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Hexamethylenetetramine carboxyborane: synthesis, structural characterization and CO releasing property

T. I. Ayudhya[a], C. C. Raymond[b], N. N. Dingra[a,†]

[a] School of Pure and Applied Sciences, Florida SouthWestern State College, Fort Myers FL 33919, USA
[b] Chemistry Department, State University of New York at Oswego, Oswego NY 13126, USA
[†]Email: ndingra@fsw.edu

Supporting Information

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§, §§ Notes from the main text.

Calculation: Percent CO released calculated from myoglobin assay [§]

Preparation of Deoxy-Myoglobin (deoxy-Mb):

Horse heart myoglobin (3.52 mg, 0.200 µmol, MM 17000) was dissolved in 300 µL sodium phosphate buffer (50 mM, pH 7.4). This was mixed with 30 µL of 100 mM (3.0 µmol) sodium dithionite and run through a desalting column (GE) to separate excess dithionite from the deoxy-Mb stock solution. Note that sodium phosphate buffer (50 mM, pH 7.4) was degassed before use by vacuum/N_2 gas several times and bubbled with N_2 gas overnight in a sealed tube. All samples were prepared in a sealed glove box with constant feed of N_2 gas.

Deoxy-Mb Stock Solution Concentration and Stability Test:

To calculate the concentration of deoxy-Mb stock, 100 µL was mixed with sodium phosphate buffer (900 µL) and the absorbance at 560 nm measured. The exact concentration of Deoxy-Mb in the solution was calculated using absorption peak intensity at 560 nm (A_{560nm} = 0.942 a.u., ε_{560nm} = extinction coefficient of deoxy-Mb = 12.92 $mM^{-1}cm^{-1}$). Calculated concentration of deoxy-Mb was 72.9 µM.

Myoglobin Assay Procedure:

Deoxy-Mb stock solution (100 µL) and HMTA-CB (4.0 µmol or 66 µL of 12 mg/mL in sodium phosphate buffer) were mixed with sodium phosphate buffer (834 µL) and placed in a sealed quartz cuvette. Control sample was set up the same way without HMTA-CB compound. A layer of mineral oil was added on top of the solution to prevent any exposure to oxygen and to prevent CO from escaping. Samples were incubated at 37 ^{o}C in N_2 Chamber. Formation of carbonmonoxy myoglobin (Mb-CO) was monitored by UV-Vis spectrophotometer (500-600 nm) at 0, 10, 20, 30, 40 and 60 minutes. The calculation for percent CO release is shown on **Table S1**.

Table S1. Absorption Values from Myoglobin Assay and Calculations for %CO Release.

Time (minutes)	A_{540}	A_{570}	A_{540} corrected[1]	A_{540} changes[2]	[Mb-CO] (M)[3]	Mb-CO (µmol)[4,5]	% CO[6]
0	0.7560	0.8591	0.7560	0.0000	0.0000	0.0000	0.0000
10	0.8103	0.8693	0.8008	0.0448	3.017E-06	0.0030	0.0754
20	0.8636	0.8604	0.8623	0.1063	7.161E-06	0.0072	0.179
30	0.9196	0.8606	0.9179	0.1619	1.090E-05	0.0109	0.2726
40	0.9632	0.8574	0.9651	0.2091	1.408E-05	0.0141	0.3521
60	1.0516	0.7843	1.1518	0.3959	2.666E-05	0.0267	0.6664

[1] A_{540} corrected = (A_{540}) x ($A_{570\ t=0}$ / $A_{570\ t=min}$)

[2] A_{540} changes = $A_{540\ t=min}$ - $A_{540\ t=0}$

[3] [Mb-CO] = A_{540} corrected / ((14850 $M^{-1}cm^{-1}$) x (1 cm)); ε_{540nm} = extinction coefficient of Mb-CO[3] = 14.85 $mM^{-1}cm^{-1}$

[4] Mb-CO = [Mb-CO] x (1.000 mL) x (1 L/1000 mL) x (10^6 µL/1 L)

[5] Mb-CO (µmol) = CO (µmol)

[6] % CO = (CO (µmol) / 4.00 (HMTA-CB µmol)) x 100

Note:

- Absorption spectrum of Deoxy-Mb sample without HMTA-CB (control sample) does not change over the period of 1 hour of incubation. We assumed that there was no degradation of deoxy-Mb nor the formation of Mb-CO under the controlled experimental condition.

- Mb-CO and deoxy-Mb spectra have four isosbestic points at 510, 550, 570, and 585 nm. The absorption values at 570 nm (A_{570}) were used to correct the maximum absorption values of Mb-CO at 540 nm (A_{540}).

Calculation: Percent CO released calculated from ^{1}H NMR [§]

Scheme 1. ^{1}H NMR spectra of HMTA-CB and HMTA with peak assignments

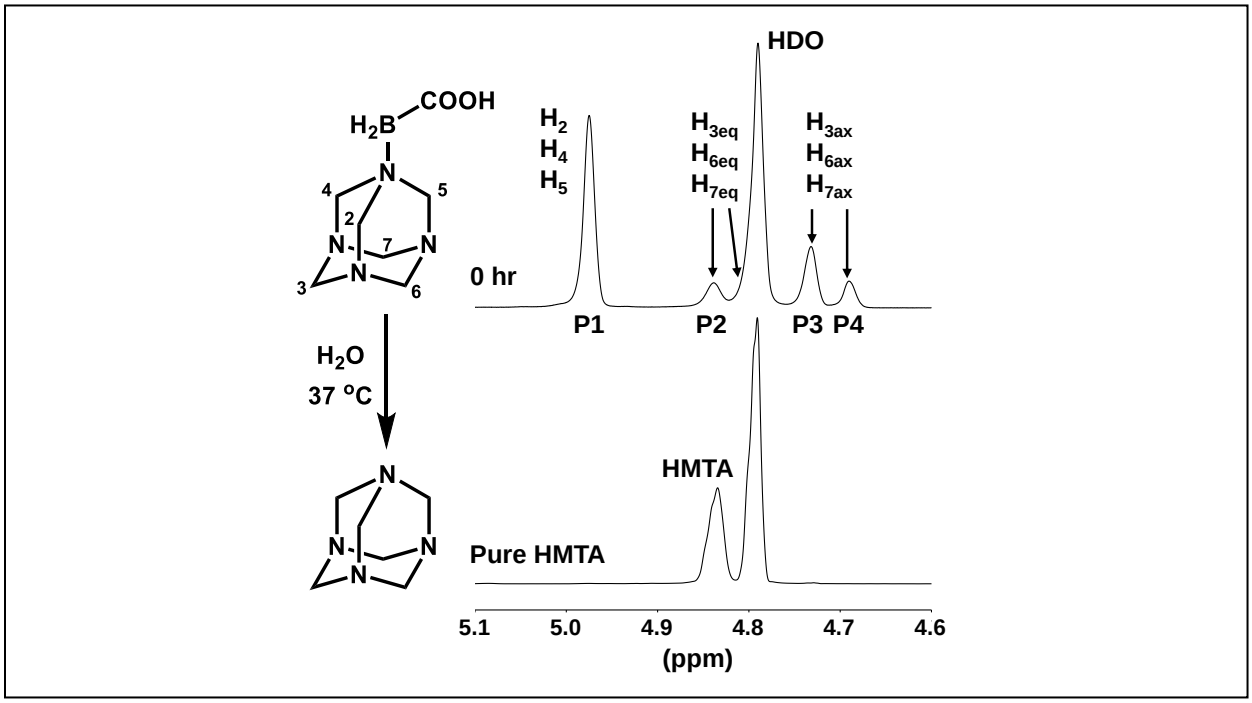


Table S2. Normalized Integrals of HMTA and HMTA-CB peaks from ^{1}H NMR spectra and calculations for % decomposition of HMTA-CB.

	Normalized Integrals[a]		
Peak	0 hour	1 hour	24 hours
P1	6.000	6.000	6.000
P2	0.969	1.047	3.486
P3	2.096	2.160	2.139
P4	0.961	0.933	0.921
P(HMTA)[b]	0[c]	0.114	2.565
% Auto-Decomposition[d]	0[c]	0.9268	17.467

[a] NMR spectra were processed by using Mnova program.

[b] P(HMTA) = P2-P4

[c] We assume that HMTA is not present at 0 hour and 1 mole of HMTA-CB gives 1 mole of CO.

[d] % Auto-Decomposition = (P(HMTA) / (P1 + 2 x (P3 + P4))) x 100

Calculation: Percent CO released calculated from meter reading [§]

Table S3. Conversion of ppm to % CO released by auto-decomposition of HMTA-CB

Sample [a] Incubation Time (hours)	CO-Meter Reading (ppm) [b]	mol% CO Released [c]
1	22	1.15
3	96	5.01
6	163	8.51
9	199	10.39
12	227	11.86
14	253	13.21
24	434	22.67

[a] 4.0 µmol of 60 mM solution was used for each trial in this experiment
[b] Averages from triplicates or more trials

[c] Example calculation for the first hour
1 ppm CO = 1.17 µg/L
Solubility of CO in water at 37 °C is 20 µg/mL, however it was ignored since the volume of the solution used (66 µL) was too small to make a difference in the results.

Convert ppm in 50 mL head space to µg: (22 ppm) * [(1.17 µg/L) / 1 ppm] * (0.050 L) = 1.29 µg
Converting µg to mol: (1.29 µg) * (1 mol /28 g) = 0.046 µmol

mol% = (0.046 µmol/4 µmol)*100 = 1.15 mol%

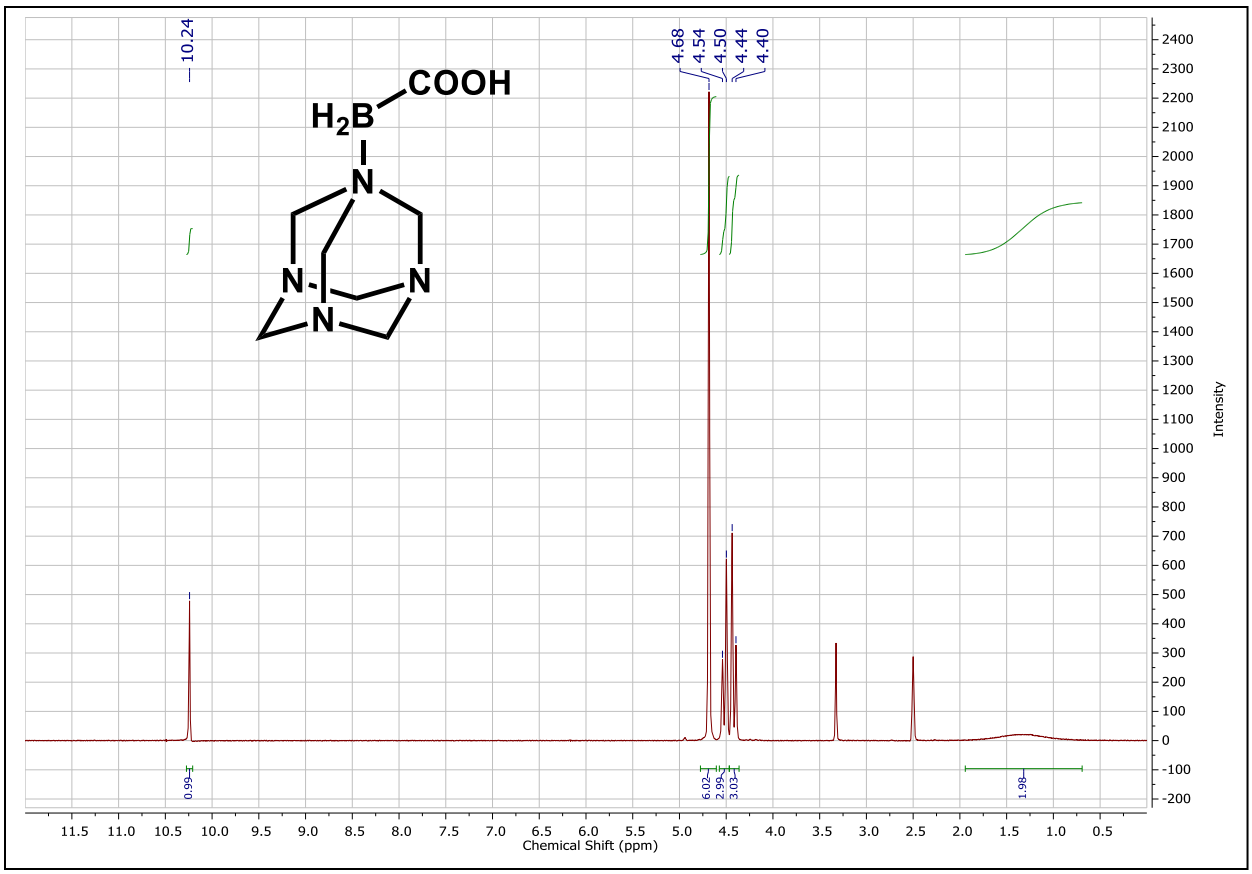


Figure S1. ^{1}H NMR of HMTA-CB in DMSO.

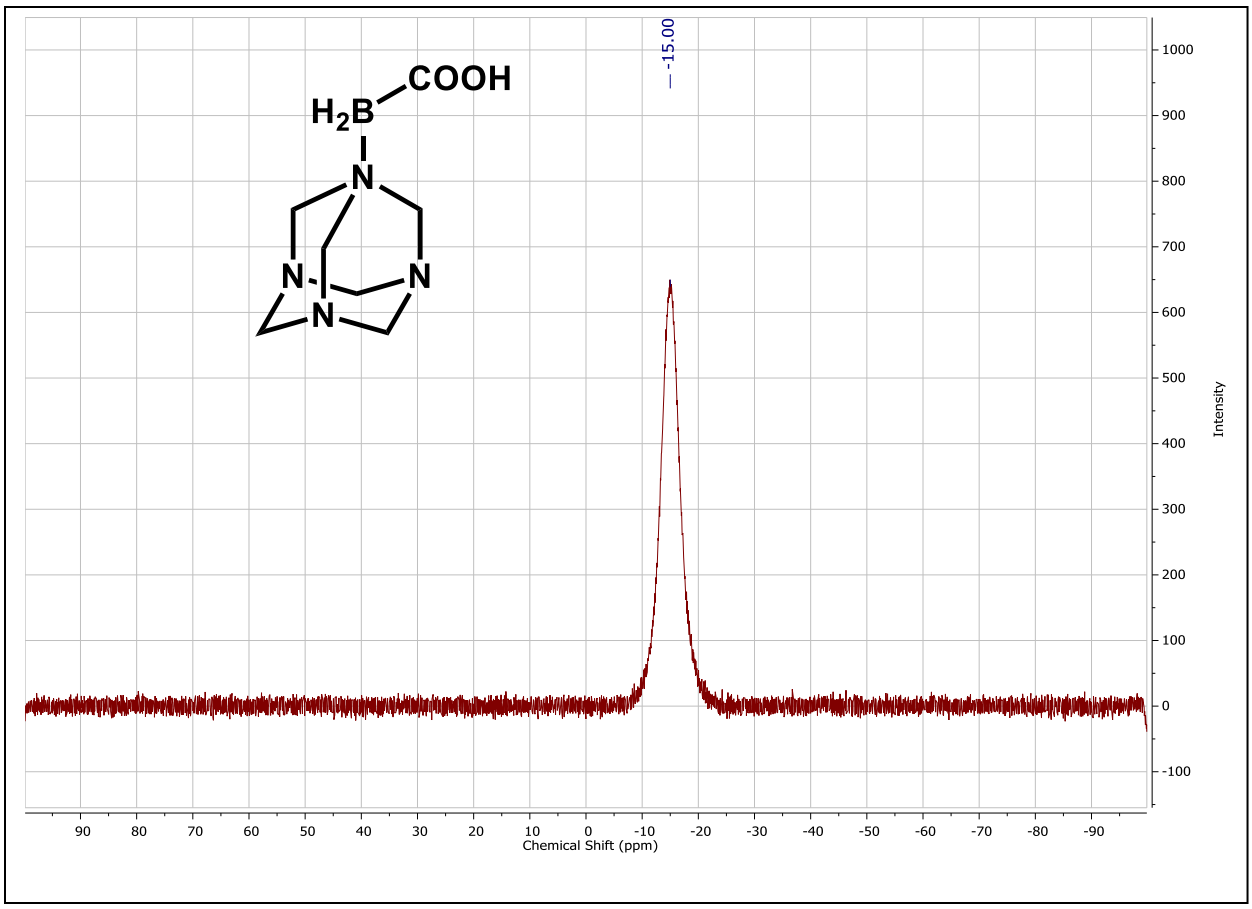


Figure S2. ^{11}B NMR of HMTA-CB in DMSO.

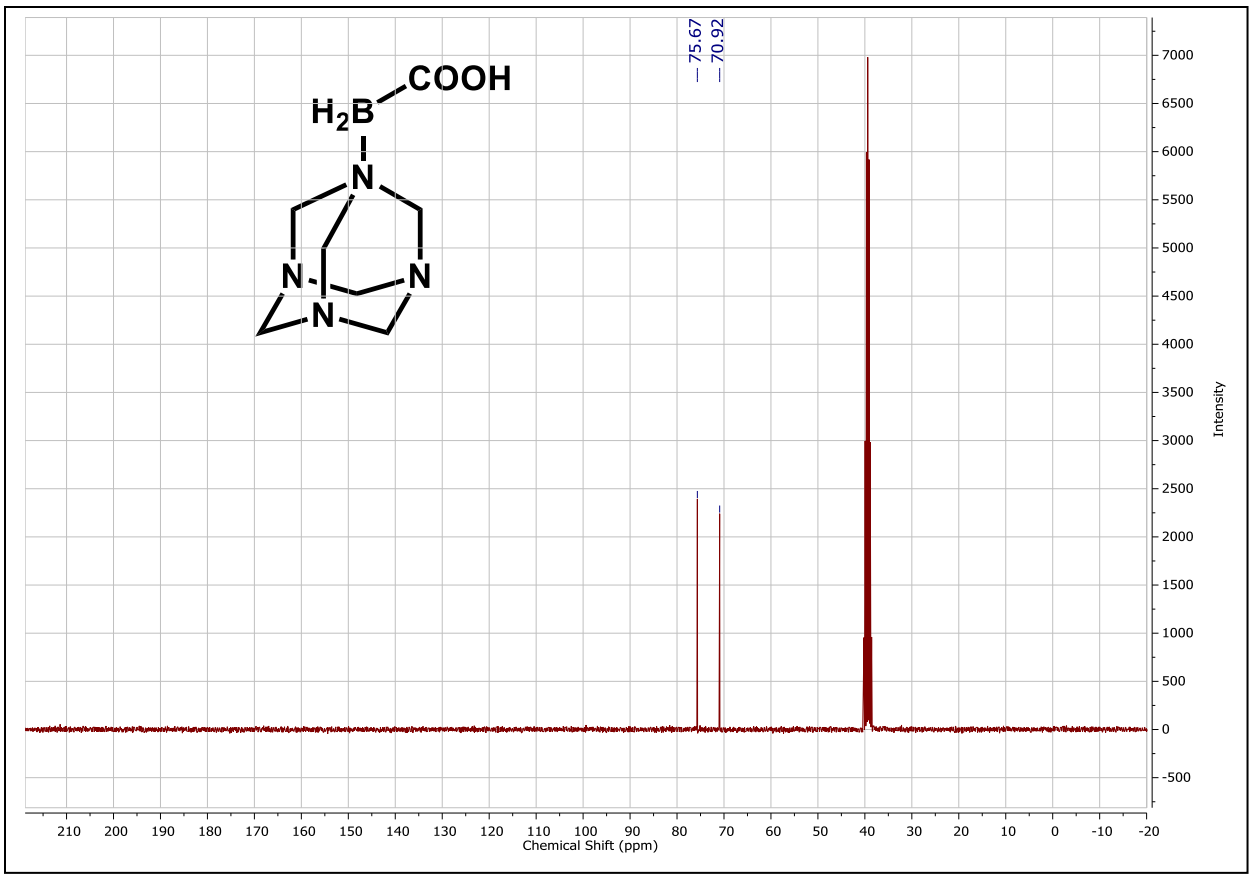


Figure S3. ^{13}C NMR of HMTA-CB in DMSO.

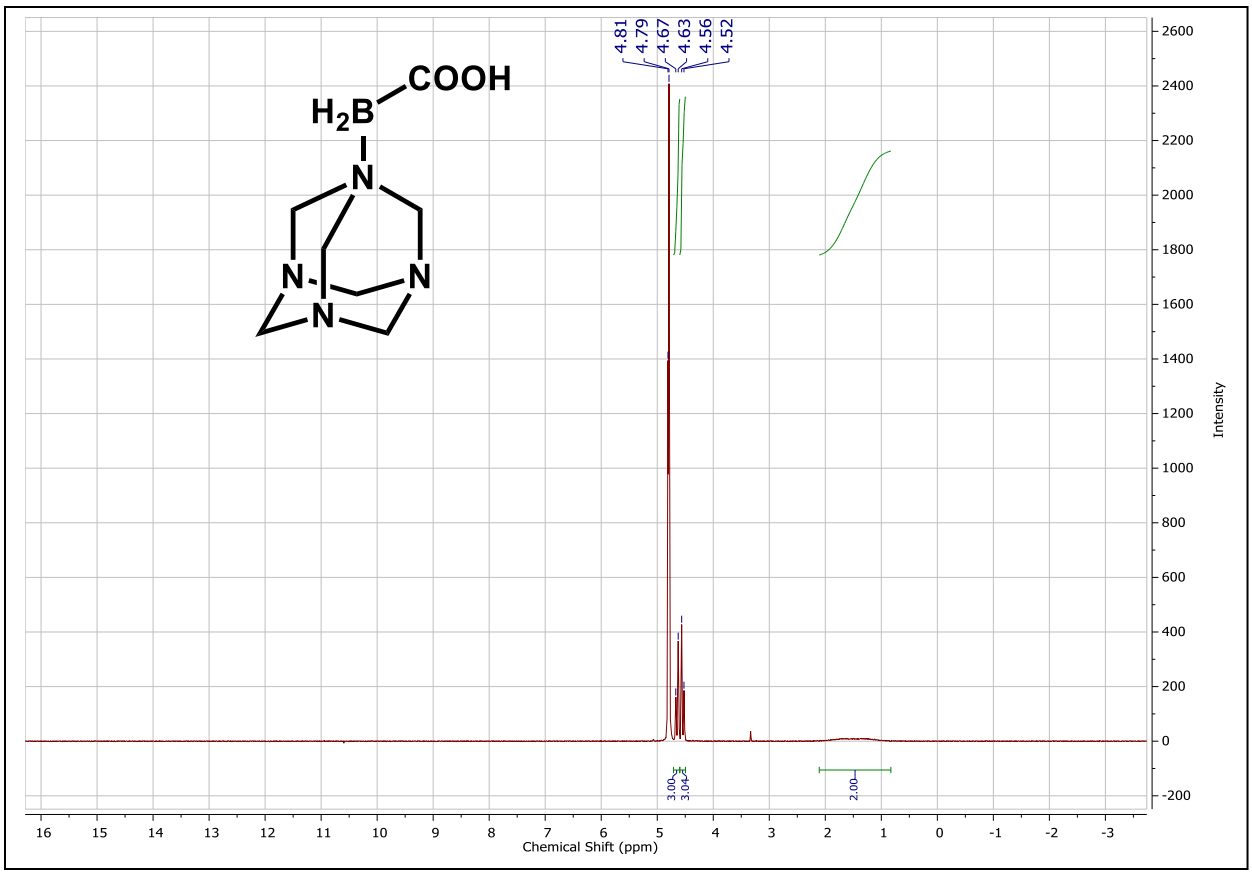


Figure S4. ^{1}H NMR of HMTA-CB in D_2O.

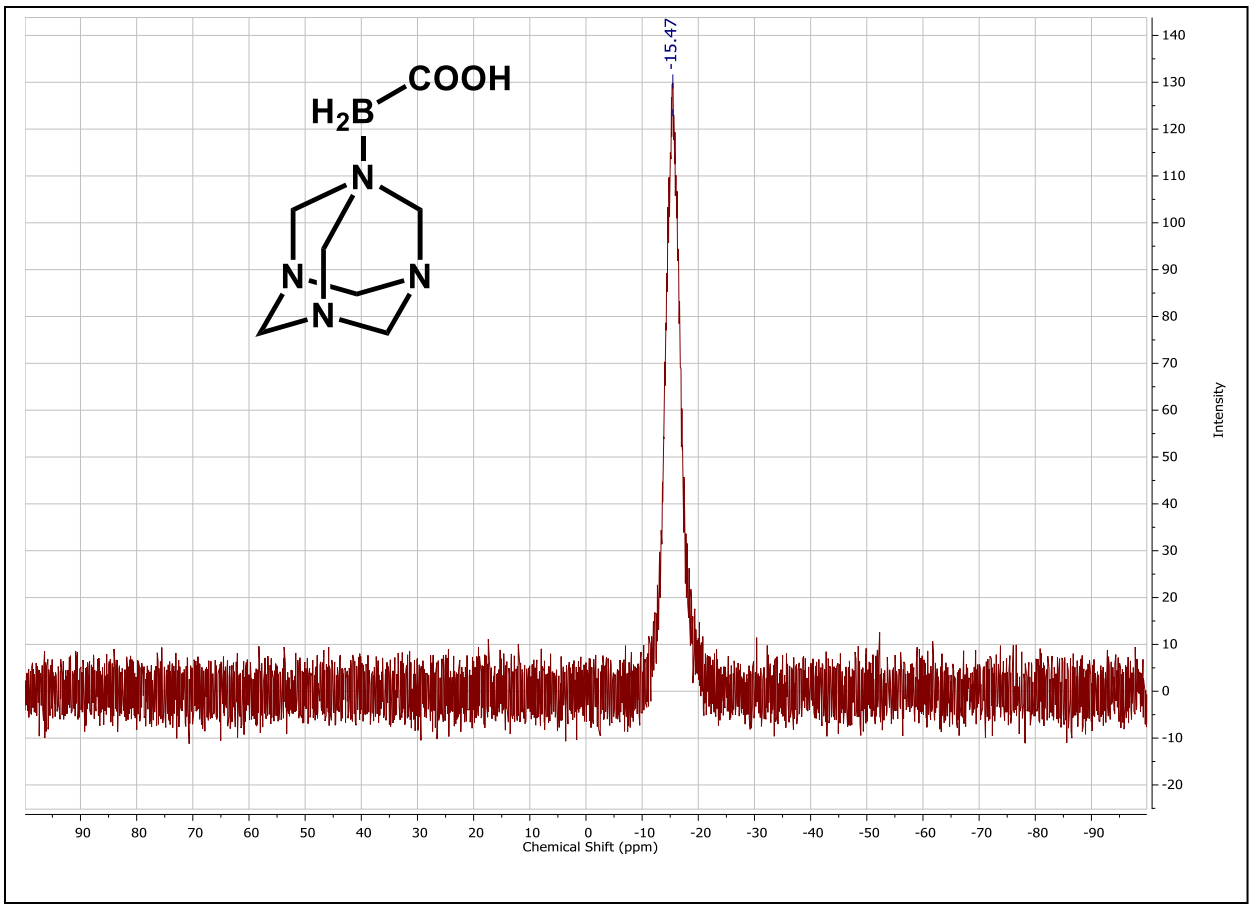


Figure S5. ^{11}B NMR of HMTA-CB in D_2O.

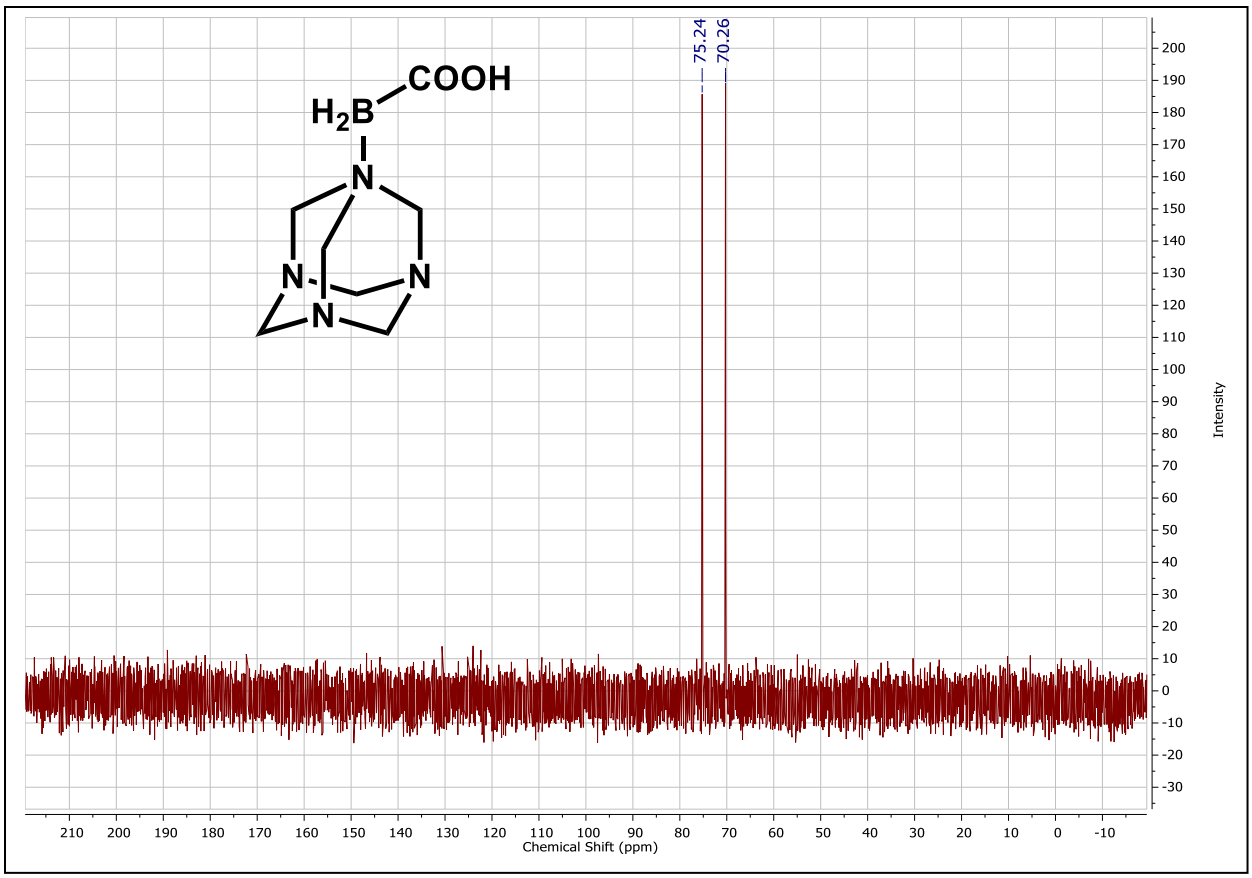


Figure S6. ^{13}C NMR of HMTA-CB in D_2O.

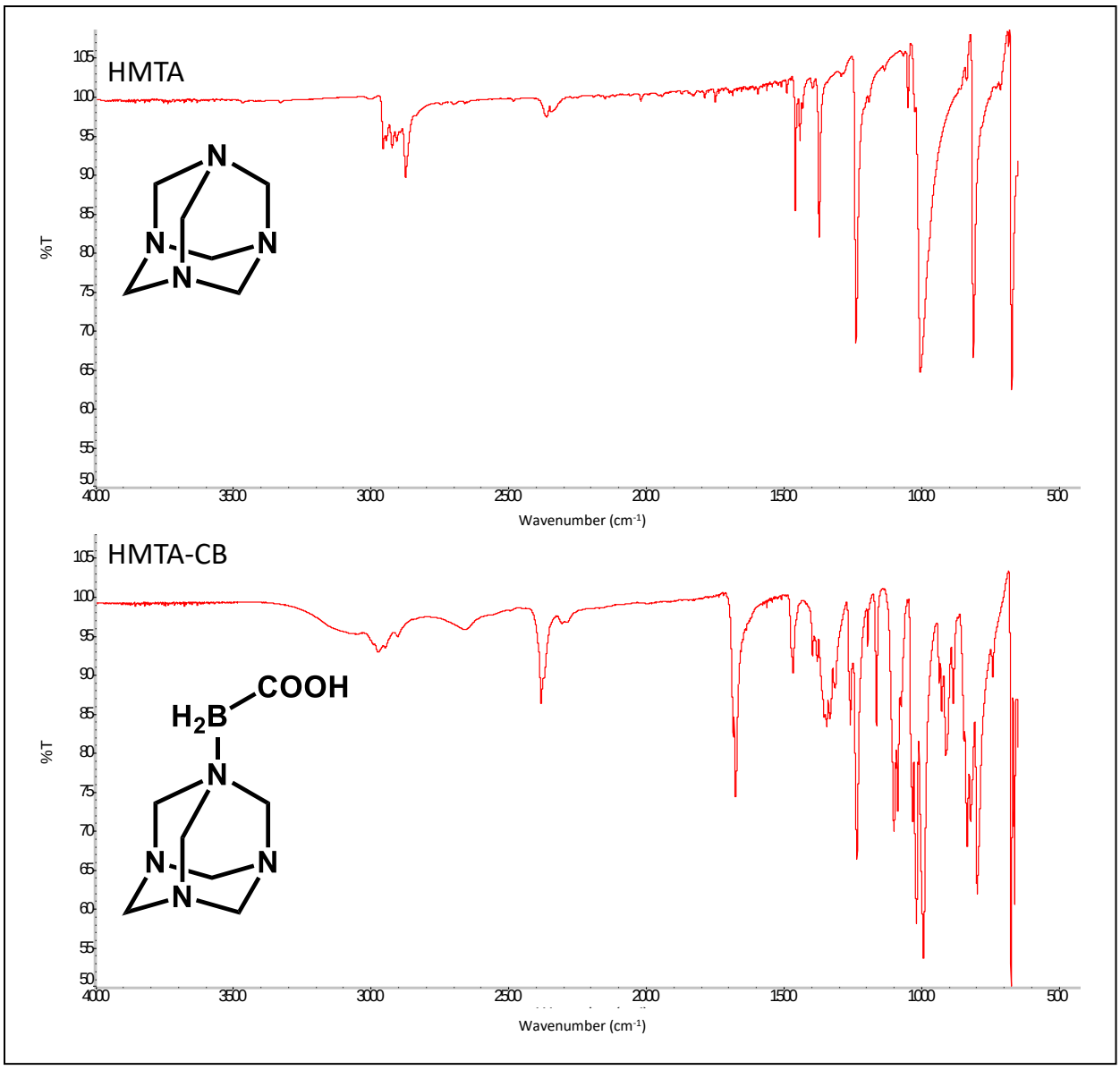


Figure S7. IR spectra of HMTA and HMTA-CB for comparison.

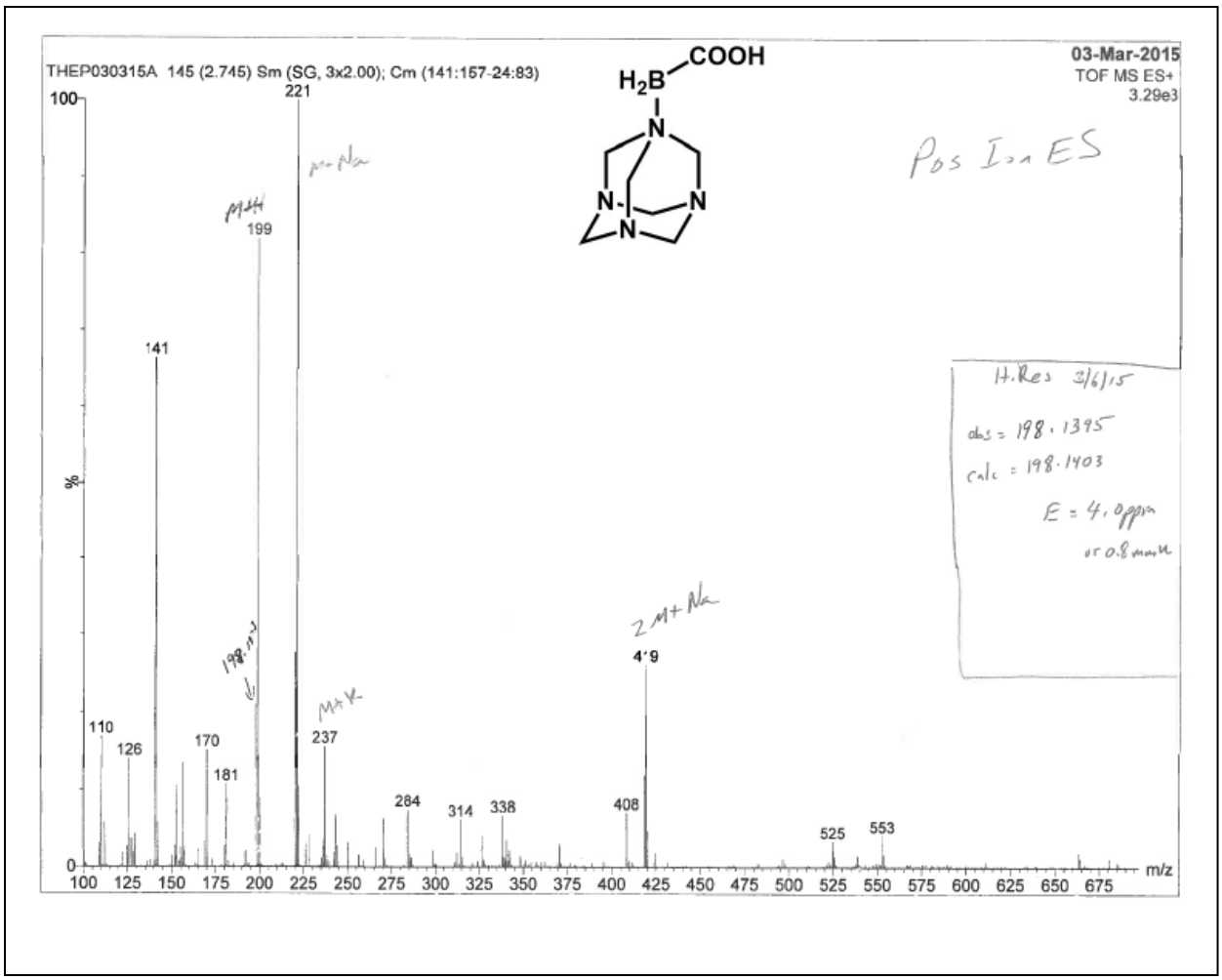
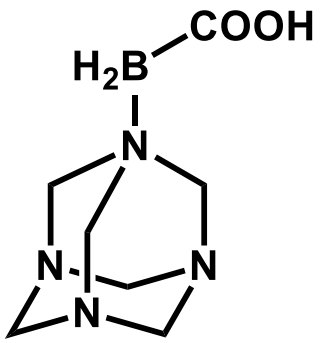


Figure S8. High Resolution Mass Spectrum of HMTA-CB.



Chemical Formula: $C_7H_{15}BN_4O_2$

Exact Mass: 198.13

Molecular Weight: 198.03

m/z: 198.13 (100.0%), 197.13 (24.8%), 199.13 (9.1%), 198.14 (1.9%)

Elemental Analysis: C, 42.46; H, 7.63; B, 5.46; N, 28.29; O, 16.16

Table S4. HMTA-CB Elemental Analysis

Elements	Calculated	Run #1	Run #2	Average	% Error
C	42.46	42.64	42.63	42.635	0.412153
H	7.63	7.66	7.65	7.655	0.327654
N	28.29	28.27	28.38	28.325	0.123719

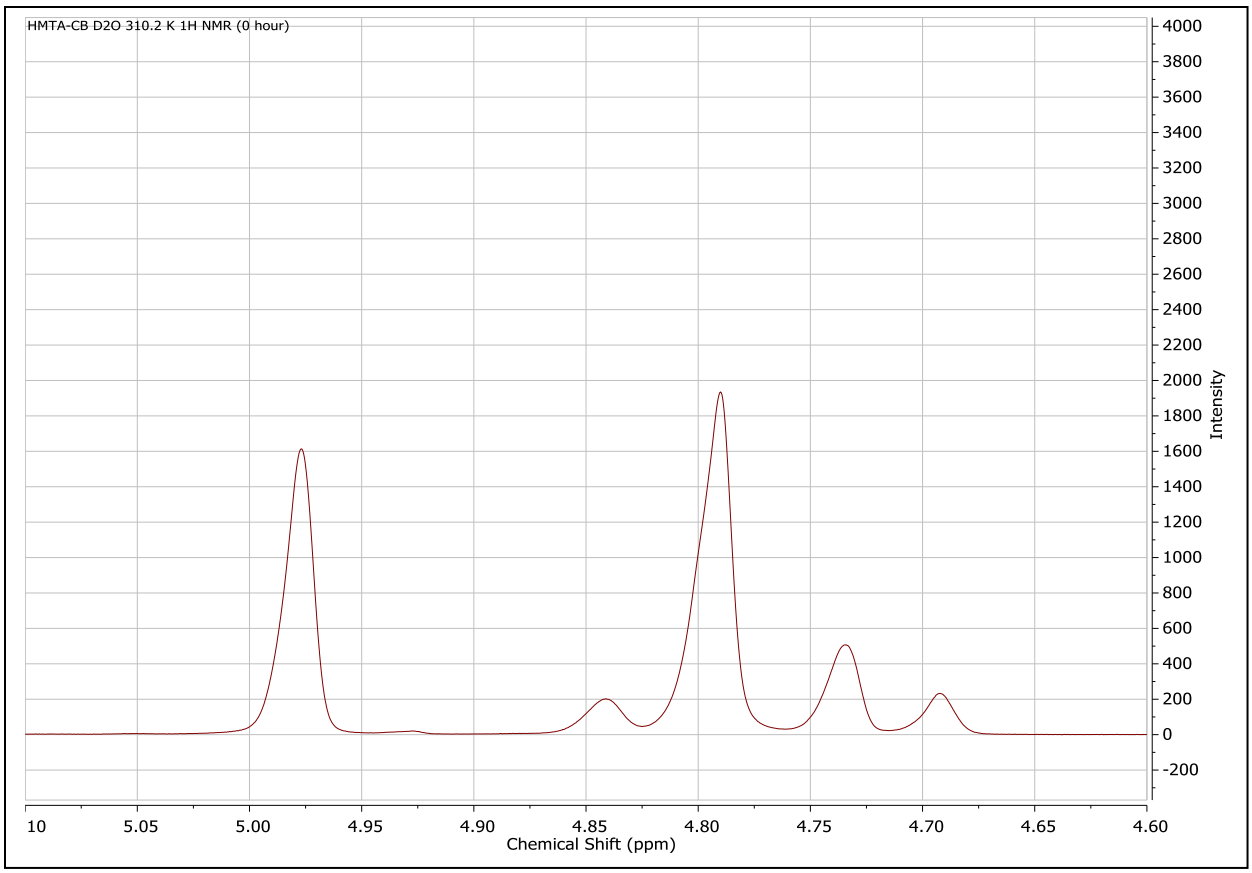


Figure S9. Decomposition of HMTA-CB monitored by ^{1}H NMR at 0 hour. HMTA-CB was dissolved in D_2O at 12 mg/mL concentration and incubated at 37 °C. Spectra were recorded at specified times. Solvent DHO at 4.79 ppm overlaps with one of the peaks from sample.

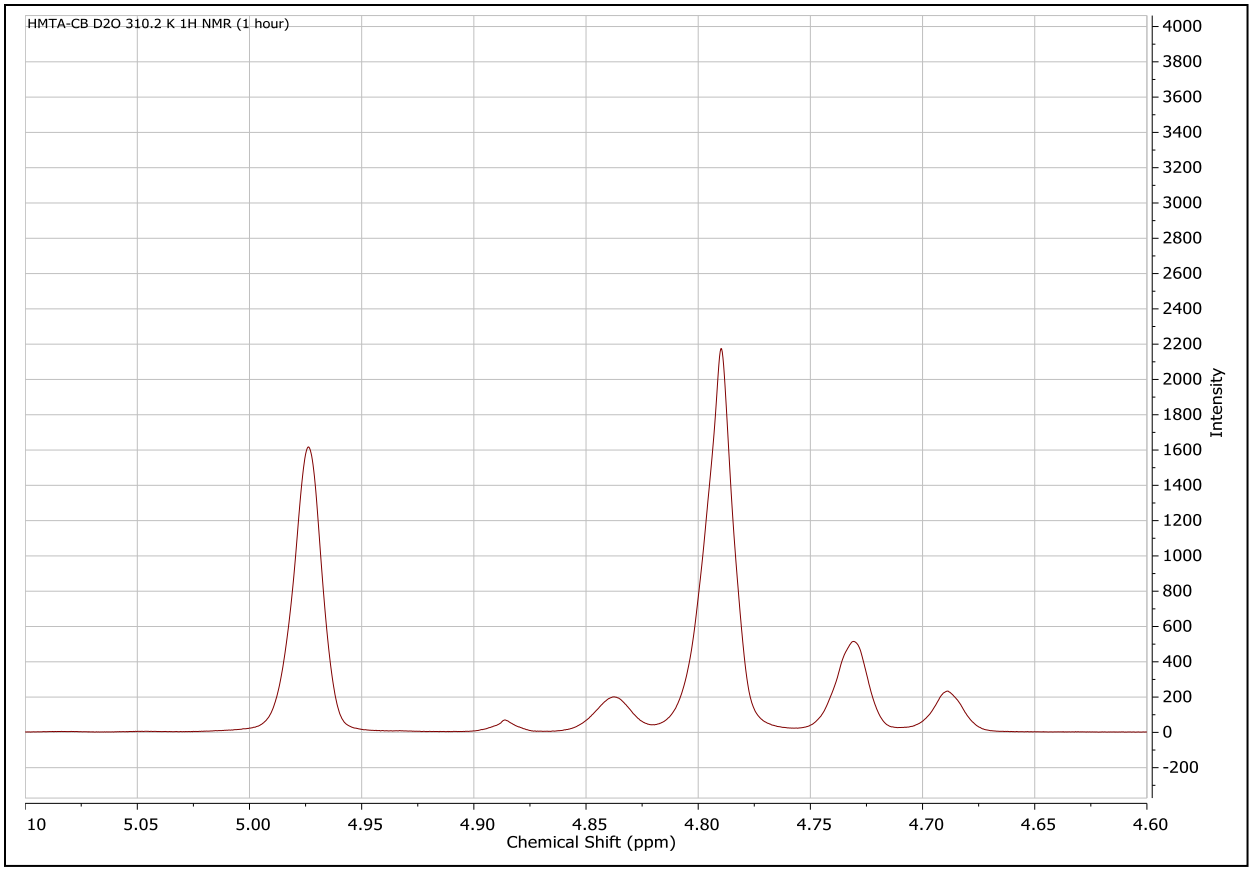


Figure S10. Decomposition of HMTA-CB monitored by ^{1}H NMR at 1 hour. HMTA-CB in D_2O at 37 °C. Solvent DHO at 4.79 ppm overlaps with one of the peaks from sample. A new peak emerges at 4.88 ppm.

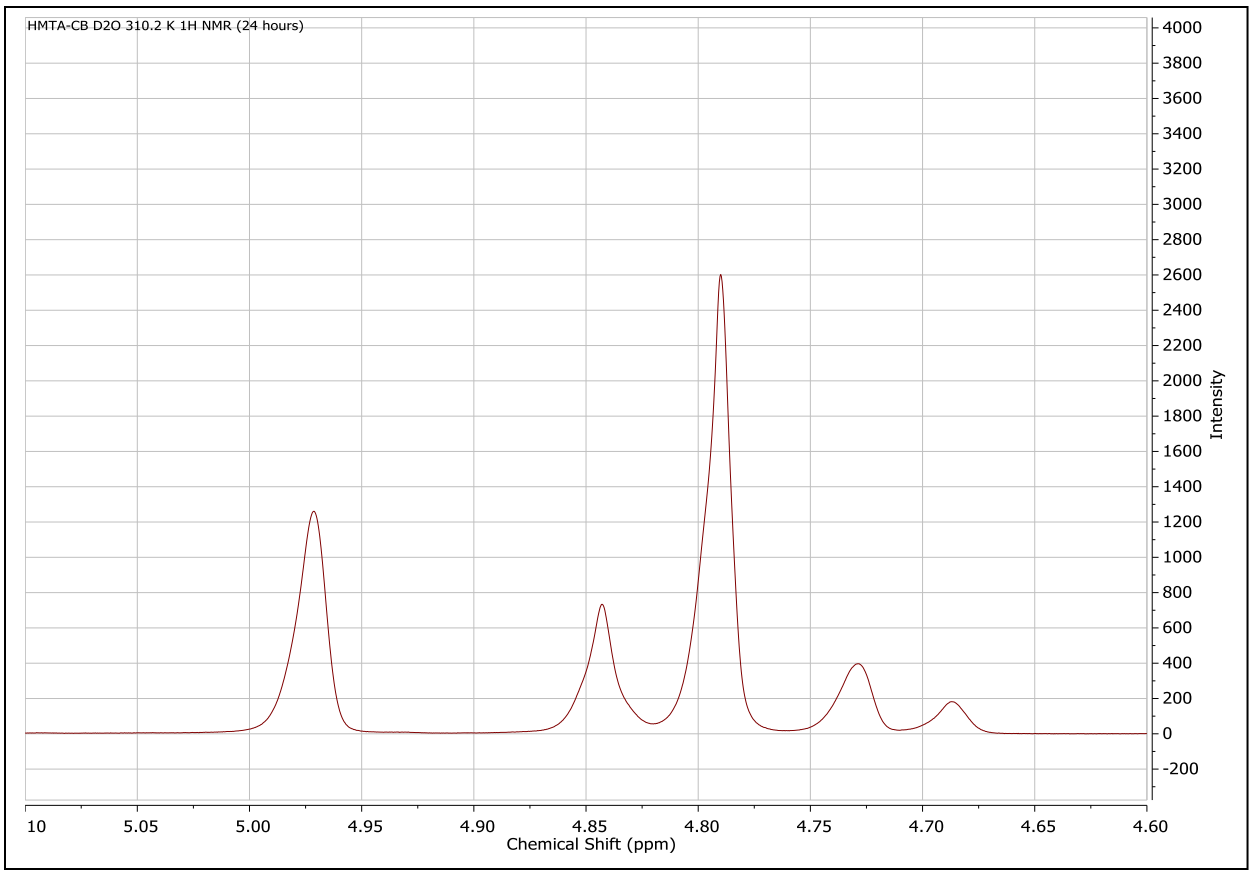


Figure S11. Decomposition of HMTA-CB monitored by ^{1}H NMR at 24 hour. HMTA-CB in D_2O at 37 °C. The new peak corresponding to the HMTA protons is now shifted to 4.84 ppm and merged with one of the peaks from HMTA-CB. For accurate estimation of the ratio, a small peak area from the far right is subtracted from the new peak.

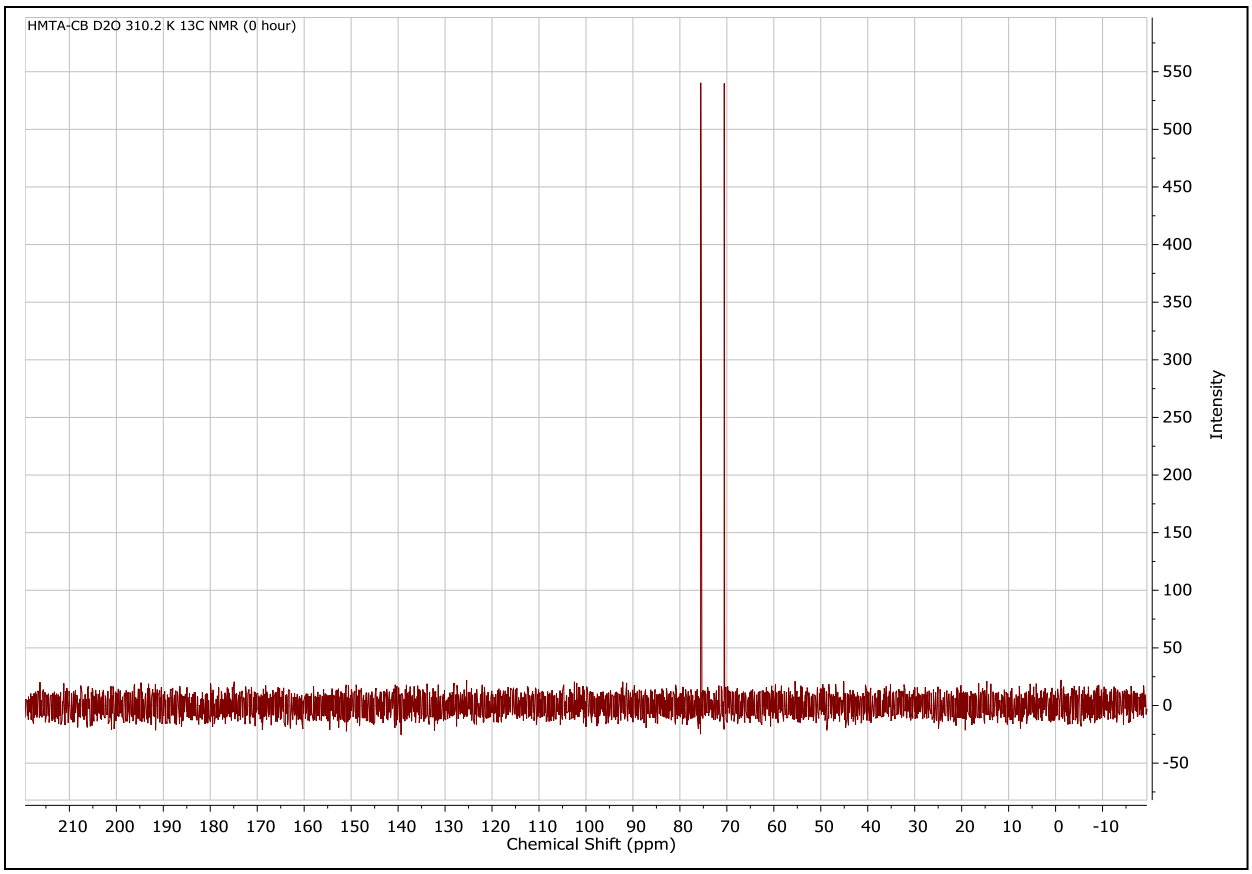


Figure S12. Decomposition of HMTA-CB monitored by ^{13}C NMR at 0 hour. HMTA-CB was dissolved in D_2O at 12 mg/mL concentration and incubated at 37 °C. Spectra were recorded at specified times.

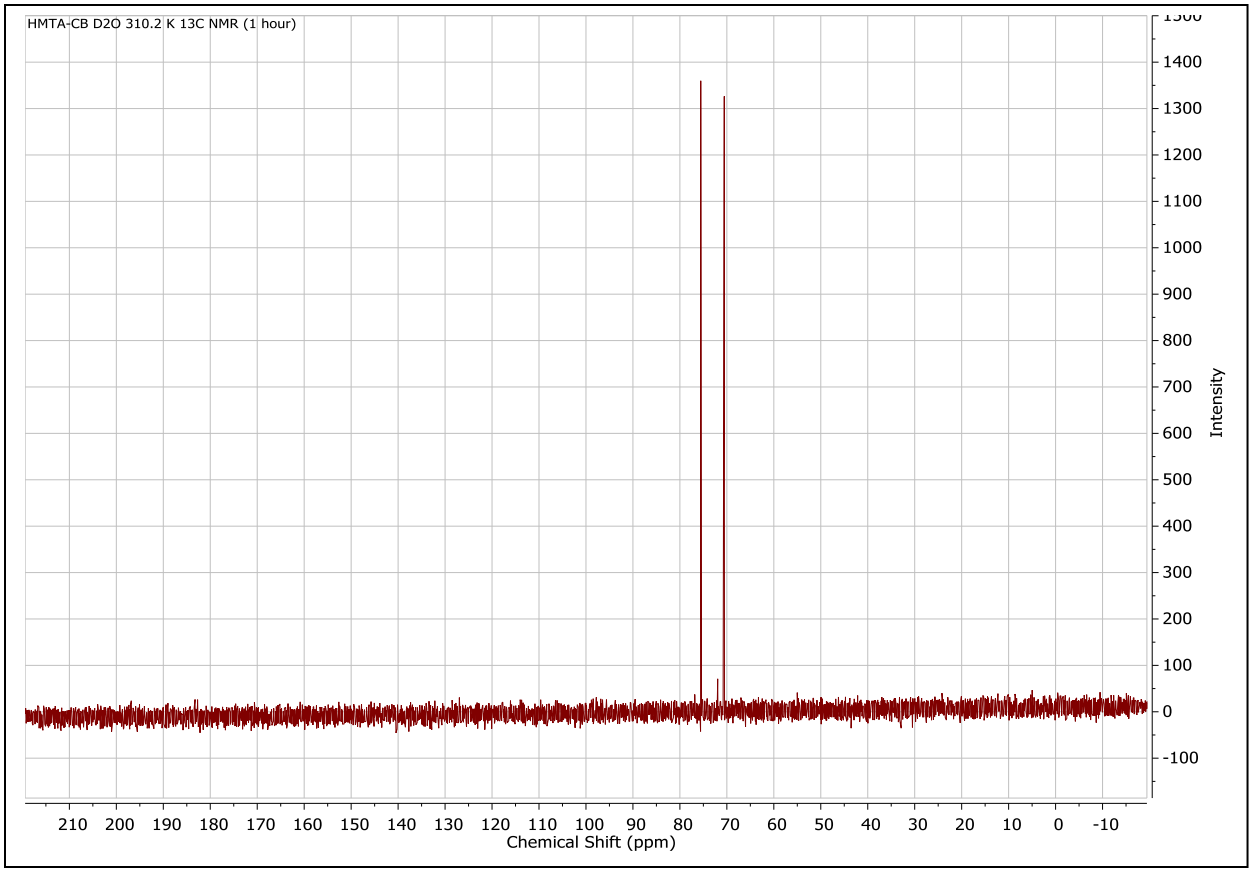


Figure S13. Decomposition of HMTA-CB monitored by ^{13}C NMR at 1 hour. HMTA-CB in D_2O at 37 °C. The presence of free HMTA was determined by the appearance of a singlet peak at 72.00 ppm.

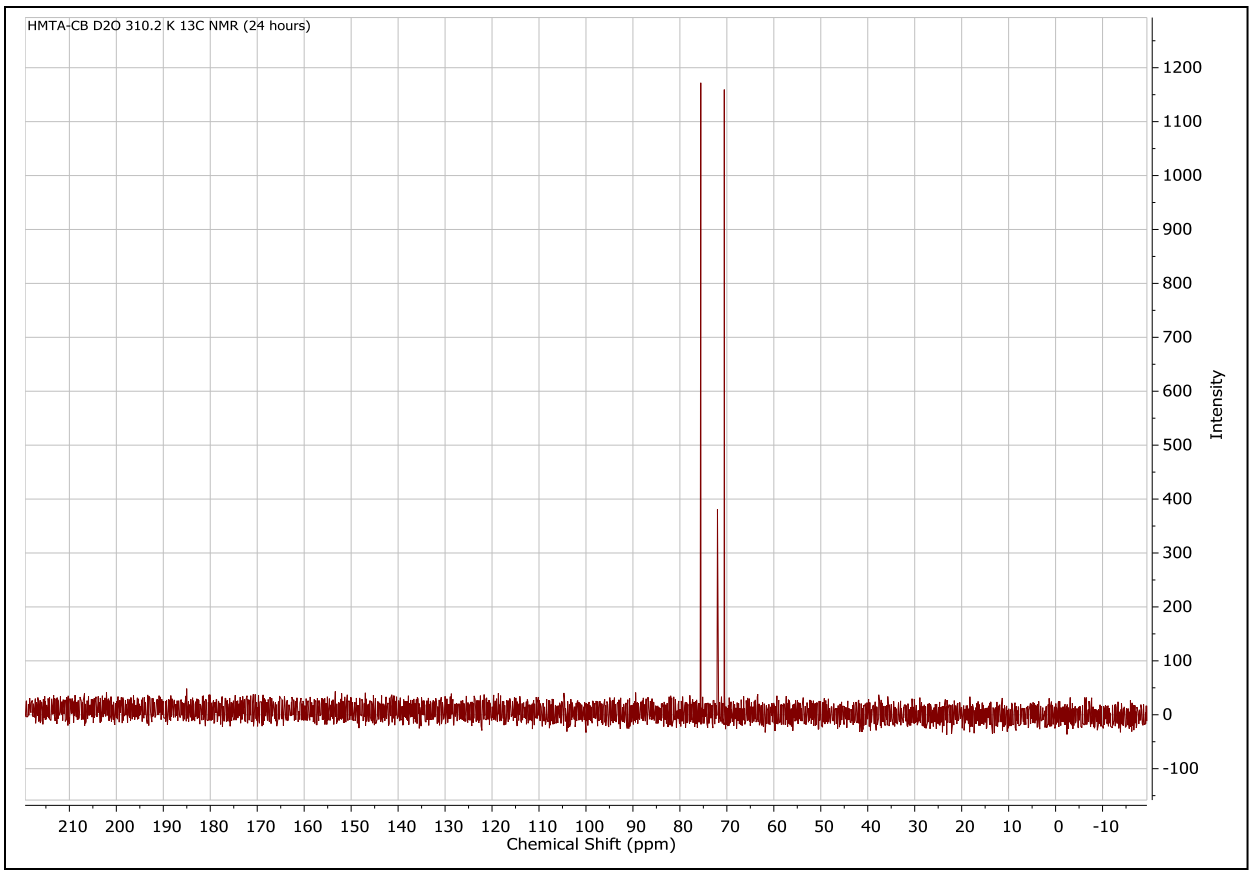


Figure S14. Decomposition of HMTA-CB monitored by ^{13}C NMR at 24 hour. HMTA-CB in D_2O at 37 °C. HMTA peak at 72.00 ppm gets taller with time.

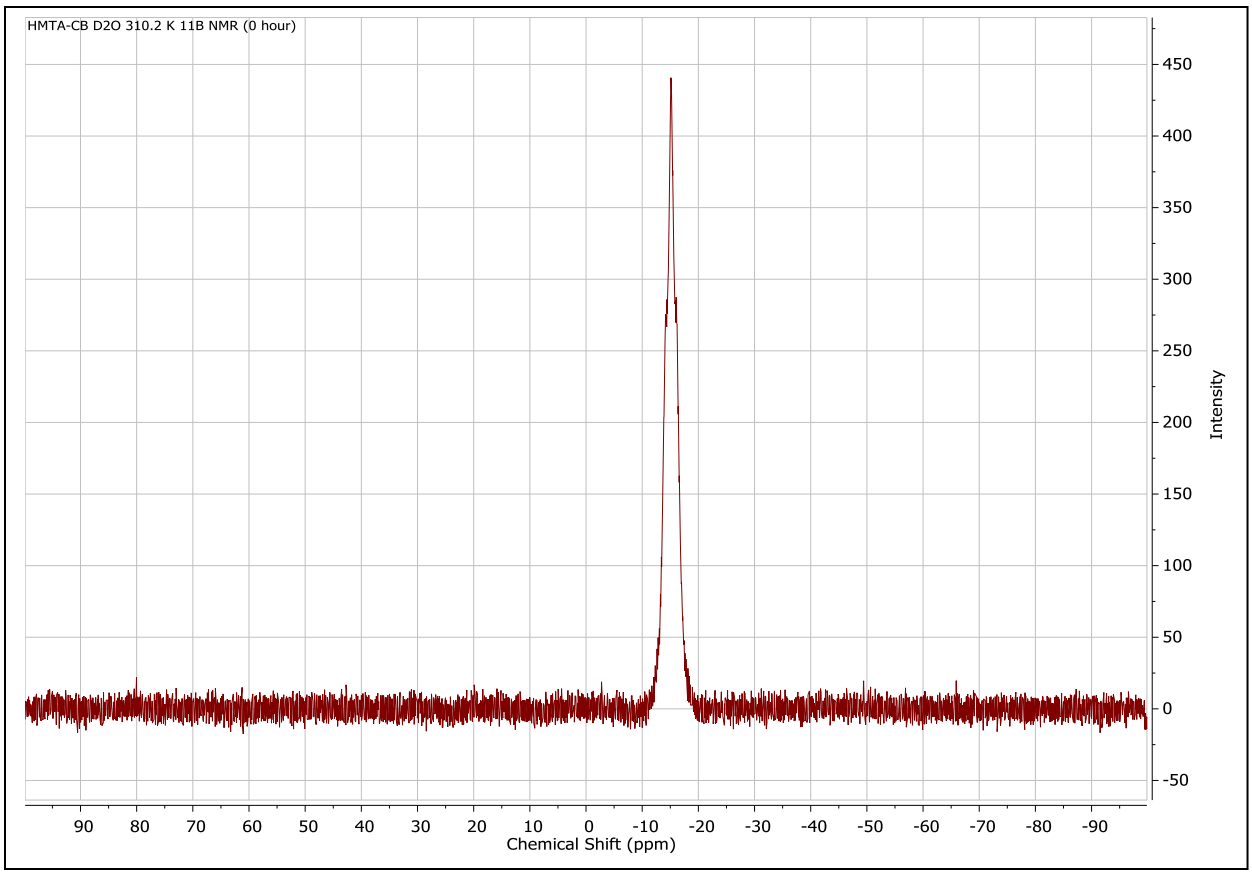


Figure S15. Decomposition of HMTA-CB monitored by ^{11}B NMR at 0 hour. HMTA-CB was dissolved in D_2O at 12 mg/mL concentration and incubated at 37 °C. Spectra were recorded at specified times.

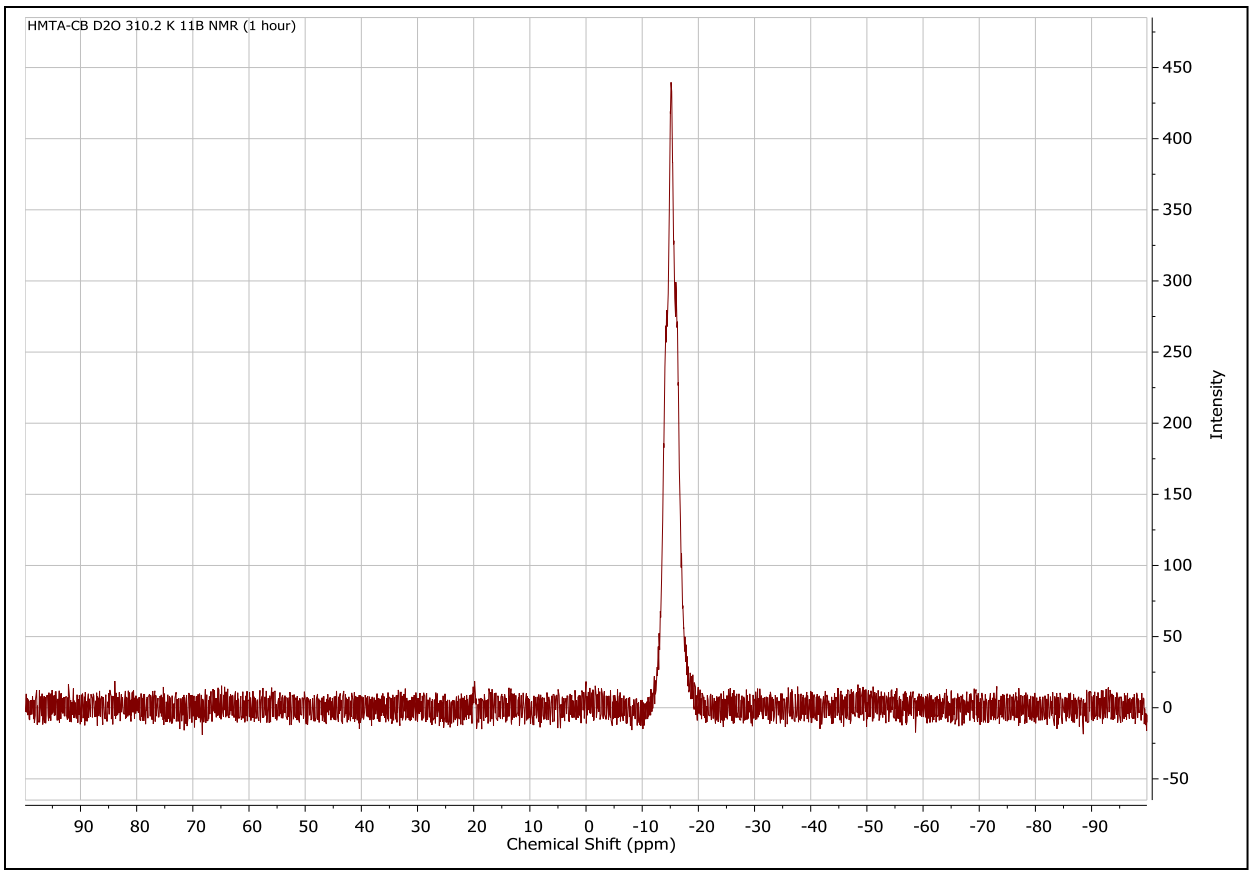


Figure S16. Decomposition of HMTA-CB monitored by ^{11}B NMR at 1 hour. HMTA-CB in D_2O at 37 °C. The boron atom on HMTA-CB is a triplet at -15.47 ppm in D_2O while the product of decomposition, boric acid, shows a weak singlet at 19.54 ppm.

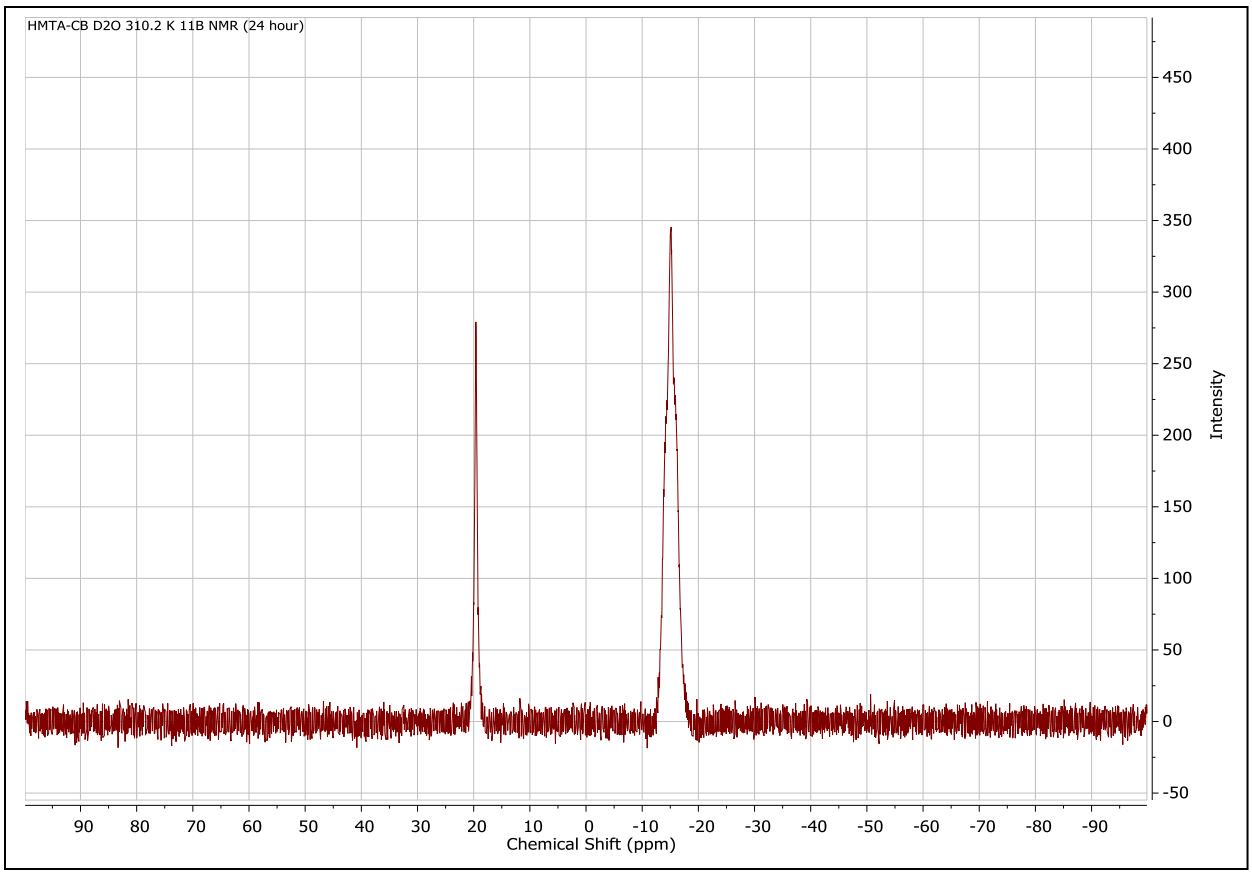


Figure S17. Decomposition of HMTA-CB monitored by ^{11}B NMR at 24 hour. HMTA-CB in D_2O at 37 °C. Boric acid peak at 19.54 ppm gets taller with time.

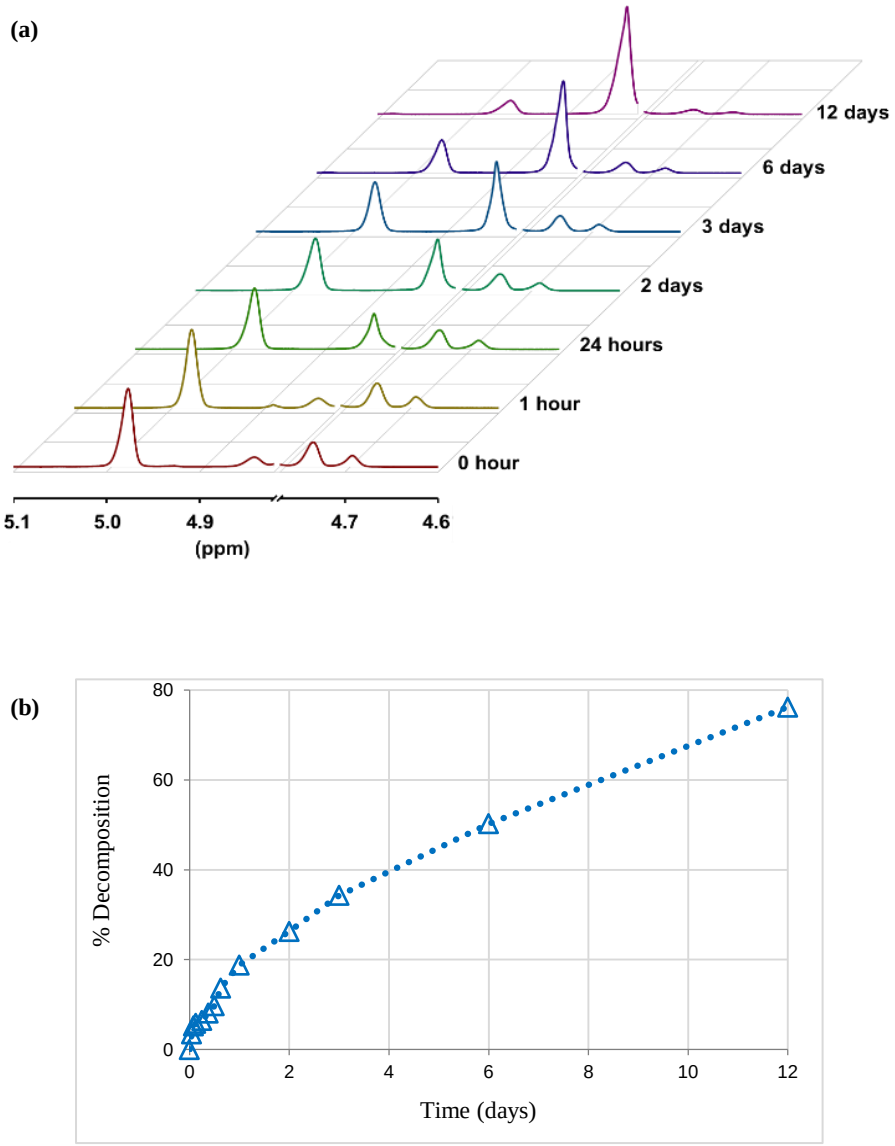


Figure S18. Percent decomposition determined by peak integration of ^{1}H NMR over a period of 12 days. 60 mM HMTA-CB solution in D_2O was incubated at 37 °C and the spectra taken at specified times. (a) Overlaid ^{1}H NMR spectra taken at 0, 1, 2, 3, 6, and 12 days. Disappearance of HMTA-CB peaks at 4.97, 4.73, and 4.69 ppm is compared with the appearance of HMTA peak at 4.84 ppm. Note: HDO peak at δ 4.79 ppm is removed from stacked spectra for clearer view of the peaks. (b) Kinetics of CO release process monitored by ^{1}H NMR. Integrated ratios of the protons are used for calculating percent decomposition.

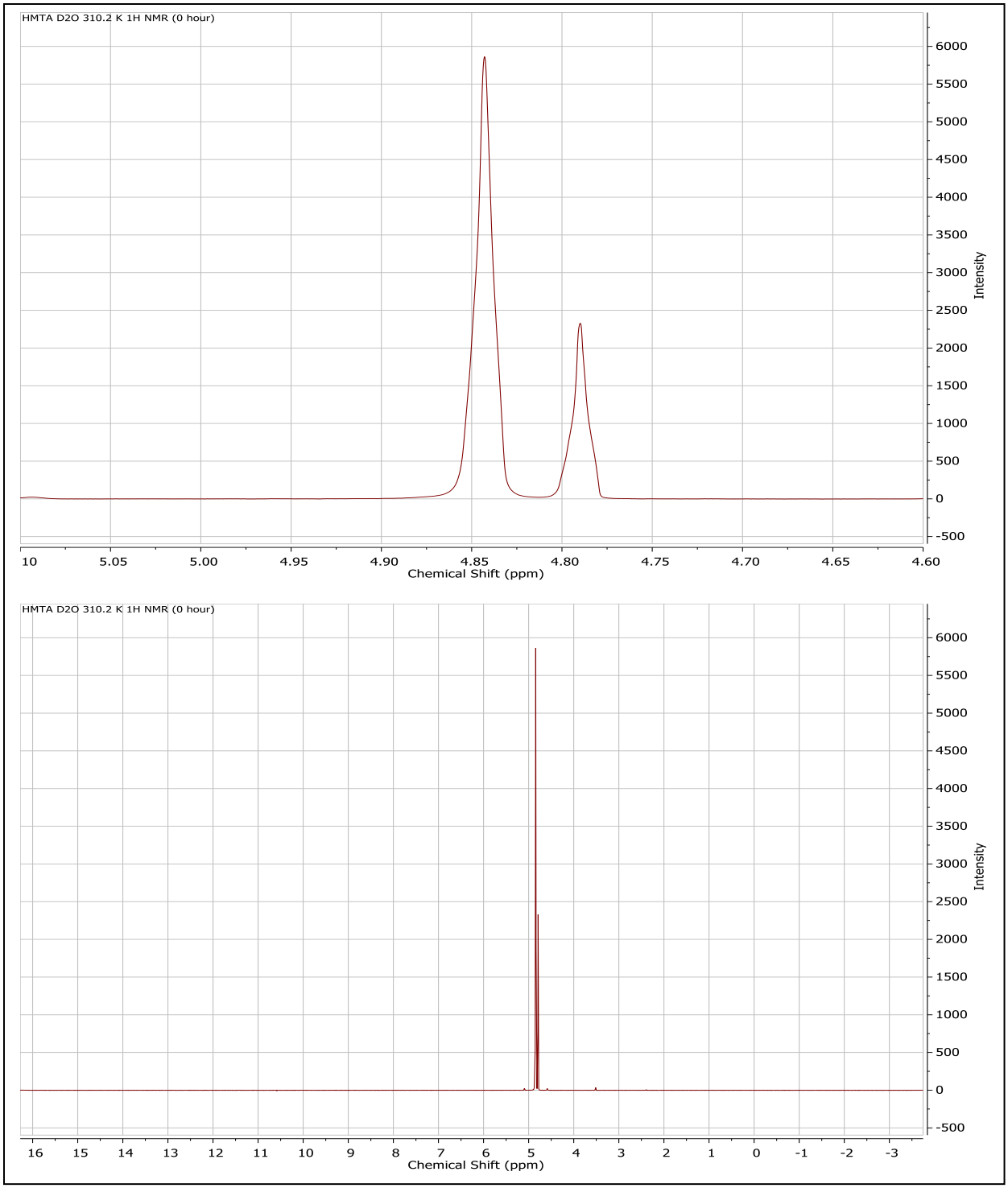


Figure S19. Stability test of HMTA monitored by ^{1}H NMR at 0 hour. HMTA was dissolved in D_2O at 12 mg/mL concentration and incubated at 37 °C. The solvent, DHO, peak shows at 4.79 ppm (top).

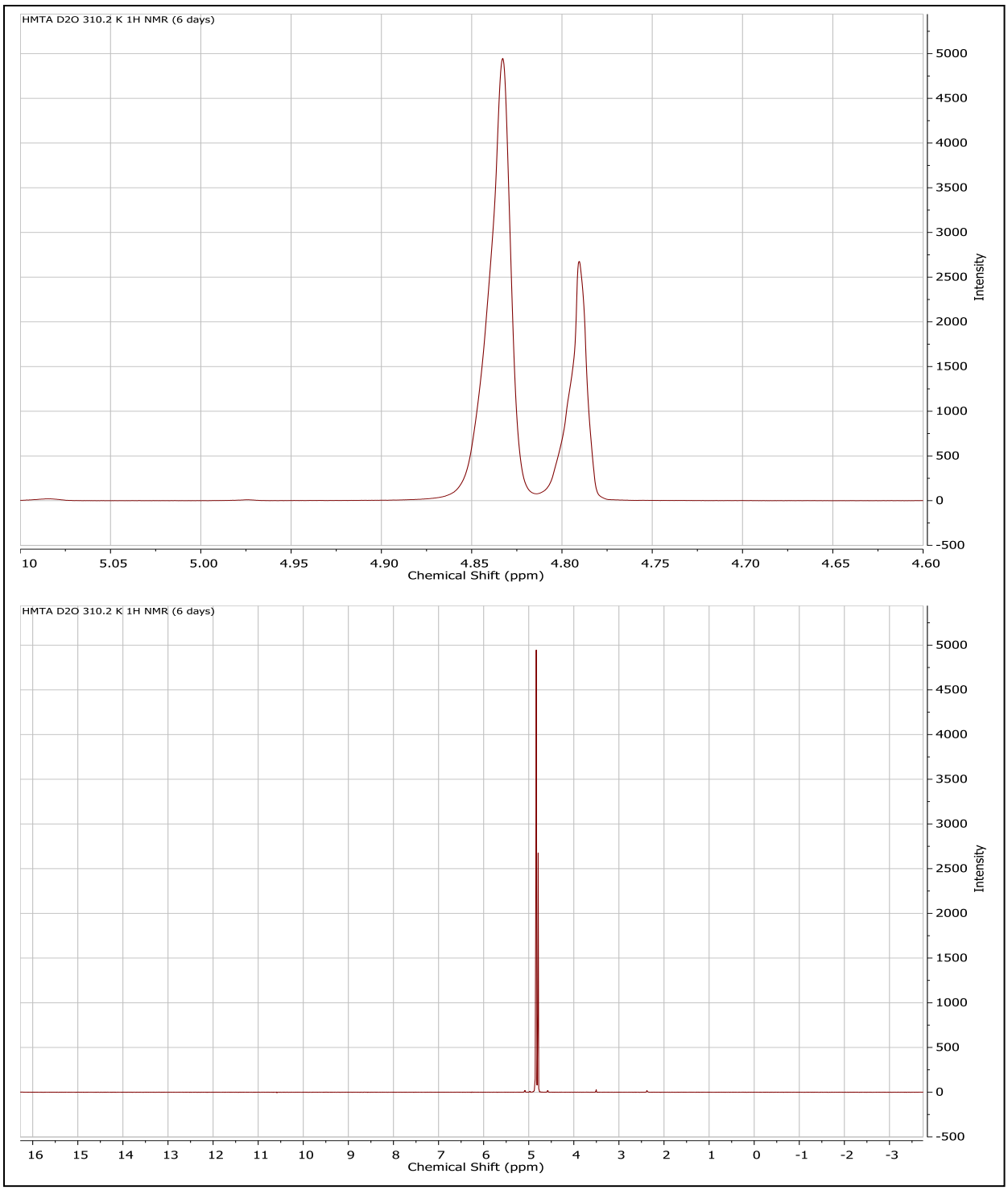


Figure S20. Stability test of HMTA monitored by ^{1}H NMR at 6 days. The solvent, DHO, peak shows at 4.79 ppm (top). No new peaks are observed on the spectrum (bottom).

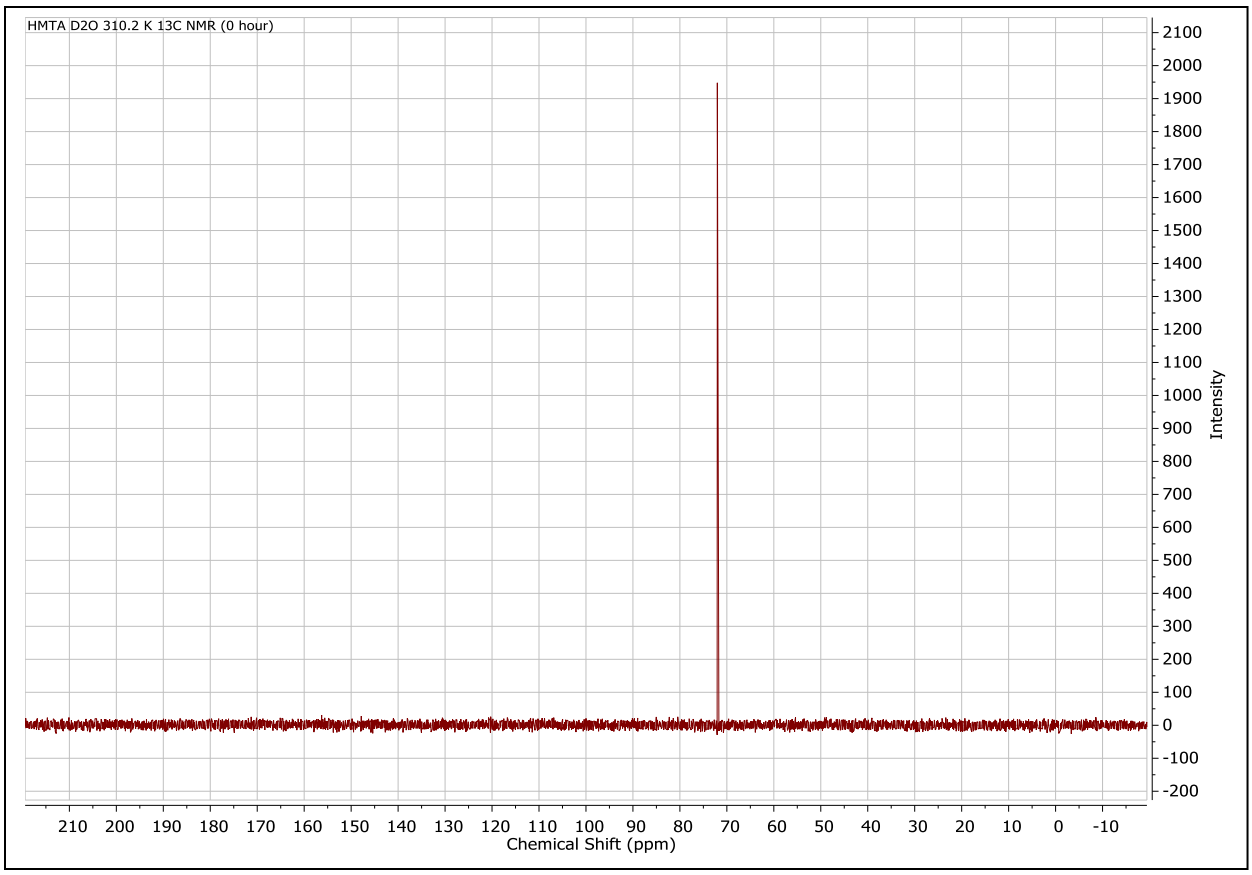


Figure S21. Stability test of HMTA monitored by ^{13}C NMR at 0 hour. HMTA was dissolved in D_2O at 12 mg/mL concentration and incubated at 37 °C. A single peak corresponding to 6 equivalent carbons of HMTA is shown at 72.00 ppm.

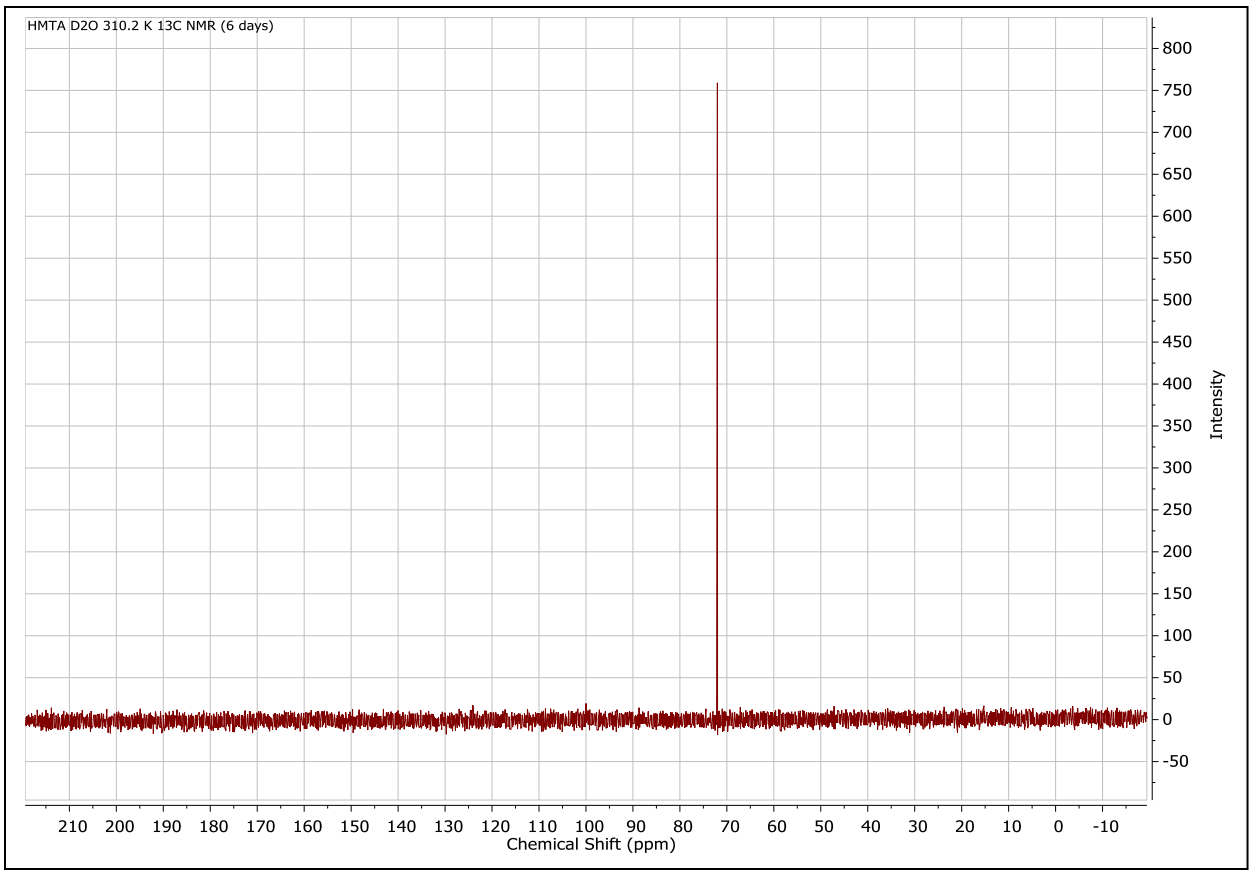


Figure S22. Stability test of HMTA monitored by ^{13}C NMR at 6 days. No new peaks are observed on the spectrum.