Investigating Hapten Clustering as a Strategy to Enhance Vaccines against Drugs of Abuse

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ELISA data

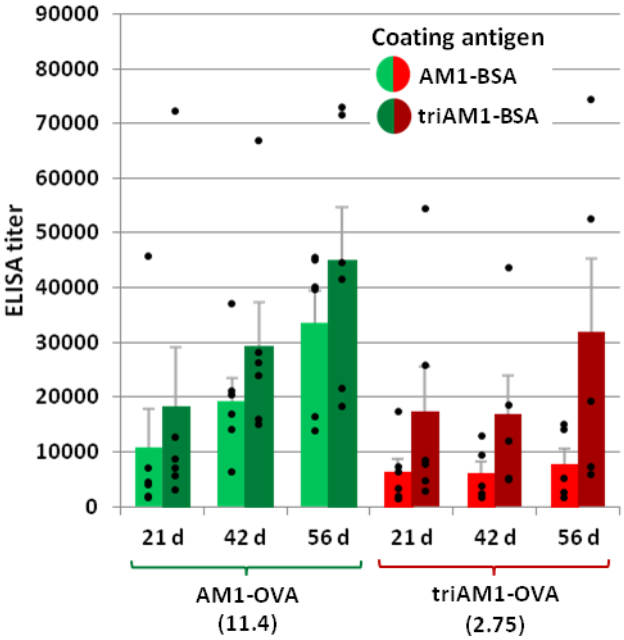


Figure S1. Midpoint antibody titers from AM1-OVA and triAM1-OVA vaccinated mice (n = 5–6) as determined by ELISA using AM1-BSA or triAM1-BSA as the coating antigen. Data were obtained in duplicate. Numbers in parentheses represent the hapten density; error bars represent SEM; individual points represent individual mouse titers.

Tables S1–S2. Midpoint Antibody Titers from AM1-OVA and triAM1-OVA Vaccinated Mice (n = 5–6) as Determined by ELISA Using AM1-BSA and triAM1-BSA Coated Plates[a]

Vaccine	Copies per OVA molecule	Titers (AM1-BSA coating antigen)		
		21 d	42 d	56 d
AM1-OVA	11.4	10842 ± 7015	19376 ± 4167	33518 ± 5885
triAM1-OVA	2.75 (≡8.24)	6297 ± 2412	6056 ± 2188	7775 ± 2857

Vaccine	Copies per OVA molecule	Titers (triAM1-BSA coating antigen)		
		21 d	42 d	56 d
AM1-OVA	11.4	18247 ± 10885	29405 ± 7816	45127 ± 9572
triAM1-OVA	2.75 (≡8.24)	17390 ± 8153	16915 ± 7149	31874 ± 13531

[a]Data were obtained in duplicate. Errors represent SEM.

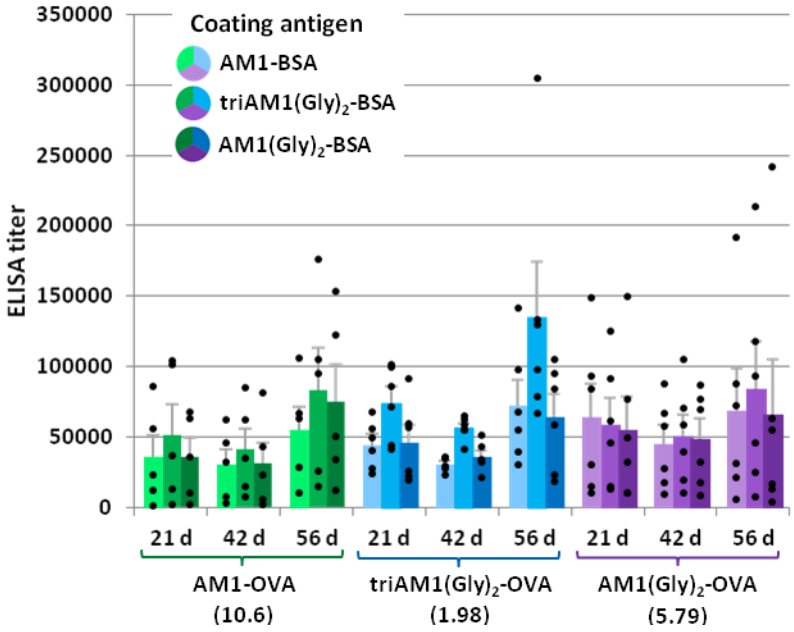


Figure S2. Midpoint antibody titers from AM1-OVA, triAM1(Gly)$_2$-OVA and AM1(Gly)$_2$-OVA vaccinated mice (n = 5–6) as determined by ELISA using AM1-BSA, triAM1(Gly)$_2$-BSA or AM1(Gly)$_2$-BSA as the coating antigen. Data were obtained in duplicate. Numbers in parentheses represent the hapten density; error bars represent SEM; individual points represent individual mouse titers.

Tables S3–S5. Midpoint Antibody Titers from AM1-OVA, triAM1(Gly)$_2$-OVA and AM1(Gly)$_2$-OVA Vaccinated Mice (n = 5–6) as Determined by ELISA Using AM1-BSA, triAM1(Gly)$_2$-BSA and AM1(Gly)$_2$-BSA Coated Plates[a]

Vaccine	Copies per OVA molecule	Titers (AM1-BSA coating antigen)		
		21 d	42 d	56 d
AM1-OVA	11.4	35617 ± 15438	30238 ± 11228	54755 ± 16564
triAM1(Gly)$_2$-OVA	1.98 (≡5.93)	44359 ± 7502	30723 ± 2273	72054 ± 18542
AM1(Gly)$_2$-OVA	5.79	63591 ± 24336	44531 ± 13887	68446 ± 30321

Vaccine	Copies per OVA molecule	Titers (triAM1(Gly)$_2$-BSA coating antigen)		
		21 d	42 d	56 d
AM1-OVA	11.4	51475 ± 21549	41039 ± 14596	83544 ± 29388
triAM1(Gly)$_2$-OVA	1.98 (≡5.93)	73908 ± 11850	56090 ± 3665	135093 ± 39113
AM1(Gly)$_2$-OVA	5.79	58454 ± 19659	50492 ± 15773	83751 ± 34018

Vaccine	Copies per OVA molecule	Titers (AM1(Gly)$_2$-BSA coating antigen)		
		21 d	42 d	56 d
AM1-OVA	11.4	35849 ± 13350	30987 ± 14505	74584 ± 27116
triAM1(Gly)$_2$-OVA	1.98 (≡1.53)	45842 ± 12611	35825 ± 4614	63915 ± 16493
AM1(Gly)$_2$-OVA	5.79	54845 ± 23717	48331 ± 14934	65423 ± 40047

[a]Data were obtained in duplicate. Errors represent SEM.

RIA data

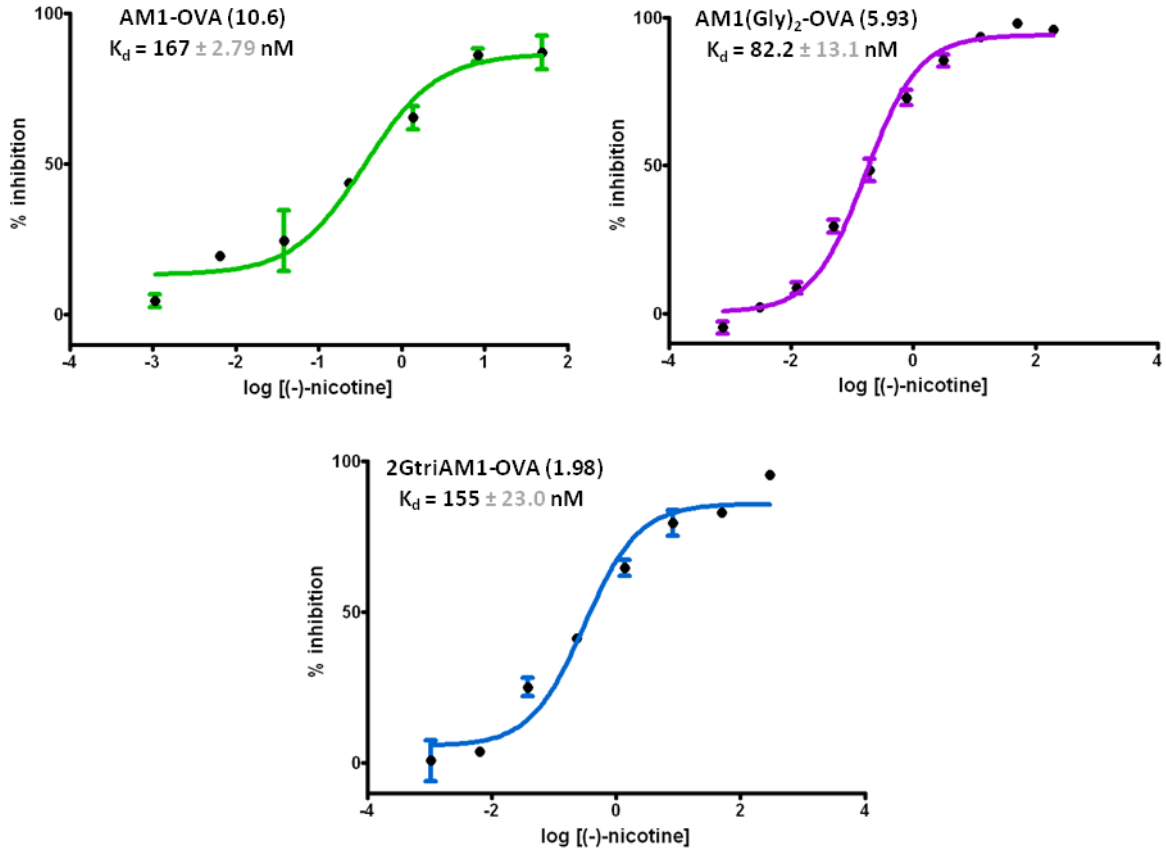
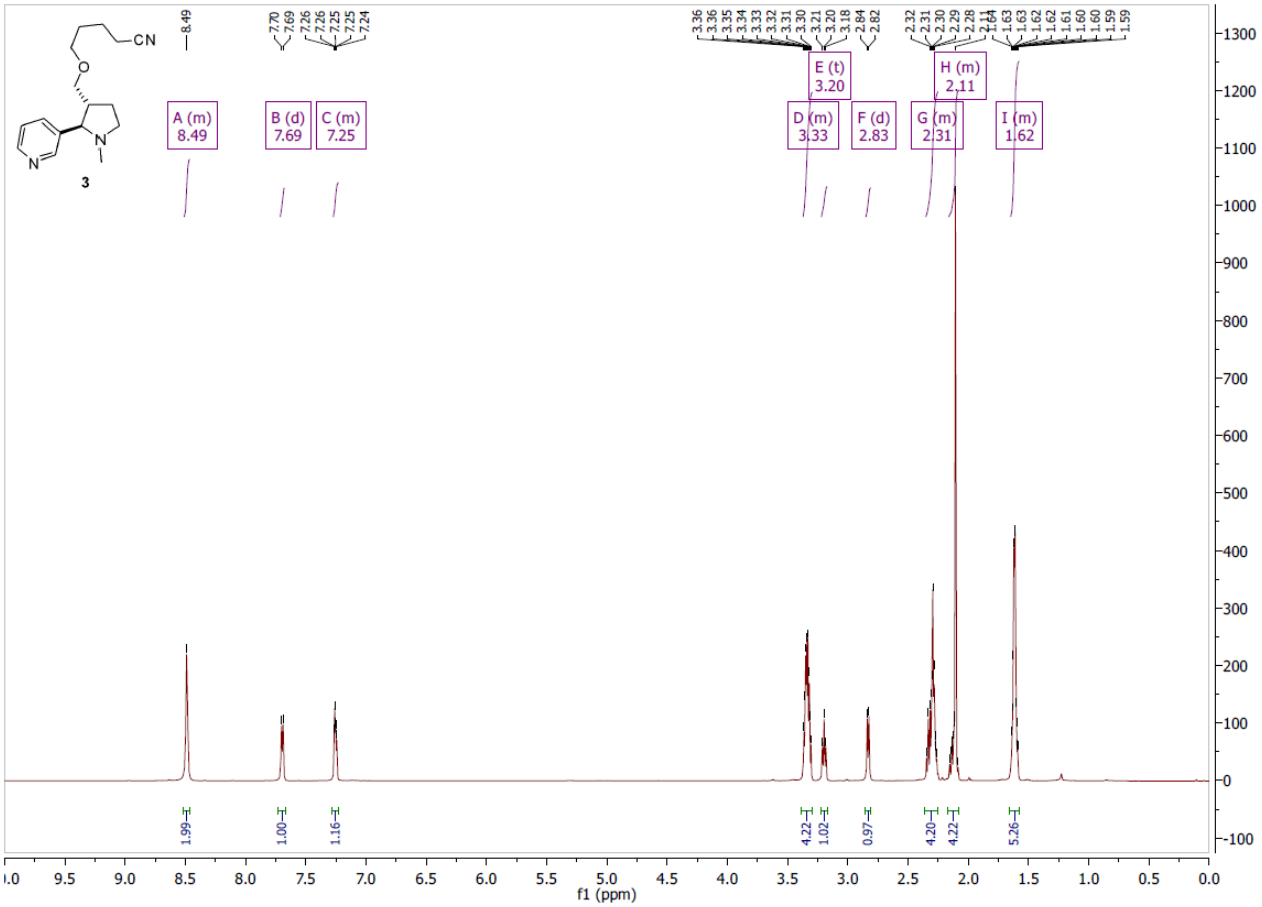
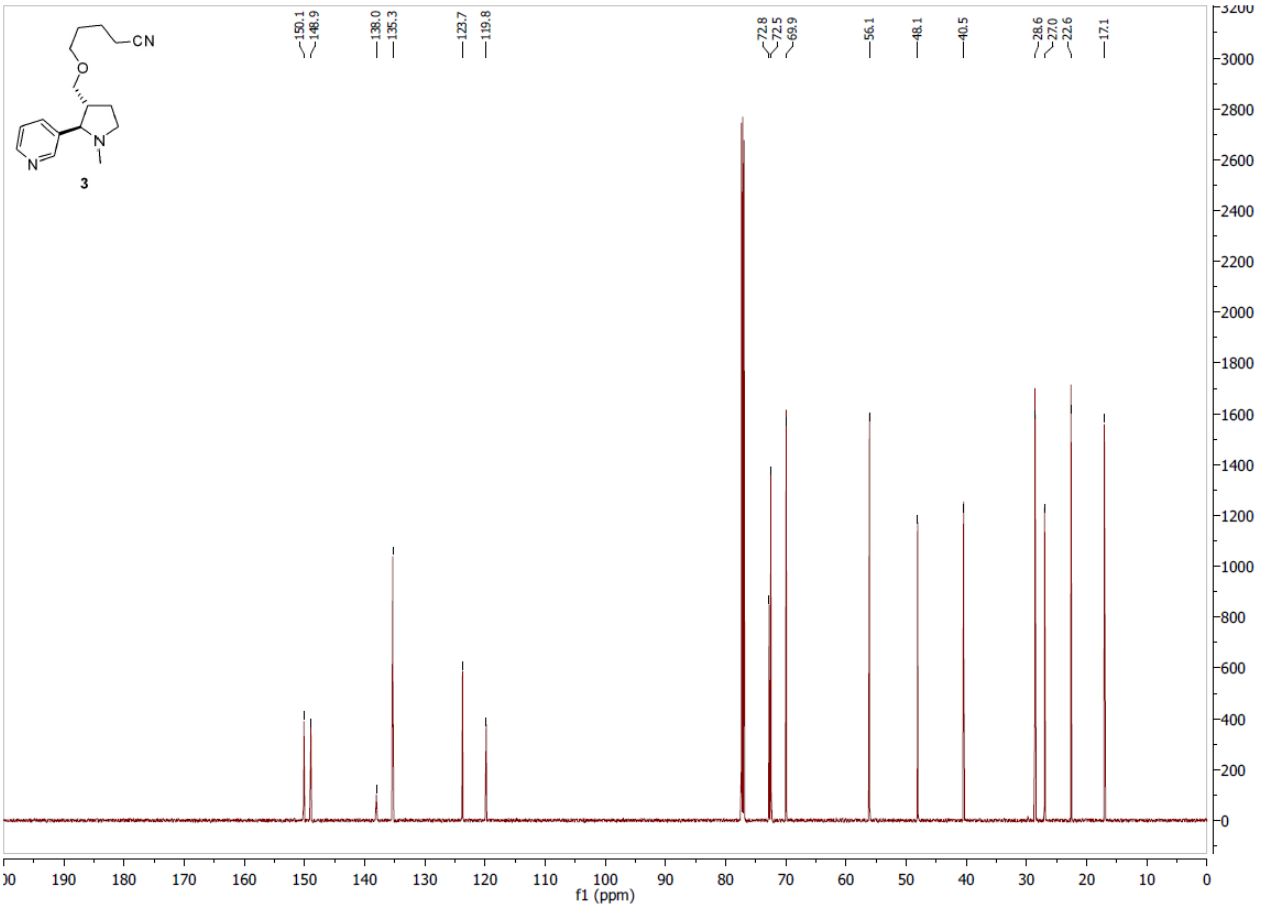


Figure S3. Anti-nicotine antibody affinities and concentrations from AM1-OVA, triAM1(Gly)$_2$-OVA and AM1(Gly)$_2$-OVA vaccinated mice (n = 5-6) using pooled plasma (56 d) as determined by competitive RIA. Data for AM1(Gly)$_2$ were obtained in triplicate, and data for AM1 and triAM1(Gly)$_2$ were obtained in duplicate. Errors represent SEM.

NMR Spectra


CN
O
N
N
3
8.49
7.70
7.69
7.26
7.26
7.25
7.25
7.24
3.36
3.36
3.35
3.34
3.33
3.32
3.31
3.30
3.21
3.20
3.18
2.84
2.82
2.32
2.31
2.30
2.29
2.28
2.11
1.64
1.63
1.63
1.62
1.62
1.61
1.60
1.60
1.59
1.59
A (m) 8.49
B (d) 7.69
C (m) 7.25
E (t) 3.20
D (m) 3.33
F (d) 2.83
H (m) 2.11
G (m) 2.31
I (m) 1.62
1.99
1.00
1.16
4.22
1.02
0.97
4.20
4.22
5.26
f1 (ppm)


CN
O
N
N
3
150.1
148.9
138.0
135.3
123.7
119.8
72.8
72.5
69.9
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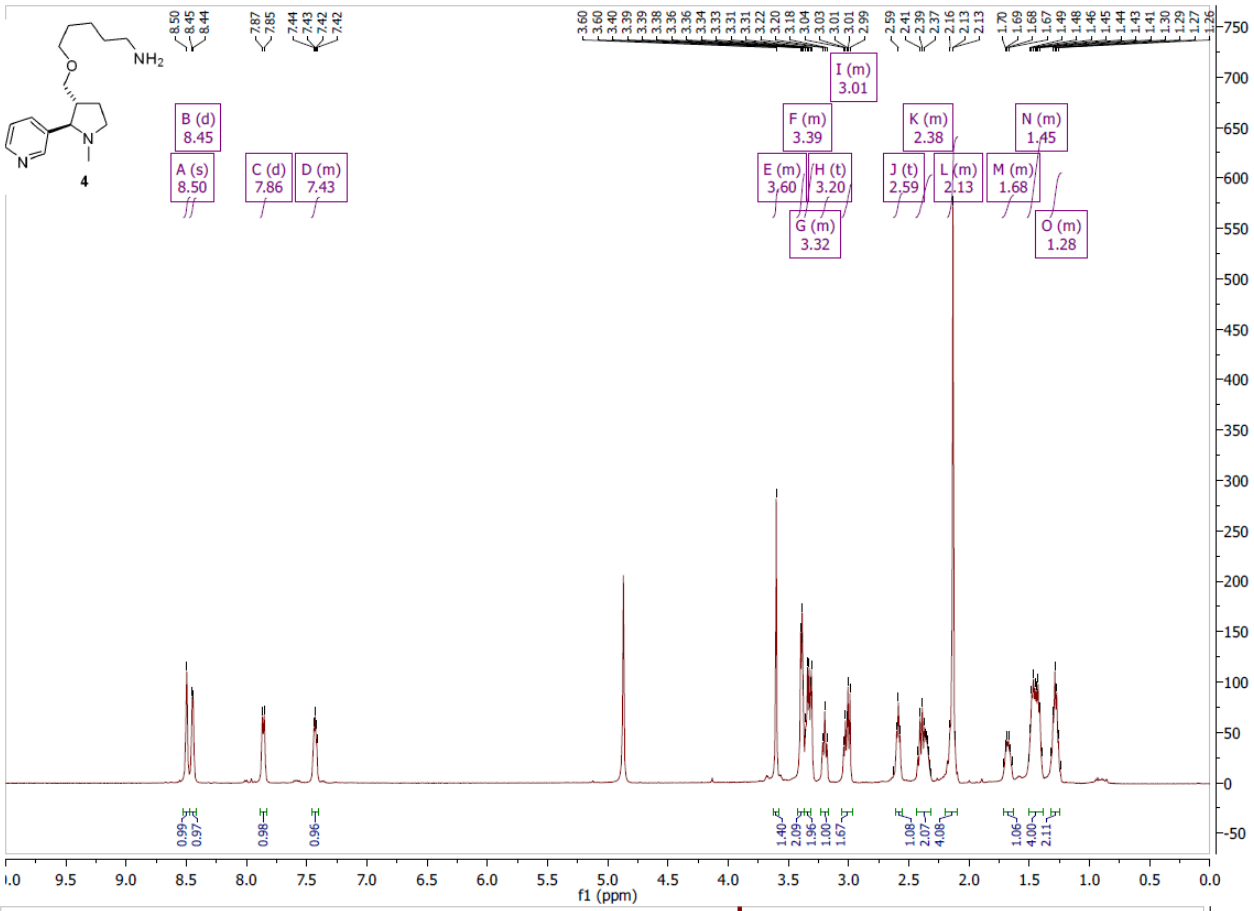
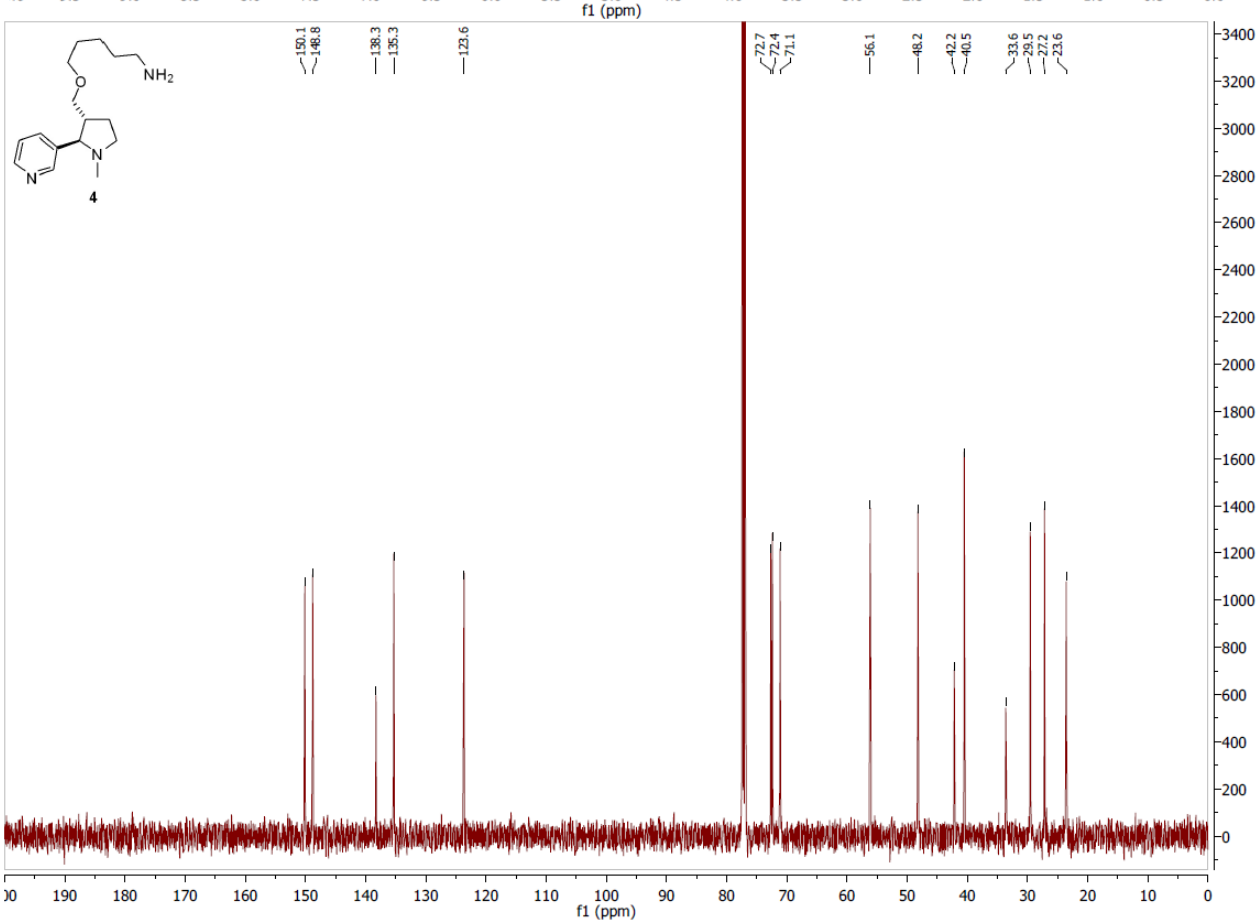
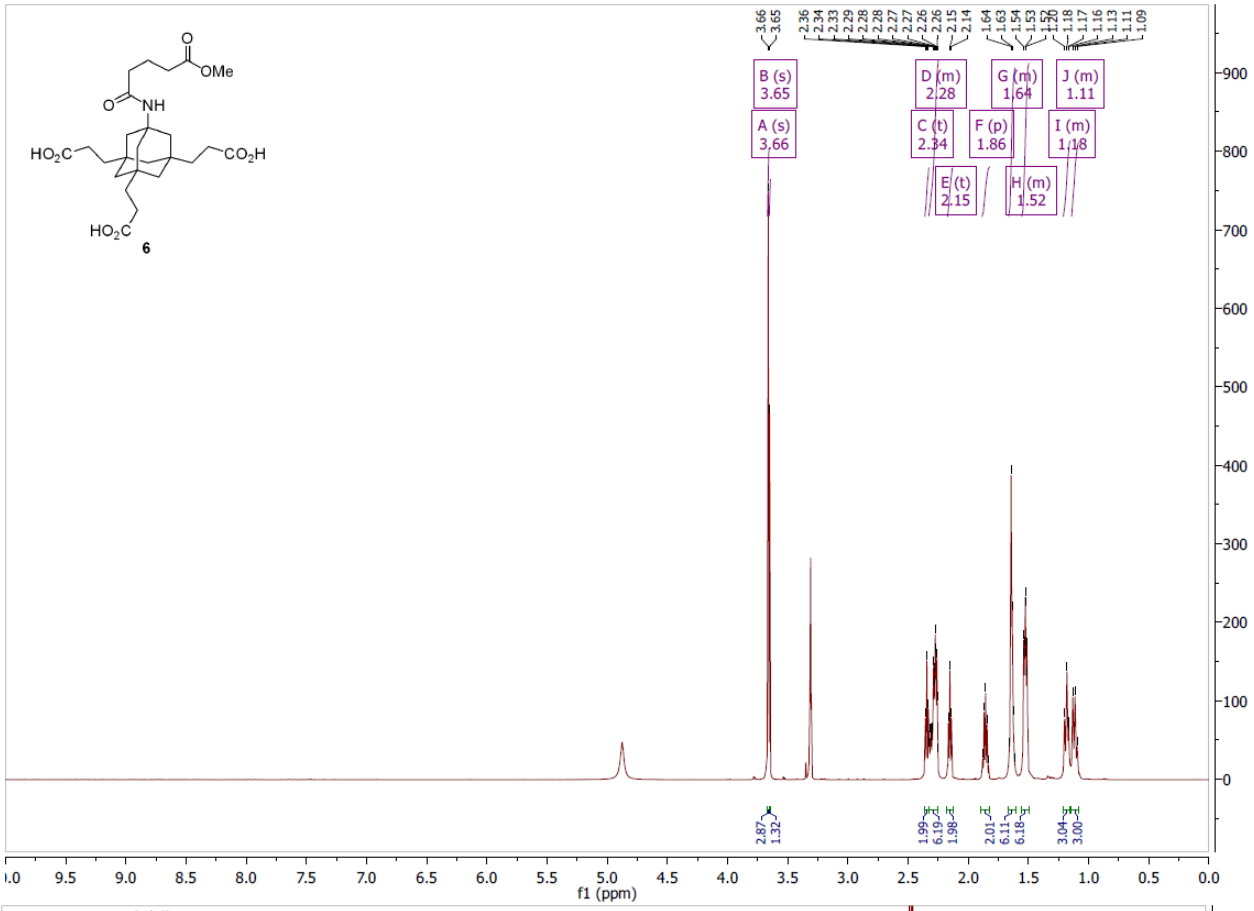
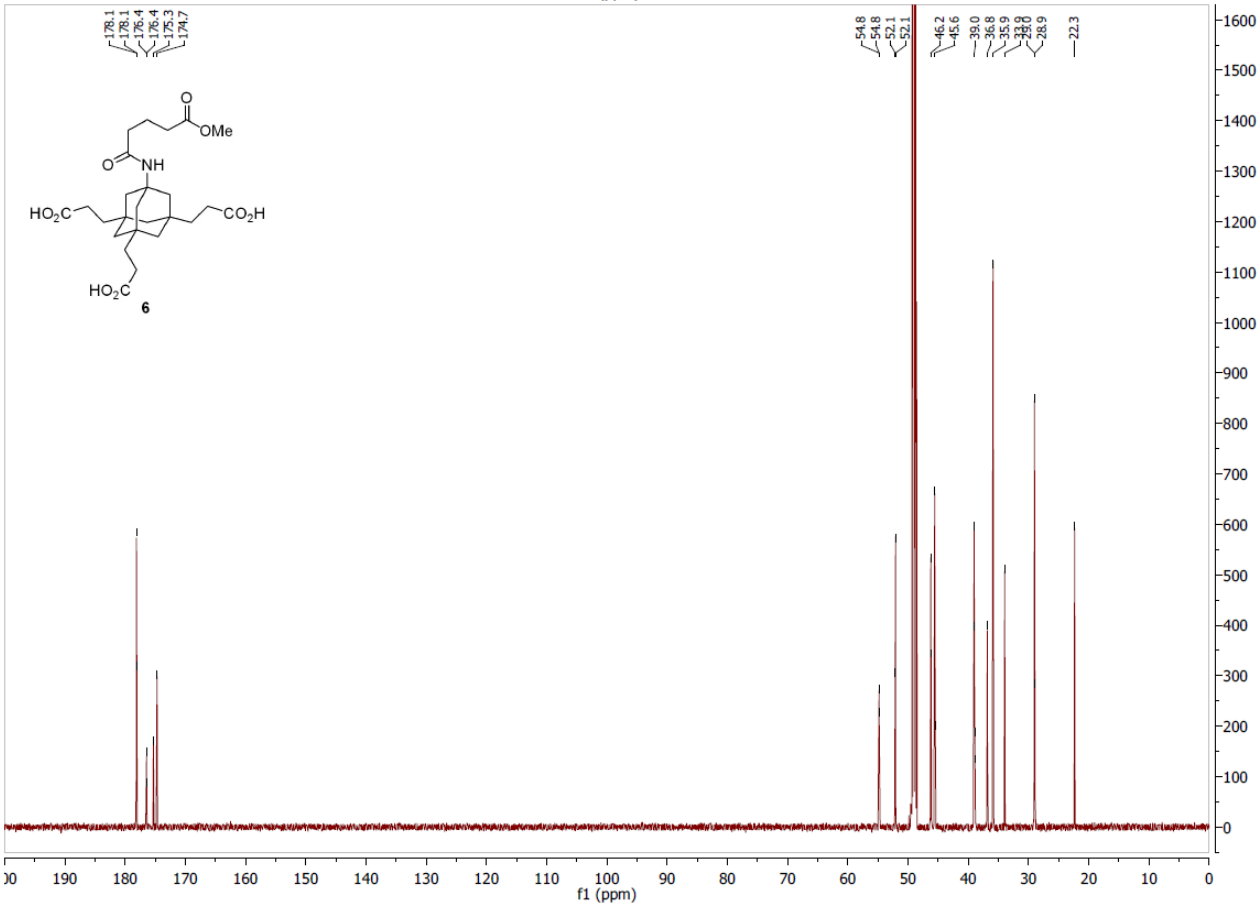
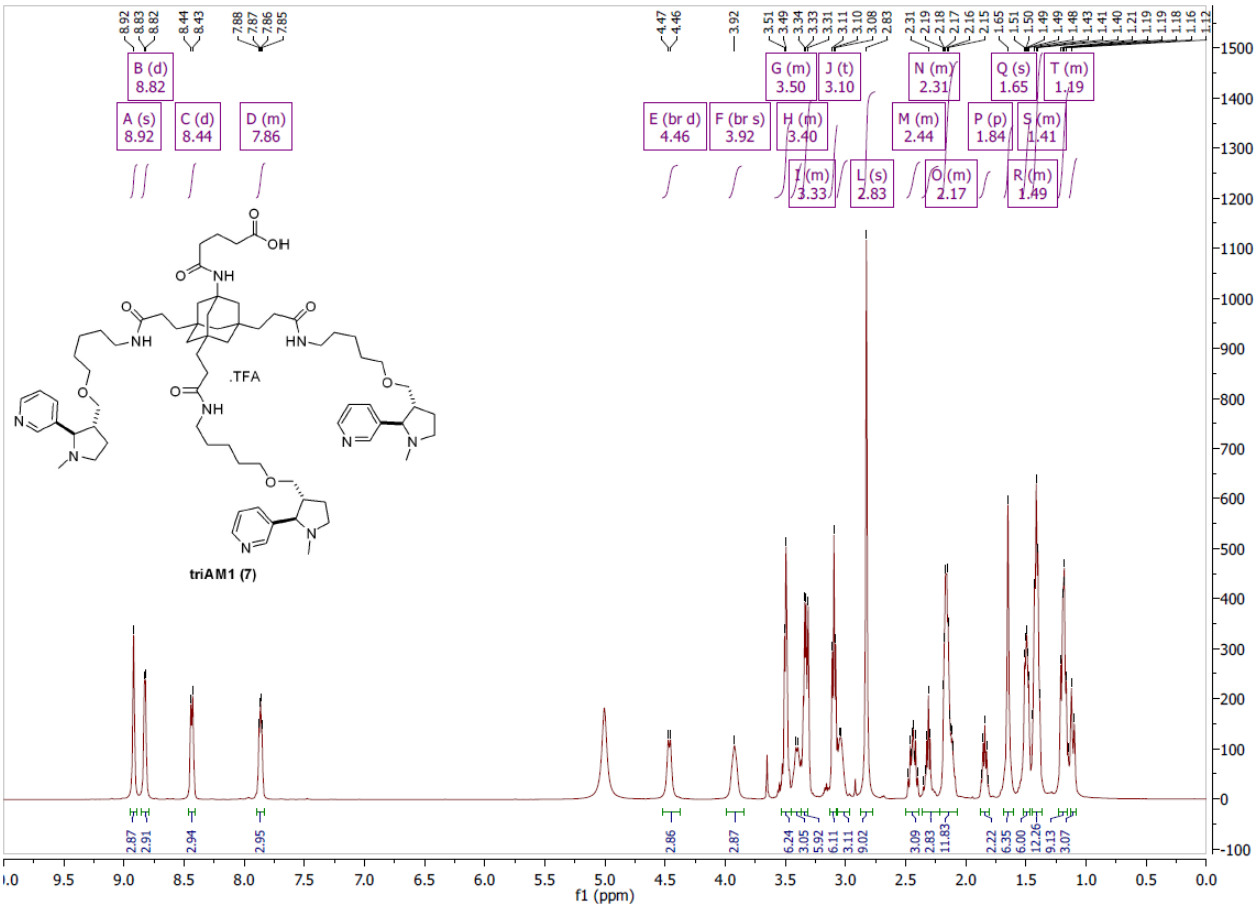
48.1
40.5
28.6
27.0
22.6
17.1
f1 (ppm)

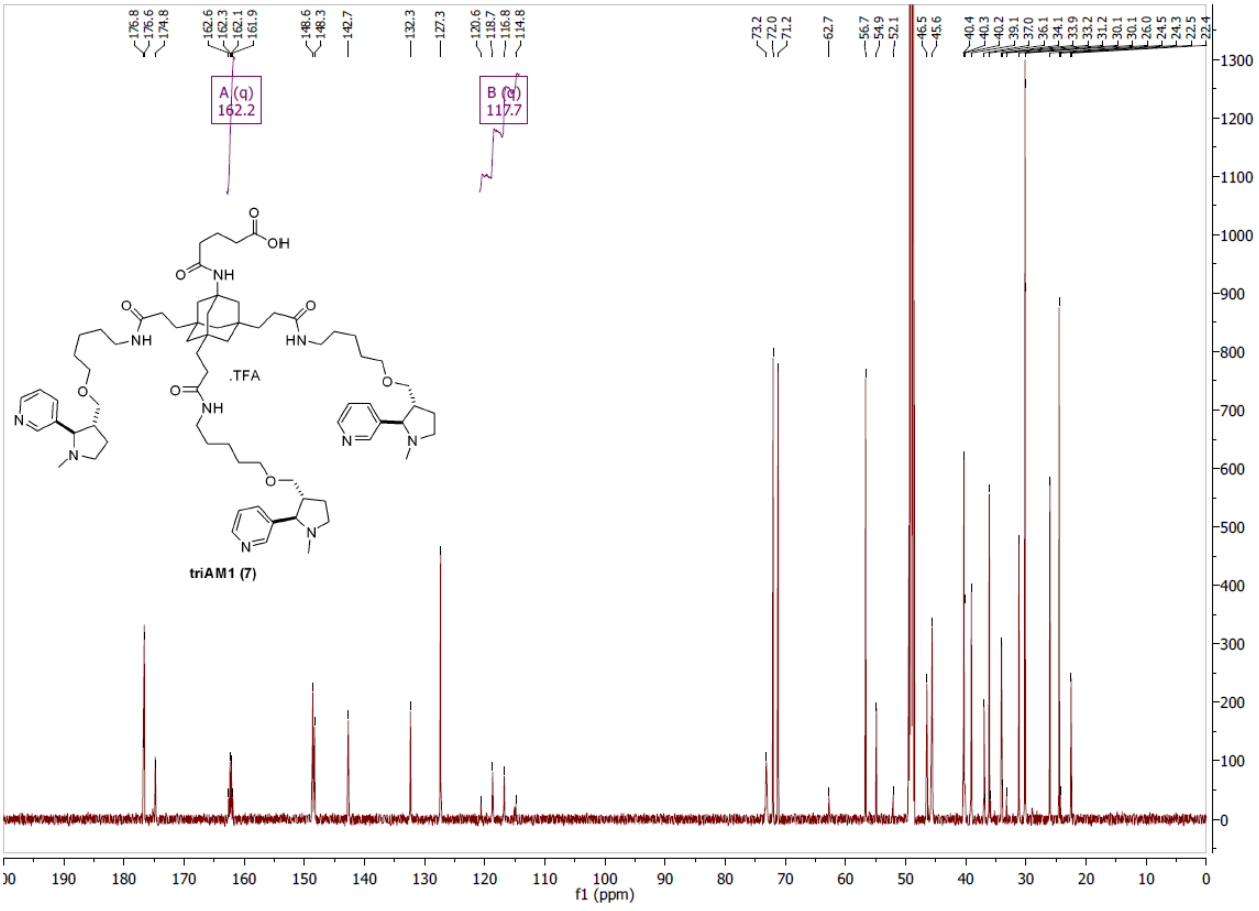

4
A (s) 8.50
B (d) 8.45
C (d) 7.86
D (m) 7.43
E (m) 3.60
F (m) 3.39
G (m) 3.32
H (t) 3.20
I (m) 3.01
J (t) 2.59
K (m) 2.38
L (m) 2.13
M (m) 1.68
N (m) 1.45
O (m) 1.28
f1 (ppm)

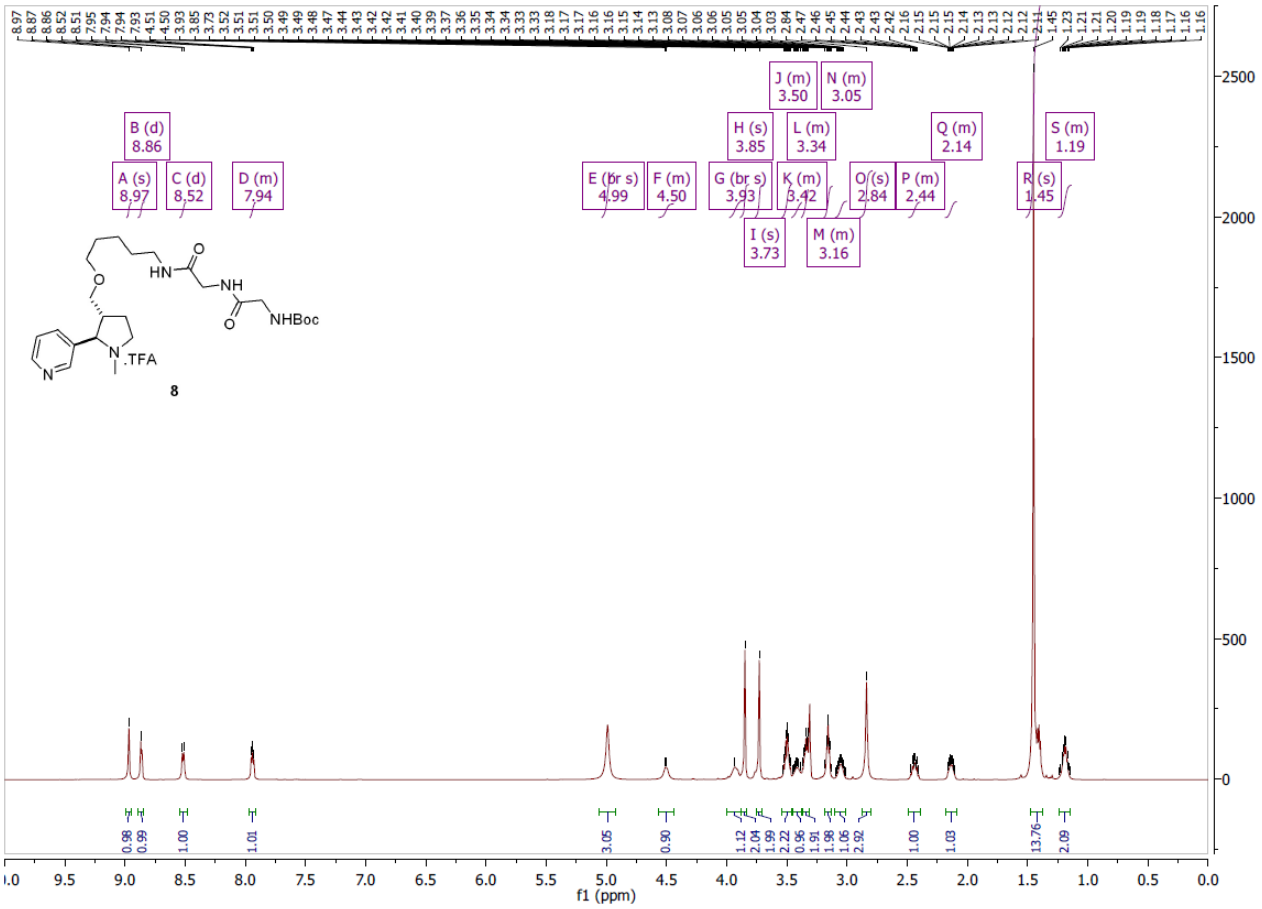

4
150.1
148.8
138.3
135.3
123.6
72.7
72.4
71.1
56.1
48.2
42.2
40.5
33.6
29.5
27.2
23.6
f1 (ppm)

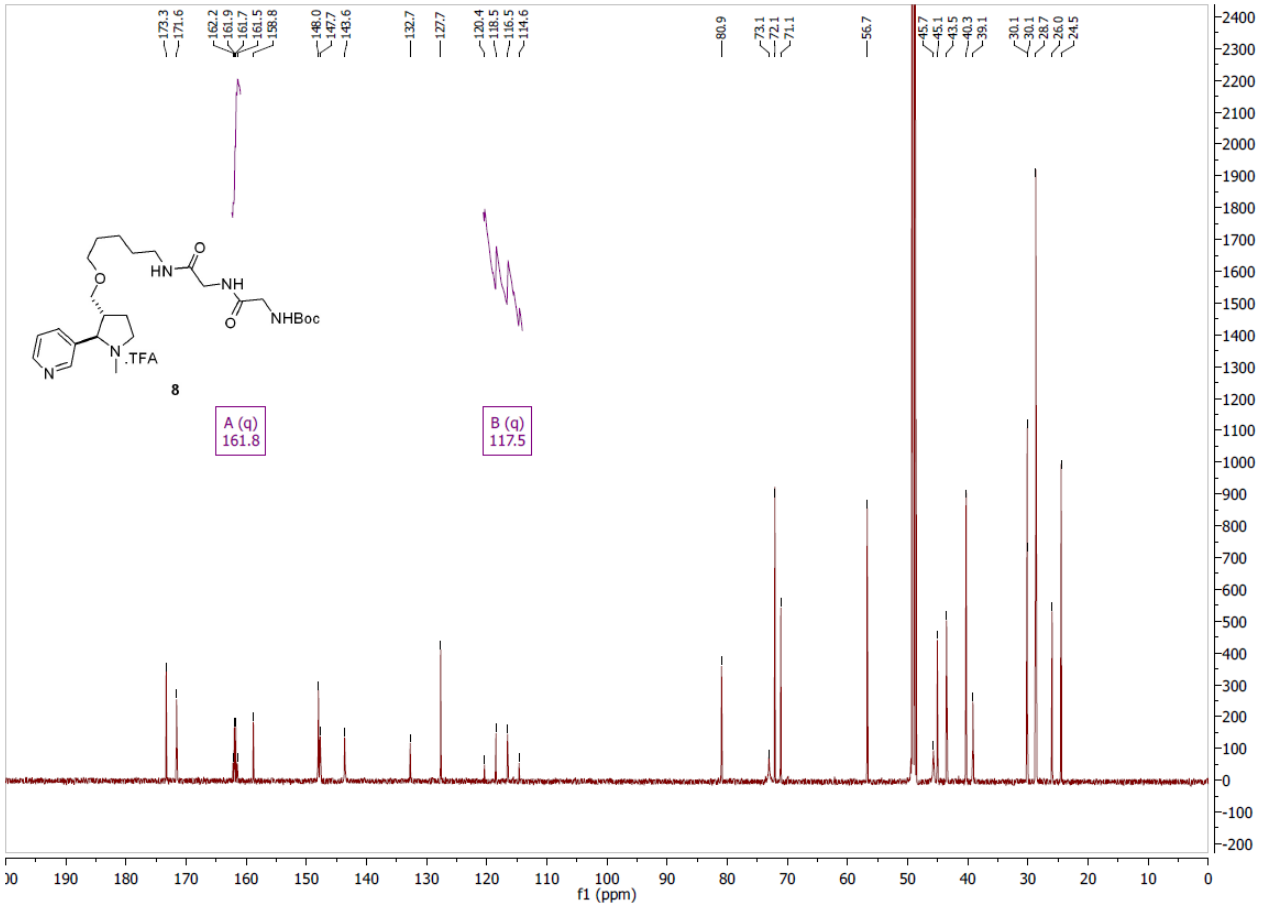

OMe
O
NH
HO2C
CO2H
HO2C
6
B (s) 3.65
A (s) 3,66
D (m) 2.28
C (t) 2.34
E (t) 2.15
G (m) 1.64
F (p) 1.86
H (m) 1.52
J (m) 1.11
I (m) 1.18
f1 (ppm)

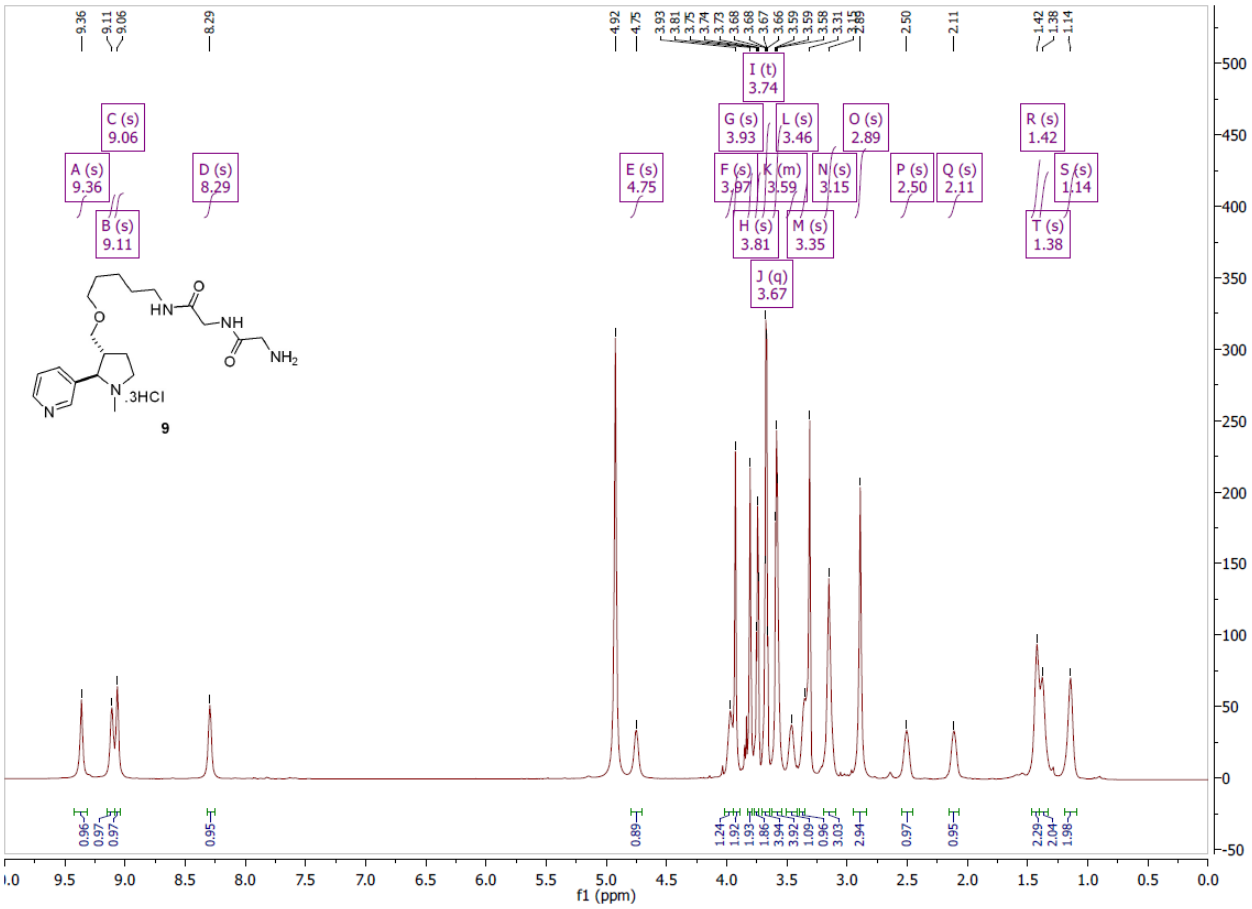

OMe
O
NH
HO2C
CO2H
HO2C
6
f1 (ppm)

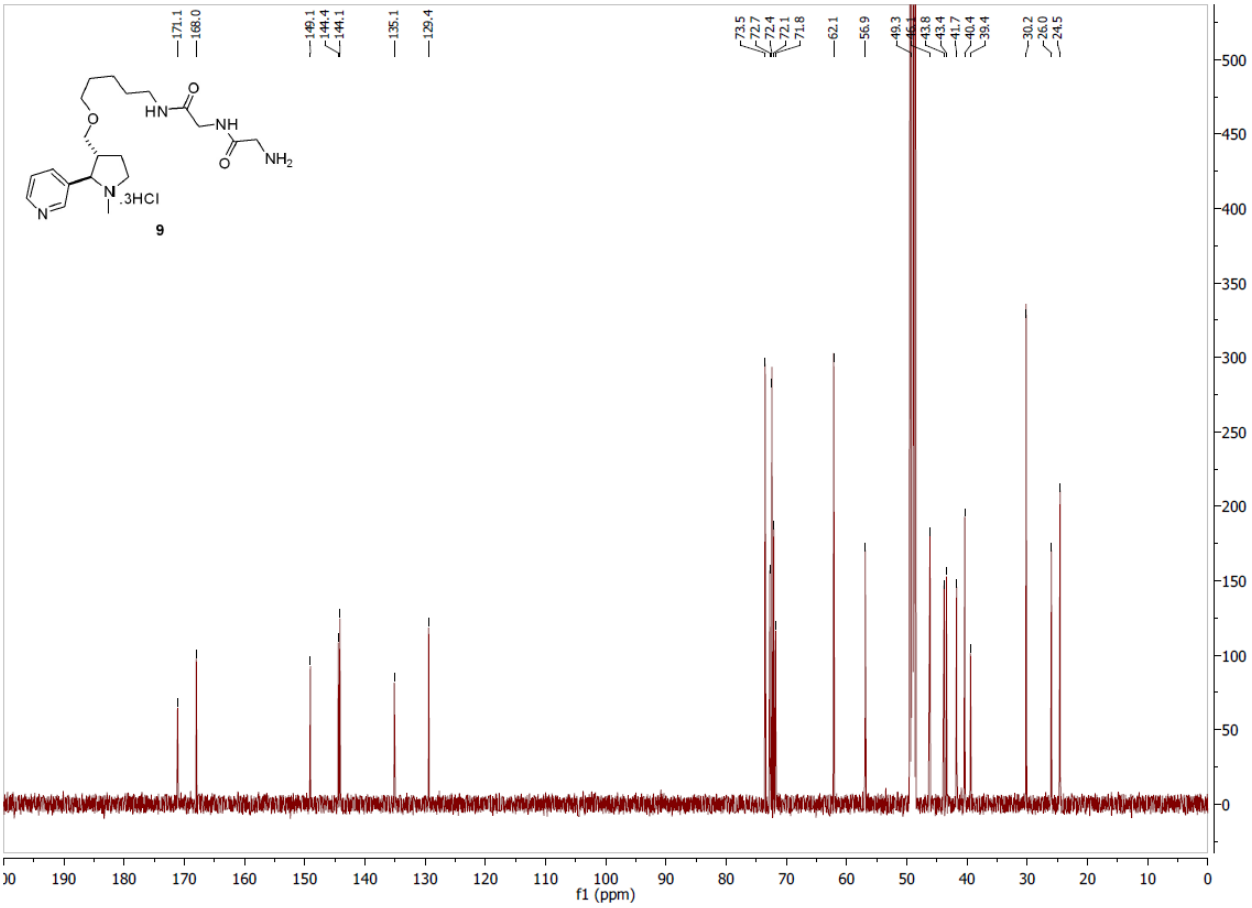

B (d) 8.82
A (s) 8.92
C (d) 8.44
D (m) 7.86
G (m) 3.50
J (t) 3.10
E (br d) 4.46
F (br s) 3.92
H (m) 3.40
I (m) 3.33
L (s) 2.83
N (m) 2.31
M (m) 2.44
Q (s) 1.65
T (m) 1.19
P (p) 1.84
S (m) 1.41
O (m) 2.17
R (m) 1.49
.TFA
triAM1 (7)
f1 (ppm)

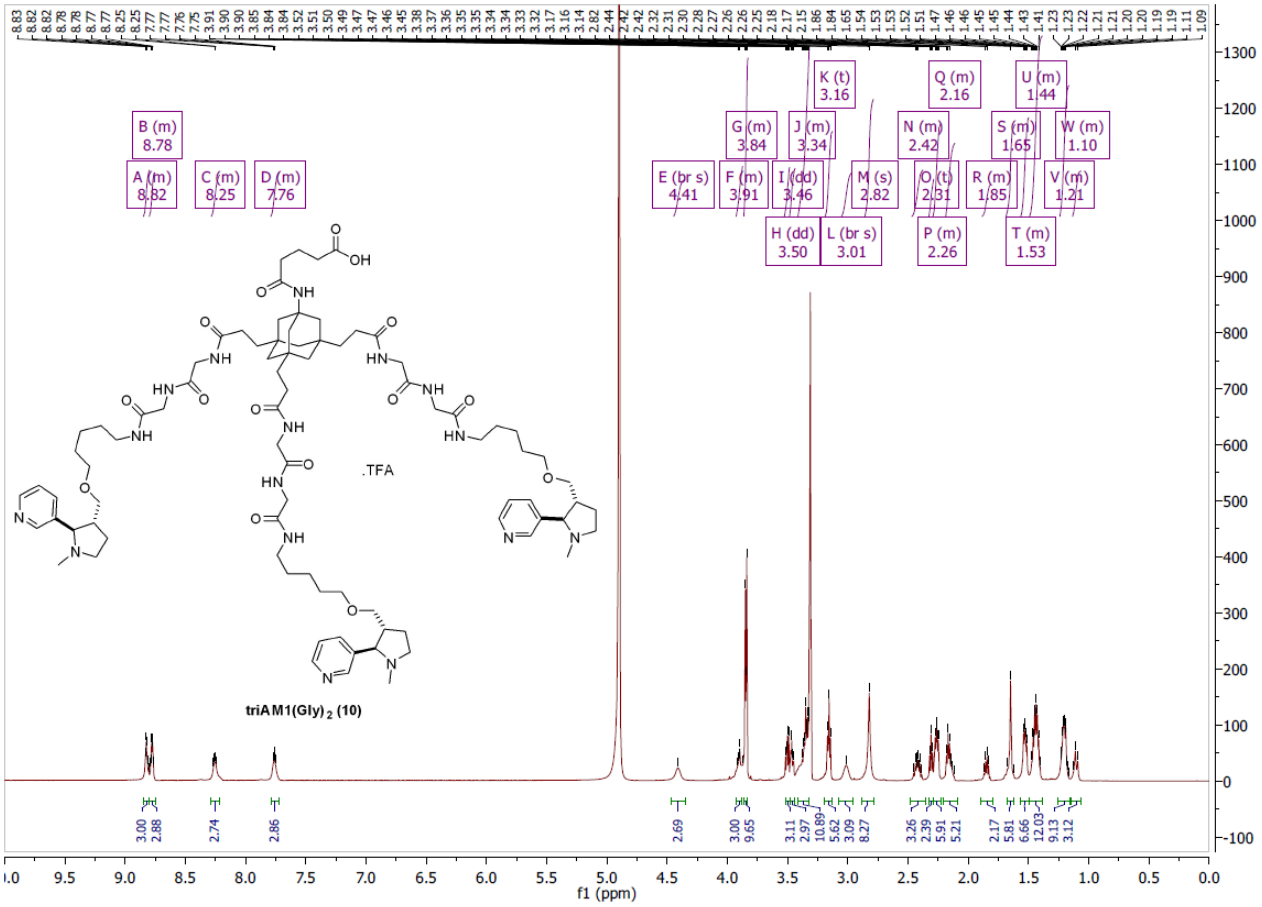

A (q) 162.2
B (q) 117.7
.TFA
triAM1 (7)
f1 (ppm)

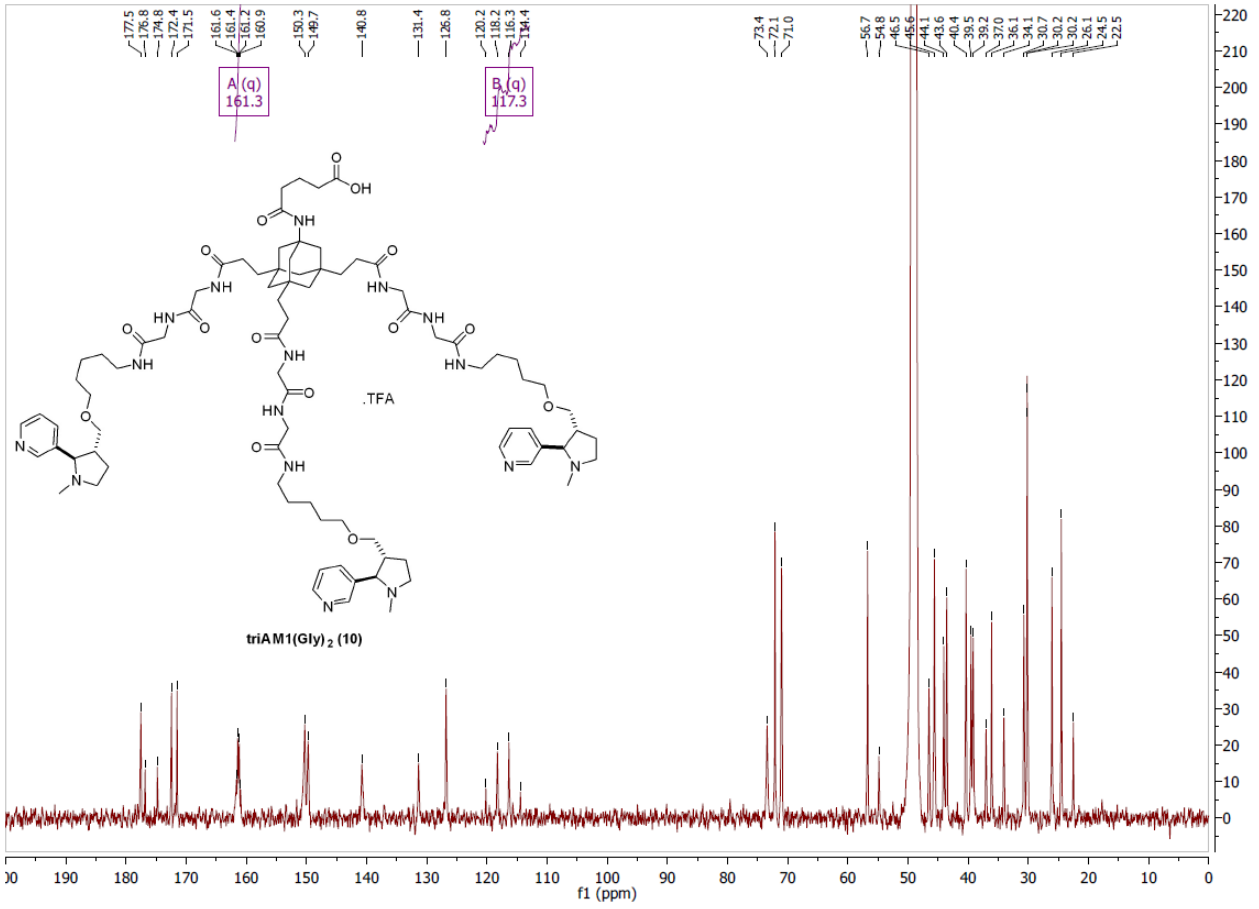

A (s) 8.97
B (d) 8.86
C (d) 8.52
D (m) 7.94
E (br s) 4.99
F (m) 4.50
G (br s) 3.93
H (s) 3.85
I (s) 3.73
J (m) 3.50
K (m) 3.42
L (m) 3.34
M (m) 3.16
N (m) 3.05
O (s) 2.84
P (m) 2.44
Q (m) 2.14
R (s) 1.45
S (m) 1.19
HN
NH
NHBoc
.TFA
8
f1 (ppm)

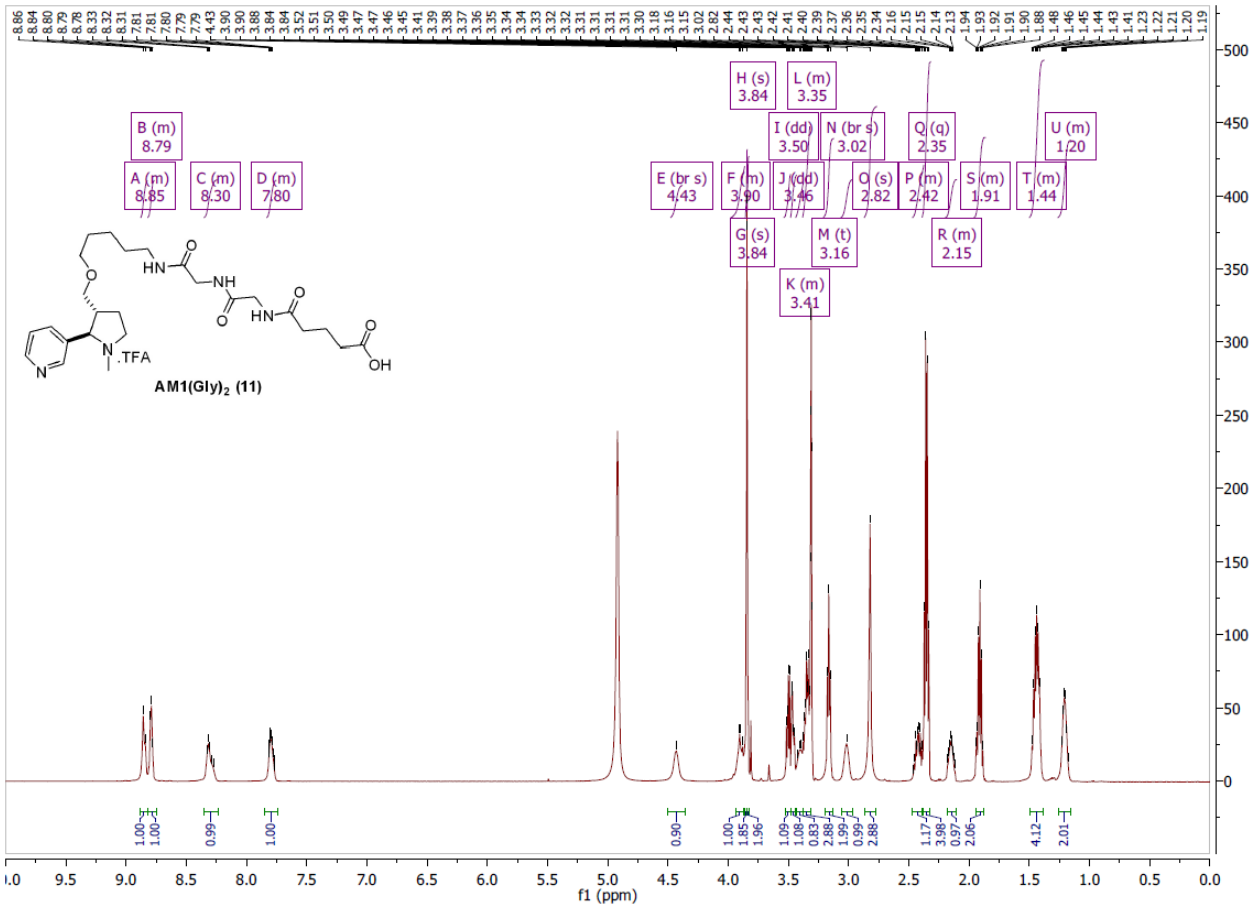

A (q) 161.8
B (q) 117.5
HN
NH
NHBoc
.TFA
8
f1 (ppm)

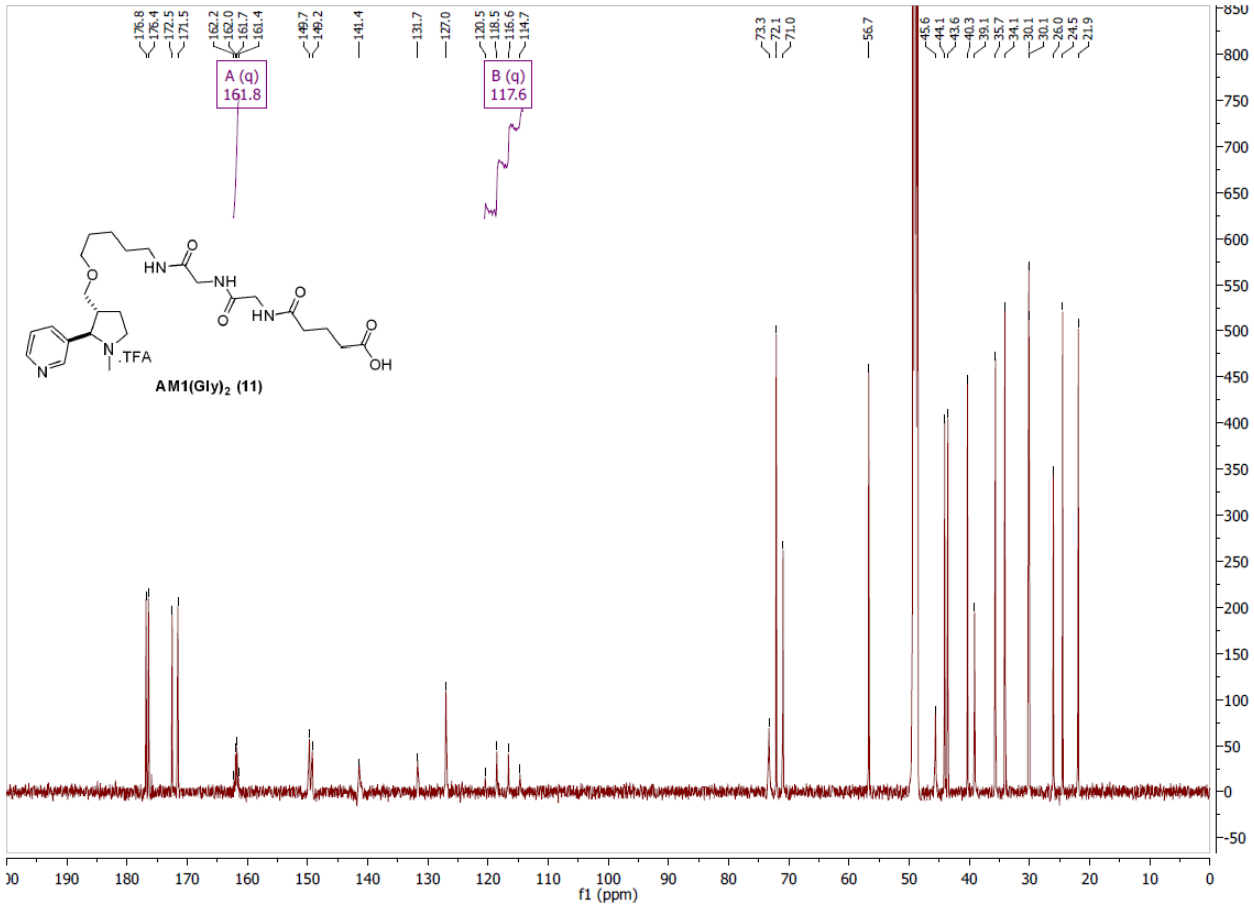

A (s) 9.36
B (s) 9.11
C (s) 9.06
D (s) 8.29
E (s) 4.75
F (s) 3.97
G (s) 3.93
H (s) 3.81
I (t) 3.74
J (q) 3.67
K (m) 3.59
L (s) 3.46
M (s) 3.35
N (s) 3.15
O (s) 2.89
P (s) 2.50
Q (s) 2.11
R (s) 1.42
S (s) 1.14
T (s) 1.38
HN
NH
NH2
.3HCl
9
f1 (ppm)


171.1
168.0
149.1
144.4
144.1
135.1
129.4
73.5
72.7
72.4
72.1
71.8
62.1
56.9
49.3
46.1
43.8
43.4
41.7
40.4
39.4
30.2
26.0
24.5
HN
NH
NH2
.3HCl
9
f1 (ppm)

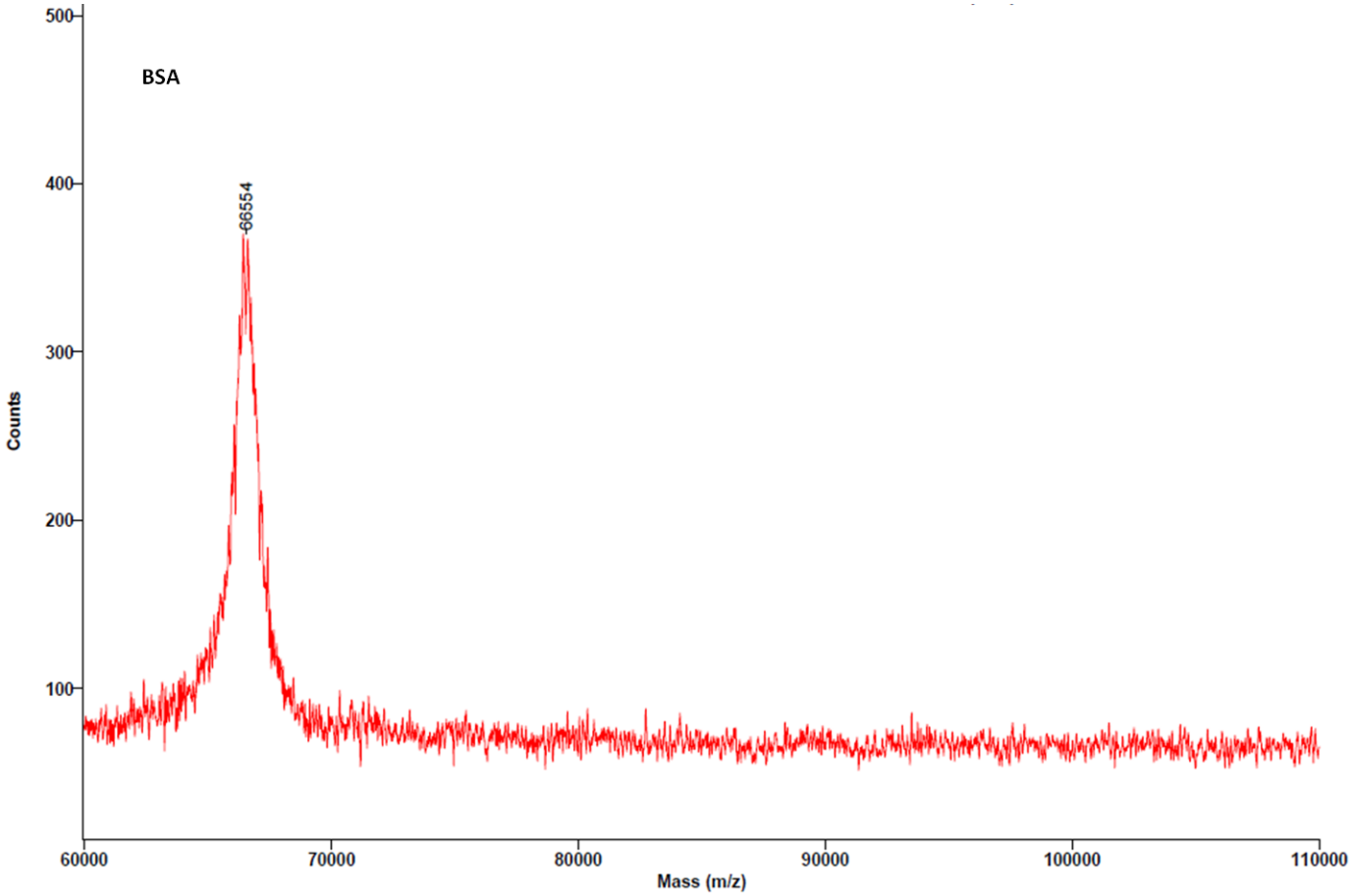

B (m) 8.78
A (m) 8.82
C (m) 8.25
D (m) 7.76
K (t) 3.16
Q (m) 2.16
U (m) 1.44
G (m) 3.84
J (m) 3.34
N (m) 2.42
S (m) 1.65
W (m) 1.10
E (br s) 4.41
F (m) 3.91
I (dd) 3.46
M (s) 2.82
O (t) 2.31
R (m) 1.85
V (m) 1.21
H (dd) 3.50
L (br s) 3.01
P (m) 2.26
T (m) 1.53
.TFA
triAM1(Gly)2 (10)
f1 (ppm)

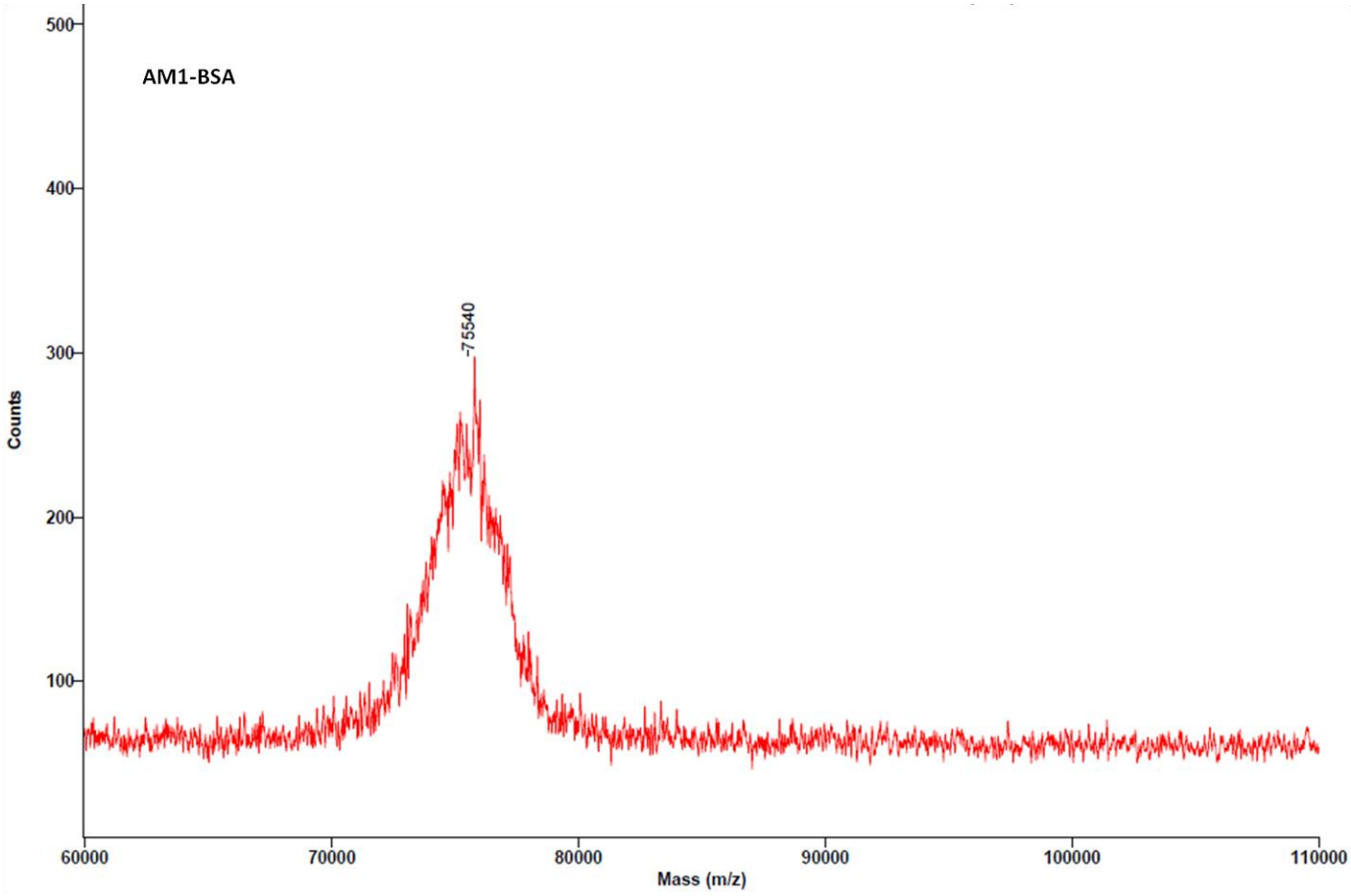

A (q) 161.3
B (q) 117.3
.TFA
triAM1(Gly)2 (10)
f1 (ppm)

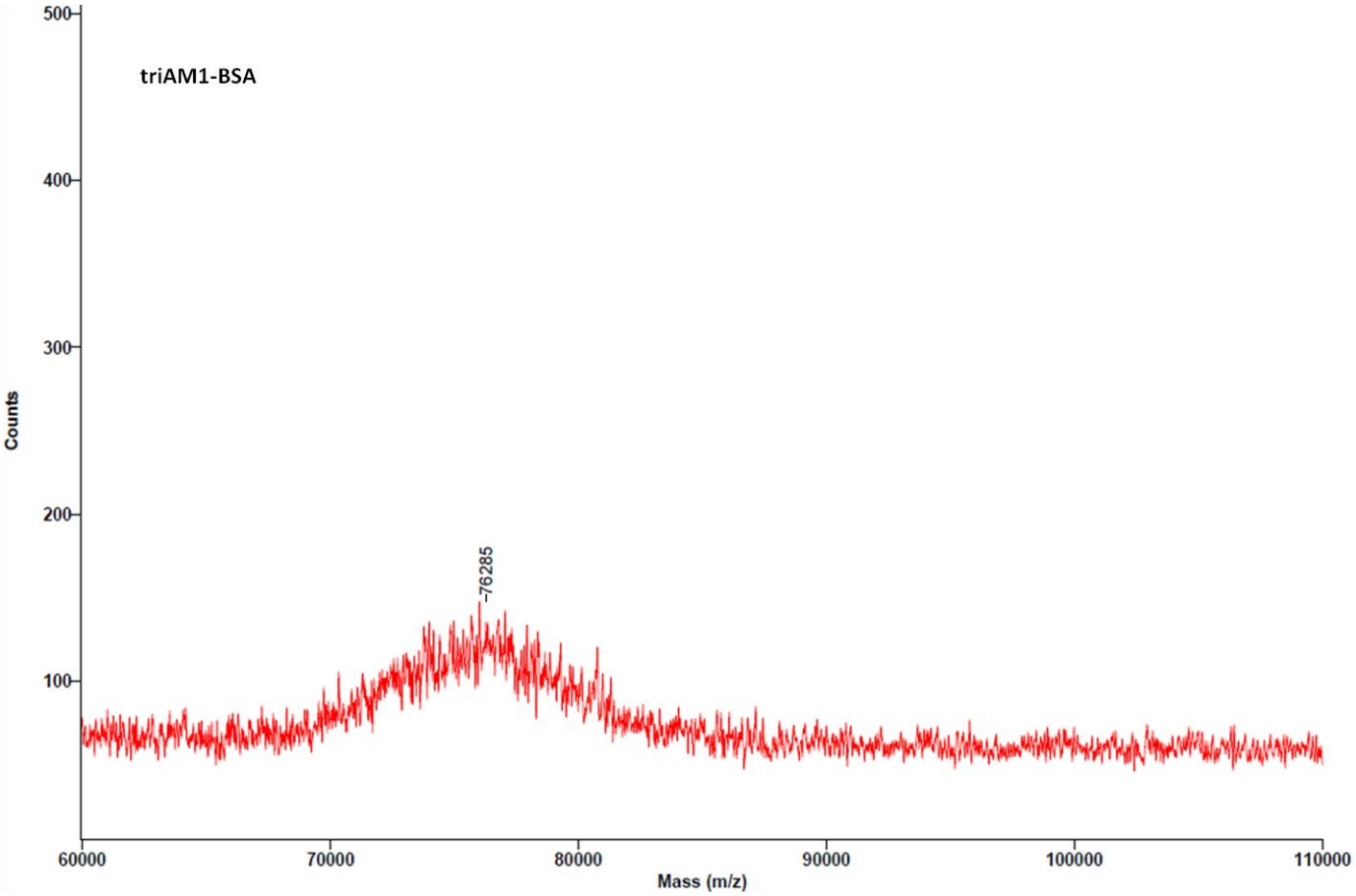

B (m) 8.79
A (m) 8.85
C (m) 8.30
D (m) 7.80
H (s) 3.84
L (m) 3.35
I (dd) 3.50
N (br s) 3.02
Q (q) 2.35
U (m) 1.20
E (br s) 4.43
F (m) 3.90
J (dd) 3.46
O (s) 2.82
P (m) 2.42
S (m) 1.91
T (m) 1.44
G (s) 3.84
M (t) 3.16
R (m) 2.15
K (m) 3.41
HN
NH
HN
OH
N
.TFA
AM1(Gly)2 (11)
f1 (ppm)

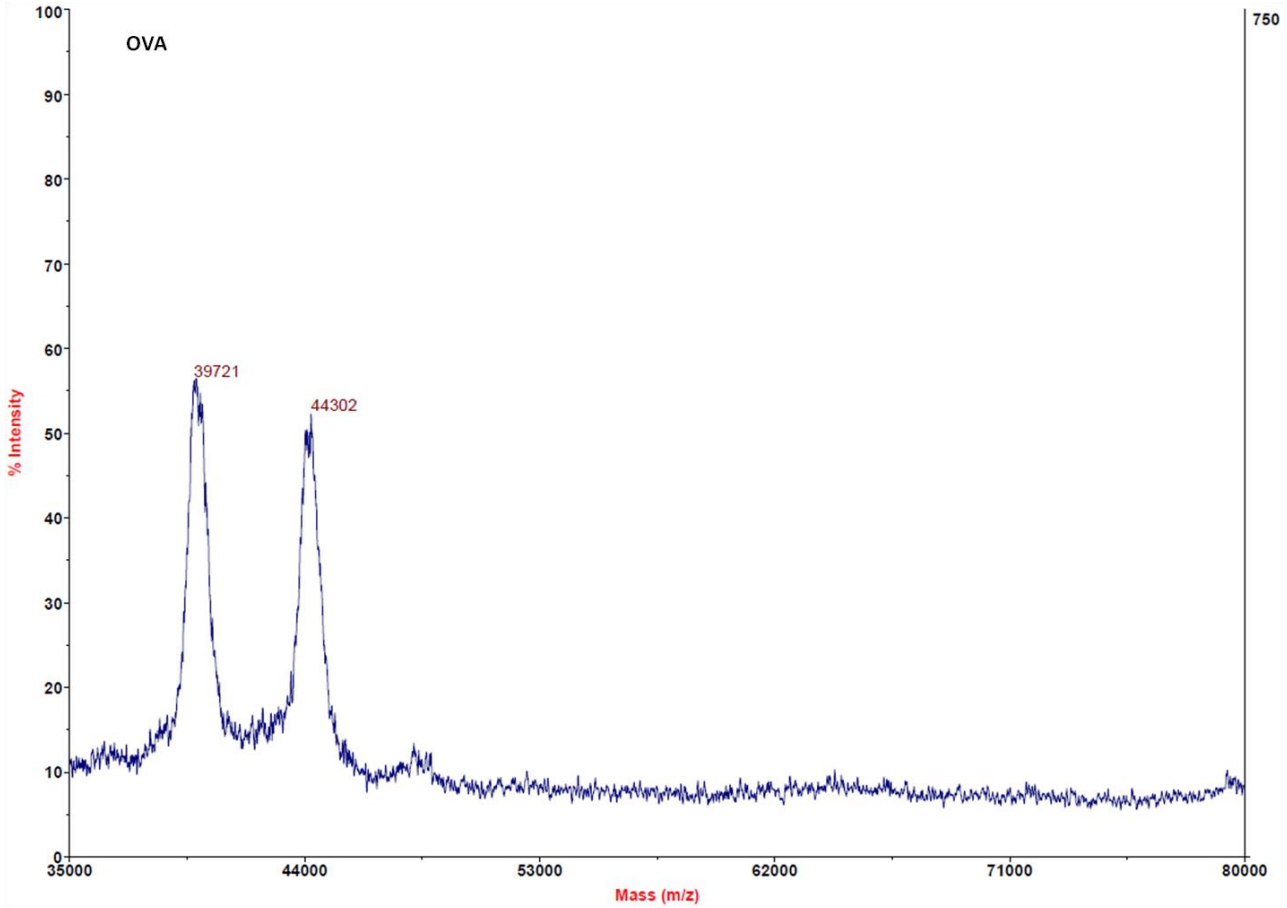

A (q) 161.8
B (q) 117.6
HN
NH
HN
OH
N
.TFA
AM1(Gly)2 (11)
f1 (ppm)

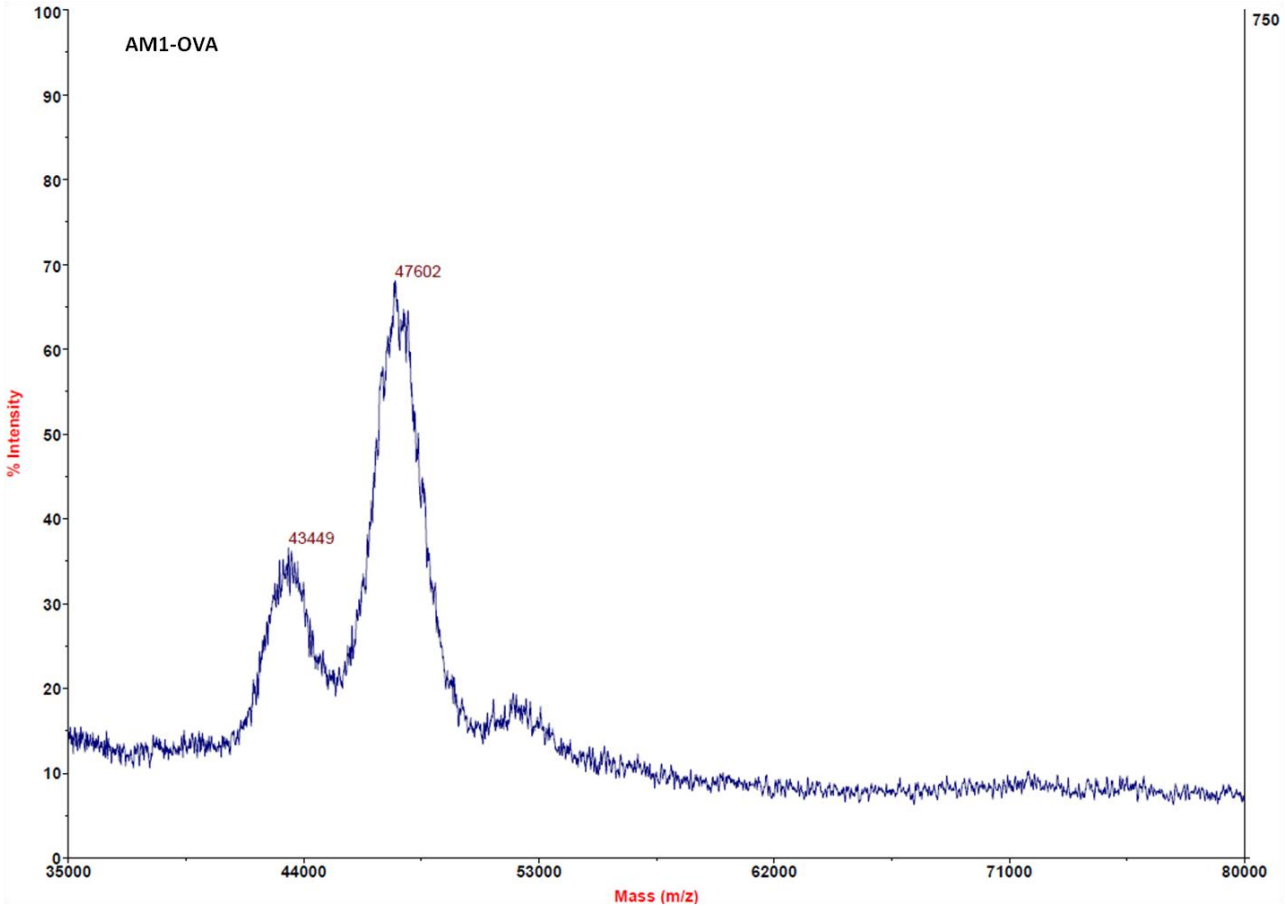
MALDI-TOF Spectra (for AM1 and triAM1 study)

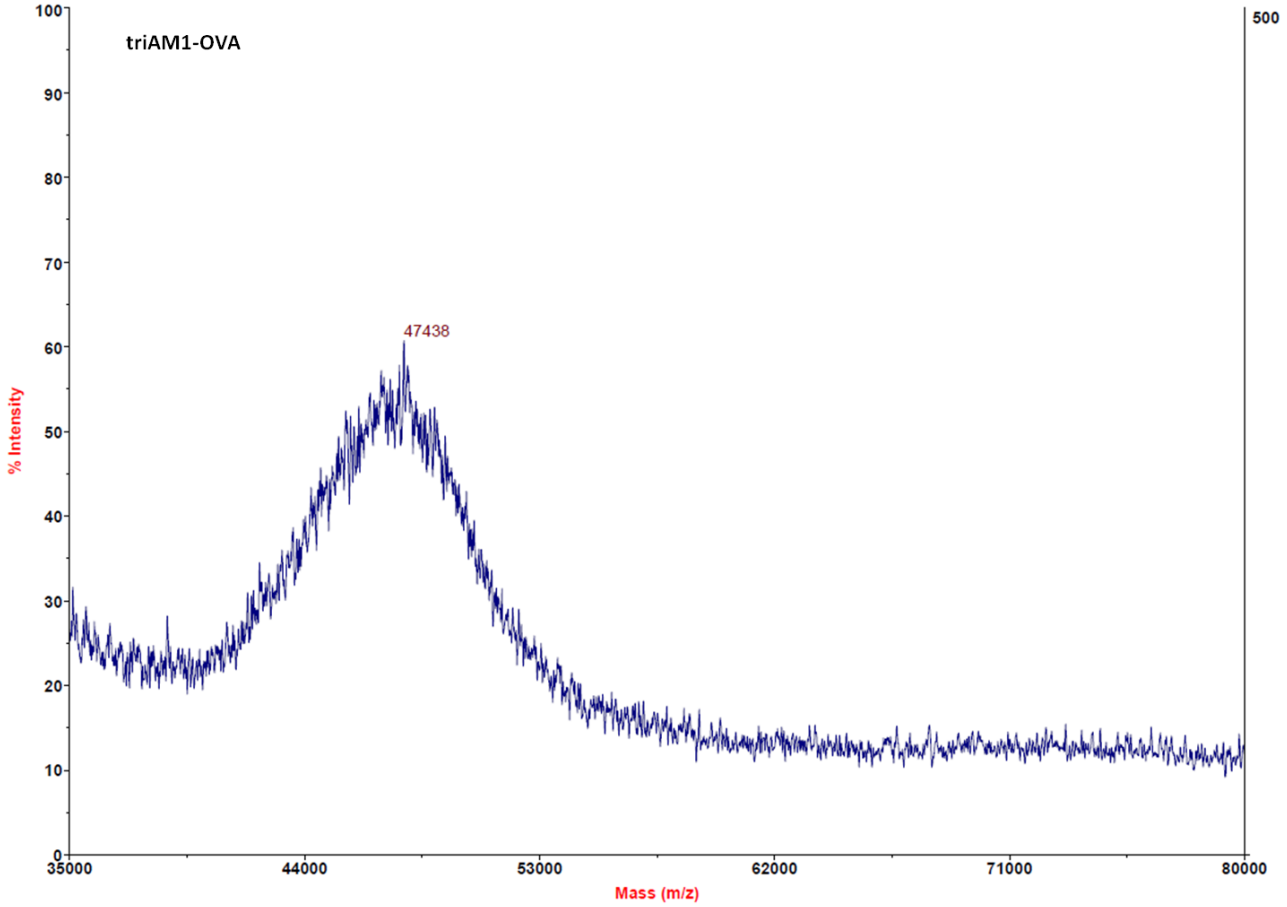




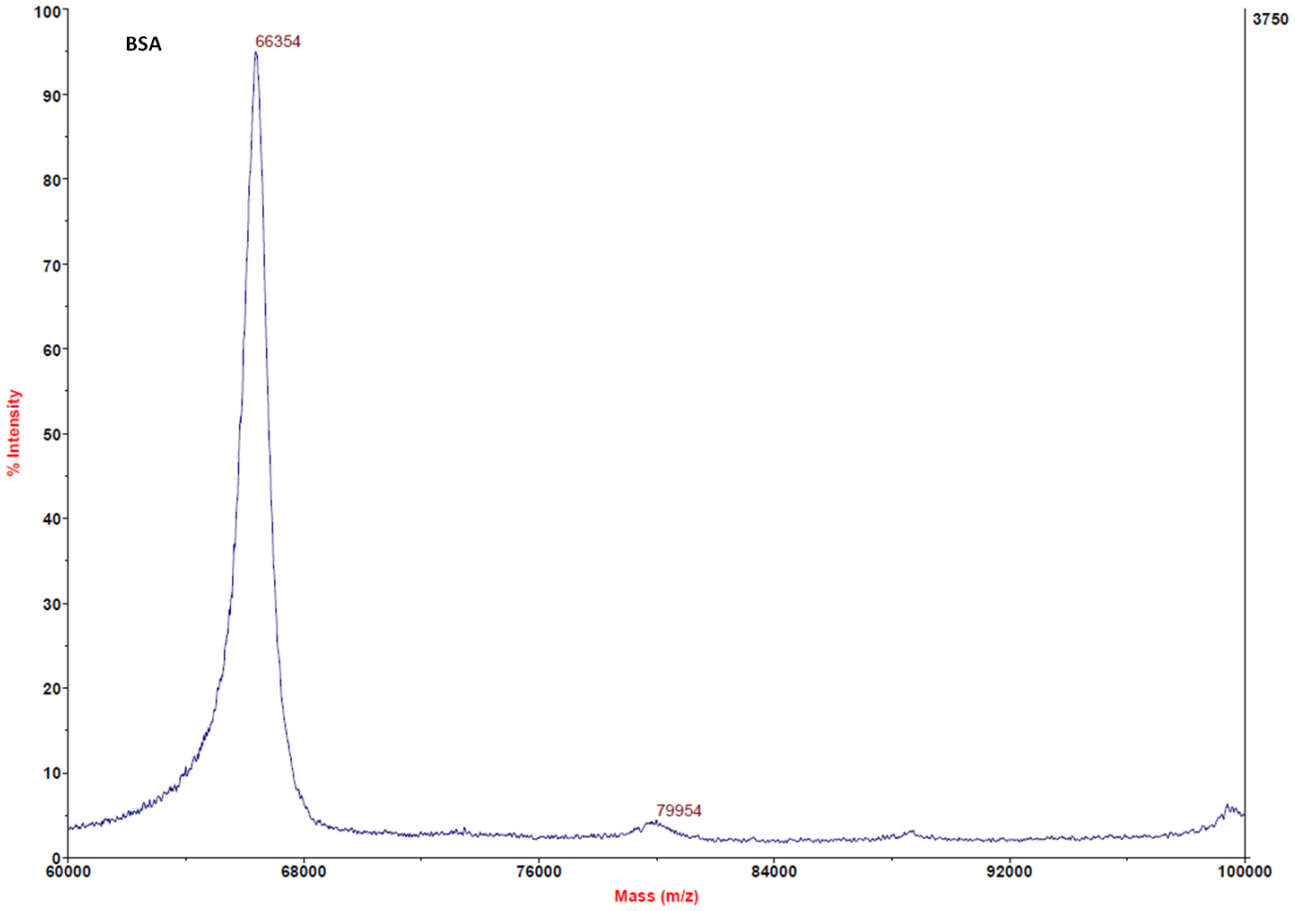

triAM1-BSA
Counts
500
400
300
200
100
76285
60000
70000
80000
90000
100000
110000
Mass (m/z)

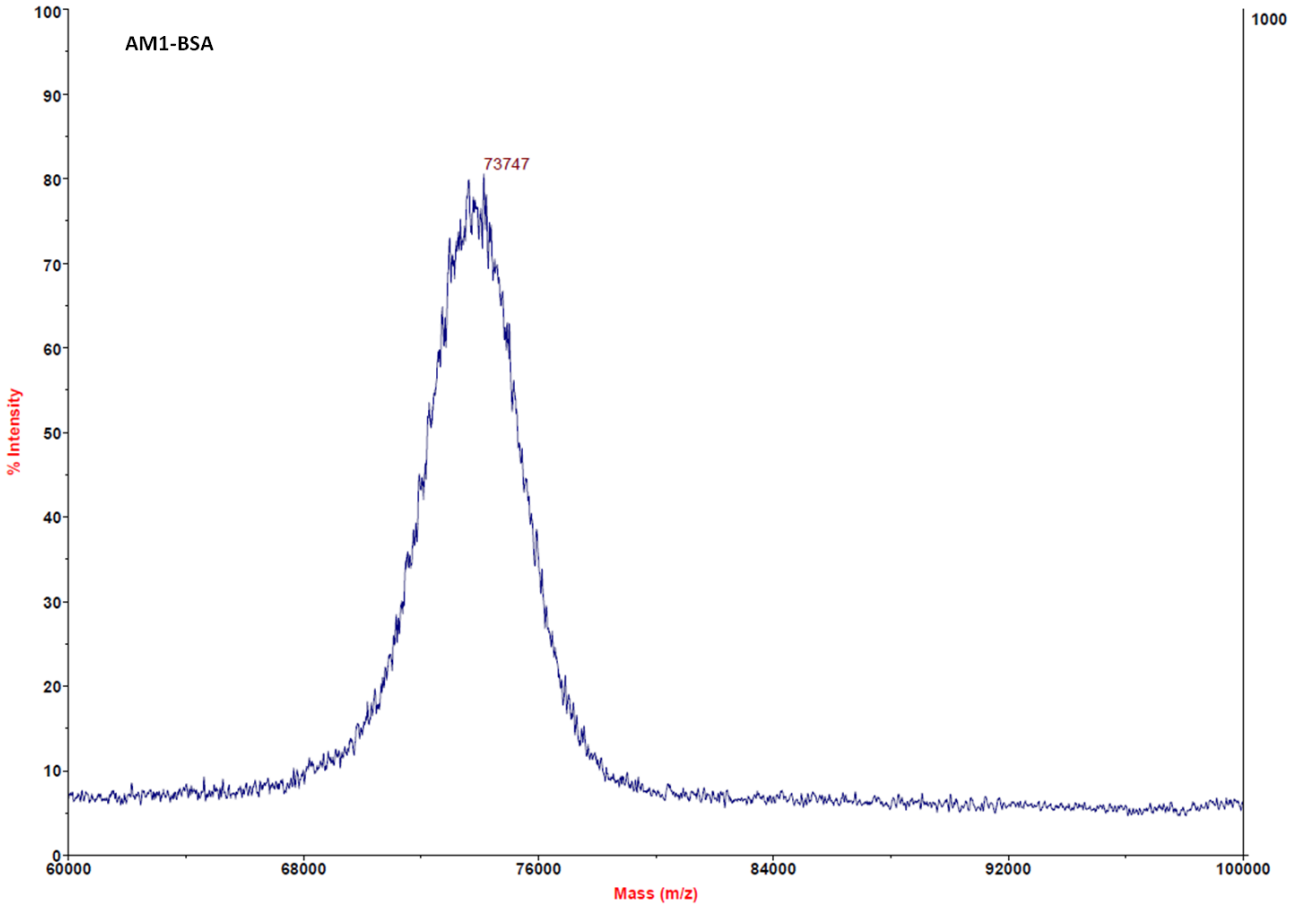

OVA
% Intensity
100
90
80
70
60
50
40
30
20
10
0
750
39721
44302
35000
44000
53000
62000
71000
80000
Mass (m/z)

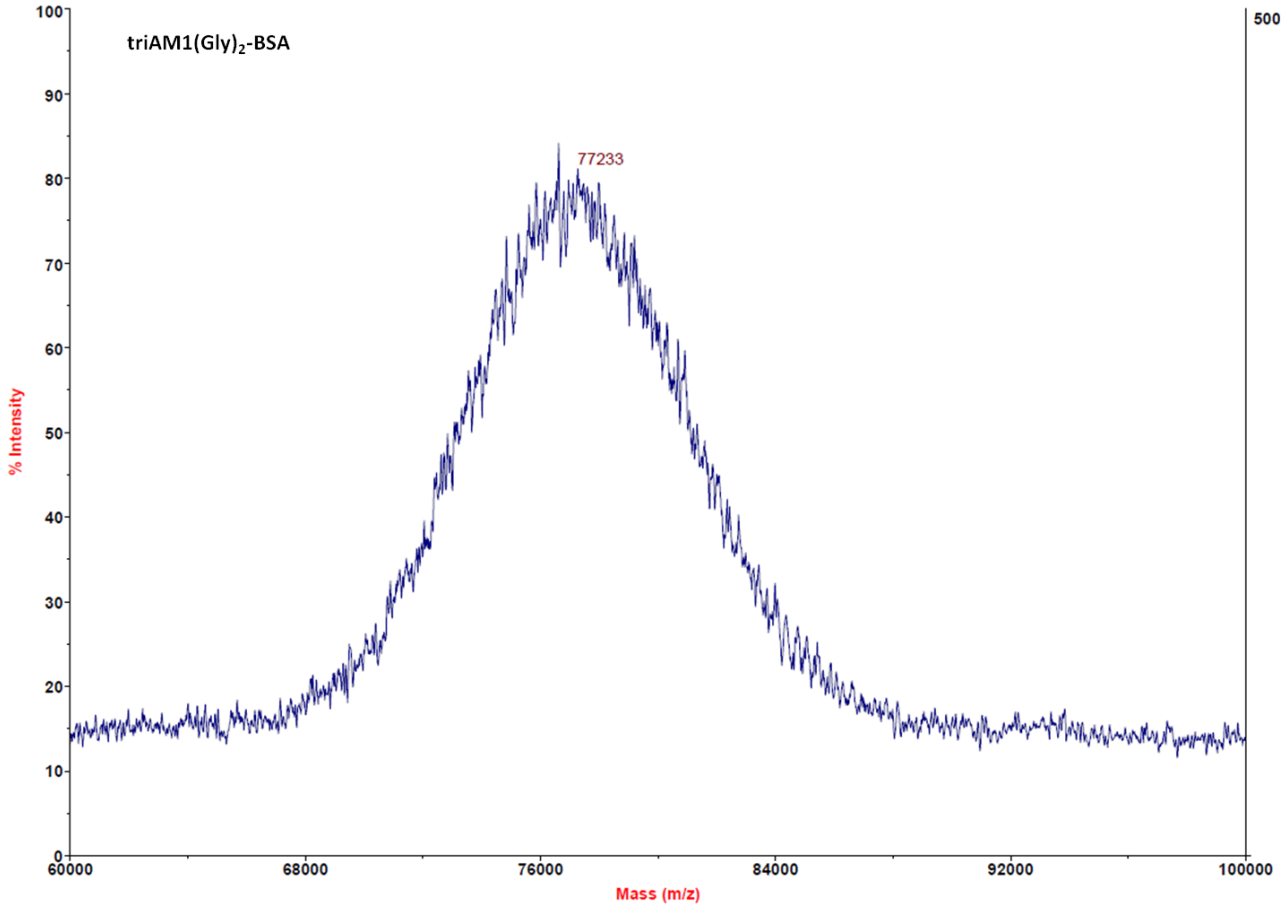

AM1-OVA
47602
43449
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
35000
44000
53000
62000
71000
80000
750

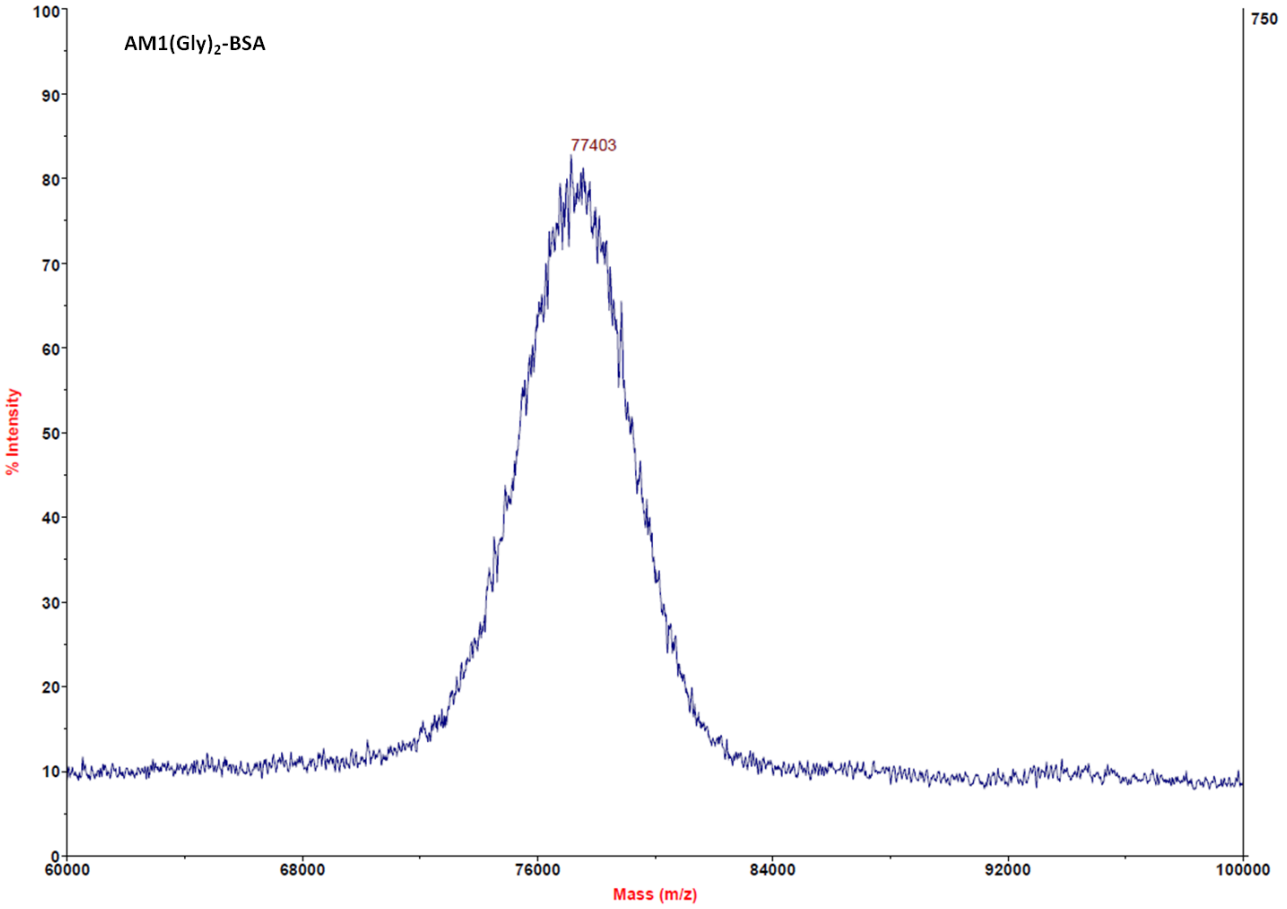

triAM1-OVA
47438
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
35000
44000
53000
62000
71000
80000
500

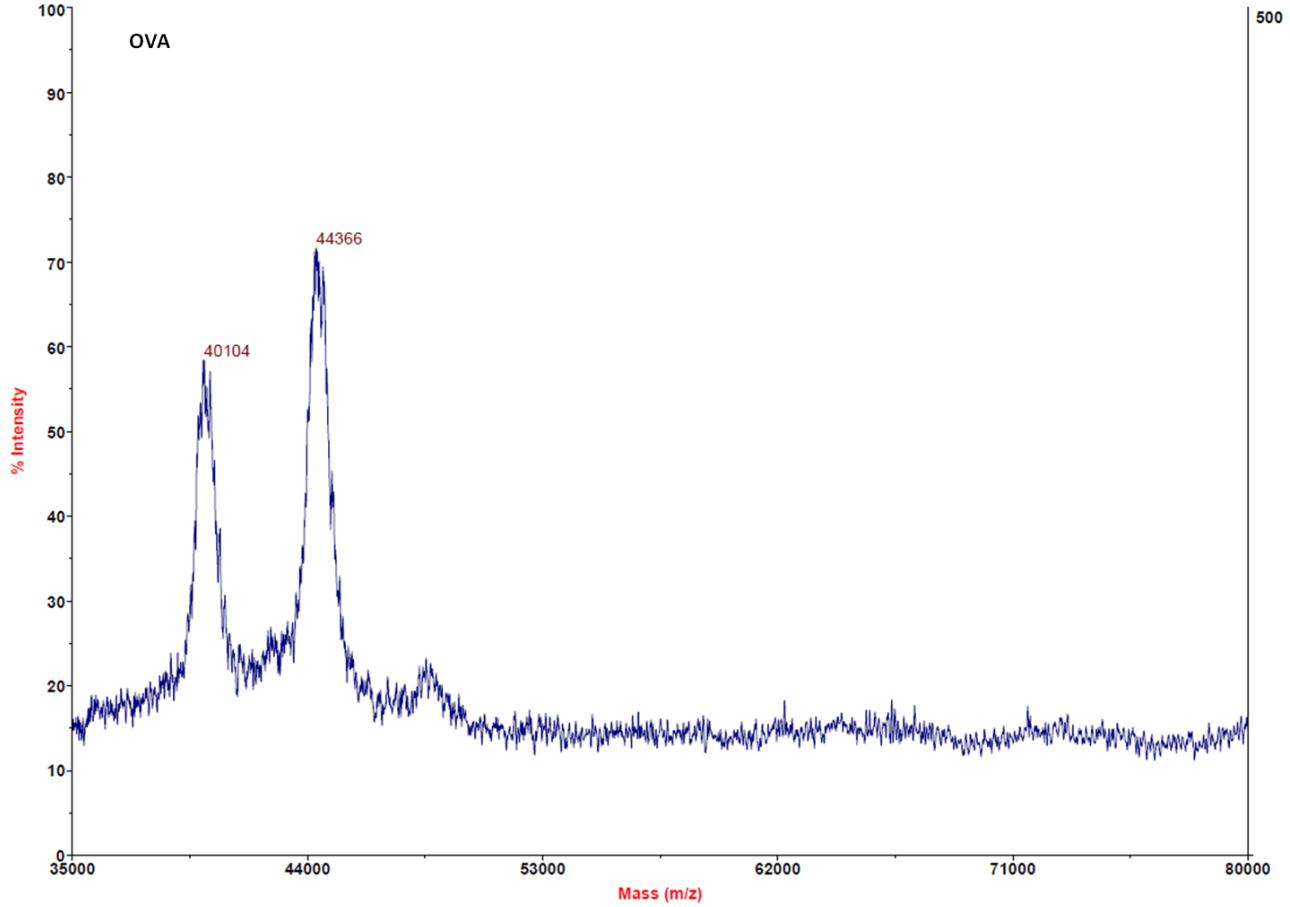
MALDI-TOF Spectra (for AM1, triAM1(Gly)$_2$ and AM1(Gly)$_2$ study)

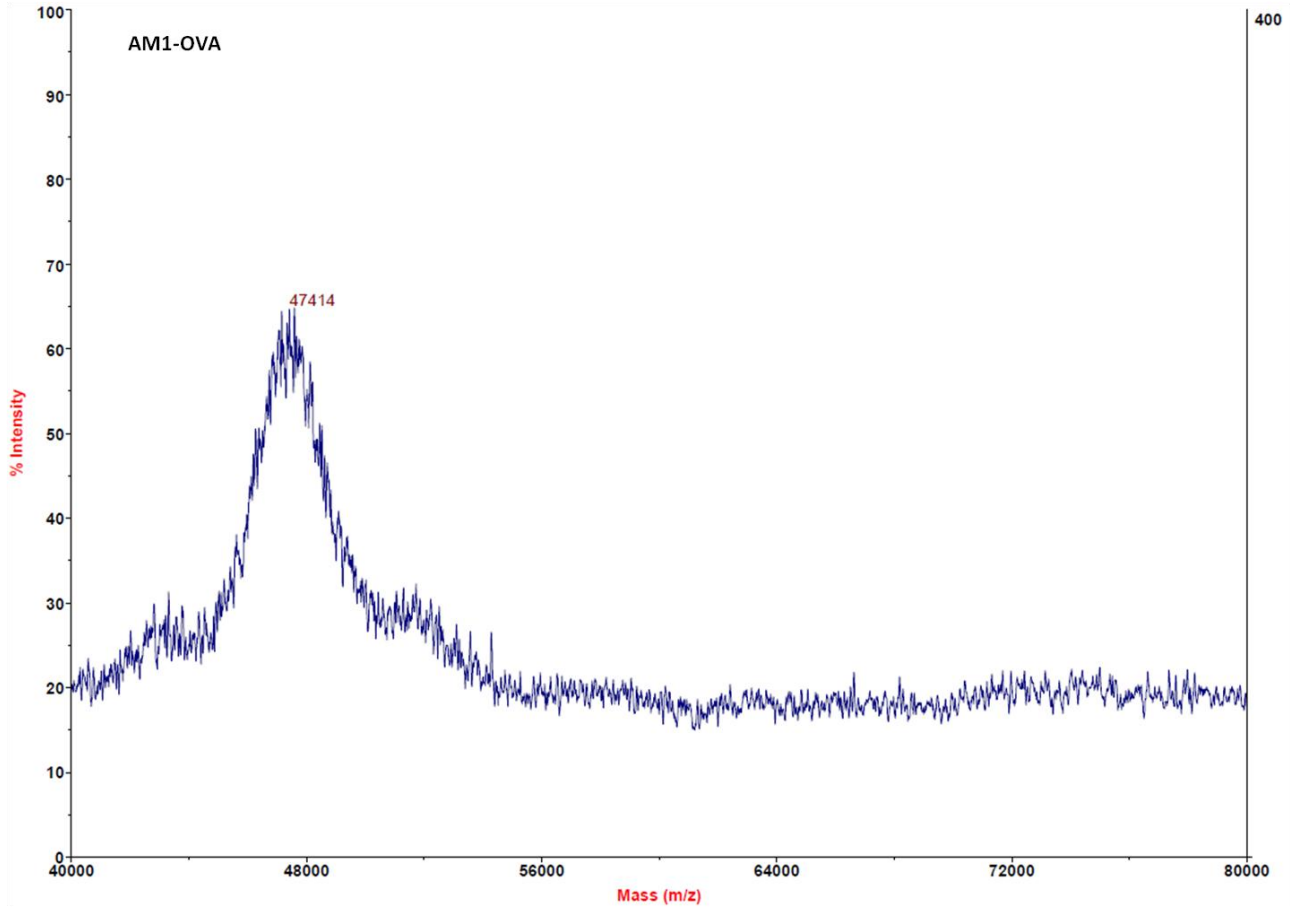


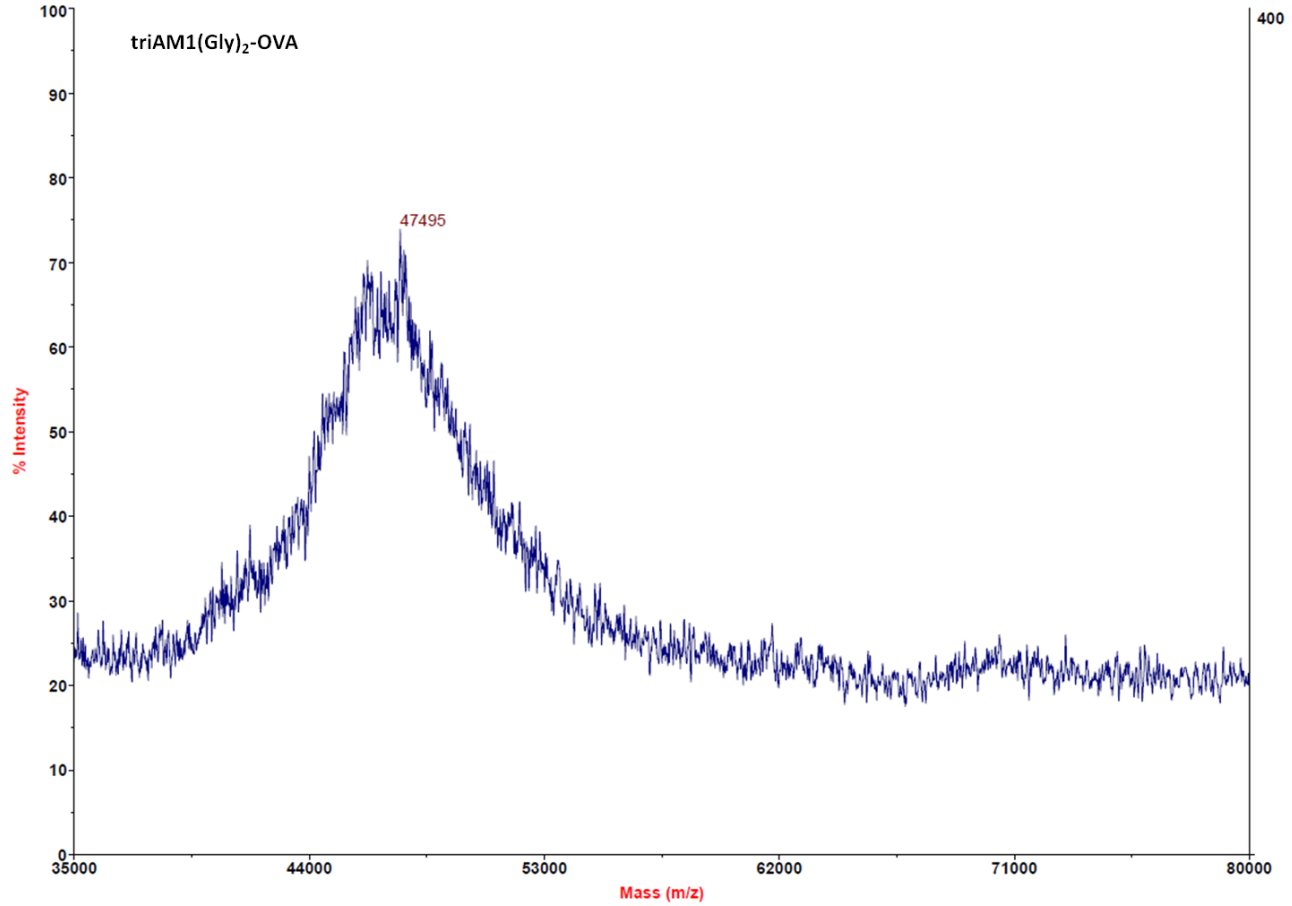


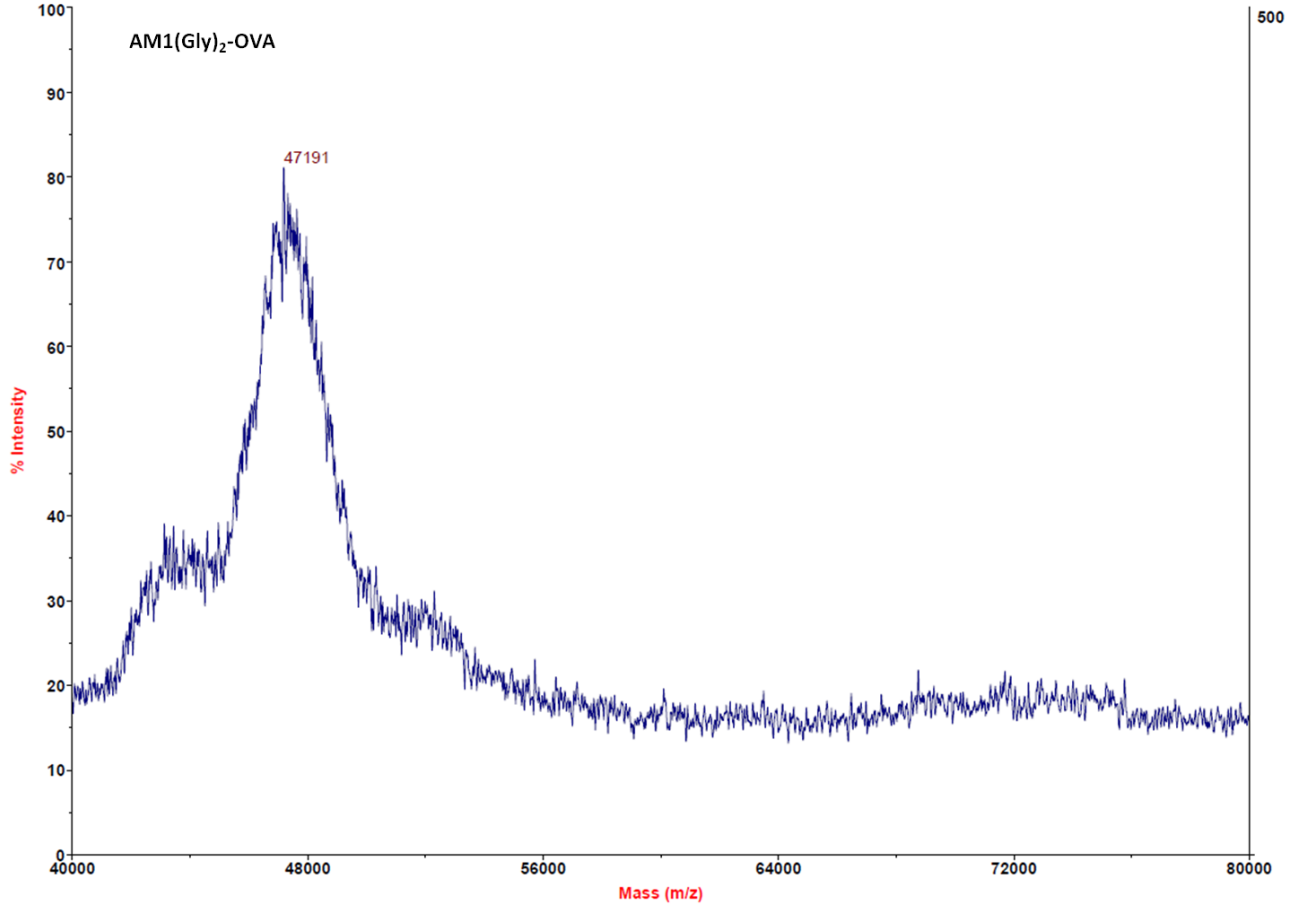

triAM1(Gly)2-BSA
77233
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
60000
68000
76000
84000
92000
100000
500


AM1(Gly)2-BSA
77403
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
60000
68000
76000
84000
92000
100000
750


OVA
100
90
80
70
60
50
40
30
20
10
0
% Intensity
44366
40104
500
35000
44000
53000
62000
71000
80000
Mass (m/z)


AM1-OVA
100
90
80
70
60
50
40
30
20
10
0
% Intensity
47414
400
40000
48000
56000
64000
72000
80000
Mass (m/z)


triAM1(Gly)2-OVA
47495
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
35000
44000
53000
62000
71000
80000
400


AM1(Gly)2-OVA
47191
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
40000
48000
56000
64000
72000
80000
500