Supplementary Information: Nuclear hyperpolarization of (1-^{13}C)-pyruvate in aqueous solution by proton-relayed side-arm hydrogenation

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ABBREVIATIONS

DCC	1,3-Dicyclohexylcarbodiimide
DMAP	4-Dimethylaminopyridine
EtOAc	Ethyl acetate
ESI-MS	Electrospray ionization mass spectrometry
Pet. Ether	Petroleum ether
THF	Tetrahydrofuran
TLC	Thin layer chromatography

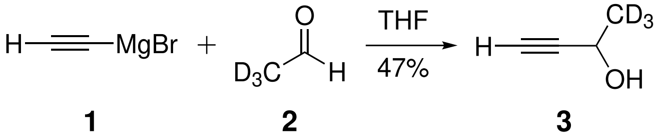
S1.SYNTHETIC PROCEDURES

General materials and methods.

Chemicals were purchased from commercial suppliers and were used without further purification. Thin layer chromatography (TLC) was performed on glass backed TLC plates and compounds were visualized by UV light. Silica gel (230-400 mesh, 60 Å) was purchased from Aldrich and used for flash chromatography. ^{1}H, ^{2}H and ^{13}C spectra were recorded on a Bruker Avance III HD spectrometer operating at 7 T. The signals of the deuterated solvents were used as internal chemical shift standards [D_2O (4.81 ppm), $CDCl_3$ (7.26 ppm)] for ^{1}H spectra. The residual ^{13}C signals from the deuterated solvent were used as references [$CDCl_3$ (77.0 ppm)] for ^{13}C spectra. All coupling constants were measured in Hertz. All moisture sensitive reactions were carried out in oven-dried glassware and under inert gas using nitrogen industrial grade cylinders unless stated otherwise. Molecular masses of the new organic compounds were determined by ESI-MS (Bruker).

Synthetic protocols.

3-Butyn-2-ol-d$_3$ (3).

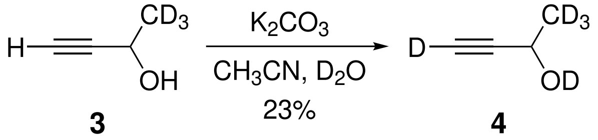


1 **2** **3**

To a solution of **2** (0.120 mL, 2.12 mmol) in THF (4 mL) was added drop-wise ethynylmagnesium bromide (10.15 mL, 0.5 M in THF, 5 mmol) at −78°C under argon atmosphere. The resulting reaction mixture was slowly warmed to

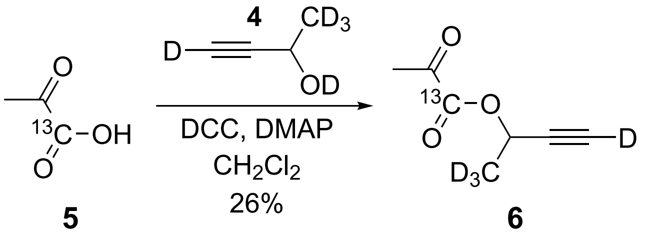
27°C and stirred at the same temperature for 2 h. The reaction was quenched with saturated NH_4Cl solution, extracted with CH_2Cl_2, dried over $MgSO_4$, filtered, and concentrated in vacuo to get **3** (0.174 g, 47%) as pale-yellow oily compound which was used as it is for the next step without the need of the purification. TLC (Silica gel, 20% EtOAc in pet. ether), $R_f(3) = 0.2$, I_2 active; ^{1}H NMR ($CDCl_3$, 298 K, 300 MHz) $\delta = 5$ (s, 1H), 4.48 (broad 1H), 2.42 (d, 1H) ppm ^{2}H NMR ($CDCl_3$, 298 K, 46.1 MHz) $\delta = 1.48$ (s, 2H) ppm.

3-Butyn-2-ol-d_5 (4).



To a solution of **3** (0.49 g, 6.69 mmol) in CH_3CN (8 mL) was added K_2CO_3 (1.38 g, 10 mmol) followed by the addition of D_2O (4 mL). The resulting suspension was stirred for 2 h at 27°C. The solvent was removed in vacuo cautiously which was then extracted with diethyl ether (3 x 10 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo to give **4** (0.114 g, 23% yield) as a yellowish liquid. TLC (Silica gel, 20% EtOAc in pet. ether), R_f (**4**) = 0.2, PMA and I_2 active. ^{1}H NMR ($CDCl_3$, 298 K, 300 MHz) $\delta = 4.48$ (s, 1H) ppm ^{2}H NMR ($CDCl_3$, 298 K, 46.1 MHz) $\delta = 2.43$ (s, ^{2}H), 1.43 (s, 2H) ppm

But-3-yn-2-yl-1,1,1,4-d_4 2-oxopropanoate-1-^{13}C (6)



To a cold solution of **5** (0.135 g, 1.51 mmol), **4** (0.114 g, 1.51 mmol) and DMAP (0.027 g, 0.23 mmol) in CH_2Cl_2 (7 mL) a solution of DCC (0.375 g, 1.82 mmol) in CH_2Cl_2 (8 mL) was added drop-wise over a period of 15 min. The resulting suspension was stirred for 1 h at 27°C. The reaction mixture was quenched by the addition of 10% $KHSO_4$ solution (15 mL) and extracted with diethyl ether (3 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo to get the crude material which was purified by flash column chromatography (silica) using a gradient elution (EtOAc:pet ether; 0:100 to 7:93) to give **6** (0.057 g, 26% yield) as a yellowish liquid. TLC (Silica gel, 20% EtOAc in pet. ether), $R_f(\mathbf{6}) = 0.5$, UV and I_2 active. ^{1}H NMR ($CDCl_3$, 298 K, 300 MHz) $\delta = 5.45$ (d, 1x 1H, $^2J_{^1H-^{13}C}$: 2.9 Hz), 2.45 (d, 3 x 1H, $^2J_{^1H-^{13}C}$: 1.5 Hz) ppm ^{2}H NMR ($CDCl_3$, 298 K, 46.1 MHz) $\delta = 2.46$ (s, 1 x 2H), 1.52 (s, 3 x 2H) ppm ^{13}C NMR ($CDCl_2$, 298 K, 75.5 MHz) $\delta = 190.94$ (d, 1 x 13C, $^1J_{^{13}C-^{13}C}$: 68 Hz), 159.68 (s, 1 x 13C), 80.24 (t, 1 x 13C, $^2J_{^{13}C-^2H}$: 7 Hz), 73.94 (t, 1 x 13C, $^1J_{^{13}C-^2H}$: 39 Hz), 62.25 (s, 1 x 13C), 26.45 (d, 1 x 13C,$^2J_{^{13}C-^{13}C}$: 17 Hz), 20.87 (m, 1 x 13C) ppm. ESI-MS: calculated for $C_7H_4D_4O_3$: 145.072, found 146.05 (M + H).

S2.CLEAVAGE OPTIMIZATION

In order to determine the optimal solution for the hydrolysis of the pyruvate precursor, various base concentrations in deuterated water were tested. The best reliability was observed using a solution of 150 mM NaOH. Lower concentrated solutions did not manage to fully cleave-off the pyruvate as seen in Figure S1.

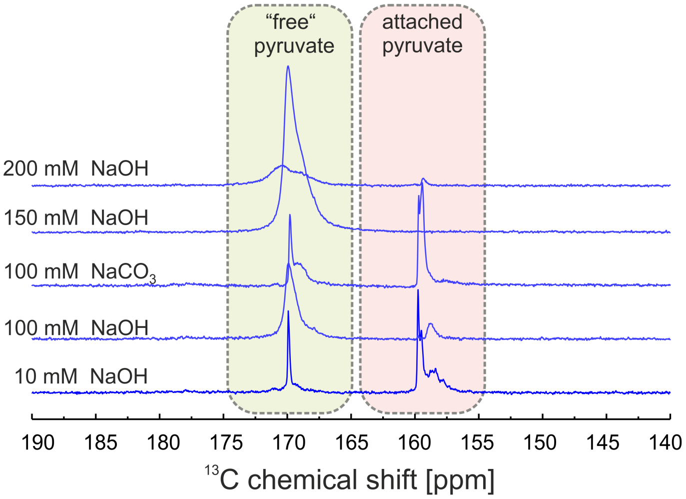


Figure S1. Hyperpolarized ^{13}C spectra of pyruvate in aqueous solution after hydrolysis using different cleaving solutions. Two moieties are observed as indicated with cleaved pyruvate and the pyruvate attached to the side-arm.

S3.DECAY OF ^{13}C POLARIZATION

The longitudinal relaxation of carbon achieved by means of the side-arm hydrogenation method assessed by a pseudo-2D experiment consisting of a small flip-angle pulse repeated every 1.5 seconds. The life-time of ^{13}C was observed to be much faster for a standard sample consisting of precursor compound and catalyst dissolved in chloroform-D without any additives. However, once 10% of solvent was replaced by deuterated ethanol, the T_1 increased from 12.2 ± 0.4 s to 62.4 ± 2.4 s. A comparable life-time was also observed in aqueous solution after the hydrolysis and extraction of the highly polarized pyruvate. The carbon polarization life-time in the final cleaved solution was measured to be 68.6 ± 1.6 s.

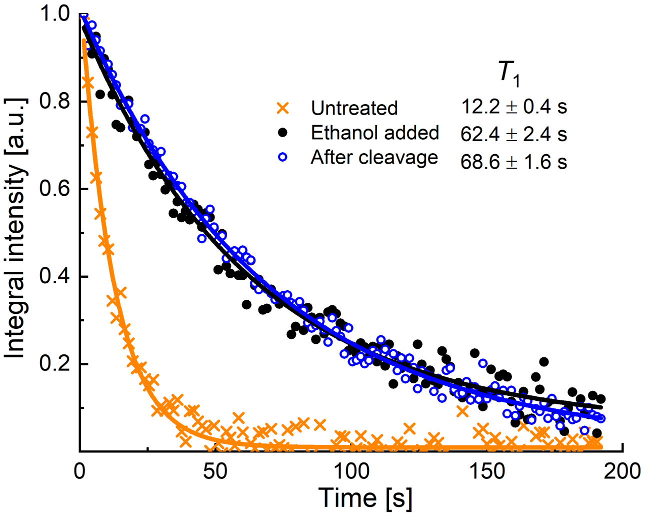


Figure S2. The decay ^{13}C hyperpolarization at different conditions. Orange crosses mark the standard solution of reacted material in chloroform-d. Black dots were collected with using a solution where 10% of the solvent was replaced with ethanol-d$_1$. Blue circles depict the polarization decay after cleaving reagent was injected into the latter solution containing ethanol-d$_1$. Time constants are indicated in the figure legend.

S4.LONG-RANGE INEPT SIMULATION

The pulse sequence depicted in the manuscript involves several INEPT blocks that address multiple J-couplings in the spin system. Refer to the Fig. 3, where delay τ_4 is normally set to $1/(4J_{23})$ if the remote scalar coupling J_{13} is negligible. This coupling of around 1 Hz in molecule **II** is comparable to the ${}^3J_{{}^{13}C-{}^1H}$ constant (3.2 Hz) which is enough to cause unwanted mixing of the spin states and diminish the polarization yield. To avoid this, the delay τ_4 needs to be adjusted such that any evolution under J_{13} makes a complete cycle. This was numerically simulated in Fig. S3 using SpinDynamica package to set the NMR pulse sequence for maximum efficiency. Several possible values of τ_4 are available for reasonable results. However, the pulse sequence efficiency slightly drops increasing all delays hence the best estimated value is 341 ms.

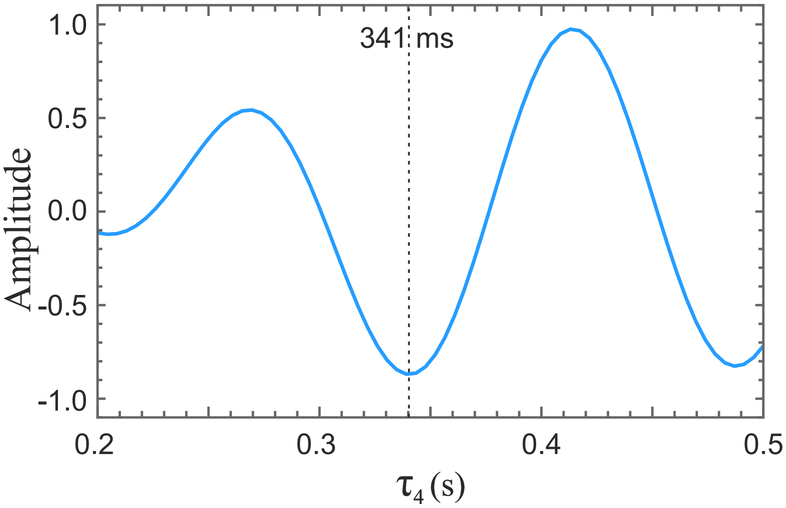


Figure S3. Numerical simulation of the NMR pulse sequence depicted in Fig. 3 efficiency as a function of delay τ_4. The initial spin density operator was set to $2I_{1z}I_{2z}$ whereas observable is the operator S_y. For spin system parameters refer to the Fig. 2 in the manuscript.