Supporting information

Electrocatalytic O-S Bonding Reaction Targeting Biological Macromolecules

Shuqiang Jiang[1], Longyu Xiao[1], Li Pan[2], Qiaoyu Huang[3], Fujin Huo[1], Meng Gao[1], Cuifen Lu[1], Pan Wu[*2], Yue Weng[*1]

[1]Ministry-of-Education Key Laboratory for the Synthesis and Application of Organic Functional Molecule, State Key Laboratory of Biocatalysis and Enzyme Engineering, School of Chemistry and Chemical Engineering, Hubei University, Wuhan, P. R. China

[2]State Key Laboratory of Biocatalysis and Enzyme Engineering, School of Life Sciences, Hubei University, Wuhan, 430062, P. R. China

[3]Ministry-of-Education Key Laboratory for the Green Preparation and Application of Functional Materials, Hubei Key Laboratory of Polymer Materials, School of Materials Science and Engineering, Hubei University, Wuhan,430062, P. R. China

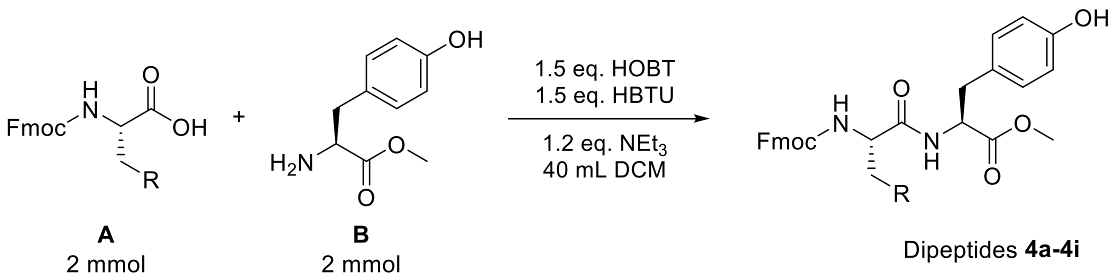
TABLE OF CONTENTS

1. General Information

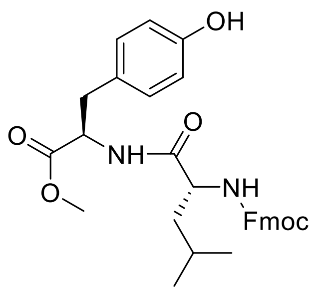
All glassware was oven dried at 110°C for hours and cooled down under vacuum. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. The instrument for electrolysis was dual display potentiostat (DJS-292B) (made in China). The anodic electrode was graphite rod (ϕ 6 mm) and cathodic electrode was platinum plate (15 mm×15 mm×0.3 mm). Thin layer chromatography (TLC) employed glass 0.25 mm silica gel plates. Flash chromatography columns were packed with 200-300 mesh silica gel. Gradient flash chromatography was conducted eluting with a continuous gradient from dichloromethane to the methanol. High resolution mass spectra (HRMS) for dipeptides were measured with a Waters Micromass GCT instrument and accurate masses were reported for the molecular ion + Sodium (M+Na). High resolution mass spectra (HRMS) for polypeptides were measured with an ABI 5800 instrument and accurate masses were reported for the molecular ion + Hydrogen (M+H) or molecular ion + Sodium (M+Na). The ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer. For ^{1}H NMR, chemical shifts (δ) were given in ppm relatives to internal standard (TMS at 0 ppm, DMSO-d_6 at 2.50 ppm, MeOH-d_4 at 3.31 ppm, Acetone-d_6 at 2.05 ppm). For ^{13}C-NMR, chemical shifts (δ) were reported in ppm using solvent as internal standard ($CDCl_3$ at 77.00 ppm, DMSO-d_6 at 39.50 ppm, MeOH-d_4 at 49.00 ppm, Acetone-d_6 at 29.84 ppm). HPLC analyses were performed on an Agilent 1260 Infinity LC system using a 100 mm Agilent Zorbax 300SB-C18 5 μm analytical column. All of the MALDI-TOF-MS and MALDI-TOF-MS/MS spectra were acquired using 5800 MALDI-MS (AB SCIEX, Concord, Canada) equipped with a 355 nm Nd: YAG laser in the reflector positive mode. Samples of 0.6 μ L mixed with 0.6 μ L freshly prepared CHCA matrix were directly loaded onto the stainless steel MALDI plate and allowed to dry in a gentle stream of warm air. Samples were ablated with a power of 3500 while the laser rastered over the target surface. A total of 2000 laser shots were employed in each sample spot. The MS and MS/MS data processing was further performed by DataExplorer 4.0 (AB SCIEX, Concord, Canada). UV-vis absorption spectra were performed on a Shimadzu UV-2700 spectrophotometer or Agilent Technologies Cary 8454. Fluorescence spectra were collected on a Hitachi F-4600 fluorescence spectrophotometer. The circular dichroism spectra were collected on Chhirascan™ CD spectroscopy (Applied Photophysics, Leatherhead, United Kingdom). CD spectra were collected from 180 nm to 280 nm and with a scanning speed of 200 nm/min. The bandwidth was 5 nm, and the response time was 2s. All spectra were taken at ambient temperature.

2. Synthesis of Starting Materials

Synthesis of starting materials dipeptides 4a-4i[1][2]

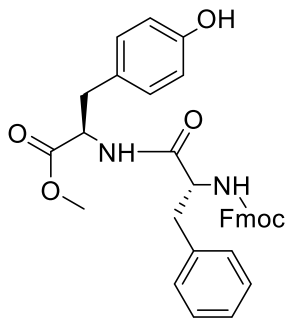


In a round bottomed flask, equipped with a stir bar, peptide **A** (2.0 mmol), HOBT (1-hydroxybenzotriazole) (3.0 mmol), HBTU (O-benzotriazole-*N, N, N', N'*-tetramethyl-uronium-hexafluorophosphate) (3.0 mmol), dichloromethane (40 mL) and triethylamine (2.4 mmol) were combined and added. The mixture was stirred for 30 min at room temperature, and then, peptide **B** (2.0 mmol) was added to the solution. The reaction was stirred overnight. After regular workup, the reaction mixture washed by saturated $NaHCO_3$ solution (40 mL x 3), 2M hydrochloric acid solution (40 mL x 3) and H_2O (40 mL x 3). The organic layers were combined, dried over Na_2SO_4, and concentrated. The resulting crude product was purified by flash chromatography (DCM/ MeOH) to afford corresponding dipeptides **4a-4i**.



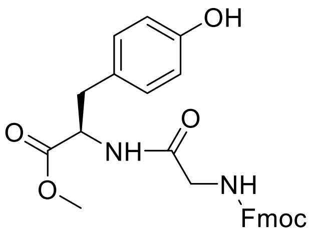
4a

Dipeptide **4a Fmoc-Leu-Tyr-OMe**, white solid. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.37 (s, 1H), 7.84 (d, J = 7.6 Hz, 2H), 7.72 – 7.68 (m, 2H), 7.63 – 7.58 (m, 1H), 7.39 (t, J = 7.6 Hz, 2H), 7.30 (td, J = 7.6, 1.2 Hz, 2H), 7.02 (d, J = 8.4 Hz, 2H), 6.84 – 6.80 (m, 1H), 6.75 (d, J = 8.2 Hz, 2H), 4.73 – 4.68 (m, 1H), 4.39 – 4.29 (m, 3H), 4.24 – 4.20 (m, 1H), 3.63 (s, 3H), 3.06 – 2.93 (m, 2H), 1.78 – 1.69 (m, 1H), 1.63 – 1.57 (m, 2H), 0.94 – 0.89 (m, 6H). ^{13}C NMR (101 MHz, Acetone-d_6) δ 173.18, 172.51, 157.06, 156.97, 145.02, 144.74, 141.95, 131.09, 128.43, 127.87, 126.08, 120.70, 115.96, 67.13, 54.64, 52.21, 47.89, 41.91, 37.34, 25.25, 23.41, 21.91.



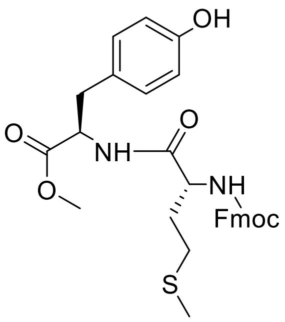
4b

Dipeptide **4b Fmoc-Phe-Tyr-OMe**, white solid. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.39 (s, 1H), 7.83 (d, J = 7.6 Hz, 2H), 7.64 – 7.62 (m, 3H), 7.39 (td, J = 7.6, 1.2 Hz, 2H), 7.31 – 7.15 (m, 7H), 7.04 – 7.00 (m, 2H), 6.81 (d, J = 8.8 Hz, 1H), 6.76 – 6.73 (m, 2H), 4.73 – 4.68 (m, 1H), 4.58 – 4.53 (m, 1H), 4.29 – 4.24 (m, 1H), 4.19 – 4.12 (m, 2H), 3.64 (s, 3H), 3.20 (dd, J = 14.0, 4.8 Hz, 1H), 3.07 – 2.90 (m, 3H). ^{13}C NMR (101 MHz, Acetone-d_6) δ 172.09, 171.71, 156.77, 156.41, 144.55, 144.47, 141.58, 138.21, 130.79, 129.85, 128.65, 128.09, 127.53, 126.85, 125.73, 120.35, 115.65, 66.87, 56.64, 54.42, 51.92, 47.44, 38.30, 37.07.



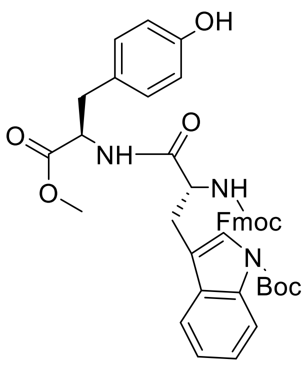
4c

Dipeptide **4c Fmoc-Gly-Tyr-OMe**, white solid. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.32 (s, 1H), 7.85 (d, J = 7.6 Hz, 2H), 7.72 (d, J = 7.6 Hz, 2H), 7.43 – 7.30 (m, 3H), 7.32 (t, J = 7.2 Hz, 2H), 7.03 (d, J = 8.4 Hz, 2H), 6.82 (t, J = 6.0 Hz, 1H), 6.75 (d, J = 8.0 Hz, 2H), 4.71 – 4.66 (m, 1H), 4.37 – 4.22 (m, 3H), 3.91 – 3.81 (m, 2H), 3.64 (s, 3H), 3.04 – 2.99 (m, 1H), 2.96 – 2.91 (m, 1H). ^{13}C NMR (101 MHz, Acetone-d_6) δ 172.53, 169.79, 157.45, 157.14, 144.97, 142.01, 131.14, 128.49, 128.05, 127.92, 126.13, 120.75, 116.03, 67.36, 54.60, 52.26, 47.88, 44.66, 37.47.



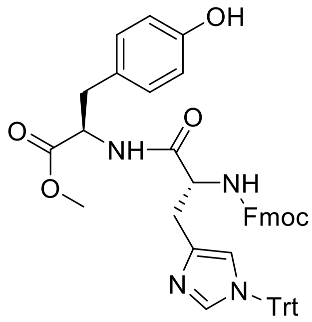
4d

Dipeptide **4d Fmoc-Met-Tyr-OMe**, white solid. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.32 (s, 1H), 7.85 (d, J = 7.6, 2H), 7.71 (t, J = 7.2 Hz, 2H), 7.52 (d, J = 7.6 Hz, 1H), 7.41 (t, J = 7.6 Hz, 2H), 7.34 – 7.30 (m, 2H), 7.05 – 6.02 (m, 2H), 6.80 (d, J = 8.4 Hz, 1H), 6.77 – 6.673 (m, 2H), 4.69 – 4.64 (m, 1H), 4.39 – 4.29 (m, 3H), 4.25 – 4.20 (m, 1H), 3.65 (s, 3H), 3.06 – 3.11 (m, 1H), 2.98 – 2.93 (m, 1H), 2.60 – 2.48 (m, 2H), 2.13 – 2.03 (m, 1H), 2.05 (s, 3H), 1.98 – 1.88 (m, 1H). ^{13}C NMR (101 MHz, Acetone-d_6) δ 172.55, 172.08, 157.13, 156.93, 145.07, 144.85, 142.03, 131.13, 128.50, 127.92, 126.14, 120.77, 116.02, 67.19, 54.82, 54.70, 52.28, 47.94, 37.26, 32.89, 30.61, 15.13.



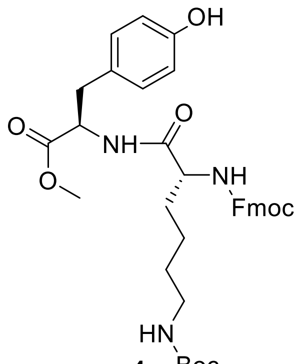
4e

Dipeptide **4e Fmoc-Trp(Boc)-Tyr-OMe**, white solid.^{1}H NMR (400 MHz, DMSO- d_6) δ 9.27 (s, 1H), 8.47 (d, J = 7.4 Hz, 1H), 8.02 (s, 1H), 7.85 (d, J = 8.0 Hz, 2H), 7.76 (d, J = 7.6 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.59 (d, J = 13.5 Hz, 3H), 7.41 – 7.30 (m, 4H), 7.27 – 7.17 (m, 3H), 7.01 (d, J = 8.5 Hz, 2H), 4.50 – 4.39 (m, 2H), 4.22 – 4.08 (m, 3H), 3.57 (s, 3H), 3.08 – 3.01 (m, 1H), 2.97 – 2.84 (m, 3H), 1.55 (s, 9H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 172.47, 172.16, 157.10, 156.79, 145.00, 144.95, 142.00, 137.55, 131.19, 128.46, 128.13, 127.94, 126.15, 124.55, 122.11, 120.73, 119.57, 119.34, 116.00, 112.15, 111.31, 67.22, 56.48, 54.75, 52.24, 47.92, 37.47, 28.77.28.09.



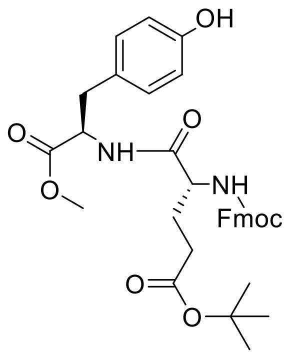
4f

Dipeptide **4f Fmoc-His(Trt)-Tyr-OMe**, white solid.^{1}H NMR (400 MHz, Chloroform-d) δ 7.73 (d, J = 7.6 Hz, 2H), 7.59 (dd, J = 7.6, 3.9 Hz, 2H), 7.44 (dd, J = 6.4, 4.7 Hz, 1H), 7.40 (d, J = 1.5 Hz, 1H), 7.37 – 7.33 (m, 2H), 7.27 – 7.24 (m, 9H), 7.06 – 7.03 (m, 6H), 6.89 (d, J = 8.5 Hz, 2H), 6.66 (s, 1H), 6.57 (d, J = 8.5 Hz, 2H), 6.32 (d, J = 7.7 Hz, 1H), 4.85 – 4.79 (m, 1H), 4.52 (s, 1H), 4.29 – 4.24 (m, 2H), 4.14 (d, J = 7.5 Hz, 1H), 3.55 (s, 3H), 3.11 – 2.90 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 171.63, 171.03, 156.40, 156.10, 143.94, 142.07, 141.22, 138.14, 136.55, 130.49, 129.73, 128.17, 128.12, 127.68, 127.15, 127.11, 126.61, 125.38, 125.33, 119.91, 119.73, 115.82, 67.30, 55.62, 53.48, 52.21, 47.10, 37.36, 31.38.



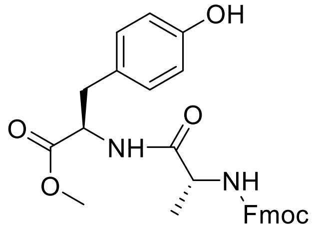
4g

Dipeptide **4g Fmoc-Lys(Boc)-Tyr-OMe**, white solid.[1]H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H), 8.23 (d, *J* = 7.4 Hz, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.75 – 7.71 (m, 2H), 7.45 – 7.40 (m, 3H), 7.33 (t, *J* = 7.4 Hz, 2H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.77 (t, *J* = 5.7 Hz, 1H), 6.65 (d, *J* = 8.4 Hz, 2H), 4.37 (q, *J* = 7.3 Hz, 1H), 4.31 – 4.14 (m, 4H), 4.03 – 3.95 (m, 1H), 3.56 (s, 3H), 2.93 – 2.82 (m, 4H), 1.63 – 1.42 (m, 3H), 1.37 (s, 9H), 1.28 – 1.16 (m, 3H).[13]C NMR (101 MHz, DMSO- d_6) δ 172.63, 172.19, 156.31, 156.05, 149.55, 149.48, 144.39, 144.22, 141.18, 134.30, 130.97, 128.09, 127.51, 125.77, 120.54, 120.06, 120.01, 77.81, 66.10, 54.87, 53.88, 52.26, 47.16, 36.25, 32.09, 29.69, 28.73, 23.20 .



4h

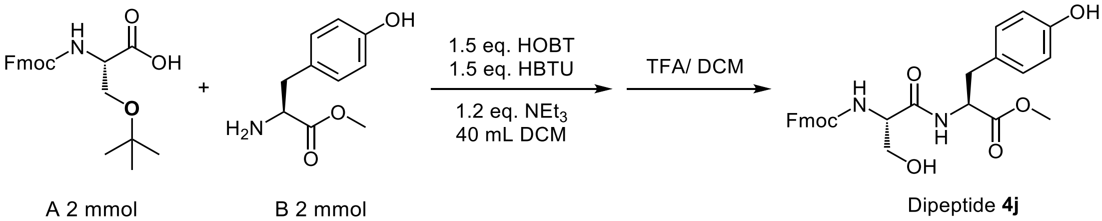
Dipeptide **4h Fmoc-Glu(tBu)-Tyr-OMe**, white solid.[1]H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H), 8.26 (d, *J* = 7.4 Hz, 1H), 7.90 (d, *J* = 7.5 Hz, 2H), 7.74 (t, *J* = 7.4 Hz, 2H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.42 (t, *J* = 6.9 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.00 (d, *J* = 8.4 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 2H), 4.39 (dt, *J* = 13.6, 6.9 Hz, 1H), 4.26 (dd, *J* = 17.2, 8.0 Hz, 3H), 4.06 (td, *J* = 8.5, 5.3 Hz, 1H), 3.58 (s, 3H), 2.92 – 2.83 (m, 2H), 2.23 (t, *J* = 8.0 Hz, 2H), 1.92 – 1.80 (m, 1H), 1.73 (dq, *J* = 16.5, 8.1 Hz, 1H), 1.40 (s, 9H).[13]C NMR (101 MHz, DMSO-d_6) δ 172.12, 172.00, 156.27, 149.55, 149.49, 144.38, 144.19, 141.19, 134.30, 130.97, 128.10, 127.51, 125.77, 120.56, 120.07, 120.03, 80.15, 66.14, 54.05, 53.93, 52.30, 47.14, 36.14, 31.69, 28.22, 27.80.



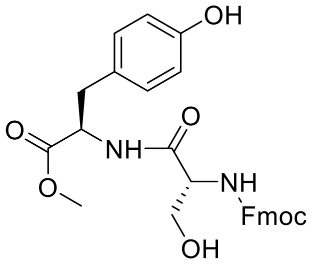
4i

Dipeptide **4i Fmoc-Ala-Tyr-OMe**,white solid.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (d, *J* = 8.1 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 7.87 (dd, *J* = 17.3, 7.5 Hz, 4H), 7.74 (d, *J* = 4.9 Hz, 1H), 7.42 (t, *J* = 7.4 Hz, 2H), 7.34 (q, *J* = 7.2 Hz, 2H), 6.98 (dd, *J* = 8.3, 1.7 Hz, 2H), 6.68 – 6.61 (m, 2H), 4.42 (tt, *J* = 8.9, 5.0 Hz, 1H), 4.27 – 4.17 (m, *J* = 12.3, 6.2 Hz, 3H), 4.04 (dt, *J* = 29.4, 7.4 Hz, 1H), 3.62 (d, *J* = 2.0 Hz, 3H), 2.97 – 2.73 (m, 2H), 1.08 – 1.02 (m, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 173.03, 172.31, 156.02, 149.51, 149.45, 144.34, 144.28, 141.17, 134.37, 131.10, 128.10, 127.53, 125.81, 120.56, 120.02, 119.97, 66.13, 53.61, 52.42, 50.23, 47.07, 36.52, 18.78.

Synthesis of starting materials dipeptides 4j[1][2]



In a round bottomed flask, equipped with a stir bar, peptide **A** (2.0 mmol), HOBT (1-hydroxybenzotriazole) (3.0 mmol), HBTU (O-benzotriazole-*N, N, N', N'*-tetramethyl-uronium-hexafluorophosphate) (3.0 mmol), dichloromethane (40 mL) and triethylamine (2.4 mmol) were combined and added. The mixture was stirred for 30 min at room temperature, and then, peptide **B** (2.0 mmol) was added to the solution. The reaction was stirred overnight. After regular workup, the reaction mixture washed by saturated $NaHCO_3$ solution (40 mL x 3), 2M hydrochloric acid solution (40 mL x 3) and H_2O (40 mL x 3). The organic layers were combined, dried over Na_2SO_4, and concentrated. Without further purification, the 95% TFA / DCM solution (8 mL) was added dropwise. The mixture was stirred for 2 h at room temperature. The resulting crude product was purified by flash chromatography (DCM / MeOH) to afford corresponding dipeptides **4j**.



4j

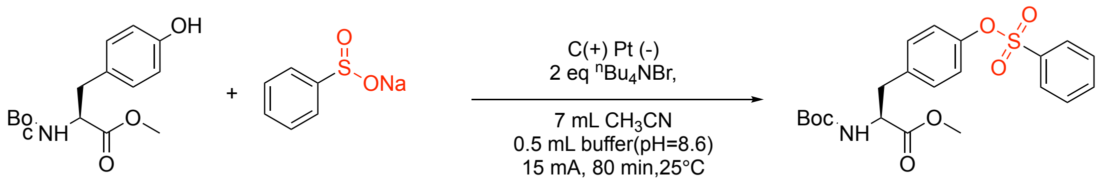
Dipeptide **4j Fmoc-Ser-Tyr-OMe**, white solid. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.32 (s, 1H), 7.88 (d, J = 7.6 Hz, 2H), 7.76 – 7.73 (m, 2H), 7.60 (d, J = 7.6 Hz, 1H), 7.43 (t, J = 7.6 Hz, 2H), 7.34 (t, J = 7.6 Hz, 2H), 7.07 (d, J = 8.0 Hz, 2H), 6.77 (d, J = 8.0 Hz, 2H), 6.69 (d, J = 8.0 Hz, 1H), 4.74 – 4.69 (m, 1H), 4.36 -4.31 (m, 3H), 4.28 – 4.22 (m, 2H), 3.86 – 3.74 (m, 2H), 3.65 (s, 3H), 3.07 – 3.02 (m, 1H), 3.02 – 2.97 (m, 1H). ^{13}C NMR (101 MHz, Acetone-d_6) δ 172.54, 171.02, 157.15, 157.05, 145.02, 144.91, 142.01, 131.20, 128.50, 127.95, 126.15, 120.76, 116.03, 67.42, 63.25, 57.53, 54.77, 52.34, 47.89, 37.35.

3. General Procedure for BioconJugation of Tyrosine and Sodium benzenesulfinate

3.1 Reaction Optimization

In an oven-dried undivided three-necked bottle (25 mL) equipped with a stir bar, protected tyrosine (0.20 mmol), Sodium benzenesulfinate (0.3 mmol), ^{n}Bu$_4$NBr (0.40 mmol) and MeCN / buffer(pH=8.6) (7.0 mL / 0.5 mL) were combined and added. The bottle was equipped graphite rod (ϕ 6 mm, about 15 mm immersion depth in solution) as the anode and platinum plate (15 mm×15 mm×0.3 mm) as the cathode . The reaction mixture was stirred and electrolyzed at constant current under room temperature. When the reaction finished,The pure product was obtained by flash column chromatography on silica gel. A summary of optimization results is presented in **Table S1** below.

Table S1. Effects of reaction parameters



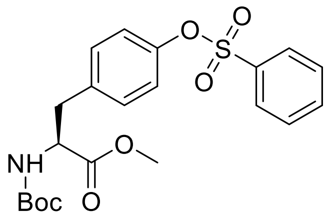
Entry	Variation from the standard conditions	Isolated yields
1	none	87%
2	8 mA instead of 15 mA, 160 min	64%
3	20 mA instead of 15 mA, 60 min	52%
4	H_2O instead of buffer	42%
5	add to 1 eq HCl	trace
6	1.5 ml buffer	64%
7	without buffer	27%
8	CH3OH instead of CH_3CN	trace
9	CH_2Cl_2 instead of CH_3CN	75%
10	C(+) C (-) instead of C(+) Pt (-)	56%
11	C(+) Ni (-) instead of C(+) Pt (-)	52%
12	Pt(+) Pt (-) instead of C(+) Pt (-)	75%
13	$^{n}Bu_4NBF_4$ instead of $^{n}Bu_4NBr$	30%
14	$^{n}Bu_4NI$ instead of $^{n}Bu_4NBr$	24%
15	$^{n}Bu_4NClO_4$ instead of $^{n}Bu_4NBr$	trace
16	KBr instead of $^{n}Bu_4NBr$	74%`
17	under N_2	83%
18	no electric current	n.r
19	Serine	n.d
20	Threonine	n.d
21	Hxdroxyproline	n.d

[a] Reaction conditions: graphite rod anode, platinum plate cathode, constant current = 15 mA, **1a** (1.0 equiv., 0.20 mmol), **2a** (1.5 equiv, 0.3 mmol), $^{n}Bu_4NBr$ (2 equiv, 0.40 mmol), 7.0 mL MeCN, 0.5 mL buffer(pH = 8.6) , 25°C. 80min. Yields of isolated products are shown. n r =no reaction. n d = no detected.

3.2 Gram-Scale Experiments

General procedure for Gram-Scale Experiments: In an oven-dried undivided three-necked bottle (250 mL) equipped with a stir bar, tyrosine (5.0 mmol), Sodium benzenesulfinate (7.5 mmol), and $^{n}Bu_4NBr$ (10.0 mmol), buffer (pH = 8.6 , 12 mL)were combined and added. Then, CH_3CN (160 mL) were injected into the tubes via syringes. The bottle was equipped with carbon rod (ϕ 6 mm) as the anode and platinum plate (15 mm×15 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at constant current of 15 mA under 25℃ overnight. The solvent was removed under vacuum. The crude product was purified by flash column chromatography on silica gel to afford pure product.

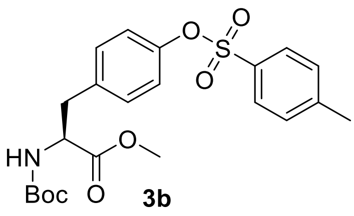
3.3 Sodium arenesulfinates scope and characterization



3a

methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-((phenylsulfonyl)oxy)phenyl)propanoate(3a);

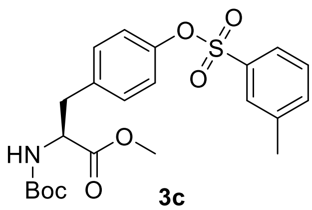
75.7 mg (yield: 87%, 0.2 mmol scale), white solid. ^{1}H NMR (400 MHz, DMSO- d_6) δ 7.86 – 7.81 (m, 2H), 7.81 – 7.77 (m, 1H), 7.65 (t, J = 7.9 Hz, 2H), 7.31 (d, J = 8.2 Hz, 1H), 7.23 (d, J = 8.7 Hz, 2H), 6.93 (d, J = 8.7 Hz, 2H), 4.21 – 4.11 (m, 1H), 3.57 (s, 3H), 2.97 (dd, J = 13.8, 5.3 Hz, 1H), 2.83 (dd, J = 13.8, 10.2 Hz, 1H), 1.30 (s, 9H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 172.84, 155.81, 148.10, 137.51, 135.39, 134.89, 131.08, 130.20, 128.61, 122.17, 78.77, 55.30, 52.24, 36.20, 28.55. HRMS (ESI) cald. for $(M+Na)^+$ $C_{21}H_{25}NO_7S$: 458.1243, found, 458.1248.



3b

methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-(tosyloxy)phenyl)propanoate(3b);

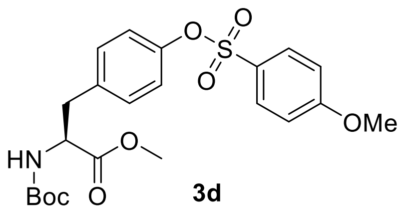
62.1 mg (yield: 69%, 0.2 mmol scale), white solid.^{1}H NMR (400 MHz, DMSO- d_6) δ 7.71 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 7.9 Hz, 2H), 7.30 (d, J = 8.2 Hz, 1H), 7.22 (d, J = 8.7 Hz, 2H), 6.92 (d, J = 8.7 Hz, 2H), 4.15 (dd, J = 18.4, 5.3 Hz, 1H), 3.57 (s, 3H), 2.96 (dd, J = 13.8, 5.3 Hz, 1H), 2.82 (dd, J = 13.9, 10.2 Hz, 1H), 2.41 (s, 3H), 1.31 (s, 9H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 172.85, 155.81, 148.15, 146.13, 137.40, 132.01, 131.05, 130.62, 128.64, 122.17, 78.77, 55.30, 52.24, 36.19, 28.55, 21.64. HRMS (ESI) cald. for $(M+Na)^+$ $C_{22}H_{27}NO_7S$: 472.1400, found, 472.1423.



3c

methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-((m-tolylsulfonyl)oxy)phenyl)propanoate(3c);

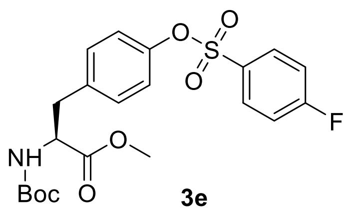
54.8 mg (yield: 61%, 0.2 mmol scale), white solid.^{1}H NMR (400 MHz, DMSO- d_6) δ 7.70 (s, 1H), 7.63 (d, J = 7.0 Hz, 2H), 7.54 (t, J = 7.7 Hz, 1H), 7.33 (d, J = 8.3 Hz, 1H), 7.25 (d, J = 8.7 Hz, 2H), 6.95 (d, J = 8.5 Hz, 2H), 4.22 – 4.14 (m, 1H), 3.60 (s, 3H), 2.99 (dd, J = 13.8, 5.1 Hz, 1H), 2.85 (dd, J = 13.8, 10.2 Hz, 1H), 2.41 (s, 3H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 172.85, 155.82, 148.12, 140.23, 137.46, 136.00, 134.87, 131.06, 129.96, 128.60, 125.78, 122.19, 78.77, 55.33, 52.23, 36.19, 28.55, 21.11. HRMS (ESI) cald. for $(M+Na)^+$ $C_{22}H_{27}NO_7S$: 472.1400, found, 472.1425.



3d

methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-(((4-methoxyphenyl)sulfonyl)oxy)phenyl)propanoate(3d);

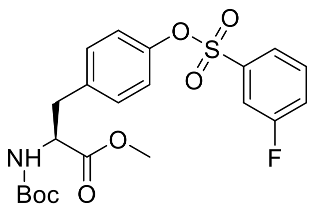
54.0 mg (yield: 58%, 0.2 mmol scale), white solid.^{1}H NMR (400 MHz, DMSO- d_6) δ 7.77 (d, J = 9.0 Hz, 2H), 7.33 (d, J = 8.2 Hz, 1H), 7.25 (d, J = 8.7 Hz, 2H), 7.16 (d, J = 9.0 Hz, 2H), 6.94 (d, J = 8.7 Hz, 2H), 4.23 – 4.14 (m, 1H), 3.87 (s, 3H), 3.60 (s, 3H), 2.99 (dd, J = 13.8, 5.3 Hz, 1H), 2.85 (dd, J = 13.9, 10.2 Hz, 1H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 172.86, 164.41, 155.83, 148.21, 137.33, 131.02, 126.11, 122.23, 115.32, 78.78, 56.34, 55.33, 52.23, 36.20, 28.54. HRMS (ESI) cald. for $(M+Na)^+$ $C_{22}H_{27}NO_8S$:488.1349, found, 488.1343.



3e

methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-(((4-fluorophenyl)sulfonyl)oxy)phenyl)propanoate(3e);

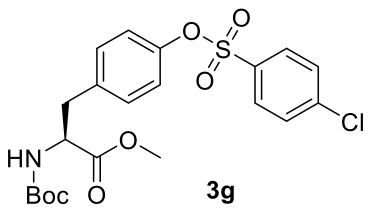
63.5 mg (yield: 70%, 0.2 mmol scale), white solid.^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 (dd, J = 8.9, 5.0 Hz, 2H), 7.48 (t, J = 8.8 Hz, 2H), 7.31 (d, J = 8.3 Hz, 1H), 7.24 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 8.7 Hz, 2H), 4.16 (dd, J = 13.4, 10.2 Hz, 1H), 3.58 (s, 3H), 2.97 (dd, J = 13.9, 5.1 Hz, 1H), 2.83 (dd, J = 13.8, 10.2 Hz, 1H), 1.30 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.83, 167.27, 164.73, 155.81, 148.02, 137.63, 132.06, 131.96, 131.14, 131.11, 131.08, 122.21, 117.69, 117.46, 78.77, 55.29, 52.24, 36.18, 28.54. 19F NMR (377 MHz, DMSO-d_6) δ -102.57. HRMS (ESI) cald. for $(M+Na)^+$ $C_{21}H_{24}FNO_7S$: 476.1149 ,found, 476.1138.



3f

methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-(((3-fluorophenyl)sulfonyl)oxy)phenyl)propanoate(3f);

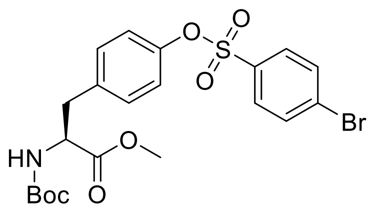
54.4 mg (yield: 60%, 0.2 mmol scale), white solid.^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75 – 7.69 (m, 4H), 7.28 (t, J = 9.3 Hz, 3H), 7.00 (d, J = 8.6 Hz, 2H), 4.24 – 4.14 (m, 1H), 3.59 (s, 3H), 3.00 (dd, J = 13.8, 5.2 Hz, 1H), 2.85 (dd, J = 13.8, 10.1 Hz, 1H), 1.34 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d6) δ 172.80, 163.44, 160.96, 155.79, 148.00, 137.71, 136.74, 136.67, 132.70, 132.62, 131.18, 125.07, 125.04, 122.85, 122.64, 122.12, 115.84, 115.59, 78.76, 55.25, 52.21, 36.22, 28.53. 19F NMR (377 MHz, DMSO-d_6) δ -57.04. HRMS (ESI) cald. for $(M+Na)^+$ $C_{21}H_{24}FNO_7S$: 476.1149, found, 476.1141.



3g

methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-(((4-chlorophenyl)sulfonyl)oxy)phenyl)propanoate(3g);

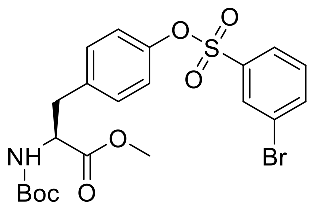
56.4 mg (yield: 60%, 0.2 mmol scale), white solid.^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86 (d, J = 8.7 Hz, 2H), 7.73 (d, J = 8.7 Hz, 2H), 7.28 (dd, J = 13.5, 8.4 Hz, 3H), 6.98 (d, J = 8.6 Hz, 2H), 4.24 – 4.12 (m, 1H), 3.59 (s, 3H), 2.99 (dd, J = 13.8, 5.1 Hz, 1H), 2.85 (dd, J = 13.9, 10.1 Hz, 1H), 1.32 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.79, 155.80, 148.01, 140.48, 137.69, 133.68, 131.18, 130.57, 130.41, 122.16, 78.77, 55.27, 52.22, 36.21, 28.55. HRMS (ESI) cald. for $(M+Na)^+$ $C_{21}H_{24}ClNO_7S$: 492.0854, found, 492.0857



3h

methyl (S)-3-(4-(((4-bromophenyl)sulfonyl)oxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate(3h);

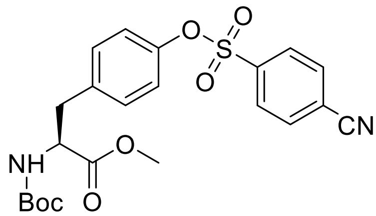
77.2 mg (yield: 75%, 0.2 mmol scale), white solid.^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86 (d, J = 8.7 Hz, 2H), 7.76 (d, J = 8.8 Hz, 2H), 7.32 (d, J = 8.2 Hz, 1H), 7.25 (d, J = 8.7 Hz, 2H), 6.97 (d, J = 8.7 Hz, 2H), 4.20 – 4.09 (m, 1H), 3.58 (s, 3H), 2.98 (dd, J = 13.8, 5.2 Hz, 1H), 2.83 (dd, J = 13.8, 10.2 Hz, 1H), 1.30 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.83, 155.81, 147.98, 137.70, 134.05, 133.36, 131.20, 130.56, 129.65, 122.18, 78.76, 55.28, 52.25, 36.17, 28.54. HRMS (ESI) cald. for $(M+Na)^+$ $C_{21}H_{24}BrNO_7S$: 536.0349, found, 536.0346.



3i

methyl (S)-3-(4-(((3-bromophenyl)sulfonyl)oxy)phenyl)-2-((tert-butoxycarbonyl) amino)propanoate(3i);

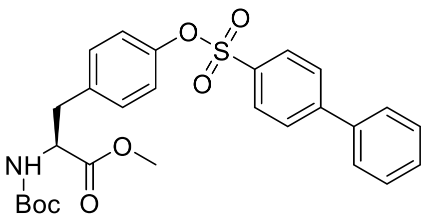
80.2 mg (yield: 78%, 0.2 mmol scale), white solid.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (ddd, J = 8.0, 2.0, 1.0 Hz, 1H), 8.00 (t, J = 1.9 Hz, 1H), 7.87 (d, J = 9.7 Hz, 1H), 7.63 (t, J = 8.0 Hz, 1H), 7.32 – 7.25 (m, 3H), 7.01 (d, J = 8.7 Hz, 2H), 4.25 – 4.16 (m, 1H), 3.60 (s, 3H), 3.00 (dd, J = 13.8, 5.3 Hz, 1H), 2.86 (dd, J = 13.8, 10.1 Hz, 1H), 1.32 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.80, 155.79, 147.95, 138.33, 137.75, 136.80, 132.38, 131.20, 130.70, 127.73, 122.98, 122.14, 78.77, 55.26, 52.22, 36.23, 28.55. HRMS (ESI) cald. for (M+Na)$^+$ $C_{21}H_{24}BrNO_7S$: 536.0349, found, 536.0342.



3j

methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-(((4-cyanophenyl)sulfonyl)oxy)phenyl)propanoate(3*J*);

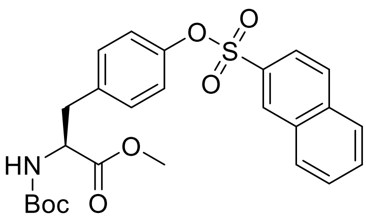
45.1 mg (yield: 49%, 0.2 mmol scale), white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (d, J = 8.6 Hz, 2H), 8.04 (d, J = 8.7 Hz, 2H), 7.27 (dd, J = 11.6, 8.4 Hz, 3H), 6.99 (d, J = 8.6 Hz, 2H), 4.18 (dd, J = 18.4, 5.1 Hz, 1H), 3.59 (s, 3H), 2.99 (dd, J = 13.9, 5.2 Hz, 1H), 2.85 (dd, J = 13.9, 10.2 Hz, 1H), 1.31 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.78, 155.80, 147.88, 138.85, 137.88, 134.30, 131.27, 129.42, 122.13, 117.74, 117.73, 78.78, 55.24, 52.24, 36.19, 28.54. HRMS (ESI) cald. for (M+Na)$^+$ $C_{22}H_{24}N_2O_7S$:483.1196, found, 483.1188.



3k

methyl (S)-3-(4-(([1,1'-biphenyl]-4-ylsulfonyl)oxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate(3k);

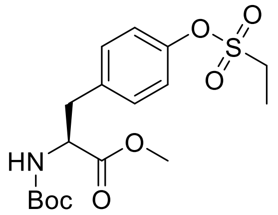
46.1 mg (yield: 45%, 0.2 mmol scale), white solid.^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (q, J = 8.7 Hz, 4H), 7.77 (d, J = 8.0 Hz, 2H), 7.54 (t, J = 7.3 Hz, 2H), 7.48 (t, J = 7.2 Hz, 1H), 7.27 (dd, J = 13.9, 8.4 Hz, 3H), 7.00 (d, J = 8.6 Hz, 2H), 4.22 – 4.12 (m, 1H), 3.58 (s, 3H), 2.99 (dd, J = 13.9, 5.2 Hz, 1H), 2.84 (dd, J = 13.9, 10.2 Hz, 1H), 1.30 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.82, 155.81, 148.14, 146.61, 138.31, 137.52, 133.61, 131.13, 129.69, 129.50, 129.30, 128.24, 127.69, 122.18, 78.77, 55.29, 52.22, 36.20, 28.54. HRMS (ESI) cald. for $(M+Na)^+$ $C_{27}H_{29}NO_7S$: 534.1556, found, 534.1558.



3l

methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-((naphthalen-2-ylsulfonyl)oxy)phenyl)propanoate(3l);

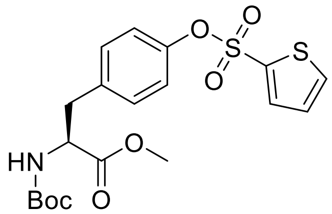
72.0 mg (yield: 74%, 0.2 mmol scale), white solid.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1H), 8.22 – 8.18 (m, 2H), 8.09 (d, J = 8.2 Hz, 1H), 7.88 (dd, J = 8.7, 2.1 Hz, 1H), 7.76 (t, J = 7.6 Hz, 1H), 7.69 (t, J = 7.5 Hz, 1H), 7.27 (d, J = 8.2 Hz, 1H), 7.22 (d, J = 8.7 Hz, 2H), 6.98 (d, J = 8.6 Hz, 2H), 4.24 – 4.13 (m, 1H), 3.55 (s, 3H), 2.97 (dd, J = 13.9, 5.2 Hz, 1H), 2.83 (dd, J = 13.9, 10.1 Hz, 1H), 1.28 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.80, 155.80, 148.20, 137.47, 135.52, 132.01, 131.93, 131.09, 130.64, 130.41, 130.37, 130.05, 128.50, 128.45, 122.96, 122.17, 78.74, 55.25, 52.16, 36.22, 28.51. HRMS (ESI) cald. for $(M+Na)^+$ $C_{25}H_{27}NO_7S$: 508.1400, found, 508.1408.



3m

methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-((ethylsulfonyl)oxy)phenyl)propanoate(3m);

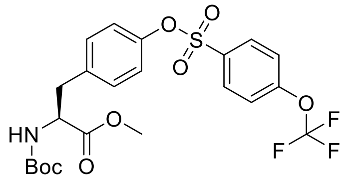
47.3 mg (yield: 61%, 0.2 mmol scale), white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39 – 7.33 (m, 3H), 7.26 (d, J = 8.7 Hz, 2H), 4.27 – 4.18 (m, 1H), 3.64 (s, 3H), 3.48 (q, J = 7.3 Hz, 2H), 3.05 (dd, J = 13.8, 5.0 Hz, 1H), 2.89 (dd, J = 13.8, 10.3 Hz, 1H), 1.38 (t, J = 7.3 Hz, 3H), 1.34 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.89, 155.87, 148.07, 137.28, 131.22, 122.26, 78.80, 55.41, 52.28, 44.90, 36.20, 28.55, 8.51. HRMS (ESI) cald. for $(M+Na)^+$ $C_{17}H_{25}NO_7S$: 410.1243, found, 410.1241.



3n

methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-((thiophen-2-ylsulfonyl)oxy)phenyl)propanoate(3n);

61.8 mg (yield: 70%, 0.2 mmol scale), white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (dd, J = 5.0, 1.4 Hz, 1H), 7.75 (dd, J = 3.9, 1.5 Hz, 1H), 7.33 (d, J = 8.2 Hz, 1H), 7.28 – 7.24 (m, 3H), 6.97 (d, J = 8.6 Hz, 2H), 4.17 (dd, J = 16.8, 6.7 Hz, 1H), 3.58 (s, 3H), 2.98 (dd, J = 13.8, 5.2 Hz, 1H), 2.84 (dd, J = 13.8, 10.2 Hz, 1H), 1.31 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.86, 155.81, 148.17, 137.76, 137.51, 136.81, 133.54, 131.14, 128.87, 122.06, 78.79, 55.32, 52.26, 36.20, 28.56. HRMS (ESI) cald. for $(M+Na)^+$ $C_{19}H_{23}NO_7S_2$: 464.0808, found, 464.0802.

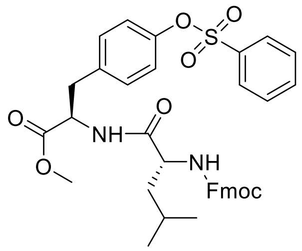


3o

methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-(((4-(trifluoromethoxy)phenyl)sulfonyl)oxy)phenyl)propanoate(3o);

70.6 mg (yield: 68%, 0.2 mmol scale), white solid.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (d, J = 9.0 Hz, 2H), 7.65 (d, J = 10.0 Hz, 2H), 7.29 (t, J = 9.4 Hz, 3H), 7.00 (d, J = 8.7 Hz, 2H), 4.27 – 4.14 (m, 1H), 3.60 (s, 3H), 3.01 (dd, J = 13.8, 5.2 Hz, 1H), 2.87 (dd, J = 13.9, 10.1 Hz, 1H), 1.32 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.78, 155.80, 152.96, 152.94, 147.99, 137.73, 133.61, 131.49, 131.17, 122.12, 122.04, 121.52, 118.95, 78.74, 55.27, 52.17, 36.20, 28.48. ^{19}F NMR (377 MHz, DMSO) δ -109.15 (t, J = 17.8 Hz). HRMS (ESI) cald. for $(M+Na)^+$ $C_{22}H_{24}F_3NO_8S$: 542.1066, found, 542.1054.

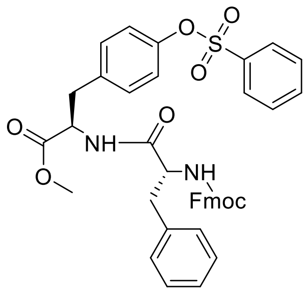
3.4 Dipeptide scope and characterization



5a

methyl (R)-2-((R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanamido)-3-(4-((phenylsulfonyl)oxy)phenyl)propanoate(5a);

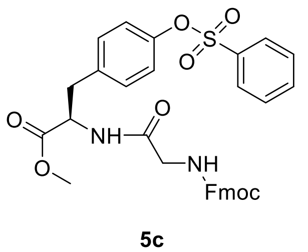
83.2 mg (yield: 62%, 0.2 mmol scale), white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (d, J = 7.5 Hz, 1H), 7.91 (d, J = 8.5 Hz, 2H), 7.83 – 7.80 (m, 2H), 7.78 (d, J = 7.4 Hz, 1H), 7.73 (dd, J = 7.5, 4.0 Hz, 2H), 7.66 – 7.61 (m, 2H), 7.47 (d, J = 8.5 Hz, 1H), 7.43 (t, J = 7.5 Hz, 2H), 7.33 (t, J = 8.6 Hz, 2H), 7.23 (d, J = 8.8 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 4.49 – 4.42 (m, 1H), 4.34 – 4.28 (m, 1H), 4.27 – 4.19 (m, 2H), 4.10 – 4.04 (m, 1H), 3.55 (s, 3H), 3.04 – 2.91 (m, 2H), 1.59 (dd, J = 17.1, 10.4 Hz, 1H), 1.48 – 1.40 (m, 1H), 1.39 – 1.32 (m, 1H), 0.89 (d, J = 6.7 Hz, 3H), 0.85 (d, J = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.99, 172.15, 156.26, 148.13, 144.42, 144.17, 141.21, 141.19, 137.08, 135.40, 134.76, 131.12, 130.20, 128.62, 128.12, 127.52, 125.76, 122.18, 120.60, 120.58, 65.99, 53.67, 53.23, 52.27, 47.16, 41.10, 36.17, 24.55, 23.45, 21.94. HRMS (ESI) cald. for $(M+Na)^+$ $C_{37}H_{38}N_2O_8S$: 693.2241, found, 693.2244.



5b

methyl (R)-2-((R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)-3-(4-((phenylsulfonyl)oxy)phenyl)propanoate(5b);

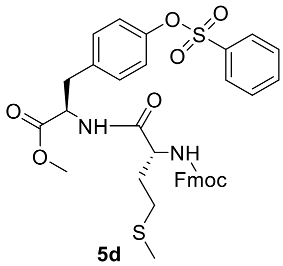
74.7 mg (yield: 53%, 0.2 mmol scale), white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (d, J = 7.6 Hz, 1H), 7.88 (d, J = 7.5 Hz, 2H), 7.81 (d, J = 10.6 Hz, 2H), 7.74 (d, J = 8.8 Hz, 1H), 7.64 (d, J = 10.2 Hz, 2H), 7.62 – 7.57 (m, 3H), 7.41 (t, J = 7.5 Hz, 2H), 7.32 (d, J = 8.7 Hz, 3H), 7.31 – 7.28 (m, 2H), 7.25 (t, J = 8.5 Hz, 4H), 7.19 (t, J = 7.2 Hz, 1H), 6.95 – 6.90 (m, 2H), 4.59 – 4.49 (m, 1H), 4.36 – 4.28 (m, 1H), 4.19 (d, J = 12.4 Hz, 1H), 4.14 (t, J = 6.2 Hz, 2H), 3.57 (s, 3H), 3.10 – 3.02 (m, 1H), 3.01 – 2.94 (m, 2H), 2.77 (dd, J = 13.8, 10.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.27, 172.08, 156.19, 148.19, 144.25, 144.15, 141.13, 138.51, 137.01, 135.36, 134.77, 131.14, 130.17, 129.71, 128.62, 128.51, 128.09, 127.52, 126.74, 125.72, 122.26, 120.55, 64.80,56.34, 53.83,52.35,47.02,37.37,34.20. HRMS (ESI) cald. for $(M+Na)^+$ $C_{40}H_{36}N_2O_8S$:727.2084, found, 727.2081.



5c

methyl (R)-2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)acetamido)-3-(4-((phenylsulfonyl)oxy)phenyl)propanoate(5c);

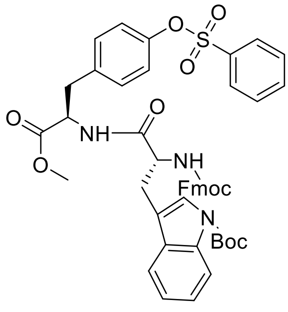
78.6 mg (yield: 64%, 0.2 mmol scale), white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (d, J = 7.8 Hz, 1H), 7.90 (s, 1H), 7.88 (s, 1H), 7.85 – 7.81 (m, 2H), 7.80 – 7.77 (m, 1H), 7.72 (d, J = 7.5 Hz, 2H), 7.65 (t, J = 8.0 Hz, 2H), 7.49 (t, J = 6.2 Hz, 1H), 7.43 (d, J = 7.5 Hz, 2H), 7.33 (t, J = 6.8 Hz, 2H), 7.21 (d, J = 8.6 Hz, 2H), 6.92 (d, J = 8.6 Hz, 2H), 4.51 – 4.44 (m, 1H), 4.29 (d, J = 8.0 Hz, 2H), 4.23 (d, J = 6.2 Hz, 1H), 3.62 (t, J = 5.1 Hz, 2H), 3.57 (s, 3H), 3.01 (dd, J = 13.8, 5.9 Hz, 1H), 2.91 (dd, J = 13.8, 8.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.17, 169.66, 156.90, 148.17, 144.30, 141.19, 137.02, 135.43, 134.73, 131.15, 130.22, 128.65, 128.11, 127.55, 125.72, 122.26, 120.59, 66.20, 53.78, 52.35, 47.07, 43.49, 36.42. HRMS (ESI) cald. for $(M+Na)^+$ $C_{33}H_{30}N_2O_8S$: 637.1615, found, 637.1619.



5d

methyl (R)-2-((R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(methylthio) butanamido)-3-(4-((phenylsulfonyl)oxy)phenyl)propanoate(5d);

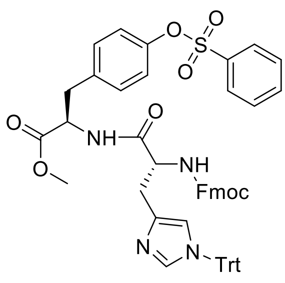
64.7 mg (yield: 48%, 0.2 mmol scale), white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (d, J = 8.2 Hz, 1H), 7.91 (d, J = 7.4 Hz, 2H), 7.86 – 7.83 (m, 2H), 7.80 (d, J = 6.1 Hz, 1H), 7.75 (d, J = 7.5 Hz, 2H), 7.68 – 7.64 (m, 2H), 7.54 (d, J = 8.5 Hz, 1H), 7.43 (t, J = 7.5 Hz, 2H), 7.34 (t, J = 7.4 Hz, 2H), 7.23 (d, J = 8.7 Hz, 2H), 6.89 (d, J = 8.7 Hz, 2H), 4.53 – 4.46 (m, 1H), 4.30 (d, J = 12.7 Hz, 1H), 4.25 – 4.21 (m, 2H), 4.12 (td, J = 8.6, 5.3 Hz, 1H), 3.62 (s, 3H), 3.06 (dd, J = 13.7, 5.0 Hz, 1H), 2.89 (dd, J = 13.7, 10.1 Hz, 1H), 2.36 – 2.30 (m, 2H), 2.01 (s, 3H), 1.70 – 1.60 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 144.34, 141.18, 137.14, 135.42, 134.82, 131.22, 130.23, 128.61, 128.13, 127.53, 120.60, 66.16, 53.99, 53.58, 52.44,47.81,37.13,33.77,31.09,14.96. HRMS (ESI) cald. for $(M+Na)^+$ $C_{36}H_{36}N_2O_8S_2$:711.1805, found, 711.1811.



5e

tert-butyl 3-((R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(((R)-1-methoxy-1-oxo-3-(4-((phenylsulfonyl)oxy)phenyl)propan-2-yl)amino)-3-oxopropyl)-1H-indole-1-carboxylate(5e);

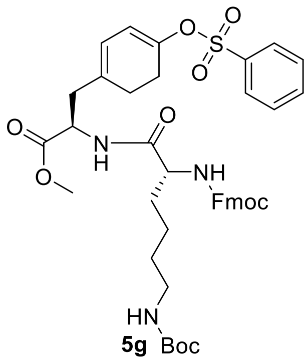
99.5 mg (yield: 59%, 0.2 mmol scale), white solid. 1H NMR (400 MHz, DMSO-d_6) δ 8.64 (d, J = 7.7 Hz, 1H), 8.07 (d, J = 8.3 Hz, 1H), 7.88 (d, J = 7.5 Hz, 2H), 7.82 (t, J = 7.4 Hz, 2H), 7.75 (t, J = 9.1 Hz, 3H), 7.62 (t, J = 7.8 Hz, 5H), 7.43 – 7.32 (m, 4H), 7.30 – 7.21 (m, 5H), 6.93 (d, J = 8.7 Hz, 2H), 4.60 – 4.51 (m, 1H), 4.49 – 4.41 (m, 1H), 4.23 – 4.10 (m, 3H), 3.58 (s, 3H), 3.12 – 2.93 (m, 4H), 1.57 (s, 9H). 13C NMR (101 MHz, DMSO-d_6) δ 172.13, 172.04, 148.19, 144.21, 144.12, 141.15, 137.04, 135.34, 134.78, 131.15, 130.74, 130.17, 128.62, 128.08, 127.47, 125.76, 125.68, 124.78, 124.61, 122.89, 122.26, 120.57, 119.97, 117.14, 115.16,85.07,63.72,55.50,53.86, 52.34, 46.02,35.13,28.08,26.35. HRMS (ESI) cald. for $(M+Na)^+$ $C_{47}H_{45}N_3O_{10}S$:866.2717, found, 866.2711.



5f

methyl (R)-2-((R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-trityl-1H-imidazol-4-yl)propanamido)-3-(4-((phenylsulfonyl)oxy)phenyl)propanoate(5f);

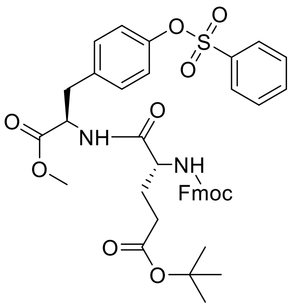
95.6 mg (yield: 51%, 0.2 mmol scale), white solid. 1H NMR (400 MHz, DMSO-d_6) δ 8.40 (d, J = 7.6 Hz, 1H), 7.90 (d, J = 7.6 Hz, 2H), 7.79 (d, J = 7.2 Hz, 2H), 7.74 (d, J = 7.5 Hz, 1H), 7.66 – 7.58 (m, 4H), 7.46 – 7.38 (m, 4H), 7.33 (s, 8H), 7.27 (d, J = 7.9 Hz, 2H), 7.20 (d, J = 8.7 Hz, 3H), 7.02 (dd, J = 6.8, 3.0 Hz, 6H), 6.88 (d, J = 8.6 Hz, 2H), 6.74 (s, 1H), 4.50 – 4.43 (m, 1H), 4.35 – 4.27 (m, 1H), 4.16 (d, *J* = 6.1 Hz, 2H), 4.11 (d, *J* = 5.9 Hz, 1H), 3.48 (s, 3H), 3.03 – 2.88 (m, 2H), 2.88 – 2.81 (m, 1H), 2.76 – 2.67 (m, 1H). 13C NMR (101 MHz, DMSO-d_6) δ 172.06, 171.94, 156.15, 148.14, 144.20, 144.17, 142.54, 141.16, 141.13, 138.14, 137.35, 137.01, 135.37, 134.72, 131.12, 130.18, 129.70, 129.64, 128.78, 128.62, 128.59, 128.47, 128.12, 127.54, 125.76, 122.23, 120.58, 119.68, 75.11, 66.26, 54.79, 53.74, 52.30, 47.05, 36.26, 31.22. HRMS (ESI) cald. for $(M+Na)^+$ $C_{56}H_{48}N_4O_8S$: 959.3085, found, 959.3092.



5g

methyl (R)-2-((R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-((tert-butoxycarbonyl)amino)pentanamido)-3-(4-((phenylsulfonyl)oxy)cyclohexa-1,3-dien-1-yl)propanoate(5g);

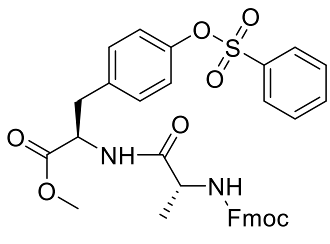
91.4 mg (yield: 58%, 0.2 mmol scale), white solid.1H NMR (400 MHz, DMSO-d_6) δ 8.31 (d, *J* = 7.6 Hz, 1H), 7.89 (d, *J* = 6.8 Hz, 2H), 7.81 (d, *J* = 8.6 Hz, 2H), 7.76 (d, *J* = 7.5 Hz, 1H), 7.74 – 7.69 (m, 2H), 7.65 – 7.60 (m, 2H), 7.44 – 7.37 (m, 3H), 7.33 (t, *J* = 7.4 Hz, 2H), 7.22 (d, *J* = 8.7 Hz, 2H), 6.90 (d, *J* = 8.7 Hz, 2H), 6.78 – 6.71 (m, 1H), 4.51 – 4.42 (m, 1H), 4.31 – 4.18 (m, 3H), 4.02 – 3.95 (m, 1H), 3.55 (s, 3H), 3.01 (dd, *J* = 14.0, 5.9 Hz, 1H), 2.93 (dd, *J* = 14.0, 8.6 Hz, 3H), 1.58 – 1.48 (m, 2H), 1.38 (s, 9H), 1.35 – 1.18 (m, 4H). 13C NMR (101 MHz, DMSO-d_6) δ 172.62, 172.10, 156.31, 156.05, 148.16, 144.39, 144.21, 141.19, 137.02, 135.35, 134.83, 131.10, 130.17, 128.59, 128.10, 127.52, 125.75, 122.17, 120.56, 77.82, 66.08, 54.83, 53.69, 52.26, 47.15, 36.26, 32.05, 29.68, 28.74, 23.20. HRMS (ESI) cald. for $(M+Na)^+$ $C_{41}H_{45}N_3O_{10}S$: 794.2717, found, 794.2712.



5h

tert-butyl (R)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-(((R)-1-methoxy-1-oxo-3-(4-((phenylsulfonyl)oxy)phenyl)propan-2-yl)amino)-5-oxopentanoate(5h);

90.6 mg (yield: 61%, 0.2 mmol scale), white solid.1H NMR (400 MHz, DMSO-d_6 δ 8.39 (d, J = 7.5 Hz, 1H), 7.91 (d, J = 7.7 Hz, 2H), 7.84 – 7.72 (m, 5H), 7.64 (t, J = 8.0 Hz, 2H), 7.52 (d, J = 8.4 Hz, 1H), 7.43 (t, J = 7.5 Hz, 2H), 7.34 (t, J = 7.5 Hz, 2H), 7.23 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 4.50 – 4.44 (m, 1H), 4.33 – 4.20 (m, 3H), 4.07 – 4.01 (m, 1H), 3.57 (s, 3H), 3.06 – 2.91 (m, 2H), 2.23 (t, J = 8.0 Hz, 2H), 1.88 – 1.69 (m, 2H), 1.41 (s, 9H). 13C NMR (101 MHz, DMSO-d_6) δ 172.13, 172.08, 172.03, 156.28, 148.14, 144.38, 144.17, 141.20, 137.04, 135.40, 134.74, 131.13, 130.20, 128.63, 128.13, 127.53, 125.77, 122.22, 120.60, 80.17, 66.13, 54.02, 53.75, 52.32, 47.11, 36.12, 31.67, 28.21, 27.77. HRMS (ESI) cald. for $(M+Na)^+$ $C_{40}H_{42}N_2O_{10}S$: 765.2452, found, 765.2439.

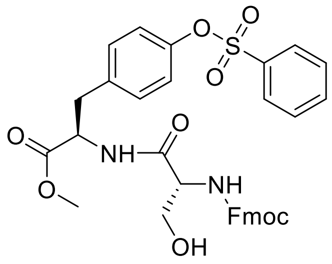


5i

methyl (R)-2-((R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-3-(4-((phenylsulfonyl)oxy)phenyl)propanoate(5i);

88.0 mg (yield:70%, 0.2 mmol scale), white solid.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (d, J = 8.3 Hz, 1H), 7.89 (d, J = 8.2 Hz, 2H), 7.84 – 7.81 (m, 2H), 7.78 (d, J = 7.5 Hz, 1H), 7.74 (dd, J = 7.4, 3.1 Hz, 2H), 7.67 – 7.62 (m, 2H), 7.47 (d, J = 7.9 Hz, 1H), 7.42 (t, J = 7.5 Hz, 2H), 7.33 (t, J = 7.5 Hz, 2H), 7.21 (d, J = 8.7 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H), 4.55 – 4.48 (m, 1H), 4.27 – 4.18 (m, 3H), 4.04 (q, J = 7.3 Hz, 1H), 3.61

(s, 3H), 3.06 (dd, J = 13.8, 4.9 Hz, 1H), 2.86 (dd, J = 13.8, 10.2 Hz, 1H), 0.99 (d, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.03, 172.23, 144.28, 143.05, 137.07, 134.77, 131.23, 130.21, 129.39, 128.61, 128.11, 127.75, 127.53, 125.79, 121.84, 120.56, 120.49, 110.19, 66.50,55.37,52.39, 50.23, 47.09,35.19,18.85. HRMS (ESI) cald. for $(M+Na)^+$ $C_{34}H_{32}N_2O_8S$: 651.1771, found,651.1778.

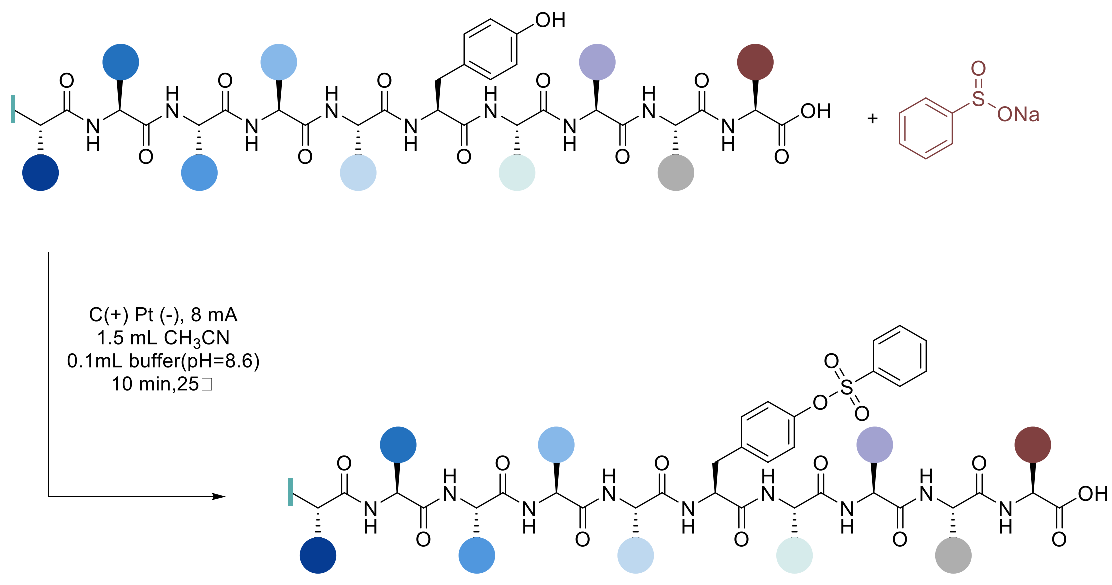


5j

methyl (R)-2-((R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-hydroxypropanamido)-3-(4-((phenylsulfonyl)oxy)phenyl)propanoate(5j);

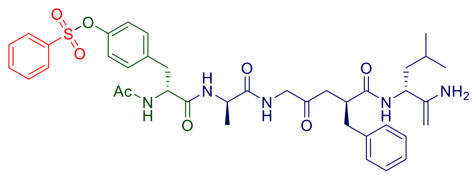
60.6 mg (yield:47%, 0.2 mmol scale), white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (d, J = 7.6 Hz, 1H), 7.90 (d, J = 7.5 Hz, 2H), 7.81 (d, J = 6.0 Hz, 2H), 7.77 (d, J = 7.4 Hz, 1H), 7.74 (dd, J = 8.1, 3.8 Hz, 2H), 7.66 – 7.62 (m, 2H), 7.42 (t, J = 7.0 Hz, 2H), 7.35 – 7.30 (m, 3H), 7.21 (d, J = 8.7 Hz, 2H), 4.89 (t, J = 5.7 Hz, 1H), 4.50 – 4.43 (m, 1H), 4.28 (d, J = 5.9 Hz, 2H), 4.22 (d, J = 6.1 Hz, 1H), 4.13 – 4.07 (m, 1H), 3.58 (d, J = 4.7 Hz, 1H), 3.55 (s, 3H), 3.47 (d, J = 11.1 Hz, 1H), 3.04 – 2.90 (m, 2H).^{13}C NMR (101 MHz, DMSO-d_6) δ 171.99, 170.68, 156.38, 148.15, 144.35, 144.24, 141.19, 137.00, 135.41, 134.72, 131.19, 130.21, 128.63, 128.13, 127.56, 125.79, 122.21, 120.59, 66.21, 62.11, 57.63, 53.76, 52.34, 47.08, 36.30. HRMS (ESI) cald. for $(M+Na)^+$ $C_{34}H_{32}N_2O_9S$: 667.1720, found, 667.1718.

3.5 Polypeptide scope and characterization



General Procedure for Bioconjugation of Tyrosine and Sodium benzenesulfite : In an oven-dried undivided three-necked bottle (15 mL) equipped with a stir bar, polypeptides (5 mg), Sodium benzenesulfite (10 mg), CH_3CN (1.5 mL),buffer (pH =8.6, 0.1mL), $^{n}Bu_4NBr$(0.08 mmol) were combined and added. The bottle was equipped graphite rod (ϕ 6 mm) as the anode and platinum plate (10 mm×10 mm×0.3 mm) as the cathode and then charged. The reaction mixture was stirred and electrolyzed at constant current of 8 mA under 25°C for 10 min. After completion of the reaction, the solution was analyzed by LC-MS/MS spectroscopy. The reaction was analyzed by reversed-phase HPLC on a 250 mm long ChromCore C18 5μm column using a gradient of 5% to 50% buffer B within 30 minutes. HPLC analysis used buffers A (water + 0.1% TFA) and B (9:1 acetonitrile : water + 0.1% TFA). Conversion reported as a % conversion as determined.

[D-ala2]-leucine encephalin:YAGFL

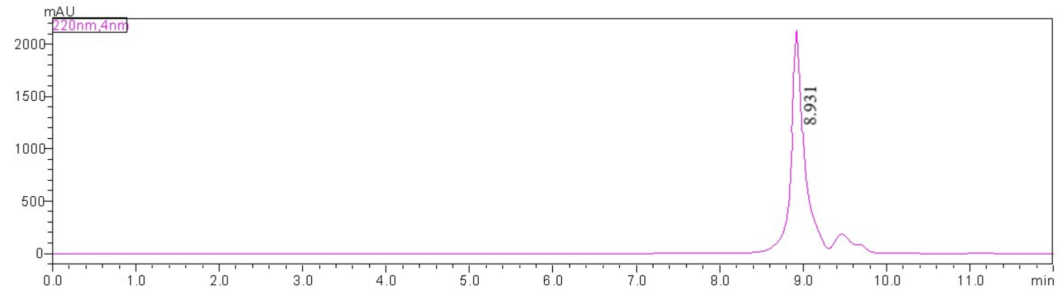


HPLC: >99% conversion.

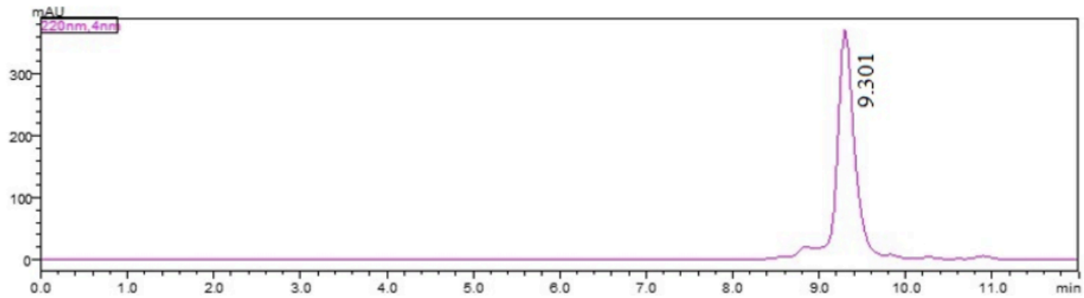
Product 6a is a peak that elute at 50% buffer B (9:1 acetonitrile: water + 0.1% TFA) with retention times of 9.301 min. Reactant is a peak that elutes at 50% buffer B with a retention time of 8.931 min.

HRMS (ESI-TOF): m/z calculated for $C_{37}H_{46}N_6O_9S$, $[M+H]^+$, 751.3119, found 751.3120.

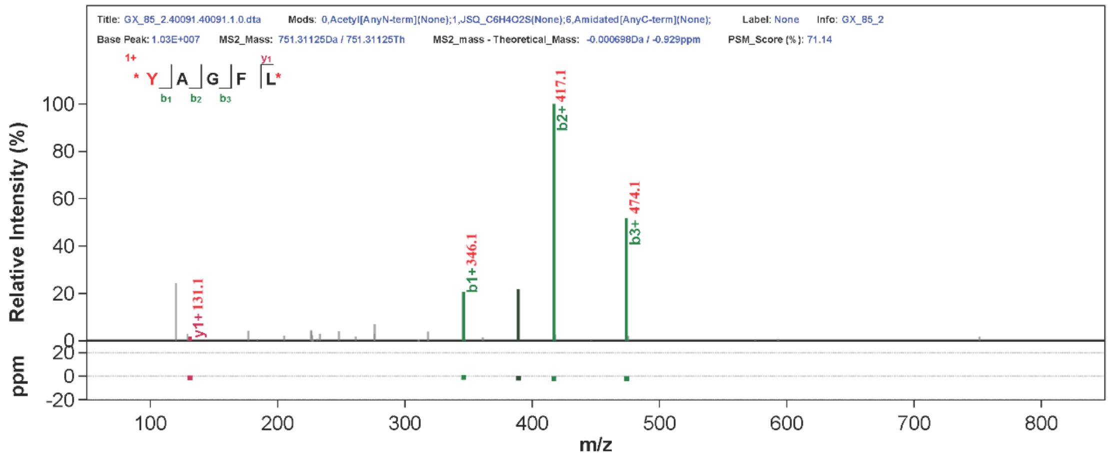
HPLC Spectra:



Data Name：2023-6-14-5 peptide product-9-1-5ul-3.lcd
Sample Name: JSQ
Sample ID:1

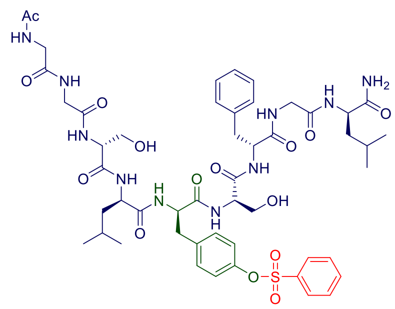


MALDI-TOF-MS/MS Spectra:



Allatostation: GGSLYSFGL

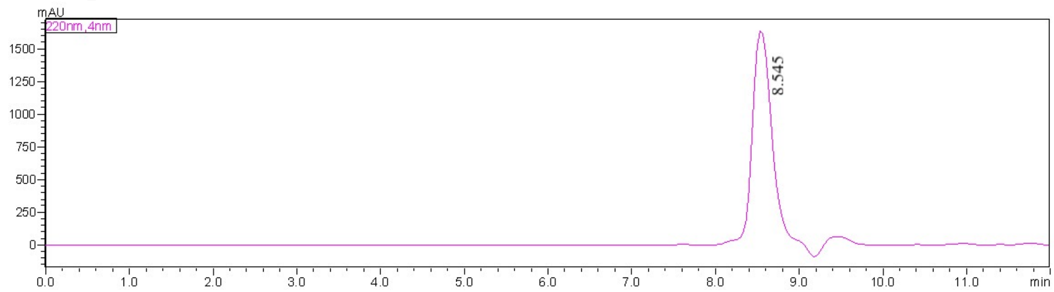
HPLC: >99% conversion.



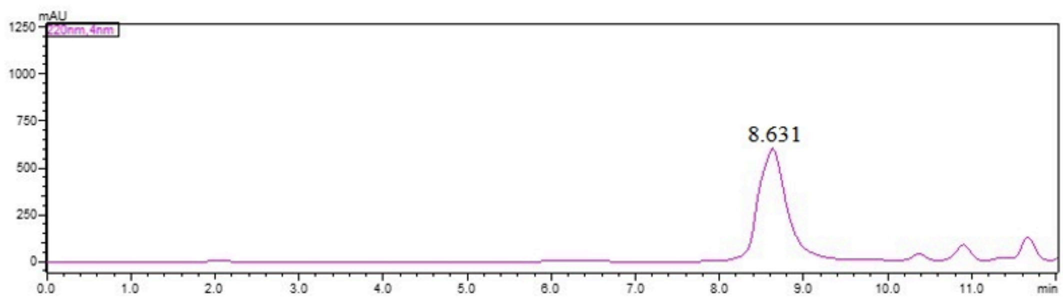
Product 6b is a peak that elute at 50% buffer B (9:1 acetonitrile: water + 0.1% TFA) with retention times of 8.631 min. Reactant is a peak that elutes at 50% buffer B with a retention time of 8.545 min.

HRMS (ESI-TOF): m/z calculated for $C_{50}H_{68}N_{10}O_{15}S$, $[M+H]^+$, 1081.4737, found 1081.4736.

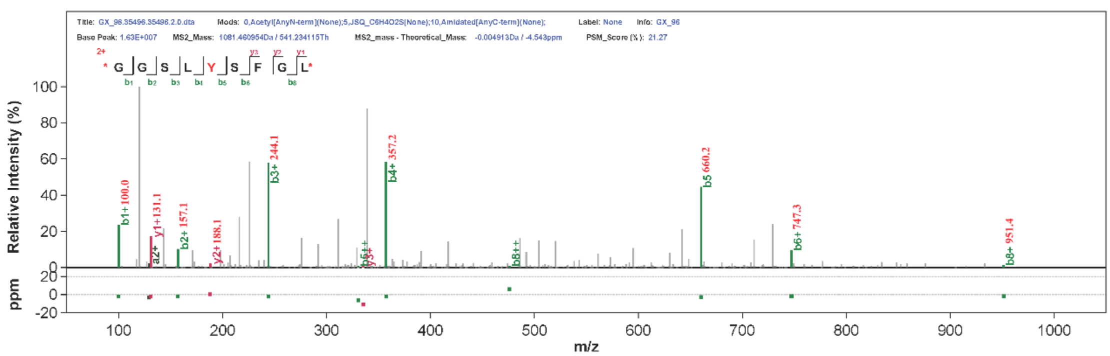
HPLC Spectra:

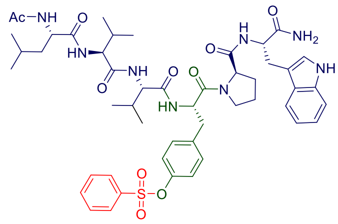


Data Name：2023-7-6-125 peptide product-1-30ul-128.Jcd
Sample Name: JSQ
Sample ID:1



MALDI-TOF-MS/MS Spectra:



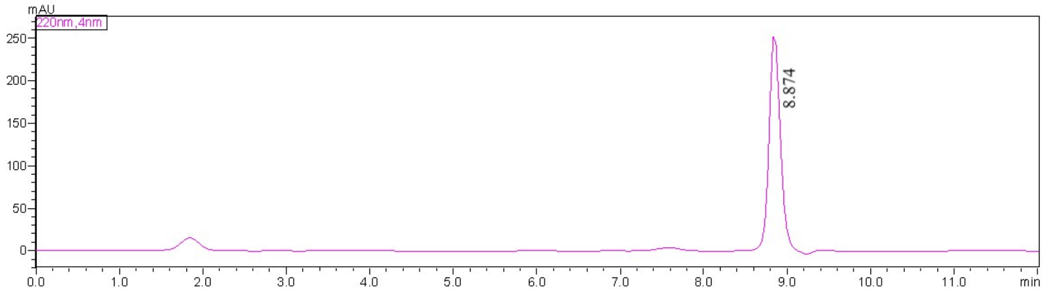


Myelopeptide-2(MP-2):LVVYPW
HPLC: >99% conversion.

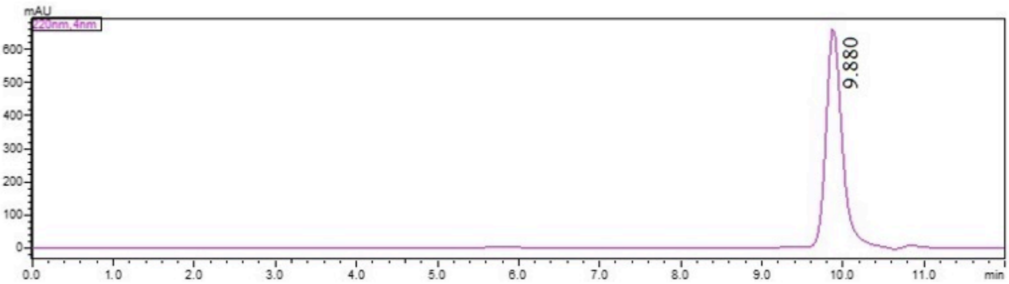
Product 6c is a peak that elute at 50% buffer B (9:1 acetonitrile: water + 0.1% TFA) with retention times of 8.874 min. Reactant is a peak that elutes at 50% buffer B with a retention time of 9. 880 min.

HRMS (ESI-TOF): m/z calculated for $C_{49}H_{64}N_8O_{10}S$, $[M+H]^+$,957.4539, found 957.4539.

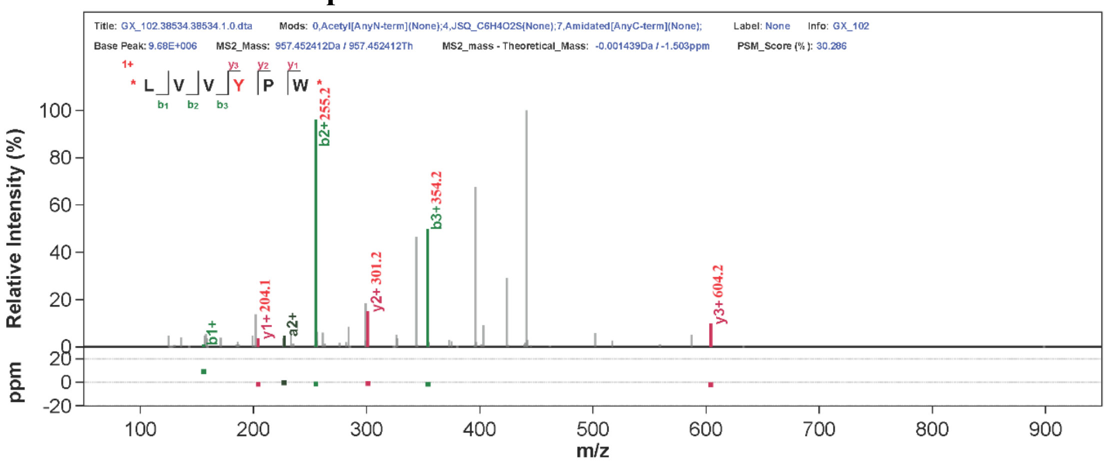
HPLC Spectra:



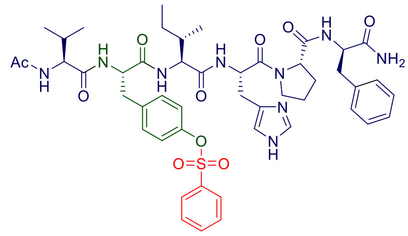
Data Name： 2023-7-11-815 peptide product-1-20ul-161.Jcd
Sample Name: JSQ
Sample ID:1



MALDI-TOF-MS/MS Spectra:



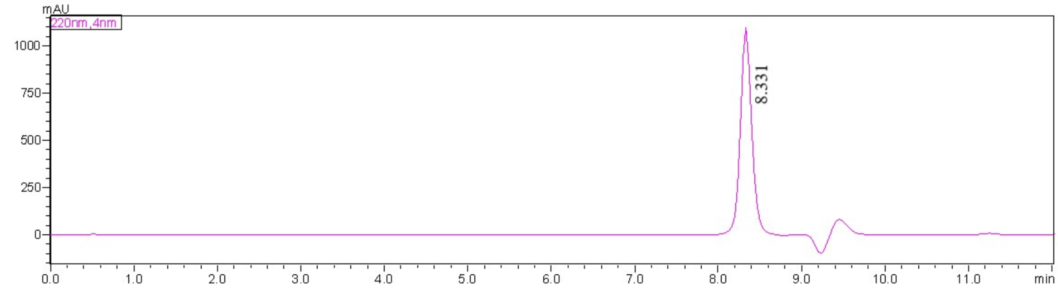
3-8-Angiotensin II:VYIHPF
HPLC: >99% conversion.



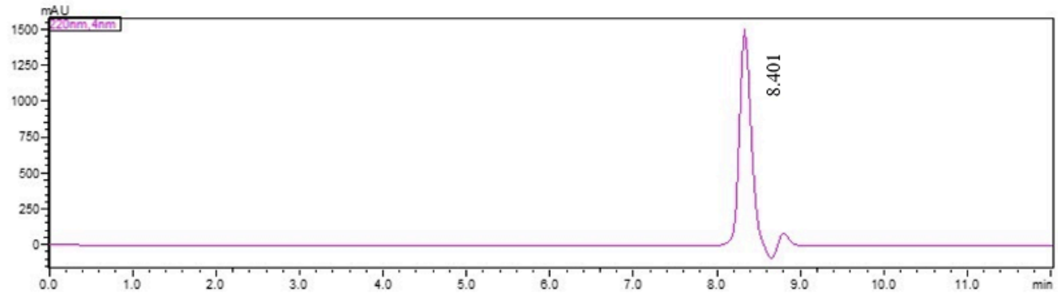
Product 6c is a peak that elute at 50% buffer B (9:1 acetonitrile: water + 0.1% TFA) with retention times of 8.401 min. Reactant is a peak that elutes at 50% buffer B with a retention time of 8.331 min.

HRMS (ESI-TOF): m/z calculated for $C_{48}H_{61}N_9O_{10}S$, $[M+H]^+$, 956.4334, found 956.4331.

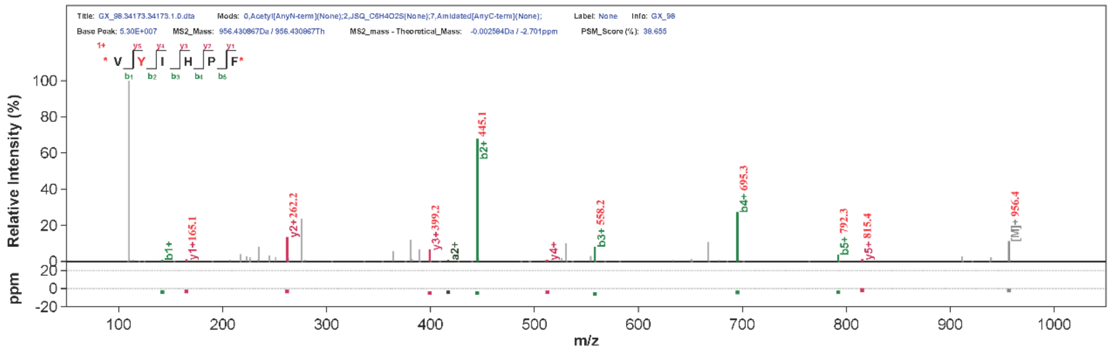
HPLC Spectra:

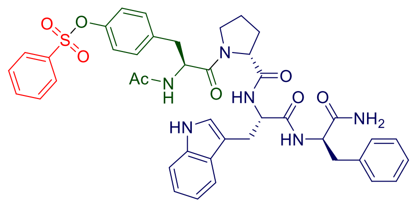


Data Name: 2023-6-29-152 peptide product-9-1-30ul-91.Jcd
Sample Name: JSQ
Sample ID:1



MALDI-TOF-MS/MS Spectra:





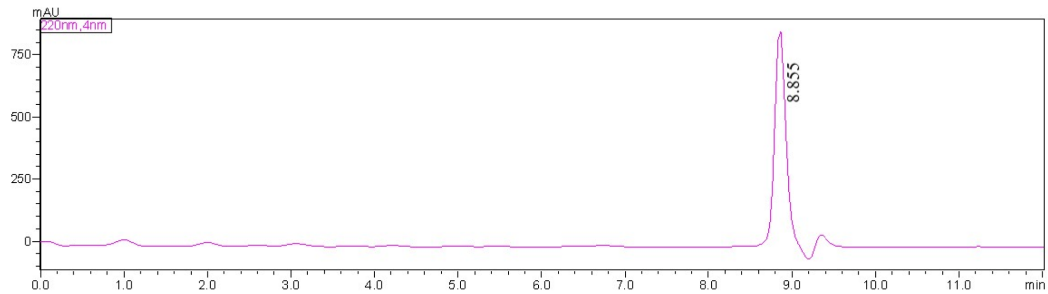
Endomorphin 1:YPWF

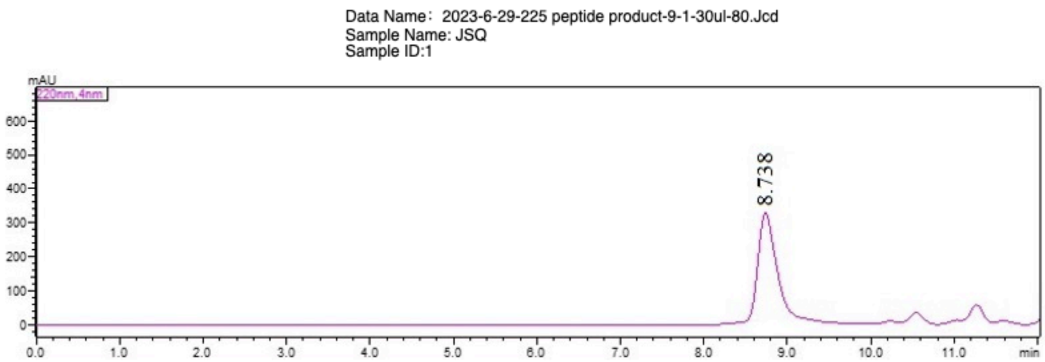
HPLC: >99% conversion.

Product 6e is a peak that elute at 50% buffer B (9:1 acetonitrile: water + 0.1% TFA) with retention times of 8.738 min. Reactant is a peak that elutes at 50% buffer B with a retention time of 8.855 min.

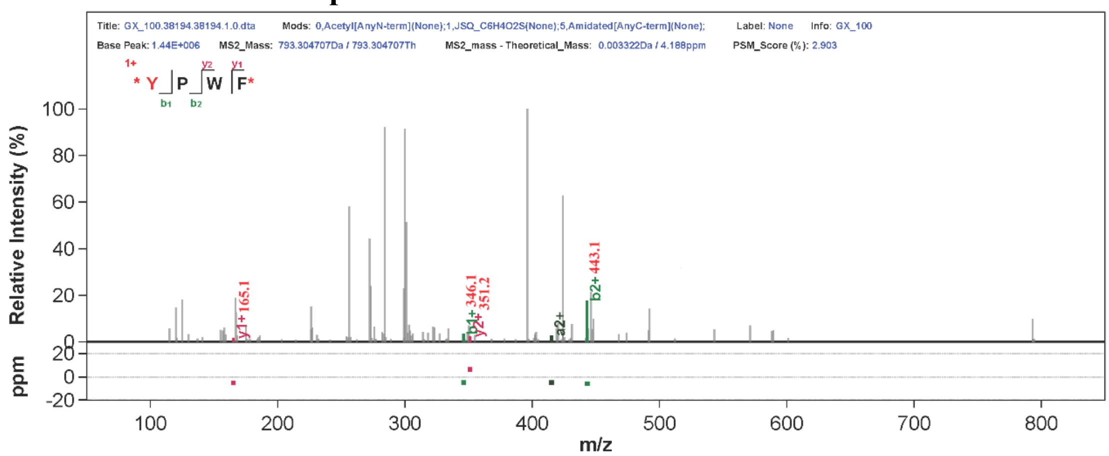
HRMS (ESI-TOF): m/z calculated for $C_{42}H_{44}N_6O_8S$, $[M+H]^+$, 793.3014, found 793.3014.

HPLC Spectra:

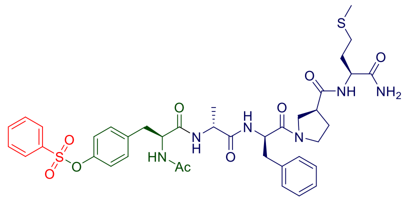




MALDI-TOF-MS/MS Spectra:



β-Casomorphin(1-5),amide,bovine:YAFPM

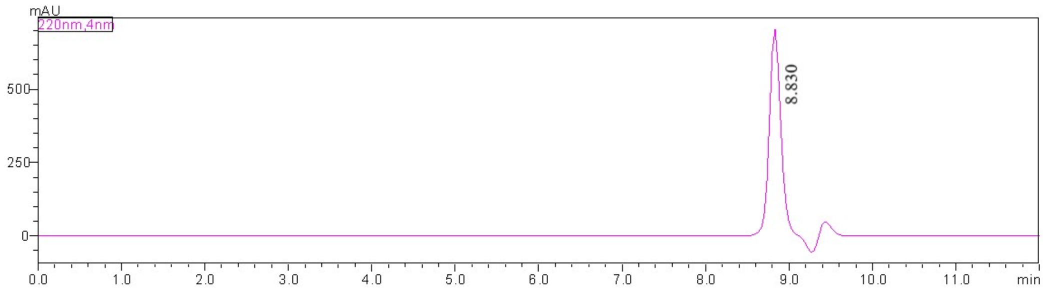


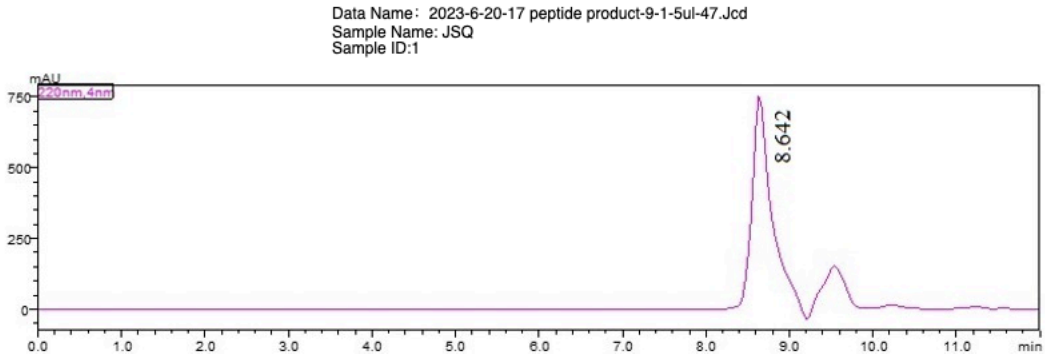
HPLC: >99% conversion.

Product 6d is a peak that elute at 50% buffer B (9:1 acetonitrile: water + 0.1% TFA) with retention times of 8.642 min. Reactant is a peak that elutes at 50% buffer B with a retention time of 8.830 min.

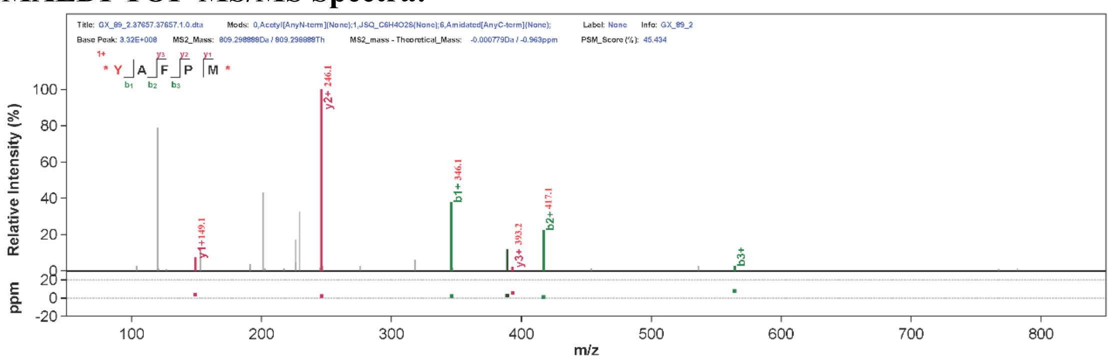
HRMS (ESI-TOF): m/z calculated for $C_{39}H_{48}N_6O_9S_2$, $[M+H]^+$, 809.2997, found 809.2997.

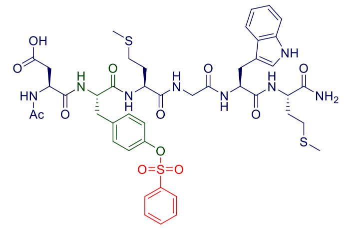
HPLC Spectra:





MALDI-TOF-MS/MS Spectra:



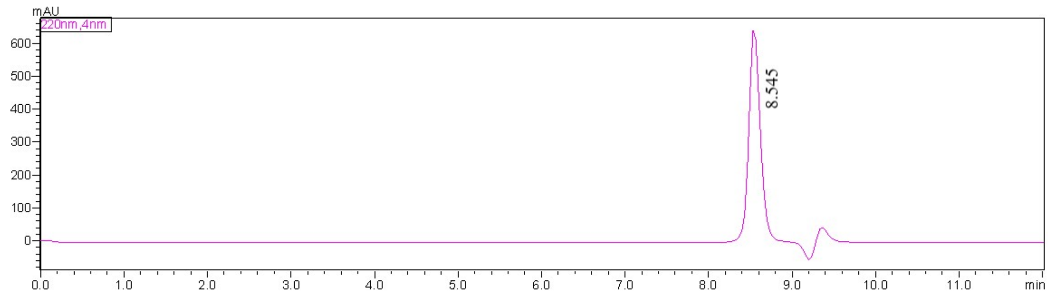


ω-Conotoxin MVIIC:DYMGWM
HPLC: >99% conversion.

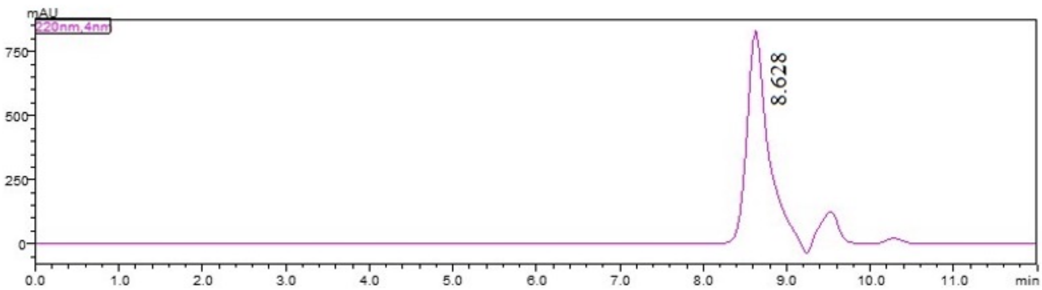
Product 6f is a peak that elute at 50% buffer B (9:1 acetonitrile: water + 0.1% TFA) with retention times of 8.628 min. Reactant is a peak that elutes at 50% buffer B with a retention time of 8.545 min.

HRMS (ESI-TOF): m/z calculated for $C_{44}H_{54}N_8O_{12}S_3$, $[M+H]^+$, 983.3096, found 983.3096

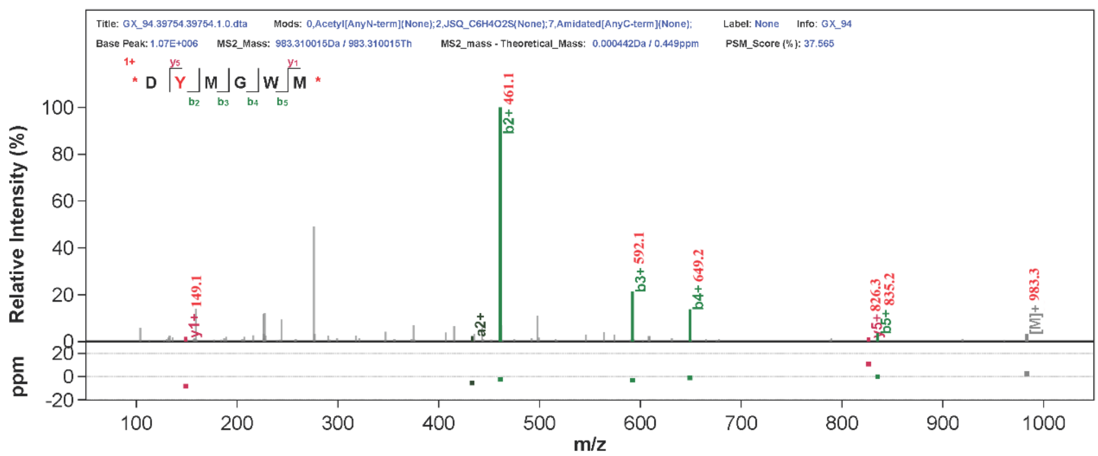
HPLC Spectra:

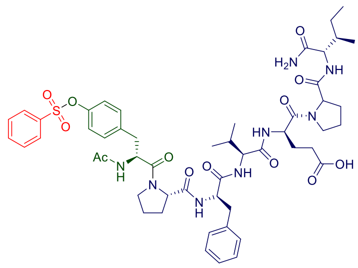


Data Name：2023-6-14-18 peptide product-9-1-20ul-27.Jcd
Sample Name: JSQ
Sample ID:1



MALDI-TOF-MS/MS Spectra:





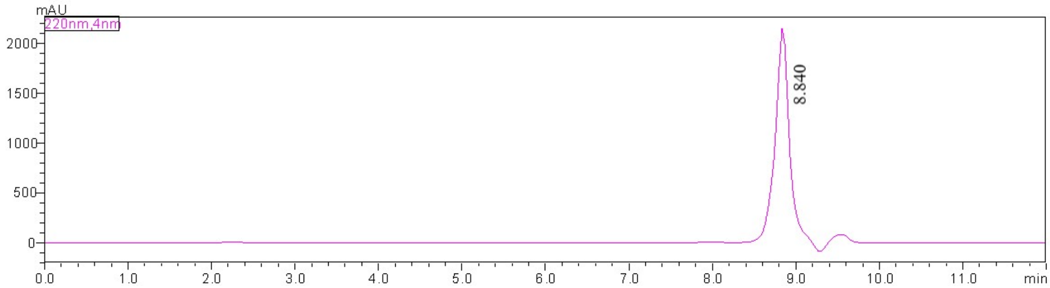
β-Casomorphin:YPFVEPI

HPLC: >99% conversion.

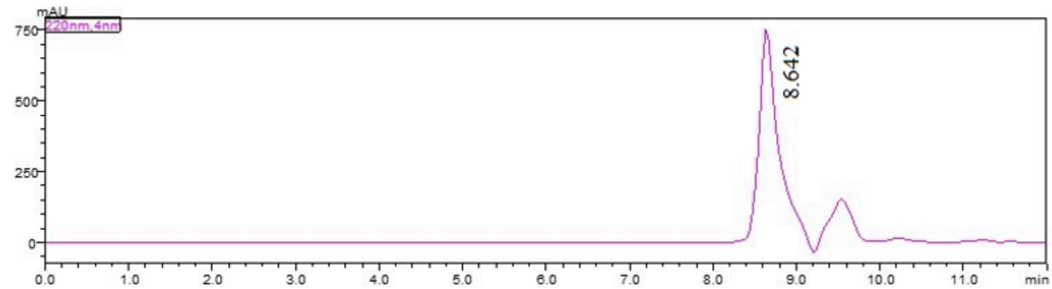
Product 6h is a peak that elute at 50% buffer B (9:1 acetonitrile: water + 0.1% TFA) with retention times of 8.642 min. Reactant is a peak that elutes at 50% buffer B with a retention time of 8.840 min.

HRMS (ESI-TOF): m/z calculated for $C_{52}H_{68}N_8O_{13}S$, $[M+H]^+$, 1045.4699, found 1045.4700.

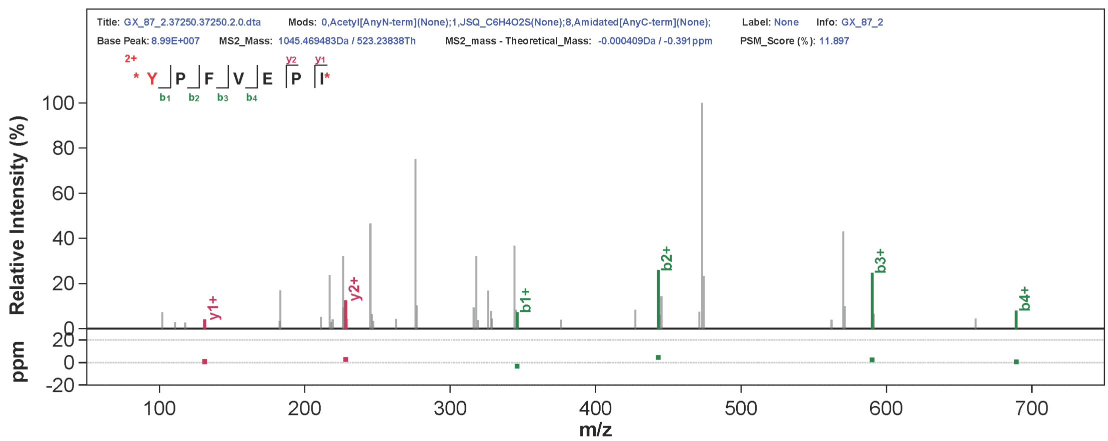
HPLC Spectra:



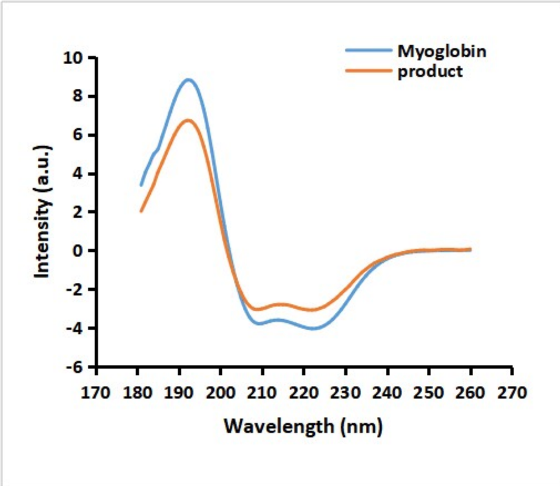
Data Name: 2023-6-20-17 peptide product-9-1-5ul-47.Jcd
Sample Name: JSQ
Sample ID:1



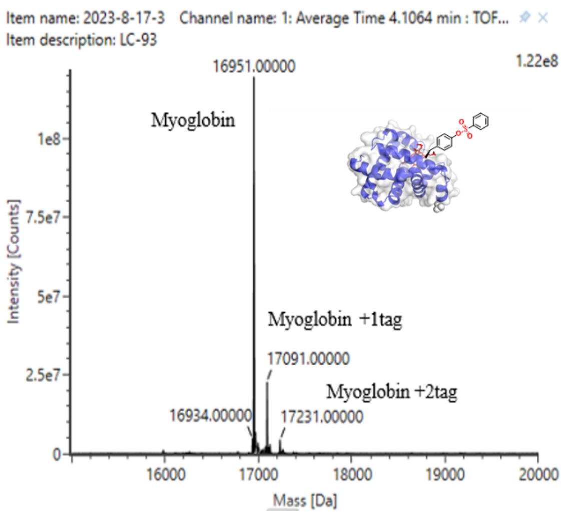
MALDI-TOF-MS/MS Spectra:



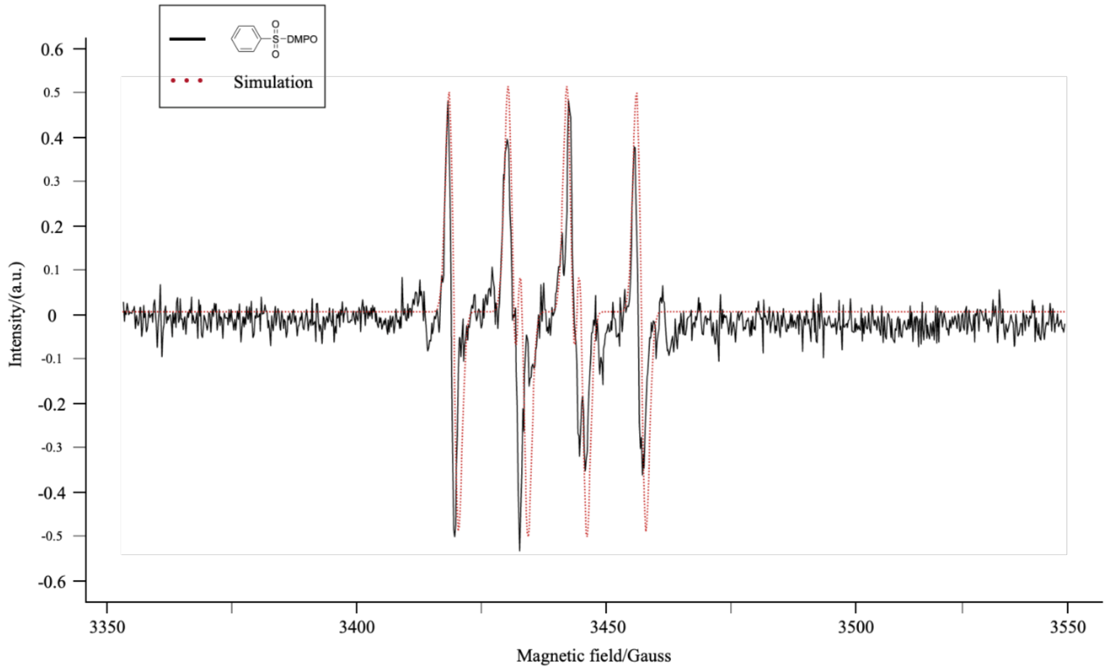
Synthesis of 7a: In an oven-dried undivided three-necked bottle (10 mL) equipped with a stir bar, Myoglobin (5 mg), Sodium benzenesulphinate (10 mg), $^nBu_4NBr$ (0.08mmol), MeCN / buffer(pH = 8.6) (1.5 mL / 0.1 mL) were combined and added. The bottle was equipped graphite rod (ϕ 6 mm) as the anode and platinum plate (10 mm×10 mm×0.3 mm) as the cathode and then charged. The reaction mixture was stirred and electrolyzed at constant current of 8 mA under 25°Cfor 10 min. After completion of the reaction, the solution was analyzed by Maldi-Tof MS.



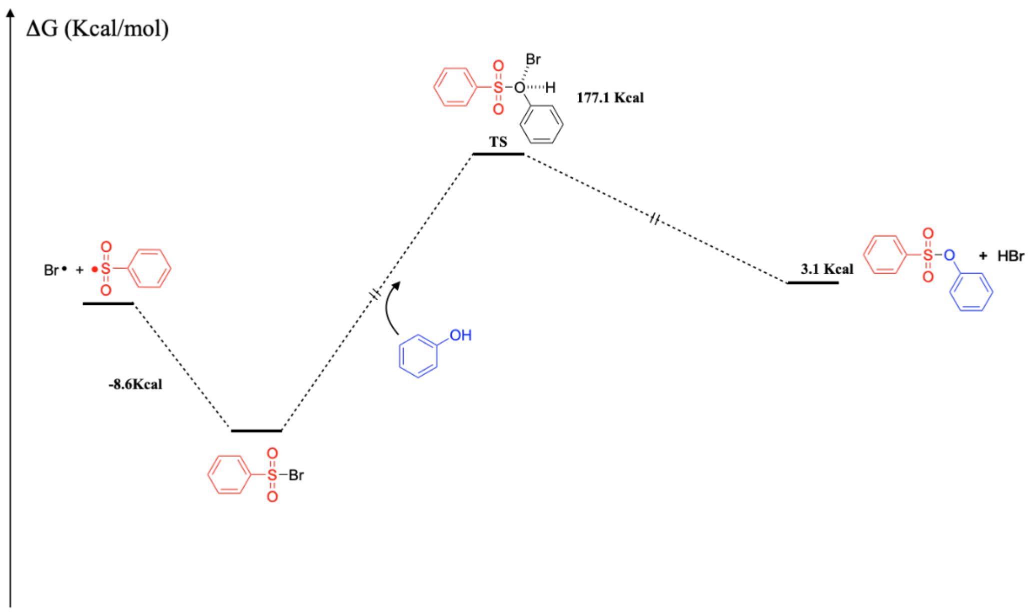
Comparison of CD spectra between Myoglobin and product (100μg/mL in buffer).



3.6 EPR and DFT

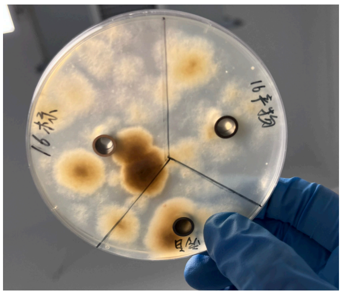


To gain additional insights into the mechanism for this reaction, we conducted DFT calculations for this reaction. The reaction diagrams were calculated at the B3LYP with 6-31G level for C and H, 6-31G+ level for S, O, and Br atoms of theory.



3.7 Anti-fungal experiment of benzenesulfonate-labeled peptide

To assess the in vivo antifungal activity of benzenesulfonate-labeled peptide, the indicator strain Alternaria alternata was cultivated in potato dextrose broth for 24 h, and 100 μL culture was added to molten potato dextrose agar cooled below 55 °C. 100 μL product 6h (3 mg/mL), substrate, solvent (water) was added to oxford cup placed onto the solidified agar respectively. Inhibition zones were recorded after 48 h at 30 °C



To assess the in vivo antifungal activity of 6h, the indicator strain Alternaria alternata was cultivated in potato dextrose broth for 24 h, and 100 μL culture was added to molten potato dextrose agar (PDA) cooled below 55 °C. 100 μL 6h (3 mg/mL), substrate, solvent (water) was added to oxford cup placed onto the solidified agar respectively. Inhibition zones were recorded after 48 h at 30 °C.

I II III IV

I: MeOH; II: blank; III: b-Casomorphin; IV: 6h.

MIC Experiment: To determine the minimal inhibitory concentration of 6h, 10 μL dilutions ranging from 0.1-3 mg/mL were added to 200μL PDA in 96 well plates. At the same time, no addition, added water or substrate as positive controls. The plates were then kept at 4 °C for 4 hours to allow the diffusion of additions. The indicator strain Alternaria alternata was then inoculated on the surface of the agar and incubated at 28 °C for 48 h. From the results, we found that the antifungal ability of 6h increases with increasing concentration.

A B C D E F

A: Water; B: no addition; C: b-Casomorphin; D:1mg/mL 6h; E: 2 mg/mL 6h; F: 3 mg/mL 6h.

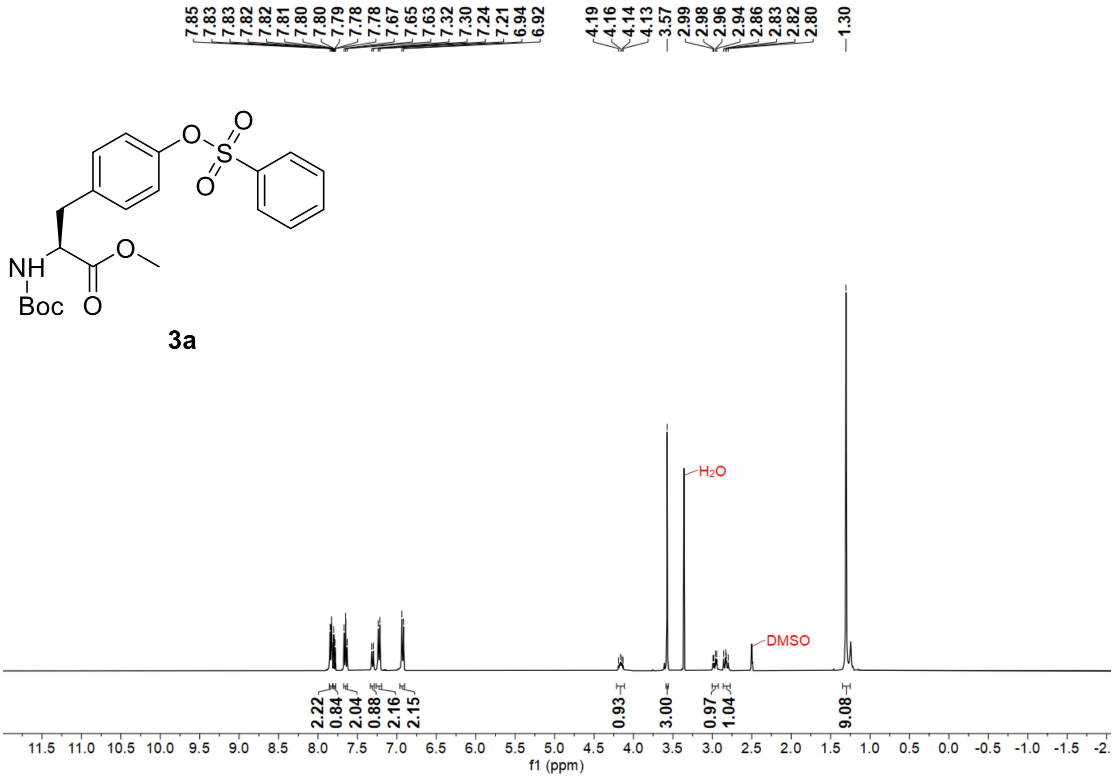
4.References

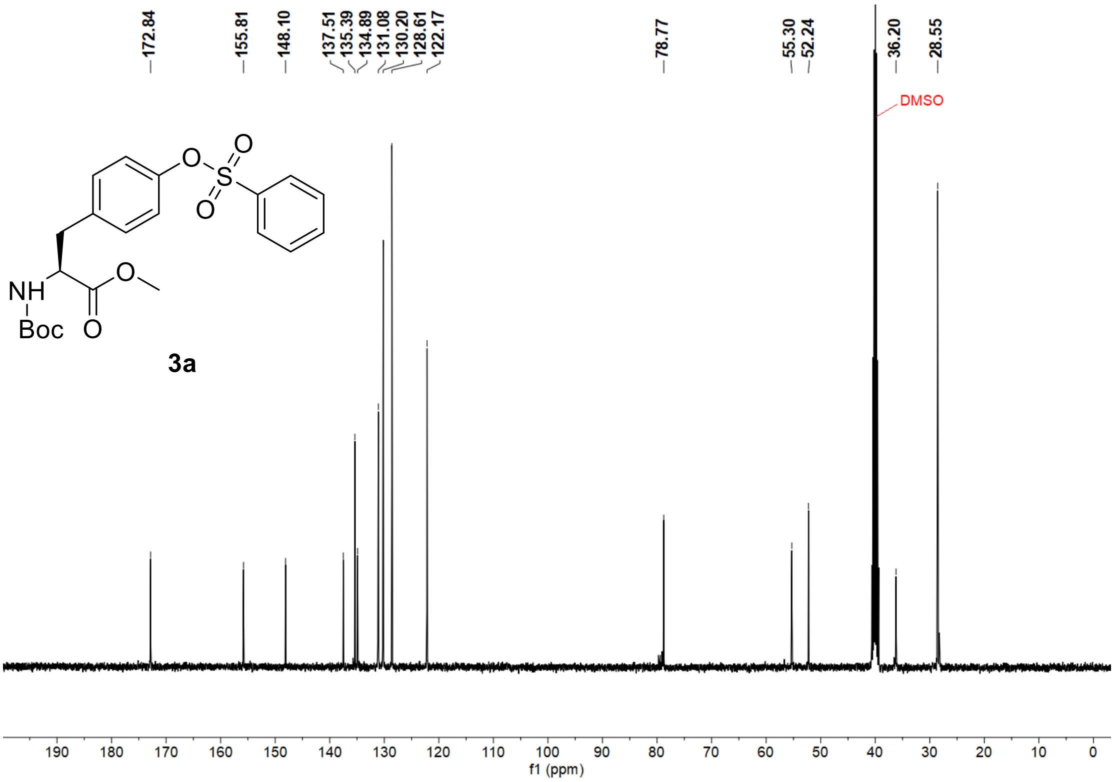
{1} R. A. Serwa, J.-M. Swiecicki, D. Homann, C. P. R. Hackenberger, Phosphoramidate peptide synthesis by solution- and solid-phase Staudinger-phosphite reactions. J. Pept. Sci.2010, 16, 563–567.

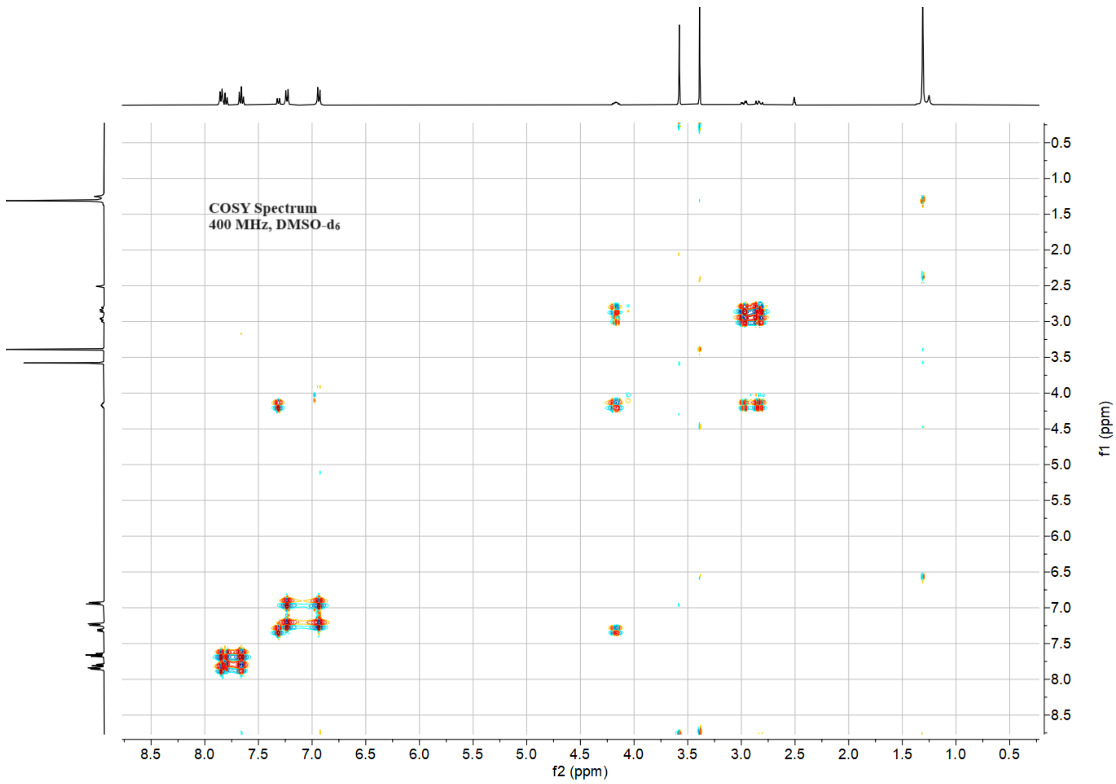
{2} Alam J, Keller T H, Loh T P. Functionalization of peptides and proteins by Mukaiyamaaldol reaction. J. Am. Chem. Soc. 2010, 132, 9546-9548.

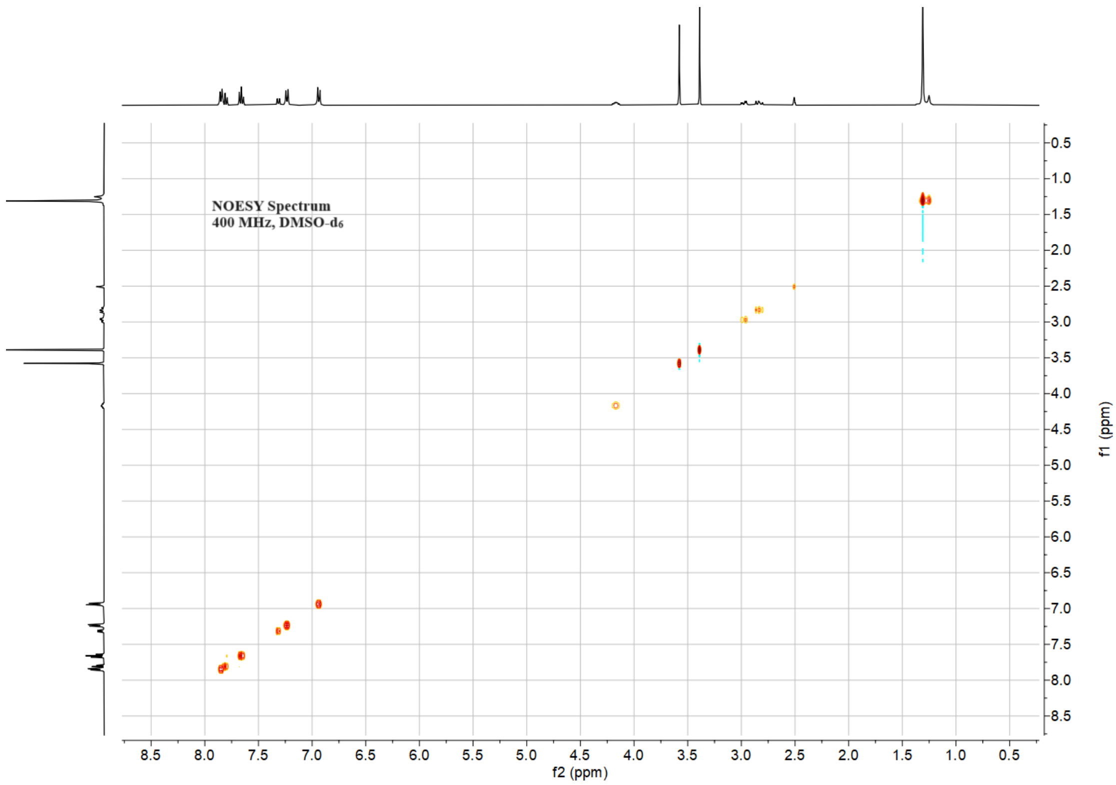
5. Spectra

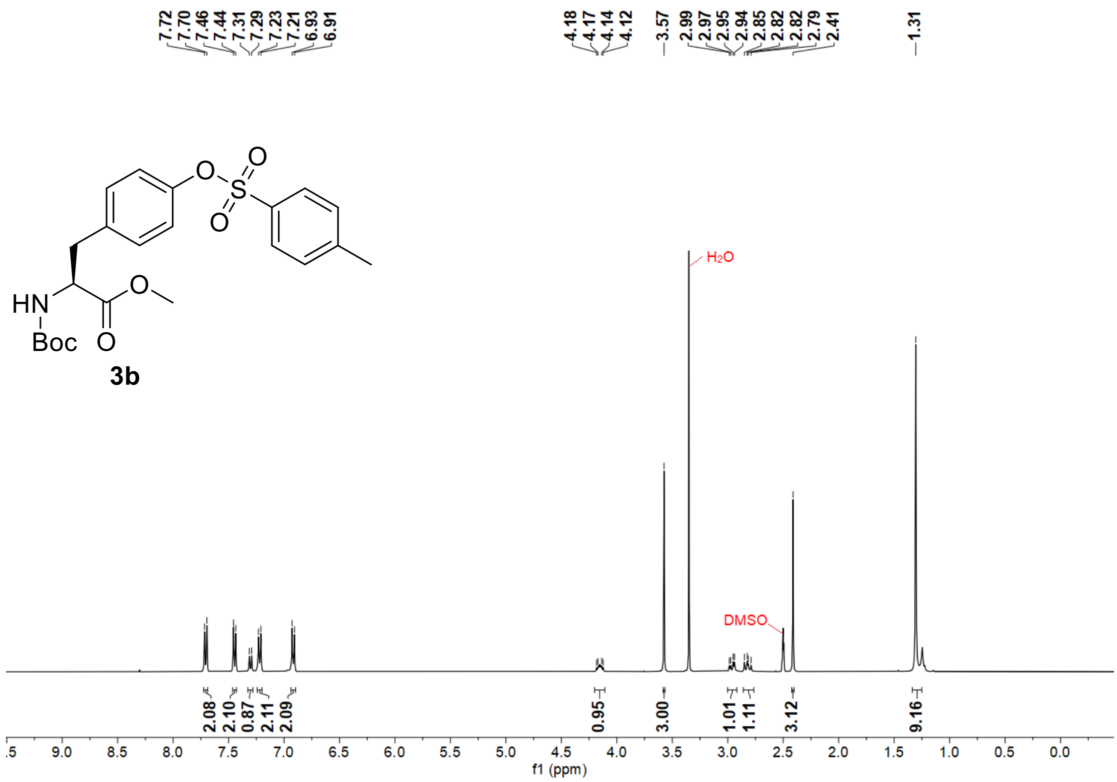
5.1 NMR Spectra of Products

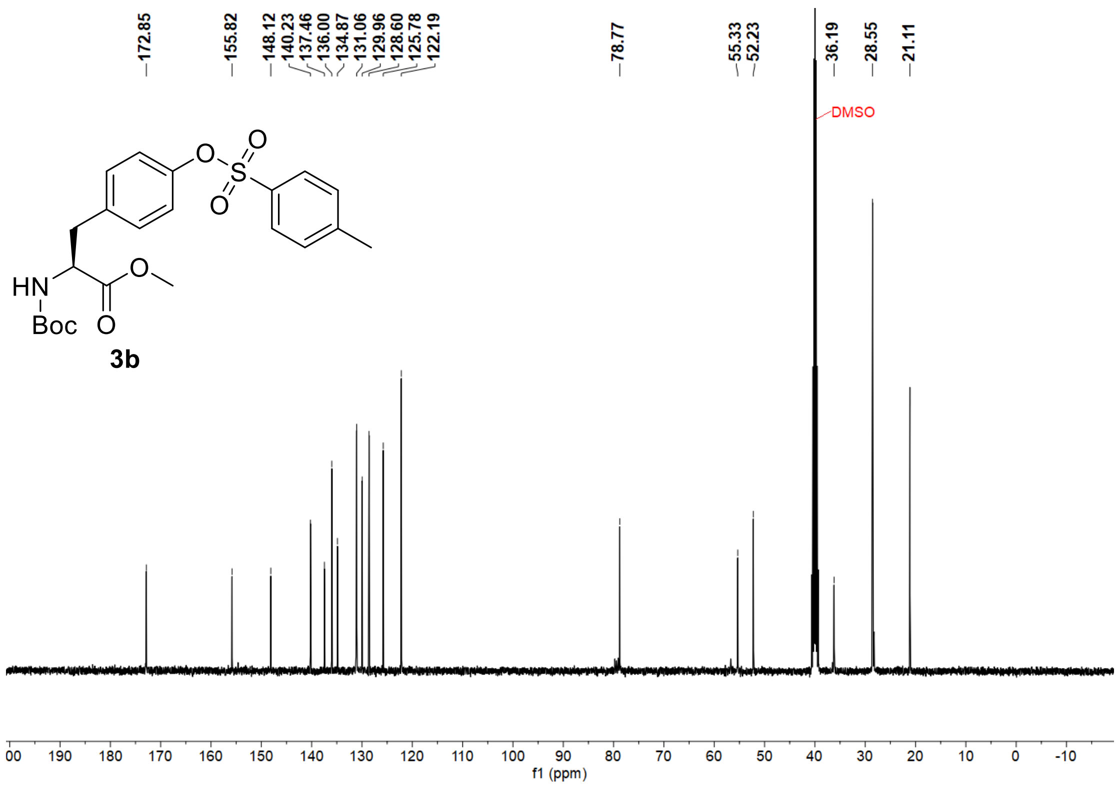


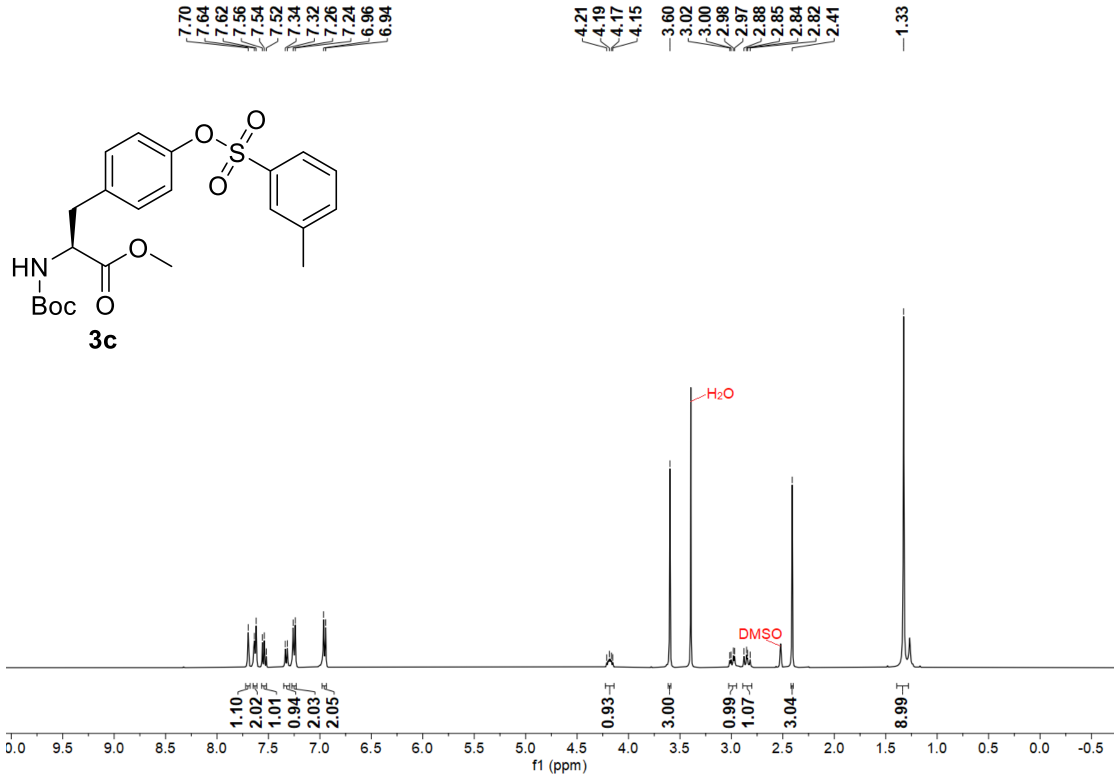


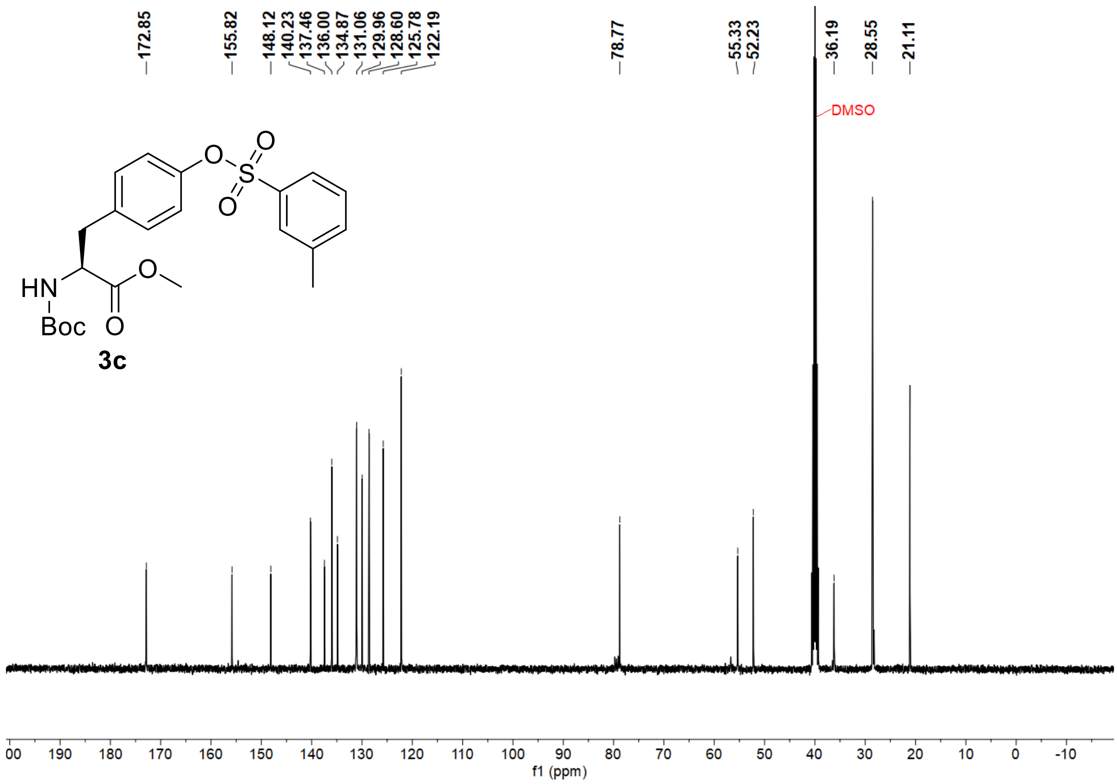

COSY Spectrum
400 MHz, DMSO-d6
f2 (ppm)
f1 (ppm)

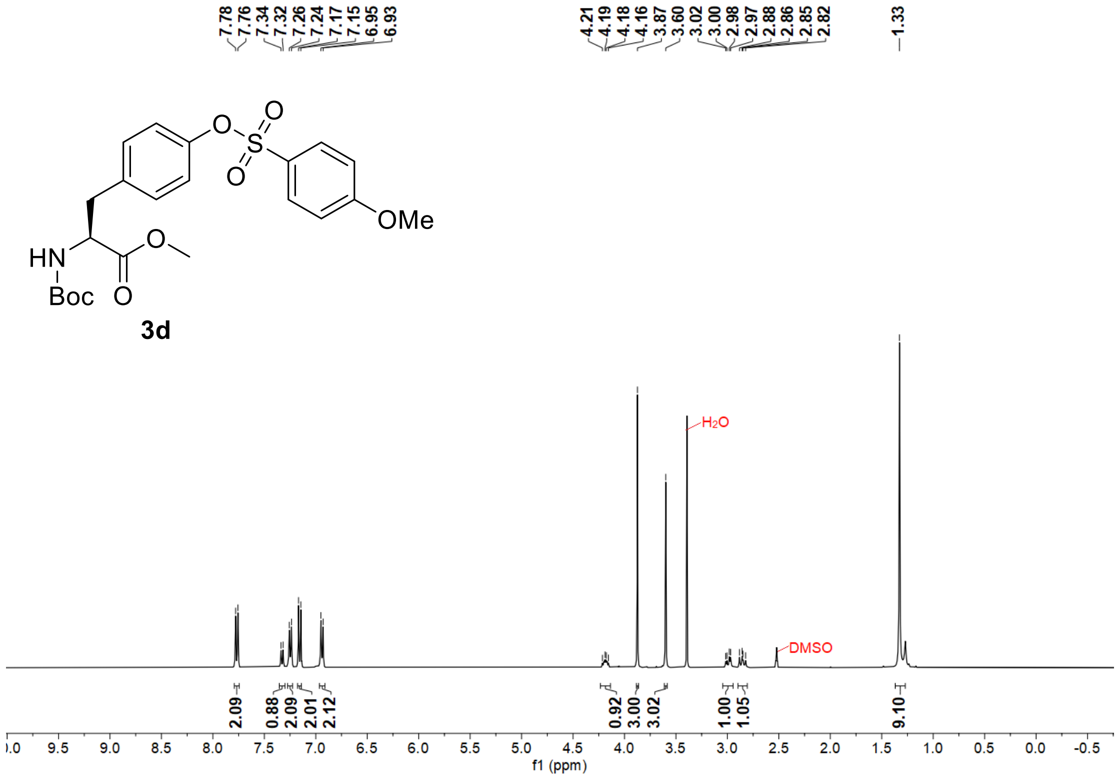

NOESY Spectrum
400 MHz, DMSO-d6
f2 (ppm)
f1 (ppm)

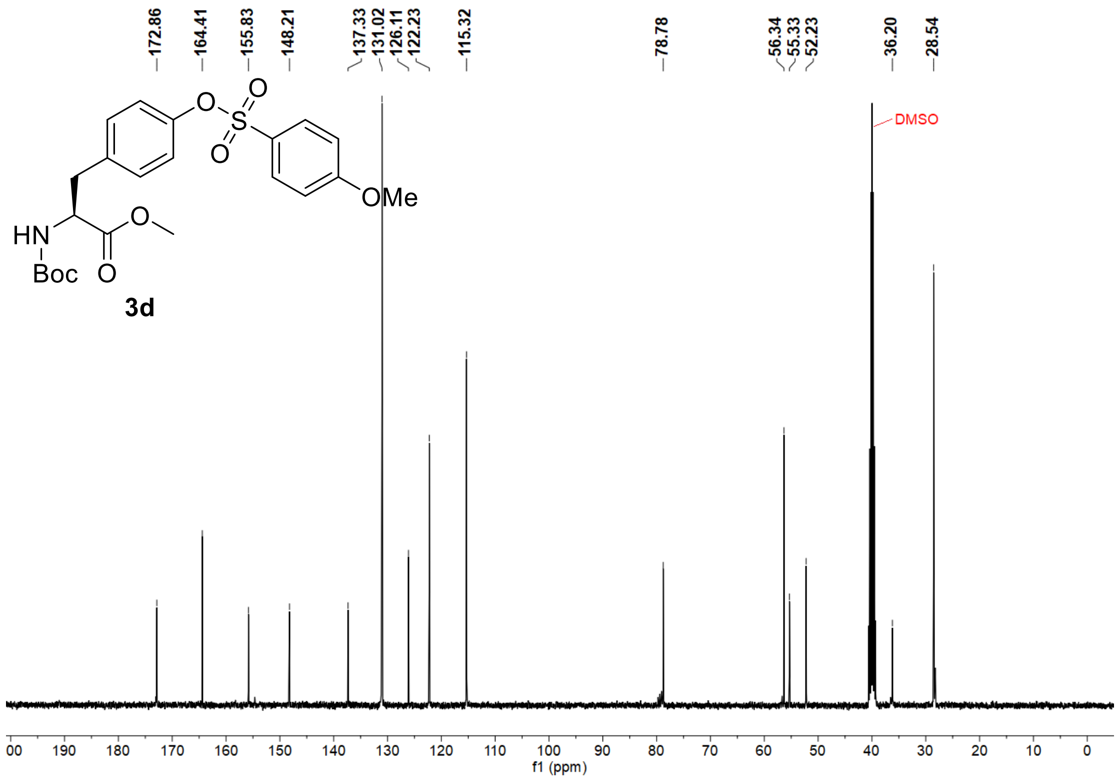

7.72
7.70
7.46
7.44
7.31
7.29
7.23
7.21
6.93
6.91
4.18
4.17
4.14
4.12
3.57
2.99
2.97
2.95
2.94
2.85
2.82
2.82
2.79
2.41
1.31
H2O
DMSO
2.08
2.10
0.87
2.11
2.09
0.95
3.00
1.01
1.11
3.12
9.16
f1 (ppm)

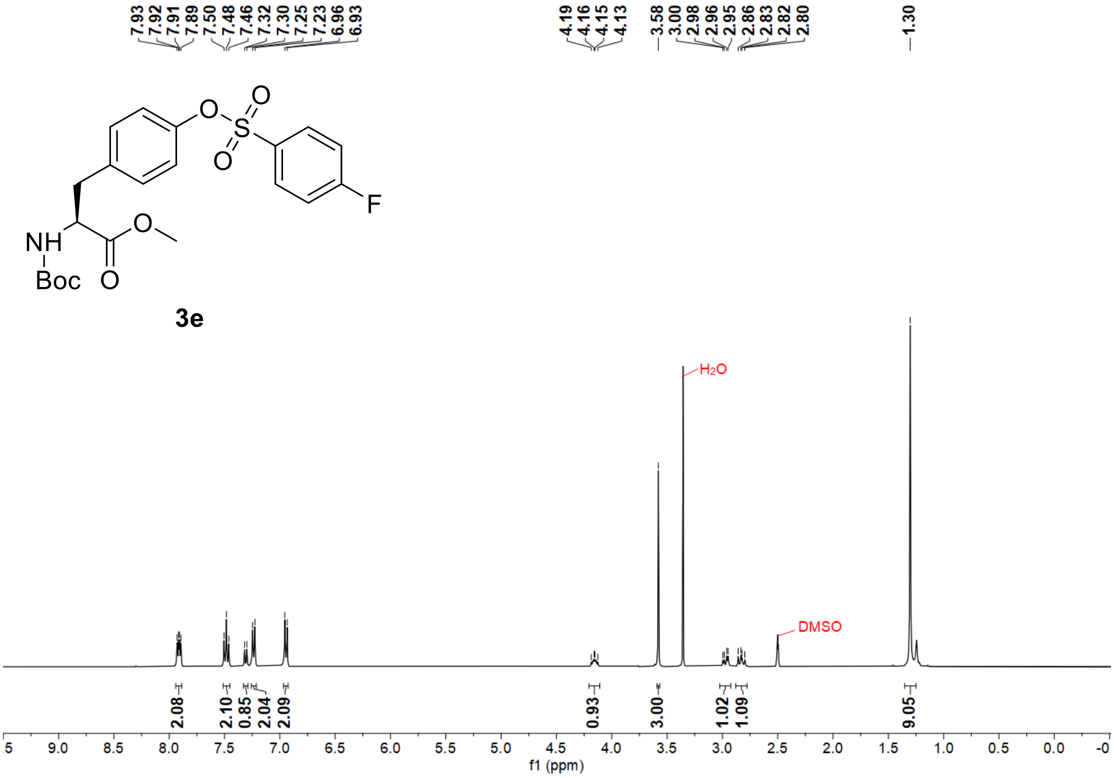

172.85
155.82
148.12
140.23
137.46
136.00
134.87
131.06
129.96
128.60
125.78
122.19
78.77
55.33
52.23
36.19
28.55
21.11
DMSO
f1 (ppm)

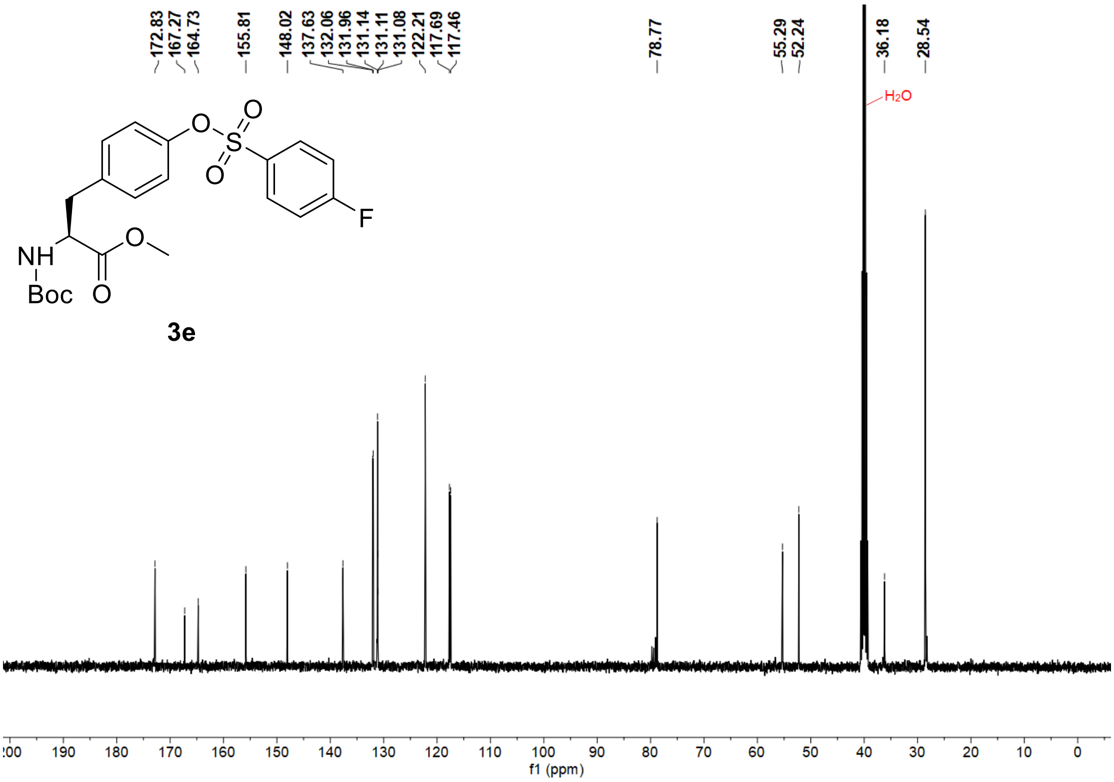

7.70 7.64 7.62 7.56 7.54 7.52 7.34 7.32 7.26 7.24 6.96 6.94
4.21 4.19 4.17 4.15
3.60 3.02 3.00 2.98 2.97 2.88 2.85 2.84 2.82 2.41
1.33
HN
Boc
3c
H2O
DMSO
1.10 2.02 1.01 0.94 2.03 2.05
0.93
3.00
0.99 1.07
3.04
8.99
f1 (ppm)

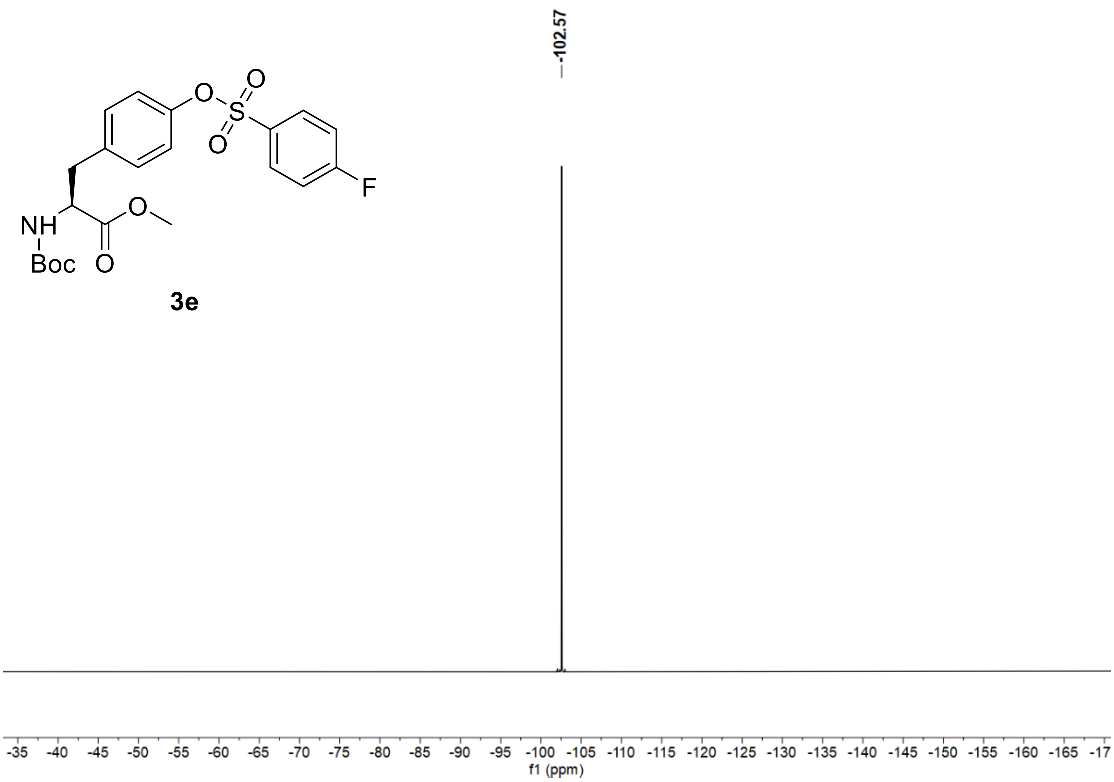

172.85
155.82
148.12 140.23 137.46 136.00 134.87 131.06 129.96 128.60 125.78 122.19
78.77
55.33 52.23
36.19
28.55
21.11
DMSO
HN
Boc
3c
f1 (ppm)



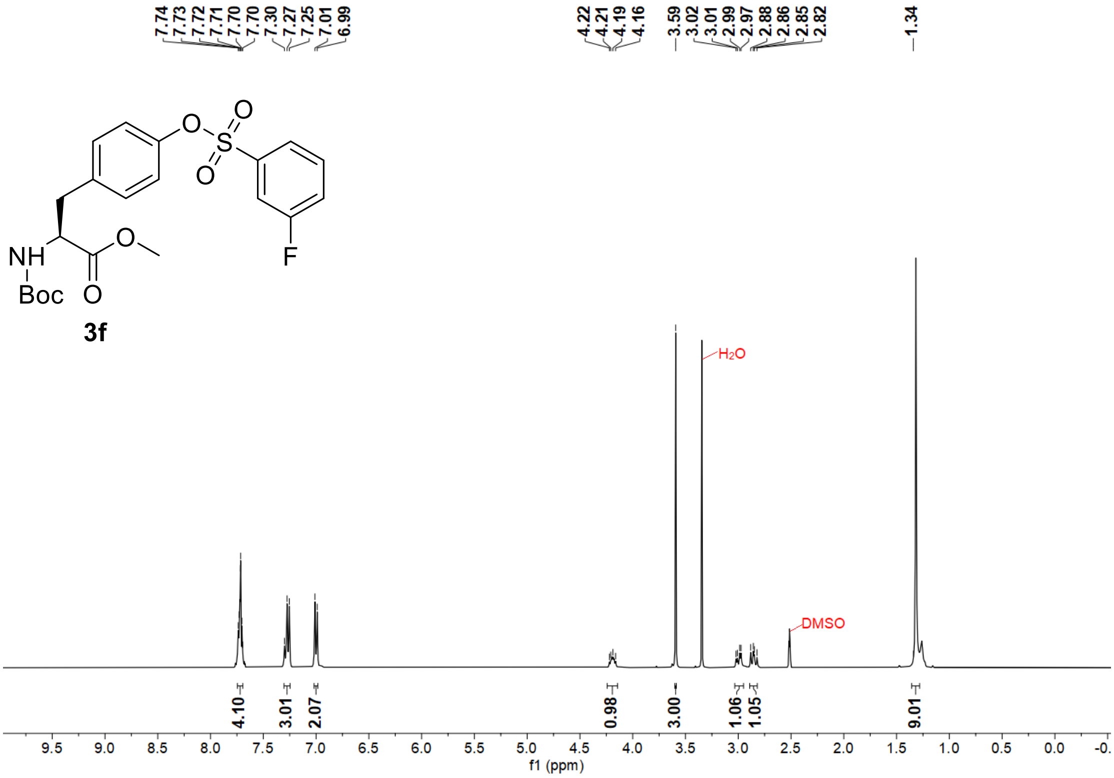


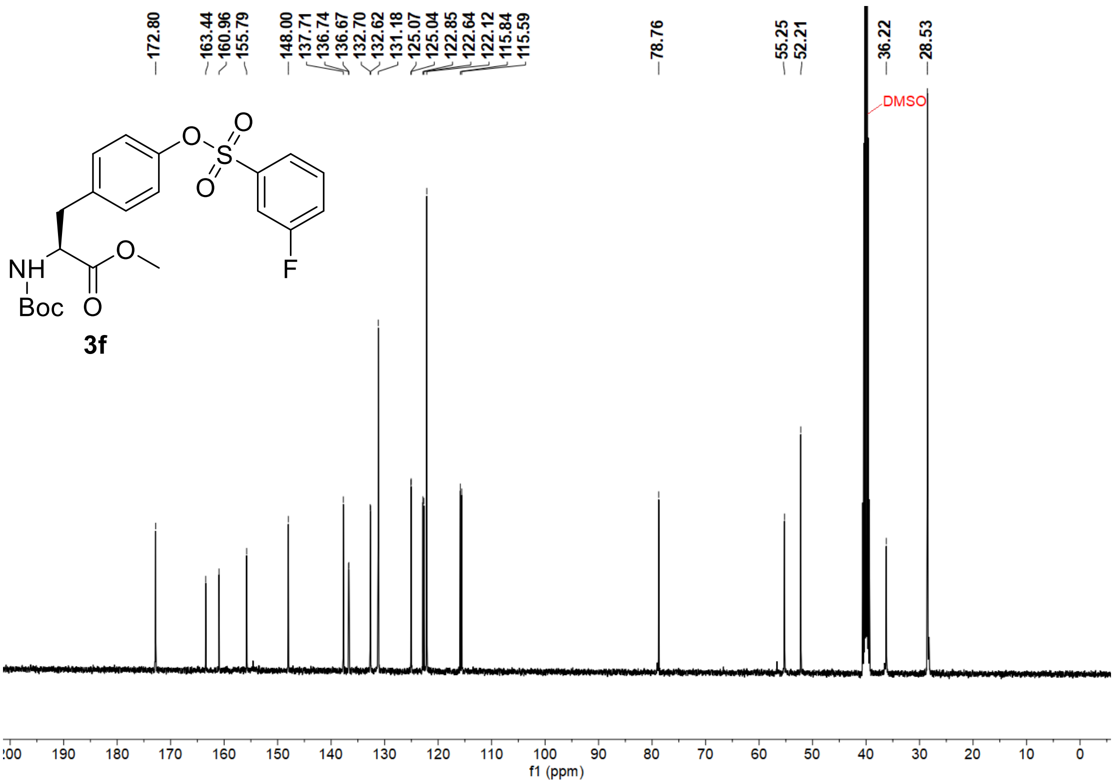

7.93 7.92 7.91 7.89 7.50 7.48 7.46 7.32 7.30 7.25 7.23 6.96 6.93
4.19 4.16 4.15 4.13
3.58 3.00 2.98 2.96 2.95 2.86 2.83 2.82 2.80
1.30
3e
H2O
DMSO
2.08 2.10 0.85 2.04 2.09 0.93 3.00 1.02 1.09 9.05
f1 (ppm)

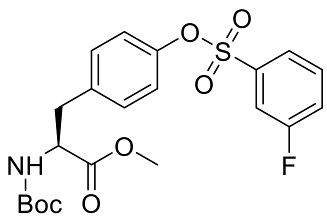

172.83 167.27 164.73 155.81 148.02 137.63 132.06 131.96 131.14 131.11 131.08 122.21 117.69 117.46
78.77
55.29 52.24
36.18 28.54
3e
H2O
f1 (ppm)



-102.57
NH
Boc
O
F
3e
-35 -40 -45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95 -100 -105 -110 -115 -120 -125 -130 -135 -140 -145 -150 -155 -160 -165 -17
f1 (ppm)

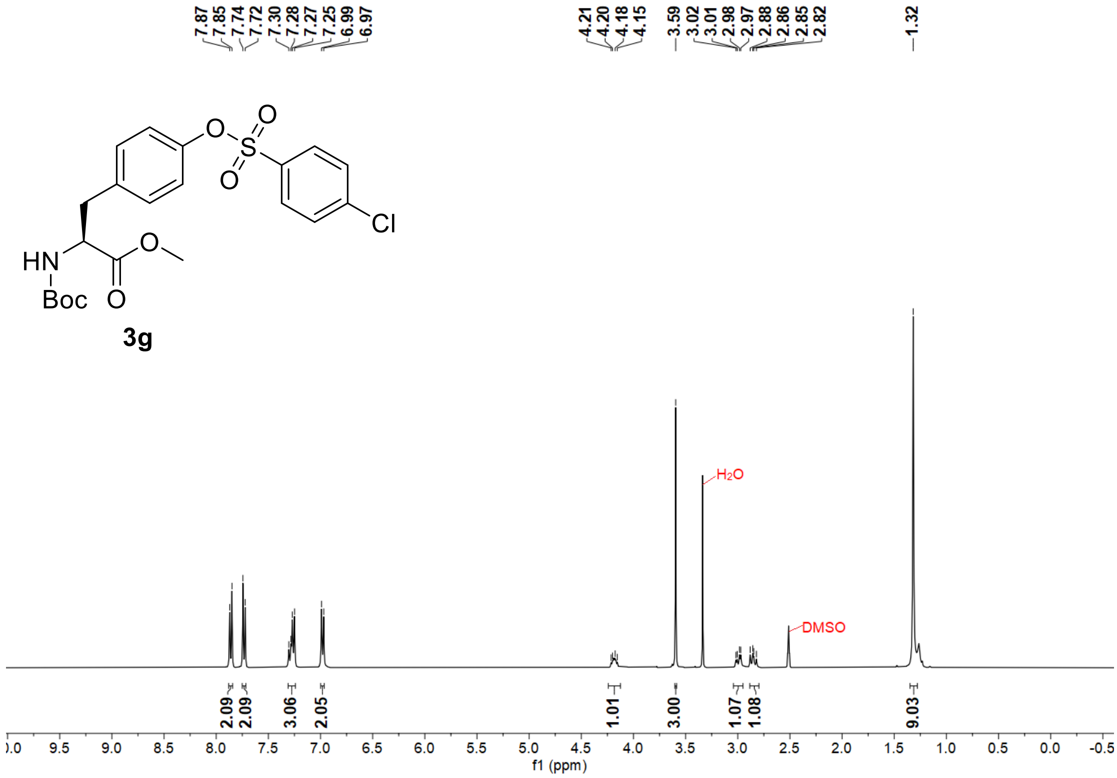


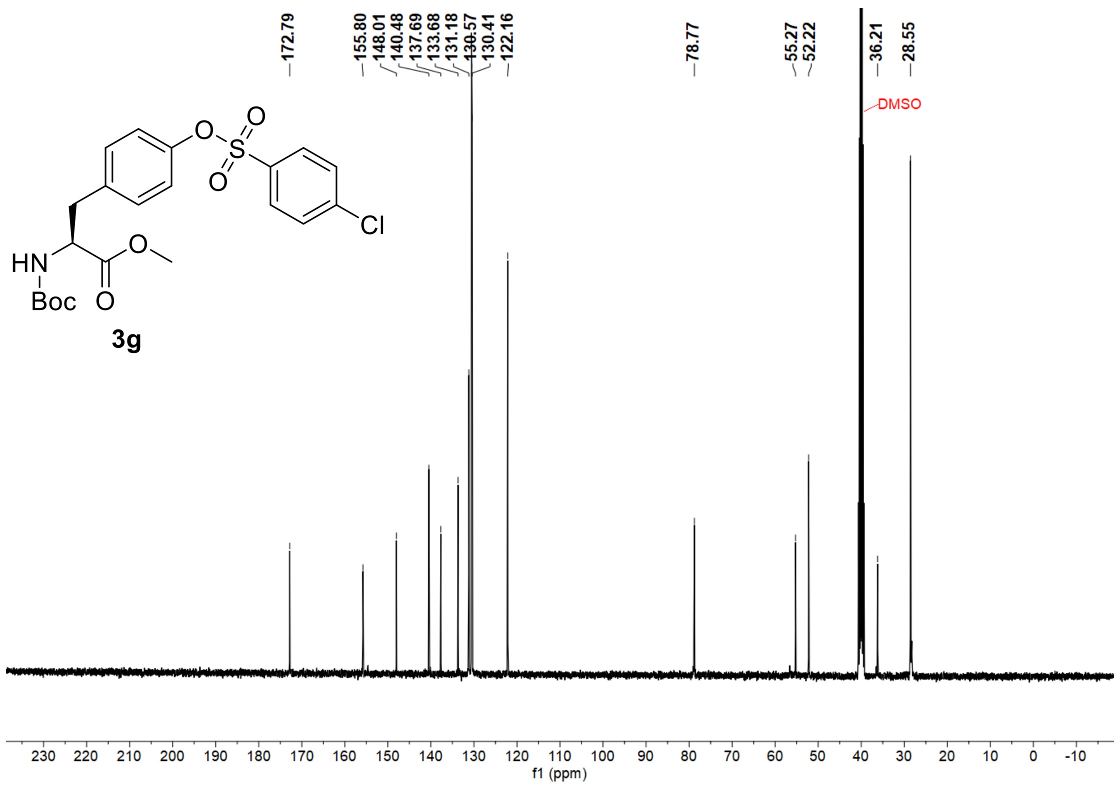


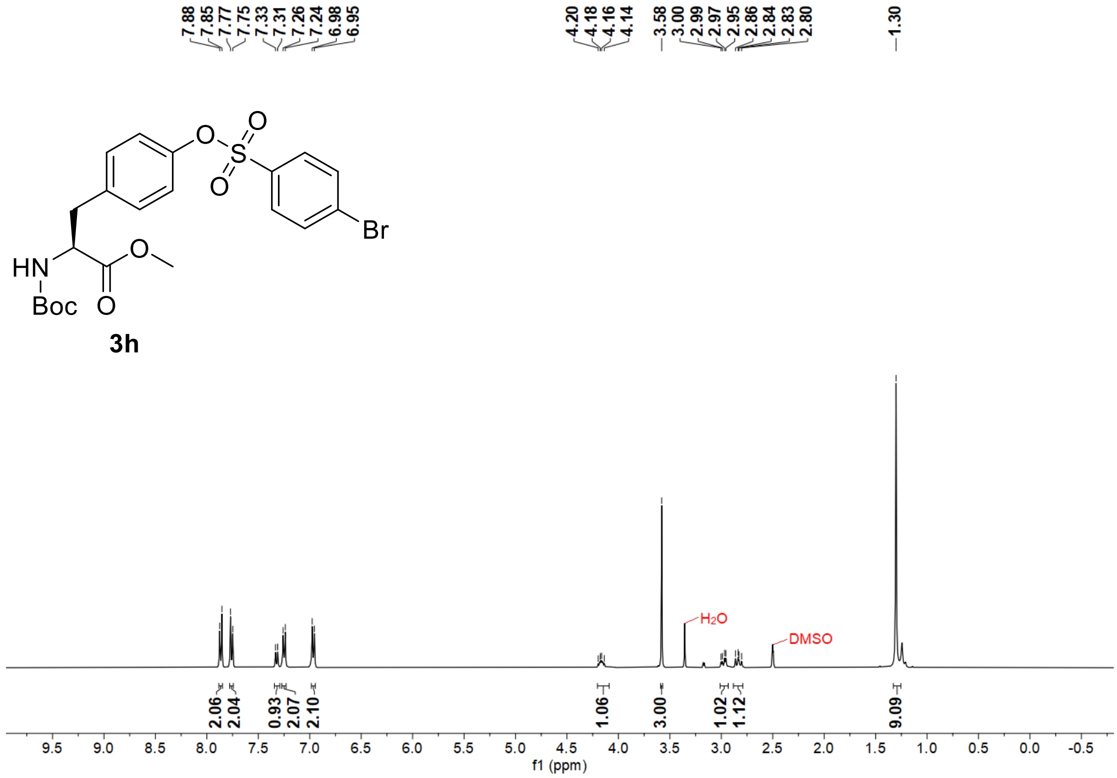

O
S
O
O
NH
Boc
O
O
F

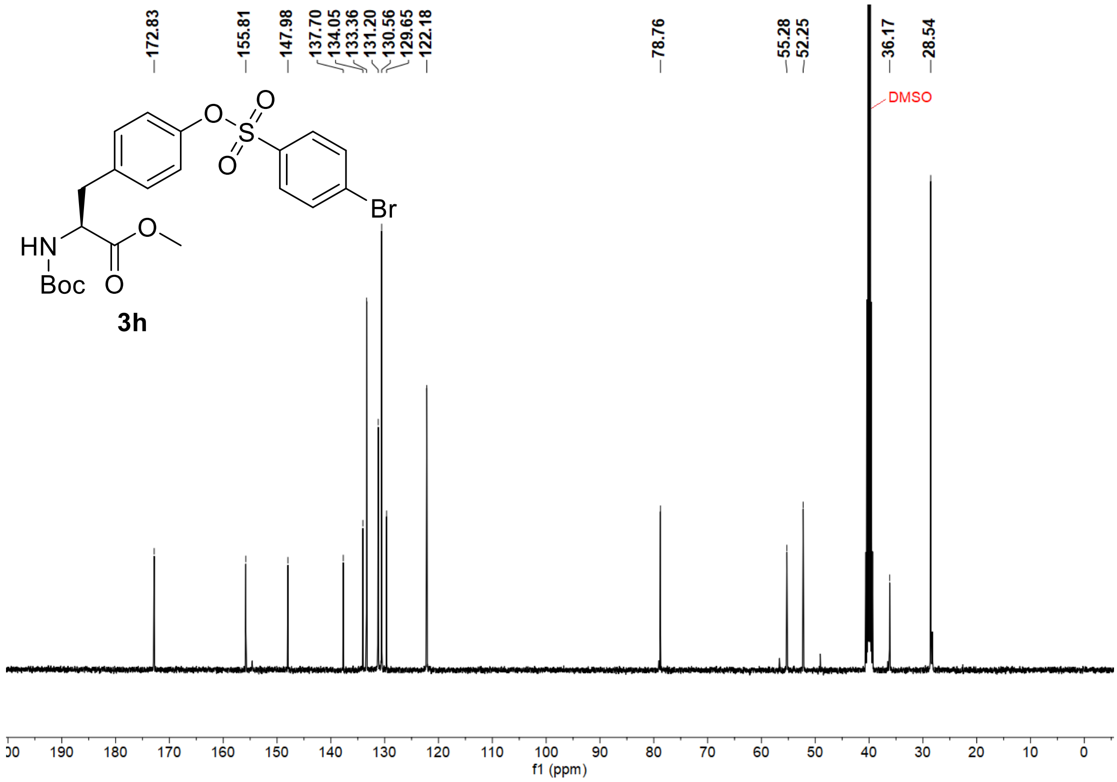
3f

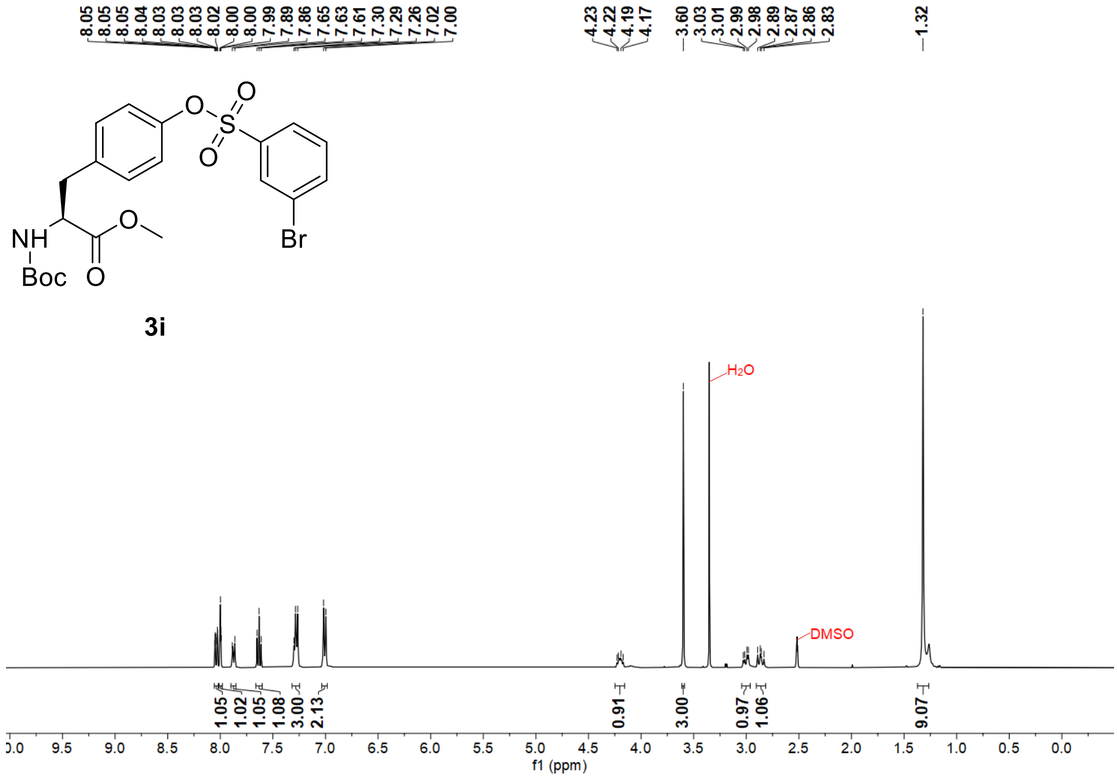

-57.04
5 0 -5 -10 -15 -20 -25 -30 -35 -40 -45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95 -100 -105 -110
f1 (ppm)


7.87
7.85
7.74
7.72
7.30
7.28
7.27
7.25
6.99
6.97
4.21
4.20
4.18
4.15
3.59
3.02
3.01
2.98
2.97
2.88
2.86
2.85
2.82
1.32
HN
Boc
O
S
Cl
3g
H2O
DMSO
2.09
2.09
3.06
2.05
1.01
3.00
1.07
1.08
9.03
f1 (ppm)

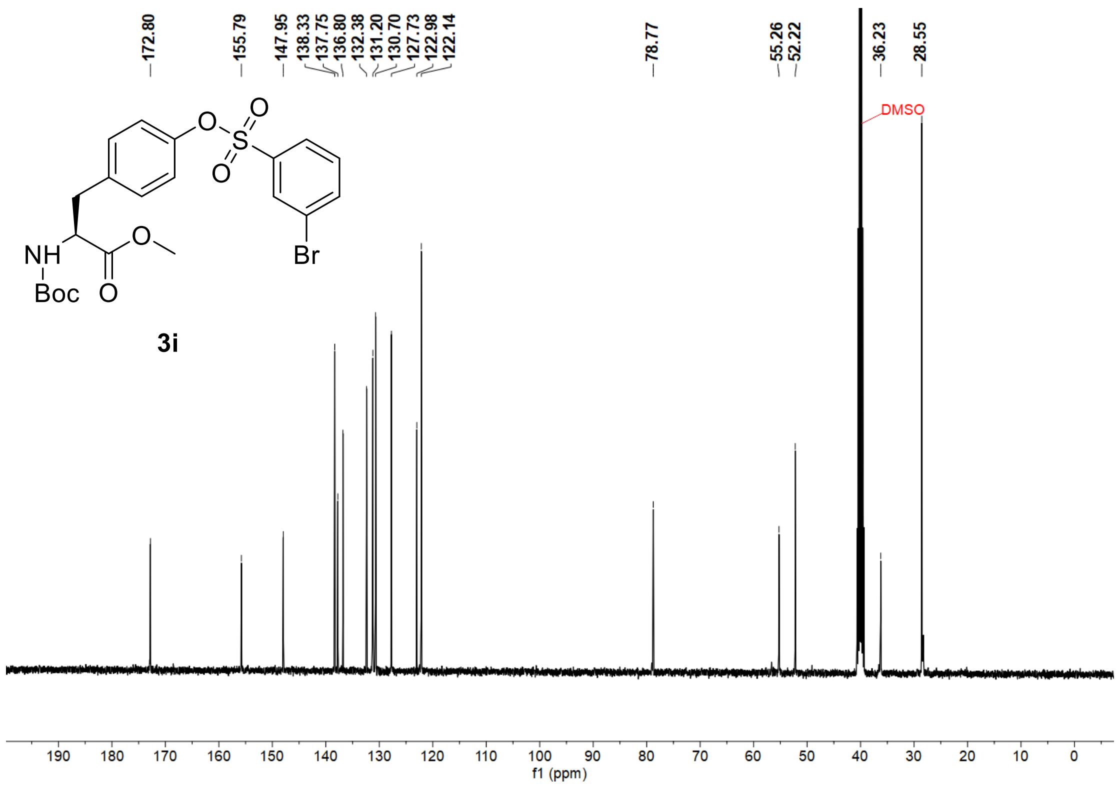

172.79
155.80
148.01
140.48
137.69
133.68
131.18
130.57
130.41
122.16
78.77
55.27
52.22
36.21
28.55
HN
Boc
O
S
Cl
3g
DMSO
f1 (ppm)


7.88
7.85
7.77
7.75
7.33
7.31
7.26
7.24
6.98
6.95
4.20
4.18
4.16
4.14
3.58
3.00
2.99
2.97
2.95
2.86
2.84
2.83
2.80
1.30
Br
HN
Boc
3h
H2O
DMSO
2.06
2.04
0.93
2.07
2.10
1.06
3.00
1.02
1.12
9.09
f1 (ppm)

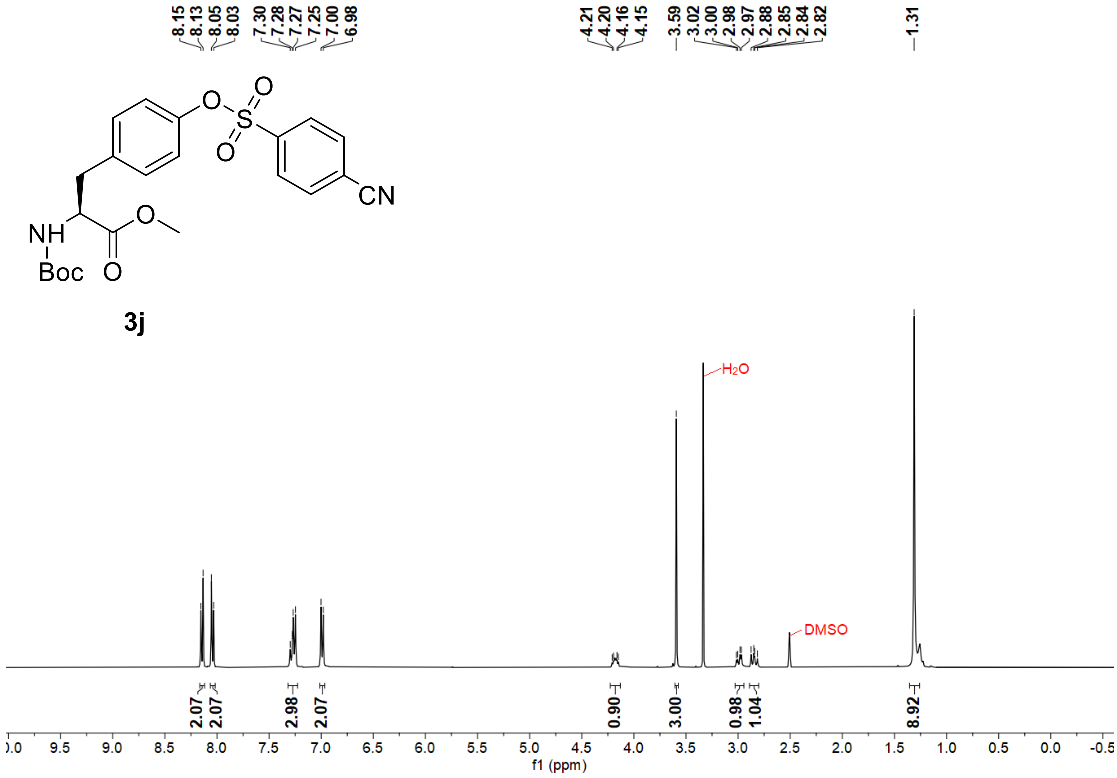

172.83
155.81
147.98
137.70
134.05
133.36
131.20
130.56
129.65
122.18
78.76
55.28
52.25
36.17
28.54
DMSO
Br
HN
Boc
3h
f1 (ppm)



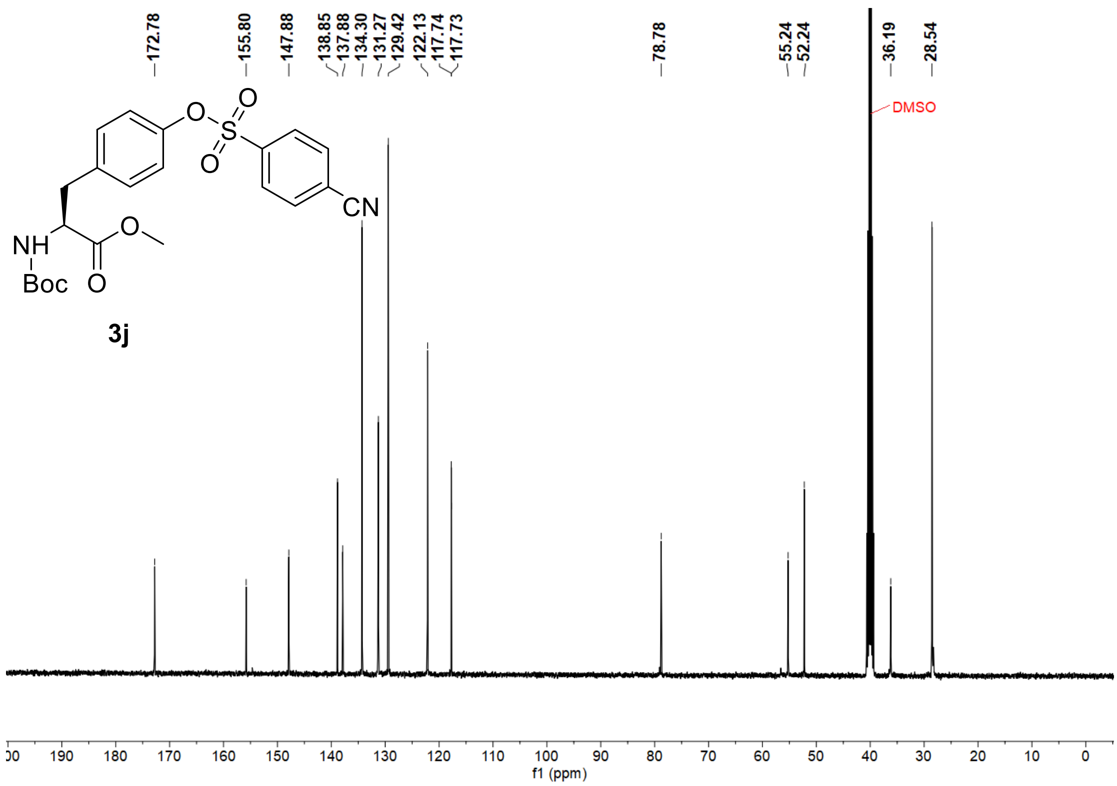
8.05
8.05
8.05
8.04
8.03
8.03
8.03
8.02
8.00
8.00
7.99
7.89
7.86
7.65
7.63
7.61
7.30
7.29
7.26
7.02
7.00
4.23
4.22
4.19
4.17
3.60
3.03
3.01
2.99
2.98
2.89
2.87
2.86
2.83
1.32
NH
Boc
O
O
O
S
O
O
Br
3i
H2O
DMSO
1.05
1.02
1.05
1.08
3.00
2.13
0.91
3.00
0.97
1.06
9.07
f1 (ppm)



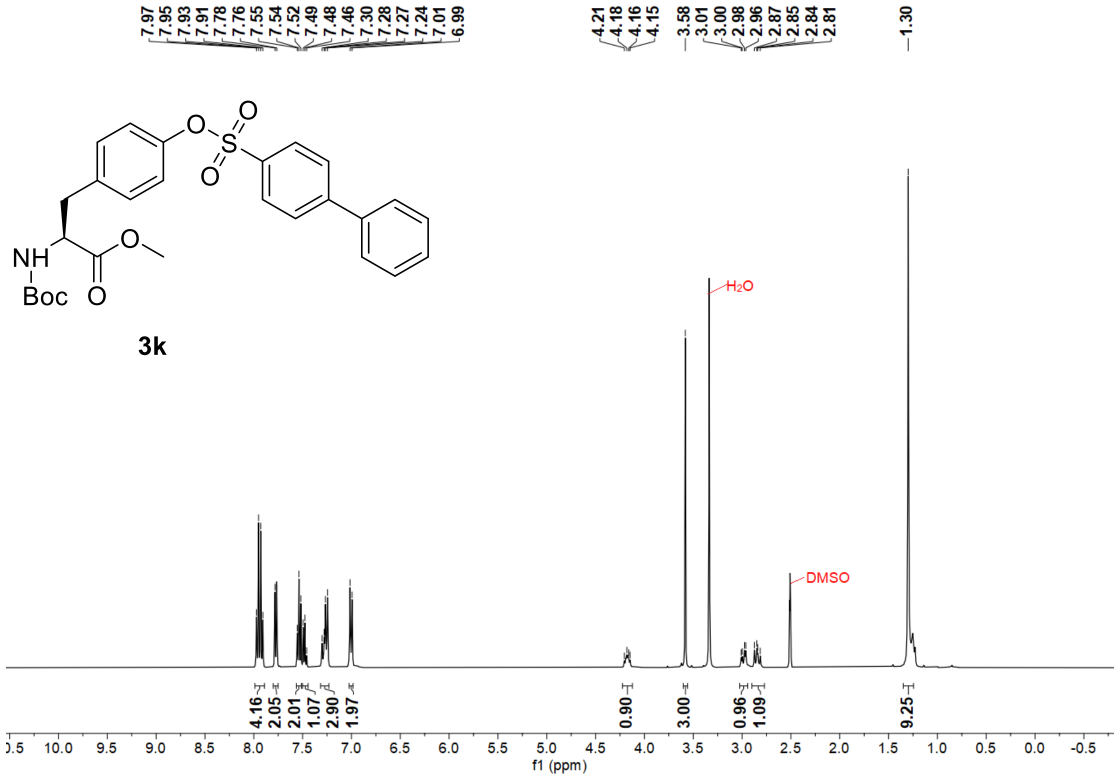
172.80
155.79
147.95
138.33
137.75
136.80
132.38
131.20
130.70
127.73
122.98
122.14
78.77
55.26
52.22
36.23
28.55
NH
Boc
O
O
O
S
O
O
Br
3i
DMSO
f1 (ppm)

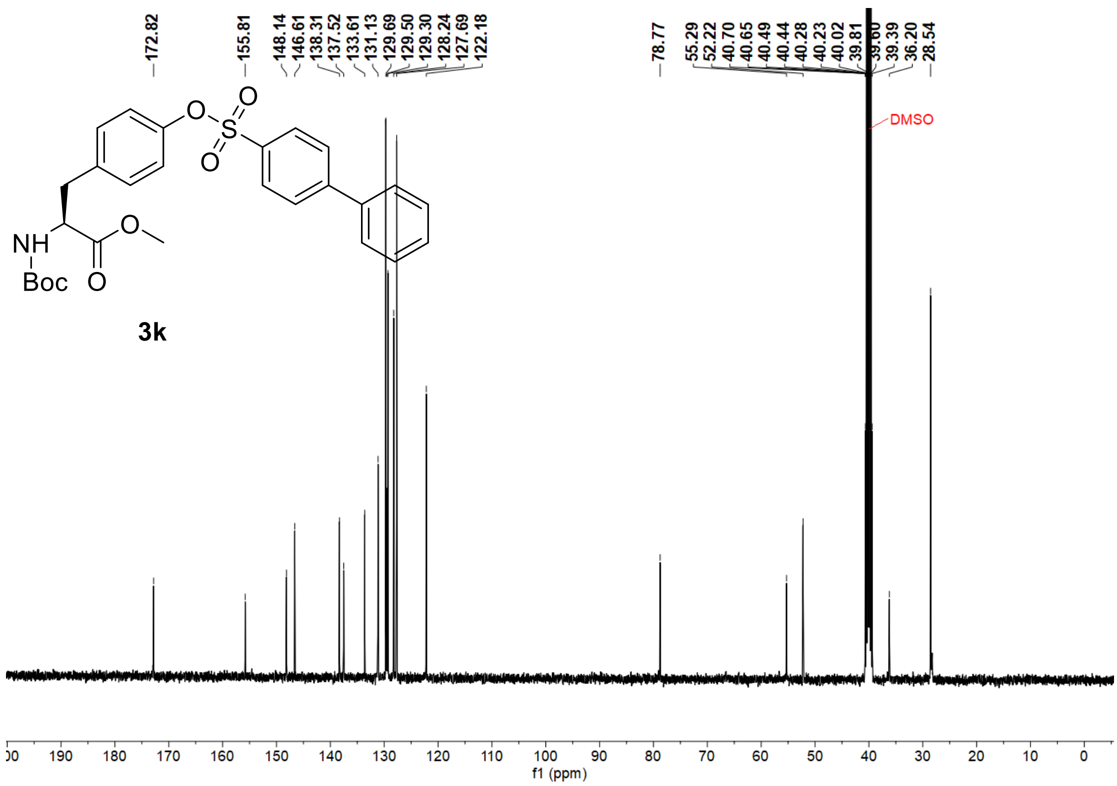


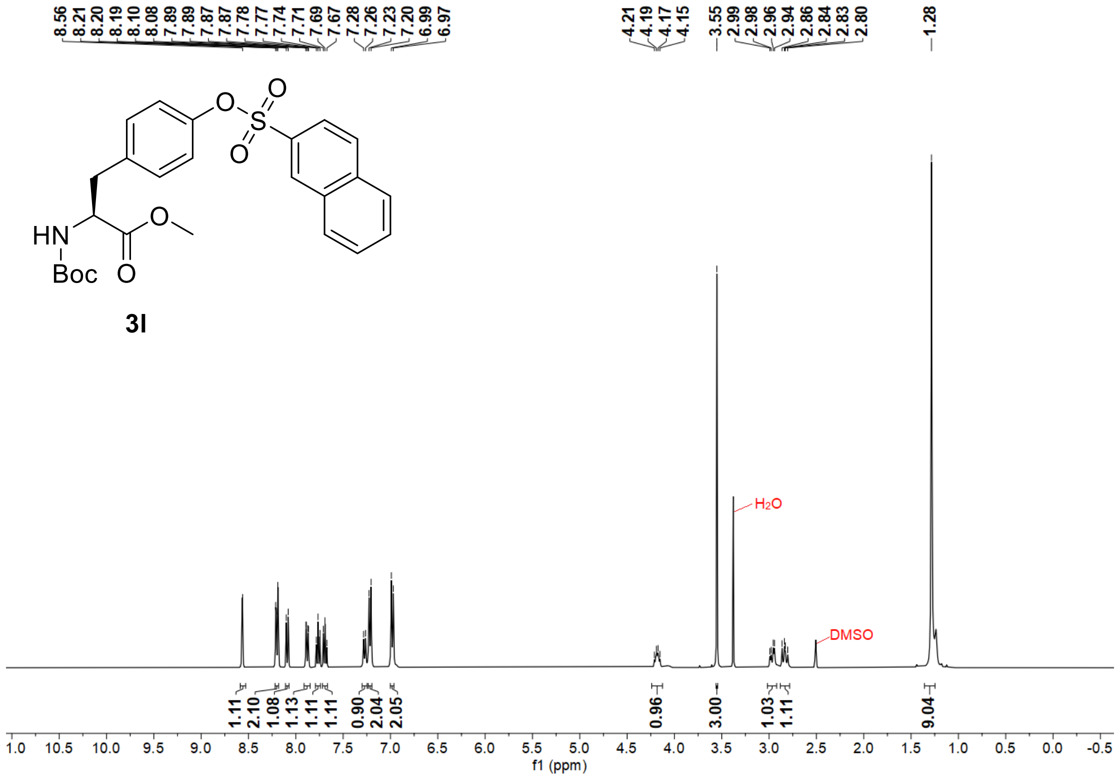
3j
H₂O
DMSO
f1 (ppm)

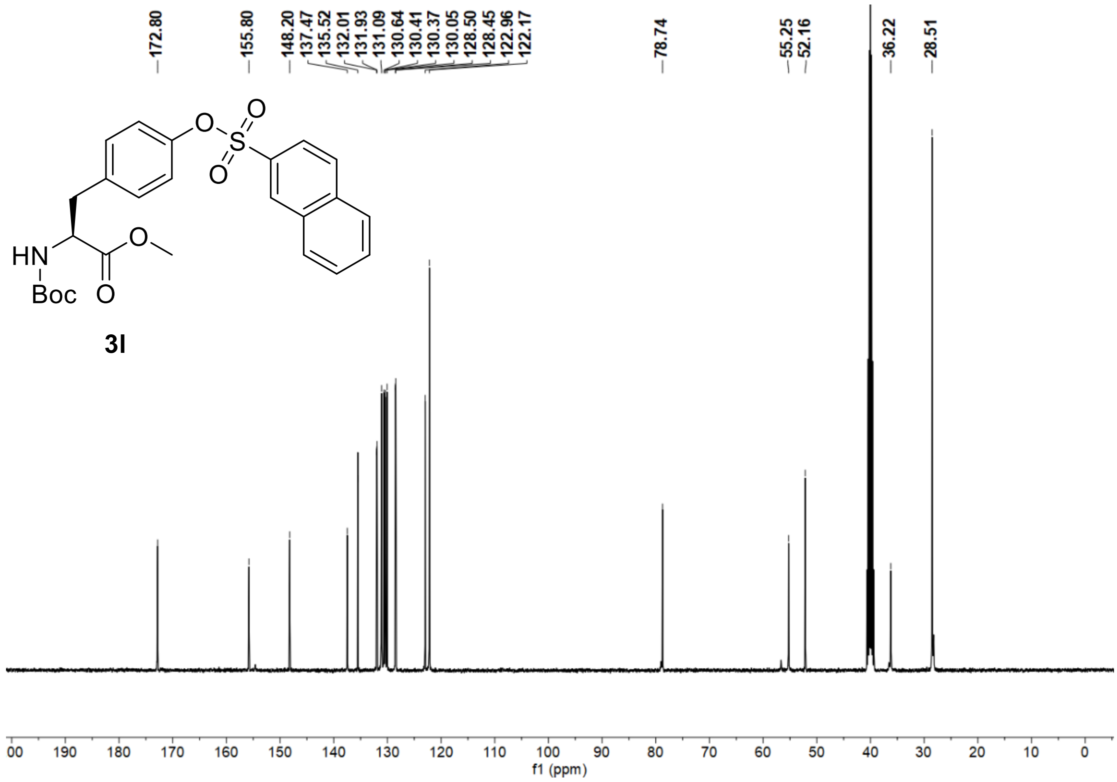


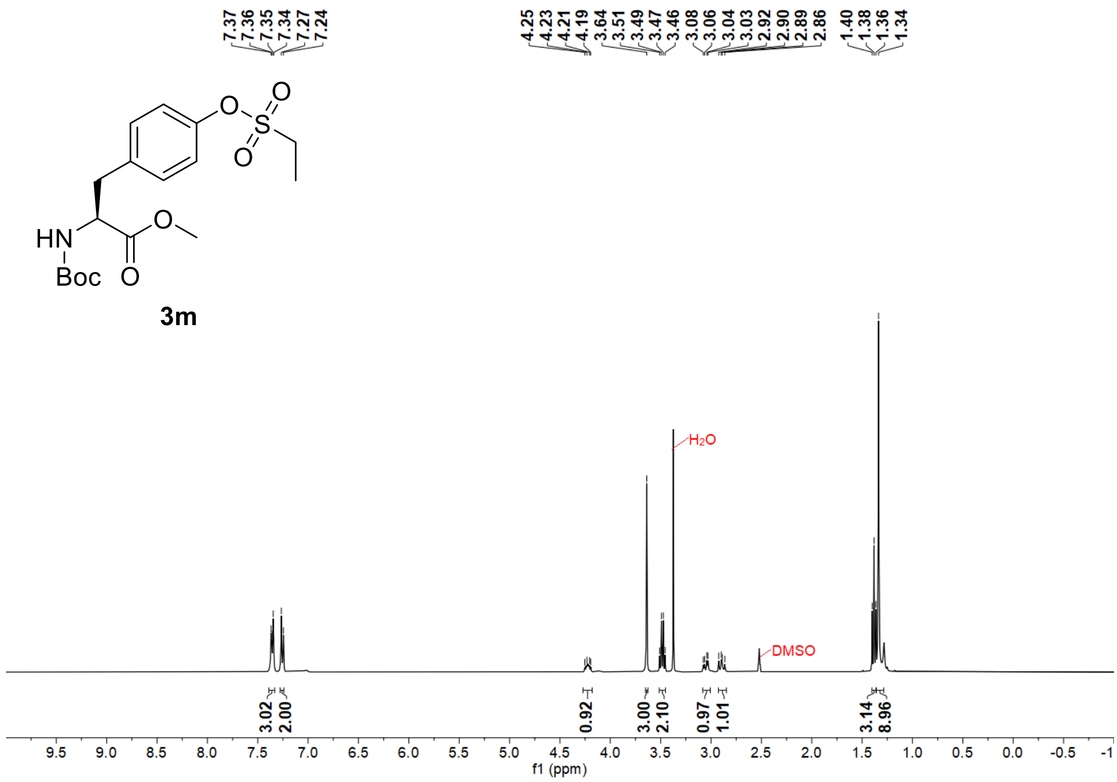
3j
DMSO
f1 (ppm)

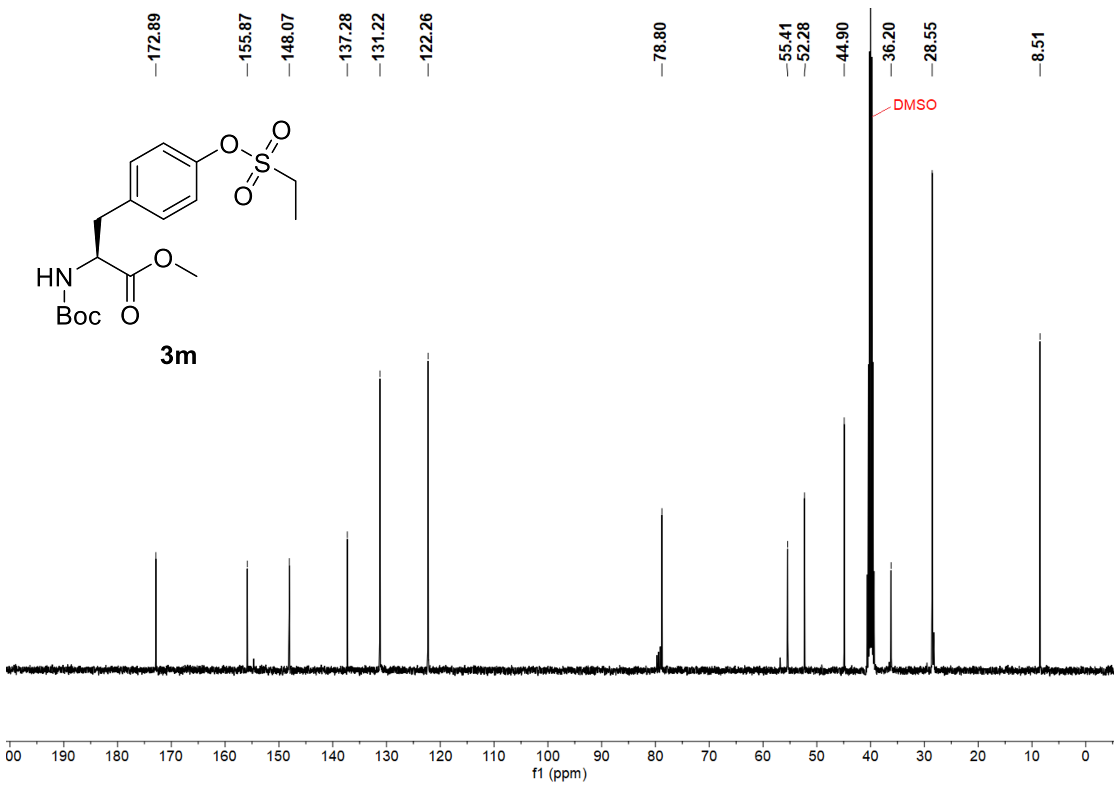


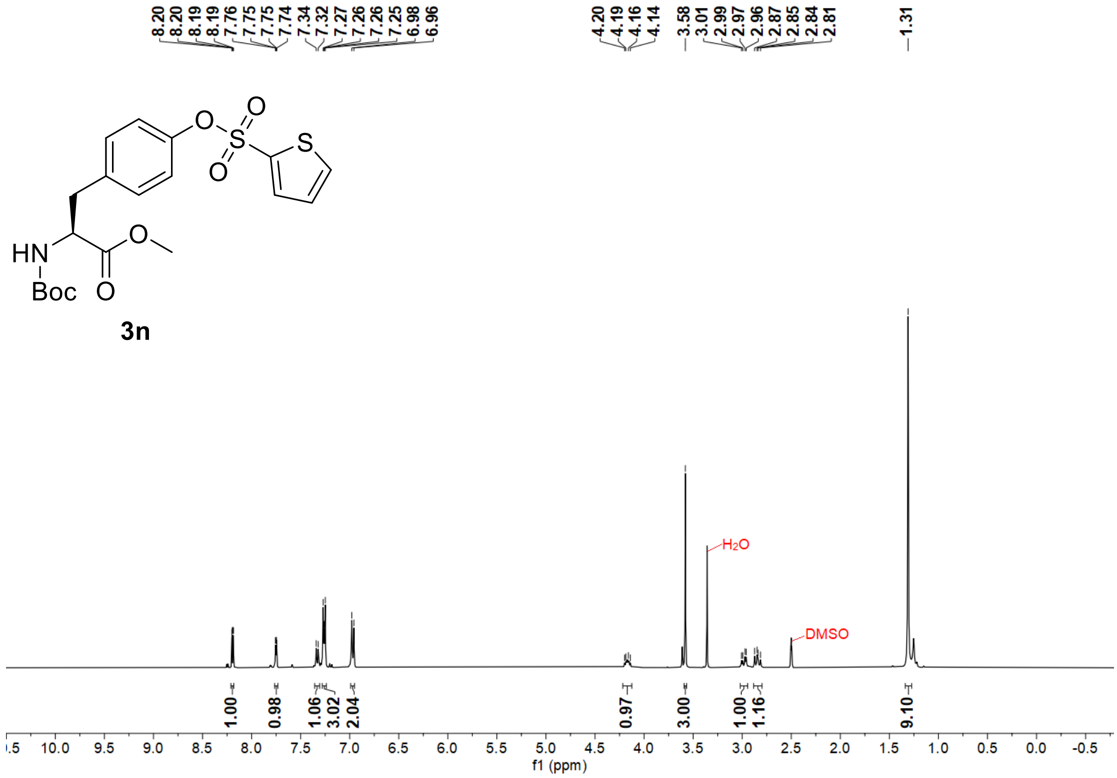



3l
H2O
DMSO
f1 (ppm)

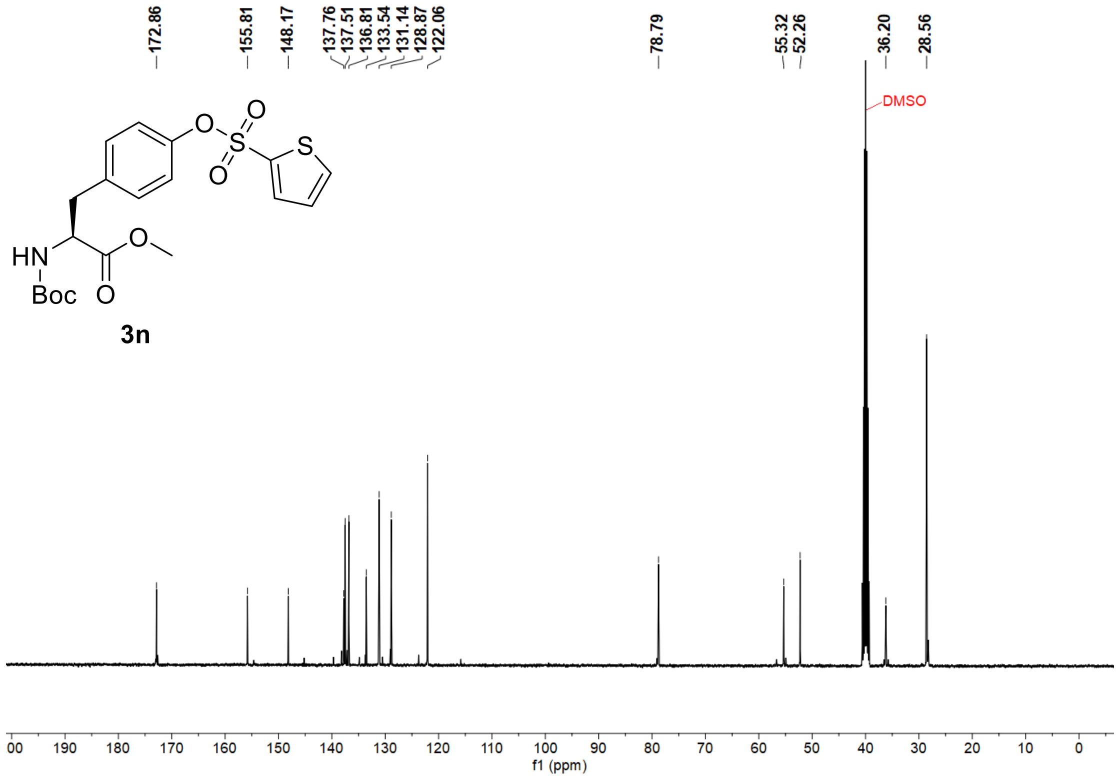

3l
f1 (ppm)


7.37
7.36
7.35
7.34
7.27
7.24
4.25
4.23
4.21
4.19
3.64
3.51
3.49
3.47
3.46
3.08
3.06
3.04
3.03
2.92
2.90
2.89
2.86
1.40
1.38
1.36
1.34
3m
H2O
DMSO
3.02
2.00
0.92
3.00
2.10
0.97
1.01
3.14
8.96
f1 (ppm)

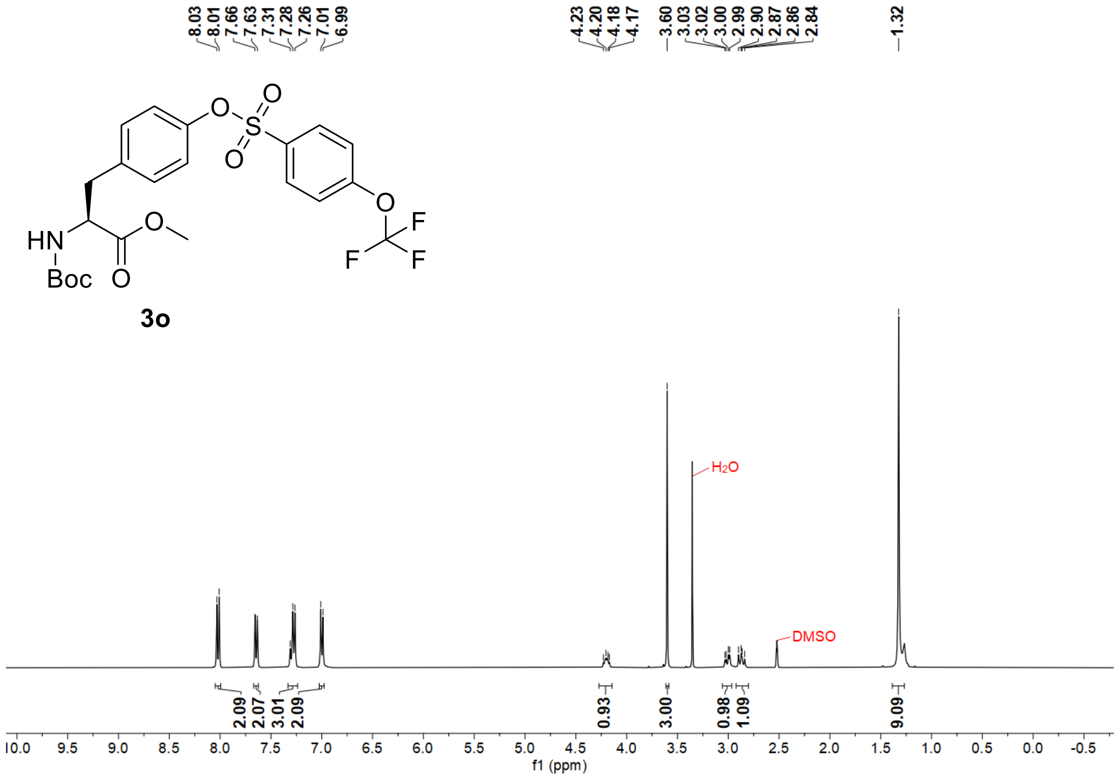

172.89
155.87
148.07
137.28
131.22
122.26
78.80
55.41
52.28
44.90
36.20
28.55
8.51
3m
DMSO
f1 (ppm)

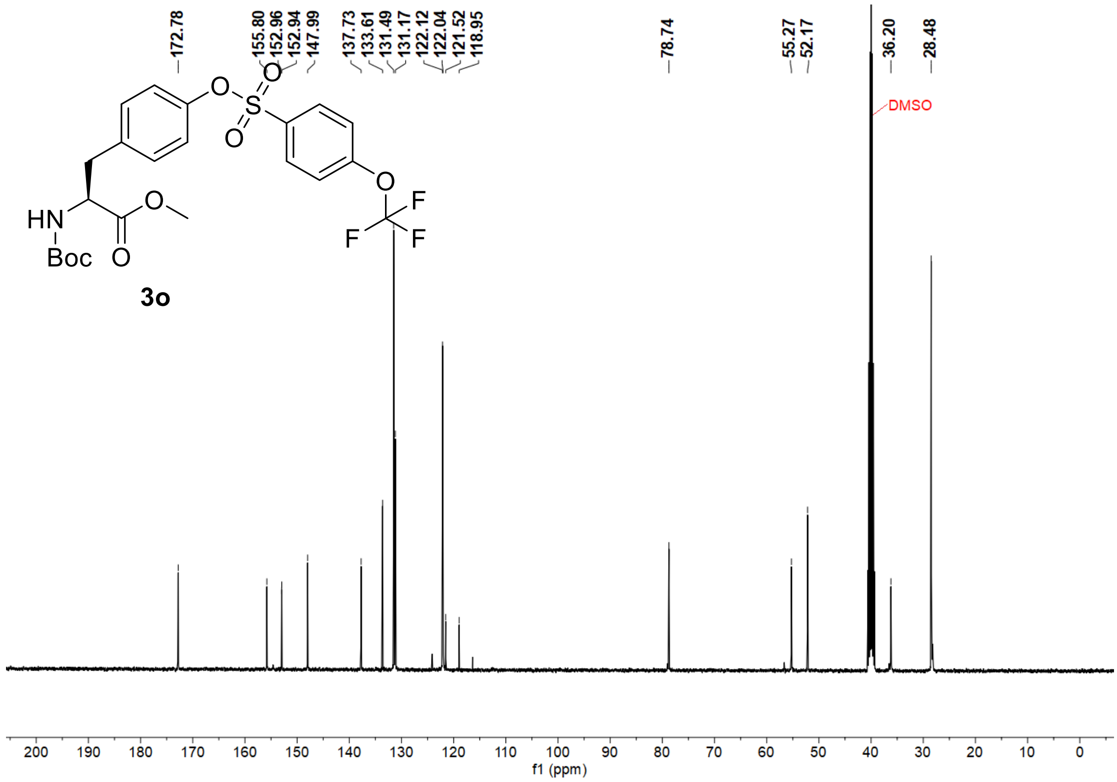


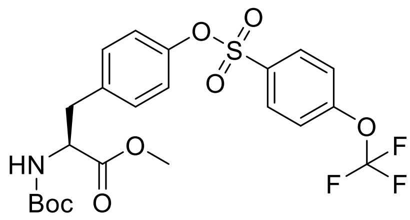
3n



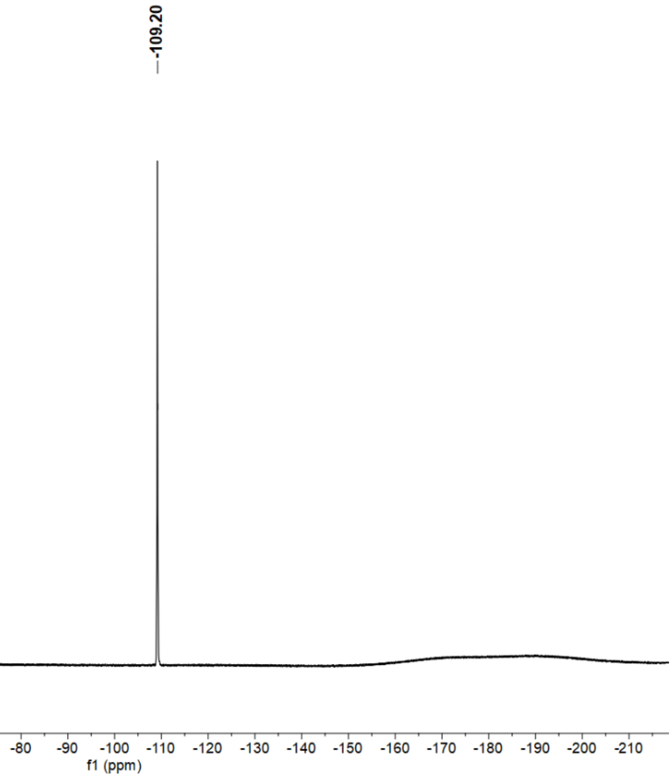
3n


8.03
8.01
7.66
7.63
7.31
7.28
7.26
7.01
6.99
4.23
4.20
4.18
4.17
3.60
3.03
3.02
3.00
2.99
2.90
2.87
2.86
2.84
1.32
HN
Boc
3o
H2O
DMSO
2.09
2.07
3.01
2.09
0.93
3.00
0.98
1.09
9.09
f1 (ppm)

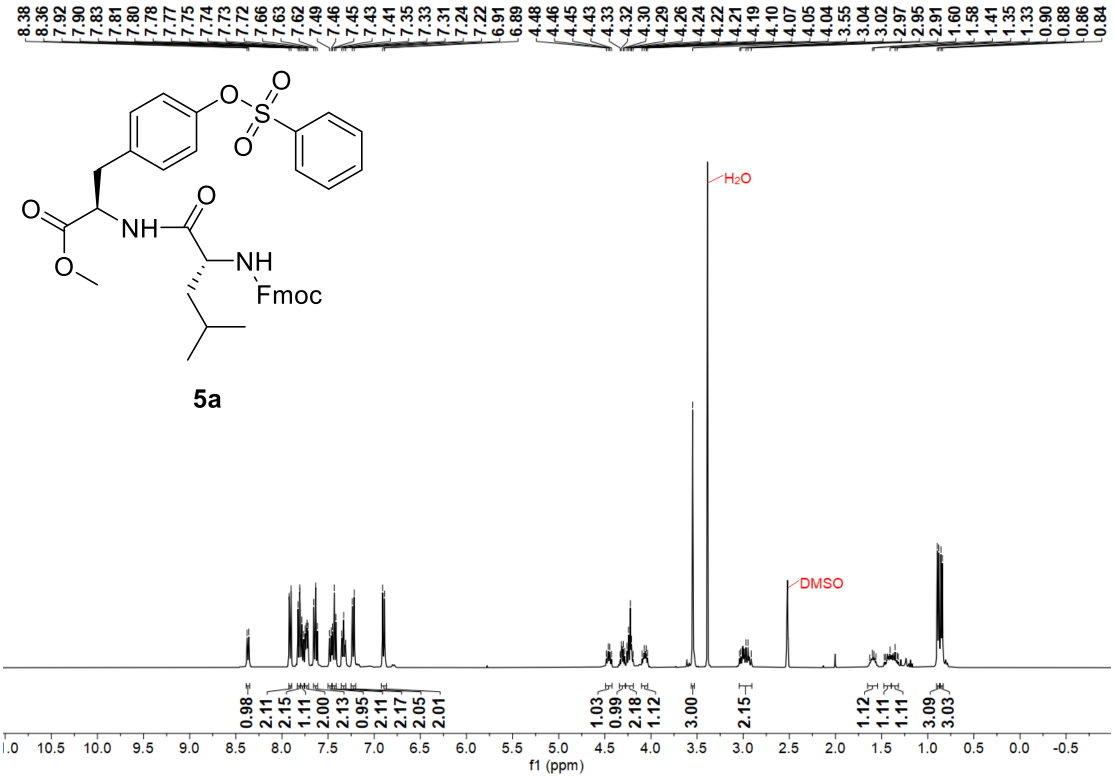

172.78
155.80
152.96
152.94
147.99
137.73
133.61
131.49
131.17
122.12
122.04
121.52
118.95
78.74
55.27
52.17
36.20
28.48
DMSO
HN
Boc
3o
f1 (ppm)

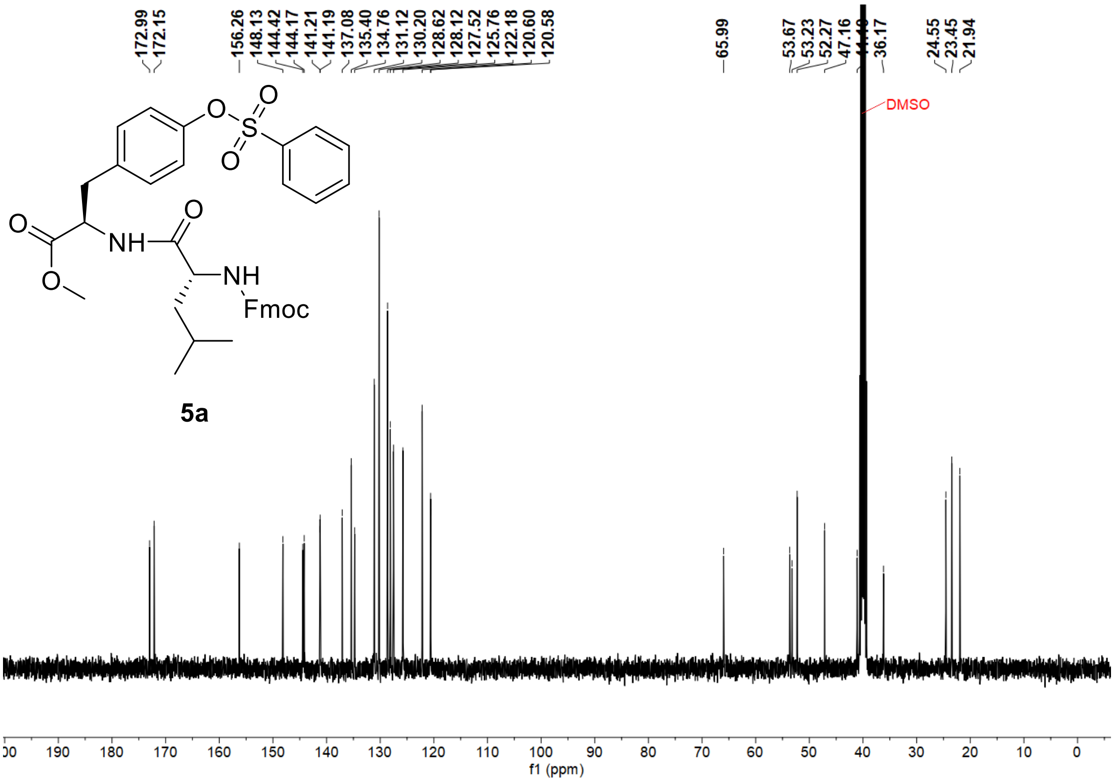


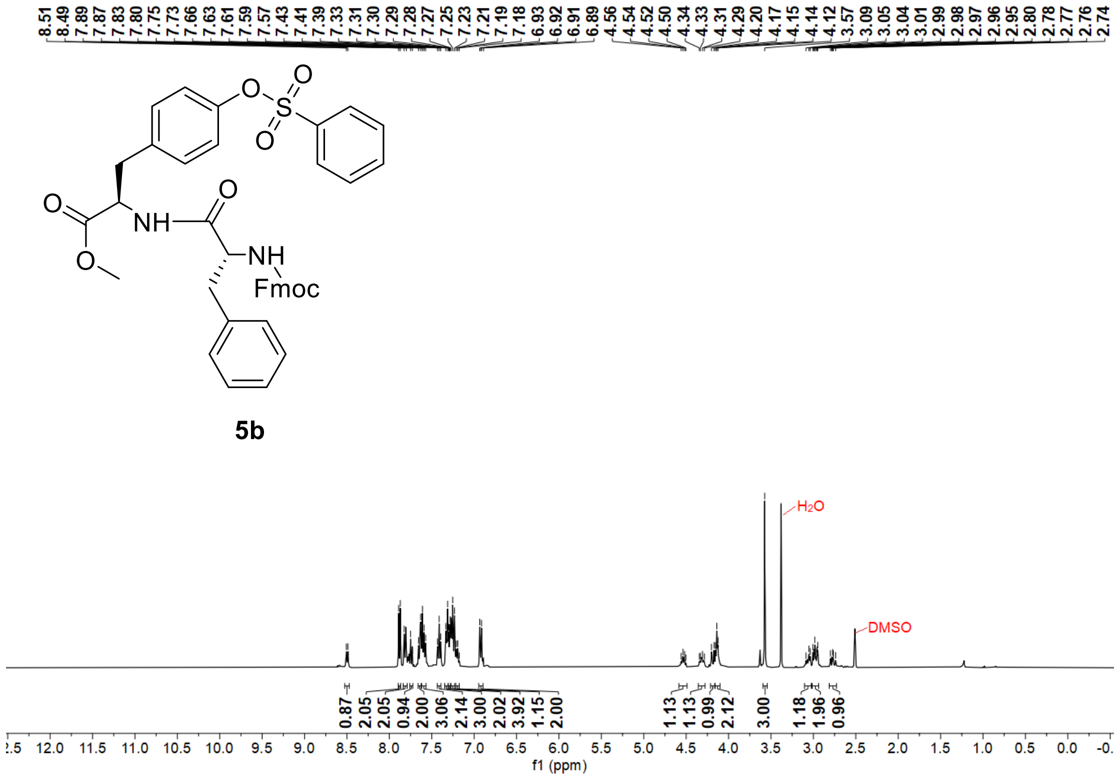
3o

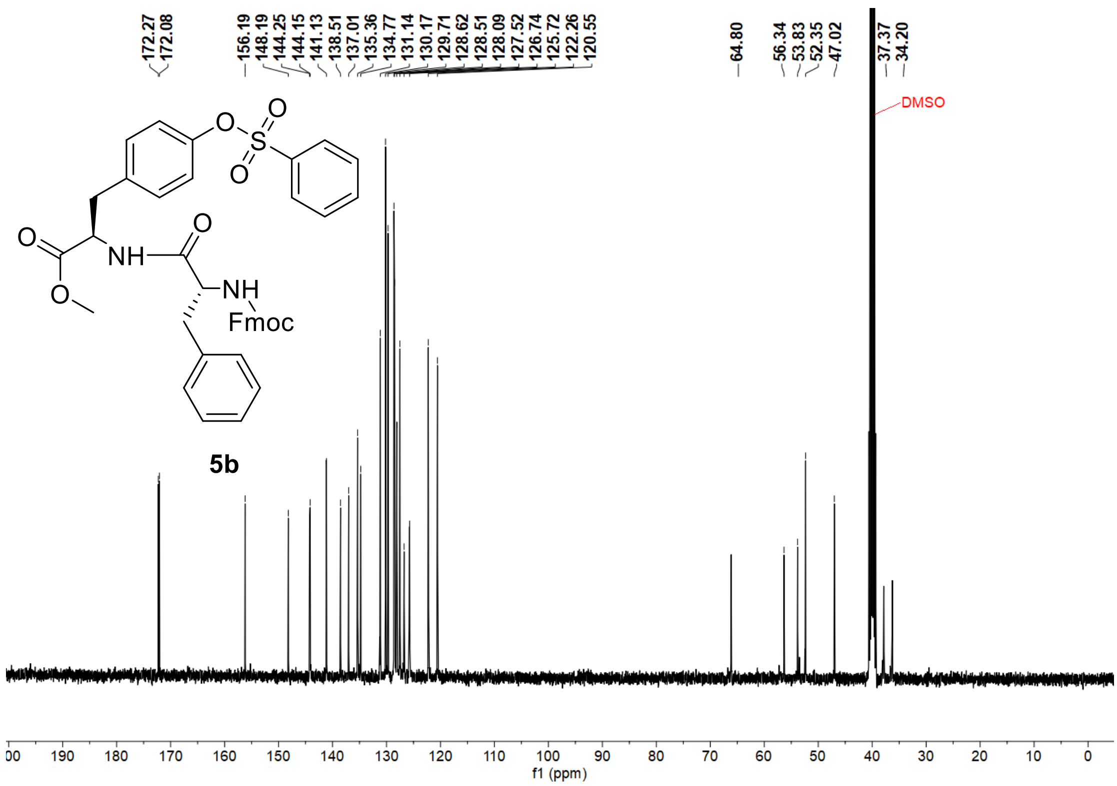


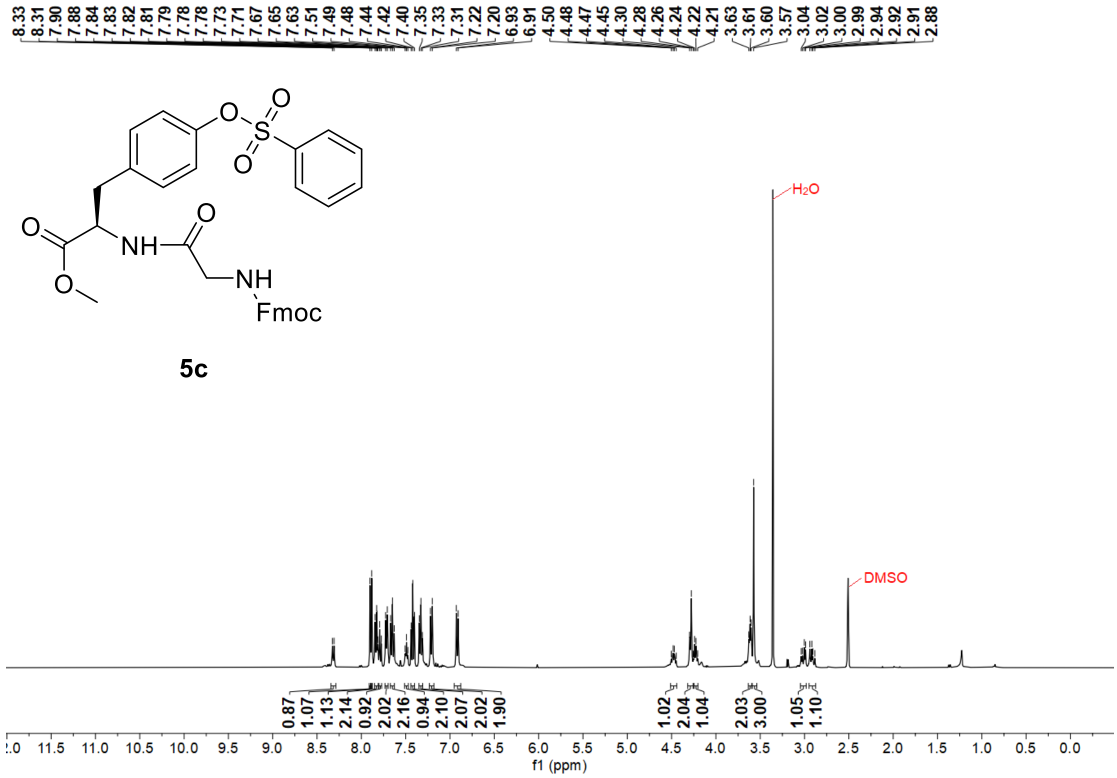
-109.20
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
f1 (ppm)

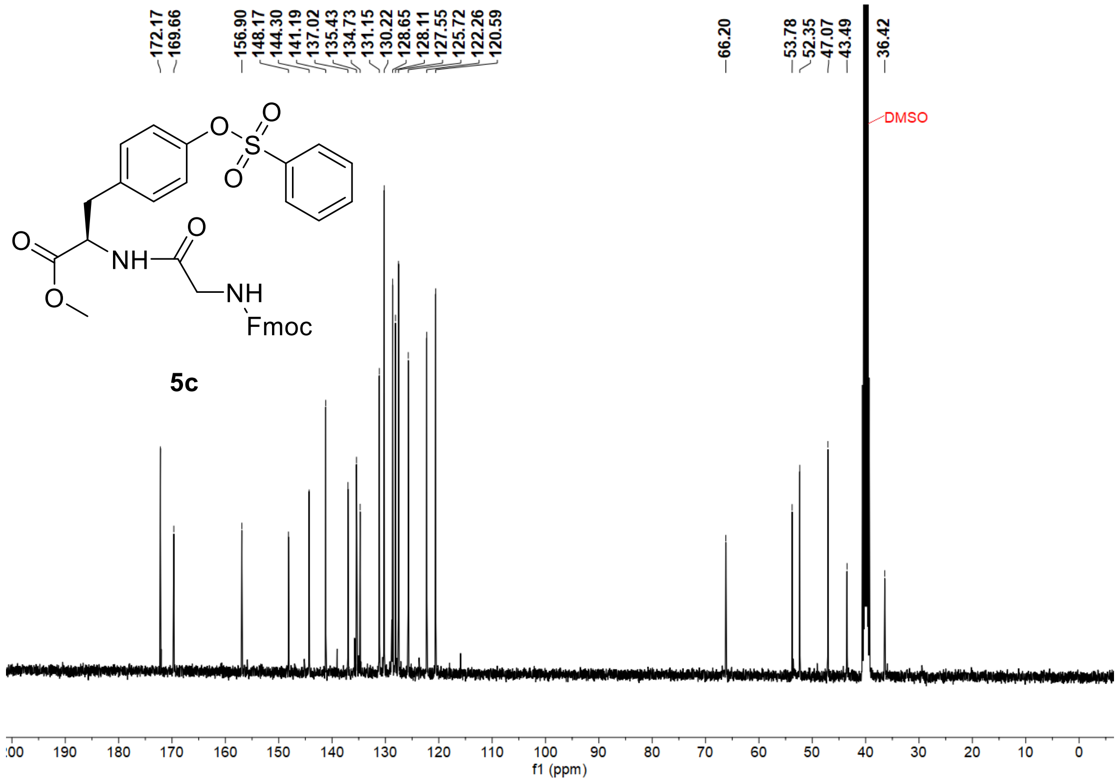

8.38 8.36 7.92 7.90 7.83 7.81 7.80 7.78 7.77 7.75 7.74 7.73 7.72 7.66 7.63 7.62 7.49 7.46 7.45 7.43 7.41 7.35 7.33 7.31 7.24 7.22 6.91 6.89 4.48 4.46 4.45 4.43 4.33 4.32 4.30 4.29 4.26 4.24 4.22 4.21 4.19 4.10 4.07 4.05 4.04 3.55 3.04 3.02 2.97 2.95 2.91 1.60 1.58 1.41 1.35 1.33 0.90 0.88 0.86 0.84
O
S
O
O
O
NH
O
O
NH
Fmoc
5a
H2O
DMSO
0.98 2.11 2.15 1.11 2.00 2.13 0.95 2.11 2.17 2.05 2.01 1.03 0.99 2.18 1.12 3.00 2.15 1.12 1.11 1.11 3.09 3.03
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

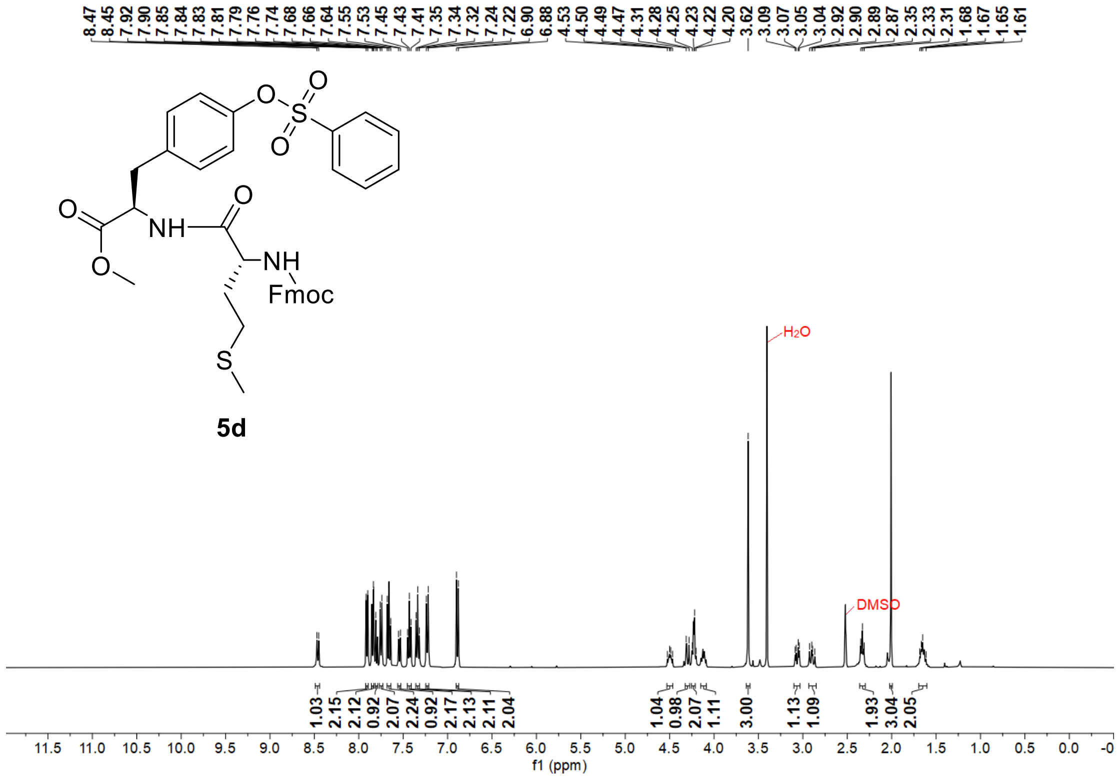

172.99 172.15 156.26 148.13 144.42 144.17 141.21 141.19 137.08 135.40 134.76 131.12 130.20 128.62 128.12 127.52 125.76 122.18 120.60 120.58 65.99 53.67 53.23 52.27 47.16 36.17 24.55 23.45 21.94
O
S
O
O
O
NH
O
O
NH
Fmoc
5a
DMSO
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

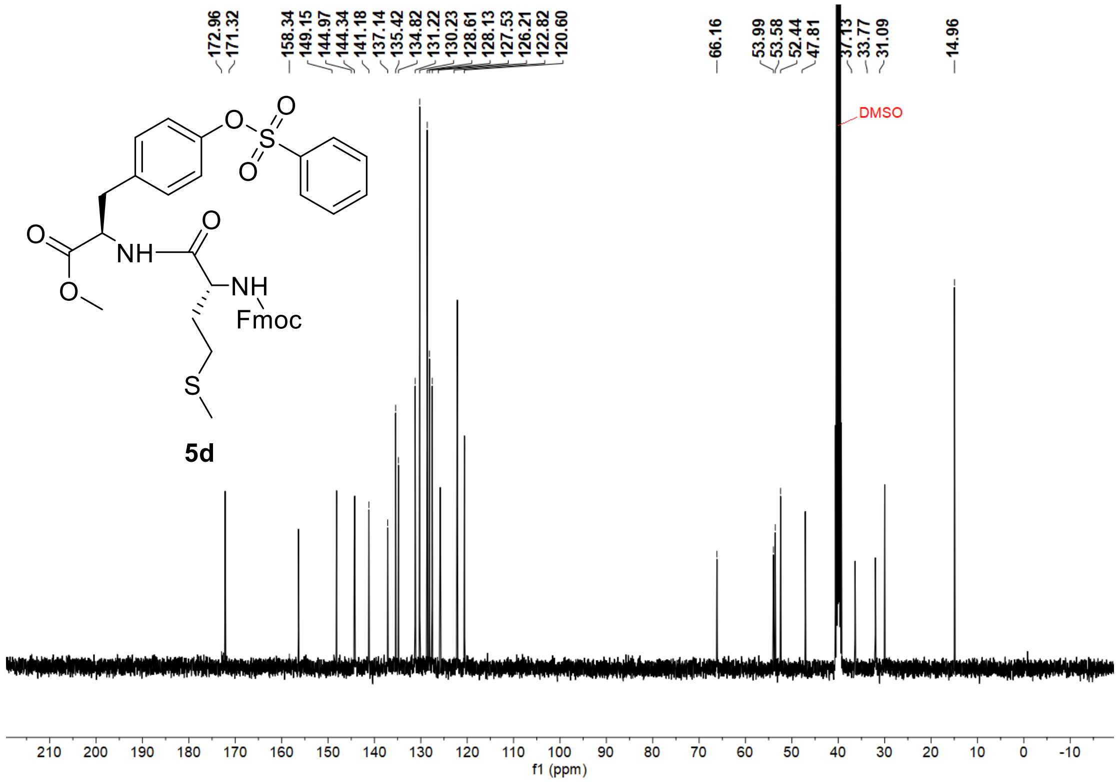

8.51 8.49 7.89 7.87 7.83 7.80 7.75 7.73 7.66 7.63 7.61 7.59 7.57 7.43 7.41 7.39 7.33 7.31 7.30 7.29 7.28 7.27 7.25 7.23 7.21 7.19 7.18 6.93 6.92 6.91 6.89 4.56 4.54 4.52 4.50 4.34 4.33 4.31 4.29 4.20 4.17 4.15 4.14 4.12 3.57 3.09 3.05 3.04 3.01 2.99 2.98 2.97 2.96 2.95 2.80 2.78 2.77 2.76 2.74
O
S
O
O
O
NH
O
NH
Fmoc
O
5b
H2O
DMSO
0.87 2.05 2.05 0.94 2.00 3.06 2.14 3.00 2.02 3.92 1.15 2.00 1.13 1.13 0.99 2.12 3.00 1.18 1.96 0.96
f1 (ppm)

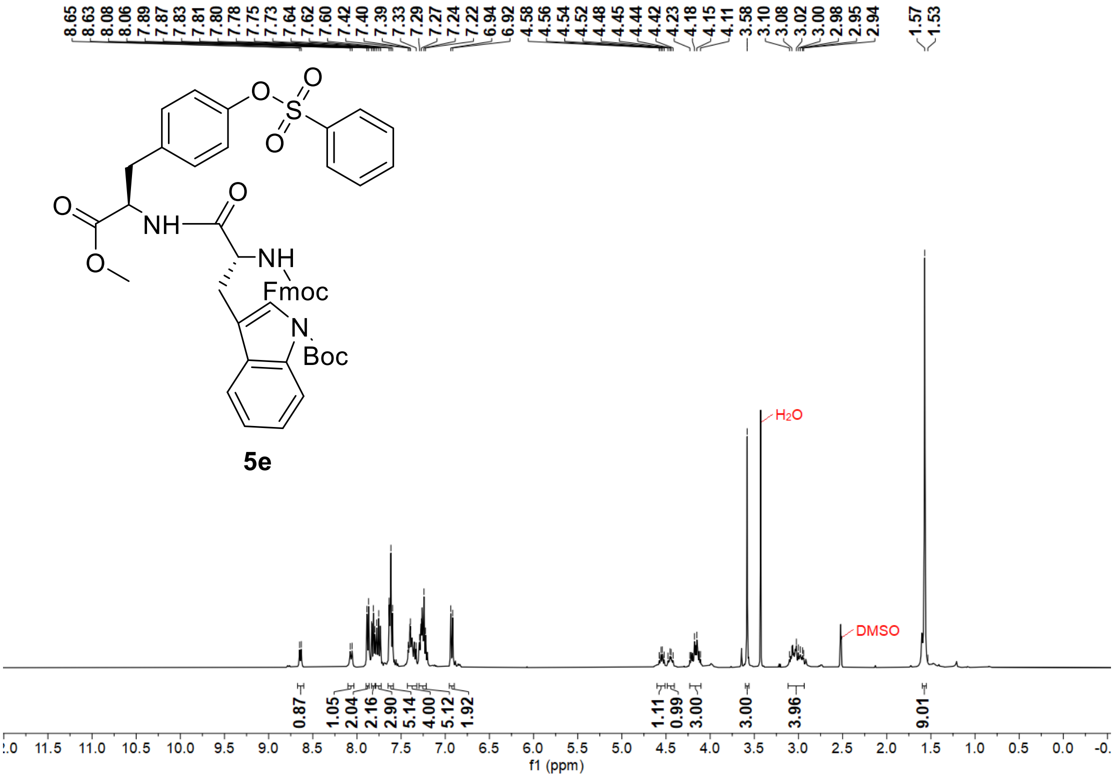

172.27 172.08 156.19 148.19 144.25 144.15 141.13 138.51 137.01 135.36 134.77 131.14 130.17 129.71 128.62 128.51 128.09 127.52 126.74 126.72 125.72 122.26 120.55 64.80 56.34 53.83 52.35 47.02 37.37 34.20
DMSO
O
S
O
O
O
NH
O
NH
Fmoc
O
5b
f1 (ppm)


H₂O
DMSO
5c
f1 (ppm)

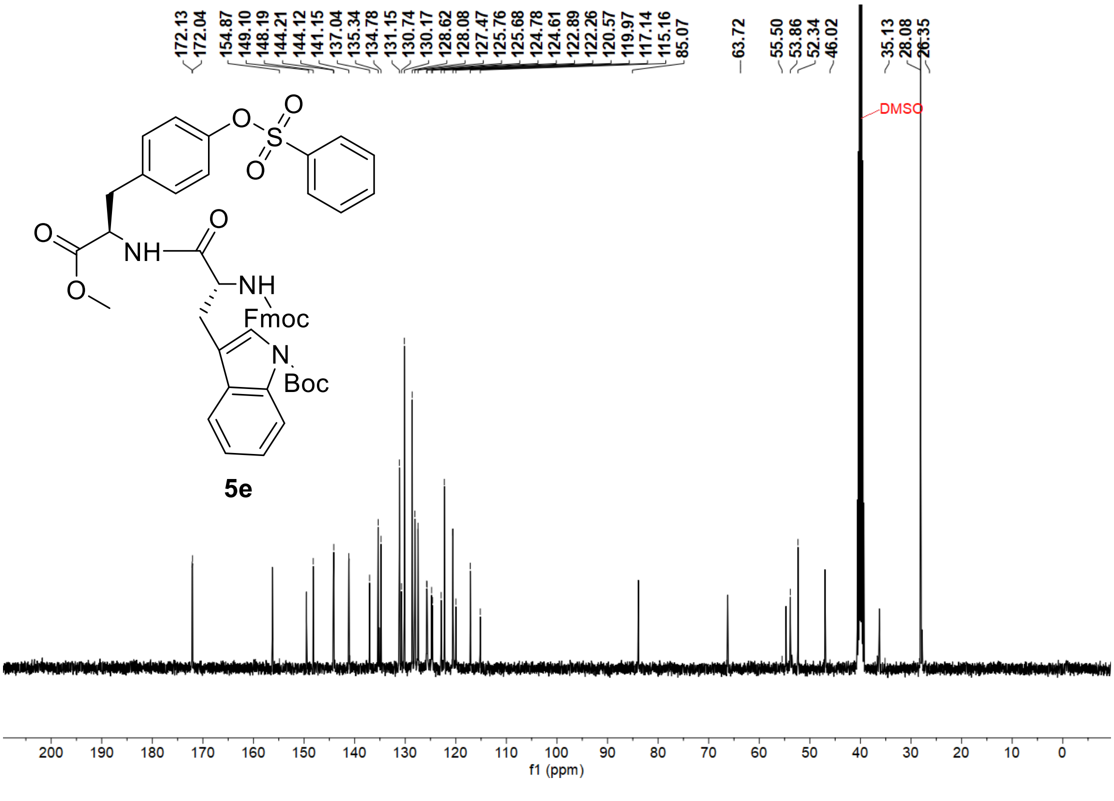

DMSO
5c
f1 (ppm)


5d
H2O
DMSO
f1 (ppm)

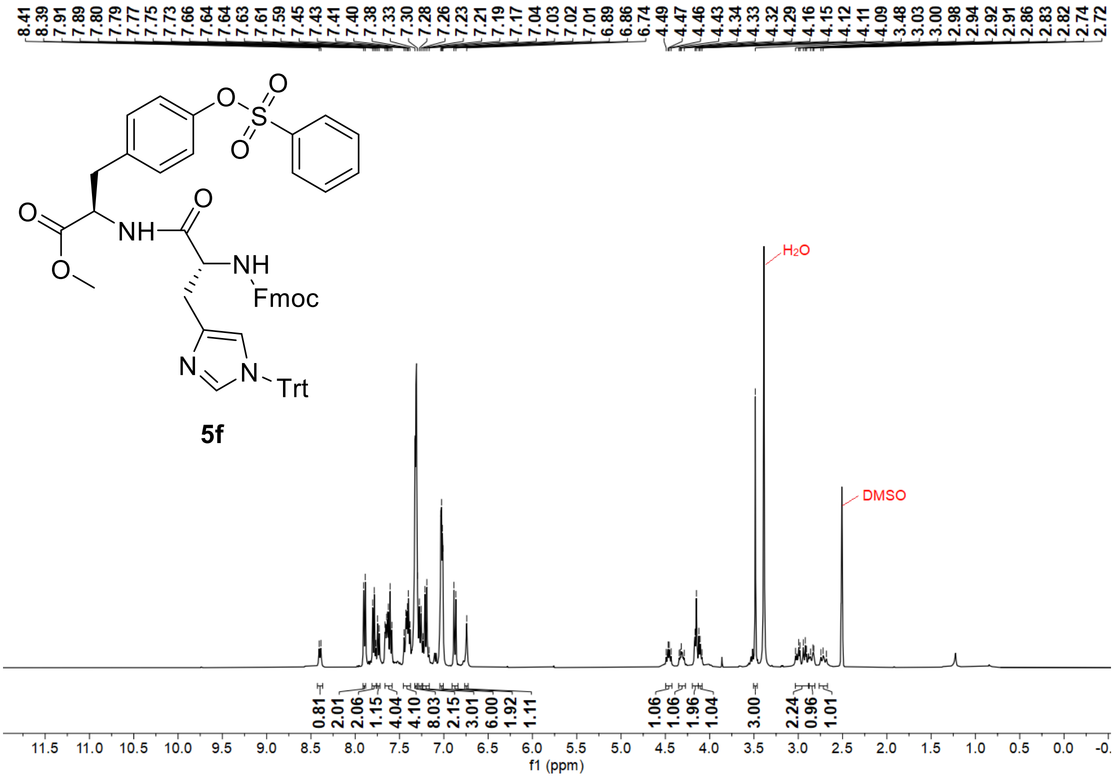

5d
DMSO
f1 (ppm)

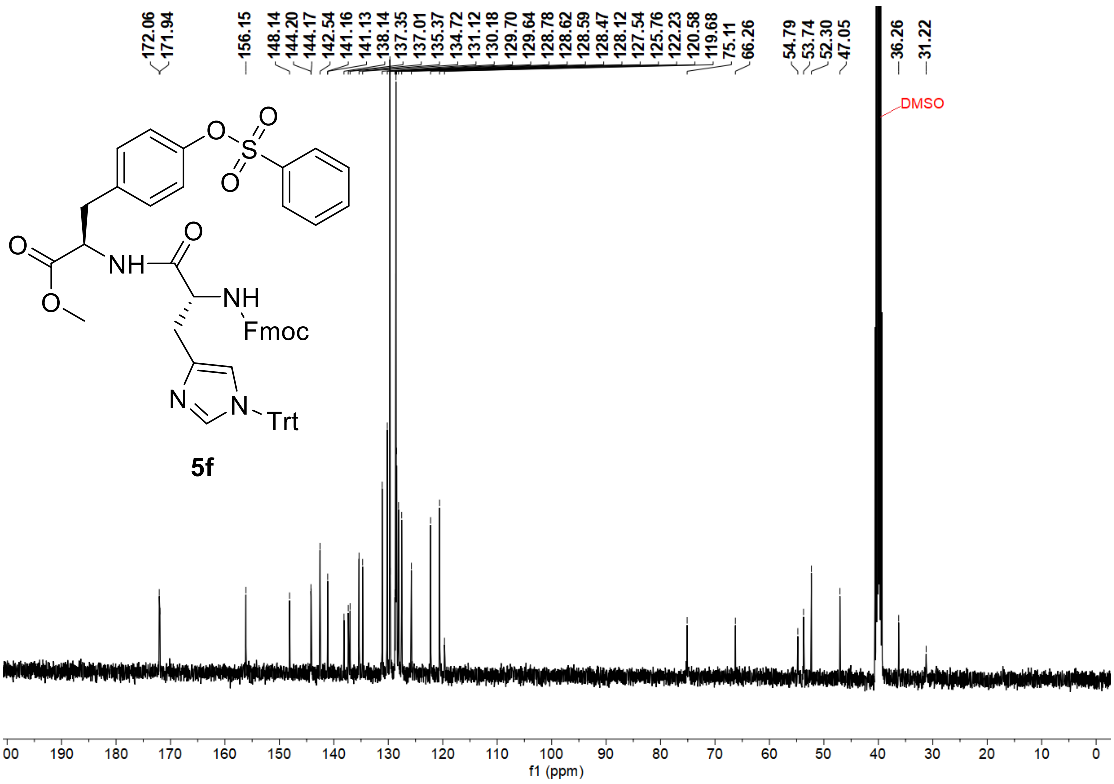


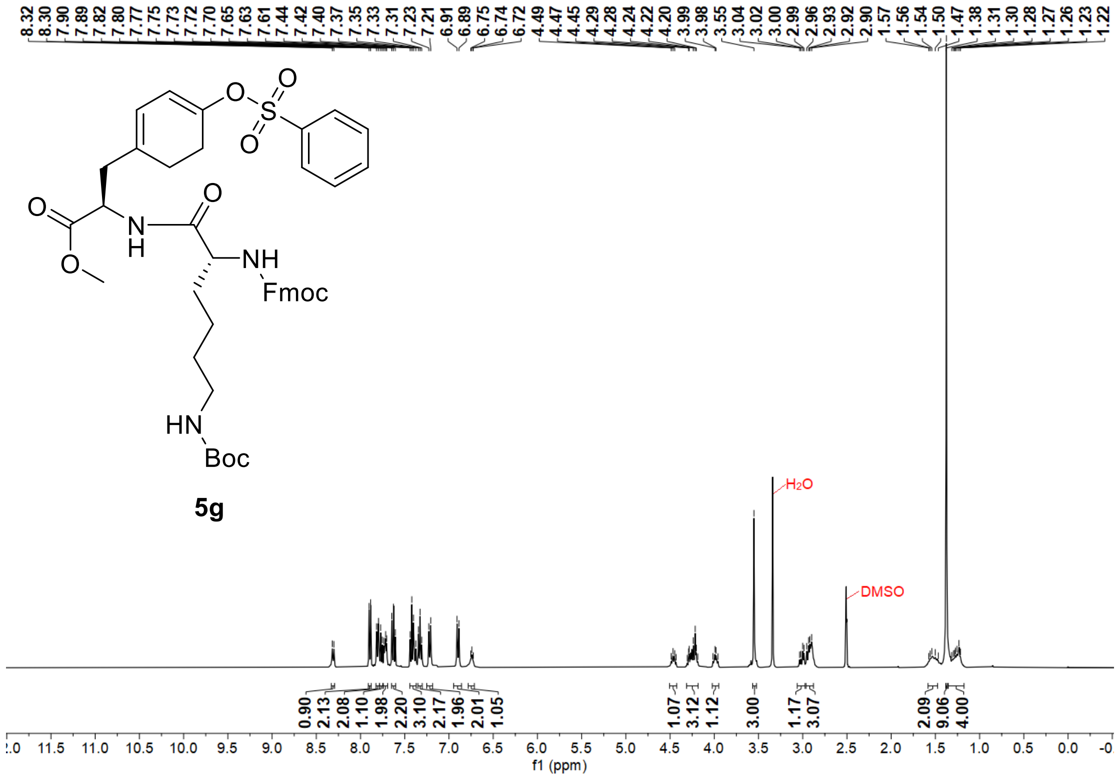
5e

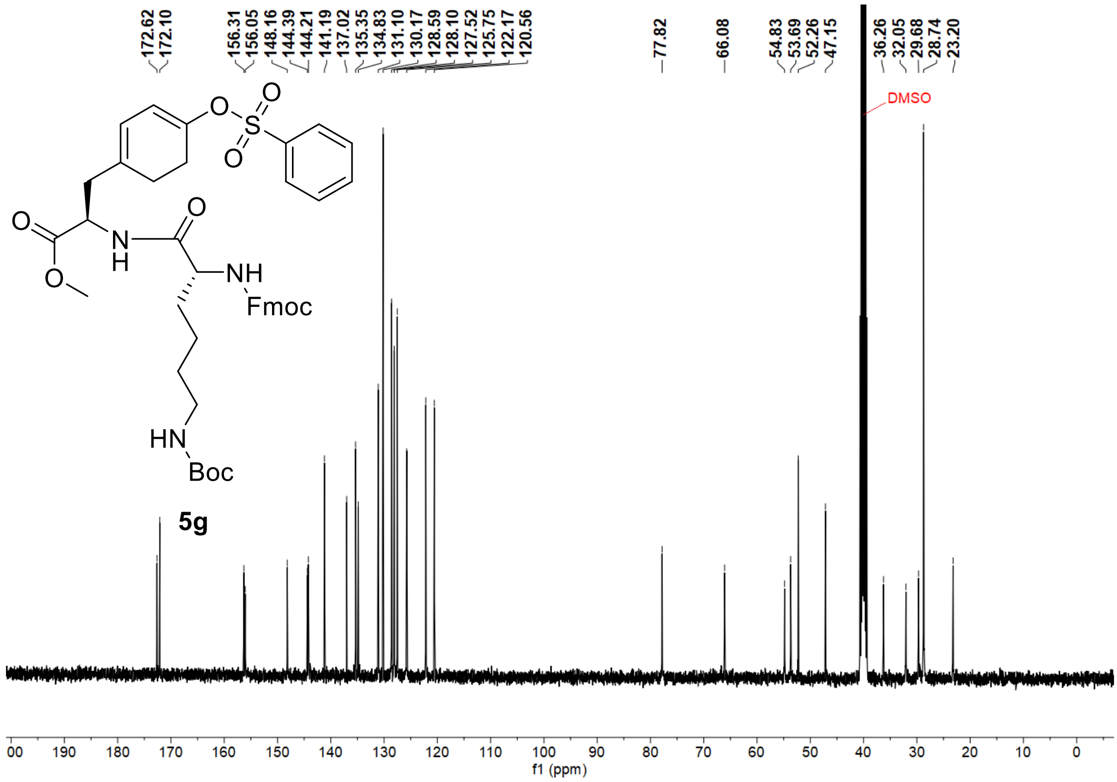


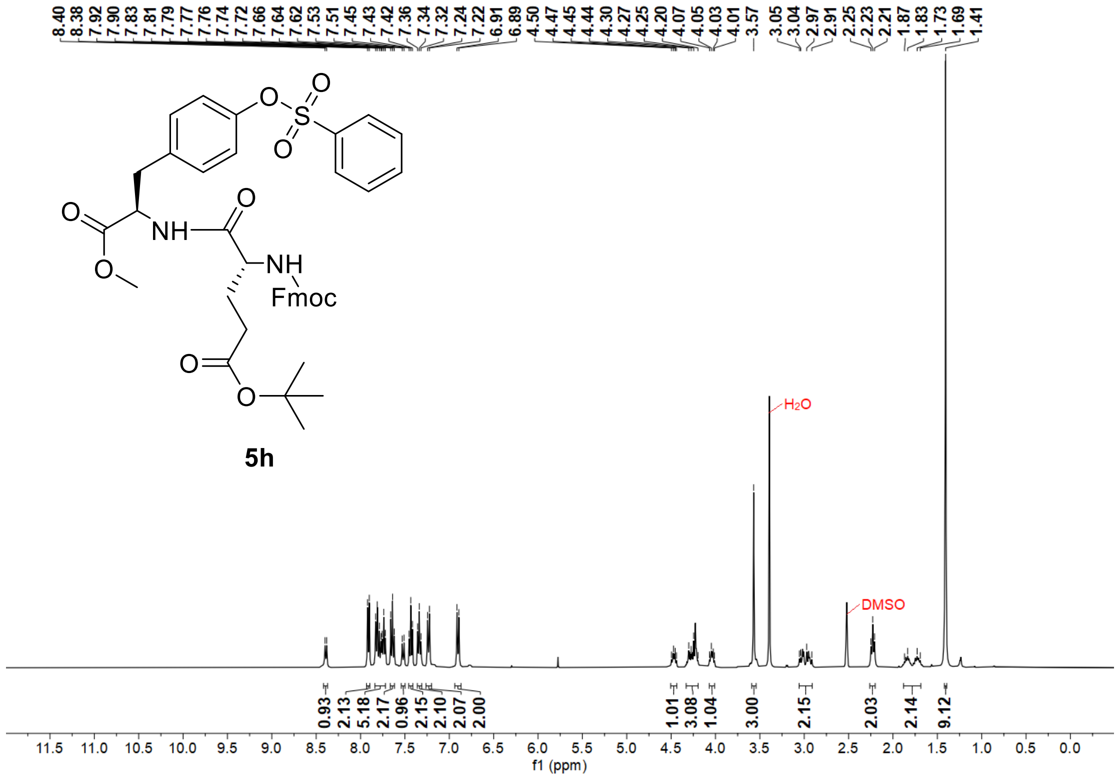
5e


8.41 8.39 7.91 7.89 7.80 7.79 7.77 7.75 7.73 7.66 7.64 7.64 7.63 7.61 7.59 7.45 7.43 7.41 7.40 7.38 7.33 7.30 7.28 7.26 7.23 7.21 7.19 7.17 7.04 7.03 7.02 7.01 6.89 6.86 6.74 4.49 4.47 4.46 4.43 4.34 4.33 4.32 4.29 4.16 4.15 4.12 4.11 4.09 3.48 3.03 3.00 2.98 2.94 2.92 2.91 2.86 2.83 2.82 2.74 2.72
NH
NH
Fmoc
N
N
Trt
5f
H2O
DMSO
0.81 2.01 2.06 1.15 4.04 4.10 8.03 2.15 3.01 6.00 1.92 1.11 1.06 1.06 1.96 1.04 3.00 2.24 0.96 1.01
f1 (ppm)

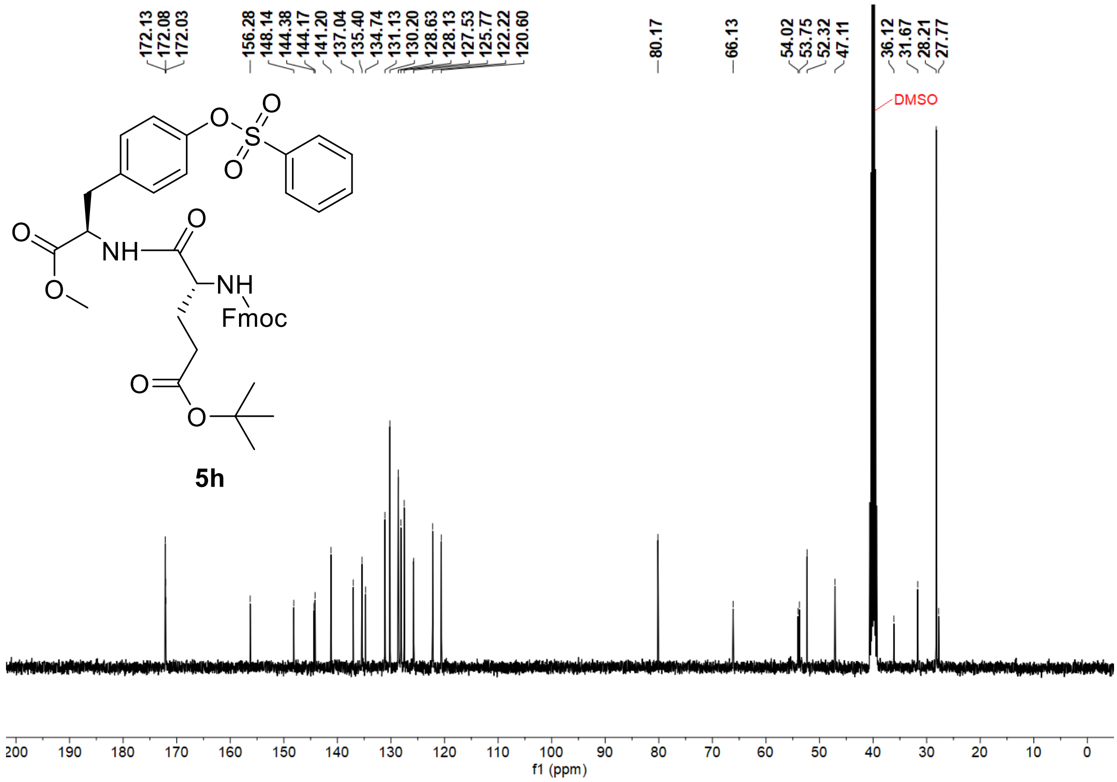

172.06 171.94 156.15 148.14 144.20 144.17 142.54 141.16 141.13 138.14 137.35 137.01 135.37 134.72 131.12 130.18 129.70 129.64 128.78 128.62 128.59 128.47 128.12 127.54 125.76 122.23 120.58 119.68 75.11 66.26 54.79 53.74 52.30 47.05 36.26 31.22
DMSO
NH
NH
Fmoc
N
N
Trt
5f
f1 (ppm)



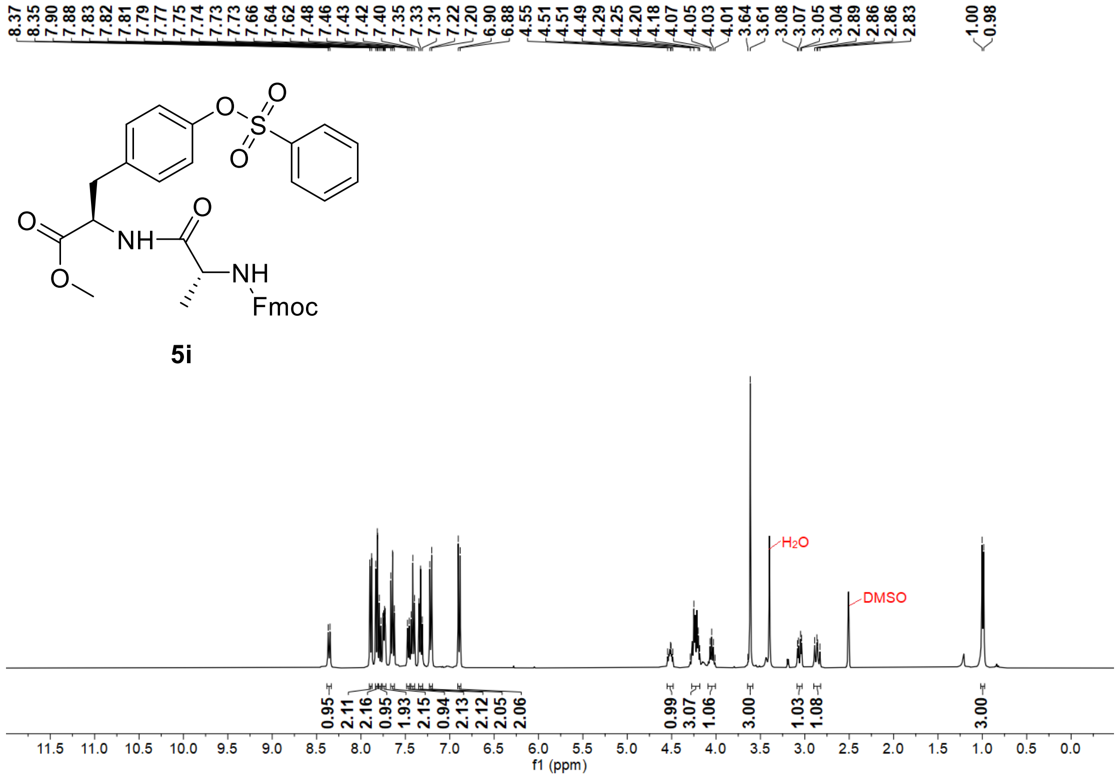


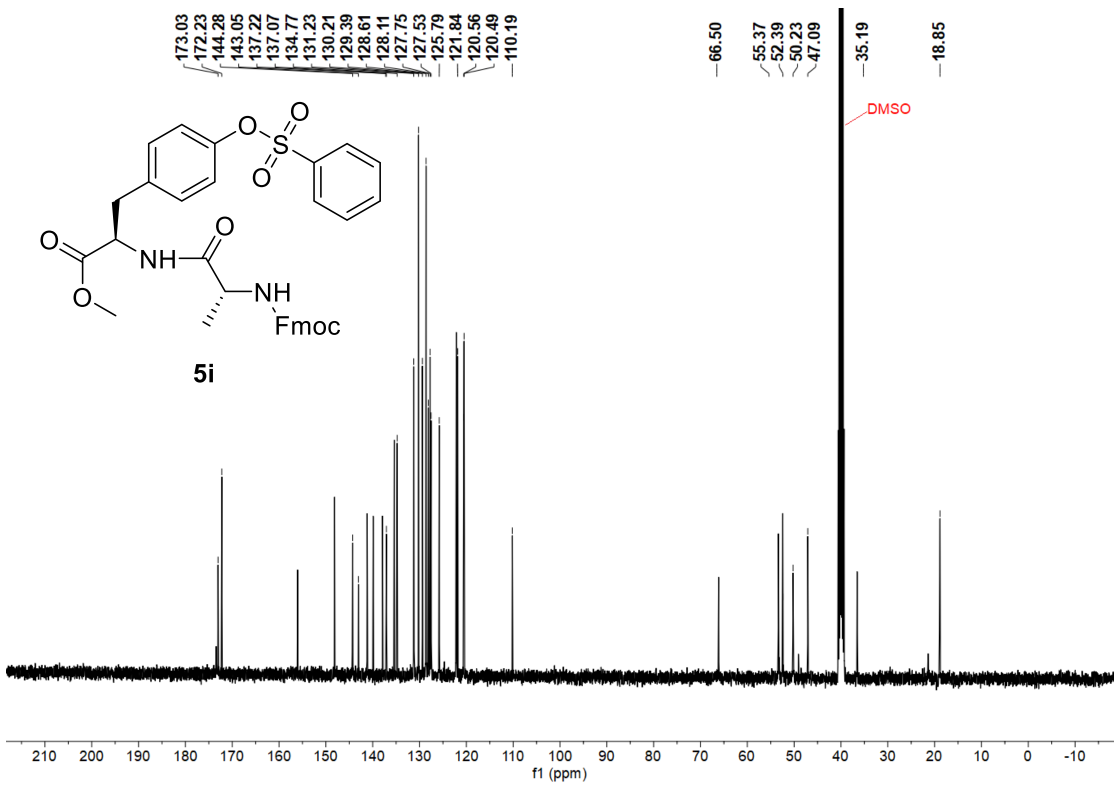

8.40
8.38
7.92
7.90
7.83
7.81
7.79
7.77
7.76
7.74
7.72
7.66
7.64
7.62
7.53
7.51
7.45
7.43
7.42
7.36
7.34
7.32
7.24
7.22
6.91
6.89
4.50
4.47
4.45
4.44
4.30
4.27
4.25
4.20
4.07
4.05
4.03
4.01
3.57
3.05
3.04
2.97
2.91
2.25
2.23
2.21
1.87
1.83
1.73
1.69
1.41
NH
Fmoc
H_2O
DMSO
0.93
2.13
5.18
2.17
0.96
2.15
2.10
2.07
2.00
1.01
3.08
1.04
3.00
2.15
2.03
2.14
9.12
f1 (ppm)

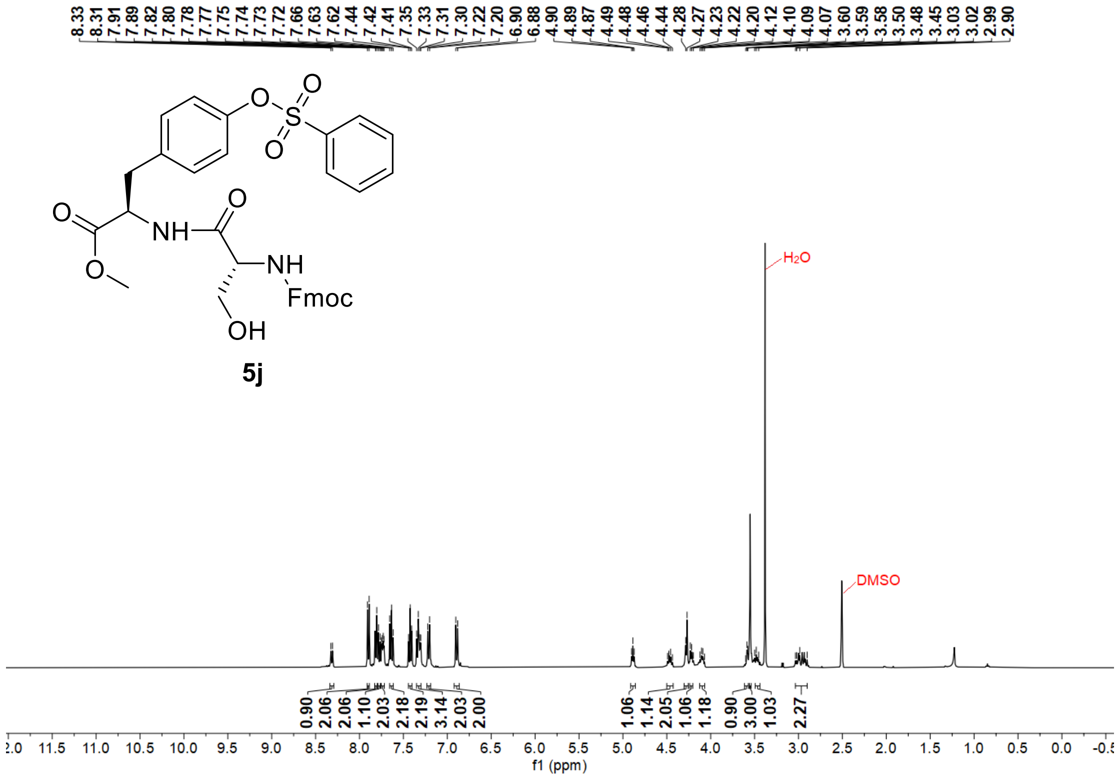
5h

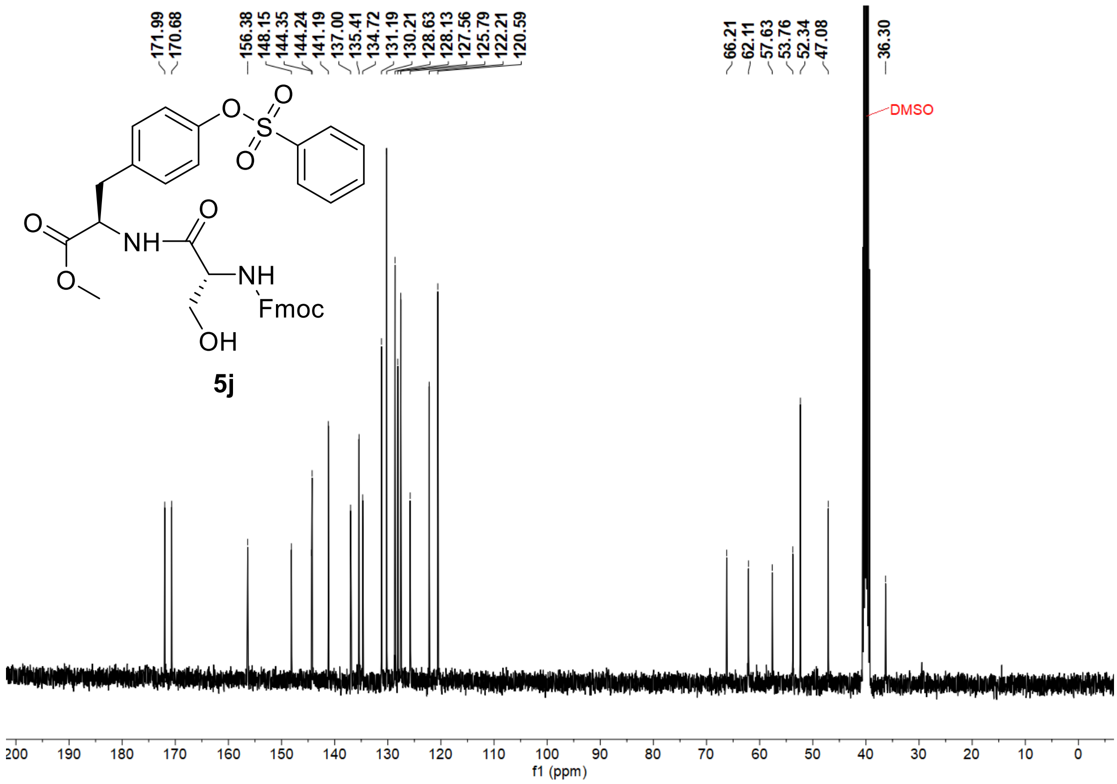

172.13
172.08
172.03
156.28
148.14
144.38
144.17
141.20
137.04
135.40
134.74
131.13
130.20
128.63
128.13
127.53
125.77
122.22
120.60
80.17
66.13
54.02
53.75
52.32
47.11
36.12
31.67
28.21
27.77
DMSO
NH
Fmoc
f1 (ppm)

5h


8.37 8.35 7.90 7.88 7.83 7.82 7.81 7.79 7.77 7.75 7.74 7.73 7.73 7.66 7.64 7.62 7.48 7.46 7.43 7.42 7.40 7.35 7.33 7.31 7.22 7.20 6.90 6.88 4.55 4.51 4.51 4.49 4.29 4.25 4.20 4.18 4.07 4.05 4.03 4.01 3.64 3.61 3.08 3.07 3.05 3.04 2.89 2.86 2.86 2.83 1.00 0.98
5i
H2O
DMSO
0.95 2.11 2.16 0.95 1.93 2.15 0.94 2.13 2.12 2.05 2.06 0.99 3.07 1.06 3.00 1.03 1.08 3.00
f1 (ppm)


173.03 172.23 144.28 143.05 137.22 137.07 134.77 131.23 130.21 129.39 128.61 128.11 127.75 127.53 125.79 121.84 120.56 120.49 110.19 66.50 55.37 52.39 50.23 47.09 35.19 18.85
DMSO
5i
f1 (ppm)


5j
H2O
DMSO
f1 (ppm)


5j
DMSO
f1 (ppm)