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

Production of highly concentrated and hyperpolarized metabolites within seconds in high and low magnetic fields

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Supplementary information

Materials and instruments

All the chemicals except vinyl acetate-d_6 (EQ Laboratories GmbH) were purchased from Sigma Aldrich and used as received. ^1H NMR and ^{13}C NMR spectra were acquired in high field on a Bruker ultrashield 300 MHz spectrometer and in low field on a Magritek Spinsolve-43 (1 T field). The high field system is equipped with temperature control system and the probeheads with Z-gradient. The low field system has a temperature control that keeps the temperature stable at 298 K inside the spectrometer. No controllable gradient system is available. Chemical shifts (δ) are given in parts per million (ppm) relative to chloroform-d (7.26 ppm for ^1H and 77.16 ppm for ^{13}C) or methanol-d_4 (3.34 ppm for ^1H and 49.86 ppm for ^{13}C).

General setup and reaction conditions

The solution for the experiments with low concentrations (<10 mM) were prepared from stock solutions of vinyl acetate-d_6 (20 mM). Solutions with higher concentrations were prepared directly in the following way: Commercially available catalyst ([1,4-Bis(diphenylphosphino)butane](1,5-cyclooctadiene)rhodium(I) tetrafluoroborate) was added to chloroform-d to achieve a 2 mM catalyst concentration. A known volume of vinyl acetate-d_6 was injected quickly into catalyst solution to prevent its evaporation. The final substrate concentration was measured after hydrogenation and compared to an external standard (500 mM benzoic acid in $CDCl_3$). Para-enriched hydrogen gas was obtained using a Bruker Para-Hydrogen Generator (BPHG 90). The nominal conversion temperature in the generator was set to 36 K providing 83% para-enriched hydrogen gas as calculated from the content of ortho-hydrogen measured in the gas phase. The content of ortho-hydrogen was quantified with respect to non-polarized hydrogen. Gaseous para-hydrogen was delivered through a capillary at 320 K using a home-built automated setup and bubbled through the solution in a 5 mm NMR tube. For smaller volume samples 3 or 2 mm capillaries were inserted into a 5 mm NMR tube. The gas delivery setup follows the idea of Ref. [1]. The para-hydrogen gas was kept at 7 bar to achieve a higher concentration of the dissolved gas and thus increase the rate of the hydrogenation reaction.

High field hydrogenation

The NMR measurements in high field were conducted in two standard inverse double resonance probeheads: 5 mm or 10 mm, in a 7 T cryo-magnet coupled to a Bruker system (Avance III HD, 300 MHz). An NMR pulse sequence with 10 s gas bubbling (described in the main text) was initiated to produce and transfer para-hydrogen spin order to observable magnetization and the FID of the enhanced ^1H or ^{13}C signal was recorded. 0.4 ml sample volume was used.

Low field hydrogenation:

The NMR measurements in low field were conducted in a 1 T low-field spectrometer (Magritek Spinsolve-43). Since no BPFGs were available in the spectrometer, we have performed experiments with 180°-refocusing pulses only. Only a 5 mm NMR tube is possible to use in the setup. Temperature inside the spectrometer is maintained at 298 K at all the time. In order to facilitate the hydrogenation reaction in the NMR tube the solution with substrate and catalyst was preheated in a water bath to 50 °C which is similar to the temperature used in high field. A sample volume of 0.6 ml was used.

Calibration of the hyperpolarization

In high field, the polarized proton signal intensity was compared with the thermally polarized product (EA) signal after reaction and enhancement factors were calculated. It works well for all concentrations. The same was true for the carbon hyperpolarization at 50 mM that could be directly compared with the thermally polarized 100 mM product (see Fig. 3c in main text). In addition, the carbon polarization calibration was done by comparing to an external reference sample (500 mM benzoic acid in chloroform-d): First, the concentration of the product molecule was found through comparison of the proton signals to the external reference sample. Second, the carbon signal of the same reference sample was compared with the hyperpolarized signal of the product to find the enhancement factor. Both carbon signal calibration procedures gave the same result. In low field, the carbon and proton hyperpolarization calculations were done through the external reference sample (500 mM benzoic acid in chloroform-d).

Hydrolysis procedure

The para-hydrogenation was conducted in methanol-d_4 solvent. After the polarization transfer was finished the pressure was released and same volume of room temperature 1 M NaOD in D_2O was injected into the 5 mm NMR tube resting in a spectrometer. Nitrogen gas was bubbled to mix the solution and after a delay the spectra of hydrolysed products together with catalyst were detected by a single RF pulse.

Influence of the gradient on the polarization of 0.8 mM vinyl acetate in $CDCl_3$ in a 5 mm sample within a 5 mm probe head

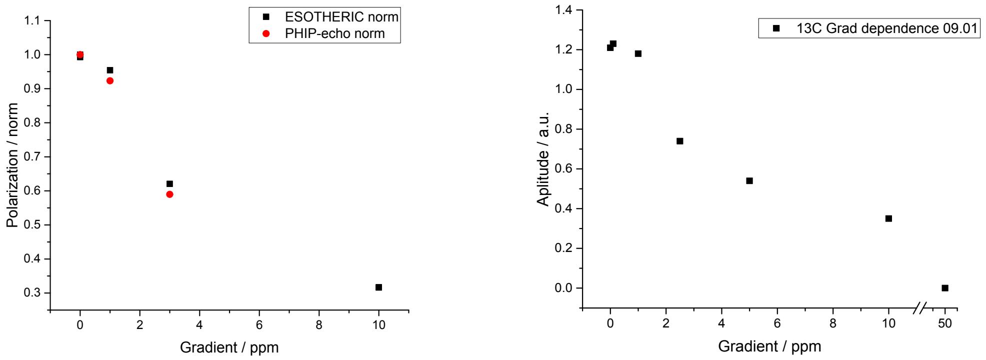


Figure S1.: Normalized polarization levels of ^{13}C (after ESOTHERIC) and ^{1}H (after PHIP-echo) in dependence on the applied gradient (in ppm of resulting linewidth) during the pulse. sequence. Note that no radiation damping at this low concentration is observed. The polarization drops with increasing gradient strengths indicating diffusion effects. 1 ppm in high field corresponds to a 50 mG/cm gradient.

Three PHIP-echo measurements in a 2 mm capillary within a 10 mm probe head

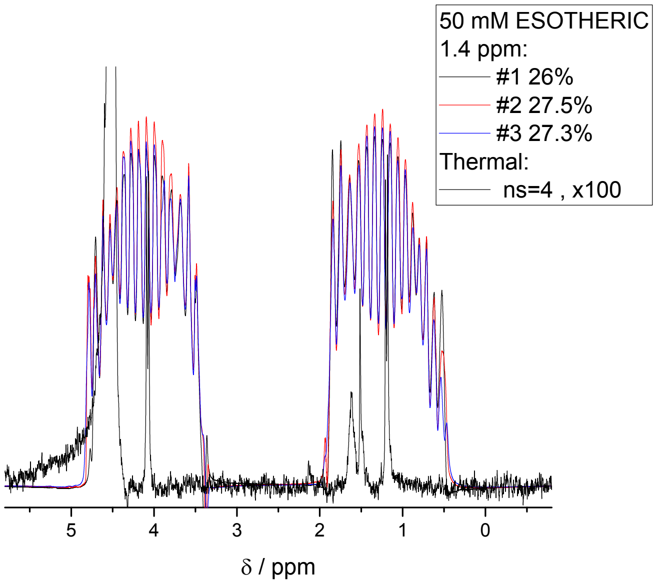


Figure S2.: Three PHIP-echo measurements of ^{1}H polarization of 50 mM ethyl acetate-d$_6$ and 2 mM catalyst in chloroform-d under a ~10 G/m Z-gradient at 320 K in comparison with the thermally polarized product. The peak at 4.7 ppm corresponds to water and the peak at ~1.5 ppm to the catalyst.

Table S1. High-field ^{1}H hyperpolarization

#	Integral	Integral	Enhancement	Enhancement	Polarization	Polarization
	4.1ppm	1.2ppm	4.1ppm	1.2ppm	4.1ppm	1.2ppm
1	7.15E7	7.57E7	11578	12259	0.25937	0.2746
2	7.6E7	7.6E7	12307	12307	0.27569	0.27569
3	7.55E7	7.48E7	12226	12113	0.27388	0.27134

P(4.1ppm) = 27.0±0.9%

P(1.2ppm)=27.4±0.2%

Three ESOTHERIC measurements in a 2 mm capillary within a 10 mm probe head

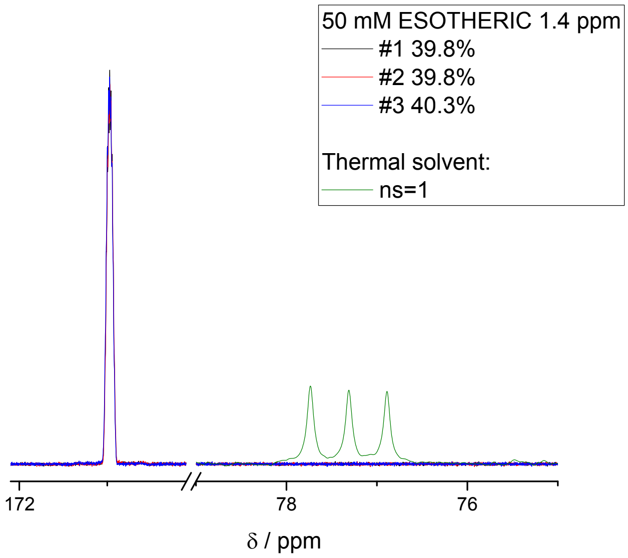


Figure S3.: Three ESOTHERIC measurements of ^{13}C polarization of 50 mM ethyl acetate-d_6 and 2 mM catalyst in chloroform-d under a ~10 G/m Z-gradient at 320 K in comparison to the thermally polarized solvent.

Table S2. High-field ^{13}C hyperpolarization.

#	^{13}C integral	Enhancement	Polarization
1	7.9904E6	70400	0.39846
2	8E6	70484	0.39894
3	8.09E6	71277	0.39894

P(^{13}C)=39.9±0.3 %

Low field experiments

Low field PASADENA starting from 1 mM vinyl acetate

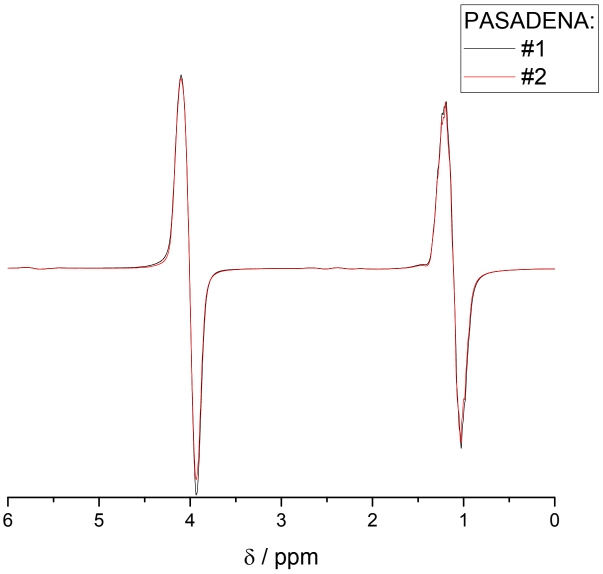


Figure S4.: Two PASADENA experiments of 1 mM ethyl acetate-d_6 and 2 mM catalyst in chloroform-d detected after a single 45° pulse. The polarization of each proton is 20.2%.

Low field PHIP-echo starting from 1 mM vinyl acetate

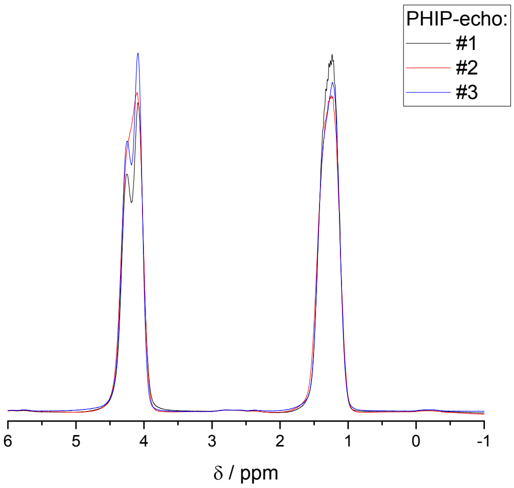


Figure S5.: Three PHIP-echo experiments utilizing 1 mM ethyl acetate-d_6 and 2 mM catalyst in chloroform-d. The polarization of the proton a 4.1 ppm is 26.1±1.4% and 28.7±0.8% of the proton at 1.2 ppm. The sum of both protons would give 54.8± 0.7% polarization that can be transferred to a ^{13}C spin.

Table S3. Low-field ^{1}H polarization

#	Integral	Integral	Enhancement	Enhancement	Polarization	Polarization
	4.1ppm	1.2ppm	4.1ppm	1.2ppm	4.1ppm	1.2ppm
1	63.61	76.96	70365	85132	0.24487	0.29626
2	70.37	73.52	77842	81327	0.27089	0.28302
3	69.76	73.14	77168	80907	0.26855	0.28156

Low field ESOTHERIC with 1 mM vinyl acetate

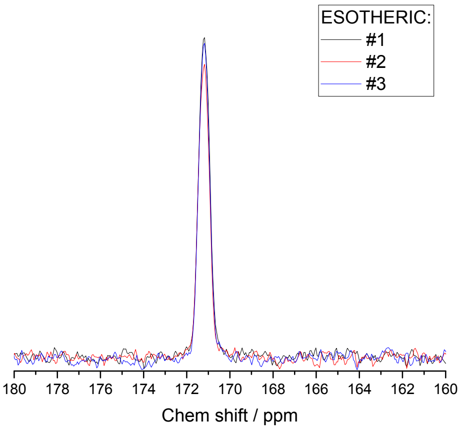


Figure S6.: Three ESOTHERIC experiments of 1 mM ethyl acetate-d_6 and 2 mM catalyst in chloroform-d. The polarization is 34±1.7%.

Table S4. Low-field ^{13}C polarization

#	Integral	Enhancement	Polarization
1	0.14369	410542	0.35964
2	0.13023	372085	0.32595
3	0.13400	382857	0.33538

Low field ESOTHERIC starting from 10 mM vinyl acetate and 1 ppm line broadening

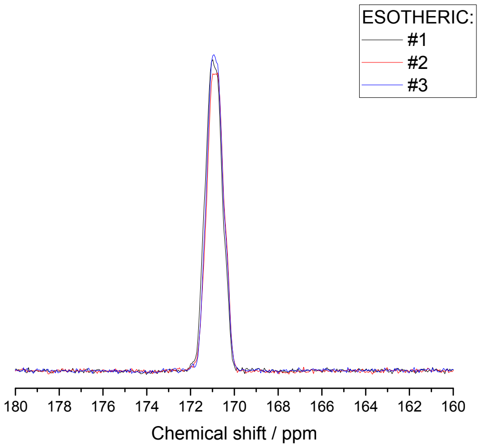


Figure S7.: Three ESOTHERIC experiments of 10 mM ethyl acetate-d_6 and 2 mM catalyst in chloroform-d. The polarization is 37.3±0.9%

Table S5. Low-field ^{13}C polarization of 10mM ethyl acetate

#	Integral	Enhancement	Polarization
1	1.52	434285	0.38043
2	1.45	414285	0.36291
3	1.5	428571	0.37543

Low field PHIP-echo starting from 50 mM vinyl acetate

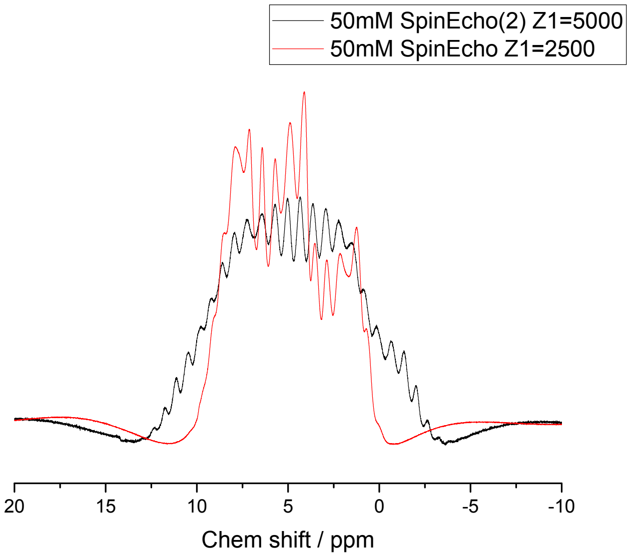


Figure S8.: Two PHIP-echo experiments of 50 mM ethyl acetate-d_6 and 2 mM catalyst in chloroform-d broadened with two different gradients. The sum of the peaks at 4.1 and 1.2 ppm result in 18% polarization under a gradient of 6.5 ppm and 19.5% under 3.25 ppm gradient.

Low field ESOTHERIC starting with 50 mM vinyl acetate

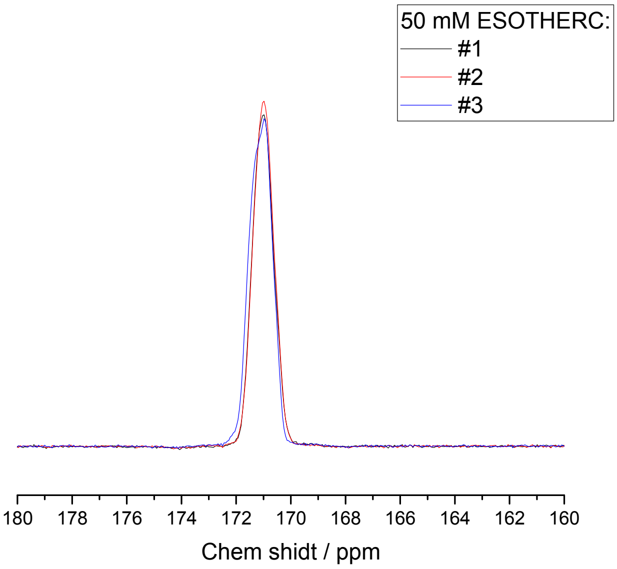


Figure S9.: Three ESOTHERIC experiments of 50 mM ethyl acetate-d_6 and 2 mM catalyst in chloroform-d under a gradient of 1 ppm. The polarization is 14.7 ± 0.5%

Table S6. Low-field ^{13}C polarization of 50mM ethyl acetate

#	Z1	LW/ppm	Integral	Enhancement	Polarization
1	500	1	2.85	162857	0.14266
2	500	1	2.92	166857	0.14617
3	500	1	3.06	174857	0.15317

Dependence of polarization on the linewidth in low field

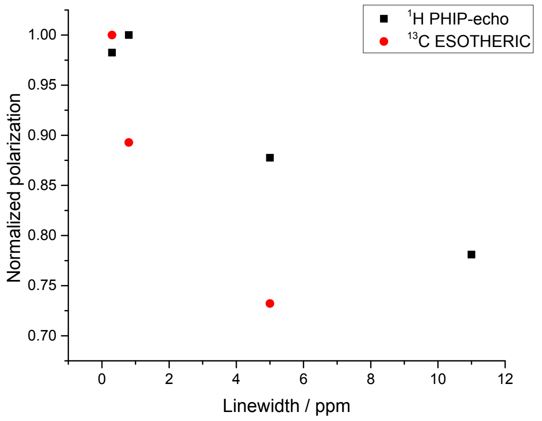


Figure S10.: Dependence of the normalized polarization on the linewidth in PHIP-echo and ESOTHERIC experiments of 1 mM ethyl acetate-d_6 and 2 mM catalyst in chloroform-d. The ESOTHERIC efficiency decays faster with an applied gradient. If a line broadening larger than 5 ppm is applied for ^{13}C, the signal vanishes.

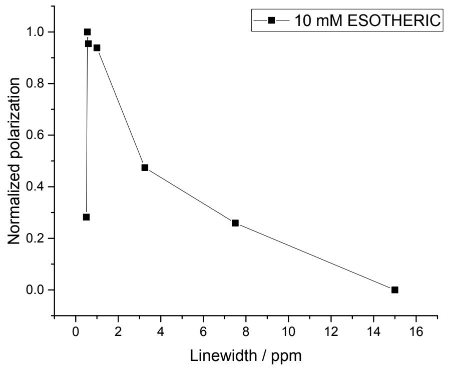


Figure S11.: Dependence of the normalized polarization on the linewidth in ESOTHERIC experiments of 10 mM ethyl acetate-d6 and 2 mM catalyst in chloroform-d. The polarization increases when a low gradient is applied because of a reduction of the radiation damping. Afterwards the polarization decays with an increasing gradient and even faster than at 1 mM concentration.

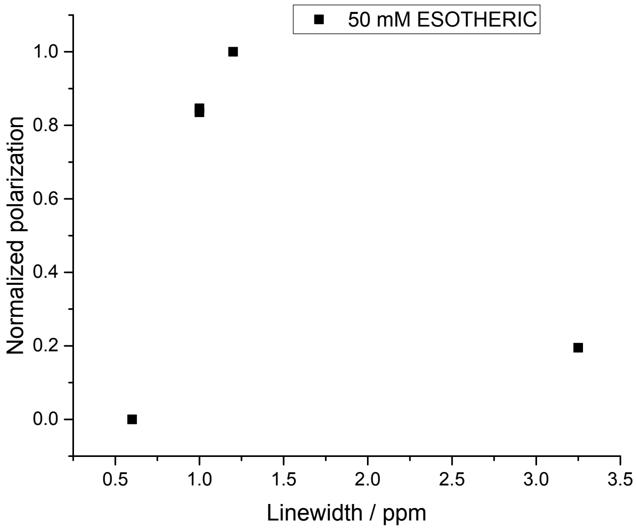
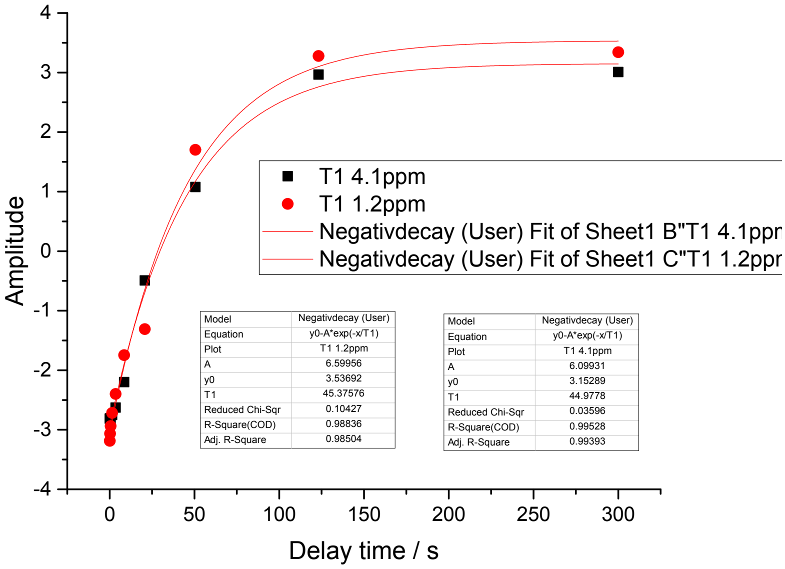


Figure S12.: Dependence of the normalized polarization on the linewidth in ESOTHERIC experiments of 50 mM ethyl acetate-d6 and 2 mM catalyst in chloroform-d. The polarization increases at low gradients because of the reduction of radiation damping and decay with increasing gradients due to diffusion.

T_1 measurements in low field

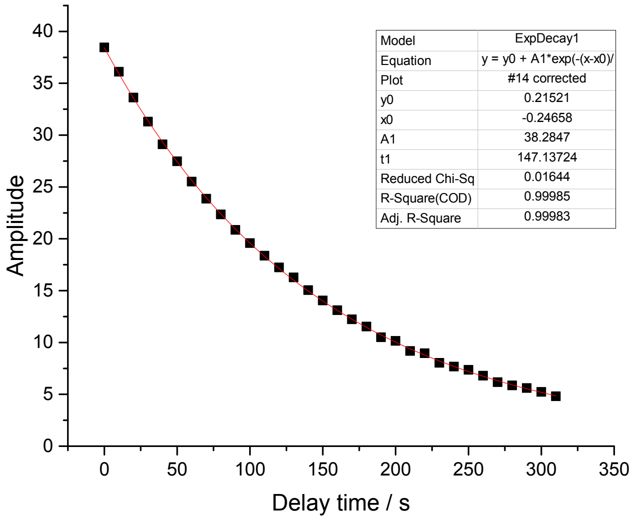


Model	Negativdecay (User)
Equation	y0-A*exp(-x/T1)
Plot	T1 1.2ppm
A	6.59956
y0	3.53692
T1	45.37576
Reduced Chi-Sqr	0.10427
R-Square(COD)	0.98836
Adj. R-Square	0.98504

Model	Negativdecay (User)
Equation	y0-A*exp(-x/T1)
Plot	T1 4.1ppm
A	6.09931
y0	3.15289
T1	44.9778
Reduced Chi-Sqr	0.03596
R-Square(COD)	0.99528
Adj. R-Square	0.99393

Figure S13.: ^{1}H inversion recovery experiment of a sample containing 50 mM ethyl acetate-d_6 and 2 mM catalyst in chloroform-d at 298 K obtained after the hydrogenation reaction. ^{1}H T_1 for both is similar and equals to 45±6 s.

T_1 measurements in high field



Model	ExpDecay1
Equation	y = y0 + A1*exp(-(x-x0)/
Plot	#14 corrected
y0	0.21521
x0	-0.24658
A1	38.2847
t1	147.13724
Reduced Chi-Sq	0.01644
R-Square(COD)	0.99985
Adj. R-Square	0.99983

Figure S14.: Detection with small flip angle (α~6°) of the decay of ^{13}C hyperpolarization of carbonyl carbon in the acetic anion. HP ethyl acetate-d_6 was obtained after para-hydrogenation of 50 mM of vinyl acetate-d_6 in methanol-d4 at 320 K and polarization transfer to ^{13}C carbonyl of the acetate moiety. The acetic anion was formed after ~80 s hydrolysis with the same volume of 1 M NaOD in D_2O. The integral data was corrected for the decay due to pulse excitation by dividing it with $\cos(\alpha)^n$. T_1 is equals to 147±2 s.

References:

[1] A.S. Kiryutin, G. Sauer, S. Hadjiali, A.V. Yurkovskaya, H. Breitzke, G. Buntkowsky, *J. Mag. Reson*. **2017**, *285*, 26.