

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**

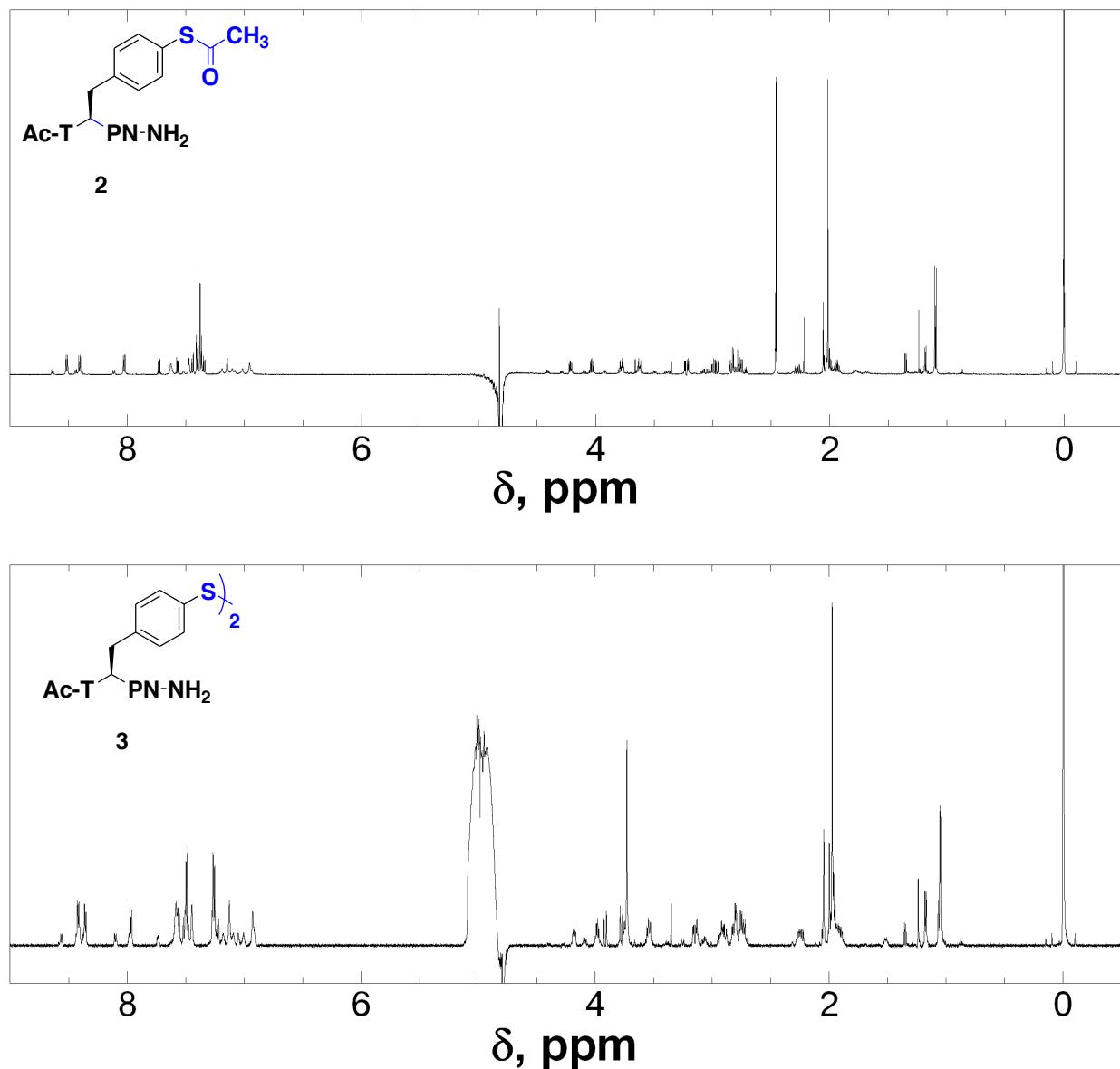


Figure S32. Full NMR spectra for peptides **2** and **3** at pH 4.0. All samples contained 5 mM phosphate and 25 mM NaCl.

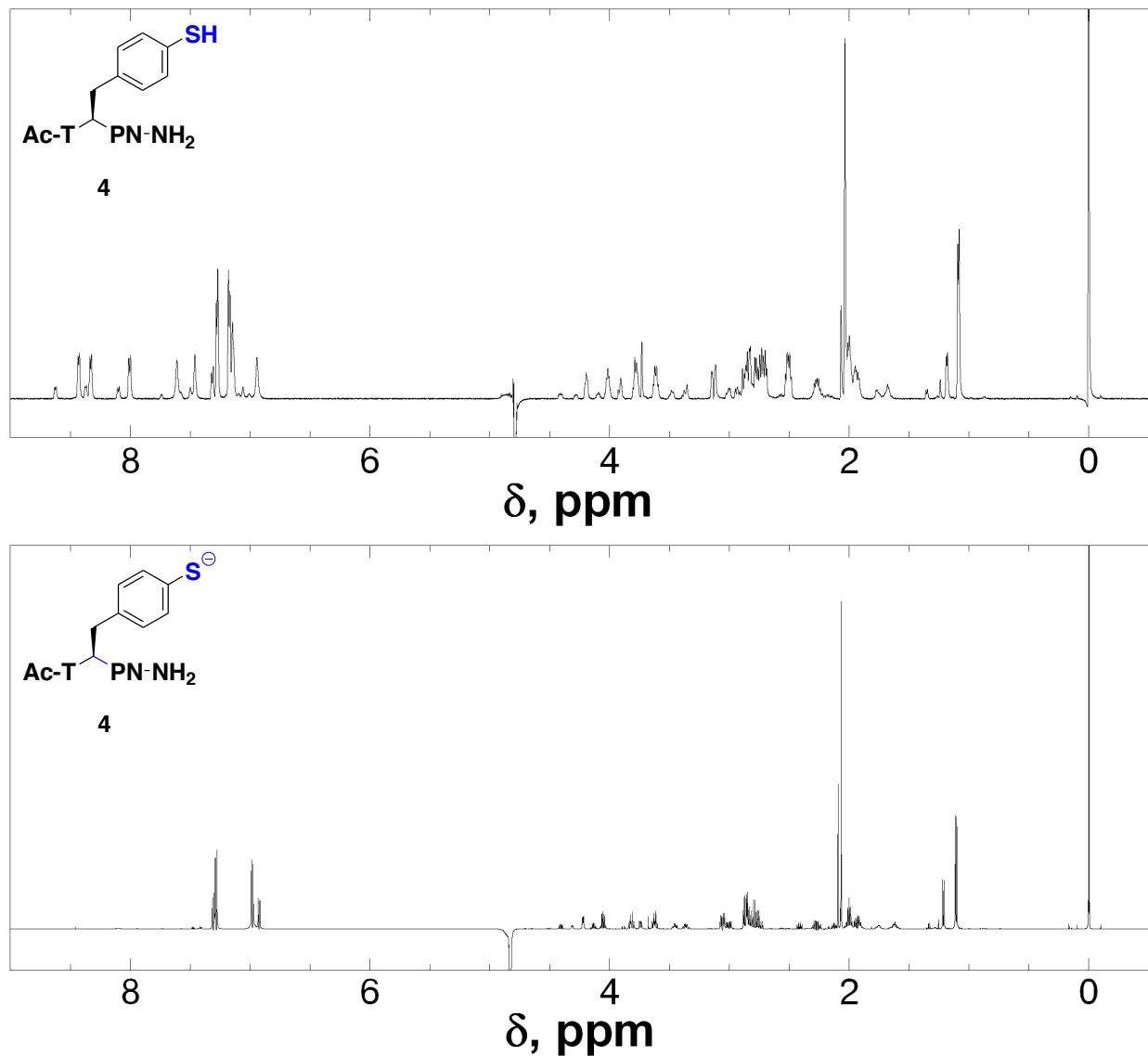


Figure S33. Full NMR spectra for peptide **4** at pH 4.0 and pH 8.5. All samples contained 5 mM phosphate and 25 mM NaCl at pH 4.0 and 0.1 mM TCEP.

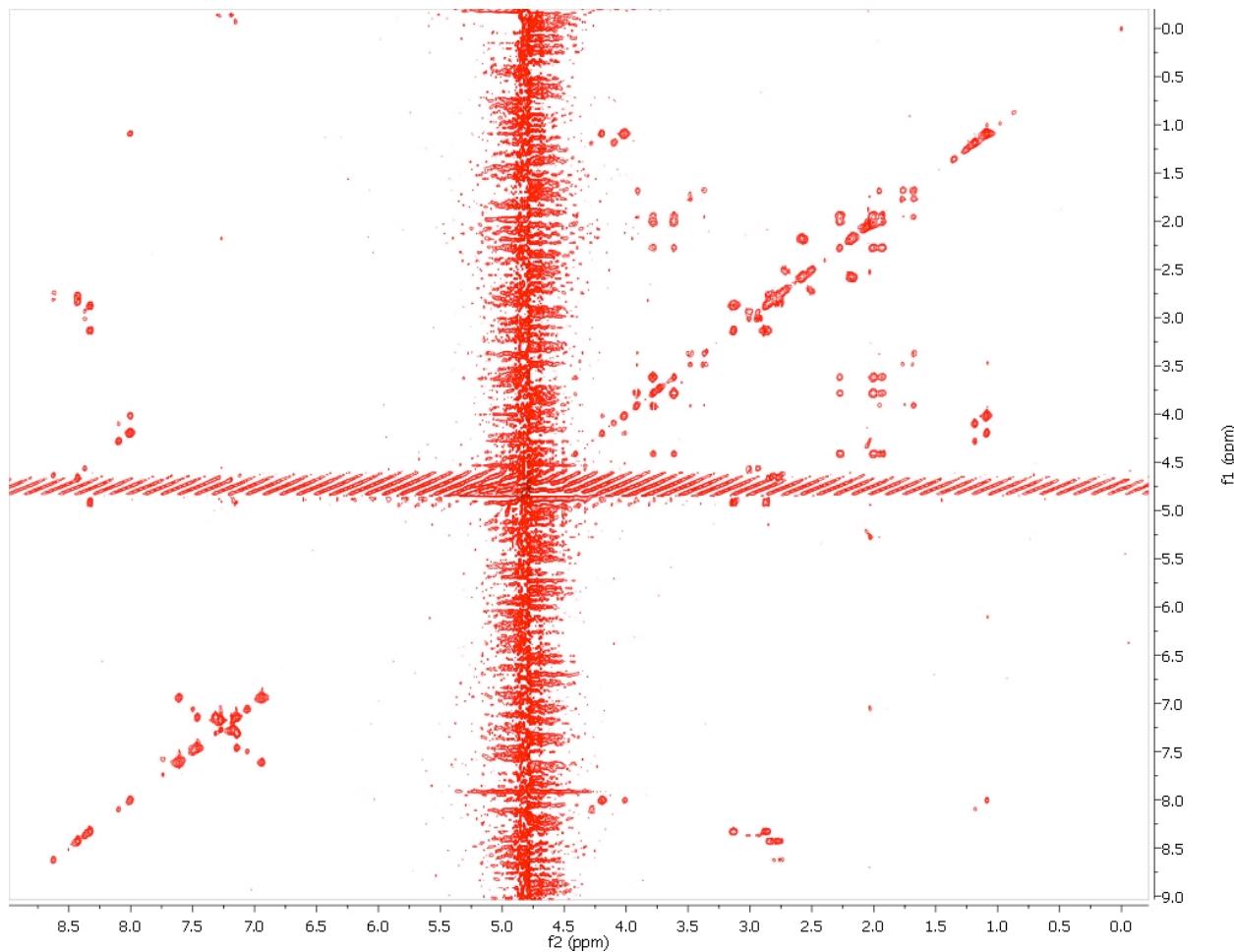


Figure S34. TOCSY spectrum for peptide **4** at pH 4.0. The solution contains 5 mM phosphate (pH 4.0), 25 mM NaCl, and 0.1 mM TCEP.

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 ($\text{Ac}_{\text{H}\alpha\text{cis}}$, s, 3H), 2.04 ($\text{Ac}_{\text{H}\alpha\text{trans}}$, s, 3H), 2.00 ($\text{Pro}_{\text{H}\gamma\text{trans}}$, m, 1H), 1.95 ($\text{Pro}_{\text{H}\beta\text{or}\gamma\text{cis}}$, m, 1H), 1.92 ($\text{Pro}_{\text{H}\gamma\text{trans}}$, m, 1H), 1.75 ($\text{Pro}_{\text{H}\beta\text{or}\gamma\text{cis}}$, m, 1H), 1.68 ($\text{Pro}_{\text{H}\beta\text{or}\gamma\text{cis}}$, 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).

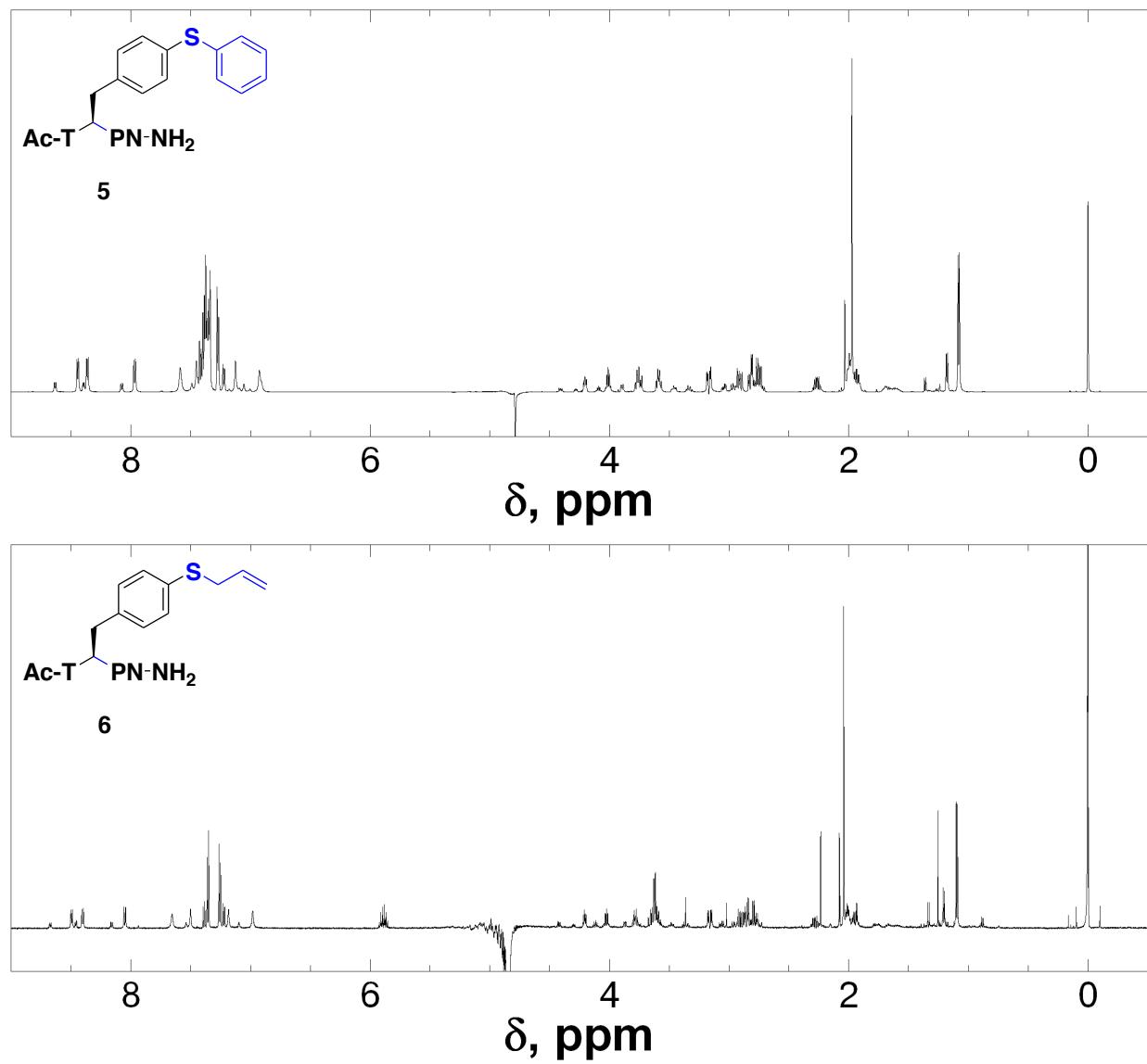


Figure S35. Full NMR spectra for peptides **5** and **6**. All samples contained 5 mM phosphate and 25 mM NaCl at pH 4.0.

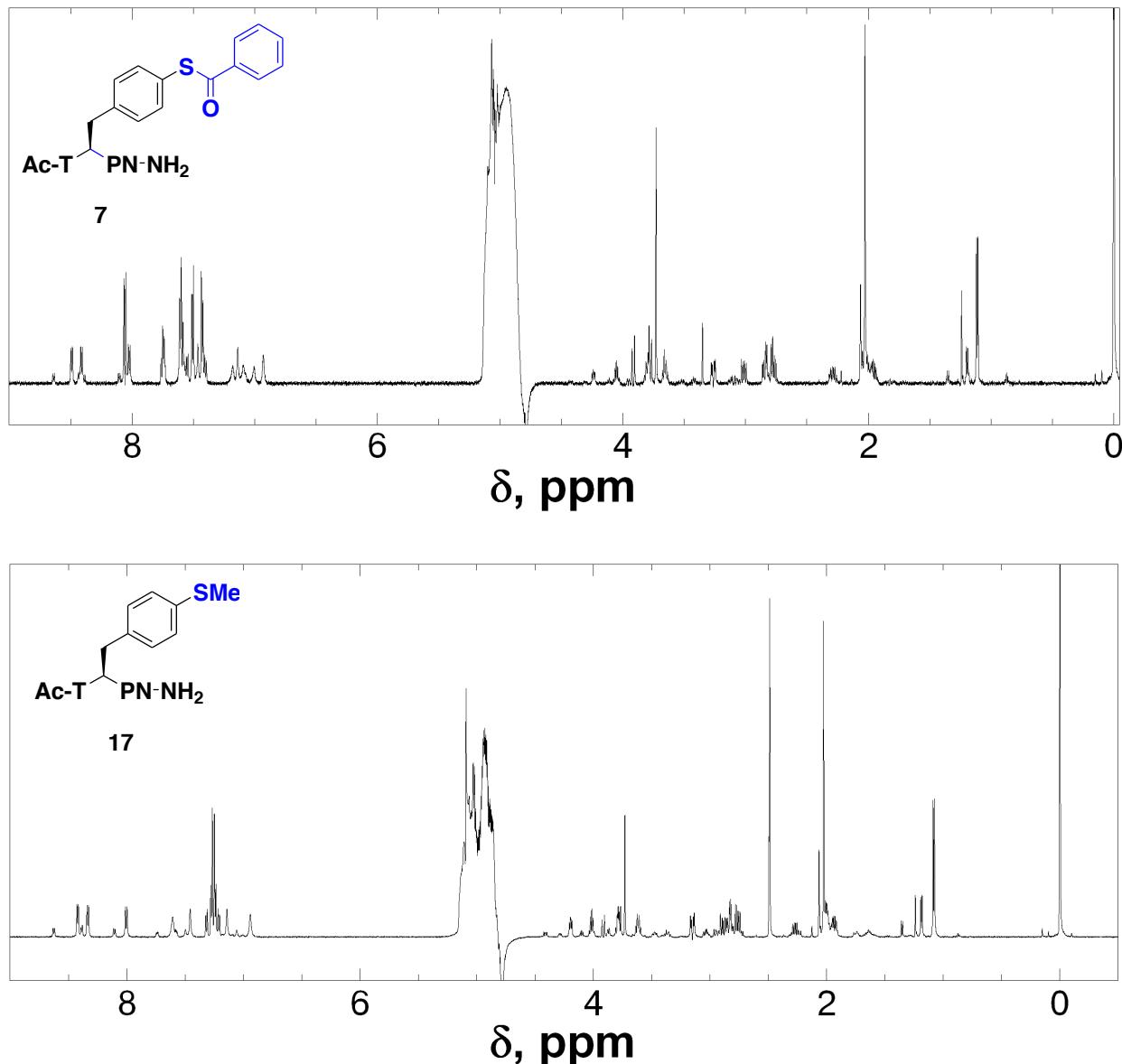


Figure S36. Full NMR spectra for peptides **7** and **17**. All samples contained 5 mM phosphate and 25 mM NaCl at pH 4.0.

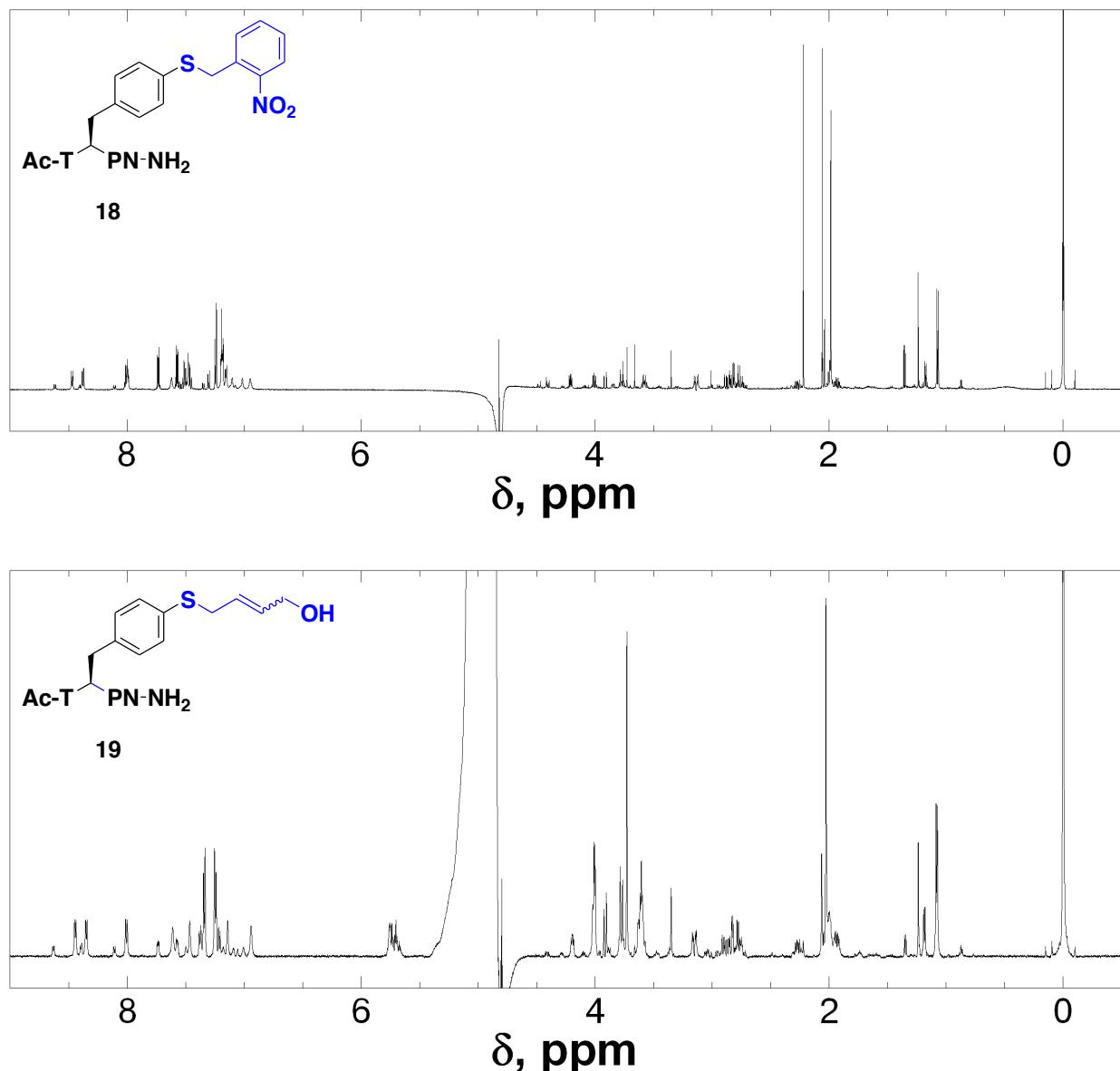


Figure S37. Full NMR spectra for peptides **18** and **19**. All samples contained 5 mM phosphate and 25 mM NaCl at pH 4.0.

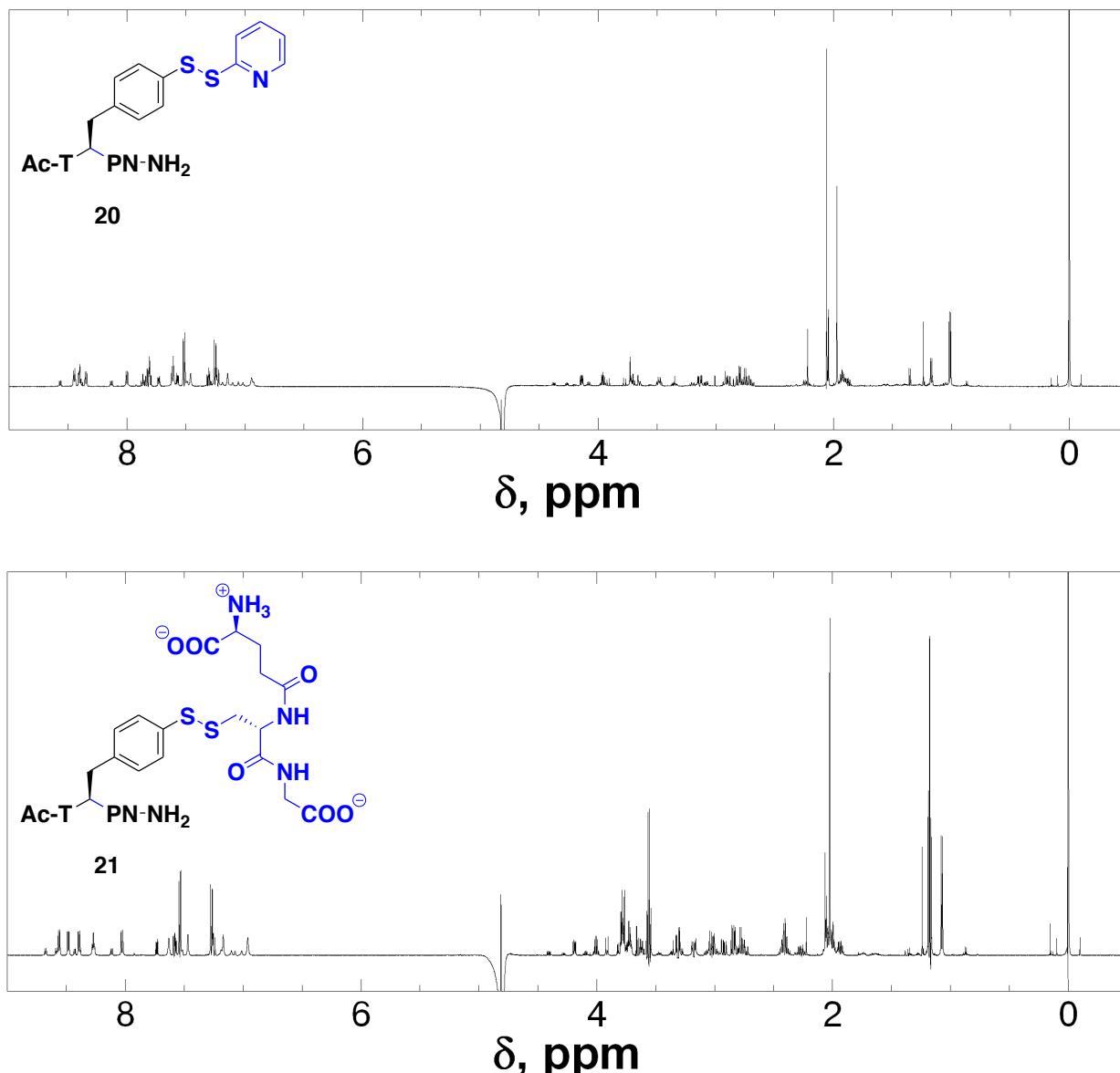


Figure S38. Full NMR spectra for peptides **20** and **21**. All samples contained 5 mM phosphate and 25 mM NaCl at pH 4.0.

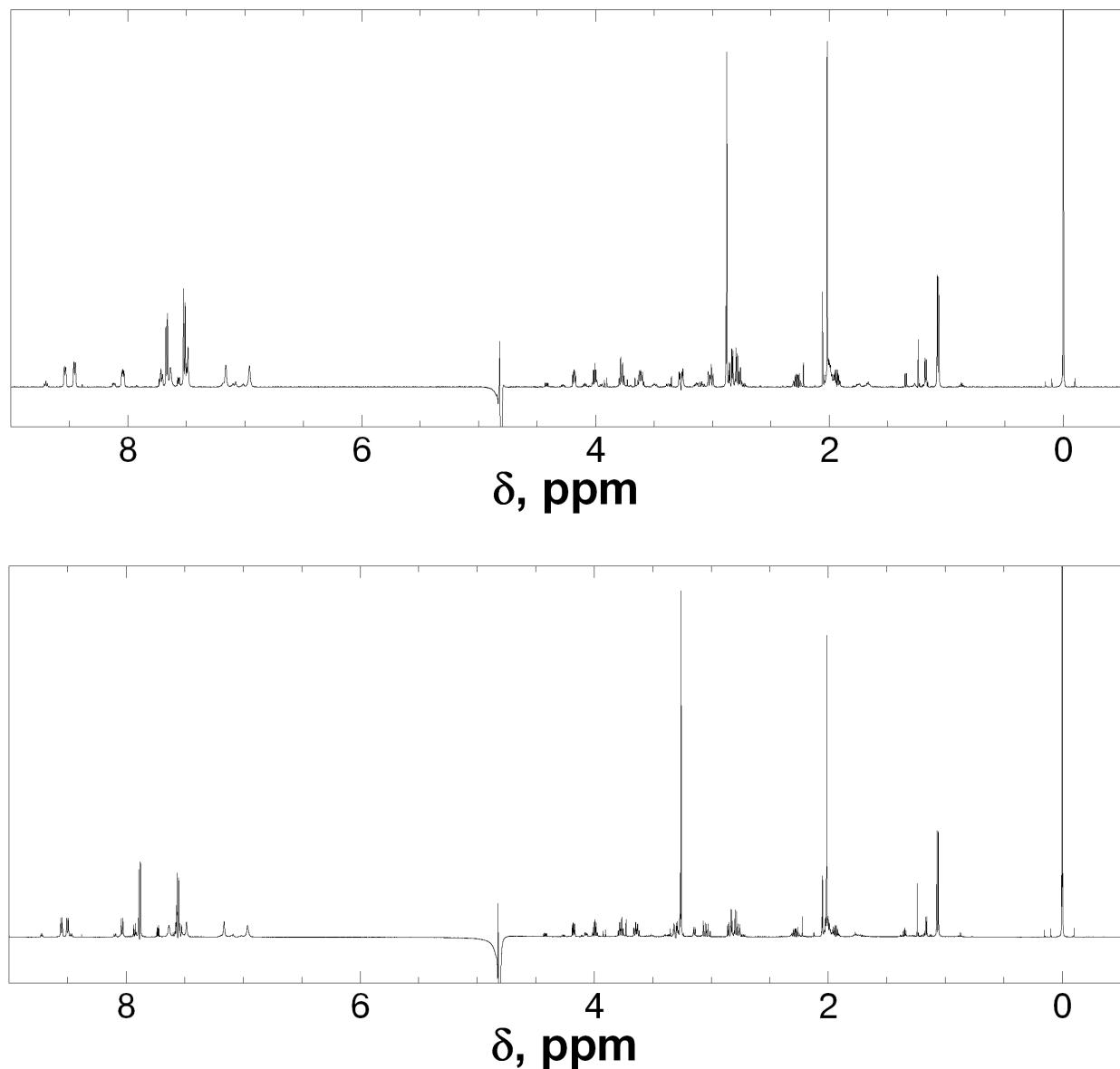


Figure S39. Full NMR spectra for peptides **22** and **23**. Peptide **22**

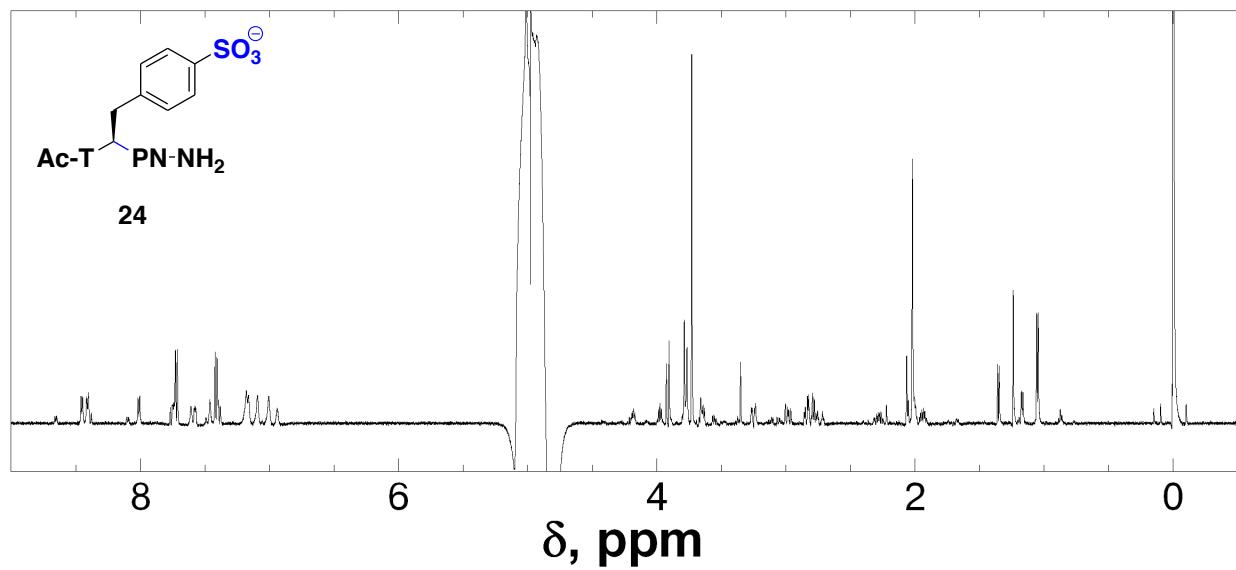


Figure S40. Full NMR spectrum for peptide 24. The solution contains 5 mM phosphate and 25 mM NaCl at pH 4.0.

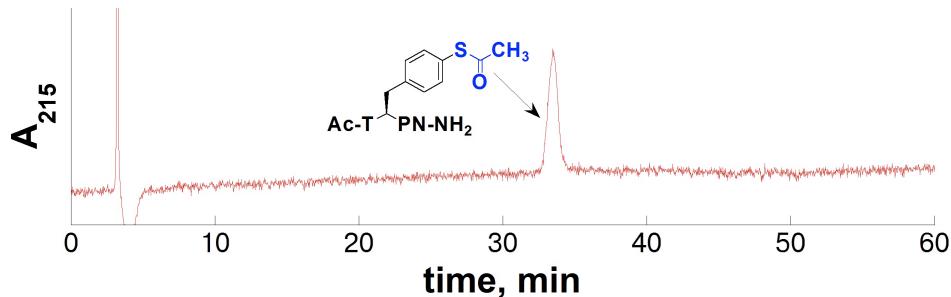


Figure S41. Reinjection of the purified peptide Ac-T(4-thioacetyl-Phe)PN-NH₂ (**2**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

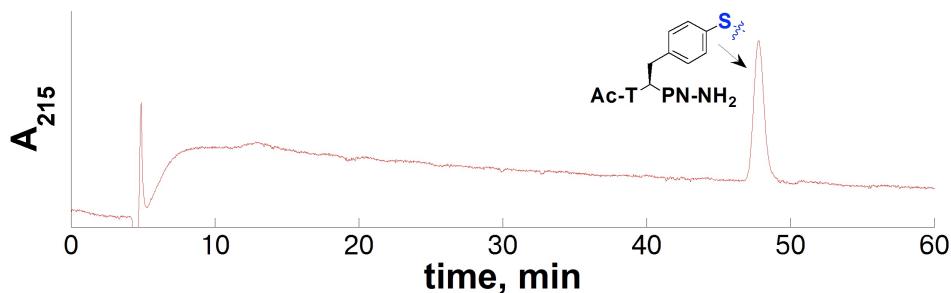


Figure S42. Reinjection of the purified thiophenylalanine disulfide peptide (**3**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

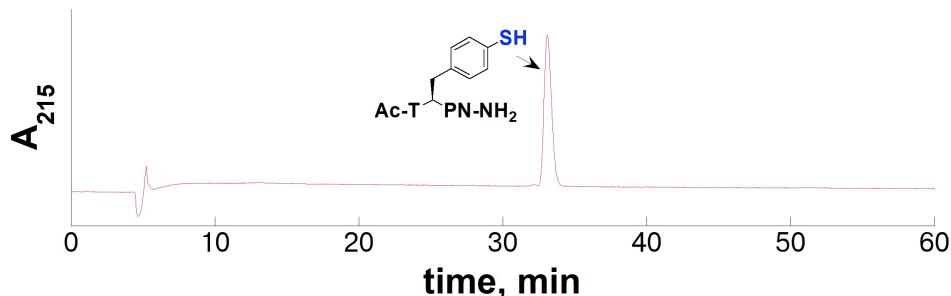


Figure S43. Reinjection of the purified peptide Ac-T(4-SH-Phe)PN-NH₂ (**4**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

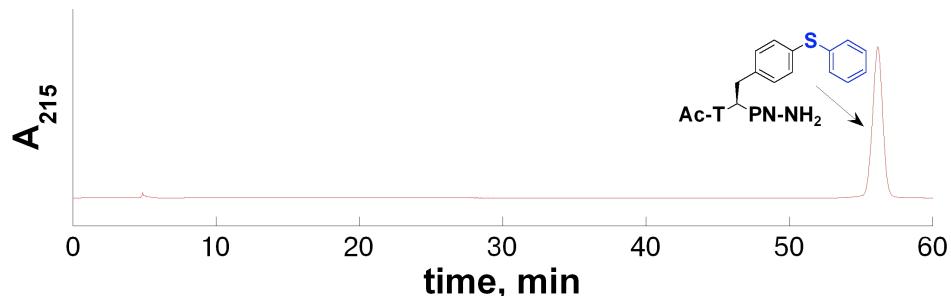


Figure S44. Reinjection of the purified peptide Ac-T(4-thiophenyl-Phe)PN-NH₂ (**5**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

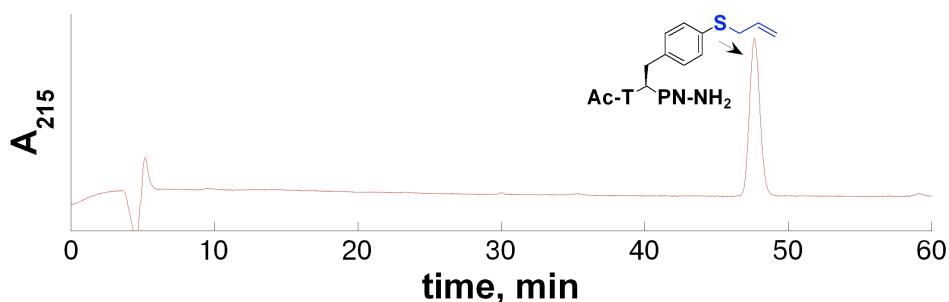


Figure S45. Reinjection of the purified peptide Ac-T(4-S-allyl-Phe)PN-NH₂ (**6**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

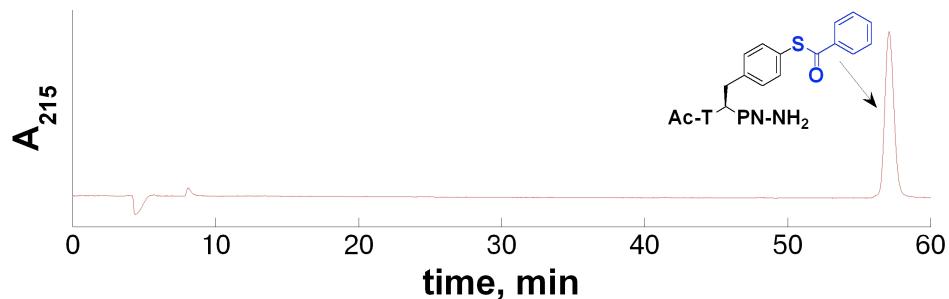


Figure S46. Reinjection of the purified peptide Ac-T(4-thiobenzoyl-Phe)PN-NH₂ (**7**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

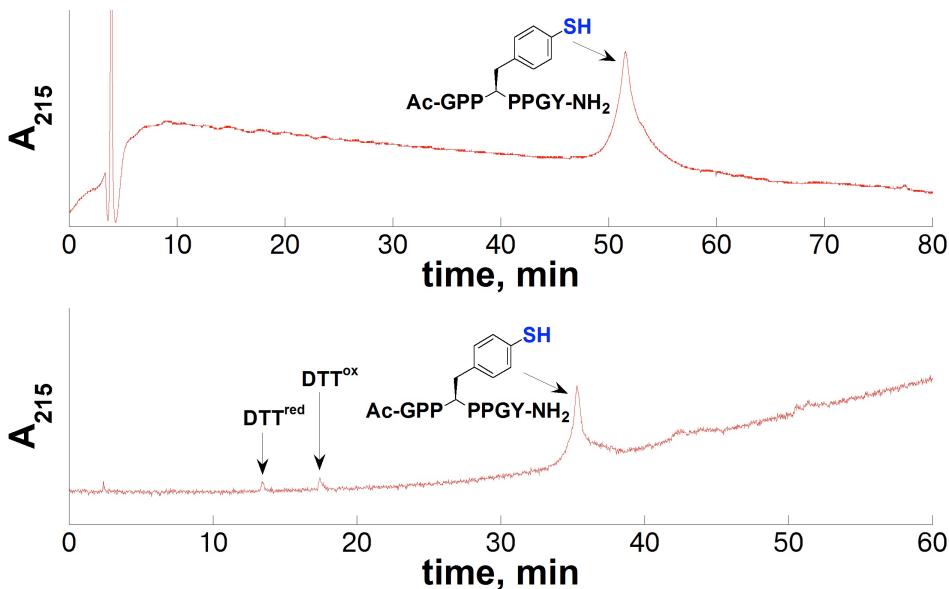


Figure S47. Reinjection of the purified peptide Ac-GPP(4-SH-Phe)PN-NH₂ (**12**) using a gradient of 0-55% buffer B in buffer A over 80 minutes (top); reinjection of the purified peptide Ac-GPP(4-SH-Phe)PN-NH₂ (**12**) with DTT (1 mM) using a gradient of 0-100% methanol in water over 60 minutes (bottom).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

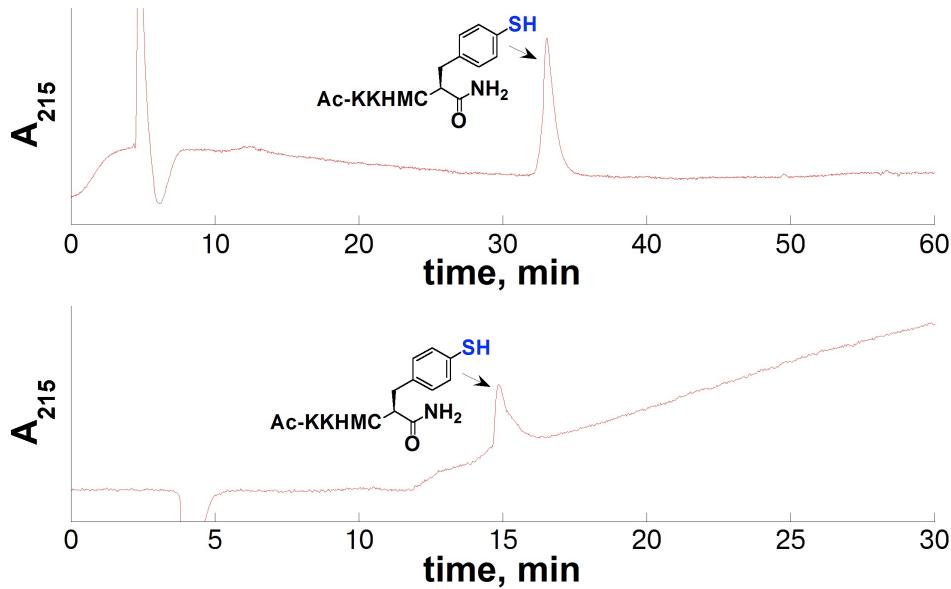


Figure S48. Reinjection of the purified peptide Ac-KKHMC(4-SH-Phe)-NH₂ (**13**) using a gradient of 0-70% buffer B in buffer A over 60 minutes (top); reinjection of the purified peptide Ac-KKHMC(4-SH-Phe)-NH₂ (**13**) using a Varian Microsorb MV C4 analytical column (250 × 4.6 mm, 5 μm particle, 300 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).

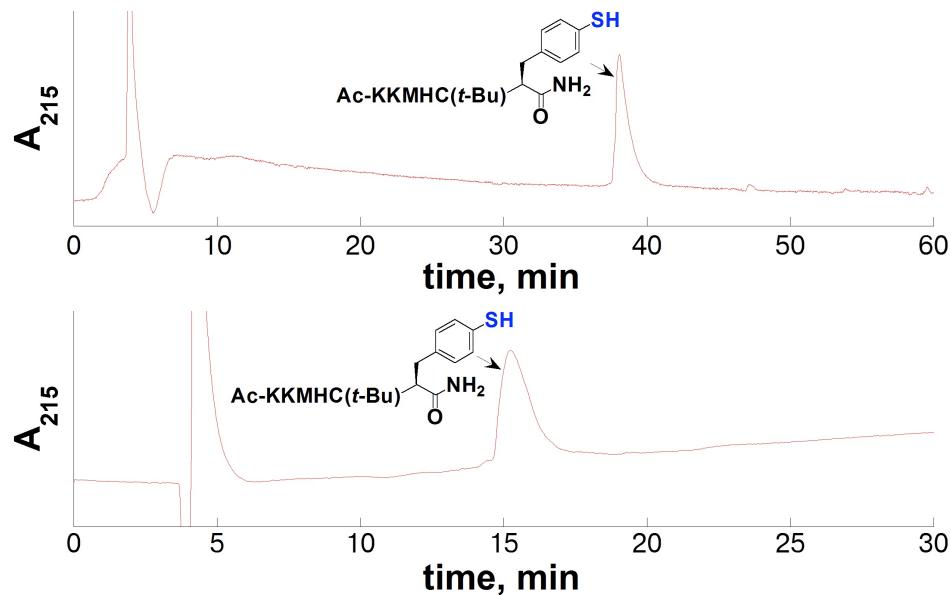


Figure S49. Reinjection of the purified peptide Ac-KKHMC(*t*Bu)(4-SH-Phe)-NH₂ (**14**) using a gradient of 0-75% buffer B in buffer A over 60 minutes (top); reinjection of the purified peptide Ac-KKHMC(*t*Bu)(4-SH-Phe)-NH₂ (**14**) using a Varian Microsorb MV C4 analytical column (250 × 4.6 mm, 5 μm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

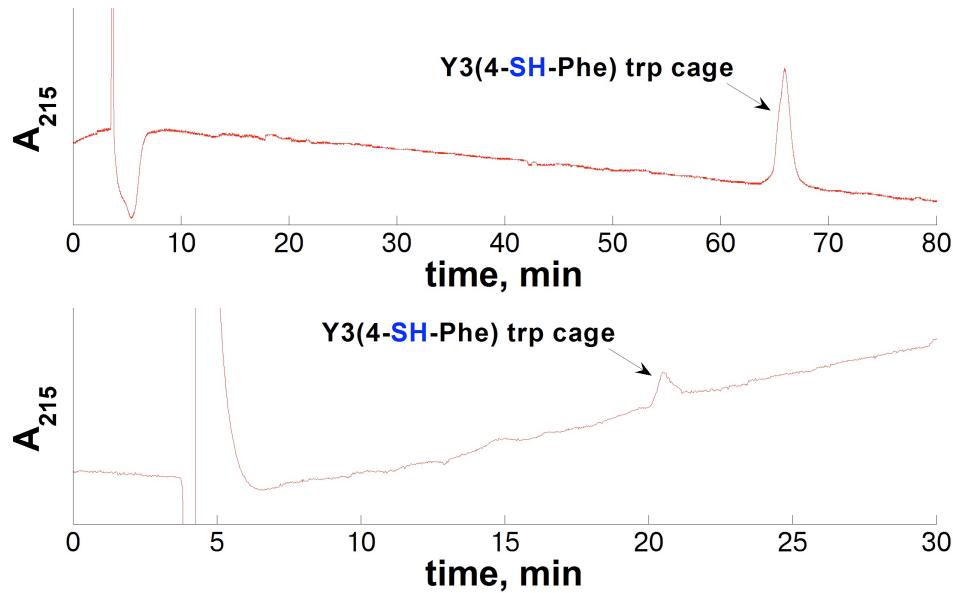


Figure S50. Reinjection of the purified Y3(4-SH-Phe) trp cage peptide (**15**) using a gradient of 0-70% buffer B in buffer A over 80 minutes (top); reinjection of the purified peptide Y3(4-SH-Phe) trp cage peptide (**15**) using a Varian Microsorb MV C4 analytical column (250 × 4.6 mm, 5 μ m particle,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

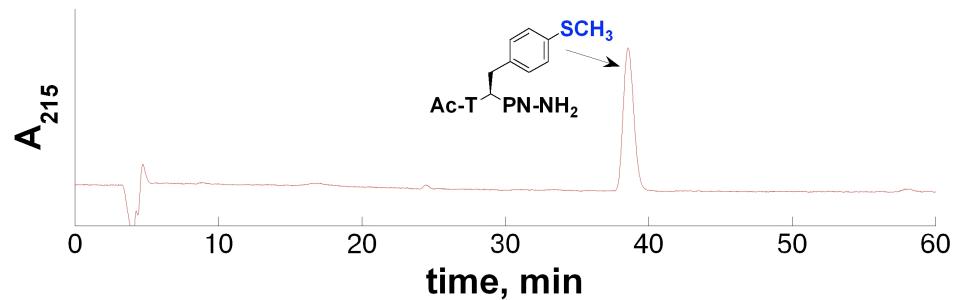


Figure S51. Reinjection of the purified peptide Ac-T(4-SMe-Phe)PN-NH₂ (**17**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

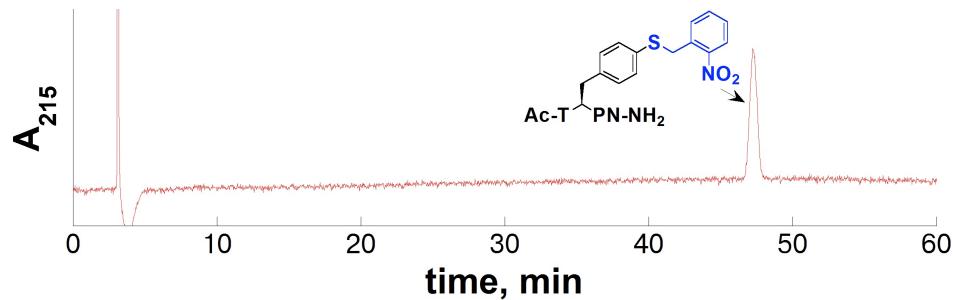


Figure S52. Reinjection of the purified peptide Ac-T(4-S-(2-nitrobenzyl)-Phe)PN-NH₂ (**18**) using a gradient of 0-50% buffer B in buffer A over 60 minutes.

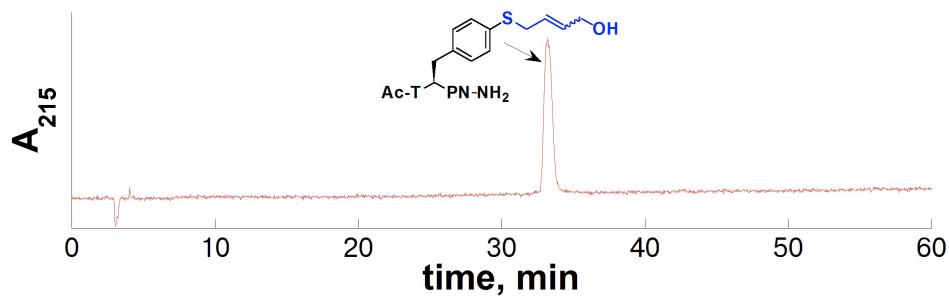


Figure S53. Reinjection of the purified peptide Ac-T(4-(SCH₂CH=CHCH₂OH)-Phe)PN-NH₂ (**19**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

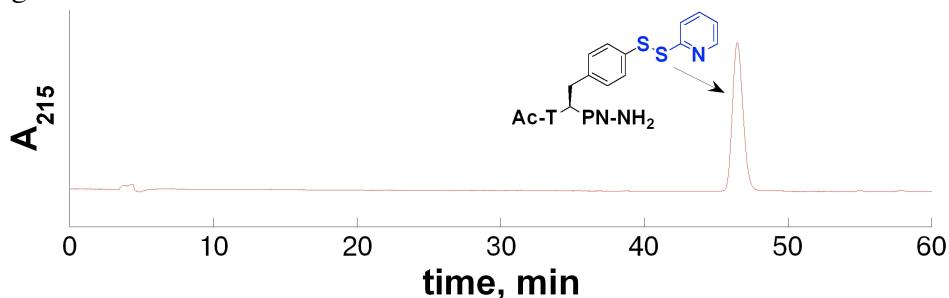


Figure S54. Re-injection of the purified peptide Ac-T(4-S-SPy-Phe)PN-NH₂ (**20**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

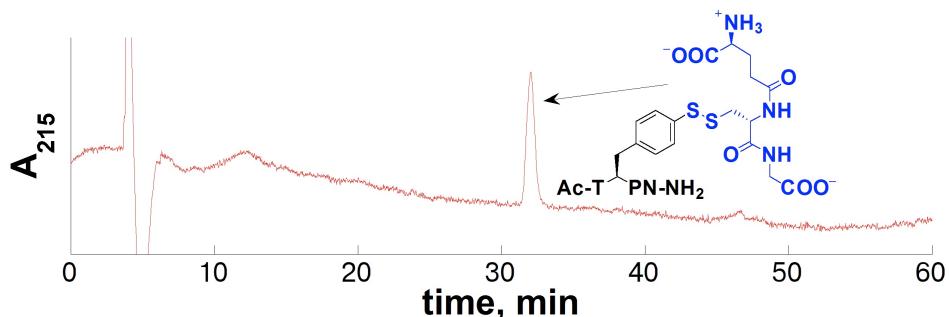


Figure S55. Re-injection of the purified peptide Ac-T(4-(glutathione disulfide)S-Phe)PN-NH₂ (**21**) using a gradient of 0-45% buffer B in buffer A over 60 minutes.

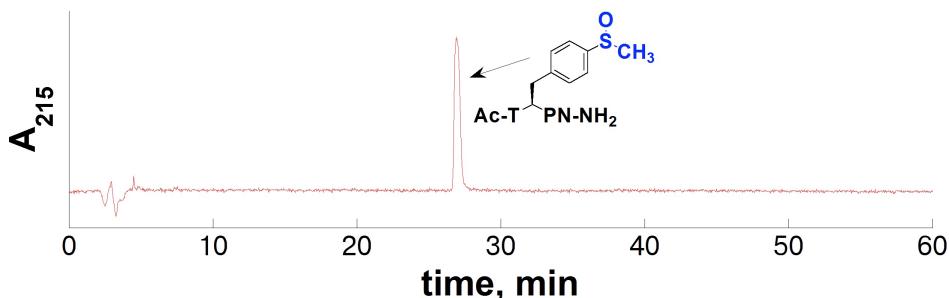


Figure S56. Re-injection of the purified peptide Ac-T(4-S(O)Me-Phe)PN-NH₂ (**22**) using a gradient of 0-20% buffer B in buffer A over 60 minutes.

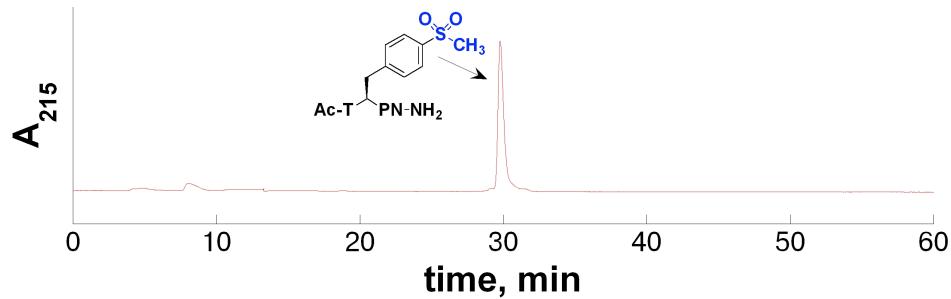


Figure S57. Reinjection of the purified peptide Ac-T(4-SO₂Me-Phe)PN-NH₂ (**23**) using a gradient of 0-20% buffer B in buffer A over 60 minutes.

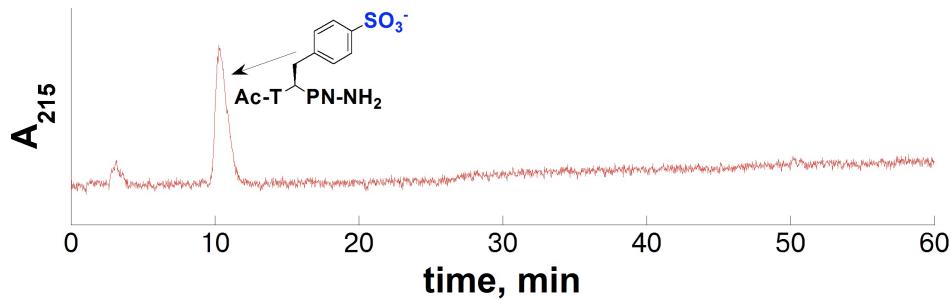


Figure S58. Reinjection of the purified peptide Ac-T(4-SO₃⁻-Phe)PN-NH₂ (**24**) using isocratic buffer A for 20 minutes followed by a gradient of 0-45% buffer B in buffer A over 40 minutes.

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11/18/2010 04:46:54 PM

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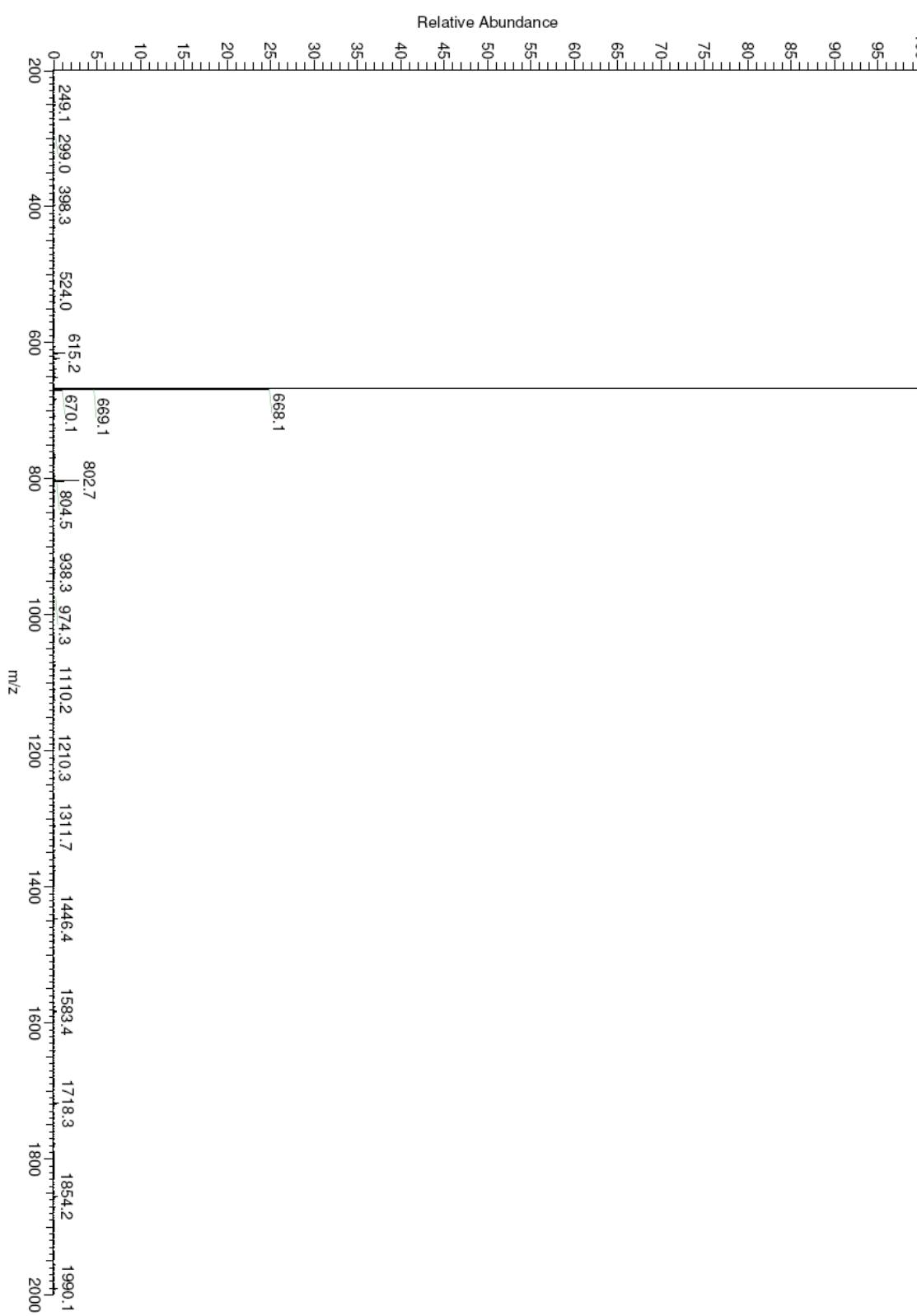


Figure S59. Mass spectrum of the peptide Ac-T(4-I-Phe)PN-NH₂ (**1**).

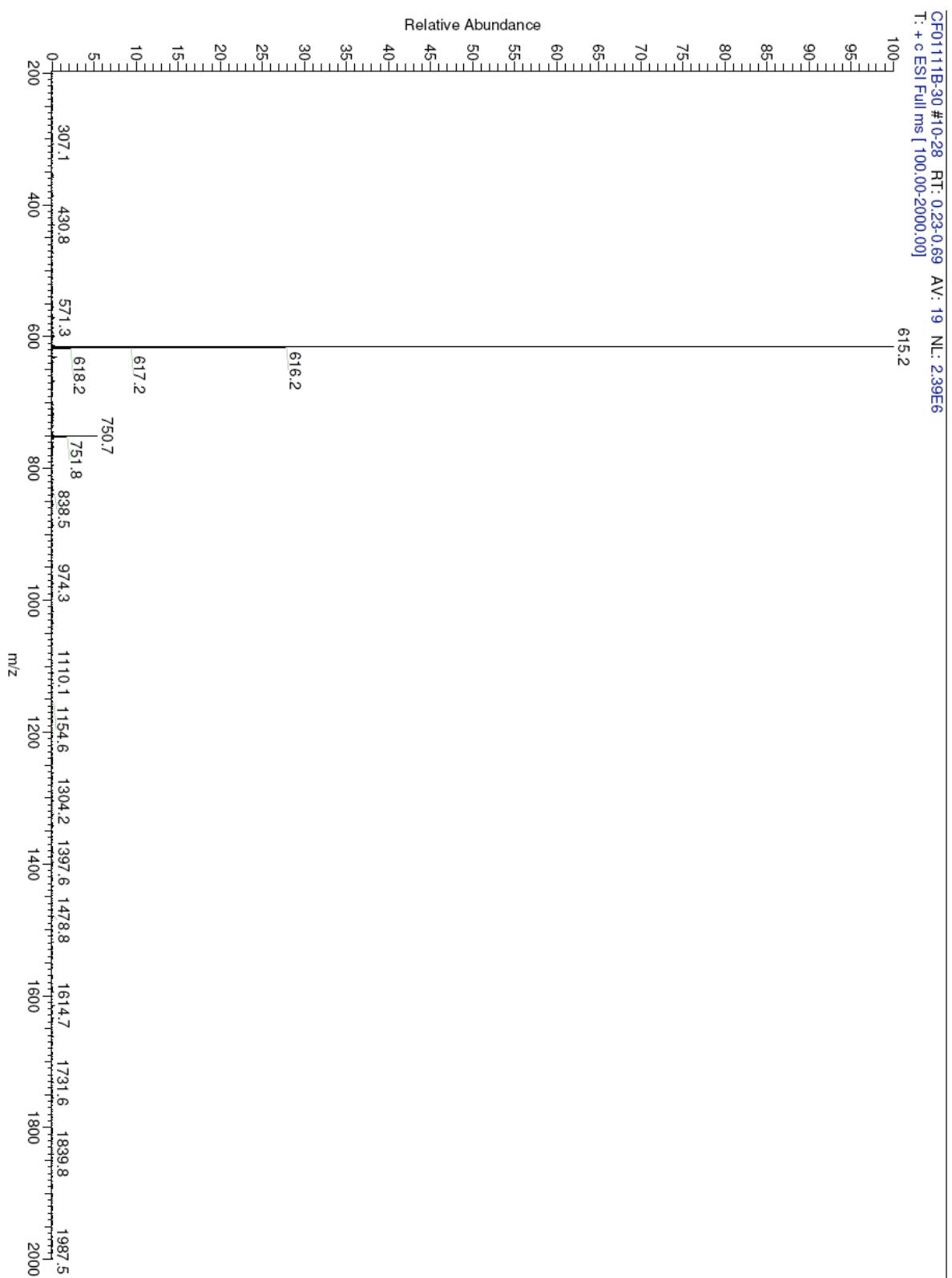


Figure S60. Mass spectrum of the peptide Ac-T(4-thioacetyl-Phe)PN-NH₂ (**2**).

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01/10/2011 07:51:59 PM

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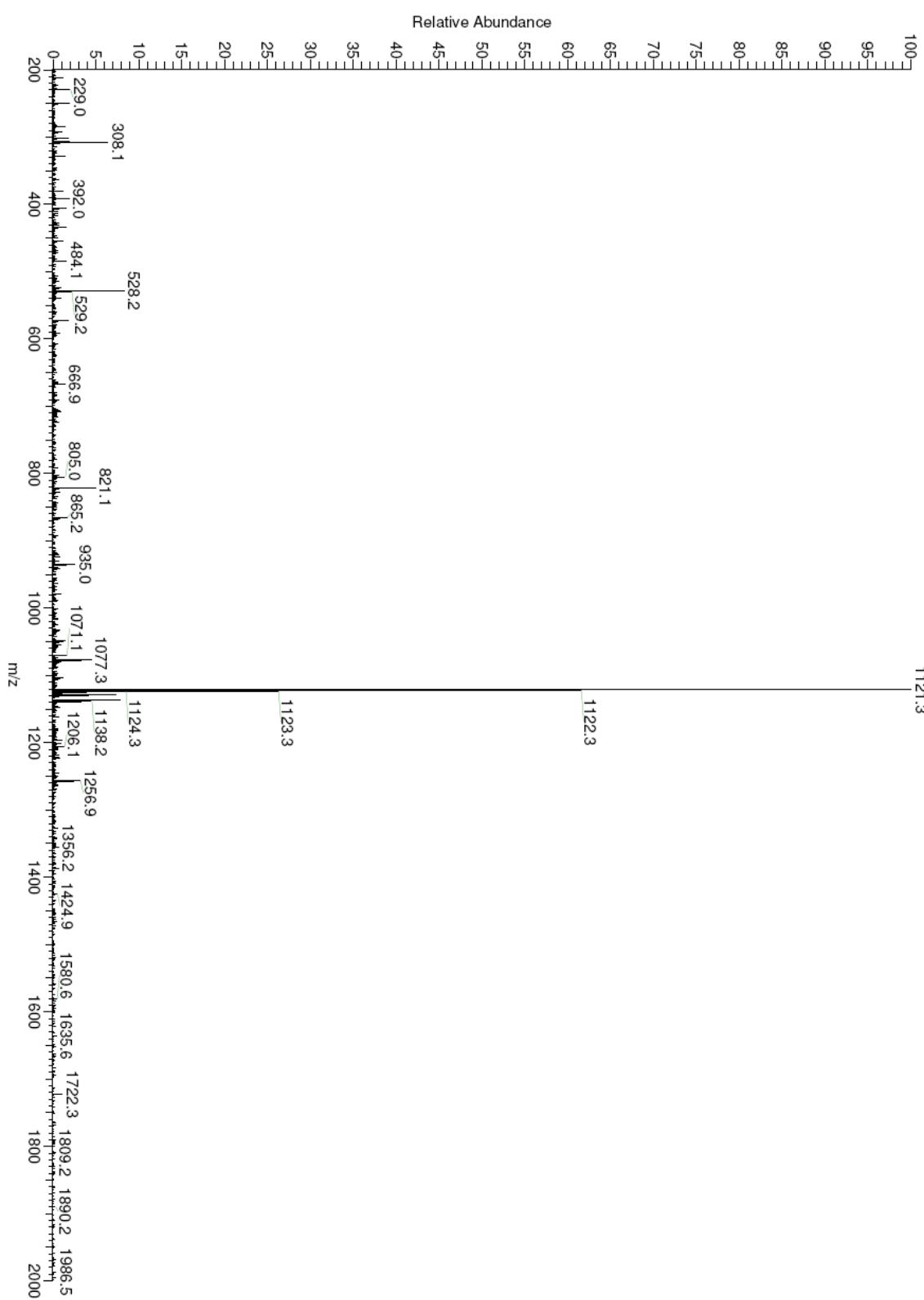


Figure S61. Mass spectrum of the thiophenylalanine disulfide peptide (**3**).

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11/19/2010 01:45:21 PM

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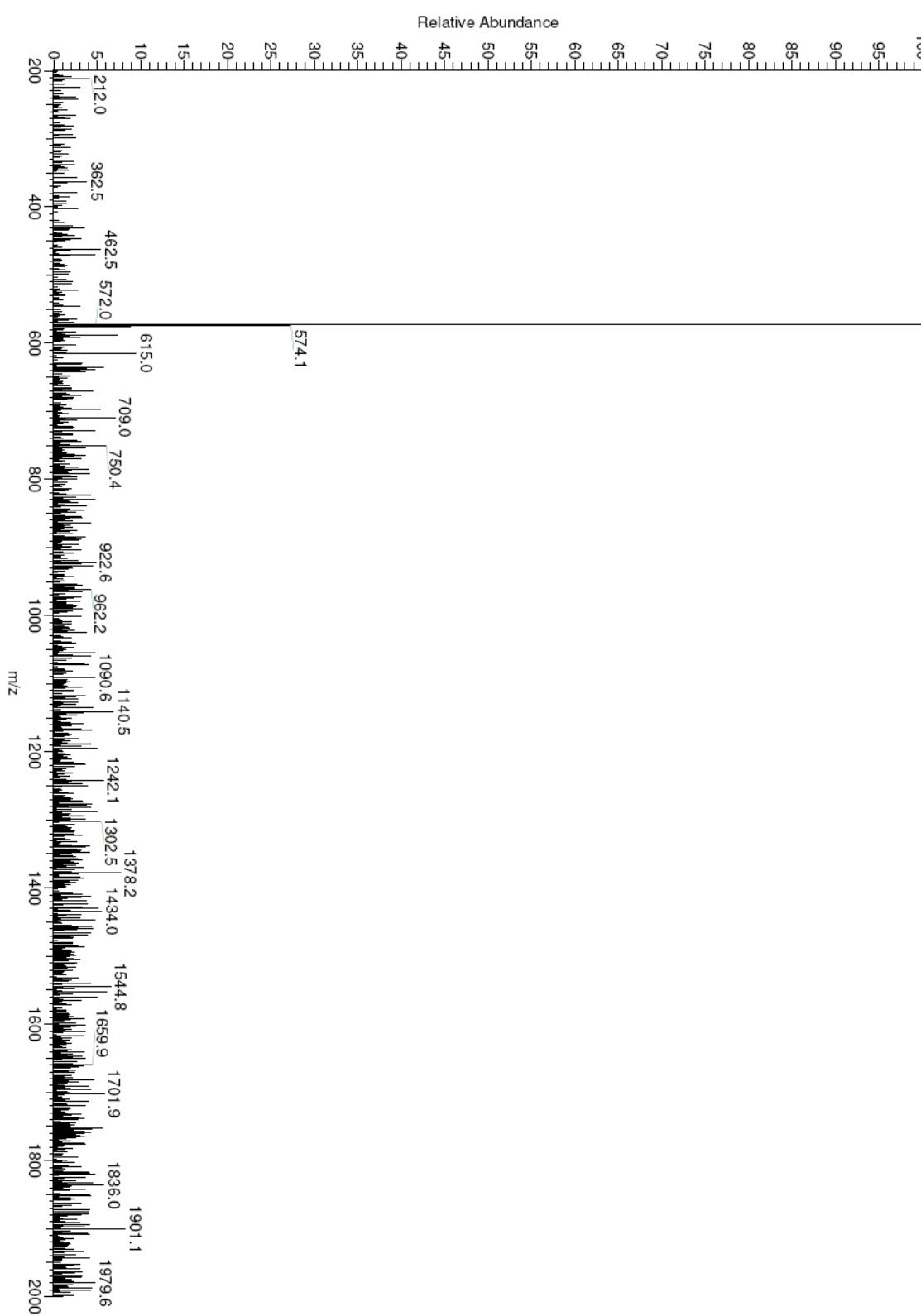


Figure S62. Mass spectrum of the peptide Ac-T(4-SH-Phe)PN-NH₂ (**4**).

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05/05/2011 05:55:16 PM

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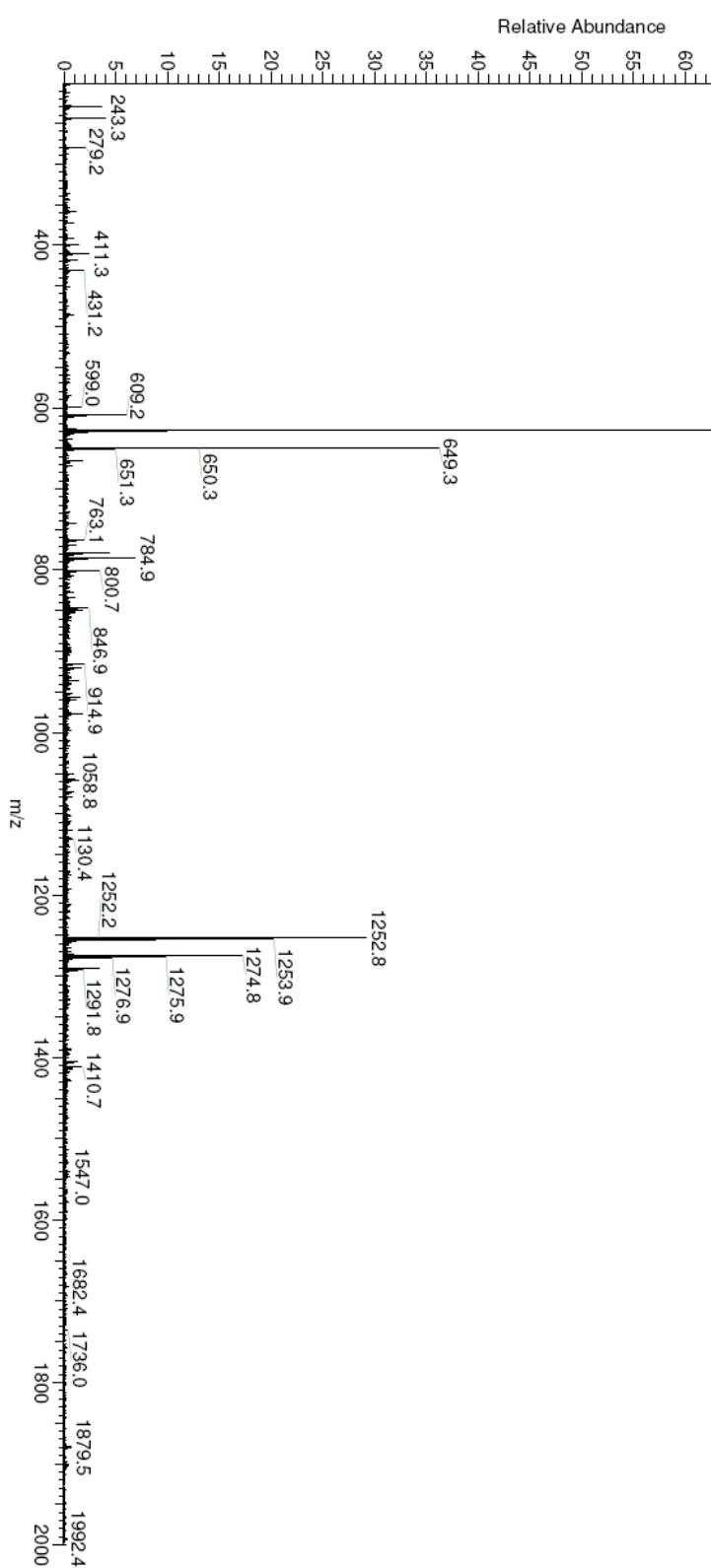


Figure S63. Mass spectrum of the peptide Ac-T(4-thiophenyl-Phe)PN-NH₂ (**5**).

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01/23/2011 02:09:40 AM

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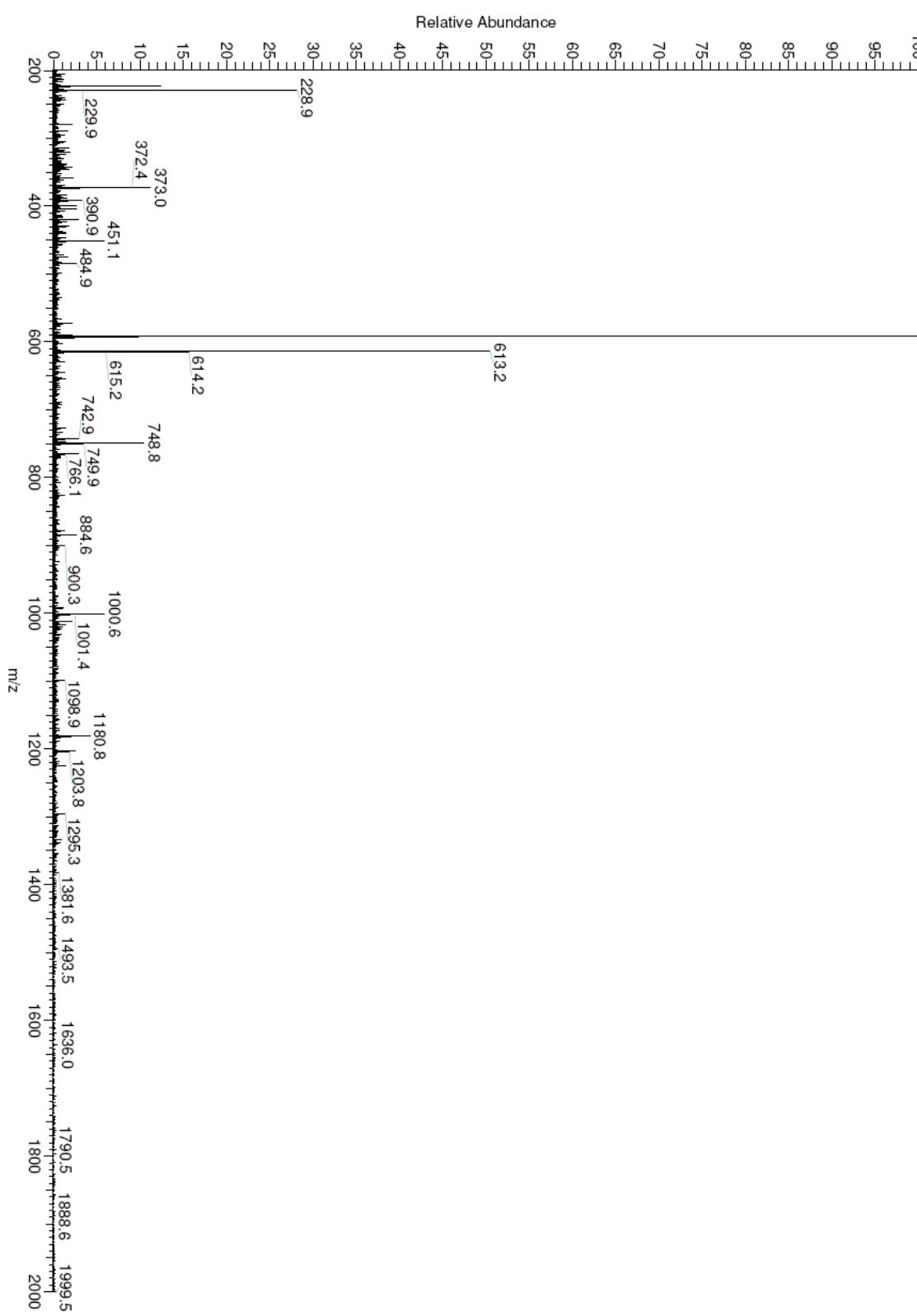


Figure S64. Mass spectrum of the peptide Ac-T(4-S-allyl-Phe)PN-NH₂ (**6**).

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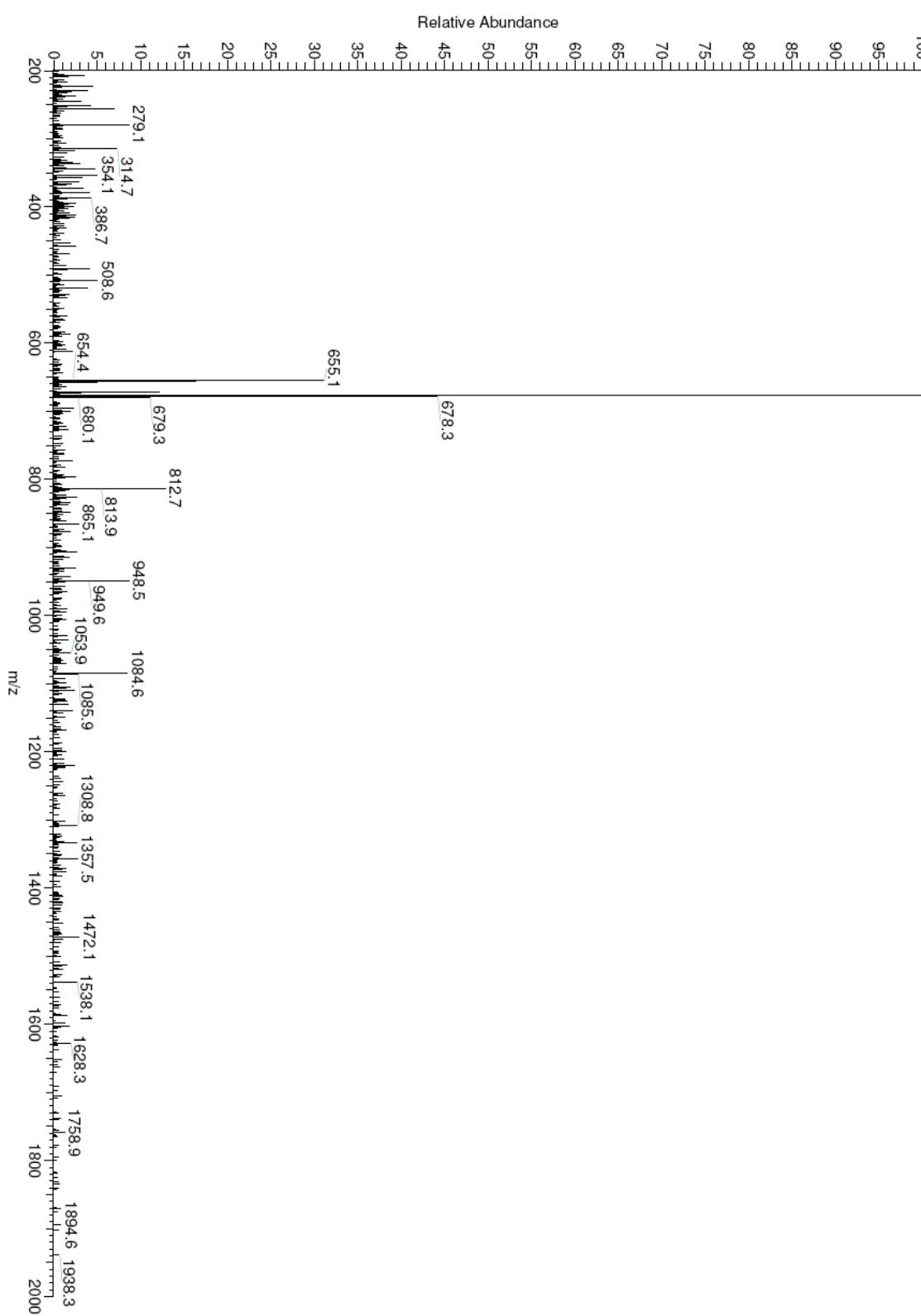


Figure S65. Mass spectrum of the peptide Ac-T(4-thiobenzoyl-Phe)PN-NH₂ (**7**).

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01/24/2011 07:37:38 PM

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998.0

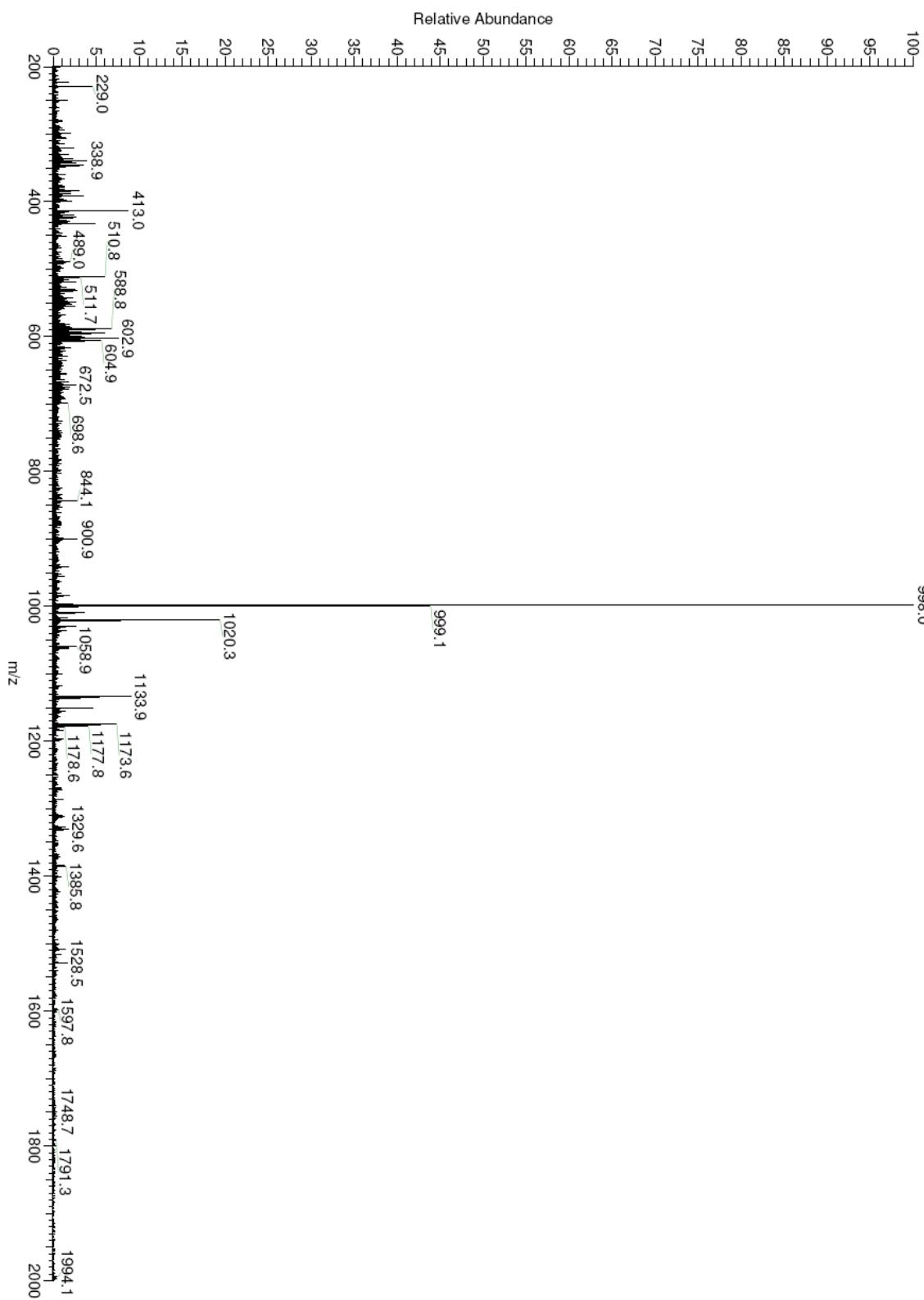


Figure S66. Mass spectrum of the peptide Ac-GPP(4-I-Phe)PPGY-NH₂ (**8**).

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03/17/2011 02:14:49 PM

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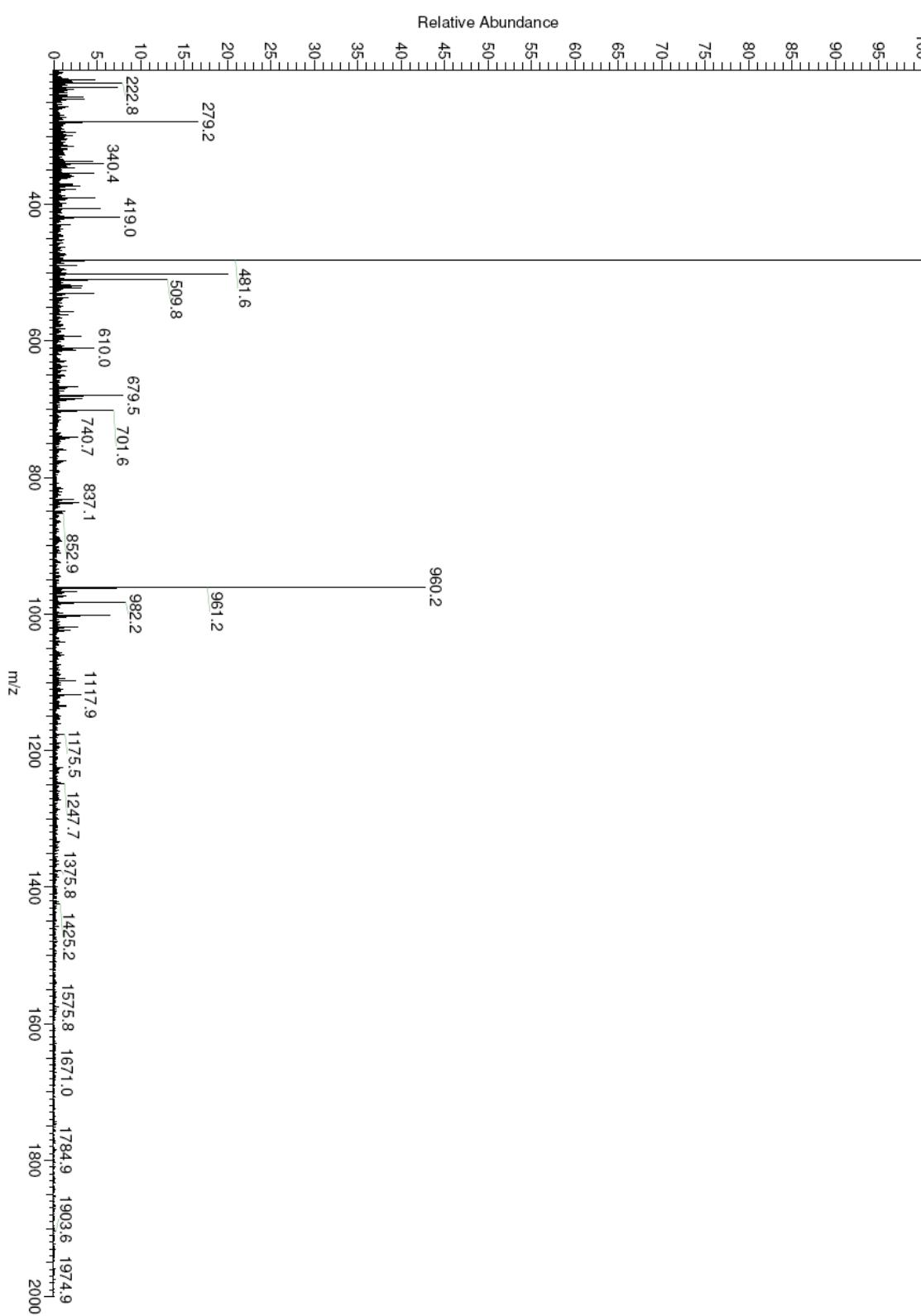


Figure S67. Mass spectrum of the peptide Ac-KKHM(4-I-Phe)-NH₂ (**9**).

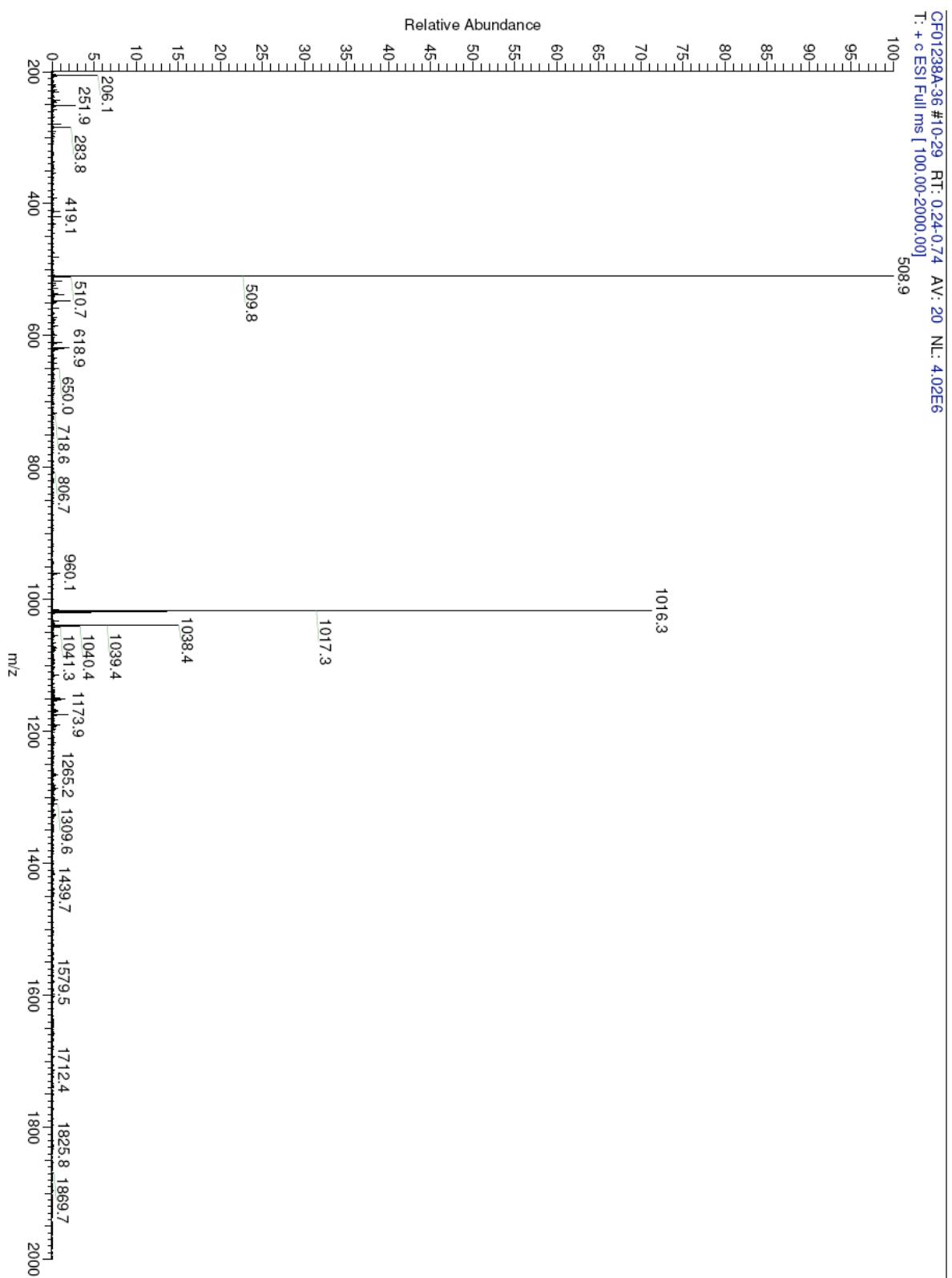


Figure S68. Mass spectrum of the peptide Ac-KKHM(*t*Bu)(4-I-Phe)-NH₂ (**10**).

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05/15/2011 04:44:59 AM

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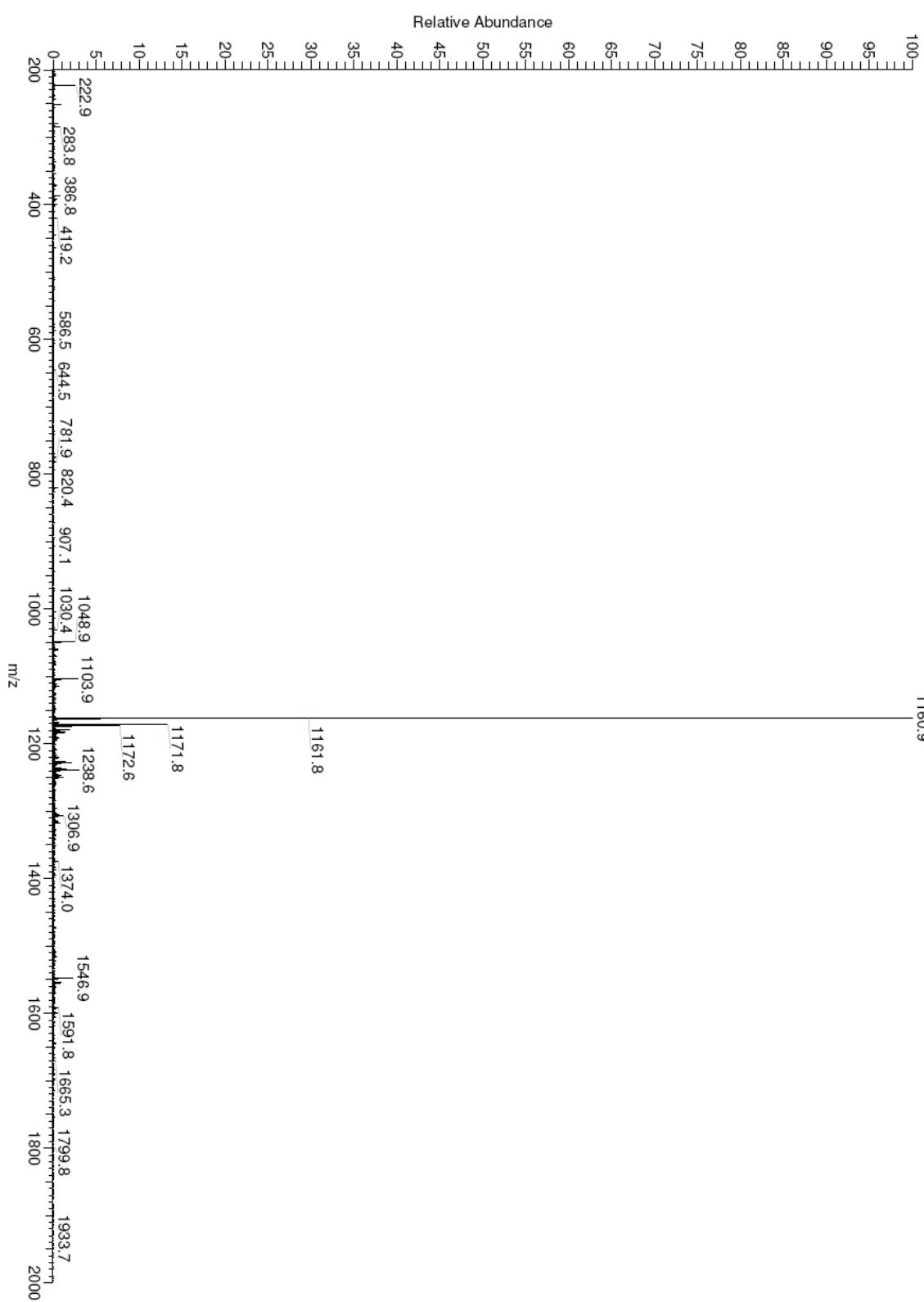


Figure S69. Mass spectrum of the Y3(4-I-Phe) trp cage peptide (**11**).

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01/25/2011 06:15:01 PM

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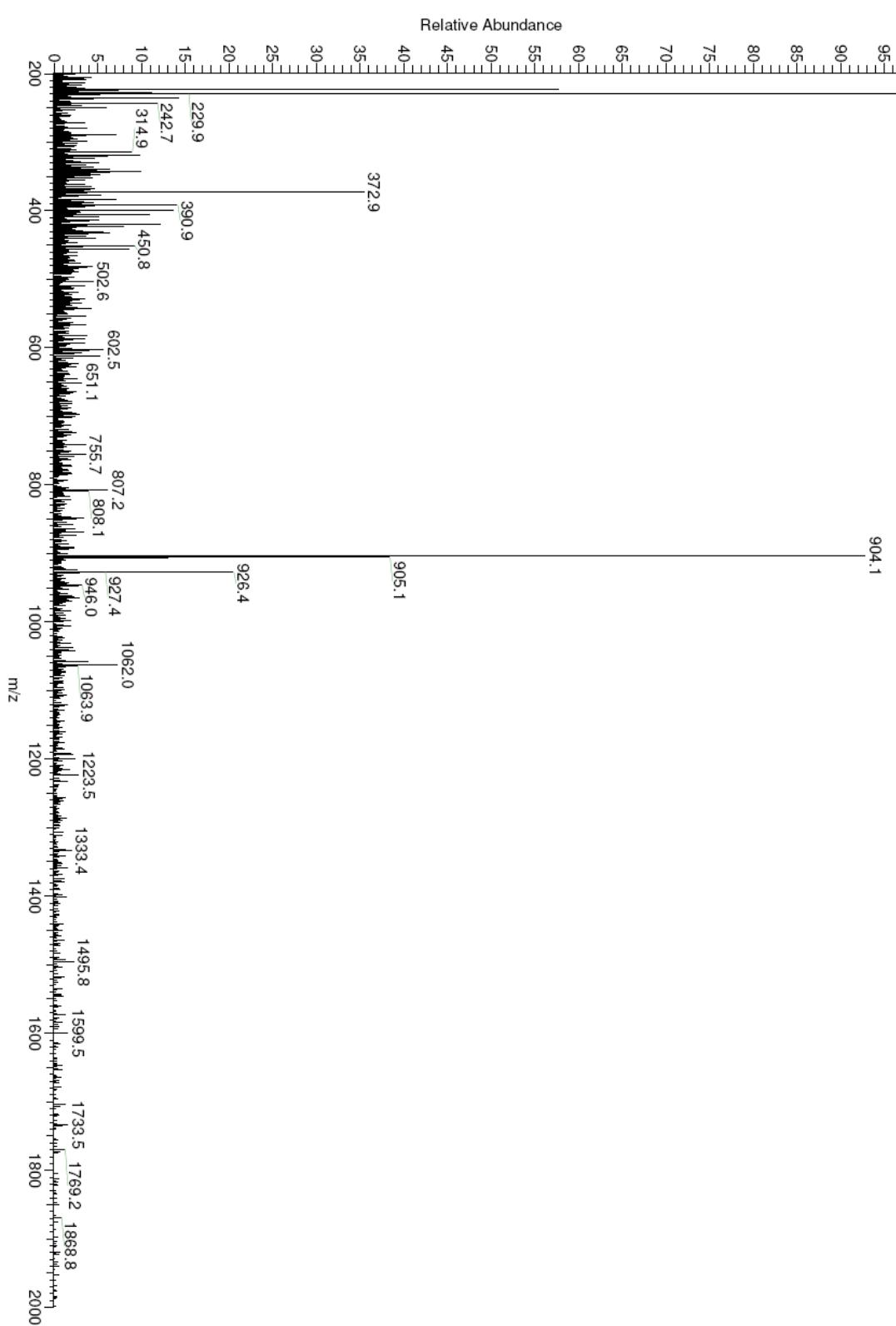


Figure S70. Mass spectrum of the peptide Ac-GPP(4-SH-Phe)PPGY-NH₂ (**12**).

C:\X\calib\data\cf\CF01277B-30

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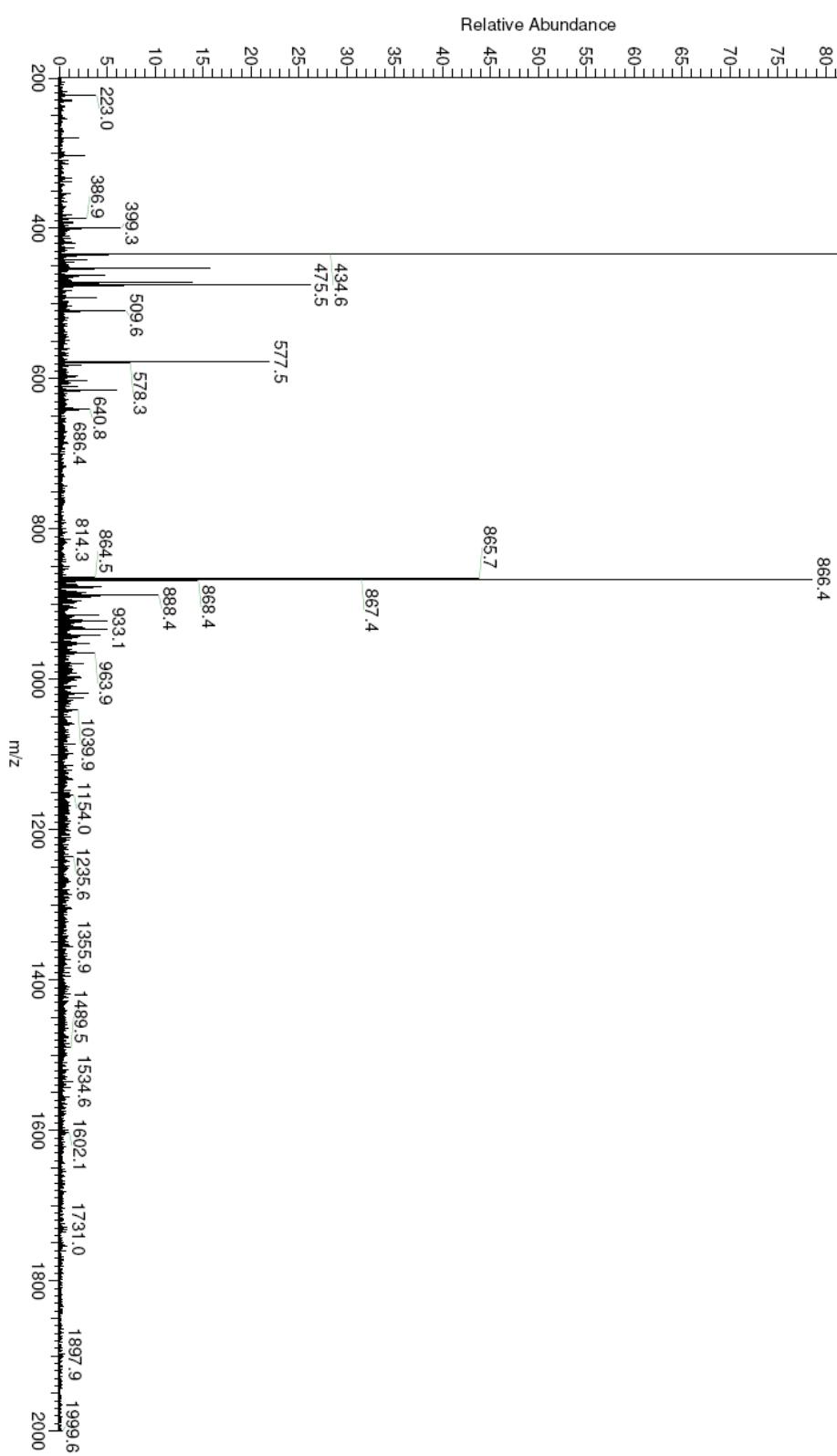


Figure S71. Mass spectrum of the peptide Ac-KKHM(4-SH-Phe)-NH₂ (**13**).

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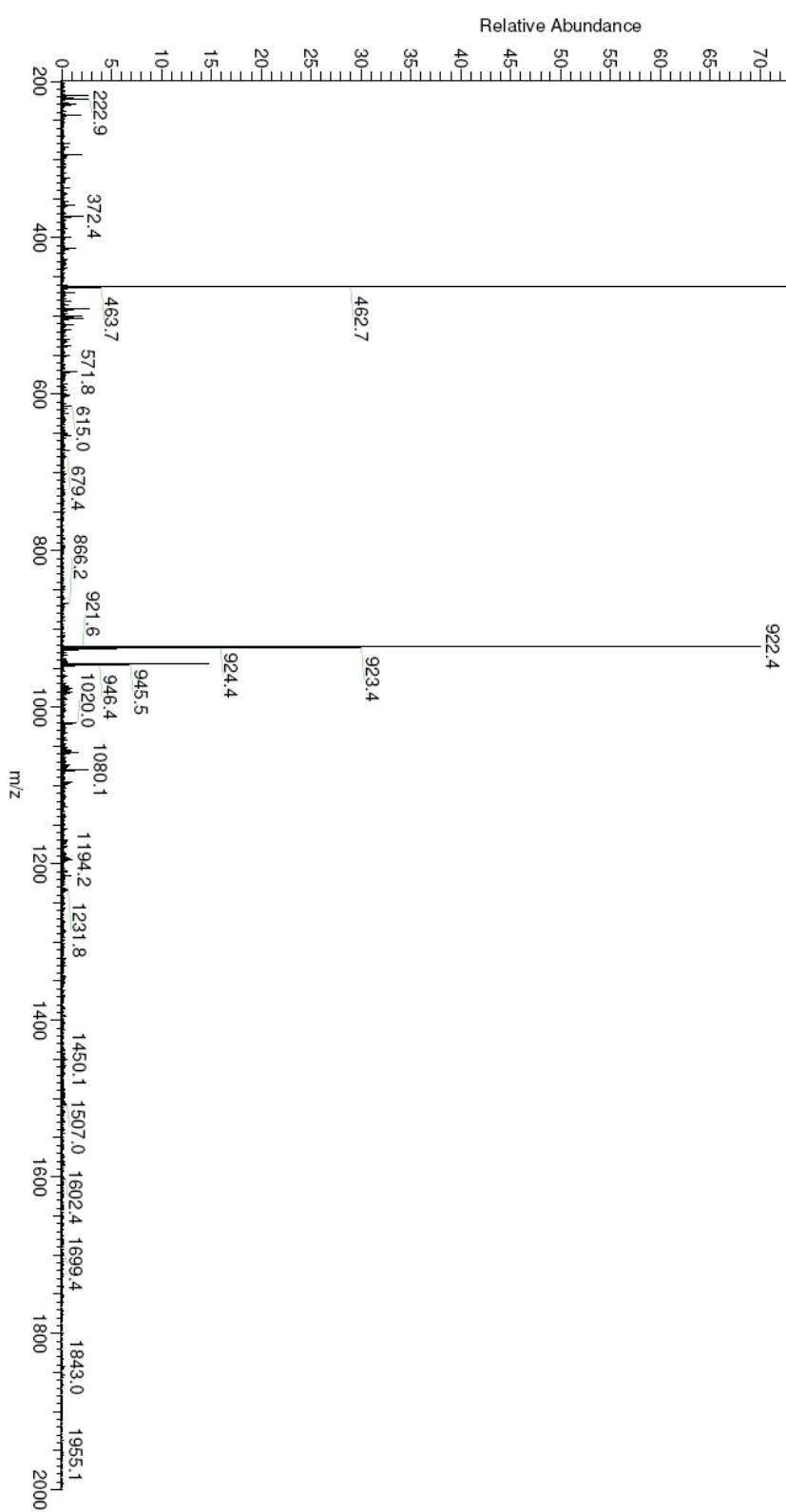


Figure S72. Mass spectrum of the peptide Ac-KKHM(*t*-Bu)(4-SH-Phe)-NH₂ (**14**).

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05/15/2011 04:36:16 AM

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

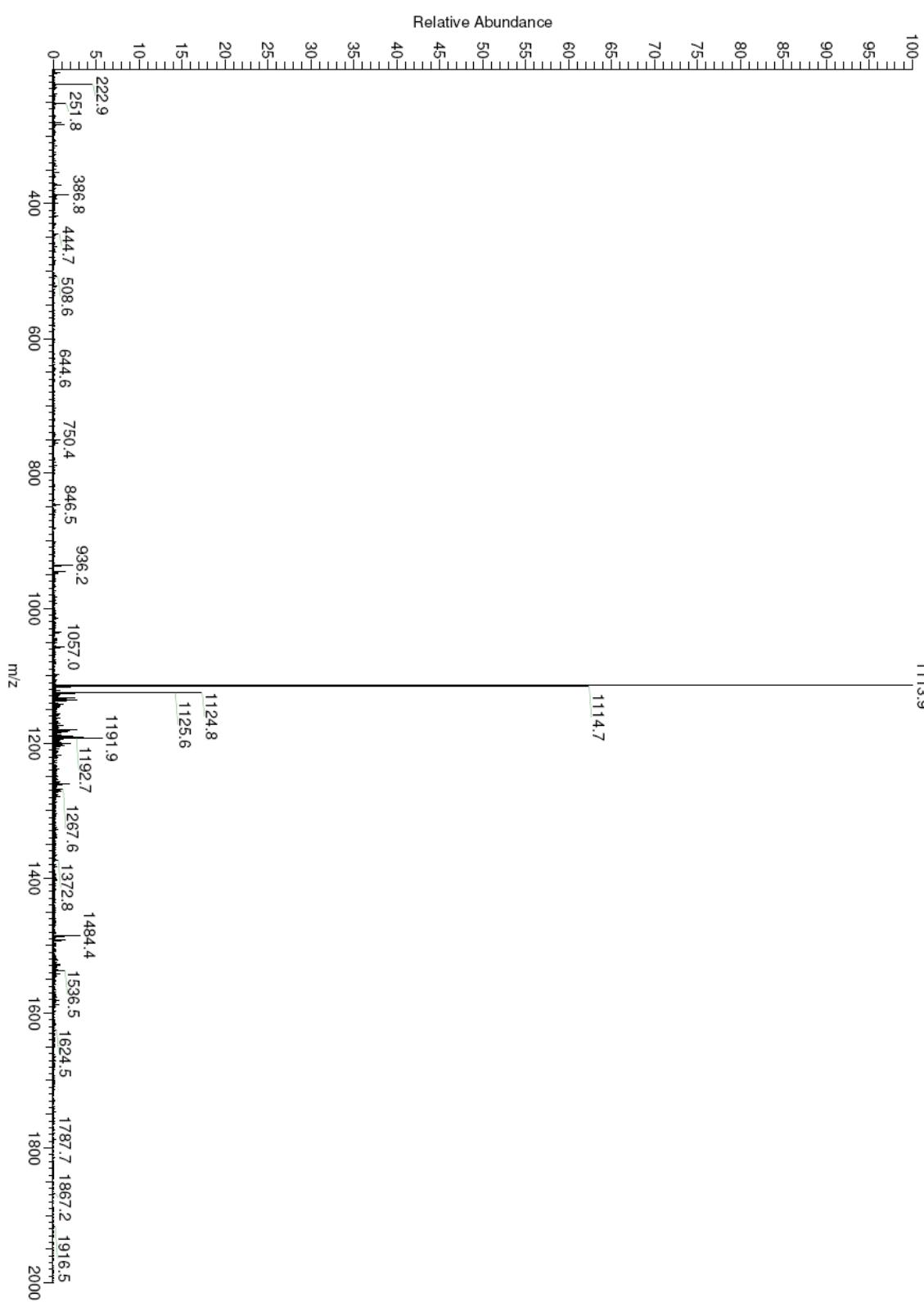


Figure S73. Mass spectrum of the Y3(4-SH-Phe) trp cage peptide (**15**).

C:\Xcalibur...\CF02078h-46_11116055910\#9-22

11/16/2011 05:59:10 AM

CF02078h-46 11116055910\#9-22 RT: 0.21-0.56 AV: 14 NL: 3.9E6

1106.2

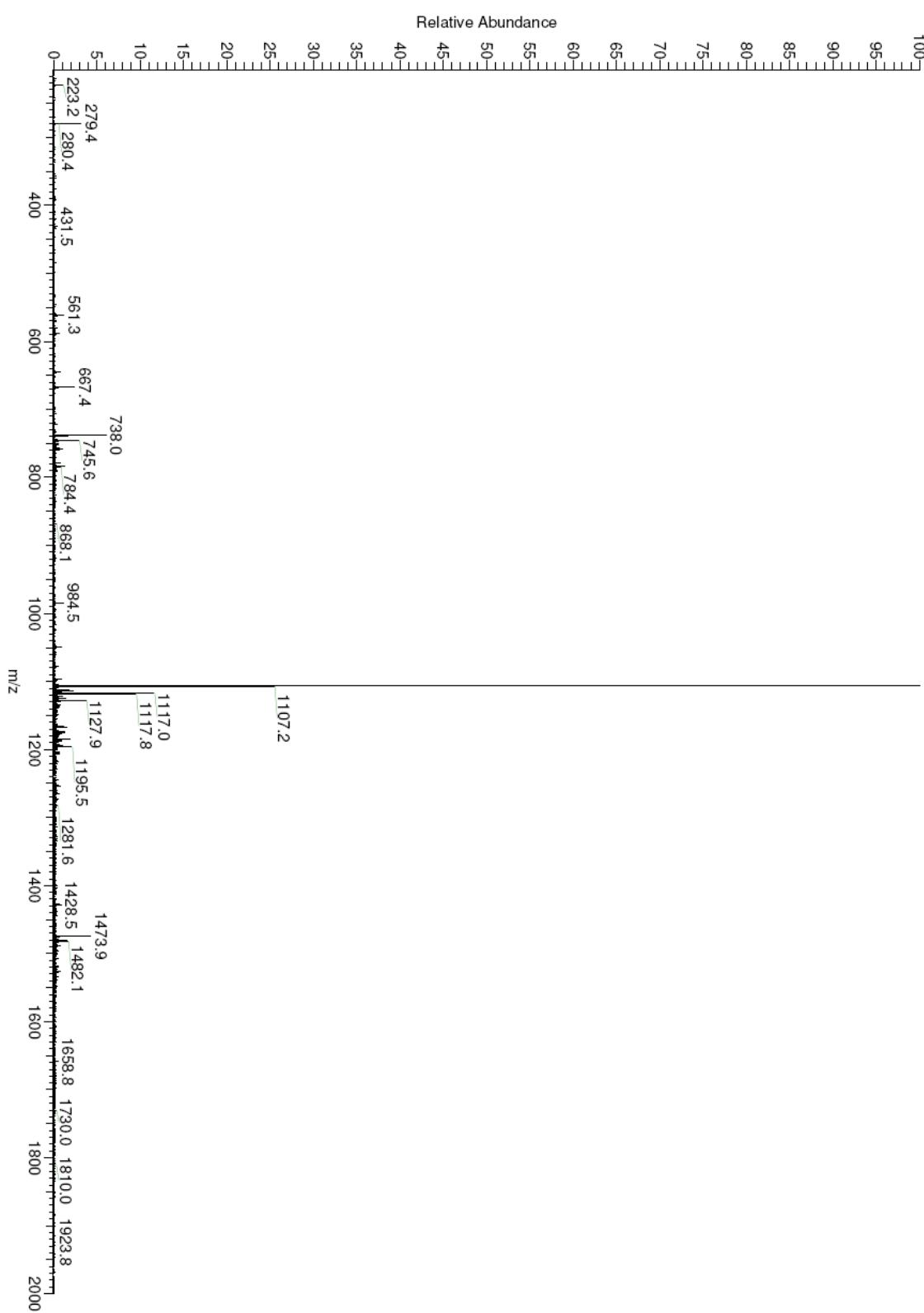


Figure S74. Mass spectrum of the trp cage peptide (**16**).

C:\Xcalibur...\CF01128a-38_101215174245#11-26

12/15/2010 05:42:45 PM

CF01128a-38 101215174245#11-26 RT: 0.25-0.64 AV: 16 NL: 6.81E5
T: + c ESI Full ms [100.00-2000.00] 587.2

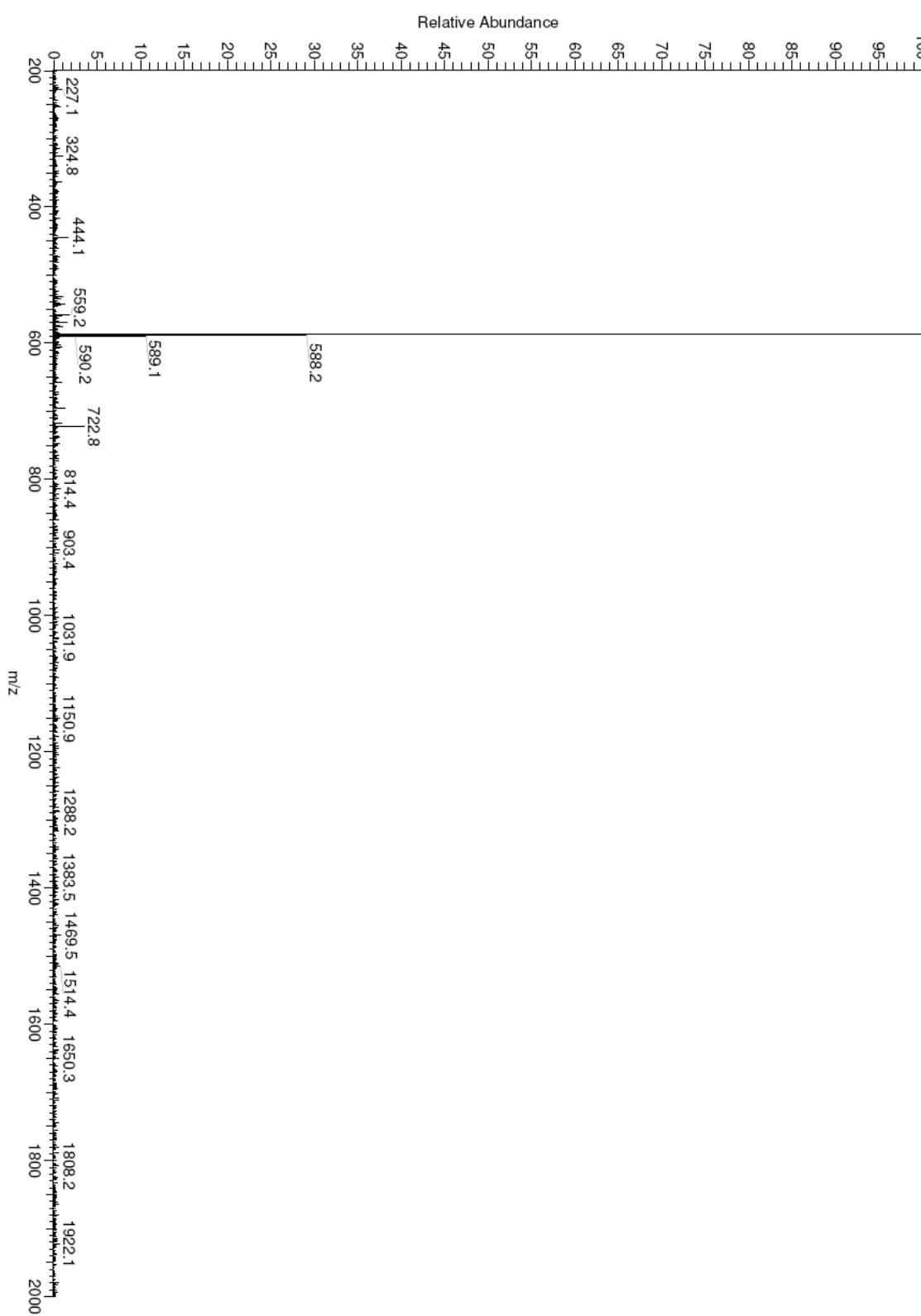


Figure S75. Mass spectrum of the peptide Ac-T(4-SMe-Phe)PN-NH₂ (**17**).

C:\Xcalibur\data\cfcl01167-a-56

01/18/2011 09:24:11 PM

c01167-a-56 #10:34 RT: 0.23-0.83 AV: 25 NL: 9.77E4
T: + c ESI Full ms [100.00-2000.00]

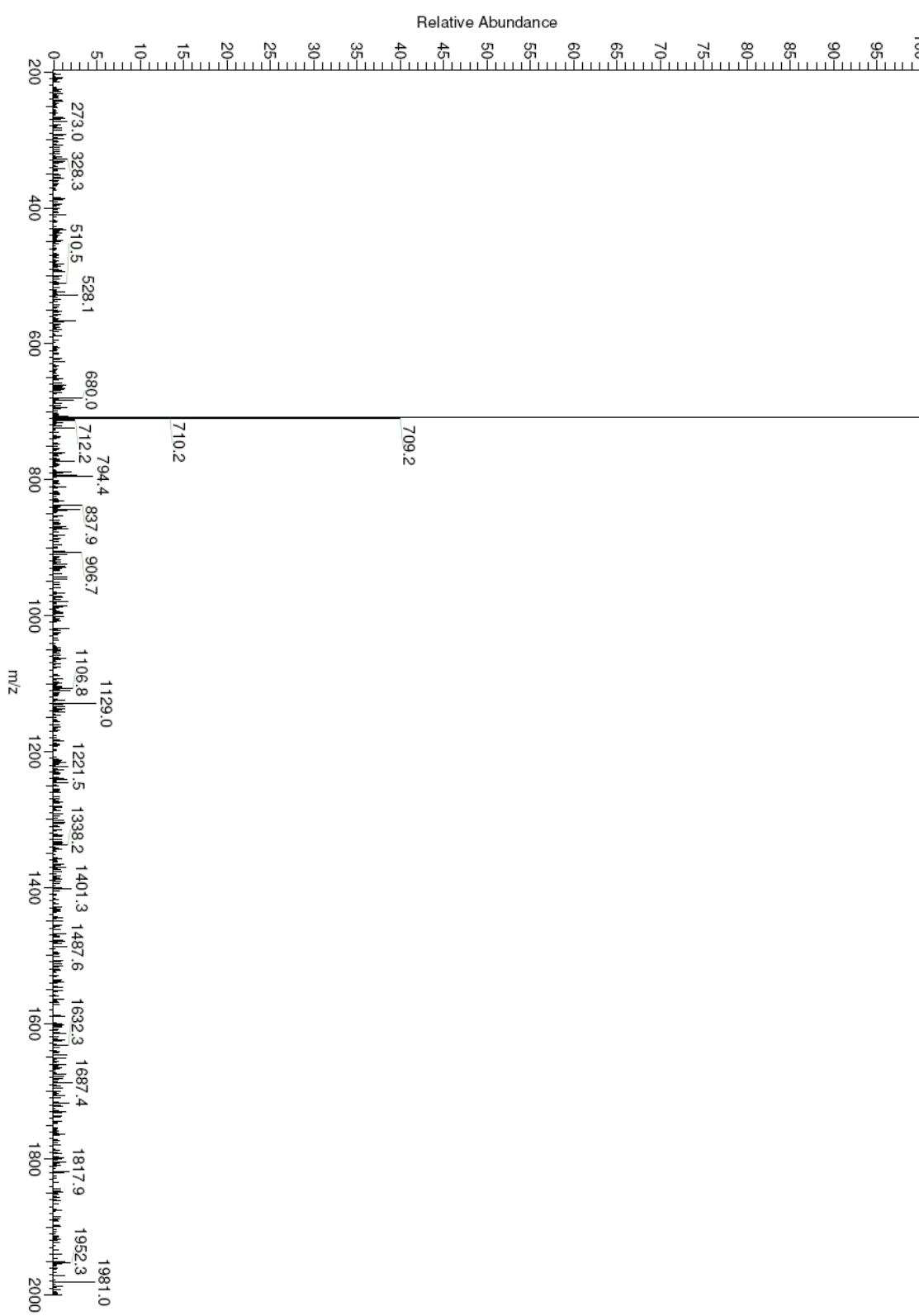


Figure S76. Mass spectrum of the peptide Ac-T(4-S-(2-nitrobenzyl)-Phe)PN-NH₂ (**18**).

C:\X\calib\data\cf\CF01225B-26

03/30/2011 02:28:22 PM

CF01225B-26 #1229 RT: 0.29-0.73 AV: 18 NL: 3.62E5
T: + c ESI Full ms [100.00-2000.00] 621.0

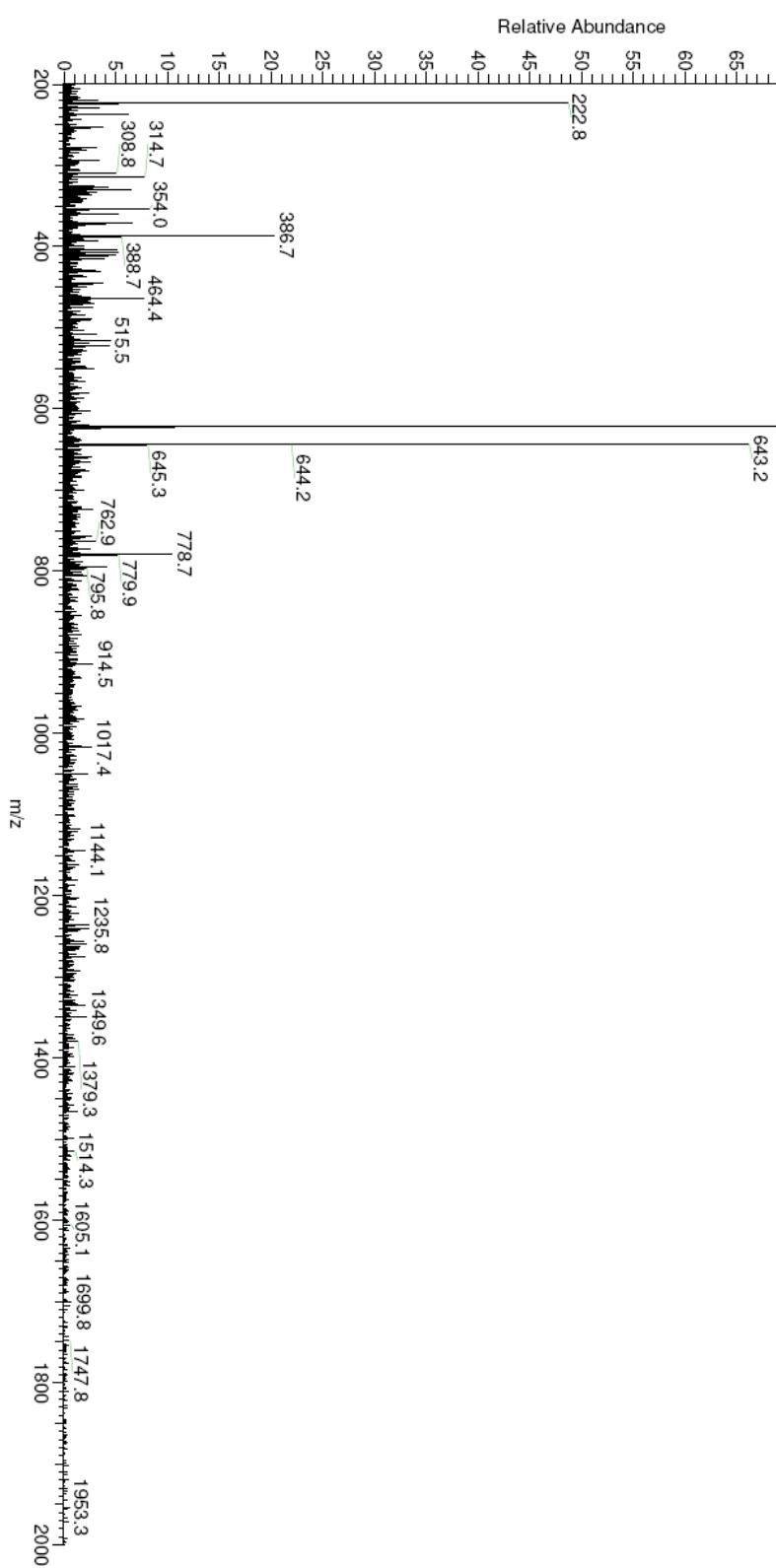


Figure S77. Mass spectrum of the peptide Ac-T(4-(SCH₂CH=CHCH₂OH)-Phe)PN-NH₂ (**19**).

C:\X\calib\data\cf\CF01216A-45

03/22/2011 05:49:27 PM

CF01216A-45 #11-30 RT: 0.26-0.76 AV: 20 NL: 2.65E6
T: + c ESI Full ms [100.00-2000.00]

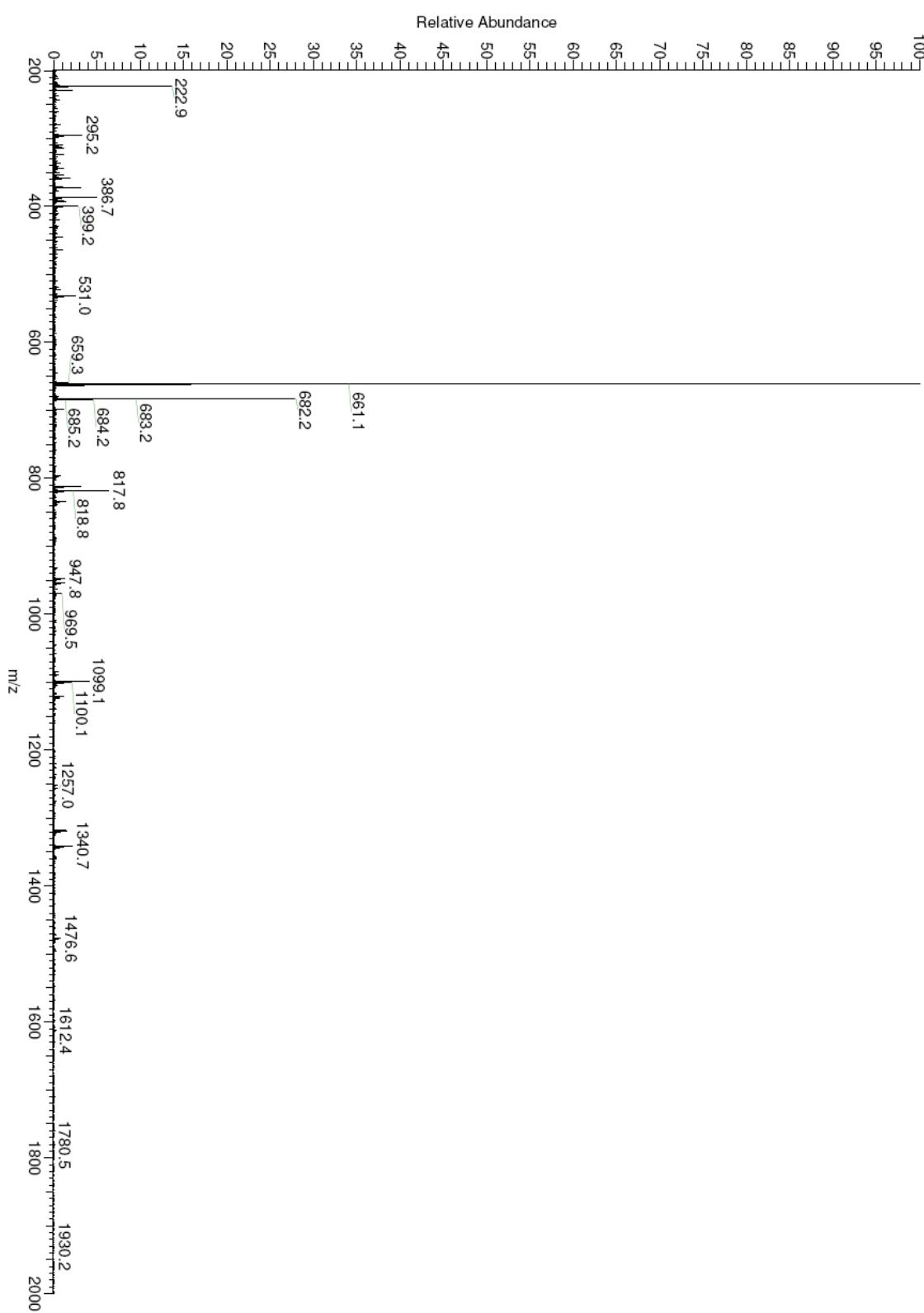


Figure S78. Mass spectrum of the peptide Ac-T(4-S-SPy-Phe)PN-NH₂ (**20**).

C:\X\calib\data\cf\CF01242B-26

04/14/2011 06:44:33 PM

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

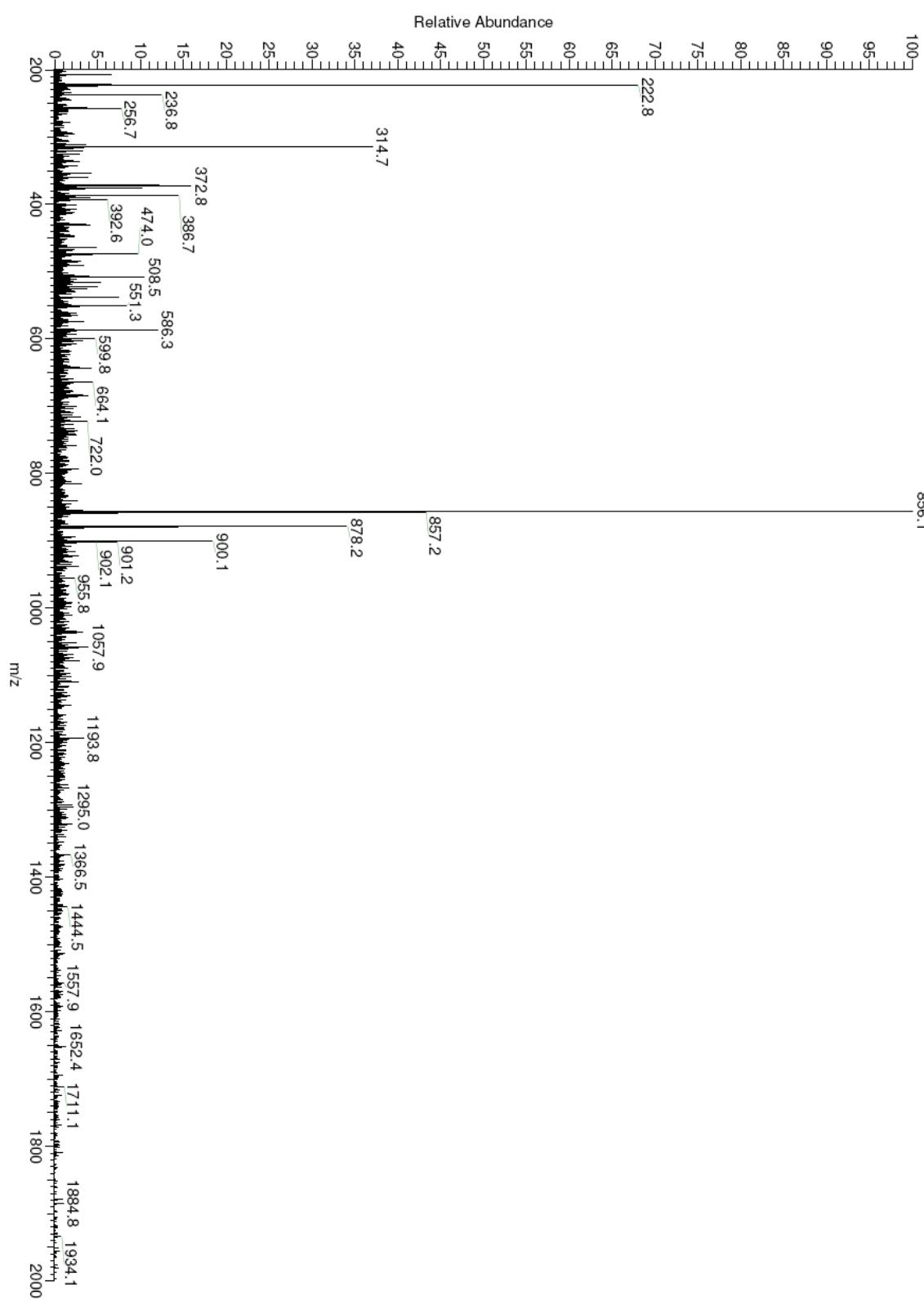


Figure S79. Mass spectrum of the peptide Ac-T(4-(glutathione disulfide)S-Phe)PN-NH₂ (**21**).

C:\Xcalibru\data\cfcf01150a-23

01/10/2011 10:50:53 AM

cf01150a-23 #11:23 RT: 0.26-0.56 AV: 13 NL: 7.12E4
T: + c ESI Full ms [100.00-2000.00]

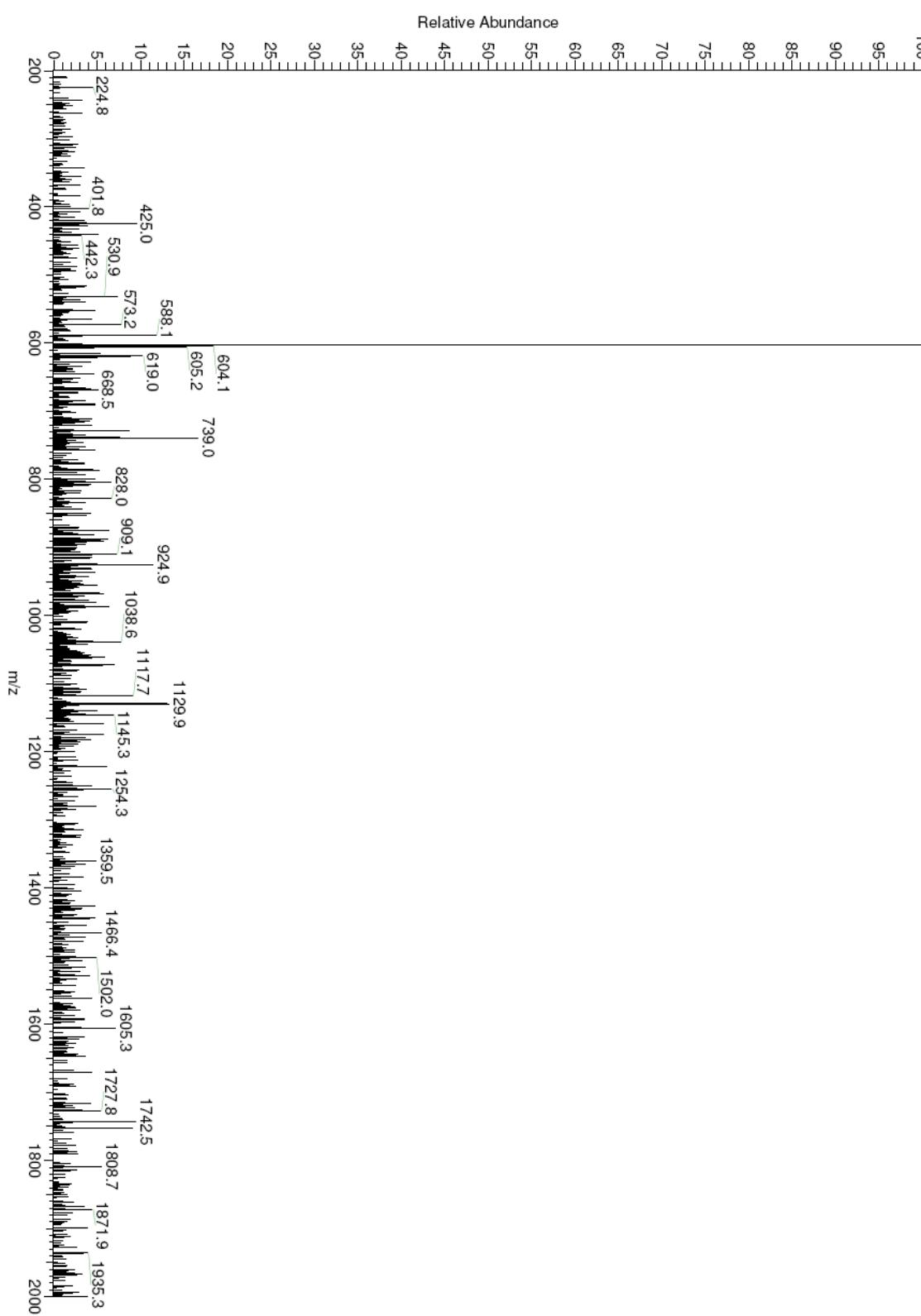


Figure S80. Mass spectrum of the peptide Ac-T(4-S(O)Me-Phe)PN-NH₂ (**22**).

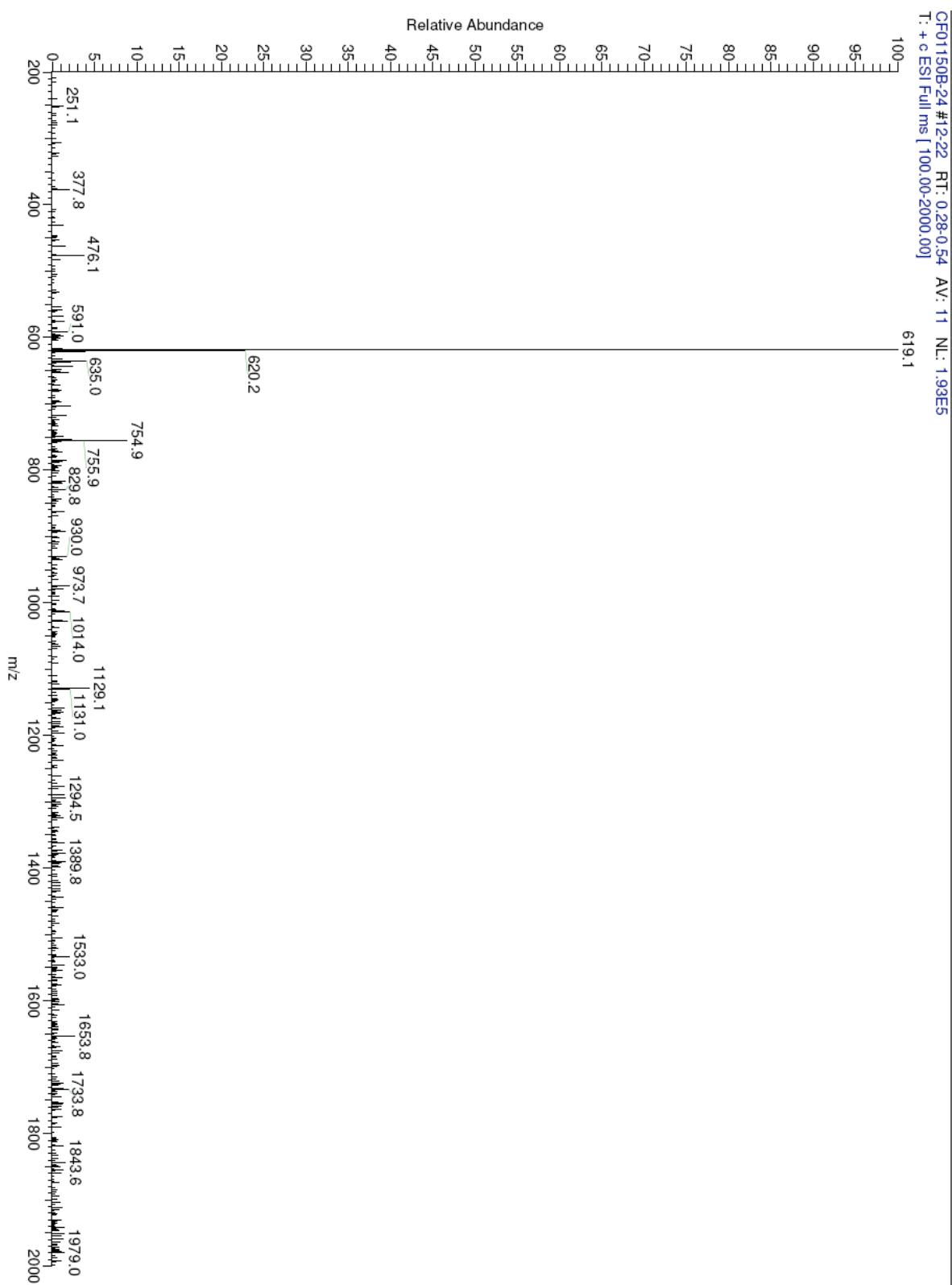


Figure S81. Mass spectrum of the peptide Ac-T(4-SO₂Me-Phe)PN-NH₂ (**23**).

C:\Xcalibur...\CF01280a3-8_110518172522

05/18/2011 05:25:22 PM

CF01280a3-8 110518172522#11-35 RT: 0.26-0.88 AV: 25 NL: 2.13E5
T: + c ESI Full ms [100.00-2000.00] 599.1

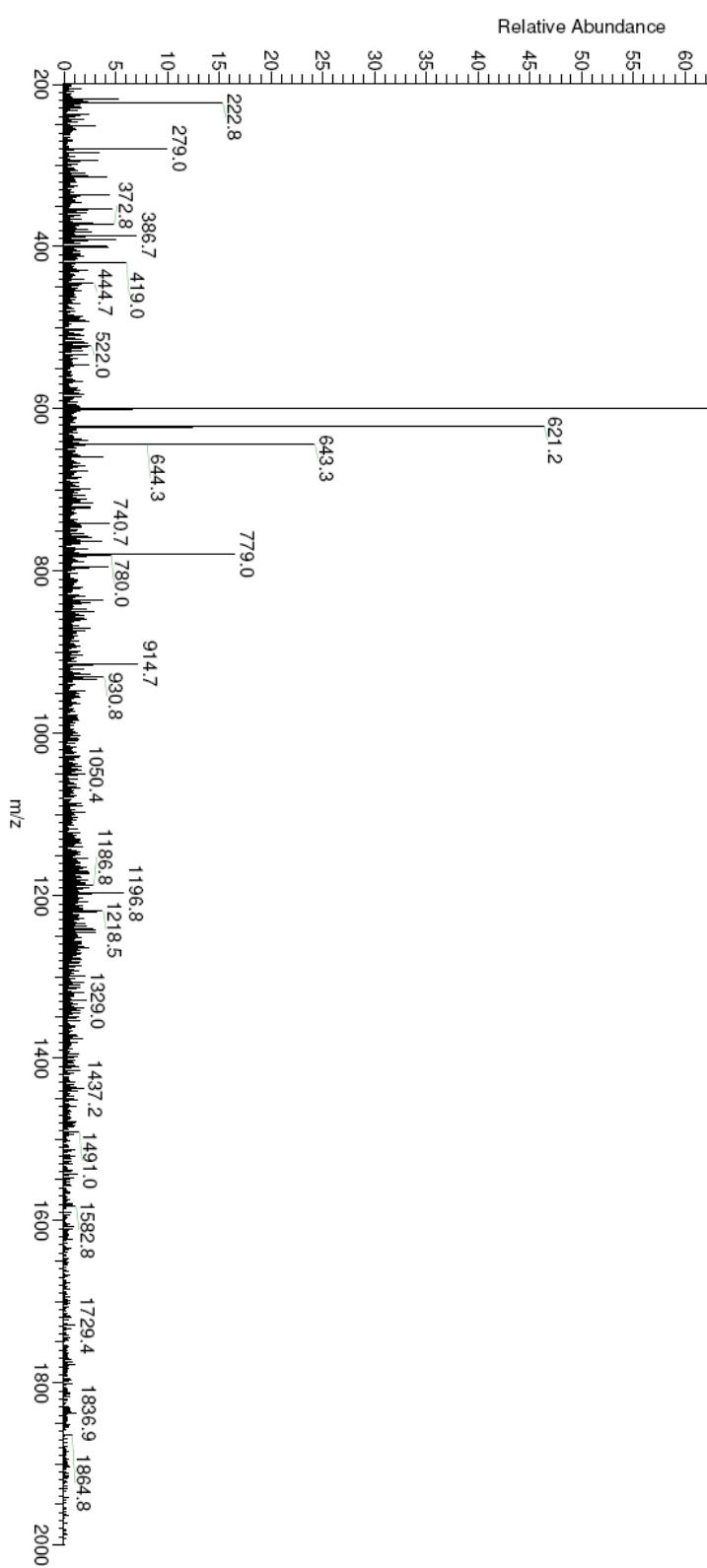


Figure S82. Mass spectrum of the peptide Ac-T(4-SO₃⁻-Phe)PN-NH₂ (**24**).

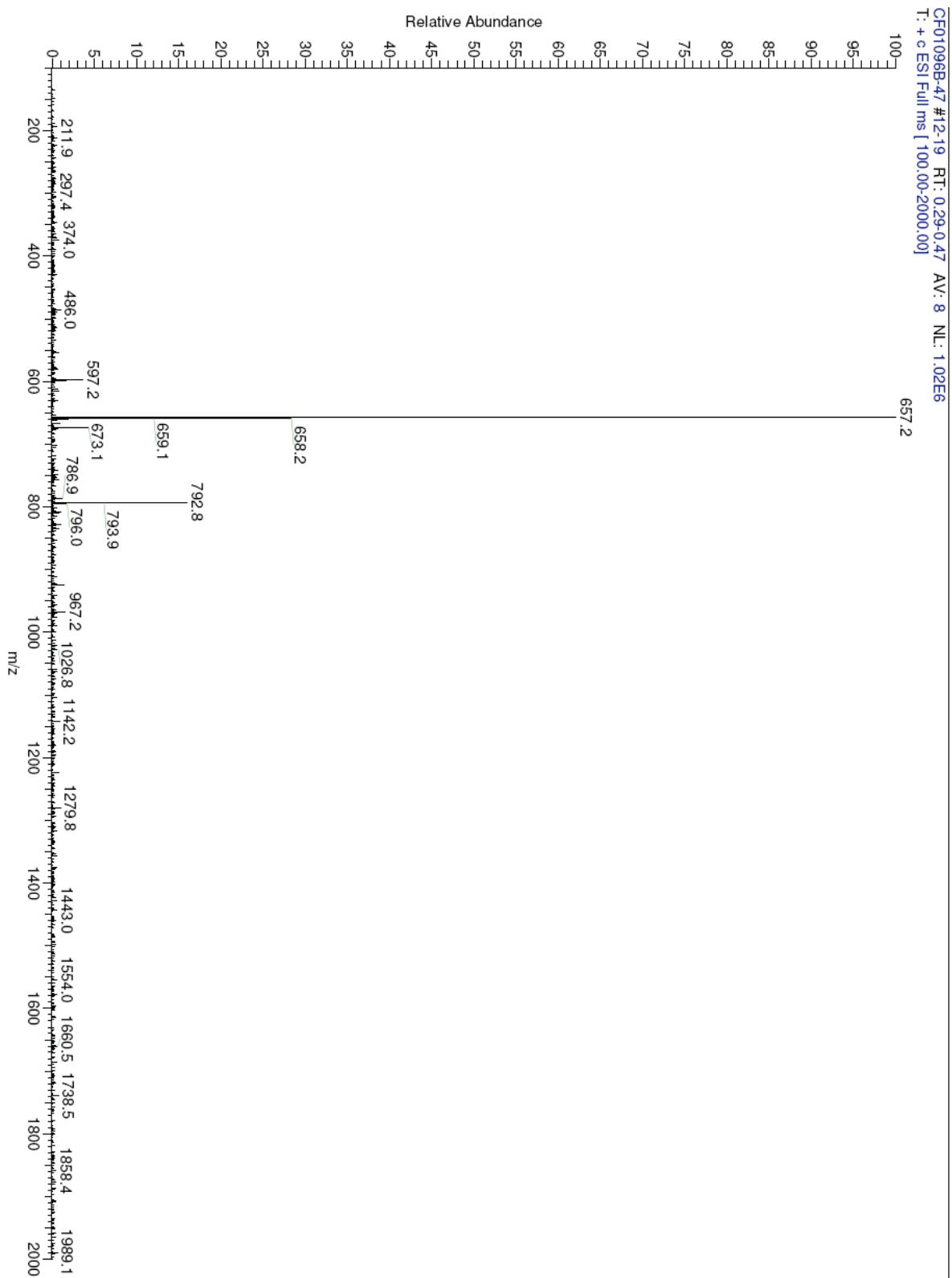


Figure S83. Mass spectrum of product at 46.9 min from solution phase cross-coupling to produce the peptide Ac-T(4-thioacetyl-Phe)PN-NH₂ (**2**). The observed mass is consistent with the peptide (**2** + Ac + Na)⁺.

CF 709-52 10102211915 #923 RT: 0.20-0.56 AV: 15 NL: 2.09E6
T: + c ESI Full ms [100.00-2000.00]

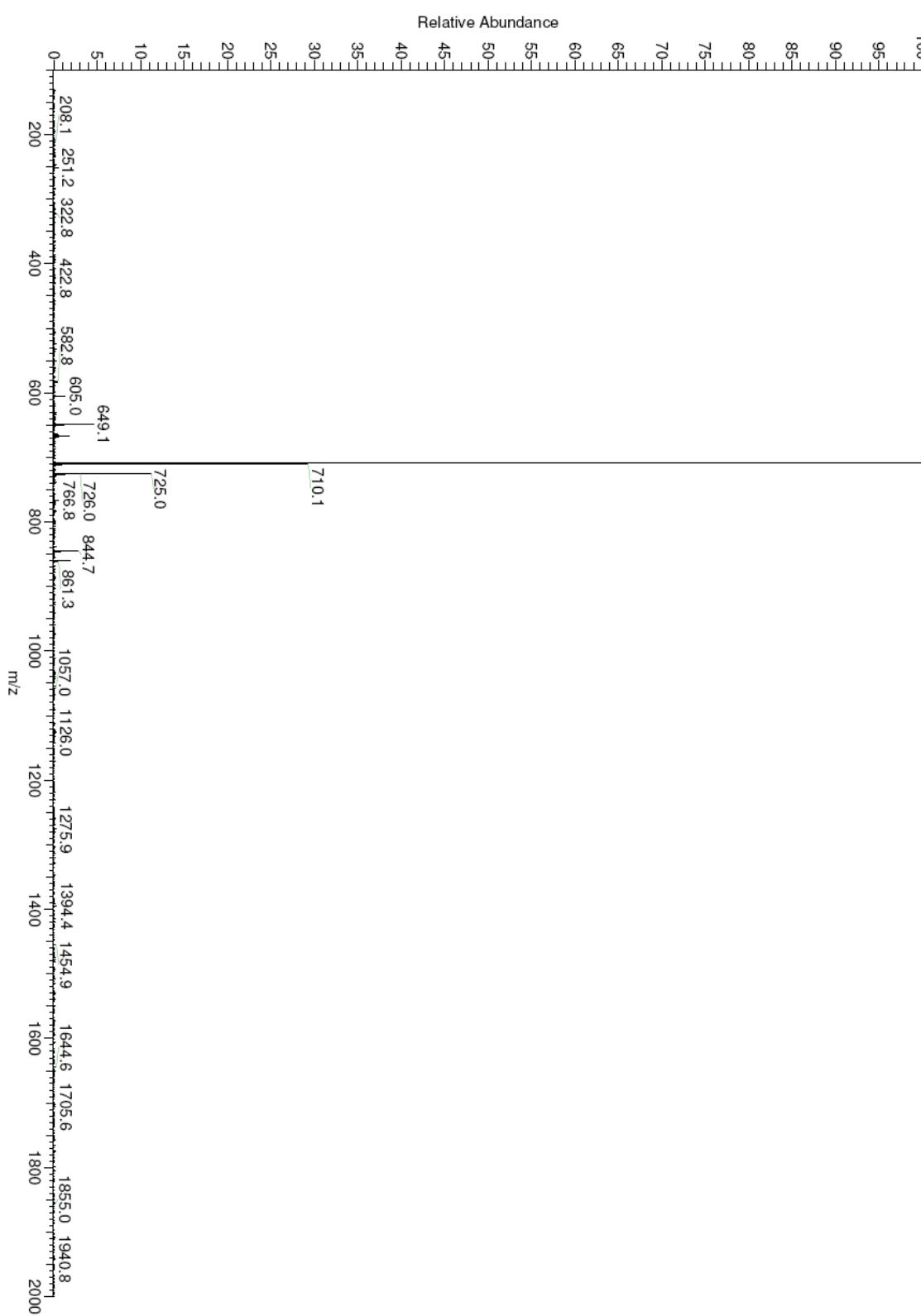


Figure S84. Mass spectrum of product at 51.7 min from solution phase cross-coupling to produce the peptide Ac-T(4-thioacetyl-Phe)PN-NH₂ (**2**). The observed mass is consistent with the peptide (**1** + Ac + Na)⁺.