

Supplementary Information

Synthesis and EPR-spectroscopic characterization of the perchlorotriarylmethyl tricarboxylic acid radical (PTMTC) and its ^{13}C labelled analogue (^{13}C -PTMTC)

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Reference numbers correspond to the reference list in the actual paper.

1. Syntheses

50% ^{13}C -Tris(2,3,5,6-tetrachlorophenyl)methane (2b).³⁴

1,2,4,5-Tetrachlorobenzene (**1**) (9.6 g, 44 mmol), AlCl_3 (0.73 g, 5.2 mmol), $^{13}\text{CHCl}_3$ (0.2 ml, 2.45 mmol) and CHCl_3 (0.2 ml, 2.45 mmol) were mixed in a glass pressure vessel, and were heated in an oil bath at 160 °C for 45 min. The mixture was then poured onto ice and HCl (1 M, 50 ml) and extracted three times with CHCl_3 . The organic layer was washed with water, aqueous NaHCO_3 , and dried over Na_2SO_4 . After evaporation, the residue was purified on silica gel eluting with heptane to give 1.26 g (39%, based on chloroform) of white crystals. $\text{C}_{19}\text{H}_4\text{Cl}_{12}$ 657.65 g/mol. *Mp*: > 280 °C. R_f = 0.67 (heptane). ^1H NMR (400 MHz, CDCl_3): δ 7.65 (s, 3H), 6.99 (s, 1H, $\text{H}^{12\text{C}}$), 6.98 (d, J = 122 Hz, 1H, $\text{H}^{13\text{C}}$). ^{13}C NMR (100 MHz, CDCl_3): δ 138.6, 134.4, 133.6, 133.3, 132.4, 130.4, 56.1. IR (KBr): ν = 3113, 3067, 2957, 2923, 1547, 1409, 1387, 1348, 1321, 1234, 1199, 1164, 1099, 974, 868, 843, 781, 758, 704, 690 cm^{-1} . EI-MS: 657.6 ($\text{C}_{19}\text{H}_4\text{Cl}_{12}$). HRMS (ESI): calcd. for $\text{C}_{19}\text{H}_3\text{Cl}_{12}$ $[\text{M} - \text{H}]^-$ 656.642; found 656.642.

50% ¹³C-Tris(4-ethoxycarbonyl-2,3,5,6-tetrachlorophenyl)methane (3b).^{18,31}

Compound **2b** (500 mg, 0.76 mmol) and TMEDA (1.15 ml, 7.6 mmol) were dissolved in dry THF (50 ml) under argon atmosphere and cooled to -78 °C. A solution of 2.5 M *n*-BuLi in *n*-hexane (3 ml, 7.6 mmol) was added in one portion and the mixture was stirred at this temperature for 1 h. Ethyl chloroformate (0.72 ml, 7.6 mmol) was added, and the reaction mixture was allowed to reach room temperature overnight. Afterwards, the solvent was evaporated and the residue was dissolved in DCM. The organic layer was washed with water and dried over Na₂SO₄. Then, the solvent was evaporated under vacuum and the residue was purified on silica gel eluting with (heptane/ethyl acetate = 12/1, V/V) to give 525 mg (79%) of colourless solid. C₂₈H₁₆Cl₁₂O₆. 873.88 g/mol. *Mp*: 170–172 °C. *R_f* = 0.26 (heptane/ethyl acetate = 10/1, V/V). ¹H NMR (400 MHz, CDCl₃): δ 7.05 (d, *J* = 122 Hz, 1H, H¹³C), 7.01 (s, 1H, H¹²C), 4.495 (q, *J* = 7.1 Hz, 6H), 1.424 (t, *J* = 7.1 Hz, 9H). ¹³C NMR (100 MHz, CDCl₃): δ 163.2, 138.4, 135.5, 135, 134, 130.5, 129.5, 63.1, 56.3, 14. IR (KBr): ν = 2981, 1741, 1555, 1465, 1370, 1341, 1298, 1259, 1224, 1207, 1113, 858, 756 cm⁻¹. EI-MS: 873.7 (C₂₈H₁₆Cl₁₂O₆). HRMS (ESI): calcd. for C₂₈H₁₇Cl₁₂O₆ [M + H]⁺ 874.720; found 874.720.

50% ¹³C-Tris(4-ethoxycarbonyl-2,3,5,6-tetrachlorophenyl)methyl radical (¹³C-PTMTE).^{18,35}

A solution 1 M Bu₄NOH in methanol (0.5 ml, 0.48 mmol, 1.2 equiv.) was added to a solution of compound **3b** (350 mg, 0.4 mmol, 1 equiv.) in freshly distilled THF (30 ml) under argon atmosphere. The mixture was stirred in the dark for 1 h. *p*-Chloranil (394 mg, 1.6 mmol, 4

equiv.) was added as a solid. The mixture was stirred overnight. Afterwards, the solvent was removed giving a purple residue, which was purified on silica gel eluting with (heptane/ethyl acetate = 80/20, V/V) to give 290 mg (83%) of red solid. $C_{28}H_{15}Cl_{12}O_6$. 872.70 g/mol. *Mp*: 160–165 °C. *R_f* = 0.26 (heptane/ethyl acetate = 10/1, V/V). IR (KBr): ν = 2955, 2916, 2849, 1741, 1466, 1378, 1342, 1284, 1224, 1010, 756 cm^{-1} . ESI-MS, $CHCl_3$: *m/z* (%): 872.81 ($[M]^-$, 100%). HRMS (ESI): calcd. for $C_{28}^{13}C_1H_{16}Cl_{12}O_6$ $[M + H]^+$ 874.712; found 874.724.

Tris(4-carboxy-2,3,5,6-tetrachlorophenyl)methyl radical (PTMTC).³³

PTMTE (200 mg, 0.23 mmol)³¹ was mixed with conc. H_2SO_4 (95%, 25 ml) and the mixture was heated at 90 °C for 12 h. The final solution was cooled and poured carefully onto cracked ice; the aqueous phase was extracted with Et_2O . The organic phase was concentrated and extracted with aqueous Na_2CO_3 . The resulting aqueous phase was acidified slowly with 5 M HCl and extracted several times with Et_2O . The organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum. The crude product was dissolved in Et_2O (5 ml) and precipitated from hexane; this process was repeated 3 times to give 154.2 mg (85%) of red powder. $C_{22}H_3Cl_{12}O_6$. 788.61 g/mol. *Mp*: > 280 °C. *R_f* = 0.2 (ethyl acetate/methanol = 5/2, V/V). IR (KBr): ν = 3702–2643, 1703, 1661, 1602, 1536, 1401, 1348, 1325, 1281, 1240, 1124, 1041, 859, 752, 724 cm^{-1} . ESI-MS, DMSO: *m/z* (%): 788.83 ($[M]^-$, 70%), 743.60 ($[M-CO_2]^-$, 100%), 698.82 ($[M-2CO_2]^-$, 60%). HRMS (ESI): calcd. for $C_{22}H_4Cl_{12}O_6$ $[M + H]^+$ 789.618; found 789.618.

50% ^{13}C -Tris(4-carboxy-2,3,5,6-tetrachlorophenyl)methyl radical (^{13}C -PTMTC).

^{13}C -PTMTE was prepared as described above for tris(4-carboxy-2,3,5,6-tetrachlorophenyl)methyl radical (PTMTC) to give 159.6 mg (88%) of red solid. $\text{C}_{22}\text{H}_3\text{Cl}_{12}\text{O}_6$. 788.61 g/mol. Mp : > 280 °C. $R_f = 0.2$ (ethyl acetate/methanol = 5/2, V/V). IR (KBr): $\nu = 3575$ – 2414 , 1698, 1660, 1601, 1394, 1323, 1240, 1143, 1037, 717 cm^{-1} . ESI-MS, DMSO: m/z (%): 789 ($[\text{M}]^-$, 85%), 701 ($[\text{C}_{20}\text{H}_3\text{Cl}_{12}\text{O}_2]^-$, 100 %). HRMS (ESI): calcd. for $\text{C}_{21}\text{H}_4\text{Cl}_{12}\text{O}_6$ $[\text{M} + \text{H}]^+$ 790.621; found 790.627.

2. Ratio of the peak-to-peak intensities of central ^{12}C line and ^{13}C doublet lines at LF as a function of glycerol obtained at 25 °C and 37 °C

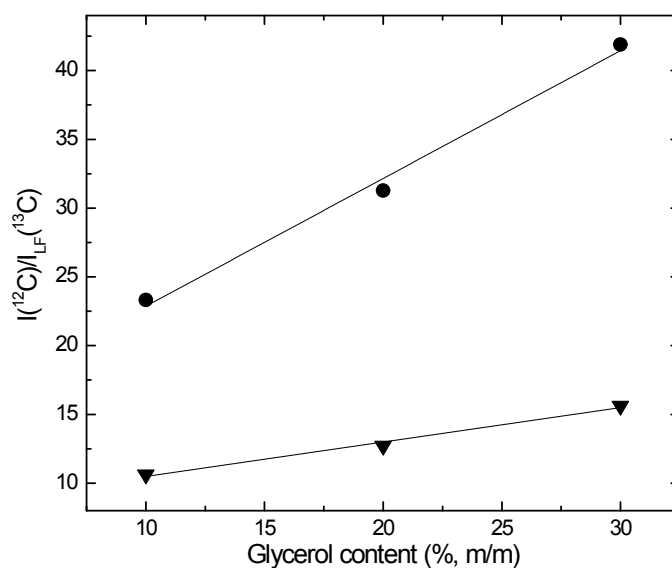


Fig. S1 Ratio of the peak-to-peak intensities of central ^{12}C line and ^{13}C doublet lines at LF as a function of glycerol obtained at two different temperatures (●) 25 °C and (▼) 37 °C. Straight lines are meant to guide the eyes.

3. The dependence of $1/T_M$ relaxation rate on water/methanol compositions

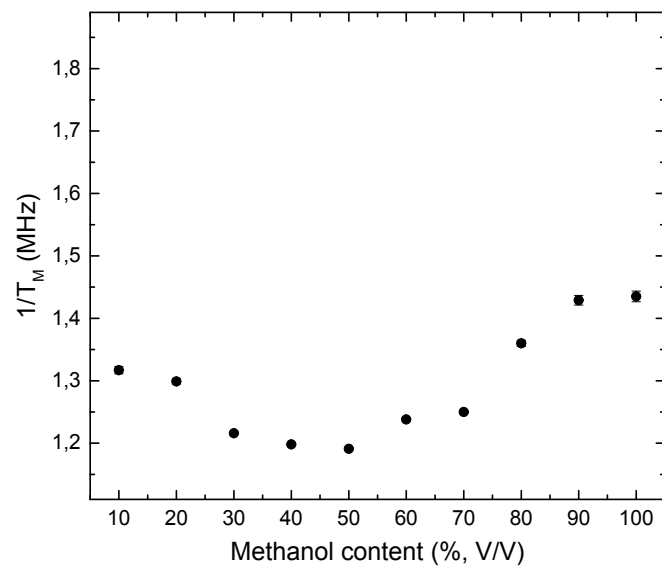


Fig. S2 Dependence of $1/T_M$ of the ^{12}C -PTMTC line on water/methanol compositions.

4. Influence of temperature on ^{13}C -PTMTC line width and intensity

The intensity of the EPR signal arising from the central line is inversely proportional to temperature according to the Curie law, whereas the intensity of EPR signals arising from the ^{13}C doublet obeys a more complex relationship. Non-Curie law behavior or the intensity enhancement is most marked with radicals where the electron spin is highly localized and isolated from nuclear interactions. This suggests that we are dealing with differential saturation where the species that produces the main $I_{pp}(^{12}\text{C})$ line in the spectrum has only weak relaxation mechanisms while the $I_{pp}(^{13}\text{C})$ labeled species have much stronger relaxation as a result of the nuclear hyperfine interaction.

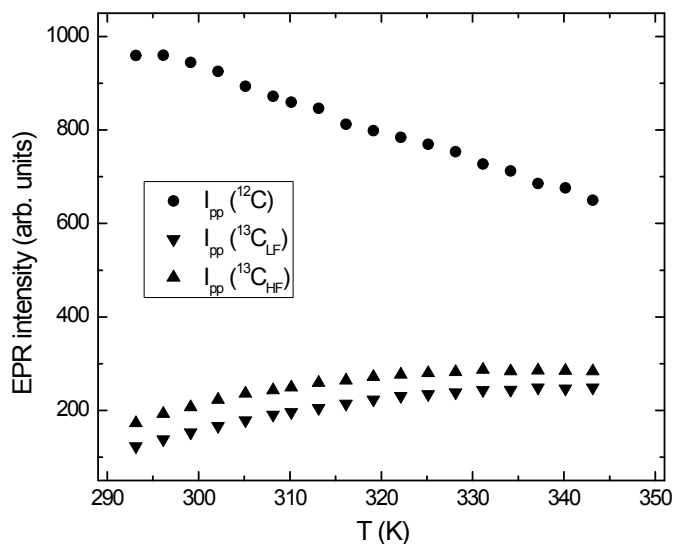


Fig. S3 Temperature dependence of the EPR intensities.

5. Ascorbic acid reduction assay

Aqueous solution of ascorbic acid ($c = 10 \text{ mM}$) was added to a solution of the ^{13}C -PTMTC radical ($c = 1 \text{ mM}$) in PB (50 mM, pH 7.4). **Fig. S4** shows a decrease of the EPR signal intensity of 50% in an hour recorded at 300 K. The process of the interaction of the ^{13}C -PTMTC and ascorbic acid is complex and can be influenced by solvent, light and concentration of oxygen. Further investigation of these effects, are indeed necessary, but they are beyond the scope of this paper.

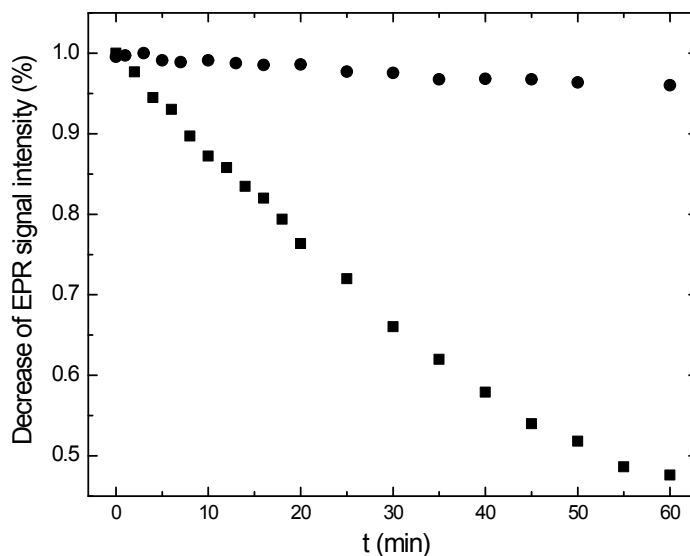


Fig. S4 Decay of ^{13}C -PTMTC recorded at 300 K (●) pure solution of ^{13}C -PTMTC and (■) solution of ^{13}C -PTMTC in the presence of ascorbic acid.