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Enzyme-Catalyzed Umpolung Strategy for the Green Synthesis of Schiff bases and their Derivatives

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1. General Information

All reactions were monitored by thin-layer chromatography (TLC), where the TLC plates were visualized under UV light for spot visualization. Column chromatography was used for product purification. Proton nuclear magnetic resonance spectroscopy was used to confirm the structures of all the compounds (see the supporting information). 1H NMR spectra were recorded on a 400 MHz spectrometer using CDCl3 as solvent. Chemical shifts of protons are reported in parts per million (ppm), downfield from tetramethylsilane, and are referenced to as the residual protium in the nuclear magnetic resonance (NMR) solvent (CHCl3=δ 7.26 ppm). 1 H NMR data are presented as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant in Hertz (Hz), and integration. The calculation of the isolated yield was based on a formula involving the division of the actual yield (the weight of the isolated product) by the theoretical yield. The result is multiplied by 100 to express the yield as a percentage.

2. Chemical Synthesis of Imine Substrates

Imine substrates can be categorized into three main types based on their functional groups, as shown in Figure 1, which forms the basis for expanding the substrate scope.

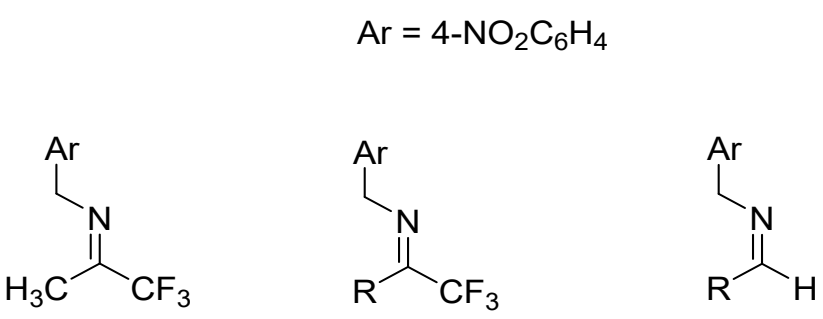


Figure S1: Synthesis of Imine Substrates

General Synthesis Procedures of Imine Substrates

Dissolve aryl benzyl bromide (1 equiv, 6 mmol, 1.4 g), phthalimide (2 eq, 2 mmol, 1.7g), potassium carbonate (2 equiv, 12 mmol, 1.6 g), and potassium iodide (2 equiv, 2 mmol, 1.9 g) in DMF (10 V/m), heat the solution to 100°C and observe the color change from yellow to reddish-purple. After 2 hours, the reaction is complete. After cooling the reaction mixture to room temperature, add an appropriate amount of water to precipitate the solid. Filter the yellow solid intermediate and dry it. Dissolve the intermediate directly in anhydrous ethanol (10 V/m), add hydrazine hydrate (20 equiv, 20 mmol, 9.9 g), and heat to 80°C. After 2 hours, the reaction is complete. After cooling to room temperature, filter off the precipitated white flocculent solid, wash the filter cake with a small amount of ethanol, collect the filtrate, and concentrate it by rotary evaporation to remove most of the ethanol. Add ethyl acetate and water, stir to separate the layers, collect the organic phase, wash with saturated NaCl (equiv), dry with anhydrous magnesium sulfate, and concentrate by rotary evaporation to obtain the corresponding aryl benzylamine. Under ice bath conditions at 0°C, add the aryl benzylamine (1 equiv, 1 mmol, 107 mg) dissolved in dichloromethane (10 V/m), and slowly add glacial acetic acid (0.2 eq, 0.6 mmol, 36 mg). A solid will form. Stir for 20 minutes, then add pre-cooled trifluoropropanone (1.5 equi, 9 mmol, 1g) and anhydrous magnesium sulfate, and heat under reflux for 3 hours. Filter off the solid magnesium sulfate, adjust the pH to neutral with an appropriate amount of saturated $NaHCO_3$ (equiv.), and extract with dichloromethane. Wash the organic phase with saturated NaCl (equiv.), dry with anhydrous magnesium sulfate, and concentrate. Purify the product by column chromatography to obtain imine substrates 1A -1C, as shown in Figure S2.

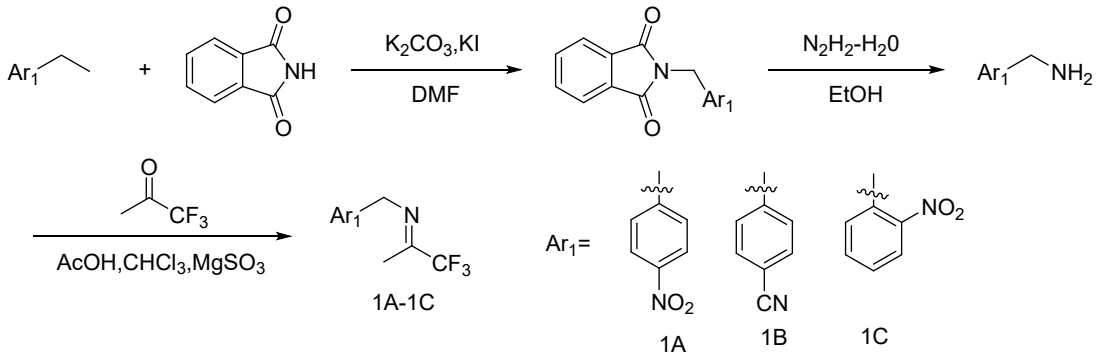


Figure S2. General Synthesis of Imine Substrates (1A-1C)

General Synthesis Procedures of Imine Substrates (2)

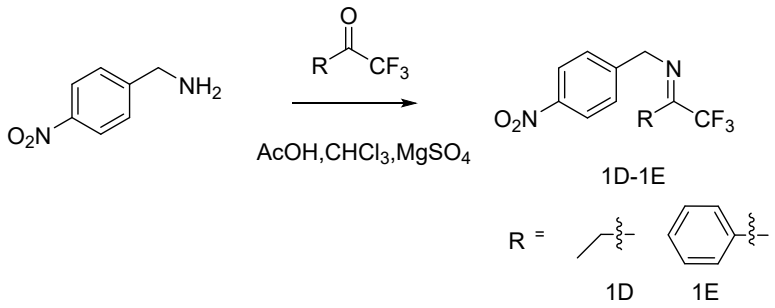


Figure S3. General Synthesis Procedures of Imine Substrates 2.

Figure S3 shows that the synthesis methods for 1D and 1E are the same as those described in Section 1.1.

General Synthesis Procedures of Imine Substrates (3)

Dissolve p-nitrobenzylamine (1 equiv, 5 mmol, 760 mg) in DCM (30 V/m) and add benzaldehyde (1 equiv, 5 mmol, 530 mg) dissolved in DCM (3 mL) at 0°C dropwise over 15 minutes. Then, add neutral alumina (8 equiv, 40 mmol, 4.3 g) and react at room temperature for 2 hours. Separate the mixture using dichloromethane and water. Collect the organic phase, wash with saturated NaCl (aquiv.), dry with anhydrous magnesium sulfate, and concentrate to obtain the crude product. Slowly add a solution of 3% EA in n-hexane to the crude product and stir vigorously for about 1 hour. Filter, wash the solid with a small amount of 3% EA in n-hexane three times, and dry to obtain high-purity imine substrates 1F-1J, as shown in Figure S4.

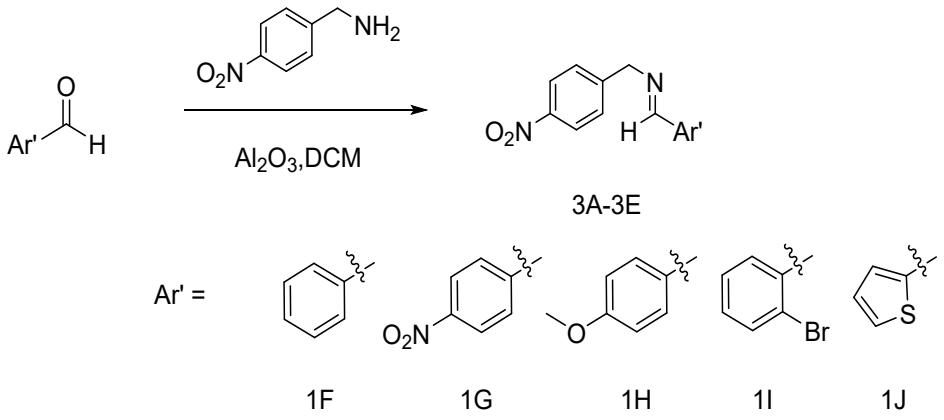


Figure S4. General Synthesis Procedures of Imine Substrates 3.

3. Chemical Synthesis of α, β-Unsaturated substrates

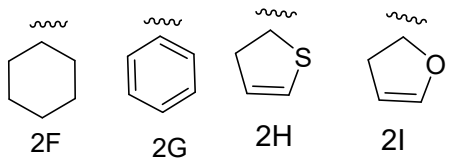


Figure S5. The Structure of α, β-Unsaturated Ketone.

Compounds 2A-2E were purchased directly, and the remaining compounds can be synthesized according to the following methods (Figure S5).

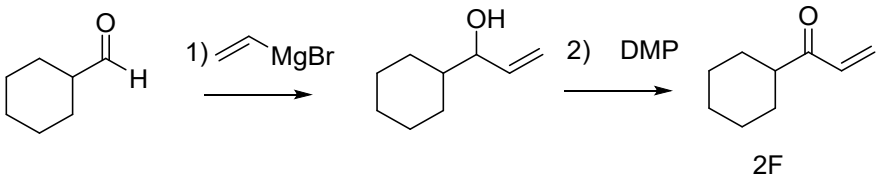


Figure S-6. Synthesis Procedures of 2F.

1-Cyclohexylprop-2-en-1-one (2F): Under anhydrous and oxygen-free conditions, add a solution of cyclohexane carboxaldehyde (1.68 g, 15 mmol) in anhydrous THF (15 mL), cool to 0°C, and slowly add vinylmagnesium bromide (2.0 M THF solution, 22.5 mmol, 2.9 g). Stir at room temperature overnight. After the reaction is complete, quench the reaction with saturated NH4Cl (aquiv.). Extract with dichloromethane-water, wash with saturated NaCl (aquiv.), dry with anhydrous magnesium sulfate, and concentrate. Dissolve the crude product in DCM, add DMP (9.54 g, 22.5 mmol), and stir at room temperature overnight. Quench the reaction with saturated NaHCO3 (aquiv.) and saturated Na2S2O3 (aquiv.), then perform liquid-liquid extraction with dichloromethane water. Collect the organic phase, wash with saturated NaCl (aq.), dry with anhydrous magnesium sulfate, and concentrate. Purify the product by column chromatography using an eluent ratio of EA= 1:10. The synthesis steps are shown in Figure S6.

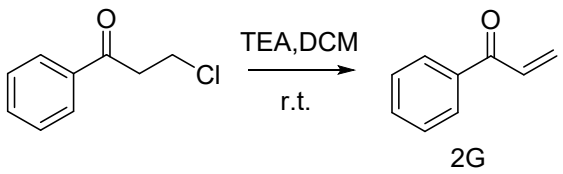


Figure S7 Synthesis Procedure of 2G.

1-Phenylprop-2-en-1-one (2G): At 0°C, slowly add TEA (3.5 mL, 25.5 mmol) dropwise to a solution of 3-chloropropiophenone (2.15 g, 12.75 mmol) in DCM (20 mL), and stir at room temperature overnight. After the reaction is complete, quench the reaction with dilute hydrochloric acid. Extract with dichloromethane-water, wash with saturated NaCl (aq), dry with anhydrous magnesium sulfate, and concentrate. Purify the product by column chromatography using an eluent ratio of EA= 1:10. The synthesis steps are shown in Figure S7.

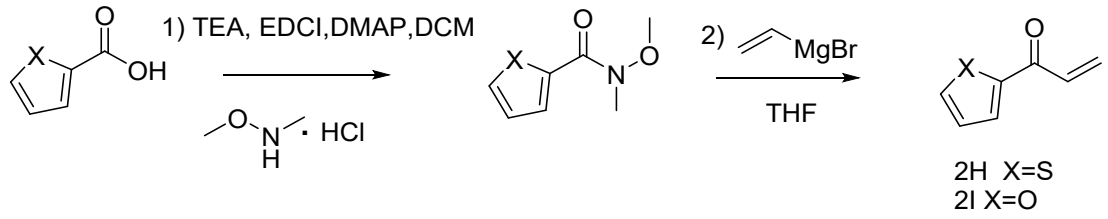


Figure S8. General Synthesis Procedures of 2H and 2I.

1-(Thiophen-2-yl) prop-2-en-1-one (2H,2I): Dissolve thiophene-2-carboxylic acid (1 equiv, 6 mmol, 762 mg), N,O-dimethylhydroxylamine hydrochloride (1.3 eq, 7.8mmol. 756mg), TEA (1.3 equiv, 7.8 mmol, 787 mg,), EDCI (1.3 equiv, 7.8 mmol, 1.2 g), and DMAP (0.1 equiv, 06 mmol, 73 mg) in DCM (15 V/m) and stir at room temperature for 2 hours. After the reaction is complete, wash thoroughly with dilute hydrochloric acid, perform liquid-liquid extraction with ethyl acetate-water, wash with saturated NaCl (aq.), dry with anhydrous magnesium sulfate, and concentrate to obtain an oily product. The crude product can be directly used for the next step without purification. Under anhydrous and oxygen-free conditions, dissolve the crude product in anhydrous THF (5 V/m), cool at 0°C, and slowly add vinylmagnesium bromide (2.0 M THF solution, 1.5 equiv, 9 mmol, 1.1 g). Stir at room temperature overnight. After the reaction is complete, quench the reaction with dilute hydrochloric acid under ice bath conditions. Extract with ethyl acetate-water, wash with saturated NaCl (aquiv.), dry with anhydrous magnesium sulfate, and concentrate. Purify the target compound by column chromatography. The synthesis steps are shown in Figure S8. The synthesis method for 1-(furan-2-yl)prop-2-en-1-one (2I) is the same as that for 2H.

Synthesis of α,β-Unsaturated Ketone Substrates (2J)

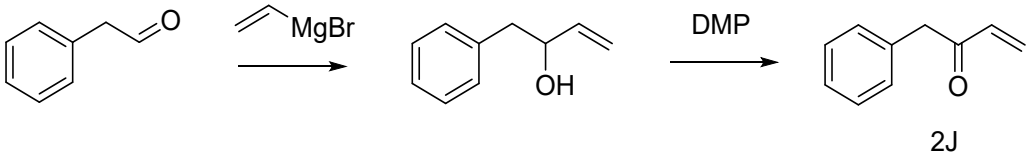


Figure S9. Synthesis Method for 2j.

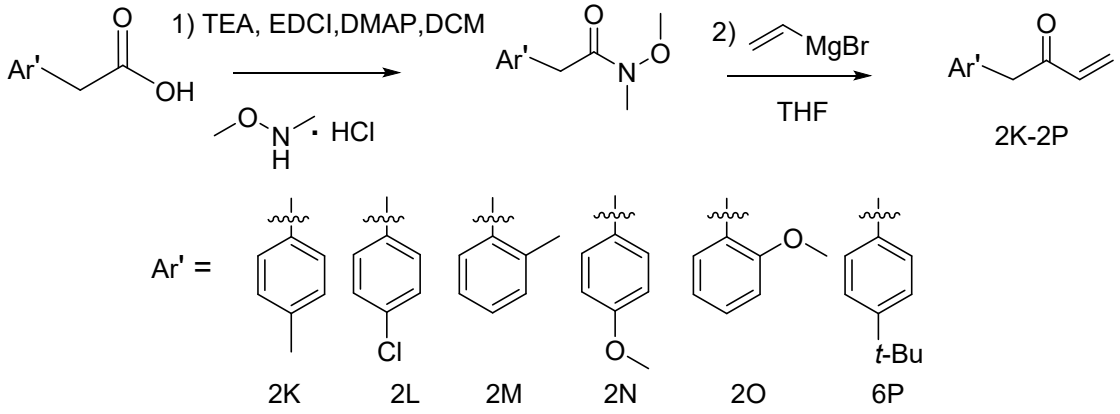


Figure S10. General Synthesis Procedures for 2K-2P

The synthesis methods for α, and β-unsaturated ketone substrates refer to the synthesis methods in Section 2. The synthesis steps are shown in Figures S9 and S10, respectively.

Synthesis of α,β-Unsaturated Ketone Substrates (2q-2v)

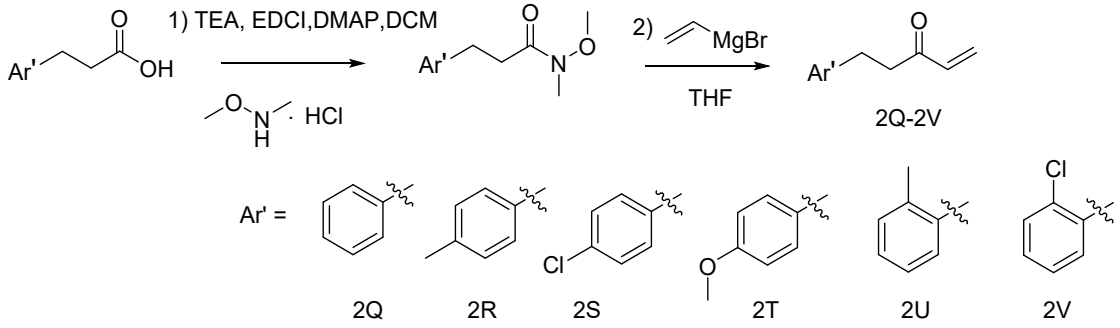


Figure S11. General Synthesis Procedures for 2Q-2V.

The synthesis method for α,β-unsaturated ketone substrates (2Q-2V) refers to the synthesis method of 2H in Section 2. The synthesis steps are shown in Figure S11.

4. General synthesis of standard product (3A)

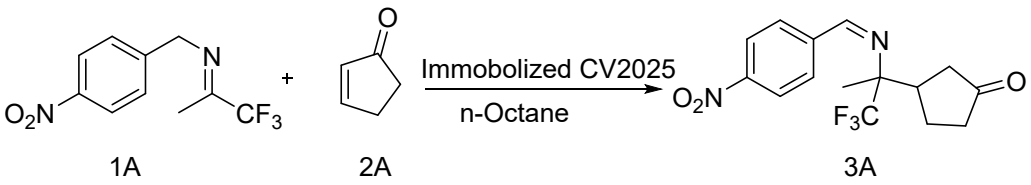


Figure S12. Synthesis Procedure of 3A

The route for synthesizing 3-(1,1,1-Trifluoro-2-((4-nitrobenzylidene)amino)prop-2-yl)cyclopentanone (3A) via an enzymatic Umpolung addition reaction 1A and 2A is outlined in Figure 12. The specific procedural steps are as follows: To create a mixture of (1A) (0.1 mmol, 1 equiv) as Imine substrate, in α, β-unsaturated ketone (2A) **(**0.6 mmol, 6 equiv**)**, in n-Octane (0.5 ml), CV2025 (500 mg) was added and stirred at 35°C for 72 hrs in a shaker. Upon completion of the reaction, the enzyme was filtered out, the reaction mass was extracted with saturated brine and ethyl acetate (EA) (3 × 5 mL). The organic layers were combined and subjected to drying by a rotary evaporator to obtain 3A. TLC monitored the progress of the reaction on Haiyang GF 254 silica gel plates. The product was purified through column chromatography using an eluent ratio of ethyl acetate (EA) to petroleum ether (PE) of 1:5 v/v. The resultant 3-(1,1,1-Trifluoro-2-((4-nitrobenzylidene)amino)prop-2-yl)cyclopentanone (3A) was structurally elucidated by ^{1}H NMR spectroscopy and compared with the reference[1] (Figure S12).

5. Characterization Data of Starting material

Imine substrates

4-Nitro-N-(2, 2, 2-trifluoro-1-methyleneethyl)benzylamine (1A): Eluent ratio EA= 1:10, yielding a pale yellow oily liquid (680 mg), ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 – 8.07 (m, 2H), 7.74 – 7.57 (m, 2H), 4.82 (s, 2H), 2.18 (s, 3H).

4-Cyano-N-(2, 2, 2-trifluoro-1-methyleneethyl)benzylamine (1B): Eluent ratio EA= 1:10, yielding a pale yellow oily liquid (428 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.65 (d, *J* = 8.3 Hz, 2H), 7.50 (d, *J* = 8.2 Hz, 2H), 4.67 (s, 2H), 2.13 (s, 3H).

2-Nitro-N-(2,2,2-trifluoro-1-methyleneethyl)benzylamine (1C): Eluent ratio EA= 1:25, yielding a colorless oily liquid (440 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.11 (dd, *J* = 8.2, 1.3 Hz, 1H), 7.88 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.69 (td, *J* = 7.6, 1.4 Hz, 1H), 7.52 – 7.46 (m, 1H), 4.96 (s, 2H), 2.20 (s, 3H).

4-Nitro-N-(1-trifluoromethylpropyl)benzylamine (1D): Eluent ratio EA= 1:10, obtained a colorless oily liquid (835 mg).^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.22 (d, *J* = 8.7 Hz, 2H), 7.55 (d, *J* = 8.7 Hz, 2H), 4.80 (s, 2H), 2.56 (q, *J* = 7.7 Hz, 2H), 1.22 (t, *J* = 7.7 Hz, 3H).

4-Nitro-N-(2,2,2-trifluoro-1-phenylethyl)benzylamine (1E): Eluent ratio EA= 1:20, obtained a colorless oily liquid (446 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.17 (d, *J* = 8.7 Hz, 2H), 7.53 – 7.48 (m, 3H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.28 – 7.22 (m, 2H), 4.66 (s, 2H).

4-Nitro-N-(benzylidene)benzylamine (1F): Colorless solid (336 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.91 (s, 1H), 7.67 (d, *J* = 8.7 Hz, 2H), 7.27 (dd, *J* = 7.5, 1.9 Hz, 2H), 7.04 – 6.84 (m, 5H), 4.37 (s, 2H).

4-Nitro-N-((4-nitrobenzylidene)benzylamine (1G): Pale yellow solid (388 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.53 (s, 1H), 8.30 (d, *J* = 8.8 Hz, 2H), 8.22 (d, *J* = 8.7 Hz, 2H), 7.98 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8.8 Hz, 2H), 4.97 (s, 2H).

4-Methoxy-N-((4-nitrobenzylidene)benzylamine (1H): Pale yellow solid (353 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.35 (d, *J* = 7.2 Hz, 1H), 8.19 (d, *J* = 8.4 Hz, 2H), 7.82 – 7.69 (m, 2H), 7.51 (d, *J* = 8.2 Hz, 2H), 7.02 – 6.90 (m, 2H), 4.86 (s, 2H), 3.85 (s, 3H).

N-((2-Bromobenzylidene)-4-nitrobenzylamine (1I): Pale yellow solid (617 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.83 (s, 1H), 8.22 (d, *J* = 8.6 Hz, 2H), 8.09 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.40 – 7.28 (m, 2H), 4.95 (s, 2H).

4-Nitro-N-(2-thienylmethylene)benzylamine (1J): Pale yellow solid (371 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.54 (s, 1H), 8.22 (d, *J* = 8.7 Hz, 2H), 7.53 (d, *J* = 8.6 Hz, 2H), 7.48 (d, *J* = 5.0 Hz, 1H), 7.41 (d, *J* = 3.5 Hz, 1H), 7.13 (dd, *J* = 5.0, 3.7 Hz, 1H), 4.89 (s, 2H).

1-(4-nitrophenyl)-N-(1,1,1-trifluoropropan-2-yl)methanimine (1K): pale yellow oily liquid ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.42 (s, 1H), 7.91 (d, *J* = 8.3 Hz, 2H), 7.73 (d, *J* = 8.3 Hz, 2H), 2.91 – 2.74 (m, 1H), 1.46 (s, 3H).

1H NMR Results of α, β-Unsaturated Ketones

1-Cyclohexylprop-2-en-1-one (2F): Eluent ratio EA= 1:20, obtained a colorless oily liquid (587 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.42 (dd, *J* = 17.5, 10.5 Hz, 1H), 6.24 (dd, *J* = 17.5, 1.4 Hz, 1H), 5.74 (dd, *J* = 10.5, 1.5 Hz, 1H), 2.60 (ddt, *J* = 11.3, 6.7, 3.3 Hz, 1H), 1.87 – 1.63 (m, 5H), 1.43 – 1.16 (m, 5H).

1-Phenylprop-2-en-1-one (2G): Eluent ratio EA= 1:20, obtained a pale yellow oily liquid (1.47 g), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.97 – 7.90 (m, 2H), 7.56 (t, *J* = 7.4 Hz, 1H),

7.47 (t, *J* = 7.6 Hz, 2H), 7.15 (dd, *J* = 17.1, 10.6 Hz, 1H), 6.43 (dd, *J* = 17.1, 1.6 Hz, 1H), 5.92 (dd, *J* = 10.6, 1.6 Hz, 1H).

1-(2-Thienyl)prop-2-en-1-one (2H): Eluent ratio EA= 1:20, obtained a pale yellow oily liquid (660 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.72 (d, *J* = 3.8 Hz, 1H), 7.62 (d, *J* = 4.9 Hz, 1H), 7.09 (t, *J* = 4.4 Hz, 1H), 7.03 (dd, *J* = 17.0, 10.4 Hz, 1H), 6.43 (dd, *J* = 17.0, 1.5 Hz, 1H), 5.81 (d, *J* = 10.4 Hz, 1H).

1-(2-Furyl)prop-2-en-1-one (2I): Eluent ratio EA= 1:20, obtained a pale yellow oily liquid (623 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.62 (s, 1H), 7.26 (d, *J* = 3.4 Hz, 1H), 7.05 (dd, *J* = 17.2, 10.5 Hz, 1H), 6.52 (d, *J* = 17.9 Hz, 2H), 5.85 (d, *J* = 10.5 Hz, 1H).

1-Phenyl-3-buten-2-one (2J): Eluent ratio EA= 1:20, obtained a pale-yellow oily liquid (228 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.34 (t, *J* = 7.2 Hz, 2H), 7.29 – 7.20 (m, 3H), 6.42 (dd, *J* = 17.6, 10.2 Hz, 1H), 6.31 (dd, *J* = 17.5, 1.4 Hz, 1H), 5.83 (dd, *J* = 10.2, 1.4 Hz, 1H), 3.88 (s, 2H).

1-(4-Methylphenyl)-3-buten-2-one (2k): Eluent ratio EA= 1:20, obtained a colorless oily liquid (632 mg),^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.13 (q, *J* = 8.1 Hz, 4H), 6.41 (dd, *J* = 17.6, 10.2 Hz, 1H), 6.30 (dd, *J* = 17.6, 1.5 Hz, 1H), 5.81 (dd, *J* = 10.3, 1.5 Hz, 1H), 3.84 (s, 2H), 2.34 (s, 3H).

1-(4-Chlorophenyl)-3-buten-2-one (2L): Eluent ratio EA= 1:20, obtained a pale yellow oily liquid (654 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.30 (d, *J* = 8.4 Hz, 2H), 7.15 (s, 2H), 6.40 (dd, *J* = 17.6, 10.2 Hz, 1H), 6.33 – 6.27 (m, 1H), 5.86 (dd, *J* = 10.2, 1.0 Hz, 1H), 3.85 (s, 2H).

1-(2-Methylphenyl)-3-buten-2-one (2M): Eluent ratio EA= 1:20, obtained a colorless oily liquid (587 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.22 – 7.11 (m, 4H), 6.44 (dd, *J* = 17.5, 10.3 Hz, 1H), 6.32 (dd, *J* = 17.5, 1.5 Hz, 1H), 5.81 (dd, *J* = 10.3, 1.5 Hz, 1H), 3.89 (s, 2H), 2.25 (s, 3H).

1-(4-Methoxyphenyl)-3-buten-2-one (2N): Eluent ratio EA= 1:20, obtained a colorless oily liquid (642 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.12 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 6.40 (dd, *J* = 17.6, 10.3 Hz, 1H), 6.29 (dd, *J* = 17.5, 1.3 Hz, 1H), 5.80 (dd, *J* = 10.3, 1.3 Hz, 1H), 3.80 (s, 2H), 3.78 (s, 3H).

1-(2-Methoxyphenyl)-3-buten-2-one (2O): Eluent ratio EA= 1:20, obtained a pale yellow oily liquid (446 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.26 (td, *J* = 7.6, 1.8 Hz, 1H), 7.15 (dd,

J = 7.5, 1.8 Hz, 1H), 6.97 – 6.84 (m, 2H), 6.42 (dd, *J* = 17.5, 10.2 Hz, 1H), 6.31 (dd, *J* = 17.6, 1.6 Hz, 1H), 5.77 (dd, *J* = 10.2, 1.6 Hz, 1H), 3.87 (s, 2H), 3.80 (s, 3H).

1-(4-tert-Butylphenyl)-3-buten-2-one (2P): Eluent ratio EA= 1:25, obtained a pale yellow oily liquid (505 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.41 – 7.34 (m, 2H), 7.20 – 7.14 (m, 2H), 6.49 – 6.27 (m, 2H), 5.81 (dd, *J* = 10.3, 1.5 Hz, 1H), 3.86 (s, 2H), 1.33 (s, 10H).

5-Phenyl-1-penten-3-one (2Q): Eluent ratio EA= 1:20, obtained a colorless oily liquid (607 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33 – 7.27 (m, 2H), 7.22 (d, *J* = 7.3 Hz, 3H), 6.37 (dd, *J* = 17.7, 10.5 Hz, 1H), 6.27 – 6.18 (m, 1H), 5.85 – 5.81 (m, 1H), 3.00 – 2.90 (m, *J* = 4.1 Hz, 4H).

5-(4-Methylphenyl)-1-penten-3-one (2R): Eluent ratio EA= 1:20, obtained a pale yellow oily liquid (583 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33 – 7.27 (m, 2H), 7.22 (d, *J* = 7.3 Hz, 3H), 6.37 (dd, *J* = 17.7, 10.5 Hz, 1H), 6.27 – 6.18 (m, 1H), 5.85 – 5.81 (m, 1H), 3.00 – 2.90 (m, *J* = 4.1 Hz, 4H).

5-(4-Chlorophenyl)-1-penten-3-one (2s): Eluent ratio EA= 1:20, obtained a colorless oily liquid (685 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.22 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 6.33 (dd, *J* = 17.7, 10.5 Hz, 1H), 6.19 (dd, *J* = 17.7, 0.9 Hz, 1H), 5.81 (dd, *J* = 10.5, 0.9 Hz, 1H), 2.94 – 2.84 (m, 4H).

5-(4-Methoxyphenyl)-1-penten-3-one (2T): Eluent ratio EA= 1:15, obtained a colorless oily liquid (642 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.14 – 7.09 (m, 2H), 6.86 – 6.79 (m, 2H), 6.35 (dd, *J* = 17.7, 10.5 Hz, 1H), 6.20 (dd, *J* = 17.7, 1.0 Hz, 1H), 5.82 (dd, *J* = 10.5, 1.1 Hz, 1H), 3.78 (s, 3H), 2.89 (s, 4H).

5-(2-Methylphenyl)-1-penten-3-one (2U): Eluent ratio EA= 1:40, obtained a colorless oily liquid (574 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.14 (d, *J* = 2.4 Hz, 4H), 6.37 (dd, *J* = 17.7, 10.5 Hz, 1H), 6.22 (dd, *J* = 17.7, 1.1 Hz, 1H), 5.84 (dd, *J* = 10.5, 1.1 Hz, 1H), 2.97 – 2.91 (m, 2H), 2.87 (ddd, *J* = 9.4, 6.2, 2.0 Hz, 2H), 2.32 (s, 3H).

5-(2-Chlorophenyl)-1-penten-3-one (2V): Eluent ratio EA= 1:40, obtained a pale yellow oily liquid (440 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.34 (dd, *J* = 7.4, 1.8 Hz, 1H), 7.27 – 7.24 (m, 1H), 7.21 – 7.12 (m, 2H), 6.36 (dd, *J* = 17.7, 10.5 Hz, 1H), 6.23 (dd, *J* = 17.7, 1.2 Hz, 1H), 5.84 (dd, *J* = 10.5, 1.2 Hz, 1H), 3.08 – 3.04 (m, 2H), 2.95 – 2.90 (m, 2H).

6. Characterization data of products

3-(1,1,1-Trifluoro-2-((4-nitrobenzylidene)amino)-2-propyl)cyclopentanone (3A): Eluent ratio EA= 1:10, obtained a colorless oily liquid (32 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.48 (s, 1H), 8.30 (d, *J* = 8.8 Hz, 2H), 7.98 (d, *J* = 8.8 Hz, 2H), 2.95 – 2.75 (m, 1H), 2.42 – 2.32 (m, 2H), 2.25 – 2.15 (m, 2H), 2.08 – 2.02 (m, 2H), 1.50 – 1.45 (m, 3H).

3-(1,1,1-Trifluoro-2-((4-nitrobenzylidene)amino)-2-propyl)cyclohexanone (3B): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (35 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.41 (s, 1H), 8.30 (d, *J* = 8.8 Hz, 2H), 7.98 (d, *J* = 8.8 Hz, 2H), 2.67 – 2.56 (m, 1H), 2.45 – 2.37 (m, 2H), 2.26 (dd, *J* = 12.8, 10.0 Hz, 2H), 2.17 (s, 1H), 1.92 (d, *J* = 10.5 Hz, 1H), 1.60 (d, *J* = 7.9 Hz, 2H), 1.47 (s, 3H).

3-(1,1,1-Trifluoro-2-((4-cyanobenzylidene)amino)-2-propyl)cyclopentanone (3C): Eluent ratio EA= 1:8, obtained a pale yellow oily liquid (18 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.42 (s, 1H), 7.91 (d, *J* = 8.3 Hz, 2H), 7.73 (d, *J* = 8.3 Hz, 2H), 2.83 (tt, *J* = 12.1, 6.5 Hz, 1H), 2.42 – 2.29 (m, 2H), 2.26 – 2.09 (m, 2H), 2.07 – 1.95 (m, 2H), 1.46 (s, 3H).

3-(1,1,1-Trifluoro-2-((4-cyanobenzylidene)amino)-2-propyl)cyclohexanone (3D): Eluent ratio EA= 1:8, obtained a pale yellow oily liquid (20 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.35 (s, 1H), 7.91 (d, *J* = 8.3 Hz, 2H), 7.74 (d, *J* = 8.3 Hz, 2H), 2.60 (d, *J* = 13.3 Hz, 1H), 2.45 – 2.34 (m, 2H), 2.30 – 2.20 (m, 2H), 2.15 – 2.05 (m, 1H), 1.91 (d, *J* = 10.1 Hz, 1H), 1.64 – 1.56 (m, 2H), 1.45 (s, 3H).

3-(1,1,1-Trifluoro-2-((2-nitrobenzylidene)amino)-2-propyl)cyclopentanone (3E): Eluent ratio EA= 1:15, obtained a pale yellow oily liquid (15 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.82 (s, 1H), 8.05 (dd, *J* = 29.1, 7.8 Hz, 2H), 7.68 (dt, *J* = 34.9, 7.1 Hz, 2H), 2.84 (dt, *J* = 11.7, 6.5 Hz, 1H), 2.38 (d, *J* = 15.5 Hz, 2H), 2.25 – 1.94 (m, 4H), 1.49 (s, 3H).

3-(1,1,1-Trifluoro-2-((2-nitrobenzylidene)amino)-2-propyl)cyclohexanone (3F): Eluent ratio EA= 1:15, obtained a pale yellow oily liquid (23 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.76 (s, 1H), 8.09 (dd, *J* = 8.1, 1.0 Hz, 1H), 8.03 – 7.98 (m, 1H), 7.72 (t, *J* = 7.5 Hz, 1H), 7.63 (td, *J* = 8.1, 1.4 Hz, 1H), 2.65 (d, *J* = 14.4 Hz, 1H), 2.40 (ddt, *J* = 15.9, 12.2, 5.2 Hz, 4H), 2.26 (s, 1H), 2.18 – 2.08 (m, 1H), 2.02 – 1.89 (m, 2H), 1.47 (s, 3H).

7,7,7-Trifluoro-6-methyl-6-((4-nitrobenzylidene)amino)heptan-3-one (3G): Eluent ratio EA= 1:10, obtained a colorless oily liquid (50 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.41 (s, 1H), 8.29 (d, *J* = 8.7 Hz, 2H), 7.95 (d, *J* = 8.7 Hz, 2H), 2.53 – 2.36 (m, 4H), 2.27 (ddd, *J* =

15.4, 9.6, 5.8 Hz, 1H), 2.14 (ddd, *J* = 16.2, 8.5, 4.5 Hz, 1H), 1.46 (s, 3H), 1.00 (t, *J* = 7.3 Hz, 3H).

8,8,8-Trifluoro-7-methyl-7-((4-nitrobenzylidene)amino)octan-4-one (3H): Eluent ratio EA= 1:10, obtained a colorless oily liquid (58 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.41 (s, 1H), 8.29 (d, *J* = 7.7 Hz, 2H), 7.96 (d, *J* = 7.7 Hz, 2H), 2.49 (qd, *J* = 10.5, 4.7 Hz, 2H), 2.41 – 2.33 (m, 2H), 2.25 (td, *J* = 9.3, 5.0 Hz, 1H), 2.19 – 2.11 (m, 1H), 1.59 – 1.53 (m, 2H), 1.46 (s, 3H), 0.87 (td, *J* = 7.4, 1.5 Hz, 3H).

7,7,7-Trifluoro-2,6-dimethyl-6-((4-nitrobenzylidene)amino)heptan-3-one (3I): Eluent ratio EA= 1:10, obtained a colorless oily liquid (27 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.42 (s, 1H), 8.28 (d, *J* = 8.8 Hz, 2H), 7.96 (d, *J* = 8.8 Hz, 2H), 2.62 – 2.49 (m, 3H), 2.23 (ddd, *J* = 15.4, 9.7, 6.2 Hz, 1H), 2.16 (d, *J* = 5.6 Hz, 1H), 1.46 (s, 3H), 1.05 (dd, *J* = 9.0, 6.9 Hz, 6H).

5,5,5-Trifluoro-1-cyclohexyl-4-methyl-4-((4-nitrobenzylidene)amino)pentan-1-one (3J): Eluent ratio EA= 1:10, obtained a colorless oily liquid (42 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.41 (s, 1H), 8.29 (d, *J* = 8.7 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 2H), 2.54 (t, *J* = 7.9 Hz, 2H), 2.43 (q, *J* = 7.3 Hz, 2H), 2.24 – 2.15 (m, 2H), 1.92 (q, *J* = 7.5 Hz, 2H), 1.03 (t, *J* = 7.3 Hz, 3H), 0.94 (t, *J* = 7.5 Hz, 3H).

5,5,5-Trifluoro-4-methyl-4-((4-nitrobenzylidene)amino)-1-phenylpentan-1-one (3K):

Eluent ratio EA= 1:5, obtained a pale yellow oily liquid (38 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.41 (s, 1H), 8.29 (d, *J* = 8.7 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 2H), 2.54 (t, *J* = 7.9 Hz, 2H), 2.43 (q, *J* = 7.3 Hz, 2H), 2.24 – 2.15 (m, 2H), 1.92 (q, *J* = 7.5 Hz, 2H), 1.03 (t, *J* = 7.3 Hz, 3H), 0.94 (t, *J* = 7.5 Hz, 3H).

5,5,5-Trifluoro-4-methyl-4-((4-nitrobenzylidene)amino)-1-(2-thienyl)pentan-1-one (3L): Eluent ratio EA= 1:5, obtained a pale yellow oily liquid (63 mg), 1H NMR (400 MHz, Chloroform-d) δ 8.47 (s, 1H), 8.27 (d, *J*=13.6 Hz, 2H), 7.93 (d, *J*=6.4 Hz, 2H), 7.71 (d, *J*=6.4 Hz, 1H), 7.55 (d, *J*=13.6 Hz, 1H), 7.12 (d, *J*=6.4 Hz, 1H), 3.03–2.94 (m, 2H), 2.50–2.32 (m, 2H), 1.55 (s, 3H).

5,5,5-Trifluoro-4-methyl-4-((4-nitrobenzylidene)amino)-1-(2-furyl)pentan-1-one (3M): Eluent ratio EA= 1:5, obtained a pale yellow oily liquid (55 mg), δ 8.46 (s, 1H), 8.28 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 8.0 Hz, 1H), 7.18 (d, J = 8.0 Hz, 1H), 6.54 (dd, J = 8.0, 2.4 Hz, 1H), 3.11–2.85 (m, 3H), 2.46 (ddd, 1H), 2.36–2.27 (m, 1H), 1.54 (s, 3H).

7,7,7-Trifluoro-6-ethyl-6-((4-nitrobenzylidene)amino)heptan-3-one (3N): Eluent ratio EA= 1:5, obtained a pale yellow oily liquid (46 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.41 (s, 1H), 8.29 (d, *J* = 8.7 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 2H), 2.54 (t, *J* = 7.9 Hz, 2H), 2.43 (q, *J* = 7.3 Hz, 2H), 2.24 – 2.15 (m, 2H), 1.92 (q, *J* = 7.5 Hz, 2H), 1.03 (t, *J* = 7.3 Hz, 3H), 0.94 (t, *J* = 7.5 Hz, 3H).

8,8,8-Trifluoro-7-ethyl-7-((4-nitrobenzylidene)amino)octan-4-one (3O): Eluent ratio EA= 1:5, obtained a pale yellow oily liquid (38 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.41 (s, 1H), 8.28 (d, *J* = 8.8 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 2H), 2.53 (t, *J* = 7.9 Hz, 2H), 2.38 (t, *J* = 7.3 Hz, 2H), 2.19 (dtd, *J* = 22.9, 14.9, 7.7 Hz, 2H), 1.92 (q, *J* = 7.5 Hz, 2H), 1.61 – 1.56 (m, 2H), 0.94 (t, *J* = 7.4 Hz, 3H), 0.89 (t, *J* = 7.4 Hz, 3H).

7,7,7-Trifluoro-6-phenyl-6-((4-nitrobenzylidene)amino)heptan-3-one (3P): Eluent ratio EA= 1:8, obtained a colorless oily liquid (30 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.42 (s, 1H), 8.31 (d, *J* = 8.6 Hz, 2H), 8.00 (d, *J* = 8.6 Hz, 2H), 7.48 (d, *J* = 7.0 Hz, 2H), 7.41 (d, *J* = 7.6 Hz, 3H), 2.76 (dt, *J* = 15.0, 7.0 Hz, 1H), 2.68 – 2.61 (m, 1H), 2.47 – 2.40 (m, 2H), 2.36 – 2.30 (m, 2H), 0.98 (s, 3H).

6-Phenyl-6-((4-nitrobenzylidene)amino)heptan-3-one (3Q): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (33 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.32 (s, 1H), 8.19 (d, *J* = 8.8 Hz, 2H), 7.79 (dd, *J* = 7.5, 2.0 Hz, 2H), 7.61 (d, *J* = 8.7 Hz, 2H), 7.44 (d, *J* = 6.7 Hz, 3H), 4.46 (dd, *J* = 7.6, 5.1 Hz, 1H), 2.42 – 2.36 (m, 4H), 2.23 – 2.18 (m, 2H), 1.03 (d, *J* = 7.3 Hz, 3H).

6-(4-Nitrophenyl)-6-((4-nitrobenzylidene)amino)heptan-3-one (3R): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (39 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.42 (s, 1H), 8.29 (d, *J* = 8.5 Hz, 2H), 8.21 (d, *J* = 8.5 Hz, 2H), 7.96 (d, *J* = 8.5 Hz, 2H), 7.60 (d, *J* = 8.6 Hz, 2H), 4.55 (t, *J* = 6.7 Hz, 1H), 2.42 – 2.37 (m, 4H), 2.24 – 2.20 (m, 2H), 1.01 (d, *J* = 7.3 Hz, 3H).

6-(4-Methoxyphenyl)-6-((4-nitrobenzylidene)amino)heptan-3-one (3S): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (27 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.24 (s, 1H), 8.19 (d, *J* = 8.8 Hz, 2H), 7.73 (d, *J* = 8.7 Hz, 2H), 7.60 (d, *J* = 8.6 Hz, 2H), 6.94 (d, *J* = 8.7 Hz, 2H), 4.41 (dd, *J* = 8.0, 5.2 Hz, 1H), 3.90 – 3.79 (m, 4H), 2.39 (dd, *J* = 14.3, 7.0 Hz, 4H), 2.17 (t, *J* = 7.8 Hz, 2H), 1.01 (t, *J* = 7.3 Hz, 3H).

6-(2-Bromophenyl)-6-((4-nitrobenzylidene)amino)heptan-3-one (3T): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (45 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.46 (s,

1H), 8.27 (d, *J* = 8.8 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 2H), 7.70 (d, *J* = 9.4 Hz, 1H), 7.56 (d, *J* = 8.0 Hz, 1H), 7.34 (t, *J* = 7.5 Hz, 1H), 7.13 (t, *J* = 7.7 Hz, 1H), 4.91 (t, *J* = 6.6 Hz, 1H), 2.42 (p, *J* = 8.2, 7.7 Hz, 4H), 2.23 (q, *J* = 7.8, 7.4 Hz, 2H), 1.02 (t, *J* = 7.3 Hz, 3H).

6-(Thiophen-2-yl)-6-((4-nitrobenzylidene)amino)heptan-3-one (3U): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (38 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.40 (s, 1H), 8.19 (d, *J* = 8.8 Hz, 2H), 7.58 (d, *J* = 8.6 Hz, 2H), 7.44 (d, *J* = 5.0 Hz, 1H), 7.33 (d, *J* = 3.6 Hz, 1H), 7.13 – 7.05 (m, 1H), 4.43 (dd, *J* = 8.1, 5.2 Hz, 1H), 2.40 (dq, *J* = 14.6, 7.3 Hz, 4H), 2.23 – 2.12 (m, 2H), 1.02 (t, *J* = 7.3 Hz, 3H).

6,6,6-Trifluoro-1-phenyl-5-methyl-5-((4-nitrobenzylidene)amino)heptan-2-one (3V): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (30 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.71 (s, 1H), 7.65 (d, *J* = 8.8 Hz, 2H), 7.27 (d, *J* = 8.8 Hz, 2H), 6.69 – 6.58 (m, 5H), 3.05 (s, 2H), 1.93 (ddd, *J* = 15.3, 9.5, 5.8 Hz, 2H), 1.63 – 1.48 (m, 2H), 0.78 (s, 3H).

6,6,6-Trifluoro-1-(4-methylphenyl)-5-methyl-5-((4 nitrobenzylidene)amino)heptan-2-one (3W): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (42 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.38 (s, 1H), 8.28 (d, *J* = 8.8 Hz, 2H), 7.94 (d, *J* = 8.8 Hz, 2H), 7.08 (d, *J* = 10.8 Hz, 2H), 7.03 (d, *J* = 11.3 Hz, 2H), 2.79 (d, *J* = 6.8 Hz, 2H), 2.30 (d, *J* = 9.5 Hz, 5H), 1.60 (s, 2H), 1.43 (s, 3H).

6,6,6-Trifluoro-1-(4-chlorophenyl)-5-methyl-5-((4-nitrobenzylidene)amino)heptan-2-one (3X): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (56 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.31 (s, 1H), 8.26 (d, *J* = 8.8 Hz, 2H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.04 (d, *J* = 8.6 Hz, 2H), 6.79 (d, *J* = 8.6 Hz, 2H), 3.59 (s, 2H), 2.53 (ddd, *J* = 17.5, 9.5, 5.9 Hz, 2H), 2.23 (ddd, *J* = 15.2, 9.6, 5.8 Hz, 1H), 2.10 (ddd, *J* = 14.5, 9.2, 5.7 Hz, 1H), 1.39 (s, 3H).

6,6,6-Trifluoro-1-(2-methylphenyl)-5-methyl-5-((4-nitrobenzylidene)amino)heptan-2-one (3Y): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (48 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.33 (s, 1H), 8.28 (d, *J* = 8.7 Hz, 2H), 7.90 (d, *J* = 8.7 Hz, 2H), 7.17 – 7.10 (m, 4H), 3.68 (s, 2H), 2.57 – 2.47 (m, 2H), 2.19 (s, 3H), 1.58 (s, 2H), 1.40 (s, 3H).

6,6,6-Trifluoro-1-(4-methoxyphenyl)-5-methyl-5-((4-nitrobenzylidene)amino)heptan-2-one (3Z): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (16 mg), yield ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.33 – 8.23 (m, 3H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.04 (d, *J* = 8.7 Hz, 2H), 6.80 (d, *J* = 8.7 Hz, 2H), 3.77 (s, 3H), 3.59 (s, 2H), 2.62 – 2.43 (m, 2H), 2.27 – 2.17 (m, 1H), 2.10 (ddd, *J* = 14.9, 9.5, 6.0 Hz, 1H), 1.40 (s, 3H).

6,6,6-Trifluoro-1-(2-methoxyphenyl)-5-methyl-5-((4-nitrobenzylidene)amino)heptan-2-one (3Aa): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (28 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.34 (s, 1H), 8.28 (d, *J* = 8.8 Hz, 2H), 7.91 (d, *J* = 8.8 Hz, 2H), 7.23 (td, *J* = 8.1, 1.7 Hz, 1H), 7.06 (dd, *J* = 7.4, 1.8 Hz, 1H), 6.88 (td, *J* = 7.5, 1.1 Hz, 1H), 6.82 (dd, *J* = 8.2, 1.1 Hz, 1H), 3.72 (s, 3H), 3.64 (s, 2H), 2.57 – 2.49 (m, 2H), 2.28 – 2.19 (m, 1H), 2.17 – 2.08 (m, 1H), 1.41 (s, 3H).

6,6,6-Trifluoro-1-(4-tert-butylphenyl)-5-methyl-5-((4-nitrobenzylidene)amino)heptan-2-one (3Ab): Eluent ratio EA= 1:10, obtained a colorless oily liquid (36 mg), 1H NMR (400 MHz, Chloroform-d) δ 8.37 (s, 1H), 8.31 (d, J = 8.5 Hz, 2H), 7.94 (d, J = 8.5 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 7.09 (d, J = 8.0 Hz, 2H), 3.66 (s, 2H), 2.68–2.49 (m, 2H), 2.25 (td, J = 7.0, 2.0 Hz, 1H), 2.18–2.08 (m, 1H), 1.43 (s, 3H), 1.31 (s, 9H).

7,7,7-Trifluoro-6-methyl-6-((4-nitrobenzylidene)amino)-1-phenylheptan-3-one (3Ac): Eluent ratio EA= 1:10, obtained a colorless oily liquid (60 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.38 (s, 1H), 8.28 (d, *J* = 8.8 Hz, 2H), 7.94 (d, *J* = 8.8 Hz, 2H), 7.24 (d, *J* = 7.5 Hz, 2H), 7.17 (d, *J* = 7.3 Hz, 1H), 7.12 (d, *J* = 7.0 Hz, 2H), 2.84 (t, *J* = 6.9 Hz, 2H), 2.75 – 2.68 (m, 2H), 2.45 (td, *J* = 9.5, 6.0 Hz, 2H), 2.24 (ddd, *J* = 15.4, 9.7, 5.8 Hz, 1H), 2.15 – 2.04 (m, 1H), 1.43 (s, 3H).

7,7,7-Trifluoro-6-methyl-6-((4-nitrobenzylidene)amino)-1-(4-methylphenyl)heptan-3-one (3Ad): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (45 mg), 1H NMR (400 MHz, Chloroform-d) δ 8.40 (s, 1H), 8.30 (d, J = 8.5 Hz, 2H), 7.96 (d, J = 8.5 Hz, 2H), 7.08 (d, J = 8.0 Hz, 2H), 7.03 (d, J = 8.0 Hz, 2H), 2.85–2.80 (m, 2H), 2.74–2.70 (m, 2H), 2.47 (td, J = 7.2, 2.0 Hz, 2H), 2.31 (s, 3H), 2.28–2.22 (m, 1H), 2.18–2.10 (m, 1H), 1.45 (s, 3H).

7,7,7-Trifluoro-6-methyl-6-((4-nitrobenzylidene)amino)-1-(4-chlorophenyl)heptan-3-one (3Ae): Eluent ratio EA= 1:10, obtained a colorless oily liquid (56 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.38 (s, 1H), 8.28 (d, J = 8.8 Hz, 2H), 7.94 (d, J = 8.8 Hz, 2H), 7.20 (d, J = 8.4 Hz, 2H), 7.05 (d, J = 8.4 Hz, 2H), 2.81 (t, J = 6.9 Hz, 2H), 2.72–2.67 (m, 2H), 2.49–2.41 (m, 2H), 2.29–2.21 (m, 1H), 2.15–2.08 (m, 1H), 1.43 (s, 3H).

7,7,7-Trifluoro-6-methyl-6-((4-nitrobenzylidene)amino)-1-(4-methoxyphenyl)heptan-3-one (3Af): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (51 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.85 (s, 1H), 11.76 (d, *J* = 8.7 Hz, 2H), 11.41 (d, *J* = 8.8 Hz, 2H), 10.74 (s, 1H), 10.51 (d, *J* = 8.6 Hz, 2H), 10.26 (d, *J* = 8.6 Hz, 2H), 7.24 (s, 3H), 6.25 (d, *J* =

6.8 Hz, 2H), 6.19 – 6.10 (m, 2H), 5.96 – 5.86 (m, 2H), 5.76 – 5.66 (m, 1H), 5.63 – 5.53 (m, 1H), 4.90 (s, 3H).

7,7,7-Trifluoro-6-methyl-6-((4-nitrobenzylidene)amino)-1-(2-methylphenyl)heptan-3-one (3Ag): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (63 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.38 (s, 1H), 8.28 (d, *J* = 8.7 Hz, 2H), 7.94 (d, *J* = 8.8 Hz, 2H), 7.14 – 7.01 (m, 4H), 2.86 – 2.79 (m, 2H), 2.70 – 2.63 (m, 2H), 2.47 (td, *J* = 9.7, 6.0 Hz, 2H), 2.30 – 2.21 (m, 4H), 2.17 – 2.07 (m, 1H), 1.44 (s, 3H).

7,7,7-Trifluoro-6-methyl-6-((4-nitrobenzylidene)amino)-1-(2-chlorophenyl)heptan-3-one (3Ah): Eluent ratio EA= 1:10, obtained a pale yellow oily liquid (58 mg), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.39 (s, 1H), 8.28 (d, *J* = 8.7 Hz, 2H), 7.94 (d, *J* = 8.7 Hz, 2H), 7.30 (d, *J* = 8.9 Hz, 1H), 7.20 – 7.10 (m, 3H), 2.95 (t, *J* = 7.5 Hz, 2H), 2.73 (t, *J* = 7.6 Hz, 2H), 2.54 – 2.39 (m, 2H), 2.31 – 2.22 (m, 1H), 2.16 – 2.07 (m, 1H), 1.44 (s, 3H).

6. Conditions optimization

The following tables summarize the optimization of various reaction conditions for the synthesis of compound 3A. These conditions include catalyst selection, solvent optimization, substrate ratio, and reaction time, each of which has been systematically evaluated to maximize the yield of the desired compounds. Table S1 presents the catalyst optimization results, showing the yield percentages of compound 3A for different catalysts. Table S2 evaluates the solvent impact on the reaction yields, while Table S3 explores the effect of varying substrates ratios. Table S4 highlights the optimization of reaction time, and finally. These optimizations provide a comprehensive understanding of how each reaction parameter influences the overall efficiency and yield of the target compounds.

Table-S1 Catalyst Optimization

Entry	Catalyst	Isolated Yield in %
1	Transaminase CV2025	78
2	IPGA	55
3	Lypozyme TLIM	34
4	Novozym 435	25
5	Lypozyme RMIM	20
6	Lipase AYS	19

7	PPL	15
8	Lipase Et 2.0	10

Table-S2 Solvent Optimization

Entry	Solvent	Isolated Yield in %
1	n-Octane	78
2	DMSO	65
3	Toluene	61
4	Isopropanol	57

Table-S3 Optimization of substrate (1A) ratio

Entry	Substrate ratio	Isolated Yield in %
1	1A= 1 equiv, 2A= 6 equiv	78
2	1.5 equiv of 1A	58
3	2.0 equiv of 1A	42
4	4.5 equiv of 2A	54
5	5.5 equiv of 2A	66

Table-S4 Optimization for the reaction time

Entry	Reaction time in hr	Isolated Yield in %
1	72	78
2	60	61
3	50	55
4	24	42
5	15	29

7. Mutagenesis and Plasmid Construction

Alanine scanning mutagenesis was employed to study the role of key residues in transaminase CV2025's catalytic activity. Residues were selected based on molecular docking analysis using AutoDock 4.2, focusing on those within 5Å of the substrate-binding pocket. protein crystal structure (PDB: 4AH3) has been fully reported [2], and can be directly obtained from the PDB website. Target residues such as Lys-288, Tyr-153, Arg-416, with others like Phe-22, Trp-60, Ser-121, and Pro-296, were identified for mutation to alanine. Specific primers were designed to introduce these mutations (Table S5), and whole plasmid PCR was performed using

recombinant plasmid pET-21K(+)-CV2025 as a template. The constructed plasmids were confirmed by sequencing, as shown in (Figure S17), and were transformed into *E.coli* BL21 (DE3) competent cells.

8. Protein Expression and Activity Analysis

Following the confirmation of successful plasmid transformation, the mutant colonies were grown in an LB medium containing kanamycin. Protein expression was induced with 1 mM IPTG when the bacterial cultures reached an OD600 of 0.6-0.8. The cultures were incubated at 16°C for 20 hours to express the mutant transaminase. The expressed proteins were purified using nickel affinity chromatography and immobilized on epoxy resin LX-1000EP via covalent bonding (Figure S18). Enzymatic activity was tested using a conjugate addition reaction between imine substrate 1A and α, β-unsaturated cyclic ketone. Using BioEdit software, the sequence results of the mutants, with the wild-type sequence were shown in (Figure S4). The activities of the mutants were compared to the wild-type CV2025 (Table S6). nzymatic activity was tested using a conjugate addition reaction between imine substrate 1A and α,β-unsaturated cyclic ketone, with activities measured via HPLC and normalized to wild-type CV2025 (100%). The relative enzyme activities reported in Table S6 were obtained from single experiments conducted under optimized conditions. These values provide a preliminary assessment of mutant performance, highlighting key residues, and are intended as indicative trends rather than statistically validated differences. Future studies may include replicates to quantify variability. Notably, the K281K and Y153A mutants exhibited increased activity (115.83% and 107.69% of wild-type activity, respectively), while the R416A mutant showed a significant decrease (62.18%), highlighting the critical role of Arg-416 in the catalytic process. All mutant activities are shown in Table S6.

Table S5 Alanine Scanning Mutation Primer

No.	Selected Site	Primer Name	Sequence (5'→3')
1	F22	F22A-T	catccgGCCaccgataccgcatcgc
		F22A-B	cggtGGCcggatgcaggtgatggg
2	W60	W60A-T	ctgGCCtgcgtgaacgtcggctacg
		W60A-B	ttcacgcaGGCcagtccggccatgc
3	N118	N111K-T	ctataccGCCtccggttccgaatcggt
		N111K-B	cggaGGCggtatagaacacgcggtcga
4	S119	S119-T	tgttctataccaatGGCggttccgaat
		S119-B	accGGCattggtatagaacacgcggtc

No.	Selected Site	Primer Name	Sequence (5'→3')
5	S121	S121A-T	ggtGCCgaatcggtggacaccatgat
		S121A-B	ccaccgattcGGCaccggaattggtata
6	E122	E122A-T	gttccGCCtcggtggacaccatgatcc
		E122A-B	ccaccgaGGCggaaccggaattggta
7	S123	S123A-T	ccgaaGCCgtggacaccatgatccgc
		S123A-B	gtgtccacGGCttcggaaccggaattgg
8	Y153	Y153A-T	gaacggcGCCcacggctccaccatc
		Y153A-B	gtgGGCgccgttccagcggccgatca
9	H154	H154A-T	ggctatGCCggctccaccatcggcg
		H154A-B	gagccGGCatagccgttccagcggc
10	S156	S156A-T	acggctatcacggcGCCaccatcg
		S156A-B	ggtGGCgccgtgatagccgttccagcg
11	Y168	Y161K-T	gaagGCCatgcacgagcagggcgac
		Y161K-B	cgtgcatGGCcttcatgccgcccag
12	D259	D259A-T	gccGCCgaagtgatctgcggcttcg
		D259A-B	cacttcGGCggccaccagcagcac
13	T285	T285A-T	ttcGCCgccgccaagggcctgtcct
		T285A-B	cttggcggcGGCgaacaggtcggg
14	K288	K281K-T	gccGCCggcctgtcctccggctatct
		K281K-B	caggccGGCggcggcggtgaac
15	S291	S291A-T	ctgGGCtccggctatctgccgataggcg
		S291A-B	gatagccggaGGCcaggcccttggc
16	P296	P296A-T	ctgGCCataggcgcggtctttgtcg
		P296A-B	cgcctatGGCcagatagccggagga
17	R416	R416A-T	catgGCCgcatgcggcgaccacatc
		R416A-B	ccgcatgcGGCcatgatcaggttgt

Table S6: Relative enzyme activity of all mutants

Entry No.	Mutant	Relative Enzyme Activity (%)

Entry No.	Mutant	Relative Enzyme Activity (%)
1	K281K	115
2	Y153A	107
3	P296A	106
4	F22A	105
5	CV2025-WT	100
6	S121A	99
7	T285A	98
8	E122A	94
9	S123A	88
10	S291A	88
11	W60A	87
12	Y161K	86
13	N111K	85
14	S119A	83
15	S156A	78
16	H154A	65
17	R416A	62

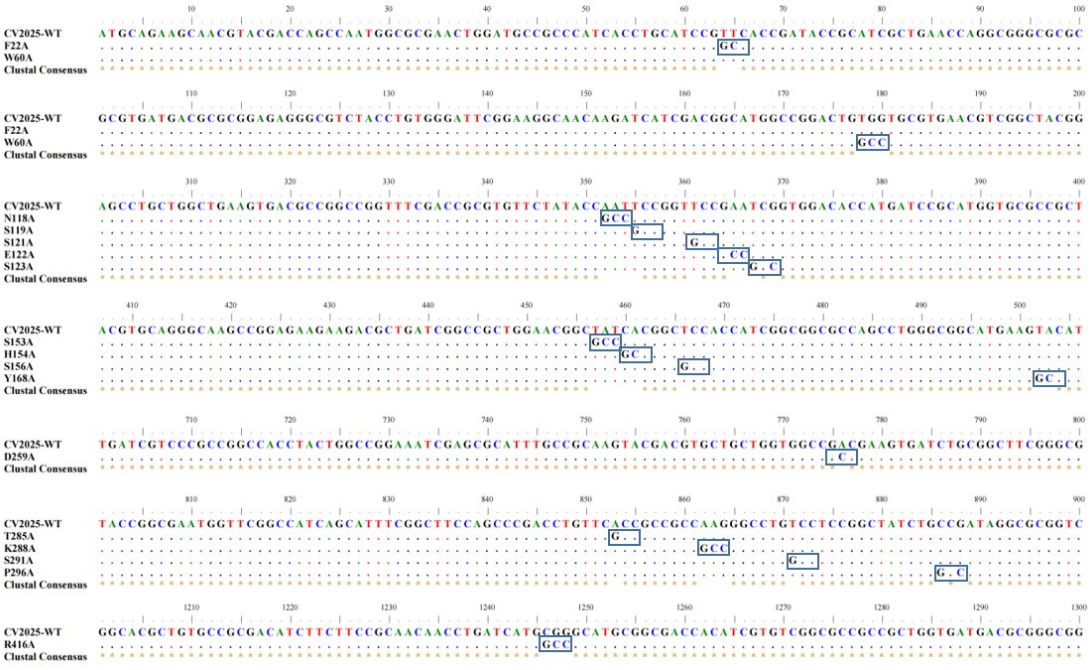


Figure S15: Sequence Alignment of wild-type CV2025 and alanine mutant.

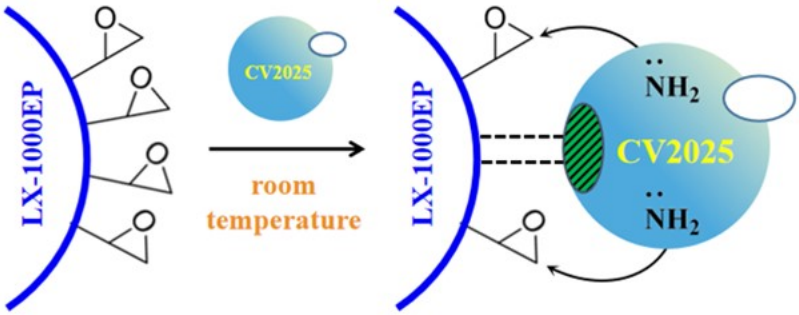


Figure S16. The Immobilization Process of CV2025 on LX-1000EP

9. Molecular docking of Transaminase CV2025 and imine substrate

Molecular docking simulations were conducted to investigate the interactions between imine substrate 1A and transaminase CV2025, using its three-dimensional structure (PDB: 4AH3) as a model (Figure S-17). AutoDock 4.2 was employed to dock 1A into the enzyme's active site, and two conformations with the lowest binding energies and distinct orientations were selected to represent the most favorable binding modes. These results were visualized and analyzed using PyMOL, revealing a spacious substrate-binding pocket capable of accommodating 1A (Figure S18). The first pose (Pose A) exhibited a binding affinity of -6.8 kcal/mol, with 1A positioned to form hydrogen bonds with Tyr153 and engage in hydrophobic interactions with Phe22, Ala231, and Lys288 (Figure S18A). The second pose (Pose B) showed a binding affinity of -6.0 kcal/mol, featuring hydrogen bonding with His154, electrostatic stabilization by Arg416, and additional contacts with Tyr153 and Asp259 (Figure S18B). Based on these docking results, key residues within 5 Å of the substrate were identified for alanine scanning mutagenesis, including hydrophilic residues (Tyr153, His154, Asp259, Thr285, Lys288, Arg416, Asn118, Ser119, Ser121, Glu122, Ser123, Ser156) and hydrophobic residues (Phe22, Trp60, Pro296). Residues slightly beyond this range, such as Tyr168 and Ser291, were also selected to probe their potential influence. These docking-derived insights into the active site configuration directly informed the mutagenesis experiments (Section 9), with the identified interactions correlating with observed catalytic roles (e.g., Arg416, Table S2).

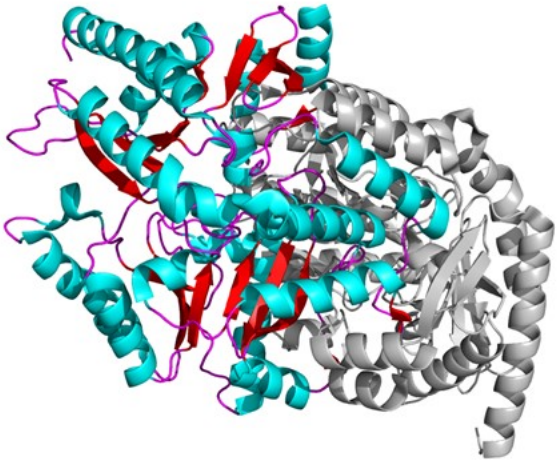


Figure S17: Transaminase CV2025 three-dimensional protein structure (PDB: 4AH3)

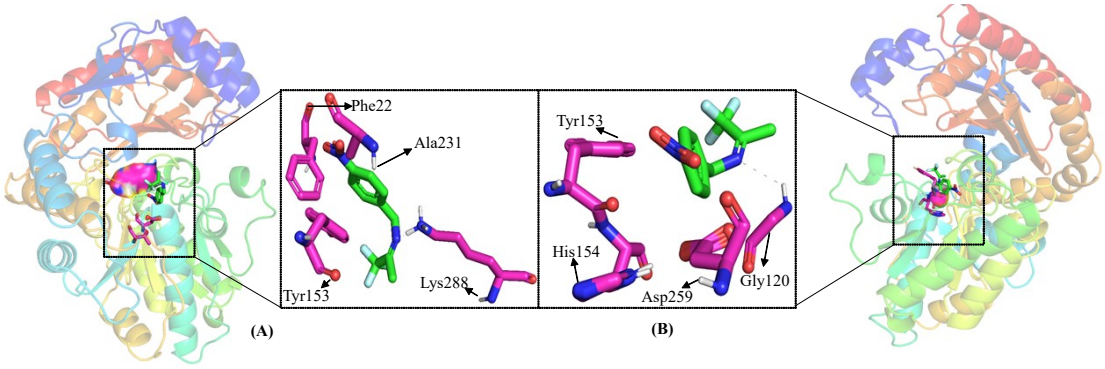


Figure S18. Molecular docking results of imines 1A and enzymes. (**A**) Docking imines 1A into the active site of CV2025 shows the interactions with residues Phe22, Ala231, Tyr153, and Lys288 (**B**) second docking result representing substrate orientation with interactions involving Tyr153, His154, Asp259, and Arg416.

10. Synthesis of 3A on a gram scale

Reaction Condition: To create a mixture of (1A, 4.07 mmol, 1.0 g) as Imine substrate, in α, β-unsaturated ketone (2a, 24.42mmol. 2.0g), in n-Octane (20 ml), CV2025 (2.5 g) was added and stirred at 35°C for 72 hours in a shaker. Upon completion of the reaction, the enzyme was filtered out, the reaction mass was extracted with saturated brine and ethyl acetate (EA) (3 × 5 mL). The organic layers were combined and subjected to drying by a rotary evaporator. TLC monitored the progress of the reaction on Haiyang GF 254 silica gel plates. The product was purified through column chromatography using an eluent ratio of ethyl acetate (EA) to petroleum ether (PE) of 1:5 v/v.

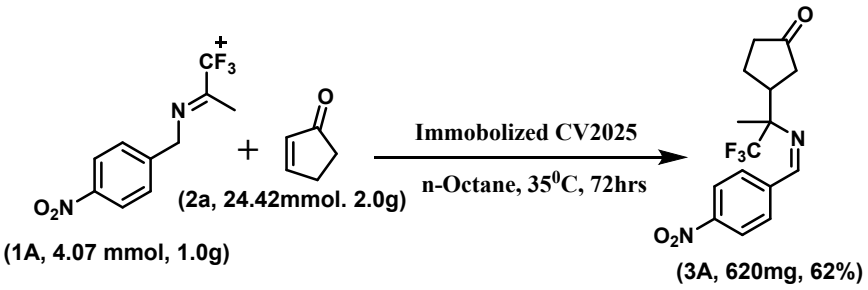


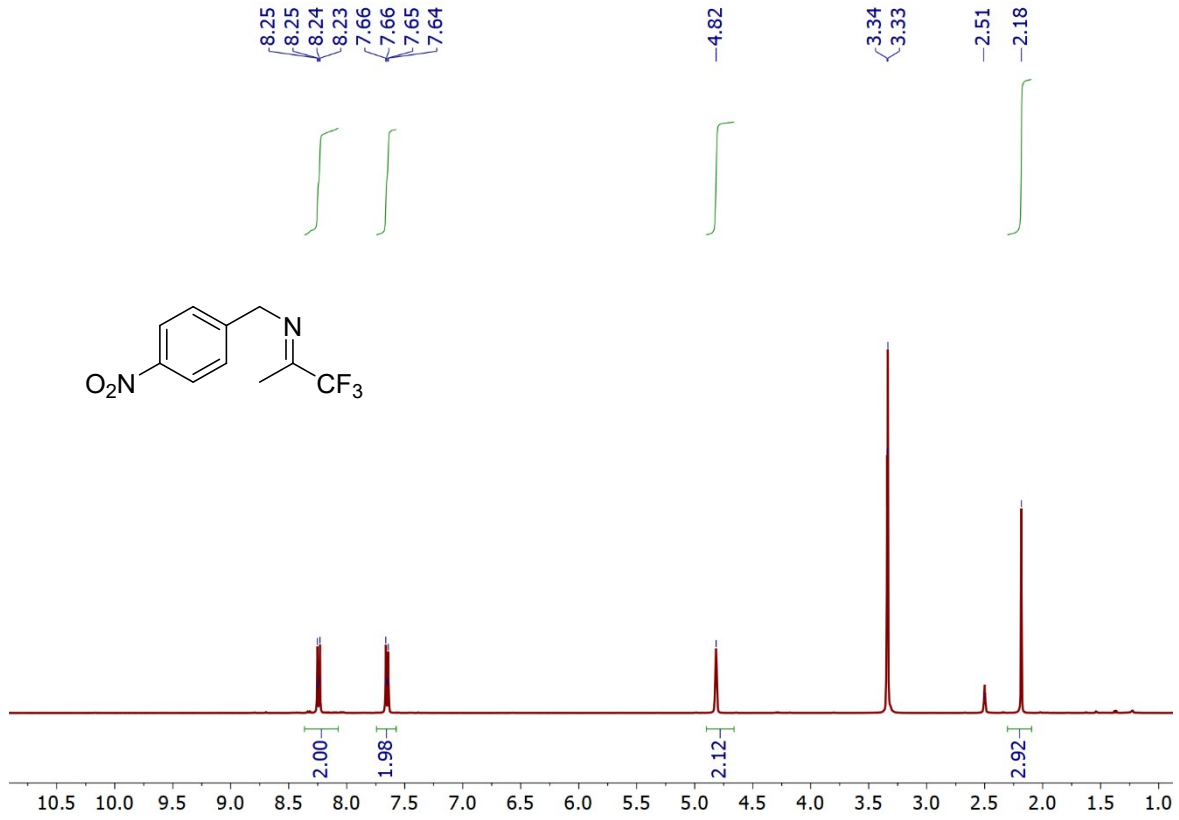
Figure S19. Efficient Gram-Scale Synthesis of 3A

References

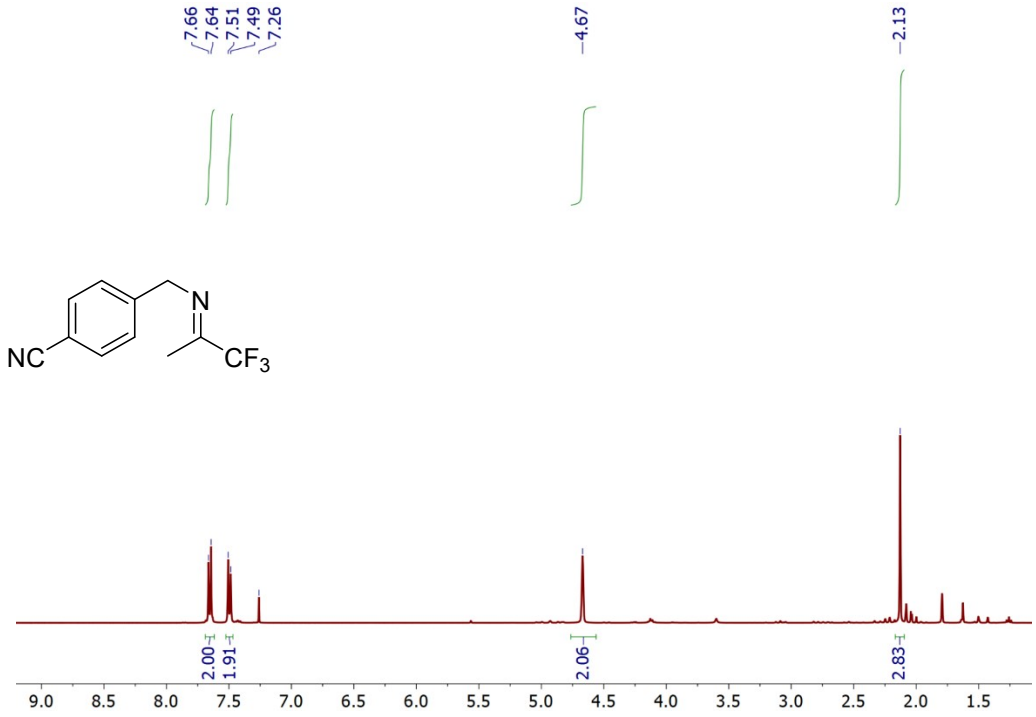
(1) Hu, L.; Wu, Y.; Li, Z.; Deng, L. Catalytic asymmetric synthesis of chiral γ-amino ketones via umpolung reactions of imines. *Journal of the American Chemical Society* **2016**, *138* (49), 15817-15820.

(2) Sayer, C.; Isupov, M. N.; Westlake, A.; Littlechild, J. A. Structural studies of Pseudomonas and Chromobacterium ω-aminotransferases provide insights into their differing substrate specificity. *Acta Crystallographica Section D: Biological Crystallography* **2013**, *69* (4), 564-576.

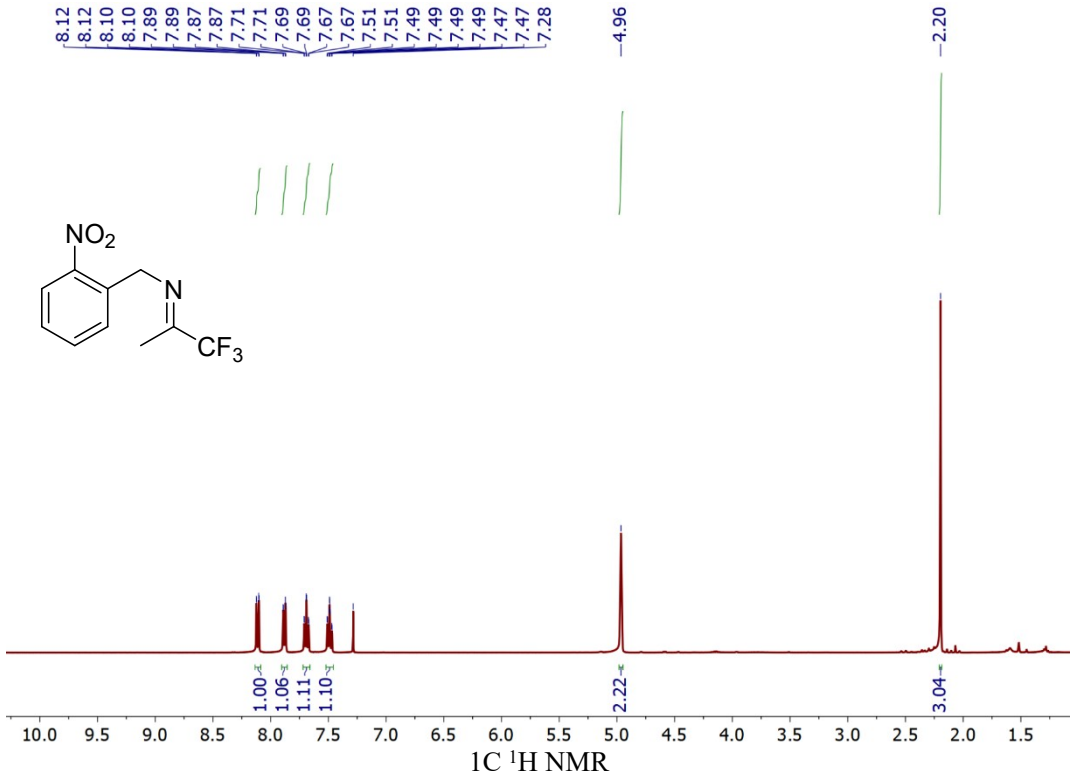
12. ^{1}H NMR Spectrum



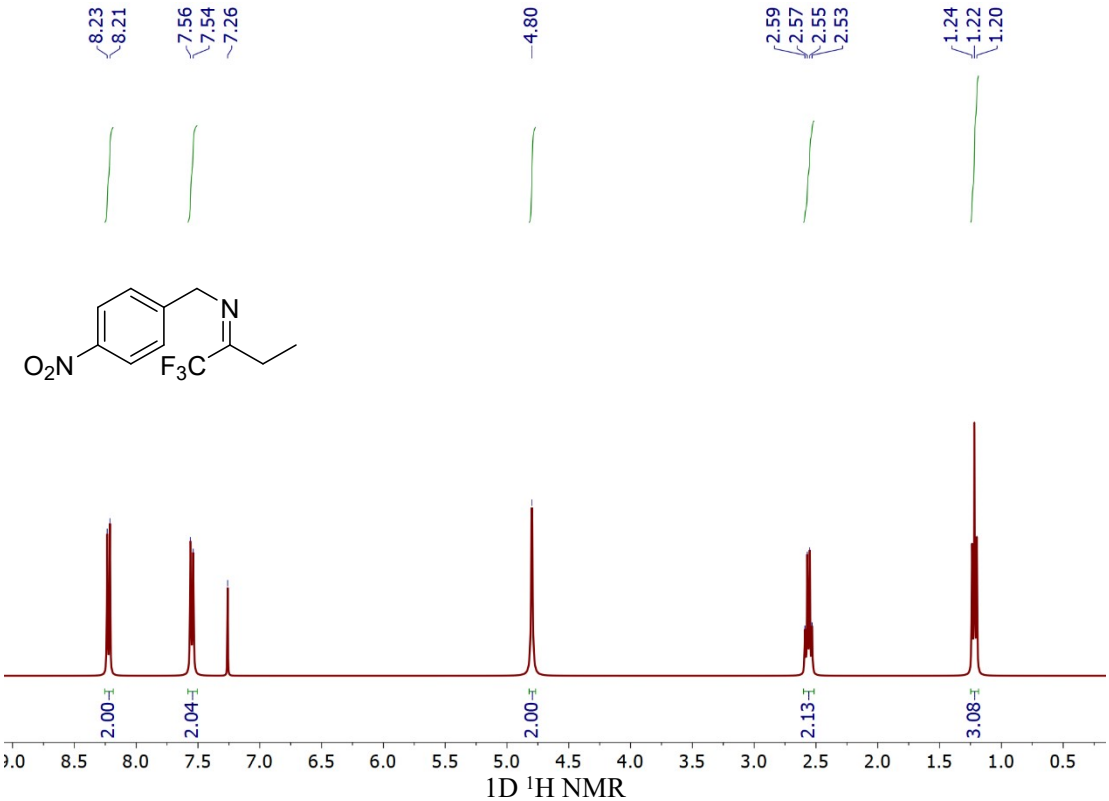
1A ^{1}H NMR



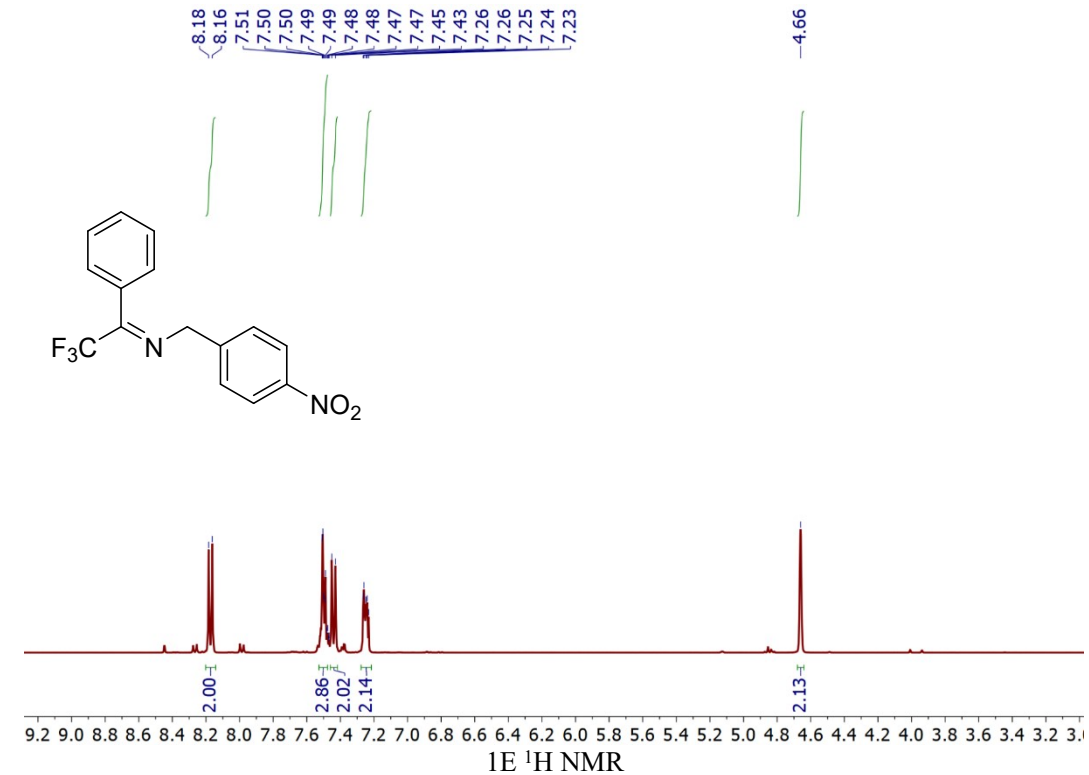
1B ^{1}H NMR



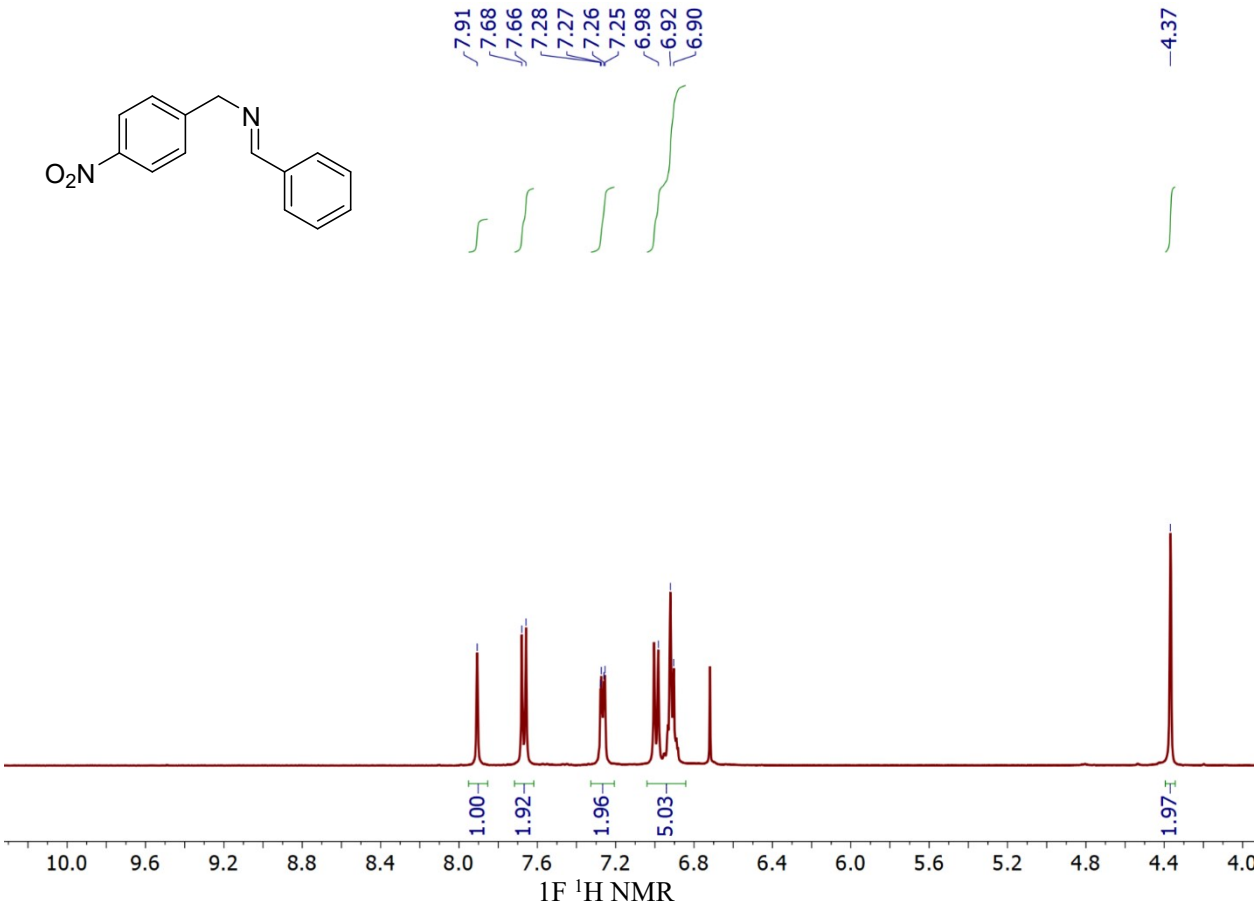
1C ^{1}H NMR



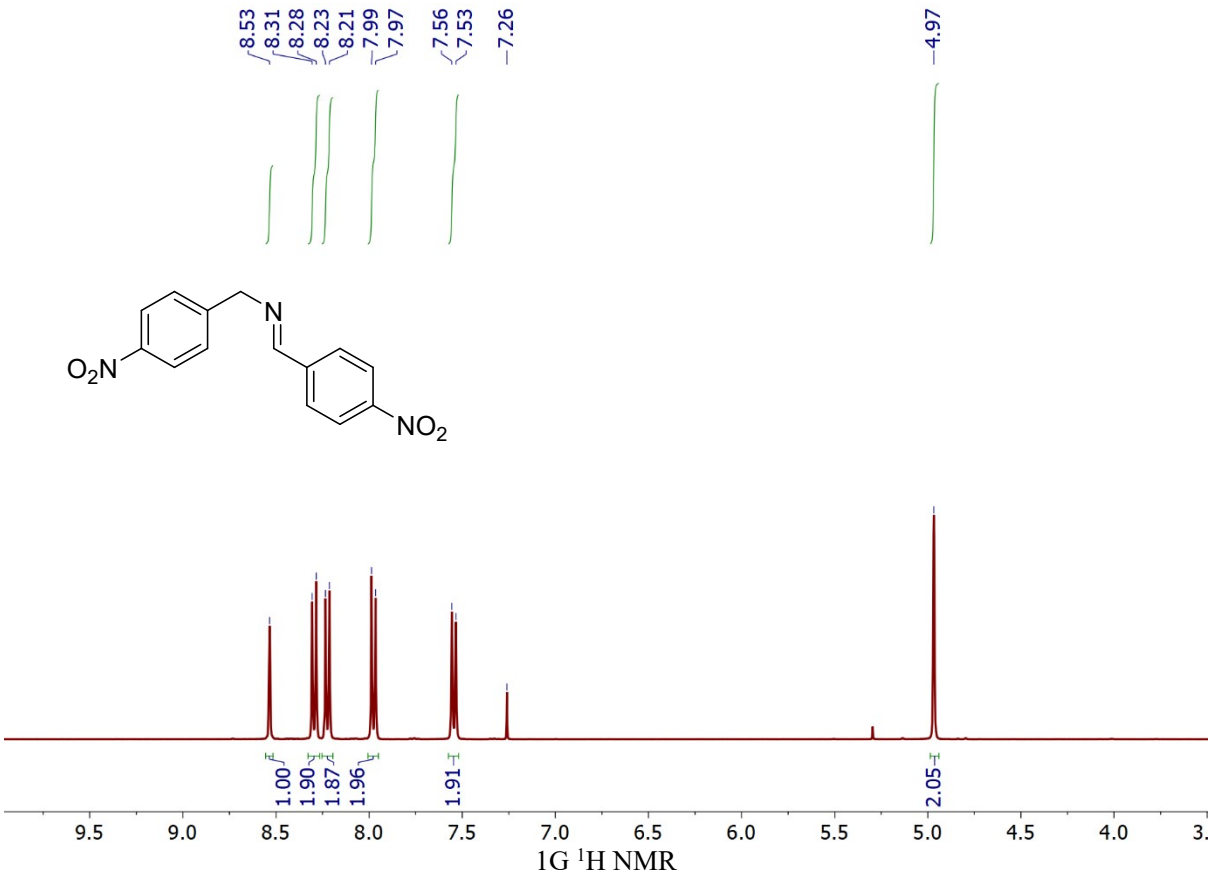
1D ^{1}H NMR



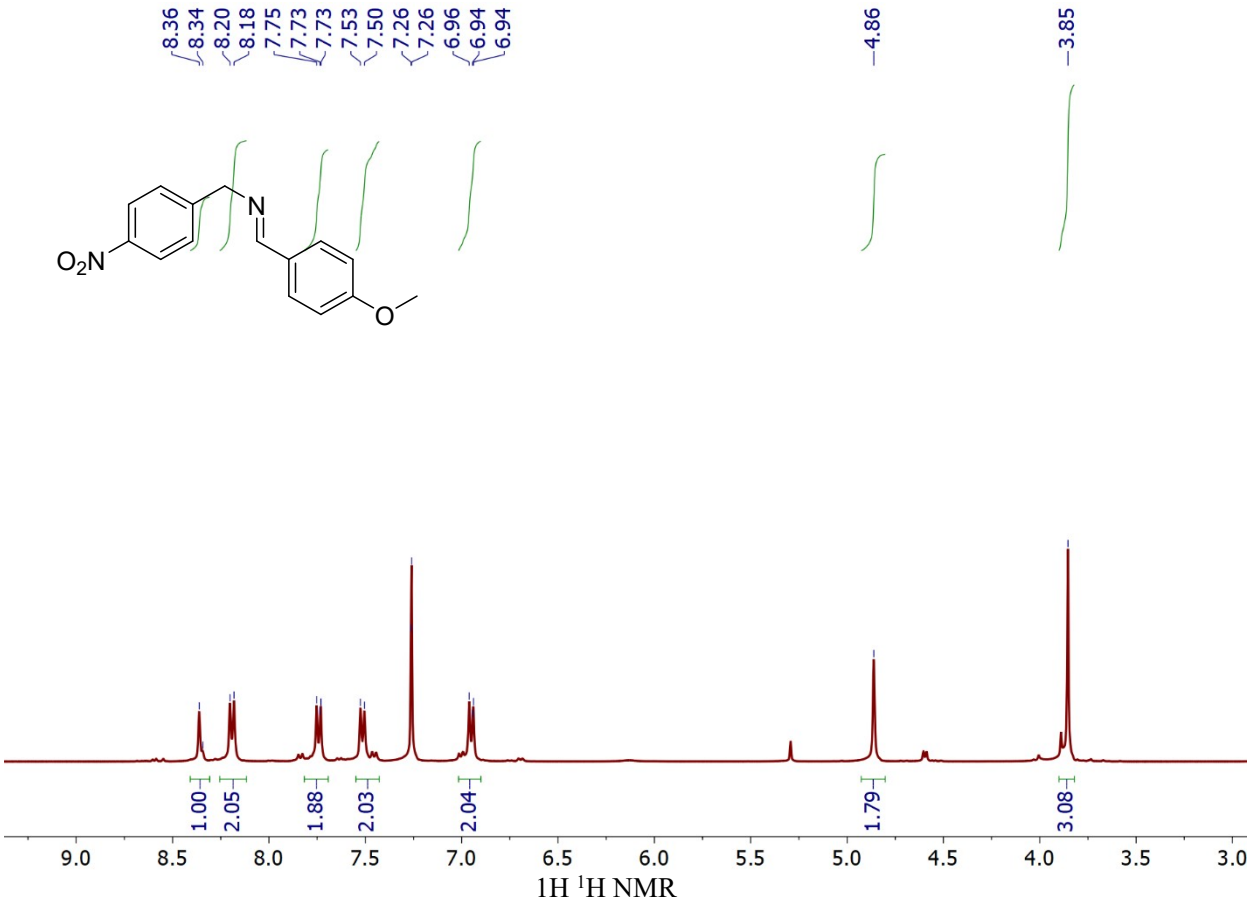
1E ^{1}H NMR



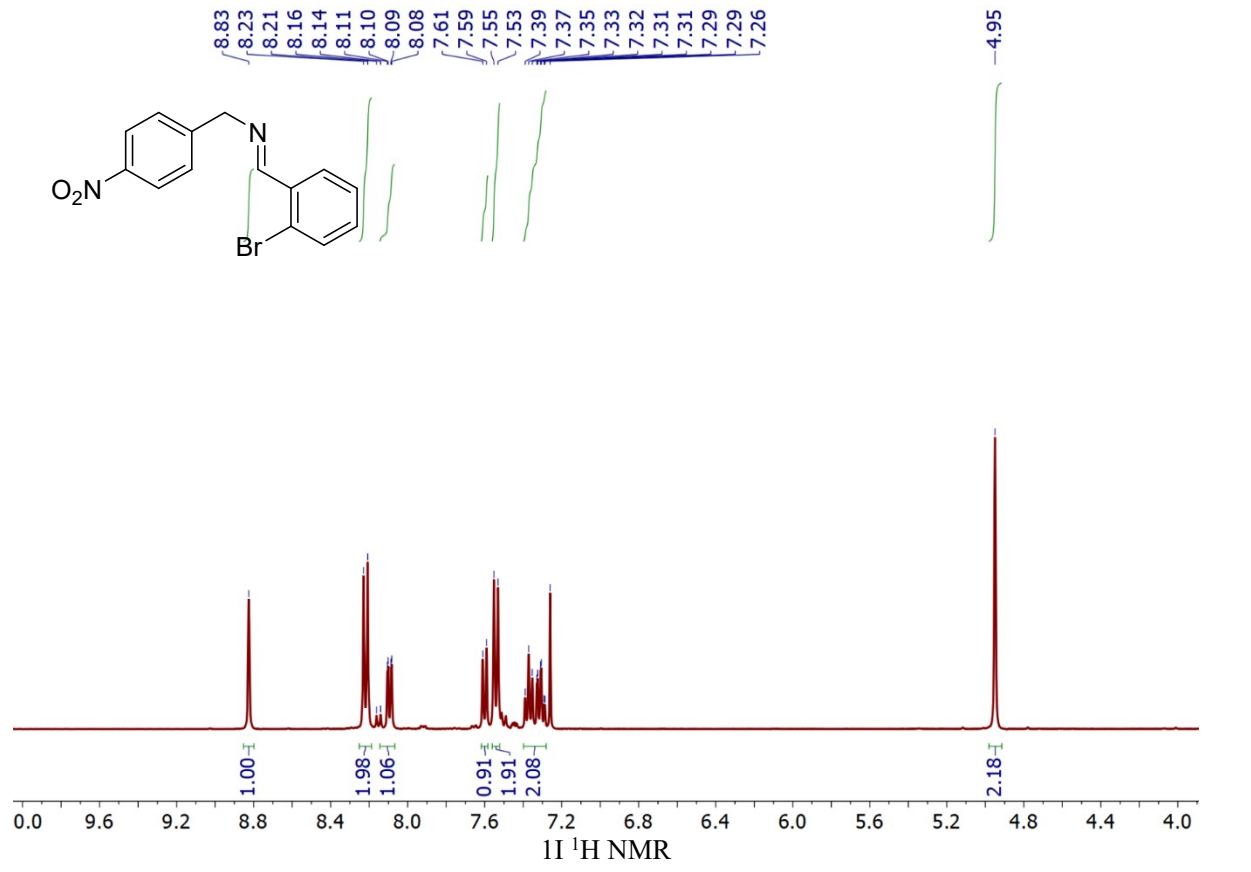
1F ^{1}H NMR



1G ^{1}H NMR



1H ^{1}H NMR



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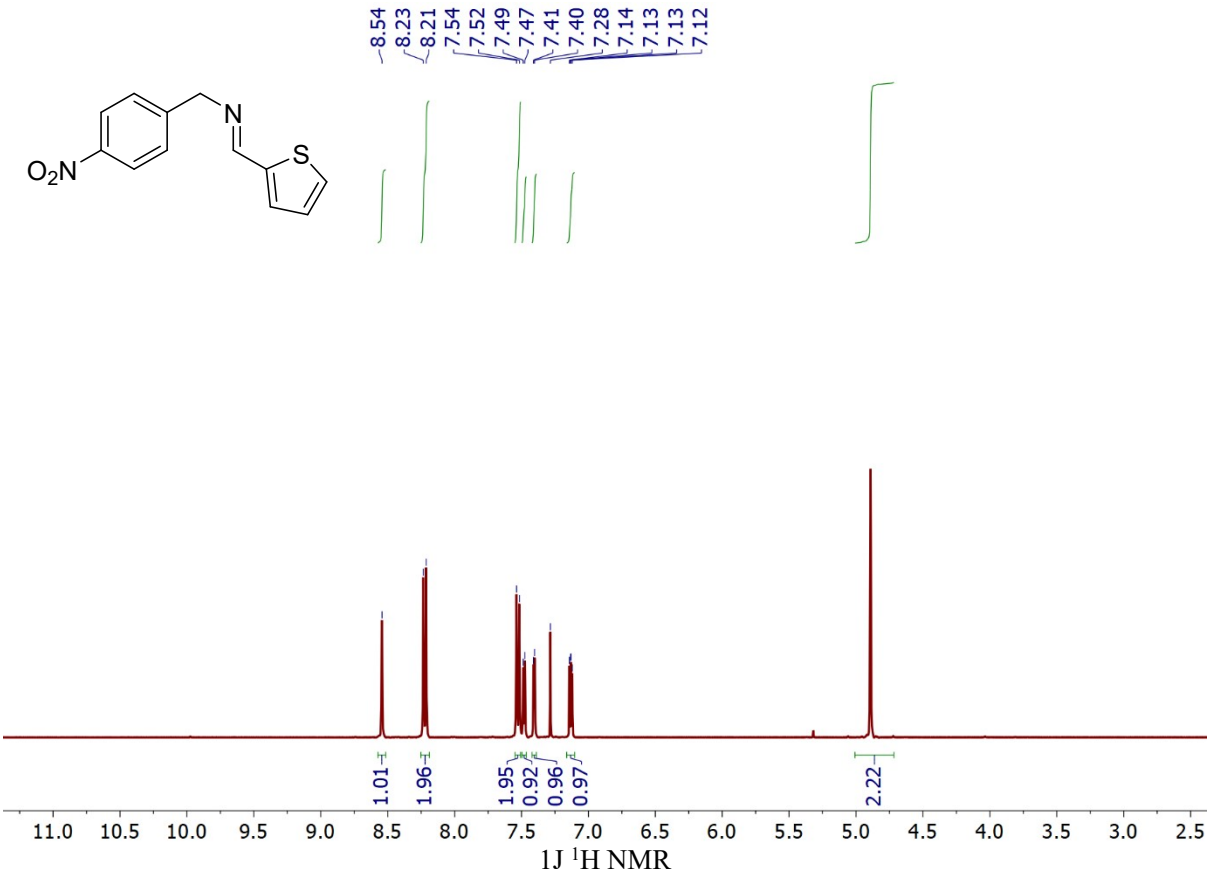

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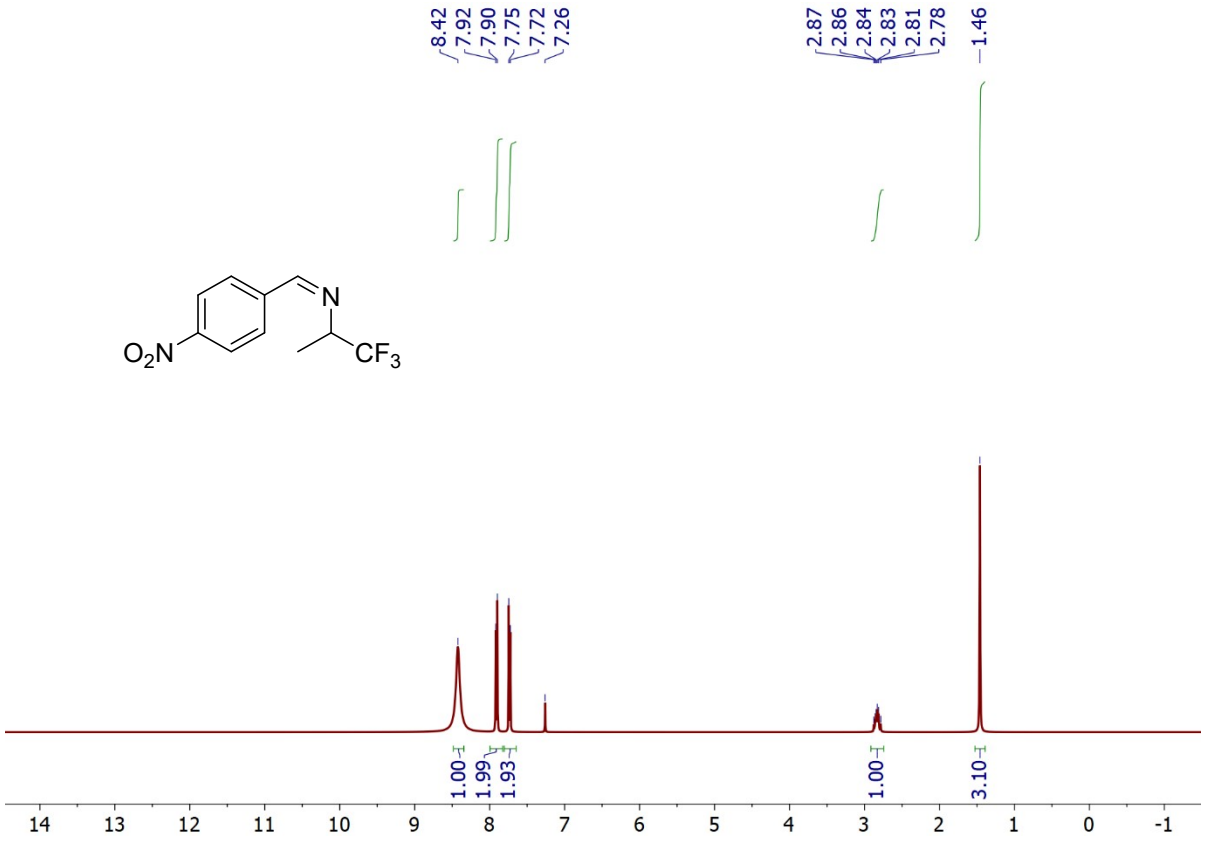
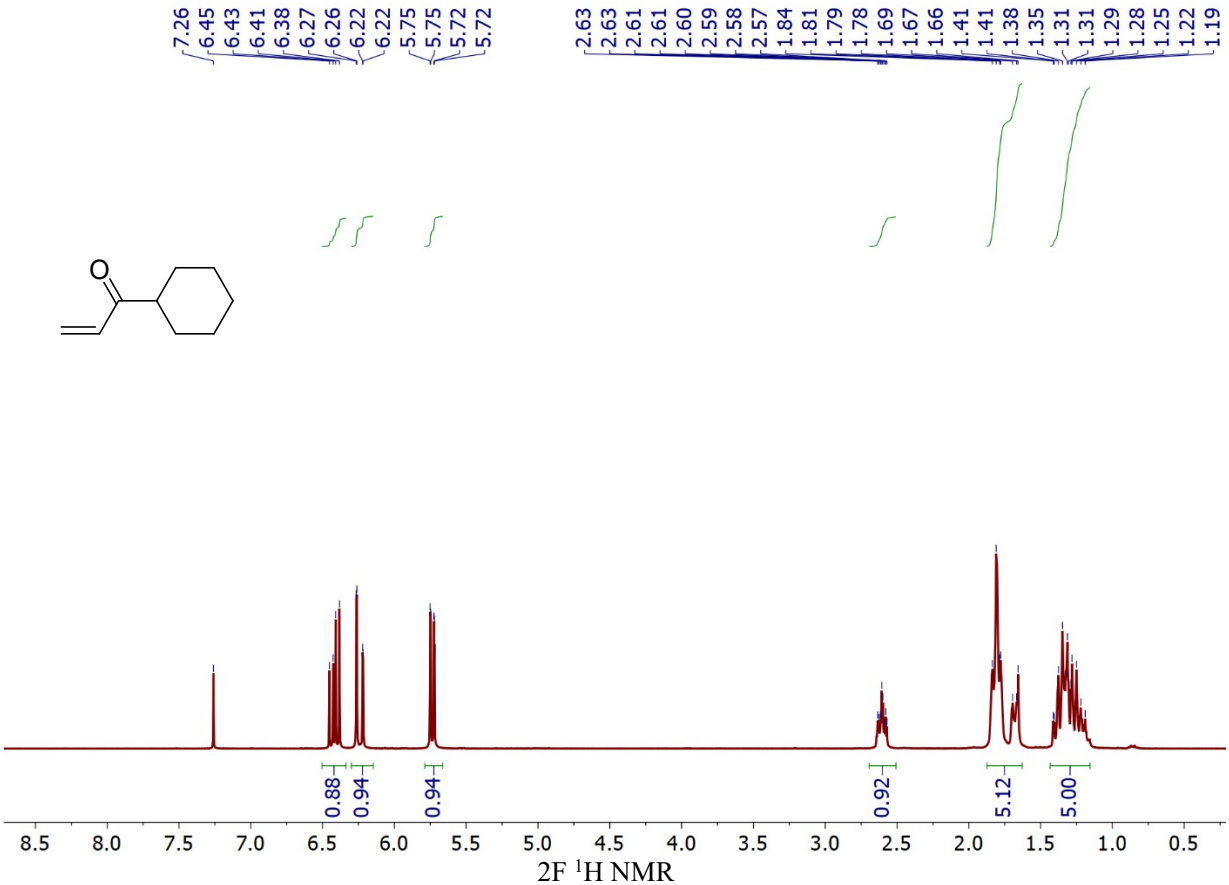
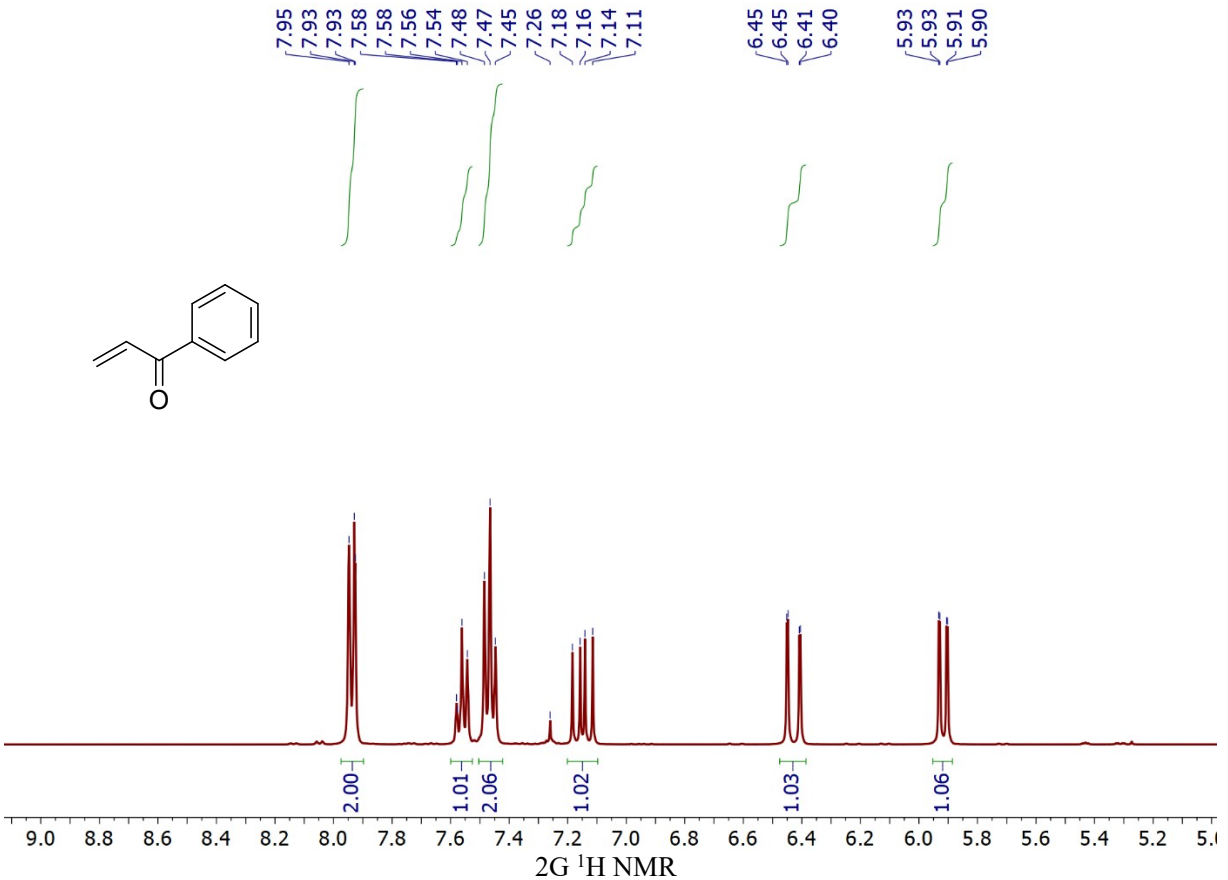


1K ^{1}H NMR



2F ^{1}H NMR



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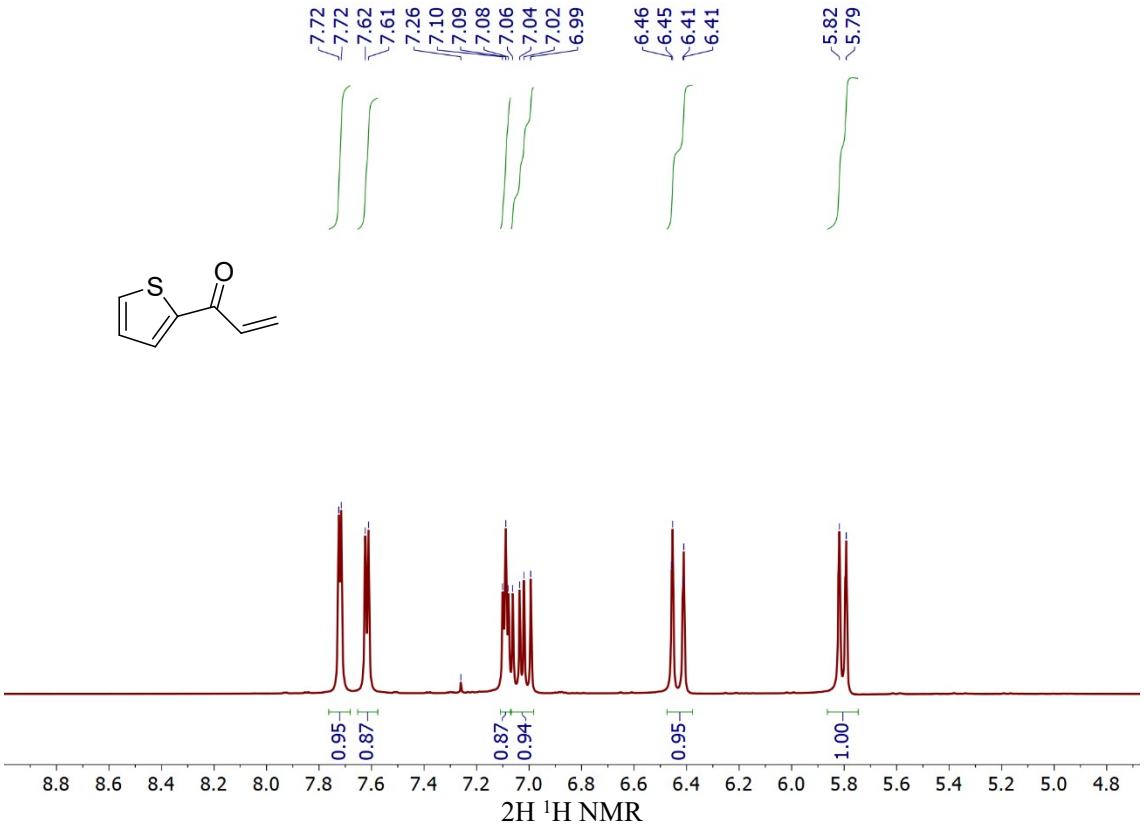

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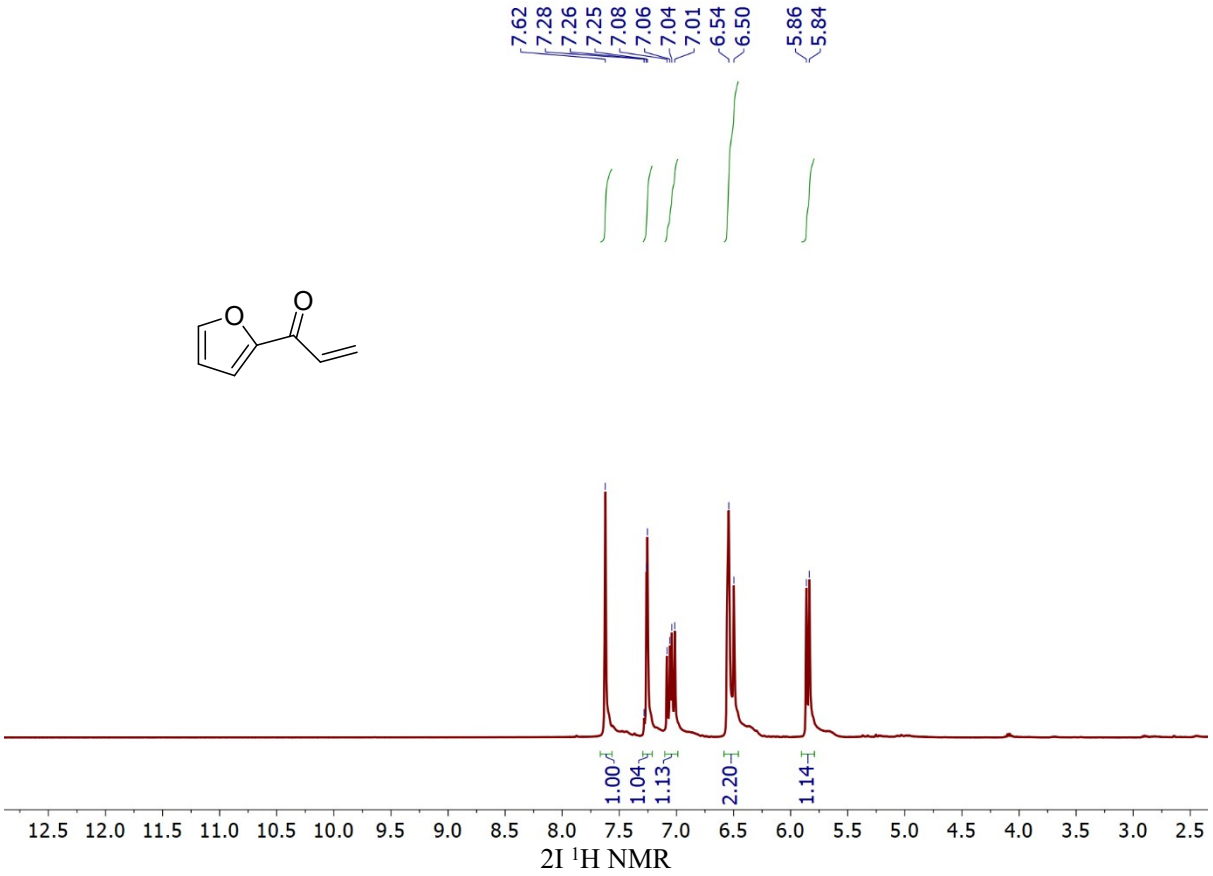

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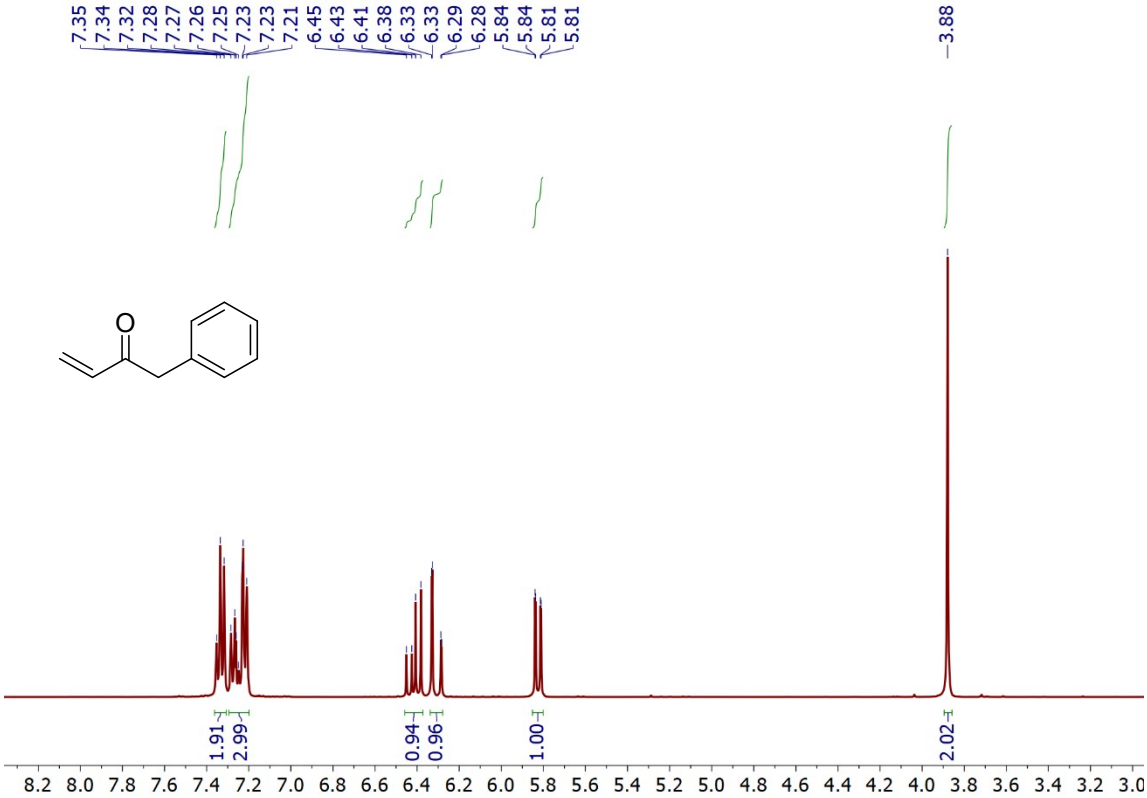

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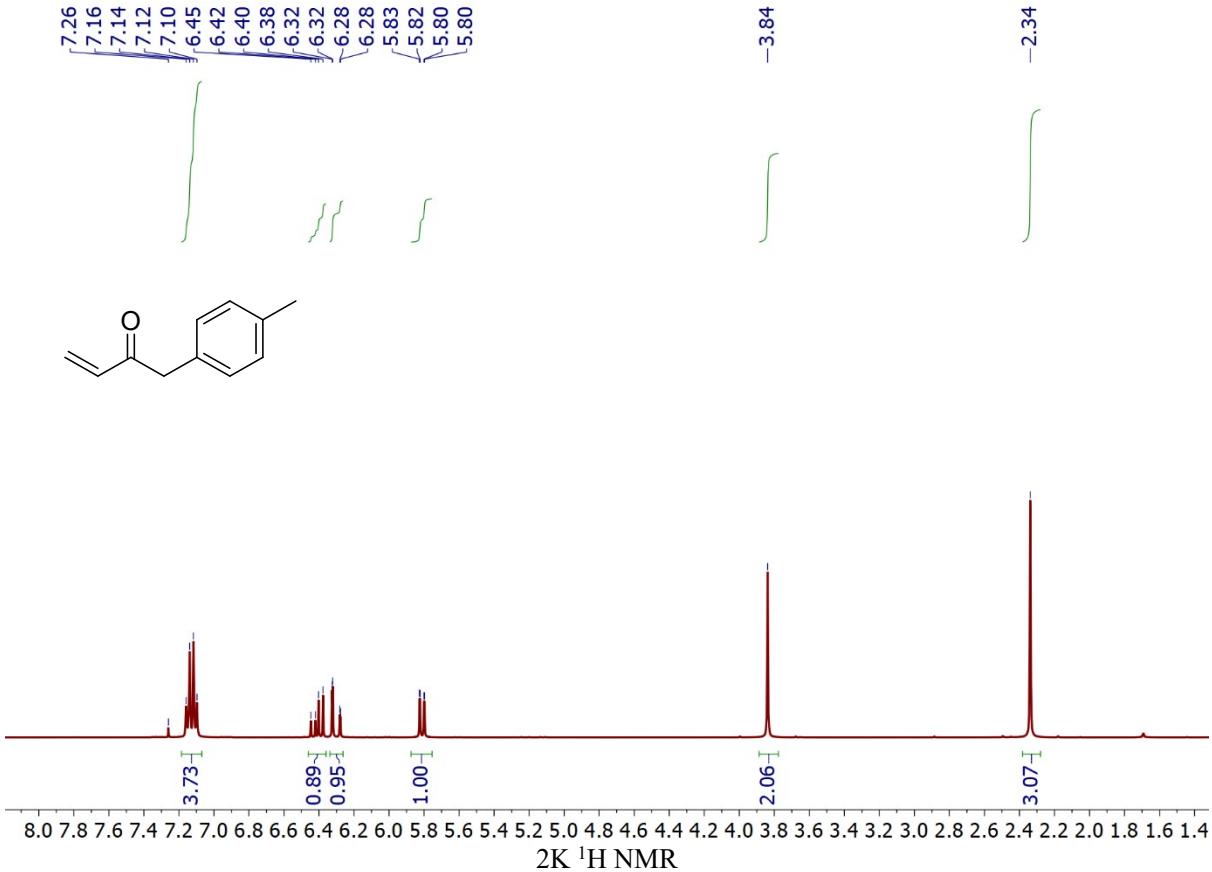

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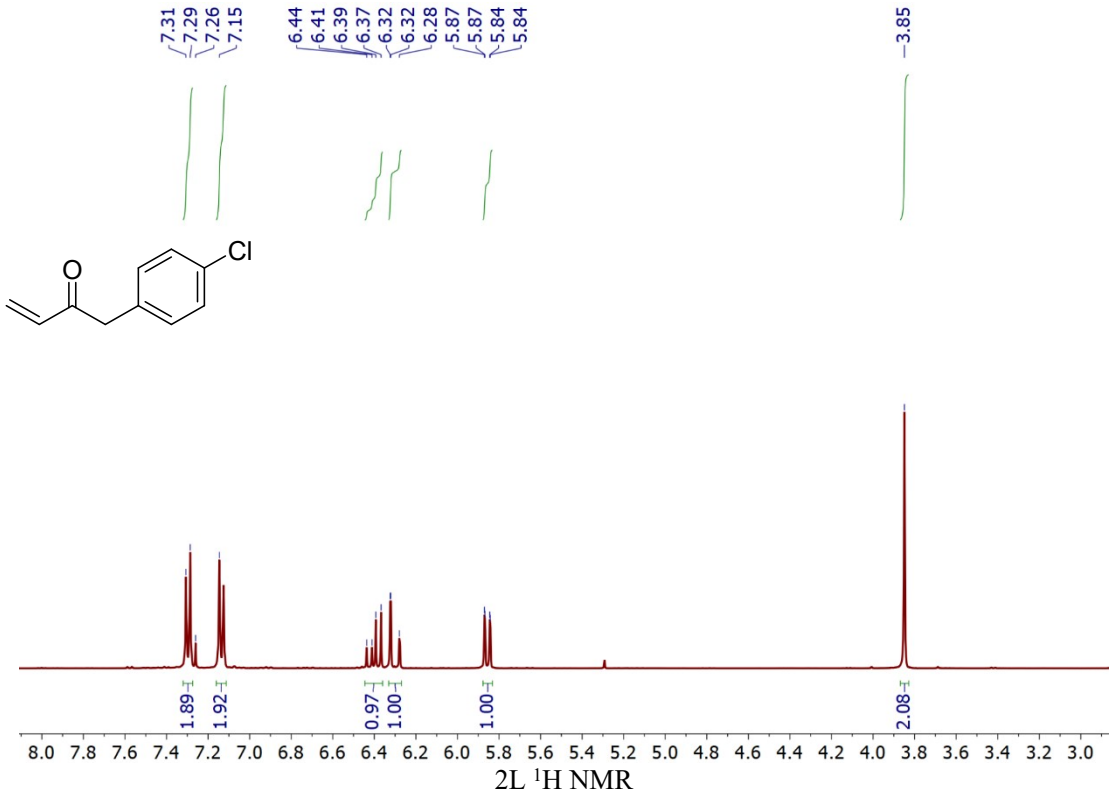
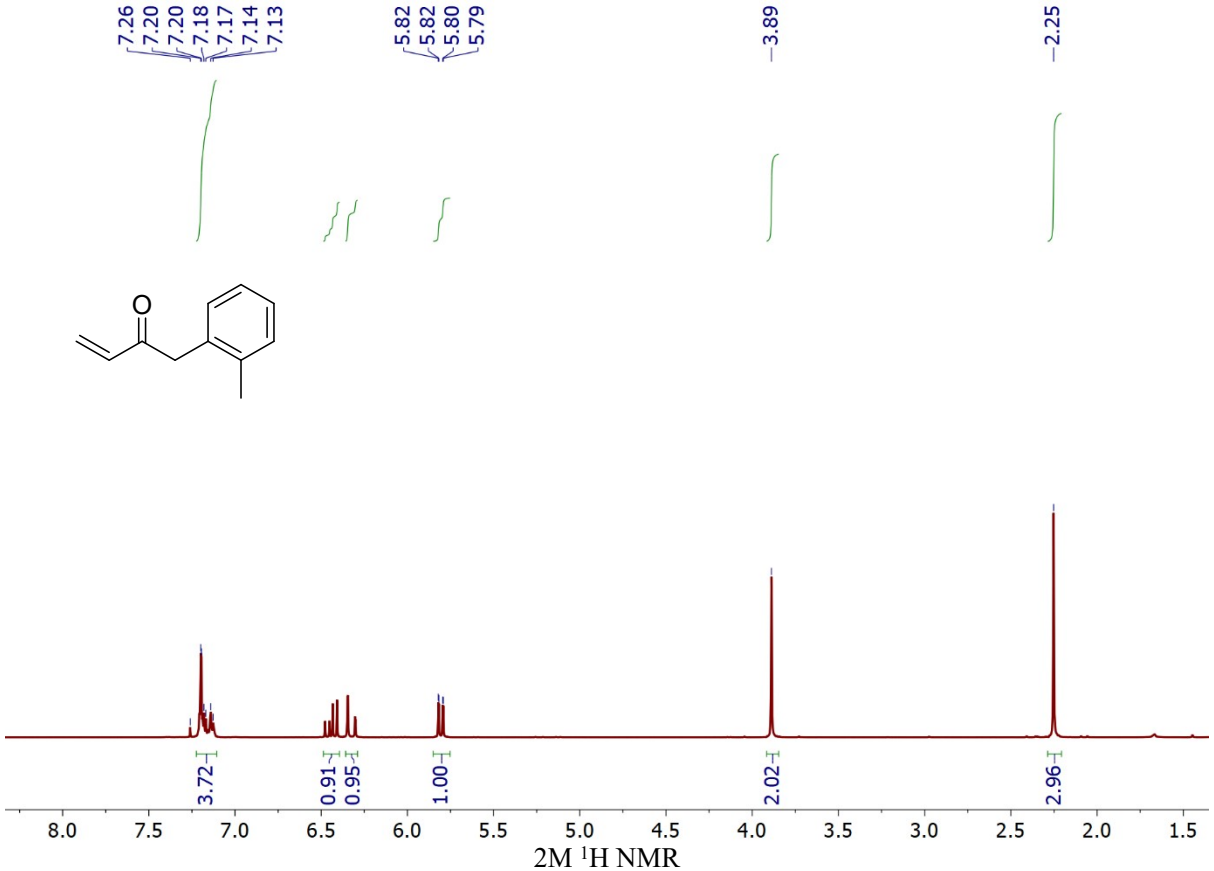


2L ^{1}H NMR



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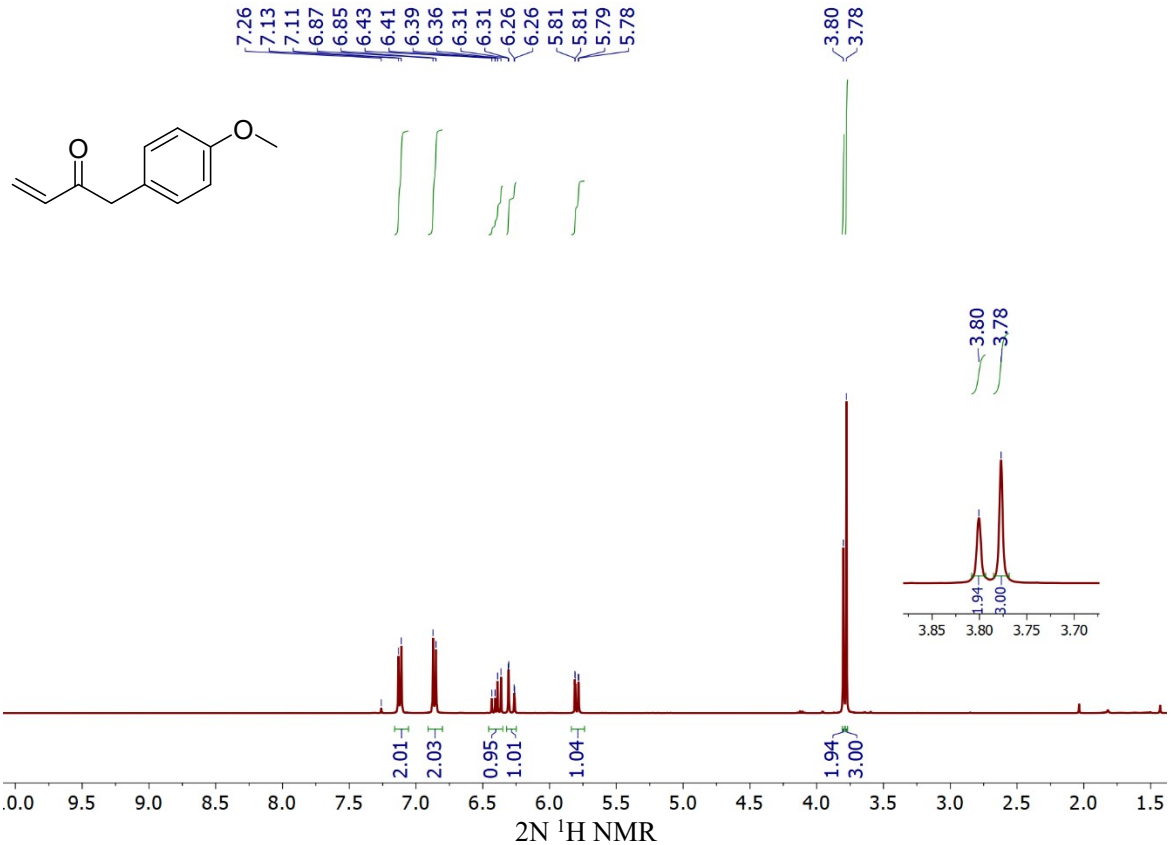
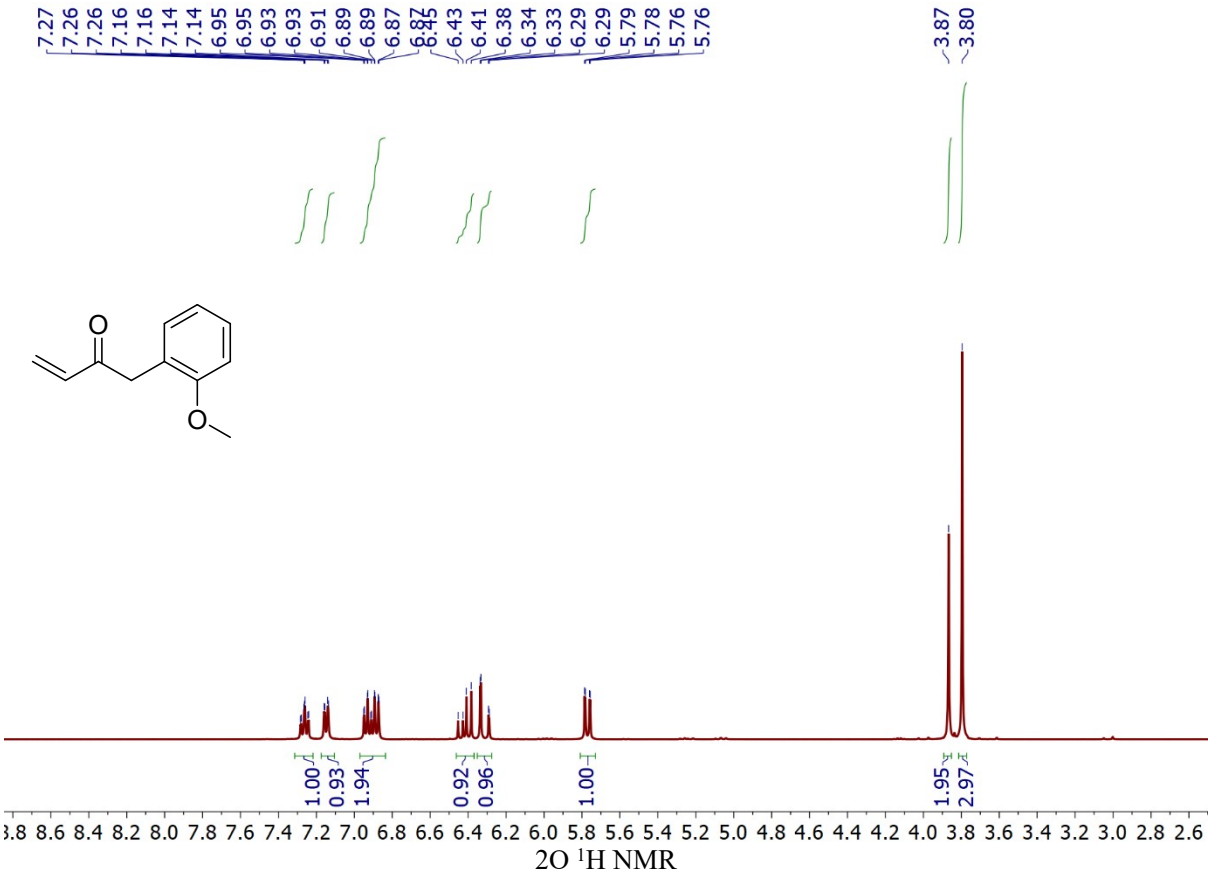
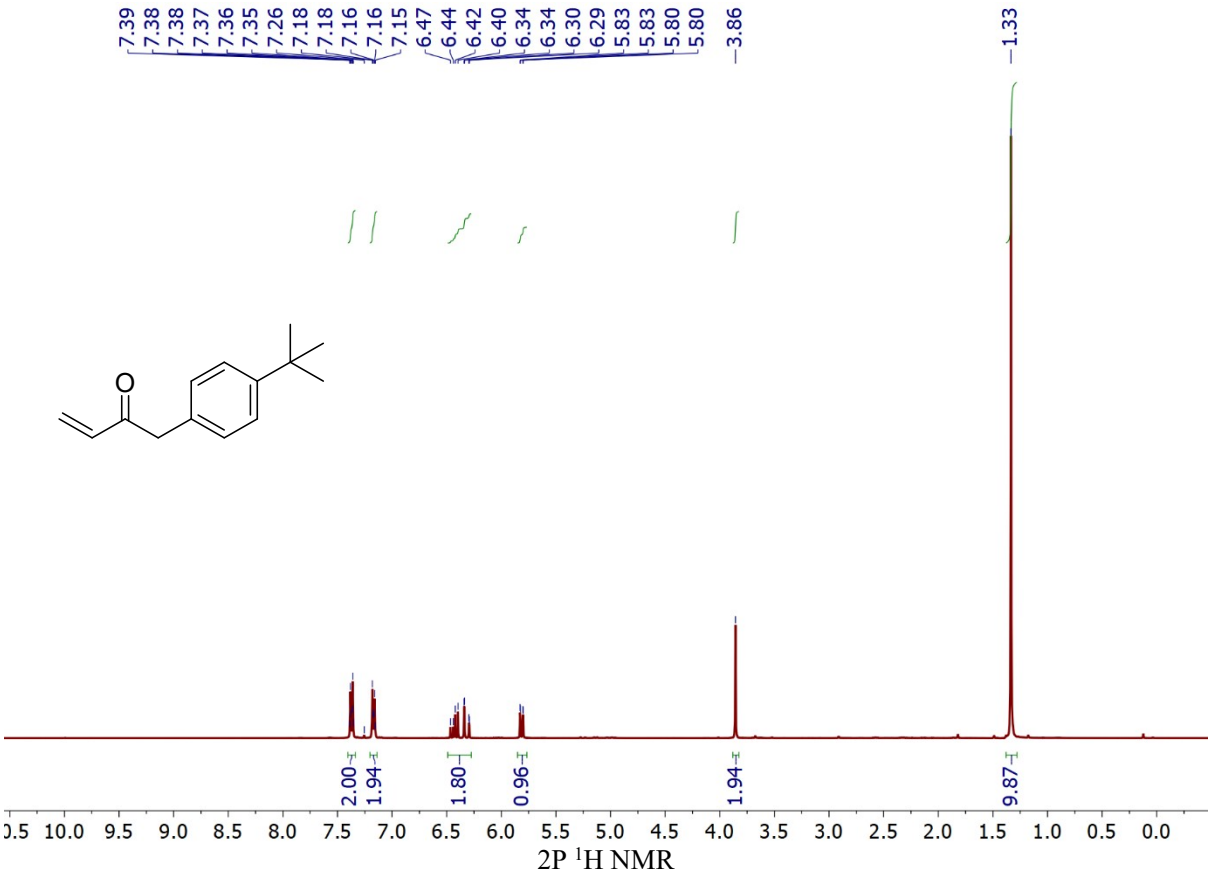


2N ^{1}H NMR



2O ^{1}H NMR



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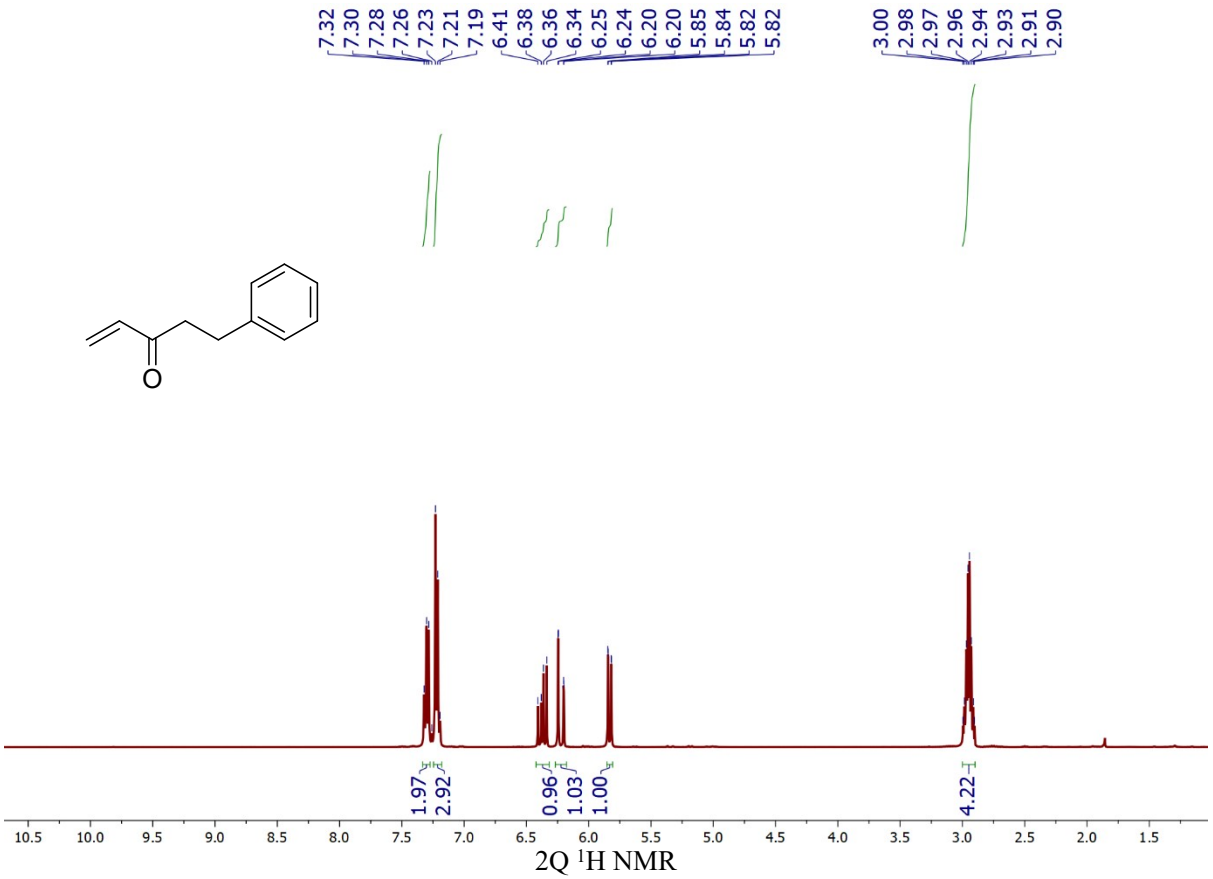

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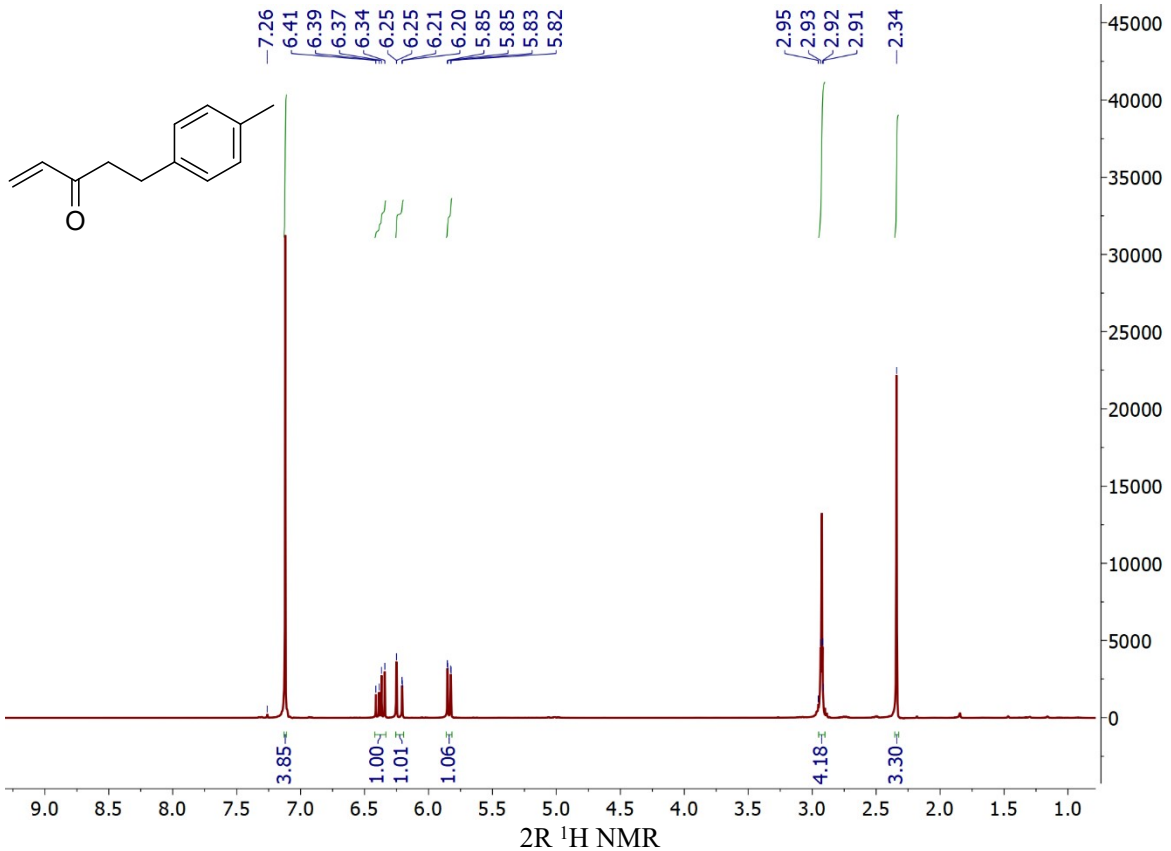

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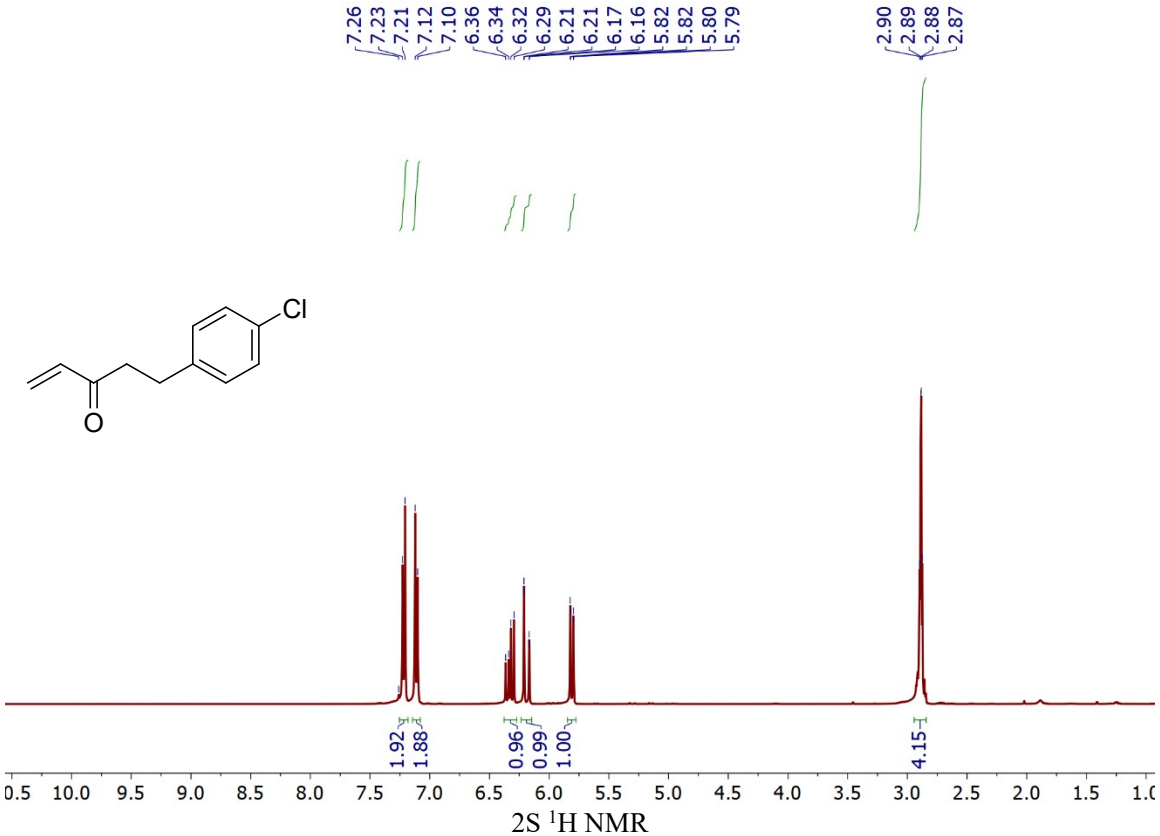
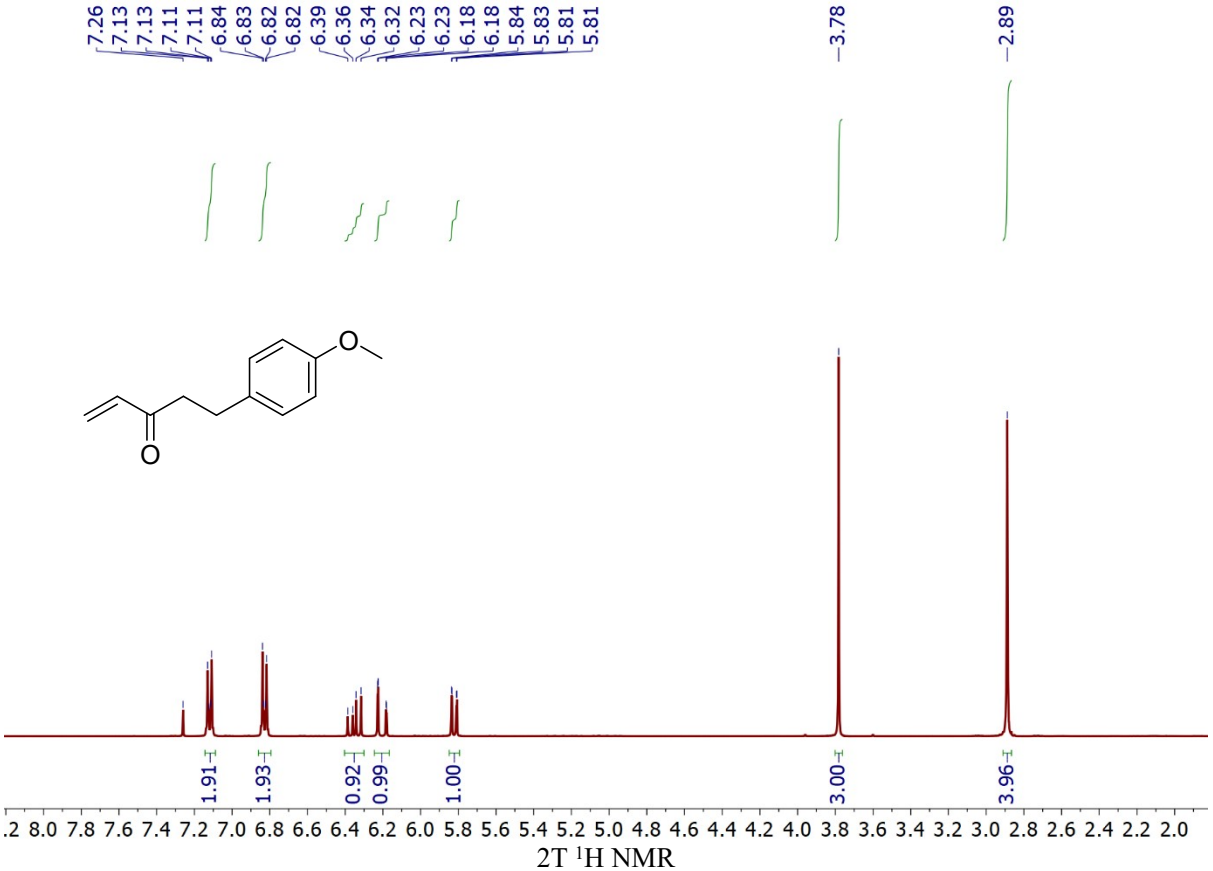
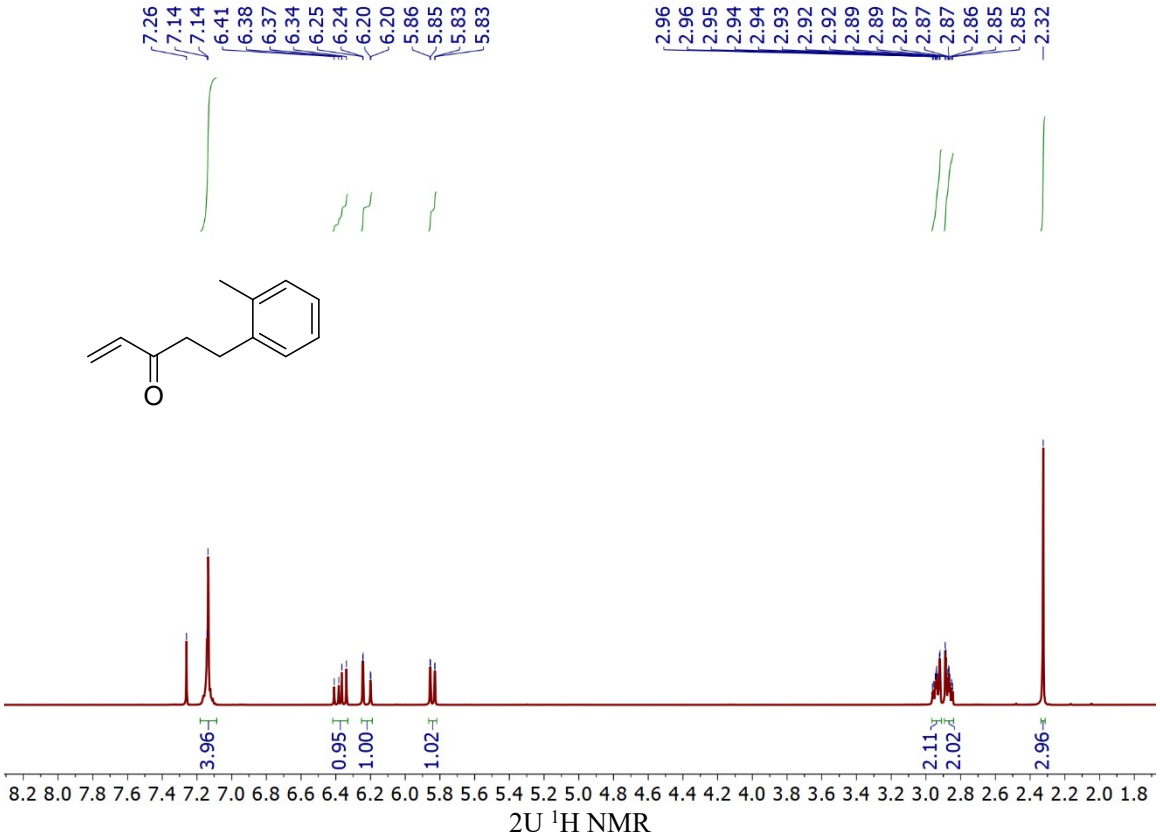


2S ^{1}H NMR



2T ^{1}H NMR



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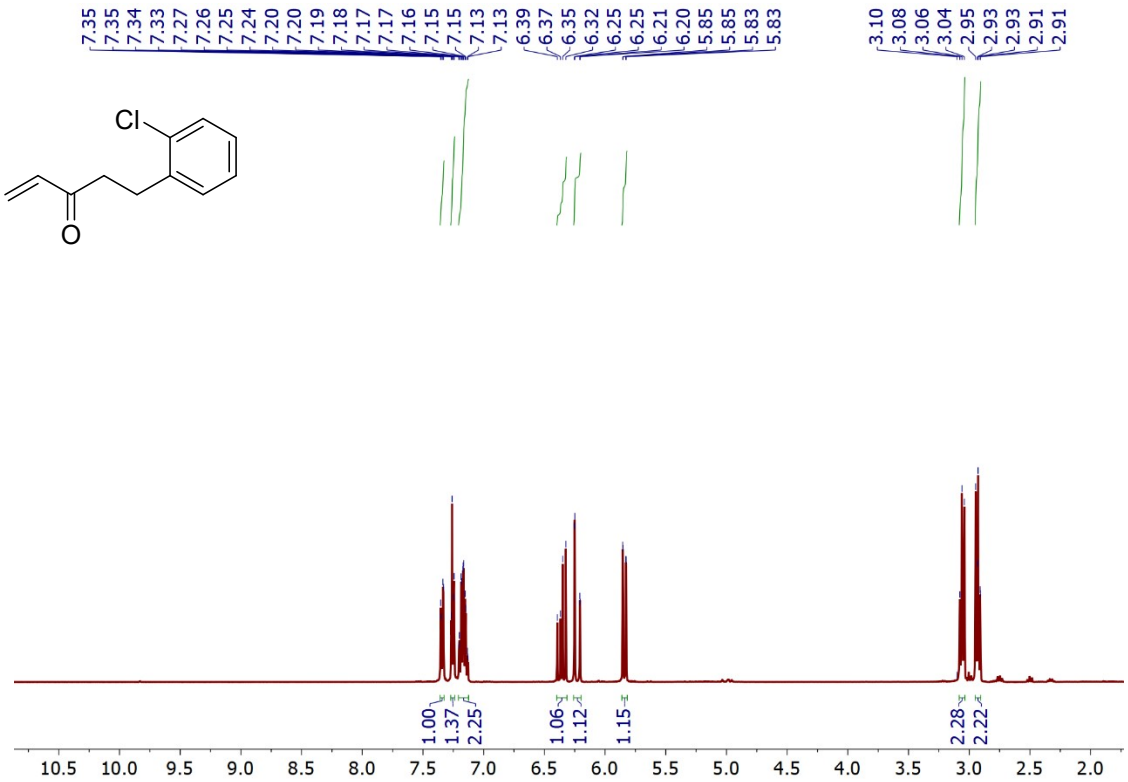
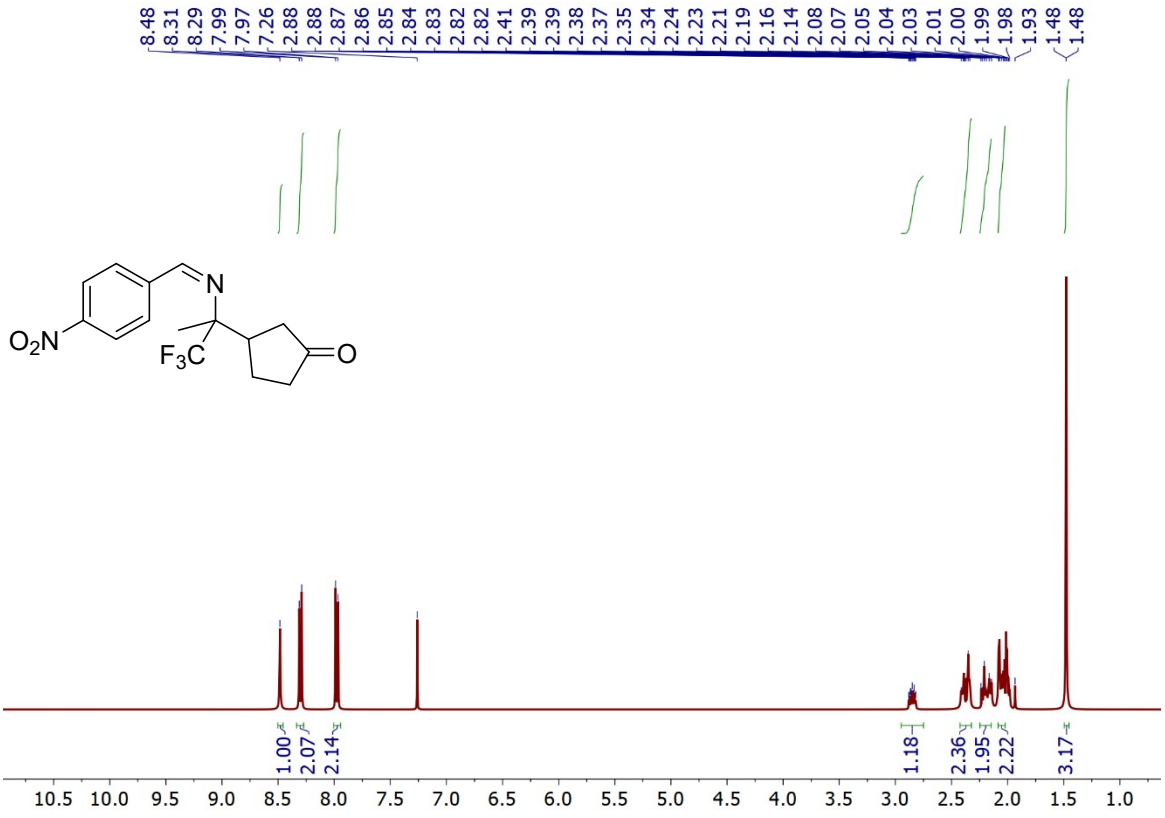
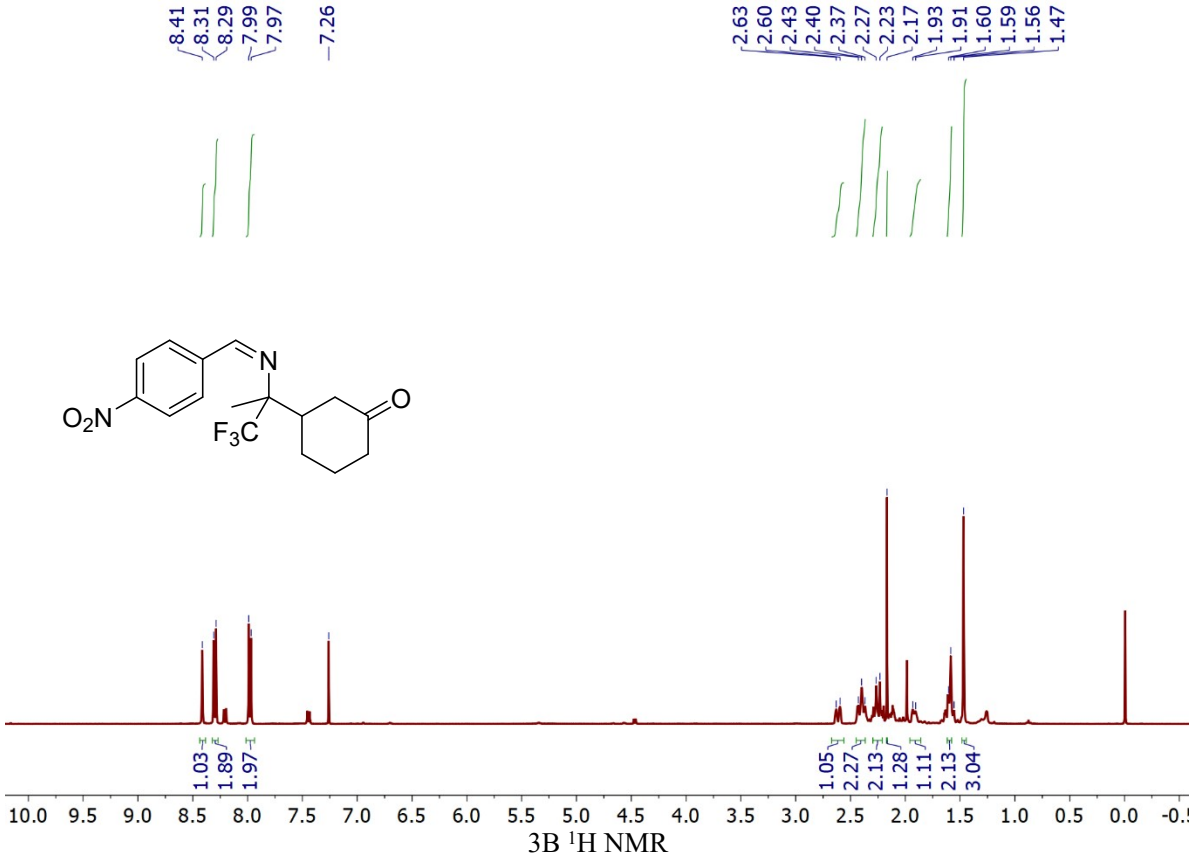


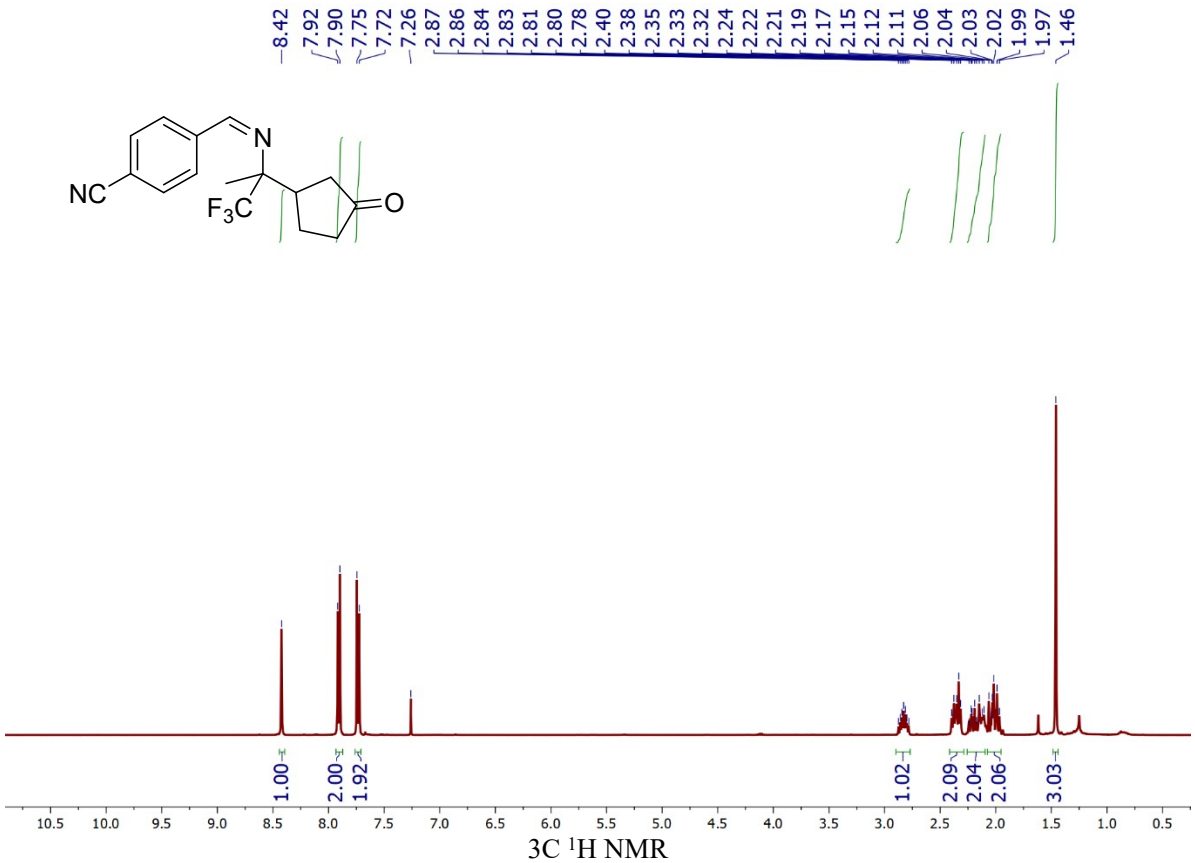
2V ^{1}H NMR



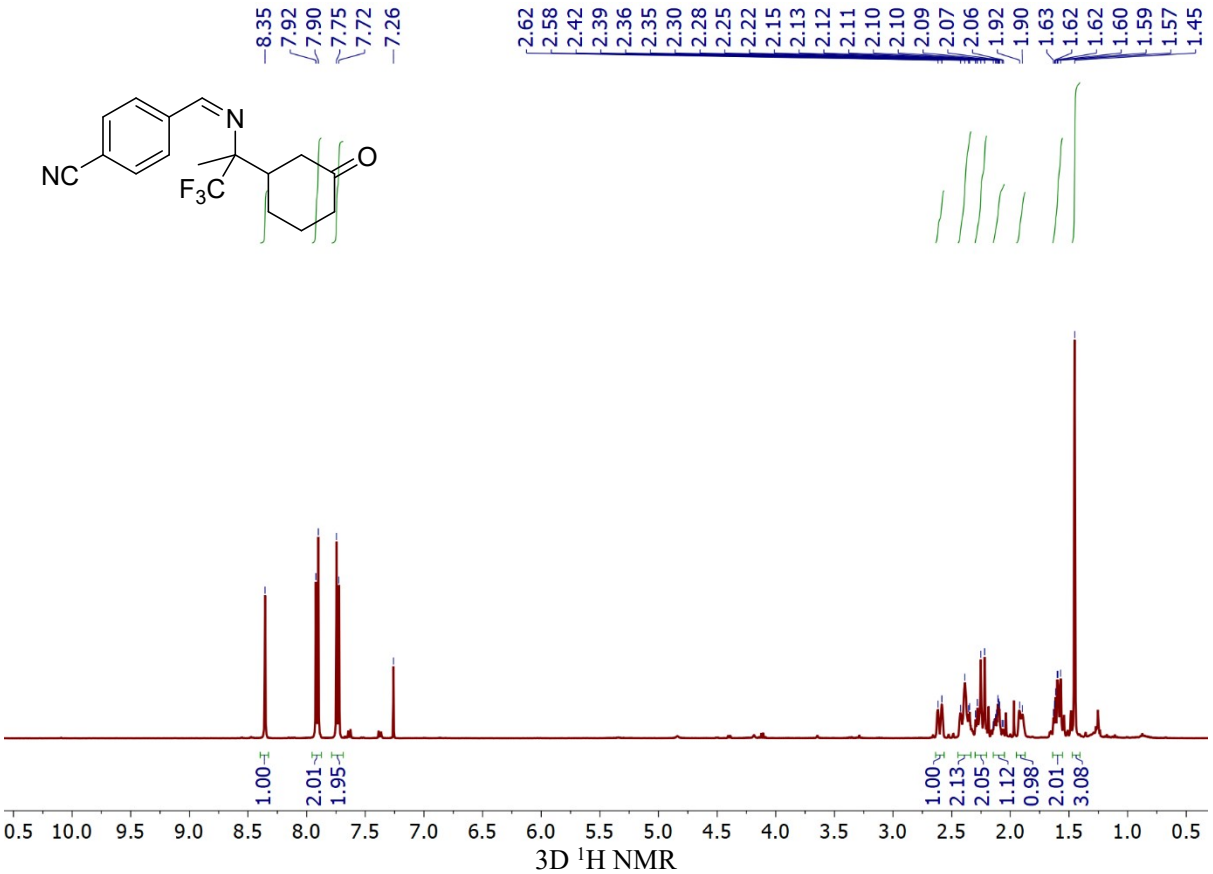
3A ^{1}H NMR



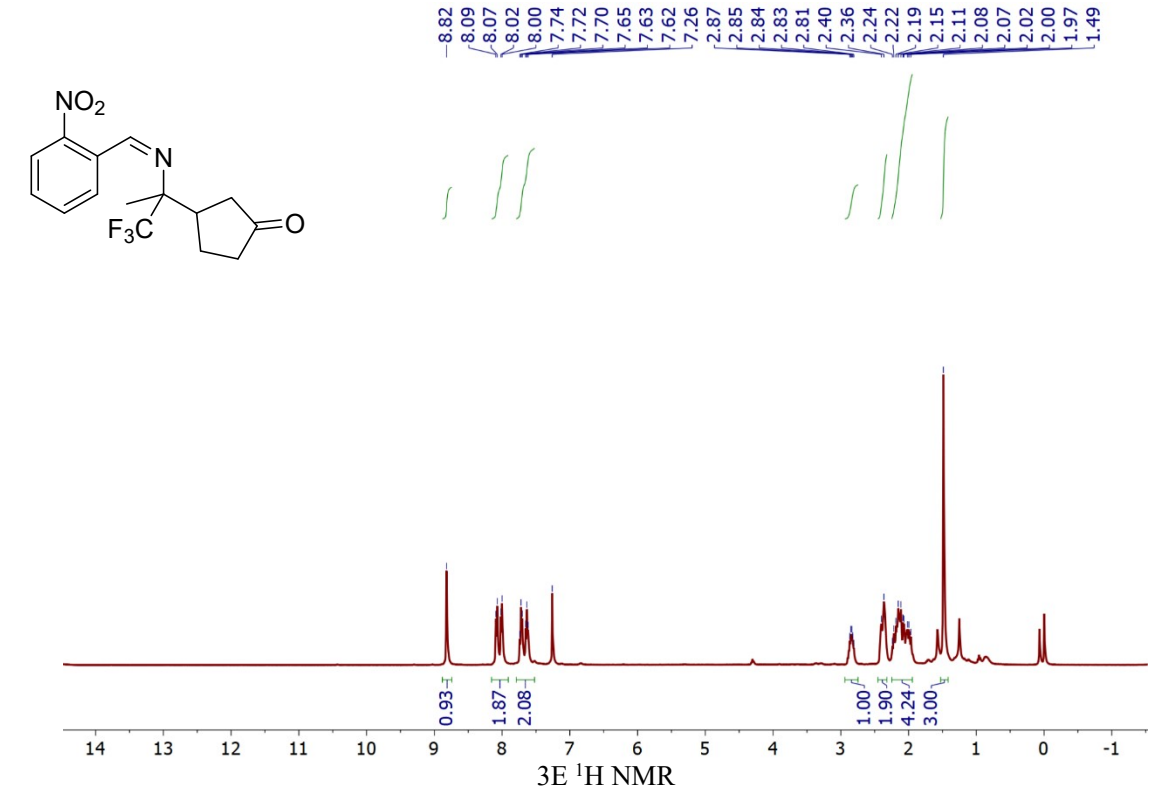
3B ^{1}H NMR



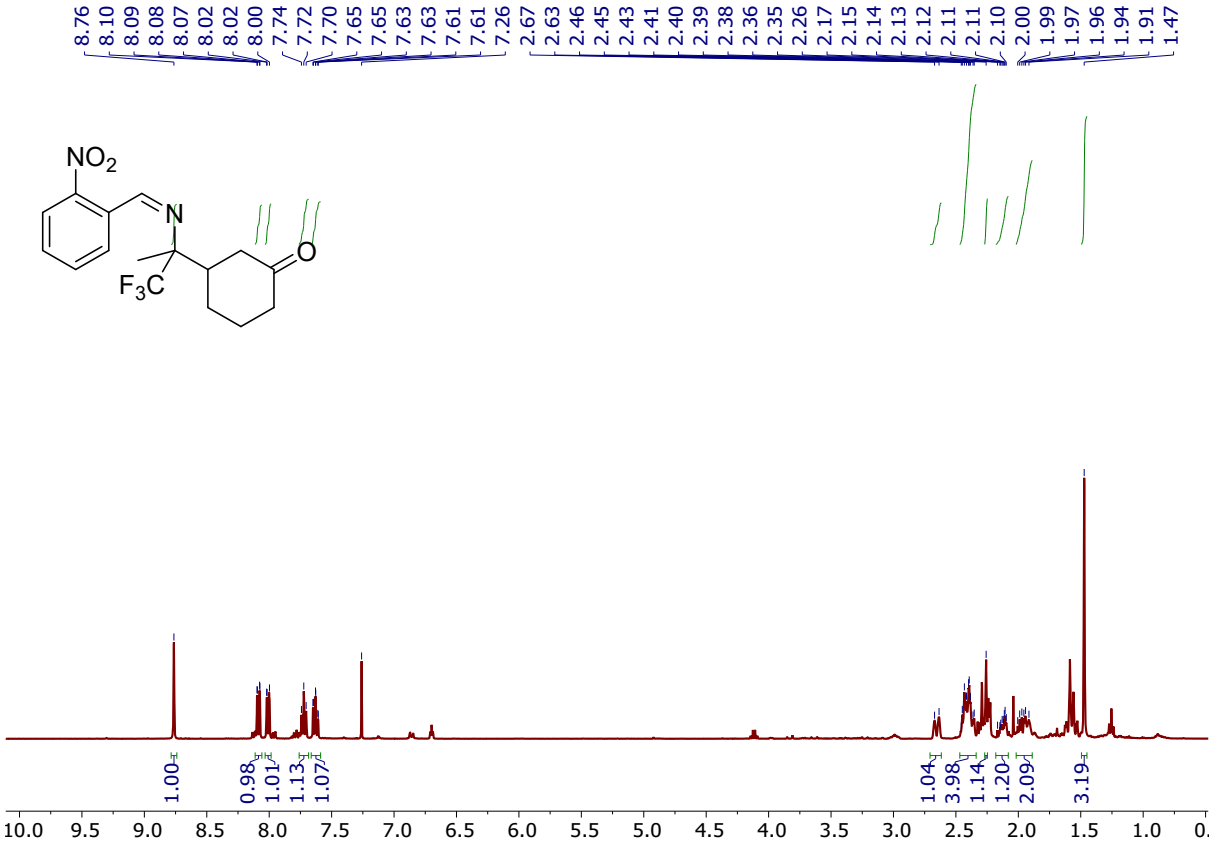
3C ^{1}H NMR



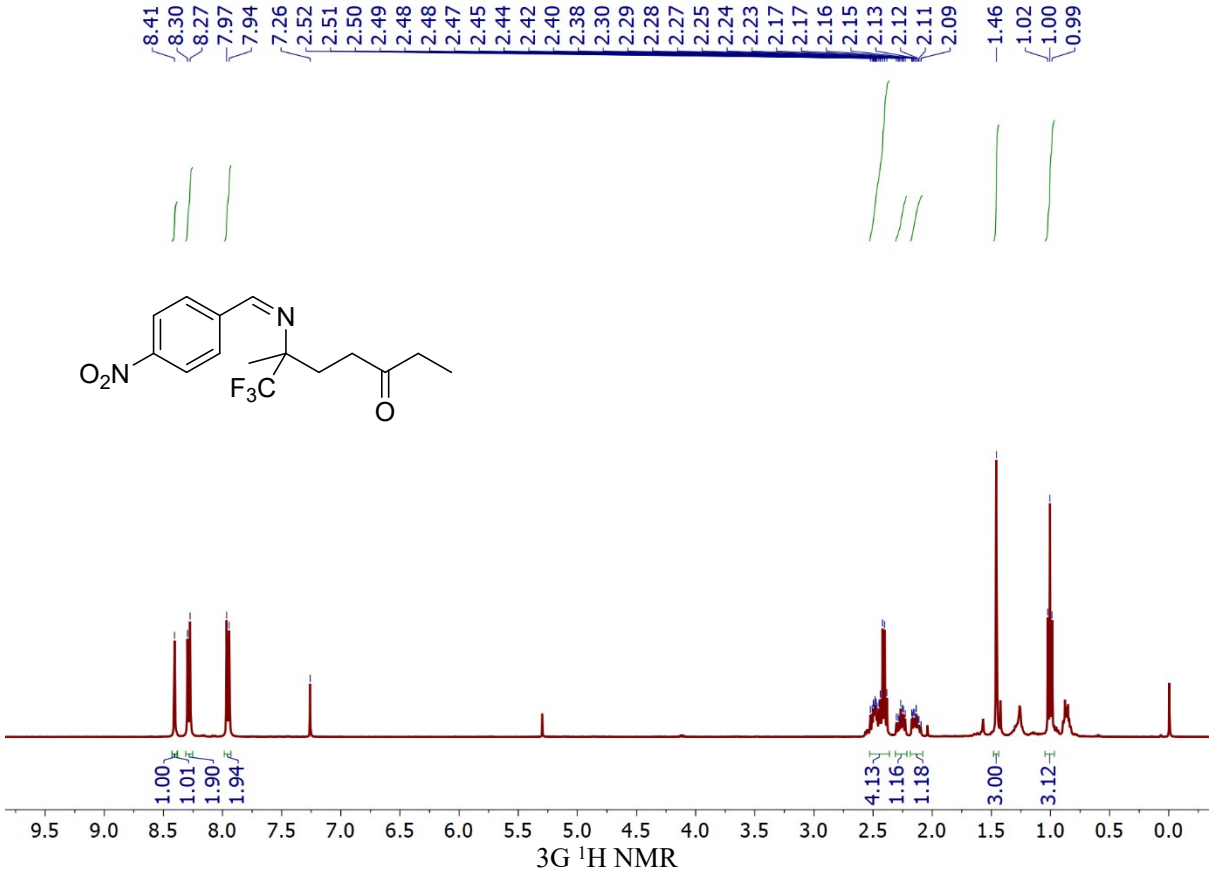
3D ^{1}H NMR



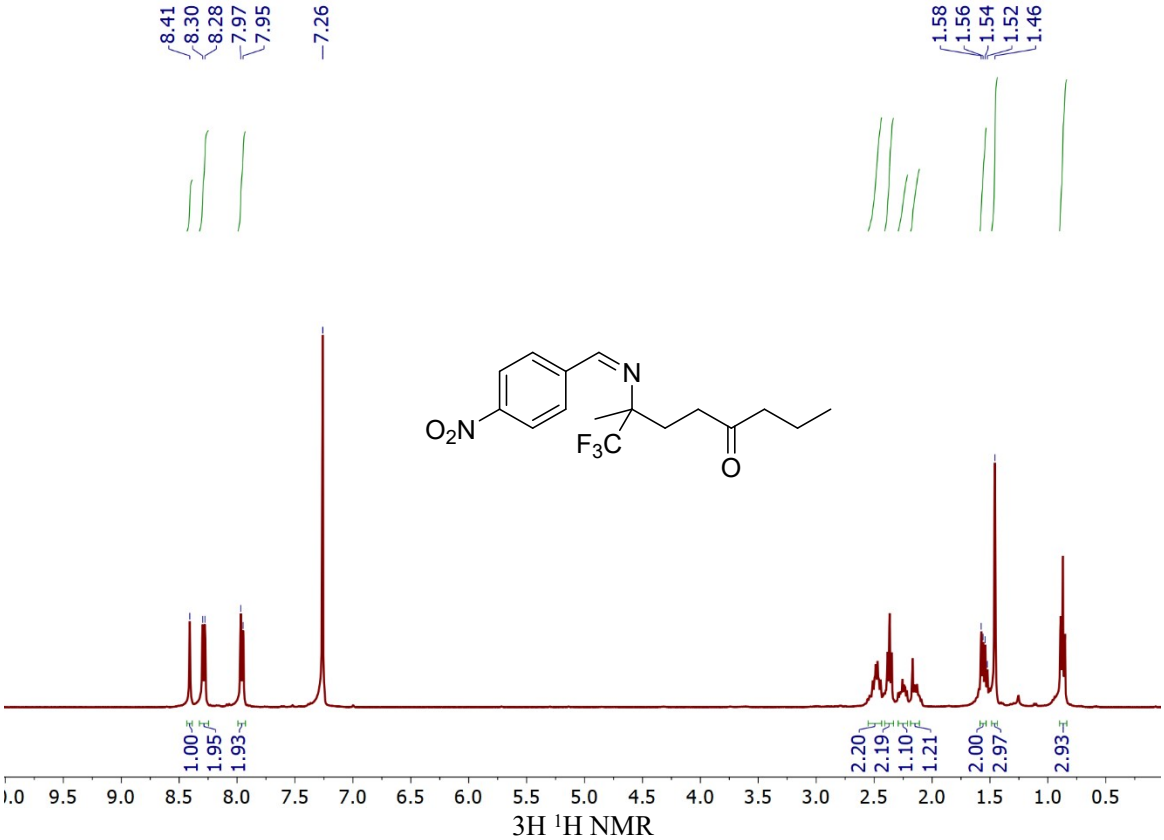
3E ^{1}H NMR



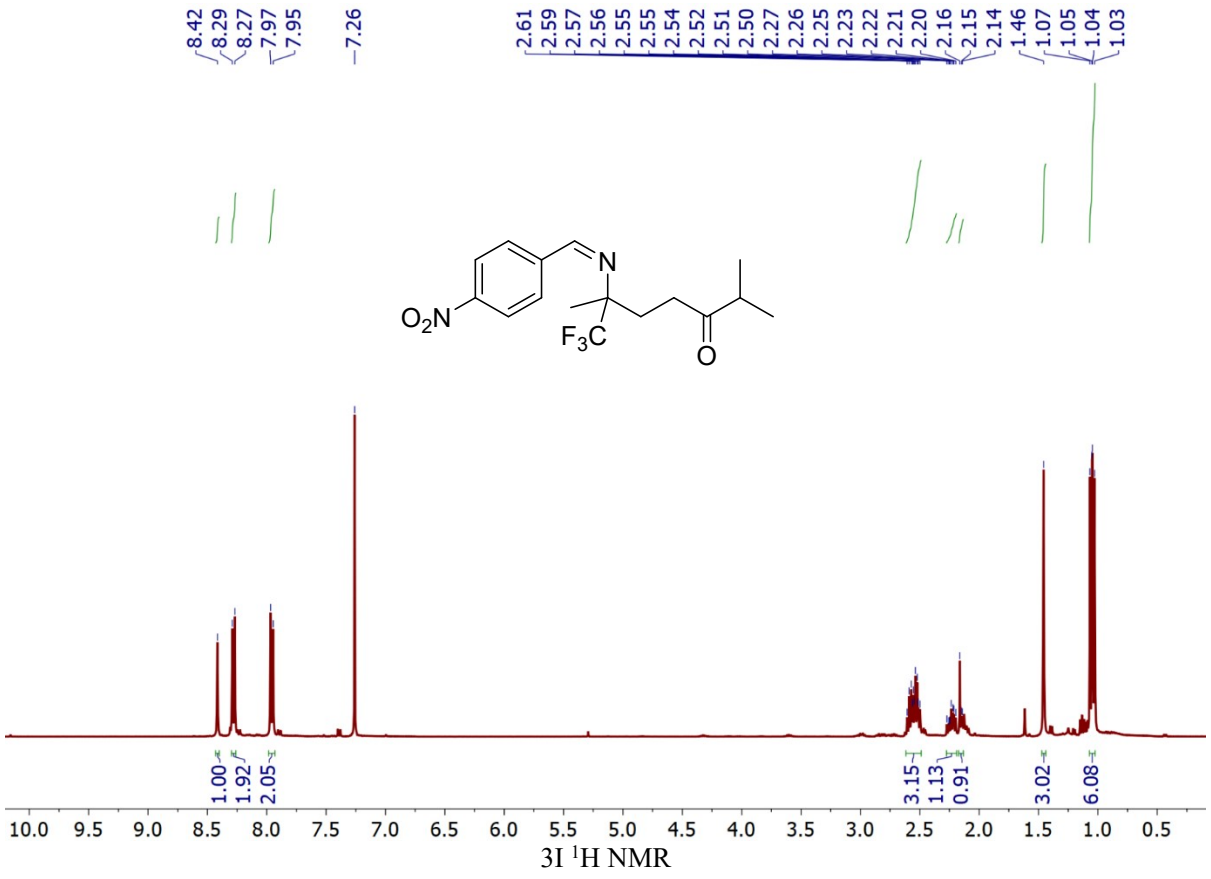
3F ^{1}H NMR



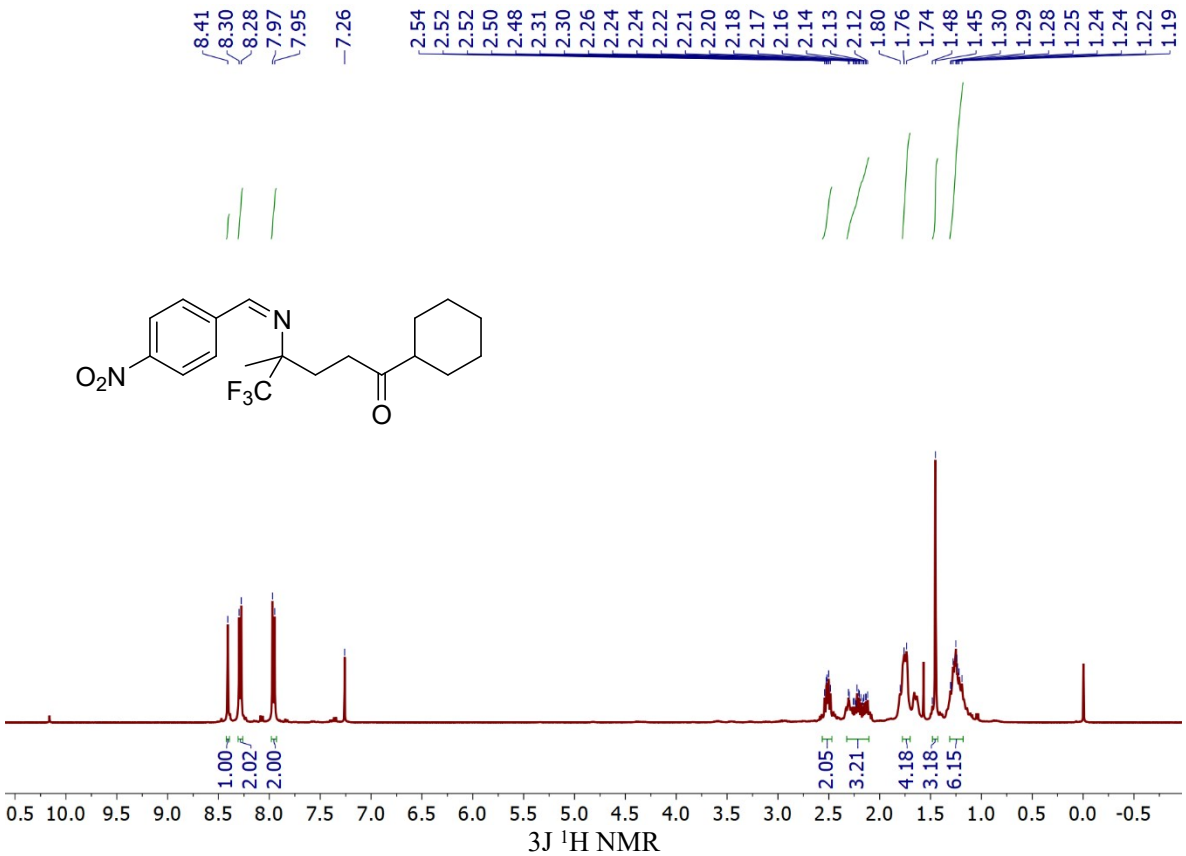
3G ^{1}H NMR



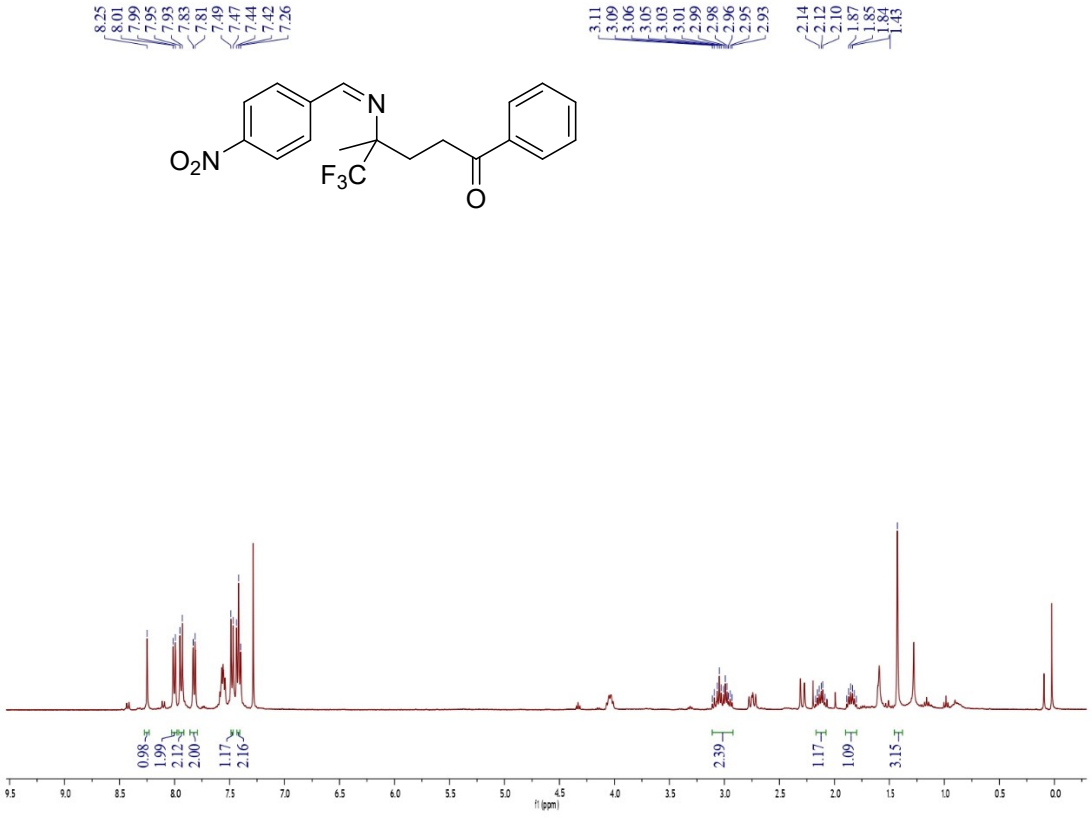
3H ^{1}H NMR



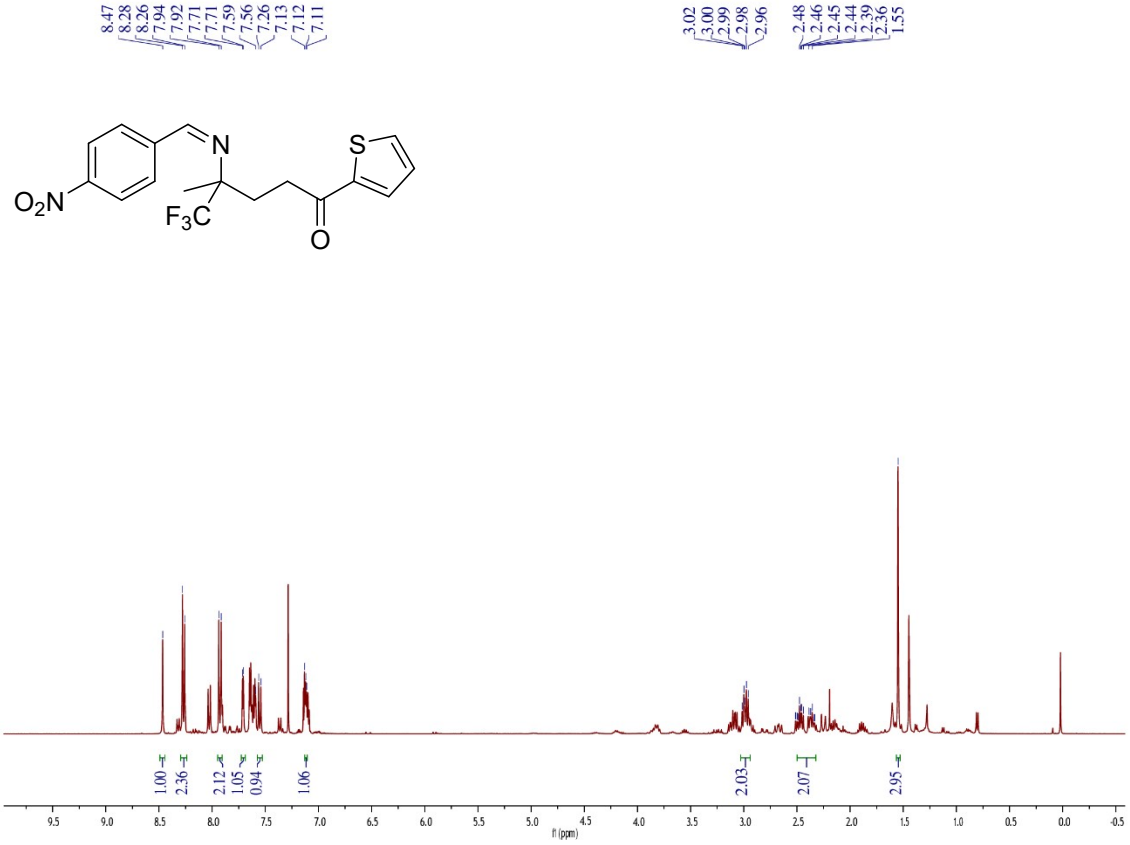
3I ^{1}H NMR


8.41
8.30
8.28
7.97
7.95
7.26
2.54
2.52
2.52
2.50
2.48
2.31
2.30
2.26
2.24
2.24
2.22
2.21
2.20
2.18
2.17
2.16
2.14
2.13
2.12
1.80
1.76
1.74
1.48
1.45
1.30
1.29
1.28
1.25
1.24
1.24
1.22
1.19
O2N
N
F3C
O
1.00
2.02
2.00
2.05
3.21
4.18
3.18
6.15
0.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5

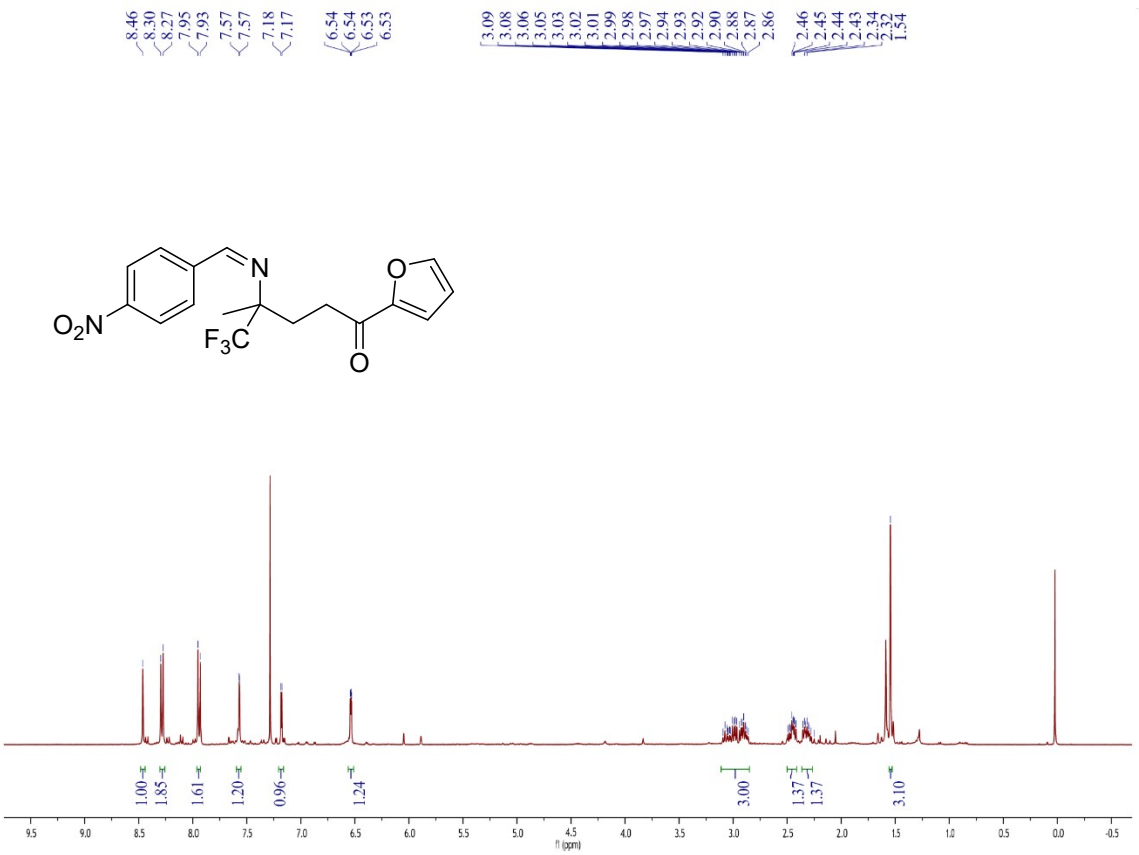
3J ^{1}H NMR


8.25
8.01
7.99
7.95
7.93
7.83
7.81
7.49
7.47
7.44
7.42
7.26
3.11
3.09
3.06
3.05
3.03
3.01
2.99
2.98
2.96
2.95
2.93
2.14
2.12
2.10
1.87
1.85
1.84
1.43
N
O2N
F3C
O
0.98
1.99
2.12
2.00
1.17
2.16
2.39
1.17
1.09
3.15
f1 (ppm)

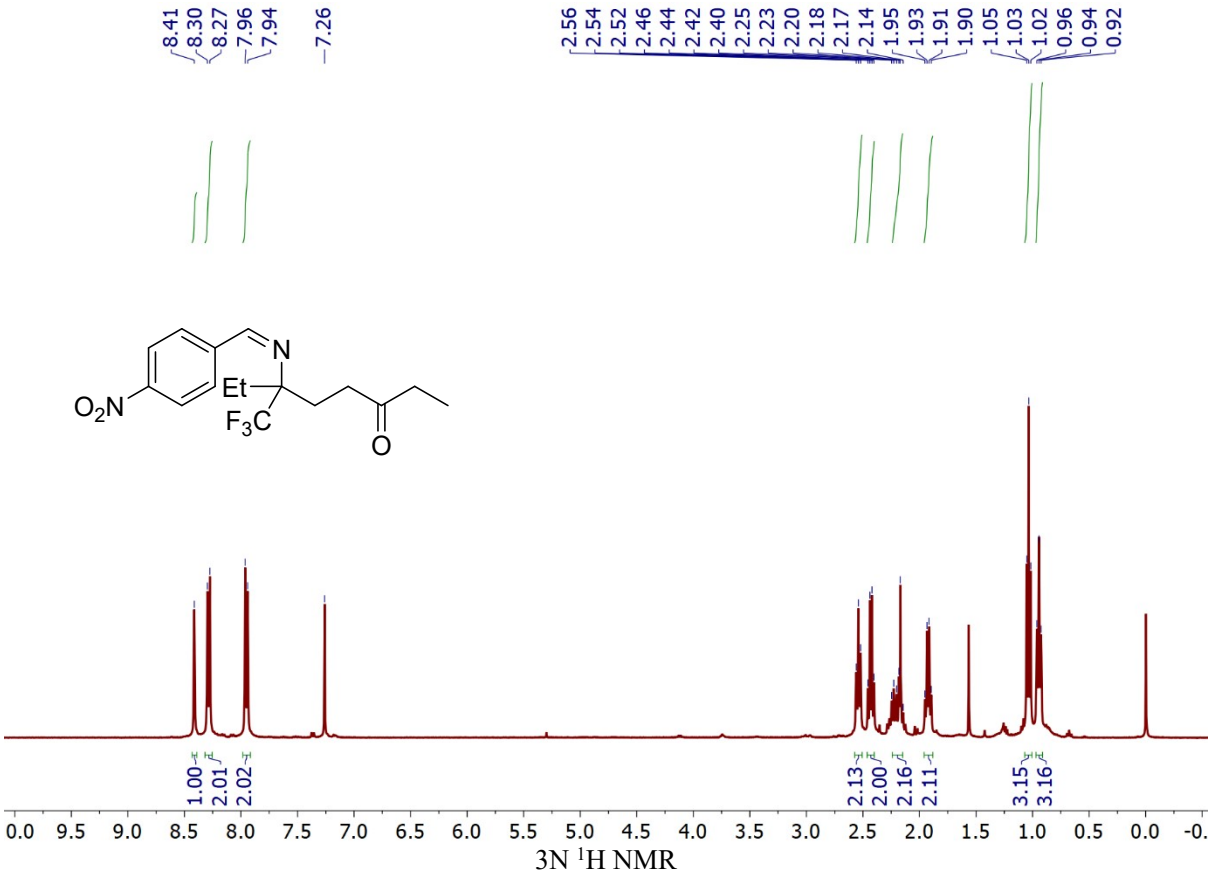
3K ^{1}H NMR



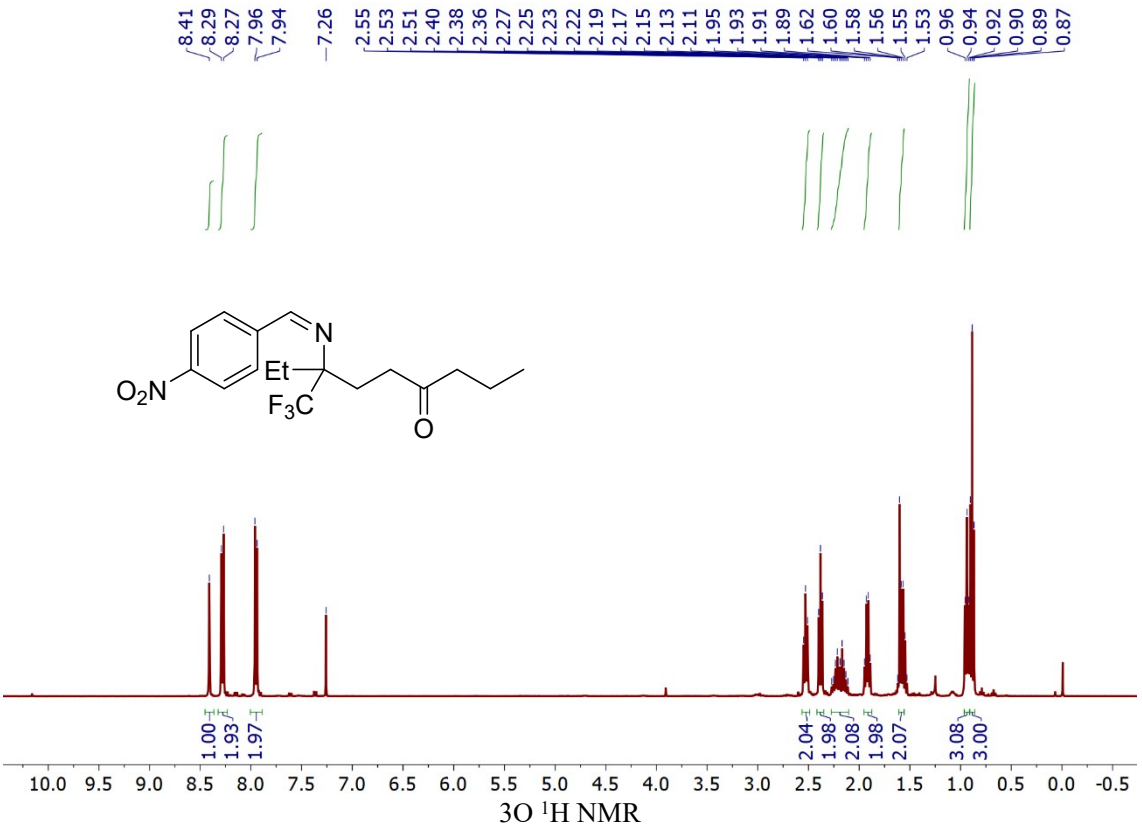
3L ^{1}H NMR



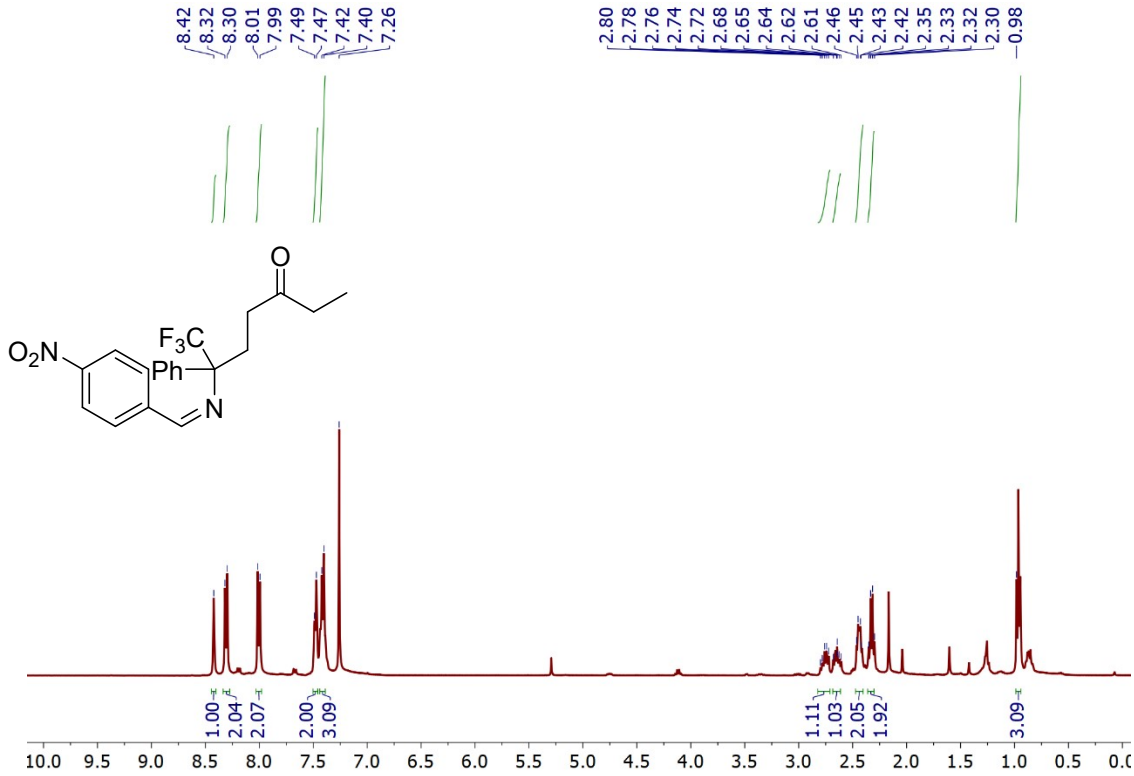
3M ^{1}H NMR



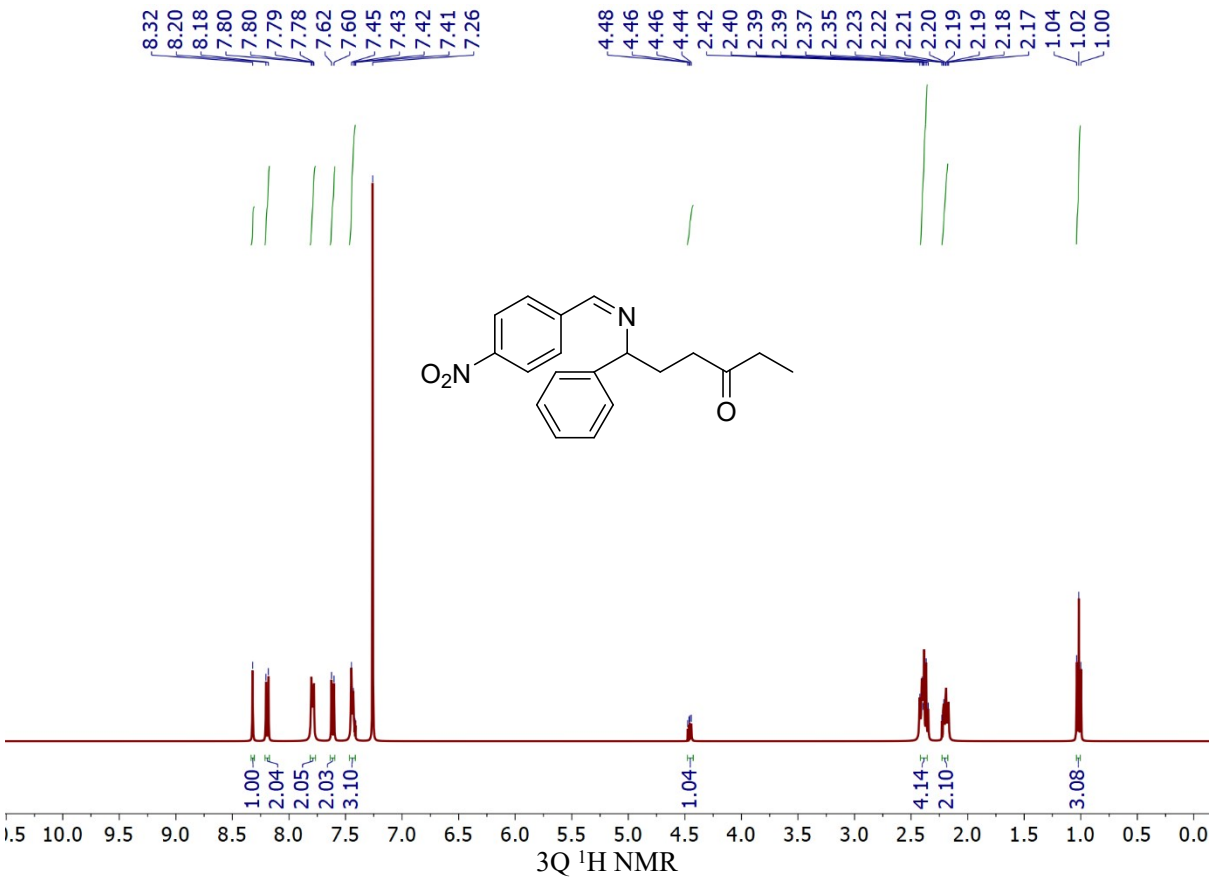
3N ^{1}H NMR



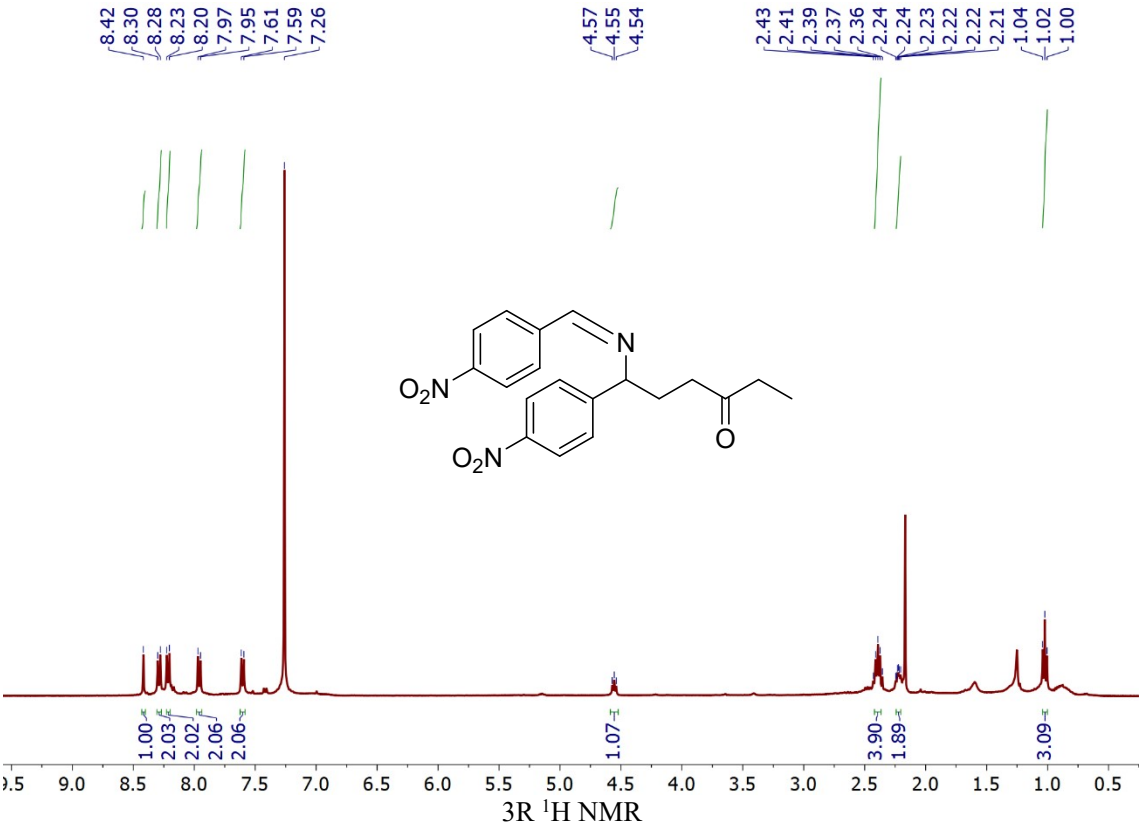
3O ^{1}H NMR



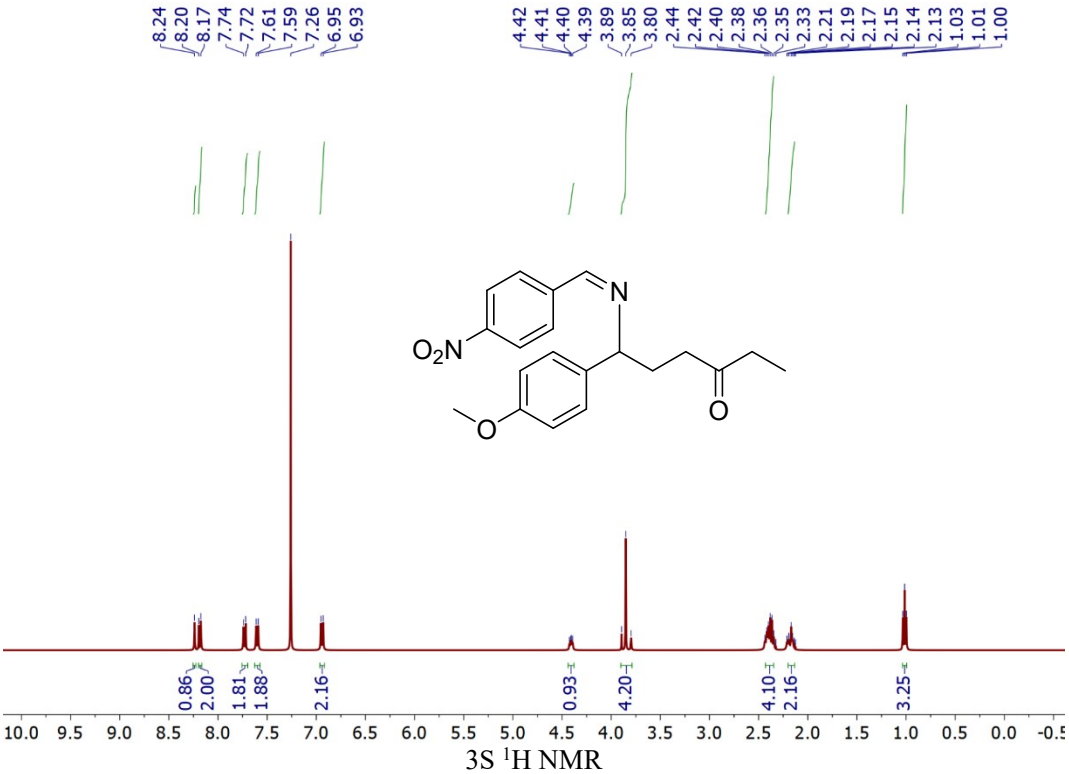
3P ^{1}H NMR



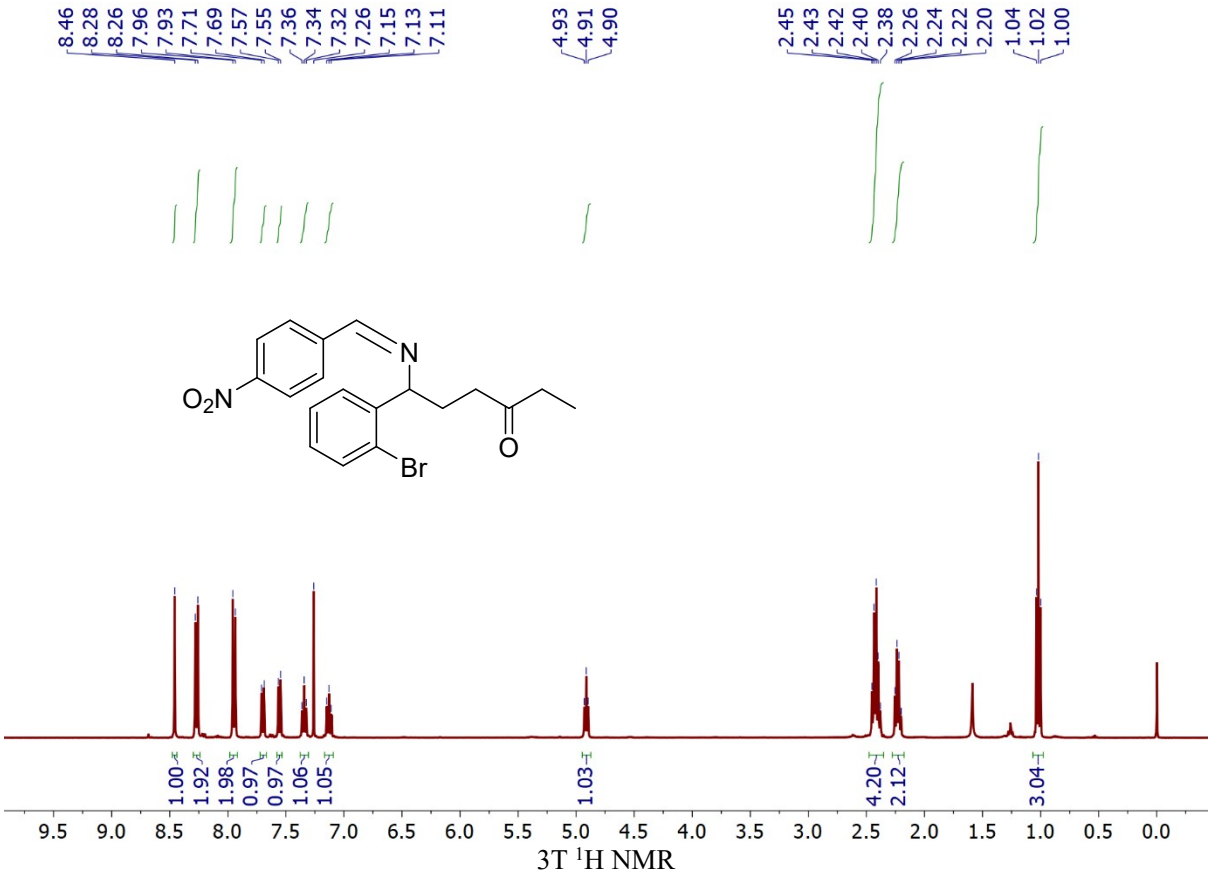
3Q ^{1}H NMR



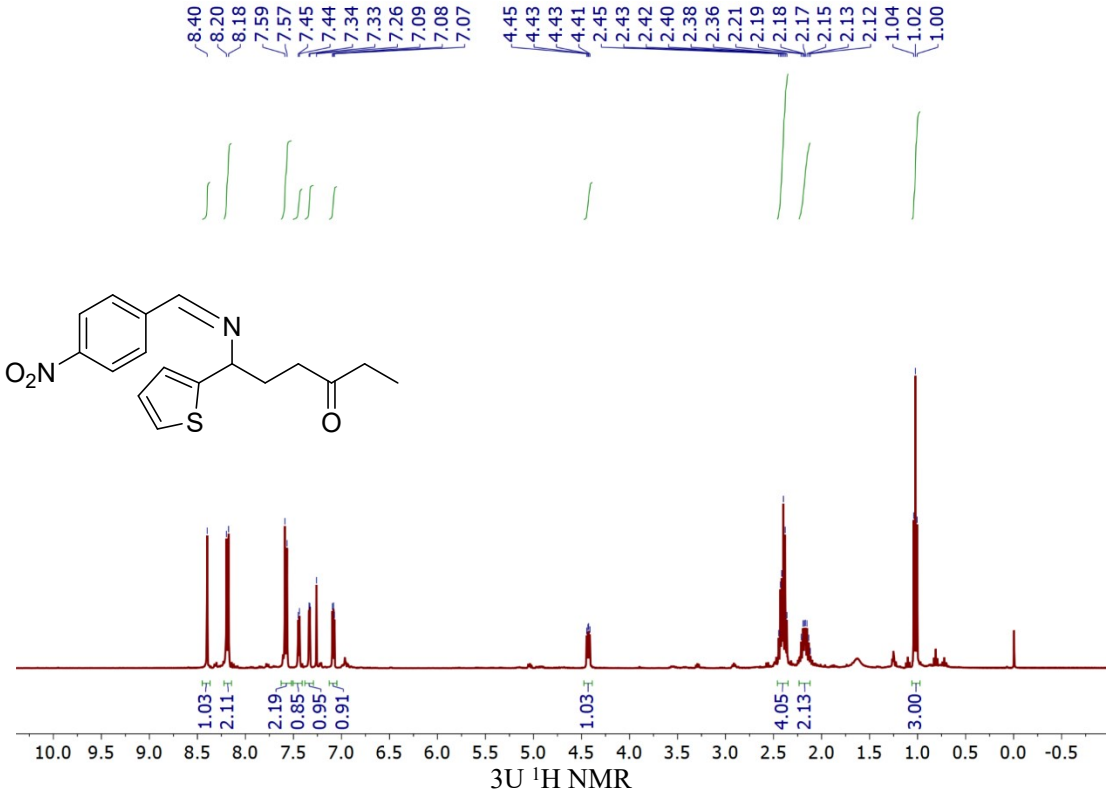
3R ^{1}H NMR



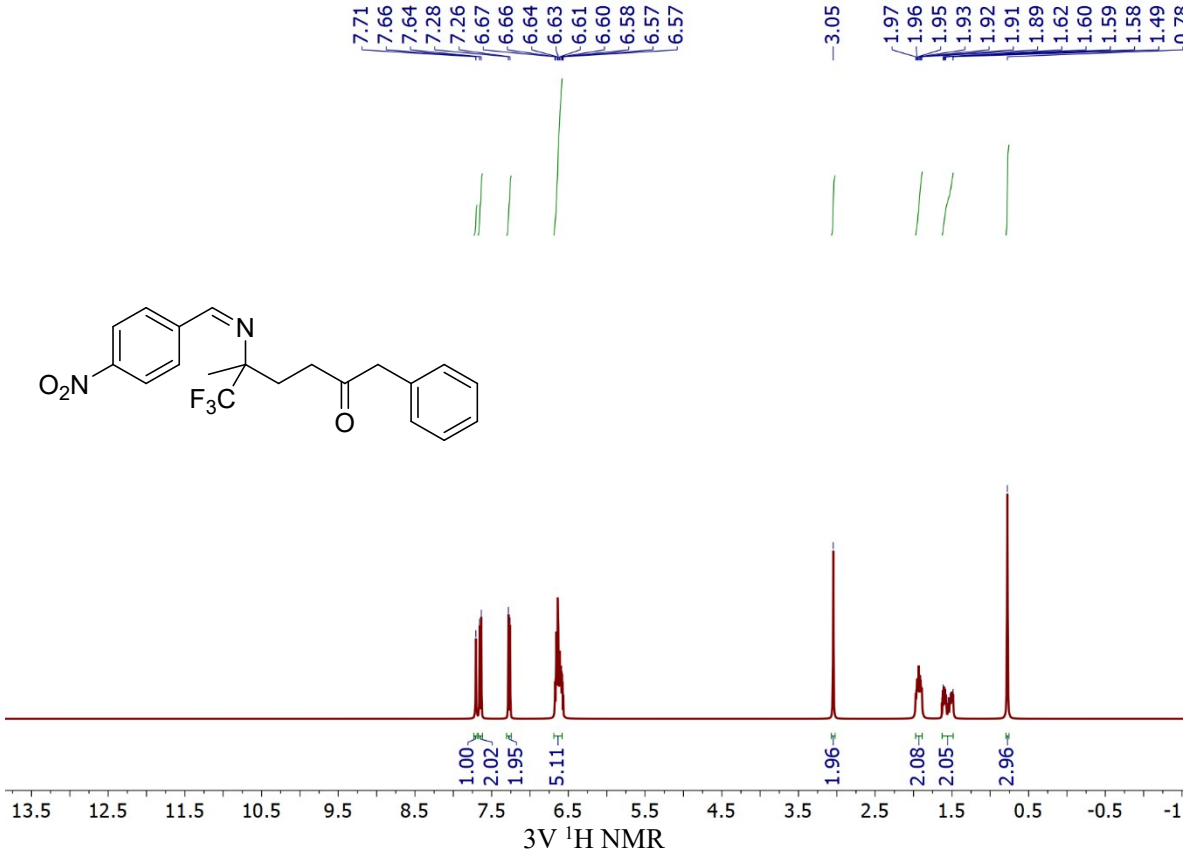
3S ^{1}H NMR



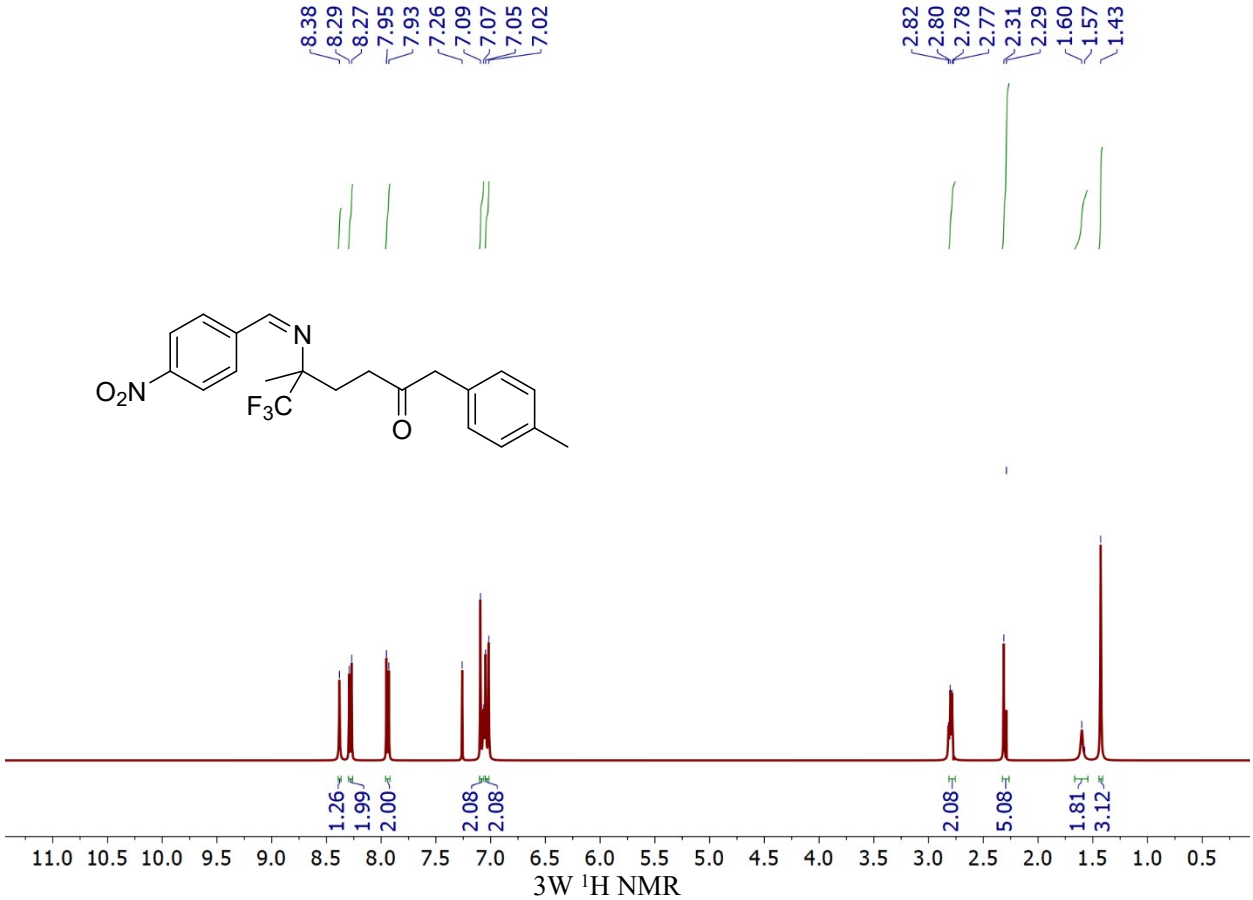
3T ^{1}H NMR



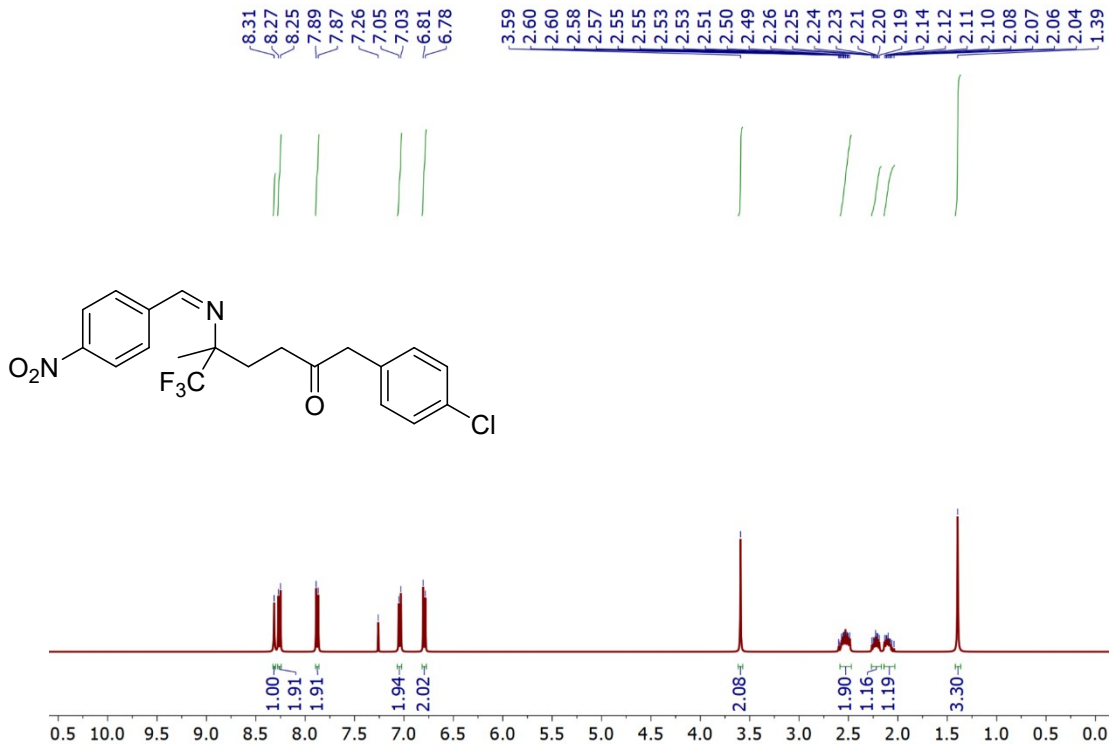
3U ^{1}H NMR



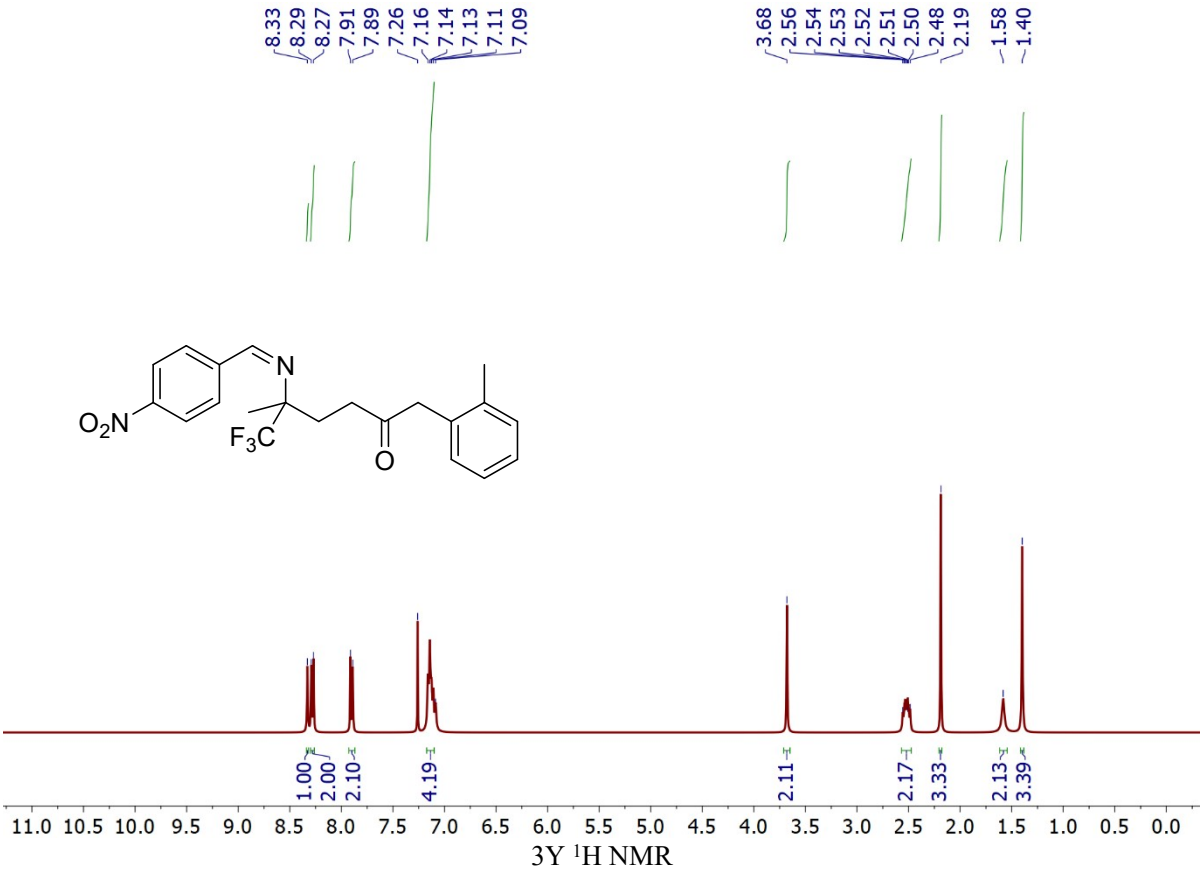
3V ^{1}H NMR



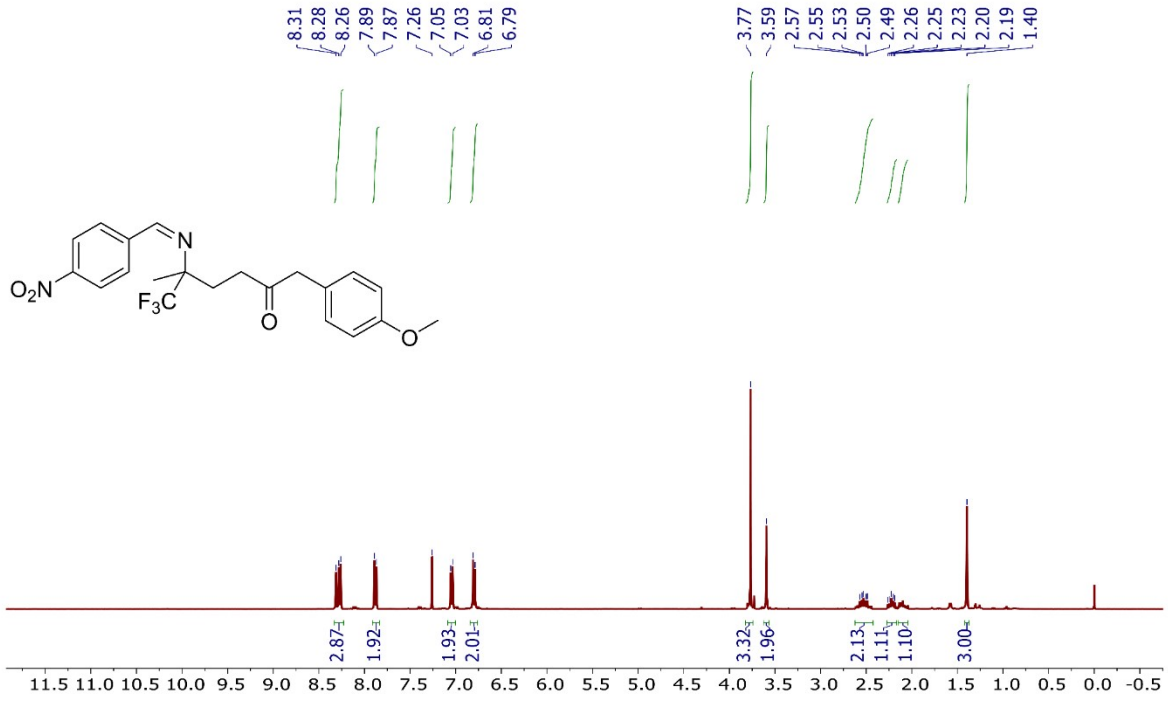
3W ^{1}H NMR



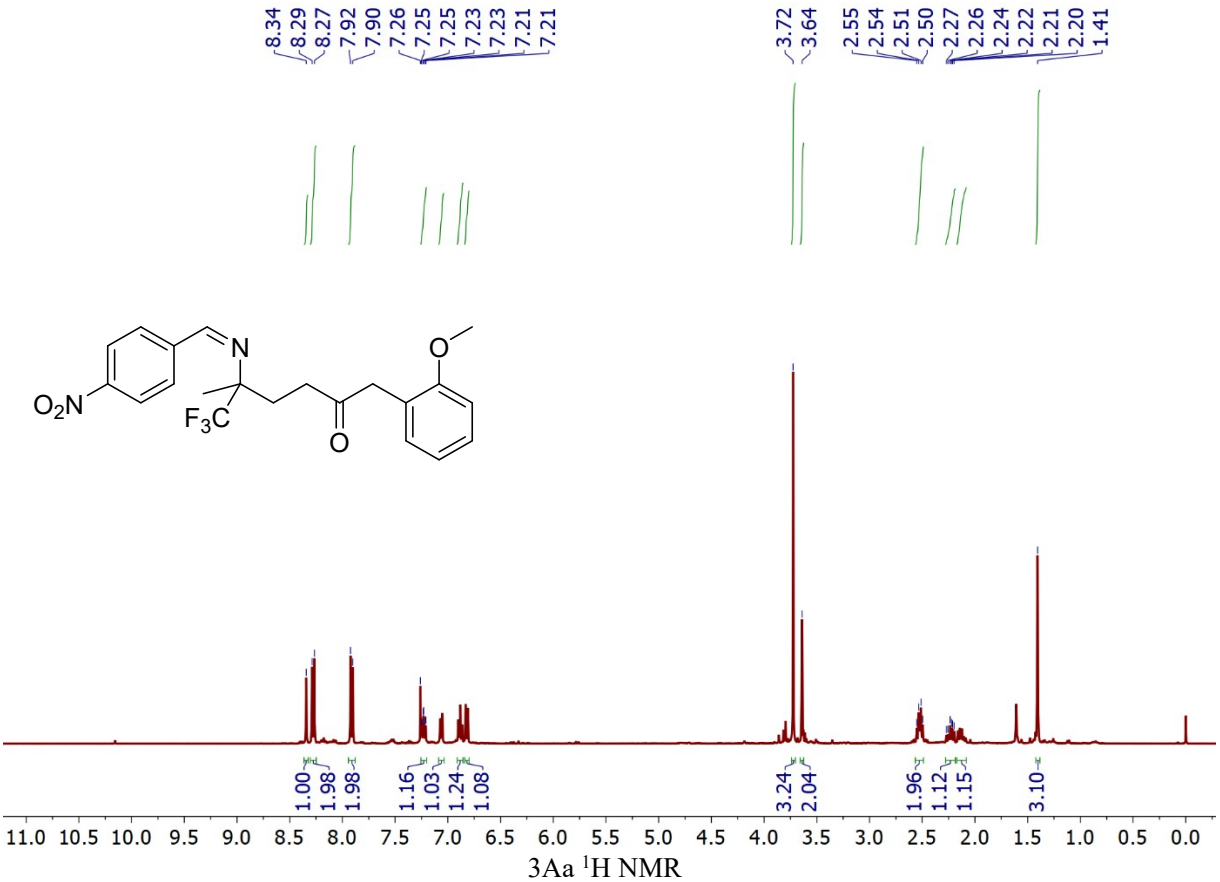
3X ^{1}H NMR



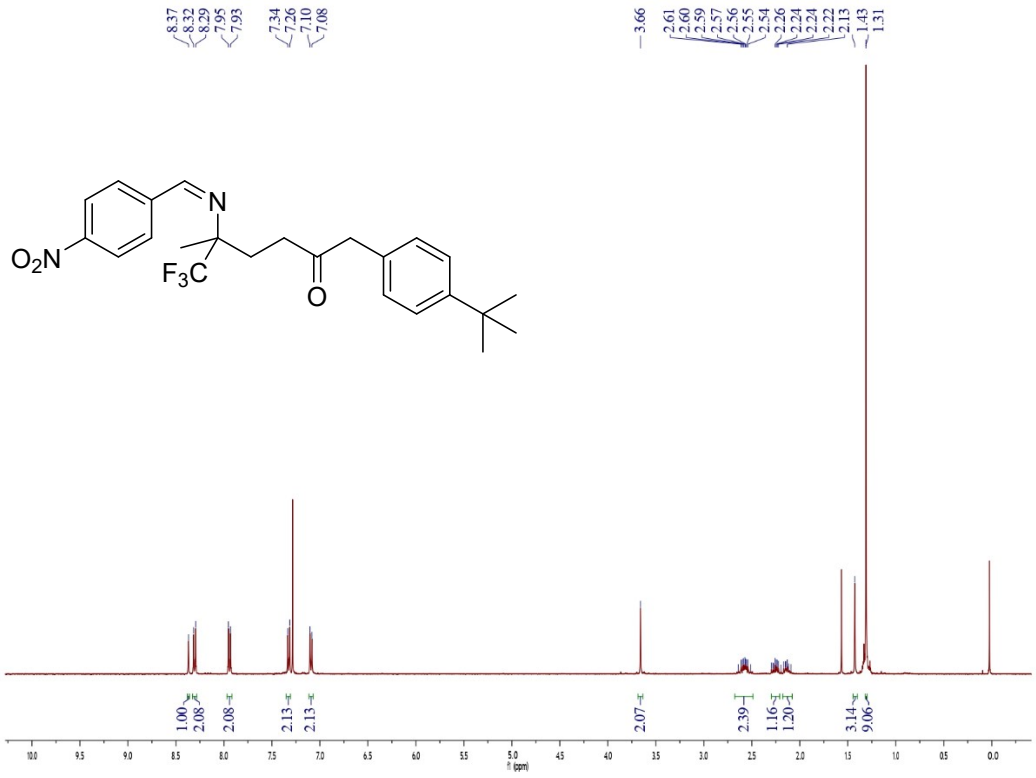
3Y ^{1}H NMR



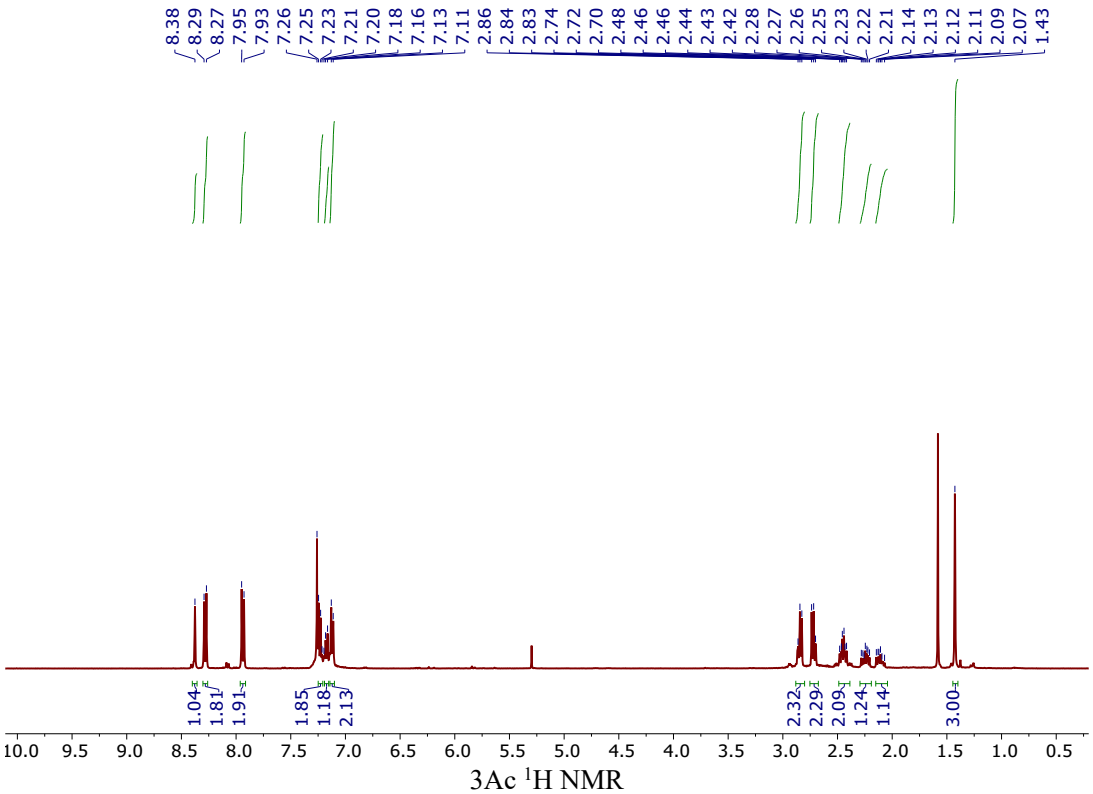
3Z ^{1}H NMR



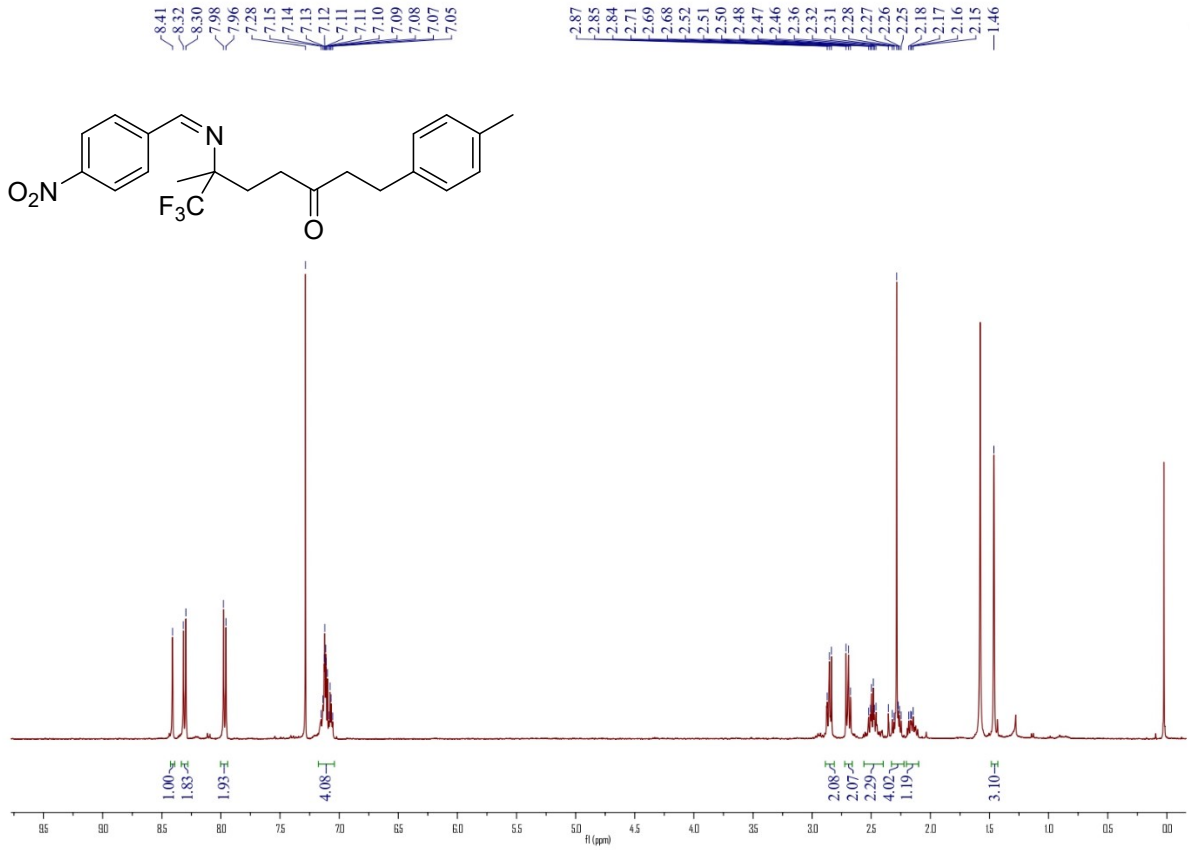
3Aa ^{1}H NMR



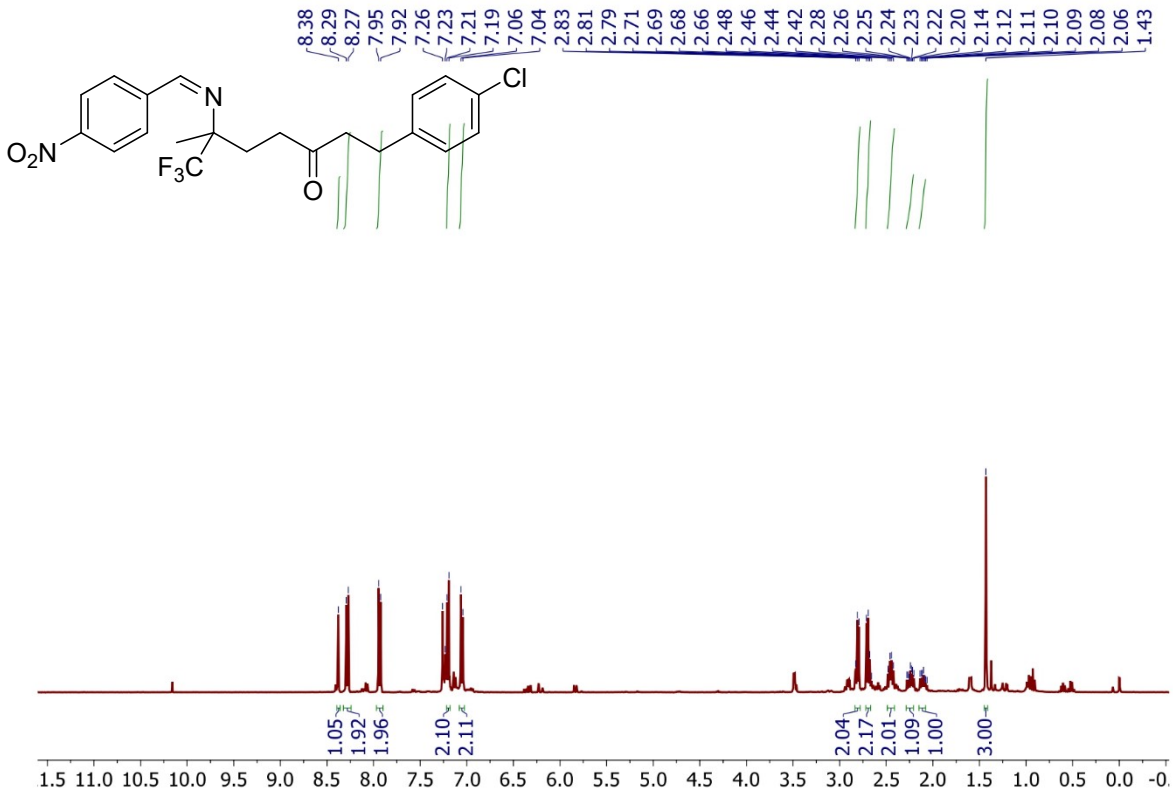
3Ab ^{1}H NMR



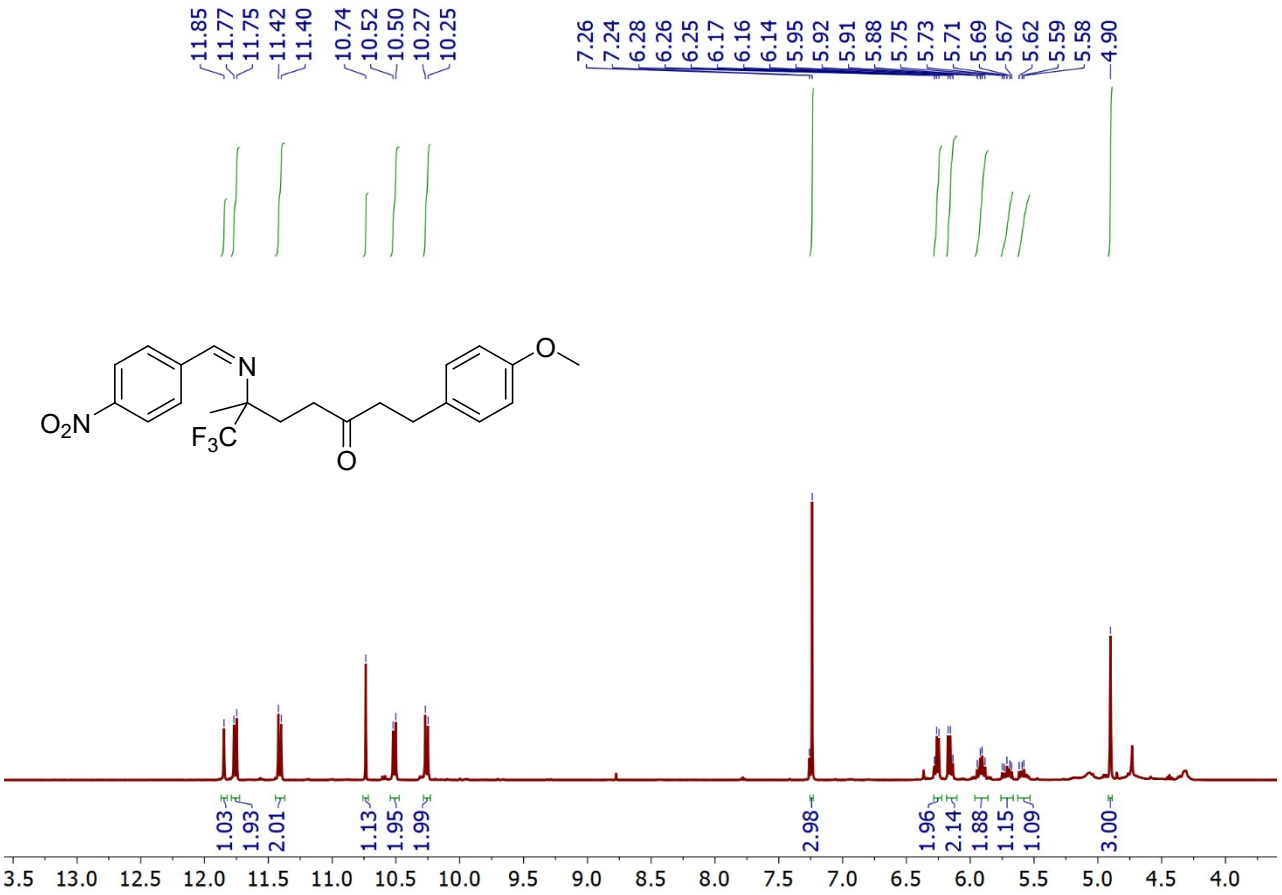
3Ac ^{1}H NMR



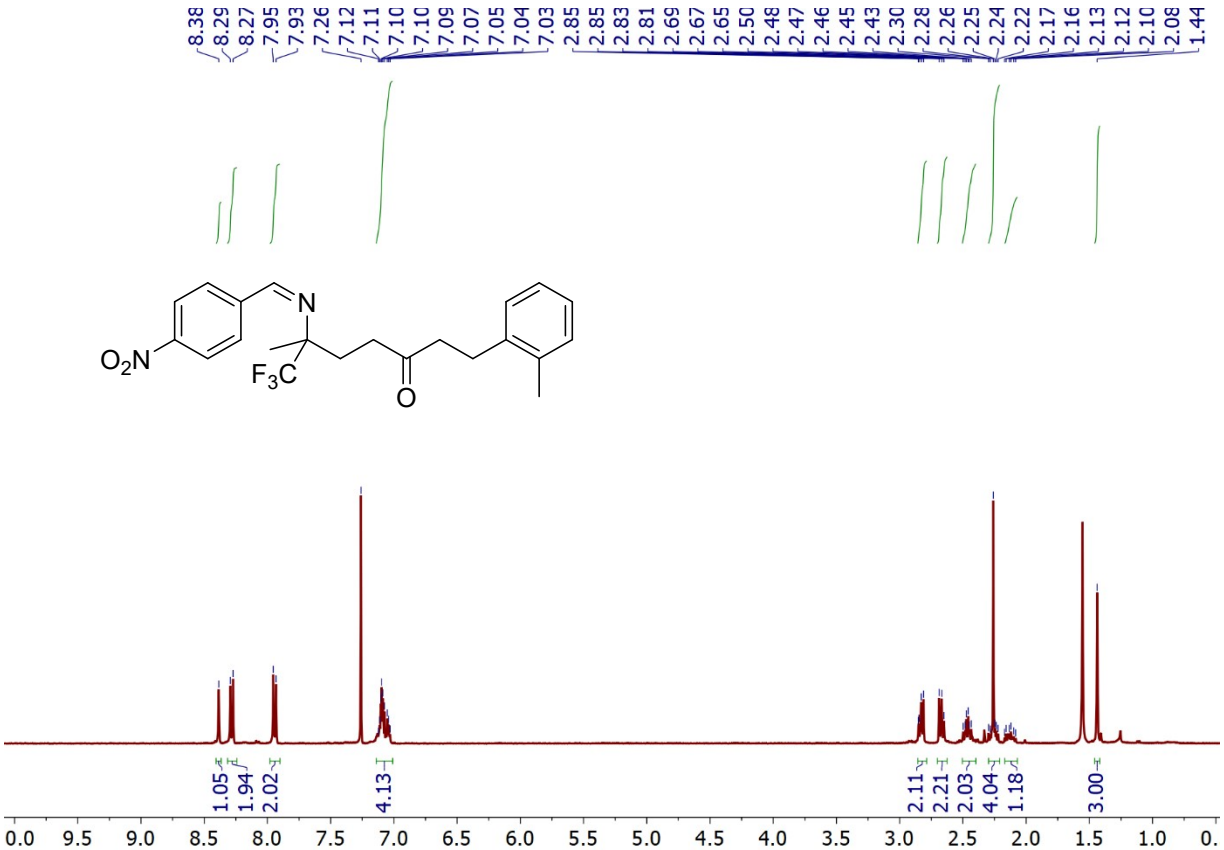
3Ad ^{1}H NMR



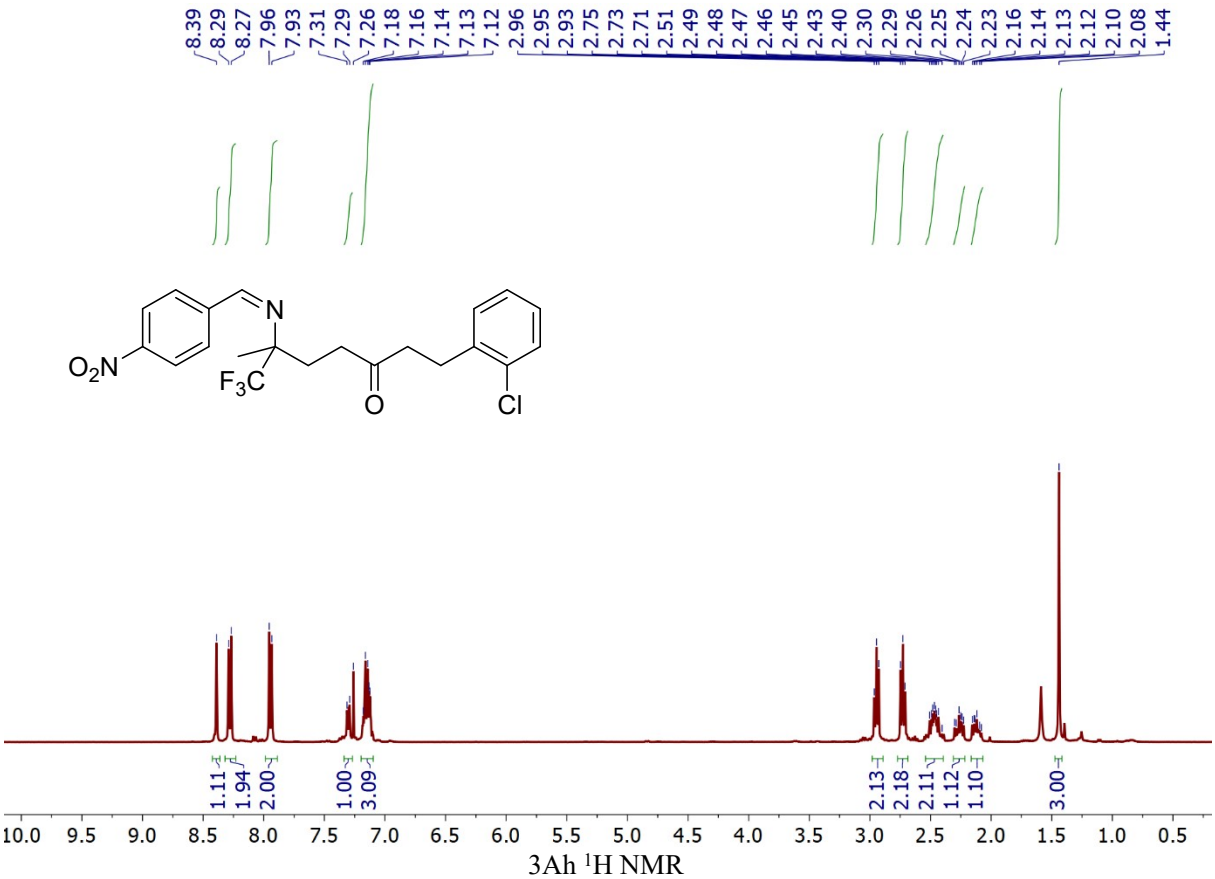
3Ae ^{1}H NMR



3Af ^{1}H NMR



3Ag ^{1}H NMR



3Ah ^{1}H NMR

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