Rational Design of Right-Handed Heterogeneous Peptidomimetics as Inhibitors of Protein−Protein Interactions

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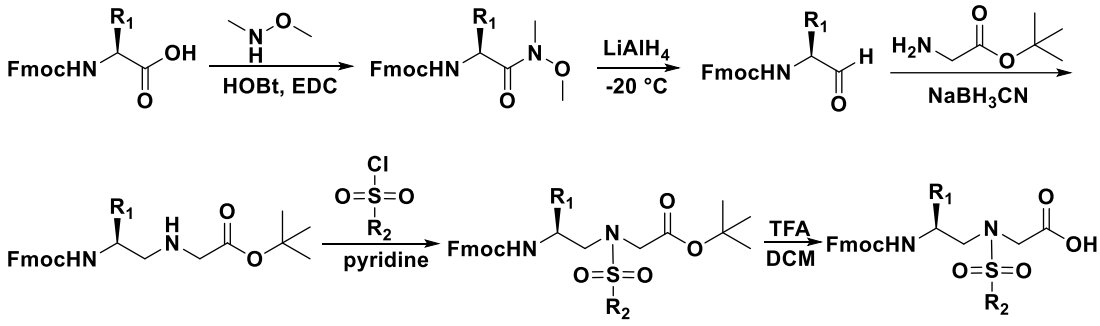
Contents

1. The Preparation of Sulfono-γ-AA Building Blocks

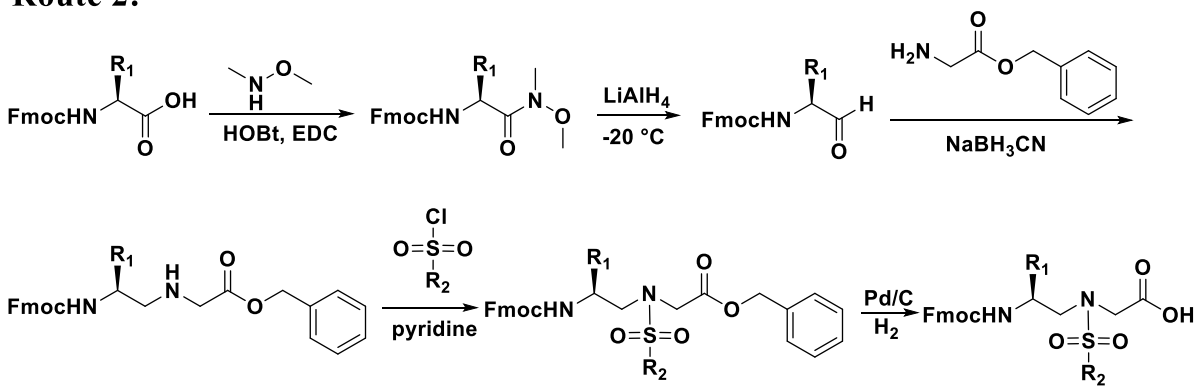
1.1 Synthetic Routes

The synthesis of the sulfono-γ-AA building blocks was based on previously report.[1] As shown in Scheme S1 the initial starting materials were Fmoc-protected amino acids. Sulfono-γ-AA Building Block **4-10, 15** was synthesized based on route 1, sulfono-γ-AA building block **1-3, 11-14** was synthesized based on route 2.

Route 1:



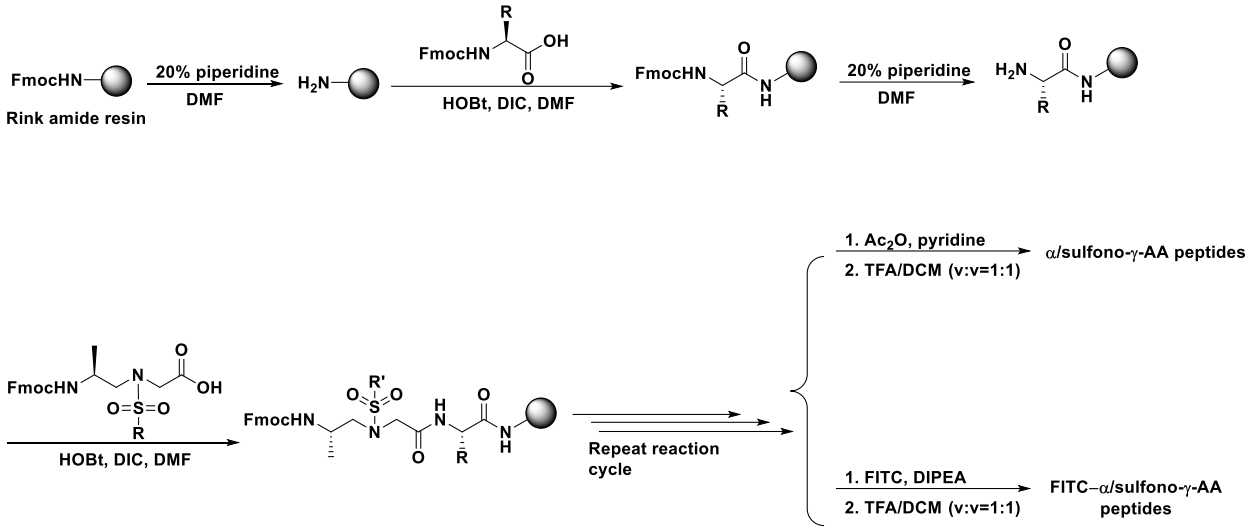
Route 2:



Scheme S1. The synthetic route of the Sulfono-γ-AA Peptides.

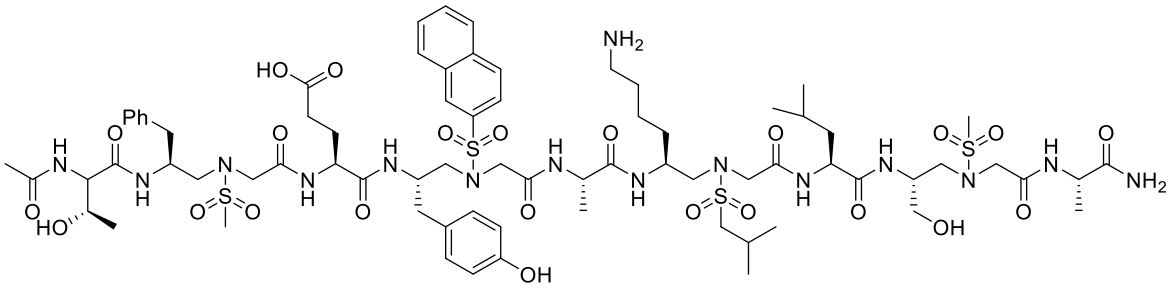
2. Preparation of α/sulfono-γ-AA Peptide

2.1 Synthetic route of the α/sulfono-γ-AA Peptides



Scheme S2. The synthetic route of the (FITC)-α/ sulfono-γ-AA Peptides.

2.2 HPLC Trace of the (FITC)-α/ sulfono-γ-AA Peptides

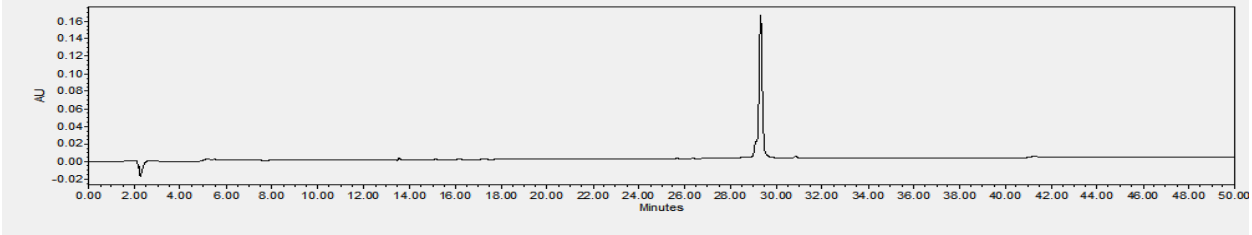


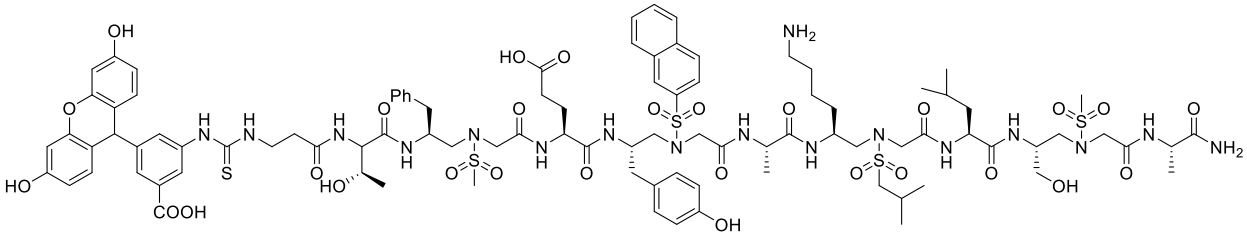
1

Chemical Formula: $C_{74}H_{113}N_{15}O_{23}S_4$

Exact Mass: 1707.7017

Observed (HR-MS (ESI)): 854.8462 $[M+2H]^{2+}$



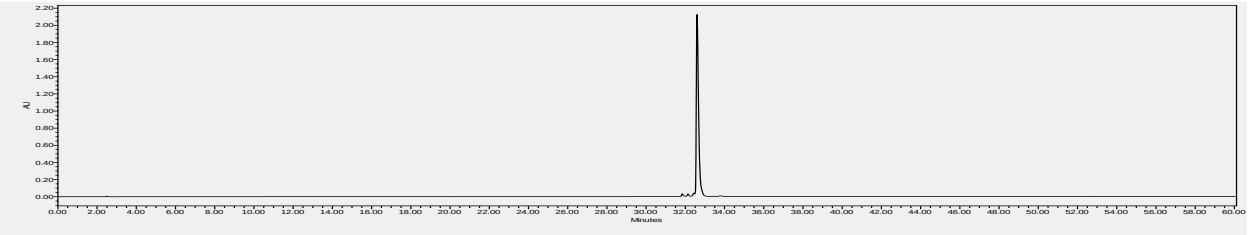


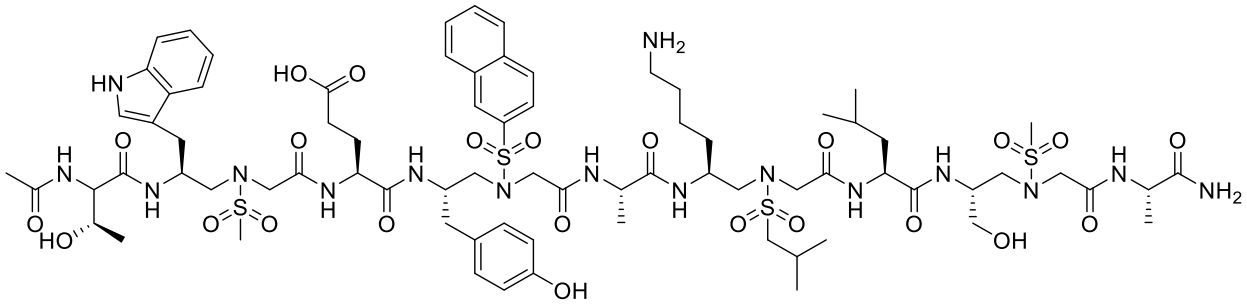
FITC-1

Chemical Formula: $C_{96}H_{129}N_{17}O_{28}S_5$

Exact Mass: 2127.7797

Observed (HR-MS (ESI)): 1064.8196 $[M+2H]^{2+}$, 710.2640 $[M+3H]^{3+}$.



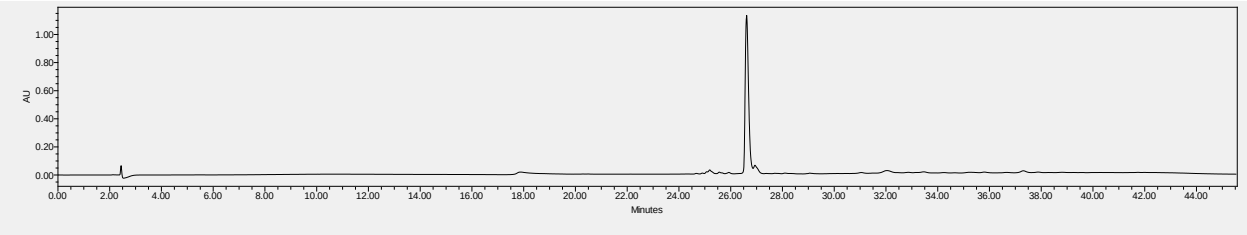


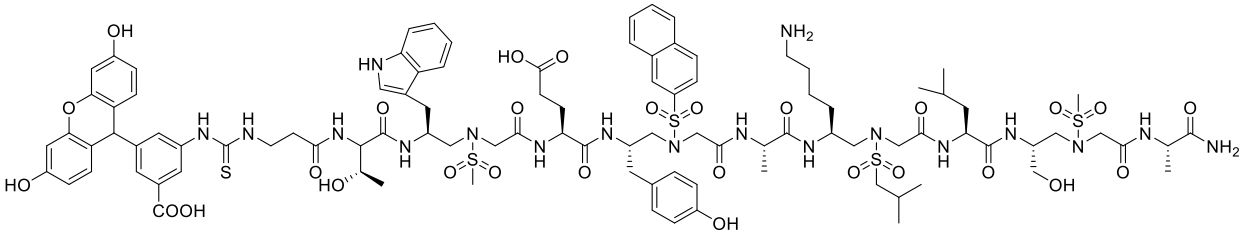
2

Chemical Formula: $C_{76}H_{114}N_{16}O_{23}S_4$

Exact Mass: 1746.7126

Observed (HR-MS (ESI)): 874.8662 $[M+2H]^{2+}$.



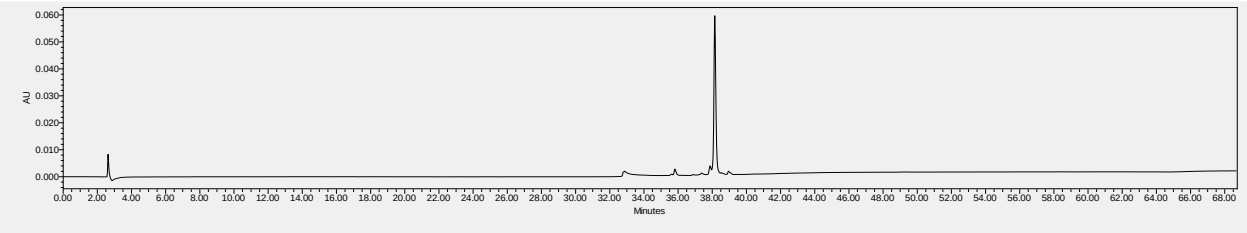


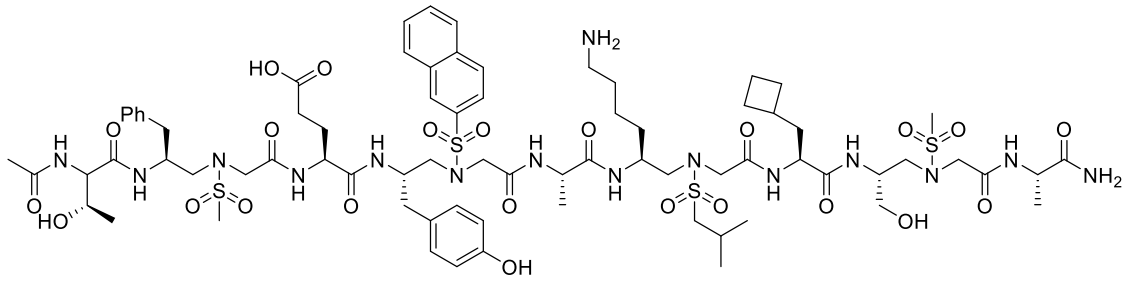
FITC-2

Chemical Formula: $C_{98}H_{130}N_{18}O_{28}S_5$

Exact Mass: 2166.7905

Observed (HR-MS (ESI): 1084.3928 $[M+2H]^{2+}$.



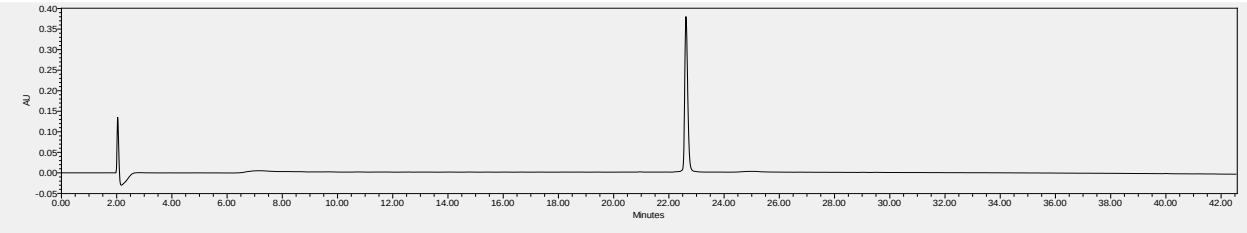


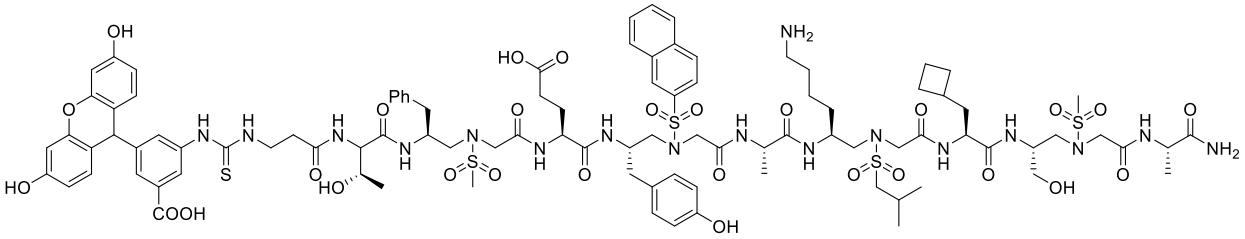
3

Chemical Formula: $C_{75}H_{113}N_{15}O_{23}S_4$

Exact Mass: 1719.7017

Observed (HR-MS (ESI)): 860.8605 $[M+2H]^{2+}$.



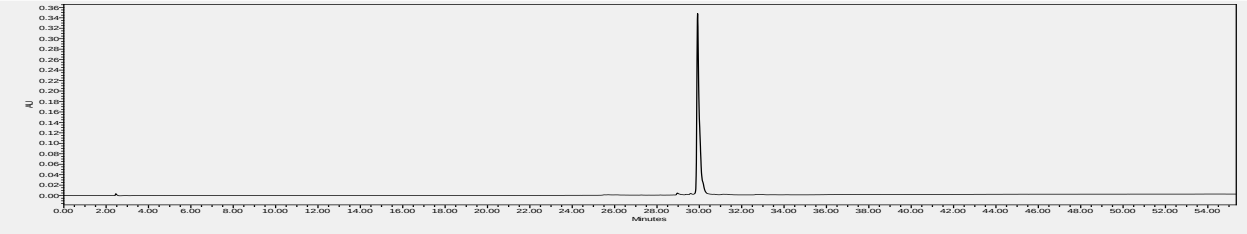


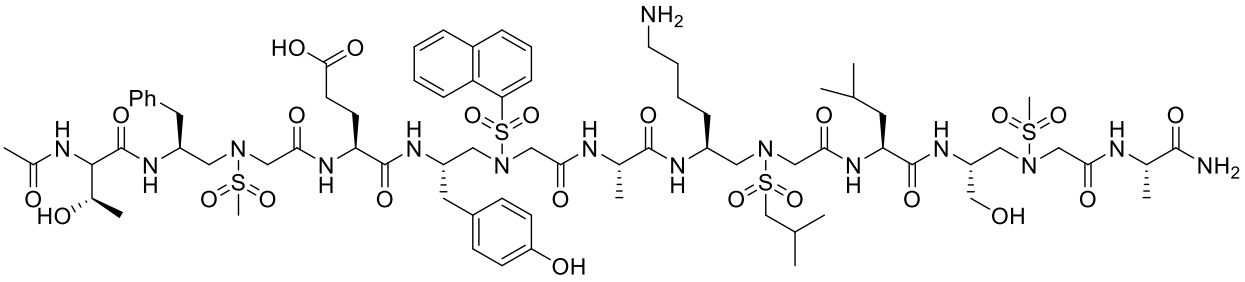
FITC-3

Chemical Formula: $C_{97}H_{129}N_{17}O_{28}S_5$

Exact Mass: 2139.7797

Observed (HR-MS (ESI)): 1070.8901 $[M+2H]^{2+}$.



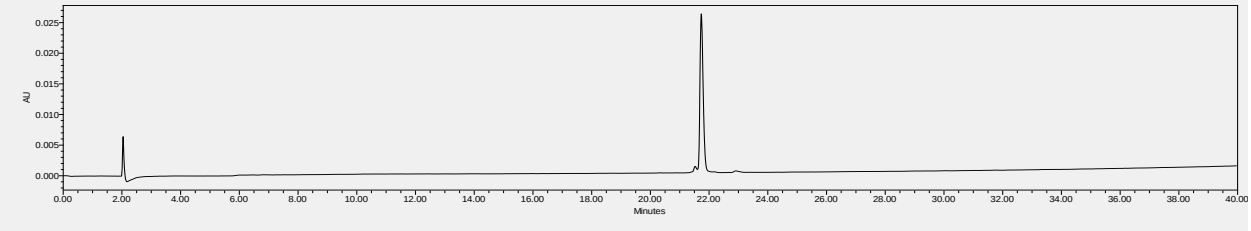


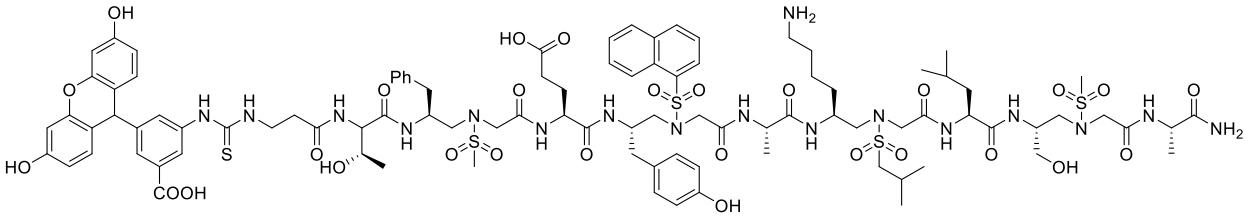
4

Chemical Formula: $C_{74}H_{113}N_{15}O_{23}S_4$

Exact Mass: 1707.7017

Observed (HR-MS (ESI)): 854.8597 $[M+2H]^{2+}$



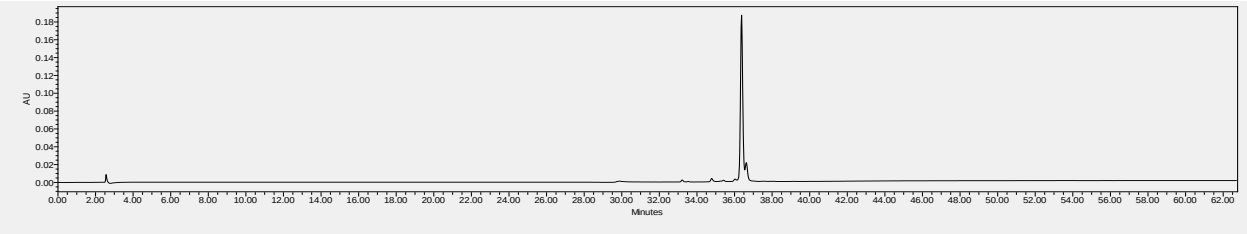


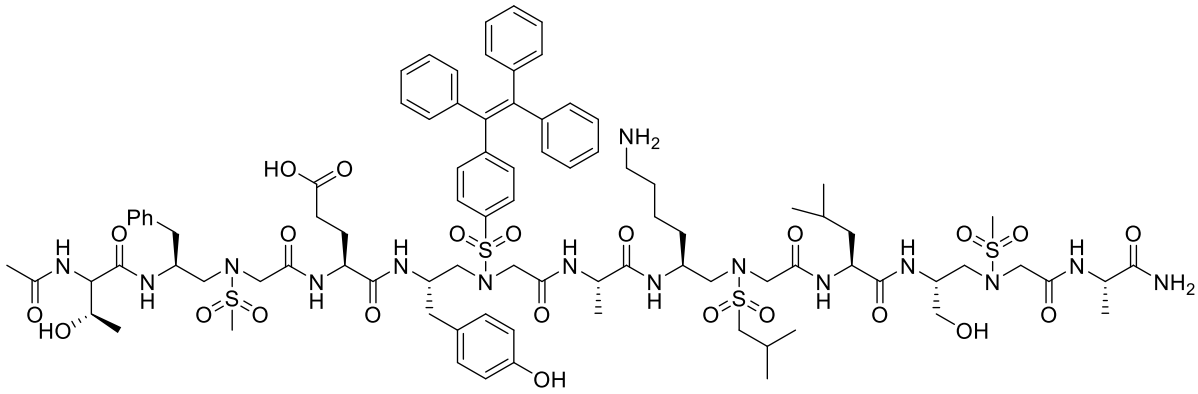
FITC-4

Chemical Formula: $C_{96}H_{129}N_{17}O_{28}S_5$

Exact Mass: 2127.7797

Observed (HR-MS (ESI)): 1064.8927 $[M+2H]^{2+}$.



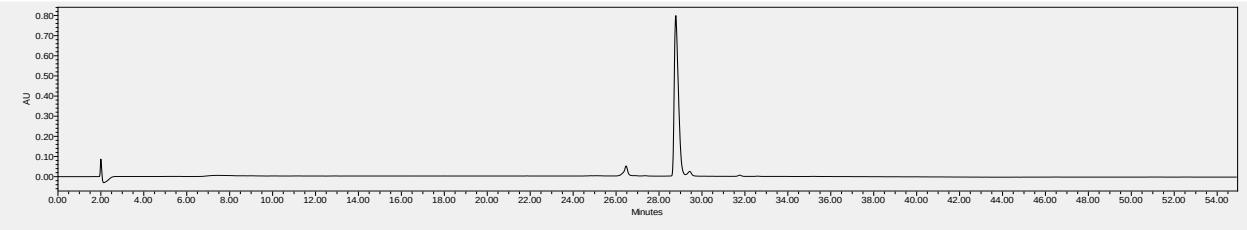


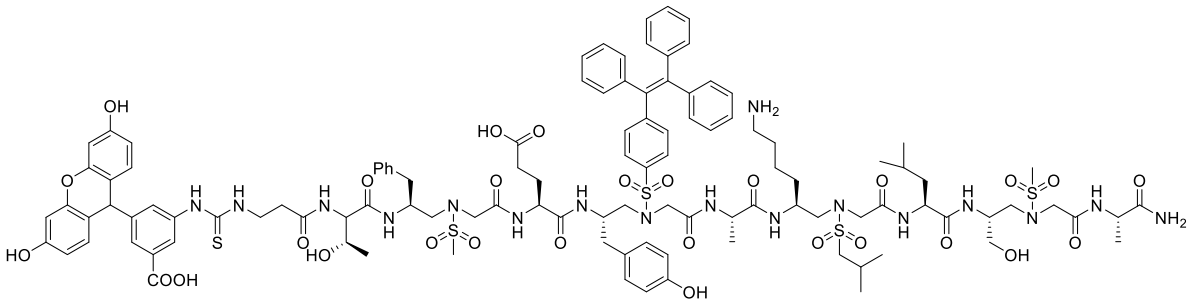
5

Chemical Formula: $C_{90}H_{125}N_{15}O_{23}S_4$

Exact Mass: 1911.7956

Observed (HR-MS (ESI)): 956.9065 $[M+2H]^{2+}$.



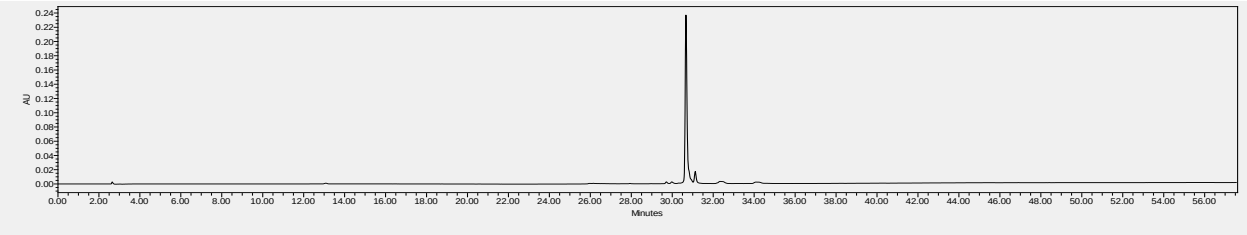


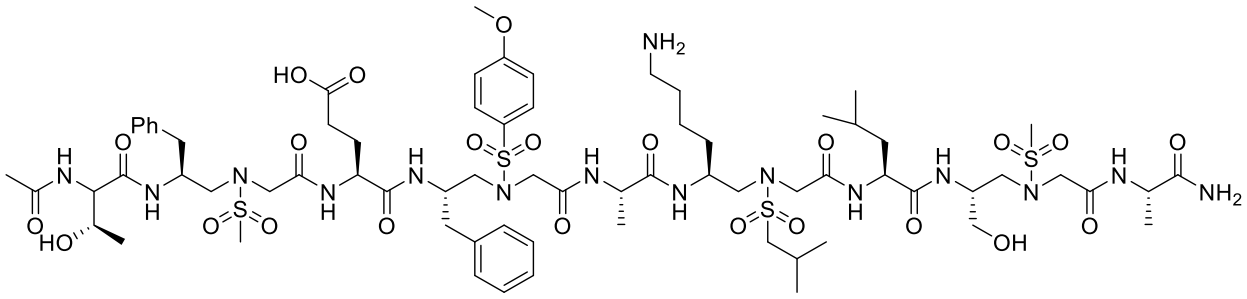
FITC-5

Chemical Formula: $C_{112}H_{141}N_{17}O_{28}S_5$

Exact Mass: 2331.8736

Observed (HR-MS (ESI)): 1166.9345 $[M+2H]^{2+}$.



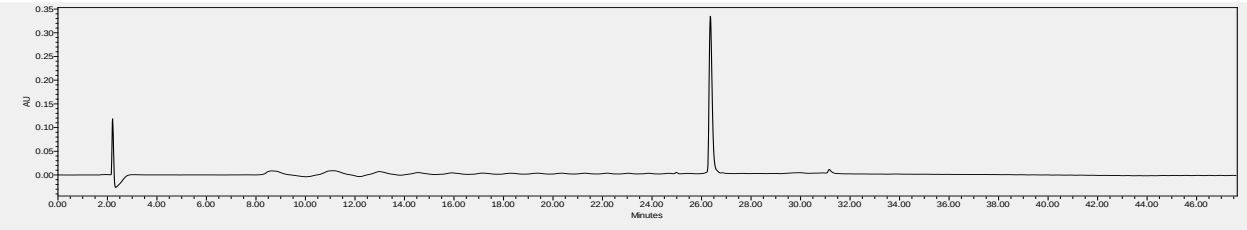


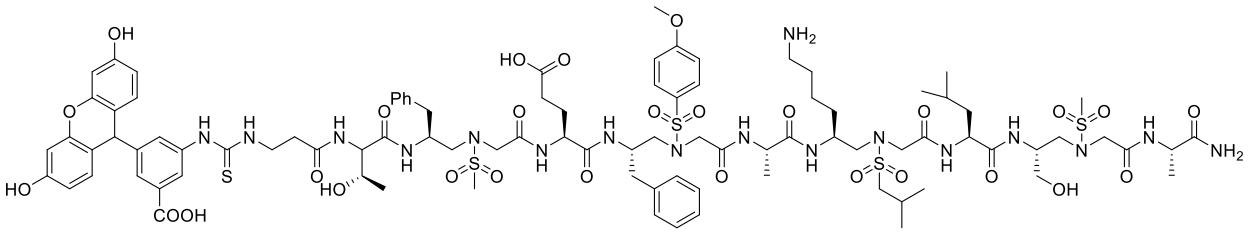
6

Chemical Formula: $C_{71}H_{113}N_{15}O_{23}S_4$

Exact Mass: 1671.7017

Observed (HR-MS (ESI)): 836.8602 $[M+2H]^{2+}$.



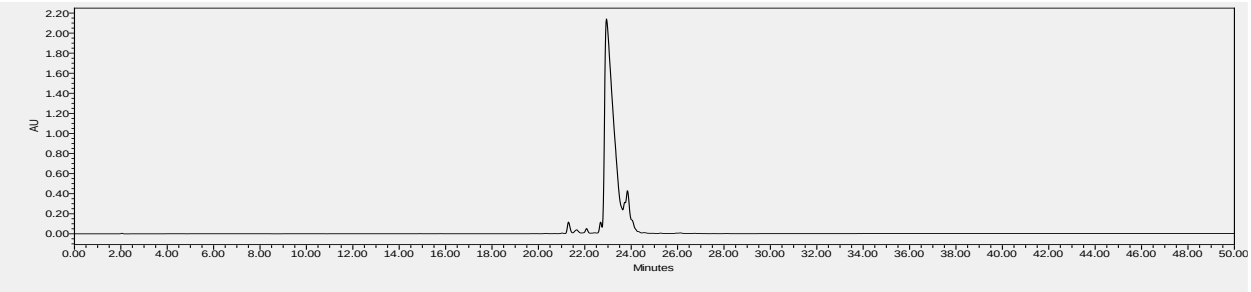


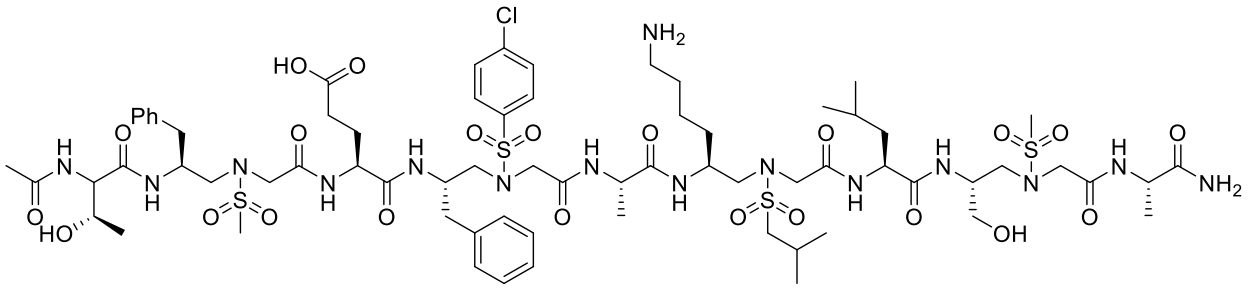
FITC-6

Chemical Formula: $C_{93}H_{129}N_{17}O_{28}S_5$

Exact Mass: 2091.7797

Observed (HR-MS (ESI)): 1046.8906 $[M+2H]^{2+}$.



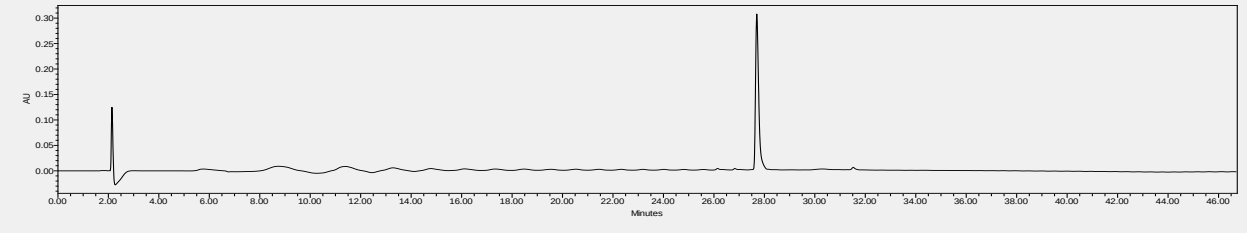


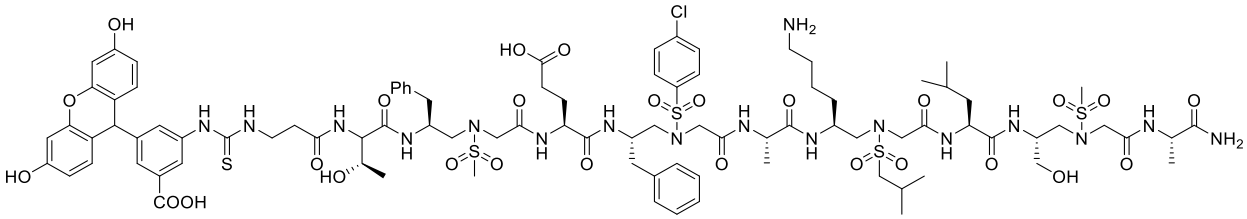
7

Chemical Formula: $C_{70}H_{110}ClN_{15}O_{22}S_4$

Exact Mass: 1675.6521

Observed (HR-MS (ESI)): 838.8336 $[M+2H]^{2+}$.



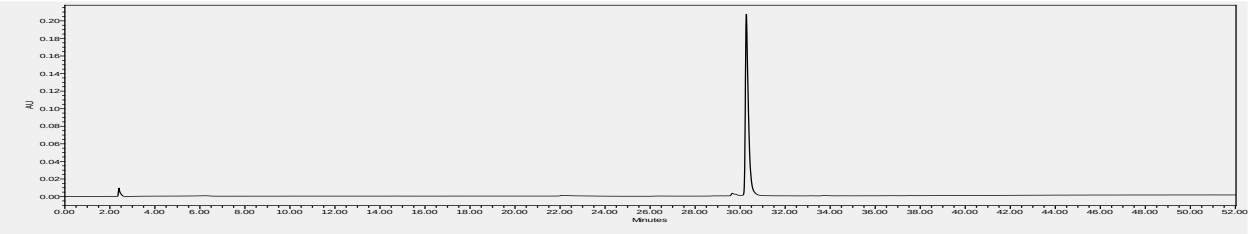


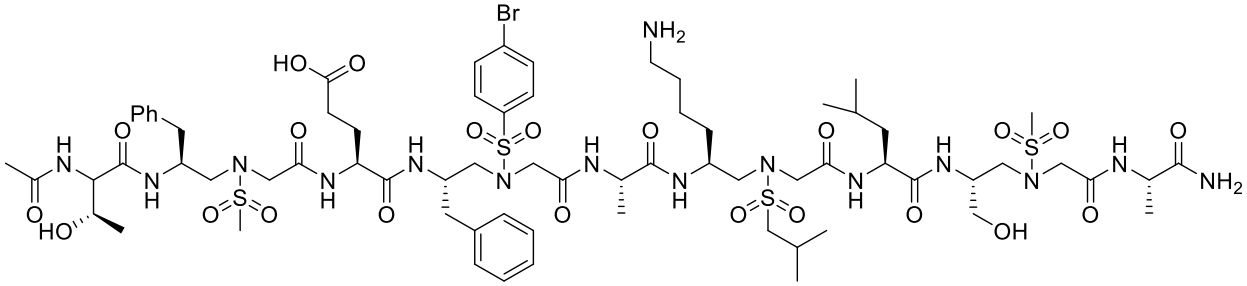
FITC-7

Chemical Formula: $C_{92}H_{126}ClN_{17}O_{27}S_5$

Exact Mass: 2095.7301

Observed (HR-MS (ESI)): 1048.8657 $[M+2H]^{2+}$.



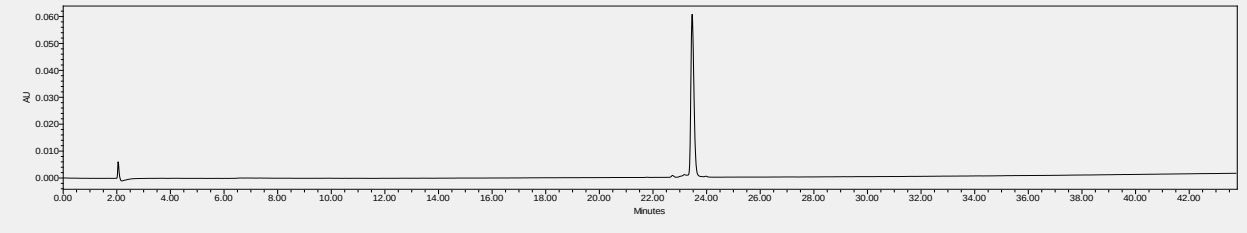


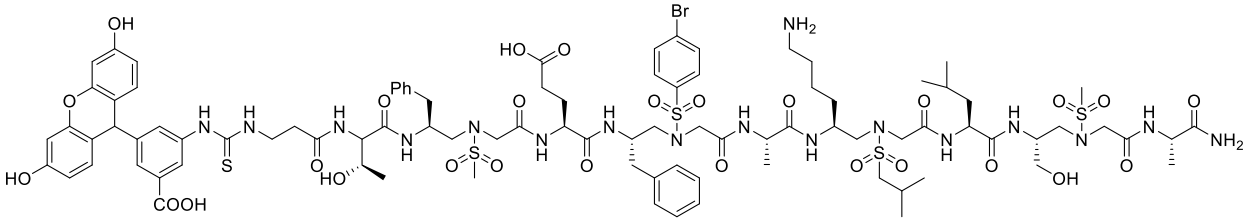
8

Chemical Formula: $C_{70}H_{110}BrN_{15}O_{22}S_4$

Exact Mass: 1719.6016

Observed (HR-MS (ESI)): 861.8081 $[M+2H]^{2+}$.



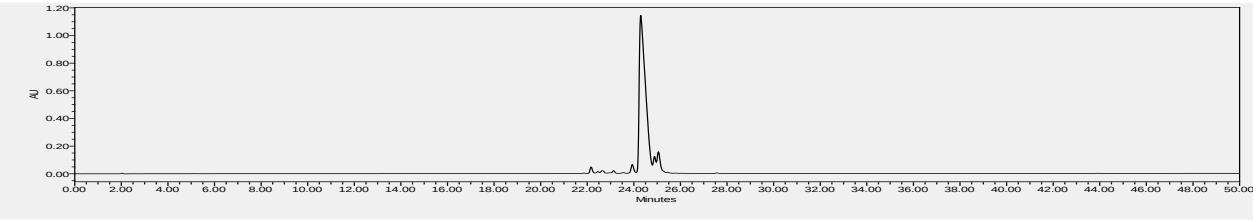


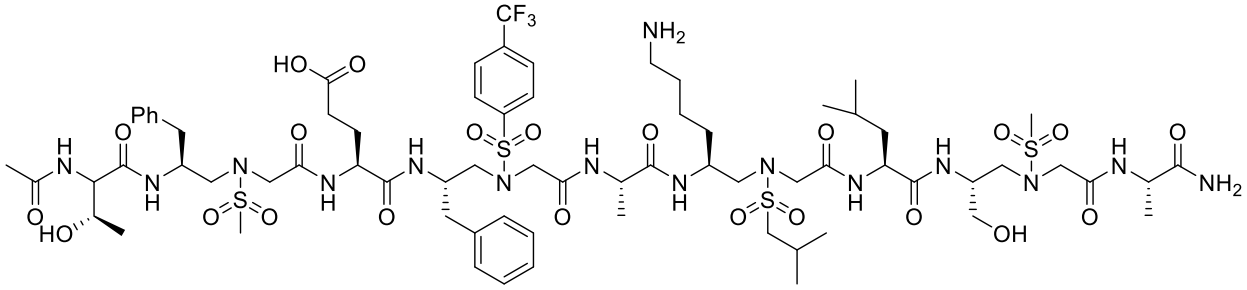
FITC-8

Chemical Formula: $C_{92}H_{126}BrN_{17}O_{27}S_5$

Exact Mass: 2139.6796

Observed (HR-MS (ESI)): 1070.8387 $[M+2H]^{2+}$.



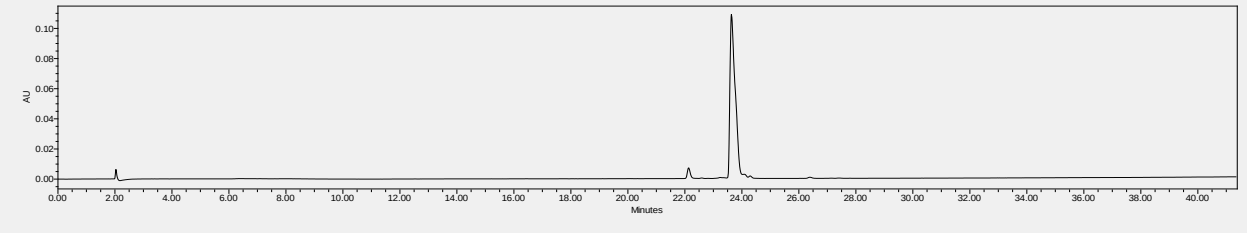


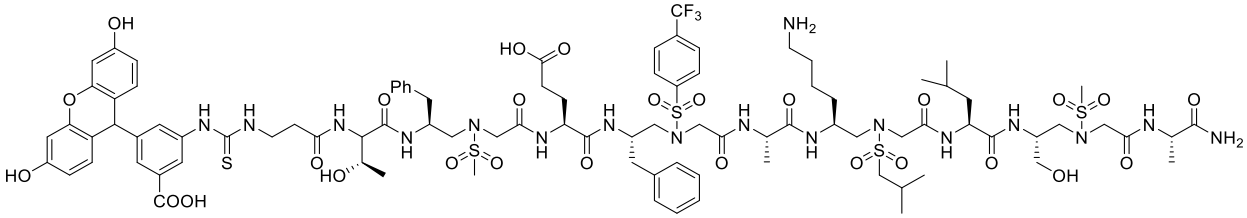
9

Chemical Formula: $C_{71}H_{110}F_3N_{15}O_{22}S_4$

Exact Mass: 1709.6785

Observed (HR-MS (ESI)): 855.8485 $[M+2H]^{2+}$.



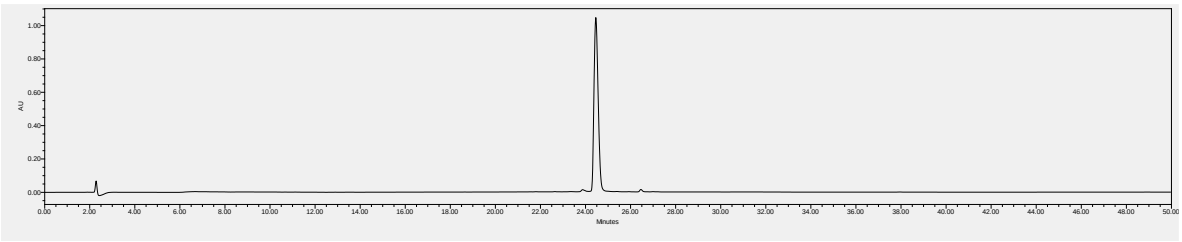


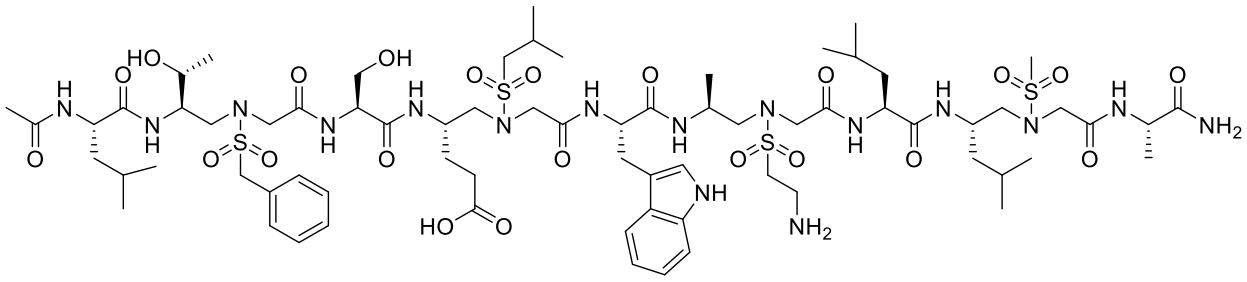
FITC-9

Chemical Formula: $C_{93}H_{126}F_3N_{17}O_{27}S_5$

Exact Mass: 2129.7565

Observed (HR-MS (ESI)): 1065.8783 $[M+2H]^{2+}$.



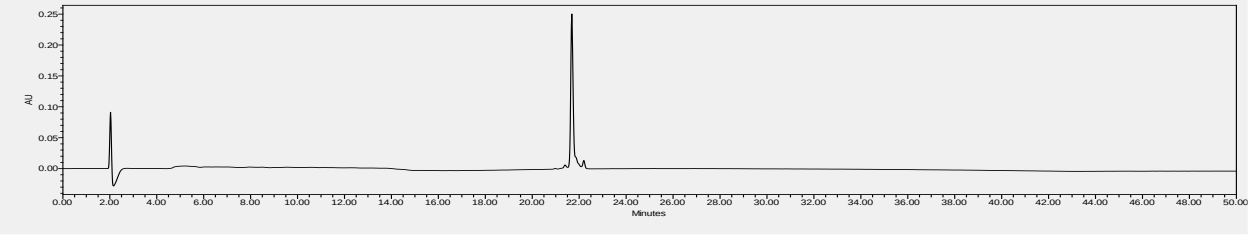


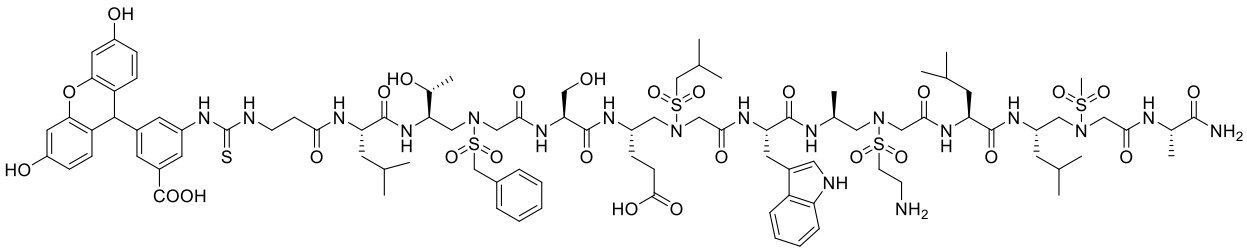
10

Chemical Formula: $C_{71}H_{118}N_{16}O_{22}S_4$

Exact Mass: 1674.7489

Observed (HR-MS (ESI)): 838.8846 $[M+2H]^{2+}$.



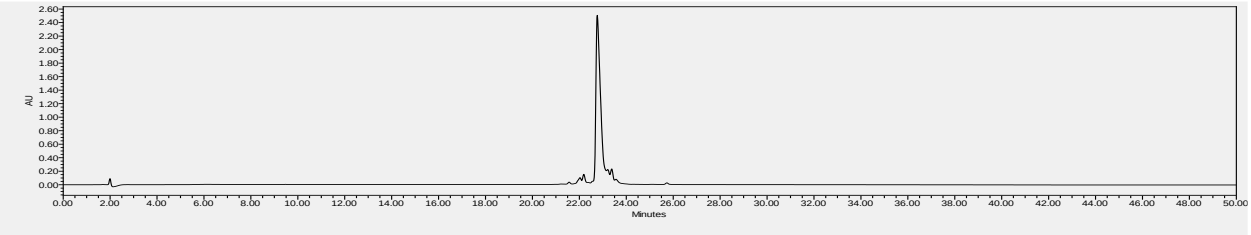


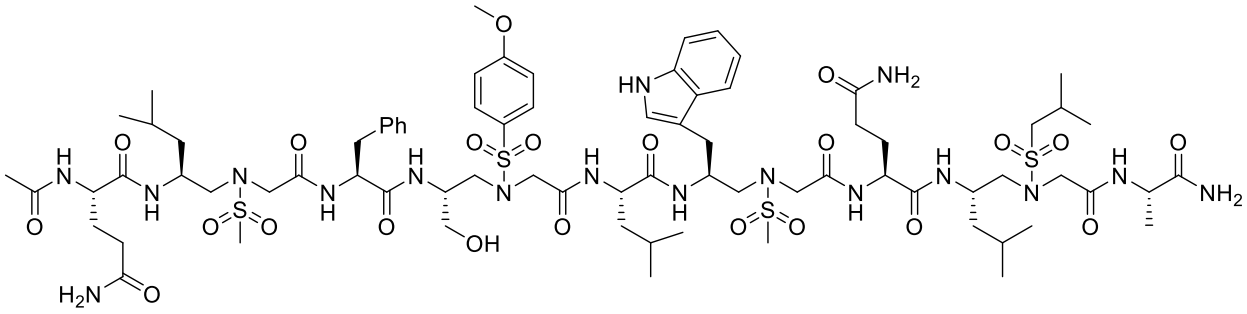
FITC-10

Chemical Formula: $C_{93}H_{134}N_{18}O_{27}S_5$

Exact Mass: 2094.8269

Observed (HR-MS (ESI)): 1048.4140 $[M+2H]^{2+}$.



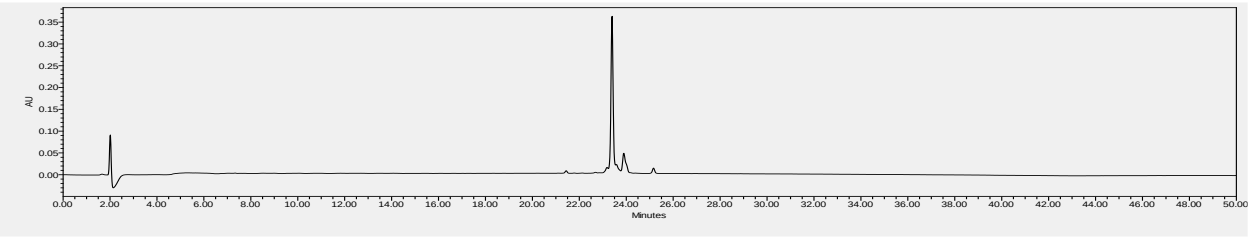


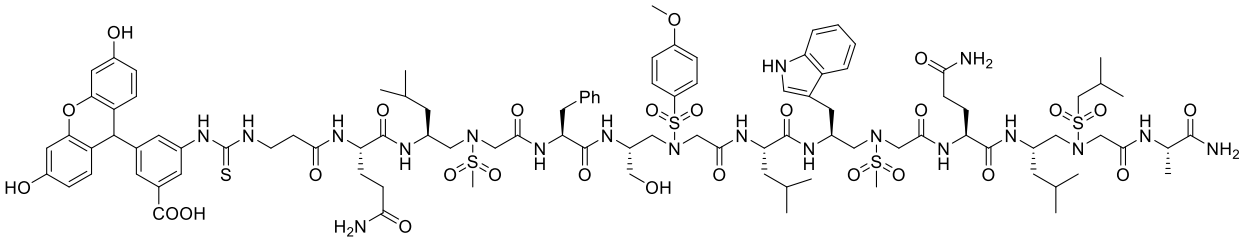
11

Chemical Formula: $C_{77}H_{121}N_{17}O_{22}S_4$

Exact Mass: 1763.7755

Observed (HR-MS (ESI)): 883.3958 $[M+2H]^{2+}$.



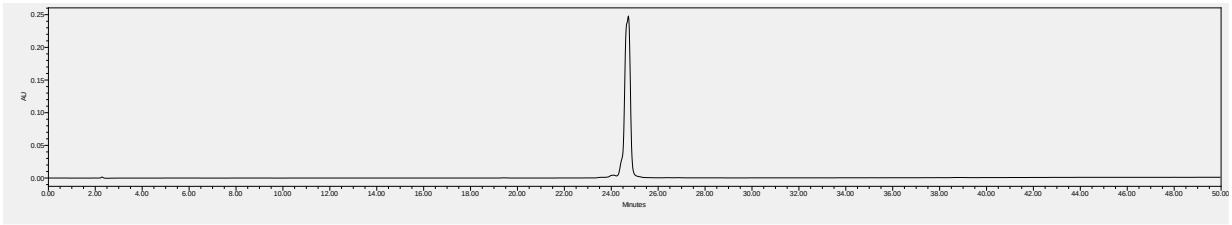


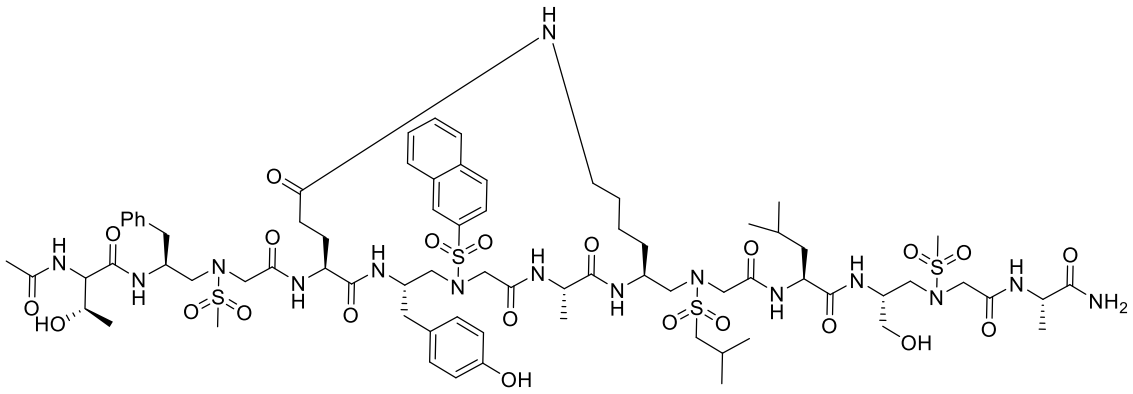
FITC-11

Chemical Formula: $C_{99}H_{137}N_{19}O_{27}S_5$

Exact Mass: 2183.8535

Observed (HR-MS (ESI)): 1092.9261 $[M+2H]^{2+}$.



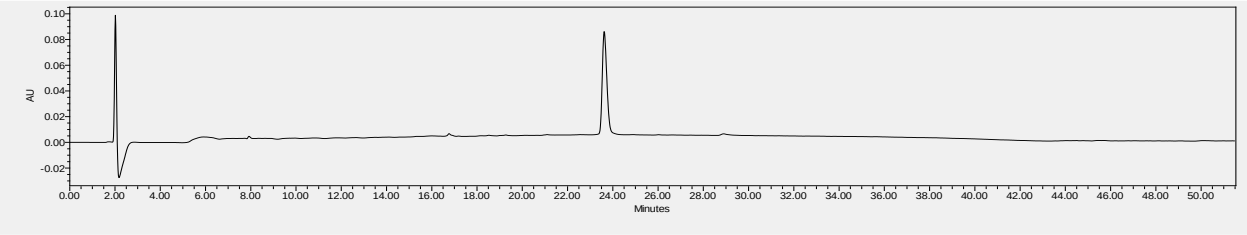


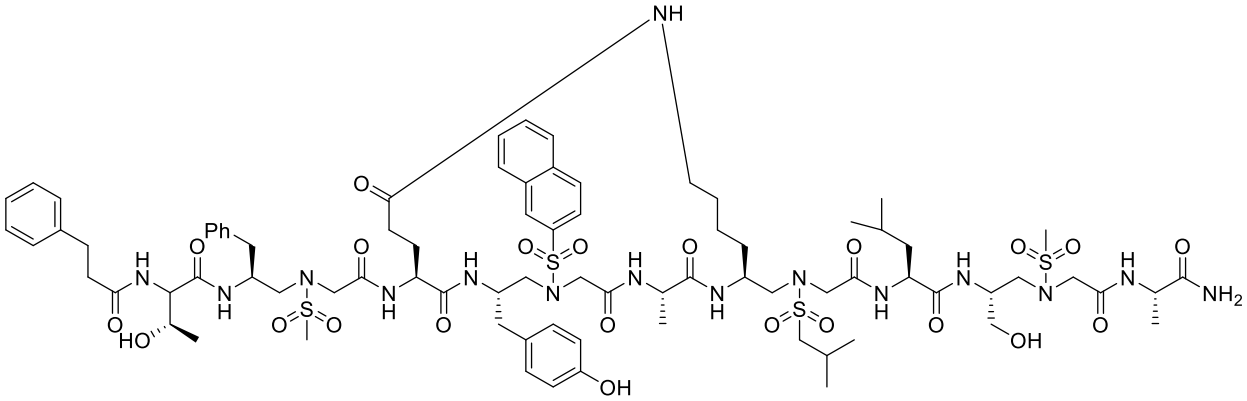
12

Chemical Formula: $C_{74}H_{111}N_{15}O_{22}S_4$

Exact Mass: 1689.6911

Observed (HR-MS (ESI)): 845.8521 $[M+2H]^{2+}$.



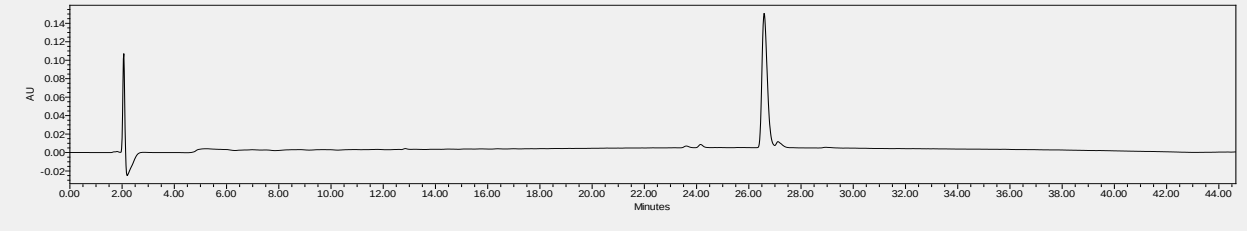


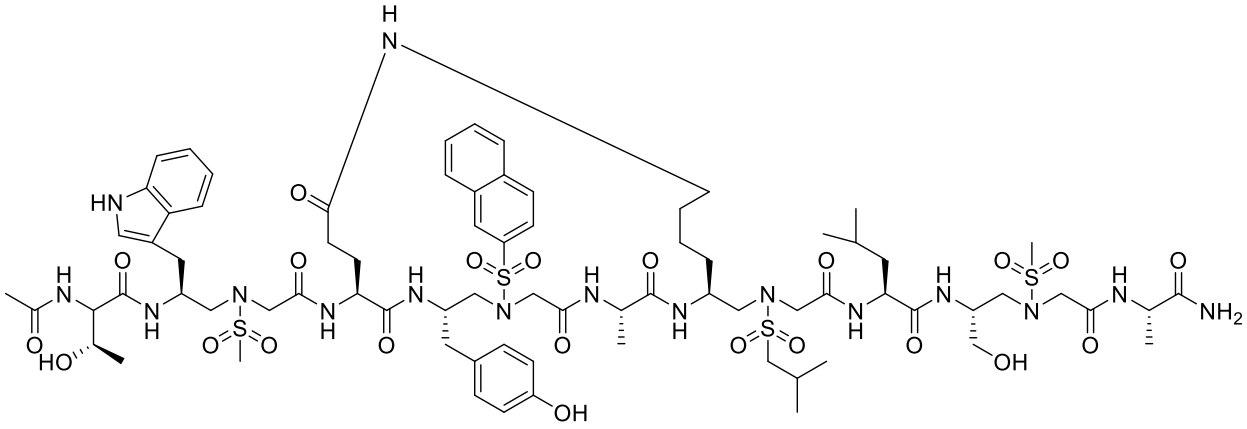
13

Chemical Formula: $C_{81}H_{117}N_{15}O_{22}S_4$

Exact Mass: 1779.7380

Observed (HR-MS (ESI)): 891.3761 $[M+2H]^{2+}$.



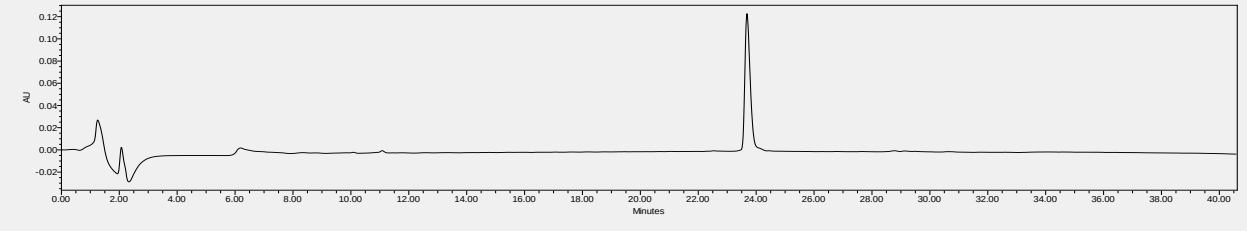


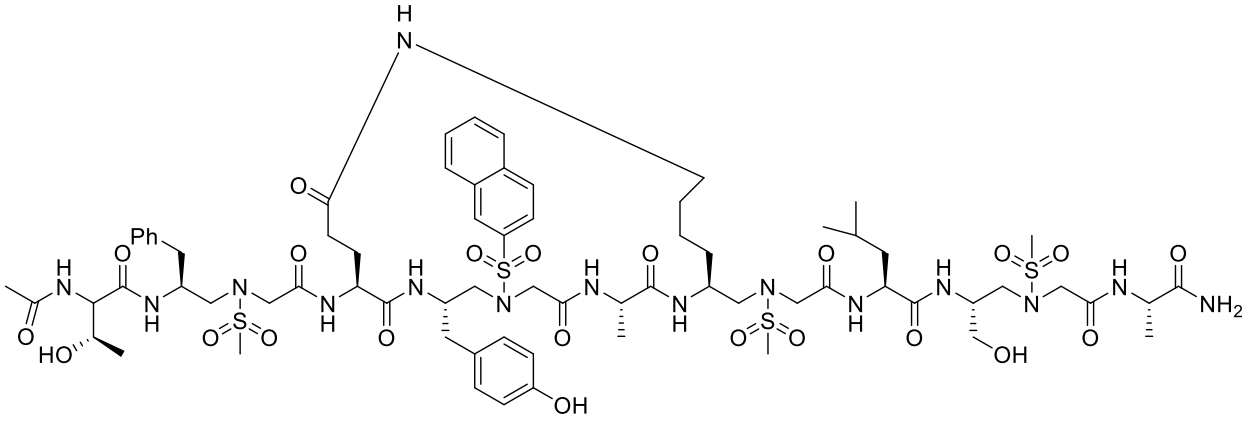
14

Chemical Formula: $C_{76}H_{112}N_{16}O_{22}S_4$

Exact Mass: 1728.7020

Observed (HR-MS (ESI)): 865.3583 $[M+2H]^{2+}$.





15

Chemical Formula: $C_{71}H_{105}N_{15}O_{22}S_4$

Exact Mass: 1647.6441

Observed (HR-MS (ESI)): 824.8300 $[M+2H]^{2+}$.

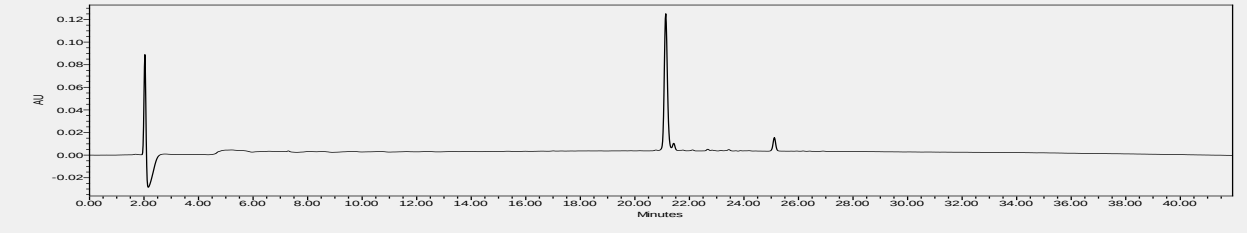


Figure S1 HPLC spectra of pure p53 and α/sulfono-γ-AApeptides.

2.3 HPLC Purities and Retention Time of Pure Peptides[a]

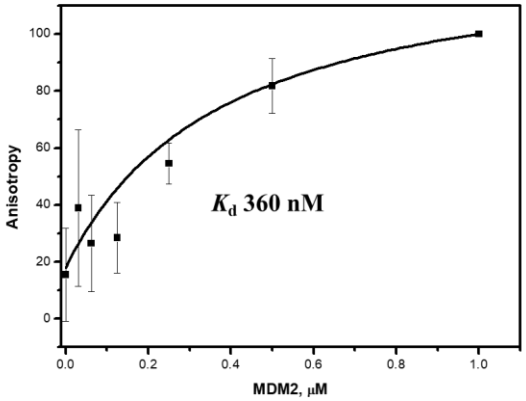
Peptide Name	Purity trace after HPLC purification (%)	Retention Time (min)
1	99.2	29.1
2	99.4	27.95
3	97.5	22.7
4	96.3	21.8
5	98.7	28.9
6	99.6	26.2
7	100	27.8
8	98.6	23.7
9	96.7	23.95
10	96.7	21.6
11	97.1	23.4
FITC-1	99.0	32.25
FITC-2	95.2	38.1
FITC-3	95.7	30.1
FITC-4	97	36.6
FITC-5	99.1	31.1
FITC-6	95.03	22.9
FITC-7	98.3	30.9
FITC-8	95.7	24.3
FITC-9	98.7	24.5
FITC-10	96.0	22.76
FITC-11	99.4	24.7
12	100	23.6
13	96.8	26.58

14	100	23.68
15	97.2	21.1

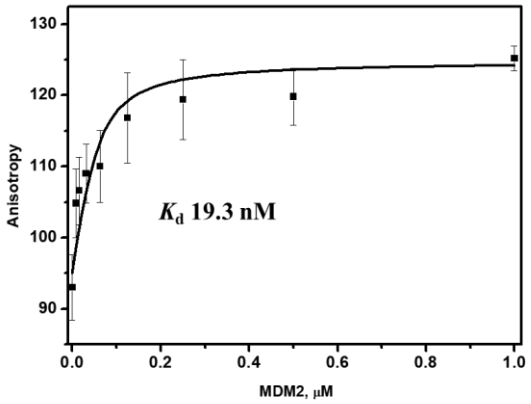
Table S1. The retention time and the purity of the 1:1 α/L-Sulfono-γ-AApeptide **1- 15.** [a]The gradient eluting method of 5% to 100% of solvent B (0.1% TFA in acetonitrile) in A (0.1% TFA in water) over 50 min was performed.

3. Fluorescence Polarization Competition Assays

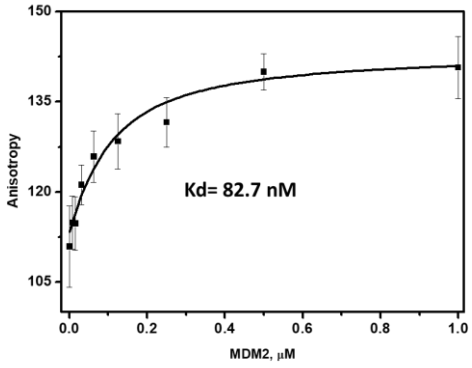
3.1 The binding affinity and inhibition to MDM2.



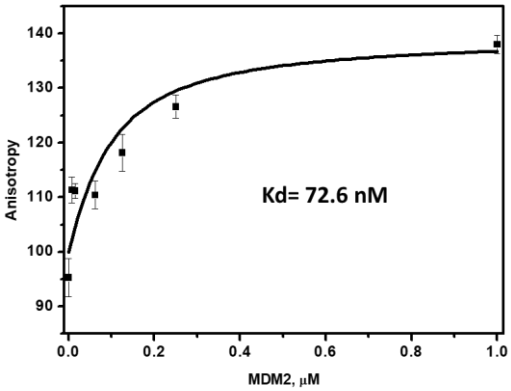
P53



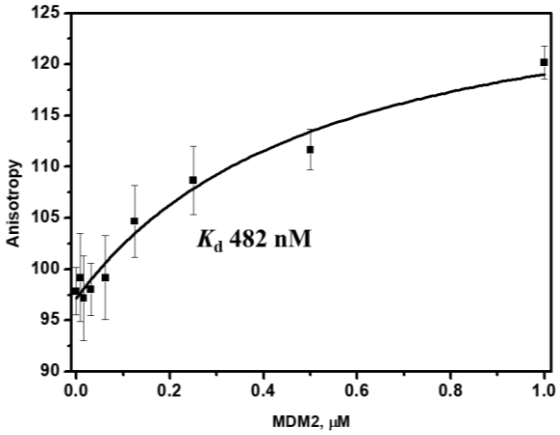
1



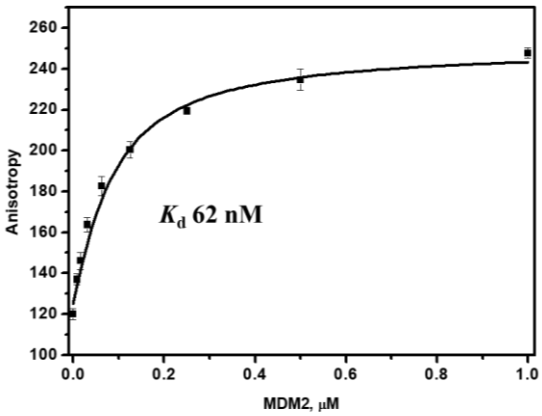
2



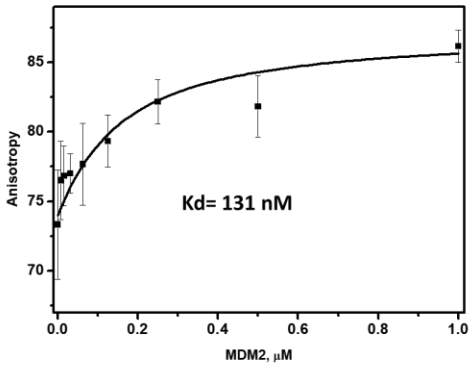
3


Anisotropy
MDM2, μM
K_d 482 nM

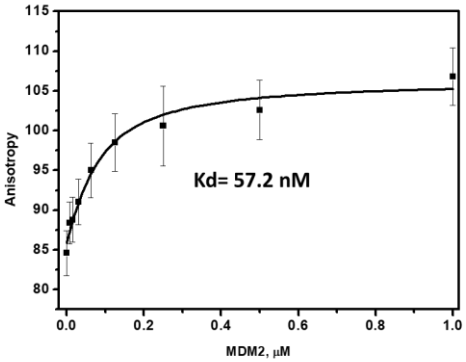
4


Anisotropy
MDM2, μM
K_d 62 nM

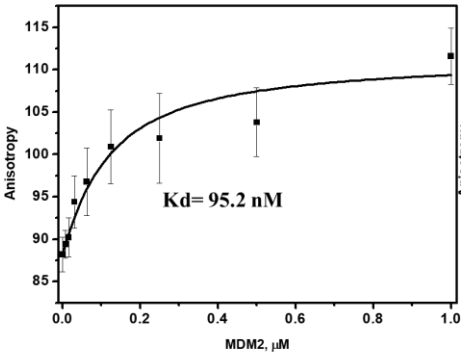
5


Anisotropy
MDM2, μM
Kd= 131 nM

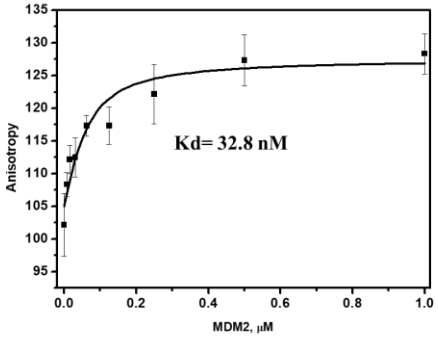
6


Anisotropy
MDM2, μM
Kd= 57.2 nM

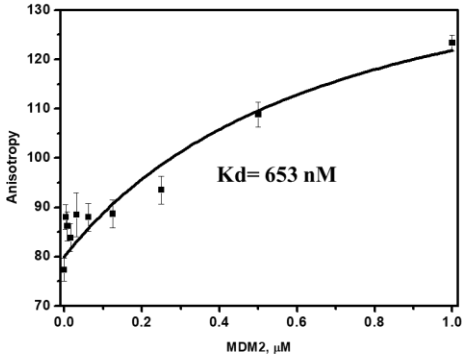
7


Anisotropy
MDM2, μM
Kd= 95.2 nM

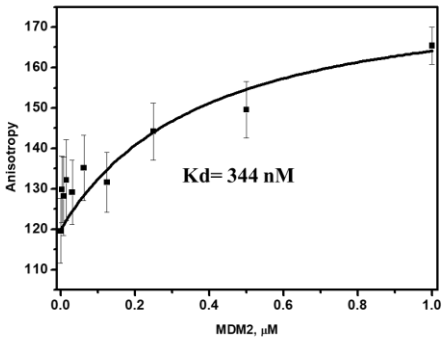
8


Anisotropy
MDM2, μM
Kd= 32.8 nM

9



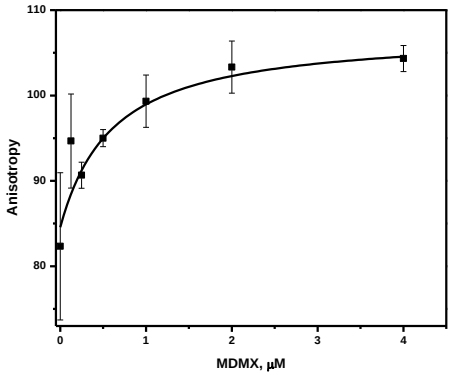
10



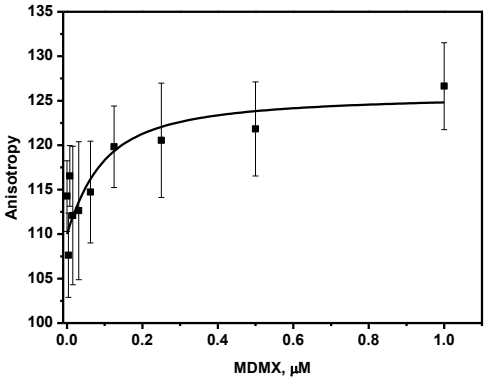
11

Figure S2. The K_d of the 1:1 α/L-Sulfono-γ-AApeptide **1- 11** to MDM2.

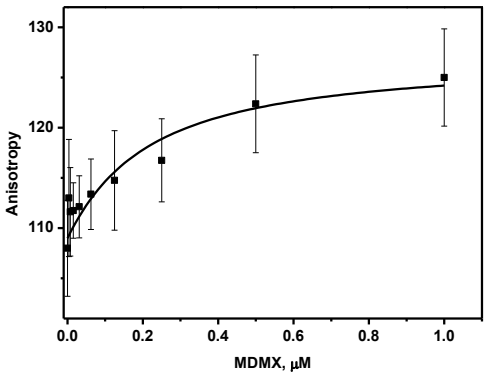
3.2 The binding affinity to MDMX.



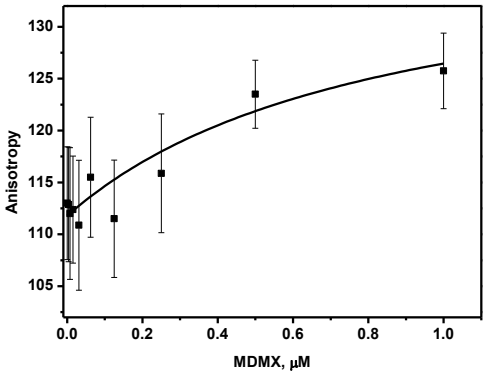
P53, K_d= 570 nM



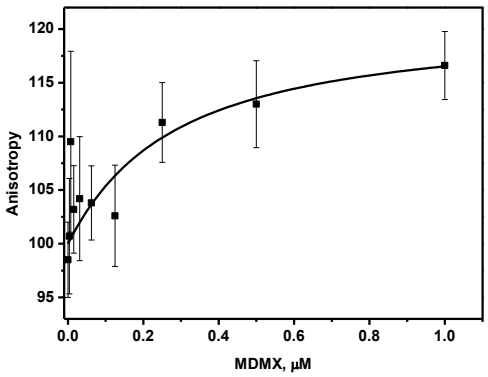
1, K_d= 66.8 nM



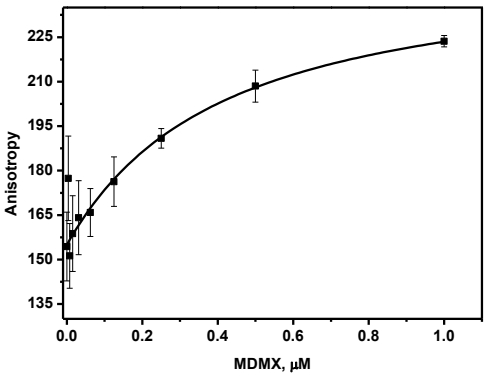
2, K_d= 187 nM



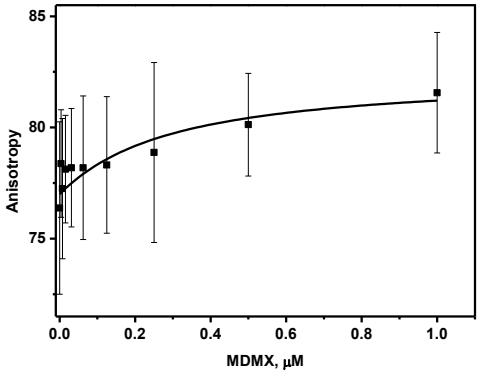
3, K_d= 781 nM



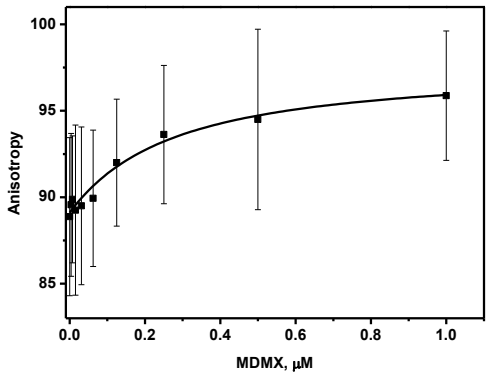
4, K_d= 251 nM



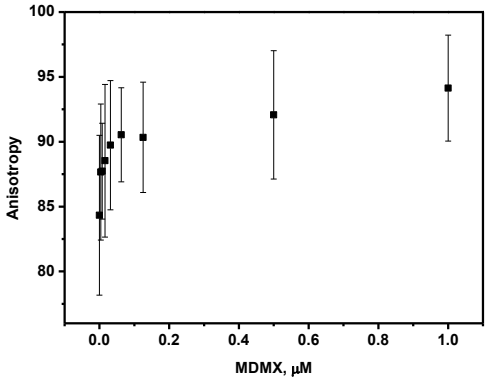
5, K_d= 372 nM



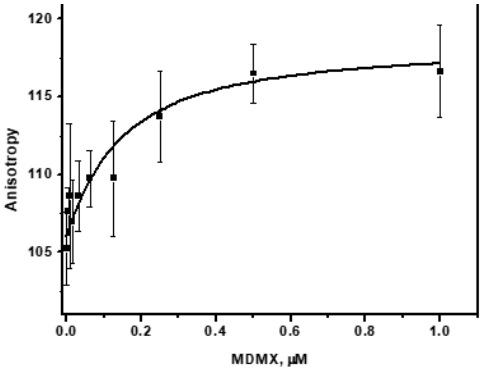
6, K_d= 265 nM



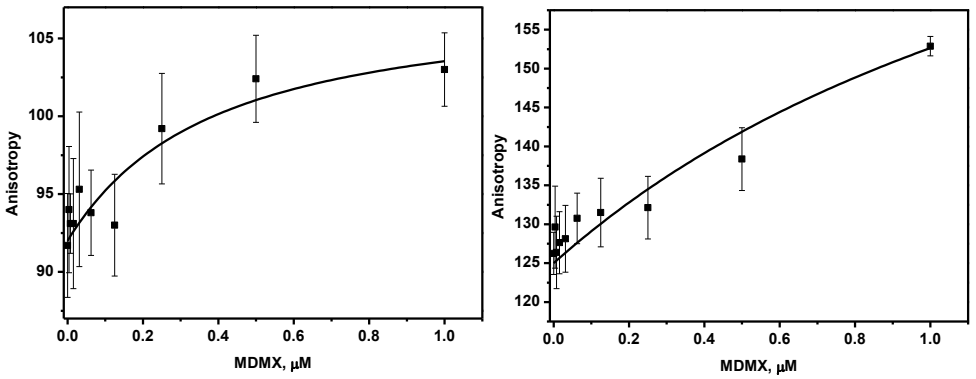
7, K_d= 242 nM



8, K_d= N.D nM



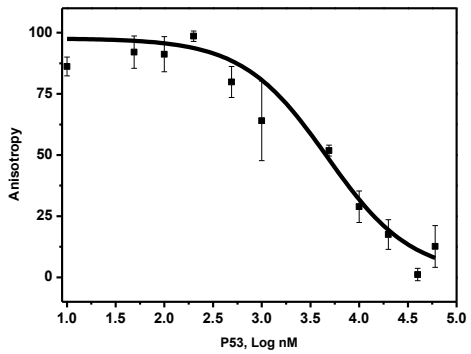
9, K_d= 115 nM



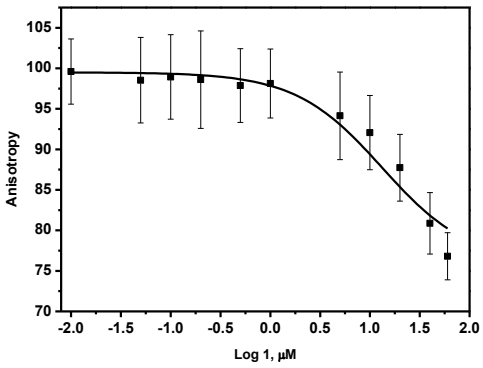
10, K_d= 348 nM **11, K_d= 1680 nM**

Figure S3. The K_d of p53 and 1:1 α/L-Sulfono-γ-AApeptide **1- 11** to MDMX.

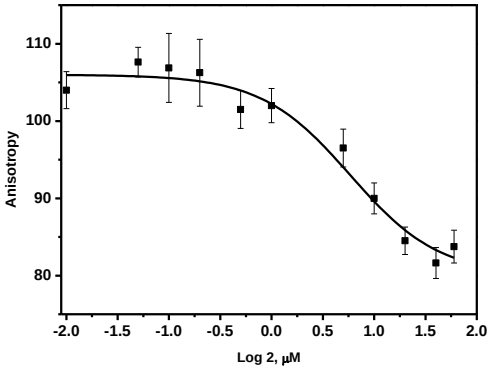
3.3 The IC_{50} of compound 1-15 against MDM2



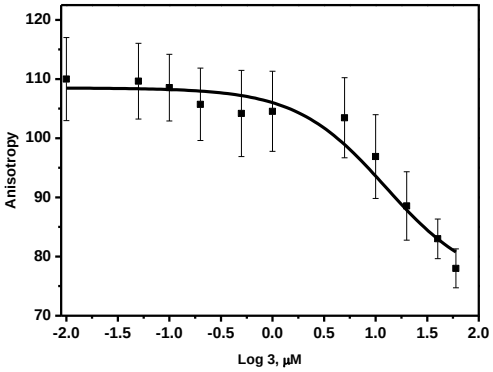
P53, IC_{50} = 4.67 μM



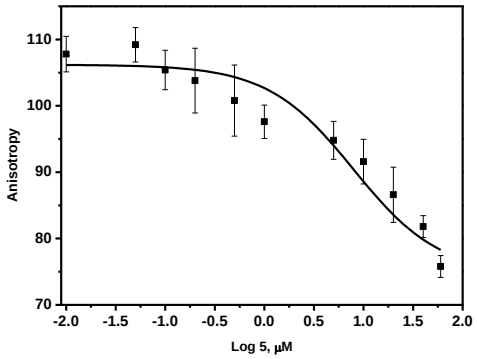
1, IC_{50} = 13.1 μM



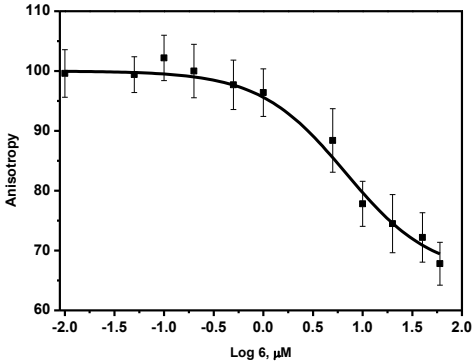
2, IC_{50} = 5.8 μM



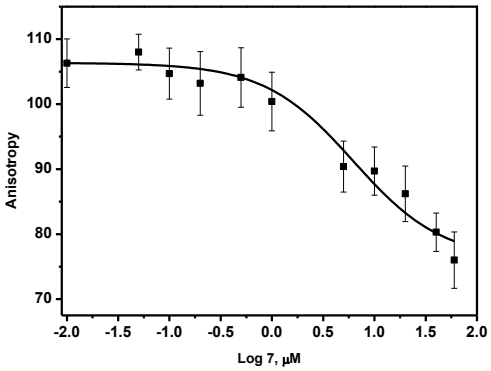
3, IC_{50} = 12.4 μM



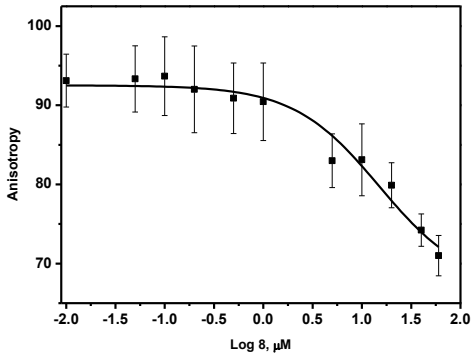
5, IC_{50} = 7.9 μM



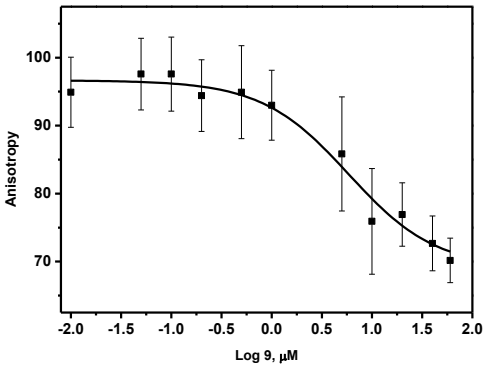
6, IC_{50} = 6.7 μM



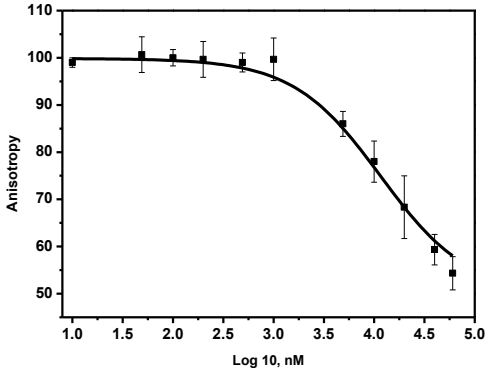
7, IC_{50} = 6.22 μM



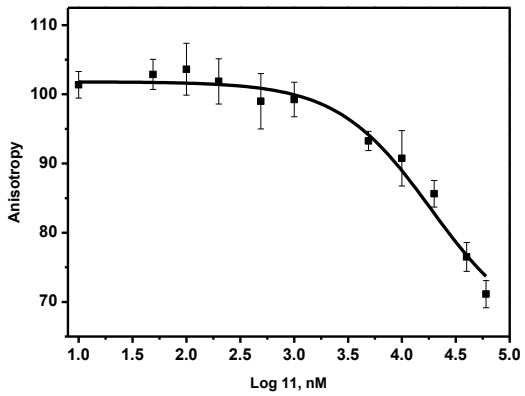
8, IC_{50} = 14.8 μM



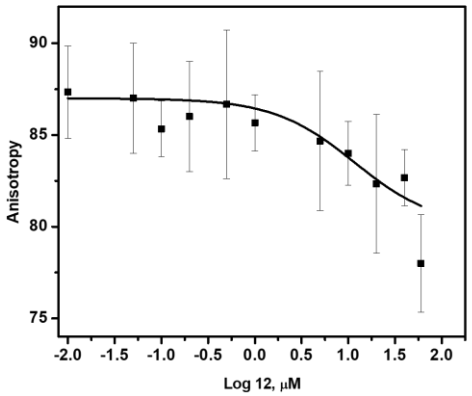
9, IC_{50} = 5.9 μM



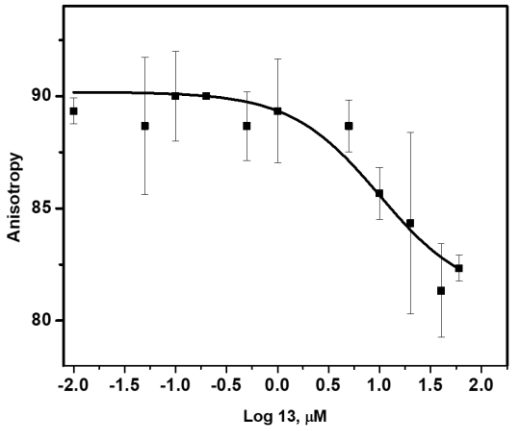
10, IC_{50} = 11.5 μM



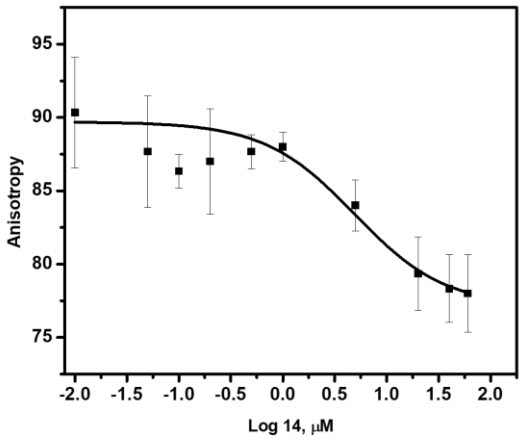
11, IC_{50} = 18.6 µM



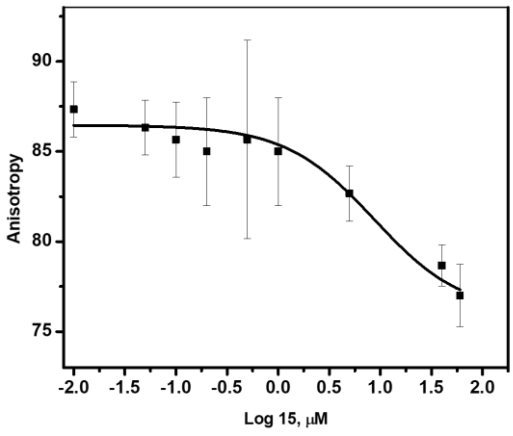
12, IC_{50} = 11.7 µM



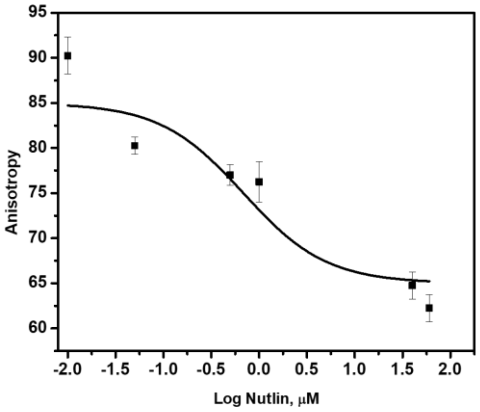
13, IC_{50} = 9.4 µM



14, IC_{50} = 4.9 µM



15, IC_{50} = 9.0 µM



Nutlin, IC_{50} = 0.68 µM

Figure S4. The IC_{50} of the 1:1 α/L-Sulfono-γ-AApeptide **1- 15** to MDM2.

4. Circular Dichroism

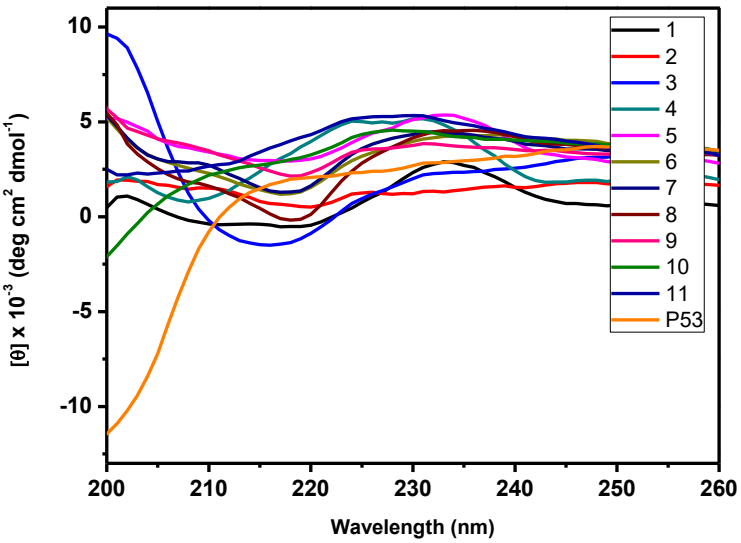


Figure S5. CD spectra of p53 and α/sulfono-γ-AApeptides (100 μM) measured at room temperature in PBS buffer.

5. Enzymatic Stability Study

5.1 HPLC Traces of P53 in Presence of Proteases

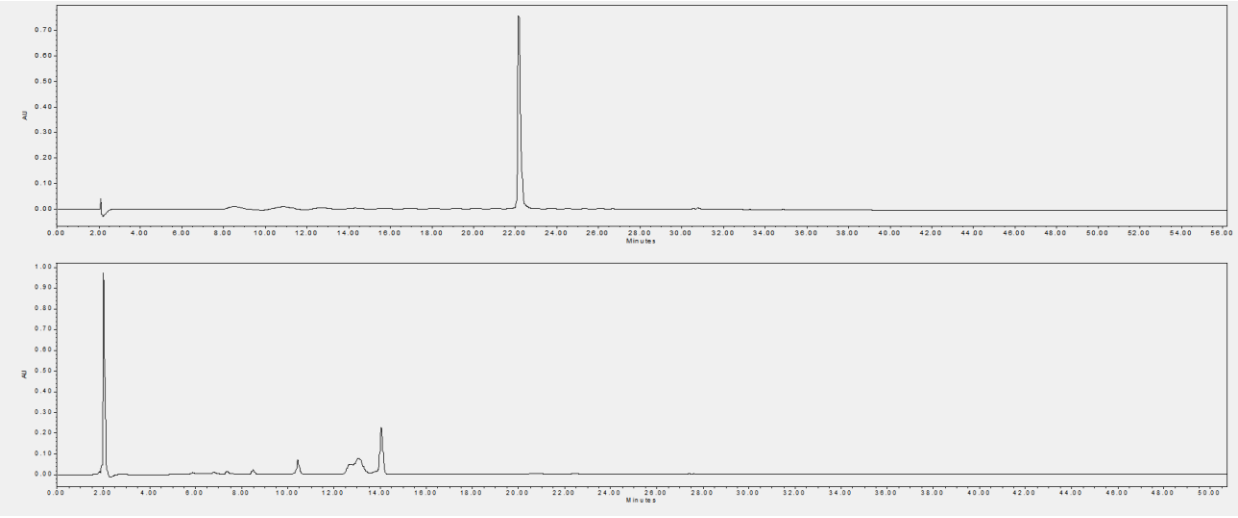


Figure S6. Analytic HPLC trace of **P53** before and after incubation with pronase (0.1 mg/mL) in 100 mM pH 7.8 ammonium bicarbonate buffer at 37 °C.

5.2 HPLC Traces of Peptide 1 in Presence of Proteases

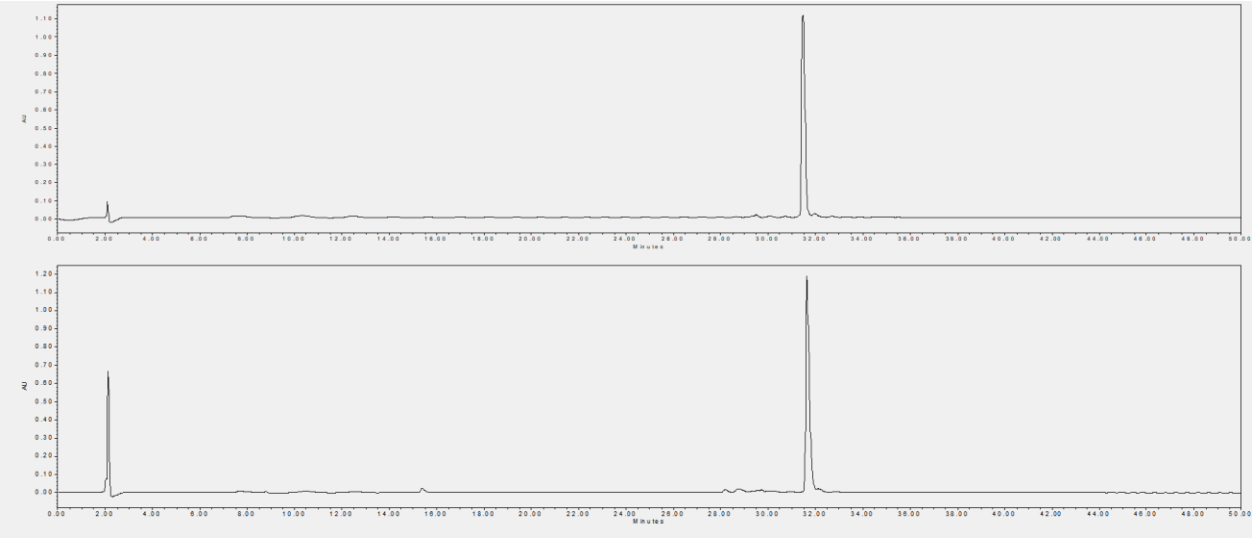


Figure S7. Analytic HPLC trace of **1** before and after incubation with pronase (0.1 mg/mL) in 100 mM pH 7.8 ammonium bicarbonate buffer at 37 °C.

6. ^{15}N-^{1}H HSQC NMR of Lead Peptide 9 in Complex with MDM2

6.1 Protein Expression and Purification

^{15}N-labeled human MDM2 residues 17–125 was expressed and purified as described in.[2]

6.2 NMR Data Collection and Analysis

Experiments for 200 μM MDM2$_{17\text{-}125}$ in the presence and absence of stochiometric excess of **9** were carried out on a Varian VNMRS 800-MHz spectrometer with a triple resonance pulse field Z-axis gradient cold probe at 30°C. ^{1}H-^{15}N heteronuclear single-quantum coherence spectroscopy experiments were performed on ^{15}N-labeled samples in 90% H_2O/ 10% D_2O. Buffer for **9** and MDM2$_{17\text{-}125}$ experiments was 50 mM NaH_2PO_4, 50 mM NaCl, 1 mM EDTA, 3% DMSO, and 0.02% NaN_3 at pH 6.8. Data were acquired in the^{1}H and ^{15}N dimension using 9689.92-Hz (t_2) x 2430.26-Hz (t_1) sweep widths and 1024 (t_2) x 128` (t_1) complex data points. Bound spectra were collected in a molar excess of the **9**.

Resonance assignments for apo MDM2$_{17\text{-}125}$ were previously made.[2] Bound chemical shifts were inferred based on the overlap of resonances in parallel titrations (**Figure S6**). Using this method,

the bound resonances for 99 residues were assigned. For the nine unassignable residues, four were prolines which are not detected, two residues, S17 and Q18, were near the N-terminus, and the remaining three were D46, L81, and N106 were not assigned in apo $MDM2_{17-125}$ [2]. In addition, residue E25 experienced line broadening during the titration and the bound peak was not observed. The combined average chemical shifts were calculated from the formula $\Delta_{ave} = [((\Delta^1H^N)^2 + (\Delta^{15}N/5)^2)/2)]^{1/2}$. All NMR spectra were processed with NMRFx and analyzed using NMRViewJ software.[3,4]

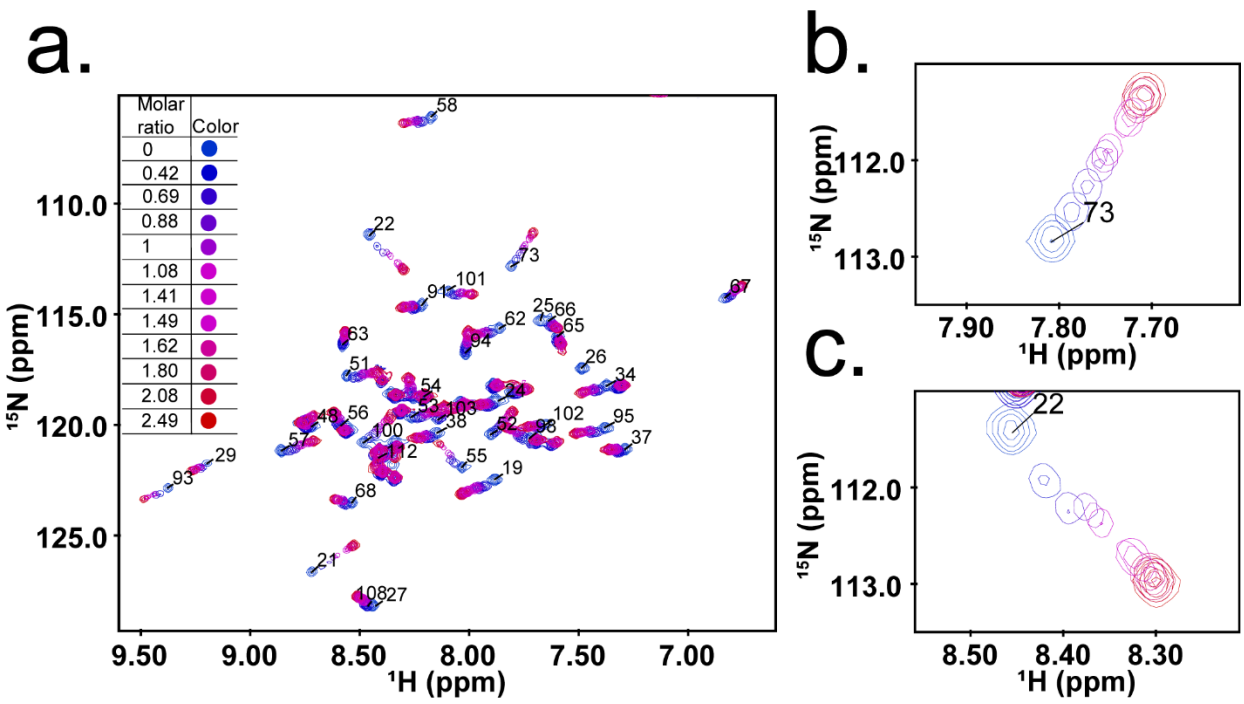


Figure S8. NMR titration results and resonance assignments of $MDM2_{17-125}$ interacting with **9**. **a.** Peak labels are shown for resonances with chemical shift changes in the presence of **9** greater than 0.048 ppm; overlay of ^1H -^{15}N HSQC spectra before (blue) and after (red) the addition of increasing concentrations of **9** (see legend for molar ratios of titration points). For clarity, residue labels are shown for resonances with chemical shift changes greater than 0.048 ppm. **b.** Chemical shifts of H73 during the titration show clear overlap of free and bound resonances. **c.** Chemical shifts of S22 during the titration show clear overlap of free and bound resonances.

7. Luciferase reporter assay.

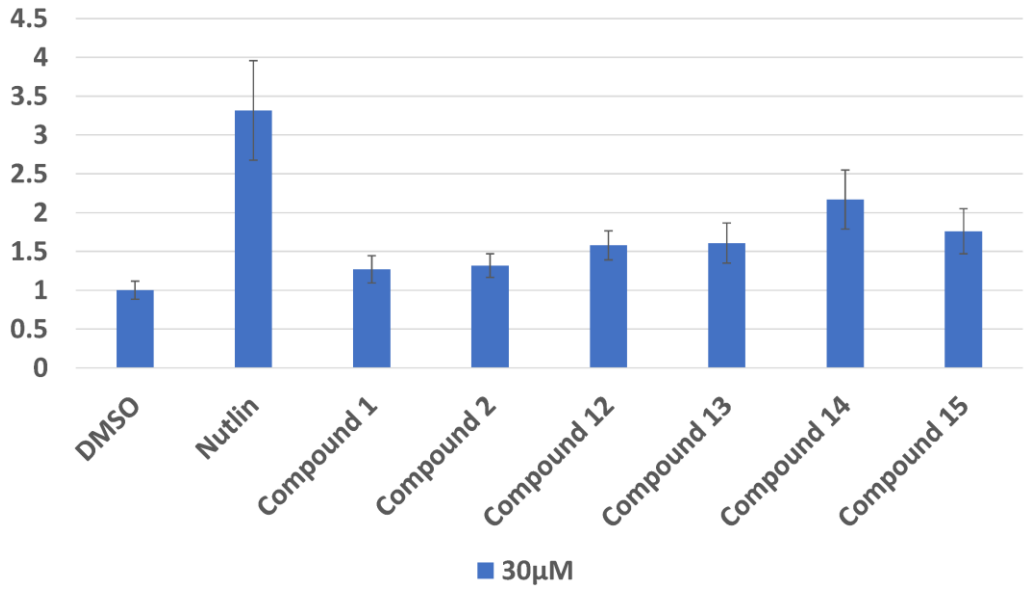


Figure S9. Luciferase reporter assay.

8. References

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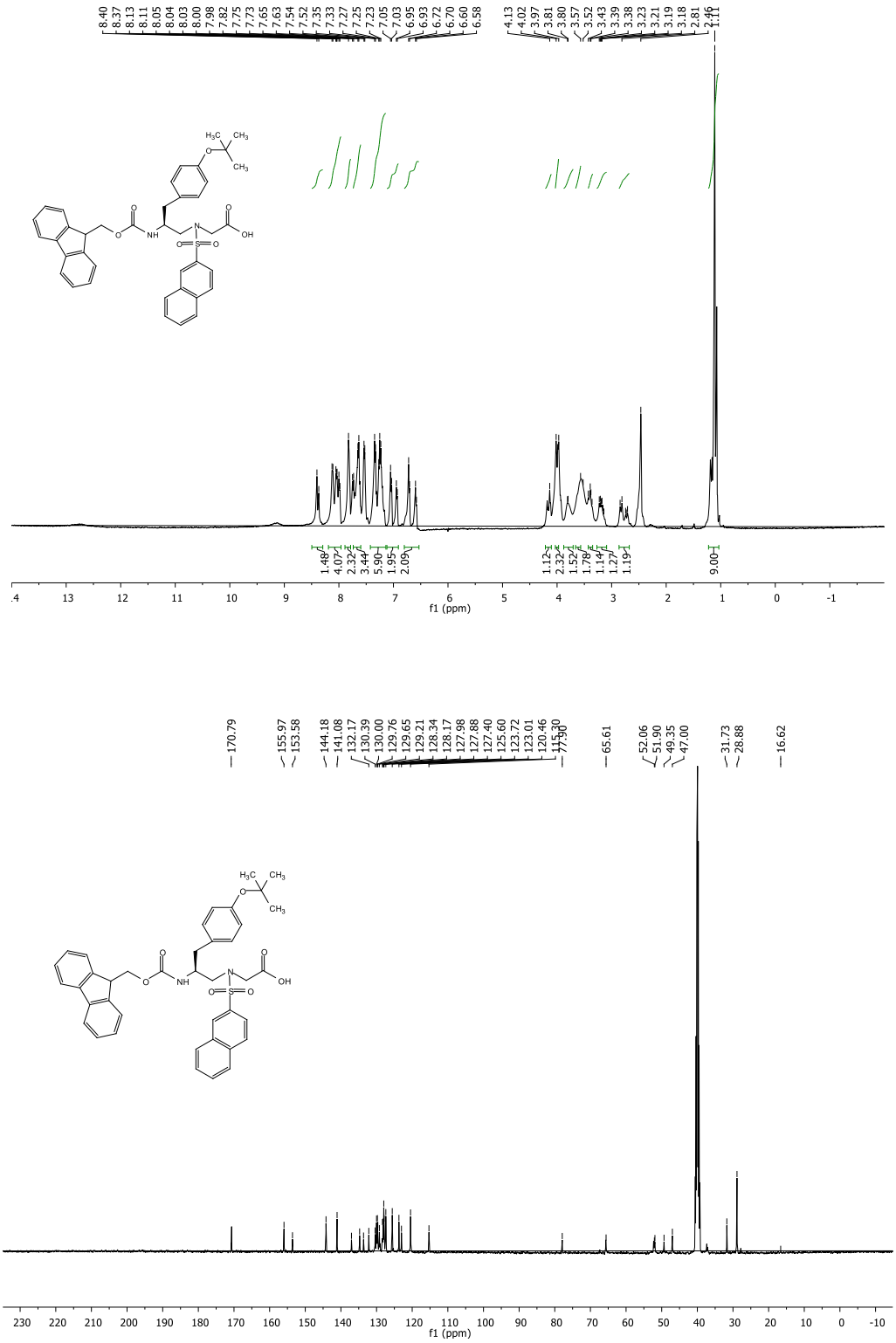
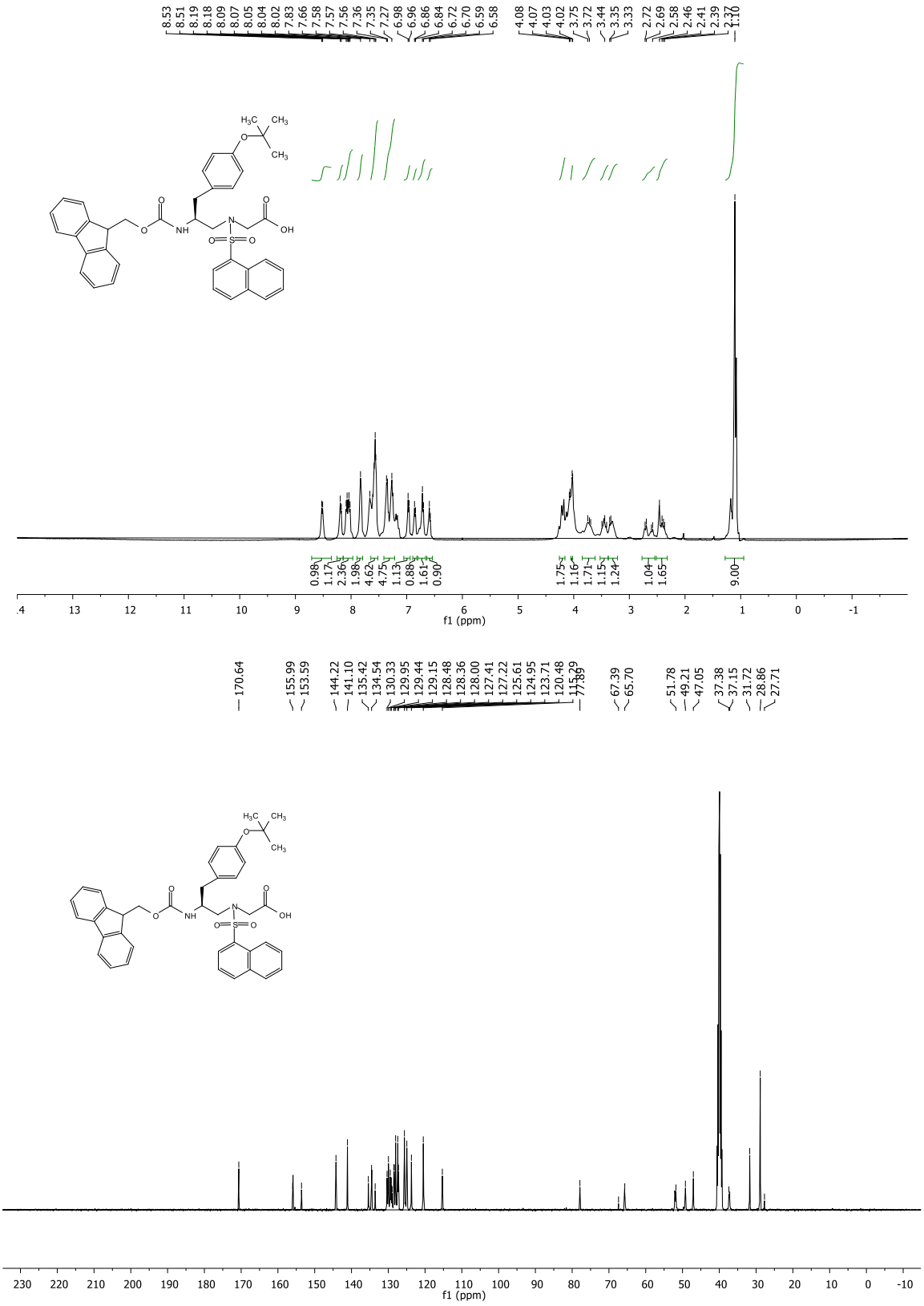
3. Johnson, B. A., Using NMRView to visualize and analyze the NMR spectra of macromolecules. In *Protein NMR Techniques*, Springer: 2004; pp 313-352.

4. Johnson, B. A.; Blevins, R. A., NMR View: A computer program for the visualization and analysis of NMR data. ***J***. *Biomol.* ***NMR*** **1994,** *4*, 603-614.

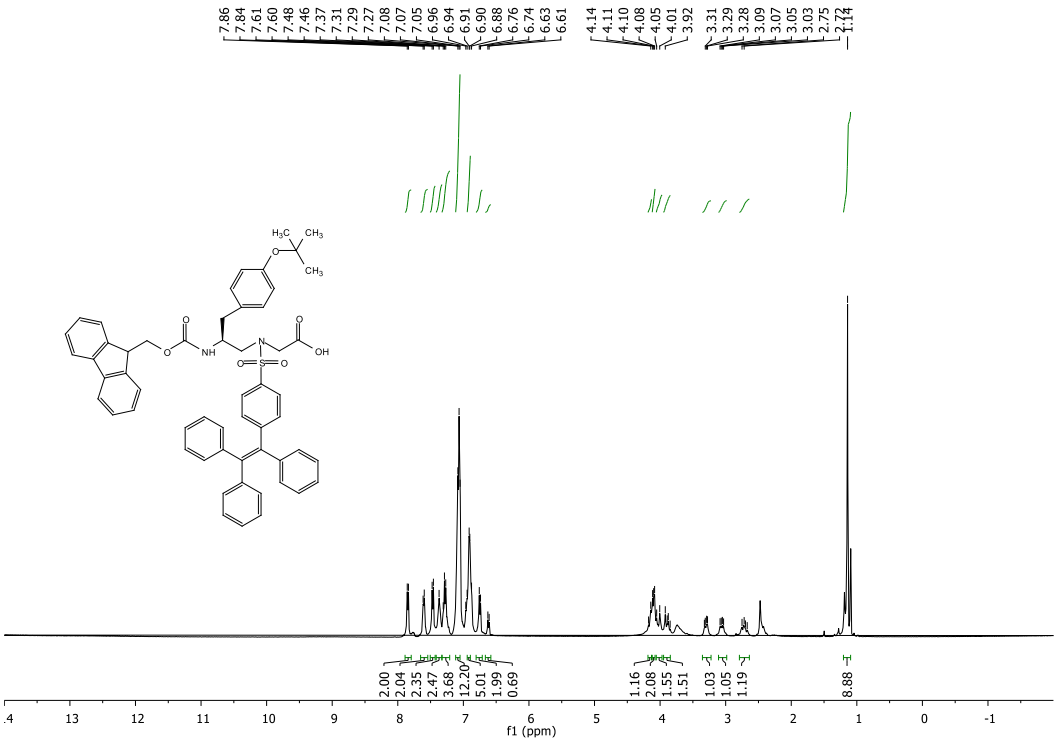
9. The ^{1}H and ^{13}C NMR Spectra of Sulfono-γ-AApeptide Building Blocks

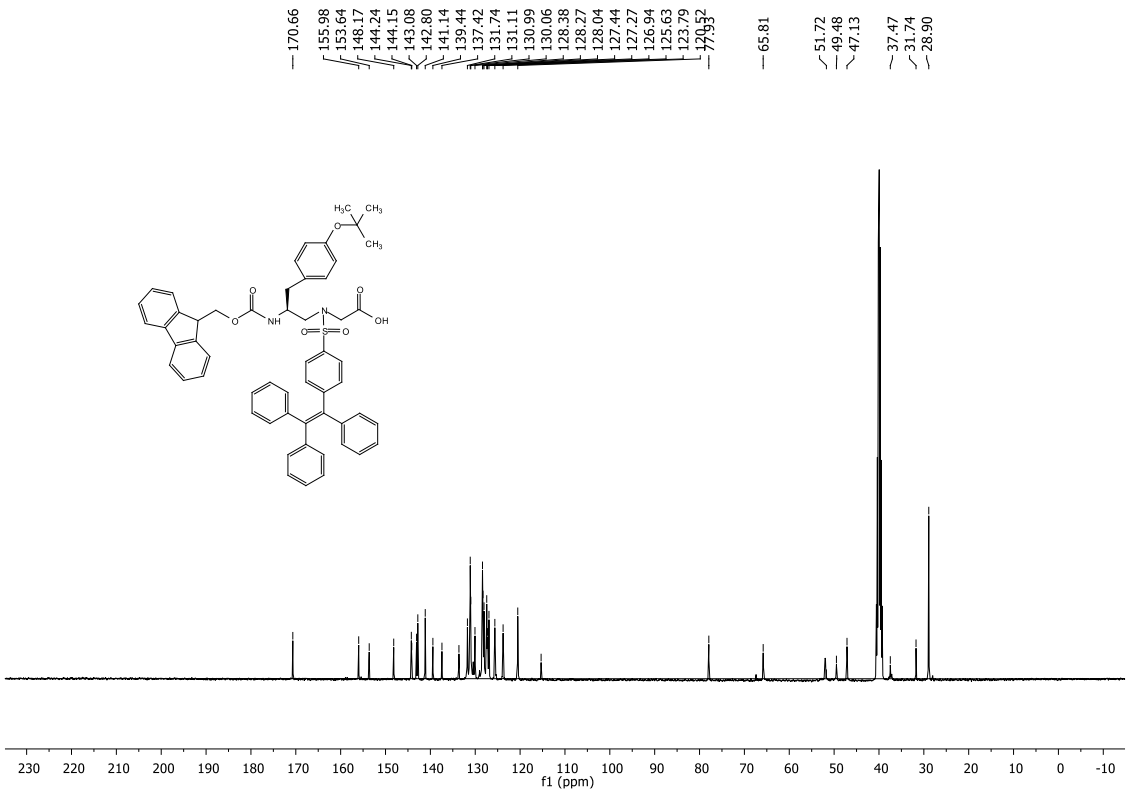


^{1}H and ^{13}C NMR Spectra of BB1

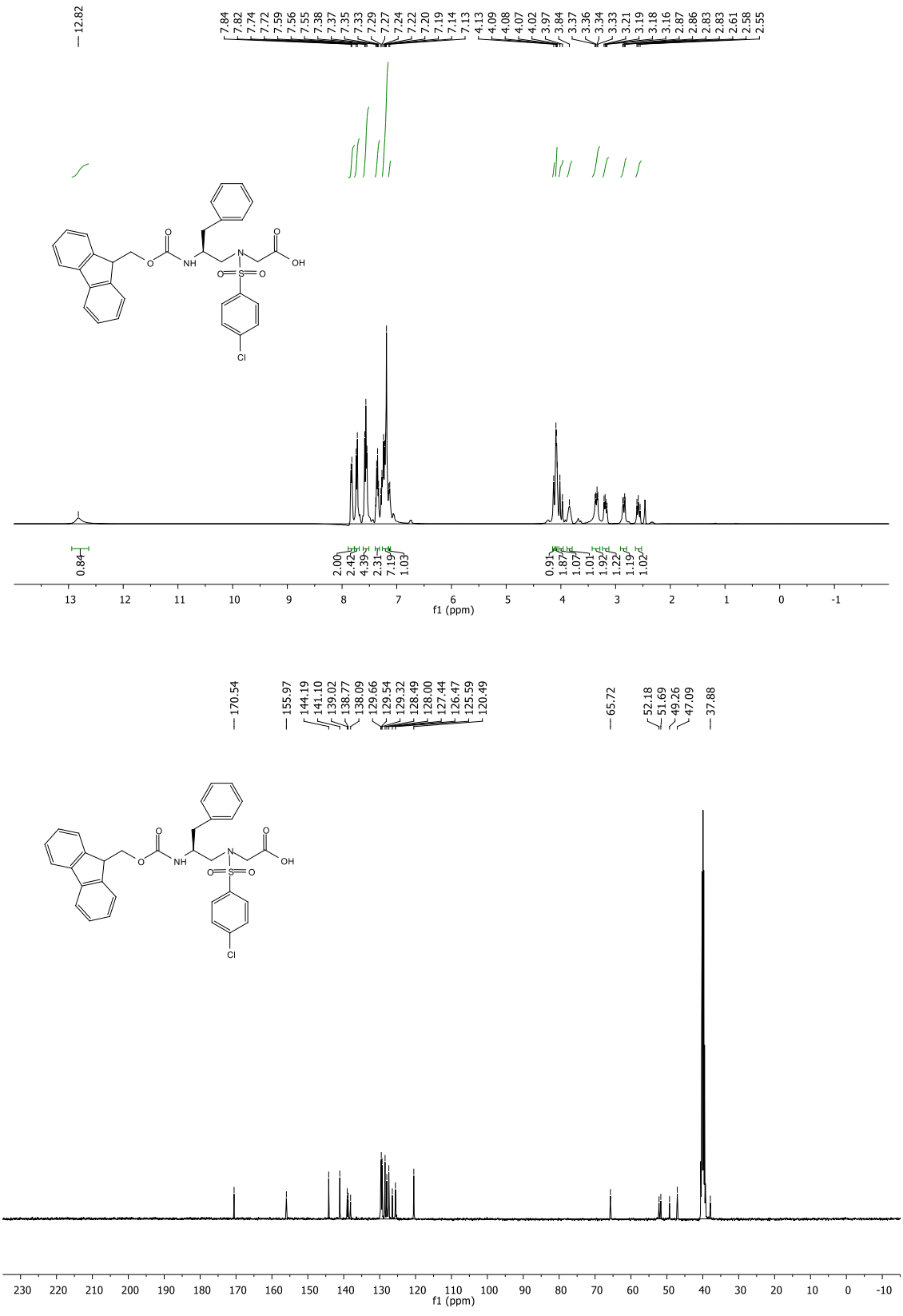


^{1}H and ^{13}C NMR Spectra of BB2





^{1}H and ^{13}C NMR Spectra of BB3



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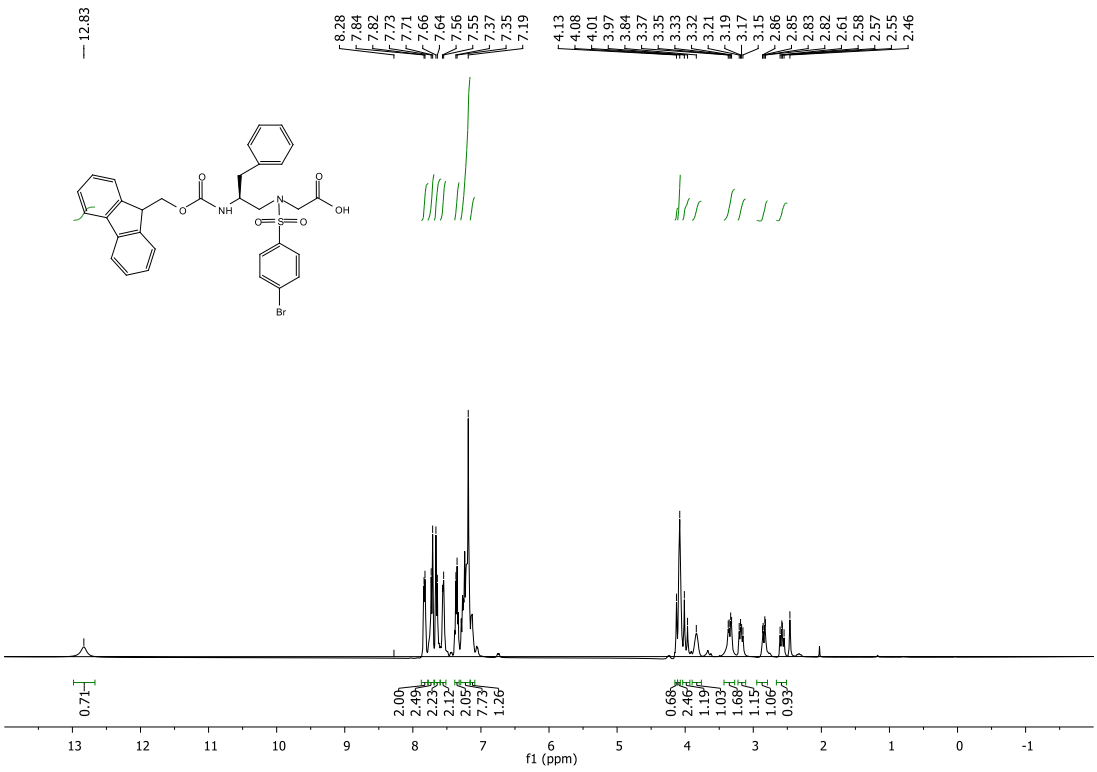
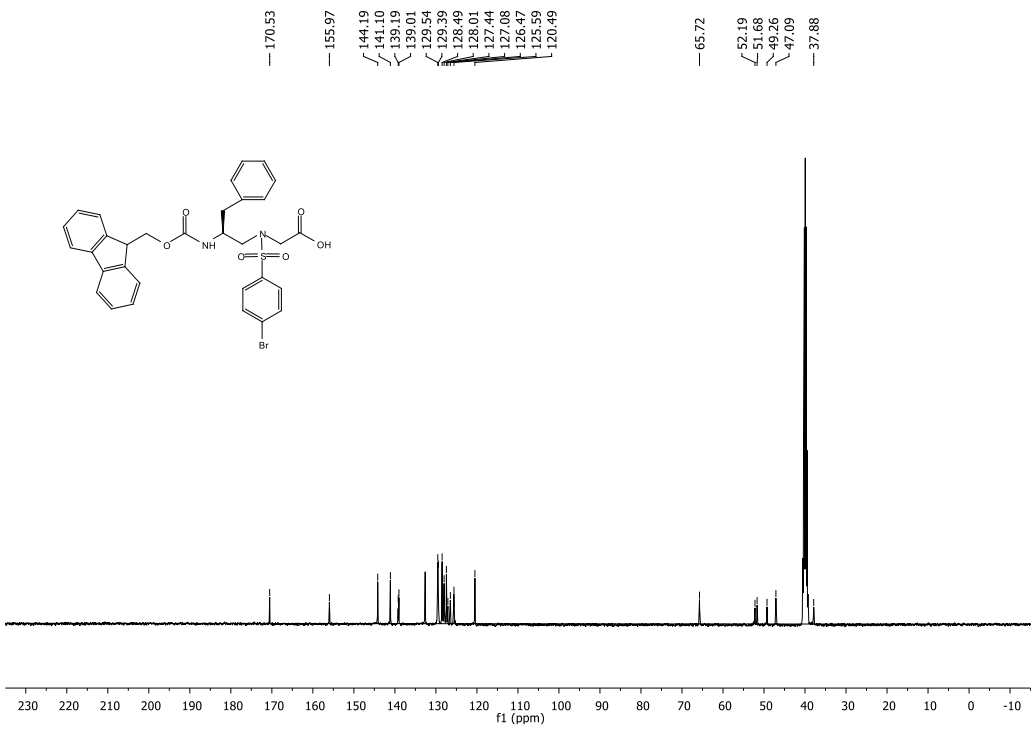
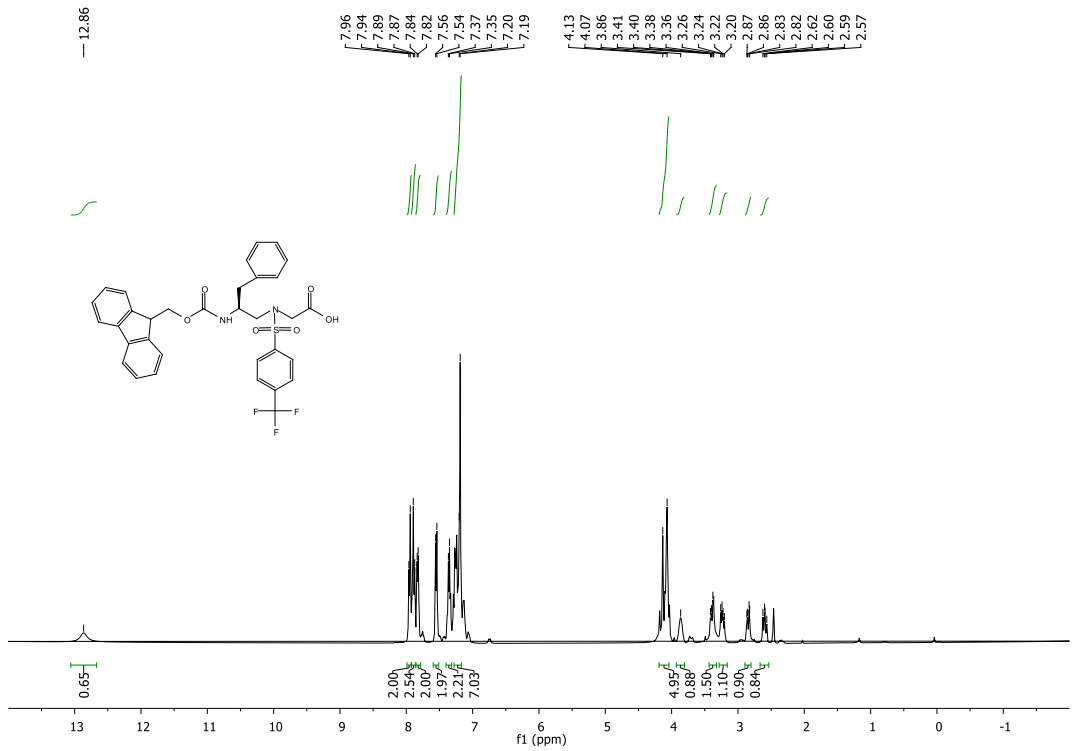
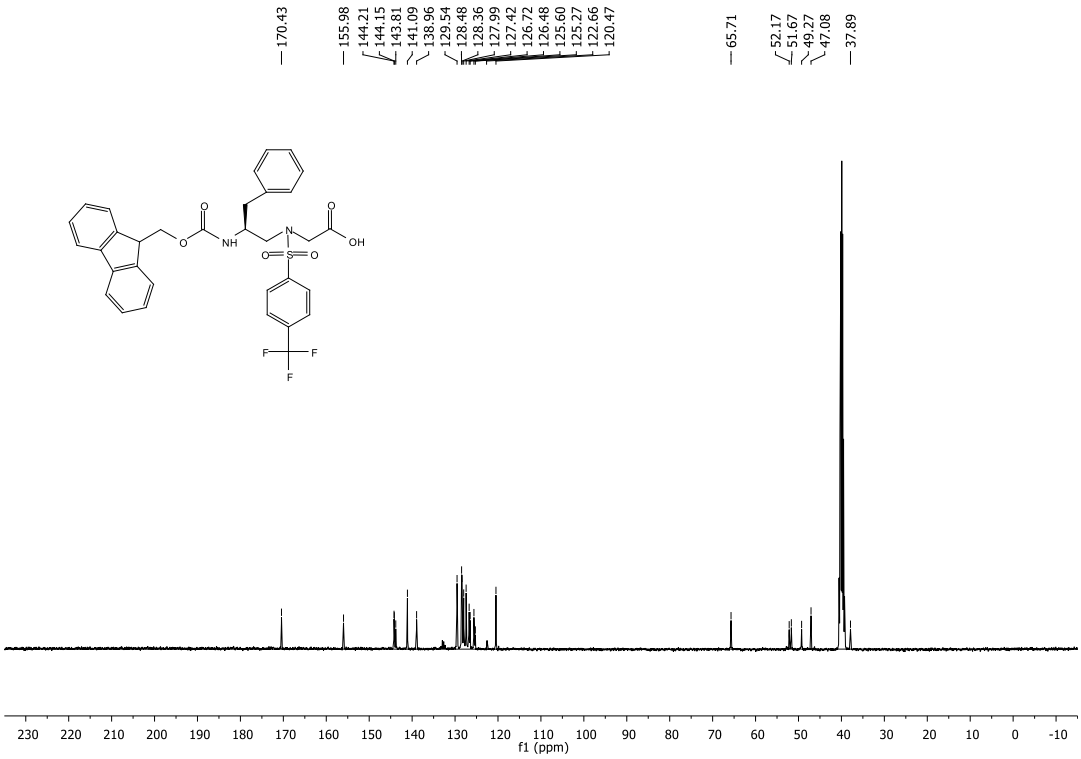


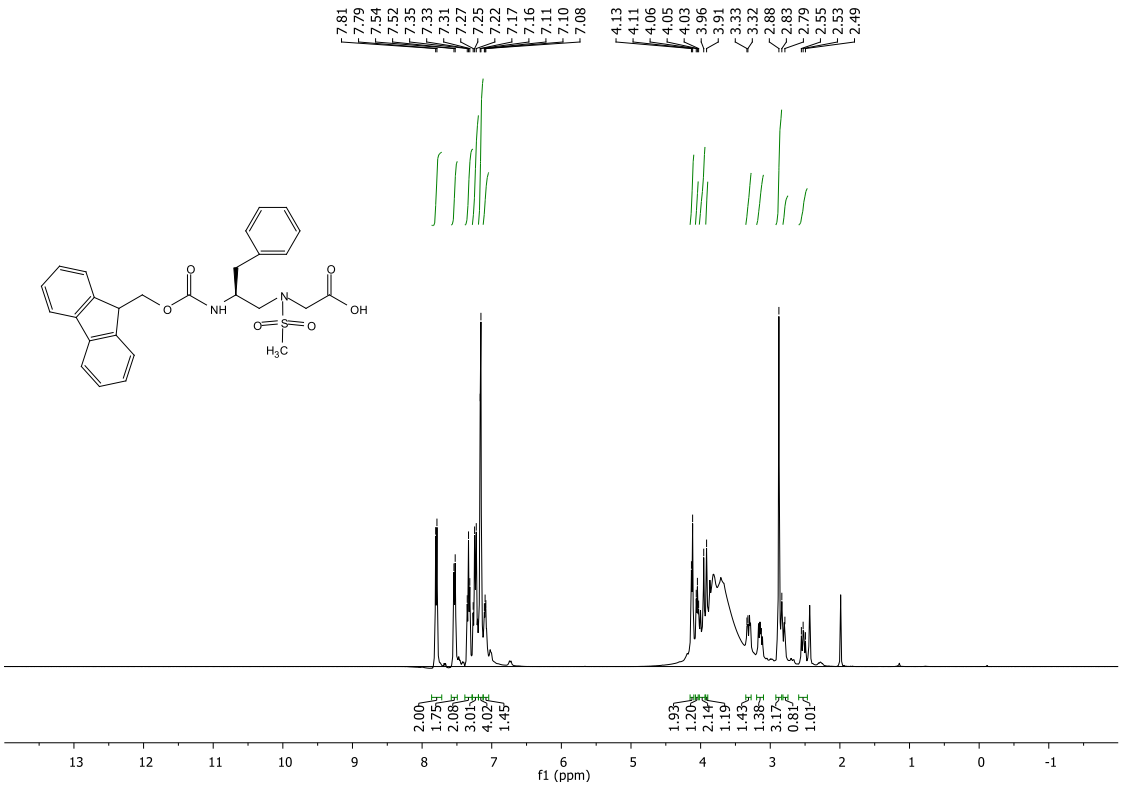


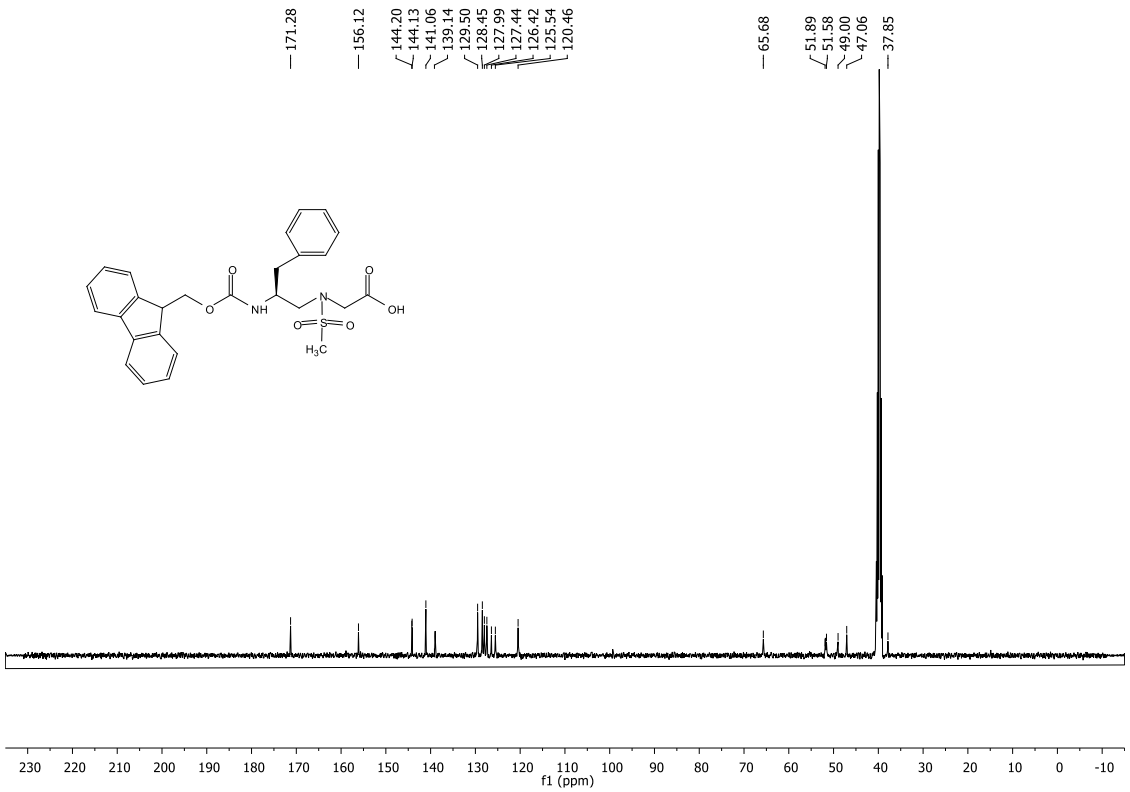
^{1}H and ^{13}C NMR Spectra of BB5



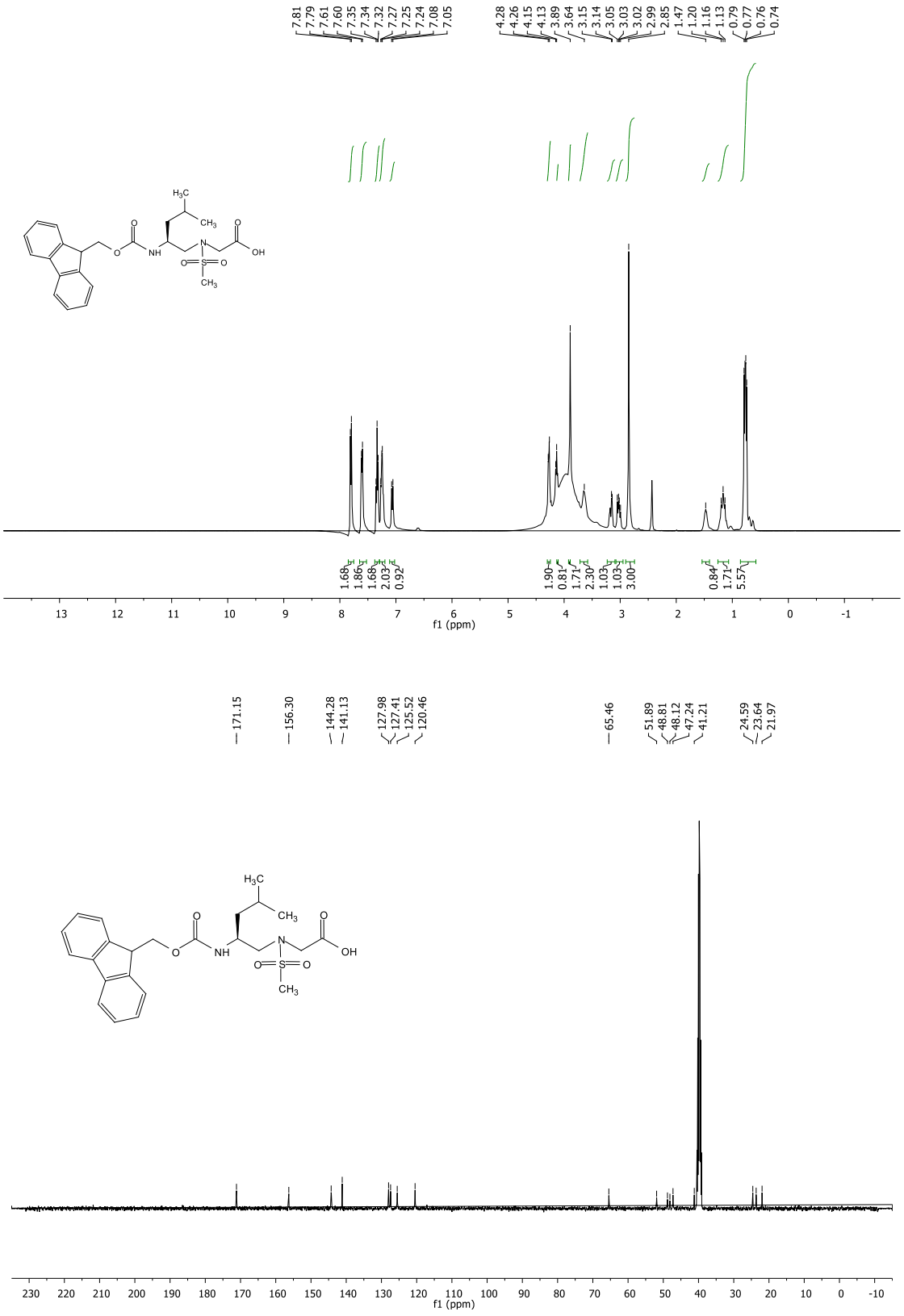


^{1}H and ^{13}C NMR Spectra of BB6

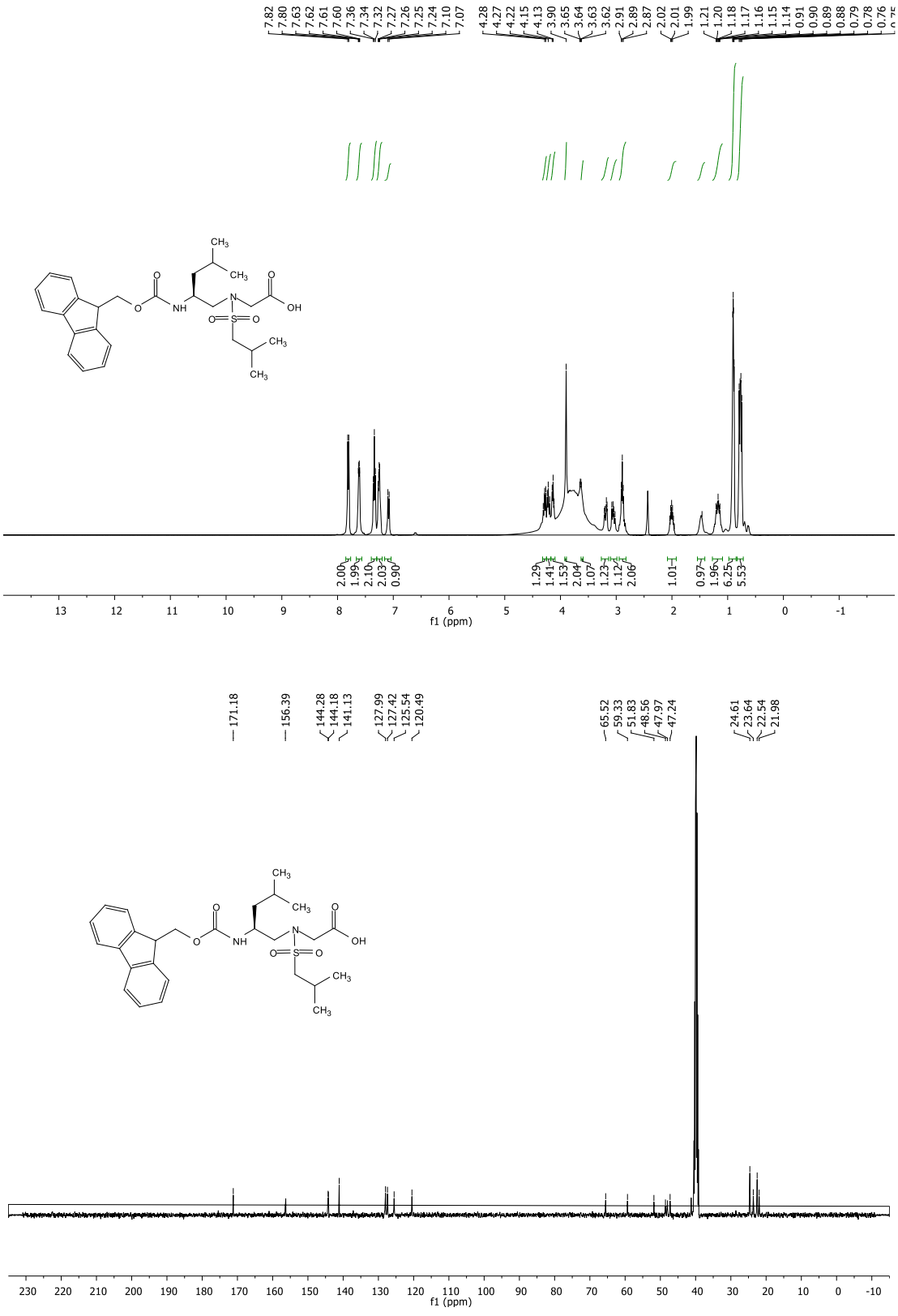




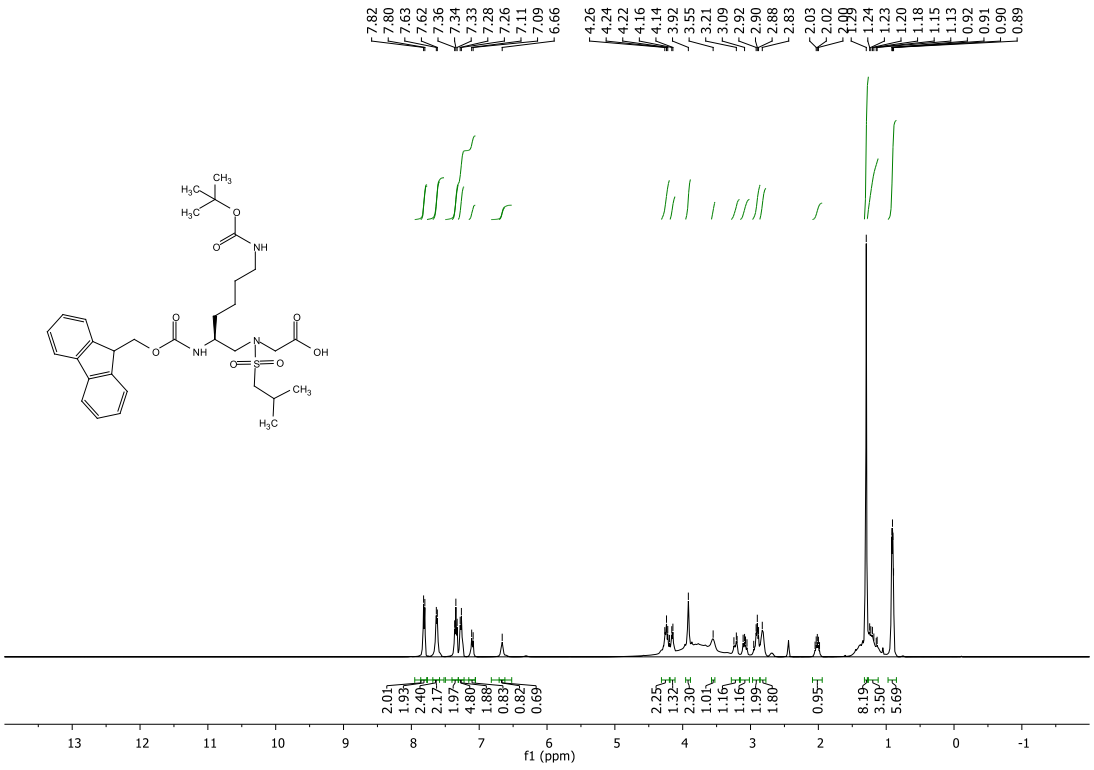
^{1}H and ^{13}C NMR Spectra of BB7

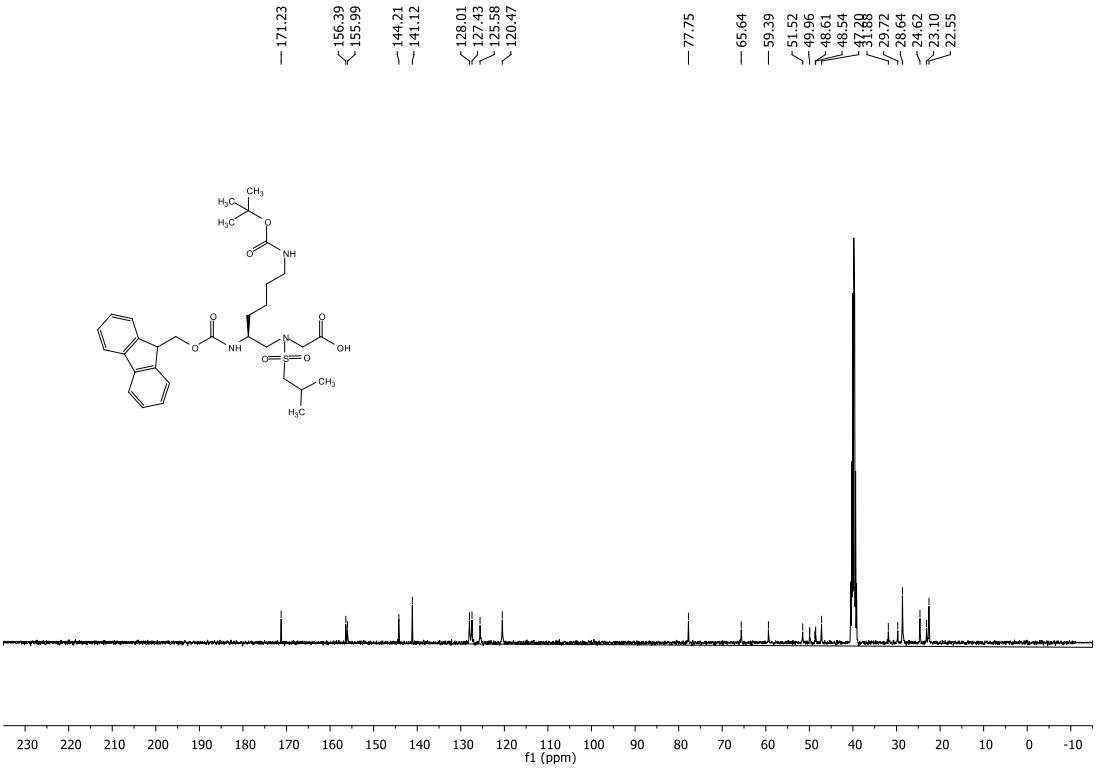


^{1}H and ^{13}C NMR Spectra of BB8

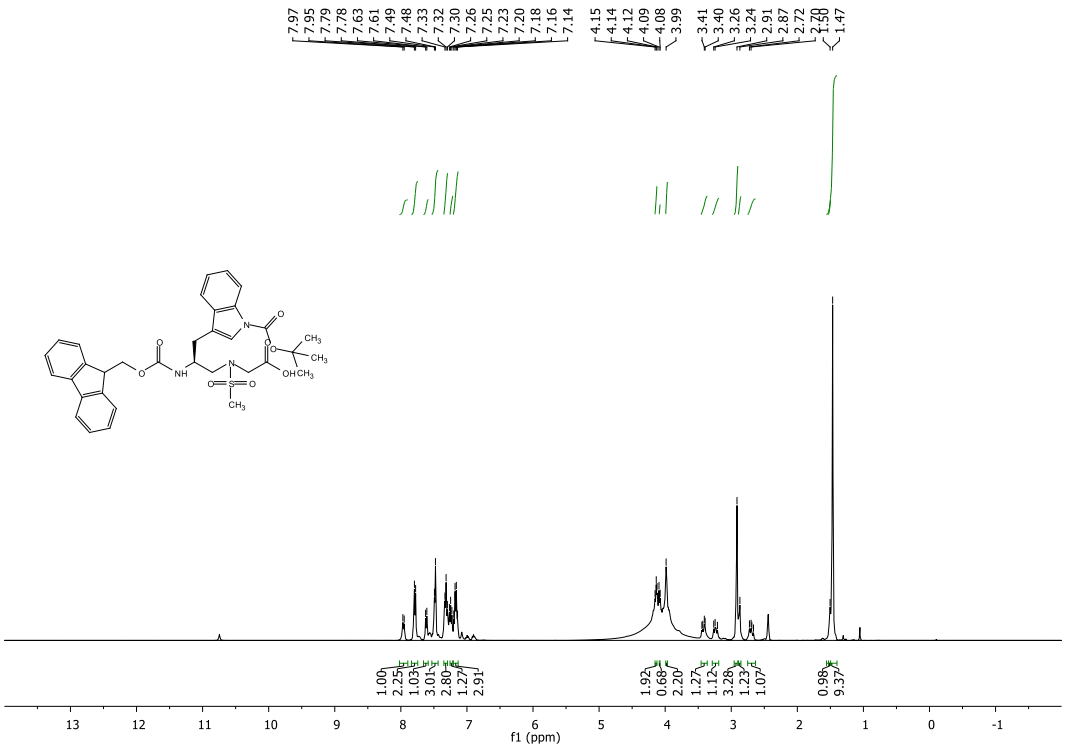


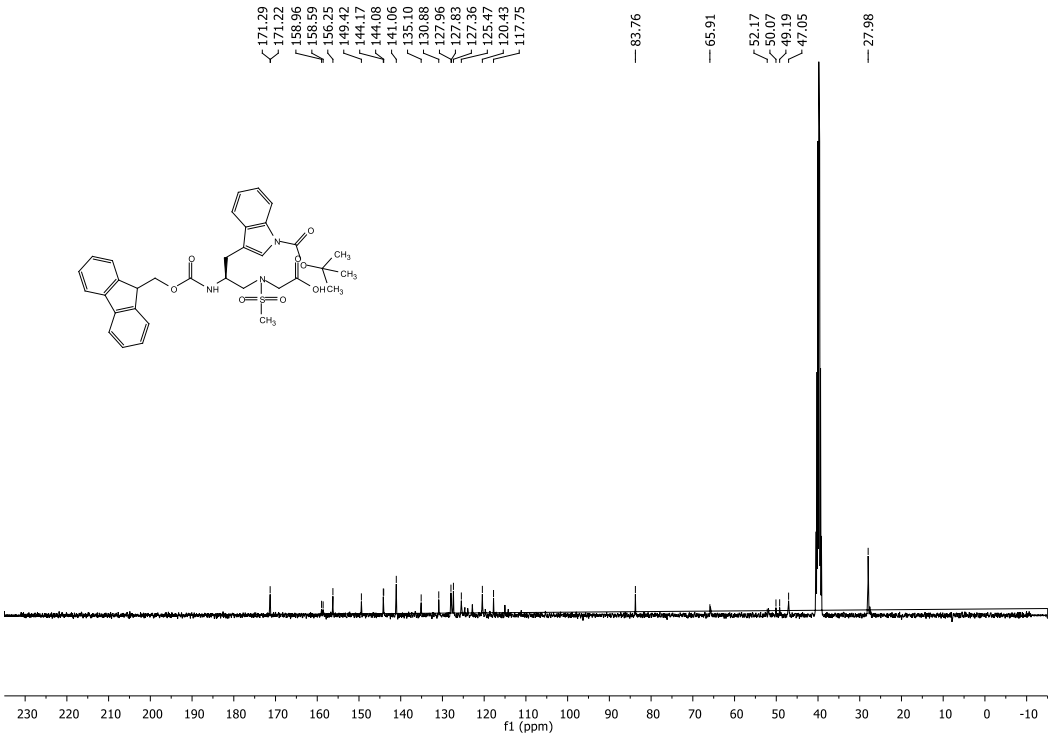
^{1}H and ^{13}C NMR Spectra of BB9



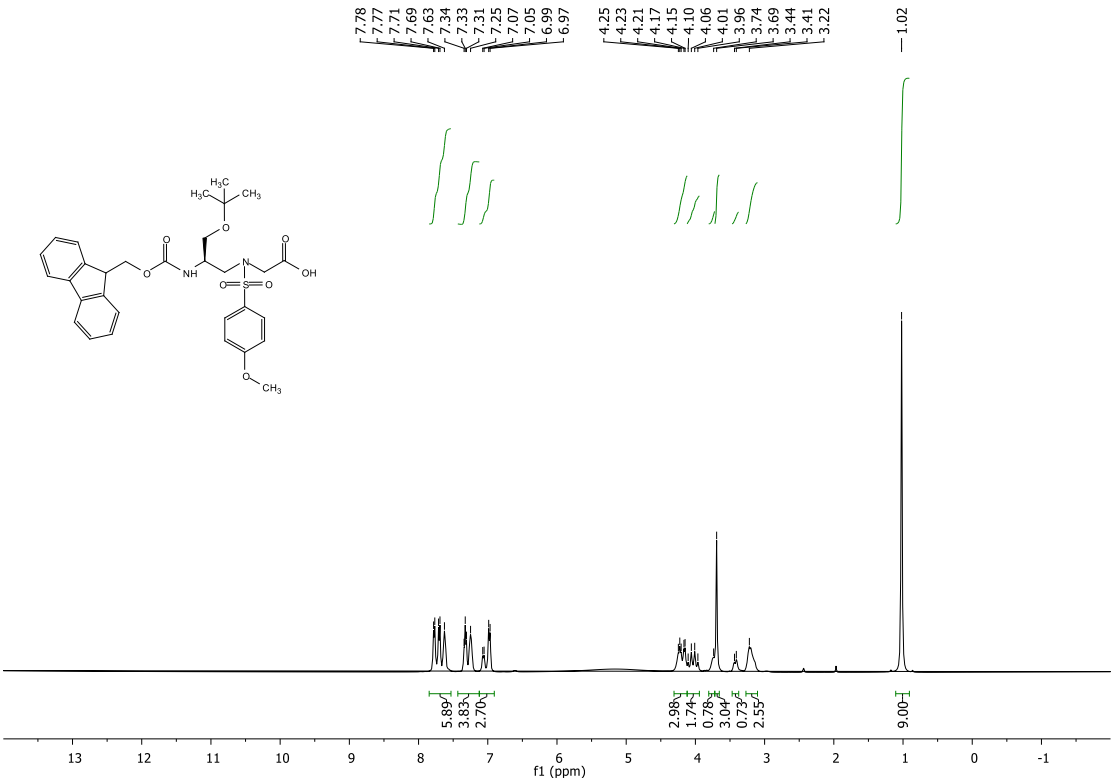


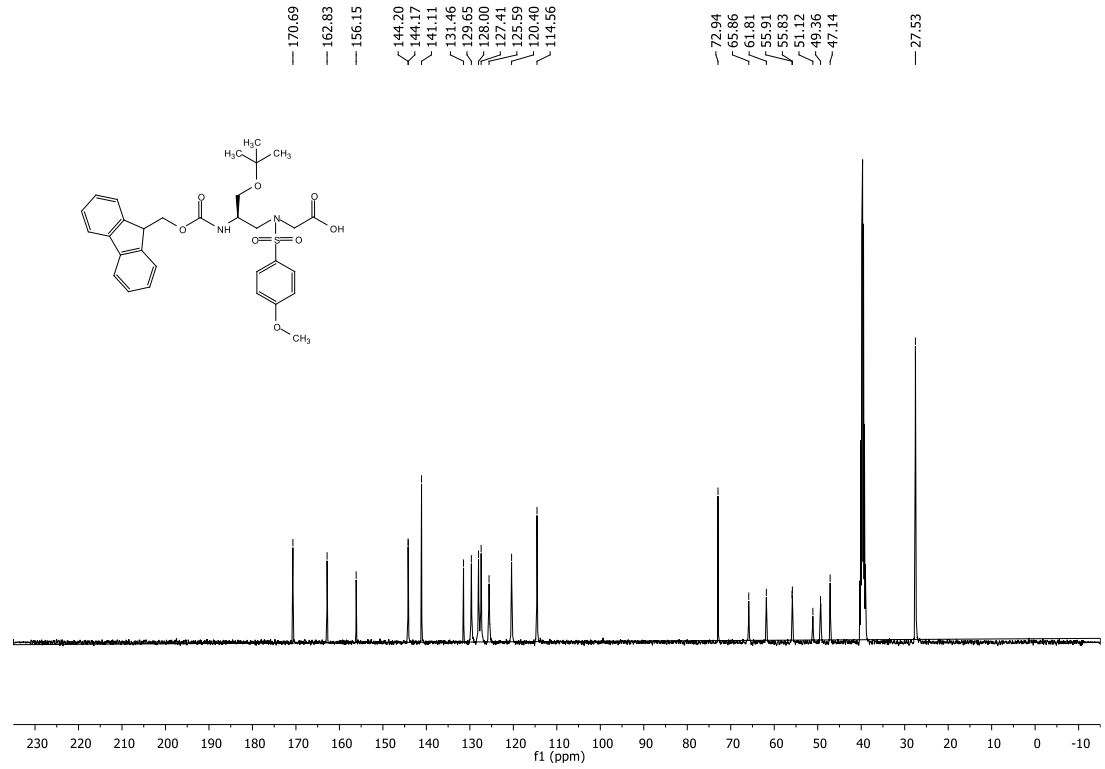
^{1}H and ^{13}C NMR Spectra of BB10



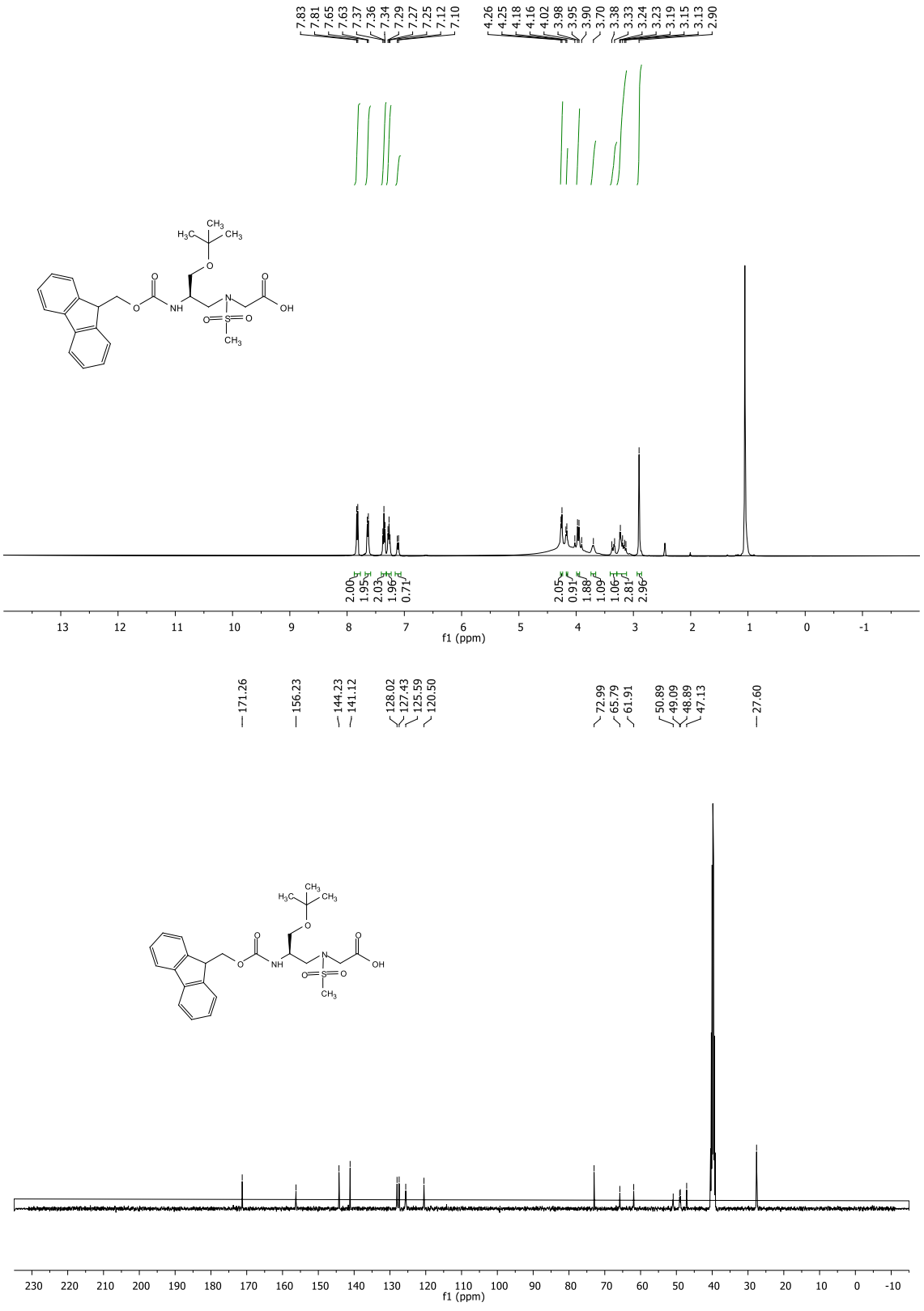


^{1}H and ^{13}C NMR Spectra of BB11

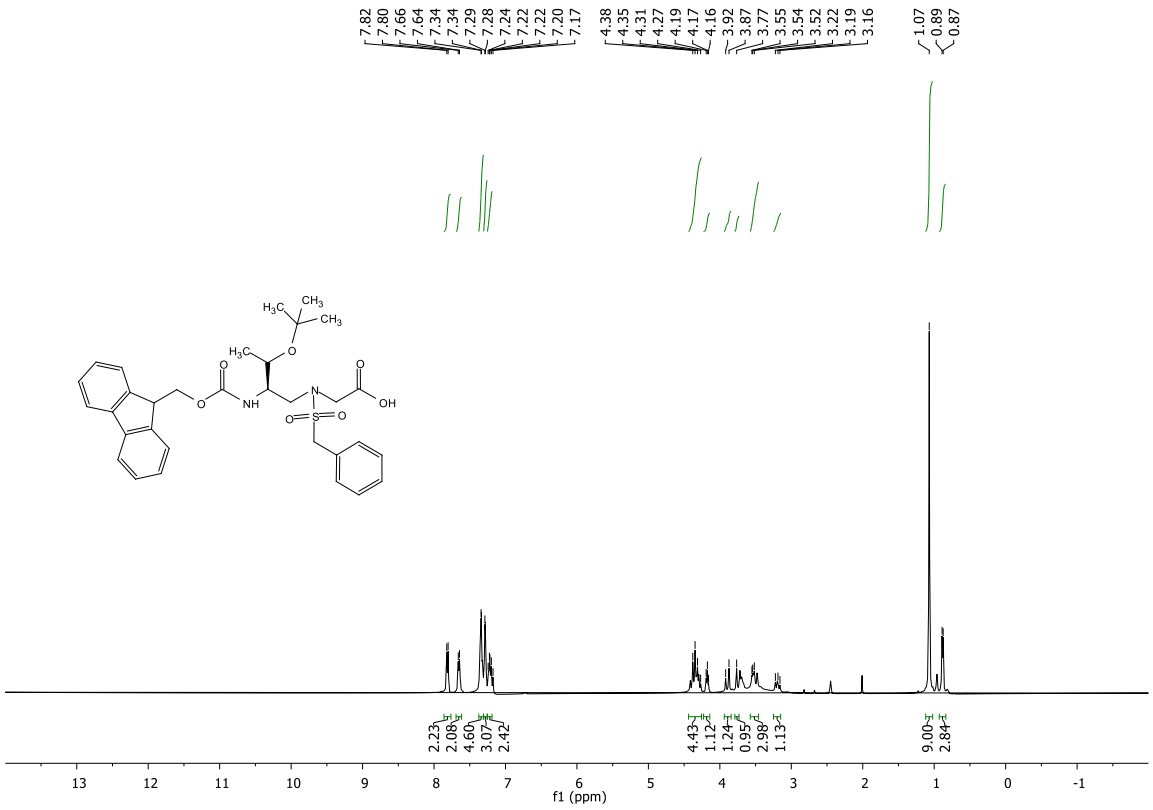


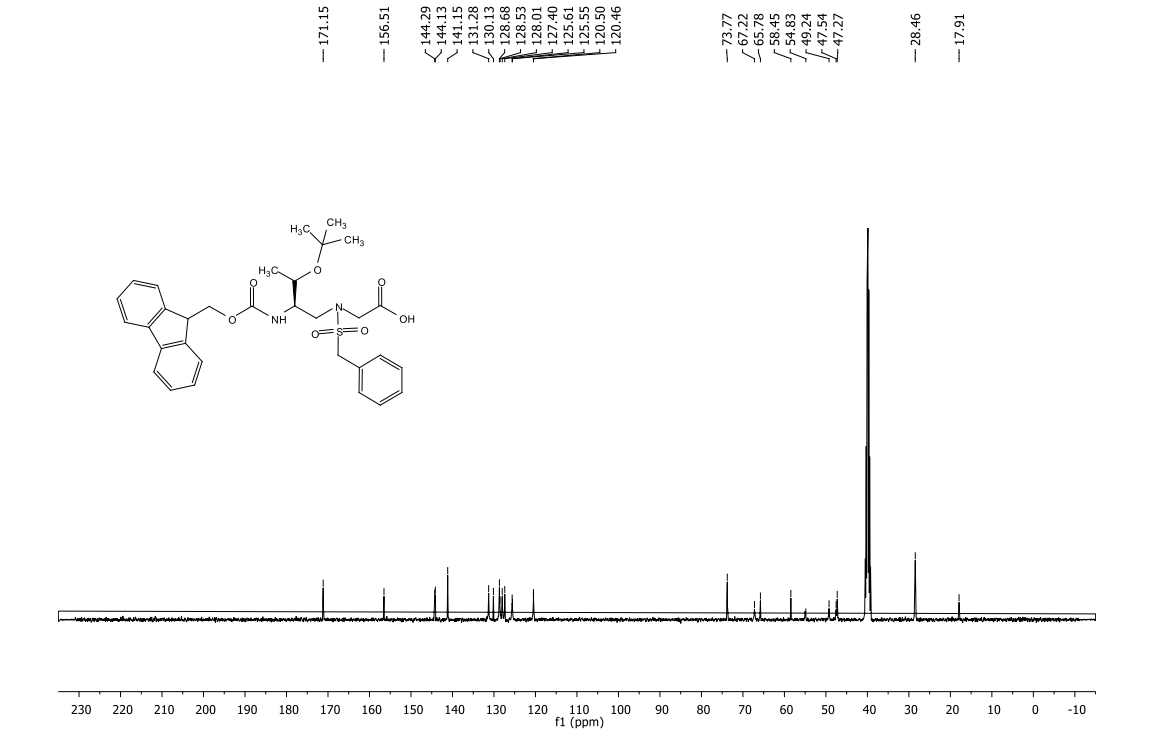


^{1}H and ^{13}C NMR Spectra of BB12

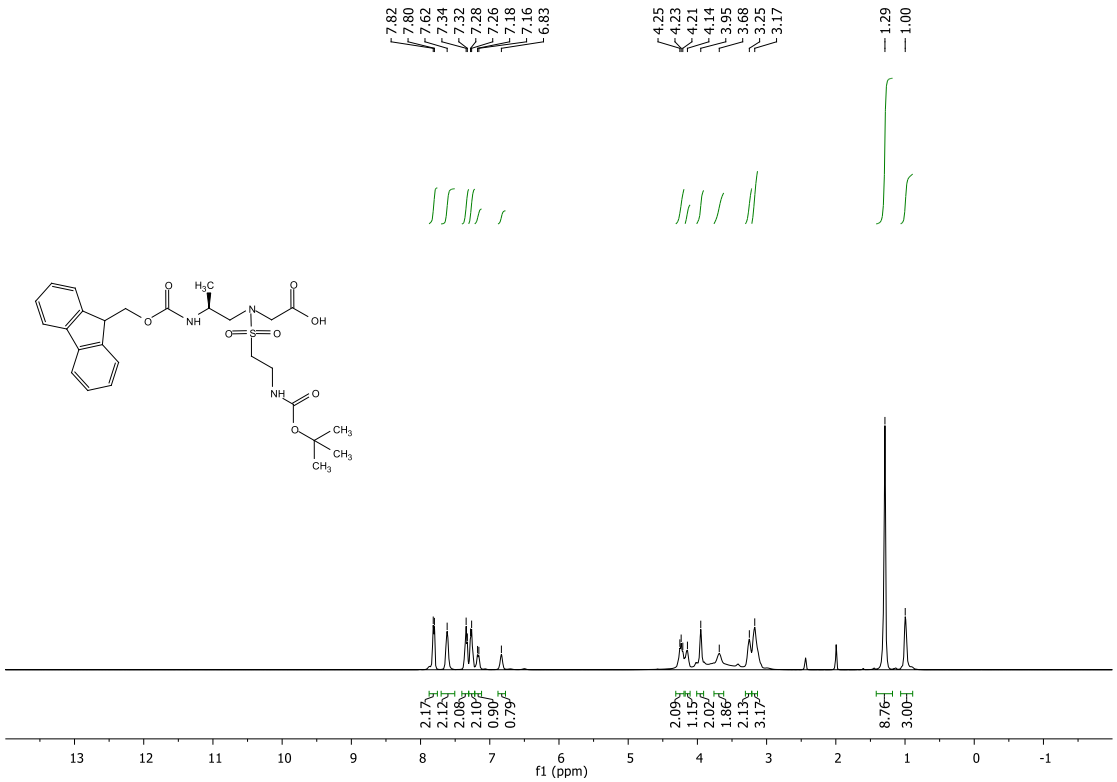


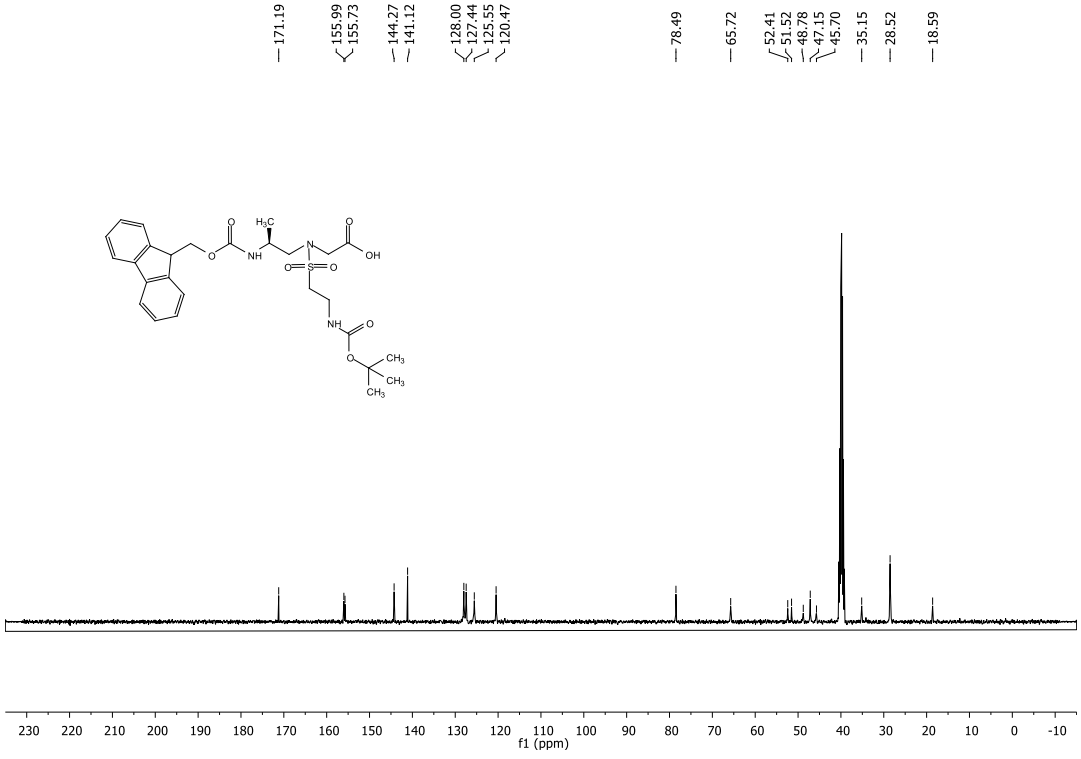
^{1}H and ^{13}C NMR Spectra of BB13





^{1}H and ^{13}C NMR Spectra of BB14





^{1}H and ^{13}C NMR Spectra of BB15