Electronic Supplementary Material (ESI) for ChemComm.

SUPPORTING INFORMATION

One-Pot Peptide Conjugation Strategy: Combination of Enzymatic Transamidation and Click Chemistry

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Materials

The plasmid pDJ1-3 was kindly provided by Professor M. Pietzsch (Martin-Luther-Universität, Halle-Wittenberg, Germany). pDJ1-3 encodes the proenzyme of MTG from *S. mobaraensis* inserted between the *Nde*I and *Xho*I restriction sites of the vector pET20b.[1] Deionized water (18Ω) was used for all experiments. HPLC solvents were of analytical grade, and products used for the expression and purification of MTG were of biological grade.

Other chemicals used were purchased from the suppliers listed below. Carboxybenzyl–L-glutaminyl–glycine (Z-Gln-Gly, or ZQG) was from Bachem (Bubendorf, Switzerland). Glutathione (reduced) and thiamine were from Bioshop (Burlington, Canada). Azidopropylamine was synthesized previously in the laboratory of Jeffrey Keillor, according to published protocols.[2] Propargylamine hydrochloride (95%) and 4-pentynoic acid (98%) were purchased from Acros Organics (New Jersey, USA). Formic acid (98 % purity) was from Fluka Analytical (St. Louis, USA). 6-Azidohexanoic acid was purchased from Merck Millipore (Darmstadt, Germany). Copper (II) sulfate pentahydrate (≥ 98%), (+)-sodium L-ascorbate (≥ 98%) and α-lactalbumin from bovine milk (calcium saturated) were purchased from Sigma Aldrich (St. Louis, MO, USA). Sulfo-cyanine5 azide (Cy5-azide) and sulfo-cynanine5 alkyne (Cy5-alkyne) were purchased from Lumiprobe (Hallandale Beach, FL, USA).

MTG Expression and Purification

MTG was expressed and purified as previously described.[3] Briefly, a 5-mL starter culture of *E. coli* BL21 (DE3) containing the plasmid pET20b-MTG, which expresses a *C*-terminally 6-His-tagged version of MTG, was propagated overnight at 37°C in ZYP-0.8G medium and shaking at 240 rpm. It was used to inoculate 500 mL of autoinducing ZYP-5052 medium. After 2h of

incubation at 37°C and 240 rpm, the temperature was reduced to 22°C overnight. Cells were collected by centrifugation and resuspended in 0.2 M Tris-HCl, pH 6.0. The cells were lysed using a Constant Systems cell disruptor set at 37 kPSI and cooled to 4°C. After further centrifugation to remove insoluble cellular matter, the inactive form of MTG was incubated with trypsin (1 mg/mL solution, 1:9 ratio of trypsin to MTG, v/v) for the purpose of cleaving its pro-sequence. Activated MTG was purified using a 5-mL His-trap nickel-nitrilotriacetic acid (Ni-NTA) column (GE Healthcare) equilibrated in 50 mM phosphate buffer, pH 8.0, with 300 mM NaCl, and eluted with an imidazole gradient (0 – 250 mM) using an Åtka FPLC (GE Healthcare). After purification, active MTG was dialyzed against 0.2 M Tris-HCl buffer, pH 6.0. The average yield was 25 mg of activated MTG per litre of culture, with ~ 85% purity as estimated by SDS-PAGE and revelation with Coomassie blue stain. Aliquots were snap frozen and stored at -80°C in 15% glycerol.

MTG Activity Assay

The activity of purified MTG was quantified using the hydroxamate assay.[5] Briefly, MTG was incubated with 30 mM Z-Gln-Gly and 100 mM hydroxamate at 37°C for 10 min. A concentrated acidic ferric chloride solution (2.0 M $FeCl_3 \cdot 6\ H_2O$, 0.3 M trichloroacetic acid, 0.8 M HCl) was used to quench the reaction, which was then vortexed and left to stand at room temperature for 10 min. The resulting iron complex was quantified by its absorbance at 525 nm. One unit (U) of MTG produces 1 μmol of L-glutamic acid and γ-monohydroxamate per min at 37°C.

Conjugation Assays

Amide, amine and complementary azide or alkyne substrates (30 or 60 mM, as indicated) were combined with 2.5 mM $CuSO_4 \cdot 5\ H_2O$, 25 mM sodium ascorbate, and 5 mM glutathione. The conjugation reaction catalyzed by MTG was initiated by the addition of 1 U/mL of MTG, where control reactions had an equivalent volume of buffer (200 mM Tris-acetate, pH 7.5) added. The final volume of each reaction was 350 µL and all were incubated at 37°C for the time indicated.

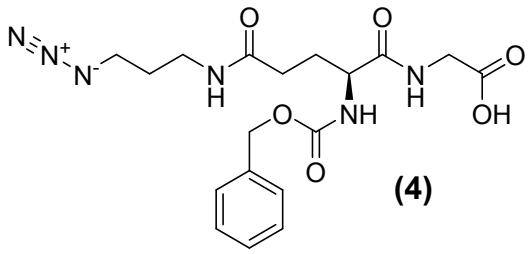
For labelling of α-lactalbumin, azidopropylamine or propargylamine (2 mM), 5 mM glutathione, 1 mM $CuSO_4$, 10 mM sodium ascorbate, and 2 mM Cy5-alkyne or Cy5-azide were mixed with α-lactalbumin such that its final concentration was 4 mg/mL, in 200 mM Tris-acetate buffer, pH 7.5. The final volume of each reaction was 200 µL. Reactions were incubated at 37°C for 24 – 48 h. The reactions were washed 6 times over a Spin-X® UF microfuge concentrator, 10k MWCO (Corning), using 200 mM Tris-acetate buffer, pH 7.5, containing 2 mM EDTA. Washed and concentrated sample (10 µL out of 75-100 µL) was resolved using tricine SDS-PAGE.[4] The fluorescent bands were visualized and recorded using a Bio Rad ChemiDoc™ MP Imaging System using an excitation filter of 625 nm with a 30 nm bandpass. The gels were then stained with Coomassie brilliant blue to reveal the protein.

Characterization of Product Formation

Samples were prepared for HPLC-MS analysis by taking an aliquot at specific time points indicated by transferring 10 µL of each reaction to a vial containing 10 µL of formic acid. 960 µL of 18.2 mΩ deionized water and 20 µL of internal standard solution (5 g/L of 4-methoxybenzamide in neat DMSO) were added and mixed by vortex. Samples (10 µL) were injected onto a Synergi

4-μm polar-RP 80 Å, 150 × 4.60 mm LC column (Phenomenex), using an Agilent 1200 series HPLC apparatus and eluted with a 5-70% MeOH/H_2O gradient. Masses were detected under positive ionization with a single quadrupole mass detector. Concentrations of amide substrate and reaction products were quantified by comparison to a standard curve constructed with the corresponding compound and the internal standard.

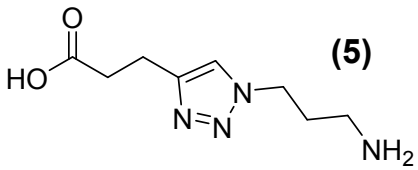
Synthesis of Products



(4)

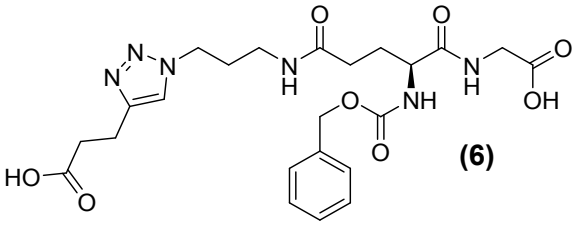
ZQG-APA (4). An aqueous solution (1 mL) buffered by 200 mM potassium phosphate, pH 7.5, containing 50 mM ZQG (1) and 90 mM azidopropylamine (3) was prepared. The conjugation reaction was initiated by adding 1 U/mL of MTG, and incubated at 37°C for a minimum of 2 hours. An additional 1 U/mL of MTG was then added to the reaction, vortexed, and incubated at the same temperature for 16 hours. The reaction volume was centrifuged in a microfuge (10 min, 13,000 rpm, 4°C) to remove insoluble debris, and the supernatant was divided into two 500 μL volumes. They acidified by adding approximately 5 μL 6M HCl to each mixture. A white precipitate formed shortly after acidification, and it was centrifuged again the supernatant transferred to new tubes. Dichloromethane (500 μL) was added to each tube and shaken vigorously. The mixtures were centrifuged, and more white precipitate was observed sitting at the interface between the two phases. The aqueous phase was removed and the precipitate transferred into a new tube. This precipitate was dried at 37°C, and its structure analyzed by NMR and its mass determined by MS.

^{1}H NMR (700 MHz, DMSO-d_6): δ 8.09 (t, *J* = 5.4 Hz, 1H), 7.88 (t, *J* = 5.3 Hz, 1H), 7.46 (d, *J* = 8.1 Hz, 1H), 7.39 – 7.31 (m, 5H), 5.04 (d, *J* = 12.6 Hz, 1H). 5.02 (d, *J* = 10.1 Hz, 1H), 4.01 (m, 1H), 3.76 (dd, *J* = 9.1, 5.6 Hz, 1H), 3.67 (dd, *J* = 9.1, 5.6 Hz, 1H), 3.34 (t, *J* = 6.8 Hz, 2H), 3.12 – 3.05 (m, 2H), 2.19 – 2.11 (m, 2H), 1.95 – 1.90 (m, 1H), 1.76 – 1.70 (m, 1H), 1.63 (quint, 6.8 Hz, 2H) ppm. ^{13}C NMR (176 MHz, DMSO-d_6): δ 172.2, 172.0, 171.6, 156.3, 137.4, 128.8, 128.25, 128.17, 65.9, 54.7, 48.9, 41.5, 36.3, 32.2, 28.9, 28.4 ppm.



(5)

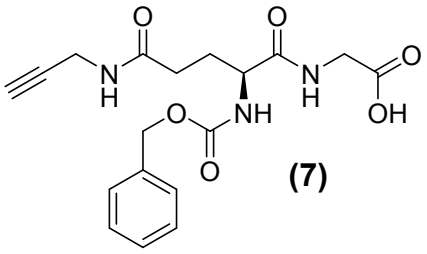
Triazole-APA (5). An aqueous solution (3 mL) containing 300 mM azidopropylamine (3), 300 mM 4-pentynoic acid (2), 5 mM $CuSO_4$ and 50 mM sodium ascorbate was prepared. The reaction was incubated at 37°C for 24 hours, and then lyophilized. ^{1}H NMR (700 MHz, D_2O): δ 7.68 (s, 1H), 4.42 (t, *J* = 6.7, 2H), 2.86 (q, *J* = 7.0 Hz, 4H), 2.442 (t, *J* = 7.2 Hz, 2H), 2.17 (quint, *J* = 7.0 Hz, 2H) ppm. ^{13}C NMR (176 MHz, D_2O): δ 181.3, 147.9, 123.1, 47.1, 36.56, 36.53, 27.3, 21.6 ppm.



(6)

ZQG-Triazole-APA (6). ZQG-APA was resuspended in 200 mM potassium phosphate, pH 7.5, to a final concentration of 100 mM, to which 110 mM 4-pentynoic acid, 2.5 mM $CuSO_4$ and 25

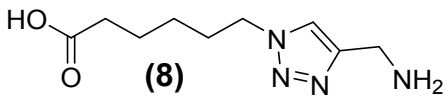
mM sodium ascorbate was also added and mixed by vortexing, and incubated for 24 hours at 37°C. A volume of methanol containing 0.5% formic acid was added in 2.5-fold excess, vortexed, and incubated at -20°C for two hours. A precipitate was observed, and the solution was centrifuged at 4°C for 10 minutes at 13,000 rpm. A small, white and blue grainy pellet was observed, and the supernatant was kept and transferred to a new microfuge tube. The volume was evaporated down to approximately 100 µL, more methanol was added in a 5-fold excess, and the procedure repeated. Acetonitrile was added in 10-fold excess and a thick brown precipitate immediately formed. The mixture was incubated on ice for 1 hour, centrifuged, and the supernatant removed. The pellet was left to dry and was subsequently analyzed by NMR and HPLC-MS. ^{1}H NMR (700 MHz, DMSO-d_6): δ 8.29 (t, J = 5.4 Hz, 1H), 7.84 (s, 1H), 7.69 (t, J = 8.5 Hz, 1H), 7.56 (br, s, 1H), 7.37 – 7.30 (m, 5H), 5.04 (d, J = 11.7 Hz, 1H), 5.10 (d, J = 12.9 Hz, 1H), 4.29 (m, 2H), 3.91 (q, J = 7, 7.6 Hz, 1H), 3.23 (s, 1H), 3.20 (dd, J = 16.5, 4.2 Hz, 2H), 2.99 (m, 1H), 2.73 (t, J = 7.4 Hz, 2H), 2.18 (br, 2H), 2.13 (t, J = 7.6 Hz, 2H), 1.89 (m, 2H), 1.76 (m, 1H) ppm. ^{13}C NMR (176 MHz, DMSO-d_6): δ 174.7, 172.4, 170.8, 159.0, 156.3, 137.5, 128.8, 128.20, 128.15, 122.6, 65.8, 63.7, 60.8, 55.0, 47.2, 44.3, 36.1, 32.3, 30.3, 28.7, 23.2 ppm.



(7)

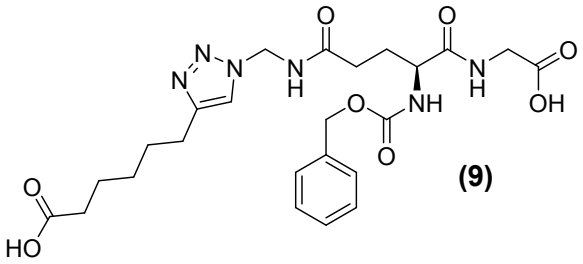
ZQG-PRO (7). An aqueous solution (3 mL) buffered by 200 mM Tris acetate, pH 7.5, containing 40 mM ZQG and 80 mM propargylamine was prepared. The reaction was initiated by adding 1 U/mL of MTG, and incubated at 37°C for 16 hours. Formic acid was added to the reaction (1%),

vortexed, and placed on ice for 3 hours. A white precipitate formed, and was separated by centrifugation for 10 minutes at 4°C and 13,000 rpm. The supernatant was discarded and the pellet was washed with ice-cold water containing 1 % formic acid four times. The pellet was left to dry and was subsequently analyzed by NMR and HPLC-MS. ^{1}H NMR (700 MHz, DMSO-d_6): δ 8.23 (t, J = 5.4 Hz, 1H), 8.18 (t, J = 5.8 Hz, 1H), 7.44 (d, J = 8.2 Hz, 1H), 7.39-7.31 (m, 5H), 5.04 (d, J = 12.6 Hz, 1H), 5.02 (d, J = 12.9 Hz, 1H), 4.02 (m, 1H), 3.83 (dd, J = 5.4, 2.5 Hz, 2H), 3.80 (dd, J = 17.8, 6.3 Hz, 1H), 3.72 (dd, J = 17.5, 5.6 Hz, 1H), 3.08 (t, J = 2.4 Hz, 1H), 2.51 (dt, J = 3.5, 1.7 Hz, 1H), 2.21-2.13 (m, 2H), 1.95-1.90 (m, 1H), 1.76-1.70 (m, 1H). ^{13}C NMR (176 MHz, DMSO-d_6): δ 172.4, 171.8, 171.6, 156.4, 137.4, 128.8, 128.26, 128.18, 81.7, 73.3, 65.9, 54.6, 41.1, 31.9, 28.3 ppm.



(8)

Triazole-PRO (8). An aqueous solution (3 mL) containing 300 mM propargylamine, 300 mM 6-azidohexanoic acid, 5 mM $CuSO_4$ and 50 mM sodium ascorbate was prepared. The reaction was incubated at 37°C for 24 hours and then lyophilized. ^{1}H NMR (700 MHz, D_2O): δ 8.00 (s, 1H), 4.36 (t, *J* = 6.9 Hz, 2H), 4.22 (s, 2H), 2.05 (t, *J* = 7.3 Hz, 2H), 1.82 (quint, *J* = 7.3 Hz, 2H), 1.45 (quint, *J* = 7.5 Hz, 2H), 1.13 (quint, *J* = 7.6 Hz, 2H) ppm. ^{13}C NMR (176 MHz, D2O): δ 183.4, 139.5, 125.2, 50.4, 37.1, 33.9, 28.9, 25.2, 24.9 ppm.



(9)

ZQG-Triazole-PRO (9). ZQG-PRO was resuspending in water to a final concentration of 150 mM, to which 150 mM 6-azidohexanoic acid, 2.5 mM $CuSO_4$ and 25 mM sodium ascorbate was also added and mixed by vortexing, and incubated for 24 hours at 37°C. A small, dark brown pellet was observed, and the supernatant was kept and transferred to a new microfuge tube. Ice-cold 300 µL water containing 1 % formic acid was added to the mixture. A white precipitate began to form after 15 minutes, and the solution was incubated on ice for 3 hours. The mixture was centrifuged once more, and the supernatant discarded. The pellet was left to dry and was subsequently analyzed by NMR and HPLC-MS. ^{1}H NMR (700 MHz, DMSO-d_6): δ 8.27 (t, J = 5.8 Hz, 1H), 8.19 (t, J = 5.7 Hz, 1H), 7.89 (s, 1H), 7.45 (d, J = 8.2 Hz, 1H), 7.37-7.30 (m, 5H), 5.02 (br, 2H), 4.28 (m, 4H), 4.02 (m, 1H), 3.80 (dd, J = 17.5, 7 Hz, 1H), 3.71 (dd, J = 17.5, 7 Hz, 1H), 2.23-2.15 (m, 4H), 1.97-1.92 (m, 1H), 1.80-1.71 (m, 3H), 1.51 (quint, J = 7.5 Hz, 2H), 1.26-1.21 (br, m, 2H). ^{13}C NMR (176 MHz, DMSO-d_6): δ 174.8, 172.4, 171.9, 171.6, 156.4, 145.3, 137.4, 128.8, 128.24, 128.16, 123.1, 65.9, 54.6, 49.6, 41.1, 34.7, 33.9, 32.1, 29.9, 28.4, 25.9, 24.3 ppm.

Nuclear Magnetic Resonance

Compounds **5** and **8** were dissolved in D_2O, and **4**, **6**, **7** and **9** in DMSO-d_6. ^{1}H and ^{13}C NMR spectra were obtained with Bruker Avance II 700 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Coupling constants are reported in Hertz (Hz).

Table S1: Relative activity of MTG in the presence of CuAAC reagents

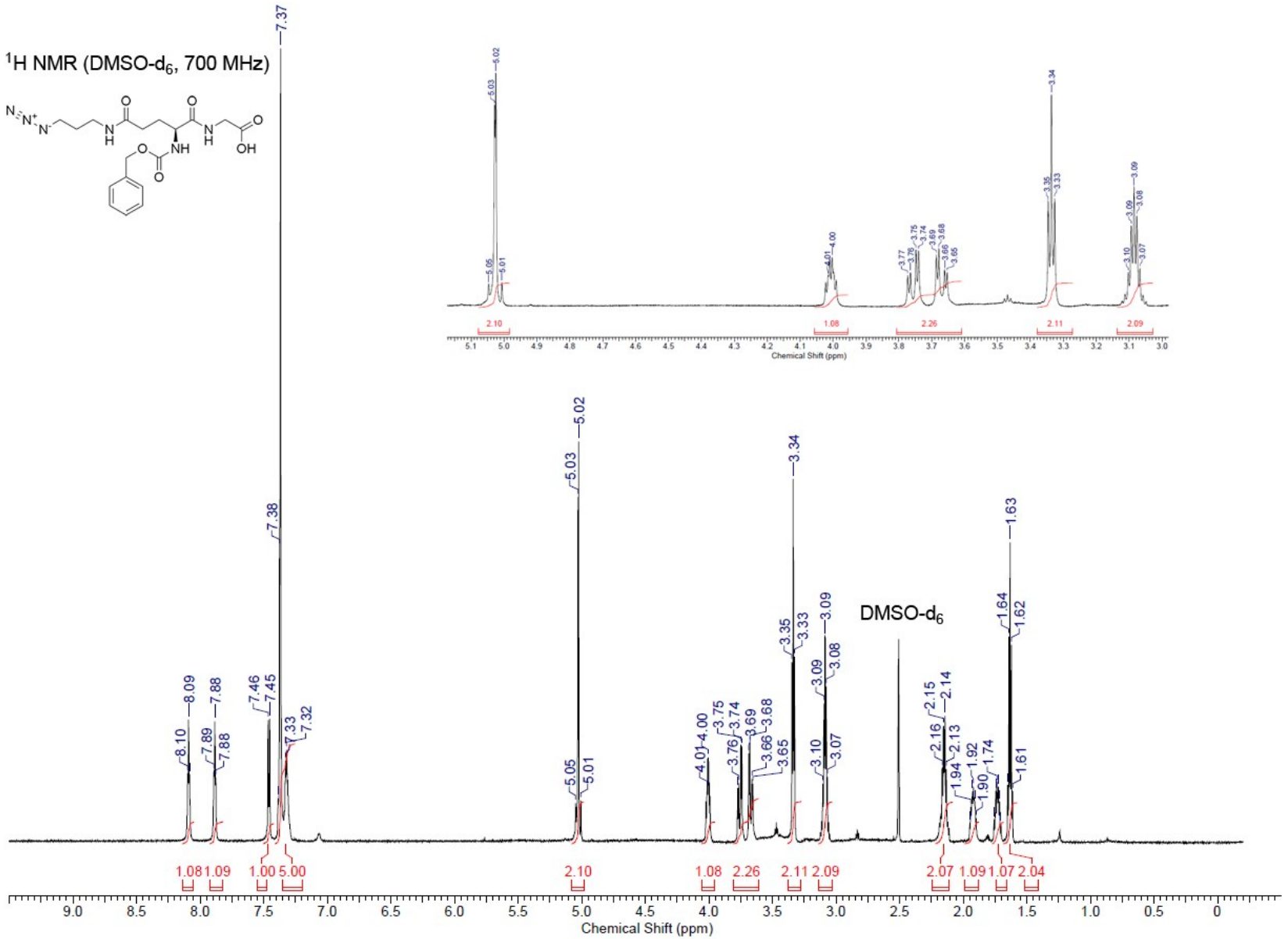
Components	Relative Activity (%)
200 mM Tris-Acetate pH 7 30 mM ZQG 100 mM hydroxylamine	100
200 mM Tris-Acetate pH 7 30 mM ZQG 100 mM hydroxylamine **+ 25 mM sodium ascorbate**	55
200 mM Tris-Acetate pH 7 30 mM ZQG 100 mM hydroxylamine **+ 2.5 mM CuSO4**	22
200 mM Tris-Acetate pH 7 30 mM ZQG 100 mM hydroxylamine **+ 25 mM sodium ascorbate, 2.5 mM CuSO4**	14
200 mM Tris-Acetate pH 7 30 mM ZQG 100 mM hydroxylamine **+ 25 mM sodium ascorbate, 2.5 mM CuSO4, 10 mM glutathione**	276

Activities were measured using the hydroxamate assay and were done in triplicate. A control reaction for each set of conditions was done containing all reaction components, excluding MTG, and subtracted from the reactions containing MTG.

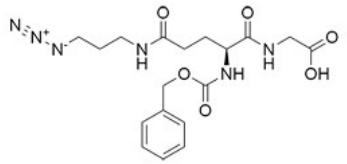
Table S2: Triazole product formation in the absence or presence of glutathione

Sample	Product formation (%)
APA Control	82.7 %
APA Reactions	94.7 % ± 1.3
PRO Control	75.7 %
PRO Reactions	86.9 ± 6.3

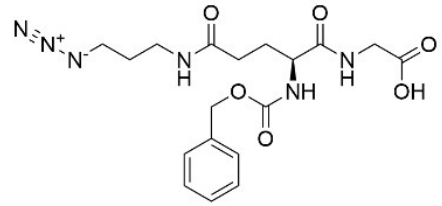
Reactions were prepared in 300 µL volumes. Each reaction contained 30 mM azidopropylamine (APA reactions) or 30 mM propargylamine (PRO reactions), 30 mM 4-pentynoic acid (APA reactions) or 30 mM 6-azidohexanoic acid (PRO reactions), 2.5 mM $CuSO_4$, 25 mM Na^+ ascorbate in 200 mM Tris acetate buffer, pH 7.5. The reactions contained 5 mM glutathione, and the control contained no glutathione; it was substituted by an equivalent volume of buffer. The reactions were done in triplicate, and the control was done as a single reaction. After 24hr incubation time at 37°C, aliquots were taken for LC-MS analysis, and the product formation quantified according to the appearance of the mass of the respective triazole product.

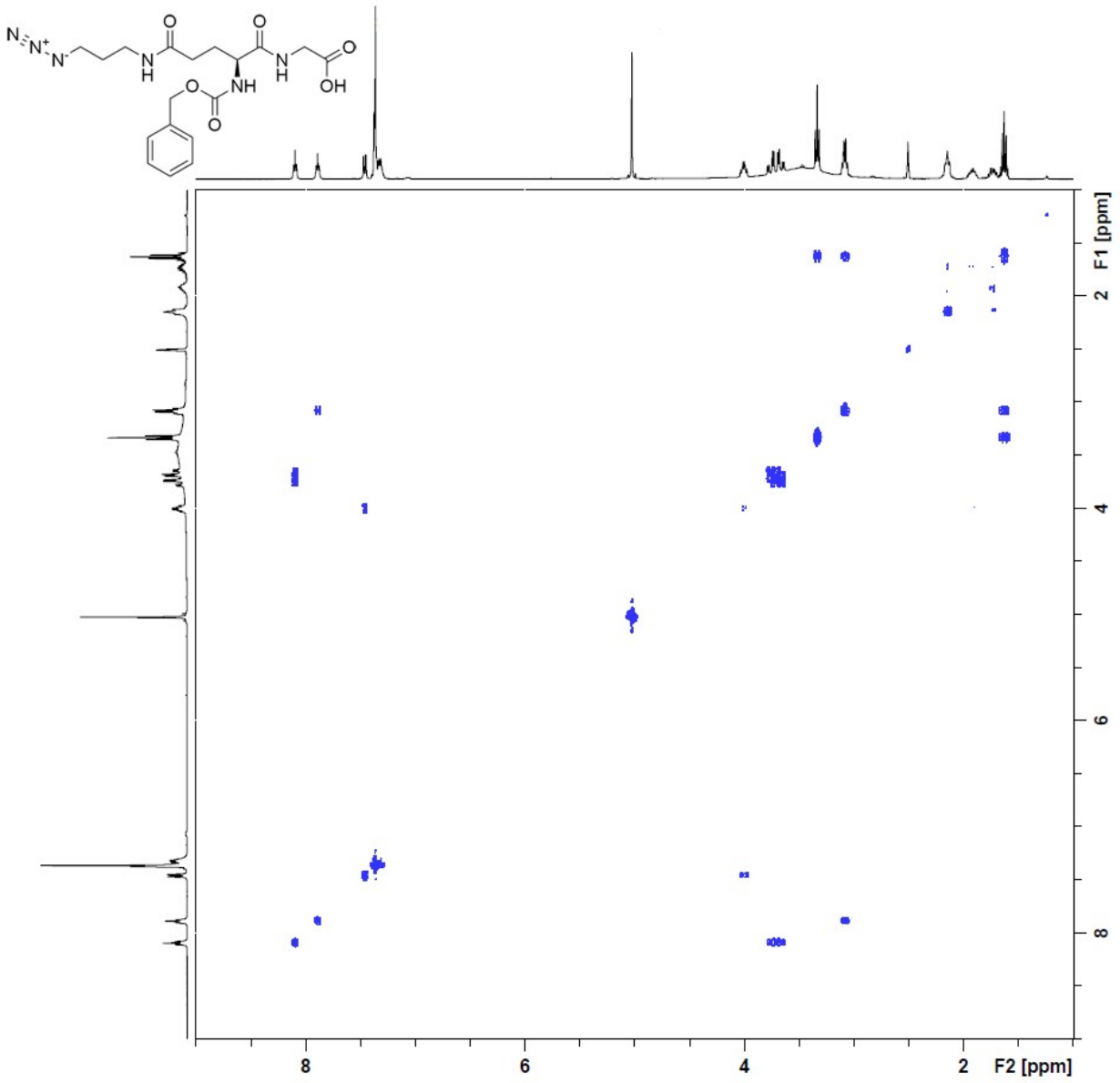

^{1}H NMR (DMSO-d$_6$, 700 MHz)
DMSO-d$_6$
Chemical Shift (ppm)

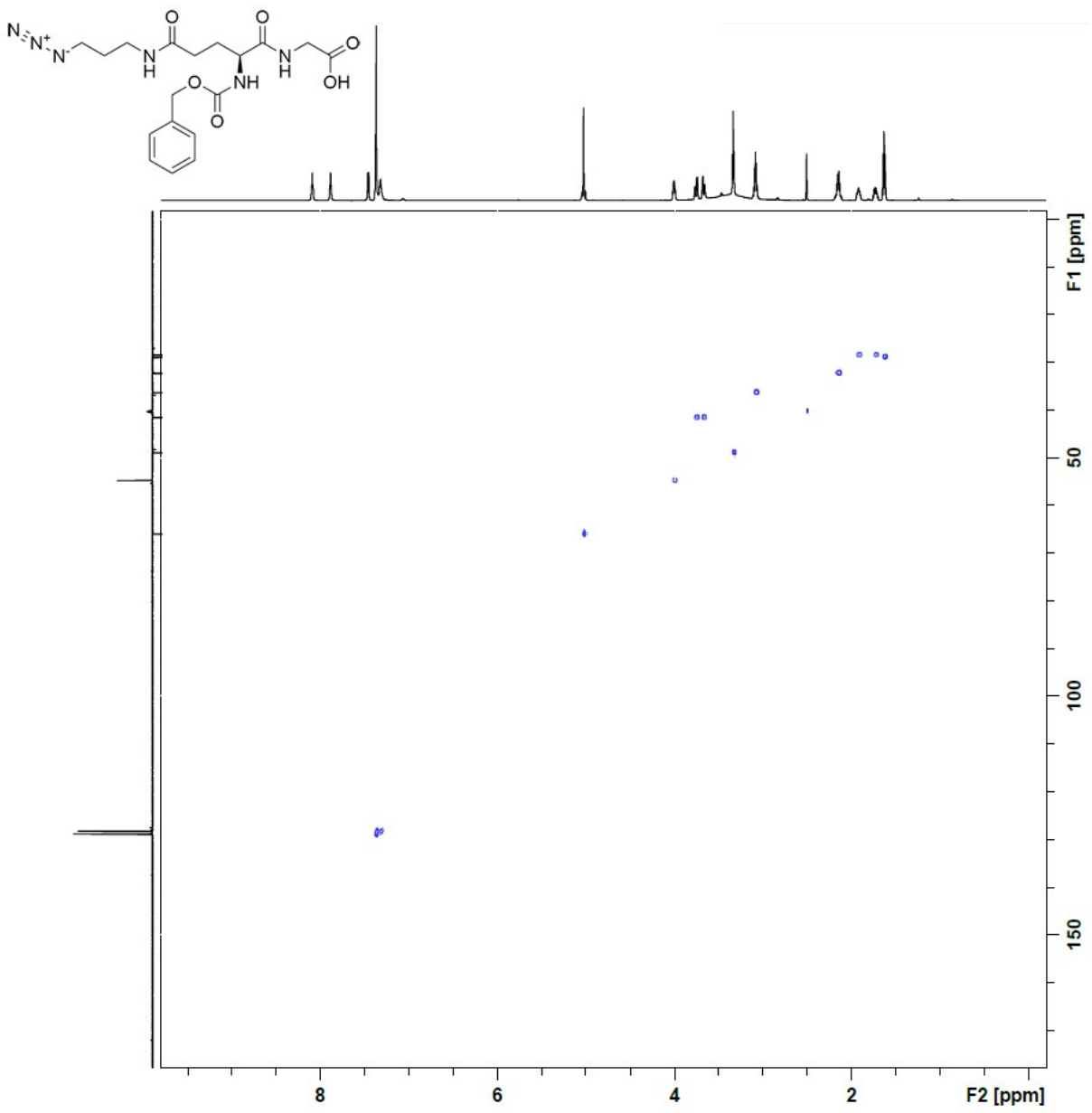
^{13}C NMR (DMSO-d_6, 176 MHz)



DEPT NMR (DMSO-d_6 , 176 MHz)




F1 [ppm]
2
4
6
8
F2 [ppm]
8
6
4
2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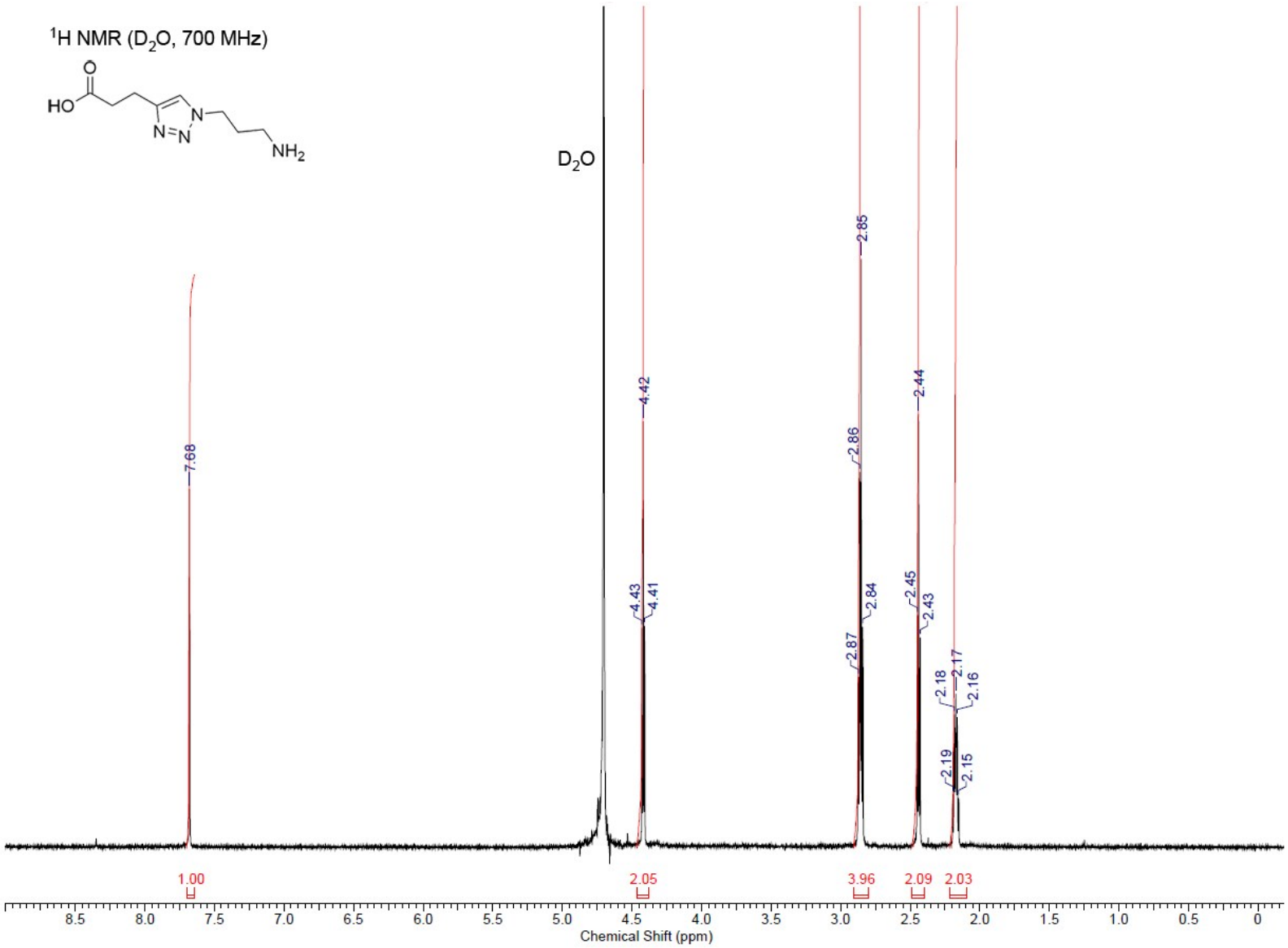
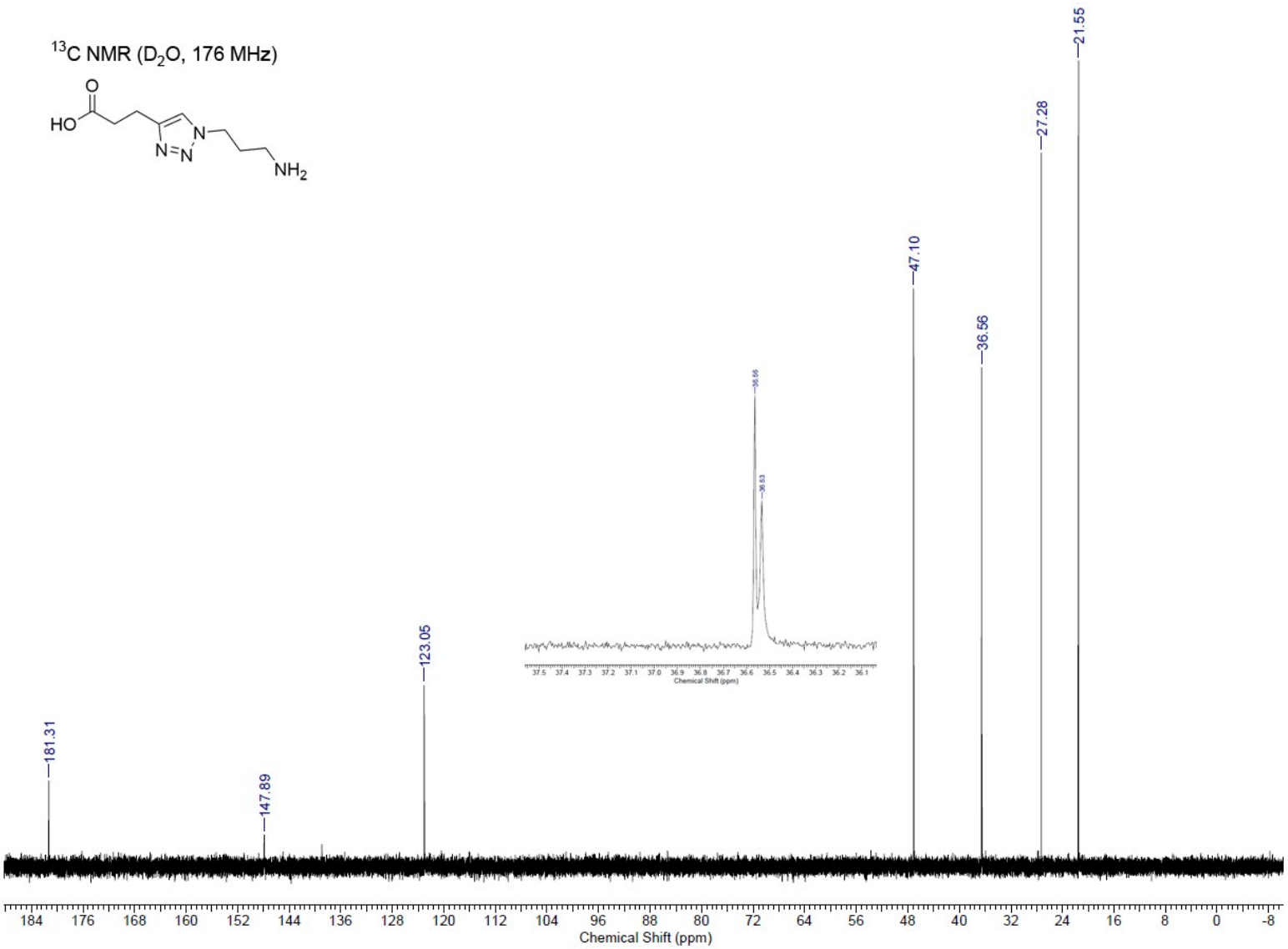
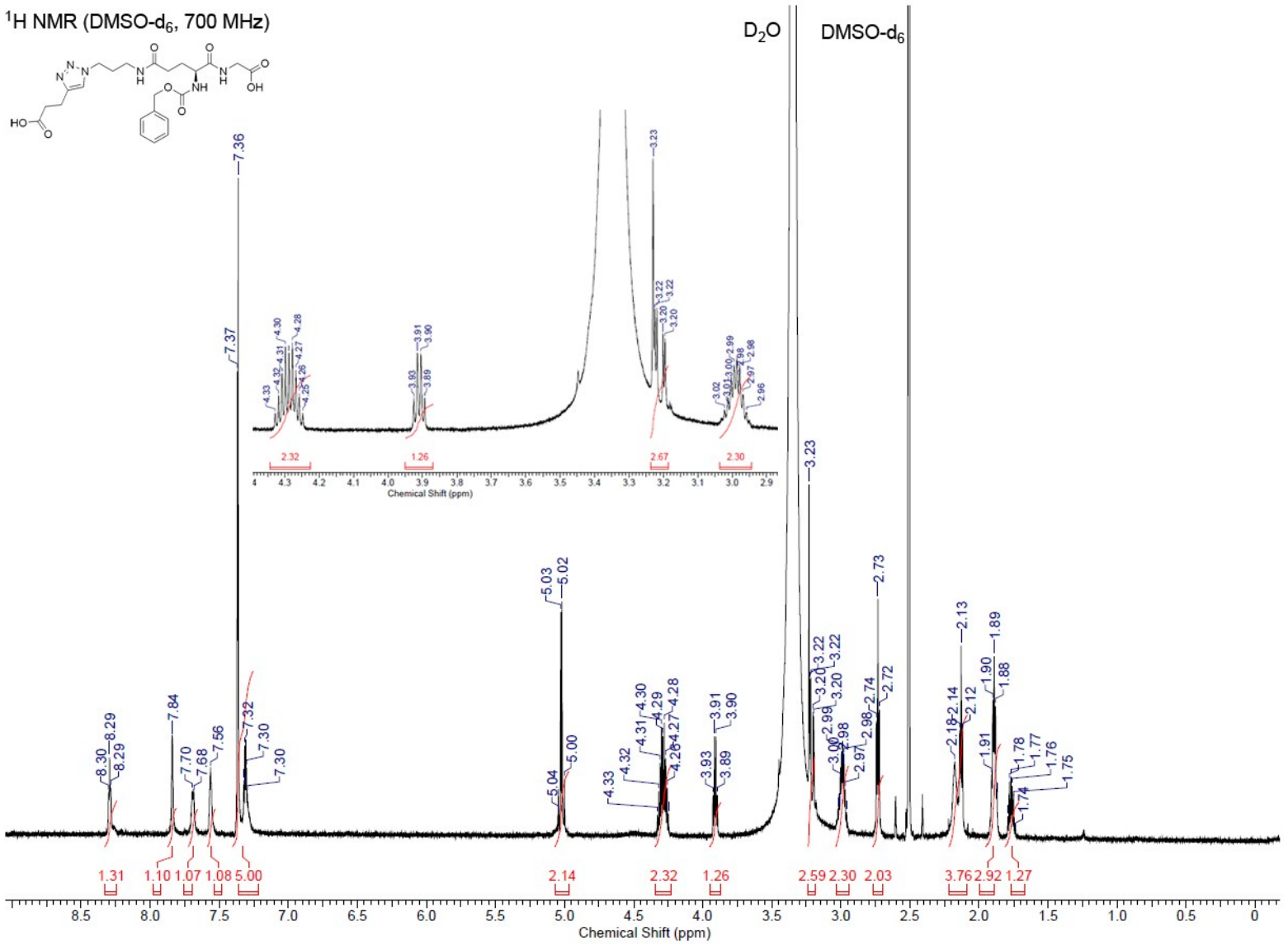
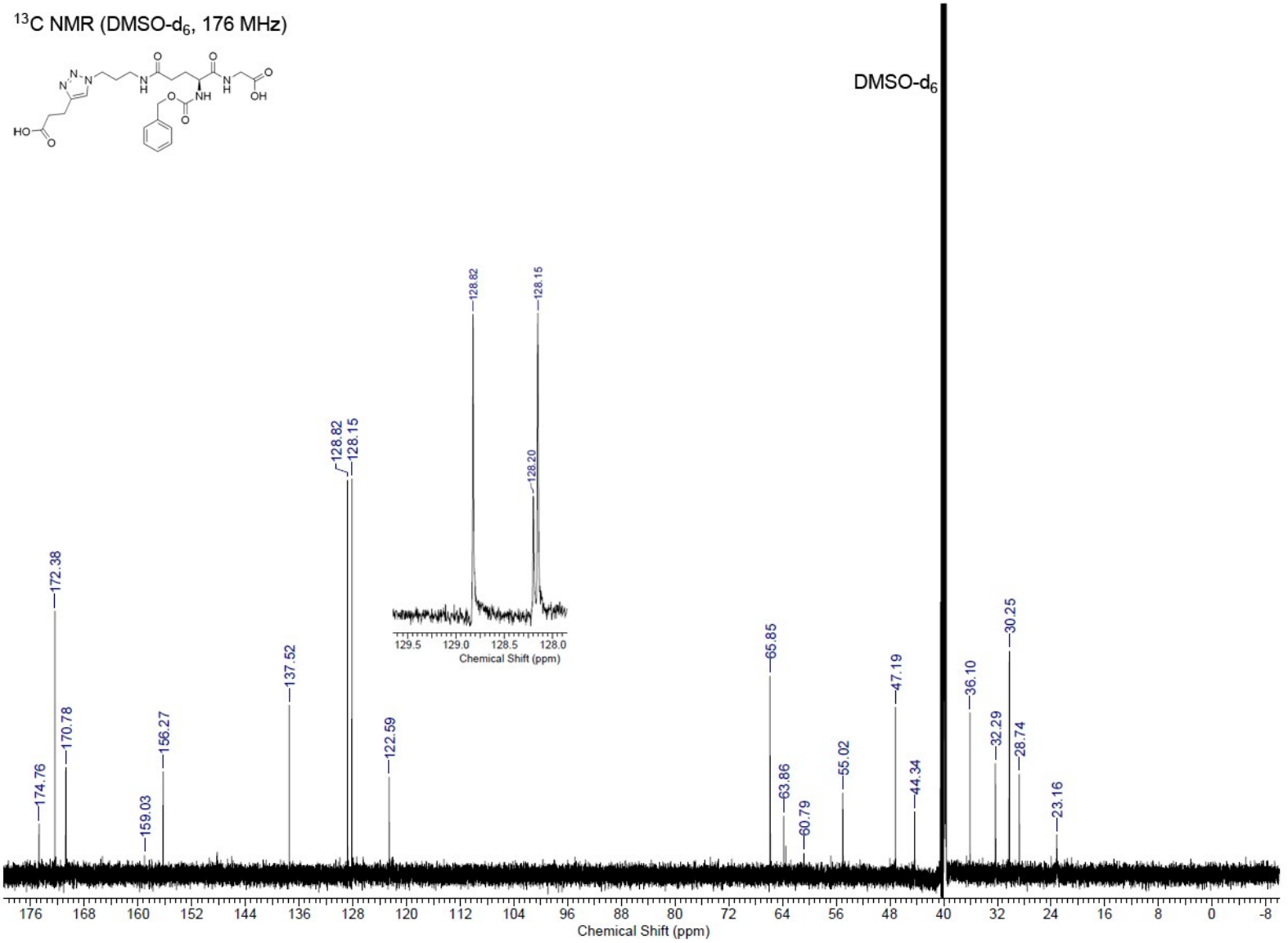
50
100
150
8
6
4
2
F2 [ppm]

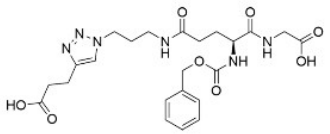

^{1}H NMR (D_2O, 700 MHz)
HO
O
N
N=N
NH$_2$
D_2O
7.68
4.43
4.42
4.41
2.87
2.86
2.85
2.84
2.45
2.44
2.43
2.19
2.18
2.17
2.16
2.15
1.00
2.05
3.96
2.09
2.03
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)

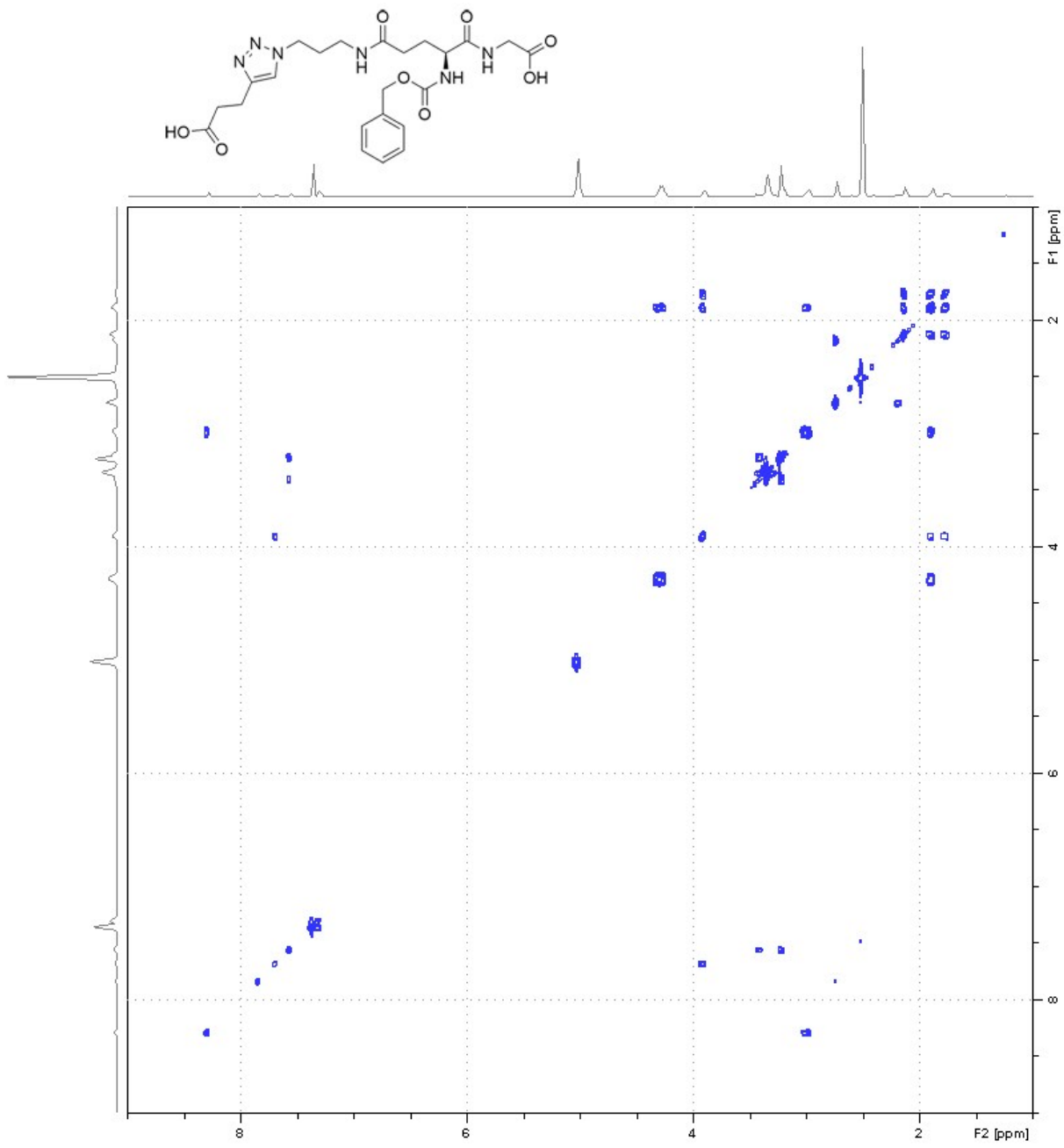

^{13}C NMR (D_2O, 176 MHz)
HO
O
N
N=N
NH2
181.31
147.89
123.05
47.10
36.56
27.28
21.55
Chemical Shift (ppm)

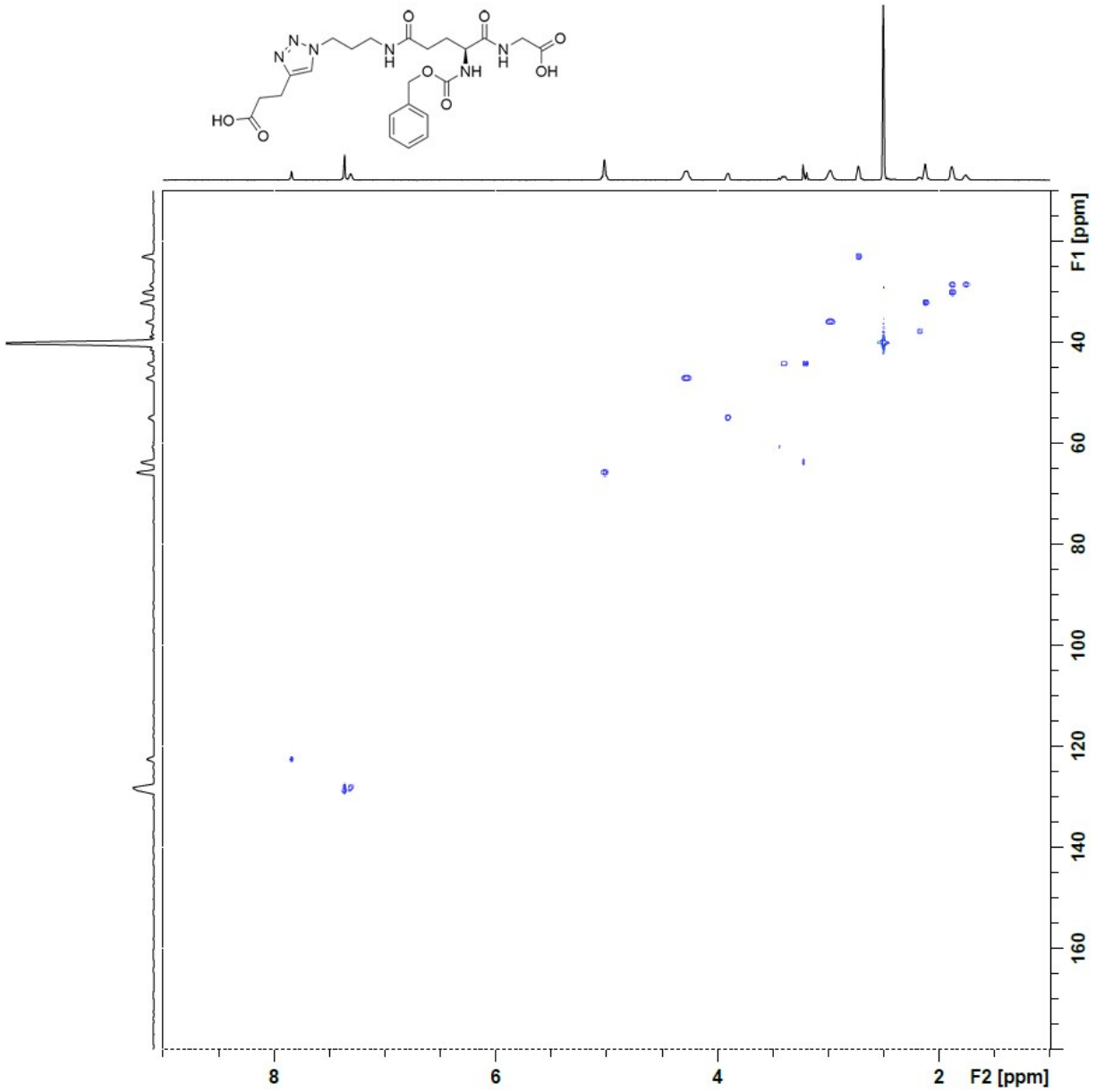

^{1}H NMR (DMSO-d$_6$, 700 MHz)
D$_2$O
DMSO-d$_6$
Chemical Shift (ppm)

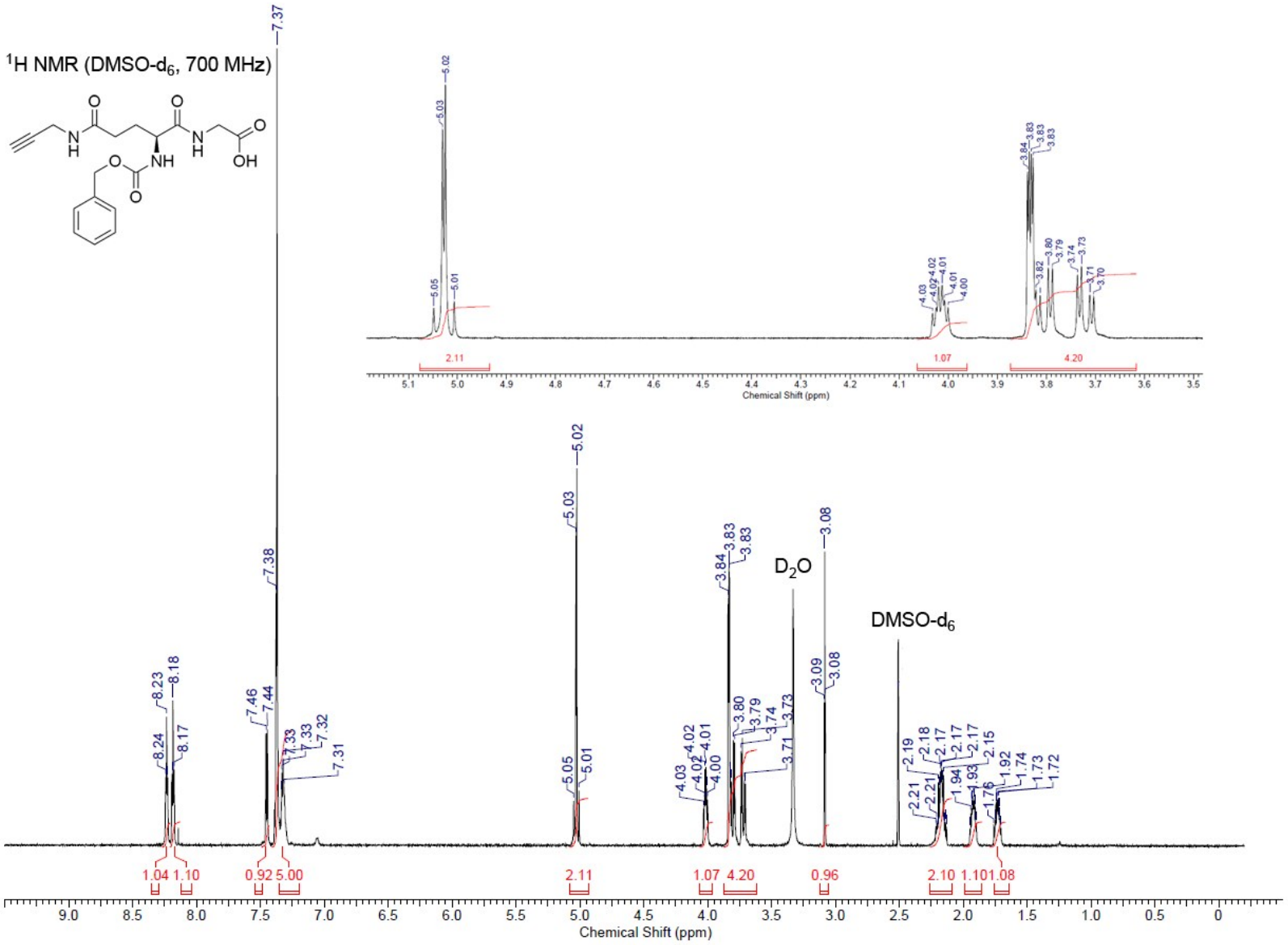

13C NMR (DMSO-d6, 176 MHz)
DMSO-d6
128.82
128.15
128.20
129.5
129.0
128.5
128.0
Chemical Shift (ppm)
172.38
170.78
174.76
159.03
156.27
137.52
128.82
128.15
122.59
65.85
63.86
60.79
55.02
47.19
44.34
36.10
32.29
30.25
28.74
23.16
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
-8
Chemical Shift (ppm)

DEPT NMR (DMSO-d_6, 176 MHz)

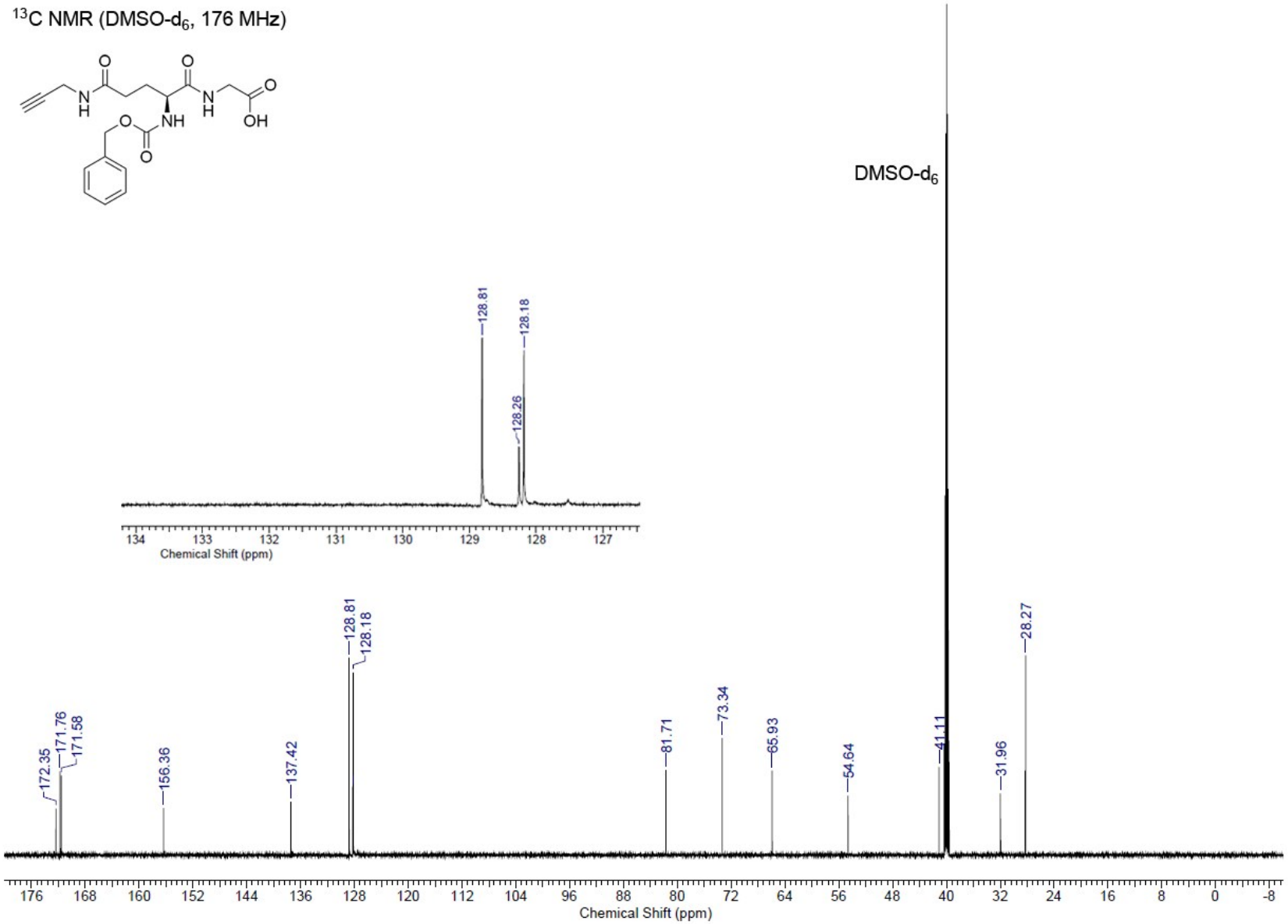


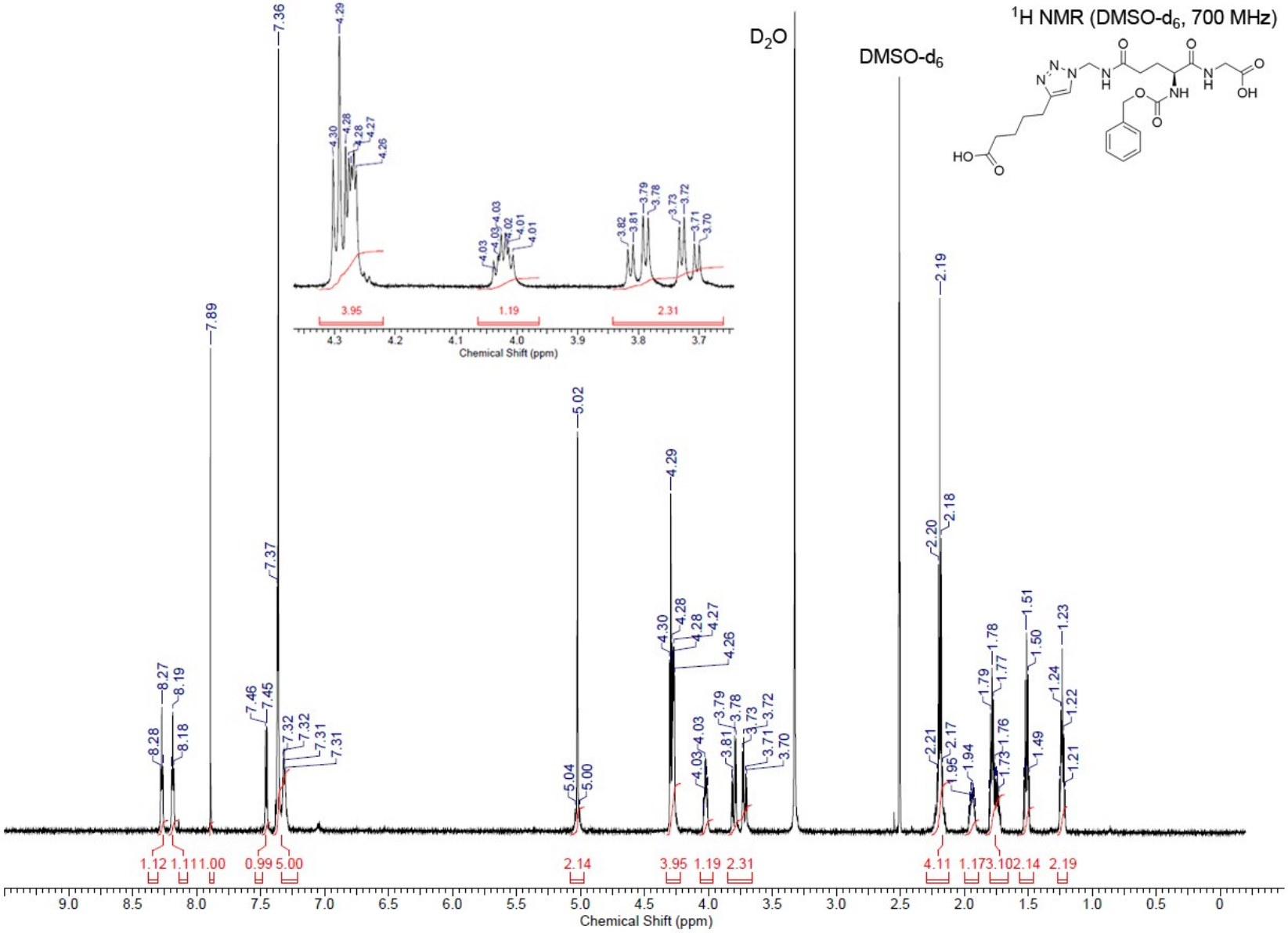

HO
O
N
N
N
H
O
NH
O
O
N
H
O
OH
F1 [ppm]
2
4
6
8
F2 [ppm]
8
6
4
2

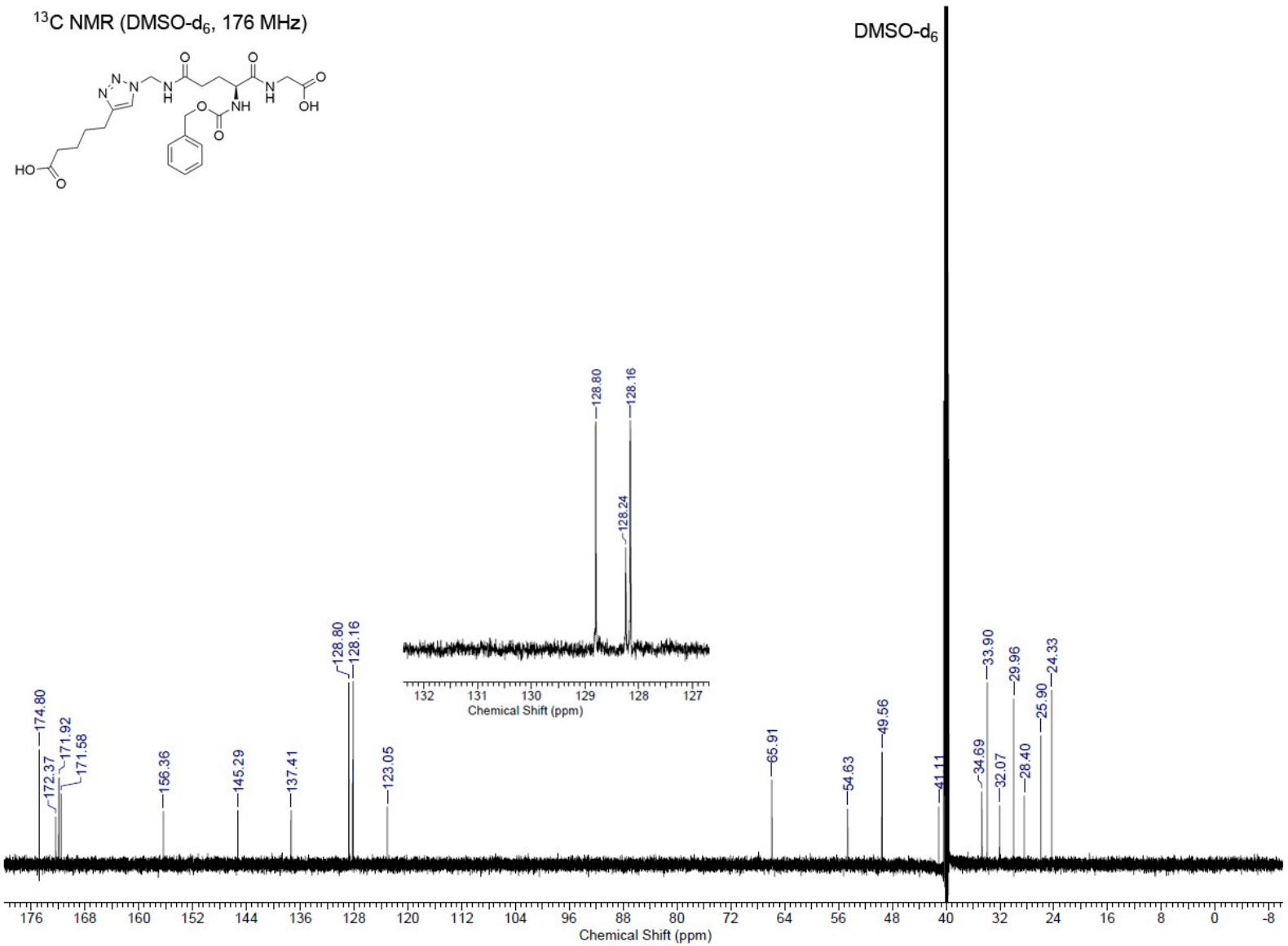

F1 [ppm]
40
60
80
100
120
140
160
8
6
4
2
F2 [ppm]

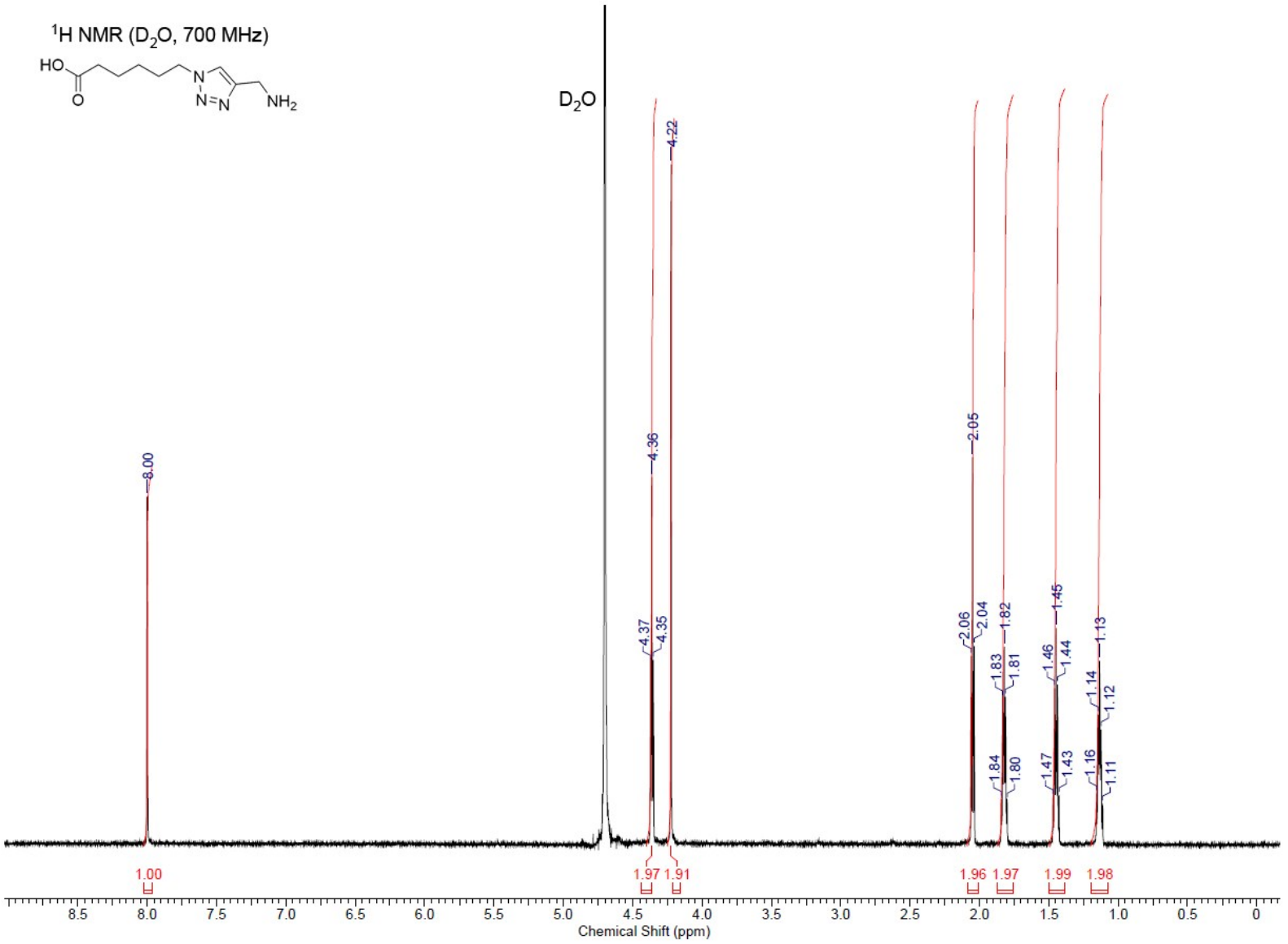

^{1}H NMR (DMSO-d$_6$, 700 MHz)
D$_2$O
DMSO-d$_6$
Chemical Shift (ppm)

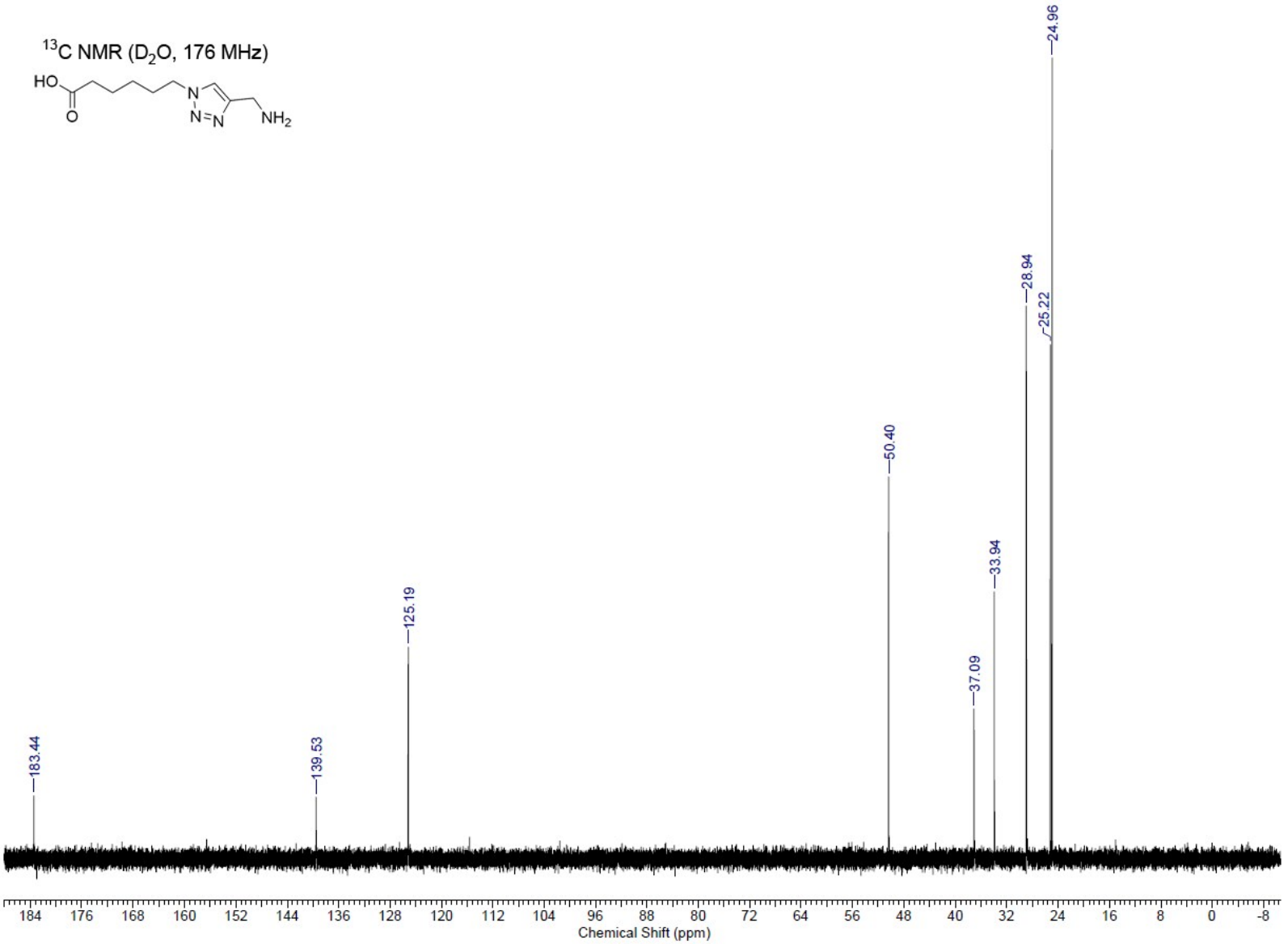
^{13}C NMR (DMSO-d_6, 176 MHz)

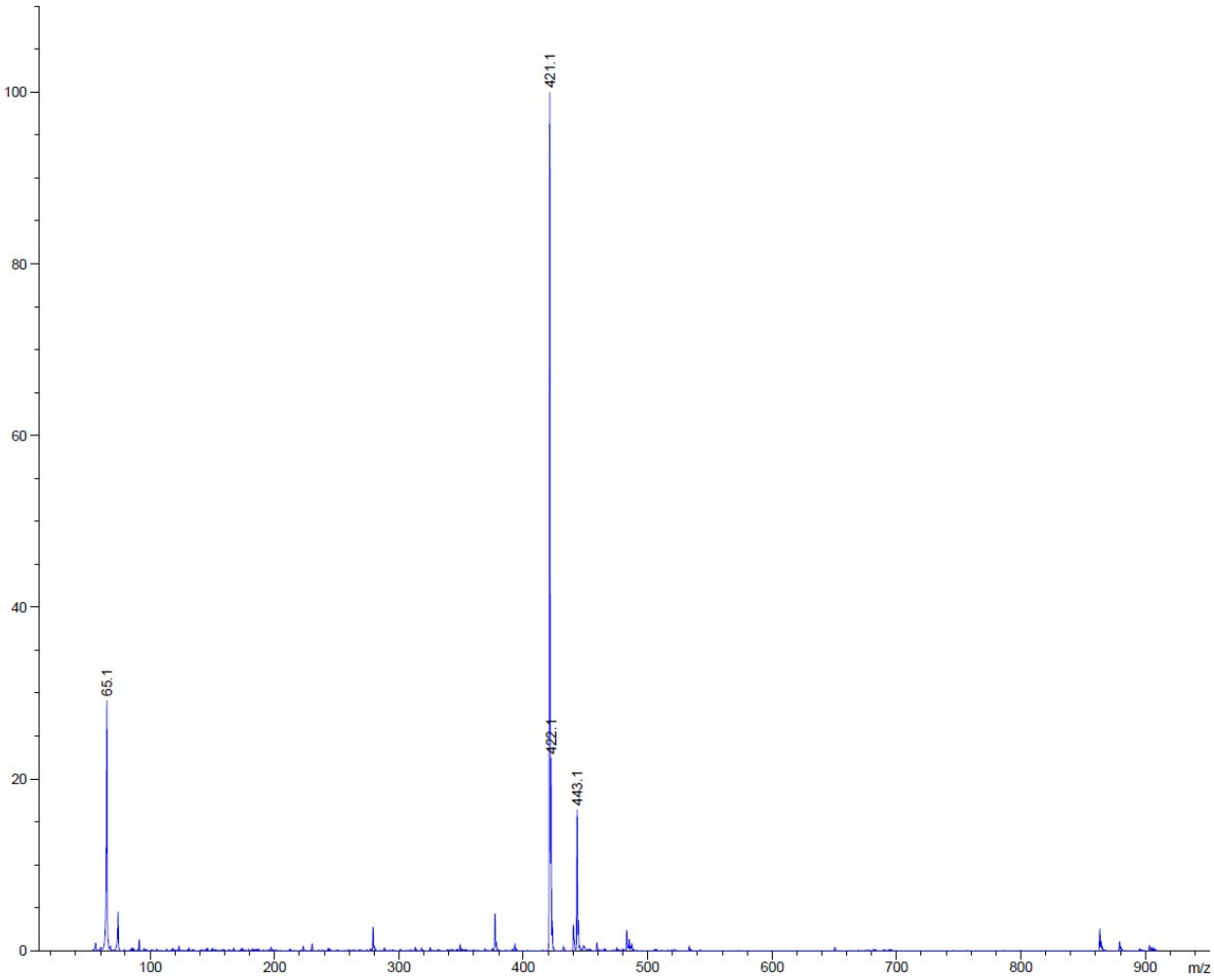


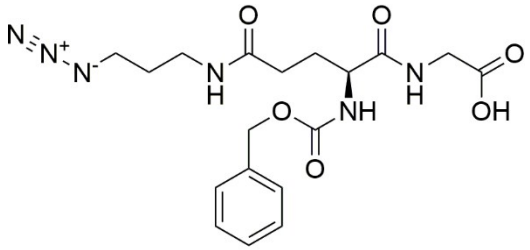

^{1}H NMR (DMSO-d$_6$, 700 MHz)
D$_2$O
DMSO-d$_6$
Chemical Shift (ppm)


13C NMR (DMSO-d6, 176 MHz)
DMSO-d6
174.80
172.37
171.92
171.58
156.36
145.29
137.41
128.80
128.16
123.05
65.91
54.63
49.56
41.11
34.69
33.90
32.07
29.96
28.40
25.90
24.33
128.80
128.16
128.24
Chemical Shift (ppm)
Chemical Shift (ppm)

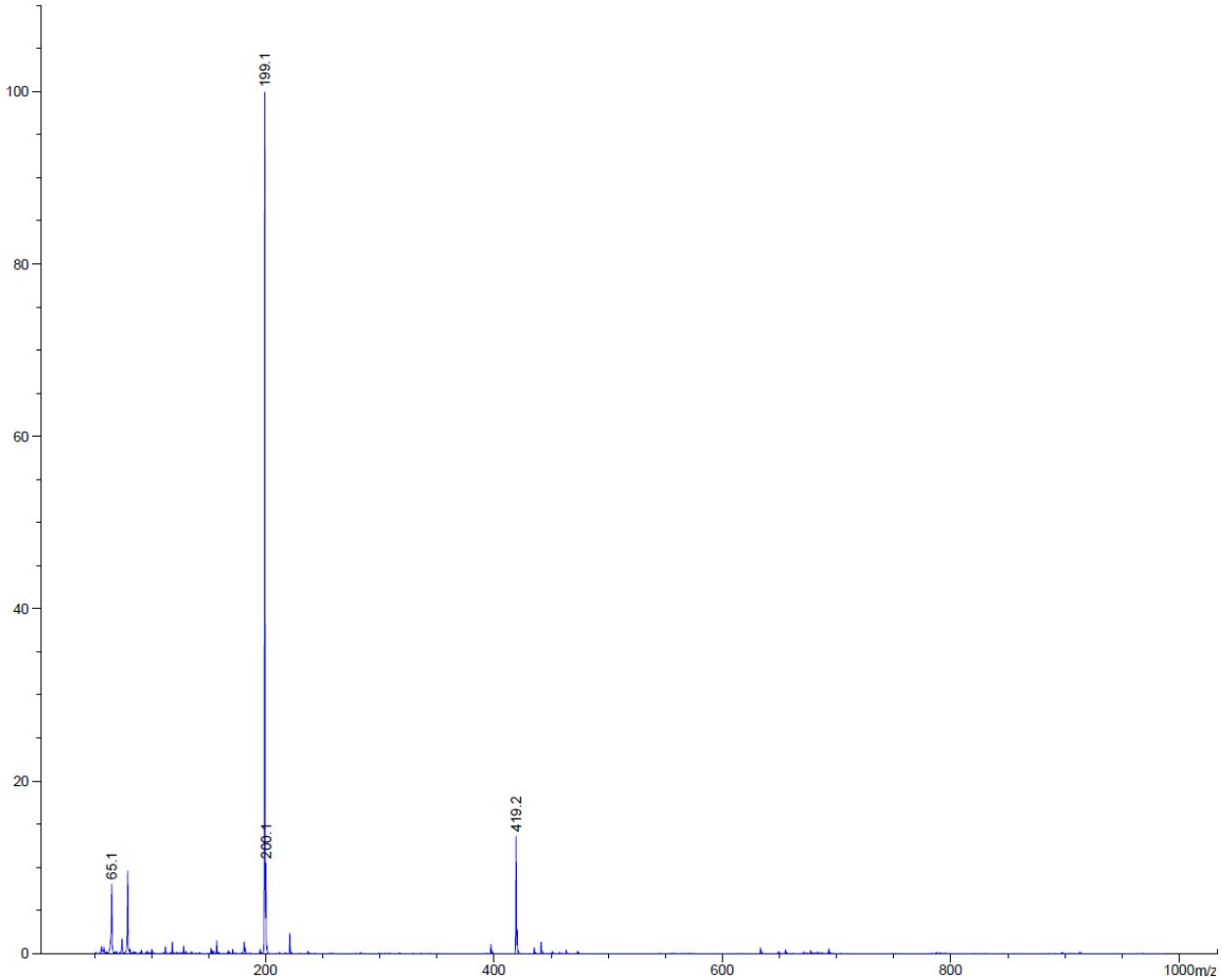

^{1}H NMR (D_2O, 700 MHz)
HO
O
N
N=N
NH$_2$
D_2O
8.00
4.36
4.22
4.37
4.35
2.05
2.06
2.04
1.82
1.83
1.81
1.45
1.46
1.44
1.13
1.14
1.12
1.84
1.80
1.47
1.43
1.16
1.11
1.00
1.97
1.91
1.96
1.97
1.99
1.98
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)


^{13}C NMR (D_2O, 176 MHz)
HO
O
N
N=N
NH2
24.96
28.94
25.22
50.40
33.94
37.09
125.19
139.53
183.44
184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0 -8
Chemical Shift (ppm)

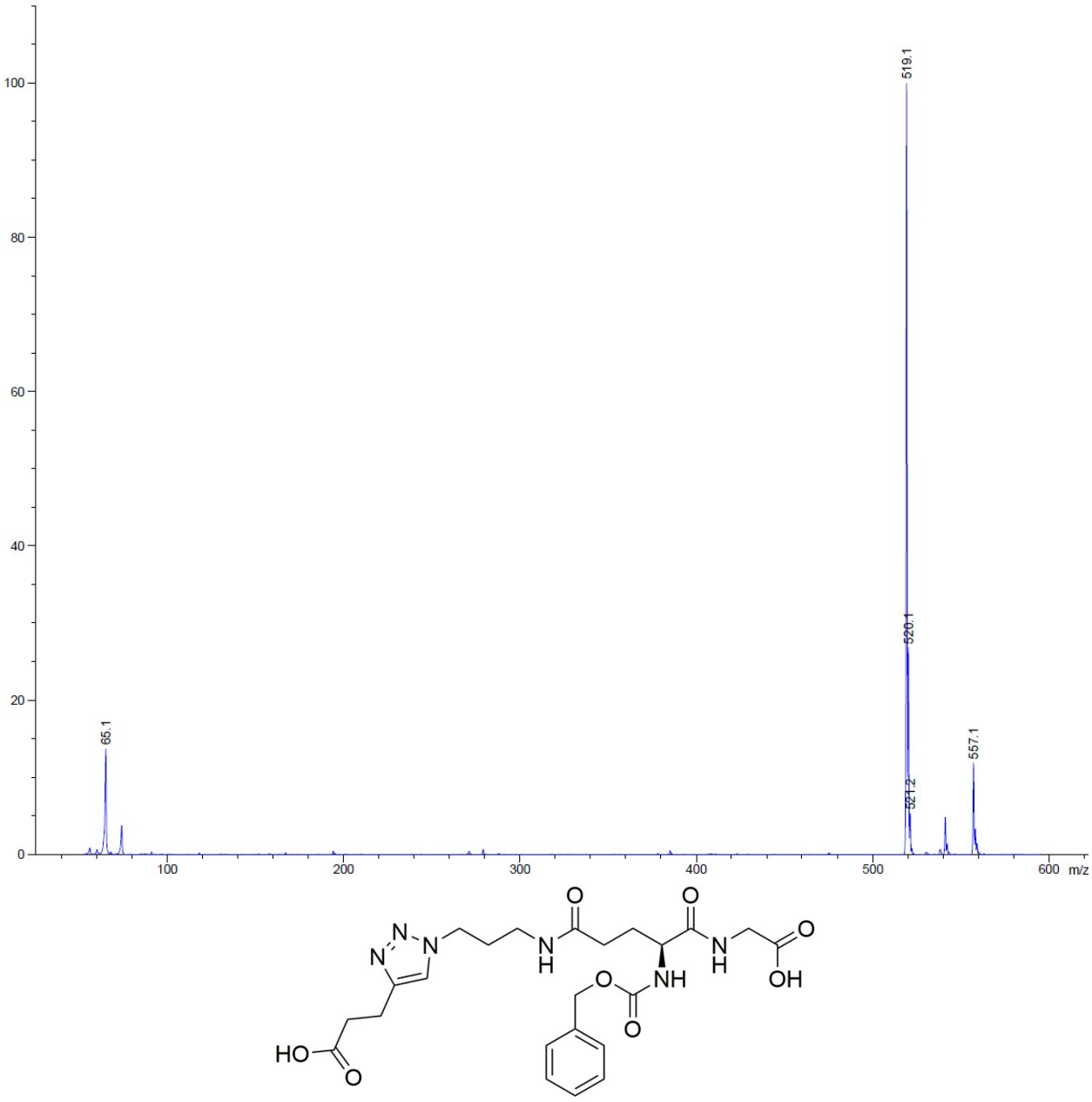




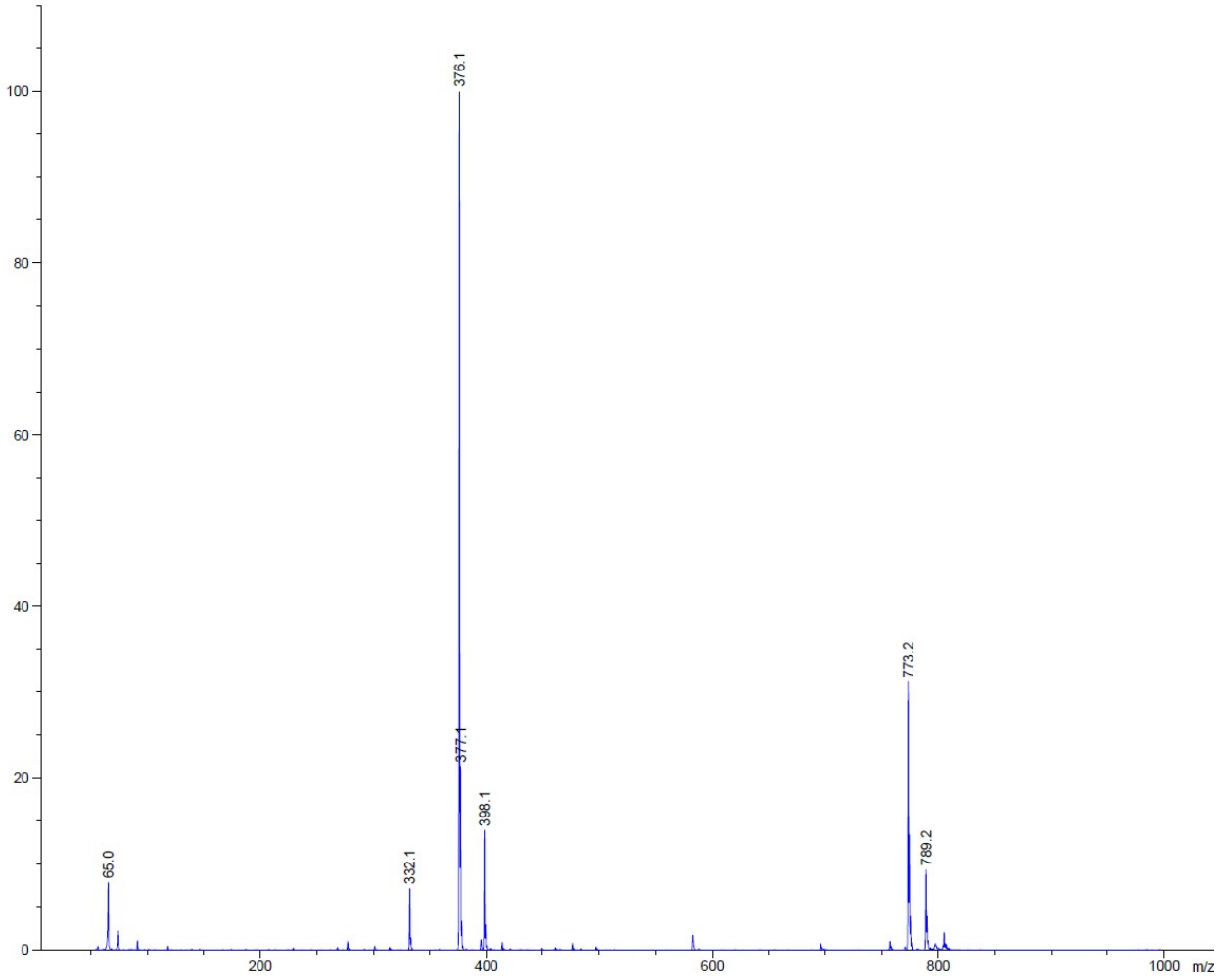
LRMS (ESI) m/z calculated for $C_{18}H_{24}N_6O_6$ $[M+H]^+$, 421.18; found: 421.1.

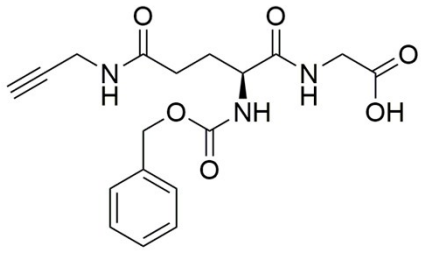


LRMS (ESI) m/z calculated for $C_8H_{14}N_4O_2$ $[M+H]^+$, 199.12; found: 199.1.

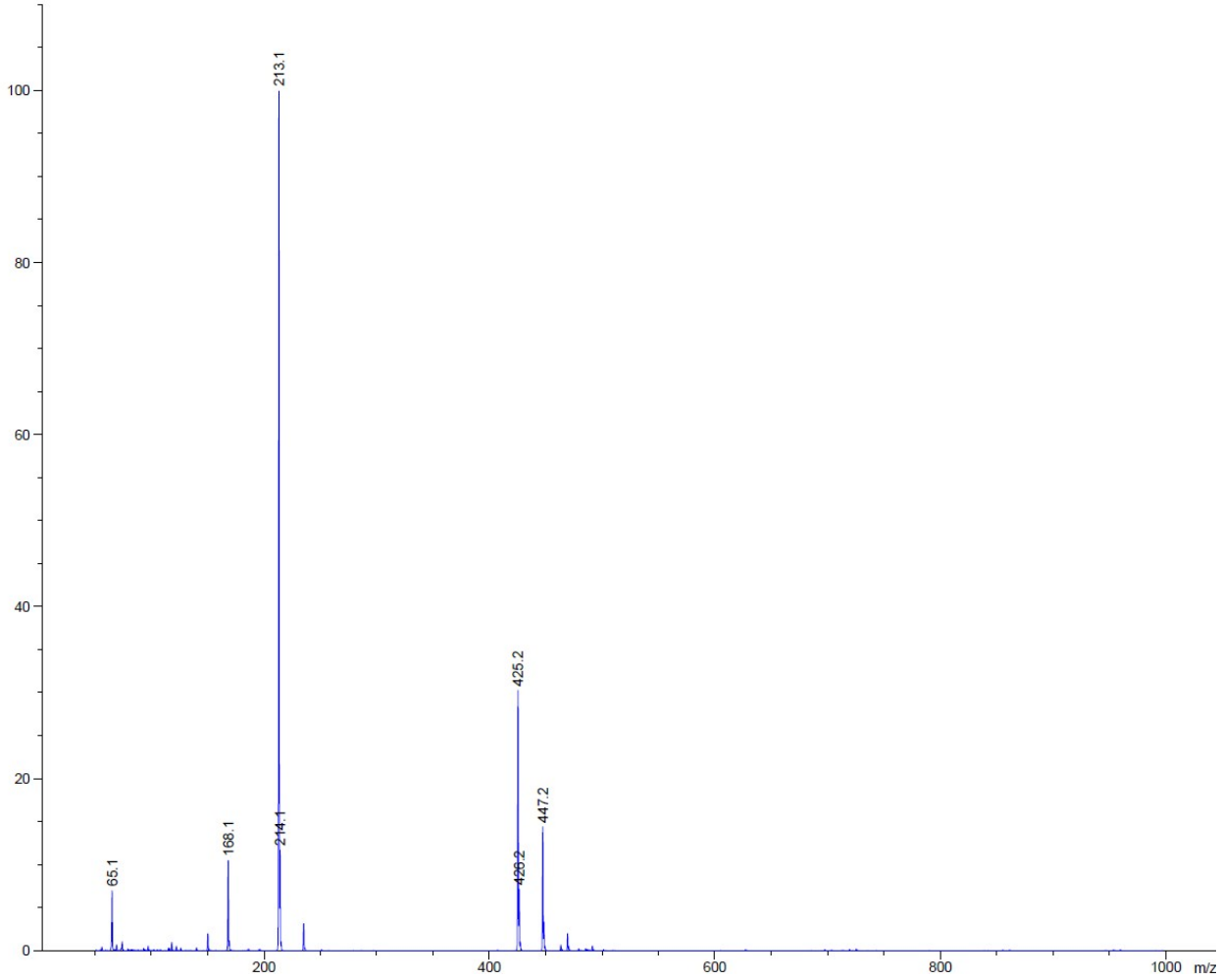


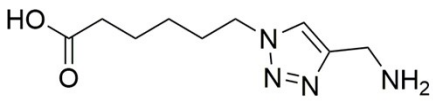
LRMS (ESI) m/z calculated for $C_{23}H_{30}N_6O_8$ $[M+H]^+$, 519.22; found: 519.1.



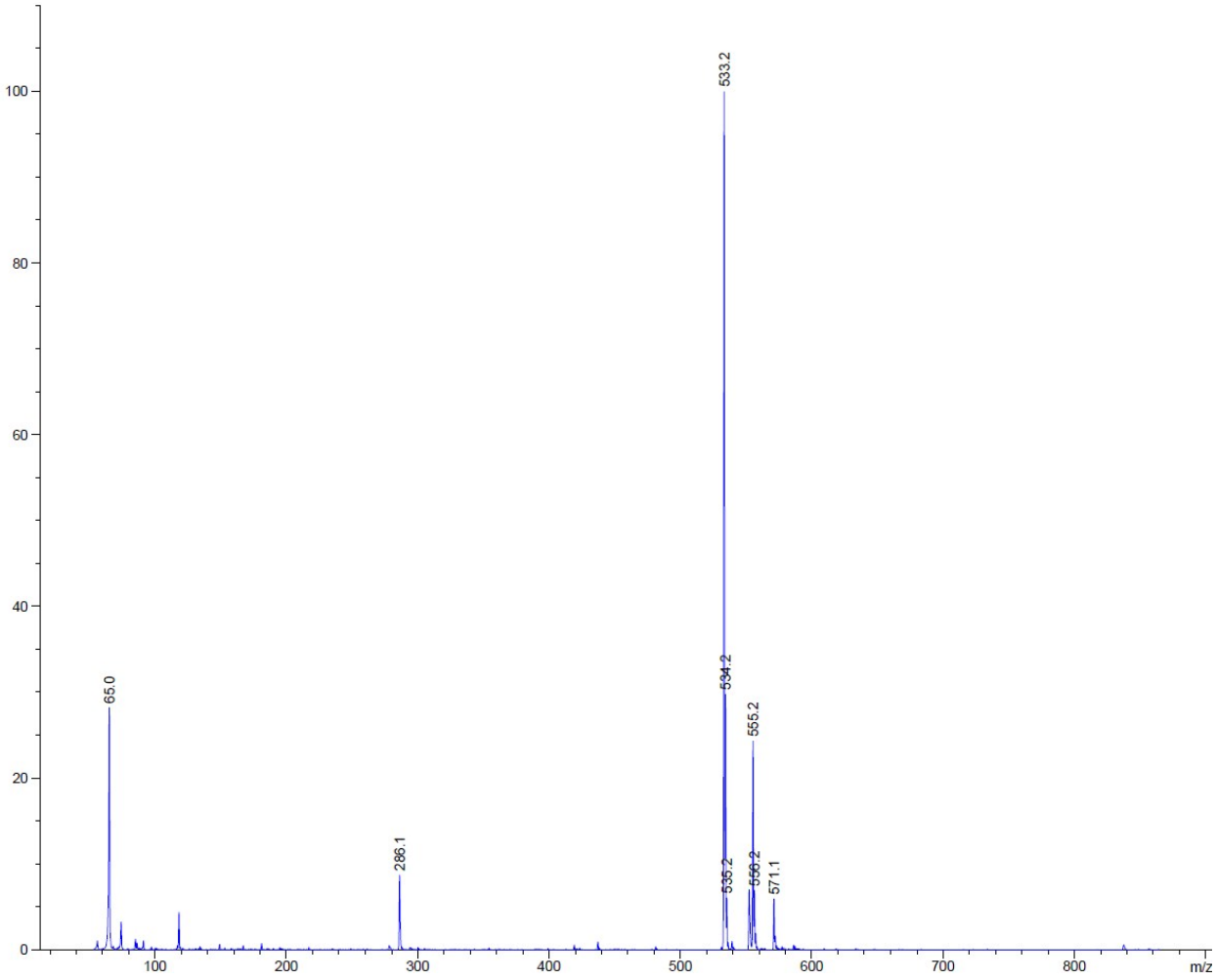


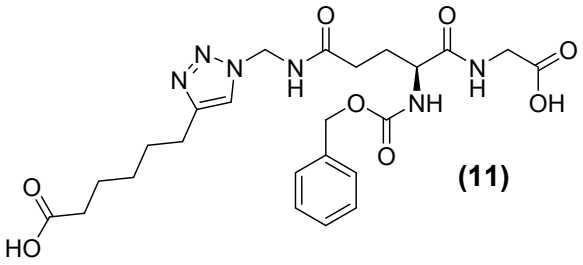
LRMS (ESI) m/z calculated for $C_{18}H_{21}N_4O_2$ $[M+H]^+$, 376.15; found: 376.1.





LRMS (ESI) m/z calculated for $C_4H_{26}N_4O_2$ $[M+H]^+$, 213.13; found: 213.1.





(11)

LRMS (ESI) m/z calculated for $C_{24}H_{32}N_6O_8$ $[M+H]^+$, 533.23; found: 533.2.

References

[1] Marx CK, Hertel TC, Pietzsch M **(2007)** Soluble expression of a pro-transglutaminase from Streptomyces mobaranesis in Escherichia coli. *Enzyme Microb Technol* 40:1543-1550

[2] Park KD, Morieux P, Salomé C, Cotten SW, Reamtong O, Eyers C, Gaskell SJ, Stables JP, Liu R, Kohn H **(2009)** Lacosamide isothiocyanate-based agents: novel agents to target and identify lacosamide receptors. *J Med Chem* 52:6897–6911

[3] Gundersen, MT, Keillor, JW, Pelletier, JN. Microbial transglutaminase displays broad acyl-acceptor substrate specificity. Appl Microbiol Biot. 98:219-230

[4] Schägger,H. and Jagow,G.V. **(1987)** *Anal. Biochem*., 166:368–379

[5] Folk JE, Cole PW **(1966)** Transglutaminase: mechanistic features of the active site as determined by kinetic and inhibitor studies. Biochim Biophys Acta Enzymol Biol Oxid 122:244–264