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Supporting Information

Over 50 % ^{1}H and ^{13}C Polarization for Generating Hyperpolarized Metabolites—A *para*-Hydrogen Approach

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Supplementary information

1. Materials and instruments

All the chemicals were purchased from commercial suppliers and used as received (Sigma Aldrich and EQ Laboratories GmbH). ^{1}H NMR and ^{13}C NMR spectra were acquired on a Bruker ultrashield 300 MHz spectrometer at 320 K. Chemical shifts (δ) are given in parts per million (ppm) relative to methanol-d_4 (3.34 ppm for ^{1}H and 49.86 ppm for ^{13}C).

2 NMR experiments

General setup and reaction conditions

The samples for the experiments were prepared from stock solutions of the vinyl acetate-d_6 (20 mM), 2 mM of commercially available catalyst ([1,4-Bis(diphenylphosphino)butane](1,5-cyclooctadiene)rhodium(I) tetrafluoroborate) and 9 mM of toluene-alpha-13C as internal standard in methanol-d_4. Final concentrations are as follows: 0.9 mM vinyl acetate-d_6, 2 mM ([1,4-Bis(diphenylphosphino)butane](1,5-cyclooctadiene)rhodium(I) tetrafluoroborate) and 9 mM toluene-α-^{13}C in 0.6 ml or 0.4 ml methanol-d_4. The experiments were performed in 5 mm NMR tubes. 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 92% para-enriched hydrogen gas. Gaseous para-hydrogen was delivered through a capillary and bubbled through the solution at 320K using a home-built automated setup. The gas delivery setup follows the idea of Ref. [1]. The para-hydrogen gas was kept at 7 bar to achieve higher concentration of the dissolved gas and increase the rate of the hydrogenation reaction. The NMR measurements were done in a standard double resonance probe-head in a 7 T cryo-magnet coupled to a Bruker Spectrometer System (Avance III HD, 300 MHz). Following a bubbling period of 10 s and a settling time of 2 s, NMR pulse sequences (as described in the main text) were initiated to transfer para-hydrogen spin order to observable magnetization and the FIDs of the enhanced ^{1}H or ^{13}C signal were recorded.

To ensure the full conversion during the hydrogenation, first experiments were performed on protonated vinyl acetate to adjust the reaction parameters. Figure S1 displays the ^{1}H NMR spectrum of reactant and product. To check stability of the internal standard under hydrogenation conditions, spectra before the reaction and afterwards were measured in the 0.6 ml (Fig. S2) and 0.4 ml (Fig. S3) samples.

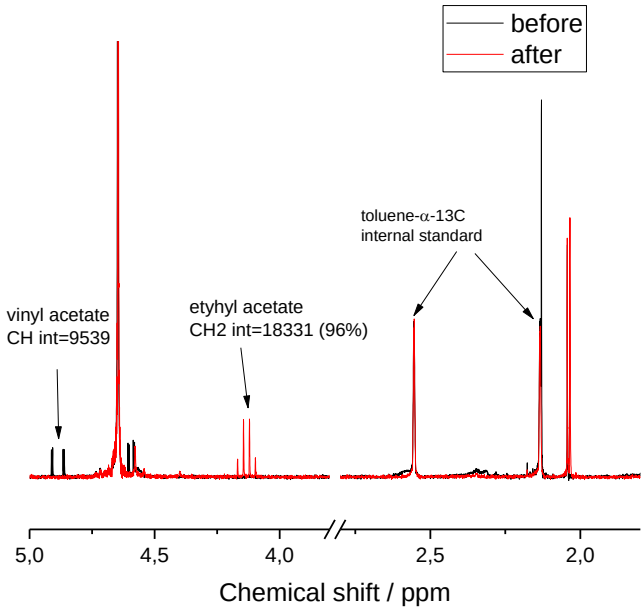

Figure S1.: ^{1}H NMR spectra at 320 K. Spectra were recorded before the hydrogenation (10 s bubbling) of 0.4 ml of 2.5 mM of vinyl acetate and 2 mM of catalyst and afterwards. Within 5 % the conversion is complete. The peaks of the internal standard toluene-α-^{13}C are not changed. Chemical shift is referenced to the solvent peak (3.34 ppm methanol).

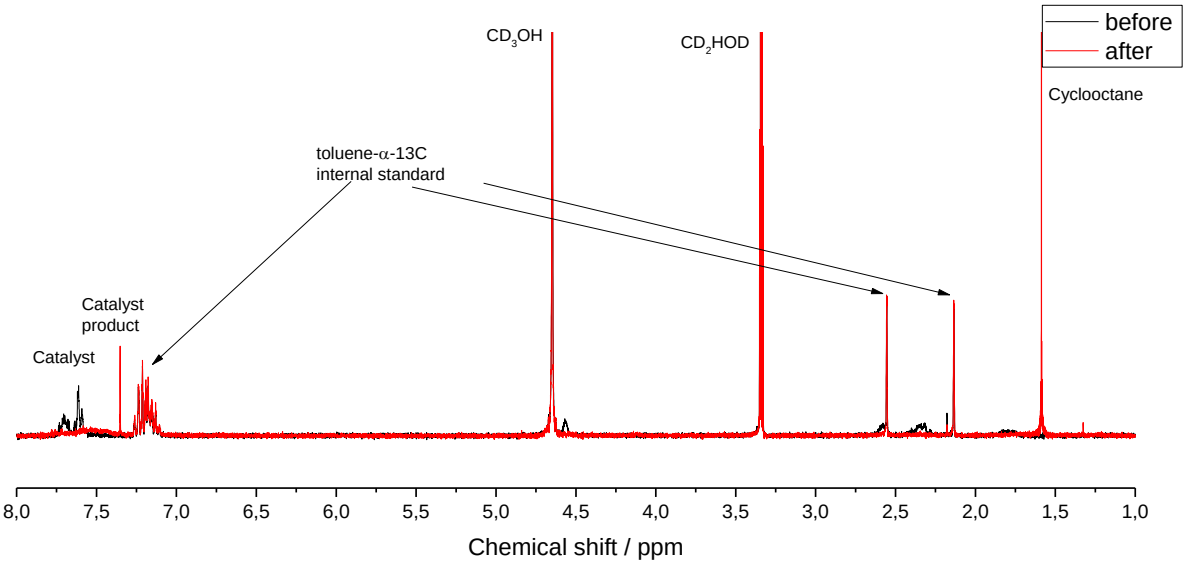

Figure S2.: ^{1}H NMR spectra at 320 K. Spectra were recorded before the hydrogenation (10 s bubbling) of 0.6 ml of 0.9 mM of vinyl acetate-d_6 and 2 mM of catalyst and afterwards. The peaks of the internal standard toluene-α-^{13}C do not change. Chemical shift is referenced to the solvent peak (3.34 ppm methanol).

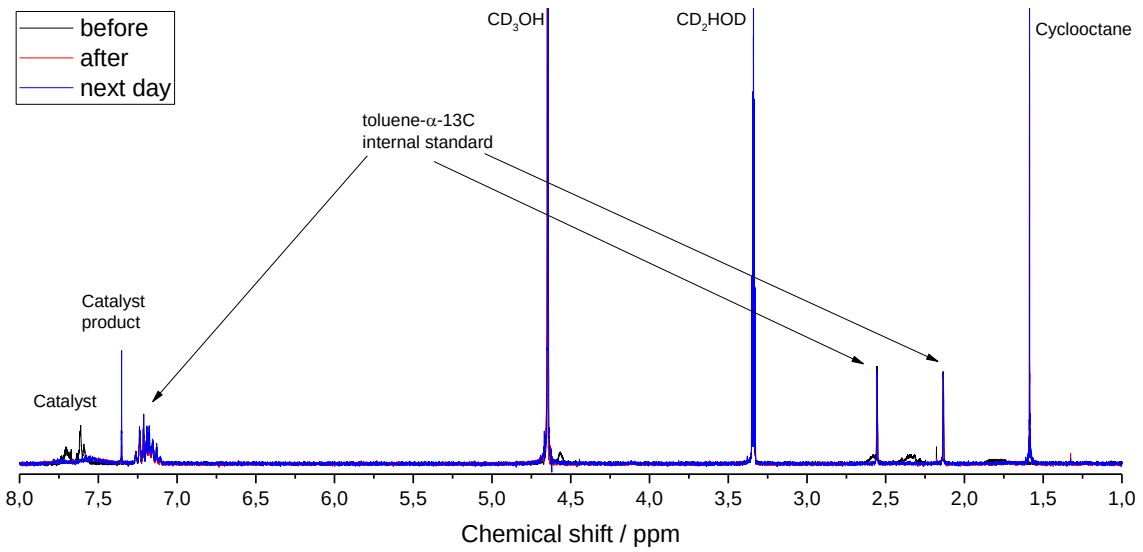

Figure S3.: ^{1}H NMR spectra at 320K. Spectra were recorded before the hydrogenation (10 s bubbling) of 0.4 ml of 0.9 mM of vinyl acetate-d_6 and 2 mM of catalyst, afterwards and on the next day after flame sealing the NMR tube. The peaks of the internal standard toluene-α-13C are not changed within 3%. Chemical shift is referenced to the solvent peak (3.34 ppm methanol).

Polarization calculations

The polarization calculations were done in the following way:

Before the reaction with para-hydrogen, control ^{1}H and ^{13}C spectra were recorded, showing only the internal standard and catalyst. After the reaction, similar spectra were recorded to make sure that no evaporation of the internal standard occurs. The sample tube was then flame sealed. The thermal spectrum of the resulting product ethyl acetate and the internal standard toluene-α-^{13}C was recorded using a 90° excitation pulse. 32 scans under ^{2}H decoupling were recorded with an inter-scan delay of 500 s for protons (Fig. S4) and 100 scans under ^{1}H decoupling with an inter-scan delay of 120 s for carbons (Fig. S5).

The signal enhancement of protons was calculated by comparing the integrals of the hyperpolarized and thermally polarized product spectra. Furthermore, the ratio of the internal standard and product was calculated. The signal enhancements of the carbon nucleus were calculated by forming the integral ratio of hyperpolarized ethyl acetate and internal standard and multiply it by the ratio of internal standard and ethyl acetate obtained from the proton spectrum in thermodynamical equilibrium. The natural abundance of ^{13}C of 0.0108 [2] was taken into account. The thermal polarization at 320 K is 22.4 ppm for protons and 5.66 ppm for carbons, respectively.

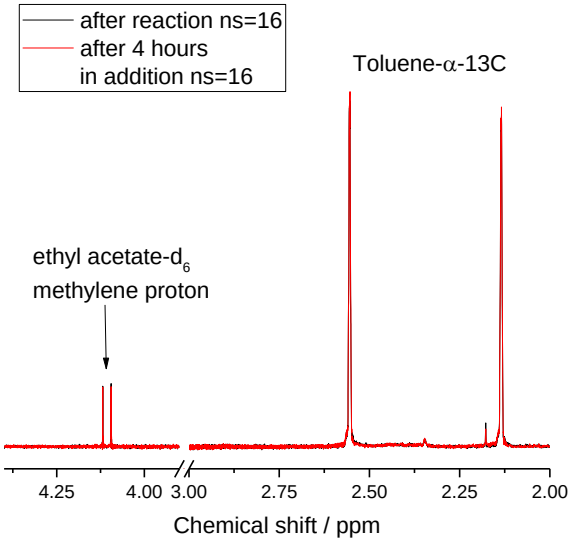

Figure S4.: ^{1}H NMR spectra under ^{2}H decoupling of the hydrogenated flame sealed sample. The peaks of the internal standard toluene-α-13C and the ethyl acetate product are not changed. The peak intensities of the product are used to calculate the proton hyperpolarization, enhancement and ratio between product and internal standard. The relaxation delay between scans was 500 s and 32 scans were acquired.

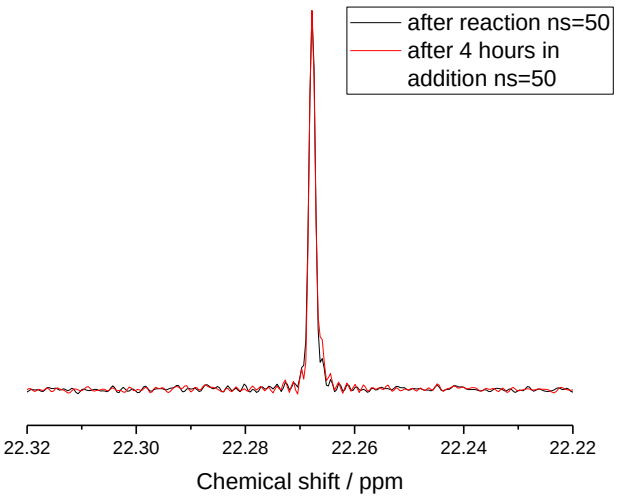

Figure S5.: ^{13}C NMR spectra with ^{1}H decoupling of the hydrogenated flame sealed sample. The peak of the internal standard toluene-α-13C is not changed even after several hours. The peak intensities of the internal standard is used to calculate the carbon hyperpolarization enhancement together with the ratio

between product and internal standard (from ^{1}H data). The relaxation delay between scans was 120 s with 100 averages.

Proton and carbon hyperpolarization experiments were repeated three times each (Fig. S6 and Fig. S7). The polarization values are summarized in the table 1.

Table 1:

Number of experiment	^{1}H integral	^{13}C integral	^{1}H polarization	^{13}C polarization
1	33309000	2142000	51.3%	57.7%
2	36717000	2193080	56.6%	59.1%
3	36935000	2246700	56.9%	60.5%

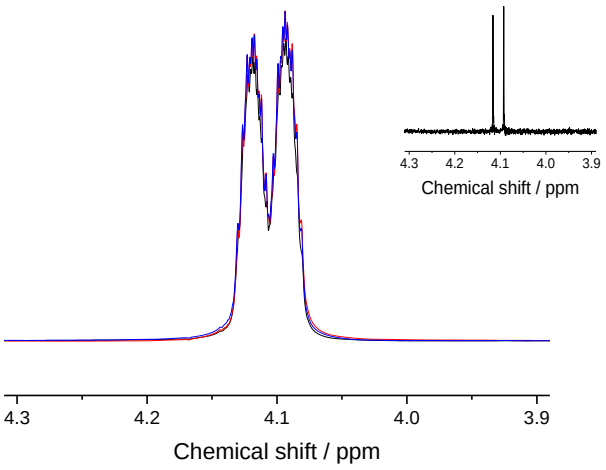

Figure S6. : ^{1}H polarized spectra of the methylene proton of ethyl acetate-d_6 after 10 s of bubbling with para-hydrogen at 320 K and applying the described pulse sequence to produce net magnetization. The inset shows a spectrum of the product ethyl acetate with ^{2}H decoupling (ns=32 d1=500 s, 90° pulse).

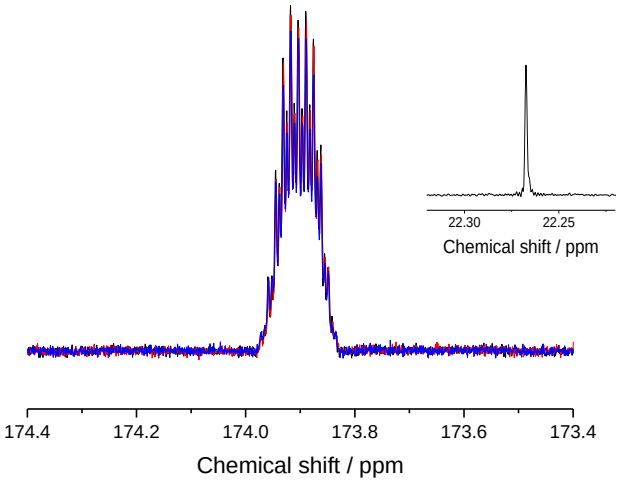

Figure S7. : ^{13}C polarized spectra of the carbonyl carbon of ethyl acetate-d_6 after 10 s of bubbling with para-hydrogen at 320 K and applying the ESOTHERIC sequence. The inset shows the spectrum of a 9mM standard (^{13}C-alpha-toluene) with ^{1}H decoupling (ns=100, d1=120 s, 90 degree pulse).

Cleavage procedure

At 320 K, the hydrogenation reaction was performed by bubbling para-hydrogen through a 0.4 ml sample. ESOTHERIC was executed to transfer the ^{1}H-polarization to the ^{13}C nuclei with a 90° pulse at the end to convert the coherence into z-magnetization. Subsequently, ~50 ul of 1 M NaOD in D_2O was added and N_2 was bubbled for 2 s through the solutions to ensure mixing. 20 s after creation of the ^{13}C polarization and subsequent cleavage, a spectrum was acquired utilizing a 90° degree pulse (see Fig. S8). The experiment was repeated three times. The tuning and matching was adjusted for the solution without NaOD and was not changed after its additions. This results in a smaller ^{13}C reference signal. The shims were adjusted to the mixture with NaOD. Variation of the line width and chemical shift is attributed to fluctuations of the amount of added NaOD and temperature that strongly influences the shims (Fig. S9).

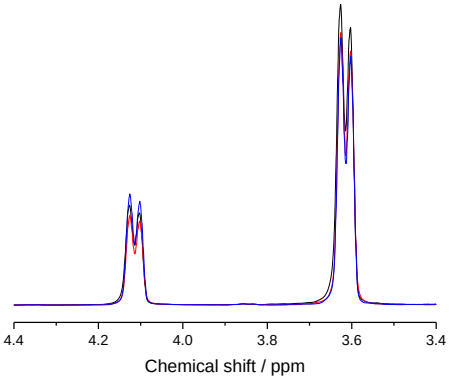

Figure S8.: ^{1}H spectra of the 0.45 ml solution 20 s after addition of NaOD. Chemical shifts are calibrated to MeOD (3.34 ppm). Non-cleaved ethyl acetate appears at ~4.1 ppm and the cleaved ethanol appears at ~3.6 ppm.

Number of cleavage experiment	^{1}H integral (sum of ethyl acetate and acetate)	^{1}H polarization
1	12061000	29.2%
2	10440000	25.2%
3	10576000	25.6%

Calculation of the ^{1}H polarization was done in reference to the product signal after reaction.

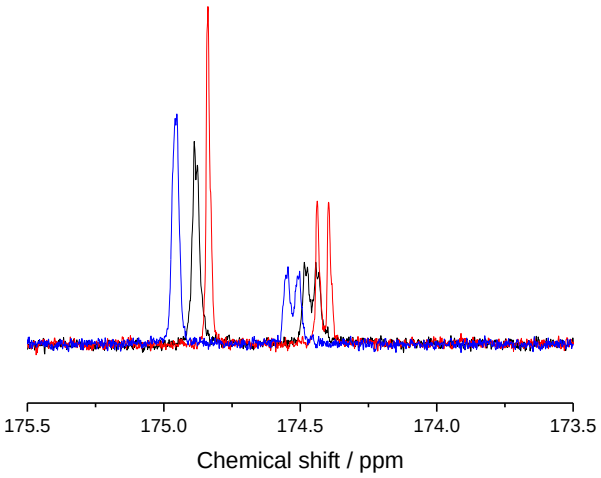

Figure S9.: ^{13}C spectra with ^{2}H decoupling of the 0.45 ml solution 20 s after addition of NaOD. The line broadening is 0.1 Hz. Chemical shifts are calibrated to MeOD (49.86 ppm). Non-cleaved ethyl acetate appears as a doublet at ~174.5 ppm and the cleaved acetate appears as a singlet at ~175 ppm.

Table 2:

Number of cleavage experiment	^{13}C integral (sum of ethyl acetate and acetate)	^{13}C polarization
1	533300	19.1%
2	542000	19.4%
3	542000	19.4%

Calculation of the polarization of ^{13}C was done in reference to the internal standard (^{13}C-α-Toluene ~9 mM). After the cleavage, a ^{13}C spectra of the solution was measured and an integral of the standard signal was obtained that is 33% smaller than the signal in a solution without NaOD. This number also accounts for dilution of the solution. Polarization losses during the cleavage can be explained as follows: Firstly, the longitudinal relaxation time of the carbons of interest is T_1 = 41 s. During the 20 s cleavage procedure a factor of 0.6 (0.74 for protons) in polarization is lost, amounting to about 36% ^{13}C and 41 % ^{1}H polarization. Additionally, only half of the sample is excited and detected during the polarization transfer and mixing (the excited volume is about 0.2 ml and the total sample volume is 0.45). Mixing of the sample with nitrogen to ensure the cleavage with NaOD will thus result in an averaged mixture of hyperpolarized and thermally polarized sample.

Relaxation studies

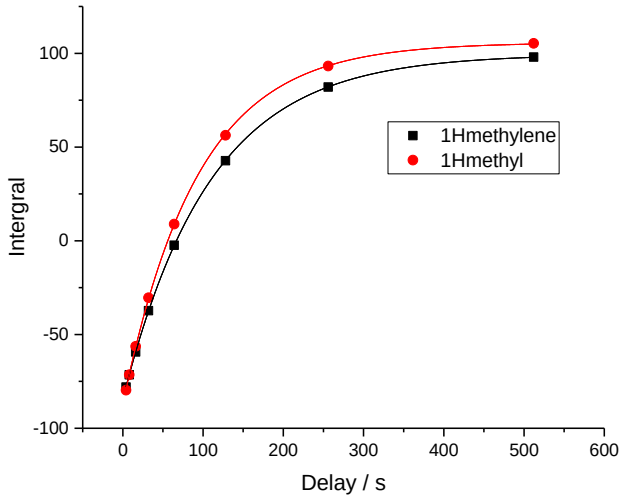

Fig. S10.: ^{1}H inversion recovery experiment of the concentrated hydrogenation product ethyl acetate-d_6 with catalyst at 7.05 T and 320 K. T_1 of the methylene proton is 108.6±0.3 s. T_1 of the methyl proton is 93.1±0.8 s in methanol-d_4

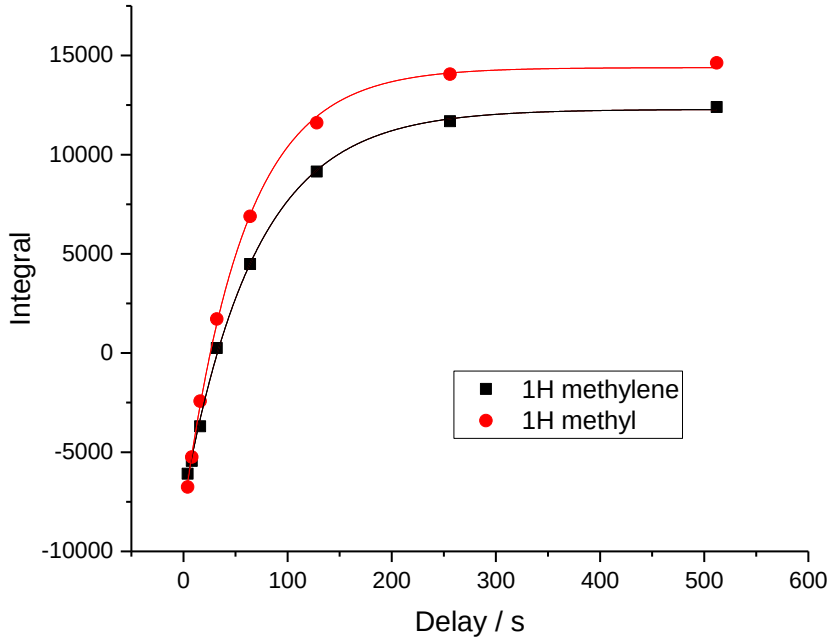

Fig. S11.: ^{1}H inversion recovery experiment of the concentrated cleaved ethanol-d_4 with catalyst at 7 T and 320 K. T_1 of the methylene proton is 68.8±2.7 s. T_1 of the methyl proton is 57.9±1.9 s in methanol-d_4 and 50 µL 1 M NaOD in D_2O.

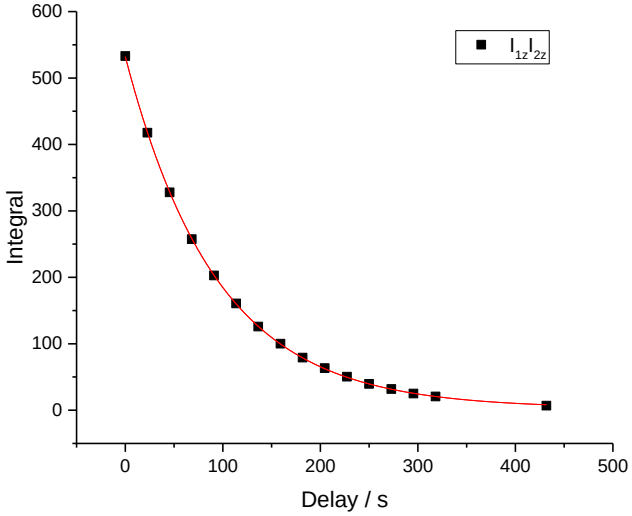

Figure S12.: The relaxation of the $I_{1z}I_{2z}$ magnetization of the ethyl acetate-d_6 protons was measured at 7 T and 320 K after the reaction with para-hydrogenation by using small flip angle pulses (4.7°). T_1= 93.6±0.3 s in methanol-d_4.

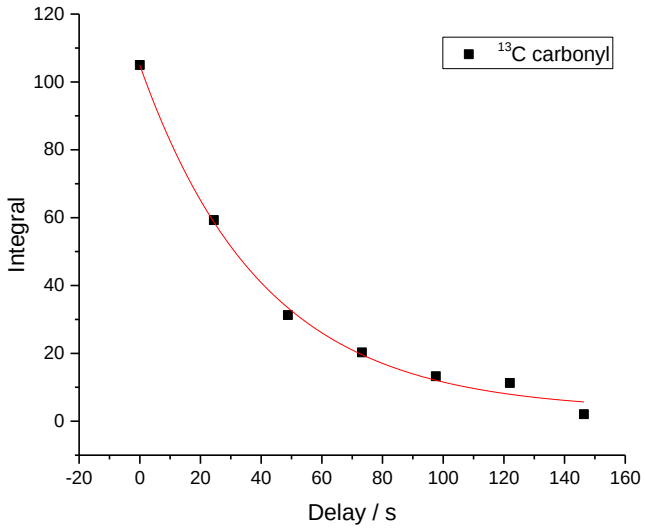

Figure S13.: The relaxation of the ^{13}C carbonyl magnetization of ethyl acetate-d_6 was measured at 7 T and 320 K after the para-hydrogen reaction by using small flip angle pulses (5.6°). T_1= 40±4s in methanol-d_4.

3 References

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

[2] J. S. Coursey, D. J., Schwab, J. J. Tsai, R. A. Dragoset, (2015), Atomic Weights and Isotopic Compositions (version 4.1). [Online] Available: http://physics.nist.gov/Comp [2018, *April, 26*]. National Institute of Standards and Technology, Gaithersburg, MD.