Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supplemental Information

High-field dissolution dynamic nuclear polarization of [1-^{13}C]pyruvic acid

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Experimental Methods

Materials

[1-^{13}C]pyruvic acid was acquired from Sigma-Aldrich (Buchs, Switzerland), and formulated with OX063 (tris(8-carboxy-2,2,6,6-tetrakis(2-hydroxyethyl)benzo[1,2-*d*:4,5-*d*']bis([1,3]dithiole)-4-yl)methyl sodium salt). When gadoteric acid (Dotarem®, Guerbet AG, Zurich, Switzerland) was used in formulation, an intermediate 46.5 mM dilution was first prepared from the 0.5 M stock and then added to the pyruvic acid to a final concentration of 1.5 mM.

Polarization Procedure

Doped [1-^{13}C]pyruvic acid (15 μl) was pipetted into a polytetrafluoroethylene (PTFE) sample cup and frozen in liquid nitrogen. A stoichiometric equivalent of 10 M sodium hydroxide (20 μl) was added separately and frozen in the cup. The polarizer cryostat was charged with liquid helium; the sample cup was then placed into a fiberglass tube sample holder and lowered inside. After installing the waveguide and microwave source, the sample was irradiated with 55 mW at 196.80 GHz while the cryostat was cooled to 1.0 K under partial vacuum. Polarization buildup was monitored with a low flip-angle radio frequency (RF) pulse every 5 minutes and the spectral peak integral fitted to an exponential curve to obtain the buildup time constant; an example is shown in Figure S2. Upon reaching >93% of the maximum polarization, the sample was rapidly dissolved in 6.0 ml of buffered D_2O (47 mM sodium phosphate, 100 mM NaCl, 2.7 mM KCl, 0.3 mM EDTA, pH 7.4) pre-heated to 11 bar, and the solution transferred though PTFE tubing with chase gas flow to a phase separator / infusion pump equipped with ^{13}C and ^{1}H RF

coils[1] placed at the isocenter of a 9.4 T horizontal bore scanner (Magnex Scientific, Oxford, UK) with a VNMRS console (Varian, Palo Alto, CA, USA). The acquisition start was triggered by the dissolution routine via a TTL port on the console and a series of 60 ^{13}C NMR spectra was acquired using a ~5° pulse every 3 s, starting 3 s after the beginning of the dissolution. The thermally polarized ^{13}C signal was acquired after the addition of Gd^{3+} to hasten its recovery. Gadoteric acid (5 μl of 0.5 M) was added via a narrow gauge polyethylene catheter to a final concentration of ~1 mM, with the solution partially withdrawn and reinjected to ensure complete mixing. To avoid any error from miscalibration of the flip angle, the same acquisition parameters used for the hyperpolarized spectra were used to acquire the thermal spectrum, except that 1024 scans were averaged with a repetition time of 1.1 s. To achieve better resolution of the unenriched 2-ketone signal from the minor [1,2-$^{13}C_2$]pyruvate isotopomer, some spectra were obtained following the automated transfer of the dissolved hyperpolarized pyruvate from the phase separator / infusion pump into a 10 mm NMR tube fitted with ^{13}C and ^{1}H coils.

Frequency Sweep

The effect of microwave frequency on polarization was measured at 3.6 K with 60 μl of [1-^{13}C]pyruvic acid containing 25 mM OX063. The sample was irradiated, starting at 196.75 GHz until the solid-state ^{13}C polarization reached a plateau, then the frequency was iteratively increased by 0.01 GHz, and the polarization level allowed to stabilize at each step.

Data Analysis

ACD/NMR Processor (ACD/Labs, Toronto, Canada) was used to quantitate the pyruvate peak integrals. Spectra were transformed with 5 Hz line broadening and baseline corrected and the integral obtained from the 5-ppm-wide region centered on the [1-^{13}C]pyruvate peak at 172.9 ppm; an example is shown in Figure S1. Polarization was calculated by the ratio of the peak integral of the first hyperpolarized spectrum to that of the thermal spectrum (averaged to the number of scans), multiplied by the thermal polarization of 0.00083% at 9.4 T and 290 K, the temperature inside the magnet bore. Statistical analyses were performed using GraphPad Prism (v. 5.04, GraphPad Software, La Jolla, CA, USA), with Student's *t*-test or one-way ANOVA where appropriate.

References

1. T. Cheng, M. Mishkovsky, J. A. M. Bastiaansen, O. Ouari, P. Hautle, P. Tordo, B. van den Brandt and A. Comment, NMR Biomed., 2013, **26**, 1582–1588.

Supplemental Figures

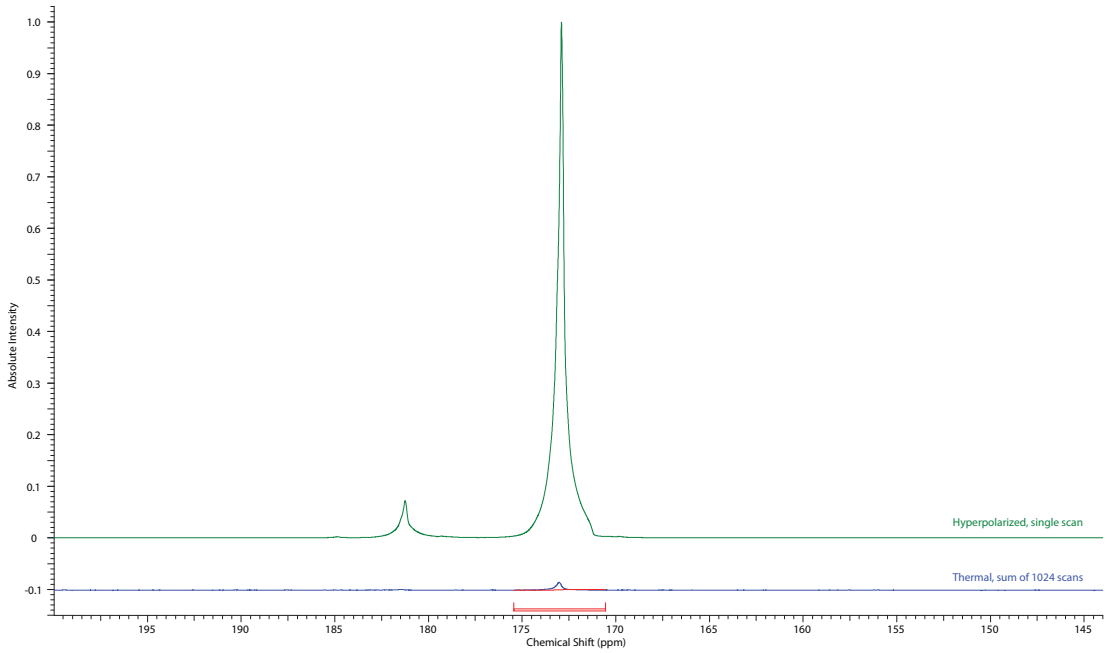

Figure S1. Example of [1-^{13}C]pyruvate signal enhancement. The indicated 5 ppm peak integration range was used for both thermal and hyperpolarized spectra. The minor [1-^{13}C]pyruvate hydrate peak appears at 181.25 ppm.

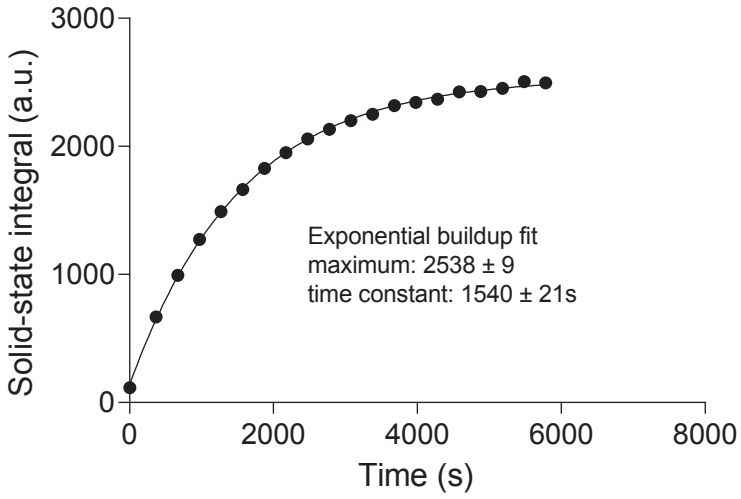

Figure S2. Example of solid-state buildup with exponential fit of a 25 mM OX063-doped [1-^{13}C]pyruvic acid sample polarized at 7 T & 1.0 K.