Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Thiourea-Catalyzed Aminolysis of N-Acyl Homoserine Lactones

Michael A. Bertucci, Stephen J. Lee and Michel R. Gagné

Department of Chemistry, University of North Carolina at Chapel Hill, Caudill Laboratories, Chapel Hill, NC, USA 27599-3290

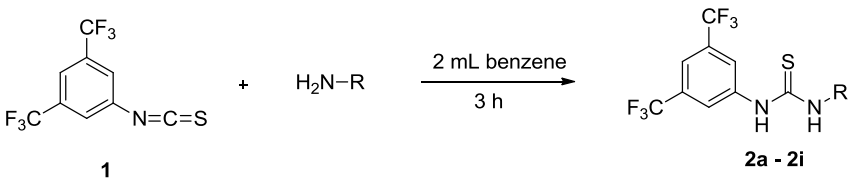
Synthetic Procedures

General

Commercial reagents were obtained from Sigma-Aldrich and Fisher Scientific. Column chromatography of all compounds, except catalyst **2f**, was completed using Fisher 230-400 Mesh Grade 60 silica gel. Catalyst **2f** was purified using a Waters semi-prep HPLC/UV-Vis with an XBridge 5μM C_{18} column as detailed in the procedure below. NMR solvents were purchased from Cambridge Isotopes Laboratories. Product characterization and kinetics experiments were completed on either a 600 MHz or 500 MHz Bruker NMR spectrometer. All related coupling constants are reported in Hertz and shifts in ppm. Elemental analyses (C, H and N) were completed by Robertson Microlit Laboratories, Inc. High resolution mass spectrometry (HRMS) was completed by the Mass Spectrometry Laboratory at the School of Chemical Sciences, University of Illinois using ESI on a Waters Q-ToF Ultima mass spectrometer.

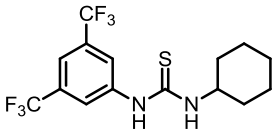
Procedure for Thiourea Catalyst Synthesis (for **2a – 2e***)*

Scheme S1



To a 25-mL schlenk flask flushed with N_2, **1** (0.336 mL, 1.84 mmol) was added and then solvated in benzene (1 mL). The corresponding amine (1.84 mmol) was added slowly to the flask. The walls of flask were washed with benzene (1 mL) and the reaction was allowed to stir at room temperature for 3 h. The reaction progress was monitored by TLC. The reaction mixture was concentrated under vacuum and redissolved in DCM. The product was purified by column chromatography with silica gel with a gradient mobile phase [DCM to MeOH/DCM (1:9)]. The percent yields reported in **Table 1** were obtained by combining pure fractions from the chromatographic separations, concentrating the combined fractions in a tared flask, and comparing the resultant mass relative to the theoretical product mass.

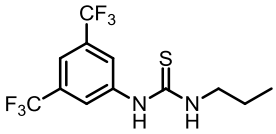
1-(3,5-bis(trifluoromethyl)phenyl)-3-cyclohexylthiourea (2a)



δ_H (DMSO-d_6, 500 MHz) 9.88 (1H, s), 8.24 (2H, s), 8.18 (1H, s), 7.72 (1H, s), 4.12 (1H, br s), 1.92 (2H, m), 1.71 (2H, m), 1.58 (1H, m), 1.35 – 1.29 (5H, m); δ_C (DMSO-d_6, 125 MHz) 179.1,

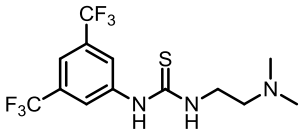
141.9, 130.0 (q, J_{CF} = 33.9 Hz), 123.2 (q, J_{CF} = 272.7 Hz), 121.6, 115.7, 52.2, 31.5, 25.0, 24.3; HRMS (ESI) calcd for $C_{15}H_{17}N_2F_6S$: 371.1017, found: 371.1014 (M + H).

1-(3,5-bis(trifluoromethyl)phenyl)-3-propylthiourea (2b)



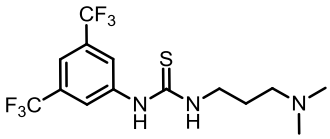
δ_H (DMSO-d_6, 500 MHz) 10.00 (1H, s), 8.23 (3H, br s), 7.69 (1H, s), 7.72 (1H, s), 3.40 (2H, m), 1.56 (2H, dt, J = 7.5 Hz), 0.89 (3H, t, J = 7.5 Hz); δ_C (DMSO-d_6, 125 MHz) 180.2, 141.7, 129.8 (q, J_{CF} = 32.7 Hz), 122.9 (q, J_{CF} = 272.9 Hz), 121.4, 115.5, 45.3, 21.4, 11.0; HRMS (ESI) calcd for $C_{12}H_{13}N_2F_6S$: 331.0712, found: 331.0704 (M + H); Anal. Calc. for $C_{12}H_{13}N_2F_6S$: C, 43.64; H, 3.66; N, 8.48; found: C, 43.56, H, 3.66, N, 8.35.

1-(3,5-bis(trifluoromethyl)phenyl)-3-(2-(dimethylamino)ethyl)thiourea (2c)



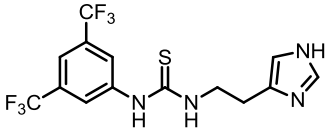
δ_H (DMSO-d_6, 500 MHz) 10.27 (1H, s), 8.26 (2H, s), 8.10 (1H, s), 7.72 (1H, s), 3.56 (2H, br q), 2.44 (br t, 2H), 2.19 (6H, s); δ_C (DMSO-d_6, 150 MHz) 180.0, 141.9, 130.2 (q, J = 33.2 Hz), 123.2 (q, J = 273.1 Hz), 121.3, 115.8, 56.8, 44.9, 41.7; HRMS (ESI) calcd for $C_{13}H_{16}N_3F_6S$: 360.0696, found: 360.0962 (M + H).

1-(3,5-bis(trifluoromethyl)phenyl)-3-(3-(dimethylamino)propyl)thiourea (2d)



δ_H (DMSO-d_6, 600 MHz) δ10.09 (1H, s), 8.33 (1H, s), 8.23 (2H, s), 7.70 (1H, s), 3.51 (2H, br s), 2.27 (2H, t, J = 6.60 Hz), 2.12 (6H, s), 1.69 (2H, p, J = 6.60 Hz); δ_C (DMSO-d_6, 150 MHz) δ180.3, 141.9, 130.2 (q, J_{CF} = 33.2 Hz), 123.2 (q, J_{CF} = 273.1 Hz), 121.8, 115.9, 56.8, 45.0, 42.6, 25.9; HRMS (ESI) calcd for $C_{14}H_{18}N_3F_6S$: 374.1126, found: 374.1126 (M + H).

1-(2-(1H-imidazol-4-yl)ethyl)-3-(3,5-bis(trifluoromethyl)phenyl)thiourea (2e)

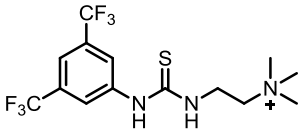


δ_H (DMSO-d_6, 500 MHz) 11.90 (1H, br s), 10.21 (1H, s), 8.27 (3H, br s), 7.72 (1H, s), 7.57 (1H, s), 6.88 (1H, s), 3.76 (2H, br q), 2.81 (2H, t, J = 7.5 Hz); δ_C (DMSO-d_6, 125 MHz) 180.2, 141.8, 134.4, 130.0 (q, J_{CF} = 32.7 Hz), 128.2, 123.1 (q, J_{CF} = 272.9 Hz), 121.6, 115.8, 43.8, 26.0; HRMS (ESI) calcd for $C_{14}H_{13}N_4F_6S$: 383.0765, found: 383.0772 (M + H); Anal. Calc. for $C_{14}H_{13}N_4F_6S$: C, 43.98, H, 3.16, N, 14.65; found: C, 44.01, H, 3.20, N, 14.61.

Scheme S2

Catalyst **2f** was synthesized through a modified version of the general procedure above. In a 2-mL eppendorf tube, (2-aminoethyl)trimethylammonium chloride hydrochloride (50 mg, 0.286 mmol) was combined with N,N-diisopropylethylamine (0.050 mL, 0.286 mmol). Isothiocyanate **1** (0.052 mL, 0.286 mmol) was then added to the tube. The reaction mixture was vortexed for 10 min in the eppendorf tube. The reaction mixture was quenched by adding 2 mL of MeOH to the tube. The reaction mixture was concentrated and redissolved in distilled water. The resultant aqueous solution was filtered and diluted to prepare for purification. The product was purified by semi-prep HPLC with a gradient mobile phase [MeCN/H_2O (1:4) to MeCN/H_2O (1:1)]. Notably, a small percentage of trifluoroacetic acid was retained by the catalyst after purification and is present in the ^{13}C NMR (page S12). Given the large excess of piperidine present in the catalyst screening, this trace acid did not significantly affect the rate of AHL aminolysis.

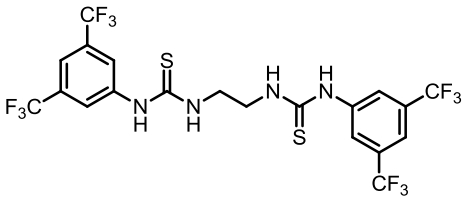
2-(3-(3,5-bis(trifluoromethyl)phenyl)thioureido)-N,N,N-trimethylethanaminium (2f)



δ_H (DMSO-d_6, 500 MHz) 10.78 (1H, br s), 8.80 (1H, s), 8.26 (2H, s), 7.80 (1H, s), 3.99 (2H, m), 3.54 (1H, t, J = 6.50), 3.14 (9H, s); δ_C (CD_3CN-d_3, 125 MHz) 182.9, 160.8 (q, J = 35.2), 142.4, 131.5 (q, J_{CF} = 32.7 Hz), 124.1 (q, J_{CF} = 271.6 Hz), 123.5, 64.8, 54.1, 38.7; HRMS (ESI) calcd for $C_{14}H_{18}N_3F_6S$: 374.1126, found: 374.1120 (M + H).

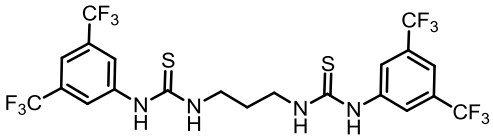
Catalysts **2g** and **2h** were synthesized following the general procedure displayed in Scheme S1 but reacting 2 eq (3.68 mmol) of **1** with 1 eq (1.84 mmol) of a diamine. Ethylenediamine was the diamine chosen to construct **2g** and 1,3 diaminopropane to make **2h**.

1,1'-(ethane-1,2-diyl)bis(3-(3,5-bis(trifluoromethyl)phenyl)thiourea) (2g)



δ_H (DMSO-d_6, 500 MHz) 10.14 (2H, s), 8.34 (2H, s), 8.22 (4H, s), 7.72 (2H, s), 3.77 (4H, br s); δ_C (DMSO-d_6, 125 MHz) 180.7, 141.4, 129.9 (q, J_{CF} = 32.7 Hz), 122.9 (q, J_{CF} = 272.9 Hz), 122.0, 116.0, 42.5; HRMS (ESI) calcd for $C_{20}H_{15}N_4F_{12}S_2$: 603.0547, found: 603.0546 (M + H); Anal. Calc. for $C_{20}H_{15}N_4F_{12}S_2$: C, 39.87; H, 2.34; N, 9.30; found: C, 40.15; H, 2.47; N, 9.06.

1,1'-(propane-1,3-diyl)bis(3-(3,5-bis(trifluoromethyl)phenyl)thiourea) (2h)



δ_H (DMSO-d_6, 600 MHz) 10.09 (2H, s), 8.26 (2H, s), 8.22 (4H, s), 7.70 (2H, s), 3.57 (4H, s), 1.89 (2H, p, J = 6.6 Hz); δ_C (DMF-d_7, 125 MHz) 181.6, 142.7, 131.1 (q, J_{CF} = 32.7 Hz), 123.8 (q, J_{CF} = 271.5 Hz), 122.6, 116.5, 42.1, 28.3; HRMS (ESI) calcd for $C_{21}H_{17}N_4F_{12}S_2$ 617.0703, found 617.0699 (M + H); Anal. Calc. for $C_{21}H_{17}N_4F_{12}S_2$: C, 40.91; H, 2.62; N, 9.09, found: C, 40.64; H, 2.58; N, 8.86.

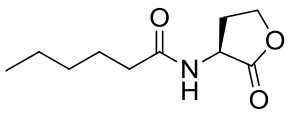
References:

1. T. Okino, Y. Hoashi, T. Furukawa, X. Xu and Y. Takemoto, *J. Am. Chem. Soc.*, 2005, **127**, 119-125.
2. B. Han, Q. Lin, R. Li, X. Tian, X. Xiong, J. Deng and Y. Chen, *Chem. Eur. J.* 2008, **14**, 8094–8097.

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

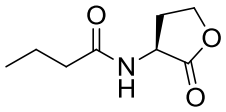
The C_4, C_6, and 3-oxo-C_{12} AHLs are known compounds and were synthesized following previously published procedures.[3-5]

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



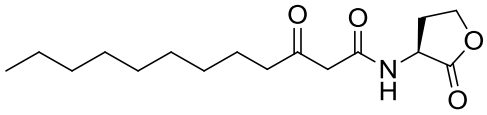
63.8% Yield; δ_H (DMSO-d_6, 500 MHz) 8.30 (1H, s), 4.49 (1H, m), 4.31 (1H, td, J_1 = 9.0 Hz, J_2 =1.5 Hz), 4.17 (1H, m), 2.35 (1H, m), 2.13-2.06 (3H, m), 1.47 (2H, m, J = 7.5 Hz), 1.26-1.21 (4H, m), 0.84 (3H, t, J = 7.0 Hz); δ_C (DMSO-d_6, 125 MHz) 175.9, 172.7, 65.7, 48.3, 35.5, 31.2, 28.7, 25.2, 22.3,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

_N_-Butyryl-L-homoserine Lactone (4)



56.1% Yield; δ_H (DMSO-d_6, 500 MHz) 8.30 (1H, s), 4.51 (1H, m), 4.32 (1H, td, J_1 = 8.5 Hz, J_2 = 2.0 Hz), 4.18 (1H, m), 2.36 (1H, m), 2.14-2.05 (3H, m), 1.59 (2H, m, J = 7.5 Hz), 0.84 (3H, t, J = 7.5 Hz); δ_C (DMSO-d_6, 150 MHz) 175.3, 172.0, 65.1, 47.7, 36.9, 28.2, 18.5, 13.4; MS (ESI) calcd for $C_8H_{13}NO_3$: 172.10, found: 172.06 (M + H).

_N_-(3-Oxododecanolyl)-L-homoserine Lactone (5)



45.2% Yield; δ_H ($CDCl_3$, 600 MHz) 7.75 (1H, d, J = 6.6 Hz), 4.62 (1H, m), 4.48 (1H, td, J_1 = 9.0 Hz, J_2 = 1.2Hz), 4.29 (1H, m), 3.48 (2H, s), 2.73 (1H, m), 2.54 (2H, t, J = 7.2 Hz), 2.27 (1H, m), 1.58 (2H, p, J = 7.2 Hz), 1.31-1.26 (12H, m), 0.88 (3H, t, J = 6.6 Hz); δ_C ($CDCl_3$, 150 MHz) 206.5, 175.0, 166.6, 65.9, 49.0, 48.3, 43.8, 31.8, 29.6, 29.4, 29.3, 29.2, 29.0, 23.4, 22.7, 14.1; MS (ESI) calcd for $C_{16}H_{27}NO_4$: 298.20, found: 298.18 (M + H).

References:

3. S. R. Chhabra, B. Philipp, L. Eberl, M. Givskov, P. Williams and M. Cámara, *Topics in Current Chemistry*, 2005, **240**, 279–315.
4. S. R. Chhabra, C. Harty, D. S. W. Hooi, M. Daykin, P. Williams, G. Telford, D. J. Pritchard and B. W. Bycroft, *J. Med. Chem.*, 2002, **46**, 97-104.
5. A. M. Pomini and A. J. Marsaioli, *J. Nat. Prod.*, 2008, **71**, 1032-1036.

NMR Kinetic Experiments

AHL-Catalyst Interaction via ^1H NMR

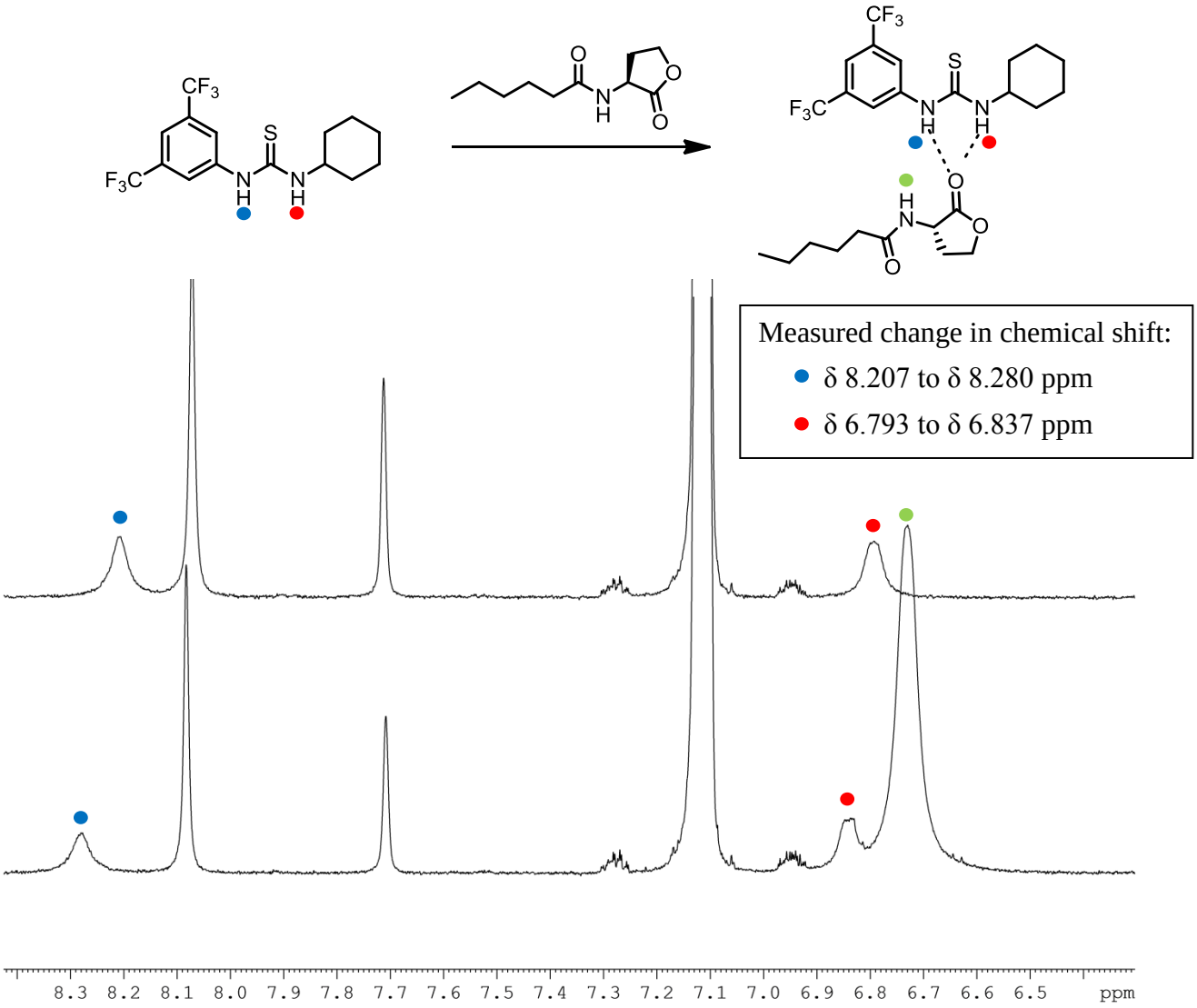
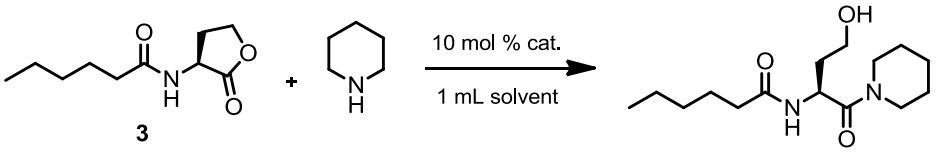


Figure S1 - Addition of C_6-AHL to a solution of catalyst **2a** in CD_3CN led to an observable downfield shift in N-H resonances of the thiourea.

General Procedure for Catalyst Screening

Scheme S3



3

In a 4-mL scintillation vial, **3** (10.0 mg, 0.0502 mmol) and the thiourea catalyst being screened (0.00502 mmol) were combined. The catalyst and substrate were then dissolved in 1 mL of the deuterated solvent of choice. As an internal standard, 1,4-difluorobenzene (5.16 μL, 0.00502 mmol) was added to the reaction mixture. Piperidine (99.0 μL, 0.1004 mmol) was transferred into the vial and the reaction mixture was vortexed and quickly transferred to an NMR tube for analysis. ^{1}H NMR experiments were acquired in a sequence with a spectra being acquired between every 376 – 669 s depending on the experiment. The integration of the α-proton resonance of the lactone was monitored over time to track conversion from starting material to product (see Figure S1).

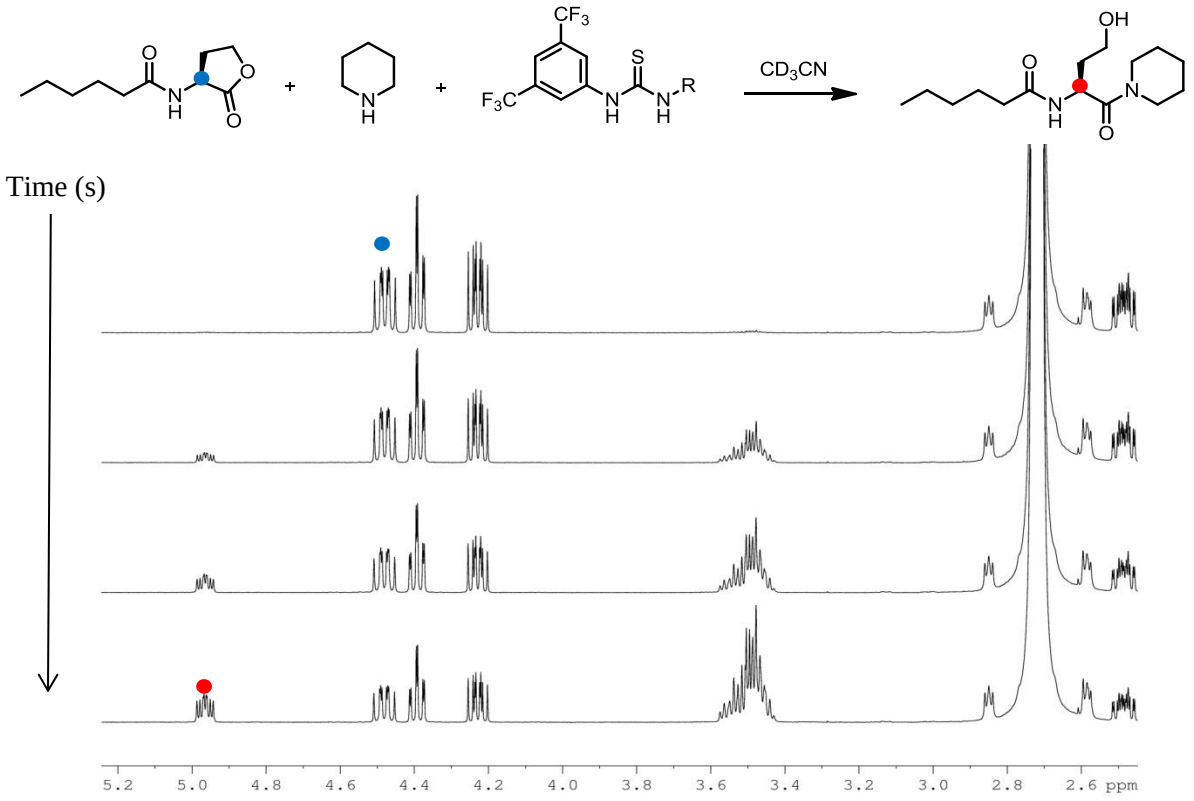


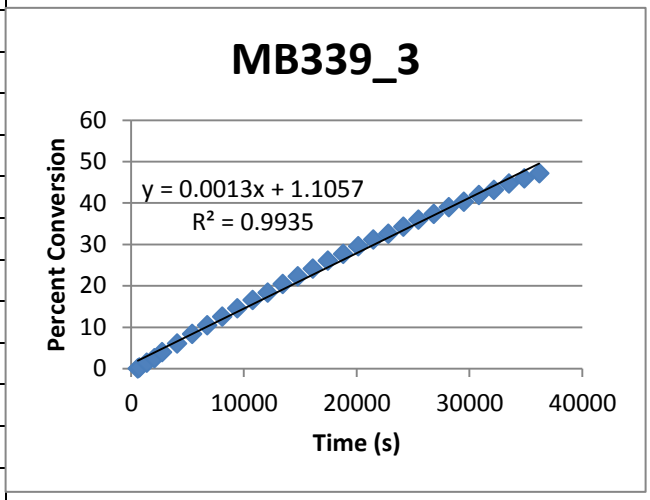
Figure S2 – Conversion of C_6-AHL to the corresponding aminolysis product

The integrations of the C_6-AHL and the corresponding aminolysis product resonances, appearing at 4.49 and 4.97 respectively, were tabulated and employed to determine percent conversion at the time that each spectra was acquired. The resultant percent conversions were then plotted vs. time (in seconds). A regression line was fit to the data points in the initial stages of reaction to determine the rate of the reaction. The regression line was solved where x = 50.0 (or 50% conversion) to determine the half-life of the stating material (C_6-AHL). A sample calculation is shown below and the results are displayed in Tables 1 and 2 of the manuscript.

Sample t_{50} Calculation

Table S1. C_6-AHL α-proton integrations changes during reaction with piperidine in CD_3CN

MB339_3	t_{50} = 37600 s		
Time (s)	Int 4.49ppm	Int 4.97ppm	% Conv
600	0.9541	0	0
729	0.930341	0.001694	0.181782
1398	0.916784	0.012678	1.364025
2067	0.909431	0.022505	2.414907
2736	0.90006	0.036206	3.867022
4074	0.880634	0.055968	5.975674
5412	0.861099	0.07793	8.299018
6750	0.842755	0.098016	10.41866
8088	0.826012	0.11798	12.49799
9426	0.804961	0.136436	14.49293
10764	0.78807	0.15608	16.53127
12102	0.765377	0.171036	18.26502
13440	0.749704	0.191816	20.37301
14778	0.730972	0.209067	22.24025
16116	0.712837	0.225932	24.06684
17454	0.697733	0.245455	26.02397
18792	0.6863	0.262463	27.66371
20130	0.669336	0.279969	29.492
21468	0.659008	0.297069	31.07166
22806	0.632959	0.304898	32.51007
24144	0.621924	0.323663	34.22879
25482	0.609451	0.341129	35.88641
26820	0.598153	0.355315	37.26554
28158	0.578194	0.367974	38.89098
29496	0.571494	0.385202	40.26378
30834	0.551948	0.397418	41.86141
32172	0.543054	0.410723	43.06279
33510	0.527491	0.425157	44.62897



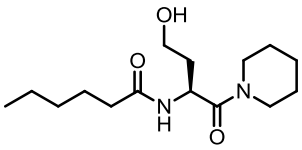
$$y = 0.0013x + 1.1057 \qquad (1)$$

$$50.0 = 0.0013x + 1.1057 \qquad (2)$$

$$x = 37600\ \text{s} = t_{1/2} \qquad (3)$$

Product Characterization

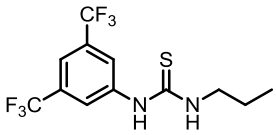
(S)-N-(4-hydroxy-1-oxo-1-(piperidin-1-yl)butan-2-yl)hexanamide



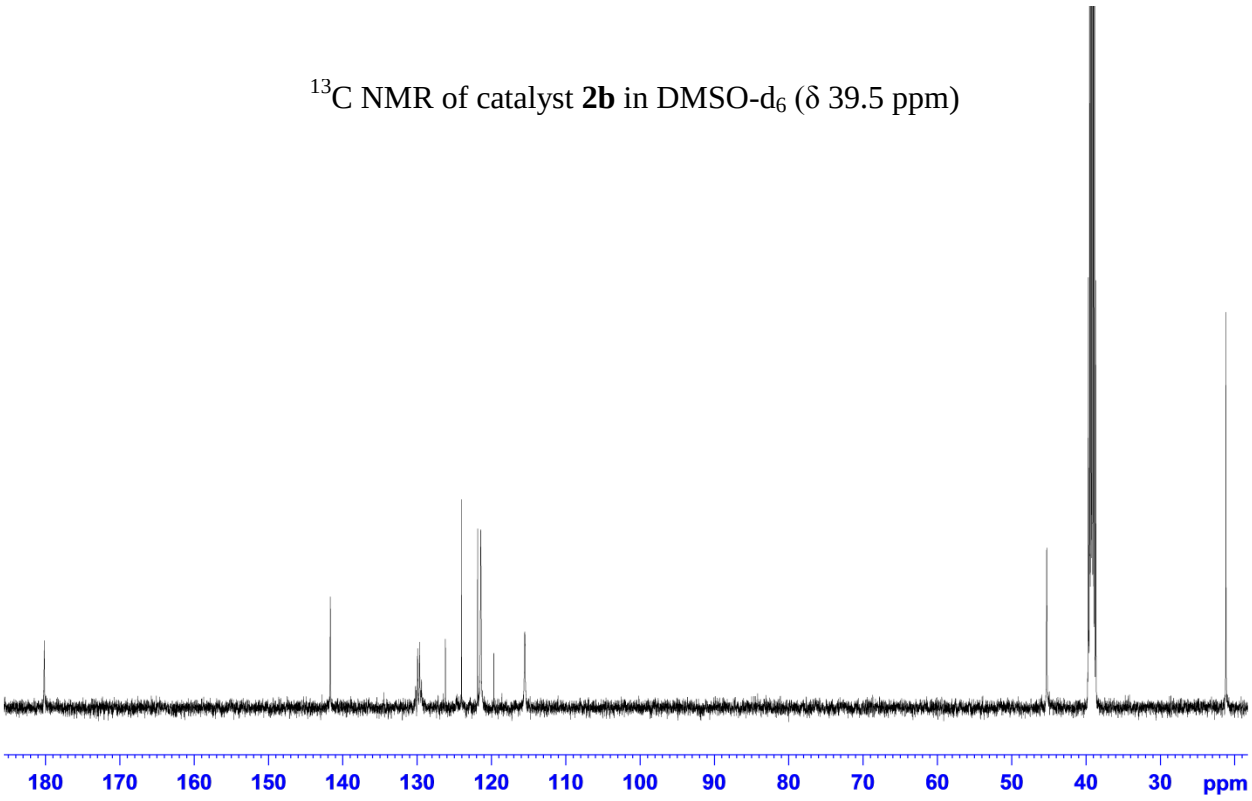
δ_H (CD_3CN-d_3, 500 MHz) 6.86 (1H, s), 4.92 (1H, m), 3.58 (1H, dd, J_1 = 2.5 Hz, J_2 = 5.0 Hz), 3.53-3.41 (6H, m), 2.21-2.17 (3H, m), 1.81 (1H, m), 1.62 (2H, m), 1.56 (4H, m), 1.50-1.45 (3H, m), 1.32-1.26 (4H, m), 0.88 (3H, t, J = 7.0 Hz); δ_C (CD_3CN-d_3, 125 MHz) 173.8, 170.0, 57.8, 46.4, 46,2, 43.0, 36.2, 36.0, 31.5, 26.4, 25.7, 25.6, 24.5, 22.5, 13.6; MS (ESI) calcd for $C_{15}H_{28}N_2O_3$: 285.22, found: 285.20 (M + H); Anal. Calc. for $C_{15}H_{28}N_2O_3$: C, 63.35; H, 9.92; N, 9.85; found: C, 63.34; H, 9.76; N, 9.80.

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

^{1}H NMR of catalyst **2b** in DMSO-d_6 (δ 2.49 ppm)

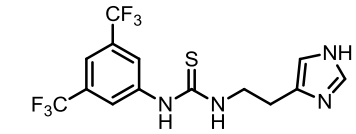


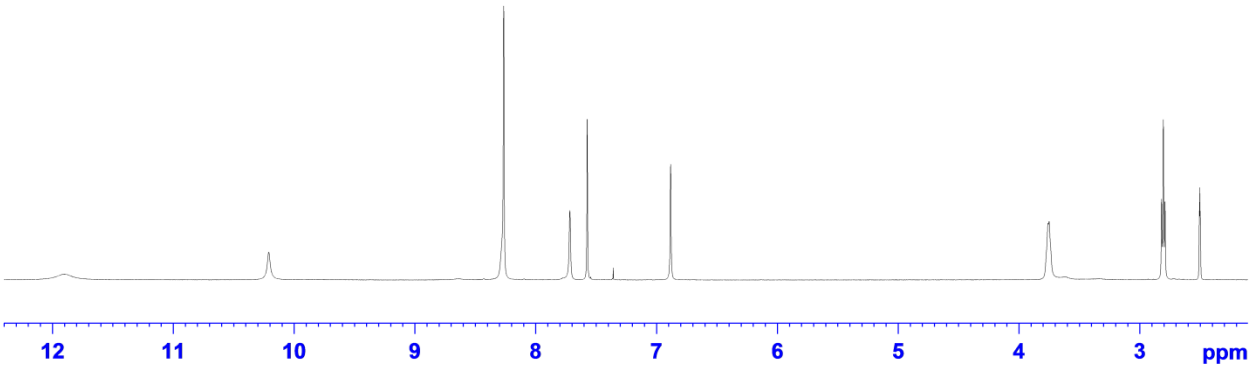
^{13}C NMR of catalyst **2b** in DMSO-d_6 (δ 39.5 ppm)



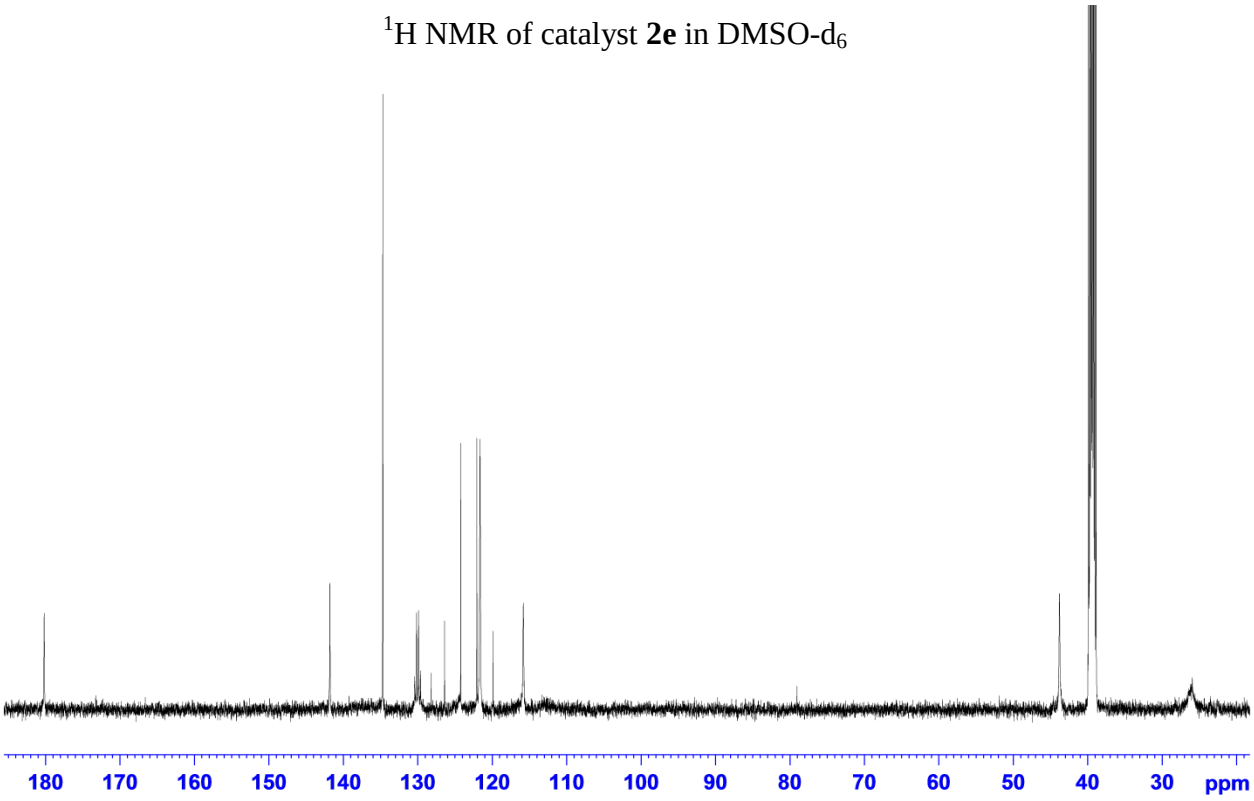
*spectral data for previously characterized compounds is not displayed

^{1}H NMR of catalyst **2e** in DMSO-d_6

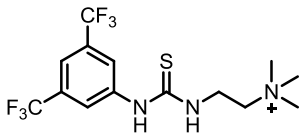




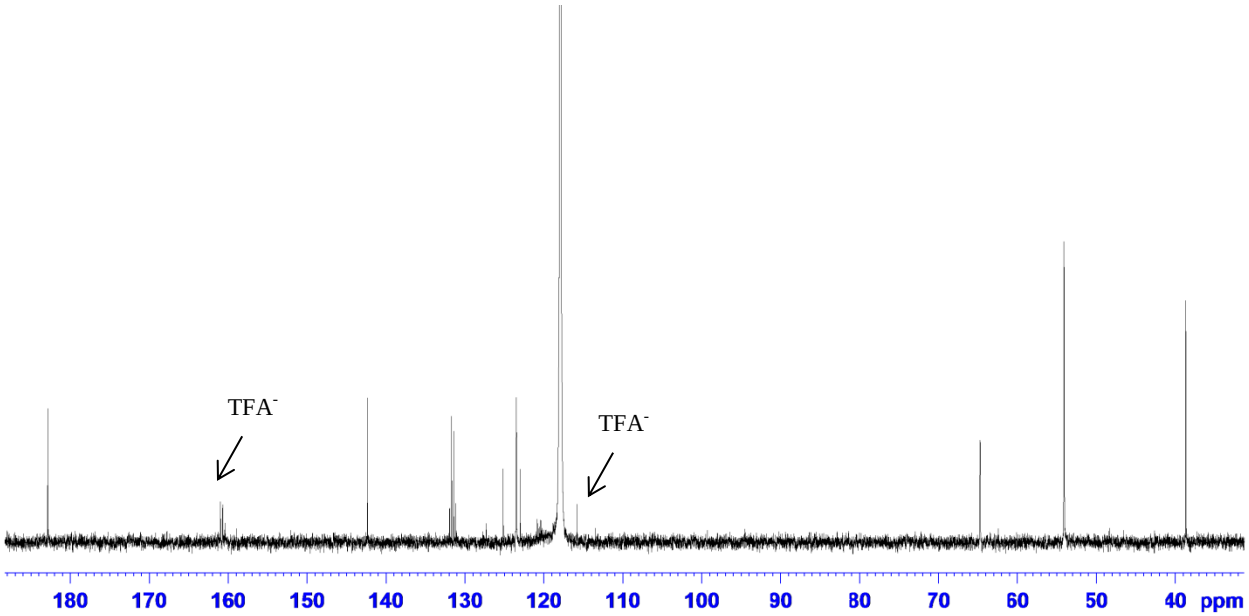
^{1}H NMR of catalyst **2e** in DMSO-d_6



^{1}H NMR of catalyst **2f** in DMSO-d_6

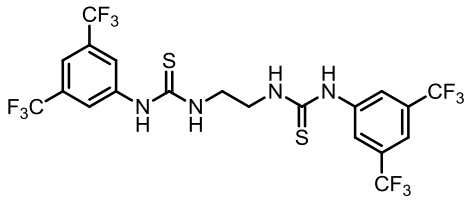


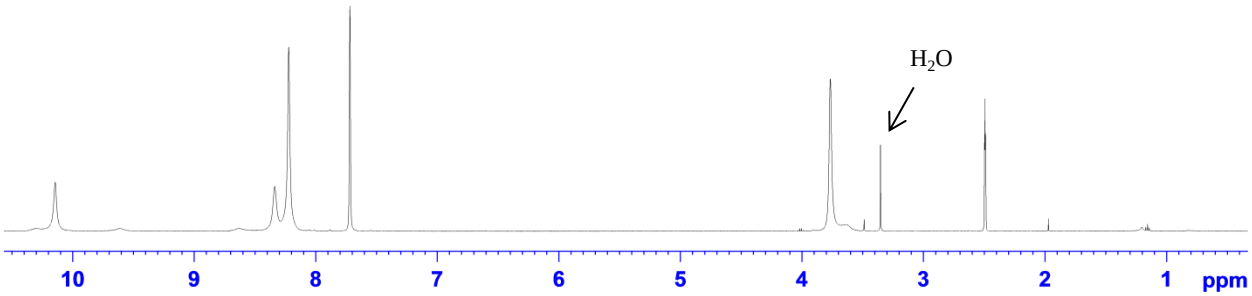
^{13}C NMR of catalyst **2f** in CD_3CN



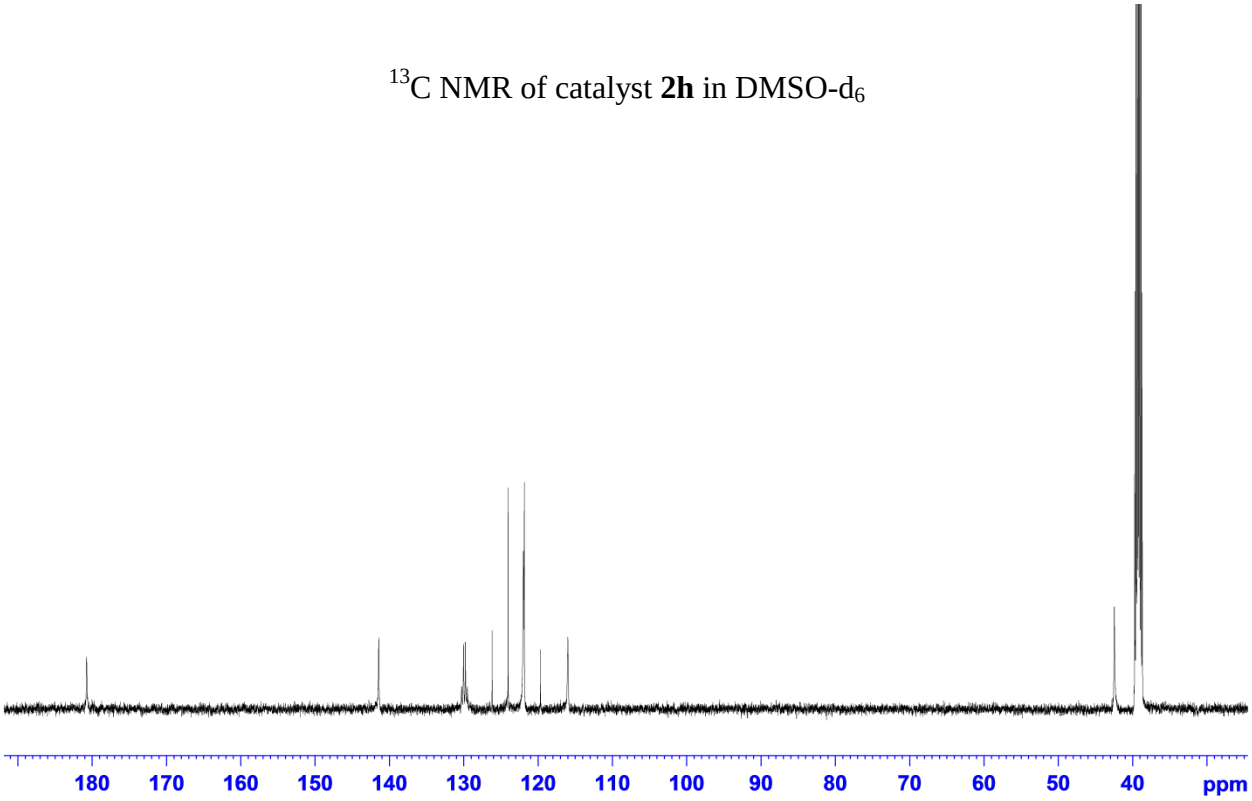
The presence of triflouroacetic acid (TFA) in the carbon spectrum is due to the acidic mobile phase of the HPLC on which the thiourea was purified. Due to the large excess of piperidine in the catalytic screenings, the acid should have had no effect on the relative rates obtained.

^{1}H NMR of catalyst **2h** in DMSO-d_6

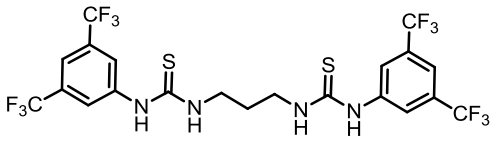


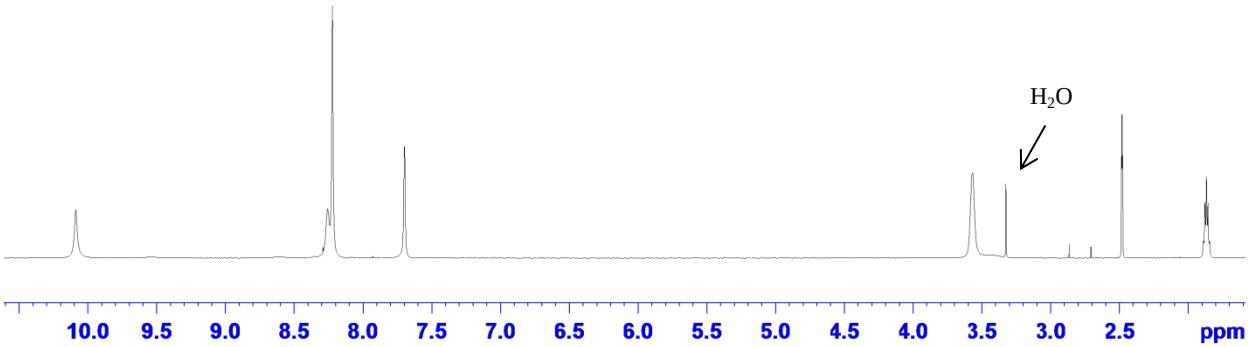


^{13}C NMR of catalyst **2h** in DMSO-d_6



^{13}H NMR of catalyst **2i** in DMSO-d_6





^{13}C NMR of catalyst **2i** in DMF-d_7 (δ162.7, 35.2, 30.1 ppm)