Supporting Information for

Chemical Synthesis and Biological Activity of Analogues of the Lantibiotic Epilancin 15X

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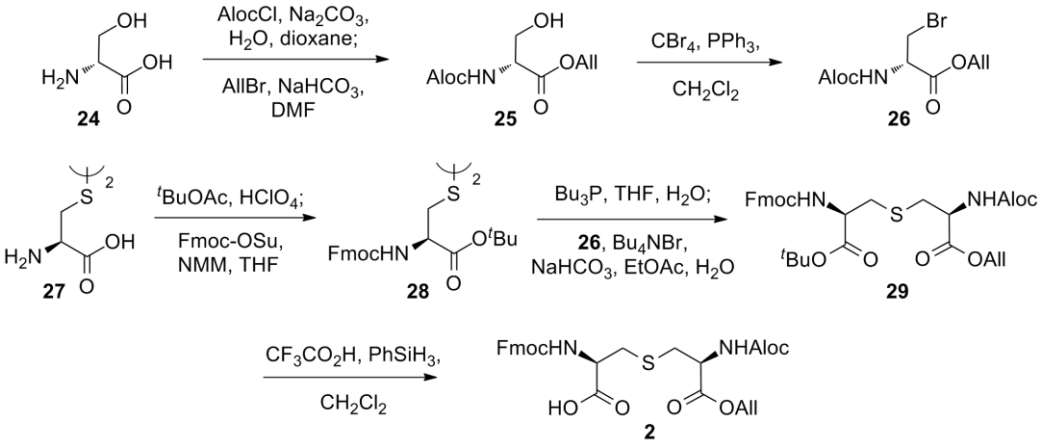
General information.

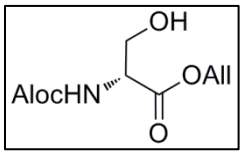
Materials, reactions and purification: Standard Fmoc-amino acids and resins for solid-phase peptide synthesis (SPPS), amino acids for solution-phase synthesis, D-lactic acid and peptide coupling reagents 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEBPT), *N*,*N'*-diisopropyl-carbodiimide (DIC), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), 1-hydroxy-7-azabenzotriazole (HOAt) and 1-hydroxybenzotriazole monohydrate (HOBt) were purchased from Chem-Impex International. 7-Azabenzotriazole-1-yloxy-*tris*-pyrrolidino-phosphonium hexafluorophosphate (PyAOP) was purchased from AAPPTec. Dimethylform-amide (DMF), dichloromethane and tetrahydrofuran (THF) were purchased at reaction grade from Fisher Scientific and dried via a solvent dispensing system prior to use. Flow cytometry dyes 3,3'-diethyloxacarbocyanine iodide ($DiOC_2(3)$) and propidium iodide (PI) were purchased from Invitrogen. Other chemical reagents and solvents were purchased from Sigma Aldrich or Alfa Aesar and used without further purification. All reactions were run under an atmosphere of N_2 unless otherwise stated. Reaction progress and chromatography fractions were monitored by thin layer chromatography (TLC) on silica-gel-coated glass plates with a F254 fluorescent indicator. Visualization was achieved by UV absorption by fluorescence quenching or permanganate stain (1.5 g $KMnO_4$, 10 g K_2CO_3, 1.25 mL 10% NaOH in 200 mL of H_2O). Flash chromatography was performed using Silicycle SiliaFlash P60, 230-400 mesh silica gel. Analytical reversed-phase high-performance liquid chromatography (RP-HPLC) was performed on an Agilent 1260 Infinity system with a Phenomenex Jupiter C12 analytical column with a flow rate of 1 mL/min and a solvent gradient of 2-100% solvent B over 45 min. Preparatory RP-HPLC was performed on a Waters 600 system with a Phenomenex Jupiter C12 preparative column with a flow rate of 10 mL/min and solvent gradients as described for each peptide. All HPLC solvents were filtered with a Millipore filtration system equipped with a 0.22 μm nylon membrane filter prior to use. HPLC solvent compositions: solvent A is 0.1% trifluoroacetic acid (TFA) in H_2O; solvent B is 80:20 MeCN/H_2O with 0.087% TFA.

Characterization: NMR spectra were recorded on a Varian Unity 400 or Unity Inova 500 spectrometer. Small molecules (MW < 1000 Da) were analyzed by electrospray ionization/time-of-flight (ESI-TOF) mass spectrometry on a Waters Quattro II quadrupole spectrometer. Peptides (MW > 800 Da) were analyzed by matrix-assisted laser desorption ionization/time-of-flight (MALDI-TOF) mass spectrometry on a Bruker Daltonics UltrafleXtreme TOF/TOF spectrometer using a matrix solution consisting of saturated α-cyano-4-hydroxycinnamic acid in 1:1:0.1 H_2O/MeCN/TFA.

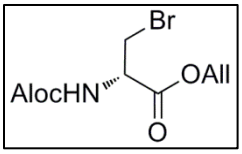
Synthetic procedures for small molecules.

Allyl-protected lanthionine building block **2**



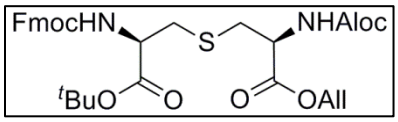


Compound **25**: D-Serine (**24**, 2.10 g, 20.0 mmol) and sodium carbonate (3.18 g, 30.0 mmol) were dissolved in water (30 mL) and MeCN (15 mL) and chilled in an ice bath. A solution of allyl chloroformate (AlocCl, 2.1 mL, 20.0 mmol) in MeCN (15 mL) was added dropwise. The reaction was stirred for 8 h, gradually warming to room temperature. The reaction was concentrated under reduced pressure, then taken up in DMF (50 mL). Sodium bicarbonate (1.68 g, 20.0 mmol) was added, followed by allyl bromide (AllBr, 3.5 mL, 40.0 mmol). The reaction was stirred as a heterogeneous mixture under N_2 for 15 h. The reaction was concentrated under reduced pressure, then partitioned between water and EtOAc. The organic layer was separated, washed with saturated aqueous $NaHCO_3$, 0.1 M $KHSO_4$ and brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (SiO_2, 2:1 hexane/EtOAc) to yield **25** (2.67 g, 11.7 mmol, 59%) as a colorless oil. R_f 0.32 (1:1 hexane/EtOAc). Spectral data match those reported previously.[1]

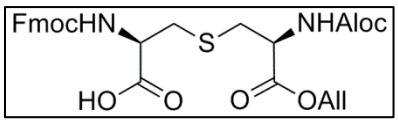


Compound **26**: Compound **25** (2.00 g, 8.73 mmol) and carbon tetrabromide (3.47 g, 10.5 mmol) were dissolved in CH_2Cl_2 (25 mL) and chilled in an ice bath. Triphenylphosphine (2.75 g, 10.5 mmol) was dissolved in CH_2Cl_2 (10 mL) and added dropwise to the chilled solution. The reaction was warmed to room temperature and stirred for 2.5 h, then washed with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Excess 4:1 hexane/EtOAc was added to precipitate phosphine oxide byproducts, which were removed via filtration through Celite. The filtrate was concentrated and purified by flash chromatography (SiO_2, 5:1 then 4:1 hexane/EtOAc) to yield **26** (1.98 g, 6.78 mmol, 78%) as a colorless oil. R_f 0.50 (3:1 hexane/EtOAc). Spectral data match those reported previously.[1]

Compound **28**: Synthesis was performed in two steps from L-cystine (**27**) as we have reported previously.[2]

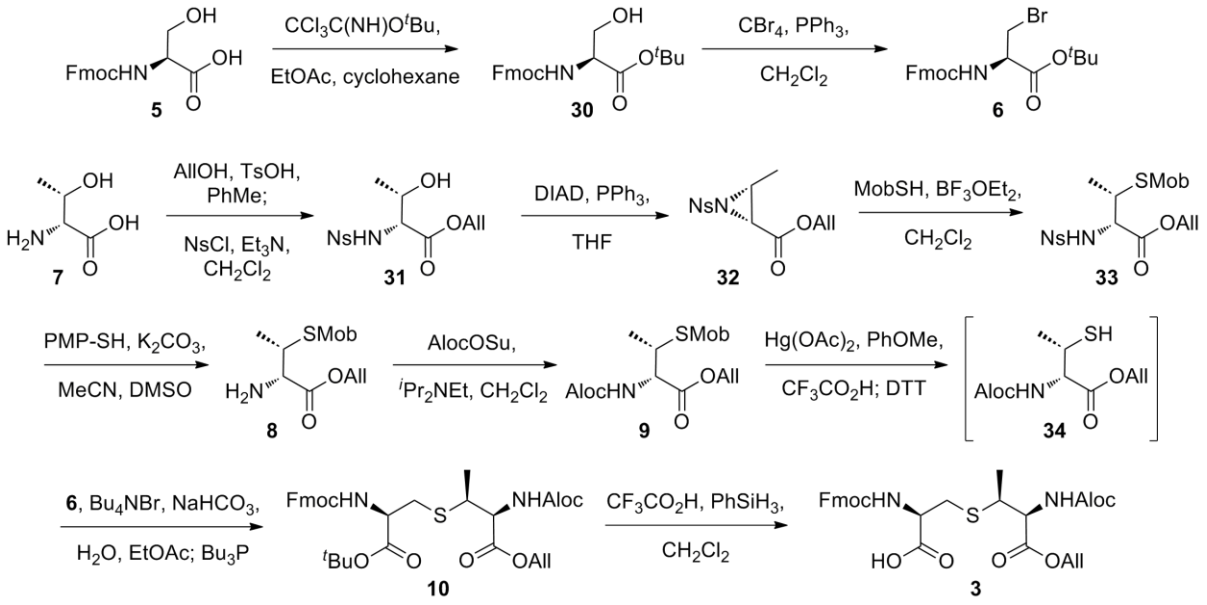


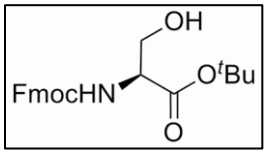
Compound **29**: Tributylphosphine (410 µL, 1.64 mmol) was added to a solution of **28** (1.09 g, 1.37 mmol) in THF (15 mL) and stirred for 15 min. Water (1.5 mL) was added, and the reaction was stirred an additional 2.5 h, and then concentrated under reduced pressure. To the resulting oil was added **26** (0.80 g, 2.74 mmol) in N_2-sparged EtOAc (15 mL). Tetrabutylammonium bromide (3.55 g, 10.0 mmol) was dissolved in N_2-sparged 0.5 M aqueous $NaHCO_3$ (pH adjusted to 8.5, 15 mL), then added to the organic solution. The biphasic mixture was stirred for 18 h, then washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography (SiO_2, 4:1 hexane/EtOAc) to yield **29** (1.29 g, 2.11 mmol, 77% over two steps) as a colorless oil. R_f 0.28 (3:1 hexane/EtOAc). Spectral data match those reported previously.[1]



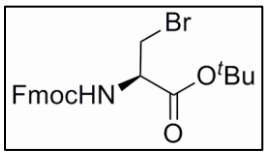
Compound **2**: To a solution of **29** (1.80 g, 2.95 mmol) in CH_2Cl_2 (15 mL) was added phenylsilane (400 µL, 3.24 mmol), followed by TFA (15 mL). The reaction was stirred for 2 h, then concentrated under reduced pressure to yield **2** (1.64 g, quant.) as a white solid after lyophilization from 1:1 benzene/MeCN. R_f 0.20 (25:1:0.1 CH_2Cl_2/MeOH/AcOH). Spectral data matched those reported previously.[1]

Allyl-protected methyllanthionine building block **3**

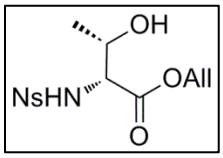




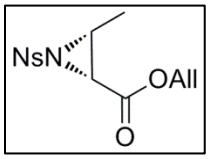
Compound **30**: To a stirring suspension of *N*-Fmoc-L-serine (**5**, 8.19 g, 25.0 mmol) in EtOAc (125 mL) was added *tert*-butyl 2,2,2-trichloroacetimidate (8.95 mL, 50.0 mmol) in cyclohexane (50 mL) by an addition funnel over 15 min. The reaction was stirred for 18 h, then washed with saturated aqueous $NaHCO_3$, water, and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography (SiO_2, 3:1 hexane/EtOAc) to yield **30** (8.90 g, 23.2 mmol, 93%) as a white solid. R_f 0.58 (1:1 hexane/EtOAc). Spectral data match those previously reported.[3]



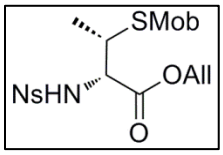
Compound **6**: Compound **30** (2.00 g, 5.22 mmol) and carbon tetrabromide (2.08 g, 6.26 mmol) were dissolved in CH_2Cl_2 (10 mL) and chilled in an ice bath. Triphenylphosphine (1.64 g, 6.26 mmol) was dissolved in CH_2Cl_2 (10 mL) and added dropwise to the chilled solution. The reaction mixture was warmed to room temperature and stirred for 2.5 h, then washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Excess 5:1 hexane/EtOAc was added to precipitate phosphine oxide byproducts, which were removed via filtration through Celite. The filtrate was concentrated and purified by flash chromatography (SiO_2, 15% EtOAc/hexane) to yield **6** (1.83 g, 4.10 mmol, 79%) as an amber oil. R_f 0.56 (3:1 hexane/EtOAc). Spectral data match those reported previously.[4]



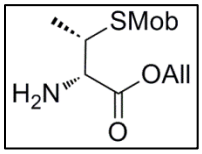
Compound **31**: D-Threonine (**7**, 4.17 g, 35.0 mmol) and *para*-toluenesulfonic acid monohydrate (7.99 g, 42.0 mmol) were combined in toluene (90 mL). Allyl alcohol (AllOH, 24 mL, 350 mmol) was added, and the reaction was refluxed in an oil bath (110 ^oC) connected to a Dean-Stark apparatus for 15 h, then concentrated under reduced pressure and dried azeotropically with benzene. The residue was taken up in CH_2Cl_2 (175 mL) and chilled in an ice bath. Triethylamine (14.6 mL, 105 mmol) was added, and the reaction was allowed to stir for 10 min. 4-Nitrobenzenesulfonyl chloride (NsCl, 8.53 g, 38.5 mmol) was added portionwise as a solid, and the reaction was stirred for 4 h at 0 ^oC. The reaction mixture was washed with 1 M NaH_2PO_4, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (SiO_2, 7:3 then 3:2 hexane/EtOAc) to yield **31** (9.48 g, 27.5 mmol, 79% over two steps) as a yellow solid. R_f 0.43 (1:1 hexane/EtOAc). Spectral data match those reported previously.[5]



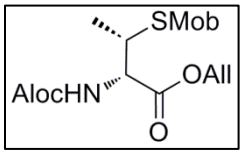
Compound **32**: A solution of **31** (2.70 g, 7.84 mmol) and triphenylphosphine (2.67 g, 10.2 mmol) in THF (30 mL) was chilled in an ice bath. Diisopropylazodicarboxylate (DIAD, 1.7 mL, 8.63 mmol) was added dropwise, and the reaction was stirred for 2.5 h at 0 ^oC, then concentrated under reduced pressure. The residue was taken up in EtOAc, washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (SiO_2, 5:1 hexane/EtOAc) to yield **32** (2.13 g, 6.53 mmol, 83%) as a yellow solid. R_f 0.54 (2:1 hexane/EtOAc). Spectral data match those reported previously.[5]



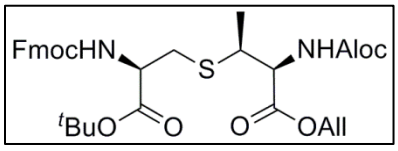
Compound **33**: A solution of **32** (0.65 g, 2.00 mmol) and 4-methoxybenzyl mercaptan (MobSH, 1.12 mL, 8.00 mmol) in CH_2Cl_2 (20 mL) was chilled in an ice bath. Boron trifluoride diethyl etherate (0.74 mL, 6.00 mmol) was added dropwise to the stirring solution. The reaction was stirred for 21 h at 4 ^oC, then washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography (SiO_2, 4:1 hexane/EtOAc) to yield **33** (0.82 g, 1.71 mmol, 86%) as a yellow solid. R_f 0.47 (2:1 hexane/EtOAc). Spectral data match those reported previously.[5]



Compound **8**: 4-Methoxybenzene thiol (PMP-SH, 1.84 mL, 15.0 mmol) and potassium carbonate (2.76 g, 20.0 mmol) were added to a stirring solution of **33** (2.40 g, 5.00 mmol) in 49:1 MeCN/dimethylsulfoxide (35 mL). The reaction was stirred as a heterogeneous mixture for 3 h, then concentrated under reduced pressure. The residue was taken up in EtOAc, washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The crude mixture was purified by flash chromatography (SiO_2, 3:2 then 2:3 hexane/EtOAc) to yield **8** (1.35 g, 4.57 mmol, 91%) as a colorless oil. R_f 0.30 (1:1 hexane/EtOAc). Spectral data match those reported previously.[5]

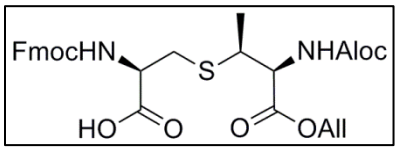


Compound **9**: Diisopropylethylamine (0.74 mL, 4.26 mmol) and allyloxycarbonyloxysuccinimide (AlocOSu, 0.74 g, 3.73 mmol) were added to a solution of **8** (1.05 g, 3.55 mmol) in CH_2Cl_2 (20 mL) and the reaction was stirred for 12 h. The reaction was washed with water, 10% citric acid and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography (SiO_2, 4:1 hexane/EtOAc) to yield **9** (1.31 g, 3.45 mmol, 97%) as a colorless oil. R_f 0.58 (2:1 hexane/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (dd, J = 9.5 Hz, 2.5 Hz, 2H), 6.84 (dd, J = 9.5 Hz, 2.5 Hz, 2H), 5.95-5.84 (m, 2H), 5.50 (d, J = 9.0 Hz, 1H), 5.37-5.20 (m, 4H), 4.66 (dd, J = 13.0 Hz, 6.0 Hz, 1H), 4.59-4.56 (m, 3H), 4.53 (dd, J = 9.5 Hz, 3.5 Hz, 1H), 3.79 (s, 3H), 3.67 (d, J = 13.0 Hz, 1H), 3.64 (d, J = 13.0 Hz, 1H), 3.31 (m, 1H), 1.30 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 158.9, 156.4, 132.7, 131.5, 130.1, 129.7, 119.3, 118.0, 114.1, 66.4, 66.1, 58.6, 55.4, 42.2, 35.2, 19.7. HRMS (ESI) calc. for $C_{19}H_{26}NO_5S$ 380.1532, found 380.1532.



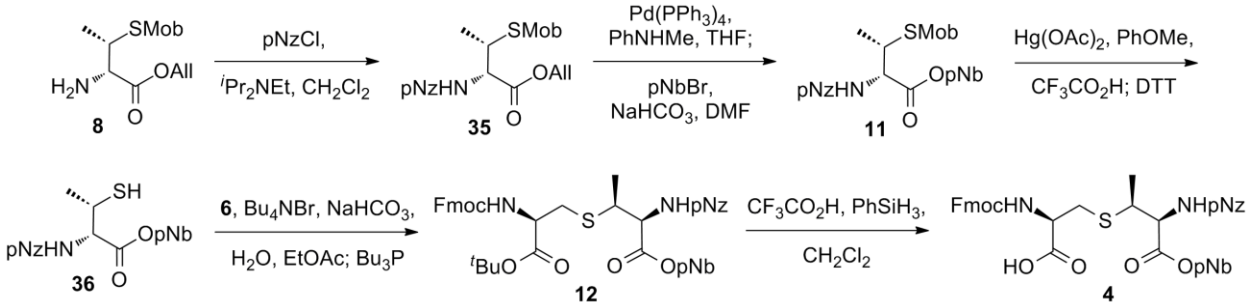
Compound **10**: Compound **9** (0.68 g, 1.80 mmol) was dissolved in TFA (10 mL) and anisole (780 μL, 7.20 mmol). Mercury(II) acetate (1.15 g, 3.60 mmol) was added as a solid, and the purple solution was stirred for 4 h. Dithiothreitol (DTT, 0.56 g, 3.60 mmol) was then added, forming a grey precipitate. This heterogeneous mixture was stirred vigorously for 15 h, then diluted with CH_2Cl_2 and centrifuged (4600 ×g, 10 min) to remove the solids. The supernatant was concentrated under reduced pressure, taken up in CH_2Cl_2 and water, and neutralized by slow addition of saturated aqueous $NaHCO_3$ to pH 7. The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (SiO_2, 15% EtOAc/hexane) to yield **34**, which was used directly for the next reaction without complete concentration or characterization due to its instability. R_f 0.52 (3:1 hexane/EtOAc). To the partially-concentrated **34** was added **6** (0.54 g, 1.20 mmol) and N_2-sparged EtOAc (6 mL). Tetrabutylammonium bromide (1.55 g, 4.80 mmol) was dissolved in N_2-sparged 0.5 M aqueous $NaHCO_3$ (pH adjusted to 8.5, 6 mL), then added to the organic solution. The biphasic reaction was stirred for 5 h, and the pH was adjusted to 8.5 as necessary with 1 M NaOH. Tributylphosphine (150 μL, 0.60 mmol) was added, and the reaction was stirred for an additional 17 h. The organic layer was isolated, washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (SiO_2, 4:1 hexane/EtOAc) to yield **10** (0.48 g, 0.77 mmol, 64% over two steps) as a colorless foam. R_f 0.34 (3:1 hexane/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 7.5 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 5.95-5.88 (m, 2H), 5.62 (d, J = 7.5 Hz, 1H), 5.57 (d, J = 9.5 Hz, 1H), 5.37-5.20 (m, 4H), 4.70-4.53 (m, 5H), 4.48-4.37 (m, 3H), 4.24 (t, J

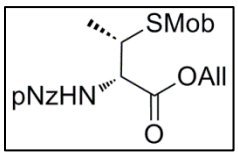
= 7.0 Hz, 1H), 3.43 (m, 1H), 3.02-2.89 (m, 2H), 1.48 (s, 9H), 1.34 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 169.4, 156.4, 155.8, 144.0, 143.9, 141.4, 132.6, 131.4, 127.9, 127.2, 125.2, 120.1, 119.6, 118.1, 83.3, 67.3, 66.5, 66.2, 58.5, 54.3, 47.2, 44.0, 34.2, 29.8, 28.1, 19.8. HRMS (ESI) calc. for $C_{33}H_{40}N_2O_8SNa$ 647.2403, found 647.2405.



Compound **3**: To a solution of **10** (0.45 g, 0.72 mmol) in CH_2Cl_2 (3 mL) was added phenylsilane (95 µL, 0.76 mmol), followed by TFA (3 mL). The reaction was stirred for 2 h, concentrated under reduced pressure and repeatedly redissolved in CH_2Cl_2 and concentrated to remove residual TFA. The crude material was purified by flash chromatography (SiO_2, 1%-2% MeOH/CH_2Cl_2) to yield **3** (0.39 g, 0.69 mmol, 96%) as a white solid after lyophilization from 1:1 benzene/MeCN. R_f 0.05 (2:1 EtOAc/hexane). ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (d, *J* = 7.5 Hz, 2H), 7.69 (d, *J* = 7.5 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 5.99-5.88 (m, 2H), 5.38-5.29 (m, 2H), 5.24-5.16 (dd, *J* = 22.5 Hz, 10.5 Hz, 2H), 4.65 (m, 2H), 4.55 (d, *J* = 5.5 Hz, 2H), 4.47 (d, *J* = 4.0 Hz, 1H), 4.41-4.30 (m, 3H), 4.25 (t, *J* = 7.0, 1H), 3.45 (m, 1H), 3.08 (dd, *J* = 13.5 Hz, 4.5 Hz, 1H), 2.83 (dd, *J* = 13.5 Hz, 8.5 Hz, 1H), 1.31 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 173.7, 171.7, 158.6, 158.4, 145.3, 145.2, 142.6, 134.2, 133.1, 128.8, 128.2, 126.3, 120.9, 119.2, 117.8, 68.2, 67.2, 66.8, 60.2, 55.3, 48.4, 43.8, 34.2, 19.8. HRMS (ESI) calc. for $C_{29}H_{33}N_2O_8S$ 569.1958, found 569.1959.

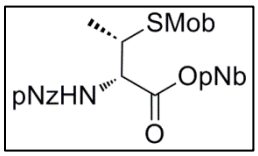
<u>Nitrobenzyl-protected methyllanthionine building block **4**</u>



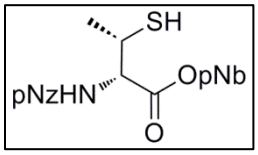


Compound **35**: Diisopropylethylamine (1.4 mL, 8.1 mmol) and 4-nitrobenzyl chloroformate (pNzCl, 1.46 g, 6.77 mmol) were added to a solution of **8** (2.00 g, 6.77 mmol) in CH_2Cl_2 (35 mL), and the reaction was stirred for 16 h. The reaction was washed with water, 10% citric acid and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography (SiO_2, 4:1 hexane/EtOAc) to yield **35** (3.10 g, 6.53 mmol, 96%) as a colorless oil. R_f 0.50 (2:1 hexane/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (d, *J* = 9.0 Hz, 2H), 7.51 (d, *J* = 9.0 Hz, 2H), 7.19 (d, *J* = 8.5 Hz, 2H), 6.83 (dt, *J* = 8.5 Hz, 2.0 Hz, 2H), 5.91-5.83 (m, 1H), 5.62 (d, *J* = 9.0 Hz, 1H), 5.36-5.32 (dd, *J* = 17.0 Hz, 1.0 Hz, 1H), 5.27 (dd, *J* = 10.5 Hz, 1.0 Hz, 1H), 5.23 (d, *J* = 13.5 Hz, 1H), 5.19 (d, *J* = 13.5 Hz, 1H), 4.66 (dd, *J* = 13.0 Hz, 6.0 Hz, 1H), 4.58 (dd, *J* = 13.0 Hz, 6.0 Hz, 1H), 4.53 (dd, *J* = 9.0 Hz, 3.5 Hz, 1H), 3.78 (s, 3H), 3.67 (d, *J* = 13.0 Hz, 1H), 3.63 (d, *J* = 13.0 Hz, 1H), 3.33 (m, 1H), 1.30 (d, *J* = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 158.9, 156.1, 147.7, 143.8,

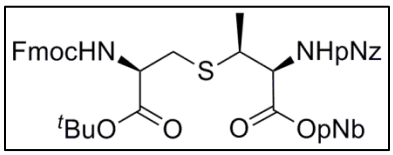
131.4, 130.0, 129.5, 128.1, 123.9, 119.4, 114.1, 66.5, 65.7, 58.7, 55.4, 42.1, 35.2, 19.8. HRMS (ESI) calc. for $C_{23}H_{27}N_2O_7S$ 475.1539, found 475.1556.



Compound **11**: Tetrakis(triphenylphosphine)palladium(0) (390 mg, 0.34 mmol) was added to a solution of **35** (3.20 g, 6.74 mmol) and *N*-methylaniline (1.5 mL, 13.5 mmol) in THF (60 mL). The reaction was stirred for 1.5 h, protected from light, then concentrated under reduced pressure. The resulting oil was taken up in DMF (25 mL). Sodium bicarbonate (1.13 g, 13.5 mmol) and 4-nitrobenzyl bromide (3.65 g, 16.9 mmol) were added as solids. The reaction was stirred for 30 h, with additional 4-nitrobenzyl bromide (pNbBr, 1.46 g, 6.74 mmol) added after 11 h. The reaction mixture was concentrated, taken up in EtOAc, washed with saturated aqueous $NaHCO_3$, water and brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (SiO_2, 30% EtOAc/hexane) to yield **11** (3.55 g, 6.23 mmol, 93% over two steps) as an amber foam. R_f 0.38 (2:1 hexane/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (app. t, J = 8.5 Hz, 4H), 7.50 (d, J = 8.5 Hz, 2H), 7.45 (d, J = 9.0 Hz, 2H), 7.13 (d, J = 8.5 Hz, 2H), 6.78 (dt, J = 8.5 Hz, 2.0 Hz, 2H), 5.60 (d, J = 9.0 Hz, 1H), 5.28-5.14 (m, 4H), 4.59 (dd, J = 9.0 Hz, 3.5 Hz, 1H), 3.75 (s, 3H), 3.64 (d, J = 13.0 Hz, 1H), 3.59 (d, J = 13.0 Hz, 1H), 3.33 (m, 1H), 1.33 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 159.0, 156.0, 147.9, 147.8, 143.6, 142.2, 129.9, 129.3, 128.7, 128.2, 123.93, 123.89, 114.1, 66.0, 65.8, 58.8, 55.3, 41.7, 35.0, 19.4. HRMS (ESI) calc. for $C_{27}H_{28}N_3O_9S$ 570.1546, found 570.1566.

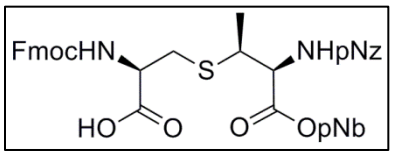


Compound **36**: Compound **11** (0.85 g, 1.50 mmol) was dissolved in TFA (6 mL) and anisole (650 μL, 6.00 mmol). Mercury(II) acetate (0.96 g, 3.00 mmol) was added, and the purple solution was stirred for 4 h. Dithiothreitol (0.46 g, 3.00 mmol) was added, immediately forming a grey precipitate. This heterogeneous mixture was stirred vigorously for 15 h, then diluted with CH_2Cl_2 and centrifuged (4600 ×*g*, 10 min) to remove the solids. The supernatant was concentrated under reduced pressure, taken up in CH_2Cl_2 and water, and neutralized by slow addition of saturated aqueous $NaHCO_3$ to pH 7. The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (SiO_2, 3:1 hexane/EtOAc) to yield **36**, which was used directly for the next reaction. R_f 0.60 (1:1 hexane/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (app. t, J = 8.5 Hz, 4H), 7.53 (d, J = 8.5 Hz, 4H), 5.66 (d, J = 9.0 Hz, 1H), 5.34-5.21 (m, 4H), 4.66 (dd, J = 9.0 Hz, 3.0 Hz, 1H), 3.65 (m, 1H), 1.68 (bs, 1H), 1.40 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 156.2, 148.3, 143.5, 142.1, 128.8, 128.3, 124.1, 124.0, 66.2, 66.0, 59.8, 37.4, 22.1. HRMS (ESI) calc. for $C_{19}H_{20}N_3O_8S$ 450.0971, found 450.0982.



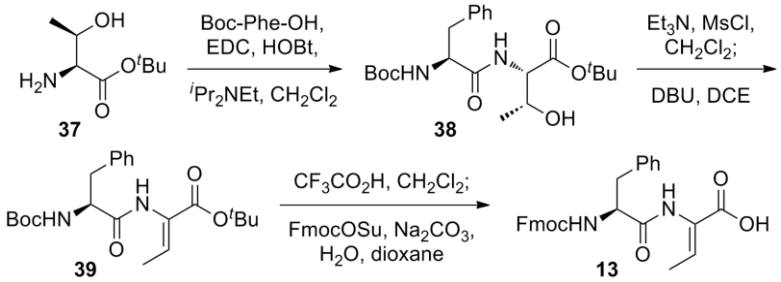
Compound **12**: Compounds **6** (0.45 g, 1.00 mmol) and **36** (assumed 1.50 mmol) were dissolved in N_2-sparged EtOAc (5 mL). Tetrabutylammonium bromide (1.29 g, 4.00 mmol) was dissolved in N_2-sparged 0.5 M aqueous $NaHCO_3$ (pH adjusted to 8.5, 5 mL), then added to the organic solution. The biphasic mixture was stirred under N_2 for 7 h, and the pH was adjusted to 8.5 as necessary with 1 M NaOH. Tributylphosphine (125 μL, 0.50 mmol) was added, and the reaction was stirred for an additional 17 h. The organic layer was isolated, washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The

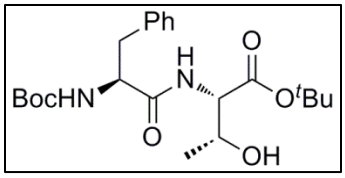
crude material was purified by flash chromatography (SiO_2, 4:1 then 2:1 hexane/EtOAc) to yield **12** (0.69 g, 0.85 mmol, 85% over two steps) as a colorless foam. R_f 0.24 (2:1 hexane/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (app. t, *J* = 8.5 Hz, 4 H), 7.75 (d, *J* = 7.5 Hz, 2H), 7.56 (m, 2H), 7.47 (app. d, *J* = 7.0 Hz, 4H), 7.39 (dt, *J* = 7.5 Hz, 3.0 Hz, 2H), 7.29 (t, *J* = 7.5 Hz, 2H), 5.78 (d, *J* = 9.0 Hz, 1H), 5.66 (d, *J* = 7.0 Hz, 1H), 5.34-5.15 (m, 4H), 4.57 (dd, *J* = 9.0 Hz, 3.0 Hz, 1H), 4.44 (m, 1H), 4.34 (d, *J* = 7.0 Hz, 2H), 4.19 (t, *J* = 7.0 Hz, 1H), 3.48 (m, 1H), 3.01 (dd, *J* = 13.5 Hz, 4.5 Hz, 1H), 2.88 (dd, *J* = 13.5 Hz, 4.5 Hz, 1H), 1.47 (s, 9H), 1.35 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 169.2, 156.1, 155.8, 148.0, 147.7, 143.8, 143.7, 143.5, 142.0, 141.4, 128.8, 128.1, 127.9, 127.2, 125.11, 125.08, 123.94, 123.87, 120.2, 83.5, 67.4, 66.1, 65.8, 58.7, 54.5, 47.1, 43.5, 33.8, 28.1, 19.7. HRMS (ESI) calc. for $C_{41}H_{42}N_4O_{12}SNa$ 837.2418, found 837.2416.



Compound **4**: To a solution of **12** (0.65 g, 0.80 mmol) in CH_2Cl_2 (3 mL) and phenylsilane (105 µL, 0.84 mmol) was added TFA (3 mL). The reaction was stirred for 2 h, concentrated under reduced pressure and repeatedly redissolved in CH_2Cl_2 and concentrated to remove residual TFA. The crude material was purified by flash chromatography (SiO_2, 1%-2% MeOH/CH_2Cl_2) to yield **4** (0.58 g, 0.76 mmol, 95%) as a white solid. R_f 0.16 (EtOAc). ^{1}H NMR (500 MHz, CD_3OD) δ 8.12 (app. t, *J* = 8.5 Hz, 4 H), 7.75 (d, *J* = 7.5 Hz, 2H), 7.63 (d, *J* = 7.5 Hz, 2H), 7.45 (app. t, *J* = 7.0 Hz, 4H), 7.35 (t, *J* = 7.5 Hz, 2H), 7.27 (t, *J* = 7.5 Hz, 2H), 5.30-5.16 (m, 4H), 4.57 (d, *J* = 4.5 Hz, 1H), 4.39 (dd, *J* = 8.5 Hz, 4.5 Hz, 1H), 4.35-4.24 (m, 2H), 4.18 (t, *J* = 7.0 Hz, 1H), 3.49 (m, 1H), 3.09 (dd, *J* = 13.5 Hz, 4.5 Hz, 1H), 2.83 (dd, *J* = 13.5 Hz, 8.5 Hz, 1H), 1.33 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 173.6, 171.5, 158.4, 158.3, 149.0, 148.8, 145.7, 145.2, 145.1, 144.2, 142.5, 129.7, 129.0, 128.8, 128.2, 126.3, 124.6, 124.5, 120.9, 68.2, 66.8, 66.4, 60.4, 55.5, 48.3, 43.7, 34.1, 19.6. HRMS (ESI) calc. for $C_{37}H_{35}N_4O_{12}S$ 759.1972, found 759.1964.

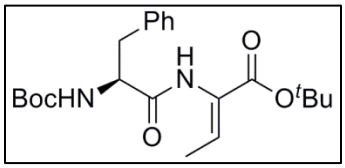
Peptide fragment **13**



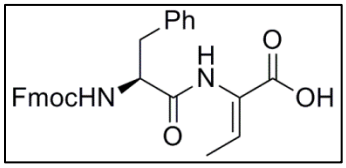


Compound **38**: L-Threonine *tert*-butyl ester hydrochloride (**37**, 0.50 g, 2.36 mmol) was dissolved in CH_2Cl_2 (12 mL) and diisopropylethylamine (620 µL, 3.54 mmol). *N*-Boc-L-phenylalanine (0.63 g, 2.36 mmol), HOBt (0.36 g, 2.36 mmol) and EDC (0.45 g, 2.36 mmol) were added as solids. The reaction was stirred for 14 h, then washed with saturated aqueous $NaHCO_3$, 10% citric acid and water. Each aqueous wash was back-extracted with CH_2Cl_2. The organic fractions were combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated, yielding **38** (0.98 g, 2.32 mmol, 98%)

as a white solid. R_f 0.82 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30-7.19 (m, 5H), 6.67 (d, J = 8.4 Hz, 1 H), 5.04 (d, J = 7.6 Hz, 1H), 4.43 (dd, J = 8.4 Hz, 3.2 Hz, 1 H), 4.38 (m, 1H), 4.19 (m, 1H), 3.16-3.03 (m, 2H), 2.45 (bs, 1H), 1.46 (s, 9H), 1.39 (s, 9H), 1.15 (d, J = 6.4 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 169.7, 155.7, 136.6, 129.5, 128.7, 127.0, 82.7, 80.4, 68.7, 58.1, 56.0, 38.1, 28.4, 28.1, 20.0. HRMS (ESI) calc. for $C_{22}H_{35}N_2O_6$ 423.2495, found 423.2499.

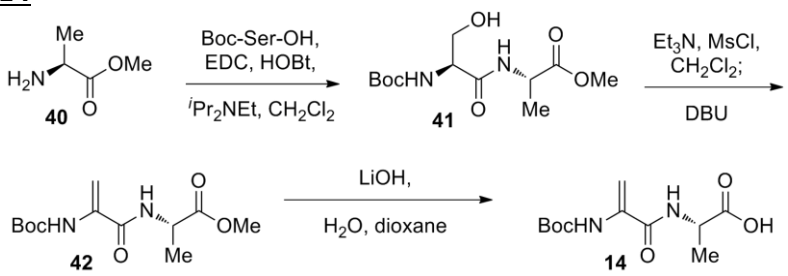


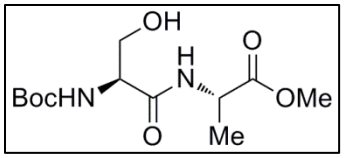
Compound **39**: A two-step dehydration selective for the *Z*-olefin was performed based on the procedure of Pattabiraman *et al*.[6] Compound **38** (1.19 g, 2.82 mmol) was dissolved in CH_2Cl_2 (30 mL) and triethylamine (0.98 mL, 7.05 mmol) and chilled in an ice bath. Methanesulfonyl chloride (MsCl, 0.44 mL, 5.64 mmol) was added dropwise, and the reaction was stirred for 1 h, gradually warming to room temperature. The reaction was concentrated under reduced pressure, then taken up in 1,2-dichloroethane (DCE, 30 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 1.69 mL, 11.3 mmol). The reaction was heated to reflux in an oil bath (90 ^{o}C) for 4 h, then concentrated. The residue was taken up in EtOAc, washed with 10% citric acid, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (SiO_2, 7:1 then 4:1 hexane/EtOAc) to yield **39** (1.03 g, 2.55 mmol, 90% over two steps) as a white solid. R_f 0.48 (2:1 hexane/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (s, 1H), 7.31-7.27 (m, 2H), 7.24-7.21 (m, 3H), 6.68 (q, J = 7.0 Hz, 1H), 5.00 (m, 1H), 4.49 (m, 1H), 3.19 (dd, J = 13.5 Hz, 6.0 Hz, 1H), 3.07 (m, 1H), 1.67 (d, J = 7.0 Hz, 3H), 1.46 (s, 9H), 1.40 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.5, 163.4, 155.6, 136.6, 132.6, 129.5, 128.8, 127.1, 126.9, 81.8, 80.5, 56.1, 38.2, 28.3, 28.1, 14.8. HRMS (ESI) calc. for $C_{22}H_{33}N_2O_5$ 405.2389, found 405.2392.



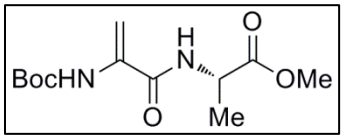
Compound **13**: Compound **39** (1.27 g, 3.14 mmol) was dissolved in CH_2Cl_2 (10 mL) and TFA (10 mL) and stirred for 1.5 h. The reaction was concentrated under reduced pressure, repeatedly taken up in CH_2Cl_2 and re-concentrated to remove residual acid. To the resulting residue was added sodium carbonate (0.67 g, 6.28 mmol), water (30 mL) and 1,4-dioxane (30 mL), and the system was chilled in an ice bath. *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (FmocOSu, 1.06 g, 3.14 mmol) was then added portionwise as a solid. The reaction was stirred for 20 h, gradually warming to room temperature. Volatile components were removed under reduced pressure, then the system was diluted with H_2O and acidified to pH 2 with 2 M HCl. The aqueous suspension was extracted with EtOAc (3x), then the combined organic layers were dried over Na_2SO_4, filtered, and concentrated to ~20 mL. Hexane (~150 mL) was added to form a precipitate, which was isolated by filtration and dried to yield **13** (1.40 g, 2.98 mmol, 95% over two steps) as a white powder. R_f 0.12 (EtOAc). ^{1}H NMR (400 MHz, CD_3OD) δ 7.75 (d, J = 7.2 Hz, 2H), 7.55 (m, 2H), 7.34 (t, J = 7.2 Hz, 2H), 7.29-7.15 (m, 7H), 6.82 (q, J = 7.2 Hz, 1H), 4.50 (dd, J = 9.6 Hz, 5.2 Hz, 1H), 4.30-4.16 (m, 2H), 4.11 (t, J = 7.0 Hz, 1H), 3.21 (dd, J = 13.8 Hz, 5.2 Hz, 1H), 2.89 (dd, J = 13.8 Hz, 9.6 Hz, 1H), 1.65 (d, J = 7.2 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 173.1, 167.2, 158.2, 145.2, 142.5, 138.6, 136.8, 130.4, 129.4, 128.7, 128.4, 128.1, 127.7, 126.3, 120.9, 68.0, 57.8, 48.3, 39.1, 14.1. HRMS (ESI) calc. for $C_{28}H_{27}N_2O_5$ 471.1920, found 471.1923.

Peptide fragment **14**

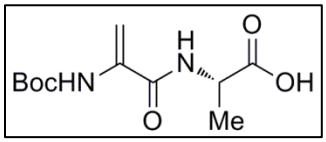




Compound **41**: L-Alanine methyl ester hydrochloride (**40**, 0.70 g, 5.00 mmol) was taken up in CH_2Cl_2 (25 mL) and diisopropylethylamine (1.3 mL, 7.50 mmol) and stirred for 5 min. *N*-Boc-L-serine (1.03 g, 5.00 mmol), HOBt (0.84 g, 5.50 mmol) and EDC (0.96 g, 5.00 mmol) were added as solids. The reaction was stirred for 21 h, then washed with saturated aqueous $NaHCO_3$, 10% citric acid, water and brine. Each aqueous wash was back-extracted with CH_2Cl_2. The organic fractions were combined, dried over Na_2SO_4, filtered, and concentrated to yield **41** (1.24 g, 4.27 mmol, 85%) as a white solid. R_f 0.45 (EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (d, *J* = 7.0 Hz, 1H), 5.69 (d, *J* = 6.5 Hz, 1H), 4.54 (pent, *J* = 7.0 Hz, 1H), 4.22 (m, 1H), 3.95 (dd, *J* = 11.0 Hz, 4.0 Hz, 1H), 3.71 (s, 3H), 3.65 (dd, *J* = 11.0 Hz, 4.0 Hz, 1H), 1.41 (s, 9H), 1.38 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4, 171.1, 156.1, 80.4, 63.1, 55.3, 52.7, 48.3, 28.3, 17.8. HRMS (ESI) calc. for $C_{12}H_{22}N_2O_6Na$ 313.1376, found 313.1377.



Compound **42**: Compound **41** (1.08 g, 3.72 mmol) was dissolved in CH_2Cl_2 (40 mL) and triethylamine (1.3 mL, 9.30 mmol) and chilled in an ice bath. Methanesulfonyl chloride (580 µL, 7.44 mmol) was added dropwise, and the reaction was allowed to warm to room temperature and stirred for 1 h. DBU (2.8 mL, 18.6 mmol) was then added, and the reaction was stirred for an additional 2 h, then concentrated under reduced pressure. The residue was taken up in EtOAc, washed with 10% citric acid, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (SiO_2, 7:3 hexane/EtOAc) to yield **42** (0.81 g, 2.97 mmol, 80% over two steps) as a pale yellow oil. R_f 0.70 (1:1 hexane/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (s, 1H), 6.69 (d, *J* =7.0 Hz, 1H), 6.03 (s, 1H), 5.12 (t, *J* = 1.5 Hz, 1H), 4.61 (pent, *J* = 7.0 Hz, 1H), 3.77 (s, 3H), 1.46-1.42 (m, 12H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 163.7, 152.7, 134.5, 98.4, 80.6, 52.7, 48.6, 28.2, 18.2. HRMS (ESI) calc. for $C_{12}H_{20}N_2O_5Na$ 295.1270, found 295.1275.

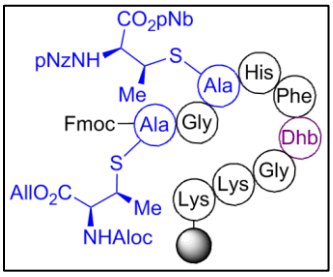


Compound **14**: To a solution of **42** (0.54 g, 2.00 mmol) in 1,4-dioxane (5 mL) was added 1 M aqueous lithium hydroxide (5 mL). The reaction was stirred for 1 h, then volatile components were removed under reduced pressure. The system was diluted with water and acidified to pH 3 with 2 M HCl. The aqueous suspension was extracted with EtOAc (10x), then the combined organic layers were dried over Na_2SO_4, filtered, and concentrated to yield **14** (0.34 g, 1.32 mmol, 66%) as a colorless foam. R_f 0.03 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (s, 1H), 6.71 (d, *J* = 7.2 Hz, 1H), 6.02 (s, 1H), 5.14 (s, 1H), 4.63 (pent, *J* = 7.2 Hz, 1H), 1.51 (d, *J* = 7.2 Hz, 3H), 1.45 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.1, 164.1, 159.9, 134.7, 110.0, 81.0, 48.2, 28.4, 18.1. HRMS (ESI) calc. for $C_{11}H_{19}N_2O_5$ 259.1294, found 259.1288.

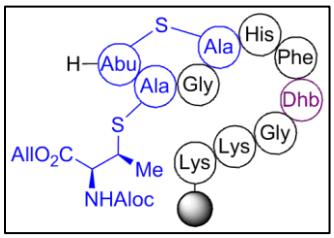
Synthetic procedures for epilancin 15X analogues.

Authentic epilancin 15X (**1**) was provided by Dr. Juan Velásquez (University of Illinois at Urbana-Champaign) after isolation from the producing organism *Staphylococcus epidermidis* 15X154 using a published procedure.[7] HRMS (MALDI-TOF) calc. for $C_{145}H_{234}N_{41}O_{33}S_3$ 3173.705, found 3173.860.

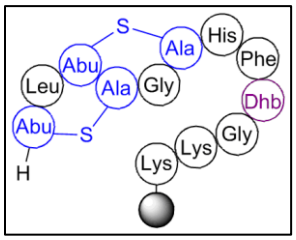
Standard SPPS procotols: Unless noted otherwise, standard cycles for SPPS were performed as follows, using a fritted glass reaction vessel equipped with a N_2 inlet for resin/reagent agitation and a suction outlet for draining. Fmoc deprotection was achieved by agitating resin with 20% piperidine in DMF for 20 min. After draining the reaction vessel, the resin was washed with DMF (3 x 30 s) and CH_2Cl_2 (2 x 30 s). The appropriately side-chain protected Fmoc-amino acid (5 equiv.) in DMF (5-10 mL) was pre-activated with DIC and HOBt (5 equiv. each) for 5 min, then added to resin and agitated for 45-60 min. After draining the reaction vessel, the resin was washed as before. The completion of all couplings was assessed by a Kaiser test; double couplings were performed as needed but were generally not necessary. Test cleavages were performed after all cyclization steps by removing a small portion of dry resin from the reaction vessel and treating with 90:5:5 TFA/H_2O/triisopropylsilane for 1 h under N_2. After removing the cleaved resin by filtration, the filtrate was concentrated under a stream of N_2. The peptide was precipitated with cold Et_2O, isolated by centrifugation and dissolved in 1:1 H_2O/MeCN. An aliquot of this solution was spotted onto a MALDI-TOF MS target for analysis, while the remainder was lyophilized to dryness, taken up in 0.1% TFA/H_2O and analyzed by analytical RP-HPLC.



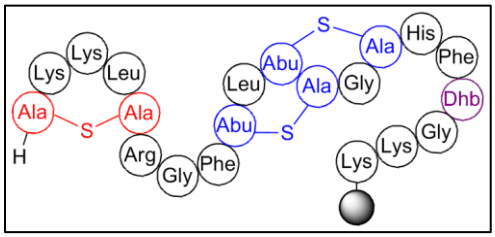
Intermediate **16**: The substitution of the Fmoc-Lys(Boc)-Wang resin (**15**, initial substitution 0.36 mmol/g) was first reduced such that 1 equiv. corresponded to 0.10 mmol/g. To ensure local as well as global reduction in resin substitution, the resin was first swelled in DMF for 20 min, followed by addition of Fmoc-Lys(Boc)-OH (1 equiv.) and Boc-Ala-OH (2 equiv.) that had been pre-activated with DIC and HOBt (3 equiv. each) for 5 min. The reaction was performed for 15 h. Any remaining free resin sites were capped with 1:2:7 Ac_2O/pyridine/DMF for 15 min. Adjusted resin substitution was calculated as follows: Fmoc-protected resin (10 mg) was agitated with 20% piperidine/DMF (1.0 mL) for 15 min. A 20 µL aliquot of this solution was diluted 100:1 with DMF. The absorbance of this solution at 301 nm was recorded after blanking with pure DMF, and the resin substitution was calculated using the equation: substitution = 101(absorbance)/7.8(resin weight).[8] After standard Fmoc deprotection and Fmoc-Gly-OH coupling/deprotection, fragment **13** (2 equiv.) was pre-activated with DIC and HOAt (2 equiv. each) and $^{i}Pr_2NEt$ (5 equiv.) in DMF for 10 min, reacted with the resin-bound peptide for 12 h, and deprotected by the standard protocol. Fmoc-His(Trt)-OH was coupled/deprotected by the standard protocol. MeLan building block **4** (1.5 equiv.) was pre-activated with DIC and HOAt (3 equiv. each) in DMF for 5 min, then reacted with the resin-bound peptide for 2 h and deprotected by the standard protocol. Fmoc-Gly-OH was coupled/deprotected by the standard protocol. MeLan building block **3** (1.5 equiv.) was pre-activated with DIC and HOAt (3 equiv.) in DMF for 5 min, then reacted with the resin-bound peptide for 2 h, but not Fmoc-deprotected, to yield resin-bound intermediate **16**.



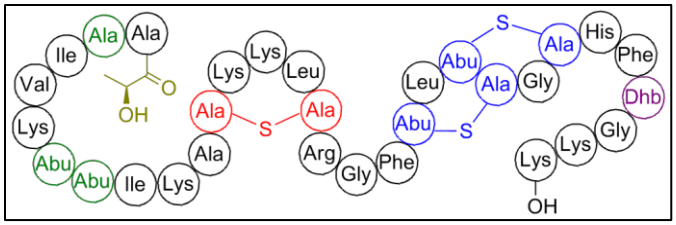
Monocyclic intermediate **17**: The nitrobenzyl protecting groups of **16** were removed with two treatments of 6 M $SnCl_2$ and 5 mM HCl/dioxane in DMF (5 mL) for 1 h each. Following the second treatment, the reaction vessel was drained, and the resin was washed with 1:1 DMF/H_2O (3 x 1 min), 1:1 THF/H_2O (3 x 1 min), DMF (3 x 30 s) and CH_2Cl_2 (2 x 30 s). The Fmoc group was removed by the standard protocol, followed by washing with DMF (5 x 30 s), CH_2Cl_2 (3 x 30 s) and DMF (2 x 30 s) to remove all traces of piperidine. Cyclization was promoted by adding PyAOP and HOAt (5 equiv. each) in DMF to the resin and agitating for 5 min, then adding 2,4,6-collidine (10 equiv.) and agitating for 1.5 h. After draining, this treatment was repeated for 1.5 h to yield **17**. Test cleavage analysis was performed to confirm completed cyclization. HRMS (MALDI-TOF) calc. for $C_{56}H_{84}N_{15}O_{15}S_2$ 1270.571, found 1270.572.



Bicyclic intermediate **18**: Fmoc-Leu-OH was coupled to **17** by the standard protocol, but not Fmoc-deprotected. The allyl protecting groups were then removed by agitating resin with tetrakis(triphenylphosphine)palladium (0) (1 equiv.) and phenylsilane (10 equiv.) in 1:1 DMF/ CH_2Cl_2 (10 mL) for 2 h, protected from light. After draining the reaction vessel, the resin was washed with CH_2Cl_2 (3 x 1 min), 0.5% diethyldithiocarbamate in DMF (3 x 1 min), DMF (3 x 30 s) and CH_2Cl_2 (2 x 30 s). The Fmoc group was removed by the standard protocol, followed by washing with DMF (5 x 30 s), CH_2Cl_2 (3 x 30 s) and DMF (2 x 30 s) to remove all traces of piperidine. Cyclization was promoted by adding PyAOP and HOAt (5 equiv. each) in DMF to the resin and agitating for 5 min, then adding 2,4,6-collidine (10 equiv.) and agitating for 2 h. After draining, this treatment was repeated for 2 h to yield **18**. Test cleavage analysis was performed to confirm completed cyclization. HRMS (MALDI-TOF) calc. for $C_{55}H_{85}N_{16}O_{13}S_2$ 1241.592, found 1241.629.



Tricyclic intermediate **20**: Fmoc-Phe-OH, Fmoc-Gly-OH and Fmoc-Arg(Pbf)-OH were coupled to **18** and deprotected by the standard protocol. Lan building block **2** (1.5 equiv.) was pre-activated with DIC and HOAt (3 equiv. each) in DMF for 5 min and coupled to the resin-bound peptide for 2 h, then deprotected by the standard protocol. Fmoc-Leu-OH and Fmoc-Lys(Boc)-OH were coupled and deprotected by the standard protocol, then a second Fmoc-Lys(Boc)-OH was coupled but not Fmoc-deprotected. Removal of the allyl and Fmoc groups, peptide cyclization and test cleavage analysis were performed as described for **18** to yield resin-bound intermediate **20** HRMS (MALDI-TOF) calc. for $C_{96}H_{152}N_{29}O_{21}S_3$ 2143.087, found 2143.117.



Analogue **21**: The next 10 residues were coupled to resin-bound **20** and deprotected by the standard protocol. The N-terminus was acylated by treatment with D-lactic acid (5 equiv.), DEPBt (5 equiv.) and $^{i}Pr_2NEt$ (10 equiv.) for 2 h. The peptide

was cleaved from resin and globally deprotected with 90:5:2.5:2.5 TFA/H_2O/triisopropylsilane/ thioanisole under N_2 for 2 h. The cleaved resin was removed by filtration, and the filtrate was concentrated under a stream of N_2. The peptide was precipitated with cold Et_2O, isolated by centrifugation, dissolved in 1:1 H_2O/MeCN and lyophilized to dryness. Crude **21** was dissolved to 10 mg/mL in 5% MeCN/H_2O with 0.1% TFA and purified by preparatory RP-HPLC using a solvent gradient of 5% std. B for 1 min, then 5-25% over 4 min, then 25-50% over 25 min, then 50-100% over 1 min. Partially-pure **21** eluted in fractions collected over 25.3-29.2 min. These fractions were concentrated and re-purified under the same conditions, with pure product eluting over 27.1-28.0 min. Lyophilization yielded **21** (2.0 mg, 0.63 μmol, 1.6% from a 40 μmol scale synthesis, 93% per step over 59 steps) as a white powder (see *Fig. S1* for HPLC and MS; see *Fig. S4* for MS/MS). HRMS (MALDI-TOF) calc. for $C_{145}H_{240}N_{41}O_{33}S_3$ 3179.752, found 3179.794.

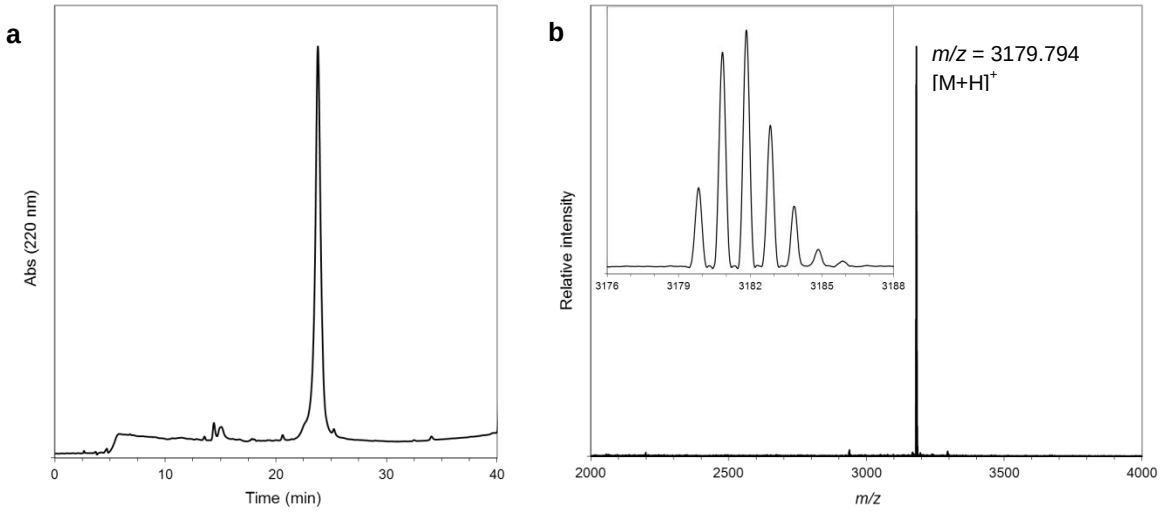
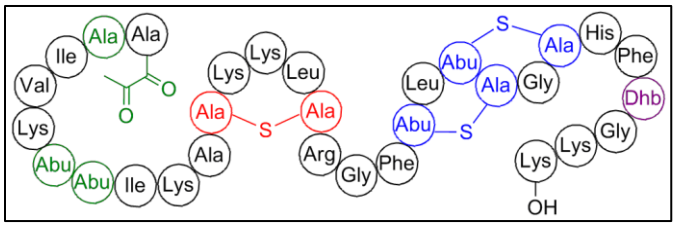


Figure S1. Characterization of analogue **21**. (a) Analytical RP-HPLC chromatogram. (b) MALDI-TOF mass spectrum (insert: zoom-in on the expected product mass).



Analogue **22**: The next 9 residues were coupled to resin-bound **20** and deprotected by the standard protocol. The N-terminal PyrAla moiety was incorporated by coupling fragment **14** (4 equiv.) with DIC and HOAt (4 equiv. each) in DMF for 3 h. To prevent reduction of the ketone formed upon Boc deprotection, anisole was used in place of triisopropylsilane in the cleavage cocktail. The peptide was cleaved from resin and globally deprotected with 90:5:2.5:2.5 TFA/H_2O/ anisole/thioanisole under N_2 for 3 h. Crude **22** was isolated and purified as described for **21**. Partially-pure **22** eluted in fractions collected over 27.2-30.0 min. These fractions were concentrated and re-purified under the same conditions, with pure product eluting over 27.5-28.5 min. Lyophilization yielded **22** (1.3 mg, 0.41 μmol, 1.6% from a 25 μmol scale synthesis, 93% per step over 57 steps) as a white powder (see *Fig. S2* for HPLC and MS). HRMS (MALDI-TOF) calc. for $C_{145}H_{238}N_{41}O_{33}S_3$ 3177.736, found 3177.765.

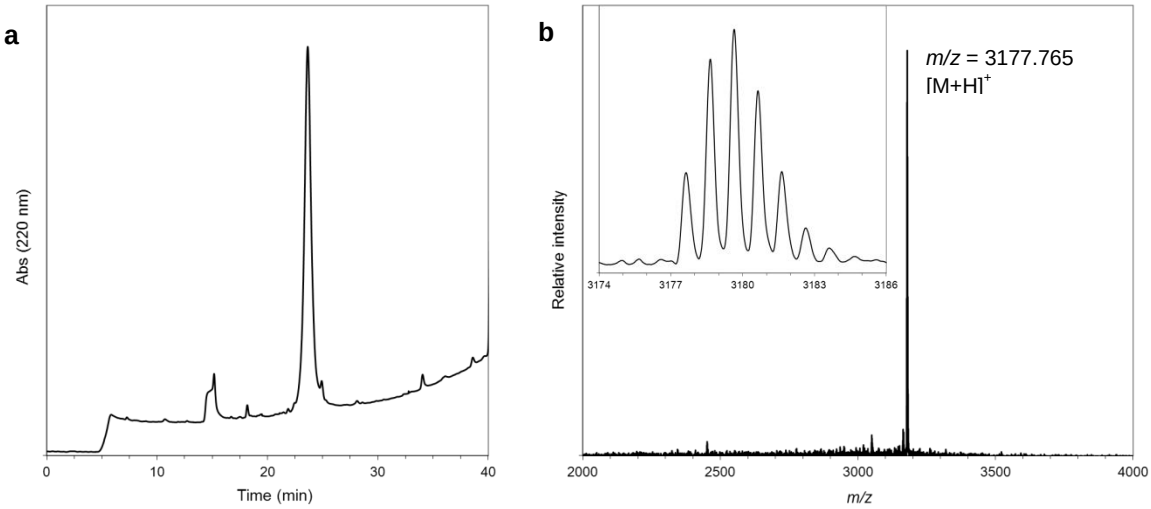
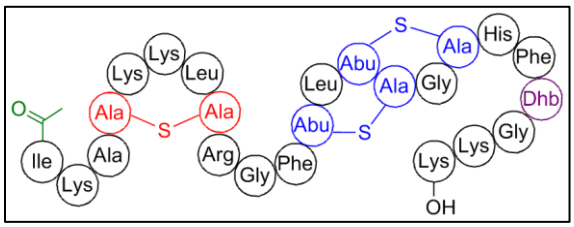


Figure S2. Characterization of analogue **22**. (a) Analytical RP-HPLC chromatogram. The peak centered at 15.0 min represents an instrument impurity. (b) MALDI-TOF mass spectrum (insert: zoom-in on the expected product mass).



Truncated analogue **23**: Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH and Fmoc-Ile-OH were coupled to resin-bound **20** and deprotected by the standard protocol. The N-terminus was acetylated with 1:2:4 Ac_2O/ pyridine/DMF for 30 min. The peptide was cleaved from resin and globally deprotected with 90:5:2.5:2.5 TFA/H_2O/ triisopropylsilane/thioanisole under N_2 for 2 h. Crude **23** was isolated and purified as for **21**. Partially-pure **23** eluted in fractions collected over 14.4-17.0 min. These fractions were concentrated and re-purified under the same conditions, with pure product eluting over 16.1-17.0 min. Lyophilization yielded **23** (1.2 mg, 0.48 μmol, 1.9% from a 25 μmol scale synthesis, 92% per step over 45 steps) as a white powder (see *Fig. S3* for HPLC and MS). HRMS (MALDI-TOF) calc. for $C_{113}H_{182}N_{33}O_{25}S_3$ 2497.314, found 2497.405.

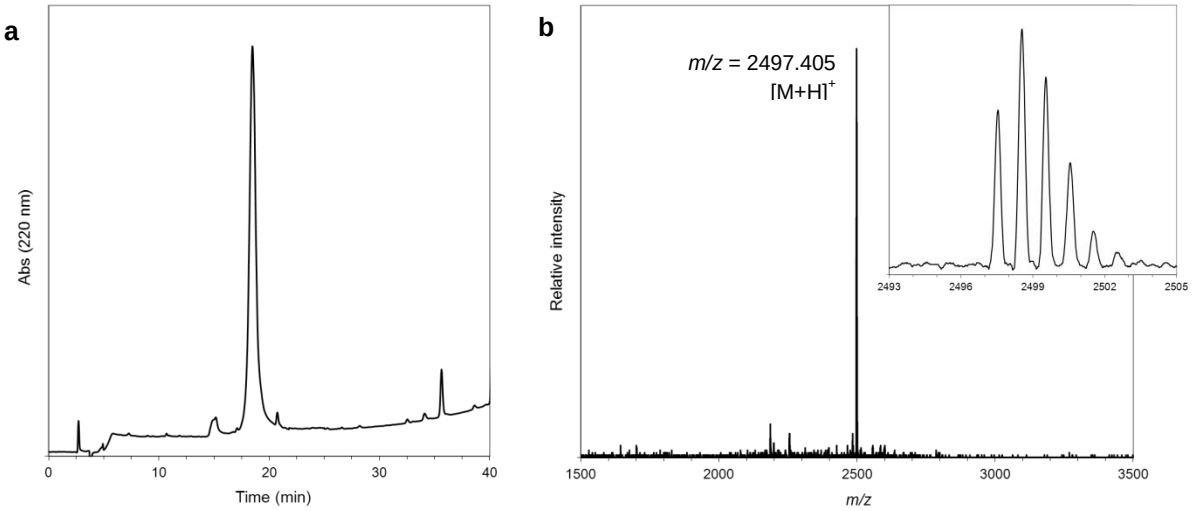


Figure S3. Characterization of analogue **23**. (a) Analytical RP-HPLC chromatogram. (b) MALDI-TOF mass spectrum (insert: zoom-in on the expected product mass).

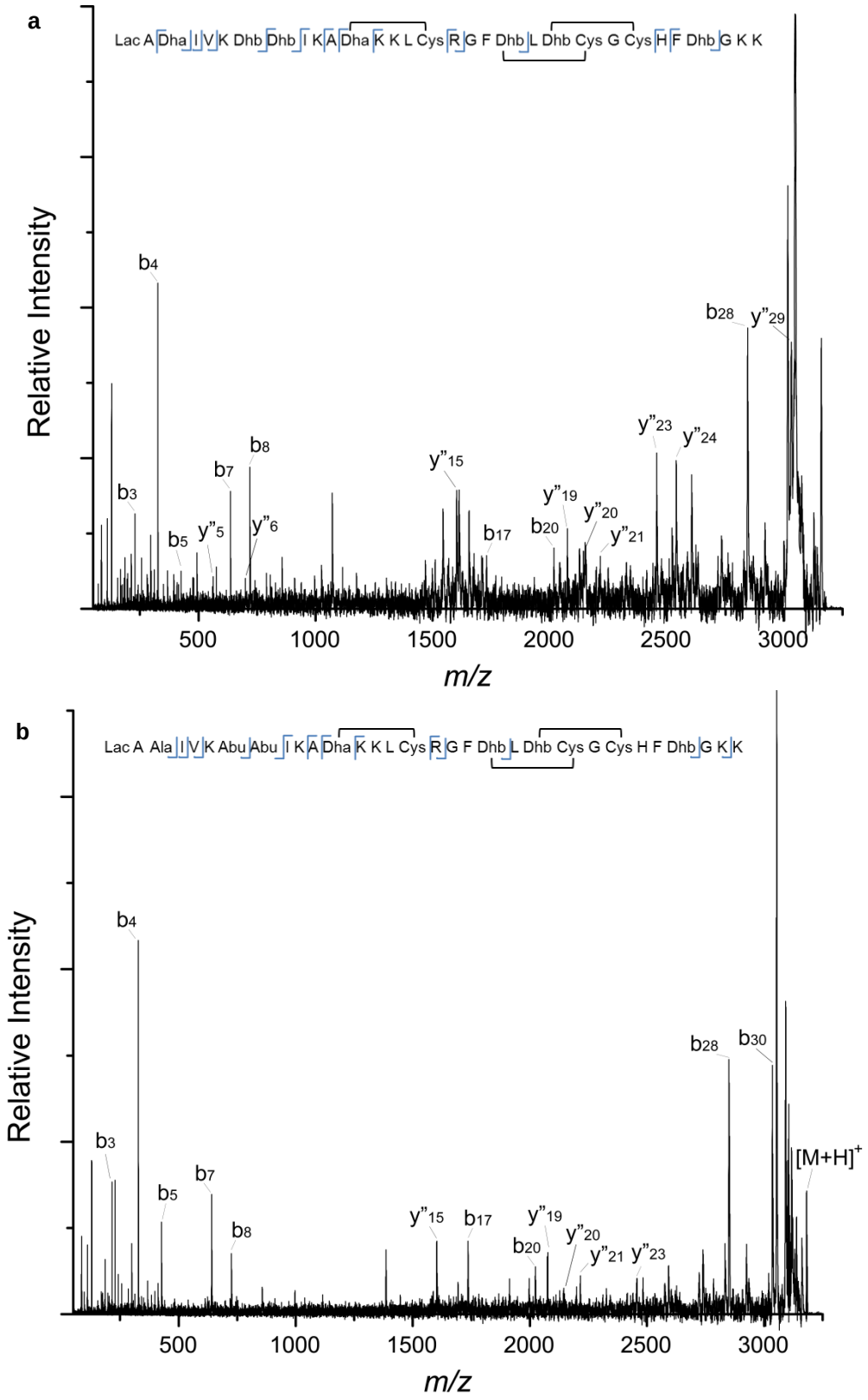


Figure S4. MALDI-MS/MS analysis of (a) authentic epilancin 15X (**1**), and (b) analogue **21**. Similar fragmentation patterns are seen for both peptides. For a discussion of tandem MS for the analysis of lantipeptides, see Li *et al*.[9]

Chiral gas chromatography-mass spectrometry analysis.

The enantiomeric purity of Lan/MeLan amino acids produced by hydrolysis of **23** was confirmed by chiral GC/MS, using a procedure modified from previous reports.[2,10,11] Lyophilized **23** (0.2 mg) was dissolved in 6 M HCl (3 mL) and heated at 100 °C in a sealed, high-pressure reaction vessel for 20 h. The reaction was cooled and concentrated with a stream of N_2 over 4 h. Methanol (3 mL) was chilled in an ice bath, and acetyl chloride (1 mL) was added dropwise. This solution was added to the dry hydrolysate, and the mixture was sealed and heated at 100 °C for 1 h. The reaction was allowed to cool, then concentrated under reduced pressure. The dry residue was suspended in CH_2Cl_2 (3 mL) and chilled in an ice bath. Pentafluoropropionic anhydride (1 mL) was added, and the mixture was sealed and heated at 100 °C for 20 min. The reaction was allowed to cool, then concentrated under reduced pressure. The residue was dissolved in methanol and re-concentrated, then dissolved again in methanol (200 μL) for analysis. Synthetic Lan/MeLan standards of differing stereochemical configurations (DD, DL and LL for Lan; DL and LL for MeLan), similarly derivatized as their pentafluoropropionamide methyl esters, were provided by Weixin Tang (University of Illinois at Urbana-Champaign) as solutions in methanol.[12]

The derivatized hydrolysate and standards were analyzed by GC-MS using an Agilent 7890A gas chromatograph equipped with an Agilent 5975C Inert XL EI/CI MS detector and a Varian CP-Chirasil-L-Val fused silica column (25 m x 250 μm x 0.12 μm). Sample solutions in methanol were introduced to the instrument via splitless injection at an inlet temperature of 200 °C and flow rate of 2 mL/min helium gas. The temperature gradient used was held at 160 °C for 5 min, then ramped from 160 °C to 180 °C at 3 °C/min, then held at 180 °C for 6 min. The MS was operated in simultaneous scan/selected-ion monitoring (SIM) mode, monitoring at known and unique fragment masses of 365 Da for Lan and 379 Da for MeLan. All standards eluted as distinct peaks. For Lan, the DD-isomer eluted at 13.8 min, the DL-isomer at 14.1 min, and the LL-isomer at 14.3 min; for MeLan, the DL-isomer eluted at 11.1 min and the LL-isomer at 11.3 min. The derivatized hydrolysate of **25** confirmed the desired DL-configuration of both Lan and MeLan (*Fig. S7*). Small amounts of non-DL-configurations in the hydrolysate are believed to result from epimerization during hydrolysis, which has been reported previously.[11,13]

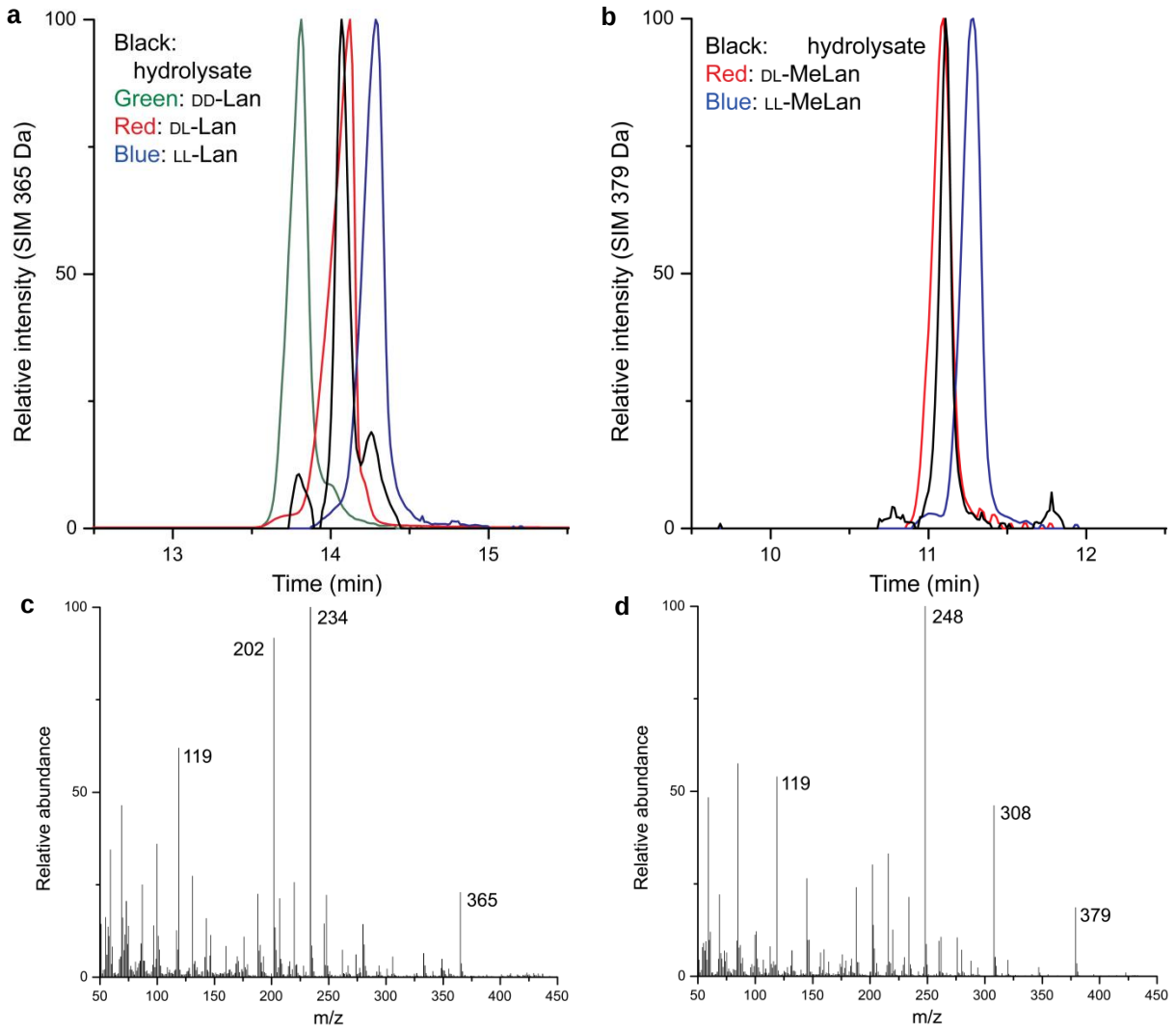


Figure S7. GC-MS analysis of derivatized hydrolysate of analogue **23**, confirming the desired DL-configuration of Lan/MeLan. (a) SIM at 365 Da of hydrolysate compared to synthetic Lan standards. (b) SIM at 379 Da of hydrolysate compared to synthetic MeLan standards. (c) Representative mass spectrum of derivatized Lan. (d) Representative mass spectrum of derivatized MeLan.

Liquid culture bioactivity assays.

Cultures of the indicator strain *Staphylococcus carnosus* TM300 (5 mL) were grown overnight at 37 ^{o}C in bovine heart infusion medium (BHI, 37 g/L), then diluted with fresh BHI to an optical density at 600 nm (OD_{600}) of 0.1. Lyophilized peptides were dissolved in sterile deionized water (SDW) to give stock solutions of 4 µM (for **1**), 10 µM (for **21** and **22**) or 100 µM (for **23**). Two-fold serial dilutions were performed for each stock solution in SDW to give 11 concentrations at 4x final assay concentration. Corning-Costar 96 well flat-bottom assay plates were used to determine the activity of each peptide against *S. carnosus* TM300, and experiments were performed in triplicate. Experimental wells contained 150 µL of diluted culture and 50 µL of 4x peptide solution. Blank wells contained 150 µL BHI and 50 µL SDW. Positive control wells

contained 150 μL diluted culture and 50 μL SDW. OD_{600} was recorded at hourly intervals using a BioTek Synergy H4 plate reader, and plates were incubated at 37 ^{o}C between readings. After subtraction of blanks from experimental measurements, plots of OD_{600} vs. peptide concentration were fitted to a dose-response curve with the equation: $y = A1 + (A2-A1) / (1 + 10^{(\log x0 - x)p})$, where p = variable Hill slope. Half maximal inhibitory concentration (IC_{50}) and minimal inhibitory concentration (MIC) values were calculated from this fit for each peptide after 5 h incubation, and triplicate calculations were averaged.

Peptide	IC_{50} (nM)	MIC (nM)
Epilancin 15X (**1**)	95 ± 9.6	250
Analogue **21**	270 ± 23	625
Analogue **22**	354 ± 8	1250
Truncated analogue **23**	Not determined	12,500

Flow cytometry analysis of pore-forming activities

Cultures of *S. carnosus* TM300 were grown as for bioactivity assays and diluted with fresh BHI to an OD_{600} of 0.1. For membrane depolarization assays using the dye 3,3'-diethyloxacarbocyanine iodide ($DiOC_2(3)$),[14] cells were combined with $DiOC_2(3)$ (final concentration 2 μM), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES, 1 mM) and glucose (1 mM) and incubated for 20 min at room temperature. Stock solutions of epilancin 15X analogues **21** or **23** were added to final concentrations of 0.1, 1.0 or 10 μM and incubated for an additional 20 min prior to analysis; water was added for the negative control. For membrane permeability assays using the dye propidium iodide (PI),[15] cells were combined with PI (final concentration 25 μM), HEPES (1 mM), glucose (1 mM) and epilancin 15X analogues (0.1, 1.0, 10 μM), incubated for 15 min at room temperature and analyzed. Changes in cell-associated dye fluorescence were measured with a BD Biosciences LSR II flow cytometer, using excitation at 488 nm with an argon laser and measurement of emission through a band-pass filter at 530/30 nm for $DiOC_2(3)$ or 695/40 nm for PI. A minimum of 25,000 events were detected for each sample, and each peptide concentration was repeated in triplicate. Data analysis to calculate the geometric mean fluorescence intensity (MFI) of each population was performed using FCS Express 3.00.0311 V Lite Stand-alone software.

References.

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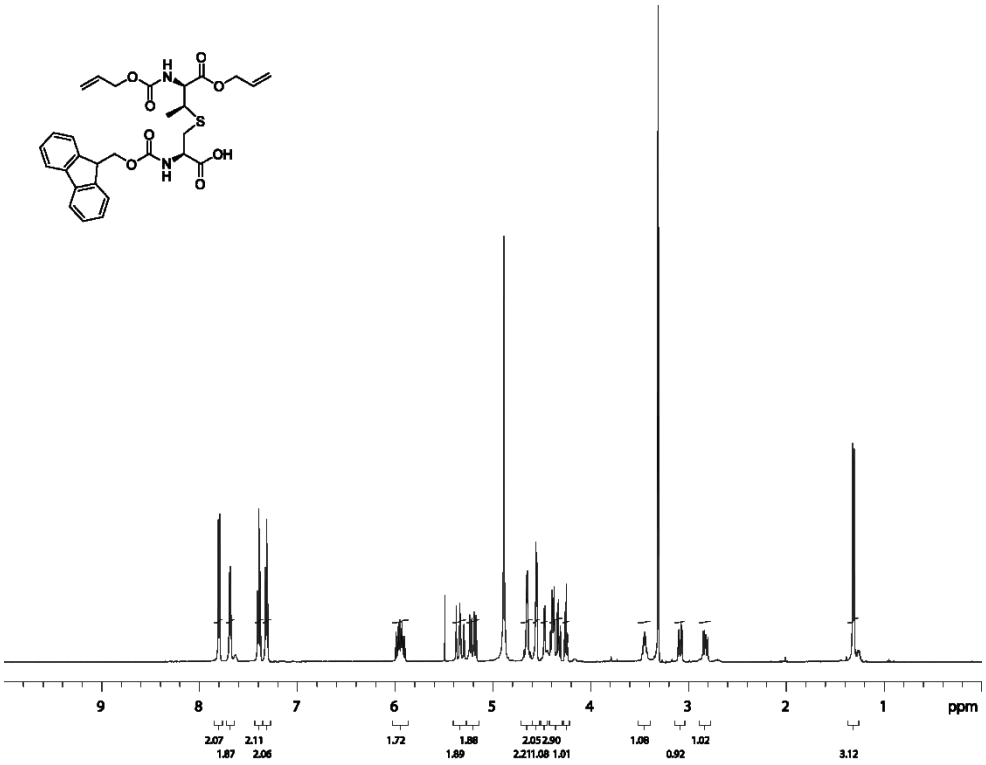
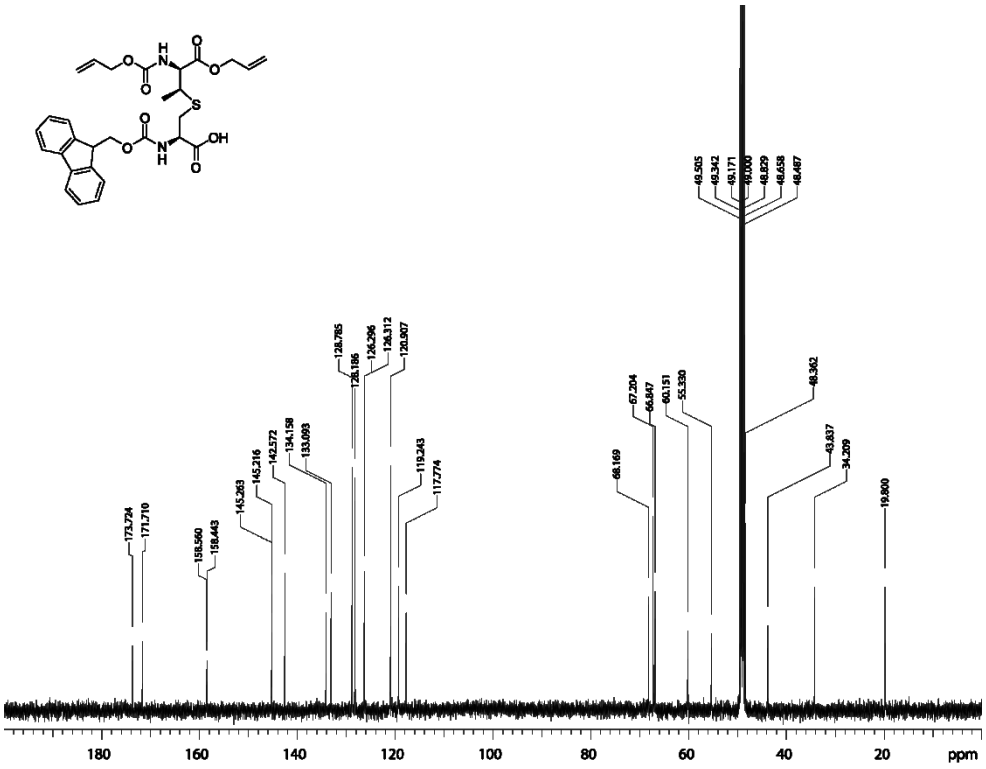
NMR spectra of novel small molecules (ordered by compound number)

Compound **3**





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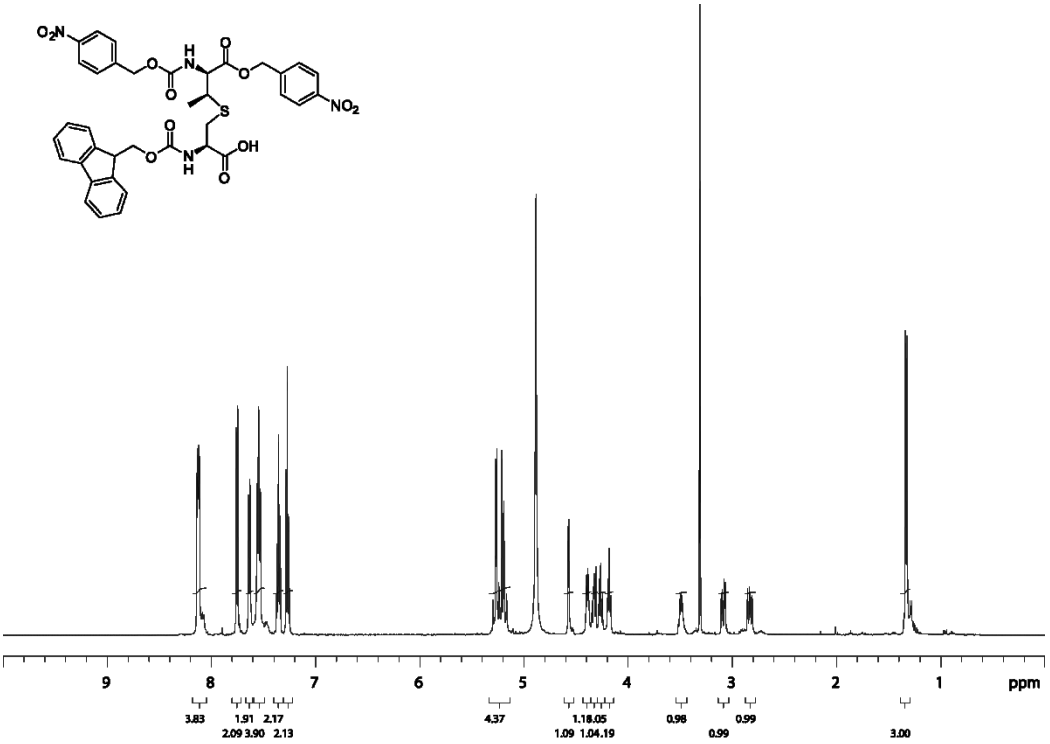
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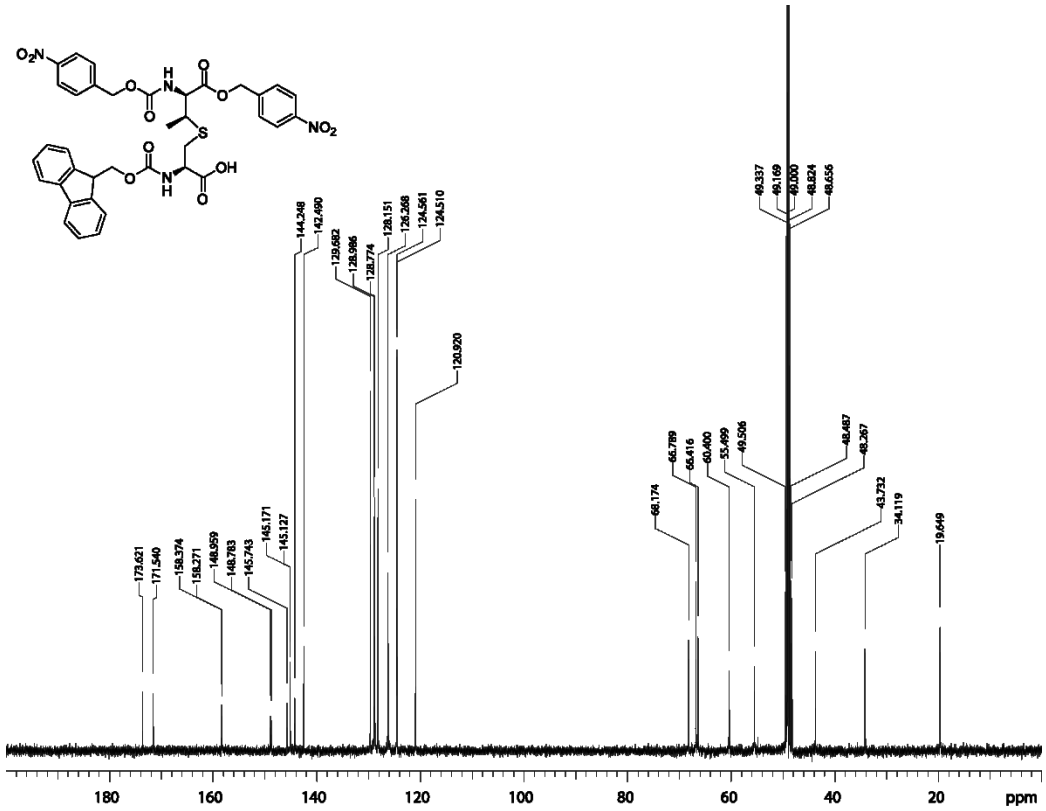
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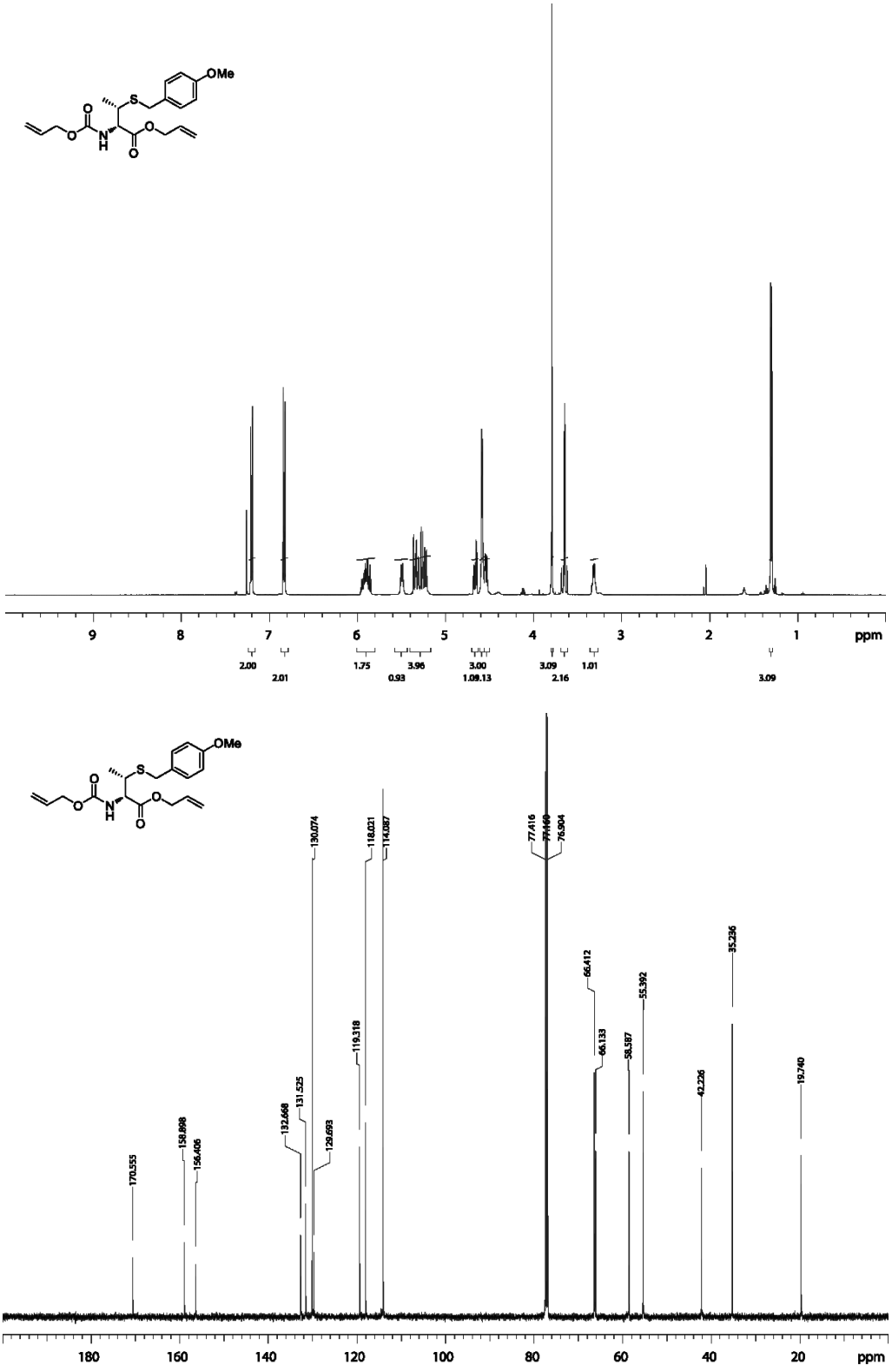
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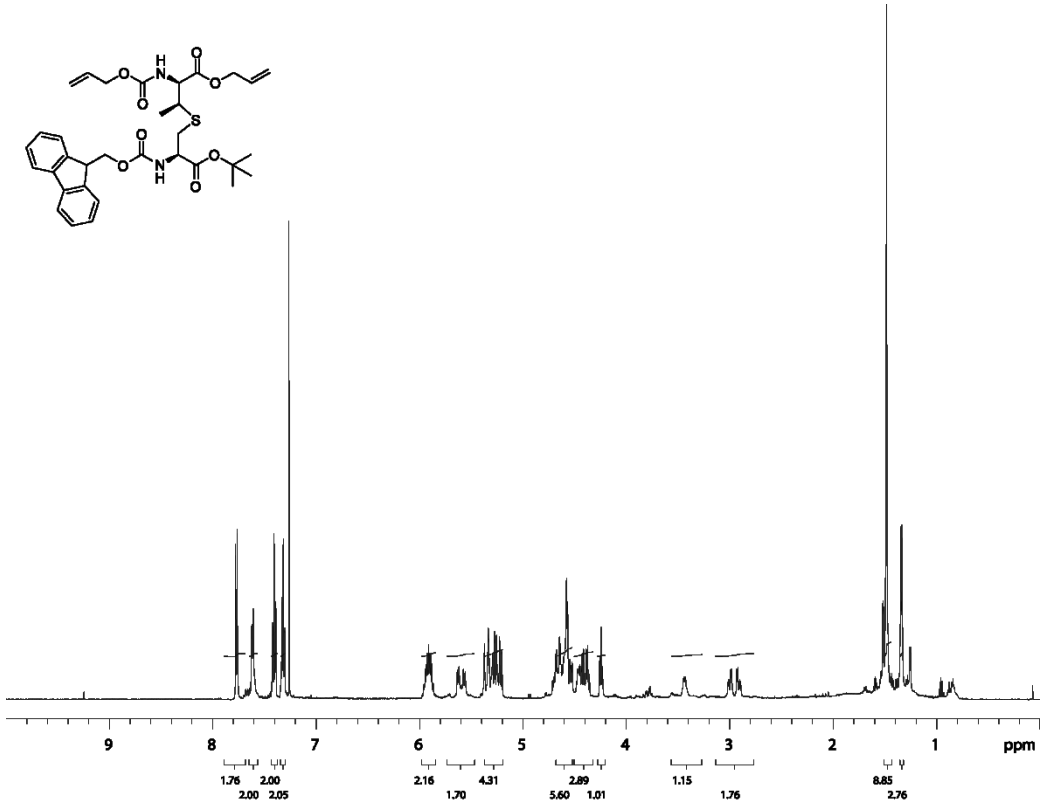
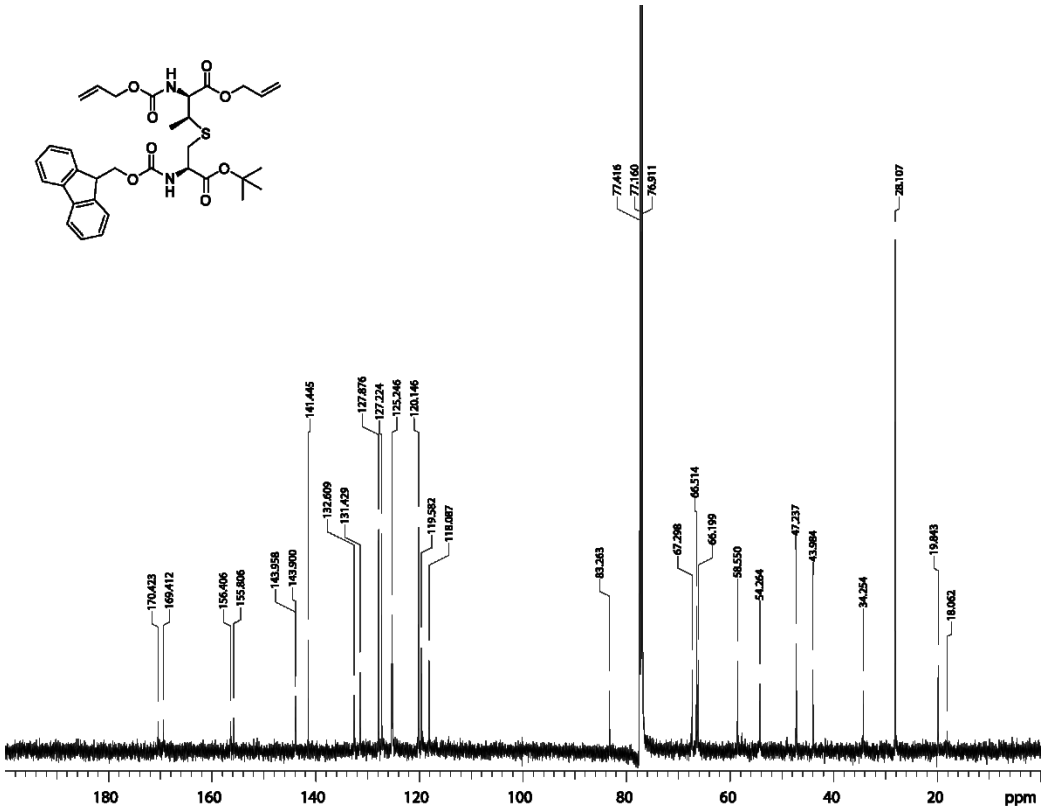
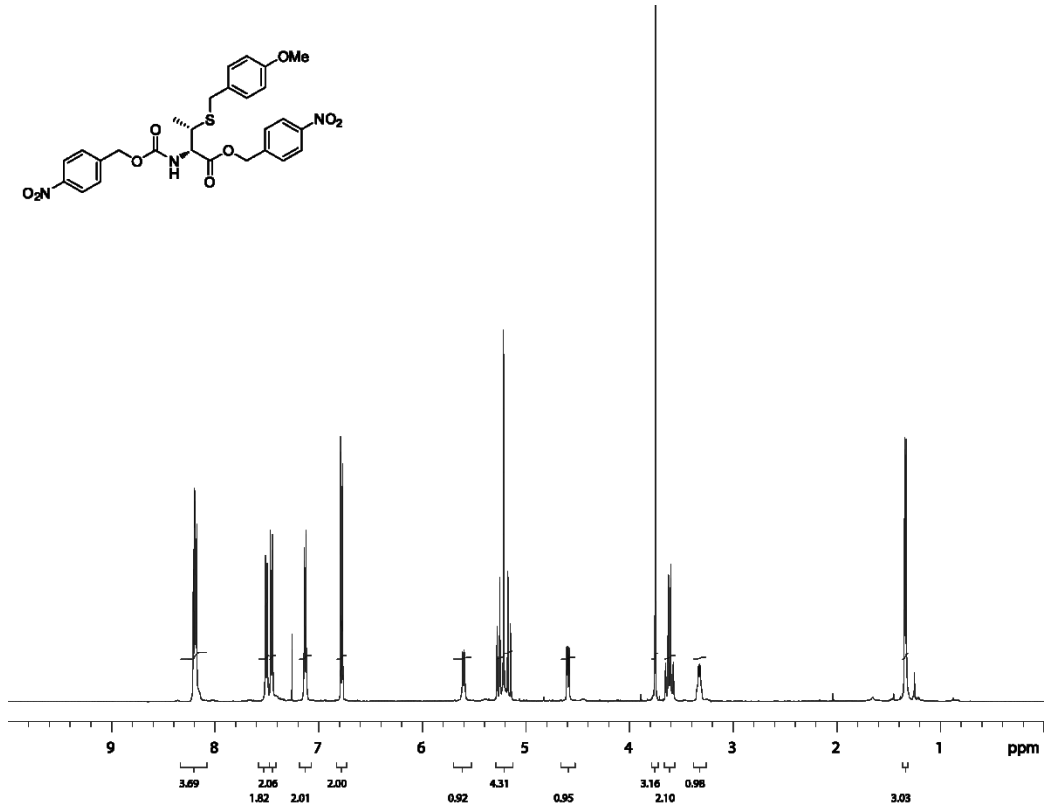
76.904
66.412
66.133
58.587
55.392
42.226
35.236
19.740
180
160
140
120
100
80
60
40
20
ppm

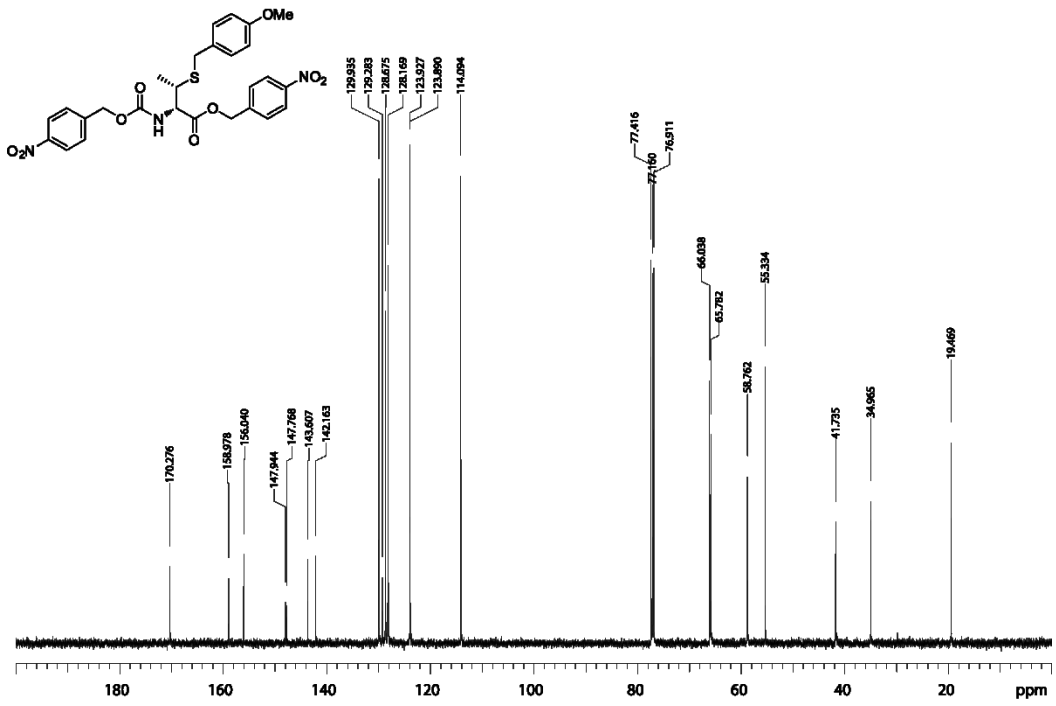
Compound **10**


9
8
7
6
5
4
3
2
1
ppm
1.76
2.00
2.00
2.05
2.16
1.70
4.31
2.89
5.60
1.01
1.15
1.76
8.85
2.76

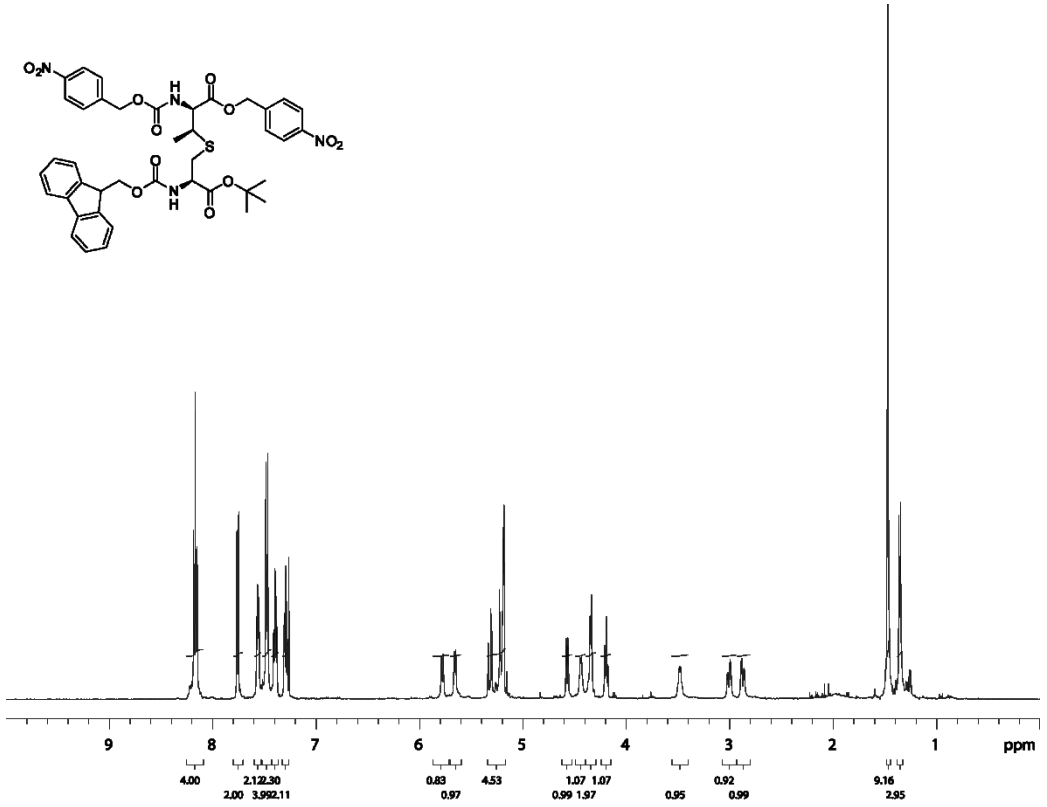

77.416
77.160
76.911
28.107
170.423
169.412
156.406
155.806
143.558
143.300
141.445
132.609
131.429
127.876
127.224
125.246
120.146
119.582
118.087
83.263
67.298
66.514
66.199
58.550
54.264
47.237
43.984
34.254
19.843
18.062
180
160
140
120
100
80
60
40
20
ppm

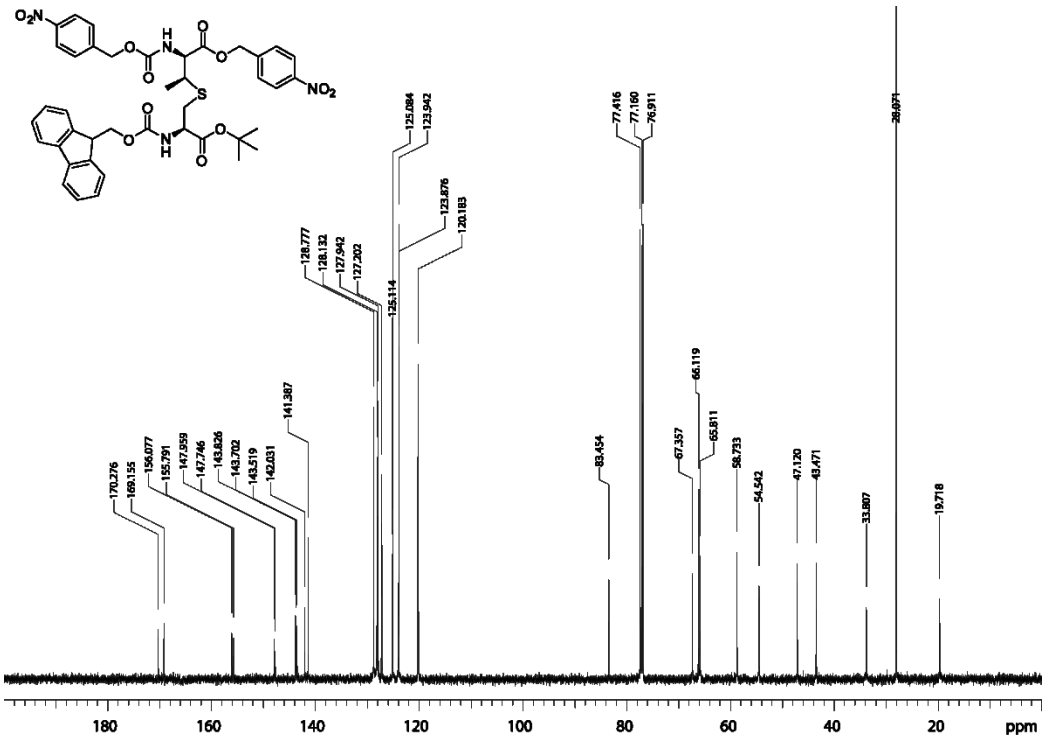
Compound **11**


OMe
S
NO2
O
N
H
O2N
9
8
7
6
5
4
3
2
1
ppm
3.69
2.06
1.82
2.01
2.00
0.92
4.31
0.95
3.16
2.10
0.98
3.03

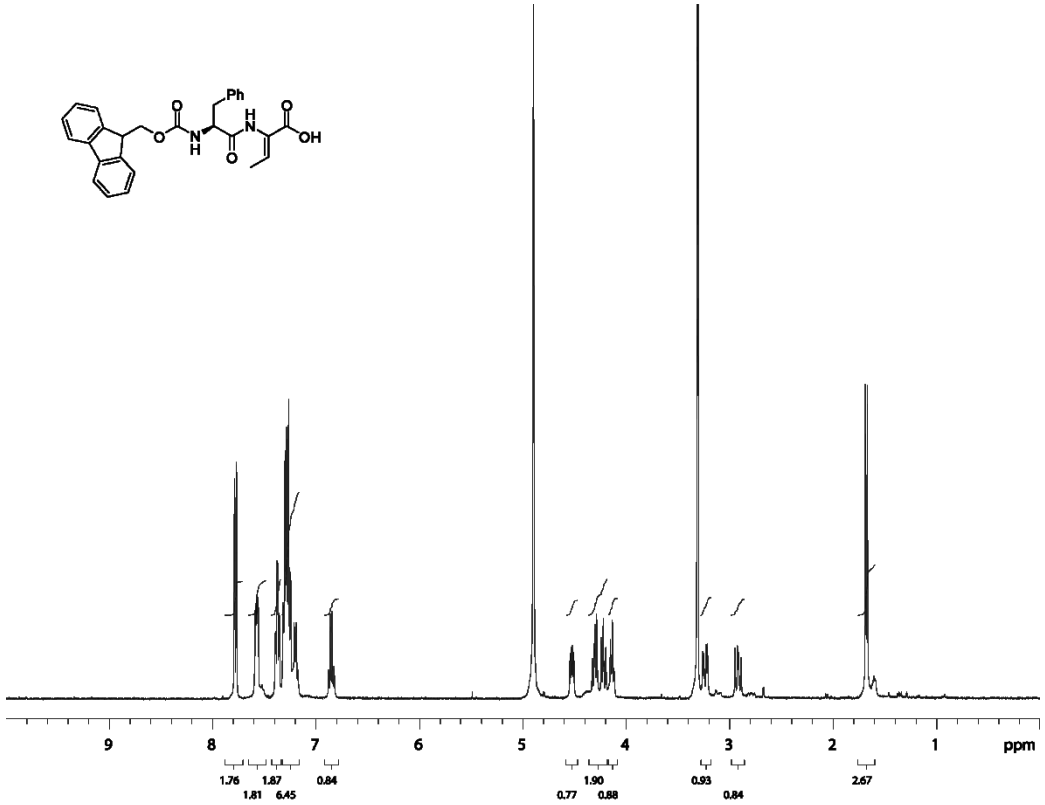

OMe
S
NO2
O
N
H
O2N
129.935
129.283
128.675
128.169
123.927
123.890
114.094
77.416
77.160
76.911
66.038
65.702
55.334
58.762
41.735
34.965
19.469
170.276
158.978
156.040
147.944
147.768
143.607
142.163
180
160
140
120
100
80
60
40
20
ppm

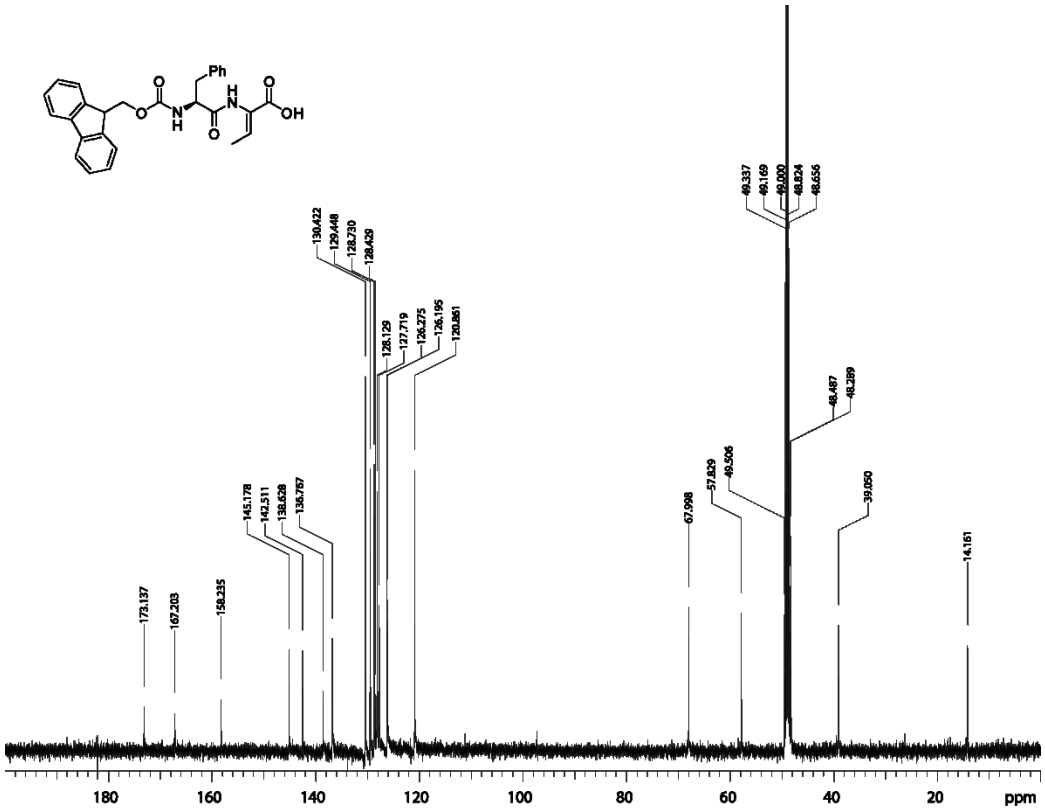
Compound **12**


9
8
7
6
5
4
3
2
1
ppm
4.00
2.12
2.30
2.00
3.99
2.11
0.83
0.97
4.53
1.07
1.07
0.99
1.97
0.95
0.92
0.99
9.16
2.95

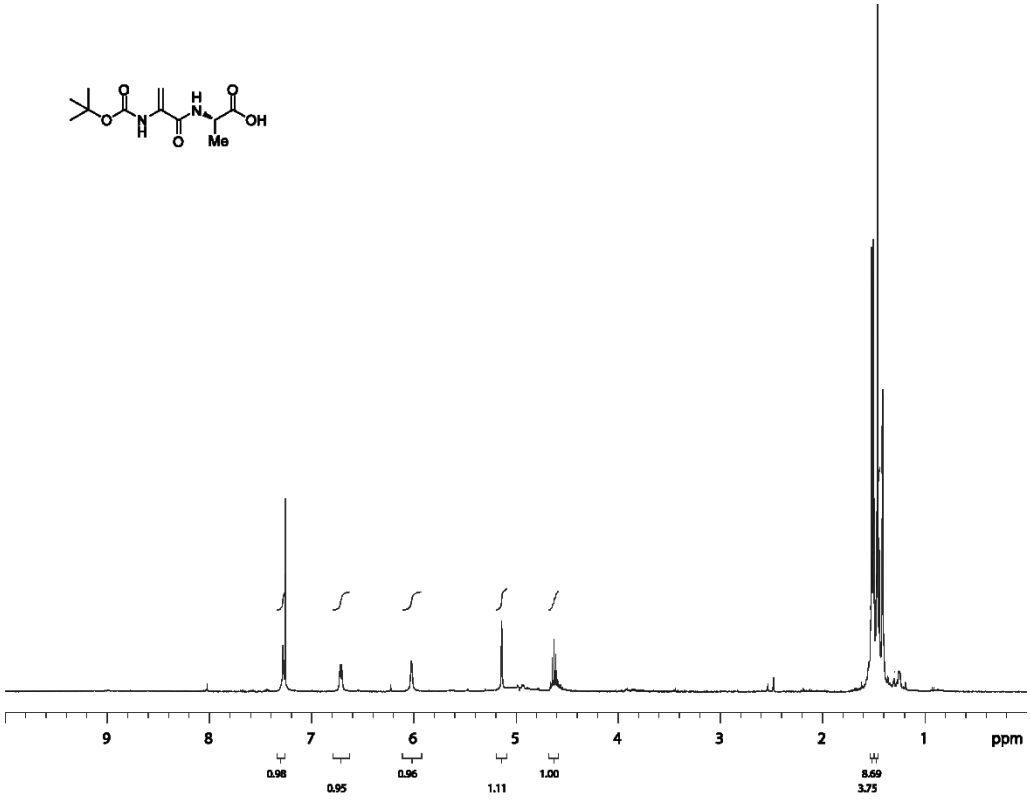

170.276
169.155
156.077
155.791
147.959
147.746
143.826
143.702
143.519
142.031
141.387
128.777
128.132
127.942
127.202
125.114
125.084
123.942
123.876
120.183
83.454
77.416
77.160
76.911
67.357
66.119
65.811
58.733
54.542
47.120
43.471
33.807
28.071
19.718
180
160
140
120
100
80
60
40
20
ppm

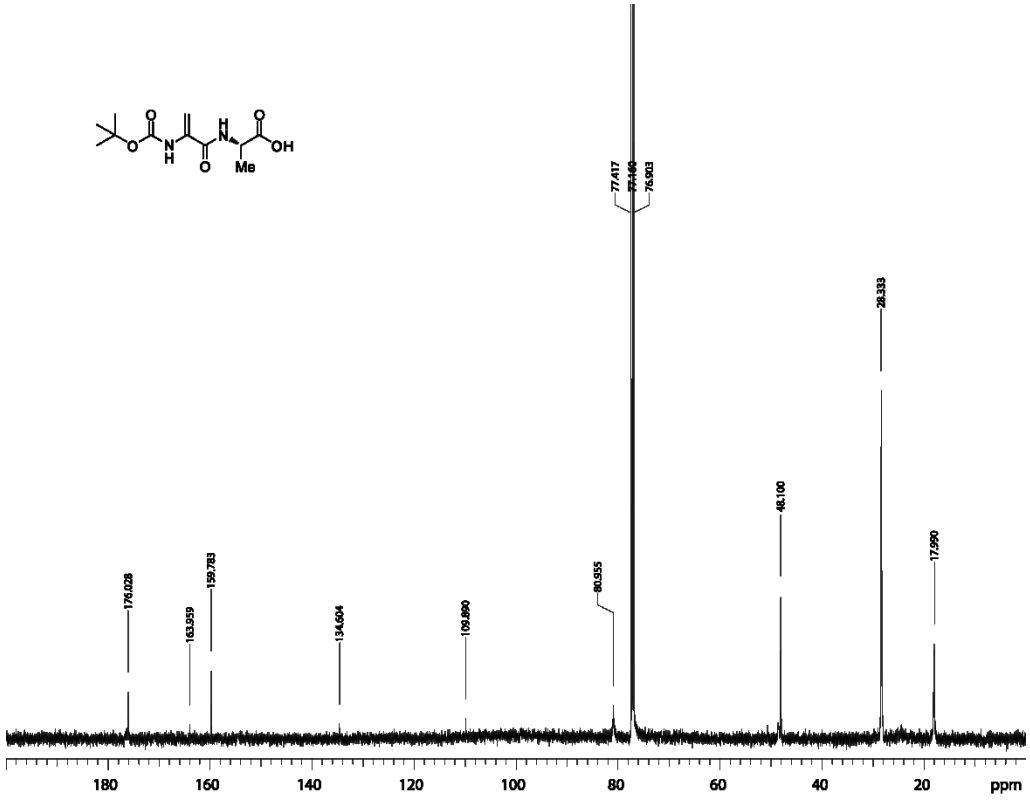
Compound **13**


Ph
O
N
H
O
O
N
H
O
OH
9
8
7
6
5
4
3
2
1
ppm
1.76
1.87
0.84
1.81
6.45
1.90
0.77
0.88
0.93
0.84
2.67

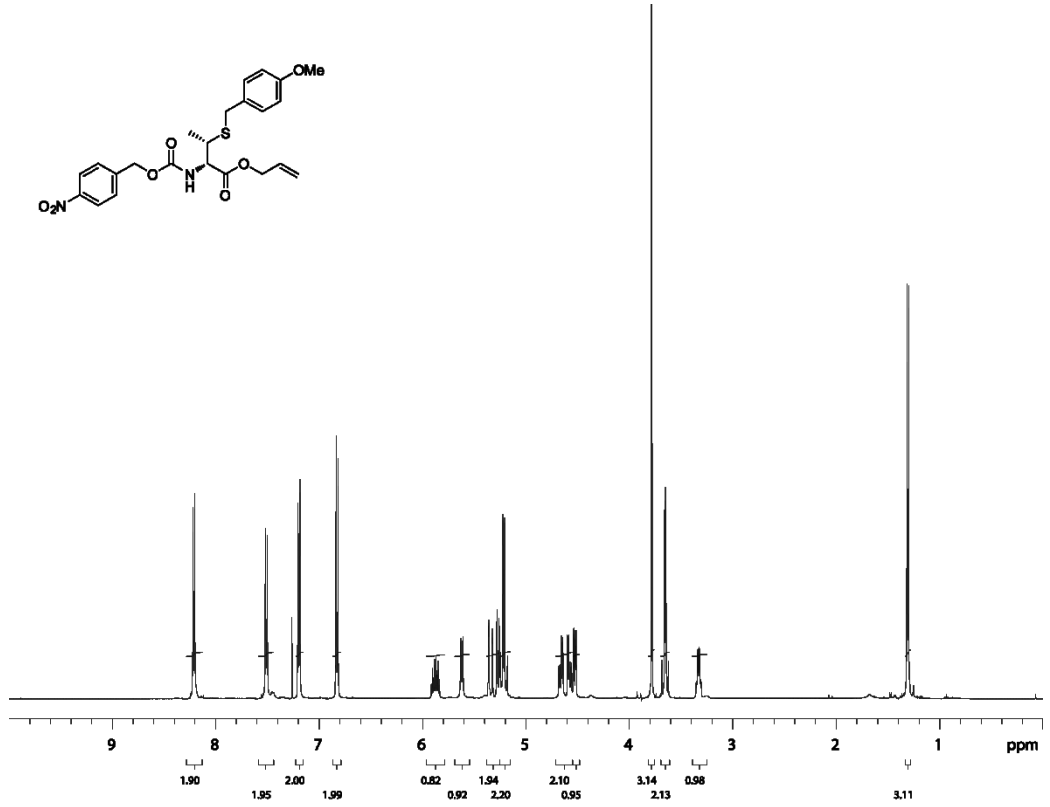

Ph
O
N
H
O
O
N
H
O
OH
130.422
129.448
128.730
128.429
128.129
127.719
126.275
126.195
120.861
145.178
142.511
138.628
136.767
173.137
167.203
158.235
49.337
49.169
49.000
48.824
48.656
48.487
48.289
67.998
57.829
49.506
39.050
14.161
180
160
140
120
100
80
60
40
20
ppm

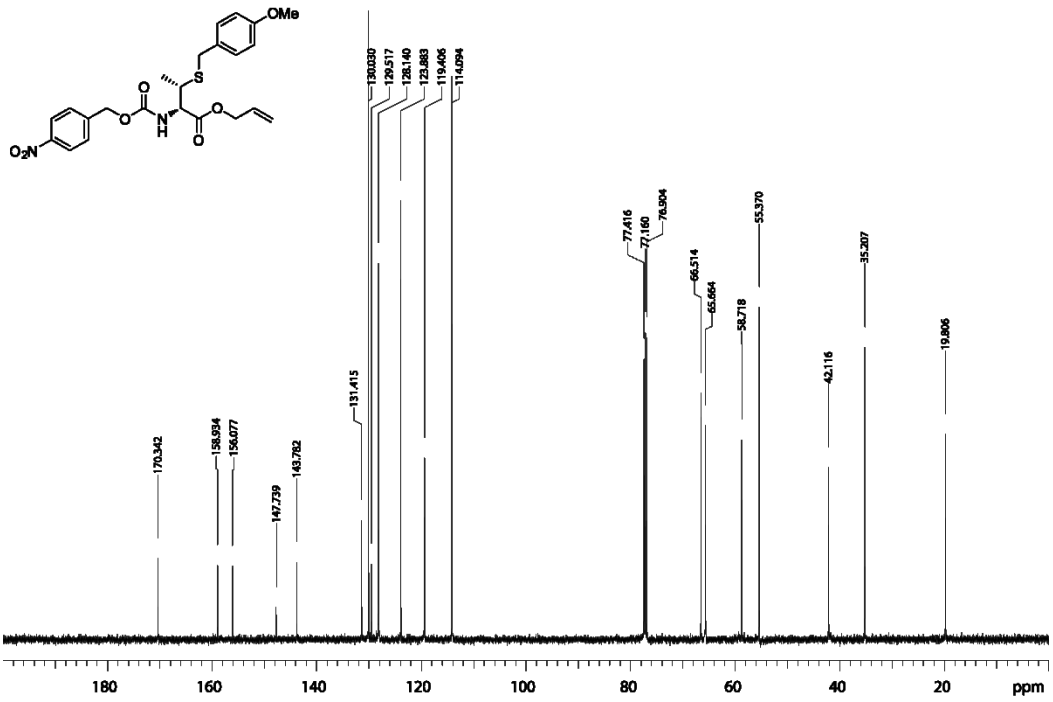
Compound **14**


9
8
7
6
5
4
3
2
1
ppm
0.98
0.95
0.96
1.11
1.00
8.69
3.75

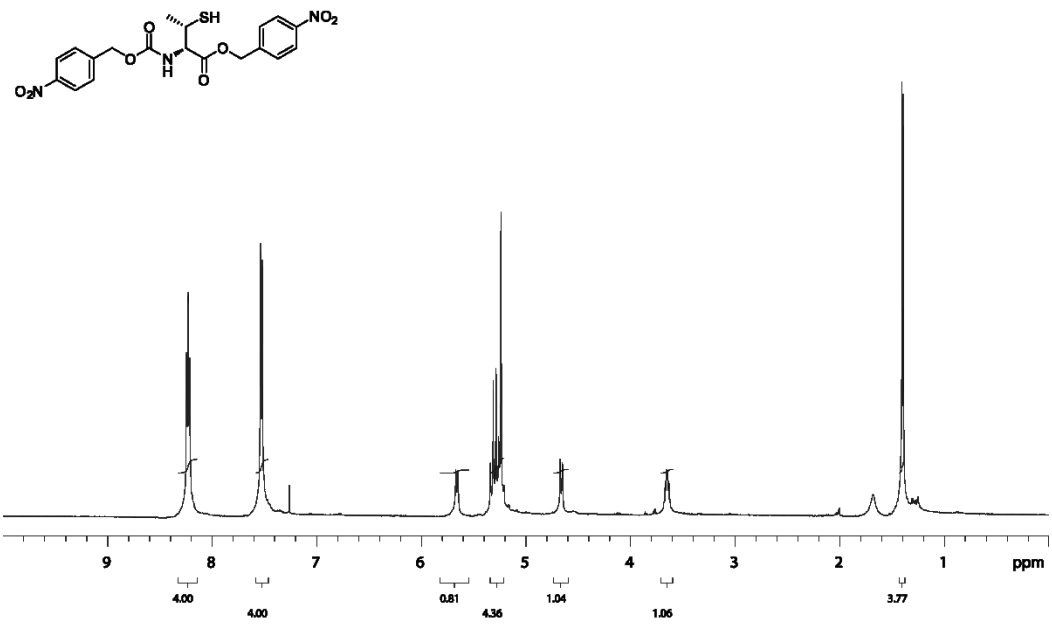

77.417
77.146
76.903
176.028
163.959
159.783
134.604
109.890
80.955
48.100
28.333
17.990
180
160
140
120
100
80
60
40
20
ppm

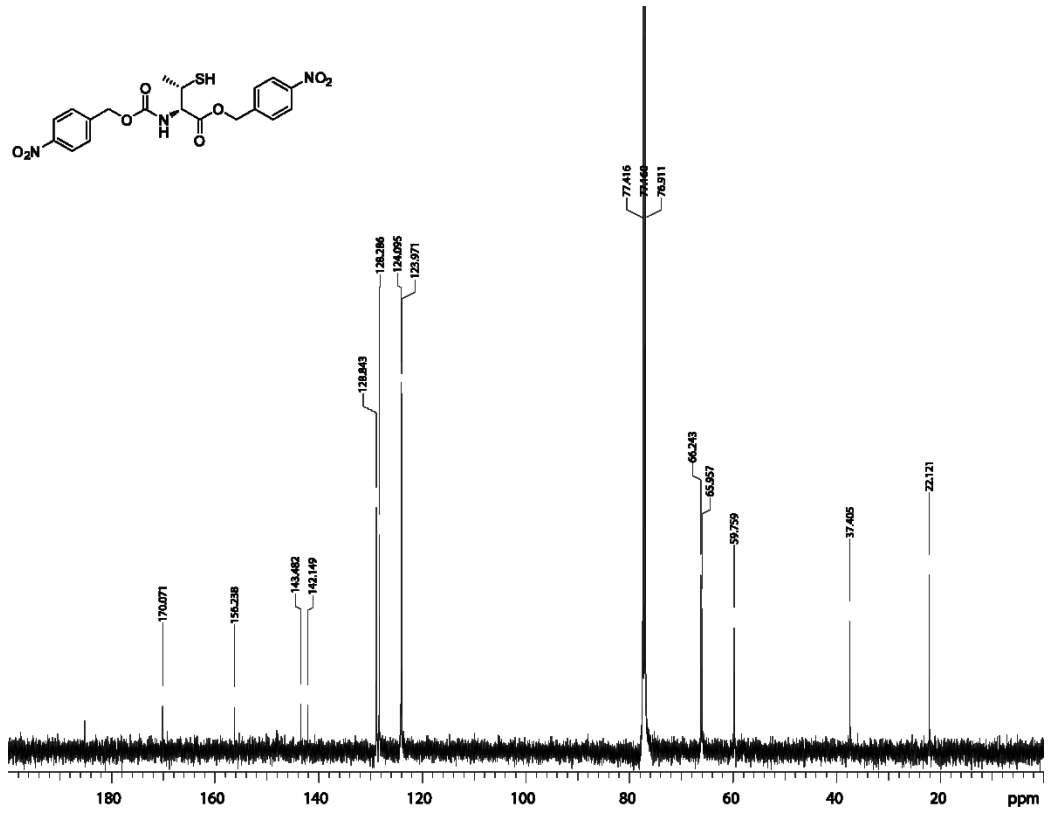
Compound **35**


OMe
O2N
9
8
7
6
5
4
3
2
1
ppm
1.90
1.95
2.00
1.99
0.82
0.92
1.94
2.20
2.10
0.95
3.14
2.13
0.98
3.11

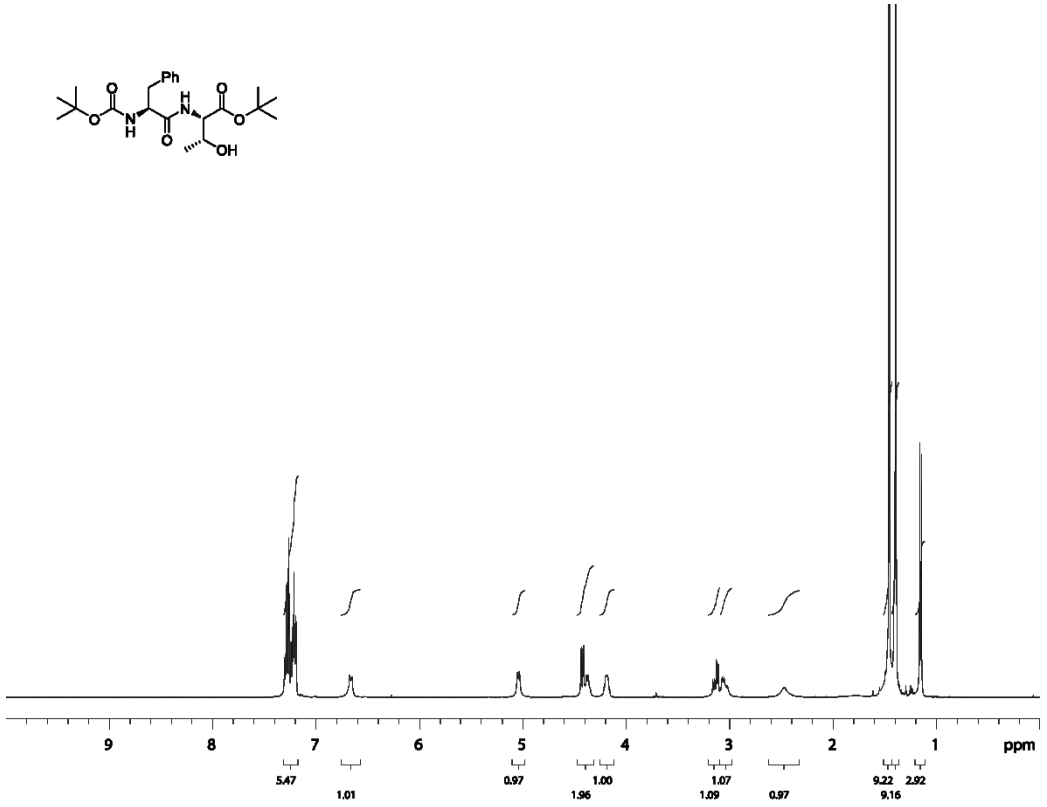

OMe
O2N
170.342
158.934
156.077
147.739
143.762
131.415
130.030
129.517
128.140
123.883
119.406
114.094
77.416
77.160
76.904
66.514
65.664
58.718
55.370
42.116
35.207
19.806
180
160
140
120
100
80
60
40
20
ppm

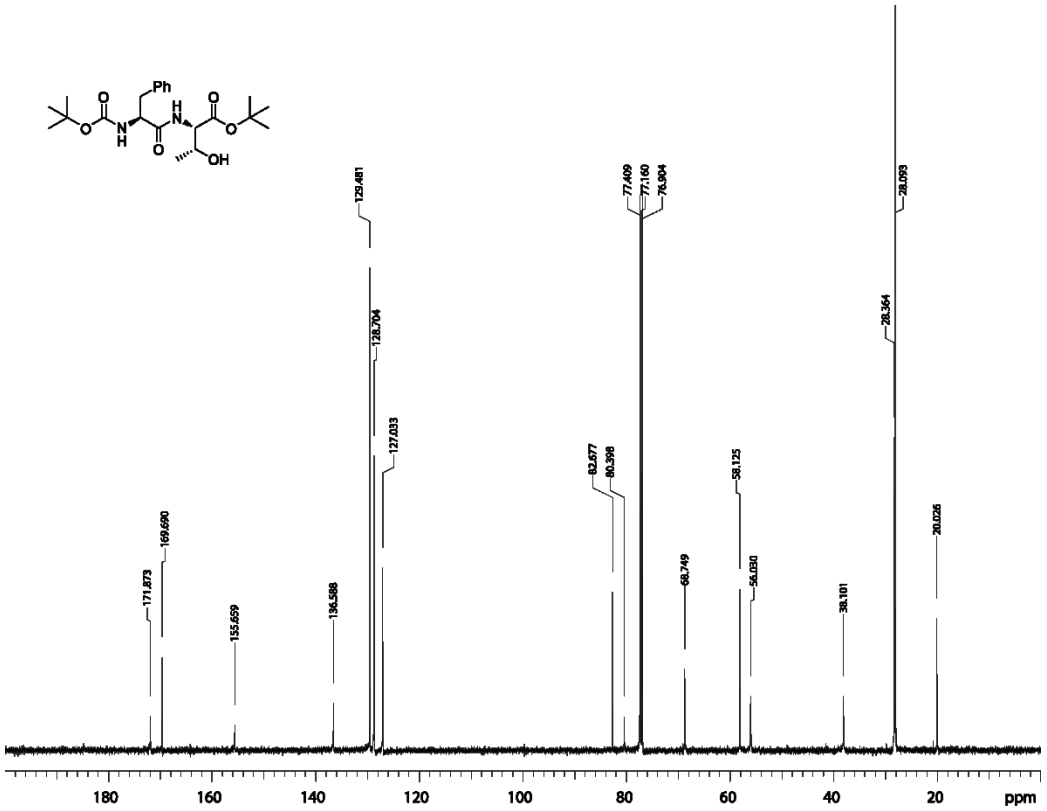
Compound **36**


SH
NO2
O2N
9
8
7
6
5
4
3
2
1
ppm
4.00
4.00
0.81
4.36
1.04
1.06
3.77

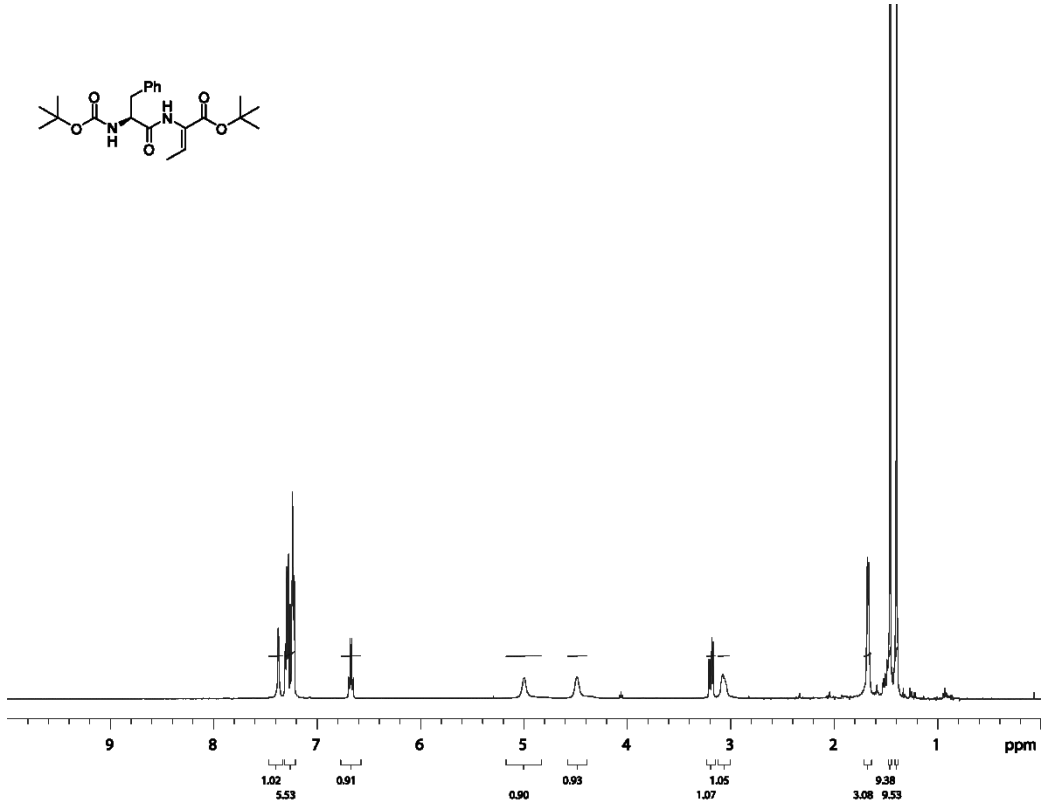

SH
NO2
O2N
77.416
77.160
76.911
129.286
124.095
123.971
128.843
66.243
65.957
59.759
37.405
22.121
170.071
156.238
143.482
142.149
180
160
140
120
100
80
60
40
20
ppm

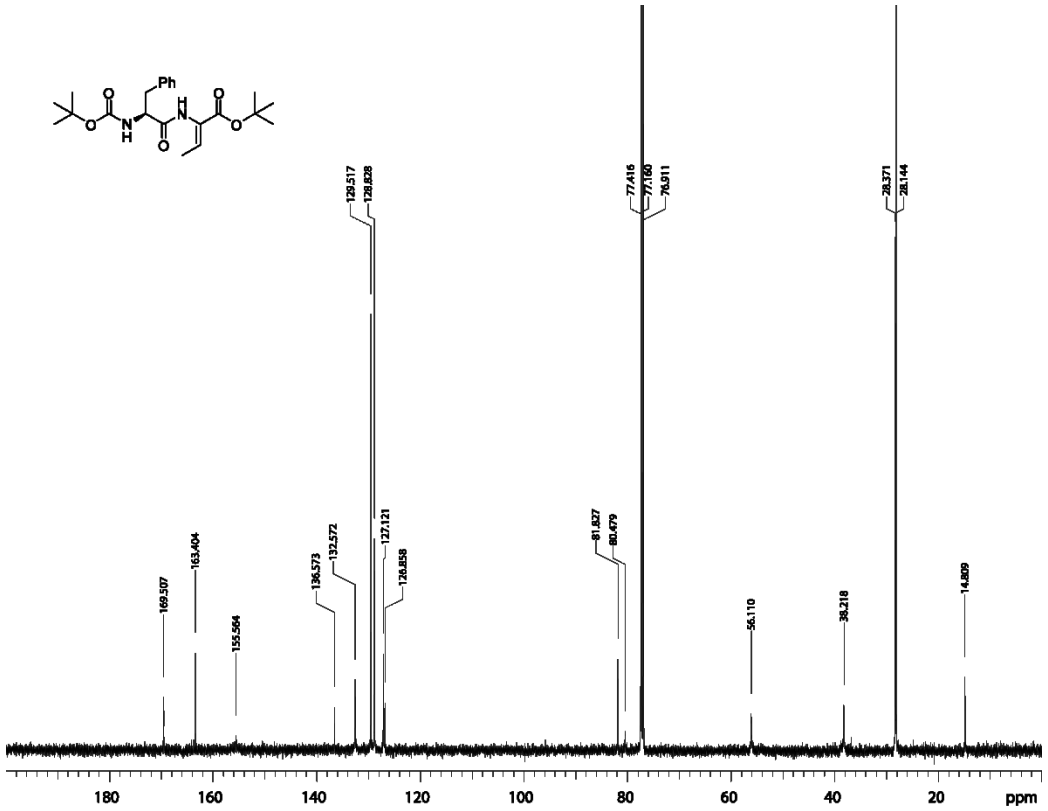
Compound **38**


Ph
O
N
H
O
O
O
OH
9
8
7
6
5
4
3
2
1
ppm
5.47
1.01
0.97
1.00
1.96
1.07
1.09
0.97
9.22
9.16
2.92

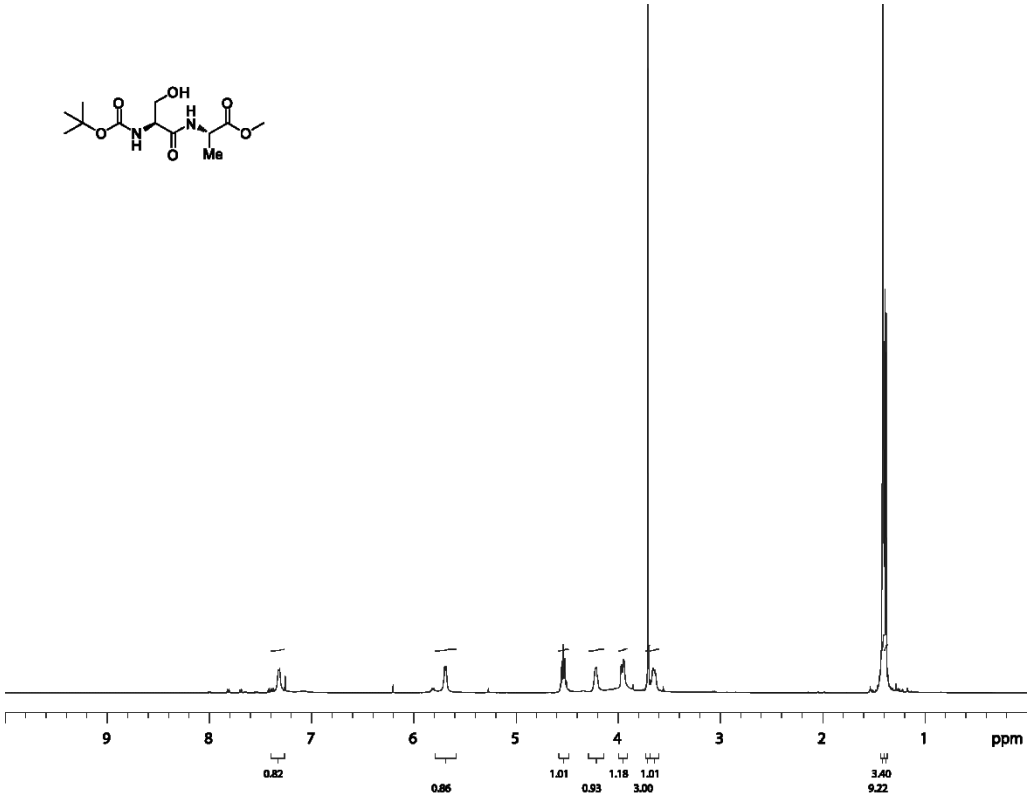

Ph
O
N
H
O
O
O
OH
171.873
169.690
155.659
136.588
129.481
128.704
127.033
82.677
80.398
77.409
77.160
76.904
68.749
58.125
54.030
38.101
28.364
28.093
20.026
180
160
140
120
100
80
60
40
20
ppm

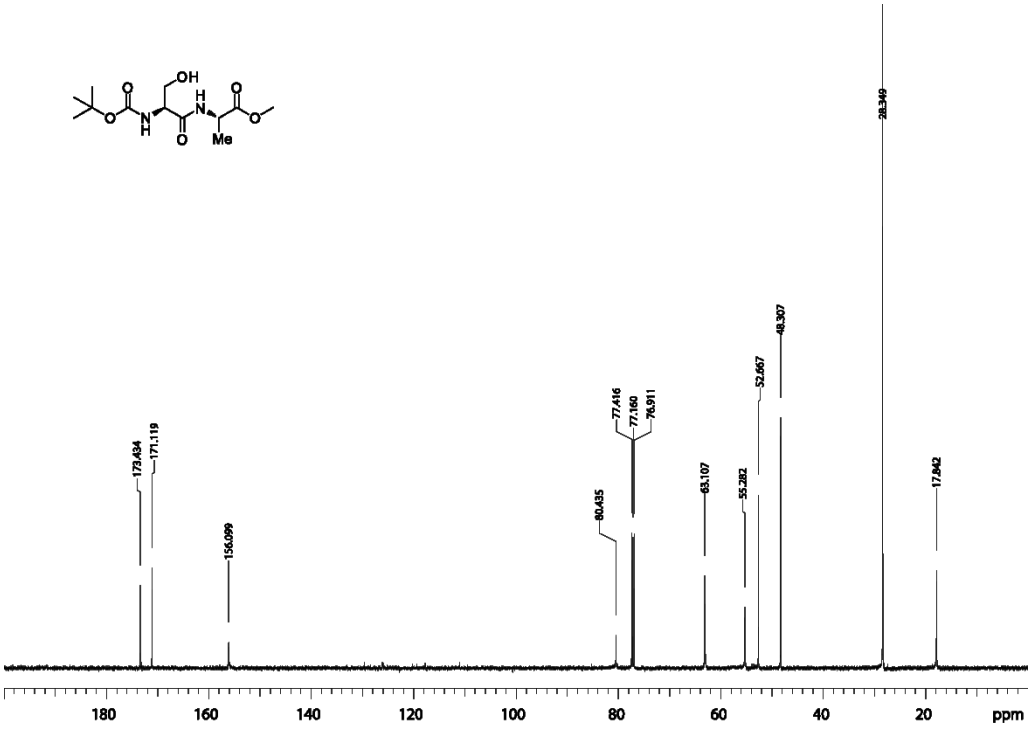
Compound **39**


Ph
9
8
7
6
5
4
3
2
1
ppm
1.02
5.53
0.91
0.90
0.93
1.05
1.07
9.38
3.08
9.53

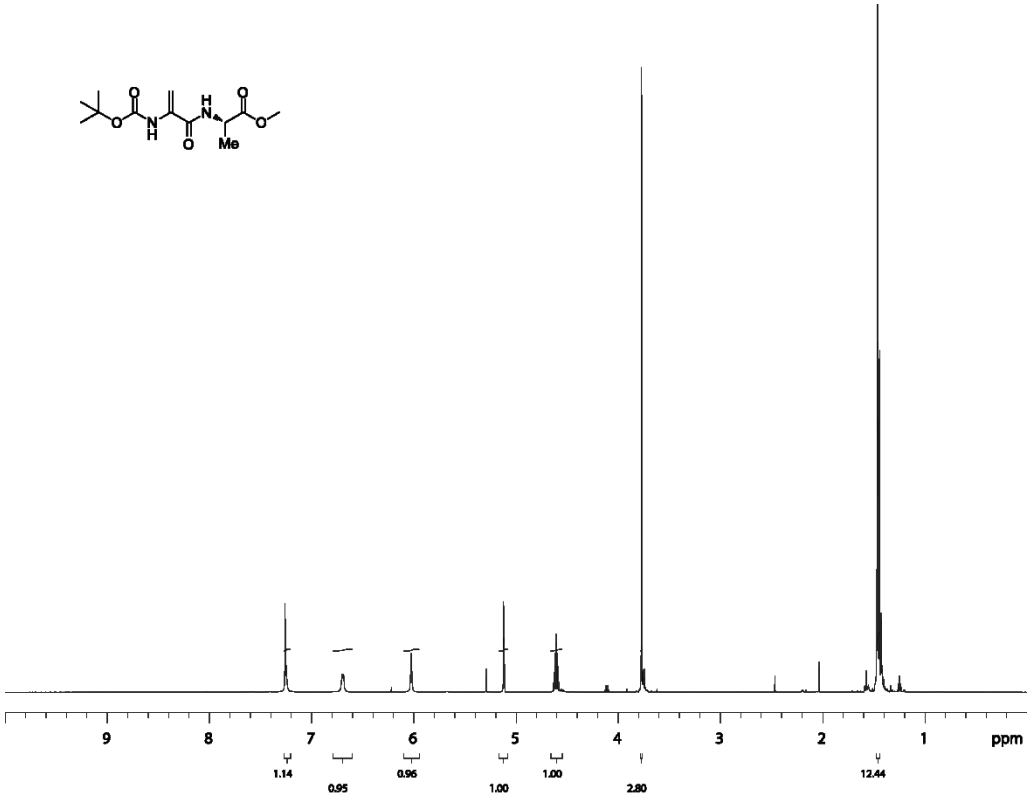

Ph
169.507
163.404
155.564
136.573
132.572
129.517
128.828
127.121
126.858
81.827
80.479
77.416
77.160
76.911
56.110
38.218
28.371
28.144
14.809
180
160
140
120
100
80
60
40
20
ppm

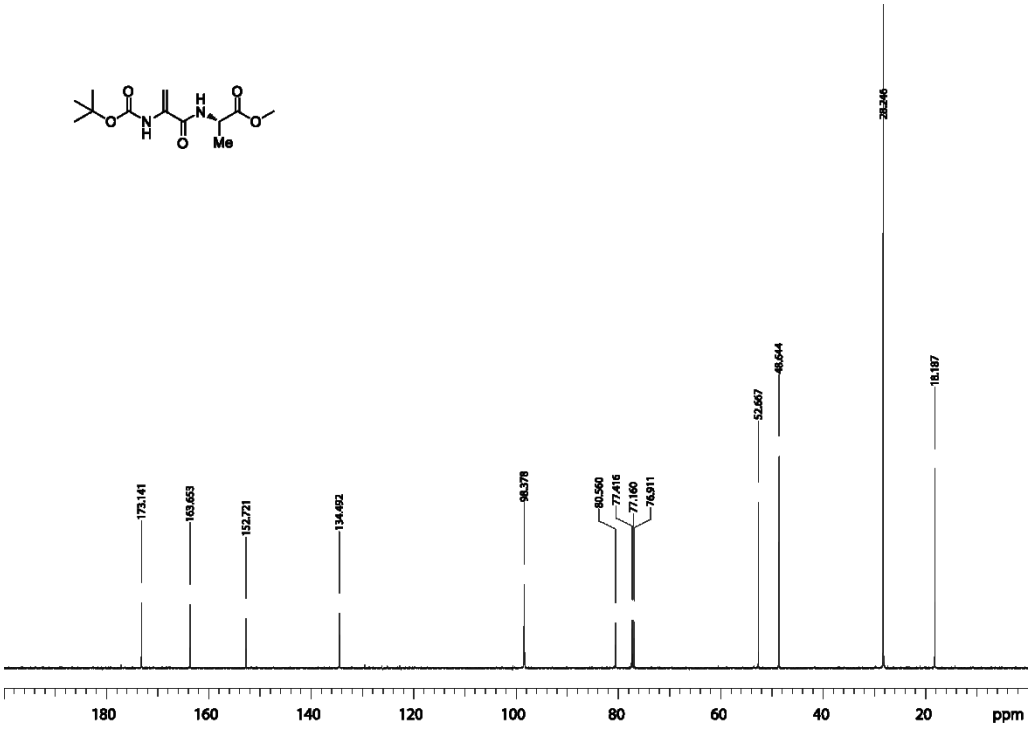
Compound **41**


OH
H
N
O
O
N
H
O
O
Me
9
8
7
6
5
4
3
2
1
ppm
0.82
0.86
1.01
0.93
1.18
1.01
3.00
3.40
9.22


OH
H
N
O
O
N
H
O
O
Me
173.434
171.119
156.099
80.435
77.416
77.160
76.911
63.107
55.282
52.667
48.307
28.349
17.842
180
160
140
120
100
80
60
40
20
ppm

Compound **42**


Me
9
8
7
6
5
4
3
2
1
ppm
1.14
0.95
0.96
1.00
1.00
2.80
12.44


Me
173.141
163.653
152.721
134.492
98.378
80.560
77.416
77.160
76.911
52.667
48.644
28.246
18.187
180
160
140
120
100
80
60
40
20
ppm