Supporting Information

Optimized Diazo Scaffold for Protein Esterification

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1. General Experimental

Materials. Silica gel (40 μm; 230–400 mesh) was from SiliCycle. Reagents were obtained from commercial sources and used without further purification. Dichloromethane and tetrahydrofuran were dried over a column of alumina. Thin-layer chromatography (TLC) was performed on plates of EMD 250 μm silica 60-F_{254}.

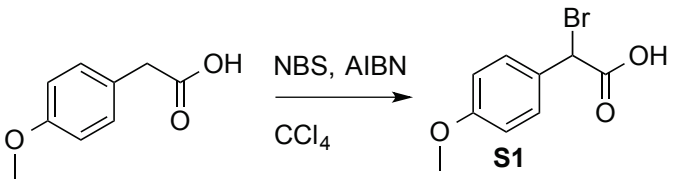
Solvent removal. The phrase "concentrated under reduced pressure" refers to the removal of solvents and other volatile materials using a rotary evaporator at water aspirator pressure (<20 torr) while maintaining a water bath below 40 °C. Residual solvent was removed from samples at high vacuum (<0.1 torr).

NMR spectroscopy. ^{1}H and ^{13}C NMR spectra for all compounds were acquired with Bruker spectrometers in the National Magnetic Resonance Facility at Madison operating at 400, 500, 600, or 750 MHz. Chemical shift data are reported in units of δ (ppm) relative to an internal standard (residual solvent or TMS).

Mass spectrometry. Electrospray ionization (ESI) mass spectrometry for small-molecule characterization was performed with a Micromass LCT at the Mass Spectrometry Facility in the Department of Chemistry at the University of Wisconsin–Madison. Matrix-assisted laser desorption-ionization–time-of-flight (MALDI–TOF) mass spectrometry for protein characterization was performed with a Voyager DE-Pro instrument at the Biophysics Instrumentation Facility at the University of Wisconsin–Madison.

2. Synthesis and Characterization Data

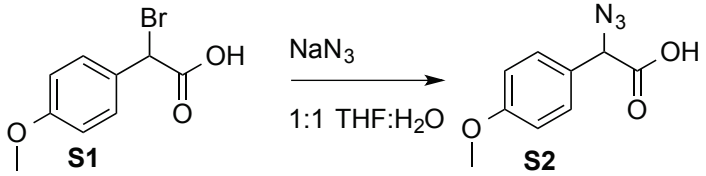
Preparation of α-Bromoacid S1



4-Methoxyphenylacetic acid (5.000 g, 30.10 mmol) was dissolved in CCl_4 (50 mL). *N*-Bromosuccinimide (5.625 g, 31.6 mmol) and AIBN (0.985 g, 6.0 mmol) were added. The resulting solution was heated to 80 °C and allowed to reflux overnight. The succinimide by-product was removed by filtration, and the solution was concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with 1:1 EtOAc/hexanes to afford **S1** (5.705 g, 78%) as a white solid.

Data for S1: ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.50 (d, 2H, J = 8.8 Hz), 6.90 (d, 2H, J = 8.8 Hz), 5.36 (s, 1H), 3.82 (s, 1H.) ^{13}C NMR (125 MHz, $CDCl_3$, δ): 173.4, 160.5, 130.2, 126.8, 114.3, 55.4, 45.9. HRMS (ESI$^-$) m/z calcd for $C_9H_9BrO_3$ $[M–H]^-$ 242.9662; found, 242.9660.

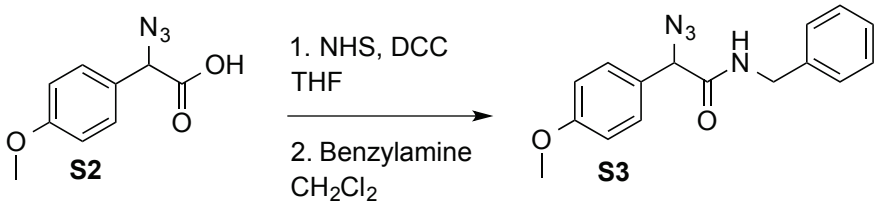
Preparation of α-Azido Acid S2



α-Bromo-4-methoxyphenylacetic acid **S1** (0.802 g, 3.3 mmol) was dissolved in 1:1 THF/H_2O (4 mL). Sodium azide (0.429 g, 6.6 mmol) was added, and the resulting solution was stirred overnight. The solution was then concentrated under reduced pressure, and the residue was dissolved in EtOAc (50 mL). The resulting solution was washed with 0.1 M HCl (2 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4(s) and concentrated under reduced pressure to afford **S2** (0.412 g, 62%) as a white solid.

Data for S2: ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.35 (d, 2H, J = 8.7 Hz), 6.95 (d, 2H, J = 8.7 Hz), 5.00 (s, 1H), 3.83 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 173.5, 160.5, 129.1, 125.2, 114.6, 64.6, 55.4. HRMS (ESI$^-$) m/z calcd for $C_9H_9N_3O_3$ $[M–H]^-$ 206.0571; found, 206.0577.

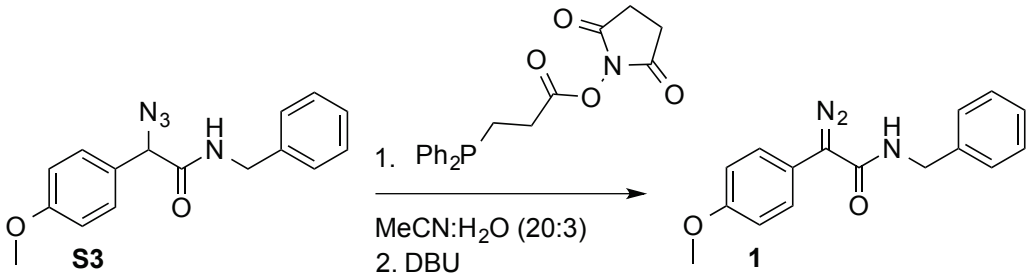
Preparation of α-azido 4-Methoxyphenylacetic Amide S3



α-Azido-4-methoxyphenylacetic acid **S2** (0.412 g, 2.0 mmol**)** was dissolved in THF (5 mL), and the resulting solutions was cooled in an ice bath. *N*-Hydroxysuccinimide (0.230 g, 2.0 mmol) was added, followed by the portion-wise addition of DCC (0.453 g, 2.2 mmol). The resulting solution was warmed to ambient temperature and stirred overnight. The slurry was removed by filtration, and the solution was concentrated under reduced pressure. The residue was dissolved in EtOAc (10 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4(s) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with 3:7 EtOAc/hexanes, and used immediately. The NHS ester (0.4 g, 1.2 mmol) was dissolved in CH_2Cl_2 (10 mL). Benzylamine (0.10 mL, 1.3 mmol) was added dropwise, and the resulting solution was stirred overnight. The solution was then concentrated under reduced pressure. The residue was dissolved in EtOAc (10 mL) and washed with 0.1 M HCl (2 × 10 mL) and saturated aqueous $NaHCO_3$ (2 × 10 mL). The organic layer was dried over anhydrous anhydrous Na_2SO_4(s) and concentrated under reduced pressure to afford **S3** (0.255 g, 43%) as a white solid.

Data for S3: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.34–7.30 (m, 4H), 7.27–7.23 (m, 3H), 6.97 (d, 2H, J = 8.8 Hz), 4.99 (s, 1H), 4.37 (m, 2H), 3.80 (s, 3H). ^{13}C NMR (125 MHz, CD_3CN, δ): 169.4, 161.0, 139.8, 130.2, 129.4, 128.4, 128.2, 128.0, 115.1, 66.6, 55.9, 43.6. HRMS (ESI$^+$) m/z calcd for $C_{16}H_{16}N_4O_2$ $[M+H]^+$ 297.1347; found, 297.1346.

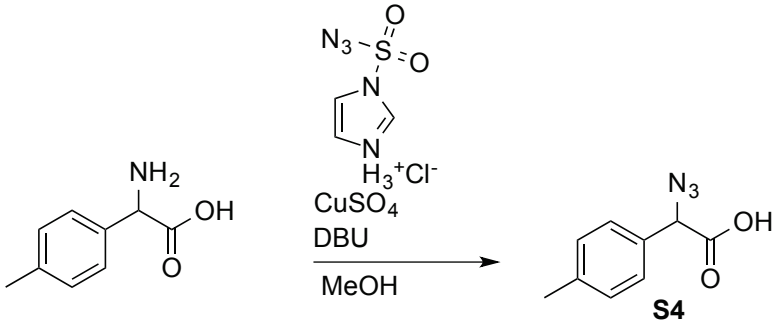
Preparation of α-Diazo Amide 1



α-Azidoamide **S3** (0.356 g, 1.2 mmol) was dissolved in 20:3 MeCN/H_2O (12 mL), and the resulting solution was cooled in an ice bath. *N*-Succinimidyl 3-(diphenylphosphino)propionate (0.440 g, 1.24 mmol) was added slowly. The solution was warmed to ambient temperature and stirred until all azide was consumed (~12 h as monitored by TLC). DBU (0.21 mL, 1.4 mmol) was added, and the solution was stirred for 1 h. The solution was then diluted with brine (10 mL) and extracted with CH_2Cl_2 (2 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4(s) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with 1:1 EtOAc/hexanes to afford **1** (0.095 g, 28%) as an orange solid.

Data for 1: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.37 (d, 2H, J = 8.9 Hz), 7.34–7.29 (m, 4H), 7.26–7.23 (m, 1H), 4.43 (d, 2H, J = 6.2 Hz), 3.80 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 165.4, 159.7, 138.4, 130.3, 128.7, 127.7, 127.5, 117.5, 115.3, 63.1, 55.4, 44.1. HRMS (ESI$^+$) *m*/*z* calcd for $C_{16}H_{15}N_3O_2$ $[M+H]^+$ 282.1238; found, 282.1232.

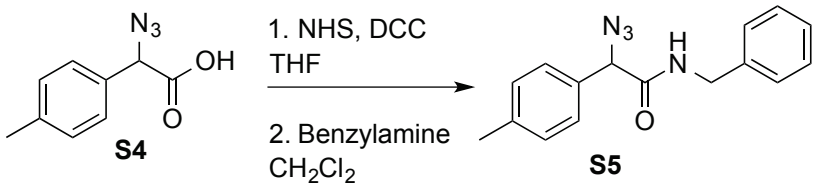
Preparation of α-Azido Acid S4



Imidazole-1-sulfonyl-azide hydrochloride was prepared as reported previously.[1] Spectral data and yields match those reported previously. α-Amino-4-methylphenylacetic acid (2.000 g, 12.1 mmol) was dissolved in MeOH (24 mL). DBU (3.61 mL, 24.2 mmol), $CuSO_4$ (0.300 g, 1.2 mmol), and azide **(**3.030 g, 14.5 mmol) were added sequentially. The resulting solution was heated to 40 °C and stirred overnight. The solution was then concentrated under reduced pressure. The residue was dissolved in EtOAc (30 mL) and washed twice with 1 M aqueous HCl (2 × 30 mL). The organic layers were combined and dried over anhydrous Na_2SO_4(s). The solution was concentrated under reduced pressure. The residue was dissolved in benzene and recrystallized from benzene and hexanes to afford **S4** (0.390 g, 17%) as a white solid.

Data for S4: ^{1}H NMR (600 MHz, $CDCl_3$, δ): 7.30 (d, 2H, J = 8.1 Hz), 7.24 (d, 2H, J = 7.8 Hz), 5.01 (s, 1H), 2.37 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$, δ): 173.4, 139.7, 130.2, 129.9, 127.6, 64.9, 21.2. HRMS (ESI^-) m/z calcd for $C_9H_9N_3O_2$ $[M–H]^-$ 190.0622; found, 190.0625.

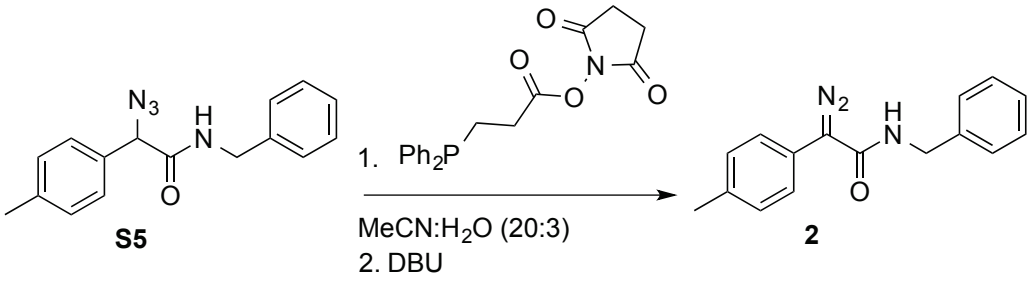
Preparation of α-Azido-methylphenylacetic Amide S5



α-Azido 4-methylphenylacetic acid **S4** (2.204 g, 11.6 mmol**)** was dissolved in THF (30 mL) and cooled in an ice bath. *N*-Hydroxysuccinimide (1.334 g, 11.6 mmol) was added, followed by portion-wise addition of DCC (2.637 g, 12.8 mmol). The resulting solution was warmed to ambient temperature and stirred overnight. The slurry was removed by filtration, and the solution was concentrated under reduced pressure. The residue was dissolved in EtOAc (30 mL). The resulting solution was washed with saturated aqueous $NaHCO_3$ (2 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4(s), concentrated under reduced pressure, and used immediately. The NHS ester (2.5 g, 8.7 mmol) was dissolved in CH_2Cl_2 (30 mL). Benzylamine (0.98 mL, 9.6 mmol) was added dropwise, and the resulting solution was stirred overnight. The solution was then concentrated under reduced pressure. The residue was dissolved in EtOAc (30 mL) and washed with 0.1 M HCl (2 × 30 mL) and saturated aqueous $NaHCO_3$ (2 × 30 mL). The organic layer was dried over anhydrous anhydrous Na_2SO_4(s) and concentrated under reduced pressure to afford **S5** (1.988 g, 61%) as a white solid.

Data for S5: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.33–7.28 (m, 4H), 7.26–7.22 (m, 5H), 5.00 (s, 1H), 4.36 (dd, 2H, J = 1.8, 6.2 Hz), 2.35 (s, 3H). ^{13}C NMR (125 MHz, CD_3CN, δ): 169.2, 140.0, 139.8, 133.5, 130.4, 129.4, 128.8, 128.2, 128.0, 66.9, 43.6, 21.1. HRMS (ESI^+) m/z calcd for $C_{16}H_{16}N_4O$ $[M+H]^+$ 281.1397; found, 281.1395.

Preparation of α-Diazo-methylphenylacetic Amide 2

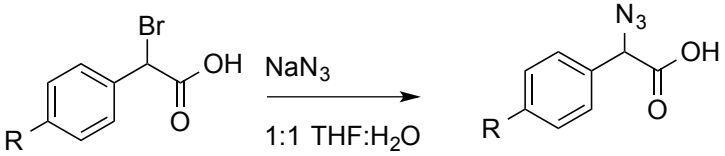


α-Azido 4-methylphenylacetic amide **S5** (1.995 g, 7.1 mmol) was dissolved in 20:3 MeCN/H_2O (50 mL), and the resulting solution was cooled in an ice bath. *N*-Succinimidyl 3-(diphenylphosphino)propionate (2.769 g, 7.8 mmol) was added slowly. The solution was warmed to ambient temperature and stirred until all azide was consumed (~24 h as monitored by TLC). DBU (1.27 mL, 8.5 mmol) was added, and the solution stirred for 45 min. The solution was then diluted with brine (10 mL) and extracted with CH_2Cl_2 (2 × 30 mL). The organic layer

was dried over anhydrous Na_2SO_4(s) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with 4:6 EtOAc/hexanes to afford **2 (**1.038 g, 55%) as an orange solid.

Data for 2: ^{1}H NMR (600 MHz, CD_3CN, δ): 7.33–7.23 (m, 9H), 6.63 (s, 1H), 4.44 (d, 2H, J = 6.2 Hz), 2.34 (s, 3H). ^{13}C NMR (150 MHz, CD_3CN, δ): 165.5, 140.7, 138.1, 130.9, 129.3, 128.2, 128.1, 127.9, 124.1, 63.74. 44.0, 21.1. HRMS (ESI$^+$) m/z calcd for $C_{16}H_{15}N_3O$ $[M+H]^+$ 266.1288; found, 266.1292.

General Procedure for Preparation of Azides S6–S8



S6 R = H
S7 R = F
S8 R = Cl

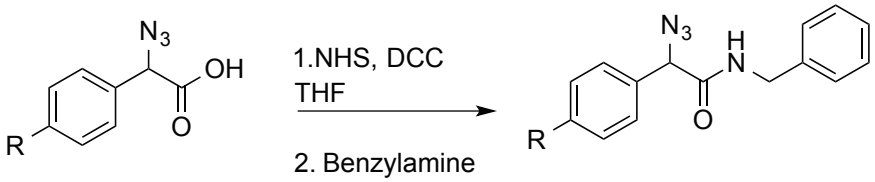
Each α-bromophenylacetic acid (23.3 mmol) was dissolved in a solution of 1:1 THF/H_2O (24 mL). Sodium azide (1.512 g, 46.5 mmol) was added, and the resulting solution was stirred overnight. The solution was then concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL), and washed with 0.1 M HCl (2 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4(s) and concentrated under reduced pressure to afford a white solid (**S6**: 4.076 g, 99%; **S7**: 4.016 g, 89%; **S8**: 3.761 g, 77%).

Data for Azide S6: ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.43 (m, 5H), 5.05 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$, δ): 174.0, 133.1, 129.6, 129.2, 127.7, 65.1. HRMS (ESI$^+$) m/z calcd for $C_8H_7N_3O_2$ $[M+H]^+$ 177.0533; found, 177.0538.

Data for Azide S7: ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.41 (dd, 2H, J = 5.1, 8.5 Hz), 7.12 (t, 2H, J = 8.4 Hz), 5.05 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 175.0, 163.5 (d, J = 249.6 Hz), 129.8 (d, J = 8.5 Hz) 129.1 (d, J = 2.6 Hz), 116.5 (d, J = 22.1 Hz), 64.5. HRMS (ESI$^-$) m/z calcd for $C_8H_6FN_3O_2$ $[M–H]^-$ 194.0371; found, 194.0378.

Data for Azide S8: ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.41 (d, 2H, J = 8.4 Hz), 7.37 (d, 2H, J = 8.3 Hz), 5.06 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 174.7, 135.8, 131.5, 129.5, 129.0, 64.3. HRMS (ESI$^-$) m/z calcd for $C_8H_6ClN_3O_2$ $[M–H]^-$ 210.0075; found, 210.0078.

General Procedure for Preparation of Amides S9–S11



S6 R = H
S7 R = F
S8 R = Cl

S9 R = H
S10 R = F
S11 R = Cl

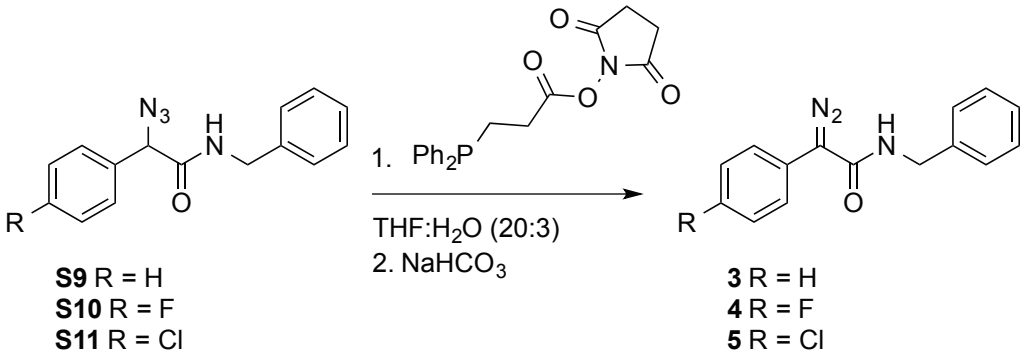
Each α-azidoacetic acid (**S6**–**S8**) (15.4 mmol) was dissolved in THF (30 mL), and the resulting solution was cooled in an ice bath. *N*-Hydroxysuccinimide (NHS) (1.772 g, 15.4 mmol) was added, followed by portion-wise addition of DCC (3.177 g, 15.4 mmol). The solution was warmed to ambient temperature and stirred overnight. The slurry was removed by filtration, and the solution was concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4(s) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with 1:1 EtOAc/hexanes. The resulting solution was then concentrated under reduced pressure and used immediately. The NHS ester (10.5 mmol) was dissolved in CH_2Cl_2 (105 mL). Benzylamine (1.16 mL, 10.6 mmol) was added drop-wise, and the resulting solution was stirred overnight. The solution was concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washed with 0.1 M HCl (2 × 50 mL) and saturated aqueous $NaHCO_3$ (2 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4(s) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with 30% EtOAc/hexanes to afford a white solid (**S9:** 2.384 g, 58% for 2 steps; **S10**: 2.062 g, 47% for 2 steps; **S11**: 2.179 g, 47% for 2 steps).

Data for Amide S9: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.43–7.42 (m, 5H), 7.31–7.29 (m, 2H), 7.26–7.22 (m, 3H), 5.06 (s, 1H), 4.37 (d, 2H, J = 6.2). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 167.8, 137.5, 134.9, 129.2, 129.1, 128.8, 127.8, 127.73, 127.67, 67.4, 43.7. HRMS (ESI^+) *m*/*z* calcd for $C_{15}H_{14}N_4O$ $[M+H]^+$ 267.1241; found, 267.1241.

Data for Amide S10: ^{1}H NMR (600 MHz, CD_3CN, δ): 7.45–7.42 (dd, 2H, J = 5.4, 8.7 Hz), 7.23–7.30 (m, 2H), 7.26–7.22 (m, 3H), 7.18–7.15 (m, 2H), 5.08 (s, 1H), 4.37 (dd, 2H, J = 3.0, 6.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 167.6, 163.1 (d, J = 249.2 Hz), 137.5, 130.9 (d, J = 2.0 Hz), 129.5 (d, J = 8.5 Hz), 128.8, 127.8, 116.2 (d, J = 21.8 Hz), 105.0, 66.6, 43.7. 43.7, HRMS (ESI^+) *m*/*z* calcd for $C_{15}H_{13}FN_4O$ $[M+H]^+$ 285.1147; found, 285.1150.

Data for Amide S11: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.44–7.39 (m, 4H), 7.33–7.27 (m, 2H), 7.25-7.22 (m, 3H), 5.08 (s, 1H), 4.36 (m, 2H). ^{13}C NMR (125 MHz, CD_3CN, δ): 168.8, 139.7, 135.5, 135.2, 130.4, 129.9, 129.4, 128.2, 128.0, 66.3, 43.6. HRMS (ESI^+) *m*/*z* calcd for $C_{15}H_{13}ClN_4O$ $[M+H]^+$ 301.0851; found, 301.0850.

General Procedure for Preparation of Diazo Compounds 3–5



Each α-azidobenzylamide (**S9**–**S11**) (7.3 mmol) was dissolved in a solution of 20:3 THF:H_2O (75 mL) and cooled in an ice bath. *N*-Succinimidyl 3-(diphenylphosphino)propionate (2.734 g,

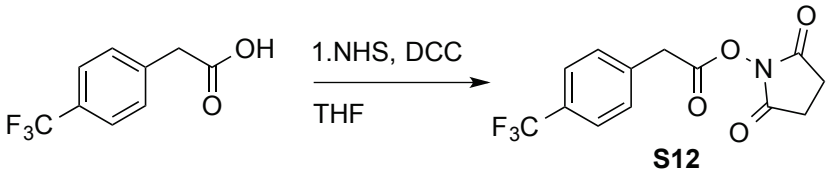
7.7 mmol) was added slowly. The resulting solution was warmed to ambient temperature and stirred until all azide was consumed (6–12 h as monitored by TLC). Saturated aqueous $NaHCO_3$ (73 mL) was added, and the solution was stirred overnight. The solution was then diluted with brine (50 mL) and extracted with CH_2Cl_2 (2 × 70 mL). The organic layer was dried over anhydrous Na_2SO_4(s) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with 1:1 EtOAc/hexanes to afford an orange solid (**3**: 1.012 g, 55%; **4**: 0.887 g, 45%; **5**: 0.877 g, 42%).

Data for Diazo 3: ^{1}H NMR (600 MHz, CD_3CN, δ): 7.46–7.41 (m, 4H), 7.34–7.28 (m, 4H), 7.28–7.23 (m, 2H), 6.73 (s, 1H), 4.44 (d, 2H, J = 6.1 Hz). ^{13}C NMR (125 MHz, CD_3CN, δ): 165.1, 140.6, 130.2, 129.3, 128.2, 127.8, 127.7, 127.6, 127.4, 64.0, 43.9. HRMS (ESI$^+$) m/z calcd for $C_{15}H_{13}N_3O$ $[M+H]^+$ 252.1132; found, 252.1125.

Data for Diazo 4: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.49–7.46 (dd, 2H, J = 5.4, 8.6 Hz), 7.34–7.29 (m, 4H), 7.26–7.23 (m, 1H), 7.20–7.16 (t, 2H, J = 8.8), 6.70 (s, 1H), 4.43 (d, 2H, J = 6.2). ^{13}C NMR (125 MHz, CD_3CN, δ): 165.2, 162.5 (d, J = 244.9 Hz), 140.6, 130.2 (d, J = 8.3 Hz), 129.2, 128.1, 127.8, 123.4 (d, J = 3.1 Hz), 116.9 (d, J = 22.1 Hz), 62.99, 43.8. HRMS (ESI$^+$) m/z calcd for $C_{15}H_{12}FN_3O$ $[M+H]^+$ 270.1038; found, 270.1032.

Data for Diazo 5: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.45 (d, 2H, J = 8.8 Hz), 7.42 (d, 2H, 8.9 Hz), 7.35–7.30 (m, 4H), 7.28-7.26 (m, 1H), 6.79 (s, 1H), 4.44 (d, 2H, J = 6.1 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 164.1, 138.1, 133.5, 129.9, 128.8, 128.5, 127.8, 127.7,124.7, 63.5, 44.2. HRMS (ESI$^+$) m/z calcd for $C_{15}H_{12}ClN_3O$ $[M+H]^+$ 286.0742; found, 286.0748.

Preparation of Ester S12

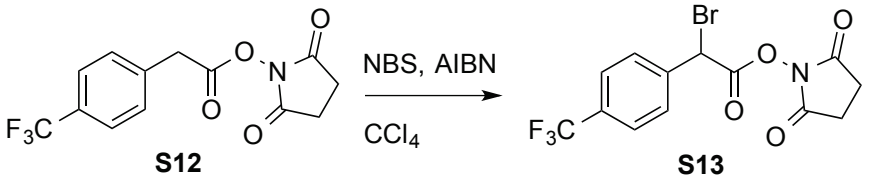


S12

4-(Trifluoromethyl)phenylacetic acid (5.000 g, 24.5 mmol) was dissolved in THF (50 mL), and the resulting solution was cooled in an ice bath. *N*-Hydroxysuccinimide (2.818 g, 24.5 mmol) was added, followed by DCC (5.047 g, 24.5 mmol). The solution was warmed to ambient temperature and stirred overnight. The slurry was removed by filtration, and the solution was concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4(s) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with 1:1 EtOAc/hexanes to afford **S12** (7.301 g, 99%) as a white solid.

Data for Ester S12: ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.63 (d, 2H, J = 7.99 Hz), 7.48 (d, 2H, J= 7.92 Hz), 4.00 (s, 2H), 2.84 (s, 4H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 168.9, 166.1, 135.27, 130.2 (q, J = 32.6 Hz), 129.7, 125.8 (q, J = 3.7 Hz), 123.9 (q, J = 272.1 Hz), 37.4, 25.6. HRMS (EI$^+$) m/z calcd for $C_{13}H_{10}F_3NO_4$ $[M+H]^+$ 301.0557; found, 301.0565.

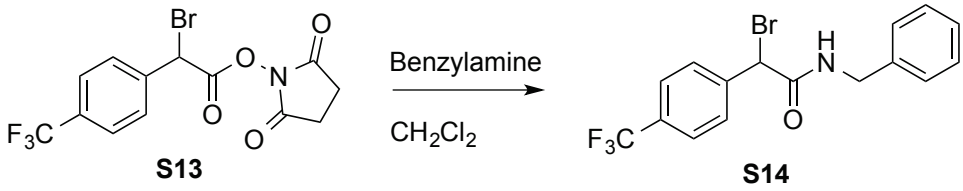
Preparation of α-Bromoester S13



Ester **S12** (3.763 g, 12.5 mmol) was dissolved in CCl_4 (25 mL). *N*-Bromosuccinimide (3.329 g, 18.7 mmol) and AIBN (0.394 g, 2.4 mmol) were added. The resulting solution was heated to 80 °C and allowed to reflux overnight. The succinimide by-product was removed by filtration, and solution was concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with 1:1 EtOAc/hexanes to afford **S13** (2.037 g, 43%) as a white solid.

Data for S13: ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.72 (d, 2H, J = 8.3 Hz), 7.69 (d, 2H, J = 8.6 Hz), 5.68 (s, 1H), 2.86 (s, 4H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 168.2, 163.8, 137.7, 131.9 (q, J = 32.8 Hz), 129.2, 126.1 (q, J = 3.7 Hz), 123.6 (q, J = 272.5 Hz), 40.7, 25.6. HRMS (EI$^+$) *m*/*z* calcd for $C_{13}H_9BrF_3NO_4$ $[M+H]^+$ 378.9662; found, 378.9667.

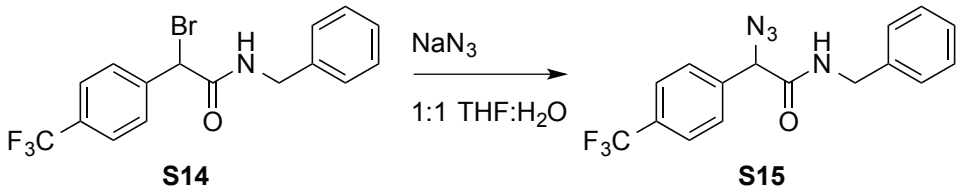
Preparation of α-Bromoamide S14



α-Bromoester **S13** (3.297 g, 8.7 mmol) was dissolved in CH_2Cl_2 (80 mL). Benzylamine (0.91 mL, 8.7 mmol) was added drop-wise, and the resulting solution was stirred overnight. The solution was concentrated under reduced pressure, and the residue was dissolved in EtOAc (50 mL). The solution was washed with 0.1 M HCl (2 × 50 mL) and saturated aqueous $NaHCO_3$ (2 × 50 mL). The organic layers were dried over anhydrous Na_2SO_4(s) and concentrated under reduced pressure. The residue was purified with chromatography on silica gel, eluting with 1:1 EtOAc/hexanes to afford **S14** (1.456 g, 45%) as a white solid.

Data for S14: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.76 (d, 2H, J = 8.3 Hz), 7.72 (d, 2H, J = 2H), 7.51 (s, 1H), 7.35 (t, 3H, J = 7.4 Hz), 7.29 (t, 3H, J = 7.7 Hz), 5.59 (s, 1H), 4.40 (m, 2H) .^{13}C NMR (125 MHz, $CDCl_3$, δ): 166.2, 141.2, 137.1, 131.1 (q, J = 32.8 Hz), 128.9, 128.8, 128.0, 127.8, 125.9 (q, J = 3.7 Hz), 123.7 (q, J = 272.3 Hz), 49.8, 44.6. HRMS (ESI$^+$) *m*/*z* calcd for $C_{16}H_{13}BrF_3NO$ $[M+H]^+$ 372.0206; found, 372.0210.

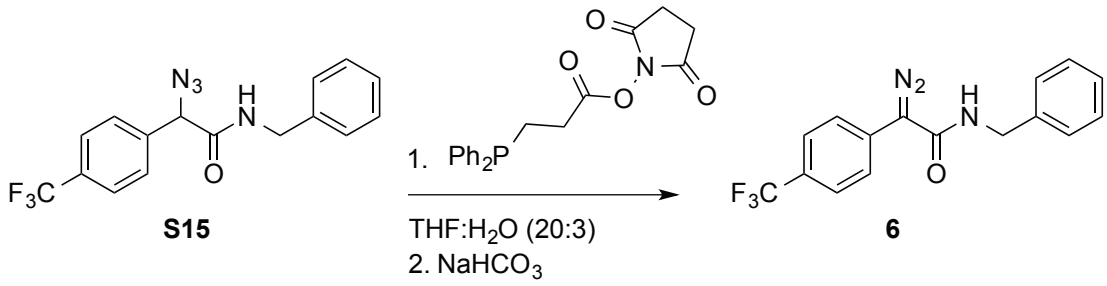
Preparation of α-Azidoamide S15



α-Bromoamide **S14** (1.823 g, 4.9 mmol) was dissolved in 1:1 THF/H_2O. Sodium azide (0.637 g, 9.8 mmol) was added, and the resulting solution was stirred overnight. The solution was concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL), and the resulting solution was washed twice with 0.1 M HCl (2 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4(s) and concentrated under reduced pressure to afford **S15** (1.018 g, 62%) as a white solid.

Data for S15: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.74 (d, 2H, J = 8.1 Hz), 7.60 (d, 2H, J = 8.0 Hz), 7.42 (s, 1H), 7.31 (m, 2H), 7.24 (m, 3H), 5.19 (s, 1H), 4.37 (d, 2H, J = 6.2 Hz). ^{13}C NMR (125 MHz, CD_3CN, δ): 170.2, 142.8, 141.4, 132.9 (q, J = 32.3 Hz), 131.2, 131.1, 130.0, 129.8, 128.5 (q, J = 3.9 Hz), 126.9 (q, J = 271.3 Hz), 68.2, 45.4. HRMS (ESI$^+$) m/z calcd for ($C_{16}H_{13}F_3N_4O$) $[M+H]^+$ 335.1115; found, 335.1112.

Preparation of α-Diazoamide 6



α-Azidoamide **S15** (1.002 g, 2.99 mmol) was dissolved in 20:3 THF/H_2O (30 mL), and the resulting solution was cooled in an ice bath. *N*-Succinimidyl 3-(diphenylphosphino)propionate (1.115 g, 3.14 mmol) was added slowly. The solution was warmed to ambient temperature and stirred until all azide was consumed (~5 h as monitored by TLC). Saturated aqueous $NaHCO_3$ (30 mL) was added, and the solution was stirred overnight. The solution was diluted with brine (30 mL) and extracted with CH_2Cl_2 (2 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4(s) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with 1:1 EtOAc/hexanes to afford **6** (0.382 g, 40%) as an orange solid.

Data for 6: ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.65 (d, 2H, J = 8.0 Hz), 7.50 (d, 2H, J = 8.1 Hz), 7.38–7.31 (m, 5H), 5.70 (s, 1H), 4.59 (d, 2H, J = 4.6 Hz). ^{13}C NMR (125 MHz, CD_3CN, δ): 164.2, 140.4, 132.9, 128.3, 127.9, 127.6 (q, J =32.4 Hz), 126.5 (q, J = 3.9 Hz), 126.3, 125.3 (q, J = 270.8 Hz), 64.0, 43.9. HRMS (ESI$^+$) m/z calcd for $C_{16}H_{12}F_3N_3O$ $[M+H]^+$ 320.1006; found, 320.0993.

3. Measurement of Reaction Rate Constants

Each diazo compound and BocGlyOH were dissolved separately in CD_3CN at a concentration of 50 mM. The solutions were combined in an NMR tube at an equimolar ratio, mixed, and then inserted immediately into an NMR spectrometer. A 16-scan ^1H NMR spectrum was acquired every 10 min. Percent conversion was monitored by disappearance of starting material and appearance of product as determined by integration of multiple ^1H NMR spectral peaks. No other products were apparent by ^1H NMR spectroscopy. The value of the second-order rate constant was determined by linear regression analysis of a plot of 1/[diazo] versus time. All reactions were performed in triplicate.

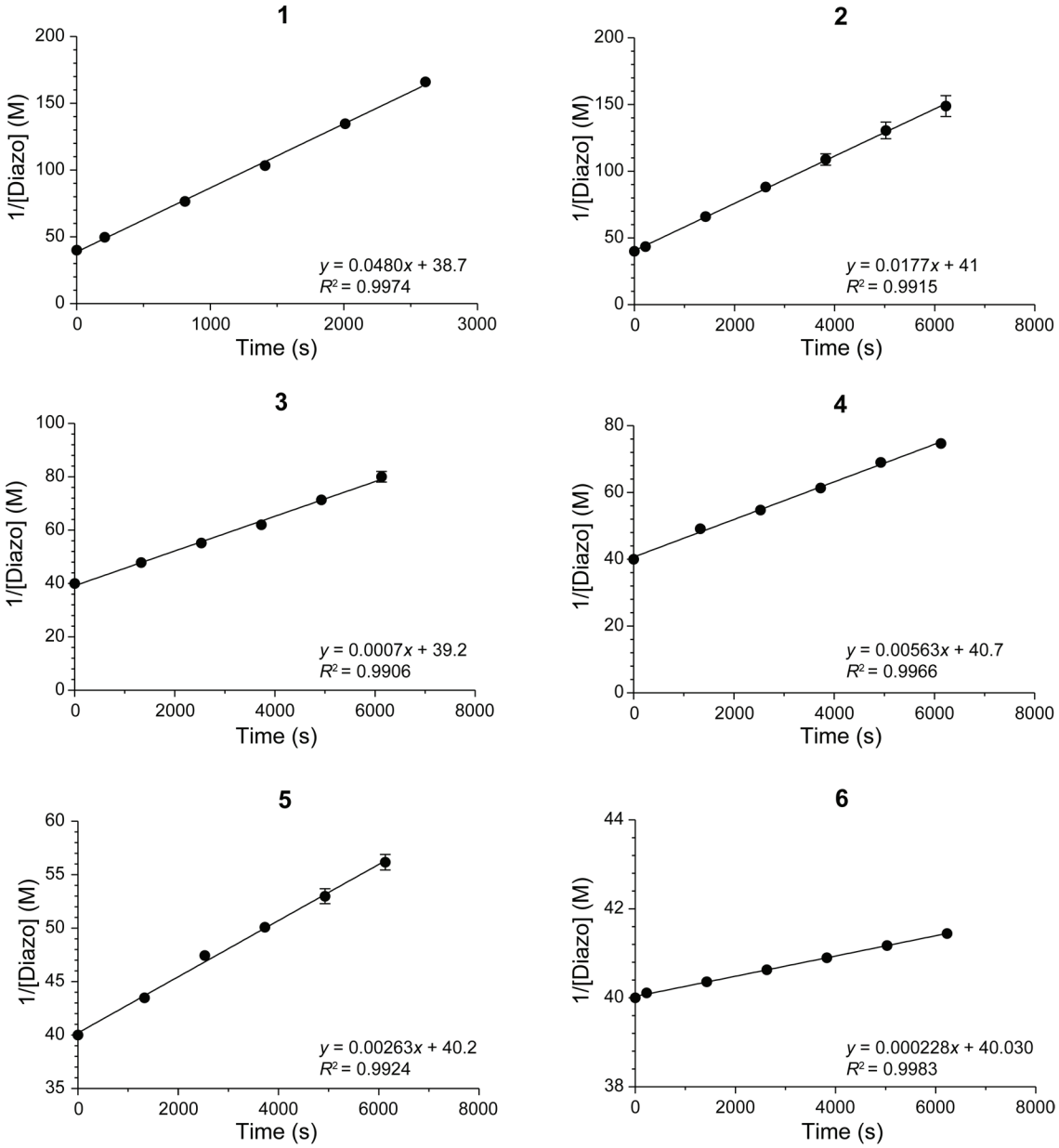
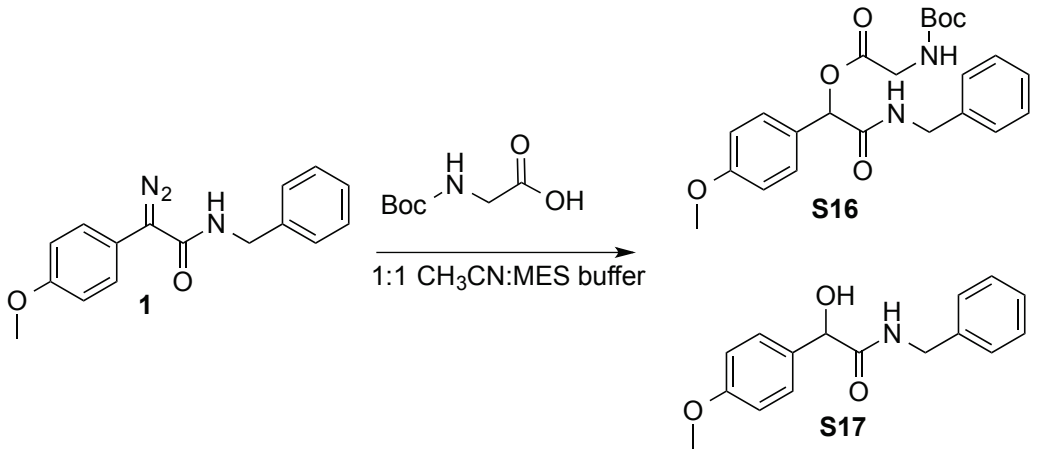


Figure S1. ^1H NMR kinetic data for reaction between compounds **1**–**6** and BocGlyOH.

4. Esterification Reactions

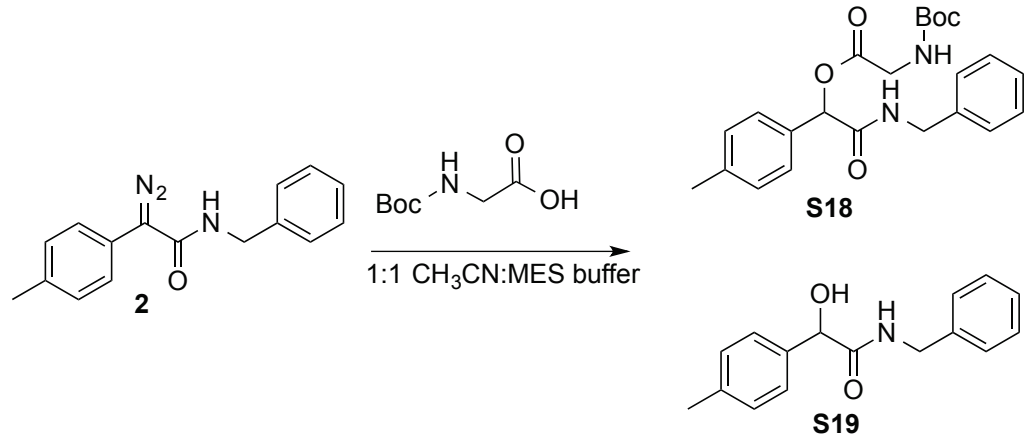
A. Esterification of BocGlyOH



Diazo compound **1** (0.005 g, 0.02 mmol) and BocGlyOH (0.003 g, 0.02 mmol) were added to a 1:1 solution of acetonitrile/100 mM MES–HCl buffer at pH 5.5, and the resulting solution was stirred for 6 h at ambient temperature. The reaction mixture was concentrated under reduced pressure, and the ratio of products was determined by integration of ^{1}H NMR spectral peaks.

Data for S16: ^{1}H NMR (400 MHz, CD_3CN, δ): 7.60 (s, 1H), 7.37–7.22 (m, 7H), 6.93 (d, 2H, J = 8.4 Hz), 5.91 (s, 1H), 5.74 (s, 1H), 4.43–4.31 (m, 2H), 3.94–3.82 (m, 2H), 3.79 (s, 3H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, CD_3CN, δ): 170.4, 169.3, 161.1, 157.4, 139.9, 129.9, 129.3, 128.6, 128.1, 127.9, 114.8, 80.3, 76.7, 55.9, 43.2, 43.2, 28.4. HRMS (ESI^+) m/z calcd for $C_{23}H_{28}N_2O_6$ $[M+H]^+$ 429.2021; found, 429.2021.

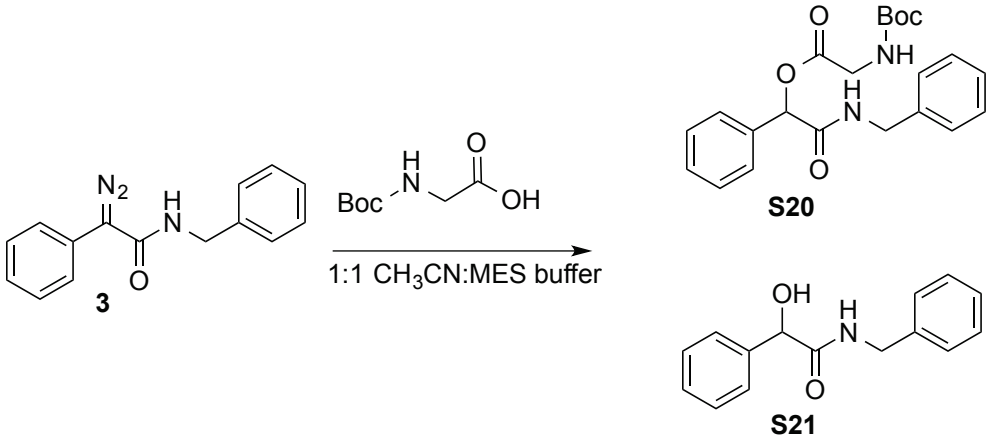
Data for S17: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.47 (s, 1H), 7.33–7.25 (m, 4H), 7.23–7.21 (m, 3H), 6.90 (d, 2H, J = 8.8 Hz), 4.97 (d, 1H, J = 4.5 Hz), 4.40–4.32 (m, 2H), 4.16 (d, 2H, J = 4.5 Hz), 3.78 (s, 3H). ^{13}C NMR (125 MHz, CD_3CN, δ): 173.3, 160.4, 140.3, 133.8, 129.3, 129.0, 128.1, 127.8, 114.5, 74.3, 55.8, 43.1. HRMS (ESI^+) m/z calcd for $C_{16}H_{17}NO_3$ $[M+H]^+$ 272.1282; found, 272.1278.



Diazo compound **2** (0.005 g, 0.02 mmol) and BocGlyOH (0.003 g, 0.02 mmol) were added to 1:1 acetonitrile/100 mM MES–HCl buffer at pH 5.5, and the resulting solution was stirred for 6 h at ambient temperature. The solution was then concentrated under reduced pressure, and the ratio of products was determined by integration of ^{1}H NMR spectral peaks.

Data for S18: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.65 (s, 1H), 7.33–7.28 (m, 4H), 7.25–7.20 (m, 5H), 5.92 (s, 1H), 5.77 (s, 1H), 4.42–4.31 (m, 2H), 3.92–3.82 (m, 2H), 2.34 (s, 3H), 1.38 (s, 9H). ^{13}C NMR (125 MHz, CD_3CN, δ) 170.4, 169.2, 157.4, 140.0, 139.8, 133.7, 130.1, 129.3, 128.3, 128.1, 127.9, 80.3, 76.8, 43.2, 43.2, 28.4, 21.2. HRMS (ESI$^+$) *m*/*z* calcd for $C_{23}H_{28}N_2O_5$ $[M+NH_4]^+$ 430.2337; found, 430.2336.

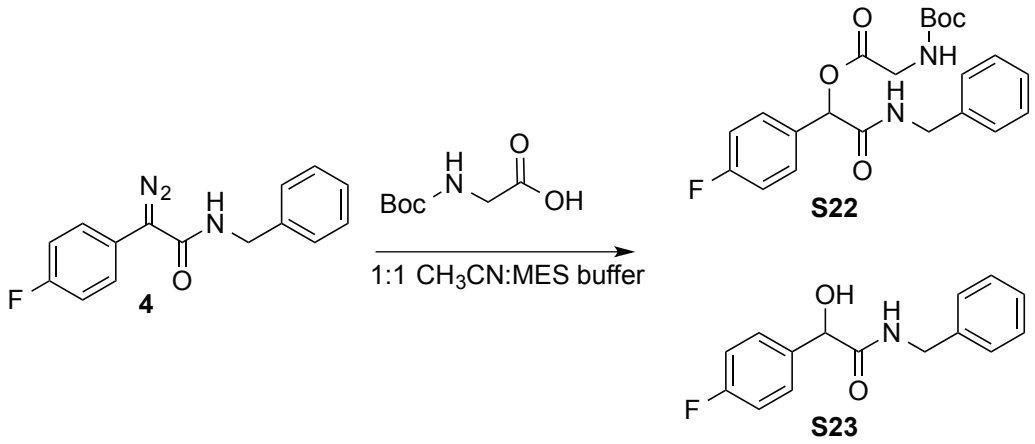
Data for S19: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.46 (s, 1H), 7.31–7.28 (m, 4H), 7.25–7.21 (m, 3H), 7.17 (d, 2H, J = 7.9 Hz), 4.99 (d, 1H, J = 4.2 Hz), 4.40–4.32 (m, 2H), 4.18 (d, 1H), J = 4.5 Hz), 2.32 (s, 1H). ^{13}C NMR (125 MHz, CD_3CN, δ): 173.3, 140.3, 138.74, 138.71, 129.8, 129.3, 128.1, 127.9, 127.6, 74.6, 43.1, 21.1. HRMS (ESI$^+$) *m*/*z* calcd for $C_{16}H_{17}NO_2$ $[M+H]^+$ 256.1333; found, 256.1330.



Diazo compound **3** (0.005 g, 0.02 mmol) and BocGlyOH (0.004 g, 0.02 mmol) were added to 1:1 acetonitrile/100 mM MES–HCl buffer at pH 5.5, and the resulting solution was stirred for 6 h at ambient temperature. The reaction mixture was then concentrated under reduced pressure, and the ratio of products was determined by integration of ^{1}H NMR spectral peaks.

Data for S20: ^{1}H NMR (750 MHz, CD_3CN, δ): 7.65 (s, 1H), 7.46 (m, 2H), 7.40 (m, 3H), 7.30 (t, 2H, J = 7.4 Hz), 7.23 (m, 3H), 5.99 (s, 1H), 5.78 (s, 1H), 4.41 (dd, 1H, J = 6.3, 15.2 Hz), 4.35 (dd, 1H, J = 6.1, 15.2 Hz), 3.92 (dd, 1H, J = 6.2, 17.9 Hz), 3.88 (dd, 1H, J = 5.7, 18.0 Hz), 1.40 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 168.7, 168.0, 156.4, 137.9, 135.0, 129.1, 128.8, 128.6, 127.8, 127.5, 127.4, 80.6, 76.2, 43.4, 43.0, 28.2. HRMS (ESI$^+$) *m*/*z* calcd for $C_{22}H_{26}N_2O_5$ $[M+H]^+$ 399.1915; found, 399.1917.

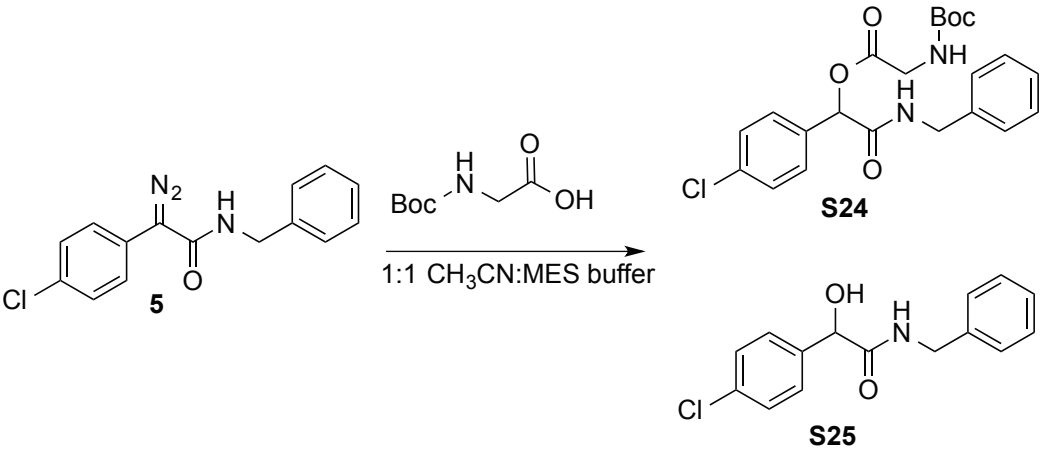
Data for S21: ^{1}H NMR (750 MHz, CD_3CN, δ): 7.48 (s, 1H), 7.43 (d, 2H, J = 7.4 Hz), 7.36 (t, 2H, J = 7.4 Hz), 7.31 (m, 3H), 7.24 (m, 3H), 5.04 (d, 1H, J = 2.8 Hz), 4.37 (m, 2H), 4.28 (d, 1H, J = 3.8 Hz). ^{13}C NMR (125 MHz, CD_3CN, δ): 173.1, 141.6, 140.3, 129.3, 129.2, 128.8, 128.1, 127.9, 127.6, 74.7, 43.1. HRMS (ESI$^+$) *m*/*z* calcd for $C_{15}H_{15}NO_2$ $[M+H]^+$ 242.1176; found, 242.1169.



Diazo **4** (0.005 g, 0.02 mmol) and BocGlyOH (0.003 g, 0.02 mmol) were added to 1:1 acetonitrile/100 mM MES–HCl buffer at pH 5.5, and the resulting solution was stirred for 6 h at ambient temperature. The reaction mixture was then concentrated under reduced pressure, and the ratio of products was determined by integration of ^{1}H NMR spectral peaks.

Data for S22: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.66 (s, 1H), 7.48 (dd, 2H, J = 5.4, 8.6 Hz), 7.30 (t, 2H, J = 7.3 Hz), 7.25–7.20 (m, 3H), 7.14 (t, 2H, J = 8.9 Hz), 5.97 (s, 1H), 5.77 (s, 1H), 4.40 (dd, 1H, J = 6.3, 15.2 Hz), 4.34 (dd, 1H, J = 6.1, 15.2 Hz), 3.94–3.84 (m, 2H), 1.38 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 168.6, 167.9, 163.1 (d, J = 248.2 Hz), 156.4, 137.8, 131.0 (d, J = 3.3 Hz), 129.4 (d, J = 8.5 Hz), 127.8, 127.5, 115.8 (d, J = 21.8 Hz), 80.7, 75.5, 43.4, 43.0, 28.2. HRMS (ESI$^+$) m/z calcd for $C_{22}H_{25}FN_2O_5$ $[M+H]^+$ 417.1821; found, 417.1816.

Data for S23: ^{1}H NMR (400 MHz, CD_3CN, δ): 7.53 (s, 1H), 7.45–7.42 (m, 2H), 7.32–7.28 (m, 2H), 7.24–7.20 (m, 3H), 7.09 (t, 2H, J = 8.9 Hz), 5.04 (s, 1H), 4.41–4.31 (m, 2H). ^{13}C NMR (125 MHz, CD_3CN, δ): 174.7, 165.0 (d, J = 243.7 Hz), 142.0, 139.6, 131.3 (d, J = 8.3 Hz), 131.1., 129.8, 129.6, 117.6 (d, J = 21.7 Hz), 75.7, 44.8. HRMS (ESI$^+$) m/z calcd for $C_{15}H_{14}FNO_2$ $[M+H]^+$ 260.1082; found, 260.1080.

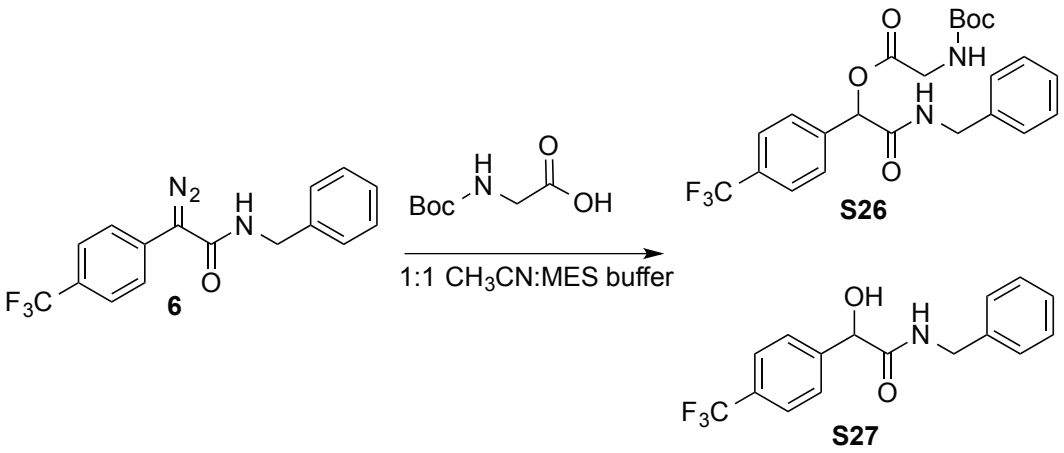


Diazo **5** (0.005 g, 0.02 mmol) and BocGlyOH (0.003 g, 0.02 mmol) were added to 1:1 acetonitrile/100 mM MES–HCl buffer at pH 5.5, and the resulting solution was stirred for 6 h at

ambient temperature. The reaction mixture was then concentrated under reduced pressure, and the ratio of products was determined by integration of ^{1}H NMR spectral peaks.

Data for S24: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.61 (s, 1H), 7.45–7.40 (m, 4H), 7.31–7.29 (m, 2H), 7.25–7.21 (m, 3H), 5.98 (s, 1H), 5.74 (s, 1H), 4.42–4.32 (m, 2H), 3.90 (m, 2H), 1.39 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 168.5, 167.6, 156.4, 137.7, 135.1, 135.6, 128.9, 128.8, 128.6, 127.8, 127.5, 80.8, 75.4, 43.4, 43.0, 28.2. HRMS (ESI^+) *m*/*z* calcd for $C_{22}H_{25}ClN_2O_5$ $[M+NH_4]^+$ 450.1791; found, 450.1785.

Data for S25: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.47 (s, 1H), 7.42 (d, 2H, J = 8.5 Hz), 7.37 (d, 2H, 8.6 Hz), 7.32–7.29 (m, 2H), 7.25–7.21 (m, 3H), 5.04 (d, 1H, J = 1.8 Hz), 4.36 (m, 2H), 4.31 (d, 1H, J = 3.4 Hz). ^{13}C NMR (125 MHz, CD_3CN, δ): 172.7, 140.5, 140.2, 134.0, 129.3, 129.21, 129.18, 128.1, 127.9, 73.9, 43.1. HRMS (ESI^+) *m*/*z* calcd for $C_{15}H_{14}ClNO_2$ $[M+H]^+$ 276.0786; found, 276.0789.

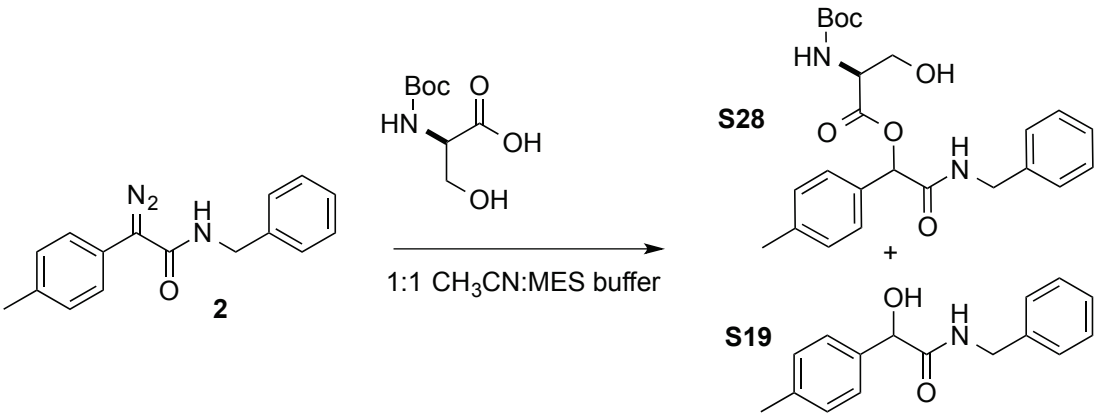


Diazo **6** (0.005 g, 0.02 mmol) and BocGlyOH (0.003 g, 0.02 mmol) were added to 1:1 acetonitrile/100 mM MES–HCl buffer at pH 5.5, and the resulting solution was stirred for 6 h at ambient temperature. The reaction mixture was then concentrated under reduced pressure, and the ratio of products was determined by integration of ^{1}H NMR spectral peaks.

Data for S26: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.73–7.71 (m, 3H), 7.65 (d, 2H, J = 8.3 Hz), 7.31–7.28 (m, 2H), 7.25–7.20 (m, 3H), 6.06 (s, 1H), 5.77 (s, 1H), 4.42–4.32 (m, 2H), 3.97–3.87 (m, 2H), 1.38 (s, 1H). ^{13}C NMR (125 MHz, CD_3CN, δ): 170.3, 168.4, 157.4, 141.1, 139.7, 131.1 (q, J = 32.4 Hz), 129.4, 128.8, 128.1, 128.0, 126.3 (q, J = 3.9 Hz), 125.1 (q, J = 271.3 Hz), 80.4, 76.1, 43.4, 43.2, 28.4. HRMS (ESI^+) *m*/*z* calcd for $C_{23}H_{25}F_3N_2O_5$ $[M+NH_4]^+$ 484.2037; found, 484.2054.

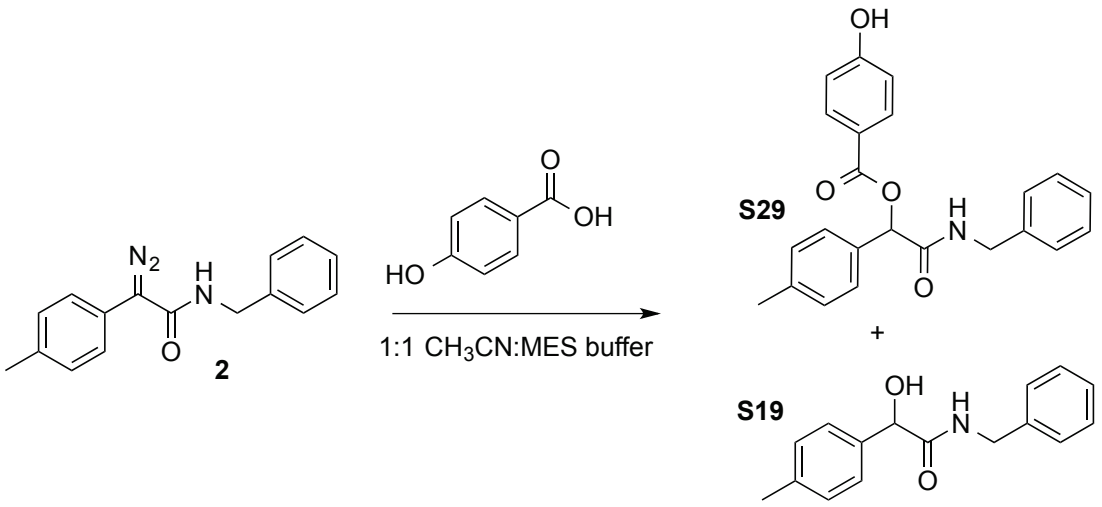
Data for S27: ^{1}H NMR (400 MHz, CD_3CN, δ): 7.69–7.62 (m, 4H), 7.56 (s, 1H), 7.31–7.20 (m, 5H), 5.54 (s, 1H), 5.14 (d, 1H, J = 4.6 Hz), 4.45 (d, 1H, J = 4.8 Hz), 4.37–4.35 (m, 2H). ^{13}C NMR (125 MHz, CD_3CN, δ): 172.3, 146.0, 140.1, 130.1 (q, J = 32.3 Hz), 129.3, 128.1, 128.9, 126.2 (q, J = 41.3 Hz), 125.3 (q, J = 271.3 Hz), 74.0, 43.1. HRMS calcd for ($C_{16}H_{14}F_3NO_2$) $[M+H]^+$ 310.1050; found, 310.1043.

B. Esterification of Other Small Molecules



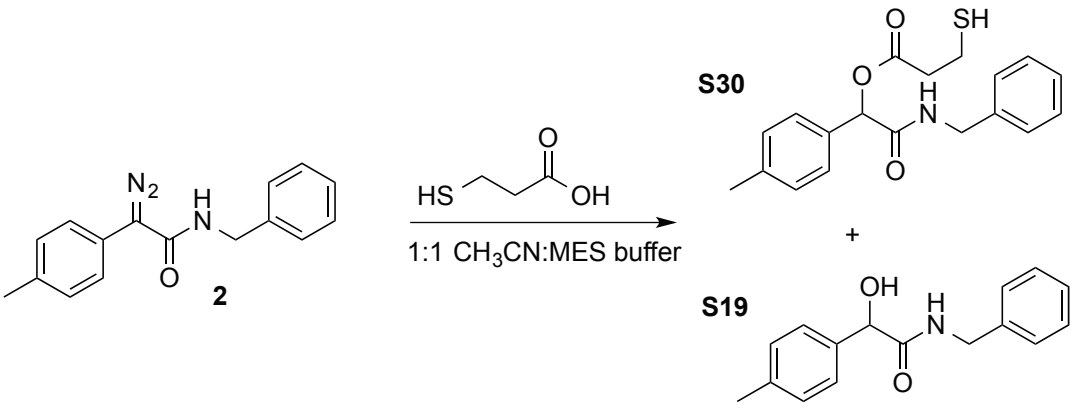
Diazo compound **2** (0.005 g, 0.02 mmol) and BocSerOH (0.004 g, 0.02 mmol) were added to 1:1 acetonitrile/100 mM MES–HCl buffer at pH 5.5, and the resulting solution was stirred for 6 h at ambient temperature. The solution was then concentrated under reduced pressure, and the ratio of products was determined by integration of ^{1}H NMR spectral peaks. Data for **S19** are reported above; data for **S28** are reported below (both diastereomers). No other products were observed by TLC or ^{1}H NMR spectroscopy.

Data for S28: ^{1}H NMR (500 MHz, CD_3CN, Diastereomer **A**, δ): 7.72 (s, 1H), 7.35 (d, 2H, J = 8.0 Hz), 7.30 (t, 2H, J = 7.3 Hz), 7.24 (t, 3H, J = 7.7 Hz), 7.18 (d, 2H, J = 7.2 Hz), 5.96 (s, 1H), 5.79 (d, 1H, J = 6.8 Hz), 4.38–4.33 (m, 2H), 4.32–4.29 (m, 1H), 4.08–4.03 (m, 1H), 3.77–3.69 (m, 2H), 2.34 (s, 3H), 1.40 (s, 9H). ^{1}H NMR (500 MHz, CD_3CN, Diastereomer **B**, δ): 7.64 (s, 1H), 7.36–7.28 (m, 4H), 7.25–7.17 (m, 5H), 5.95 (s, 1H), 5.84 (d, 1H, J = 7.8 Hz), 4.41–4.30 (m, 2H), 4.28–4.25 (m, 1H), 3.86–3.82 (m, 1H), 3.79–3.72 (m, 1H), 3.41 (t, 3H, J = 5.7 Hz), 2.34 (s, 3H), 1.36 (s, 9H). ^{13}C NMR (125 MHz, CD_3CN, Diasteromer **A**, δ): 171.3, 169.7, 157.0, 140.2, 139.6, 133.2, 130.2, 129.3, 128.5, 128.1, 128.0, 80.3, 77.0, 63.3, 57.1, 43.4, 28.4, 21.2. ^{13}C NMR (125 MHz, CD_3CN, Diastereomer **B**, δ): 171.2, 169.3, 156.7, 139.9, 139.8, 133.6, 130.1, 129.3, 128.4, 128.1, 127.9, 80.3, 77.0, 62.8, 57.1, 43.3, 28.4, 21.1. HRMS (ESI$^+$) m/z calcd for $C_{24}H_{30}N_2O_6$ $[M+H]^+$ 443.2177; found, 443.2185 (Diastereomer **A**), 443.2183 (Diastereomer **B**).



Diazo compound **2** (0.005 g, 0.02 mmol) and *p*-hydroxybenzoic acid (0.003 g, 0.02 mmol) were added to 1:1 acetonitrile/100 mM MES–HCl buffer at pH 5.5, and the resulting solution was stirred for 6 h at ambient temperature. The solution was then concentrated under reduced pressure, and the ratio of products was determined by integration of ^{1}H NMR spectral peaks. Data for **S19** are reported above; data for **S29** are reported below. No other products were observed by TLC or ^{1}H NMR spectroscopy.

Data for S29: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.98 (d, 2H, J = 8.8 Hz), 7.76 (s, 1H), 7.44 (d, 2H, J = 8.1 Hz), 7.39 (s, 1H), 7.29–7.18 (m, 7H), 6.89 (d, 2H, J = 8.8 Hz), 6.06 (s, 1H), 4.36 (d, 2H, J = 6.2 Hz), 2.35 (s, 3H). ^{13}C NMR (125 MHz, CD_3CN, δ): 169.7, 165.8, 162.6, 140.0, 139.8, 134.2, 133.0, 130.1, 129.3, 128.3, 128.0, 127.9, 121.9, 116.1, 76.8, 43.1, 21.2. HRMS (ESI^+) m/z calcd for $C_{23}H_{21}NO_4$ $[M+H]^+$ 376.1544; found, 376.1539.



Diazo compound **2** (0.005 g, 0.02 mmol) and 3-mercaptopropanoic acid (0.002 g, 0.02 mmol) were added to 1:1 acetonitrile/100 mM MES–HCl buffer at pH 5.5, and the resulting solution was stirred for 6 h at ambient temperature. The solution was then concentrated under reduced pressure, and the ratio of products was determined by integration of ^{1}H NMR spectral peaks. Data for **S19** are reported above; data for **S30** are reported below. No other products were observed by TLC or ^{1}H NMR spectroscopy.

Data for S30: ^{1}H NMR (500 MHz, CD_3CN, δ): 7.38 (s, 1H), 7.34 (d, 2H, J = 8.1 Hz), 7.29 (t, 2H, J = 7.3 Hz), 7.25–7.19 (m, 5H), 5.91 (s, 1H), 4.35 (d, 2H, J = 6.2 Hz), 2.80–2.70 (m, 4H), 2.34 (s, 3H), 1.89 (t, 1H, J = 8.2 Hz). ^{13}C NMR (125 MHz, CD_3CN, δ): 171.5, 169.4, 139.9, 139.8, 133.9, 130.1, 129.3, 128.3, 128.1, 127.9, 76.6, 43.1, 39.1, 21.1, 20.2. HRMS (ESI^+) m/z calcd for ($C_{19}H_{21}NO_3S$) $[M+H]^+$ 344.1315; found, 344.1315.

Diazo compound **2** (0.005 g, 0.02 mmol) and AlaOH (0.002 g, 0.02 mmol) were added to 1:1 acetonitrile/100 mM MES–HCl buffer at pH 5.5, and the resulting solution was stirred for 6 h at ambient temperature. The solution was then concentrated under reduced pressure, and the crude reaction mixture was analyzed by ^{1}H NMR spectroscopy (Figure S2) and LC–MS (Figure S3), which revealed no reaction.

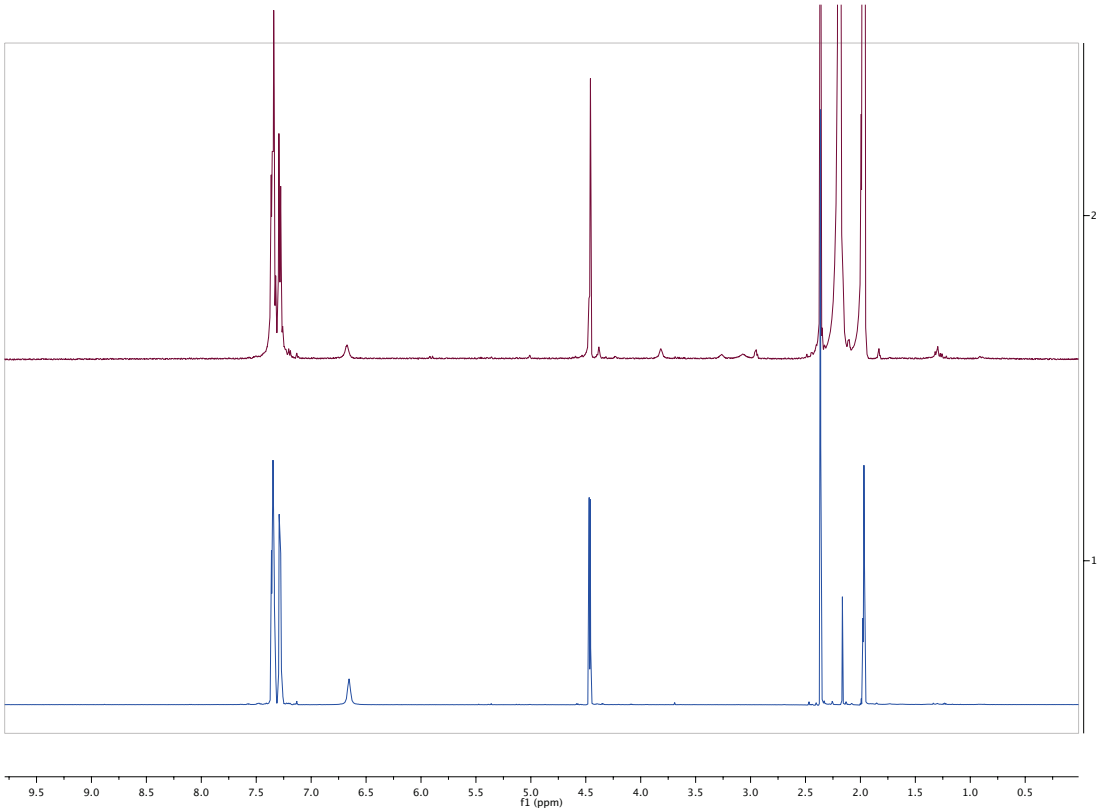


Figure S2. ^{1}H NMR (400 MHz, CD_3CN) overlay of diazo compound **2** (bottom, blue) and a crude reaction mixture of diazo compound **2** treated with AlaOH in 1:1 acetonitrile/100 mM MES–HCl buffer at pH 5.5 (top, red).

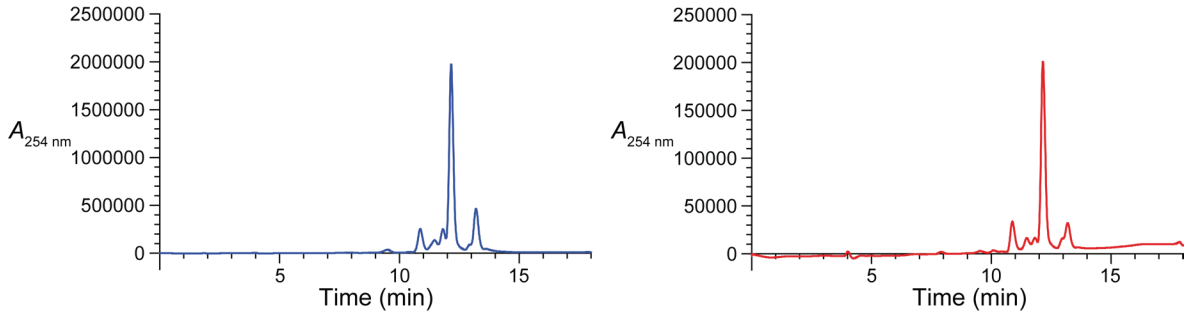
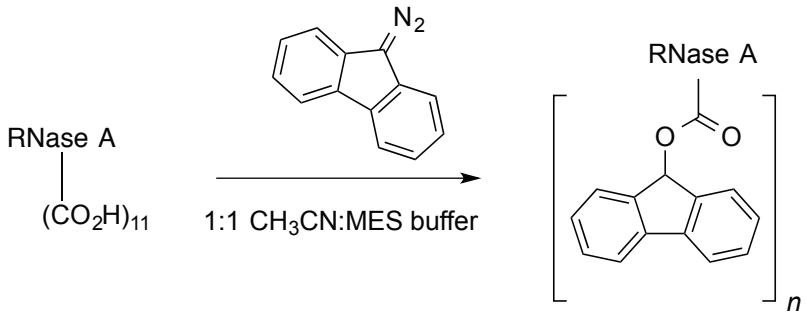
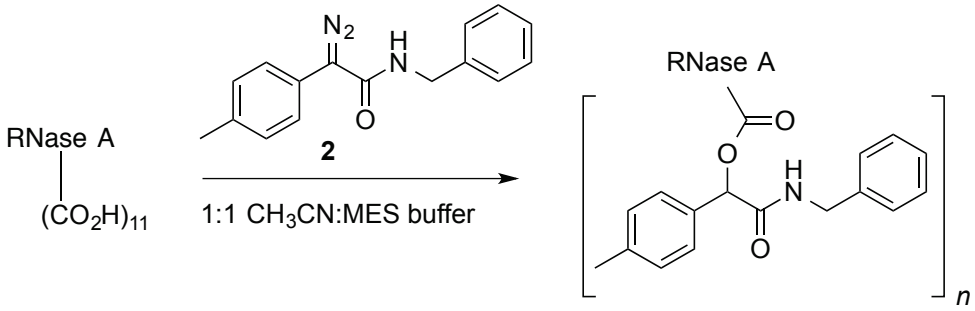


Figure S3. LC–MS chromatograms of diazo compound **2** (left, blue) and a crude reaction mixture of diazo compound **2** treated with AlaOH in 1:1 acetonitrile/100 mM MES–HCl buffer at pH 5.5 (right, red). The trace impurities with retention times of 11 and 13 min are present in both chromatograms and are likely decomposition products of diazo compound **2** in the acidic conditions used for chromatography.

5. Protein Labeling



9-Diazofluorene was prepared as described previously.[2] Yields and spectra matched the published data. Ribonuclease A (0.010 g, 0.73 μmol) was dissolved in 1 mL of 10 mM MES–HCl buffer at pH 5.5. 9-Diazofluorene (0.007 g, 0.036 mmol) was dissolved in 5 mL of CH_3CN. A 100-μL aliquot of the diazo stock solution was added to a 100-μL aliquot of the RNase A stock solution. The resulting mixture was mixed by nutation for 4 h at 37 °C. Any remaining diazo compound was then quenched by addition of 10 μL of 17.4 M acetic acid. Acetonitrile was removed by concentration under reduced pressure, and the aqueous solution of labeled protein was analyzed by MALDI–TOF mass spectrometry (Figures S2 and 5).



Ribonuclease A (0.010 g, 0.73 μmol) was dissolved in 1 mL of 10 mM MES–HCl buffer at pH 5.5. Diazo compound **2** (0.095 g, 0.036 mmol) was dissolved in 5 mL of CH_3CN. A 100-μL aliquot of the diazo stock solution was added to a 100-μL aliquot of the RNase A stock solution. The resulting mixture was mixed by nutation for 4 h at 37 °C. Any remaining diazo compound was then quenched by addition of 10 μL of 17.4 M acetic acid. Acetonitrile was removed by concentration under reduced pressure, and the aqueous solution of labeled protein was analyzed by MALDI–TOF mass spectrometry (Figures S2 and 5).

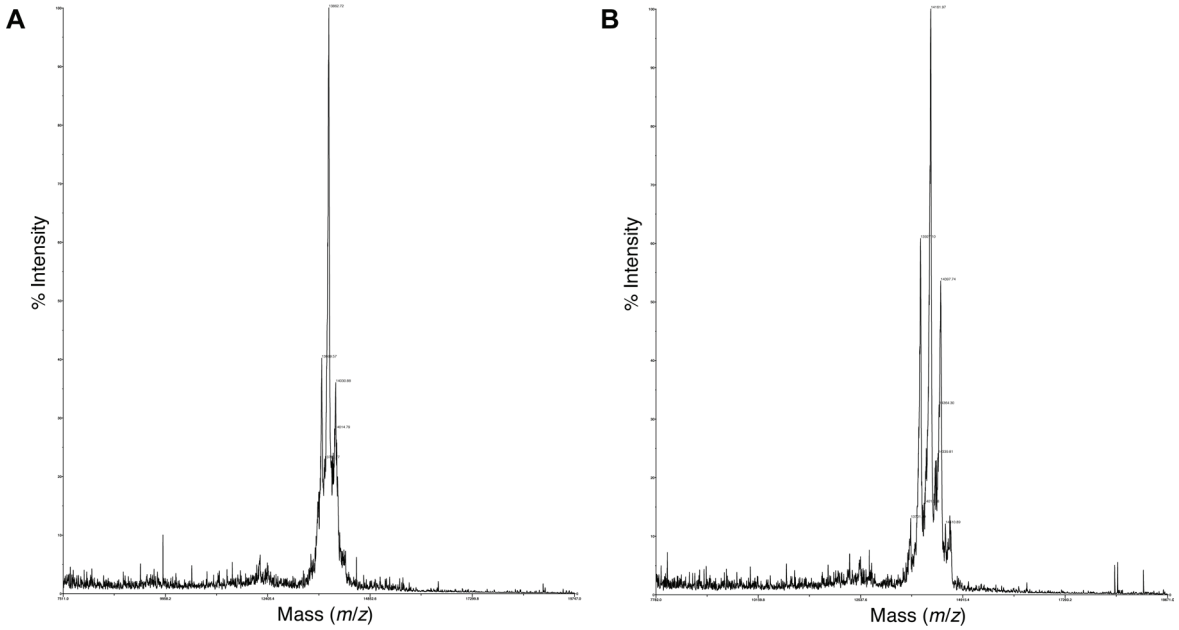


Figure S4. Raw MALDI–TOF mass spectrometry data for esterification of RNase A with (A) 9-diazofluorene or (B) diazo compound **2**. A truncated version of these data is depicted in Figure 5.

6. Ultraviolet Spectra of Diazo Compound 2

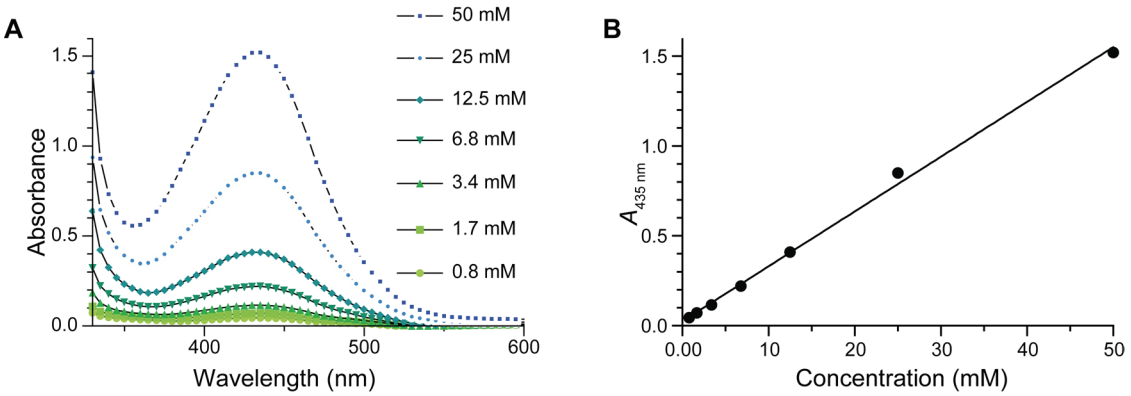


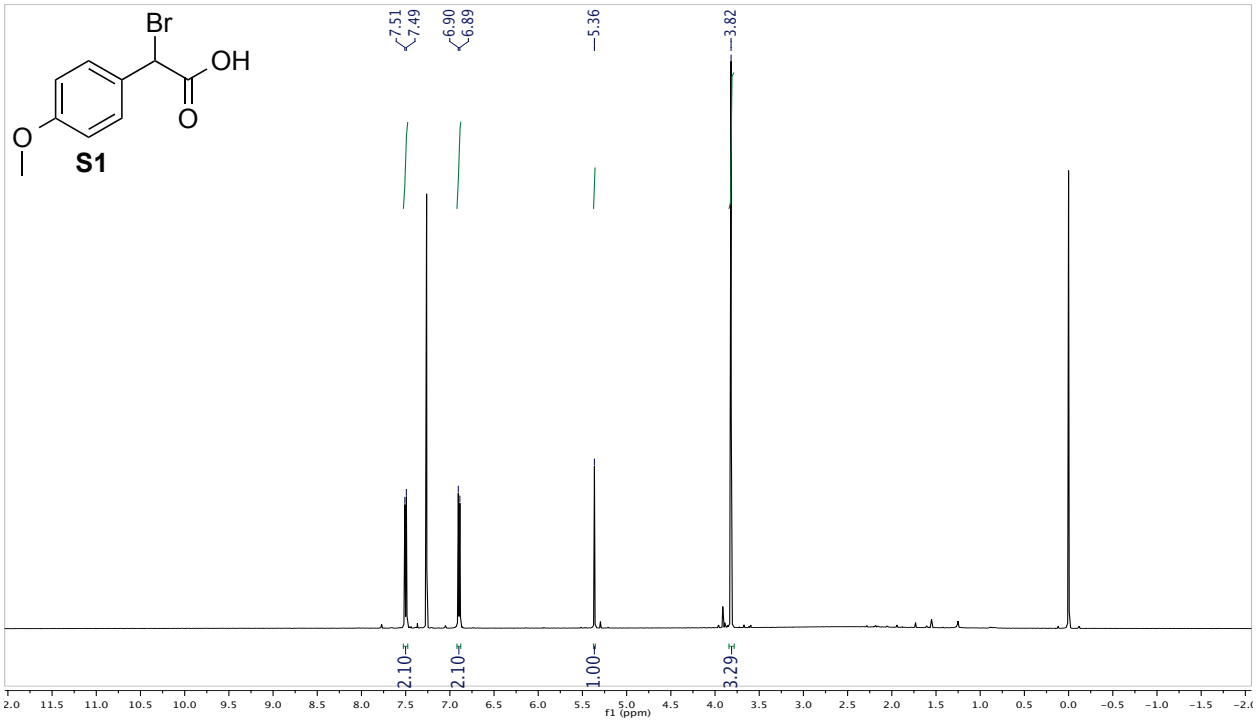
Figure S5. (A) Ultraviolet spectra of diazo compound **2** (0.8–50 mM). (B) Plot of the concentration dependence of the absorbance of diazo compound **2** (0.8–50 mM) at λ_{max} = 435 nm, giving ε = 30.5 $M^{-1}cm^{-1}$.

7. References

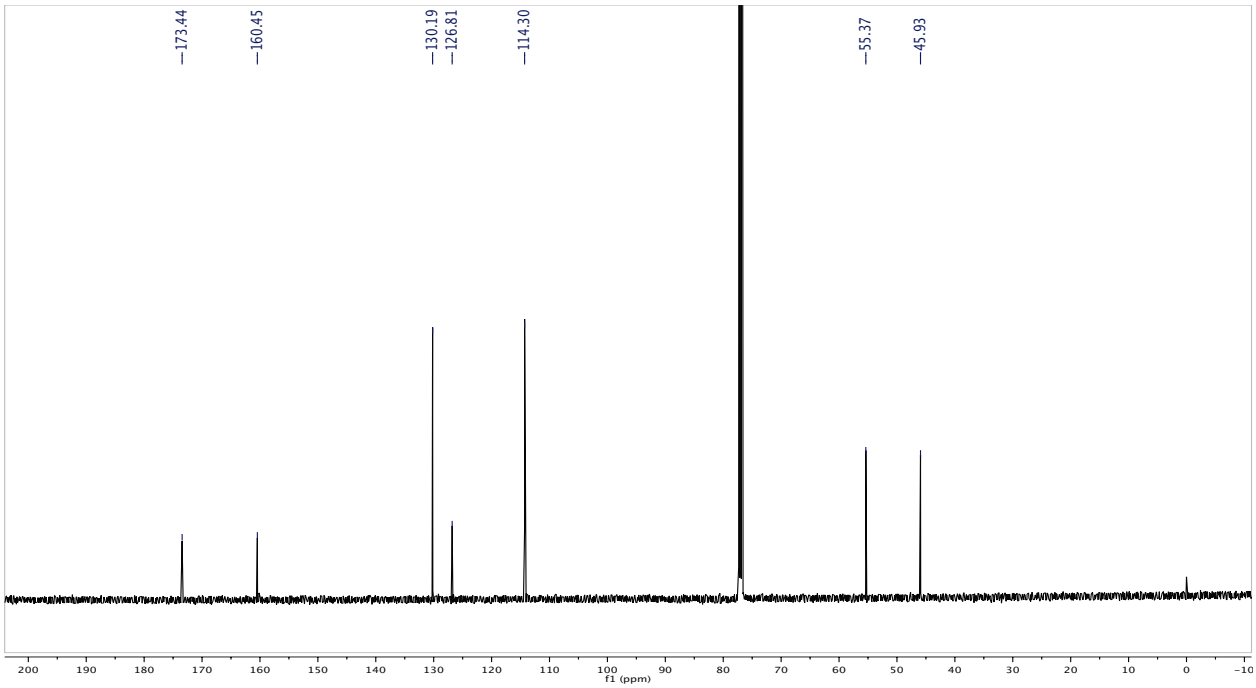
(1) Goddard-Borger, E. D.; Stick, R.V. *Org. Lett.* **2007**, *9*, 3797–3800.
(2) Myers, E. L.; Raines, R. T. *Angew. Chem. Int. Ed.* **2009**, *48*, 2359–2363.

8. NMR Spectra

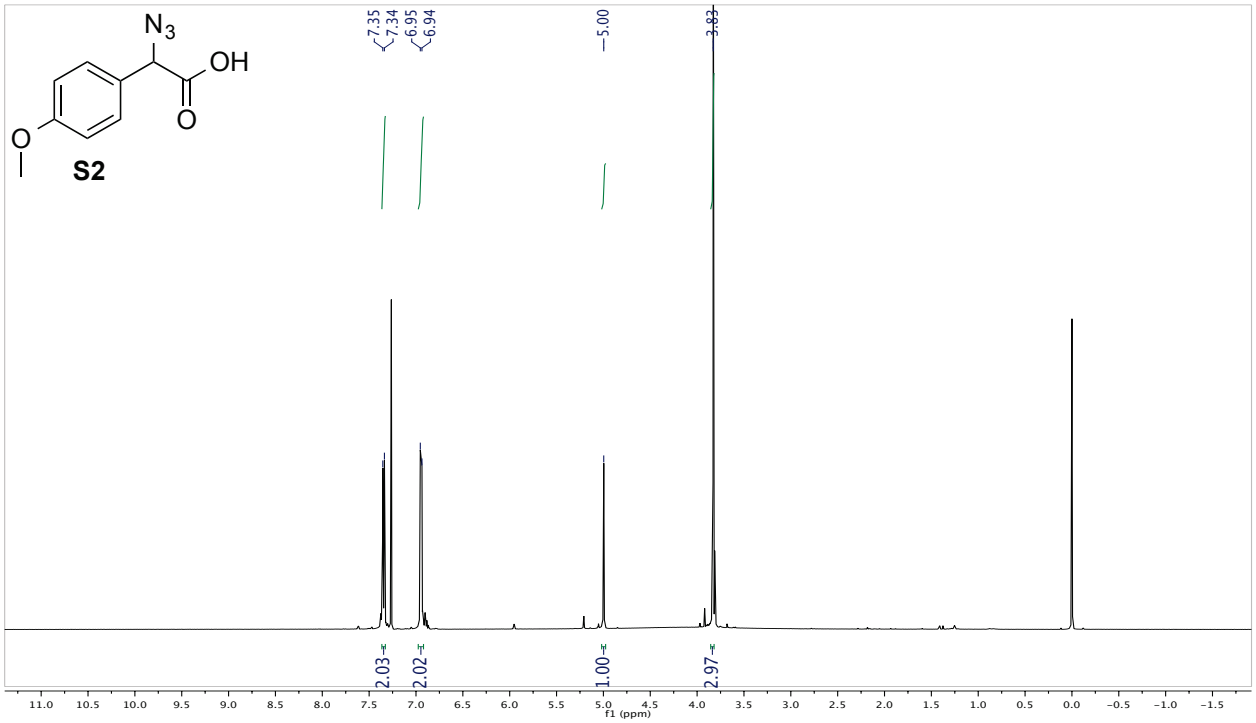
^{1}H NMR of S1 in $CDCl_3$ (500 MHz):



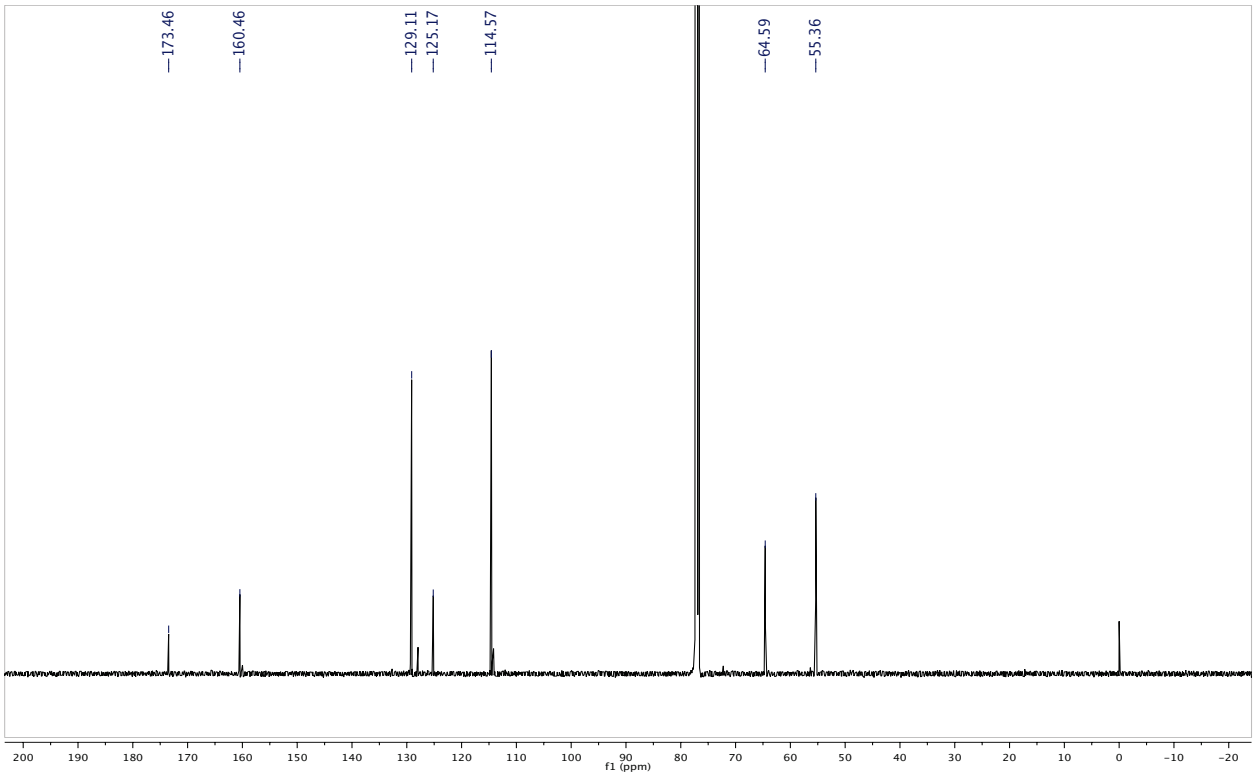
^{13}C NMR of S1 in $CDCl_3$ (125 MHz):



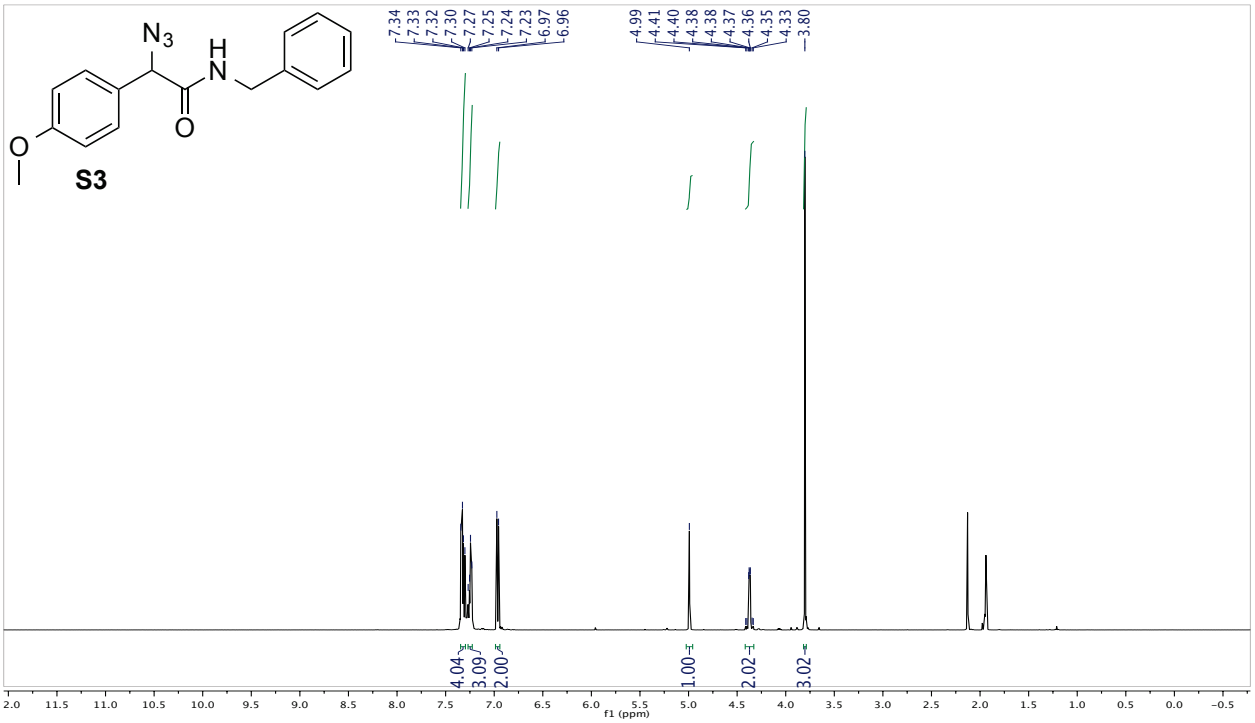
^{1}H NMR of S2 in $CDCl_3$ (500 MHz):



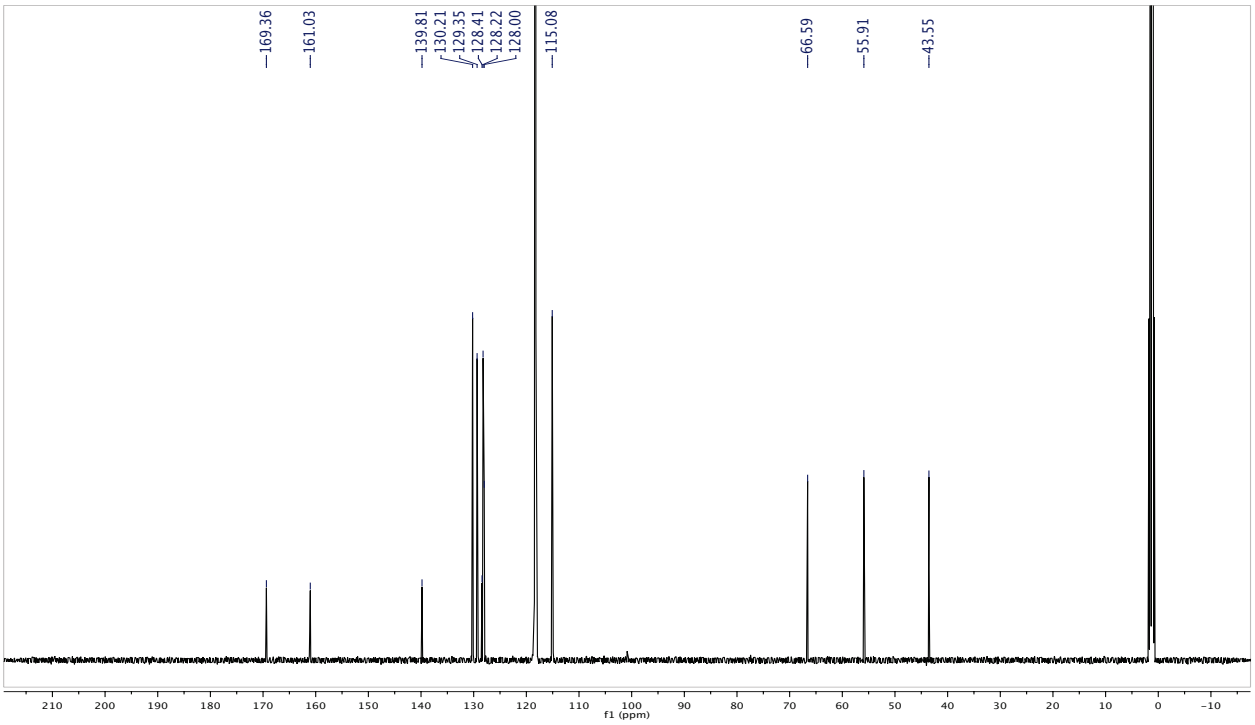
^{13}C NMR of S2 in $CDCl_3$ (125 MHz):



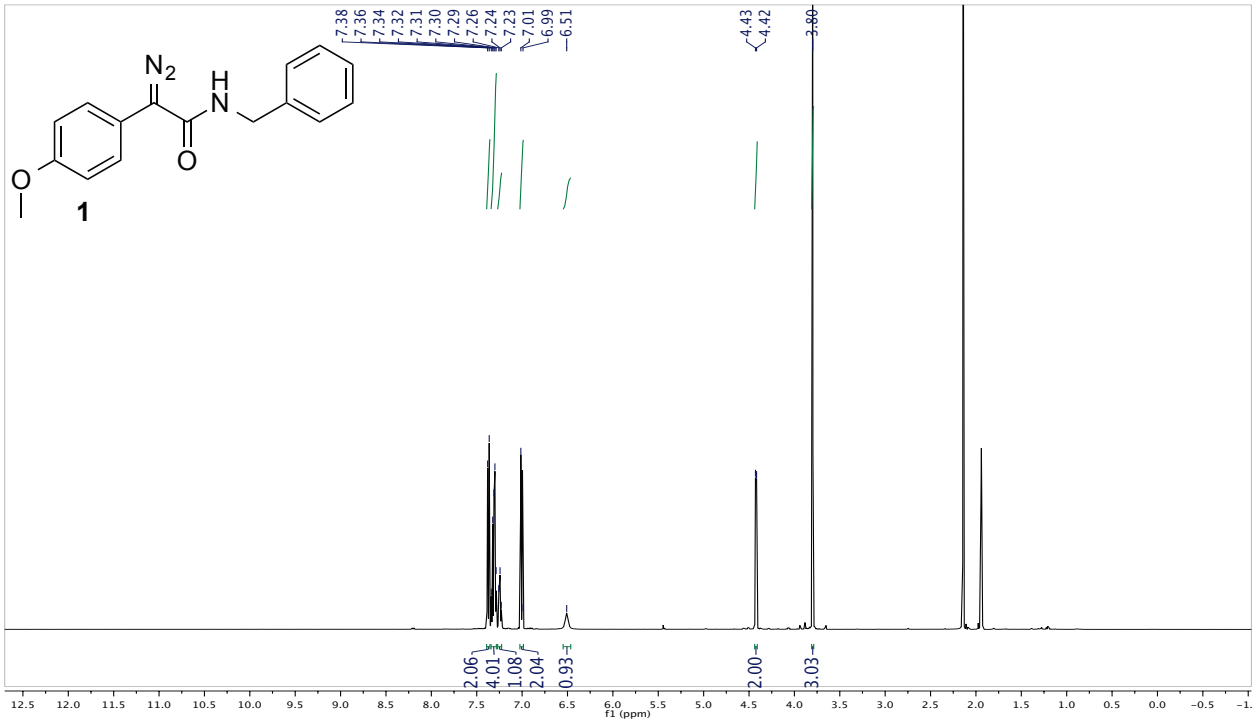
^{1}H NMR of S3 in CD_3CN (500 MHz):



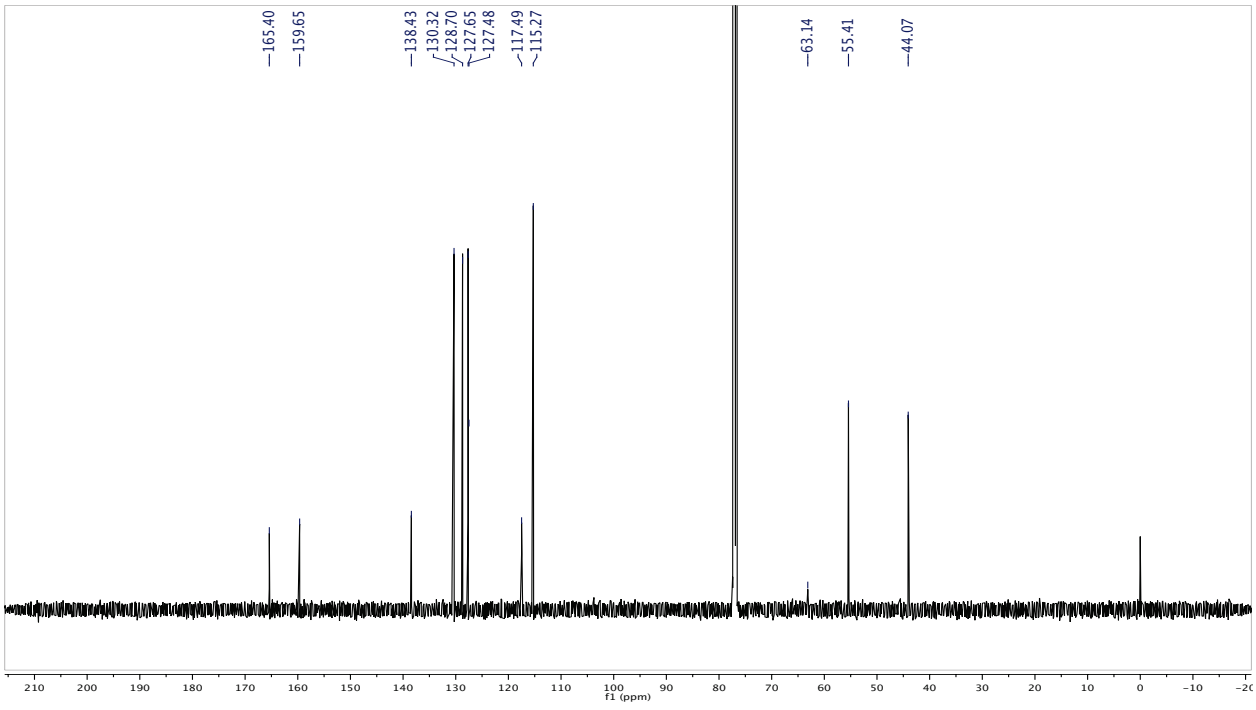
^{13}C NMR of S3 in CD_3CN (125 MHz):



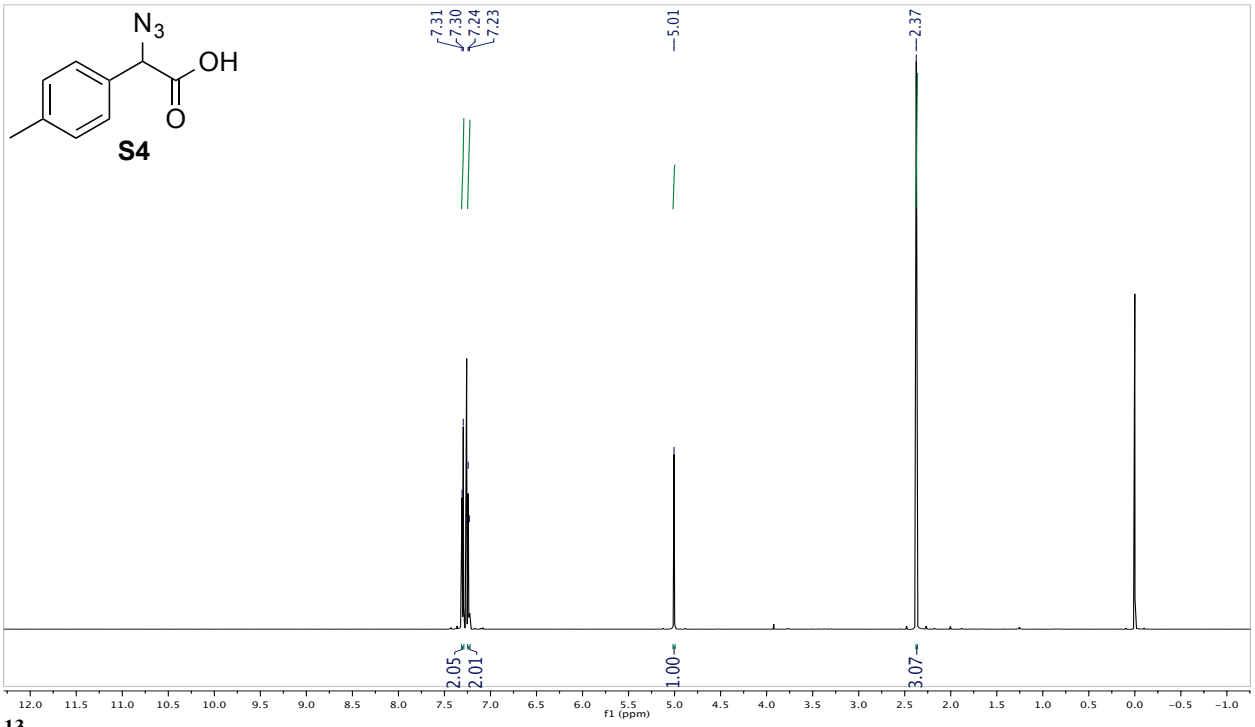
^{1}H NMR of 1 in CD_3CN (500 MHz):



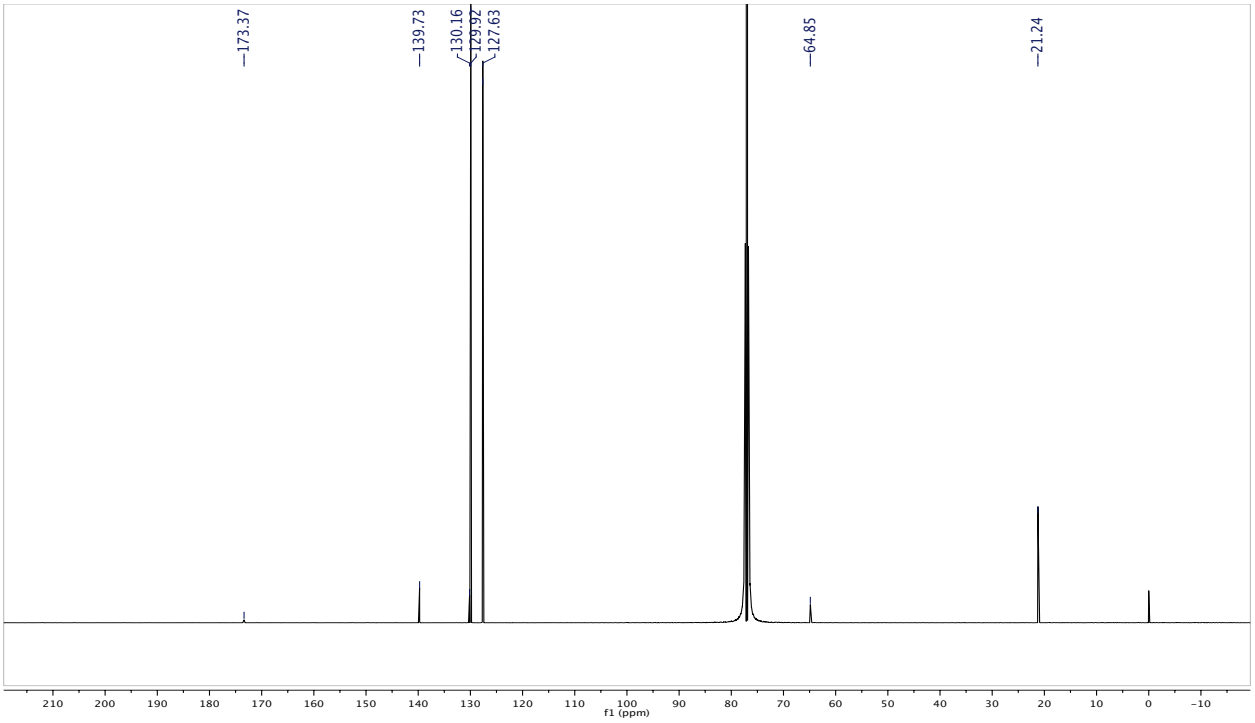
^{13}C NMR of 1 in $CDCl_3$ (125 MHz):



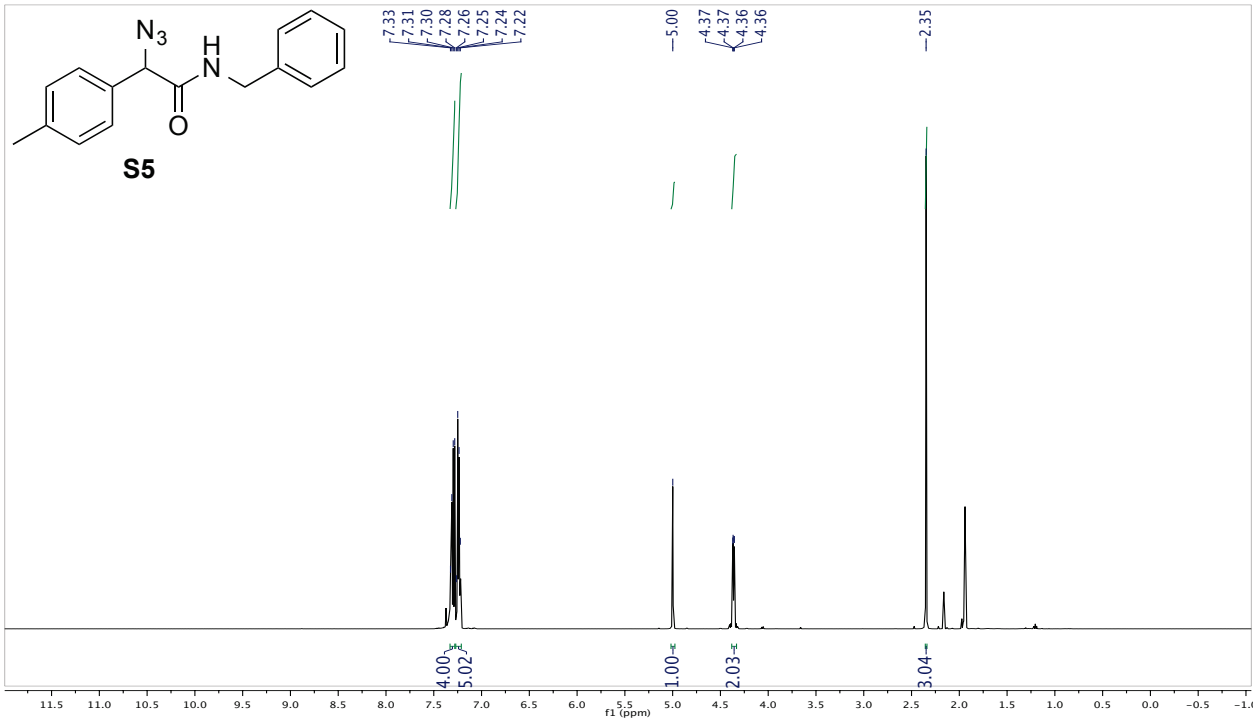
^{1}H NMR of S4 in $CDCl_3$ (600 MHz):



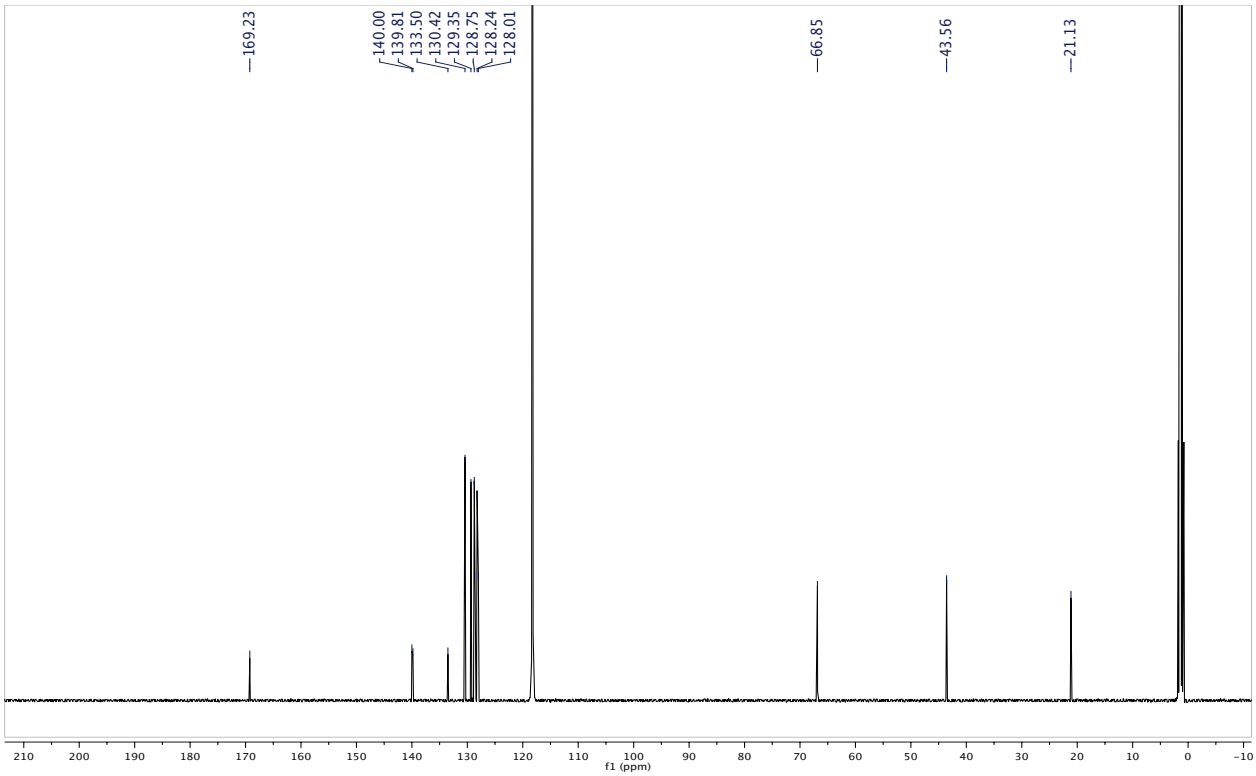
^{13}C NMR of S4 in $CDCl_3$ (150 MHz)



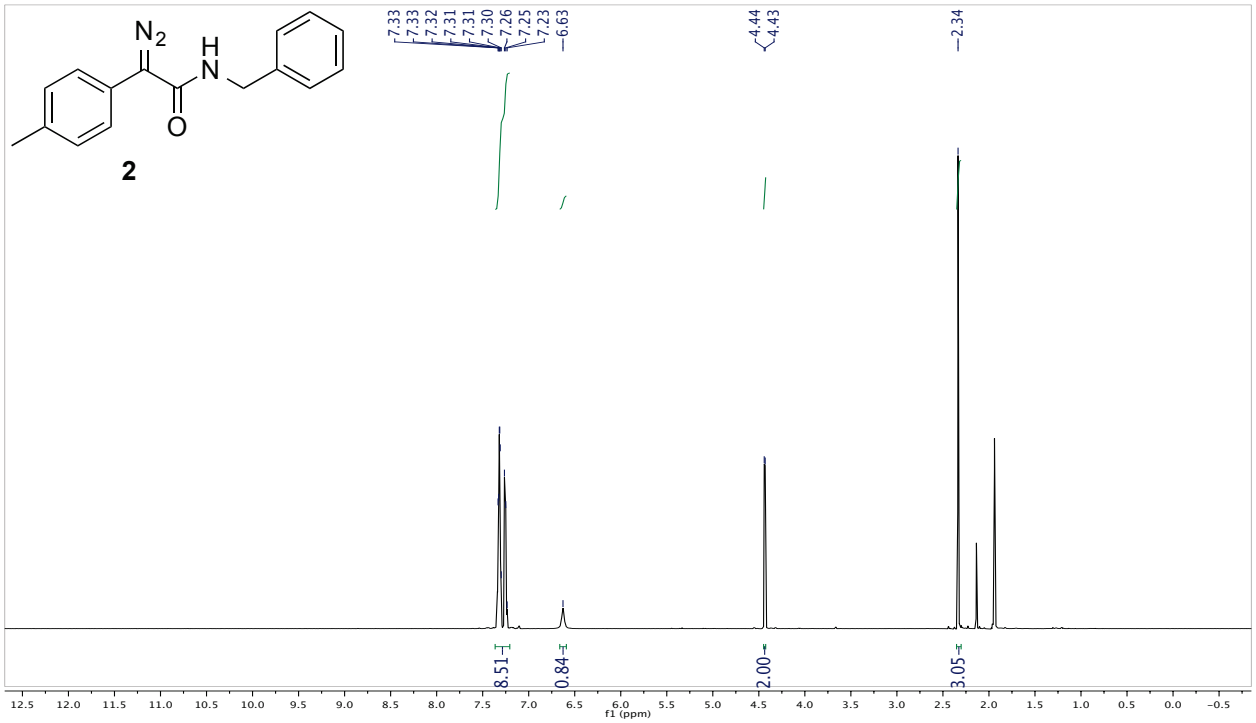
^{1}H NMR of S5 in CD_3CN (500 MHz):



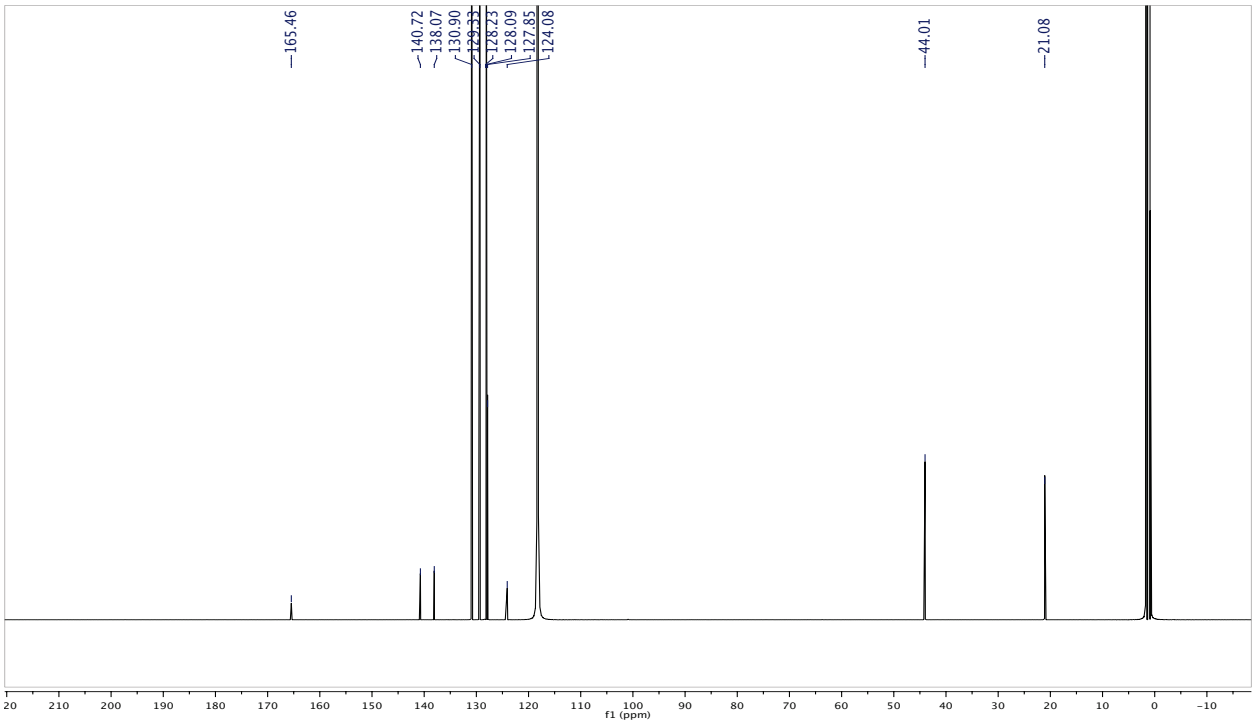
^{13}C NMR of S5 in CD_3CN (125 MHz):



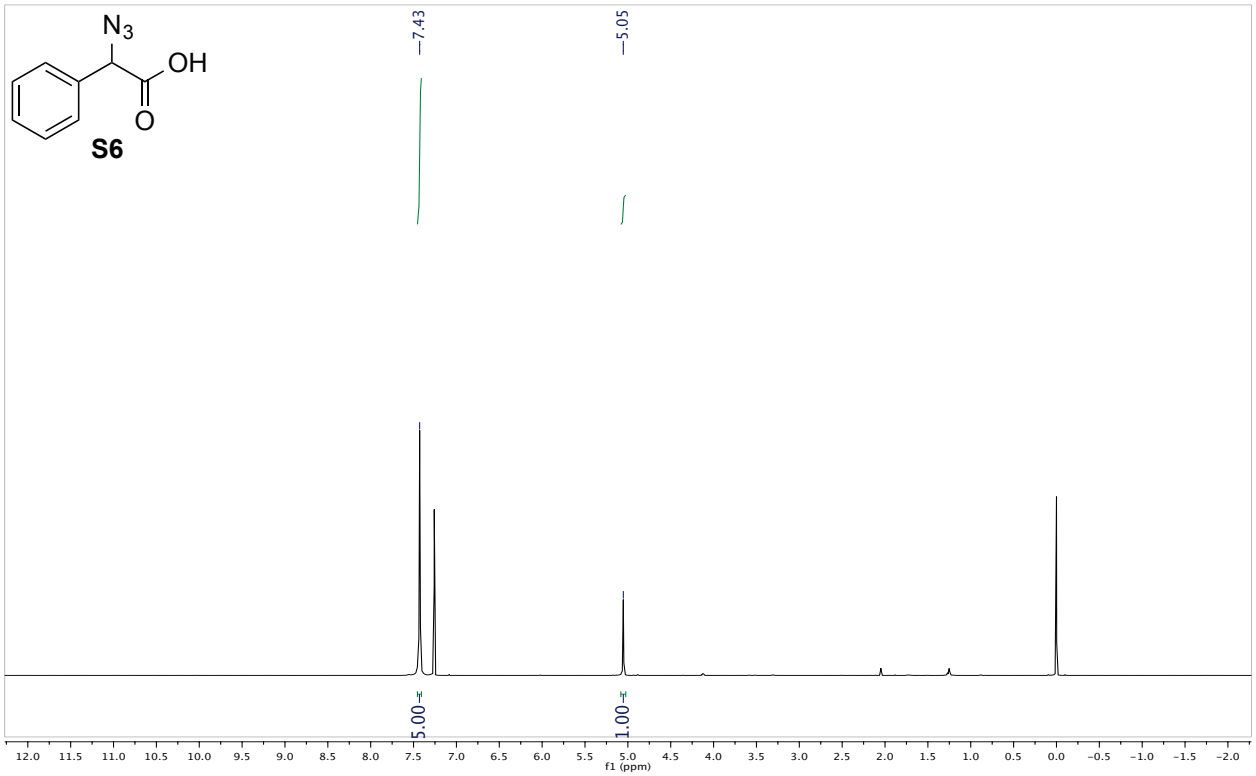
^{1}H NMR of 2 in CD_3CN (600 MHz):



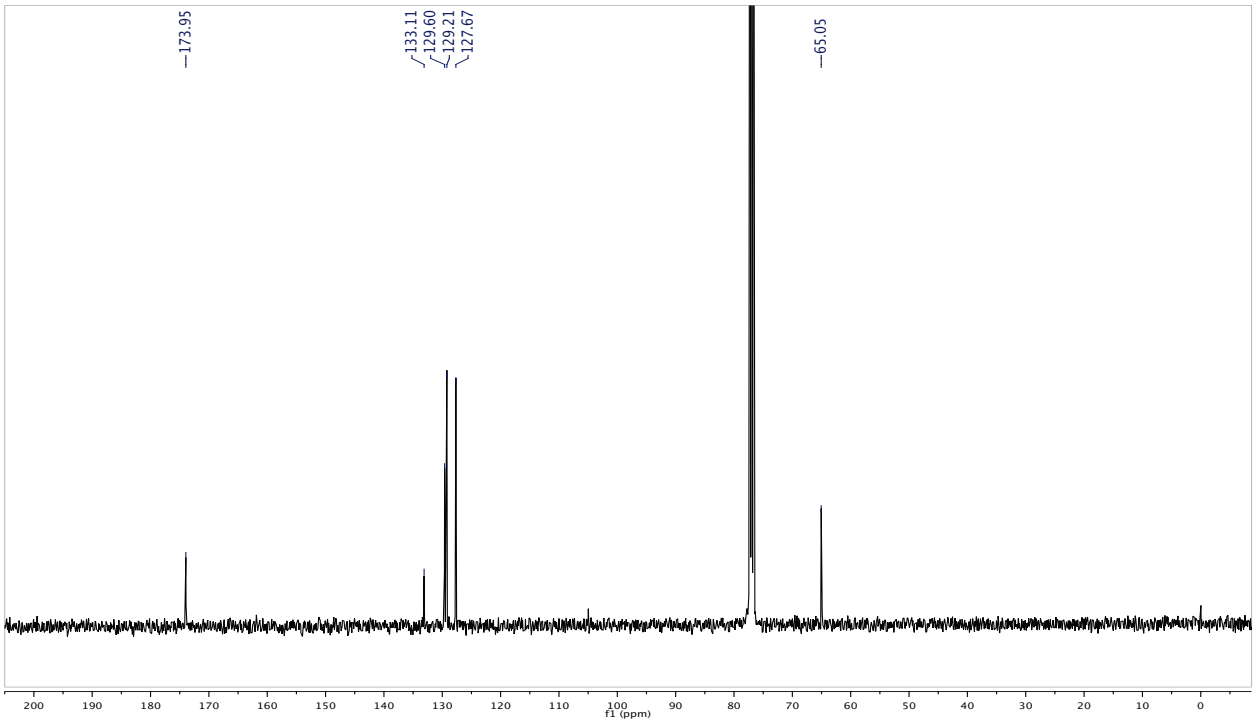
^{13}C NMR of 2 in CD_3CN (150 MHz):



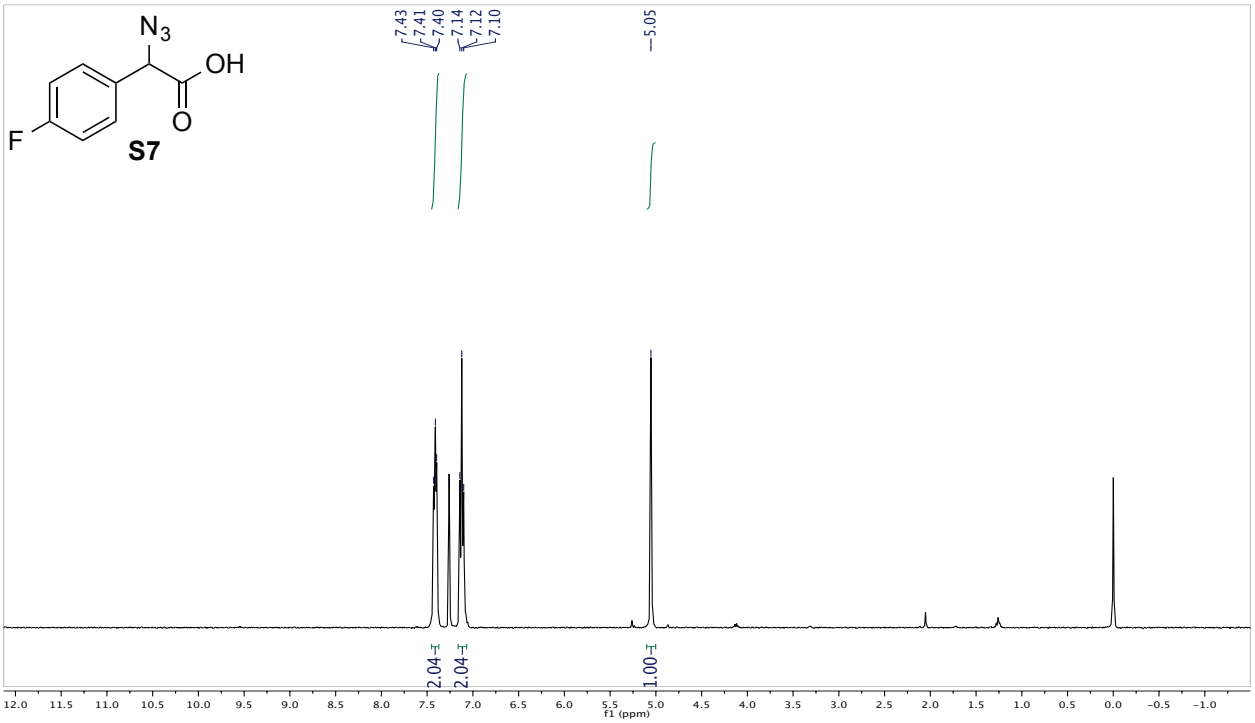
^{1}H NMR of S6 in $CDCl_3$ (400 MHz):



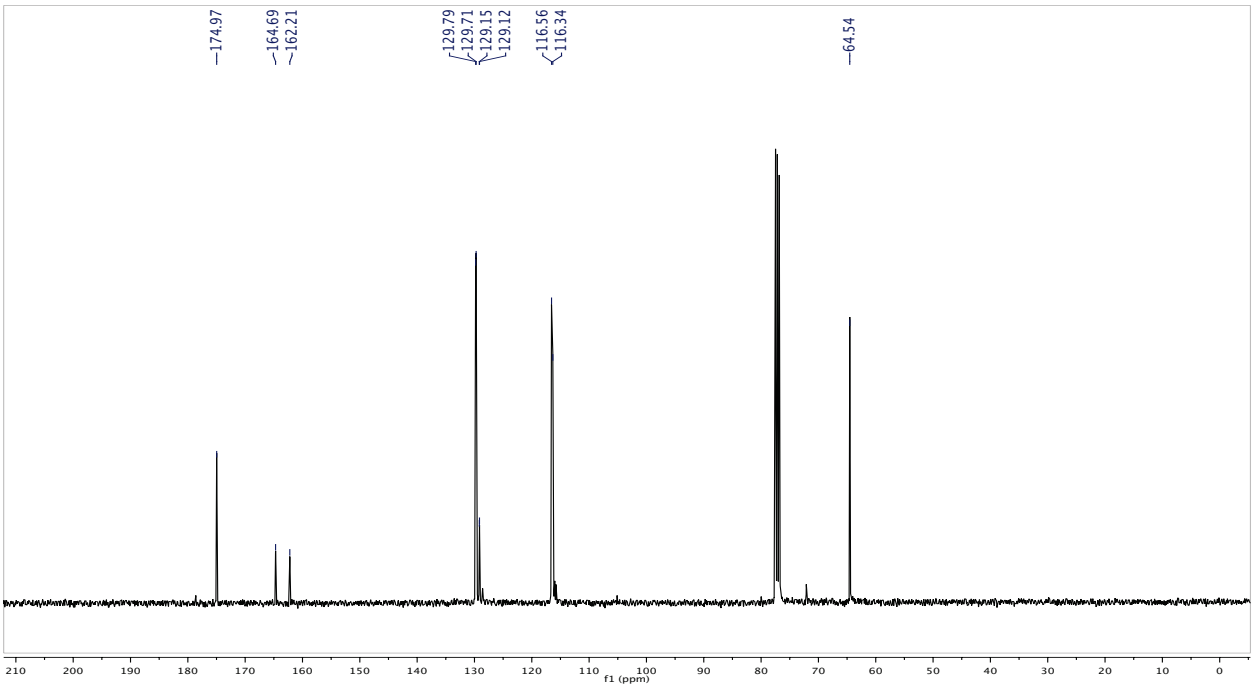
^{13}C NMR of S6 in $CDCl_3$ (100 MHz):



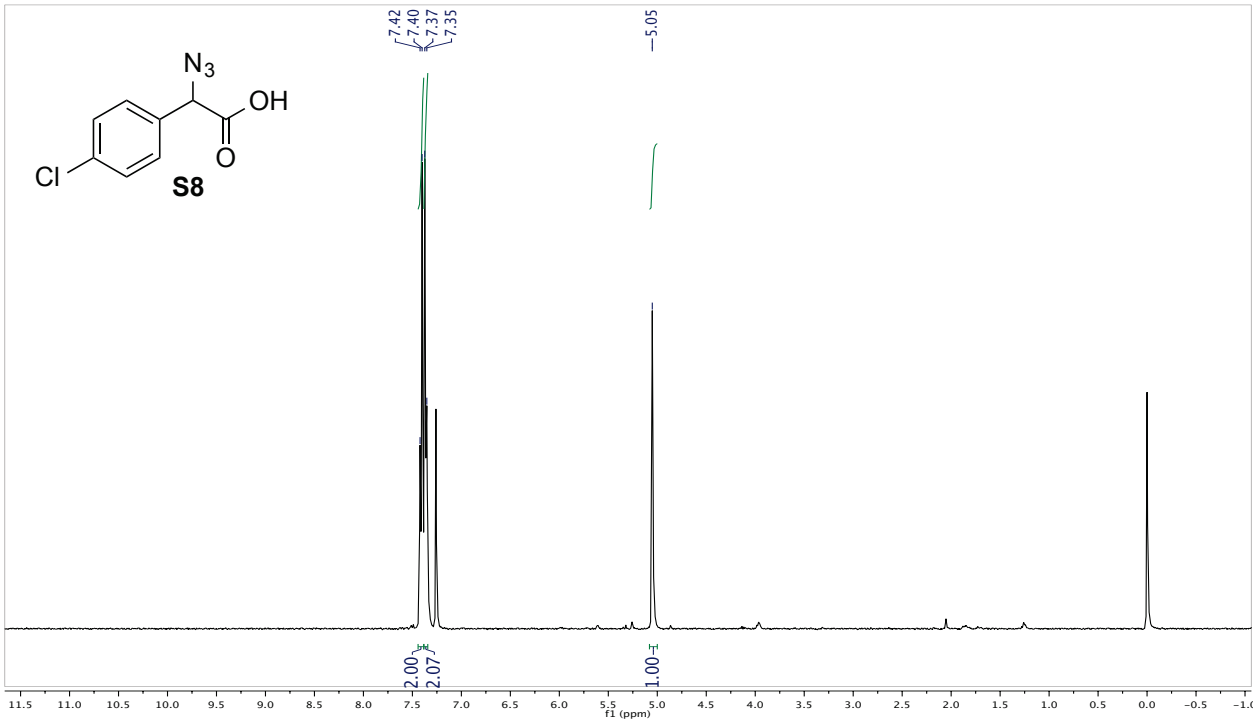
^{1}H NMR of S7 in $CDCl_3$ (400 MHz):



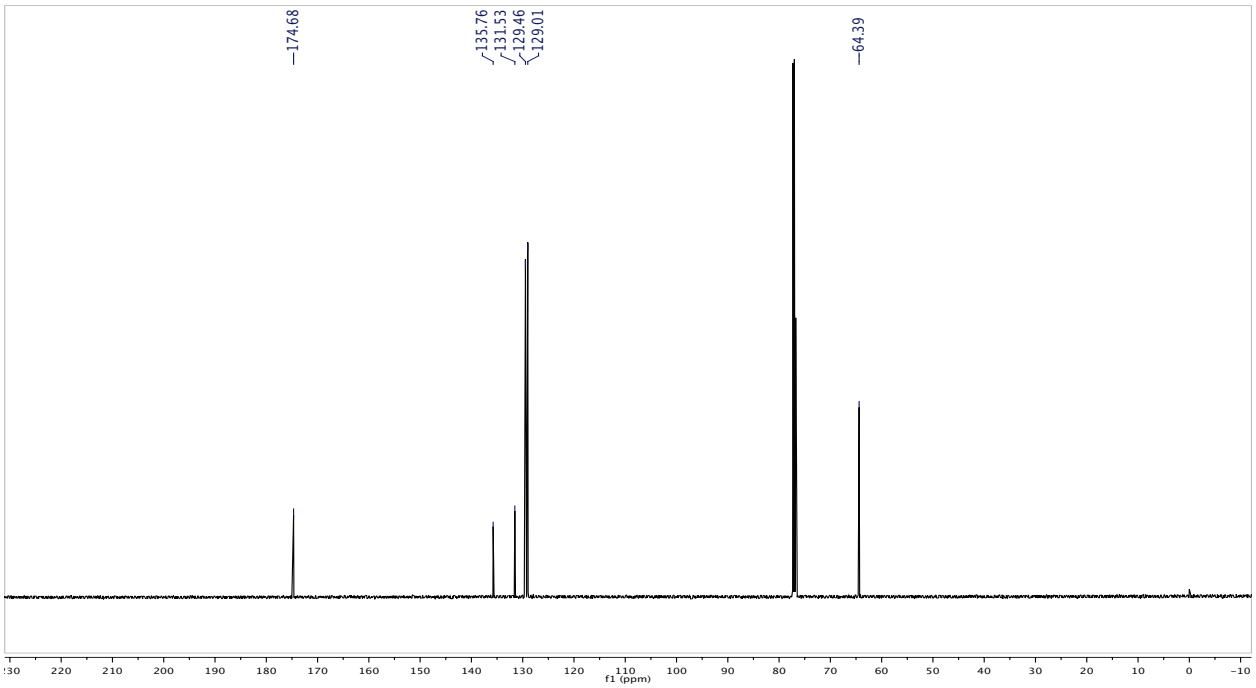
^{13}C NMR of S7 in $CDCl_3$ (100 MHz):



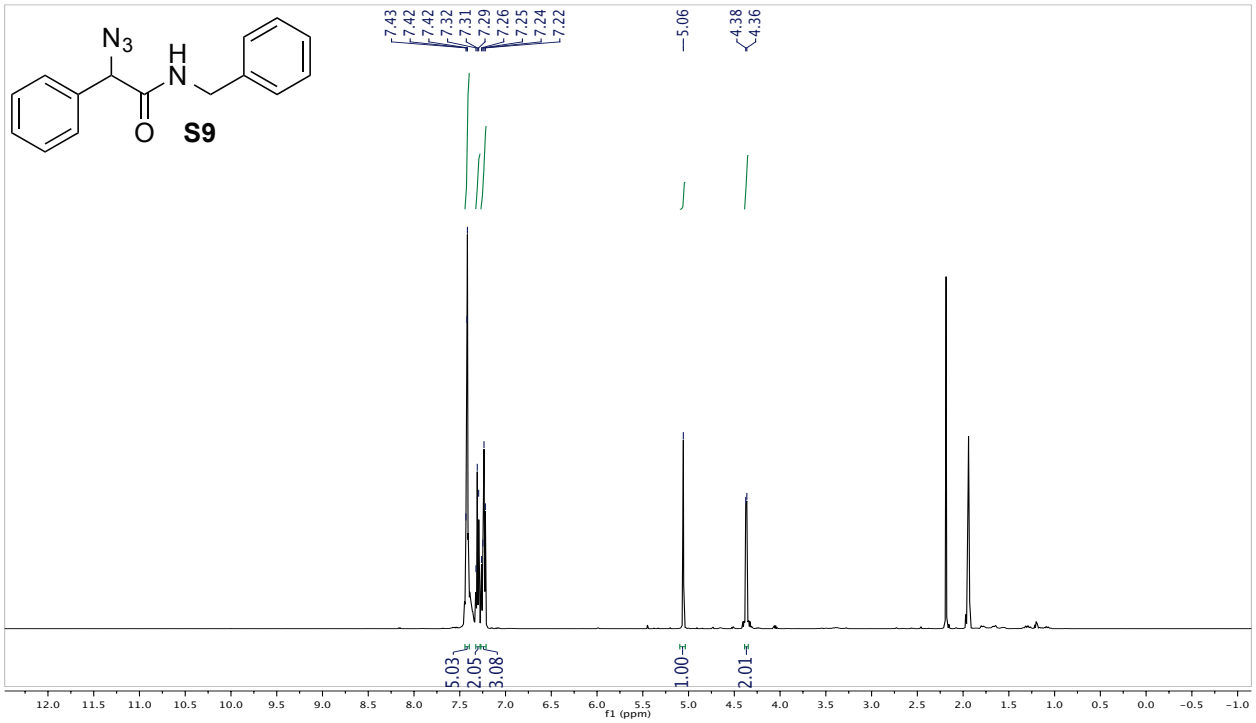
^{1}H NMR of S8 in $CDCl_3$ (400 MHz):



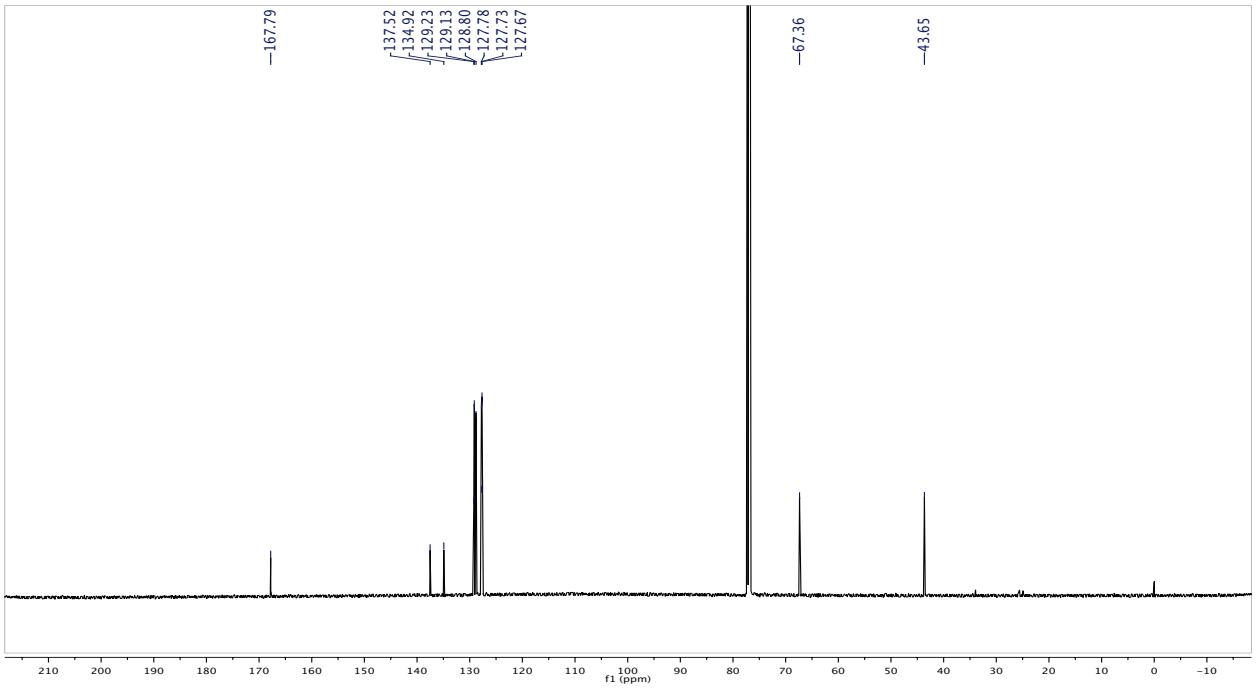
^{13}C NMR of S8 in $CDCl_3$ (125 MHz):



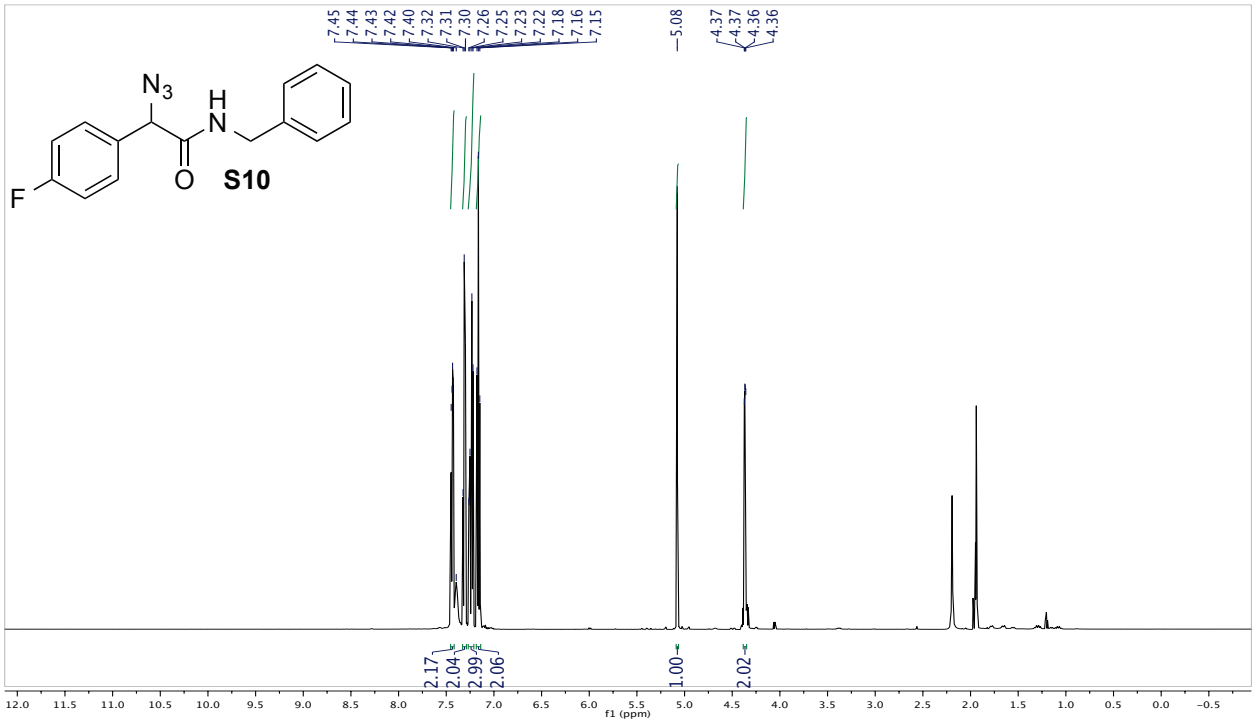
^{1}H NMR of S9 in CD_3CN (500 MHz):



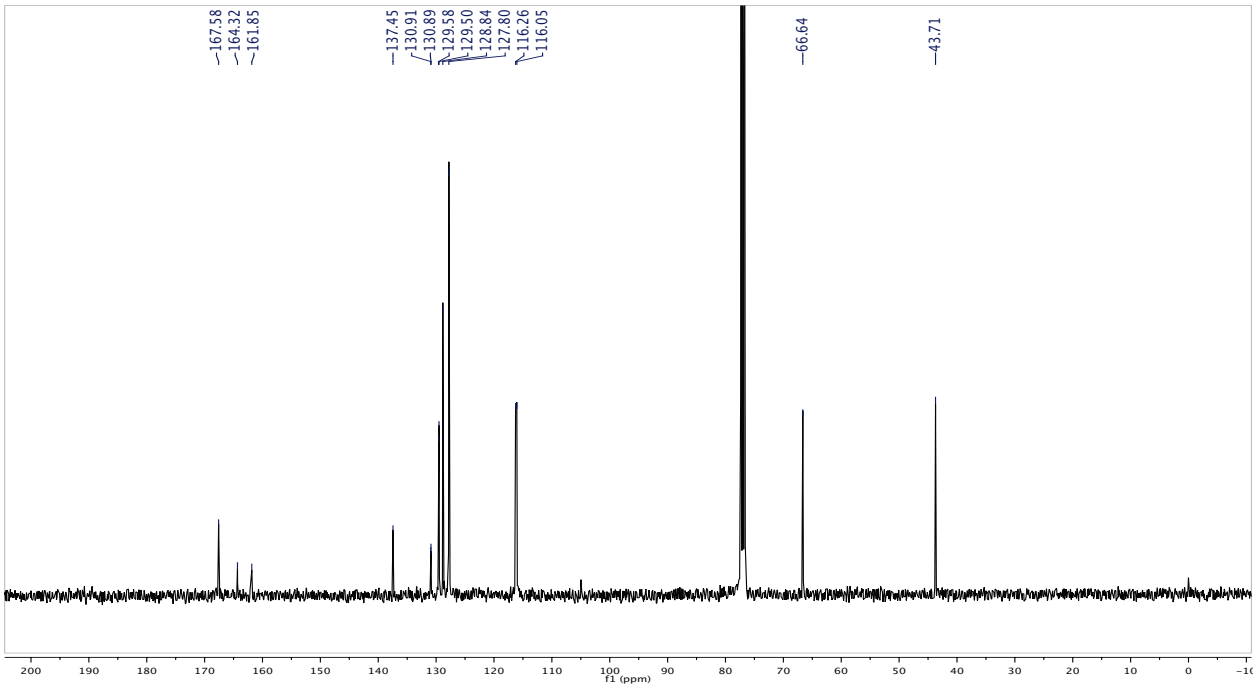
^{13}C NMR of S9 in $CDCl_3$ (125 MHz):



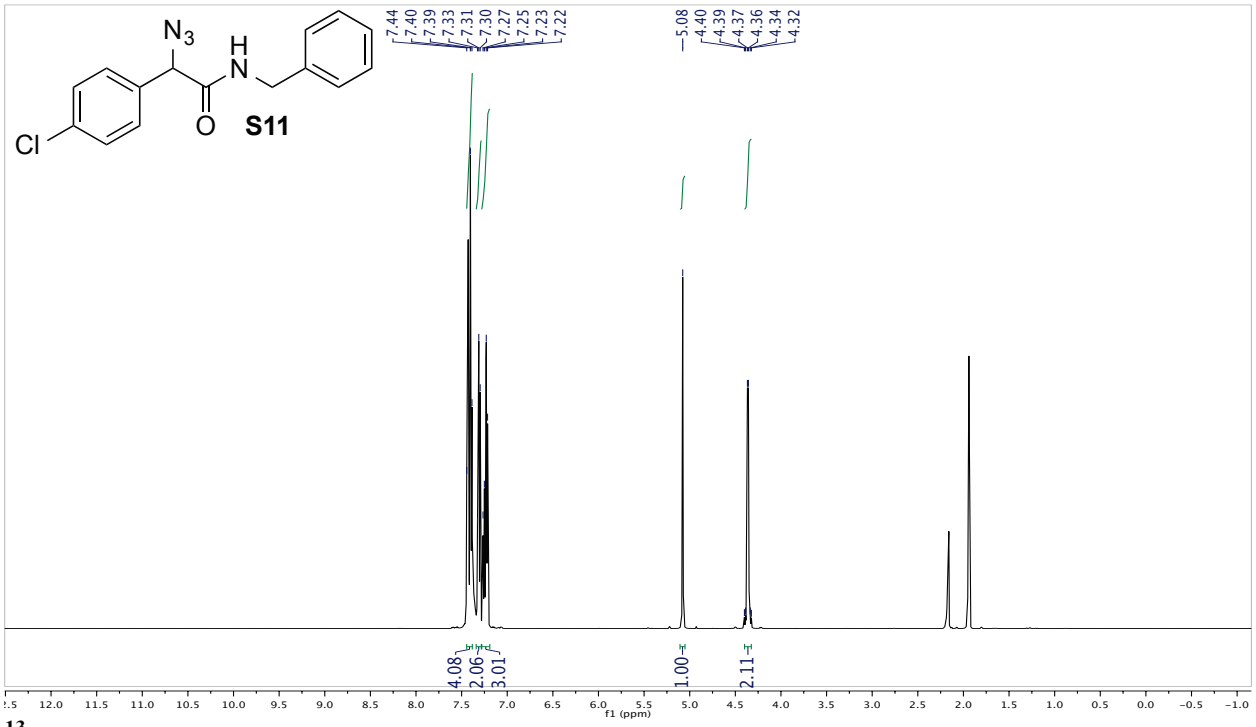
^{1}H NMR of S10 in CD_3CN (600 MHz):



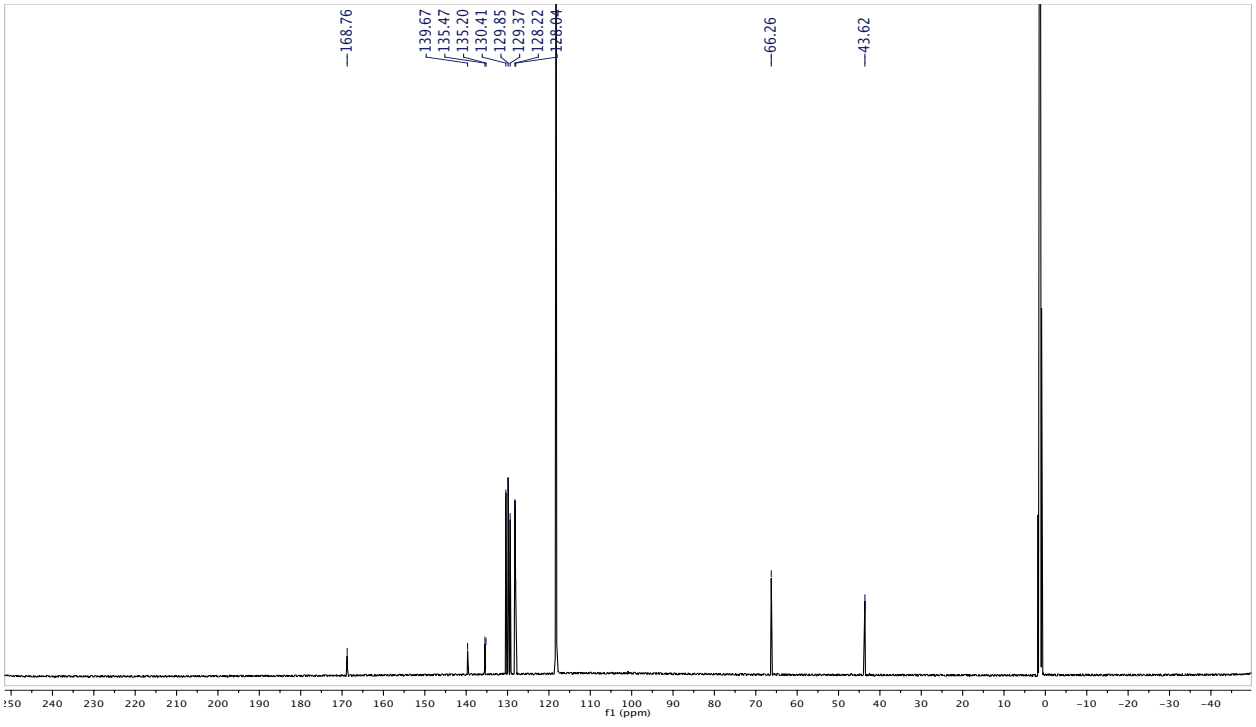
^{13}C NMR of S10 in $CDCl_3$ (100 MHz):



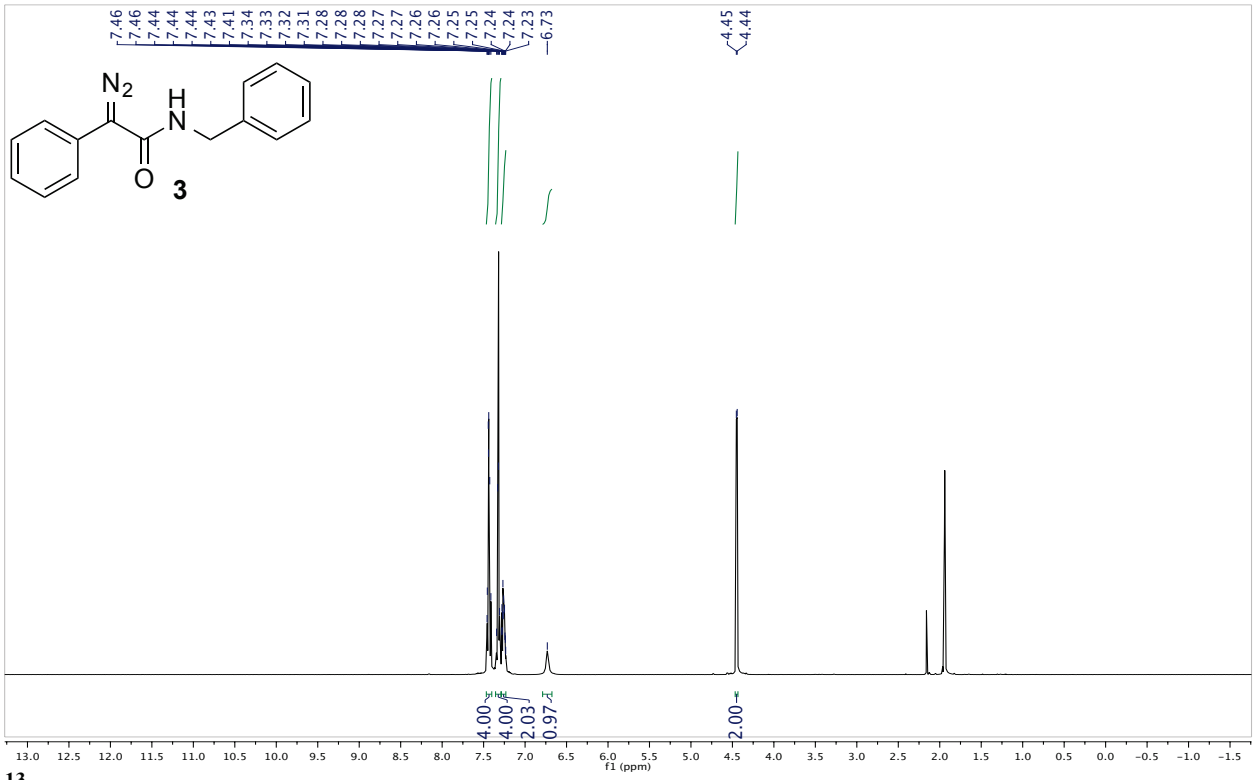
^{1}H NMR of S11 in CD_3CN (500 MHz):



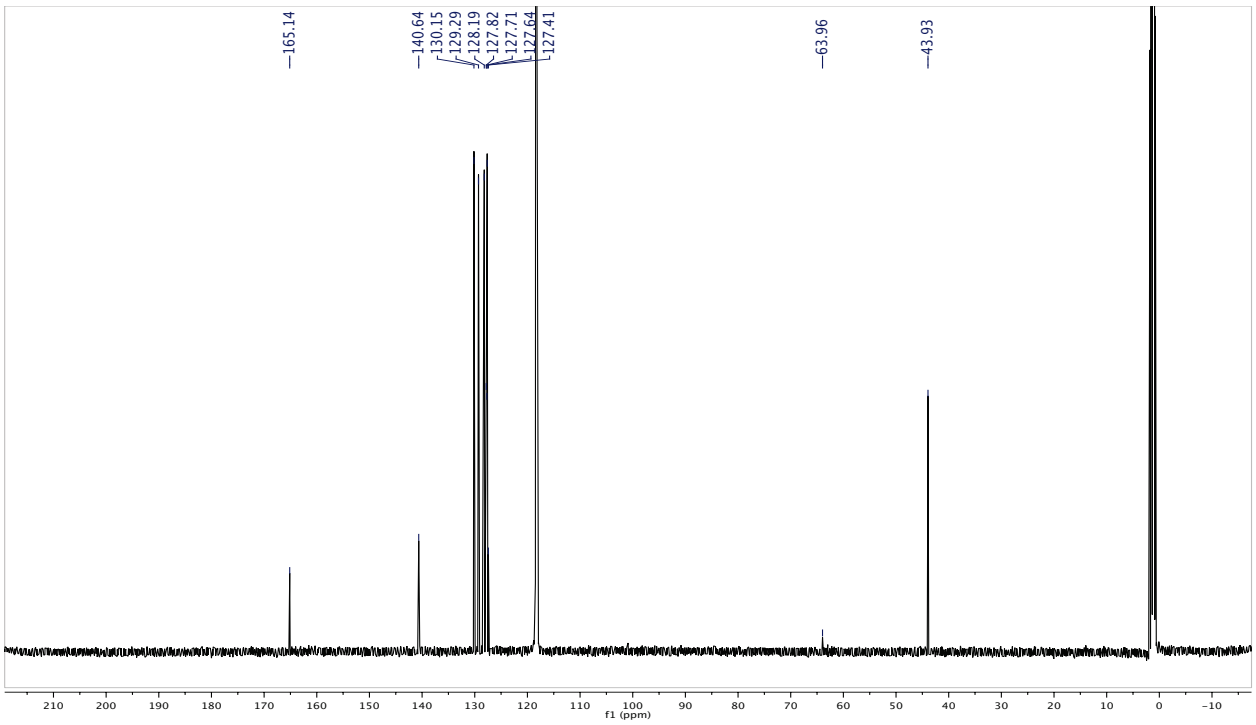
^{13}C NMR of S11 in CD_3CN (125 MHz):



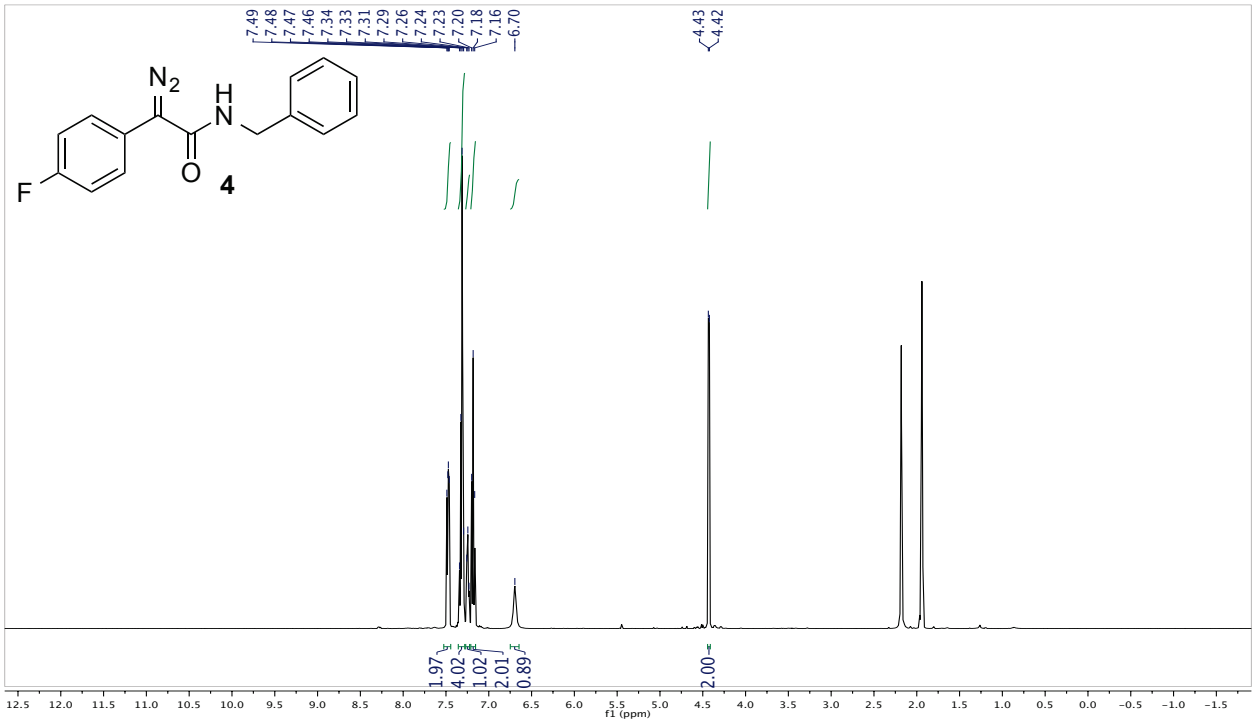
^{1}H NMR of 3 in CD_3CN (600 MHz):



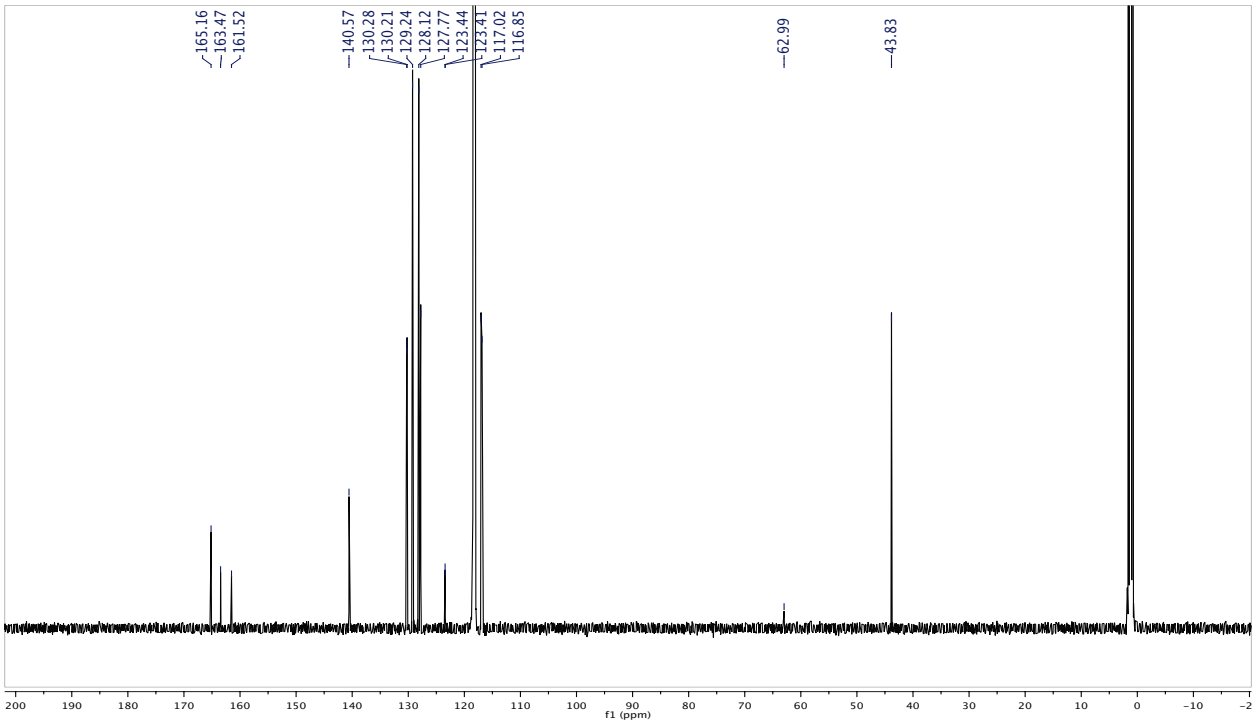
^{13}C NMR of 3 in CD_3CN (125 MHz):



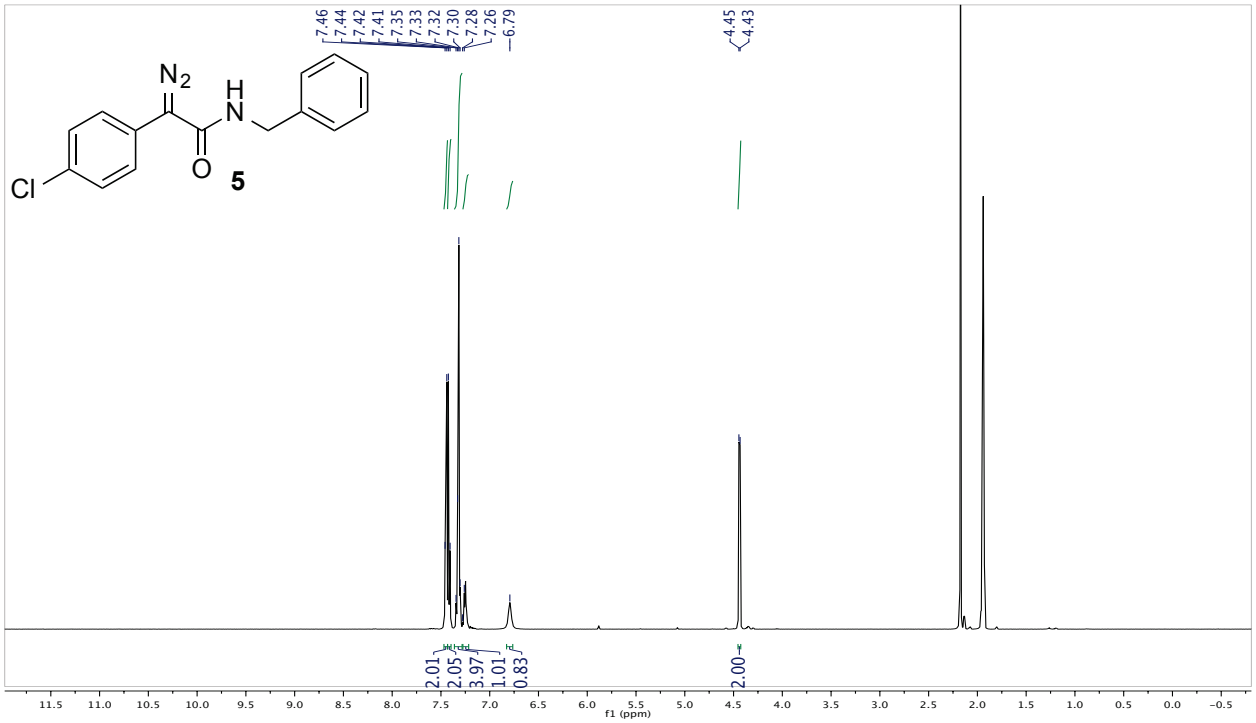
^{1}H NMR of 4 in CD_3CN (500 MHz):



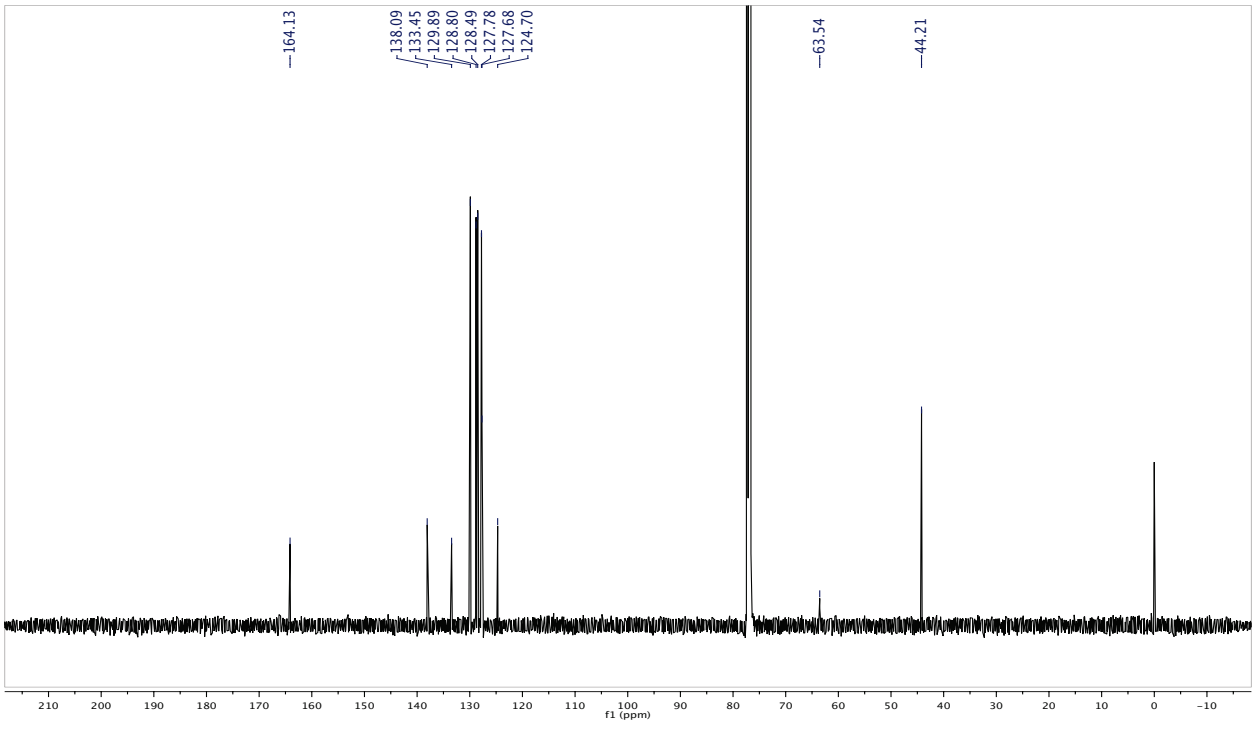
^{13}C NMR of 4 in CD_3CN (125 MHz):



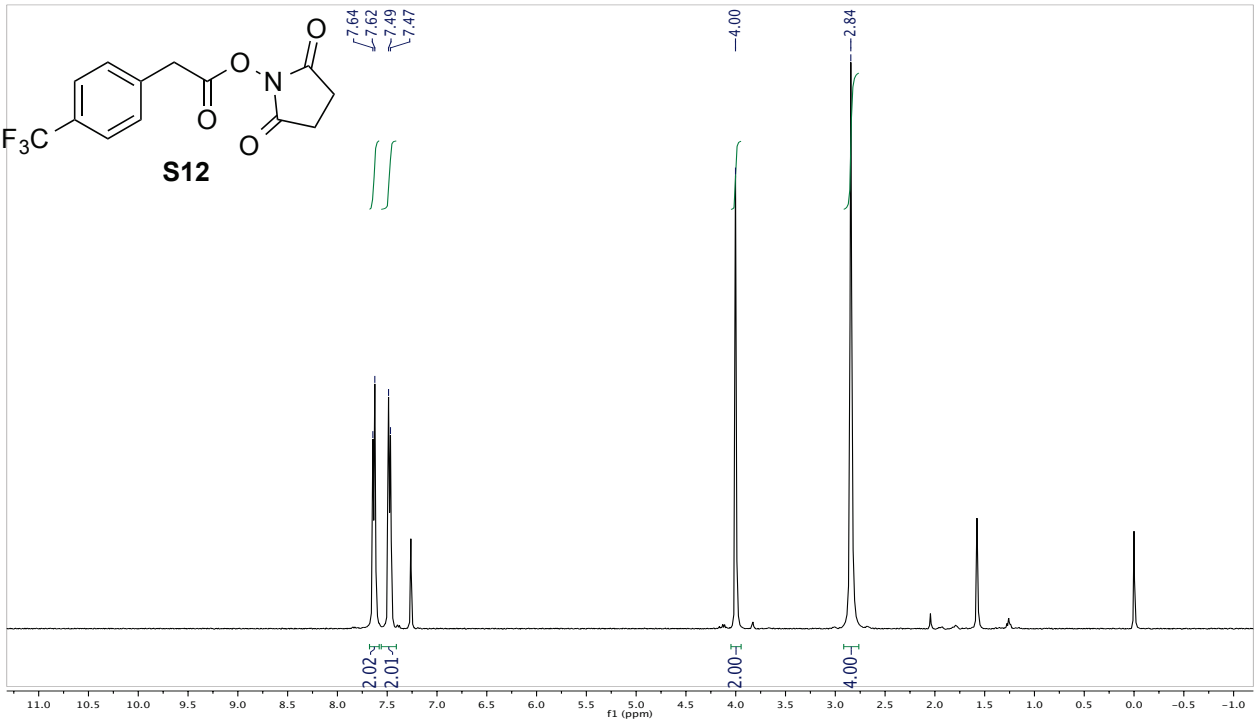
^{1}H NMR of 5 in CD_3CN (500 MHz):



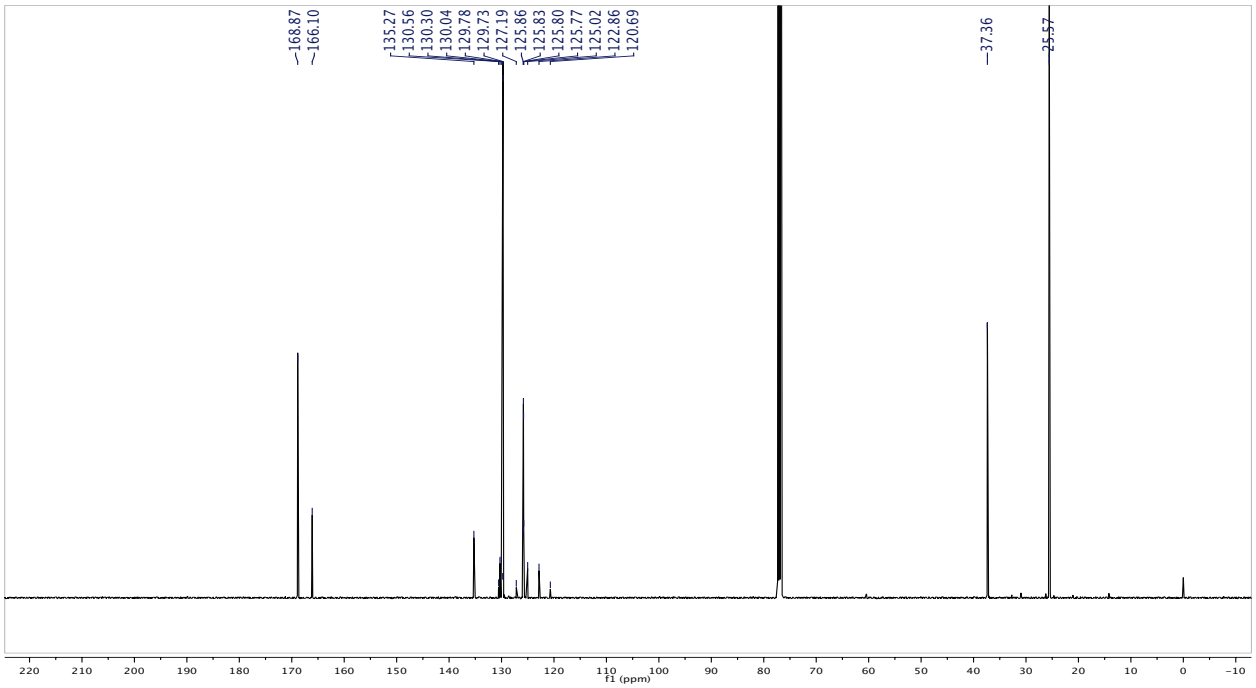
^{13}C NMR of 5 in $CDCl_3$ (125 MHz):



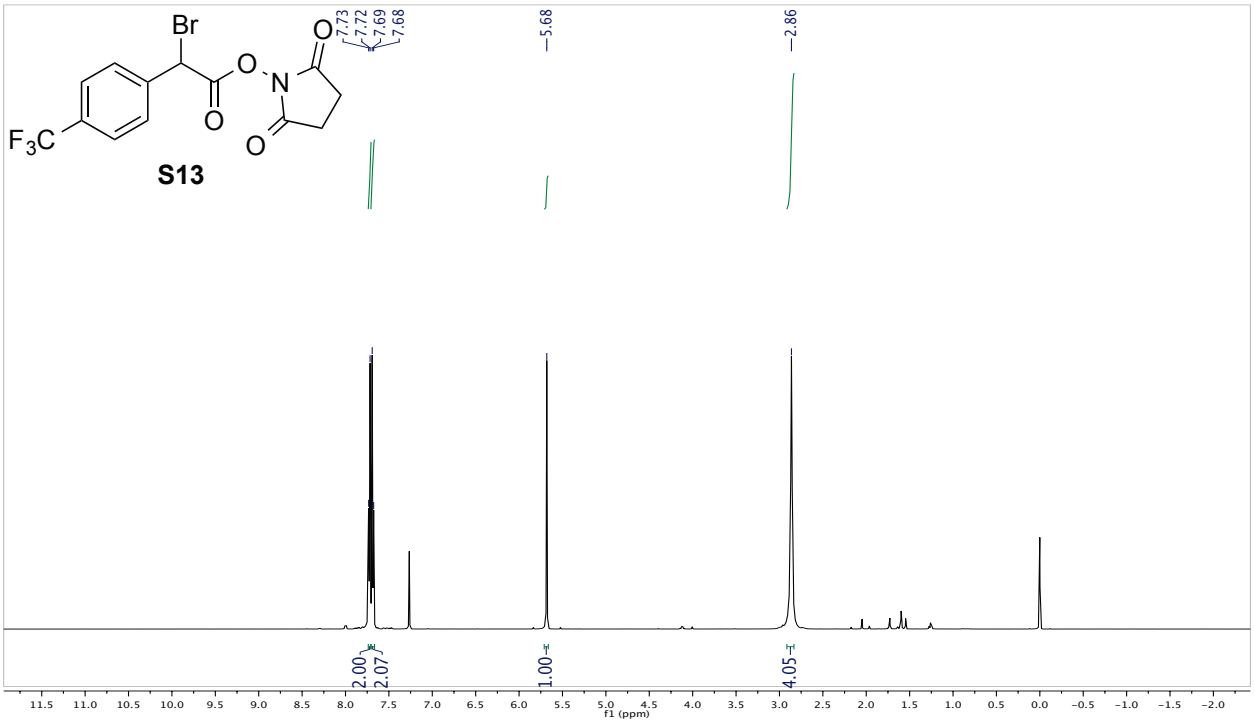
^{1}H NMR of S12 in $CDCl_3$ (400 MHz):



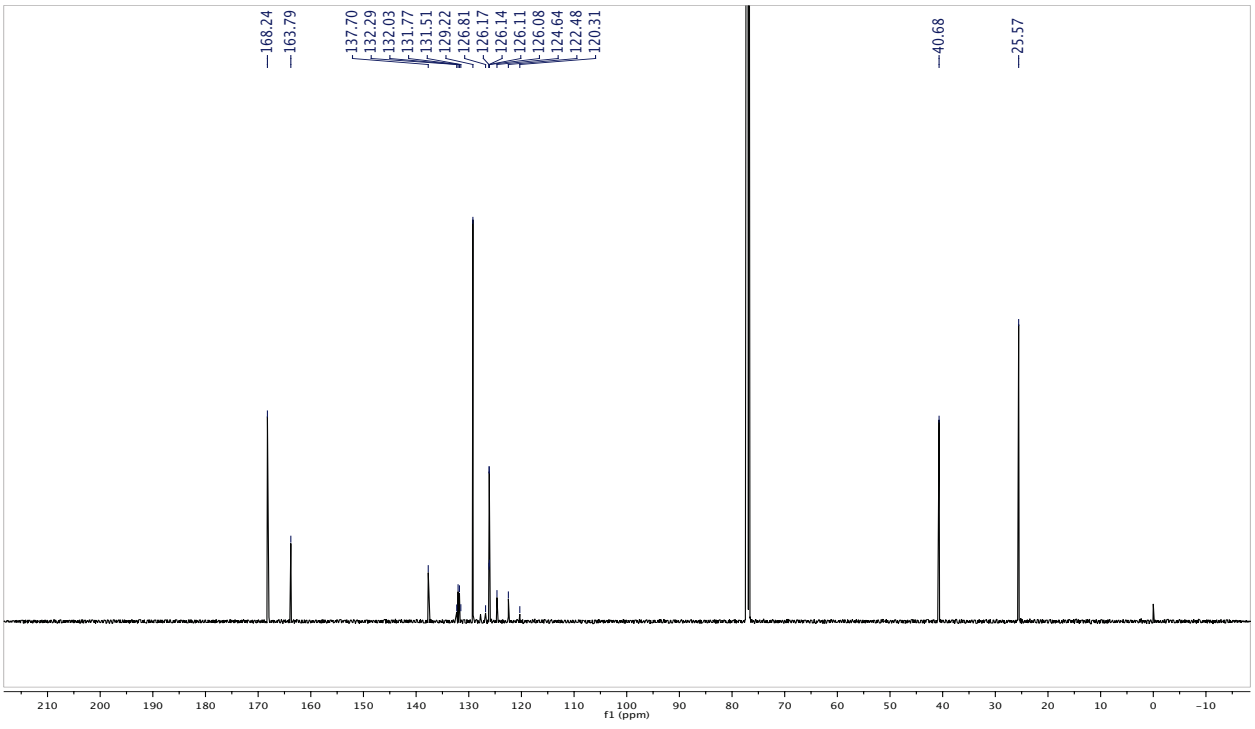
^{13}C NMR of S12 in $CDCl_3$ (125 MHz):



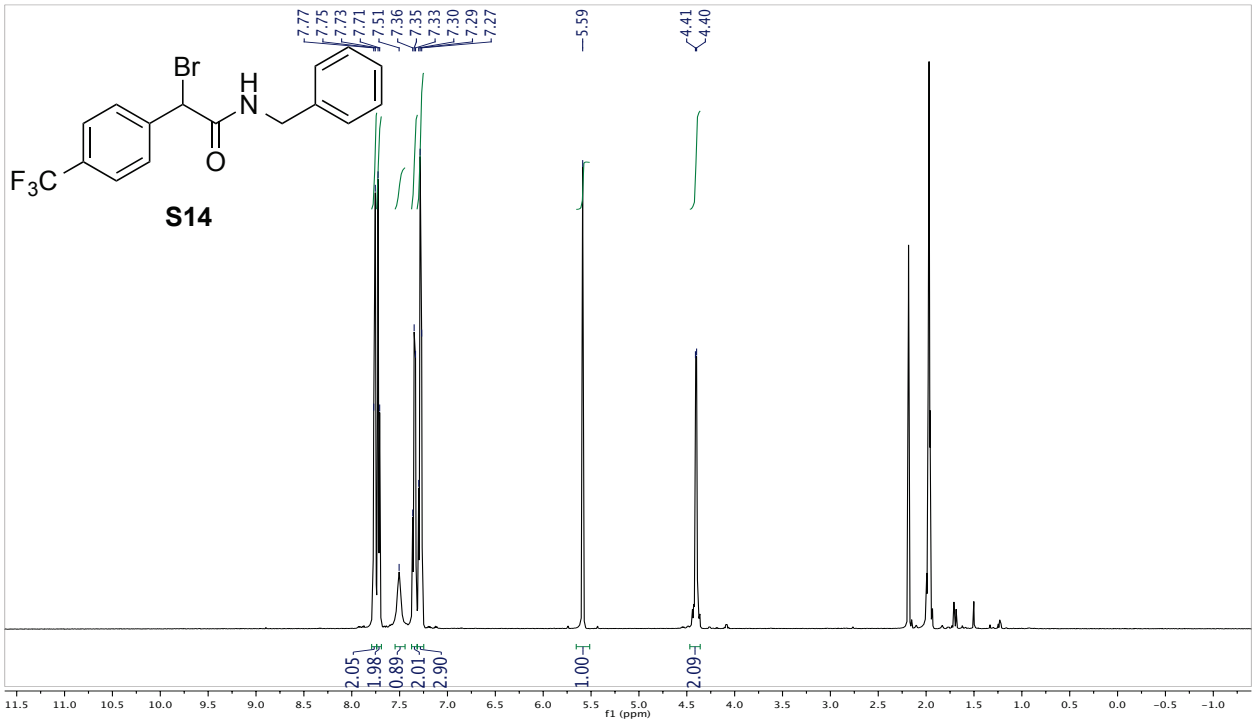
^{1}H NMR of S13 in $CDCl_3$ (500 MHz):



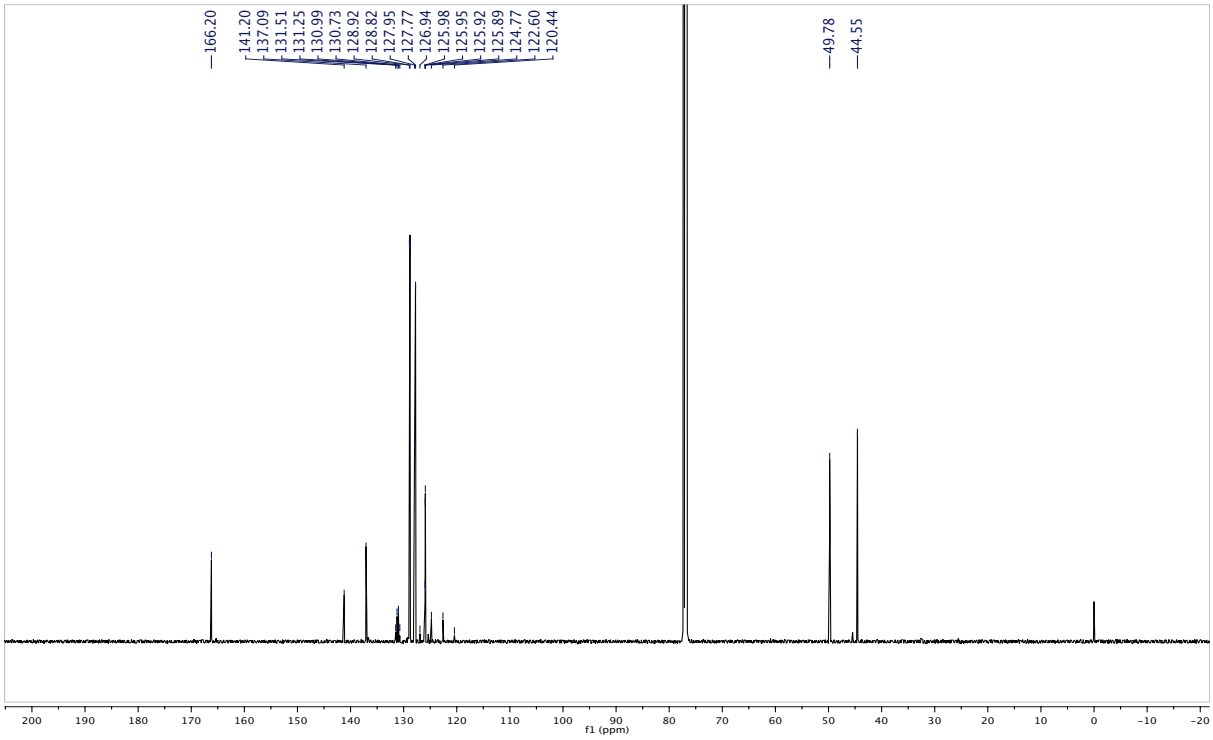
^{13}C NMR of S13 in $CDCl_3$ (125 MHz):



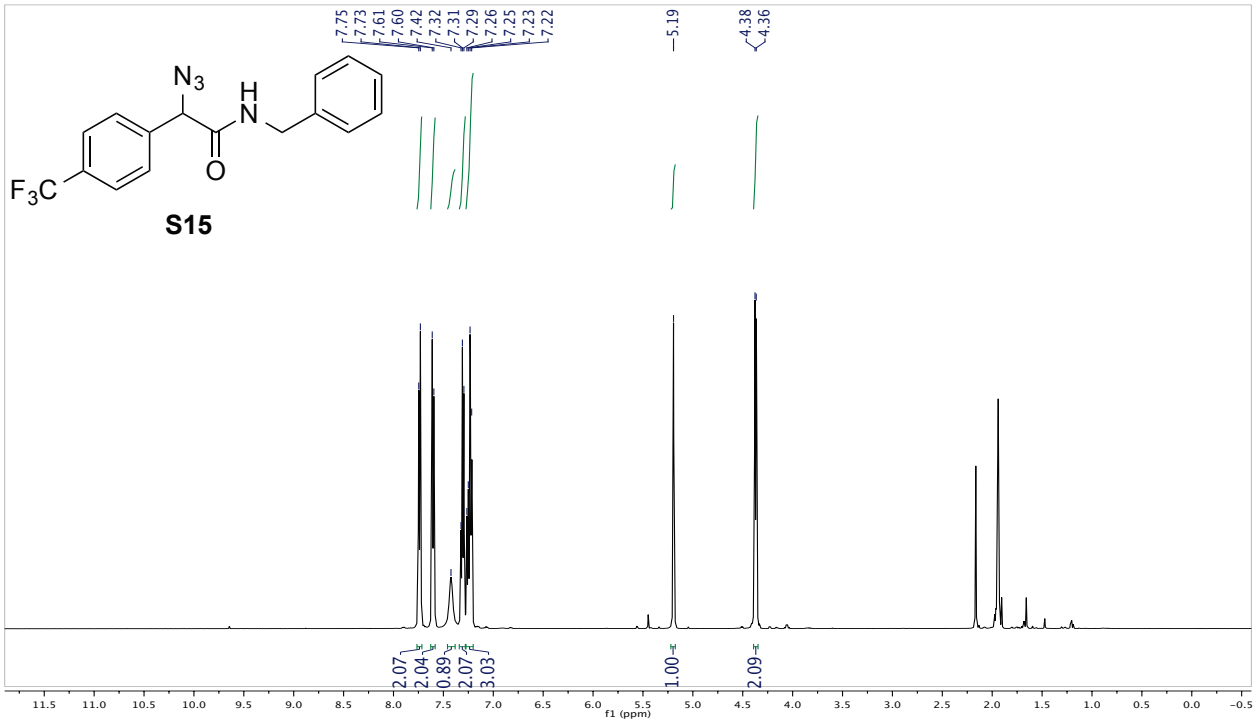
^{1}H NMR of S14 in CD_3CN (500 MHz):



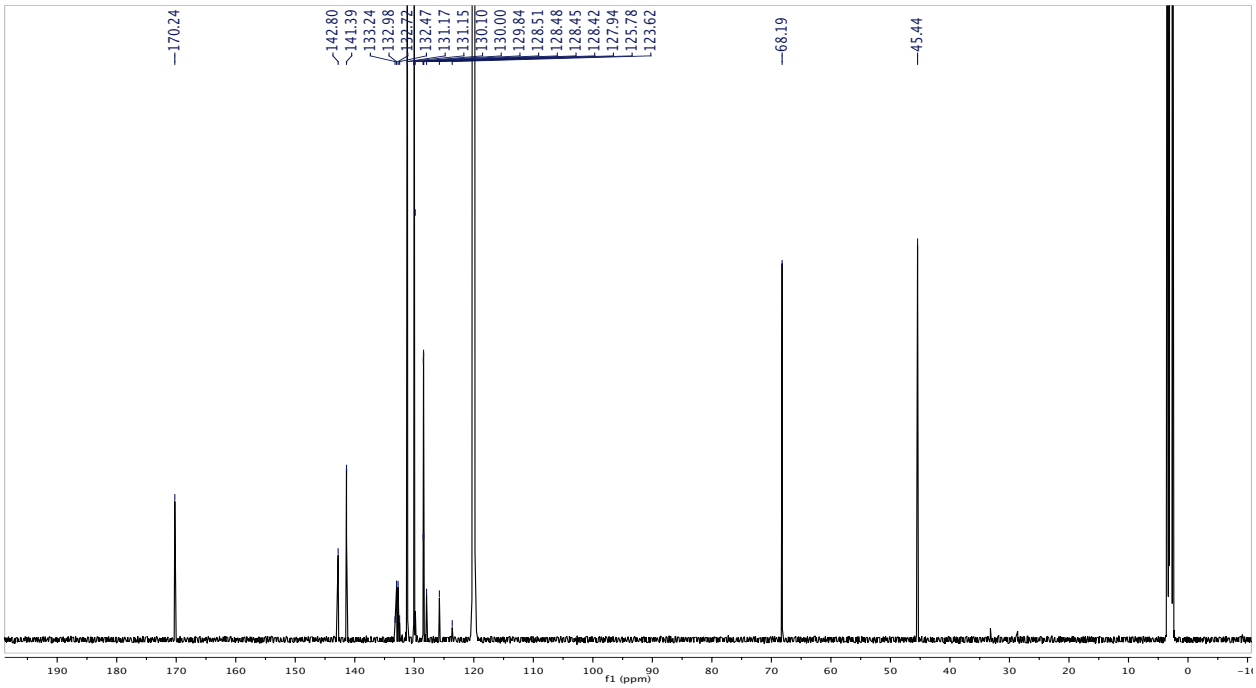
^{13}C NMR of S14 in $CDCl_3$ (125 MHz):



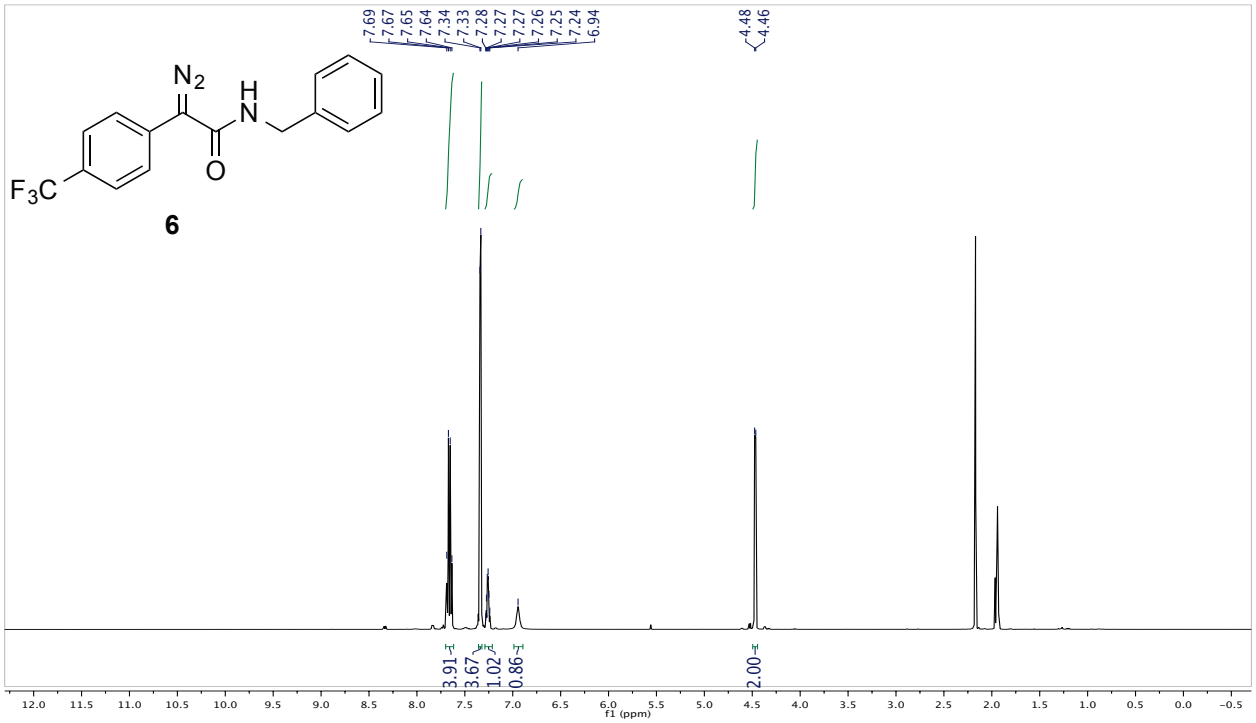
^{1}H NMR of S15 in CD_3CN (500 MHz):



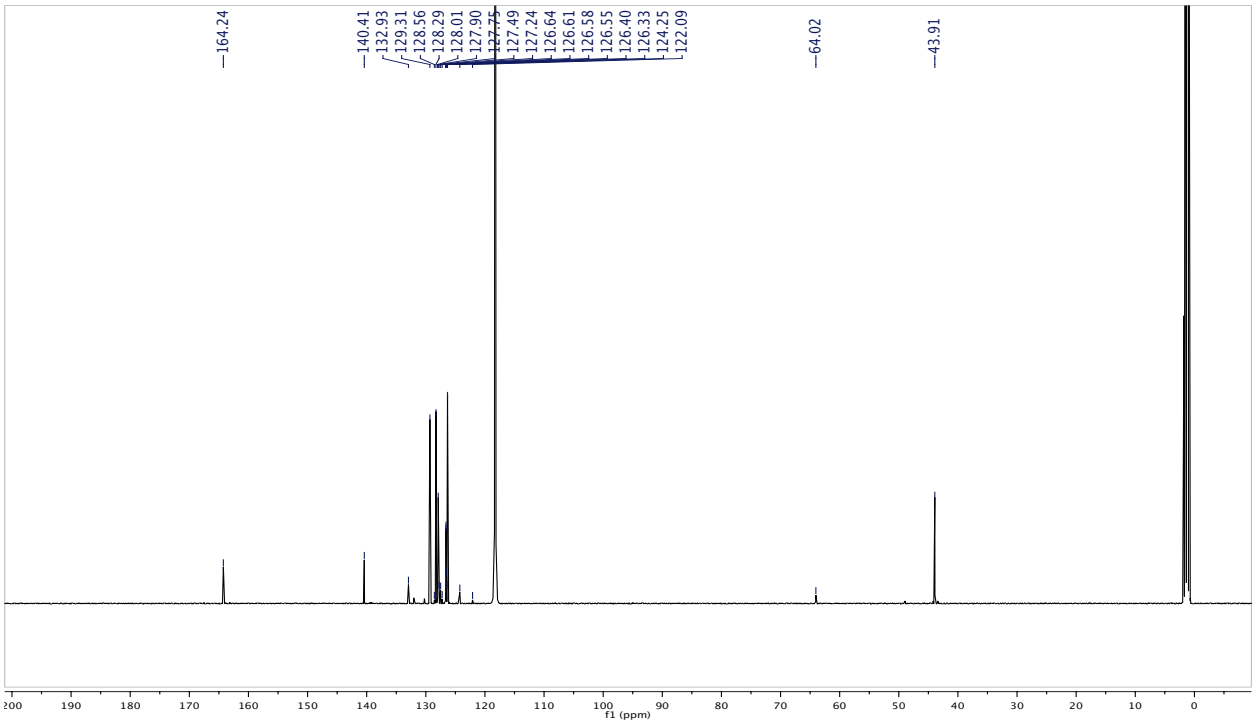
^{13}C NMR of S15 in CD_3CN (125 MHz):



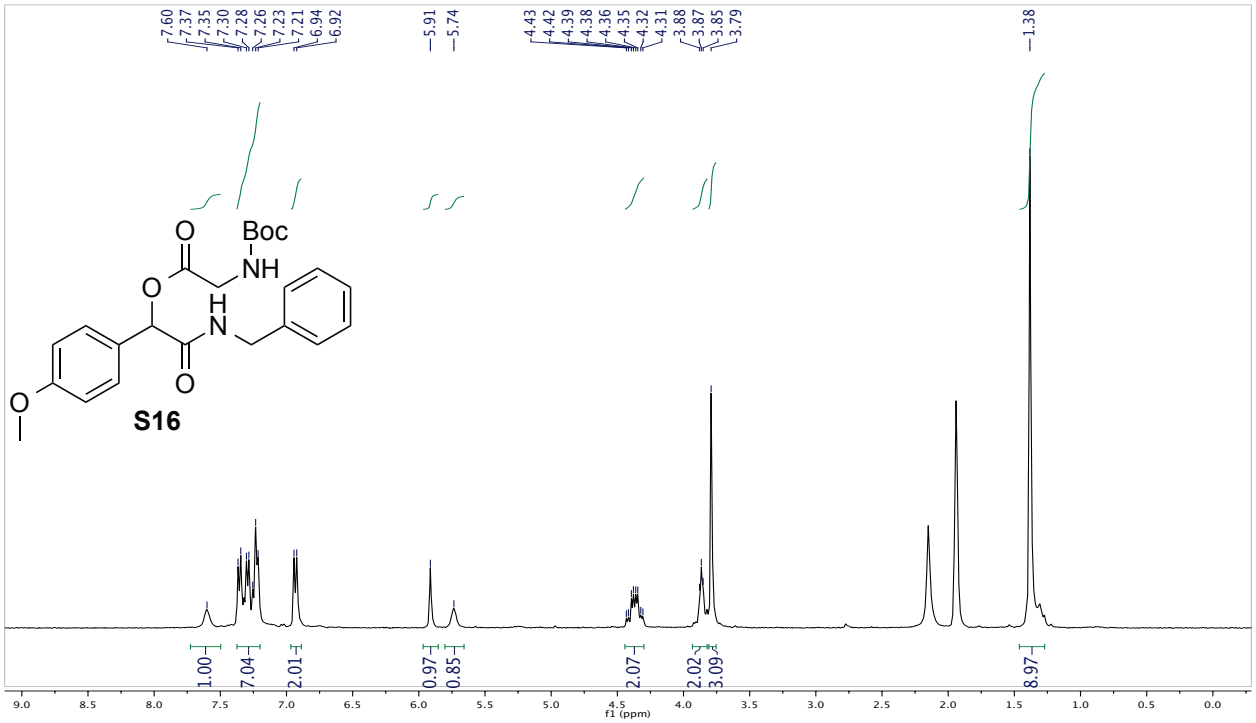
^{1}H NMR of 6 in $CDCl_3$ (400 MHz):



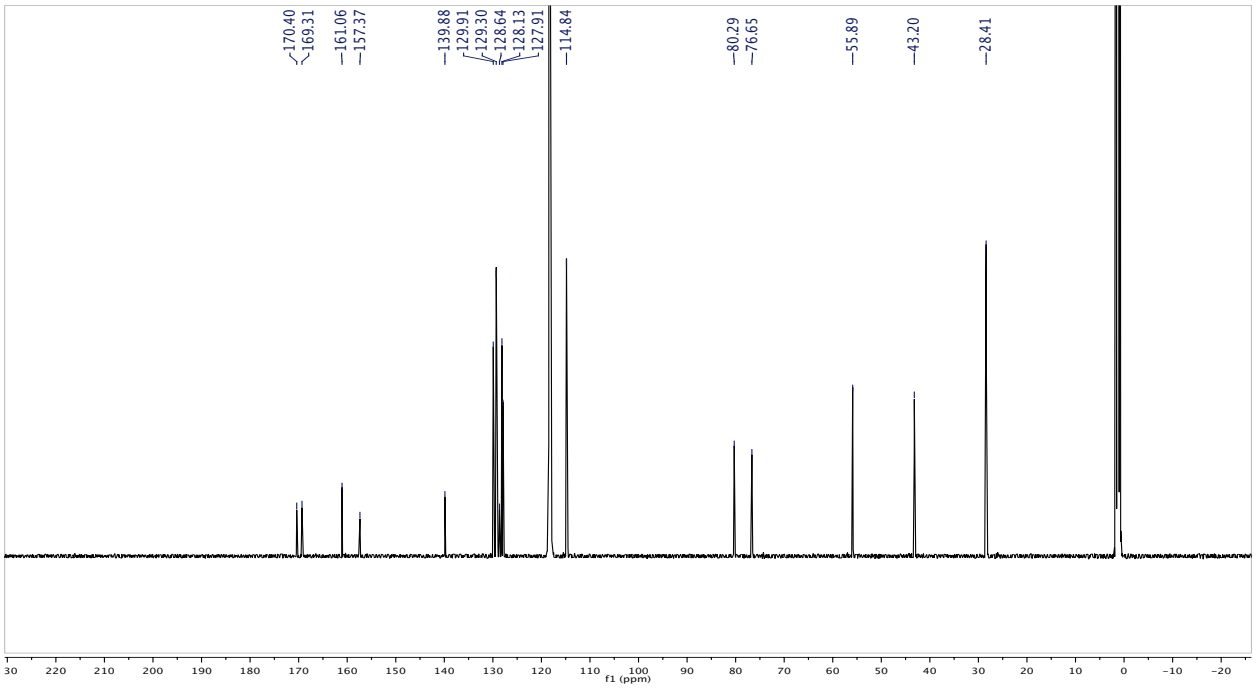
^{13}C NMR of 6 in CD_3CN (125 MHz):



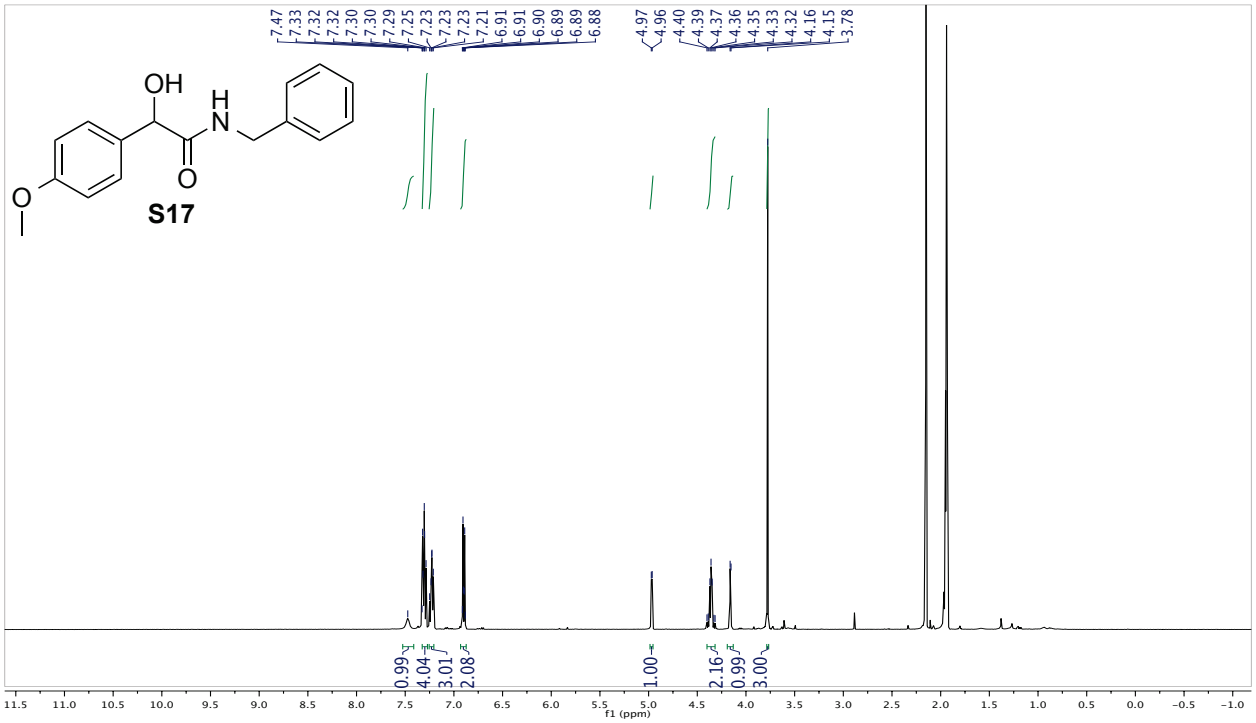
^{1}H NMR of S16 in CD_3CN (400 MHz):



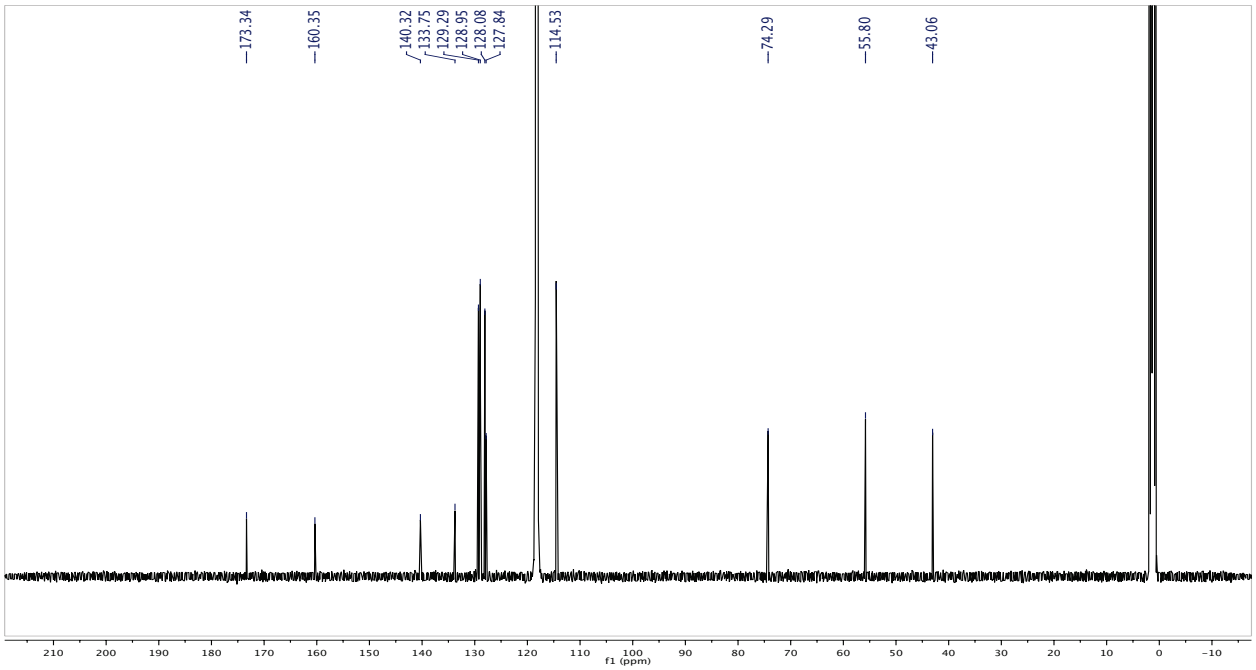
^{13}C NMR of S16 in CD_3CN (100 MHz):



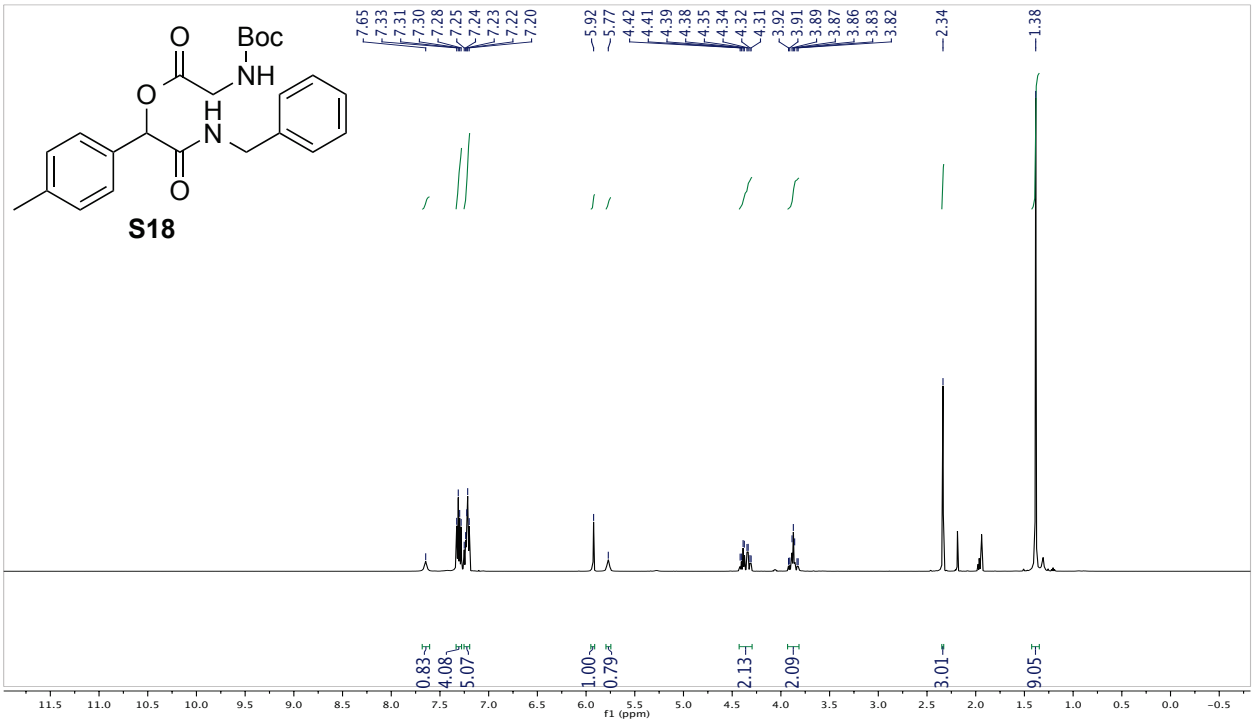
^{1}H NMR of S17 in CD_3CN (500 MHz):



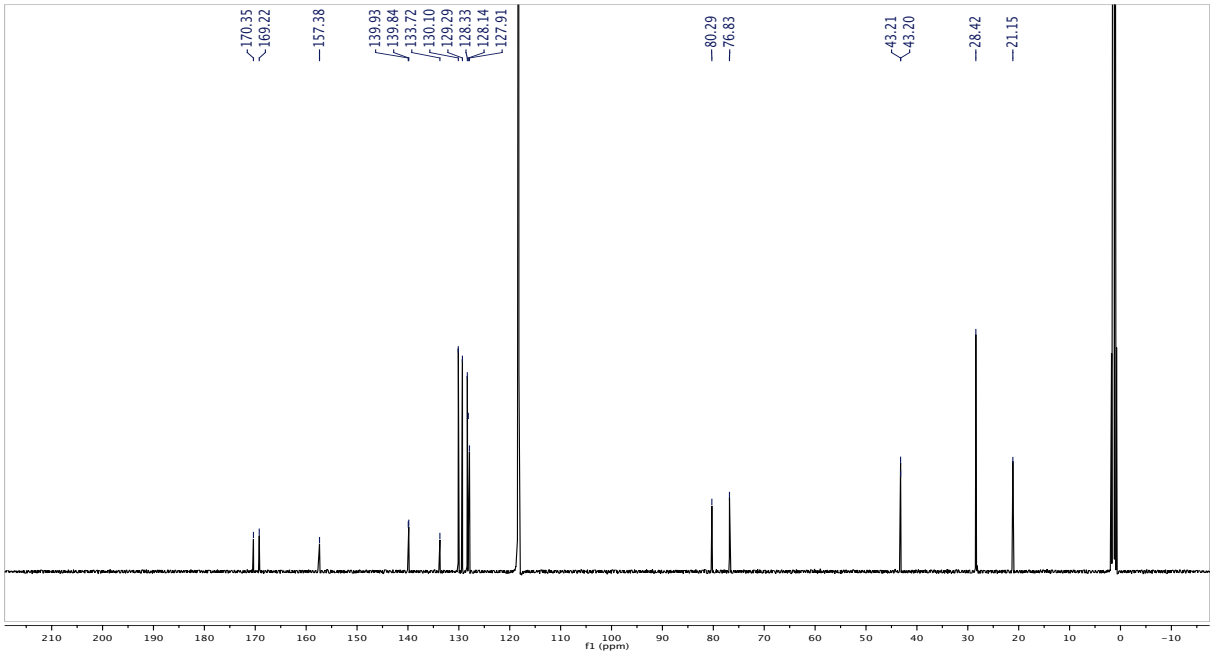
^{13}C NMR of S17 in CD_3CN (125 MHz):



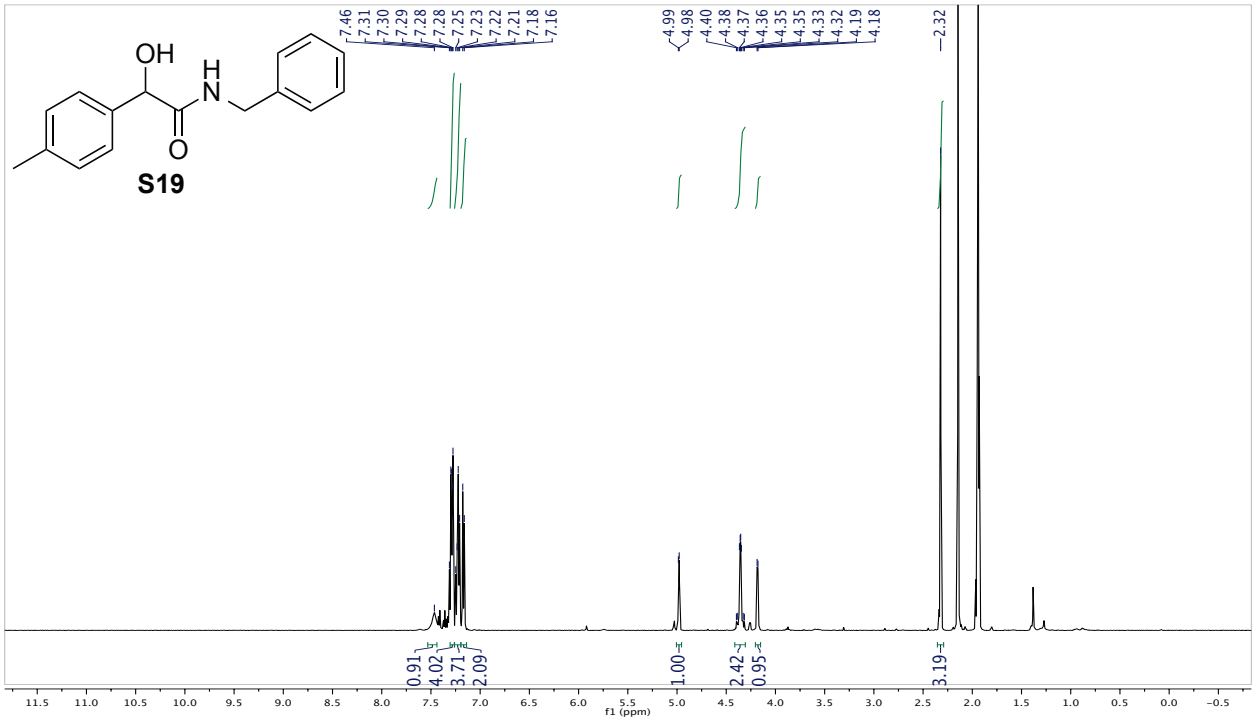
^{1}H NMR of S18 in CD_3CN (500 MHz):



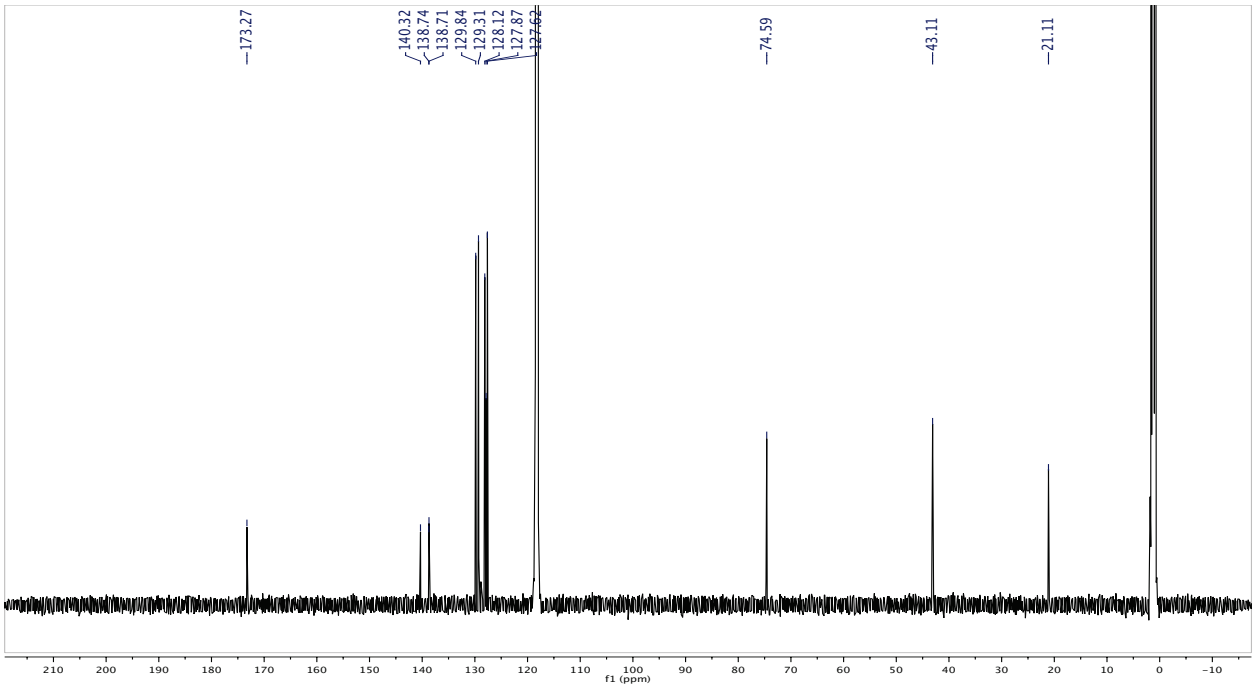
^{13}C NMR of S18 in CD_3CN (125 MHz):



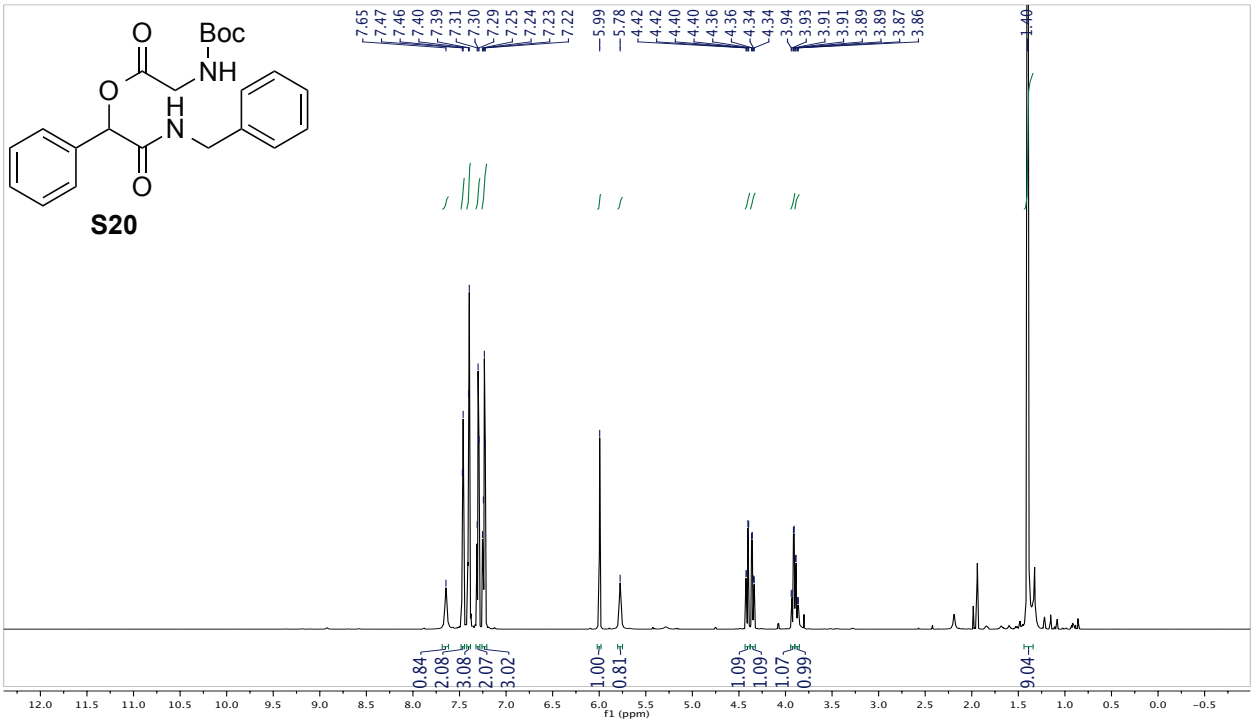
^{1}H NMR of S19 in CD_3CN (500 MHz):



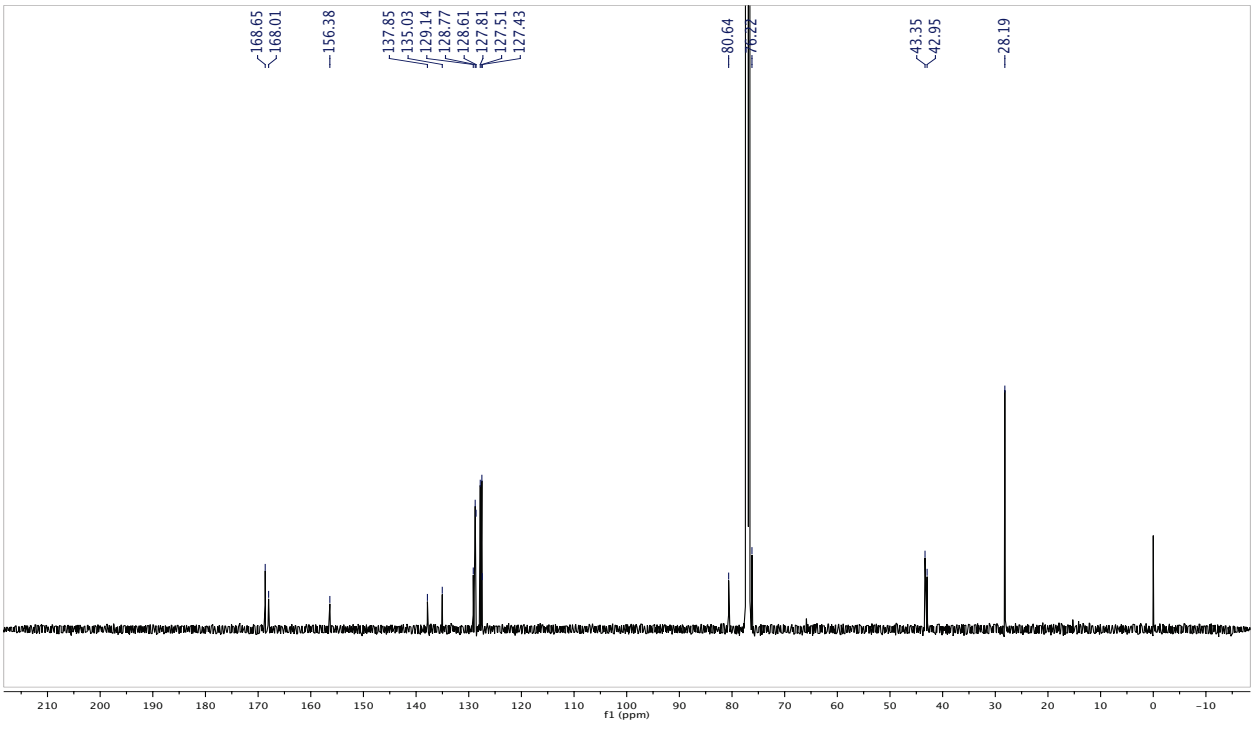
^{13}C NMR of S19 in CD_3CN (125 MHz):



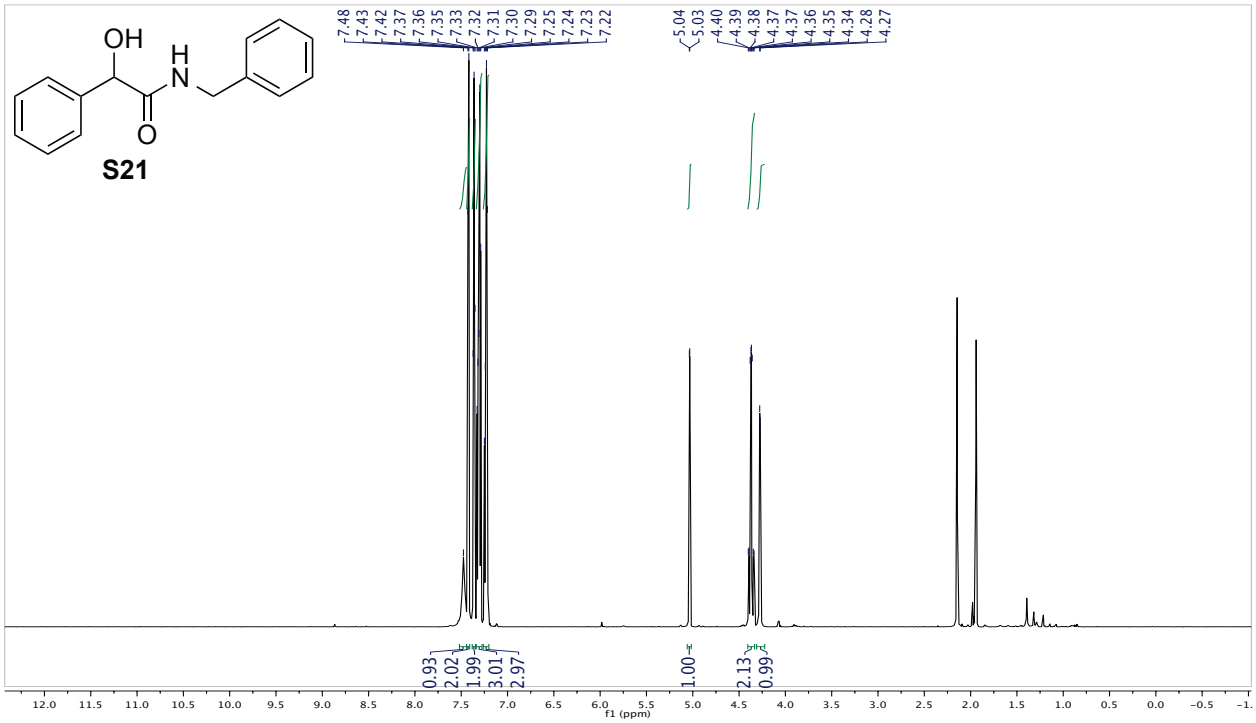
^{1}H NMR of S20 in CD_3CN (750 MHz):



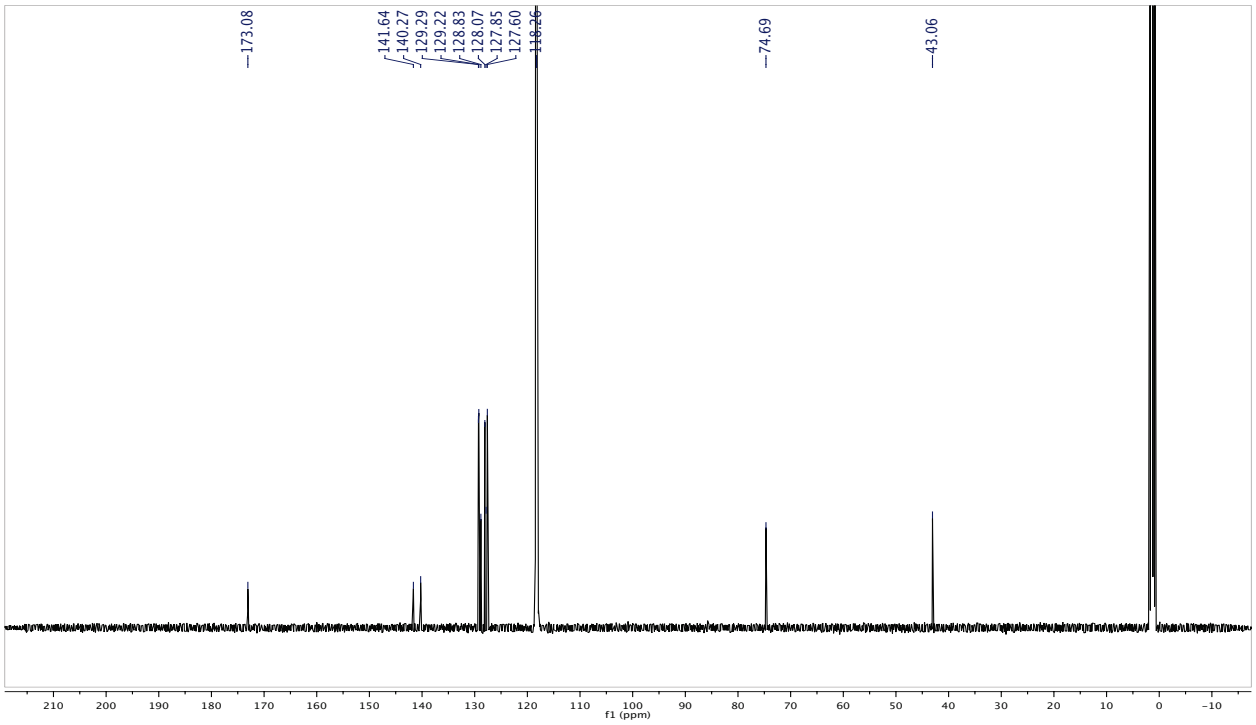
^{13}C NMR of S20 in $CDCl_3$ (125 MHz):



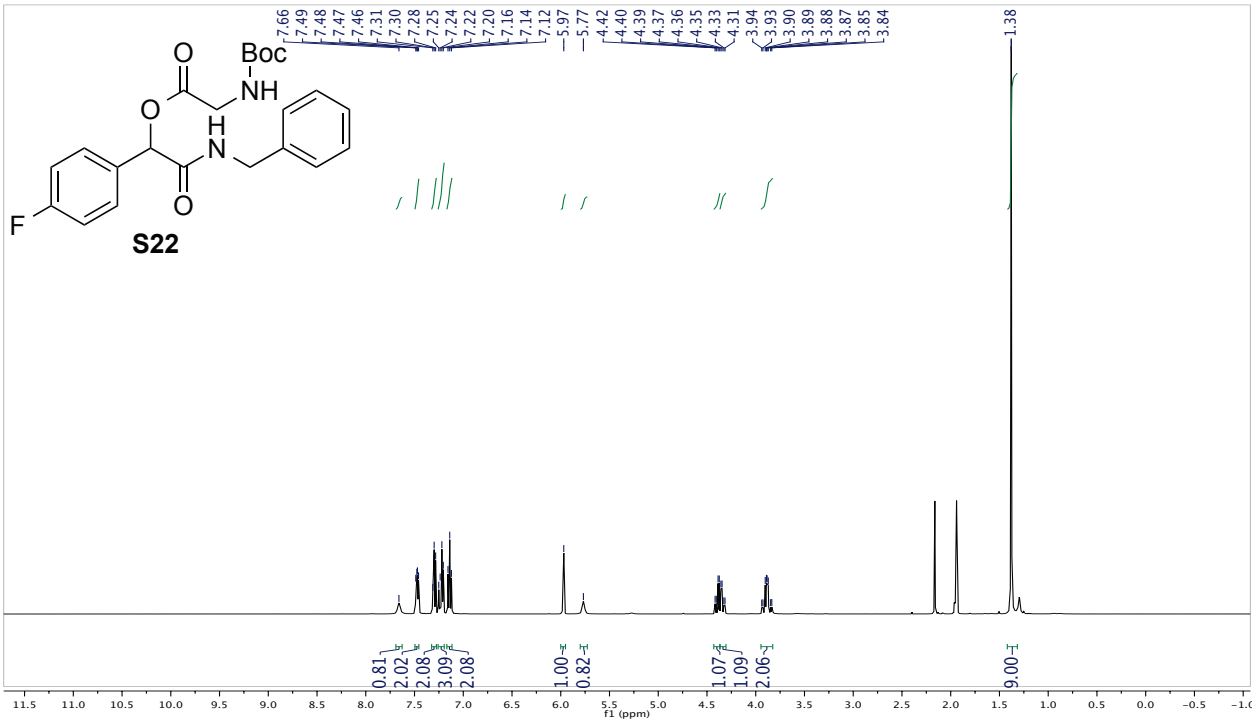
^{1}H NMR of S21 in CD_3CN (750 MHz):



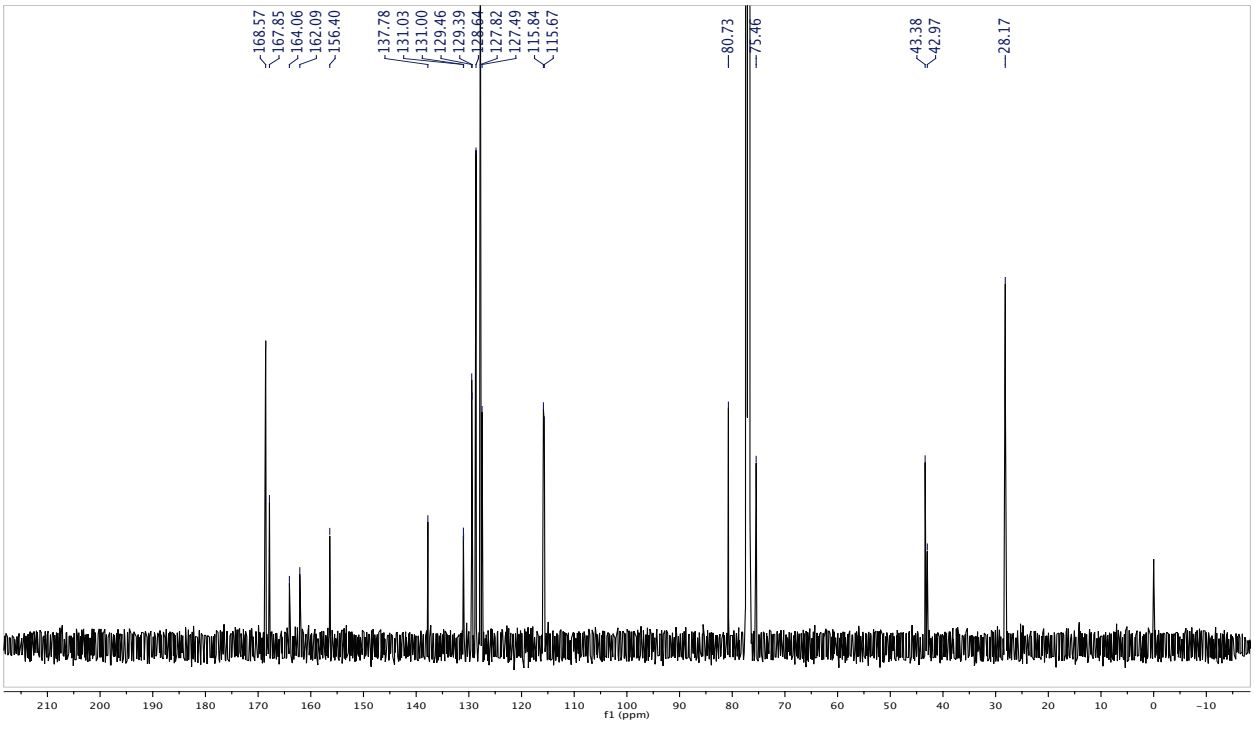
^{13}C NMR of S21 in CD_3CN (125 MHz):



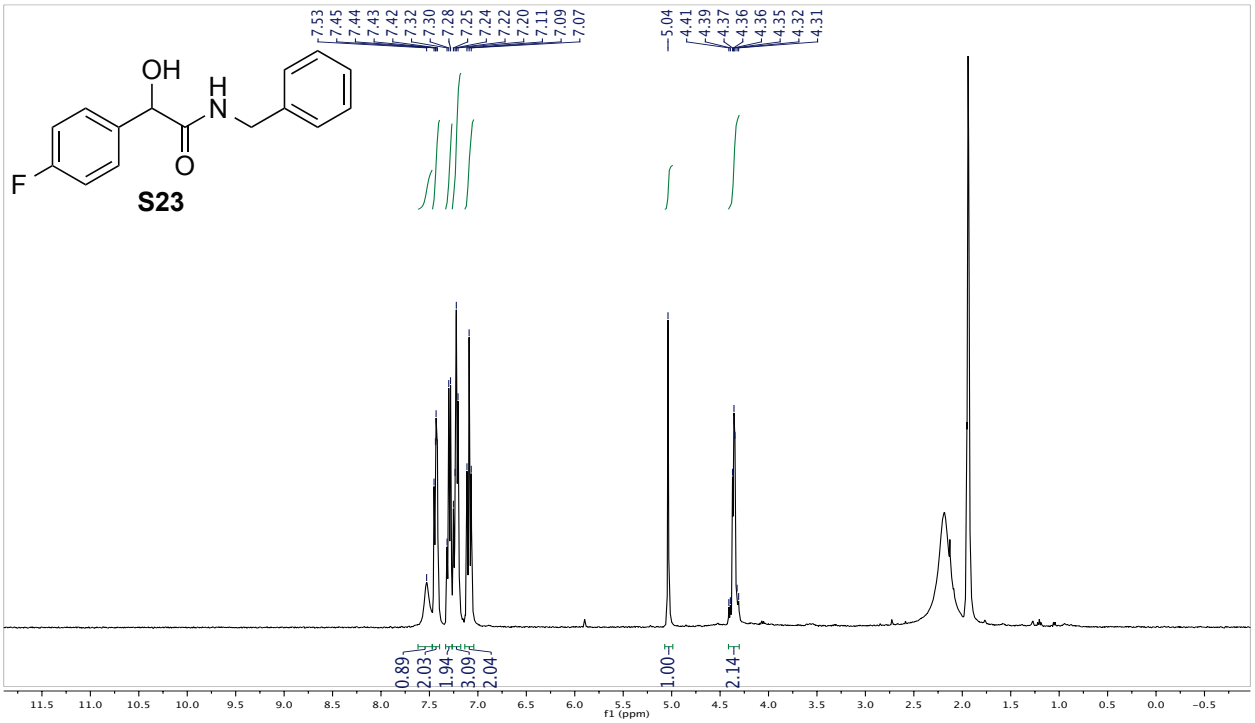
^{1}H NMR of S22 in CD_3CN (500 MHz):



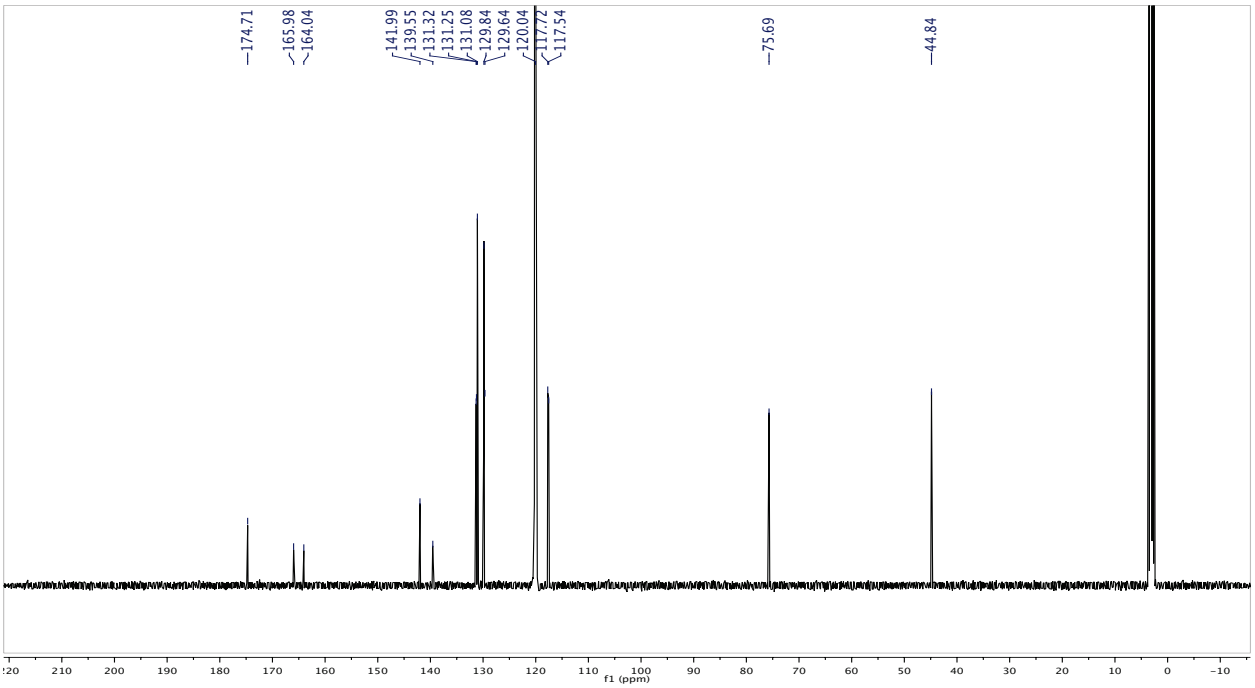
^{13}C NMR of S22 in $CDCl_3$ (125 MHz):



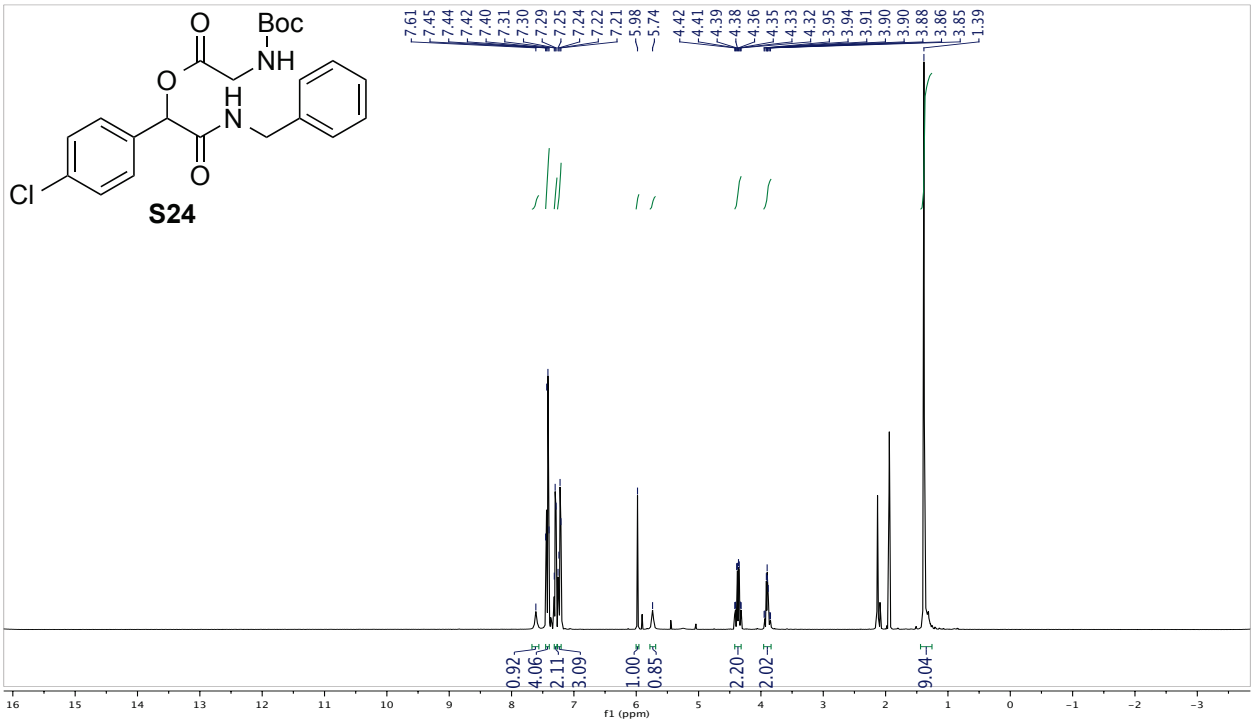
^{1}H NMR of S23 in CD_3CN (600 MHz):



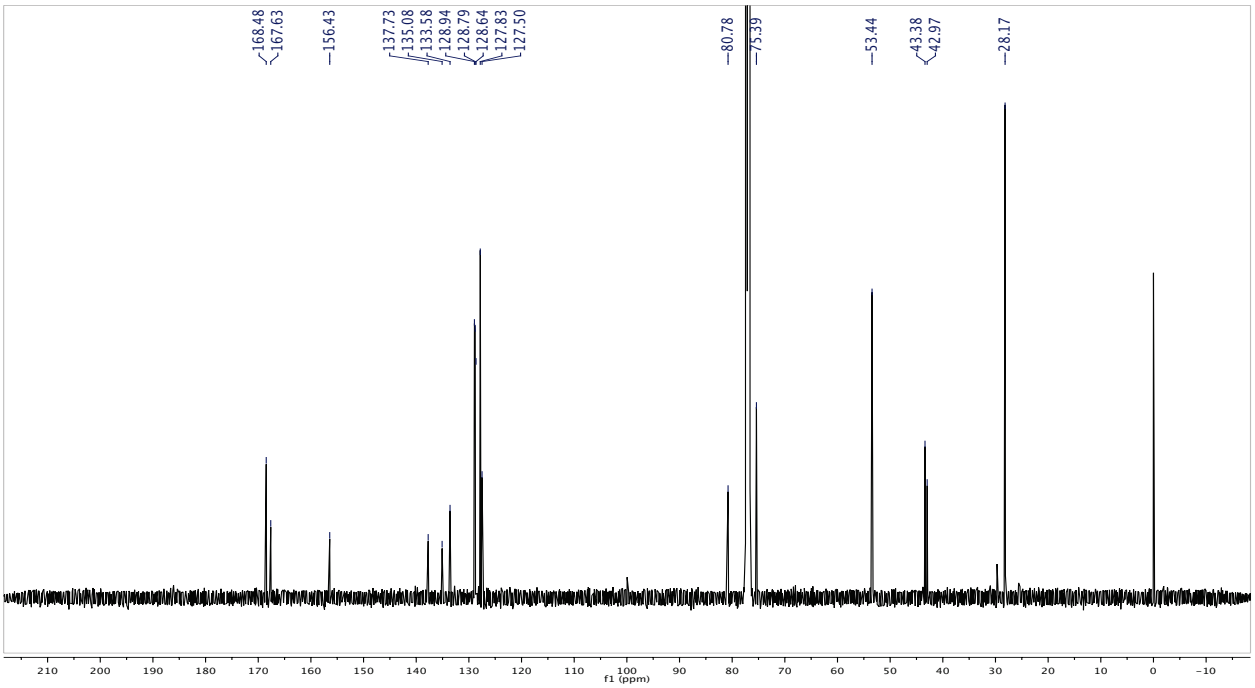
^{13}C NMR of S23 in CD_3CN (125 MHz):



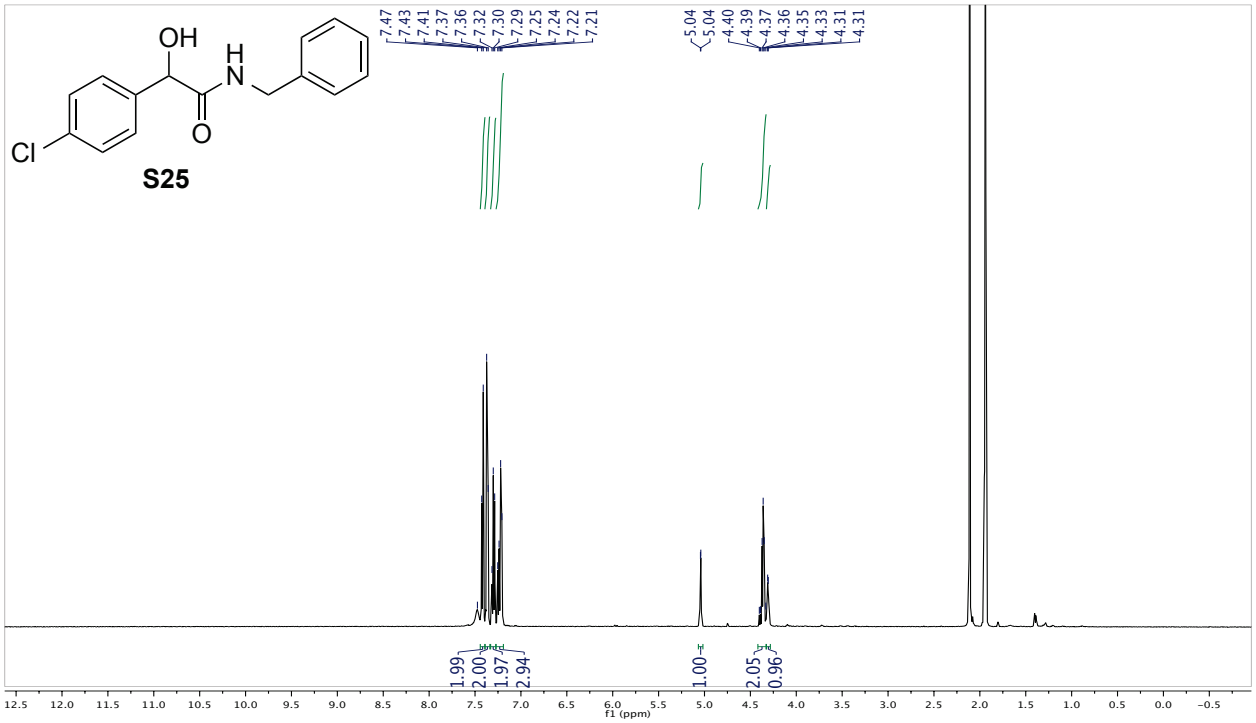
^{1}H NMR of S24 in CD_3CN (500 MHz):



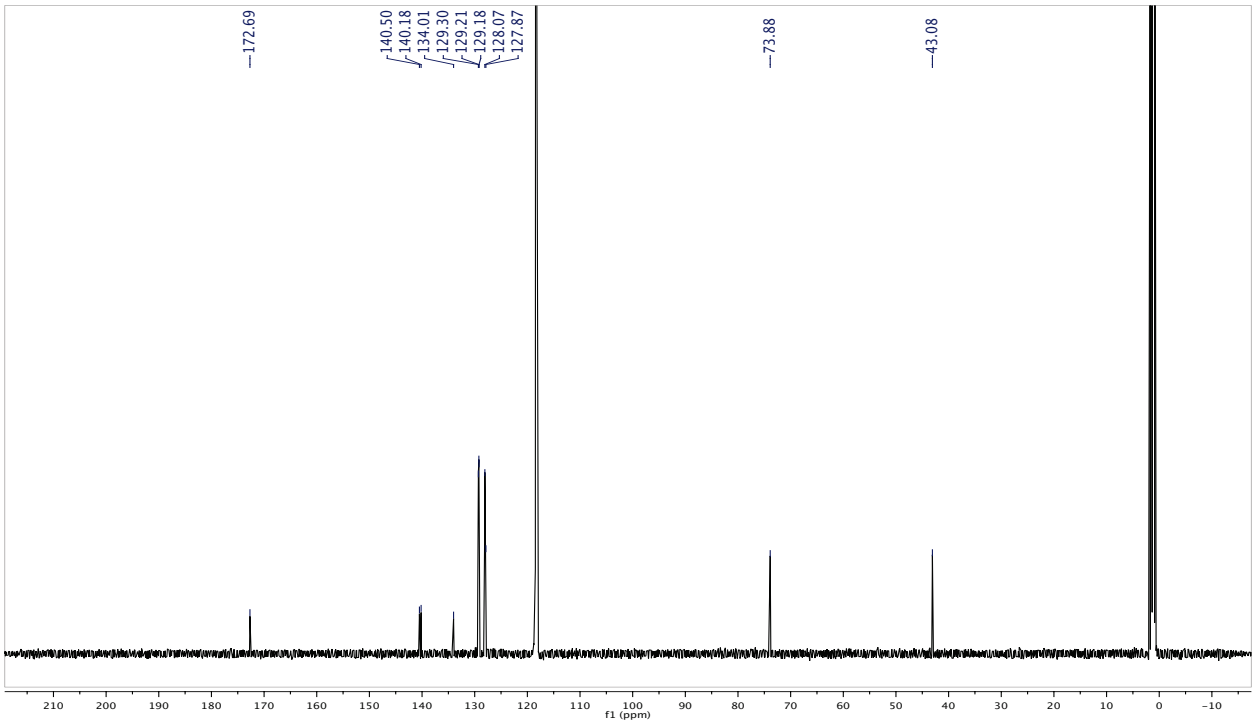
^{13}C NMR of S24/CH_2Cl_2 in $CDCl_3$ (125 MHZ):



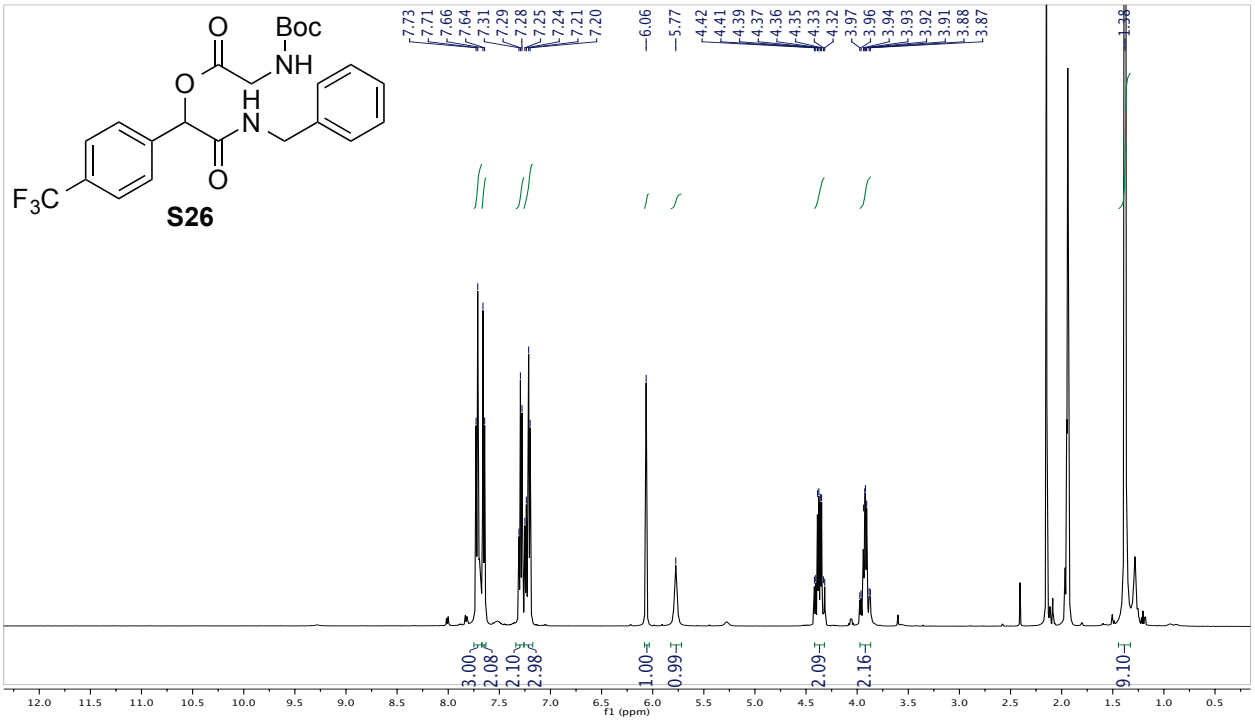
^{1}H NMR of S25 in CD_3CN (500 MHz):



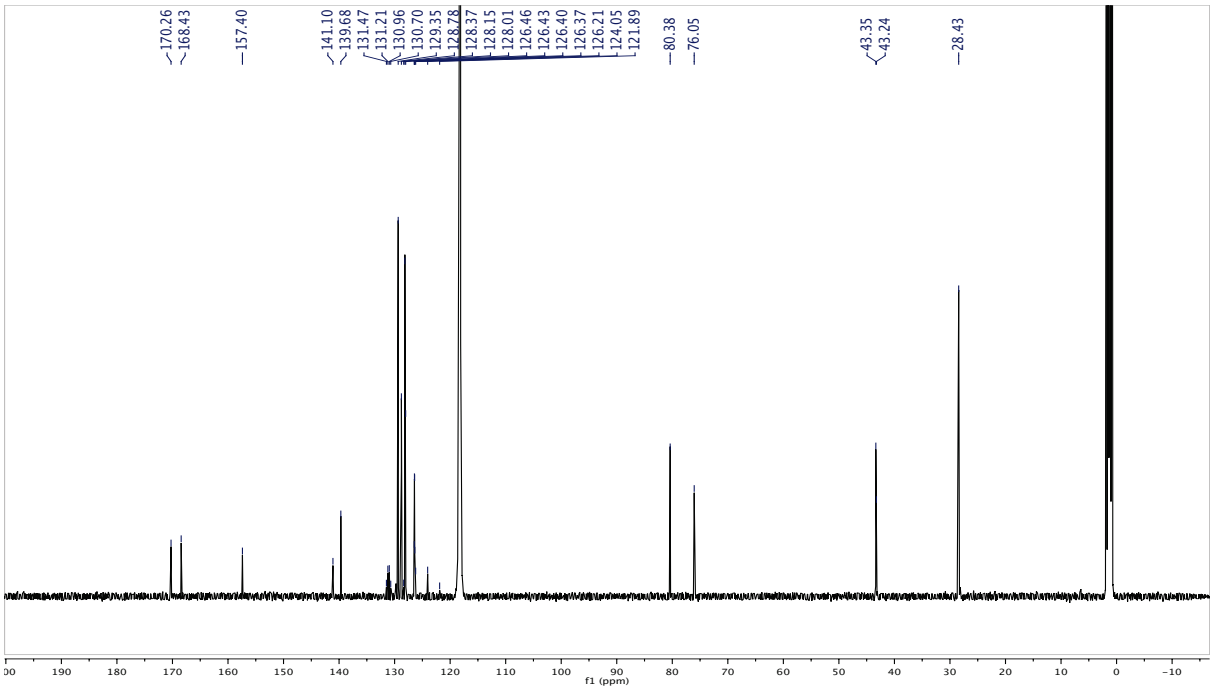
^{13}C NMR of S25 in $CDCl_3$ (125 MHz):



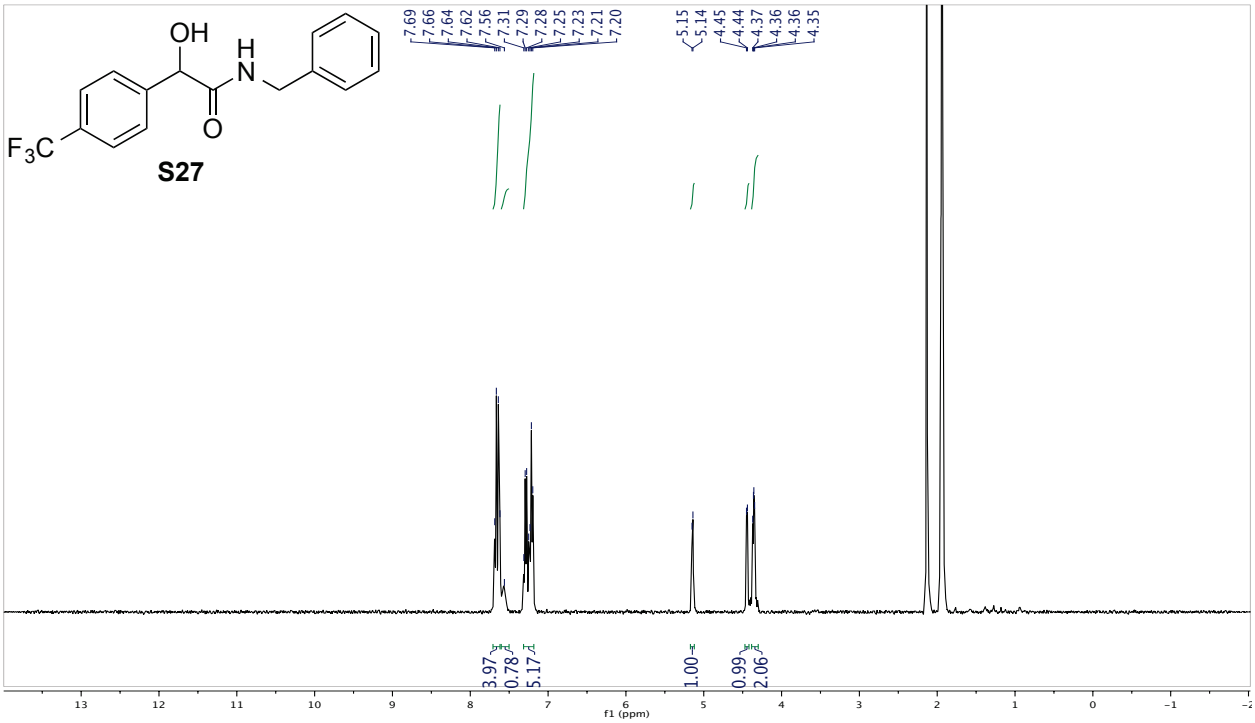
^{1}H NMR of S26 in CD_3CN (500 MHz):



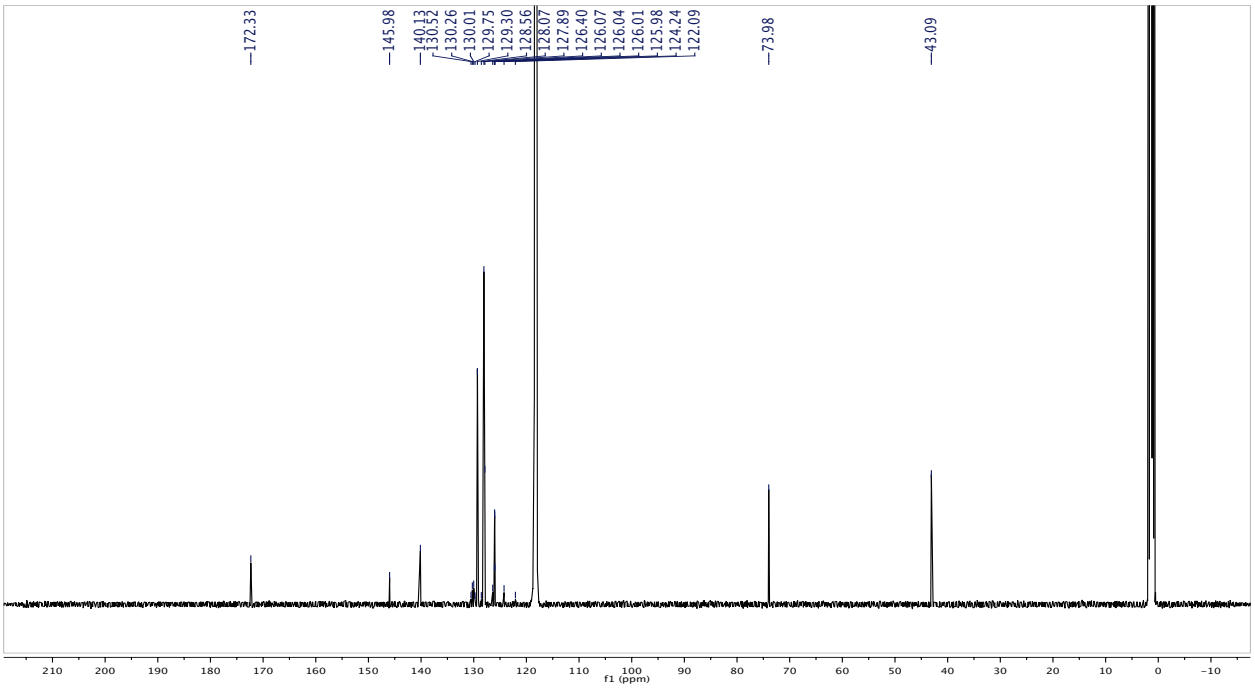
^{13}C NMR of S26 in CD_3CN (125 MHz):



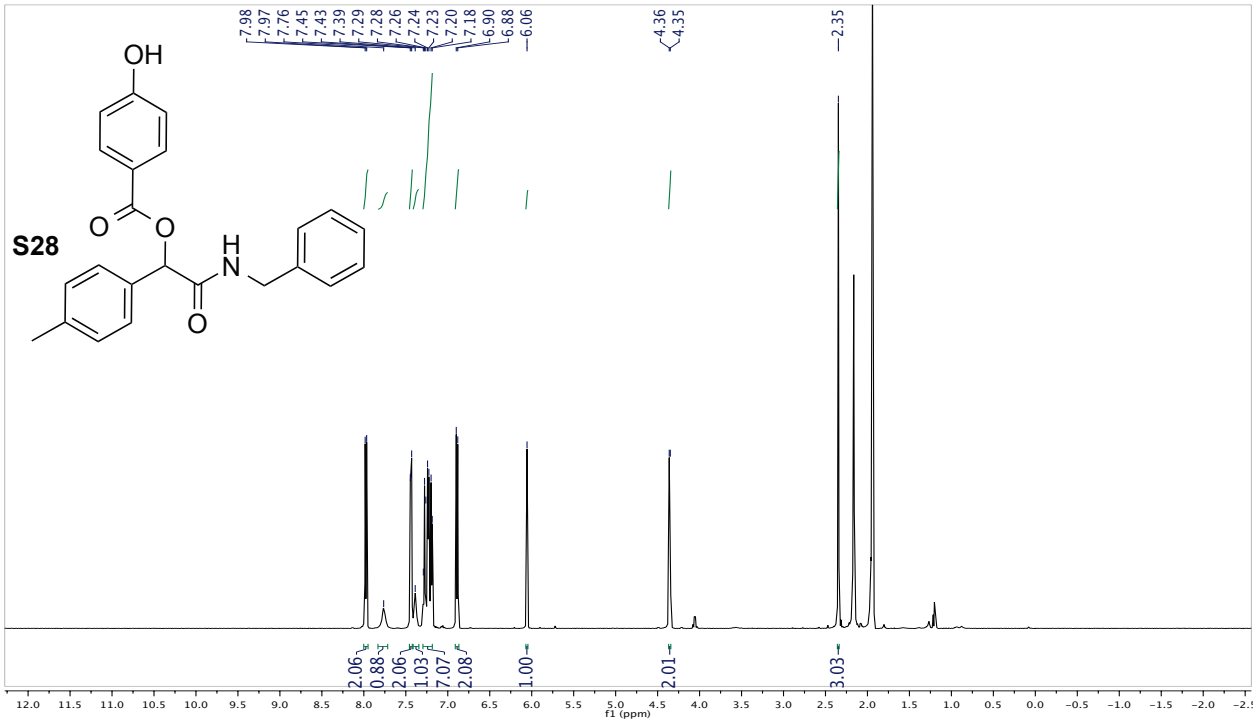
^{1}H NMR of S27 in CD_3CN (500 MHz):



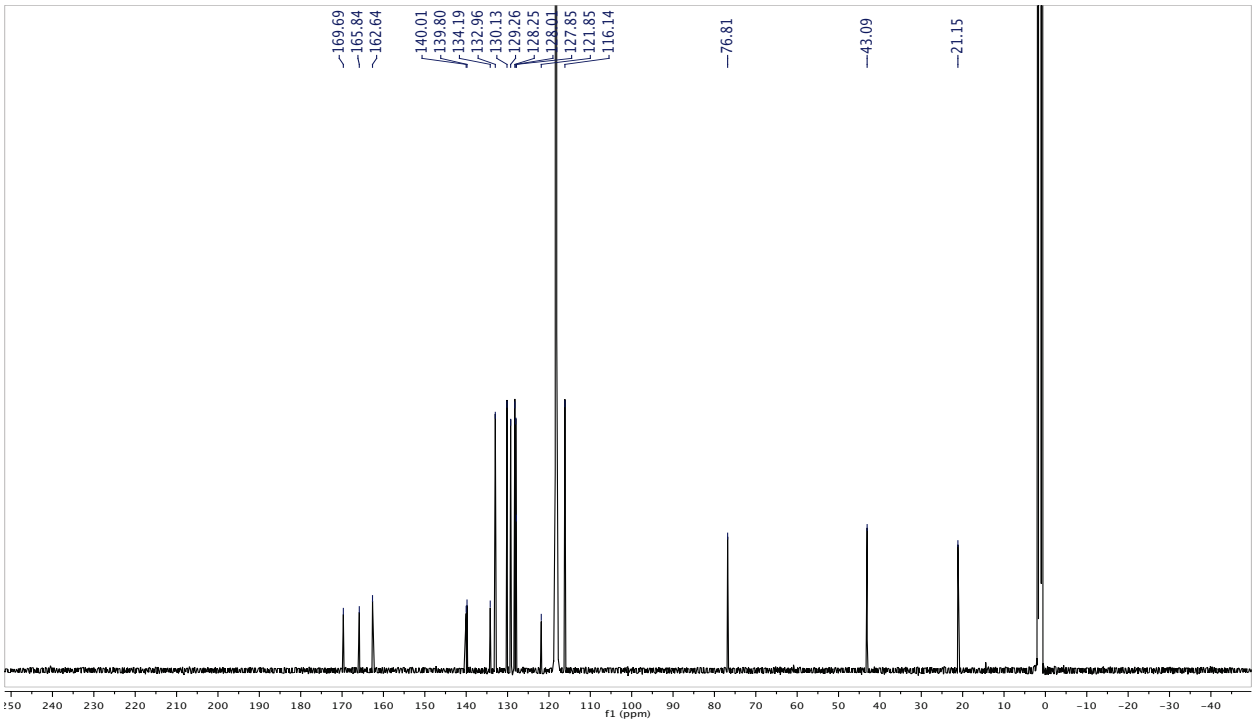
^{13}C NMR of S27 in CD_3CN (125 MHz):



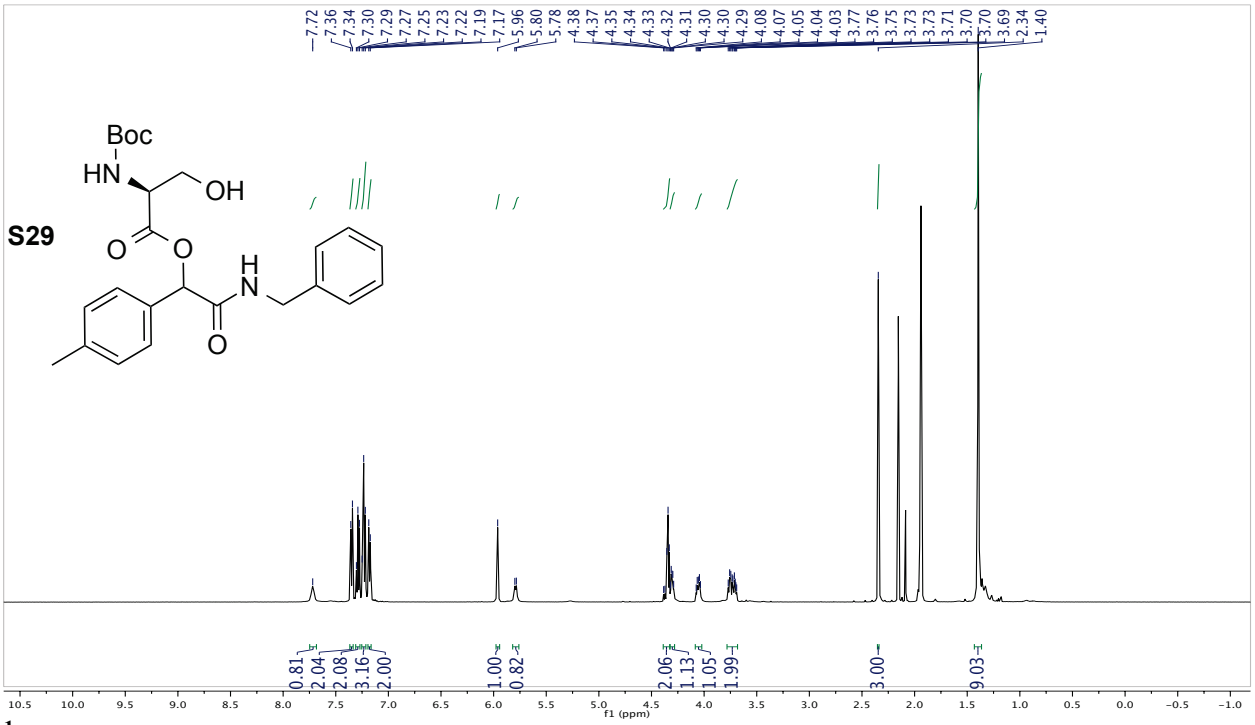
^{1}H NMR of S28 in CD_3CN (500 MHz):



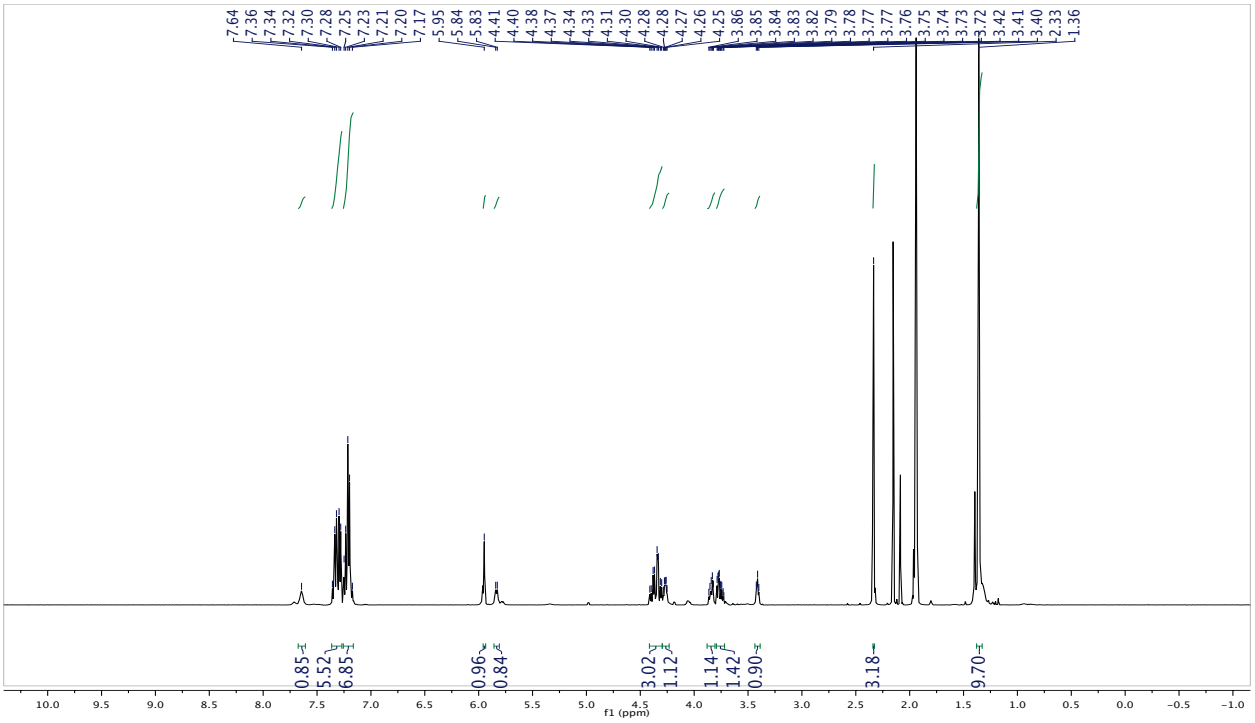
^{13}C NMR of S28 in CD_3CN (125 MHz):



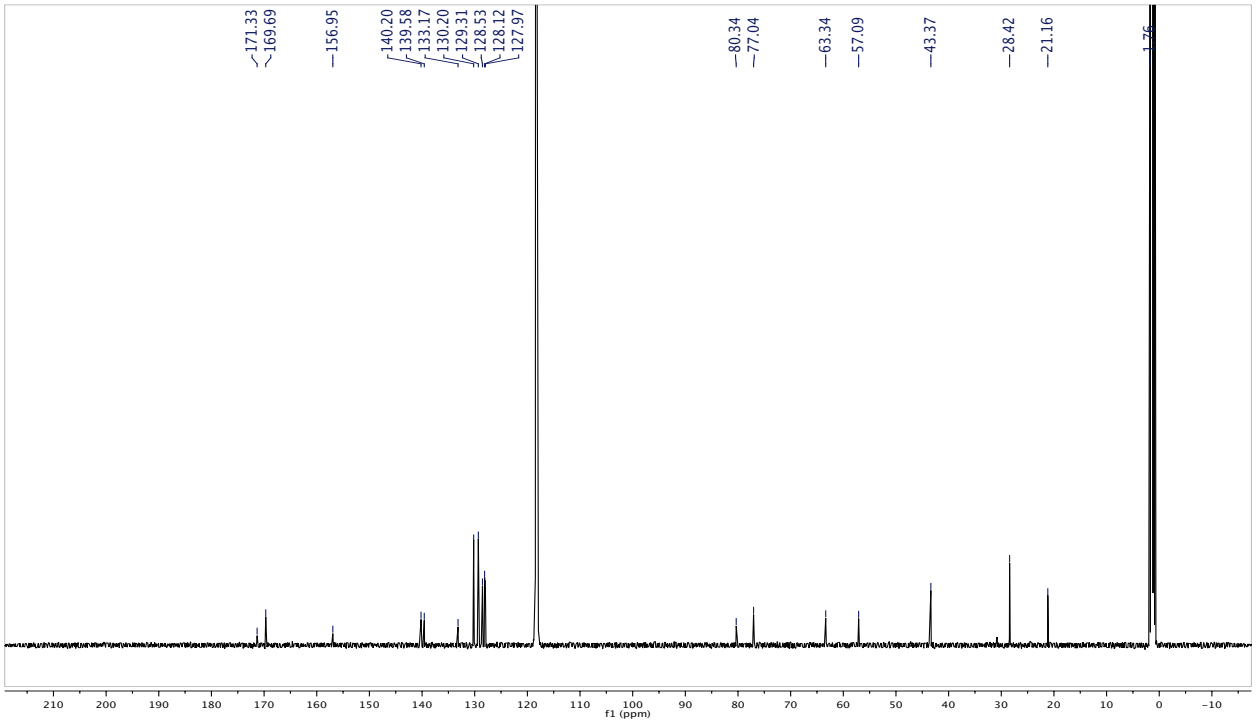
^{1}H NMR of S29 (Diastereomer A) in CD_3CN (500 MHz):



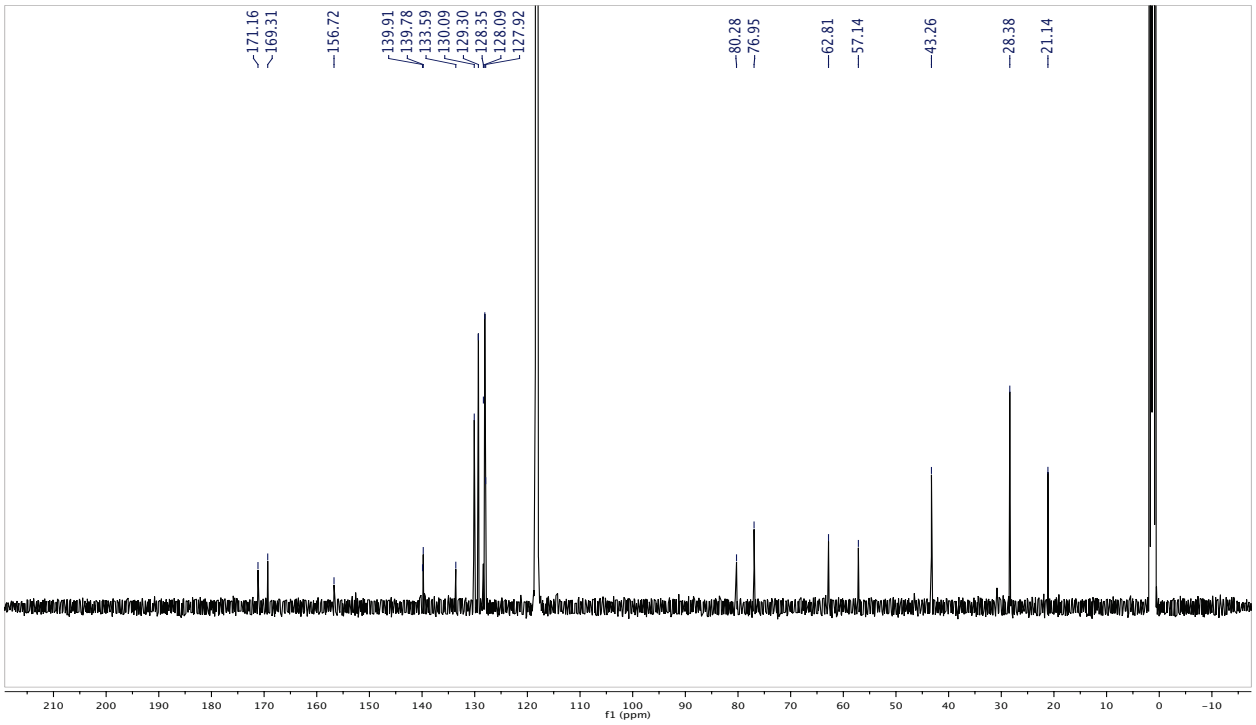
^{1}H NMR of S29 (Diastereomer B) in CD_3CN (500 MHz):



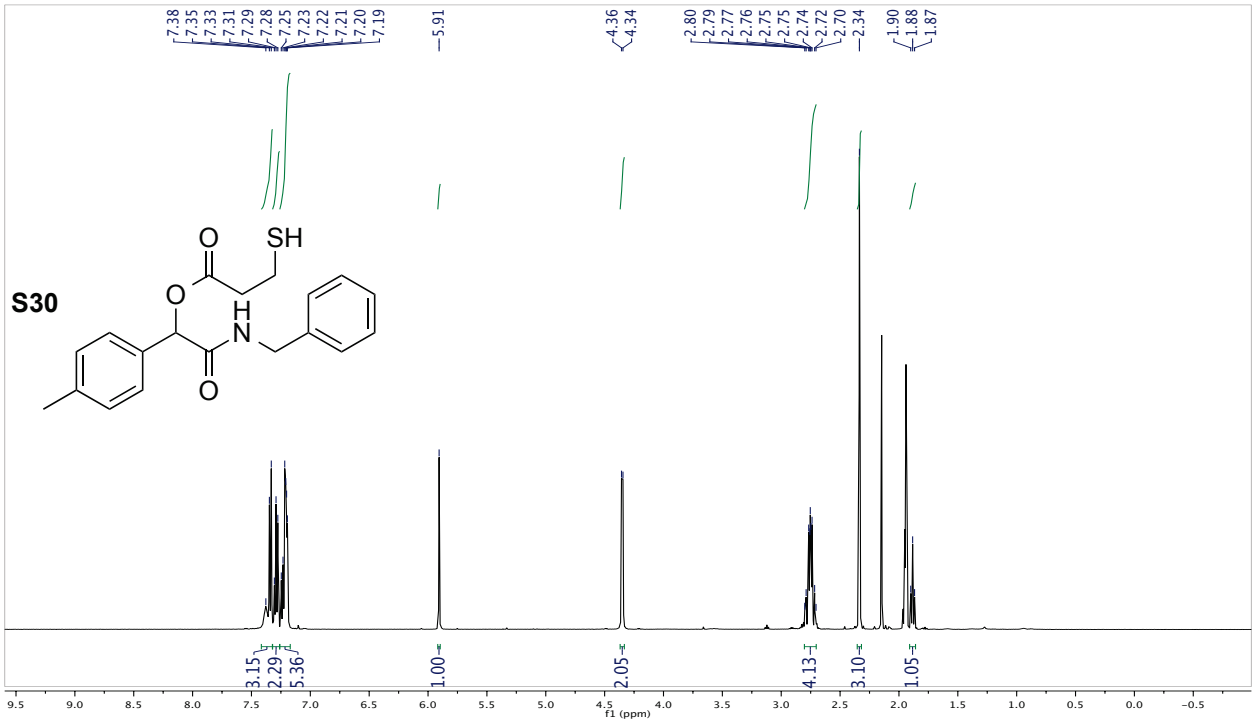
^{13}C NMR of S29 (Diastereomer A) in CD_3CN (100 MHz):



^{13}C NMR of S29 (Diastereomer B) in CD_3CN (100 MHz):



^{1}H NMR of S30 in CD_3CN (500 MHz):



^{13}C NMR of S30 in CD_3CN (125 MHz):