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Supplementary Information

Selective transamidation of the 3-oxo-N-acyl homoserine lactones by hydrazine derivatives

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General Synthetic Procedures & Characterization

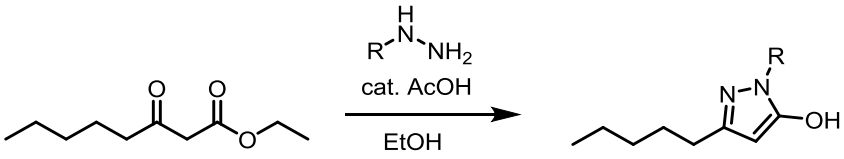
General Reagents

Commercial reagents and solvents, including hydrazine derivatives, were purchased from Sigma-Aldrich and Fisher Scientific. Reaction kinetics were monitored on an HP 1100 Series analytical HPLC/UV-Vis using a 2.7μM HALO C_{18} column at 210 nm with masses confirmed by an Agilent 1200 Series LC-MS using a 2.7μM HALO C_{18} column in positive ion mode with electrospray ionization. Both HPLC and LC-MS data were analyzed using Agilent Chemstation software. Hydroxypyrazoles **6a, 6b, 6c,** and **6g** were purified using a Waters semi-prep HPLC/UV-Vis with a Waters 4.2μM C_{18} column. Product characterization (^{1}H and ^{13}C) was completed on a 500 or 600 MHz Bruker NMR spectrometer with Bruker TOPSPIN NMR software (V 3.2). All related coupling constants are reported in Hertz (Hz) and chemical shifts in ppm. NMR solvents were purchased from Cambridge Isotopes Laboratories. High resolution mass spectra were obtained on an Agilent Accurate LC-TOF Mass Spectrometer (Agilent Series 6220) operating in positive ion mode with an electrospray ionization source (fragmentor = 175 V). The data was analyzed using Agilent MassHunter Workstation Software, Qualitative Analysis (V. B.02.00).

General Procedure for Preparation of Hydroxypyrazole Products for Characterization

To a 20-mL scintillation vial, compound **1** (12.1 mg, 0.0501 mmol) was added to 10.0 mL of 200 mM PBS buffer at pH 7.4 and vortexed. The hydrazine derivative (0.250 mmol) was then added to the reaction mixture. The reaction was allowed to stir at RT and reaction progress was monitored by LC-MS. Upon maximum conversion to the hydroxypyrazole product, the reaction mixture was directly purified by semi-prep HPLC under neutral conditions. Separations were performed in a mobile phase of 20 to 50% B over 20 minutes where A = 100% water, 0.1% methanol and B = 100% acetonitrile. Products were then confirmed by HRMS and ^{1}H and ^{13}C NMR in the case of **6a**. NMR spectra were obtained for **6b, 6c,** and **6g** through the synthesis of authentic products as verified by LC-MS and NMR spectroscopy (see below). Several of the screened hydrazines did not progress to the corresponding hydroxypyrazole products by LC-MS (Table 2, **6d, 6e, 6f**) and therefore were not isolated for characterization.

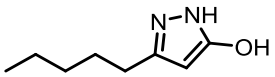
General Procedure for Authentic Product Synthesis



To a 10-mL round-bottom flask, ethyl 3-oxooctanoate (132 mg, 0.709 mmol) was dissolved in 2.0 mL of EtOH. The corresponding hydrazine (0.709 mmol) was then added to the reaction mixture followed by a drop of acetic acid. The reaction mixture was brought to reflux for five hours at 80 °C. The reaction mixture was concentrated and purified directly by column chromatography.

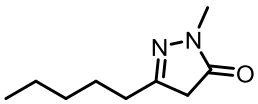
The transamidation products display solvent dependent keto-enol tautomerism. The keto-form is favored when in $CDCl_3$ while the enol-form is favored in polar protic solvents such as DMSO-d_6 (see NMR spectra of **6b** for comparison). As our reactions proceed in aqueous media, we have drawn our products as the enol tautomer in the publication, however the spectra below in $CDCl_3$ display the keto tautomer.

3-pentyl-1H-pyrazol-5-ol (6a)

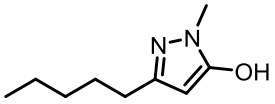


δ_H (DMSO-d_6, 600 MHz) 11.2 (1H, s), 9.26 (1H, s), 5.21 (1H, s), 2.41 (2H, t, *J* = 6.6 Hz), 1.52 (2H, p, *J* = 7.8 Hz), 1.29 (4H, m), 0.86 (3H, t, *J* = 6.6 Hz); δ_C (DMSO-d_6, 150 MHz) 161.4, 144.7, 88.3, 31.3, 28.8, 26.1, 22.3, 14.4; HRMS (ESI) calcd for $C_8H_{14}N_2O$: m/z 155.1179, found: m/z 155.1181 (M + H).

1-methyl-3-pentyl-1H-pyrazol-5-ol (6b)

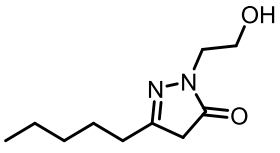


δ_H ($CDCl_3$, 600 MHz) 3.29 (3H, s), 3.18 (2H, s), 2.43 – 2.37 (2H, s), 1.65 – 1.54 (2H, m), 1.41 – 1.25 (4H, m), 0.92 (3H, t, *J* = 6.8 Hz). δ_C ($CDCl_3$, 151 MHz) 172.2, 159.5, 40.0, 31.4, 31.1, 31.1, 26.4, 22.3, 13.9.



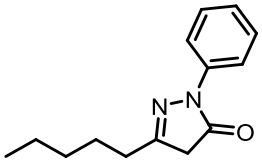
δ_H (DMSO-d_6, 600 MHz) 5.13 (1H, s), 3.41 (3H, s), 2.31 (2H, t, *J* = 6.0 Hz), 1.49 (2H, p, *J* = 7.2 Hz) 1.27 (4H, m), 0.87 (3H, t, *J* = 7.2 Hz); δ_C (DMSO-d_6, 150 MHz) 152.6, 150.2, 84.8, 33.1, 31.5, 29.1, 29.0, 22.4, 14.4; HRMS (ESI) calcd for $C_9H_{16}N_2O$: m/z 169.1335, found: m/z 169.1336 (M + H).

1-(2-hydroxyethyl)-3-pentyl-1H-pyrazol-5-ol (6c)



δ_H ($CDCl_3$, 600 MHz) 3.92 – 3.82 (4H, m), 3.25 (2H, s), 2.44 – 2.37 (2H, m), 1.60 (p, *J* = 7.4 Hz, 2H), 1.41 – 1.25 (4H, m), 0.93 (3H, t, *J* = 6.8 Hz). δ_C ($CDCl_3$, 151 MHz) 172.8, 160.3, 61.3, 47.2, 40.3, 31.4, 31.1, 26.3, 22.3, 13.9; HRMS (ESI) calcd for $C_{10}H_{18}N_2O_2$: m/z 199.1441, found: m/z 199.1446 (M + H).

3-pentyl-1-phenyl-1H-pyrazol-5-ol (6g)

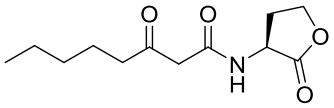


δ_H ($CDCl_3$, 600 MHz) 7.90 (2H, dd, *J* = 8.5, 1.3 Hz), 7.45 – 7.37 (2H, m), 7.25 – 7.17 (1H, m), 3.45 (2H, m), 2.55 – 2.49 (2H, m), 1.68 (2H, tt, *J* = 7.3, 5.9 Hz), 1.40 (4H, dq, *J* = 7.2, 3.8 Hz), 0.98 – 0.88 (3H, m). δ_C ($CDCl_3$, 151 MHz) 170.6, 160.1, 138.1, 128.8, 125.0, 118.9, 41.8, 31.4, 31.2, 26.3, 22.4, 14.0; HRMS (ESI) calcd for $C_{14}H_{18}N_2O$: m/z 231.1492, found: m/z 231.1495 (M + H).

General Procedure for N-Acyl Homoserine Lactone (AHL) Synthesis

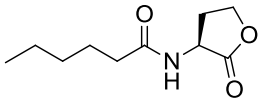
The C_6, 3-OH-C_8 and 3-oxo-C_{12} AHLs are known compounds and were synthesized following previously published procedures.[1,2]

***N*-(3-Oxooctanoyl)-L-homoserine Lactone (1)**



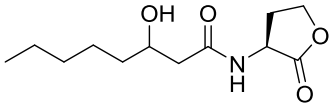
53.1% Yield; δ_H (CD_3CN, 500 MHz) 7.21 (1H, s), 4.48 (1H, m), 4.35 (1H, td, J_1 = 7.5 Hz, J_2 = 1.5 Hz), 4.20 (1H, m), 3.34 (2H, s), 2.50-2.46 (3H, m), 2.22 (1H, m), 1.52 (2H, p, *J* = 7.0 Hz), 1.31-1.20 (4H, m), 0.87 (3H, t, *J* = 7.0 Hz); δ_C (DMSO-d_6, 150 MHz) 210.0, 175.5, 166.7, 65.8, 50.7, 48.6, 42.3, 31.1, 28.7, 23.0, 22.4, 14.3; MS (ESI) calcd for $C_{12}H_{19}NO_4$: m/z 242.1, found: m/z 242.1 (M + H).

***N*-Hexanolyl-L-homoserine Lactone (3)**



63.8% Yield; δ_H (DMSO-d_6, 500 MHz) 8.30 (1H, s), 4.49 (1H, m), 4.31 (1H, td, J_1 = 9.0 Hz, J_2 =1.5 Hz), 4.17 (1H, m), 2.35 (1H, m), 2.13-2.06 (3H, m), 1.47 (2H, m, *J* = 7.5 Hz), 1.26-1.21 (4H, m), 0.84 (3H, t, *J* = 7.0 Hz); δ_C (DMSO-d_6, 125 MHz) 175.9, 172.7, 65.7, 48.3, 35.5, 31.2, 28.7, 25.2, 22.3, 14.3; MS (ESI) calcd for $C_{10}H_{17}NO_3$: m/z 200.13, found: m/z 200.12 (M + H).

***N*-(3-hydroxyoctanoyl)-L-homoserine Lactone (4)**



51.3% Yield; δ_H ($CDCl_3$, 600 MHz) 6.85-6.80 (1H, br d), 4.56 (1H, m), 4.47 (1H, m), 4.28 (1H, m), 4.00 (1H, br s), 3.38-3.31 (1H, br d), 2.76 (1H, m), 2.44 (1H, m), 2.33 (1H, m), 2.22 (1H, m), 1.43 (1H, m), 1.32 (2H, m), 1.29 (5H, m), 0.88 (3H, t); δ_C ($CDCl_3$, 150 MHz) 175.7, 173.0, 68.6, 66.2, 49.1, 42.6, 36.9, 31.9, 30.0, 25.1, 22.6, 14.0; MS (ESI) calcd for $C_{12}H_{21}NO_4$: m/z 244.2, found: m/z 244.2 (M + H).

HPLC Experiments

Procedure for AHL Competition Experiment

In a 20-mL scintillation vial, the C_6 AHL (1.99 mg, 10.0 μmol), 3-OH-C_8 AHL (2.43 mg, 10.0 μmol), and 3-oxo-C_8 AHL (2.41 mg, 10.0 μmol) were all combined and dissolved in 10.0 mL of NaOAc buffer at pH 4.5. Hydrazine (0.32 μL, 10. μmol) was then added to the reaction mixture. The reaction progress was monitored by analytical HPLC with automated injection every 40 minutes. Separations were performed in a mobile phase of 25 to 45% B over 25 minutes where A = 95:5 water:acetonitrile and B = 0:100 water:acetonitrile with a flow rate of 4.00 mL/min. After 44 h, the products were analyzed by LC-MS.

General Procedure for Comparative Kinetics (pH Dependence & Hydrazine Derivatives)

Stock solutions of both the 3-oxo-C_8 AHL (2.0 mM) and the corresponding hydrazine (100 mM) were prepared in distilled water and 400 mM PBS or NaOAc buffer, respectively. The hydrazine was then diluted 10x in buffer to a final concentration of 10 mM. In a 2.0-mL vial, 500 μL of both the AHL and hydrazine stock solutions were combined. The reaction progress was monitored by analytical HPLC. Separations were performed in a mobile phase of 30 to 50% B for phenylhydrazine and hydrazide derivatives (**5e-5j**) and a mobile phase of 5 to 40% B over 22 minutes for the remaining hydrazines. Percent conversions were obtained by integration of the peak corresponding to the AHL after each injection with concentrations determined by a prepared standard curve. Products were analyzed by LC-MS. The second order rate constants displayed in Table 3 were calculated by using eq. (1) under the assumption that the initial rate of the reaction (hydrazone formation by our proposed scheme) is second order and the contribution of the reverse reaction to the rate equation is initially negligible.[3]

$$-\frac{d[AHL]}{dt} = k[AHL][hydrazine] \quad (1)$$

LC-TOF Experiments

General Procedure for Product Identification at Reduced Concentration by LC-TOF

Stock solutions of both the 3-oxo-C_8 AHL (1.0 mM) and phenylhydrazine (5.0 mM) were prepared in distilled water in PBS buffer. In a 20-mL vial, 2.0 mL of the hydrazine stock was diluted with buffer and combined with either 100 μL or 5.0 μL of the AHL stock solutions to achieve a final concentration of 1.0 mM hydrazine and 10 μM or 500 nM AHL, respectively. The initial rate for the 10 μM reaction was obtained by analytical HPLC (see *Comparative Kinetics*). The progress of both the 10 μM and 500 nM experiments was also monitored by LC-TOF due to the detection limits of the LC-MS and HPLC for the 500 nM trial. Separation was

performed at a flow rate of 0.300 mL/min on a 20 – 100% acetonitrile:water gradient for 13.1 minutes followed by a 100% MeCN flush from 13.1 to 18.0 minutes. HRMS data of both the corresponding hydrazone and hydroxypyrazole were obtained and reaction progress was qualitatively assessed by changes in the extracted ion chromatogram (EIC).

References:

1. Chhabra, S. R., Harty, C., Hooi, D. S. W., Daykin, M., Williams, P., Telford, G., Pritchard, D. I., and Bycroft, B. W. *J. Med. Chem.* **2003**, 46, 97–104.
2. Chhabra, S. R, Stead, P., Bainton, N., Salmond, G., Stewart, G., Williams, P., & Bycroft, B. *The Journal of Antibiotics* **1993**, *3*, 441–454.
3. Kool, E. T., Park, D., Crisalli, P. *J. Am. Chem. Soc.* **2013**, 47, 17663-17666.

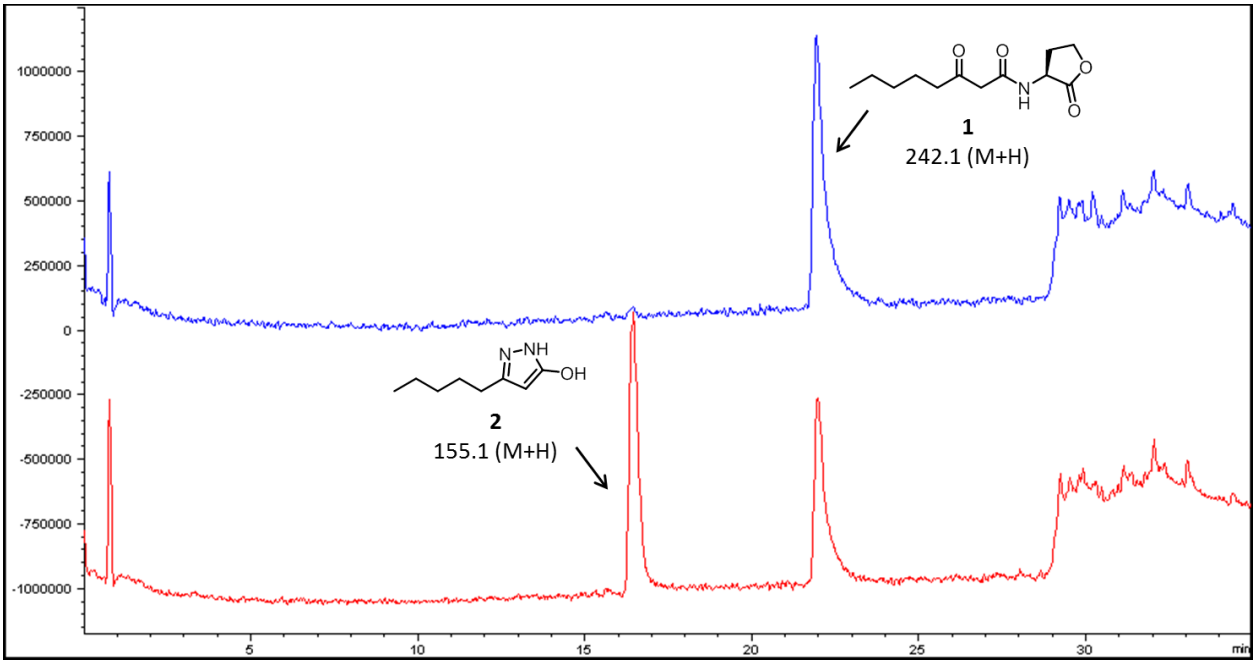


Figure S1. Total ion chromatogram of hydrazine-mediated AHL transamidation. The trace displays clean conversion of the 3-oxo-C_8 AHL (**1**) (1.0 mM) to the corresponding hydroxypyrazole (**2**) product at pH 4.5 in NaOAc buffer.

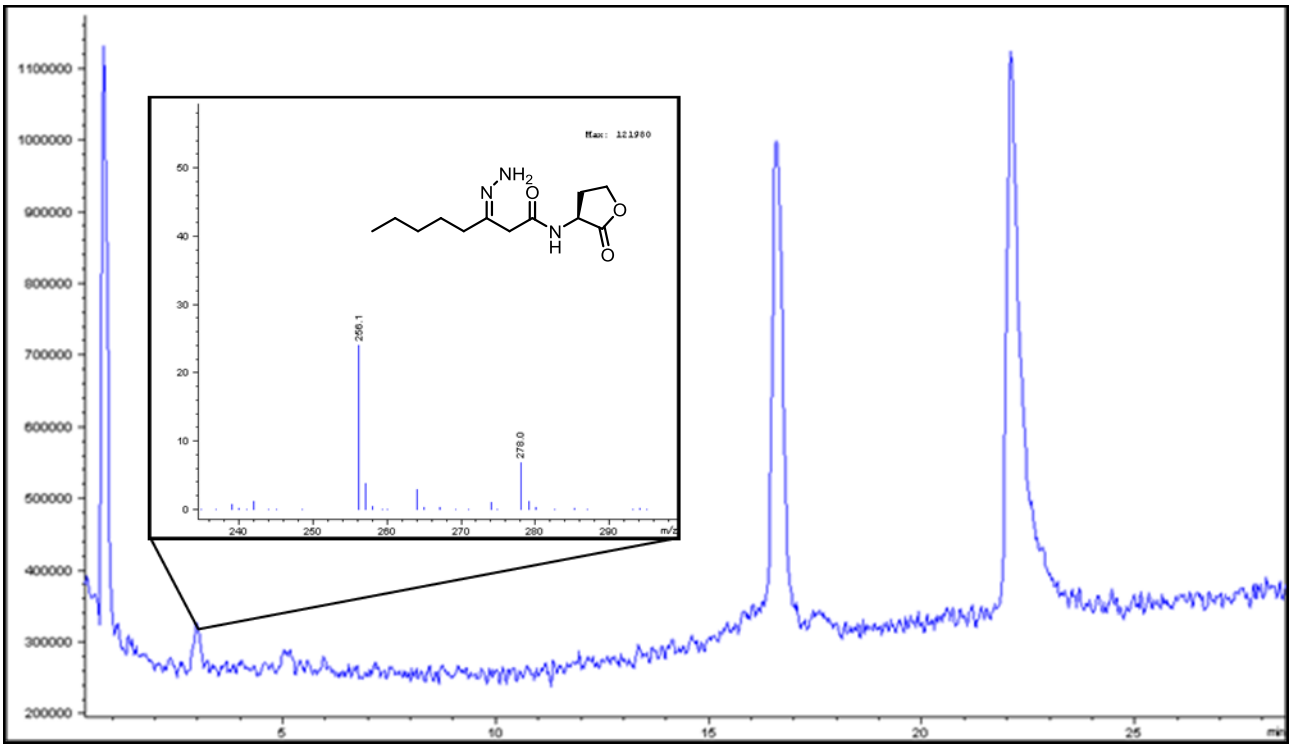
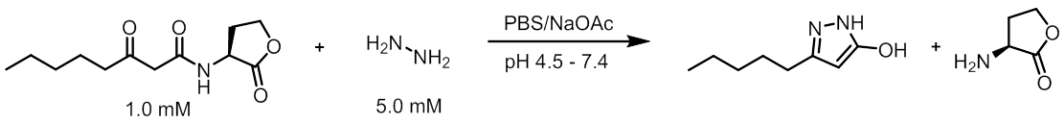
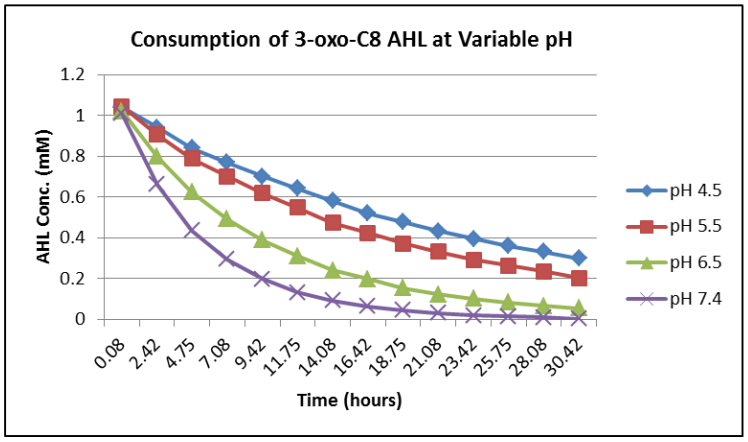


Figure S2. Detection of hydrazone product (256.1 m/z) as a putative intermediate in hydroxypyrazole formation in total ion chromatogram LC-MS.





pH	k_{rel}
7.4	4.3
6.5	2.5
5.5	1.4
4.5	1.0

Figure S3 - pH dependence on 3-oxo-C_8 AHL rates of consumption with hydrazine. Reaction progress was monitored by analytical HPLC at 210 nm with a 5:1 ratio of hydrazine to AHL.

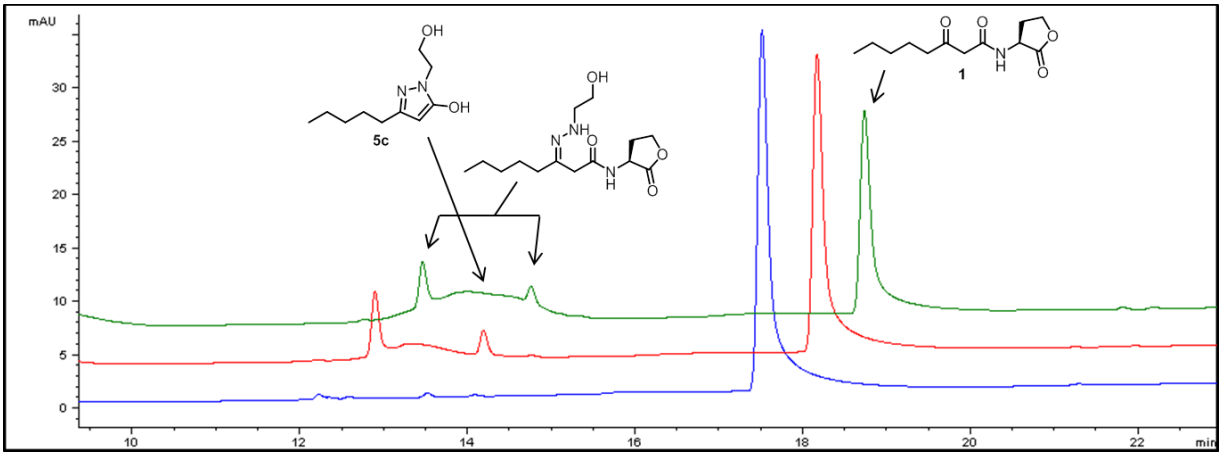


Figure S4 – Representative HPLC trace for the determination of rates of consumption as depicted in Table 2 from 0 h (blue) to 10.7 h (green). The integration of (**1**) was monitored over time to determine rates of consumption by HPLC with masses of the products confirmed by LC-MS. The axes are offset for clarity.

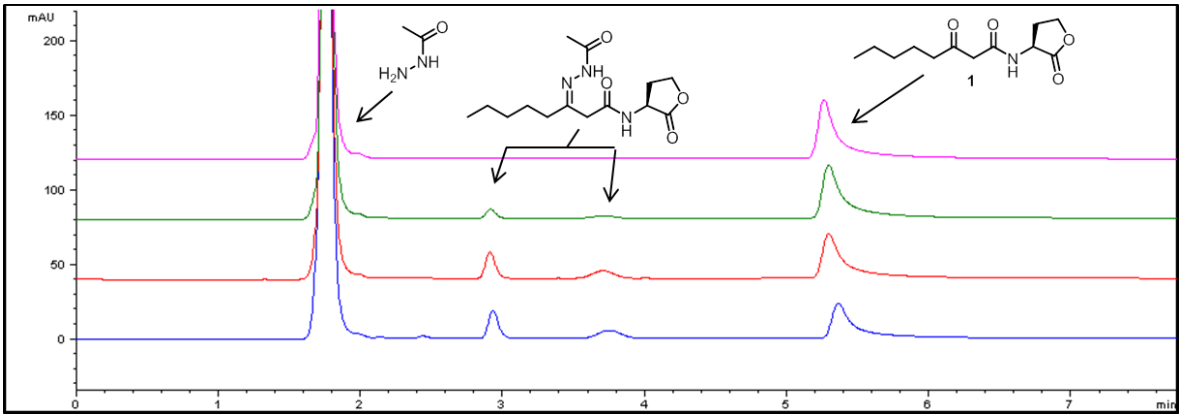


Figure S5 –Representative HPLC trace for the determination of rates of consumption of hydrazides as depicted in Table 2 from 0 h (pink) to 30 h (blue). The two product peaks were determined to be the corresponding hydrazone isomers by LC-MS with no hydroxylpyrazole formation detected.

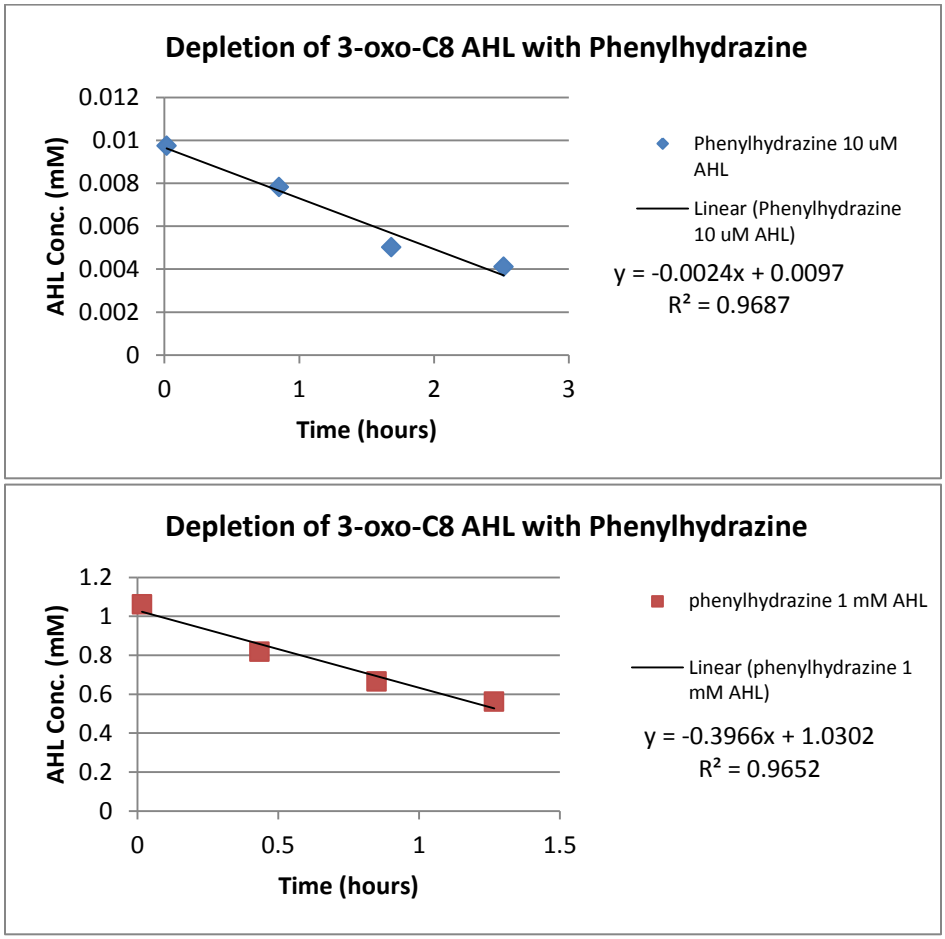


Figure S6. Initial rates of AHL depletion at 10 μM (blue) compared to 1.0 mM (red) by phenylhydrazine as determined by HPLC at 210 nm. The rates, as represented by the slope of the linear regression, are consistent with a two order of magnitude change in AHL concentration.

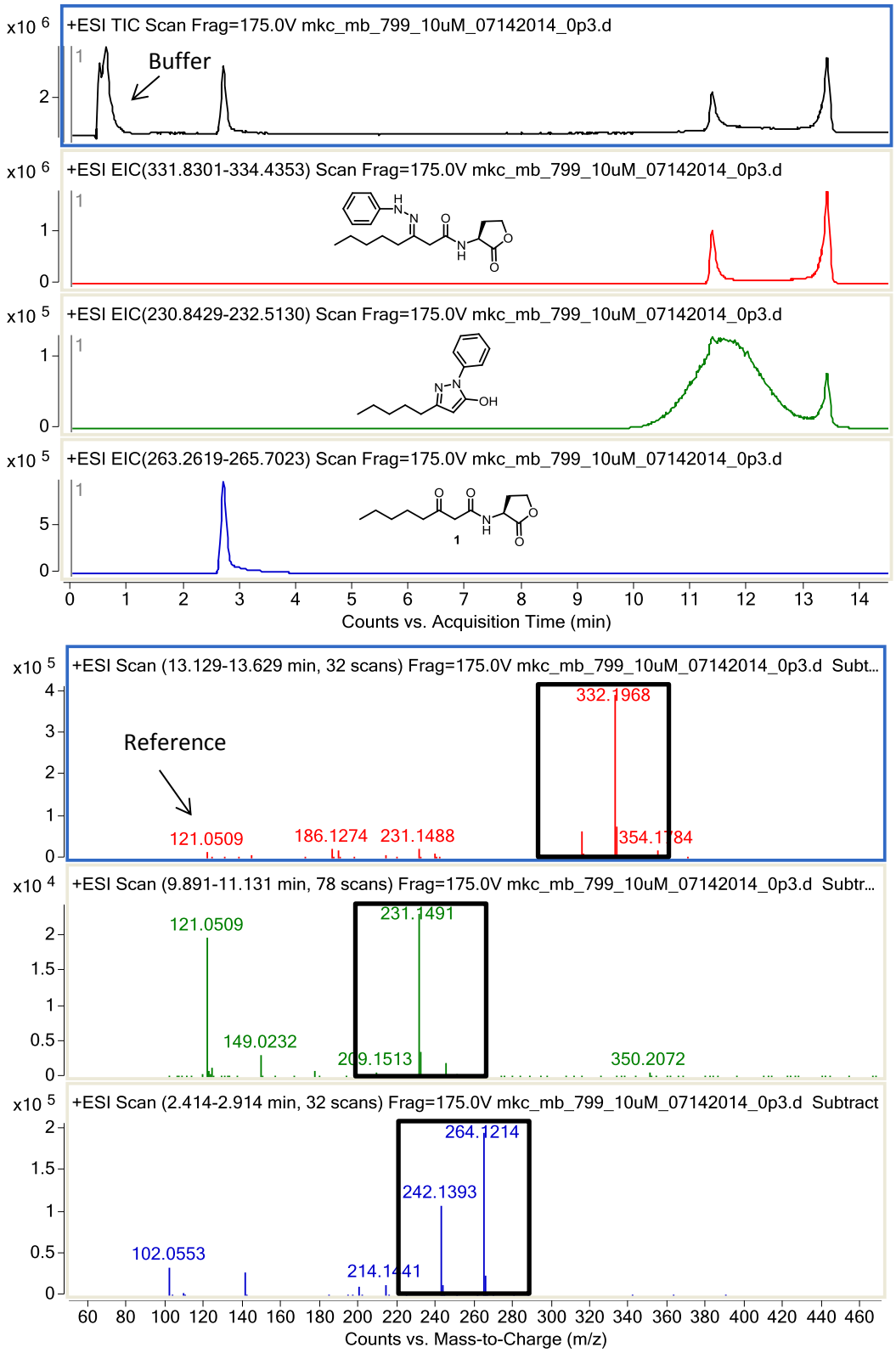


Figure S7. LC-TOF analysis of the phenylhydrazine (1.0 mM) and **1** (10 μM) reaction mixture at 80 minutes, including the total ion chromatogram (TIC) (black) and corresponding extracted ion chromatograms (EIC) and HRMS for the hydrazone (red), hydroxypyrazole (green) and **1** (blue).

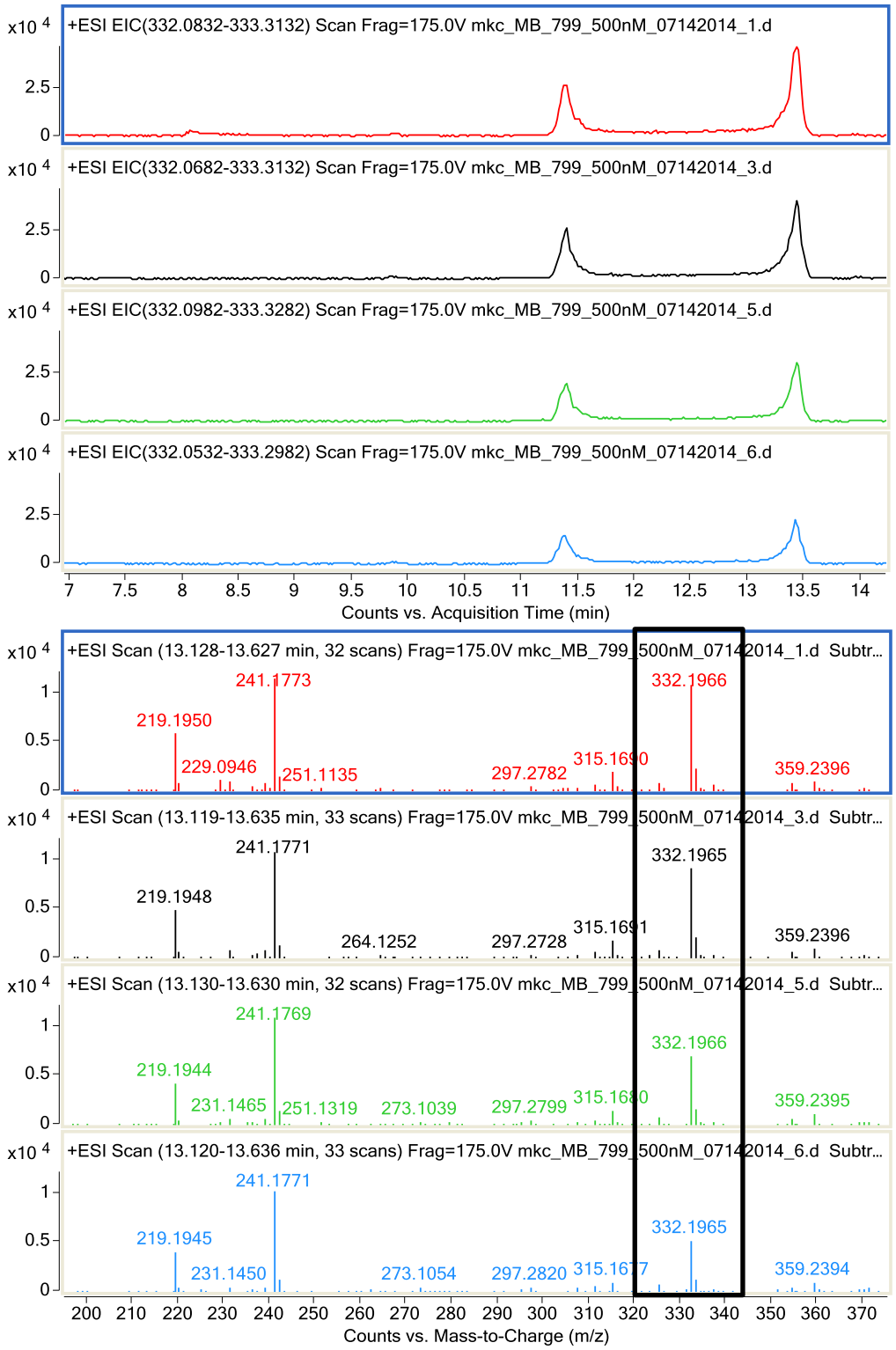


Figure S8. Time dependent EIC of the phenylhydrazone in the reaction of phenylhydrazine and **1** (500 nM) at 2.78 h (red), 5.03 h (black), 7.25 h (green), and 9.48 h (blue). Both the retention times and masses correlate with the 10 μM experiment above.

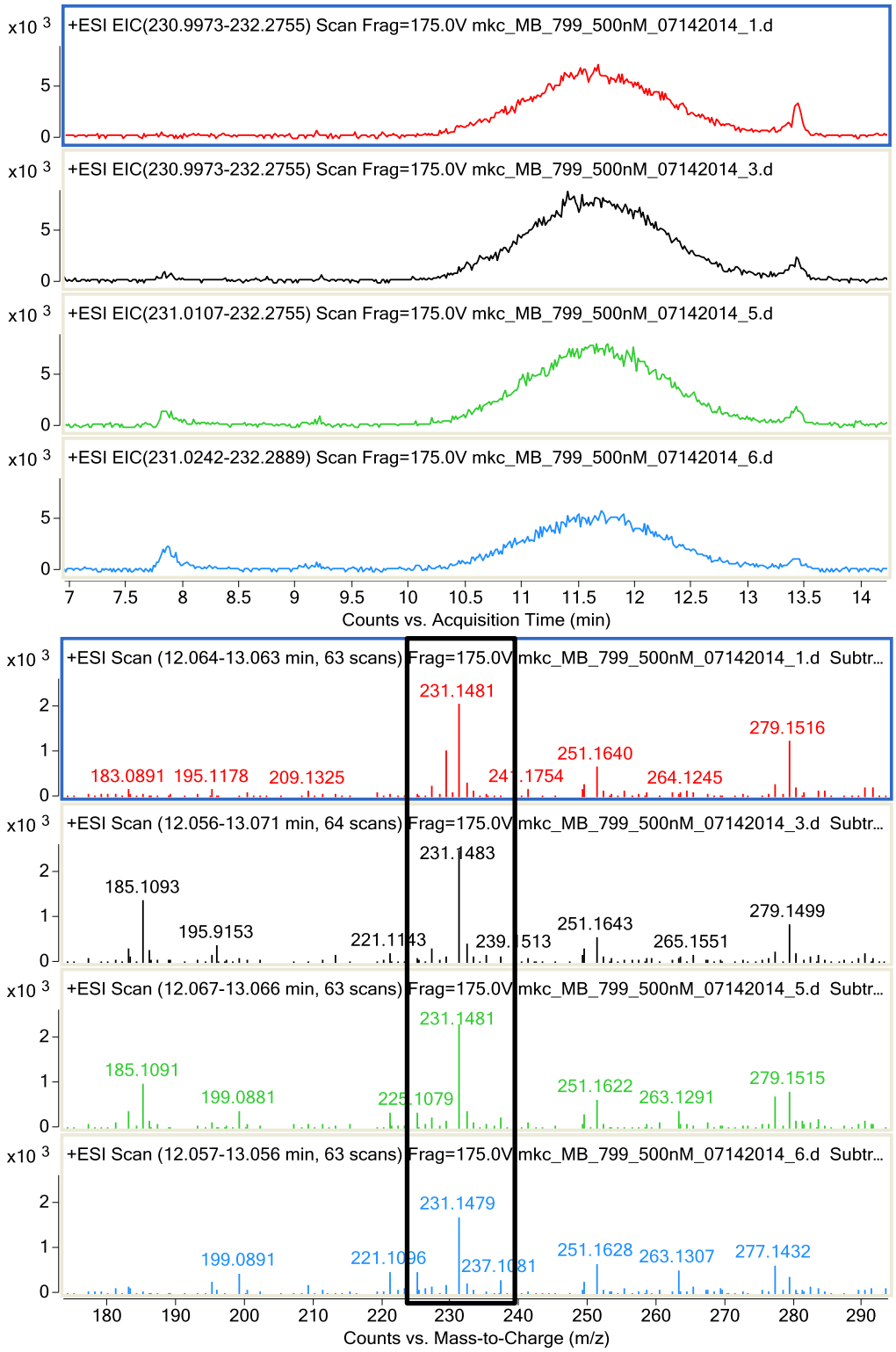
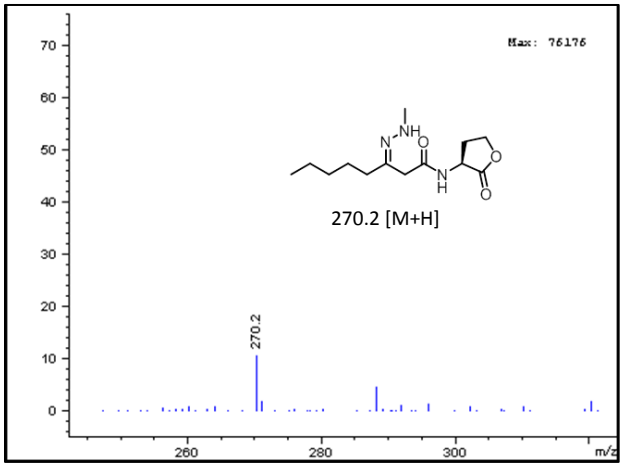
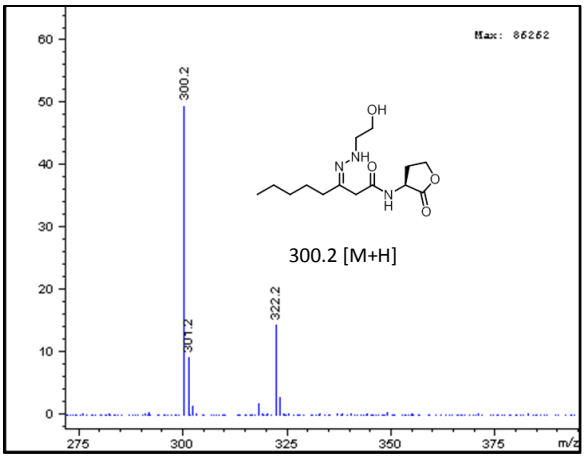
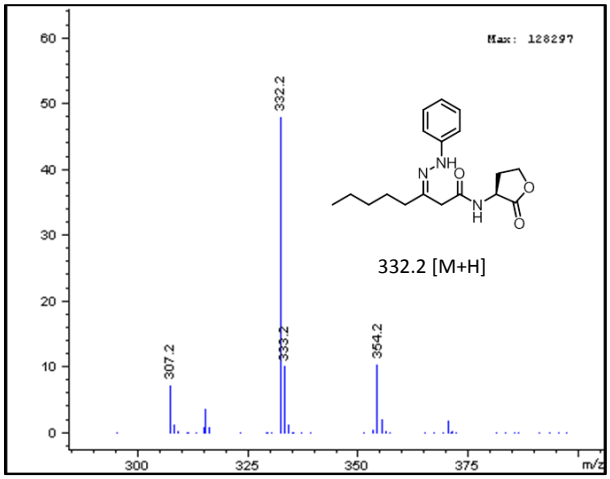


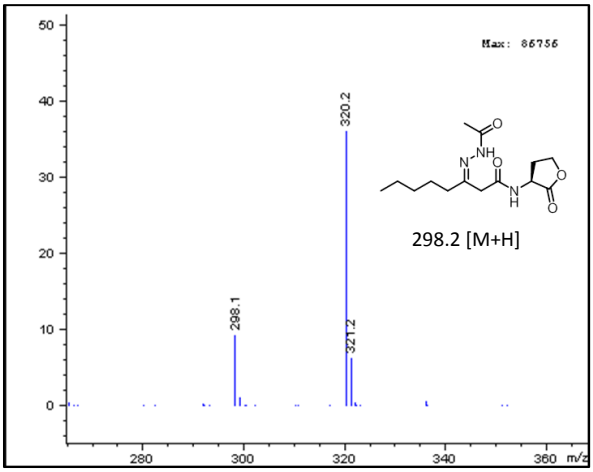
Figure S9. Time dependent EIC of the phenylhydroxypyrazole in the reaction of phenylhydrazine and **1** (500 nM) at 2.78 h (red), 5.03 h (black), 7.25 h (green), and 9.48 h (blue). Both retention times and masses correlate with the 10 μM experiment above.

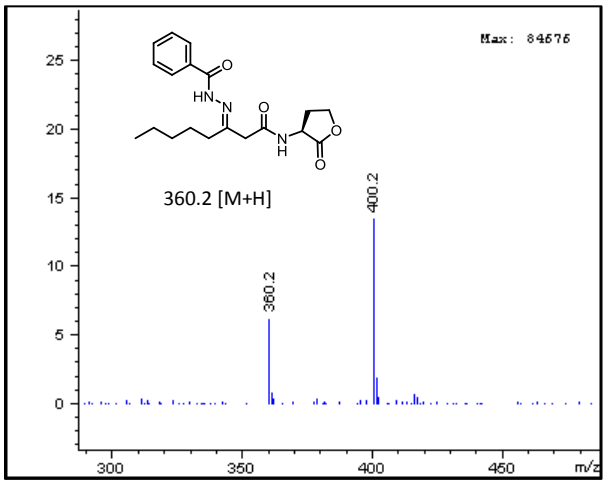
*Detected hydrazone masses by LC-MS**

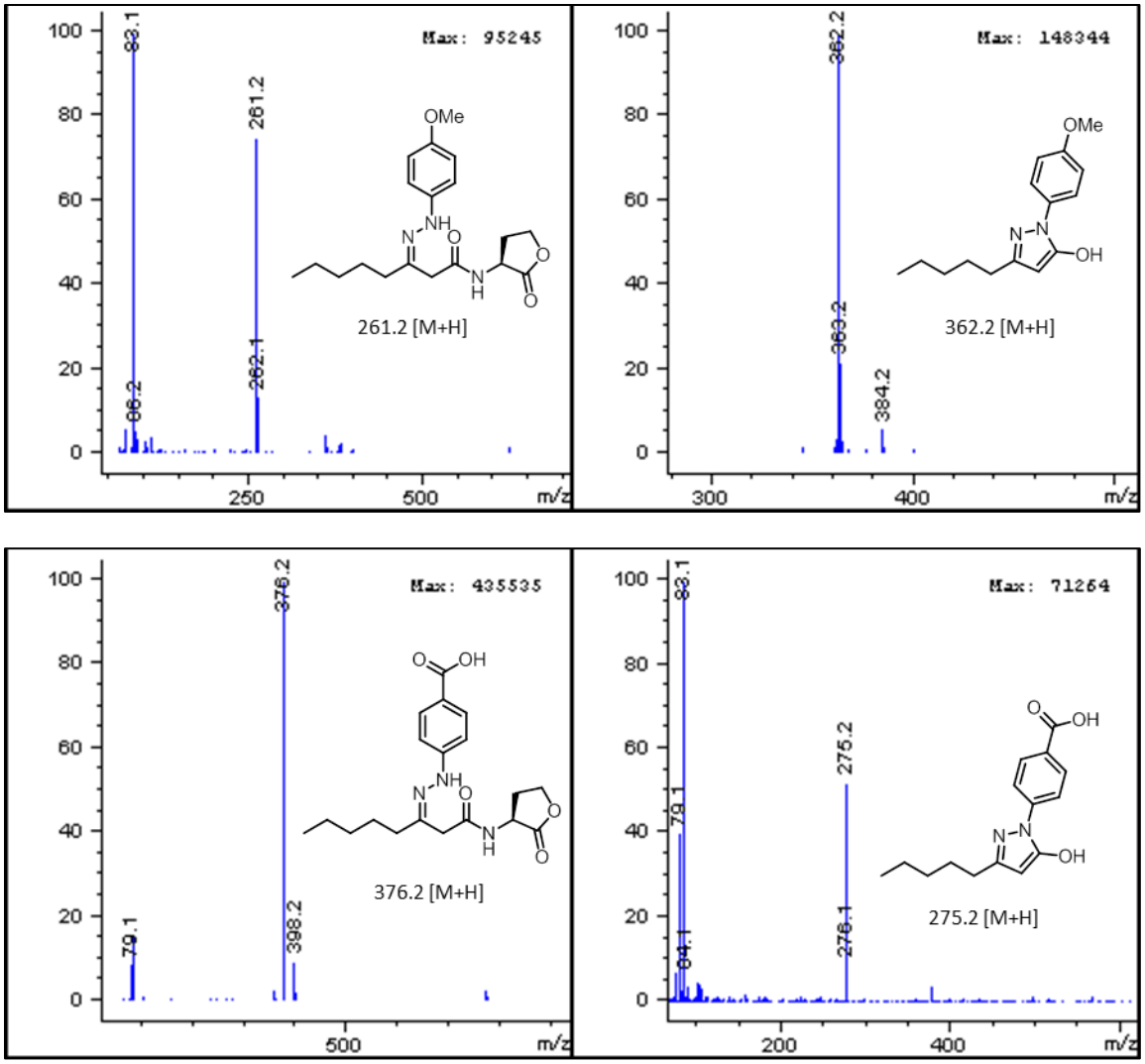








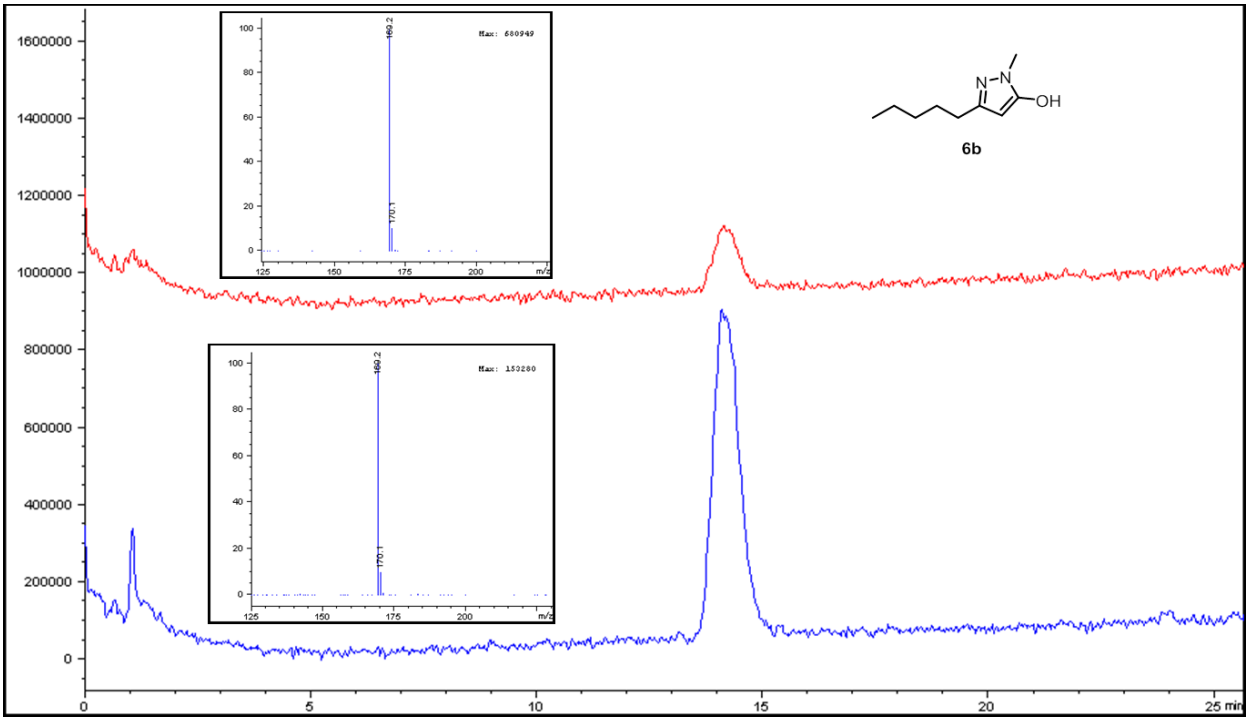


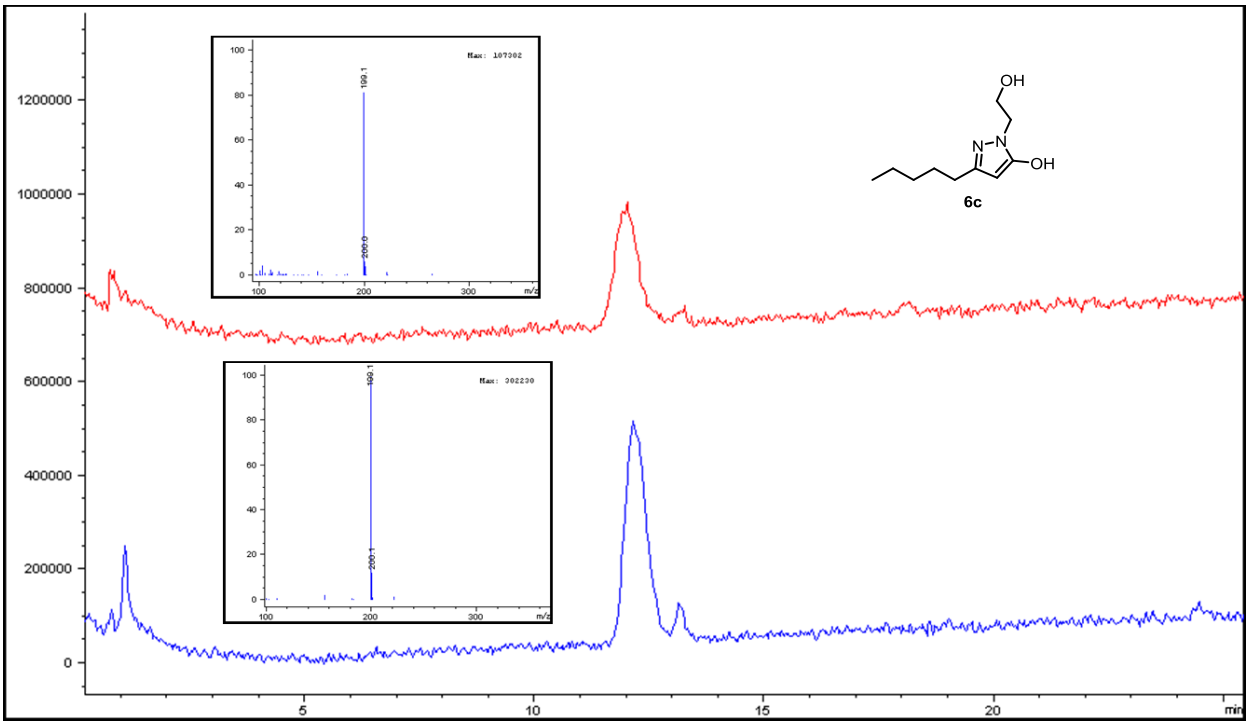


*HRMS data of the other detected hydroxypyrazoles is included in the spectral characterization data (*vide infra*).

Verification of Authentic Products by LC-MS

The top trace indicates product obtained by *General Procedure for Preparation of Hydroxypyrazole Products for Characterization* (see above) and the bottom trace is the authentic product.


1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
0
5
10
15
20
25 min
OH
N
N
OH
6b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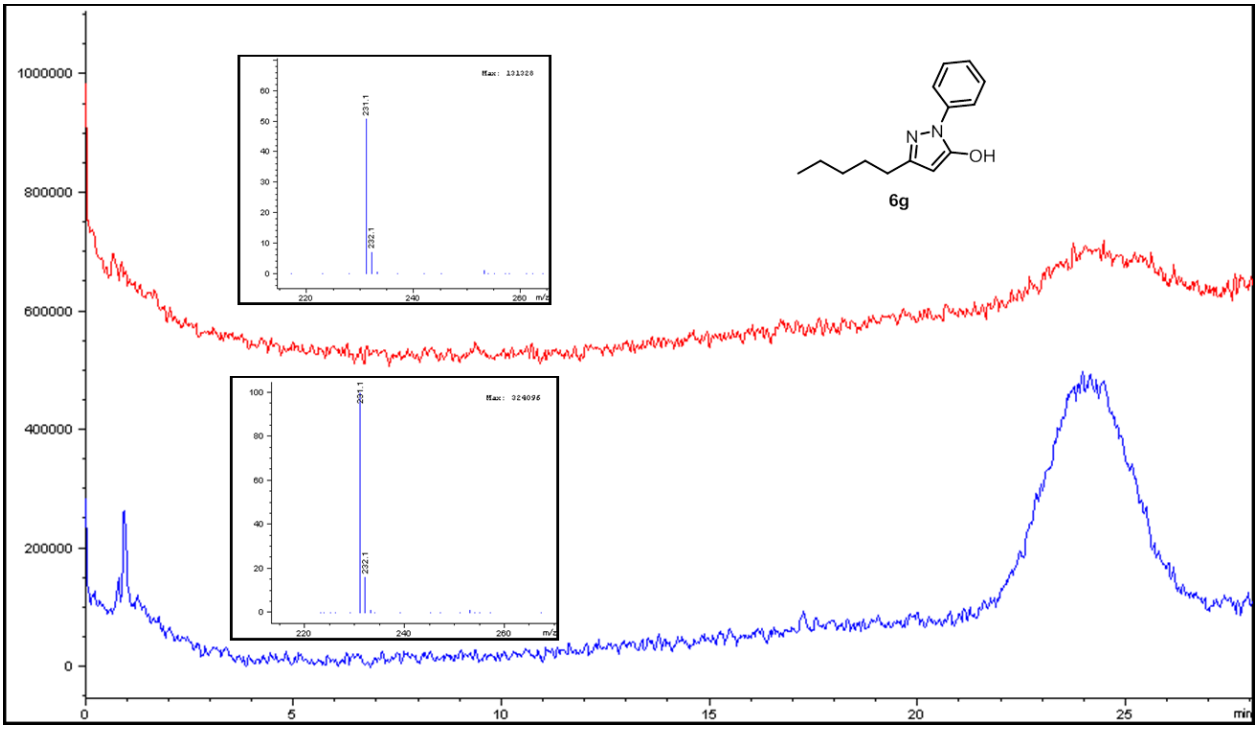
800000
600000
400000
200000
0
5
10
15
20
min
OH
N
N
OH
6c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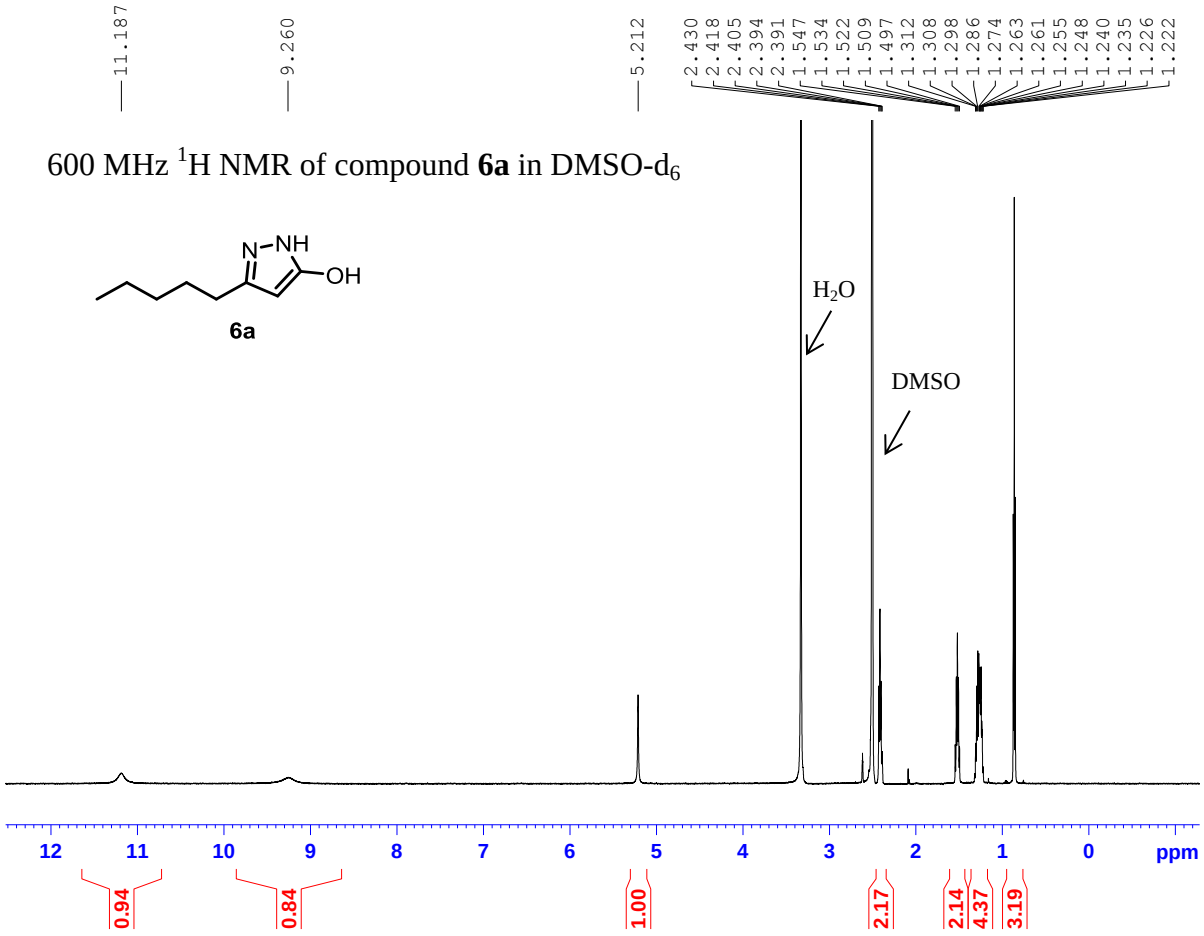
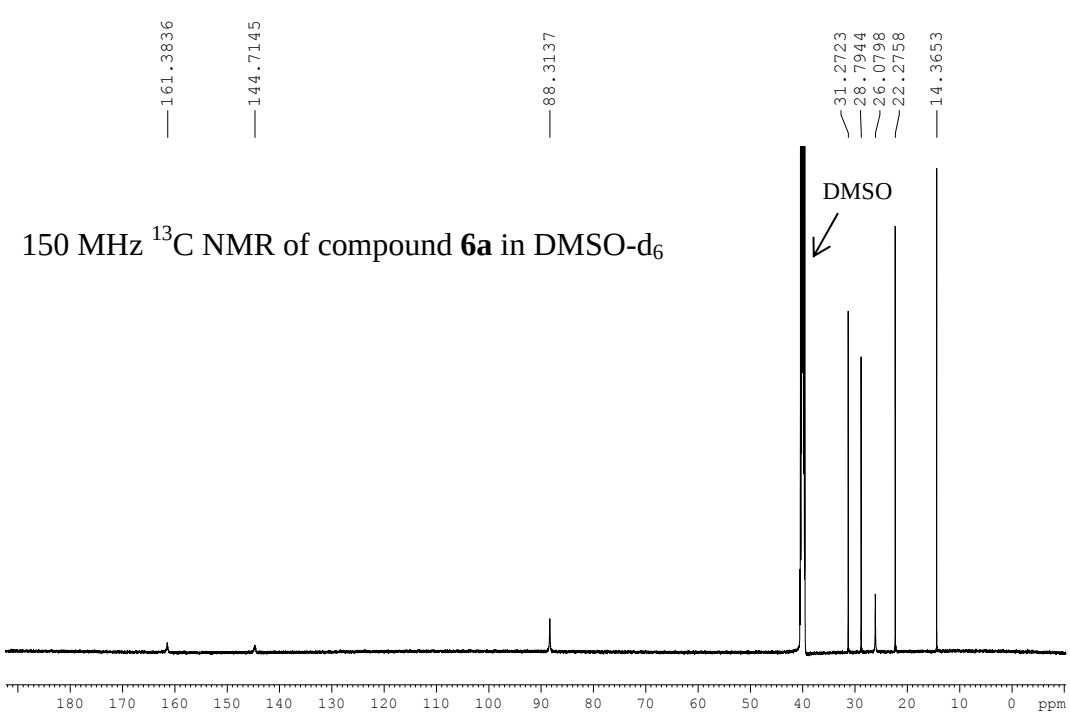
800000
600000
400000
200000
0
0
5
10
15
20
25
min
231.1
232.1
291.1
232.1
N
N
OH
6g

Spectral Data – ^{1}H & ^{13}C NMR Spectra



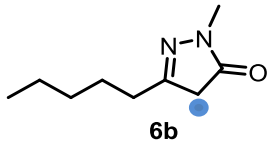
600 MHz ^{1}H NMR of compound **6a** in DMSO-d_6

6a



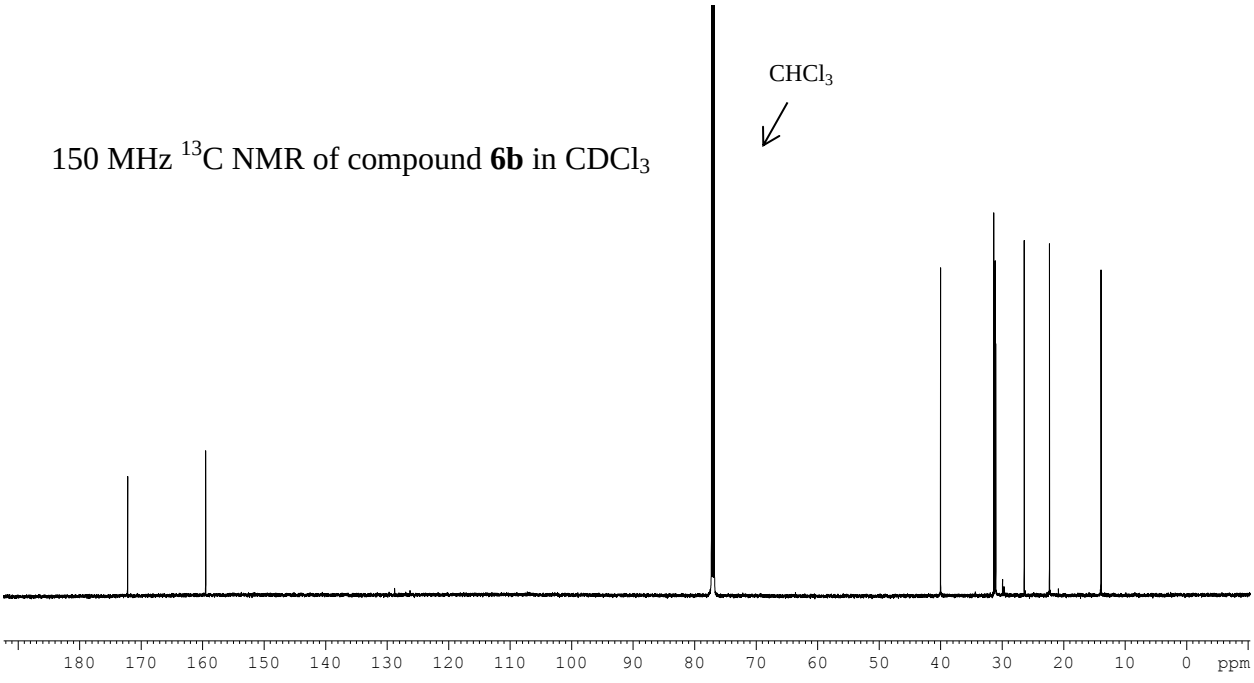
150 MHz ^{13}C NMR of compound **6a** in DMSO-d_6

600 MHz ^{1}H NMR of compound **6b** in $CDCl_3$

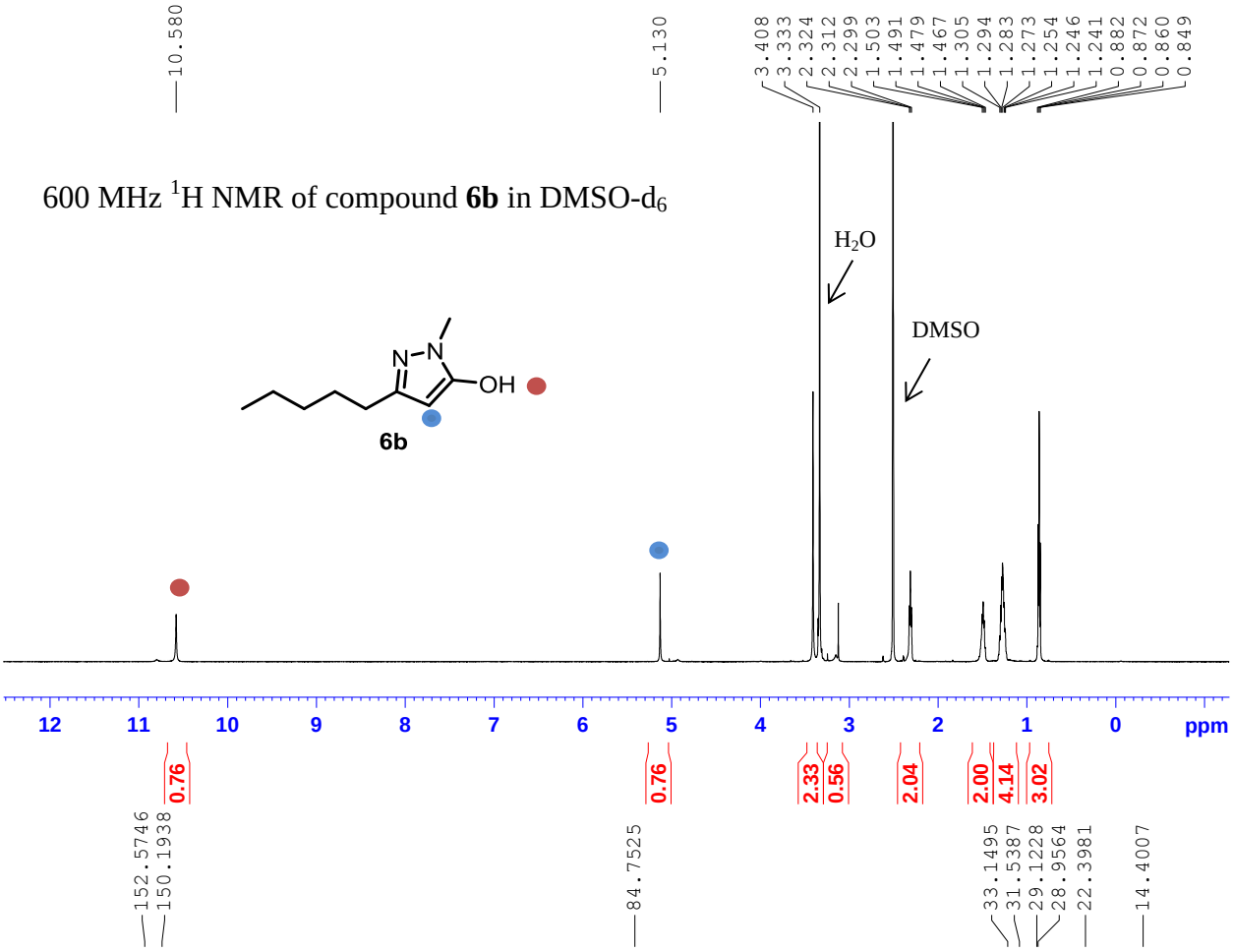


6b

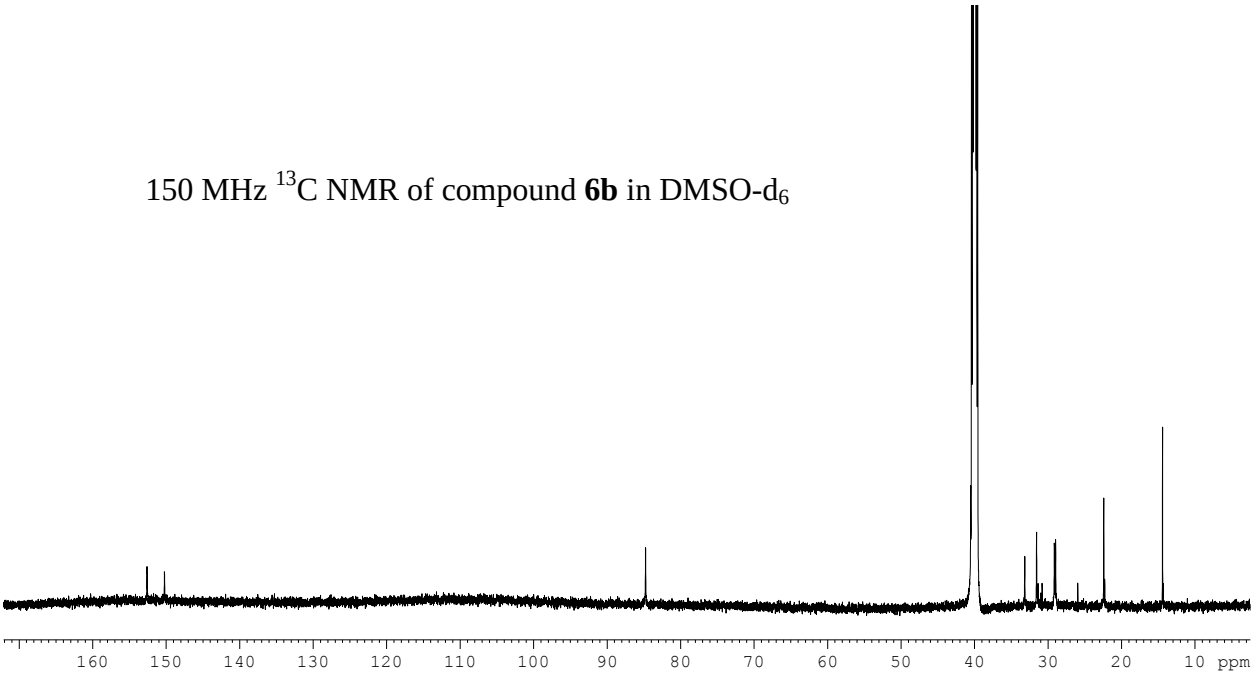
150 MHz ^{13}C NMR of compound **6b** in $CDCl_3$

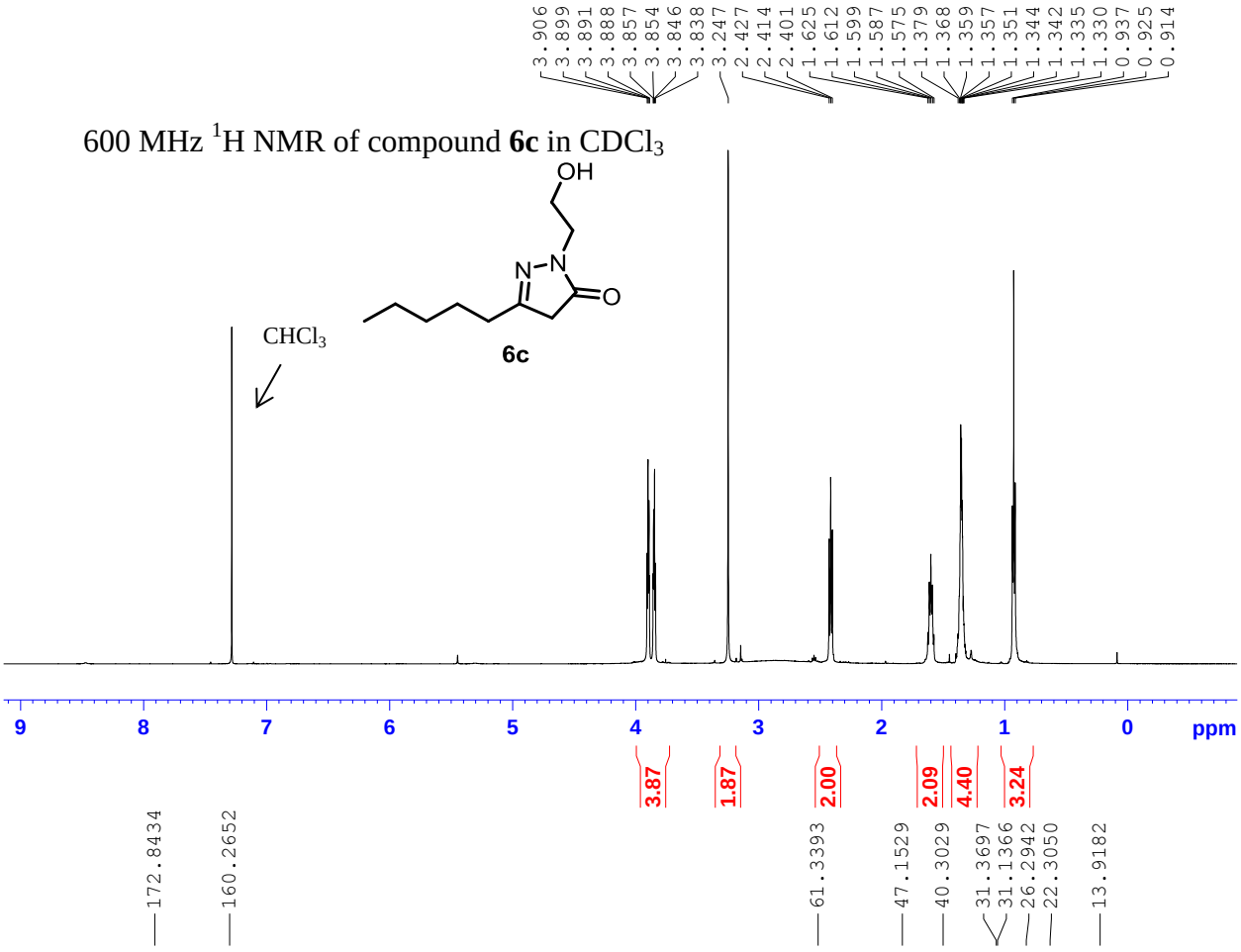


600 MHz ^{1}H NMR of compound **6b** in DMSO-d_6

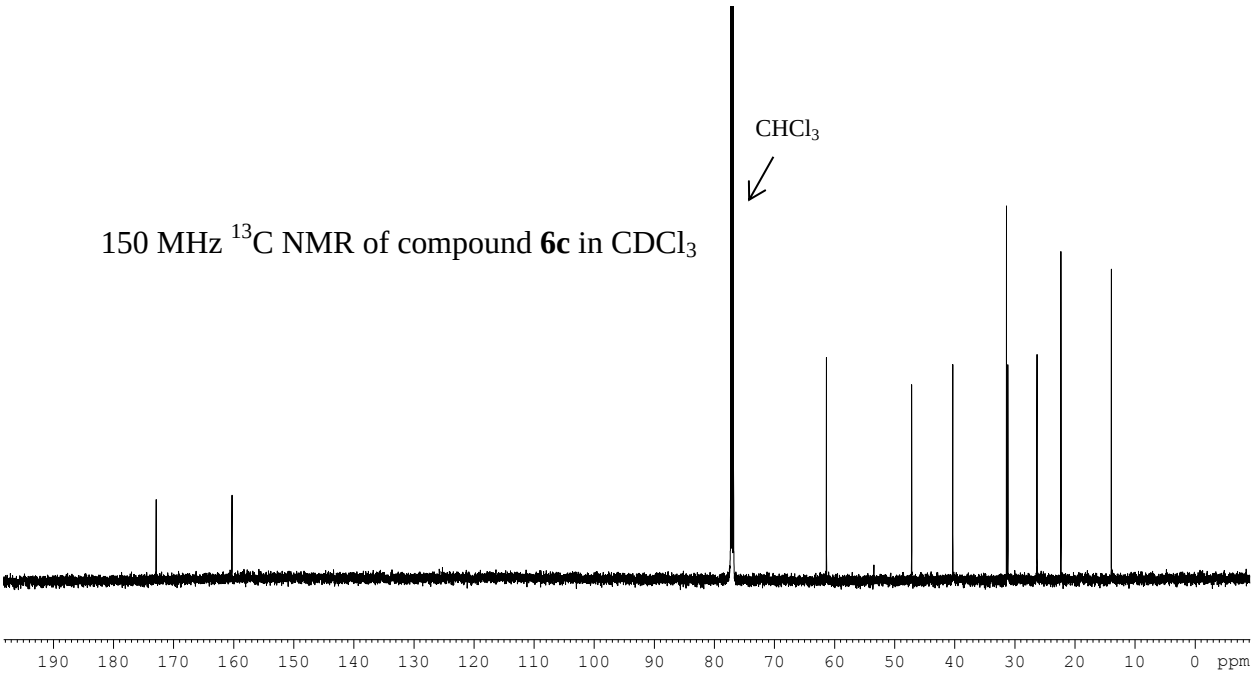


150 MHz ^{13}C NMR of compound **6b** in DMSO-d_6

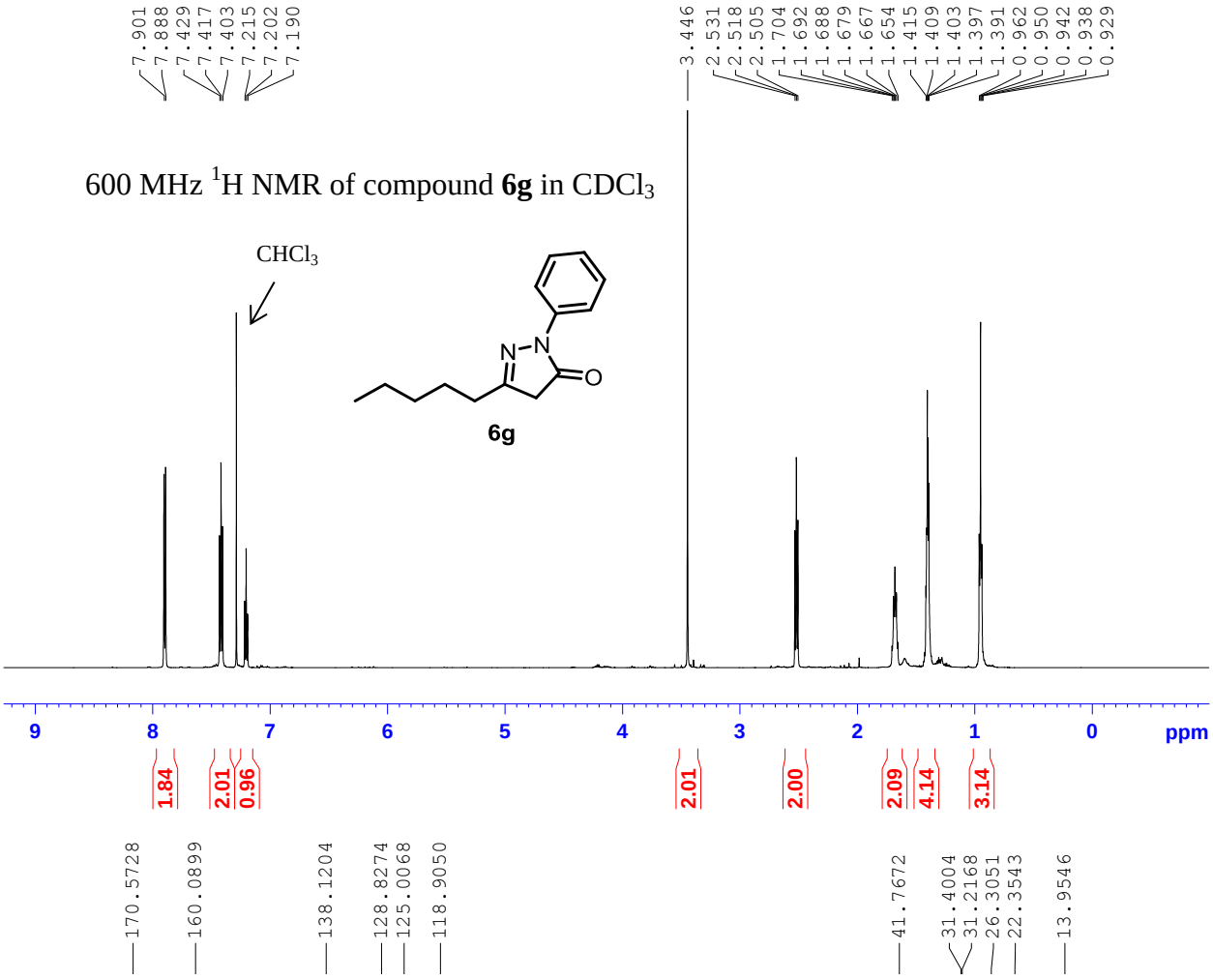




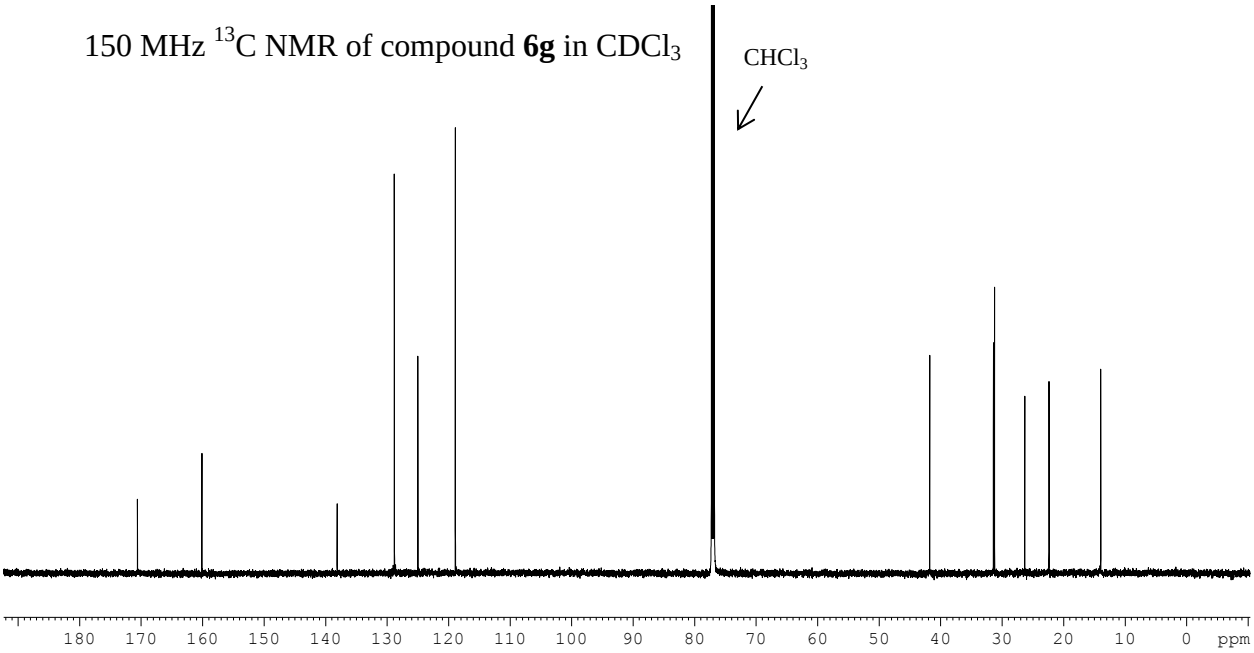
600 MHz ^{1}H NMR of compound **6c** in $CDCl_3$



150 MHz ^{13}C NMR of compound **6c** in $CDCl_3$



600 MHz ^{1}H NMR of compound **6g** in $CDCl_3$



150 MHz ^{13}C NMR of compound **6g** in $CDCl_3$