Supporting Informations

Synthetic and structural studies on Syringolin A and B reveal critical determinants of selectivity and potency of proteasome inhibition

Jérôme Clerc,[a] Michael Groll,[b] Damir J. Illich,[a] André S. Bachmann,[c,d] Robert Huber,[e,f,g] Barbara Schellenberg,[h] Robert Dudler,[h] and Markus Kaiser[a]*

[a] Chemical Genomics Centre der Max-Planck-Gesellschaft, Otto-Hahn-Str. 15, 44227 Dortmund (Germany),
Fax: (+49) 231-9742-6479; eMail: markus.kaiser@cgc.mpg.de

[b] Center for Integrated Protein Science at the Department Chemie, Lehrstuhl für Biochemie, Technische Universtität München, Lichtenbergstr. 4, 85747 Garching (Germany)

[c] Cancer Research Center of Hawaii, University of Hawaii at Manoa, 1236 Lauhala Street, Honolulu, Hawaii 96813 (USA)

[d] Department of Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawaii at Manoa, 651 Ilalo Street, Honolulu, Hawaii 96813 (USA)

[e] Max-Planck-Institut für Biochemie, Am Klopferspitz 18a, 82152 Martinsried (Germany)

[f] School of Biosciences, Cardiff University, Cardiff CF10 3US (United Kingdom)

[g] Zentrum für medizinische Biotechnologie, Universität Duisburg-Essen, 45117 Essen (Germany)

[h] Zurich-Basel Plant Science Center, Institute of Plant Biology, University of Zurich, Zollikerstr. 107, 8008 Zürich (Switzerland)

Index of Contens

A. Synthesis

A-1. General

Unless otherwise noted, all reagents and solvents were purchased from Acros, Fluka, Sigma, Aldrich or Merck and used without further purification. Dry solvents were purchased as anhydrous reagents from commercial suppliers.

For comparison with synthetic syringolin A and B, the natural products syringolin A and B were obtained by natural product isolation. Syringolin A was isolated from the bacterial strain *Pseudomonas syringae* pv. *syringae* B301D-R as described in H. Amrein *et al.*, *Mol. Plant Microbe Interact.* **2004**, *17*, 90-97. Syringolin B was isolated from the same strain as described in U. Wäspi *et al.*, *Microbiol. Res.* **1999**, *154*, 89-93. Glidobactin A was obtained from an unknown species of the order Burkholderiales as described in B. Schellenberg *et al.*, *Enivron. Microbiol.* **2007**, *9*, 1640-1650.

LC-MS analyses were performed on an HPLC system from Agilent (1200 series) with a Eclipse XDB-C18, 5 μm (column dimensions: 150×4.60 mm) column from Agilent and a Thermo Finnigan LCQ Advantage Max ESI-Spectrometer. Two gradients were used for the analyses using H_2O with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B) at a flow of 1 mL/min. Gradient 1: from 0 to 1 min: 75% solvent A/25% solvent B; from 1 to 10 min: from 75% solvent A/25% solvent B to 0% solvent A/100% solvent B; from 10 to 12 min: 0% solvent A/100% solvent B; from 12 to 15 min: from 0% solventA/100% solvent B to 90% solvent A/10% solvent B. Gradient 2: from 0 to 1 min: 90% solvent A/10% solvent B; from 1 to 10 min: from 90% solvent A/10% solvent B to 0% solvent A/100% solvent B; from 10 to 12 min: 0% solventA/100% solvent B; from 12 to 15 min: from 0% solvent A/100% solvent B to 90% solvent A/10% solvent B.

The chiral purity of Syringolin A and B was checked with the chiral column Chiralcel® OD-R (column dimensions: 250 × 4.60 mm) from Daicel/Chiral Technologies.

Preparative HPLC was conducted on a Varian HPLC system (Pro Star 215) with a VP 250/21 Nucleosil C18PPN-column from Macherey-Nagel. The corresponding gradient is described in the synthesis section.

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury 400 system (400 MHz for ^{1}H- and 100 MHz for ^{13}C-NMR), a Bruker Avance DRX 500 system (500 MHz for ^{1}H- and 125 MHz for ^{13}C-NMR) or a Varian Unity Inova 600 system (600 MHz for ^{1}H- and 150 MHz for ^{13}C-NMR). ^{1}H NMR spectra are reported in the following manner: chemical shifts calculated with reference to solvent standards based on tetramethylsilane, multiplicity (s, singulet; d, doublet; t, triplet; q, quartet; sept; septuplet, m, multiplet), coupling constant(s) in Hz, and number of protons. As especially the NH shifts of SylA and SylB are highly susceptible to residual trifluoroacetic acid, thereby limiting reproducibility of the corresponding NMR spectra, a small portion of trifluoracetic acid was added prior to spectra accumulation.

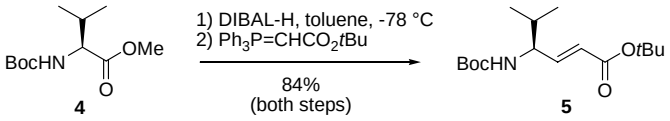
TLC analyses were performed with TLC aluminium sheets 20×20 cm silica gel 60 F_{254} from Merck.

HRMS measurements were performed on a LC-HR/ESI-FTMS machine from Thermo Electron Corporation.

The microwave-assisted reactions were conducted using a focused microwave unit (Discover® Reactor from CEM Corporation). The instrument consists of a continuous focused microwave power delivery system with operator-selectable power output from 0-300 W. In all experiments, the microwave power was held constant to ensure reproducibility. Reactions were performed in 10-mL glass vessels, which were sealed with a septum and locked into a pressure device, which controlled the pressure in the reaction vessel (maximum 10 bars). The specified reaction time corresponds to the total irradiation time. The temperature was monitored by an infrared temperature sensor positioned below the reaction vessel. The indicated temperature correlates with the maximum temperature reached during each experiment.

A-2. Synthesis of Syringolin B (SylB, 2)

Synthesis of *tert*-butyl (*S*,E)-1-(tert-butoxycarbonyl)-4-methylpent-1-en-3-ylcarbamate (5)

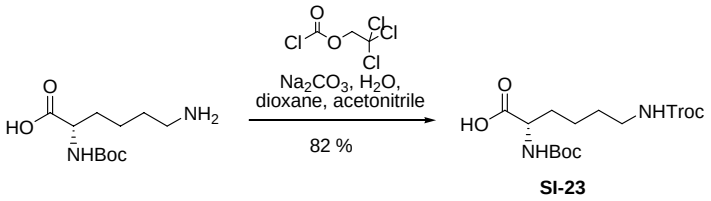


N-(*tert*-Butoxycarbonyl)-(L)-valine methyl ester **4** (500 mg, 2.16 mmol, 1 eq.) was dissolved in toluene (22 mL) under argon in a 100 mL flame-dried flask. The solution was cooled to -78 °C and a 1 M solution of DIBAL-H in toluene (4.4 mL, 4.32 mmol, 2 eq.) was slowly added over 2 hours. After further 2 hours of stirring, the mixture was quenched with a 1.2 M solution of potassium sodium tartrate (25 mL) and vigorously stirred at room temperature for further 2 hours. The resulting mixture was extracted with dichloromethane and the organic layers were dried with Na_2SO_4. The solution was filtered and concentrated to give *N*-(*tert*-butoxycarbonyl)-(L)-valinal which was directly used in the next step without further purification.

Crude *N*-(*tert*-butoxycarbonyl)-(L)-valinal was dissolved in dichloromethane (22 mL) and (*tert*-butoxycarbonylmethylene)triphenylphosphorane (1.21 g, 3.24 mmol, 1.5 eq.) was added in one portion. After 12 hours of stirring the mixture was concentrated and purified by flash column chromatography (10% ethyl acetate in cyclohexane) to afford 466 mg (1.81 mmol, 84%) of **5** as colorless crystals.

TLC (15% ethyl acetate in cyclohexane) Rf 0.49; HPLC (gradient 1) t_R 10.77 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.73 (dd, J = 15.6, 5.6 Hz, 1 H), 5.82 (dd, J = 15.6, 1.6 Hz, 1 H), 4.55 (br s, 1 H), 4.15 (br s, 1 H), 1.79-1.88 (m, 1 H), 1.47 (s, 9 H), 1.43 (s, 9 H), 0.92 (d, J = 6.8 Hz, 3 H), 0.89 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.66, 155.45, 146.10, 123.30, 80.50, 79.65, 56.66, 32.40, 28.44, 28.20, 18.94, 18.05; HRMS (ESI) *m/z* calcd for $C_{16}H_{29}O_4NH^+$ $[M + H]^+$ 300.2169, found 300.2171.

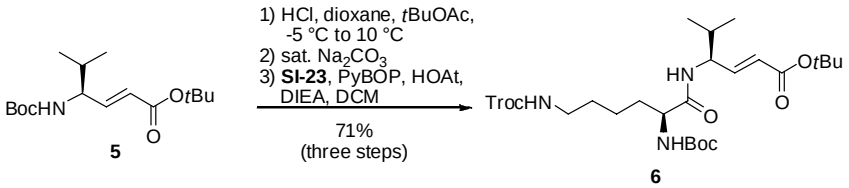
Synthesis of Boc-Lys(Troc)-OH (SI-23)



Boc-Lys-OH (3.00 g, 12.18 mmol, 1 eq.) and Na_2CO_3 (1.30 g, 12.18 mmol, 1 eq.) were dissolved in water/dioxane/acetonitrile (19:14:12, 450 mL) in a 1 L flask. The solution was cooled to 0 °C and a solution of 2,2,2-trichloroethyl chloroformate (1.8 mL, 13.40 mmol, 1.1 eq.) in dioxane (160 mL) was slowly added. The resulting mixture was stirred overnight at room temperature, concentrated to dryness and redissolved in a saturated aqueous solution of ammonium chloride. Crude **SI-23** was extracted from the aqueous phase with dichloromethane (3×200 mL), dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by flash column chromatography (dichloromethane/methanol/acetic acid = 38:1:1) to yield 4.19 g (9.91 mmol, 82%) pure **SI-23** as a colorless solid.

TLC (methanol/acetic acid/dichloromethane = 1:1:38) R_f 0.30; HPLC (gradient 2) t_R 9.11 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.10 (br s, 1 H), 4.72 (d, J = 1.6 Hz, 2 H), 4.31 (br s, 1 H), 3.26 (t, J = 6.4 Hz, 1 H), 3.24 (t, J = 6.8 Hz, 1 H), 1.80-1.93 (m, 1 H), 1.66-1.78 (m, 1 H), 1.53-1.64 (m, 2 H), 1.45-1.50 (m, 2 H), 1.45 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.24, 155.83, 154.92, 95.66, 80.27, 74.51, 53.13, 40.80, 31.97, 29.07, 28.32, 22.32; HRMS (ESI) *m/z* calcd for $C_{14}H_{23}O_6N_2Cl_3H^+$ $[M + H]^+$ 421.0695, found 421.0695.

Synthesis of 6



5 (1.07 g, 3.57 mmol, 1 eq.) was dissolved under argon in *tert*-butyl acetate (12 mL, dried over 4Å molecular sieves) in a 100 mL flame-dried flask. The resulting solution was cooled to -5 °C, a 4 M solution of HCl in dioxane (12 mL) was slowly added and the resulting mixture was stirred overnight at 10 °C. Evaporation to dryness provided the crude hydrogenchloride salt which was subsequently recrystallized in cyclohexane. The crystals were filtered and washed with small portions of cyclohexane, redissolved in saturated Na_2CO_3 solution and the free amine was extracted from the aqueous phase with dichloromethane. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness to give the pure deprotected **5** in 596 mg (2.99 mmol, 84%) yield.

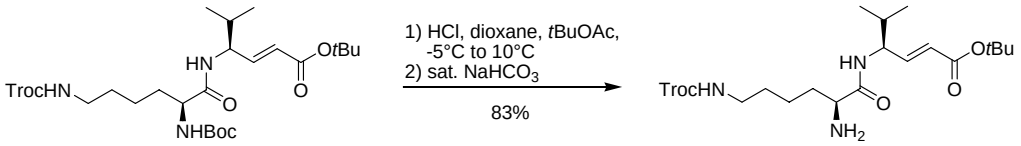
TLC (7% methanol in dichloromethane) R_f 0.33; HPLC (gradient 2) t_R 5.86 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.79 (dd, J = 15.6, 6.8 Hz, 1 H), 5.82 (dd, J = 15.6, 1.6 Hz, 1 H), 3.22 (ddd, J = 6.8, 5.6, 1.2 Hz, 1 H), 1.66-1.75 (m, 1 H), 1.46 (s, 9 H), 1.30 (br s, 2 H), 0.91 (d, J = 6.8 Hz, 3 H), 0.90 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.02, 150.00, 122.52, 80.31, 58.46, 33.69, 28.21, 18.85, 18.28; HRMS (ESI) *m/z* calcd for $C_{11}H_{21}O_2NH^+$ $[M + H]^+$ 200.1645, found 200.1644.

This intermediate (484 mg, 2.43 mmol, 1 eq.) was dissolved in dichloromethane (2 mL) in a 25 mL flask and cooled to 0 °C. A solution of **SI-23** (1.74 g, 4.13 mmol, 1.7 eq.), PyBop (3.80 g, 7.30 mmol, 3 eq.), HOAt (994 mg, 7.30 mmol, 3 eq.) and *N*,*N*-diisopropylethylamine (2.65 mL, 14.60 mmol, 6 eq.) in dichloromethane (8 mL) was added and stirred overnight at room temperature. After evaporation to dryness, the crude product was purified by flash column chromatography (30% ethyl acetate in cyclohexane) to yield 1.25 g (2.07 mmol, 85%) of **6** as a colorless solid.

TLC (30% ethyl acetate in cyclohexane) R_f 0.23; HPLC (gradient 2) t_R 11.35 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.72 (dd, J = 15.6, 5.6 Hz, 1 H), 6.66 (br s, 1 H), 5.78 (dd, J = 15.6, 1.6 Hz, 1 H), 5.46 (br s, 1 H), 5.32 (br s, 1 H), 4.70 (d, J = 12.0 Hz, 1 H), 4.65 (d, J = 12.0 Hz, 1 H), 4.42-4.48 (m, 1 H), 4.03 (br s, 1 H), 3.19 (t, J = 6.8 Hz, 1 H), 3.18 (t, J = 6.8 Hz, 1 H), 1.76-1.87 (m, 2 H), 1.57-1.66 (m, 1 H), 1.49-1.57 (m, 2 H), 1.43 (s, 9 H), 1.40 (s, 9 H), 0.87 (d, J = 6.8 Hz, 3 H), 0.85 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.86, 165.52, 156.06, 154.85, 145.45, 123.39, 95.72, 80.58, 80.19, 74.43, 54.99, 54.55, 40.61, 32.12, 31.08, 29.32, 28.34, 28.12, 22.67, 18.97, 17.98; HRMS (ESI)

m/z calcd for $C_{25}H_{42}O_7N_3{}^{35}Cl_3H^+$ $[M + H]^+$ 602.2161, found 602.2160 and calcd for $C_{25}H_{42}O_7N_3{}^{37}Cl_3H^+$ $[M + H]^+$ 604.2132, found 604.2130.

Synthesis of SI-24

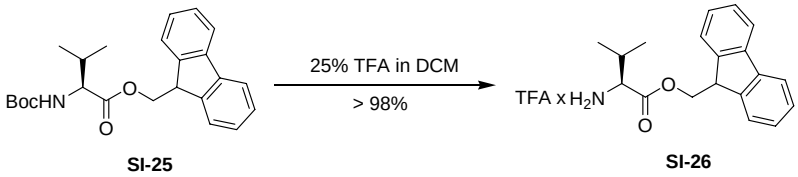


6 **SI-24**

3 (935 mg, 1.55 mmol, 1 eq.) was dissolved under argon in *tert*-butyl acetate (12 mL, dried over 4Å molecular sieves) in a 100 mL flame-dried flask and cooled to -5 °C. A solution of 4 M HCl in dioxane (12 mL) was slowly added and stirred overnight at 10 °C. The resulting mixture was evaporated to dryness and recrystallized in cyclohexane. The crystals were filtered, washed with small portions of cyclohexane and redissolved in saturated aqueous Na_2CO_3 solution. The free amine was extracted from the aqueous phase with ethyl acetate (3×50 mL), dried over Na_2SO_4, filtered and concentrated to dryness to give 628 mg (1.29 mmol, 83%) of **SI-24** as a colorless oil.

TLC (7% methanol in dichloromethane) R_f 0.33; HPLC (gradient 2) t_R 7.56 min; ^1H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, J = 9.6 Hz, 1 H), 6.75 (dd, J = 15.6, 5.6 Hz, 1 H), 5.77 (dd, J = 15.6, 1.6 Hz, 1 H), 5.20 (br s, 1 H), 4.70 (s, 2 H), 4.42-4.48 (m, 1 H), 3.41 (dd, J = 8.0, 4.4 Hz, 1 H), 3.24 (t, J = 6.8 Hz, 1 H), 3.23 (t, J = 6.4 Hz, 1 H), 1.80-1.92 (m, 4 H), 1.52-1.63 (m, 3 H), 1.46 (s, 9 H), 1.39-1.47 (m, 2 H), 0.92 (d, J = 6.8 Hz, 3 H), 0.91 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.38, 165.64, 154.76, 145.57, 123.48, 95.77, 80.63, 74.52, 55.10, 54.68, 40.92, 34.61, 32.16, 29.59, 28.19, 22.80, 19.08, 18.16; HRMS (ESI) *m/z* calcd for $C_{20}H_{34}O_5N_3Cl_3H^+$ $[M + H]^+$ 502.1637, found 502.1633.

Synthesis of SI-26

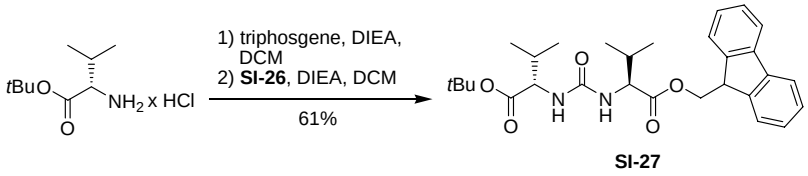


SI-25 **SI-26**

SI-25 (3.96 g, 10.0 mmol, 1 eq., prepared according to Henkel B *et al* (1997) *Liebigs Annalen/Receuil* 10:2161-2168.) was dissolved in dichloromethane (75 mL) in a 250 mL flask and trifluoroacetic acid (25 mL) was slowly added. The mixture was stirred for 30 minutes, followed by evaporation to dryness. Addition of toluene and re-evaporation to dryness yielded 4.10 g **(**10.0 mmol, >98%) of **SI-26** as a colorless solid.

TLC (60% ethyl acetate in cyclohexane) R_f 0.17; HPLC (gradient 2) t_R 7.09 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 6.0 Hz, 1 H), 7.71 (d, J = 5.6 Hz, 1 H), 7.51 (dd, J = 8.0, 0.8 Hz, 1 H), 7.49 (dd, J = 7.2, 0.8 Hz, 1 H), 7.38 (t, J = 7.6 Hz, 1 H), 7.35 (t, J = 8.0 Hz, 1 H), 7.28 (td, J = 7.6, 1.2 Hz, 1 H), 7.26 (td, J = 7.6, 1.2 Hz, 1 H), 4.61 (dd, J = 10.8, 6.0 Hz, 1 H), 4.51 (dd, J = 10.8, 5.6 Hz, 1 H), 4.16 (dd, J = 6.0, 5.6 Hz, 1 H), 3.76 (d, J = 4.0 Hz, 1 H), 2.08-2.17 (m, 1 H), 0.87 (d, J = 6.8 Hz , 3 H), 0.79 (d, J = 6.8 Hz , 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.22, 143.21, 142.86, 141.52, 141.40, 128.12, 128.06, 127.38, 127.34, 124.73, 120.16, 67.72, 58.48, 46.70, 29.66, 17.47, 17.46; HRMS (ESI) *m/z* calcd for $C_{19}H_{21}O_2NH^+$ $[M + H]^+$ 296.1645, found 296.1646.

Synthesis of SI-27



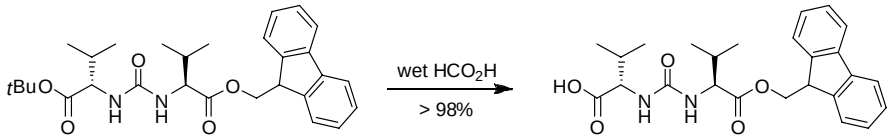
SI-27

Triphosgene (110 mg, 0.37 mmol, 1.11 eq.) was dissolved under argon in dichloromethane (2 mL) in a 25 mL flame-dried flask and a solution of commercially available valine *tert*-butylester hydrochloride (210 mg, 1.00 mmol, 1.00 eq.) and *N*,*N*-

diisopropylethylamine (385 µL, 2.20 mmol, 2.20 eq.) in dichloromethane (3.5 mL) was added over 30 minutes. The mixture was stirred for further five minutes, then a mixture of **SI-26** (410 mg, 1.00 mmol, 1.00 eq.) and *N*,*N*-diisopropylethylamine (385 µL, 2.20 mmol, 2.20 eq.) in dichloromethane (2.0 mL) was added in one portion. The resulting mixture was stirred for 10 minutes, concentrated to dryness, the residue was redissolved in ethyl acetate and successively washed with a 10% aq. $KHSO_4$ solution, a 5% aq. $NaHCO_3$ solution and with brine. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. The resulting crude product was purified by flash column chromatography (15% ethyl acetate in cyclohexane) to yield 299 mg (0.61 mmol, 61%) of **SI-27** as colorless crystals.

TLC (15% ethyl acetate in cyclohexane) R_f 0.16; HPLC (gradient 1) t_R 11.55 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 7.2 Hz, 1 H), 7.74 (d, J = 7.2 Hz, 1 H), 7.61 (dd, J = 7.2, 0.8 Hz, 1 H), 7.59 (dd, J = 7.2, 0.8 Hz, 1 H), 7.39 (t, J = 7.2 Hz, 1 H), 7.37 (t, J = 7.2 Hz, 1 H), 7.30 (td, J = 7.2, 1.2 Hz, 1 H), 7.28 (td, J = 7.2, 1.2 Hz, 1 H), 5.40 (br s, 2 H), 4.47-4.53 (m, 3 H), 4.31-4.35 (m, 1 H), 4.20 (dd, J = 6.8, 6.4 Hz, 1H), 1.99-2.14 (m, 2 H), 1.46 (s, 9 H), 0.92 (d, J = 6.8 Hz, 3 H), 0.90 (d, J = 6.8 Hz, 3 H), 0.84 (d, J = 6.8 Hz, 3 H), 0.76 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.65, 172.54, 157.53, 143.71, 143.53, 141.39, 141.35, 127.85, 127.22, 127.19, 125.09, 125.07, 120.02, 120.00, 81.72, 66.94, 58.37, 58.14, 46.86, 31.69, 31.47, 28.13, 19.11, 19.05, 17.63; HRMS (ESI) *m/z* calcd for $C_{29}H_{38}O_5N_2H^+$ $[M + H]^+$ 495.2854, found 495.2846.

Synthesis of 9



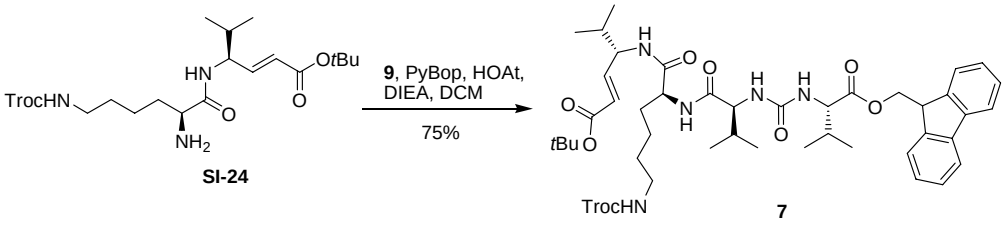
SI-27 **9**

SI-27 (299 mg, 0.61 mmol) was dissolved in formic acid (4 mL) in a 25 mL flask. Some drops of water were added and the mixture was stirred overnight. Evaporation to dryness,

addition of toluene and re-evaporation yielded 262 mg (0.60 mmol, >98%) of **9** as a colorless solid.

TLC (methanol/acetic acid/dichloromethane = 1:1:38) R_f 0.26; HPLC (gradient 2) t_R 9.99 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.00 (br s, 1 H), 7.73 (d, J = 7.2 Hz, 1 H), 7.72 (d, J = 7.6 Hz, 1 H), 7.59 (d, J = 7.2 Hz, 1 H), 7.57 (d, J = 7.2 Hz, 1 H), 7.38 (t, J = 7.2 Hz, 1 H), 7.36 (t, J = 7.2 Hz, 1 H), 7.29 (td, J = 7.2, 1.2 Hz, 1 H), 7.27 (td, J = 7.2, 1.2 Hz, 1 H), 5.94 (br s, 2 H), 4.50 (dd, J = 10.8, 6.8 Hz, 1 H), 4.45-4.48 (m, 1 H), 4.44 (dd, J = 10.8, 6.4 Hz, 1 H), 4.38-4.42 (m, 1 H), 4.19 (dd, J = 6.8, 6.4 Hz, 1 H), 2.12-2.21 (m, 1 H), 1.98-2.06 (m, 1 H), 0.95 (d, J = 7.2 Hz, 3 H), 0.89 (d, J = 7.2 Hz, 3 H), 0.87 (d, J = 7.2 Hz, 3 H), 0.73 (d, J = 7.2 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.91, 173.77, 158.27, 143.53, 143.36, 141.34, 141.28, 127.85, 127.19, 127.16, 124.99, 120.00, 119.99, 67.07, 58.34, 58.24, 46.74, 31.35, 31.05, 19.02, 18.92, 17.63, 17.47; HRMS (ESI) *m/z* calcd for $C_{25}H_{30}O_5N_2H^+$ $[M + H]^+$ 439.2228, found 439.2224.

Synthesis of 7

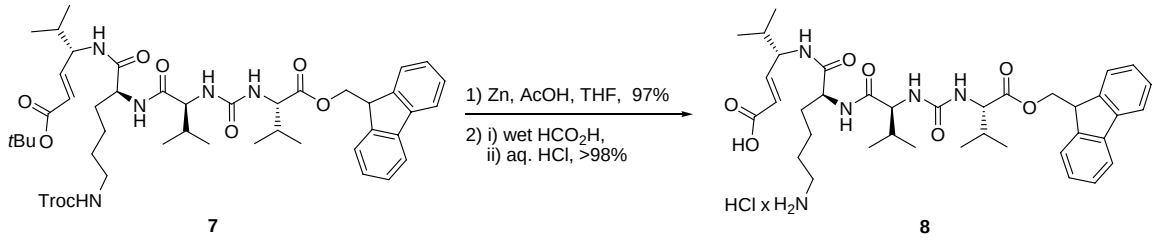


SI-24 (29 mg, 58 µmol, 1 eq.) was dissolved in dichloromethane (1 mL) in a 10 mL flask and cooled to 0°C. A solution of **9** (31 mg, 69 µmol, 1.2 eq.), PyBop (46 mg, 87 µmol, 1.5 eq.), HOAt (12 mg, 87 µmol, 1.5 eq.) and *N*,*N*-diisopropylethylamine (32 µL, 180 µmol, 3 eq.) in dichloromethane (1 mL) were added and the resulting mixture was stirred overnight at room temperature. After evaporation, the crude product was purified by flash column chromatography (60% ethyl acetate in cyclohexane) to yield 40 mg (43 µmol, 75%) of **7** as a colorless solid.

TLC (60% ethyl acetate in cyclohexane) R_f 0.35; HPLC (gradient 2) t_R 11.33 min; ^{1}H NMR (400 MHz, d^6-DMSO) δ 7.95 (d, J = 8.0 Hz, 1 H), 7.89 (d, J = 7.6 Hz, 1 H), 7.87

(d, J = 7.6 Hz, 1 H), 7.86 (d, J = 9.6 Hz, 1 H), 7.71 (d, J = 8.0 Hz, 1 H), 7.68 (d, J = 7.6 Hz, 1 H), 7.62-7.66 (m, 1 H), 7.42 (t, J = 7.2 Hz, 1 H), 7.41 (t, J = 7.2 Hz, 1 H), 7.33 (td, J = 6.0, 0.8 Hz, 1 H), 7.31 (td, J = 6.0, 1.2 Hz, 1 H), 6.70 (dd, J = 15.6, 5.6 Hz, 1 H), 6.46 (d, J = 8.8 Hz, 1 H), 6.30 (d, J = 8.8 Hz, 1 H), 5.75 (dd, J = 15.6, 1.6 Hz, 1 H), 4.77 (s, 2 H), 4.52 (dd, J = 10.8, 6.4 Hz, 1 H), 4.39 (dd, J = 10.8, 6.4 Hz, 1 H), 4.21-4.30 (m, 2 H), 4.23 (dd, J = 6.4, 6.4 Hz, 1 H), 4.00-4.07 (m, 2 H), 2.99 (t, J = 6.8 Hz, 1 H), 2.98 (t, J = 6.4 Hz, 1 H), 1.86-1.95 (m, 1 H), 1.74-1.86 (m, 2 H), 1.49-1.65 (m, 2 H), 1.37-1.46 (m, 2 H), 1.42 (s, 9 H), 1.16-1.33 (m, 2 H), 0.80-0.90 (m, 9 H), 0.77 (d, J = 6.8 Hz, 3 H), 0.76 (d, J = 6.8 Hz , 3 H), 0.67 (d, J = 7.2 Hz, 3 H); ^{13}C NMR (600 MHz, d^6-DMSO) δ 172.43, 171.60, 171.19, 164.79, 157.59, 154.28, 146.74, 143.60, 143.50, 140.76, 140.73, 127.67, 127.59, 127.11, 127.02, 125.07, 122.32, 122.26, 120.03, 96.31, 79.80, 73.25, 65.47, 57.76, 57.64, 54.57, 52.70, 46.38, 40.35, 31.51, 31.13, 30.13, 28.87, 27.67, 22.68, 19.20, 18.99, 18.94, 18.19, 17.51, 17.49; HRMS (ESI) *m/z* calcd for $C_{45}H_{62}O_9N_5{}^{35}Cl_3H^+$ [M + H]$^+$ 922.3686, found 922.3698 and calcd for $C_{45}H_{62}O_9N_5{}^{37}Cl_3H^+$ [M + H]$^+$ 924.3656, found 924.3673.

Synthesis of 8



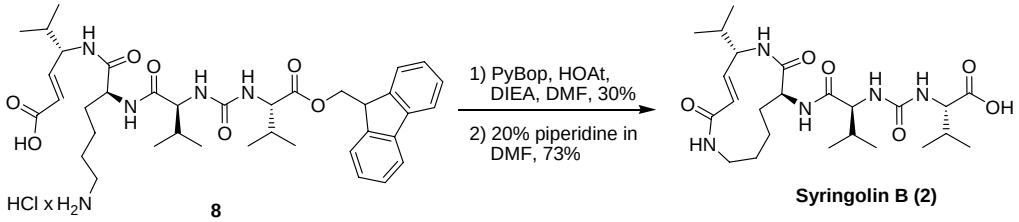
7 **8**

7 (60 mg, 65 µmol, 1 eq.) was dissolved in tetrahydrofurane (2 mL) in a 10 mL flask. Acetic acid was added (2 mL), followed by zinc powder (638 mg, 9.76 mmol, 150 eq.) which was added in portions over 30 minutes. After 3 hours of vigorous stirring, the mixture was filtered over a small plug of Celite and washed with ethyl acetate. After evaporation to dryness, 47 mg (63 µmol, 97%) of the deprotected amine was obtained which was used in the next step without further purification.

The cleaved intermediate (47 mg, 63 µmol) was dissolved in formic acid (4 mL) in a 10 mL flask and some drops of water were added. The resulting mixture was stirred overnight, concentrated to dryness, redissolved in diluted aq. HCl and re-evaporated to dryness. Addition of toluene and concentration to dryness yielded 45 mg (62 µmol, >98%) of **8** as a colorless solid.

HPLC (gradient 2) t_R 7.75 min; ^{1}H NMR (400 MHz, d^6-DMSO) δ 8.24 (s, 1 H), 7.98 (d, J = 8.0 Hz, 1 H), 7.92 (d, J = 9.2 Hz, 1 H), 7.89 (d, J = 7.6 Hz, 1 H), 7.88 (d, J = 7.6 Hz, 1 H), 7.71 (d, J = 7.2 Hz, 1 H), 7.68 (d, J = 7.6 Hz, 1 H), 7.42 (t, J = 7.2 Hz, 1 H), 7.41 (t, J = 7.6 Hz, 1 H), 7.28-7.36 (m, 2 H), 6.60 (dd, J = 15.6, 5.6 Hz, 1 H), 6.47 (d, J = 9.2 Hz, 1 H), 6.31 (d, J = 8.8 Hz, 1 H), 5.76 (dd, J = 15.6, 2.4 Hz, 1 H), 4.54 (dd, J = 10.8, 6.4 Hz, 1 H), 4.39 (dd, J = 10.8, 6.4 Hz, 1 H), 4.21-4.32 (m, 2 H), 4.23 (dd, J = 6.4, 6.4 Hz, 1 H), 4.05 (dd, J = 8.4, 5.2 Hz, 1 H), 4.00 (dd, J = 8.8, 4.8 Hz, 1H), 3.40 (br s, ~5H), 2.73 (t, J = 7.6 Hz, 2 H), 1.86-1.95 (m, 1 H), 1.72-1.84 (m, 2 H), 1.47-1.67 (m, 4 H), 1.20-1.34 (m, 2 H), 0.80-0.86 (m, 9 H), 0.77 (d, J = 6.8 Hz, 3 H), 0.75 (d, J = 6.8 Hz, 3 H), 0.66 (d, J = 7.2 Hz, 3 H); ^{13}C NMR (400 MHz, d^6-DMSO) δ 172.43, 171.67, 171.00, 165.39, 157.63, 143.61, 143.50, 140.78, 140.75, 128.89, 127.09, 125.09, 125.04, 121.34, 120.04, 119.99, 65.46, 57.74, 57.54, 54.67, 52.43, 46.37, 44.28, 31.60, 31.35, 31.19, 30.14, 22.35, 19.22, 19.00, 18.97, 18.26, 17.49, 17.47; HRMS (ESI) *m/z* calcd for $C_{38}H_{53}O_7N_5H^+$ $[M + H]^+$ 692.4018, found 692.4016.

Synthesis of Syringolin B (2)



PyBOP (339 mg, 651 µmol, 3eq.), HOAt (89 mg, 651 µmol, 3 eq.) and *N*,*N*-diisopropylethylamine (114 µL, 651 µmol, 3eq.) were dissolved under argon in dimethylformamide (114 mL) in a 500 mL flame-dried flask. A solution of **8** (150 mg,

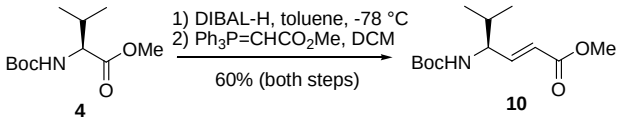
217 μmol, 1 eq.) and *N*,*N*-diisopropylethylamine (114 μL, 651 μmol, 3eq.) in *N*,*N*-dimethylformamide (58 mL) was slowly added over 8 hours with a syringe pump and stirred for further 24 hours. The reaction was quenched by addition of a 20% aq. citric acid solution and extracted with ethyl acetate. The organic layers were washed with water (2×50 mL) and dried over Na_2SO_4, filtered and evaporated to dryness. The remaining residue was purified by flash column chromatography (4% methanol in ethyl acetate) to yield 44 mg (65 μmol, 30%) of the cyclized product.

The cyclized product (7.70 mg, 11.4 μmol, 1 eq.) was dissolved in *N*,*N*-dimethylformamide (800 μL) in a 10 mL flask and piperidine (200 μL) was added. The mixture was stirred for one hour and then evaporated to dryness. The remaining residue was purified by preparative HPLC (using H_2O with 0.1% TFA (solvent A) and acetonitrile with 0.1% TFA (solvent B) at a flow of 25 mL/min. Gradient: from 0 to 10 min: 90% solvent A/10% solvent B; from 10 to 30 min: from 90% solvent A/10% solvent B to 70% solvent A/30% solvent B; from 30 to 50 min: from 70% solvent A/30% solvent B to 40% solvent A/60% solvent B; from 50 to 60 min: from 40% solvent A/60% solvent B to 0% solvent A/100% solvent B; from 60 to 80 min: 0% solvent A/100% solvent B) to yield 4.11 mg (8.3 μmol, 73%) of Syringolin B (SylB, **2**) as a colorless powder.

TLC (2% acetic acid + 15 % methanol in dichloromethane) R_f 0.40; HPLC (gradient 2) t_R 6.12 min; ^{1}H NMR (600 MHz, d^6-DMSO) δ 12.44 (br s, 1H), 8.26 (d, J = 7.8 Hz, 1 H), 7.63 (d, J = 7.8 Hz, 1 H), 7.37 (dd, J = 7.2, 7.2 Hz, 1 H), 6.77 (dd, J = 15.6, 4.8 Hz, 1 H), 6.33 (d, J = 8.4 Hz, 1 H), 6.32 (d, J = 8.4 Hz, 1 H), 6.21 (d, J = 15.6 Hz, 1 H), 4.55-4.60 (m, 1 H), 4.08-4.13 (m, 1 H), 4.03 (dd, J = 8.4, 4.8 Hz, 1 H), 3.99 (dd, J = 9.0, 4.8 Hz, 1H), 3.23-3.32 (m, 2H), 2.91-2.98 (m, 1 H), 2.03-2.10 (m, 1 H), 1.94-2.02 (m, 2 H), 1.73-1.80 (m, 1 H), 1.58-1.65 (m, 1 H), 1.40-1.46 (m, 1 H), 1.21-1.33 (m, 2 H), 0.95 (d, J = 6.6 Hz, 3 H), 0.92 (d, J = 6.6 Hz , 3 H), 0.86 (d, J = 6.6 Hz, 3 H), 0.84 (d, J = 7.2 Hz, 3 H), 0.83 (d, J = 6.6 Hz, 3 H), 0.78 (d, J = 6.6 Hz, 3 H); ^{13}C NMR (125 MHz, d^6-DMSO) δ 173.97, 171.22, 170.82, 165.87, 157.75, 144.85, 119.79, 57.87, 57.53, 56.00, 51.33, 38.03, 31.13, 30.53, 30.29, 30.19, 29.88, 19.86, 19.26, 19.16, 17.61, 17.38; HRMS (ESI) *m/z* calcd for $C_{24}H_{41}O_6N_5H^+$ $[M + H]^+$ 496.3130, found 496.3123.

A-3. Synthesis of Syringolin A (SylA, 1)

Synthesis of 10



N-(*tert*-Butoxycarbonyl)-(L)-valine methyl ester **4** (5.64 g, 24.39 mmol, 1 eq.) was dissolved under argon in toluene (245 mL) in a 500 mL flame-dried flask. The solution was cooled to -78 °C and a 1 M solution of DIBAL-H in toluene (49 mL, 48.78 mmol, 2 eq.) was slowly added over 2 hours. After stirring for further 2 hours, the mixture was quenched with a 1.2 M solution of potassium sodium tartrate (150 mL) and stirred vigorously at room temperature for 2 hours. The resulting solution was extracted with dichloromethane and the organic layers were dried over Na_2SO_4, filtered and concentrated to dryness to give *N*-(*tert*-butoxycarbonyl)-(L)-valinal which was directly used in the next step without further purification.

Crude *N*-(*tert*-butoxycarbonyl)-(L)-valinal was dissolved in dichloromethane (245 mL) and (methoxycarbonylmethylene)triphenylphosphorane (9.38 g, 28.05 mmol, 1.15 eq.) was added in one portion. After stirring for 12 hours, the mixture was successively washed with a 10% aq. $KHSO_4$ solution, a 5% aq. $NaHCO_3$ solution and with brine. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. The resulting crude product was purified by flash column chromatography (10% ethyl acetate in cyclohexane) to afford 3.78 g (14.69 mmol, 60%) of **10** as colorless crystals.

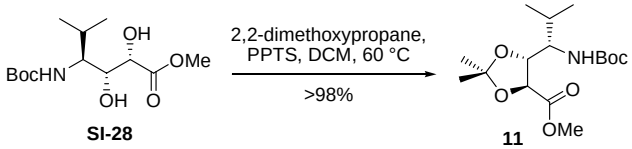
TLC (15% ethyl acetate in cyclohexane) R_f 0.31; HPLC (gradient 1) t_R 8.93 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86 (dd, J = 15.6, 5.6 Hz, 1 H), 5.92 (dd, J = 15.6, 1.6 Hz, 1 H), 4.50-4.62 (m, 1 H), 4.11-4.23 (m, 1 H), 3.73 (s, 3 H), 1.80-1.91 (m, 1 H), 1.44 (s, 9 H), 0.93 (d, J = 6.8 Hz, 3 H), 0.90 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.75, 155.42, 147.74, 121.19, 79.78, 56.79, 51.66, 32.32, 28.44, 18.92, 18.10; HRMS (ESI) *m/z* calcd for $C_{13}H_{23}O_4NH^+$ $[M + H]^+$ 258.1700, found 258.1702.

Synthesis of SI-28

10 (643 mg, 2.50 mmol, 1 eq.) was dissolved in acetone/water (2:1, 22.5 mL) in a 100 mL flask. 4-Methylmorpholine N-oxide (440 mg, 3.75 mmol, 1.5 eq.) and osmium tetroxide solution (4% wt/H_2O, 764 µL, 125 µmol, 0.05 eq.) were added consecutively. The flask was flushed with argon and the reaction was stirred for 2 days. The reaction was quenched by addition of a saturated aq. $NaHSO_3$ solution and the acetone was evaporated *in vacuo*. Ethyl acetate and further water were added, separated in a funnel, and the organic layer was dried over Na_2SO_4, filtered over Celite and concentrated to dryness to give a crude mixture of diastereoisomers. **SI-28** was obtained by recrystallization from cyclohexane to yield 583 mg (2.00 mmol, 80 %) of a pure single diastereoisomer **SI-28** as colorless crystals. The residual mixture was then purified by flash column chromatography (70% diethyl ether in petroleum ether) to afford another 38 mg (0.13 mmol, 5%) of **SI-28** as colorless crystals (=overall yield of 85%).

TLC (70% diethyl ether in petroleum ether) R_f 0.23; HPLC (gradient 2) t_R 7.55 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.78 (d, J = 10.0 Hz, 1 H), 4.32 (br s, 1 H), 4.01 (br s, 1 H), 3.81-3.86 (m, 1 H), 3.81 (s, 3 H), 3.52-3.59 (m, 1 H), 2.59 (br s, 1 H), 2.08-2.18 (m, 1 H), 1.43 (s, 9 H), 0.98 (d, J = 6.8 Hz, 3 H), 0.91 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.04, 157.49, 80.36, 72.16, 71.17, 57.35, 52.73, 28.38, 27.99, 20.11, 16.67; HRMS (ESI) *m/z* calcd for $C_{13}H_{25}O_6NH^+$ $[M + H]^+$ 292.1755, found 292.1757.

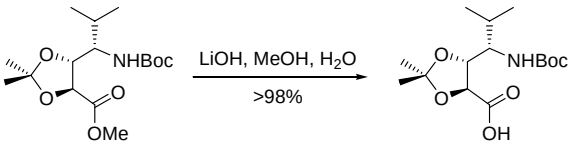
Synthesis of 11



SI-28 (3.53 g, 12.12 mmol, 1 eq.) was dissolved in dichloromethane (45 mL) in a 250 mL flame-dried flask and 2,2-dimethoxypropane (45 mL, 364.00 mmol, 30 eq.) and pyridinium *p*-toluenesulfonate (153 mg, 0.61 mmol, 0.05 eq.) were added. The flask was flushed with argon and the solution was heated to reflux for 5 hours. After evaporation to dryness, 3.93 g (11.88 mmol, >98%) of the desired product **11** was obtained as a colorless solid.

TLC (15% ethyl acetate in cyclohexane) R_f 0.26; HPLC (gradient 1) t_R 9.78 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.46 (d, J = 6.0 Hz, 1 H), 4.41-4.45 (m, 1 H), 4.11 (dd, J = 9.2, 6.4 Hz, 1 H), 3.76 (s, 3 H), 3.69-3.76 (m, 1 H), 2.09 (septd, J = 6.8, 3.6 Hz, 1 H), 1.44 (s, 3 H), 1.42 (s, 9 H), 1.41 (s, 3 H), 0.94 (d, J = 6.8 Hz, 3 H), 0.86 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.87, 156.07, 111.88, 79.50, 77.97, 57.50, 52.47, 28.52, 28.39, 27.23, 26.02, 19.83, 15.70; HRMS (ESI) *m/z* calcd for $C_{16}H_{29}O_6NH^+$ $[M + H]^+$ 332.2068, found 332.2069.

Synthesis of SI-29

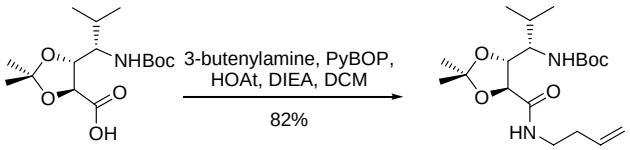


11 (1.40 g, 4.23 mmol, 1 eq.) was dissolved in methanol/water (1:1, 20 mL) in a 50 mL flask and a 1 M aq. lithium hydroxide solution (13 mL, 533 mg, 12.69 mmol, 3 eq.) was added at 0 °C. The mixture was stirred for further 30 min at room temperature. After evaporation of the methanol, a 20% aq. citric acid solution was added to acidify the reaction mixture. Extraction with dichloromethane (3×50 mL), drying over Na_2SO_4, filtering and concentration to dryness yielded 1.31 g (4.15 mmol, >98%) of **SI-29** as a white powder.

TLC (methanol/acetic acid/dichloromethane = 1:1:38) R_f 0.33; HPLC (gradient 2) t_R 9.05 min; ^{1}H NMR (500 MHz, CD_3OD) δ 4.23 (dd, J = 7.5, 5.5 Hz, 1 H), 4.14 (d, J = 5.5 Hz, 1 H), 3.54 (dd, J = 8.0, 4.5 Hz, 1 H), 1.97-2.05 (m, 1 H), 1.43 (s, 9 H), 1.40 (s, 3 H), 1.38

(s, 3 H), 0.94 (d, J = 7.0 Hz, 3 H), 0.89 (d, J = 7.0 Hz, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 178.61, 158.72, 111.38, 80.72, 80.34, 79.98, 59.62, 30.54, 28.79, 27.86, 26.72, 20.49, 17.31; HRMS (ESI) *m/z* calcd for $C_{15}H_{27}O_6NH^+$ $[M + H]^+$ 318.1911, found 318.1913.

Synthesis of 12

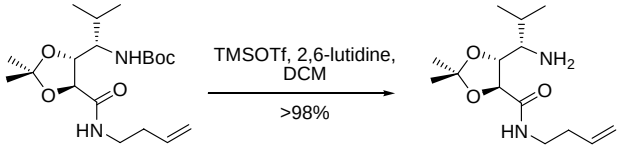


SI-29 **12**

SI-29 (1.33 g, 4.20 mmol, 1 eq.), 3-butenylamine hydrochloride (0.54 g, 5.10 mmol, 1.2 eq.), HOAt (858 mg, 6.30 mmol, 1.5 eq.) and PyBop (3.28 g, 6.30 mmol, 1.5 eq.) were dissolved in dichloromethane (5 mL) in a 10 mL flask. *N*,*N*-Diisopropylethylamine (1.46 mL, 8.40 mmol, 2 eq.) was added at 0 °C and the resulting mixture was stirred overnight at room temperature. The reaction was stopped by quenching with a 20% aq. citric acid solution and **12** was extracted from the mixture with chloroform (3×50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated to dryness. The crude product was purified by flash column chromatography (20% ethyl acetate in cyclohexane) to afford 1.27 g (3.43 mmol, 82%) of **12** as a colorless solid.

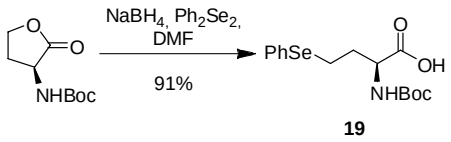
TLC (30% ethyl acetate in cyclohexane) R_f 0.49; HPLC (gradient 2) t_R 10.79 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.63 (br s, 1 H), 5.75 (ddt, J = 17.2, 10.4, 6.8 Hz, 1 H), 5.19 (d, J = 9.2 Hz, 1 H), 5.06-5.13 (m, 2 H), 4.30 (d, J = 6.0 Hz, 1 H), 4.06 (dd, J = 9.2, 6.0 Hz, 1 H), 3.64-3.73 (m, 1 H), 3.37-3.46 (m, 1 H), 3.24-3.33 (m, 1 H), 2.24-2.31 (m, 2 H), 2.01-2.11 (m, 1 H), 1.45 (s, 3 H), 1.43 (s, 9 H), 1.36 (s, 3 H), 0.96 (d, J = 7.2 Hz, 3 H), 0.87 (d, J = 7.2 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.40, 156.60, 135.05, 117.49, 111.36, 79.17, 78.36, 58.11, 37.99, 33.77, 29.59, 28.47, 27.28, 26.32, 19.81, 16.25; HRMS (ESI) *m/z* calcd for $C_{19}H_{34}O_5N_2H^+$ $[M + H]^+$ 371.2541, found 371.2540.

Synthesis of SI-30



12 (710 mg, 1.92 mmol, 1 eq.) was dissolved under argon in dichloromethane (2 mL) in a 10 mL flame-dried flask. 2,6-Lutidine (446 µL, 3.84 mmol, 2 eq.) and trimethylsilyl trifluoro methanesulfonate (522 µL, 2.88 mmol, 1.5 eq.) were added and the resulting mixture was stirred for further 15 minutes. The reaction was quenched upon addition of a saturated aq. NH_4Cl solution. The pH of the water phase was adjusted to 9 by addition of a 2 M aq. NaOH solution and was extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness, yielding 508 mg (1.88 mmol, >98%) of the desired product **SI-30** as a colorless solid.
TLC (7% methanol in dichloromethane) R_f 0.38; HPLC (gradient 2) t_R 5.88 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (br s, 1 H), 5.75 (ddt, J = 17.2, 10.0, 6.8 Hz, 1 H), 5.06-5.13 (m, 2 H), 4.34 (d, J = 6.8 Hz, 1 H), 3.99 (dd, J = 7.6, 6.8 Hz, 1 H), 3.27-3.43 (m, 2 H), 2.95 (br s, 2 H), 2.85 (dd, J = 7.6, 4.4 Hz, 1 H), 2.24-2.31 (m, 2 H), 2.06 (septd, J = 6.8, 4.4 Hz, 1 H), 1.44 (s, 3 H), 1.37 (s, 3 H), 1.02 (d, J = 7.2 Hz, 3 H), 0.92 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.27, 135.10, 117.47, 110.29, 80.35, 78.77, 59.17, 37.99, 33.71, 29.07, 27.04, 25.88, 19.87, 16.03; HRMS (ESI) *m/z* calcd for $C_{14}H_{26}O_3N_2H^+$ $[M + H]^+$ 271.2016, found 271.2017.

Synthesis of 19

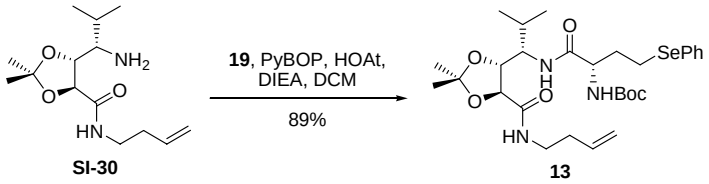


Sodium borohydride (125 mg, 3.3 mmol, 4.4 eq.) was disposed under argon in a 100 mL flame dried flask. A solution of diphenyl diselenide (937 mg, 3.0 mmol, 1 eq.) in

dimethylformamide (20 mL) was added, followed by addition of a solution of Boc-homoserine lactone (603 mg, 3.0 mmol, 1 eq.) in dimethylformamide (20 mL). The resulting mixture was heated to 100 °C for 90 minutes. After cooling to 0 °C, methanol (5 mL) was added and the mixture was stirred for an hour. The solvents were removed *in vacuo* and the remaining residue was partitioned between diethyl ether (150 mL) and 100 mM NaOAc buffer (pH 5.0). The aqueous layer was extracted twice more with diethyl ether (150 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (40% ethyl acetate in cyclohexane) to afford 973 mg (2.72 mmol, 91%) of **19** as a colorless solid.

TLC (2% acetic acid + 58% ethyl acetate in cyclohexane) R_f 0.56; HPLC (gradient 2) t_R 9.54 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (br s, 1 H), 7.48-7.52 (m, 2 H), 7.23-7.28 (m, 3 H), 5.07 (d, J = 6.4 Hz, 1 H), 4.35 (br s, 1 H), 2.93 (t, J = 8.0 Hz, 2 H), 2.16-2.32 (m, 1 H), 1.98-2.15 (m, 1 H), 1.44 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.32, 155.68, 133.04, 129.54, 129.24, 127.26, 80.56, 53.56, 33.23, 28.38, 23.21; HRMS (ESI) *m/z* calcd for $C_{15}H_{21}O_4N^{80}SeH^+$ $[M + H]^+$ 360.0709, found 360.0710 and calcd for $C_{15}H_{21}O_4N^{78}SeH^+$ $[M + H]^+$ 358.0716, found 358.0719.

Synthesis of 13



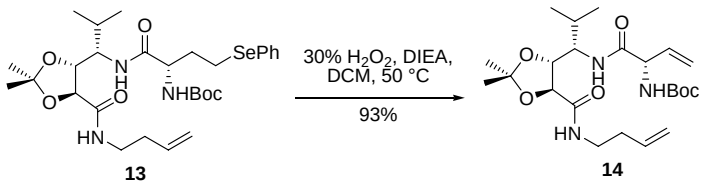
SI-30 **13**

SI-30 (512 mg, 1.90 mmol, 1 eq.), **19** (878 mg, 2.45 mmol, 1.3 eq.), PyBop (1.48 g, 2.85 mmol, 1.5 eq.) and HOAt (388 mg, 2.85 mmol, 1.5 eq.) were dissolved in dichloromethane (10 mL) in a 25 mL flask. The solution was cooled to 0 °C and *N*,*N*-diisopropylethylamine (662 μL, 3.80 mmol, 2 eq.) was added. The reaction was stirred overnight at room temperature, quenched by addition of a 20% aq. citric acid solution and extracted with chloroform (3×50 mL). The combined organic layers were dried over

Na_2SO_4, filtered and concentrated to dryness. The crude product was purified by flash column chromatography (20% ethyl acetate in cyclohexane) to afford 1.03 g (1.69 mmol, 89%) of **13** as a colorless solid.

TLC (25% ethyl acetate in cyclohexane) R_f 0.27; HPLC (gradient 2) t_R 11.68 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.46 (m, 2 H), 7.17-7.23 (m, 3 H), 6.97 (d, J = 8.4 Hz, 1 H), 6.64 (t, J = 5.6 Hz, 1 H), 5.72 (ddt, J = 17.6, 9.6, 6.8 Hz, 1 H), 5.19 (d, J = 8.0 Hz, 1 H), 5.04-5.10 (m, 2 H), 4.16-4.25 (m, 1 H), 4.10 (d, J = 6.8 Hz, 1 H), 4.03 (dd, J = 8.8, 6.8 Hz, 1 H), 3.96 (ddd, J = 8.8, 3.6, 3.6 Hz, 1 H), 3.33-3.42 (m, 1 H), 3.17-3.26 (m, 1 H), 2.83-2.90 (m, 2 H), 2.16-2.28 (m, 3 H), 2.07 (septd, J = 6.8, 3.6 Hz, 1 H), 1.91-2.00 (m, 1 H), 1.42 (s, 3 H), 1.40 (s, 9 H), 1.33 (s, 3 H), 0.90 (d, J = 6.8 Hz, 3 H), 0.87 (d, J = 7.2 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.58, 171.15, 155.38, 134.87, 132.51, 130.08, 129.01, 126.79, 117.49, 111.29, 79.76, 78.41, 78.24, 56.85, 54.96, 37.90, 33.64, 32.98, 29.90, 28.33, 26.95, 25.88, 23.22, 19.57, 16.40; HRMS (ESI) *m/z* calcd for $C_{29}H_{45}O_6N_3{}^{80}SeH^+$ $[M + H]^+$ 612.2546, found 612.2543 and calcd for $C_{29}H_{45}O_6N_3{}^{78}SeH^+$ $[M + H]^+$ 610.2554, found 610.2558.

Synthesis of 14

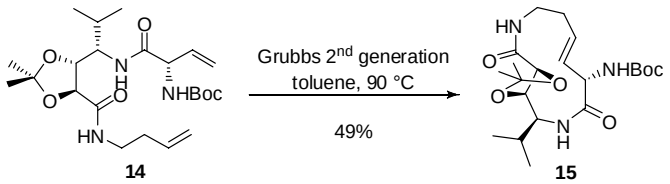


13 (925 mg, 2.04 mmol) was dissolved in dichloromethane (85 mL) in a 250 mL flask. Hydrogen peroxide (30% in water, 10 mL) and *N*,*N*-diisopropylethylamine (10 mL) were added and the resulting mixture was heated to 50 °C for 3 hours. The reaction was quenched by addition of a saturated aq. $CuSO_4$ solution. Addition of ethyl acetate (50 mL) and a 10% aq. $KHSO_4$ solution (50 mL) generated a biphasic mixture which was separated in a funnel. The organic phase was washed with a 5% aq. $NaHCO_3$ solution (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and concentrated to dryness. The

crude product was purified by flash column chromatography (20% ethyl acetate in cyclohexane) to afford 861 mg (1.90 mmol, 93%) of **14** as a colorless solid.

TLC (25% ethyl acetate in cyclohexane) R_f 0.16; HPLC (gradient 2) t_R 10.27 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (d, J = 8.8 Hz, 1 H), 6.67 (t, J = 5.6 Hz, 1 H), 5.93 (ddd, J = 17.2, 10.4, 6.4 Hz, 1 H), 5.76 (ddt, J = 17.6, 9.6, 6.8 Hz, 1 H), 5.49 (br s, 1 H), 5.38 (ddd, J = 17.2, 1.2, 1.2 Hz, 1 H), 5.23 (ddd, J = 10.4, 1.2, 1.2 Hz, 1 H), 5.08-5.14 (m, 2 H), 4.67 (br s, 1 H), 4.15 (d, J = 6.4 Hz, 1 H), 4.06 (dd, J = 9.2, 6.8 Hz, 1 H), 3.98 (ddd, J = 9.2, 3.6, 3.6 Hz, 1 H), 3.36-3.45 (m, 1 H), 3.25-3.34 (m, 1 H), 2.25-2.31 (m, 2 H), 2.09 (septd, J = 6.8, 3.6 Hz, 1 H), 1.45 (s, 3 H), 1.44 (s, 9 H), 1.36 (s, 3 H), 0.94 (d, J = 7.2 Hz, 3 H), 0.90 (d, J = 7.2 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.31, 170.21, 155.08, 134.78, 134.20, 117.41, 117.22, 111.33, 79.61, 78.45, 78.02, 57.51, 56.89, 37.90, 33.59, 30.03, 28.29, 26.90, 25.82, 19.50, 16.49; HRMS (ESI) *m/z* calcd for $C_{23}H_{39}O_6N_3H^+$ $[M + H]^+$ 454.2912, found 454.2906.

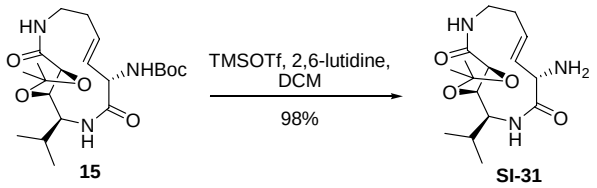
Synthesis of 15



14 (737 mg, 1.620 mmol, 1 eq.) was dissolved under argon in toluene (800 mL) in a 1 L flame-dried flask and heated to 90 °C. A solution of Grubbs' 2nd generation catalyst (207 mg, 0.243 mmol, 0.15 eq.) in toluene (25 mL) was added over 8 hours with a syringe pump to the preheated mixture. The resulting solution was stirred for further 10 hours at 90 °C. After concentration to dryness, the crude product was purified by flash column chromatography (50% ethyl acetate in cyclohexane) to afford 335 mg (0.787 mmol, 49%) of **15** as a light brown solid. The product was pure enough to be used in the next step without further purification. Nevertheless, a second flash column chromatography can be performed to completely eliminate the remaining traces of ruthenium residues.

TLC (60% ethyl acetate in cyclohexane) R_f 0.29; HPLC (gradient 2) t_R 8.56 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.63-5.77 (m, 2 H), 5.47-5.55 (m, 2 H), 5.12 (ddd, J = 15.2, 10.0, 0.8 Hz, 1 H), 4.46 (dd, J = 10.0, 8.4 Hz, 1 H), 4.34 (t, J = 8.0 Hz, 1 H), 3.83-3.95 (m, 2 H), 3.68 (d, J = 8.0 Hz, 1 H), 2.82-2.90 (m, 1 H), 2.36-2.44 (m, 1 H), 1.98-2.04 (m, 1 H), 1.83-1.94 (m, 1 H), 1.36 (s, 9 H), 1.34 (s, 3 H), 1.33 (s, 3 H), 0.88 (d, J = 6.8 Hz, 3 H), 0.86 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.86, 169.67, 154.82, 131.54, 130.49, 111.65, 79.93, 79.81, 76.14, 58.04, 56.71, 37.07, 34.62, 30.09, 28.43, 26.90, 26.27, 19.75, 15.98; HRMS (ESI) *m/z* calcd for $C_{21}H_{35}O_6N_3H^+$ $[M + H]^+$ 426.2599, found 426.2596.

Synthesis of SI-31

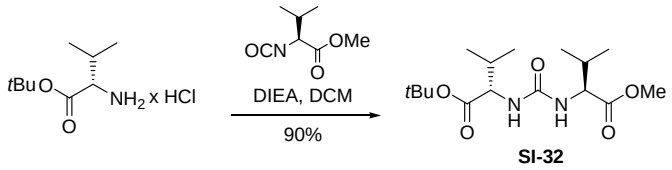


15 (295 mg, 0.69 mmol, 1 eq.) was dissolved in dichloromethane (4 mL) under argon in a 10 mL flame-dried flask. 2,6-Lutidine (161 µL, 1.38 mmol, 2 eq.) and trimethylsilyl trifluoro methanesulfonate (188 µL, 1.04 mmol, 1.5 eq.) were added at room temperature and the resulting mixture was stirred for 15 minutes. Addition of a saturated aq. NH_4Cl solution quenched the reaction. The pH was adjusted to 9 by addition of a 2 M NaOH solution and the desired product was extracted from the water phase with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness to yield 221 mg (0.68 mmol, 98 %) of **SI-31** as a colorless solid.

TLC (15% methanol in dichloromethane) R_f 0.20; HPLC (gradient 2) t_R 6.18 min; ^{1}H NMR (400 MHz, CD_3OD) δ 5.58 (ddd, J = 15.2, 10.8, 4.0 Hz, 1 H), 5.31 (ddd, J = 15.2, 9.2, 0.8 Hz, 1 H), 4.43 (dd, J = 10.4, 8.0 Hz, 1 H), 3.95 (dd, J = 10.4, 3.6 Hz, 1 H), 3.86 (d, J = 9.2 Hz, 1 H), 3.84 (d, J = 8.0 Hz, 1 H), 3.79-3.88 (m, 1 H), 2.88 (ddd, J = 13.2, 5.2, 1.6 Hz, 1 H), 2.32-2.38 (m, 1 H), 1.98-2.13 (m, 2 H), 1.40 (s, 3 H), 1.39 (s, 3 H),

0.93 (d, J = 6.8 Hz, 6 H); ^{13}C NMR (100 MHz, CD_3OD) δ 176.23, 170.80, 136.34, 130.29, 111.99, 81.71, 78.22, 58.80, 57.56, 37.46, 33.98, 31.02, 27.14, 26.48, 20.12, 16.23; HRMS (ESI) *m/z* calcd for $C_{16}H_{27}O_4N_3H^+$ $[M + H]^+$ 326.2074, found 326.2074.

Synthesis of SI-32

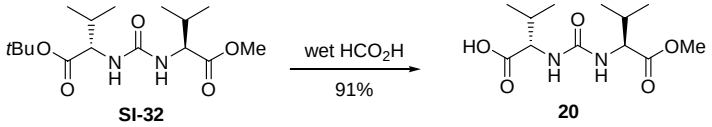


SI-32

Methyl (S)-(-)-2-isocyanato-3-methylbutyrate (431 µL, 3.00 mmol, 1 eq.) was dissolved under argon in dichloromethane (10 mL) in a 25 mL flame-dried flask. A solution of *tert*-butyl valine hydrochloride (629 mg, 3.00 mmol, 1 eq.) and *N*,*N*-diisopropylethylamine (1.05 mL, 6.00 mmol, 2 eq.) in dichloromethane (5 mL) was added and the resulting mixture was stirred overnight at room temperature. The reaction was quenched by addition of a 20% aq. citric acid solution and the desired product was extracted with chloroform (2×20 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by flash column chromatography (15% ethyl acetate in cyclohexane) to yield 892 mg (2.70 mmol, 90%) of **SI-32** as colorless crystals.

TLC (25% ethyl acetate in cyclohexane) R_f 0.21; HPLC (gradient 1) t_R 8.72 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.17 (d, J = 8.8 Hz, 1 H), 5.14 (d, J = 9.2 Hz, 1 H), 4.41 (dd, J = 8.8, 4.8 Hz, 1 H), 4.29 (dd, J = 8.4, 4.4 Hz, 1 H), 3.73 (s, 3 H), 2.04-2.16 (m, 2 H), 1.46 (s, 9 H), 0.94 (d, J = 6.8 Hz, 3 H), 0.92 (d, J = 6.8 Hz, 3 H), 0.88 (d, J = 6.8 Hz , 3 H), 0.86 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.94, 172.41, 157.48, 81.80, 58.45, 58.19, 52.13, 31.69, 31.48, 28.16, 19.10, 19.03, 17.86, 17.64; HRMS (ESI) *m/z* calcd for $C_{16}H_{30}O_5N_2H^+$ $[M + H]^+$ 331.2228, found 331.2229.

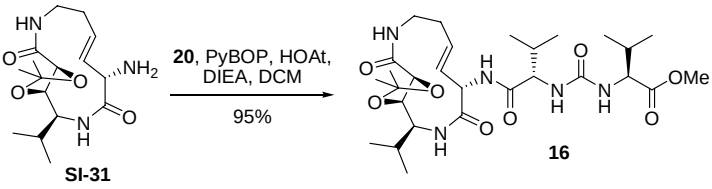
Synthesis of 20



SI-32 (892 mg, 2.70 mmol) was dissolved in formic acid (6 mL) in a 25 mL flask. Some drops of water were added and the mixture was stirred overnight. After concentration to dryness and co-evaporation with toluene, crude **20** was obtained which was purified by flash column chromatography (70% ethyl acetate in cyclohexane) to yield 674 mg (2.46 mmol, 91%) of **20** as a colorless solid.

TLC (2% acetic acid + 78% ethyl acetate in cyclohexane) R_f 0.51; HPLC (gradient 2) t_R 6.82 min; ^{1}H NMR (400 MHz, CD_3OD) δ 4.85 (br s, 3 H), 4.20 (d, J = 5.2 Hz, 1 H), 4.19 (d, J = 4.8 Hz, 1 H), 3.71 (s, 3 H), 2.07-2.19 (m, 2 H), 0.98 (d, J = 6.8 Hz, 3 H), 0.97 (d, J = 6.8 Hz, 3 H), 0.94 (d, J = 6.8 Hz, 3 H), 0.92 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.93, 174.76, 160.48, 59.57, 59.29, 32.09, 32.04, 19.64, 19.52, 18.03, 17.80; HRMS (ESI) *m/z* calcd for $C_{12}H_{22}O_5N_2H^+$ $[M + H]^+$ 275.1602, found 275.1603.

Synthesis of 16

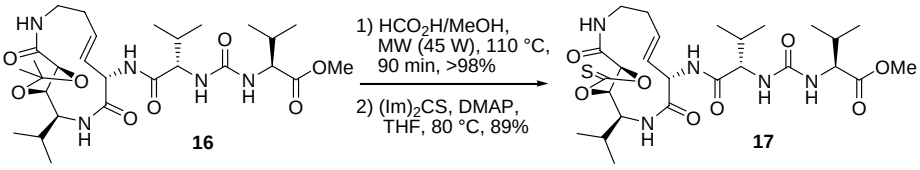


SI-31 (161 mg, 495 µmol, 1 eq.), **20** (190 mg, 693 µmol, 1.4 eq.), PyBOP (387 mg, 743 µmol, 1.5 eq.) and HOAt (102 mg, 743 µmol, 1.5 eq.) were dissolved in dichloromethane (10 mL) in a 10 mL flask. The solution was cooled to 0 °C and *N*,*N*-diisopropylethylamine (173 µL, 990 µmol, 2 eq.) was added. The reaction was stirred overnight at room temperature, was diluted with methanol/dichloromethane (1:9, 25 mL) and then washed with a 20% aq. citric acid solution and a 5% aq. $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness. The crude

product was purified by flash column chromatography (5% methanol in ethyl acetate) to yield 273 mg (469 mmol, 95%) of **16** as a colorless solid.

TLC (5% methanol in ethyl acetate) R_f 0.67; HPLC (gradient 2) t_R 8.07 min; ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$ = 1:1) δ 8.25 (d, J = 6.0 Hz, 1 H), 8.02 (d, J = 8.4 Hz, 1 H), 7.49 (d, J = 10.4 Hz, 1 H), 6.52 (d, J = 9.2 Hz, 1 H), 6.47 (d, J = 8.8 Hz, 1 H), 5.65 (ddd, J = 14.8, 11.2, 4.0 Hz, 1 H), 5.41 (dd, J = 15.2, 10.0, 1 H), 4.92 (dd, J = 9.2, 6.8 Hz, 1 H), 4.41 (dd, J = 10.0, 7.6 Hz, 1 H), 4.25-4.30 (m, 1 H), 4.08-4.14 (m, 1 H), 3.94 (ddd, J = 10.0, 10.0, 3.2 Hz, 1 H), 3.84-3.90 (m, 1 H), 3.82 (d, J = 8.0 Hz, 1 H), 3.73 (s, 3 H), 2.85-2.92 (m, 1 H), 2.31-2.38 (m, 1 H), 1.94-2.12 (m, 4 H), 1.41 (s, 3 H), 1.38 (s, 3 H), 0.83-0.94 (m, 18 H); ^{13}C NMR (100 MHz, $CD_3OD/CDCl_3$ = 1:1) δ 174.14, 172.90, 172.84, 169.72, 158.95, 131.85, 130.01, 111.04, 80.72, 77.21, 58.63, 58.30, 56.94, 56.38, 51.83, 36.54, 32.91, 32.16, 31.20, 29.75, 26.46, 25.74, 19.45, 18.91, 18.85, 17.57, 17.38, 15.34; HRMS (ESI) *m/z* calcd for $C_{28}H_{47}O_8N_5H^+$ $[M + H]^+$ 582.3497, found 582.3494.

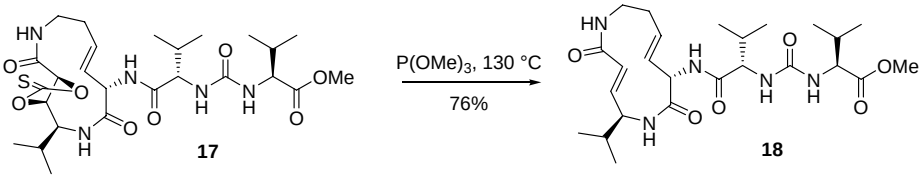
Synthesis of 17



In a 10 mL vessel was placed **16** (5 mg, 8.6 μmol), formic acid/methanol (6:4, 5 mL) and a magnetic stirring bar. The vessel was sealed with a septum, placed into the MW cavity, and locked with the pressure device. Constant microwave irradiation of 45 W as well as a simultaneous air-cooling (300 kPa, 45 Psi) were used during the entire reaction time (90 min, 110 °C, resulting reaction pressure 6 bar). After cooling to room temperature, the solvent was removed under reduced pressure to afford 4.5 mg (8.4 μmol, >98%) of the dihydroxyl intermediate as a colorless solid. The product was pure enough to be used in the next step without further purification (intermediate characterization: HPLC (gradient 2) t_R 6.49 min; HRMS (ESI) *m/z* calcd for $C_{25}H_{43}O_8N_5H^+$ $[M + H]^+$ 542.3184, found 542.3179).

After performing this reaction several times, all product fractions were pooled and the resulting residue of the dihydroxyl derivative (92 mg, 170 µmol, 1 eq.) was dissolved under argon in tetrahydrofurane (50 mL) in a 100 mL flame-dried flask. To this solution was added thiocarbonyl diimidazole (303 mg, 1.70 mmol, 10 eq.) and 4-(dimethylamino)pyridine (208 mg, 1.70 mmol, 10 eq.). The resulting reaction mixture was heated to 80 °C and stirred at this temperature overnight. After recooling to room temperature, a small portion of silica gel was added and the solvent was removed under vacuo. The adsorbed crude product was purified by flash column chromatography (4% methanol in dichloromethane) to yield 88 mg (151 µmol, 89%) of **17** as a colorless solid.
TLC (6% methanol in dichloromethane) R_f 0.32; HPLC (gradient 2) t_R 8.38 min; ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$ = 1:4) δ 8.30 (d, J = 9.6 Hz, 1 H), 8.20-8.25 (m, 1 H), 7.72 (d, J = 9.1 Hz, 1 H), 5.58 (ddd, J = 15.5, 11.5, 4.2 Hz, 1 H), 5.39 (dd, J = 15.5, 9.4, 1 H), 5.07 (dd, J = 10.2, 10.2 Hz, 1 H), 4.85-4.92 (m, 1 H), 4.62 (d, J = 9.6 Hz, 1 H), 4.30 (d, J = 4.9 Hz, 1 H), 4.17-4.24 (m, 1 H), 4.05 (d, J = 6.9 Hz, 1 H), 3.79-3.87 (m, 1 H), 3.70 (s, 3 H), 2.90-2.96 (m, 1 H), 2.31-2.39 (m, 1 H), 1.99-2.13 (m, 3 H), 1.83-1.91 (m, 1 H), 0.78-0.90 (m, 18 H); ^{13}C NMR (100 MHz, $CD_3OD/CDCl_3$ = 1:4) δ 190.46, 176.03, 174.32, 173.30, 172.93, 164.17, 131.91, 130.03, 83.95, 82.19, 58.59, 58.21, 56.74, 54.93, 52.26, 37.01, 32.75, 31.29, 29.21, 26.78, 19.24, 19.11, 18.95, 18.10, 17.64, 15.41; HRMS (ESI) *m/z* calcd for $C_{26}H_{41}O_8N_5SH^+$ $[M + H]^+$ 584.2749, found 584.2757.

Synthesis of 18

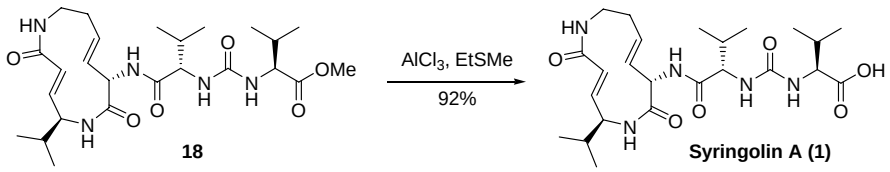


17 (20.0 mg, 34 µmol, 1 eq.) was dissolved under argon in trimethyl phosphite (2 mL) in a 10 mL flame-dried flask. The resulting mixture was refluxed for 3 hours at 130 °C. After concentration to dryness, the crude product was purified by flash column

chromatography (10% methanol in dichloromethane) to yield 13.1 mg (26 μmol, 76%) of **18** as a colorless solid.

TLC (8% methanol in dichloromethane) R_f 0.19; HPLC (gradient 2) t_R 6.84 min; ^{1}H NMR (500 MHz, d^6-DMSO) δ 8.04 (d, J = 7.2 Hz, 1 H), 8.00 (d, J = 8.7 Hz, 1 H), 7.44 (t, J = 7.1 Hz, 1 H), 6.68 (dd, J = 15.5, 5.5 Hz, 1 H), 6.42 (d, J = 8.8 Hz, 1 H), 6.22 (d, J = 9.1 Hz, 1 H), 6.09 (d, J = 15.5 Hz, 1 H), 5.60 (dt, J = 15.6, 7.7, 1 H), 5.41 (dd, J = 15.9, 7.7 Hz, 1 H), 4.86 (t, J = 7.4 Hz, 1 H), 4.00-4.11 (m, 3 H), 3.61 (s, 3 H), 3.10-3.24 (m, 2 H), 2.24-2.32 (m, 1 H), 1.86-2.02 (m, 3 H), 1.69-1.78 (m, 1 H), 0.95 (d, J = 6.7, 3 H), 0.90 (d, J = 6.7, 3 H), 0.82-0.87 (m, 9 H), 0.78 (d, J = 6.8, 3 H); ^{13}C NMR (125 MHz, d^6-DMSO) δ 190.46, 176.03, 174.32, 173.30, 172.93, 164.17, 131.91, 130.03, 83.95, 82.19, 58.59, 58.21, 56.74, 54.93, 52.26, 37.01, 32.75, 31.29, 29.21, 26.78, 19.24, 19.11, 18.95, 18.10, 17.64, 15.41; HRMS (ESI) *m/z* calcd for $C_{25}H_{41}O_6N_5H^+$ $[M + H]^+$ 508.3130, found 508.3134.

Synthesis of Syringolin A (1)



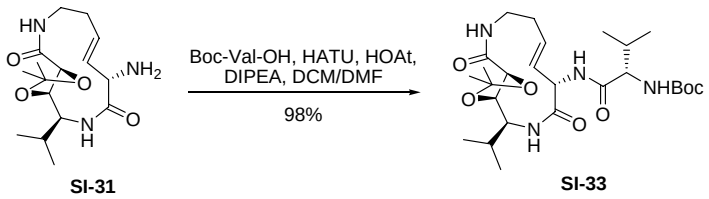
18 (8.0 mg, 16 μmol, 1 eq.) and aluminium trichloride (17.1 mg, 128 μmol, 8 eq.) were dissolved under argon in ethyl methyl sulfide (400 μL) in a 10 mL flame-dried flask. The resulting mixture was stirred for 1 hour at room temperature. After concentration to dryness, the crude product was purified by flash column chromatography (2% acetic acid + 15% methanol in dichloromethane) to yield 7.3 mg (15 μmol, 92%) of **1** (SylA) as a colorless solid.

TLC (2% acetic acid + 15% methanol in dichloromethane) R_f 0.32; HPLC (gradient 2) t_R 6.13 min; ^{1}H NMR (400 MHz, d^6-DMSO) δ 12.35 (br s, 1 H), 8.03 (d, J = 8.4 Hz, 1 H), 7.99 (d, J = 6.7 Hz, 1 H), 7.40-7.48 (m, 1 H), 6.68 (dd, J = 15.2, 4.3 Hz, 1 H), 6.32 (d, J = 8.9 Hz, 1 H), 6.25 (d, J = 9.0 Hz, 1 H), 6.10 (d, J = 15.4 Hz, 1 H), 5.59 (dt, J = 15.5, 7.1,

1 H), 5.40 (dd, J = 15.5, 7.5 Hz, 1 H), 4.82-4.88 (m, 1 H), 4.01-4.10 (m, 2 H), 3.97 (dd, J = 8.7, 4.7 Hz, 1 H), 3.07-3.25 (m, 2 H), 2.23-2.32 (m, 1 H), 1.86-2.03 (m, 3 H), 1.69-1.78 (m, 1 H), 0.94 (d, J = 6.2, 3 H), 0.90 (d, J = 6.3, 3 H), 0.80-0.88 (m, 9 H), 0.77 (d, J = 6.5, 3 H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 174.00, 171.41, 168.88, 166.23, 157.62, 143.20, 133.04, 125.92, 121.50, 57.52, 57.36, 55.43, 53.57, 42.50, 34.98, 31.40, 31.07, 30.16, 19.72, 19.23, 19.19, 19.14, 17.60, 17.56; HRMS (ESI) *m/z* calcd for $C_{24}H_{39}O_6N_5H^+$ $[M + H]^+$ 494.2973, found 494.2978.

A-3. Synthesis of lipophilic Syringolin A derivative (21)

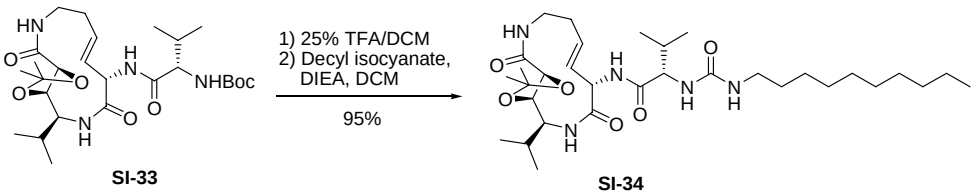
Synthesis of SI-33



SI-31 (61 mg, 188 µmol, 1 eq.), Boc-Val-OH (49 mg, 226 µmol, 1.2 eq.), HATU (107 mg, 282 µmol, 1.5 eq.) and HOAt (39 mg, 282 µmol, 1.5 eq.) were dissolved in dichloromethane/N,N-dimethylformamide (1:1, 3 mL) in a 10 mL flask. The solution was cooled to 0 °C and *N*,*N*-diisopropylethylamine (66 µL, 376 µmol, 2 eq.) was added. The reaction was stirred 1 h at room temperature and concentrated to dryness. The crude product was purified by flash column chromatography (4% methanol in dichloromethane) to yield 97 mg (185 mmol, 98%) of **SI-33** as a colorless solid.

TLC (5% methanol in dichloromethane) R_f 0.30; HPLC (gradient 2) t_R 8.85 min; ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$ = 1:1) δ 7.94 (d, J = 7.1 Hz, ~1 H), 7.43 (d, J = 9.8 Hz, ~1 H), 5.66 (ddd, J = 14.9, 11.1, 3.9 Hz, 1 H), 5.34 (dd, J = 15.3, 9.7, 1 H), 4.77-4.82 (m, 1 H), 4.40 (dd, J = 10.3, 8.0 Hz, 1 H), 3.83-3.93 (m, 3 H), 3.79 (d, J = 7.9 Hz, 1 H), 2.84-2.90 (m, 1 H), 2.32-2.39 (m, 1 H), 1.98-2.10 (m, 3 H), 1.41 (s, 9 H), 1.40 (s, 3 H), 1.37 (s, 3 H), 0.85-0.93 (m, 12 H); ^{13}C NMR (100 MHz, $CD_3OD/CDCl_3$ = 1:1) δ 173.01, 170.12, 170.05, 157.14, 132.38, 130.48, 111.52, 81.00, 80.37, 77.41, 60.32, 57.37, 56.73, 37.04, 33.54, 31.76, 30.19, 28.57, 27.00, 26.31, 19.85, 19.45, 18.09, 15.82; HRMS (ESI) *m/z* calcd for $C_{26}H_{44}O_7N_4H^+$ $[M + H]^+$ 525.3283, found 525.3278.

Synthesis of SI-34

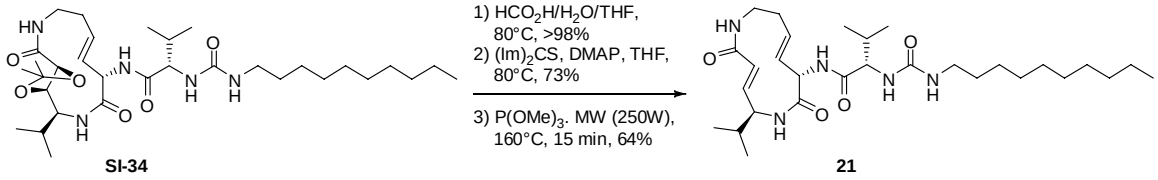


SI-33 (56 mg, 107 μmol, 1 eq.) was dissolved in dichloromethane (375 μL) in a 10 mL flask. Trifluoroacetic acid (125 μL) was then added and the solution was stirred 30 min at room temperature and concentrated at room temperature to dryness. The product was pure enough to be used in the next step without further purification (intermediate characterization: HPLC (gradient 2) t_R 5.54 min; HRMS (ESI) *m/z* calcd for $C_{21}H_{36}O_5N_4H^+$ $[M + H]^+$ 425.2759, found 425.2755).

The resulting ammonium salts were dissolved in dichloromethane (2 mL). *N*,*N*-diisopropylethylamine (37 μL, 214 μmol, 2 eq.) and decyl isocyanate (27 μL, 129 μmol, 1.2 eq.) were added. The reaction was stirred 1 h at room temperature and concentrated to dryness. The crude product was purified by flash column chromatography (6% methanol in dichloromethane) to yield 62 mg (102 μmol, 95%) of **SI-34** as a colorless solid.

TLC (8% methanol in dichloromethane) R_f 0.33; HPLC (gradient 2) t_R 11.25 min; ^{1}H NMR (400 MHz, d^6-DMSO) δ 7.98 (d, J = 5.9 Hz, 1 H), 7.95 (d, J = 9.8 Hz, 1 H), 7.51 (d, J = 10.2 Hz, 1 H), 5.99-6.03 (m, 1 H), 5.87 (d, J = 9.3 Hz, 1 H), 5.58 (ddd, J = 15.0, 11.1, 4.0 Hz, 1 H), 5.35 (dd, J = 15.2, 9.6, 1 H), 4.66 (dd, J = 9.7, 6.0, 1 H), 4.33 (dd, J = 10.4, 7.7 Hz, 1 H), 4.02 (dd, J = 8.8, 5.5, 1 H), 3.81 (ddd, J = 10.4, 10.3, 3.4, 1 H), 3.73 (d, J = 7.6 Hz, 1 H), 3.65-3.74 (m, 1H), 2.90-2.97 (m, 2 H), 2.71-2.77 (m, 1 H), 2.23-2.29 (m, 1 H), 1.84-2.03 (m, 3 H), 1.33 (s, 3 H), 1.32 (s, 3 H), 1.21-1.28 (m, 16 H), 0.86 (t, J = 6.7 Hz, 1 H), 0.85 (d, J = 6.9 Hz, 1 H), 0.82 (d, J = 6.8 Hz, 1 H), 0.78 (d, J = 7.0 Hz, 1 H), 0.76 (d, J = 6.8 Hz, 1 H); ^{13}C NMR (100 MHz, d^6-DMSO) δ 171.22, 171.16, 167.71, 157.78, 131.19, 129.87, 109.64, 79.91, 76.83, 57.08, 55.99, 55.02, 53.57, 41.82, 35.63, 32.21, 31.26, 31.14, 29.94, 29.01, 28.92, 28.75, 28.66, 26.73, 26.33, 25.97, 22.06, 18.06, 17.53, 16.71, 15.81, 13.92; HRMS (ESI) *m/z* calcd for $C_{32}H_{57}O_6N_5H^+$ $[M + H]^+$ 608.4382, found 608.4380.

Synthesis of lipophilic SylA derivative (21)



SI-34 (25 mg, 41 μmol) was dissolved in formic acid/water/tetrahydrofurane (1:1.1, 10 mL) and the mixture was heated to 80°C overnight. After cooling, the solvents were removed under reduced pressure to afford 23 mg (40 μmol, >98%) of the dihydroxyl intermediate as a colorless solid. The product was pure enough to be used in the next step without further purification (intermediate characterization: HPLC (gradient 2) t_R 9.24 min; HRMS (ESI) *m/z* calcd for $C_{29}H_{53}O_6N_5H^+$ $[M + H]^+$ 568.4069, found 568.4065).

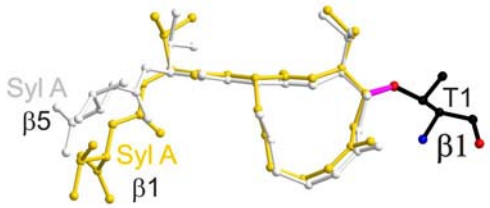
The dihydroxyl derivative (16 mg, 29 μmol, 1 eq.) was dissolved under argon in tetrahydrofurane (5 mL) in a 100 mL flame-dried flask. To this solution was added thiocarbonyl diimidazole (52 mg, 290 μmol, 10 eq.) and 4-(dimethylamino)pyridine (35 mg, 290 μmol, 10 eq.). The resulting reaction mixture was heated to 80 °C and stirred at this temperature overnight. After cooling to room temperature, a small portion of silica gel was added and the solvent was removed under vacuo. The adsorbed crude product was purified by flash column chromatography (6% methanol in dichloromethane) to yield 12.5 mg (21 μmol, 73%) of the thiocarbonate derivative as a colorless solid. The product was pure enough to be used in the next step without further purification (intermediate characterization: HPLC (gradient 2) t_R 11.15 min; HRMS (ESI) *m/z* calcd for $C_{30}H_{51}O_6N_5SH^+$ $[M + H]^+$ 610.3633, found 610.3631).

In a 10 mL vessel was placed the thiocarbonate derivative (5.4 mg, 8.8 μmol), trimethyl phosphite (300 μL) and a magnetic stirring bar. The vessel was sealed with a septum, placed into the MW cavity, and locked with the pressure device. Constant microwave irradiation of 250 W as well as a simultaneous air-cooling (300 kPa, 45 Psi) were used during the entire reaction time (15 min, 160 °C, resulting reaction pressure 2.5 bar). After concentration to dryness, the crude product was purified by flash column chromatography

(9% methanol in dichloromethane) to yield 3.0 mg (5.6 μmol, 64%) of **21** as a colorless solid.

TLC (10% methanol in dichloromethane) R_f 0.30; HPLC (gradient 2) t_R 9.81 min; ^{1}H NMR (400 MHz, d^6-DMSO) δ 7.99-8.03 (m, 2 H), 7.44 (t, J = 7.5 Hz, 1 H), 6.68 (dd, J = 15.4, 5.5 Hz, 1 H), 6.10 (d, J = 15.7 Hz, 1 H), 6.00-6.04 (m, 1 H), 5.90 (d, J = 9.0, 1 H), 5.60 (dt, J = 15.0, 7.7 Hz, 1 H), 5.41 (dd, J = 15.5, 7.8 Hz, 1 H), 4.86 (dd, J = 7.3, 7.3 Hz, 1 H), 4.03-4.10 (m, 2 H), 3.12-3.21 (m, 2 H), 2.92-2.98 (m, 2 H), 2.24-2.32 (m, 1 H), 1.94-2.01 (m, 1 H), 1.86-1.94 (m, 1 H), 1.69-1.78 (m, 1 H), 1.19-1.36 (m, 18H), 0.95 (d, J = 6.7, 3 H), 0.90 (d, J = 6.6, 3 H), 0.85 (t, J = 7.3 Hz, 3 H), 0.83 (d, J = 6.8, 3 H), 0.76 (d, J = 6.8, 3 H); ^{13}C NMR (500 MHz, d^6-DMSO) δ 170.24, 166.26, 162.92, 157.72, 142.45, 133.03, 125.19, 121.82, 57.33, 55.44, 52.98, 42.27, 33.95, 31.44, 31.27, 31.08, 29.95, 29.03, 28.94, 28.77, 28.68, 26.33, 22.07, 19.76, 19.28, 19.16, 17.59, 13.94; HRMS (ESI) *m/z* calcd for $C_{29}H_{51}O_4N_5H^+$ $[M + H]^+$ 534.4014, found 534.4011.

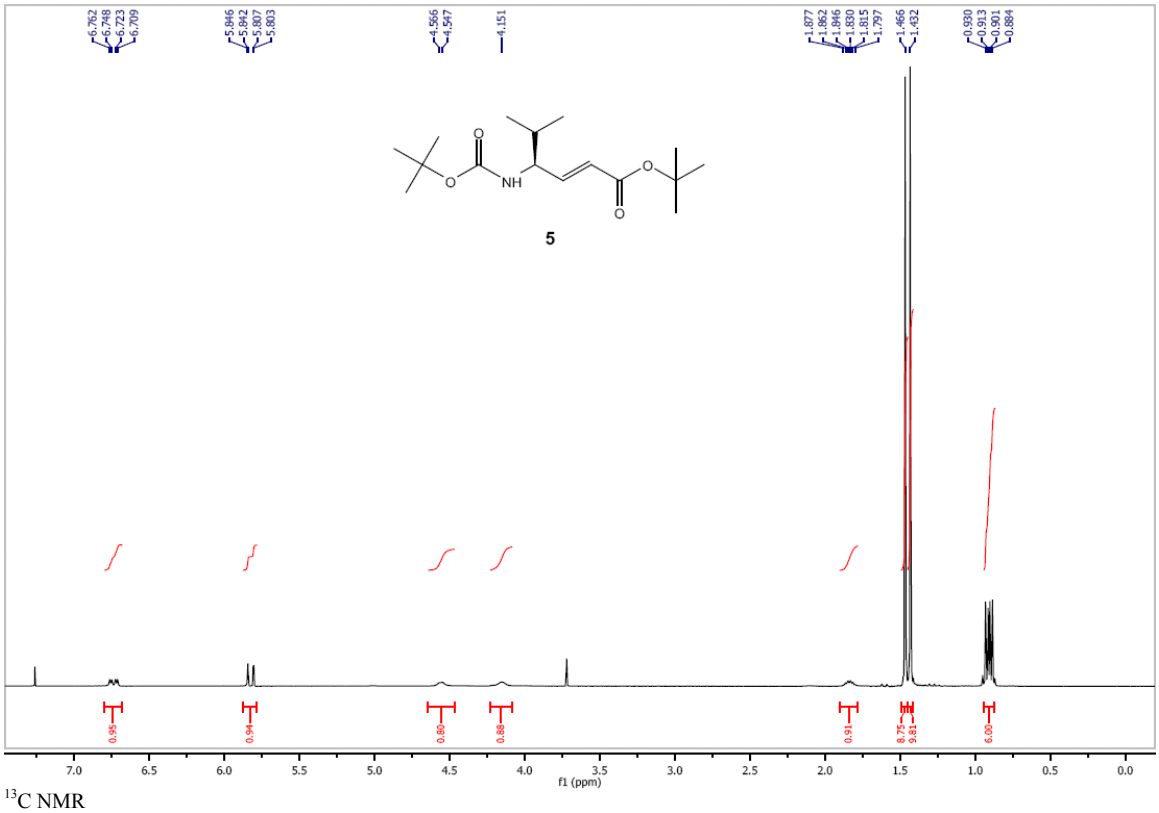
B. Supplementary Figures



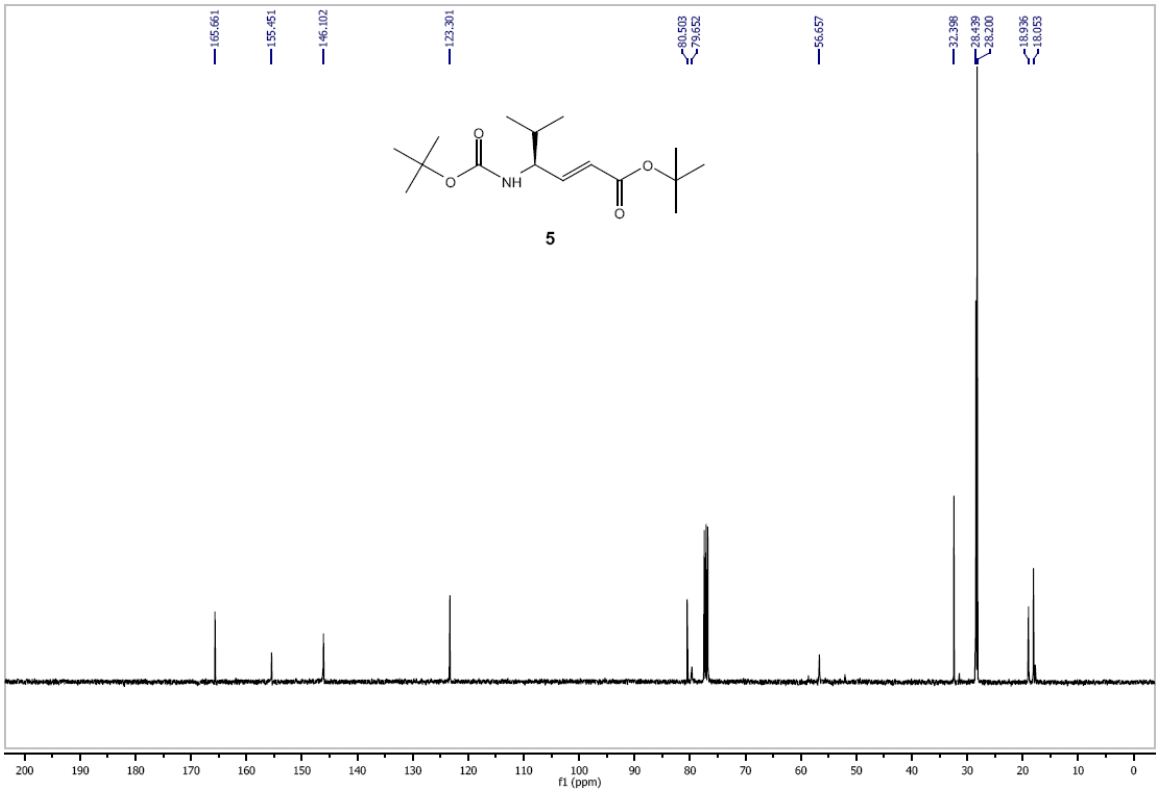
Supporting Figure 1. Structural superposition of SylA bound to subunit β5 with SylA bound to subunit β1. The r.m.s.-fit was determined only for the N-terminal threonine residues of subunits β1 and β5. The calculated rotation matrix and translation vector was applied to SylA including Thr1 of subunit β5 (colored in grey). The figure illustrates identical arrangement of both lactam-rings, which significantly alters in the superposition of SylA, SylB and GlbA (see Fig. 4d). The structure of SylA obtained from the experimental electron density is colored in yellow, both threonines are colored in black and the covalent bond between protein and inhibitor molecules is highlighted in magenta. Structures are shown as balls-and-sticks-representation

C. NMR Spectra

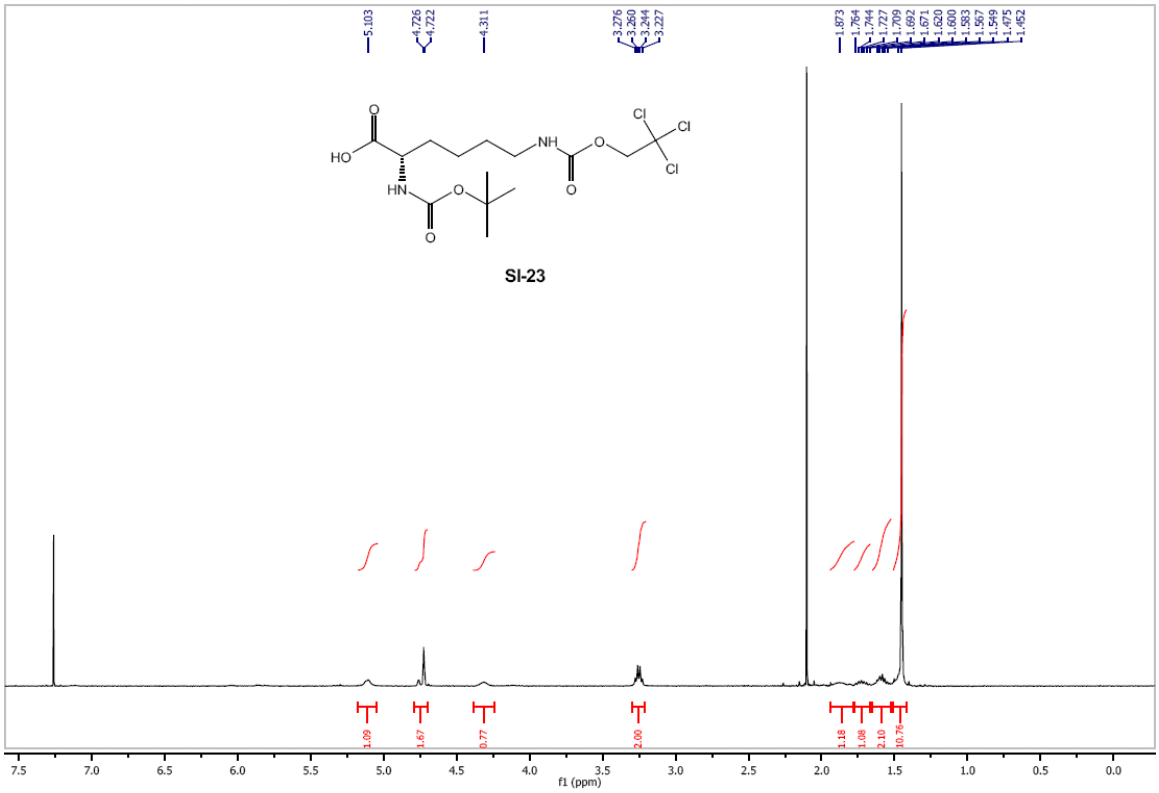
^{1}H NMR



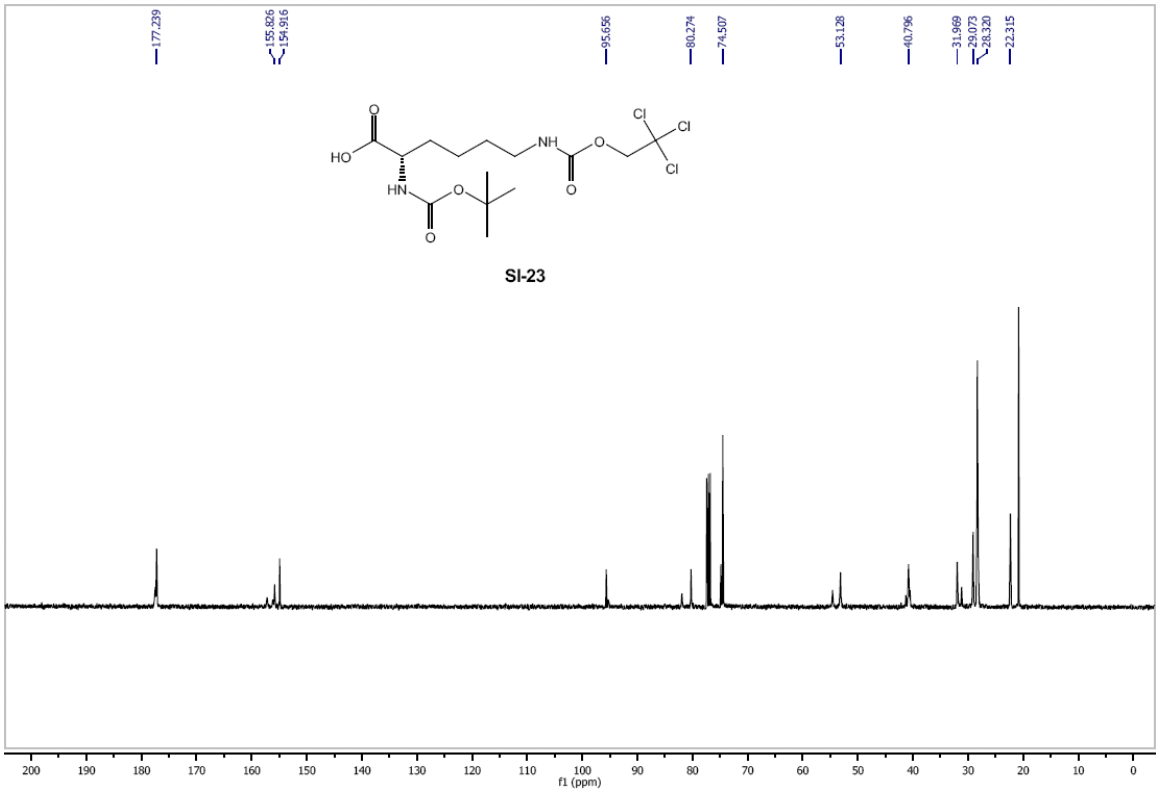
^{13}C NMR



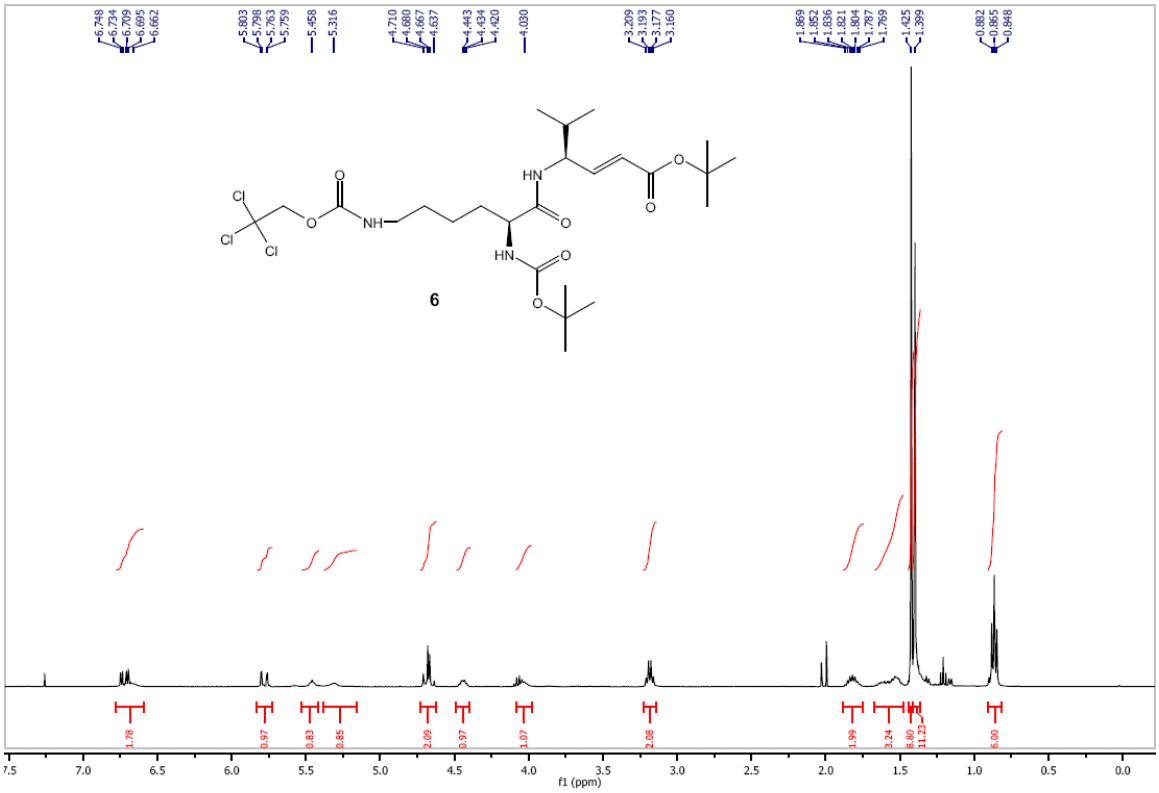
^{1}H NMR



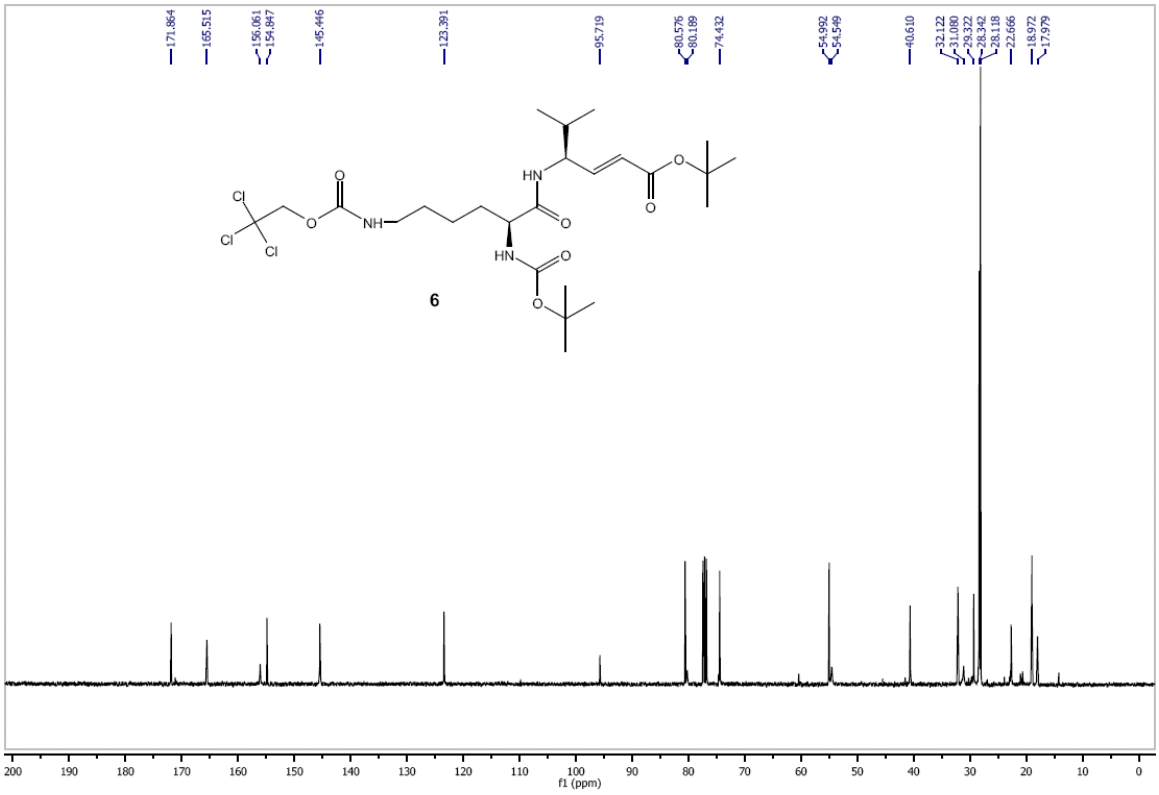
^{13}C NMR



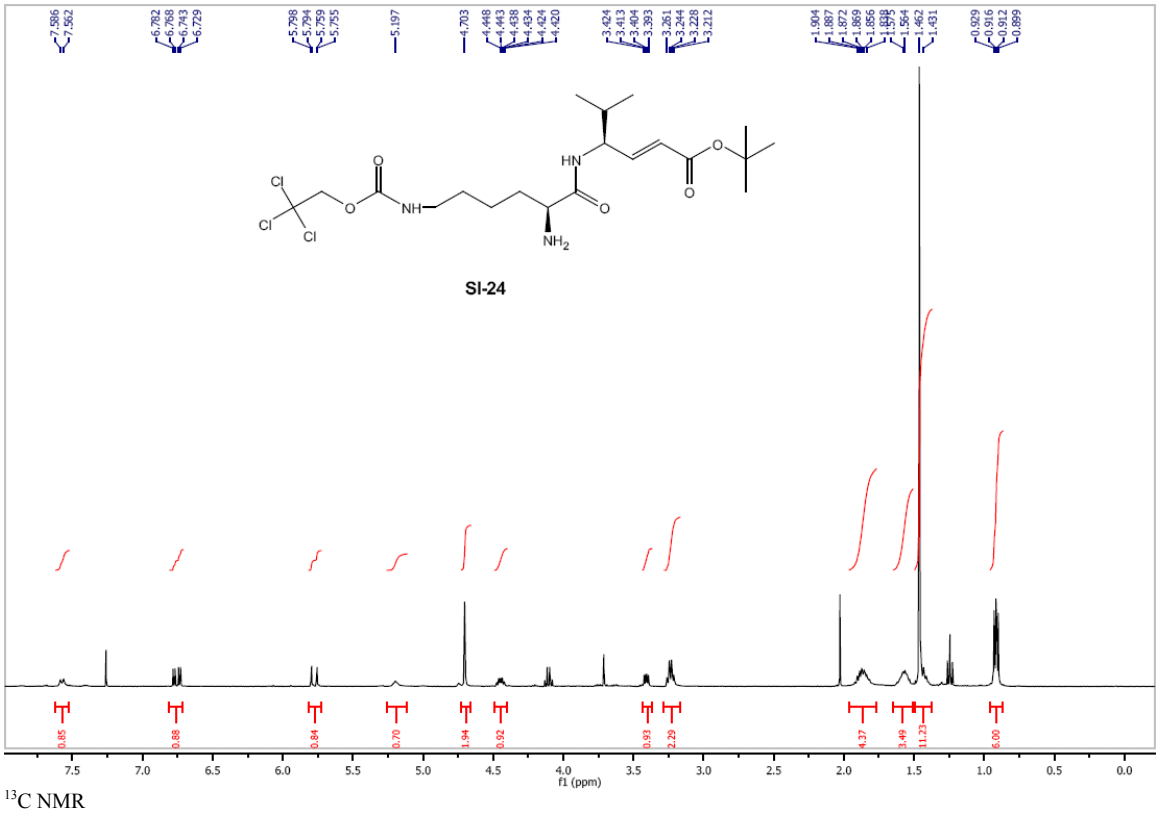
^{1}H NMR



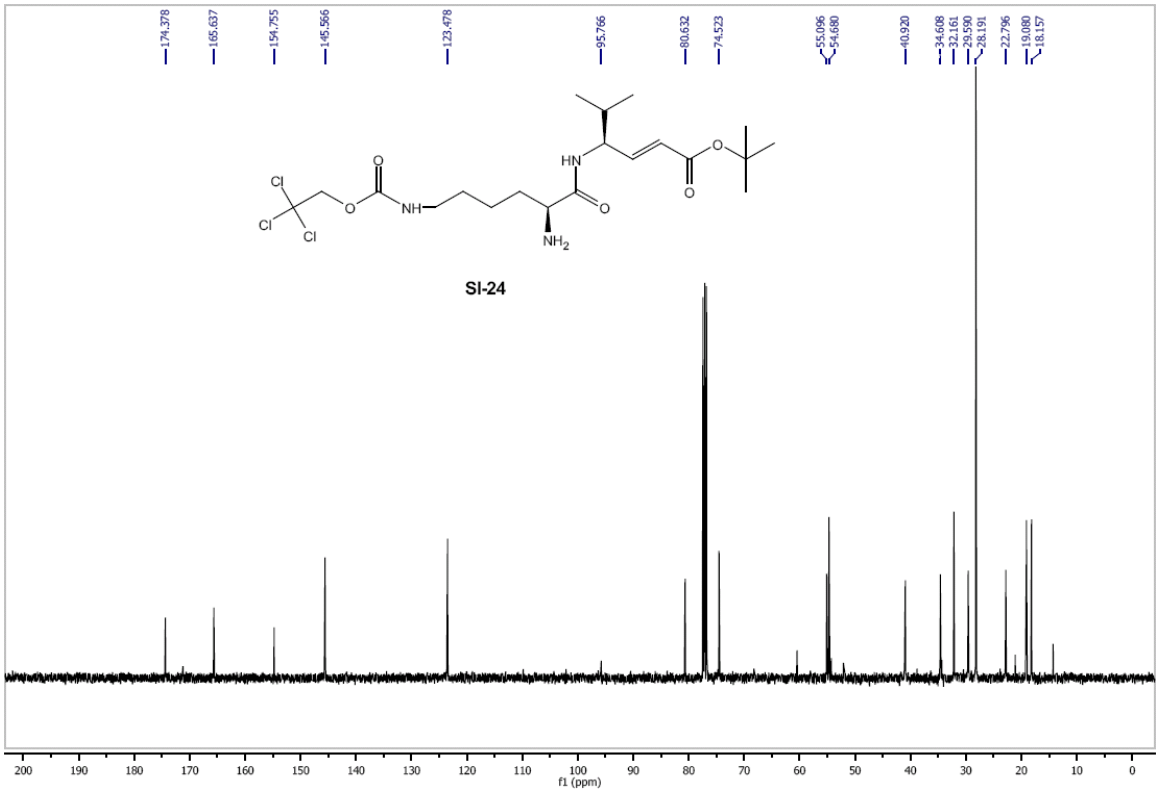
^{13}C NMR



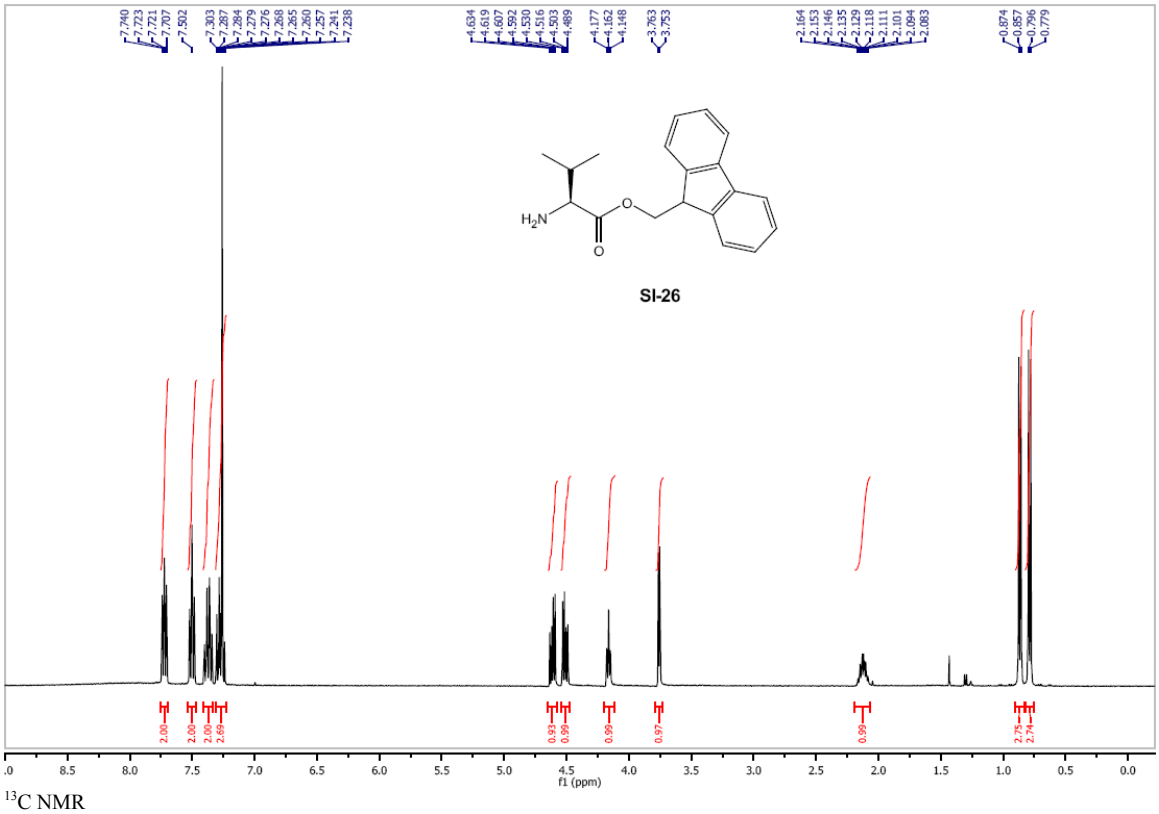
^{1}H NMR



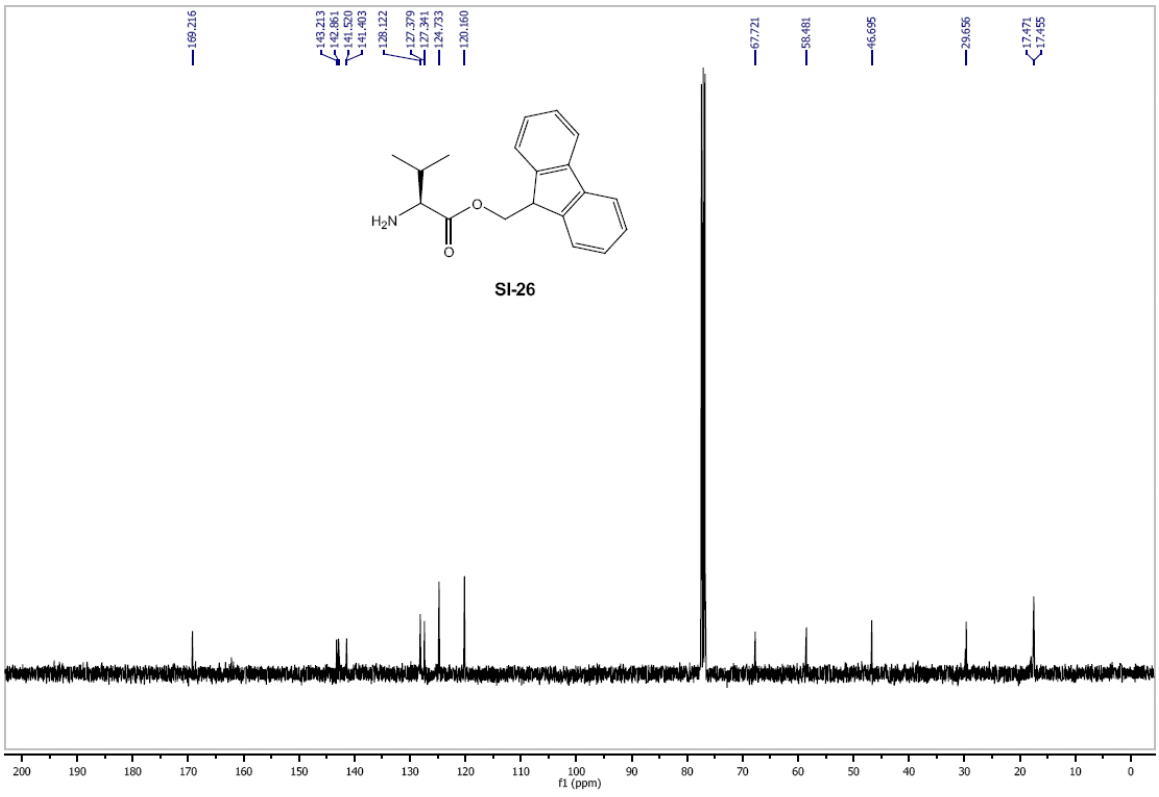
^{13}C NMR



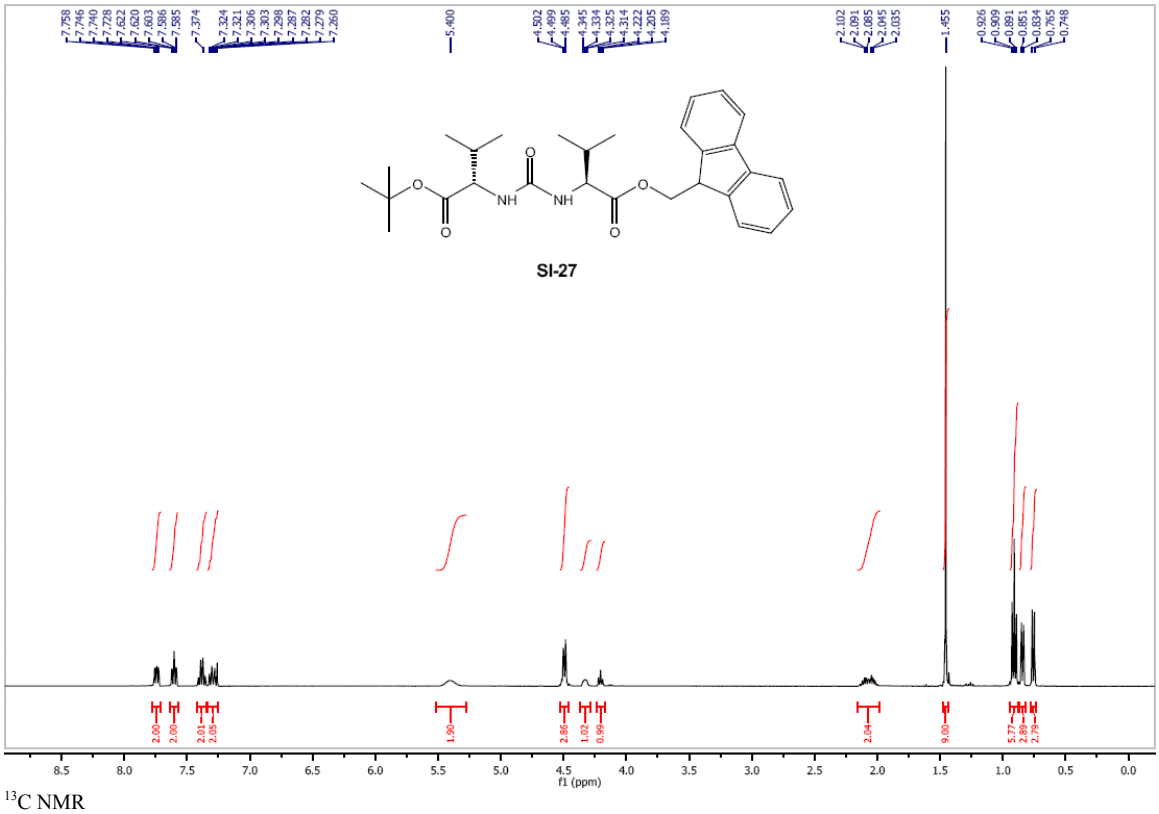
^{1}H NMR



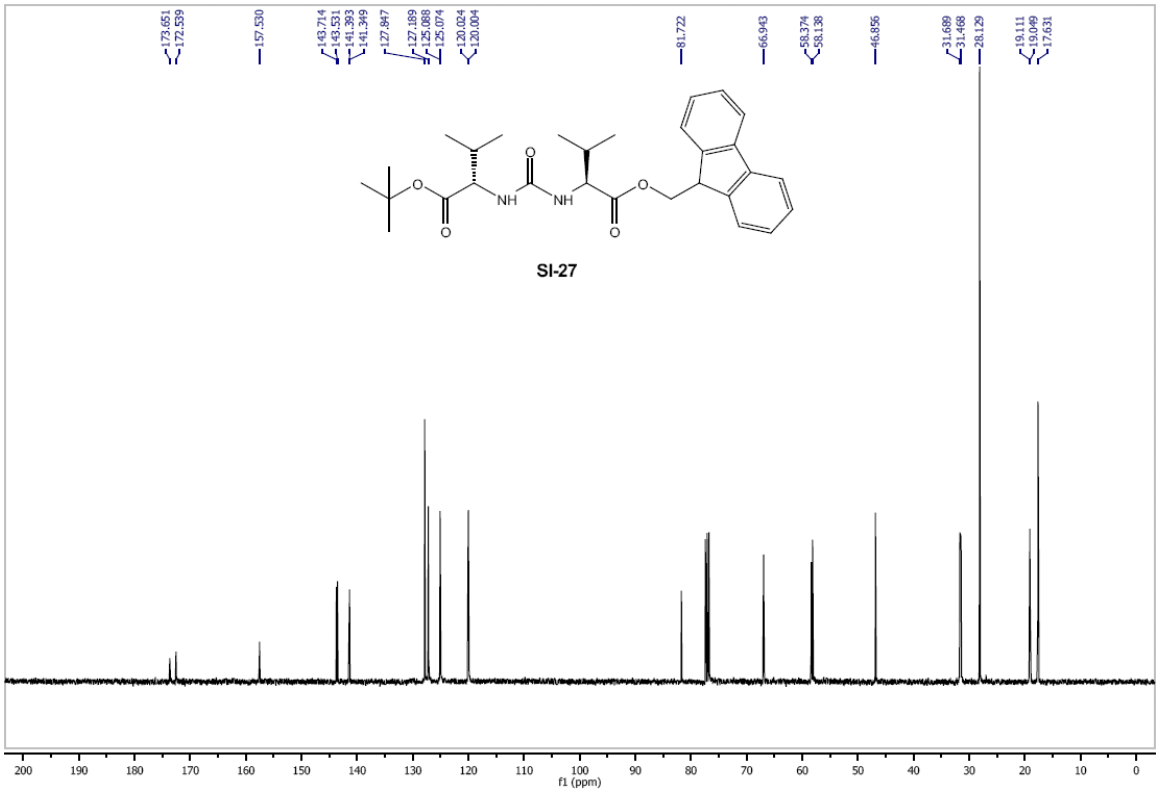
^{13}C NMR



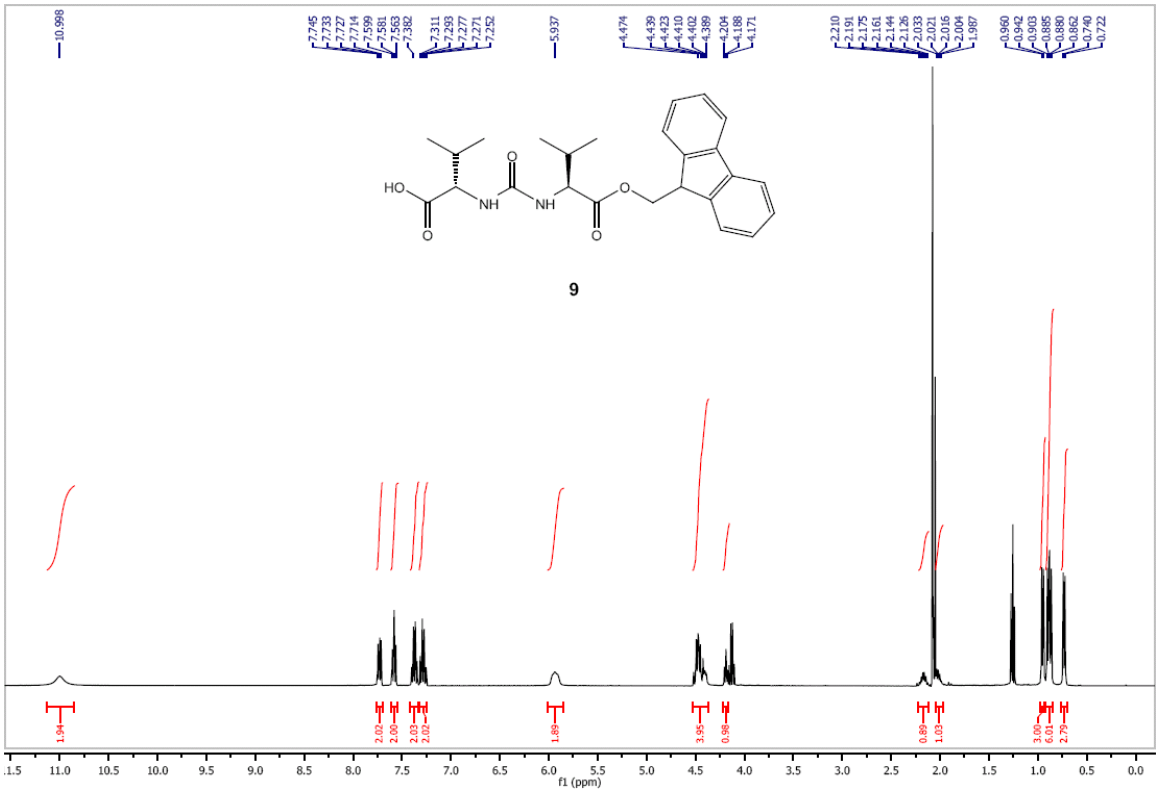
^{1}H NMR



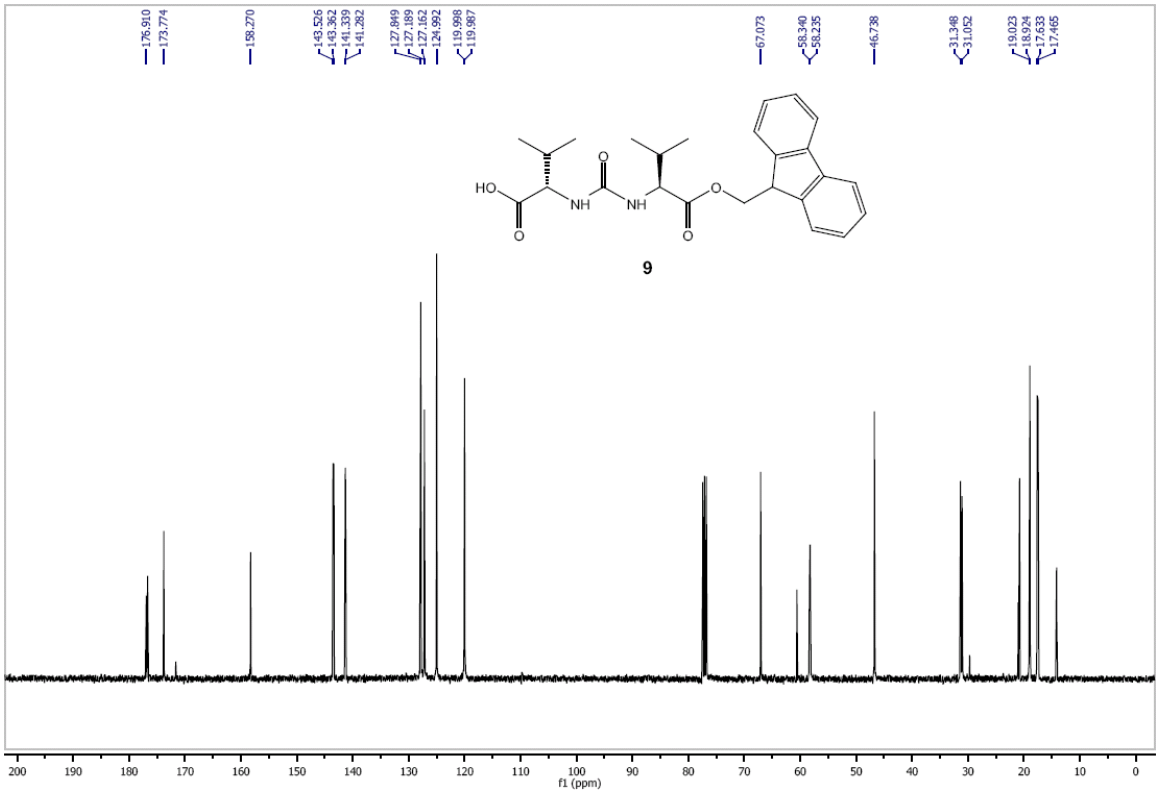
^{13}C NMR



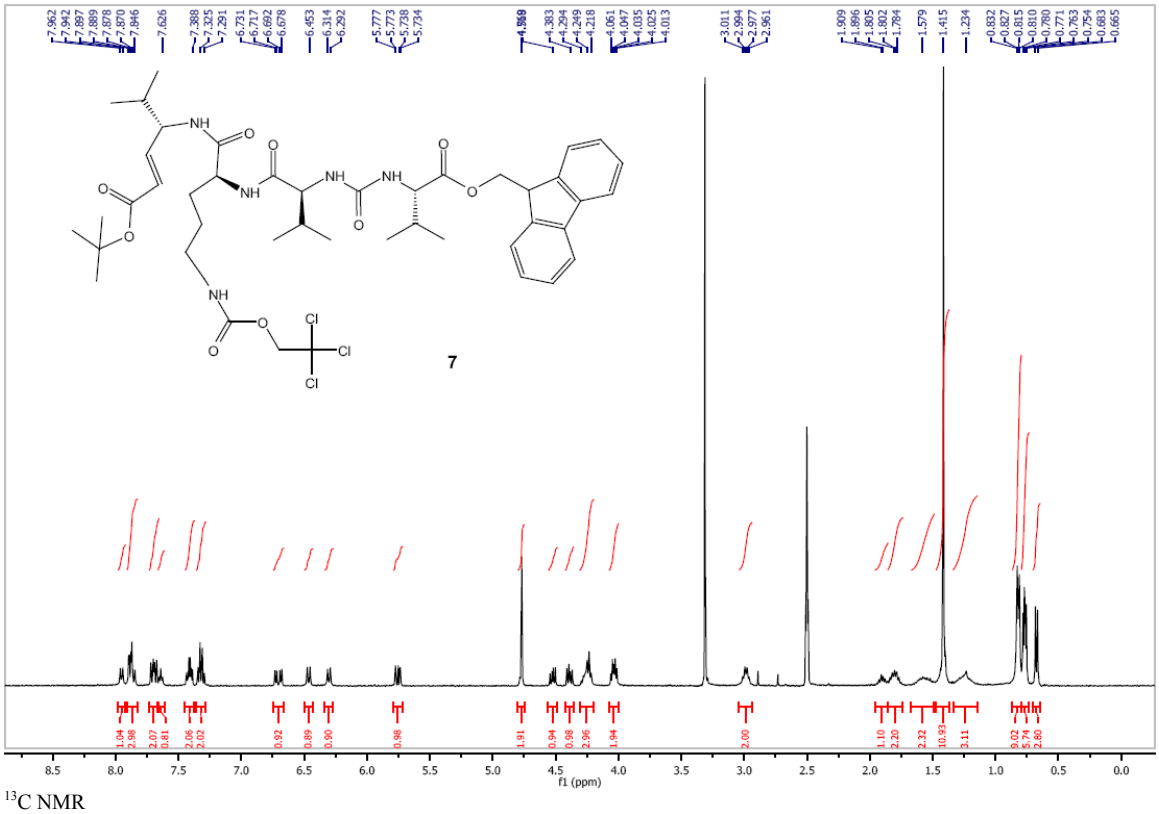
^{1}H NMR



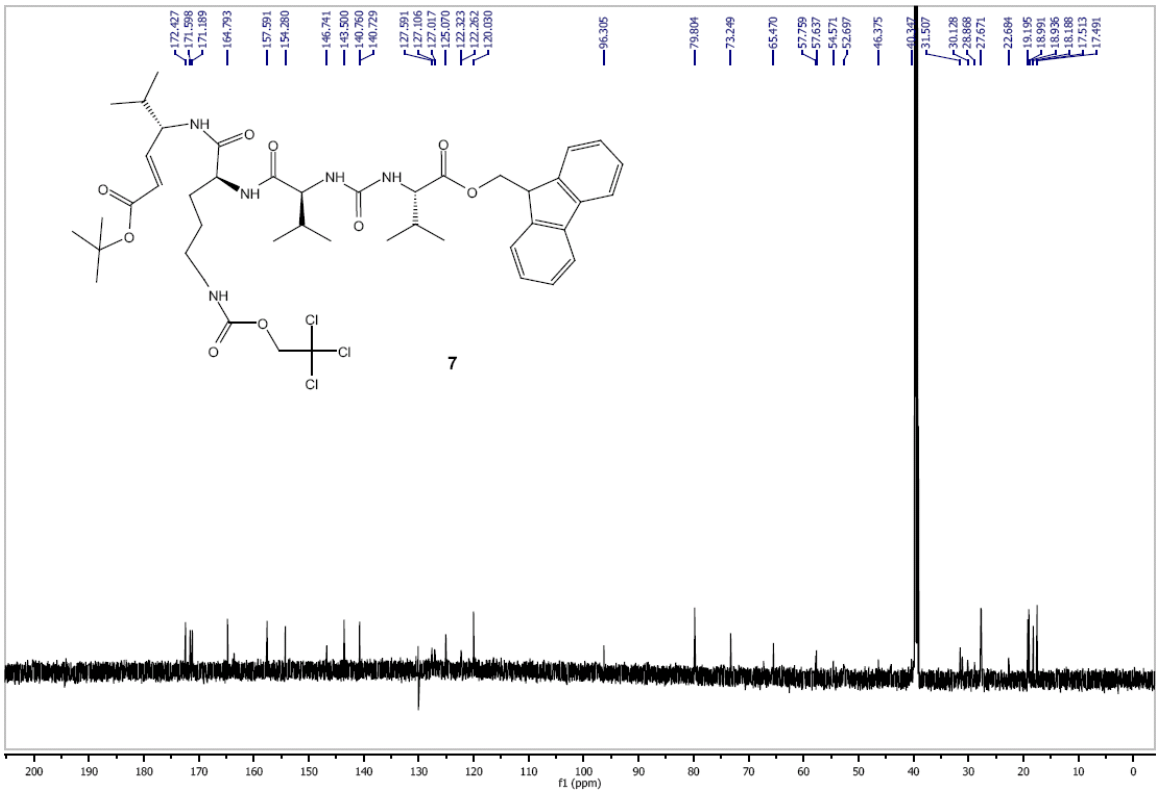
^{13}C NMR



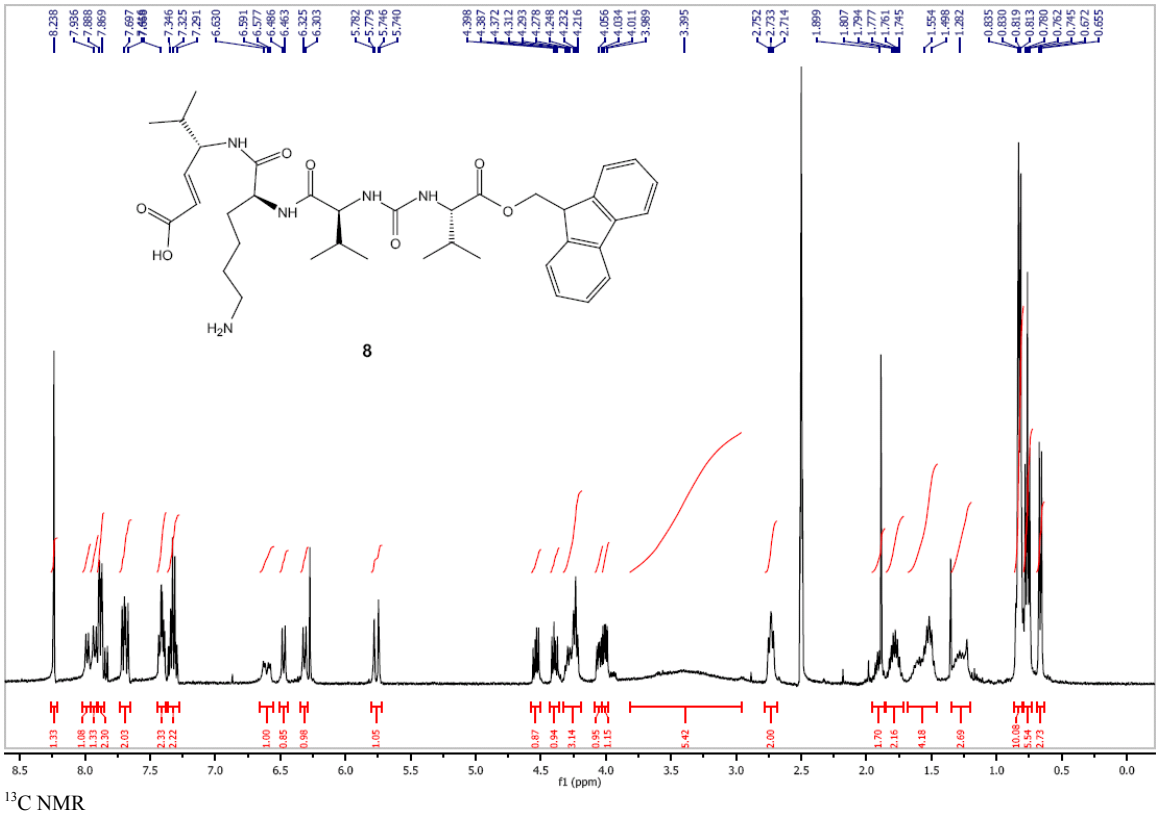
^{1}H NMR



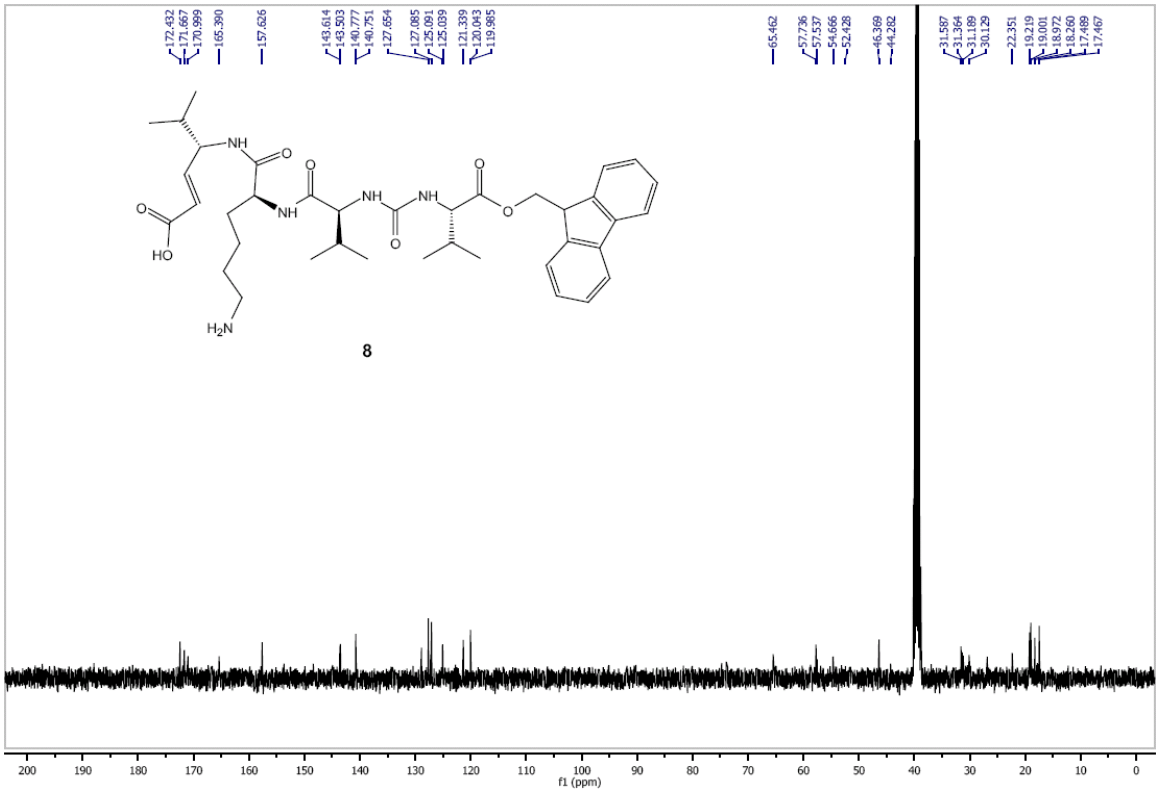
^{13}C NMR



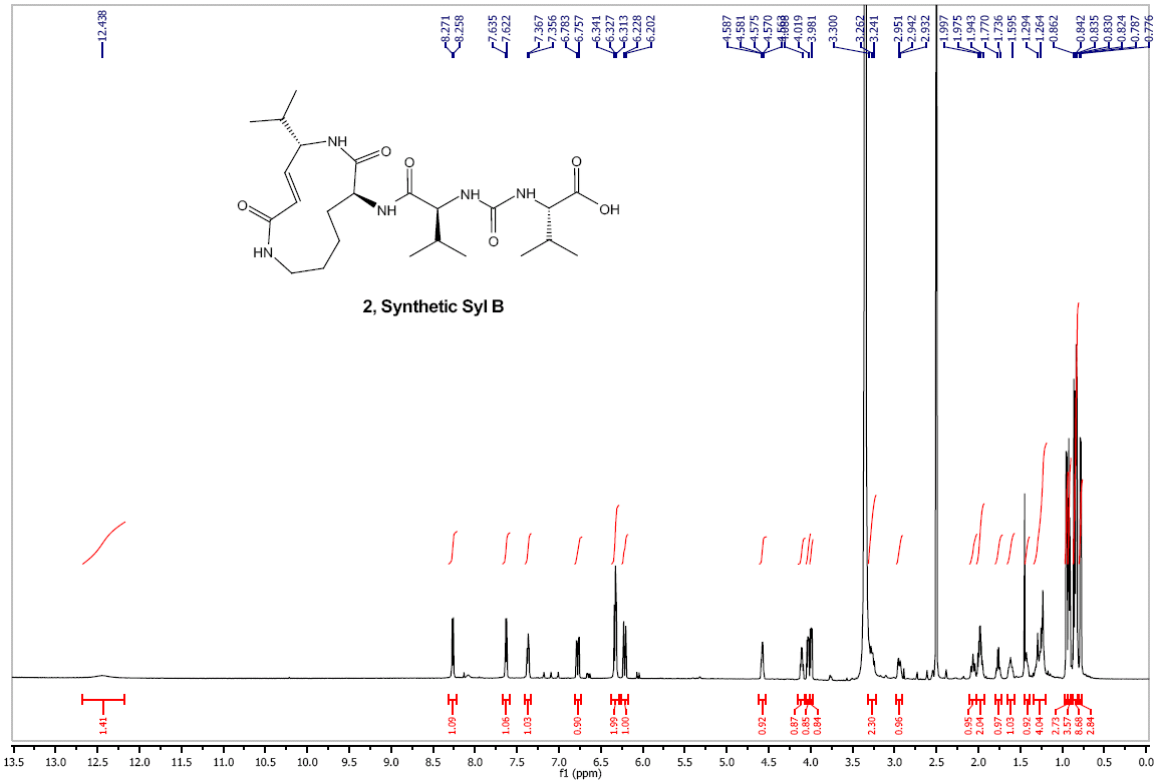
^{1}H NMR



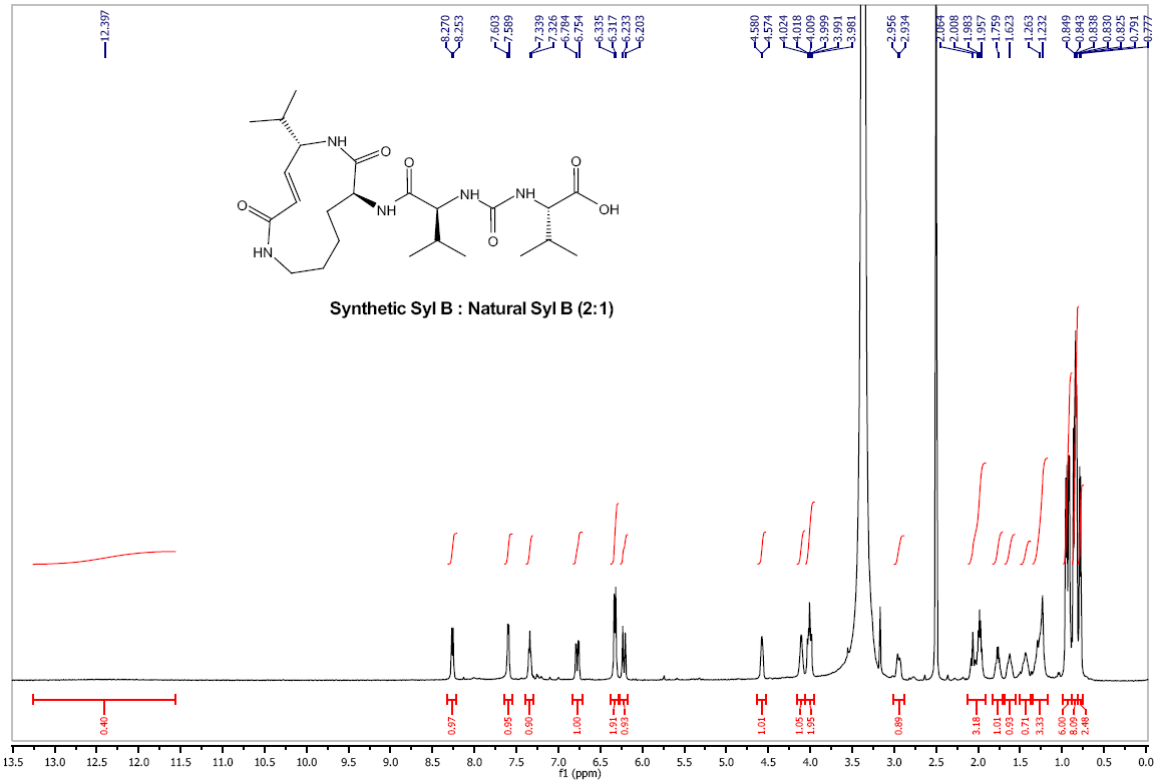
^{13}C NMR



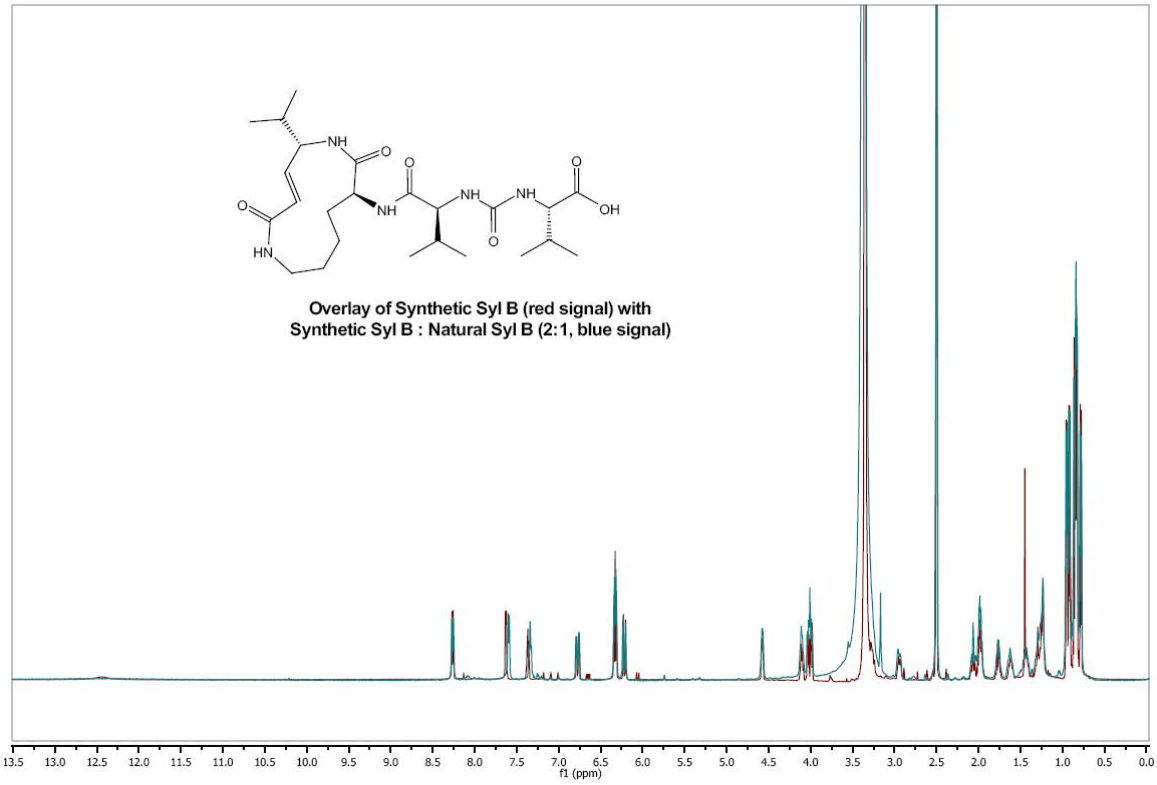
^{1}H NMR


2, Synthetic Syl B
f1 (ppm)

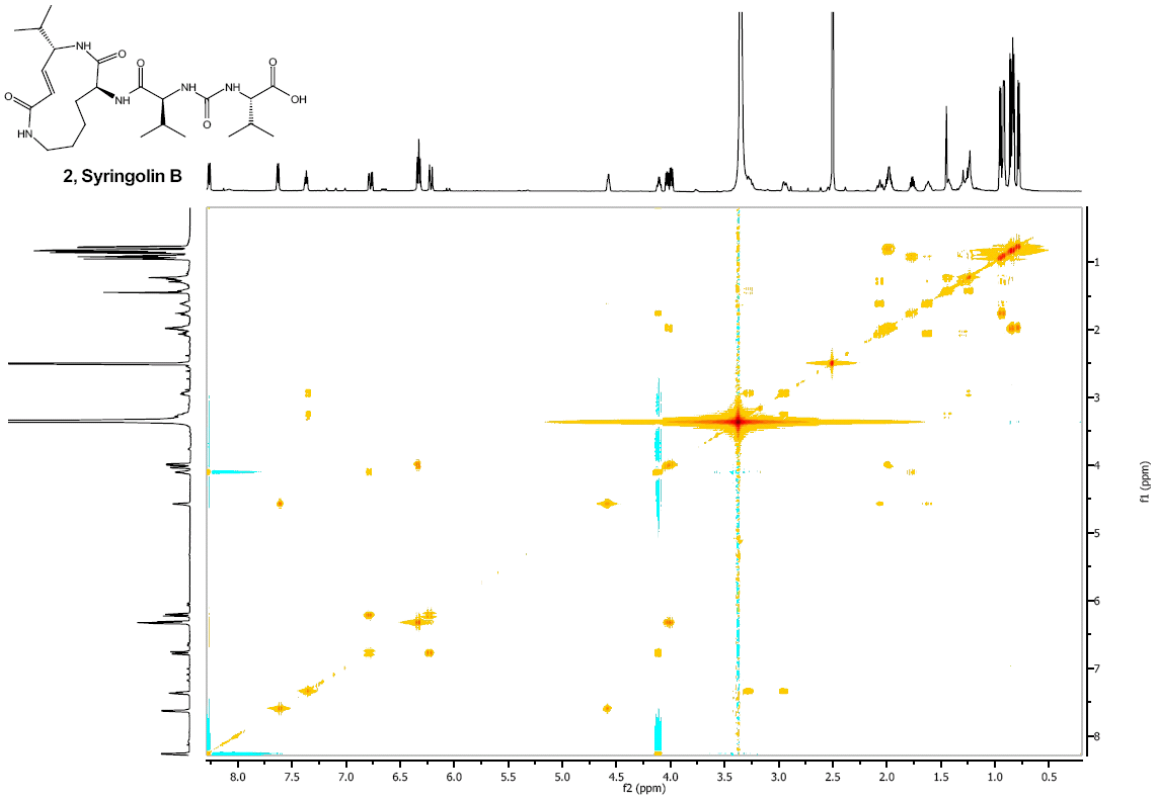
^{1}H NMR


Synthetic Syl B : Natural Syl B (2:1)
f1 (ppm)

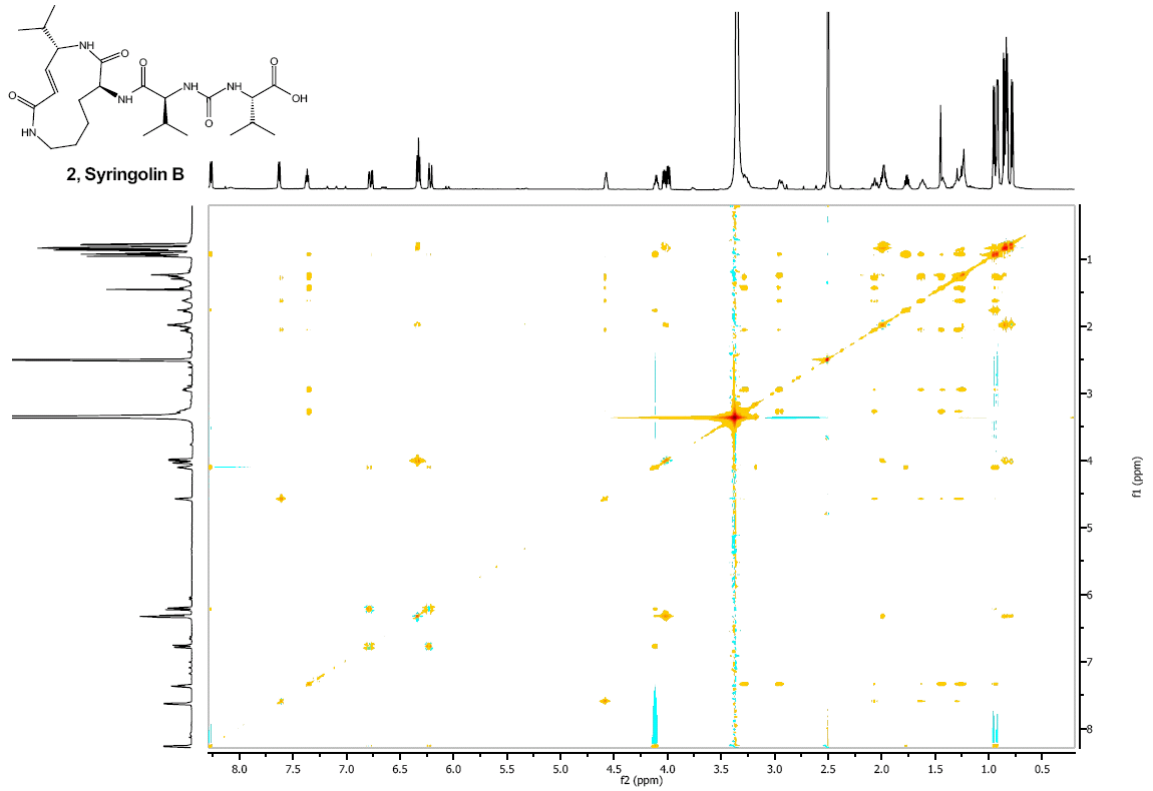
^{1}H NMR (overlay)



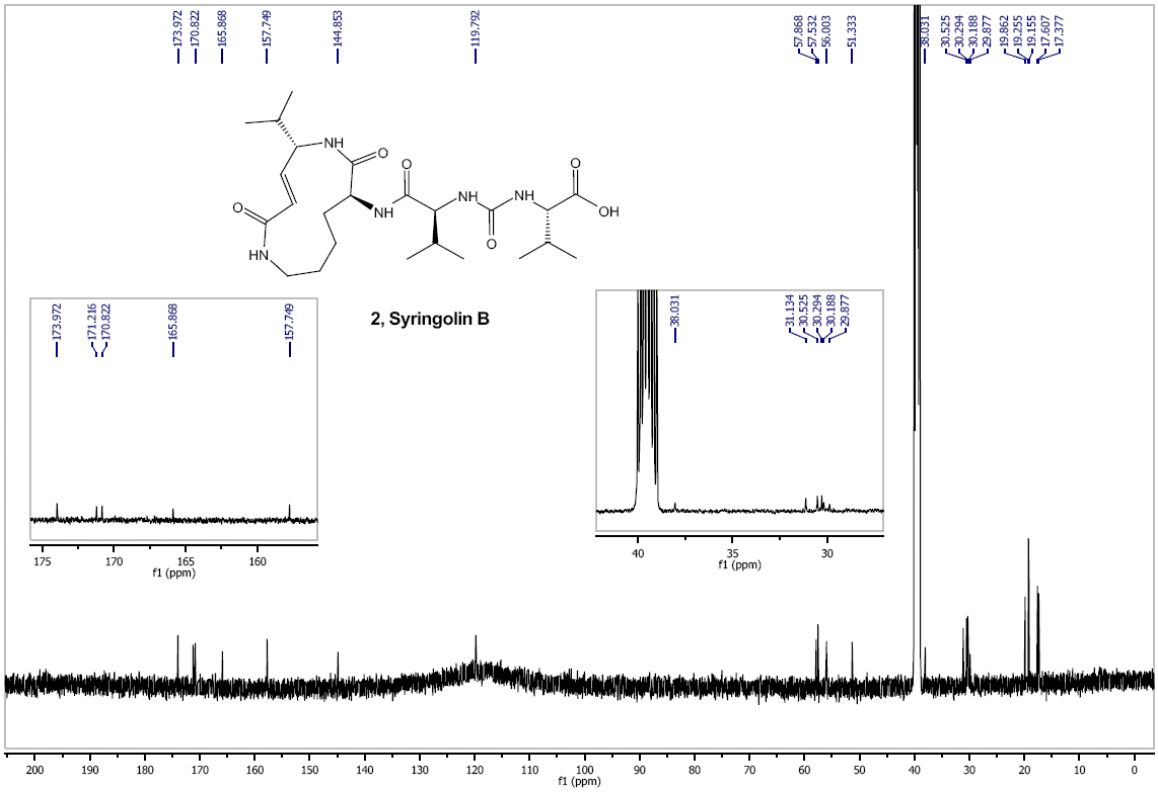
COSY



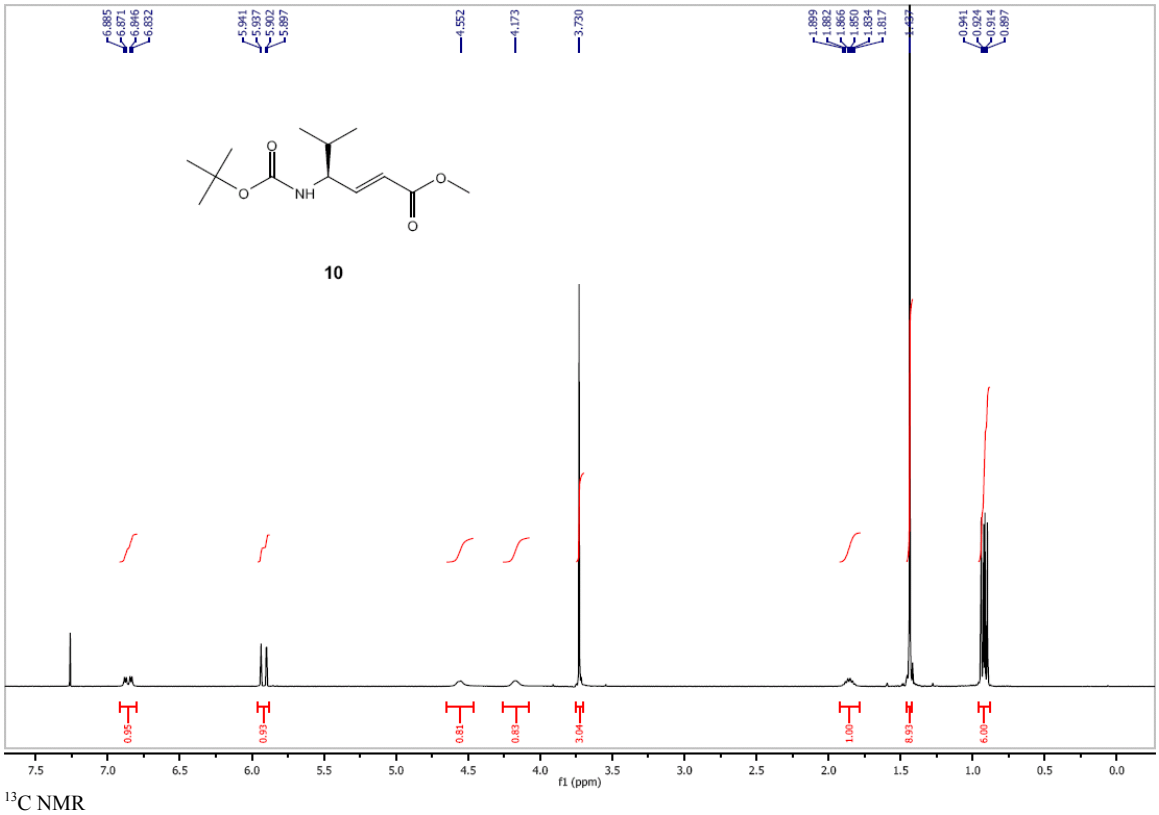
TOCSY



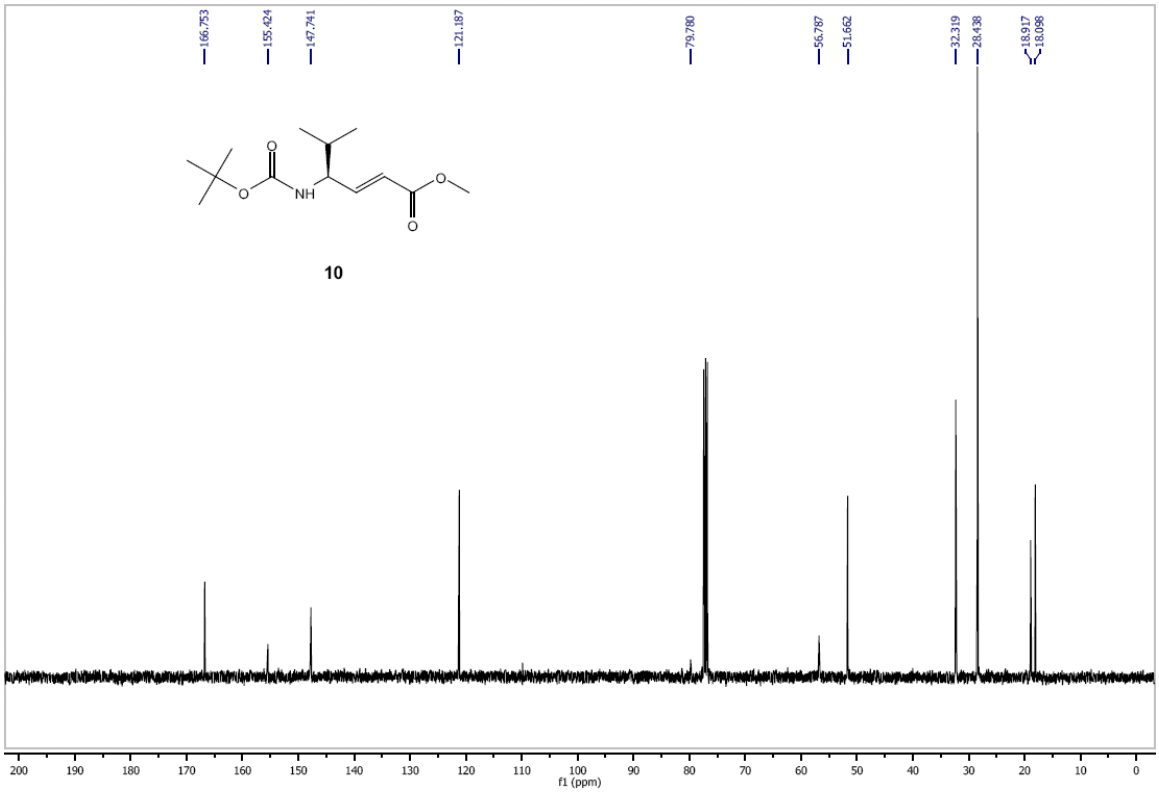
^{13}C NMR



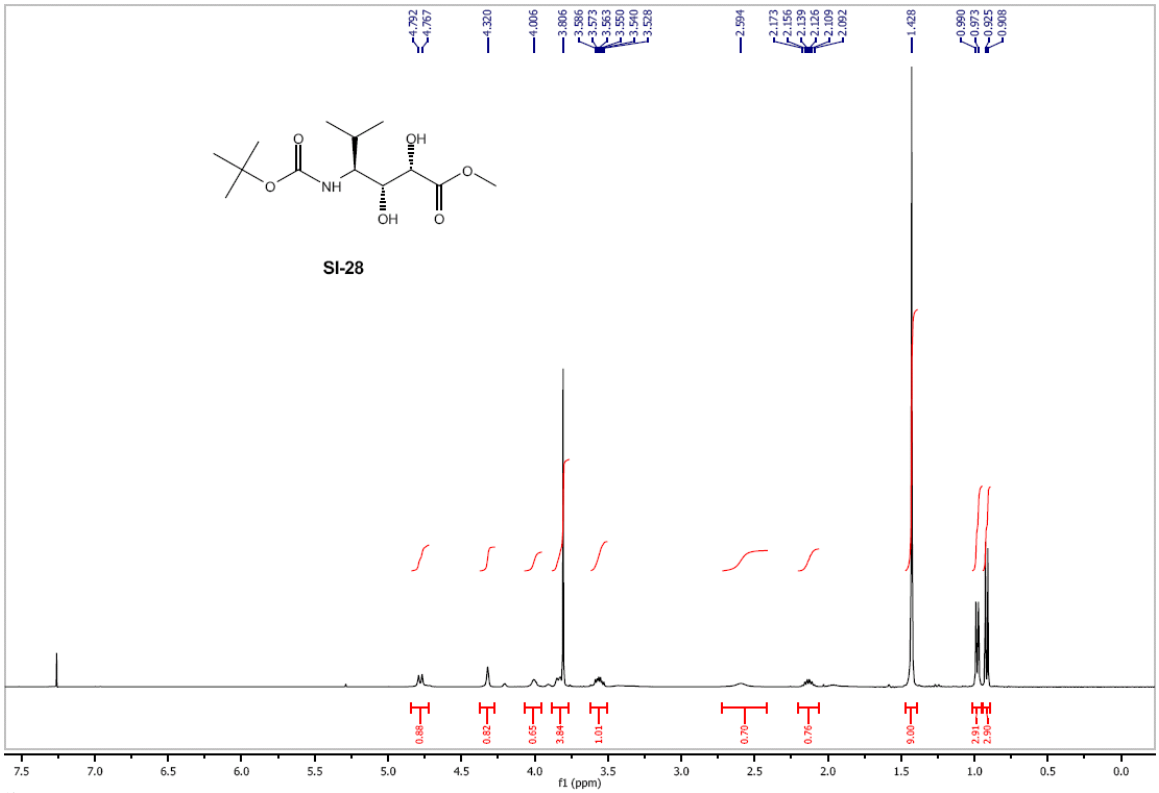
^{1}H NMR



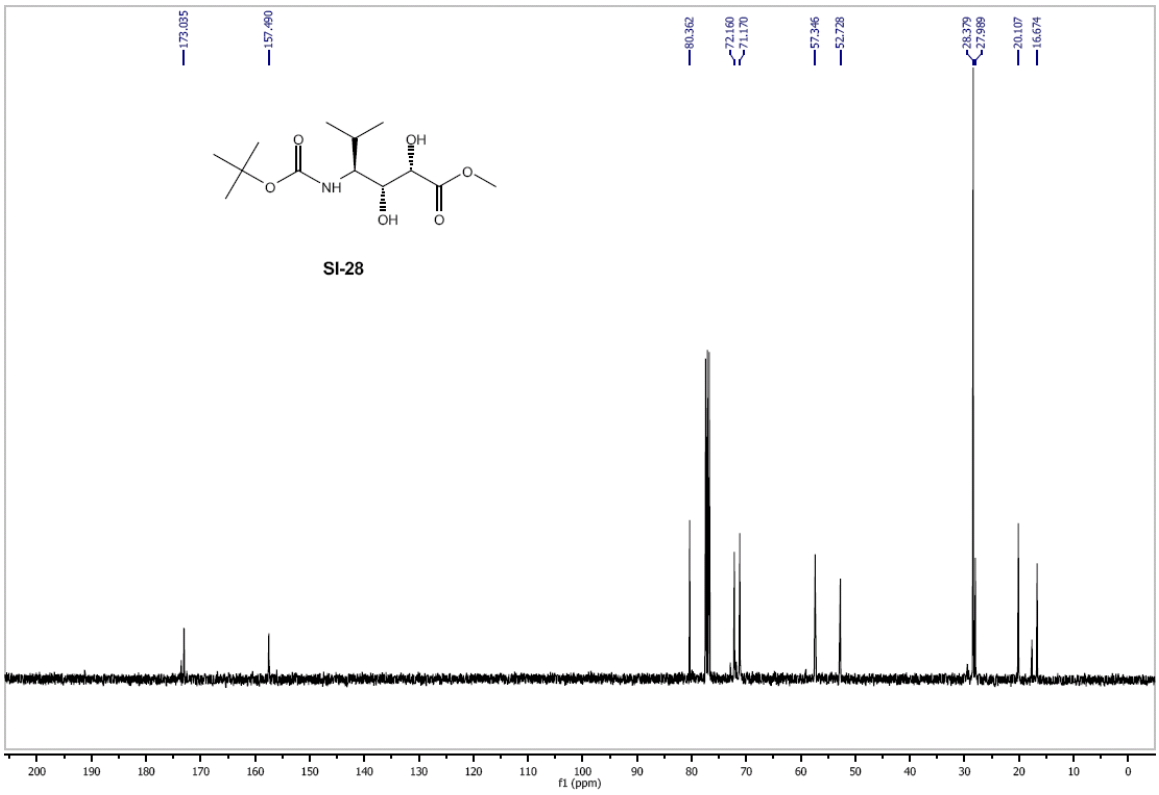
^{13}C NMR



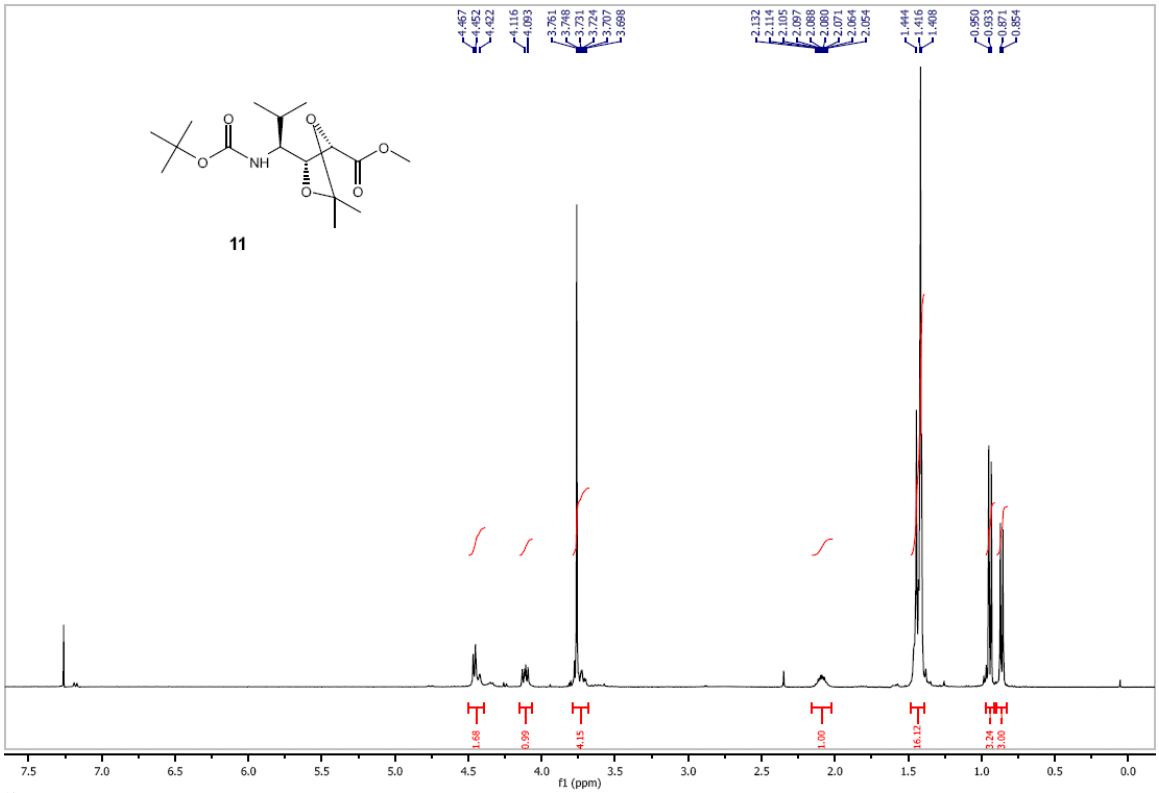
^{1}H NMR



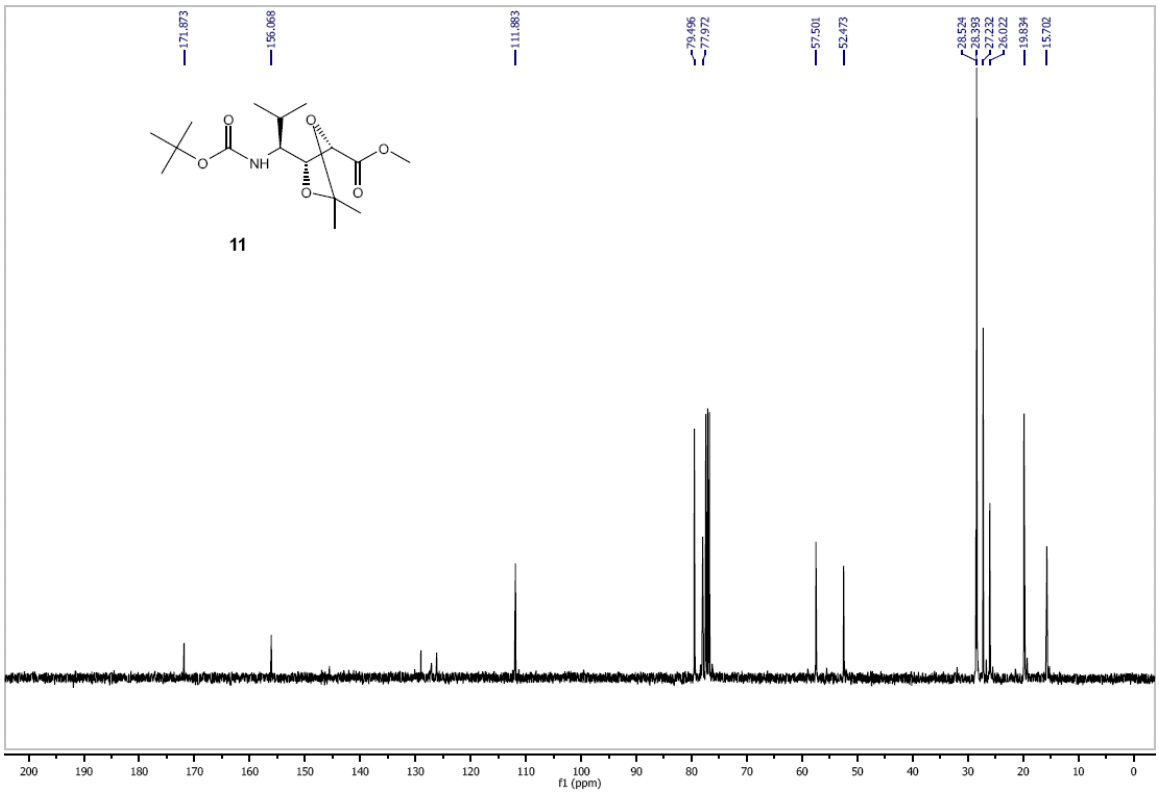
^{13}C NMR



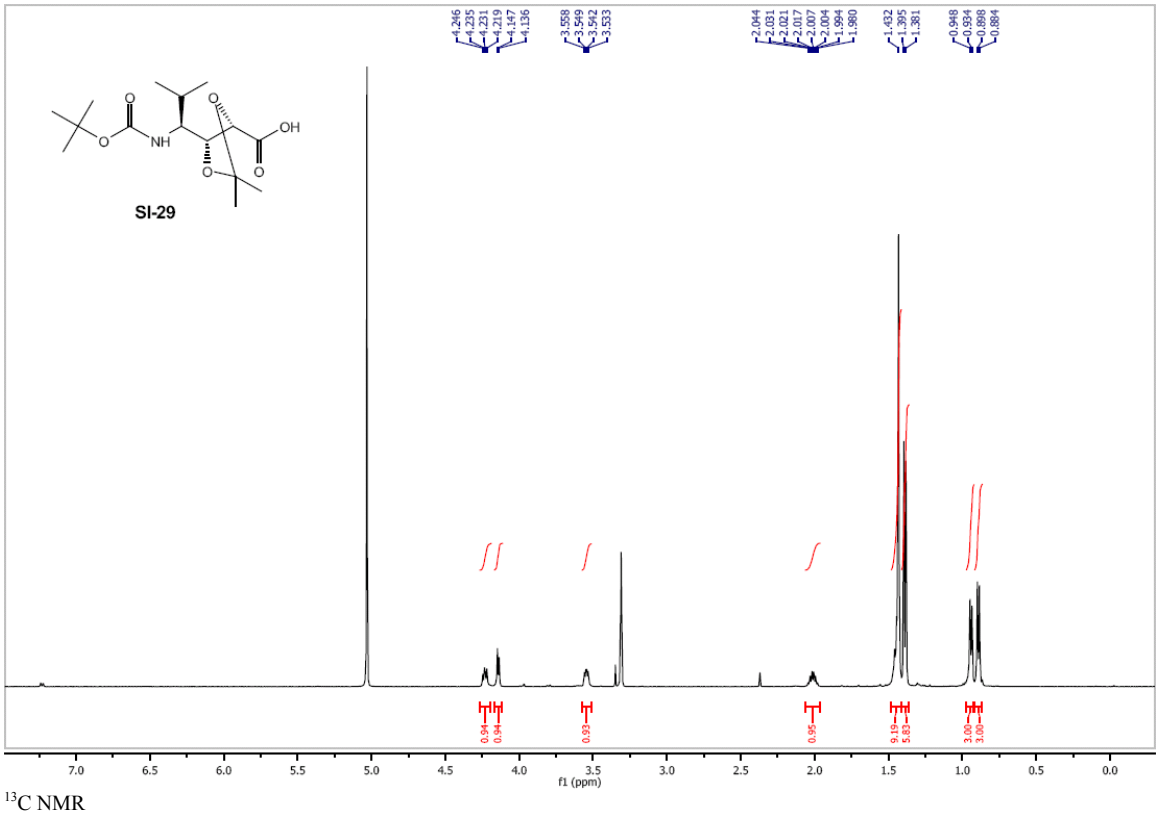
^{1}H NMR



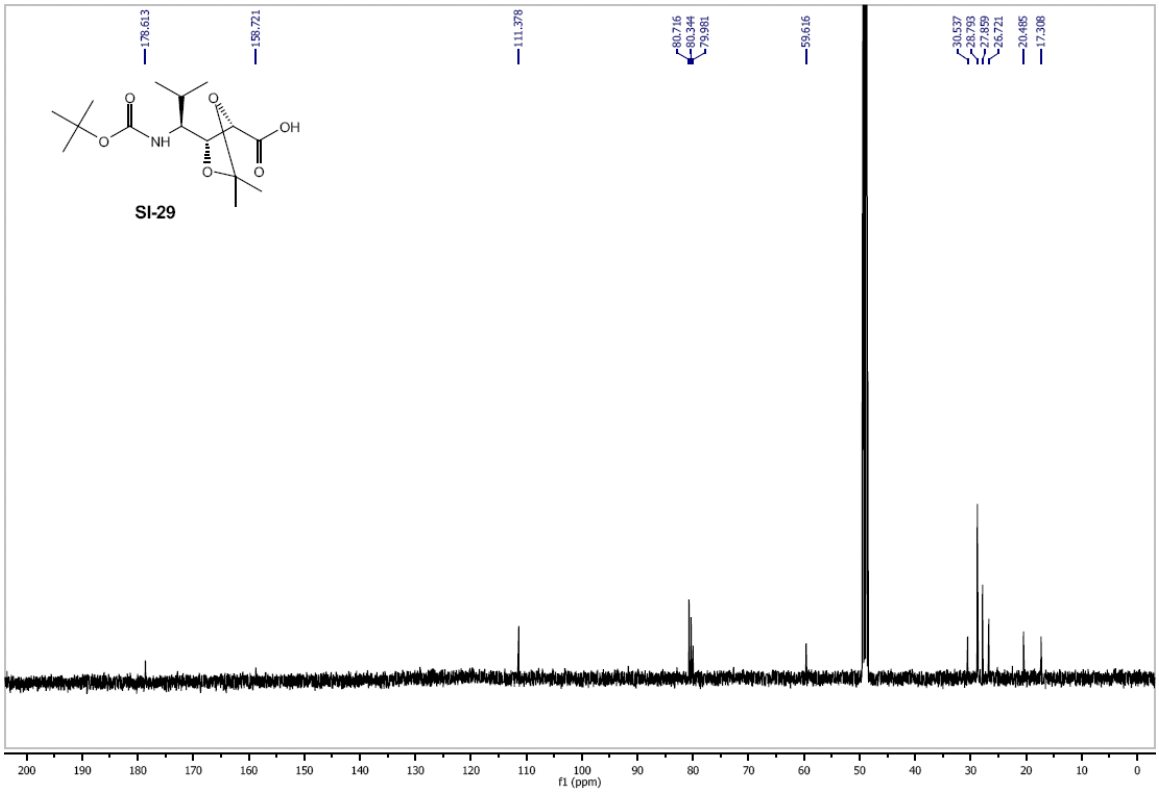
^{13}C NMR



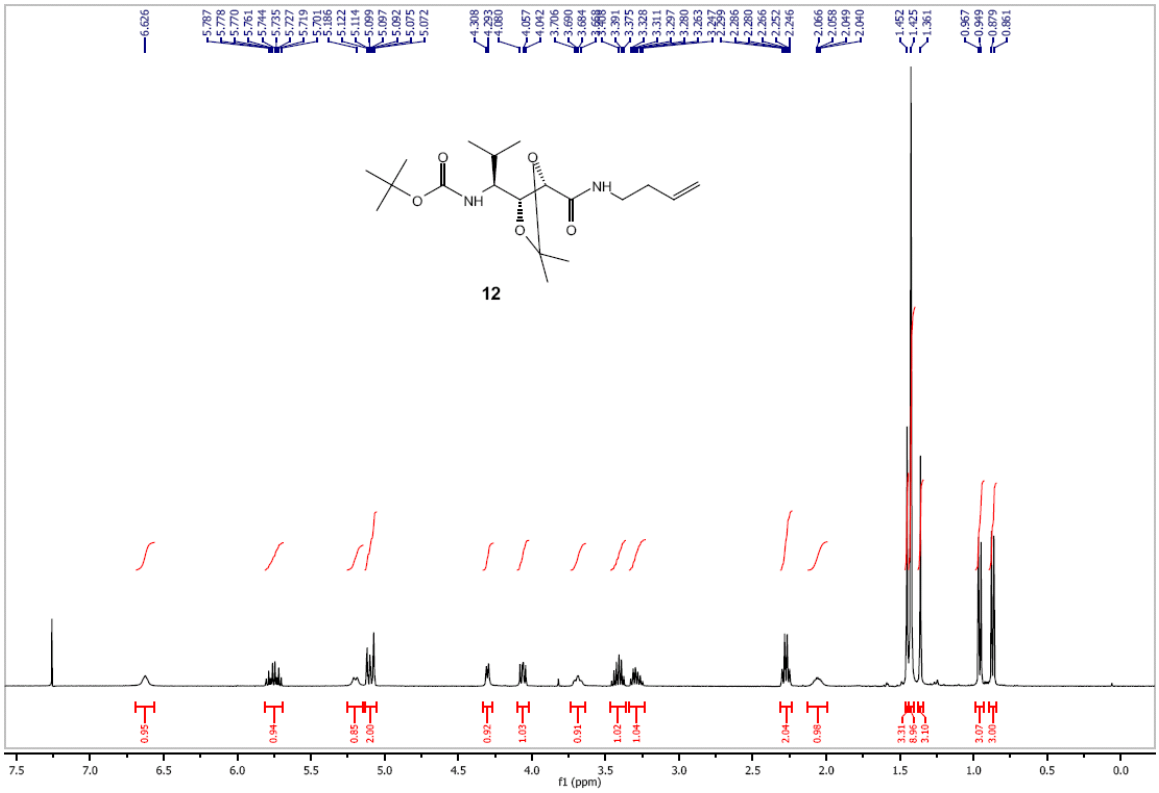
^{1}H NMR



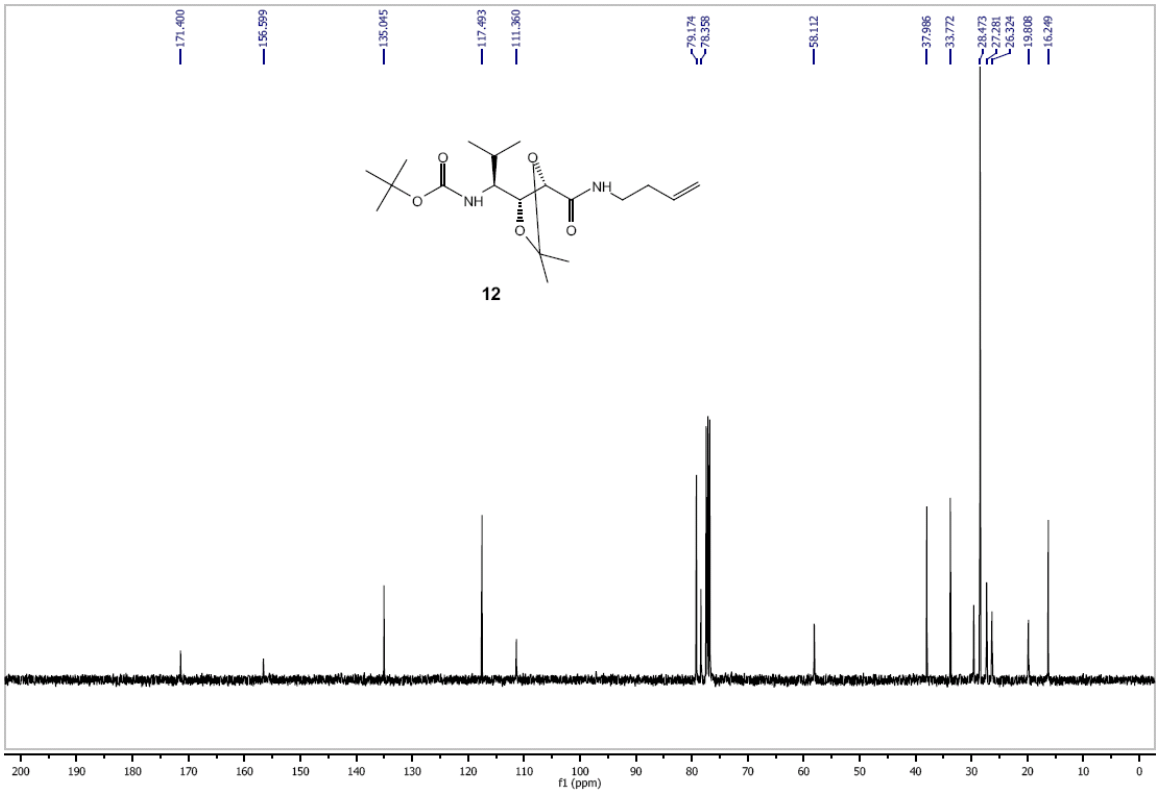
^{13}C NMR



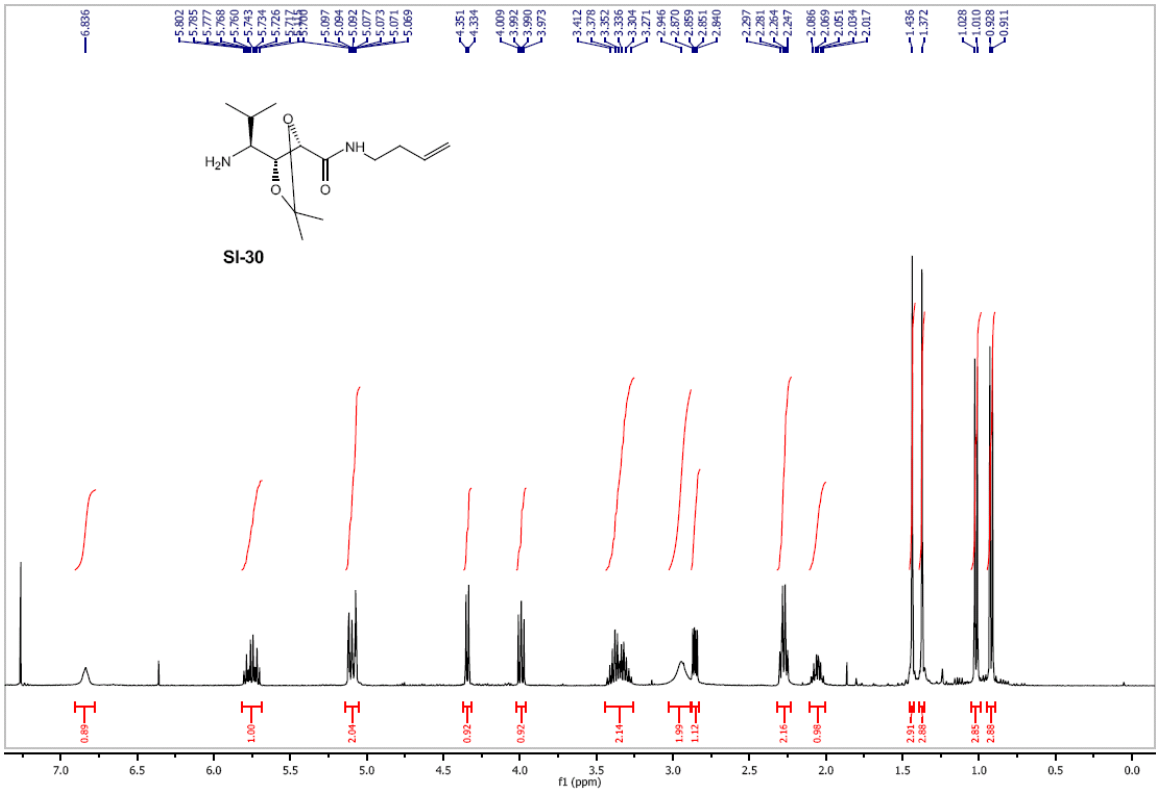
^{1}H NMR



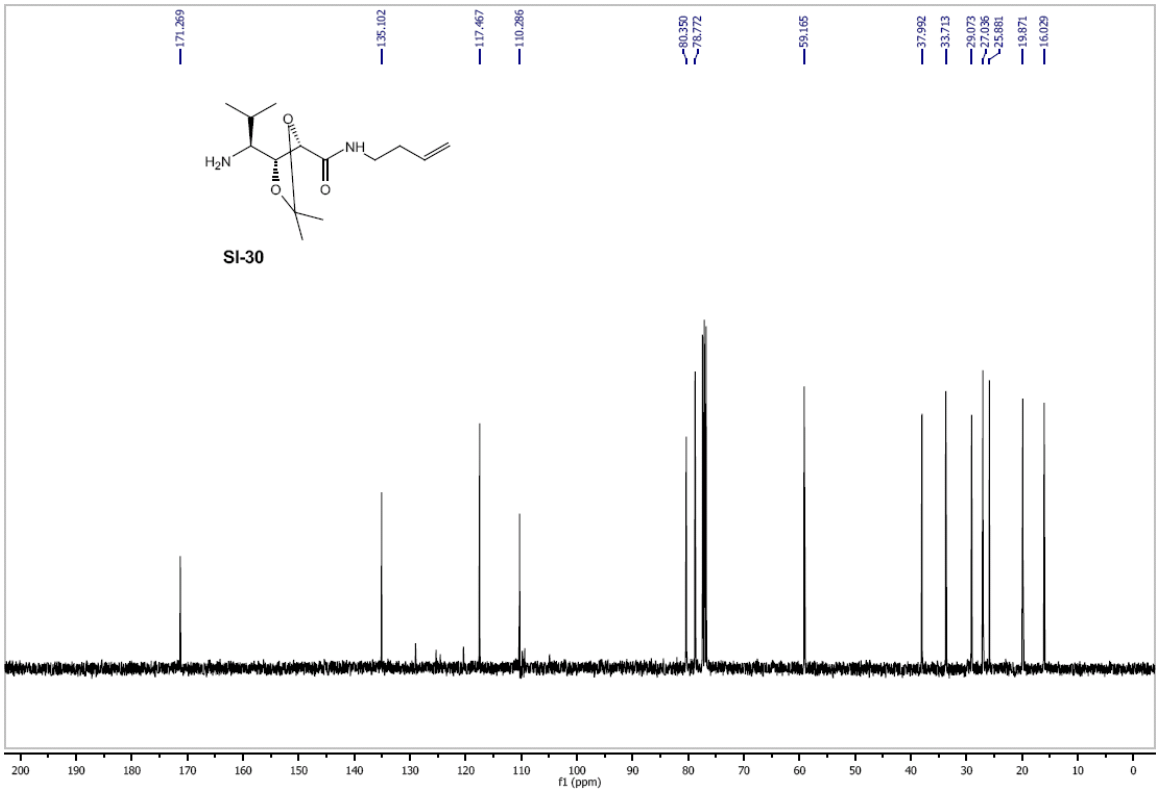
^{13}C NMR



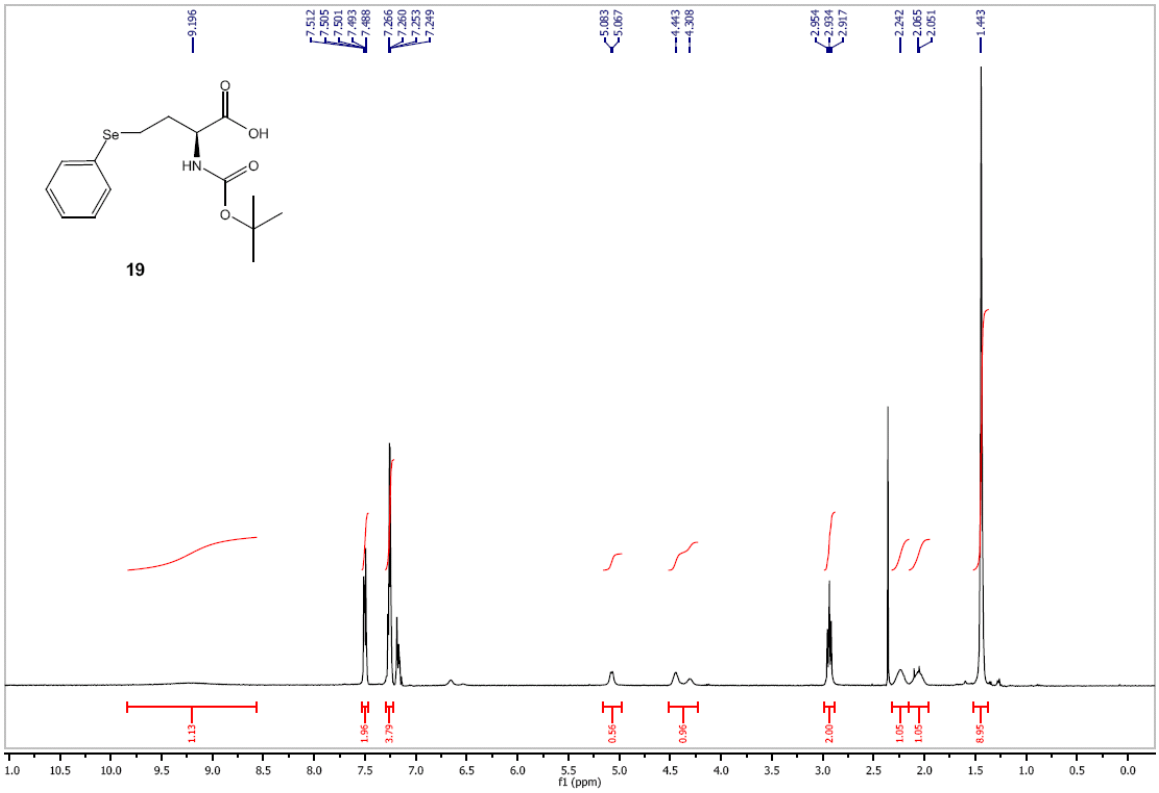
^{1}H NMR



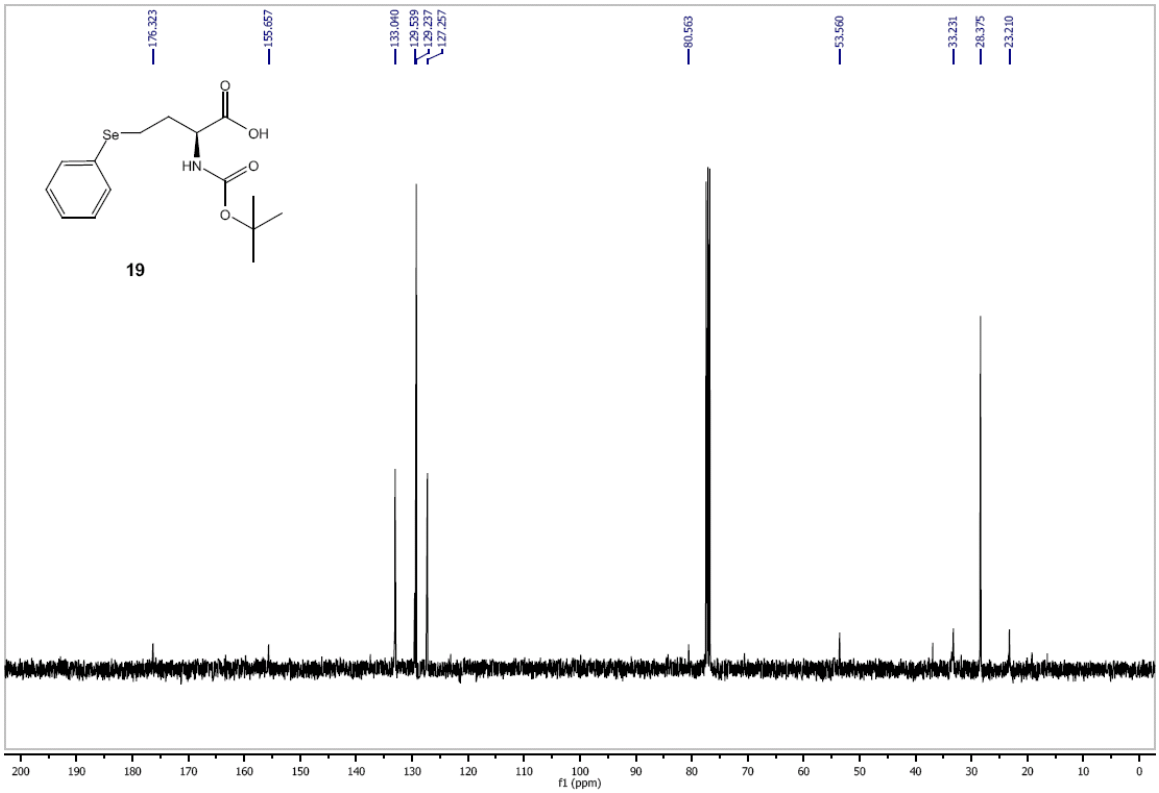
^{13}C NMR



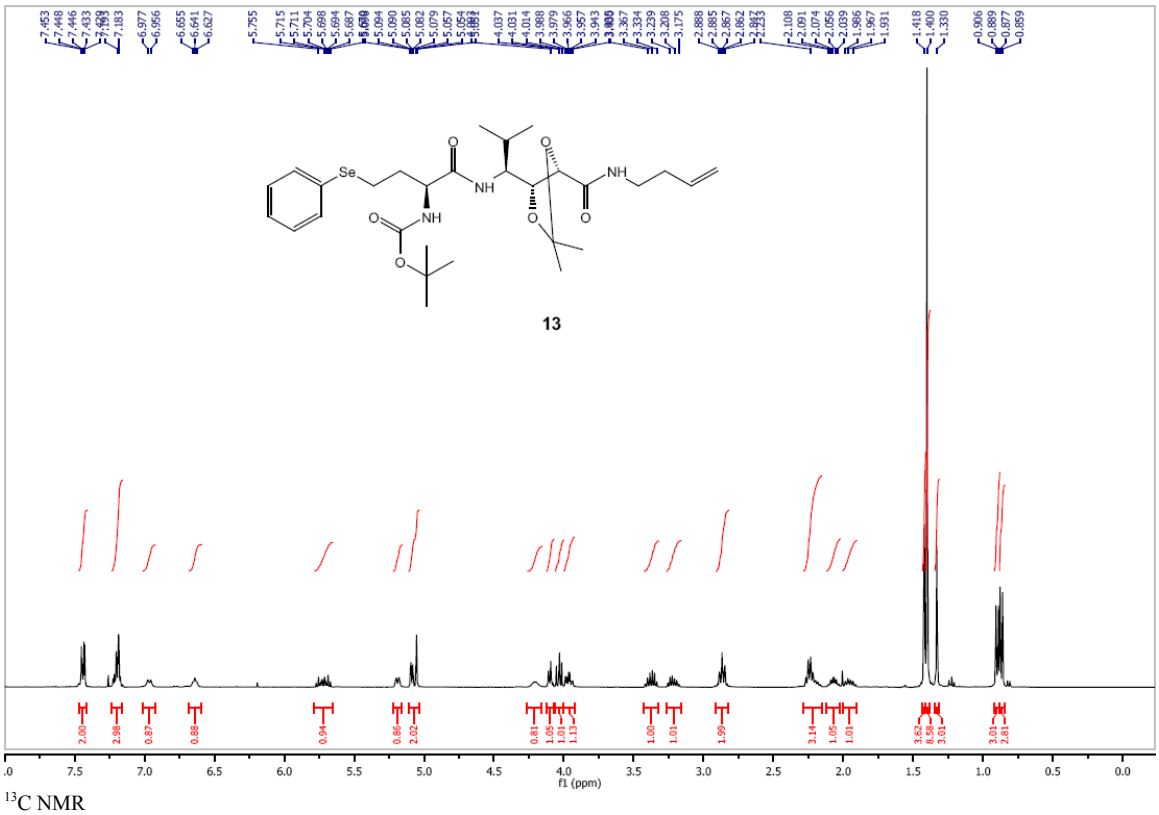
^{1}H NMR



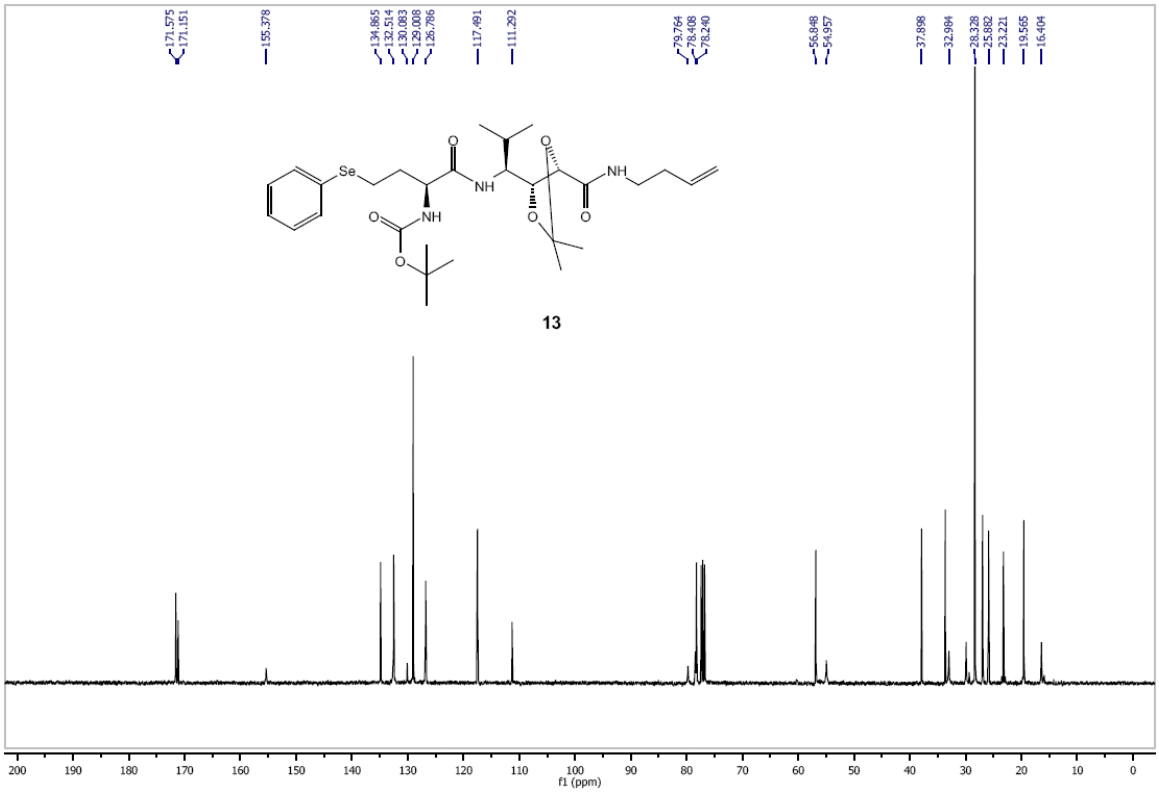
^{13}C NMR



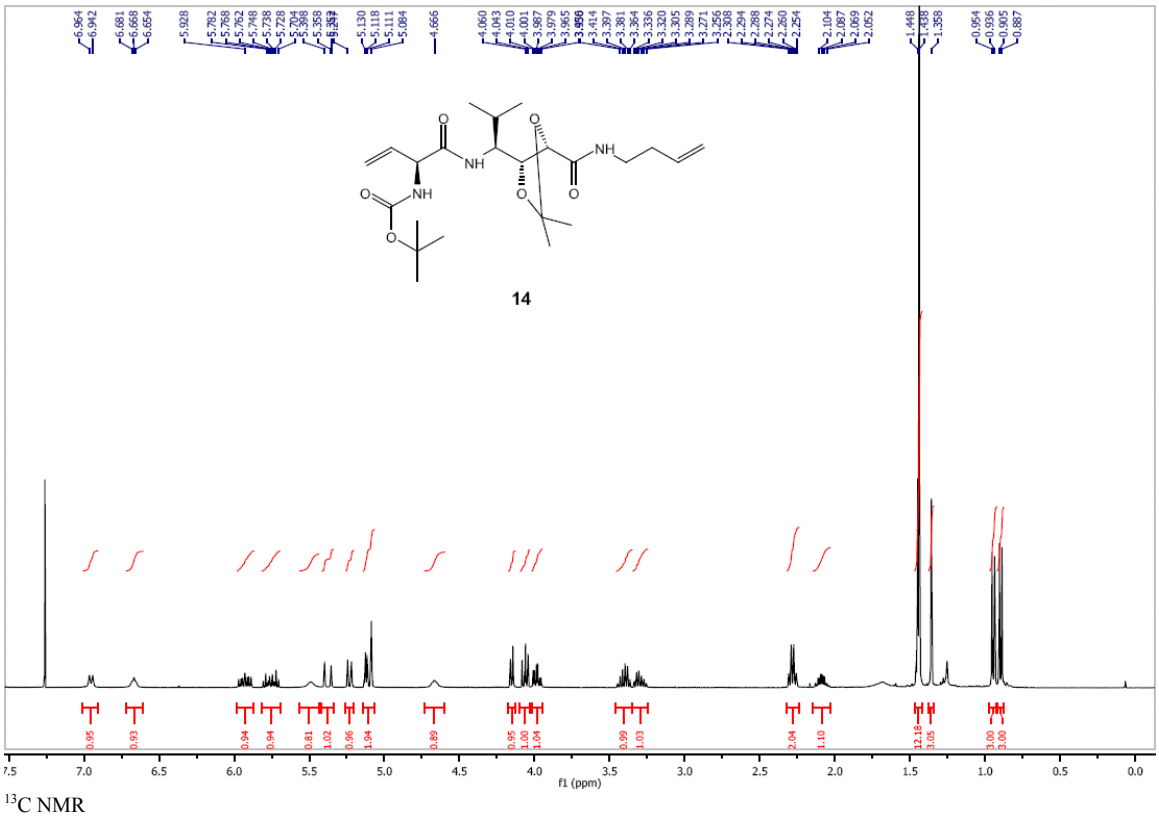
^{1}H NMR



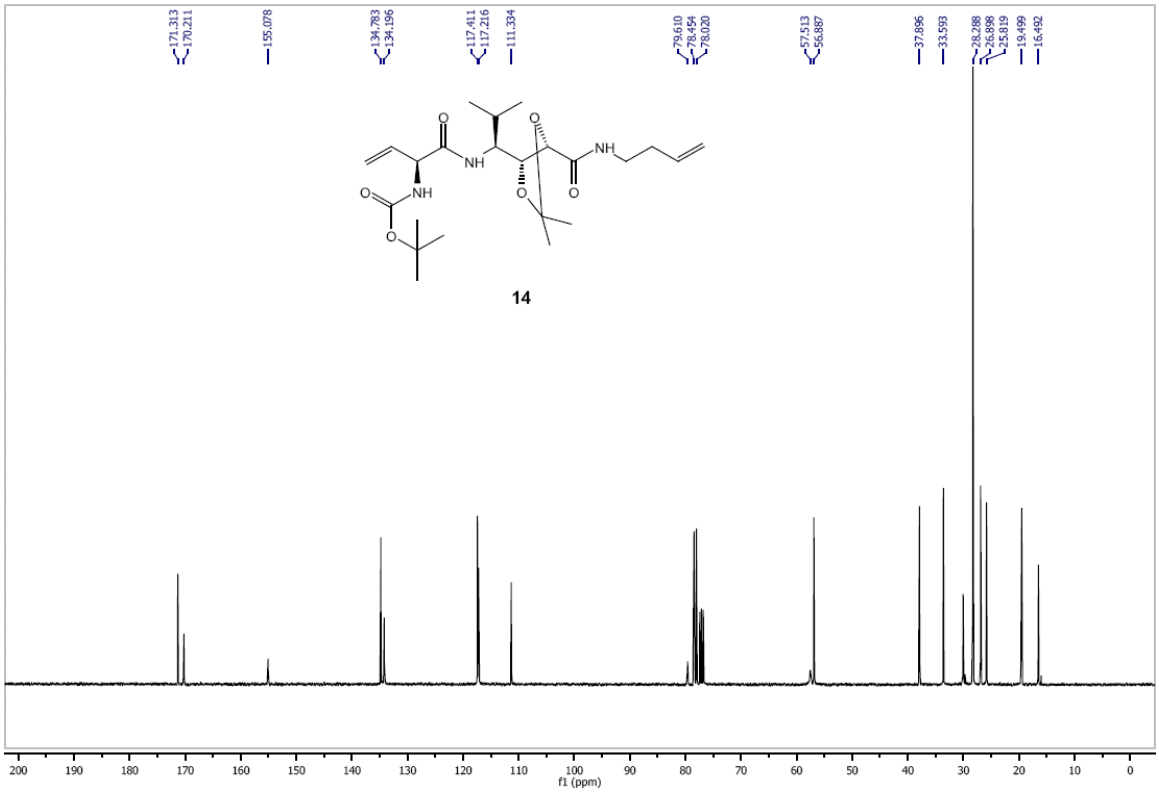
^{13}C NMR



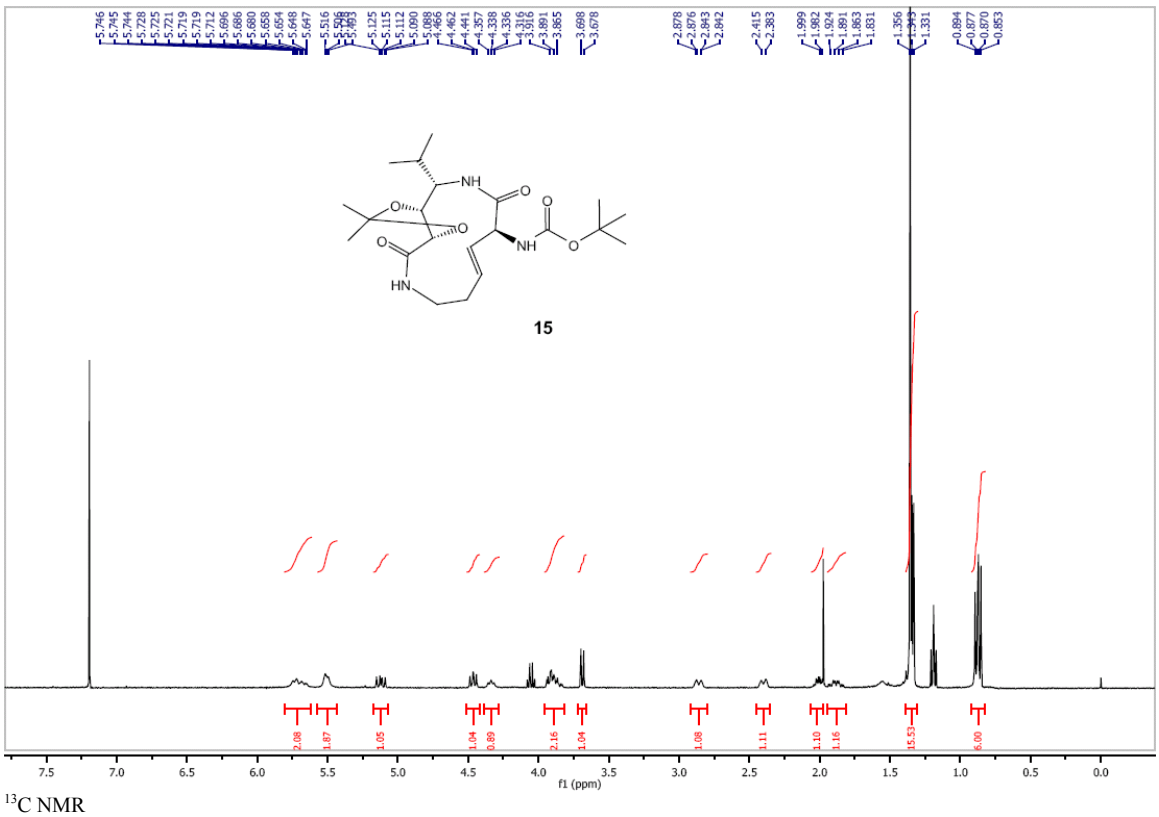
^{1}H NMR



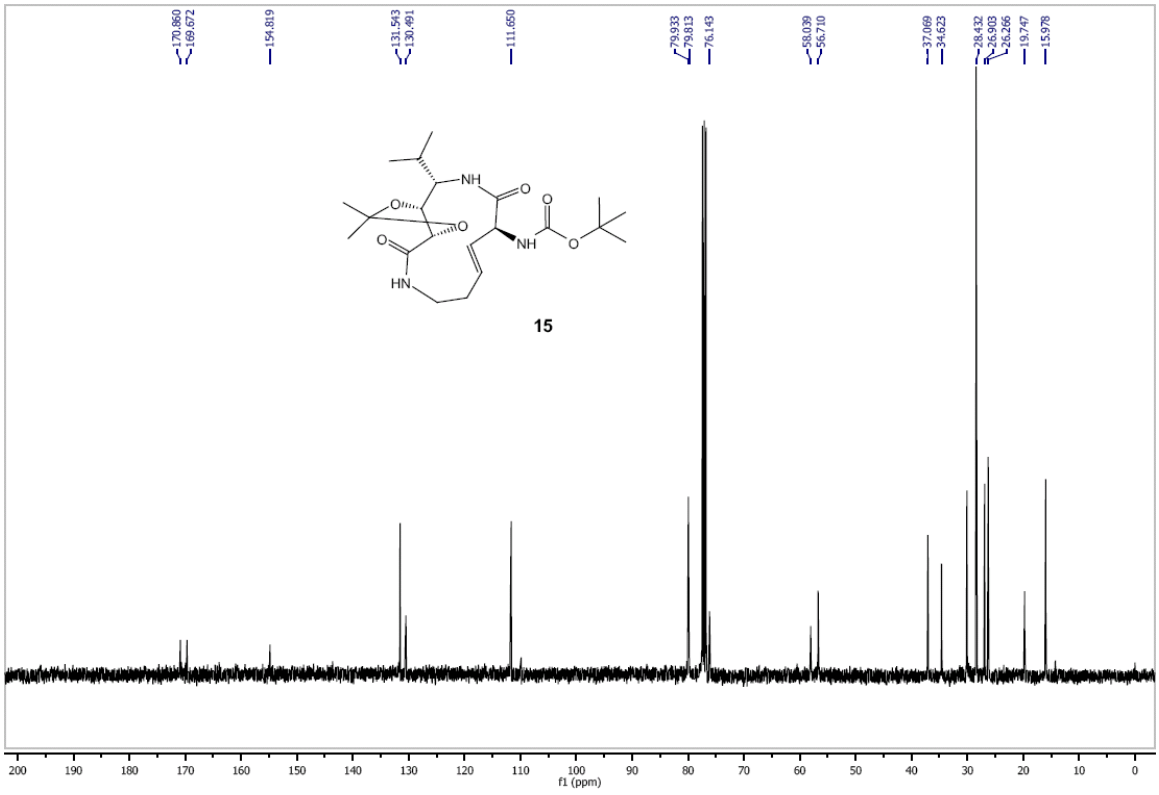
^{13}C NMR



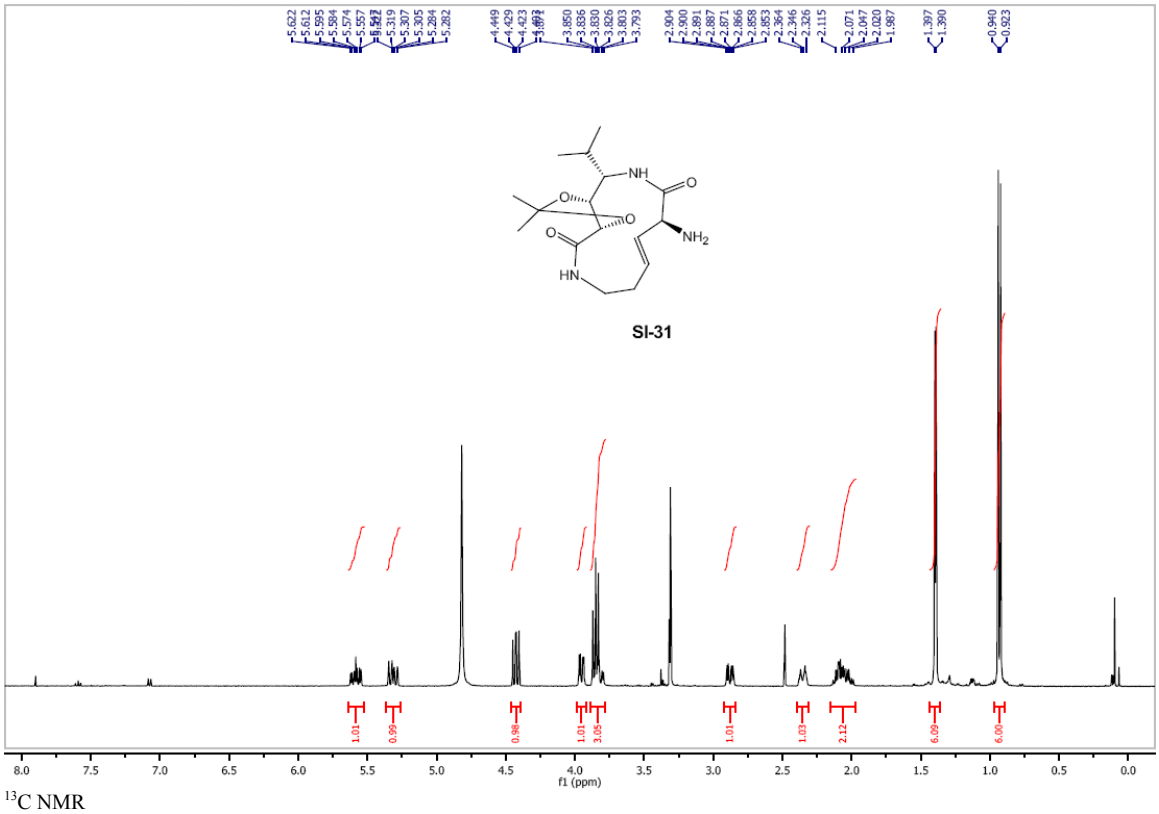
^{1}H NMR



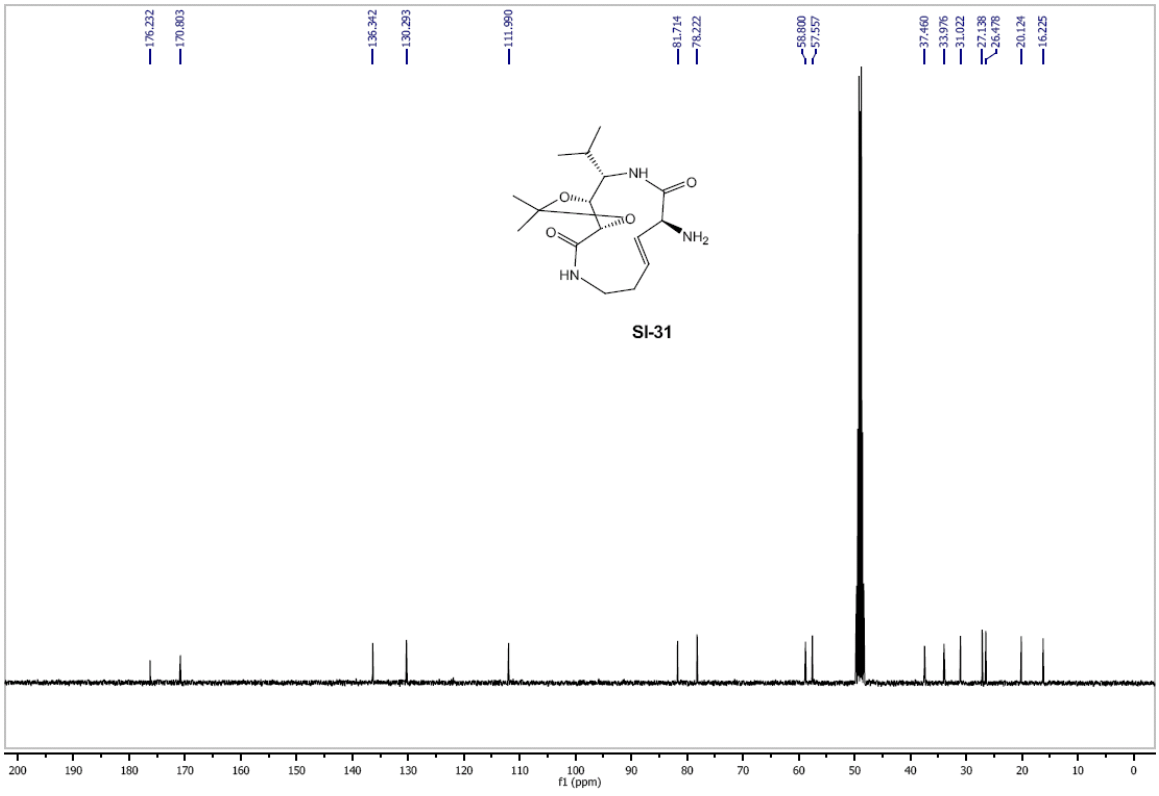
^{13}C NMR



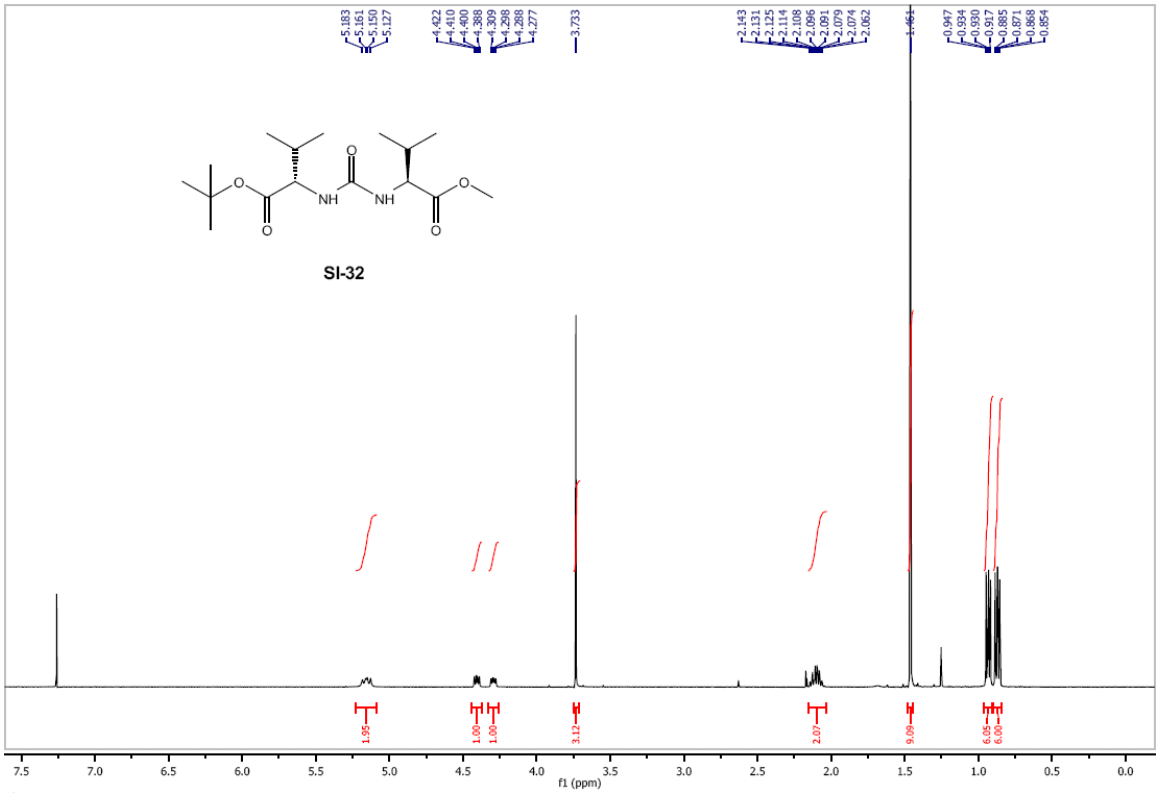
^{1}H NMR



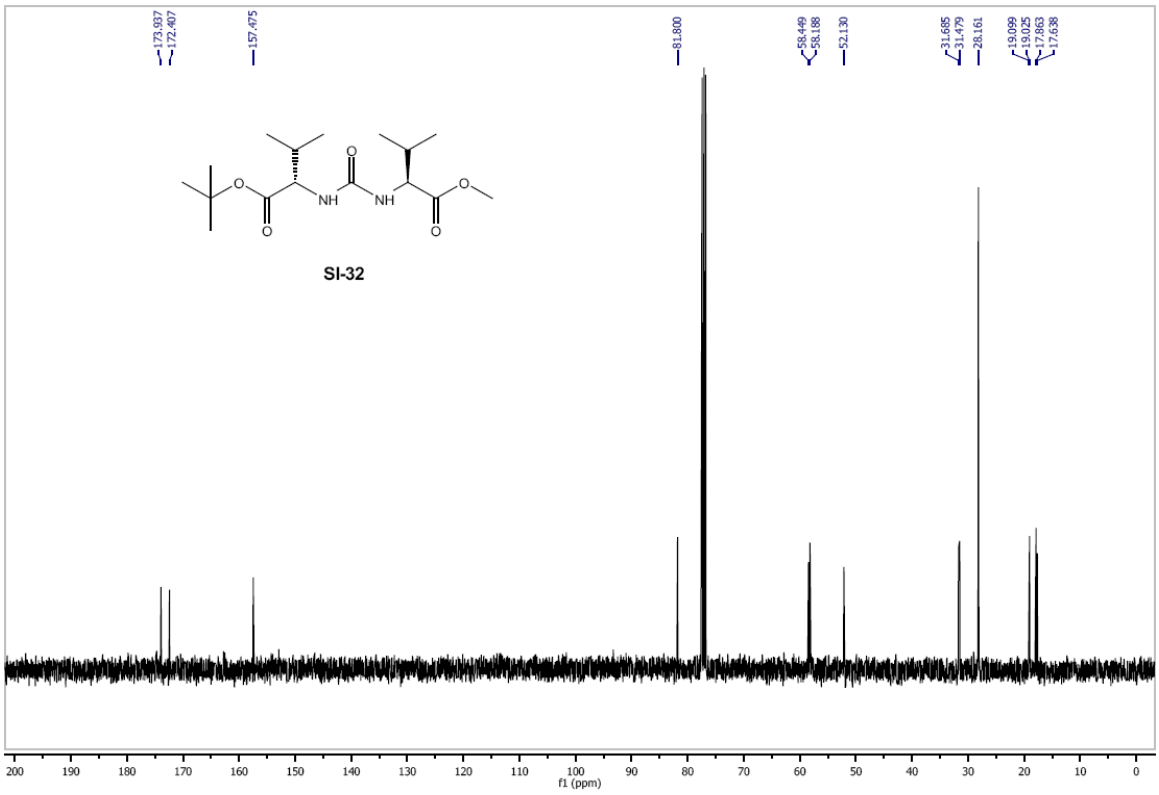
^{13}C NMR



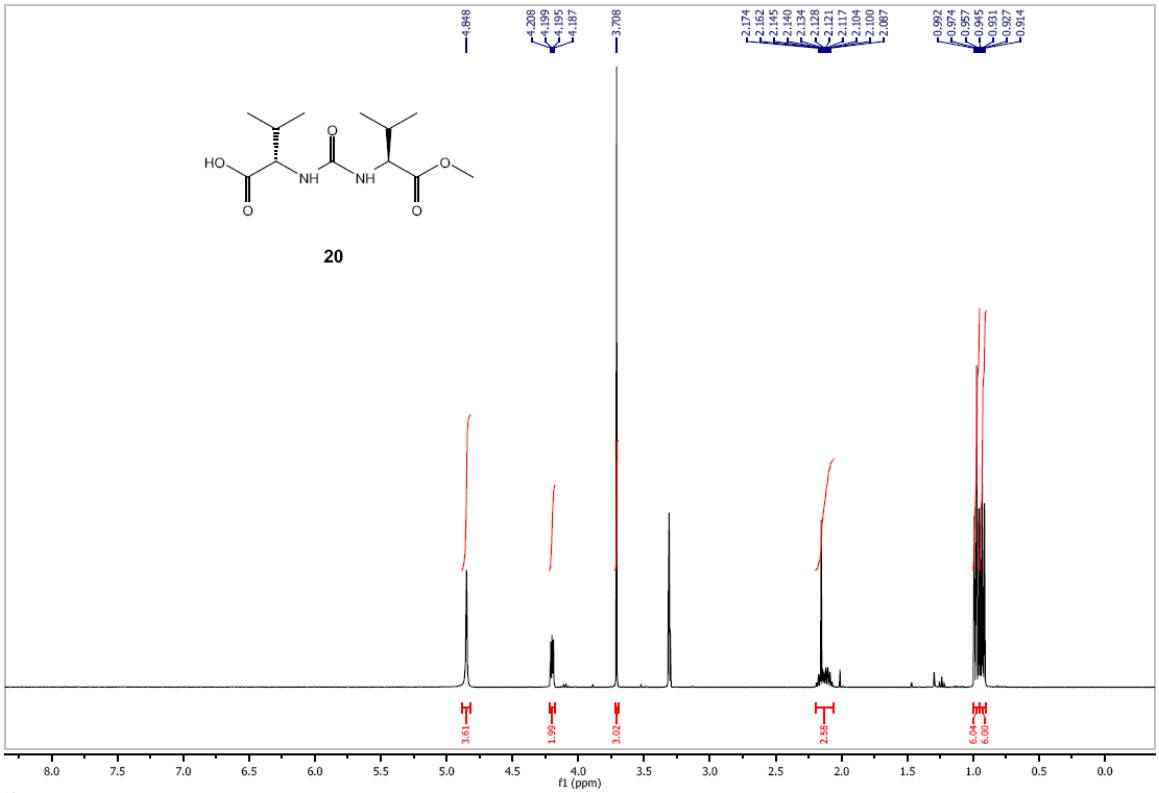
^{1}H NMR



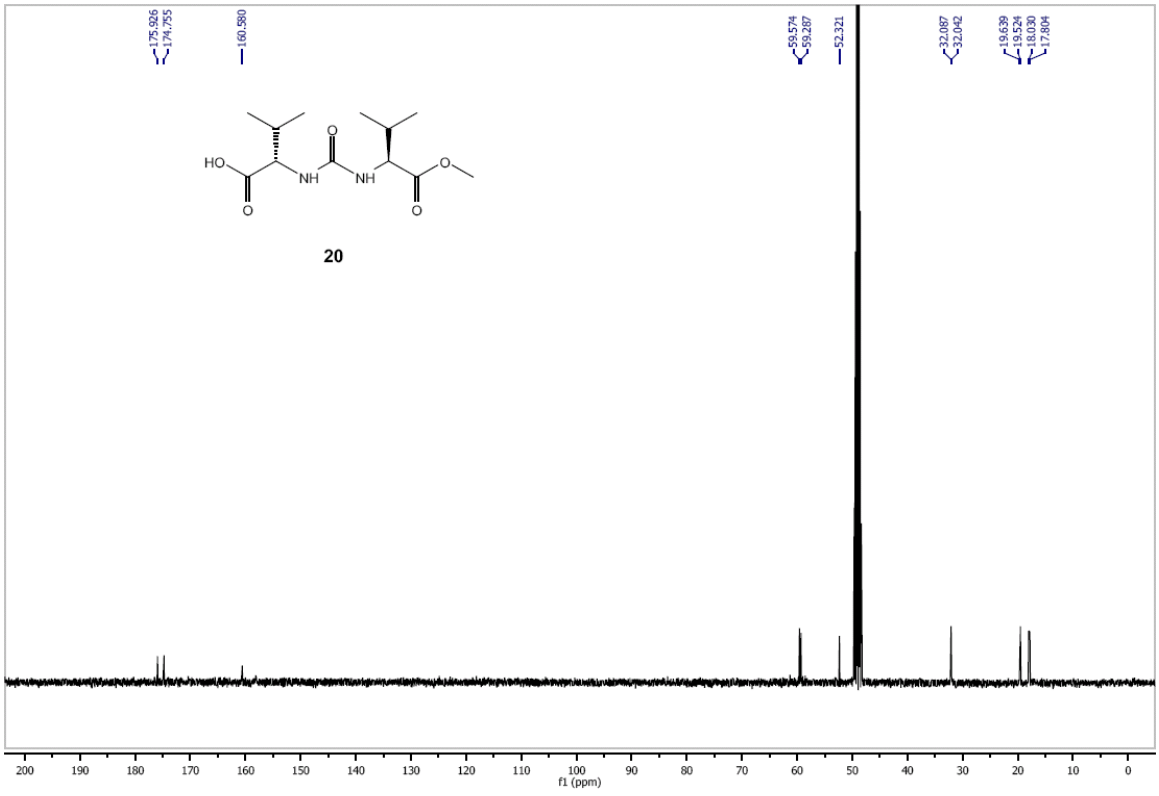
^{13}C NMR



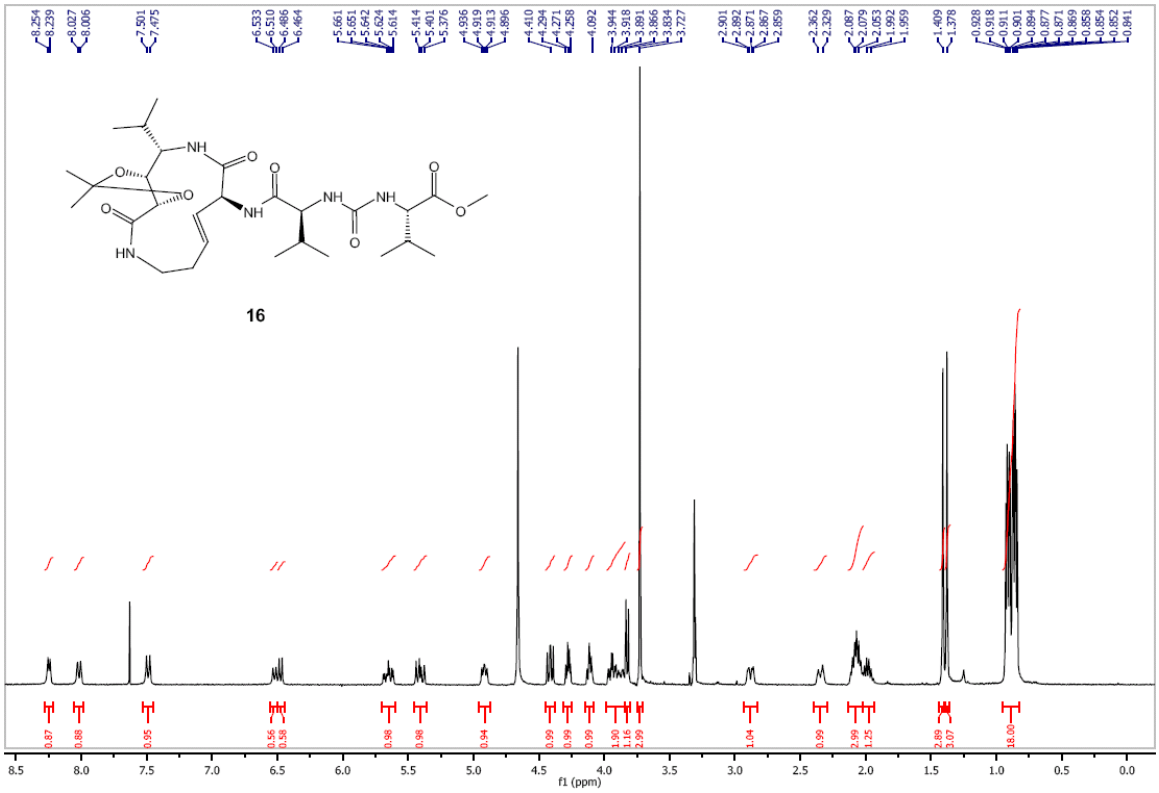
^{1}H NMR



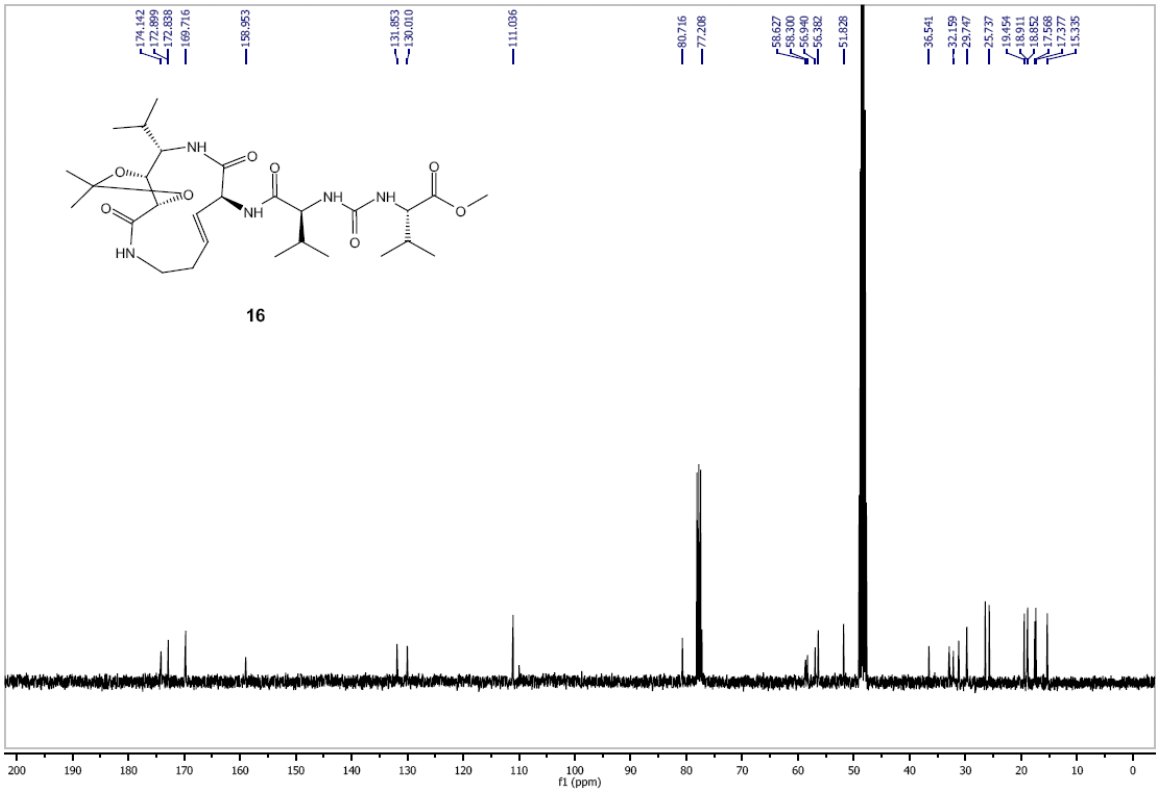
^{13}C NMR



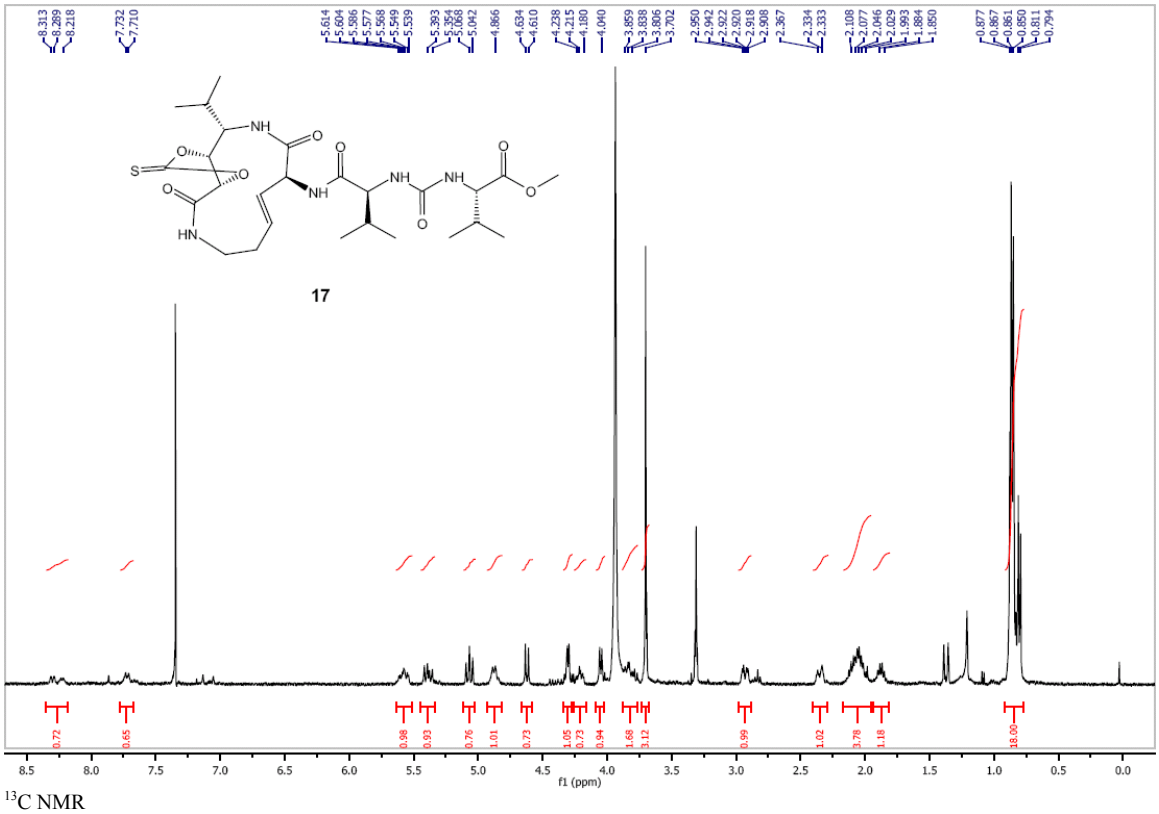
^{1}H NMR



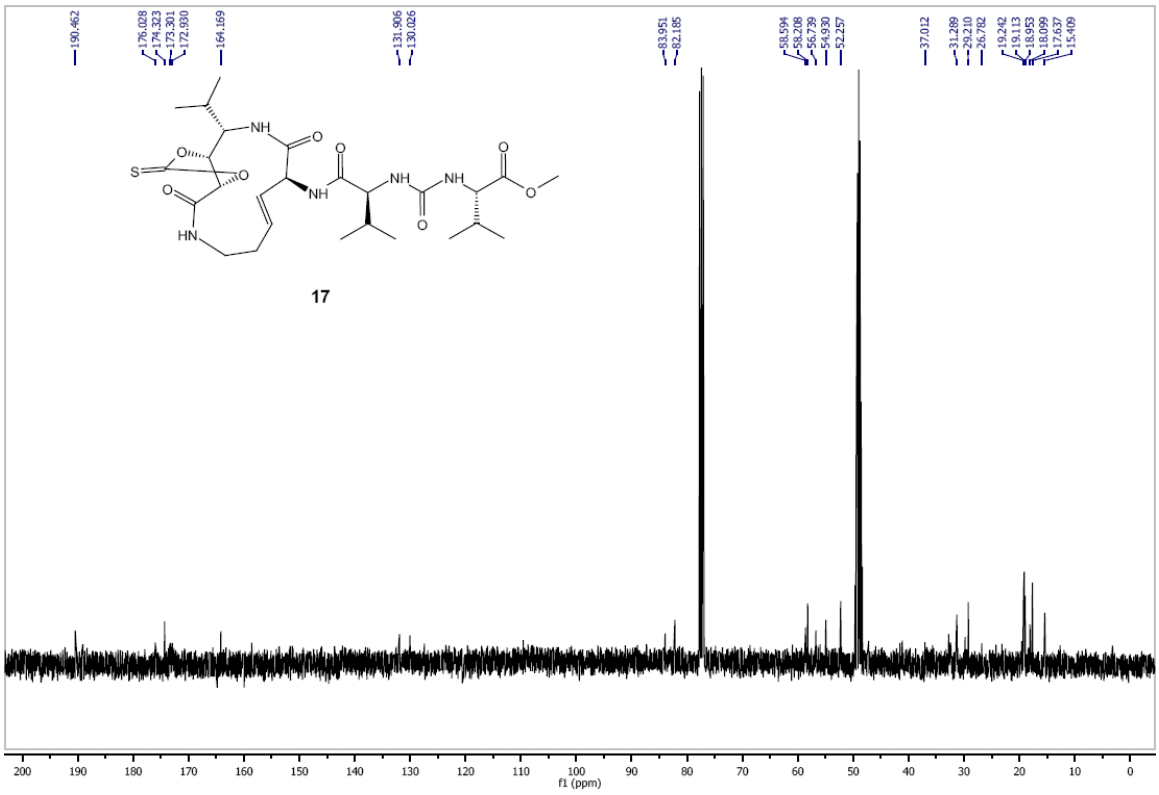
^{13}C NMR



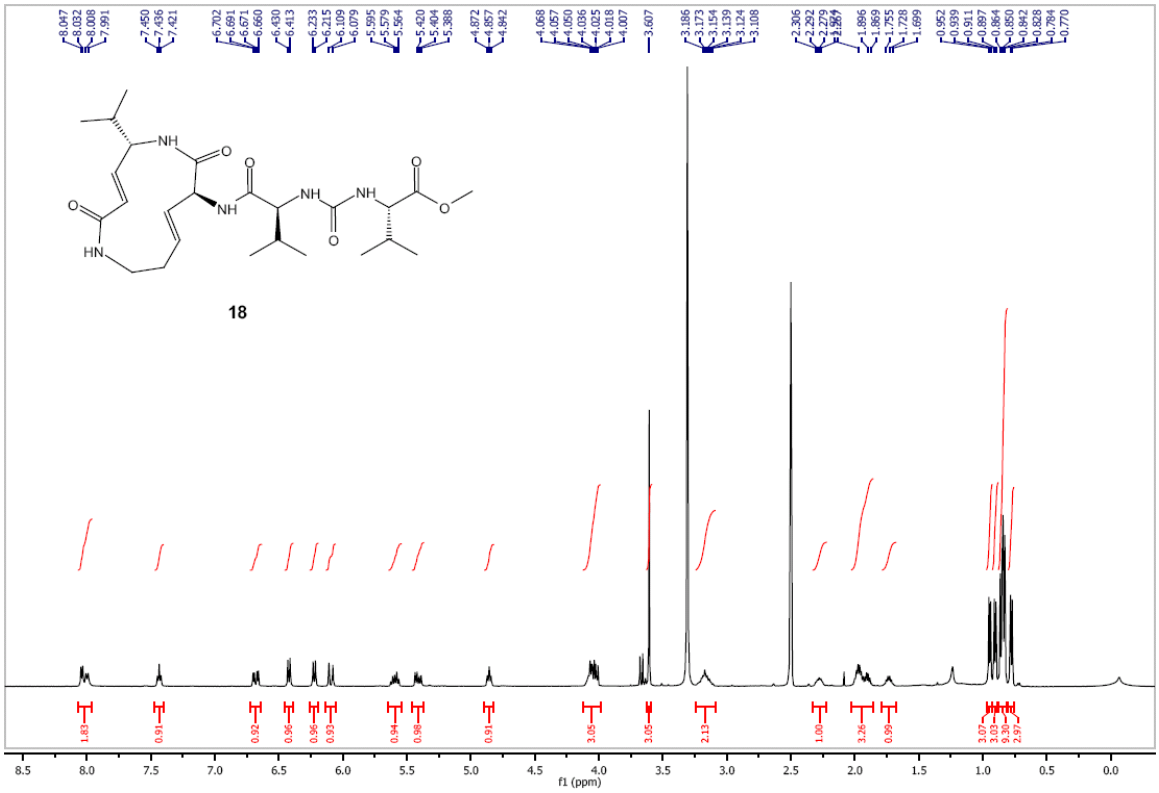
^{1}H NMR



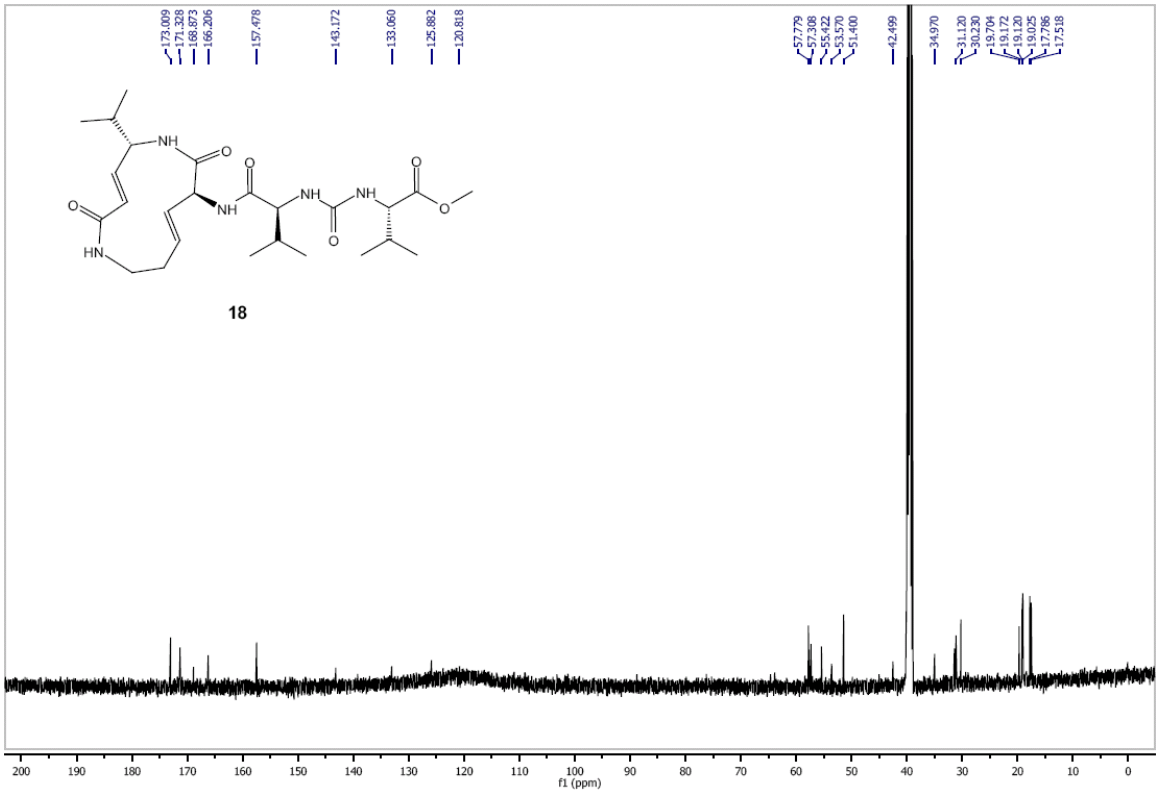
^{13}C NMR



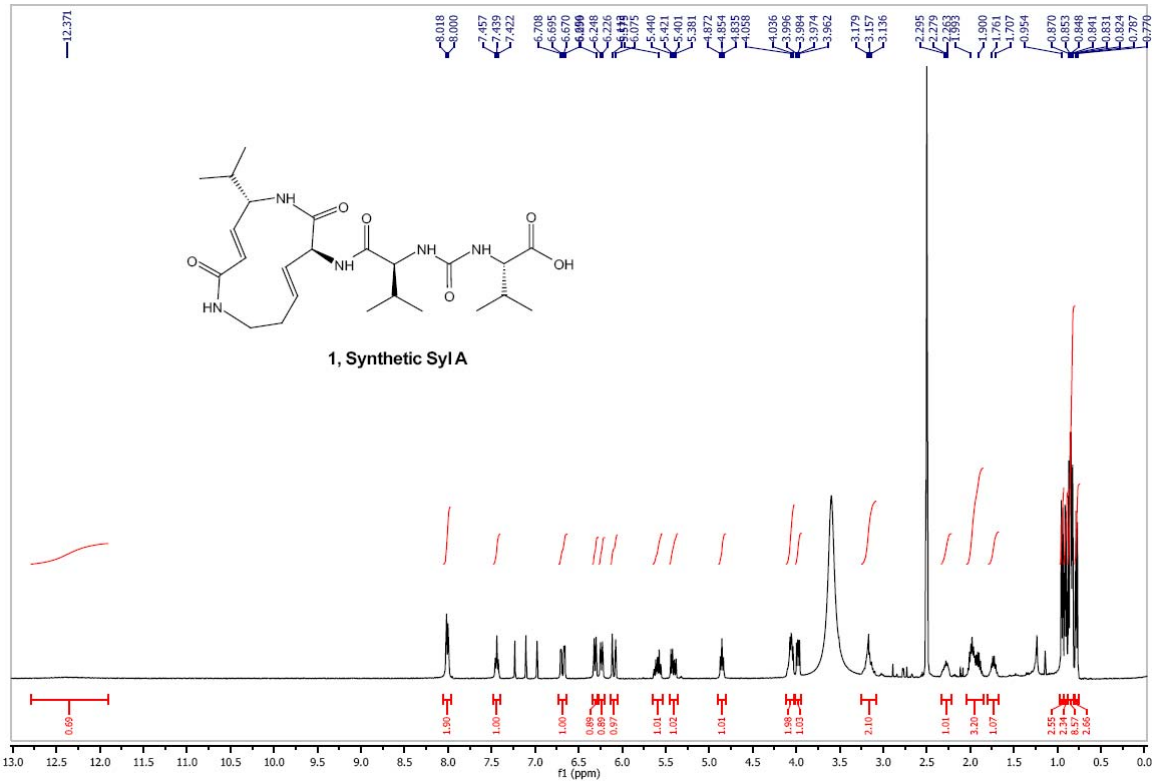
^{1}H NMR



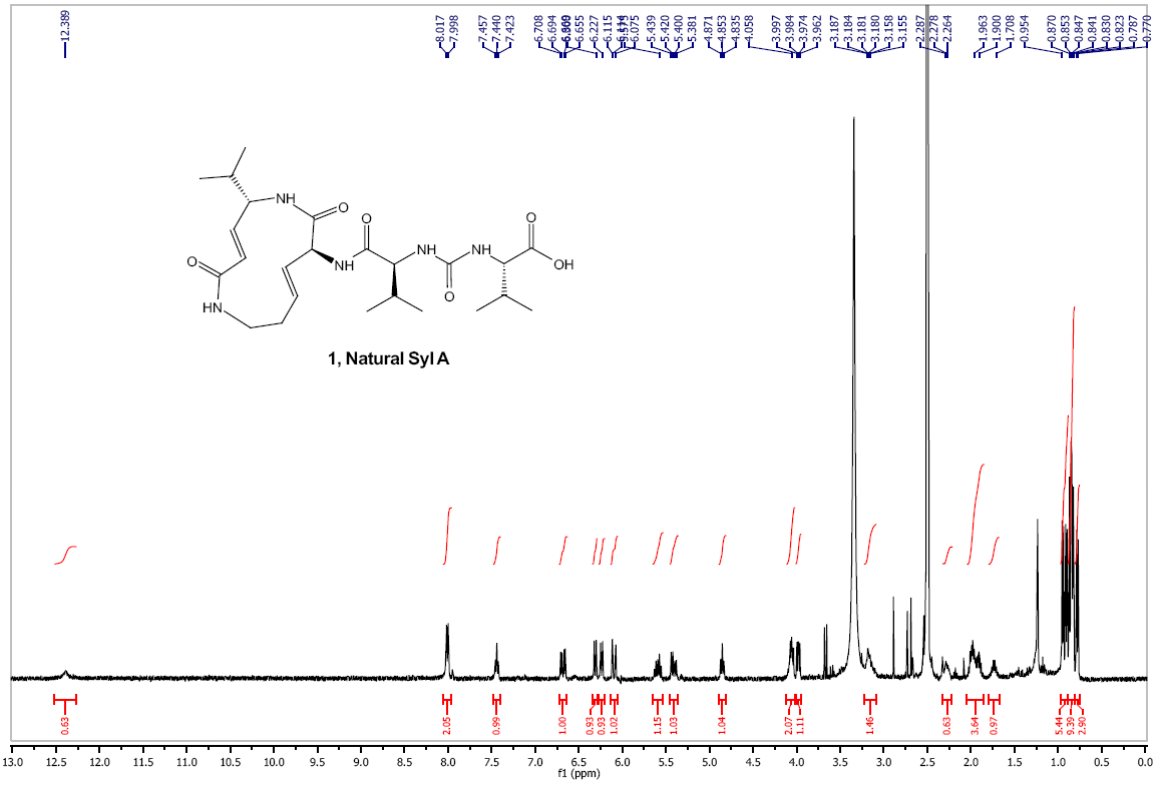
^{13}C NMR



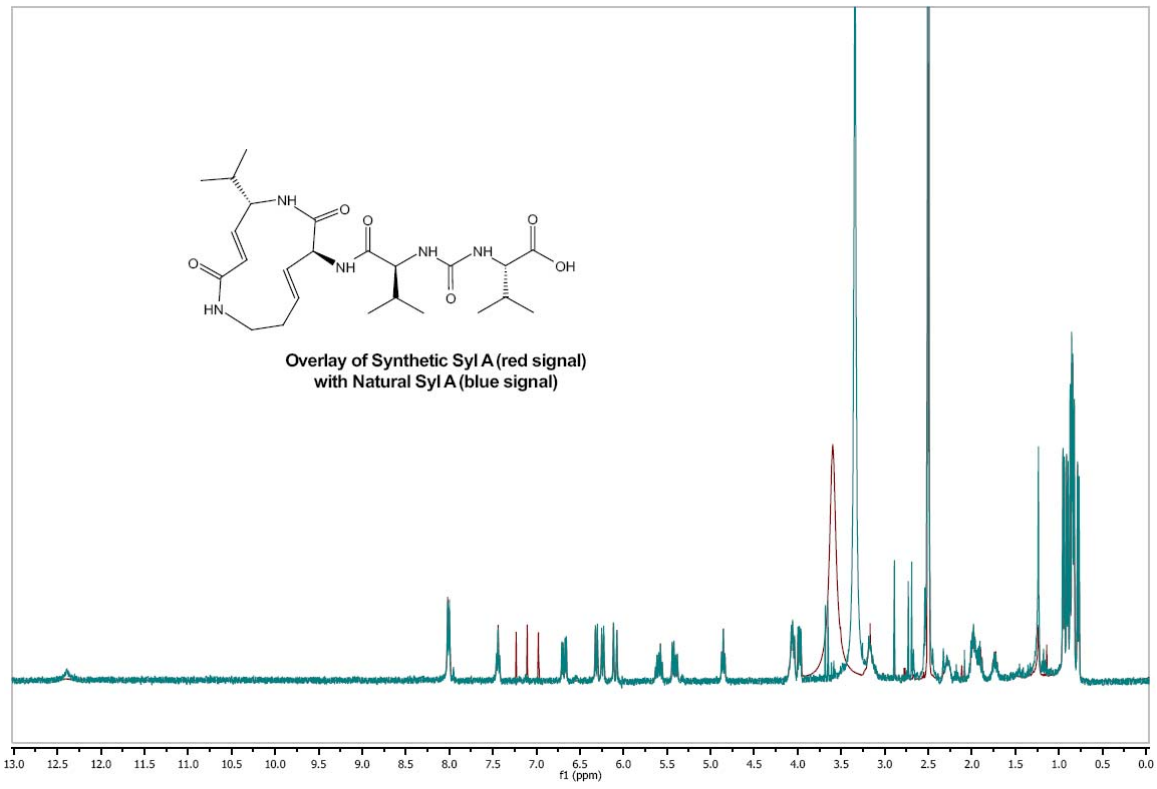
^{1}H NMR



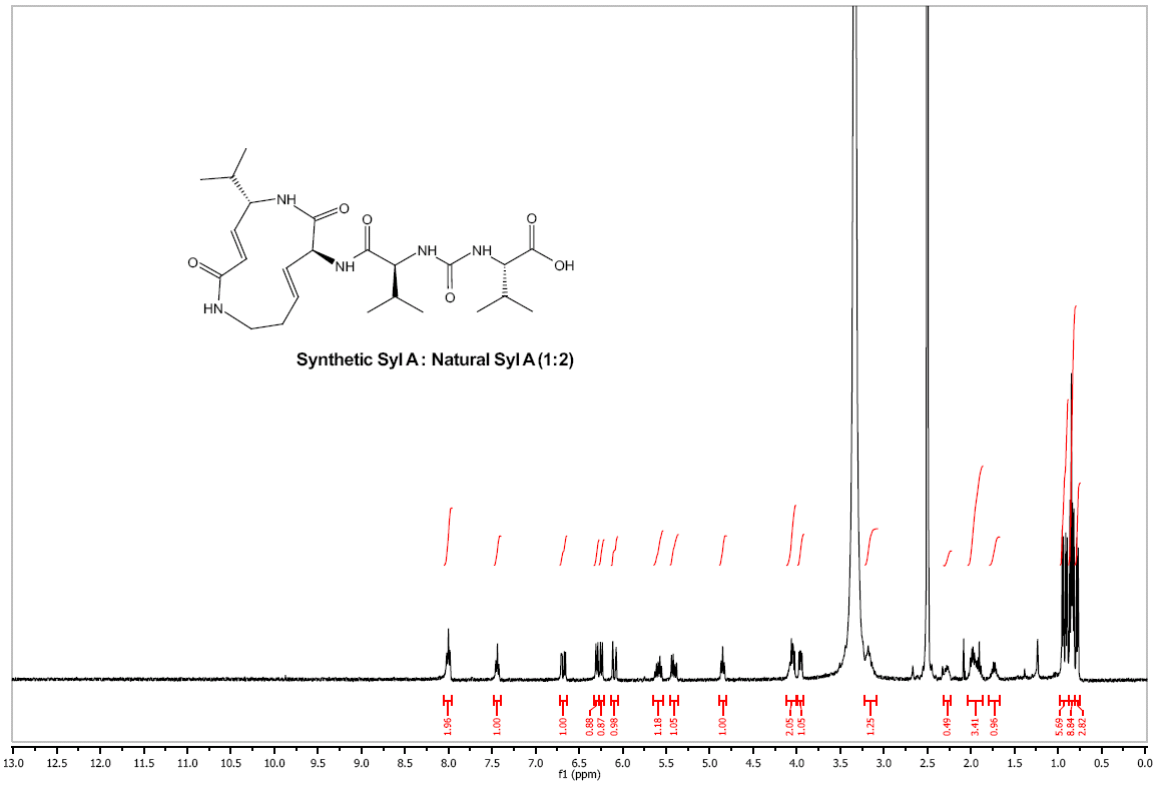
^{1}H NMR



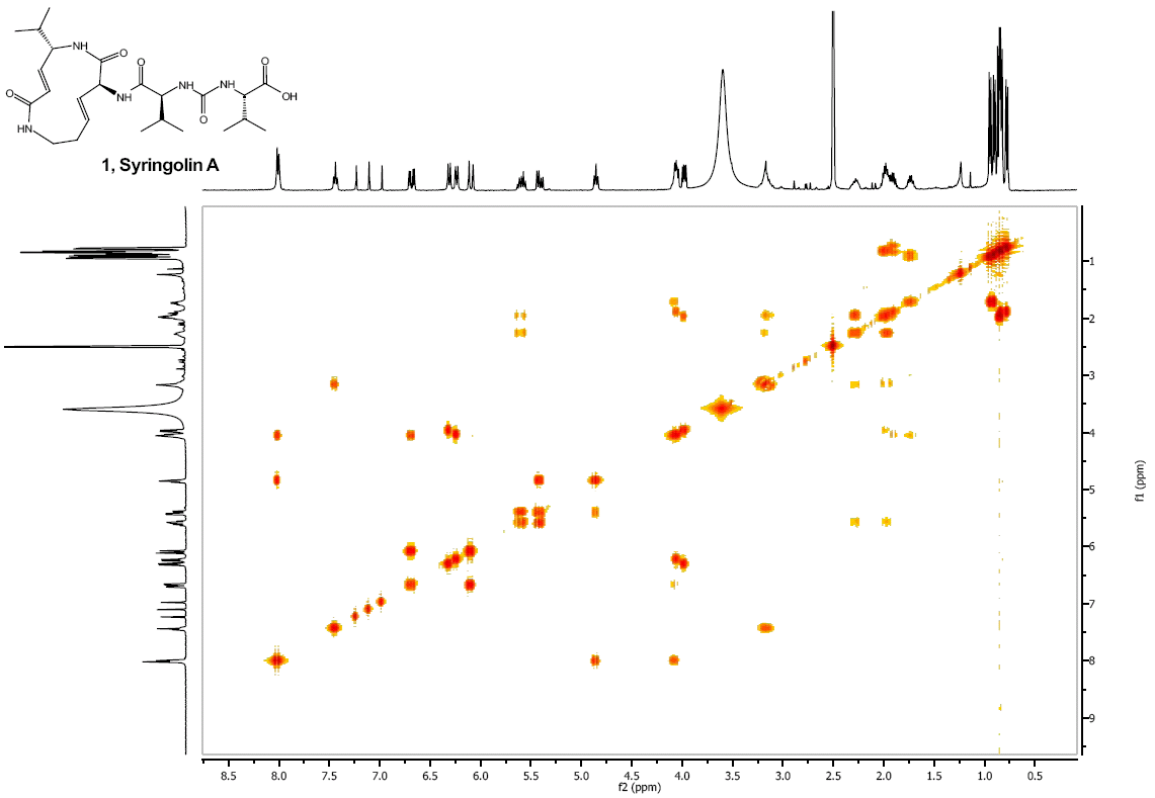
^{1}H NMR (overlay)



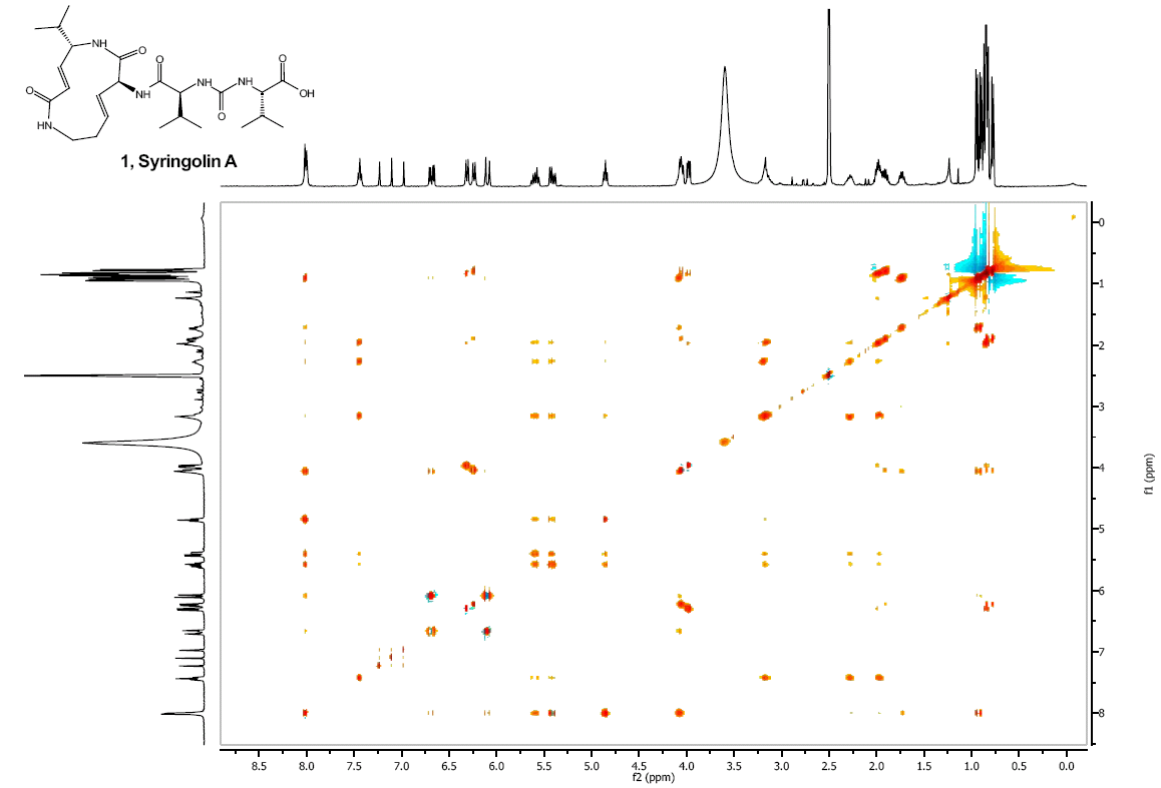
^{1}H NMR



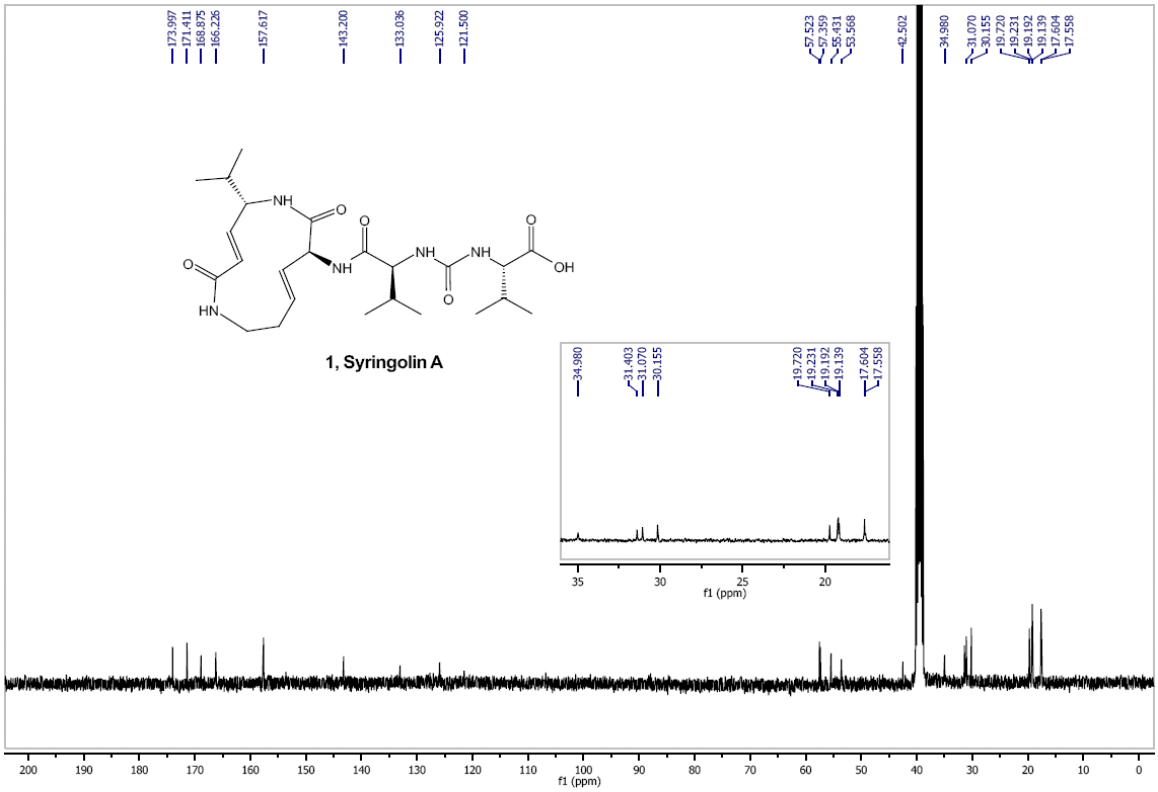
COSY



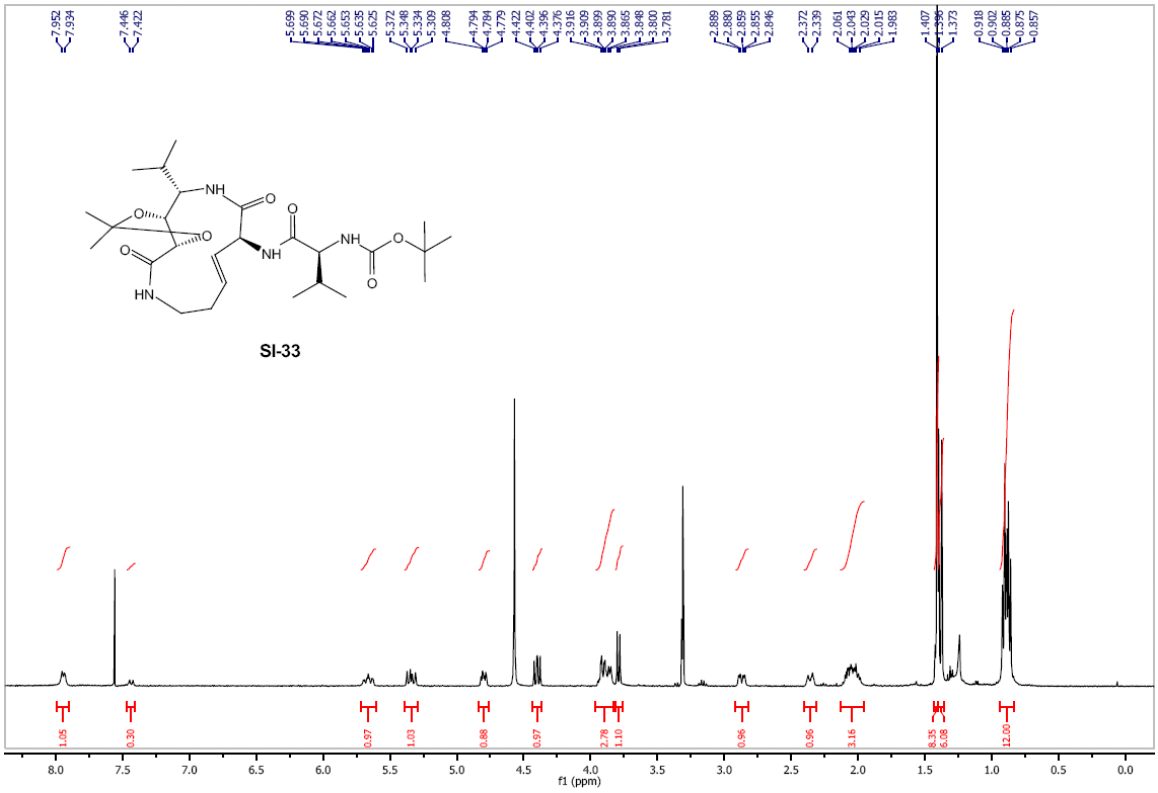
TOCSY



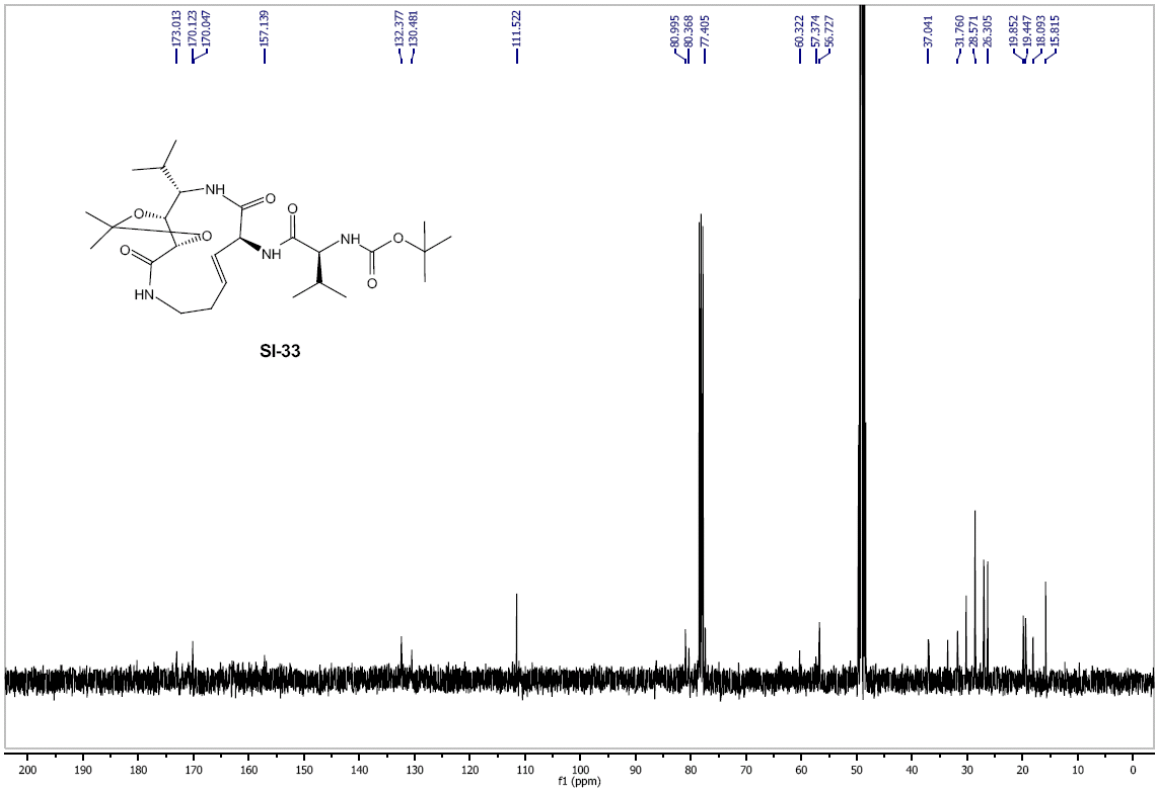
^{13}C NMR



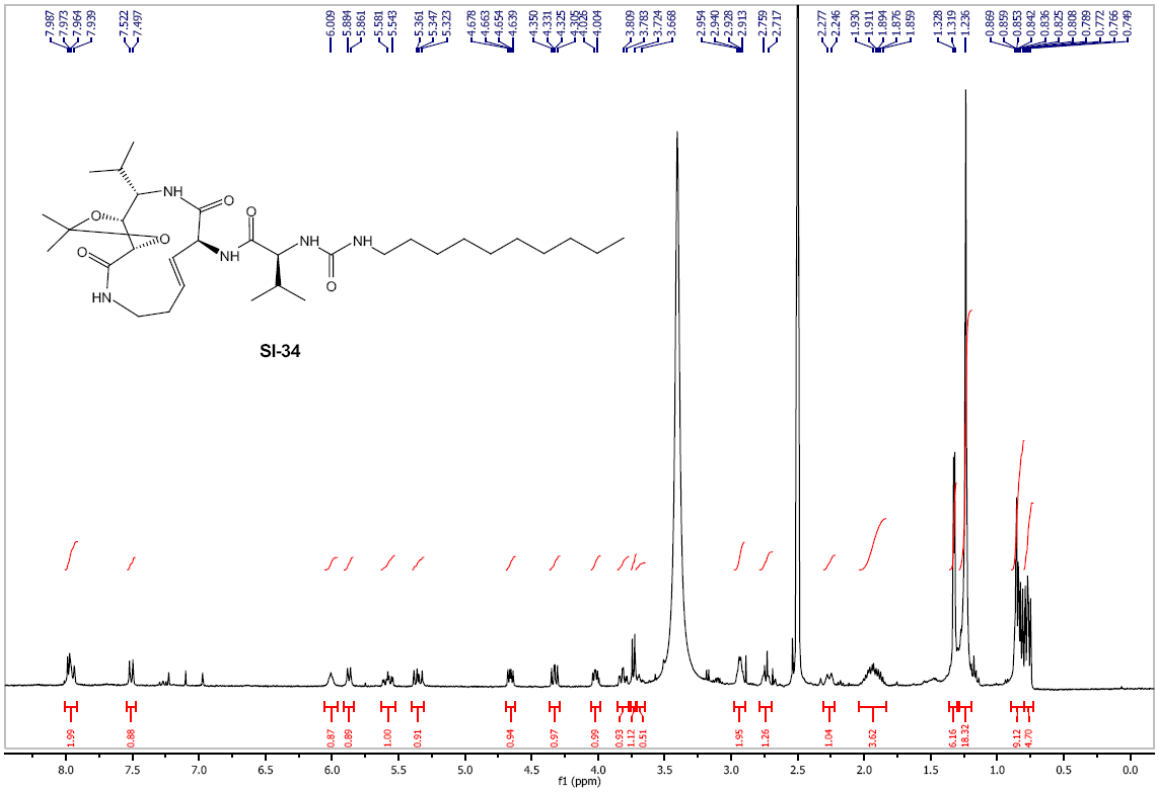
^{1}H NMR



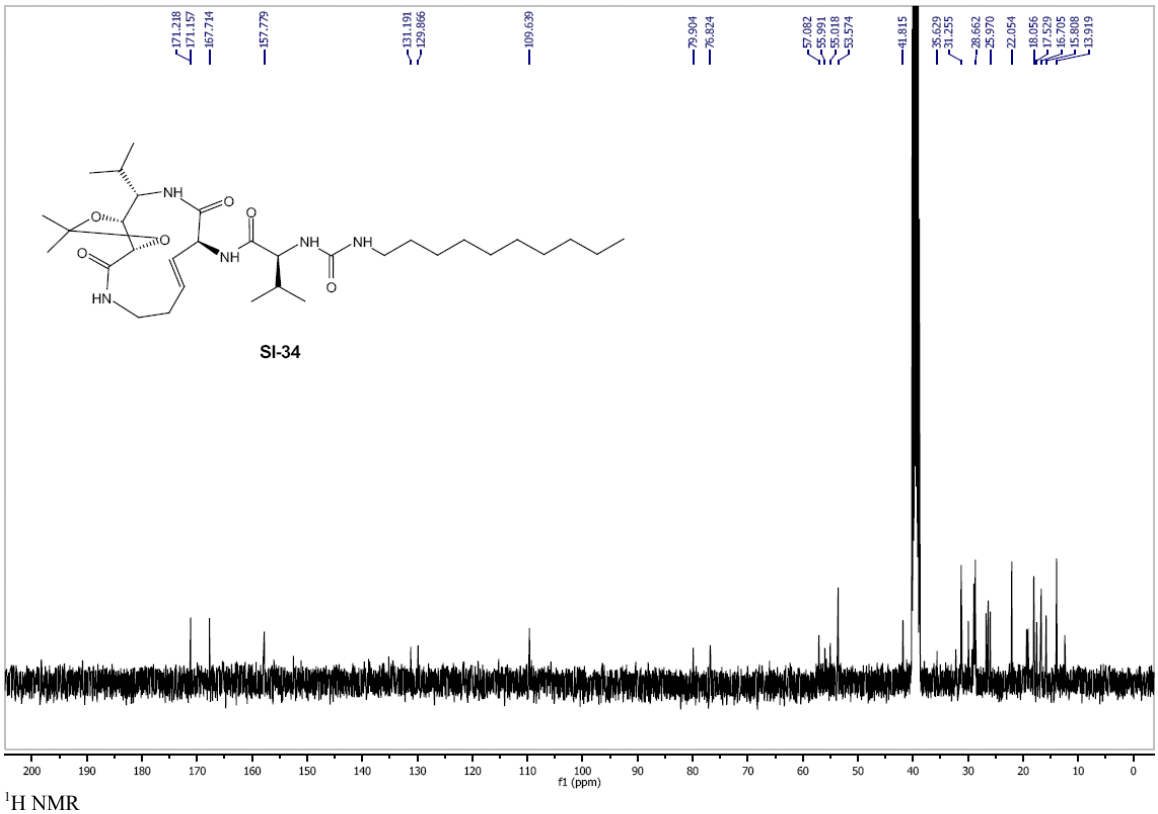
^{13}C NMR



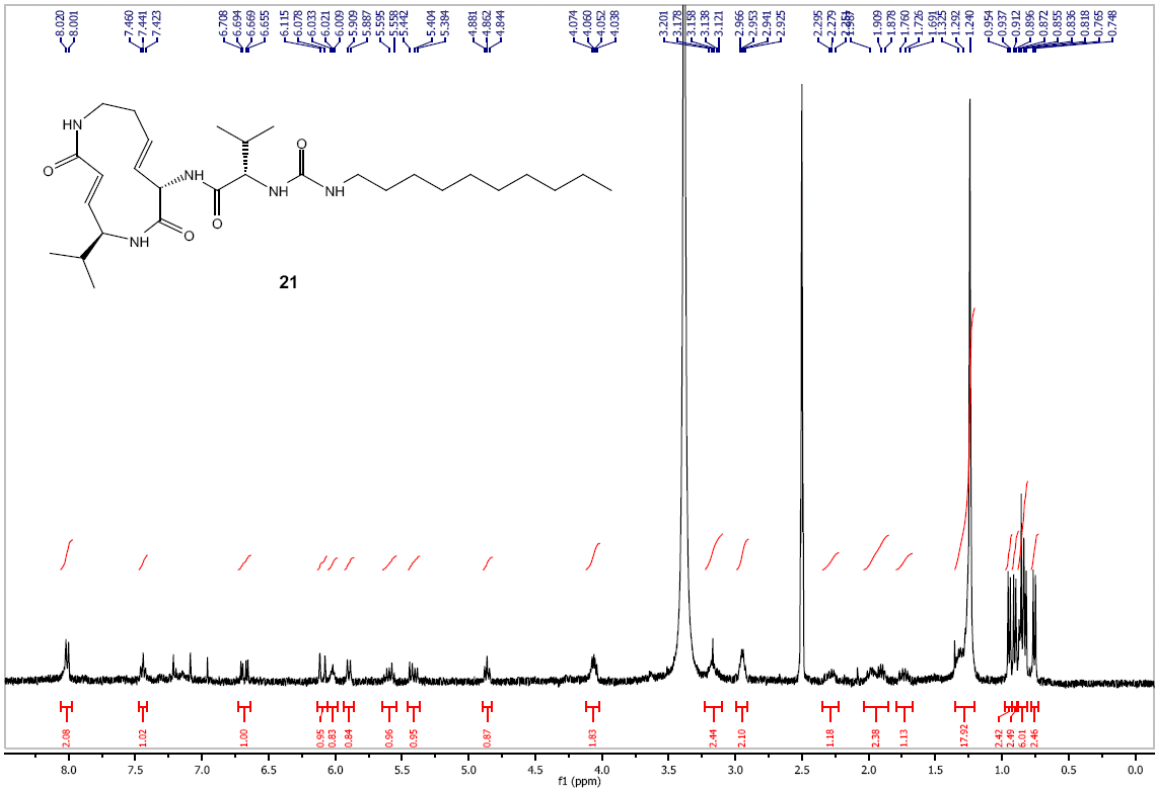
^{1}H NMR



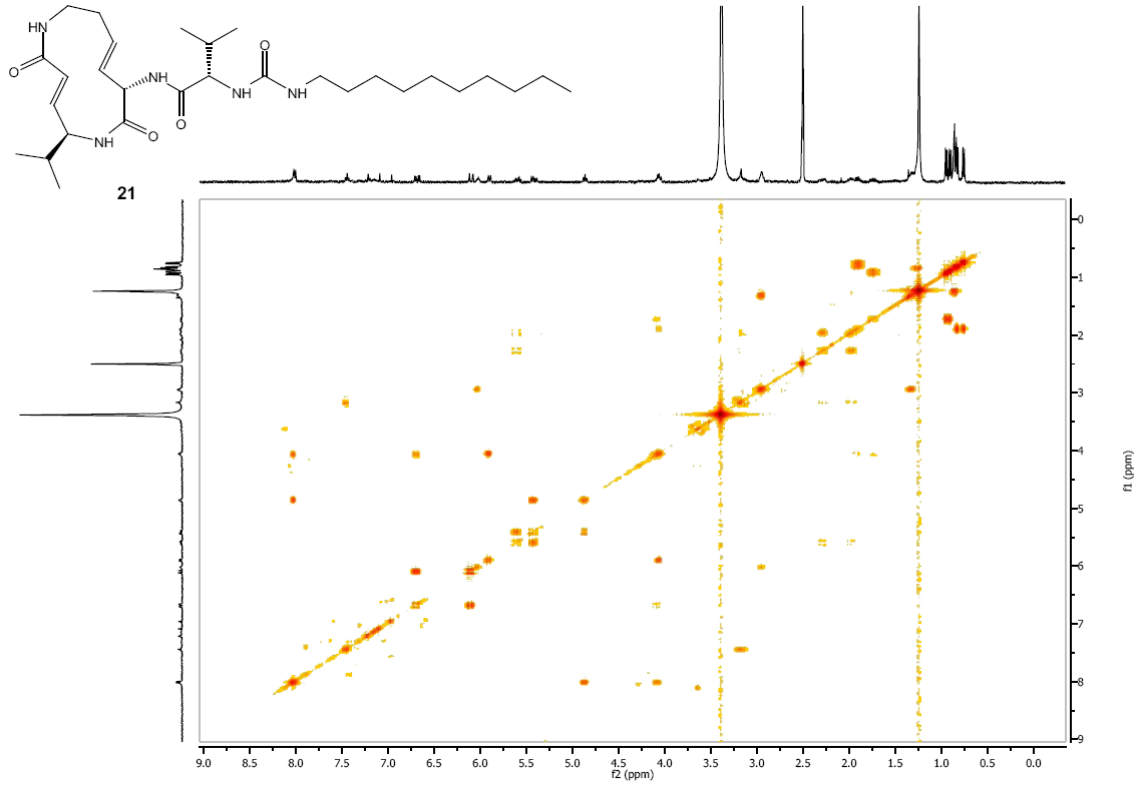
^{13}C NMR



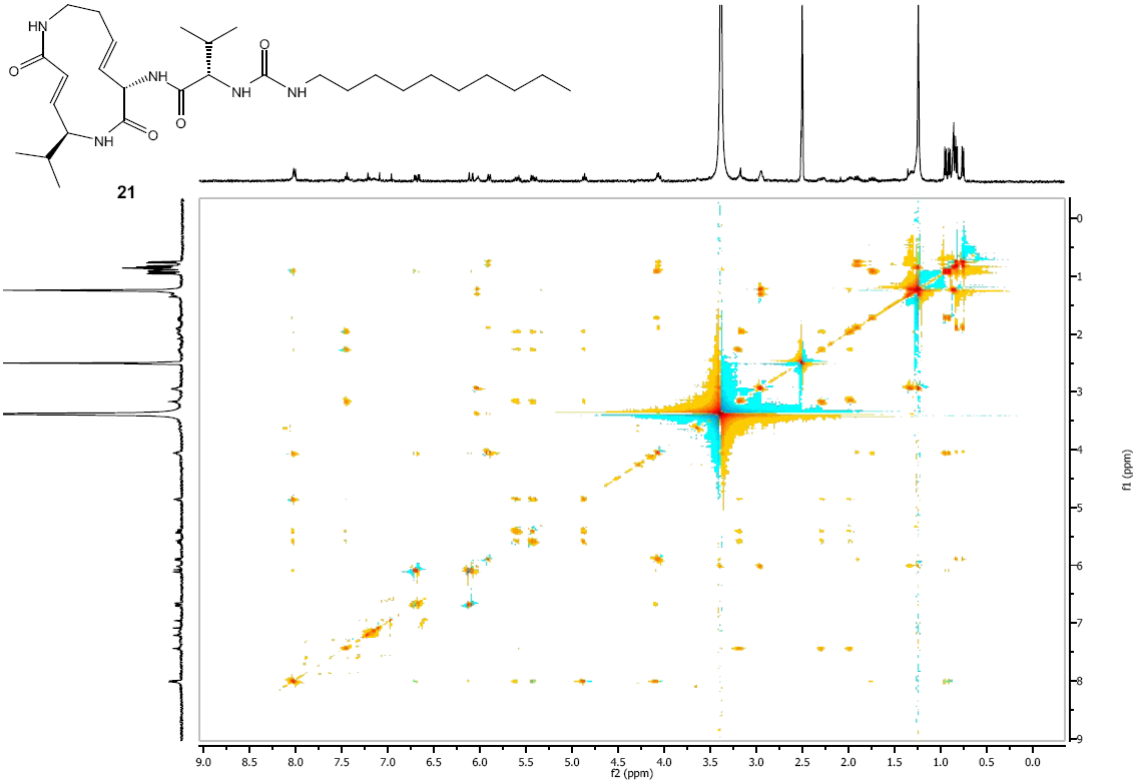
^{1}H NMR



COSY



TOCSY



HSQC