SUPPORTING INFORMATION

Importance of *ortho* Proton Donors in Catalysis of Hydrazone Formation

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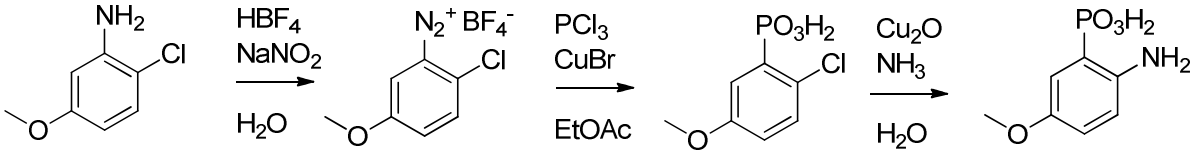
Experimental

Chemicals and Reagents. Chemicals were purchased from Sigma-Aldrich, TCI America, AK Scientific and Acros Organics and used without further purification. Solvents were purchased from Acros Organics and used as received. Compounds **1**, **2**, **5** and **7** are commercially available. Compounds **3**[1], **4**[2], **6**[3], **8**[4], **9**[5], **10**[6] and **12**[7] were prepared by published methods.

Instrumentation. All UV/Vis work was performed on a Cary 100 Bio UV-Visible Spectrophotometer. ^{1}H, ^{13}C and ^{31}P-NMR spectra were recorded on a Varian 400 MHz NMR spectrometer and internally referenced to the residual solvent signal; *J* values are reported in Hz. ESI MS data were measured by the Vincent Coates Foundation Mass Spectrometry Laboratory, Stanford University.

Preparation of 2-amino-5-methoxybenzenephosphonic acid (**11**).



(1) 2.5 mL of 2-chloro-5-methoxyaniline was dissolved in a solution of 9 mL water and 7 mL 48% aqueous HBF_4 and stirred at 0 °C. Dropwise, a solution of 1.45 g sodium nitrite in 3.6 mL water was added, providing a thick red suspension. The suspension was stirred at 0 °C for 30 min, then filtered and washed twice with aqueous 5% HBF_4, twice with methanol and four times with diethyl ether. The filtered diazonium material was dried overnight under vacuum.

(2) 4.71 g of the crude diazonium tetrafluoroborate salt was suspended in 23 mL ethyl acetate and stirred at room temperature. 1.8 mL phosphorous trichloride was added followed by 0.395 g CuBr. The resulting suspension was stirred at room temperature for one hour, then heated to 50 °C until gas evolution was completed (approximately two hours). 5 mL water was slowly added to quench the reaction, then the reaction was further diluted with water and concentrated *in vacuo*. The crude material was reconcentrated *in vacuo* twice from 30 mL water and resuspended in 20 mL boiling water, then filtered and washed with boiling water. The filtrate was then concentrated to approximately 5 mL and allowed to cool, then filtered and washed with water to provide crude 2-chloro-5-methoxybenzenephosphonic acid. The crude product was dissolved in approximately 6 mL of 20% aqueous sodium hydroxide and filtered to remove precipitated copper hydroxide. The filtrate was stirred with decolorizing carbon for ten minutes, filtered and washed with water. The filtrate was acidified with concentrated hydrochloric acid to afford a white solid, which was filtered and washed with water to provide 1.81 g of pure 2-chloro-5-methoxybenzenephosphonic acid.

ESI MS (Calc M – H = 220.98): 221.17, 223.33

^{1}H NMR (D_2O/NaOD, 400 MHz): 3.62 (s, 3H), 6.71 (d, 1H, J = 8.8 Hz), 7.11 (m, 1H), 7.23 (d, 1H, J = 13.2 Hz)

^{13}C NMR (DMSO-d_6, 100 MHz): 55.6, 117.8, 119.4, 126.3, 131.4, 132.7, 134.5, 157.4 (some splitting by phosphonic acid observed)

^{31}P NMR (D_2O/NaOD, 170 MHz): 7.20

Melting Point: 190 - 191°C

(3) 1.68 g of 2-chloro-5-methoxybenzenephosphonic acid was added to a mixture of 30 mL concentrated aqueous ammonia and 1.35 g Cu_2O. The resulting solution was stirred at 80 – 90 °C for 21 hours, then cooled to room temperature and adjusted to pH 4 with hydrochloric acid to afford a brown precipitate, which was filtered and washed with water. The crude material was dissolved in 4 M hydrochloric acid, and excess sodium sulfide nonahydrate was added to precipitate copper sulfide. The suspension was filtered and washed with 4 M hydrochloric acid and the filtrate stirred with decolorizing carbon for ten minutes, then again filtered. The filtrate was adjusted to pH 4 by the addition of sodium carbonate and a small amount of EDTA to eliminate residual copper. The formed yellow-green precipitate was filtered, washed with water and dried overnight to afford 2-amino-5-methoxybenzenephosphonic acid.

ESI MS (Calc M + H = 204.04): 204.56

^{1}H NMR (D_2O/NaOD, 400 MHz): 3.21 (s, 3H), 6.22 (m, 1H), 6.31 (d, 1H, J = 8 Hz), 6.62 (d, 1H, J = 13.6 Hz)

^{13}C NMR: Could not be obtained (poor solubility in common NMR solvents)

^{31}P NMR (D_2O/NaOD, 170 MHz): 10.33

Melting Point: 186 - 194°C (Decomposes)

Preparation of 5-(2-amino-5-methoxyphenyl)tetrazole (**13**). 1.36 g of 2-amino-5-methoxybenzonitrile[8] was dissolved in 10 mL toluene. 1.64 g (1.3 eq) of triethylamine hydrochloride was added followed by 0.776 g (1.3 eq) sodium azide. The resulting suspension was heated at 90 – 100 °C for 23 hours to give a biphasic mixture. After cooling, the mixture was extracted with 20 mL of water. The aqueous layer was acidified with 0.94 mL concentrated hydrochloric acid to afford a gray precipitate, which was filtered, washed with water, and dried to afford 1.43 g of the desired product.

ESI MS (Calc M – H = 190.07): 190.27

^{1}H NMR (DMSO-d_6, 400 MHz): 3.71 (s, 3H), 6.83 – 6.92 (m, 2H), 7.32 (d, 1H, J = 3.6 Hz), 9.8 (br s, 2H).

^{13}C NMR (DMSO-d_6, 100 MHz): 55.6, 104.6, 111.2, 118.1, 120.1, 141.7, 150.1, 154.9.

Melting Point: 154 - 157°C

General hydrazone reaction screening procedure.[9] 590 μL of 10:1 PBS (pH 7.4):DMF was prepared in a UV-Vis cuvette. 1.2 μL of a 500 mM solution of catalyst in DMF was added (final concentration 1 mM) and the baseline was collected. For catalysts **2, 4** and **12**, 60 μL of a 10 mM stock solution in 100 mM sodium phosphates (pH 7.4) was added to 531 μL 10:1 PBS (pH 7.4:DMF) and the baseline collected. 1.5 μL of a concentrated solution of NBD Hydrazine was added (final concentration 18 μM) and the solution well mixed by pipetting. The reaction was initiated by the addition of 7.6 μL of a concentrated solution of 4-nitrobenzaldehyde (final concentration 1 mM) and the absorbance at 504 nm was monitored for two hours. The absorbance data was converted to concentration (in μM) of hydrazone product by dividing by the extinction coefficient of the hydrazone product (14,100 L mol^{-1} cm^{-1}, path length 1 cm) and yields were determined by dividing the concentration at the given time point by 18 μM (starting concentration). The yield was assumed to be zero at the starting point. All experiments were performed in triplicate and averaged. For reactions with different carbonyl substrates, the same procedure was used and only the carbonyl substrate was varied. These reactions were monitored at the given wavelength for each substrate noted in the figures below.

Reactions with varied concentrations of hydrazine. For reactions with different amount of hydrazine compound, the same conditions as those in the general section were employed, with hydrazine concentration varied at 11 μM, 14 μM, 24 μM, or 36 μM.

Equations used for fitting kinetic data[9]

Determining pseudo-second-order rate constants. Data was converted appropriately and fit in Microsoft Excel 2007 to the linear solution (y = mx + b) for a second-order reaction (equation 2)

$$\ln\frac{[\mathbf{1}]}{[4NBA]} = kt([\mathbf{1}]_o - [4NBA]_o) + \ln\frac{[\mathbf{1}]_o}{[4NBA]_o} \quad (1)$$

Where **1** is NBD Hydrazine and 4NBA is 4-nitrobenzaldehyde.
This fit assumes that there is a low concentration of the intermediate imine present and that the reaction goes to completion with the large excess of 4-nitrobenzaldehyde.

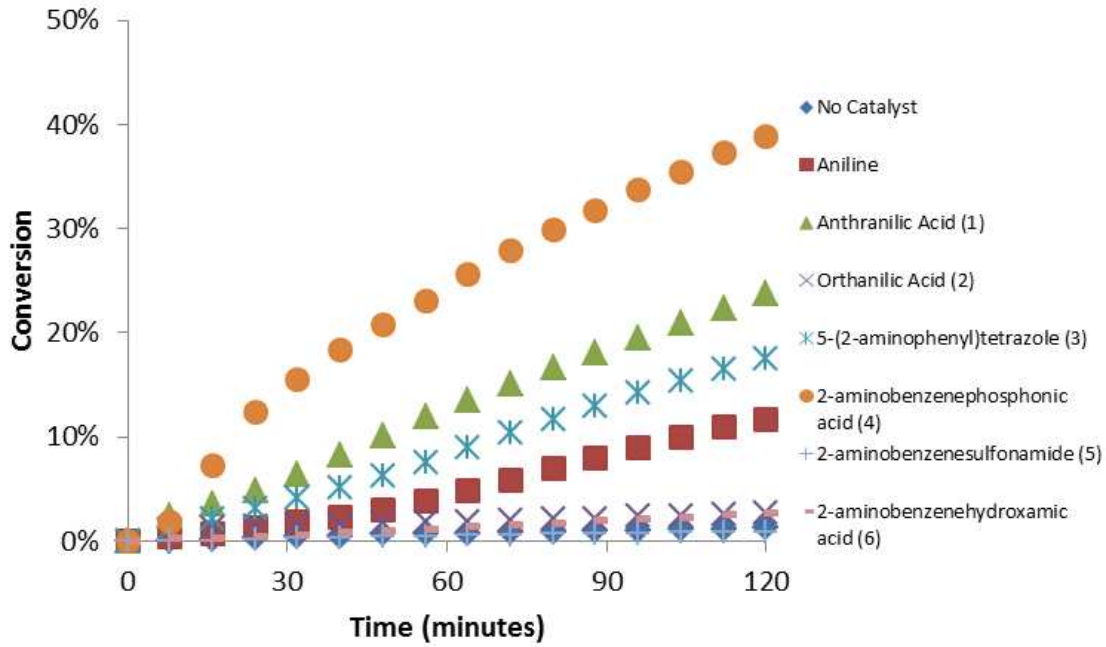


Figure S1: Hydrazone formation between 18 µM NBD Hydrazine and 1 mM 4-nitrobenzaldehyde catalyzed by 1 mM various acid catalysts in 10:1 PBS (pH 7.4):DMF. The reaction was monitored at 504 nm.

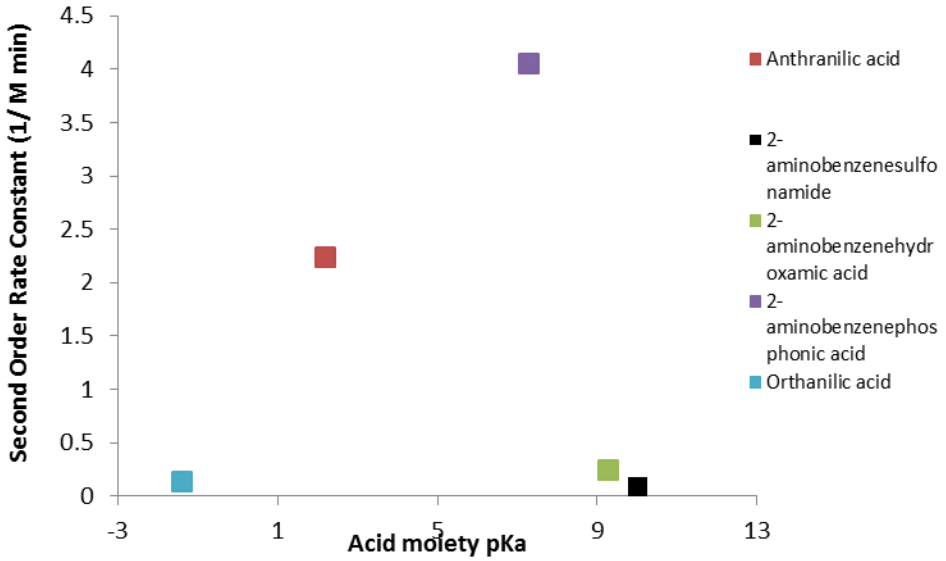


Figure S2: Dependence of 2nd order rate constant for hydrazone formation on pK_a of *ortho* acid moiety

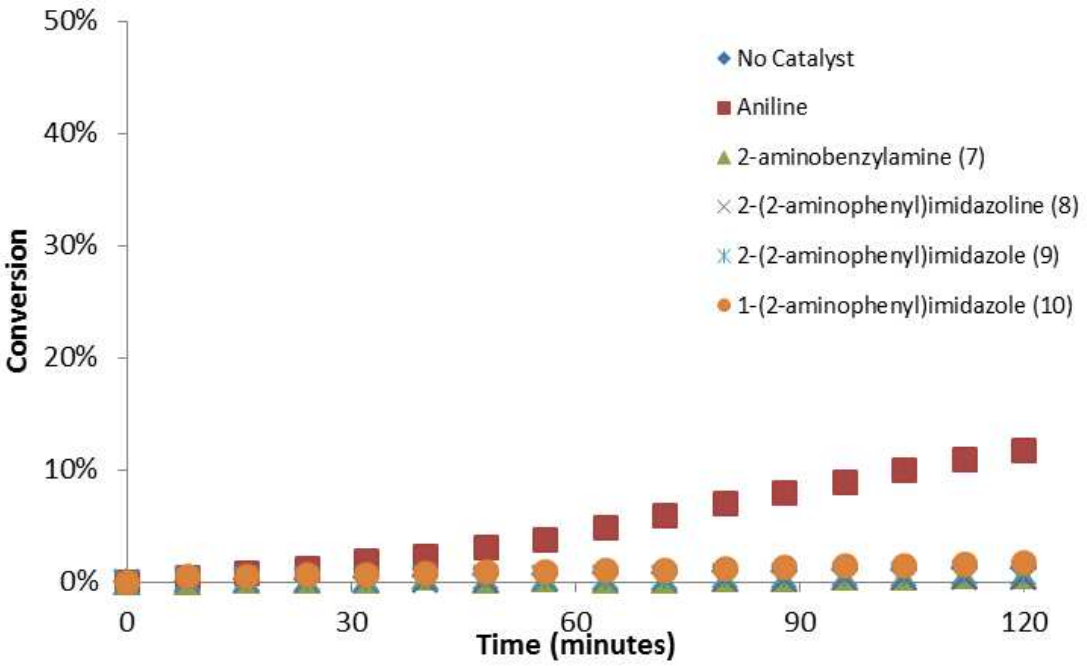


Figure S3: Hydrazone formation between 18 μM NBD Hydrazine and 1 mM 4-nitrobenzaldehyde catalyzed by 1 mM various conjugate acid catalysts in 10:1 PBS (pH 7.4):DMF. The reaction was monitored at 504 nm.

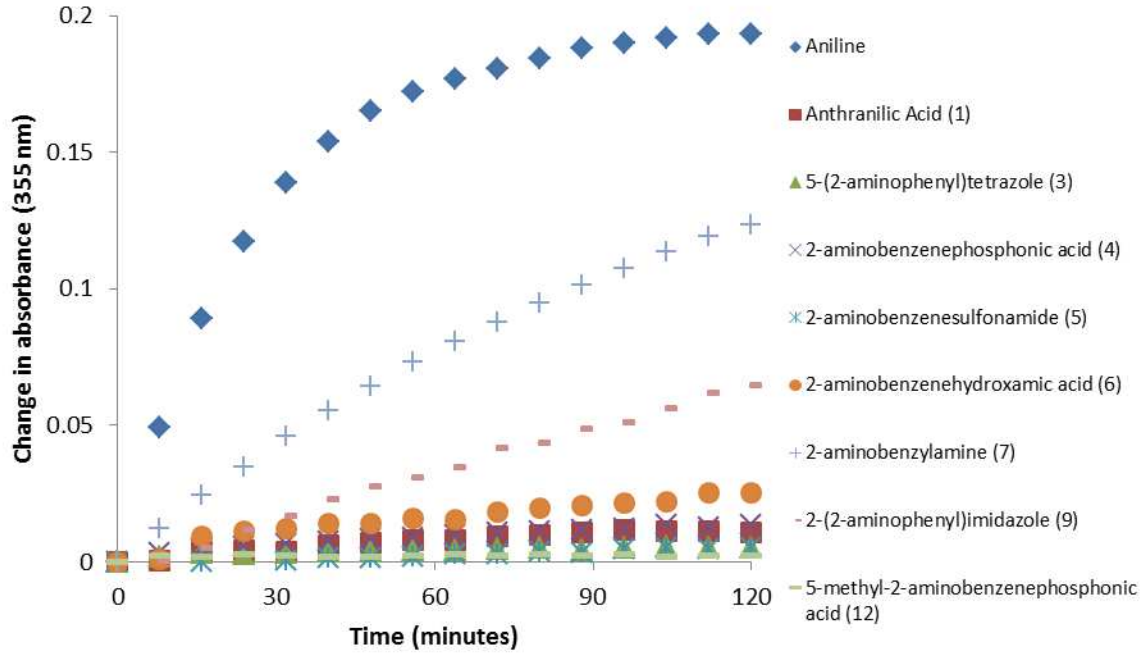


Figure S4: Monitoring imine formation (355 nm) between 1 mM 4-nitrobenzaldehyde and 1 mM catalyst in 10:1 PBS (pH 7.4):DMF

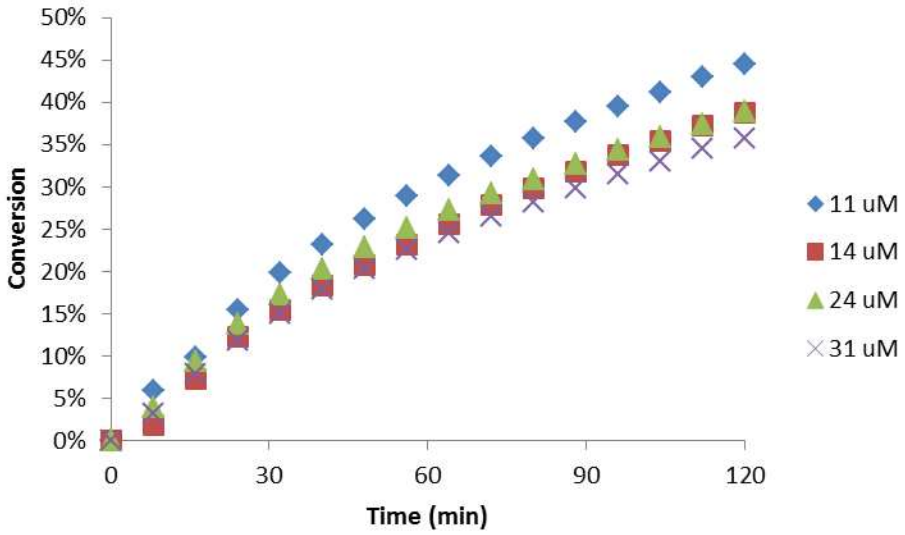


Figure S5: Reaction of varied concentration of NBD Hydrazine with 1 mM 4-nitrobenzaldehyde and 1 mM 2-aminobenzenephosphonic acid (**5**) in 10:1 PBS (pH 7.4):DMF. Reaction conversion was monitored at 504 nm.

Table S1: 2nd order rate constants (M^{-1} min^{-1}) for hydrazone formation with different concentrations of NBD hydrazine.[1]

Concentration (μM)	Conversion	2nd order rate constant
11 ± 2	44 ± 1%	4.8 ± 0.2
13.8 ± 0.2	39 ± 1%	4.1 ± 0.2
24 ± 1	39 ± 2%	4.0 ± 0.3
31 ± 2	36 ± 1%	3.7 ± 0.1

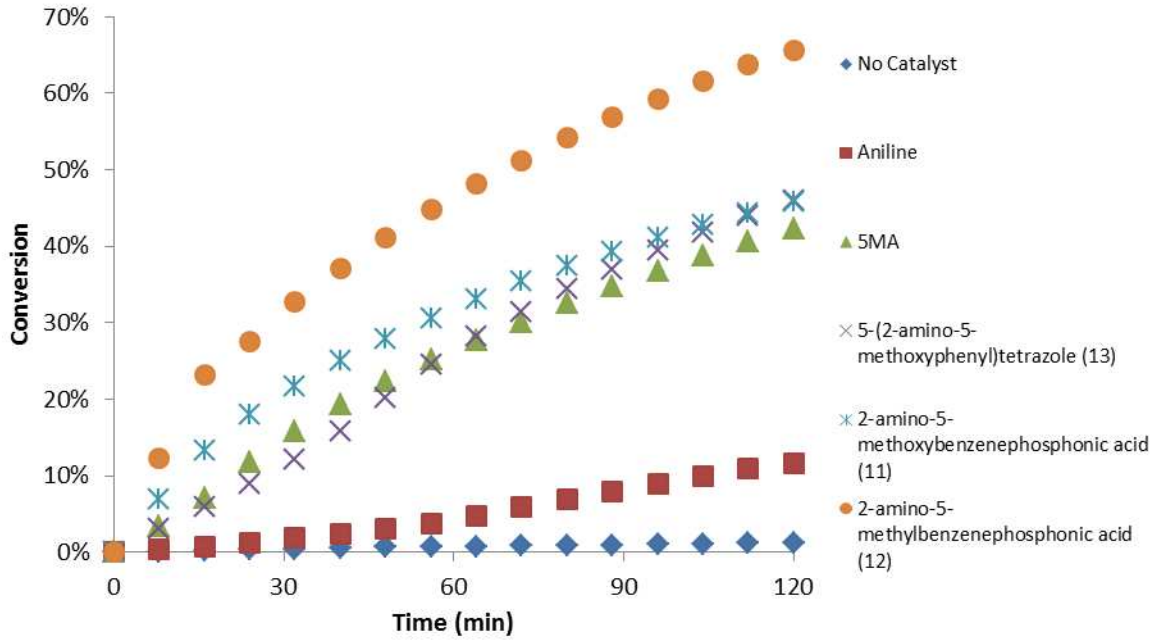


Figure S6: Hydrazone formation between 18 μM NBD Hydrazine and 1 mM 4-nitrobenzaldehyde in 10:1 PBS (pH 7.4):DMF containing 1 mM of different catalyst. Reaction conversion was monitored at 504 nm.

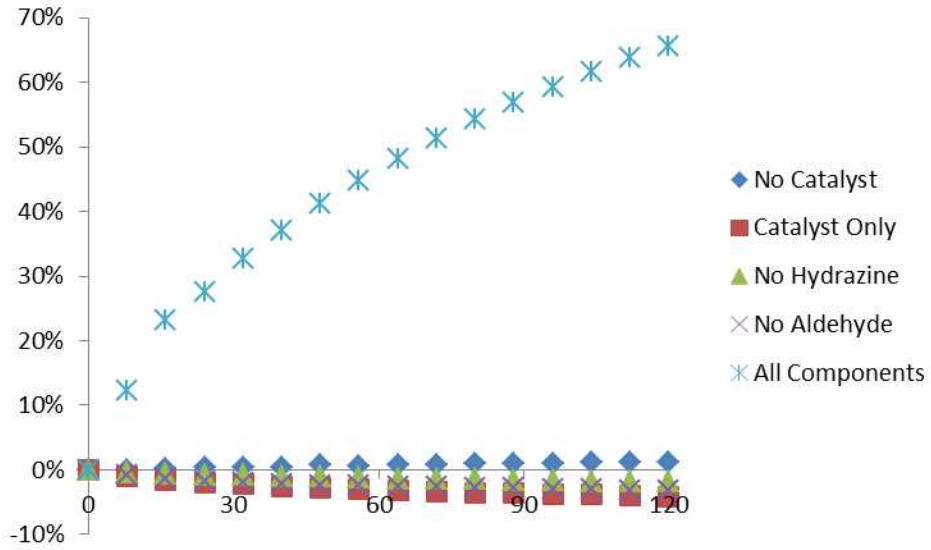


Figure S7: Hydrazone formation catalyzed by 2-amino-5-methylbenzenephosphonic acid requires all components for the reaction to provide a signal at 504 nm.

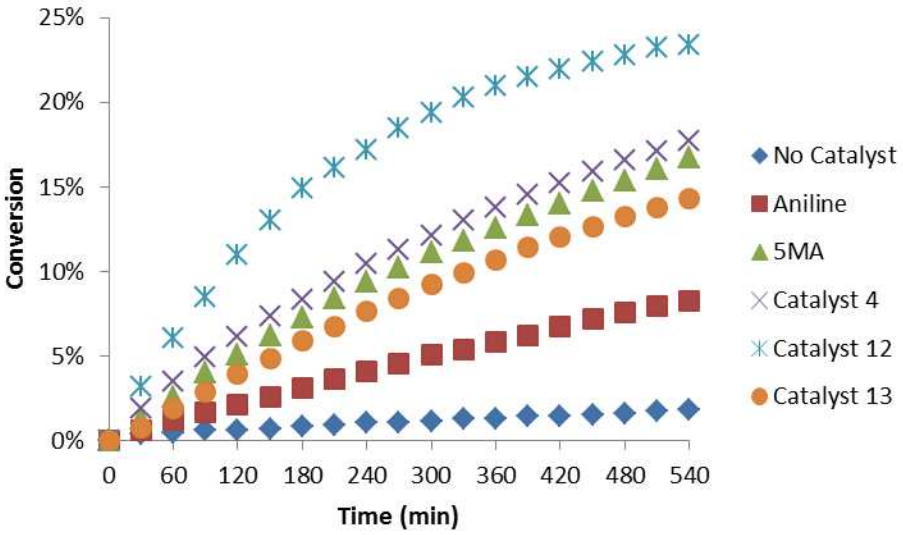


Figure S8: Reaction of 18 μM NBD Hydrazine with 1 mM benzaldehyde in the presence of 1 mM of different catalysts in 10:1 PBS (pH 7.4):DMF. Reaction conversion monitored at 470 nm.

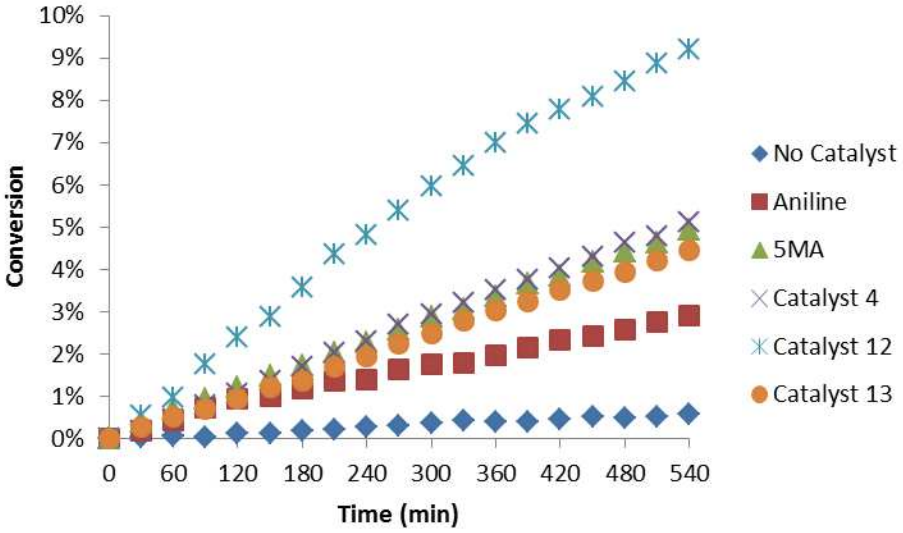


Figure S9: Reaction of 18 μM NBD Hydrazine with 1 mM *p*-anisaldehyde in the presence of 1 mM of different catalysts in 10:1 PBS (pH 7.4):DMF. Reaction conversion monitored at 480 nm.

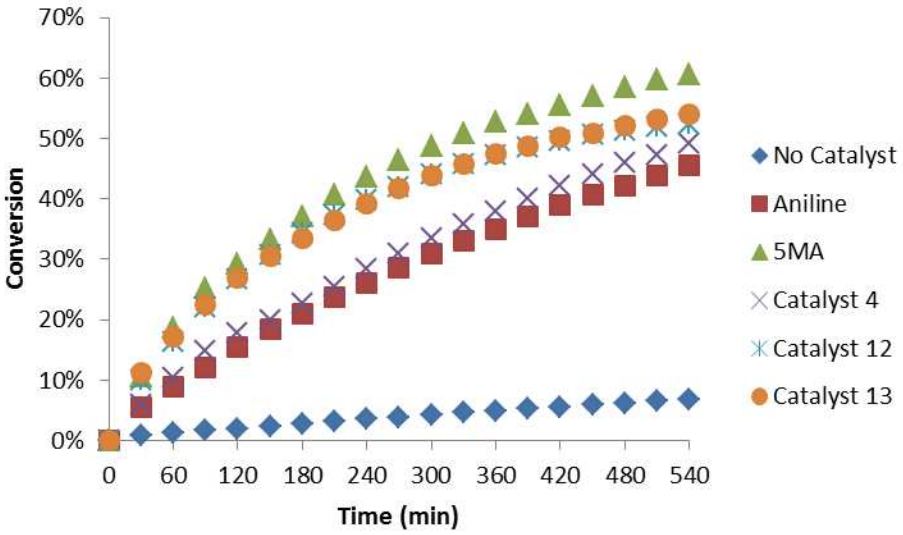


Figure S10: Reaction of 18 μM NBD Hydrazine with 1 mM 4-carboxybenzaldehyde in the presence of 1 mM of different catalysts in 10:1 PBS (pH 7.4):DMF. Reaction conversion monitored at 480 nm.

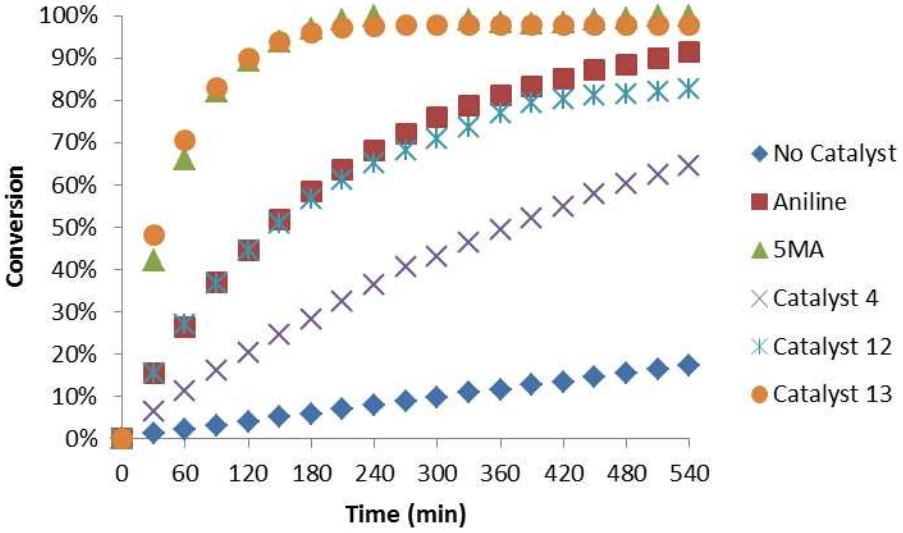


Figure S11: Reaction of 18 μM NBD Hydrazine with 1 mM 2-carboxybenzaldehyde in the presence of 1 mM of different catalysts in 10:1 PBS (pH 7.4):DMF. Reaction conversion monitored at 470 nm.

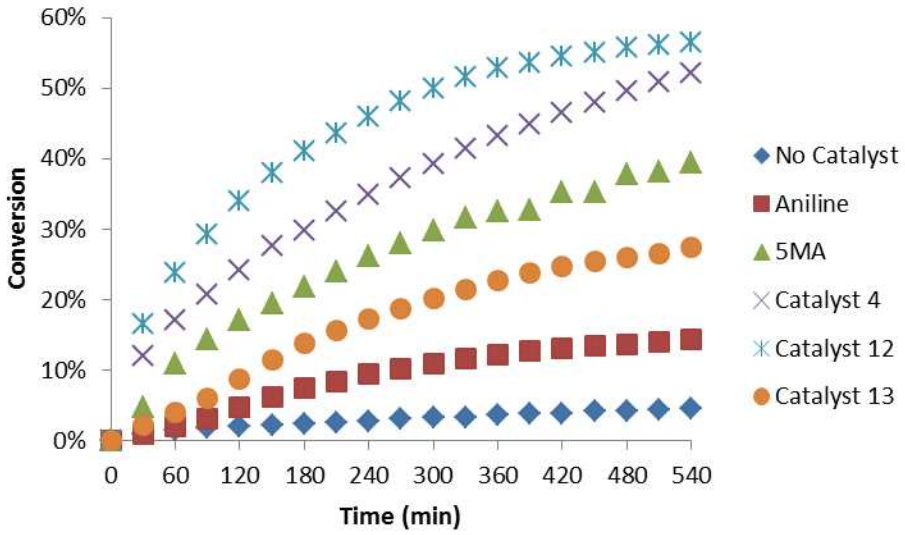


Figure S12: Reaction of 18 μM NBD Hydrazine with 1 mM 4-chlorobenzaldehyde in the presence of 1 mM of different catalysts in 10:1 PBS (pH 7.4):DMF. Reaction conversion monitored at 475 nm.

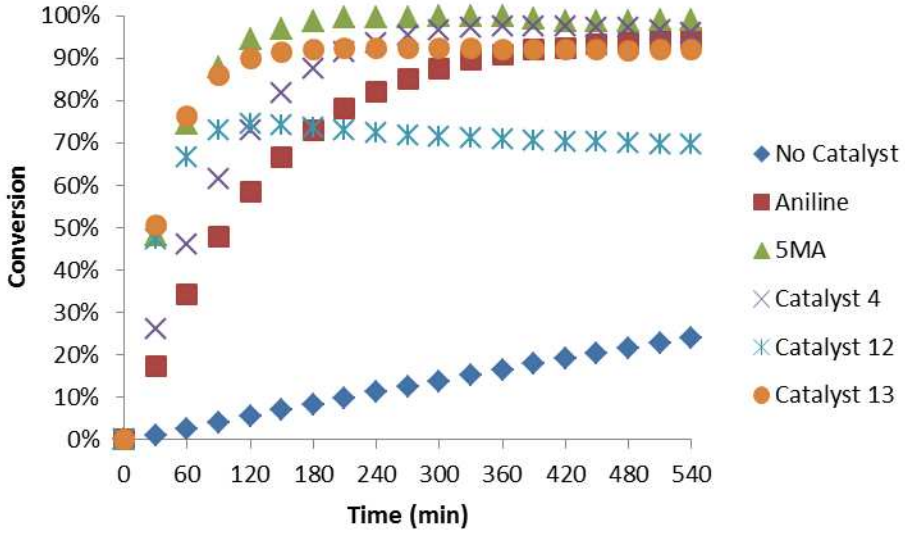


Figure S13: Reaction of 18 μM NBD Hydrazine with 1 mM 2-formylpyridine in the presence of 1 mM of different catalysts in 10:1 PBS (pH 7.4):DMF. Reaction conversion monitored at 490 nm.

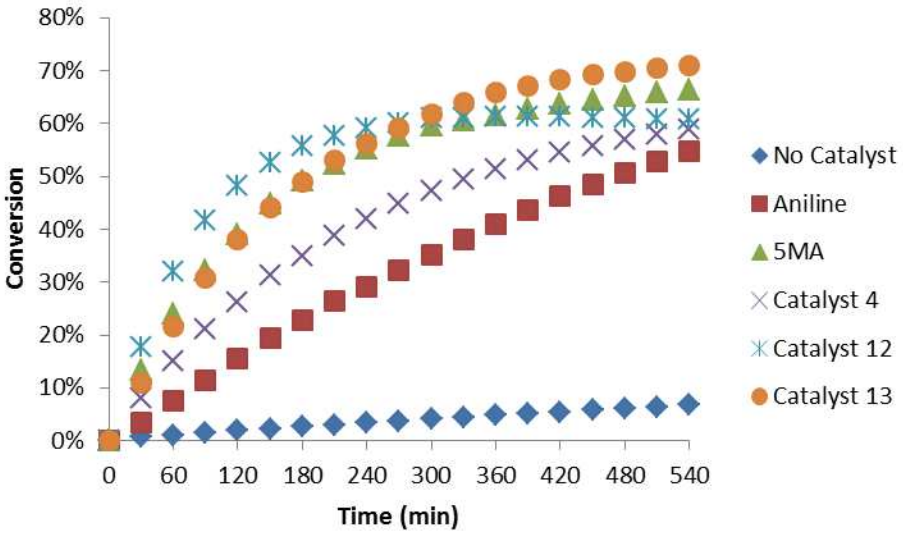


Figure S14: Reaction of 18 μM NBD Hydrazine with 1 mM 4-formylpyridine in the presence of 1 mM of different catalysts in 10:1 PBS (pH 7.4):DMF. Reaction conversion monitored at 490 nm.

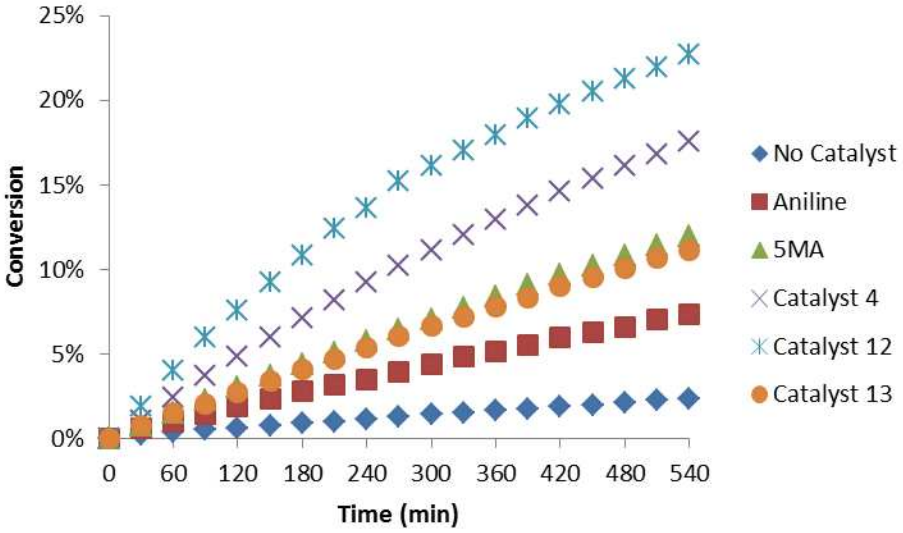


Figure S15: Reaction of 18 μM NBD Hydrazine with 1 mM cinnamaldehyde in the presence of 1 mM of different catalysts in 10:1 PBS (pH 7.4):DMF. Reaction conversion monitored at 490 nm.

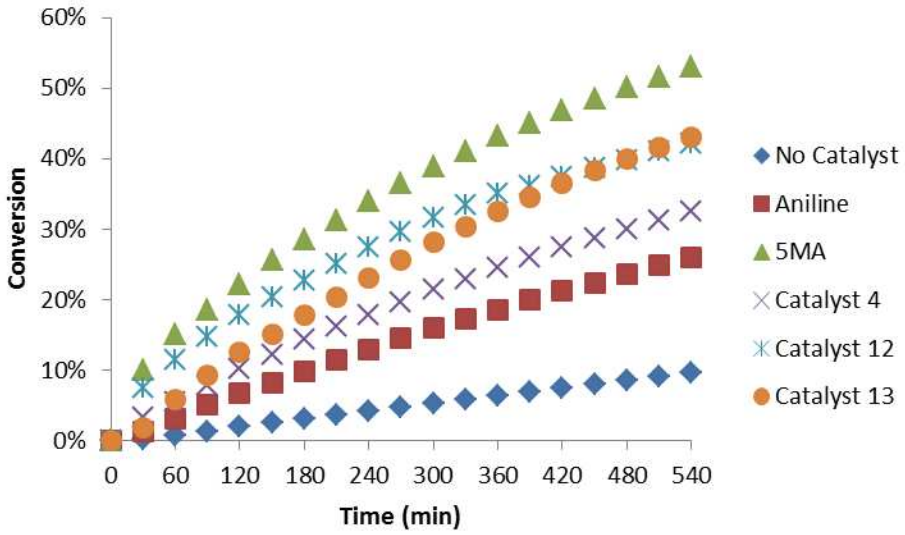


Figure S16: Reaction of 18 µM NBD Hydrazine with 1 mM salicylaldehyde in the presence of 1 mM of different catalysts in 10:1 PBS (pH 7.4):DMF. Reaction conversion monitored at 490 nm.

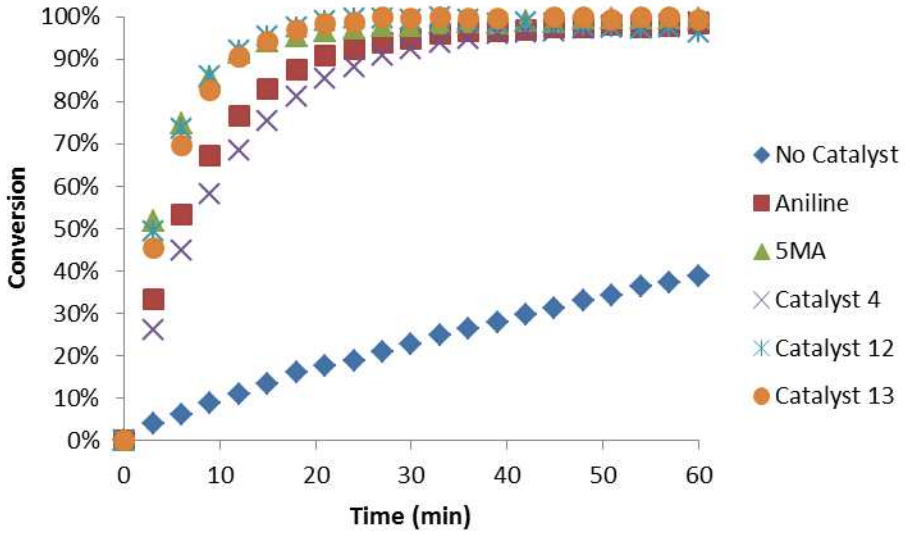


Figure S17: Reaction of 18 µM NBD Hydrazine with 1 mM butyraldehyde in the presence of 1 mM of different catalysts in 10:1 PBS (pH 7.4):DMF. Reaction conversion monitored at 450 nm.

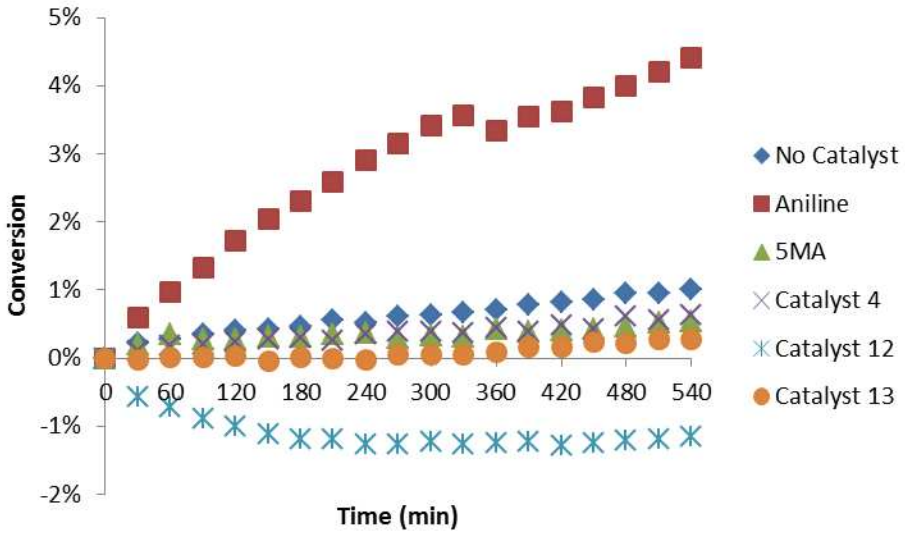
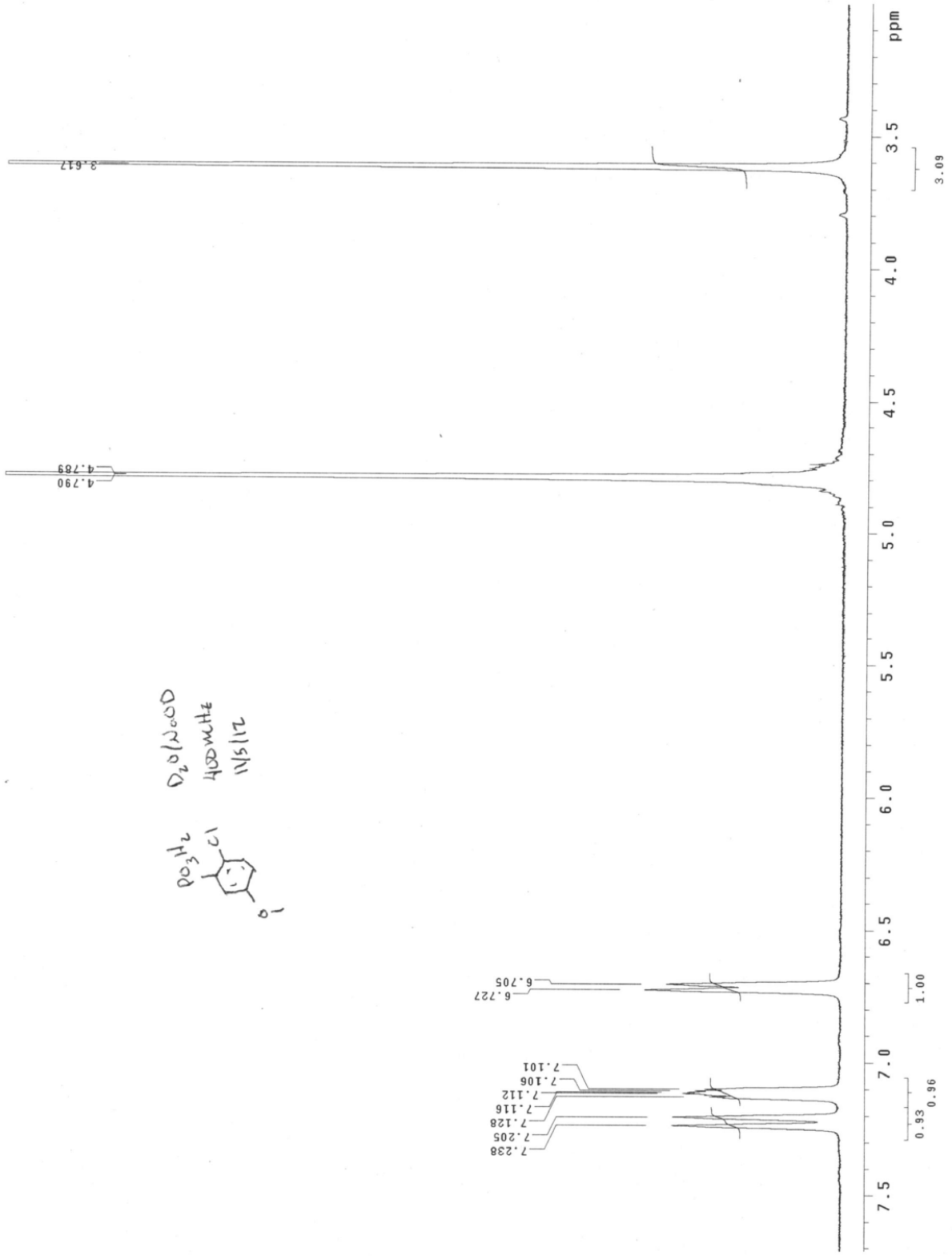
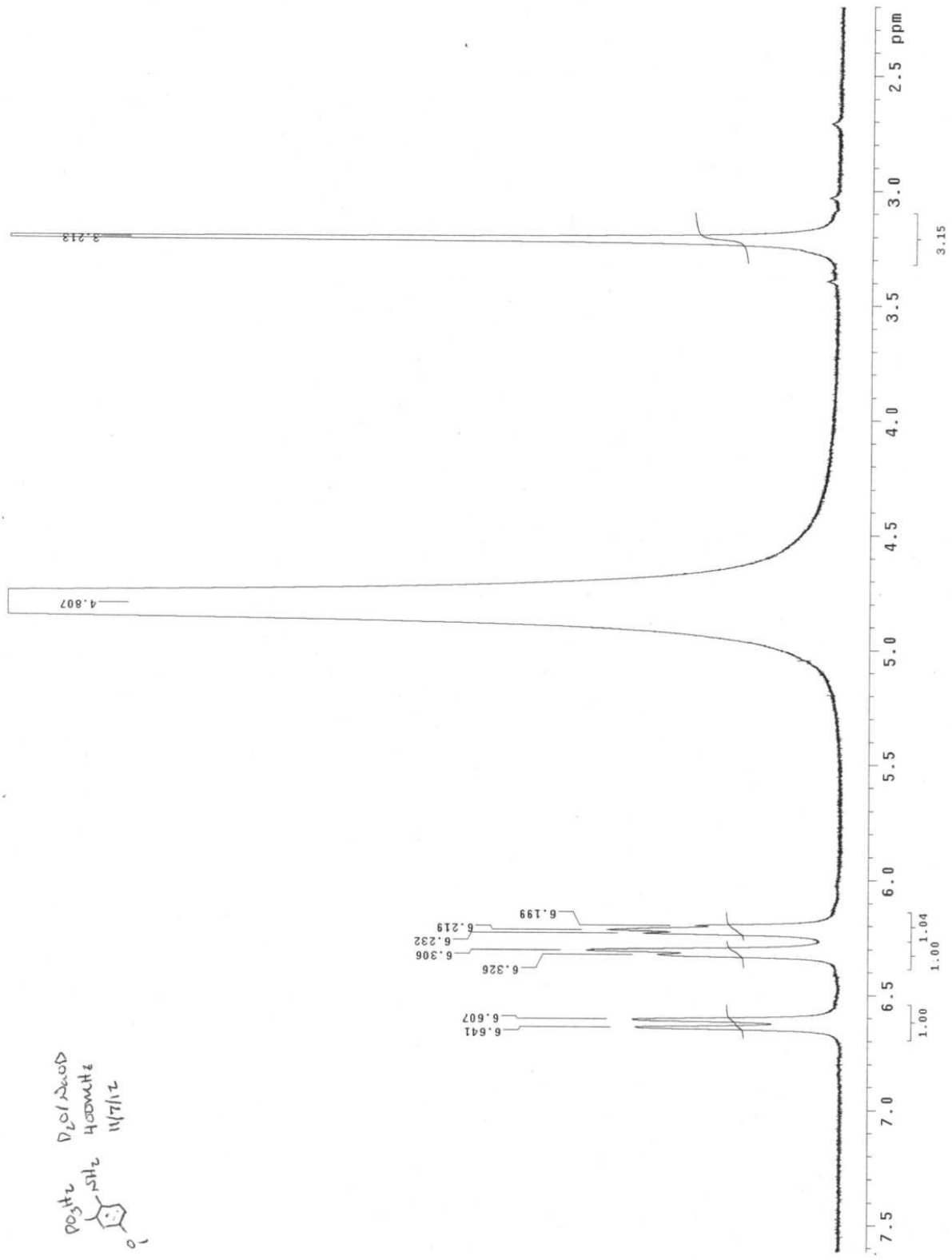


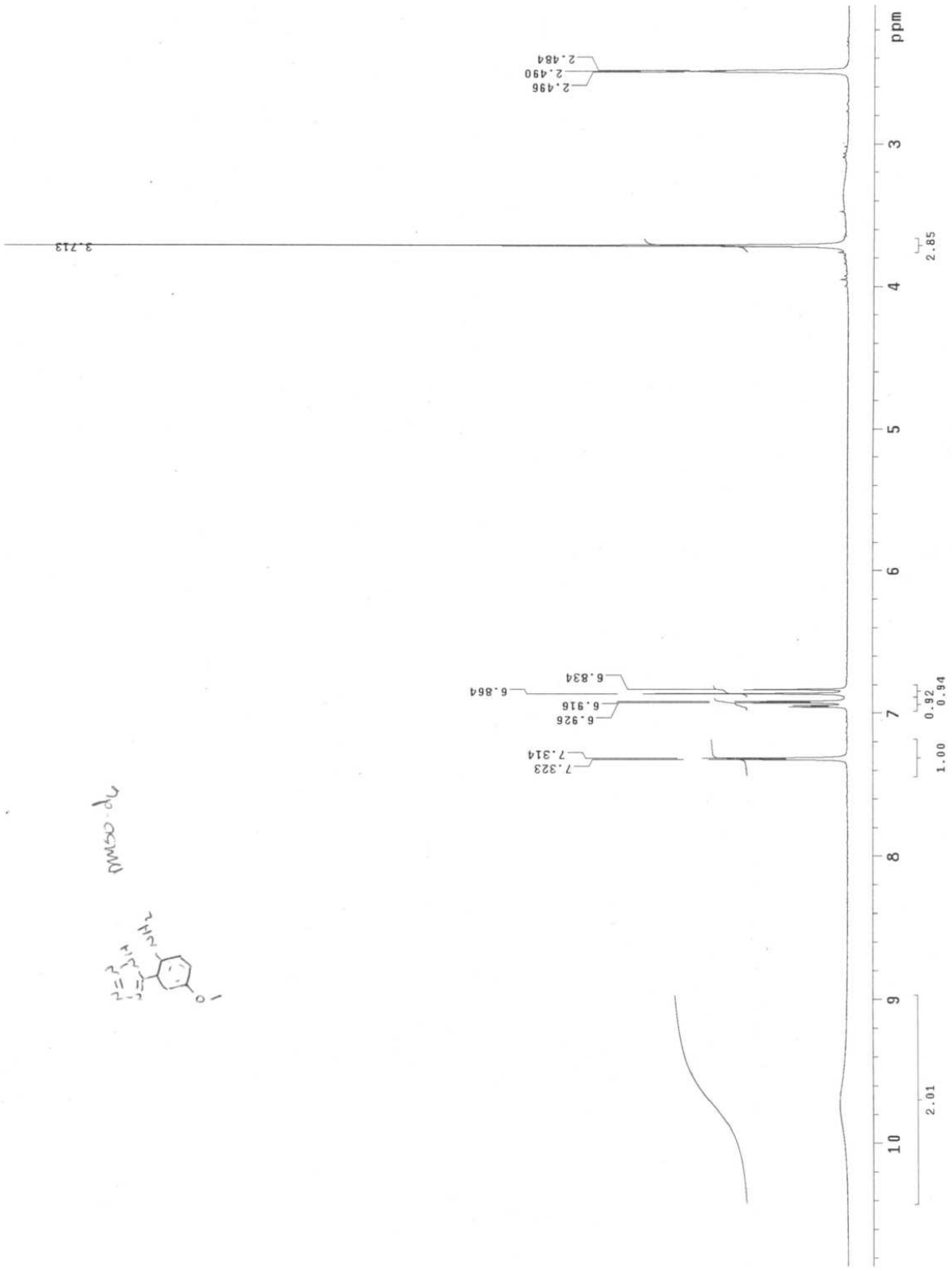
Figure S18: Reaction of 18 μM NBD Hydrazine with 1 mM acetophenone in the presence of 1 mM of different catalysts in 10:1 PBS (pH 7.4):DMF. Reaction conversion monitored at 470 nm.



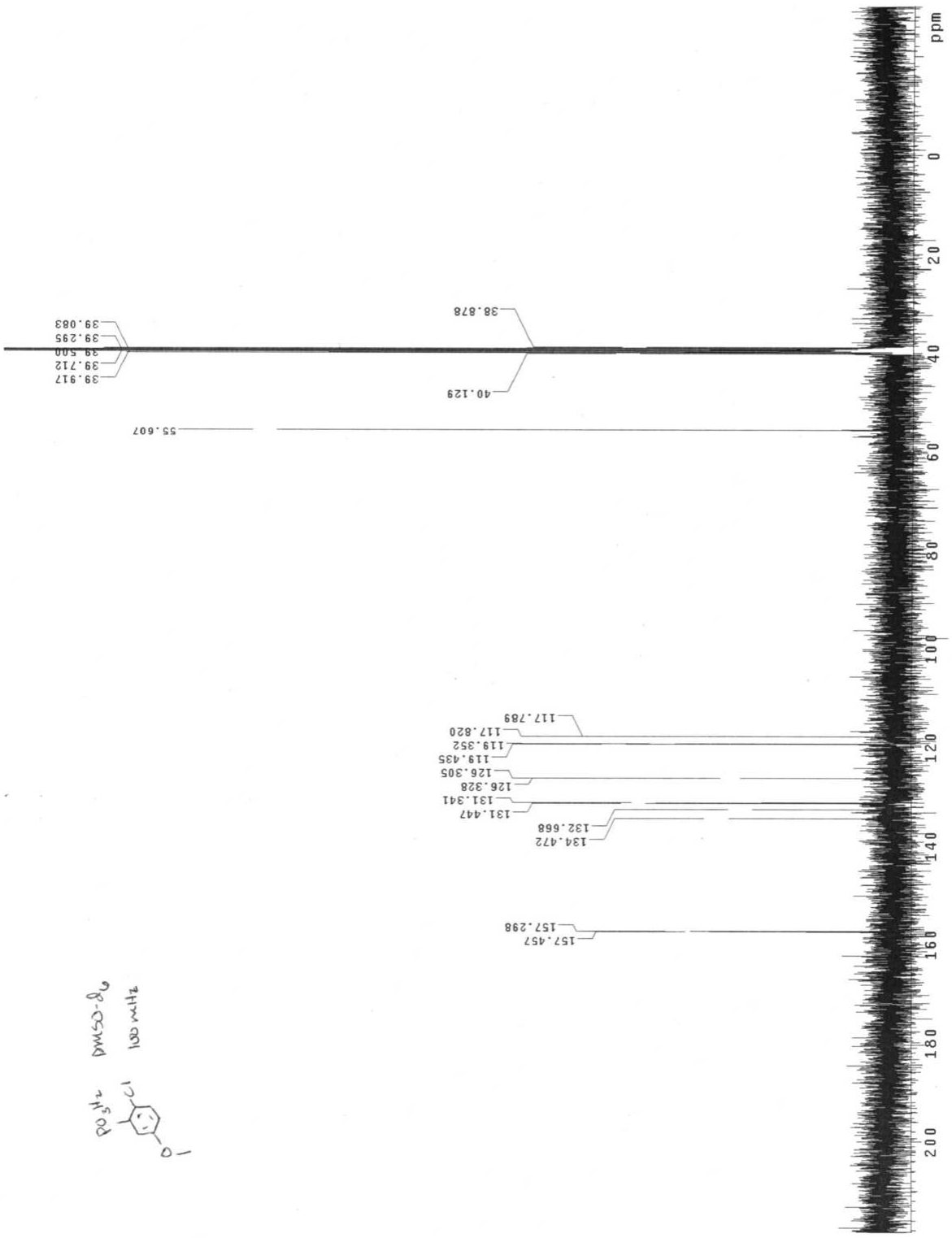
[1]H NMR Spectrum of intermediate in synthesis of **11** (400 MHz, D_2O/NaOD)



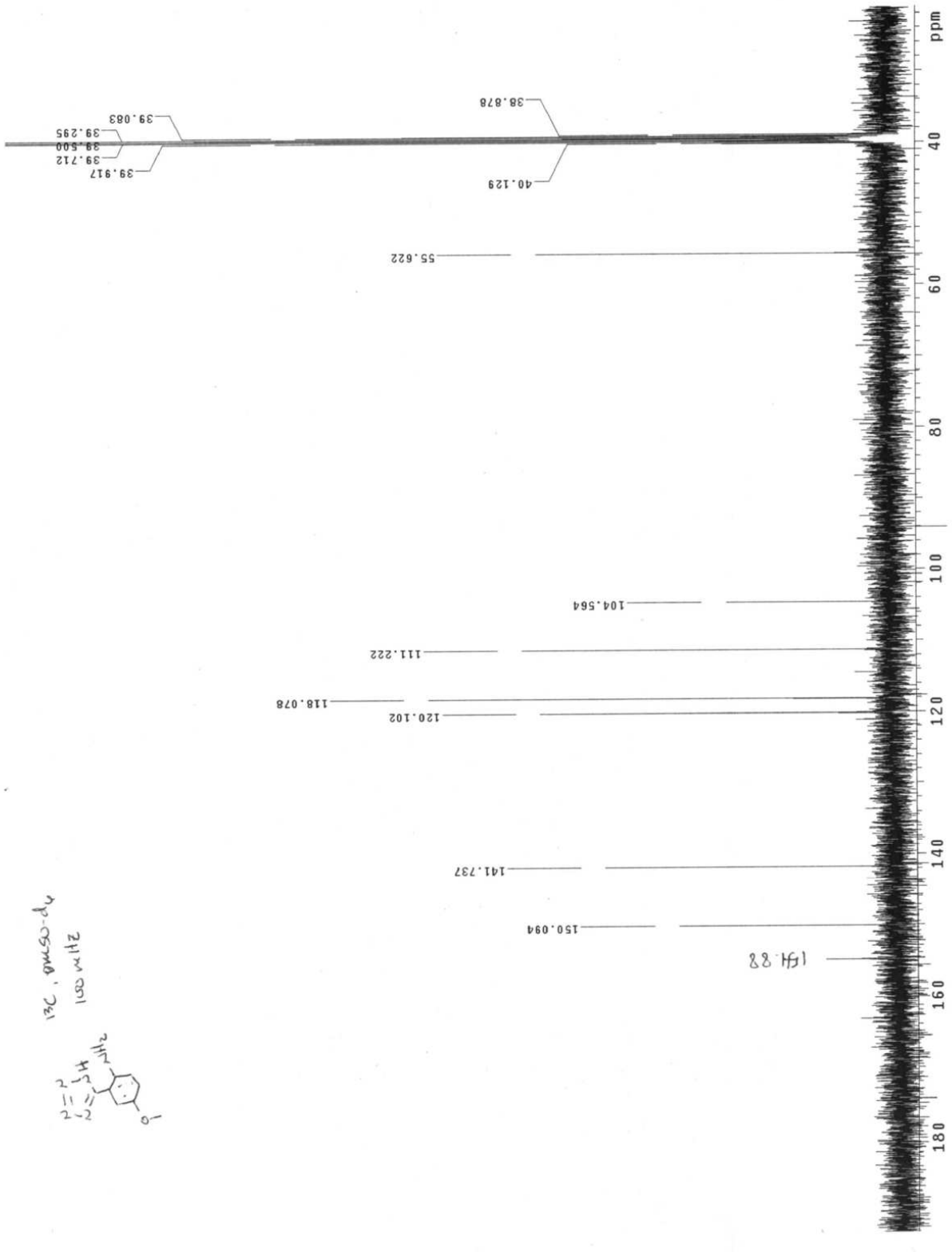
^{1}H NMR Spectrum of **11** (400 MHz, D_2O/NaOD)



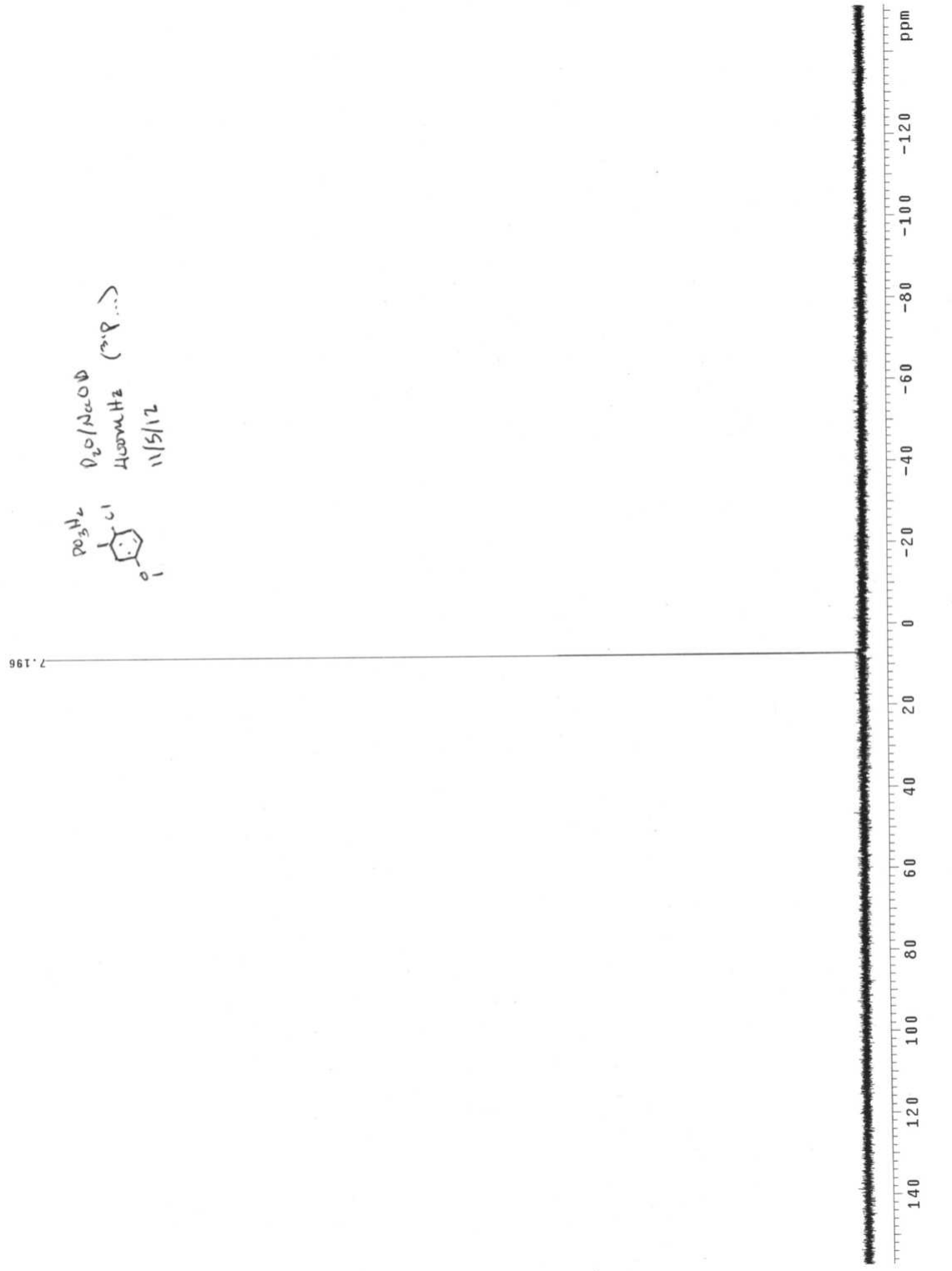
^{1}H NMR Spectrum of **13** (400 MHz, DMSO-d_6)



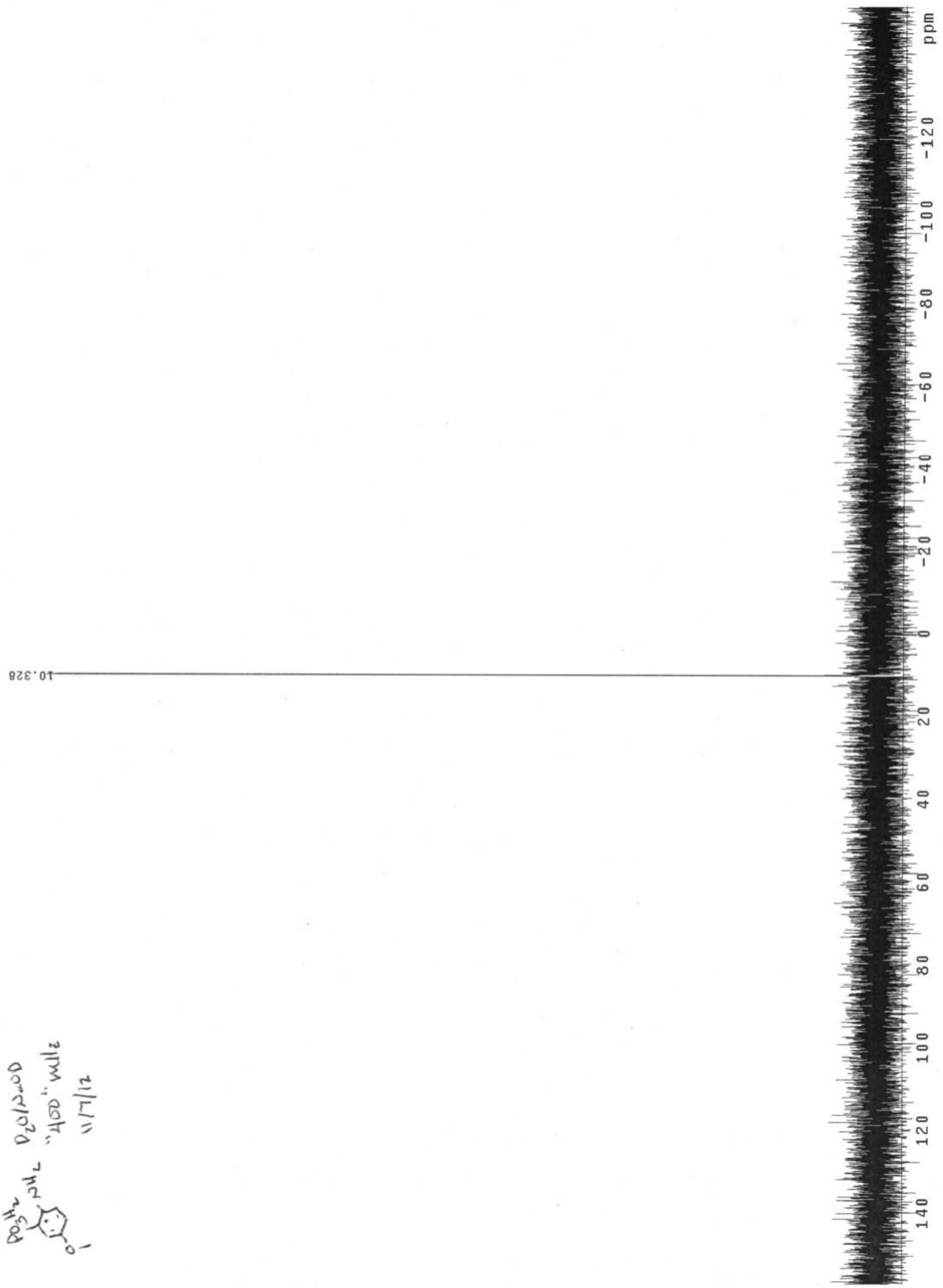
^{13}C NMR Spectrum of intermediate in synthesis of **11** (100 MHz, DMSO-d_6)



^{13}C NMR Spectrum of **13** (100 MHz, DMSO-d_6)



^{31}P NMR Spectrum of intermediate in synthesis of **11** (170 MHz, D_2O/NaOD)



^{31}P NMR Spectrum of **11** (170 MHz, D_2O/NaOD)

References

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