Supplementary Information

Thiopeptide Pyridine Synthase TbtD Catalyzes an Intermolecular Formal Aza-Diels-Alder Reaction

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I. General Information

All reactions were carried out in an oven-dried round-bottomed-flask under an inert nitrogen atmosphere with stirring. Solvents, reagents, and chemicals were purchased through Fisher Scientific and used as received unless otherwise noted. Amino acids and their protected derivatives, 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), and 1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDC*HCl) were purchased from ChemPep. *N,N*-diisopropylethyl amine (DIPEA), 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), and 1-Hydroxybenzotriazole hydrate (HOBt*H_2O) were purchased from Sigma-Aldrich.

Spectra for ^{1}H and ^{13}C NMR were recorded at room temperature, unless otherwise noted, with a Varian Inova 400 (400 MHz and 100 MHz, respectively) or Varian Inova 500 (500 MHz and 125 MHz, respectively) spectrometers. Chemical shifts are reported in δ (ppm) relative units to residual solvent peaks $CDCl_3$ (7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C) and DMSO-*d*6 (2.50 ppm for ^{1}H and 39.5 ppm for ^{13}C). Splitting patterns are assigned as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), multiplet (m), dd (doublet of doublets), and td (triplet of doublets).

LC/MS measurements were recorded using an Agilent 6520 Accurate-Mass Q-TOF ESI positive in high-resolution mode, 350 ^{o}C temperature and 250 V fragmentor, with a 100 x 2.10 mm Kinetex® 2.6 A C8 100 Å column or a 50 x 2.1 mm Kinetex® 2.6 A C18 100 Å column; both were purchased from Phenomenex. MS-MS was performed with collision energy of 45 V or 60 V. Predicted masses were extracted to $\pm$ 5 ppm. Two methods were utilized:

Method A:

Time	**% Solvent B**
0 - 2 min	2%
2 - 5 min	2 - 45%
5 – 22 min	45 - 60%
22 - 23 min	60 – 95%
23 - 24 min	95%
24 – 25 min	95 - 2%

Method B:

Time	**% Solvent B**
0 -2 min	2%
2- 13 min	2 - 98%
13– 15 min	98%

Microwave assisted peptide couplings were programmed and synthesized in a Biotage Initiator + Alstra™ with Rink Amide ChemMatrix purchased from Biotage. Solutions utilized were freshly prepared just before synthesis. Flash chromatography was performed on a Biotage Isolera™ system with a hexanes/ethyl acetate solvent system or a dichloromethane/methanol system. Thin-layer-chromatography glass plates were purchased from Sorbent Technologies, Inc.® with a fluorescent indicator at λ = 254 nm. Preparatory HPLC was performed in a Shimadzu UFLC CBM-20A with a dual channel wavelength detector at 220 nm and 254 nm with a LUNA 10®

C18(2) 100 Å, AXIA (Phenomenex) semi-preparatory column with a 15 mL/min flow rate. Purifications were carried out with a two solvent system (solvent A = 0.1% trifluoroacetic acid in water; solvent B = 0.1% trifluoroacetic acid in acetonitrile). Analytical HPLC was performed with a dual channel wavelength detector at 254 and 220 or 350 nm on a Kinetex 10® C18 100 Å, (Phenomenex) column with a 0.5 mL/min flow rate.

Method A:

Time	**% Solvent B**
0 -2 min	5%
2- 8 min	5 - 35%
8 – 23 min	35-55%
23- 25 min	55 – 100%
25 – 28.5 min	100%
28.5 – 30 min	100 – 5%
30 – 32 min	5%

Method B:

Time	**% Solvent B**
0 -2 min	5%
2- 25 min	5 - 95%
25 - 27 min	95%
27 - 28 min	95 – 5%
28 – 30 min	5%

Method C:

Time	**% Solvent B**
0 -2 min	5%
2- 15 min	5 - 95%
15 - 18 min	95%
18 - 19 min	95 – 5%
19 – 20 min	5%

II. Synthetic Procedures

General Procedure A (TBTU Coupling):
The Boc-protected amino acid was dissolved in DCM (0.15 M) and cooled to 0°C. To this, NH2-Ser(OH)-OMe (1.0 equiv.) and TBTU (1.0 equiv.) were added followed by slow addition of DIPEA (3.0 equiv.). The mixture was slowly warmed to room temperature and stirred overnight. The reaction was monitored by TLC and upon consumption of the Boc-protected amino acid the solution was concentrated *in vacuo* to give a clear crude oil. The residue was purified via silica gel chromatography to give the desired compound as a white solid.

General Procedure B (Oxazole):
Starting material was dissolved in dry DCM (.2 M), put under N_2 and cooled to -78°C. DAST (1.1 equiv.) was added and the temperature was increased to -20°C and the reaction was stirred for 4 h. To the cooled solution DBU (3.0 equiv.) then $CBrCl_3$ (4.5 equiv.) were added and the mixture was allowed to reach room temperature. The reaction was stirred overnight and monitored by TLC. Upon consumption of the starting material the solution was concentrated *in vacuo* to give a crude black oil. The residue was purified via silica gel chromatography to give the desired compound as a white solid.

General Procedure C (Saponification/Deprotection/Protection):
The starting material was dissolved in a 1:1 mixture of methanol and tetrahydrofuran (0.1 M) and cooled to 0°C. 0.4 M aq. NaOH (3.13 equiv.) was added dropwise and the reaction was stirred for 4 h at room temperature. The reaction was monitored by TLC and upon consumption of starting material the solution was concentrated *in vacuo*. The remaining aqueous solution was acidified with 1 N HCl until pH = 3.0. Acid derivative was extracted with ethyl acetate unless otherwise noted, dried with Na_2SO_4 and concentrated to dryness. Crude acid was dissolved in dichloromethane (0.5 M), cooled to 0°C and an equal volume of trifluoroacetic acid was added dropwise. The reaction was stirred for 2 h at 0°C and 1h at room temperature. The crude solution was then concentrated *in vacuo* and washed several times with dichloromethane, methanol and toluene to remove any residual trifluoroacetic acid. The intermediate amine was dissolved in equal parts tetrahydrofuran and water (0.2 M) and cooled to 0°C. $NaHCO_3$ (2.5 equiv.) was added scoop-wise, followed by Fmoc-OSu (1.0 equiv.). The reaction was stirred overnight at room temperature. The reaction was monitored by TLC and after all starting material was consumed tetrahydrofuran was removed under reduced pressure. The remaining aqueous layer was acidified with 1 N HCl until pH = 3.0 and extracted with ethyl acetate unless otherwise noted. The organic layers were combine, dried by Na_2SO_4 and concentrated *in vacuo.* The crude material was then purified via silica gel chromatography.

General Procedure D (Thiazole):
Starting material acid was dissolved in tetrahydrofuran (0.2 M), cooled to 0 °C and put under N_2. N-methyl morpholine (1.1 equiv.) was added dropwise to this solution followed by isobutyl chloroformate (1.1 equiv.). After 30 min, TLC shows full consumption of the starting material and the solution was again cooled to 0°C. Aqueous NH_4OH (28%, 5.0 equiv.) was added dropwise. After 45 min. TLC shows full consumption of the intermediate. The crude material was concentrated and then extracted with ethyl acetate and brine/citric acid. The combined organic

layer was dried ($MgSO_4$), filtered, and concentrated yielding a white solid. Crude material was immediately carried to the next step without further purification.

Intermediate amide and Lawesson's reagent (0.5 equiv.) were suspended in benzene (0.4 M), put under N_2 and refluxed (100 °C) for 1 h. After 1 h the now yellow solution is cooled and assumed complete. The crude material was concentrated and extracted with ethyl acetate and brine/water/bicarb. The organic layers were combine, dried ($MgSO_4$) and concentrated *in vacuo* yielding an off-white, foamy solid. The crude material was run through a small silica plug to remove any unreacted Lawesson's reagent. The crude thioamide was immediately carried to the next step without any further purification.

A flask was charged with the crude thioamide, calcium carbonate (1.5 equiv.) and bromopyruvic acid (1.5 equiv.) and put under N_2. Dry ethanol (0.1 M) was added via syringe at room temperature and allowed to stir overnight. After ~12 h TLC showed full consumption of the thioamide and the solution was filtered to remove the suspended calcium carbonate. The solution was concentrated under reduced pressure, diluted with brine and neutralized to pH 3.0 with 1 N HCl. The crude material was then extracted with 10% methanol in dichloromethane. Organic layers were combine, dried ($MgSO_4$) and concentrate under reduced pressure to yield a foamy off white solid. The crude thiazole was then purified by silica gel chromatography to yield a tan to white solid.

General Procedure E (Deprotection/Coupling):
Starting material Fmoc-protected amino acid was dissolved in dichloromethane (0.2 M) and cooled to 0°C. Diethylamine (equal volume as dichloromethane) was added dropwise. The reaction was monitored by TLC and after 2 h all starting material was consumed. The crude solution was concentrated and washed several times with dichloromethane, methanol and toluene to remove any residual diethylamine.

Crude amine was then dissolved in dichloromethane (0.15 M) and TBTU (1.0 equiv.) and the corresponding acid building block (1.1 equiv.) were added as solids, scoop-wise and the solution was cooled to 0°C. Finally, DIPEA (3.0 equiv.) was added dropwise, the reaction was warmed to room temperature and stirred overnight. After ~12 h TLC showed all starting material had been consumed and the reaction was concentrated and immediately purified by silica gel chromatography to yield the desired compound.

General Procedure F (Deprotection/Acylation):
Starting material Fmoc-protected amino acid was dissolved in dichloromethane (0.2 M) and cooled to 0°C. Diethylamine (equal volume as dichloromethane) was added dropwise. The reaction was monitored by TLC and after 2 h all starting material was consumed. The crude solution was concentrated and washed several times with dichloromethane, methanol and toluene to remove any residual diethylamine.

The intermediate amine was suspended in dichloromethane (0.15 M) and cooled to 0°C. Acetic Anhydride (1.1 equiv.) followed by DIPEA (2.0 equiv.) were added dropwise. The reaction mixture stirred for 0.5 h when TLC show full consumption of the intermediate amine. The reaction was then concentrated under reduced pressure and moved to the next step without further purification.

General Procedure G (Deprotection/Elimination 1):
The protected diol starting material was dissolved in 5% triisopropylsilane in trifluoroacetic acid (0.2 M) and stirred for 2 h. The reaction was assumed complete after 2 h and the intermediate diol was concentrated under reduced pressure and washed several times with dichloromethane and toluene to remove any residual trifluoroacetic acid.

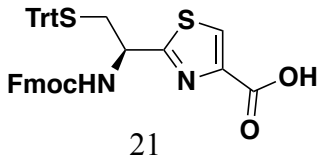
The residue was dissolved in dichloromethane (0.2 M), cooled to 0°C and put under N_2. To this, triethylamine (2.5 equiv.) was added followed by dropwise addition of methanesulfonyl chloride (2.0 equiv.). The reaction was stirred overnight and monitored by TLC. After 12 h the TLC shows full consumption of starting material and the reaction was concentrated under reduced pressure. The product was then purified via reverse-phase chromatography and lyophilized to yield the desired final product.

General Procedure H (Deprotection/Elimination 2):
The protected dithiol starting material was dissolved in 5% triisopropylsilane in trifluoroacetic acid (0.2 M) and stirred for 30 min. The reaction was assumed complete after 30 min and the intermediate dithol was concentrated under reduced pressure and washed several times with dichloromethane and toluene to remove any residual trifluoroacetic acid.

Intermediate dithiol was dissolved in equal parts tetrahydrofuran and water (633 μM) and reduced with TCEP (2.0 equiv.) for 15 min at room temperature. The crude material was then cooled to 0°C and K_2CO_3 (100.0 equiv.) was added scoopwise followed by dropwise addition of methyl 2,5-dibromovalerate (100.0 equiv.). The reaction was heated to 37°C and stirred for 3 h when the reaction was assumed complete. The solution was then concentrated under reduced pressure and extracted with dichloromethane and brine. Organic layers were combine, dried ($MgSO_4$) and concentrate under reduced pressure. The crude material was then purified via reverse-phase chromatography and lyophilized to yield the desired final product.

Building Block Synthesis:

(S)-2-(1-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(tritylthio)ethyl)thiazole-4-carboxylic acid (21)

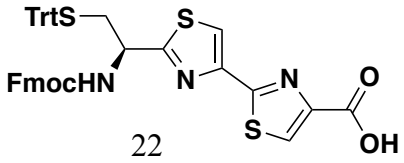


21

We have previously described the synthesis of compound 21.[1] The ^{1}H NMR is in good agreement with that previously reported.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.31 (d, J = 8.5 Hz, 1H), 8.26 (s, 1H), 7.88 (d, J = 7.6 Hz, 2H), 7.70 (d, J = 7.6 Hz, 2H), 7.39 (dt, J = 8.0, 4.2 Hz, 2H), 7.33 (d, J = 6.6 Hz, 12H), 7.30 – 7.21 (m, 5H), 4.53 (td, J = 8.9, 5.3 Hz, 1H), 4.34 (d, J = 7.1 Hz, 2H), 4.23 (t, J = 7.0 Hz, 1H), 2.83 (dd, J = 12.7, 9.6 Hz, 1H), 2.67 (dd, J = 12.7, 5.3 Hz, 1H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 172.13, 162.57, 155.96, 144.66, 144.14, 141.20, 129.58, 128.62, 128.10, 127.54, 127.52, 127.35, 125.69, 125.66, 120.59, 66.96, 66.17, 52.97, 47.11, 35.92.
LC-MS ESI: $C_{40}H_{32}N_2NaO_4S_2^+$ $[M+Na]^+$ Exact Mass: 691.1696; Observed: 691.1689

(*S*)-2'-(1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-(tritylthio)ethyl)-[2,4'-bithiazole]-4-carboxylic acid (22)

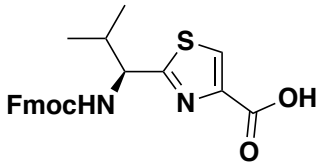


22

We have previously described the synthesis of compound 22.[1] The ^{1}H NMR is in good agreement with that previously reported.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.48 (s, 1H), 8.40 (d, J = 8.3 Hz, 1H), 8.20 (s, 1H), 7.88 (d, J = 7.6 Hz, 2H), 7.73 (dd, J = 7.6, 3.3 Hz, 2H), 7.42 – 7.31 (m, 16H), 7.28 – 7.23 (m, 4H), 4.56 (td, J = 9.1, 4.8 Hz, 1H), 4.44 (dd, J = 10.6, 7.1 Hz, 1H), 4.38 (dd, J = 10.8, 6.8 Hz, 1H), 4.27 (t, J = 7.0 Hz, 1H), 2.95 (dd, J = 13.0, 9.9 Hz, 1H), 2.70 (dd, J = 13.0, 4.6 Hz, 1H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 173.93, 162.53, 156.13, 148.60, 147.97, 144.68, 144.16, 141.25, 129.64, 128.62, 128.13, 127.57, 127.55, 127.37, 125.70, 125.62, 120.60, 118.56, 66.18, 53.55, 47.20, 36.24.
LC-MS ESI: $C_{43}H_{33}N_3NaO_4S_3^+$ $[M+Na]^+$ Exact Mass: 774.1525; Observed: 774.1526

(*S*)-2-(1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylpropyl)thiazole-4-carboxylic acid (23)

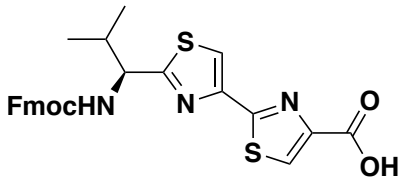


23

We have previously described the synthesis of compound 23.[1] The ^{1}H NMR is in good agreement with that previously reported.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (d, J = 0.6 Hz, 1H), 8.22 (d, J = 8.6 Hz, 1H), 7.89 (d, J = 7.6 Hz, 2H), 7.76 – 7.69 (m, 2H), 7.44 – 7.38 (m, 2H), 7.32 (t, J = 7.4 Hz, 2H), 4.67 (t, J = 7.9 Hz, 1H), 4.41 – 4.28 (m, 2H), 4.23 (t, J = 7.1 Hz, 1H), 2.26 (h, J = 6.8 Hz, 1H), 0.93 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 6.7 Hz, 3H).
LC-MS ESI: $C_{23}H_{23}N_2O_4S^+$ $[M+H]^+$ Exact Mass: 423.1373; Observed: 243.1352

(*S*)-2'-(1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylpropyl)-[2,4'-bithiazole]-4-carboxylic acid (24)

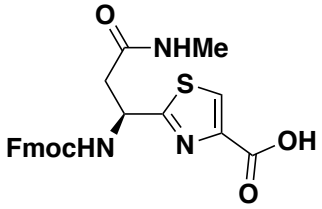


24

We have previously described the synthesis of compound 24.[1] The ^{1}H NMR is in good agreement with that previously reported.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 – 8.13 (m, 2H), 7.84 (d, J = 7.6 Hz, 2H), 7.68 (dd, J = 7.9, 3.9 Hz, 2H), 7.40 – 7.31 (m, 2H), 7.26 (t, J = 7.5 Hz, 2H), 4.65 (t, J = 7.9 Hz, 1H), 4.36 – 4.25 (m, 2H), 4.19 (t, J = 7.2 Hz, 1H), 2.22 (q, J = 6.9 Hz, 1H), 0.91 (d, J = 6.7 Hz, 3H), 0.85 (d, J = 6.7 Hz, 3H).
LC-MS ESI: $C_{26}H_{24}N_3O_4S_2^+$ $[M+H]^+$ Exact Mass: 506.1203; Observed: 506.1202

(*S*)-2-(1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(methylamino)-3-oxopropyl)thiazole-4-carboxylic acid (25)

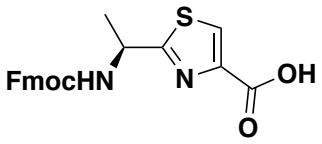


25

We have previously described the synthesis of compound 25.[1] The ^{1}H NMR is in good agreement with that previously reported.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H), 8.23 (d, J = 8.1 Hz, 1H), 7.89 (d, J = 7.2 Hz, 3H), 7.70 (dd, J = 7.6, 4.5 Hz, 2H), 7.41 (t, J = 7.8 Hz, 2H), 7.32 (td, J = 7.6, 2.5 Hz, 2H), 5.26 (td, J = 8.3, 5.4 Hz, 1H), 4.39 (dd, J = 10.6, 7.2 Hz, 1H), 4.32 (dd, J = 10.6, 6.8 Hz, 1H), 4.24 (t, J = 6.9 Hz, 1H), 2.86 (dd, J = 15.2, 5.4 Hz, 1H), 2.70 (dd, J = 15.1, 8.6 Hz, 1H), 2.57 (d, J = 4.5 Hz, 3H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 174.09, 169.48, 162.50, 156.04, 147.32, 144.27, 144.12, 141.22, 129.21, 128.12, 127.59, 127.56, 125.68, 125.64, 120.61, 66.14, 50.77, 47.15, 26.03.
LC-MS ESI: $C_{23}H_{22}N_3O_5S^+$ $[M+H]^+$ Exact Mass: 452.1275; Observed: 452.1266

(*S*)-2-(1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)thiazole-4-carboxylic acid (26)

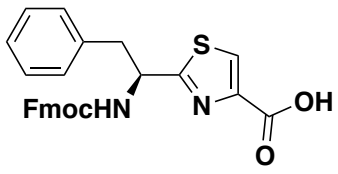


26

We have previously described the synthesis of compound 26.[1] The ^{1}H NMR is in good agreement with that previously reported.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.36 (s, 1H), 8.29 (d, J = 7.7 Hz, 1H), 7.89 (d, J = 7.6 Hz, 2H), 7.73 (d, J = 7.5 Hz, 2H), 7.41 (d, J = 7.8 Hz, 2H), 7.34 (t, J = 7.5 Hz, 2H), 4.96 (p, J = 7.2 Hz, 1H), 4.45 (dd, J = 10.6, 7.0 Hz, 1H), 4.35 (dd, J = 10.6, 6.9 Hz, 1H), 4.25 (t, J = 6.8 Hz, 1H), 1.52 (d, J = 7.0 Hz, 3H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 176.00, 162.59, 156.14, 147.47, 144.26, 144.20, 141.25, 128.83, 128.12, 127.56, 125.68, 125.61, 120.60, 66.07, 49.50, 47.21, 20.99.
LC-MS ESI: $C_{21}H_{19}N_2O_4S^+$ $[M+H]^+$ Exact Mass: 395.1060; Observed: 395.1055

(*S*)-2-(1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-phenylethyl)thiazole-4-carboxylic acid (27)

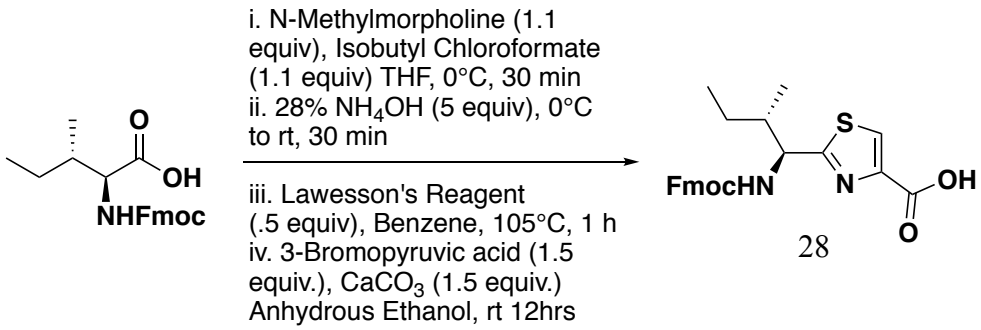


27

We have previously described the synthesis of compound 27.[1] The ^{1}H NMR is in good agreement with that previously reported.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (d, J = 14.9 Hz, 2H), 7.88 (d, J = 7.5 Hz, 2H), 7.61 (t, J = 7.4 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.36 – 7.25 (m, 6H), 7.24 – 7.17 (m, 1H), 5.05 (ddd, J = 10.9, 8.6, 4.3 Hz, 1H), 4.28 (dd, J = 10.5, 7.2 Hz, 1H), 4.22 (dd, J = 10.5, 6.8 Hz, 1H), 4.15 (t, J = 7.0 Hz, 1H), 3.38 (d, J = 4.4 Hz, 1H), 3.34 (d, J = 4.5 Hz, 2H), 3.06 (dd, J = 13.8, 10.9 Hz, 1H).
LC-MS ESI: $C_{27}H_{23}N_2O_4S^+$ $[M+H]^+$ Exact Mass: 471.1373; Observed: 471.1371

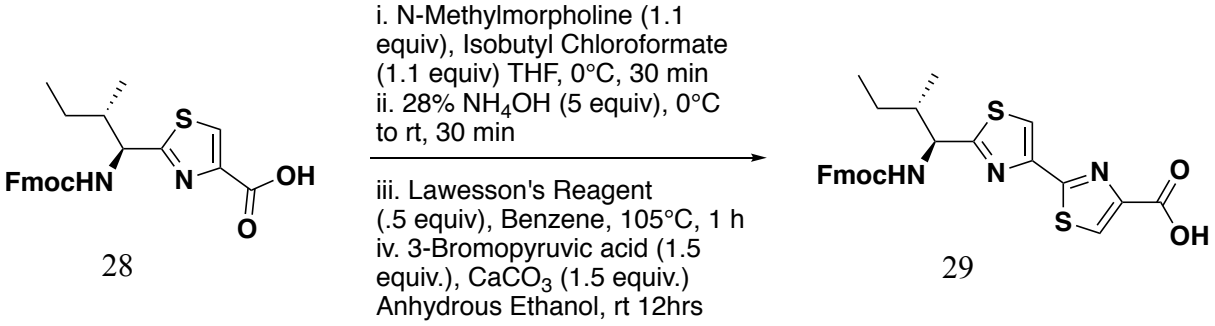
2-((1*S*,2*S*)-1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylbutyl)thiazole-4-carboxylic acid (28)



Compound 28 was prepared via general procedure D. Crude compound was purified via silica gel chromatography, eluting in 5% methanol in dichloromethane to give the titled compound as a fluffy white solid (1.8g, 63% yield over 3 steps).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (s, 1H), 8.17 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 7.6 Hz, 2H), 7.66 (dt, J = 9.3, 4.6 Hz, 2H), 7.36 (dt, J = 9.0, 4.6 Hz, 2H), 7.26 (t, J = 7.6 Hz, 2H), 4.66 (t, J = 8.0 Hz, 1H), 4.33 – 4.23 (m, 2H), 4.17 (t, J = 7.2 Hz, 1H), 1.96 (d, J = 8.9 Hz, 1H), 1.49 – 1.36 (m, 1H), 1.28 – 1.18 (m, 1H), 0.77 (dt, J = 20.4, 7.2 Hz, 6H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 173.98, 162.53, 156.47, 147.19, 144.15, 141.15, 128.85, 128.05, 127.46, 125.65, 120.53, 66.05, 58.23, 47.14, 38.79, 25.65, 25.01, 16.05, 11.30.
LC-MS ESI: $C_{24}H_{25}N_2O_4S^+$ $[M+H]^+$ Exact Mass: 437.1530; Observed: 437.1499

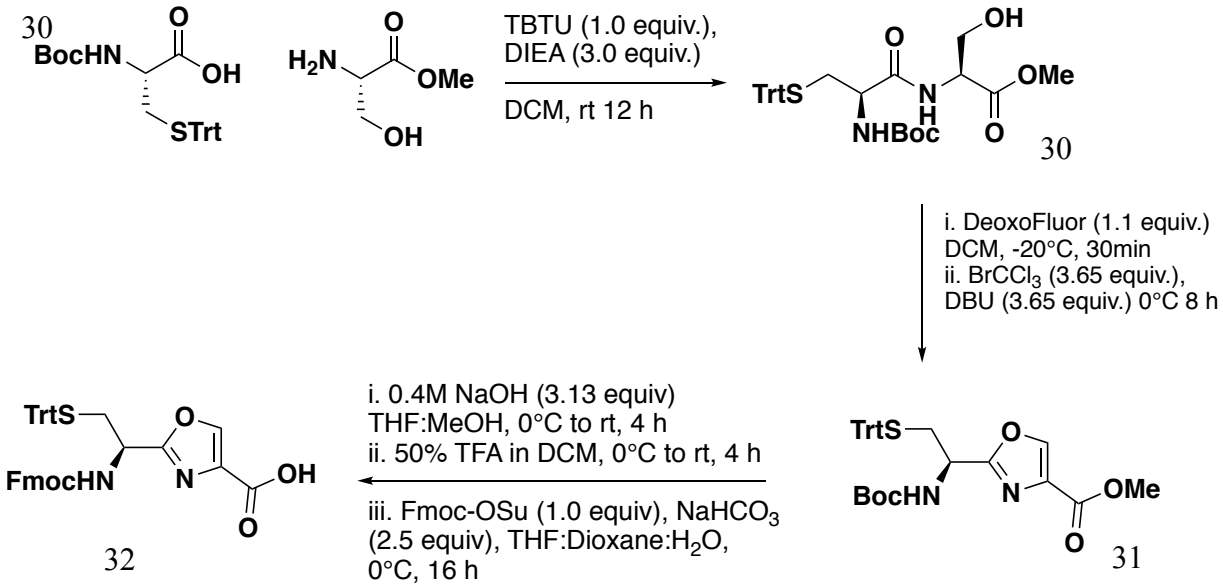
2'-((1*S*,2*S*)-1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylbutyl)-[2,4'-bithiazole]-4-carboxylic acid (29)



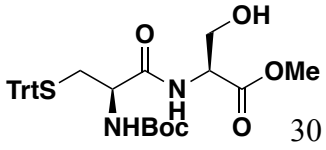
Compound 29 was prepared from 28 via general procedure D. Crude compound was purified via silica gel chromatography, eluting in 3% methanol in dichloromethane to give the titled compound as a fluffy white solid (0.85g, 55% yield over 3 steps).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (s, 1H), 8.26 (d, J = 9.8 Hz, 2H), 7.88 (d, J = 7.6 Hz, 2H), 7.72 (dd, J = 7.6, 4.1 Hz, 2H), 7.41 (td, J = 7.5, 2.7 Hz, 2H), 7.31 (t, J = 7.5 Hz, 2H), 4.77 (t, J = 7.9 Hz, 1H), 4.42 – 4.31 (m, 2H), 4.24 (t, J = 7.1 Hz, 1H), 2.04 (q, J = 9.7 Hz, 1H), 1.57 – 1.45 (m, 1H), 1.28 (dq, J = 15.9, 8.7, 8.2 Hz, 1H), 0.86 (t, J = 7.8 Hz, 6H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 175.25, 162.67, 162.46, 156.52, 148.55, 147.62, 144.16, 141.17, 129.35, 128.06, 127.46, 125.65, 120.53, 118.27, 66.07, 58.17, 47.16, 38.90, 25.15, 16.05, 11.36.
LC-MS ESI: $C_{27}H_{26}N_3O_4S_2^+$ $[M+H]^+$ Exact Mass: 520.1359; Observed: 520.1366



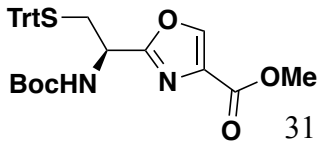
methyl N-(tert-butoxycarbonyl)-S-trityl-L-cysteinyl-L-serinate (30)



Compound 30 was prepared following general procedure A. The crude material was purified via silica gel chromatography, eluting in 45% ethyl acetate in hexanes to give the titled compound as a white solid (10.84g, 89% yield).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.44 – 7.41 (m, 5H), 7.34 – 7.20 (m, 10H), 6.79 (s, 1H), 4.87 (d, *J* = 7.3 Hz, 1H), 4.57 – 4.50 (m, 1H), 3.95 (d, *J* = 12.1 Hz, 1H), 3.86 (dd, *J* = 11.7, 3.5 Hz, 1H), 3.75 (d, *J* = 0.9 Hz, 3H), 3.56 (s, 1H), 2.77 (t, *J* = 10.5 Hz, 1H), 2.57 (dd, *J* = 13.2, 5.0 Hz, 1H), 1.40 (s, 9H).
^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.56, 170.45, 144.26, 129.52, 128.12, 127.92, 126.99, 67.28, 62.48, 55.02, 53.94, 52.71, 33.40, 28.27, 28.22.
LC-MS ESI: $C_{31}H_{36}N_2NaO_6S^+$ $[M+Na]^+$ Exact Mass: 587.2186; Observed: 587.2191

methyl (*R*)-2-(1-((*tert*-butoxycarbonyl)amino)-2-(tritylthio)ethyl)oxazole-4-carboxylate (31)

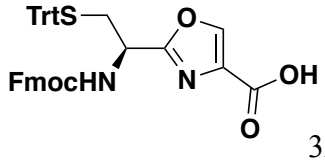


31

Compound 31 was prepared from 30 following General Procedure B. The crude material was purified by silica gel chromatography, eluting in 35% ethyl acetate in hexanes to afford the desired compound as a white solid (4.58g, 79% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.76 (s, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.37 – 7.22 (m, 15H), 4.47 (q, J = 7.8 Hz, 1H), 3.78 (s, 3H), 2.66 (qd, J = 12.5, 7.5 Hz, 2H), 1.35 (s, 9H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 164.06, 161.45, 155.18, 146.10, 144.60, 132.57, 129.54, 128.78, 128.59, 127.35, 79.10, 66.93, 52.26, 48.52, 34.42, 28.54.
LC-MS ESI: $C_{31}H_{32}N_2NaO_5S^+$ $[M+Na]^+$ Exact Mass: 567.1924; Observed: 567.1927

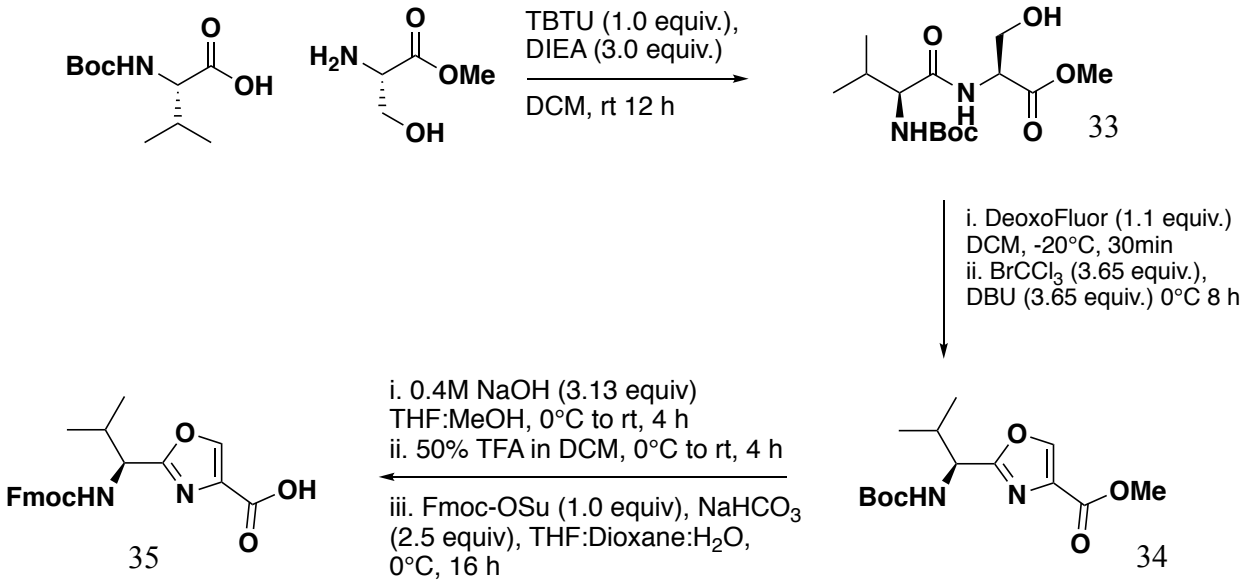
(*R*)-2-(1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-(tritylthio)ethyl)oxazole-4-carboxylic acid (32)



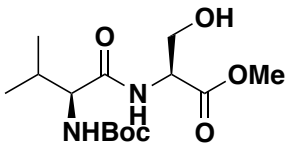
32

Compound 32 was prepared from 31 following general procedure C. The crude material was purified by silica gel chromatography, eluting in 5% methanol in dichloromethane to afford the desired compound as a fluffy white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.66 (s, 1H), 8.15 (d, J = 8.5 Hz, 1H), 7.88 (d, J = 7.5 Hz, 2H), 7.75 – 7.64 (m, 2H), 7.39 (tt, J = 7.7, 3.2 Hz, 2H), 7.37 – 7.28 (m, 12H), 7.28 – 7.22 (m, 4H), 4.47 (td, J = 8.6, 6.2 Hz, 1H), 4.35 – 4.24 (m, 2H), 4.22 (t, J = 7.1 Hz, 1H), 2.78 (dd, J = 12.7, 8.9 Hz, 1H), 2.66 (dd, J = 12.7, 6.3 Hz, 1H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 163.47, 162.32, 155.89, 145.84, 144.58, 144.17, 144.11, 141.17, 133.57, 129.56, 128.62, 128.11, 128.09, 127.52, 127.38, 125.71, 120.57, 66.96, 66.26, 48.87, 47.04, 34.32.
LC-MS ESI: $C_{40}H_{32}N_2NaO_5S^+$ $[M+Na]^+$ Exact Mass: 675.1924; Observed: 675.1928



methyl (*tert*-butoxycarbonyl)-*L*-valyl-*L*-serinate (33)

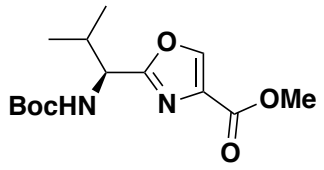


33

Compound 33 was prepared following general procedure A. The crude material was purified via silica gel chromatography, eluting in 40% ethyl acetate in hexanes to give the titled compound as a white solid (9.0g, 88% yield).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.38 – 7.33 (m, 1H), 5.50 – 5.42 (m, 1H), 4.63 (dt, *J* = 7.7, 3.5 Hz, 1H), 3.95 (ddt, *J* = 20.2, 10.9, 4.9 Hz, 2H), 3.83 (dt, *J* = 11.1, 2.8 Hz, 1H), 3.71 (d, *J* = 1.4 Hz, 3H), 2.04 (h, *J* = 6.3 Hz, 1H), 1.38 (s, 9H), 0.94 (dd, *J* = 6.8, 1.2 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H).
^{13}C NMR (126 MHz, Chloroform-*d*) δ 172.17, 170.80, 165.76, 156.19, 79.89, 62.53, 59.88, 54.65, 52.43, 38.58, 31.17, 28.26, 19.09, 17.89.
LC-MS ESI: $C_{14}H_{26}N_2NaO_6^+$ $[M+Na]^+$ Exact Mass: 341.1683; Observed: 341.1694.

methyl (*S*)-2-(1-((*tert*-butoxycarbonyl)amino)-2-methylpropyl)oxazole-4-carboxylate (34)

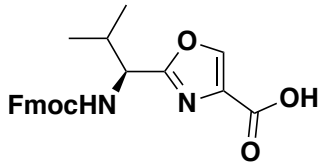


34

Compound 34 was prepared from 33 following general procedure B. The crude material was purified via silica gel chromatography, eluting in 20% ethyl acetate in hexanes to give the titled compound as a white solid (7.6g, 82% yield).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.19 (s, 1H), 5.30 (d, *J* = 9.3 Hz, 1H), 4.80 (dd, *J* = 9.4, 6.0 Hz, 1H), 3.92 (s, 3H), 2.20 (h, *J* = 6.9 Hz, 1H), 1.43 (s, 9H), 0.92 (t, *J* = 7.5 Hz, 6H).
^{13}C NMR (126 MHz, Chloroform-*d*) δ 165.18, 161.58, 155.36, 143.83, 133.16, 80.03, 54.26, 52.22, 32.93, 28.27, 18.68, 17.95.
LC-MS ESI: $C_{14}H_{22}N_2NaO_5^+$ $[M+Na]^+$ Exact Mass: 321.1421; Observed: 321.1425

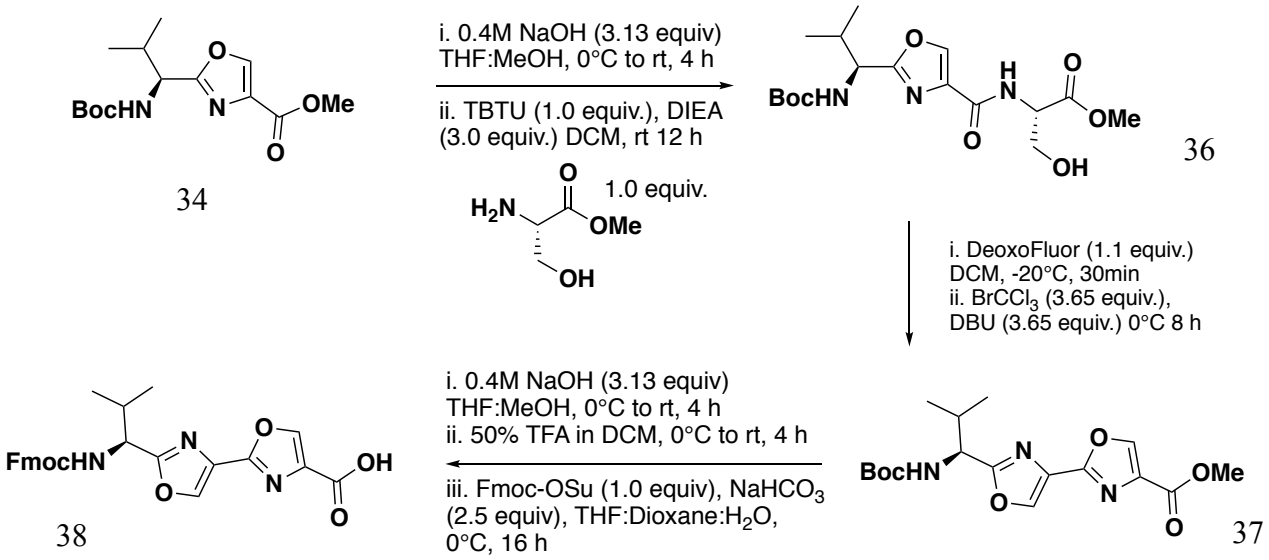
(*S*)-2-(1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylpropyl)oxazole-4-carboxylic acid (35)



35

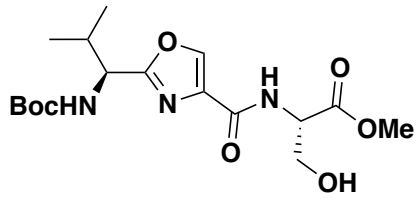
Compound 35 was prepared from 34 following general procedure C. The crude material was purified via silica gel chromatography, eluting in 2% methanol in dichloromethane to give the title compound as a white powder (13.0g, 88% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72 (d, *J* = 4.0 Hz, 1H), 8.14 (d, *J* = 8.6 Hz, 1H), 7.88 (d, *J* = 7.8 Hz, 2H), 7.73 (t, *J* = 8.1 Hz, 2H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.31 (d, *J* = 7.7 Hz, 2H), 4.53 (t, *J* = 8.2 Hz, 1H), 4.31 (dt, *J* = 28.0, 9.9 Hz, 2H), 4.24 (d, *J* = 7.2 Hz, 1H), 2.20 (h, *J* = 7.0 Hz, 1H), 0.96 (d, *J* = 6.7 Hz, 3H), 0.82 (d, *J* = 6.8 Hz, 3H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 164.76, 162.53, 156.58, 145.52, 144.22, 141.20, 141.18, 133.58, 128.12, 128.09, 127.52, 125.76, 120.58, 120.56, 66.23, 55.50, 47.14, 31.45, 19.48, 19.14.
LC-MS ESI: $C_{23}H_{22}N_2NaO_5^+$ $[M+Na]^+$ Exact Mass: 429.1421; Observed: 429.1425



34 36 37 38

methyl (2-((*S*)-1-((*tert*-butoxycarbonyl)amino)-2-methylpropyl)oxazole-4-carbonyl)-*L*-serinate (36)



36

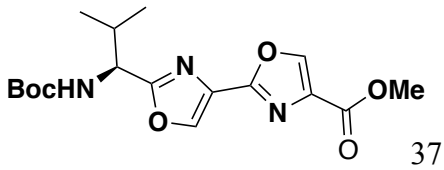
Compound 34 was dissolved in a 1:1 mixture of methanol and tetrahydrofuran (0.1 M) and cooled to 0°C. 1 M aq. NaOH (3.13 equiv.) was added dropwise and reaction stirred for 4 h at room temperature. The reaction was monitored by TLC and upon consumption of starting material the solution was concentrated *in vacuo*. The remaining aqueous solution was acidified with 1 N HCl until pH = 3.0. Acid derivative was extracted with ethyl acetate, dried with Na_2SO_4 and concentrated to yield a solid white powder.

The intermediate acid was then dissolved in dichloromethane (0.15 M) and TBTU (1.0 equiv.) and NH2-Ser(OH)-OMe (1.1 equiv.) were added as solids, scoop-wise and the solution was cooled to 0°C. Finally, DIPEA (3.0 equiv.) was added dropwise, the reaction was warmed to room temperature and stirred overnight. After ~12 h TLC showed all starting material had been consumed and the reaction was concentrated and immediately purified by silica gel chromatography (eluting at 40% ethyl acetate in hexanes) to yield the desired compound (36) as a white powder (10.0g, 97% yield).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.11 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 5.53 (d, *J* = 9.4 Hz, 1H), 4.81 (dt, *J* = 8.0, 3.8 Hz, 1H), 4.74 (dd, *J* = 9.3, 6.5 Hz, 1H), 4.13 – 4.05 (m, 1H), 4.00 (dd, *J* = 11.4, 3.4 Hz, 1H), 3.80 (s, 3H), 3.16 – 3.10 (m, 1H), 2.13 (h, *J* = 6.9 Hz, 1H), 1.45 (s, 9H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 170.59, 163.90, 160.82, 155.59, 141.48, 135.41, 80.46, 62.95, 54.57, 54.37, 52.70, 32.68, 28.35, 18.77, 18.10.
LC-MS ESI: $C_{17}H_{27}N_3NaO_7^+$ $[M+Na]^+$ Exact Mass: 408.1741; Observed: 408.1742

methyl (*S*)-2'-(1-((*tert*-butoxycarbonyl)amino)-2-methylpropyl)-[2,4'-bioxazole]-4-carboxylate (37)

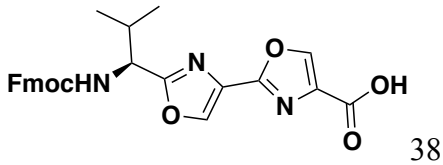


37

Compound 37 was prepared from 36 following general procedure B. The crude material was purified via silica gel chromatography, eluting 20% in ethyl acetate in hexanes to give the titled compound as a white solid (8.3g, 87% yield).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.29 (s, 1H), 8.27 (s, 1H), 5.33 (d, *J* = 9.4 Hz, 1H), 4.81 (dd, *J* = 9.5, 6.1 Hz, 1H), 3.92 (s, 3H), 2.24 – 2.17 (m, 1H), 1.41 (d, *J* = 4.2 Hz, 9H), 0.92 (t, *J* = 7.4 Hz, 6H).
^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.66, 161.30, 155.36, 143.71, 139.36, 134.29, 129.59, 80.07, 54.31, 52.29, 42.41, 32.85, 28.26, 18.67, 17.98.
LC-MS ESI: $C_{17}H_{23}N_3NaO_6^+$ $[M+Na]^+$ Exact Mass: 388.1479; Observed: 388.1490

(*S*)-2'-(1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylpropyl)-[2,4'-bioxazole]-4-carboxylic acid (38)

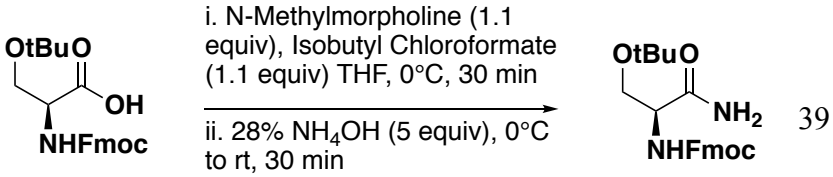


38

Compound 38 was prepared from 37 following general procedure C. The crude material was purified via silica gel chromatography, eluting in 5% methanol in dichloromethane to give the titled compound as a fluffy white solid (3.3g, 63% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.85 (s, 1H), 8.19 (d, *J* = 8.5 Hz, 1H), 7.87 (d, *J* = 7.6 Hz, 2H), 7.73 (t, *J* = 7.6 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.31 (td, *J* = 7.5, 2.3 Hz, 2H), 4.59 (t, *J* = 8.2 Hz, 1H), 4.36 – 4.28 (m, 2H), 4.23 (t, *J* = 7.1 Hz, 1H), 2.24 (hept, *J* = 6.9 Hz, 1H), 0.98 (d, *J* = 6.7 Hz, 3H), 0.85 (d, *J* = 6.7 Hz, 3H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 165.53, 162.26, 156.63, 155.48, 145.57, 144.21, 141.19, 141.18, 141.08, 134.75, 129.42, 128.10, 127.51, 125.75, 120.55, 66.25, 55.52, 47.14, 31.45, 19.47, 19.15.
LC-MS ESI: $C_{26}H_{24}N_3O_6^+$ $[M+Na]^+$ Exact Mass: 474.1660; Observed: 474.1662

(9*H*-fluoren-9-yl)methyl (*S*)-(1-amino-3-(*tert*-butoxy)-1-oxopropan-2-yl)carbamate (39)

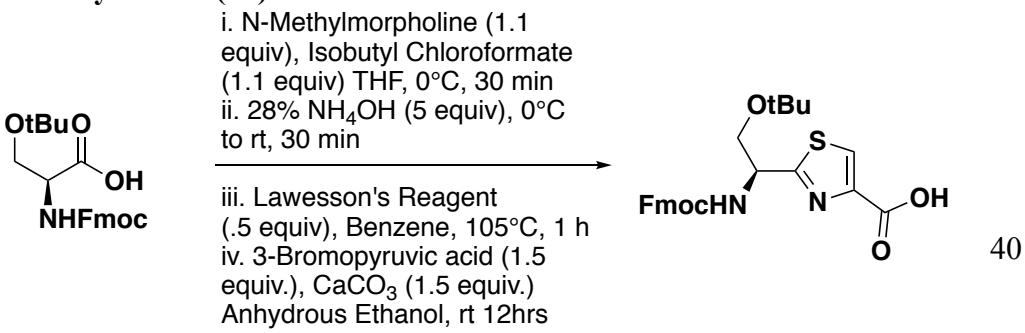


39

Starting material acid was dissolved in tetrahydrofuran (0.2 M), cooled to 0 °C and put under N_2. N-methyl morpholine (1.1 equiv.) was added dropwise to this solution followed by isobutyl chloroformate (1.1 equiv.). After 30 min, TLC shows full consumption of the starting material and the solution was again cooled to 0°C. Aqueous NH_4OH (28%, 5.0 equiv.) was added dropwise. After 30 min. TLC shows full consumption of the intermediate. The crude material was concentrated and then extracted with ethyl acetate and brine/citric acid. The combined organic layer was dried ($MgSO_4$), filtered, and concentrated yielding a white solid. Crude material was immediately carried to the next step without further purification. (2.0g, 98% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.87 (d, J = 7.6 Hz, 2H), 7.74 (d, J = 7.5 Hz, 2H), 7.41 (td, J = 7.5, 1.1 Hz, 2H), 7.36 (s, 1H), 7.32 (tt, J = 7.5, 1.5 Hz, 2H), 7.24 (d, J = 8.6 Hz, 1H), 7.17 (s, 1H), 4.34 – 4.21 (m, 3H), 4.11 (dt, J = 8.5, 5.7 Hz, 1H), 3.57 – 3.47 (m, 2H), 1.12 (s, 9H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 172.40, 156.33, 144.33, 144.27, 141.20, 141.18, 128.11, 127.52, 125.82, 125.79, 120.55, 73.20, 66.25, 62.44, 55.84, 47.14, 27.71.
LC-MS ESI: $C_{22}H_{26}N_2NaO_4^+$ $[M+Na]^+$ Exact Mass: 405.1785; Observed: 405.1793

(*S*)-2-(1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-(*tert*-butoxy)ethyl)thiazole-4-carboxylic acid (40)

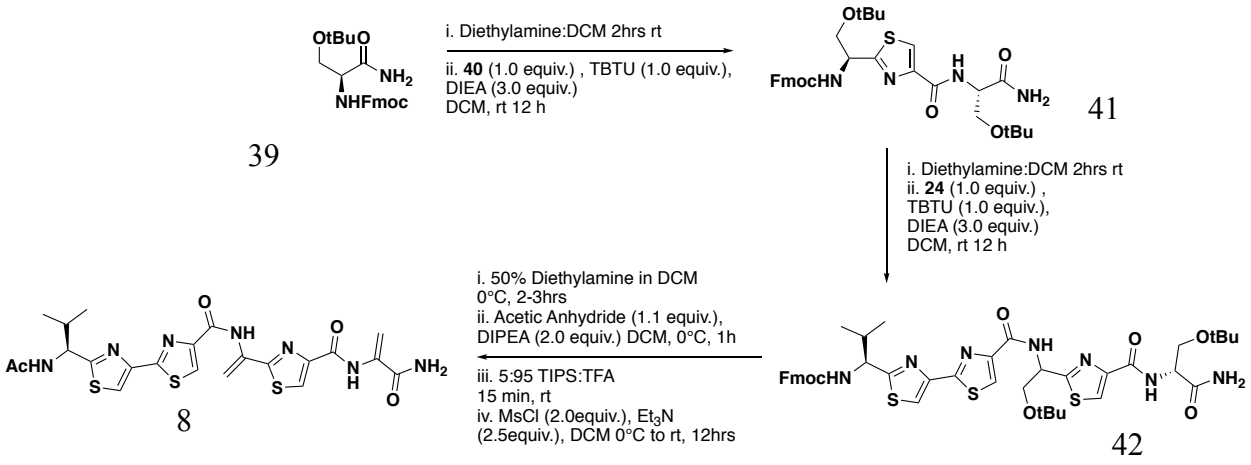


40

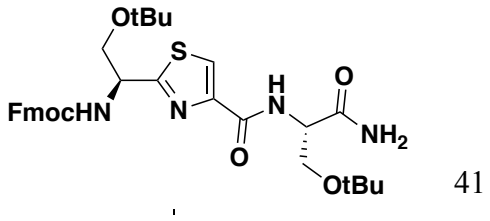
Compound 40 was prepared via general procedure D. Crude compound 40 was purified via silica gel chromatography, eluting in 5% methanol in dichloromethane to give the titled compound as a fluffy white solid (2.0g, 68% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.36 (s, 1H), 8.16 (d, J = 8.2 Hz, 1H), 7.89 (d, J = 7.6 Hz, 2H), 7.73 (d, J = 7.6 Hz, 2H), 7.40 (d, J = 8.0 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 4.92 (q, J = 7.0 Hz, 1H), 4.36 (q, J = 8.0, 5.8 Hz, 2H), 4.25 (t, J = 7.2 Hz, 1H), 3.75 (dd, J = 9.5, 5.3 Hz, 1H), 3.64 (t, J = 8.5 Hz, 1H), 1.12 (s, 9H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 171.97, 162.59, 156.44, 147.43, 144.23, 144.18, 141.20, 129.15, 128.12, 127.53, 125.71, 120.59, 73.63, 66.22, 63.52, 54.67, 47.13, 27.70.
LC-MS ESI: $C_{25}H_{26}N_2NaO_5S^+$ $[M+Na]^+$ Exact Mass: 489.1455; Observed: 489.1455.

4π Synthesis:



(9*H*-fluoren-9-yl)methyl((*S*)-1-(4-(((*S*)-1-amino-3-(*tert*-butoxy)-1-oxopropan-2-yl)carbamoyl)thiazol-2-yl)-2-(*tert*-butoxy)ethyl)carbamate (41)

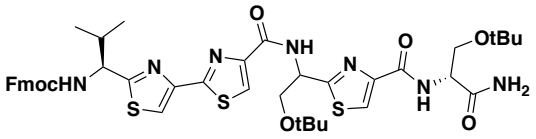


41

Compound 41 was prepared from 39 and 40 following general procedure E. The crude material was purified via silica gel chromatography, eluting in 100% ethyl acetate to give the titled compound as a white solid (1.3g, 54% yield).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.11 (d, *J* = 7.0 Hz, 1H), 8.05 (s, 1H), 7.75 (d, *J* = 13.1 Hz, 2H), 7.65 – 7.55 (m, 2H), 7.39 (q, *J* = 7.5, 6.9 Hz, 2H), 7.31 (q, *J* = 7.7, 6.8 Hz, 2H), 6.71 (s, 1H), 5.89 (d, *J* = 7.9 Hz, 1H), 5.62 (s, 1H), 5.17 (s, 1H), 4.63 (td, *J* = 7.6, 4.0 Hz, 1H), 4.53 – 4.42 (m, 2H), 4.26 (s, 1H), 3.93 (dd, *J* = 15.4, 6.4 Hz, 2H), 3.72 (dd, *J* = 9.0, 4.5 Hz, 1H), 3.47 (d, *J* = 8.4 Hz, 1H), 1.26 – 1.20 (m, 9H), 1.14 (s, 9H).
^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.57, 161.06, 141.31, 127.74, 127.08, 124.05, 120.01, 74.40, 73.98, 63.21, 61.25, 53.87, 52.89, 47.19, 27.46, 27.32, 27.25.
LC-MS ESI: $C_{32}H_{41}N_4O_6S^+$ $[M+H]^+$ Exact Mass: 609.2741; Observed: 607.2743

(9*H*-fluoren-9-yl)methyl ((1*S*)-1-(4-((1-(4-(((*R*)-1-amino-3-(*tert*-butoxy)-1-oxopropan-2-yl)carbamoyl)thiazol-2-yl)-2-(*tert*-butoxy)ethyl)carbamoyl)-[2,4'-bithiazol]-2'-yl)-2-methylpropyl)carbamate (42)

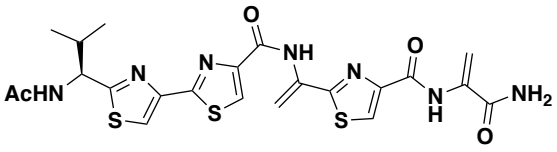


42

Compound 42 was prepared from 41 and 24 following general procedure E. The crude material was purified via silica gel chromatography, eluting in 100% ethyl acetate to give the titled compound as a white powder. (152mg, 67.2% yield).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.13 – 8.11 (m, 1H), 8.03 (d, *J* = 1.2 Hz, 1H), 7.93 (s, 1H), 7.72 – 7.67 (m, 2H), 7.55 (d, *J* = 7.5 Hz, 2H), 7.31 (d, *J* = 7.7 Hz, 2H), 7.23 (t, *J* = 7.3 Hz, 2H), 5.50 (t, *J* = 4.5 Hz, 1H), 4.85 (d, *J* = 6.1 Hz, 1H), 4.57 (dd, *J* = 7.2, 4.0 Hz, 1H), 4.42 (d, *J* = 6.1 Hz, 2H), 4.17 (t, *J* = 6.8 Hz, 1H), 3.97 (dd, *J* = 9.1, 3.7 Hz, 1H), 3.82 (dd, *J* = 8.9, 3.8 Hz, 1H), 3.75 (dd, *J* = 9.1, 5.3 Hz, 1H), 3.47 (dd, *J* = 8.9, 7.0 Hz, 1H), 2.32 (d, *J* = 6.1 Hz, 1H), 1.18 – 1.09 (m, 18H), 0.94 – 0.86 (m, 6H).
^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.84, 170.30, 162.81, 156.37, 149.63, 148.63, 148.26, 143.71, 143.62, 141.25, 127.67, 127.65, 127.02, 124.89, 124.60, 124.44, 119.90, 116.78, 74.12, 74.10, 66.68, 63.09, 61.42, 58.39, 51.79, 49.52, 49.31, 49.10, 48.88, 48.67, 47.22, 38.47, 33.22, 27.27, 27.23, 27.21, 19.26, 17.53.
LC-MS ESI: $C_{43}H_{52}N_7O_7S_3^+$ $[M+H]^+$ Exact Mass: 874.3085; Observed: 874.3094

(*S*)-2'-(1-acetamido-2-methylpropyl)-*N*-(1-(4-((3-amino-3-oxoprop-1-en-2-yl)carbamoyl)thiazol-2-yl)vinyl)-[2,4'-bithiazole]-4-carboxamide (8)

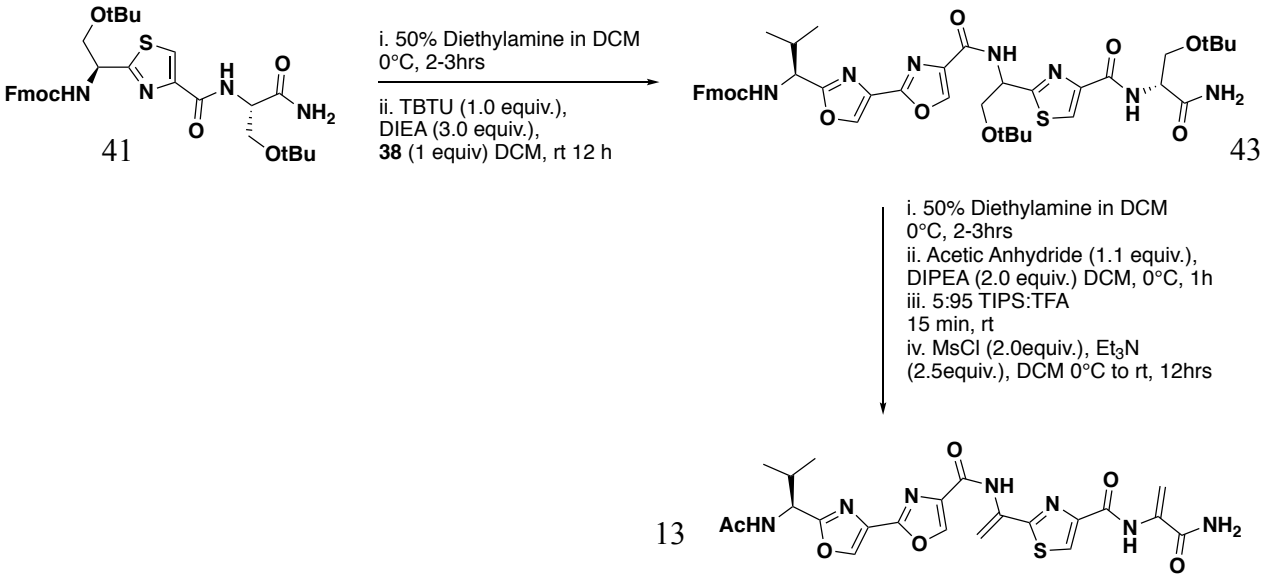


8

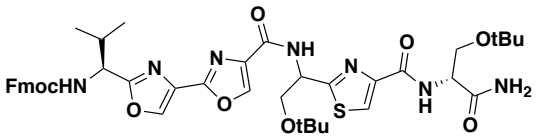
Compound 8 was prepared from 42 following general procedure F and G. The crude material was purified via reverse-phase chromatography (acetonitrile and water with 0.1% trifluoroacetic acid as solvents), eluting in 100% acetonitrile. The material was concentrated and diluted with water and then lyophilized to afford the title compound as a fluffy white solid (100mg, 53% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.07 (d, *J* = 1.3 Hz, 1H), 9.95 (d, *J* = 1.3 Hz, 1H), 8.58 (d, *J* = 8.3 Hz, 1H), 8.53 (s, 1H), 8.52 (s, 1H), 8.51 (s, 1H), 8.22 (s, 1H), 7.71 (s, 1H), 6.51 (dd, *J* = 9.5, 1.3 Hz, 2H), 5.81 (dt, *J* = 13.2, 1.3 Hz, 2H), 5.00 (dd, *J* = 8.3, 6.7 Hz, 1H), 2.31 (dt, *J* = 13.5, 6.7 Hz, 1H), 1.96 (s, 3H), 0.95 (dd, *J* = 21.7, 6.8 Hz, 6H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 175.08, 170.15, 165.93, 165.41, 159.56, 158.80, 149.89, 149.48, 147.33, 134.08, 133.99, 127.22, 126.84, 118.83, 105.52, 103.46, 56.75, 32.54, 31.13, 22.86, 19.78, 18.55.
LC-MS ESI: $C_{22}H_{24}N_7O_4S_3^+$ $[M+H]^+$ Exact Mass: 546.1046; Observed: 546.1040



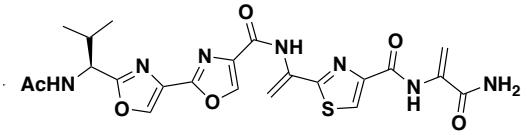
(9*H*-fluoren-9-yl)methyl((1*S*)-1-(4-((1-(4-(((*R*)-1-amino-3-(*tert*-butoxy)-1-oxopropan-2-yl)carbamoyl)thiazol-2-yl)-2-(*tert*-butoxy)ethyl)carbamoyl)-[2,4'-bioxazol]-2'-yl)-2-methylpropyl)carbamate (43)


43

Compound 43 was prepared from 41 and 38 following general procedure E. The crude material was purified via silica gel chromatography, eluting in 100% ethyl acetate to give the title compound as a white solid (156mg, 72% yield).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.31 (s, 1H), 8.28 (d, *J* = 3.2 Hz, 1H), 8.19 – 8.13 (m, 1H), 8.06 (d, *J* = 1.7 Hz, 1H), 7.75 – 7.69 (m, 2H), 7.58 (d, *J* = 7.4 Hz, 2H), 7.40 – 7.31 (m, 2H), 7.29 – 7.23 (m, 2H), 6.61 (s, 1H), 6.17 (d, *J* = 9.4 Hz, 1H), 5.98 (s, 1H), 5.52 (q, *J* = 5.8 Hz, 1H), 4.87 (dd, *J* = 9.2, 7.0 Hz, 1H), 4.65 (td, *J* = 7.2, 3.7 Hz, 1H), 4.48 – 4.43 (m, 1H), 4.39 (dd, *J* = 10.7, 6.7 Hz, 1H), 4.20 (t, *J* = 6.7 Hz, 1H), 3.96 (ddt, *J* = 21.5, 8.7, 3.9 Hz, 2H), 3.66 (dd, *J* = 9.0, 6.1 Hz, 1H), 3.52 – 3.45 (m, 1H), 2.31 – 2.21 (m, 1H), 1.25 – 1.12 (m, 16H), 0.99 (d, *J* = 6.7 Hz, 2H), 0.96 – 0.88 (m, 3H).
^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.79, 165.83, 160.99, 156.17, 143.77, 143.65, 141.53, 141.29, 139.47, 136.80, 129.80, 127.70, 127.05, 125.04, 124.29, 119.96, 74.10, 66.96, 63.13, 61.34, 55.05, 52.92, 51.91, 47.19, 38.66, 32.84, 27.45, 27.42, 27.36, 18.82, 18.30.
LC-MS ESI: $C_{43}H_{52}N_7O_9S^+$ $[M+H]^+$ Exact Mass: 842.3542; Observed: 842.3587.

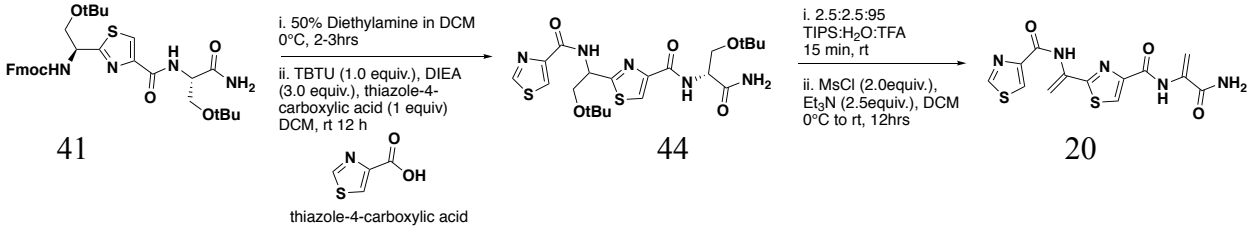
(*S*)-2'-(1-acetamido-2-methylpropyl)-*N*-(1-(4-((3-amino-3-oxoprop-1-en-2-yl)carbamoyl)thiazol-2-yl)vinyl)-[2,4'-bioxazole]-4-carboxamide (13)



13

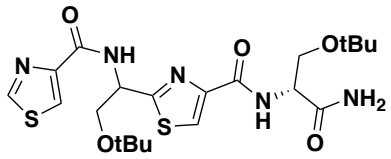
Compound 13 was prepared from 43 following general procedure F then G. The crude material was purified via reverse-phase chromatography, eluting in 54% acetonitrile in water (0.1% trifluoroacetic acid), and lyophilized to afford the title compound as a fluffy white solid (112mg, 35% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.94 (d, J = 1.3 Hz, 1H), 9.91 (s, 1H), 9.09 (s, 1H), 8.93 (s, 1H), 8.53 (d, J = 8.4 Hz, 1H), 8.49 (s, 1H), 8.21 (s, 1H), 7.74 (s, 1H), 6.52 (d, J = 1.1 Hz, 1H), 6.30 (d, J = 1.4 Hz, 1H), 5.81 (dt, J = 4.3, 1.2 Hz, 2H), 4.87 (dd, J = 8.4, 7.5 Hz, 1H), 2.20 (dq, J = 13.7, 6.8 Hz, 1H), 1.92 (s, 3H), 0.96 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 6.8 Hz, 3H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 169.92, 165.81, 165.57, 165.52, 159.12, 158.77, 149.34, 143.58, 141.44, 136.66, 134.07, 133.98, 129.24, 127.09, 106.69, 103.22, 52.92, 41.80, 31.68, 31.58, 22.75, 19.41, 19.35, 18.99, 11.45.
LC-MS ESI: $C_{22}H_{24}N_7O_6S^+$ $[M+H]^+$ Exact Mass: 514.1503; Observed: 514.1512



41 44 20

_N_-((_R_)-1-amino-3-(_tert_-butoxy)-1-oxopropan-2-yl)-2-(2-(_tert_-butoxy)-1-(thiazole-4-carboxamido)ethyl)thiazole-4-carboxamide (44)



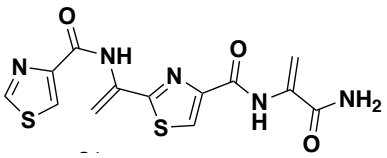
44

Compound 44 was prepared from 41 and thiazole-4-carboxylic acid following general procedure E. The crude material was purified via silica gel chromatography, eluting in 100% ethyl acetate to give the title compound as a white solid (101mg, 78% yield).

^{1}H NMR (400 MHz, Chloroform-d) δ 8.78 (dd, J = 2.1, 0.8 Hz, 1H), 8.20 (dd, J = 2.2, 1.0 Hz, 1H), 8.02 (d, J = 0.9 Hz, 1H), 5.52 (td, J = 3.9, 2.0 Hz, 1H), 4.57 (ddd, J = 7.0, 3.9, 1.3 Hz, 1H), 3.95 (ddd, J = 9.0, 3.8, 1.2 Hz, 1H), 3.84 (ddd, J = 8.9, 4.0, 1.6 Hz, 1H), 3.74 (ddd, J = 9.2, 4.9, 0.9 Hz, 1H), 3.48 (dd, J = 9.0, 7.0 Hz, 1H), 2.96 (s, 4H), 1.18 – 1.14 (m, 9H), 1.13 (d, J = 1.1 Hz, 9H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.43, 161.26, 160.87, 153.23, 150.06, 148.62, 124.43, 124.29, 74.12, 74.08, 63.07, 61.35, 52.92, 51.87, 51.78, 49.43, 49.24, 49.03, 48.79, 38.50, 27.27, 27.22.
LC-MS ESI: $C_{21}H_{31}N_5NaO_5S_2^+$ $[M+H]^+$ Exact Mass: 520.1659; Observed: 520.1670

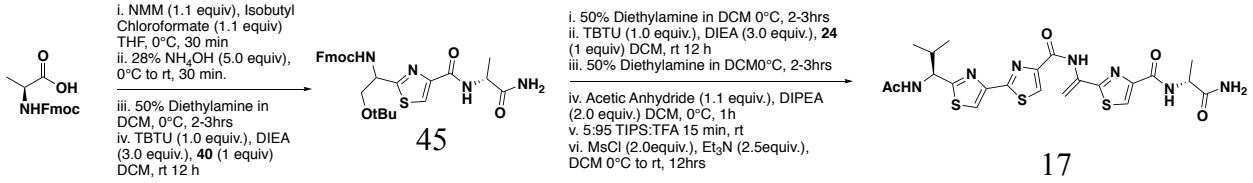
***N*-(3-amino-3-oxoprop-1-en-2-yl)-2-(1-(thiazole-4-carboxamido)vinyl)thiazole-4-carboxamide (20)**



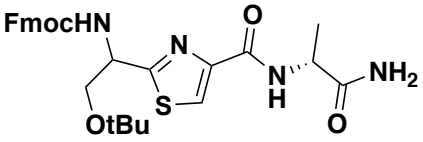
20

Compound 20 was prepared from 44 following general procedure G. The crude material was purified via silica gel chromatography, eluting in 50% ethyl acetate in hexanes to give the title compound as a white solid (37mg, 26% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.37 (s, 1H), 9.93 (s, 1H), 9.17 (dd, J = 2.0, 0.9 Hz, 1H), 8.54 (dd, J = 2.1, 0.9 Hz, 1H), 8.46 (d, J = 0.9 Hz, 1H), 8.10 (s, 1H), 7.61 (s, 1H), 6.48 (s, 1H), 6.29 (d, J = 1.3 Hz, 1H), 5.75 (d, J = 1.3 Hz, 2H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 165.81, 165.28, 159.65, 158.80, 155.84, 150.02, 149.23, 134.23, 134.14, 127.06, 126.42, 105.59, 102.99.
LC-MS ESI: $C_{13}H_{11}N_5NaO_3S_2^+$ $[M+Na]^+$ Exact Mass: 372.0196; Observed: 372.0196



(9*H*-fluoren-9-yl)methyl (1-(4-(((*R*)-1-amino-1-oxopropan-2-yl)carbamoyl)thiazol-2-yl)-2-(*tert*-butoxy)ethyl)carbamate (45)



45

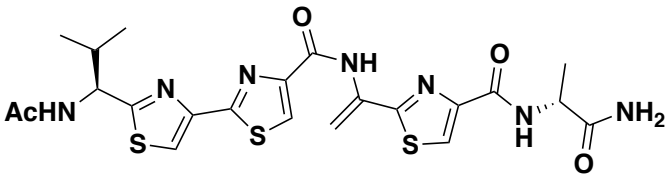
Starting material acid was dissolved in tetrahydrofuran (0.2 M), cooled to 0 °C and put under N_2. N-methyl morpholine (1.1 equiv.) was added dropwise to this solution followed by isobutyl chloroformate (1.1 equiv.). After 30 min, TLC shows full consumption of the starting material and the solution was again cooled to 0°C. Aqueous NH_4OH (28%, 5.0 equiv.) was added dropwise. After 30 min. TLC shows full consumption of the intermediate. The crude material was concentrated and then extracted with ethyl acetate and brine/citric acid. The combined organic

layer was dried ($MgSO_4$), filtered, and concentrated yielding a white solid. Crude material was immediately carried to the next step without further purification.

This was followed by procedure E, using 40 as the coupling partner. The crude material was purified via silica gel chromatography, eluting in 90% ethyl acetate in hexanes to give the title compound as a white solid. (153mg, 88% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 (s, 1H), 8.16 (d, J = 8.1 Hz, 1H), 8.10 (d, J = 7.6 Hz, 1H), 7.89 (d, J = 7.6 Hz, 2H), 7.75 – 7.70 (m, 2H), 7.58 – 7.55 (m, 1H), 7.41 (h, J = 4.7, 4.2 Hz, 2H), 7.33 (t, J = 7.6 Hz, 2H), 7.20 – 7.17 (m, 1H), 4.94 (q, J = 6.9 Hz, 1H), 4.46 – 4.41 (m, 1H), 4.37 (t, J = 7.3 Hz, 2H), 4.25 (t, J = 6.9 Hz, 1H), 3.75 (dd, J = 9.3, 5.5 Hz, 1H), 3.66 (t, J = 8.3 Hz, 1H), 1.34 (d, J = 7.0 Hz, 3H), 1.13 (s, 9H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 174.16, 171.98, 160.02, 156.43, 149.51, 144.22, 141.22, 128.12, 127.54, 125.71, 124.77, 120.60, 73.67, 66.22, 63.61, 54.65, 48.31, 47.14, 38.71, 27.71, 19.52.
LC-MS ESI: $C_{28}H_{32}N_4NaO_5S^+$ $[M+Na]^+$ Exact Mass: 559.1986; Observed: 559.1996

2'-((*S*)-1-acetamido-2-methylpropyl)-*N*-(1-(4-(((*R*)-1-amino-1-oxopropan-2-yl)carbamoyl)thiazol-2-yl)vinyl)-[2,4'-bithiazole]-4-carboxamide (17)

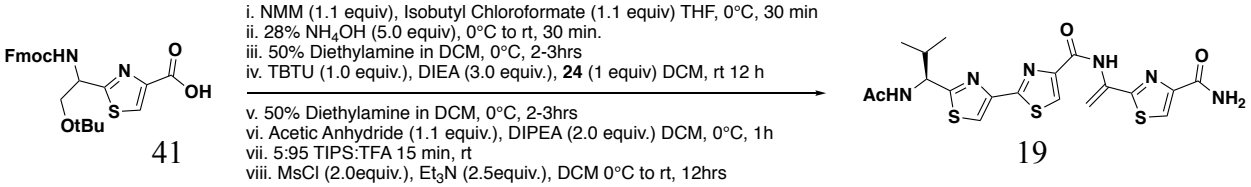


17

Compound 17 was prepared from 45 and 24 following general procedure E. Crude intermediate material was pushed through a silica plug with ethyl acetate. The intermediate material is moved to the through the next steps immediately, following procedures F then G. The crude material was purified via reverse-phase chromatography, eluting in 55% acetonitrile in water (0.1% trifluoroacetic acid), and lyophilized to afford the title compound as a fluffy white solid (56 mg, 41% yield over 6 steps).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.21 (d, J = 2.5 Hz, 1H), 8.60 – 8.51 (m, 2H), 8.49 (s, 1H), 8.37 (d, J = 1.3 Hz, 1H), 8.20 – 8.11 (m, 1H), 7.70 (s, 1H), 7.28 (d, J = 16.8 Hz, 1H), 6.50 (dd, J = 4.2, 1.4 Hz, 1H), 5.71 (s, 1H), 4.98 (ddt, J = 8.8, 5.3, 2.5 Hz, 1H), 4.60 – 4.48 (m, 1H), 2.35 – 2.25 (m, 1H), 1.36 (d, J = 7.0 Hz, 3H), 0.99 – 0.84 (m, 6H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 175.39, 174.31, 170.19, 170.15, 165.34, 163.04, 159.55, 159.39, 149.87, 149.60, 147.42, 134.10, 126.61, 126.23, 126.20, 119.01, 104.19, 104.14, 56.74, 48.24, 48.22, 32.56, 22.86, 22.84, 19.77, 19.75, 18.54, 18.45.
LC-MS ESI: $C_{22}H_{26}N_7O_4S_3^+$ $[M+H]^+$ Exact Mass: 548.1203; Observed: 548.1209

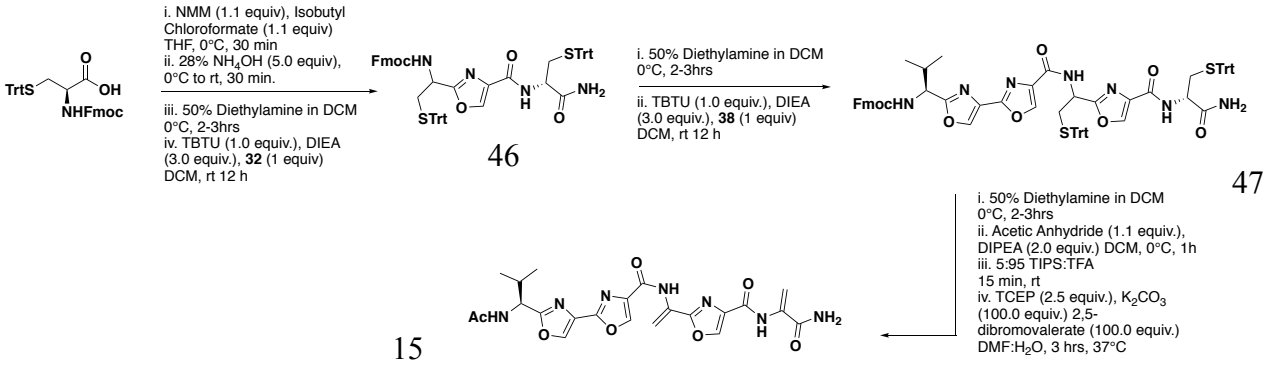
(*S*)-2'-(1-acetamido-2-methylpropyl)-*N*-(1-(4-carbamoylthiazol-2-yl)vinyl)-[2,4'-bithiazole]-4-carboxamide (19)



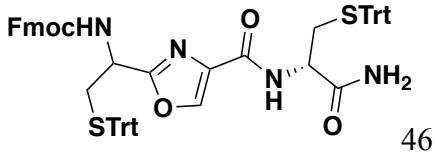
Starting material 41 was dissolved in tetrahydrofuran (0.2 M), cooled to 0 °C and put under N_2. N-methyl morpholine (1.1 equiv.) was added dropwise to this solution followed by isobutyl chloroformate (1.1 equiv.). After 30 min, TLC shows full consumption of the starting material and the solution was again cooled to 0°C. Aqueous NH_4OH (28%, 5.0 equiv.) was added dropwise. After 30 min. TLC shows full consumption of the intermediate. The crude material was concentrated and then extracted with ethyl acetate and brine/citric acid. The combined organic layer was dried ($MgSO_4$), filtered, and concentrated yielding a white solid. Crude material was immediately carried to the next step without further purification.

Intermediate amide was deprotected and coupled to 24 following general procedure E. Crude intermediate material was pushed through a silica plug with ethyl acetate. The intermediate material is moved to the through the next steps immediately, following procedures F then G. The crude material was purified via reverse-phase chromatography, eluting in 55% acetonitrile in water (0.1% trifluoroacetic acid), and lyophilized to afford the title compound as a fluffy white solid (20 mg, 16% yield over 8 steps).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.60 (d, J = 8.4 Hz, 1H), 8.52 (s, 1H), 8.33 (s, 1H), 8.33 (s, 1H), 7.85 (s, 1H), 7.72 (s, 1H), 6.53 (d, J = 1.9 Hz, 1H), 6.35 (d, J = 1.3 Hz, 1H), 5.76 (t, J = 1.2 Hz, 1H), 5.00 (dd, J = 8.4, 6.7 Hz, 1H), 2.34 – 2.28 (m, 1H), 1.96 (s, 3H), 0.97 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 175.34, 170.31, 170.29, 164.95, 162.78, 162.45, 159.59, 150.34, 150.00, 147.65, 134.28, 134.21, 129.85, 126.48, 125.86, 119.45, 118.30, 104.91, 56.79, 32.58, 32.55, 22.81, 19.73, 19.70, 18.53, 14.64.
LC-MS ESI: $C_{19}H_{21}N_6O_3S_3^+$ $[M+H]^+$ Exact Mass: 477.0832; Observed: 477.0836



(9*H*-fluoren-9-yl)methyl(1-(4-(((*S*)-1-amino-1-oxo-3-(tritylthio)propan-2-yl)carbamoyl)oxazol-2-yl)-2-(tritylthio)ethyl)carbamate (46)



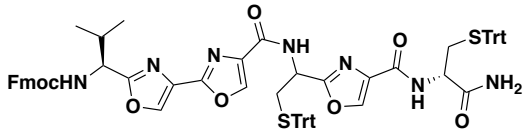
46

Starting material Fmoc-Cys(Trt)-OH was dissolved in tetrahydrofuran (0.2 M), cooled to 0 °C and put under N_2. N-methyl morpholine (1.1 equiv.) was added dropwise to this solution followed by isobutyl chloroformate (1.1 equiv.). After 30 min, TLC shows full consumption of the starting material and the solution was again cooled to 0°C. Aqueous NH_4OH (28%, 5.0 equiv.) was added dropwise. After 30 min. TLC shows full consumption of the intermediate. The crude material was concentrated and then extracted with ethyl acetate and brine/citric acid. The combined organic layer was dried ($MgSO_4$), filtered, and concentrated yielding a white solid. Crude material was immediately carried to the next step without further purification.

This was followed by procedure E with coupling partner 32. The crude material was purified via silica gel chromatography, eluting in 50% ethyl acetate in hexanes to give the title compound as a white solid. (300mg, 70% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.61 (s, 1H), 8.23 (d, J = 8.3 Hz, 1H), 7.87 (d, J = 7.6 Hz, 2H), 7.82 (d, J = 8.2 Hz, 1H), 7.68 (dd, J = 7.5, 2.9 Hz, 2H), 7.60 (s, 1H), 7.38 (td, J = 7.5, 2.7 Hz, 2H), 7.34 – 7.18 (m, 32H), 4.54 (dq, J = 23.6, 7.9, 7.4 Hz, 2H), 4.31 (qd, J = 10.6, 7.2 Hz, 2H), 4.21 (t, J = 7.1 Hz, 1H), 2.81 (dd, J = 12.7, 8.4 Hz, 1H), 2.69 (dd, J = 12.6, 6.7 Hz, 1H), 2.60 – 2.47 (m, 3H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 171.29, 163.10, 159.43, 144.66, 144.52, 144.16, 144.12, 142.79, 141.19, 129.55, 129.50, 128.61, 128.50, 128.11, 128.08, 127.51, 127.36, 127.23, 125.69, 120.56, 66.28, 50.98, 49.03, 47.06, 34.88, 34.47.
LC-MS ESI: $C_{62}H_{53}N_4O_5S_2^+$ $[M+H]^+$ Exact Mass: 997.3452; Observed: 997.3479

(9*H*-fluoren-9-yl)methyl ((1*S*)-1-(4-((1-(4-(((*S*)-1-amino-1-oxo-3-(tritylthio)propan-2-yl)carbamoyl)oxazol-2-yl)-2-(tritylthio)ethyl)carbamoyl)-[2,4'-bioxazol]-2'-yl)-2-methylpropyl)carbamate (47)

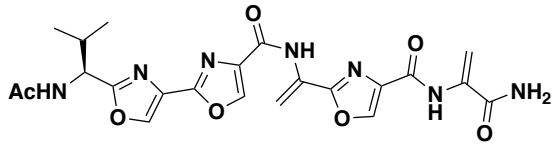


47

Compound 47 was prepared from 46 and 38 following general procedure E. The crude material was purified via silica gel chromatography, eluting in 65% ethyl acetate in hexanes to give the title compound as a white solid. (300mg, 90% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.13 (d, J = 8.4 Hz, 1H), 8.88 – 8.80 (m, 1H), 8.75 (d, J = 2.6 Hz, 1H), 8.60 (d, J = 2.3 Hz, 1H), 8.16 (d, J = 8.2 Hz, 1H), 7.88 (d, J = 7.8 Hz, 2H), 7.82 (d, J = 8.1 Hz, 1H), 7.73 (t, J = 6.6 Hz, 2H), 7.55 (d, J = 16.2 Hz, 1H), 7.40 (t, J = 8.0 Hz, 2H), 7.34 – 7.21 (m, 31H), 5.02 (q, J = 7.7 Hz, 1H), 4.58 (q, J = 9.5, 8.9 Hz, 1H), 4.54 (d, J = 6.9 Hz, 1H), 4.38 (dd, J = 10.4, 7.0 Hz, 1H), 4.29 (dd, J = 10.4, 7.3 Hz, 1H), 4.23 (t, J = 6.9 Hz, 1H), 3.06 (dd, J = 12.5, 8.3 Hz, 1H), 2.84 (dt, J = 16.1, 7.8 Hz, 1H), 2.24 (h, J = 6.8, 6.3 Hz, 1H), 0.98 (d, J = 6.8 Hz, 3H), 0.85 (d, J = 6.8 Hz, 3H).
^{13}C NMR (126 MHz, Chloroform-*d*) δ 149.40, 149.34, 148.95, 145.95, 134.33, 134.24, 133.34, 133.23, 132.86, 132.25, 132.10, 131.95, 130.49, 125.32, 70.98, 51.90, 45.23, 45.06, 44.90, 44.73, 44.56, 44.39, 44.23, 43.44, 24.23, 23.93.
LC-MS ESI: $C_{73}H_{64}N_7O_8S_2^+$ $[M+H]^+$ Exact Mass: 1230.4252; Observed: 1230.4257

(*S*)-2'-(1-acetamido-2-methylpropyl)-*N*-(1-(4-((3-amino-3-oxoprop-1-en-2-yl)carbamoyl)oxazol-2-yl)vinyl)-[2,4'-bioxazole]-4-carboxamide (15)

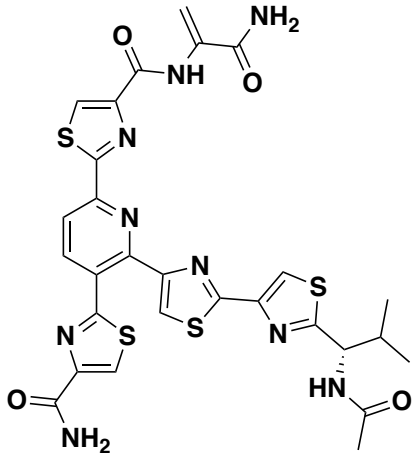


15

Compound 15 was prepared via general procedure F then H. The crude material was purified via flash column chromatography, eluting in 10% methanol in dichloromethane and concentrated to afford the title compound as a fluffy white solid (24 mg, 33% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.84 (s, 1H), 9.63 (s, 1H), 9.00 (d, J = 2.8 Hz, 1H), 8.92 (d, J = 2.8 Hz, 1H), 8.89 (s, 1H), 8.54 (d, J = 8.3 Hz, 1H), 8.19 (s, 1H), 7.70 (s, 1H), 6.47 (s, 1H), 6.24 (s, 1H), 5.80 (d, J = 17.6 Hz, 2H), 4.87 (t, J = 8.0 Hz, 1H), 2.20 (h, J = 7.3 Hz, 1H), 1.92 (d, J = 2.8 Hz, 3H), 0.97 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 169.93, 165.73, 165.33, 159.13, 158.39, 158.31, 155.14, 144.08, 143.58, 136.64, 136.51, 133.95, 129.27, 128.77, 107.73, 103.23, 52.97, 52.91, 31.69, 22.77, 19.43, 19.02.
LC-MS ESI: $C_{22}H_{24}N_7O_7^+$ $[M+H]^+$ Exact Mass: 498.1732; Observed: 498.1736.

(*S*)-2-(6-(2'-(1-acetamido-2-methylpropyl)-[2,4'-bithiazol]-4-yl)-5-(4-carbamoylthiazol-2-yl)pyridin-2-yl)-*N*-(3-amino-3-oxoprop-1-en-2-yl)thiazole-4-carboxamide (9)



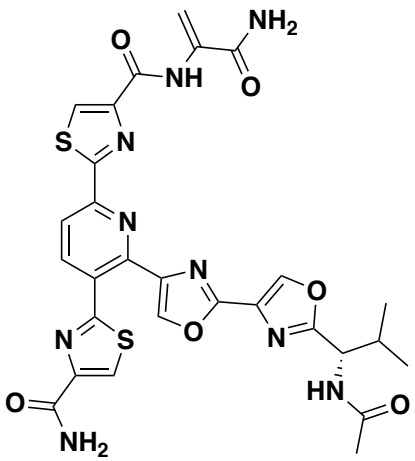
Compound 9 was prepared by procedure described in section X.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.11 (s, 1H), 8.78 (d, J = 8.2 Hz, 1H), 8.63 (s, 1H), 8.60 (d, J = 8.3 Hz, 1H), 8.33 (s, 1H), 8.31 (d, J = 8.7 Hz, 1H), 8.17 (s, 1H), 7.89 (s, 1H), 7.83 (s, 1H), 7.68 (d, J = 11.9 Hz, 1H), 7.63 (s, 1H), 6.49 (s, 1H), 5.79 (s, 1H), 4.99 – 4.95 (m, 1H), 2.31 – 2.26 (m, 1H), 1.94 (s, 3H), 0.94 (dd, J = 22.0, 6.8 Hz, 6H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 175.24, 170.26, 167.99, 165.42, 163.74, 162.65, 162.55, 159.03, 153.08, 151.37, 151.04, 150.46, 150.15, 148.03, 140.42, 134.27, 130.00, 128.98, 127.38, 123.38, 118.89, 116.53, 63.51, 56.84, 32.57, 22.83, 19.81, 18.60.

LC-MS ESI: $C_{28}H_{26}N_9O_4S_4^+$ $[M+H]^+$ Exact Mass: 680.0985; Observed: 680.0987

(*S*)-2-(6-(2'-(1-acetamido-2-methylpropyl)-[2,4'-bioxazol]-4-yl)-5-(4-carbamoylthiazol-2-yl)pyridin-2-yl)-*N*-(3-amino-3-oxoprop-1-en-2-yl)thiazole-4-carboxamide (14)



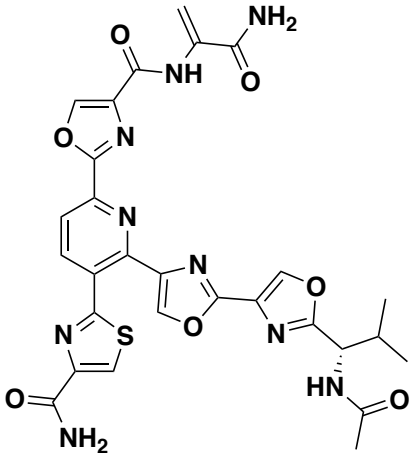
Compound 14 was prepared by procedure described in section X.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.11 (s, 1H), 8.78 (s, 1H), 8.76 (d, J = 8.3 Hz, 1H), 8.67 (s, 1H), 8.64 (s, 1H), 8.53 (d, J = 8.5 Hz, 1H), 8.37 (s, 1H), 8.31 (d, J = 8.3 Hz, 1H), 8.16 (s, 1H), 7.92 (s, 1H), 7.67 (s, 2H), 6.49 (s, 1H), 5.79 (s, 1H), 4.85 (t, J = 8.0 Hz, 1H), 2.20 – 2.15 (m, 1H), 1.91 (s, 3H), 0.95 (d, J = 6.7 Hz, 3H), 0.85 (d, J = 6.7 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 169.46, 167.36, 165.08, 164.93, 162.52, 162.11, 158.54, 158.06, 154.70, 150.61, 150.29, 149.87, 148.00, 140.16, 139.90, 138.57, 133.82, 129.80, 129.09, 128.58, 127.05, 102.70, 52.44, 31.21, 22.31, 18.95, 18.56.

LC-MS ESI: $C_{28}H_{26}N_9O_6S_2^+$ $[M+H]^+$ Exact Mass: 648.1442; Observed: 648.1447

(*S*)-2-(6-(2'-(1-acetamido-2-methylpropyl)-[2,4'-bioxazol]-4-yl)-5-(4-carbamoylthiazol-2-yl)pyridin-2-yl)-*N*-(3-amino-3-oxoprop-1-en-2-yl)oxazole-4-carboxamide (16)



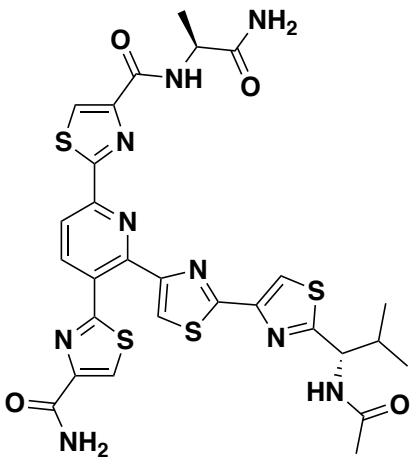
Compound 16 was prepared by procedure described in section X.

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.68 (s, 1H), 9.04 (s, 1H), 8.79 (s, 1H), 8.74 (d, J = 8.3 Hz, 1H), 8.64 (s, 1H), 8.53 (d, J = 8.5 Hz, 1H), 8.38 (s, 1H), 8.33 (d, J = 8.3 Hz, 1H), 8.16 (s, 1H), 7.94 (s, 1H), 7.69 (s, 1H), 7.66 (s, 1H), 6.47 (s, 1H), 5.78 (s, 1H), 4.85 (t, J = 7.9 Hz, 1H), 2.16 (dt, J = 13.8, 6.9 Hz, 1H), 1.91 (s, 3H), 0.94 (d, J = 6.7 Hz, 3H), 0.85 (d, J = 6.7 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 169.93, 165.51, 165.27, 162.86, 162.55, 159.39, 158.54, 155.13, 150.81, 148.71, 145.19, 144.96, 140.60, 140.29, 140.21, 139.02, 137.72, 134.02, 130.34, 129.54, 127.66, 122.67, 103.39, 52.86, 31.66, 22.76, 19.41, 19.00.

LC-MS ESI: $C_{28}H_{26}N_9O_7S^+$ $[M+H]^+$ Exact Mass: 632.1670; Observed: 632.1669

2-(6-(2'-((*S*)-1-acetamido-2-methylpropyl)-[2,4'-bithiazol]-4-yl)-5-(4-carbamoylthiazol-2-yl)pyridin-2-yl)-*N*-((*S*)-1-amino-1-oxopropan-2-yl)thiazole-4-carboxamide (18)



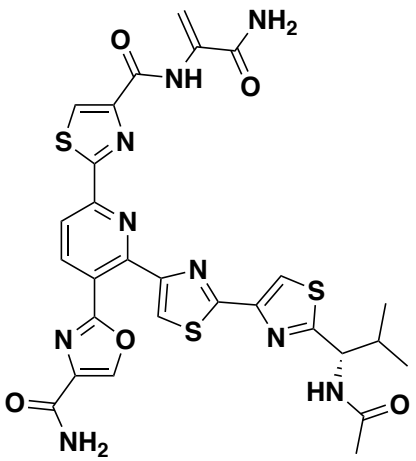
Compound 18 was prepared by procedure described in section X.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.77 (dd, J = 8.2, 2.2 Hz, 1H), 8.59 (dd, J = 8.5, 4.7 Hz, 1H), 8.50 (s, 1H), 8.47 (dd, J = 8.2, 1.1 Hz, 1H), 8.44 (dd, J = 8.0, 2.1 Hz, 1H), 8.33 (s, 1H), 8.30 (d, J = 1.5 Hz, 1H), 7.91 (s, 1H), 7.85 (d, J = 1.6 Hz, 1H), 7.63 (s, 1H), 7.58 (s, 1H), 7.21 – 7.16 (m, 1H), 6.54 (s, 1H), 4.97 (dd, J = 8.4, 6.8 Hz, 1H), 4.50 (p, J = 7.2 Hz, 1H), 2.29 (dq, J = 13.6, 6.8 Hz, 1H), 1.94 (s, 3H), 1.41 (d, J = 7.1 Hz, 3H), 0.95 (dd, J = 22.1, 6.7 Hz, 5H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 175.24, 174.30, 170.18, 167.54, 163.75, 162.62, 162.56, 160.05, 153.14, 151.46, 151.33, 150.49, 148.05, 140.18, 129.88, 127.83, 127.34, 123.32, 119.69, 117.26, 56.82, 48.56, 32.57, 22.86, 19.83, 19.21, 18.62.

LC-MS ESI: $C_{28}H_{28}N_9O_4S_4^+$ $[M+H]^+$ Exact Mass: 682.1142; Observed: 682.1126

(*S*)-2-(2-(2'-(1-acetamido-2-methylpropyl)-[2,4'-bithiazol]-4-yl)-6-(4-((3-amino-3-oxoprop-1-en-2-yl)carbamoyl)thiazol-2-yl)pyridin-3-yl)oxazole-4-carboxamide (11)



Compound 11 was prepared by procedure described in section X.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.10 (s, 1H), 8.68 (dd, J = 4.5, 0.9 Hz, 1H), 8.58 (d, J = 8.4 Hz, 1H), 8.51 – 8.45 (m, 2H), 8.29 (d, J = 8.1 Hz, 1H), 8.16 (s, 1H), 7.80 (s, 1H), 7.66 (s, 1H), 7.55 (d, J = 0.9 Hz, 1H), 6.50 (s, 1H), 6.31 (s, 1H), 5.79 (s, 1H), 4.96 (dd, J = 8.1, 6.9 Hz, 1H), 2.30 – 2.26 (m, 1H), 1.94 (s, 3H), 0.93 (dd, J = 22.8, 6.8 Hz, 6H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 175.22, 170.17, 167.90, 165.36, 162.33, 162.02, 159.68, 159.00, 158.53, 158.28, 153.38, 151.11, 148.15, 143.04, 141.95, 137.87, 134.27, 123.12, 123.12, 118.99, 118.66, 116.60, 32.47, 22.86, 19.79, 18.60.

LC-MS ESI: $C_{28}H_{26}N_9O_5S_3^+$ $[M+H]^+$ Exact Mass: 664.1214; Observed: 664.1214

III. Solid Phase Peptide Synthesis (SPPS)

Intramolecular substrates (3 and 4), FITC-labeled TbtA (5), TbtA 16-residue N-terminal leader peptide fragment (6), all 2π components and all TclM 4π components were synthesized by microwave assisted-SPPS on the Biotage Initiator + Alstra™ with Rink Amide ChemMatrix resin. Specifically, 3, 4 and 5, core peptides were assembled from thiazole building blocks and standard Fmoc-protected SPPS amino acids following the general microwave SPPS methods described in our previous publication and below.[1] Leader peptides were synthesized by direct extension off of the core peptides using method 1 (below). With the exception of **22,** all building blocks were coupled using Method 1. Compound **22** was coupled using Method 2. The TbtA leader peptide variants were synthesized in parallel at room temperature using Method 3 described below. All peptides were cleaved and purified following the "general peptide cleavage method."

General Microwave SPPS, Method 1:

Resin (20-100 mg) was initially swollen in DMF (1.5-6 mLs) for 20 min at 70°C.
(i) Coupling: Fmoc-AA-OH (5.0 equiv., 0.5 M in DMF), HATU (5.0 equiv., 0.5 M in DMF), and DIEA (10.0 equiv., 1.0 M in DMF) were added to the swollen resin in that order. The resulting suspension was heated under microwave irradiation: 5 min at 75 °C. The reaction vessel is then drained and resin is thoroughly washed with DMF four times.
(ii) Fmoc removal: Following coupling, excess 20% piperidine is added to the reaction vessel and allowed to incubate for 3 min while stirring at rt. The reaction vessel is drained, washed with DMF and excess 20% piperidine is introduced again and allowed to stir at rt for 10 min. The reaction vessel is drained and resin is thoroughly washed with DMF four times.

General Microwave SPPS, Method 2:

(i) Coupling: Fmoc-AA-OH (5.0 equiv., 0.5 M in DMF), TBTU (5.0 equiv., 0.5 M in DMF), HOBt (5.0 equiv., 0.5 M in DMF) and DIEA (10.0 equiv., 1.0 M in DMF) were added to the resin in that order. The resulting suspension was heated under microwave irradiation: 5 min at 75 °C. The reaction vessel is then drained and resin is thoroughly washed with DMF four times.
(ii) Fmoc removal: Same as Fmoc removal in **Method 1**.

General Room Temperature SPPS, Method 3:

Resin (20-100 mg) was initially swollen in DMF (1.5-6 mLs) for 30 min at rt.
(i) Coupling: Fmoc-AA-OH (5.0 equiv., 0.5 M in DMF), HATU (5.0 equiv., 0.5 M in DMF), and DIEA (10.0 equiv., 1.0 M in DMF) were added to the swollen resin in that order. The resulting suspension was stirred 15 min at rt. The reaction vessel is then drained and resin is thoroughly washed with DMF four times.
(ii) Fmoc removal: Following coupling, excess 20% piperidine is added to the reaction vessel and allowed to incubate for 10 min while stirring at rt. The reaction vessel is drained and resin is thoroughly washed with DMF four times.

General Peptide Cleavage Method:

The resin was washed several times with DMF and DCM. The resin was dried and cleaved using a cleavage cocktail (TFA/TIS/H_2O, 95:2.5:2.5) at 37°C for 1 h. The resin was then filtered from the cleavage cocktail solution. The solution was dried by blowing N_2. Coooled diethyl ether (at least 10 times the volume of the residual TFA) was added to crash out the crude peptide. The crude peptide was pelleted by centrifugation (10 min at 15,000 rpm at 4°C) and then decanted. The crude

peptide was dissolved in 1:1 acetonitrile:water and purified by reverse-phase Prep HPLC using method A or B (page S3). Fractions containing peptide of interest were analyzed by LCMS to verify their identities and lyophilized.

IV. Conversion of Cysteines to Dehydroalanines

The cysteine-containing peptide substrates was dissolved to a final concentration of 0.63 mM in a solution containing 50% aq. DMF, 1.26 mM TCEP, and 63 mM K_2CO_3. The mixture was allowed to incubate for 15 min at 37°C, after which methyl 2,5-dibromovalerate was added to a final concentration of 63 mM. The reaction was allowed to stir at 37°C for 3 h. The crude peptide was dissolved in 1:1 acetonitrile:water and purified by reverse-phase Prep HPLC using method A or B (page S3). Fractions containing peptide of interest were analyzed by LCMS to verify their identities and lyophilized.

V. Protein Expression and Purification

The purification of both TclM and TbtD have been previously described.[1] Both enzymes was heterologously expressed in *E. coli* BL21 (DE3) cells and purified separately as follows. 1 L of Luria-Bertani (LB) medium, supplemented with ampicillin (100 μg/mL) was inoculated with 5 mL of an overnight seed and grown to an OD_{600} between 0.6-0.8 at 37°C, at which point 400 μL of a 1 M stock of IPTG was added and culture grown for 18 h at 16°C. Cells were pellet and resuspended in 40 mL of lysis/binding buffer (50 mM $KHPO_4$ at pH = 7.00, 250 mM KCl, 10 mM imidazole, and 10% glycerol) supplemented with 250 μg/mL lysozyme, 2 mM PMSF, and 1-protease inhibitor tablet (ROCHE). Cells were lysed by sonicating twice with a 20% maximum amplitude intermittent pulses for 1:30 min. Cell debris was removed by centrifugation (20 min at 15,000 rpm at 4°C) and supernatant collected and cold filtered through a 0.44 μm filter. Supernatant was loaded into a 5-mL HisTrap (Ni^{2+}) IMAC column and washed (5 CV) to remove non-specific binding. 6xHis-MBP-TbtD was eluted with an elution buffer gradient (50 mM $KHPO_4$ at pH = 7.00, 250 mM KCl, 500 mM imidazole, and 10% glycerol) from 0 – 100% (6 CV). Fractions containing eluted protein are pooled and concentrated to 5 mL or less utilizing a Centricon (30,000 Da MWCO) concentrator (EMD Millipore). The resulting concentrated protein was buffer-exchanged into 50 mM $KHPO_4$ at pH = 7.00, 250 mM KCl, and 10% glycerol utilizing a PD-10 column (GE Healthcare Life Sciences). The concentration of each 6xHis-MBP-enzyme was determined by UV absorbance at 280nm. Enyzmes were used for all assay directly from the PD-10 elution.

VI. Intramolecular Cyclization Kinetics of TclM and TbtD

The pH-dependent rate enhancement: All reactions were performed with a 1 mL plastic cuvette and analyzed using the DeNovix DS-11 table top UV spectrophotometer. All reactions were performed at ambient temperature (25°C) with a total volume of 50 μL. An aliquot of TbtA or TclE (compounds 4 or 3, respectively) substrate was taken from a 5 mM (in DMF) stock and diluted with pHed buffer to 100 μM in the cuvette. This was used to blank the spec. TbtD or TclM was then added to a final concentration on 1 μM, quickly mixed (pipette up and down several times) and absorbance readings at 350 nm were taken every 10 sec for 15 min. All reactions had 2% DMF and were performed in triplicate.

In experiments with the 16-residue N-terminal LP fragment (6) the procedure described above was followed with the exception that, 100 uM of the LP fragment was added and the mixture of this fragment with the TbtA substrate in buffer was used to blank the spec. before the addition of TbtD.

Buffers used:
pH 7.2 Buffer: 50 mM HEPES, 150 mM NaCl, pH 7.2
pH 9.0 Buffer: 50 mM HEPES, 150 mM NaCl, pH 9.0

Determination of k_{cat} at each pH: All reactions were performed with a 1mL quartz cuvette and analyzed using the DeNovix DS-11 table top UV spectrophotometer. All reactions were performed at ambient temperature (25°C) with a total volume of 300 μL. An aliquot of TbtA substrate (4) was taken from a 10 mM (in DMF) stock and diluted with pHed buffer in the cuvette to a volume of 200 μL. This was used to blank the spectrophotometer. TbtD was then added, quickly mixed and absorbance readings at 350 and 315 nm were taken every 10 sec for 30 min. For pH 7.2, a final concentration of 2.5 μM was used for TbtD and for pH 8.0 and 9.0, 0.5 μM was the final concentration of TbtD. All reactions contained 2% DMF. Reactions were run with 10, 20, 50, 100, 200 and 500 μM TbtA for each pH. All measurements were performed in triplicate.

Buffers Used:
pH 7.2 Buffer: 50 mM HEPES, 150 mM NaCl, pH 7.2
pH 8.0 Buffer: 50 mM HEPES, 150 mM NaCl, pH 8.0
pH 9.0 Buffer: 50 mM HEPES, 150 mM NaCl, pH 9.0

These procedures are associated with the Figure 2 in the main text and Figure S1 and S3.

VII. Intramolecular Cyclization Assays of TbtA LP Variants

Cyclization assays were performed using 20 μM substrate and 10 μM MBP-TbtD in a total volume of 50 μL using either pH 7.2 or pH 9.0 buffers listed below. Reactions were incubated at 25°C on the bench top for 20 h. After 20 h, 100 μL of methanol was added to each reaction to crash out MBP-TbtD. Insoluble material was then removed by centrifugation (15,000 rpm, 5 min, 25°C) and the samples were analyzed LC-MS using method B. Controls lacking enzyme were also run for each LP mutant.

Efficiency of cycloaddition was measured via percent conversion. Area under the 254 nm peaks for substrate and product was calculated. The area of the product peak was divided by the sum of both peaks (substrate and product) to get a % conversion.

Buffers Used:
pH 7.2 Buffer: 50 mM HEPES, 150 mM NaCl, pH 7.2
pH 9.0 Buffer: 50 mM CHES, 150 mM NaCl, pH 9.0

These procedures are associated with the Table 1 in the main text and Figure S4.

VIII. Intermolecular Cyclization Assays of TclM and TbtD

Cyclization assays were performed using 50 μM 2π, 50 μM 4π and 5 μM MBP-TbtD in a total volume of 50 μL using either pH 7.2 or pH 9.0 buffers listed below. Reactions were incubated at

25°C on the bench top for 20 h. After 20 hrs, 100 μL of methanol was added to each reaction to crash out MBP-TbtD. Insoluble material was then removed by centrifugation (15,000 rpm, 5 min, 25°C) and the samples were analyzed LC-MS using method B.

Efficiency of cycloaddition was measured via percent conversion. Area under the 254 nm peaks for 4π and product was calculated. The area of the product peak was divided by the sum of both peaks (4π and product) to get a % conversion.

Buffers Used:
pH 7.2 Buffer: 50 mM HEPES, 150 mM NaCl, pH 7.2
pH 9.0 Buffer: 50 mM CHES, 150 mM NaCl, pH 9.0

These procedures are associated with the Figures 3 and 4 in the main text and Figure S5 and Table S1.

IX. Intermolecular Cyclization Kinetics of TbtD

All reactions were performed with a 1 mL plastic cuvette and analyzed using the DeNovix DS-11 table top UV spectrophotometer. All reactions were performed at ambient temperature (25°C) with a total volume of 50 μL. Aliquots of 2π and 4π were taken from 10 mM stocks and diluted to 50 μM (each) with pH 9.0 buffer (50 mM CHES, 150 mM NaCl, pH 9.0) in the cuvette. This was used to blank the spec. TbtD was then added to a final concentration on 5 μM, quickly mixed (pipette up and down several times) and absorbance readings at 350 nm were taken every 10 sec for 30 min. All reactions had 2% DMF and were performed in triplicate.

These procedures are associated with the Figure 4 in the main text and Figure S6.

X. Large Scale Intermolecular Cyclization with TbtD

Cyclization assays were performed using 100 μM 2π, 100 μM 4π and 10 μM MBP-TbtD in a total volume of 50 mL of reaction buffer: 50 mM CHES, 150 mM NaCl, pH 9.0 (the low aqueous solubility of the 4π components prevented more concentrated reactions). Reactions were incubated at room temperature on the bench top for 20 h. After 20 h, smooth conversion was observed. The MBP-TbtD was crashed out of the reaction by the addition of 100 mLs of MeOH. The solution was spun down and decanted to remove TbtD. The solution was then concentrated to 5 mLs under reduced pressure and purified by Prep-HPLC using method A, monitoring 254 and 350nm. Peaks of interested were collected, analyzed by MS and lyophilized.

XI. FITC-labeling 15-res LP TbtA-Ala Core

Labeling

3 mg of 15-res LP TbtA-Ala Core (compound 50) was dissolved in 11 mLs of 1x PBS pH 8.0 (100 μM final concentration). 4.3 mg of FITC was dissolved in 100 μL of DMF and added, dropwise, to a stirring scintillation vial. The vial was wrapped in tin foil and the reaction was stirred vigorously at room temperature for 12 h.

Scavenger Resin Prep

50mg of ChemMatrix Rink Amide Resin (~24 μmol) was swollen and Fmoc-deprotected on the peptide synthesizer using standard protocols (see SPPS protocols above).

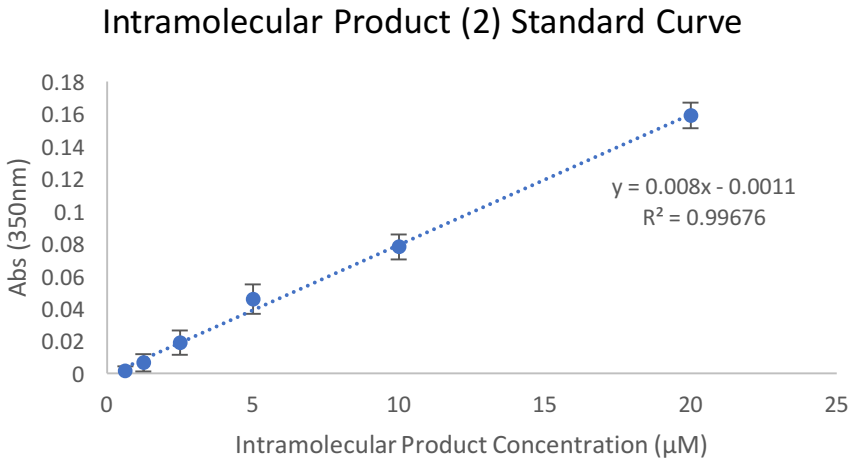
Scavenger Reaction
The swollen, deprotected resin was suspended in 3 mL DMF and added dropwise to the labeling reaction vial (the resin turned a slightly darker orange) and stirred overnight at room temperature. The crude reaction was dried under reduced pressure. The resulting foamy/film was dissolved in 4.5 mLs of 1:1 MeCN:H2O and purified by HPLC in a single injection using method A. LCMS was taken of each fraction of interest. Fractions containing product were lyophilized to yield 1.62 mg of FITC labeled peptide (compound 5 in 47% yield).

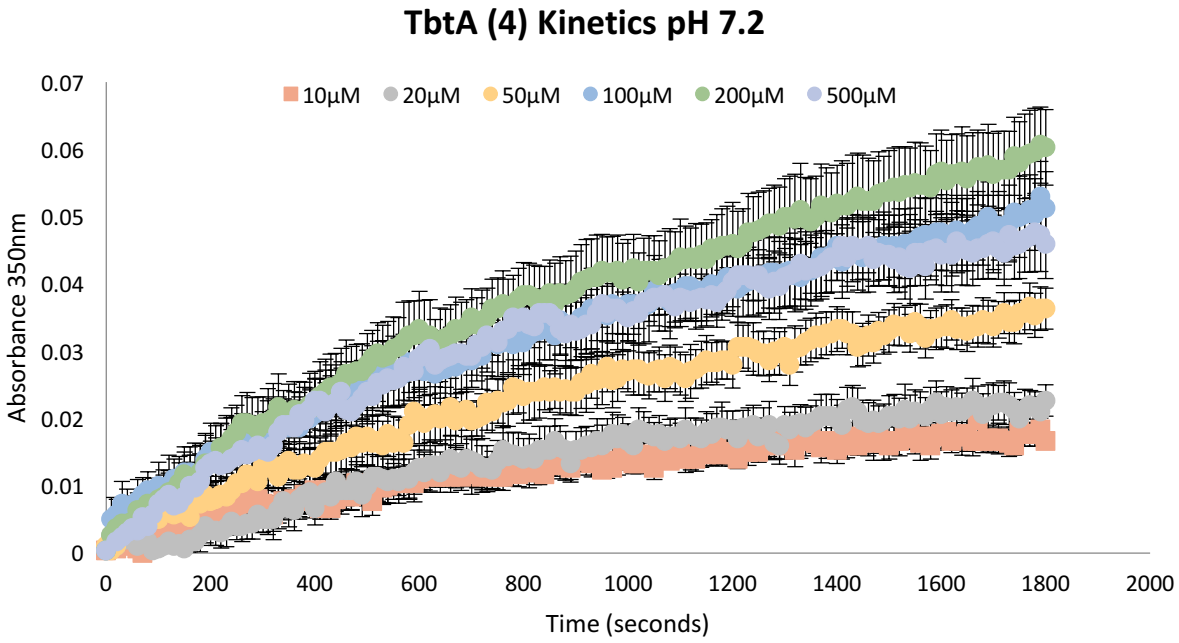
XII. Fluorescence Polarization Assay

The interaction between the FITC-15-res LP TbtA-Ala Core (compound 5) and TbtD was measured using fluorescence polarization by adapting a known procedure.[2] MBP-TbtD was serially diluted into buffer (50 mM HEPES, pH 7.2, 150 mM NaCl, 2.5% (v/v) glycerol) and mixed with 25 nM of the FITC labeled peptide (5). Dilutions were equilibrated at 25°C for 30min shaking very slowly (60 rev/min). Binding assays were performed in a non-binding surface, 384-well plate (Black, Corning) and measured using the Perkin Elmer EnVision 2103 Multilevel Reader with λ_{ex} = 485 nm and λ_{em} = 538 nm. Wells containing only MBP-TbtD were measured and these signals were subtracted from the mP signals to remove background fluorescence. This binding assay was performed in triplicate and dissociation constant (K_d) values were calculated from the 50% saturation point using a dose-response curve fit in GraphPad Prism 5.

This procedure is associated with the Figure 2 in the main text and Figure S2.

XIII. Figure S1: Intramolecular Kinetics of TbtA (4) at pH 7.2, 8.0 and 9.0





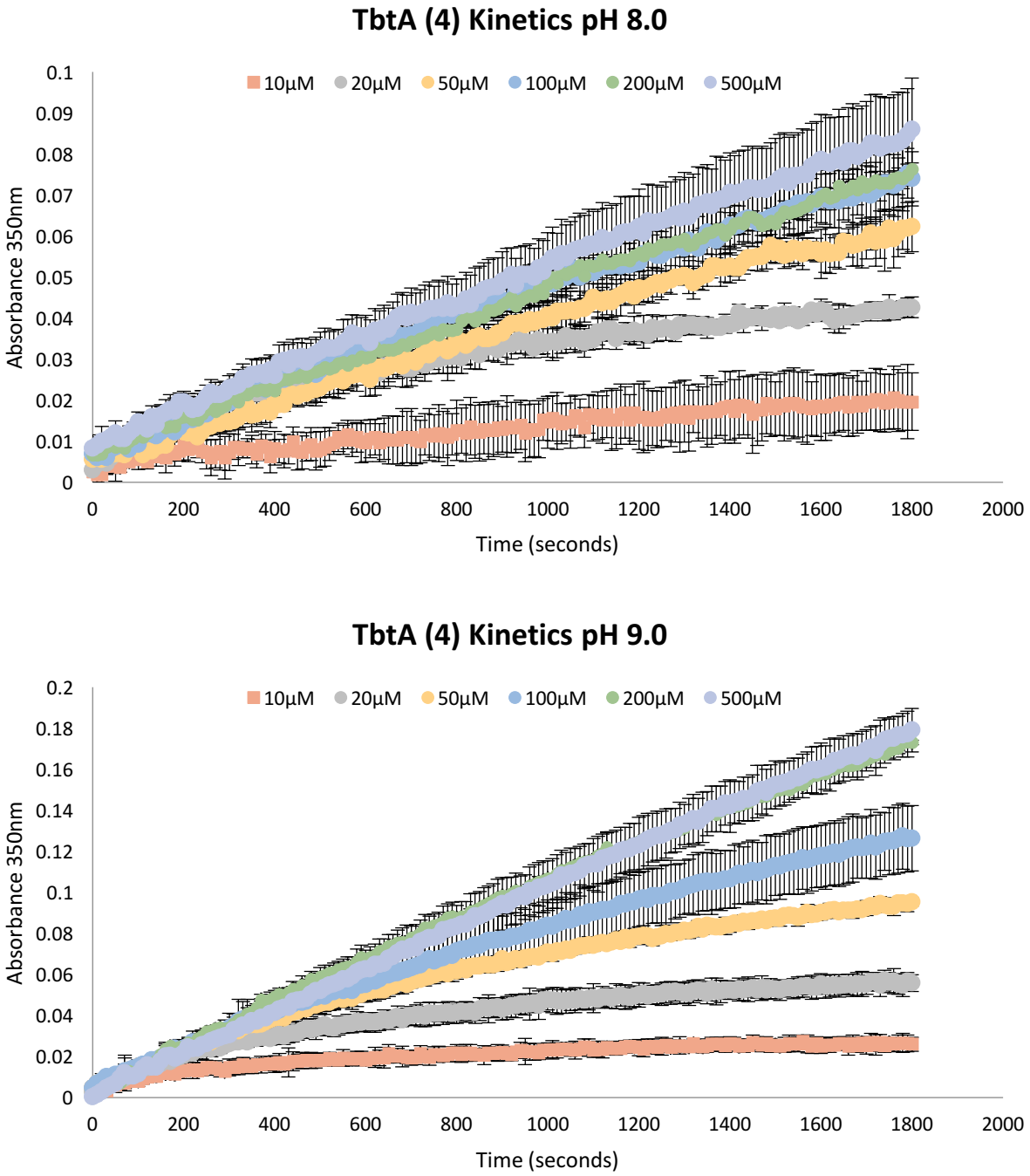


Figure S1. Standard Curve and time courses of TbtA (**4**) cyclizations at pH 7.2, 8.0 and 9.0 used to calculate k_{cat}.

XIV. Figure S2: Fluorescence Polarization of Min. LP TbtA and TbtD

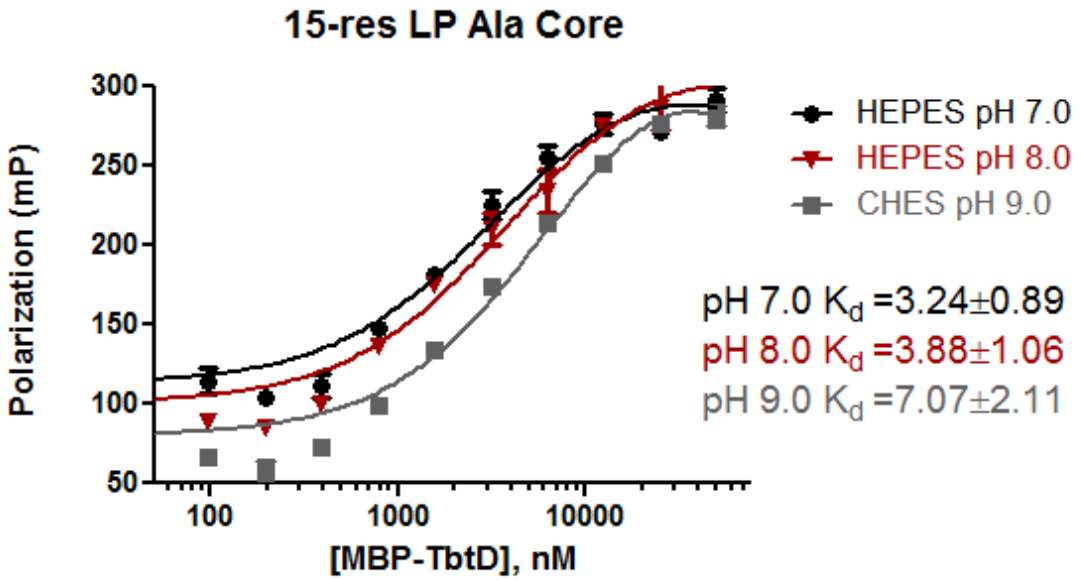


Figure S2. Binding of compound 5 to TbtD across pHs 7.2, 8.0 and 9.0. Measured by Fluorescence polarization in triplicate. A 2-fold drop in K_d is observed between pH 7.2 and 9.0. pH has a low impact on compound 5 binding TbtD.

XV. Figure S3: Effect of 16-res N-terminal LP Fragment on Cyclization

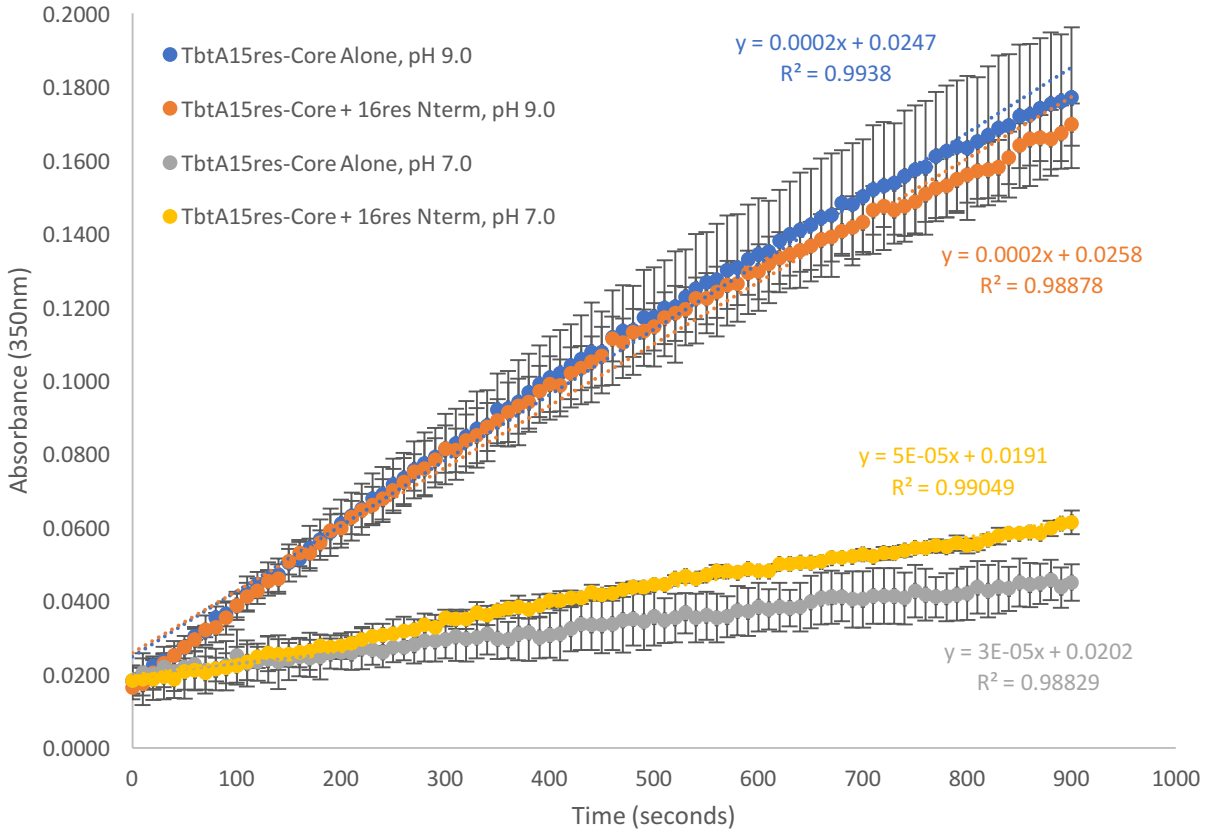
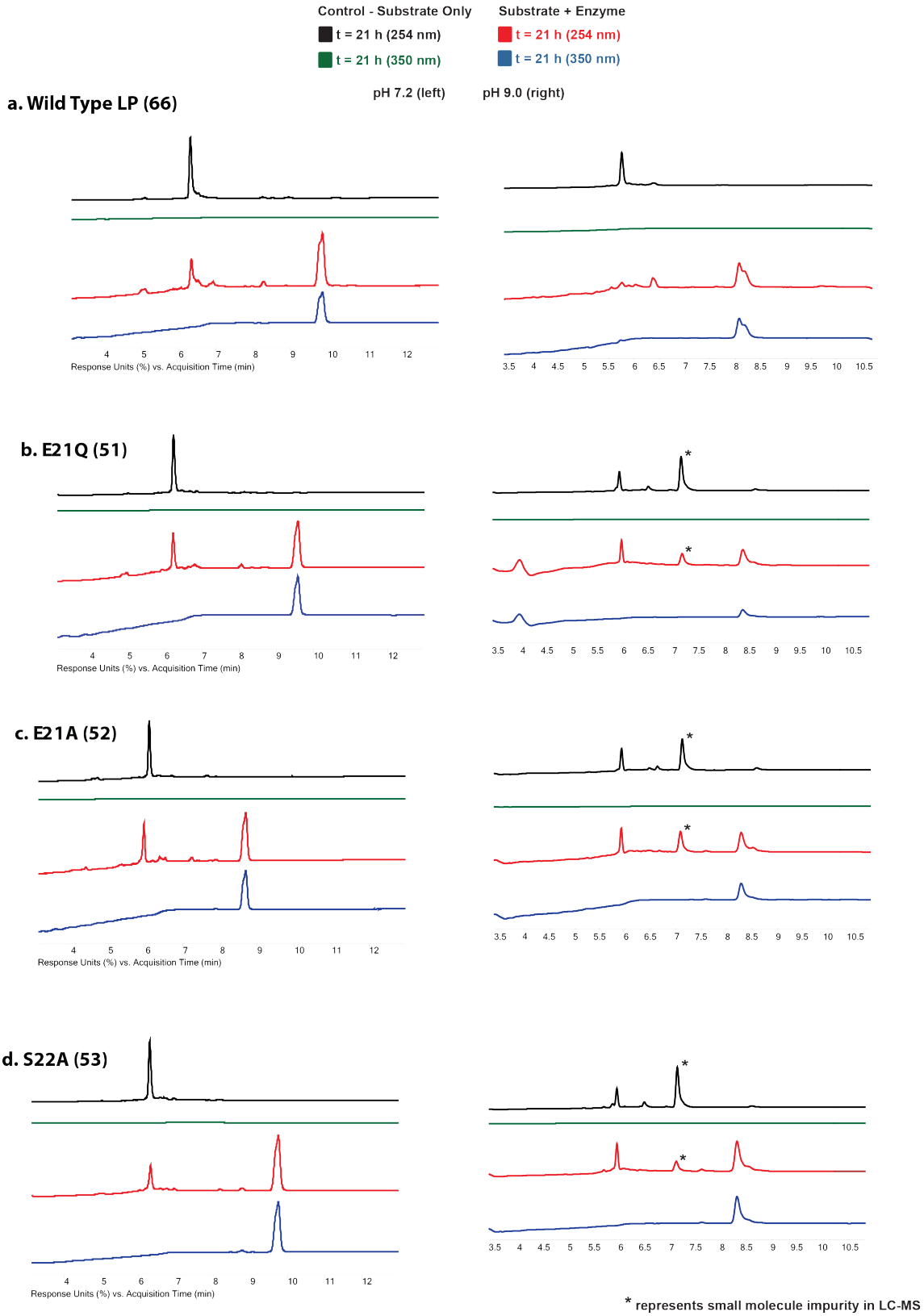
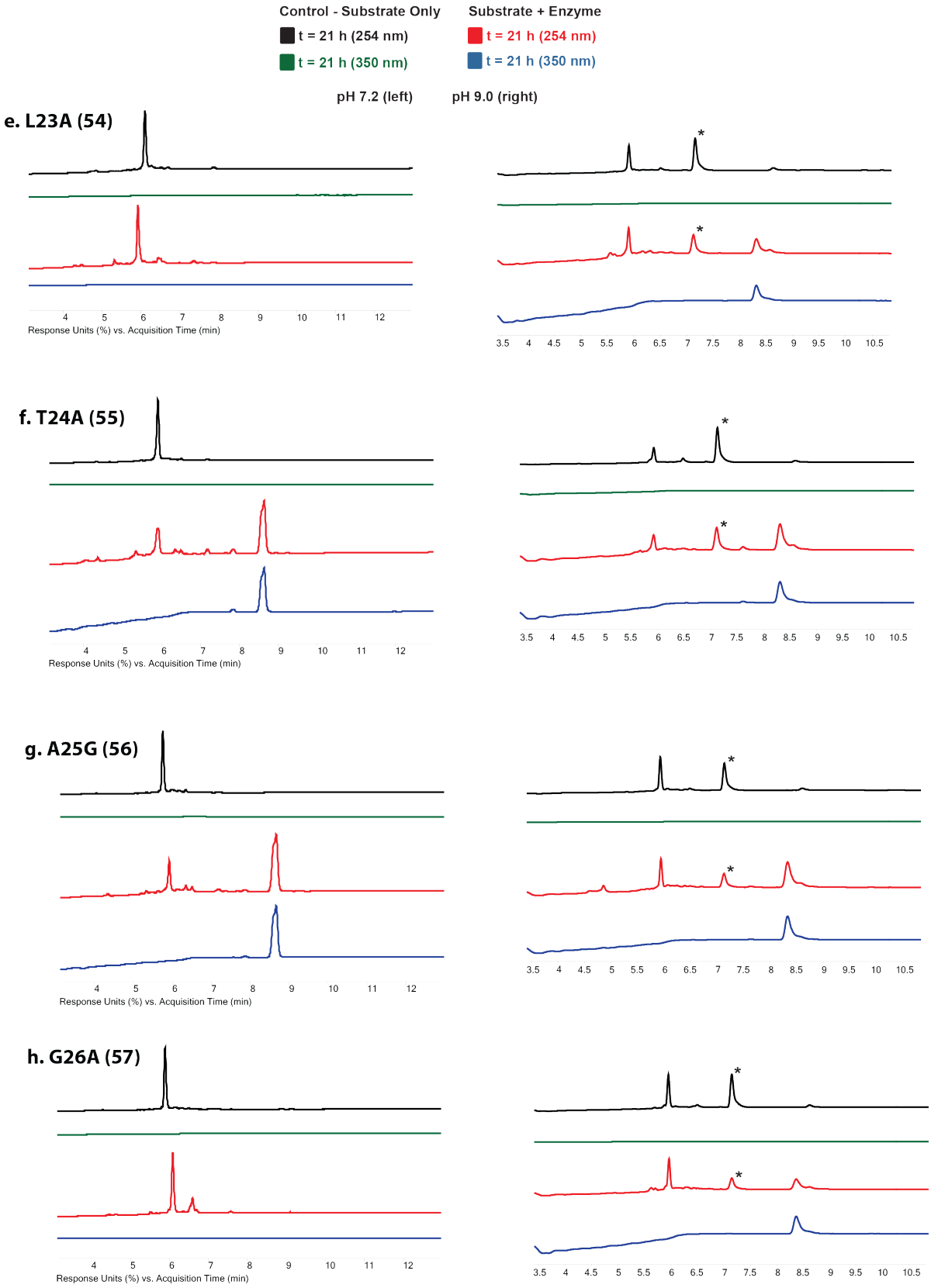


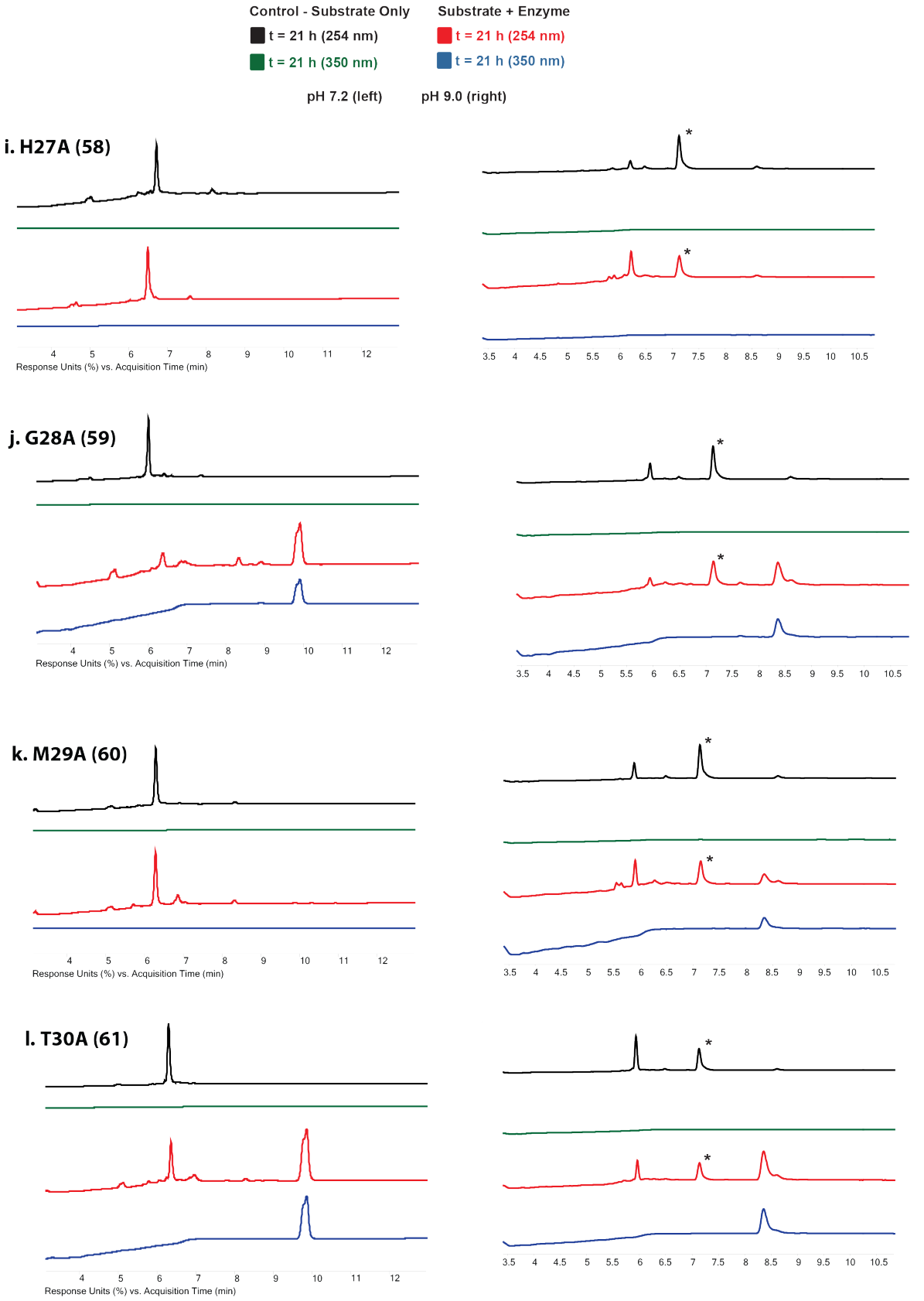
Figure S3. The rate of TbtD-catalyzed pyridine formation was measured for compound **4** (TbtA 15res Core) with and without an equimolar amount of the 16res N-terminal TbtA LP fragment (compound **6**) at pH 7.2 and pH 9.0. Assays were conducted in triplicate. No significant change in the rate of the reaction when compound **6** is present.

XVI. Figure S4: Intramolecular Cyclization Assays with TbtA LP Variants

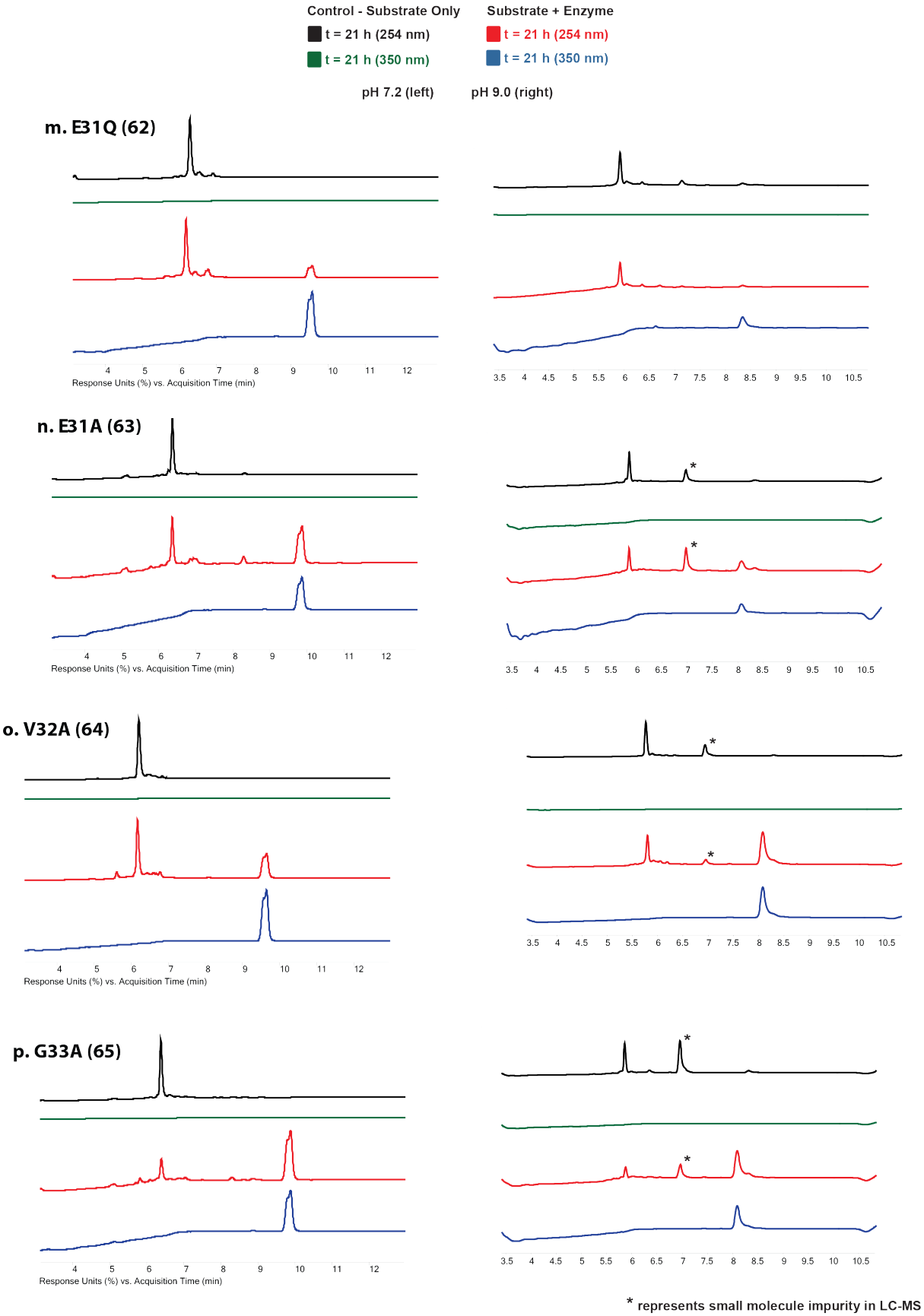




* represents small molecule impurity in LC-MS



* represents small molecule impurity in LC-MS



* represents small molecule impurity in LC-MS

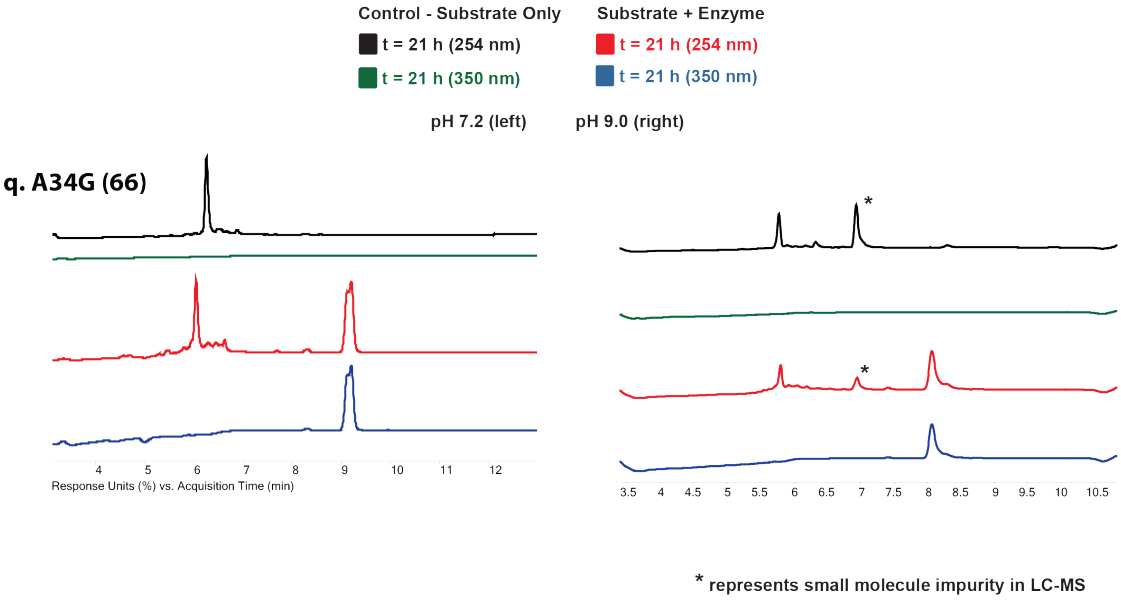
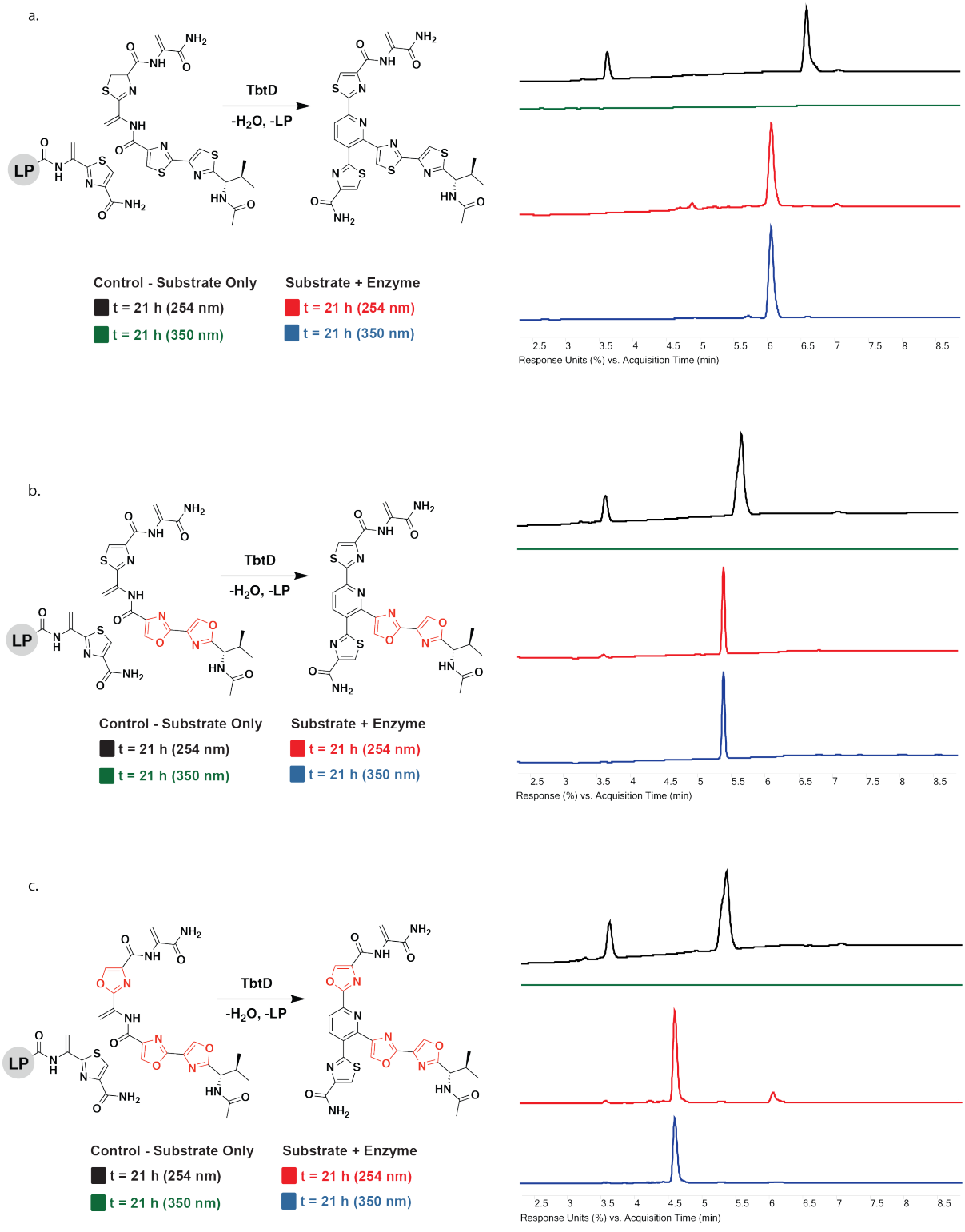
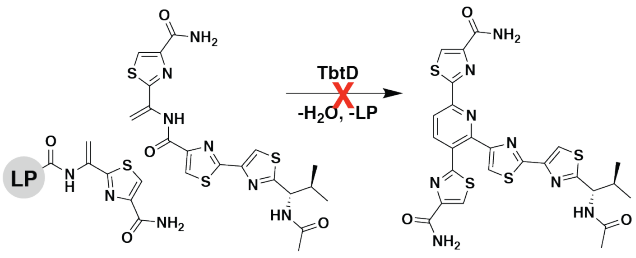


Figure S4. Intramolecular cycloaddition assays with TbtD and TbtA LP variants were analyzed by LC-MS under neutral (left) and basic (right) pHs. In each set of UV traces, the black and green traces (measuring 254 nm and 350 nm, respectively) represent the control reaction lacking TbtD after 21 h. The red and blue traces (254 nm and 350 nm, respectively) represent the reaction with TbtD after 21 h. A new peak with the characteristic 350 nm absorbance of the tri-substituted pyridine emerges and was confirmed by LCMS to be the cyclized product. Cyclization efficiency/percent conversion was measured by dividing the area under the product peak by the total area under both the product and starting material substrate peaks. Cyclization efficiency is rescued under basic conditions in several substrates (e, h, k).

XVII. Figure S5: TbtD-Catalyzed Intermolecular Cyclization Assays

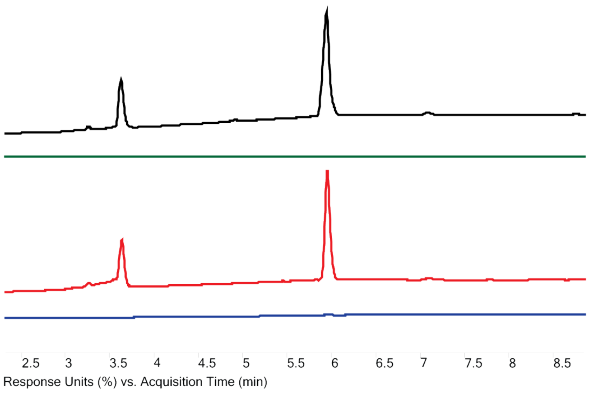


d.

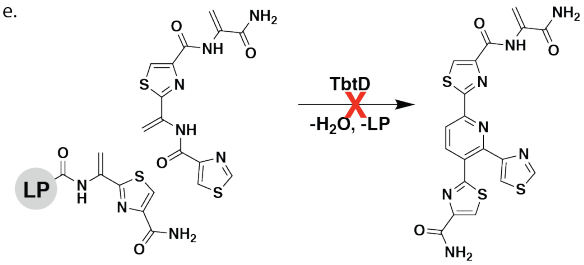


Control - Substrate Only
t = 21 h (254 nm)
t = 21 h (350 nm)

Substrate + Enzyme
t = 21 h (254 nm)
t = 21 h (350 nm)

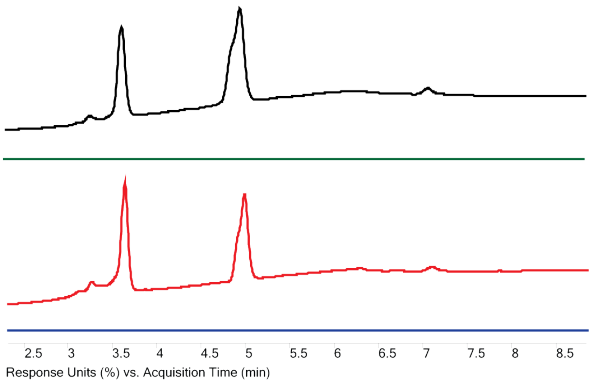


e.

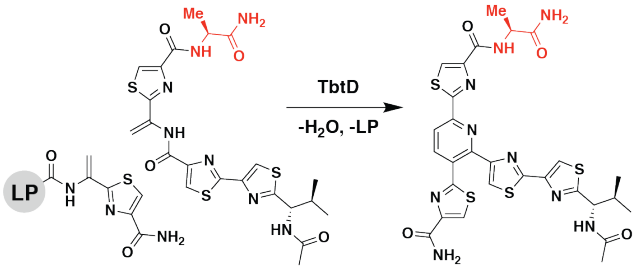


Control - Substrate Only
t = 21 h (254 nm)
t = 21 h (350 nm)

Substrate + Enzyme
t = 21 h (254 nm)
t = 21 h (350 nm)

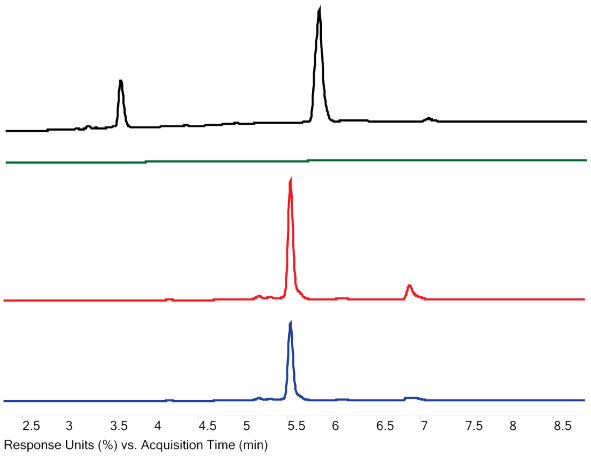


f.



Control - Substrate Only
t = 21 h (254 nm)
t = 21 h (350 nm)

Substrate + Enzyme
t = 21 h (254 nm)
t = 21 h (350 nm)



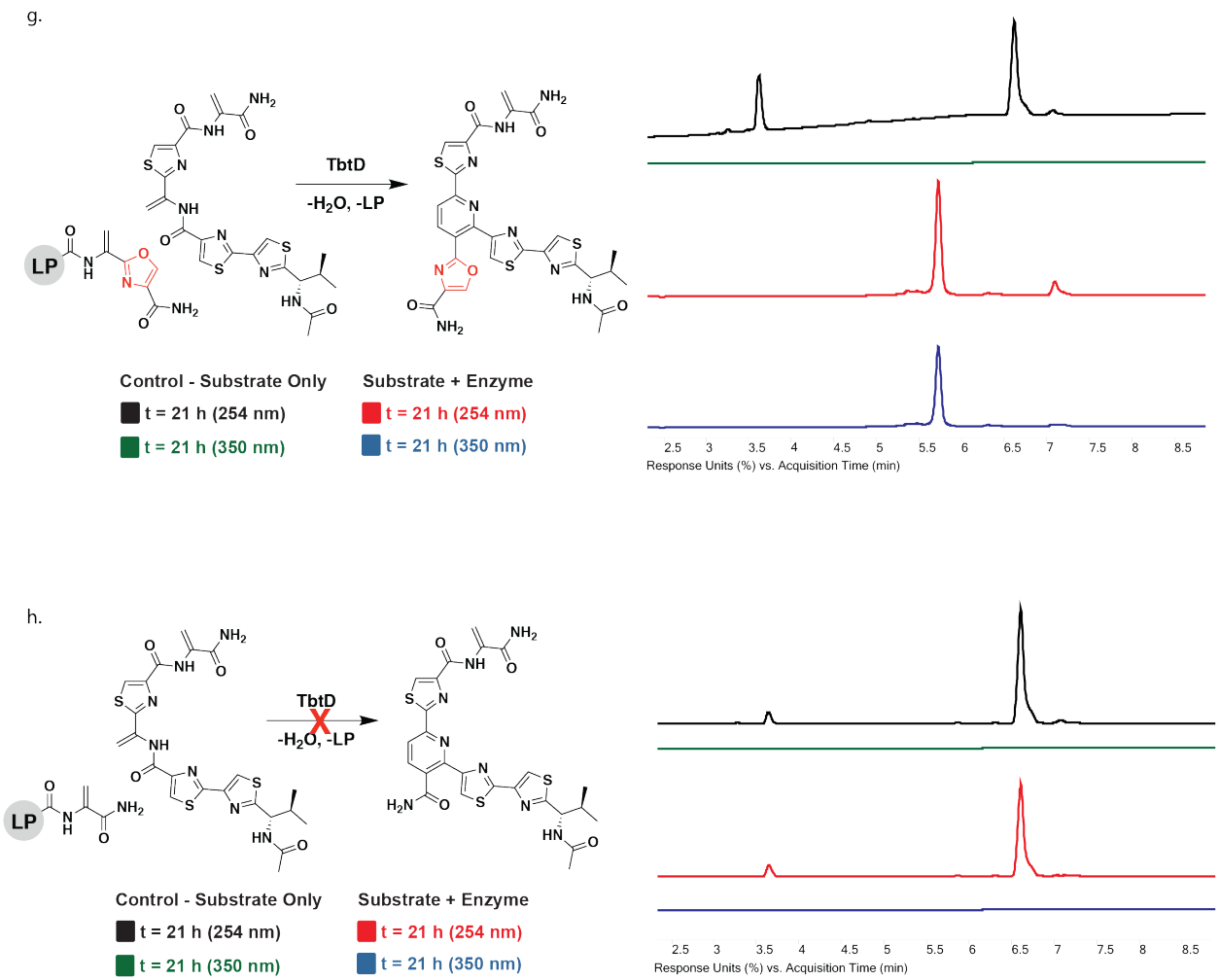


Figure S5. Intermolecular cycloaddition assays with TbtD were analyzed by LC-MS. In each set of UV traces, the black and green traces (measuring 254 nm and 350 nm, respectively) represent the control reaction lacking TbtD after 21 h. The red and blue traces (254 nm and 350 nm, respectively) represent the reaction with TbtD after 21 h. A new peak with the characteristic 350 nm absorbance of the tri-substituted pyridine emerges and was confirmed by LCMS and NMR to be the cyclized intermolecular product.

XVIII. Table S1: Substrate Scope of TclM-Catalyzed Intermolecular Cyclization

2π Component	4π Component	% Conv.
10-residue LP-DhaThz	Ac-AlaThzDhaThzThz	<1
10-residue LP-DhaThz	Ac-AlaThzDhaThzThzDha	<1
10-residue LP-DhaThz	Ac-AlaThzDhaThzThzAlaGly	<1
10-residue LP-DhaThz	Ac-AlaThzDhaThzThzDhaThr	<1
20-residue LP-DhaThz	Ac-AlaThzDhaThzThz	<1
20-residue LP-DhaThz	Ac-AlaThzDhaThzThzDha	<1
20-residue LP-DhaThz	Ac-AlaThzDhaThzThzAlaGly	<1
20-residue LP-DhaThz	Ac-AlaThzDhaThzThzDhaThr	<1

Table S1. Percent conversions for the TclM-catalyzed intermolecular cycloadditions. Results reflect the conversion observed at both pH 7.2 and 9.0. TclM does not catalyze the intermolecular cycloaddition to any significant extend using these substrates.

XIX. Figure S6: Intermolecular Kinetics at 9.0

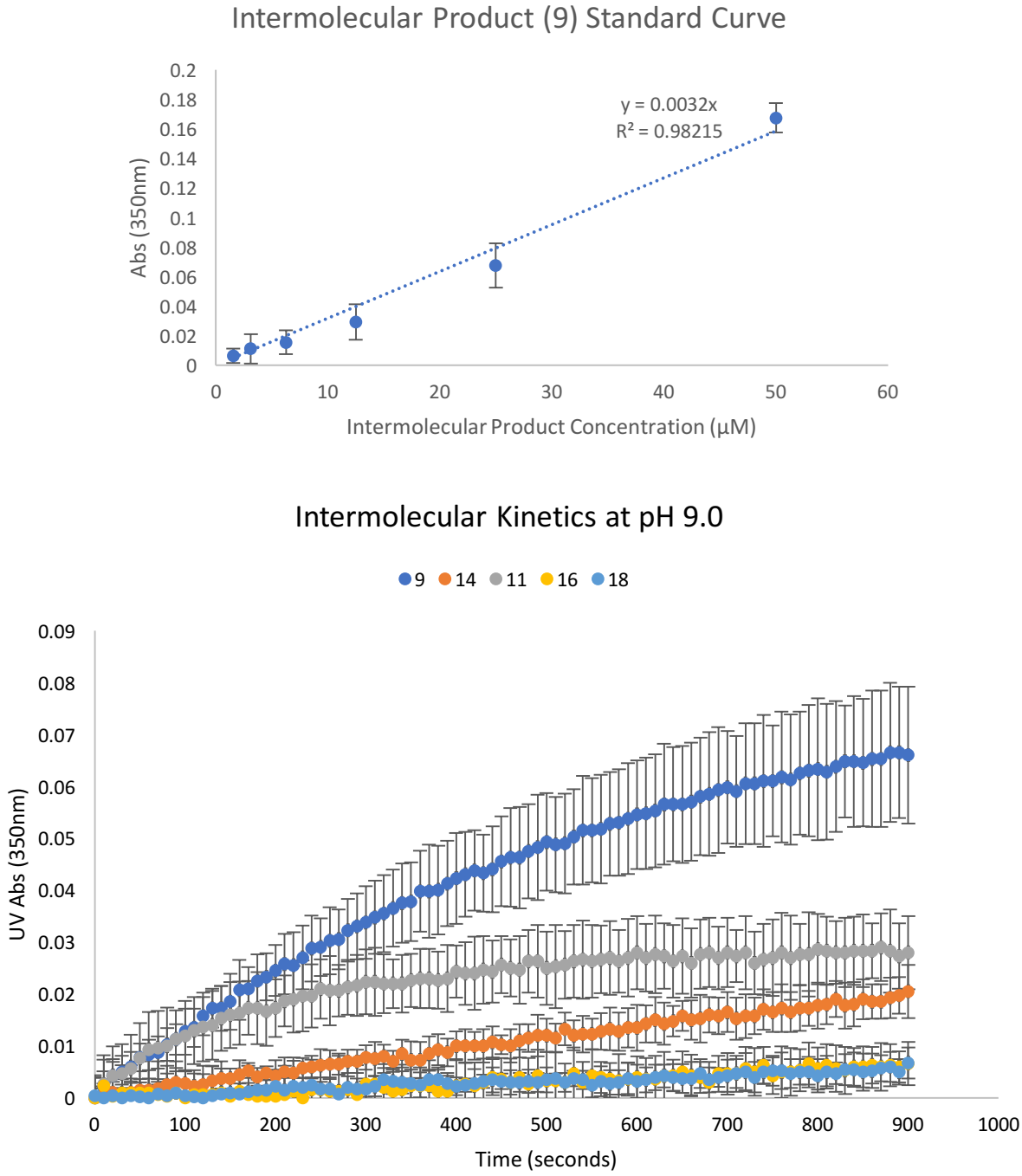
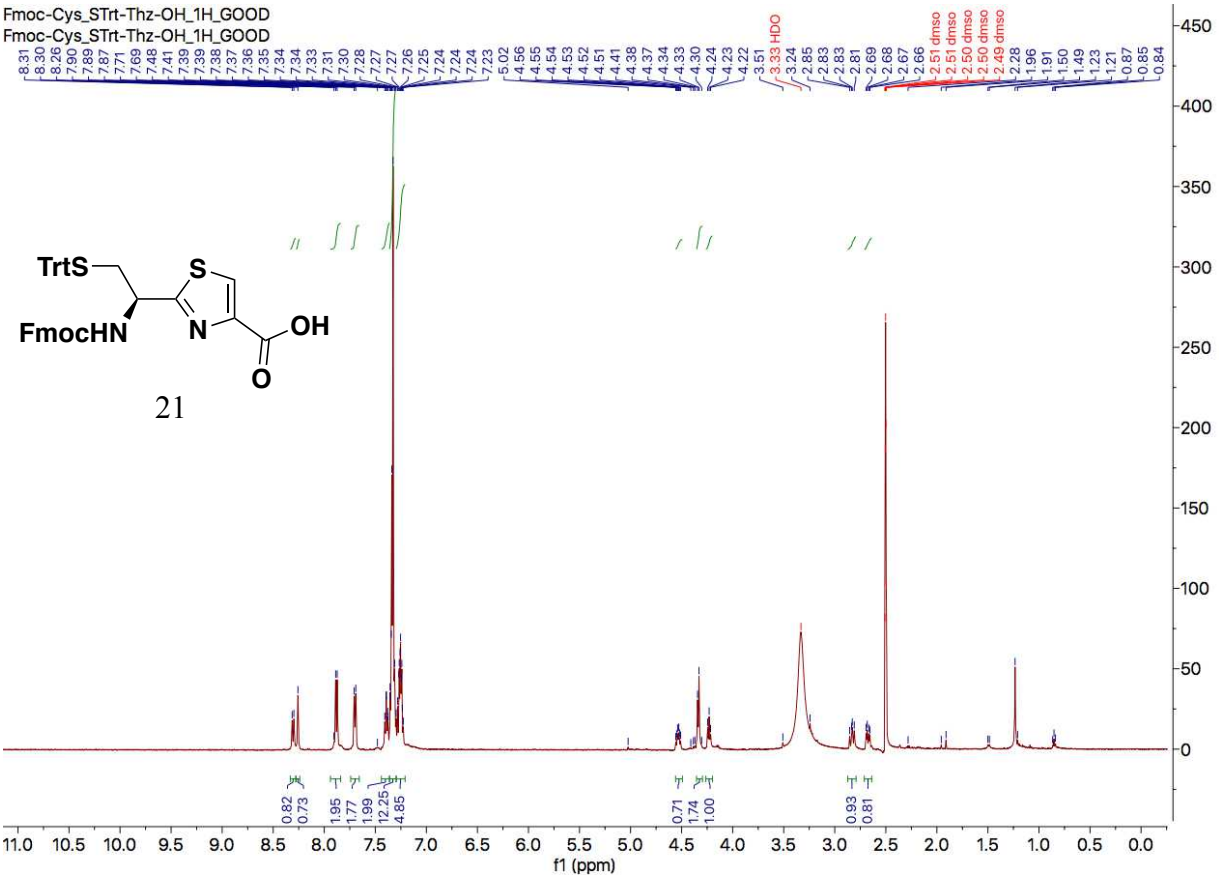
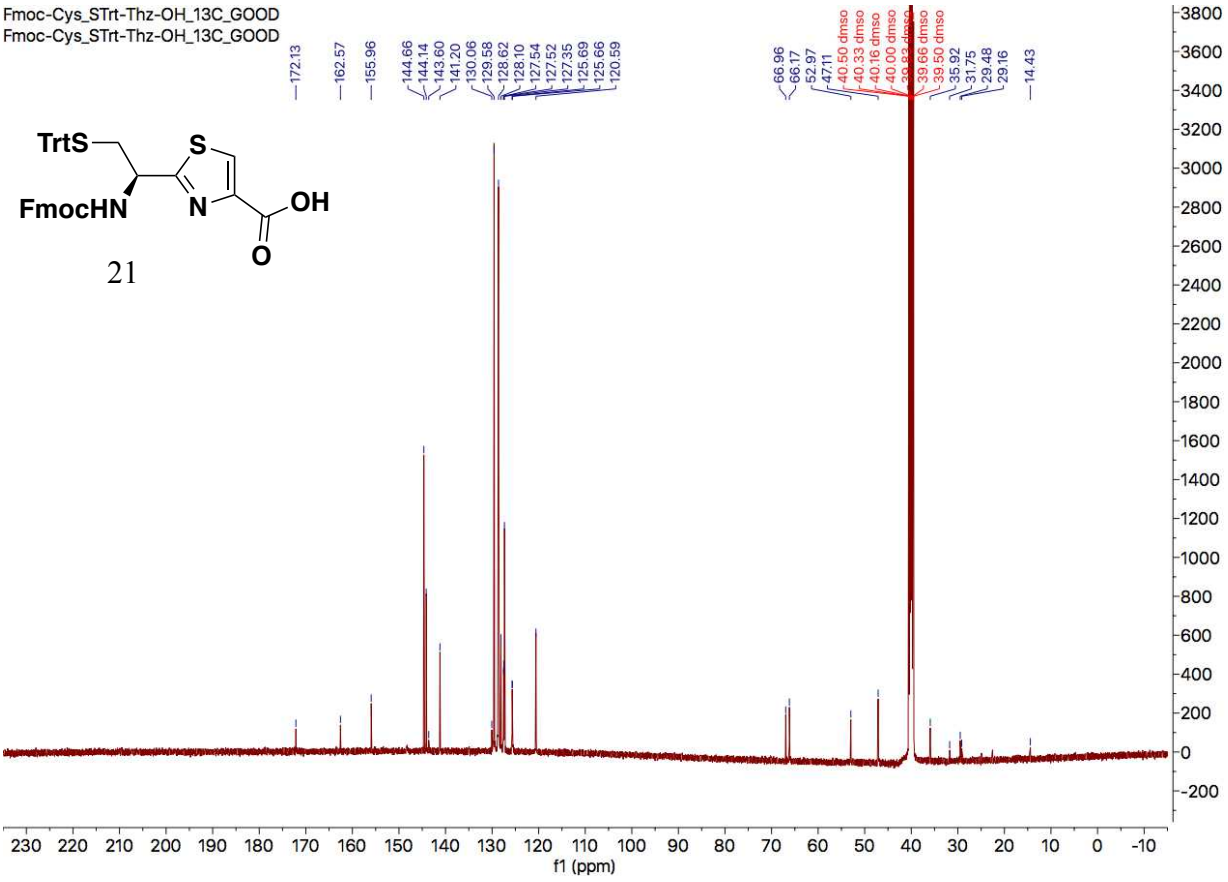


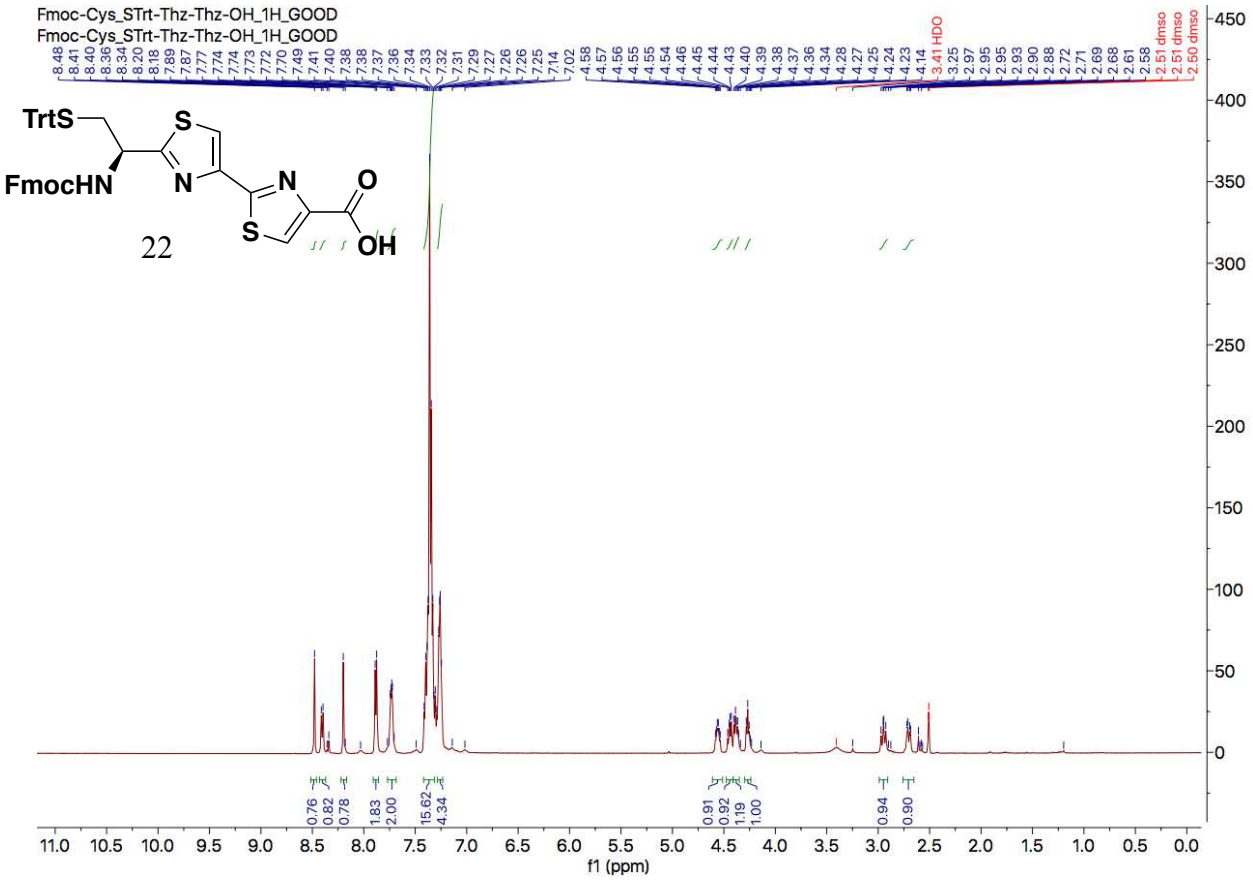
Figure S6. Standard curve and time course of TbtD-catalyzed intermolecular reactions used to calculate k_{obs}.

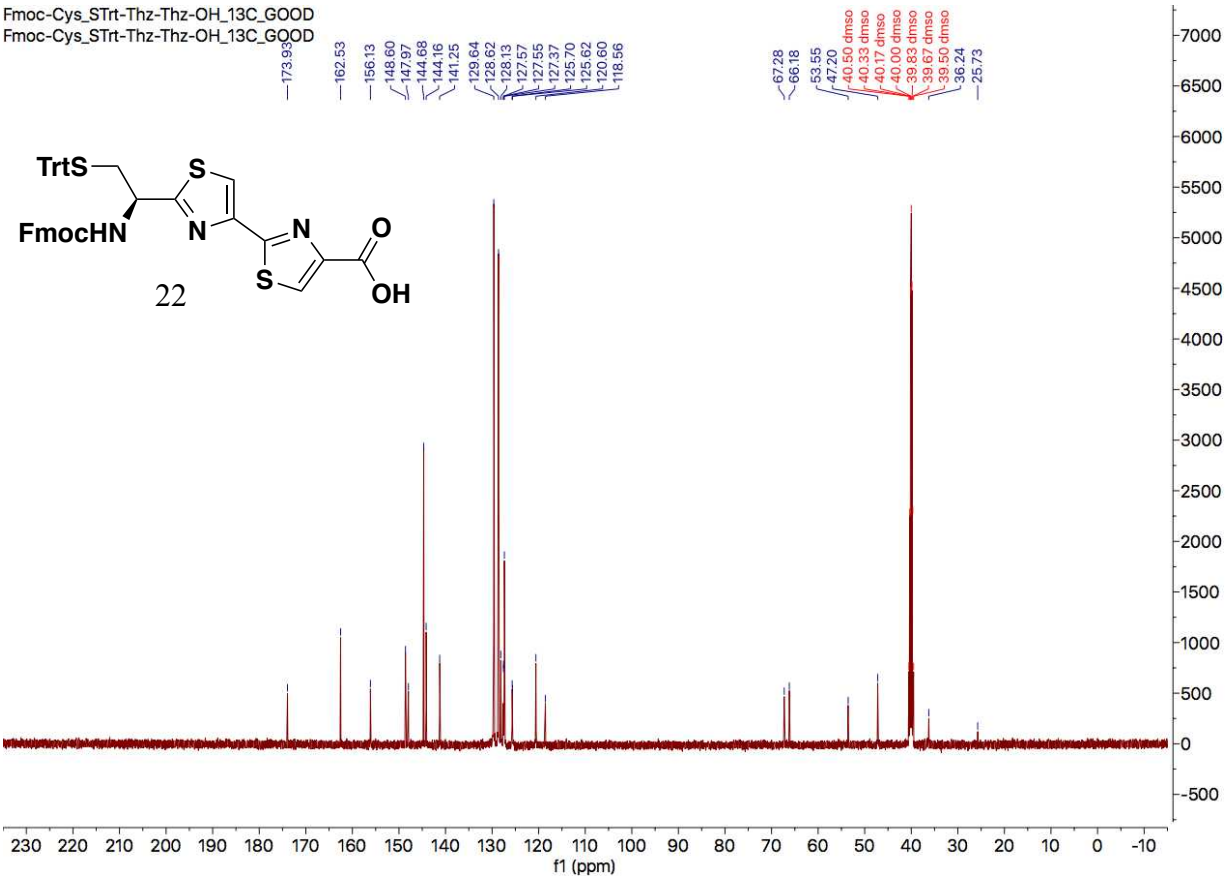
XX. NMR Spectra

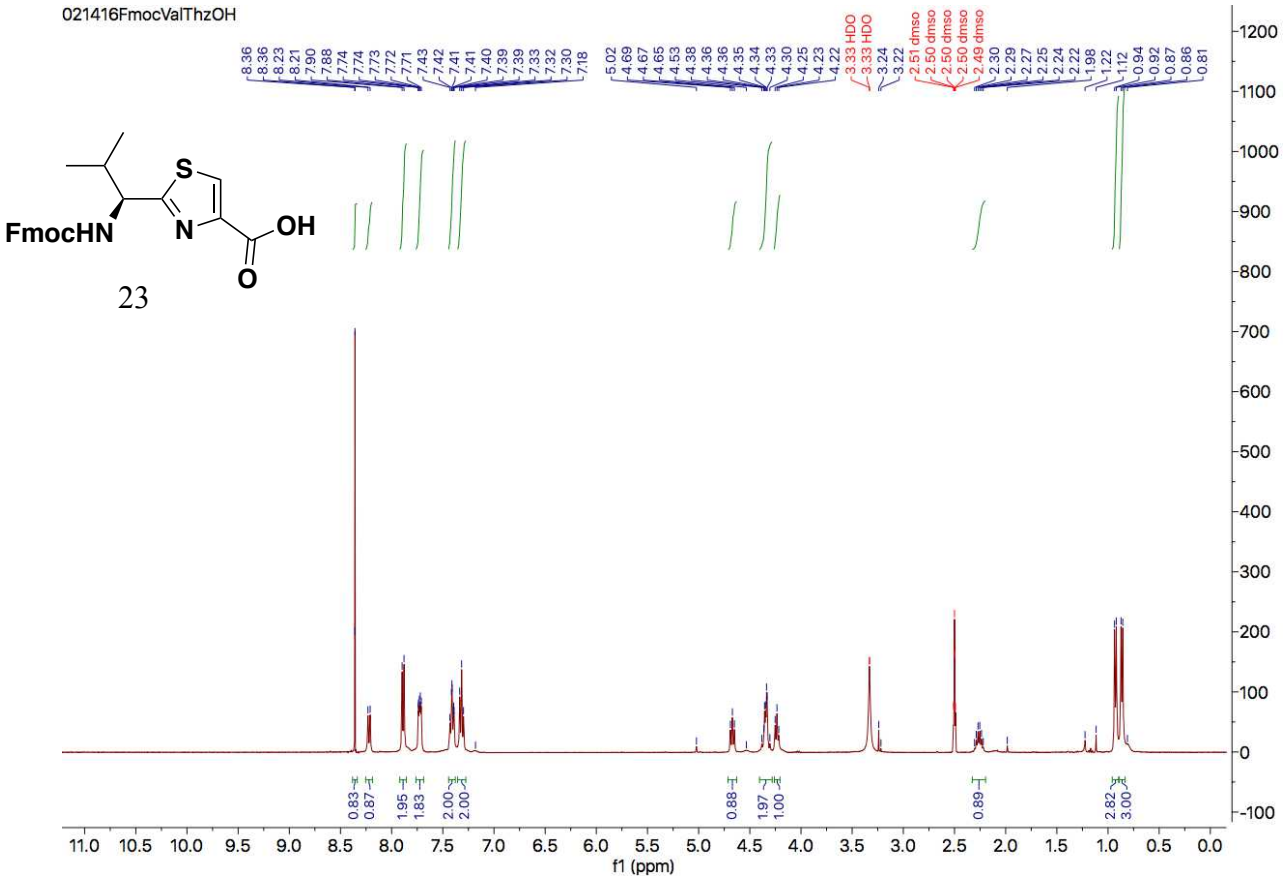


21

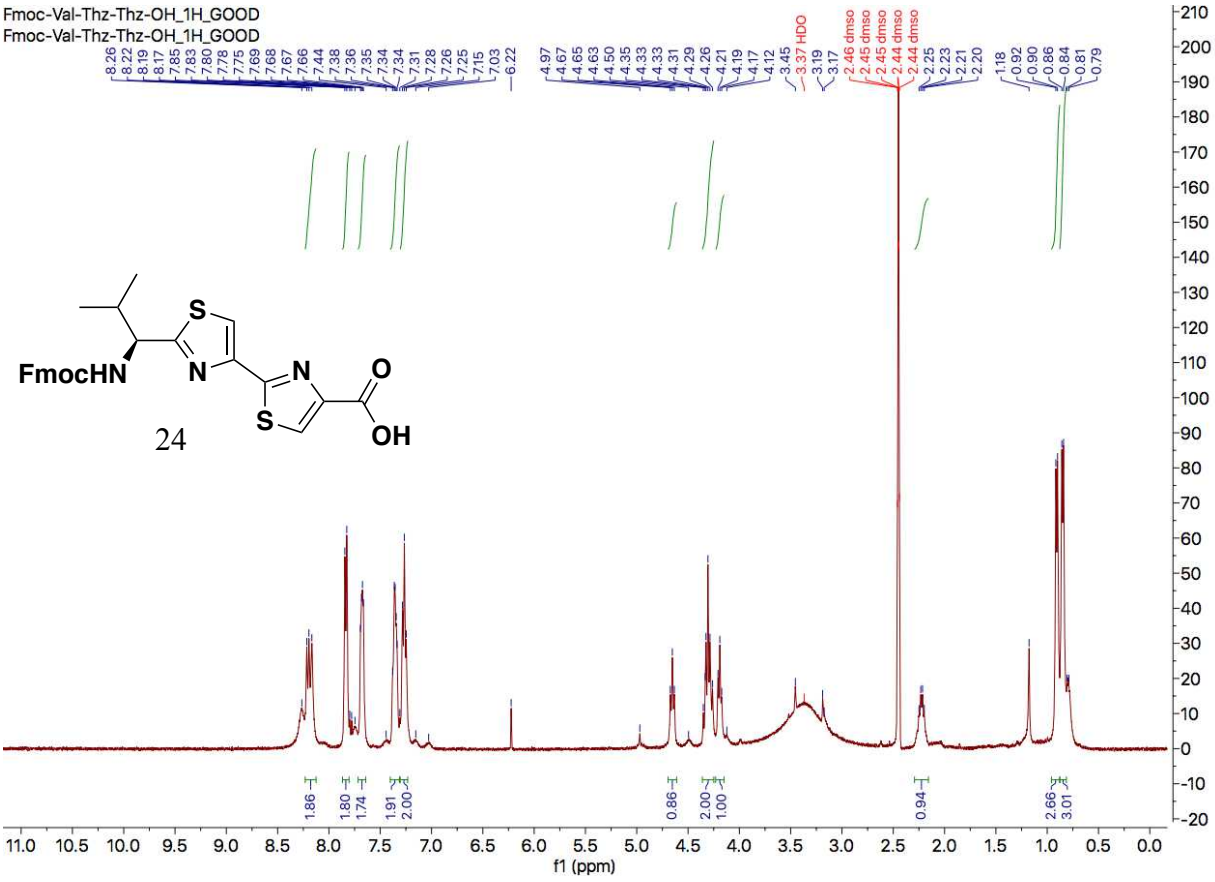

Fmoc-Cys_STrt-Thz-OH_13C_GOOD
Fmoc-Cys_STrt-Thz-OH_13C_GOOD
TrtS
FmocHN
S
N
OH
O
21
172.13
162.57
155.96
144.66
144.14
143.60
141.20
130.06
129.58
128.62
128.10
127.54
127.52
127.35
125.69
125.66
120.59
66.96
66.17
52.97
47.11
40.50 dmso
40.33 dmso
40.16 dmso
40.00 dmso
39.83 dmso
39.66 dmso
39.50 dmso
35.92
31.75
29.48
29.16
14.43
f1 (ppm)

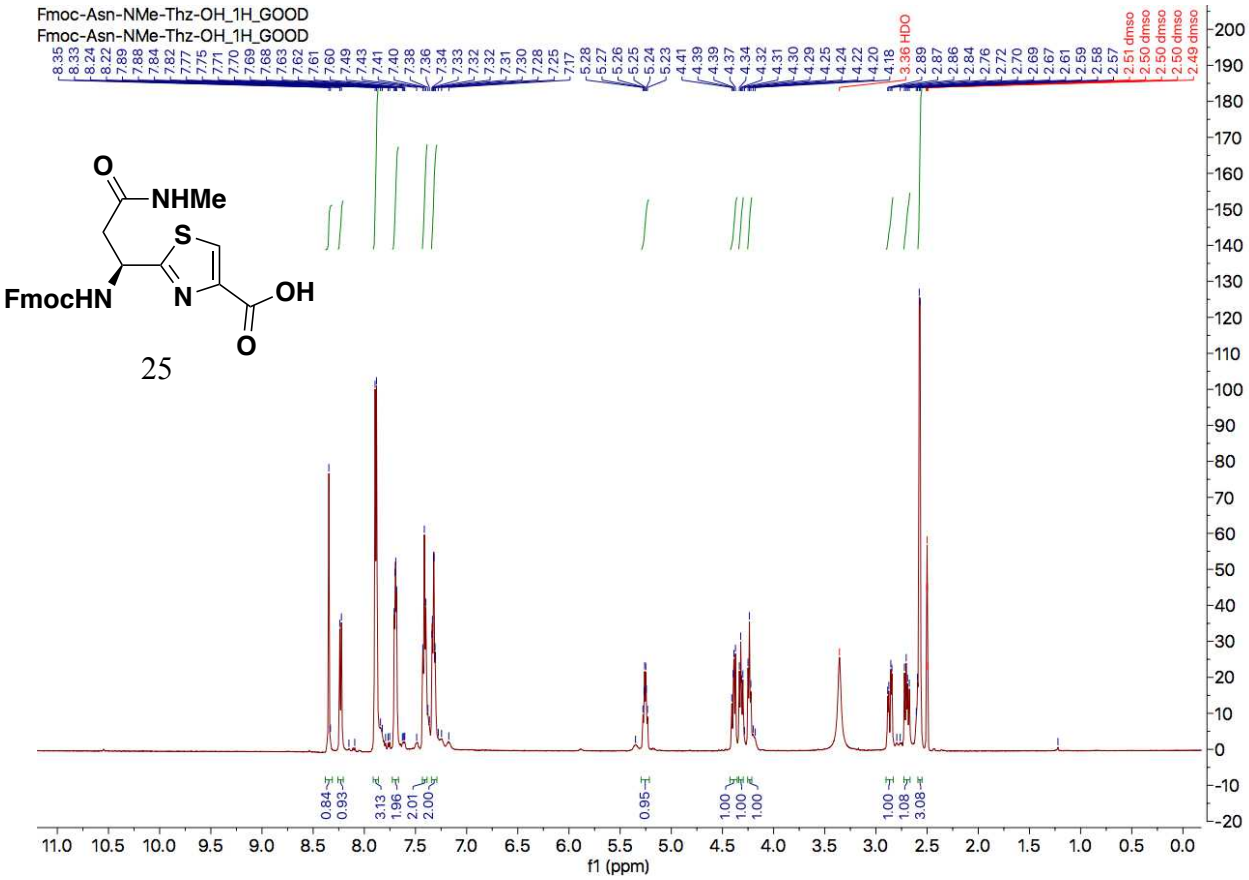

Fmoc-Cys_STrt-Thz-Thz-OH_1H_GOOD
Fmoc-Cys_STrt-Thz-Thz-OH_1H_GOOD
TrtS
FmocHN
S
N
N
S
O
OH
22
f1 (ppm)


Fmoc-Cys_STrt-Thz-Thz-OH_13C_GOOD
Fmoc-Cys_STrt-Thz-Thz-OH_13C_GOOD
173.93
162.53
156.13
148.60
147.97
144.68
144.16
141.25
129.64
128.62
128.13
127.57
127.55
127.37
125.70
125.62
120.60
118.56
67.28
66.18
53.55
47.20
40.50 dmso
40.33 dmso
40.17 dmso
40.00 dmso
39.83 dmso
39.67 dmso
39.50 dmso
36.24
25.73
TrtS
FmocHN
S
N
N
O
S
OH
22
f1 (ppm)

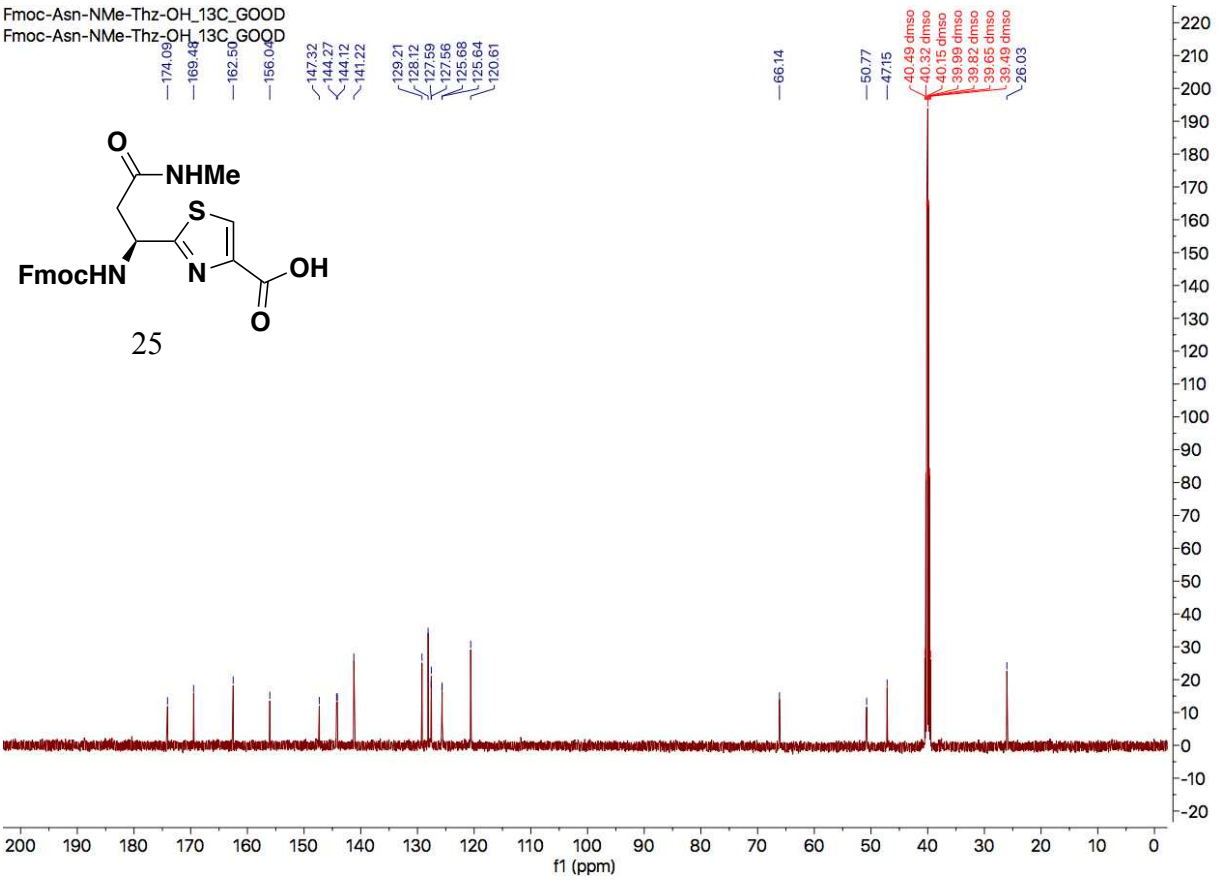


23

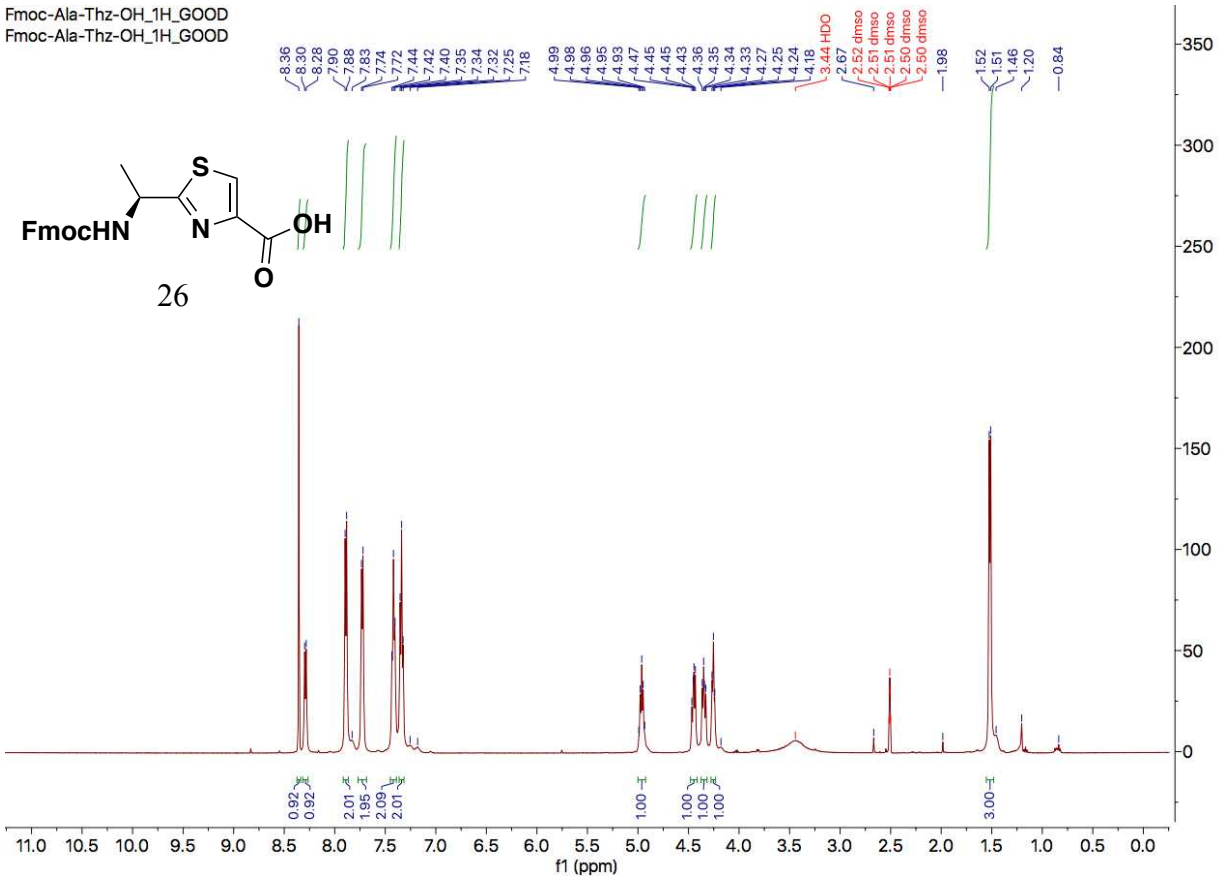

Fmoc-Val-Thz-Thz-OH_1H_GOOD
Fmoc-Val-Thz-Thz-OH_1H_GOOD
FmocHN
S
N
N
S
O
OH
24
f1 (ppm)


Fmoc-Asn-NMe-Thz-OH_1H_GOOD
Fmoc-Asn-NMe-Thz-OH_1H_GOOD
O
NHMe
S
FmocHN
N
OH
O
f1 (ppm)

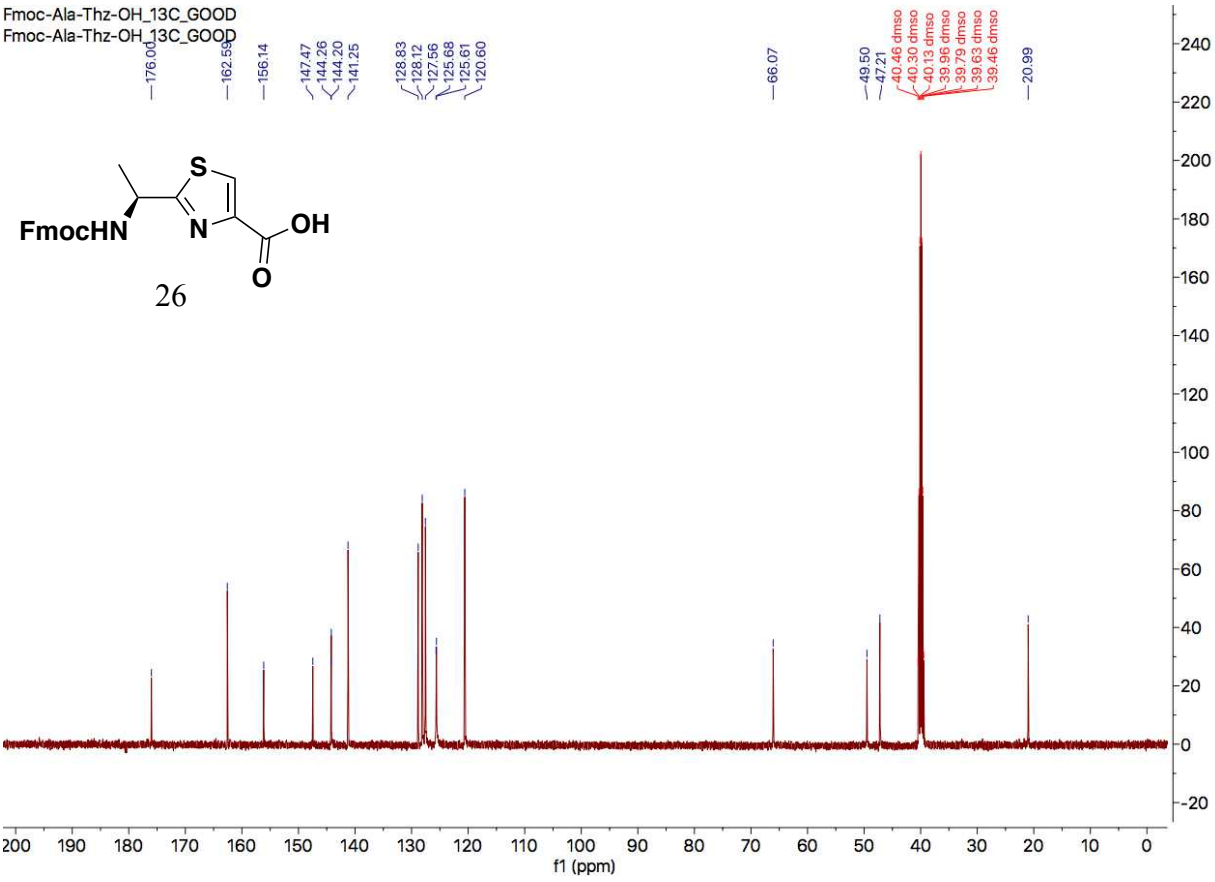
25


Fmoc-Asn-NMe-Thz-OH_13C_GOOD
Fmoc-Asn-NMe-Thz-OH_13C_GOOD
174.09
169.48
162.50
156.04
147.32
144.27
144.12
141.22
129.21
128.12
127.59
127.56
125.68
125.64
120.61
66.14
50.77
47.15
40.49 dmso
40.32 dmso
40.15 dmso
39.99 dmso
39.82 dmso
39.65 dmso
39.49 dmso
26.03
O
NHMe
S
FmocHN
N
OH
O
f1 (ppm)

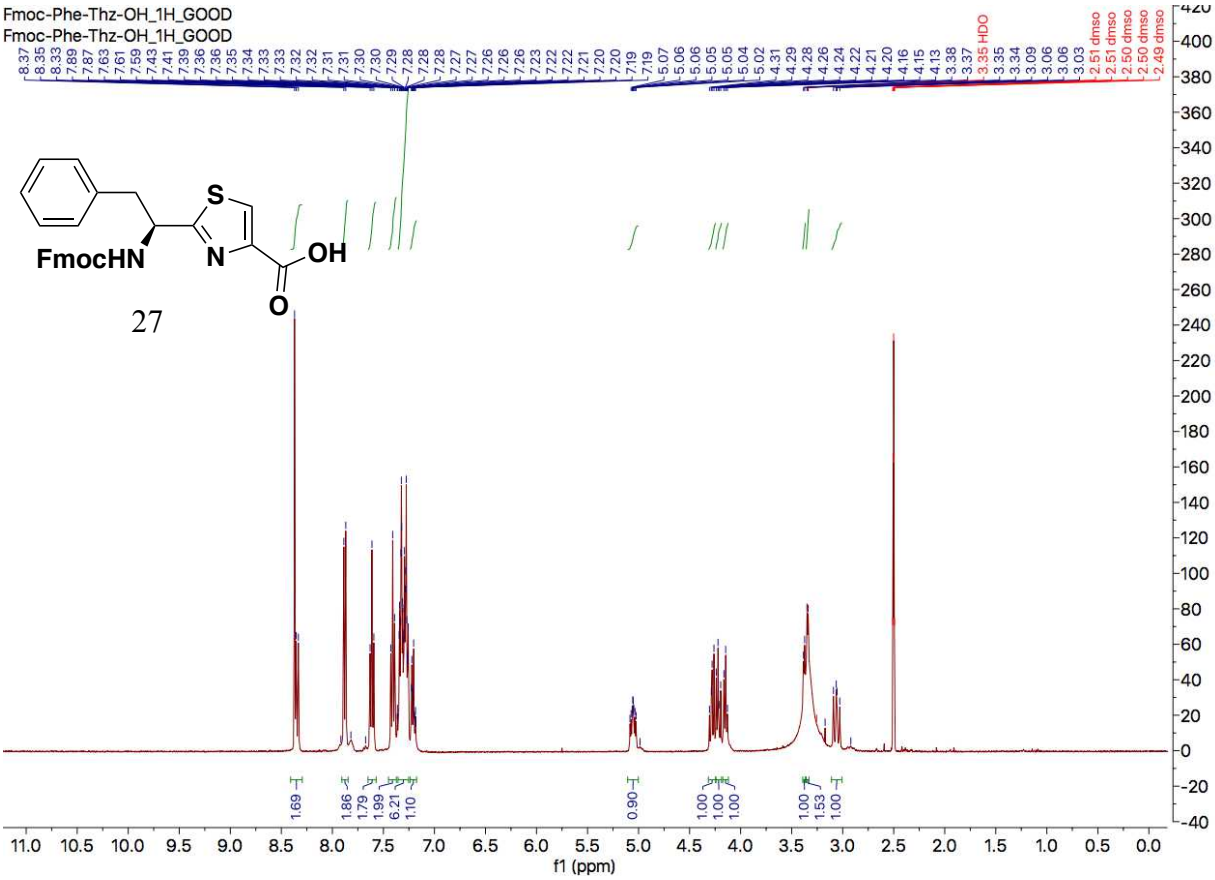
25

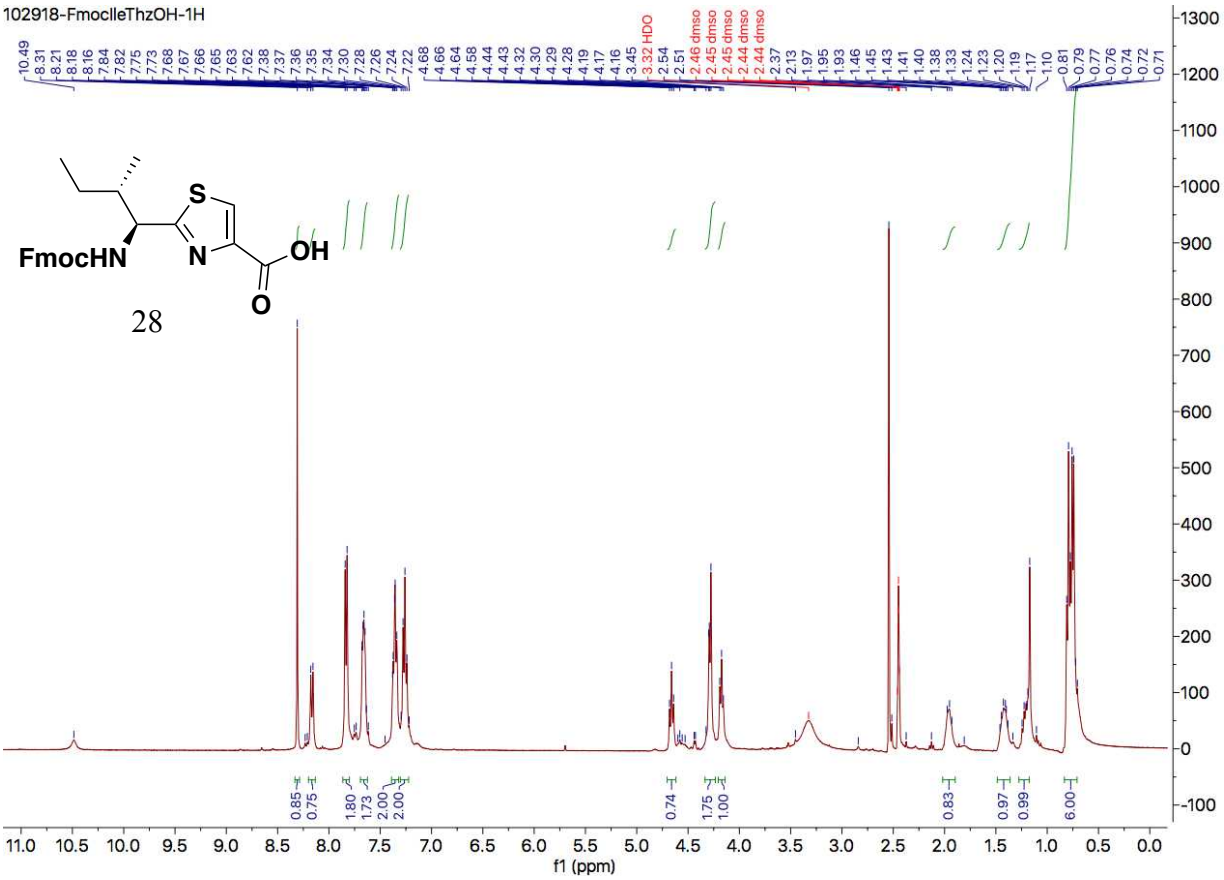

Fmoc-Ala-Thz-OH_1H_GOOD
Fmoc-Ala-Thz-OH_1H_GOOD
FmocHN
S
N
OH
O
f1 (ppm)

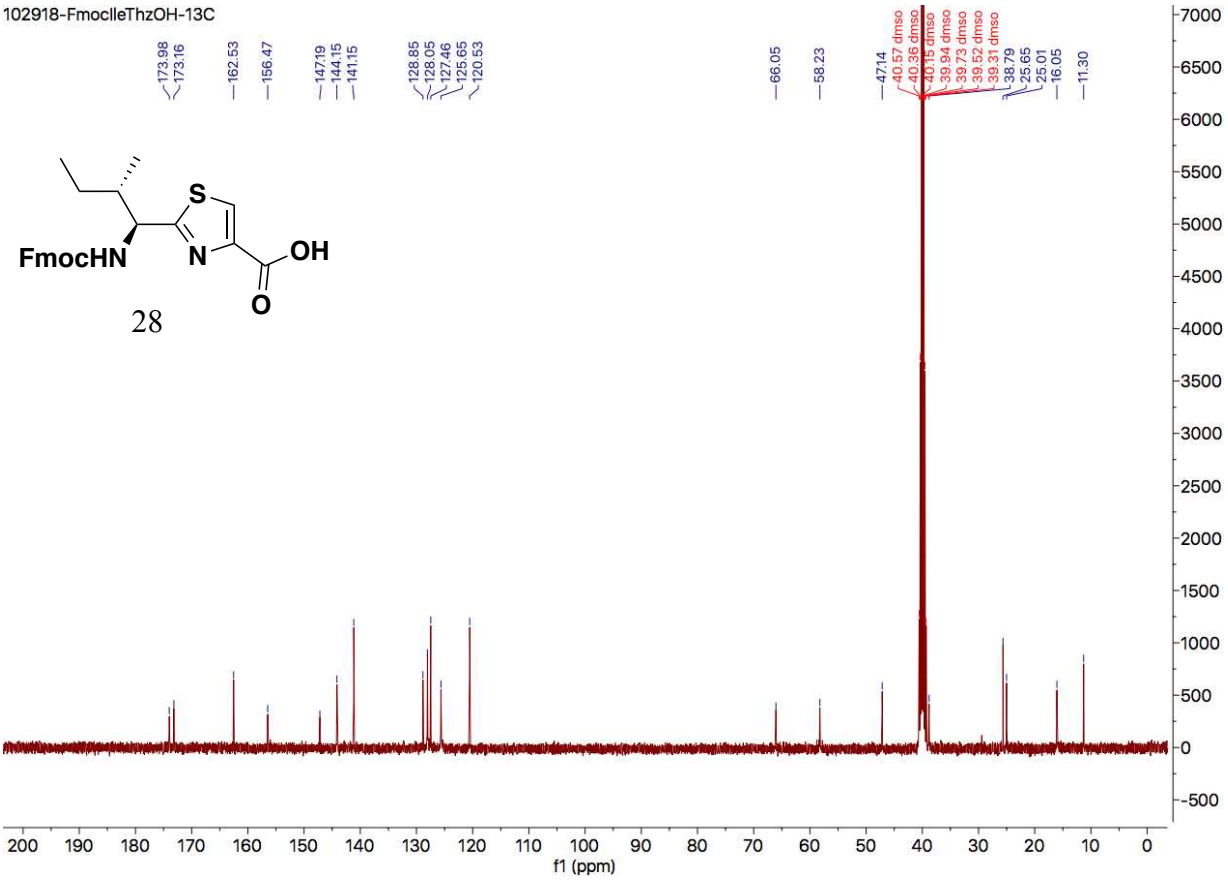
26


Fmoc-Ala-Thz-OH_13C_GOOD
Fmoc-Ala-Thz-OH_13C_GOOD
176.00
162.59
156.14
147.47
144.26
144.20
141.25
128.83
128.12
127.56
125.68
125.61
120.60
66.07
49.50
47.21
40.46 dmso
40.30 dmso
40.13 dmso
39.96 dmso
39.79 dmso
39.63 dmso
39.46 dmso
20.99
FmocHN
S
N
OH
O
f1 (ppm)

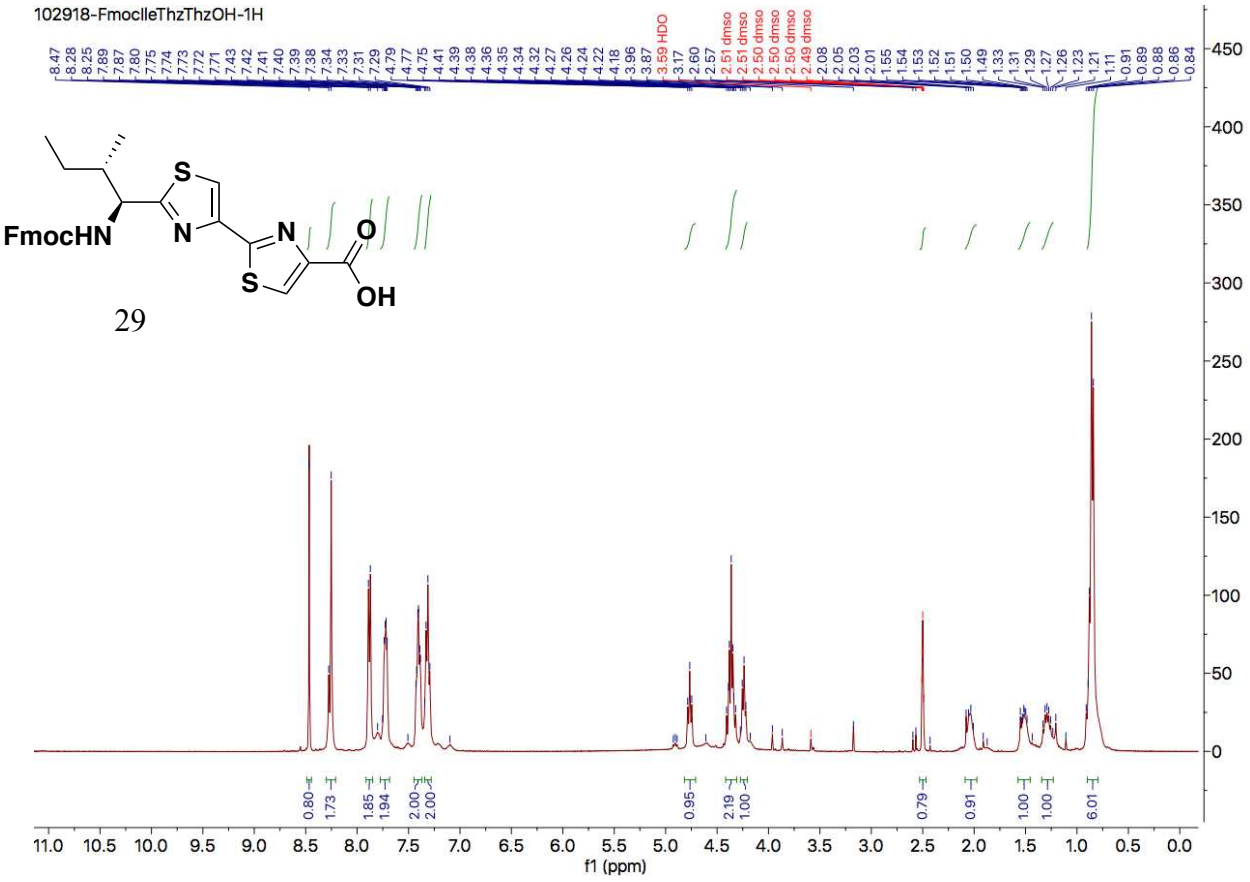
26


Fmoc-Phe-Thz-OH_1H_GOOD
Fmoc-Phe-Thz-OH_1H_GOOD
FmocHN
S
N
OH
O
27
f1 (ppm)


102918-FmocIleThzOH-1H
FmocHN
S
N
OH
O
28
f1 (ppm)


102918-FmocIleThzOH-13C
173.98
173.16
162.53
156.47
147.19
144.15
141.15
128.85
128.05
127.46
125.65
120.53
66.05
58.23
47.14
40.57 dmso
40.36 dmso
40.15 dmso
39.94 dmso
39.73 dmso
39.52 dmso
39.31 dmso
38.79
25.65
25.01
16.05
11.30
FmocHN
S
N
OH
O
f1 (ppm)

28



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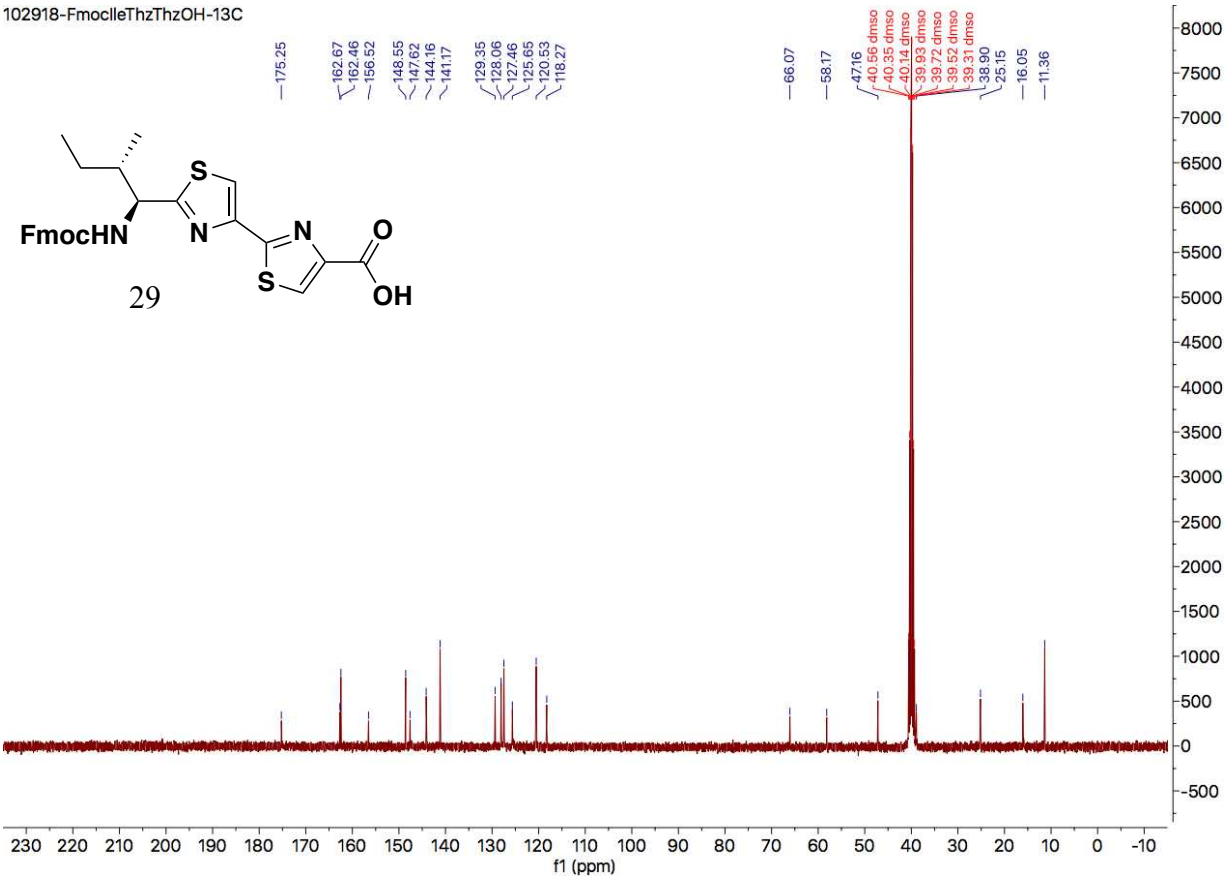
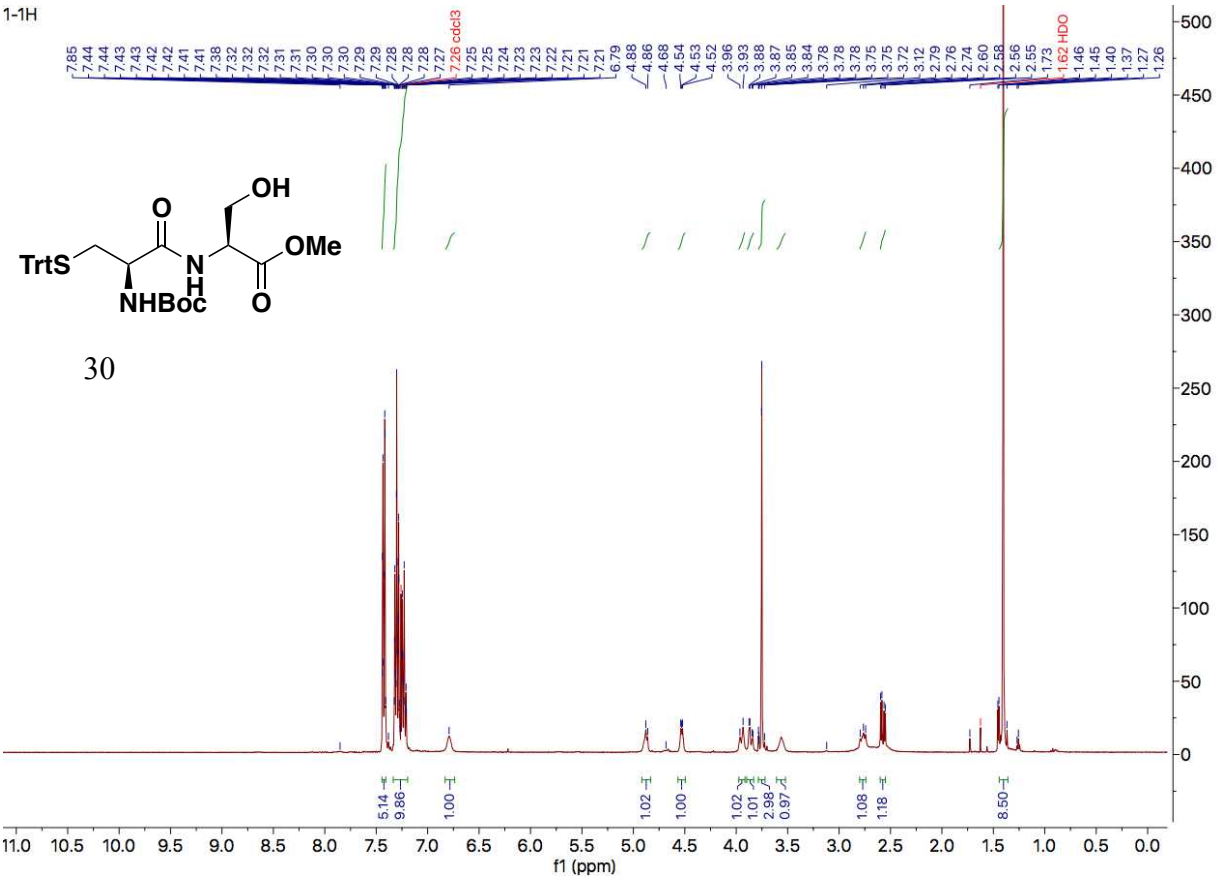
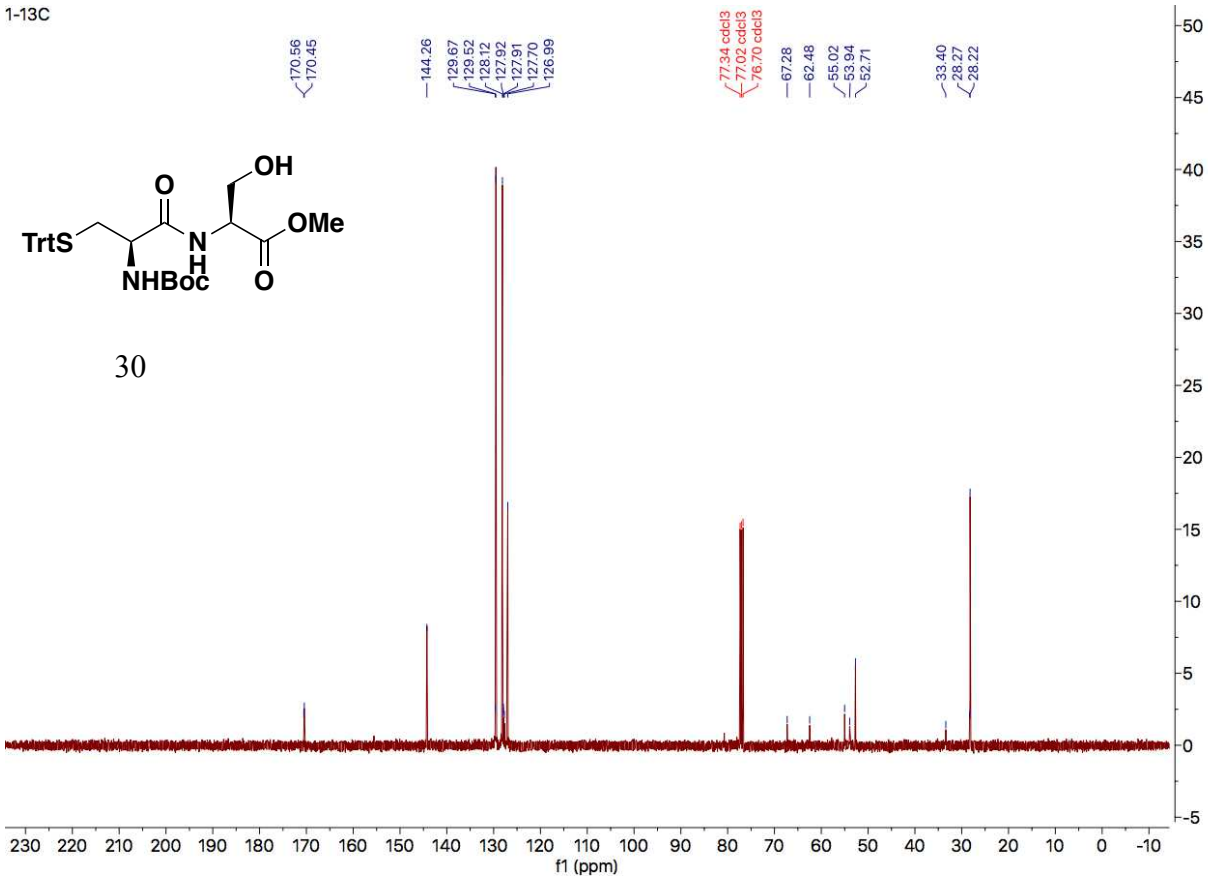
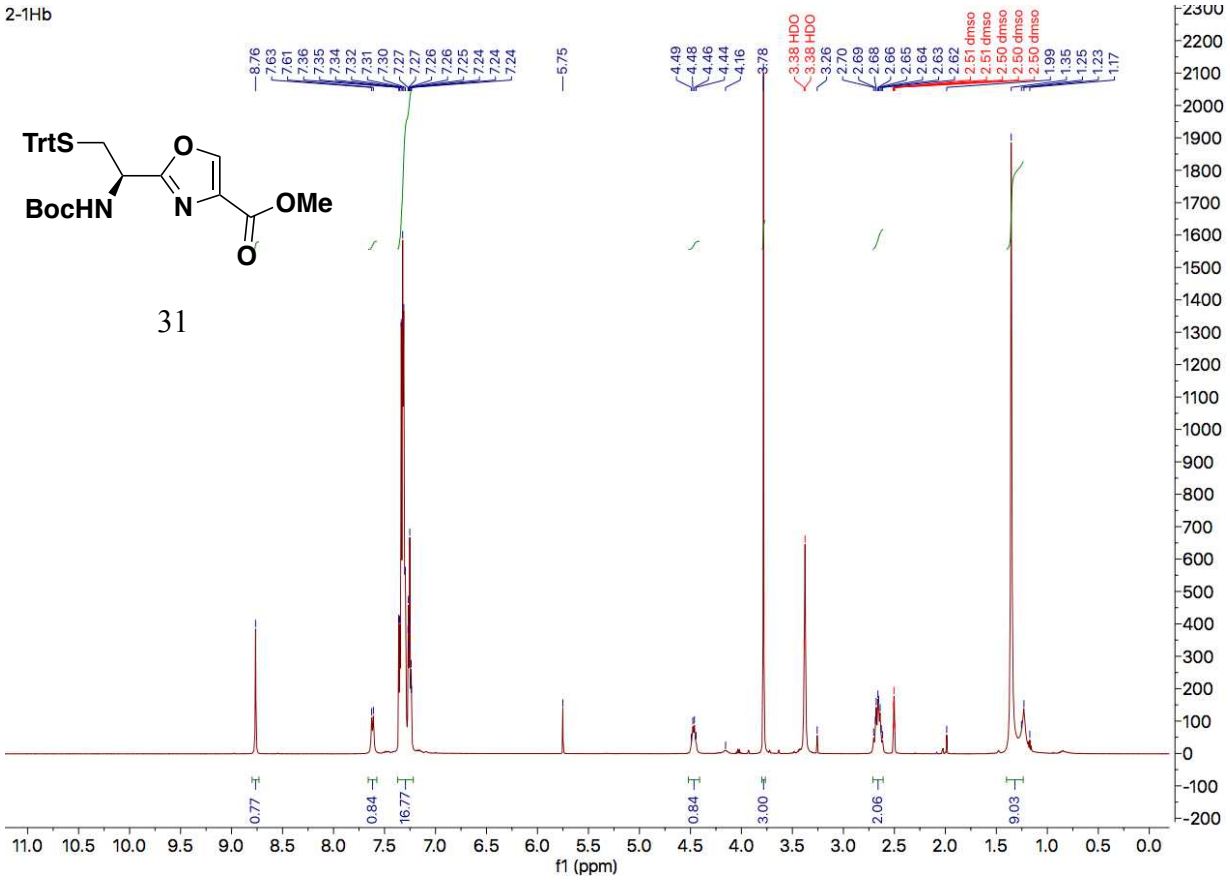

102918-FmocIleThzThzOH-13C
175.25
162.67
162.46
156.52
148.55
147.62
144.16
141.17
129.35
128.06
127.46
125.65
120.53
118.27
66.07
58.17
47.16
40.56 dmso
40.35 dmso
40.14 dmso
39.93 dmso
39.72 dmso
39.52 dmso
39.31 dmso
38.90
25.15
16.05
11.36
FmocHN
S
N
N
O
S
OH
29
f1 (ppm)

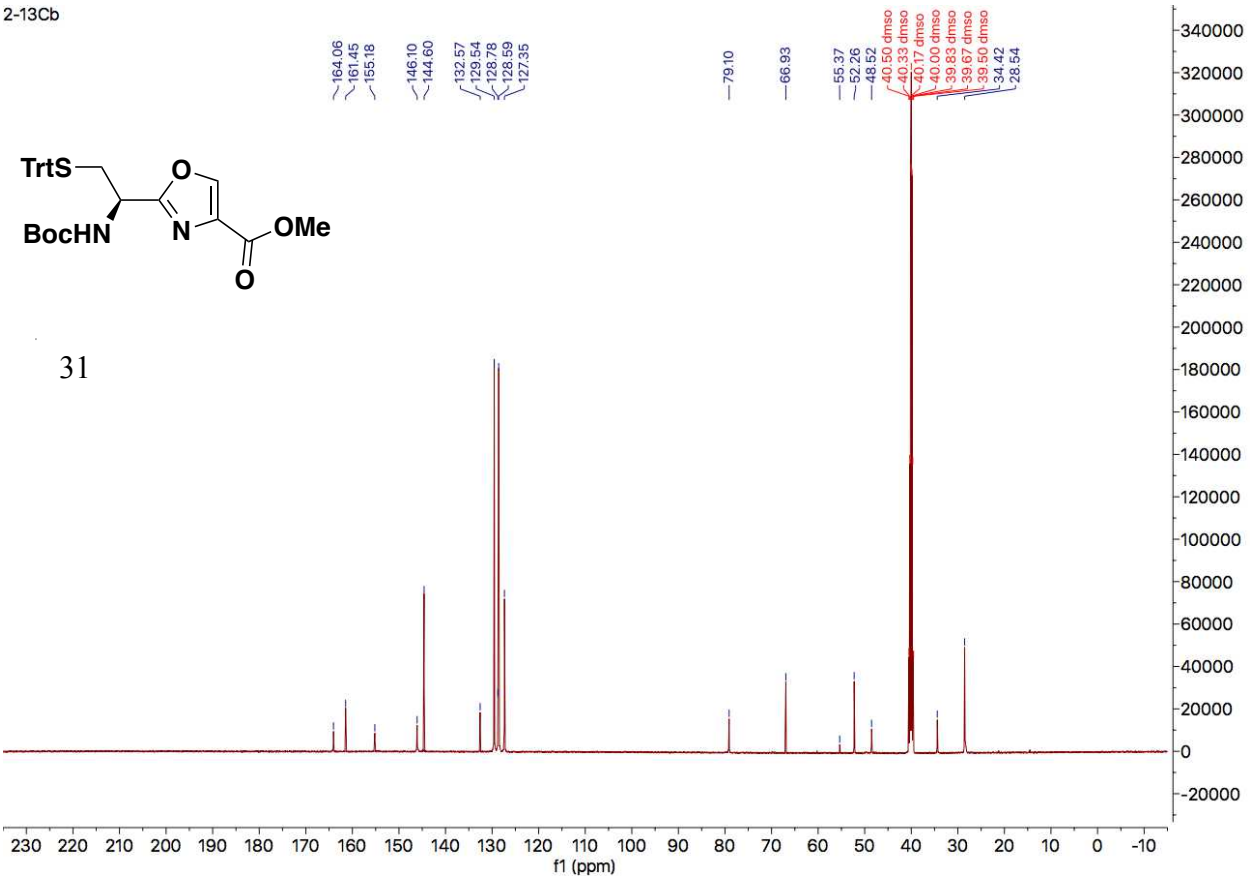


30

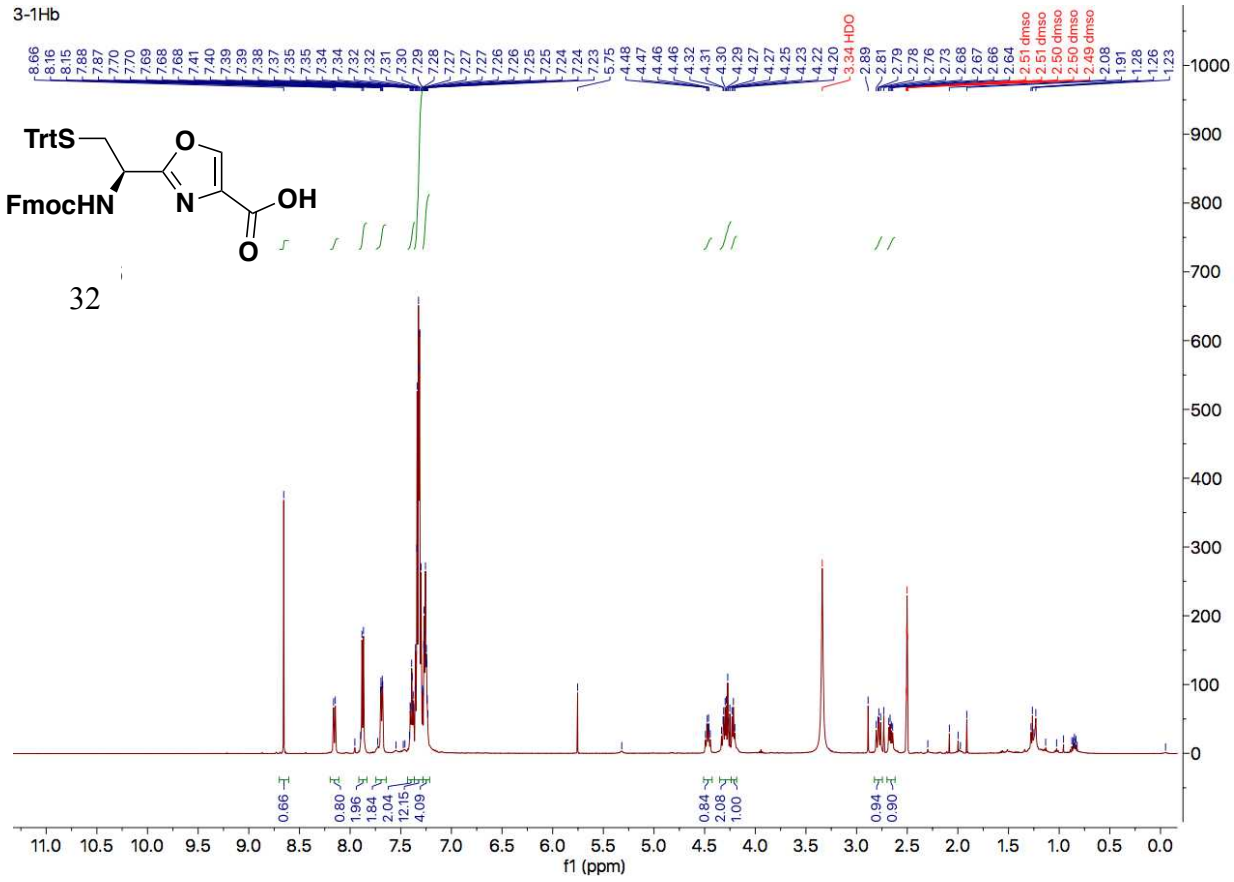

1-13C
170.56
170.45
144.26
129.67
129.52
128.12
127.92
127.91
127.70
126.99
77.34 cdcl3
77.02 cdcl3
76.70 cdcl3
67.28
62.48
55.02
53.94
52.71
33.40
28.27
28.22
OH
O
OMe
TrtS
N
H
NHBoc
O
f1 (ppm)

30

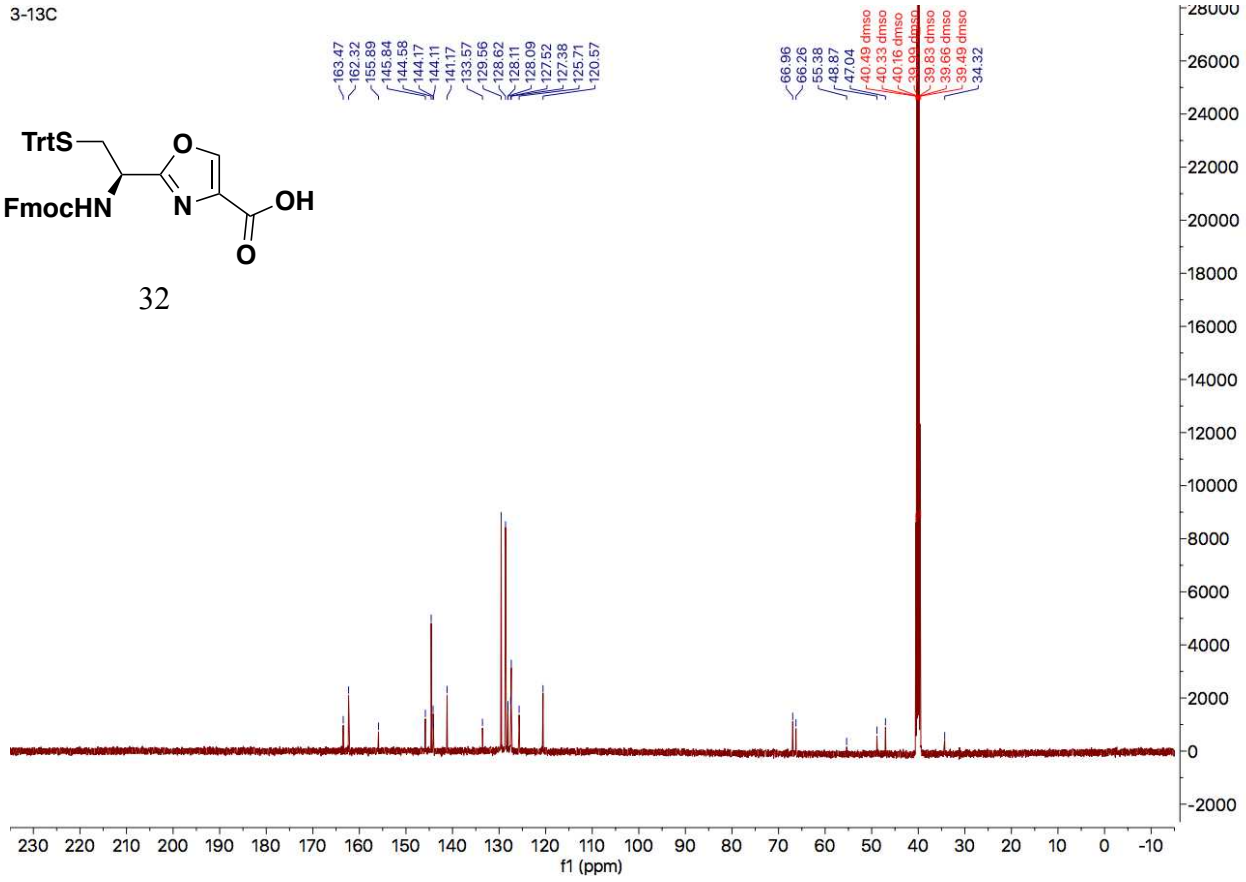

2-1Hb
TrtS
BocHN
O
N
OMe
O
31
f1 (ppm)


2-13Cb
TrtS
BocHN
O
N
OMe
O
164.06
161.45
155.18
146.10
144.60
132.57
129.54
128.78
128.59
127.35
79.10
66.93
55.37
52.26
48.52
40.50 dmso
40.33 dmso
40.17 dmso
40.00 dmso
39.83 dmso
39.67 dmso
39.50 dmso
34.42
28.54
f1 (ppm)

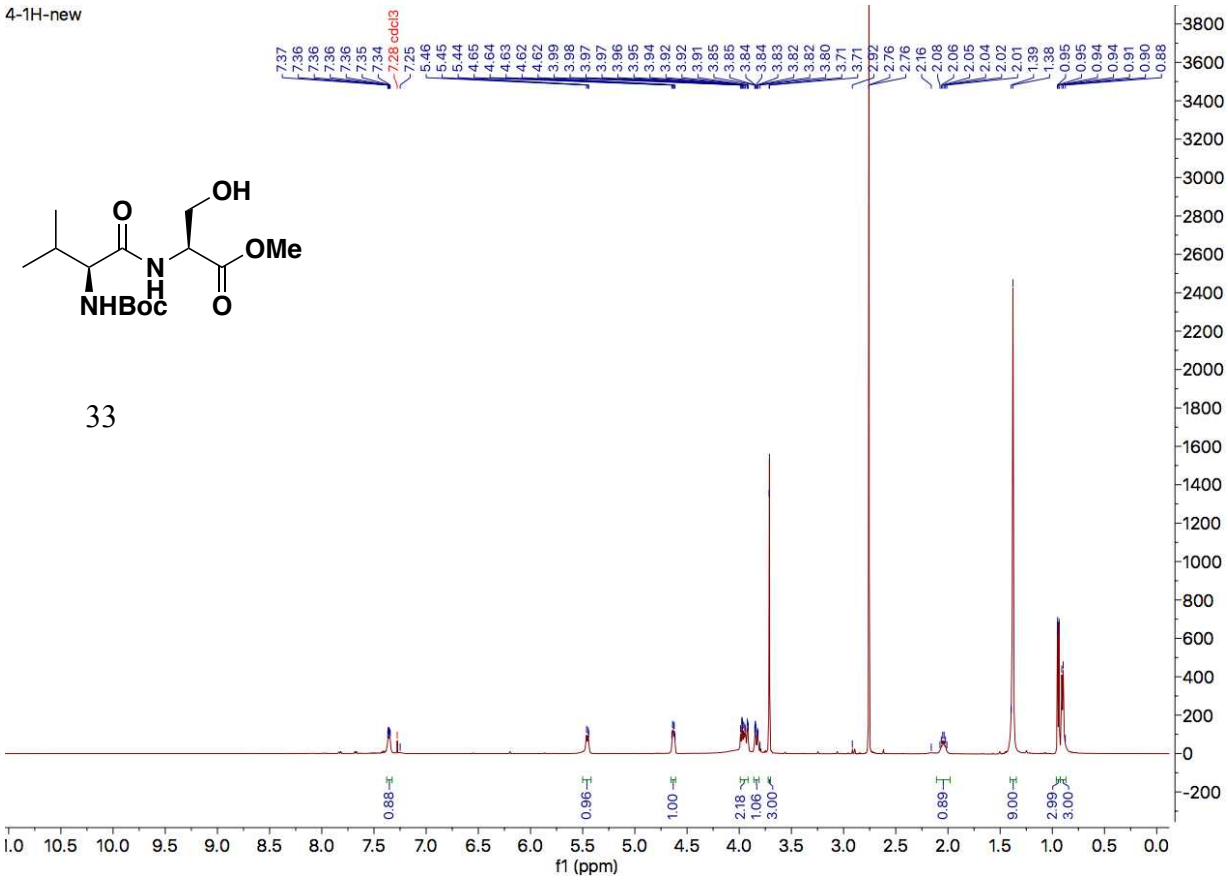
31



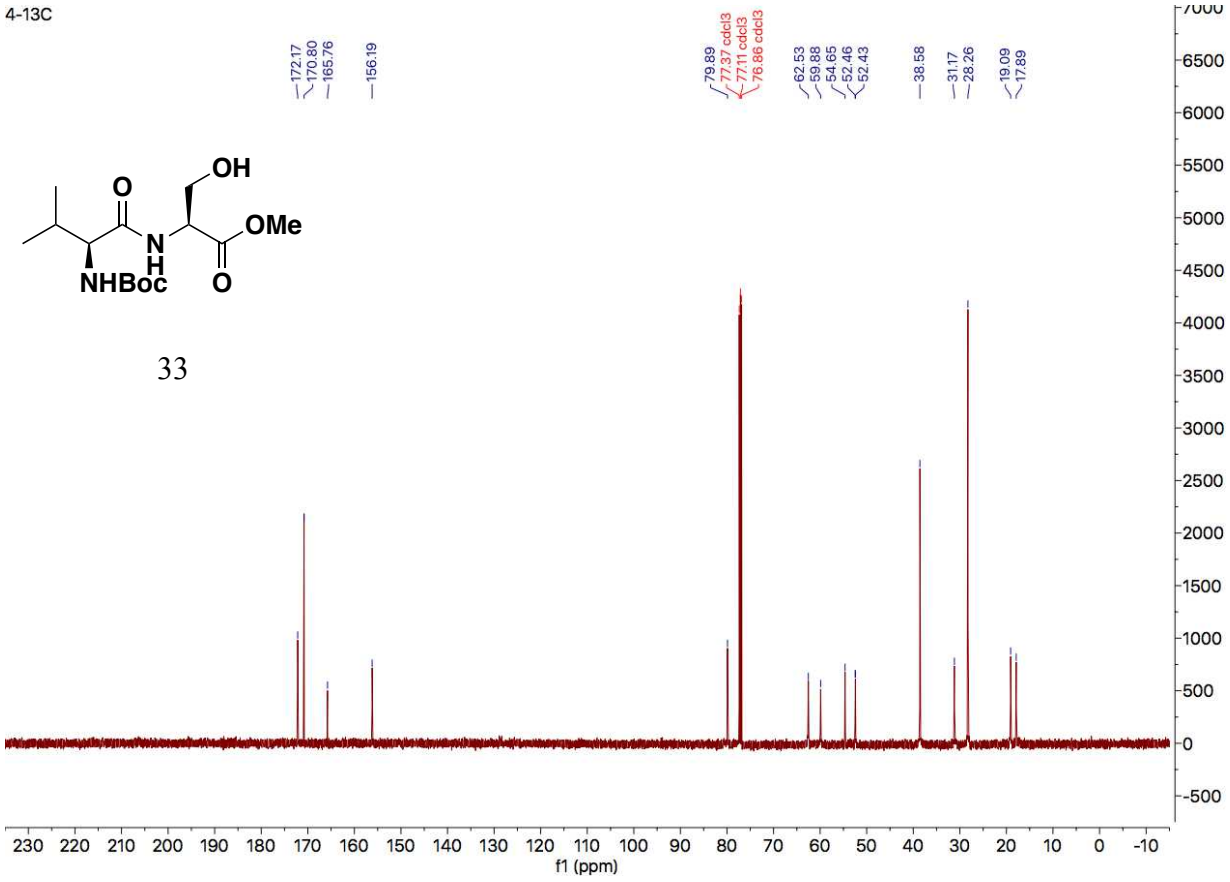
32


3-13C
TrtS
FmocHN
O
N
OH
O
163.47
162.32
155.89
145.84
144.58
144.17
144.11
141.17
133.57
129.56
128.62
128.11
128.09
127.52
127.38
125.71
120.57
66.96
66.26
55.38
48.87
47.04
40.49 dmso
40.33 dmso
40.16 dmso
39.99 dmso
39.83 dmso
39.66 dmso
39.49 dmso
34.32
f1 (ppm)

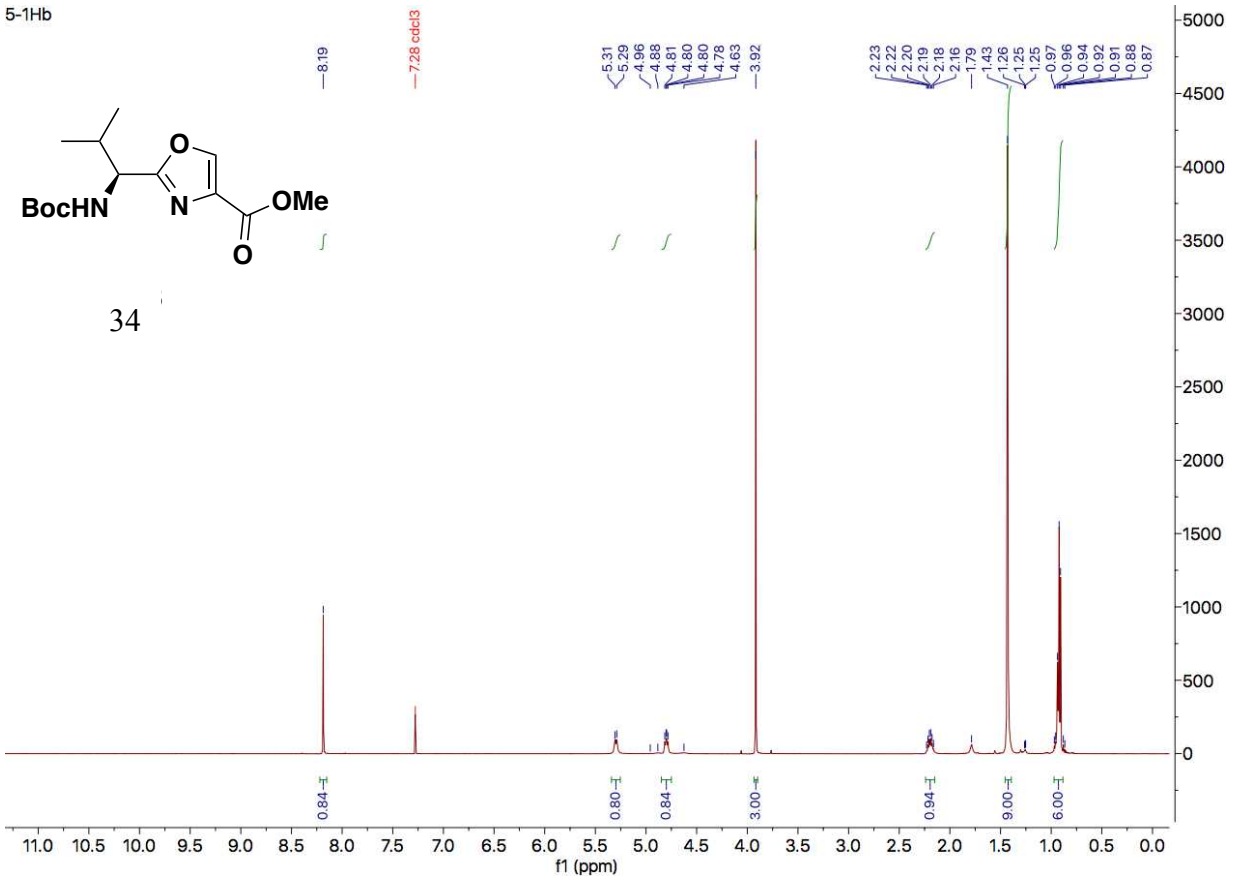
32

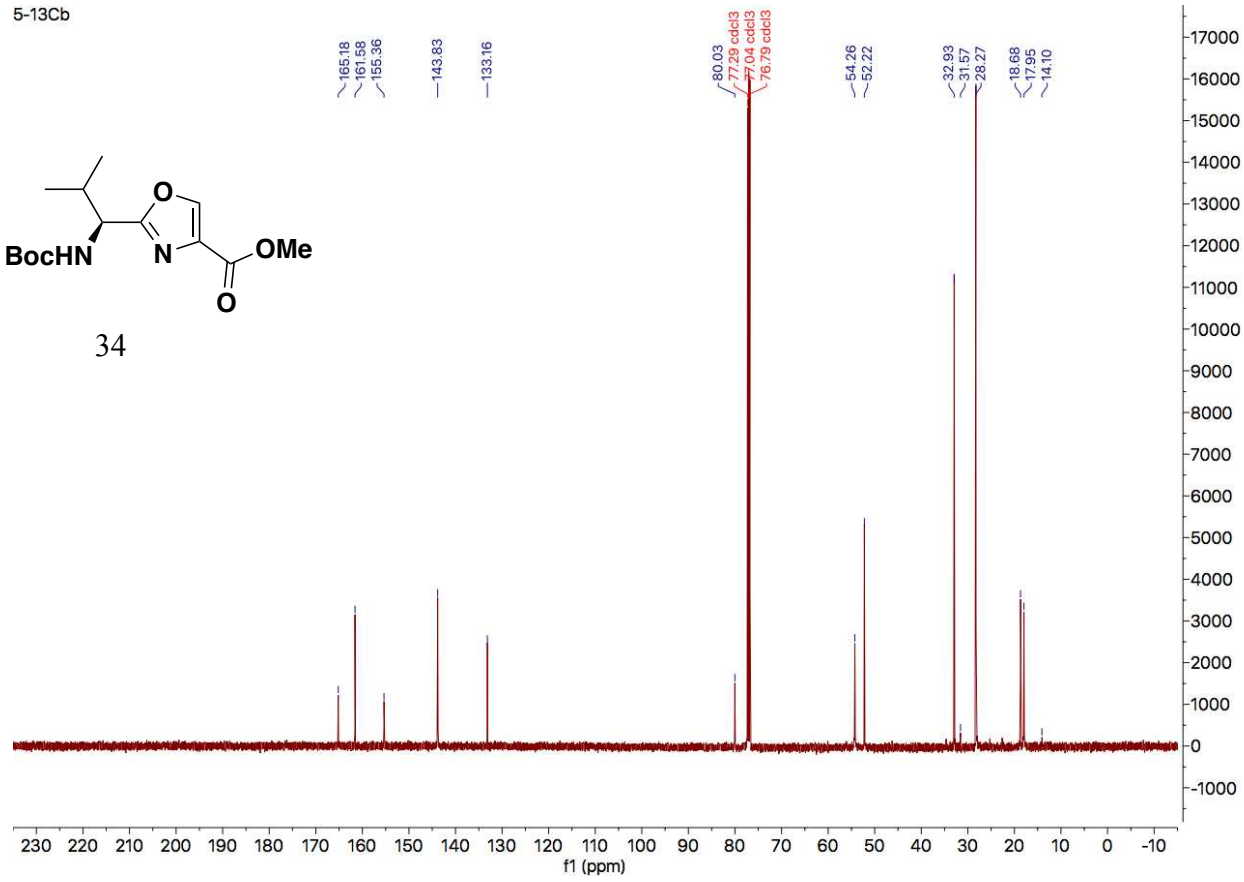


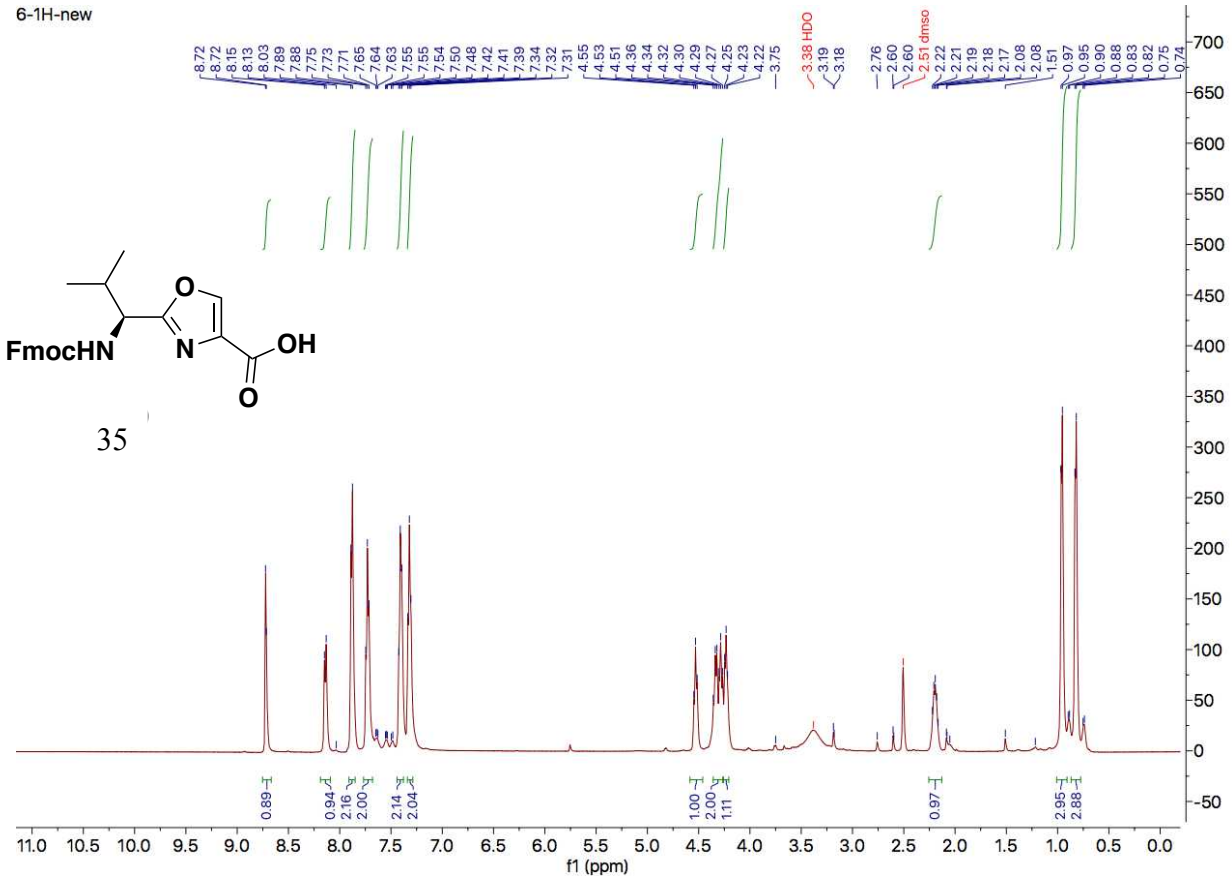
33

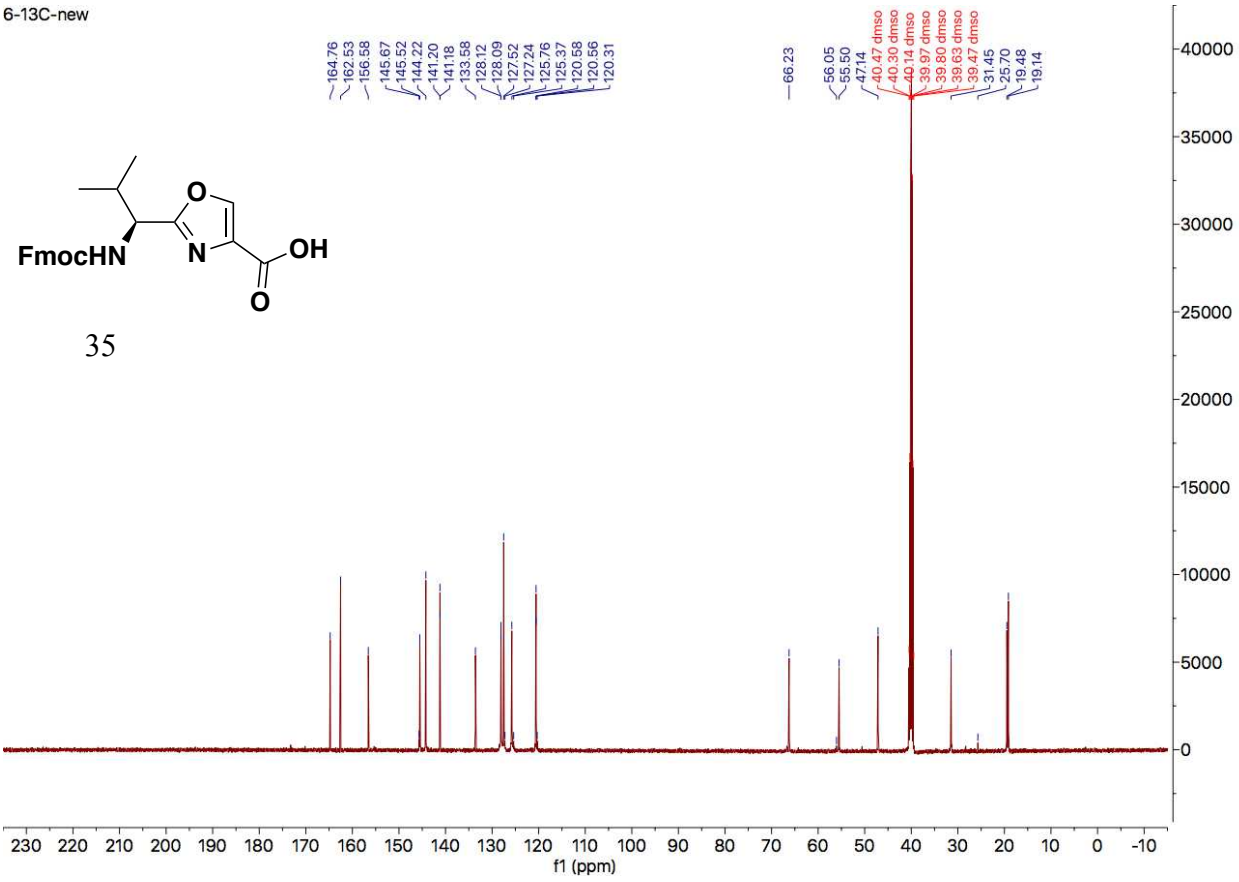

4-13C
172.17
170.80
165.76
156.19
79.89
77.37 cdcl3
77.11 cdcl3
76.86 cdcl3
62.53
59.88
54.65
52.46
52.43
38.58
31.17
28.26
19.09
17.89
OH
O
OMe
N
H
NHBoc
O
f1 (ppm)

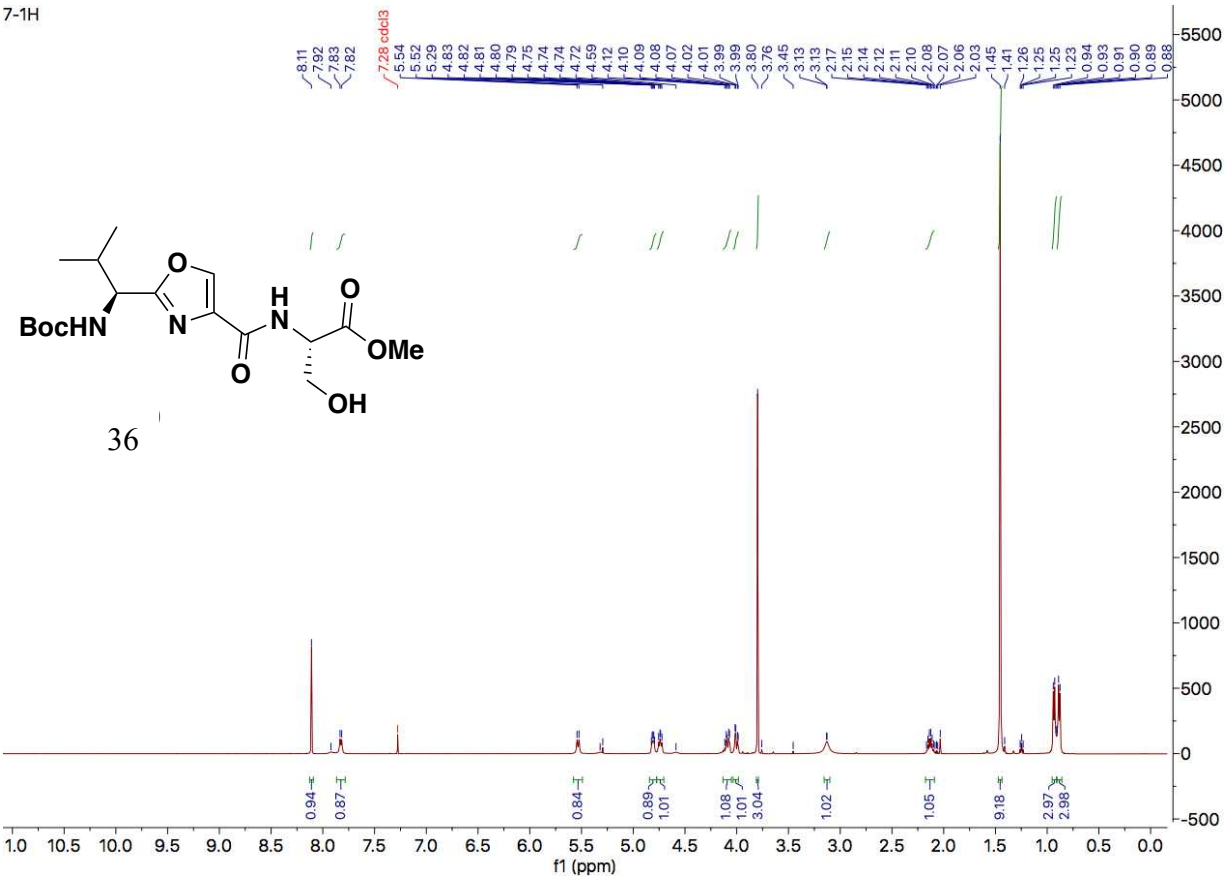
33


5-1Hb
BocHN
N
O
OMe
O
34
8.19
7.28 cdcl3
5.31
5.29
4.96
4.88
4.81
4.80
4.80
4.78
4.63
3.92
2.23
2.22
2.20
2.19
2.18
2.16
1.79
1.43
1.26
1.25
1.25
0.97
0.96
0.94
0.92
0.91
0.88
0.87
0.84
0.80
0.84
3.00
0.94
9.00
6.00
f1 (ppm)

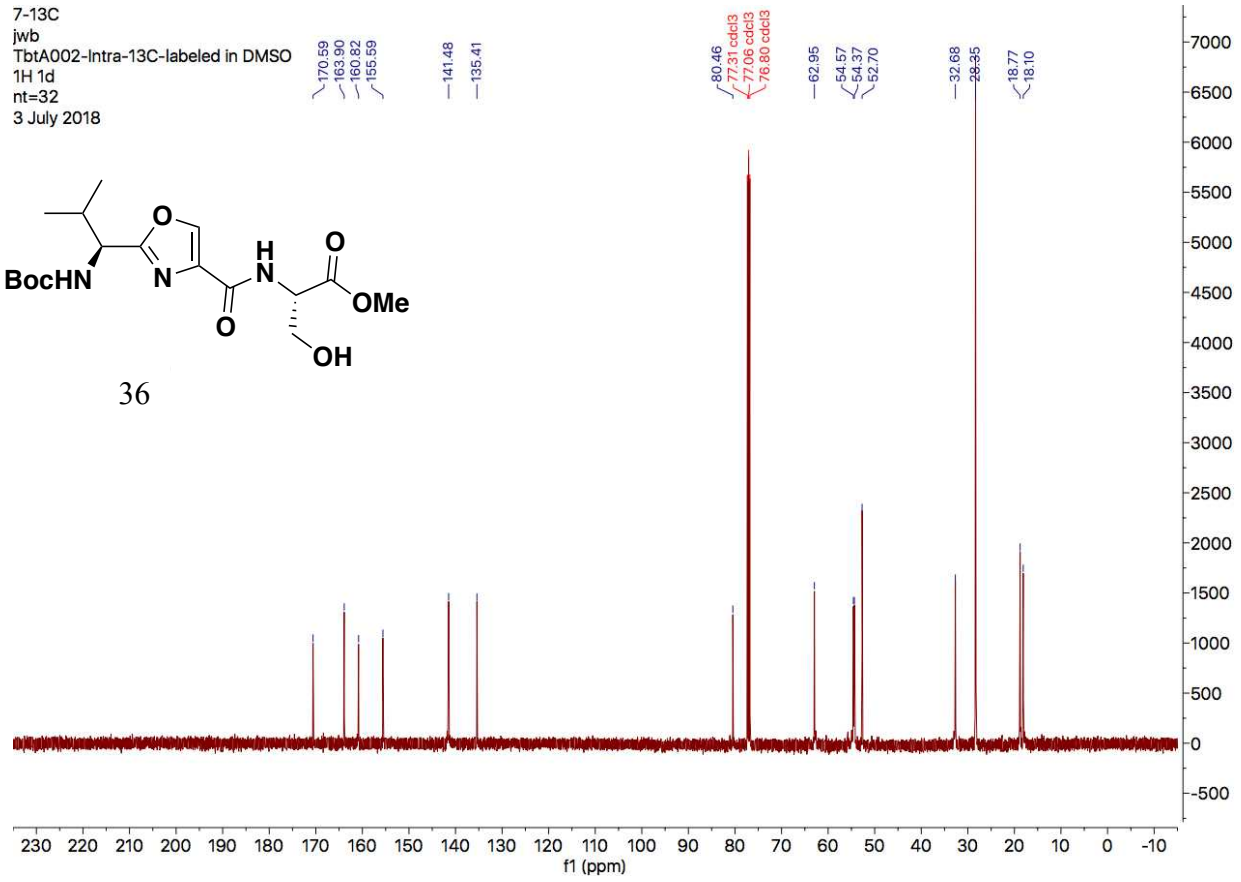

5-13Cb
165.18
161.58
155.36
143.83
133.16
80.03
77.29 cdcl3
77.04 cdcl3
76.79 cdcl3
54.26
52.22
32.93
31.57
28.27
18.68
17.95
14.10
BocHN
O
N
OMe
O
34
f1 (ppm)


6-1H-new
FmocHN
N
O
OH
O
35
f1 (ppm)

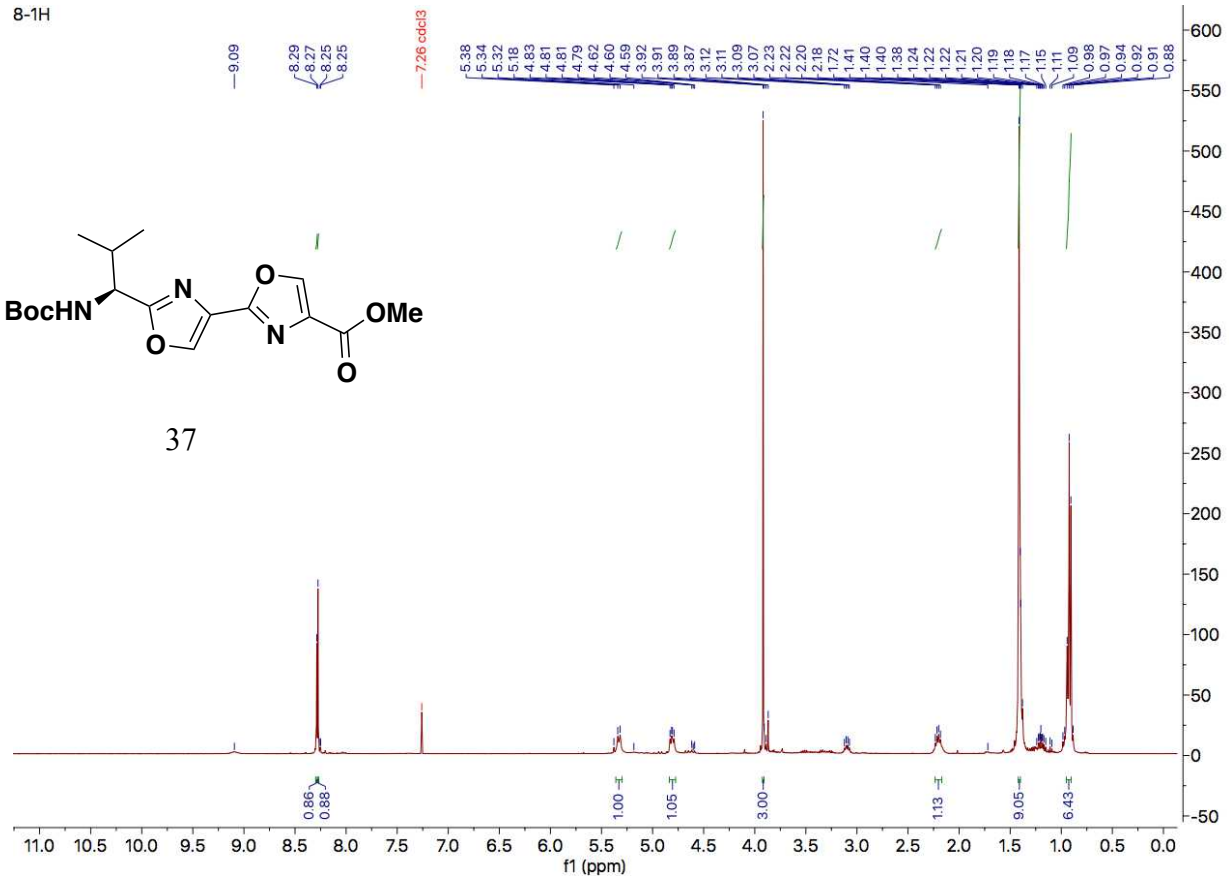

6-13C-new
164.76
162.53
156.58
145.67
145.52
144.22
141.20
141.18
133.58
128.12
128.09
127.52
127.24
125.76
125.37
120.58
120.56
120.31
66.23
56.05
55.50
47.14
40.47 dmso
40.30 dmso
40.14 dmso
39.97 dmso
39.80 dmso
39.63 dmso
39.47 dmso
31.45
25.70
19.48
19.14
FmocHN
O
N
OH
O
35
40000
35000
30000
25000
20000
15000
10000
5000
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

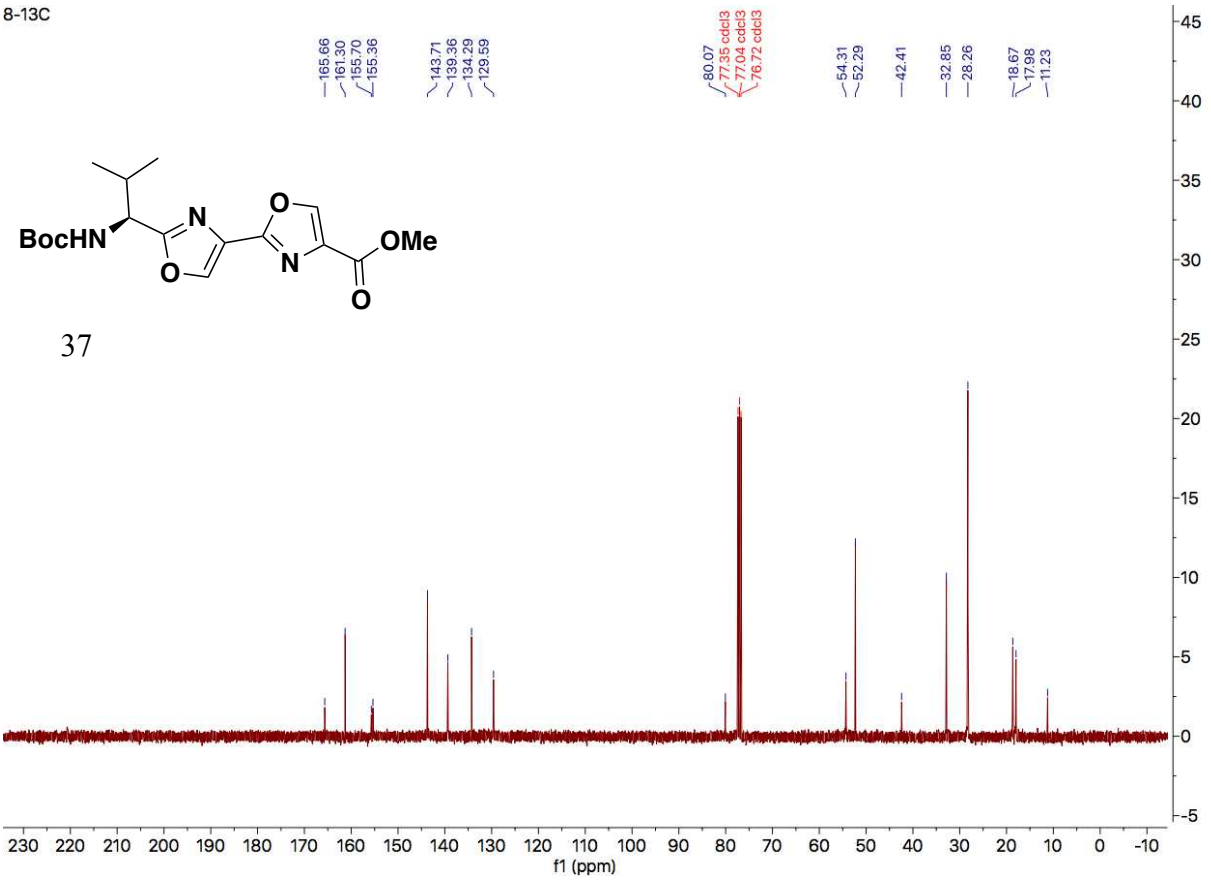


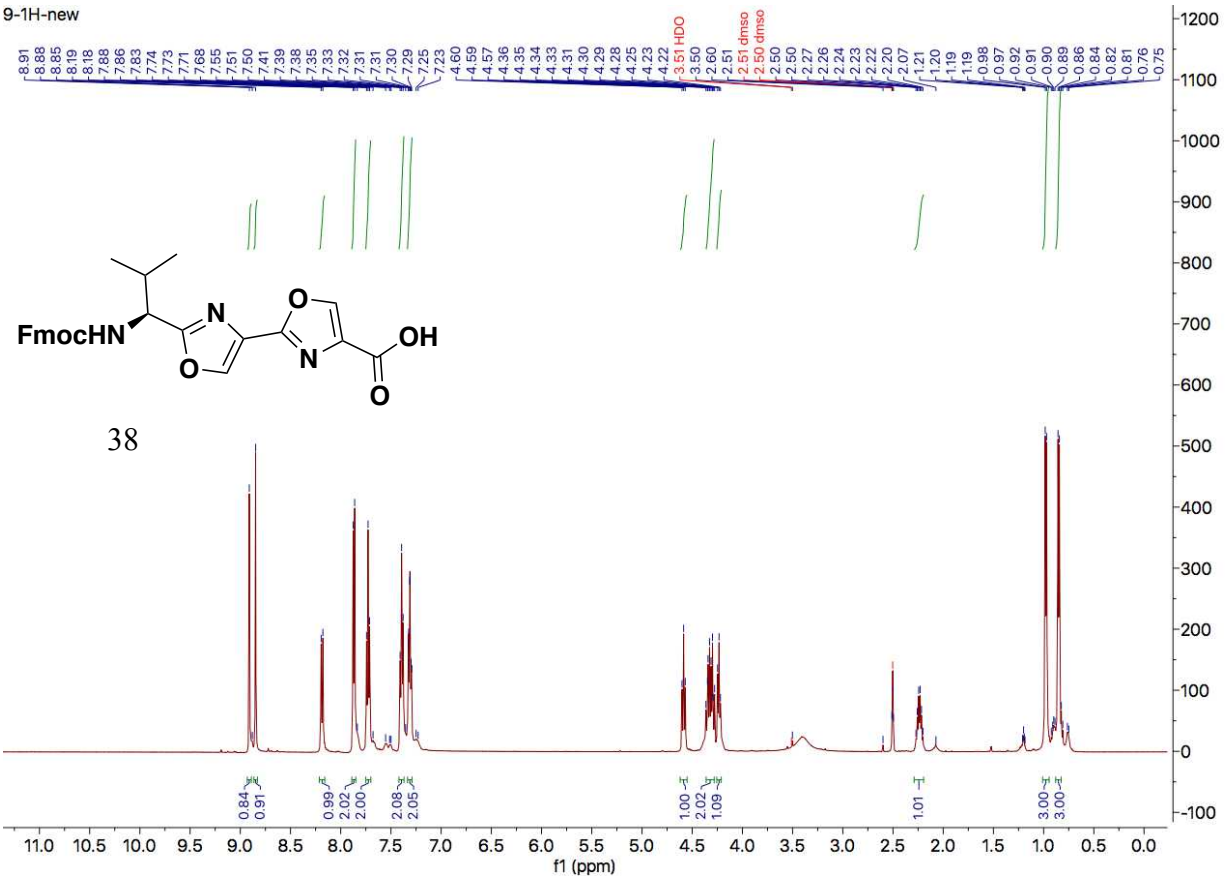
36

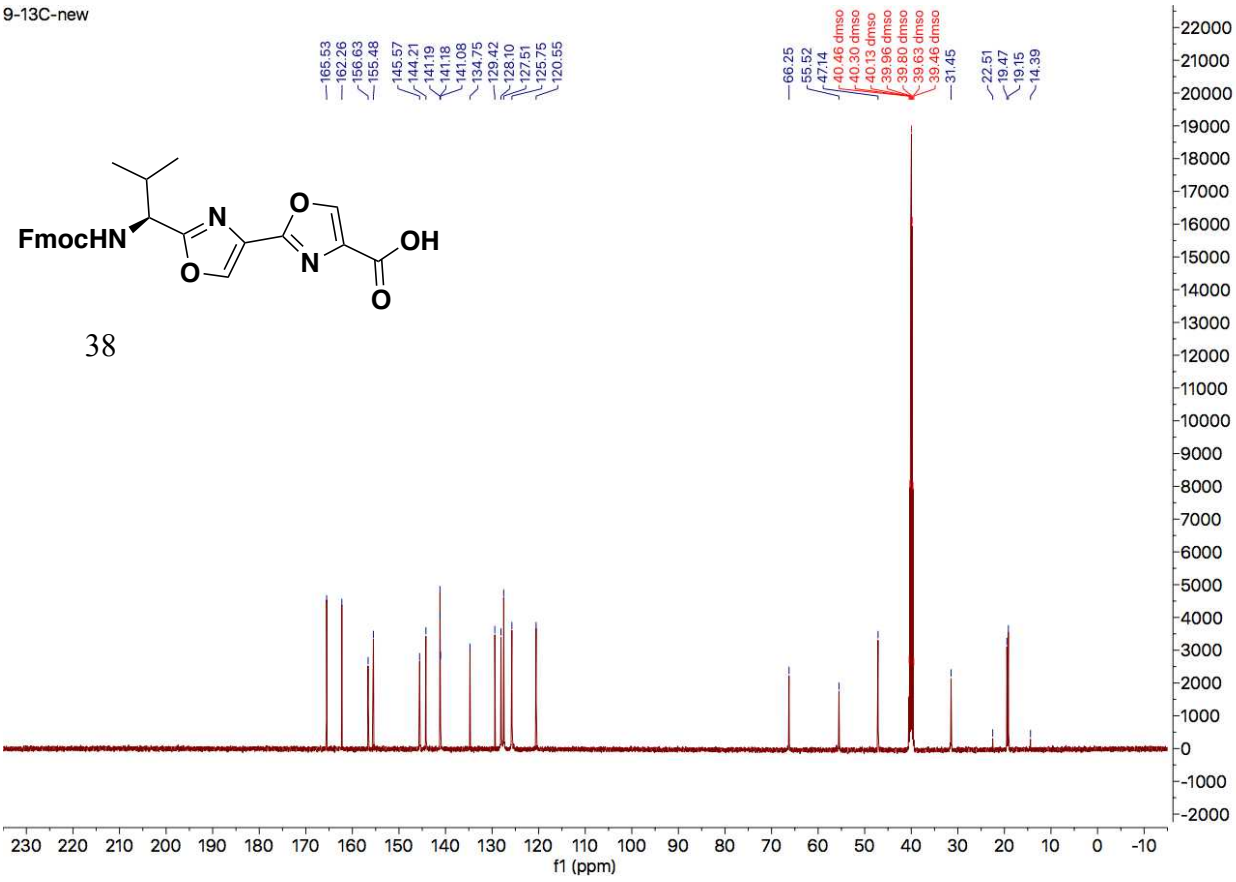

7-13C
jwb
TbtA002-Intra-13C-labeled in DMSO
1H 1d
nt=32
3 July 2018
170.59
163.90
160.82
155.59
141.48
135.41
80.46
77.31 cdcl3
77.06 cdcl3
76.80 cdcl3
62.95
54.57
54.37
52.70
32.68
28.35
18.77
18.10
BocHN
O
N
H
N
O
O
OMe
OH
f1 (ppm)

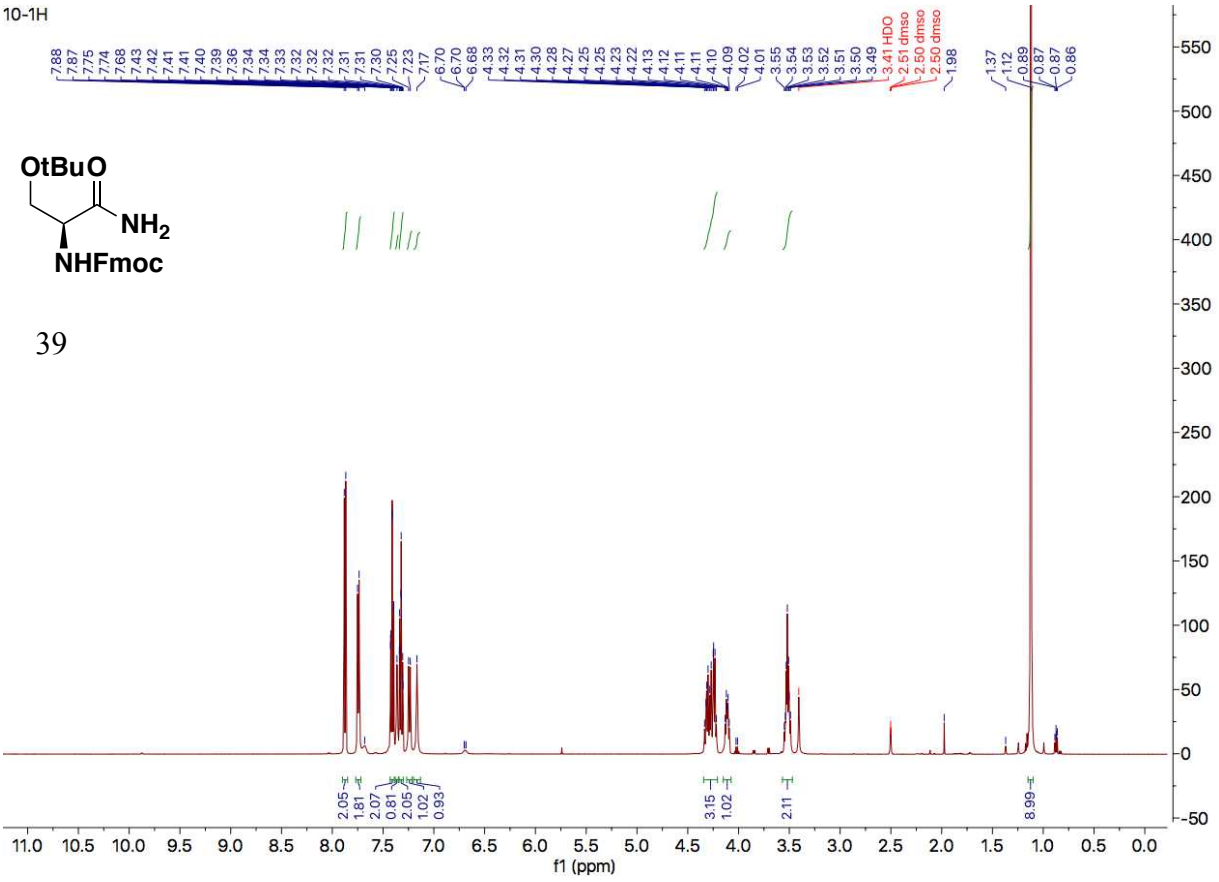
36


8-1H
BocHN
N
O
O
N
O
OMe
O
37
f1 (ppm)

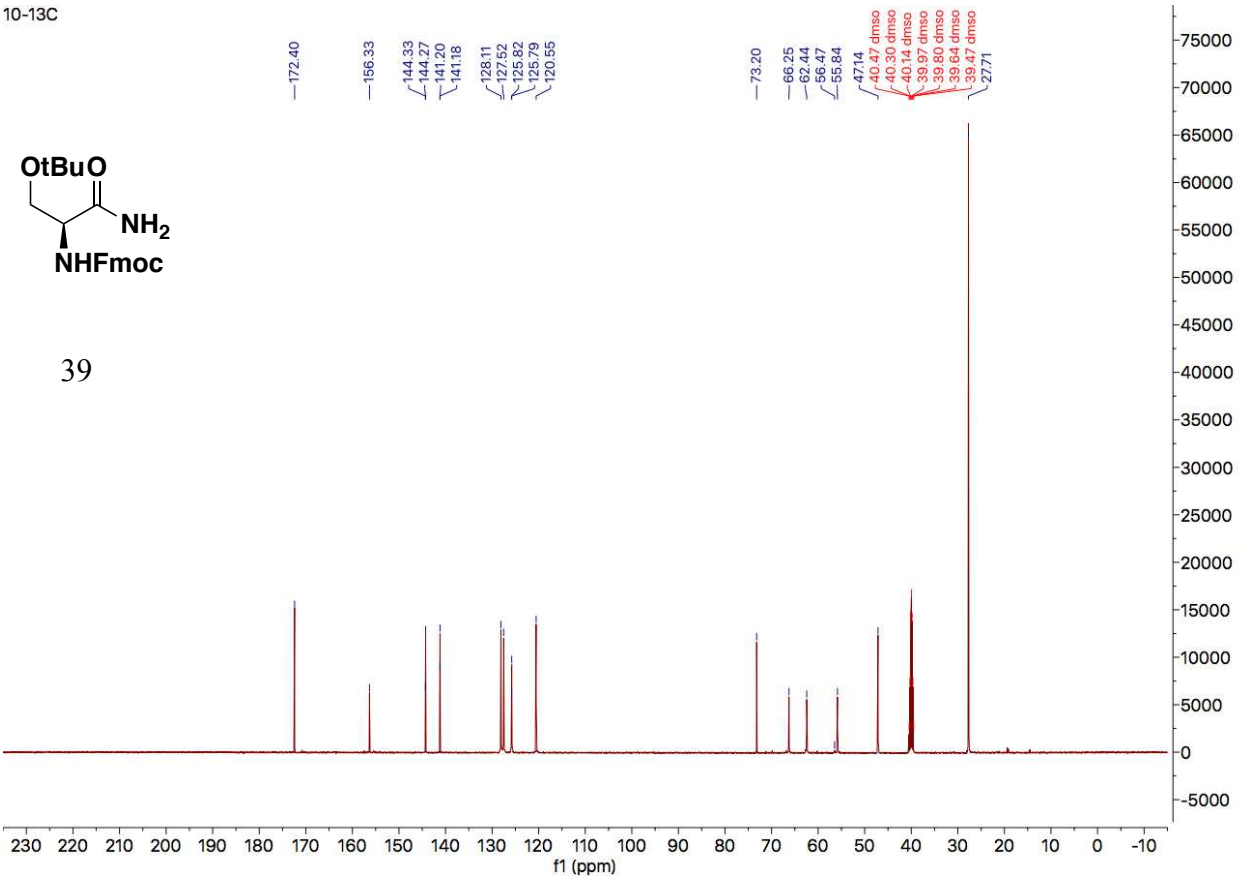

8-13C
165.66
161.30
155.70
155.36
143.71
139.36
134.29
129.59
80.07
77.35 cdcl3
77.04 cdcl3
76.72 cdcl3
54.31
52.29
42.41
32.85
28.26
18.67
17.98
11.23
BocHN
N
O
O
N
O
OMe
O
37
f1 (ppm)


9-1H-new
FmocHN
O
N
O
N
O
OH
38
f1 (ppm)

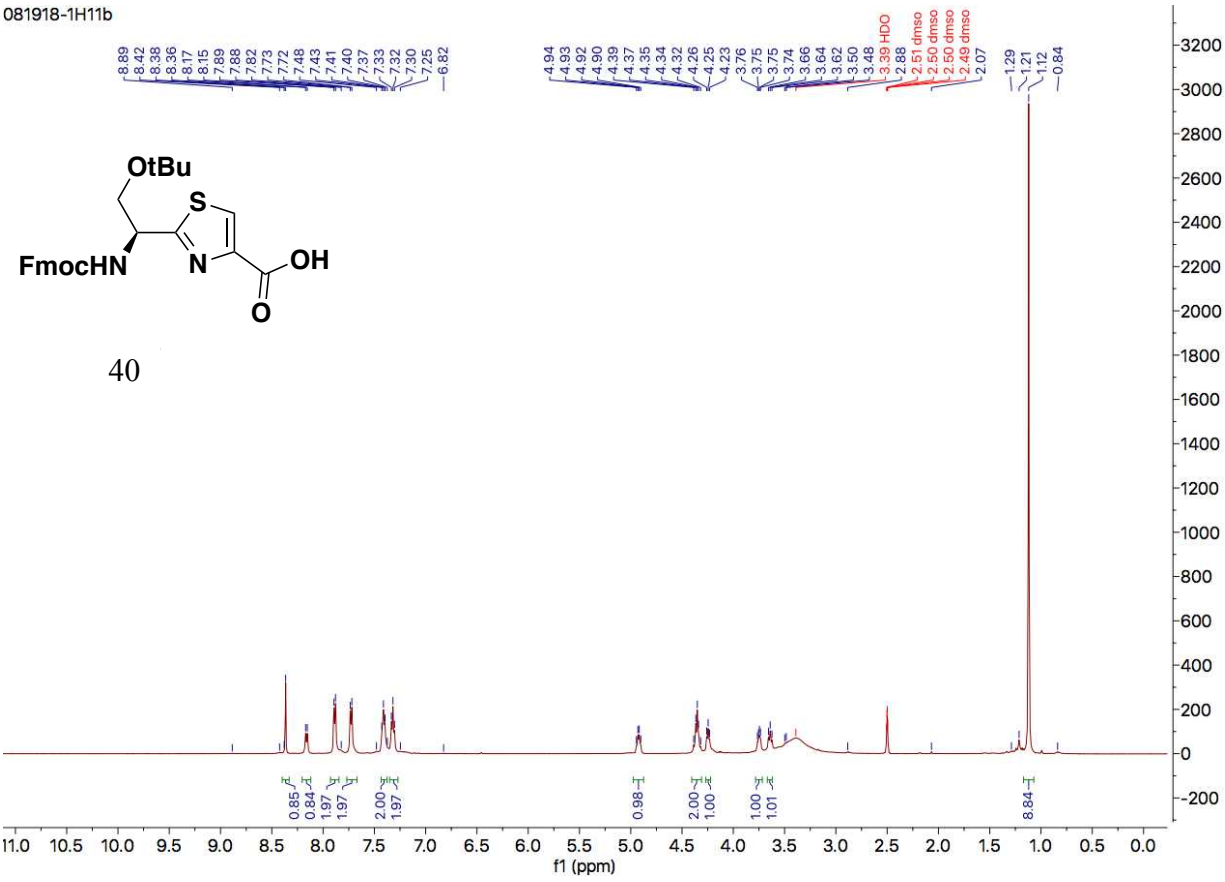

9-13C-new
165.53
162.26
156.63
155.48
145.57
144.21
141.19
141.18
141.08
134.75
129.42
128.10
127.51
125.75
120.55
66.25
55.52
47.14
40.46 dmso
40.30 dmso
40.13 dmso
39.96 dmso
39.80 dmso
39.63 dmso
39.46 dmso
31.45
22.51
19.47
19.15
14.39
FmocHN
O
N
O
O
N
OH
O
38
f1 (ppm)



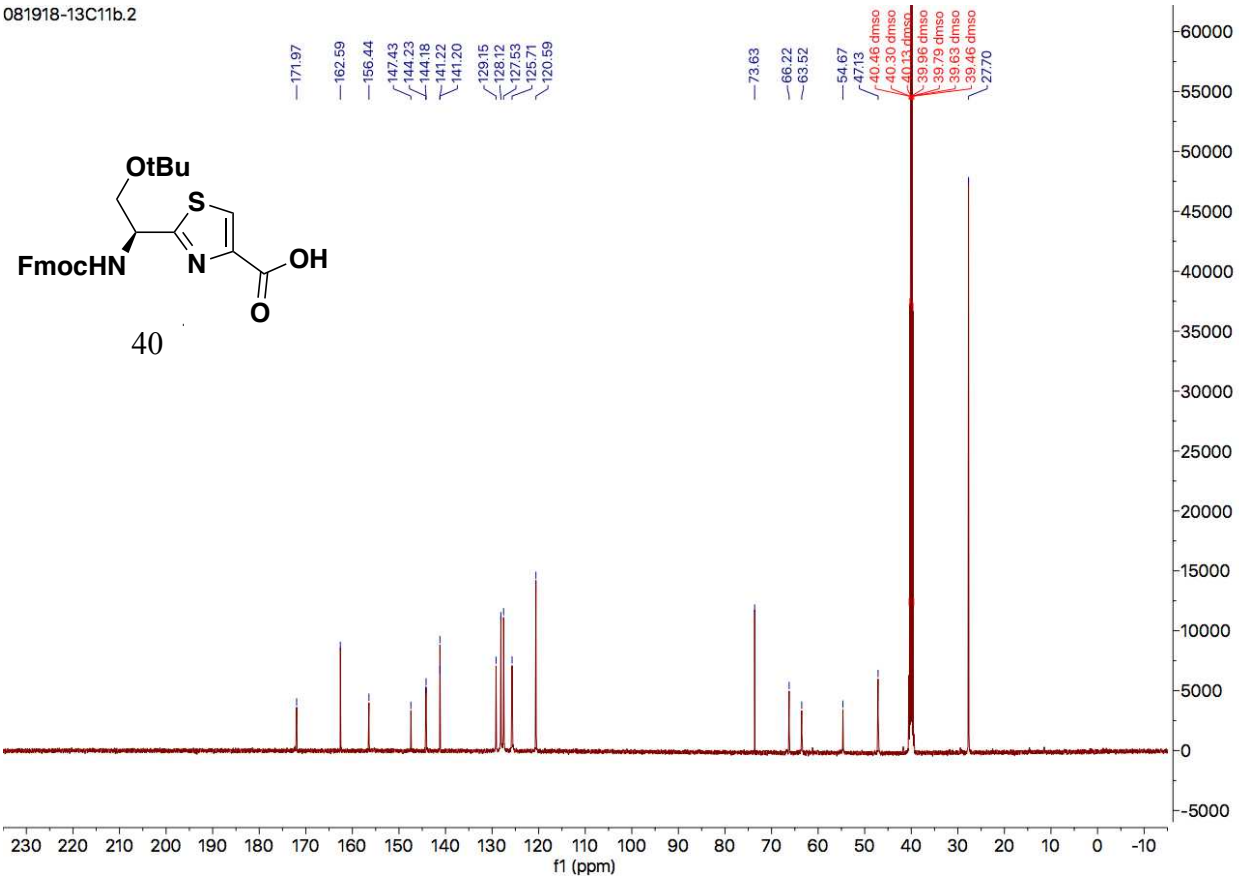
39


10-13C
OtBuO
NH2
NHFmoc
172.40
156.33
144.33
144.27
141.20
141.18
128.11
127.52
125.82
125.79
120.55
73.20
66.25
62.44
56.47
55.84
47.14
40.47 dmso
40.30 dmso
40.14 dmso
39.97 dmso
39.80 dmso
39.64 dmso
39.47 dmso
27.71
f1 (ppm)

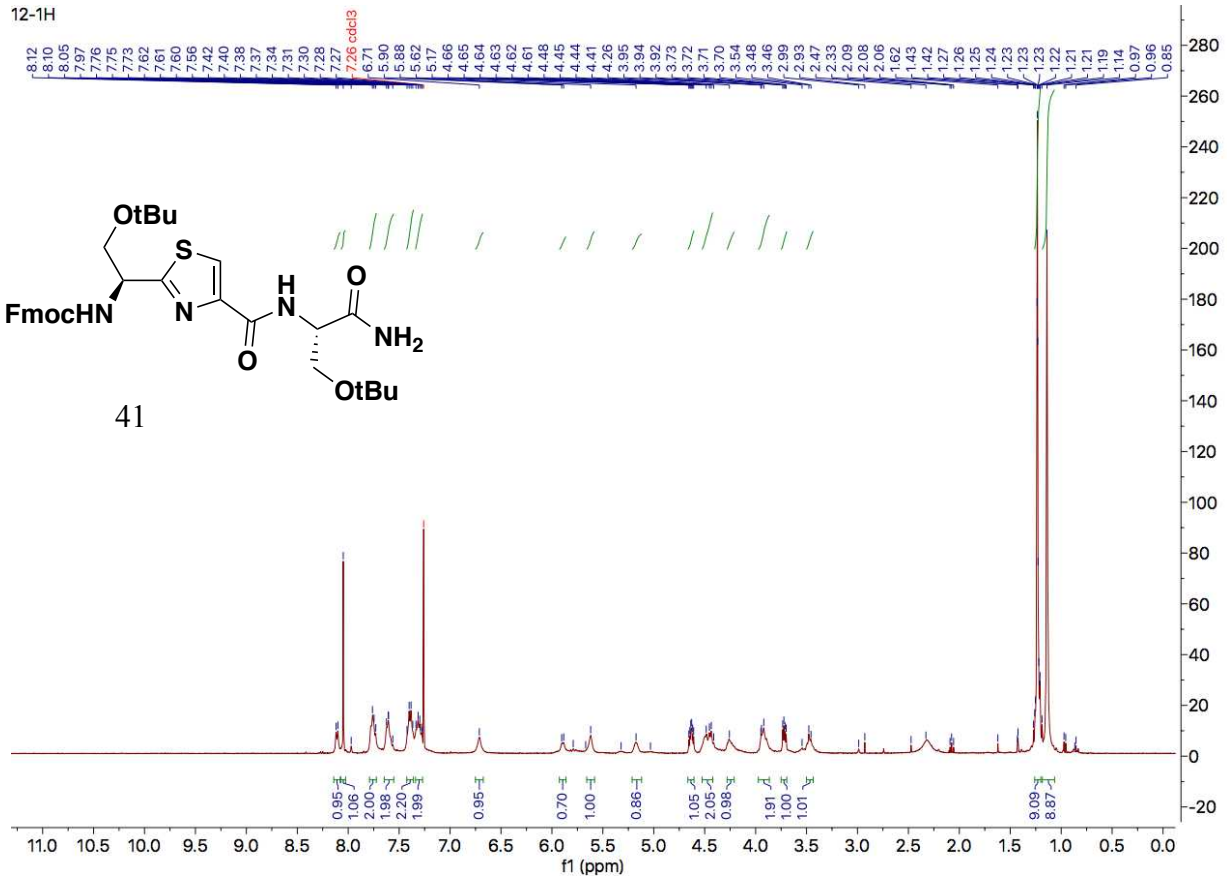
39



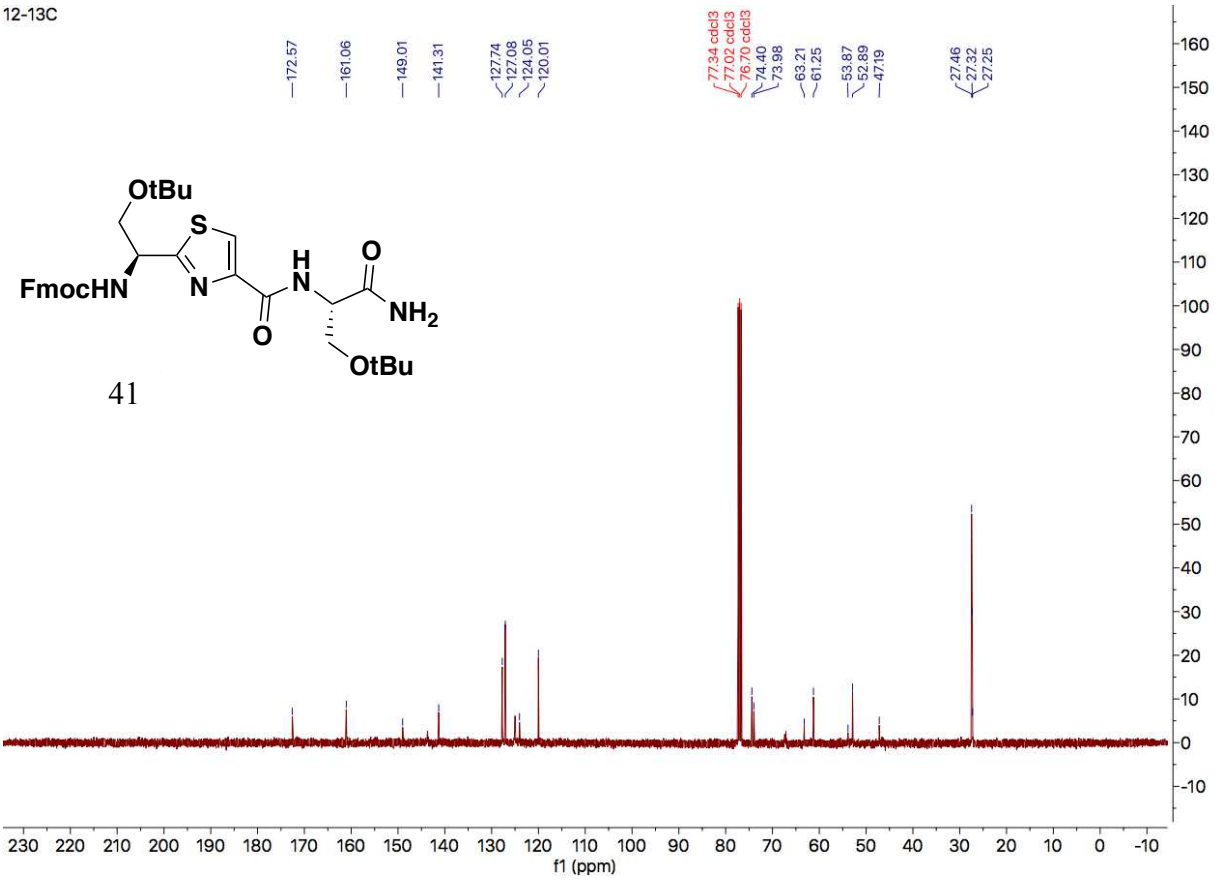
40


081918-13C11b.2
171.97
162.59
156.44
147.43
144.23
144.18
141.22
141.20
129.15
128.12
127.53
125.71
120.59
73.63
66.22
63.52
54.67
47.13
40.46 dmso
40.30 dmso
40.13 dmso
39.96 dmso
39.79 dmso
39.63 dmso
39.46 dmso
27.70
OtBu
S
FmocHN
N
OH
O
f1 (ppm)

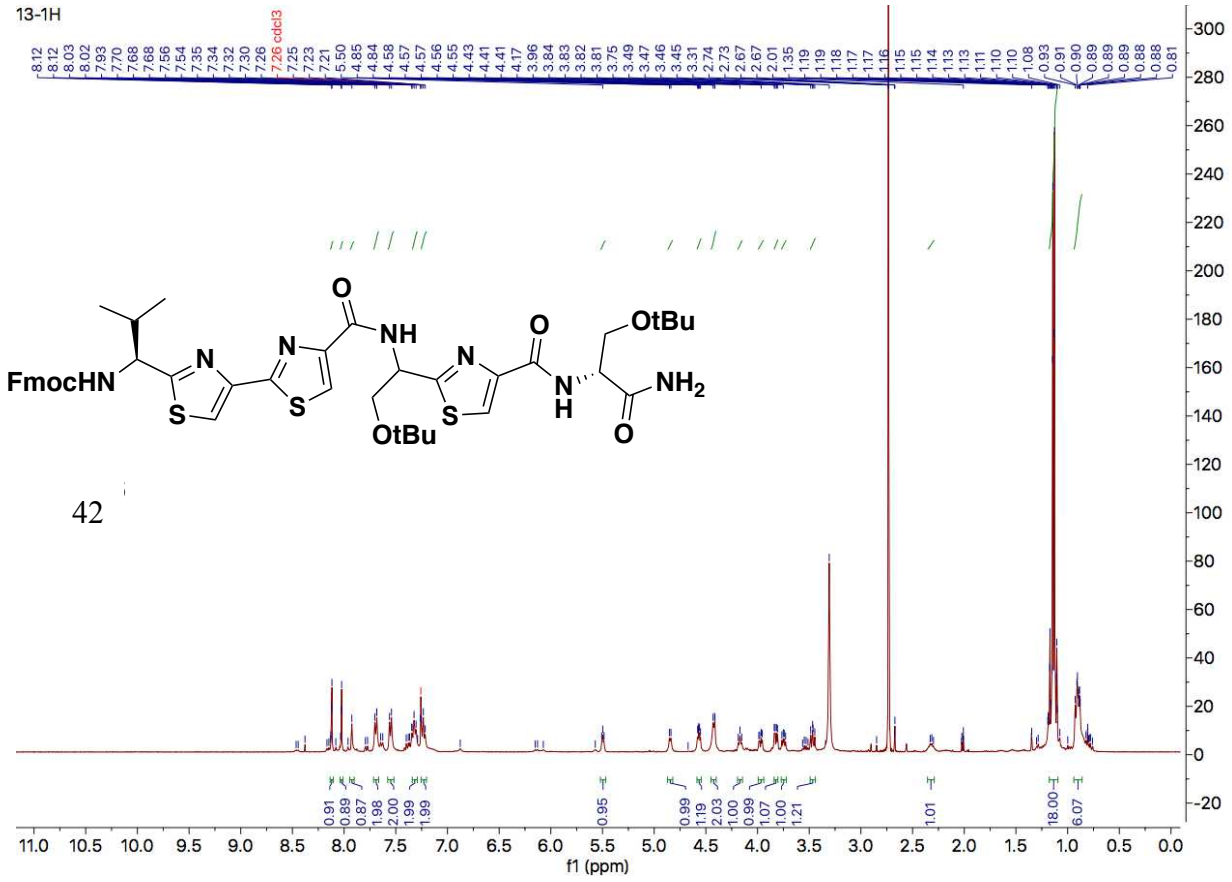
40



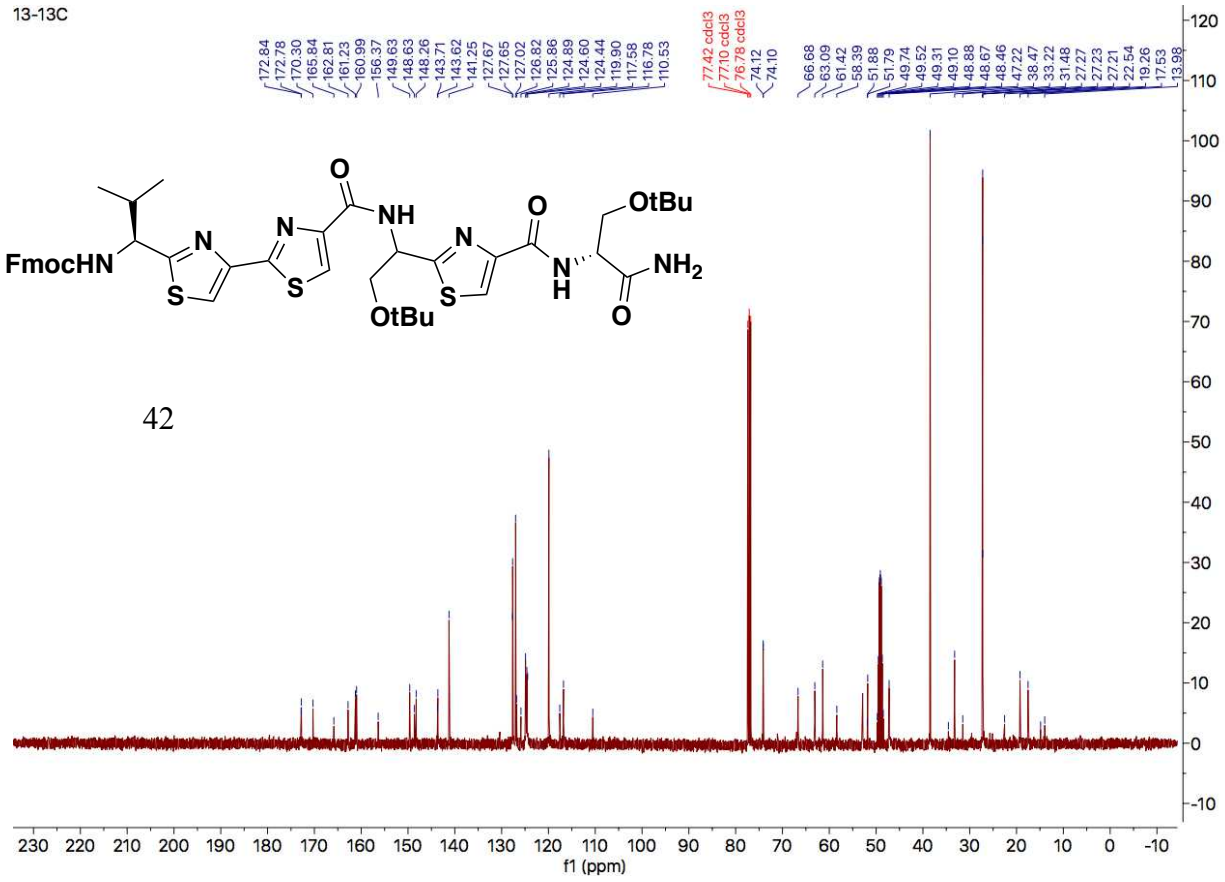
41


12-13C
172.57
161.06
149.01
141.31
127.74
127.08
124.05
120.01
77.34 cdcl3
77.02 cdcl3
76.70 cdcl3
74.40
73.98
63.21
61.25
53.87
52.89
47.19
27.46
27.32
27.25
OtBu
S
FmocHN
N
H
N
O
O
NH2
OtBu
f1 (ppm)

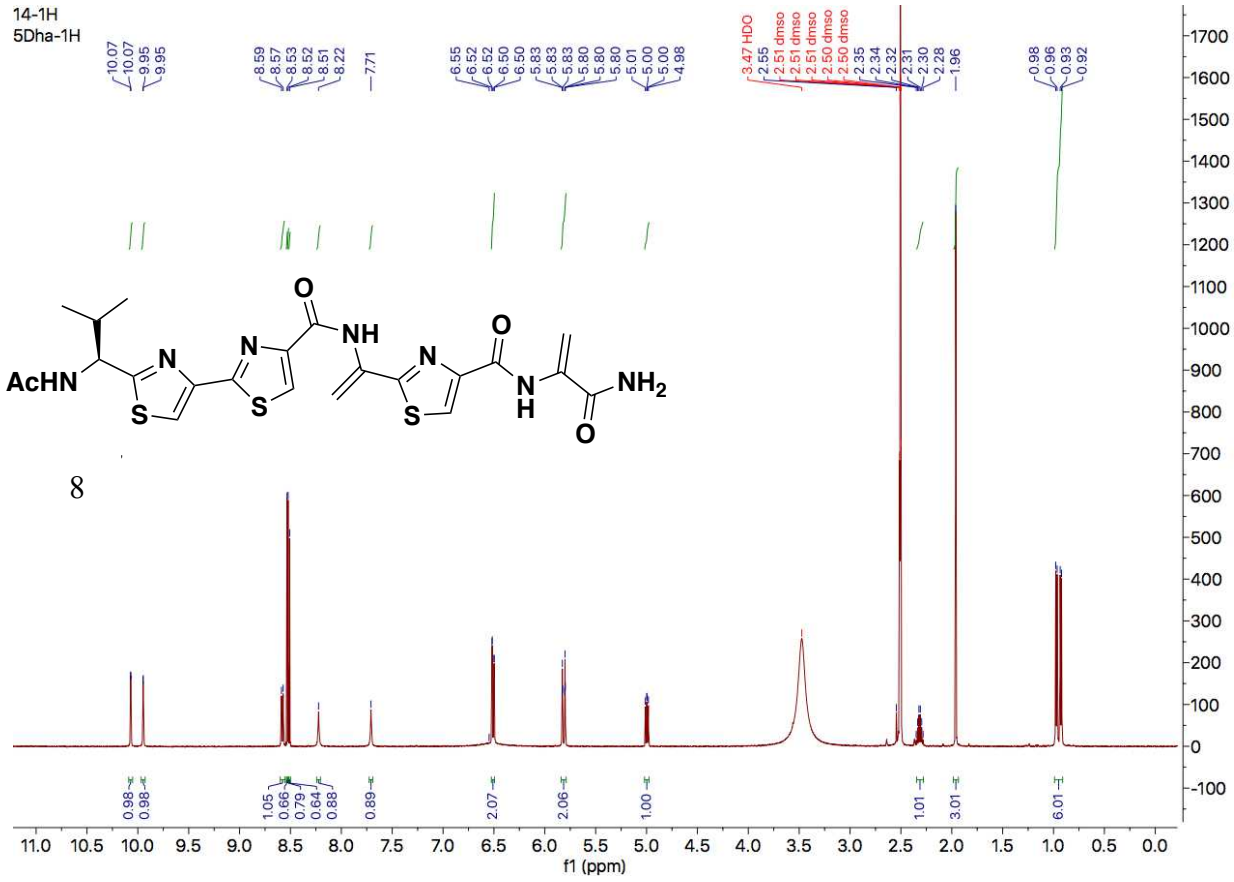
41

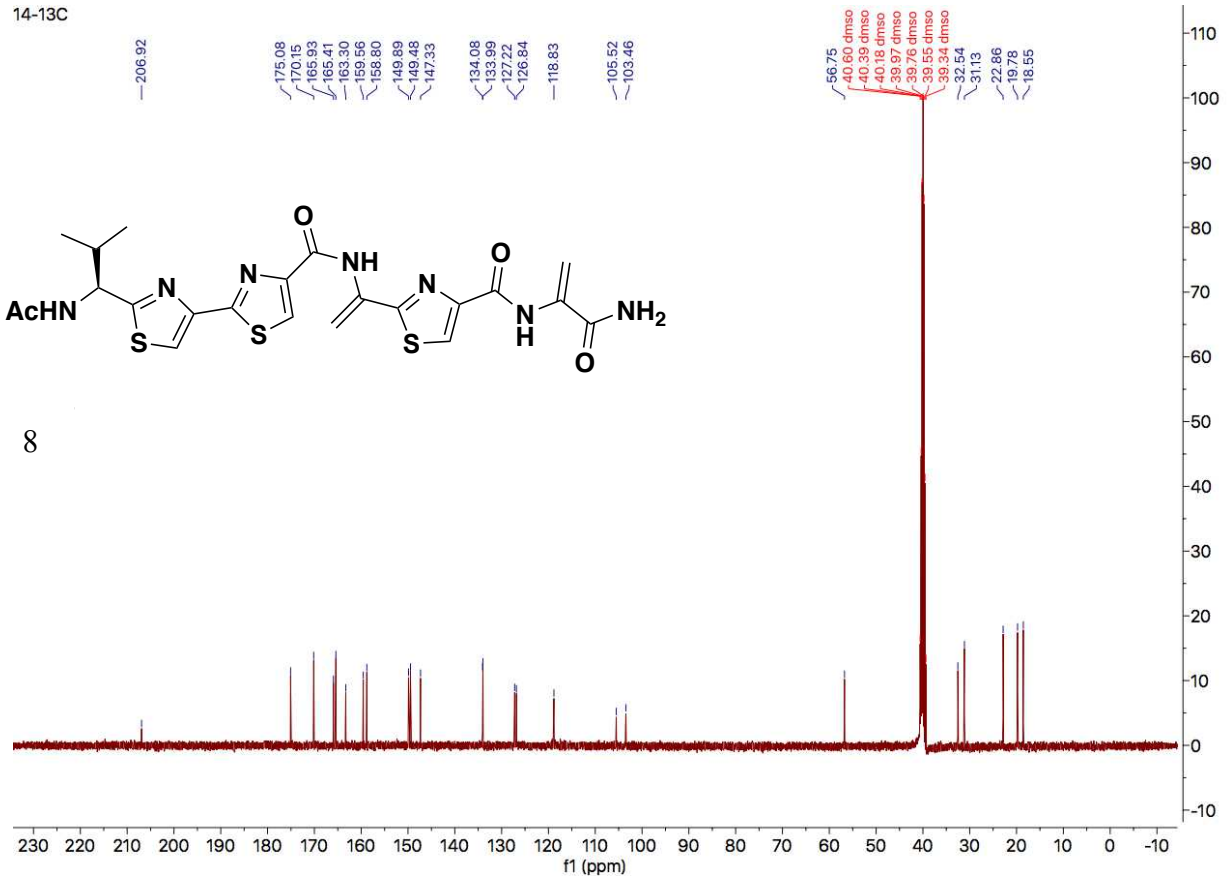


42

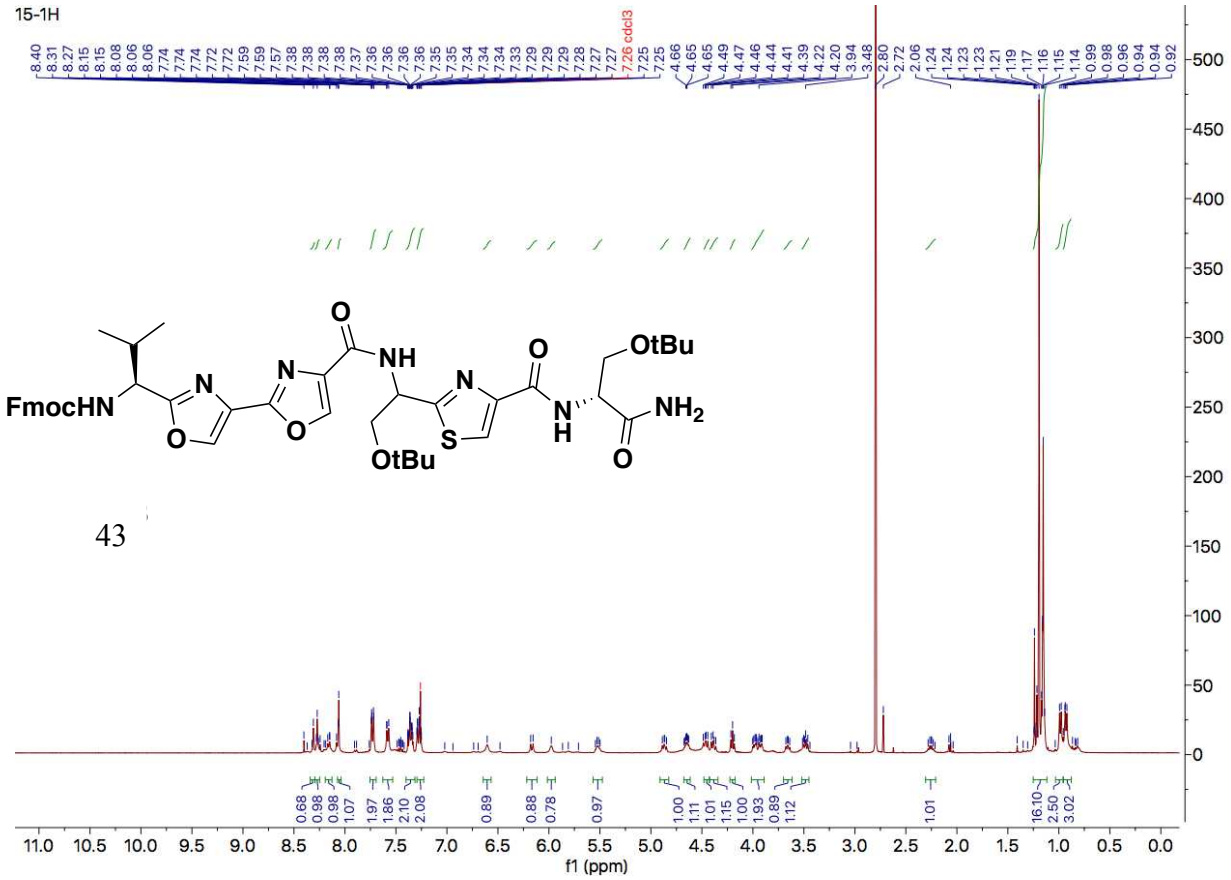


42

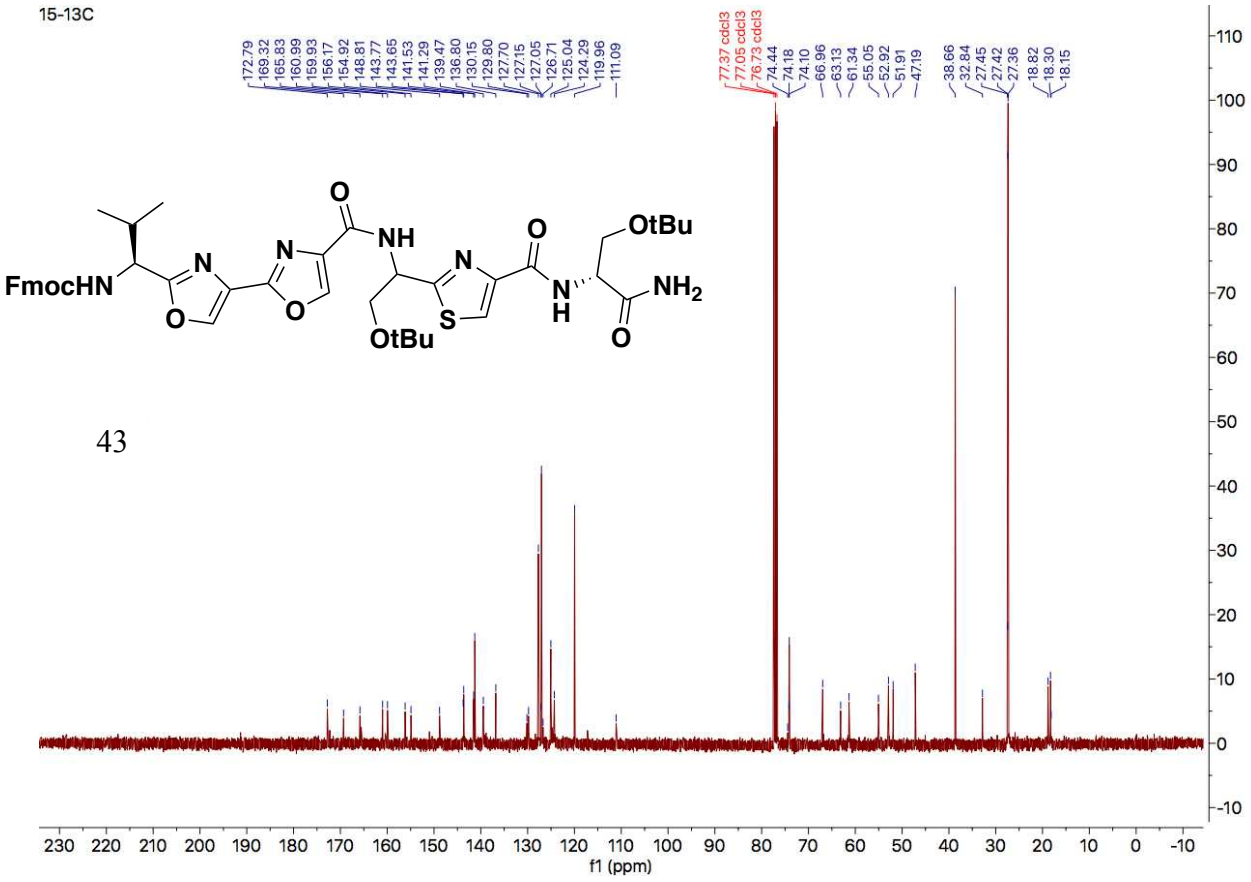

14-1H
5Dha-1H
AcHN
NH
NH2
8
f1 (ppm)


14-13C
206.92
175.08
170.15
165.93
165.41
163.30
159.56
158.80
149.89
149.48
147.33
134.08
133.99
127.22
126.84
118.83
105.52
103.46
56.75
40.60 dmso
40.39 dmso
40.18 dmso
39.97 dmso
39.76 dmso
39.55 dmso
39.34 dmso
32.54
31.13
22.86
19.78
18.55
AcHN
NH
NH2
f1 (ppm)

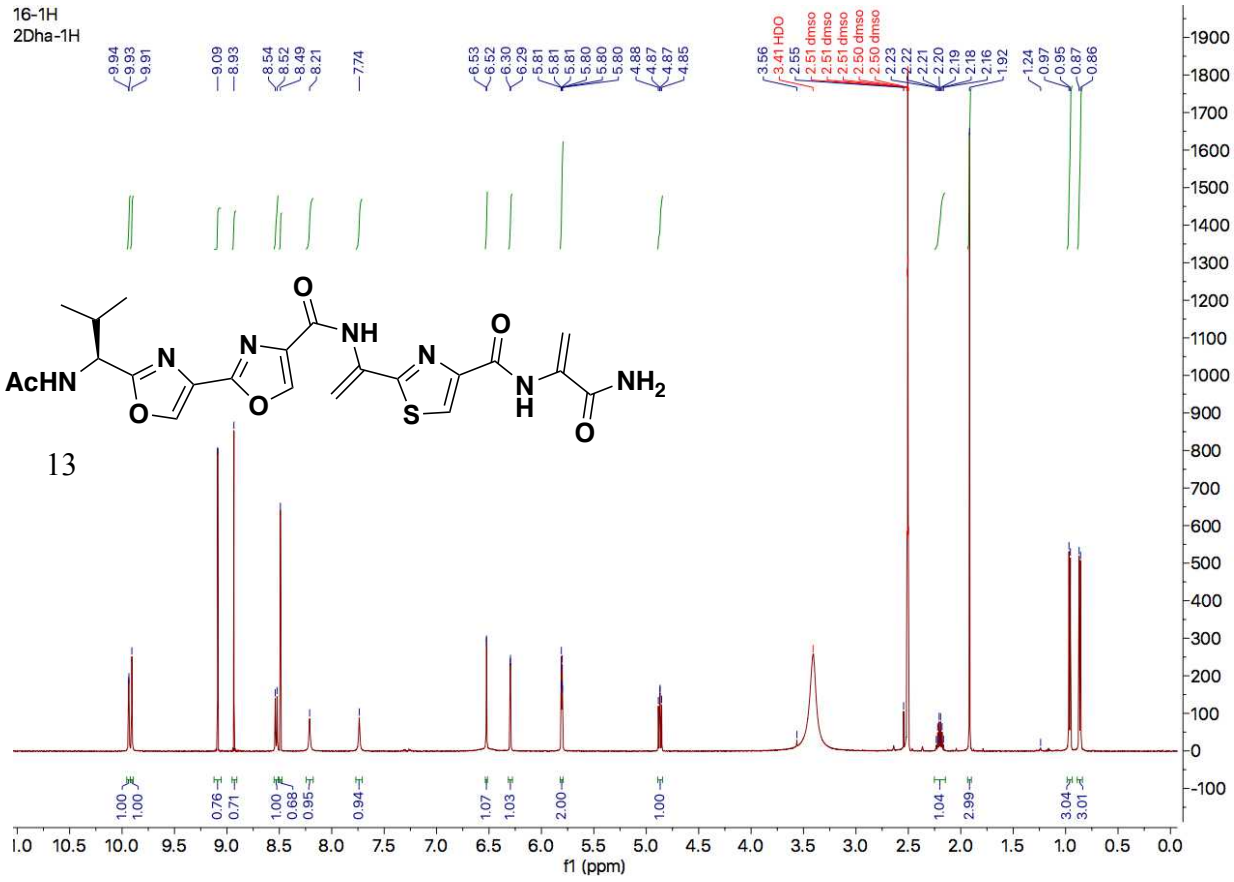
8



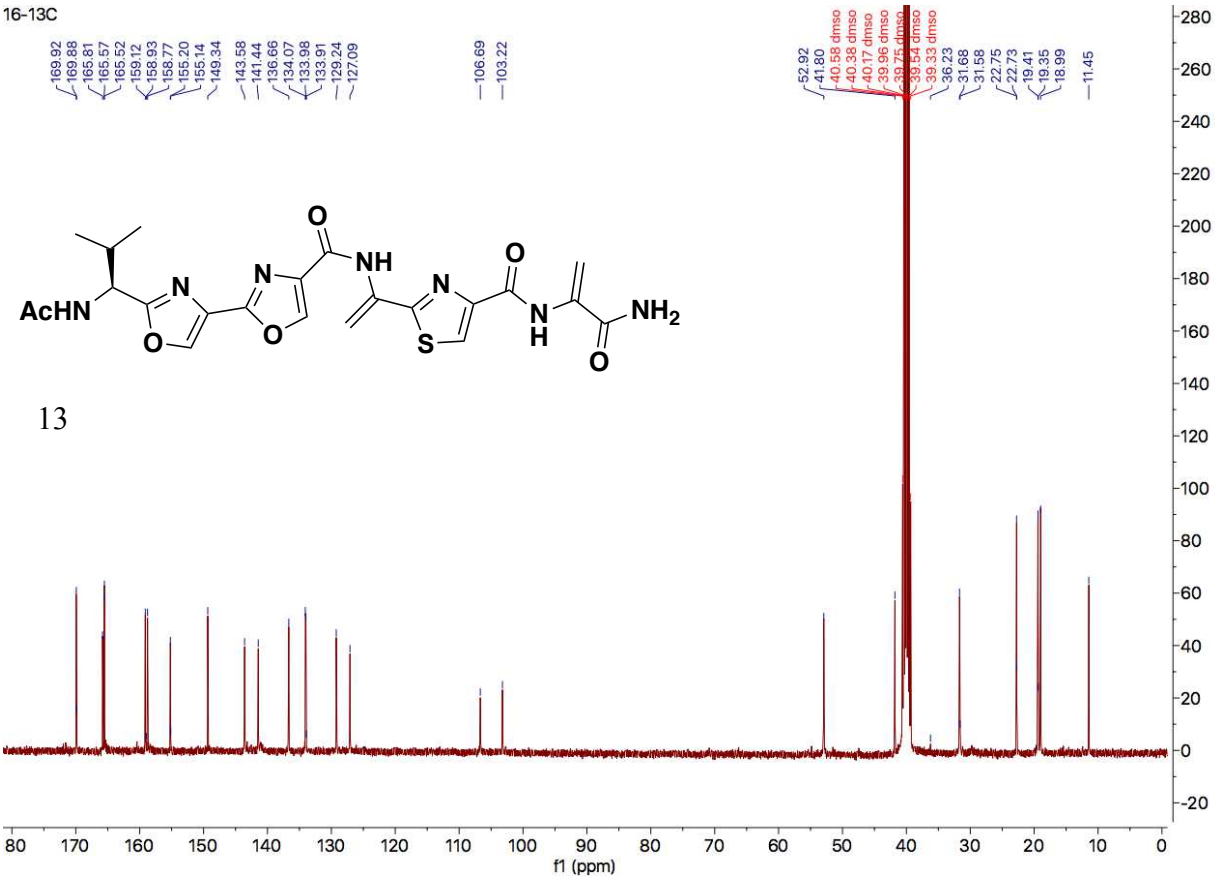
43


15-13C
172.79
169.32
165.83
160.99
159.93
156.17
154.92
148.81
143.77
143.65
141.53
141.29
139.47
136.80
130.15
129.80
127.70
127.15
127.05
126.71
125.04
124.29
119.96
111.09
77.37 cdcl3
77.05 cdcl3
76.73 cdcl3
74.44
74.18
74.10
66.96
63.13
61.34
55.05
52.92
51.91
47.19
38.66
32.84
27.45
27.42
27.36
18.82
18.30
18.15
FmocHN
O
N
O
N
O
NH
O
OtBu
N
S
OtBu
N
H
O
NH2
f1 (ppm)

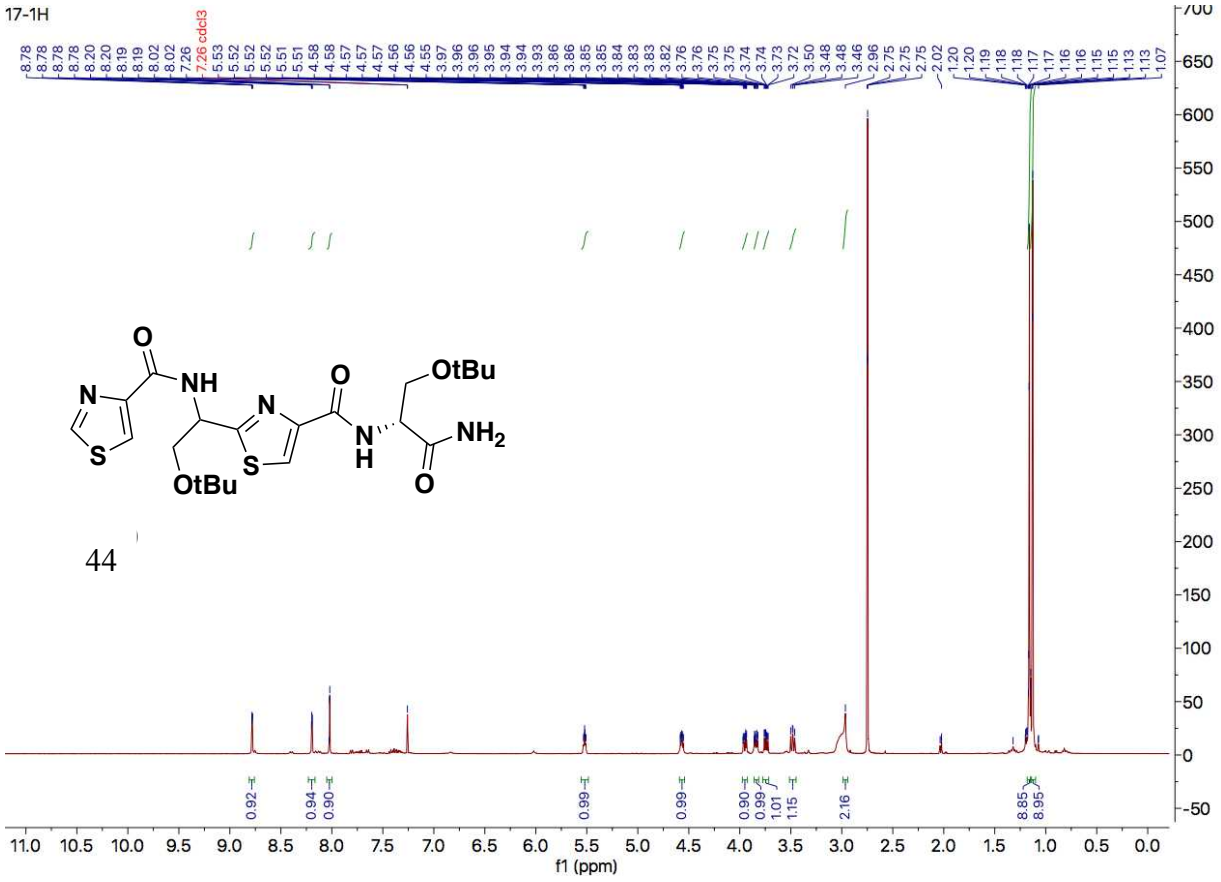
43

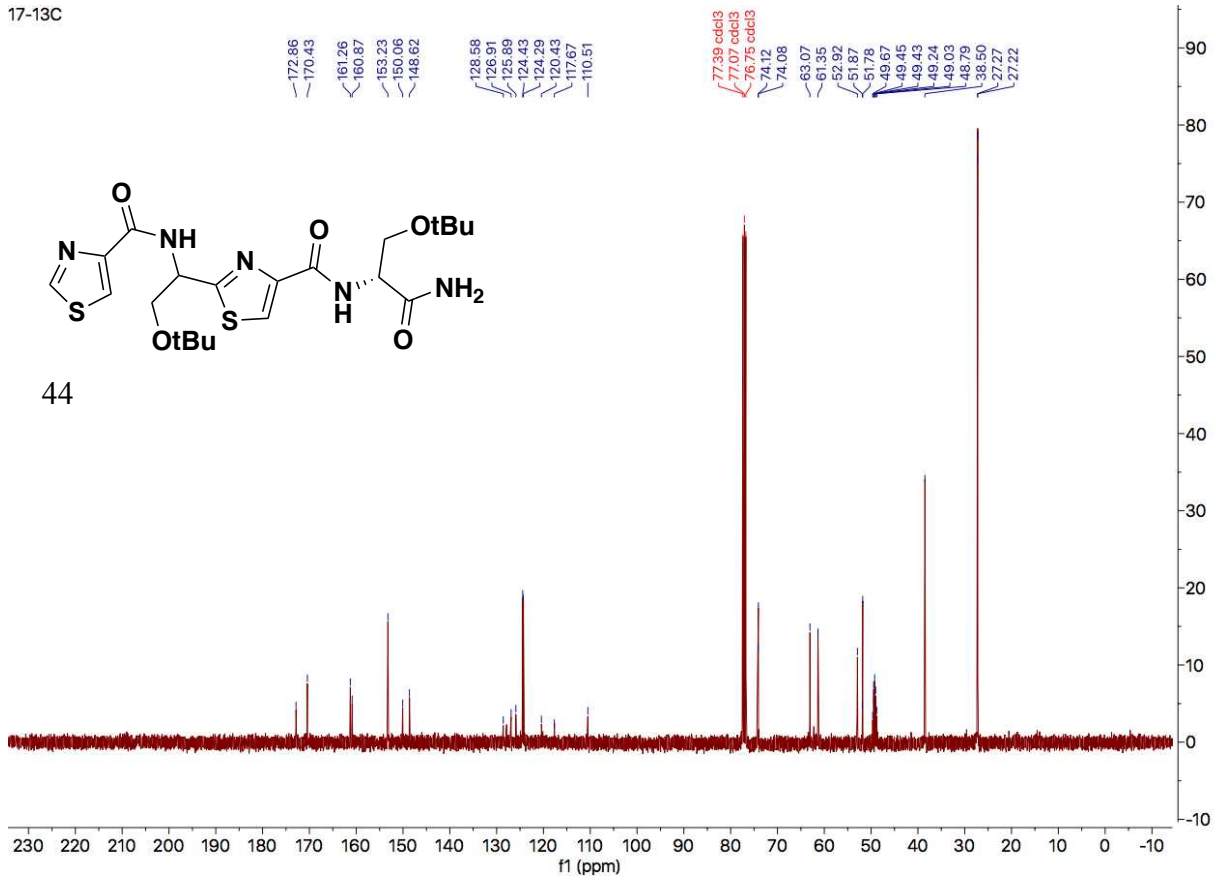


13

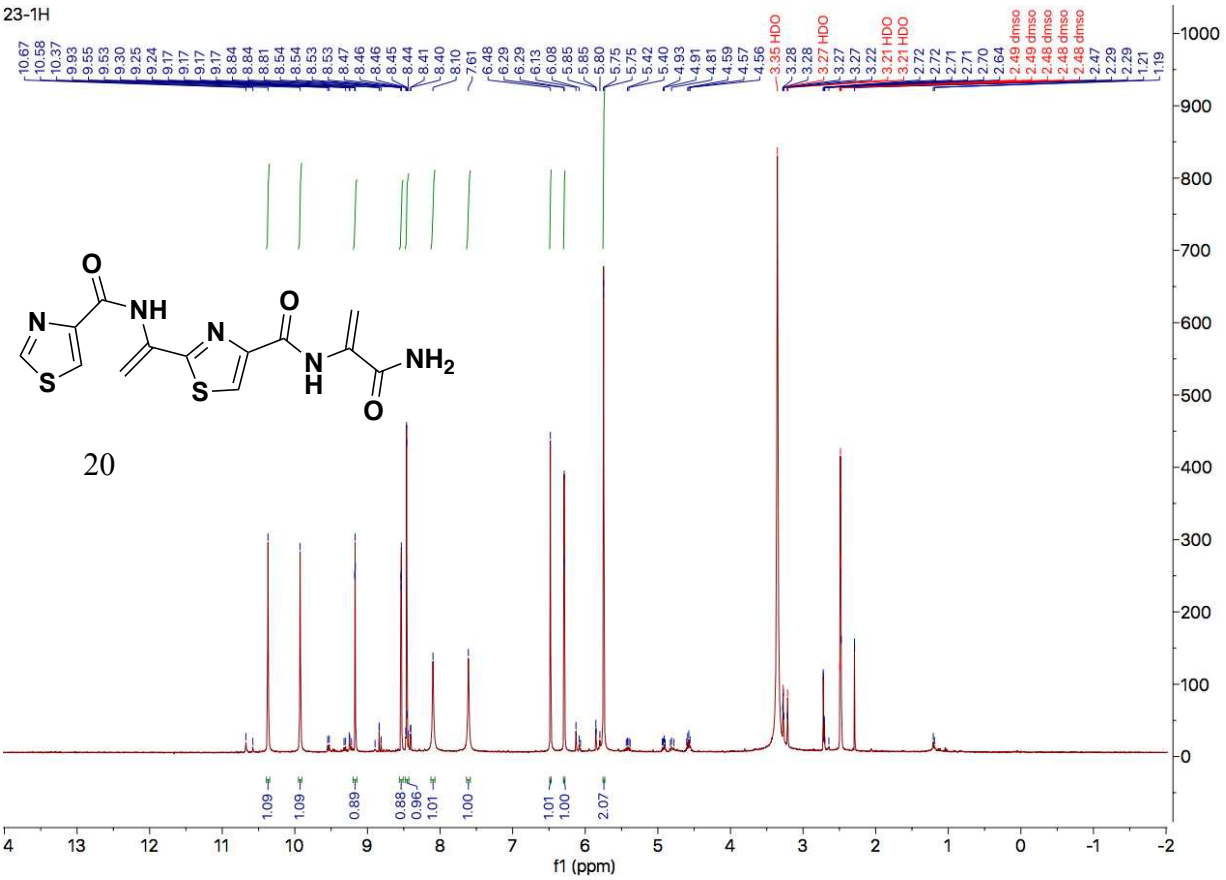

16-13C
169.92
169.88
165.81
165.57
165.52
159.12
158.93
158.77
155.20
155.14
149.34
143.58
141.44
136.66
134.07
133.98
133.91
129.24
127.09
106.69
103.22
52.92
41.80
40.58 dmso
40.38 dmso
40.17 dmso
39.96 dmso
39.75 dmso
39.54 dmso
39.33 dmso
36.23
31.68
31.58
22.75
22.73
19.41
19.35
18.99
11.45
AcHN
O
N
O
N
NH
O
N
S
N
H
O
NH2
f1 (ppm)

13

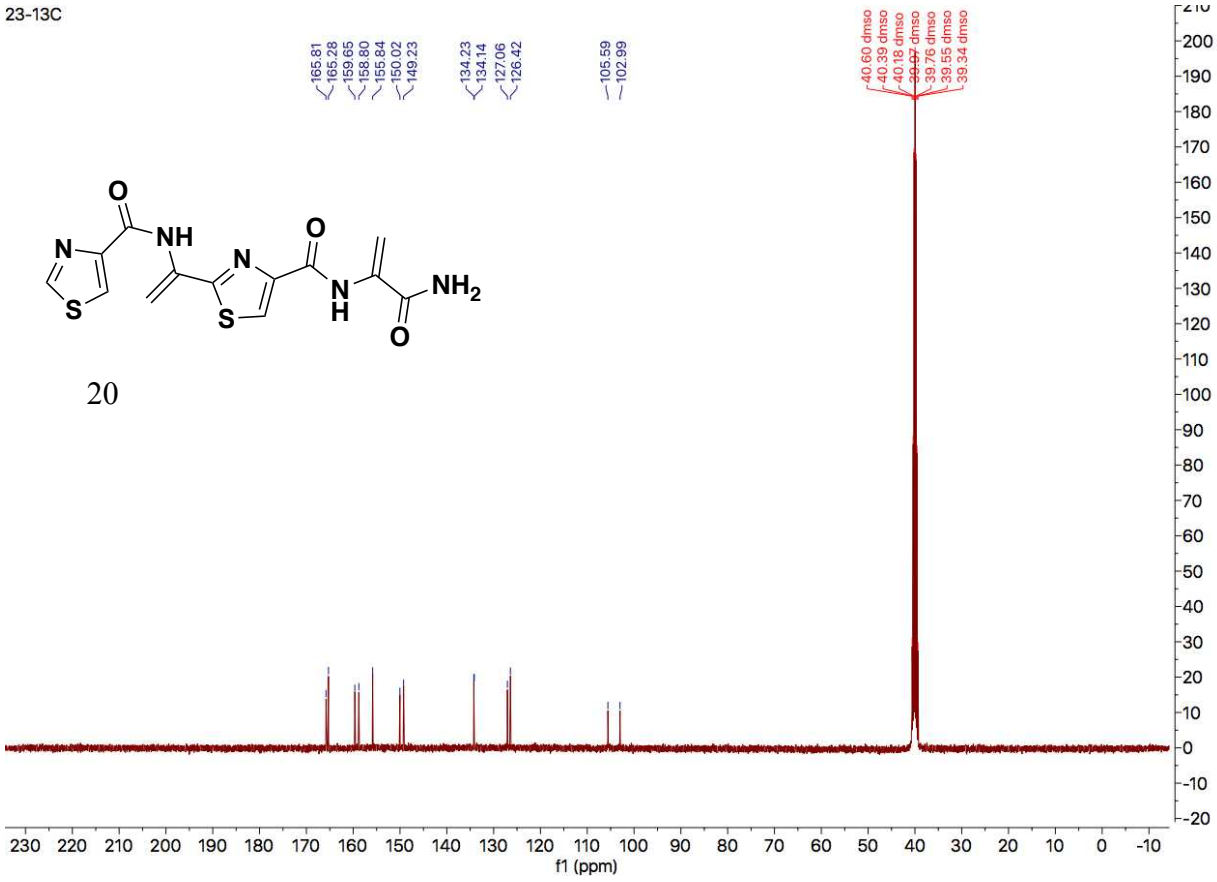

17-1H
O
NH
N
S
N
S
O
N
H
OtBu
OtBu
NH2
O
44
f1 (ppm)


17-13C
172.86
170.43
161.26
160.87
153.23
150.06
148.62
128.58
126.91
125.89
124.43
124.29
120.43
117.67
110.51
77.39 cdcl3
77.07 cdcl3
76.75 cdcl3
74.12
74.08
63.07
61.35
52.92
51.87
51.78
49.67
49.45
49.43
49.24
49.03
48.79
38.50
27.27
27.22
O
NH
N
S
N
S
O
N
H
OtBu
NH2
O
OtBu
f1 (ppm)

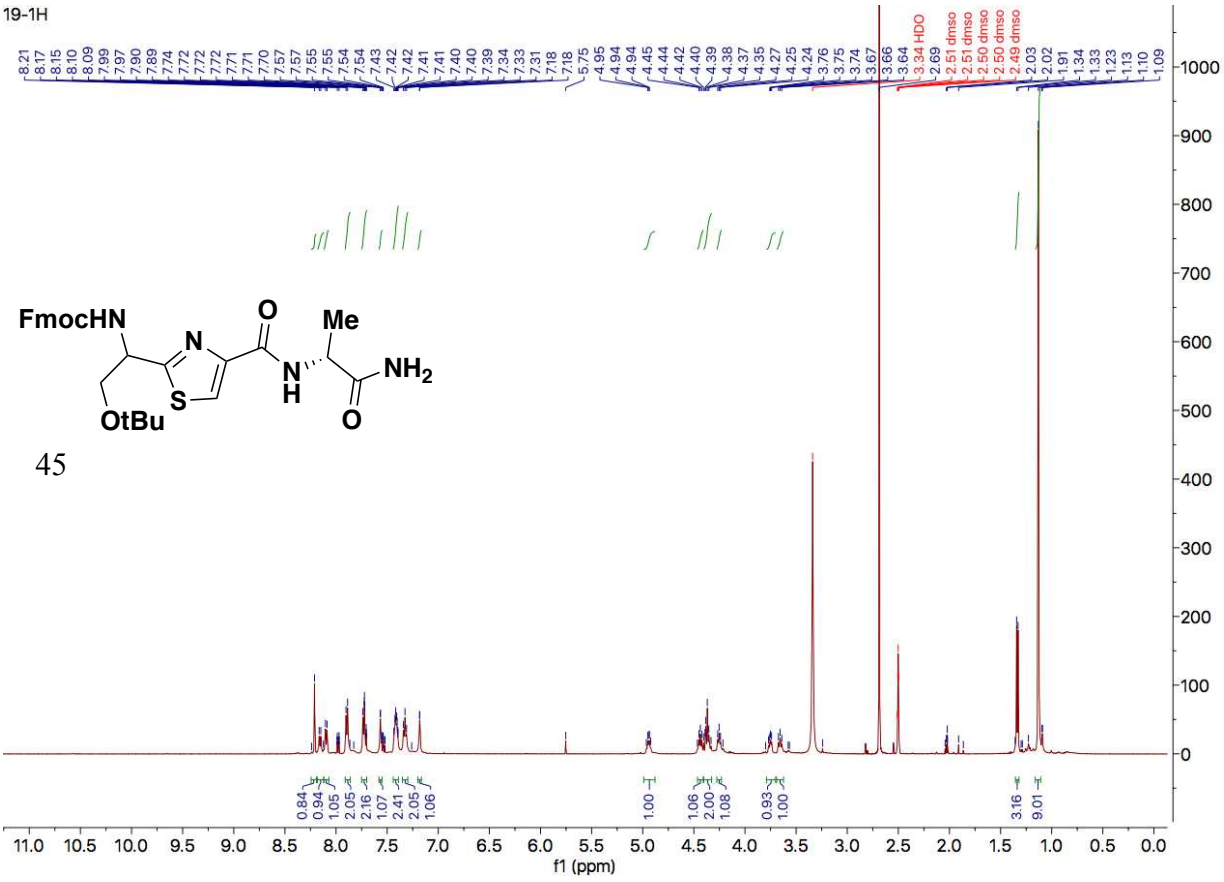
44

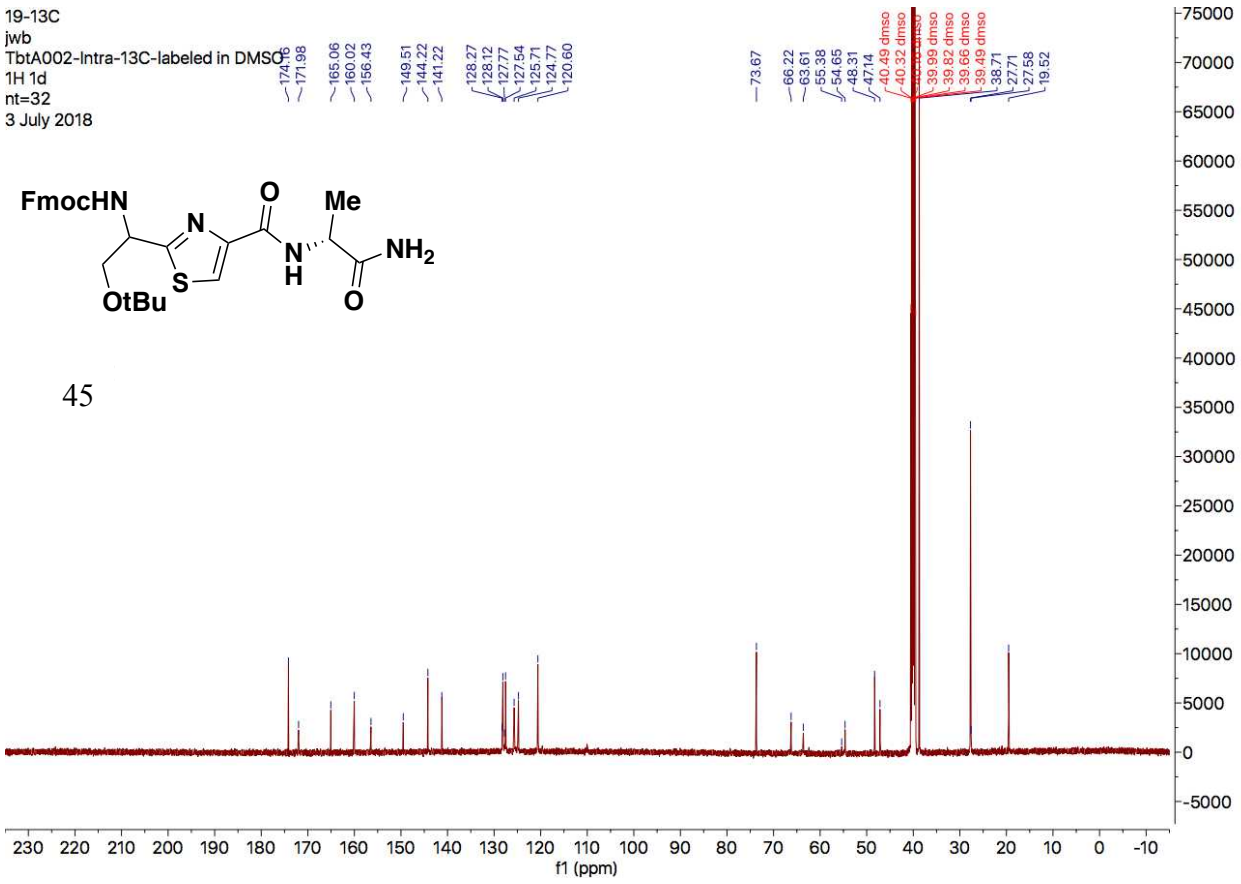


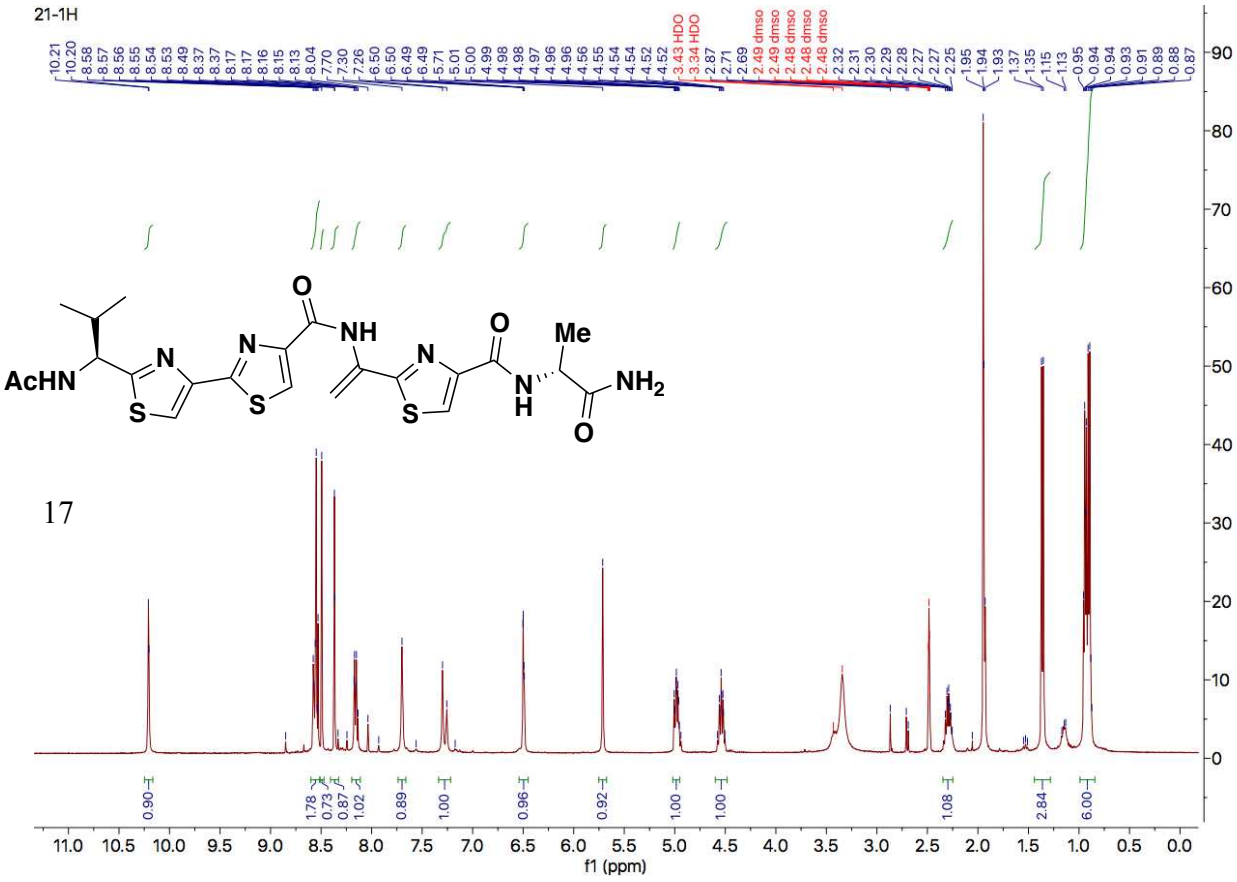
20

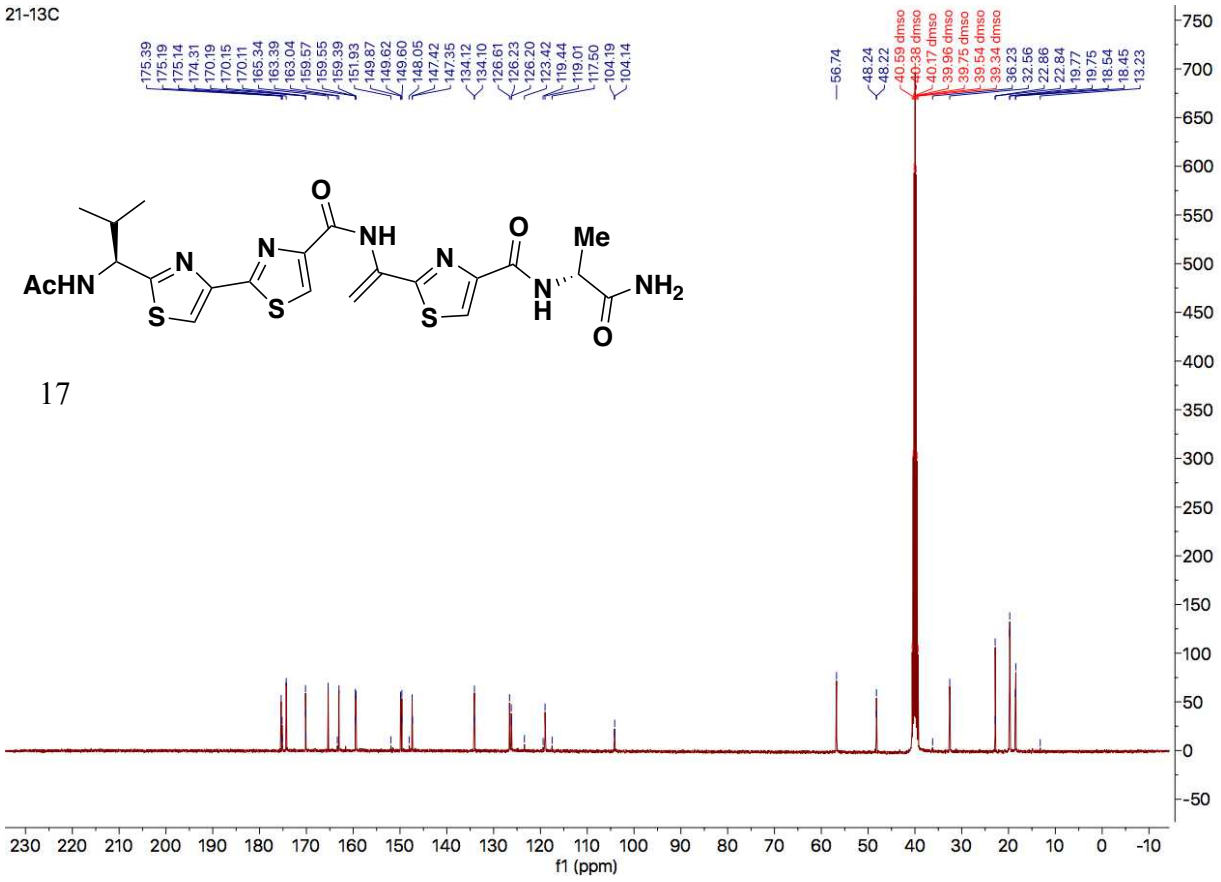

23-13C
165.81
165.28
159.65
158.80
155.84
150.02
149.23
134.23
134.14
127.06
126.42
105.59
102.99
40.60 dmso
40.39 dmso
40.18 dmso
39.97 dmso
39.76 dmso
39.55 dmso
39.34 dmso
O
NH
N
S
N
S
O
N
H
O
NH2
f1 (ppm)

20

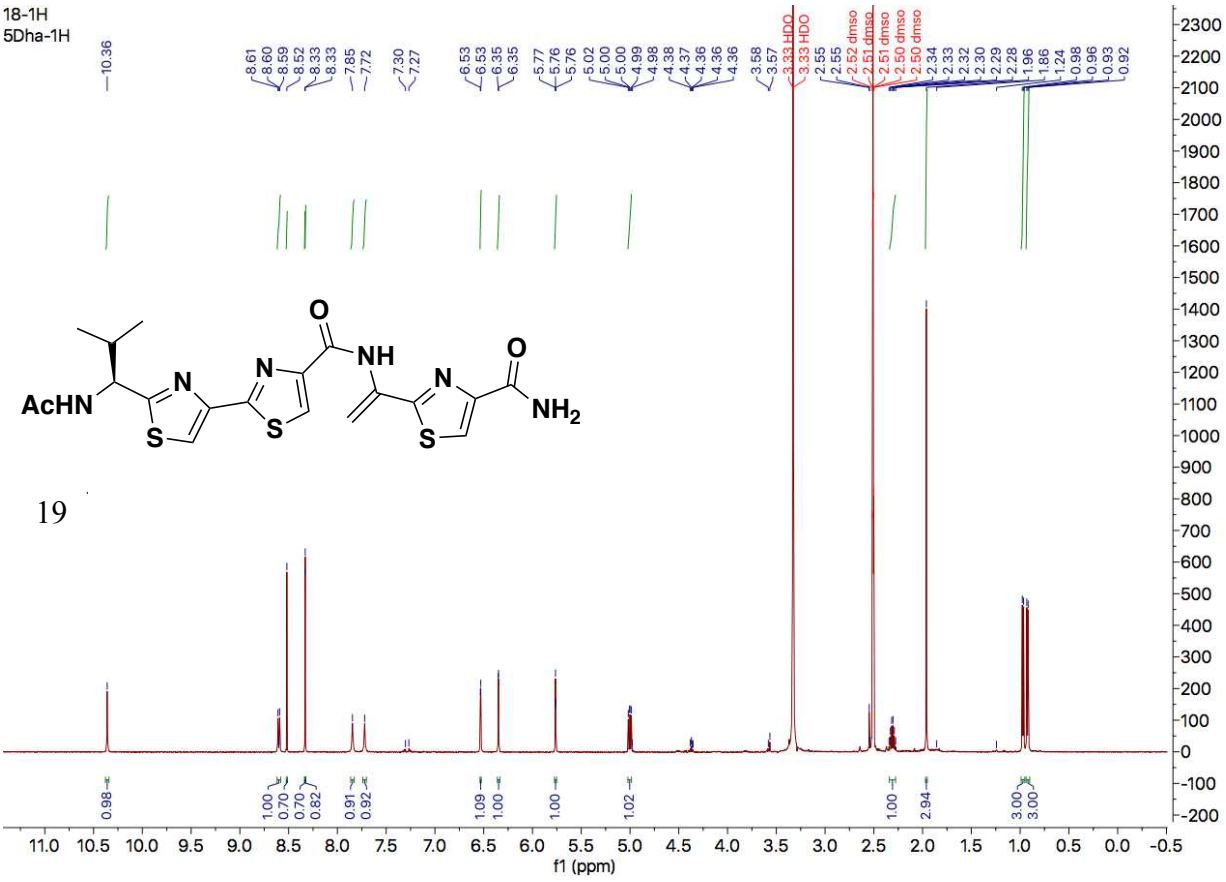

19-1H
FmocHN
N
O
Me
S
N
H
NH2
OtBu
O
45
f1 (ppm)


19-13C
jwb
TbtA002-Intra-13C-labeled in DMSO
1H 1d
nt=32
3 July 2018
FmocHN
O
N
Me
N
H
NH2
S
OtBu
O
45
174.76
171.98
165.06
160.02
156.43
149.51
144.22
141.22
128.27
128.12
127.77
127.54
125.71
124.77
120.60
73.67
66.22
63.61
55.38
54.65
48.31
47.14
40.49 dmso
40.32 dmso
39.99 dmso
39.82 dmso
39.66 dmso
39.49 dmso
38.71
27.71
27.58
19.52
f1 (ppm)

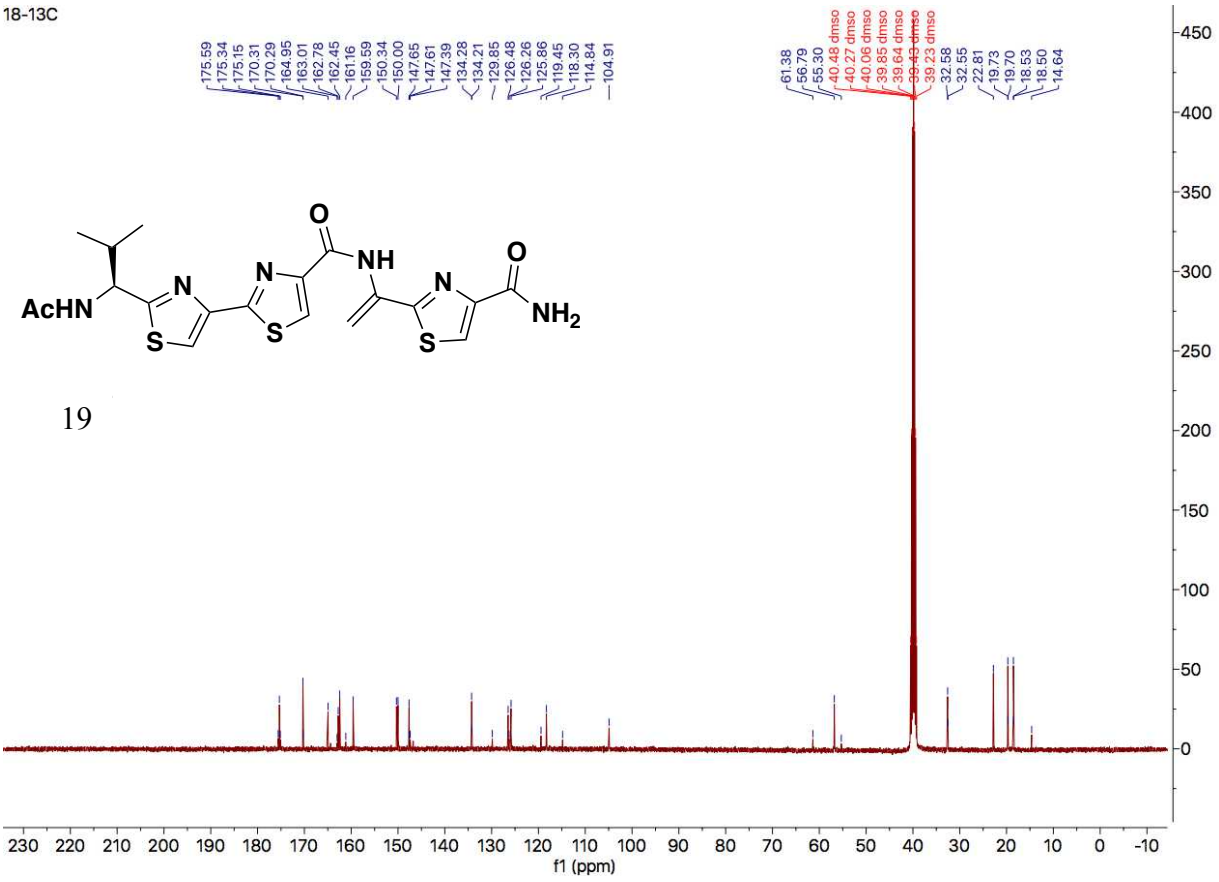

21-1H
AcHN
NH
Me
NH2
17
f1 (ppm)


21-13C
175.39
175.19
175.14
174.31
170.19
170.15
170.11
165.34
163.39
163.04
159.57
159.55
159.39
151.93
149.87
149.62
149.60
148.05
147.42
147.35
134.12
134.10
126.61
126.23
126.20
123.42
119.44
119.01
117.50
104.19
104.14
56.74
48.24
48.22
40.59 dmso
40.38 dmso
40.17 dmso
39.96 dmso
39.75 dmso
39.54 dmso
39.34 dmso
36.23
32.56
22.86
22.84
19.77
19.75
18.54
18.45
13.23
AcHN
NH
Me
NH2
f1 (ppm)

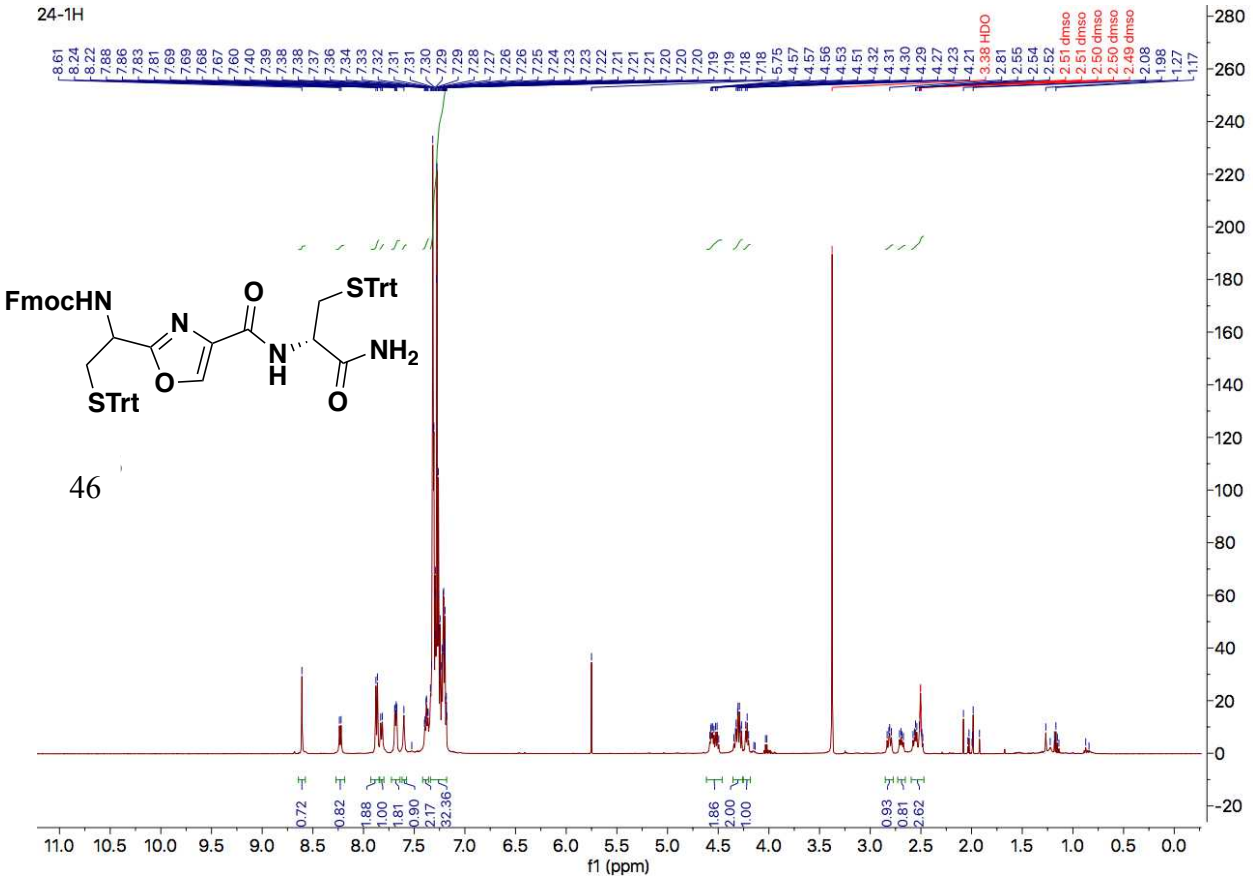
17

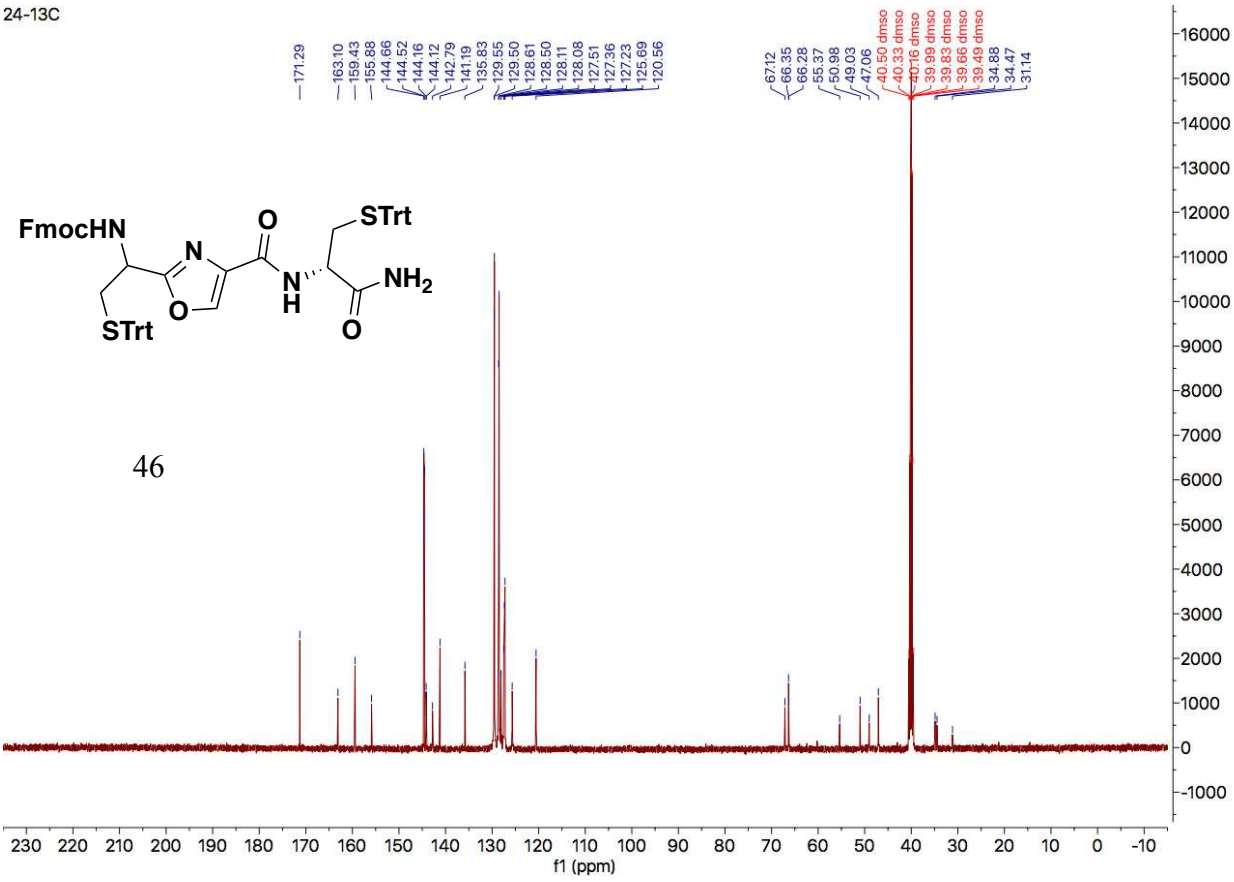


19

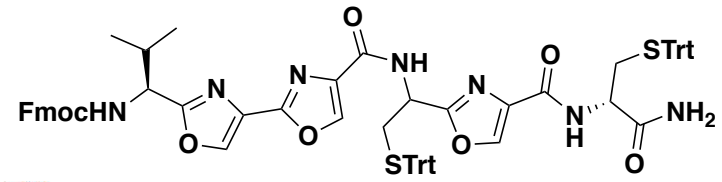

18-13C
175.59
175.34
175.15
170.31
170.29
164.95
163.01
162.78
162.45
161.16
159.59
150.34
150.00
147.65
147.61
147.39
134.28
134.21
129.85
126.48
126.26
125.86
119.45
118.30
114.84
104.91
61.38
56.79
55.30
40.48 dmso
40.27 dmso
40.06 dmso
39.85 dmso
39.64 dmso
39.43 dmso
39.23 dmso
32.58
32.55
22.81
19.73
19.70
18.53
18.50
14.64
AcHN
N
S
N
S
O
NH
N
S
O
NH2
450
400
350
300
250
200
150
100
50
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

19

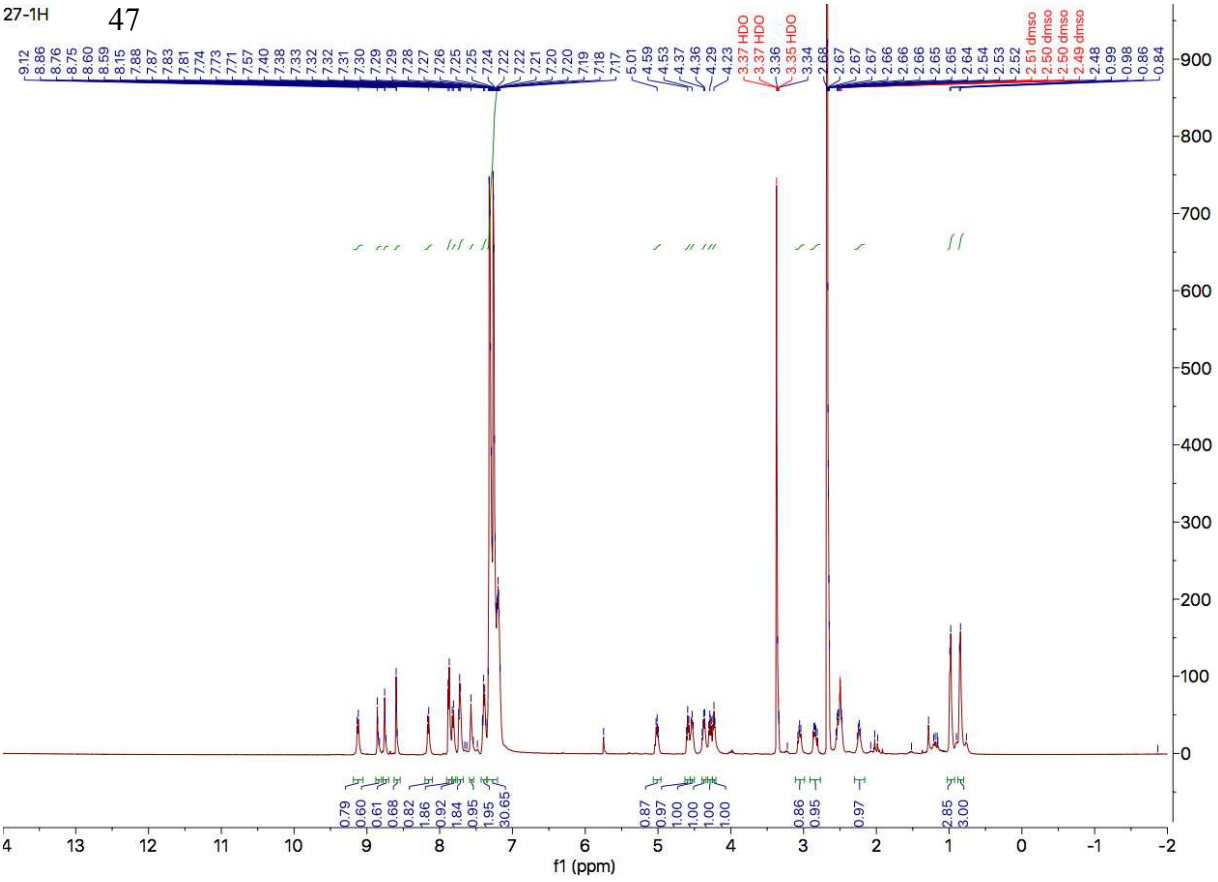

24-1H
FmocHN
STrt
STrt
NH2
46
f1 (ppm)

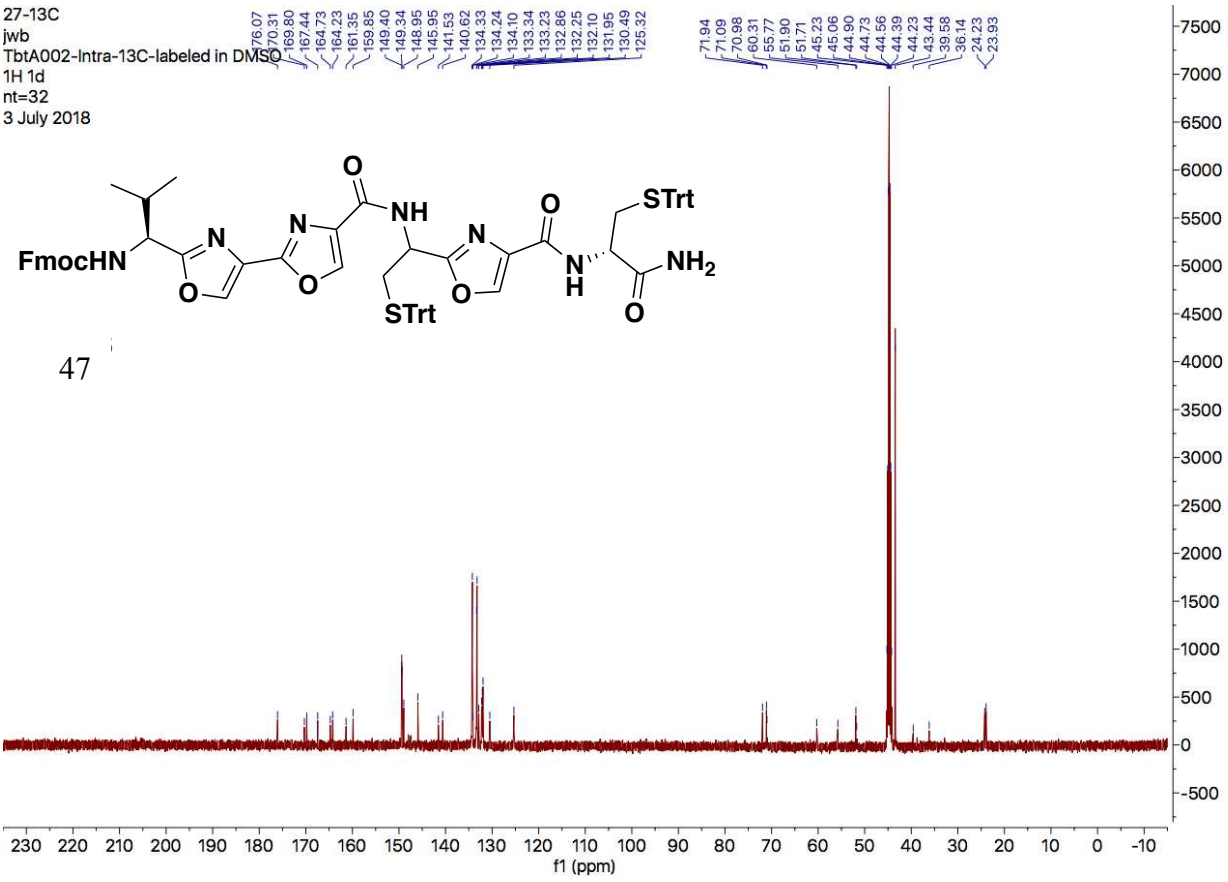

24-13C
171.29
163.10
159.43
155.88
144.66
144.52
144.16
144.12
142.79
141.19
135.83
129.55
129.50
128.61
128.50
128.11
128.08
127.51
127.36
127.23
125.69
120.56
67.12
66.35
66.28
55.37
50.98
49.03
47.06
40.50 dmso
40.33 dmso
40.16 dmso
39.99 dmso
39.83 dmso
39.66 dmso
39.49 dmso
34.88
34.47
31.14
FmocHN
O
N
STrt
N
H
NH2
O
O
STrt
f1 (ppm)

46

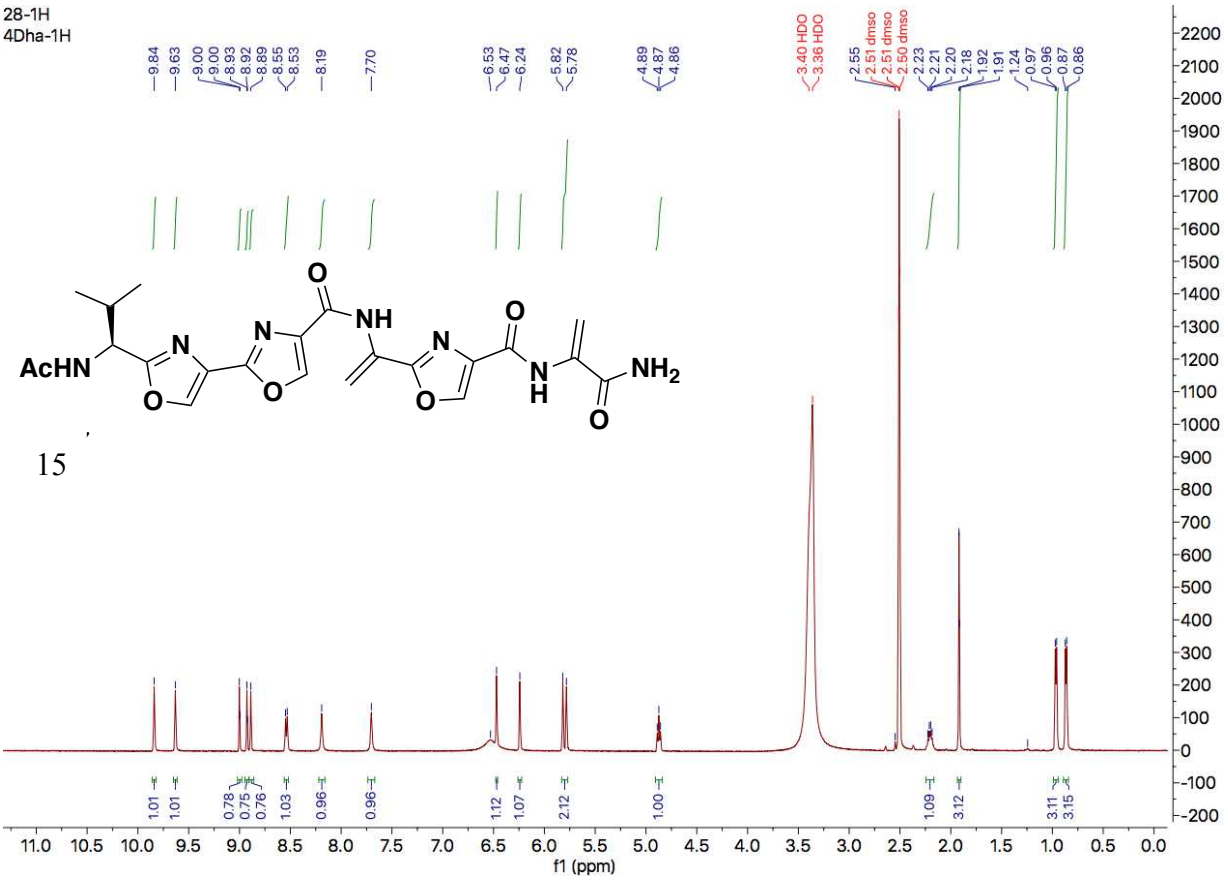


47

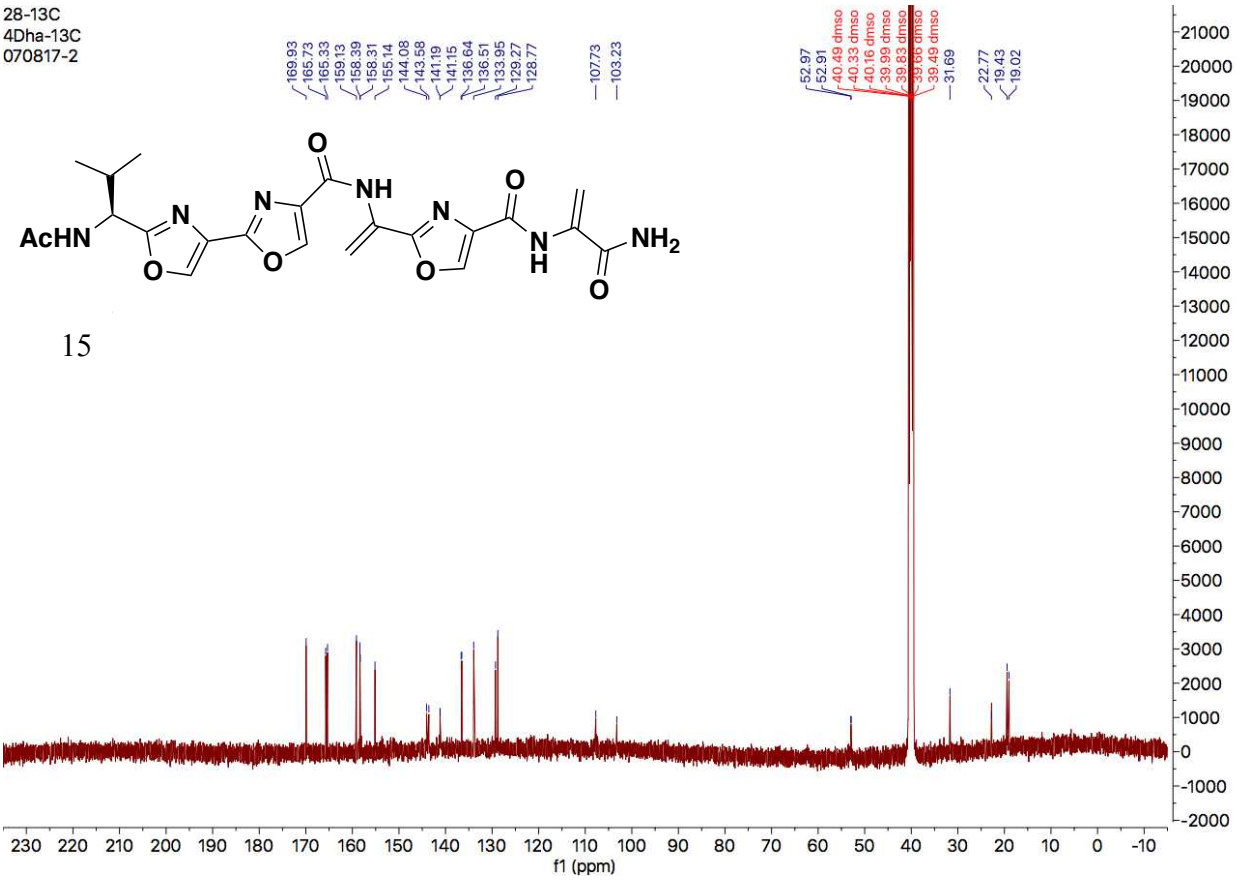


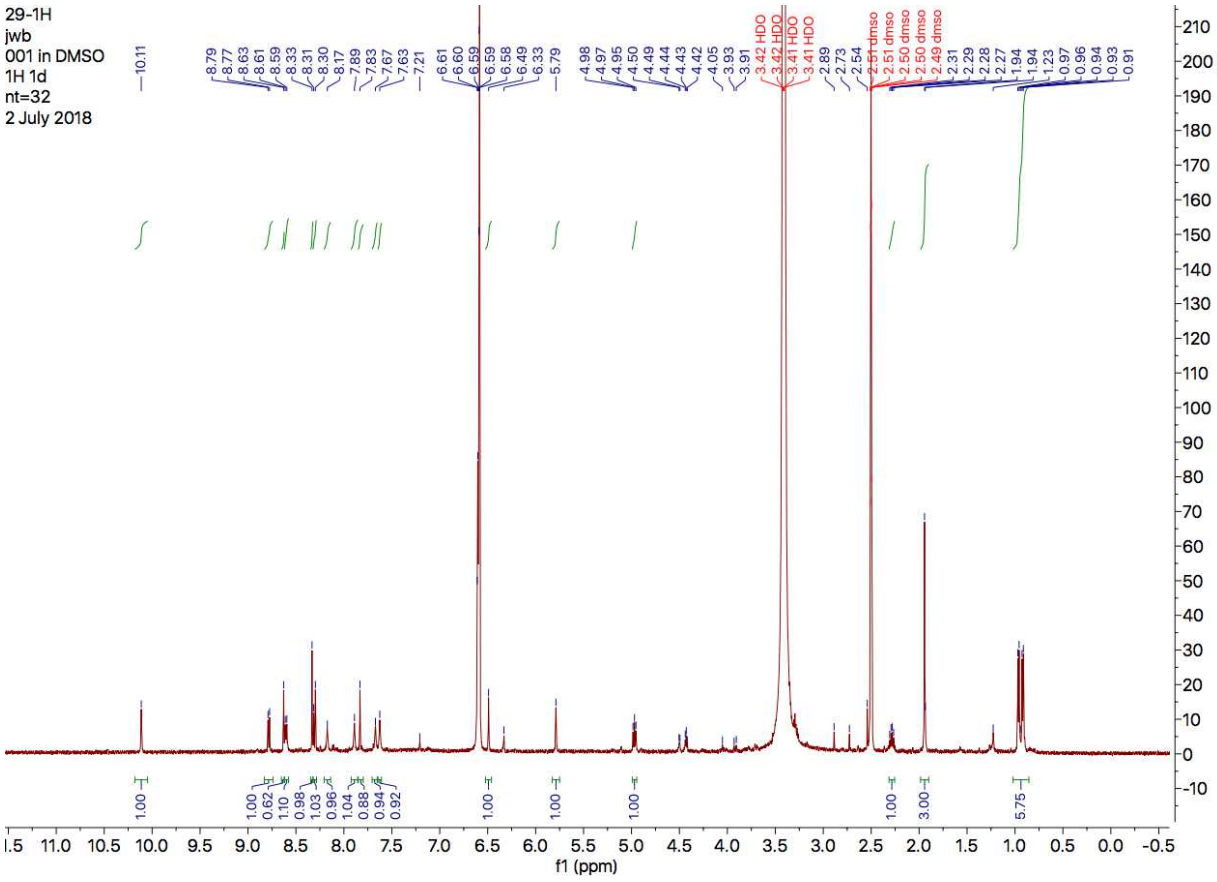

27-13C
jwb
TbtA002-Intra-13C-labeled in DMSO
1H 1d
nt=32
3 July 2018
FmocHN
NH
STrt
STrt
NH2
f1 (ppm)

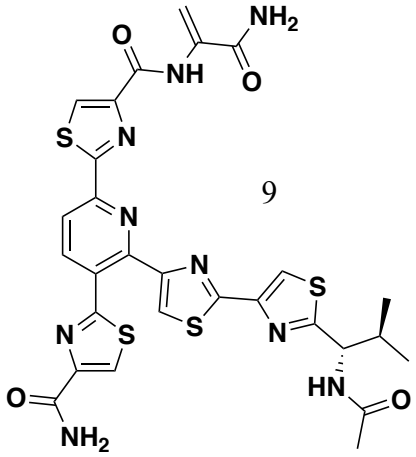
47



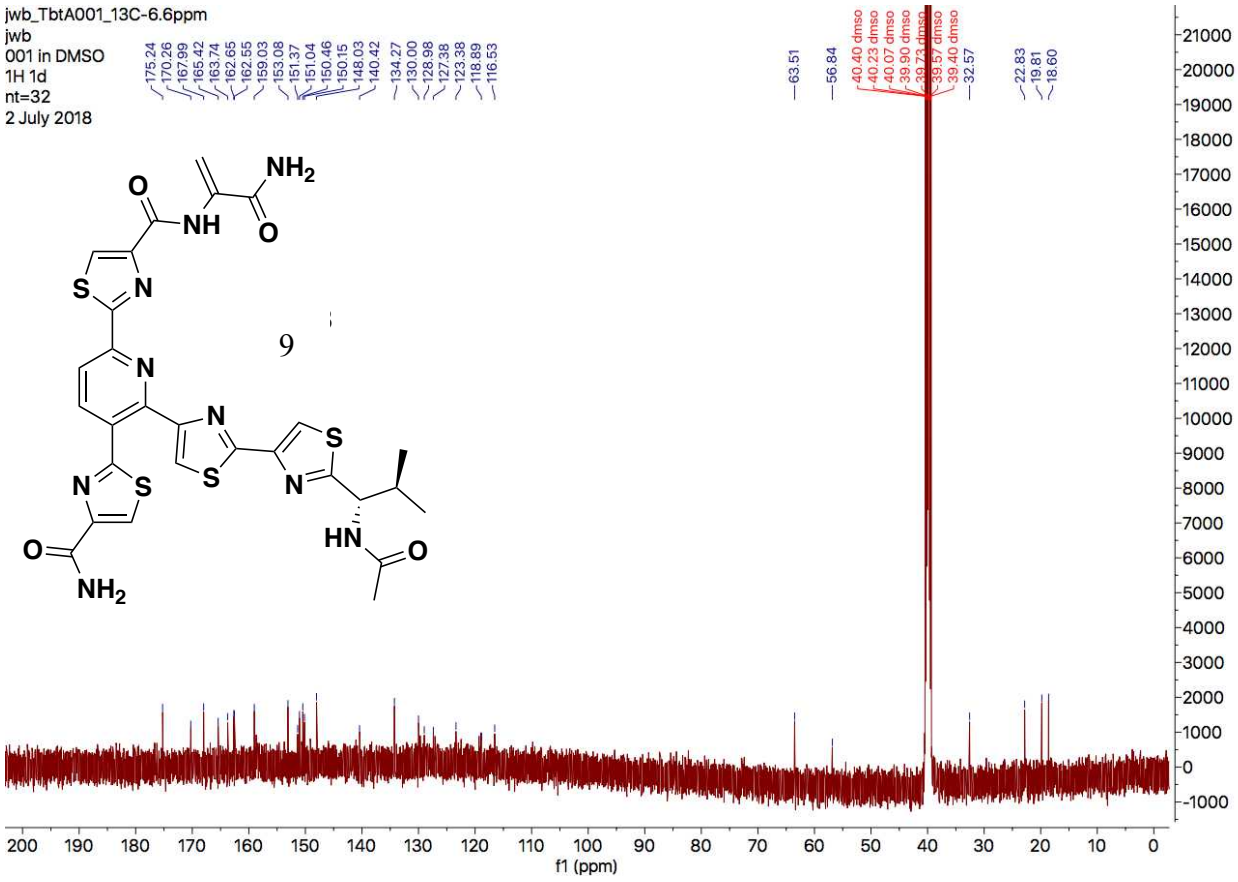
15

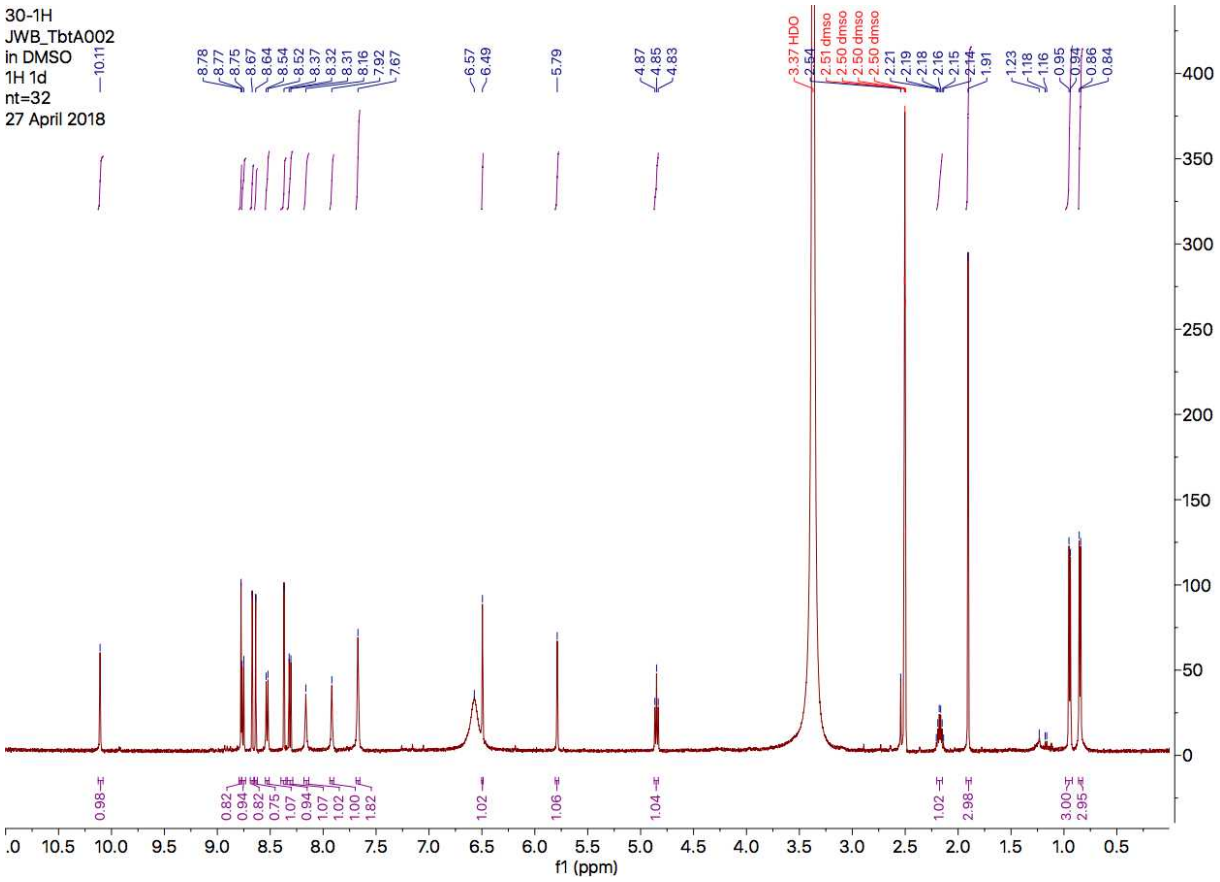

28-13C
4Dha-13C
070817-2
169.93
165.73
165.33
159.13
158.39
158.31
155.14
144.08
143.58
141.19
141.15
136.64
136.51
133.95
129.27
128.77
107.73
103.23
52.97
52.91
40.49 dmso
40.33 dmso
40.16 dmso
39.99 dmso
39.83 dmso
39.66 dmso
39.49 dmso
31.69
22.77
19.43
19.02
AcHN
O
N
O
N
O
NH
N
O
O
N
H
NH2
O
15
f1 (ppm)

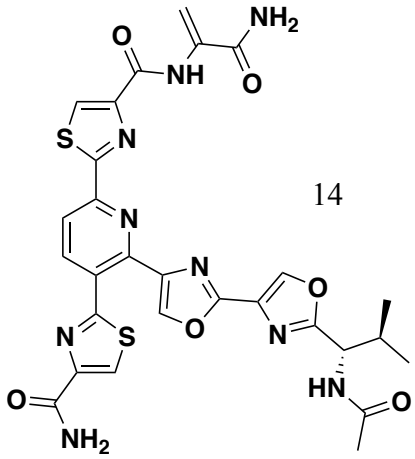




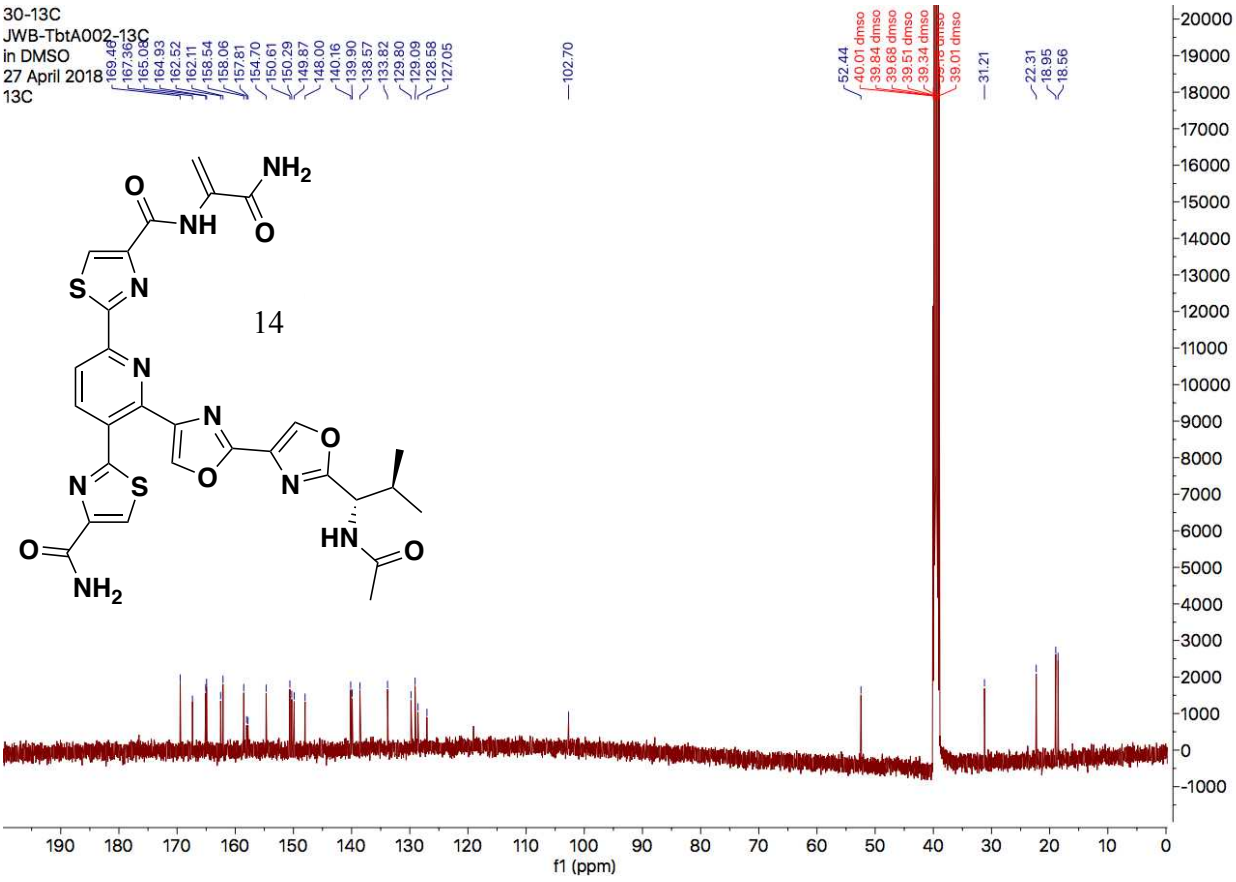
9


jwb_TbtA001_13C-6.6ppm
jwb
001 in DMSO
1H 1d
nt=32
2 July 2018
175.24
170.26
167.99
165.42
163.74
162.65
162.55
159.03
153.08
151.37
151.04
150.46
150.15
148.03
140.42
134.27
130.00
128.98
127.38
123.38
118.89
116.53
63.51
56.84
40.40 dmso
40.23 dmso
40.07 dmso
39.90 dmso
39.73 dmso
39.57 dmso
39.40 dmso
32.57
22.83
19.81
18.60
NH2
O
NH
O
S
N
N
N
S
N
S
N
S
O
NH2
HN
O
f1 (ppm)

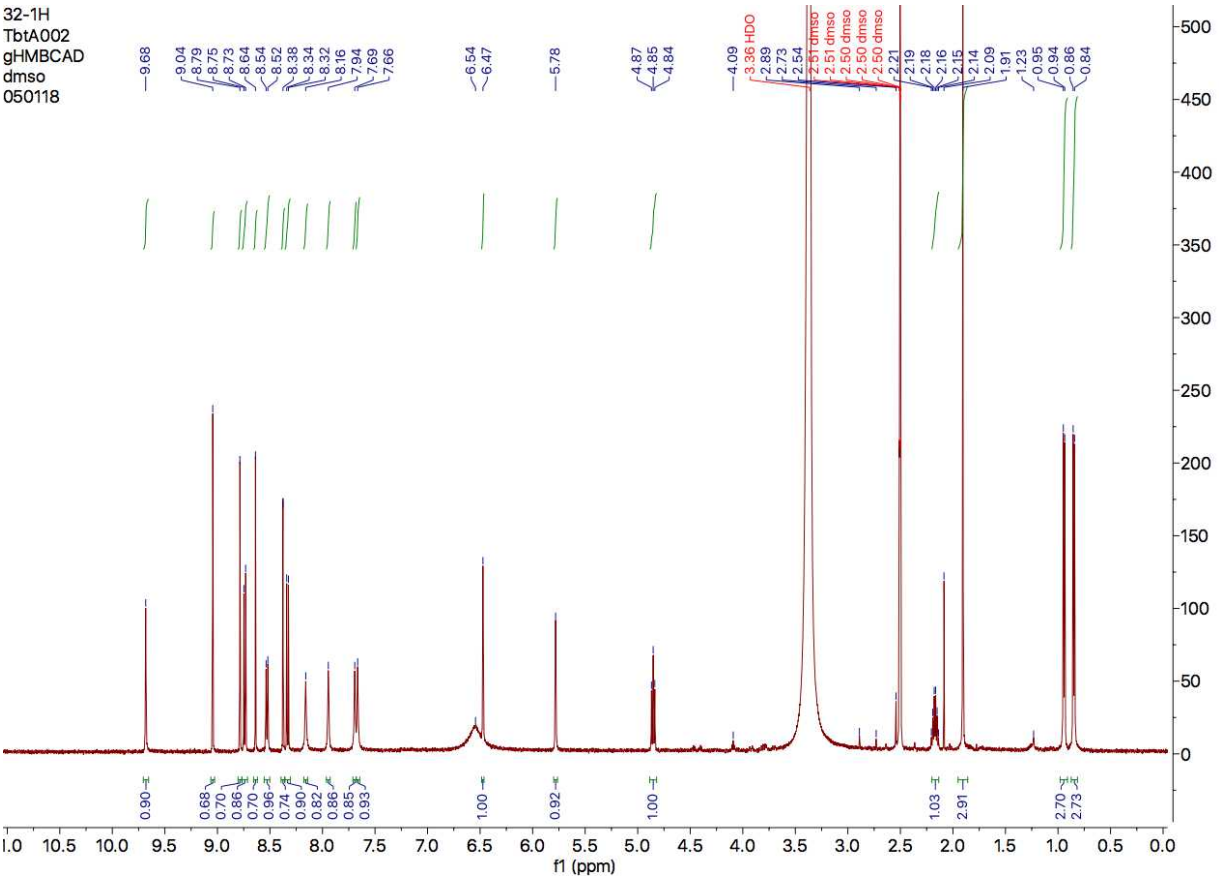


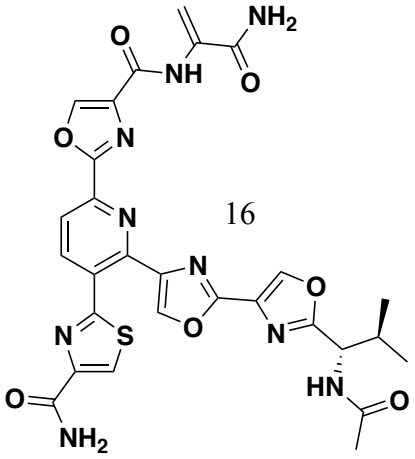


14

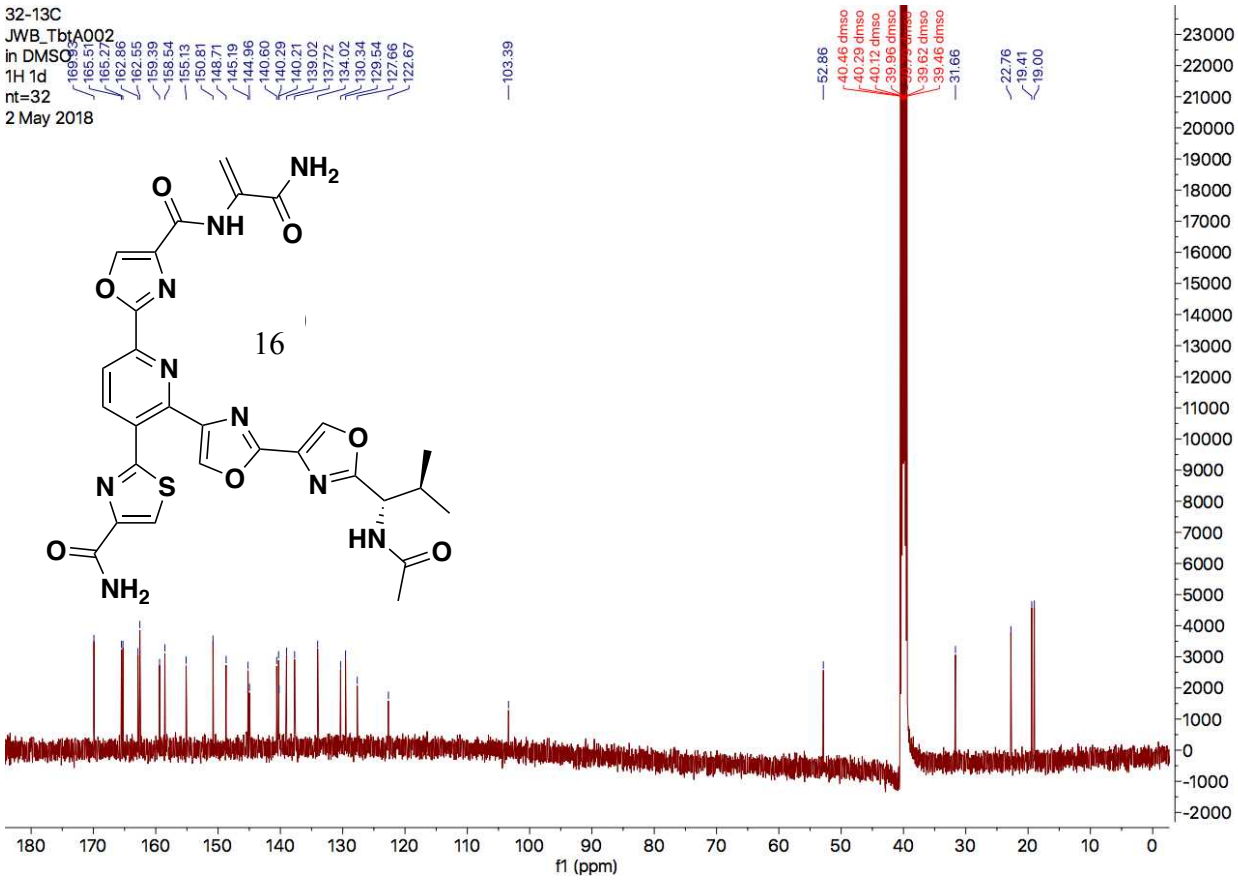

30-13C
JWB-TbtA002-13C
in DMSO
27 April 2018
13C
169.48
167.36
165.08
164.93
162.52
162.11
158.54
158.06
157.81
154.70
150.61
150.29
149.87
148.00
140.16
139.90
138.57
133.82
129.80
129.09
128.58
127.05
102.70
52.44
40.01 dmso
39.84 dmso
39.68 dmso
39.51 dmso
39.34 dmso
39.01 dmso
31.21
22.31
18.95
18.56
NH2
O
NH
O
S
N
N
N
O
N
O
N
S
HN
O
O
NH2
f1 (ppm)

14

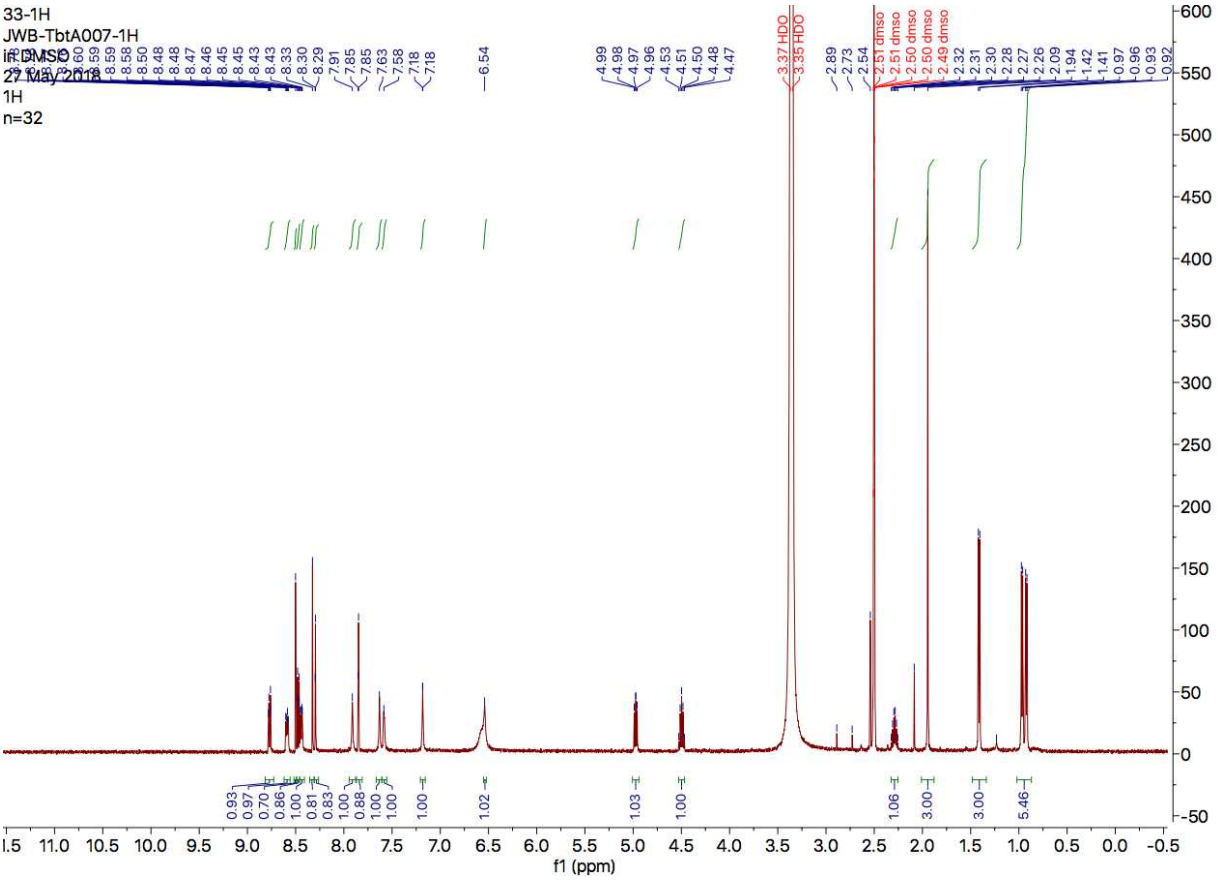


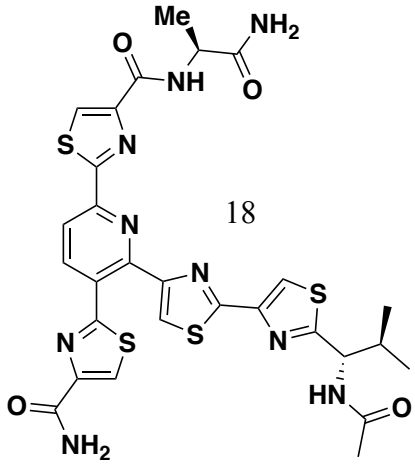


16

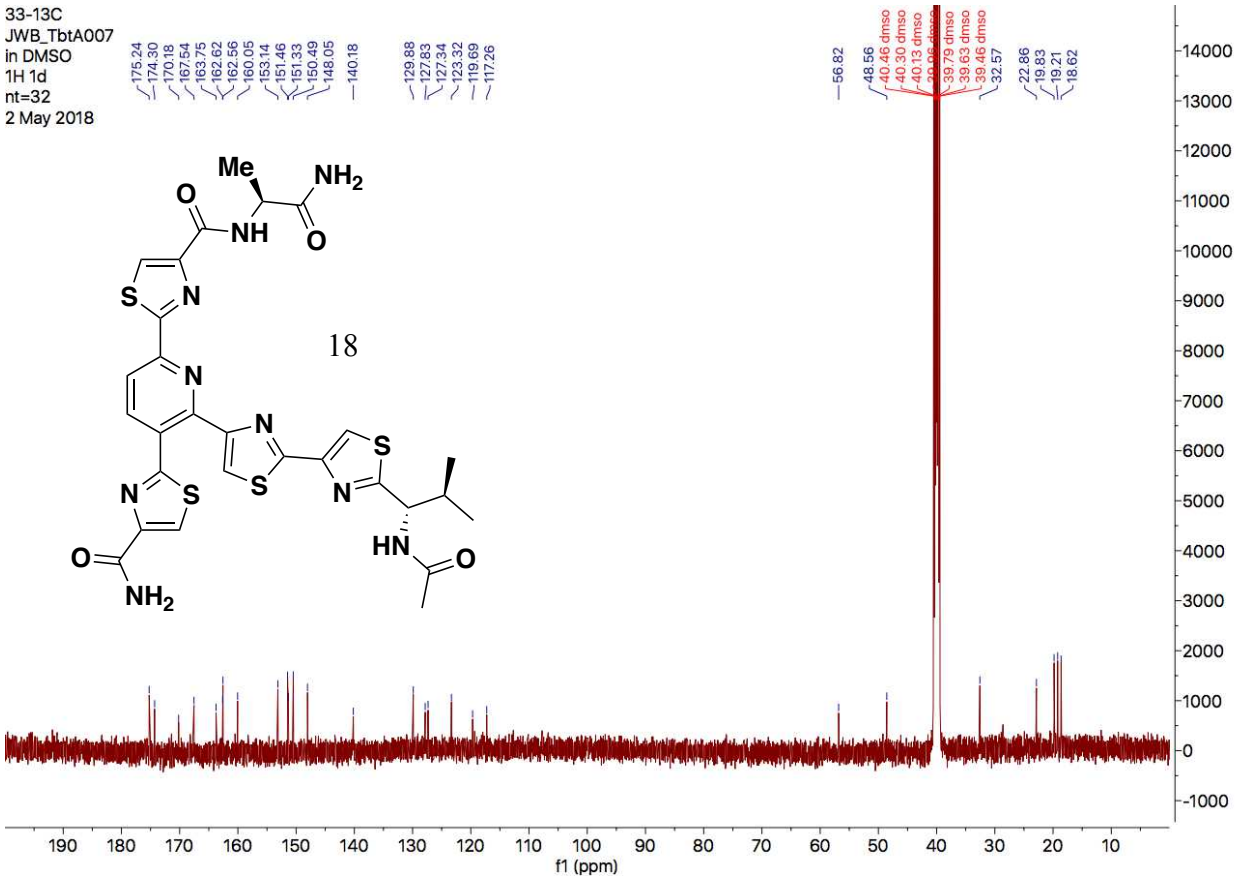

32-13C
JWB_TbtA002
in DMSO
1H 1d
nt=32
2 May 2018
169.36
165.51
165.27
162.86
162.55
159.39
158.54
155.13
150.81
148.71
145.19
144.96
140.60
140.29
140.21
139.02
137.72
134.02
130.34
129.54
127.66
122.67
103.39
52.86
40.46 dmso
40.29 dmso
40.12 dmso
39.96 dmso
39.79 dmso
39.62 dmso
39.46 dmso
31.66
22.76
19.41
19.00
NH2
O
NH
O
O
N
N
N
O
N
S
O
NH2
O
N
HN
O
f1 (ppm)

16

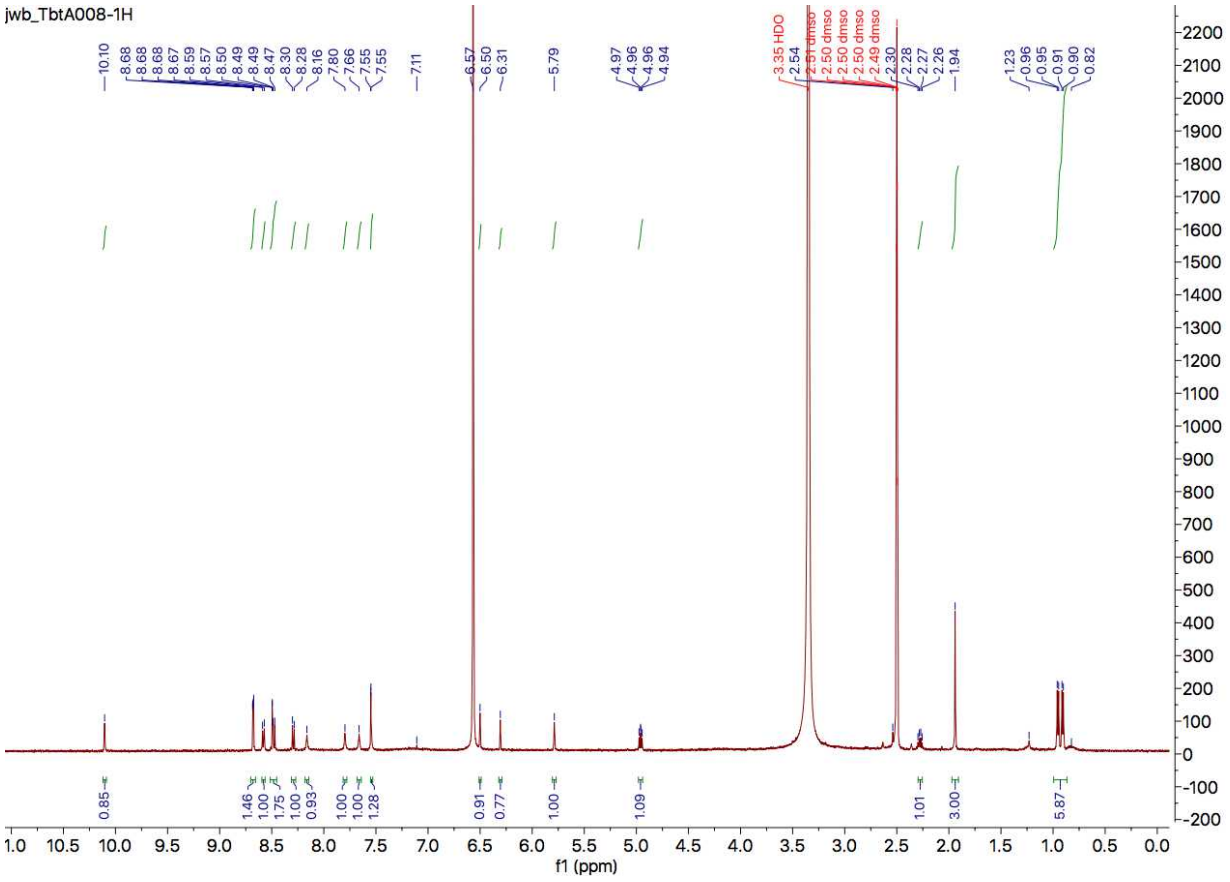


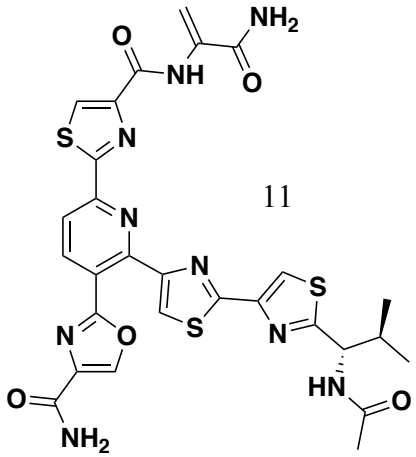


18

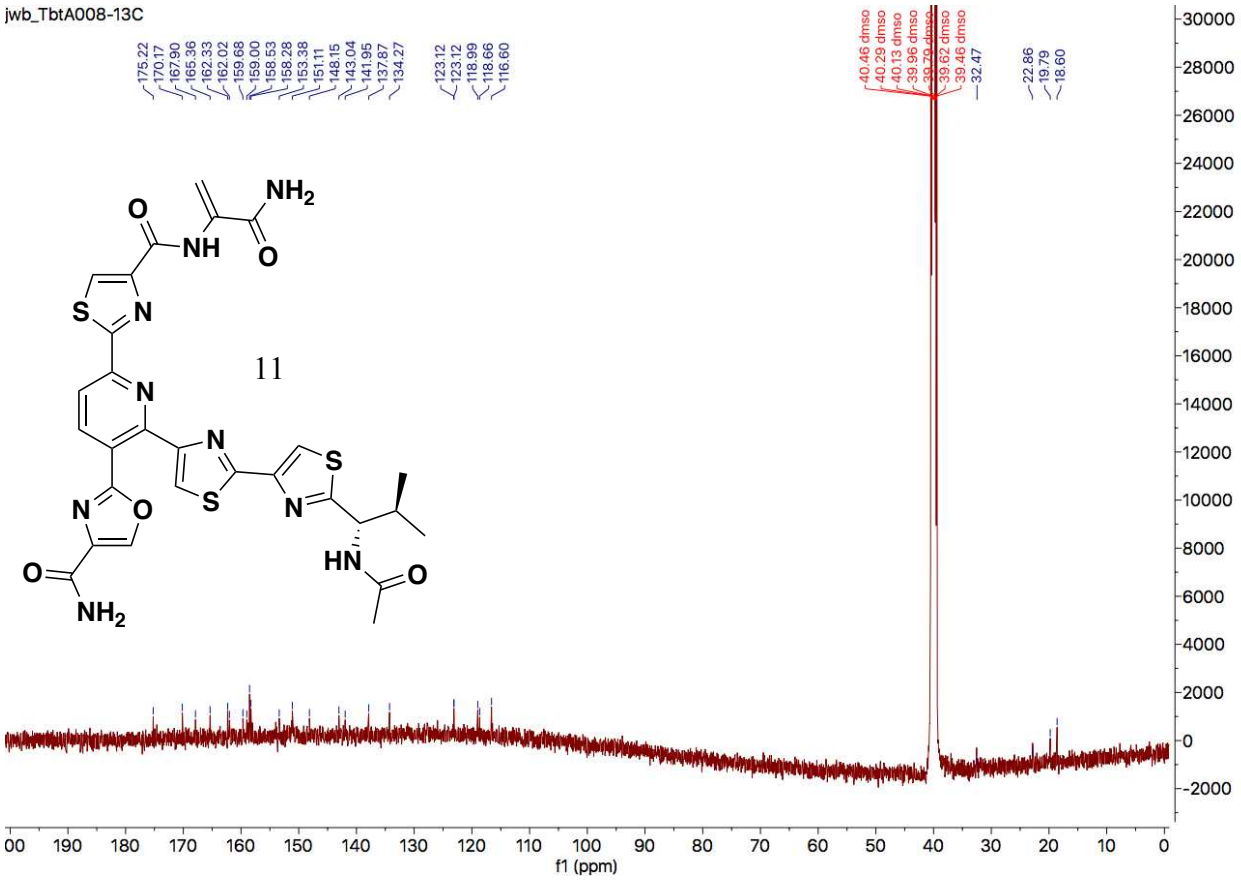

33-13C
JWB_TbtA007
in DMSO
1H 1d
nt=32
2 May 2018
175.24
174.30
170.18
167.54
163.75
162.62
162.56
160.05
153.14
151.46
151.33
150.49
148.05
140.18
129.88
127.83
127.34
123.32
119.69
117.26
56.82
48.56
40.46 dmso
40.30 dmso
40.13 dmso
39.79 dmso
39.63 dmso
39.46 dmso
32.57
22.86
19.83
19.21
18.62
Me
NH2
O
NH
O
S
N
N
N
S
S
N
S
N
S
HN
O
O
NH2
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

18



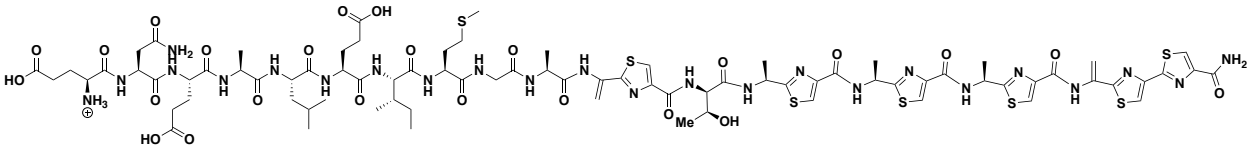


11

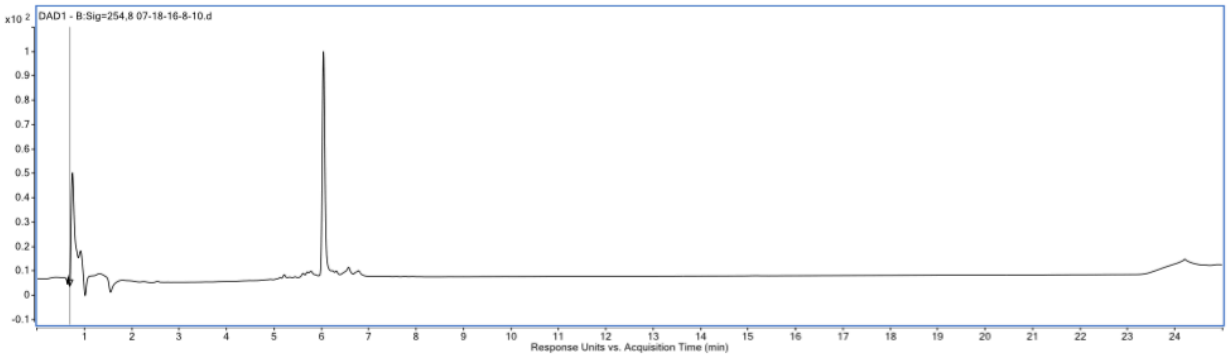

jwb_TbtA008-13C
175.22
170.17
167.90
165.36
162.33
162.02
159.68
159.00
158.53
158.28
153.38
151.11
148.15
143.04
141.95
137.87
134.27
123.12
123.12
118.99
118.66
116.60
40.46 dmso
40.29 dmso
40.13 dmso
39.96 dmso
39.79 dmso
39.62 dmso
39.46 dmso
32.47
22.86
19.79
18.60
11
f1 (ppm)

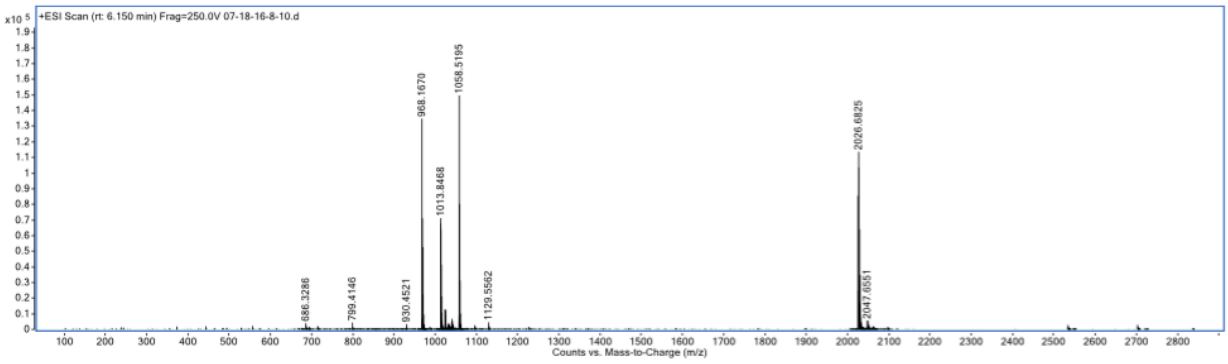
XXI. Peptide Characterization

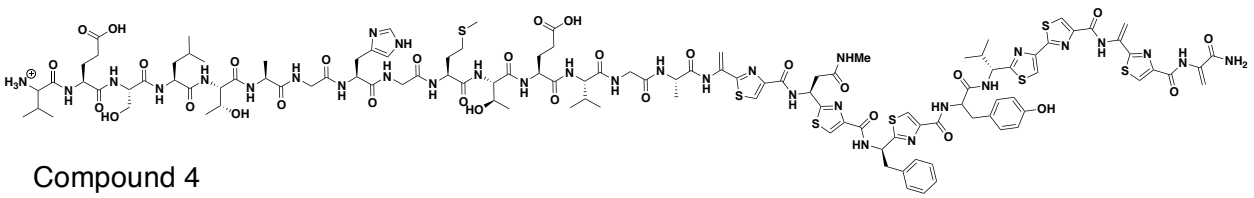
Compound 3



Exact Mass: 2025.6086
Molecular Weight: 2027.3265

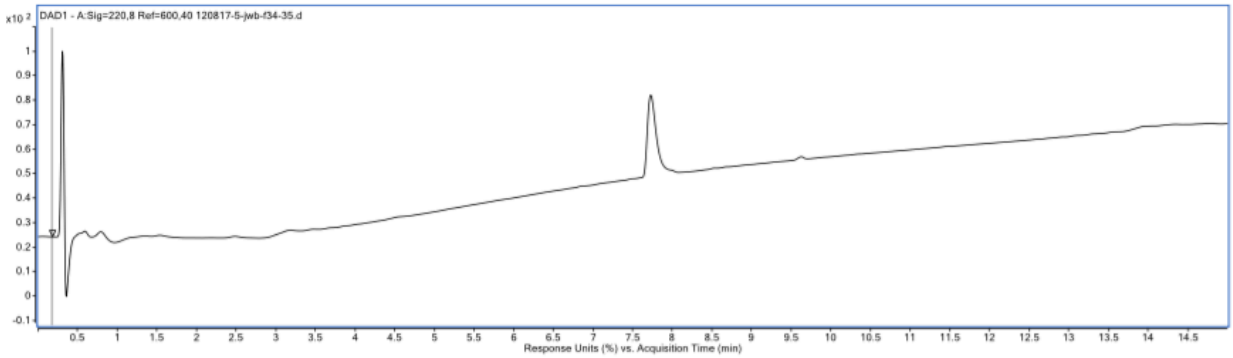


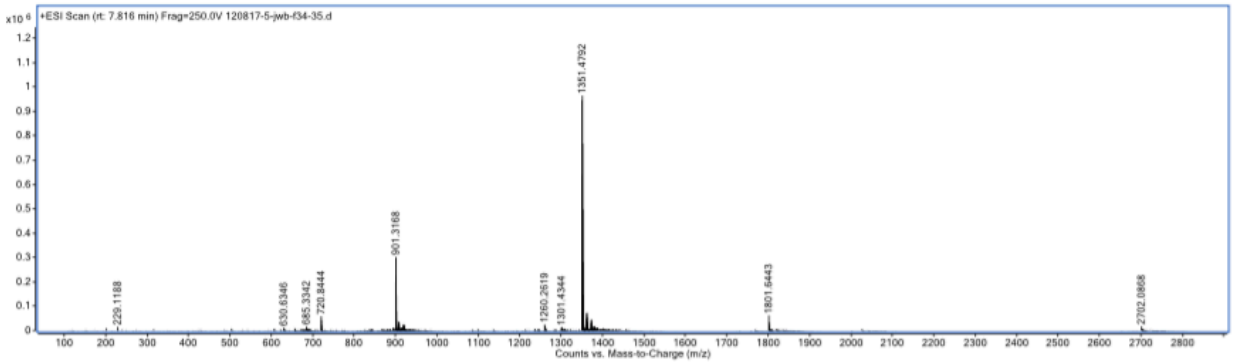


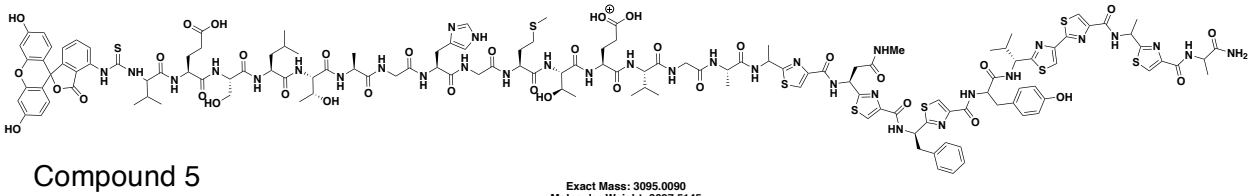


Compound 4

Exact Mass: 2699.9262
Molecular Weight: 2702.0855

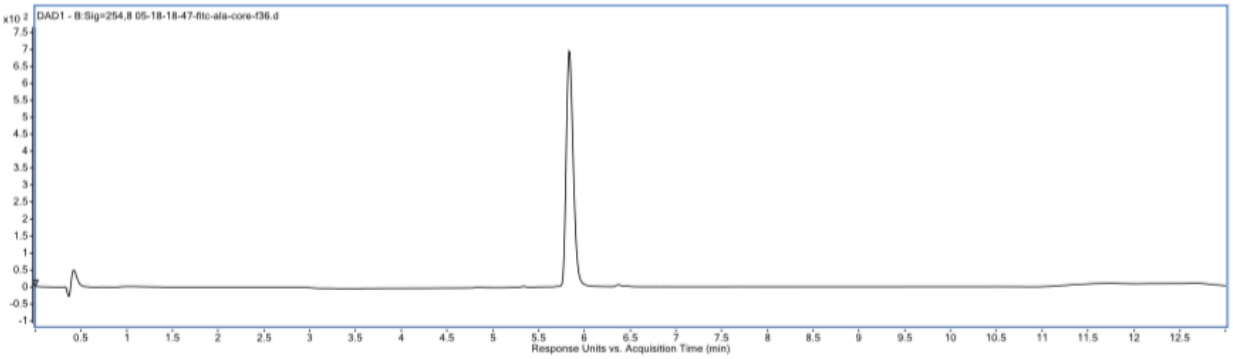


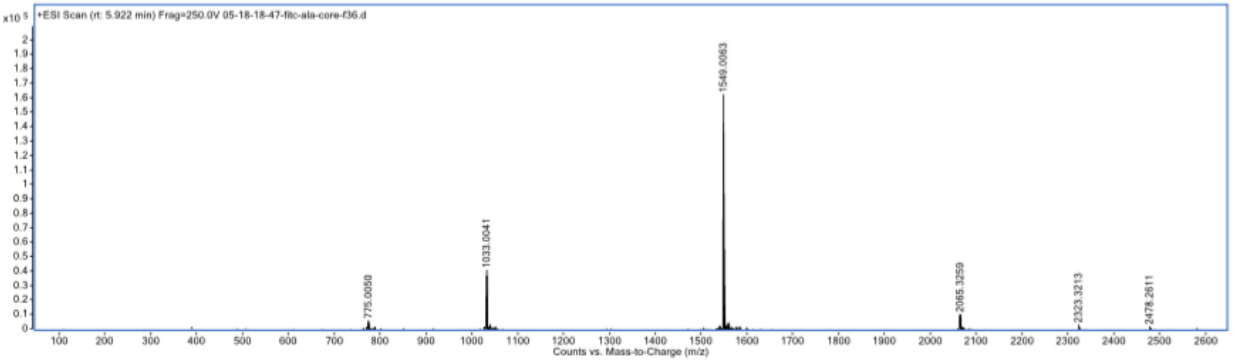


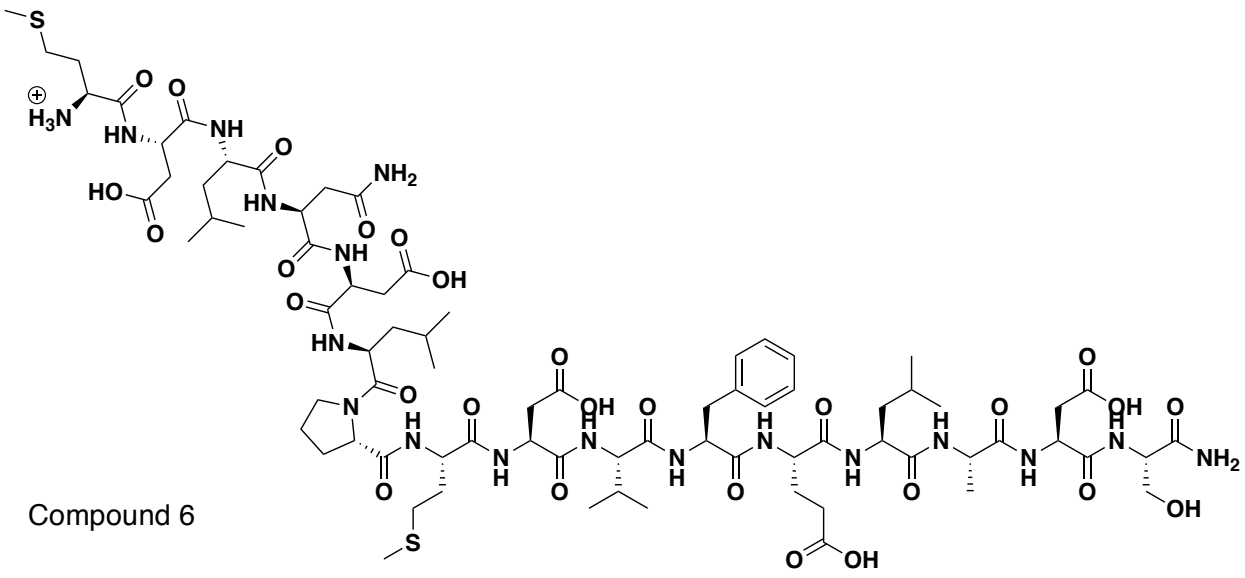


Compound 5

Exact Mass: 3095.0090
Molecular Weight: 3097.5145

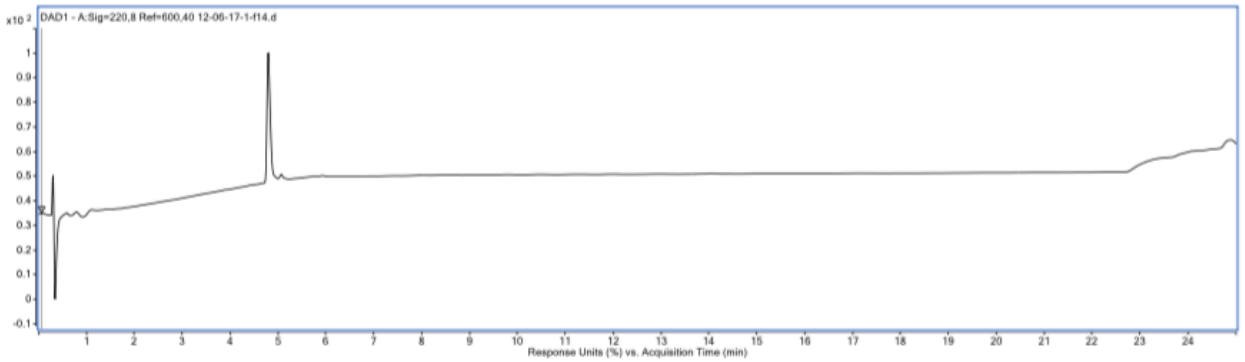


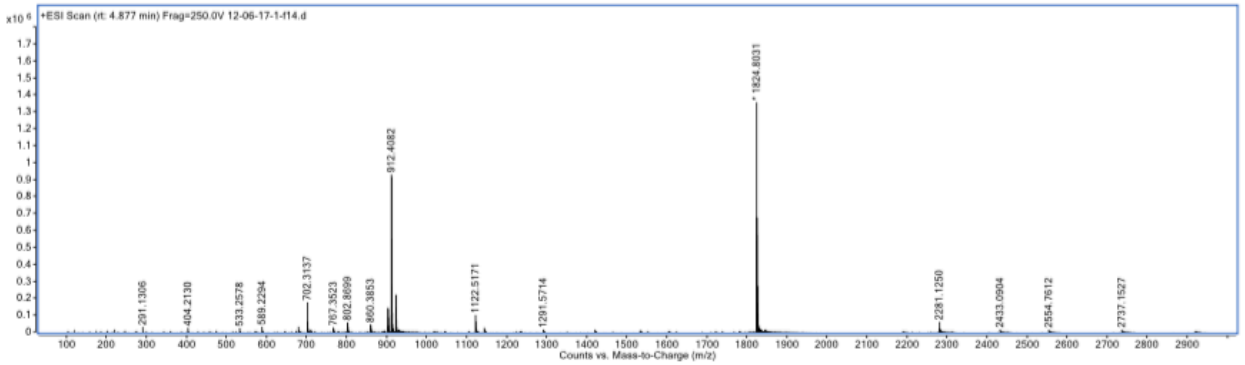


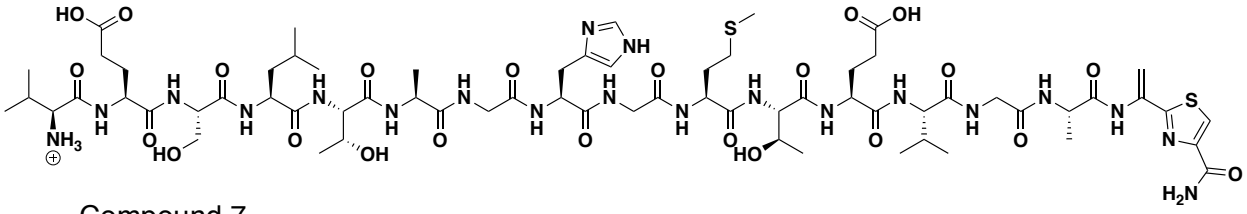


Compound 6

Exact Mass: 1823.8190
Molecular Weight: 1825.0595

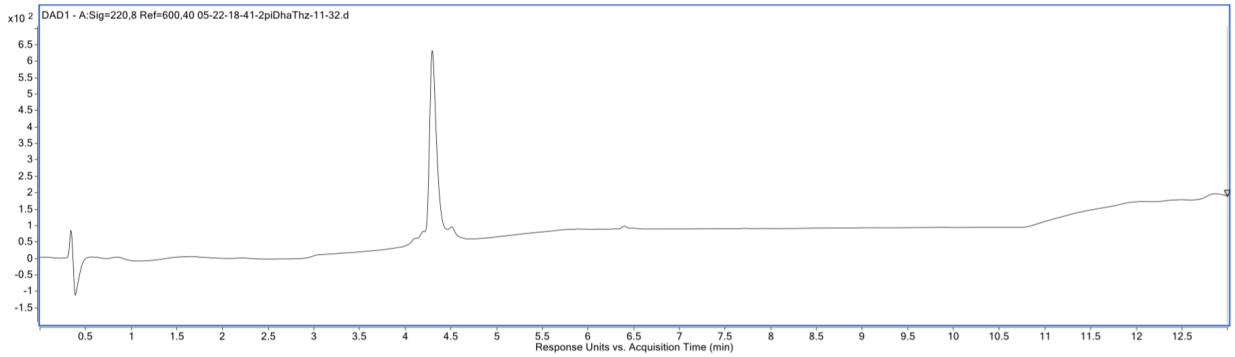


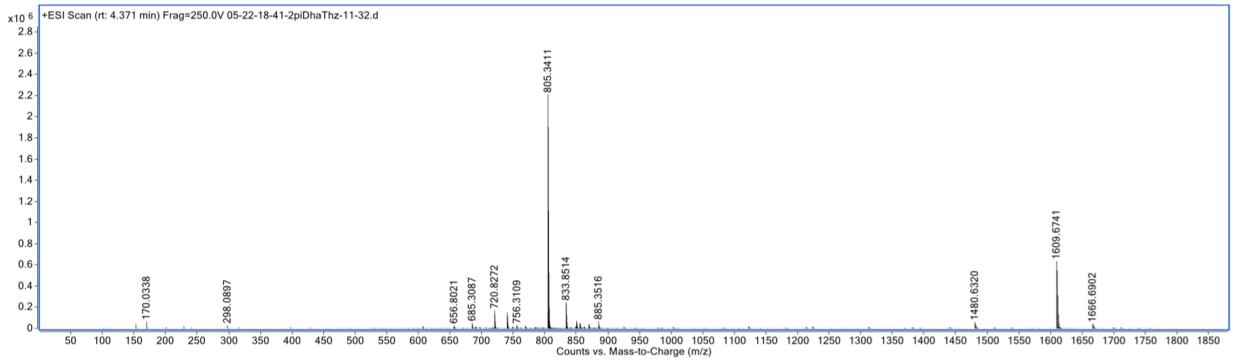


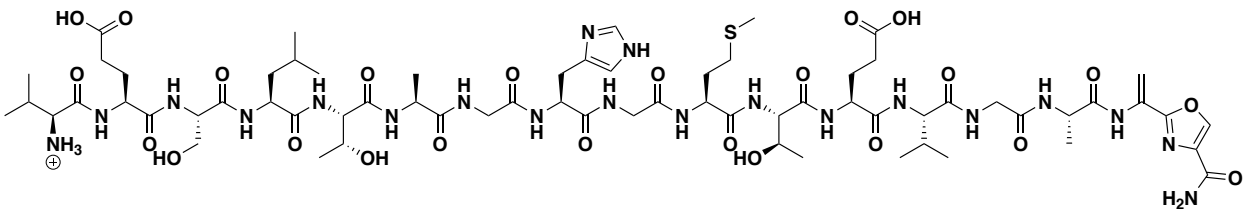


Compound 7

Exact Mass: 1609.7097
Molecular Weight: 1610.8025

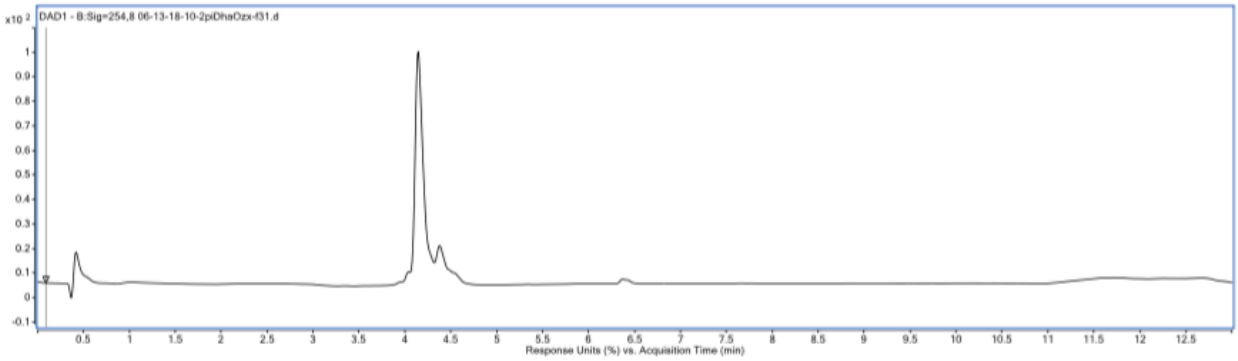


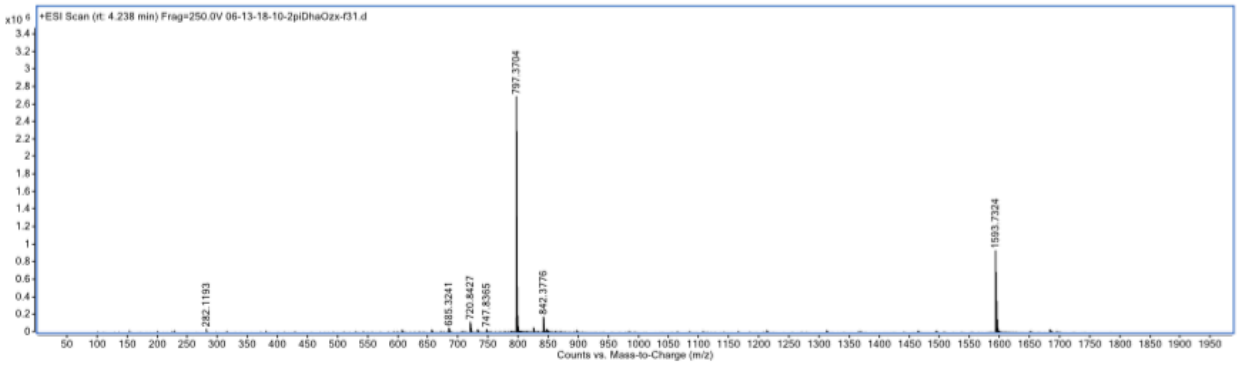


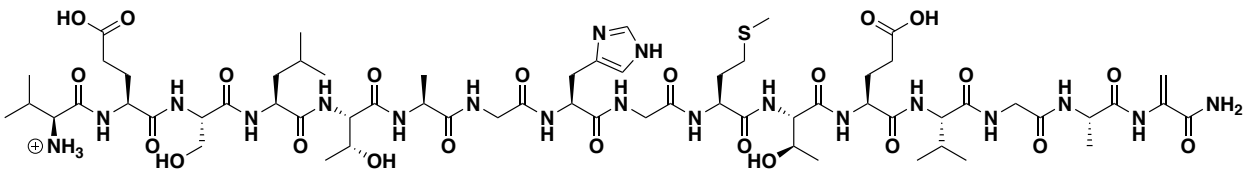


Compound 10

Exact Mass: 1593.7326
Molecular Weight: 1594.7415

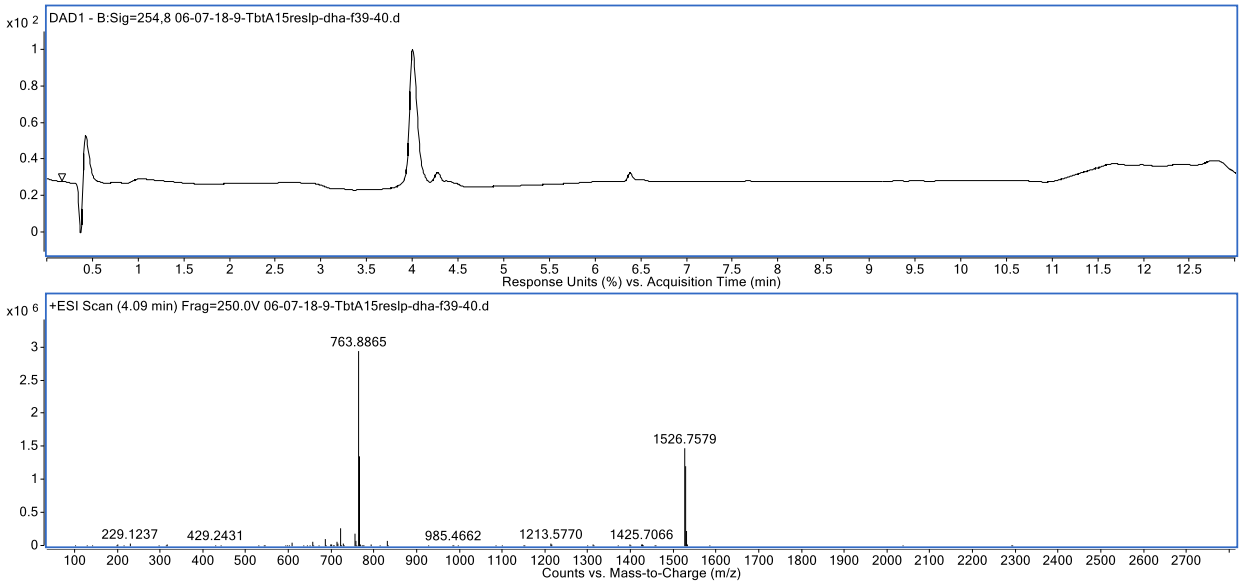


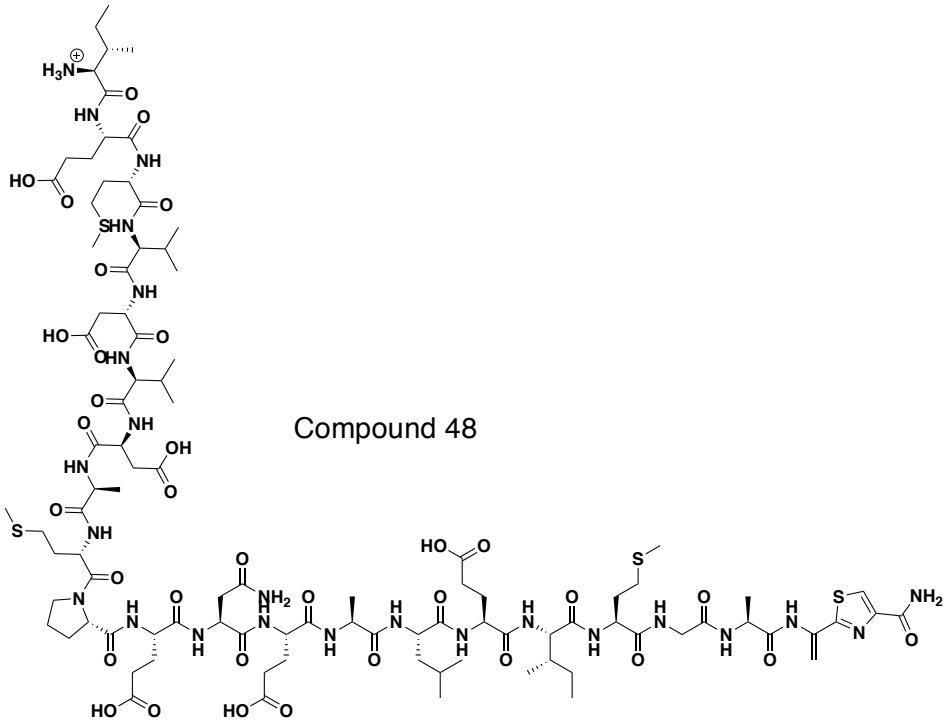




Exact Mass: 1526.7268
Molecular Weight: 1527.6945

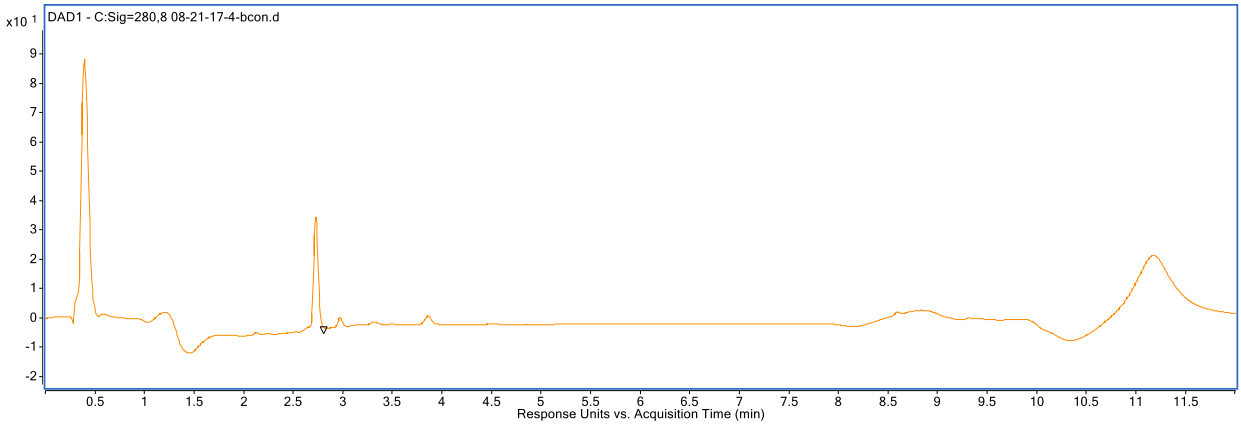
Compound 12

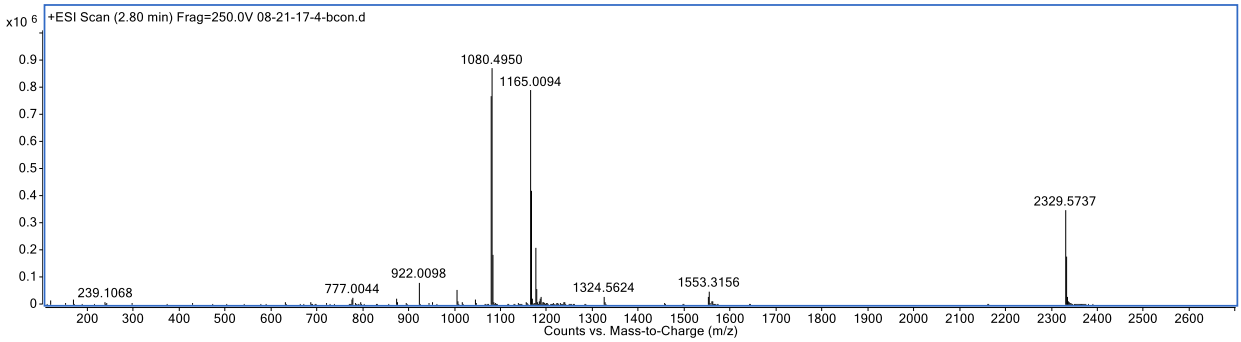


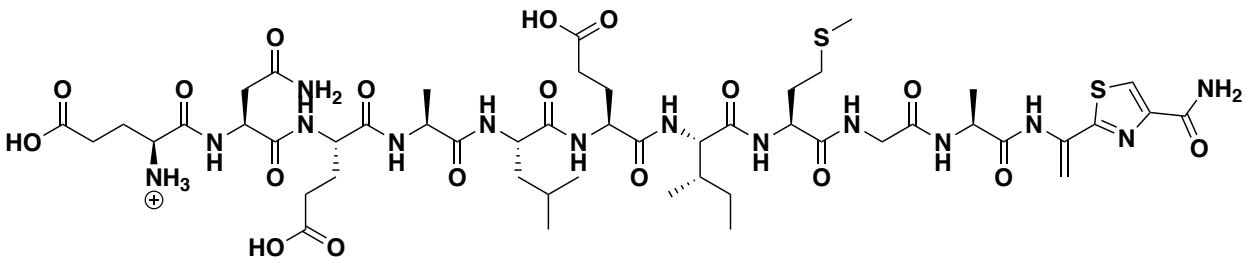


Compound 48

Chemical Formula: $C_{97}H_{155}N_{24}O_{34}S_4^+$
Exact Mass: 2328.0015
Molecular Weight: 2329.6805

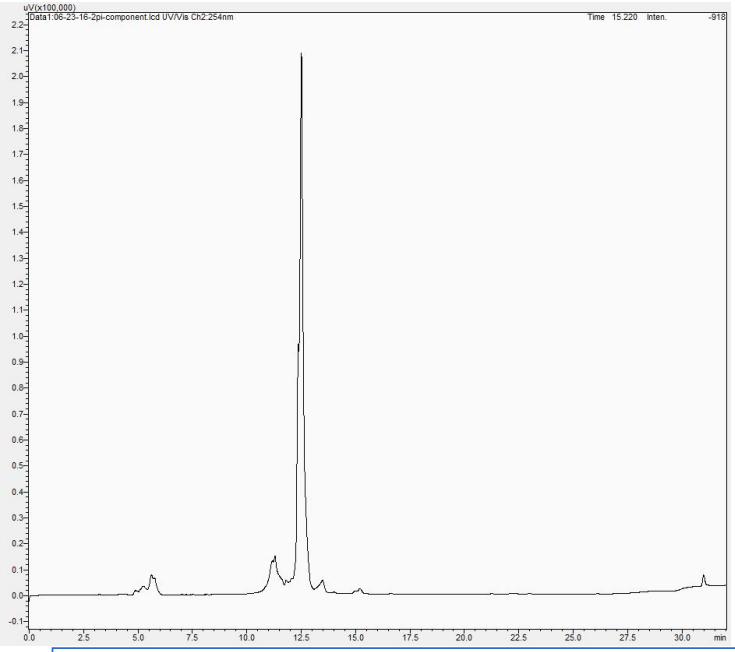


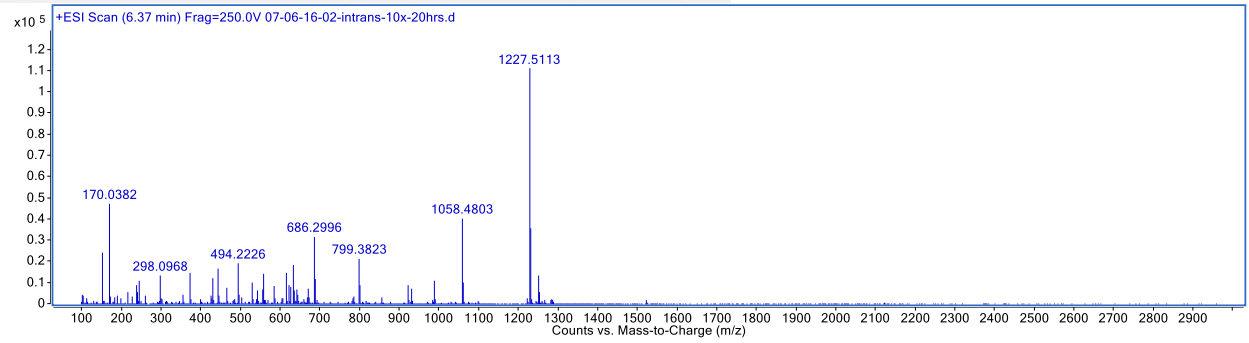


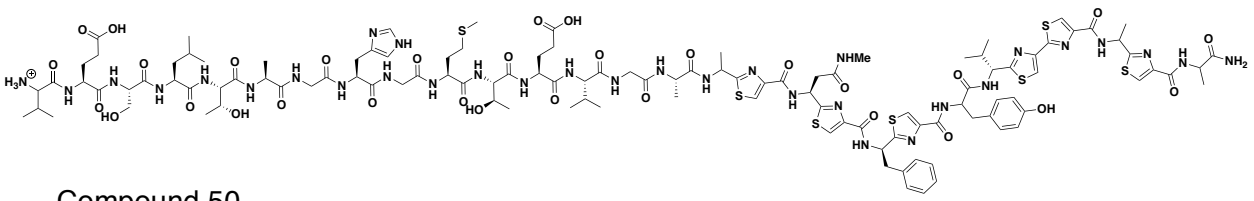


Chemical Formula: $C_{50}H_{79}N_{14}O_{18}S_2^+$
Exact Mass: 1227.5133
Molecular Weight: 1228.3815

Compound 49

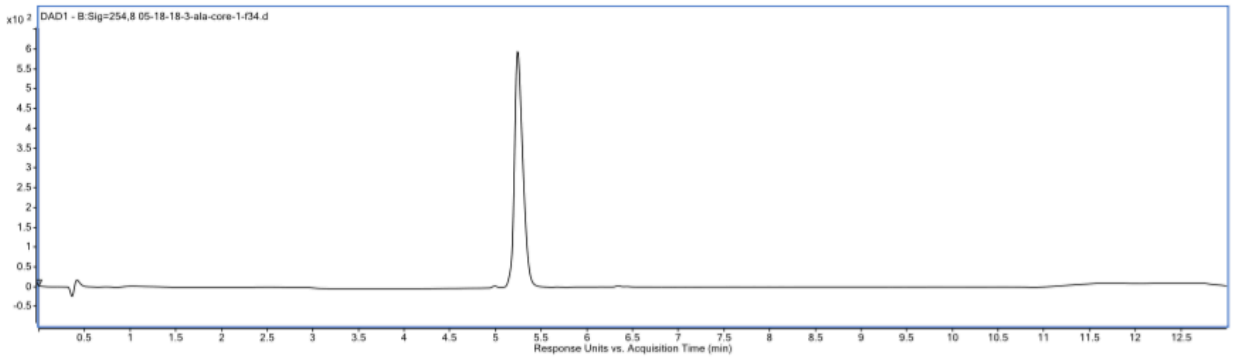


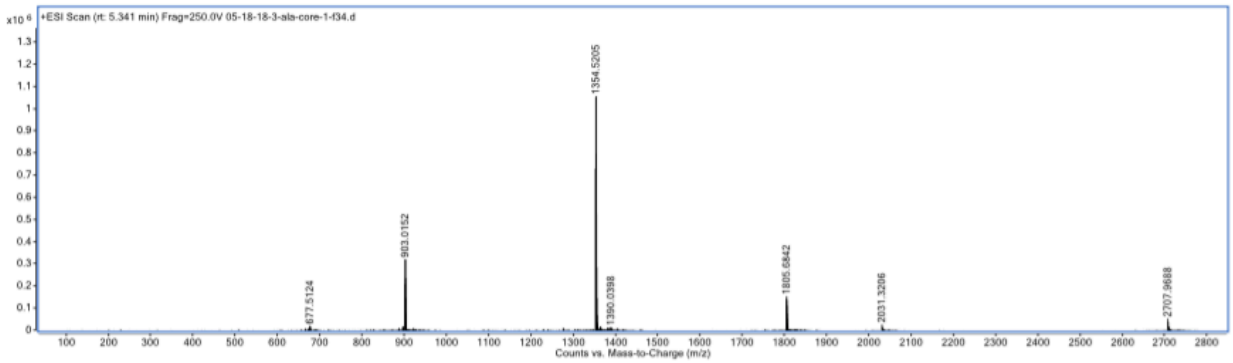




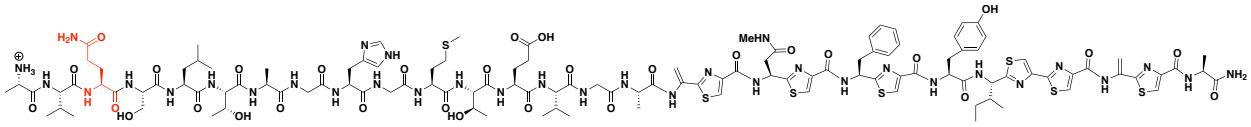
Compound 50

Exact Mass: 2705.9732
Molecular Weight: 2708.1335

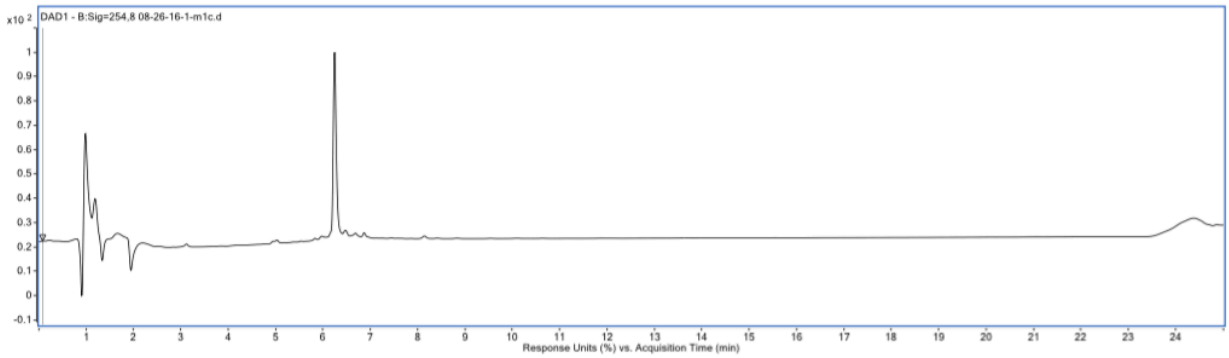


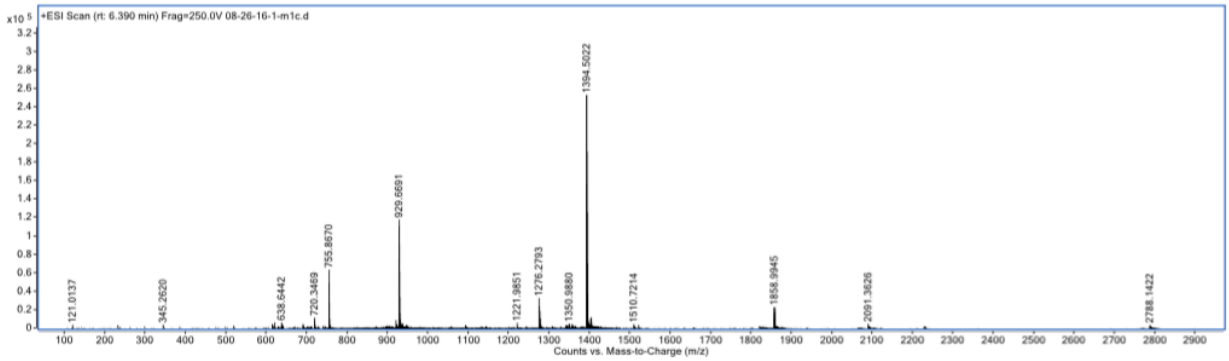


Compound 51 E21Q

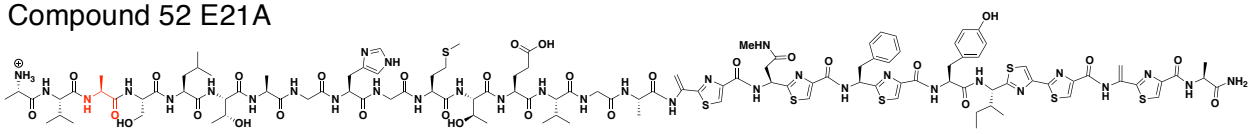


Exact Mass: 2786.0106
Molecular Weight: 2788.2235

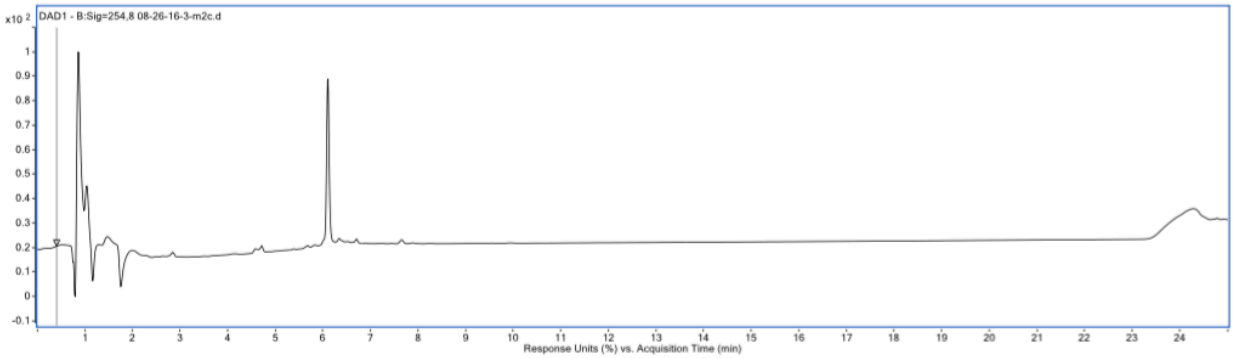


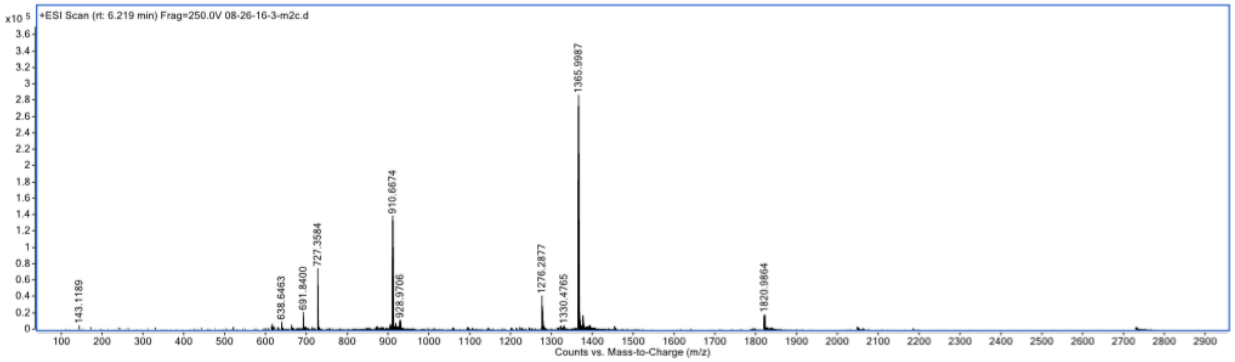


Compound 52 E21A

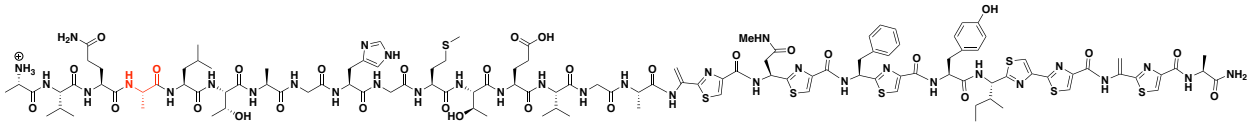


Exact Mass: 2728.9892
Molecular Weight: 2731.1715

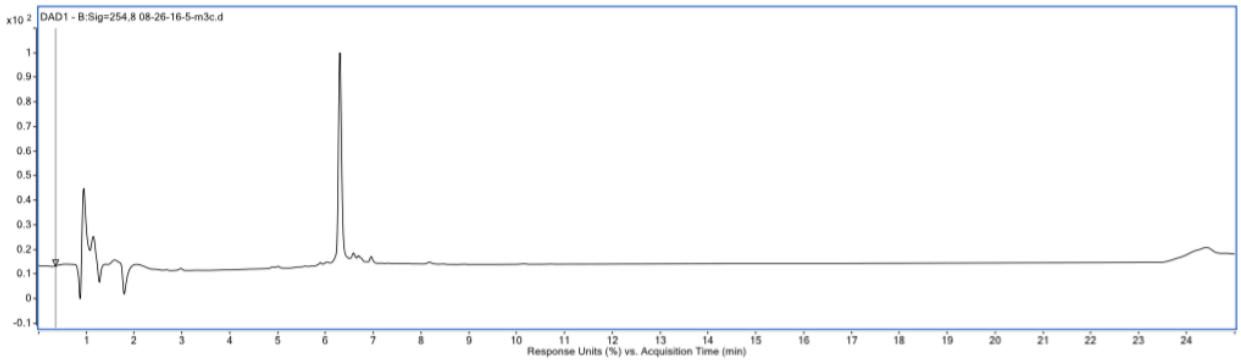


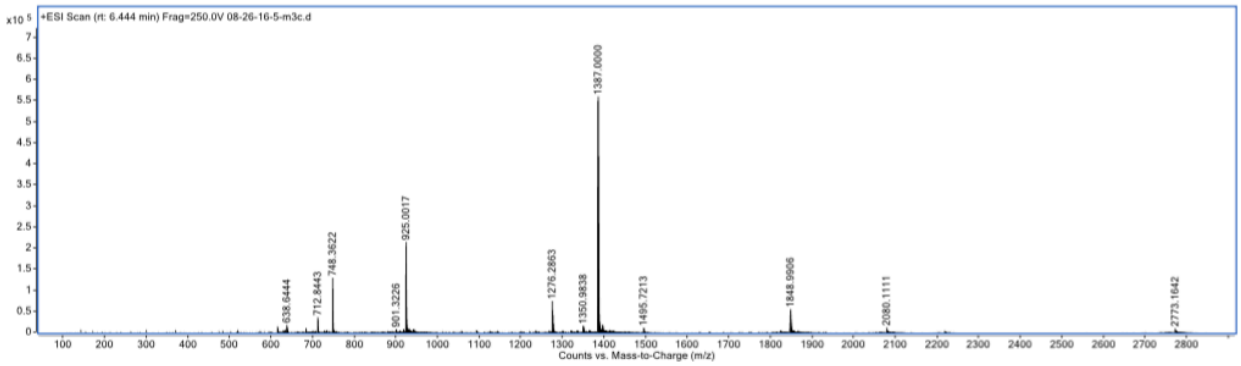


Compound 53 S22A

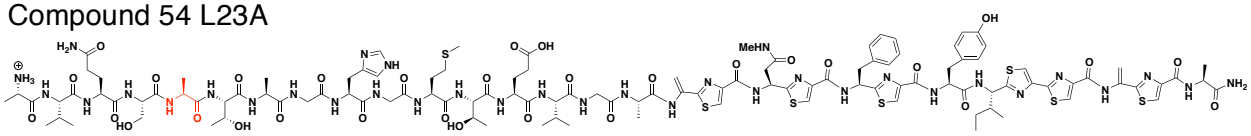


Exact Mass: 2770.0157
Molecular Weight: 2772.2245

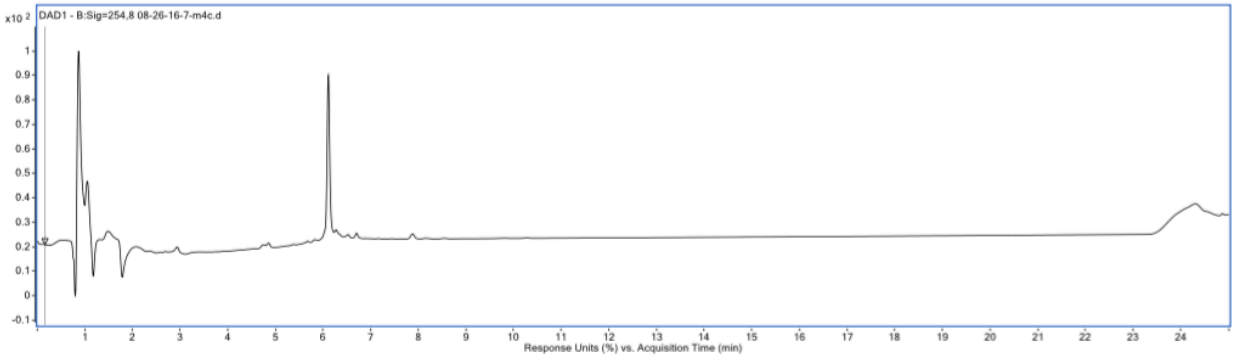


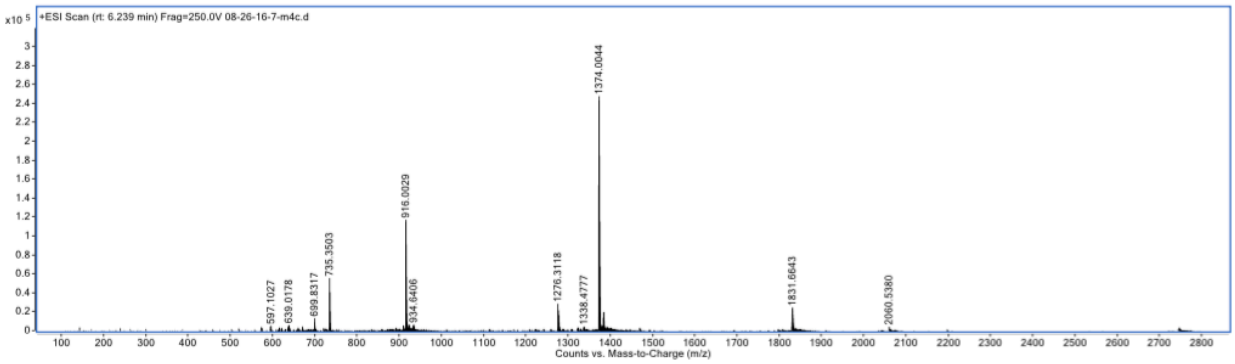


Compound 54 L23A

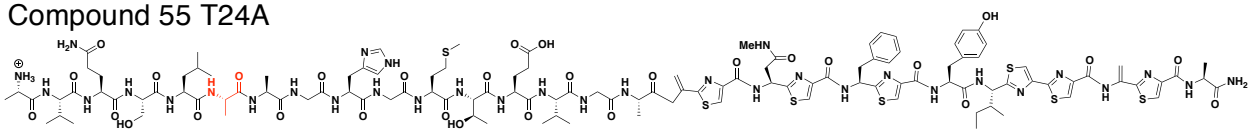


Exact Mass: 2743.9637
Molecular Weight: 2746.1425

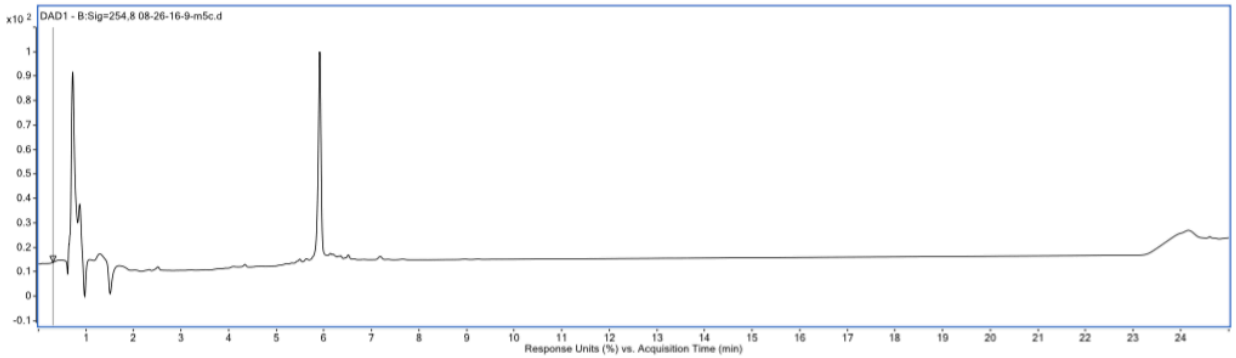


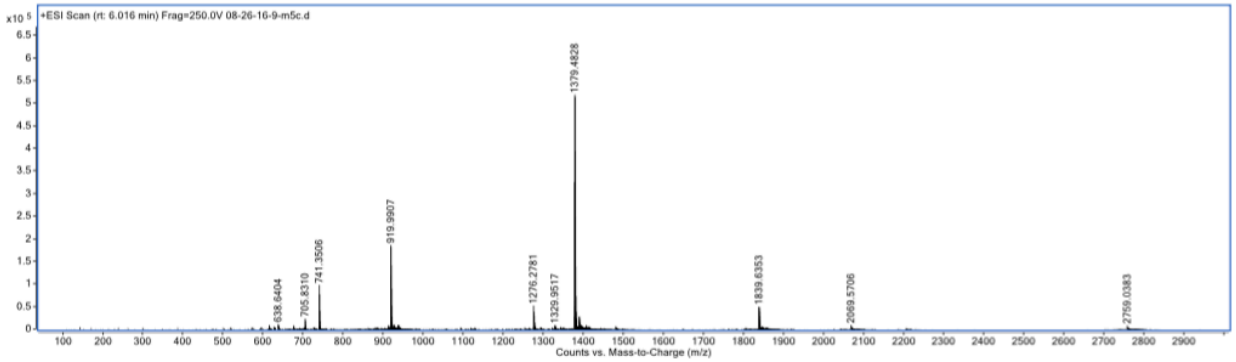


Compound 55 T24A

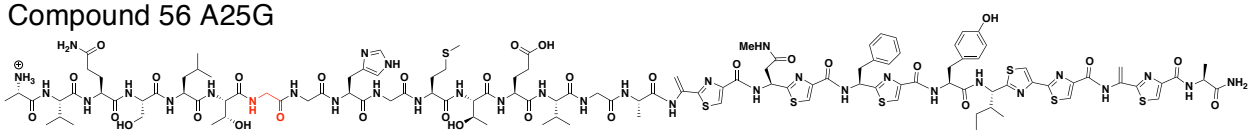


Exact Mass: 2755.0048
Molecular Weight: 2757.2095

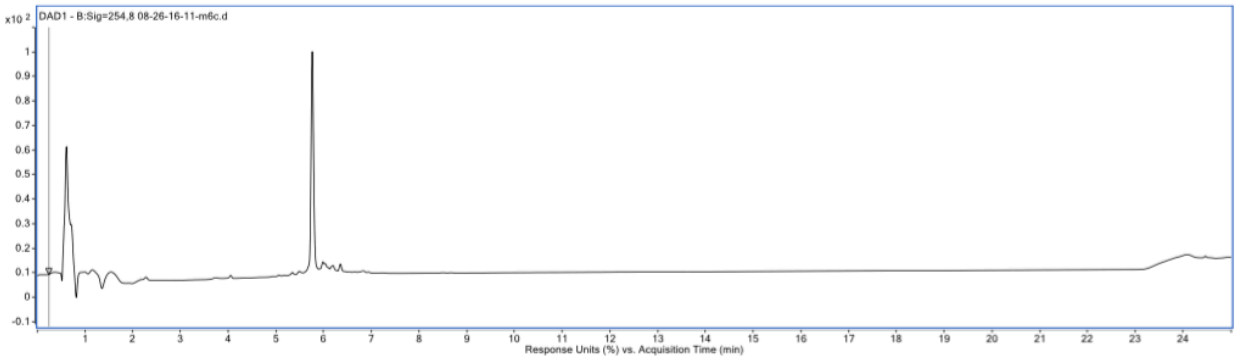


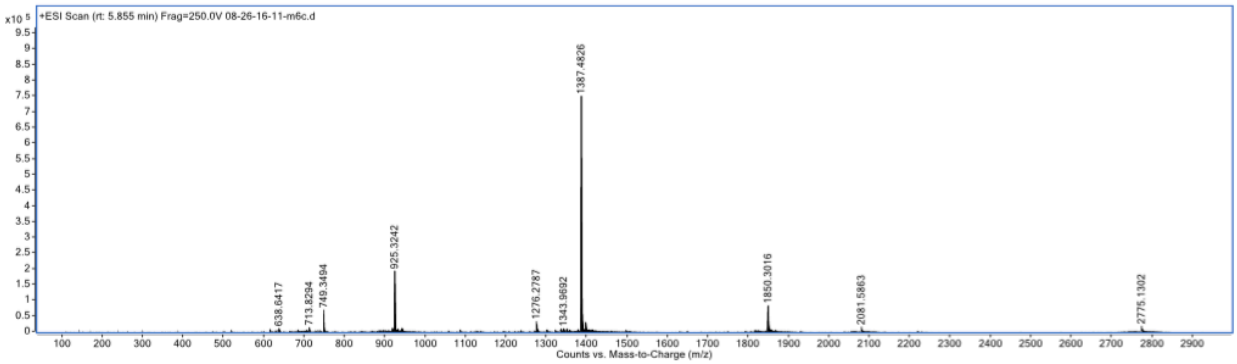


Compound 56 A25G

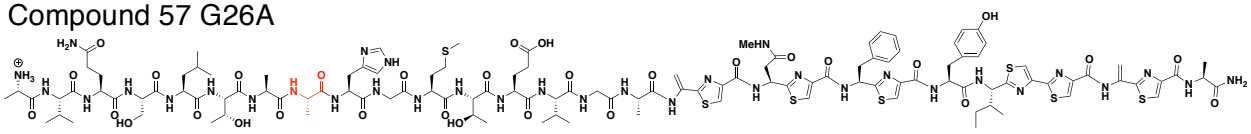


Exact Mass: 2771.9950
Molecular Weight: 2774.1965

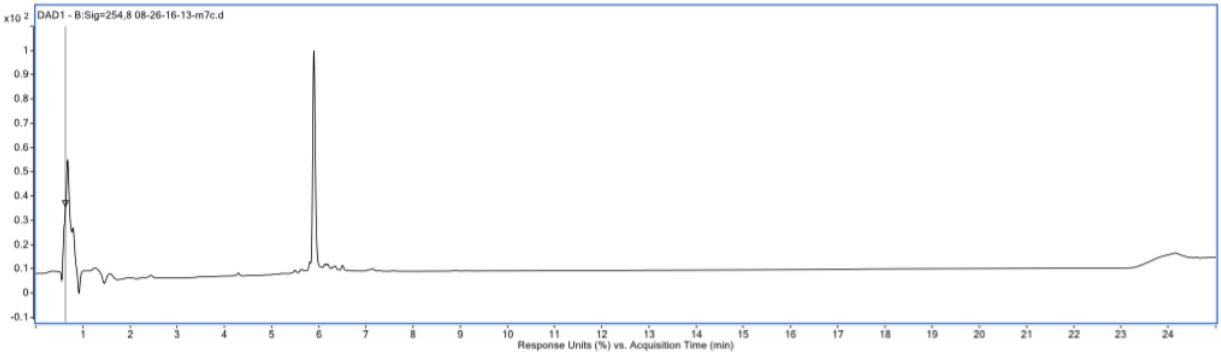


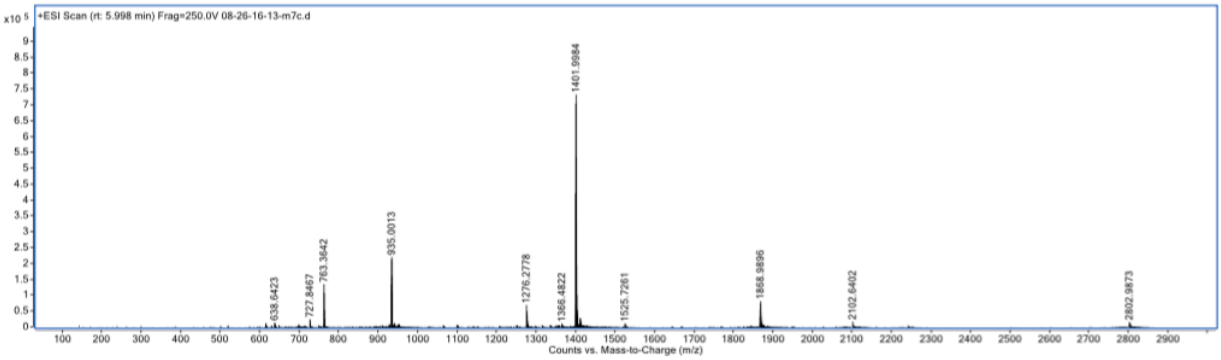


Compound 57 G26A

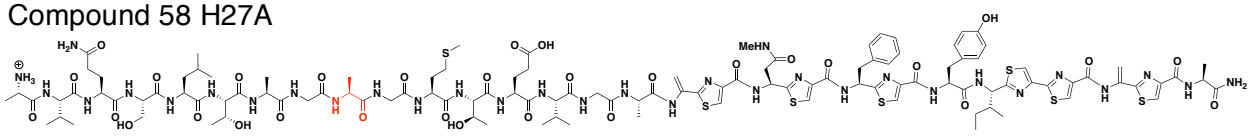


Exact Mass: 2800.0263
Molecular Weight: 2802.2505

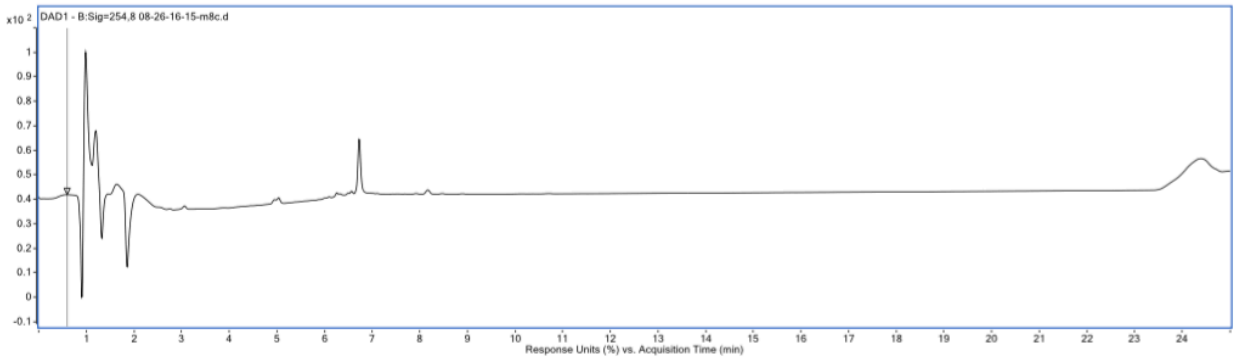


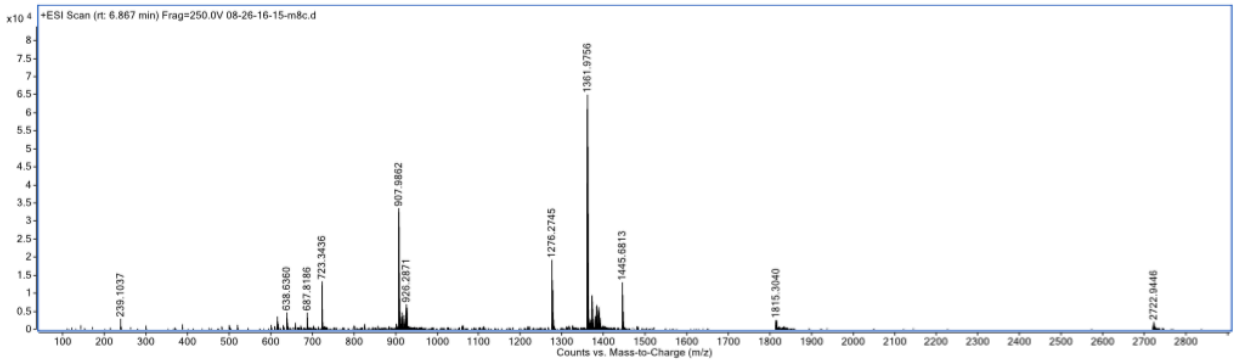


Compound 58 H27A

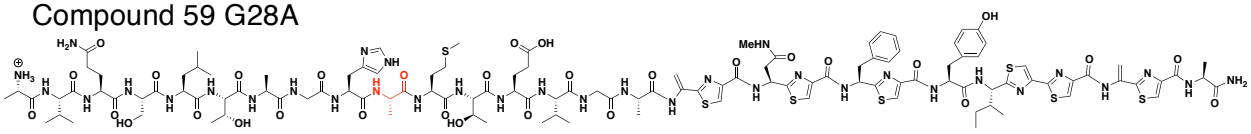


Exact Mass: 2719.9888
Molecular Weight: 2722.1605

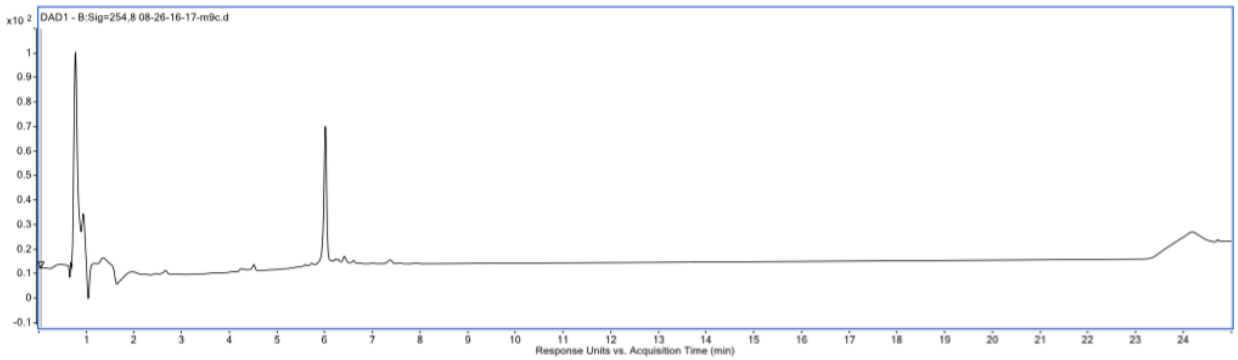


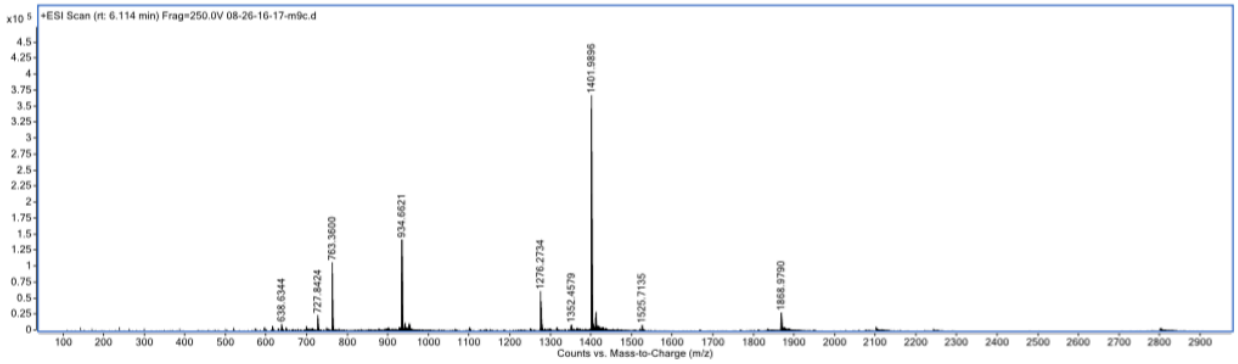


Compound 59 G28A

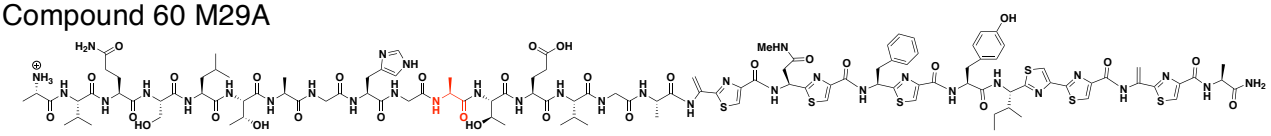


Exact Mass: 2800.0263
Molecular Weight: 2802.2505

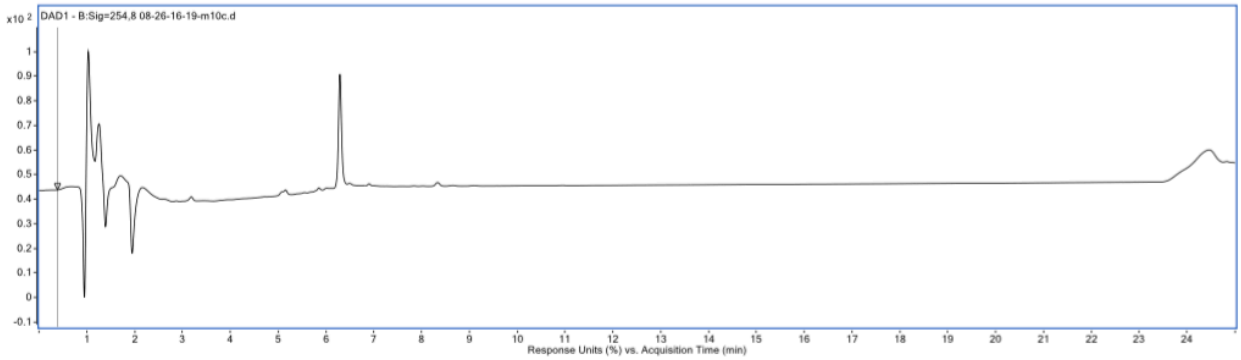


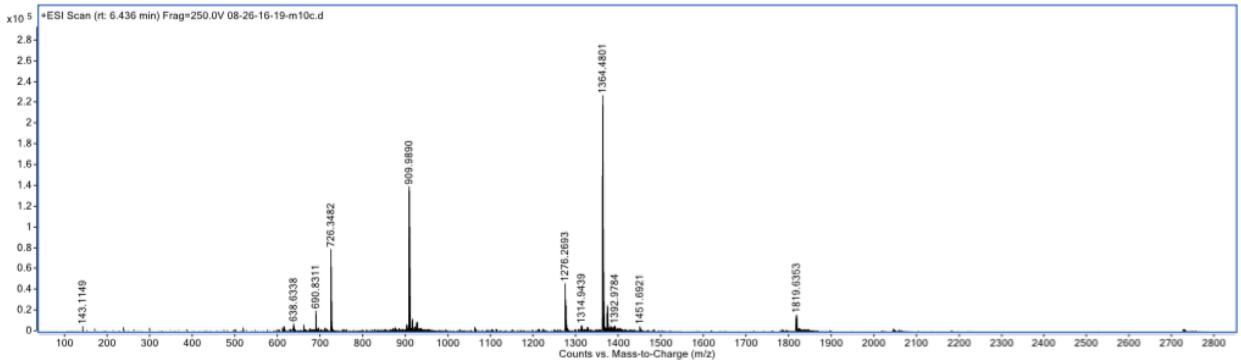


Compound 60 M29A

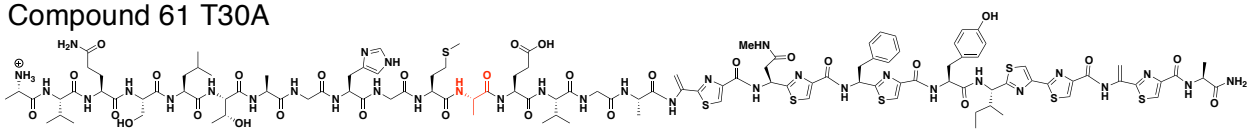


Exact Mass: 2726.0073
Molecular Weight: 2728.1095

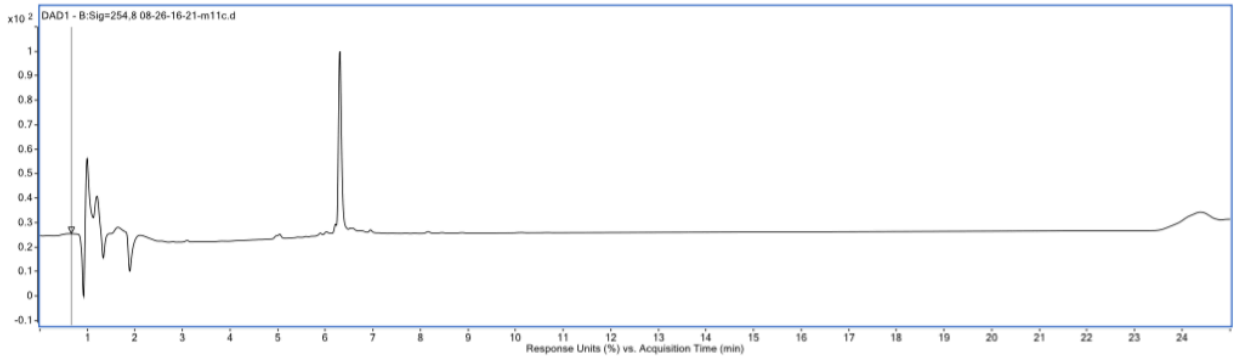


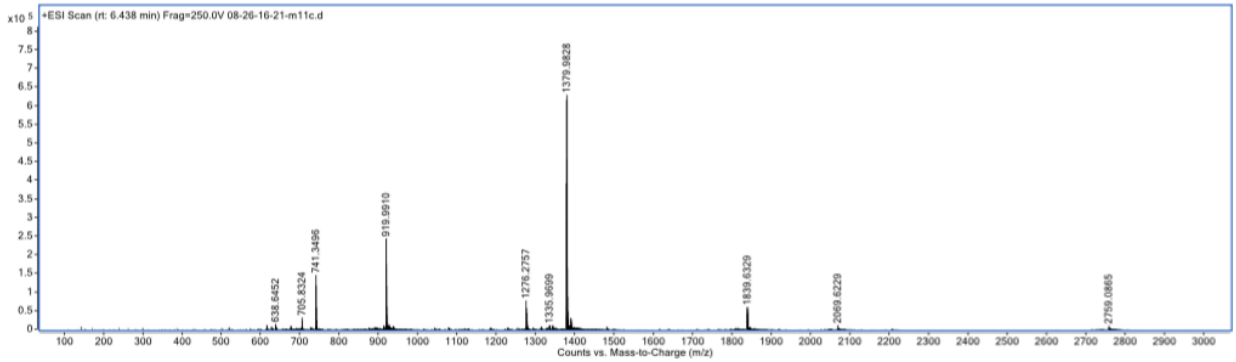


Compound 61 T30A

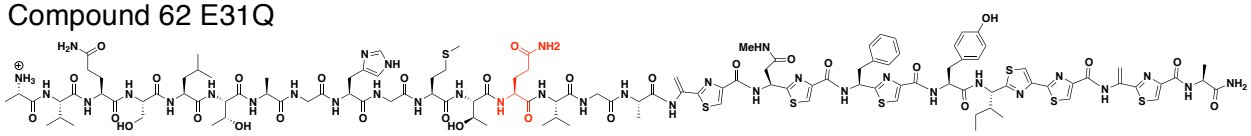


Exact Mass: 2756.0001
Molecular Weight: 2758.1975

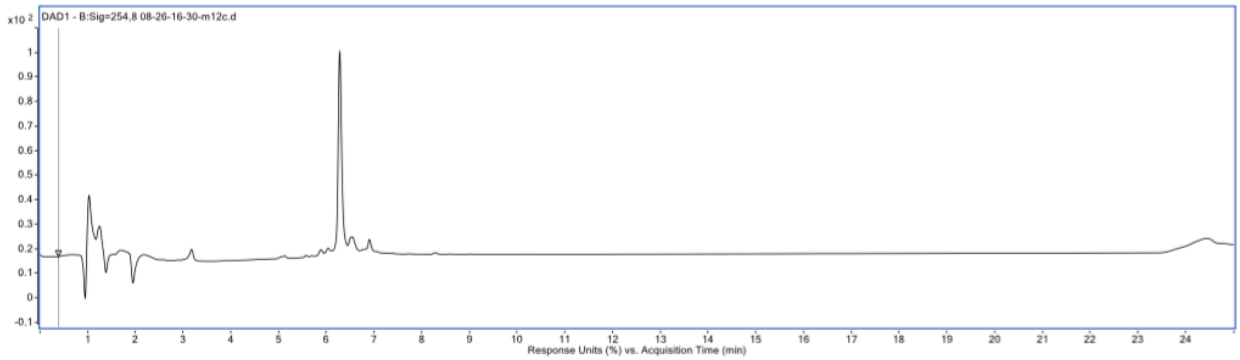


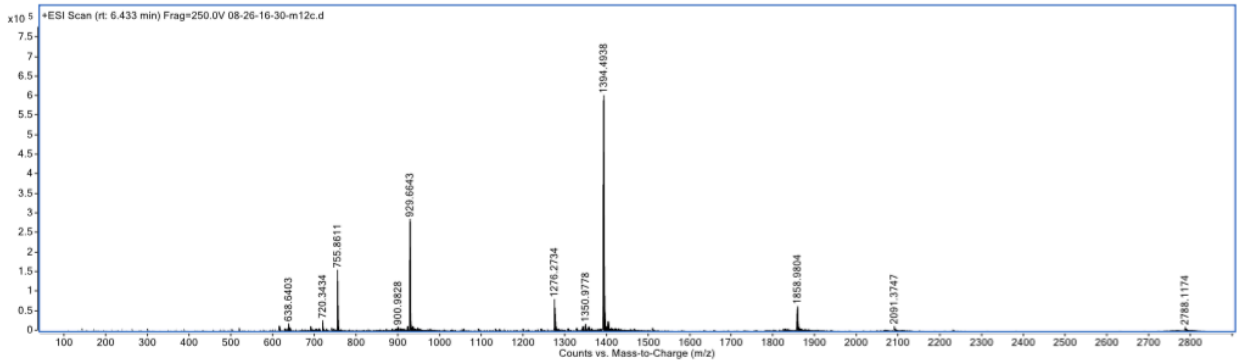


Compound 62 E31Q

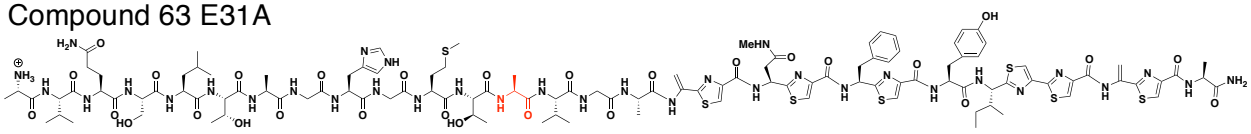


Exact Mass: 2785.0266
Molecular Weight: 2787.2395

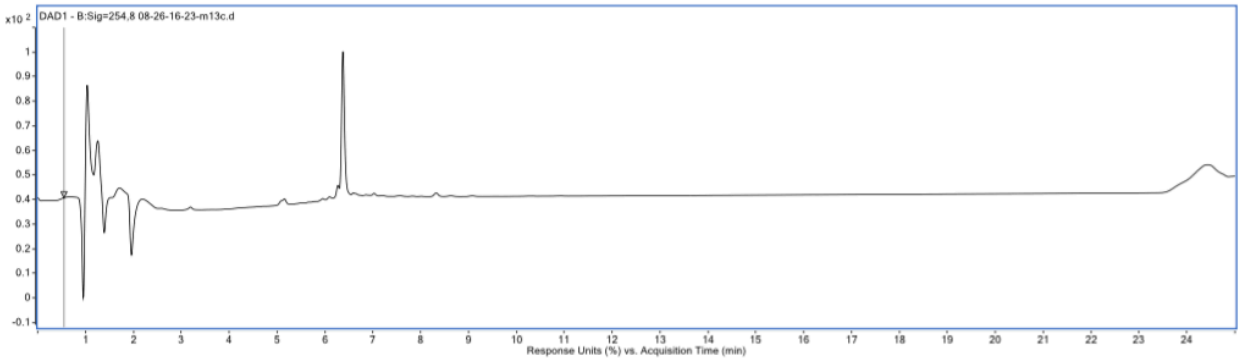


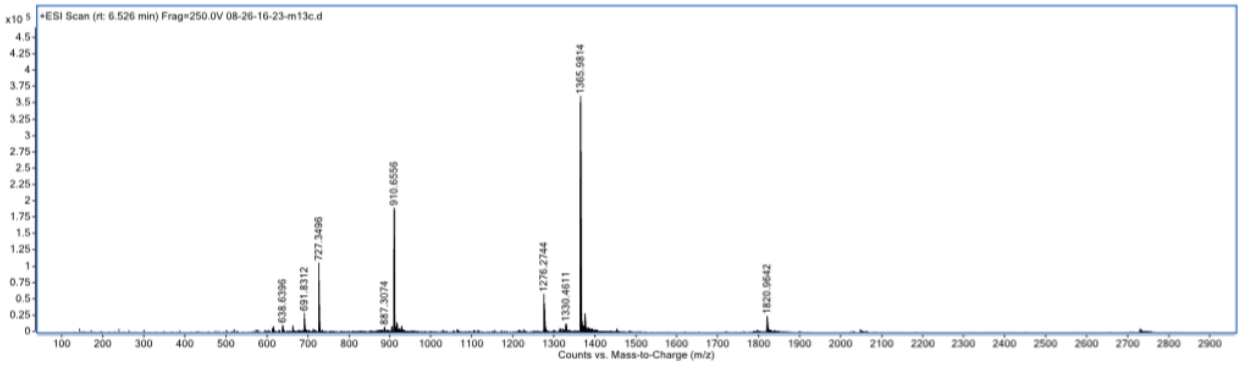


Compound 63 E31A

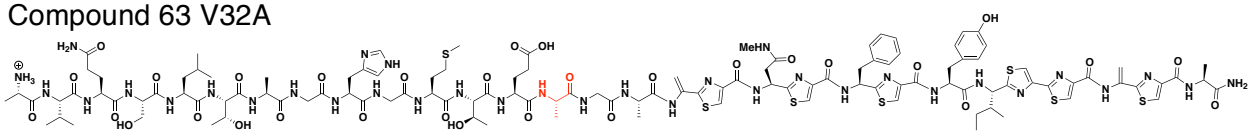


Exact Mass: 2728.0052
Molecular Weight: 2730.1875

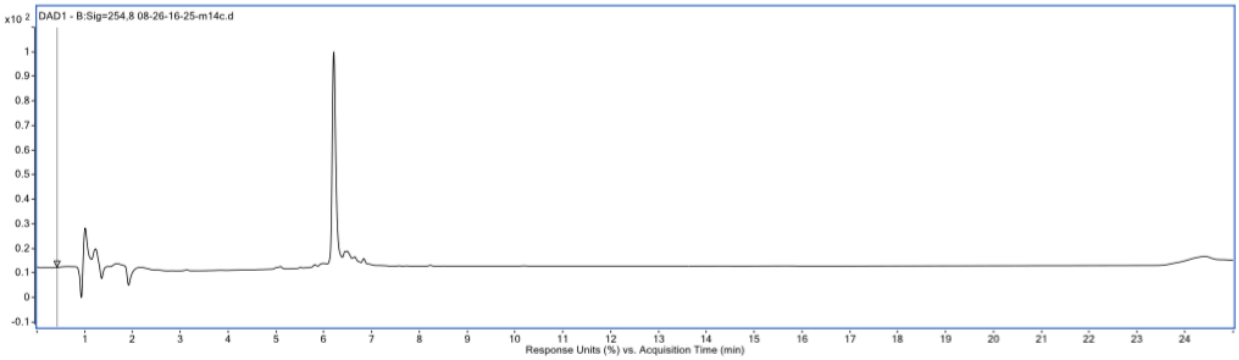


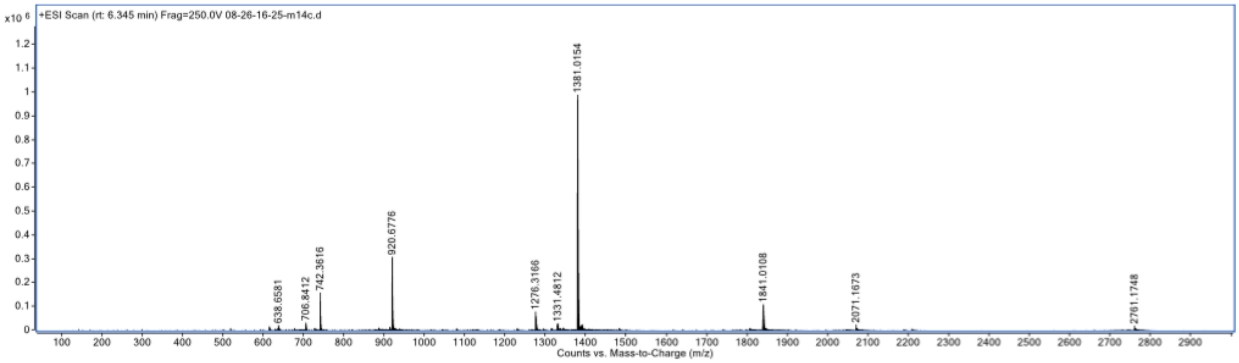


Compound 63 V32A

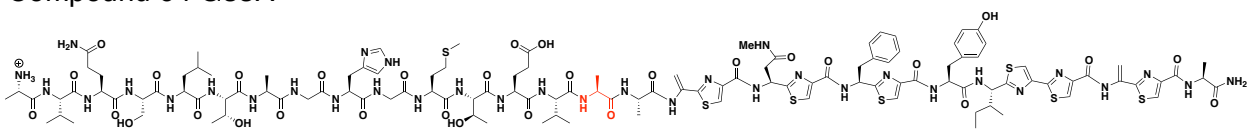


Exact Mass: 2757.9793
Molecular Weight: 2760.1695

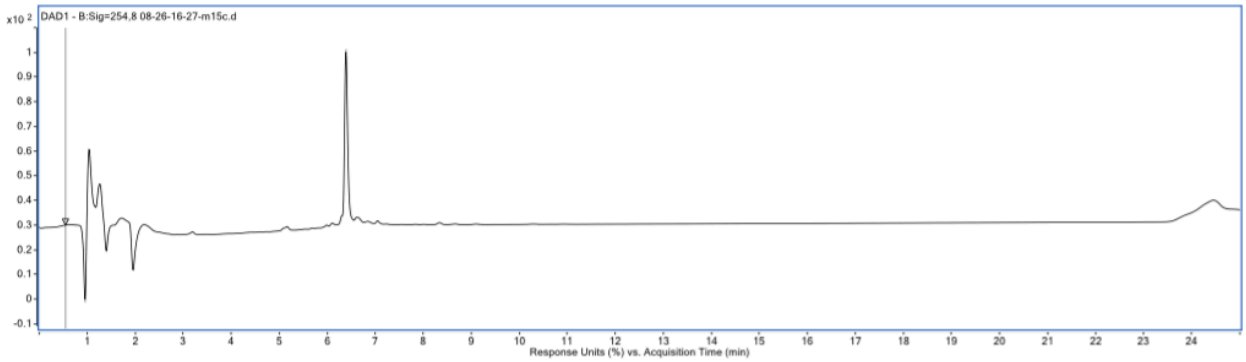


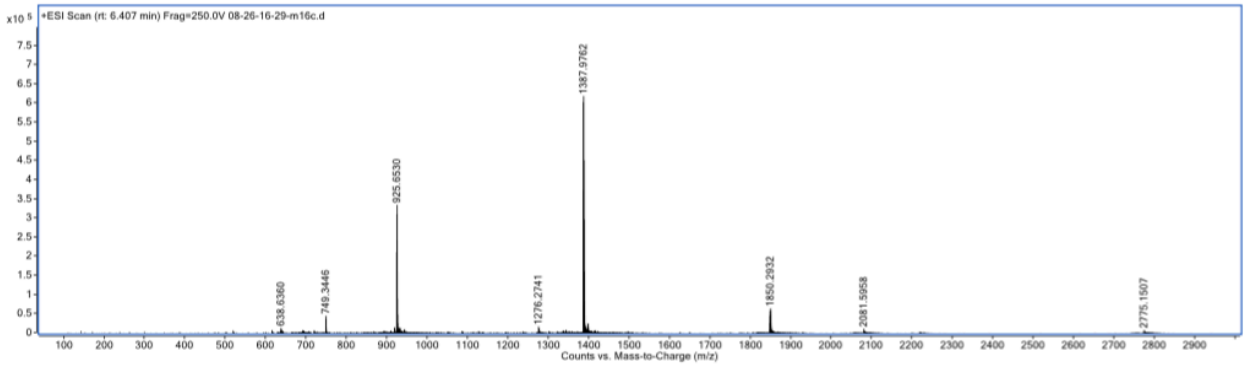


Compound 64 G33A

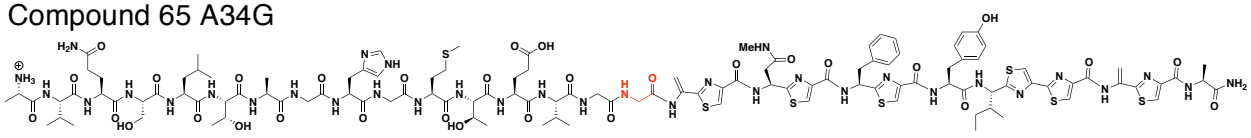


Exact Mass: 2800.0263
Molecular Weight: 2802.2505

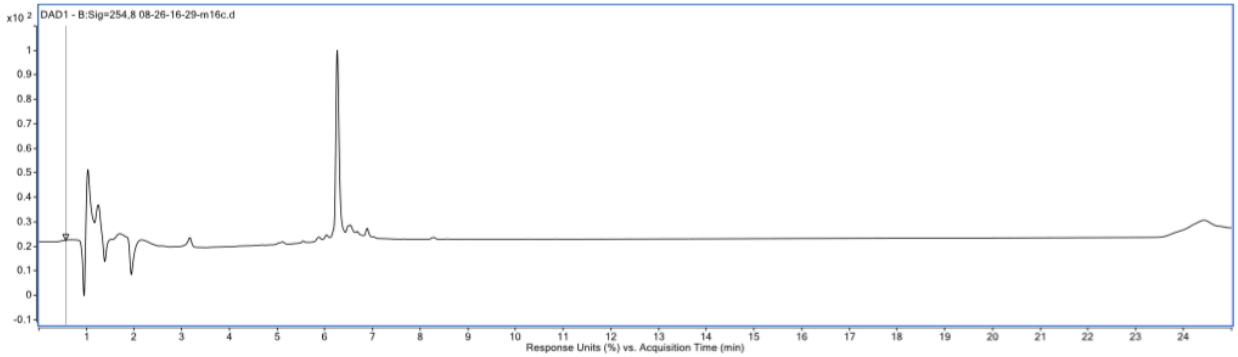


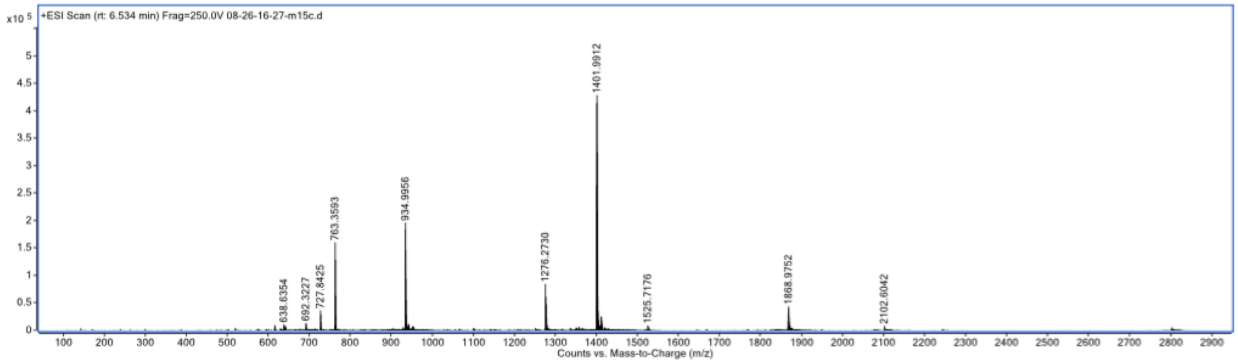


Compound 65 A34G

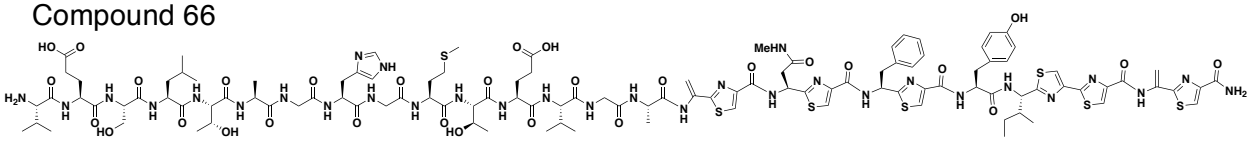


Exact Mass: 2771.9950
Molecular Weight: 2774.1965

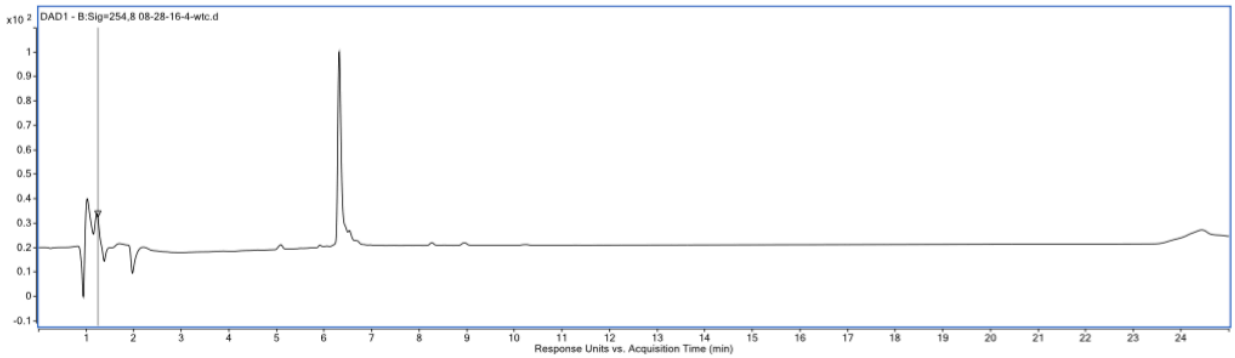


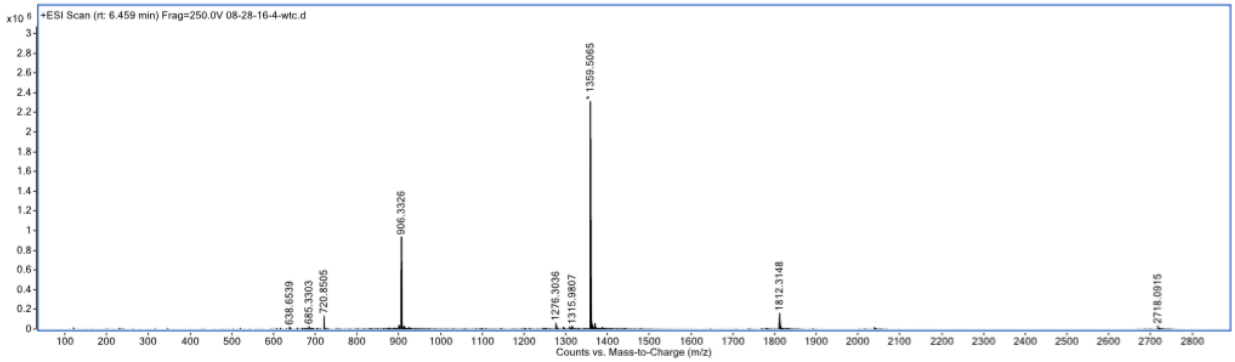


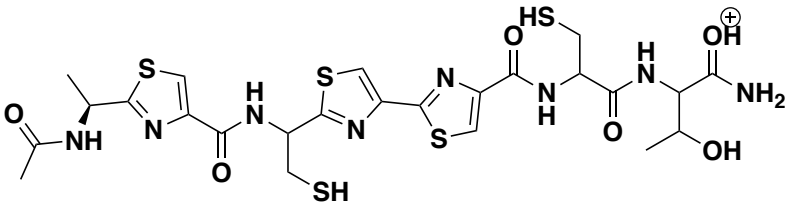
Compound 66



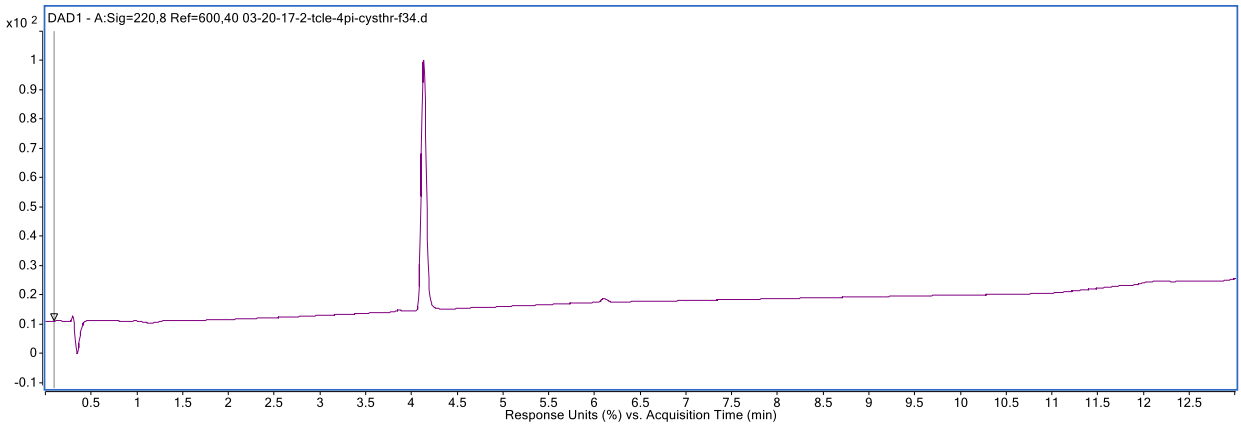
Exact Mass: 2643.9132
Molecular Weight: 2646.0420

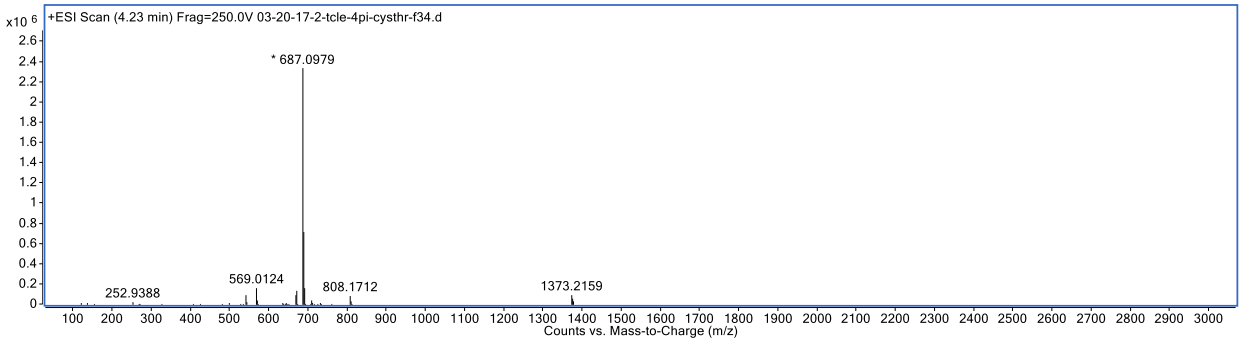


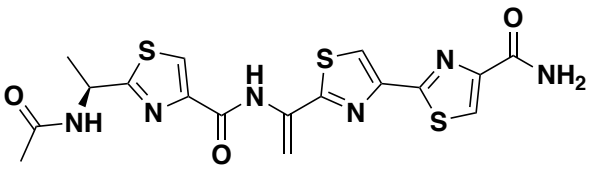




Chemical Formula: $C_{24}H_{31}N_8O_6S_5^+$ Compound 68
Exact Mass: 687.0965
Molecular Weight: 687.8615

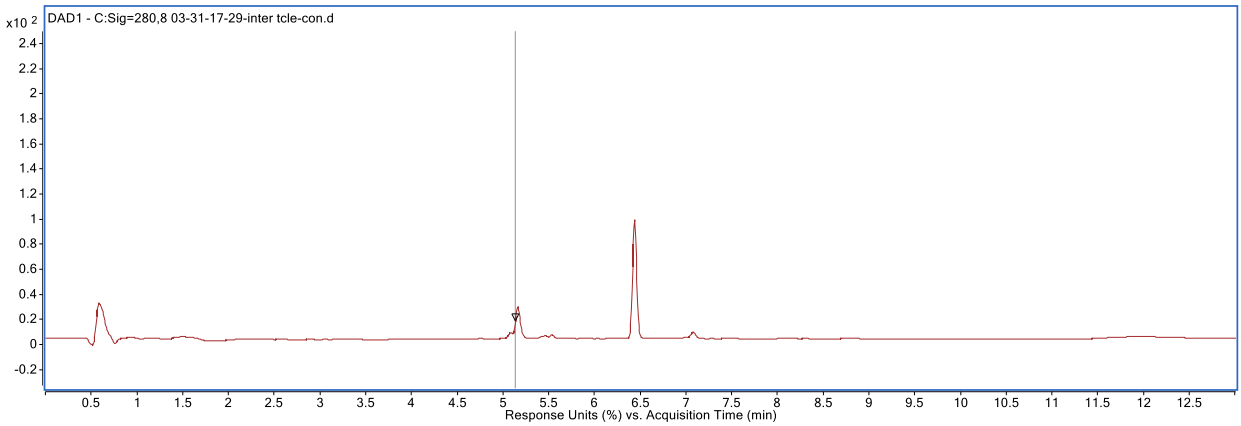

x10 2
DAD1 - A:Sig=220,8 Ref=600,40 03-20-17-2-tcle-4pi-cysthr-f34.d
Response Units (%) vs. Acquisition Time (min)

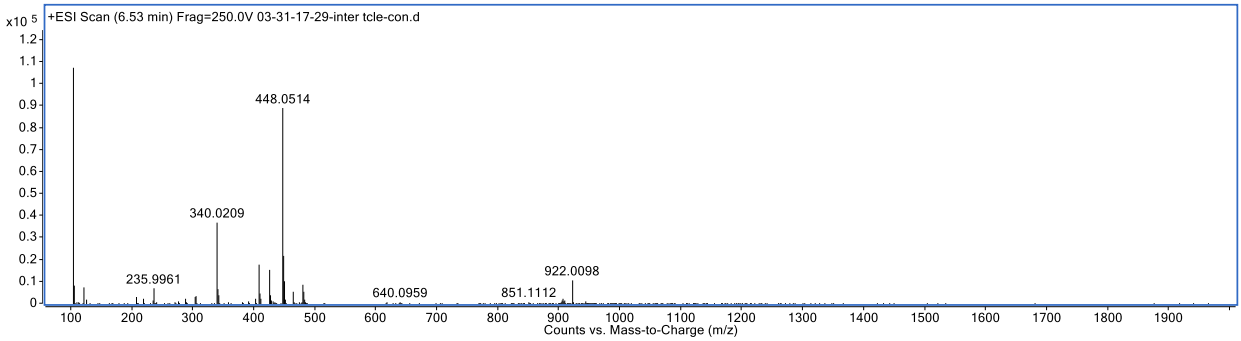

x10 6
+ESI Scan (4.23 min) Frag=250.0V 03-20-17-2-tcle-4pi-cysthr-f34.d
* 687.0979
569.0124
252.9388
808.1712
1373.2159
Counts vs. Mass-to-Charge (m/z)

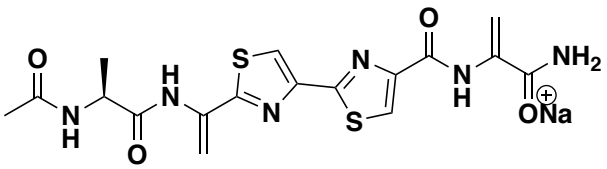


Chemical Formula: $C_{17}H_{16}N_6O_3S_3$
Exact Mass: 448.0446
Molecular Weight: 448.5340

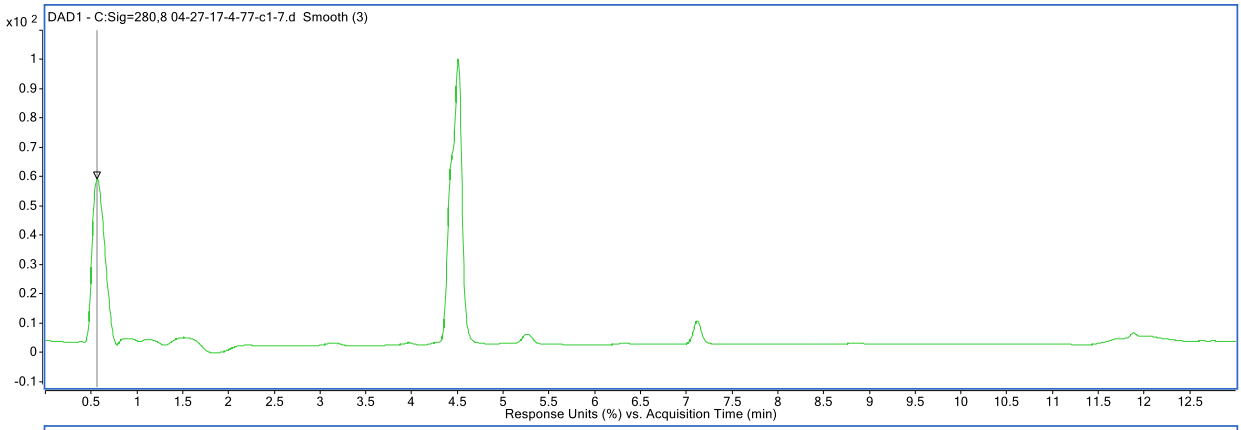
Compound 69

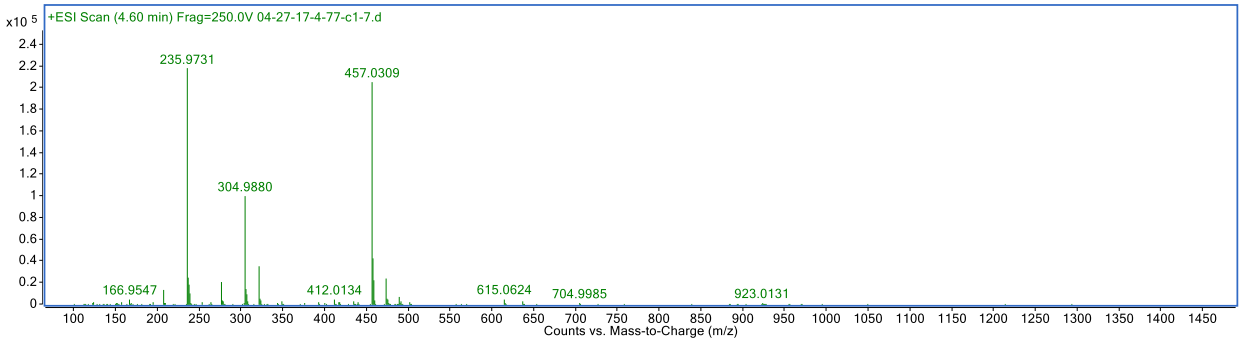


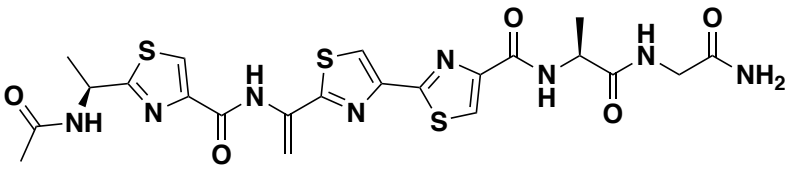




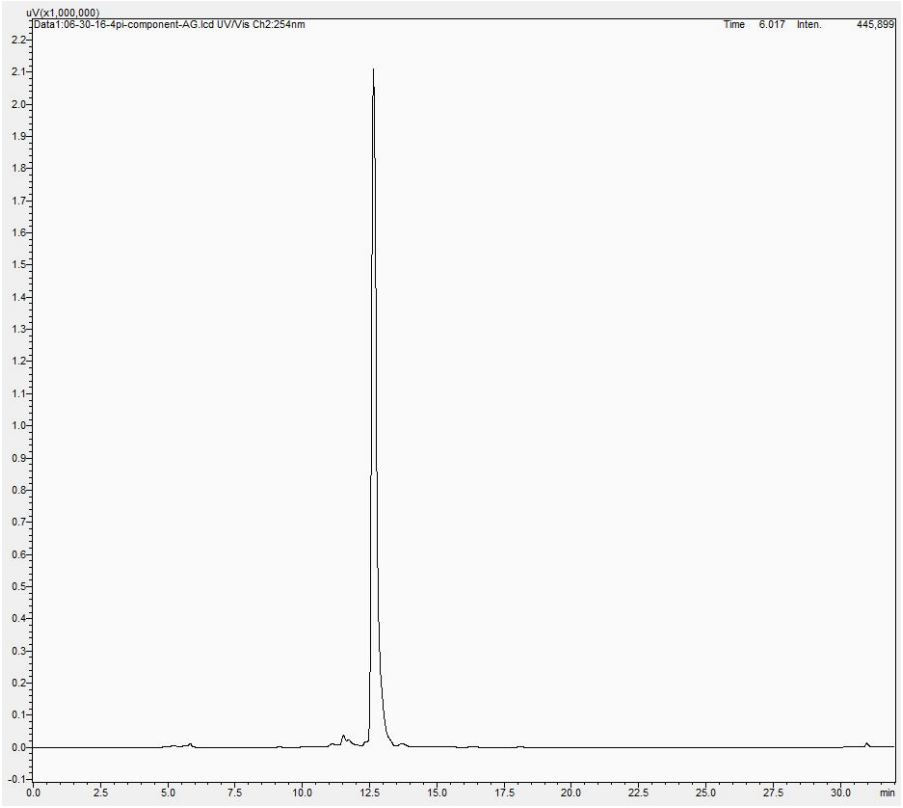
Chemical Formula: $C_{17}H_{18}N_6NaO_4S_2^+$ Compound 69
Exact Mass: 457.0723
Molecular Weight: 457.4782

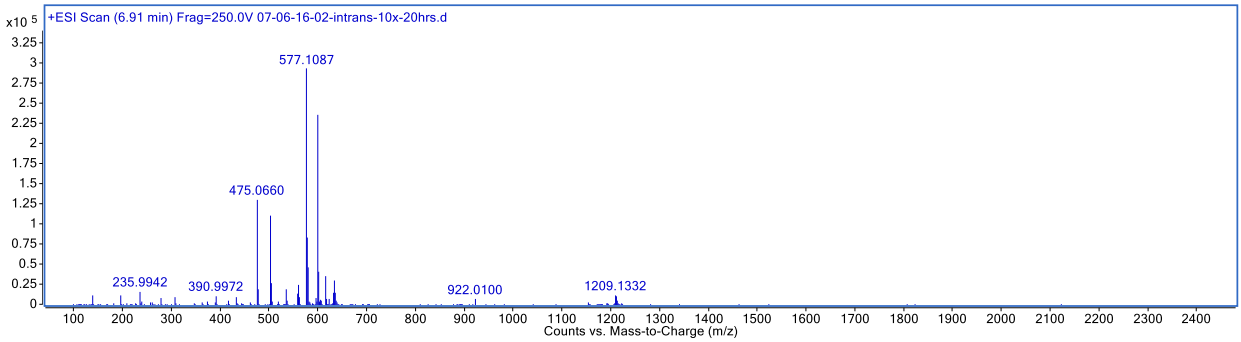






Chemical Formula: $C_{22}H_{24}N_8O_5S_3$ Compound 70
Exact Mass: 576.1032
Molecular Weight: 576.6650





XXII. References

1. Wever, W. J., Bogart, J. W., & Bowers, A. A. (2016). Identification of Pyridine Synthase Recognition Sequences Allows a Modular Solid-Phase Route to Thiopeptide Variants. *Journal of the American Chemical Society*, jacs.6b05389. http://doi.org/10.1021/jacs.6b05389
2. Zhang, Z., Hudson, G. A., Mahanta, N., Tietz, J. I., van der Donk, W. A., & Mitchell, D. A. (2016). Biosynthetic Timing and Substrate Specificity for the Thiopeptide Thiomuracin. *Journal of the American Chemical Society*, *138*(48), 15511–15514. http://doi.org/10.1021/jacs.6b08987