

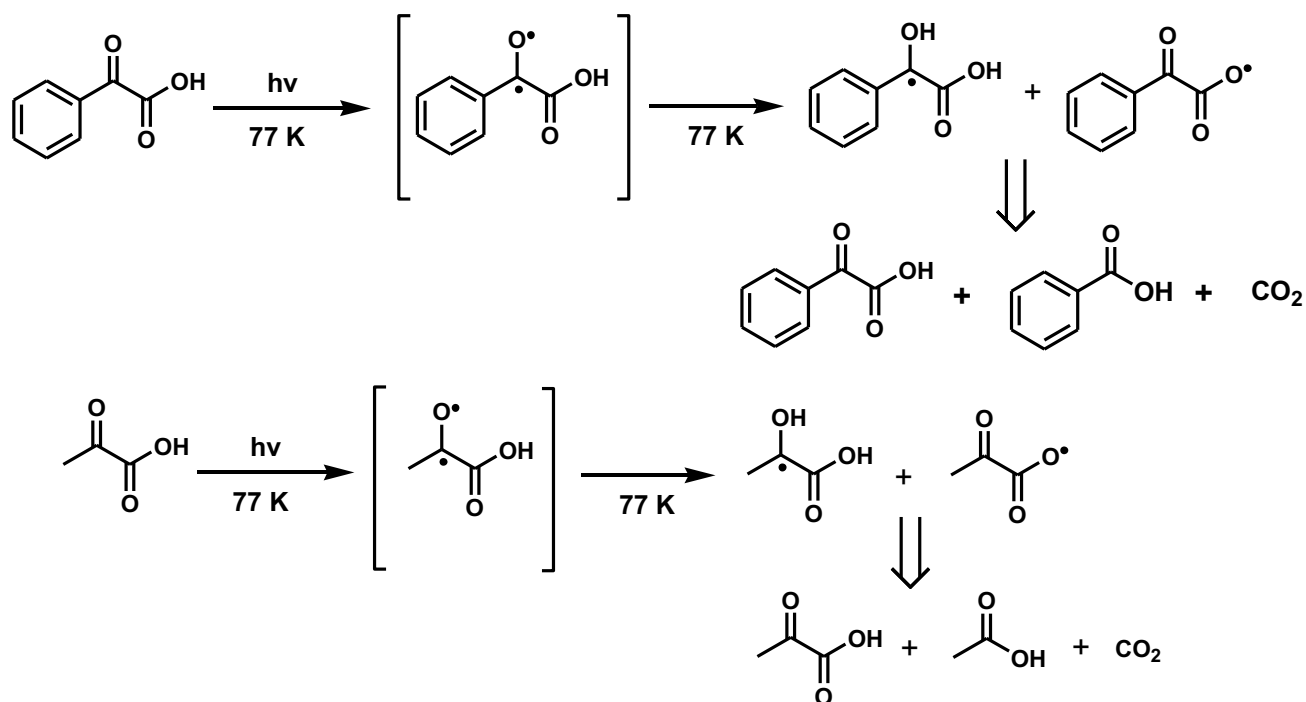
## Supporting Information

### Photo-generated radical in phenylglyoxylic acid for in vivo hyperpolarized $^{13}\text{C}$ MR with photo-sensitive metabolic substrates

Irene Marco-Rius,<sup>a</sup> Tian Cheng,<sup>a</sup> Adam P. Gaunt,<sup>a</sup> Saket Patel,<sup>b</sup> Felix Kreis,<sup>a</sup> Andrea Capozzi,<sup>c</sup> Alan J. Wright,<sup>a</sup> Kevin M. Brindle,<sup>a</sup> Olivier Ouari,<sup>b</sup> and Arnaud Comment<sup>a,d</sup>

<sup>a</sup> University of Cambridge, Cambridge, UK ; <sup>b</sup> Aix-Marseille University, CNRS, ICR, Marseille, France ;  
<sup>c</sup> Department of Electrical Engineering, Center for Hyperpolarization in Magnetic Resonance, Technical University of Denmark, Lyngby, Denmark; <sup>d</sup> General Electric Healthcare, Chalfont St Giles, UK.

## Photochemistry



Scheme 1. Radical production upon photo-irradiation of phenylglyoxylic acid (top) and pyruvic acid (bottom) at 77 K.

## Hyperpolarized signal corrected by flip angle and T<sub>1</sub> relaxation

The hyperpolarized signal was corrected by flip angle and T<sub>1</sub> relaxation, as per eq. 1:

$$S_n = S_1 \cos(\theta_{HP})^{n-1} e^{-((n-1) \cdot TR / T_1)}, \quad (1)$$

where  $S_n$  is the signal amplitude of the  $n^{\text{th}}$  spectrum,  $\theta_{HP}$  is the flip angle, and TR is the repetition time.

To calculate the liquid-state polarization, the thermal <sup>13</sup>C-NMR signal was acquired with the same parameters as above but repetition time  $5 \times T_1$ . The polarization,  $P$ , at the time of the first acquisition ( $n = 1$ ) was then calculated using eq. 2:

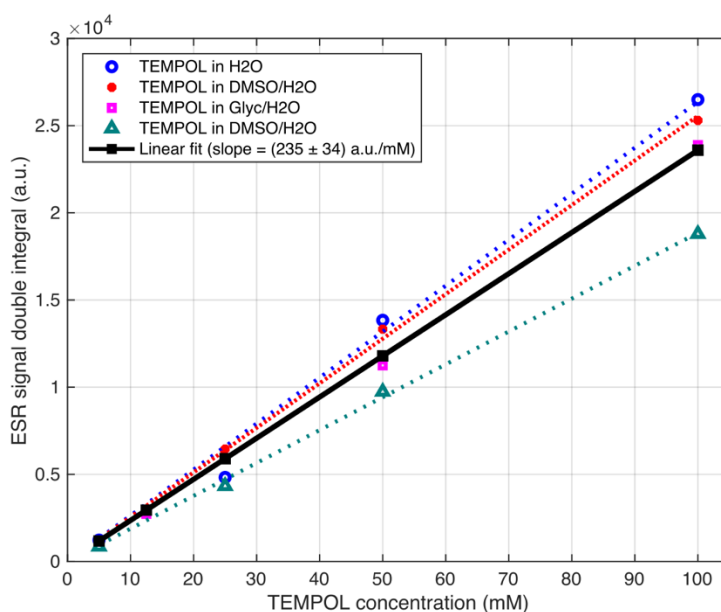
$$P = \frac{\sin(\theta_{TP})}{\sin(\theta_{HP})} \frac{I_{HP}}{I_{TP}/N_{TP}} P_{eq}, \quad (2)$$

where  $\theta_{TP}$  and  $\theta_{HP}$  are the flip angles used during the thermal and hyperpolarized signal acquisition, respectively.  $I_{HP}$  (proportional to  $S_1$ ) is the integral of the  $[2-^{13}\text{C}]$ DHAc peak in the first spectrum,  $I_{TP}$  is the integral of the thermally polarized  $[2-^{13}\text{C}]$ DHAc peak using the same frequency limits as for  $I_{HP}$ ,  $N_{TP}$  is the number of averages of the thermal signal and  $P_{eq}$  is the predicted thermal equilibrium polarization from eq. 3:

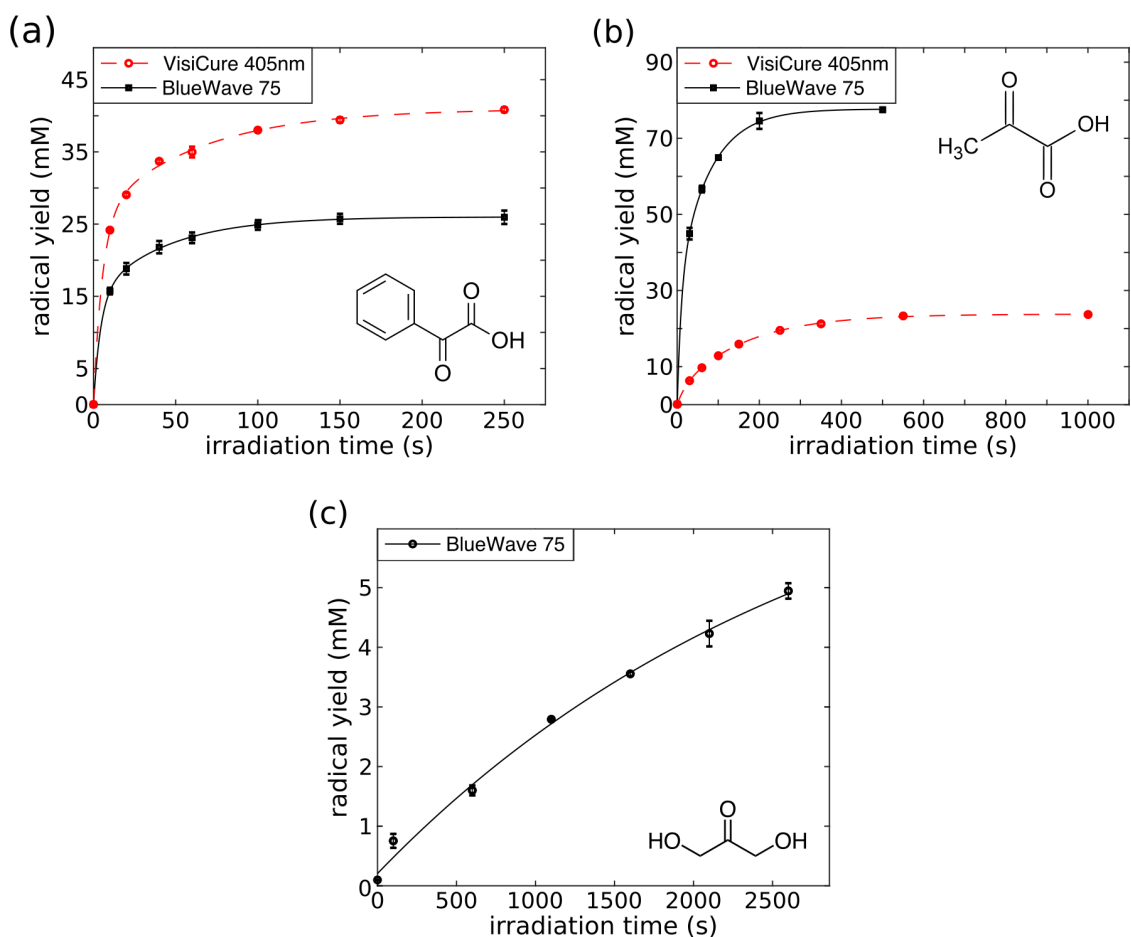
$$P_{eq} = \tanh\left(\frac{\hbar\gamma B_0}{2\kappa T}\right) \times 100\% , \quad (3)$$

where  $\hbar$  is the Planck constant,  $\gamma$  is the  $^{13}\text{C}$  gyromagnetic ratio,  $B_0$  is the magnetic field strength,  $T$  is the temperature, and  $\kappa$  is Boltzmann's constant. In this paper, all values for  $P$  and  $P_{eq}$  are quoted in percent. At  $B_0 = 7\text{ T}$  and  $T = 298\text{ K}$ ,  $P_{eq} = 6 \times 10^{-4}\%$ .

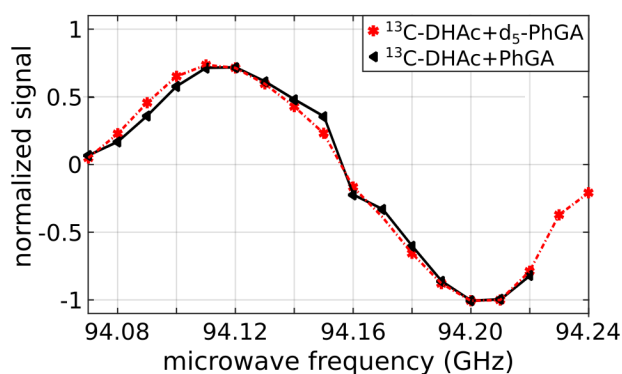
## Supporting Figures



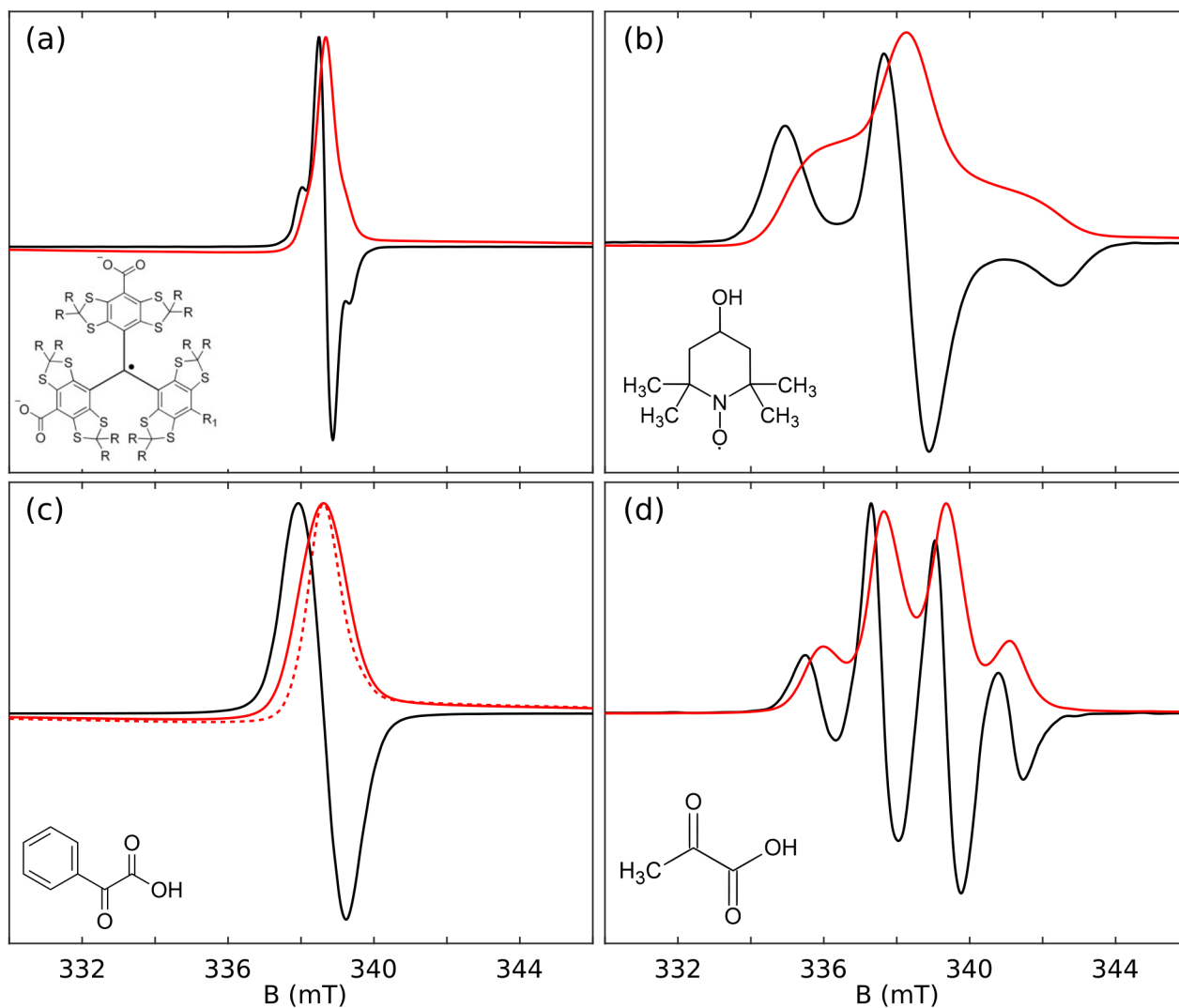
Supporting Figure 1. ESR spectrometer calibration curve. The double integral of the ESR signal of known concentrations of the stable radical 4-hydroxy-TEMPO (TEMPOL) dissolved in H<sub>2</sub>O, DMSO/H<sub>2</sub>O (v/v) and glyc/H<sub>2</sub>O (v/v) was linearly fitted. The average of the four linear fits is shown as a solid black line. The average slope ( $235 \pm 34$ ) mM<sup>-1</sup> was used to convert the volume-corrected ESR signal of the photo-generated radicals into a concentration estimate. Abbreviations: DMSO = dimethyl sulfoxide; glyc = glycerol.



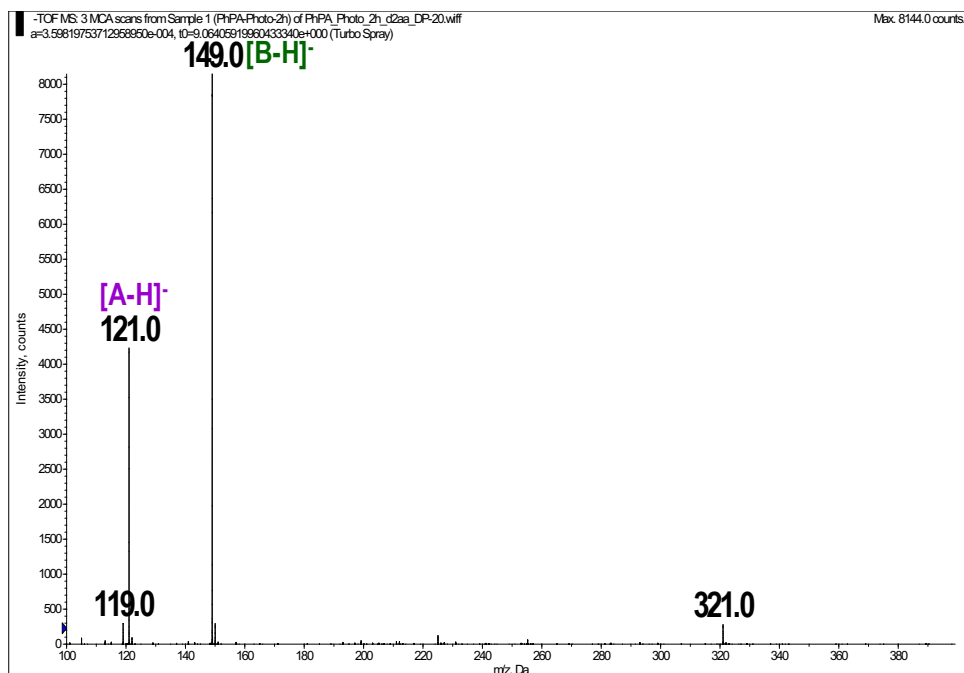
Supporting Figure 2. Radical yield as a function of photo-irradiation time of (a) phenylglyoxylic acid, (b) pyruvic acid and (c) dihydroxyacetone. Each measurement was performed twice. Lines are guides for the eye. Note: VisiCure 405 = Dymax BlueWave LED VisiCure 405; BlueWave75 = Dymax BlueWave 75.



Supporting Figure 3. Solid-state microwave spectra of  $[2-^{13}\text{C}]$  dihydroxyacetone (DHAc) doped with radicals of unlabeled and perdeuterated phenylglyoxylic acid (PhGA). Data acquired in a 3.35 T polarizer at 1.25 K (HyperSense, Oxford Instruments, Abingdon, UK).

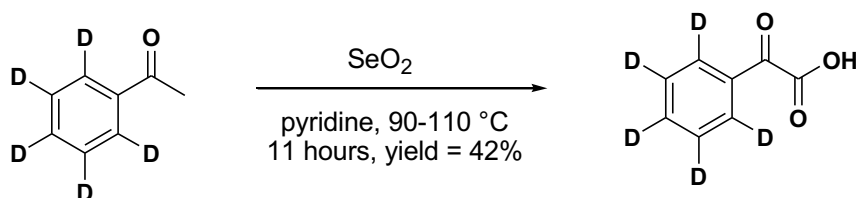


Supporting Figure 4. First derivative (black) and absorption (red) ESR spectra of solutions containing (a) OX063, (b) 4-hydroxy-TEMPO, (c) photo-irradiated phenylglyoxylic acid (PhGA) along with  $d_5$ -PhGA in dashed line, and (d) photo-irradiated pyruvic acid.



Supporting Figure 5. Electrospray ionization mass spectrometry (ESI MS) analysis of the mixture after 2h of photoirradiation of PhGA (50% in volume in EtOH) has shown the presence of the starting material PhGA and benzoic acid. No other product can be detected.

## Synthesis data for the synthesis of deuterated PhGA



Scheme 2. Synthesis of 2-Oxo-2-(phenyl- $d_5$ )acetic acid

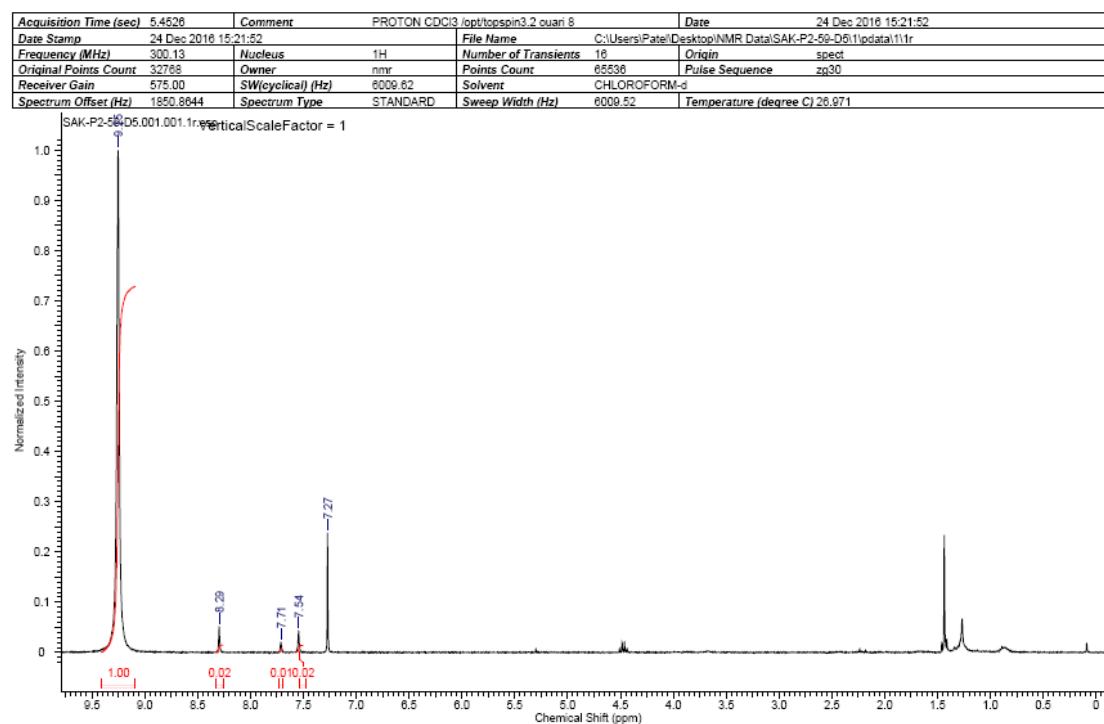
**(Phenyl- $d_5$ )glyoxylic acid ( $d_5$ -PhGA):**<sup>1-3</sup> - In a 50 ml round bottom flask acetophenone- (phenyl- $d_5$ ) (1.5 g, 12 mmol, 1 equiv., 99 atom % deuterated from Sigma Aldrich ),  $\text{SeO}_2$  (2.0 g, 18 mmol, 1.5 equiv.) was introduced and 30 ml of pyridine was added. The reaction mixture was heated at 110 °C for 1 hour under argon atmosphere and then the temperature of the reaction was reduced to 90 °C. The progress of the reaction was monitored using thin layer chromatography (TLC). After the completion of the reaction, reaction mixture was filtered to remove the selenium powder and washed with chloroform. The filtrate was treated with 50 ml of 1 N HCl and the organic phase was removed. The aqueous phase was extracted with chloroform (3\*50 ml) three

times. The combined organic phases were treated with 25 ml of 1 N NaOH and aqueous phase was separated. The organic phase was again extracted with 50 ml of water and the combined aqueous phase was acidified using 1 N HCl up to pH ~1 and extracted with chloroform (3\*50 ml). The combined organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The purification over silica gel by using 5% acetic acid in ethanol as the eluent provided the product as a white powder in 42 % yield (0.770 g, 99 atom% D).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 9.25 (s, 1H), 8.29 (s, 0.02 H), 7.71 (s, 0.01H), 7.54 (s, 0.02 H).

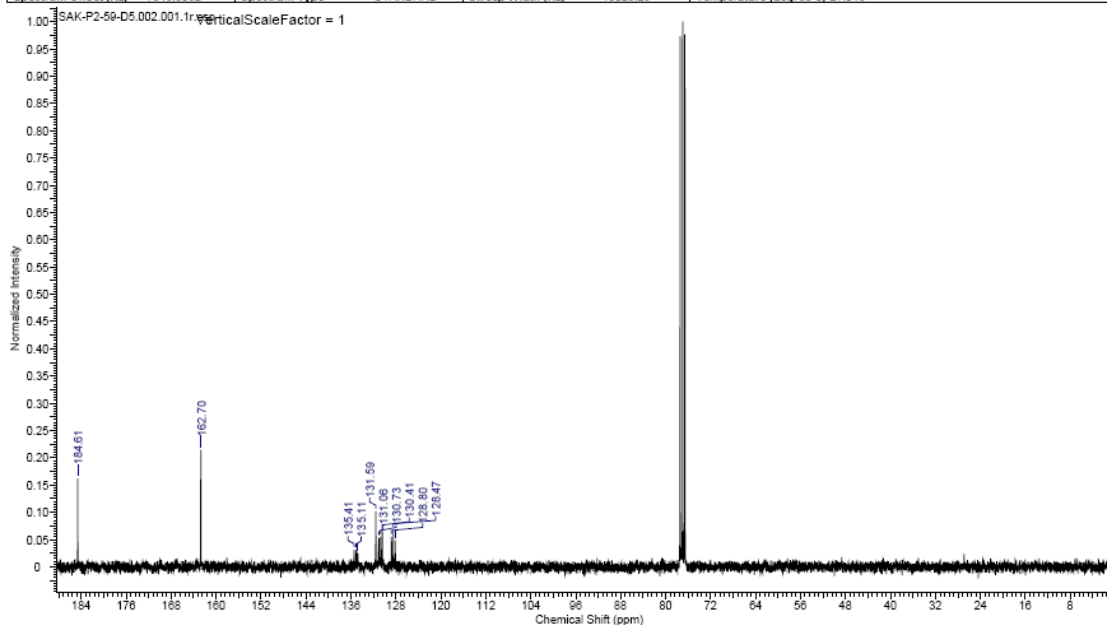
<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 184.6, 162.7, 135.1 (t, J = 27.2 Hz), 131.6, 130.41 (t, J = 25.6 Hz), 128.5 (t, J = 24.0 Hz).

HRMS-ESI: *m/z* calcd for C<sub>8</sub>D<sub>5</sub>O<sub>3</sub>H [M-H]<sup>-</sup> 154.0553, found 154.0558.

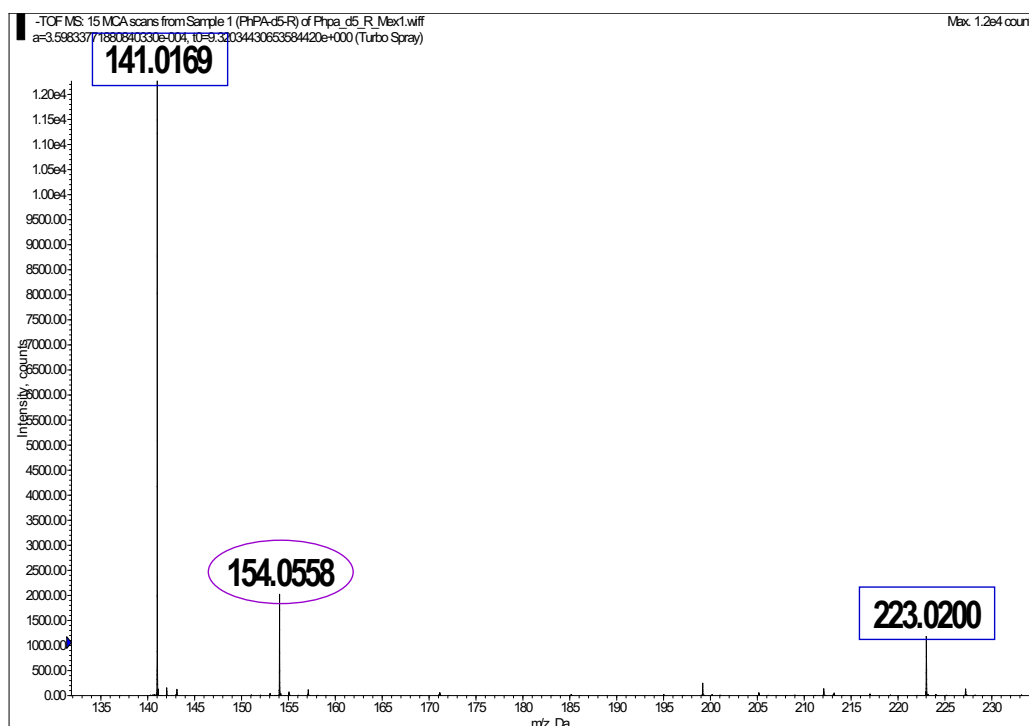


Supporting Figure 6: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) of d<sub>5</sub>-PhGA.

Acquisition Time (sec)	1.8175	Comment	C13CPD-nuit CDCl3 (opt/topspin3.2 suari 8	Date	24 Dec 2016 16:56:00
Date Stamp	24 Dec 2016 15:56:00	File Name	C:\Users\Patel\Desktop\NMR Data\SAK-P2-59-D52\data111r		
Frequency (MHz)	75.47	Nucleus	<sup>13</sup> C	Number of Transients	512
Original Points Count	32768	Owner	nmr	Points Count	32768
Receiver Gain	1820.00	SW(cyclical) (Hz)	18028.86	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	7546.3032	Spectrum Type	STANDARD	Sweep Width (Hz)	18028.29
				Temperature (degree C)	27.013



Supporting Figure 7. <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>) of d<sub>5</sub>-PhGA.



Supporting Figure 8. HRMS ESI in negative mode of d<sub>5</sub>-PhGA sample. Peaks at m/z 141.0169 and m/z 223.0200 are internal references (QStar Elite from Applied Biosystems SCIEX, Spectropole, Marseille).



## REFERENCES

- (1) R. Howe, B. S. Rao, H. Heyneker, 2,3-Dihydro-2-hydroxybenzo[b]furan-3-one, the cyclic hemiacetal of 2-hydroxyphenylglyoxal, *J. Chem. Soc.* **1967**, 2510–2514;
- (2) D. Nardi, E. Massarini, S. Rossi, L. Degen, P. Altucci, G. Catalano, and G. L. Varone, Antiviral compounds (XVI) p-alkylthiophenylglyoxals and their derivatives, *Farmaco* **1974**, 29, 473–481.
- (3) K. Wadhwa, C. Yang, P. R. West, K. C. Deming, S. R. Chemburkar, and R. E. Reddy, Synthesis of Arylglyoxylic Acids and Their Collision-Induced Dissociation, *Synthetic Communications*, **2008**, 38, 4434-4444.