Electronic Supplementary Information (ESI) for

Dicarboxylic Acids as pH Sensors for Hyperpolarized ^{13}C Magnetic Resonance Spectroscopic Imaging

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Contents

Chemicals

Diethylmalonic acid, mellitic acid, trans-1,2-cyclohexanedicarboxylic acid, (±)-cis-cyclopentane-dicarboxylic acid, and tert-butan-1-^{13}C, d_9-ol were purchased from Aldrich (St. Louis, MO). Dimethylmalonic acid, phthalic acid, 1,1-cyclopropanedicarboxylic acid, cis-1,2-cyclohexanedicarboxylic acid, and 1,1-cyclobutanedicarboxylic acid were purchased from Combi-Blocks (San Diego, CA). Trans-cyclopentane-1,2-dicarboxylic acid was purchased from Ark Pharm (Libertyville, IL). Cis-1,2-cyclohexanedicarboxylic acid was purchased from TCI America (Portland, OR). Succinic acid was purchased from EMD Millipore (Billerica, MA). Tetramethylsuccinic acid was purchased from Apollo Scientific Ltd (Denton, Manchester, UK). All substrates for chemical synthesis, including [2-^{13}C]diethylmalonate, [D_5]bromoethane, and [D_4]1,2-dibromoethane, were purchased from Aldrich and were used without further purification. The OX063 and Finland trityl radicals [1] were purchased from GE Healthcare (Menlo Park, CA). Other chemicals and solvents were purchased from Aldrich (St. Louis, MO).

^{13}C-NMR spectroscopy of dicarboxylic acids at 11.7 T

Each of the compounds listed in Table 1 below was dissolved in deionized H_2O at 250 mM along with an equal concentration of urea. None of the compounds were isotopically enriched at any position. Each solution was titrated with HCl or NaOH to pH 6.5 and pH 7.4, as measured using a standard laboratory pH meter. The solution was then transferred to a capped 5 mm NMR tube and placed inside a 37 °C temperature-regulated 500 MHz Varian INOVA spectrometer (Agilent Technologies, Palo Alto, CA) equipped with a 5 mm triple-tuned, direct-detect, triple-axis gradient-equipped broadband probe (Agilent Technologies, Palo Alto, CA). ^{13}C NMR was performed (TR = 7 s, 28° hard pulses, 30 kHz spectral width, 60k FID points, 16-103 transients), the acquired spectra were referenced to ^{13}C-urea at 163.7 ppm, and the chemical shift change between 6.5 and 7.4 was measured for each ^{13}C nucleus. Tetramethyl succinate was not measured due to insufficient solubility in H_2O.

Table S1: Dicarboxylic acids and reported pK_a values

Molecule name	Dimethyl-malonic acid	Diethyl-malonic acid	1,1-cyclopropane-dicarboxylic acid	1,1-cyclobutane-dicarboxylic acid	1,2-cyclopentane-dicarboxylic acid		1,2-cyclohexane-dicarboxylic acid	
					cis	trans	cis	trans
$pK_{a,2}$	6.06	7.29	7.43	*	6.51	5.91	6.76	5.93
Ref	[2]	[2]	[3]	*	[2]	[2]	[2]	[2]

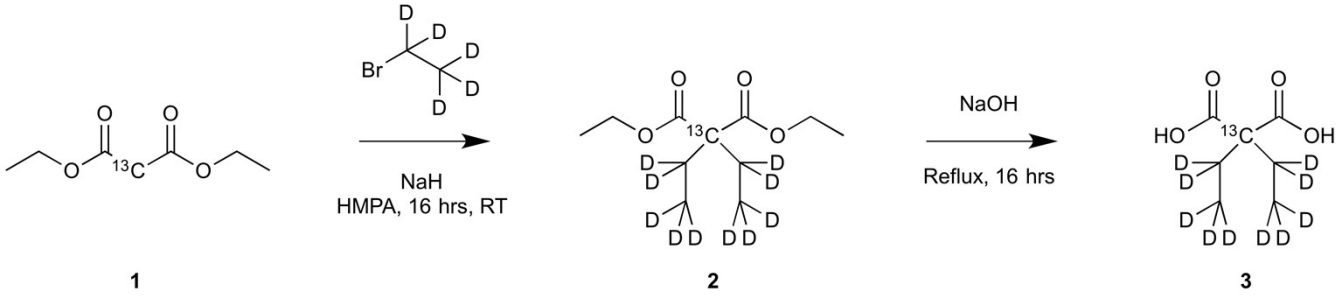
Molecule name	Succinic acid	Phthalic acid	Mellitic acid
$pK_{a,2}$	5.48	5.41	2.19
$pK_{a,6}$	----------	----------	6.96
Ref	[2]	[2]	[2]

*No literature value for $pK_{a,2}$

Syntheses of [2-^{13}C,D_{10}]diethylmalonic acid (DEMA) and [2-^{13}C,D_4]cyclopropane-1,1-dicarboxylic acid (CPDA)

^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 MHz spectrometer. ^{13}C spectra were acquired with ^{1}H decoupling on. Chemical shifts are reported in parts per million (ppm) downfield from residual solvent peaks and coupling constants are reported in Hertz (Hz). Splitting patterns are designated as singlet (s), doublet (d), triplet (t), or quadruplet (q). Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m). High resolution mass spectrometry was performed by the Notre-Dame mass spectrometry facility on a microTOF instrument (Notre Dame, IN). Deuterium incorporation was confirmed by mass spectrometry and by the decrease of ^{1}H and ^{13}C NMR intensities at the specified positions compared to the starting material. All NMR spectra are located in the Appendix.

*[2-^{13}C,D_{10}]2,2-diethylmalonic acid **3**:*



1 **2** **3**

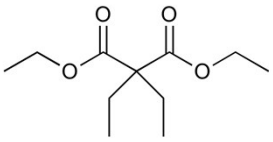
Scheme S1: Synthesis of [2-^{13}C,D_{10}]2,2-diethylmalonic acid **3**.

Synthesis of [2-^{13}C,D_{10}]diethyl 2,2-diethylmalonate 2: This method was based on a previously described protocol.[4] To 900 mg of sodium hydride, 60% dispersion in mineral oil (22.5 mmol), was added 20 mL of anhydrous hexamethylphosphoramide (HMPA), under nitrogen atmosphere. To the resulting solution was added 1 g (6.3 mmol) [2-^{13}C]diethylmalonate **1**, dissolved in 8 mL of anhydrous HMPA. The solution was stirred under nitrogen atmosphere for 30 minutes. To this solution was added 1.801 g (1.179 mL, 15.8 mmol) of [D_5]bromoethane. The resulting mixture was stirred for 16 hours at room temperature. Then, 25 mL of an ice/water mixture was added to the solution. The resulting aqueous solution was extracted twice with hexanes. The organic fraction was dried over magnesium sulfate and the solvent evaporated, yielding a yellowish oil. Purification by column chromatography on silica gel (hexane/AcOEt 98:2) afforded [2-^{13}C,D_{10}]diethyl 2,2-diethylmalonate **2** as a clear oil (1.07 g, 4.71 mmol, 75%).

^{1}H NMR (400 MHz, DMSO-d6): δ ppm 1.15 (t, J = 7.06 Hz, 6 H, 2 CH_3) 4.11 (q, J = 7.06 Hz, 4 H, 2 CH_2)

^{13}C NMR (100 MHz, DMSO-d6): δ ppm 13.92, 57.25 (enriched), 60.58, 170.98 (d, J = 56 Hz)

Reference material, diethyl 2,2-diethylmalonate 2':



2'

^{1}H NMR (400 MHz, DMSO-d6): δ ppm 0.73 (t, J = 7.55 Hz, 6 H, 2 CH_3) 1.16 (t, J = 7.06 Hz, 6 H, 2 CH_3) 1.80 (q, J = 7.47 Hz, 4 H, 2 CH_2) 4.11 (q, J = 7.06 Hz, 4 H, 2 CH_2)

^{13}C NMR (100 MHz, DMSO-d6): δ ppm 8.07, 13.92, 24.06, 57.60, 60.60, 170.93

Synthesis of [2-^{13}C,D_{10}]2,2-diethylmalonic acid 3: The intermediate **2** was dissolved in 25 mL methanol and 15 mL 50% sodium hydroxide, and then stirred under reflux for 6 hours. The solution was diluted in 40 mL water and the organic phase was washed with ether. The aqueous phase was acidified with concentrated HCl and extracted with ether. The ether was removed in a rotary evaporator to give [2-^{13}C,D_{10}]2,2-diethylmalonic acid **3** as a white solid (683 mg, 4.0 mmol, 85%).

^{1}H NMR (400 MHz, DMSO-d6): no peaks

^{13}C NMR (100 MHz, DMSO-d6): δ ppm 57.05 (enriched), 173.06 (d, J = 60 Hz)

HRMS-ES+ (m/z): calculated for $C_6{}^{13}CH_3D_{10}O_4$ 172.1470 found 172.1455 ($[M+H]^+$)

Reference material, 2,2-diethylmalonic acid 3':

^{1}H NMR (400 MHz, DMSO-d6): δ ppm 0.74 (t, J = 7.55 Hz, 6 H, 2 CH_3) 1.75 (q, J = 7.55 Hz, 4 H, 2 CH_2) 12.59 (br s, 2 H, 2 OH)

^{13}C NMR (100 MHz, DMSO-d6): δ ppm 8.37, 23.93, 57.44, 173.04

*[2-^{13}C,D_4]cyclopropane-1,1-dicarboxylic acid **5**:*

Scheme S2: Synthesis of [2-^{13}C,D_4]cyclopropane-1,1-dicarboxylic acid **5**.

Synthesis of [2-^{13}C,D_4]diethyl cyclopropane-1,1-dicarboxylate 4: To 448 mg of sodium hydride, 60% dispersion in mineral oil (11.2 mmol), was added 10 mL of anhydrous HMPA, under nitrogen atmosphere. To the resulting solution was added 500 mg (3.1 mmol) [2-^{13}C]diethylmalonate **1**, dissolved in 4 mL of anhydrous HMPA. The solution was stirred under nitrogen atmosphere for 30 minutes. To this solution was added 1.786 g (0.820 mL, 9.3 mmol) of [D_4]1,2-dibromoethane. The resulting mixture was stirred for 16 hours at room temperature. Then, 20 mL of an ice/water mixture was added to the solution. The resulting aqueous solution was extracted twice with hexanes. The organic fraction was dried over magnesium sulfate and the solvent evaporated, yielding a yellowish oil. Purification by column chromatography on silica gel (hexane/AcOEt 98:2) afforded [2-^{13}C,D_4]diethyl cyclopropane-1,1-dicarboxylate **4** as a clear oil (338 mg, 1.77 mmol, 57%).

^{1}H NMR (400 MHz, DMSO-d6): δ ppm 1.19 (t, J = 7.18 Hz, 6 H, 2 CH_3), 4.11 (q, J = 7.06 Hz, 4 H, 2 CH_2)

^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 13.86, 27.83 (enriched), 60.98, 168.58 (d, J = 3 Hz)

Reference material, diethyl cyclopropane-1,1-dicarboxylate 4':

^{1}H NMR (400 MHz, DMSO-d6): δ ppm 1.19 (t, *J* = 7.18 Hz, 6 H, 2 CH_3) 1.33 (s, 4 H, 2 CH_2) 4.12 (q, *J* = 7.06 Hz, 4 H, 2 CH_2)

^{13}C NMR (100 MHz, DMSO-d6): δ ppm 13.86, 14.97, 28.13, 60.99, 168.98

Synthesis of [2-^{13}C,D_4]cyclopropane-1,1-dicarboxylic acid 5: The intermediate **4** was dissolved in 10 mL methanol and 6 mL 50% sodium hydroxide, and then stirred under reflux for 6 hours. The solution was diluted in 20 mL water and the organic phase was washed with ether. The aqueous phase was acidified with concentrated HCl and extracted with ether. The ether was removed in a rotary evaporator to give [2-^{13}C,D_4]cyclopropane-1,1-dicarboxylic acid **5** as a white solid (188 mg, 1.38 mmol, 79%).

^{1}H NMR (400 MHz, DMSO-d6): no peaks

^{13}C NMR (100 MHz, DMSO-d6): δ ppm 26.89 (enriched), 171.73 (d, J = 73 Hz)

HRMS-ES+ (m/z): calculated for $C_4{}^{13}CH_3D_4O_4$ 136.0623 found 136.0597 ($[M+H]^+$)

Reference material, cyclopropane-1,1-dicarboxylic acid 5':

^{1}H NMR (400 MHz, DMSO-d6): δ ppm 1.28 (s, 4 H, 2 CH_2)

^{13}C NMR (100 MHz, DMSO-d6): δ ppm 16.15, 27.25, 171.71

Formulation of [2-^{13}C,D_{10}]DEMA, [2-^{13}C,D_4]CDPA, and [1-^{13}C,D_9]*tert*-butanol (tBuOH) for hyperpolarization

Each compound in the study was formulated at the maximum concentration that formed a glassy state upon immediate immersion in liquid nitrogen. Formulation glassing was determined by visual inspection; if the flash-frozen formulation without radical added was transparent, then it was determined to be in a glassy state. All formulations were made with the isotopically enriched molecules of interest.

[2-^{13}C,D_{10}]DEMA

225.5 mg of [2-^{13}C,D_{10}]diethylmalonic acid (DEMA) were added to 225.2 mg of N,N-dimethylacetamide, vortexed to mix, and spun down in a microcentrifuge. The mixture was sonicated for 10 minutes at 37 °C, 7.3 mg of OX063 trityl radical were added, and after vortexing and spinning down the solution was sonicated for another 8-9 minutes at 37 °C. To 300 µL of the resulting formulation was added 1.2 uL of 500 mM Gd-DOTA (Guerbet, Bloomington, IN). The final concentration of DEMA in the formulation was ~3.8 M.

[2-^{13}C,D_4]CDPA

29.9 mg of [2-^{13}C,D_4]cyclopropane-1,1-dicarboxylic acid (CPDA) were added to 32.8 mg of dimethyl sulfoxide, vortexed to mix, and spun down in a microcentrifuge. The mixture was sonicated at room temperature until fully dissolved. 1.3 mg of OX063 trityl radical were added, and after vortexing and spinning down the solution was sonicated again until fully dissolved. The final concentration of CPDA in the formulation was ~4.0 M.

[1-^{13}C,D_9]tBuOH

Tert-butanol was formulated as previously described.[5] Briefly, 500 uL each of [1-^{13}C,D_9]*tert*-butanol and glycerol were mixed together, and 16.0 mg of Finland trityl radical were dissolved with sonication. Gd-DOTA was added to a final concentration of 1 mM.

^{13}C-NMR titration curve of [2-^{13}C,D_{10}]DEMA at 11.7 T and 37 °C

[2-^{13}C,D_{10}]DEMA and [1-^{13}C,D_9]tBuOH were prepared at 5 mM in solution and were titrated to several pH values between 2.5 and 8.8 using NaOH and HCl with a conventional pH electrode (Ion 500 series, Oakton Instruments, Vernon Hills, IL). A ^{13}C NMR spectrum was acquired for each pH value (TR = 22 s, 40° hard pulses, 30 kHz spectral width, 60k FID points, 8-90 transients) using a 37 °C temperature-regulated 500 MHz Varian INOVA spectrometer (Agilent Technologies, Palo Alto, CA) equipped with a 5 mm triple-tuned, direct-detect, triple-axis, gradient-equipped broadband probe (Agilent Technologies, Palo Alto, CA). The chemical shift difference between the labeled carbons was measured, plotted versus pH, and fitted to a sigmoidal curve in MATLAB (MathWorks, Natick, MA) using a previously-reported equation.[6]

Hyperpolarized (HP) ^{13}C-NMR spectroscopy at 11.7 T of [2-^{13}C,D_{10}]DEMA, [2-^{13}C,D_4]CDPA, and [1-^{13}C,D_9]tBuOH

HP [2-^{13}C,D_{10}]DEMA + [1-^{13}C,D_9]tBuOH

5 µL of each formulated compound were placed in a sample cup and polarized at 94.085 GHz at a temperature of 1.3-1.4 K for 2.5 hours using a HyperSense DNP polarizer (Oxford Instruments, Abingdon, UK). The sample was then dissolved using 4-4.3 mL of 0.3 mM EDTA solution and ejected into a glass flask containing 160-225 µL of 0.1 M NaOH to neutralize the compounds. The solution was briefly swirl-mixed, then transferred to a capped 5 mm NMR tube and inserted into a pre-tuned, pre-shimmed, 37 °C temperature-regulated 500 MHz Varian INOVA spectrometer (Agilent Technologies, Palo Alto, CA). ^{13}C NMR spectra were acquired using a 5 mm triple-tuned, direct-detect, triple-axis, gradient-equipped broadband probe (Agilent Technologies, Palo Alto, CA). The final concentration of DEMA in the dissolution was ~5 mM. The time between the sample leaving the polarizer and the start of acquisition was 19-25 seconds. A series of hyperpolarized spectra was then acquired (TR = 3 s, 5° hard pulses, 30 kHz spectral width, 60k FID points, 100 timepoints). For processing, the data were 2 Hz line-broadened, Fourier transformed, phased, referenced, and integrated using VnmrJ 3.2A software (Agilent Technologies, Palo Alto, CA). The chemical shift separation between the HP DEMA and tBuOH peaks at the 50th timepoint was measured and used with the equation derived from the NMR titration curve in order to calculate the pH. For the n = 5 measurements in Figure 3c, the chemical shift separation between DEMA and tBuOH changed 0.04 ± 0.02 ppm from the 1st to 50th timepoints, resulting in a measured pH difference of 0.03 ± 0.02 unit between the two timepoints. Peak areas from the hyperpolarized spectra were flip angle-corrected and fit to a decaying exponential to approximate T_1 values. The polarization was determined with reference to a spectrum at thermal equilibrium (TR = 5 * [measured T_1], 90° hard pulse, same spectral window/resolution, 16-64 transients), accounting for differences in flip angle and the transfer time between the polarizer and spectrometer.

HP [2-^{13}C,D_4]CPDA

For the HP CPDA experiments, only T_1 was measured. 10 µL of formulated compound were placed in a sample cup and polarized at 94.075 GHz at a temperature of 1.3-1.4 K for 30 minutes using a HyperSense DNP polarizer. The sample was then dissolved using 4-4.3 mL of 0.3 mM EDTA solution and ejected into a glass flask containing 380-420 µL of 0.1 M NaOH to neutralize the compound (pH between 6.6 and 6.8). The dissolution and NMR protocol used was identical to that used with HP DEMA, described in the preceding section.

HP DEMA T_1 fluctuation with pH over the physiologic rangeFigure S1 presents the DEMA T_1 measured during HP ^{13}C NMR as a function of dissolution pH (n = 3). The T_1 fluctuation was less than 10% across all pH values measured. As such, the T_1 value presented in the manuscript is the average ± s.d. of these three measurements.

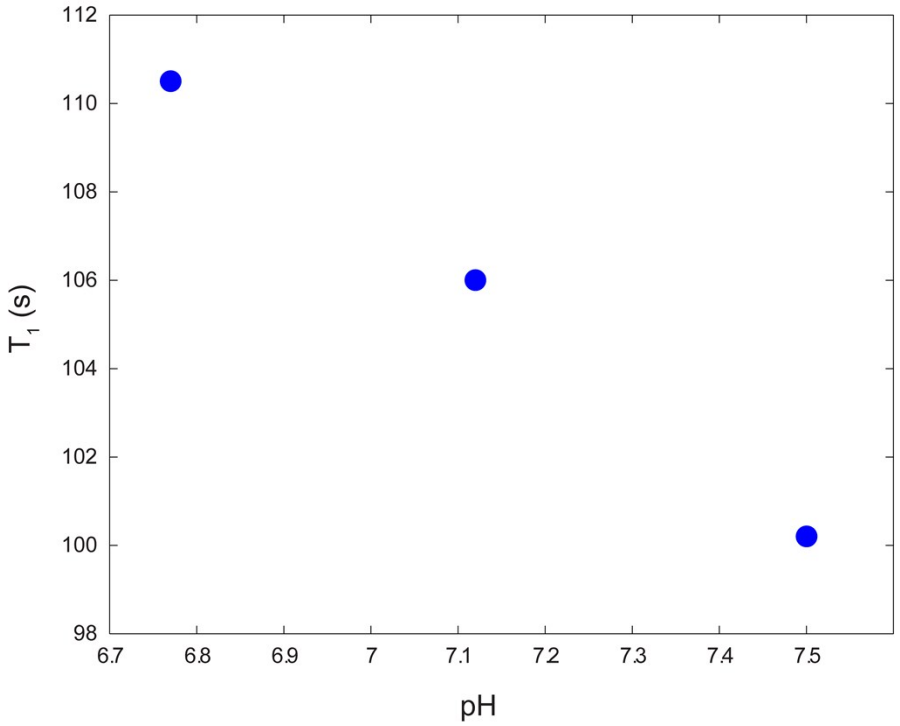


Figure S1: Plot of HP [2-^{13}C,D_{10}]DEMA ^{13}C T_1 vs. pH.

HP phantom imaging for pH quantification at 3 T

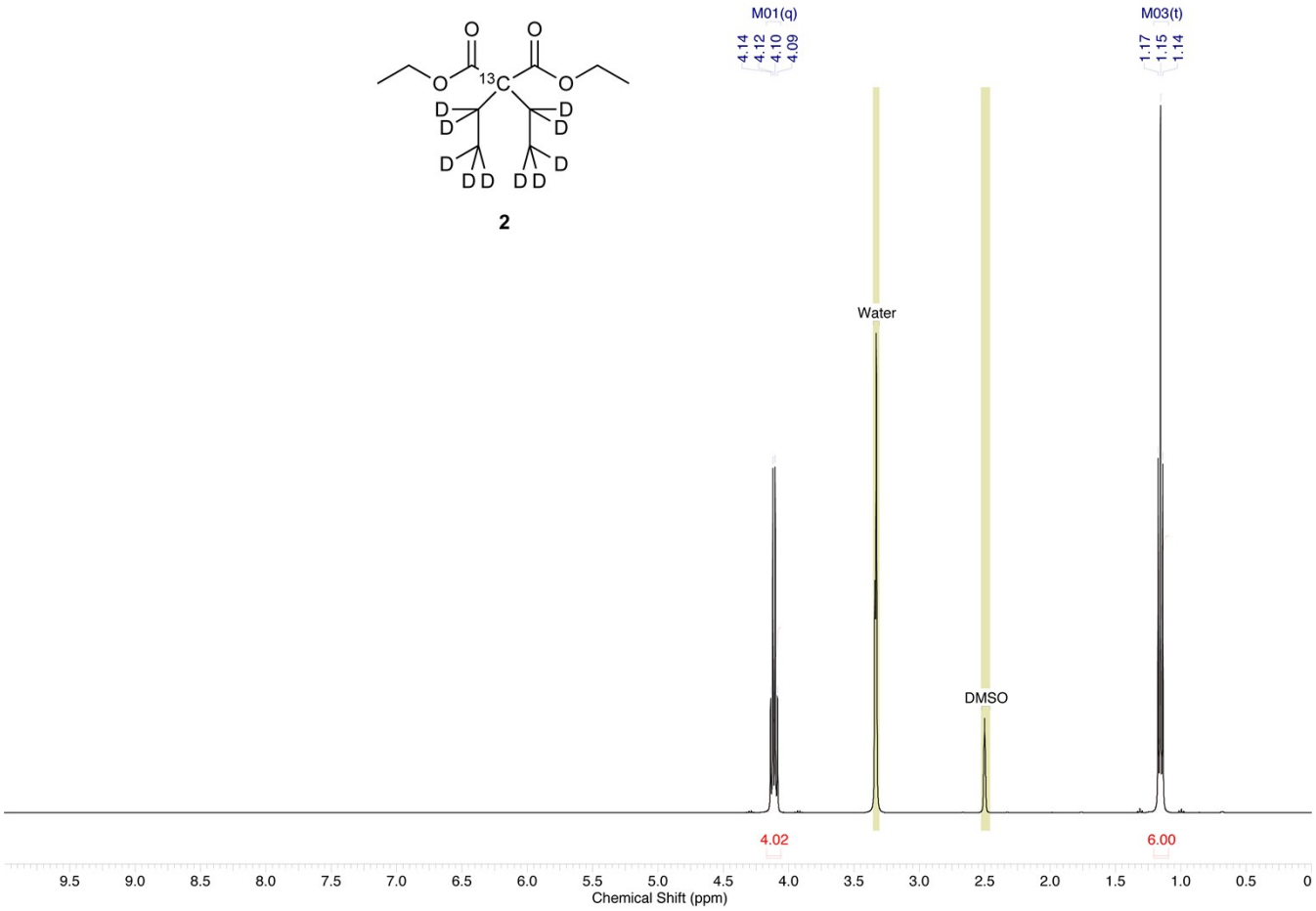
75 µL each of [2-^{13}C,D_{10}]DEMA and tBuOH formulations were polarized, as described previously, and dissolved using 5 mL of 0.3 mM EDTA solution. ~1 mL of HP solution was added to each of five 15 mL NMR tubes filled with 14 mL of water and NaOH at various concentrations. The final DEMA concentration in each tube was ~4 mM. Tubes were kept at 37 ℃ just prior to dissolution. The tubes were briefly vortexed to mix and inserted into a 3 T clinical imaging system (GE Healthcare, Milwaukee, WI) equipped with a custom-built 8 mm ^{13}C solenoid coil. The system was tuned and shimmed prior to HP imaging, and a T_2-weighted ^{1}H spin-echo axial image of the five tubes was acquired for co-registration with the ^{13}C data. The ^{13}C transmitter frequency for HP DEMA imaging was calculated using a ^{13}C-urea phantom. The transfer time between the polarizer and the magnet was ~1 minute. The pulse sequence was a ^{13}C 2D CSI sequence (10 x 10 x 2048 matrix size, centric encoding, 10° hard pulses, FOV = 75 x 75 mm, 25 kHz spectral width, TR = 105 ms, total imaging time = 10.5 s). After imaging, the pH of each tube in the phantom was measured at 37 ℃ outside the magnet using a conventional pH electrode (Ion 500 series, Oakton Instruments, Vernon Hills, IL) to compare with the pH measured via spectroscopy. Shortly after imaging, the DEMA T_1 was measured at 3 T with the remaining HP magnetization via ^{13}C NMR spectroscopy (10° hard pulses, TR = 3 s).

The HP data were Fourier-transformed and visualized using an open-source software, SIVIC (Sourceforge.net). The voxel grid was shifted ½ voxel to the left by applying a linear phase factor along the horizontal direction. The chemical shift differences per voxel between DEMA and tBuOH were used for analysis. Spectra with a DEMA or tBuOH SNR ≤ 5 were excluded from analysis.

Appendix

*[2-^{13}C,D$_{10}$]diethyl 2,2-diethylmalonate **2***

^{1}H NMR, [2-^{13}C,D$_{10}$]diethyl 2,2-diethylmalonate **2**, DMSO-d$_6$, 400 MHz



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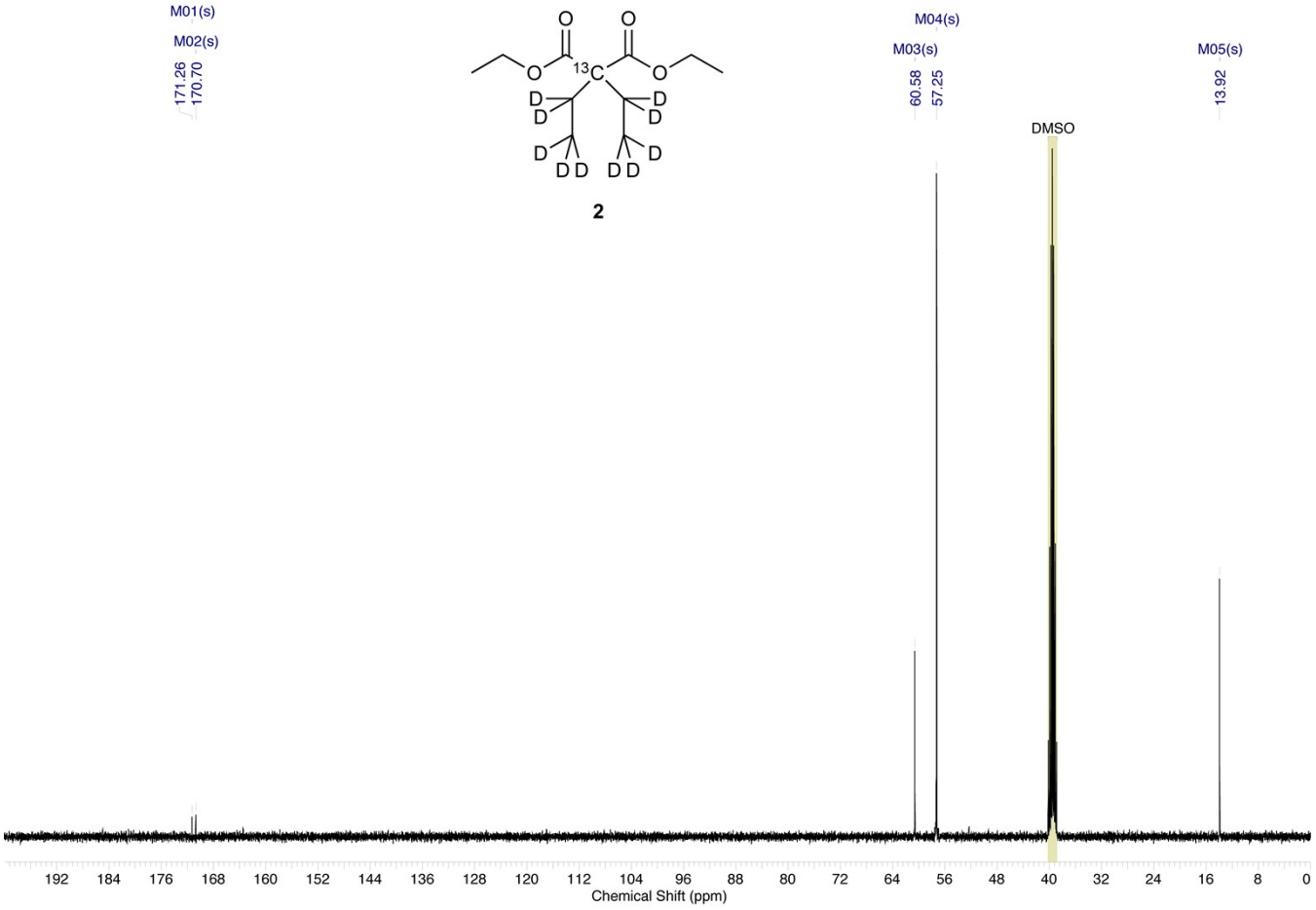
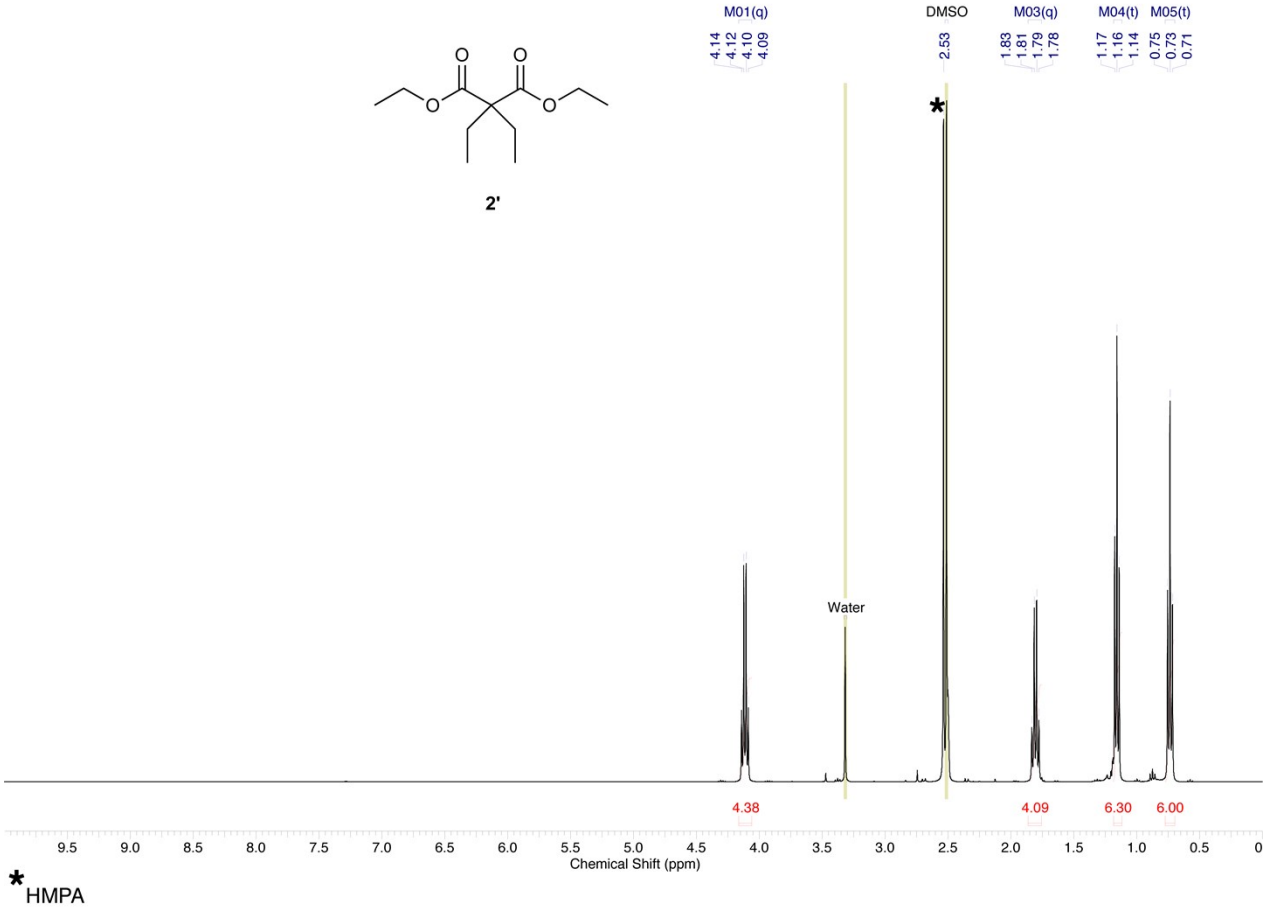
^{13}C NMR, [2-^{13}C,D_{10}]diethyl 2,2-diethylmalonate **2**, DMSO-d_6, 100 MHz



Diethyl 2,2-diethylmalonate ***2'****, protonated reference*

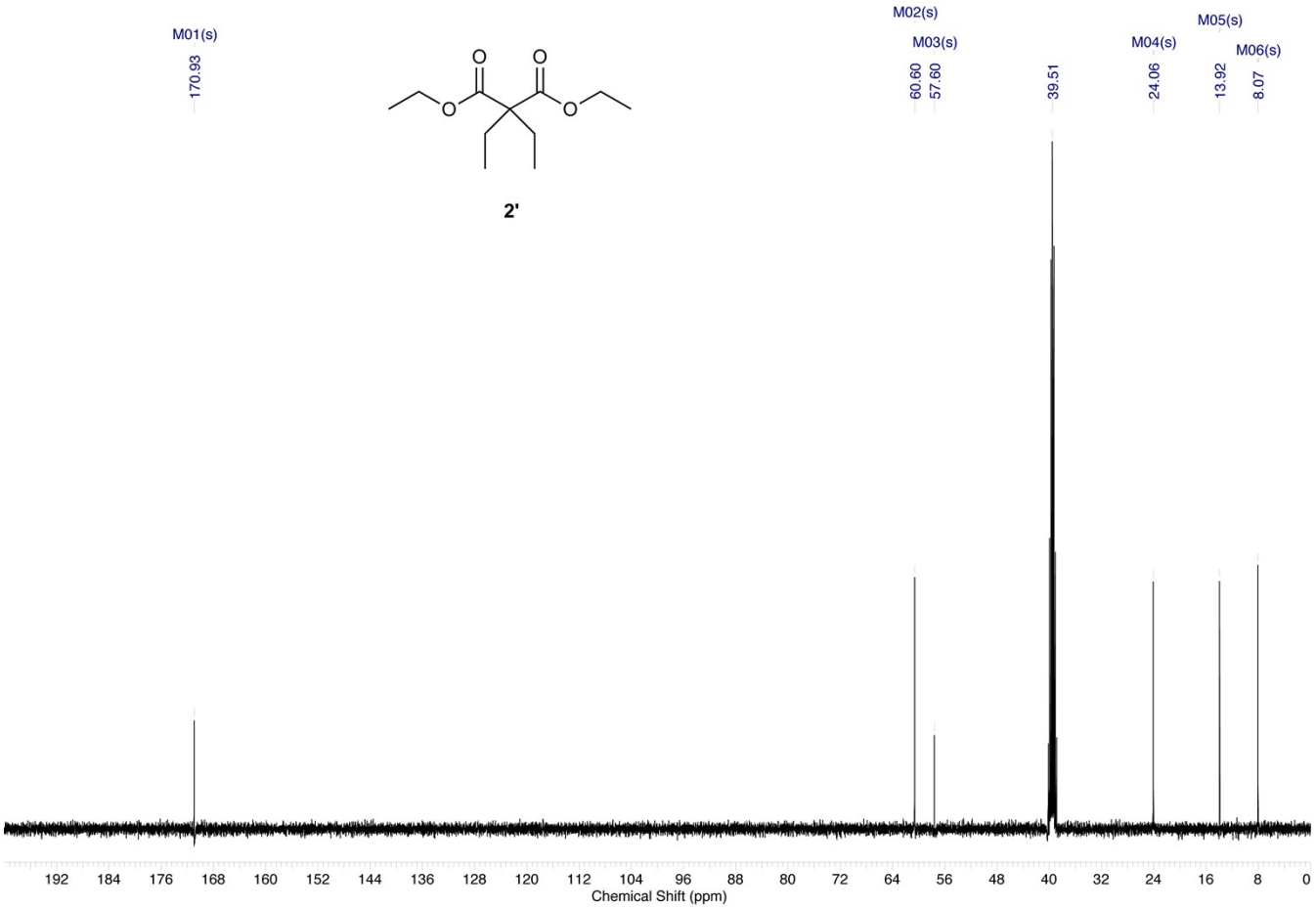
^{1}H NMR, diethyl 2,2-diethylmalonate **2'** (protonated), DMSO-d_6, 400 MHz



2'

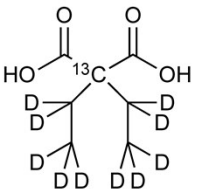
* HMPA

^{13}C NMR, diethyl 2,2-diethylmalonate 2' (protonated), DMSO-d_6, 100 MHz



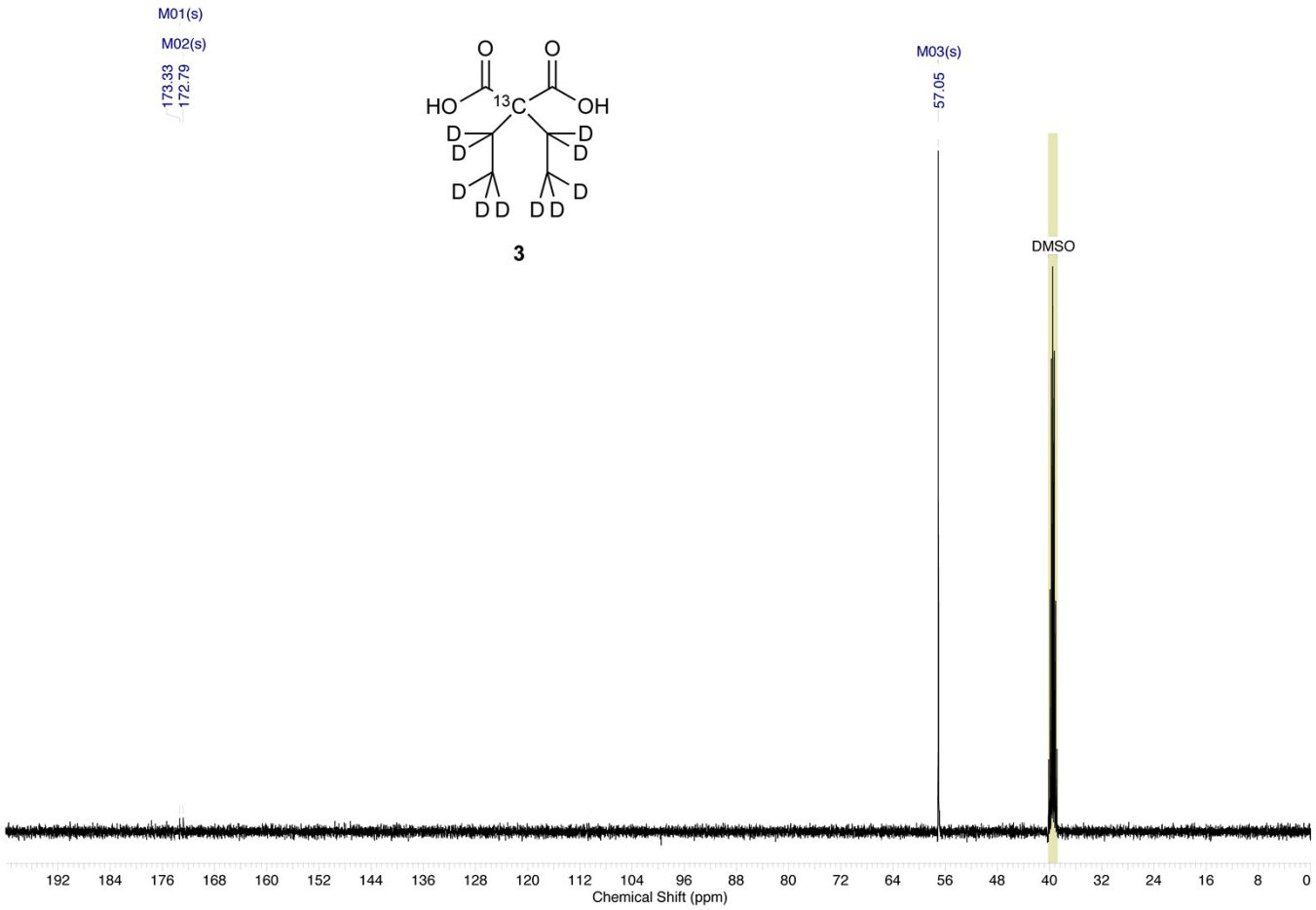
*[2-^{13}C,D_{10}]2,2-diethylmalonic acid **3***

^{1}H NMR, [2-^{13}C,D_{10}]2,2-diethylmalonic acid **3**, DMSO-d_6, 400 MHz



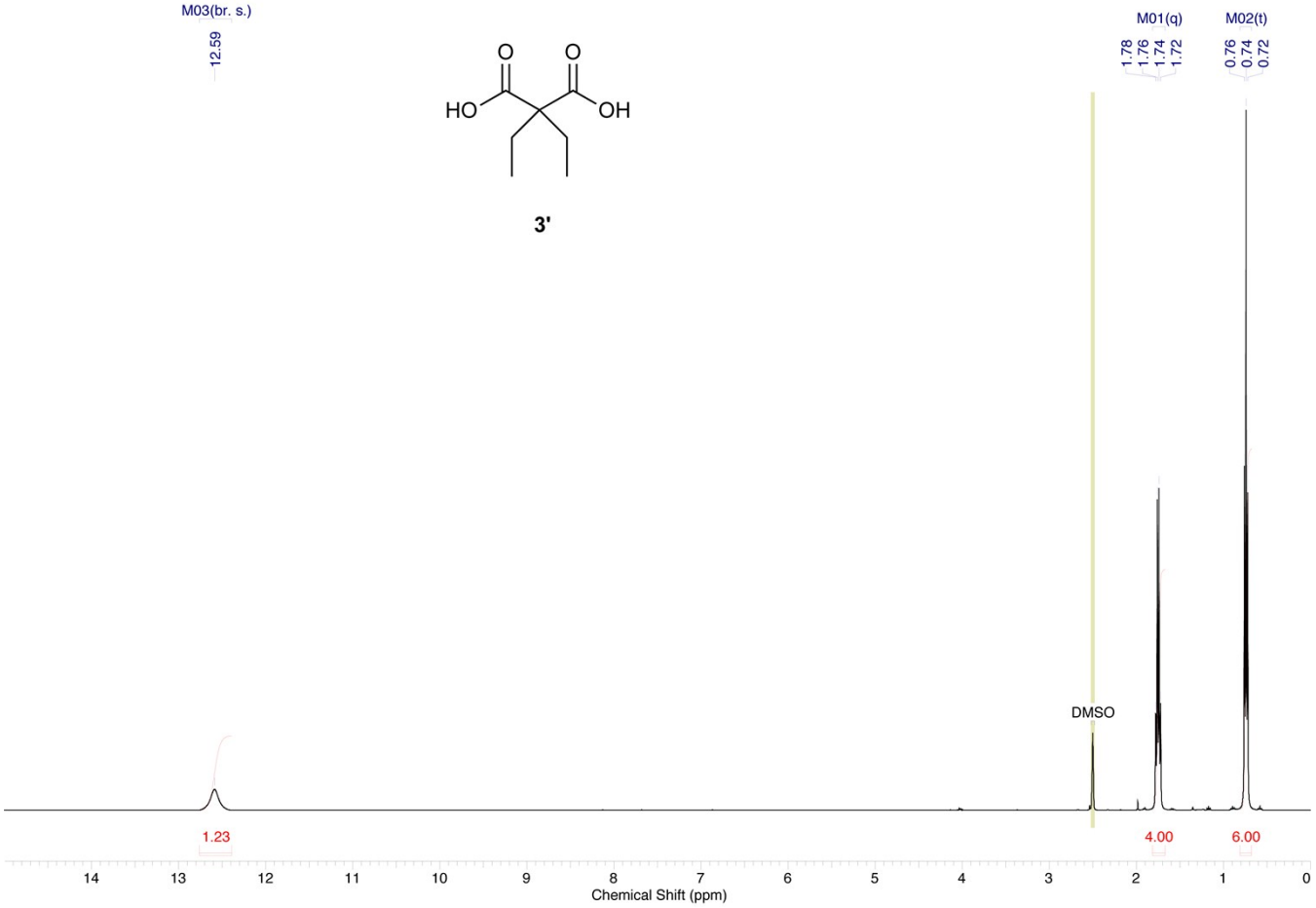
3

^{13}C NMR, [2-^{13}C,D$_{10}$]2,2-diethylmalonic acid **3**, DMSO-d$_6$, 100 MHz

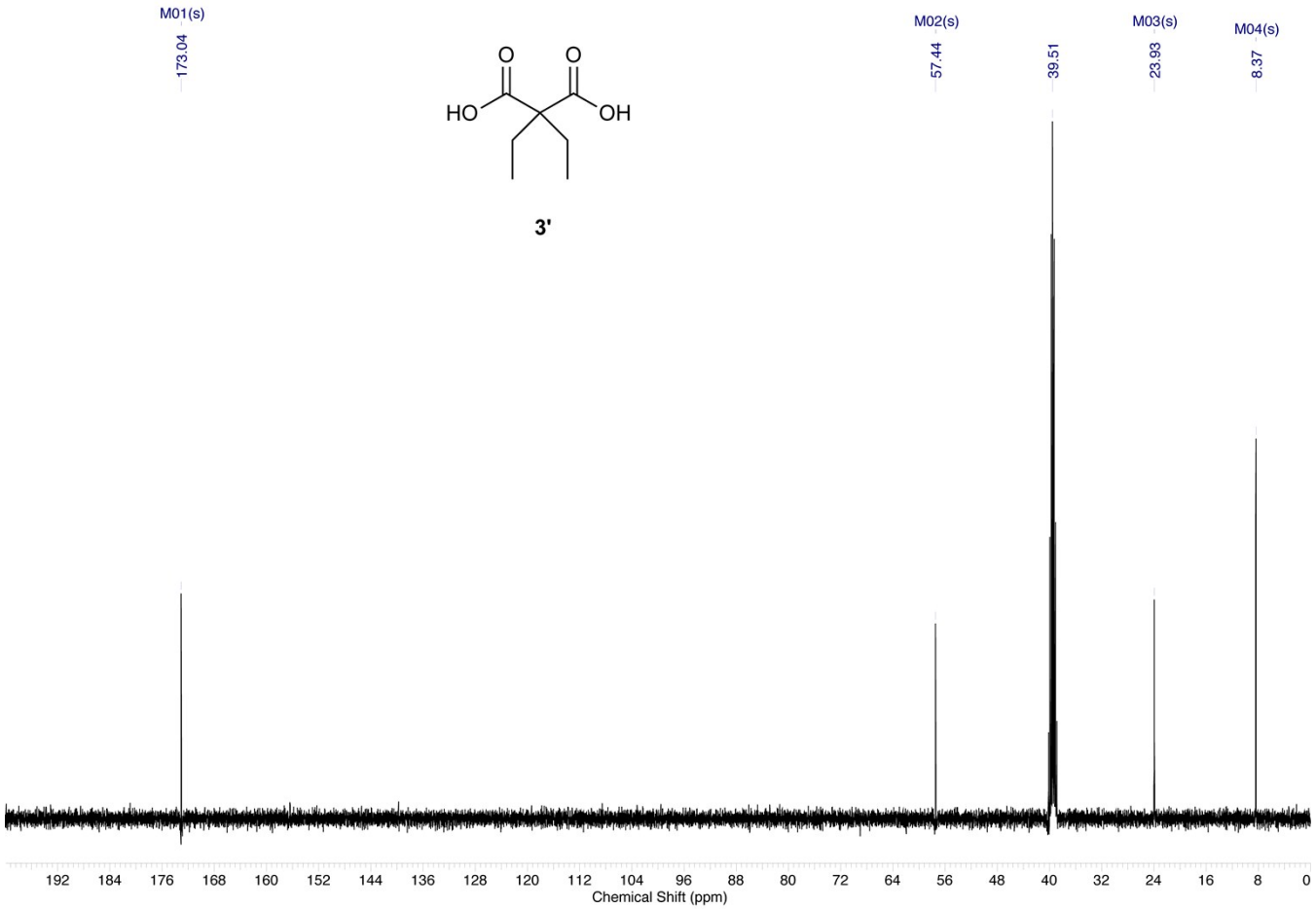


*2,2-diethylmalonic acid **3'**, protonated reference*

^{1}H NMR, 2,2-diethylmalonic acid **3'** (protonated), DMSO-d_6, 400 MHz

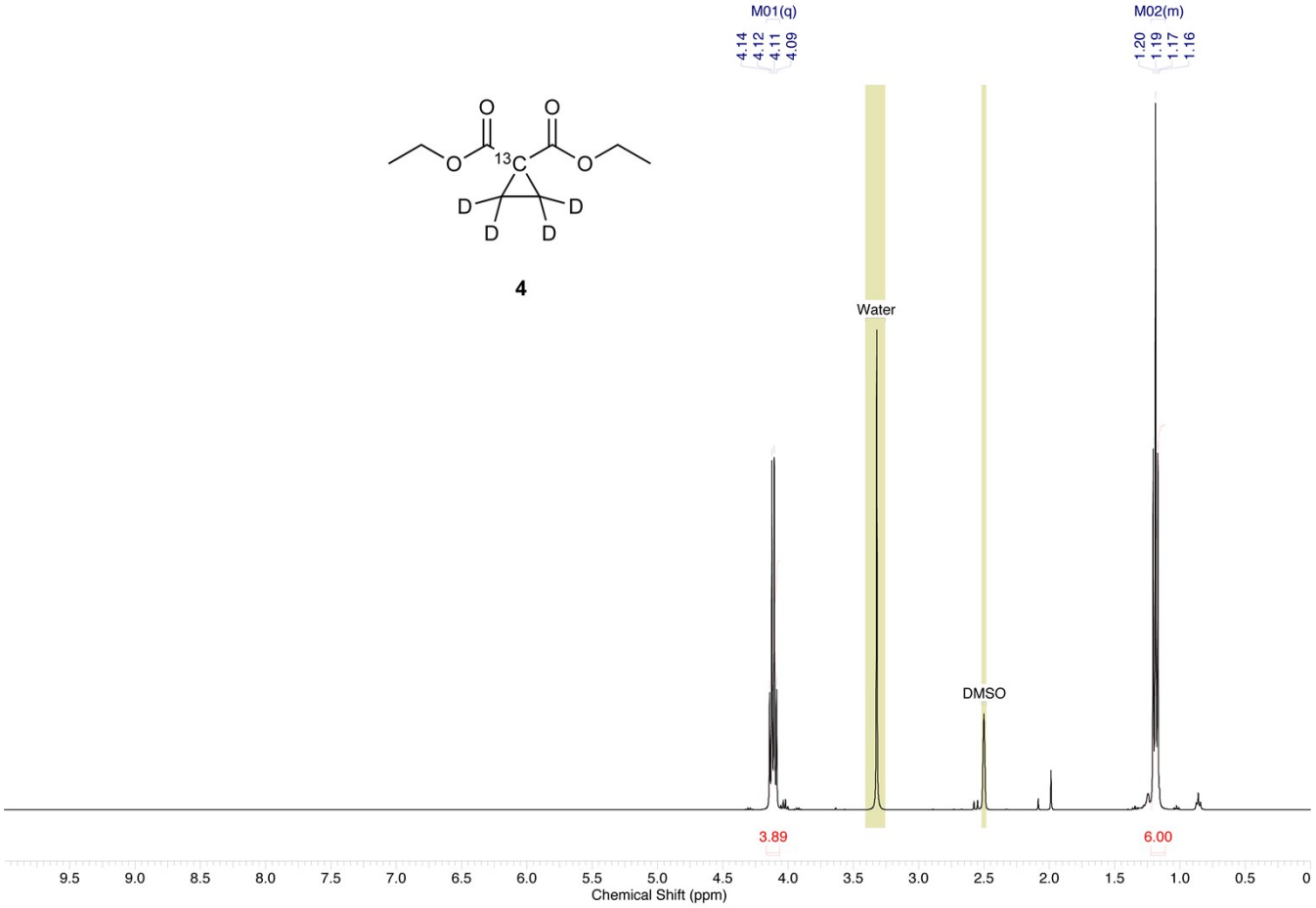


^{13}C NMR, 2,2-diethylmalonic acid **3'** (protonated), DMSO-d_6, 100 MHz



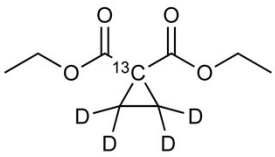
*[2-$^{13}C,D_4$]diethyl cyclopropane-1,1-dicarboxylate **4***

^{1}H NMR, [2-^{13}C,D$_4$]diethyl cyclopropane-1,1-dicarboxylate **4**, DMSO-d$_6$, 400 MHz



4

^{13}C NMR, [2-^{13}C,D$_4$]diethyl cyclopropane-1,1-dicarboxylate **4**, DMSO-d$_6$, 100 MHz



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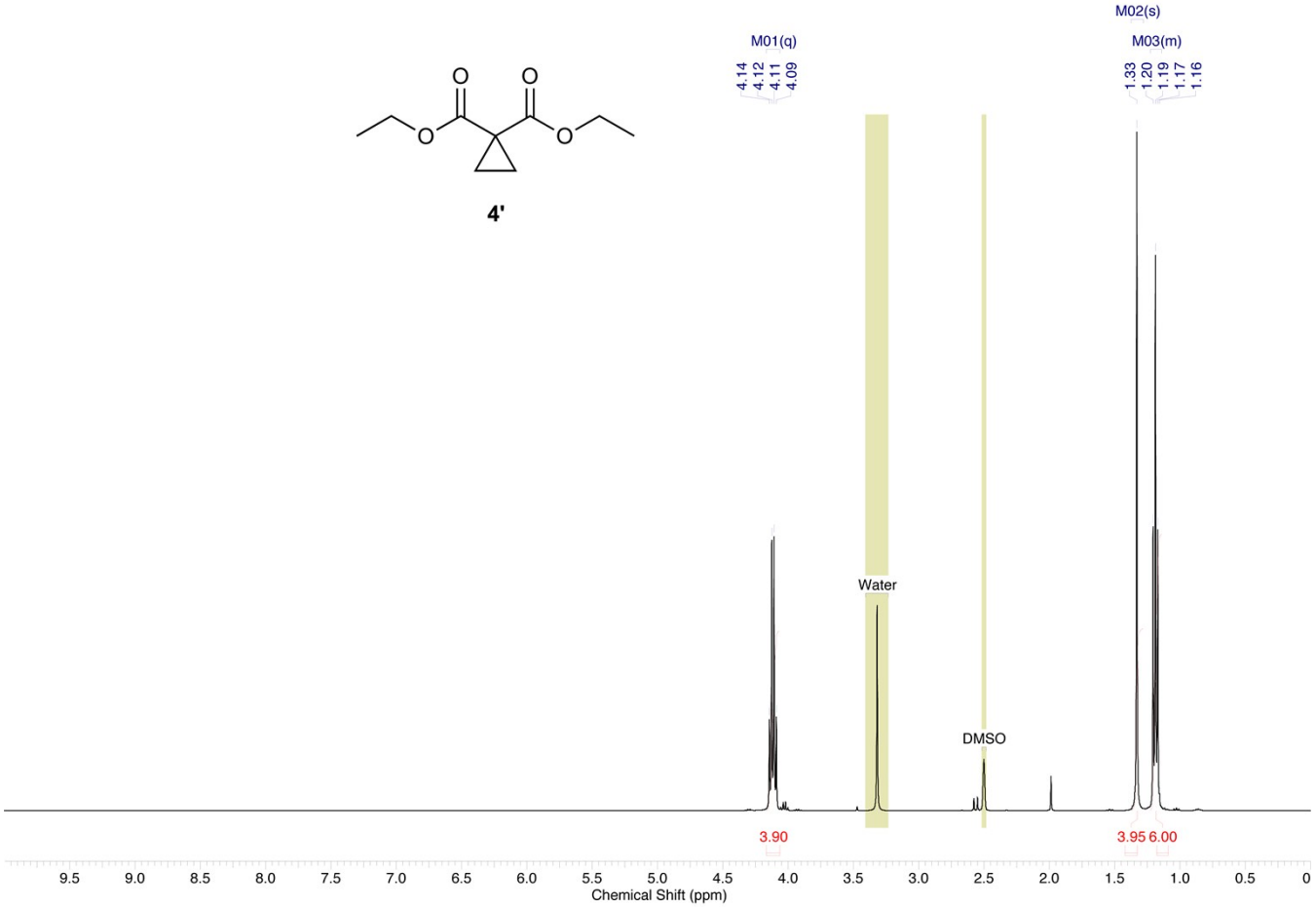
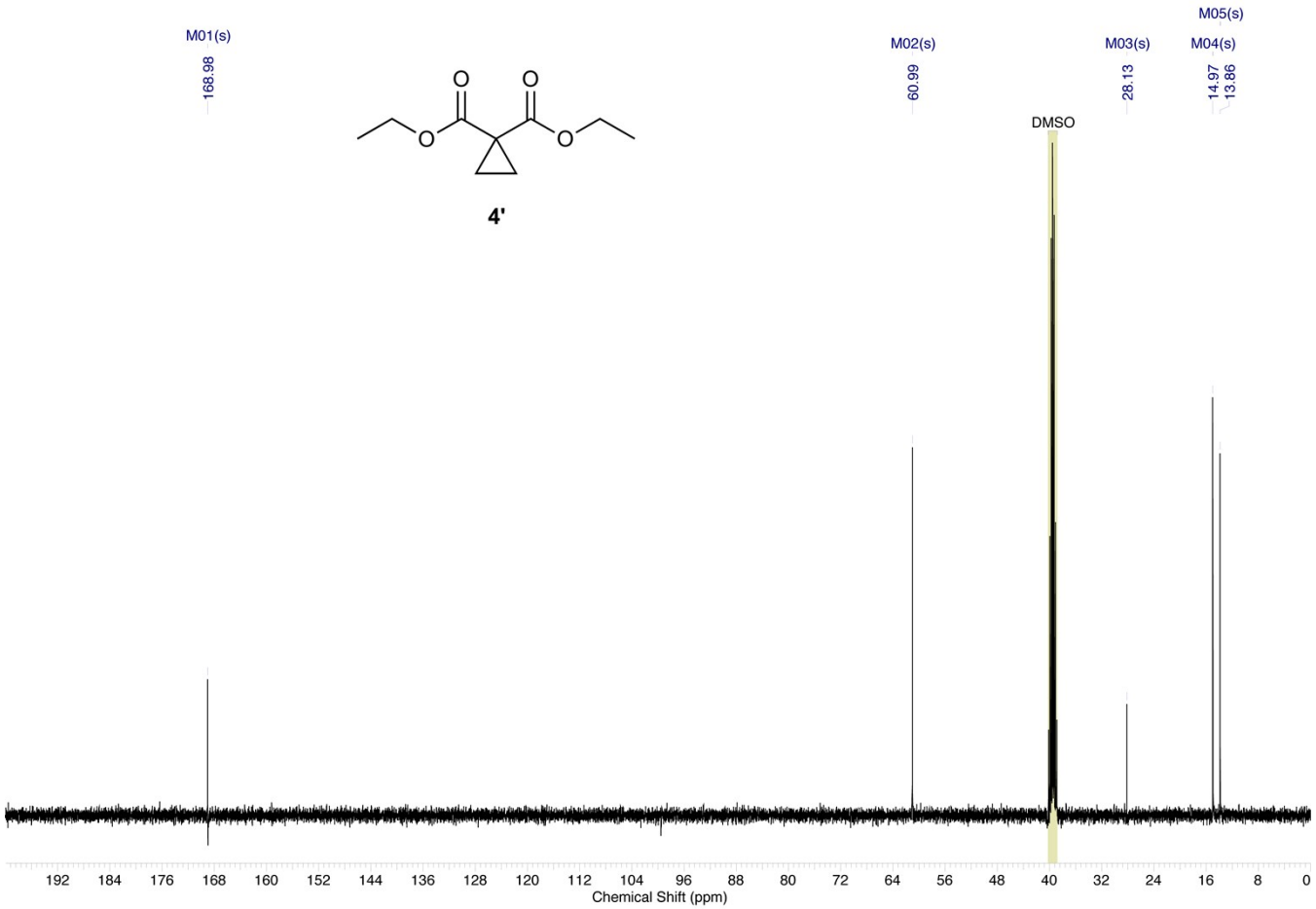
* Presence of residual malonic acid

*Diethyl cyclopropane-1,1-dicarboxylate **4'**, protonated reference*

^{1}H NMR, diethyl cyclopropane-1,1-dicarboxylate **4'** (protonated), DMSO-d_6, 400 MHz

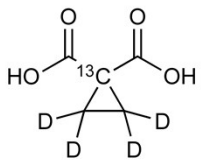


^{13}C NMR, diethyl cyclopropane-1,1-dicarboxylate **4'** (protonated), DMSO-d_6, 100 MHz



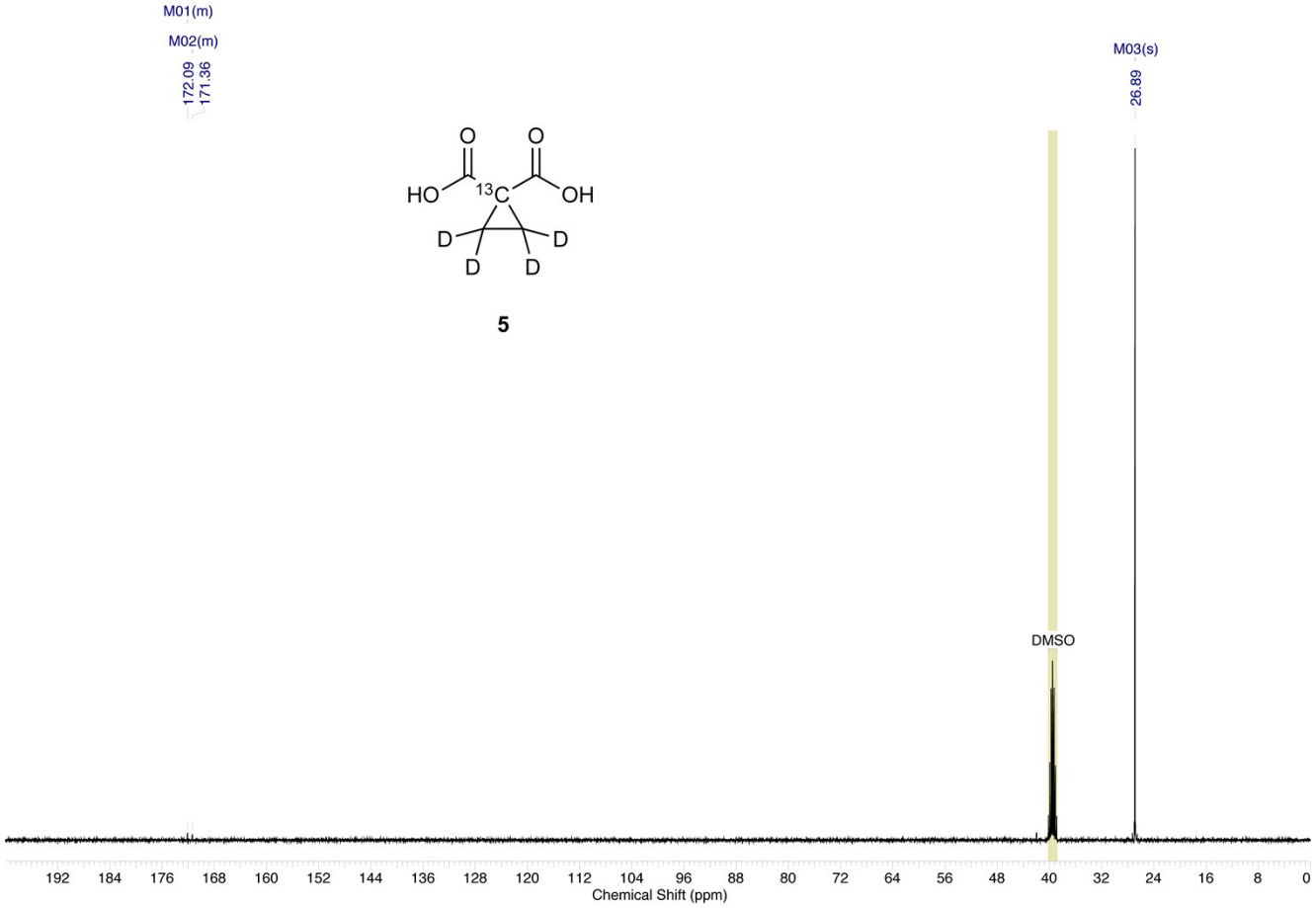
*[2-^{13}C,D$_4$]cyclopropane-1,1-dicarboxylic acid **5***

^{1}H NMR, [2-^{13}C,D$_4$]cyclopropane-1,1-dicarboxylic acid **5**, DMSO-d$_6$, 400 MHz



5

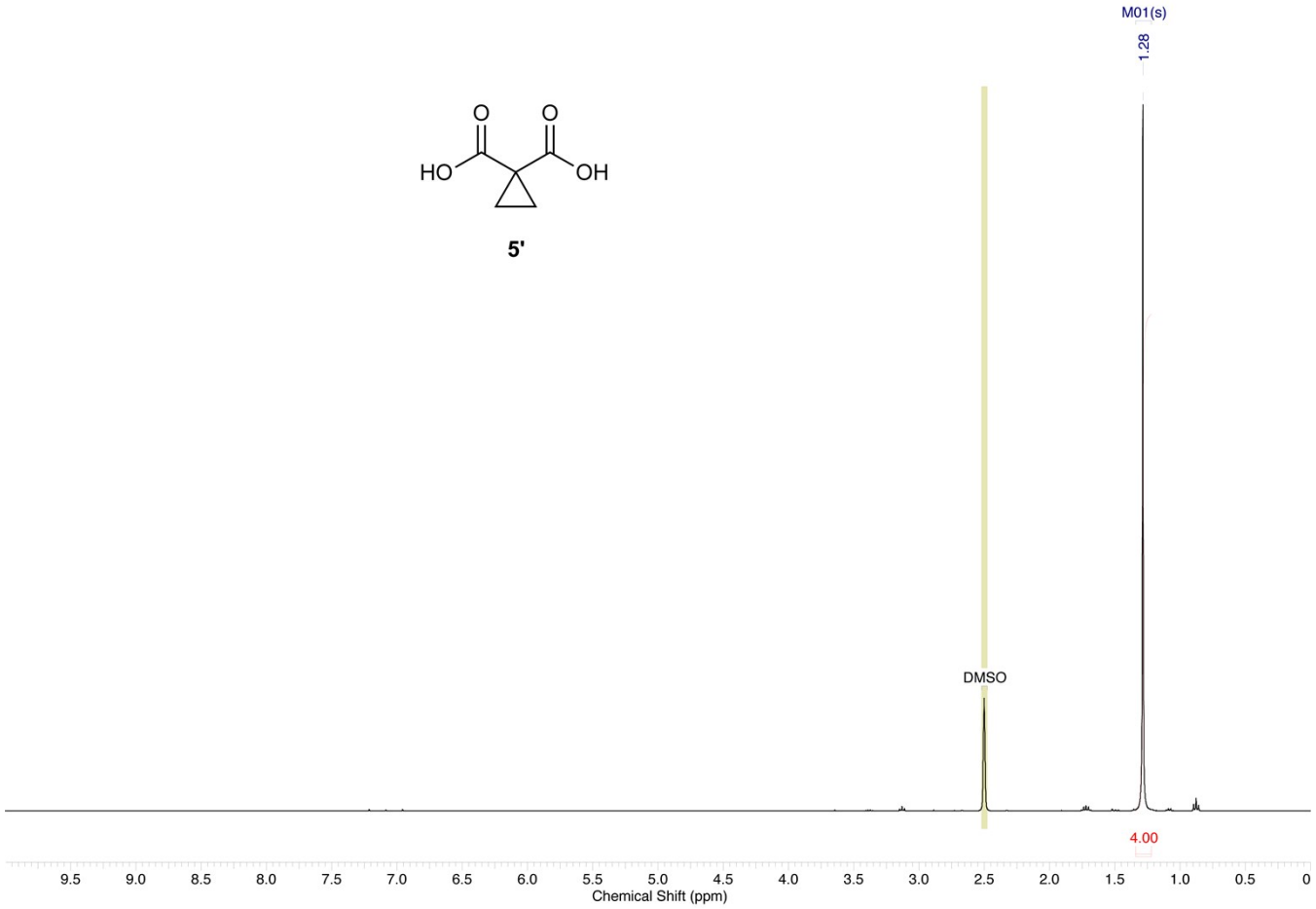
^{13}C NMR, [2-^{13}C,D$_4$]cyclopropane-1,1-dicarboxylic acid **5**, DMSO-d$_6$, 100 MHz



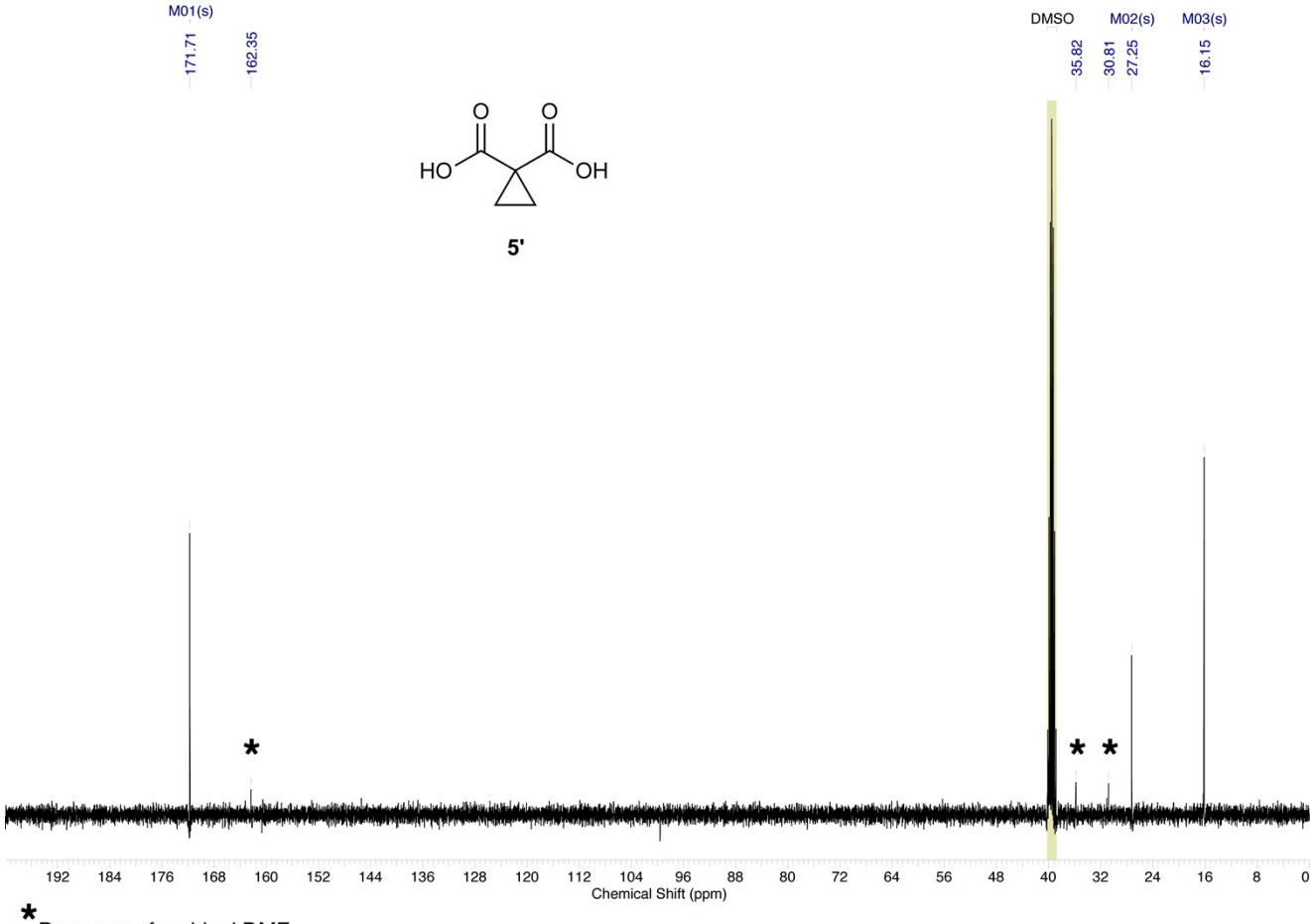
5

*Cyclopropane-1,1-dicarboxylic acid **5'**, protonated reference*

^{1}H NMR, cyclopropane-1,1-dicarboxylic acid **5'** (protonated), DMSO-d_6, 400 MHz



^{13}C NMR, cyclopropane-1,1-dicarboxylic acid **5'** (protonated), DMSO-d_6, 100 MHz



* Presence of residual DMF

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