Supporting Information

Inhibition of β−Catenin/ B-Cell Lymphoma 9 Protein−Protein Interaction using α-Helix-Mimicking Sulfono-γ-AApeptide Inhibitors

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Table of Contents

1. Sulfono-γ-AApeptide Building Block Preparation

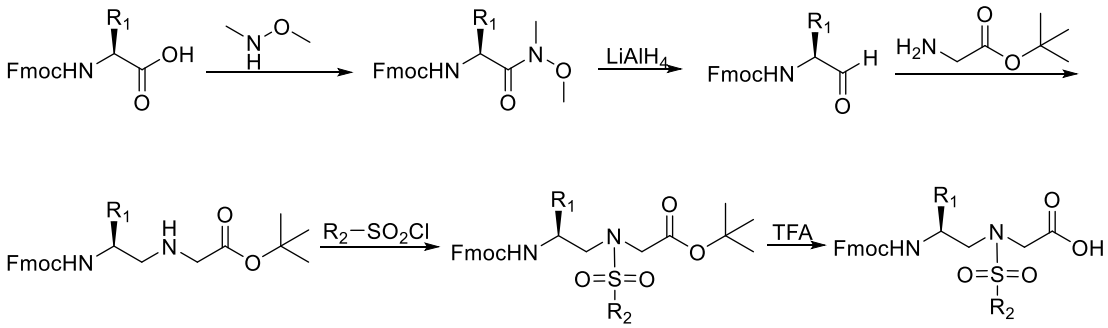
1.1 General Information

Fmoc-protected amino acids and Fluorescein isothiocyanate (FITC) were purchased from Chem-impex (Wood Dale, IL). D-biotin was purchased from Sigma-Aldrich, Inc. Rink Amide-MBHA resin (0.646 mmol/g) was purchased from GL Biochem (Shanghai) Ltd. 1-Hydroxybenzotriazole wetted with no less than 20% wt. water (HOBt), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*, *N*-Diisopropylethylamine (DIPEA) were purchased from Oakwood Chemical (Estill, SC). Thin layer chromatography was performed on Sorbtech TLC plates (silica gel w/UV254), visualizing with UV-light 254 nm. Flash column chromatography was performed with ICN silica gel (60 Å, 230-400 mesh, 32-63 μm). ^{1}H NMR spectra were recorded at 400 MHz using TMS as internal standard. ^{13}C NMR spectra were recorded at 100 MHz using TMS as internal standard. The multiplicities are reported as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), multiplet (m). Coupling constants are reported in Hertz (Hz). High resolution mass spectra were obtained on an Agilent 6220 using electrospray ionization time-of-flight (ESI-TOF). Other chemicals and all solvents were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher and used without further purification.

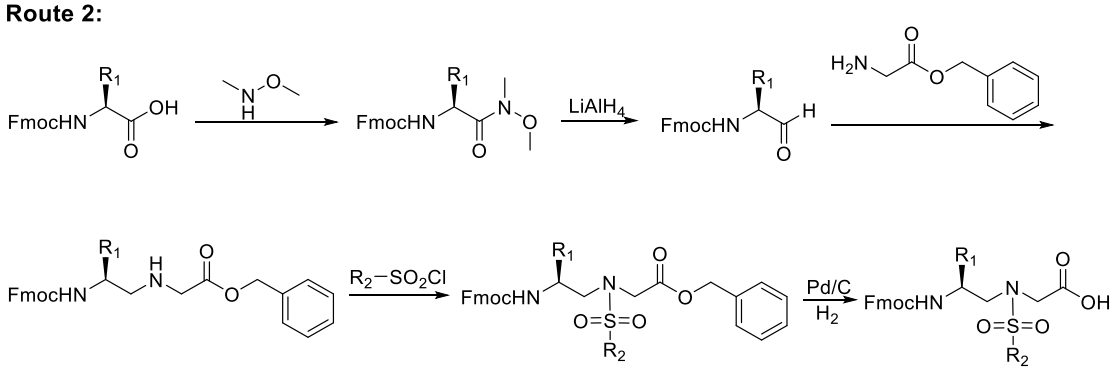
1.2 Synthetic Routes

The sulfono-γ-AApeptide building blocks **BB1-14** were synthesized based on previously report and Fmoc-protected amino acids were used as the initial starting materials.[1] **BB1-7** were synthesized based on route **1**, **BB8-10** were synthesized based on route **2**, **BB11-14** were synthesized based on route **3** (Scheme S1).

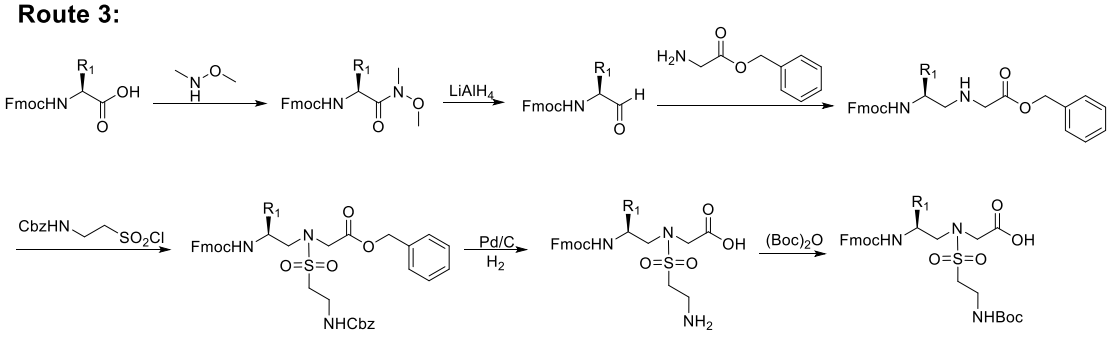
Route 1:



Route 2:

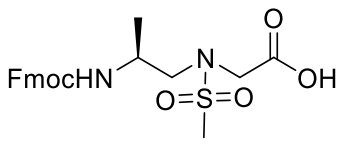


Route 3:



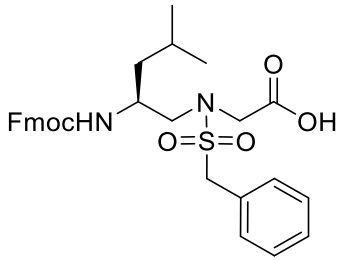
Scheme S1. General synthetic route to prepare sulfono-γ-AApeptide building blocks.

1.3 Characterization of Sulfono-γ-AApeptide Building Blocks



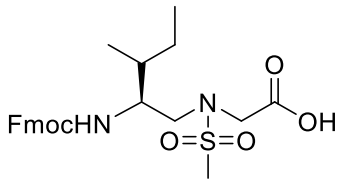
BB1

(S)-*N*-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propyl)-*N*-(methylsulfonyl)glycine (BB1). ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.85 (d, *J* = 7.60 Hz, 2H), 7.65 (d, *J* = 6.40 Hz, 2H), 7.38 (t, *J* = 7.20 Hz, 2H), 7.30 (t, *J* = 6.80 Hz, 2H), 7.18 (d, *J* = 8.40 Hz, 1H), 4.24-4.33 (m, 2H), 4.19 (d, *J* = 6.40 Hz, 1H), 3.96 (s, 2H), 3.72-3.74 (m, 1H), 3.13-3.18 (m, 2H), 2.91 (s, 3H), 1.01 (d, *J* = 6.00 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 171.3, 156.0, 144.3, 141.2, 128.0, 127.5, 125.6, 120.5, 65.7, 52.5, 49.0, 47.2, 45.8, 18.6. HRMS (ESI) ([M+H]$^{+}$) Calcd. for $C_{21}H_{25}N_2O_6S$: 433.1433, found: 433.1434.



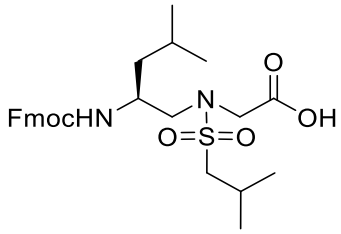
BB2

(S)-*N*-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentyl)-N-(benzy lsulfonyl)glycine (BB2). ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.83 (d, *J* = 7.60 Hz, 2H), 7.64 (d, *J* = 7.20 Hz, 2H), 7.36-7.38 (m, 3H), 7.34 (s, 1H), 7.31 (t, *J* = 4.00 Hz, 3H), 7.25 (t, *J* = 7.20 Hz, 2H), 7.17 (d, *J* = 9.20 Hz, 1H), 4.41 (t, *J* = 13.60 Hz, 2H), 4.34 (q, *J* = 6.40, 3.60 Hz, 1H), 4.28 (q, *J* = 10.40, 6.80 Hz, 1H), 4.17 (t, *J* = 6.40 Hz, 1H), 3.91 (q, *J* = 28.00, 18.80 Hz, 2H), 3.66-3.71 (m, 1H), 3.24 (dd, *J* = 14.80, 5.60 Hz, 1H), 3.07 (q, *J* = 14.40, 8.80 Hz, 1H), 1.46-1.55 (m, 1H), 1.18-1.28 (m, 2H), 0.81 (q, *J* = 10.00, 6.00 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 171.2, 156.4, 144.3, 144.2, 141.2, 131.3, 130.1, 128.7, 128.5, 128.0, 127.4, 125.6, 120.5, 65.6, 57.8, 52.7, 49.3, 48.2, 47.3, 41.3, 24.6, 23.7, 22.0. HRMS (ESI) ([M+H]$^{+}$) Calcd. for $C_{30}H_{35}N_2O_6S$: 551.2216, found: 551.2211.



BB3

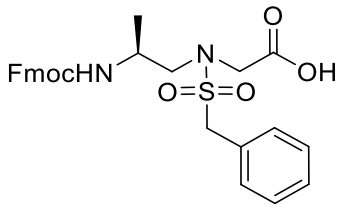
***N*-((2S,3R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylpentyl)-N-(methylsulfonyl)glycine (BB3).** ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.84 (d, *J* = 7.20 Hz, 2H), 7.67 (d, *J* = 7.60 Hz, 2H), 7.37 (t, *J* = 7.60 Hz, 2H), 7.26-7.31 (m, 2H), 7.17 (d, *J* = 9.60 Hz, 1H), 4.31 (d, *J* = 7.20 Hz, 2H), 4.19 (t, *J* = 6.40 Hz, 1H), 3.97 (q, *J* = 21.20, 19.20 Hz, 2H), 3.56-3.61 (m, 1H), 3.42 (dd, *J* = 14.40, 2.80 Hz, 1H), 3.09 (q, *J* = 14.40, 10.40 Hz, 1H), 2.91 (s, 3H), 1.31-1.40 (m, 2H), 0.99-1.08 (m, 1H), 0.80 (t, *J* = 6.80 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 171.3, 156.5, 144.3, 141.2, 128.0, 127.4, 125.6, 120.5, 65.6, 54.4, 48.9, 48.7, 47.3, 37.6, 25.1, 15.6, 11.8. HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{24}H_{31}N_2O_6S$: 475.1903, found: 475.1912.



BB4

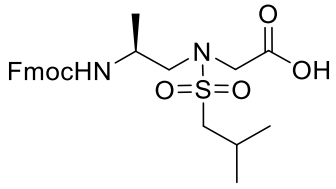
(S)-*N*-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentyl)-*N*-(isobu tylsulfonyl)glycine (BB4). ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.85 (d, *J* = 7.20 Hz, 2H), 7.66 (t, *J* = 3.20 Hz, 2H), 7.38 (t, *J* = 6.80 Hz, 2H), 7.27-7.31 (m, 2H), 7.13 (d, *J* = 8.80 Hz, 1H), 4.33 (q, *J* = 10.00, 7.20 Hz, 1H), 4.26 (t, *J* = 6.80 Hz, 1H), 4.16 (t, *J* = 6.40 Hz, 1H), 3.94 (s, 2H), 3.68-3.69 (m, 1H), 3.22-3.26 (m, 1H), 3.09 (q, *J* = 14.00, 8.80 Hz, 1H), 2.88-2.99 (m, 2H), 2.02-2.08 (m, 1H), 1.52 (brs, 1H), 1.18-1.25 (m, 2H), 0.93-0.95 (m, 6H), 0.81 (dd, *J* = 12.00, 6.40 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 171.2, 156.3, 144.3, 144.2, 141.2, 128.0, 127.4, 125.6, 120.5, 65.6, 59.4,

51.9, 48.6, 48.1, 47.3, 41.3, 24.7, 23.7, 22.6, 22.0. HRMS (ESI) ([M+H]$^+$) Calcd. for $C_{27}H_{37}N_2O_6S$: 517.2372, found: 517.2370.



BB5

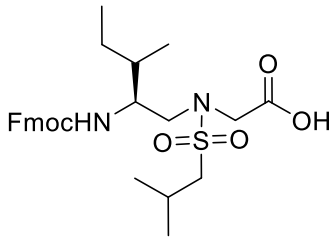
(S)-*N*-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propyl)-N-(benzylsulfonyl) glycine (BB5). ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.84 (d, *J* = 7.60 Hz, 2H), 7.64 (d, *J* = 7.20 Hz, 2H), 7.31-7.38 (m, 7H), 7.25 (q, *J* = 15.20, 7.60 Hz, 3H), 4.41 (q, *J* = 22.80, 9.60 Hz, 2H), 4.28 (d, *J* = 6.80 Hz, 2H), 4.17 (t, *J* = 6.40 Hz, 1H), 3.90 (q, *J* = 32.00, 18.40 Hz, 1H), 3.72 (q, *J* = 13.20, 6.40 Hz, 1H), 3.10-3.24 (m, 2H), 1.02 (d, *J* = 6.40 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 171.3, 156.1, 144.3, 141.1, 131.3, 130.1, 128.7, 128.6, 128.0, 127.5, 125.6, 120.5, 65.8, 57.7, 53.2, 49.5, 47.2, 45.9, 32.0, 18.7. HRMS (ESI) ([M+H]$^+$) Calcd. for $C_{27}H_{29}N_2O_6S$: 509.1746, found: 509.1740.



BB6

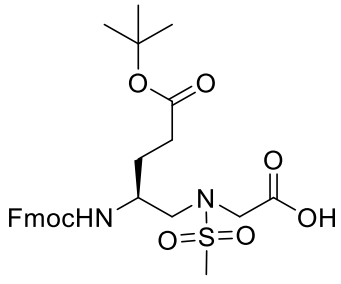
(S)-*N*-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propyl)-N-(isobutylsulfony l)glycine (BB6). ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.81 (d, *J* = 7.60 Hz, 2H), 7.62 (d, *J* = 6.40 Hz, 2H), 7.34 (t, *J* = 7.20 Hz, 2H), 7.26 (t, *J* = 7.20 Hz, 2H), 7.16 (d, *J* = 9.60 Hz, 1H), 4.22 (d, *J* = 6.40 Hz, 2H), 4.14 (d, *J* = 6.40 Hz, 1H), 3.92 (s, 2H), 3.68 (t, *J* = 6.40 Hz, 1H), 3.11-3.16 (m, 2H), 2.91 (t, *J* = 6.00 Hz, 2H), 1.97-2.07 (m, 1H), 0.97 (d, *J* = 6.40 Hz, 3H), 0.92 (d, *J* = 1.60 Hz, 3H), 0.90 (s, 3H). ^{13}C NMR (100

MHz, DMSO-*d*6): δ 171.2, 156.0, 144.2, 141.1, 128.0, 127.4, 125.6, 120.5, 65.7, 59.3, 52.4, 48.7, 47.1, 45.7, 24.6, 22.5, 18.7. HRMS (ESI) ([M+H]$^+$) Calcd. for $C_{24}H_{31}N_2O_6S$: 475.1903, found: 475.1908.



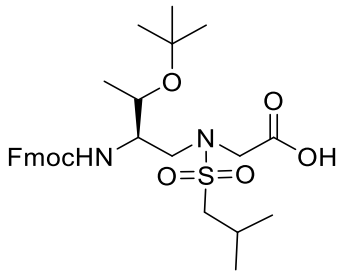
BB7

***N*-((2S,3S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylpentyl)-N-(isobutylsulfonyl)glycine (BB7).** ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.84 (d, *J* = 7.20 Hz, 2H), 7.67 (d, *J* = 7.20 Hz, 2H), 7.37 (t, *J* = 7.20 Hz, 2H), 7.26-7.30 (m, 2H), 7.19 (d, *J* = 9.60 Hz, 1H), 4.31 (q, *J* = 10.00, 7.20 Hz, 1H), 4.24 (t, *J* = 6.80 Hz, 1H), 4.18 (t, *J* = 6.80 Hz, 1H), 3.95 (q, *J* = 28.40, 18.40 Hz, 2H), 3.54-3.59 (m, 1H), 3.43 (dd, *J* = 14.80, 2.80 Hz, 1H), 3.10 (q, *J* = 14.40, 10.40 Hz, 1H), 2.98 (q, *J* = 14.00, 6.40 Hz, 1H), 2.88 (q, *J* = 14.00, 6.80 Hz, 1H), 1.99-2.09 (m, 1H), 1.28-1.40 (m, 2H), 0.99-1.09 (m,1H), 0.92 (d, *J* = 6.40 Hz, 6H), 0.75-0.81 (m, 5H), 0.62-0.70 (m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 171.3, 156.5, 144.3, 144.2, 141.2, 128.0, 127.4, 125.6, 120.5, 65.7, 59.7, 54.3, 48.6, 47.3, 37.6, 25.1, 24.7, 22.6, 22.5, 15.6, 11.8. HRMS (ESI) ([M+H]$^+$) Calcd. for $C_{27}H_{37}N_2O_6S$: 517.2372, found: 517.2380.



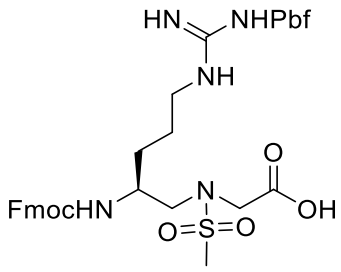
BB8

(S)-*N*-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-(tert-butoxy)-5-oxopentyl)-*N*-(methylsulfonyl)glycine (BB8). ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.87 (d, *J* = 7.60 Hz, 2H), 7.68 (d, *J* = 6.80 Hz, 2H), 7.40 (t, *J* = 6.80 Hz, 2H), 7.32 (t, *J* = 7.20 Hz, 2H), 7.17 (d, *J* = 8.80 Hz, 1H), 4.33 (d, *J* = 6.80 Hz, 2H), 4.22 (d, *J* = 6.40 Hz, 1H), 3.97 (s, 2H), 3.67 (brs, 1H), 3.27-3.30 (m, 1H), 3.14 (q, *J* = 14.40, 8.40 Hz, 1H), 2.93 (s, 3H), 2.18-2.20 (m, 2H), 1.74 (s, 1H), 1.38 (s, 1H), 1.38 (brs, 10 H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 172.3, 171.2, 156.4, 144.3, 141.2, 128.1, 127.5, 125.6, 120.6, 80.0, 65.7, 51.3, 49.4, 48.7, 47.3, 31.8, 28.2, 27.6. HRMS (ESI) ([M+H]$^{+}$) Calcd. for $C_{27}H_{35}N_2O_8S$: 547.2114, found: 547.2119.



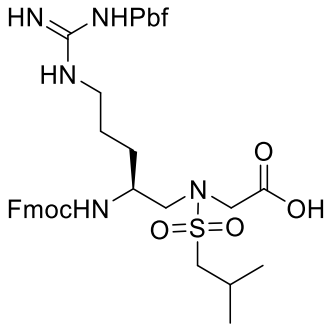
BB9

***N*-((2R,3S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)butyl)-N-(isobutylsulfonyl)glycine (BB9).** ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.84 (d, *J* = 7.20 Hz, 2H), 7.67 (d, *J* = 7.20 Hz, 2H), 7.37 (t, *J* = 7.20 Hz, 2H), 7.27-7.31 (m, 2H), 7.19 (d, *J* = 9.20 Hz, 1H), 4.26-4.35 (m, 2H), 4.19 (t, *J* = 6.80 Hz, 1H), 4.04 (d, *J* = 18.80 Hz, 1H), 3.89 (d, *J* = 18.40 Hz, 1H), 3.65-3.68 (m, 1H), 3.51-3.57 (m, 2H), 3.18 (q, *J* = 14.80, 10.80 Hz, 1H), 2.89-3.01 (m, 2H), 2.01-2.11 (m, 1H), 1.09 (s, 9H), 0.94 (d, *J* = 6.80 Hz, 6H), 0.90 (d, *J* = 6.00 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 171.3, 156.4, 144.3, 144.1, 141.2, 128.0, 127.4, 125.7, 125.6, 120.5, 73.8, 67.2, 65.7, 59.9, 55.1, 48.8, 47.3, 46.7, 28.4, 24.7, 22.6, 22.6, 17.7. HRMS (ESI) ([M+H]$^{+}$) Calcd. for $C_{29}H_{41}N_2O_7S$: 561.2634, found: 561.2639.



BB10

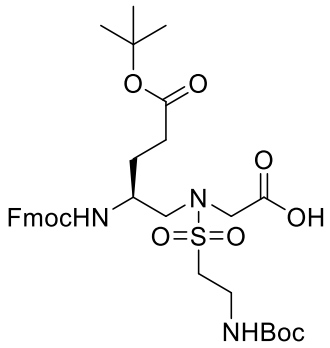
(S)-*N*-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentyl)-N-(methylsulfonyl)glycine (BB10). ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.83 (d, *J* = 7.20 Hz, 2H), 7.64 (d, *J* = 7.20 Hz, 2H), 7.36 (t, *J* = 7.20 Hz, 2H), 7.26-7.30 (m, 2H), 7.13 (d, *J* = 9.20 Hz, 1H), 6.39-6.68 (m, 2H), 4.26-4.34 (m, 3H), 4.18 (t, *J* = 6.40 Hz, 2H), 3.95 (s, 2H), 3.61 (brs, 1H), 3.23 (dd, *J* = 14.40, 4.80 Hz, 1H), 3.10 (q, *J* = 14.40, 8.80 Hz, 1H), 2.94-3.00 (m, 2H), 2.89 (s, 5H), 2.47 (s, 3H), 2.40 (s, 3H), 1.97 (s, 3H), 1.38 (d, *J* = 4.00 Hz, 1H), 1.34 (s, 6H), 1.18-1.29 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 171.2, 157.9, 156.4, 144.3, 141.2, 137.8, 134.5, 131.9, 128.0, 127.5, 125.6, 124.8, 120.5, 116.7, 86.7, 65.6, 51.5, 49.9, 48.8, 47.2, 42.9, 29.6, 28.7, 19.4, 18.0, 12.7. HRMS (ESI) ([M+H]$^{+}$) Calcd. for $C_{37}H_{48}N_5O_9S_2$: 770.2893, found: 770.2899.



BB11

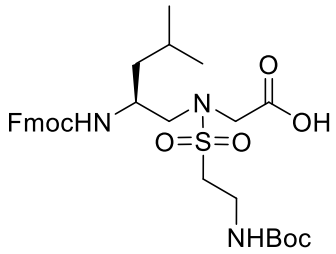
(S)-*N*-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentyl)-N-(isobutylsulfonyl)glycine (BB11). ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.82 (d, *J* = 7.60 Hz, 2H), 7.65 (q, *J* = 7.20, 3.60 Hz, 2H), 7.36 (t, *J* = 7.20 Hz, 2H), 7.26-7.30 (m, 2H), 7.16 (d, *J* = 9.20

Hz, 1H), 6.41-6.67 (m, 2H), 4.25-4.33 (m, 2H), 4.18 (t, *J* = 6.80 Hz, 1H), 3.97 (t, *J* = 19.60 Hz, 2H), 3.62 (brs, 1H), 3.27 (dd, *J* = 14.40, 4.40 Hz, 1H), 3.14 (q, *J* = 14.40, 8.80 Hz, 1H), 2.97-3.01 (m, 2H), 2.93 (q, *J* = 9.60, 2.80 Hz, 2H), 2.88 (s, 2H), 2.48 (s, 3H), 2.41 (s, 3H), 2.02-2.10 (m, 1H), 1.97 (s, 3H), 1.39-1.41 (m, 2H), 1.34 (s, 6H), 1.23-1.29 (m, 2H), 0.94 (q, *J* = 6.40, 3.20 Hz, 6H), 0.77-0.80 (m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 171.3, 156.4, 144.3, 144.2, 141.2, 137.9, 132.0, 128.0, 127.4, 125.6, 124.8, 120.5, 117.0, 116.8, 86.7, 65.7, 59.5, 51.4, 49.9, 48.6, 47.3, 42.9, 29.7, 28.6, 24.7, 22.6, 19.4, 18.0, 12.6. HRMS (ESI) ([M+H]$^+$) Calcd. for $C_{40}H_{54}N_5O_9S_2$: 812.3363, found: 812.3360.



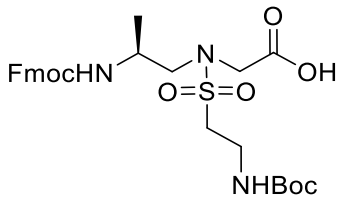
BB12

(S)-*N*-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-(tert-butoxy)-5-oxopentyl)-N-((2-((tert-butoxycarbonyl)amino)ethyl)sulfonyl)glycine (BB12). ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.84 (d, *J* = 7.60 Hz, 2H), 7.65 (t, *J* = 6.80 Hz, 2H), 7.37 (t, *J* = 7.20 Hz, 2H), 7.29 (t, *J* = 7.20 Hz, 2H), 7.16 (d, *J* = 9.20 Hz, 1H), 6.84 (d, *J* = 5.20 Hz, 1H), 4.29 (d, *J* = 6.80 Hz, 2H), 4.18 (t, *J* = 6.80 Hz, 1H), 3.98 (s, 2H), 3.63 (brs, 1H), 3.28 (q, *J* = 12.80, 5.60 Hz, 3H), 3.21 (q, *J* = 10.00, 5.60 Hz, 2H), 3.14 (q, *J* = 14.40, 8.40 Hz, 1H), 2.09-2.22 (m, 2H), 1.71-1.73 (m, 1H), 1.44-1.50 (m, 1H), 1.35 (s, 9H), 1.32 (s, 9H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 172.3, 171.1, 156.4, 155.7, 144.3, 144.2, 141.2, 128.0, 127.4, 125.6, 120.5, 80.0, 78.5, 65.7, 51.7, 51.2, 49.3, 47.2, 35.2, 31.8, 28.6, 28.1, 27.5. HRMS (ESI) ([M+H]$^+$) Calcd. for $C_{33}H_{46}N_3O_{10}S$: 676.2904, found: 676.2901.



BB13

(S)-*N*-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentyl)-N-((2-((tert-butoxycarbonyl)amino)ethyl)sulfonyl)glycine (BB13). ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.78 (d, *J* = 7.20 Hz, 2H), 7.60 (t, *J* = 6.00 Hz, 2H), 7.32 (t, *J* = 7.20 Hz, 2H), 7.21-7.26 (m, 2H), 7.08 (d, *J* = 9.20 Hz, 1H), 6.80 (brs, 1H), 4.25 (d, *J* = 5.60 Hz, 2H), 4.12 (t, *J* = 6.80 Hz, 1H), 3.93 (s, 2H), 3.64-3.65 (m, 1H), 3.23 (t, *J* = 5.60 Hz, 2H), 3.16 (t, *J* = 4.00 Hz, 2H), 3.05 (q, *J* = 14.00, 8.40 Hz, 1H), 1.99 (s, 1H), 1.43-1.48 (m, 1H), 1.27 (s, 9H), 1.11-1.23 (m, 2H), 0.76 (q, *J* = 12.40, 6.80 Hz, 6H), 0.66 (dd, *J* = 22.40, 4.80 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 171.1, 156.2, 155.7, 144.3, 144.1, 141.1, 127.9, 127.4, 125.5, 120.4, 78.4, 65.5, 51.8, 51.6, 48.7, 48.1, 47.2, 41.2, 35.1, 31.0, 28.5, 24.6, 23.6, 21.9. HRMS (ESI) ([M+H]$^{+}$) Calcd. for $C_{30}H_{42}N_3O_8S$: 604.2693, found: 604.2689.



BB14

(S)-*N*-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propyl)-N-((2-((tert-butoxycarbonyl)amino)ethyl)sulfonyl)glycine (BB14). ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.84 (d, *J* = 7.60 Hz, 2H), 7.65 (t, *J* = 5.20 Hz, 2H), 7.37 (t, *J* = 7.20 Hz, 2H), 7.29 (t, *J* = 7.20 Hz, 2H), 7.19 (d, *J* = 8.40 Hz, 1H), 6.86 (brs, 1H), 4.24-4.31 (m, 2H), 4.16-4.21 (m, 1H), 3.98 (s, 2H), 3.70-3.73 (m, 1H), 3.28 (t, *J* = 6.00 Hz, 2H), 3.20 (t, *J* = 8.00 Hz, 3H), 2.02 (s, 1H), 1.32 (s, 9H), 1.02 (d, *J* = 6.40 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 171.2, 156.0, 155.7, 144.3, 144.3, 141.1, 128.0, 127.5, 125.5,

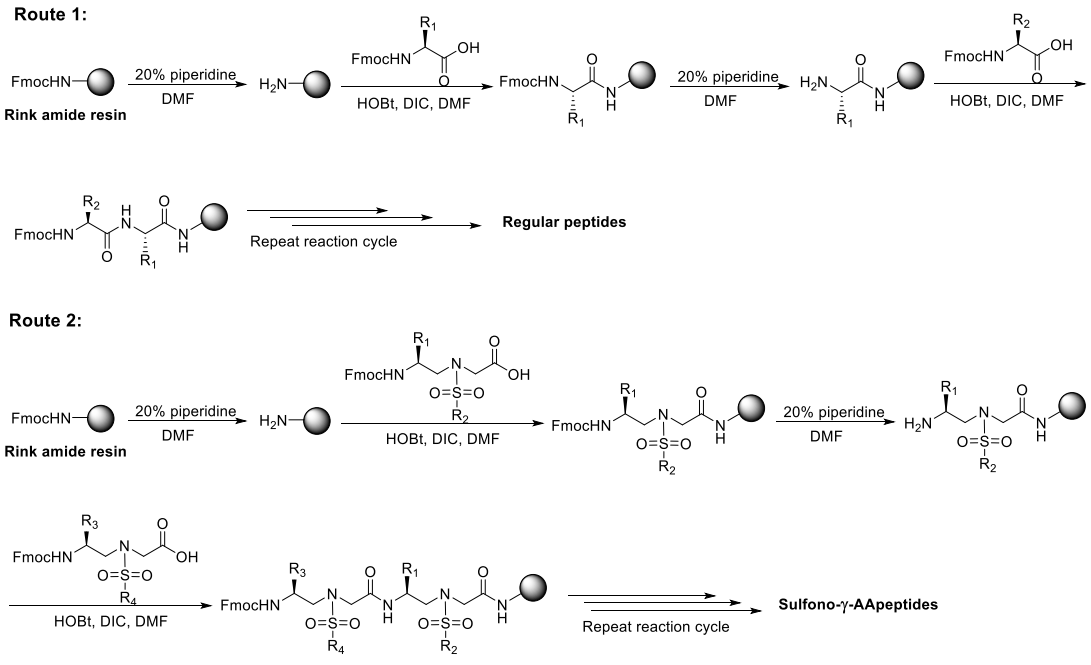
120.5, 78.5, 65.7, 52.4, 51.5, 48.8, 47.2, 45.8, 35.2, 28.6, 18.6. HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{27}H_{36}N_3O_8S$: 562.2223, found: 562.2217.

2. Preparation of BCL9 Peptides and Sulfono-γ-AApeptides

2.1 General Information

Solid-phase synthesis of the peptides were conducted in the peptide synthesis vessels on a Burrell Wrist-Action shaker. All peptides were analyzed and purified on a Waters Breeze 2 HPLC system installed with both analytic module (1 mL/min) and preparative module (16 mL/min), by employing a method using 5–100% linear gradient of solvent B (0.1% TFA in acetonitrile) in solvent A (0.1% TFA in water) over 35 min, followed by 100% solvent B over 15 min. The pure products were then collected and lyophilized on a Labcono lyophilizer, and the purity of the compounds was determined to be >95% by analytical HPLC. Masses of γ-AApeptides were obtained on an Agilent 6220 using electrospray ionization time-of-flight (ESI-TOF).

2.2 General Synthetic Route



Scheme S2. General synthetic route to prepare regular peptides and sulfono-γ-AApeptides.

The regular peptides were prepared based on route 1, the sulfono-γ-AApeptides were prepared based on route 2. The synthesis was carried out on 100 mg Rink Amide-MBHA resin (0.646 mmol/g) under room temperature at atmosphere. The resin was swelled in DMF for 5 min before use, followed by treatment with 20% piperidine/DMF solution (2 mL) for 15 min (×2) to remove Fmoc protecting group, afterwards washed DCM (×3) and DMF (×3). A premixed solution of the sulfono-γ-AApeptide Building Block (2 equiv.), HOBt (4 equiv.), and DIC (4 equiv.) in 2 mL DMF was added to the resin and shaken for 4 h to complete the coupling reaction. After wash with DCM and DMF, the resin was treated with 20% piperidine/DMF solution for 15 min (×2). Another Fmoc protected regular amino acid /sulfono-γ-AApeptide building block (2 equiv.) was attached on the resin following the procedure in the first coupling step, and Fmoc protecting group was removed after the coupling reaction was done. The reaction cycles were repeated until the desired sulfono-γ-AApeptides were synthesized. For the capped sequence, the N-terminus of the sequence was capped with acetic anhydride (1 mL) in pyridine (2 mL) (15 min×2), followed by treatment with TFA/DCM (6 mL, 1:1, v/v) for 3 h. The cleavage solution was collected, and the beads were washed with DCM (3 mL×2). The solution was combined and evaporated under air flow to give the crude product, which was analyzed and purified by Water HPLC system, at the 1 mL/min and 16 mL/min flow rate for analytic and preparative HPLC, respectively. 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. All the sulfono-γ-AApeptides were obtained with decent yield (39.39–46.72%) with a purity > 95% after prep-HPLC purification.

For the FITC-labeled sulfono-γ-AApeptides synthesis, after installation of the last sulfono-γ-AApeptide building block, the Fmoc protecting group was then removed, afterwards washed with DCM (×3) and with DMF (×3). A premixed solution of

Fmoc-β-Ala-OH (2 equiv.), HOBt (4 equiv.), and DIC (4 equiv.) in 2 mL DMF was added to the resin and shaken for 2 h to complete the coupling reaction. The Fmoc protecting group was then removed, FITC (2 equiv.) in 2 mL DMF and DIPEA (6 equiv.) was added to the resin and shaken for overnight to complete the reaction. After wash with DMF (×3) and DCM (×3), the resin was cleaved using TFA/DCM (6 mL, 1:1, v/v) for 3 h. The pure FITC-labeled sulfono-γ-AApeptides (>95%) were obtained using the same abovementioned method by HPLC.

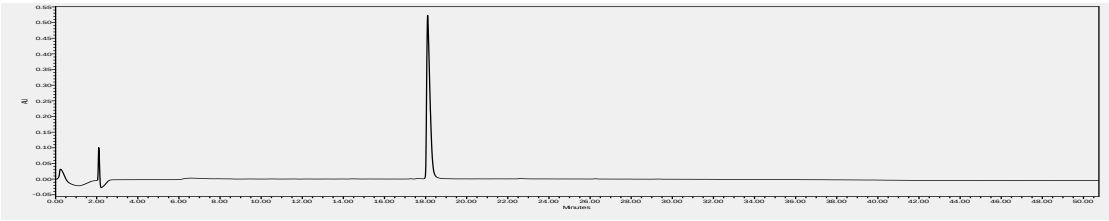
For the biotinylated sulfono-γ-AApeptides synthesis, a premixed solution of biotin (2 equiv.), HOBt (4 equiv.), and DIC (4 equiv.) in 2 mL DMF was added to the resin after dealloc and shaken for 24 h to complete the coupling reaction. After wash with DMF (×3) and DCM (×3), the resin was cleaved using TFA/DCM (6 mL, 1:1, v/v) for 3 h. The pure biotinylated sulfono-γ-AApeptides (>95%) were obtained using the same abovementioned method by HPLC.

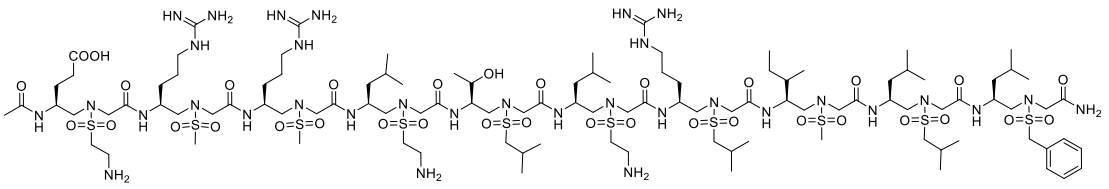
2.3 HPLC Trace

Ac-SQEQLEHRERSLQTLRDIQRMLF-NH_2

1

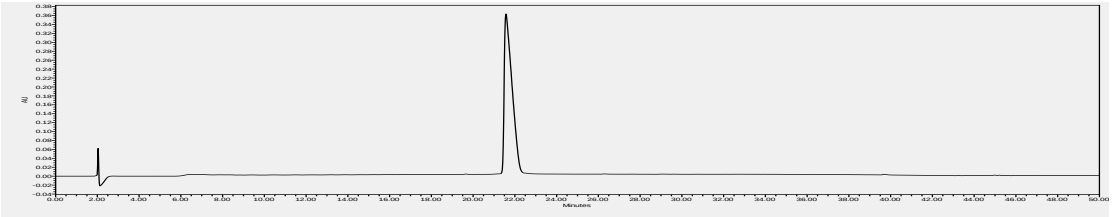
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{125}H_{209}N_{42}O_{39}S$: 2954.5383, found: 591.9159$[M+5H]^{5+}$, 739.6416$[M+4H]^{4+}$, 985.8539$[M+3H]^{3+}$, 1478.2743$[M+2H]^{2+}$.

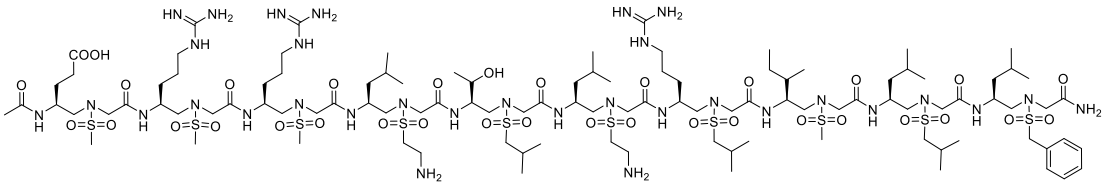




2

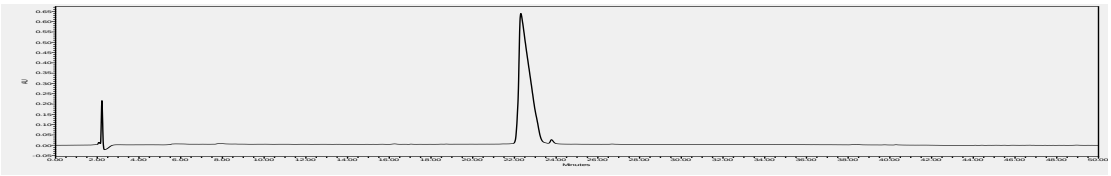
HRMS (ESI) ([M+H]$^{+}$) Calcd. for $C_{107}H_{212}N_{33}O_{34}S_{10}$: 2823.3082, found: 707.0802[M+4H]$^{4+}$, 942.4420[M+3H]$^{3+}$, 1413.1570[M+2H]$^{2+}$.

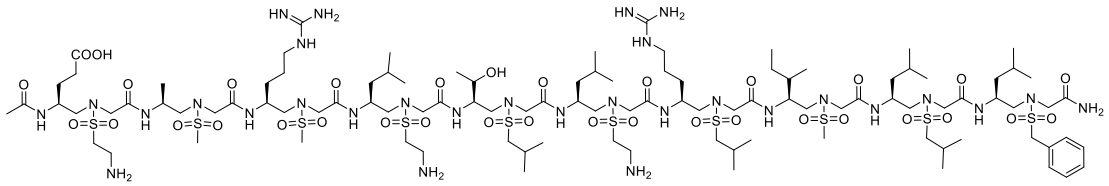




3

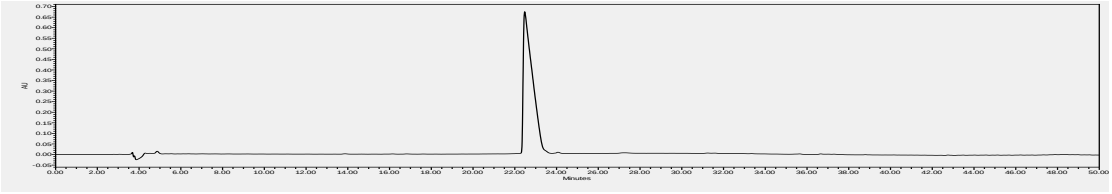
HRMS (ESI) ([M+H]$^{+}$) Calcd. for $C_{106}H_{209}N_{32}O_{34}S_{10}$: 2794.2816, found: 699.8256[M+4H]$^{4+}$, 932.7664[M+3H]$^{3+}$, 1398.6429[M+2H]$^{2+}$.

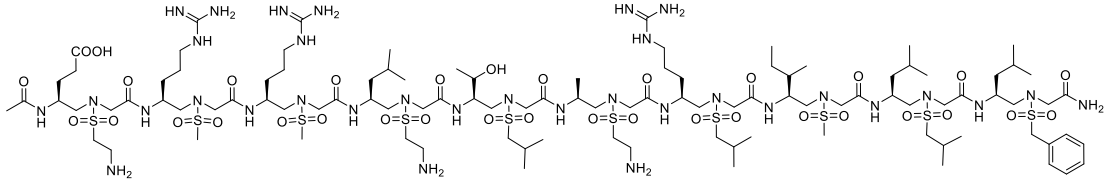




4

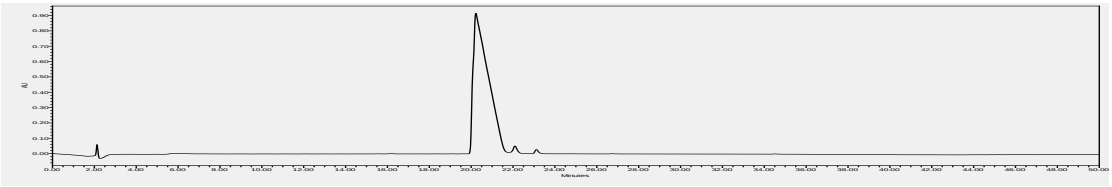
HRMS (ESI) ([M+H]$^{+}$) Calcd. for $C_{104}H_{205}N_{30}O_{34}S_{10}$: 2738.2442, found: 548.8560[M+5H]$^{5+}$, 685.8159[M+4H]$^{4+}$, 914.0856[M+3H]$^{3+}$.

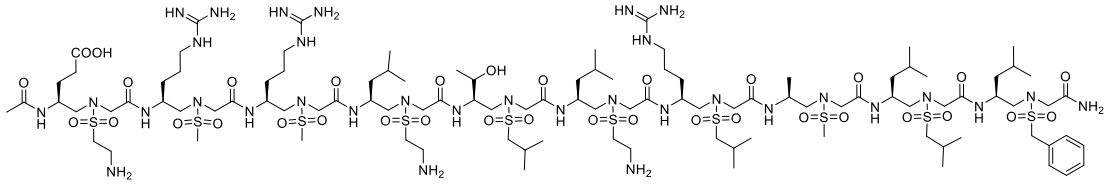




5

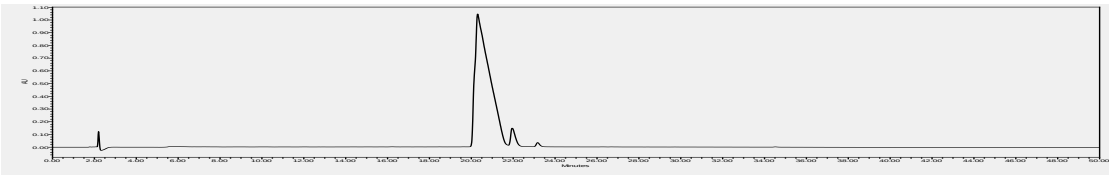
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{104}H_{206}N_{33}O_{34}S_{10}$: 2781.2612, found: 696.5706$[M+4H]^{4+}$, 928.4252$[M+3H]^{3+}$, 1392.1317$[M+2H]^{2+}$.

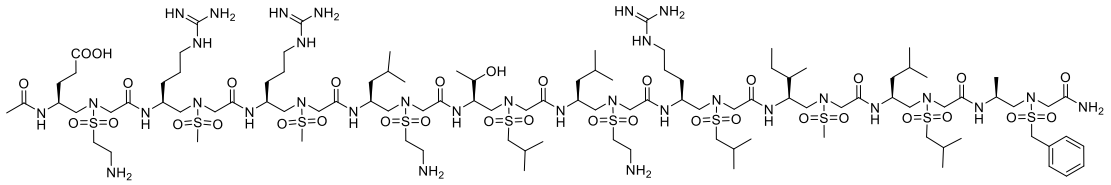




6

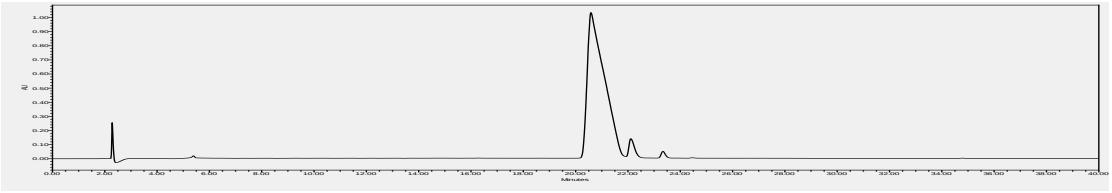
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{104}H_{206}N_{33}O_{34}S_{10}$: 2781.2612, found: 696.5704$[M+4H]^{4+}$, 928.4238$[M+3H]^{3+}$, 1392.1294$[M+2H]^{2+}$.

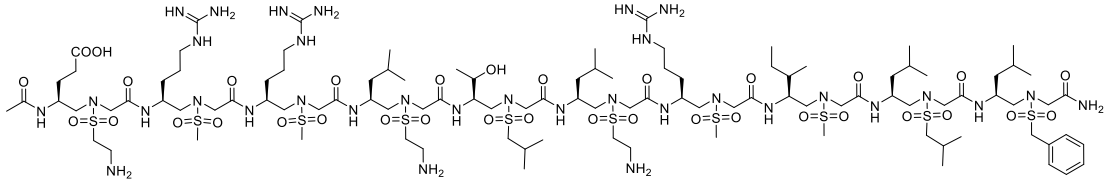




7

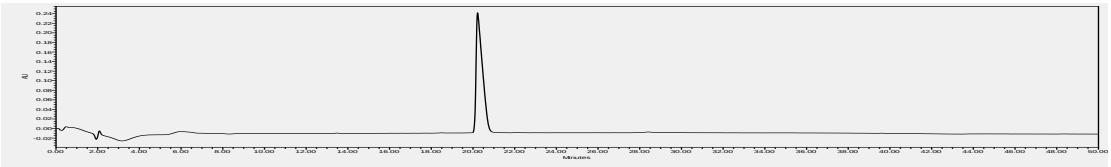
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{104}H_{206}N_{33}O_{34}S_{10}$: 2781.2612, found: 696.5699$[M+4H]^{4+}$, 928.4243$[M+3H]^{3+}$, 1392.1294$[M+2H]^{2+}$.

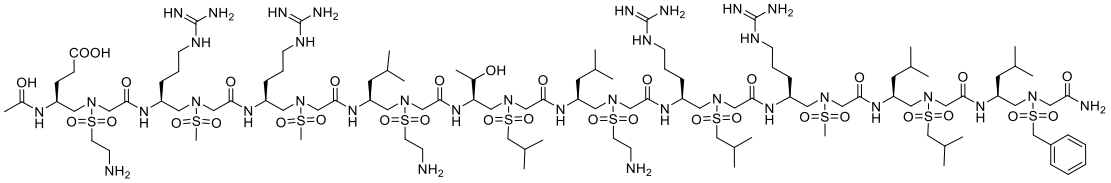




8

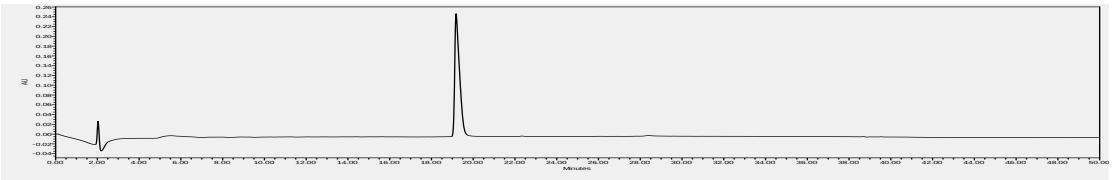
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{104}H_{206}N_{33}O_{34}S_{10}$: 2781.2612, found: 696.5706$[M+4H]^{4+}$, 928.4256$[M+3H]^{3+}$, 1392.1323$[M+2H]^{2+}$.

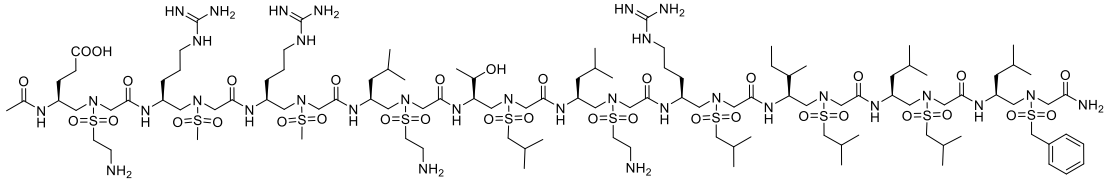




9

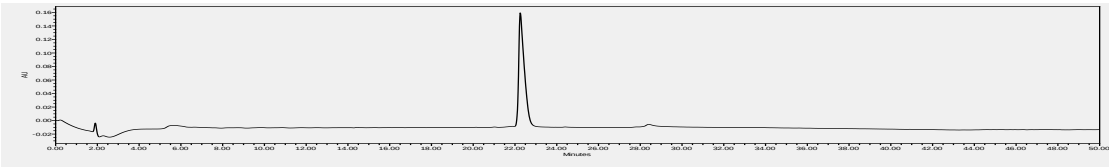
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{107}H_{213}N_{36}O_{34}S_{10}$: 2866.3252, found: 717.8380$[M+4H]^{4+}$, 956.7816$[M+3H]^{3+}$, 1434.6666$[M+2H]^{2+}$.

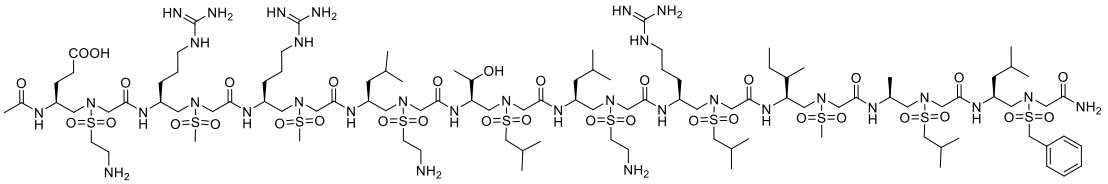




10

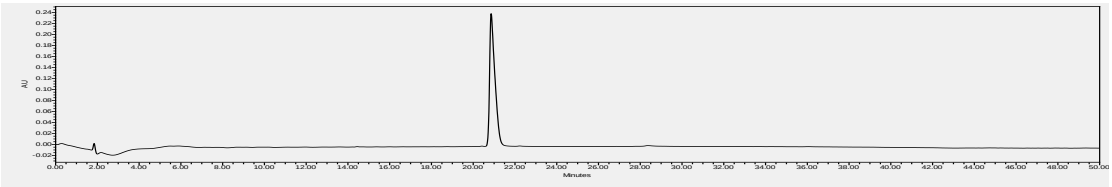
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{110}H_{218}N_{33}O_{34}S_{10}$: 2865.3551, found: 717.5952$[M+4H]^{4+}$, 956.4579$[M+3H]^{3+}$, 1434.1813$[M+2H]^{2+}$.

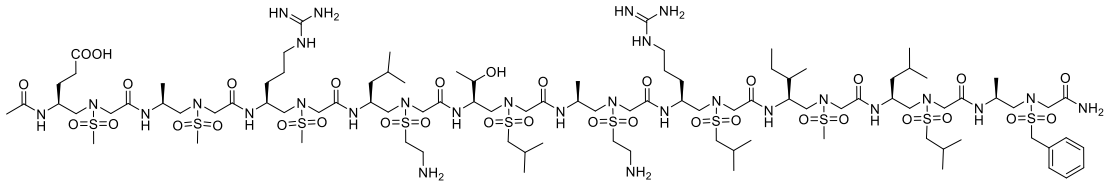




11

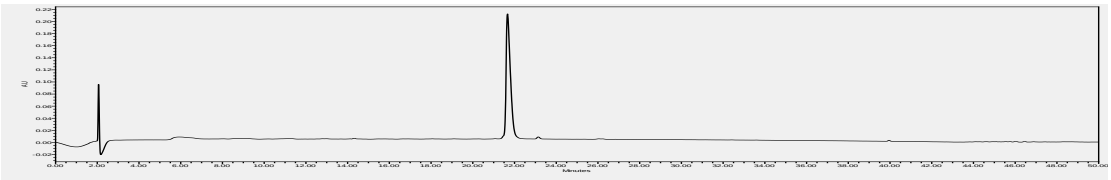
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{104}H_{206}N_{33}O_{34}S_{10}$: 2781.2612, found: 696.5718$[M+4H]^{4+}$, 928.4270$[M+3H]^{3+}$, 1392.1340$[M+2H]^{2+}$.





12

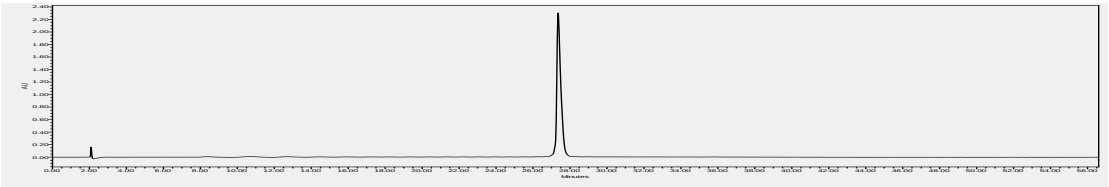
HRMS (ESI) ([M+H]$^{+}$) Calcd. for $C_{97}H_{190}N_{29}O_{34}S_{10}$: 2625.1237, found: 657.5362[M+4H]$^{4+}$, 876.3809[M+3H]$^{3+}$, 1314.0647[M+2H]$^{2+}$.

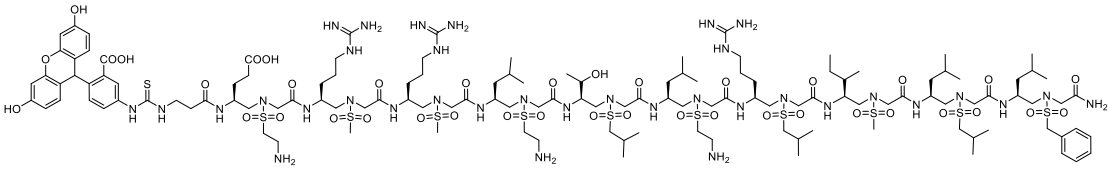


Flu-Beta-Ala-SQEQLEHRERSLQTLRDIQRMLF

1-FITC

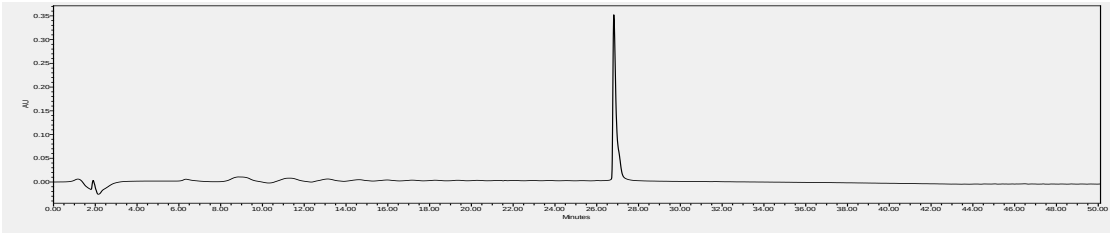
HRMS (ESI) ([M+H]$^{+}$) Calcd. for $C_{147}H_{225}N_{44}O_{44}S_{2}$: 3374.6163, found: 675.7287[M+5H]$^{5+}$, 844.4084[M+4H]$^{4+}$, 1125.5406[M+3H]$^{3+}$, 1687.8041[M+2H]$^{2+}$.

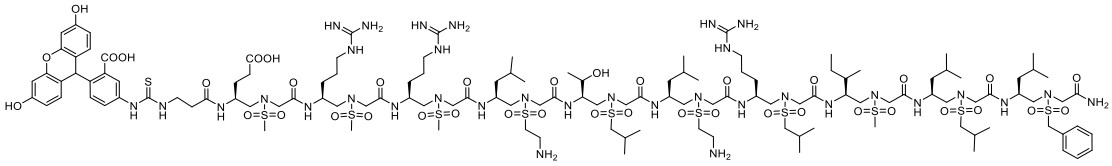




2-FITC

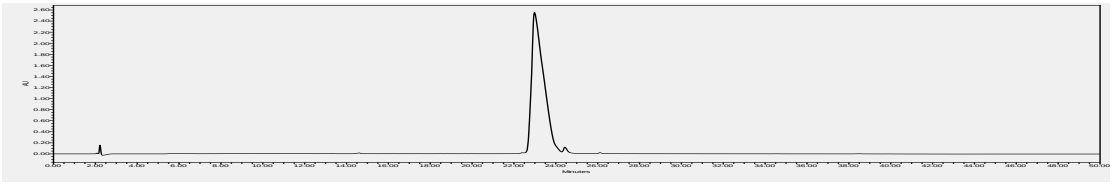
HRMS (ESI) ([M+H]$^{+}$) Calcd. for $C_{129}H_{228}N_{35}O_{39}S_{11}$: 3243.3861, found: 649.4835[M+5H]$^{5+}$, 811.6012[M+4H]$^{4+}$, 1081.7983[M+3H]$^{3+}$, 1622.1922[M+2H]$^{2+}$.

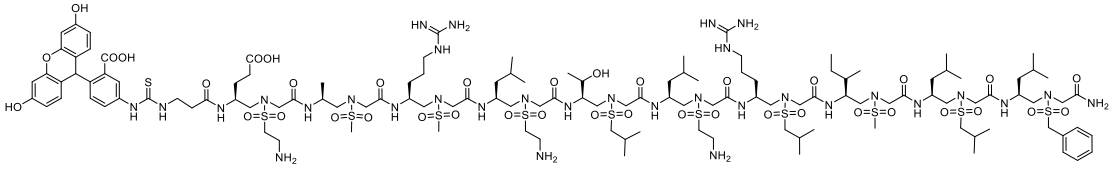




3-FITC

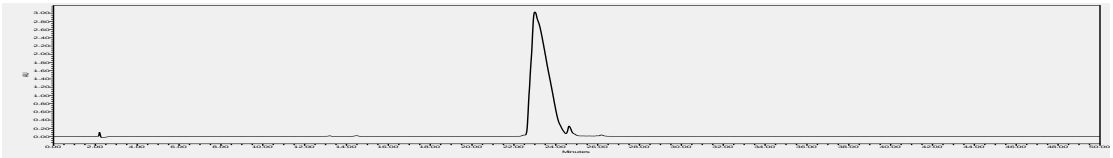
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{128}H_{225}N_{34}O_{39}S_{11}$: 3214.3596, found: 643.6762$[M+5H]^{5+}$, 804.3418$[M+4H]^{4+}$, 1072.1186$[M+3H]^{3+}$, 1607.6726$[M+2H]^{2+}$.

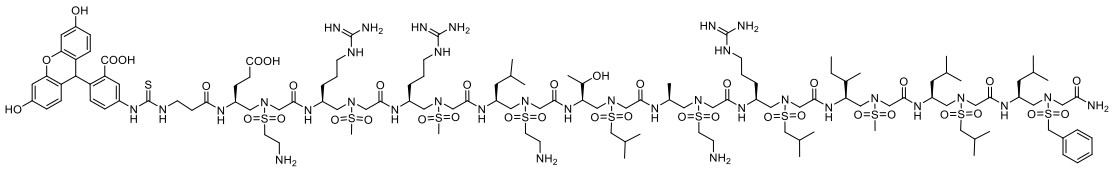




4-FITC

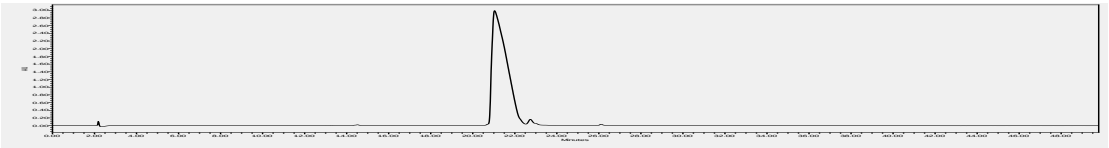
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{126}H_{221}N_{32}O_{39}S_{11}$: 3158.3221, found: 632.4701$[M+5H]^{5+}$, 790.3345$[M+4H]^{4+}$, 1053.4426$[M+3H]^{3+}$, 1579.6599$[M+2H]^{2+}$.

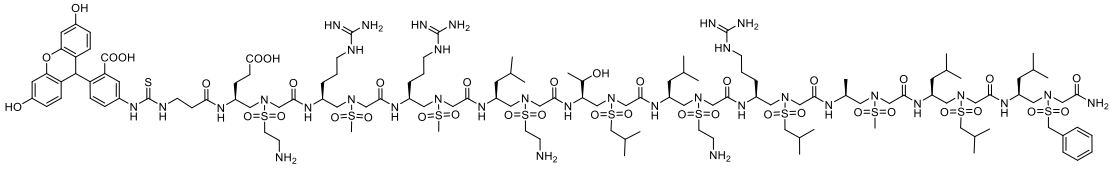




5-FITC

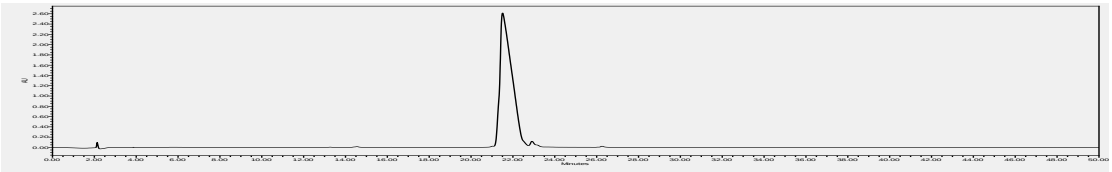
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{126}H_{222}N_{35}O_{39}S_{11}$: 3201.3392, found: 641.0726$[M+5H]^{5+}$, 801.0880$[M+4H]^{4+}$, 1067.7806$[M+3H]^{3+}$, 1601.1656$[M+2H]^{2+}$.

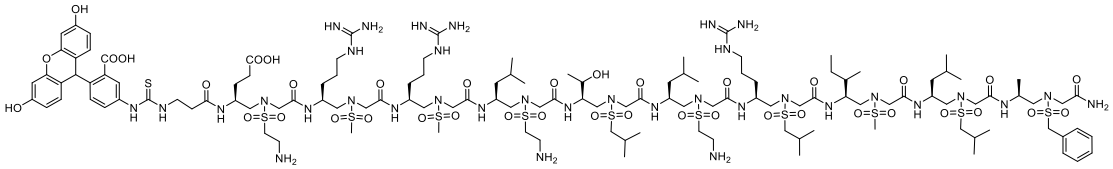




6-FITC

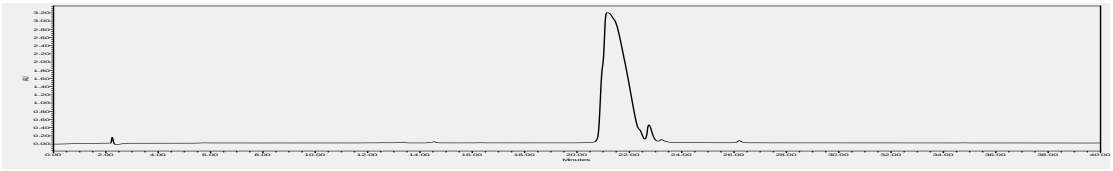
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{126}H_{222}N_{35}O_{39}S_{11}$: 3201.3392, found: 641.0728$[M+5H]^{5+}$, 801.0878$[M+4H]^{4+}$, 1067.7799$[M+3H]^{3+}$, 1601.1623$[M+2H]^{2+}$.

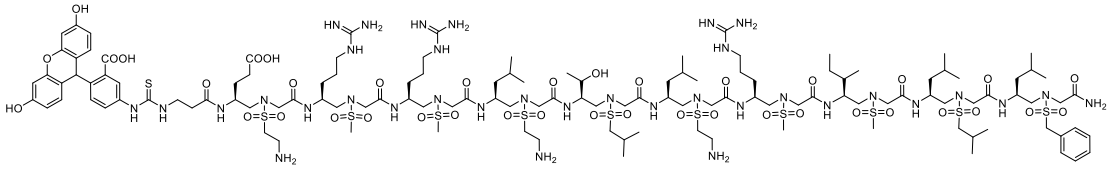




7-FITC

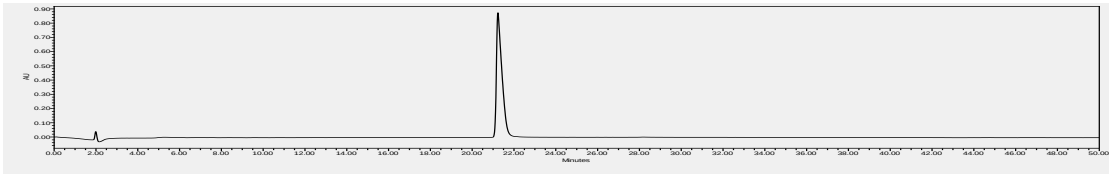
HRMS (ESI) ([M+H]$^+$) Calcd. for $C_{126}H_{222}N_{35}O_{39}S_{11}$: 3201.3392, found: 641.0739[M+5H]$^{5+}$, 801.0890[M+4H]$^{4+}$, 1067.7820[M+3H]$^{3+}$, 1601.1668[M+2H]$^{2+}$.

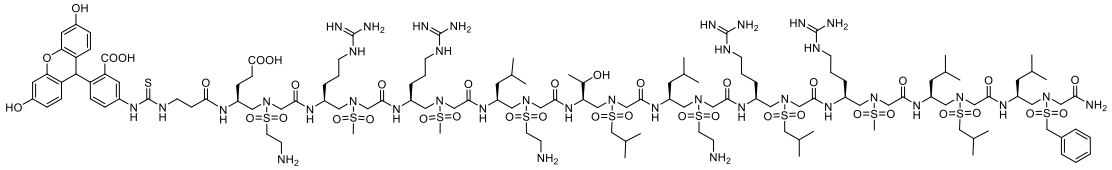




8-FITC

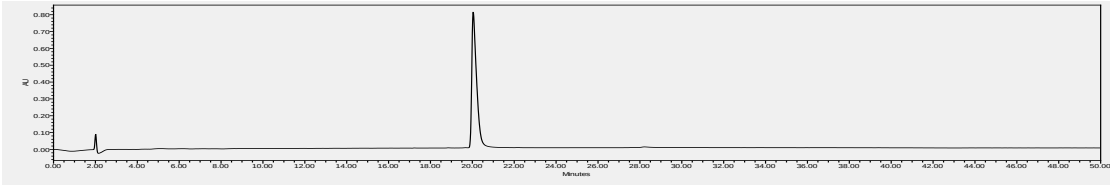
HRMS (ESI) ([M+H]$^+$) Calcd. for $C_{126}H_{222}N_{35}O_{39}S_{11}$: 3201.3392, found: 641.0731[M+5H]$^{5+}$, 801.0882[M+4H]$^{4+}$, 1067.7811[M+3H]$^{3+}$, 1601.1670[M+2H]$^{2+}$.

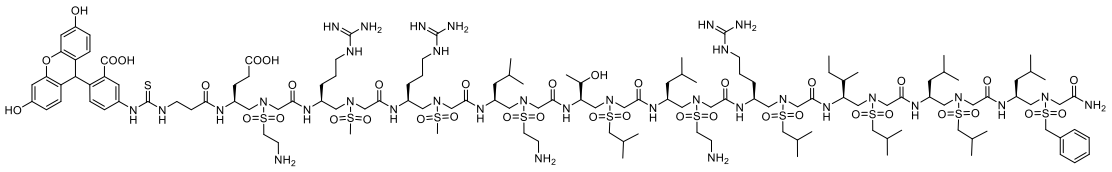




9-FITC

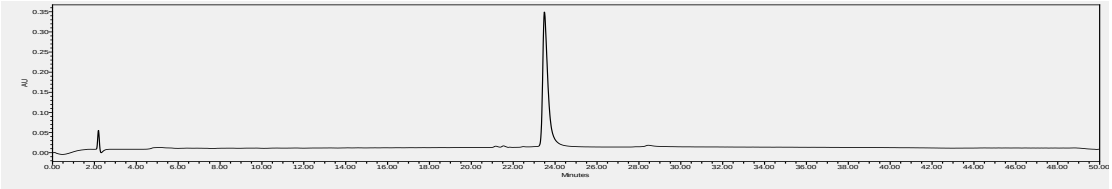
HRMS (ESI) ([M+H]$^+$) Calcd. for $C_{129}H_{229}N_{38}O_{39}S_{11}$: 3286.4032, found: 658.0845[M+5H]$^{5+}$, 822.3523[M+4H]$^{4+}$, 1096.1327[M+3H]$^{3+}$, 1644.1916[M+2H]$^{2+}$.

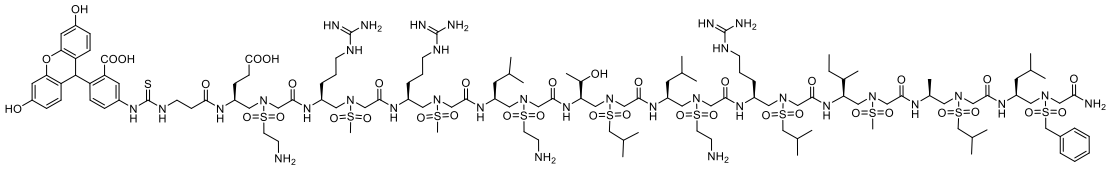




10-FITC

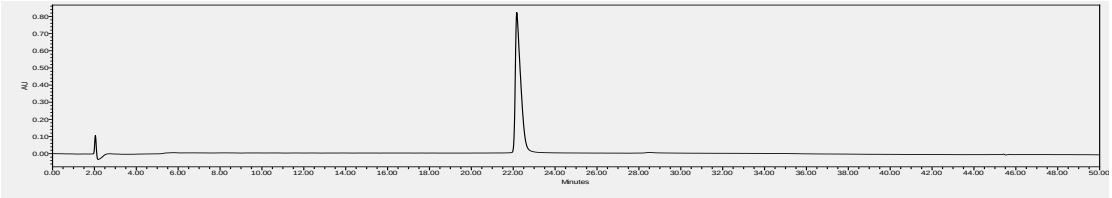
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{132}H_{234}N_{35}O_{39}S_{11}$: 3285.4331, found: 657.8915$[M+5H]^{5+}$, 822.1112$[M+4H]^{4+}$, 1095.8114$[M+3H]^{3+}$, 1643.2136$[M+2H]^{2+}$.

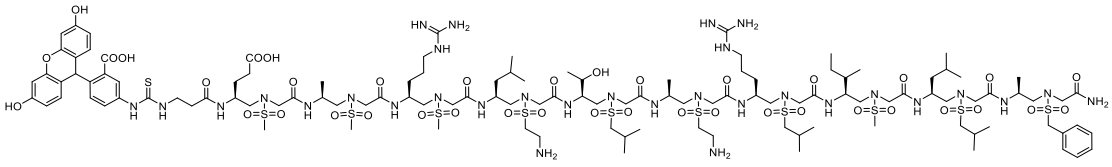




11-FITC

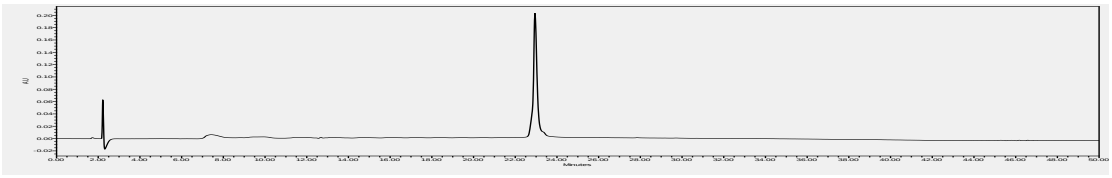
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{126}H_{222}N_{35}O_{39}S_{11}$: 3201.3392, found: 641.0734$[M+5H]^{5+}$, 801.0887$[M+4H]^{4+}$, 1067.7812$[M+3H]^{3+}$, 1601.1666$[M+2H]^{2+}$.

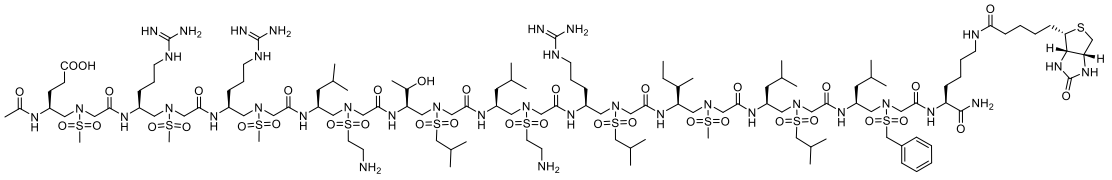




12-FITC

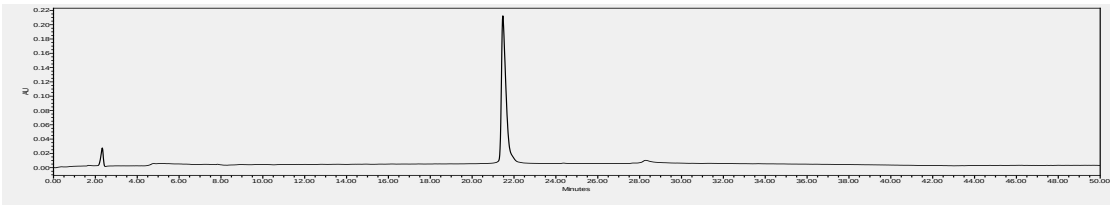
HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{119}H_{206}N_{31}O_{39}S_{11}$: 3045.2017, found: 784.5418$[M+4Na]^{4+}$.

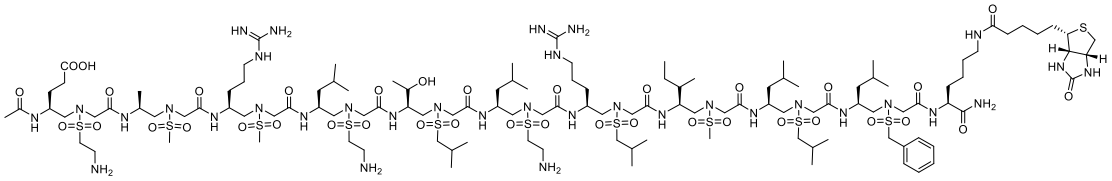




3-Biotin

HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{122}H_{235}N_{36}O_{37}S_{11}$: 3148.4542, found: 630.8994$[M+5H]^{5+}$, 788.3710$[M+4H]^{4+}$, 1050.8246$[M+3H]^{3+}$, 1575.7312$[M+2H]^{2+}$.





4-Biotin

HRMS (ESI) ($[M+H]^+$) Calcd. for $C_{120}H_{231}N_{34}O_{37}S_{11}$: 3092.4167, found: 619.6883$[M+5H]^{5+}$, 774.3566$[M+4H]^{4+}$, 1032.1391$[M+3H]^{3+}$, 1547.7030$[M+2H]^{2+}$.

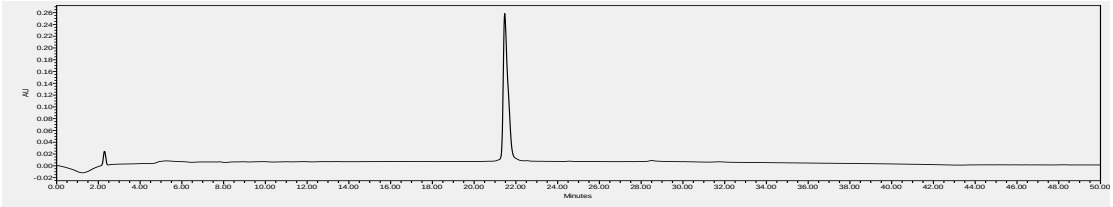


Figure S1. HPLC spectra of regular peptide and sulfono-γ-AApeptides.

2.4 HPLC Purities and Retention Time of Pure Peptides.

Table S1. HPLC purities and retention time of regular peptide and sulfono-γ-AApeptides.[a]

Peptide Name	Purity trace after HPLC purification (%)	Retention Time (min)
1	100.0%	18.10
2	100.0%	21.58
3	99.09%	22.31
4	100.0%	22.48
5	98.76%	20.22
6	97.98%	20.30
7	97.87%	20.59
8	100.0%	22.23
9	100.0%	19.18
10	98.96%	22.26
11	99.76%	20.86
12	100.0%	21.66
1-FITC	100.0%	19.20
2-FITC	100.0%	22.60
3-FITC	99.10%	22.98
4-FITC	98.79%	23.00
5-FITC	98.99%	21.04
6-FITC	99.01%	21.51
7-FITC	98.07%	21.16
8-FITC	99.99%	21.23
9-FITC	99.42%	20.04
10-FITC	99.19%	23.49
11-FITC	99.83%	22.17
12-FITC	100.0%	22.95

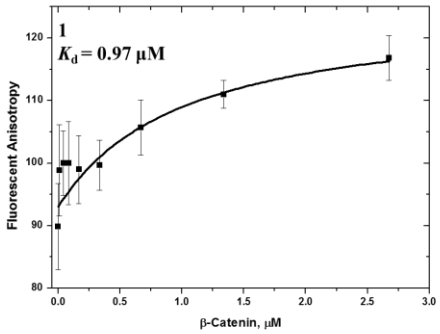
3-Biotin	98.71%	21.47
4-Biotin	100.0%	21.47

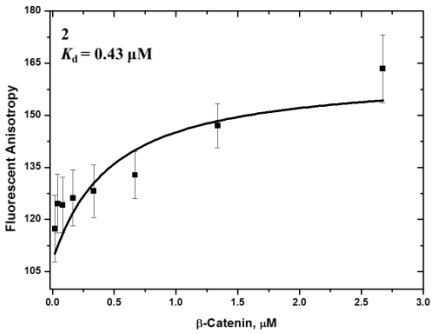
[a] The gradient eluting method of 5% to 100% of solvent B (0.1% TFA in acetonitrile) in A (0.1% TFA in water) over 50 min was performed.

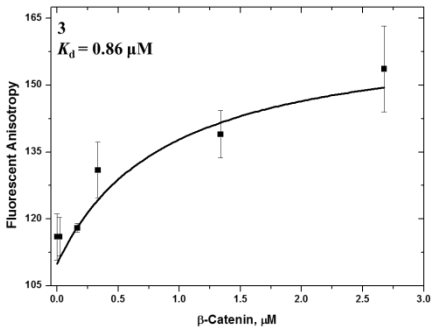
3. FP Assay to Measure the Binding of the Regular Peptide and Sulfono-γ-AApeptides with β-catenin (K_d)

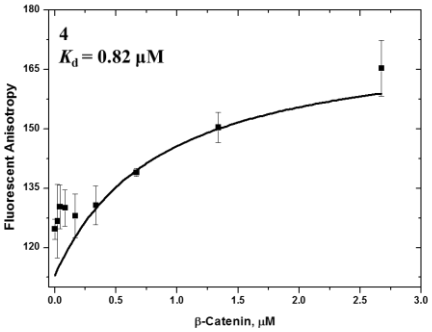
The binding affinity (K_d) of the regular amino acid and sulfono-γ-AApeptides was investigated by fluorescence polarization (FP). FP experiment was carried out by incubating 50 nM FITC labeled AApeptide with β-catenin (0.02 to 2.6 μM) in 1×PBS. Dissociation constants (K_d) were determined by plotting the fluorescence anisotropy values as a function of protein concentration, and the plots were fitted to the following equation. The L_{st} is the concentration of the peptide and the x stands for the concentration of the protein. The experiments were performed in triplicates and repeated for three times.

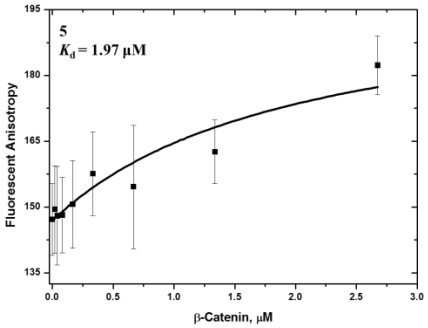
$$Y = [\text{FPmin} + (FPmin - FPmin)]\frac{(Kd + \text{L}_{st} + \text{x}) - \sqrt{(Kd + L_{st} + x)^2 - 4L_{st}x}}{2L_{st}}$$

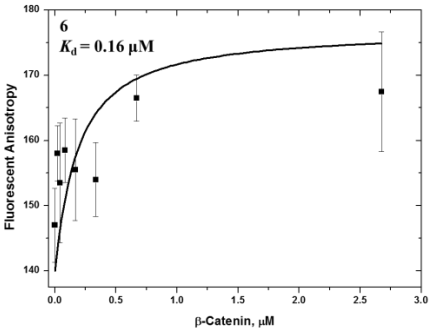


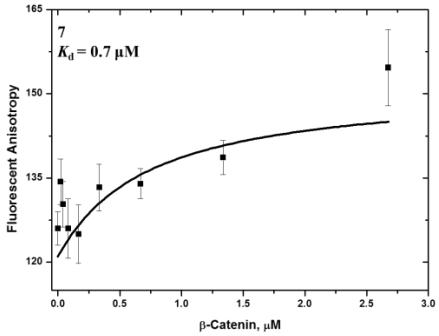


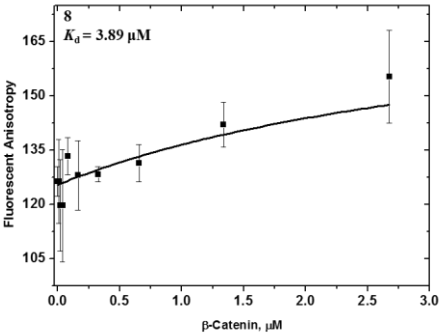

3
K_d = 0.86 μM
Fluorescent Anisotropy
β-Catenin, μM

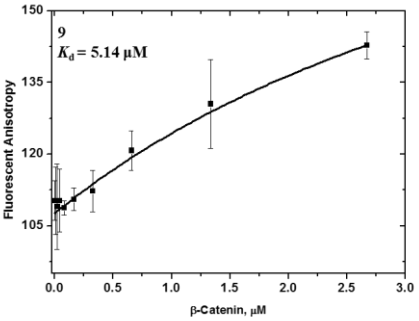

4
K_d = 0.82 μM
Fluorescent Anisotropy
β-Catenin, μM

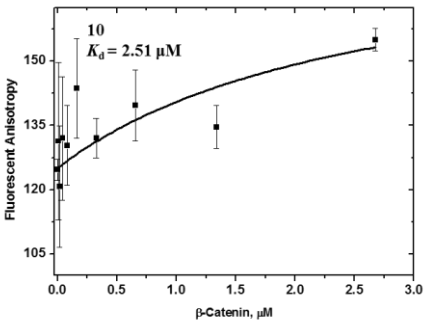

5
K_d = 1.97 μM
Fluorescent Anisotropy
β-Catenin, μM


6
K_d = 0.16 μM
Fluorescent Anisotropy
β-Catenin, μM


7
K_d = 0.7 μM
Fluorescent Anisotropy
β-Catenin, μM


8
K_d = 3.89 μM
Fluorescent Anisotropy
β-Catenin, μM


9
K_d = 5.14 μM
Fluorescent Anisotropy
β-Catenin, μM


10
K_d = 2.51 μM
Fluorescent Anisotropy
β-Catenin, μM

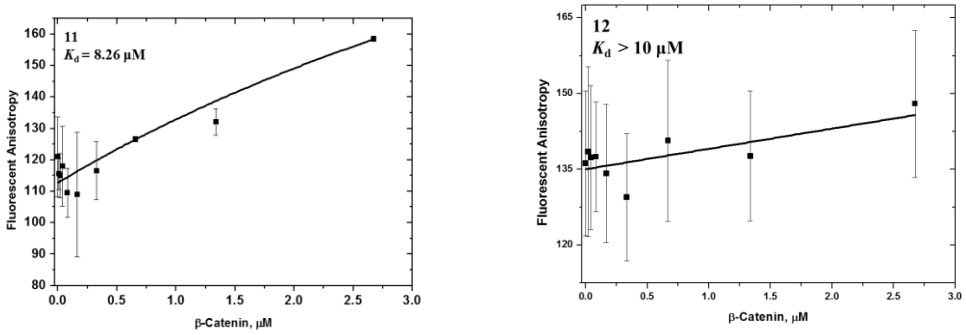


Figure S2. The K_d data of regular peptide **1** and sulfono-γ-AApeptides **2-12**.

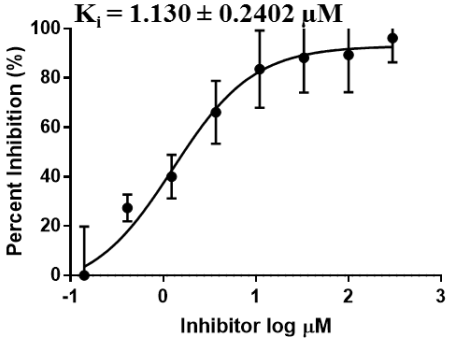
4. Alphascreen Results of β-Catenin and BCL9 Interaction

For the competitive inhibition assays of β-catenin/BCL9 PPI, the negative control (equivalent to 0% inhibition) refers to 5.0 nM biotinylated BCL9, 40 nM His6-tagged FL-β-catenin, and 10 μg/mL of donor and acceptor beads in a final volume of 25 μL of assay buffer, but no tested inhibitor present. The positive control (equivalent to 100% inhibition) refers to 5.0 nM biotinylated BCL9 and 10 μg/mL of donor and acceptor beads in a final volume of 25 μL of assay buffer. For the β-catenin/BCL9 assay, 5 nM biotinylated BCL9 and 40 nM His6-tagged FL-β-catenin were incubated in assay buffer at 4 °C for 30min. Different concentrations of the tested inhibitor were added and incubated in 20 μL of assay buffer at 4 °C for another 1 h. All of the above assay plates were covered and gently mixed on an orbital shaker. The donor and acceptor beads were then added to the plates to a final concentration of 10 μg/mL in 25 μL of assay buffer. The mixture was incubated for 1 h at 4 °C before detection. The IC_{50} value was determined by nonlinear least-squares analysis of GraphPad Prism 5.0. The K_i values were derived from the IC_{50} values using a method reported by Nikolovska-Coleska et al.[2] The assays were conducted under the conditions reported by Nikolovska-Coleska et al.'s equation for determining the K_i values. All of the experiments were performed in triplicate. The results were expressed as mean ± standard deviation.

1

IC_{50} = 1.275 ± 0.2874 µM

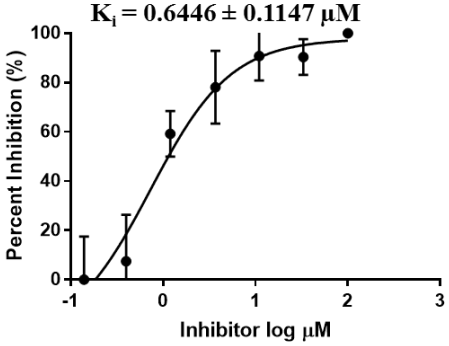
K_i = 1.130 ± 0.2402 µM



2

IC_{50} =0.736 ± 0.1482 µM

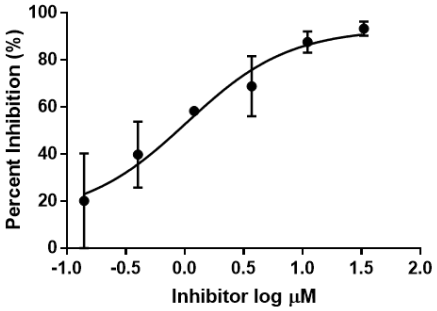
K_i = 0.6446 ± 0.1147 µM



3

IC_{50} =1.038 ± 0.2063 µM

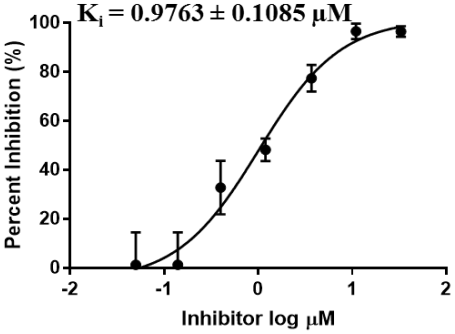
K_i = 0.9168 ± 0.1671 µM



4

IC_{50} =1.104 ± 0.1413µM

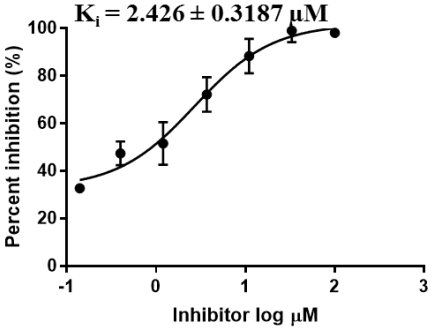
K_i = 0.9763 ± 0.1085 µM



5

IC_{50} =2.712 ± 0.3745 µM

K_i = 2.426 ± 0.3187 µM



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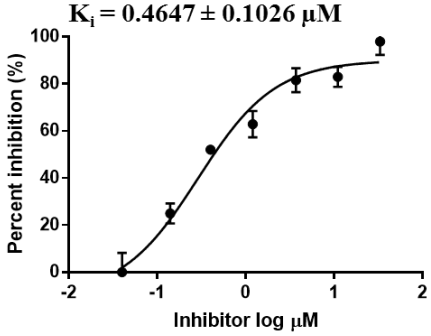
IC_{50} =0.5365 ± 0.1347 µM

K_i = 0.4647 ± 0.1026 µM



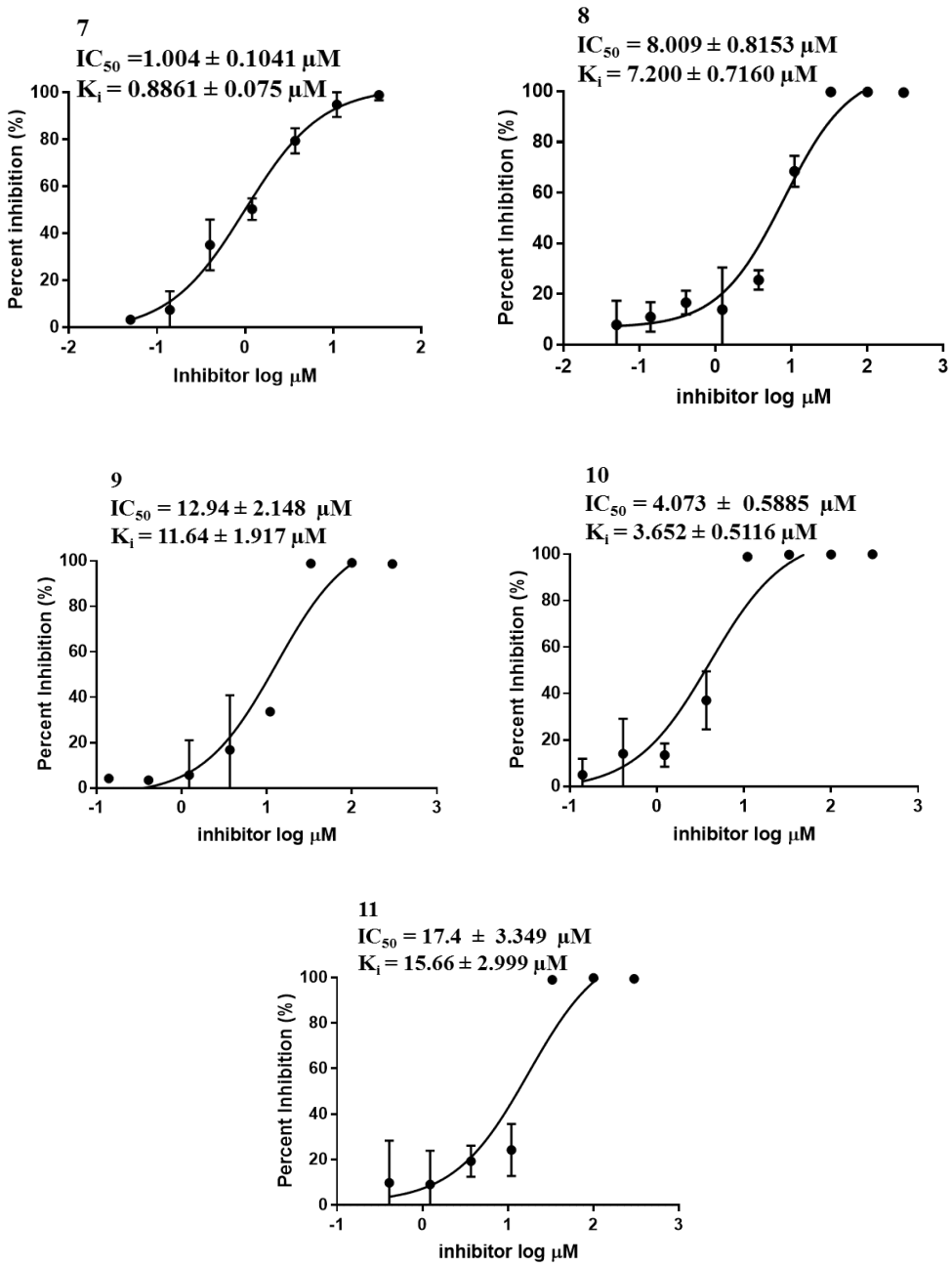


Figure S3. The K_i and IC_{50} data of regular peptide **1** and sulfono-γ-AApeptides **2-11**.

5. Circular Dichroism

Circular Dichroism (CD) spectra were measured on an Aviv 215 circular dichroism spectrometer using a 1 mm path length quartz cuvette, and compound solutions in PBS buffer were prepared using dry weight of the lyophilized solid followed by dilution to give the desired concentration (100 μM) and solvent combination. 10 scans were averaged for each sample, and 3 times of independent experiments were

conducted and the spectra were averaged. The final spectra were normalized by subtracting the average blank spectra. Molar ellipticity [θ] (deg·cm^2·dmol^{-1}) was calculated using the equation:

$$[\theta] = \theta_{obs}/(n \times l \times c \times 10)$$

Where θ_{obs} is the measured ellipticity in millidegrees, while n is the number of side groups, l is path length in centimeter (0.1 cm), and c is the concentration of the sulfono-γ-AA peptide in molar units.

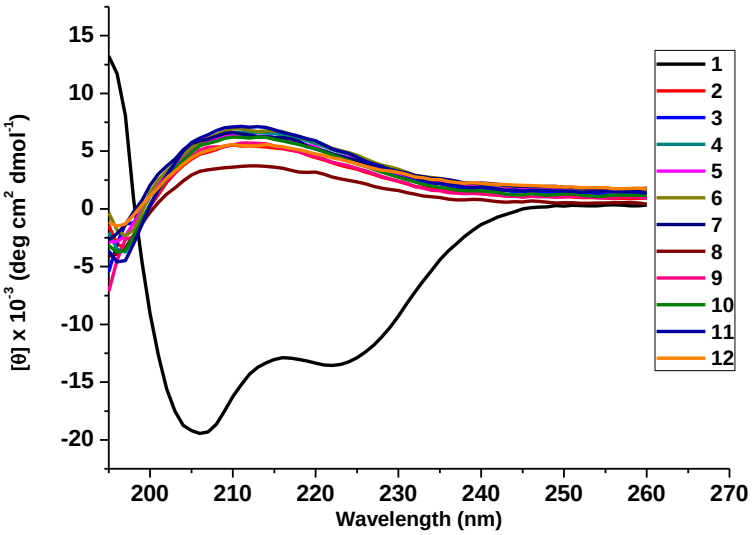


Figure S4. Circular dichroism spectra of regular peptide **1** and sulfono-γ-AApeptides **2-12** (100 μM) measured at room temperature in PBS buffer.

6. Cell Permeability Test

SW480 cells were plated on confocal dishes with 50% confluent and serum starved overnight, and then treated with different FITC-labeled peptides of 1 μM, and 10 μM for 2 hours. Then the cells were washed twice with PBS for 2 min. Next the cells were fixed with 100% MeOH for 5 min at room temperature, washed three times with PBS for 5 min. After which 0.1 μg/ml DAPI/PBS was added directly to cells in well,

incubated for 15 min, washed with PBS, kept from light and observed by inverted microscope.

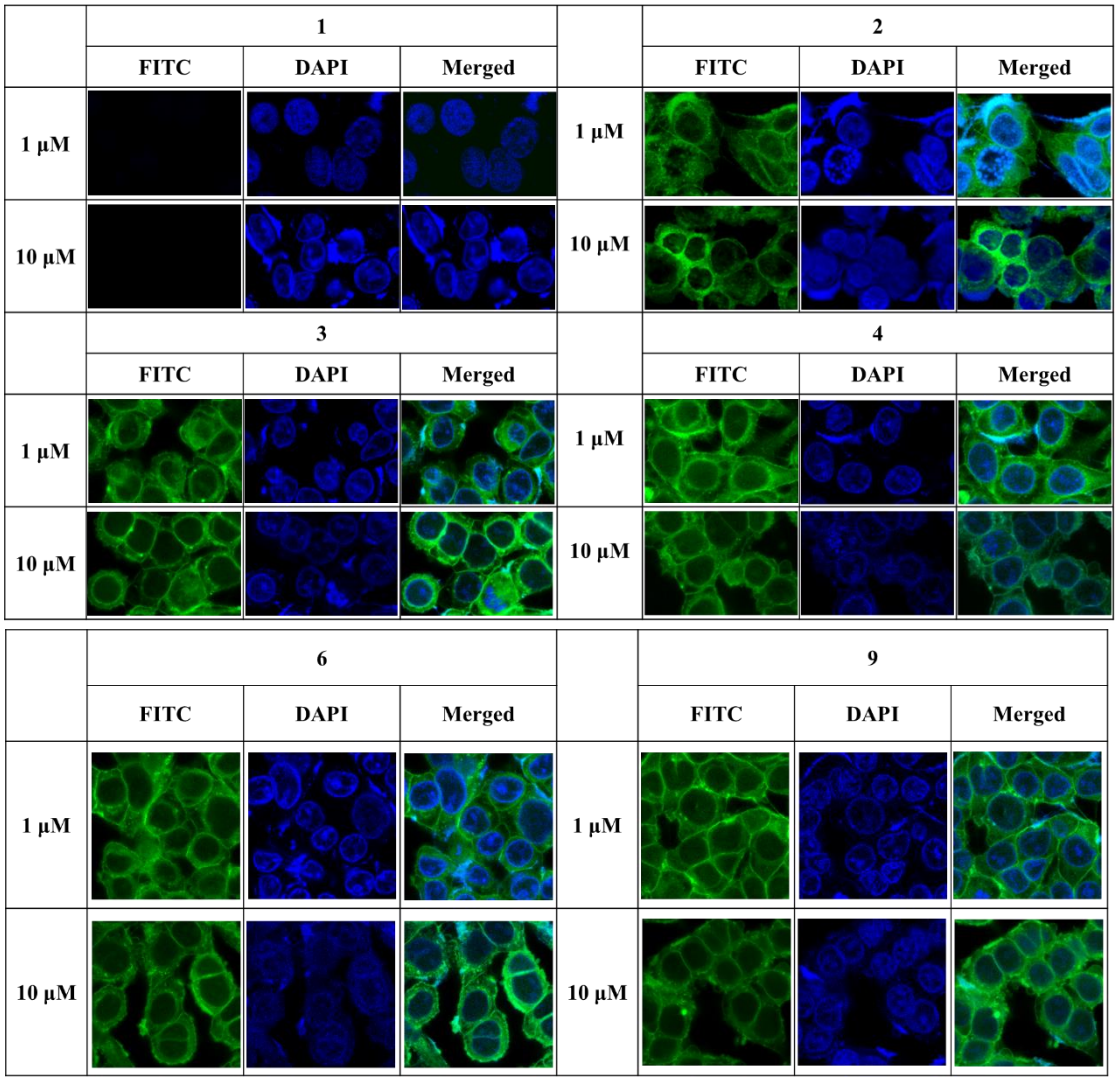


Figure S5. Fluorescent microscopy images of SW480 cells treated with 1 μM and 10 μM of the FITC-labeled peptide **1** and sulfono-γ-AApeptides **2-4**, **6** and **9** for 2 h.

7. MTs Cell Viability Assay

Colorectal cancer cell lines SW480 were seeded in 96-well plates at 5 $\times 10^3$ cells/well, maintained overnight at 37 °C, and incubated in the presence of inhibitors at various concentrations. Cell viability was monitored after 72 h using a freshly prepared mixture of one-part phenazine methosulfate (PMS, Sigma) solution (0.92 mg/mL) and

19 parts MTs agent (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt, Promega) solution (2 mg/mL). Cells were incubated in 10 μL of this solution at 37 °C for 3 h, and A490 was measured. The effect of each compound is expressed as the concentration required to reduce A490 by 50% (IC_{50}) relative to vehicle-treated cells. Experiments were performed in triplicate and repeated for three times.

8. TOPFlash/FOPFlash Luciferase Reporter Assay

FuGENE6 (E269A, Promega) 96-well plate format was used for the transfection of SW480 cells according to the manufacturer's instruction. SW480 cells were co-transfected with 60 ng of the TOPFlash or FOPFlash firefly luciferase reporter gene and 40 ng of renilla luciferase pCMV-RL normalization reporter. Cells were cultured in DMEM and 10% FBS at 37 °C for 24 h, and different concentrations of inhibitors or DMSO were added. After 24 h, the luciferase reporter activity was measured using the Dual-Glo system (E2940, Promega). Normalized luciferase activity in response to the treatment with inhibitors was compared with that obtained from the cells treated with DMSO. Experiments were performed in triplicate.

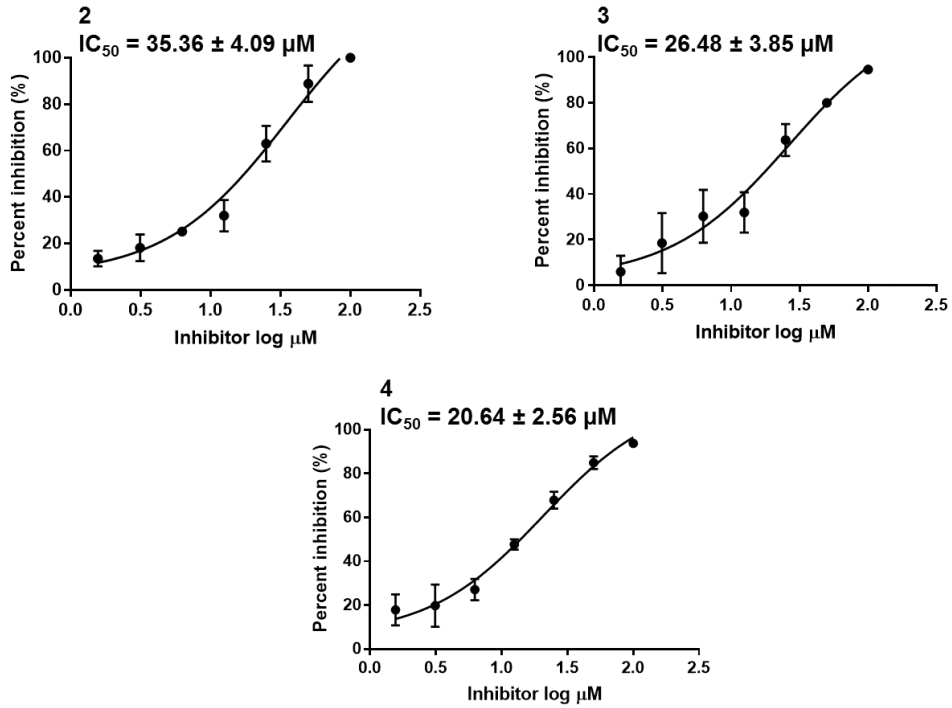


Figure S6. Wnt-responsive TOPFlash luciferase reporter assay results of inhibitors **2-4** in β-catenin activated SW480 cells.

Table S2. Results of renilla luciferase reporter, the internal control of TOPFlash/FOPFlash reporter assays.

Average renilla values of TOPFlash luciferase reporter assays

Compound	0 μM	12.5 μM	25 μM	50 μM
1	237	227	234	238
2	237	266	257	248
3	237	268	270	245
4	237	279	285	282

Average Renilla values of FOPFlash luciferase reporter assays

Compound	0 μM	12.5 μM	25 μM	50 μM
1	279	268	281	246
2	279	256	274	261
3	279	240	231	245
4	279	249	265	258

9. Pull-Down Experiments

Adherent β-catenin signaling hyperactive SW480 cancer cells (70-80% confluency) in T75 flask were lysed first in 1 mL buffer A containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Nonidet P-40, 2 mM EDTA, and protease inhibitors. Cell debris was removed by centrifugation at 10,000 g for 20 min at 4 °C. In 500 µL SW480 cell lysates, 1 µM biotinylated inhibitor was added in and incubated at 4 °C for 3 h. Then, 25 µL Streptavidin Sepharose beads (S-1638, Sigma) were added to the lysate mixture and rotated at 4 °C for 2 h. The lysate mixture was centrifuged at 4000 rpm for 2 min at 4 °C. The beads were washed with buffer B (20 mM Tris pH 7.4,150 mM NaCl, 0.05% NP-40) for 4 times. The beads were resuspended in 60 µL of 2x SDS sample buffer. After boiling, the samples were loaded onto 8% SDS polyacrylamide gel for electrophoretic analysis. Separated proteins were transferred onto nitrocellulose membranes for immunoblot analysis. The antibody against β-catenin (610153, BD Biosciences) were incubated with the membranes. IRDye 680LT goat anti-mouse IgG (827−11080, LiCOR) was used as the secondary antibody. The images were detected by the Odyssey Infrared Imaging System (LiCOR). Experiments were performed in duplicate.

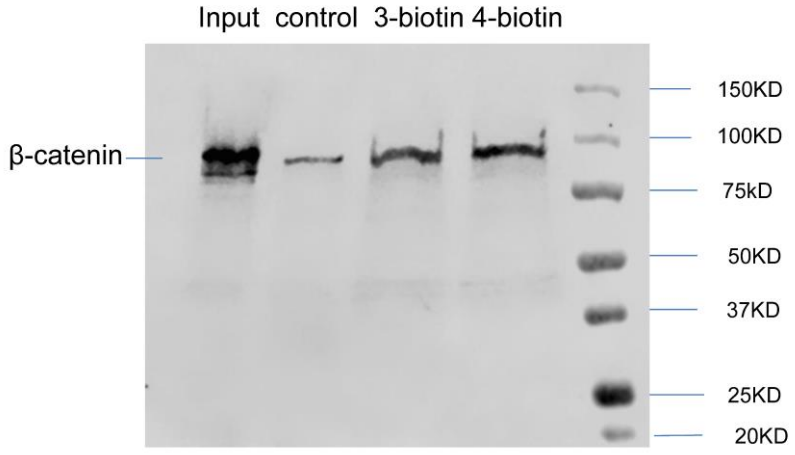


Figure S7. Original image for the pull-down experiments.

10. Co-Immunoprecipitation Experiments

β-Catenin signaling hyperactive HCT116 cnacer cells at 1 × 10^6/mL were treated with different concentrations of the inhibitor for 24 h. Cells were then lysed in buffer containing 50 mM Tris, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 2 mM EDTA, and protease inhibitors. The cell lysates were then preadsorbed to A/G plus agarose (sc-2003, Santa Cruz Biotechnology) at 4 °C for 1 h. Preadsorbed lysates were incubated with a specific primary antibody against β-catenin (610153, BD Biosciences) overnight at 4 °C. A/G plus agarose was then added to the lysate mixture and incubated for 3 h. The beads were washed four times with the lysis buffer at 4 °C. The bound protein was eluted by boiling in the SDS sample buffer and loaded onto 8% SDS polyacrylamide gel for electrophoretic analysis. Separated proteins were transferred onto nitrocellulose membranes for immunoblot analysis. The antibodies against BCL9 (ab37305, Abcam), β-catenin (610153, BD Biosciences), and β-tubulin (sc-55529, Santa Cruz Biotechnology, Inc.) were incubated with the membranes, respectively. IRDye 680LT goat anti mouse IgG (827−11080, LiCOR) and IRDye 800CW goat anti rabbit IgG (926-32211, LiCOR) were used as the secondary antibodies. The images were detected by the Odyssey Infrared Imaging System (LiCOR). Experiments were performed in duplicate.

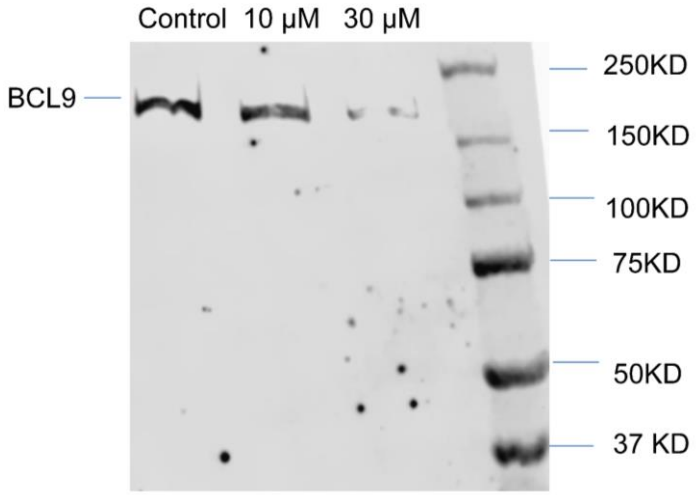


Figure S8. Original image for β-catenin immunoprecipitation (IP) and then BCL9 immunoblotting (IB).

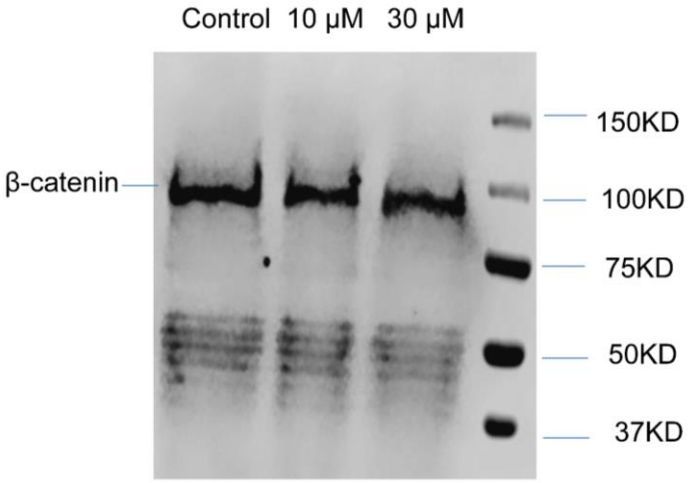


Figure S9. Original image for β-catenin immunoprecipitation (IP) and then β-catenin immunoblotting (IB).

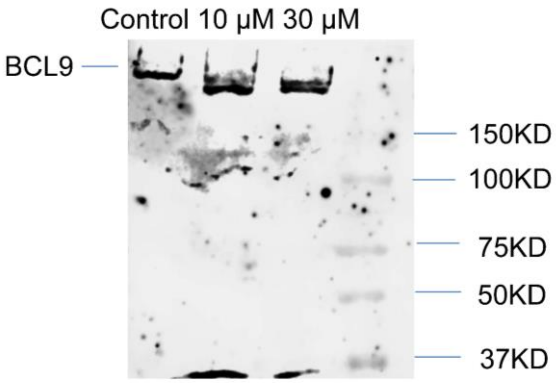


Figure S10. Original image for BCL9 immunoblotting as the input.

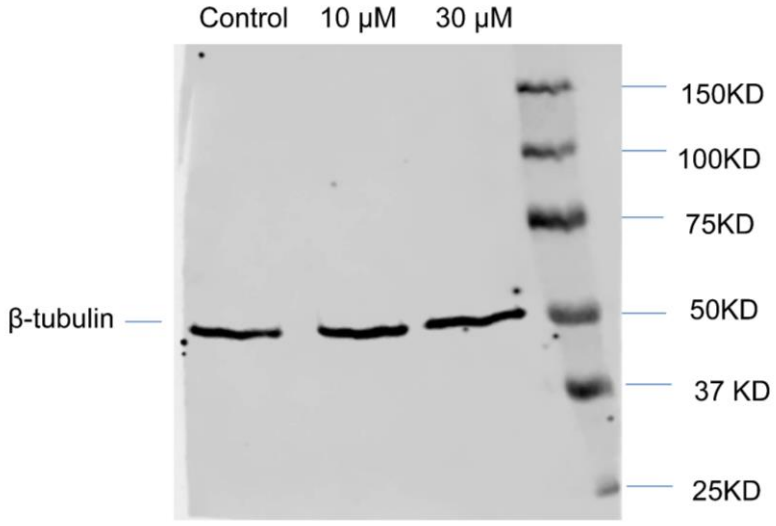


Figure S11. Original image for β-tubulin immunoblotting as the input.

11. Enzymatic Stability Study

Lead Compounds **2-4** and peptide control **1** (0.1 mg/mL) were incubated with 0.1 mg/mL protease in 100 mM ammonium bicarbonate buffer (pH 7.8) at 37 °C for 24 h. Then, the reaction mixtures were concentrated in a speed vacuum at medium temperature to remove water and ammonium bicarbonate. The resulting residues were re-dissolved in H_2O/MeCN and analyzed on a Waters analytical HPLC system with 1 mL/min flow rate and 5% to 100% linear gradient of solvent B (0.1% TFA in acetonitrile) in A (0.1% TFA in water) over the duration of 50 min. The UV detector was set to 215 nm.

11.1 HPLC Traces of 1 in Presence of Pronase

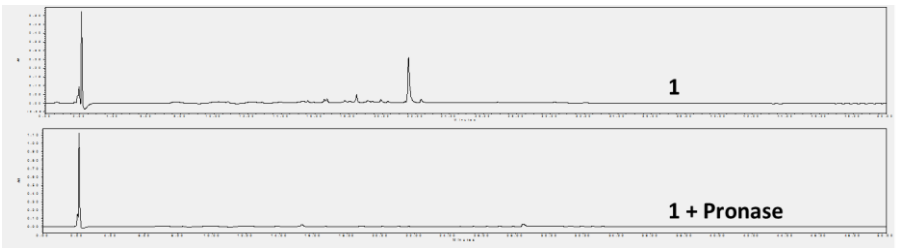


Figure S12. Analytic HPLC trace of **1** before and after incubation with Pronase (0.1 mg/mL) in 100 mM pH 7.8 ammonium bicarbonate buffer at 37 °C.

11.2 HPLC Traces of Lead Peptides in Presence of Proteases

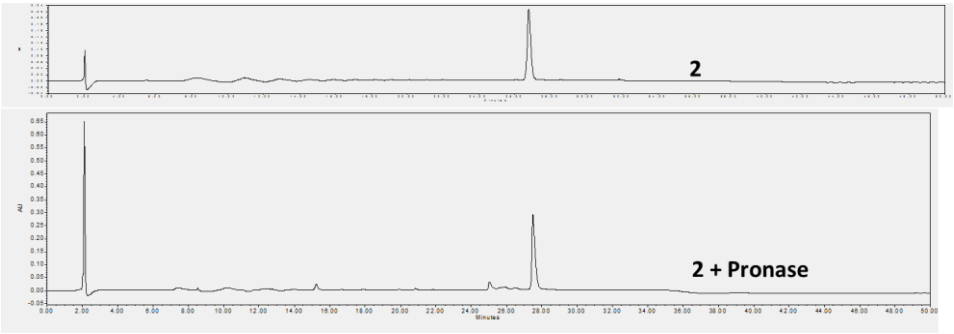


Figure S13. Analytic HPLC trace of **2** before and after incubation with Pronase (0.1 mg/mL) in 100 mM pH 7.8 ammonium bicarbonate buffer at 37 °C.

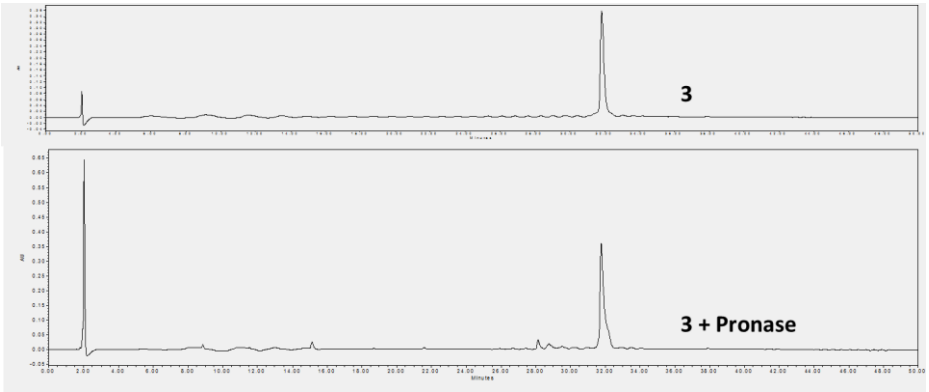


Figure S14. Analytic HPLC trace of **3** before and after incubation with Pronase (0.1 mg/mL) in 100 mM pH 7.8 ammonium bicarbonate buffer at 37 °C.

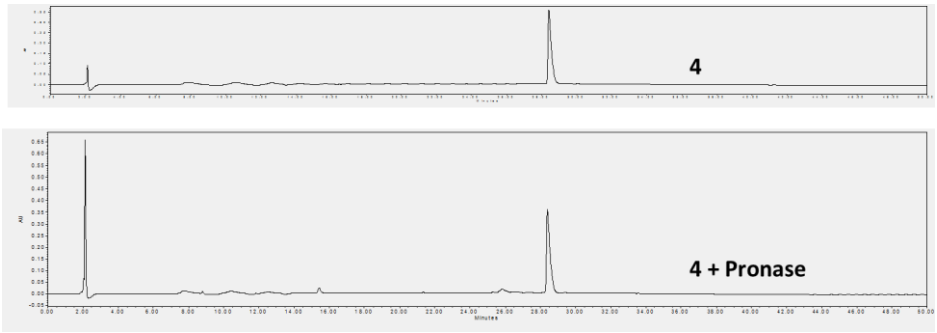


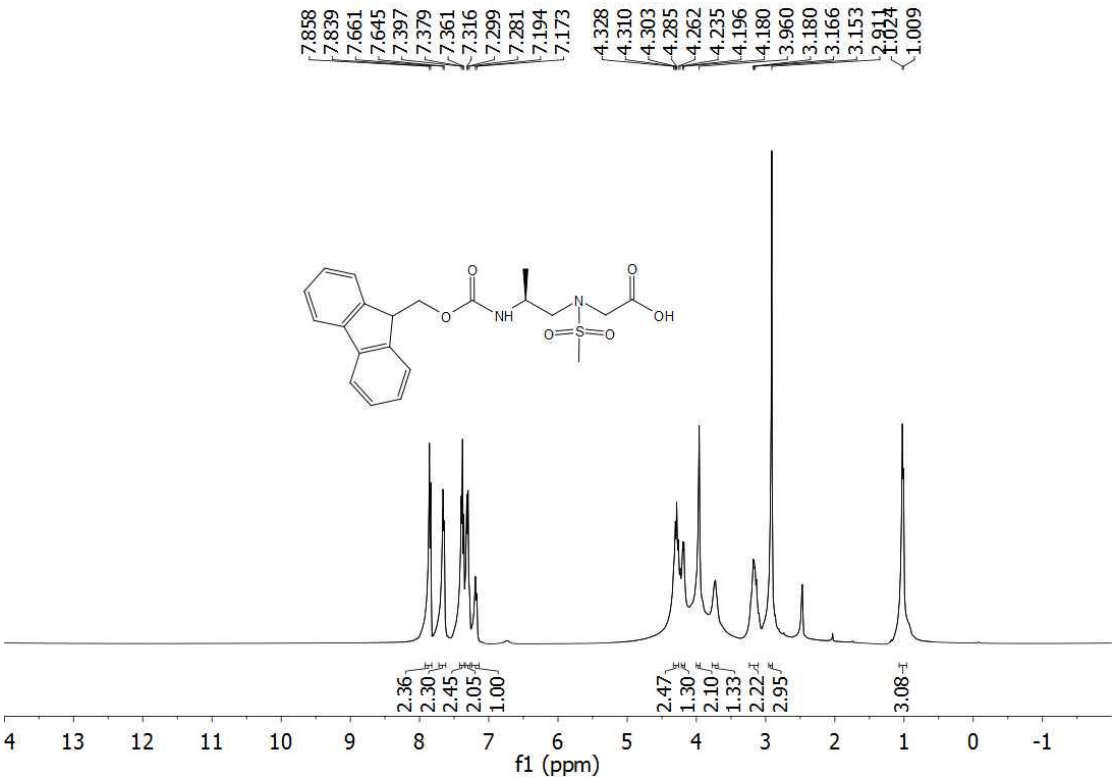
Figure S15. Analytic HPLC trace of **4** before and after incubation with Pronase (0.1 mg/mL) in 100 mM pH 7.8 ammonium bicarbonate buffer at 37 °C.

12. References

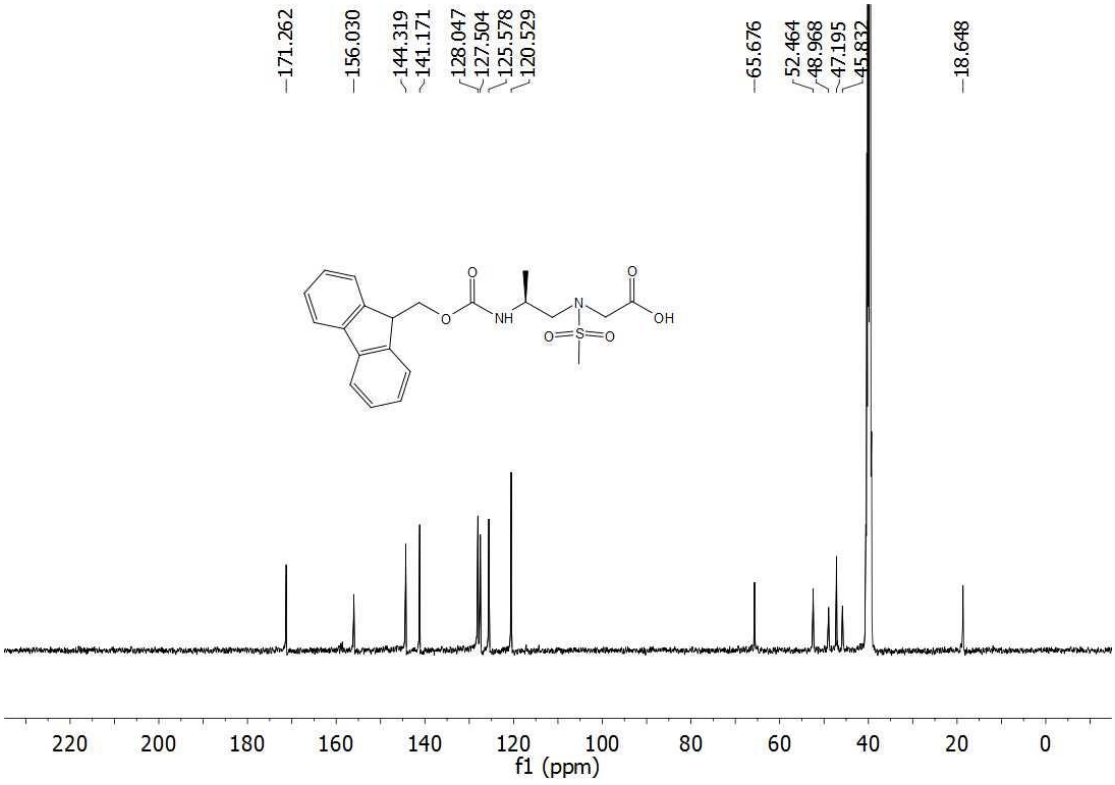
1. Teng P, et al. (2017) Right-Handed Helical Foldamers Consisting of De Novo d-AApeptides. *J. Am. Chem. Soc.* 139(21):7363-7369.

2. Nikolovska-Coleska Z, et al. (2004) Development and optimization of a binding assay for the XIAP BIR3 domain using fluorescence polarization. *Anal. Biochem.* 332(2):261-273.

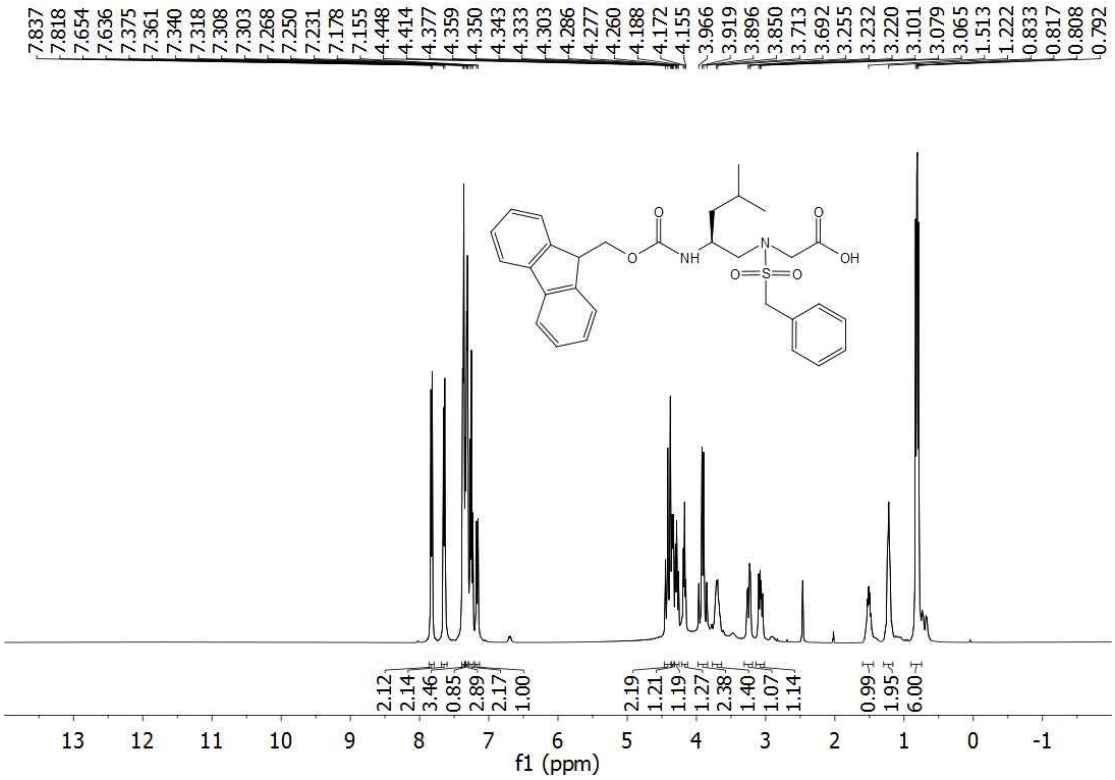
13. The ^{1}H and ^{13}C NMR Spectra of Sulfono-γ-AApeptide Building Blocks



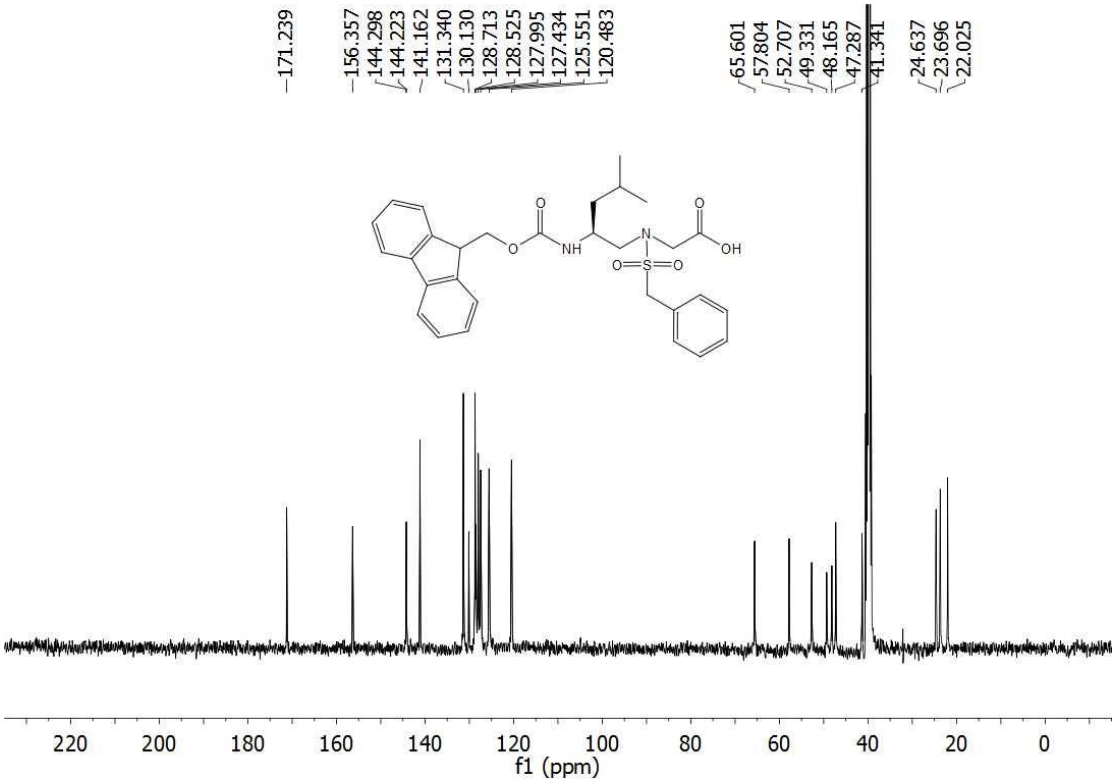
^{1}H NMR of BB1



^{13}C NMR of BB1



^{1}H NMR of BB2



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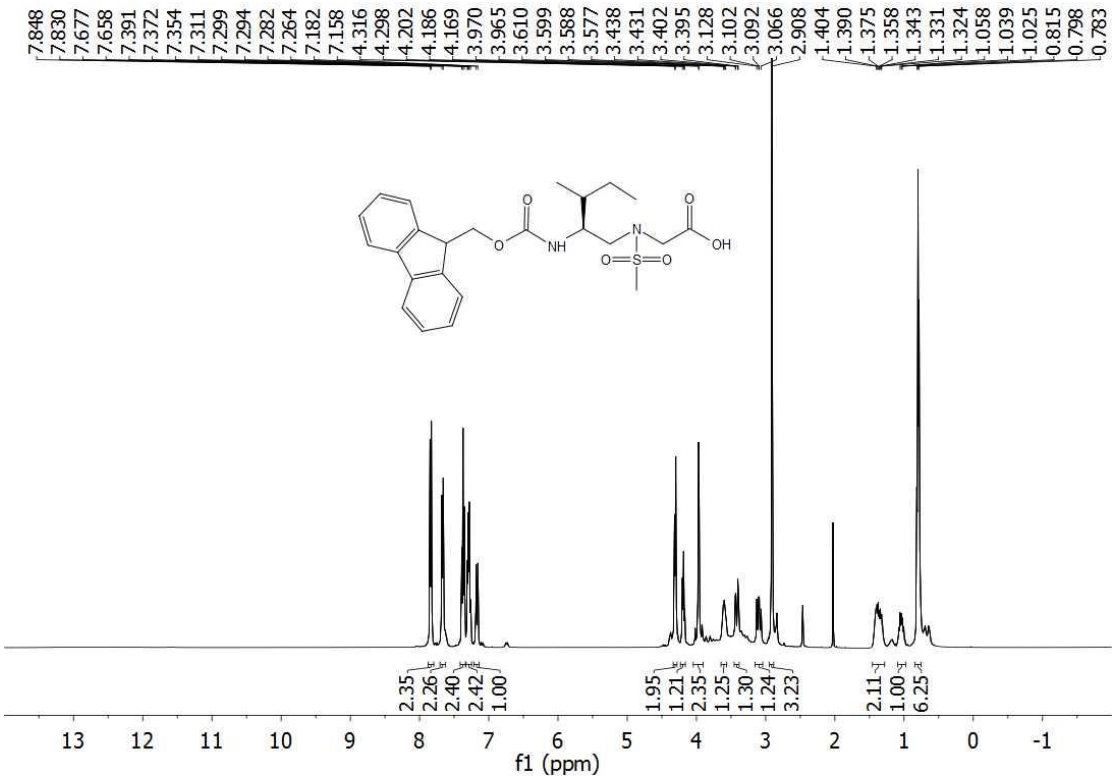
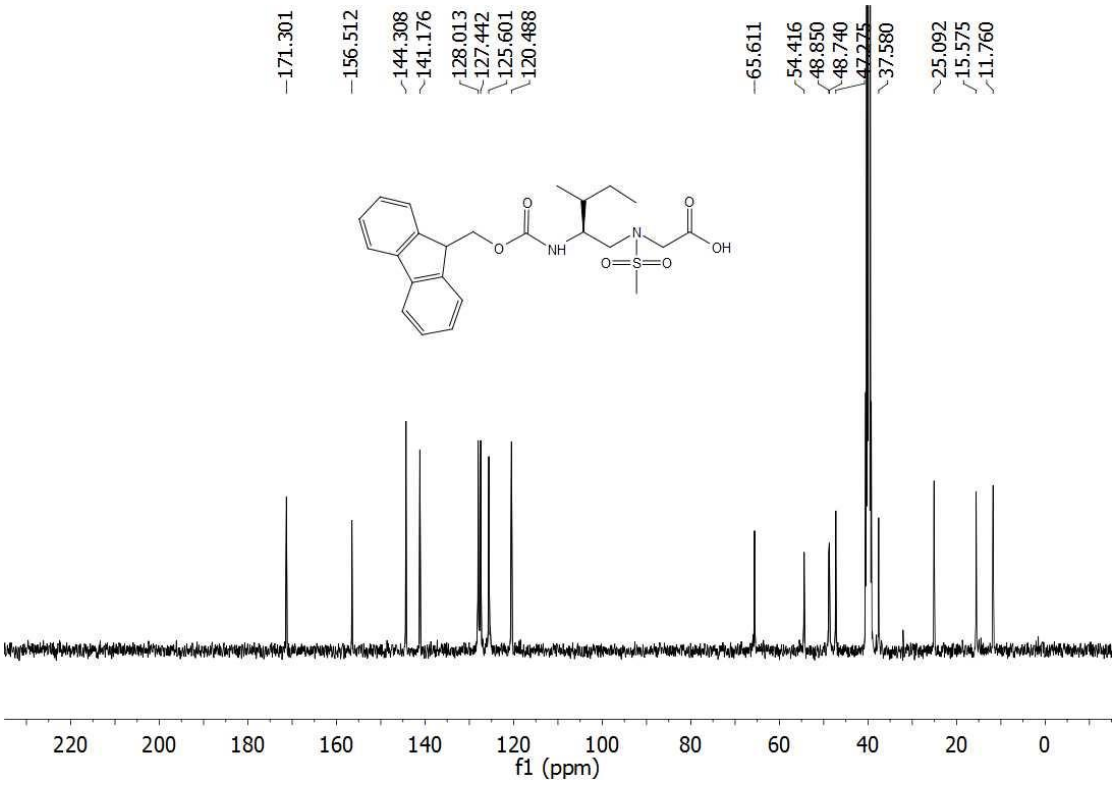
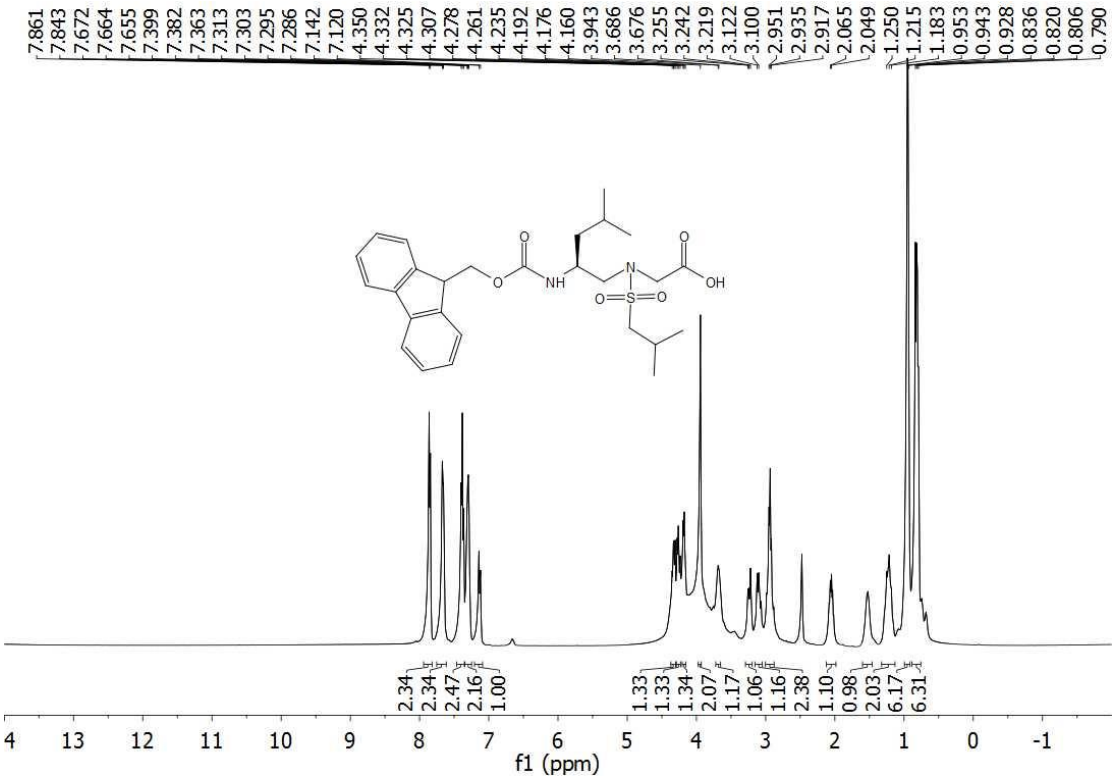


^{1}H NMR of BB3



^{13}C NMR of BB3



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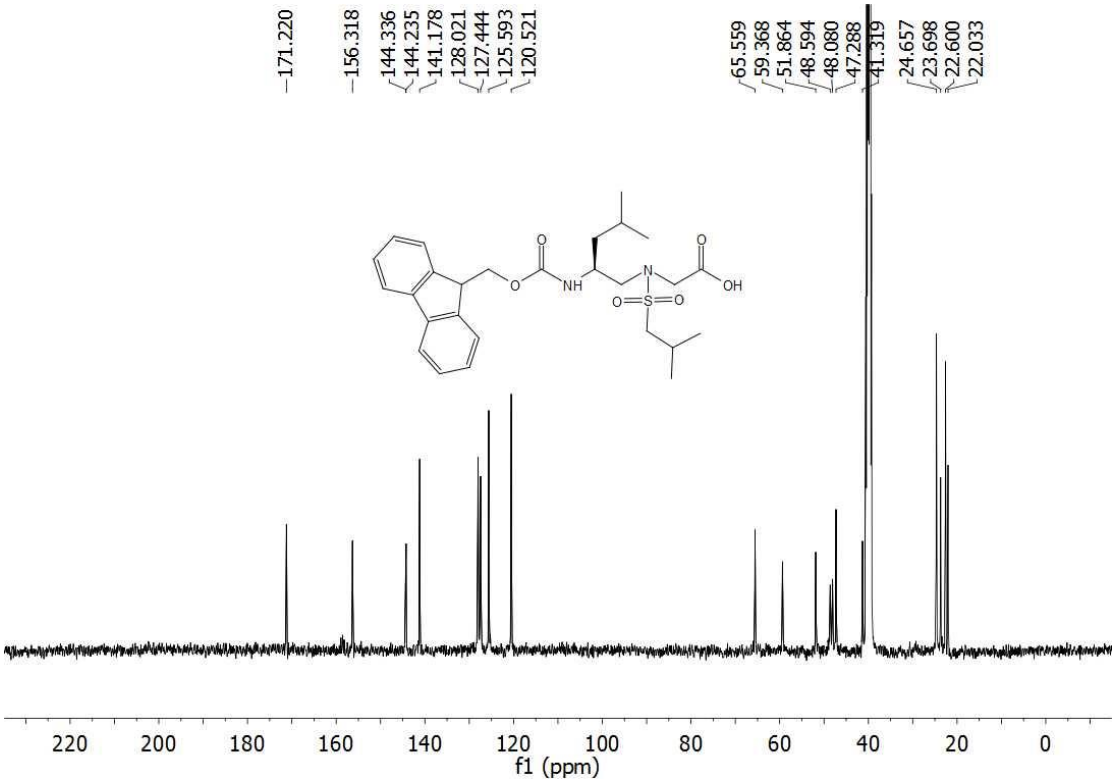
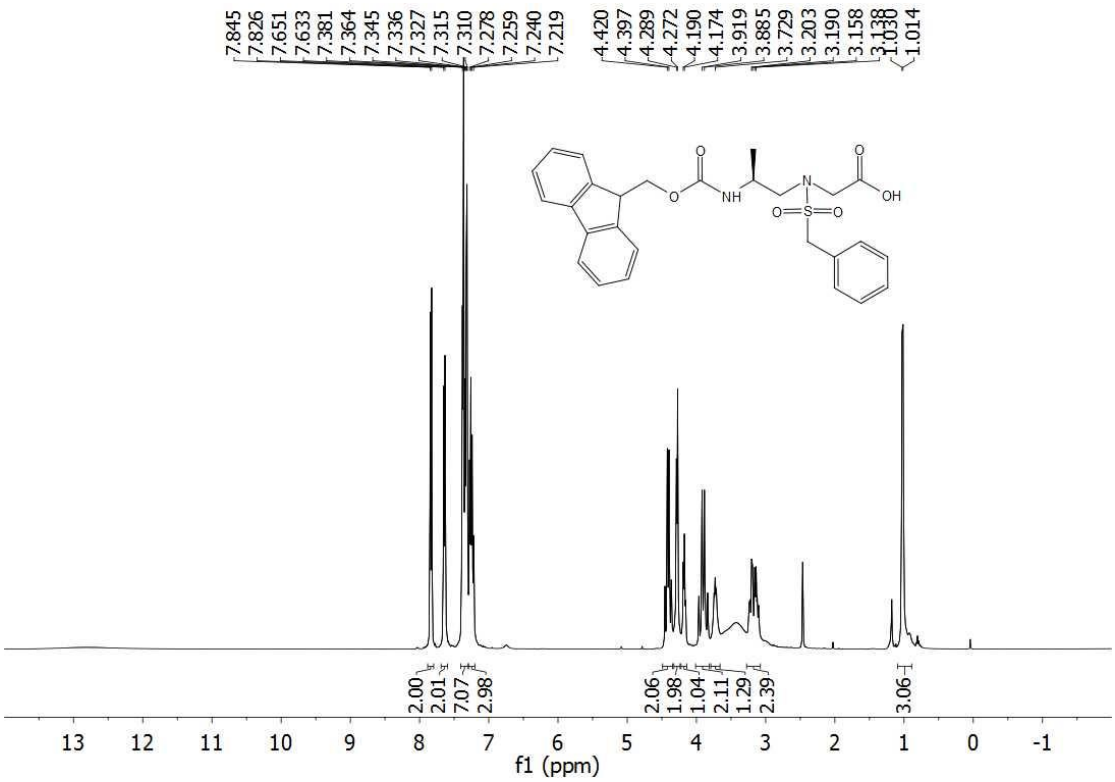


^{13}C NMR of BB4



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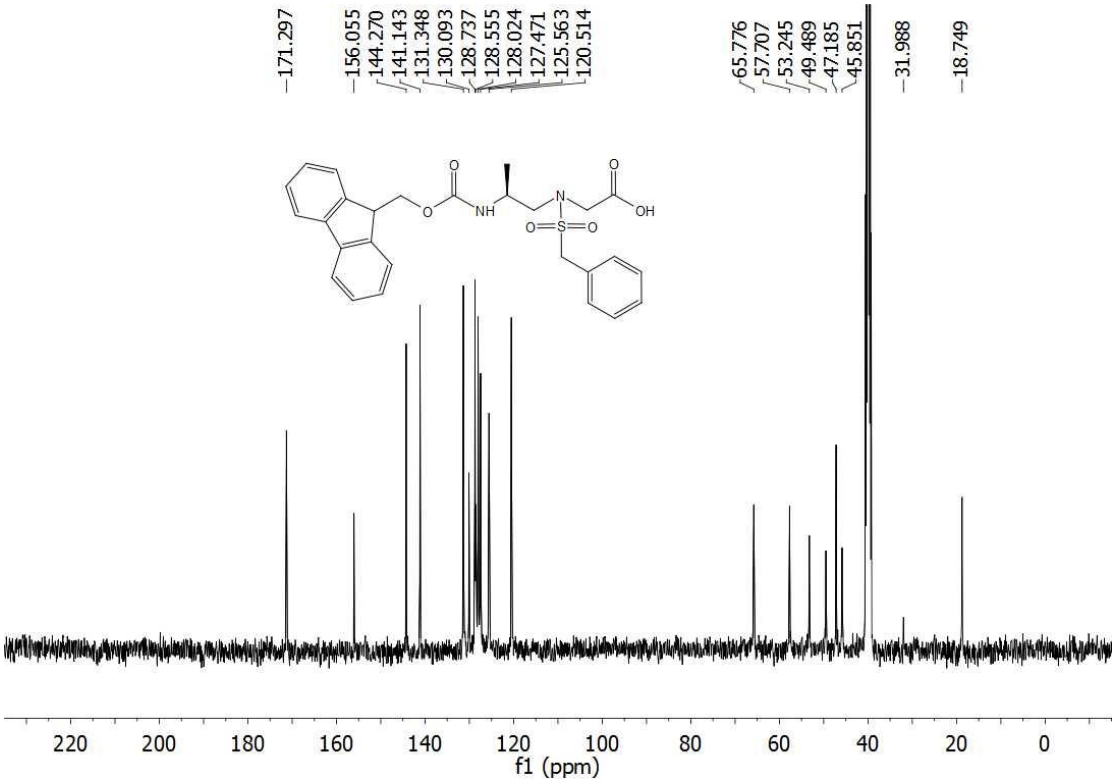

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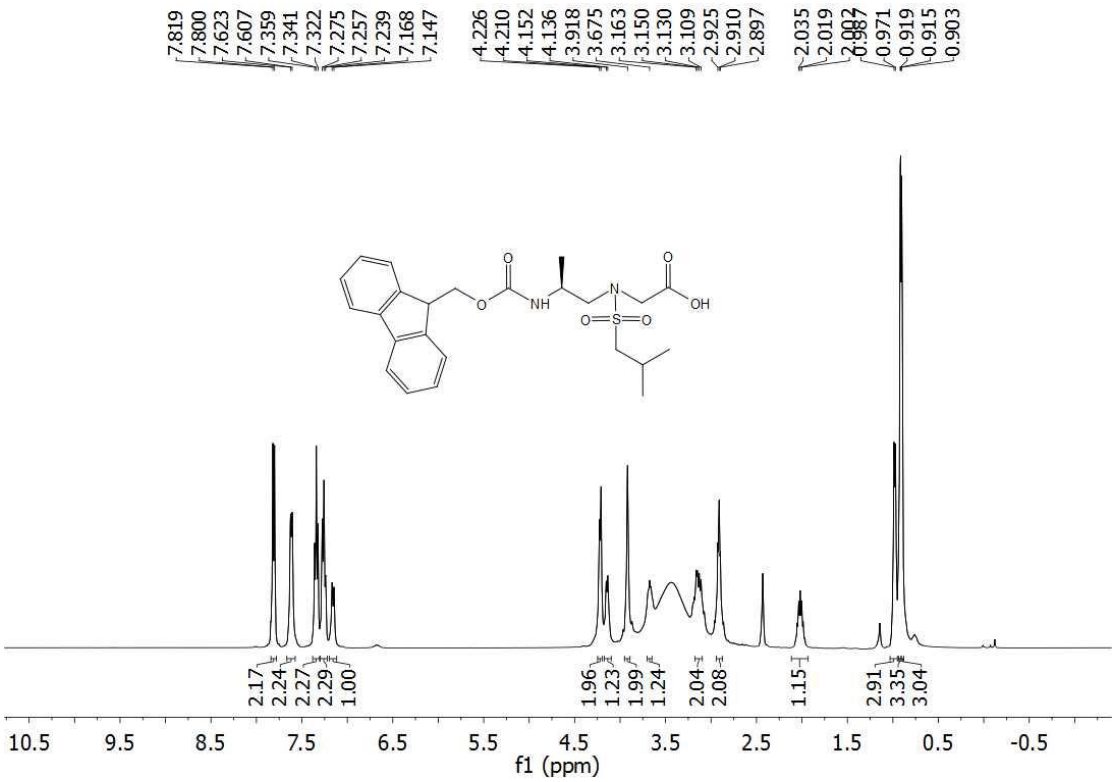
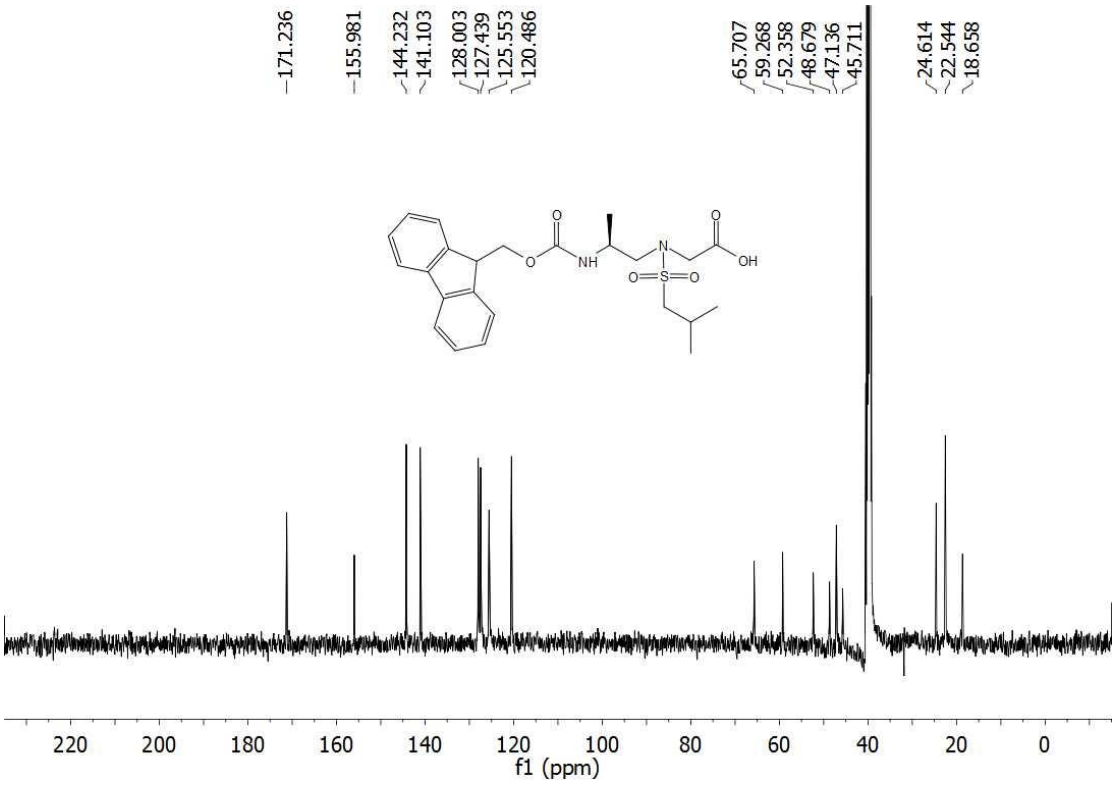
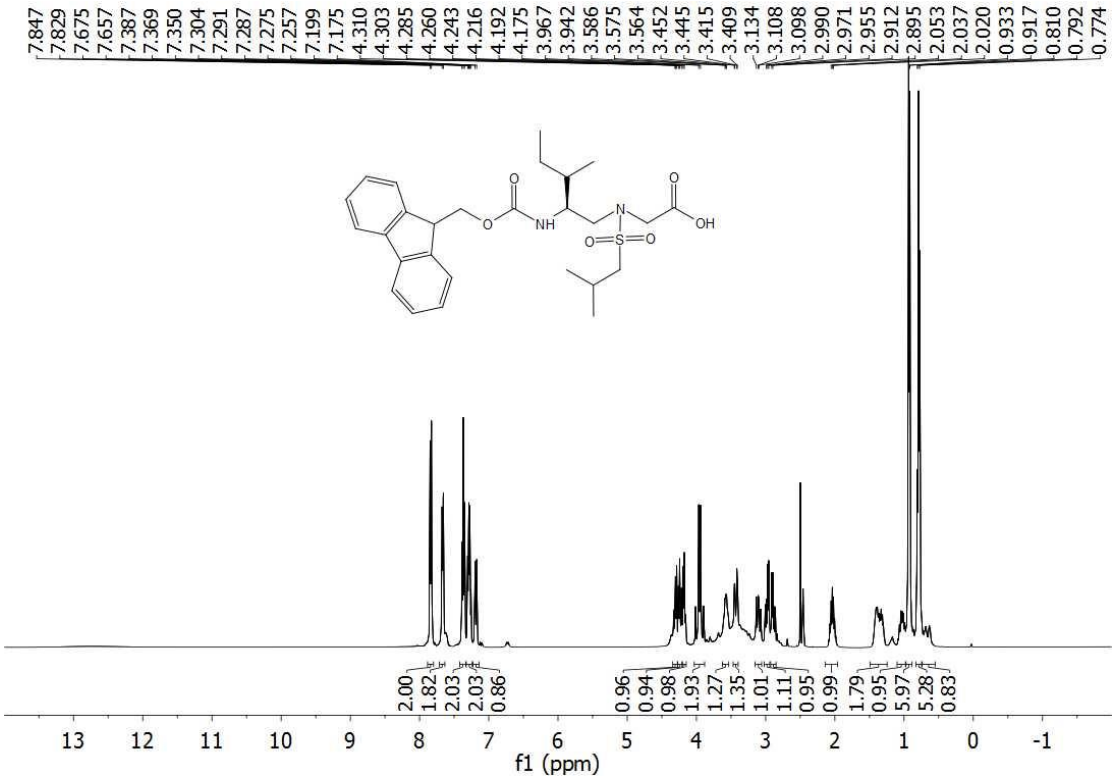


^{1}H NMR of BB6



^{13}C NMR of BB6



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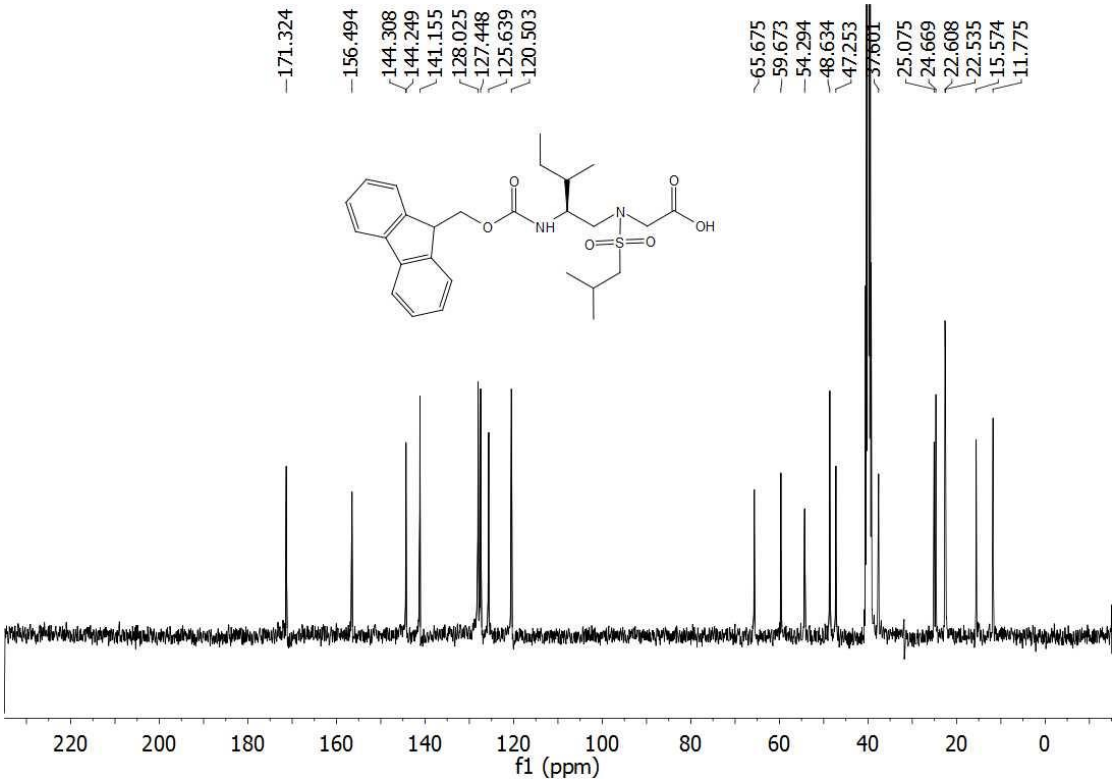

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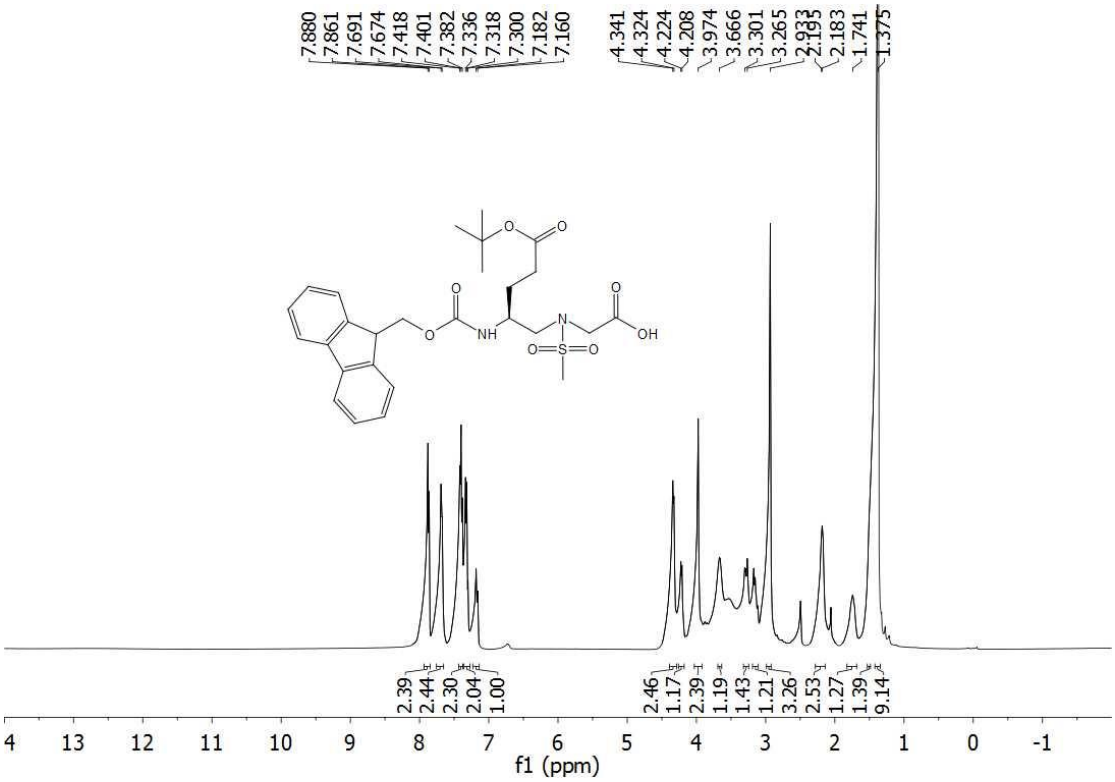
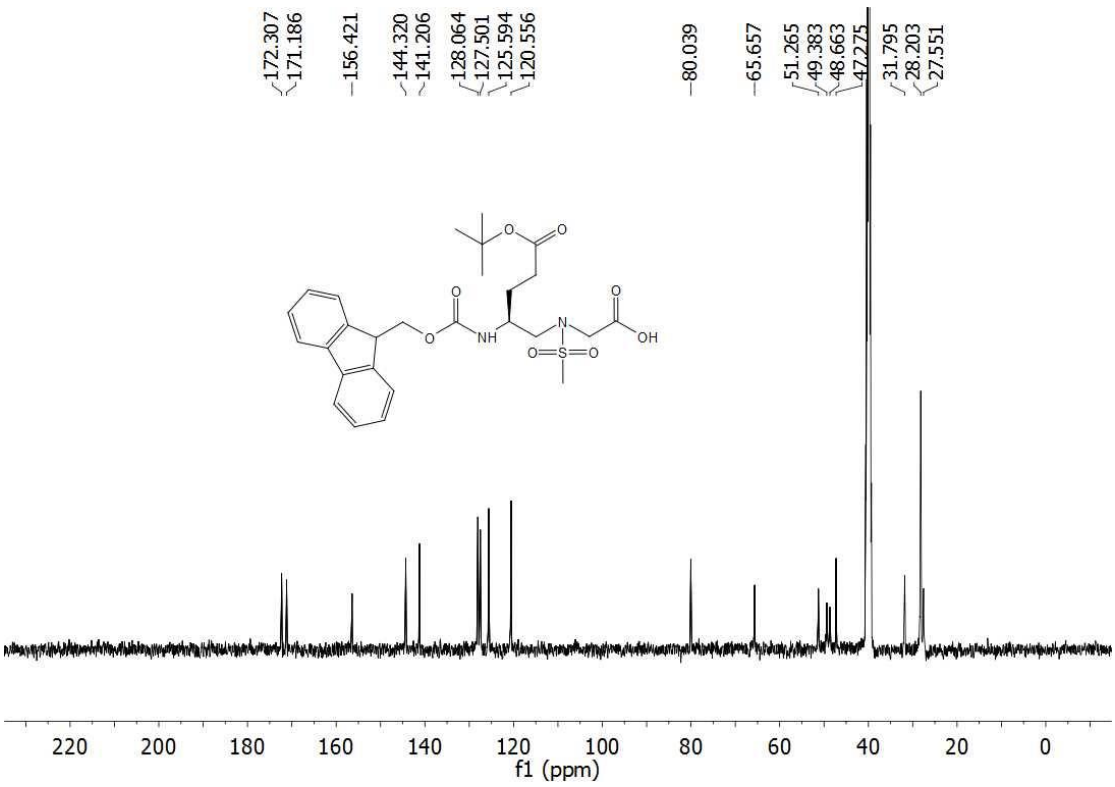


^{1}H NMR of BB8



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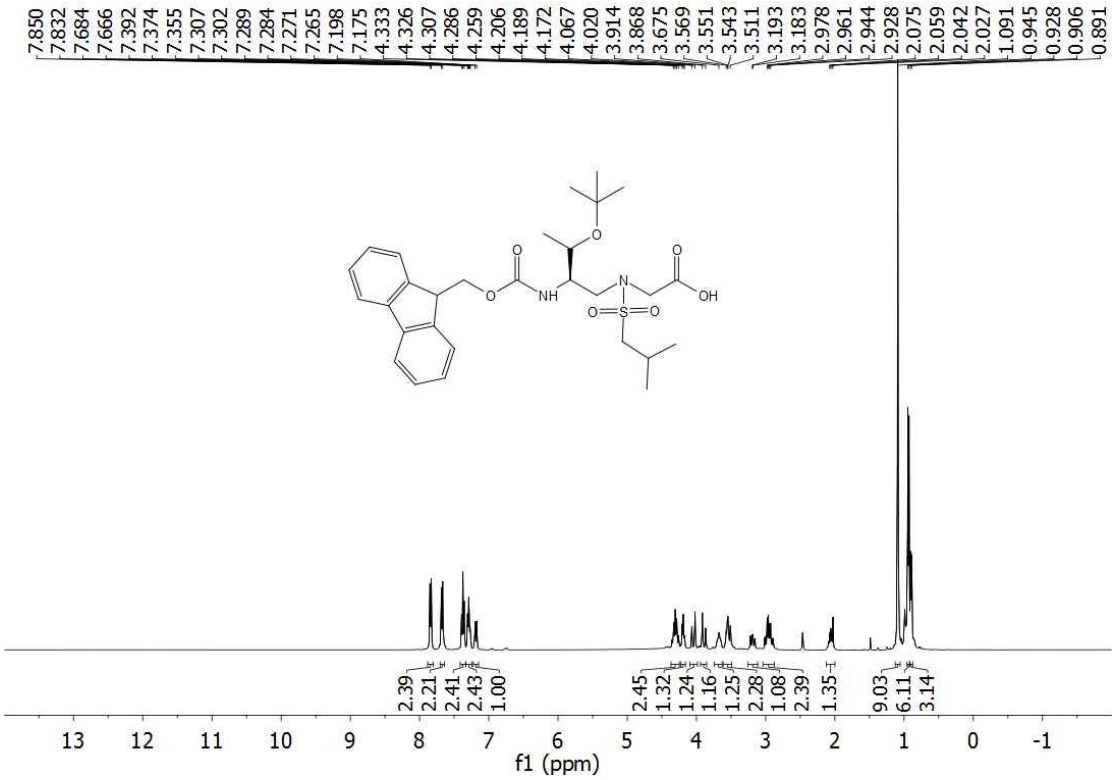
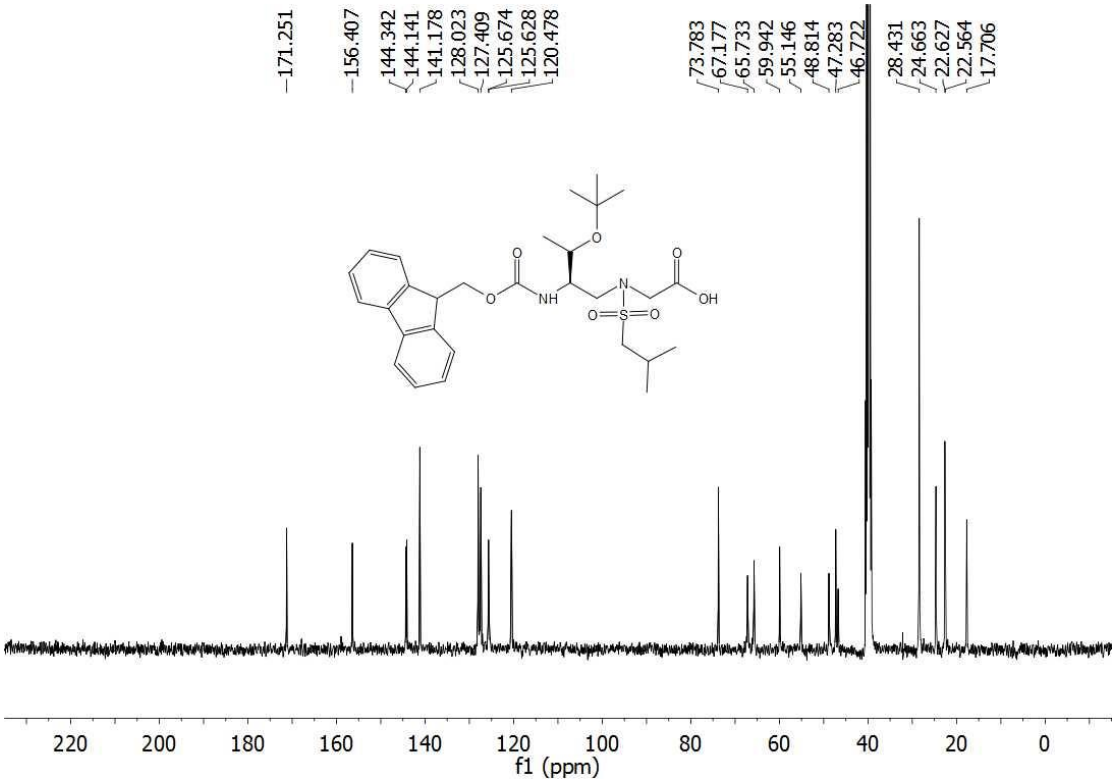


^{1}H NMR of BB9



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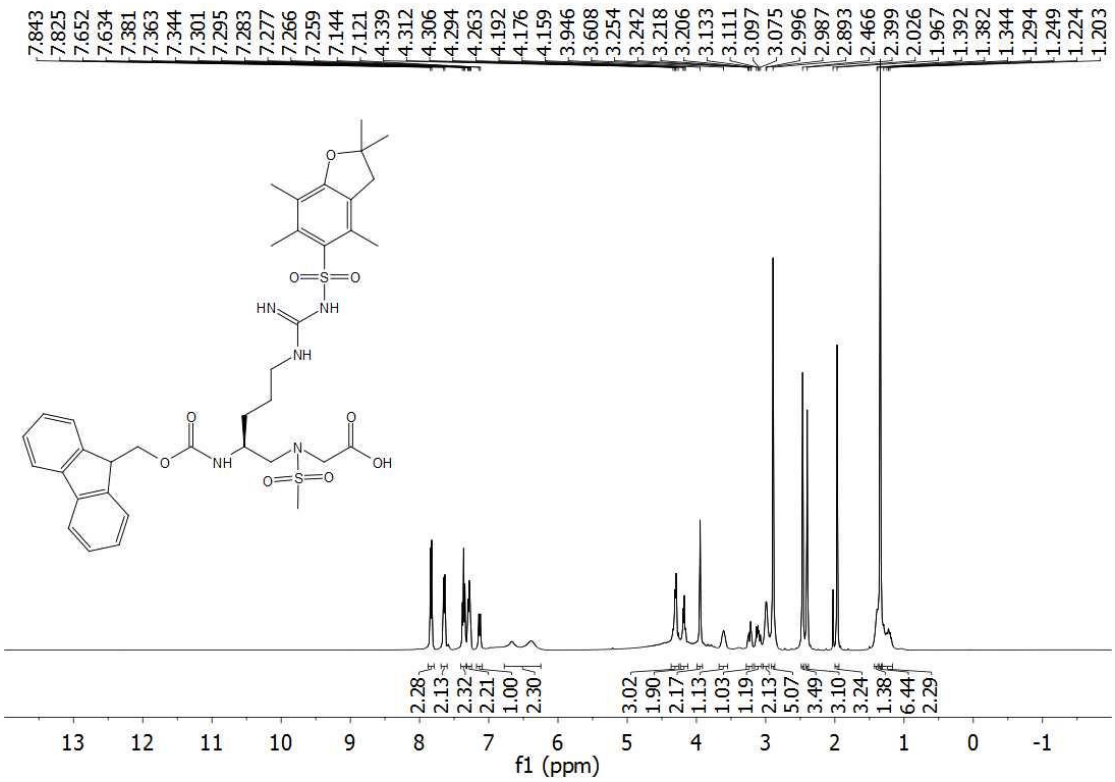

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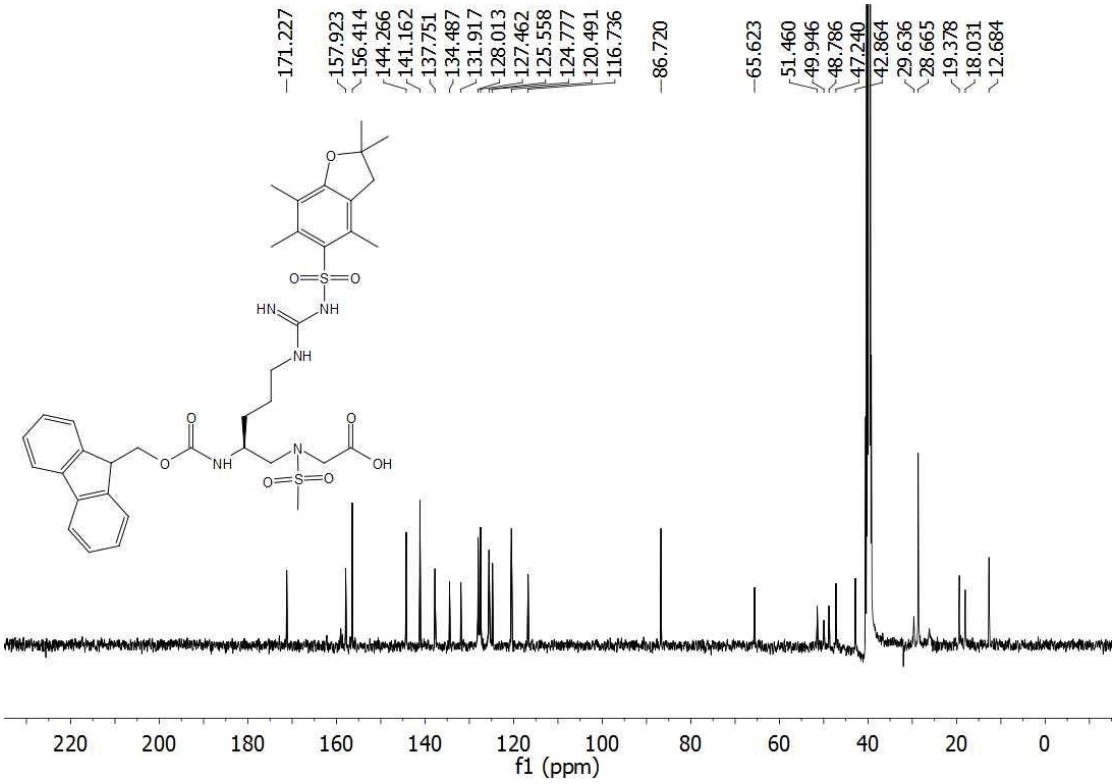

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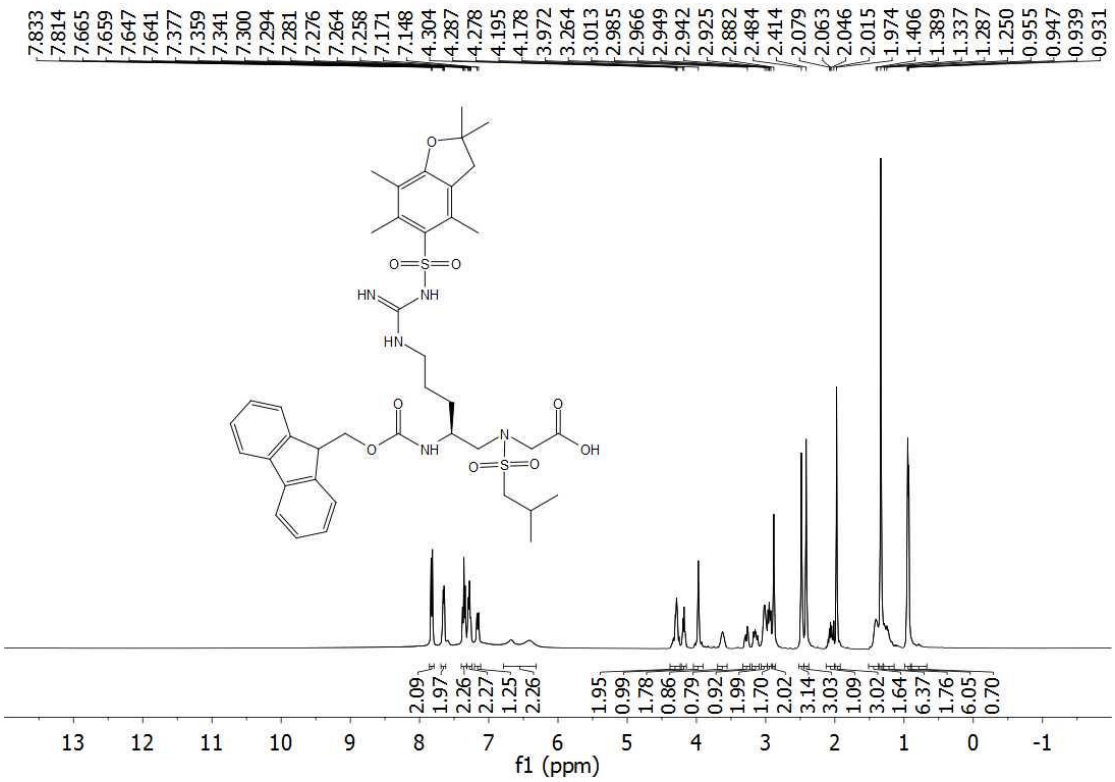

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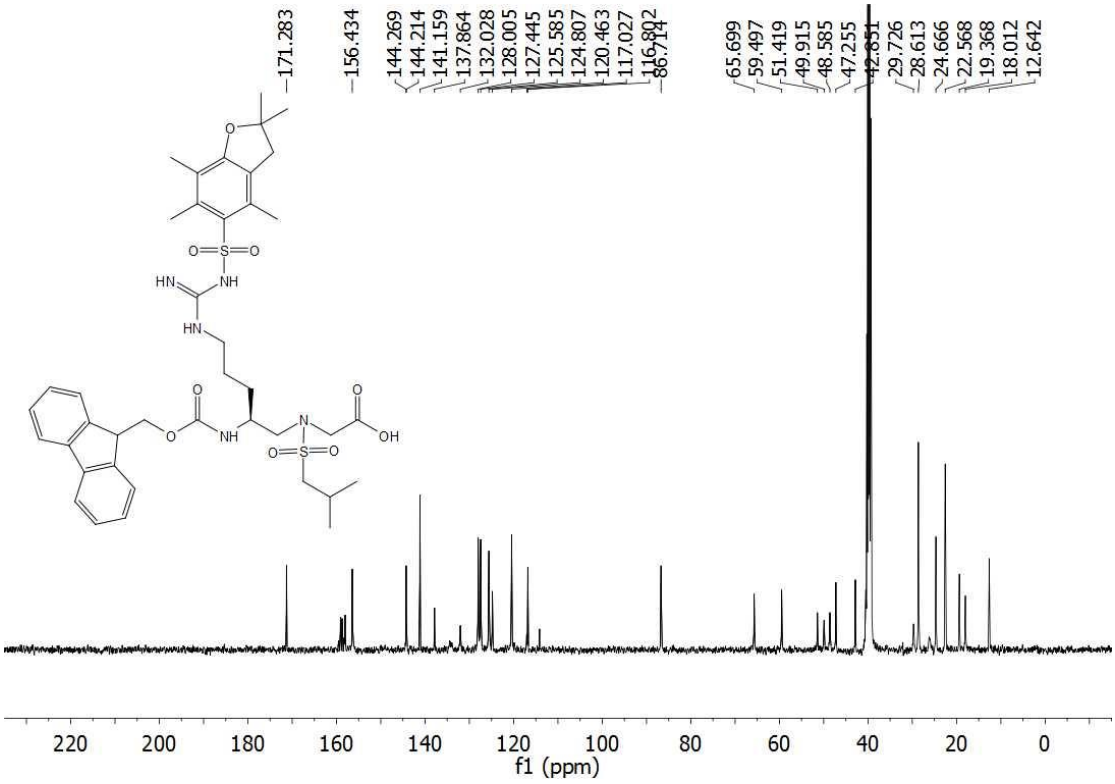

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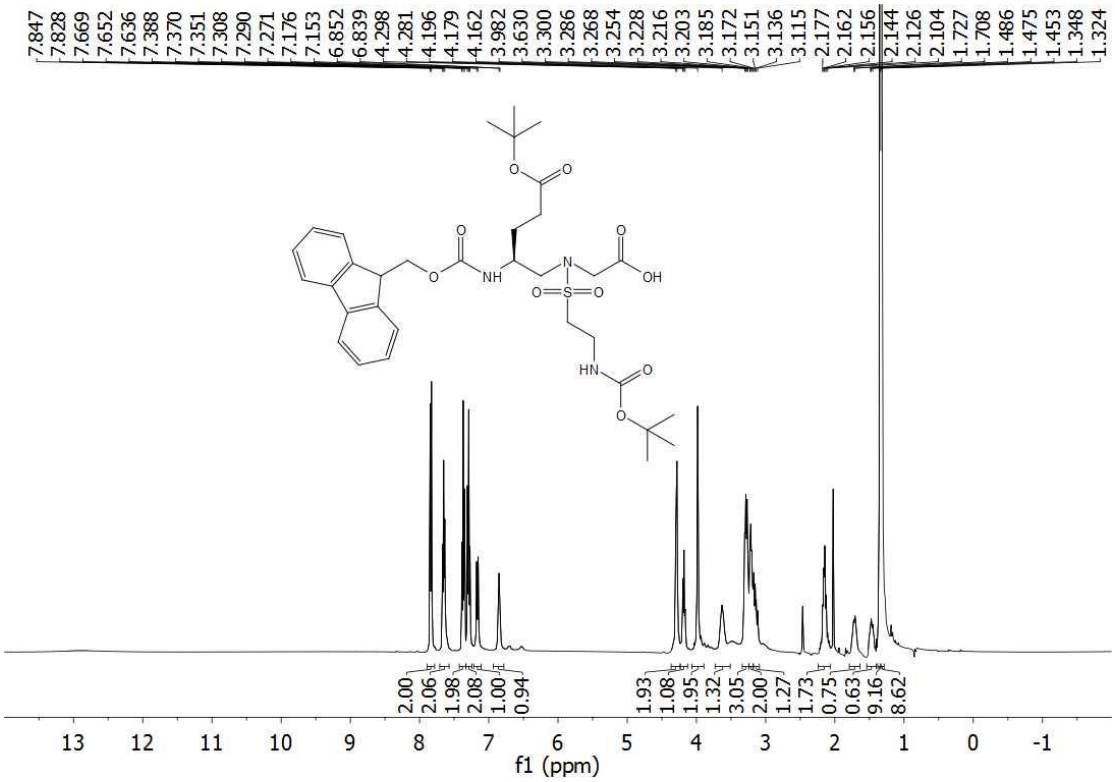
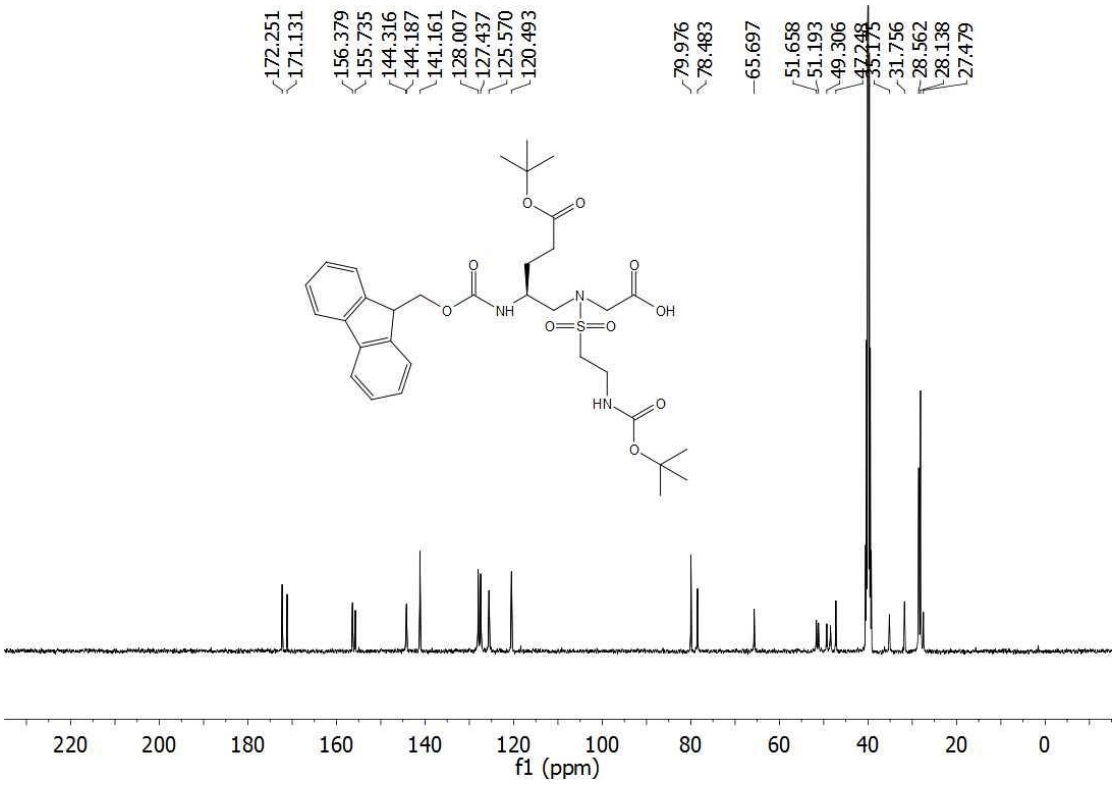
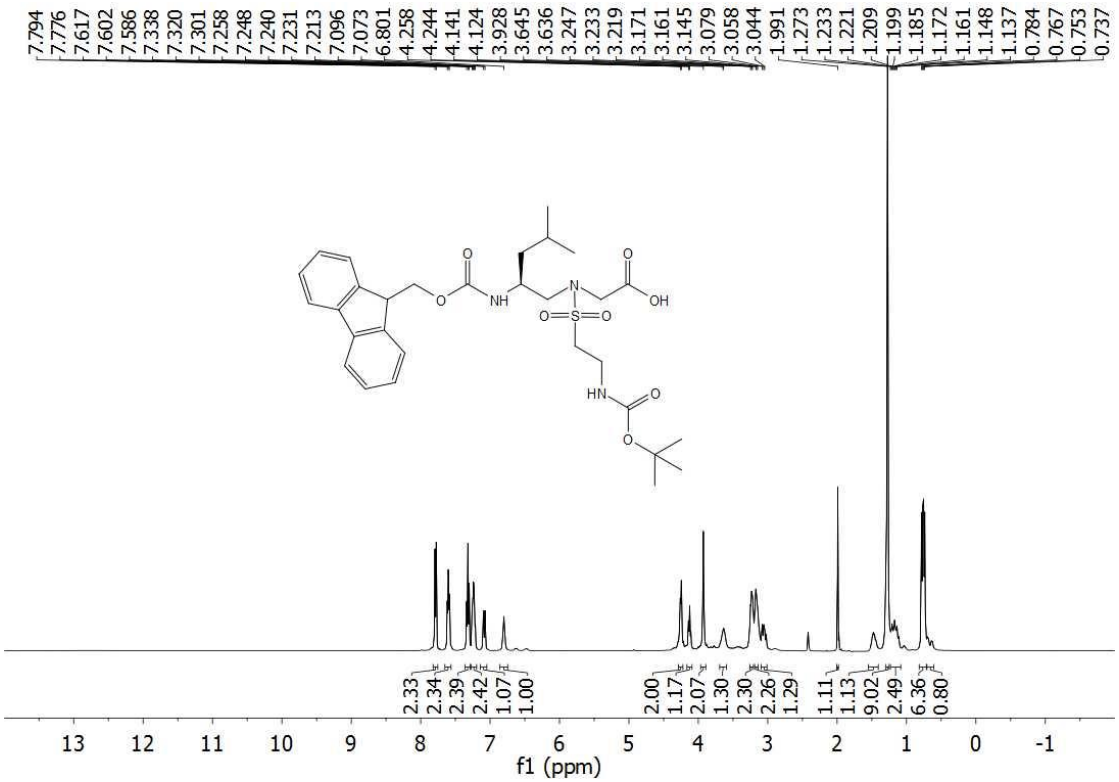


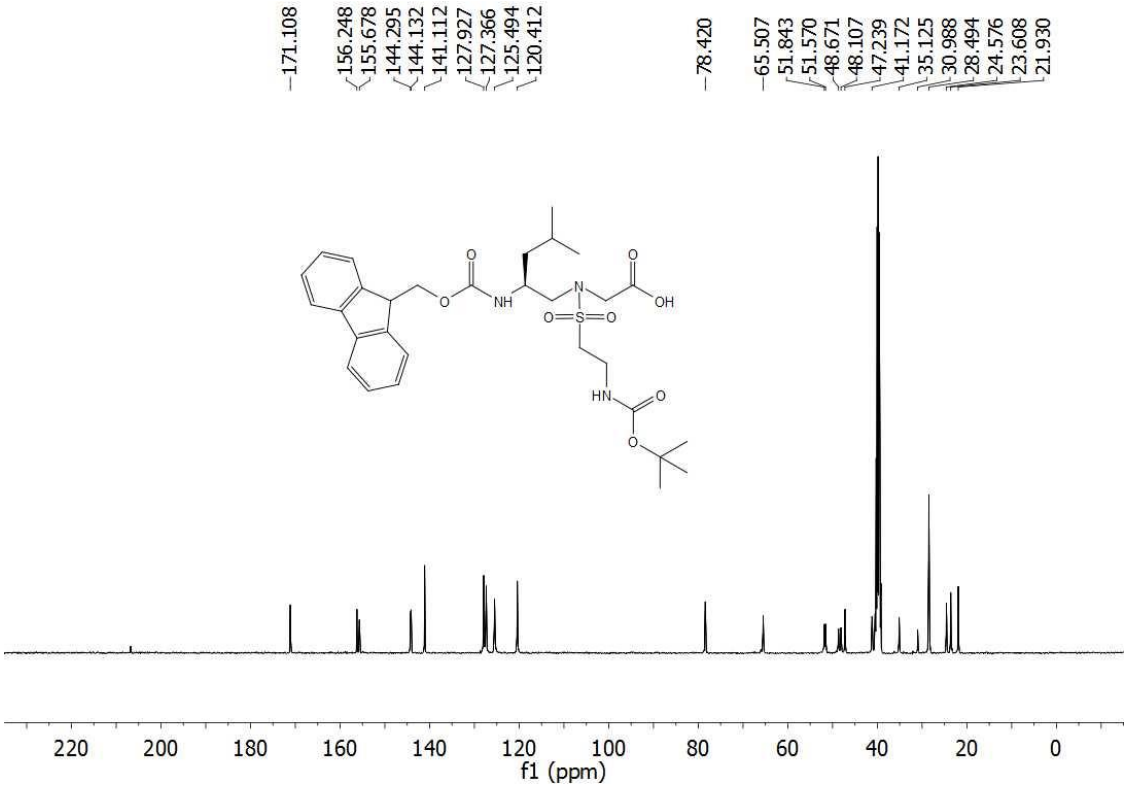
^{1}H NMR of BB12



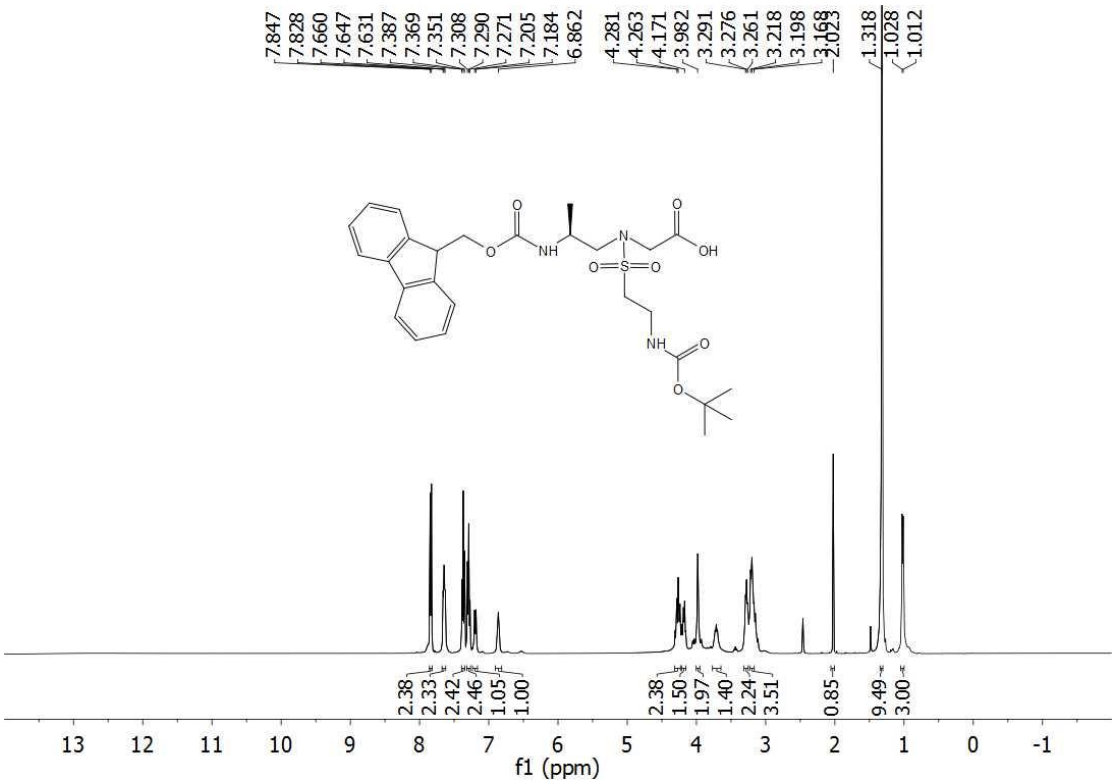
^{13}C NMR of BB12



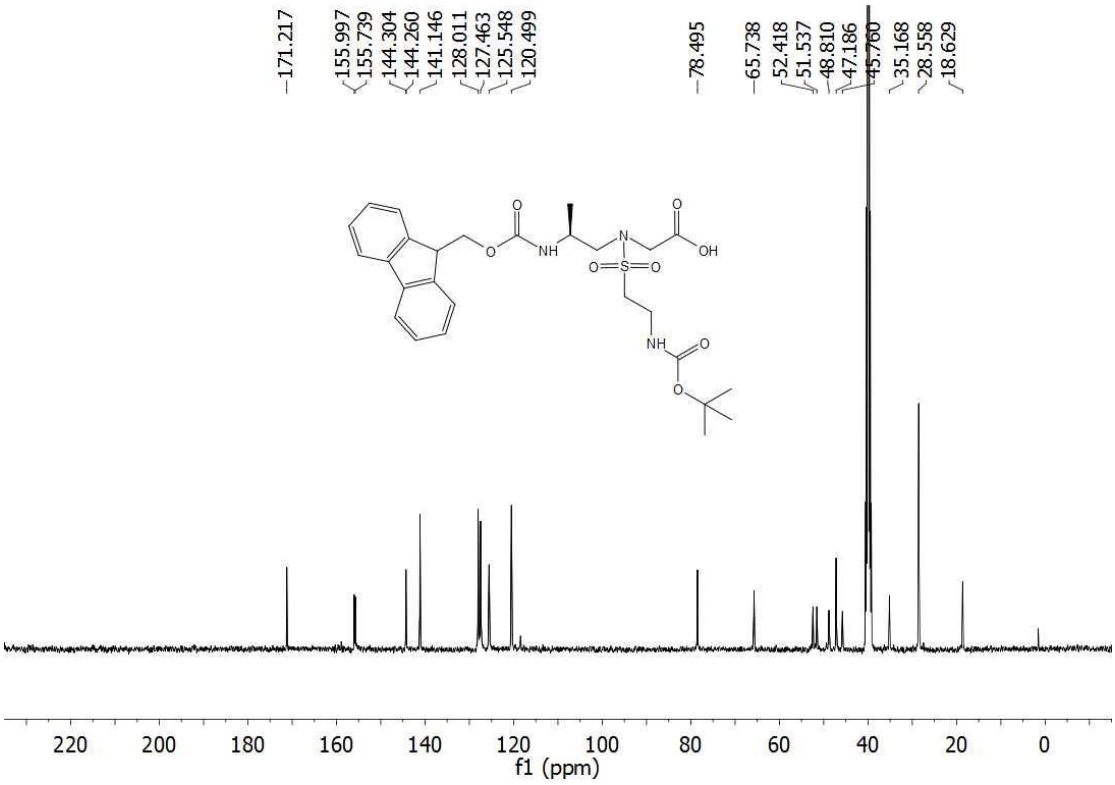
^{1}H NMR of BB13



^{13}C NMR of BB13



^{1}H NMR of BB14



^{13}C NMR of BB14