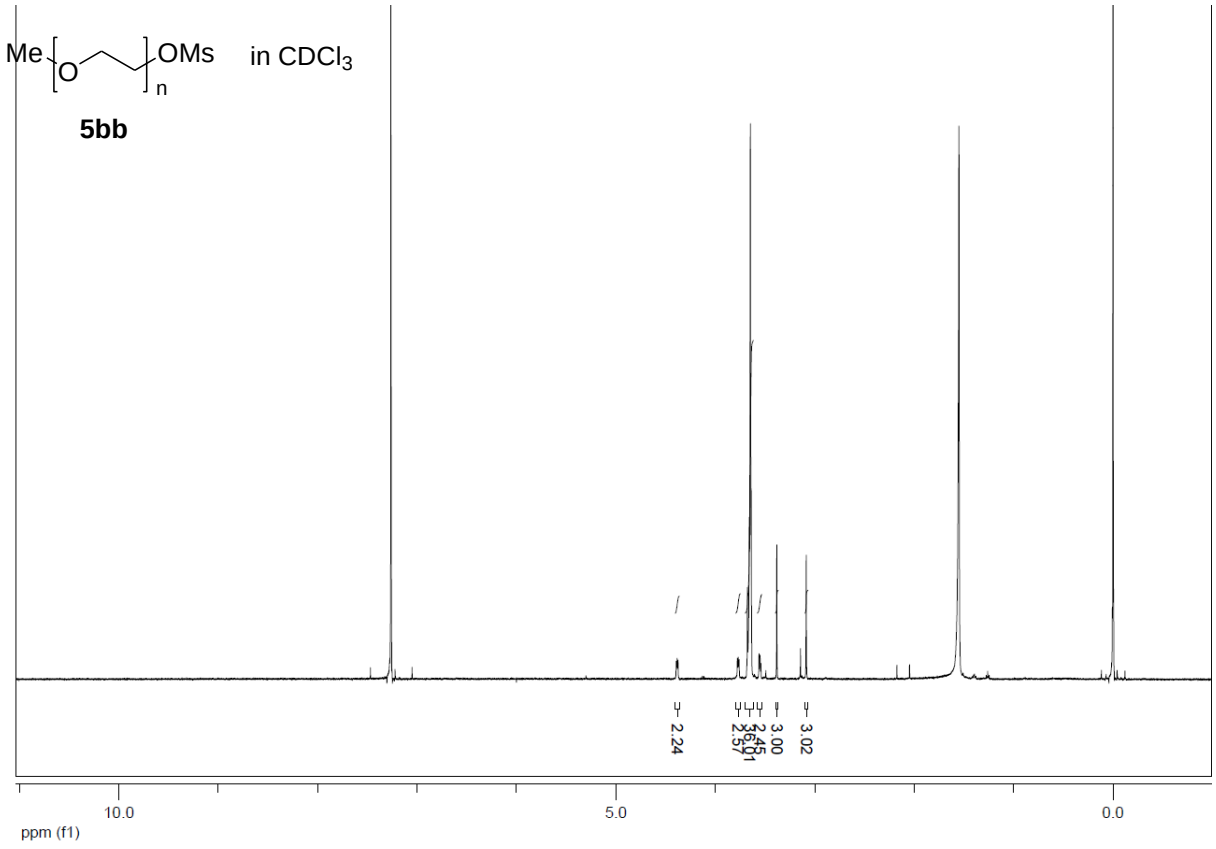


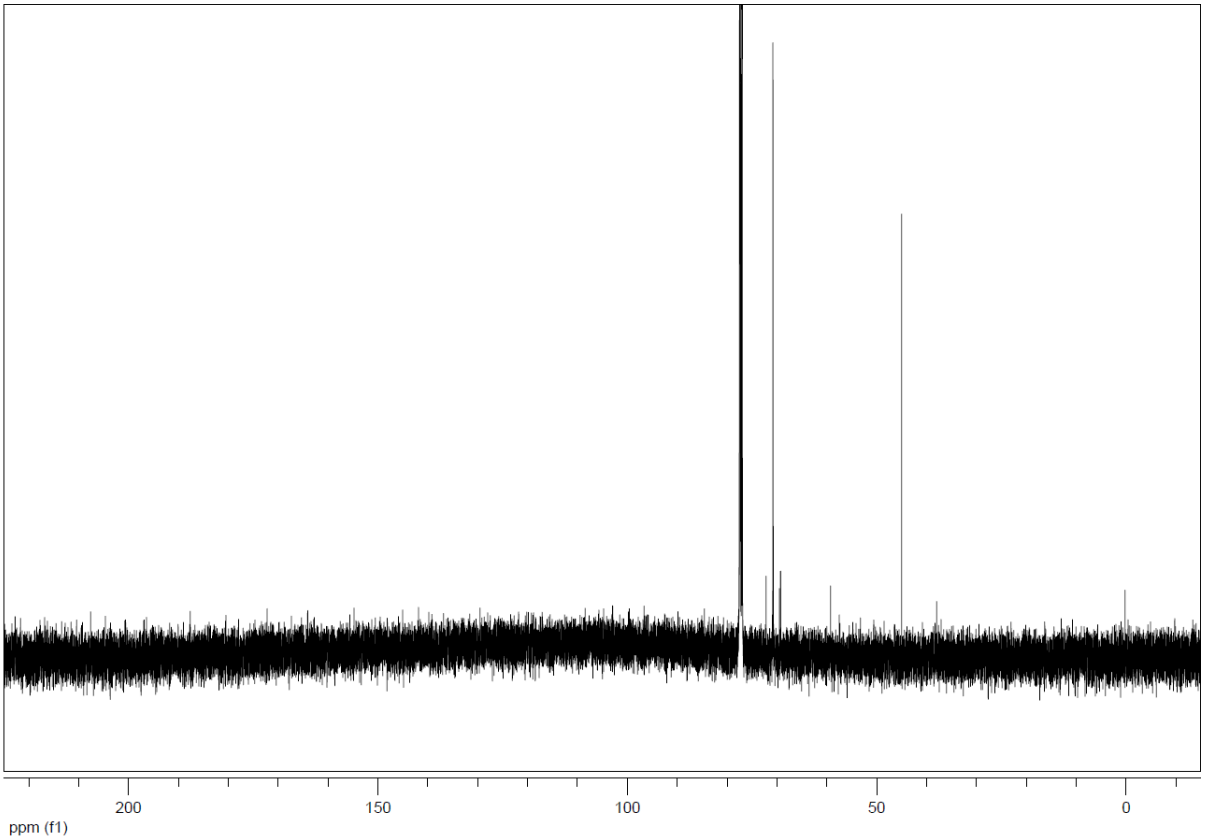
NMR Spectra of 5a



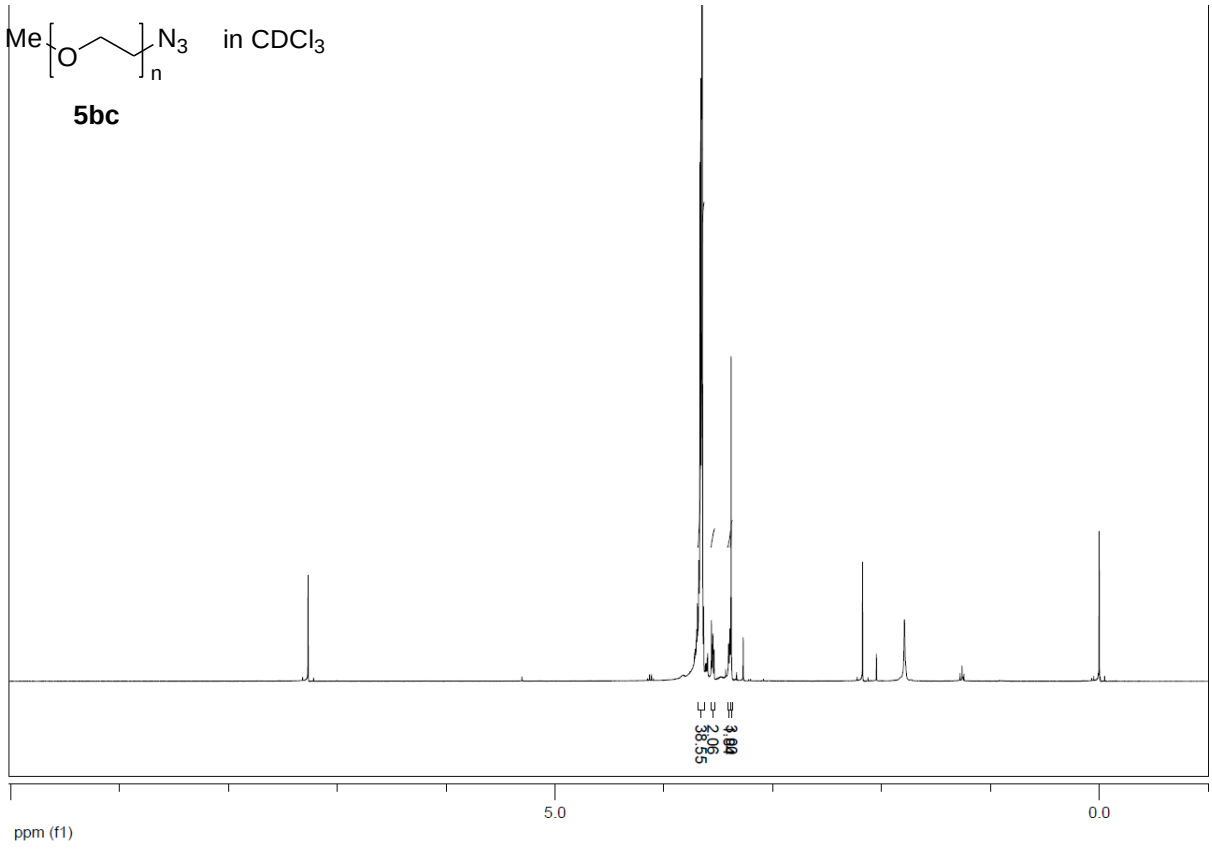


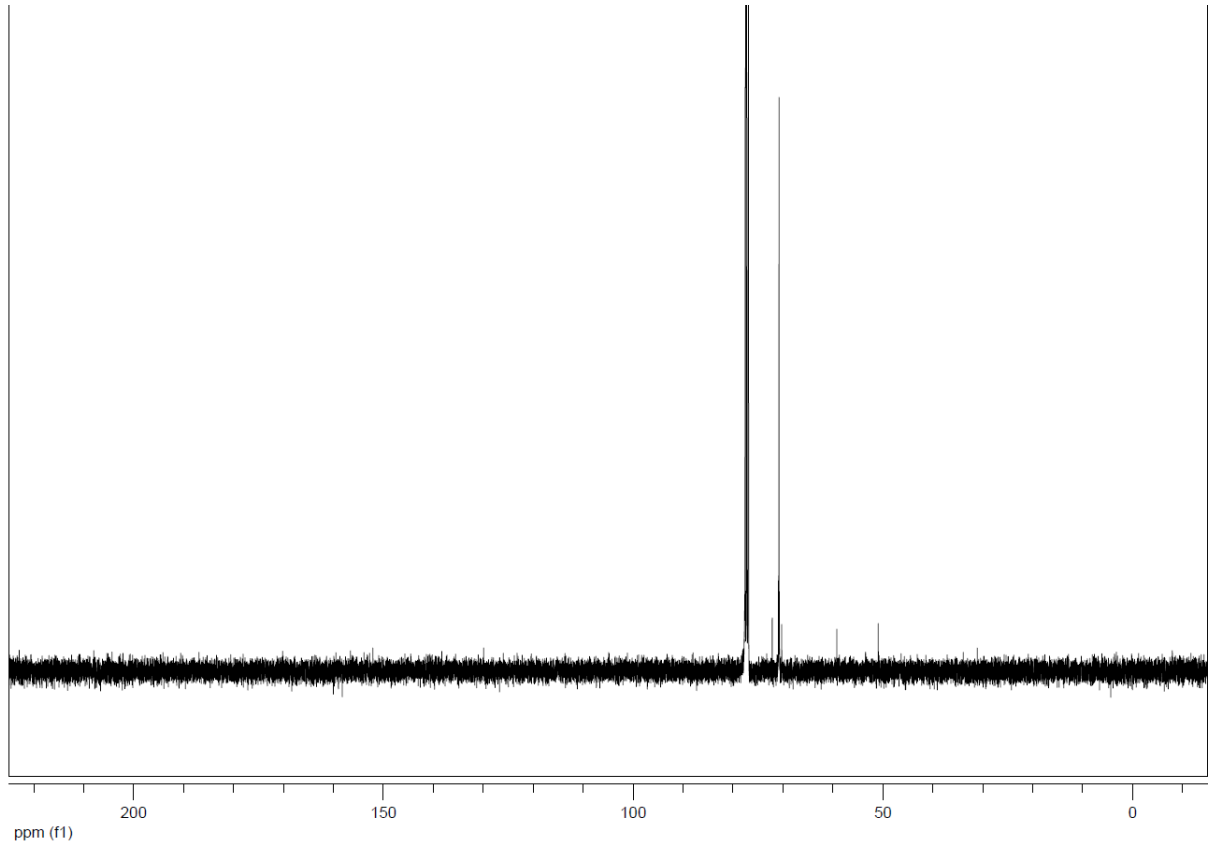
NMR Spectra of 5bb



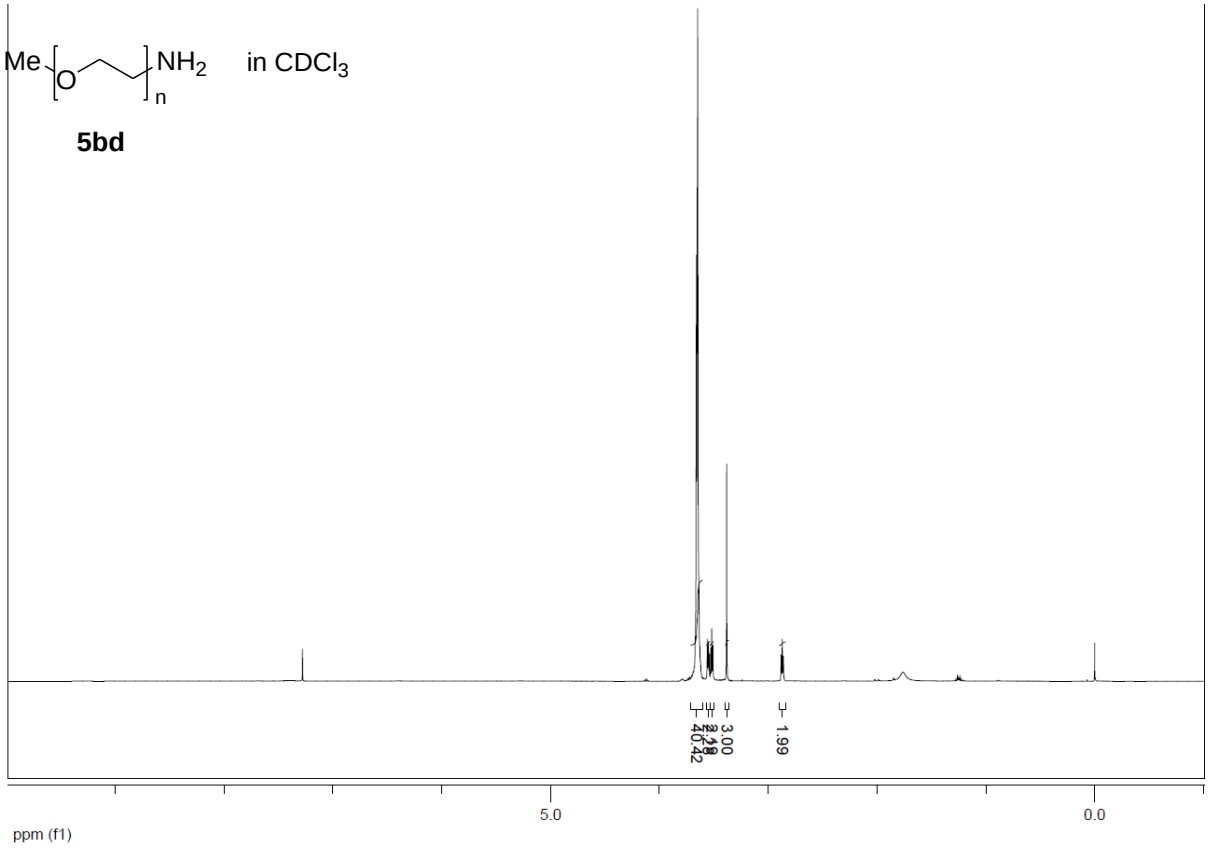


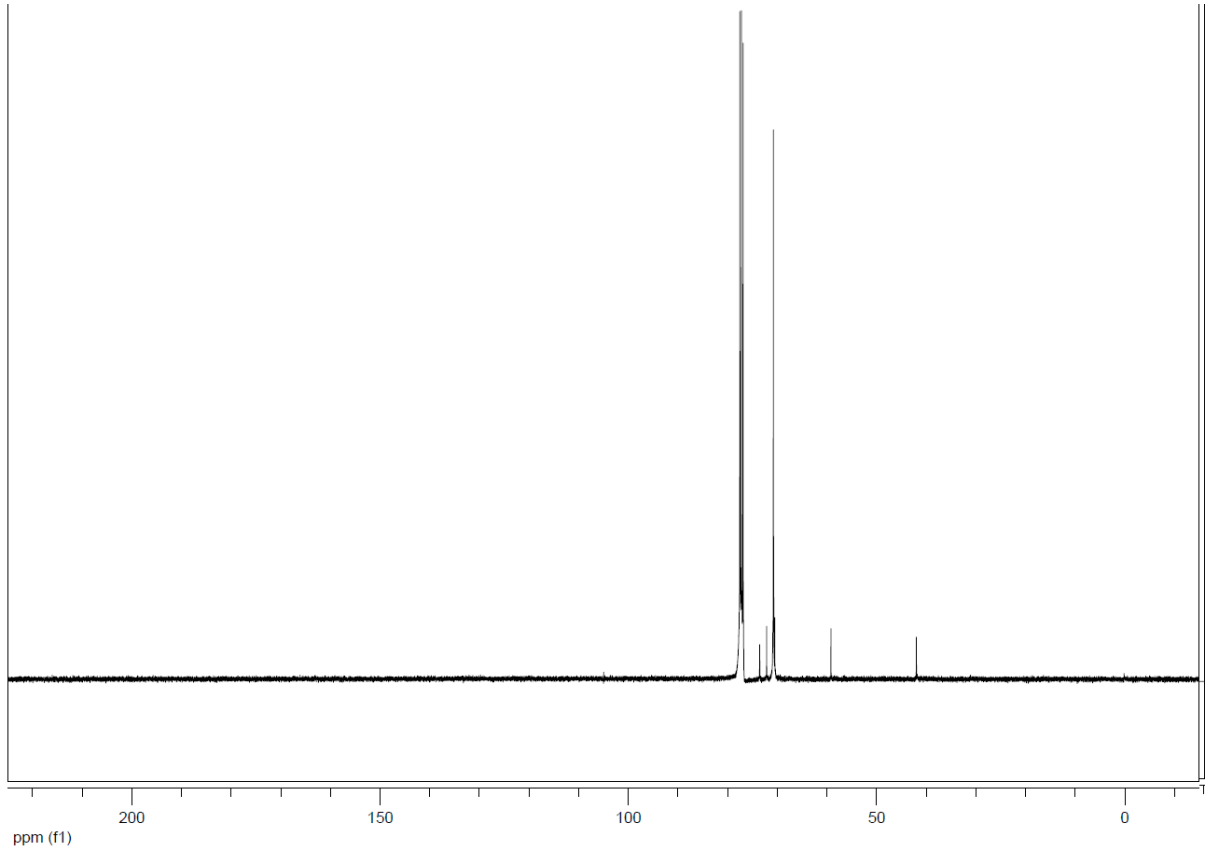
NMR Spectra of 5bc



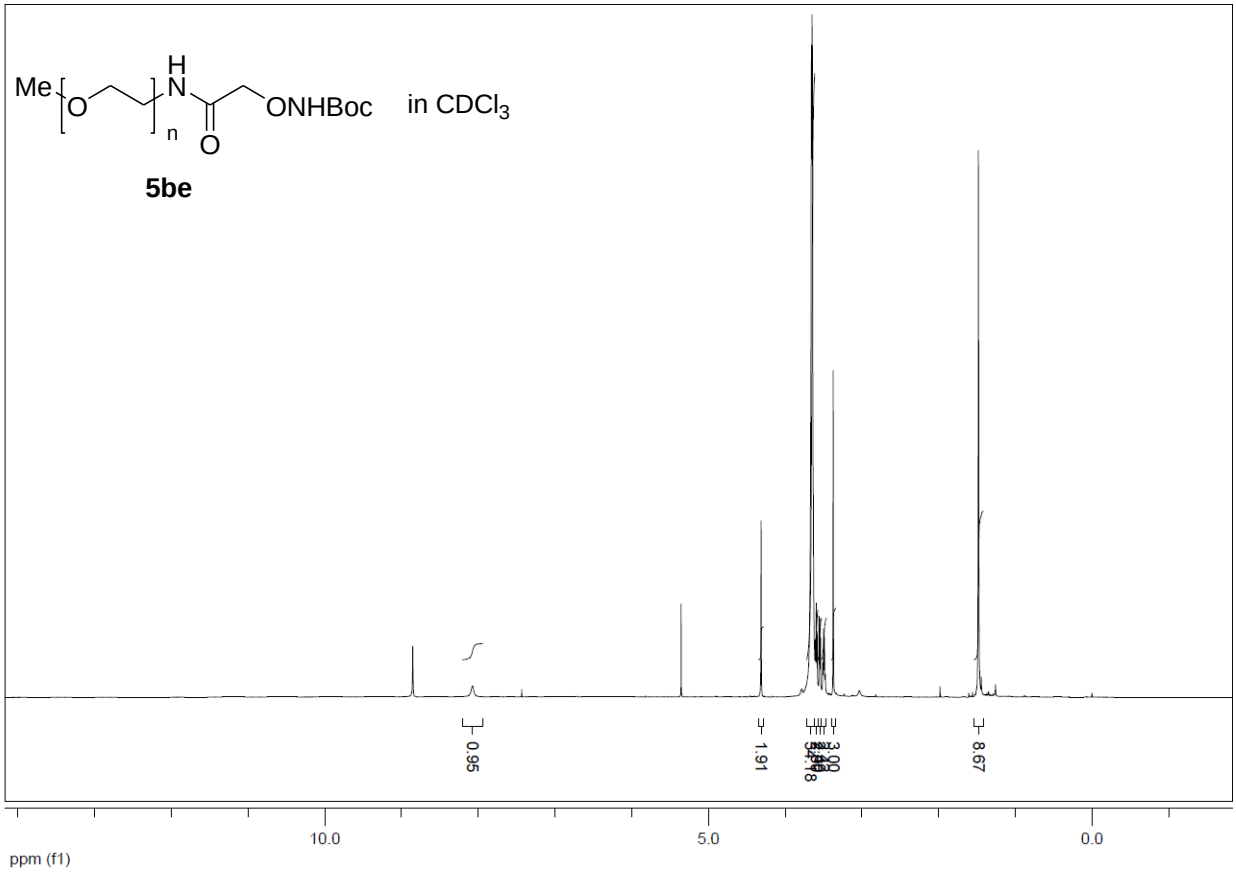


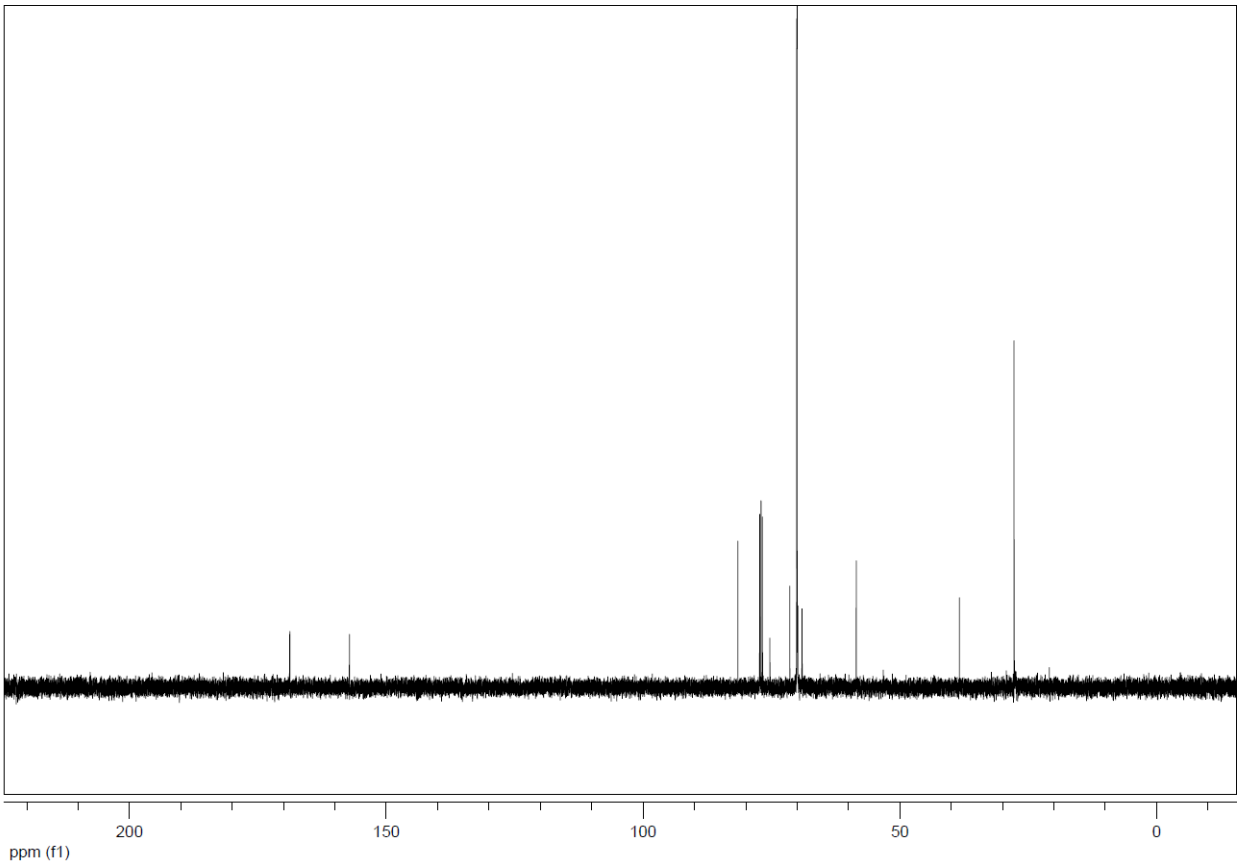
NMR Spectra of 5bd



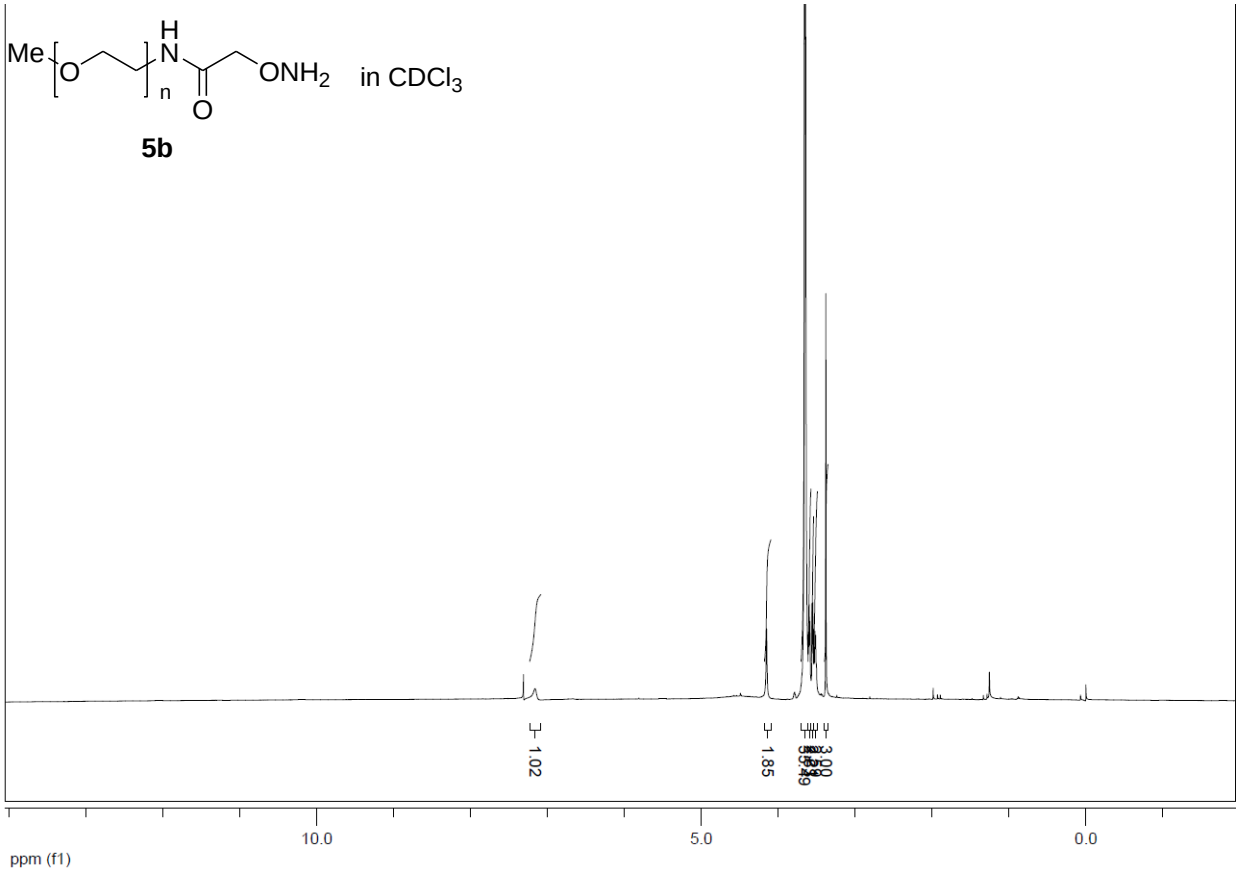


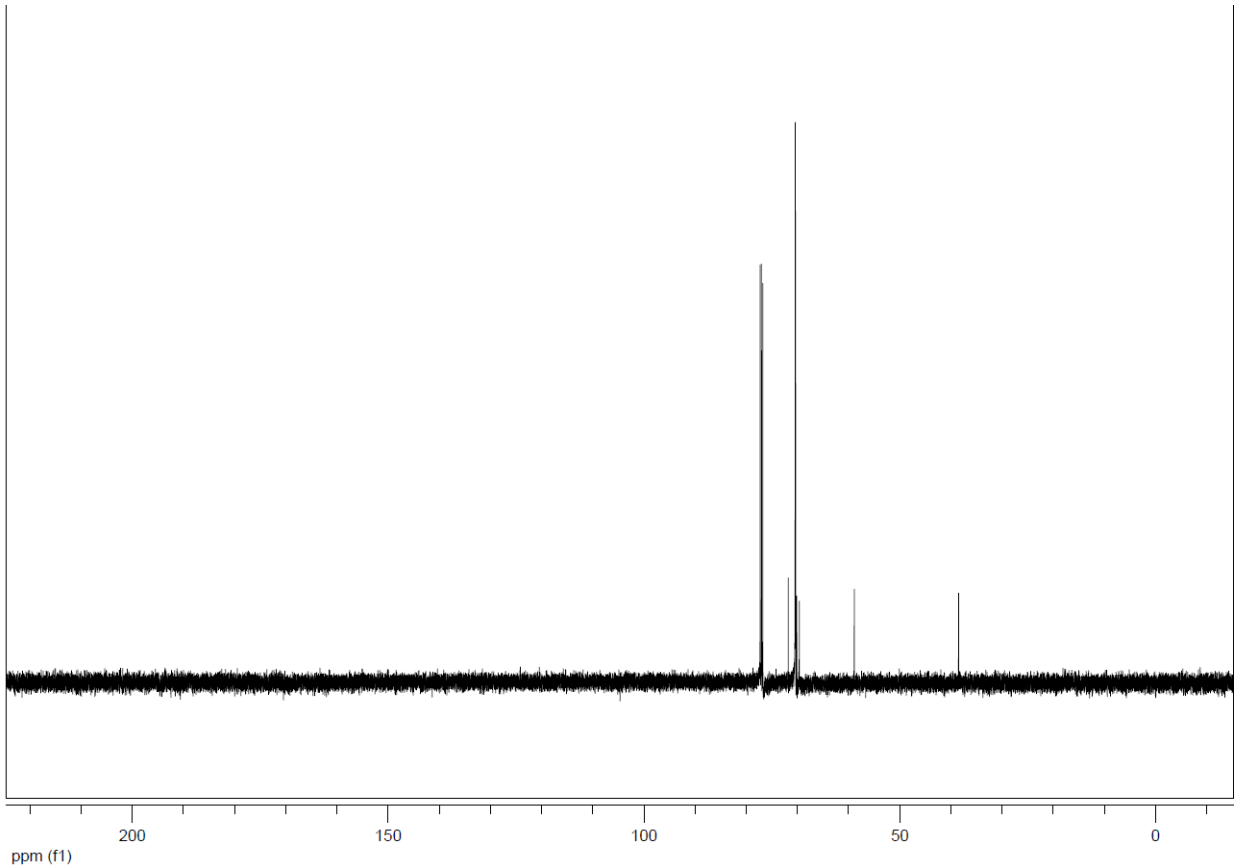
NMR Spectra of 5be



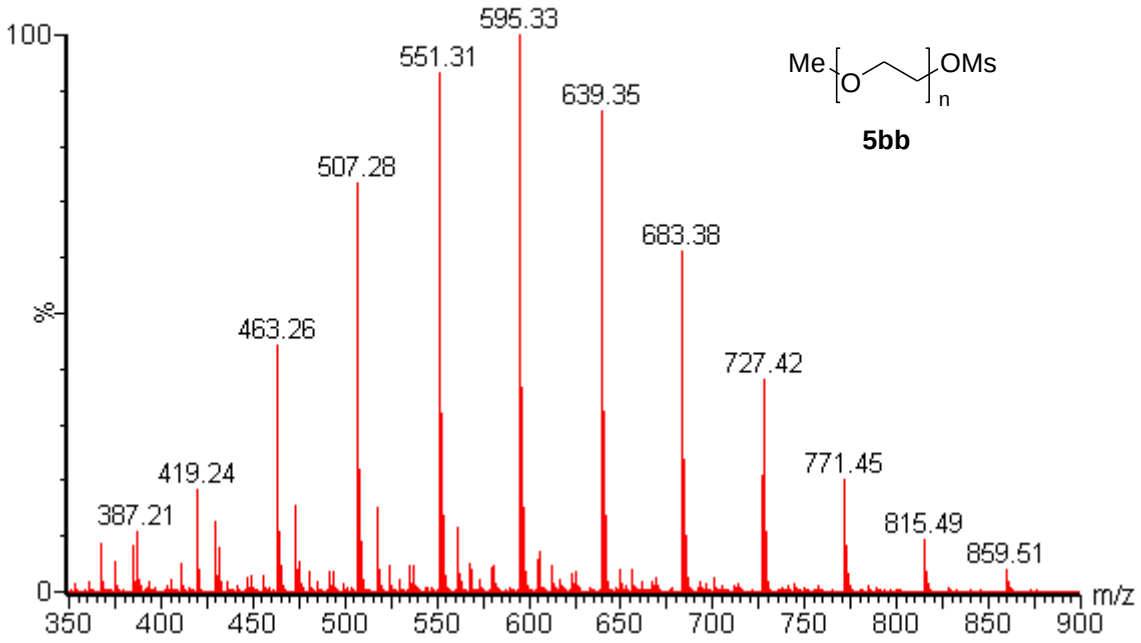


NMR Spectra of 5b



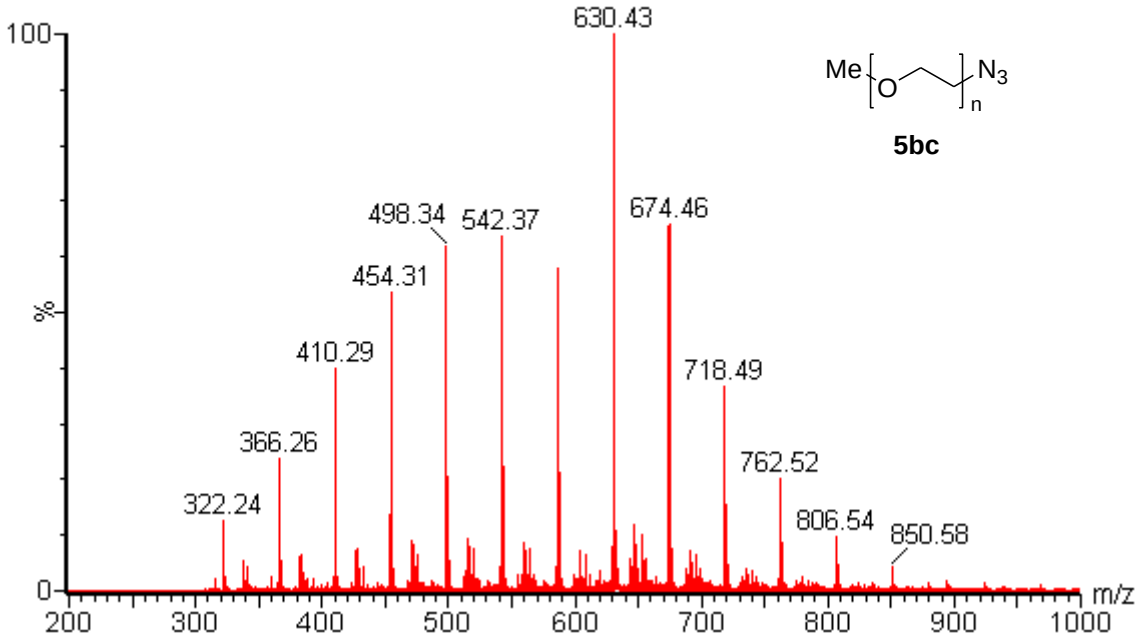


ESI-MS Spectrum of 5bb



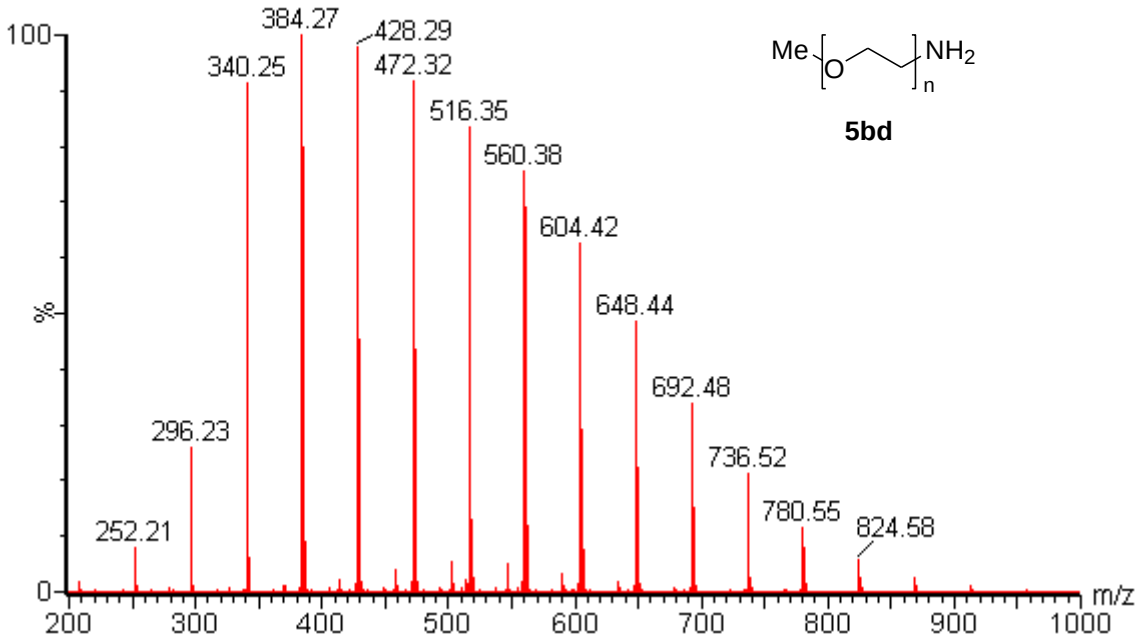
5bb

ESI-MS Spectrum of 5bc



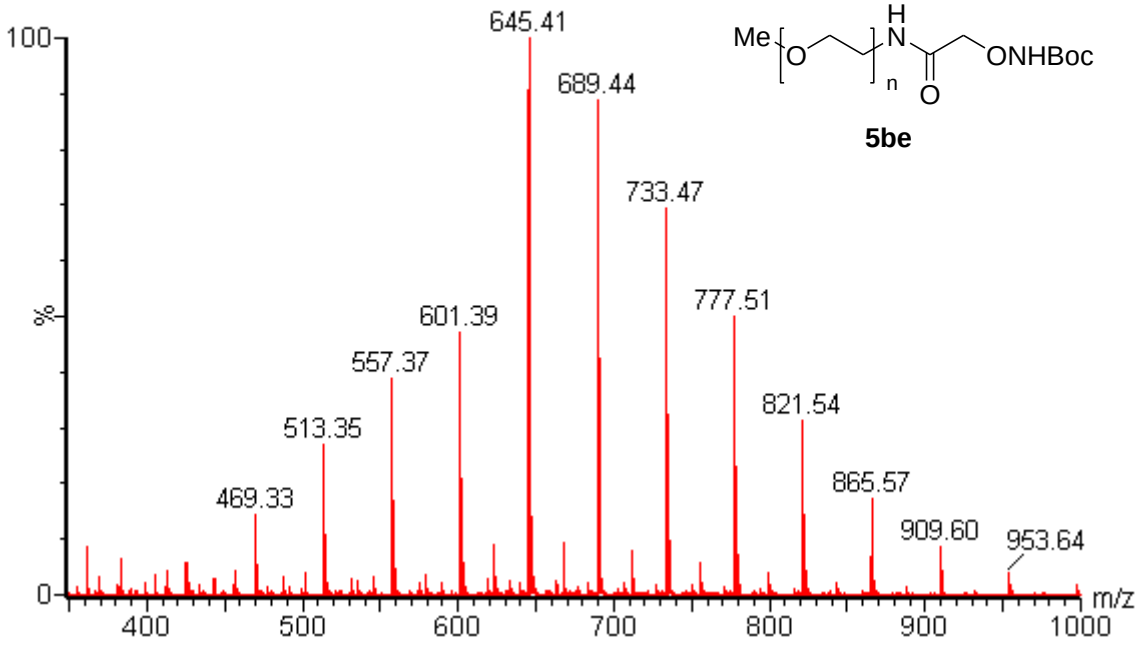
5bc

ESI-MS Spectrum of 5bd



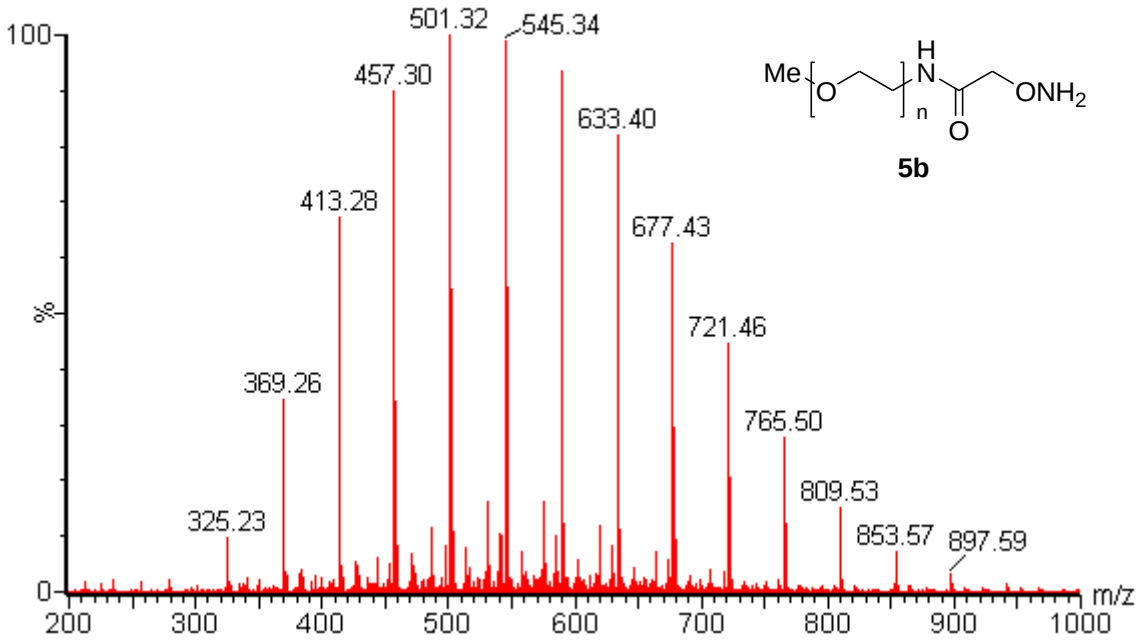
5bd

ESI-MS Spectrum of 5be



5be

ESI-MS Spectrum of 5b



5b