Supporting Information

Synthesis of the CILAT reagents

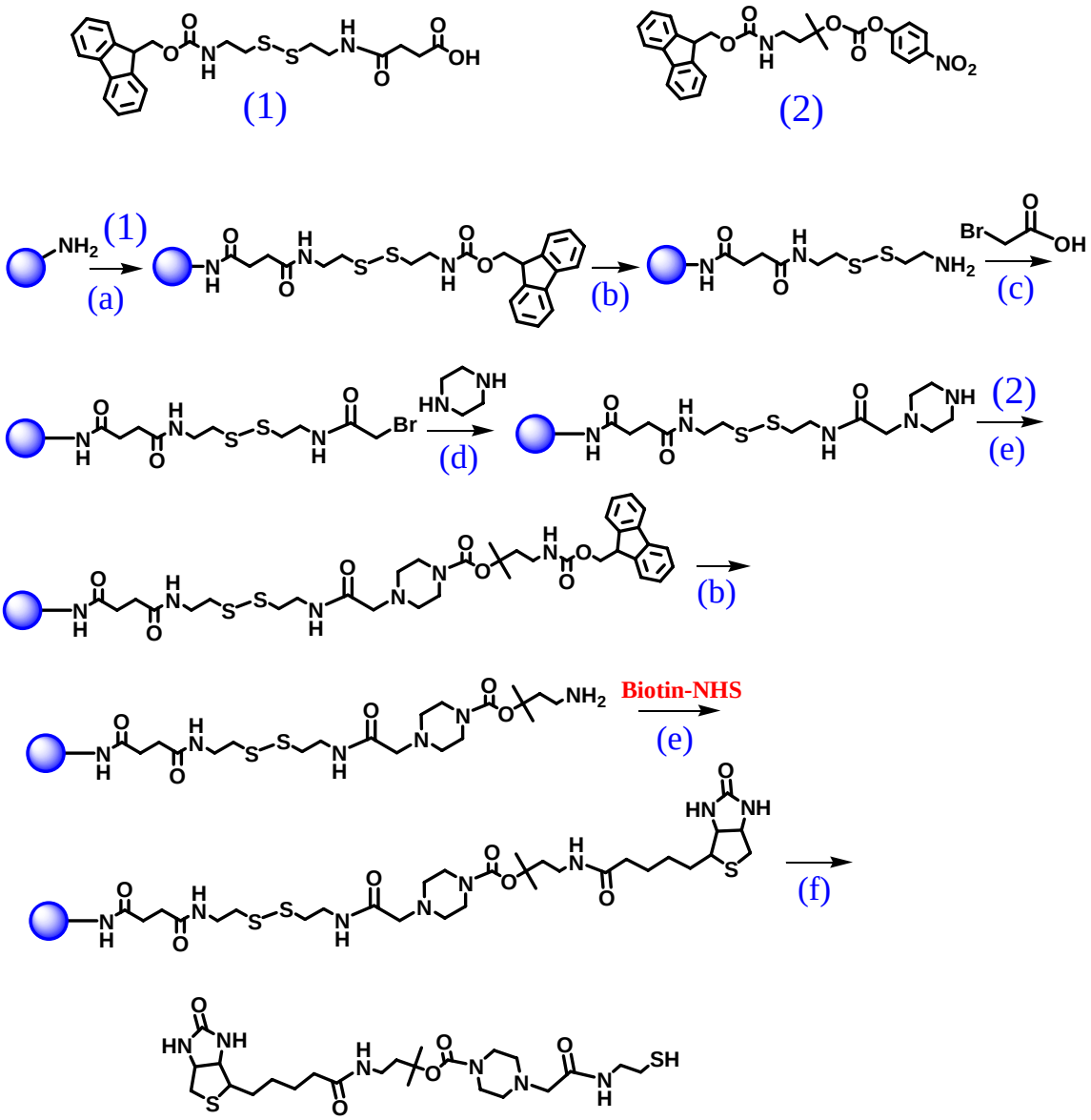


Figure 1: Synthetic route of the CILAT reagents. Conditions: (a) HBTU/HOBt/DIPEA; (b) piperidine; (c) DIC; (d) TEA; (e) HOBt/DIPEA; (f) TCEP.

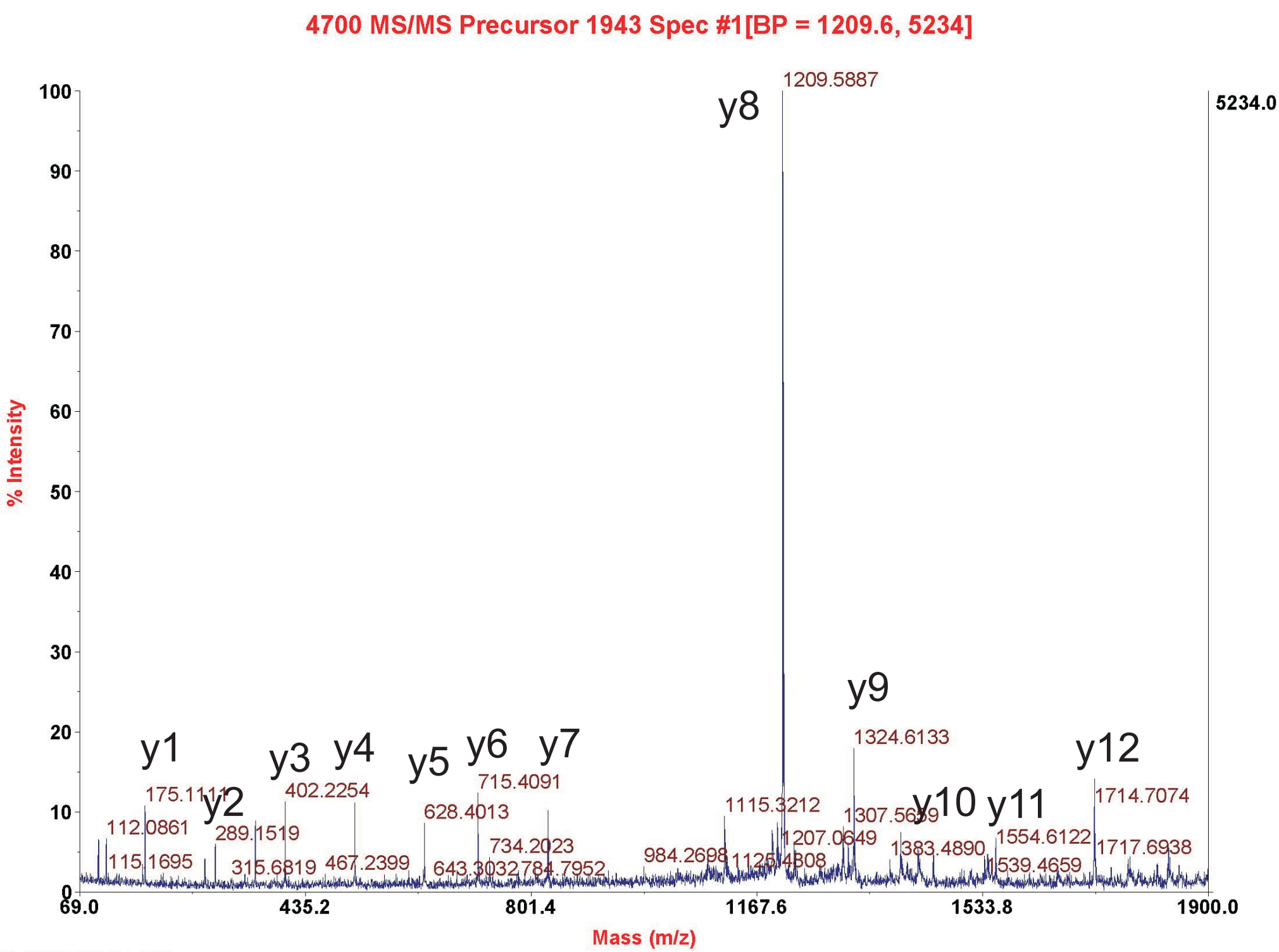
The CILAT reagents were prepared on a 433A peptide synthesizer automatically (Applied Biosystems) using a modified program. NovaSyn HL amine resin (20 μmol loading, Novabiochem) was used as solid support to coupled with five-fold excess of compound-**1** (compared to the resin substitution, see reference #12) using standard peptide coupling condition. After Fmoc group was deprotected with 20% piperidine in NMP (N-methylpyrrolidinone), 15-fold excess of either 1-^{13}C or 2-^{13}C coded bromoacetic acid (Cambridge Isotope Laboratories) was mixed with equal amount of

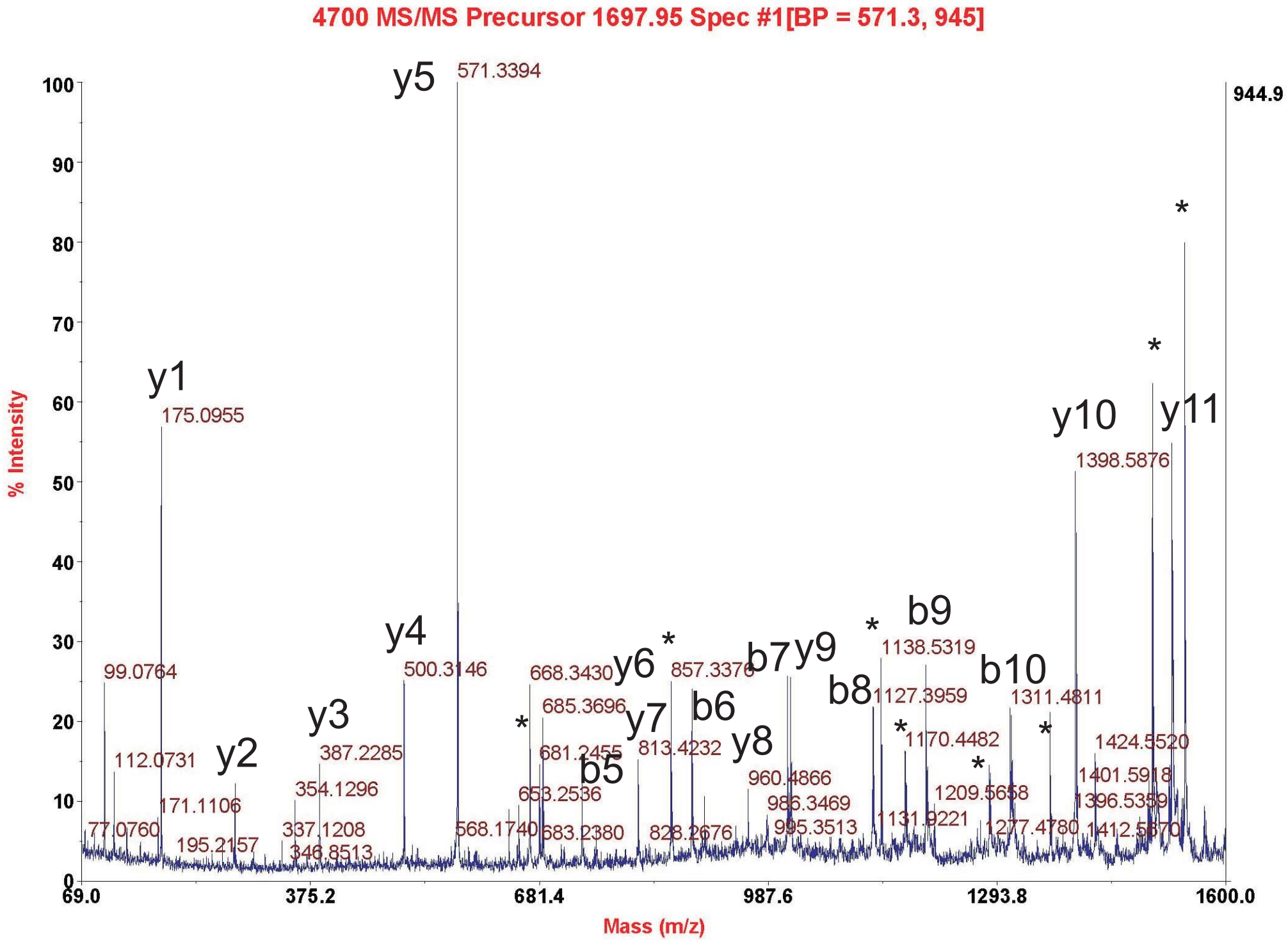
DIC (diisopropylcarbodiimide) in NMP to react with the resin for 45 mins. Then, 35-fold excess of piperazine (compared to the resin substitution) was mixed with 35-fold excess of TEA (triethylamine) in 85:15 NMP: methanol to react with resin for another 45 mins. Next, five-fold excess of compound-**2** (see reference #12) was mixed with equal amount of HOBt and DIPEA (diisopropylethylamine) to react with the resin for 45 mins. Fmoc group was then removed again with 20% piperidine, followed by the reaction with the mixture of five-fold excess of biotin-NHS, HOBt, and DIPEA for 45 mins. The final product was cleaved from the resin with cleavage solution (250 mM TCEP in 50% methanol, pH = 4.6) and purified by HPLC. ESI-MS: $(M+H)^+$ = 559.5. Calculated $(M+H)^+$ = 560.3

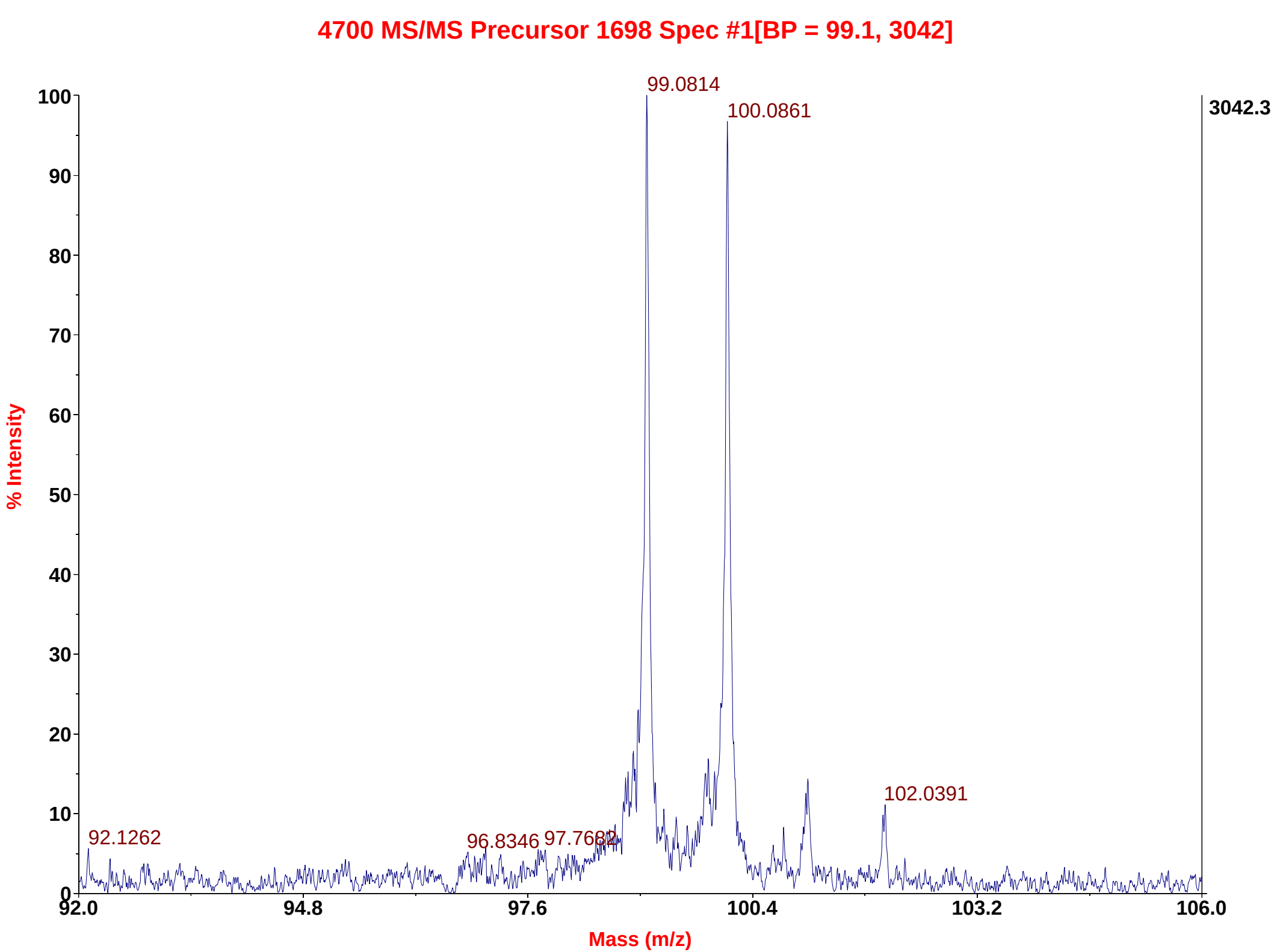
Procedure

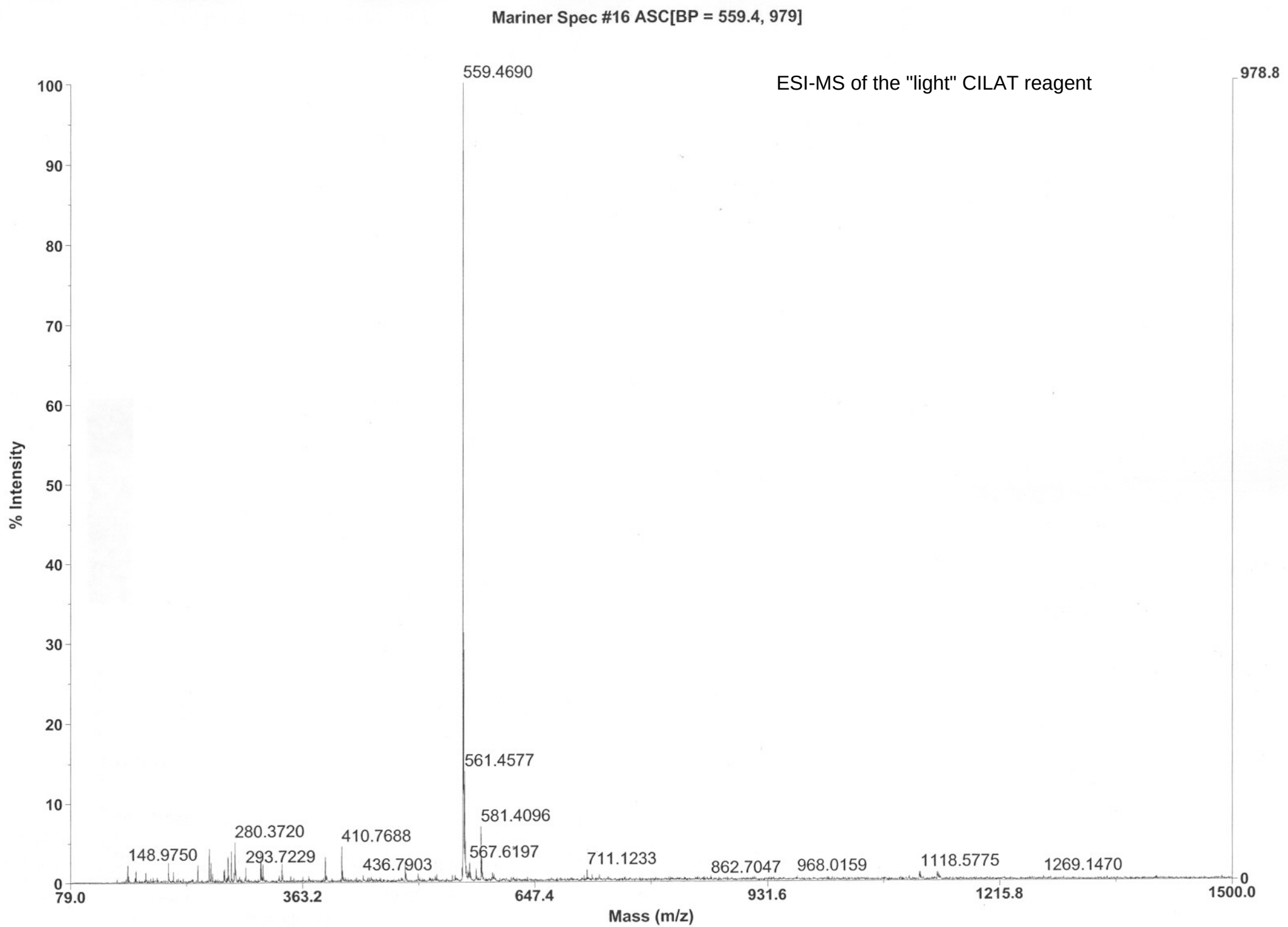
Two tubes of bovine serum albumin (BSA) were treated exactly the same as described below. 200 μg BSA (20 μL, 10 mg/mL) was mixed with 40 μL denaturing buffer (200 mM Tris, pH=8.3; 8M urea) and 15 μL reducing buffer (TCEP, 25 mM, pH=4.6), sealed, and then heated at 100 °C for 10 mins. Bromoacetamide solution (500 mM, 1.5 μL) was added and incubated at 37 °C for 2 hrs in dark. DTT solution (1M, 1.5μL) was then added and incubated at 37 °C for 30 mins to quench the extra alkylation reagent. After the mixture was diluted with water (322 μL), 10 μg trypsin (sequencing grade) was added and the digestion was kept at 37 °C for 20 hrs. The resulting solution was then desalted with a C_{18} reverse phase column, dried in a SpeedVac and redissolved in 500 μL phosphate buffer (50 mM, pH=8.0). 10 μg trypsin was added again and the mixture was incubated for 3 hrs at 37 °C to make sure complete digestion of proteins. The trypsin was then removed by incubating with immobilized trypsin inhibitor (SimgaAldrich, 100 μL bed volume, capacity: 1.7 mg/mL), which was filtered out after 30 mins. 100 μL solution was taken out from each tube and added with 2 μL "light" and "heavy" tags (5 mM) respectively, together with 3 μL tyrosinase. The reaction was kept at room temperature for 1 hr and two tubes were mixed together. The extra labeling reagents were removed with a disposable SCX column (Fisher). The biotinylated peptides were enriched with immobilized monoavidin (Pierce) and eluted with 30% acetonitrile containing 1 mM biotin and 0.4% TFA. The elution was dried in a SpeedVac and the residue was deprotected with a cocktail (100 μL, 88:5:5:2 TFA:water:phenol:TIS) for 2 hrs. After the solution was removed in a lyophilizer, the peptides were dissolved in 50% acetonitrile for MALDI-MS/MS assay.

Annotated MS/MS of the precursor ions at 1697.8 and 1942.9 are attached as below. There are several peaks labeled with an asterisk (*) that are not identified on the spectrum of 1697.8. These peaks might be the fragments of a contaminating precursor ion at 1700.0, which could be removed if the sample were fractionated with LC prior to MS experiment.
The expanded region including the signature peaks of the precursor ion at 1697.8 is also included.


4700 MS/MS Precursor 1943 Spec #1[BP = 1209.6, 5234]
1209.5887
y8
5234.0
100
90
80
70
60
50
40
30
20
10
0
% Intensity
y1
y2
y3
y4
y5
y6
y7
y9
y10
y11
y12
175.1111
112.0861
115.1695
289.1519
315.6819
402.2254
467.2399
628.4013
643.3032
715.4091
734.2023
784.7952
984.2698
1115.3212
1125.4808
1207.0649
1307.5659
1324.6133
1383.4890
1539.4659
1554.6122
1714.7074
1717.6938
69.0
435.2
801.4
1167.6
1533.8
1900.0
Mass (m/z)
S:\...\MSMS-1943-CID off.T2D
Acquired:


4700 MS/MS Precursor 1697.95 Spec #1[BP = 571.3, 945]
y5
571.3394
944.9
y1
175.0955
y10
y11
1398.5876
y4
500.3146
y6
857.3376
b7y9
b9
1138.5319
b8
1127.3959
b10
1311.4811
y3
387.2285
y2
112.0731
b5
y7
813.4232
b6
y8
960.4866
99.0764
354.1296
171.1106
77.0760
195.2157
337.1208
346.8513
653.2536
568.1740
685.3696
681.2455
683.2380
828.2676
986.3469
995.3513
1131.9221
1170.4482
1209.5658
1277.4780
1401.5918
1396.5359
1412.5870
1424.5520
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
69.0
375.2
681.4
987.6
1293.8
1600.0
S:\...\MSMS-1695.50-40000.T2D
Acquired:


4700 MS/MS Precursor 1698 Spec #1[BP = 99.1, 3042]
99.0814
100.0861
3042.3
92.1262
96.8346
97.7682
102.0391
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
92.0
94.8
97.6
100.4
103.2
106.0
S:\...\MSMS-1698-CID on.T2D
Acquired:


Mariner Spec #16 ASC[BP = 559.4, 979]
ESI-MS of the "light" CILAT reagent
559.4690
978.8
100
90
80
70
60
50
40
30
20
10
0
% Intensity
561.4577
581.4096
280.3720
410.7688
148.9750
293.7229
567.6197
436.7903
711.1233
862.7047
968.0159
1118.5775
1269.1470
79.0
363.2
647.4
931.6
1215.8
1500.0
Mass (m/z)
Mariner Mass Spectrum
C:\...\1202b-1.dat
Acquired: Dec 02 15:57:00 2006