Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.
This journal is © The Royal Society of Chemistry 2015

Electronic Supplementary Information

A Benzyl Alcohol Derivative of BDPA Radical for Fast Dissolution Dynamic Nuclear Polarization NMR Spectroscopy

J. L. Muñoz-Gómez,[a] E. Monteagudo,[b] V. Lloveras,[a] T. Parella,[b] J. Veciana,*[,a] and J. Vidal-Gancedo*[,a]

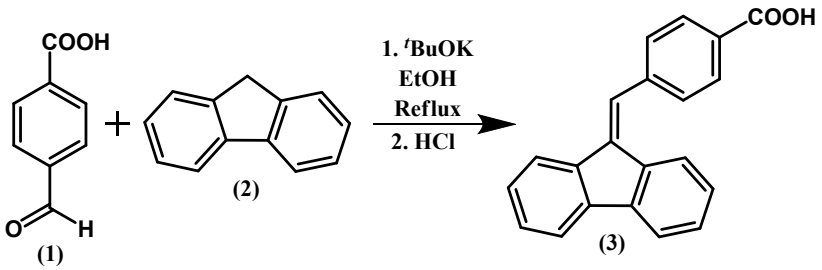
* [a]Institut de Ciència de Materials de Barcelona, (ICMAB-CSIC) Campus Universitari de Bellaterra, E-08193 Cerdanyola del Vallès, Spain and Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Barcelona, Spain.

[b]Servei de Ressonància Magnètica Nuclear, Facultat de Ciències i Biociències, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Barcelona (Catalonia), Spain

Table of contents

1. Synthesis and characterization of BA-BDPA free radical

1.1. Compound 3



Scheme S1. Synthesis of compound **3**

To an oven-dried two necked 250 ml round bottom flask containing a magnetic stir-bar 2.68 g (16.2 mmol, 1 equiv) of fluorene **2** and 6.0 g (53.3 mmol, 2.75 equiv) of ^{t}BuOK were added, followed by 150 ml of absolute ethanol. The flask was fitted with a water-cooler reflux condenser and a rubber septum. The mixture was heated to reflux with vigorous stirring and then 3.0 g (19.4 mmol, 1.2 equiv) of 4-formylbenzoic acid **1** was added. The flask was allowed to reflux overnight. After cooling to room temperature, the mixture was poured into a flask containing excess of cold 1 M HCl. This mixture was extracted with three portions of ethyl acetate. The organic phase was dried over anhydrous magnesium sulfate and the solvent was removed under vacuum. The product was purified by recristalization on toluene. The pure product **3** was isolated with further purification as a yellow powder (1.8 g, 91%).

Product Characterization:

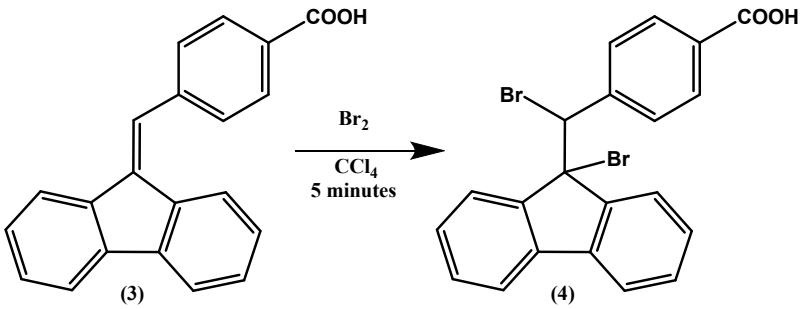
^{1}H NMR (600 MHz, d^{6}-acetone): δ 11, 28 (1H, br), 8.20 (2H, *d*, J= 6 Hz),7.96 (1H, *d*, J= 6 Hz), 7.91 (1H, *s*), 7.85 (2H, *d*, J= 6 Hz), 7.77 (2H, *d*, J= 6 Hz), 7.56 (1H, *d*, J= 6 Hz), 7.44 (1H, *t*, J= 6 Hz), 7.39 (2H, *t*, J= 6 Hz), 7.13 (1H, *t*, J= 6 Hz) ppm.

^{13}C NMR (125 MHz, d^{6}-dimethylsulfoxide):167.4, 140.9, 140.7, 138.8, 138.5, 136.5, 135.5, 130.9, 129.7, 129.3, 129.1, 128.7, 127.37, 127.35, 127.1, 123.9, 121.0, 120.3, 119.9.

MS (MALDI-TOF, positive mode): m/z 299 $[M+H]^{+}$ (calculated for $C_{21}H_{14}O_{2}$: 298.3).

ATR-IR(ν_{max} (cm^{-1}): 1679, 1602, 1445, 1422, 1290, 722.

1.2. Compound 4



Scheme S2. Synthesis of compound **4**

To an oven-dried 100 ml round-bottom flask equipped with a magnetic stir-bar 3 g of compound **3** (10.1 mmol, 1 equiv), and 60 ml of CCl_4 were added. The resulting suspension was introduced in an ultrasound bath for 5 minutes and then, bromine was added (0.62 ml, 1.93 g, 12.1 mmol, 1.2 equiv). The solution became a homogeneous red solution and was stirred for 10 minutes at room temperature. After this time, the mixture was poured into a flask containing excess of cold 1 M $NaHSO_3$ in order to eliminate the excess of bromine. The pH was adjusted to 4 with Na_2CO_3, and the solution was extracted with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate and the solvent was removed under vacuum. The pure product **4** was isolated as white powder (4.05 mg, 88%) and was used in the next step without further purification.

Product Characterization:

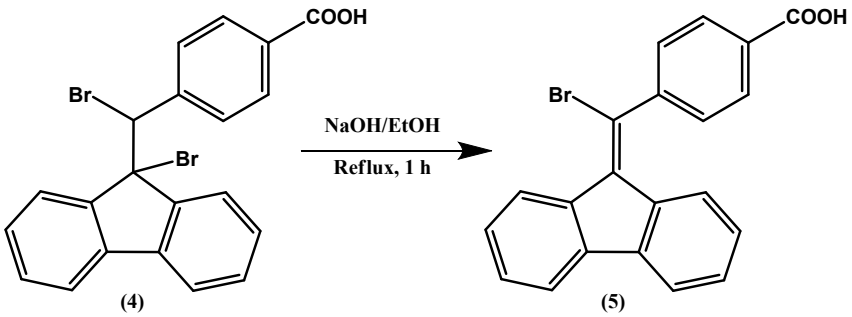
^{1}H NMR (400 MHz, d^6-acetone): δ 8.3 (1H, *d*, J= 7.2 Hz), 7.84 (1H, *d*, J= 7.2 Hz), 7.72 (1H, 1H, *d*, J= 7.2 Hz), 7.65 (2H, *d*, J= 7.2 Hz), 7.58 (1H, *d*, J= 7.2 Hz), 7.53 (2, *d*, J= 7.2 Hz), 7.38-7.31 (2H, *m*), 7.12 (2H, *d*, J= 7.2 Hz), 6.36 (1H, *s*) ppm.

^{13}C NMR (100 MHz, d^6-dimethylsulfoxide): δ 166.6, 144.7, 144.4, 141.2, 139.0, 138.1, 130.5, 130.2, 129.8, 129.1, 128.2, 128.09, 129.05, 126.4, 126.0, 120.5, 120.4, 66.2, 60.4 ppm.

MS (MALDI-TOF, positive mode): m/z 456/458 $[M+H]^+$ (calculated for $C_{21}H_{14}Br_2O_2$: 458).

ATR-IR (ν_{max} (cm^{-1}): 1688, 1610, 1426, 1288, 741, 728.

1.3. Compound 5



Scheme S3. Synthesis of compound **5**

To an oven-dried two necked 250 ml round-bottom flask equipped with a magnetic stir-bar 3.0 g of compound **4** (6.5 mmol, 1 equiv) and 60 ml of absolute ethanol were added. The flask was fitted with a water-cooler reflux condenser and with a rubber septum. In a 100 ml round-bottom flask equipped with a magnetic stir-bar 1.05 g of NaOH (26.2 mmol, 4 equiv) and 75 ml of absolute ethanol were added. After dissolution of NaOH in ethanol, it was added to the 250ml flask and refluxed for 1 hour. Then it was allowed to cool down to room temperature and the mixture was acidified with diluted hydrochloric acid. Afterwards, it was extracted with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate and the solvent was removed under vacuum. The pure product **5** was isolated without further purification as a yellow powder (2.42 g, 98%).

Product Characterization:

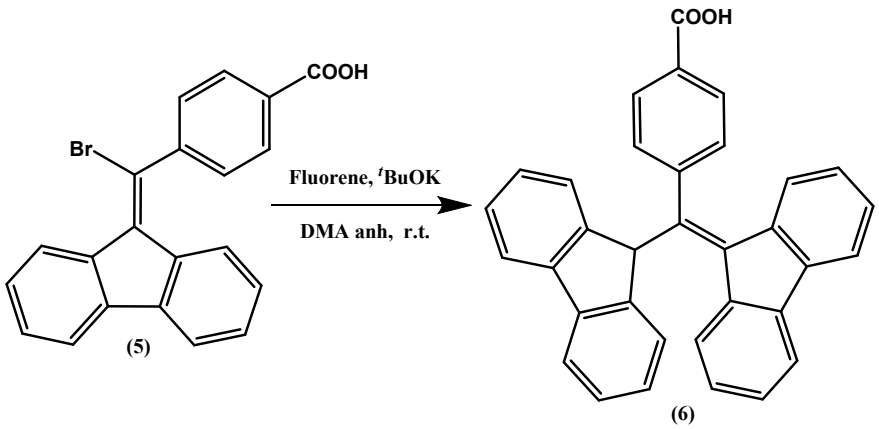
^{1}H NMR (400 MHz, d^{6}-acetone): δ 8.87 (1H, *d*, J= 8 Hz), 7.27 (2H, *d*, J= 8 Hz), 7.91 (1H, *d*, J= 8 Hz), 7.82 (1H, *d*, J= 8 Hz), 7.68 (2H, *d*, J= 8 Hz), 7.54 (1H, *t*, J= 8 Hz), 7.47 (1H, *t*, J= 8 Hz), 7.30 (1H, *t*, J= 8 Hz), 6.93 (1H, *t*, J= 8 Hz), 6.26 (1H, *d*, J= 8 Hz) ppm.

^{13}C NMR (100 MHz, d^{6}-dimethylsulfoxide): δ 166.7, 146.0, 140.7, 139.3, 137.0, 136.8, 135.5, 131.6, 130.5, 129.8, 128.8, 128.6, 127.3, 125.5, 124.0, 123.0, 120.3, 120.0 ppm.

MS (MALDI-TOF, positive mode): m/z 377/379 $[M+H]^{+}$ (calculated for $C_{21}H_{13}BrO_2$: 377).

ATR-IR(ν_{max} (cm^{-1}): 1673, 1418, 1268, 1177, 776, 765, 726.

1.4. Compound 6



Scheme S4. Synthesis of compound **6**

To an oven-dried 250 ml Schlenk flask equipped with a stir-bar and a rubber septum 0.5 g (1.33 mmol, 1 equiv) of compound **5** was added. The flask was evacuated under vacuum and refilled with argon for three times and then 18.5 mL of dry dimethylacetamide (DMA) were added. In a separate oven-dried 100 ml Schlenk flask equipped with a stir-bar and a rubber septum 330 mg (1.99 mmol, 1.5 equiv) of fluorene and 744 mg (6.63 mmol, 5 equiv) of ^{t}BuOK were added. The flask was also evacuated under vacuum and refilled with argon for three times and 68.5 mL of dry dimethylacetamide (DMA) were added. The solution appeared dark red. The dark red solution was slowly added to the compound **5** flask by cannulation. The reaction turned deep blue and then it was stirred for 1.5 hours at room temperature. After that, the flask was cooled down in an ice bath and 1 M HCl in ether was added until the blue color disappeared. The solution was diluted with water and extracted with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate, the solvent was removed under vacuum and then with high vacuum to eliminate the dimethylacetamide. The organic solid obtained was purified by column chromatography on silica gel eluting with dichloromethane. The pure product **6** was isolated as a yellow powder (330 mg, 54%).

Product Characterization:

^{1}H NMR (400 MHz, CD_2Cl_2): δ 8.46 (1H, *d*, J= 7.6 Hz), 7.90 (1H, *d*, J= 7.6 Hz), 7.76 (1H, *d*, J= 7.6 Hz), 7.769 (4H, *m*), 7.62 (2H, *d*, J= 7.6 Hz), 7.49 (1H, *t*, J= 7.6 Hz), 7.41-7.35 (3H, *m*), 7.29 (2H, *t*, J= 7.6 Hz), 7.23 (1H, *t*, J= 7.6 Hz), 6.82-6.78 (3H, *m*), 6.53 (1H, *s*), 5.89 (1H, *d*, J= 8 Hz) ppm.

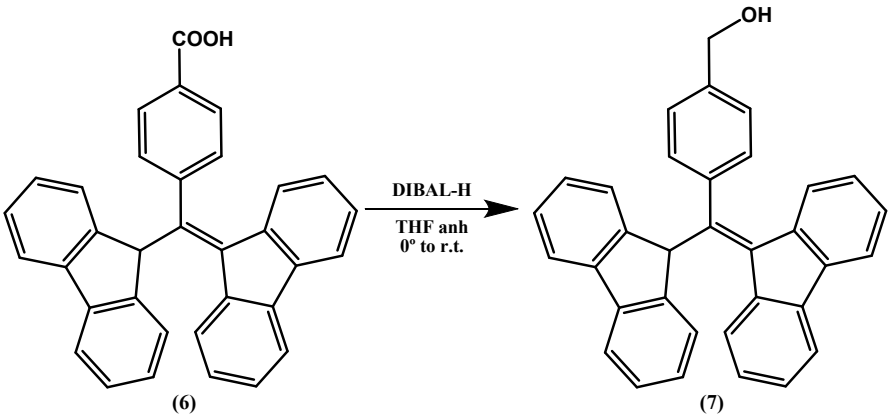
^{13}C NMR (125 MHz, $CDCl_3$): δ 169.9, 144.3, 143.7, 143.4, 141.9, 141.4, 139.9, 138.4, 138.4, 136.1, 129.6, 128.7, 128.5, 127.74, 127,69, 127.4, 127.1, 126.7, 125.9, 125.28, 125.26, 125.1, 120.1, 120.0, 119.2, 52.3 ppm.

MS (MALDI-TOF, positive mode): m/z 497.5 $[M-H]^-$ (calculated for $C_{33}H_{21}Br$: 496.1).

ATR-IR (ν_{max} (cm^{-1}): 1589, 1444, 1075, 1006 and 729.

UV-Vis: Anion: 610 nm (ε = 28600 M^{-1} cm^{-1}), radical: 494 nm (ε = 29200 M^{-1} cm^{-1}), 859 nm (ε = 1410 M^{-1} cm^{-1}).

1.5. Compound 7



Scheme S5. Synthesis of compound **7**

To an oven-dried 25 ml Schlenk flask equipped with a stir-bar and a rubber septum 50 mg (108 µmol, 1 equiv) of compound **6** was added. The flask was evacuated under vacuum and refilled with argon for three times and then 4 mL of dry tetrahydrofuran (THF) were added. The Schlenk was cooled down in an ice bath, after that 540 µl (540 µmol, 5 eq) of diisobutylaluminium hydride (DIBAL-H) were slowly added. The reaction was allowed to warm to room temperature and was stirred for 1.5 hours at this temperature. After that, the flask was cooled down in an ice bath and 1 M HCl in ether was added until pH of 4. The solution was diluted with water and extracted with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate, the solvent was removed under vacuum and the organic solid obtained was purified by column chromatography on silica gel eluting with dichloromethane. The pure product **7** was isolated as a off yellow powder (43 mg, 90 %).

Product Characterization:

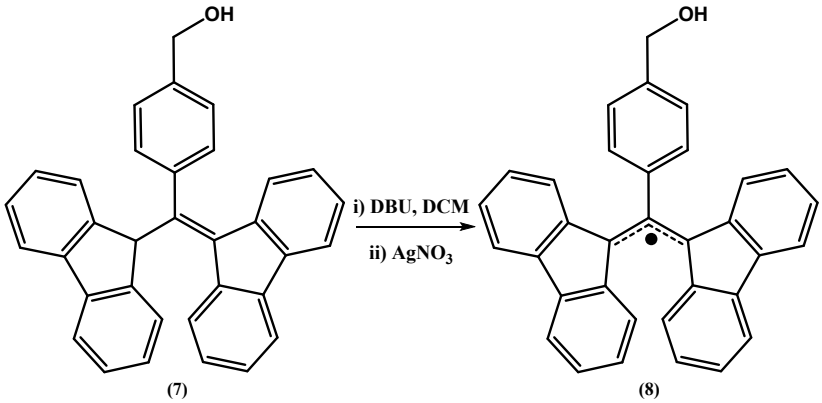
^{1}H NMR (500 MHz, d^{6}-acetone): δ 8.56 (1H, *d*, J= 8 Hz), 8.02 (1H, *d*, J= 8 Hz), 7.87 (1H, *d*, J= 8 Hz), 7.78 (2H, *d*, J= 8 Hz), 7.70 (2H, *d*, J= 8 Hz), 7.53 (1H, *t*, J= 8 Hz), 7.45 (1H, *t*, J= 8 Hz),7.39 (2H, *t*, J= 8 Hz), 7.34 (2H, *t*, J= 8 Hz), 7.25 (1H, *t*, J= 8 Hz), 7.25 (2H, *d*, J= 8 Hz), 7.05 (2H, *d*, J= 8 Hz),), 6.80 (1H, *t*, J= 8 Hz), 7.71 (2H, *d*, J= 8 Hz),7.55 (1H, *s*), 5.86 (1H, *d*, J= 8 Hz), 4.51 (2H, *d*, J= 8 Hz), 4.13 (1H, *t*, J= 6 Hz),ppm.

^{13}C NMR (125 MHz, d^{6}-acetone): 145.7, 144.7, 142.2, 142.1, 141.6, 140.2, 139.3, 138.9, 137.7, 136.5, 128.68, 128.67,127, 95, 127.93, 127.90, 127.4, 126.8, 126.4, 126.1, 125.8, 125.7, 120.4, 120.3, 119.5, 63.7, 53.1 ppm.

MS (MALDI-TOF, positive mode): m/z 447.2 $[M-H]^-$ (calculated for $C_{34}H_{24}O$: 448.6).

ATR-IR (ν_{max} (cm^{-1}): 2923, 1444, 1013, 1006, 782 and 727.

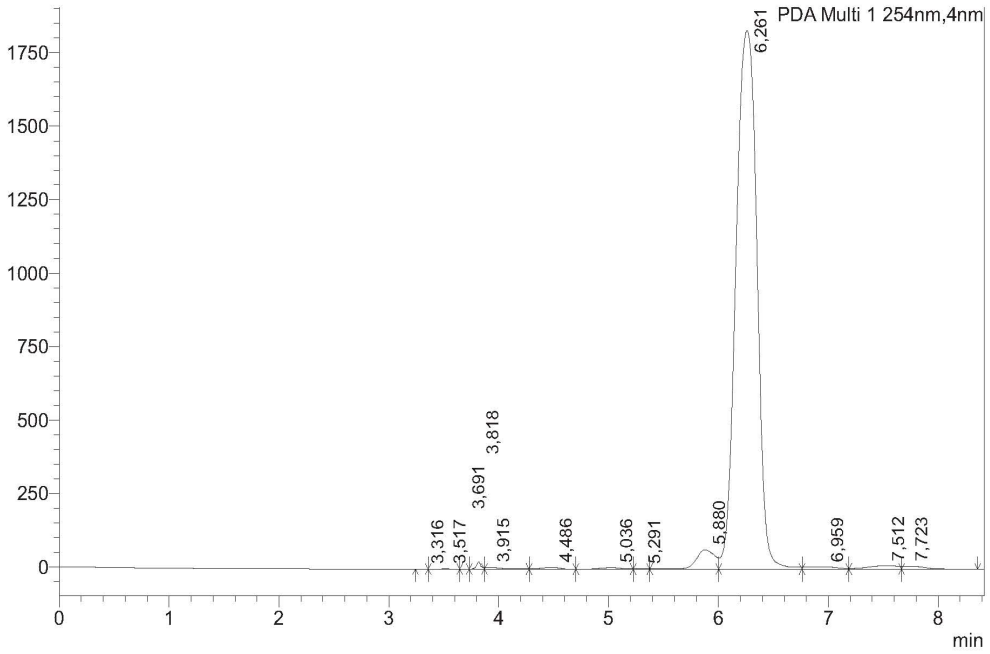
1.6. Benzyl alcohol-BDPA radical (BA-BDPA, 8)



Scheme S6. Synthesis of compound **8**

To an oven-dried 25 ml Schlenk flask equipped with a stir-bar and a rubber septum 100 mg (223 µmol, 1 equiv) of acid-compound **7** was added. The flask was evacuated under vacuum and refilled with argon for three times and then 5 ml of dry DCM were added. After that 70 µl (468 µmol, 2 eq.) of 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) were slowly added. The reaction turned into a deep blue colour. The blue solution was stirred at room temperature for five minutes, then a solution of $AgNO_3$ (42 mg, 247 µmol, 1.1 equiv) in ACN was added, and the oxidation was monitored by UV-Visible. The organic phase was removed under vacuum and the organic solid obtained was purified by column chromatography on silica gel eluting with dichloromethane to dichloromethane /methanol 98:2. The pure product **8** was isolated as an off red powder (98 mg, 98 %). The purity cheked by HPLC was of 97 %.

Product Characterization: EPR: g: 2.0024; a(4H): 1.988 G, a(4H): 1.827 G, a(4H):0.539 G, a(4H): 0.365 G, a(2H): 0.065 G and a(2H): 0.062 G; line width: 0.31. ATR-IR (ν_{max} (cm^{-1}): 2923, 1444, 1013, 1006, 782 and 727. UV-Vis: 491 nm, ε = 17600 M^{-1} cm^{-1}. HPLC: Column: TRACER EXCEL 120 ODSA 5µm. Solvent: CH_3CN. λ = 254 nm. Peak 1: 5.88 min. (area %: 3.1); Peak 2: 6.26 min. (area % : 96.9).



2. EPR spectra of BA-BDPA 8 free radical in dichloromethane.

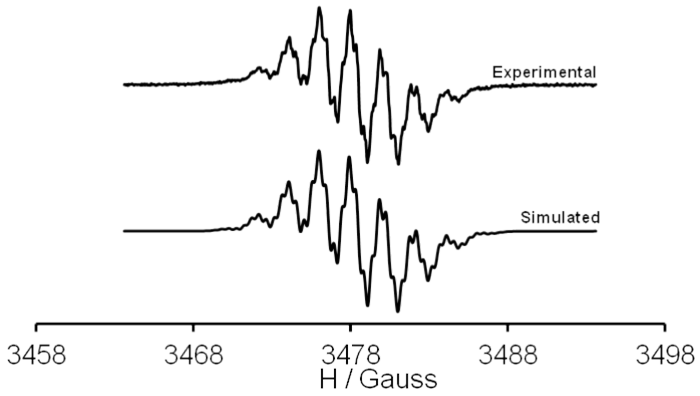


Fig. S1. EPR spectra of **8** in DCM. Experimental (up) and simulated (down).

The room temperature X-band EPR of BA-BDPA free radical **8** at g-value of 2.0024 (**Fig. S1**) shows the typical spectrum of BDPA compound due to the electronic density of the singly occupied orbital (SOMO) is mainly distributed over the fluorenyls rings.[1]

3. Polarization build-up curves for BA-BDPA 8 radical at different concentrations

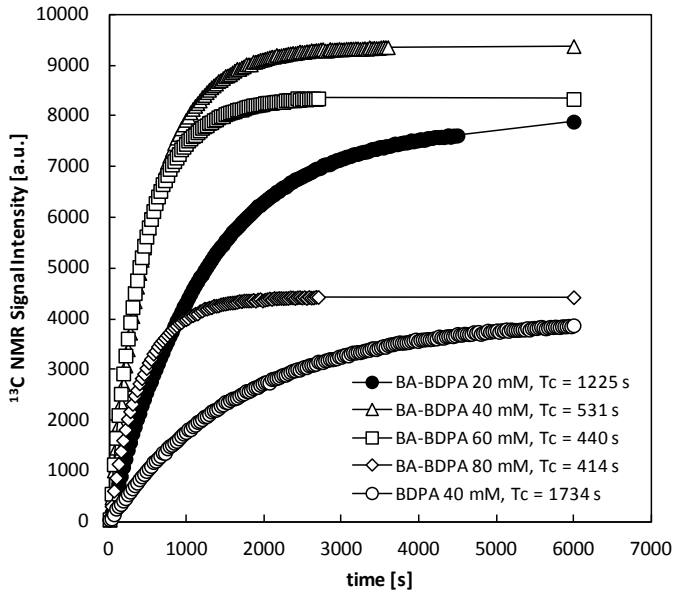


Fig. S2. ^{13}C polarization build-up curves for samples of 20 µl [1-^{13}C]pyruvic acid doped with BA-BDPA 20 mM (solid circles), 40 mM (open triangles), 60 mM (open squares) and 80 mM (open diamonds). The curve of 20 ml 1:1 (v/v) [1-^{13}C]pyruvic acid:sulfolane doped with BDPA 40 mM (open circles) is also shown for comparison.

The optimum concentration of BA-BDPA radical **8** was 40 mM. Additionally a change in polarization transfer mechanism could be observed from 20 mM to 40 mM as can be seen in **Fig. S3**.[2]

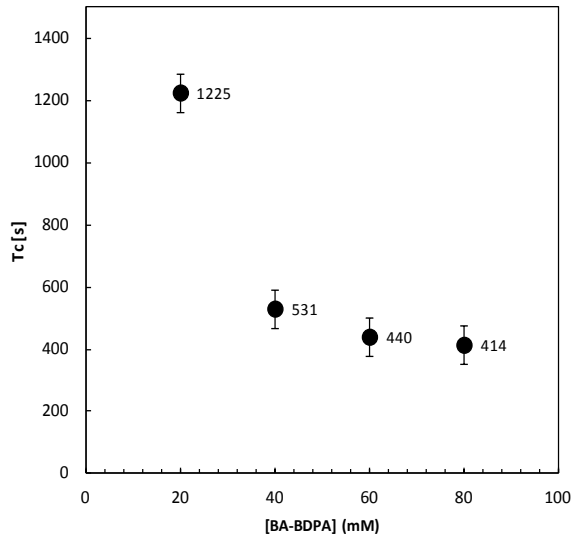


Fig. S3. Build-up time constant (Tc) versus concentration of **8** radical.

4. Removal of BA-BDPA 8 radical from the dissolution liquid.

For fast filtration, a home-built in line filter was used. The filter is placed in the dissolution line of the equipment. **Fig. S4** shows a filter before and after using, consisting on a high pressure adapter to a syringe filled with cotton.

Fig. S4. Filters used in the transfer process from the DNP to the NMR to remove the residual radical traces from the hyperpolarized solution.

5. UV-Visible and EPR of in-line filtered dissolution liquids.

The absence of BA **8** free radical in the filtered dissolution liquids was confirmed by UV-Vis spectrophotometry and by EPR. radical **8**, as all the BDPA derivatives, has a strong UV-Vis absorbance at ~480 nm. **Fig. S5** shows the absorbance of a filtered and unfiltered solution of BA-BDPA **8**. As can be observed, the elimination of the radical by filtration was highly effective And the remaining radical concentration after the filtration is lower than 10^{-7} M.

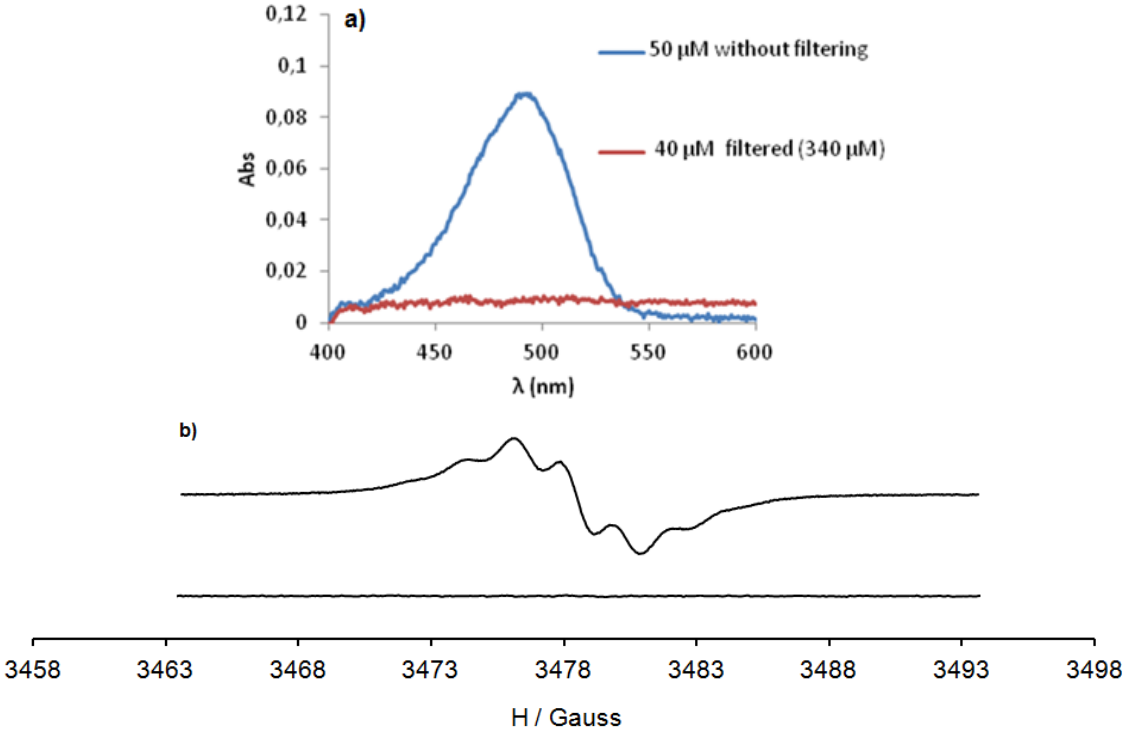


Fig. S5: a) Uv-Vis spectra of BA-BDPA. In blue, a 50 µM of BA-BDPA radical in CH_2Cl_2 unfiltered. In red, 40 µM of a BA-BDPA in-line filtered polarized sample. The theoretical final concentration of BA-BDPA in the Water/Na_2EDTA (670 µM) hyperpolarized solution was 340 µM. b) EPR of BA-BDPA. Up, a 50 µM of BA-BDPA radical in CH_2Cl_2 unfiltered. Down, 40 µM of a BA-BDPA in-line filtered polarized sample. The theoretical final concentration of BA-BDPA in the Water/Na_2EDTA (670 µM) hyperpolarized solution was 340 µM.

6. Cleaning of the dissolution line of the polarizer.

The PTFE dissolution line of the HyperSense polarizer was cleaned after each DNP experiment. The dissolution line was washed with methanol (3 x 4 ml) and then with Water/Na_2EDTA (670 µM) (3 x 4 ml).

7. Experimental linewidths

The experimental linewidth of the ^{13}C NMR signal observed for the hyperpolarized sample is typically 4 to 7 Hz although sometimes widths up to 20 Hz can be obtained. This is not due to changes in relaxation time T2 but probably due to the inhomogeneity of the sample and also for the possible presence of air bubbles generated by the rapid injection from the DNP to the NMR probe and subsequent fast data sampling without deuterium shimming stability. In thermal equilibrium conditions, the measurement is made after making the sample stand, and conventional linewidths between 1-2 Hz are obtained. Enhancement factors have been calculated by area integration and not by signal-to-noise ratio, and therefore the effect of line broadening can be considered minimal.

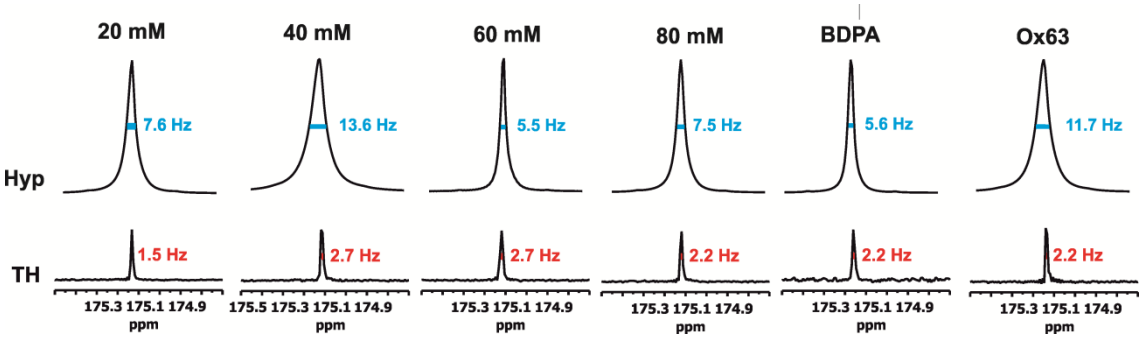


Fig. S6: Experimental linewidths (Hz) measured from the hyperpolarized DNP (Hyp) and thermal equilibrium (TH) 13C NMR spectra of [1-13C]pyruvic acid after filtration. Samples were doped with different concentrations of radical 8 (20, 40, 60 and 80 mM), BDPA (40 mM) and OX63 (15 mM).

8. References

1. Müllegger,S.; Rashidi, M.; Fattinger, M. Koch, R. *J. Phys. Chem. C* **2012**, *116*, 22587-22594.

2. a) Hartmann, G.; Hubert, D.; Mango, S.; Morehouse, C. C. Plog, K. *Nucl. Instrum. Methods* **1973**, *106*, 9-12. b) Jannin, S.; Bornet, A.; Melzi, R.; Bodenhausen, G. *Chem. Phys. Lett.* **2012**, *549*, 99-102.