Supplementary Information

Internal Standards for Quantitative ^{1}H NMR (qNMR) Spectroscopy in Concentrated Sulfuric Acid

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Supplementary Experimental

General Considerations. CAS = Chemical Abstracts Service registry number. All compounds were purchased from MilliporeSigma (www.sigmaaldrich.com) and AmBeed (www.ambeed.com) unless noted otherwise. Tetramethylsilane and 2,3,4,5,6-pentafluorotoluene were purchased from TCI America (www.tcichemicals.com). Malonic acid (CAS [141-82-2]) was purchased from MilliporeSigma. Lithium 3-oxobutanoate (CAS [3483-11-2]) and 3-methoxy-2,2-dimethyl-3-oxopropanoic acid (CAS [13051-21-3]) were purchased from AmBeed. Sulfuric acid-d_2 solution (96–98 wt. % in D_2O, 99.5 atom D, CAS [13813-19-9]) was purchased from Cambridge Isotope Laboratories (www.isotope.com) or MilliporeSigma and used without further purification.

NMR Spectroscopy in Deuterated Sulfuric Acid. Data were collected on 300 MHz and 400 MHz Bruker instruments in the Department of Chemistry at Saint Louis University. All samples were prepared in standard 5 mm NMR tubes with sulfuric acid-d_2 (D_2SO_4) as the solvent. The instrument did not have a solvent profile for sulfuric acid, so a profile was created in the Bruker TopSpin software by copying the existing profile for D_2O and making adjustments to the lock parameters (loop power, loop gain, loop time, loop filter) and shim set. Shimming was performed on the deuterium (^2H) signal instead of the ^1H signal to enhance the sensitivity. Locking and shimming of samples had to be performed manually and was more difficult than for typical NMR solvents (e.g., D_2O or $CDCl_3$). The process took several hours. Supplementary shimming commands such as CONVCOMP and ORDMAX=8 were used to improve the shimming process. Quantitative ^1H NMR spectroscopy requires setting up parameters like relaxation delay (d1), pulse width selection (pw), receiver gain (rga), and acquisition time.[1] For our qNMR experiments, we set the delay time (d1) to 7 sec—over 5 times the longest T_1 signal present—to ensure quantitative integration of the peaks. The number of scans (ns) was set to 32. Other parameters were: RG (receiver gain) = 101, AQ (acquisition time) = 5.238 sec, TD (size of FID) = 95238, SW (spectral width) = 30.2898 ppm, and p1 (pulse width) = 15.00. All spectra were referenced to tetramethylsilane (TMS) at δ 0.00 with the solvent peak ($HDSO_4$) at δ 10.46.

Measurement of T_1 Values. A specialized NMR spectroscopy experiment known as inverse recovery pulse sequence is often carried out to measure the spin-lattice relaxation time (T_1) of the protons in a signal.[2] The PROTON pulse program experiment zg30 was modified into zg and used to determine the maximum p1 value by entering the command pulsecal in TopSpin. Following this step, a T_1 experiment, referred to as t1ir1d, was conducted to identify the variable delay termed D7. Subsequently, a 16-point variable delay list (VDLIST) was created, typically covering a time range that extends beyond the anticipated T_1 value. This VDLIST was used to execute a 2D T_1 experiment using the t12r experiment on TopSpin.

Initial Screen of Candidate Internal Standards for Solubility and Stability to Decomposition. Our initial screen comprised 16 compounds: tetramethylsilane (**1**, CAS [75-76-3]), 1,4-dioxane (**2**, CAS [123-91-1]), 2,3,4,5,6-pentafluorotoluene (**3**, CAS [771-56-2]), dimethyl sulfone (**4**, CAS [67-71-0]), 1,4-dinitrobenzene (**5**, CAS [100-25-4]), duroquinone (**6**, CAS [527-17-3]), potassium hydrogen phthalate (**7**, CAS [877-24-7]), tetraphenylphosphonium bromide (**8**, CAS [2751-90-8]), *tert*-butanol (CAS [75-65-0]), triphenylphosphine (CAS [603-35-0]), ammonium formate (CAS [540-69-2]), sodium formate (CAS [141-53-7]), diethyl ether (CAS [60-29-7]), tetrahydrofuran (CAS [109-99-9]), hydroquinone (CAS [123-31-9]), and 1,3,5-trioxane (CAS [110-88-3]).

A sample of each compound (roughly, 10−35 mg) was introduced to 500−700 μL of D_2SO_4 in a vial to gauge solubility. Each mixture was vortexed to speed dissolution. Once the compound dissolved or the solvent appeared saturated, the solution was transferred into an NMR tube and a ^{1}H NMR spectrum was collected. The solutions were maintained at room temperature (23 ± 3 °C) and monitored periodically by NMR spectroscopy over two weeks to assess the extent of decomposition, if any.

Second Screen (at Elevated Temperatures) of Candidates for Stability. The stabilities of compounds **2−8** were tested at elevated temperatures to ascertain whether these compounds would remain stable for 5 days at 50, 70, and 100 °C. Stability was judged by the original signals maintaining ≥99% of their integrated intensity relative to the total integration from δ 0–9. Signals were integrated over the range ±0.25 ppm from the peak maximum, ≥20 times the peak width at half height for the peak integrated for each candidate.

Further Stability Testing of the Final Three Candidates. The three best internal standard candidates—1,4-dinitrobenzene, KHP, and dimethyl sulfone—were mixed in 1:1:1 molar concentration in 50 mL deuterated sulfuric acid. This solution was divided into 12 vials. Sets of 3 vials (triplicate trials) were maintained at constant temperatures of 23 °C (room temperature), 50 °C, 70 °C, and 100 °C for a period of 7 days. Aliquots were taken at time intervals of 1 day, 3 days, 5 days, and 7 days, then analyzed by NMR spectroscopy. We ran each experiment in triplicate and acquired NMR spectra with 32 scans and a recycle delay of 7 seconds. The close proximity of some signals in the mixed sample necessitated shortening the integration ranges. Each peak was integrated over the range ±0.02 ppm from its maximum, ≥5 times the peak width at half height for each integrated peak.

Measurement of the First-Order Rate Constants for Decarboxylation Reactions. The progress of each decarboxylation reaction was monitored by observing the losses in the intensity of isolated methyl signals (**β** and **OCH_3**) over time relative to the signals from the unreactive internal standards. The close proximity of some signals for the compounds in the reaction mixture necessitated shortening the integration ranges. For the kinetics experiments, each peak was integrated over the range ±0.02 ppm from its maximum, ≥5 times the peak width at half height for each integrated peak. When KHP was used as the internal standard, only its peak at δ 8.3 was integrated (the downfield/left peak). These qNMR experiments used a delay time (d1) set to 7 sec, which was over 5 times the longest T_1 signal in the reaction mixture to ensure quantitative integration of the signals. The analyte peaks were integrated relative to the peak of the internal standard. A plot of the negative natural logarithm of the ratio between the integrated value at time t and the initial integrated value was generated against time (in seconds) to produce a first-order kinetics plot, as shown in Figure 4 in the main paper. For measuring the rate of the decarboxylation of compound **9**, dimethyl sulfone and 1,4-dinitrobenzene were used as internal standards as a mixed pair in triplicate trials at 50 °C.

Supplemental Discussion

Longitudinal Spin-Lattice (T_1) Measurements by NMR Spectroscopy. Quantitative NMR spectroscopy relies on the accuracy of the intensity of the signals, which should be directly proportional to the number of nuclei giving rise to the signal.[3-4] The intensity of each signal is measured by integrating the area under it. We performed a series of spin-lattice relaxation time (T_1) measurements to determine the recycle delay time required to ensure the quantitative integration of signals. The T_1 measurements of the candidate internal standards are reported in Table 1 in the main paper and copied in Table S1, below. The T_1 times for 3-methoxy-2,2-

dimethyl-3-oxopropanoic acid were measured as 0.460 sec (**OCH_3** peak, δ 3.77) and 0.304 sec (**β** peak, δ 1.31).

Initial Screen of Candidate Internal Standards. Eight of the original sixteen compounds screened were rejected from further consideration for poor solubility (hydroquinone), decomposition in deuterated sulfuric acid (tetrahydrofuran, diethyl ether, sodium formate, ammonium formate, 1,3,5-trioxane, and *tert*-butanol), and/or complicated signal patterns (triphenylphosphine and hydroquinone).

Table S1. Data for eight compounds examined as internal standards for qNMR spectroscopy in concentrated D_2SO_4. Symbols: *s* = singlet; *dd* = doublet of doublets; *m* = multiplet; m.p. = melting point; b.p. = boiling point. Melting point and boiling point data obtained from reference #5 in the Supplementary Information.

#	**Name**	**Chemical Shifts & Multiplicity**	***T₁* (sec)**	**Solubility (mg/mL)**	**m.p. (°C)**	**b.p. (°C)**
1	tetramethylsilane (TMS)	0.00 (*s*)	1.27	>70		26–28
2	1,4-dioxane	3.79 (*s*)	0.36	>70		100–102
3	pentafluorotoluene (PFT)	1.79 (*s*)	0.89	>70		117
4	dimethyl sulfone (DMSul)	2.77 (*s*)	0.63	>70		238
5	1,4-dinitrobenzene (DNB)	8.03 (*s*)	1.36	>40		183 (at 34 mmHg)
6	duroquinone	1.85 (*s*)	0.51	>70	110–112	
7	potassium hydrogen phthalate (KHP)	8.24 (*dd*) 7.65 (*dd*)	0.75 1.15	>60	210 (for phthalic acid)	300
8	tetraphenylphosphonium bromide	7.50 (*m*) 7.33 (*m*) 7.21 (*m*)	0.71 0.94 0.90	>70	295–298	

Investigating the Remaining Eight Candidate Internal Standards at Room Temperature. The remaining 8 candidates were held for 14 days at room temperature (23 ± 3 °C) in D_2SO_4 and analyzed by qNMR spectroscopy. Literature data on the melting and boiling points of the candidates are presented in Table S1, along with our experimental estimates of solubility in D_2SO_4.[5] The ^1H spectrum of tetramethylsilane (**1**, TMS) showed one sharp singlet emerging from 12 protons at 0.0 ppm upon referencing (the TMS peak came in at −5 ppm before re-referencing). After 1 day, the signal intensity of the δ 0.0 peak decreased and a new signal had appeared at −0.31 ppm (see Figure S1). TMS is not a good choice for an internal standard in deuterated sulfuric acid because it decomposes.

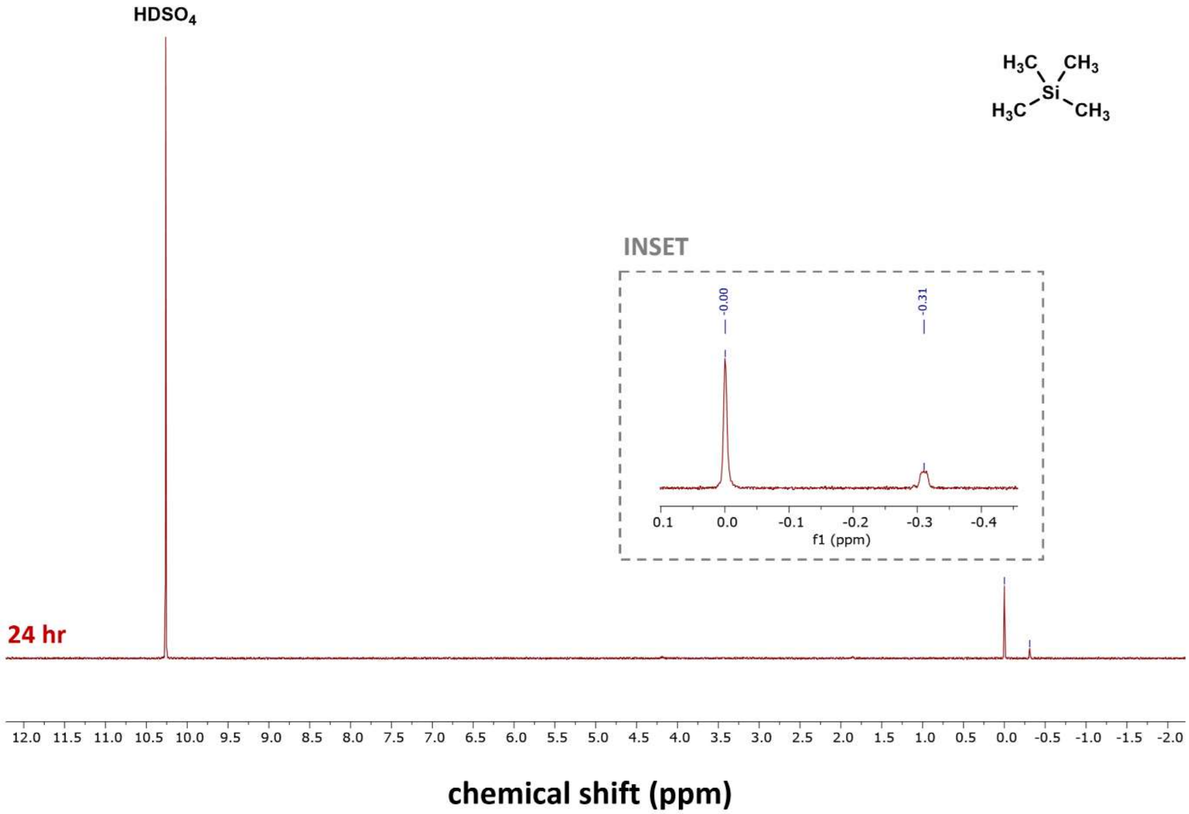

Figure S1. ^{1}H NMR spectrum of tetramethylsilane (TMS) in deuterated sulfuric acid after 1 day at room temperature. There is obvious partial decomposition of the TMS—evidenced by the new signal at −0.31 ppm.

The remaining seven compounds (**2–8**) were observed to be soluble, though KHP and 1,4-dinitrobenzene were not as soluble as the others. 1,4-dinitrobenzene required heat (~ 40 °C) to dissolve, while time and vortexing was sufficient for dissolving KHP. Compounds **2–8** were stable to decomposition over 14 days at 23 °C. No new peaks appeared in their ^{1}H NMR spectra after 14 days. The spectra for these compounds appear in Figure S2, and relevant spectral data are summarized in Table 1 in the main paper.

Second Screen (at Elevated Temperatures) of Candidates for Stability to Decomposition. The stabilities of compounds **2–8** were tested at elevated temperatures to ascertain whether these compounds would remain stable for 5 days at 50, 70, and 100 °C. Stability was judged by the original signals maintaining ≥99% of their integrated intensity relative to the entire integration over the range δ 0–9. Figure S3 shows example spectra for one candidate internal standard (**7**, KHP) at the three elevated temperatures (50, 70, and 100 °C). Similar spectra were acquired for all seven compounds (**2–8**). The integration data for these spectra are tabulated in the Source Data File (available as Supplementary Information as an Excel file).

Further Stability Testing of the Final Three Candidates. Figure S4 shows spectra taken when mixed samples of 1:1:1 1,4-dinitrobenzene:dimethyl sulfone:KHP were maintained at constant temperatures of 23 °C (room temperature), 50 °C, 70 °C, and 100 °C for 7 days. Each frame represents one replicate (of three). Table S2 shows the analysis of the tabulated integration data from the three replicate trials. All of the integrated data (for the three replicate experiments) appears in the Source Data File. The rationale for this extra test of stability was that the prior stability experiments that examined one compound at a time—while good at detecting decomposition—would not easily detect loss of internal standard to outgassing (of a volatile compound) or H/D exchange (where ^{1}H lost would simply blend into the solvent peak). From Table S2, the ratio of the integrations for dimethyl sulfone and 1,4-dinitrobenzene were remarkably stable over 7 days, changing by ≤2.0% at 23, 50, and 70 °C. At 100 °C, the change was 11.94 ± 4.96%. We cannot determine whether this difference arose from outgassing, H/D exchange, or another source. Still, dimethyl sulfone and 1,4-dinitrobenzene appear remarkably stable as internal standards when judged in the context of having to remain at constant concentration in hot sulfuric acid over seven days.

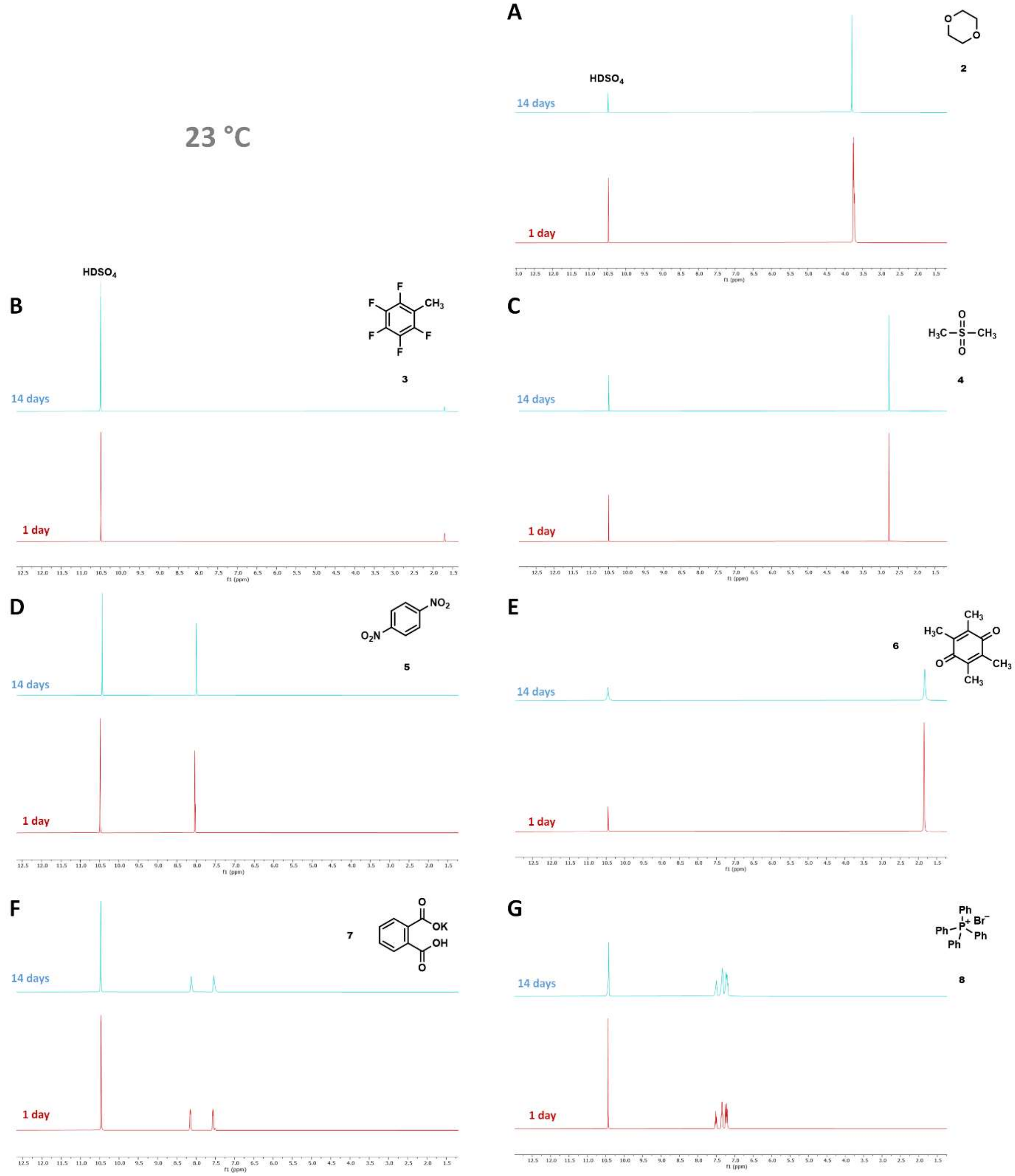

Figure S2. Example ^{1}H NMR spectra collected to monitor the thermal stability to composition of the candidate internal standards. Specifically, spectra of 1,4-dioxane (A), 2,3,4,5,6-pentafluorotoluene (B), dimethyl sulfone (C), 1,4-dinitrobenzene (D), duroquinone (E), potassium hydrogen phthalate (F), and tetraphenylphosphonium bromide (G) in deuterated sulfuric acid monitored for 2 weeks at 23 °C. The red spectra correspond to 1 day and the blue spectra to 14 days.

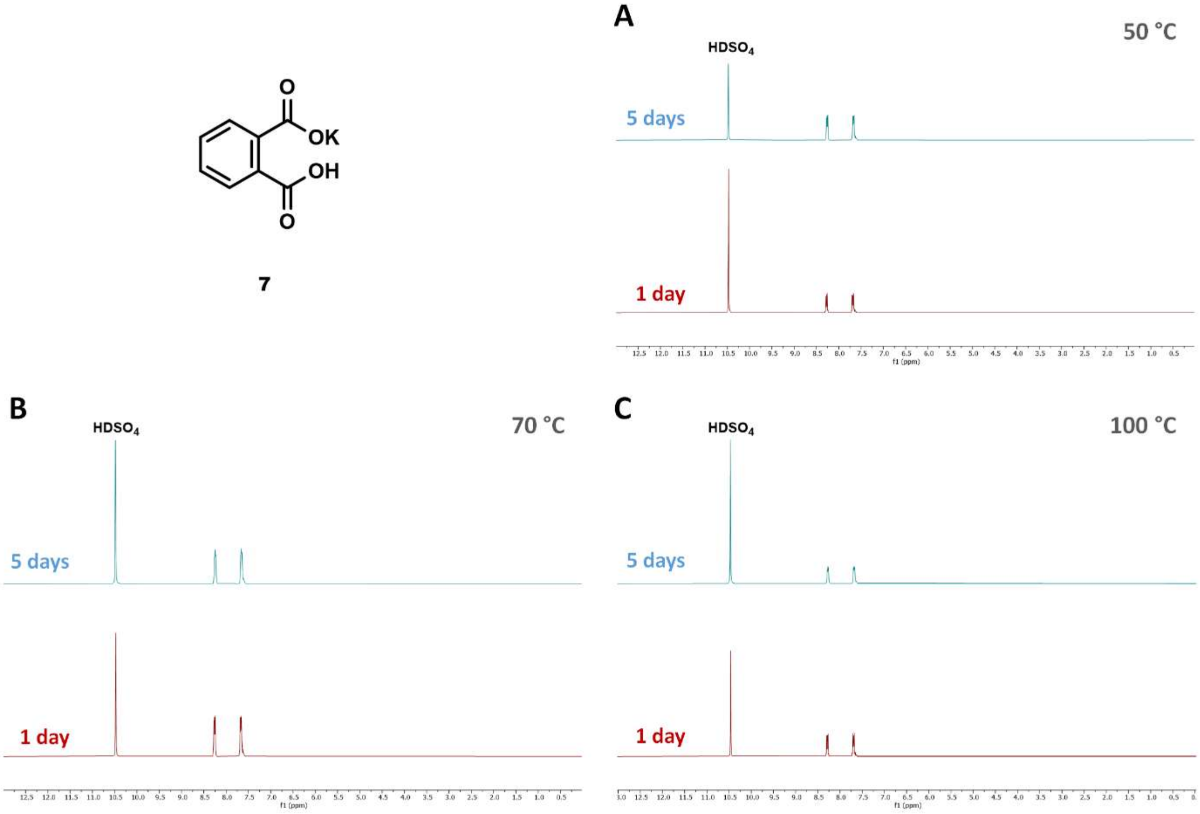

Figure S3. Example ^{1}H NMR spectra collected to judge the thermal stability to decomposition of the candidate internal standards. Specifically, spectra of potassium hydrogen phthalate (**7**, KHP) in deuterated sulfuric acid taken after 1 day and 5 days at 50 °C (A), 70 °C (B), and 100 °C (C). The red spectra correspond to 1 day and the blue spectra to 5 days. The absence of new peaks is indicative of the stability of phthalate to decomposition.

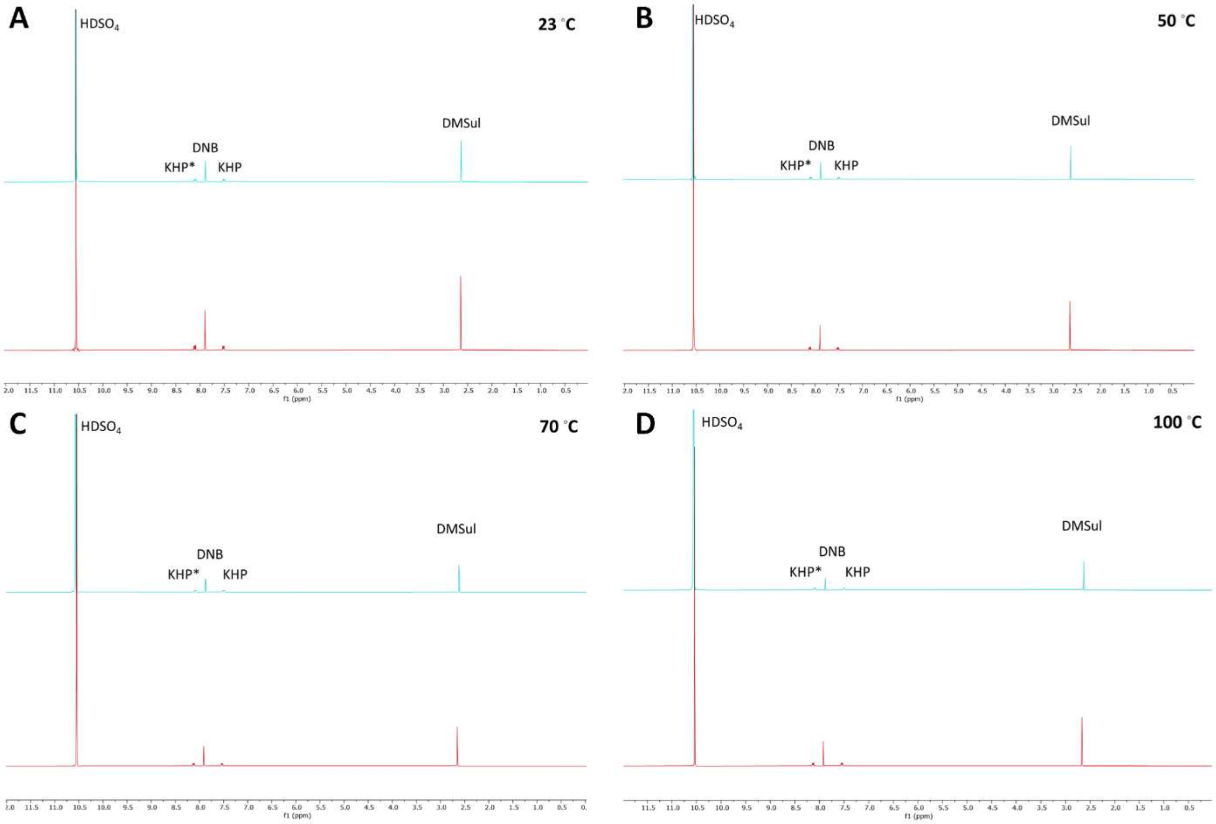

Figure S4. ^{1}H NMR spectra of a mixture of potassium hydrogen phthalate (KHP), 1,4-dinitrobenzene (DNB), and dimethyl sulfone (DMSul) in deuterated sulfuric acid monitored for 7 days at 23 °C (A), 50 °C (B), 70 °C (C) and 100 °C (D). Each stack of spectra represents one trial (of three trials total) at the indicated temperature. Red spectra are the initial measurement and blue spectra are after 7 days.

Table S2. Integration data and analysis of the experiments testing the thermal stability of dimethyl sulfone (DMSulf) and 1,4-dinitrobenzene (DNB) in a mixture with potassium hydrogen phthalate (KHP). The change in the ratio of the integrated peaks for the two compounds over 7 days is reported for 4 temperatures (23, 50, 70, and 100 °C) as 95% confidence intervals calculated from three replicate experiments.

	T/°C	Trial 1 DMSul/DNB	Trial 2 DMSul/DNB	Trial 3 DMSul/DNB	Trial 1 % chng	Trial 2 % chng	Trial 3 % chng	AVG % change (95% CI)
Initial	**23**	2.03	2.02	2.00				
Day 7	**23**	2.01	2.03	2.01	0.53	0.33	0.17	0.34 ± 0.46
Initial	**50**	2.03	2.02	2.02				
Day 7	**50**	2.08	2.03	2.03	2.60	0.55	0.56	1.23 ± 2.93
Initial	**70**	2.03	2.01	2.03				
Day 7	**70**	2.07	2.07	2.05	2.06	2.69	1.33	2.03 ± 1.70
Initial	**100**	2.04	2.03	2.03				
Day 7	**100**	2.33	2.31	2.23	13.90	12.00	9.92	11.94 ± 4.96

Selection of a Model Reaction to Evaluate the Effectiveness of the Internal Standards. In selecting a reaction to demonstrate the effectiveness of the internal standards in quantitative NMR spectroscopy, we wanted a straightforward reaction that proceeded cleanly (with no side reactions) in D_2SO_4 with a rate that could be measured conveniently (within a matter of days). We attempted the three decarboxylation reactions shown in Figure S5. The decarboxylation of malonic acid required elevated temperature (130 °C), at which point the stability of the internal standards (to decomposition, H/D exchange, and/or outgassing) was a concern. The decarboxylation of lithium 3-oxobutanoate was too fast to monitor conveniently—even at room temperature. We found that the decarboxylation of 3-methoxy-2,2-dimethyl-3-oxoproponoic acid (**9**) proceeded at a convenient rate at 50 °C. Furthermore, the methyl signals of **9** have less potential to be impacted by H/D exchange than the signals in the other two reactions, which arise from α-hydrogens.

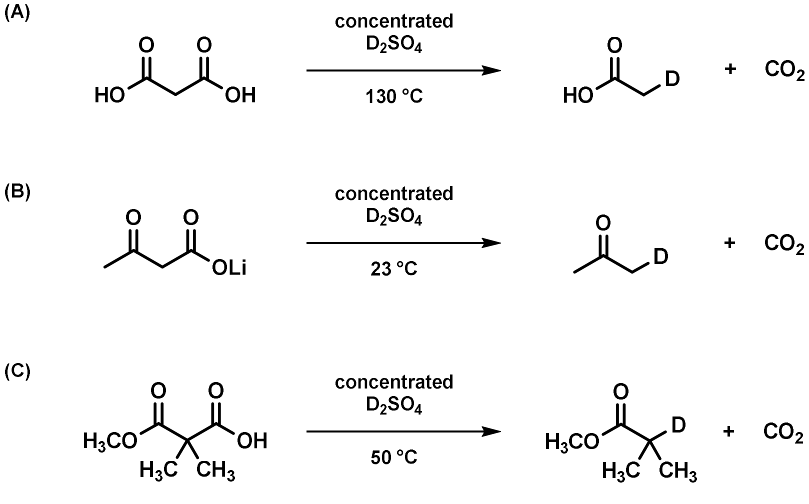

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Figure S5. Decarboxylation reactions of β-ketocarboxylic acids examined in this study: the decarboxylations of malonic acid (A), lithium 3-oxobutanoate (B), and 3-methoxy-2,2-dimethyl-3-oxopropanoic acid (C).

Measurement of the First-Order Rate Constant for Decarboxylation of 3-Methoxy-2,2-Dimethyl-3-Oxopropanoic Acid. In the main paper, dimethyl sulfone and 1,4-dinitrobenzene were the internal standards used to monitor the decarboxylation reaction. The molar ratio was 2:1:1 for 3-methoxy-2,2-dimethyl-3-oxopropanoic acid:dimethyl sulfone:1,4-dinitrobenzene in the reaction mixture. The reaction was set up on a magnetic hot plate in a 20 mL scintillation vial placed in an aluminium heat block (17-position, with 24 mm hole depth) at 50 °C. Aliquots removed from the reaction mixture were transferred into NMR tubes at elapsed times of 30 minutes, 5.5 hours, 24 hours, 48 hours, 72 hours, and 96 hours. This experiment was run in triplicate, and the results are discussed in the main paper.

Supplementary References

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