

Supporting Information

Synthesis of Thiophenylalanine-Containing Peptides via Cu(I)-Mediated Cross-Coupling

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Part 2

S31-S39. Complete 1-D ^1H NMR spectra for thiophenylalanine-derived Ac-TXPN-NH₂ peptides **2-7**, **17-24**

S33. TOCSY spectrum and complete resonance assignments for peptide **4**

S40-S45. HPLC reinjection chromatograms for purified thiophenylalanine-derived peptides **2-7**, **12-15**, **17-24**

S46-S71. Mass spectra for peptides **1-24**

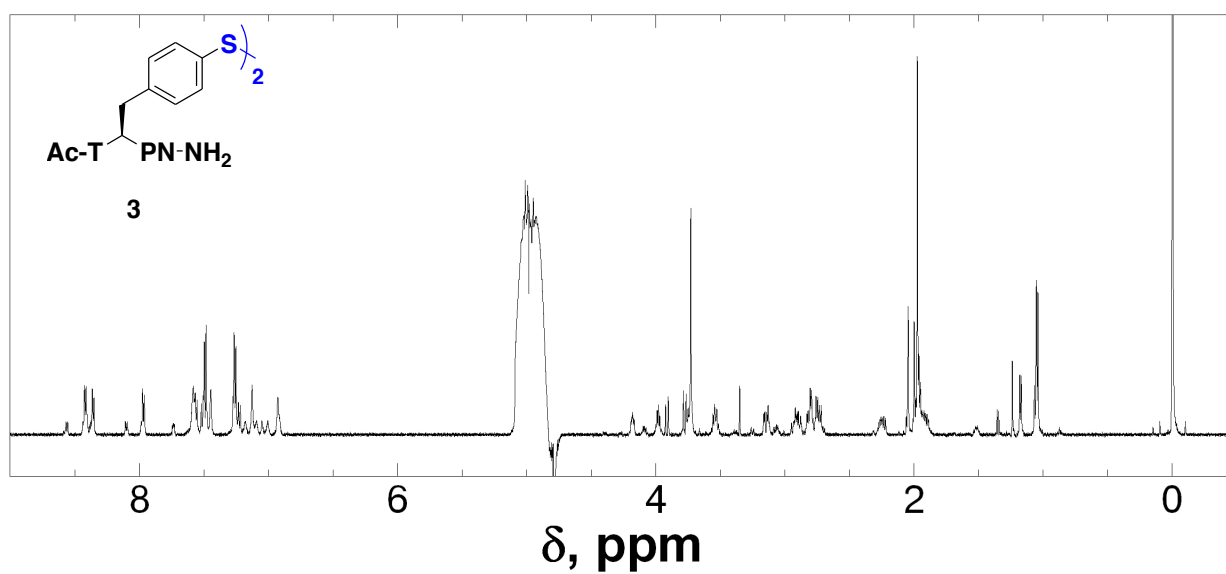
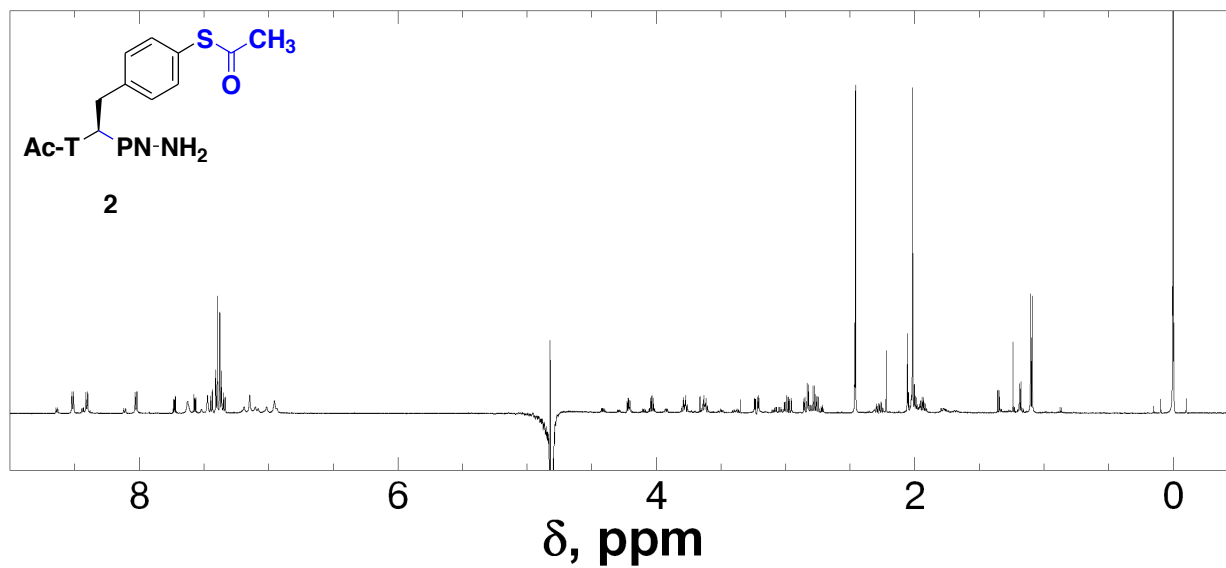


Figure S32. Full NMR spectra for peptides **2** and **3** at pH 4.0. All samples contained 5 mM phosphate and 25 mM NaCl.

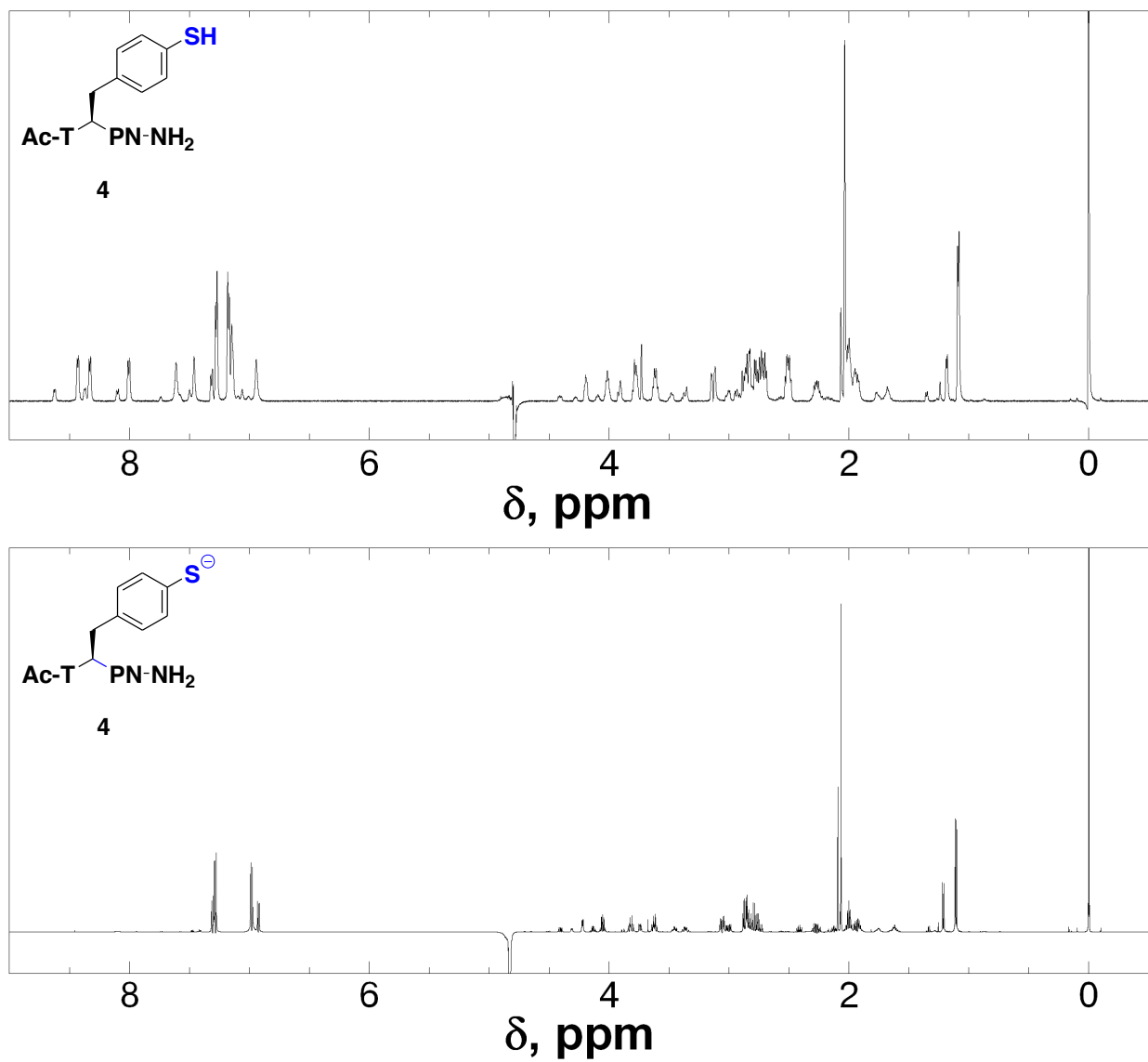


Figure S33. Full NMR spectra for peptide 4 at pH 4.0 and pH 8.5. All samples contained 5 mM phosphate and 25 mM NaCl at pH 4.0 and 0.1 mM TCEP.

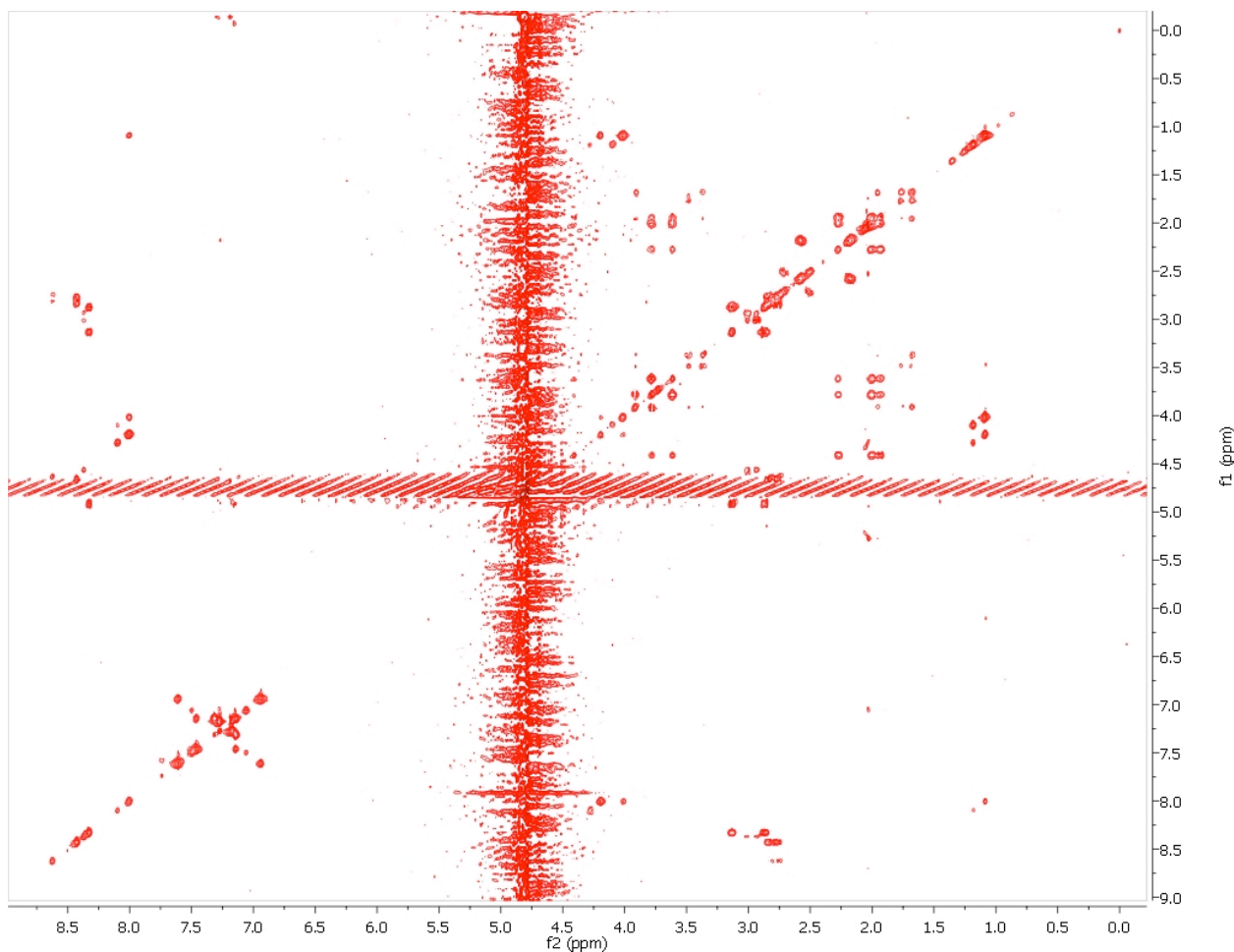


Figure S34. TOCSY spectrum for peptide **4** at pH 4.0. The solution contains 5 mM phosphate (pH 4.0), 25 mM NaCl, and 0.1 mM TCEP.

Peptide **4**, with a 3.5:1 ratio of trans:cis amide bond: ^1H NMR (600 MHz, 90% $\text{H}_2\text{O}/10\%$ D_2O) δ 8.62 ($\text{Asn}_{\text{HNcis}}$, d, $J = 7.8$ Hz, 1H), 8.43 ($\text{Asn}_{\text{HNtrans}}$, d, $J = 7.0$ Hz, 1H), 8.37 (4-SH-Phe $_{\text{HNcis}}$, d, $J = 5.8$ Hz, 1H), 8.33 (4-SH-Phe $_{\text{HNtrans}}$, d, $J = 7.5$ Hz, 1H), 8.10 ($\text{Thr}_{\text{HNcis}}$, d, $J = 8.0$ Hz, 1H), 8.01 ($\text{Thr}_{\text{HNtrans}}$, d, $J = 8.1$ Hz, 1H), 7.61 ($\text{Asn}_{\text{CONH2trans}}$, s, 1H), 7.58 ($\text{Asn}_{\text{CONH2cis}}$, s, 1H), 7.50 ($\text{Terminus}_{\text{CONH2cis}}$, s, 1H), 7.46 ($\text{Terminus}_{\text{CONH2trans}}$, s, 1H), 7.35-7.12 (4-SH-Phe $_{\text{aromatic}}$, m, 5H), 7.15 ($\text{Terminus}_{\text{CONH2trans}}$, s, 1H), 7.06 ($\text{Terminus}_{\text{CONH2cis}}$, s, 1H), 6.94 ($\text{Asn}_{\text{CONH2trans}}$, s, 1H), 6.94 ($\text{Asn}_{\text{CONH2cis}}$, s, 1H), 4.91 (4-SH-Phe $_{\text{H}\alpha\text{trans}}$, m, 1H), 4.67 ($\text{Asn}_{\text{H}\alpha\text{trans}}$, m, 1H), 4.63 ($\text{Asn}_{\text{H}\alpha\text{cis}}$, m, 1H), 4.57 (4-SH-Phe $_{\text{H}\alpha\text{cis}}$, m, 1H), 4.41 ($\text{Pro}_{\text{H}\alpha\text{trans}}$, m, 1H), 4.28 ($\text{Thr}_{\text{H}\alpha\text{cis}}$, m, 1H), 4.20 ($\text{Thr}_{\text{H}\alpha\text{trans}}$, m, 1H), 4.11 ($\text{Thr}_{\text{H}\beta\text{cis}}$, m, 1H), 4.02 ($\text{Thr}_{\text{H}\beta\text{trans}}$, m, 1H), 3.91 ($\text{Pro}_{\text{H}\alpha\text{cis}}$, m, 1H), 3.78 ($\text{Pro}_{\text{H}\delta\text{trans}}$, m, 1H), 3.61 ($\text{Pro}_{\text{H}\delta\text{trans}}$, m, 1H), 3.48 ($\text{Pro}_{\text{H}\delta\text{cis}}$, m, 1H), 3.37 ($\text{Pro}_{\text{H}\delta\text{cis}}$, m, 1H), 3.13 (4-SH-Phe $_{\text{H}\beta\text{trans}}$), 3.01 (4-SH-Phe $_{\text{H}\beta\text{cis}}$), 2.93 (4-SH-Phe $_{\text{H}\beta\text{cis}}$), 2.87 (4-SH-Phe $_{\text{H}\beta\text{trans}}$), 2.84-2.77 ($\text{Asn}_{\text{H}\beta\text{trans}}$, m, 2H), 2.82-2.74 ($\text{Asn}_{\text{H}\beta\text{cis}}$, m, 2H), 2.27 ($\text{Pro}_{\text{H}\beta\text{trans}}$, m, 2H), 2.07 (Ac_{Hcis} , s, 3H), 2.04 ($\text{Ac}_{\text{Htrans}}$, s, 3H), 2.00 ($\text{Pro}_{\text{H}\gamma\text{trans}}$, m, 1H), 1.95 ($\text{Pro}_{\text{H}\beta\text{orycis}}$, m, 1H), 1.92 ($\text{Pro}_{\text{H}\gamma\text{trans}}$, m, 1H), 1.75 ($\text{Pro}_{\text{H}\beta\text{orycis}}$, m, 1H), 1.68 ($\text{Pro}_{\text{H}\beta\text{orycis}}$, m, 2H), 1.19 ($\text{Thr}_{\text{H}\gamma\text{cis}}$, d, $J = 7.0$ Hz, 3H), 1.09 ($\text{Thr}_{\text{H}\gamma\text{trans}}$, d, $J = 7.0$ Hz, 3H).

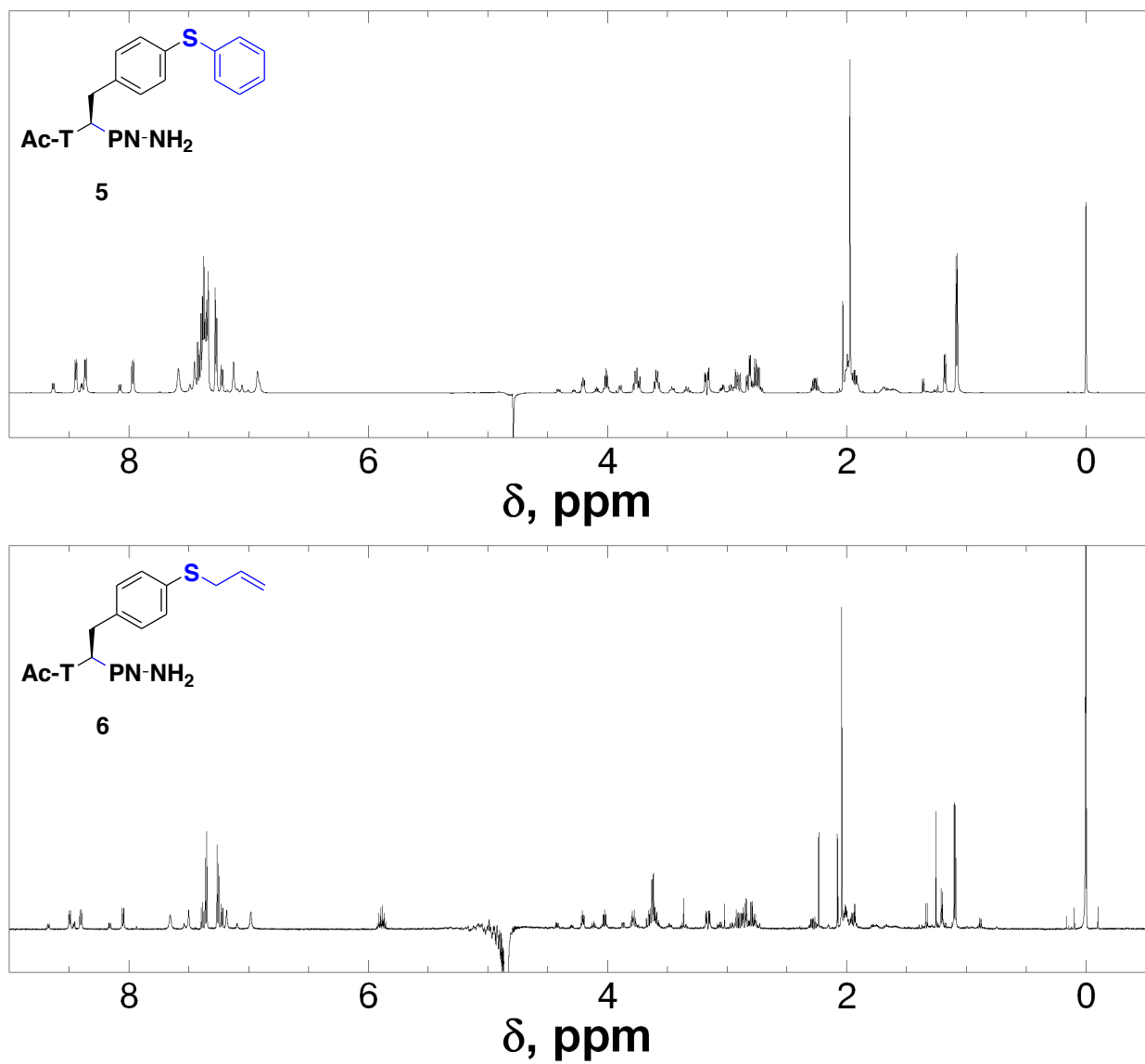


Figure S35. Full NMR spectra for peptides **5** and **6**. All samples contained 5 mM phosphate and 25 mM NaCl at pH 4.0.

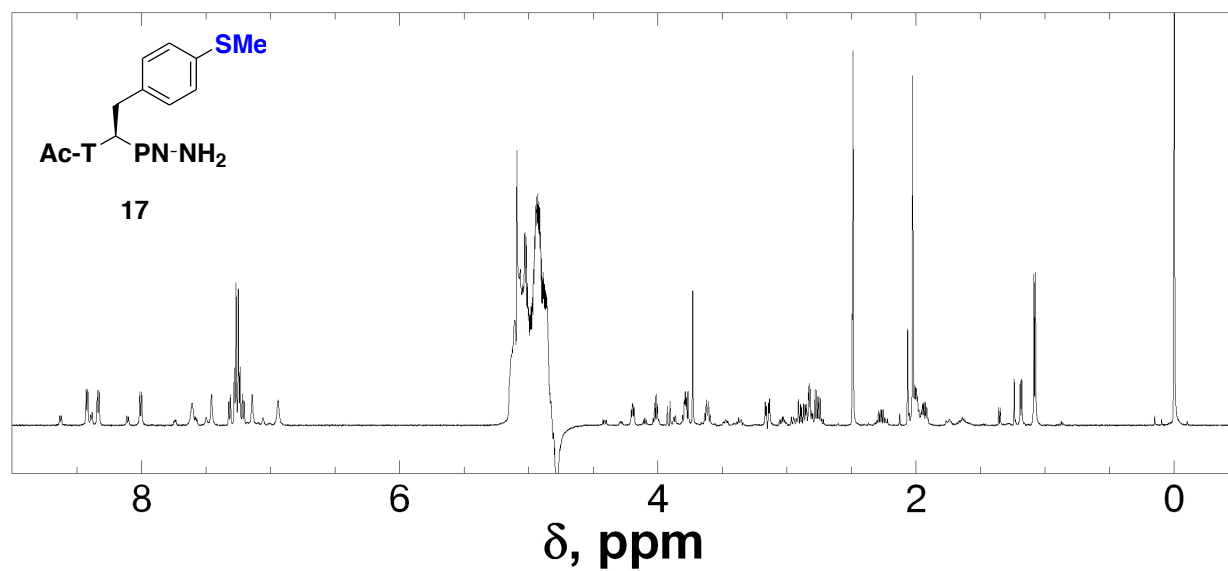
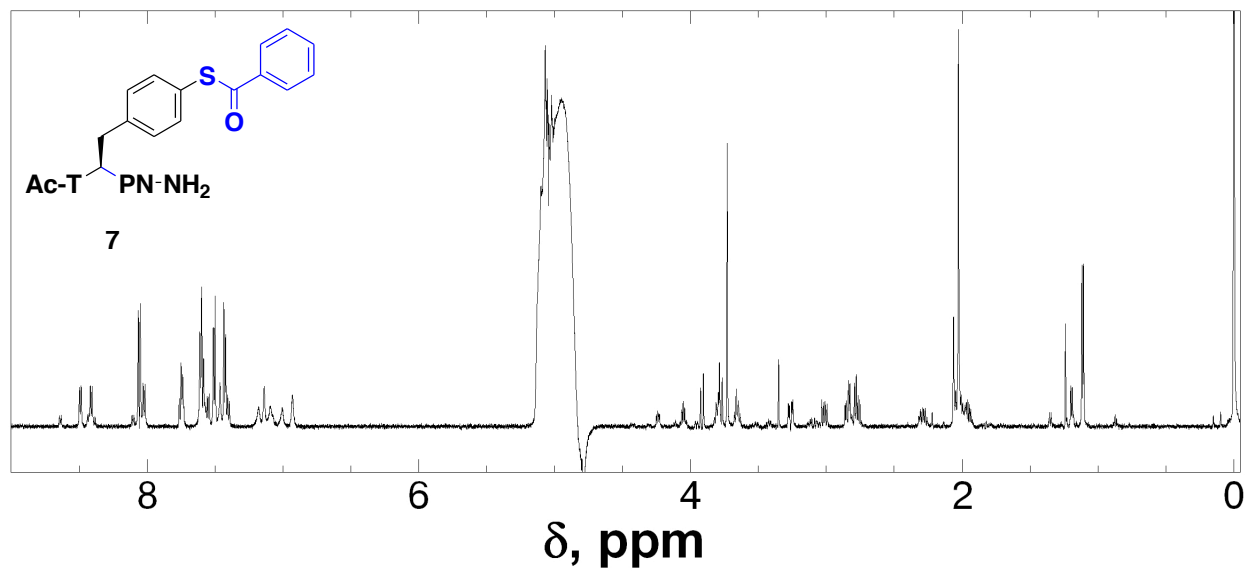


Figure S36. Full NMR spectra for peptides **7** and **17**. All samples contained 5 mM phosphate and 25 mM NaCl at pH 4.0.

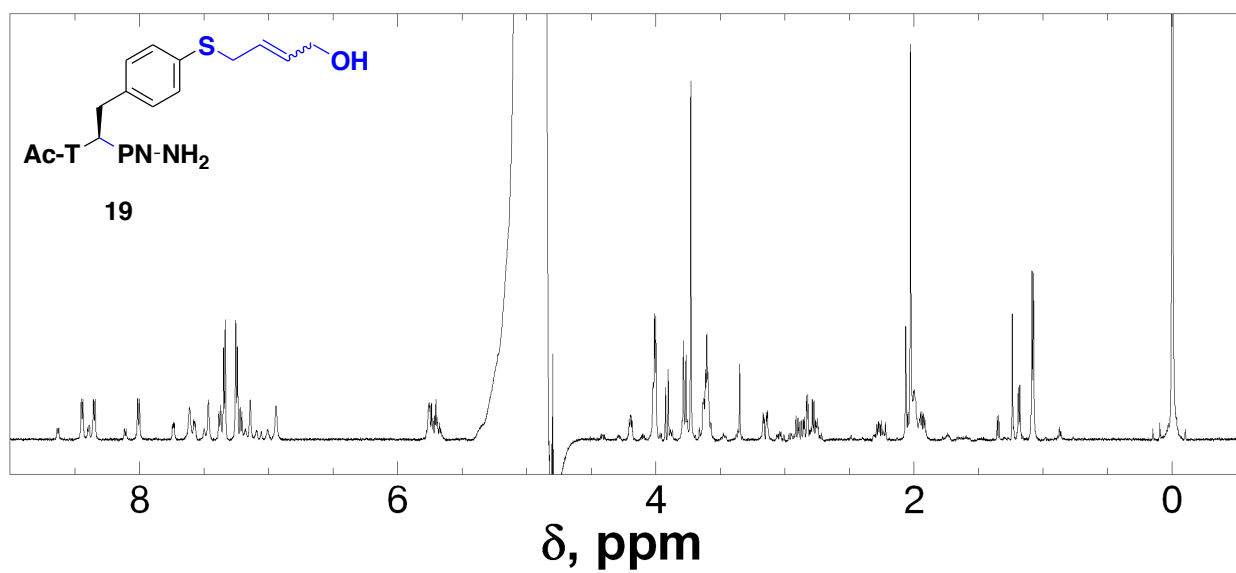
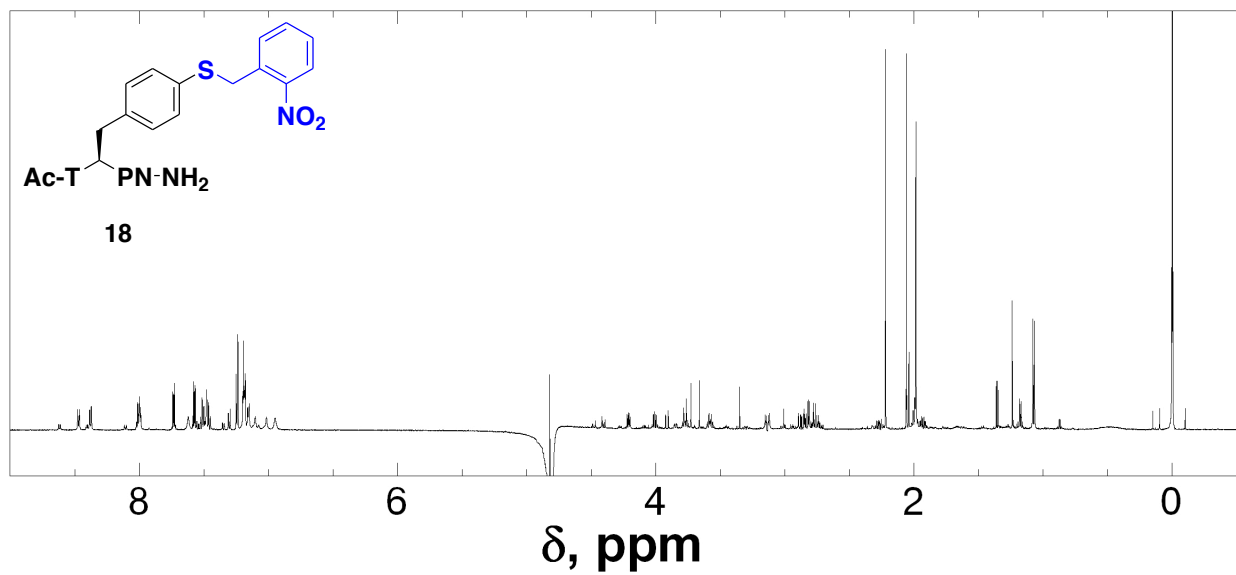


Figure S37. Full NMR spectra for peptides **18** and **19**. All samples contained 5 mM phosphate and 25 mM NaCl at pH 4.0.

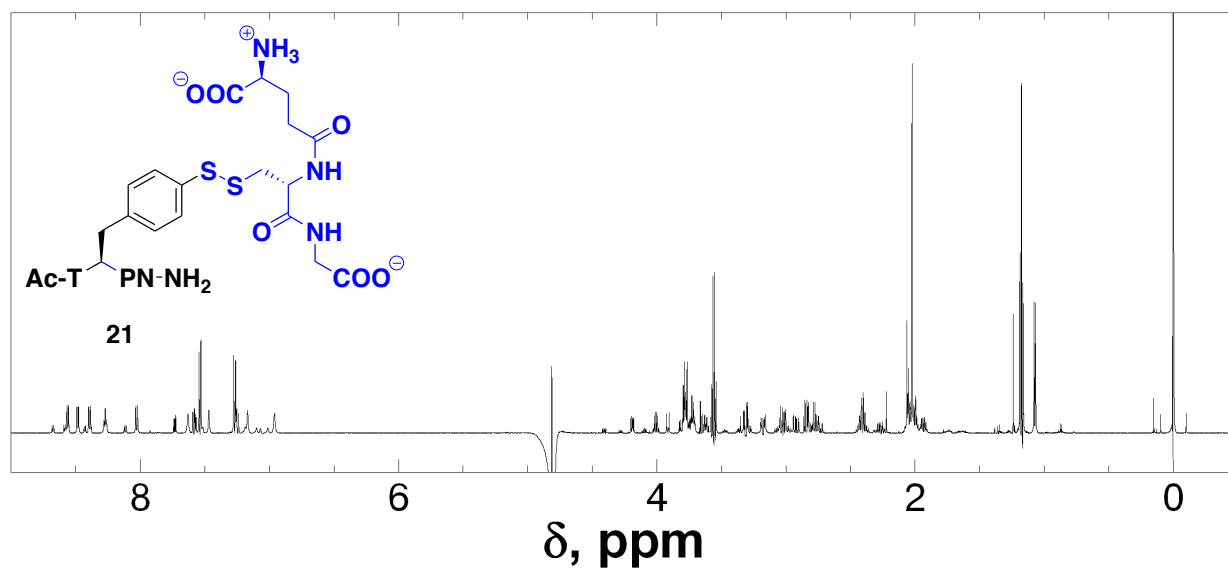
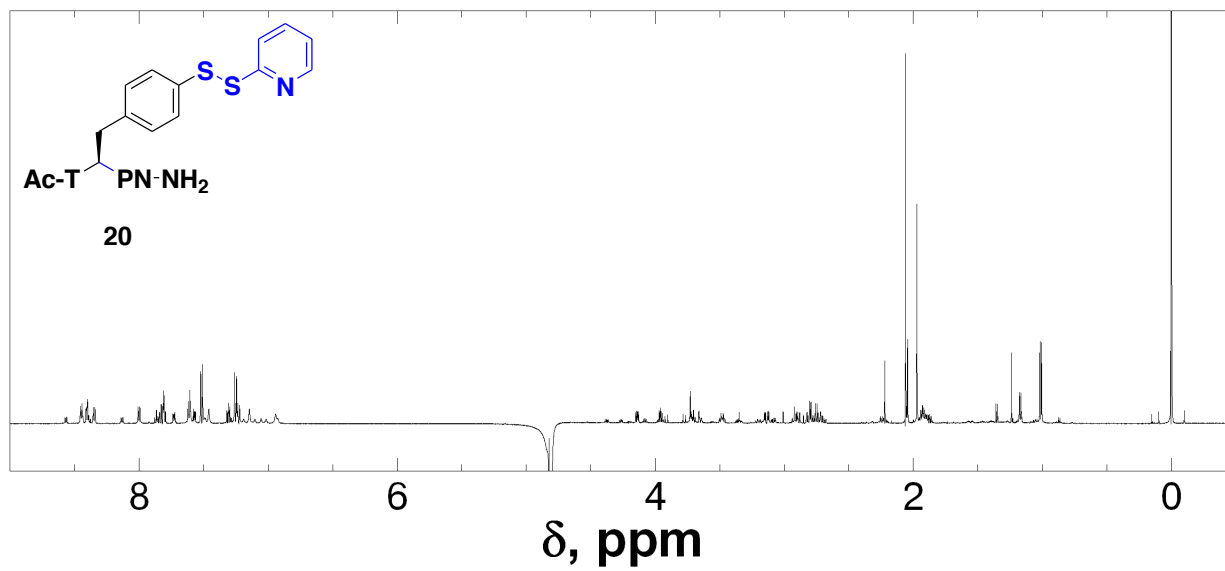


Figure S38. Full NMR spectra for peptides **20** and **21**. All samples contained 5 mM phosphate and 25 mM NaCl at pH 4.0.

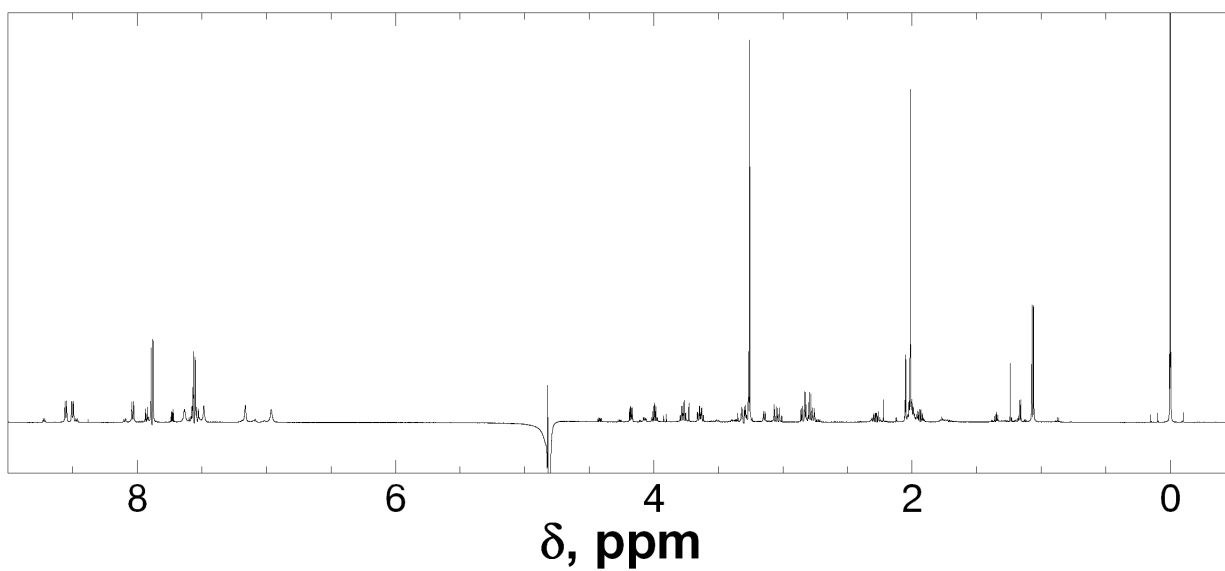
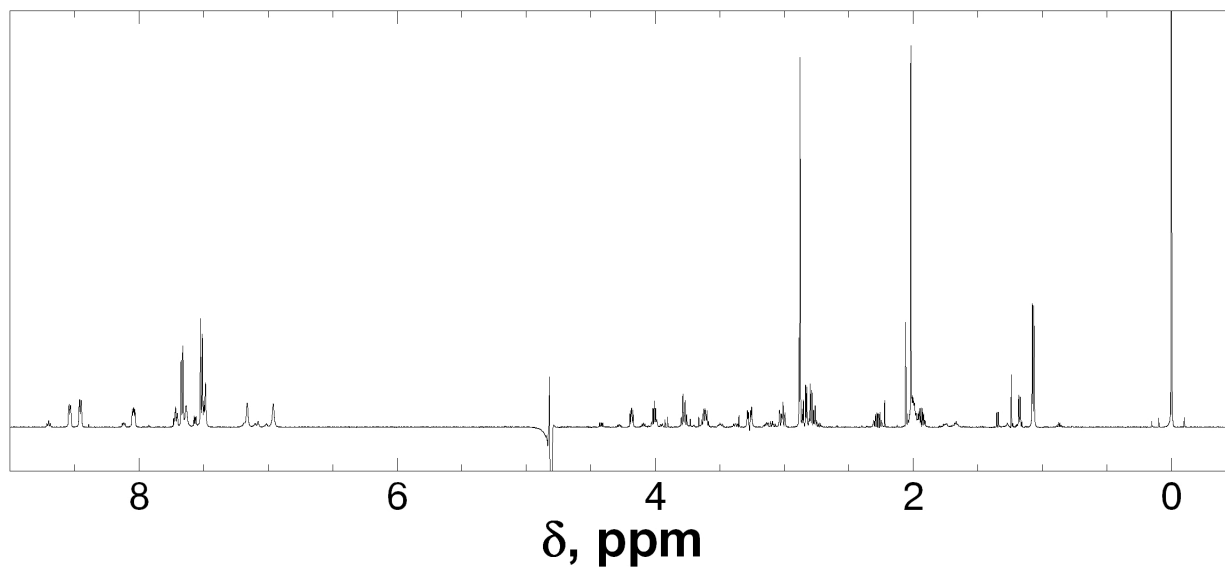


Figure S39. Full NMR spectra for peptides **22** and **23**. Peptide **22**

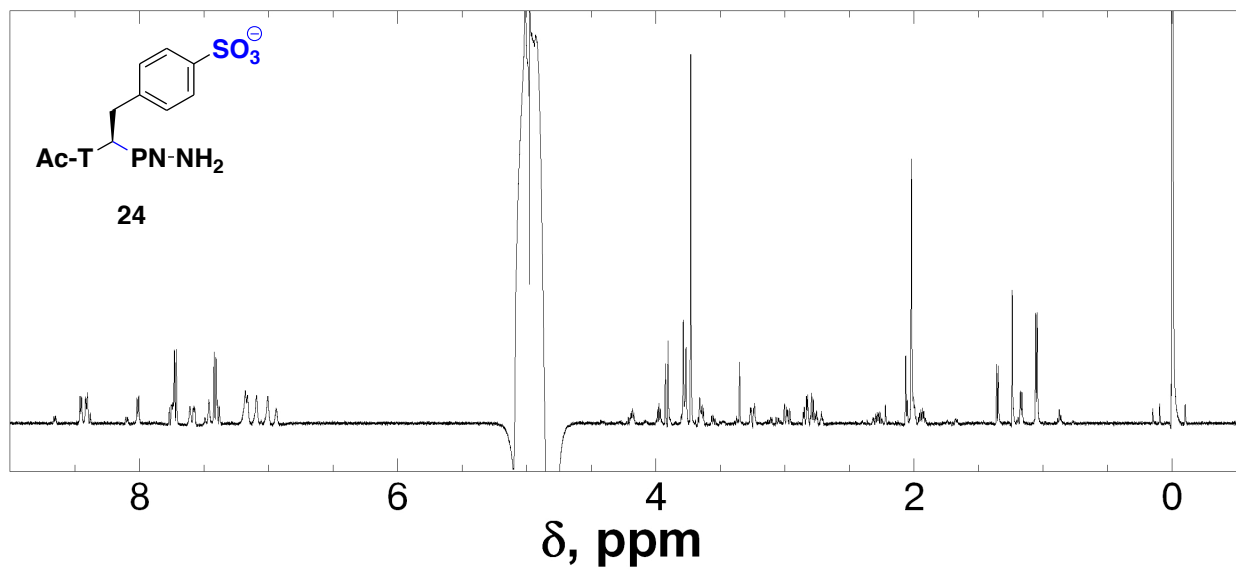


Figure S40. Full NMR spectrum for peptide **24**. The solution contains 5 mM phosphate and 25 mM NaCl at pH 4.0.

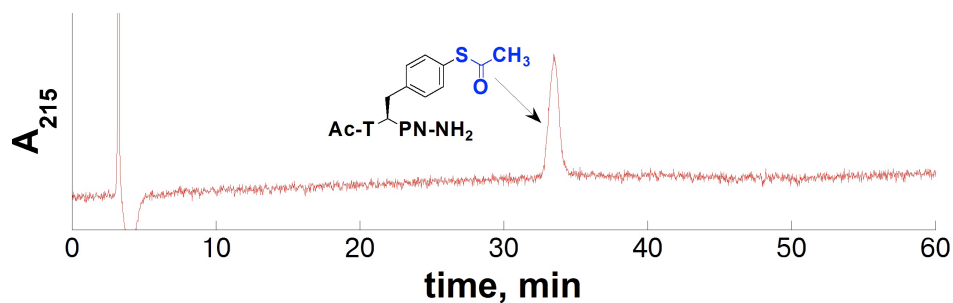


Figure S41. Reinjection of the purified peptide Ac-T(4-thioacetyl-Phe)PN-NH₂ (**2**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

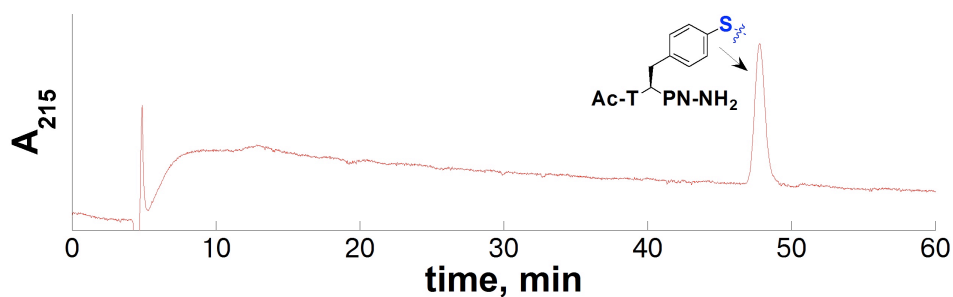


Figure S42. Reinjection of the purified thiophenylalanine disulfide peptide (**3**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

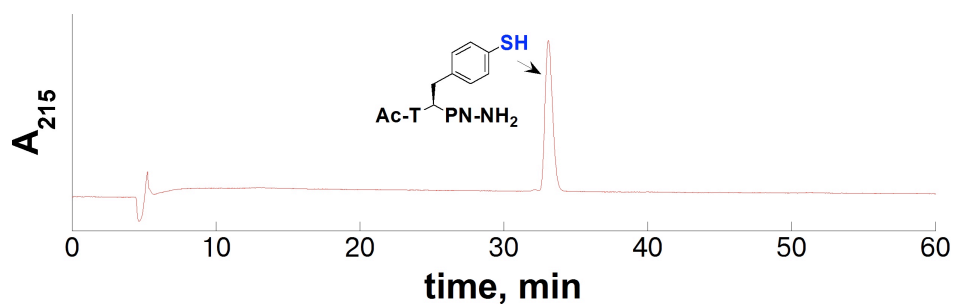


Figure S43. Reinjection of the purified peptide Ac-T(4-SH-Phe)PN-NH₂ (**4**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

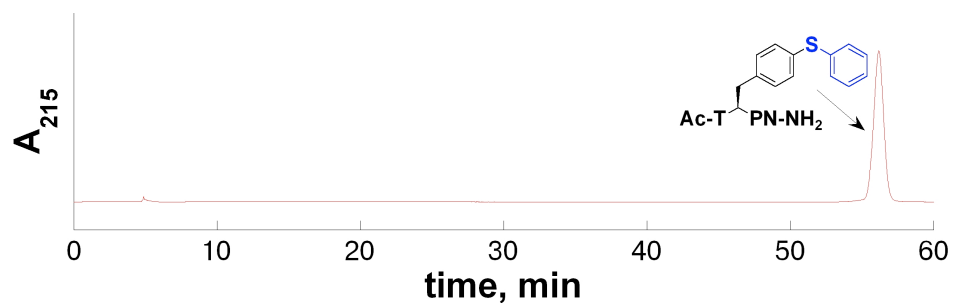


Figure S44. Reinjection of the purified peptide Ac-T(4-thiophenyl-Phe)PN-NH₂ (**5**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

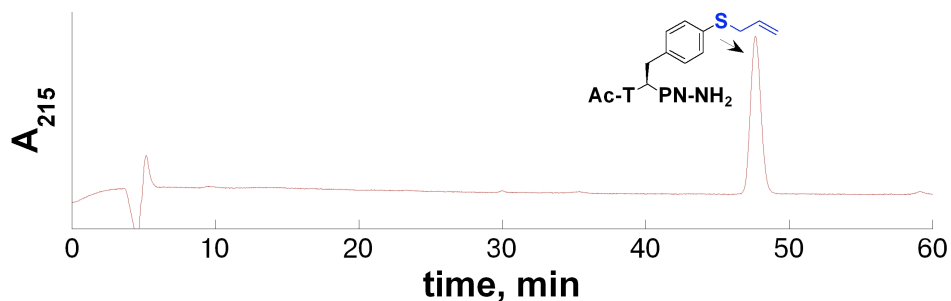


Figure S45. Reinjection of the purified peptide Ac-T(4-S-allyl-Phe)PN-NH₂ (6) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

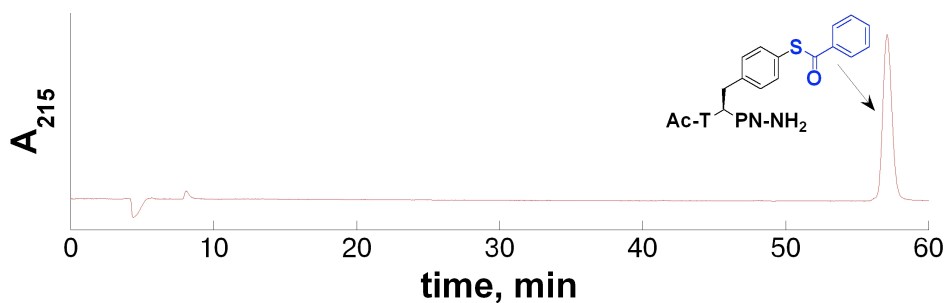


Figure S46. Reinjection of the purified peptide Ac-T(4-thiobenzoyl-Phe)PN-NH₂ (7) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

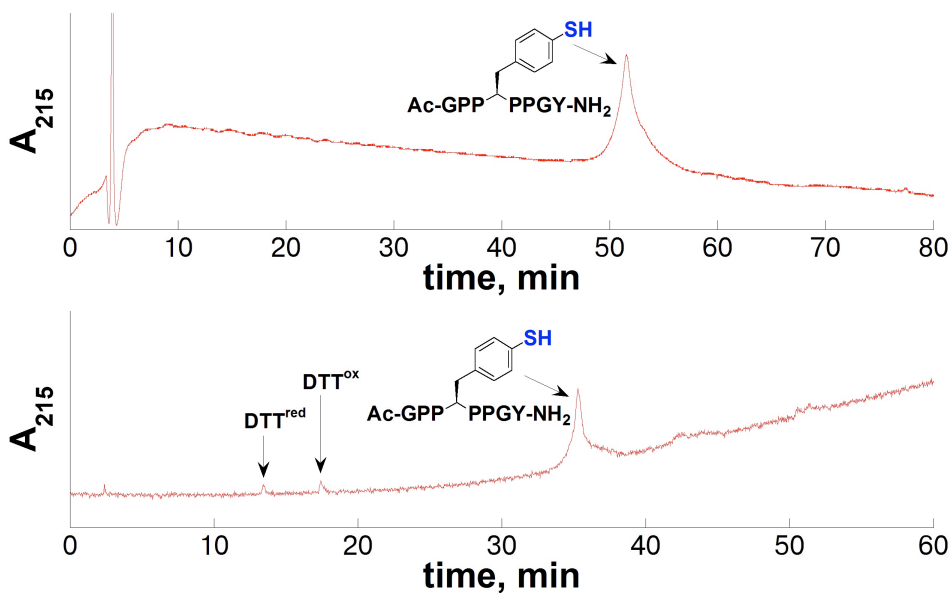


Figure S47. Reinjection of the purified peptide Ac-GPP(4-SH-Phe)PN-NH₂ (12) using a gradient of 0-55% buffer B in buffer A over 80 minutes (top); reinjection of the purified peptide Ac-GPP(4-SH-Phe)PN-NH₂ (12) with DTT (1 mM) using a gradient of 0-100% methanol in water over 60 minutes (bottom). Residual peaks at 40-55 minutes were present in blank chromatograms.

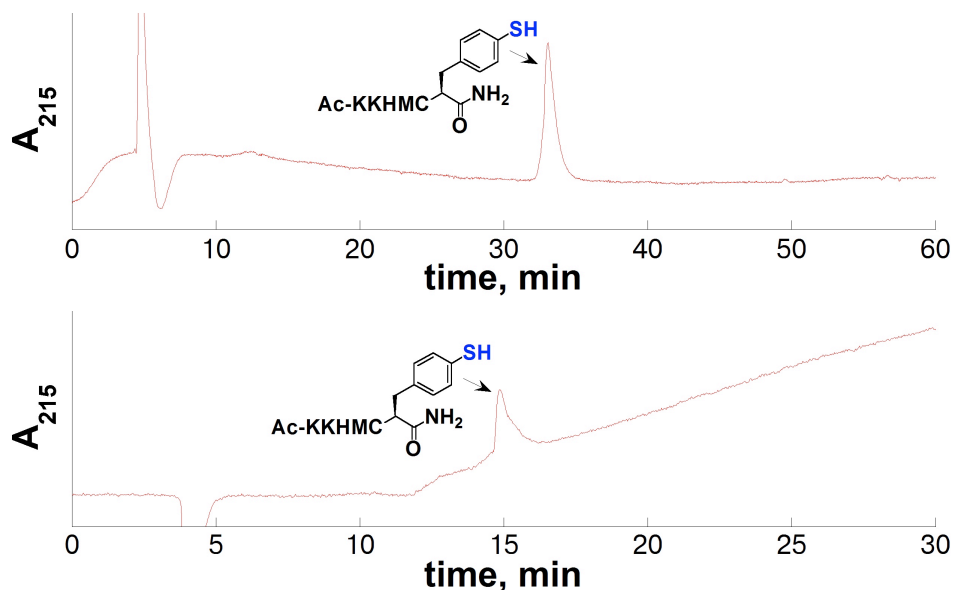


Figure S48. Reinjection of the purified peptide Ac-KKHMC(4-SH-Phe)-NH₂ (**13**) using a gradient of 0-70% buffer B in buffer A over 60 minutes (top); reinjection of the purified peptide Ac-KKHMC(4-SH-Phe)-NH₂ (**13**) using a Varian Microsorb MV C4 analytical column (250 × 4.6 mm, 5 μm particle, 300 Å pore) with a gradient of 0-100% buffer B' (20% water, 80% MeCN, 0.1% heptafluorobutyric acid (HFB)) in buffer A' (98% water, 2% MeCN, 0.1% HFB) over 30 minutes (bottom).

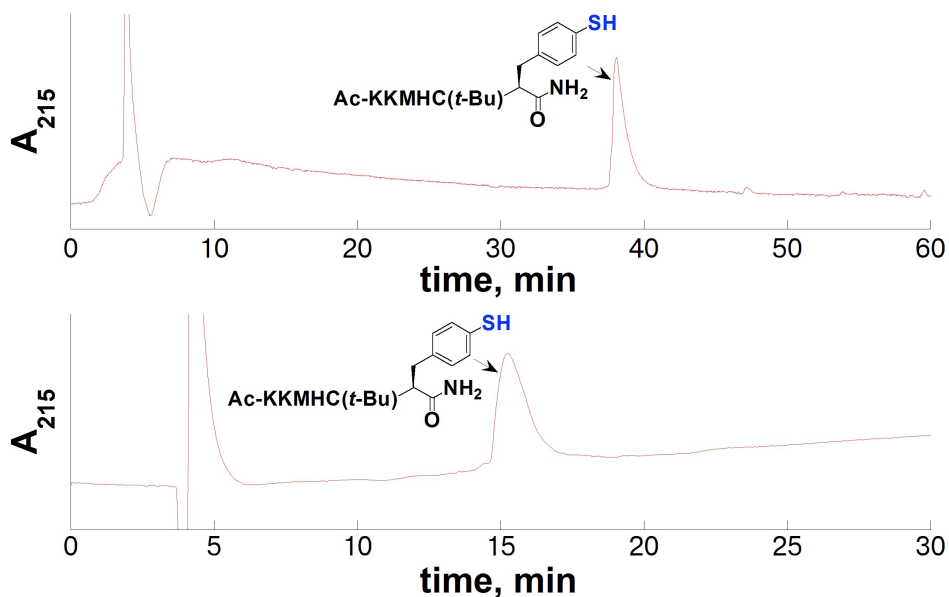


Figure S49. Reinjection of the purified peptide Ac-KKHMC(*t*Bu)(4-SH-Phe)-NH₂ (**14**) using a gradient of 0-75% buffer B in buffer A over 60 minutes (top); reinjection of the purified peptide Ac-KKHMC(*t*Bu)(4-SH-Phe)-NH₂ (**14**) using a Varian Microsorb MV C4 analytical column (250 × 4.6 mm, 5 μm particle, 300 Å pore) with a gradient of 0-100% buffer B' in buffer A' over 30 minutes (bottom).

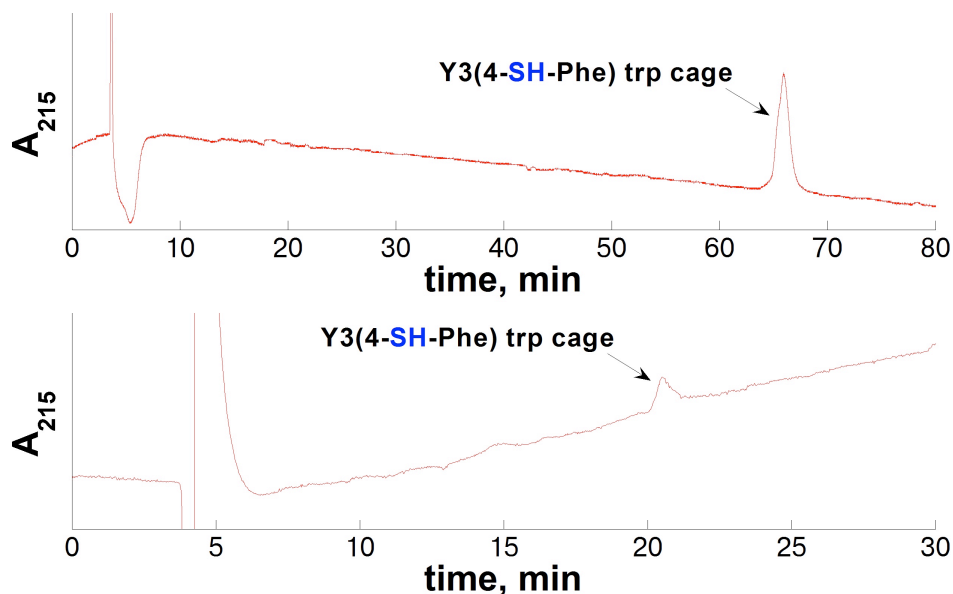


Figure S50. Reinjection of the purified Y3(4-SH-Phe) trp cage peptide (**15**) using a gradient of 0-70% buffer B in buffer A over 80 minutes (top); reinjection of the purified peptide Y3(4-SH-Phe) trp cage peptide (**15**) using a Varian Microsorb MV C4 analytical column (250 × 4.6 mm, 5 μm particle, 300 Å pore) with a gradient of 0-100% buffer B' in buffer A' over 30 minutes (bottom).

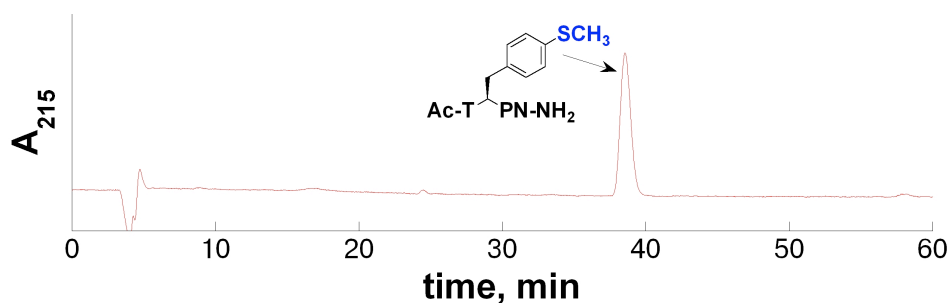


Figure S51. Reinjection of the purified peptide Ac-T(4-SMe-Phe)PN-NH₂ (**17**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

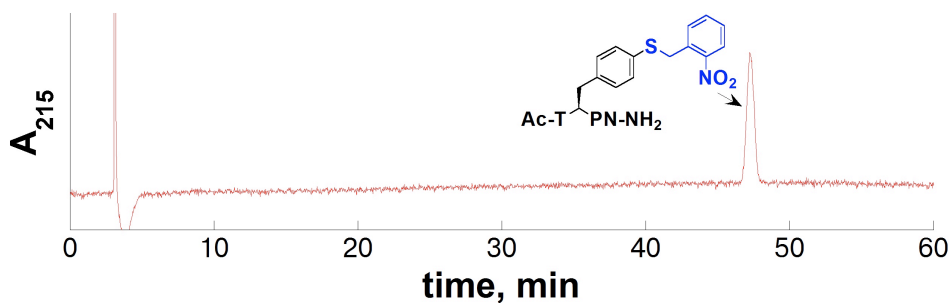


Figure S52. Reinjection of the purified peptide Ac-T(4-S-(2-nitrobenzyl)-Phe)PN-NH₂ (**18**) using a gradient of 0-50% buffer B in buffer A over 60 minutes.

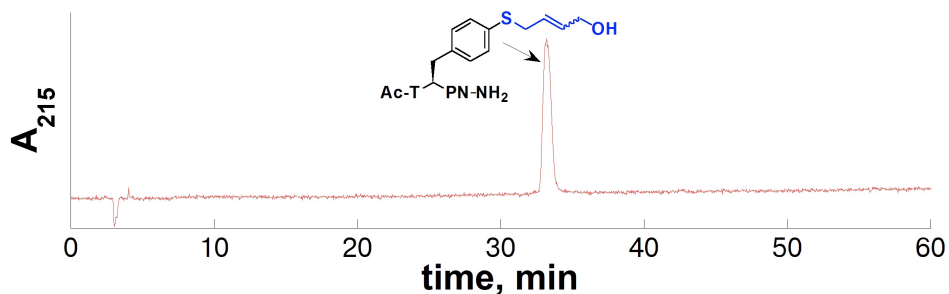


Figure S53. Reinjection of the purified peptide Ac-T(4-(SCH₂CH=CHCH₂OH)-Phe)PN-NH₂ (**19**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

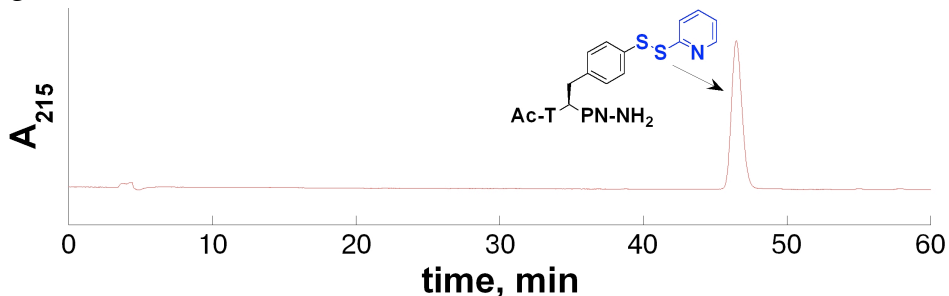


Figure S54. Reinjection of the purified peptide Ac-T(4-S-SPy-Phe)PN-NH₂ (**20**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

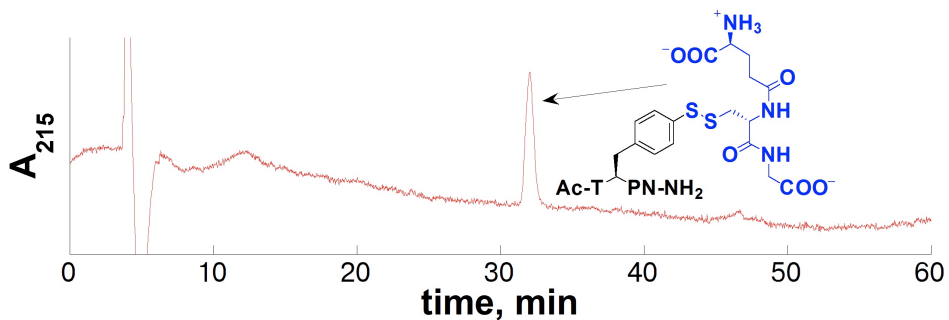


Figure S55. Reinjection of the purified peptide Ac-T(4-(glutathione disulfide)S-Phe)PN-NH₂ (**21**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

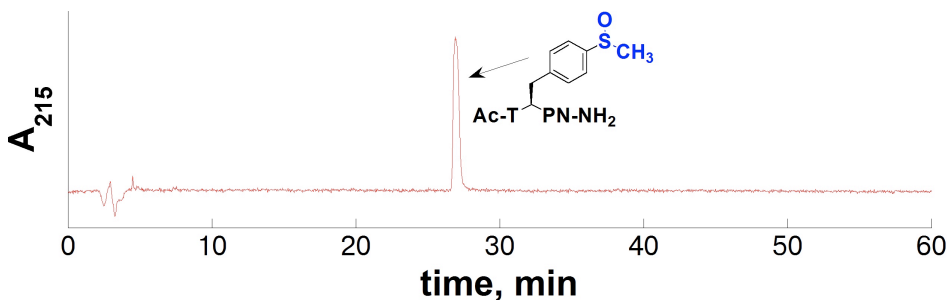


Figure S56. Reinjection of the purified peptide Ac-T(4-S(O)Me-Phe)PN-NH₂ (**22**) using a gradient of 0-20% buffer B in buffer A over 60 minutes.

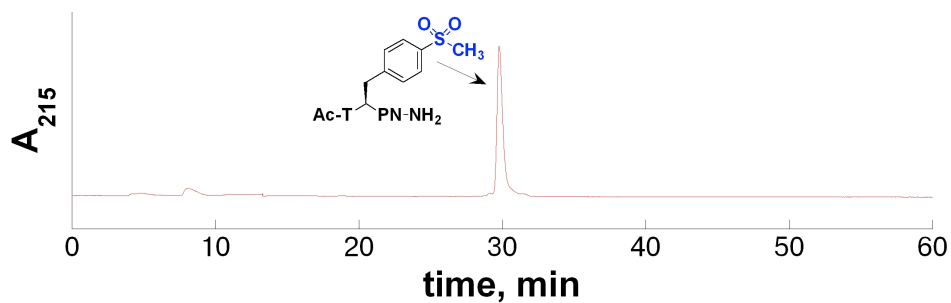


Figure S57. Reinjection of the purified peptide Ac-T(4-SO₂Me-Phe)PN-NH₂ (**23**) using a gradient of 0-20% buffer B in buffer A over 60 minutes.

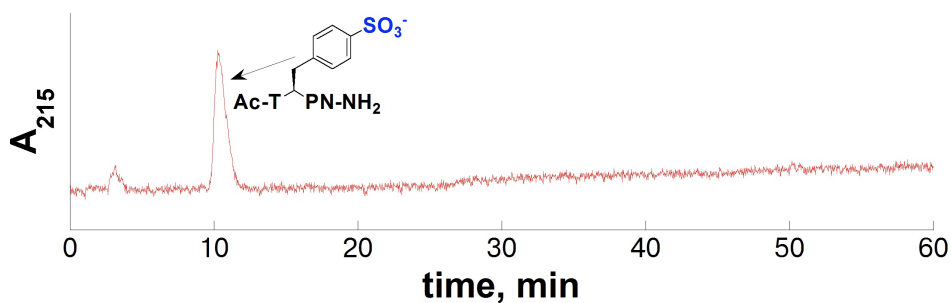


Figure S58. Reinjection of the purified peptide Ac-T(4-SO₃⁻-Phe)PN-NH₂ (**24**) using isocratic buffer A for 20 minutes followed by a gradient of 0-45% buffer B in buffer A over 40 minutes.

CF0111B-35 #10-27 RT: 0.23-0.66 AV: 18 NL: 2.98E6
T: + c ESI Full ms [100.00-2000.00]

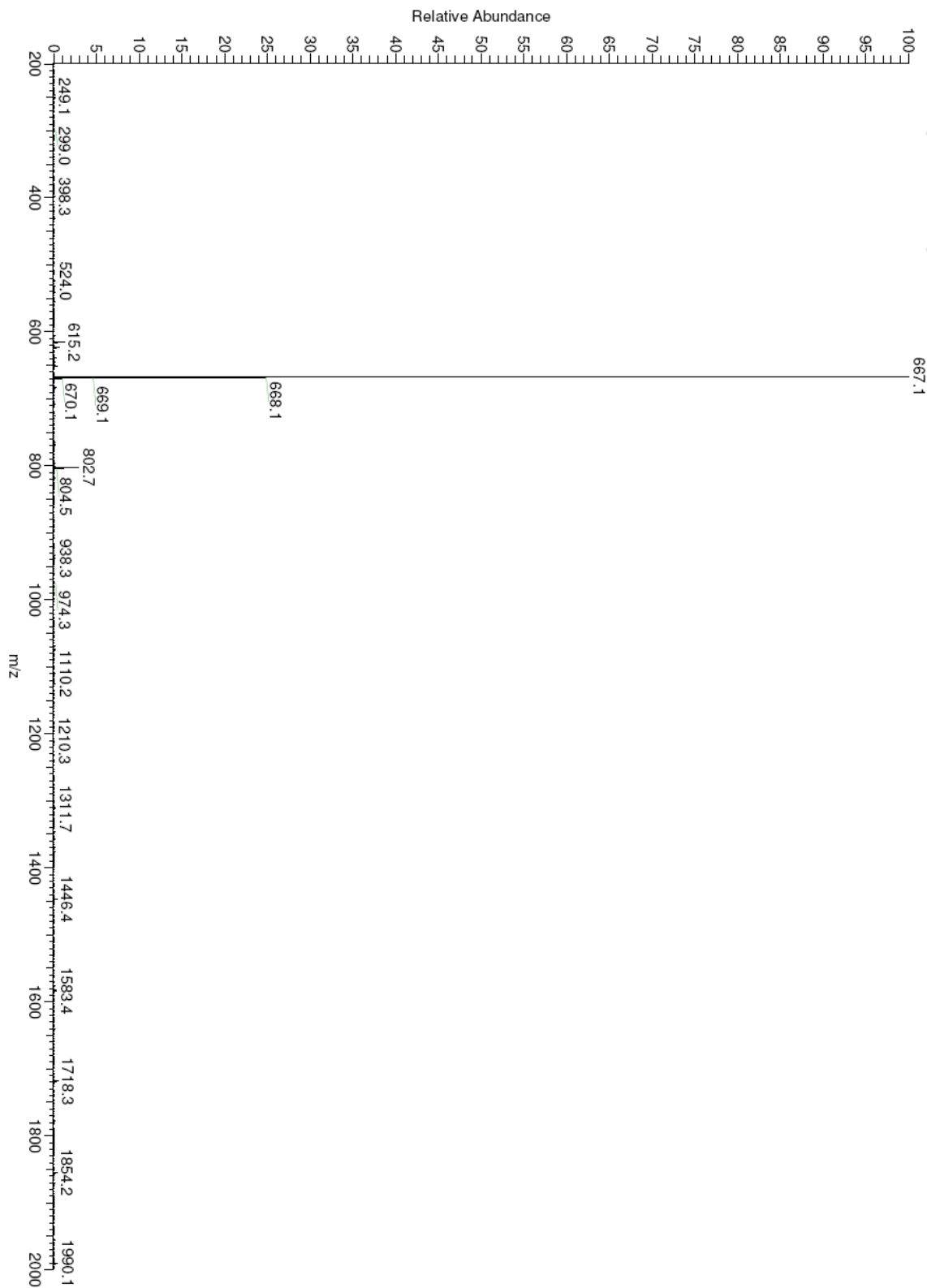


Figure S59. Mass spectrum of the peptide Ac-T(4-I-Phe)PN-NH₂ (1).

CF0111B-30 #10-28 RT: 0.23-0.69 AV: 19 NL: 2.39E6
T: + c ESI Full ms [100.00-2000.00]

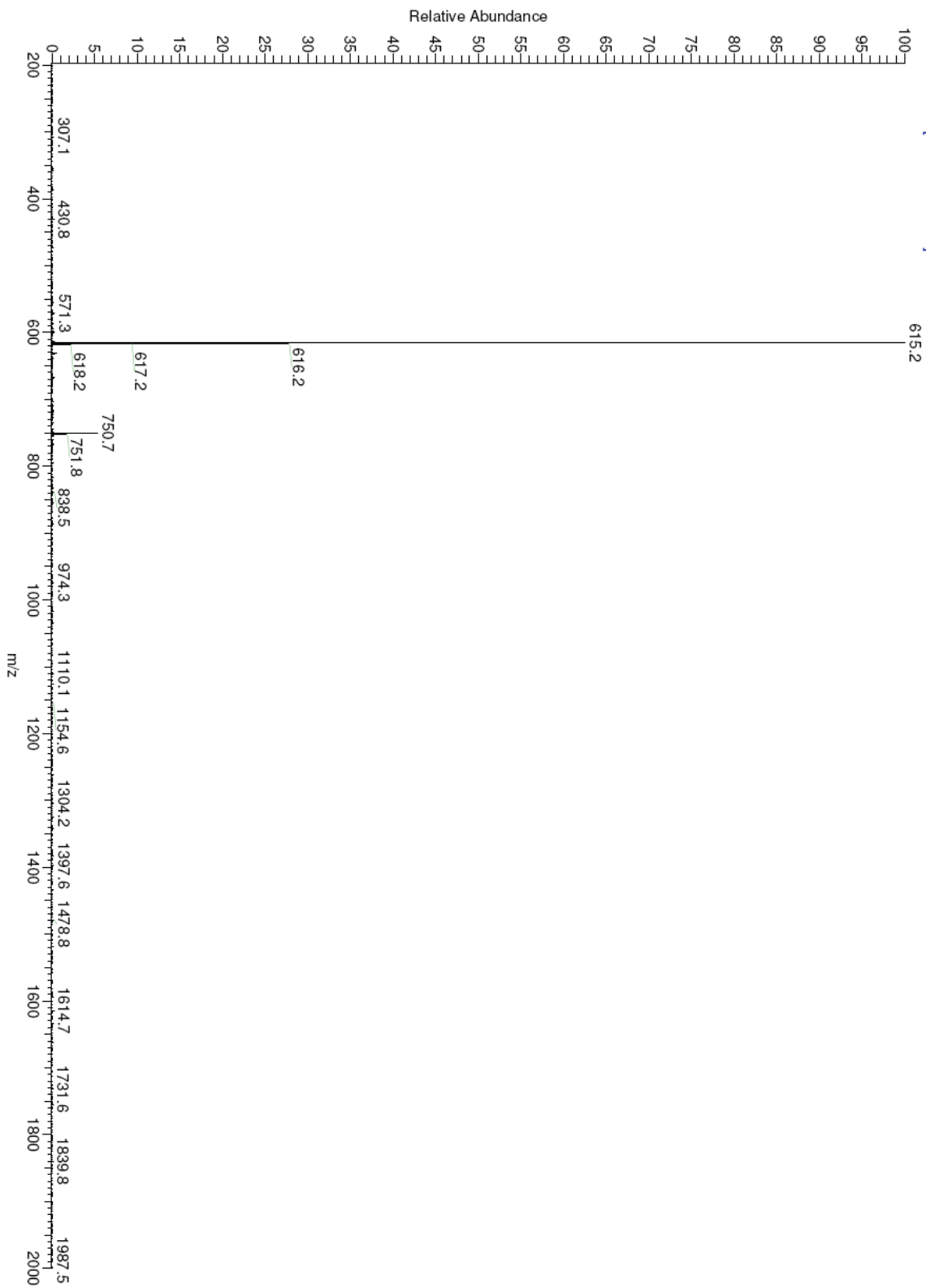


Figure S60. Mass spectrum of the peptide Ac-T(4-thioacetyl-Phe)PN-NH₂ (**2**).

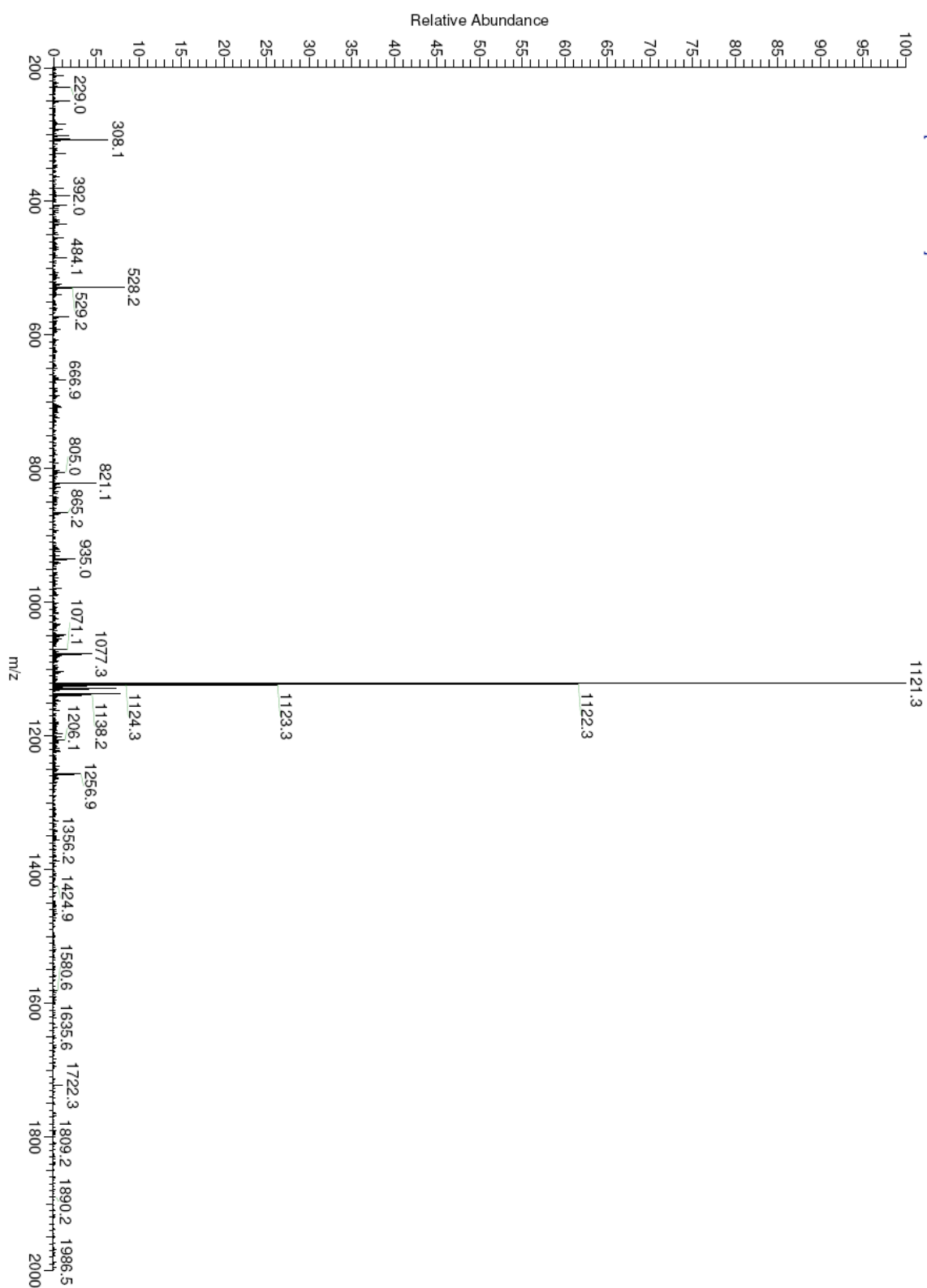
CF01147C-48 #11-29 RT: 0.26-0.71 AV: 19 NL: 5.98E5
T: + c ESI Full ms [100.00-2000.00]

Figure S61. Mass spectrum of the thiophenylalanine disulfide peptide (**3**).

CF01111C-38 #11-24 RT: 0.25-0.58 AV: 14 NL: 8.03E4
T: + c ESI Full ms [100.00-2000.00]

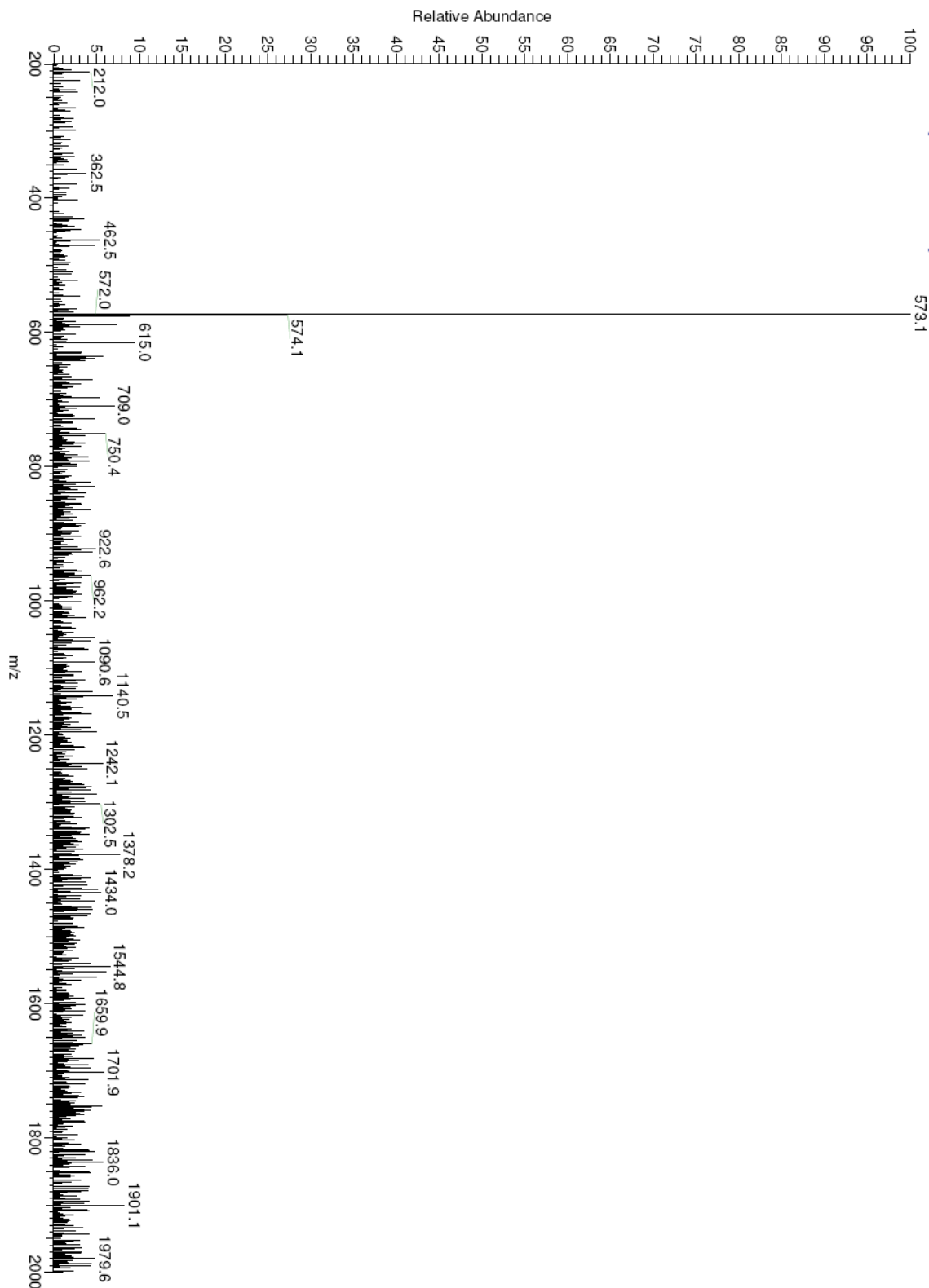


Figure S62. Mass spectrum of the peptide Ac-T(4-SH-Phe)PN-NH₂ (4).

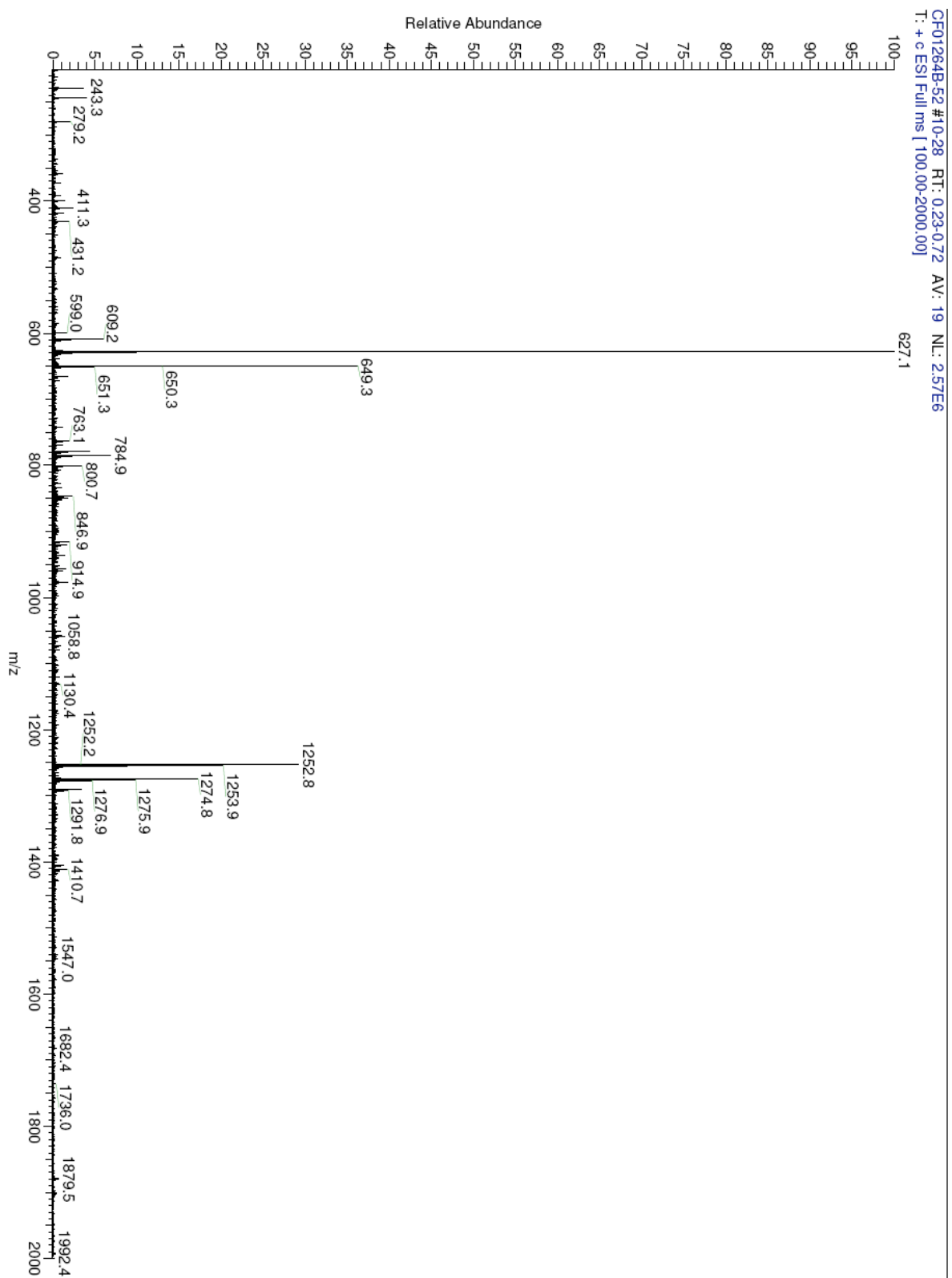


Figure S63. Mass spectrum of the peptide Ac-T(4-thiophenyl-Phe)PN-NH₂ (**5**).

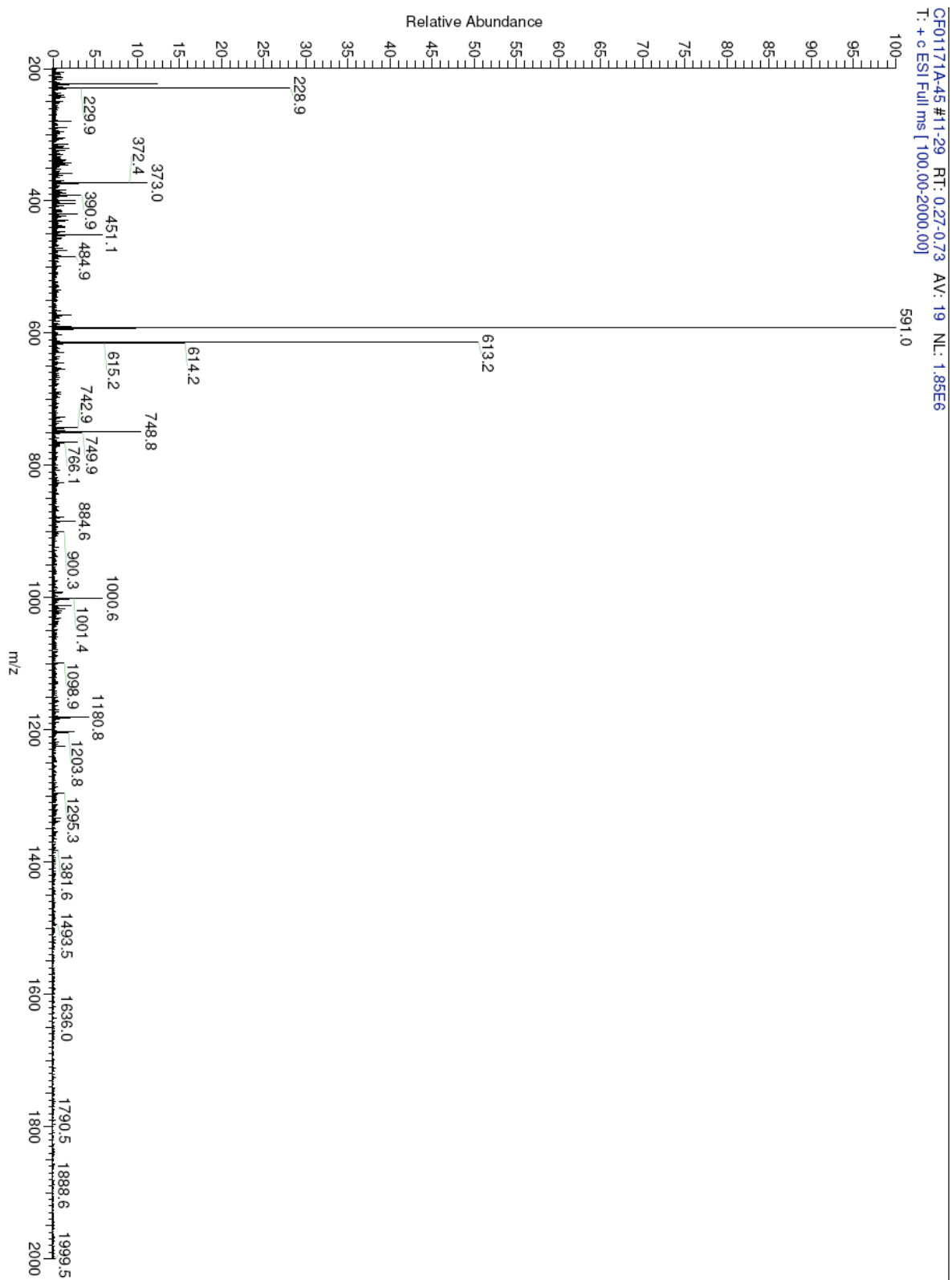


Figure S64. Mass spectrum of the peptide Ac-T(4-S-allyl-Phe)PN-NH₂ (**6**).

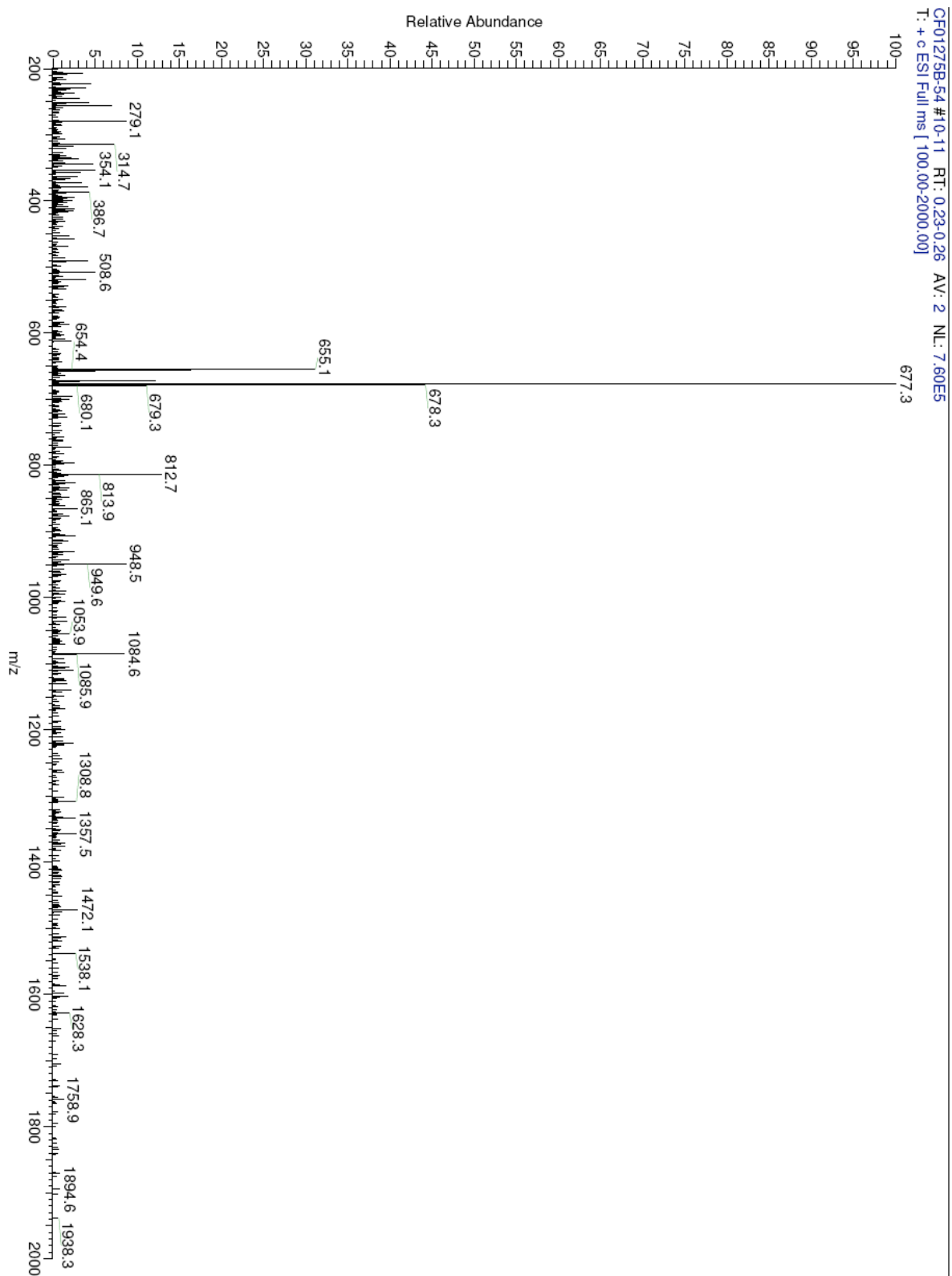


Figure S65. Mass spectrum of the peptide Ac-T(4-thiobenzoyl-Phe)PN-NH₂ (7).

CF01173B-70 #10-27 RT: 0.25-0.68 AV: 18 NL: 7.09E6
T: + c ESI Full ms [100.00-2000.00]

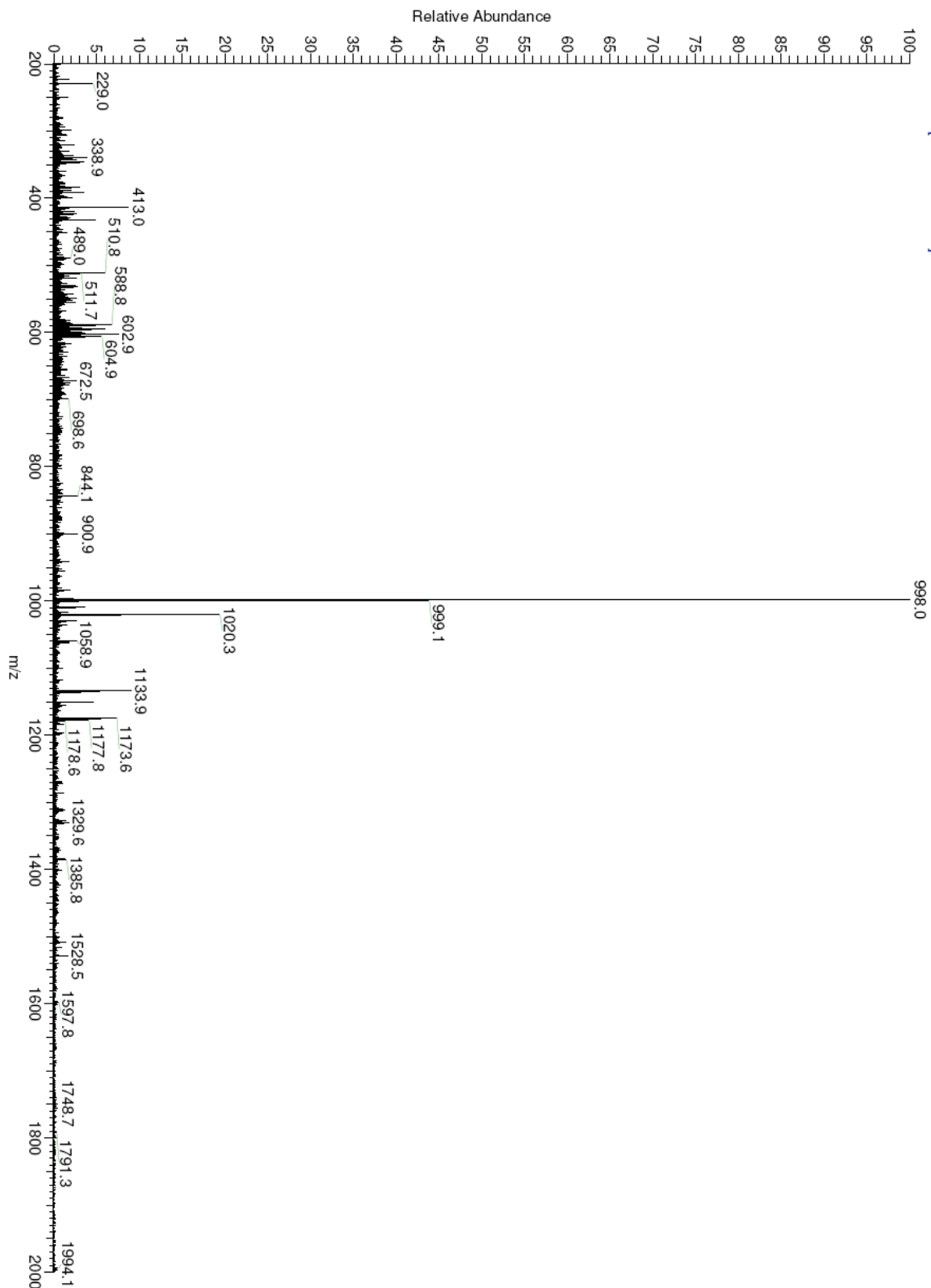


Figure S66. Mass spectrum of the peptide Ac-GPP(4-I-Phe)PPGY-NH₂ (8).

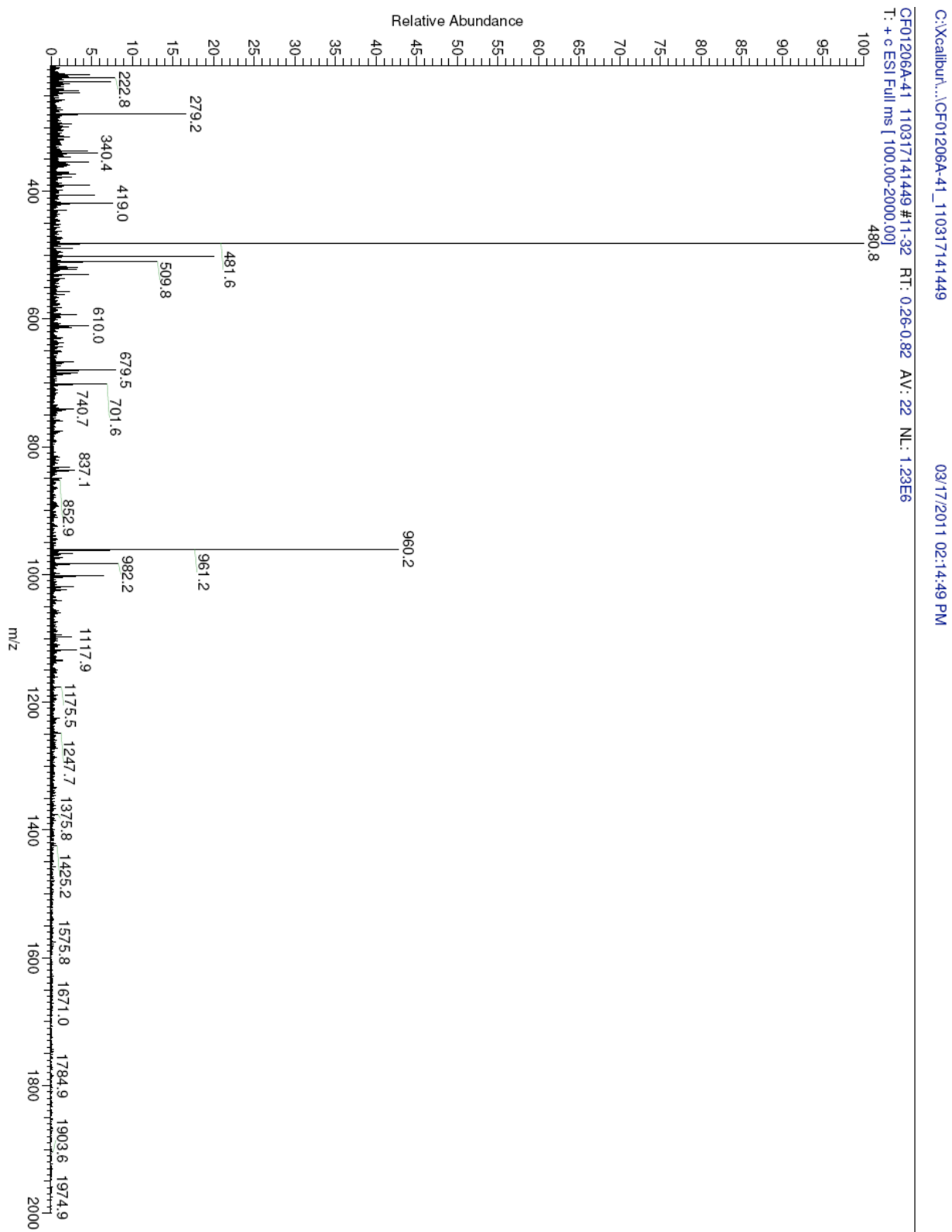


Figure S67. Mass spectrum of the peptide Ac-KKHMC(4-I-Phe)-NH₂ (**9**).

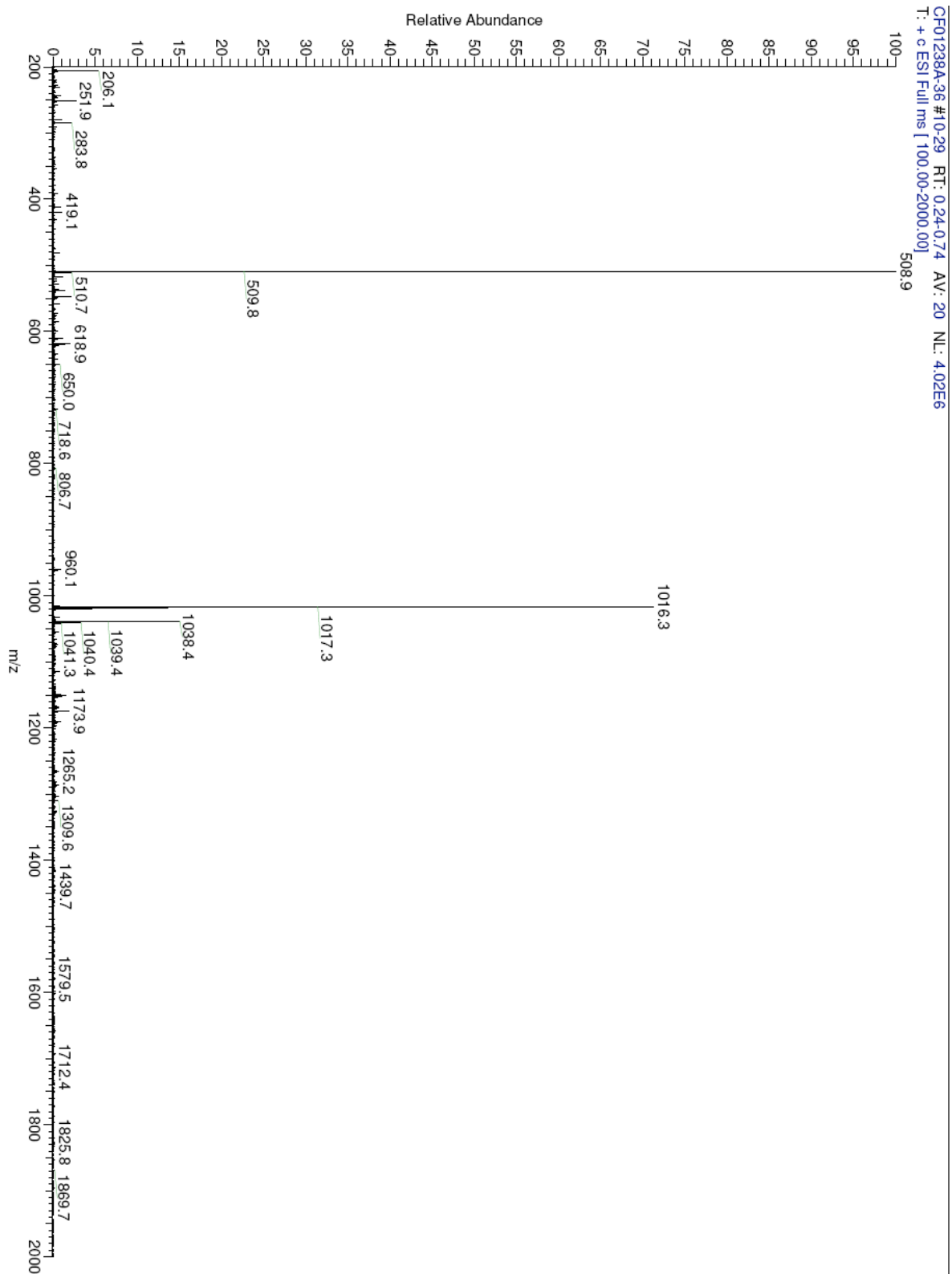


Figure S68. Mass spectrum of the peptide Ac-KKHMC(*t*Bu)(4-I-Phe)-NH₂ (**10**).

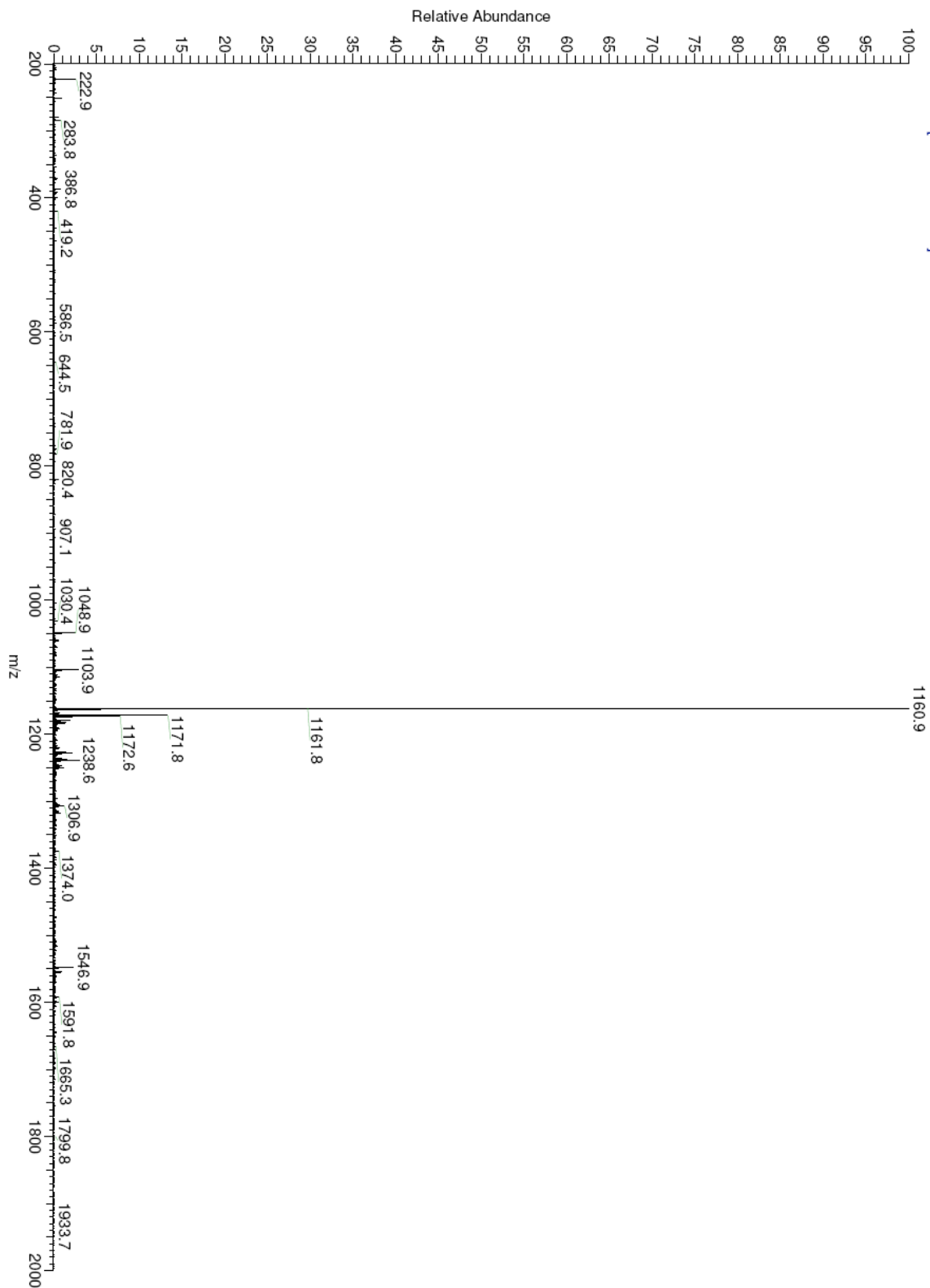
CF01272H-65 #10-30 RT: 0.23-0.75 AV: 21 NL: 2.81E6
T: + c ESI Full ms [100.00-2000.00]

Figure S69. Mass spectrum of the Y3(4-I-Phe) trp cage peptide (**11**).

CF01175C-41 110125181501 #12-18 RT: 0.29-0.44 AV: 7 NL: 5.16E5
T: + c ESI Full ms [100.00-2000.00]

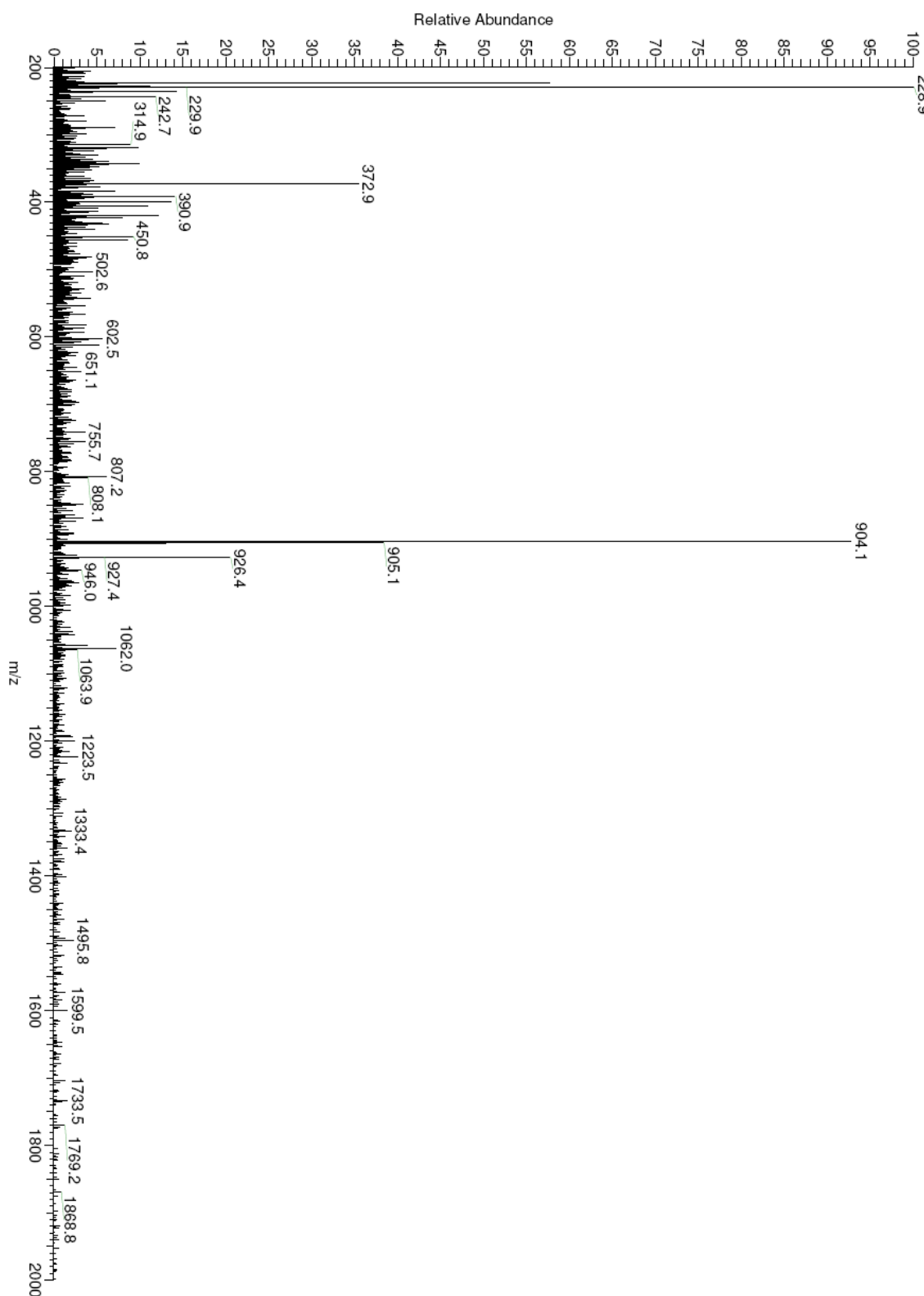


Figure S70. Mass spectrum of the peptide Ac-GPP(4-SH-Phe)PPGY-NH₂ (12).

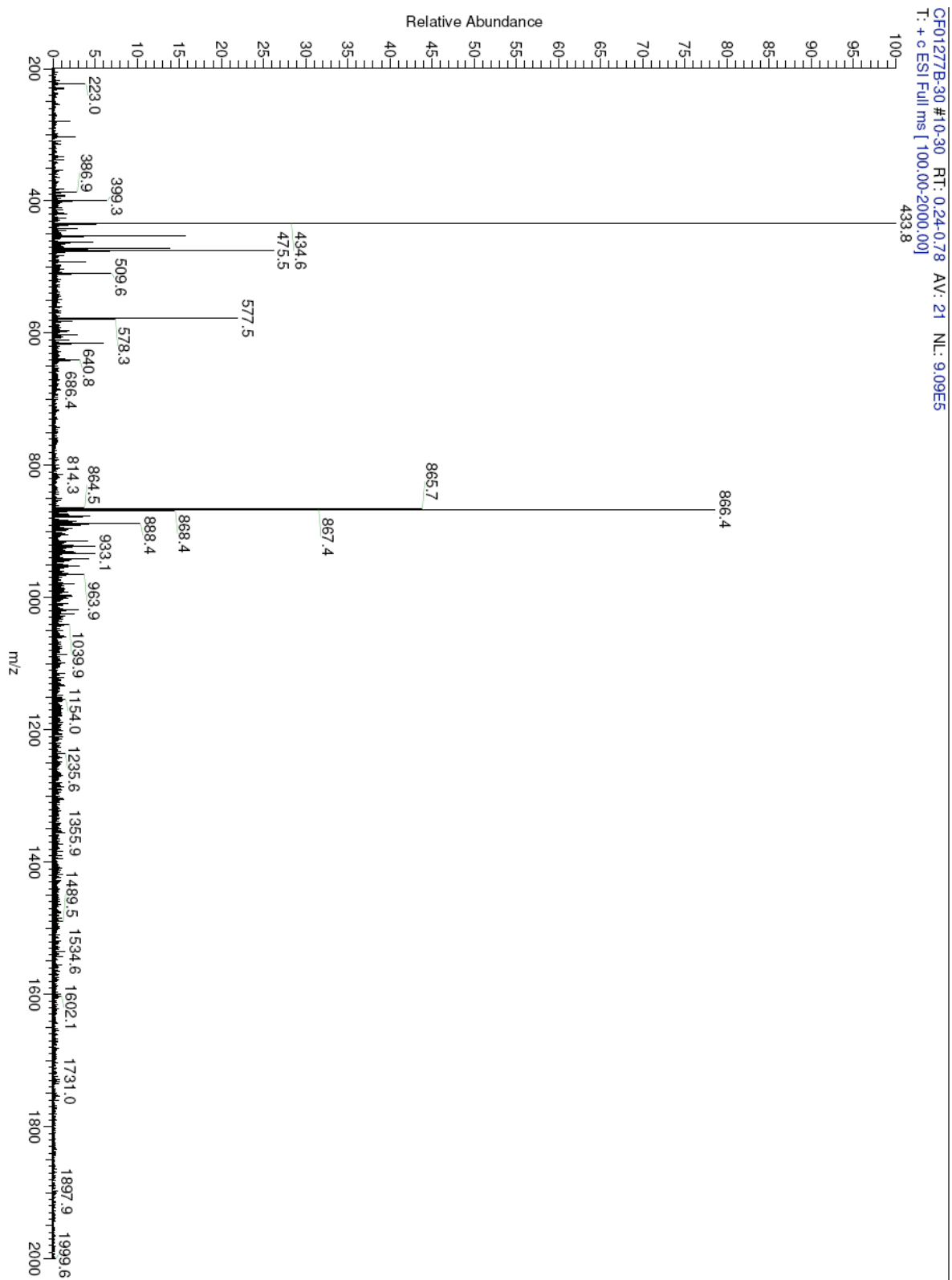


Figure S71. Mass spectrum of the peptide Ac-KKHMC(4-SH-Phe)-NH₂ (**13**).

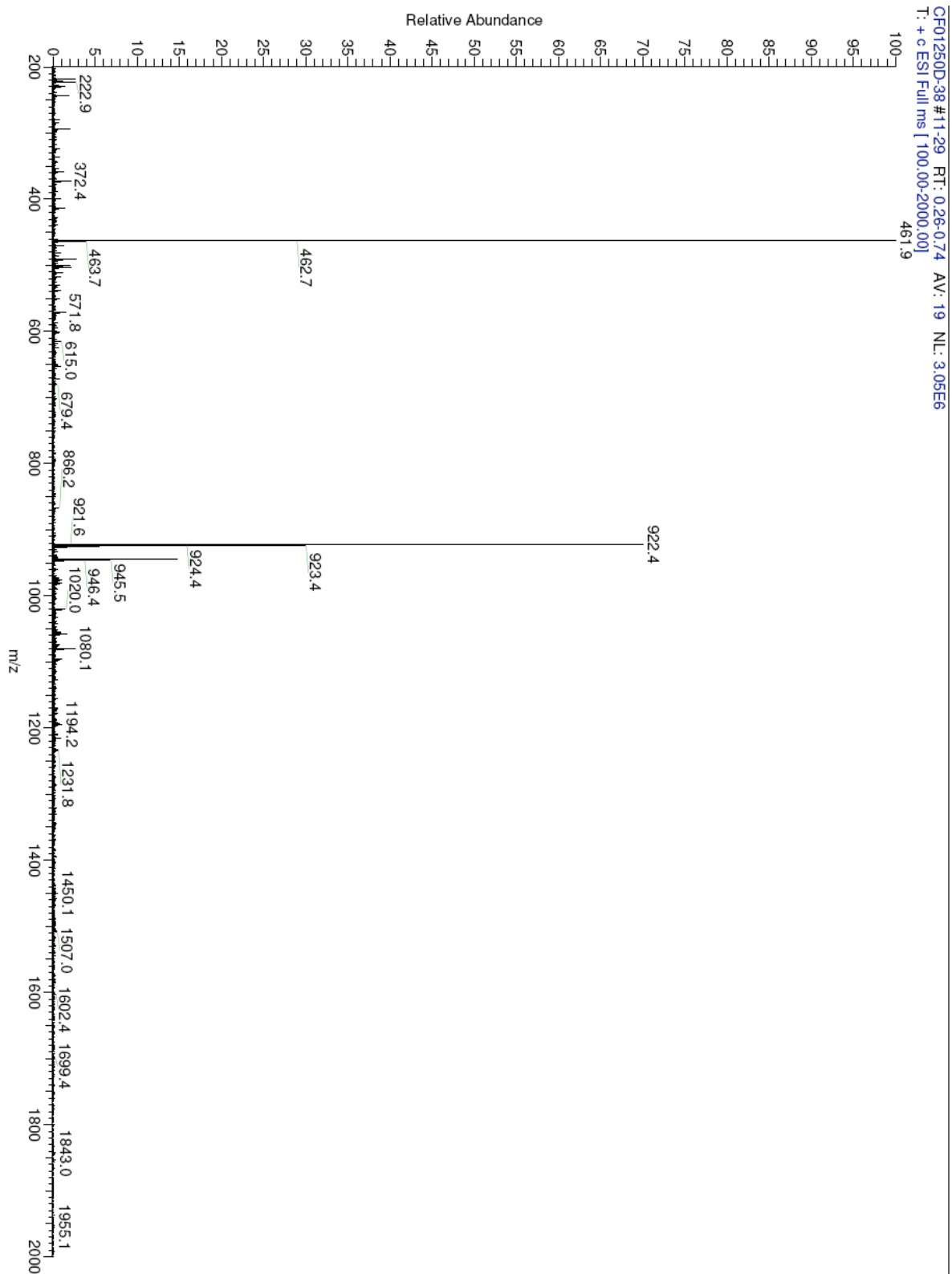


Figure S72. Mass spectrum of the peptide Ac-KKHMC(*t*-Bu)(4-SH-Phe)-NH₂ (**14**).

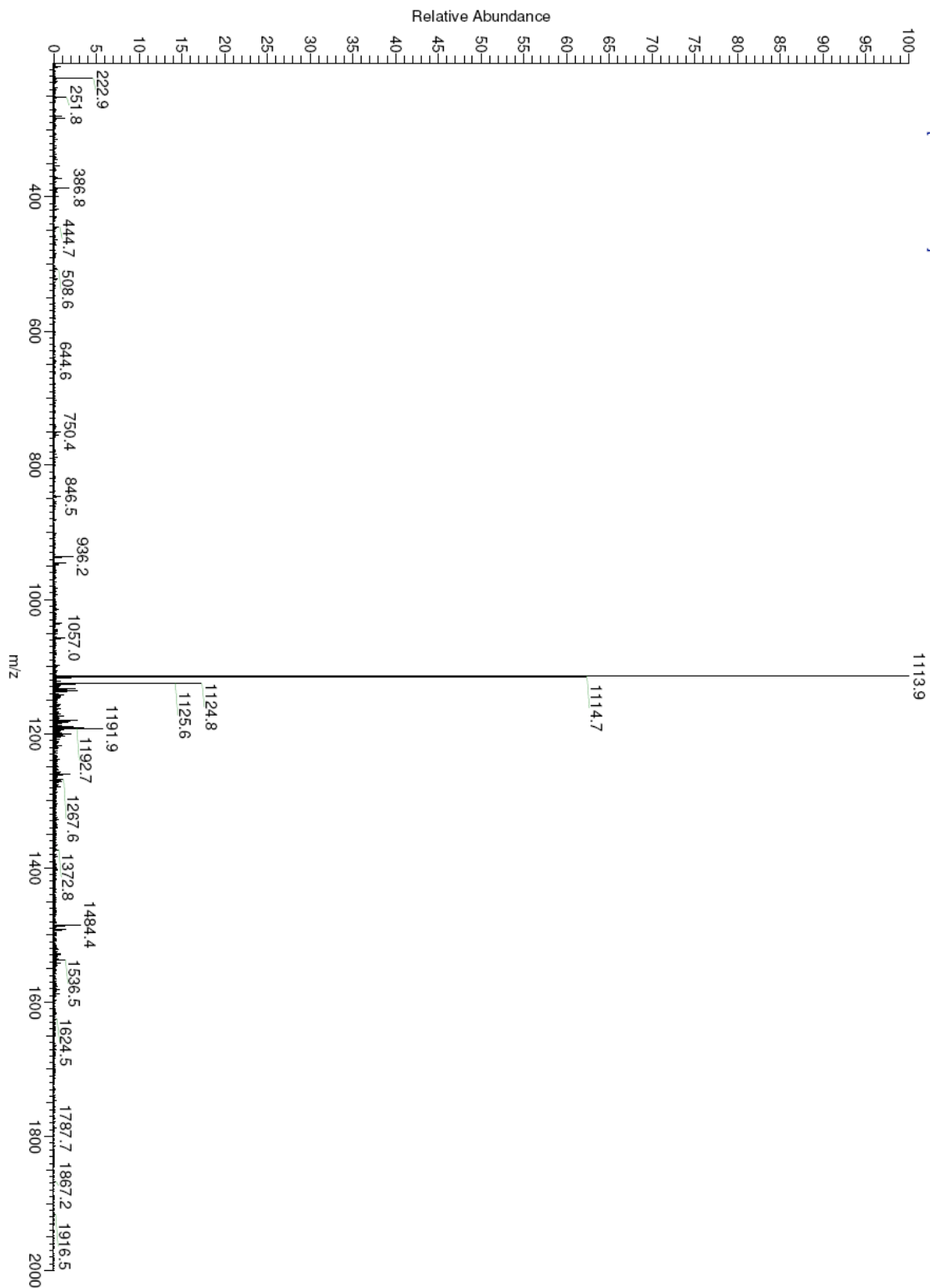
CF01272H-61 #10-31 RT: 0.24-0.79 AV: 22 NL: 1.71E6
T: + c ESI Full ms [100.00-2000.00]

Figure S73. Mass spectrum of the Y3(4-SH-Phe) trp cage peptide (**15**).

CF02078h-46_111116055910 #9-22 RT: 0.21-0.56 AV: 14 NL: 3.91E6
T: + c ESI Full ms [100.00-2000.00]

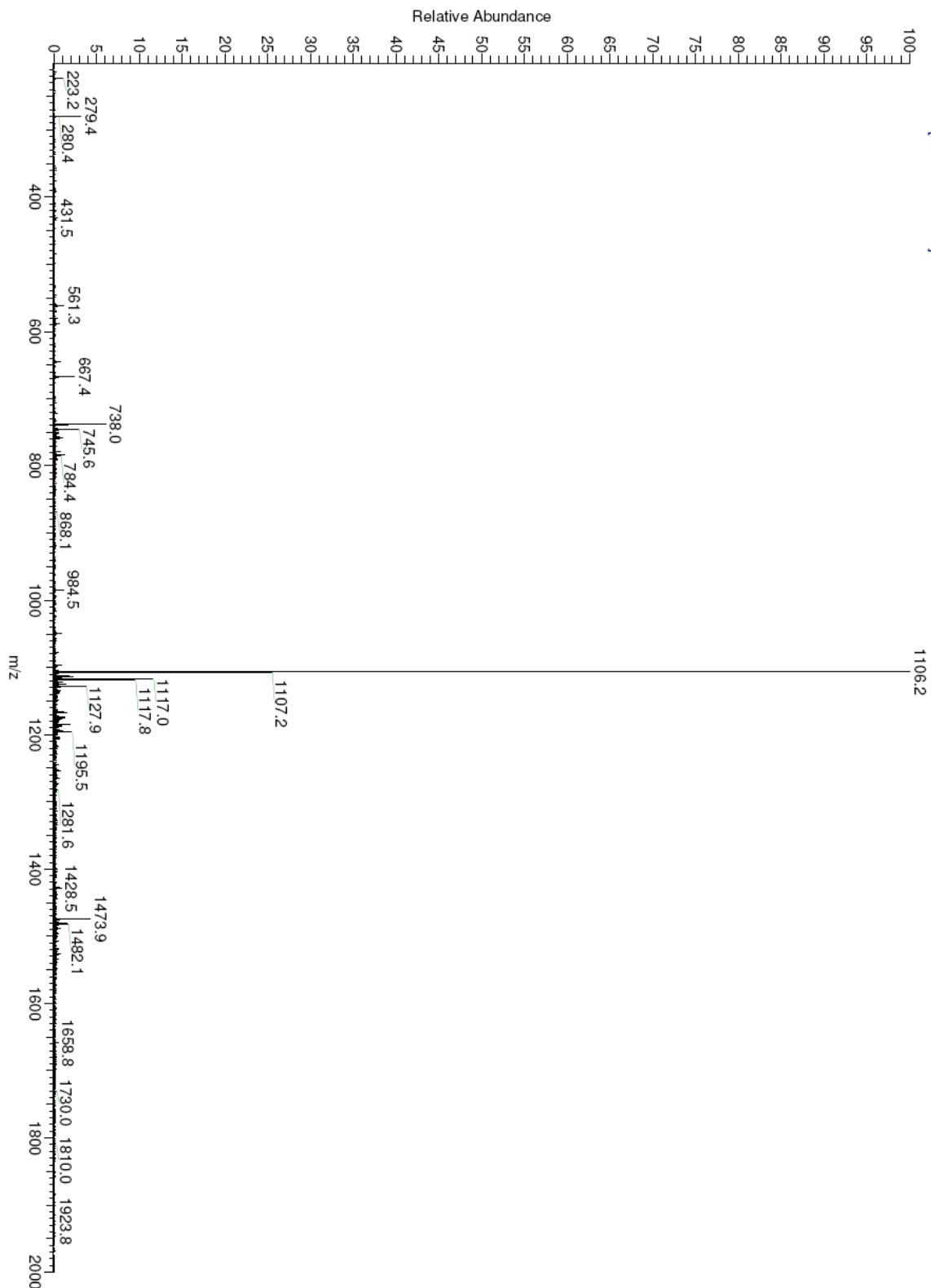


Figure S74. Mass spectrum of the trp cage peptide (16).

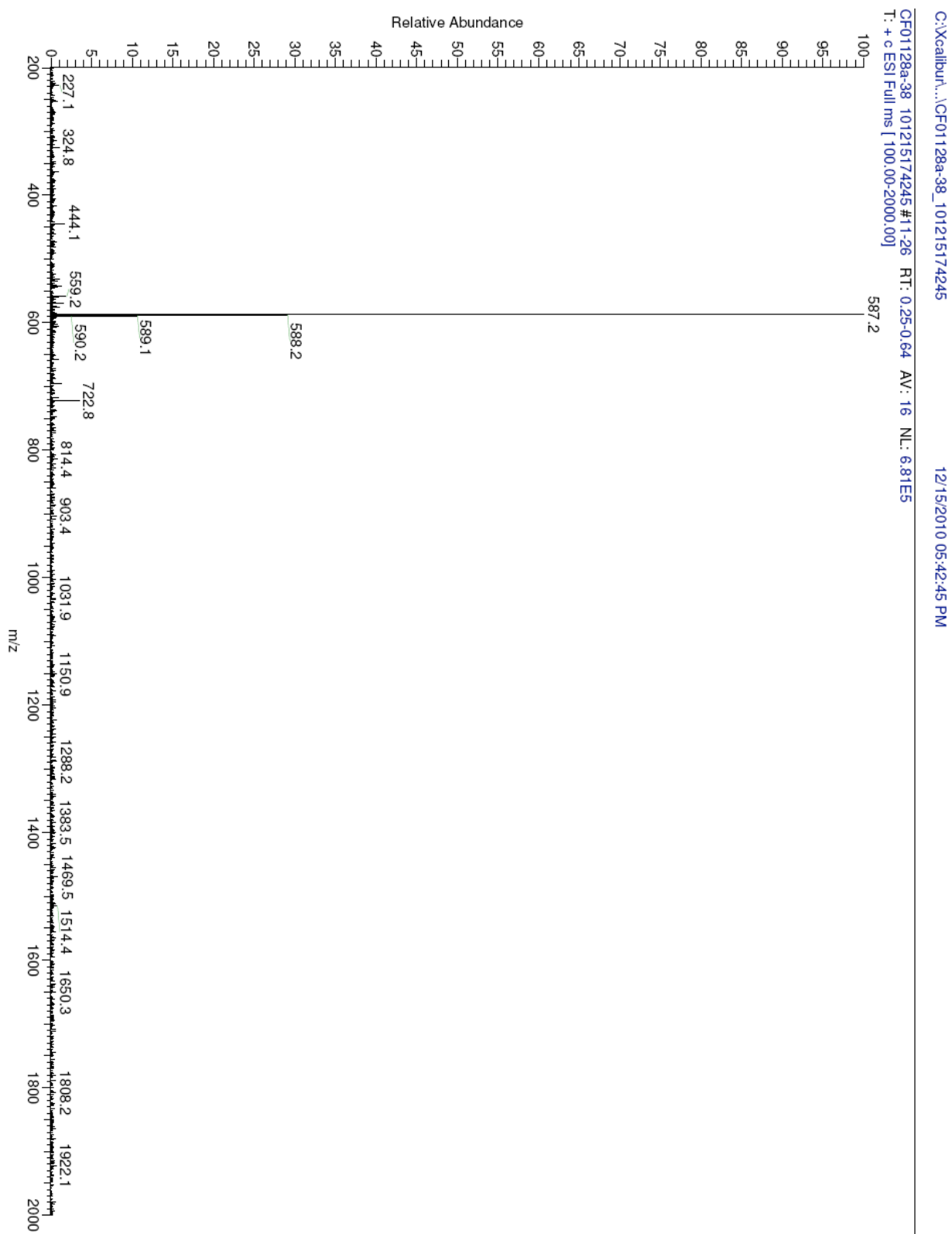


Figure S75. Mass spectrum of the peptide Ac-T(4-SMe-Phe)PN-NH₂ (**17**).

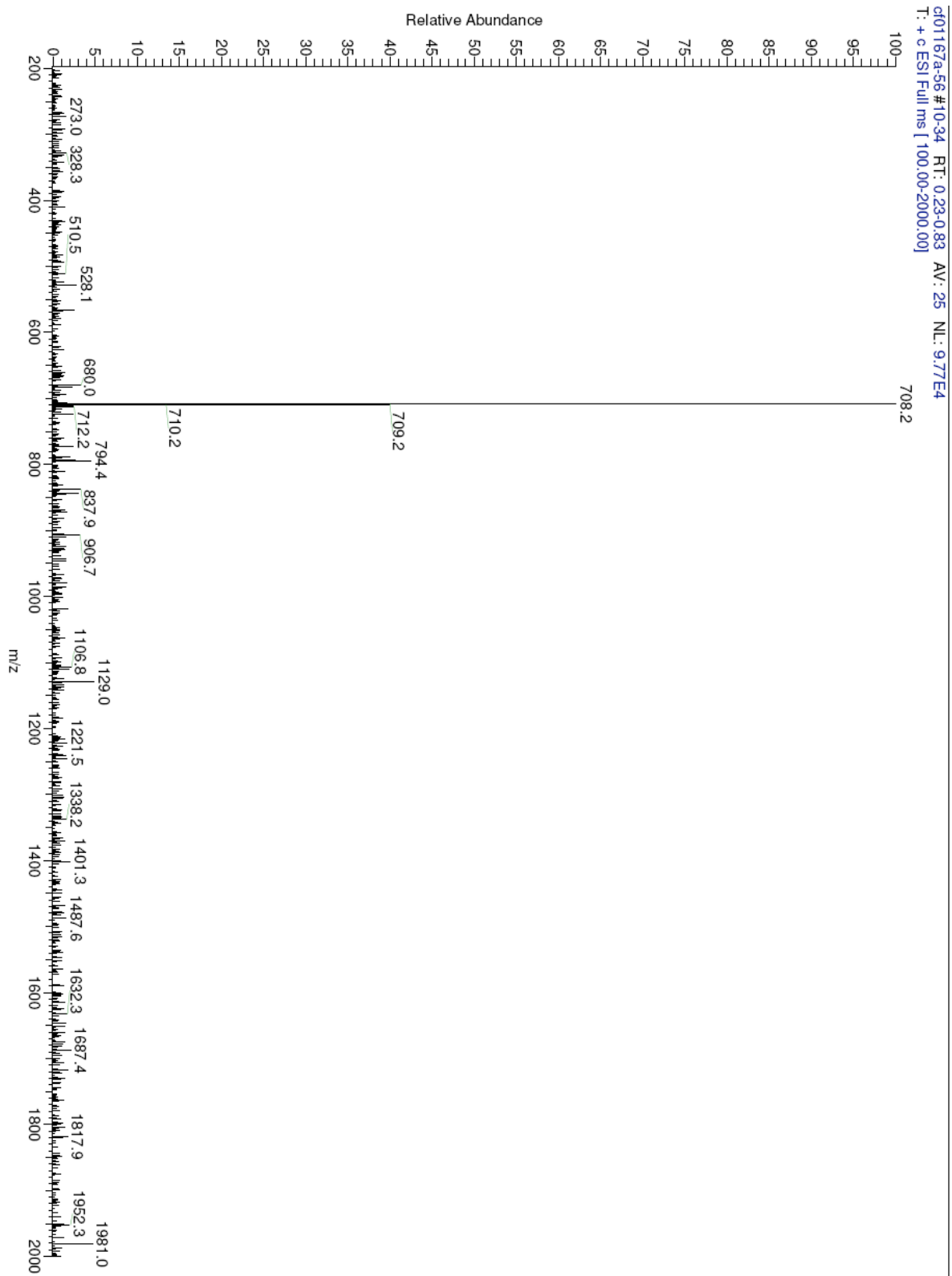


Figure S76. Mass spectrum of the peptide Ac-T(4-S-(2-nitrobenzyl)-Phe)PN-NH₂ (**18**).

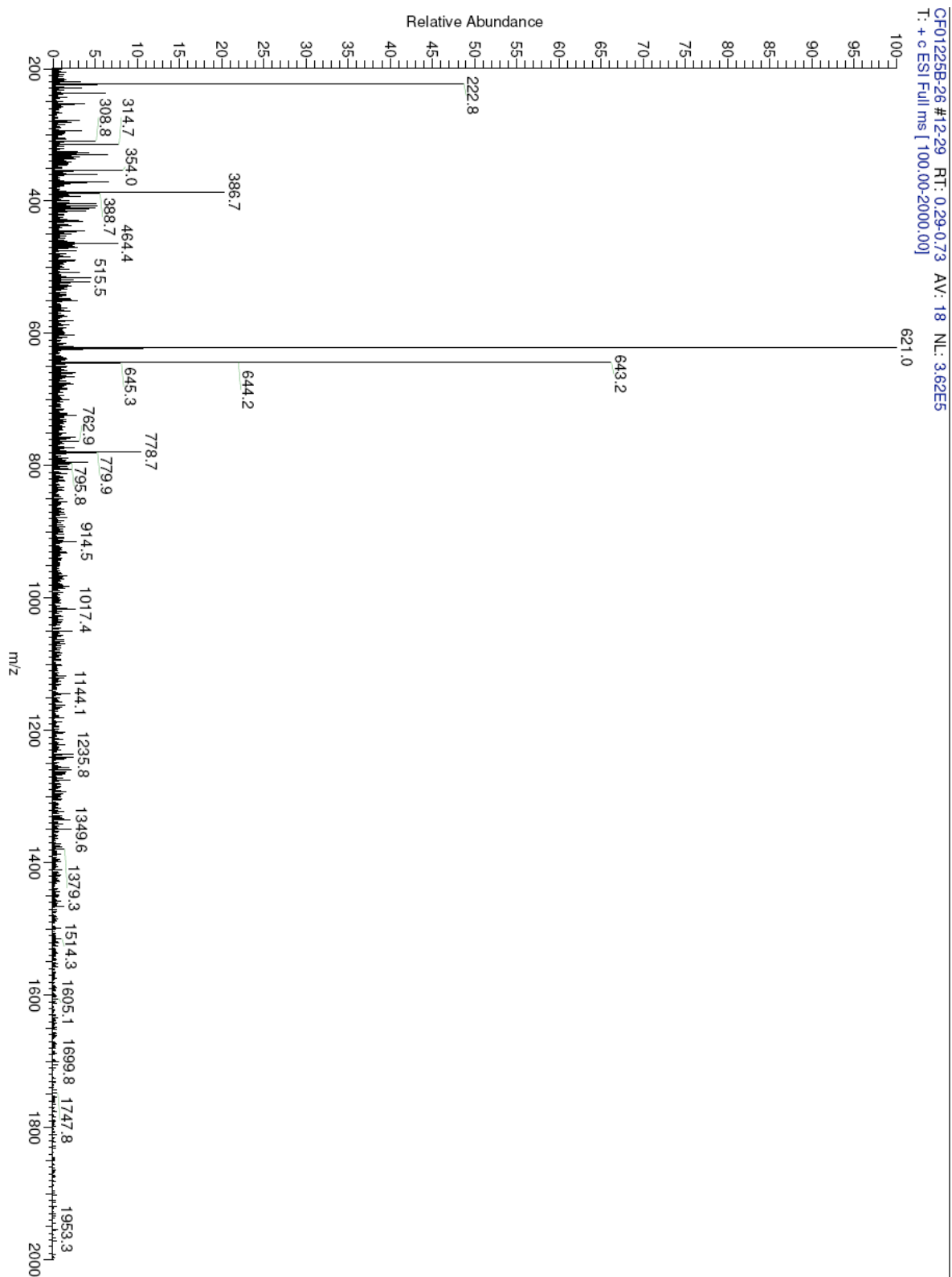


Figure S77. Mass spectrum of the peptide Ac-T(4-(SCH₂CH=CHCH₂OH)-Phe)PN-NH₂ (**19**).

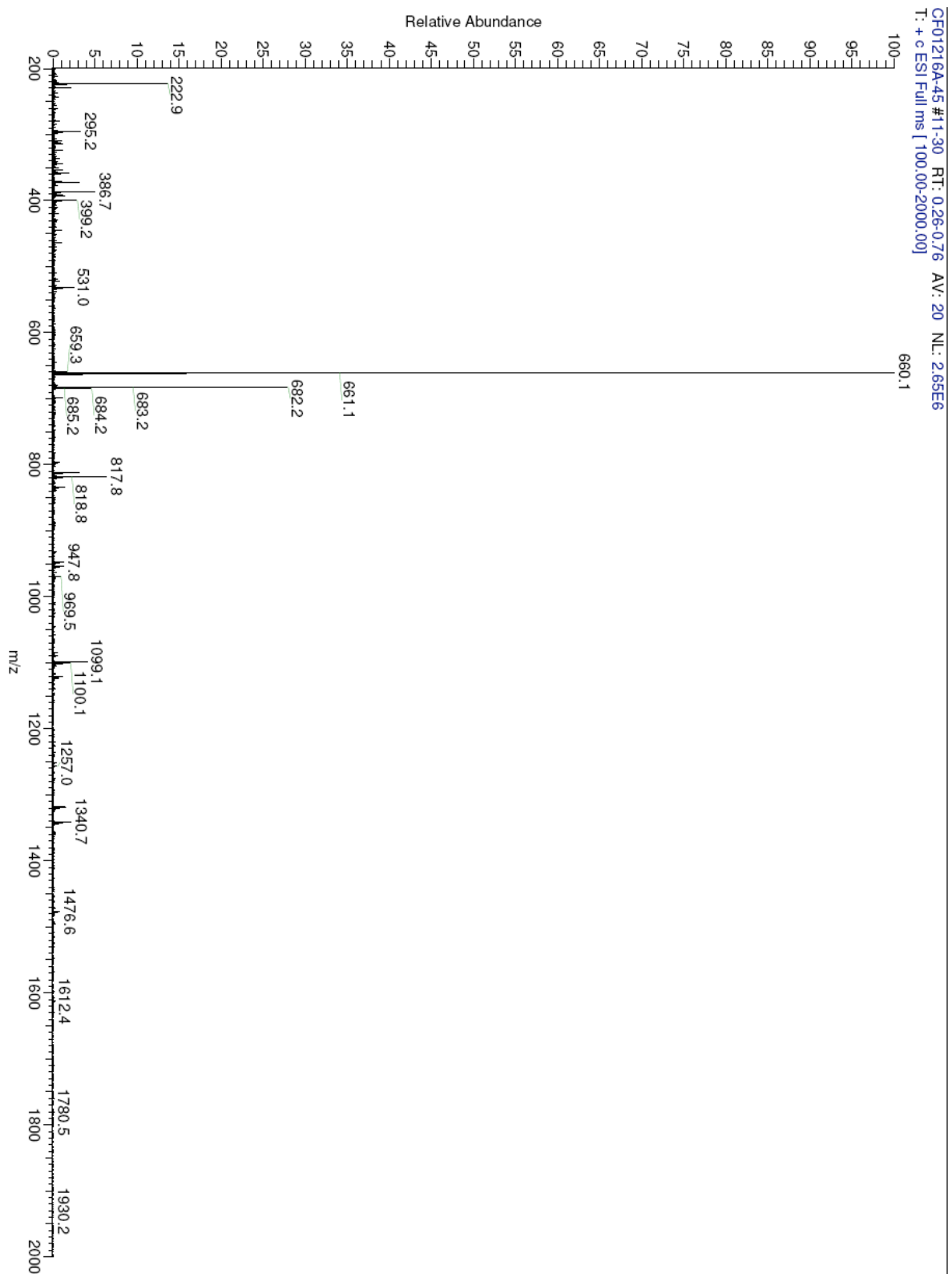


Figure S78. Mass spectrum of the peptide Ac-T(4-S-SPy-Phe)PN-NH₂ (**20**).

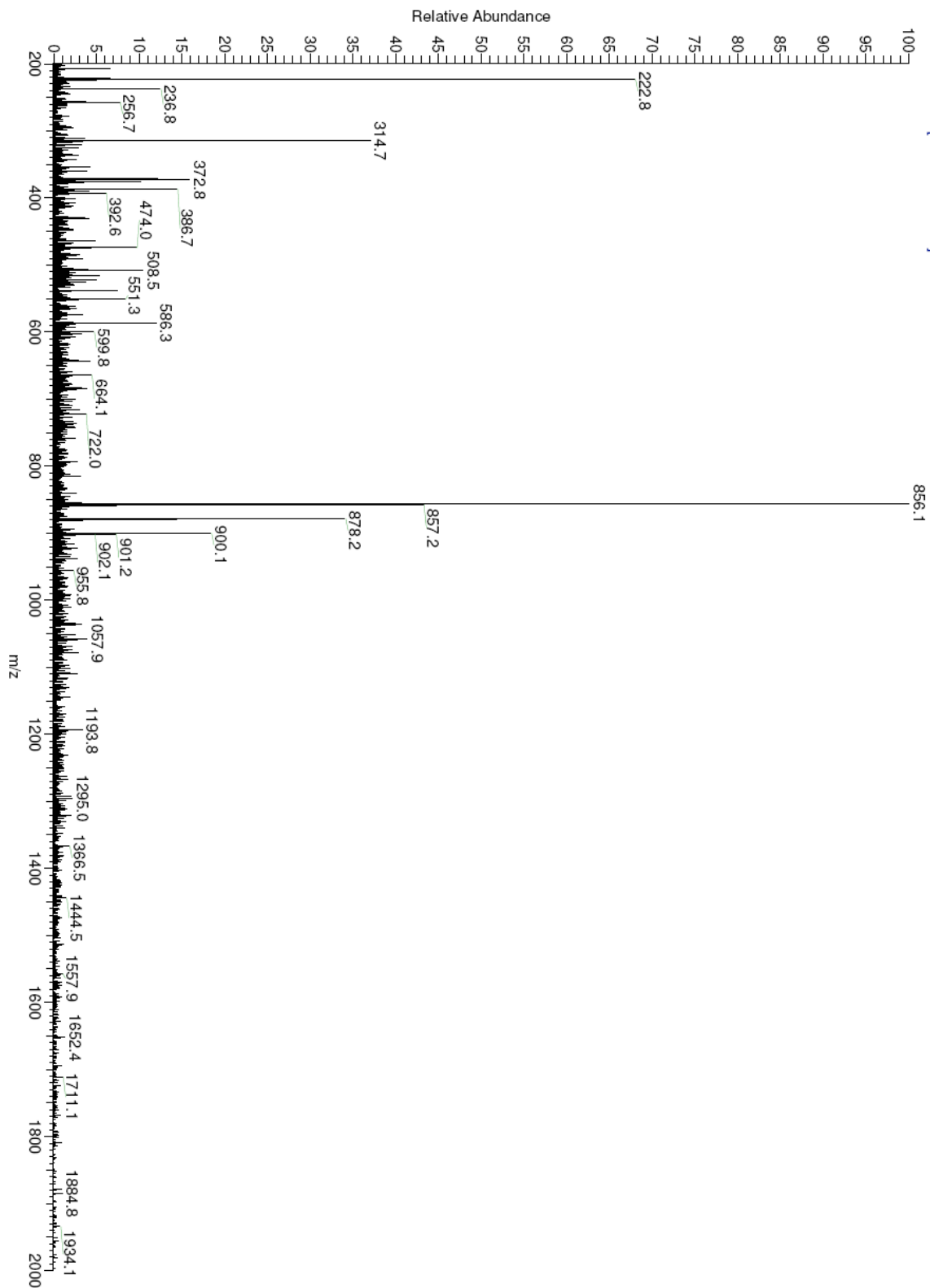
CF01242B-26 #16-28 RT: 0.40-0.72 AV: 13 NL: 4.37E5
T: + c ESI Full ms [100.00-2000.00]

Figure S79. Mass spectrum of the peptide Ac-T(4-(glutathione disulfide)S-Phe)PN-NH₂ (**21**).

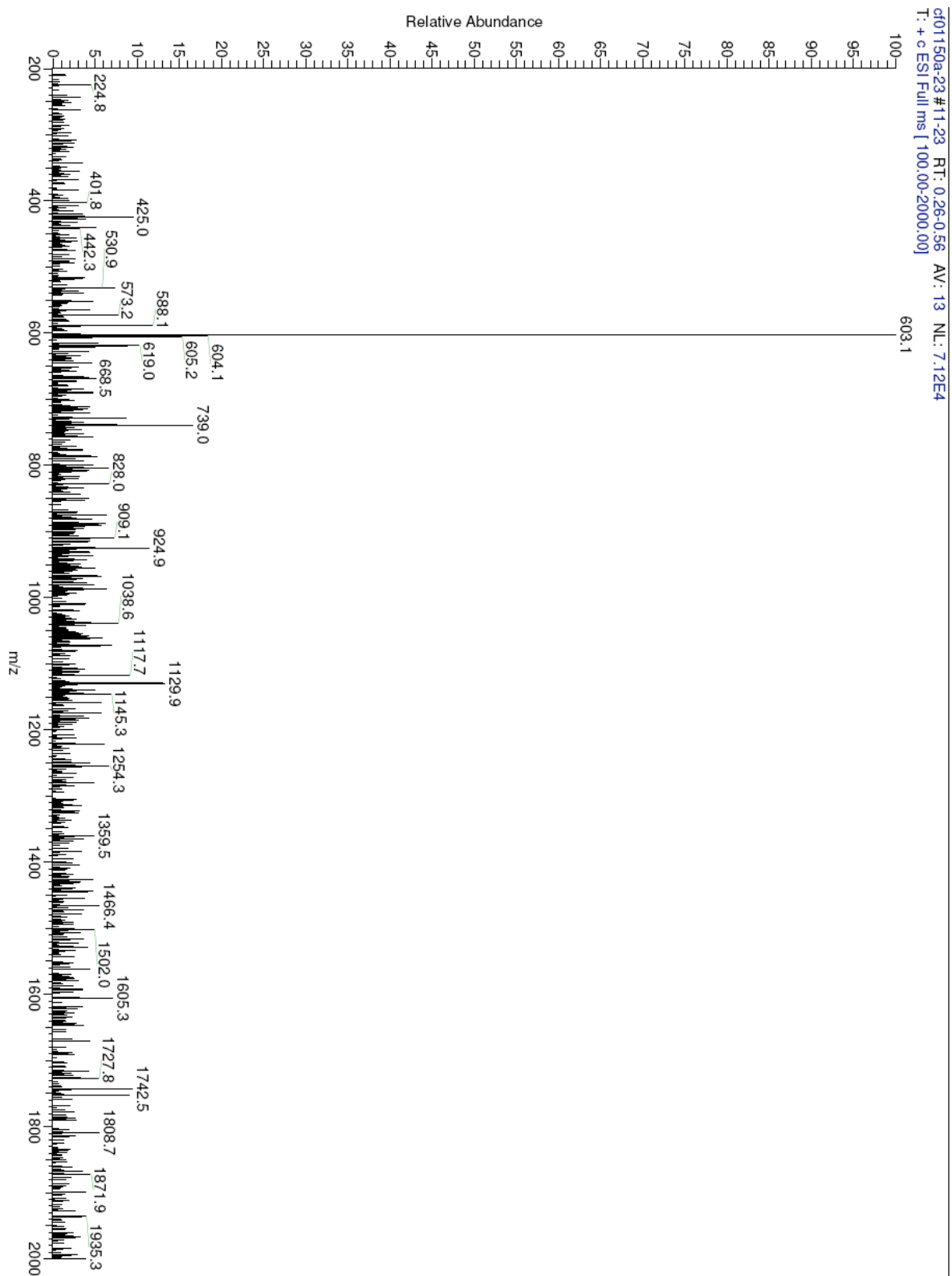


Figure S80. Mass spectrum of the peptide Ac-T(4-S(O)Me-Phe)PN-NH₂ (**22**).

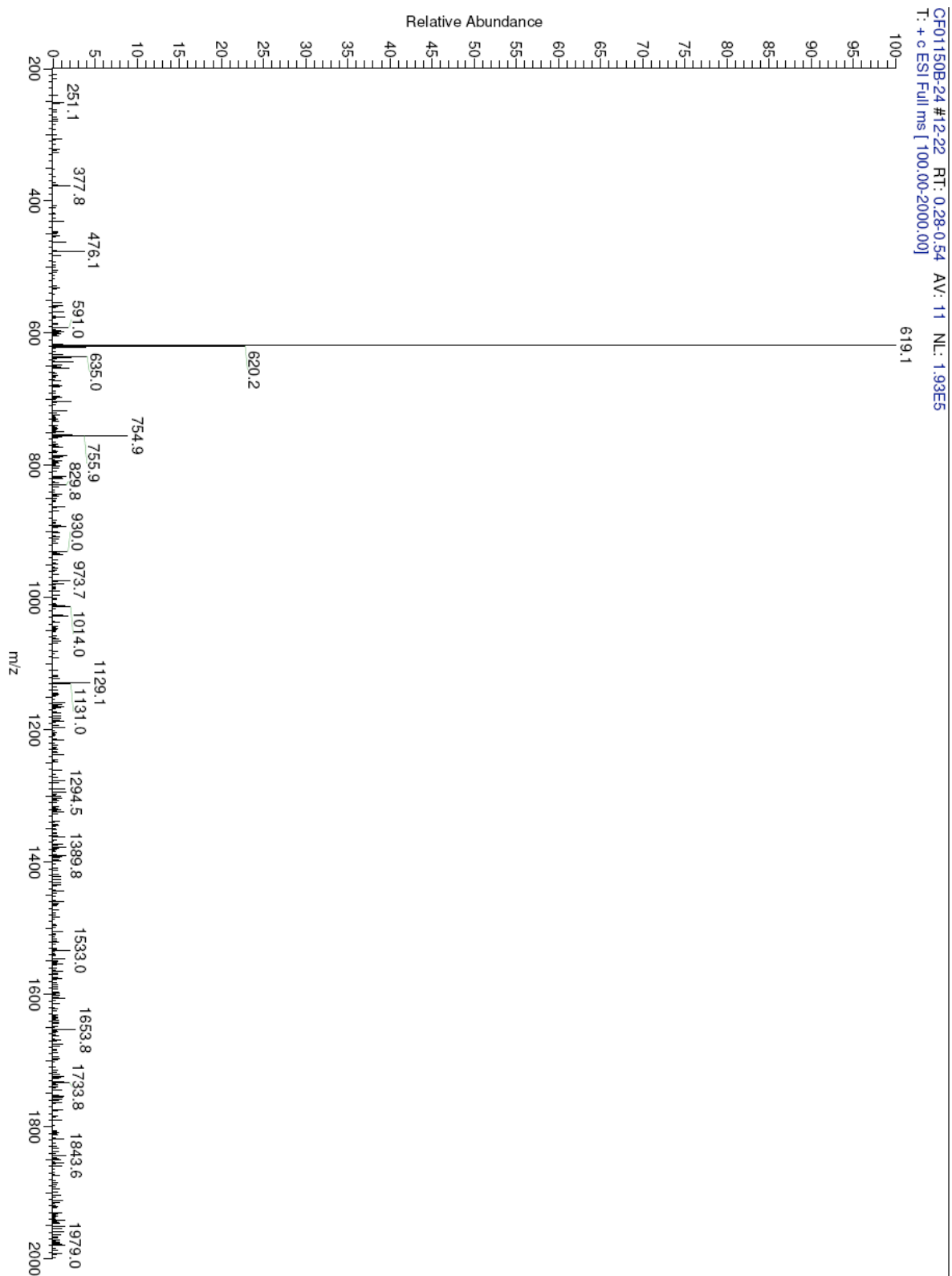


Figure S81. Mass spectrum of the peptide Ac-T(4-SO₂Me-Phe)PN-NH₂ (**23**).

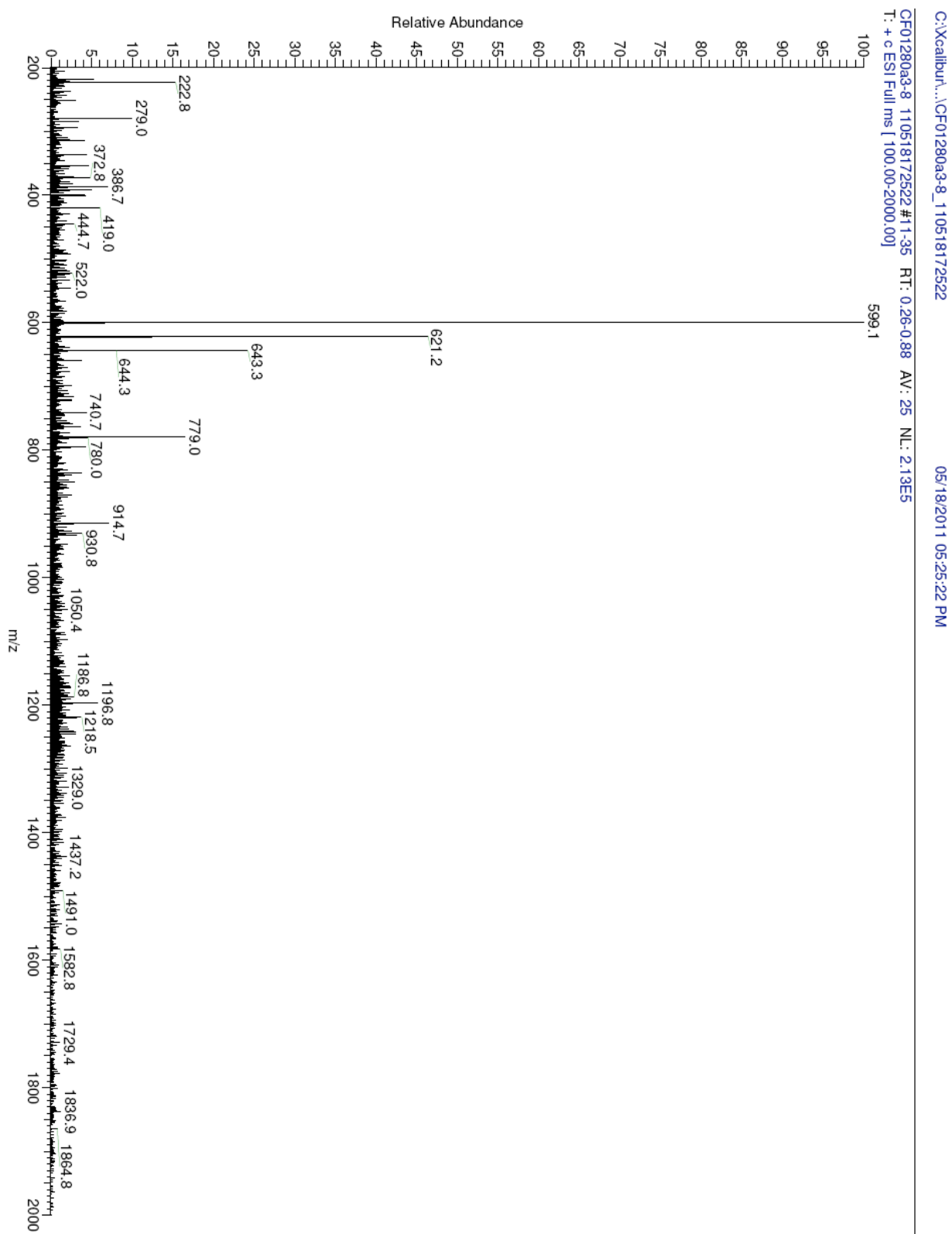


Figure S82. Mass spectrum of the peptide Ac-T(4-SO₃⁻-Phe)PN-NH₂ (**24**).

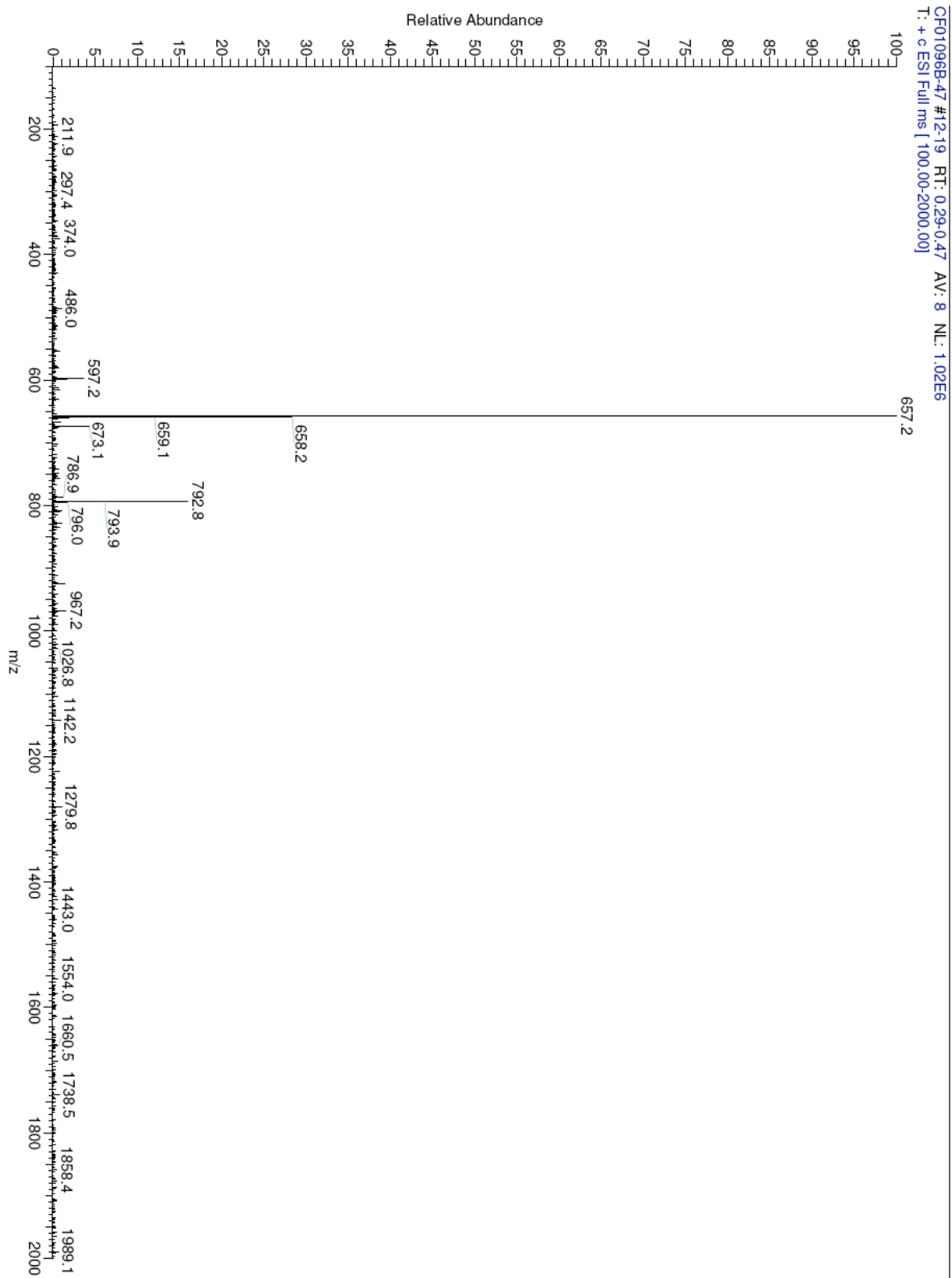


Figure S83. Mass spectrum of product at 46.9 min from solution phase cross-coupling to produce the peptide Ac-T(4-thioacetyl-Phe)PN-NH₂ (**2**). The observed mass is consistent with the peptide (**2** + Ac + Na)⁺.

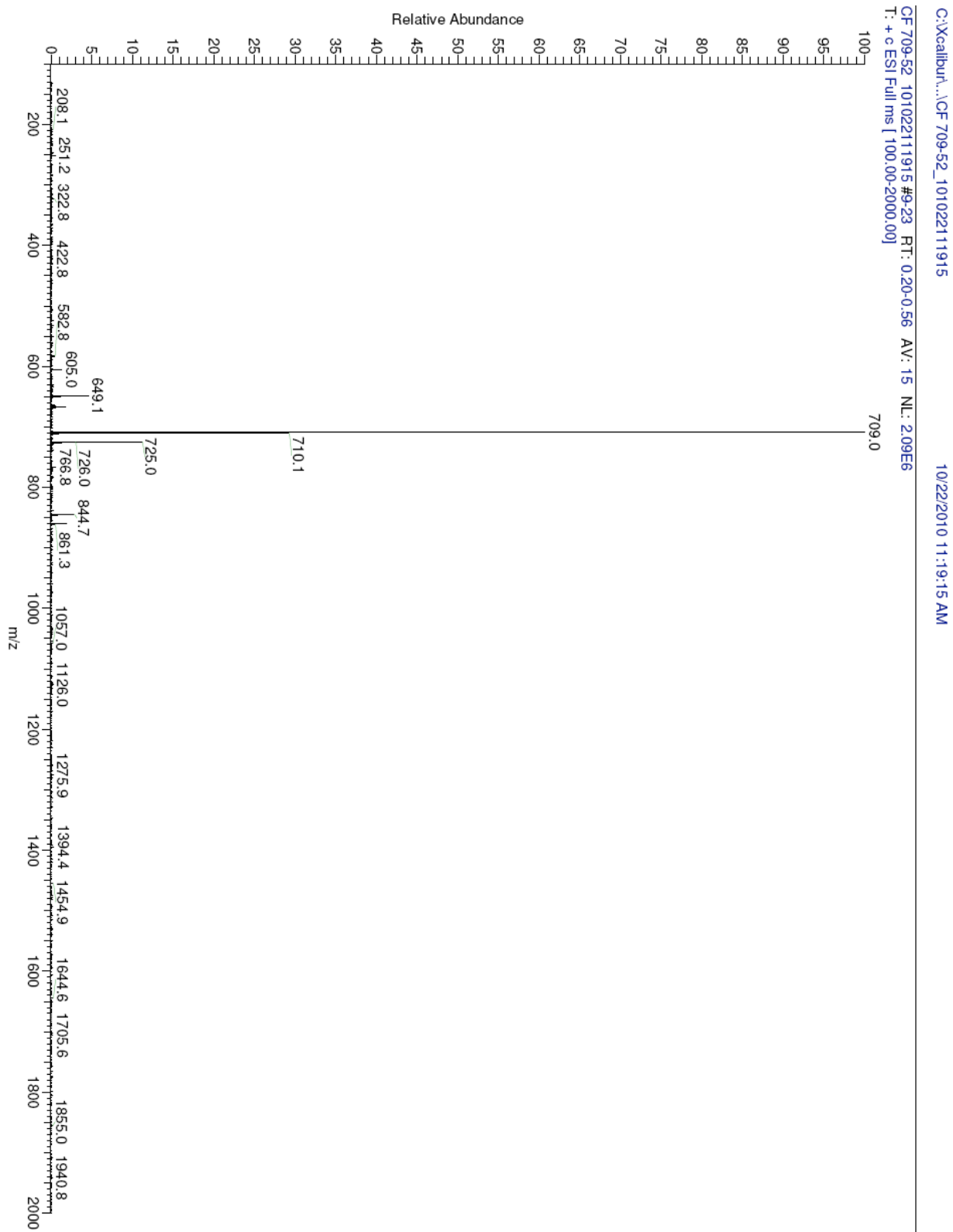


Figure S84. Mass spectrum of product at 51.7 min from solution phase cross-coupling to produce the peptide Ac-T(4-thioacetyl-Phe)PN-NH₂ (**2**). The observed mass is consistent with the peptide (**1** + Ac + Na)⁺.