Supporting information

Efficient Hyperpolarization of U-^{13}C-Glucose using Narrow-line UV-generated Labile Free Radicals

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d_9-TriPA synthesis

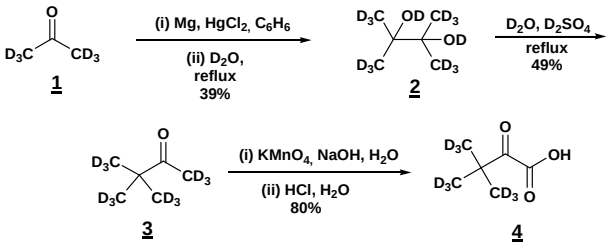

Figure S1. Synthetic pathway for d_9-TriPA preparation.

Synthesis of Pinacol-d_{14} (2):[1]
In a two necked, flame dried round bottom flask, 20 mL of dry benzene (20 mL) was taken and magnesium (1.63 g, 68.0 mmol) was added. The suspension was stirred for 10 minutes at room temperature under argon atmosphere. The solution of mercury (II) chloride (1.85 g, 6.8 mmol) in acetone-d_6 (68 mmol, 5 ml, 99.9% in D) was added dropwise to the stirring suspension and the mixture was heated to 50 °C and stirred until the vigorous reaction was complete. Then 5 mL of acetone-d_6 and benzene (1:1) was added to the mixture and reaction flask was heated at reflux for 2 h. Deuterium oxide (D_2O, 4 mL) was added to the reaction mixture and the mixture was stirred for additional 1 hour at reflux. The mixture was cooled to ambient temperature and filtered. H_2O (10 mL) was added to the filtrate and the resulting precipitate was collected by filtration and dried under high vacuum to give the desired product **2** (3.5 g; yield = 39%). ^{13}C NMR (75 MHz, $CDCl_3$) δ 74.6, 23.8 (*sept, J* = 19 Hz); ESI-MS analysis performed in ethanol and due to hydrogen-deuterium exchange we observe the mass of $C_6H_2D_{12}O_2$ instead of $C_6D_{14}O_2$. ESI-MS: *m/z* = calcd for $[M+Li]^+$ 137.2, found 137.1; calcd for $[M+Na]^+$ 153.2, found 153.1; calcd for $[2M+Li]^+$ 267.3, found 267.4.

3,3-dimethyl-2-butanone-d_{12} (3):[2]
To a 50 mL round bottom flask, pinacol-d_{14} (3.0 g, 22.7 mmol) was taken and D_2O (4.5 mL) was added to the flask. Then sulfuric acid-d_2 (1.6 mL, 22.7 mmol) was added to the solution and reaction flask was equipped with distillation assembly. The flask was heated to 120 °C and the upper layer of the collected liquid was separated and dried over Na_2SO_4 to give 3,3-dimethyl-2-butanone-d_{12} (**3**) as a liquid (1.5 g; yield = 59 %). ^{13}C NMR: (75 MHz, $CDCl_3$) δ 214.3, 43.6, 25.2 (*sept J* =20.3 Hz), 23.8 (*sept, J* = 19.2 Hz). ESI-MS *m/z* ($C_6D_{12}O$) = calcd for $[M+Li]^+$ 119.2, found 119.2.

d_9-3, 3, 3- Trimethyl pyruvic acid (4):[3]
To a 100 mL round bottom flask, compound **3** (1.4 g, 12.5 mmol) was taken in 10 mL of water and NaOH (1.0 g, 25 mmol) was added to it. The mixture was stirred for 5 minutes and then cooled down to 0 °C. To the reaction mixture, $KMnO_4$ (3.9 g, 25 mmol) was added in portions over the period of 1.5 hour. After the complete addition of $KMnO_4$, the reaction was stirred at 0 °C for 3 hours and additional 12 hours at room temperature. The resulting suspension was filtered and the solid was washed with H_2O (3 x 10 mL). The resulting filtrate was cooled down in ice bath, acidified up to pH ~1 using concentrated. HCl solution and extracted with diethyl ether (3 X 100 mL). The combined organic layers were washed with saturated NaCl solution and dried over Na_2SO_4. The solvent was removed under reduced pressure to give a pale yellow liquid. The obtained liquid was purified by distillation (27 mbar; 90 °C) to give compound **4** (1.5 g, 86%) as a colorless oil. ^{13}C NMR: (75 MHz, $CDCl_3$) δ 201.3, 163.9, 41.9, 24.6 (*sept*, *J* = 19.8 Hz). HRMS: (ESI) *m/z* = calcd for $C_6HD_9O_3$ $[M-H]^+$ 138.1107, found 138.1122.

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Fitting of ESR spectra

ESR spectra were measured at two different experimental conditions: X-band/77 K and 6.7 T/1.1 K. All fits were obtained using the MATLAB-based software EASYSPIN.[4] Because of the random distributions of the radicals inside the glassing matrix the PEPPER routine for powder spectra was employed. A spin system S = ½ was assumed in all cases. It was previously demonstrated that the ESR-line dipolar broadening is radical-concentration independent, but mainly due to dipolar coupling to the protons in the sample matrix.[5] Being the solvent composition and volume ratio to the precursor the same for all samples, a phenomenological Gaussian dipolar broadening of 1 mT was used in all cases. In order to get the best estimation of the A-tensor and g-tensor we proceeded as follows.

The A-tensor was calculated from X-band spectra assuming an isotropic g-tensor of 2.0037 according to the Landolt–Börnstein reference.[6]

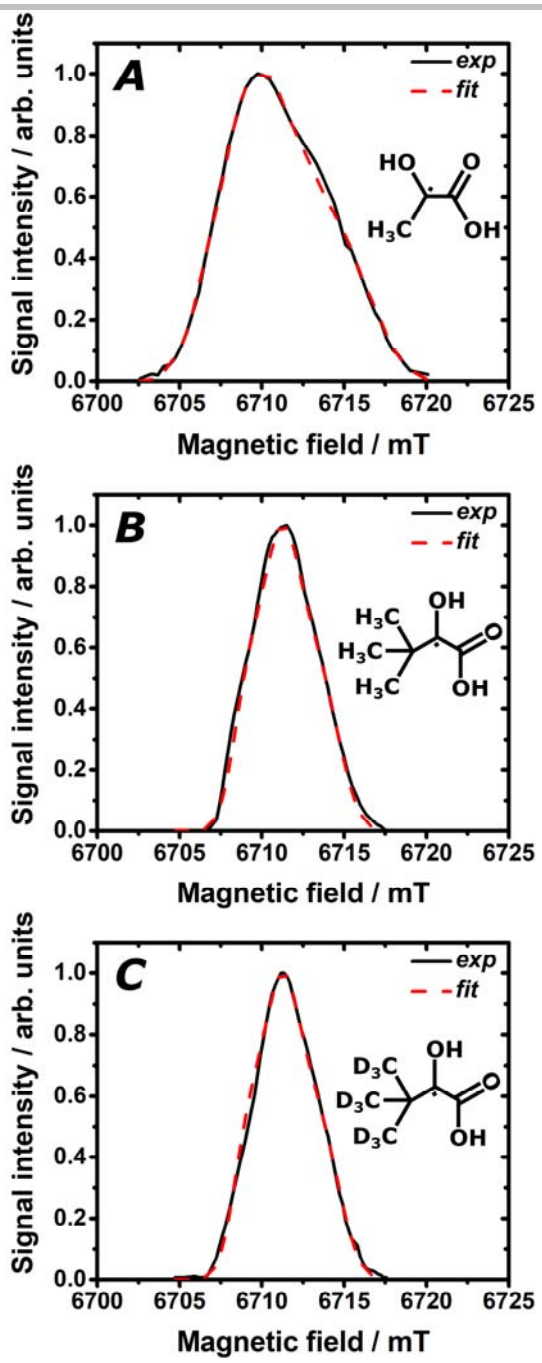

Figure S2. 6.7 T/1.1 K ESR measurement (black line) and ESR spectrum fit (dashed red line) after 5 min UV-light irradiation at 77 K of a hundred 4.0±0.2 µL beads of a frozen solution containing PA:GW55 1:9 (v/v) (**A**); TriPA:GW55 1:9 (v/v) (**B**); TriPA:GW55 1:9 (v/v) (**C**). In each inset the radical molecular structure is showed.

The obtained A-tensor was then used to fit data at 6.7 T/ 1.1 K (see Fig S2), keeping the three principal values of the g-tensor as free parameters. The calculated g-tensor was then employed at X-band to improve the fit quality (see Fig 1 in the main text) and verify the robustness of the method. UV-[1-^{13}C]PA and UV-[2-^{13}C]PA were not measured at 6.7 T. For them the g-tensor of UV-PA was used. Results are reported below.

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UV-PA:
g-tensor = [2.0036 2.0027 2.0007]; A-tensor = [1.67] mT.

UV-[1-^{13}C]PA:
g-tensor = [2.0036 2.0027 2.0007]; A-tensor(^{1}H) = [1.67] mT;]; A-tensor(^{13}C) = [1.07] mT.

UV-[2-^{13}C]PA:
g-tensor = [2.0036 2.0027 2.0007]; A-tensor(^{1}H) = [1.07 1.07 1.67] mT;]; A-tensor(^{13}C) = [1.43 1.43 5.60] mT.

UV-TriPA:
g-tensor = [2.0031 2.0022 2.0012]; A-tensor(^{1}H) = [0.14 0.14 0.71] mT;

UV-d_9-TriPA:
g-tensor = [2.0031 2.0022 2.0012]; A-tensor(^{2}H) = [0.02 0.02 0.11] mT;

It is worth noticing that the different molecular structure between PA and TriPA affected not only the hyperfine interaction, but also the Zeeman one: the same g-tensor could be used to fit all spectra of the UV-PA series and a different one to fit the UV-TriPA and UV-d_9-TriPA.
While UV-PA and UV-[1-^{13}C]PA showed an isotropic hyperfine interaction, for UV-[2-^{13}C]PA it was not possible to get good fitting results unless an axial tensor was used. Currently, we do not have an explanation for this phenomenon.
An axial A-tensor characterized also TriPA and d_9-TriPA. Moreover the tensor elements scaled according to the ration $\gamma(^{1}H)/\gamma(^{2}H)$. In the main text, for the sake of simplifying the discussion, only the isotropic component of the hyperfine interaction was reported for [2-^{13}C]PA, TriPA and d_9-TriPA. This information was sufficient to prove that the photo-generated radical was localized on the C2 and, in the case of TriPA and d_9-TriPA, that the hyperfine interaction was reduced.

Free radical *vs* precursor concentration and spatial distribution

During the process of optimization of the UV-irradiated samples, the amount of radical precursor was titrated. In Figure S3A the radical concentration as a function of TriPA concentration (red circles) and d_9-TriPA concentration (black circles) is reported. For each series of data, the experimental points correspond to a precursor amount of 5%, 10% and 20% of the sample volume. In all cases the precursor was mixed with a solution of 2M glucose in GW55. All samples were UV-irradiate until the radical concentration plateau was achieved (n = 3).
We verified the distribution homogeneity of the UV-induced radicals for *TriPA_DNP-sample* of the main text (0.7 M TriPA with 2M glucose dissolved in GW55). Results are reported in Figure S3B. As previously described,[7] we measured the ESR signal, after UV-irradiation, of frozen beads with different volumes (n = 3). The ESR signal increased linearly ($\propto r^3$) proving a homogenous distribution of the paramagnetic centres.

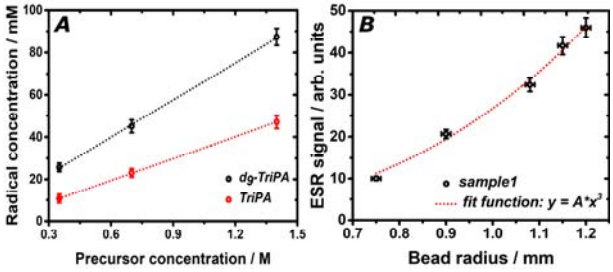

Figure S3. UV-radical precursor titration (**A**) and paramagnetic centres distribution homogeneity inside the sample volume (**B**).

Electron spin-lattice relaxation time (T_{1e})

We measured the electron spin-lattice relaxation time (T_{1e}) for *TriPA_DNP-sample* and *d_9-TriPA_DNP-sample* at DNP conditions (6.7 T and 1.1 K) by means of the LOD-ESR probe developed in house.[7] Results are reported in Figure S4.

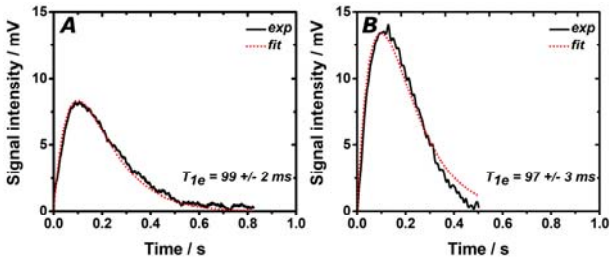

Figure S4. T_{1e} measurements for TriPA_DNP-sample (**A**) and d_9-TriPA_DNP-sample (**B**).

^{13}C NMR hydrate amount estimation

The amount of precursor in its hydrate form was measured via ^{13}C NMR for mixtures of 0.7 M TriPA and d_9-TriPA with 2 M glucose dissolved in GW55, respectively *TriPA_DNP-sample* and *d_9-TriPA_DNP-sample* of the main text before UV-irradiation. Within experimental errors, the hydrate-to-keto form ratio was the same for both samples (20/80). Results are reported in Figure S5.

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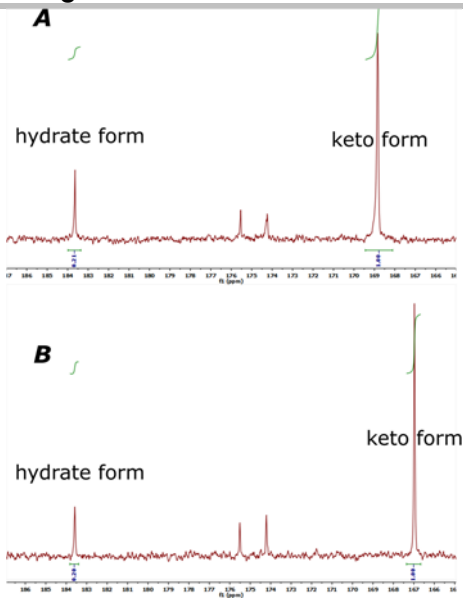

Figure S5. Liquid state ^{13}C NMR spectra of *TriPA_DNP-sample* (**A**) and *d_9-TriPA_DNP-sample* (**B**) before freezing and UV-irradiation. It is shown the spectral region where the C1 carbon peaks appear at different position depending of the hydrate or keto form.

Trityl ESR spectrum and microwave frequency sweep

The microwave frequency corresponding to the best DNP enhancement for *Trityl_DNP-sample* (30 mM trityl and 2 M glucose dissolved in GW55) was 187.94 GHz (see Figure S5, red curve). For the same sample we measured the ESR spectrum at DNP conditions (see Figure S5, grey curve).

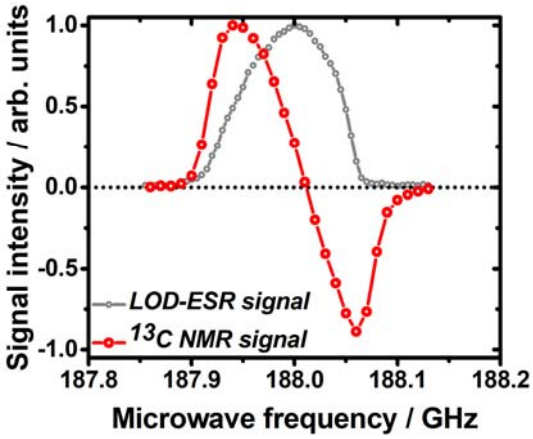

Figure S5. The ESR spectrum and corresponding ^{13}C microwave frequency sweep were measured for *Trityl_DNP-sample* at 6.7 T and 1.1 K.

HP001 polarization and dissolution

The experiment to test the improved DNP performance of the new UV-radicals on 1,1-bis(hydroxymethyl)cyclopropane-1-$^{13}C,d_8$ (HP001) is reported in Figure S6. 2 M HP001 replaced the labelled glucose in the preparation of *d9-TriPA_DNP-sample*. DNP was performed at identical conditions.

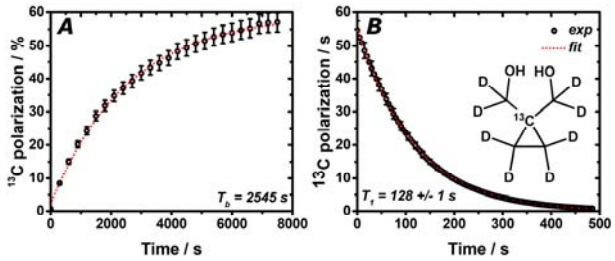

Figure S6. Polarization build up (**A**) and liquid-state relaxation (**B**) of a sample containing 0.7 M d_9-TriPA with 2M HP001 dissolved in GW55. Solid-state measurements were performed at 6.7 T and 1.1 K by mw irradiation at 188.19 GHz (20 MHz amplitude modulation and 1 kHz frequency modulation). Liquid-state measurements were acquired on 9.4 T high resolution magnet equipped with a 5 mm NMR probe. In the inset the molecular structure of HP001 is reported.

Glycolysis probing with TriPA hyperpolarised [$^{13}C_6$-d_7]glucose in human prostate adenocarcinoma cells

Human prostate adenocarcinoma cells (PC3) were grown to confluence. Cells were grown in flasks (75 cm^2) in an environment with 5% CO_2, at 37 ° C in RPMI-1640 medium with FBS and antibiotics. Cells were harvested by trypsination, washed, and resuspended in 40mM phosphate buffer (pH 7.3) to a concentration of 10 million cells/100 μl. 100 μl of suspension was transferred to a shigemi NMR tube and equipped with a connecting inlet tubing (dead volume 0.9 ml).

Twelve 4 μl drops of a solution containing 0.7 M TriPA with 2 M [$^{13}C_6$-d_7]glucose in GW55 (TriPA_DNP-sample) were poured in liquid nitrogen and UV-irradiated for 300 s as described in ref [5]. The sample, polarized at optimal conditions, was dissolved in 10 ml 40 mM phosphate buffer (pH 7.3). The dissolved hyperpolarized [$^{13}C_6$-d_7]glucose had a concentration of 20 mM of which 1.1 ml was injected through the injection line resulting in approx. 7 million cells in the active volume and a substrate concentration of approx. 13 mM. The NMR acquisition was performed sending 20° rf pulses every second.

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