Singlet oxygen photosensitisation by the fluorescent probe Singlet Oxygen Sensor Green

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Purity of SOSG

Liquid Chromatography (HPLC) was performed with an Agilent 1200 series liquid chromatograph equipped with a diode array detector. SOSG (100 µg diluted in 0.5 mL of MeOH / H_2O 70:30) was analyzed on a 12.5 mm x 4.6 mm, 5 µm particle, Lichrospher 100 RP-18 column. Detection was achieved at 254, 355 and 532 nm. All experiments were carried out at 25 ºC with a mobile phase flow rate of 1.0 mL min^{-1}. A gradient elution was performed from 40:60 to 70:30 MeOH/H_2O for the mobile phase (Table S1):

Table S1. Chromatographic conditions for the gradient elution of SOSG.

Time / min	%MeOH	% H_2O
0	40	60
2	40	60
15	70	30
20	70	30
22	40	60
27	40	60

As shown in Figure S1, four different peaks could be observed at 254, 355 and 532 nm. The relative chromatographic areas are collected in Table S2.

Table S2. Relative areas of the peaks obtained by liquid chromatography of SOSG

Chromatogram	λ_{obs} / nm	Peak 1 (%)	Peak 2 (%)	Peak 3 (%)	Peak 4 (%)
A	254	0.1	0.2	94.9	4.8
B	355	0.1	0.2	95.3	4.4
C	532	0.1	0.2	94.9	4.8

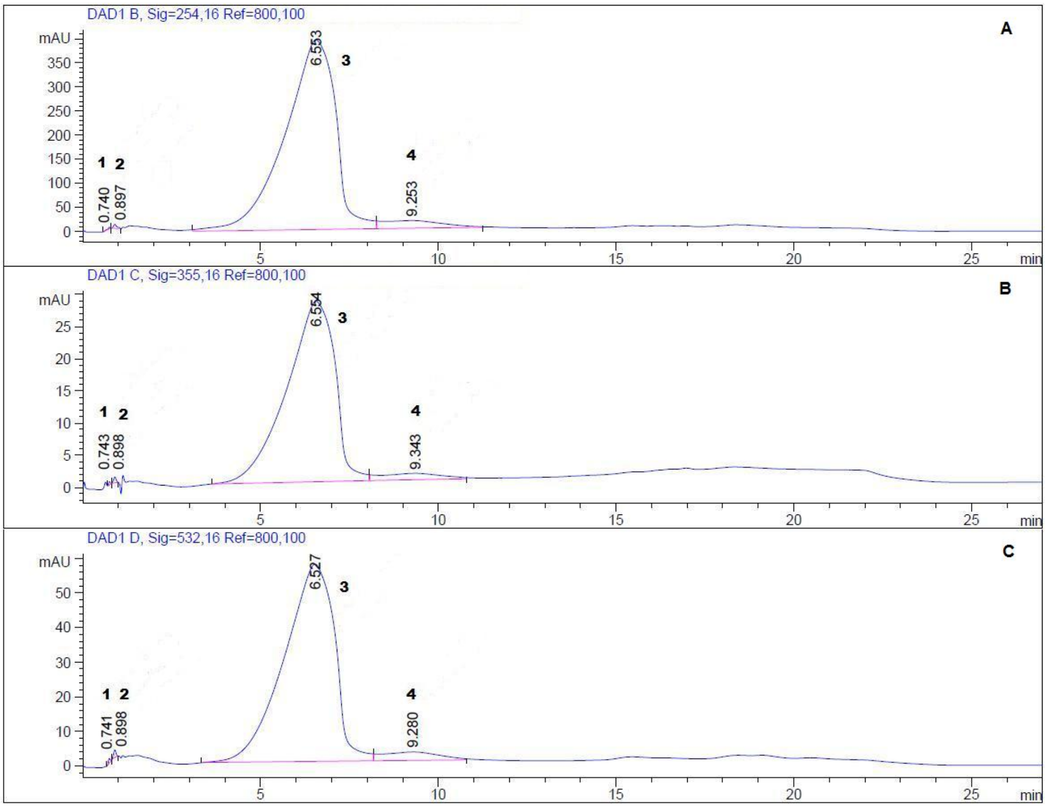

Figure S1. Liquid chromatography of SOSG at (A) 254 nm, (B) 355 nm and (C) 532 nm.

Peaks **3** and **4** account for more than 99.5% of the total chromatographic area and show the same UV-Vis spectrum as SOSG (Figure S2). This suggests that SOSG exists as a mixture of isomers and/or species with different degree of ionization.

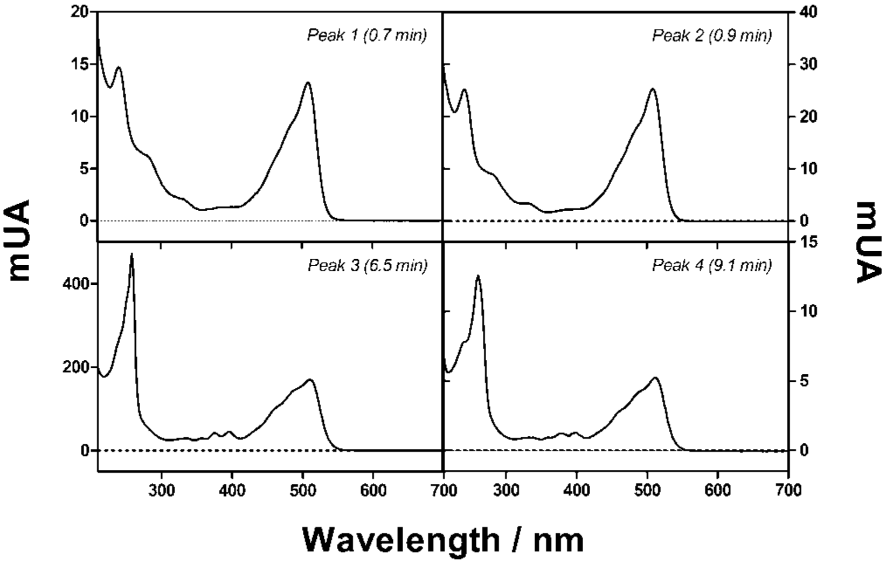

Figure S2. Absorption spectra of the different peaks obtained by liquid chromatography of SOSG.

Singlet oxygen experiments

Lifetime of singlet oxygen in methanol-d_4

The singlet oxygen lifetime observed in a methanol-d_4 solution of SOSG 17 μM is 113 μs. This value is consistent with that of 149 μs calculated from the lifetime of singlet oxygen in neat methanol-d_4 (227 μs; M.A.J Rodgers, J. Am. Chem. Soc. 1983, 105: 6201-6205) and a rate constant for reaction of singlet oxygen with dimethylanthracene of 6.9 x 10^7 $M^{-1}\cdot s^{-1}$ (F. Wilkinson et al., J. Phys. Chem. Ref. Data 1995, 24: 663-1021):

Sodium azide addition

Addition of increasing volumes of a 10 mM sodium azide solution to SOSG dissolved in methanol-d_4 induced a progressive decrease of the singlet oxygen decay lifetime τ_Δ (Figure S3). As shown in the inset of figure S3, the rate constant for singlet oxygen decay (= 1/ τ_Δ) increases linearly with the concentration of azide, yielding a quenching rate constant of 2.6 x 10^8 $M^{-1}s^{-1}$, in excellent agreement with the reported value of 2.2 x 10^8 $M^{-1}s^{-1}$ (F. Wilkinson et al., J. Phys. Chem. Ref. Data 1995, 24: 663-1021)

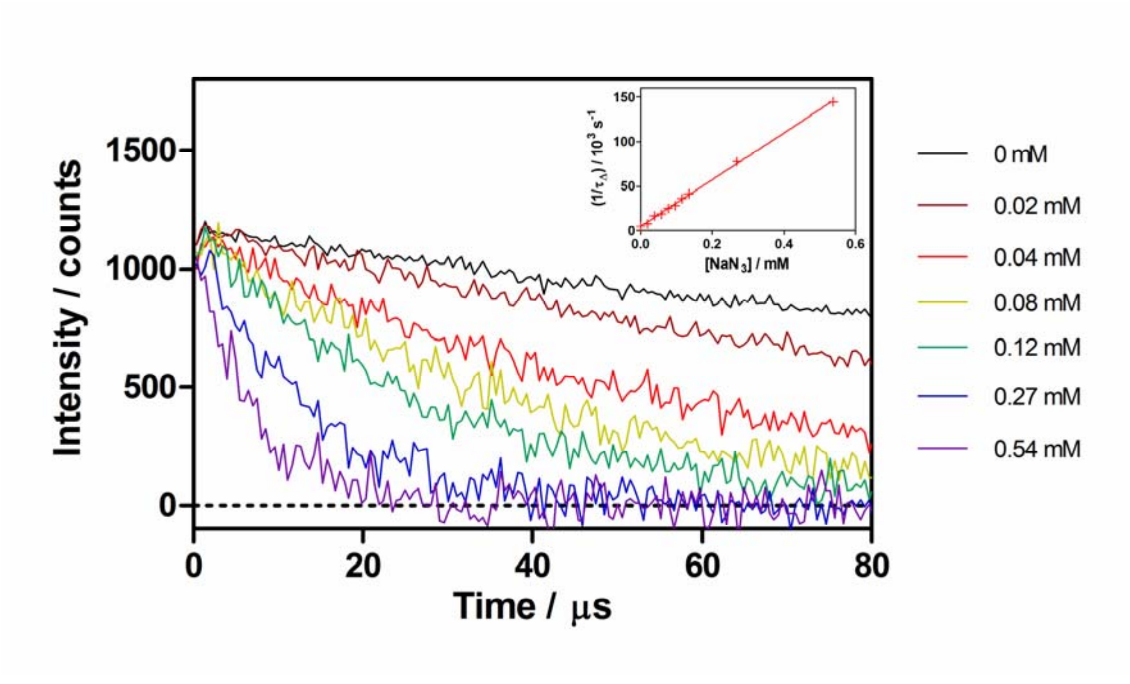

Figure S3. Singlet oxygen phosphorescence photosensitised by SOSG in air-saturated methanol-d_4 solutions containing variable amounts of sodium azide. The solution was irradiated with 1-ns laser pulses at 355 nm (ca. 1 μJ per pulse, repetition rate 10 kHz) and the luminescence was observed at 1275 nm. Inset: Stern-Volmer Plot.

Blank controls

Exclusion of either oxygen or SOSG completely eliminated the singlet oxygen signal (Fig. 1 trace c of the manuscript and Fig. S4, respectively).

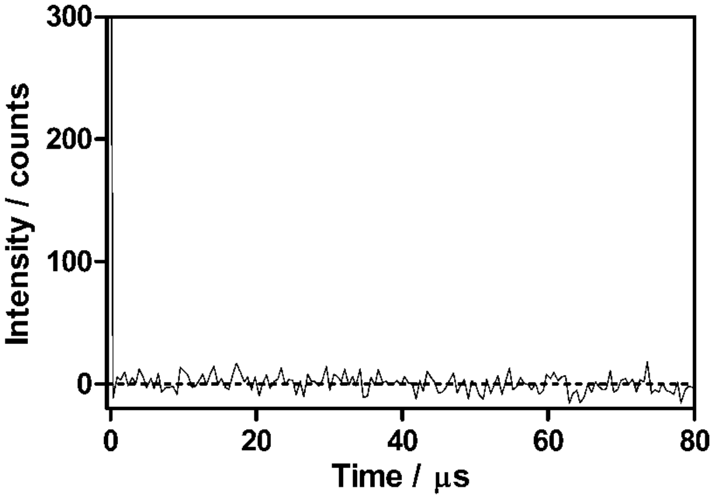

Figure S4. Luminescence observed for a control air-equilibrated methanol-d_4 sample in the absence of SOSG. The sample was irradiated with 1-ns laser pulses at 355 nm (ca. 1 μJ per pulse, repetition rate 10 kHz) and the luminescence was observed at 1275 nm.

Quantum yield of $^1O_2$ formation (Φ_Δ)

Production of singlet oxygen was verified in four different samples of SOSG.

In a triplet-photosensitized experiment, the time profile of $^1O_2$ phosphorescence, *S(t)*, is given by Eq. (1):

$$S(t)=S(0)\cdot\frac{\tau_\Delta}{\tau_T-\tau_\Delta}\cdot\left(e^{-t/\tau_T}-e^{-t/\tau_\Delta}\right) \qquad (1)$$

where $S(0)$ is a parameter proportional to Φ_Δ, and τ_T and τ_Δ are the actual lifetimes of the photosensitiser triplet state and singlet oxygen, respectively.

The pre-exponential factor $S(0)$ was determined by fitting Eq. 1 to the time-resolved phosphorescence intensity at 1275 nm. The quantum yields of $^1O_2$ production by SOSG were determined from the comparison of its $S(0)$ value to that produced by an optically-matched reference in the same solvent and at the same excitation wavelength and intensity (Eq. 2).

$$\Phi_\Delta(SOSG) = \frac{S(0)_{SOSG}}{S(0)_{ref}} \cdot \Phi_\Delta(ref) \qquad (2)$$

Using phenalenone (PN) as reference at 355 nm and Rose Bengal (RB) as reference at 532 nm, ${}^1O_2$ phosphorescence signals were obtained in MeOH (Figure S5) and in D_2O/MeOH 95:5 (Figure S6).

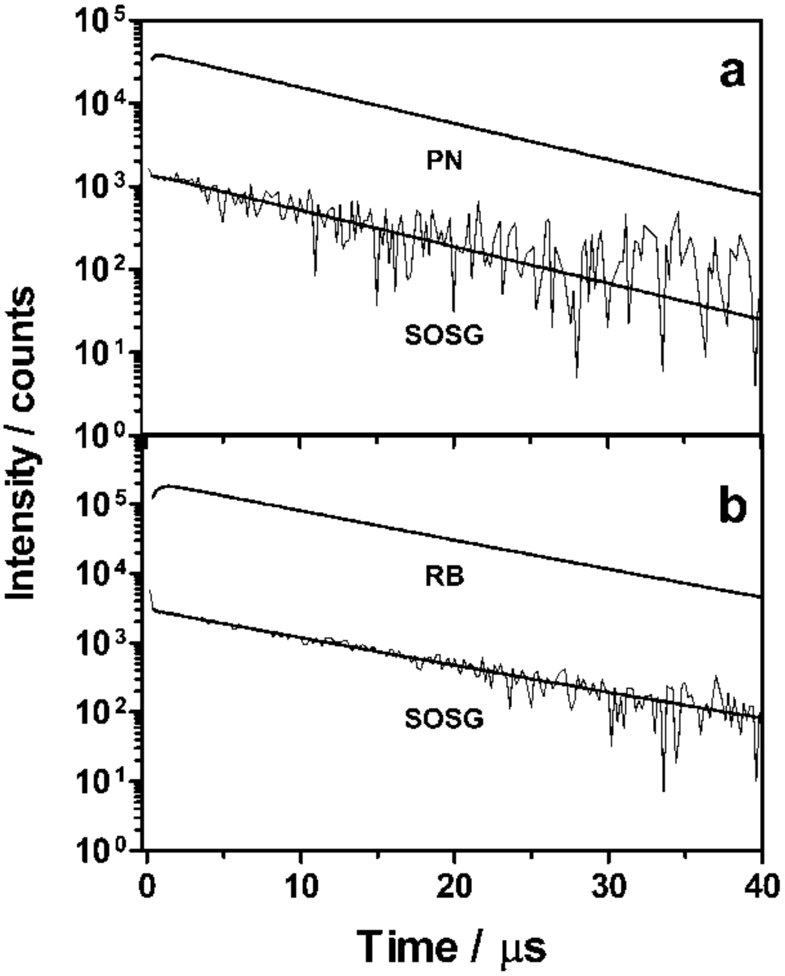

Figure S5. Singlet oxygen phosphorescence photosensitised by SOSG in MeOH observed at 1275 nm. The solution was irradiated with 1-ns laser pulses at (a) 355 nm using phenalenone (PN) as reference, and (b) 532 nm using rose bengal (RB) as reference.

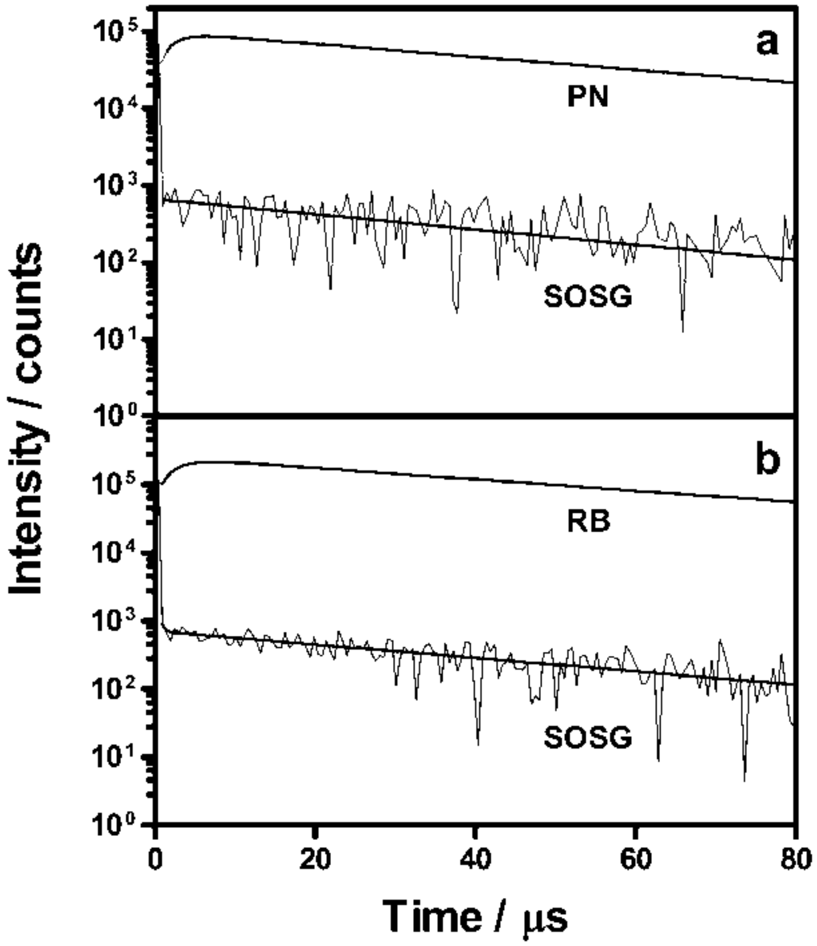

Figure S6. Singlet oxygen phosphorescence photosensitised by SOSG in D_2O-MeOH 95:5 observed at 1275 nm. The solution was irradiated with 1-ns laser pulses at (a) 355 nm using phenalenone (PN) as reference, and (b) 532 nm using rose bengal (RB) as reference.

UV-Vis spectrum

Fig. S7 shows the absorption spectra of dimethylanthracene and fluorescein (panel A) and of SOSG (panel B).

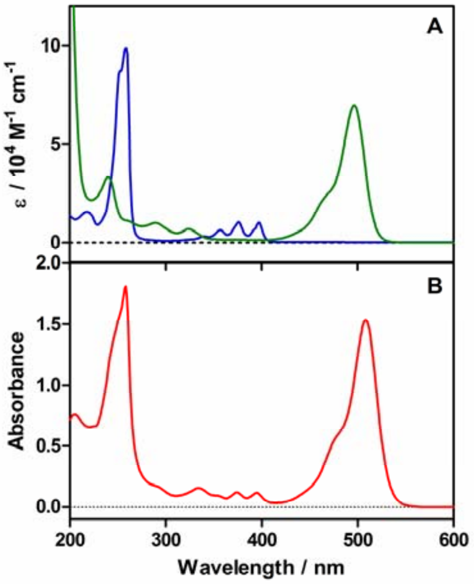

Figure S7. UV-Visible absorption spectrum of (A) dimethylanthracene (grey line) and fluorescein (black line). (B) spectrum of SOSG.

Mass spectrometry

High resolution mass spectrometry was done to know the exact mass of SOSG. A TOF spectrometer with ESI gave a major peak of m/z = 601.1050 (Figure S5) corresponding to an empirical formula of $C_{36}H_{22}ClO_7$.

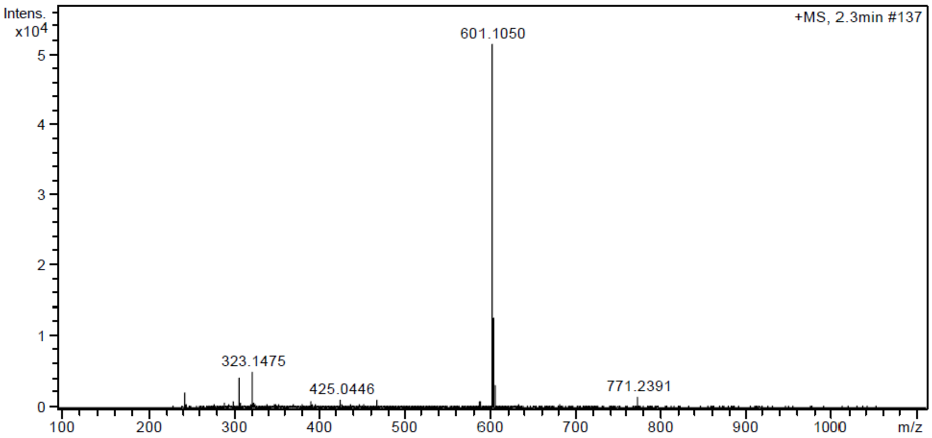

Figure S8. ESI-TOF high resolution mass spectrometry of SOSG.

NMR Spectroscopy

^{1}H-NMR

δ_H (800 MHz; CD_3OD) 3.10 (6 H_{ant}, s, Me), 6.47 (1 H_{fluo}, s), 6.50 (1 H_{fluo}, s), 6.72 (1 H_{fluo}, s), 6.74 (1 H_{fluo}, s), 6.94 (1 H_{fluo}, s), 6.95 (1 H_{fluo}, s), 7.01 (1 H_{fluo}, s), 7.02 (1 H_{fluo}, s), 7.20 (1 H_{ant}, dd), 7.23 (1 H_{ant}, dd), 7.32 – 7.43 (7 $H_{6ant\ +\ 1fluo}$, m), 7.47 (2 H_{ant}, dd), 7.56 (1 H_{ant}, d), 7.58 (1 H_{ant}, d), 7.90 (1 H_{fluo}, d), 7.91 (1 H_{fluo}, d), 8.16 (1 H_{fluo}, d), 8.30 (2 H_{ant}, d), 8.32 (1 H_{fluo}, d), 8.34 (2 H_{ant}, d), 8.43 (1 H_{fluo}, s).

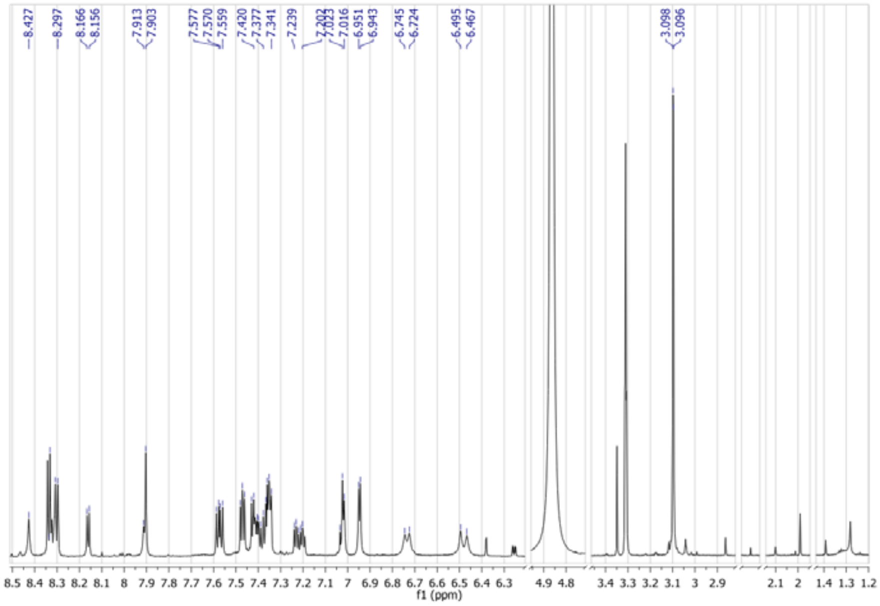

Figure S9. ^{1}H-NMR of SOSG in methanol-d_4.

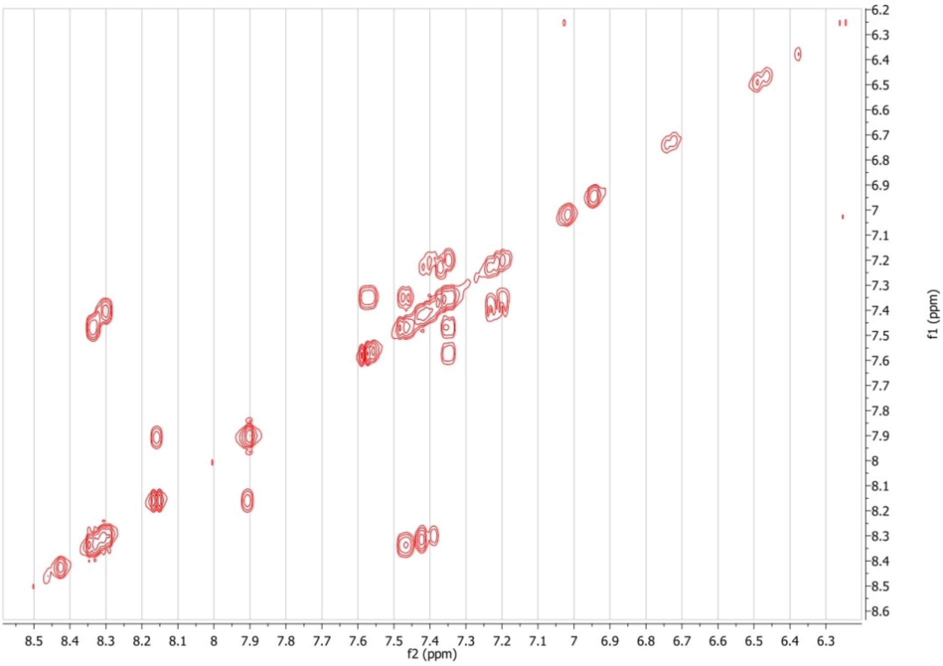

Figure S10. 2D-COSY of SOSG in methanol-d_4.

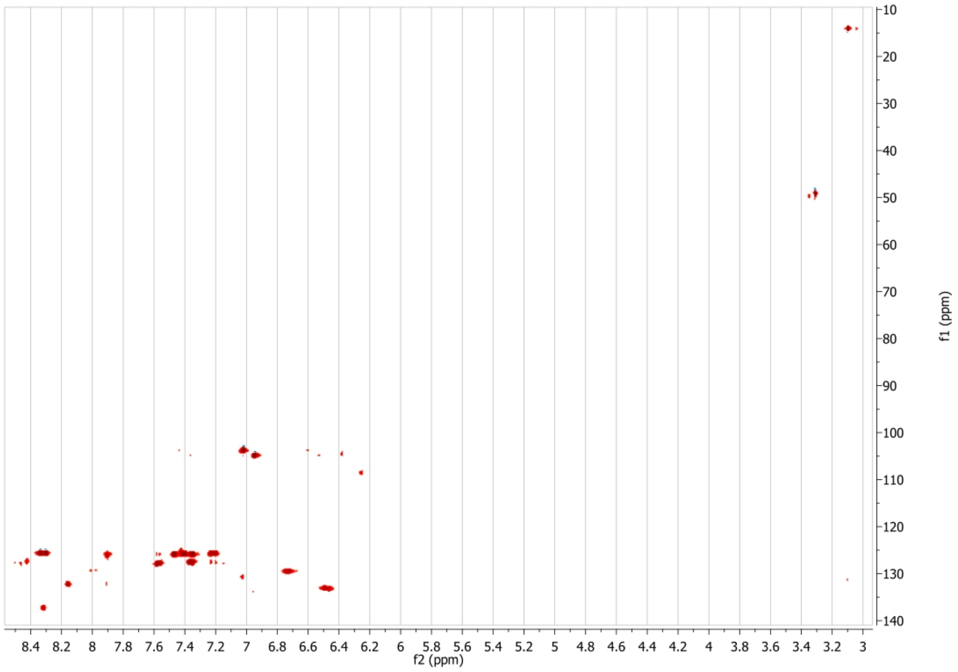

Figure S11. ^{1}H-^{13}C HSQC of SOSG in methanol-d_4.