Supplementary Information

Direct and chemoselective transformation of cysteine to dehydroalanine on peptides

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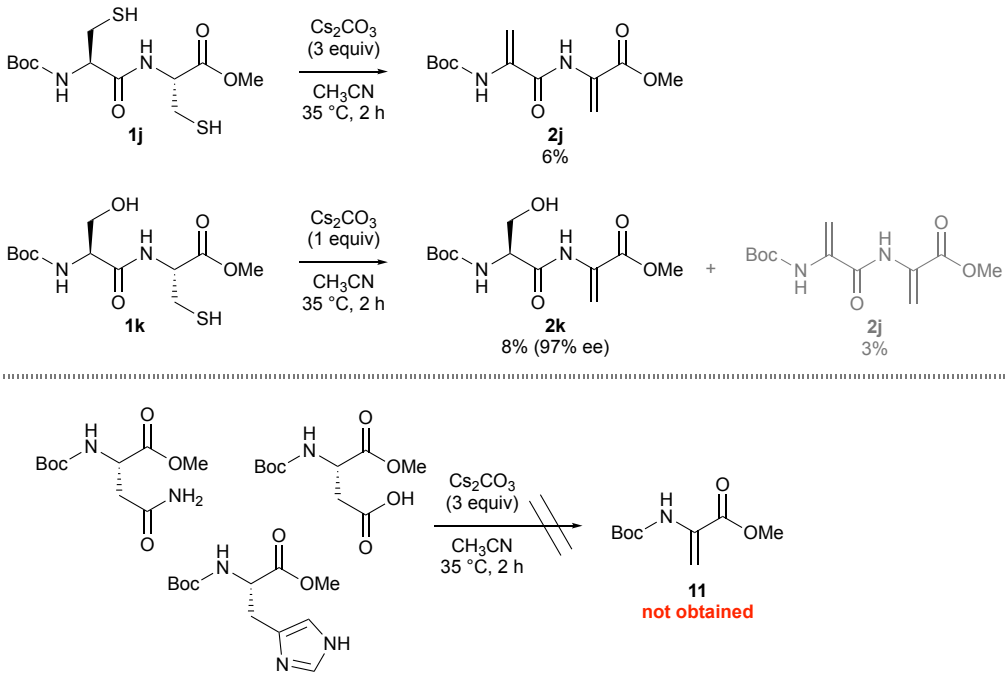


Scheme S1 Additional reactions.

General Information

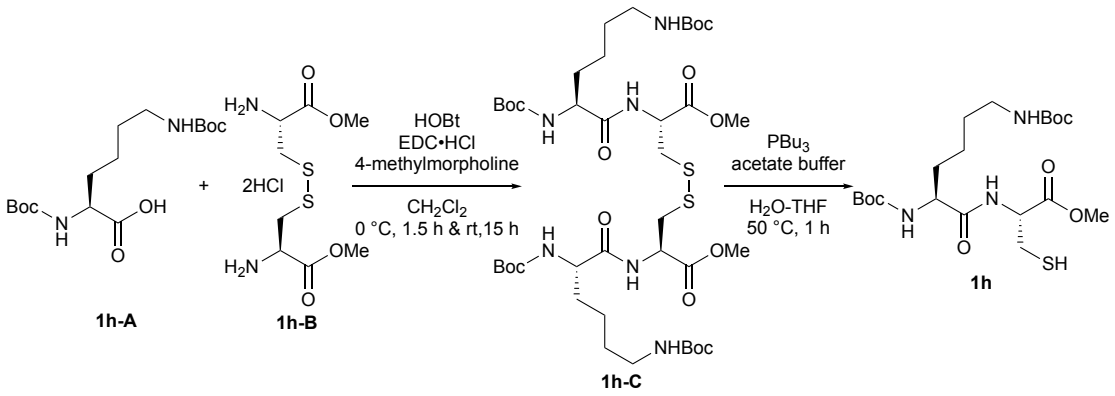
^{1}H and ^{13}C NMR spectra were measured on a JEOL JNM-ECZ 400R NMR instrument (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR). Tetramethylsilane (TMS) served as the internal standard (0 ppm) for ^{1}H NMR, and solvent peaks served as the internal standard (77.0 ppm for $CDCl_3$; 49.0 ppm for CD_3OD; 39.5 ppm for DMSO-d_6) for ^{13}C NMR. The following abbreviations were used to express the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. High-resolution mass spectra (HRMS) were measured on a JEOL JMS-700N. Infrared spectra (IR) were measured on a JASCO FT/IR-4200 spectrometer. Optical rotations were measured on a JASCO P-2100 polarimeter. High performance liquid chromatography (HPLC) was performed on Shimadzu LC-20AT and SPD-20A instruments using Daicel Chiralpak AD-3, ID-3, or IE-3 columns (4.6 mm × 250 mm). All reactions were monitored by thin-layer chromatography using Merck precoated TLC plates (silica gel 60GF-254, 0.25 mm), with visualization by the use of UV lamp (254 nm) or dyes. The products were purified by flash column chromatography on silica gel.

Experimental Section

1. Preparation of substrates

Dipeptide substrates [**1** (except **1h**), **3**, and **5**] and amnio acid-derived substrates [**6** and **10**] are known and commercially available compounds.

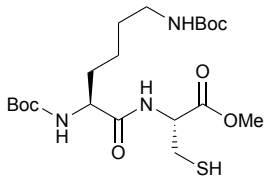
Dipeptide substrate **1h** and tripeptide substrate **8** were prepared according to the general synthetic methods for peptides as shown in below.



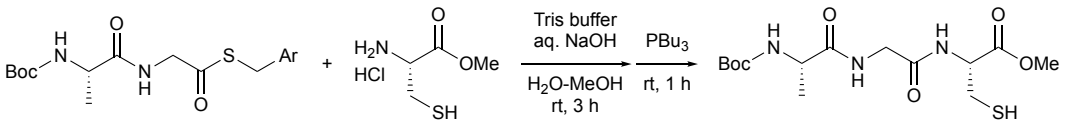
To a solution of Boc-protected lysine derivative **1h-A** (0.35 g, 1.0 mmol) in CH_2Cl_2 (5 mL) was added 1-hydroxybenzotriazole (HOBt, 0.14 g, 1.0 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC•HCl, 0.19 g, 1.0 mmol) at 0 ℃. The reaction mixture was stirred for 1.5 hours at 0 ℃. After 1.5 hours, 4-methylmorpholine (0.12 g, 1.2 mmol) and cystine dimethyl ester dihydrochloride **1h-B** (0.15 g, 0.45 mmol) were added to the reaction mixture at 0 ℃, and the resulting mixture was stirred for 1.5 hours at 0 ℃. The mixture was then warmed to room temperature and stirred for additional 15 hours. After 15 hours, H_2O (5 mL) was added to the reaction mixture at room temperature. After evaporation to remove CH_2Cl_2, the reaction products were extracted three times with ethyl acetate (5 mL × 3). The combined ethyl acetate extracts were washed with saturated aqueous $NaHCO_3$ for three times (5 mL × 3) and brine (5 mL × 1). The resulting ethyl acetate solution was dried over Na_2SO_4. Following filtration to remove Na_2SO_4, the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 10:1–1:2 as the eluent) to afford disulfide **1h-C** (0.35 g, 0.38 mmol).

To a solution of disulfide **1h-C** (0.28 g, 0.30 mmol) in THF (2 mL) was added 0.1M acetate (AcOH•AcONa) buffer solution [pH 4.7] (1 mL) and tributylphosphine (67

mg, 0.33 mmol) at room temperature. The reaction mixture was warmed to 50 °C and stirred for 1 hour at 50 °C. After 1 hour, the reaction mixture was cooled to room temperature. H_2O (5 mL) was added to the resulting solution at room temperature and the reaction products were extracted three times with ethyl acetate (5 mL × 3). The combined ethyl acetate extracts were dried over Na_2SO_4. Following filtration to remove Na_2SO_4, the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 10:1–1:2 as the eluent) to afford dipeptide **1h** in 91% yield (0.25 g, 0.54 mmol, white solid).

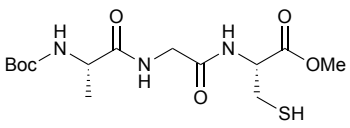


1h: $[\alpha]^{27}_D$ +1.2 (*c* = 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (d, *J* = 6.9 Hz, 1H), 5.19 (br, 1H), 4.87–4.83 (m, 1H), 4.65 (br, 1H), 4.12–4.06 (br m, 1H), 3.79 (s, 3H), 3.15–3.10 (br m, 2H), 3.03–2.99 (m, 2H), 1.88–1.38 (m, 7H), 1.45 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 170.1, 155.9, 155.6, 79.5, 78.6, 54.1, 53.6, 52.4, 39.6, 31.4, 29.3, 28.1, 28.0, 26.2, 22.2; IR (neat): 3312, 2979, 2933, 1693, 1507, 1366, 1248, 1166, 907, 727 cm^{-1}; HRMS (FAB) calcd for $C_{20}H_{38}N_3O_7S$: 464.2430 ($[M+H]^+$), found 464.2431.



A solution of peptide **8-A** (0.57 g, 1.4 mmol) in methanol (5 mL) was added to a mixture of cysteine methyl ester hydrochloride **8-B** (0.70 g, 4.1 mmol), H_2O (3 mL), 1M TRIS buffer solution [pH 8.0] (1 mL), and 5M aqueous NaOH (1 mL) at room temperature. The mixture was stirred for 3 hours at room temperature. After 3 hours, tributylphosphine (0.34 g, 1.7 mmol) was added to the reaction mixture at room temperature. The resulting mixture was stirred for 1 hour at room temperature. After 1 hour, the reaction products were extracted three times with ethyl acetate (5 mL × 3). The combined ethyl acetate extracts were washed with saturated aqueous NH_4Cl (10 mL × 1) and brine (10 mL × 1). The resulting ethyl acetate solution was dried over Na_2SO_4. Following filtration to remove Na_2SO_4, the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 2:1–1:10

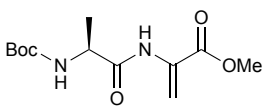
as the eluent) to afford tripeptide **8** in 75% yield (0.37 g, 1.0 mmol, white solid).



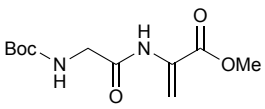
8: $[\alpha]^{24}_{D}$ –22.7 (*c* = 0.80, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 4.65 (t, *J* = 5.7 Hz, 1H), 4.04 (q, *J* = 7.2 Hz, 1H), 3.94 (d, *J* = 16.7 Hz, 1H), 3.87 (d, *J* = 16.9 Hz, 1H), 3.74 (s, 3H), 2.95 (dd, *J* = 14.0, 4.8 Hz, 1H), 2.89 (dd, *J* = 14.0, 6.8 Hz, 1H), 1.44 (s, 9H), 1.33 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 176.2, 171.7, 171.3, 157.6, 80.5, 55.9, 53.0, 51.6, 43.4, 28.7, 26.5, 18.1; IR (neat): 3301, 2979, 1660, 1522, 1249, 1167 cm^{-1}; HRMS (EI) calcd for $C_{14}H_{25}N_3O_6S$: 363.1464 ($[M]^+$), found 363.1471.

2. General methods for the preparation of dehydroamino acid derivatives [2, 4, 7, and 9] (Schemes 2 and 3)

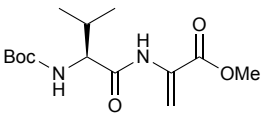
A solution of dipeptide **1** [or **3**, or **5**, or threonine derivative **6**, or tripeptide **8**] (0.30 mmol) in acetonitrile (3.0 mL) was warmed to 35 °C and stirred for 5 min. Cesium carbonate (98 mg, 0.30 mmol, 1 equiv) was then added to the warmed solution. The reaction mixture was stirred for 2 hours at 35 °C under open air conditions. After 2 hours, H_2O (5 mL) was added to the reaction mixture. The reaction products were initially extracted three times with ethyl acetate (5 mL × 3). After acidifying the aqueous phase with 1N HCl, further extraction was performed three times with ethyl acetate (5 mL × 3). The combined ethyl acetate extracts were dried over Na_2SO_4. Following filtration to remove Na_2SO_4, the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate as the eluent) to afford dehydroamino acid derivative **2** [or **4**, or **7**, or **9**].



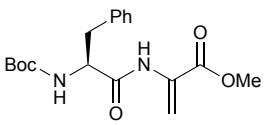
2a:[1–3] 56% yield (46 mg, 0.17 mmol, white solid). $[\alpha]^{20}_{D}$ –73.6 (*c* = 1.1, $CHCl_3$, >99% ee); HPLC analysis: Daicel Chiralpak IE-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm; retention time: 16.8 min (minor) and 22.6 min (major). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (br, 1H), 6.61 (s, 1H), 5.91 (d, *J* = 1.4 Hz, 1H), 4.94 (br, 1H), 4.26 (br, 1H), 3.85 (s, 3H), 1.46 (s, 9H), 1.41 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 164.2, 155.4, 130.8, 109.2, 80.5, 52.9, 50.9, 28.2, 17.9; IR (neat): 3332, 2979, 1683, 1515, 1442, 1323, 1248, 1162, 733 cm^{-1}; HRMS (EI) calcd for $C_{12}H_{20}N_2O_5$: 272.1372 ($[M]^+$), found 272.1372.



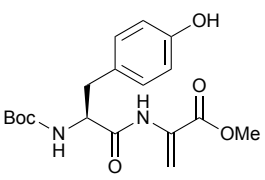
2b:[1,3,4] 57% yield (44 mg, 0.17 mmol, colorless viscous oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (br, 1H), 6.62 (s, 1H), 5.92 (d, *J* = 1.4 Hz, 1H), 5.13 (br, 1H), 3.90 (d, *J* = 5.7 Hz, 2H), 3.85 (s, 3H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 164.2, 156.0, 130.5, 109.3, 80.5, 52.9, 45.1, 28.2; IR (neat): 3379, 2979, 1684, 1514, 1327, 1250, 1202, 1162 cm^{-1}; HRMS (EI) calcd for $C_{11}H_{18}N_2O_5$: 258.1216 ([M]$^+$), found 258.1216.



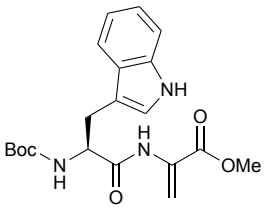
2c:[5] 54% yield (49 mg, 0.16 mmol, white solid). $[\alpha]^{18}_D$ –25.5 (*c* = 1.2, $CHCl_3$, >99% ee); HPLC analysis: Daicel Chiralpak IE-3, hexane/2-propanol = 5:1, flow rate = 0.5 mL/min, 254 nm; retention time: ~35 min [not detected] (minor) and 38.3 min (major). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (br, 1H), 6.63 (s, 1H), 5.92 (d, *J* = 1.4 Hz, 1H), 5.04 (br d, *J* = 7.1 Hz, 1H), 4.08–4.01 (br m, 1H), 3.85 (s, 3H), 2.26–2.18 (m, 1H), 1.46 (s, 9H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.93 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 164.3, 155.8, 130.5, 109.3, 80.2, 60.6, 53.0, 30.7, 28.2, 19.3, 17.5; IR (neat): 3313, 2971, 1726, 1675, 1522, 1366, 1322, 1204, 1168 cm^{-1}; HRMS (EI) calcd for $C_{14}H_{24}N_2O_5$: 300.1685 ([M]$^+$), found 300.1685.



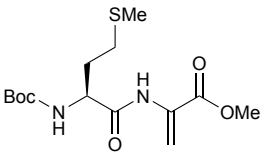
2d:[3,5,6] 62% yield (65 mg, 0.19 mmol, white solid). $[\alpha]^{19}_D$ –26.5 (*c* = 1.1, $CHCl_3$, 94% ee); HPLC analysis: Daicel Chiralpak IE-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm; retention time: 21.0 min (minor) and 23.9 min (major). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (br, 1H), 7.33–7.29 (m, 2H), 7.26–7.18 (m, 3H), 6.62 (s, 1H), 5.90 (d, *J* = 1.1 Hz, 1H), 4.96 (br, 1H), 4.44 (br, 1H), 3.80 (s, 3H), 3.16–3.08 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 164.0, 155.3, 136.2, 130.5, 129.1, 128.7, 127.0, 109.3, 80.5, 56.5, 52.9, 38.1, 28.2; IR (neat): 3313, 2979, 1723, 1677, 1521, 1500, 1440, 1367, 1324, 1251, 1203, 1165 cm^{-1}; HRMS (EI) calcd for $C_{18}H_{24}N_2O_5$: 348.1685 ([M]$^+$), found 348.1683.



2e:[5,7] 45% yield (49 mg, 0.14 mmol, white solid). $[\alpha]^{21}_{D}$ –4.1 (*c* = 0.99, CH_3OH, 96% ee); HPLC analysis: Daicel Chiralpak AD-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm; retention time: 16.7 min (minor) and 19.8 min (major). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (s, 1H), 9.19 (s, 1H), 7.17 (d, *J* = 8.2 Hz, 1H), 7.08 (d, *J* = 8.2 Hz, 2H), 6.65 (d, *J* = 8.5 Hz, 2H), 6.26 (s, 1H), 5.72 (s, 1H), 4.27–4.21 (m, 1H), 3.77 (s, 3H), 2.89 (dd, *J* = 13.8, 4.0 Hz, 1H), 2.64 (dd, *J* = 13.6, 10.6 Hz, 1H), 1.32 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.8, 163.8, 155.8, 155.5, 132.3, 130.2, 127.9, 114.8, 109.1, 78.4, 56.7, 52.8, 35.9, 28.1; IR (neat): 3340, 2979, 2930, 1701, 1515, 1365, 1222, 1203, 1163, 733 cm^{-1}; HRMS (FAB) calcd for $C_{18}H_{25}N_2O_6$: 365.1713 ([M+H]$^+$), found 365.1699.

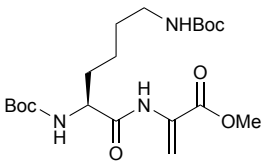


2f:[5,7] 62% yield (72 mg, 0.19 mmol, white solid). $[\alpha]^{22}_{D}$ –8.0 (*c* = 1.2, $CHCl_3$, >99% ee); HPLC analysis: Daicel Chiralpak IE-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm; retention time: 16.6 min (minor) and 19.0 min (major). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16–8.09 (br m, 2H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.36 (dt, *J* = 8.0, 1.0 Hz, 1H), 7.20 (td, *J* = 8.0, 1.0 Hz, 1H), 7.12 (td, *J* = 8.0, 1.0 Hz, 1H), 7.06 (d, *J* = 2.3 Hz, 1H), 6.61 (s, 1H), 5.87 (s, 1H), 5.12 (br, 1H), 4.54 (br, 1H), 3.73 (s, 3H), 3.40–3.21 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 163.8, 155.5, 136.2, 130.6, 127.3, 123.1, 122.2, 119.7, 118.7, 111.2, 110.0, 109.2, 80.4, 55.9, 52.8, 28.2, 27.9; IR (neat): 3385, 2980, 2954, 2930, 1682, 1518, 1440, 1327, 1202, 1162, 907, 728 cm^{-1}; HRMS (FAB) calcd for $C_{20}H_{25}N_3O_5$: 387.1794 ([M]$^+$), found 387.1795.

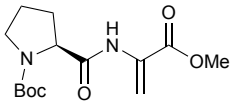


2g: 57% yield (57 mg, 0.17 mmol, white solid). $[\alpha]^{21}_{D}$ –26.5 (*c* = 1.2, $CHCl_3$, 95% ee); HPLC analysis: Daicel Chiralpak IE-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm; retention time: 26.1 min (major) and 36.5 min (minor). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (br, 1H), 6.60 (s, 1H), 5.93 (d, *J* = 1.4 Hz, 1H), 5.18 (br d, *J* = 6.2 Hz, 1H), 4.42–4.33 (br m, 1H), 3.85 (s, 3H), 2.64–2.52 (m, 2H), 2.20–2.12 (m, 1H), 2.12 (s, 3H), 2.00–1.91 (m, 1H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 164.1, 155.5, 130.7, 109.5, 80.5, 54.2, 53.0, 31.3, 30.2, 28.2, 15.2; IR (neat): 3313, 2979, 2919, 1678, 1515, 1440, 1322, 1248, 1202, 1161, 732 cm^{-1}; HRMS (FAB) calcd for

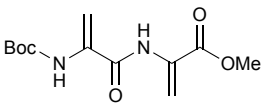
$C_{14}H_{25}N_2O_5S$: 333.1484 ([M+H]$^+$), found 333.1484.



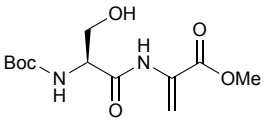
2h: 56% yield (72 mg, 0.17 mmol, white solid). $[\alpha]^{27}_D$ –31.7 (*c* = 0.90, $CHCl_3$, >99% ee); HPLC analysis: Daicel Chiralpak IE-3, hexane/2-propanol = 1:1, flow rate = 0.5 mL/min, 254 nm; retention time: 18.2 min (major) and ~29 min [not detected] (minor). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (br, 1H), 6.60 (s, 1H), 5.91 (d, *J* = 1.1 Hz, 1H), 5.20 (br, 1H), 4.60 (br, 1H), 4.15 (br, 1H), 3.84 (s, 3H), 3.15–3.10 (m, 2H), 1.94–1.25 (m, 6H), 1.46 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 164.0, 156.0, 155.7, 130.6, 109.1, 79.9, 78.7, 55.1, 52.7, 39.5, 31.1, 29.5, 28.2, 28.0, 22.3; IR (neat): 3384, 3331, 2979, 2933, 1683, 1509, 1366, 1249, 1163, 909, 729 cm^{-1}; HRMS (FAB) calcd for $C_{20}H_{36}N_3O_7$: 430.2553 ([M+H]$^+$), found 430.2542.



2i:[8] 54% yield (48 mg, 0.16 mmol, white solid). $[\alpha]^{24}_D$ –92.2 (*c* = 1.1, $CHCl_3$, >99% ee); HPLC analysis: Daicel Chiralpak ID-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm; retention time: 13.6 min (minor) and 20.6 min (major). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20–8.15 (br m, 1H), 6.60 (s, 1H), 5.90 (s, 1H), 4.50–4.15 (br m, 1H), 3.84 (s, 3H), 3.60–3.25 (br m, 2H), 2.45–1.86 (m, 4H), 1.54–1.39 (br m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6 & 171.0, 164.2, 155.8 & 154.3, 131.2 & 130.6, 108.9, 80.7, 61.9 & 60.8, 52.8, 47.1, 30.8 & 29.6, 28.4 & 28.2, 24.4 & 23.8; IR (neat): 3388, 2977, 1690, 1518, 1390, 1366, 1319, 1201, 1159, 1120 cm^{-1}; HRMS (EI) calcd for $C_{14}H_{22}N_2O_5$: 298.1529 ([M]$^+$), found 298.1532.

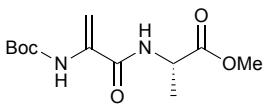


2j: 6% yield (4.9 mg, 0.018 mmol, white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (br, 1H), 6.62 (s, 1H), 6.12 (br, 1H), 5.97 (d, *J* = 1.4 Hz, 1H), 5.21–5.20 (m, 1H), 3.88 (s, 3H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3, 162.3, 152.6, 134.8, 130.5, 109.4, 98.7, 80.8, 53.2, 28.2; IR (neat): 3406, 2979, 1678, 1495, 1340, 1161 cm^{-1}; HRMS (EI) calcd for $C_{12}H_{18}N_2O_5$: 270.1216 ([M]$^+$), found 270.1216.

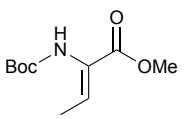


2k: 8% yield (6.9 mg, 0.024 mmol, white solid). $[\alpha]^{21}_D$ –42.7 (*c* = 0.73, $CHCl_3$, 97% ee); HPLC analysis: Daicel Chiralpak IE-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm;

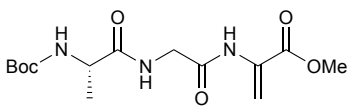
retention time: 17.1 min (minor) and 32.3 min (major). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (br, 1H), 6.58 (s, 1H), 5.95 (d, J = 1.6 Hz, 1H), 5.55 (br, 1H), 4.32–4.17 (br m, 2H), 3.85 (s, 3H), 3.75–3.68 (m, 1H), 2.47–2.44 (m, 1H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 164.0, 156.1, 130.9, 109.7, 80.9, 62.5, 55.7, 53.0, 28.2; IR (neat): 3384, 2979, 2927, 1721, 1683, 1522, 1367, 1329, 1165 cm^{-1}; HRMS (FAB) calcd for $C_{12}H_{21}N_2O_6$: 289.1400 ([M+H]$^+$), found 289.1401.



4:[9] 33% yield (27 mg, 0.099 mmol, white solid). $[\alpha]^{17}_D$ +19.8 (c = 0.77, $CHCl_3$, 93% ee); HPLC analysis: Daicel Chiralpak IE-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm; retention time: 11.4 min (major) and 12.5 min (minor). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.66 (d, J = 7.3 Hz, 1H), 6.04 (s, 1H), 5.12 (t, J = 1.7 Hz, 1H), 4.65–4.58 (m, 1H), 3.79 (s, 3H), 1.48 (s, 9H), 1.46 (d, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 163.5, 152.7, 134.4, 98.1, 80.5, 52.7, 48.6, 28.2, 18.3; IR (neat): 3386, 2981, 1731, 1660, 1656, 1627, 1493, 1456, 1367, 1243, 1214, 1155, 1071 cm^{-1}; HRMS (EI) calcd for $C_{12}H_{20}N_2O_5$: 272.1372 ([M]$^+$), found 272.1372.



(*Z*)-**7**:[1,6] 28% yield (18 mg, 0.084 mmol, white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (q, J = 7.1 Hz, 1H), 5.97 (br, 1H), 3.77 (s, 3H), 1.81 (d, J = 7.1 Hz, 3H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 153.1, 132.1, 126.6, 80.4, 52.2, 28.1, 14.3; IR (neat): 3040, 2979, 2954, 1707, 1496, 1367, 1273, 1245, 1159, 1047 cm^{-1}; HRMS (EI) calcd for $C_{10}H_{17}NO_4$: 215.1158 ([M]$^+$), found 215.1159.

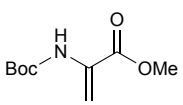


9: 43% yield (42 mg, 0.13 mmol, white solid). $[\alpha]^{24}_D$ –16.7 (c = 1.3, $CHCl_3$, >99% ee); HPLC analysis: Daicel Chiralpak IE-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm; retention time: 30.0 min (major) and ~33 min [not detected] (minor).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 6.89 (br, 1H), 6.57 (s, 1H), 5.92 (d, J = 1.4 Hz, 1H), 5.00 (br, 1H), 4.29–4.18 (br m, 1H), 4.08 (dd, J = 16.9, 5.7 Hz, 1H), 4.02 (dd, J = 16.9, 5.5 Hz, 1H), 3.85 (s, 3H), 1.45 (s, 9H), 1.40 (d, J = 4.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 167.7, 164.2, 155.6, 130.7, 109.7, 80.2, 53.0, 50.0, 43.9, 28.2, 18.4; IR (neat): 3310, 2979, 1666, 1516, 1442, 1366, 1323, 1248, 1203, 1164, 731 cm^{-1};

HRMS (EI) calcd for $C_{14}H_{23}N_3O_6$: 329.1587 ([M]$^+$), found 329.1586.

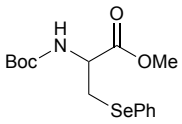
3. Preparation and transformations of dehydroalanine derivative 11 (Scheme 4)

A solution of cysteine derivative **10** (0.71 g, 3.0 mmol) in acetonitrile (30 mL) was warmed to 35 °C and stirred for 5 min. Cesium carbonate (0.98 g, 3.0 mmol, 1 equiv) was then added to the warmed solution. The reaction mixture was stirred for 2 hours at 35 °C under open air conditions. After 2 hours, H_2O (50 mL) was added to the reaction mixture. The reaction products were extracted three times with ethyl acetate (50 mL × 3). The combined ethyl acetate extracts were dried over Na_2SO_4. Following filtration to remove Na_2SO_4, the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 100:1–20:1 as the eluent) to afford dehydroalanine derivative **11** in 58% yield (0.35 g, 1.7 mmol, colorless viscous oil).



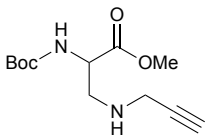
11:[1–4] 58% yield (0.35 g, 1.7 mmol, colorless viscous oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 (br, 1H), 6.16 (s, 1H), 5.73 (d, J = 1.6 Hz, 1H), 3.83 (s, 3H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4, 152.5, 131.2, 105.1, 80.7, 52.8, 28.2; IR (neat): 3422, 2980, 1715, 1508, 1325, 1155, 1066 cm^{-1}; HRMS (EI) calcd for $C_9H_{15}NO_4$: 201.1001 ([M]$^+$), found 201.1001.

To a solution of dehydroalanine derivative **11** (20 mg, 0.10 mmol) in acetone (0.50 mL) was added 0.1M phosphate buffer solution [pH 8.0] (0.50 mL) at room temperature. Phenylselenol (47 mg, 0.30 mmol, 3 equiv) was then added to the solution of **11**. The reaction mixture was warmed to 40 °C and stirred for 24 hours at 40 °C. After 24 hours, saturated aqueous NH_4Cl (5 mL) was added to the reaction mixture. The reaction products were extracted three times with ethyl acetate (5 mL × 3). The combined ethyl acetate extracts were dried over Na_2SO_4. Following filtration to remove Na_2SO_4, the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 50:1–10:1 as the eluent) to afford selenocysteine derivative **12** in 89% yield (32 mg, 0.089 mmol, white solid).



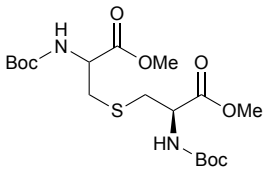
12:[10–13] 89% yield (32 mg, 0.089 mmol, white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57–7.52 (m, 2H), 7.28–7.24 (m, 3H), 5.35 (br d, J = 7.5 Hz, 1H), 4.69–4.64 (br m, 1H), 3.49 (s, 3H), 3.33 (d, J = 4.8 Hz, 2H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 154.9, 133.7, 129.1, 128.8, 127.5, 80.0, 53.2, 52.2, 30.6, 28.2; IR (neat): 3363, 2978, 1746, 1713, 1501, 1366, 1215, 1165, 739 cm^{-1}; HRMS (FAB) calcd for $C_{15}H_{21}NO_4Se$: 359.0636 ([M]$^+$), found 359.0636.

To a solution of dehydroalanine derivative **11** (20 mg, 0.10 mmol) in acetone (0.50 mL) was added 0.1M phosphate buffer solution [pH 8.0] (0.50 mL) at room temperature. Propargylamine (6.1 mg, 0.11 mmol, 1.1 equiv) was then added to the solution of **11**. The reaction mixture was warmed to 35 °C and stirred for 48 hours at 35 °C. After 48 hours, the reaction mixture was concentrated. The resulting residue was purified by flash column chromatography on silica gel (dichloromethane/methanol = 100:1–50:1 as the eluent) to afford amino acid product **13** in 55% yield (14 mg, 0.055 mmol, white solid).



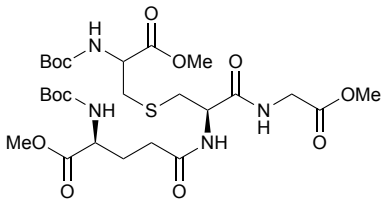
13:[14] 55% yield (14 mg, 0.055 mmol, white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 (br, 1H), 4.40 (br, 1H), 3.77 (s, 3H), 3.47–3.37 (m, 2H), 3.10 (dd, J = 11.9, 4.8 Hz, 1H), 3.02 (dd, J = 12.3, 4.8 Hz, 1H), 2.22 (t, J = 2.4 Hz, 1H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 155.5, 81.5, 80.0, 71.7, 53.5, 52.5, 49.5, 38.1, 28.3; IR (neat): 3300, 2979, 2932, 1743, 1706, 1366, 1250, 1207, 1162 cm^{-1}; HRMS (EI) calcd for $C_{12}H_{21}N_2O_4$: 257.1501 ([M+H]$^+$), found 257.1503.

To a solution of dehydroalanine derivative **11** (20 mg, 0.10 mmol) in methanol (1.0 mL) was added cysteine derivative **10** (35 mg, 0.15 mmol) and triethylamine (15 mg, 0.15 mmol) at room temperature. The reaction mixture was stirred for 2 hours at room temperature. After 2 hours, the reaction mixture was concentrated. The resulting residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 20:1–1:1 as the eluent) to afford lanthionine derivative **14** in >99% yield (43 mg, 0.099 mmol, white solid).



14:[15–17] >99% yield (43 mg, 0.099 mmol, white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.50–5.00 (br m, 2H), 4.65–4.27 (br m, 2H), 3.77 (s, 6H), 3.18–2.91 (m, 4H), 1.45 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 155.1, 80.2, 53.23 & 53.16, 52.6, 35.3 & 35.2, 28.2; IR (neat): 3372, 2978, 1700, 1501, 1366, 1248, 1213, 1160, 730 cm^{-1}; HRMS (EI) calcd for $C_{18}H_{33}N_2O_8S$: 437.1958 ([M+H]$^+$), found 437.1958.

To a solution of dehydroalanine derivative **11** (20 mg, 0.10 mmol) in acetone (0.50 mL) was added 0.1M phosphate buffer solution [pH 8.0] (0.50 mL) at room temperature. Glutathione derivative **15** (48 mg, 0.11 mmol, 1.1 equiv) was then added to the solution of **11**. The reaction mixture was warmed to 35 °C and stirred for 7 hours at 35 °C. After 7 hours, H_2O (2 mL) was added to the reaction mixture. The reaction products were extracted three times with ethyl acetate (5 mL × 3). The combined ethyl acetate extracts were dried over Na_2SO_4. Following filtration to remove Na_2SO_4, the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (dichloromethane/methanol = 100:1–25:1 as the eluent) to afford peptide **16** in 87% yield (56 mg, 0.087 mmol, white solid).



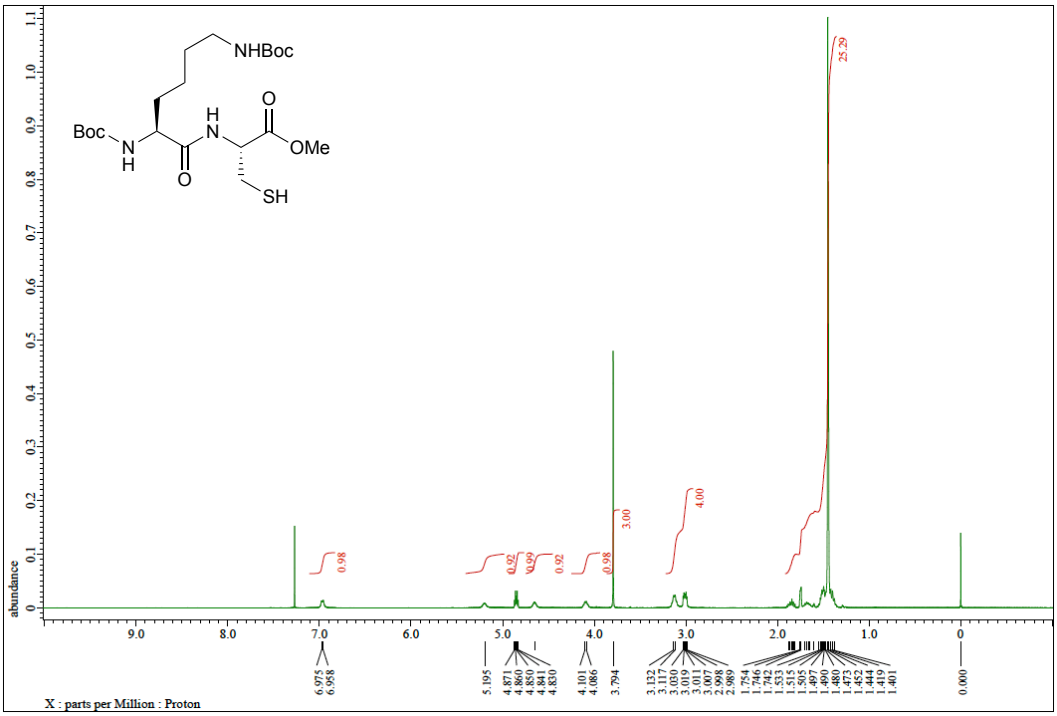
16: 87% yield (56 mg, 0.087 mmol, white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23–6.78 (br m, 2H), 5.58–5.25 (br m, 2H), 4.70–4.35 (br m, 3H), 4.13–3.99 (m, 2H), 3.79–3.75 (m, 9H), 3.09–2.84 (m, 4H), 2.42–2.33 (m, 2H), 2.28–2.15 (br m, 1H), 2.05–1.90 (br m, 1H), 1.45–1.44 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.89, 172.86, 172.3, 172.2, 171.6, 171.4, 170.7, 170.51, 170.45, 169.94, 169.89, 155.7, 155.4, 80.4, 80.1, 53.7, 53.2, 52.72, 52.67, 52.5, 52.4, 52.3, 52.0, 51.9, 41.25, 41.18, 35.6, 35.3, 34.6, 34.4, 32.1, 31.9, 28.5, 28.3; IR (neat): 3320, 2979, 2955, 1745, 1703, 1653, 1520, 1213, 1167 cm^{-1}; HRMS (EI) calcd for $C_{26}H_{45}N_4O_{12}S$: 637.2755 ([M+H]$^+$), found 637.2756.

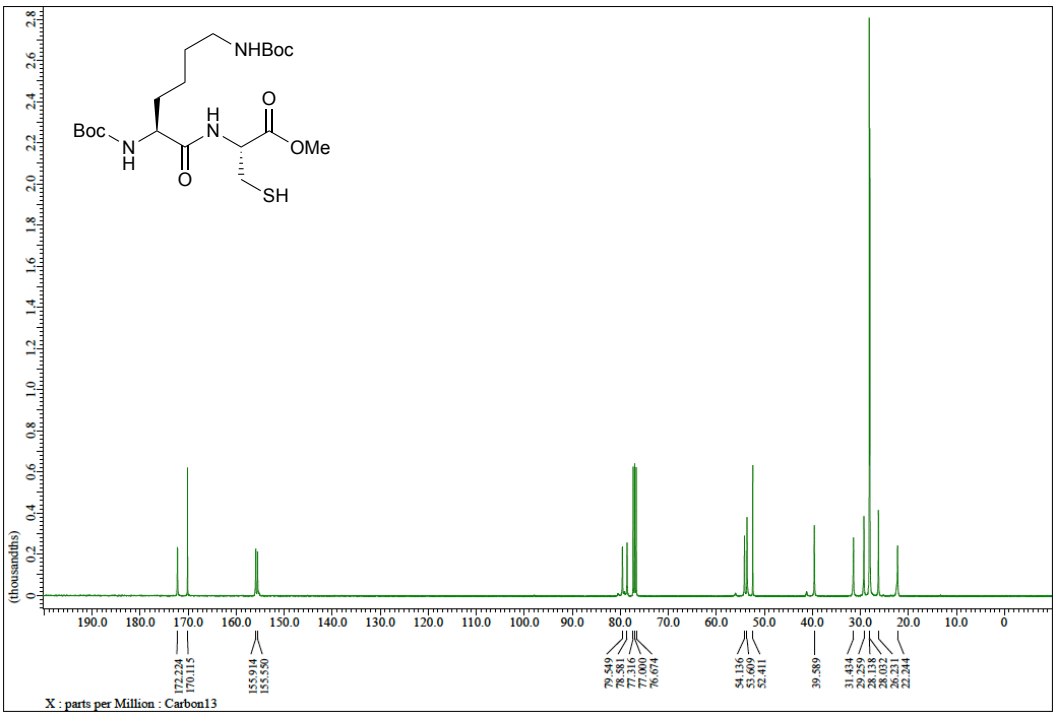
References

1. P. M. T. Ferreira, L. S. Monteiro, G. Pereira, L. Ribeiro, J. Sacramento and L. Silva, *Eur. J. Org. Chem.* 2007, 5934–5949.
2. J. M. Chalker, S. B. Gunnoo, O. Boutureira, S. C. Gerstberger, M. Fernández-González, G. J. L. Bernardes, L. Griffin, H. Hailu, C. J. Schofield and B. G. Davis, *Chem. Sci.*, 2011, **2**, 1666–1676.
3. K. M. Reddy and G. Mugesh, *RSC Adv.*, 2019, **9**, 34–43.
4. H. Wang, J. Zhang and M. Xian, *J. Am. Chem. Soc.*, 2009, **131**, 13238–13239.
5. C. J. Chapman, J. D. Hargrave, G. Bish and C. G. Frost, *Tetrahedron*, 2008, **64**, 9528–9539.
6. R. Ramesh, K. De and S. Chandrasekaran, *Tetrahedron*, 2007, **63**, 10534–10542.
7. C. J. Chapman, A. Matsuno, C. G. Frost and M. C. Willis, *Chem. Commun.*, 2007, 3903–3905.
8. L. Gentilucci, L. Cerisoli, R. De Marco and A. Tolomelli, *Tetrahedron Lett.*, 2010, **51**, 2576–2579.
9. P. J. Knerr and W. A. van der Donk, *J. Am. Chem. Soc.*, 2012, **134**, 7648–7651.
10. A. L. Braga, P. H. Schneider, M. W. Paixão, A. M. Deobald, C. Peppe and D. P. Bottega, *J. Org. Chem.*, 2006, **71**, 4305–4307.
11. Y. Fang, T. Rogge, L. Ackermann, S.-Y. Wang and S.-J. Ji, *Nat. Commun.*, 2018, **9**, 2240.
12. K. M. Reddy and G. Mugesh, *Chem. – Eur. J.*, 2019, **25**, 8875–8883.
13. Z. Yu, Y. Kong, B. Li, S. Su, J. Rao, Y. Gao, T. Tu, H. Chen and K. Liao, *Chem. Commun.*, 2023, **59**, 2935–2938.
14. V. S. Sudhir, N. Y. P. Kumar, R. B. N. Baig and S. Chandrasekaran, *J. Org. Chem.*, 2009, **74**, 7588–7591.
15. C. Dugave and A. Ménez, *Tetrahedron: Asymmetry*, 1997, **8**, 1453–1465.
16. A. P. Galow, F. Rominger and M. Mastalerz, *Eur. J. Org. Chem.*, 2023, **26**, e202201383.
17. J. M. Hammond, M. G. Gardiner and L. R. Malins, *Org. Lett.*, 2023, **25**, 3157–3162.

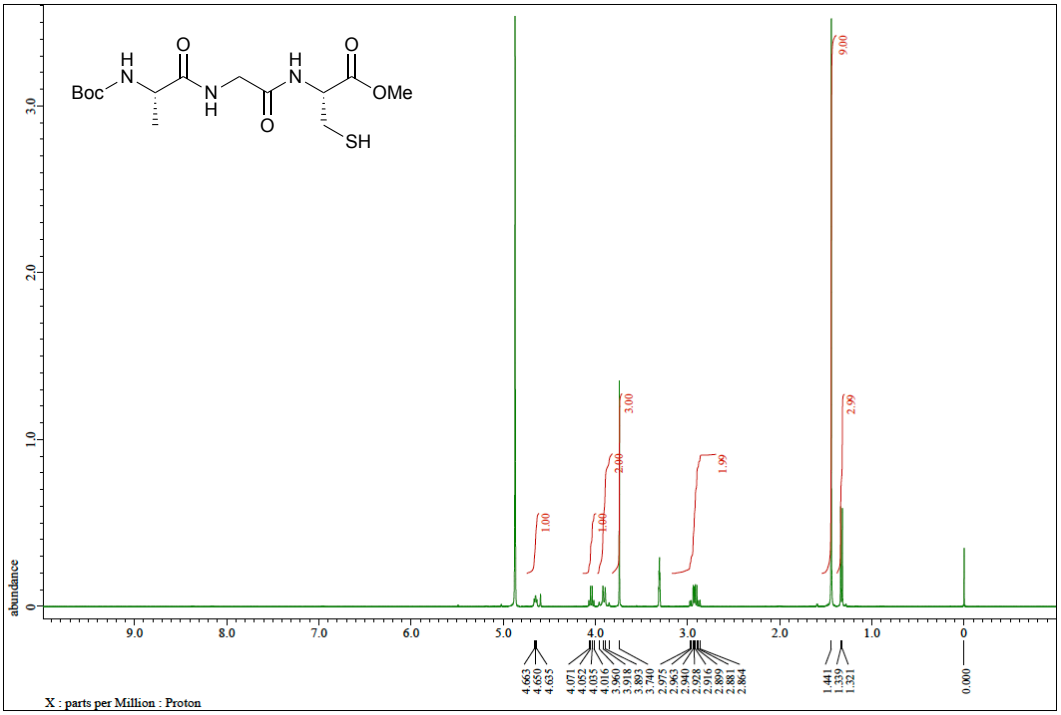
NMR Charts

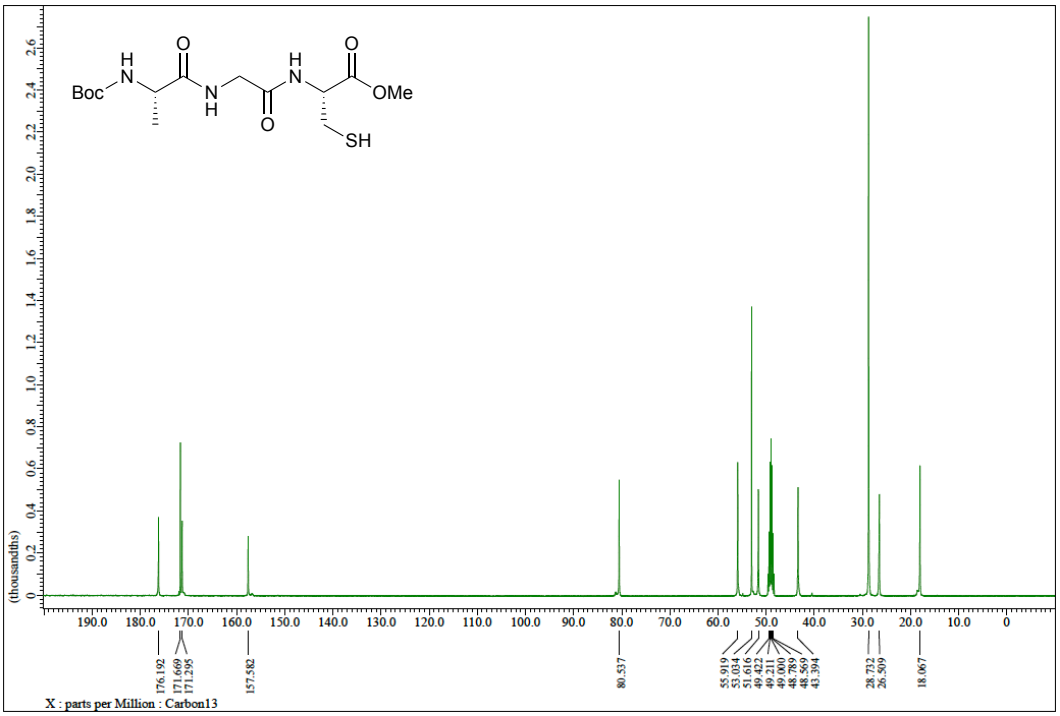
1h: ^{1}H and ^{13}C NMR ($CDCl_3$)



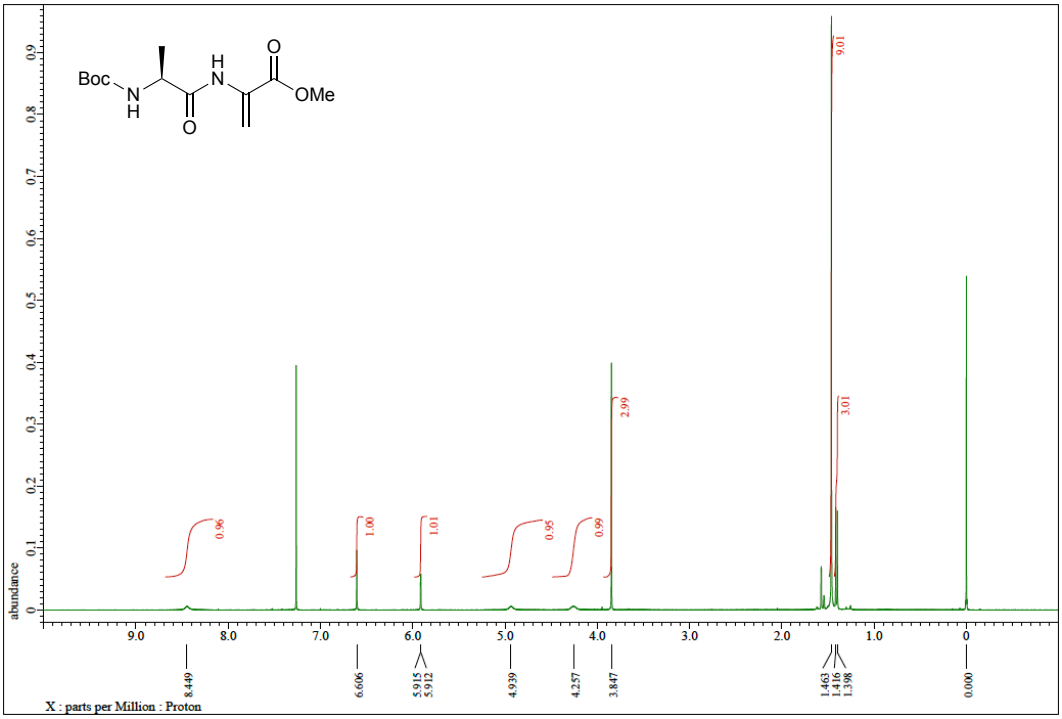


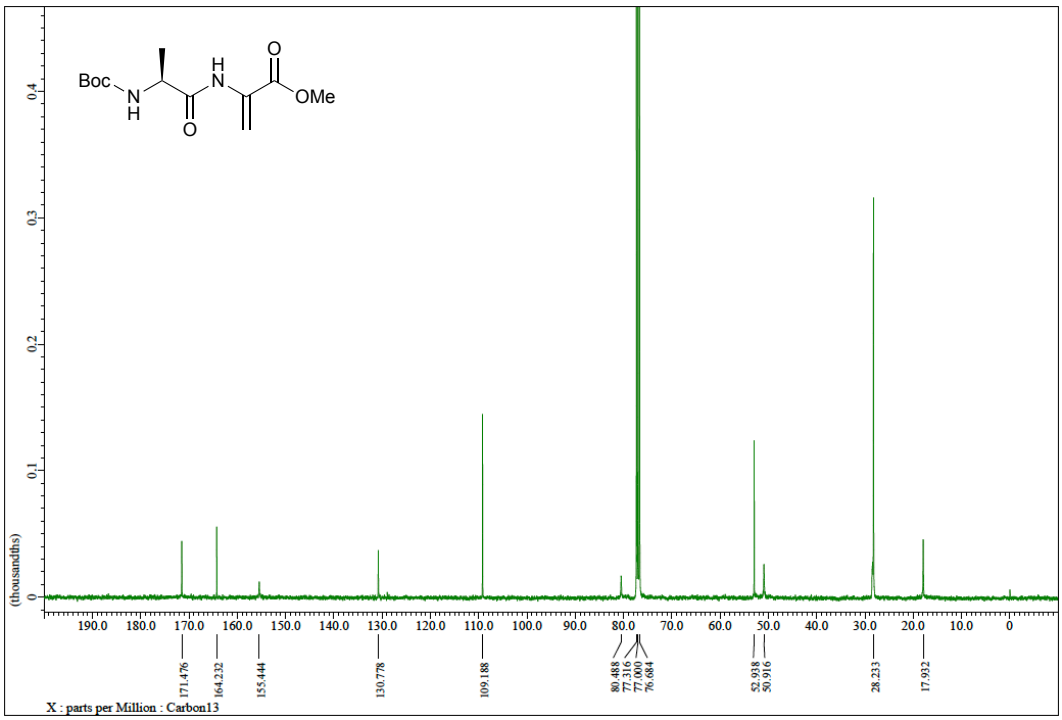
8: ^{1}H and ^{13}C NMR (CD_3OD)



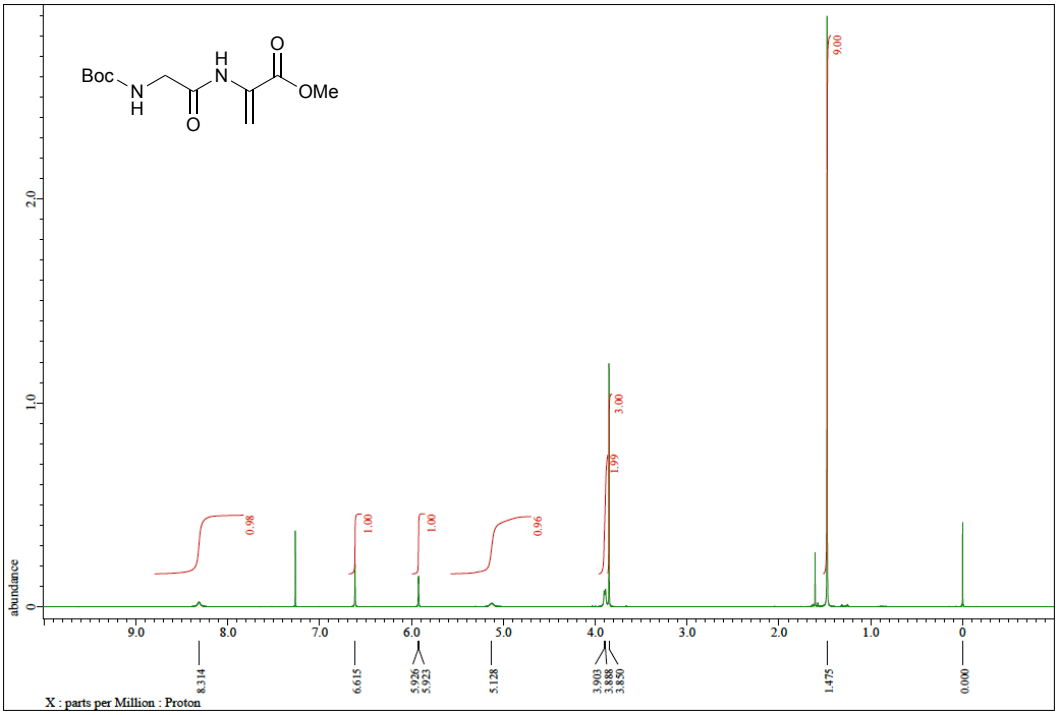


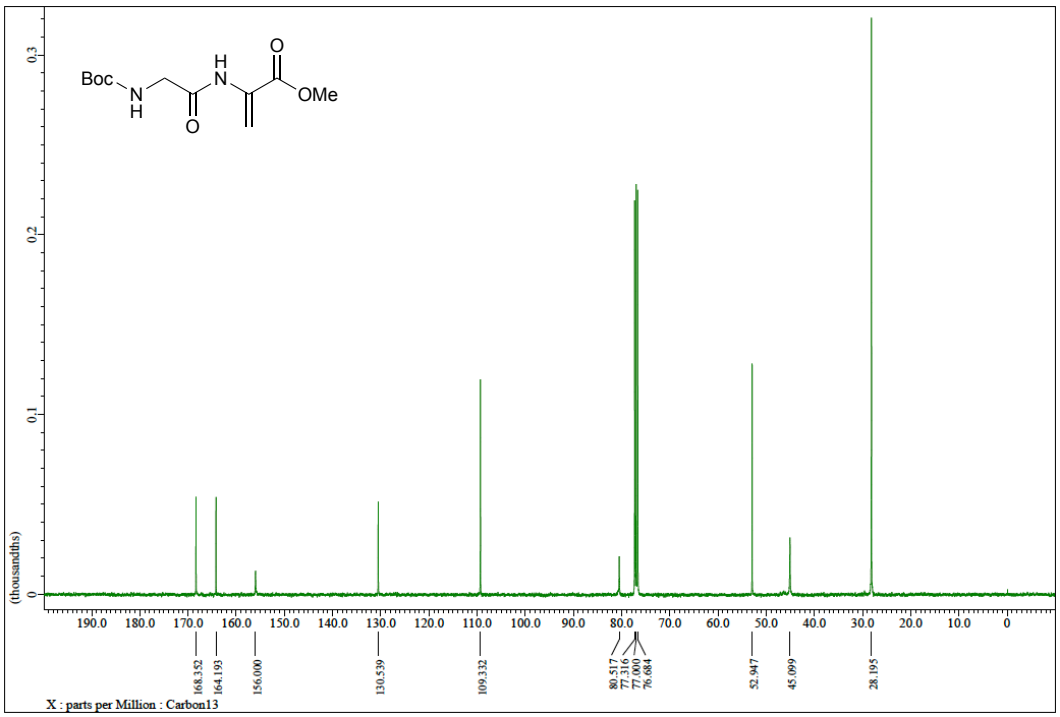
2a: ^{1}H and ^{13}C NMR ($CDCl_3$)



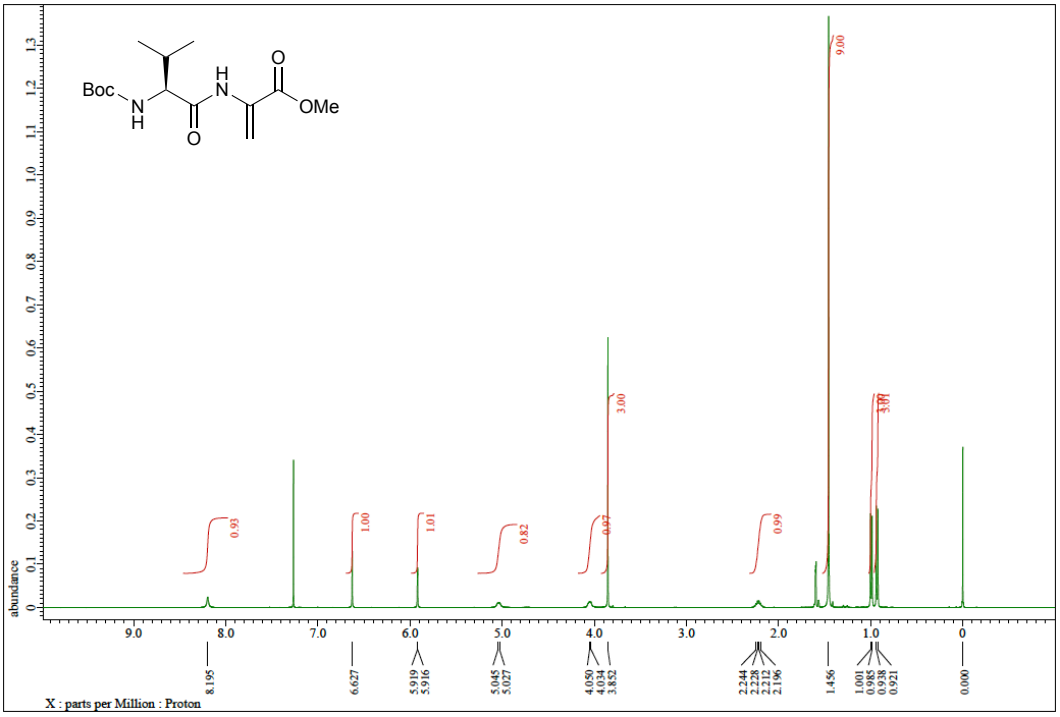


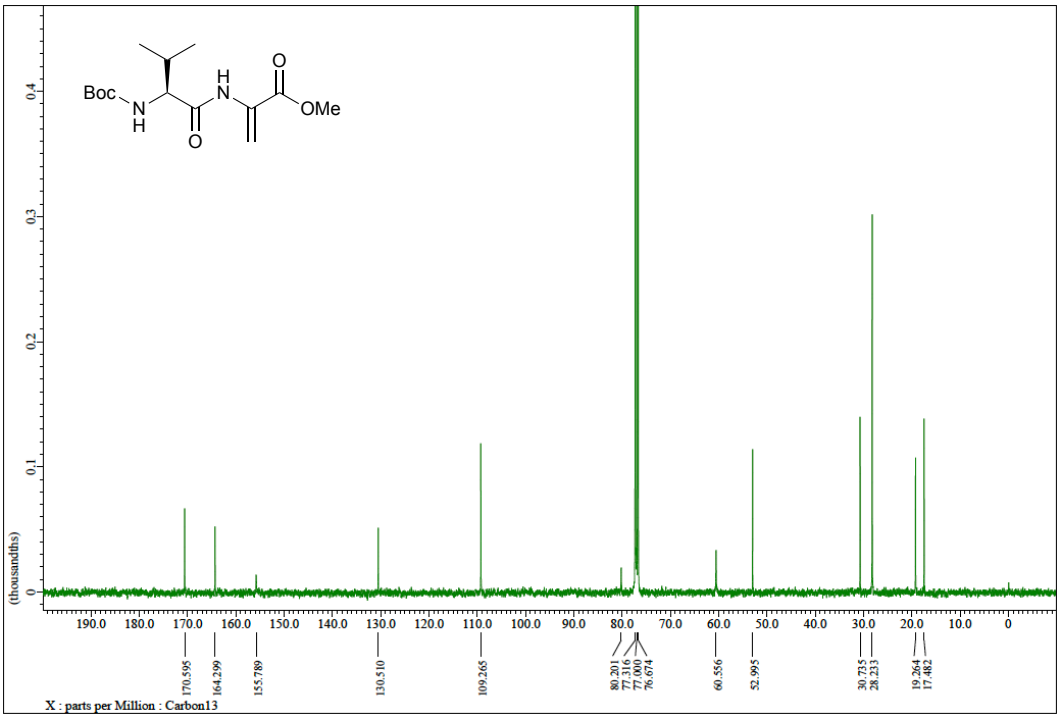
2b: ^{1}H and ^{13}C NMR ($CDCl_3$)



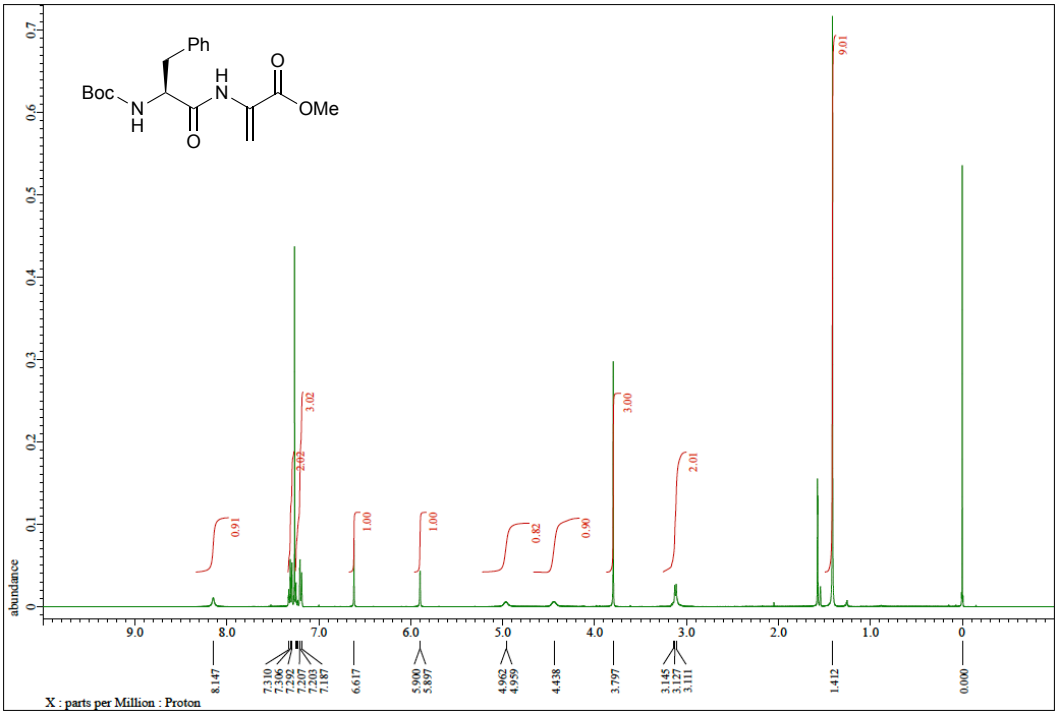


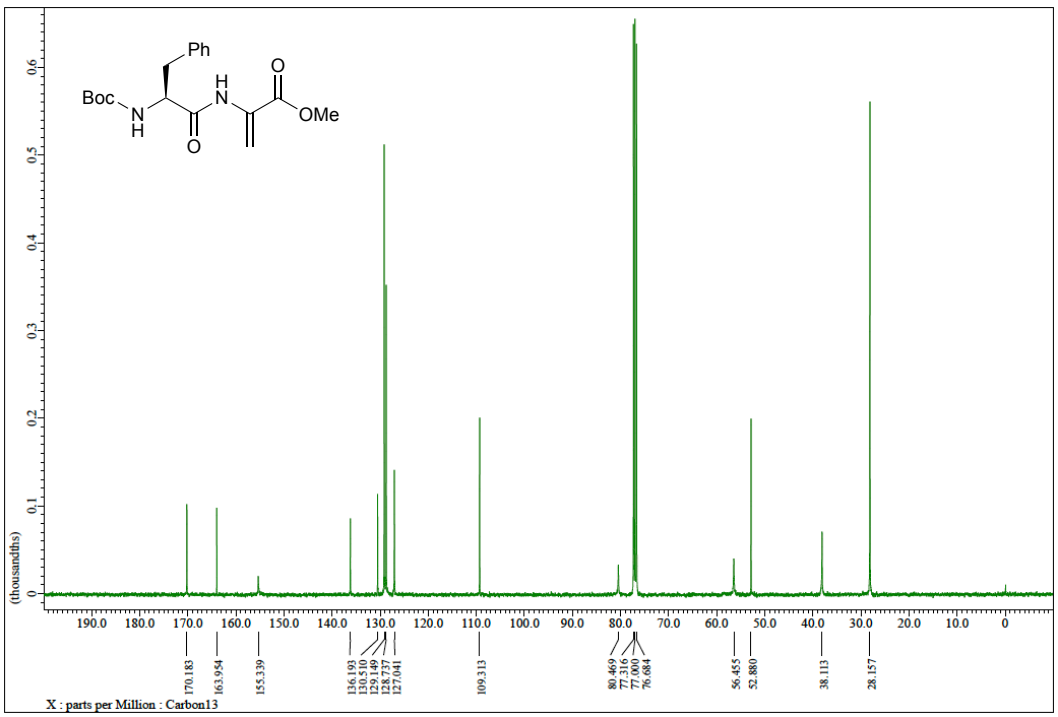
2c: ^{1}H and ^{13}C NMR ($CDCl_3$)



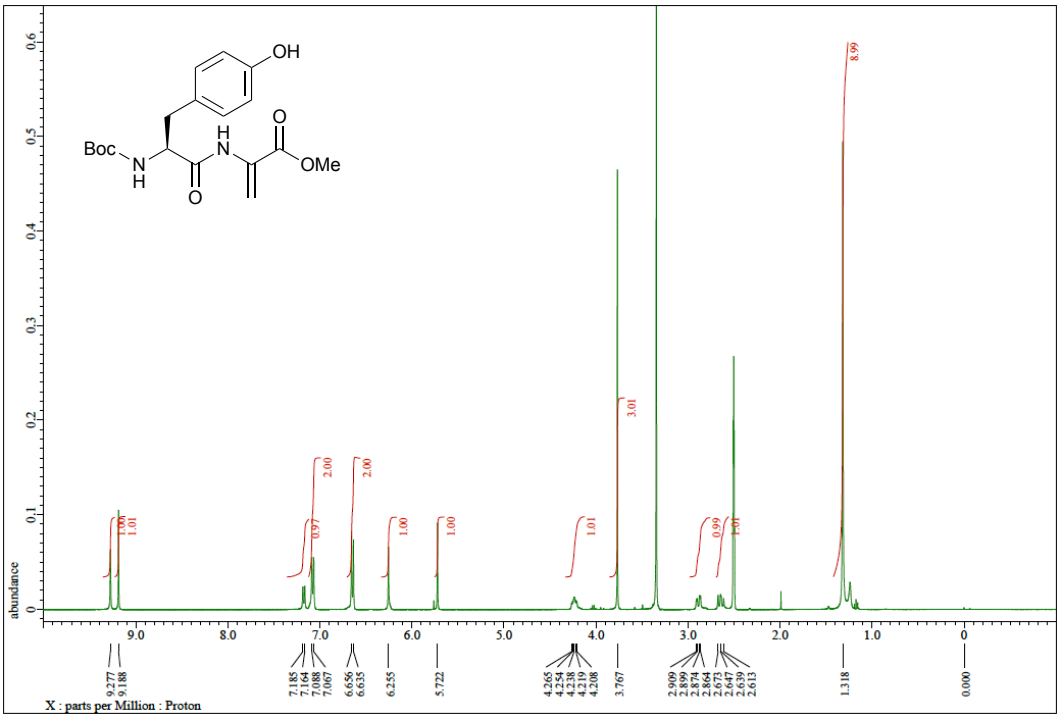


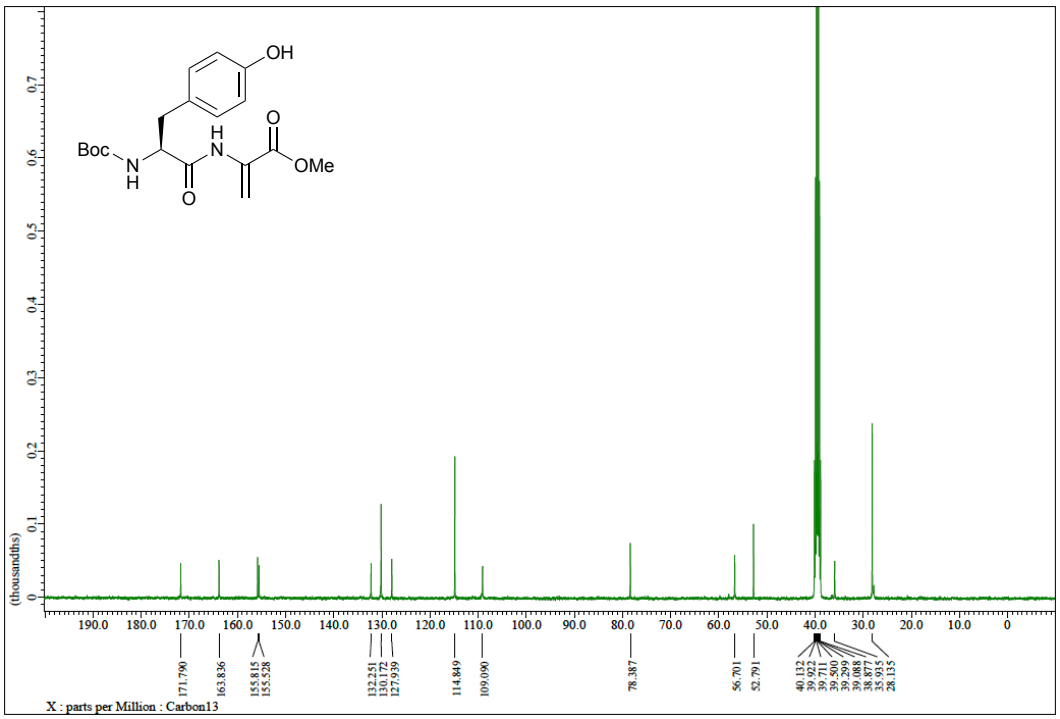
2d: ^{1}H and ^{13}C NMR ($CDCl_3$)



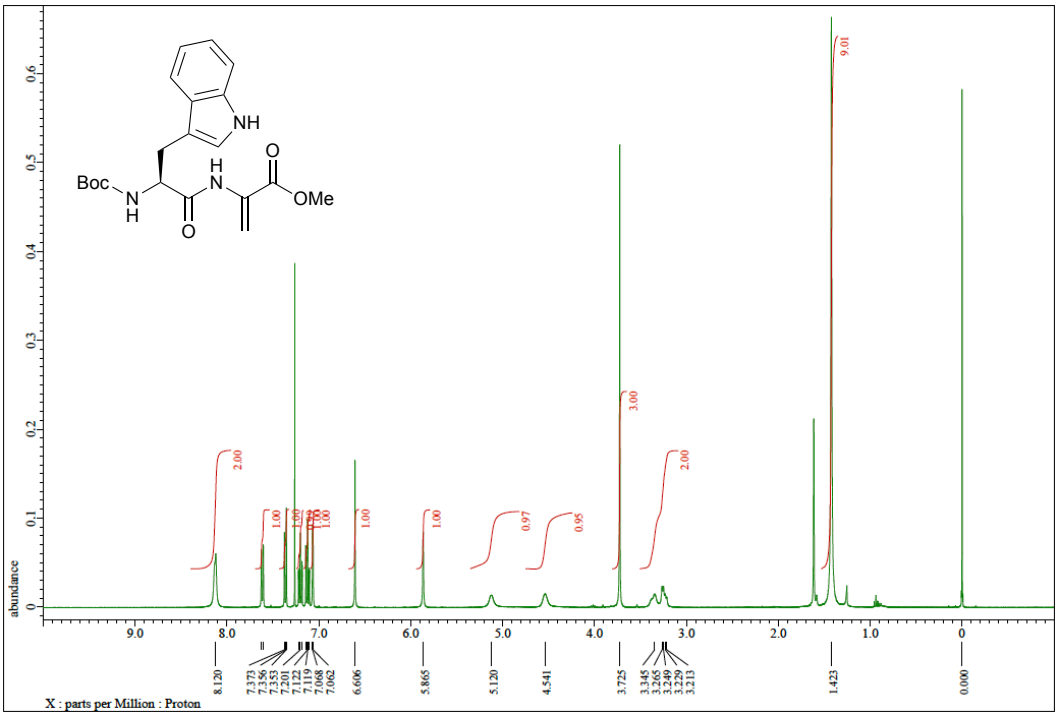


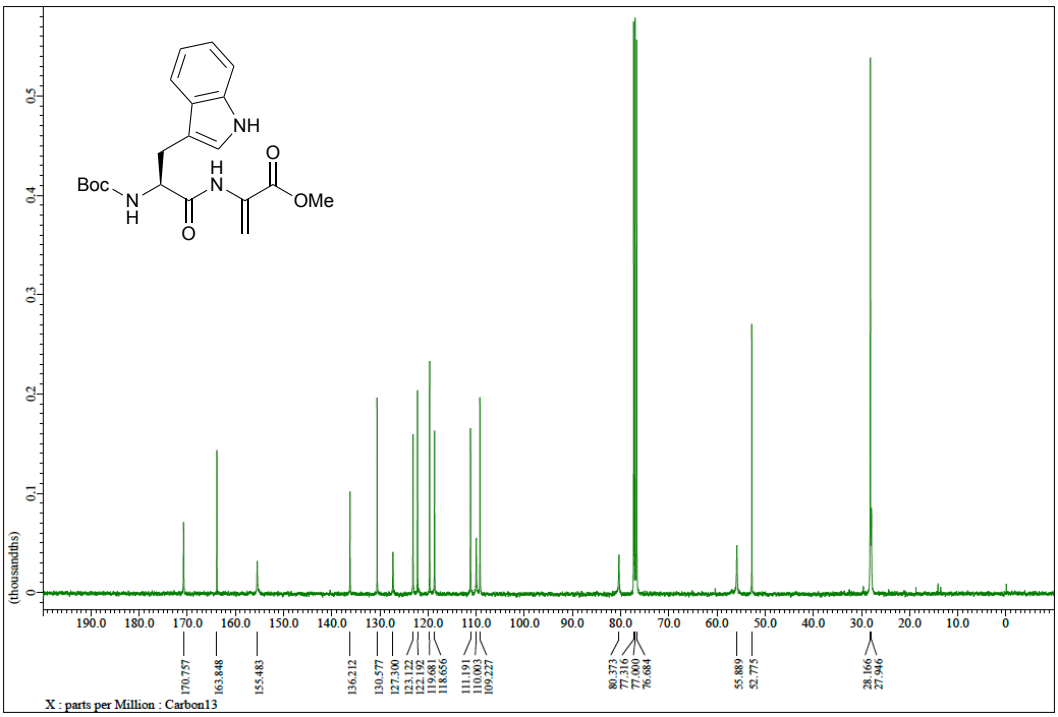
2e: ^{1}H and ^{13}C NMR (DMSO-d_6)



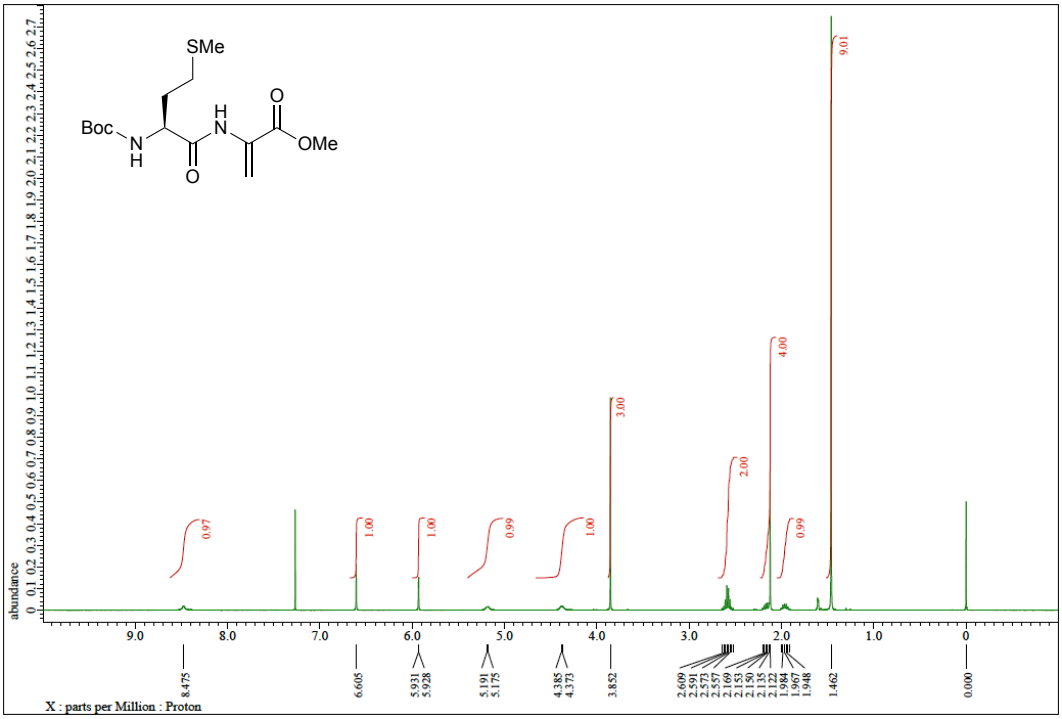


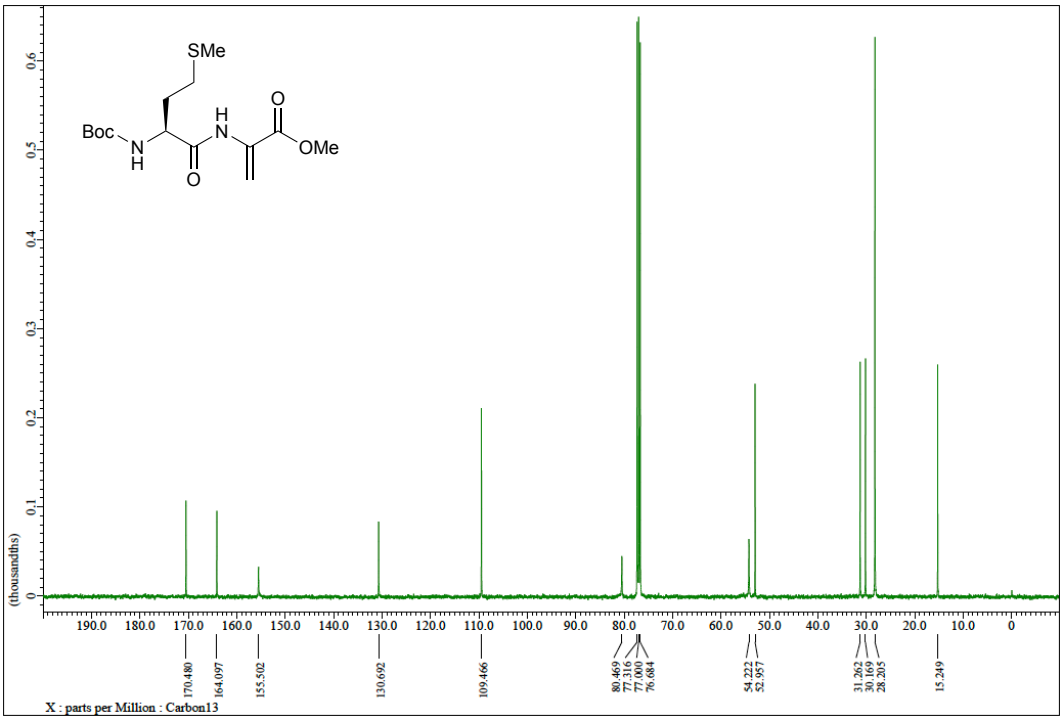
2f: ^{1}H and ^{13}C NMR ($CDCl_3$)



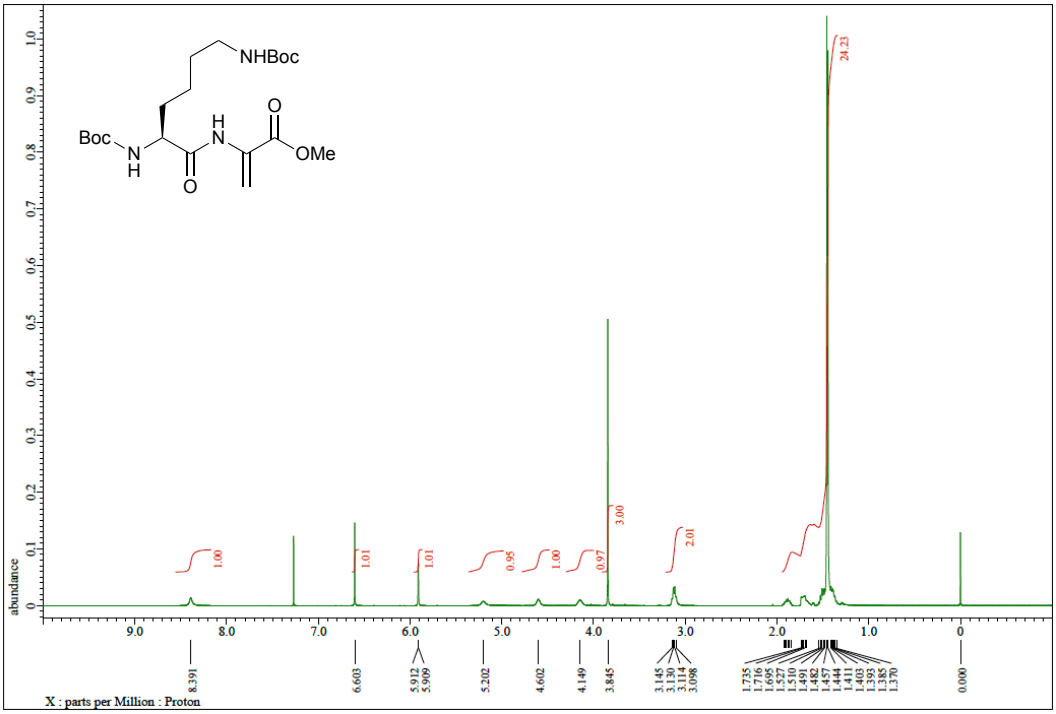


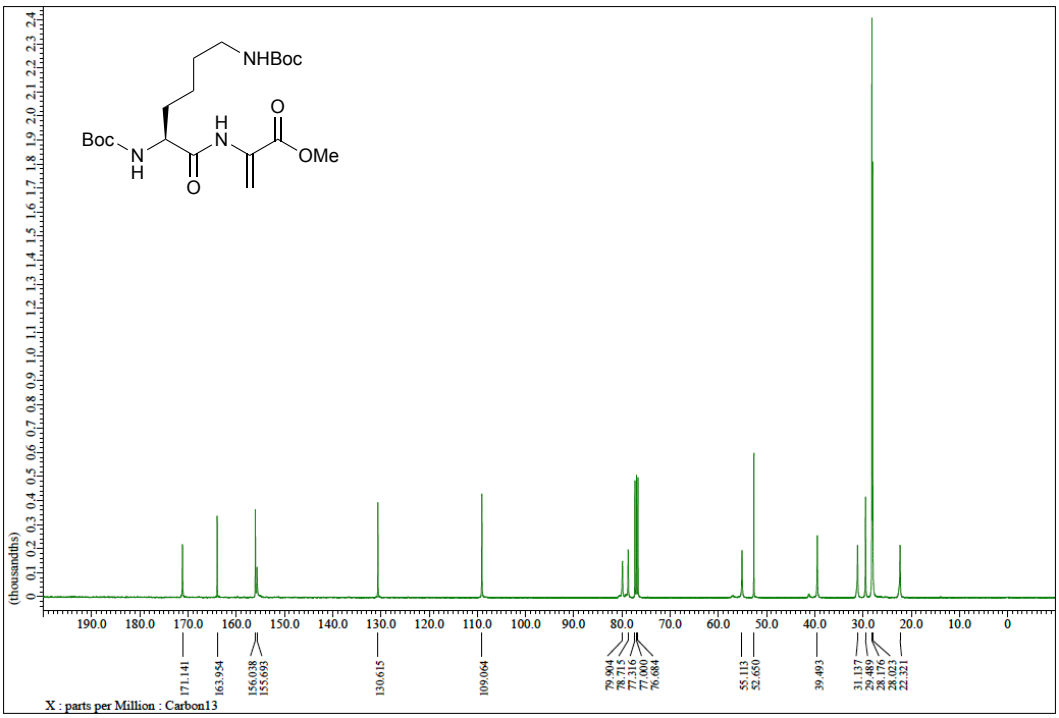
2g: ^{1}H and ^{13}C NMR ($CDCl_3$)



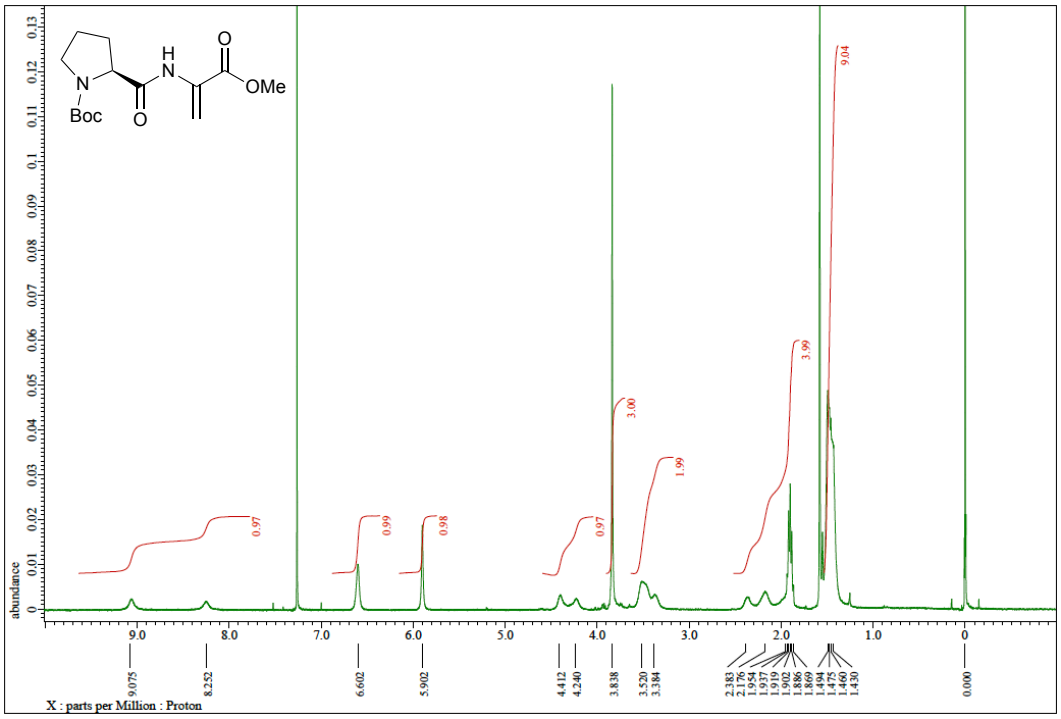


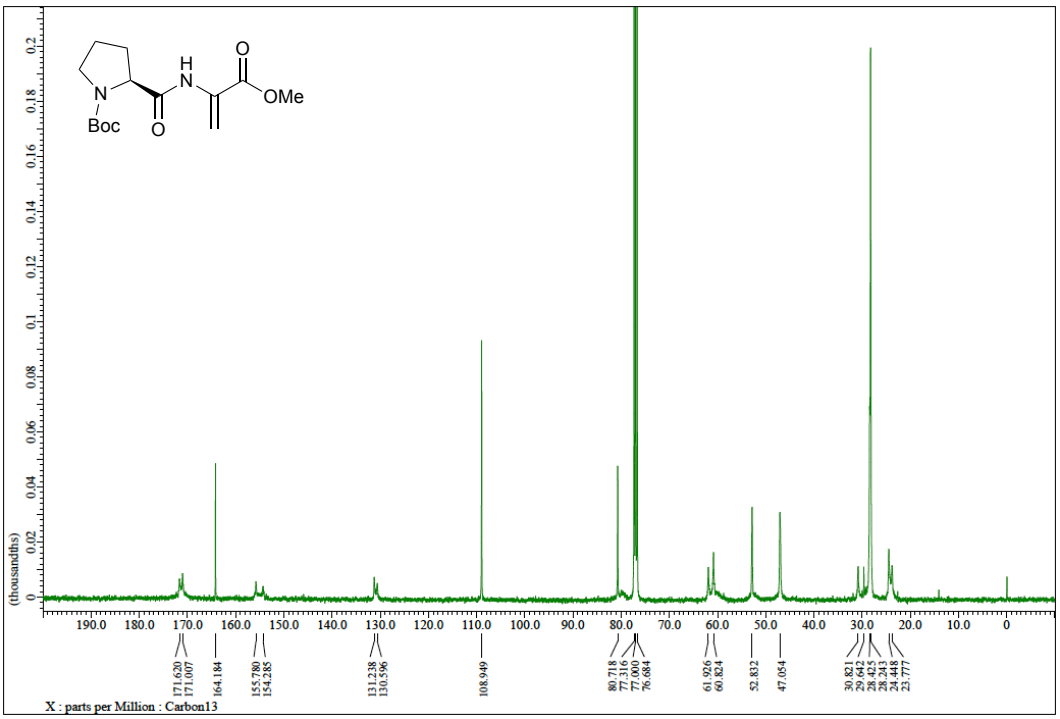
2h: ^{1}H and ^{13}C NMR ($CDCl_3$)



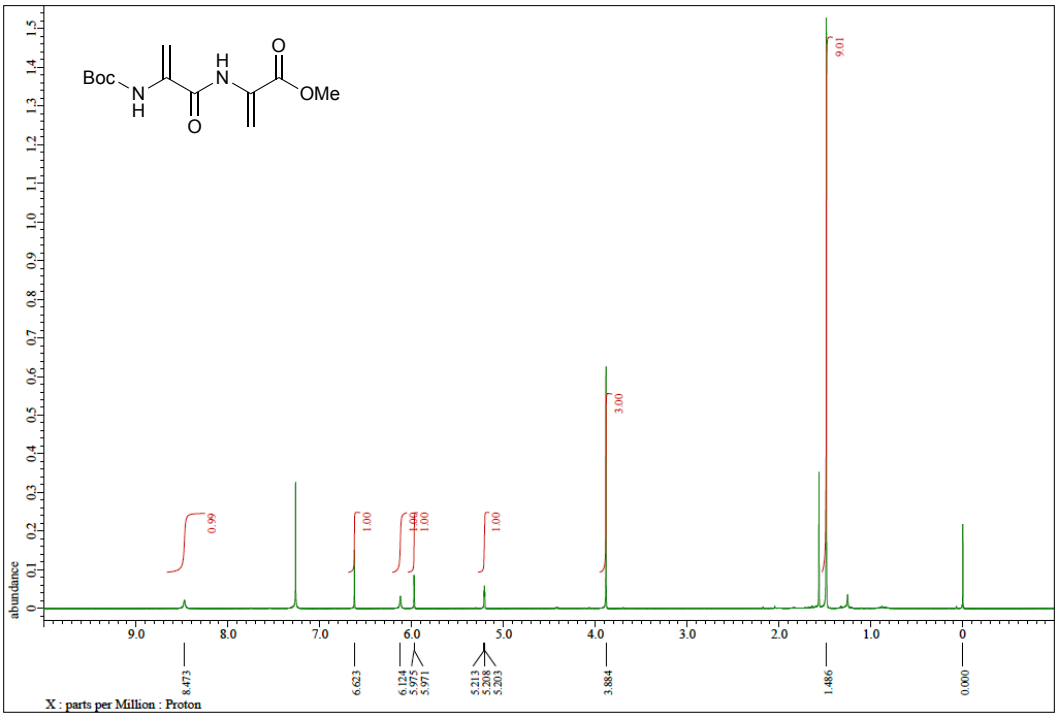


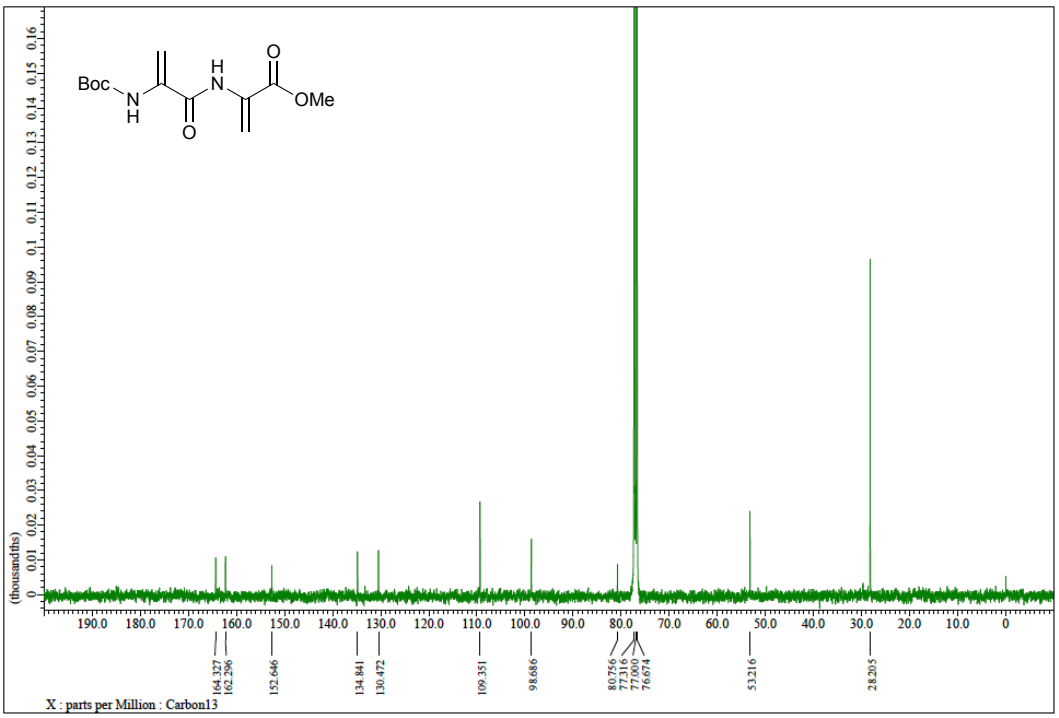
2i: ^{1}H and ^{13}C NMR ($CDCl_3$)



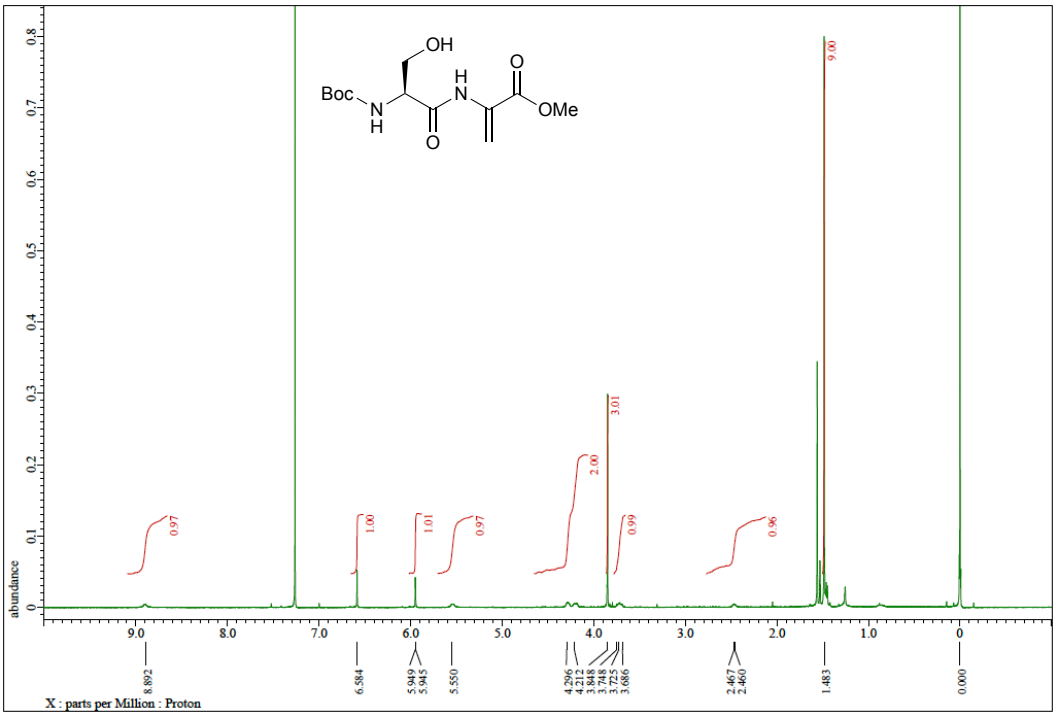


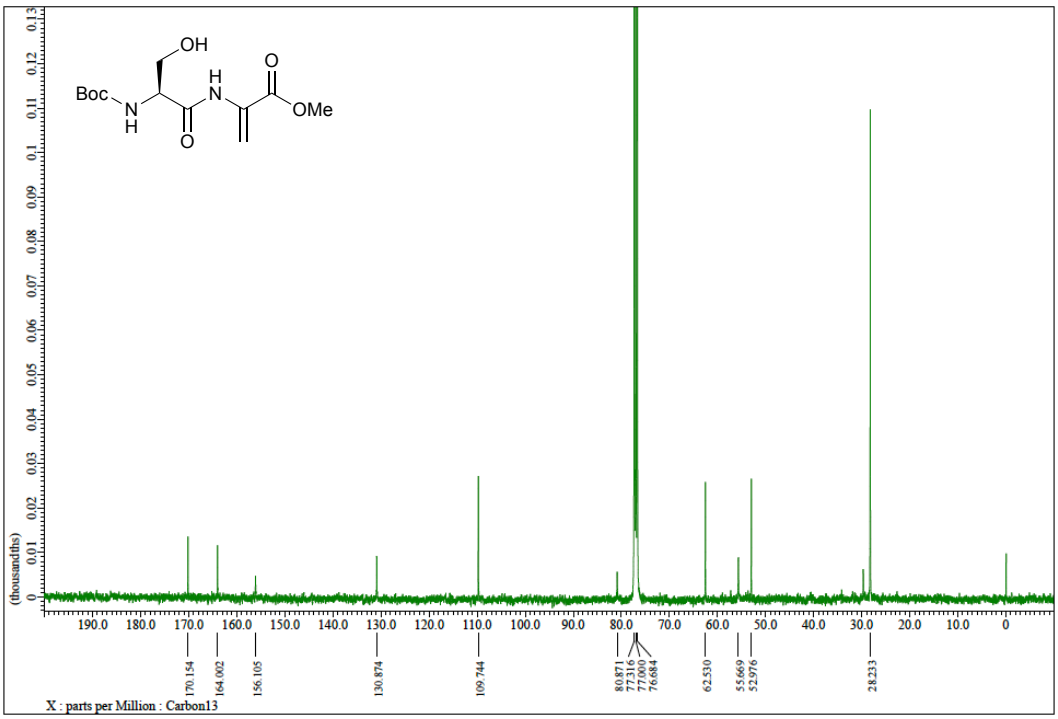
2j: ^{1}H and ^{13}C NMR ($CDCl_3$)



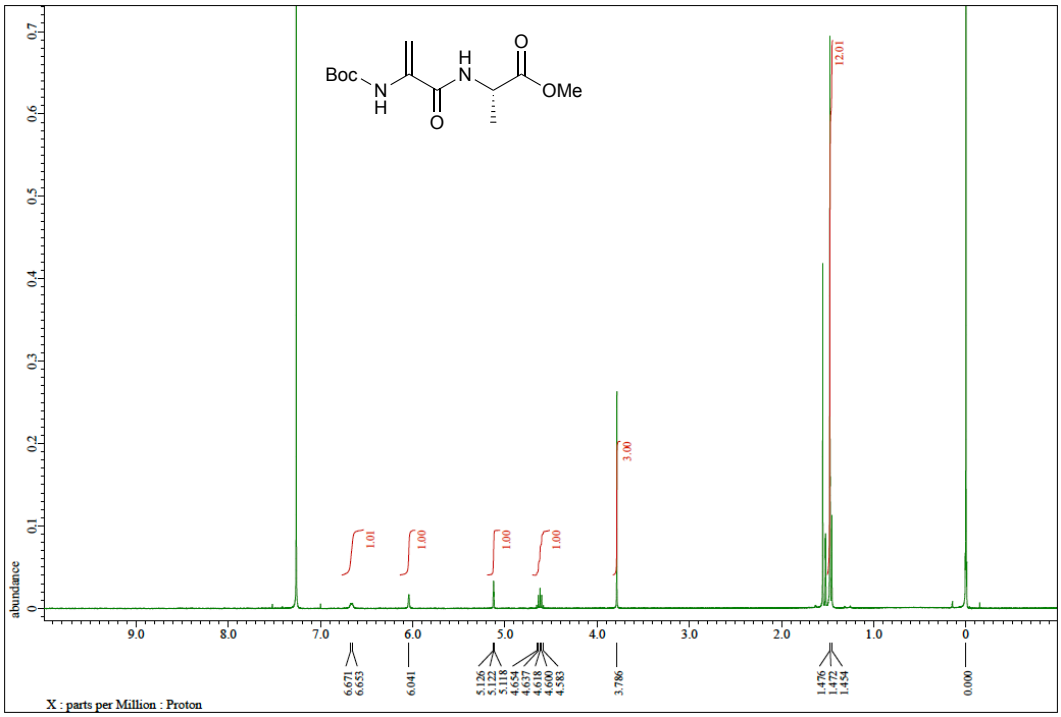


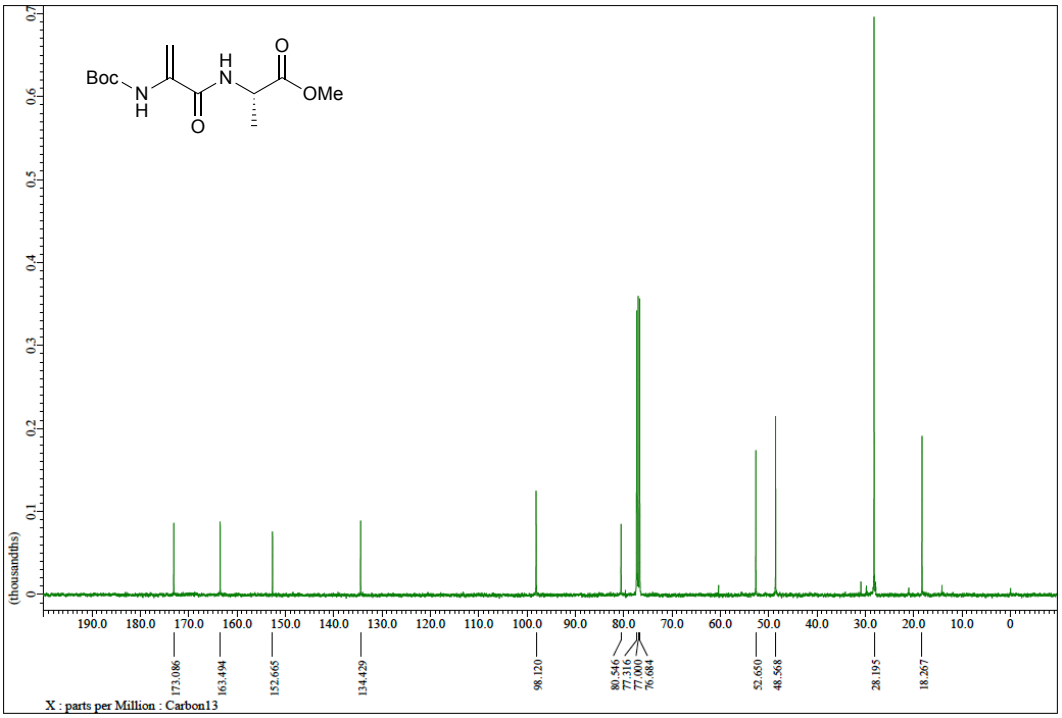
2k: ^{1}H and ^{13}C NMR ($CDCl_3$)



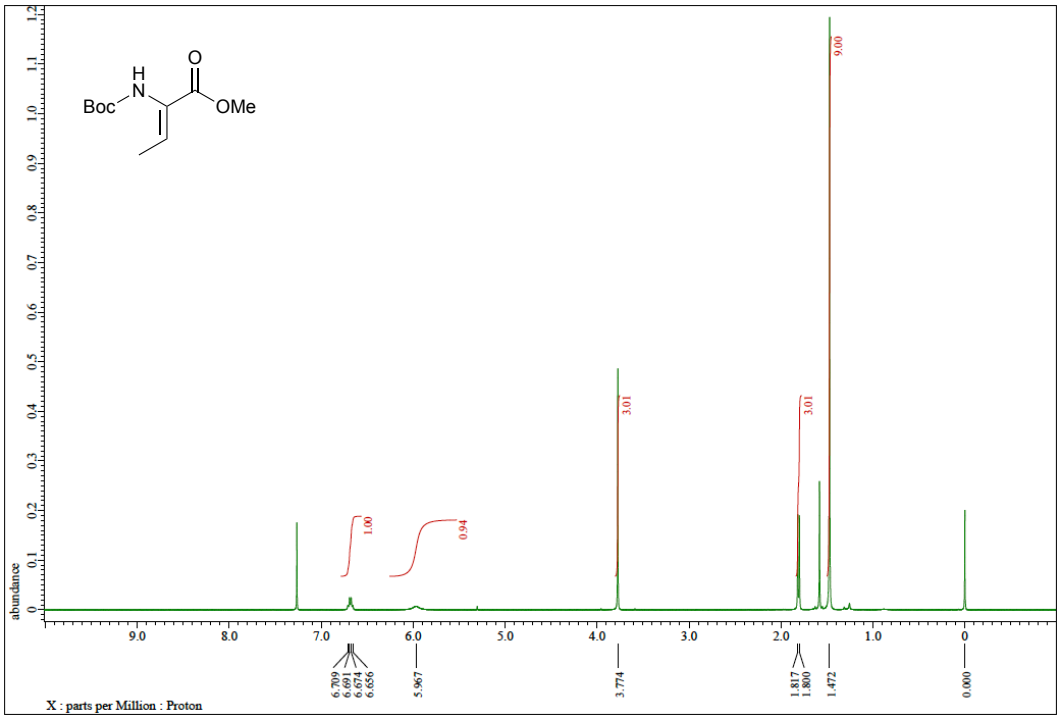


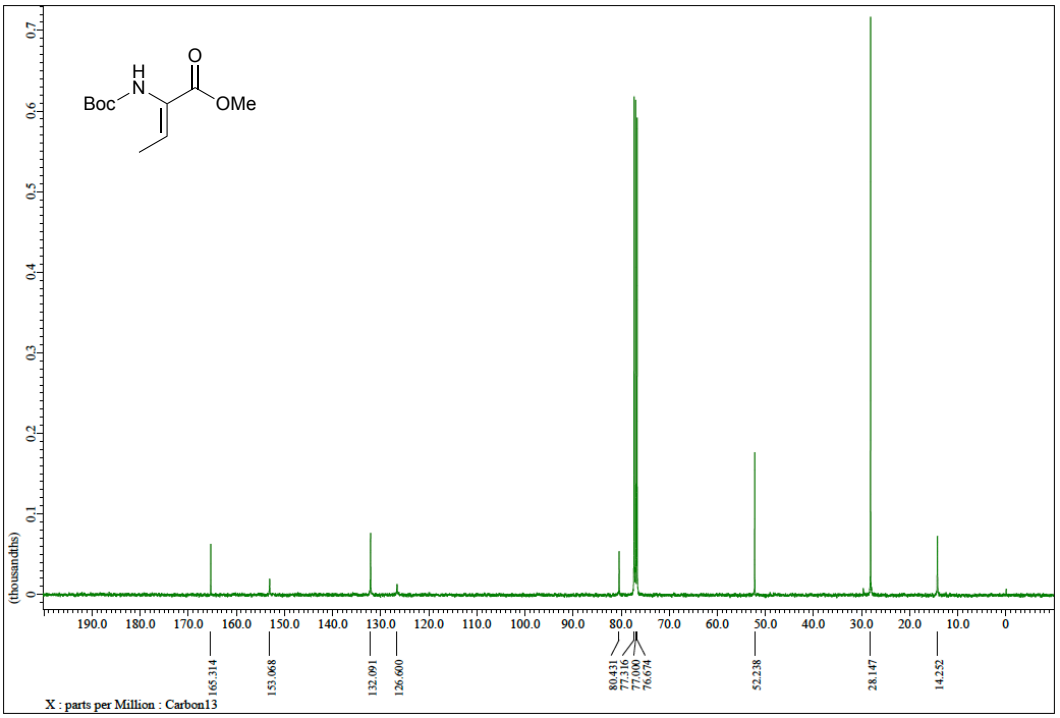
4: ^{1}H and ^{13}C NMR ($CDCl_3$)



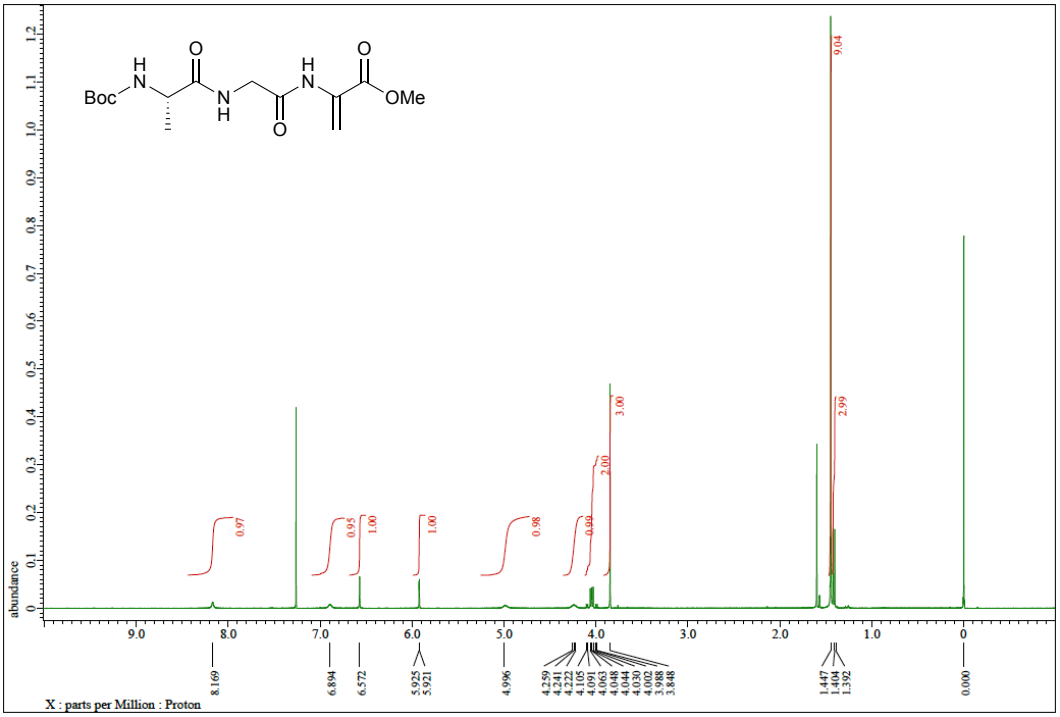


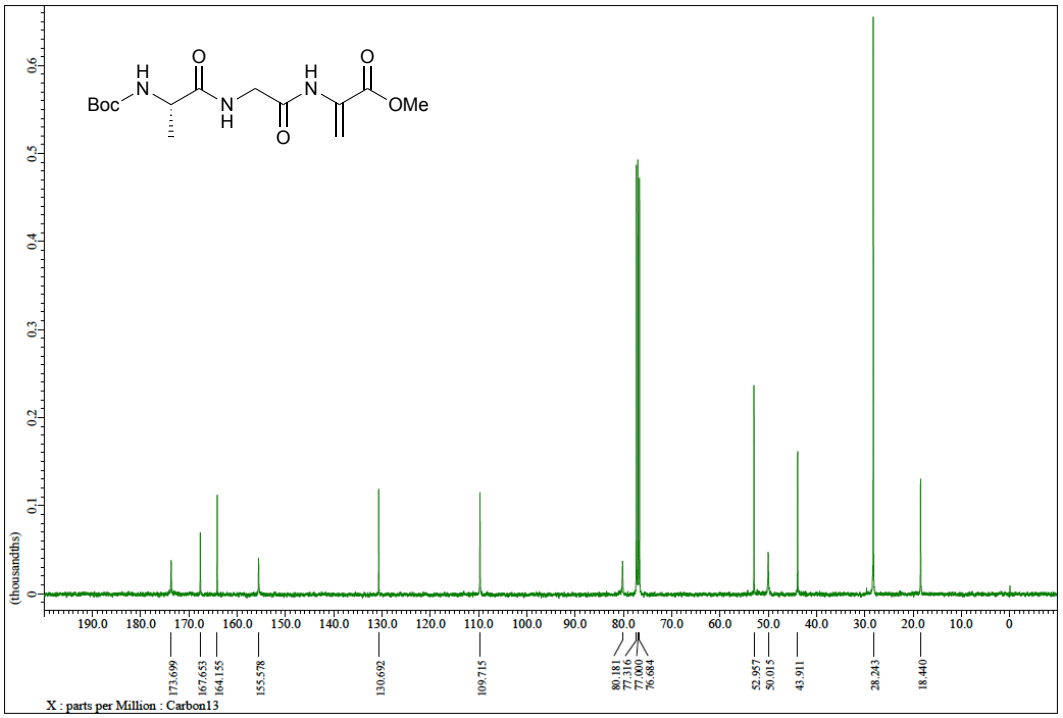
(*Z*)-**7**: ^{1}H and ^{13}C NMR ($CDCl_3$)



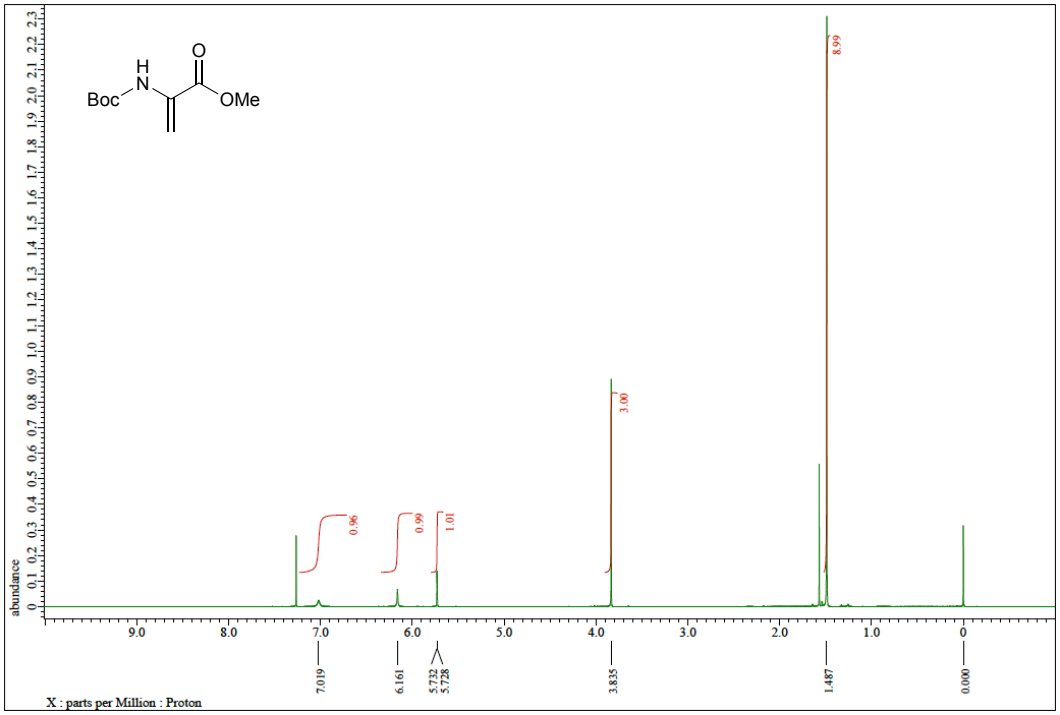


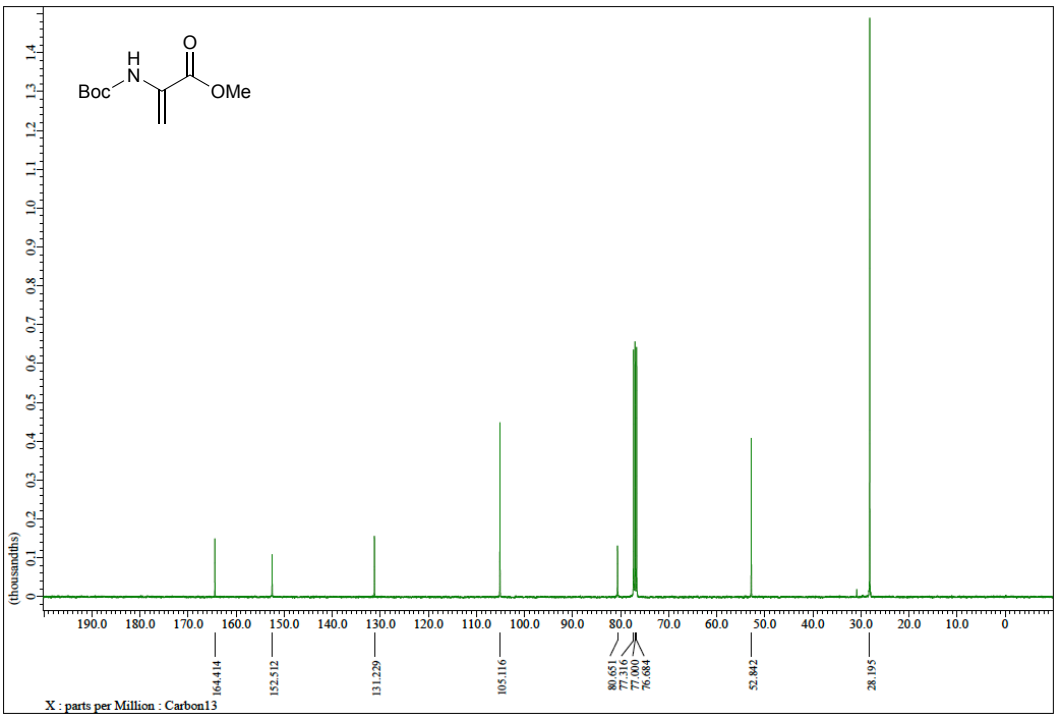
9: ^{1}H and ^{13}C NMR ($CDCl_3$)



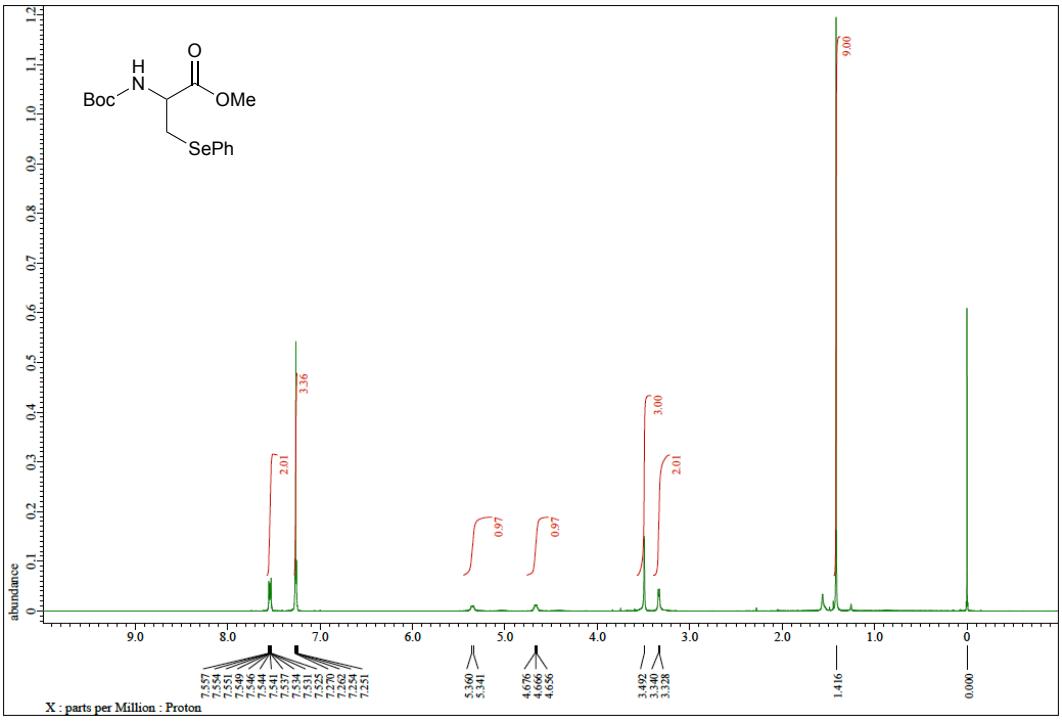


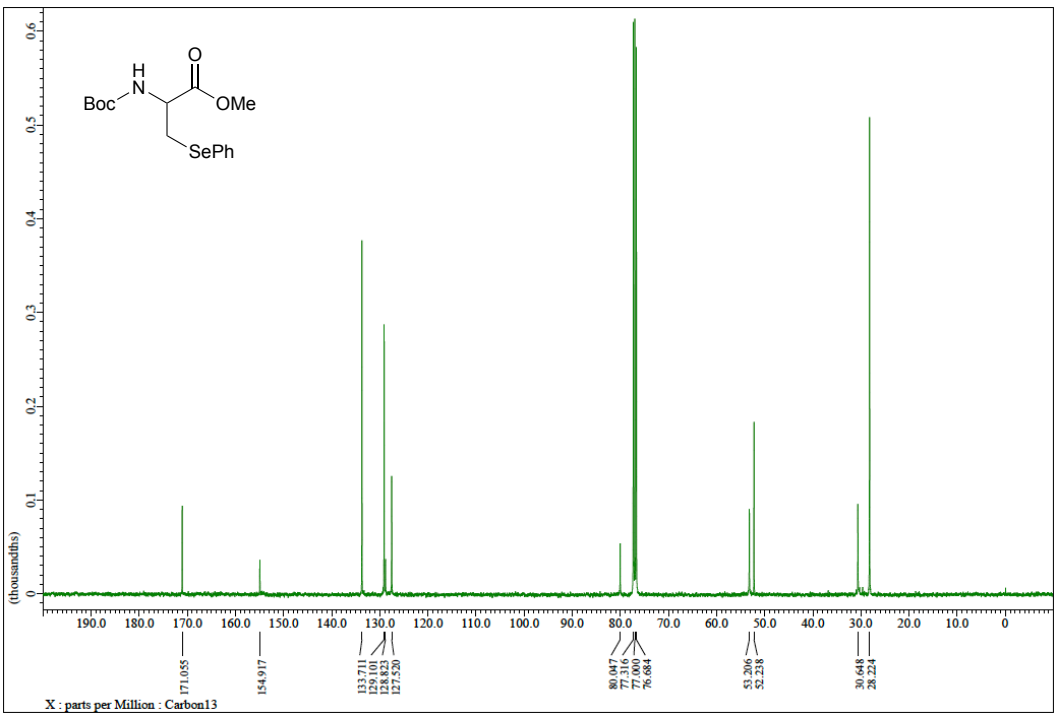
11: ^{1}H and ^{13}C NMR ($CDCl_3$)



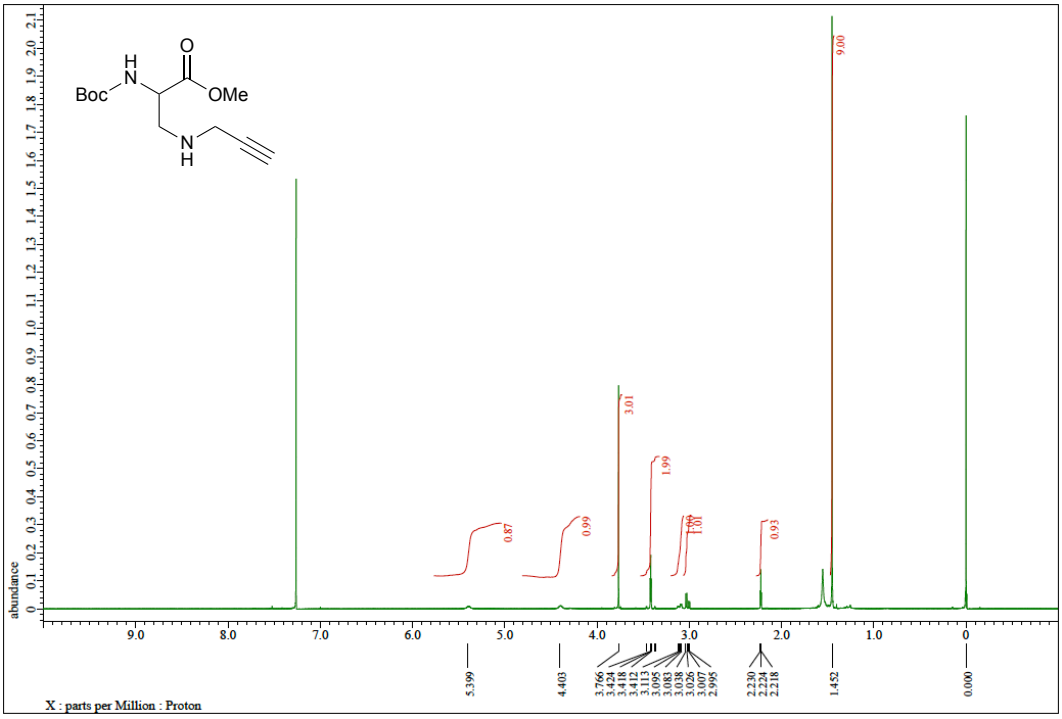


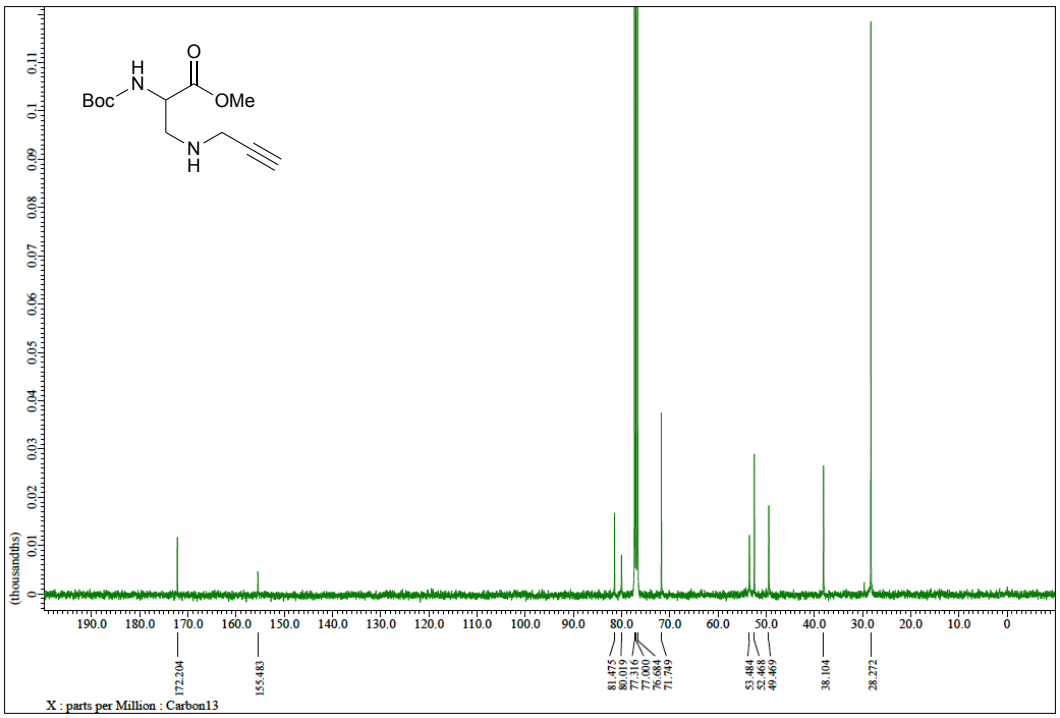
12: ^{1}H and ^{13}C NMR ($CDCl_3$)



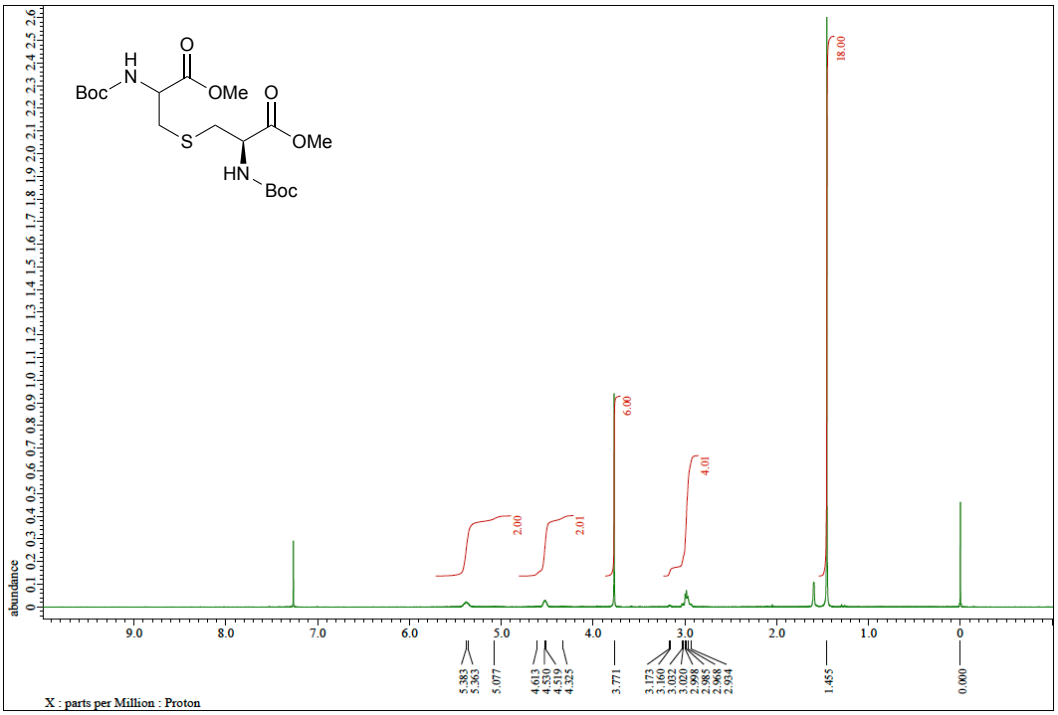


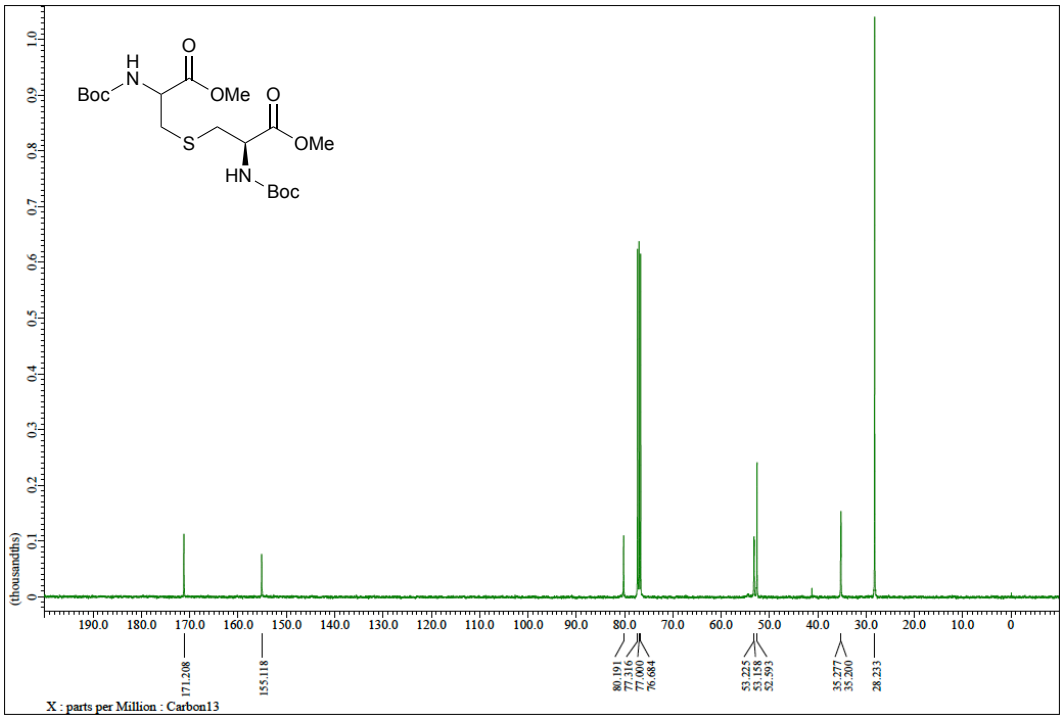
13: ^{1}H and ^{13}C NMR ($CDCl_3$)



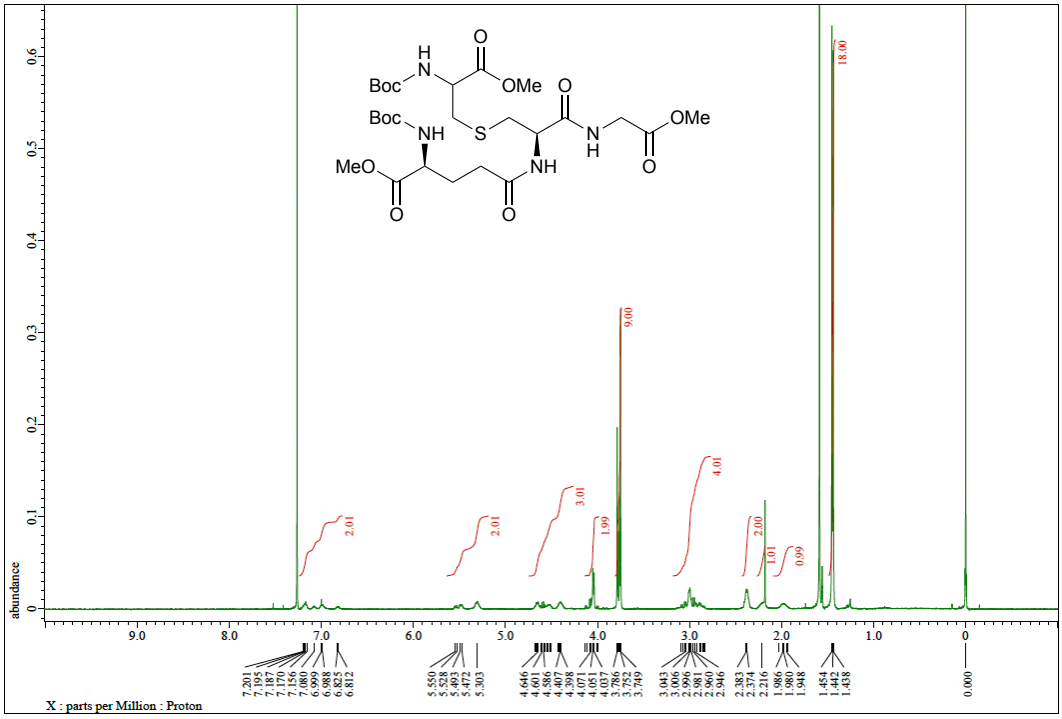


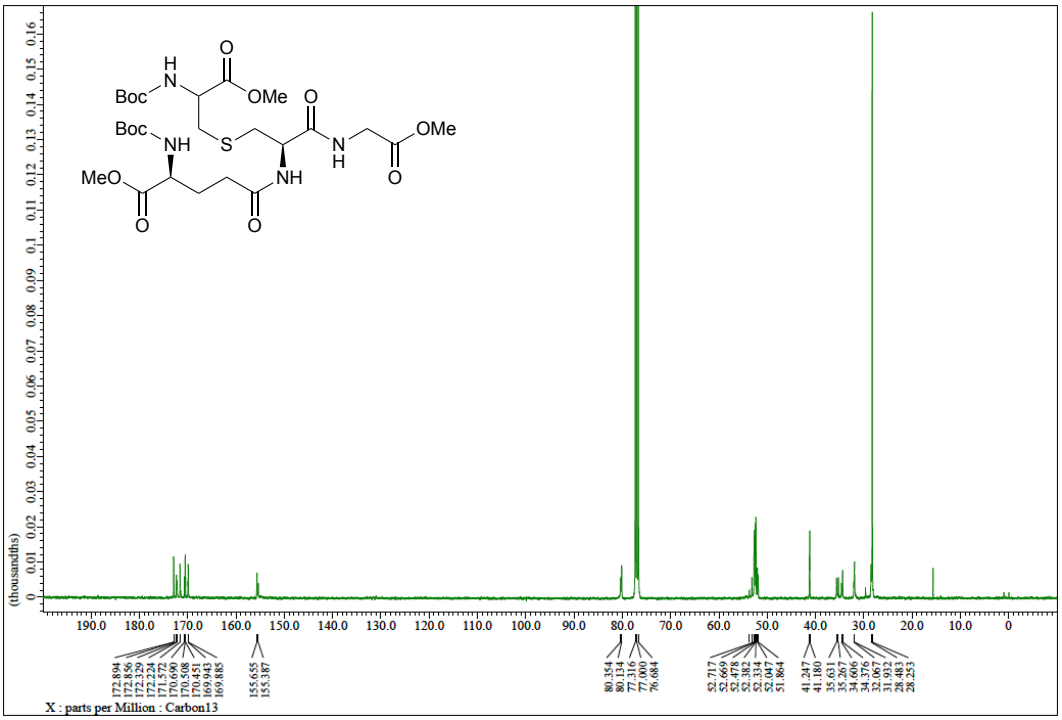
14: ^{1}H and ^{13}C NMR ($CDCl_3$)





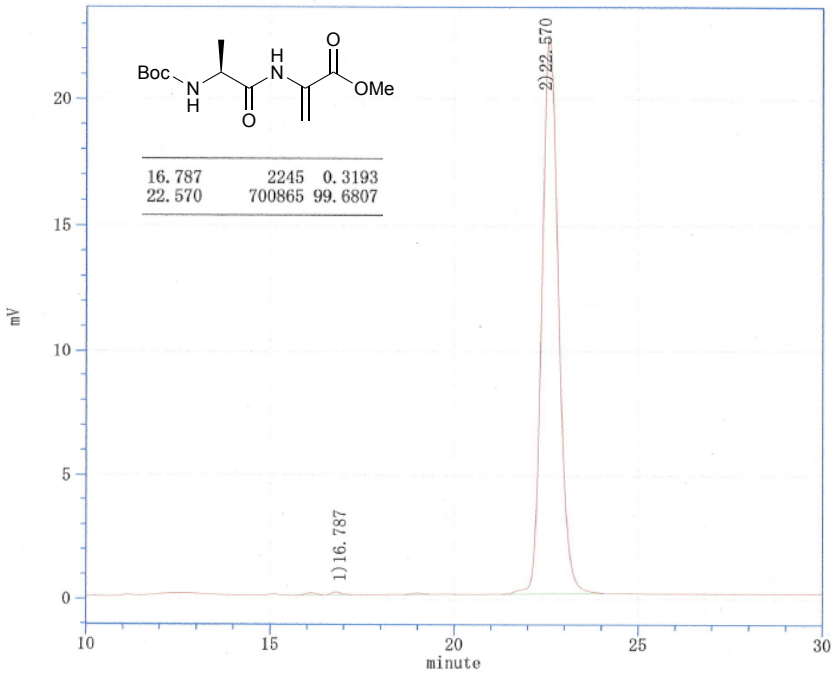
16: ^{1}H and ^{13}C NMR ($CDCl_3$)

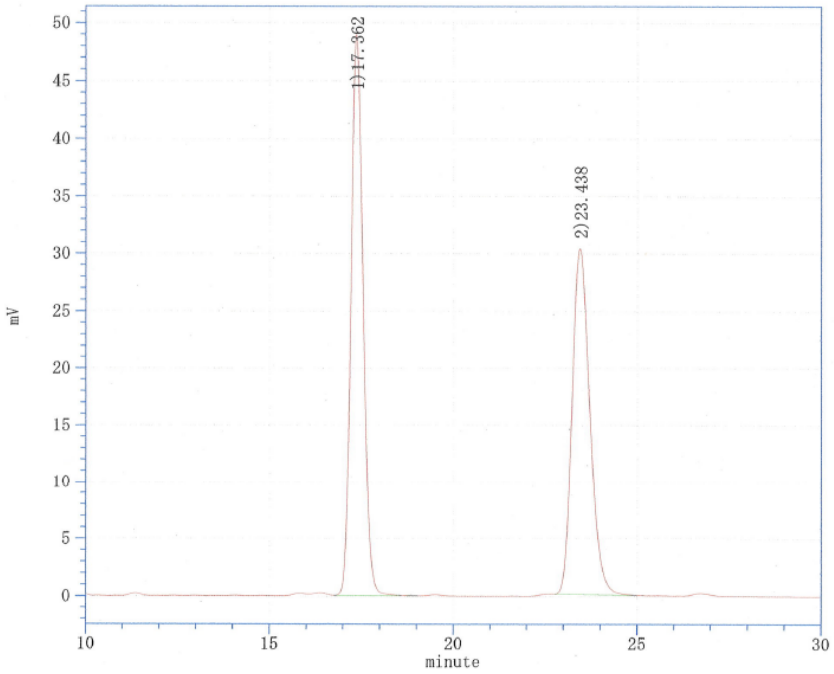




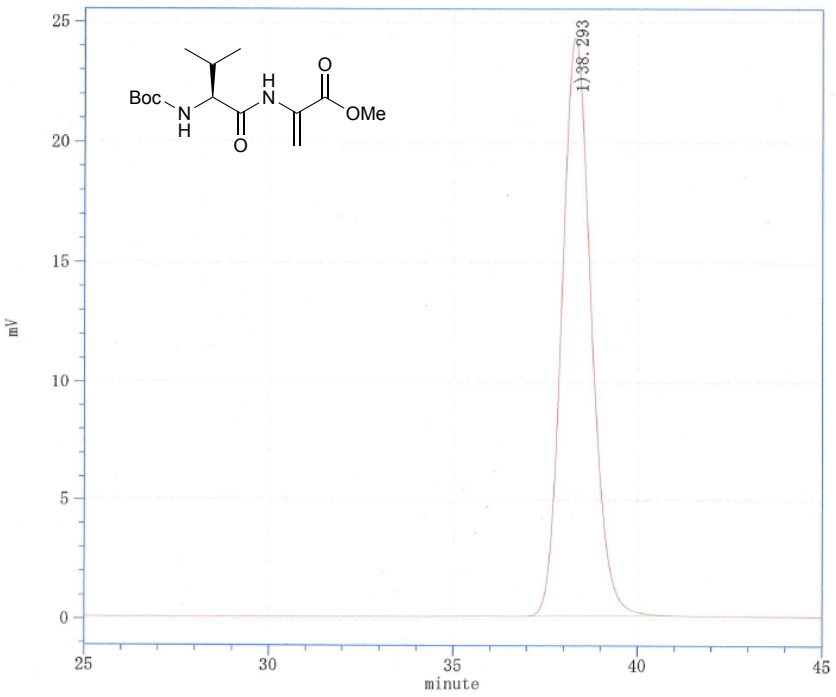
HPLC Charts

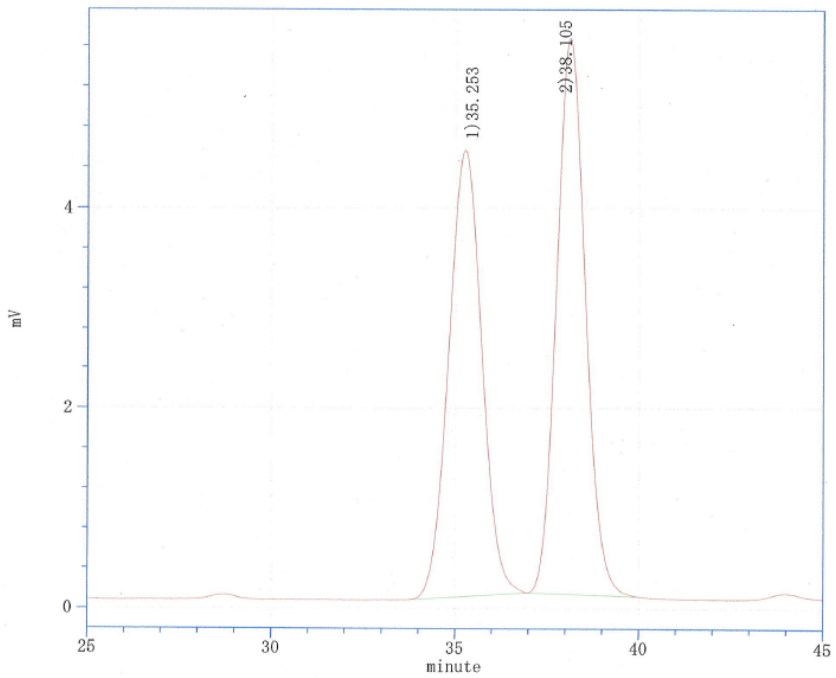
2a: Daicel Chiralpak IE-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm



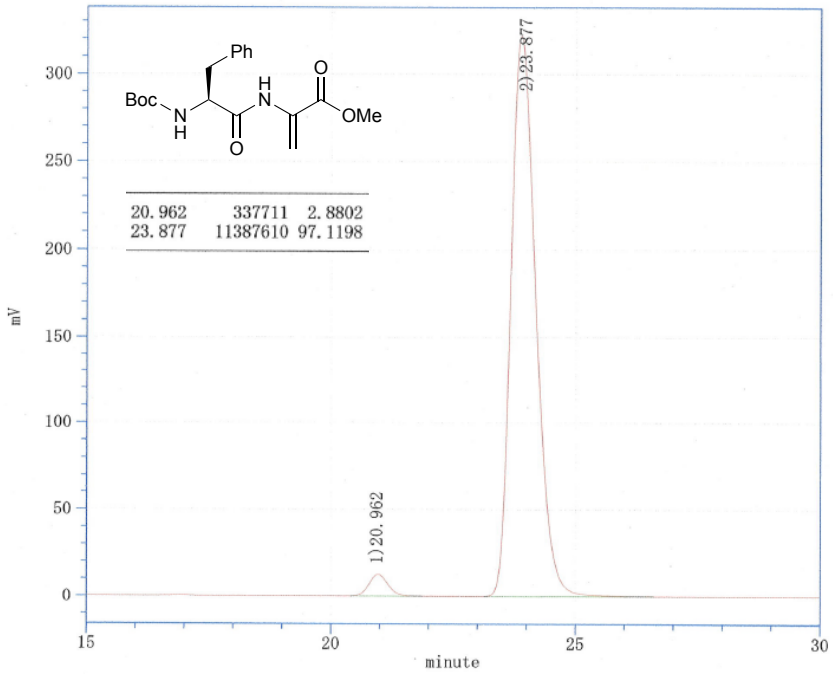


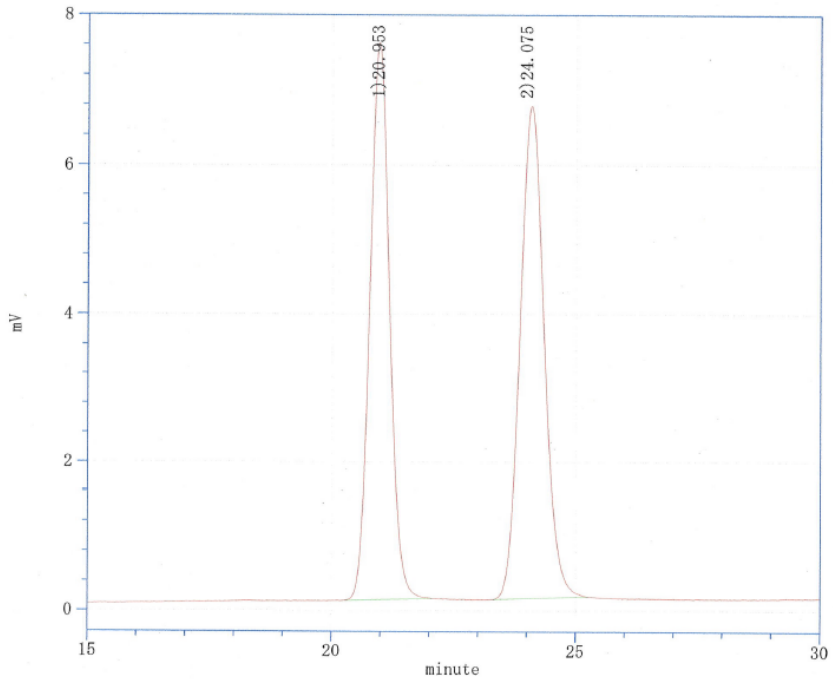
2c: Daicel Chiralpak IE-3, hexane/2-propanol = 5:1, flow rate = 0.5 mL/min, 254 nm



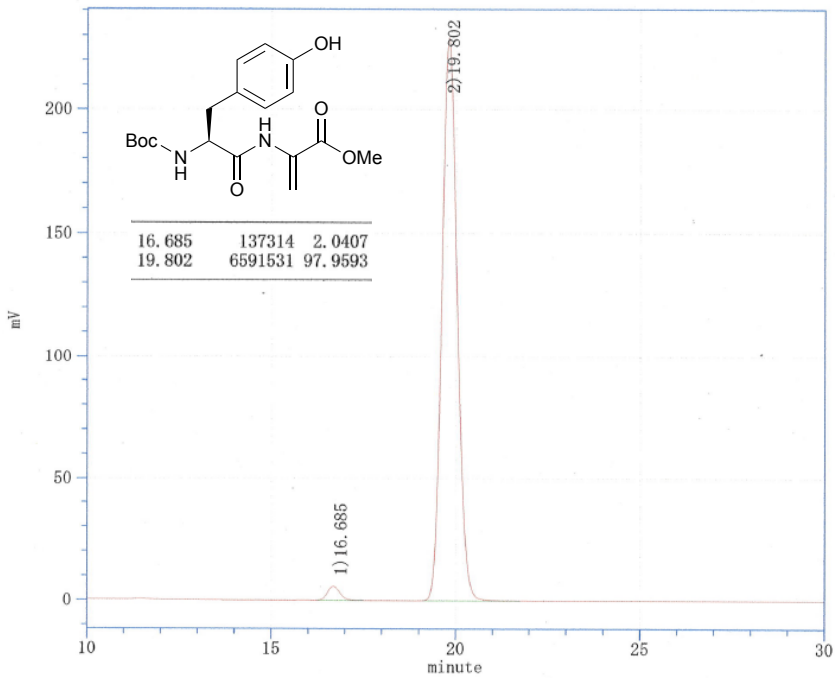


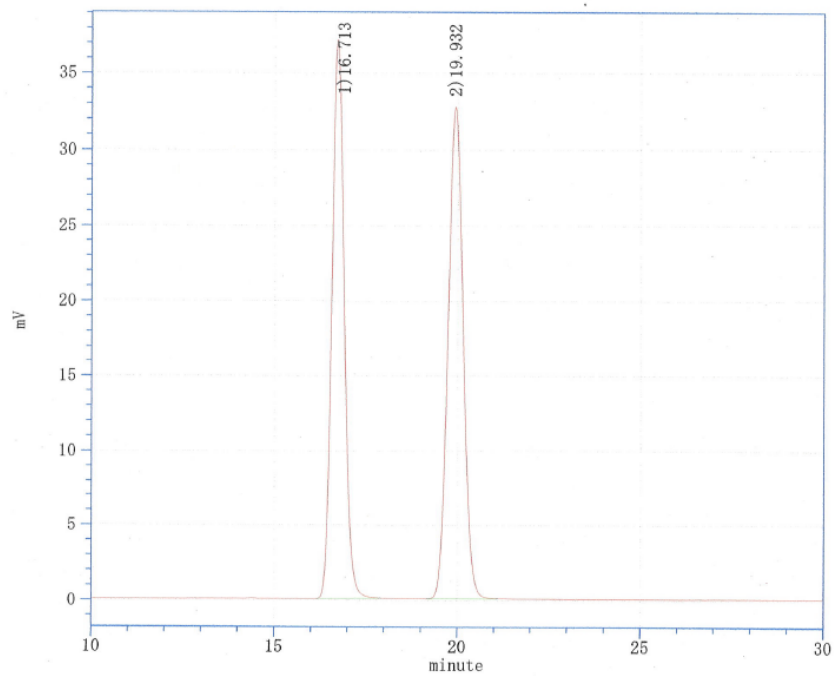
2d: Daicel Chiralpak IE-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm



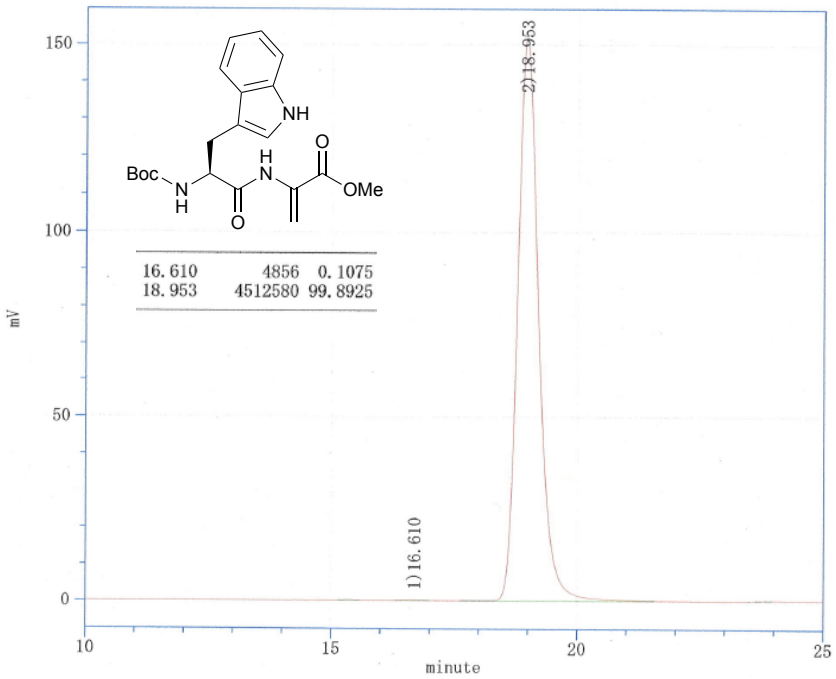


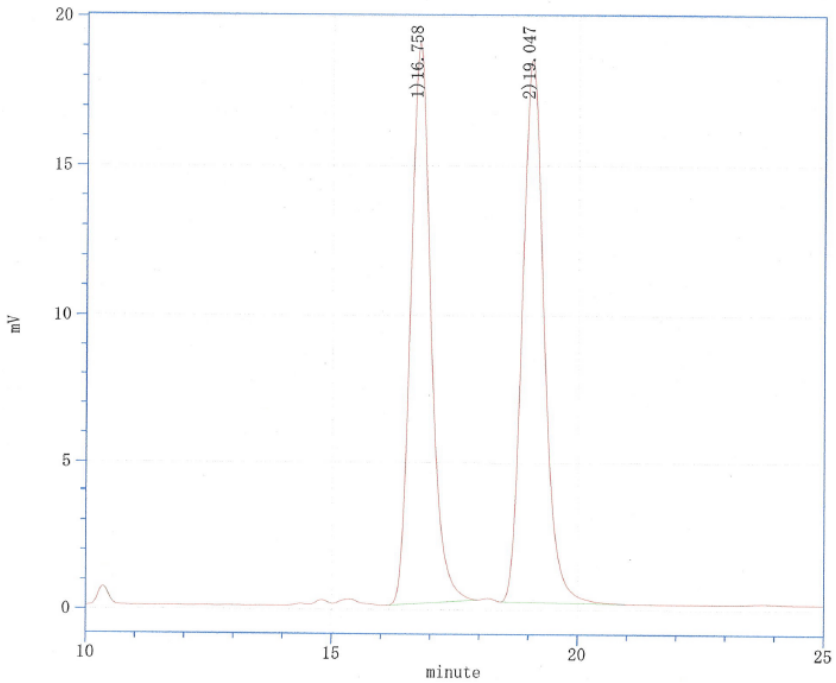
2e: Daicel Chiralpak AD-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm



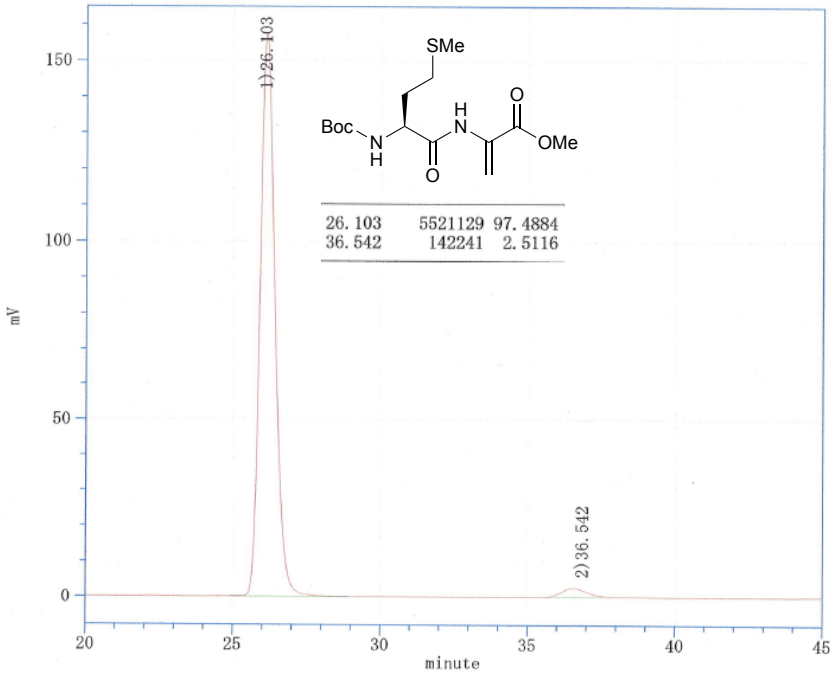


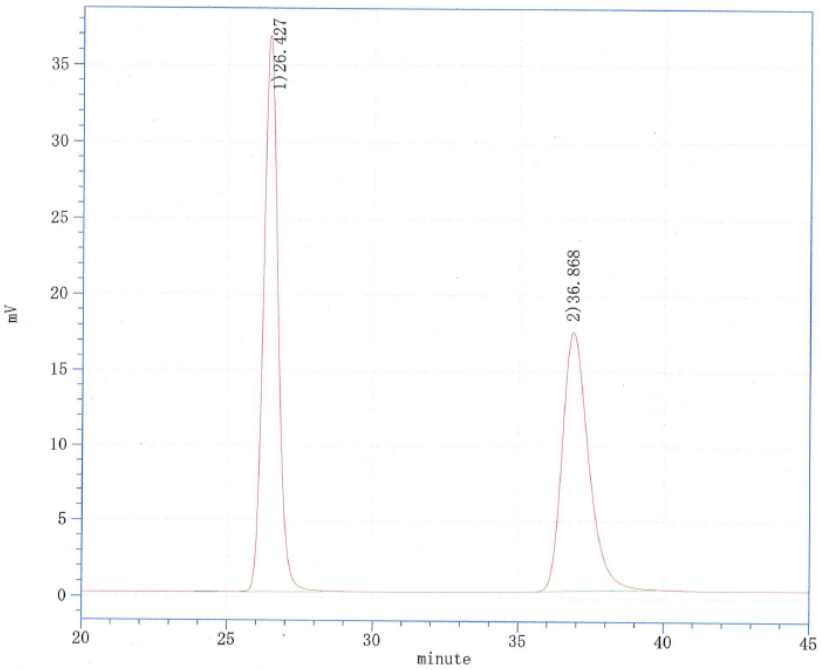
2f: Daicel Chiralpak IE-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm



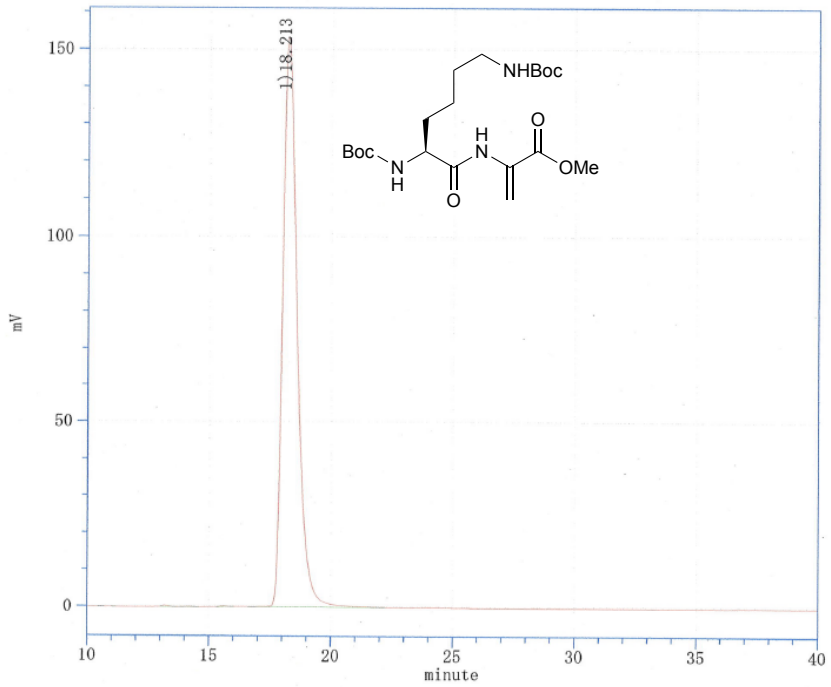


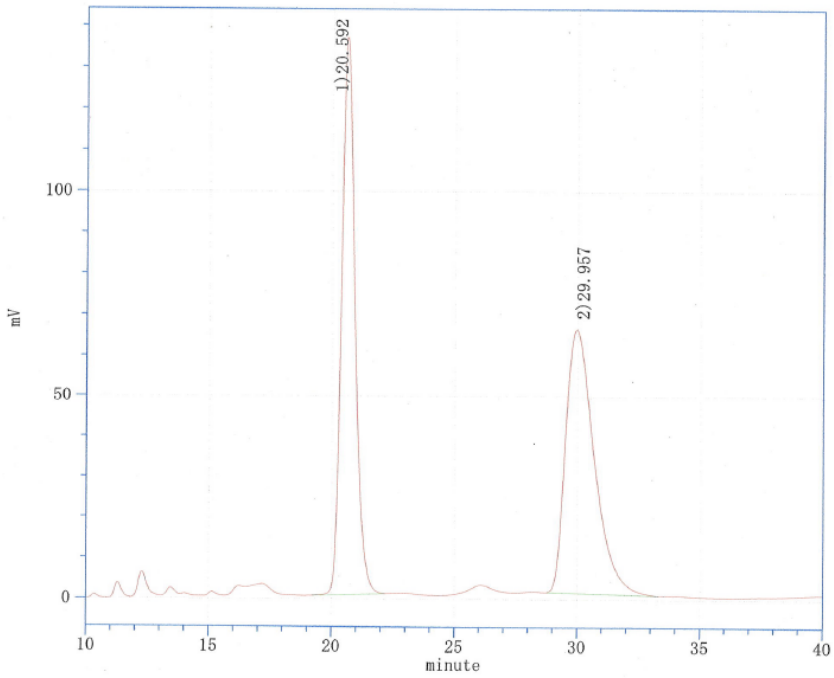
2g: Daicel Chiralpak IE-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm



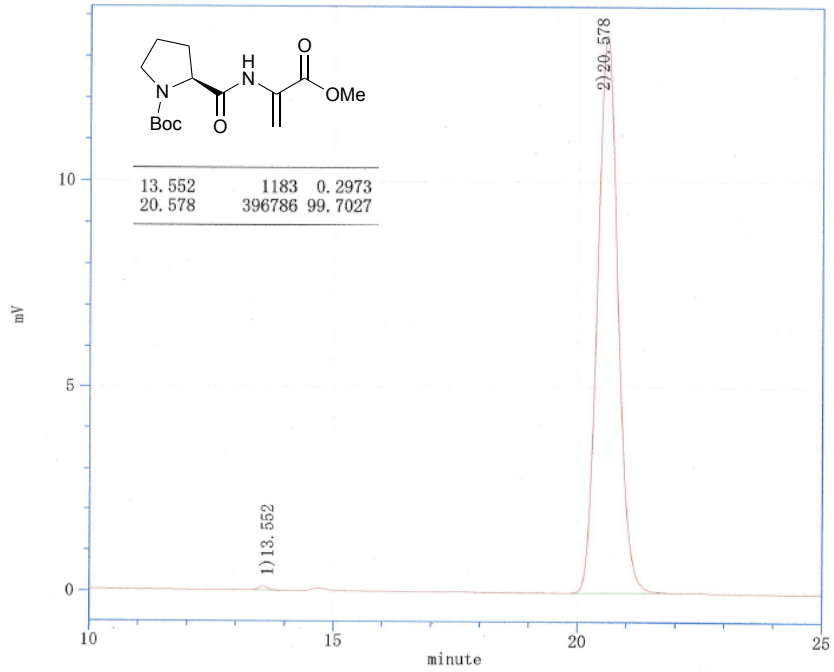


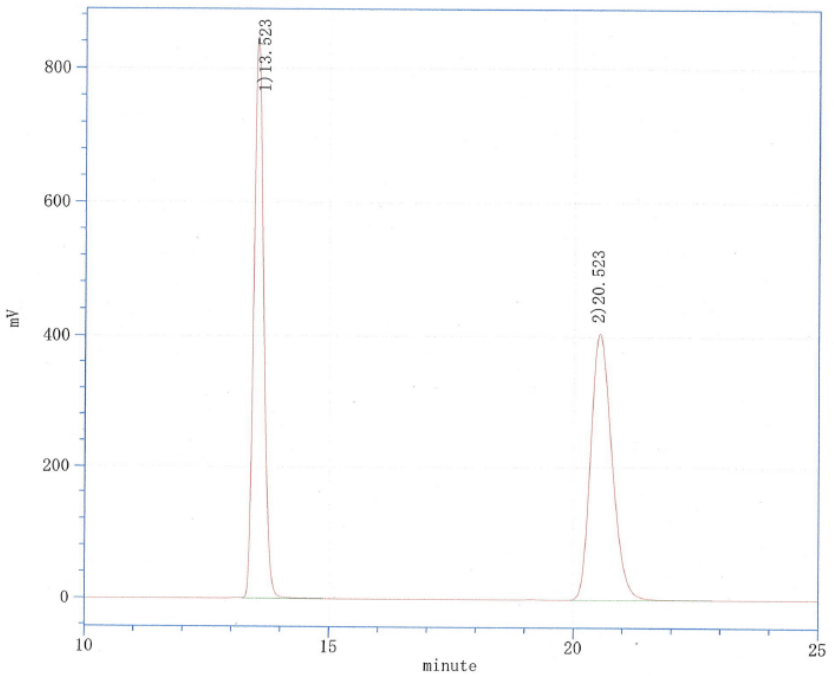
2h: Daicel Chiralpak IE-3, hexane/2-propanol = 1:1, flow rate = 0.5 mL/min, 254 nm



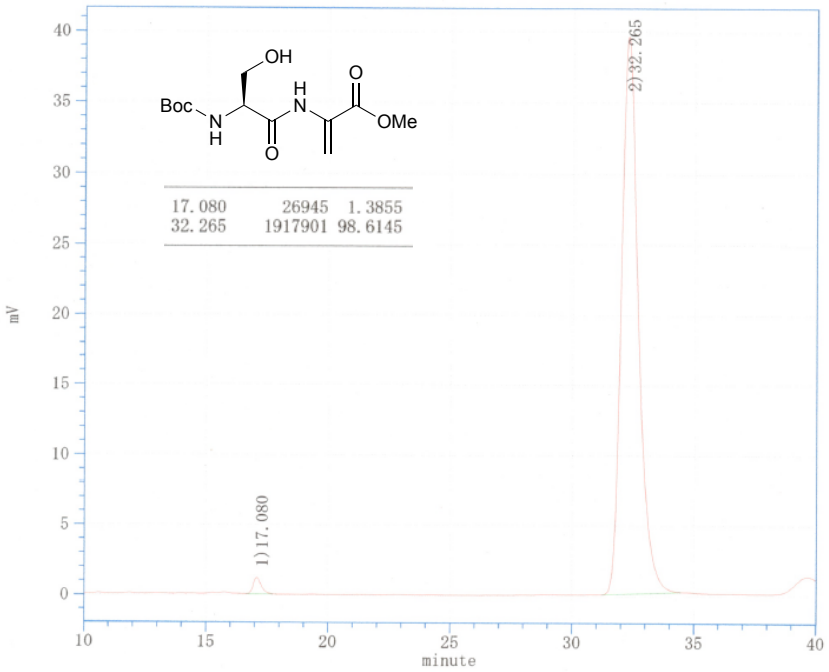


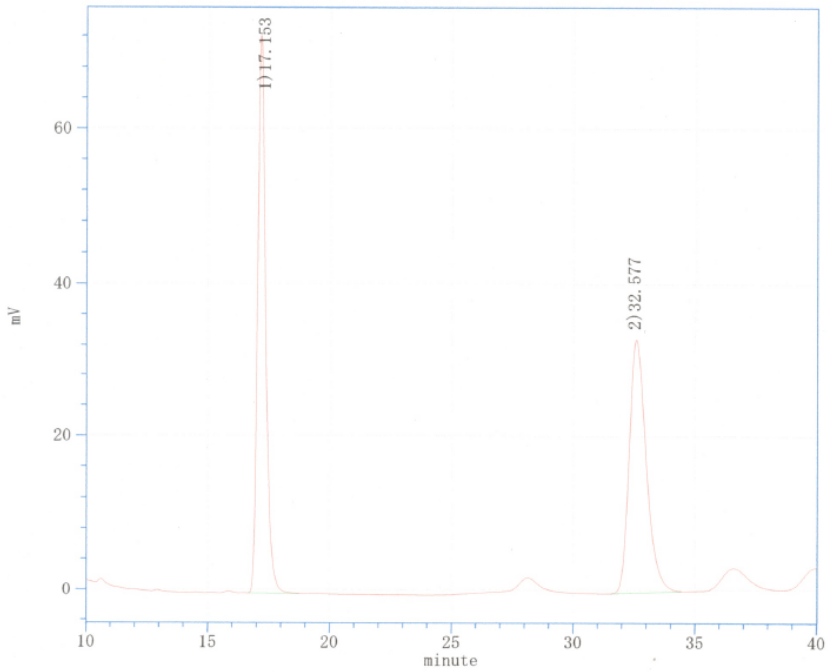
2i: Daicel Chiralpak ID-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm



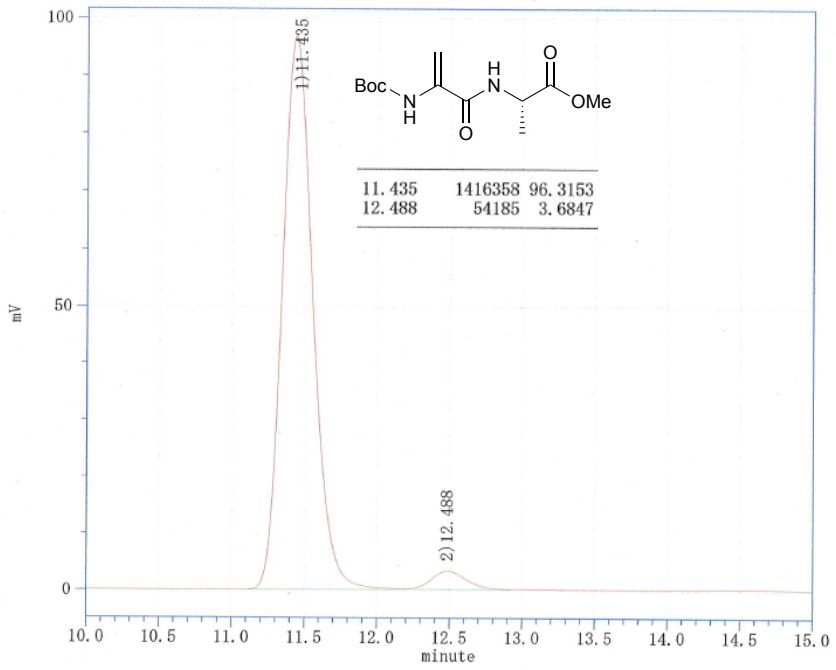


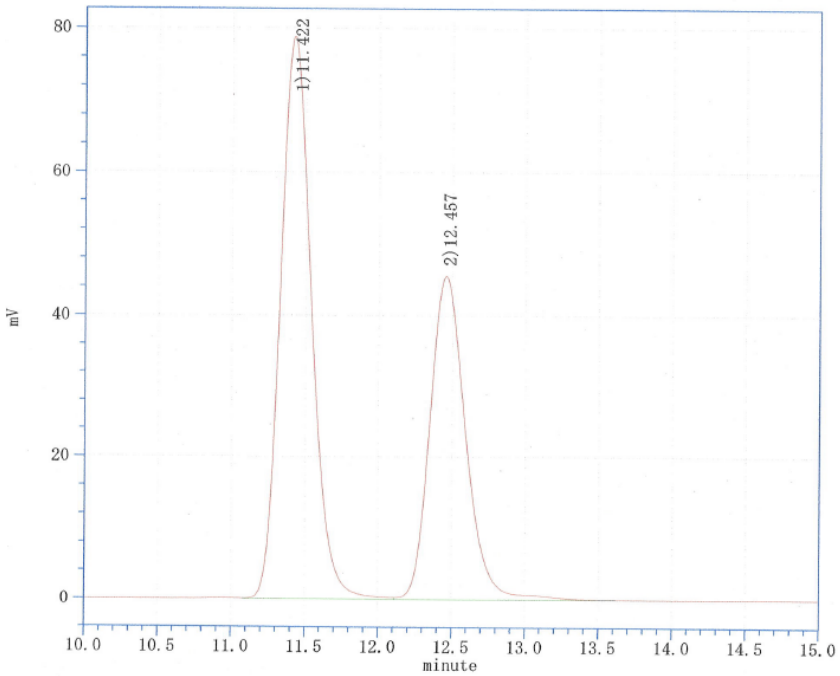
2k: Daicel Chiralpak IE-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm





4: Daicel Chiralpak IE-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm





9: Daicel Chiralpak IE-3, hexane/2-propanol = 2:1, flow rate = 0.5 mL/min, 254 nm