Electronic Supplementary Information

Oxidation of Tris-(*p*-carboxyltetrathiaaryl)methyl Radical EPR Probes: Evidence for their Oxidative Decarboxylation and Molecular Origin of their Specific Ability to React with $O_2^{\bullet-}$

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I- General procedures

a- Chemicals

Hydrogen peroxide, xanthine (X), xanthine oxidase (XO), superoxide dismutase (SOD), ethylenediaminetetraacetate (EDTA), oxone ($KHSO_5$), sodium periodate ($NaIO_4$), *tert*-butylhydroperoxide (*t*BuOOH), and 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) were purchased from Sigma.

b- NMR experiments

All NMR experiments (^1H, ^{13}C, HMBC and HSQC) were carried out at room temperature on a Bruker Biospin Advance II 500 MHz spectrometer with a multinuclear probe (5mm ^1H/BB/Reverse Gradient on Z axis). Chemical shifts are reported in ppm (δ) relative to TMS.

c- UV-Vis experiments

UV-Vis spectra were recorded at 37°C in 1-cm pathlength quartz cuvettes (200 µL final volume) by repetitive scanning betwen 380 and 880 nm on a UVIKON 942 (Kontron Biotech) spectrophotometer.

d- HPLC analyses

Separation of TAM **1b** and QM **2b** was performed at room temperature on a 150 mm × 3.9 mm Novapak C_{18} column using a Spectra Physics HPLC system. The mobile phase comprised mixtures of solvent A (water + 0.1% formic acid) and solvent B (acetonitrile + 0.1% formic acid) as follows: 0 - 2 min: isocratic elution with 95% A; 2 - 32 min: linear increase from 5 to 95% B; 32 - 35 min: isocratic elution with 95% B; 35 - 38 min: linear decrease from 95 to 5% B; 38 – 45 min: reequilibration at 5% B. The flow rate was 1 mL/min. The absorbance was monitored at 270 nm and recorded using the Borwin data acquisition system. Under these conditions, the retention times for **1b** and **2b** were 20.1 and 22.8 min respectively.

Products were further identified by LC-MS using a Surveyor ThermoQuest system coupled to a LCQ Advantage mass spectrometer fitted with an Electrospray Ionization (ESI) source. Separations were performed as described above. MS detection was done using the negative and positive modes and scanning in full scan mode (*m/z* from 200 to 2000). Data were recorded and analyzed with the XCalibur acquisition system.

d- IR analysis

IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer.

II- Preparation of quinone-methide 2b

a- Experimenal procedure

QM **2b** was obtained by reaction of TAM **1b** with superoxide radical generated by the xanthine (X)-xanthine oxidase (XO) system. TAM **1b** (10 mg, 9.4 µmol) was dissolved in 100 mL phosphate buffer (0.1 M, pH = 7.4), containing 0.1 mM EDTA, 1 mM X and 0.04 U/mL XO. The solution was kept at 37°C for 90 min under stirring with a slow bubbling of dioxygen. The organic products were extracted with a diethyl ether / acetonitrile mixture (1/1, v/v; 3 × 100 mL) and solvents were evaporated under vacuum. The crude product was purified by RP-flash chromatography over a pre-packed C18 column (AIT, France) using a gradient from 5/95 to 20/80 of acetonitrile / water mixture to afford 9.0 mg of pure compound as a purple solid.

b- UV-Vis monitoring of the oxidation of **1b** into **2b** by superoxide anion generated by the X-XO system

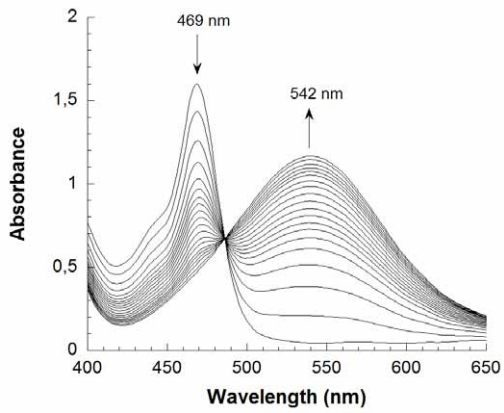

Fig. S1 Monitoring of TAM **1b** oxidation into QM **2b** by superoxide anion generated from the X-XO system (spectra recorded every 4 min, from 0 to 76 min).

c- QM **2b** characterization

- HPLC profile

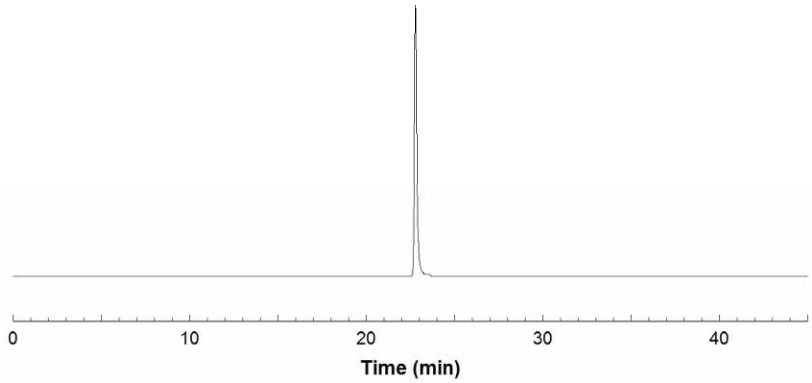

Fig. S2 HPLC profile of QM **2b** after purification by RP-flash chromatography.

- ESI-MS

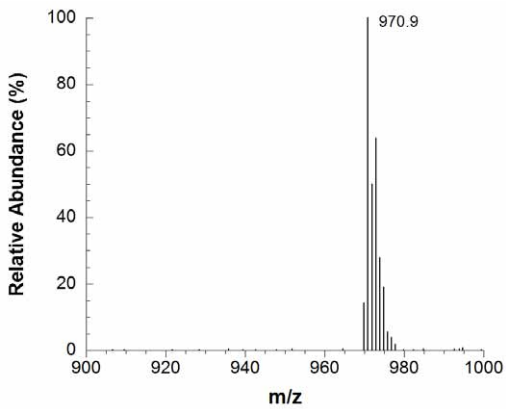

Fig. S3 ESI mass spectrum (positive mode) of QM **2b** (molecular ion corresponding to [**2b** + 3H]$^+$).

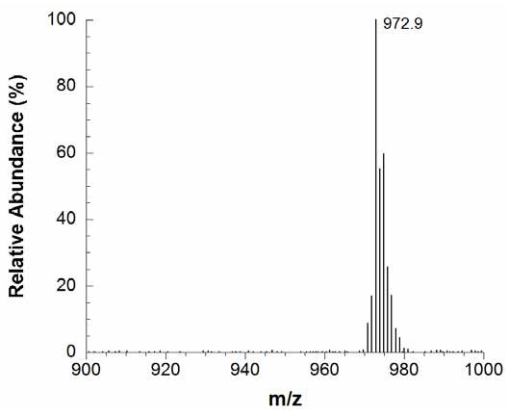

Fig. S4 ESI mass spectrum (positive mode) of the ^{18}O labeled product of **2b** obtained from oxidation of **1b** into **2b** with the X-XO system under an $^{18}O_2$ atmosphere (molecular ion corresponding to [**2b** + 3H]$^+$).

- ^{1}H NMR

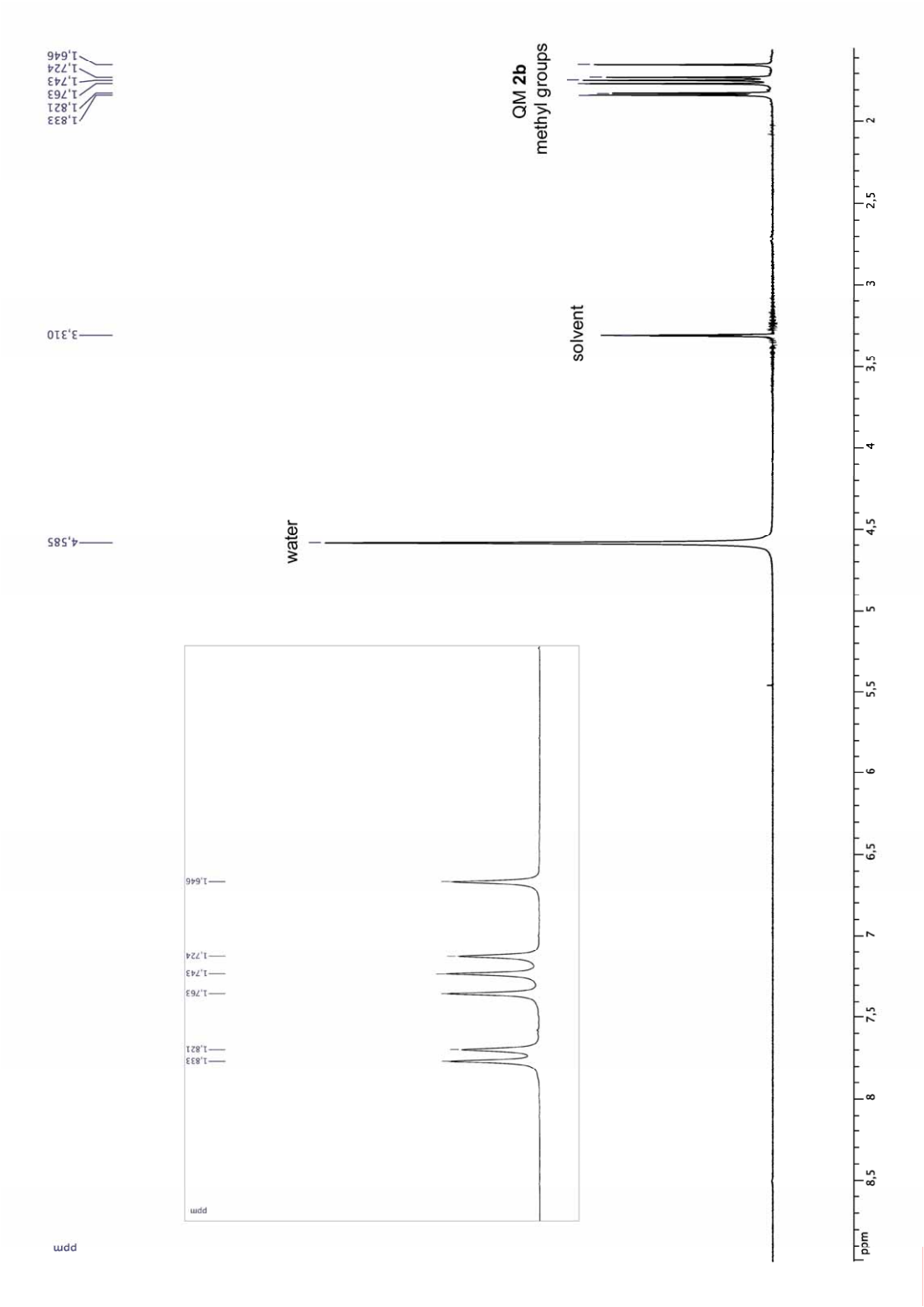

Fig. S5 ^{1}H NMR spectrum of QM **2b** in CD_3OD.

- ^{13}C NMR

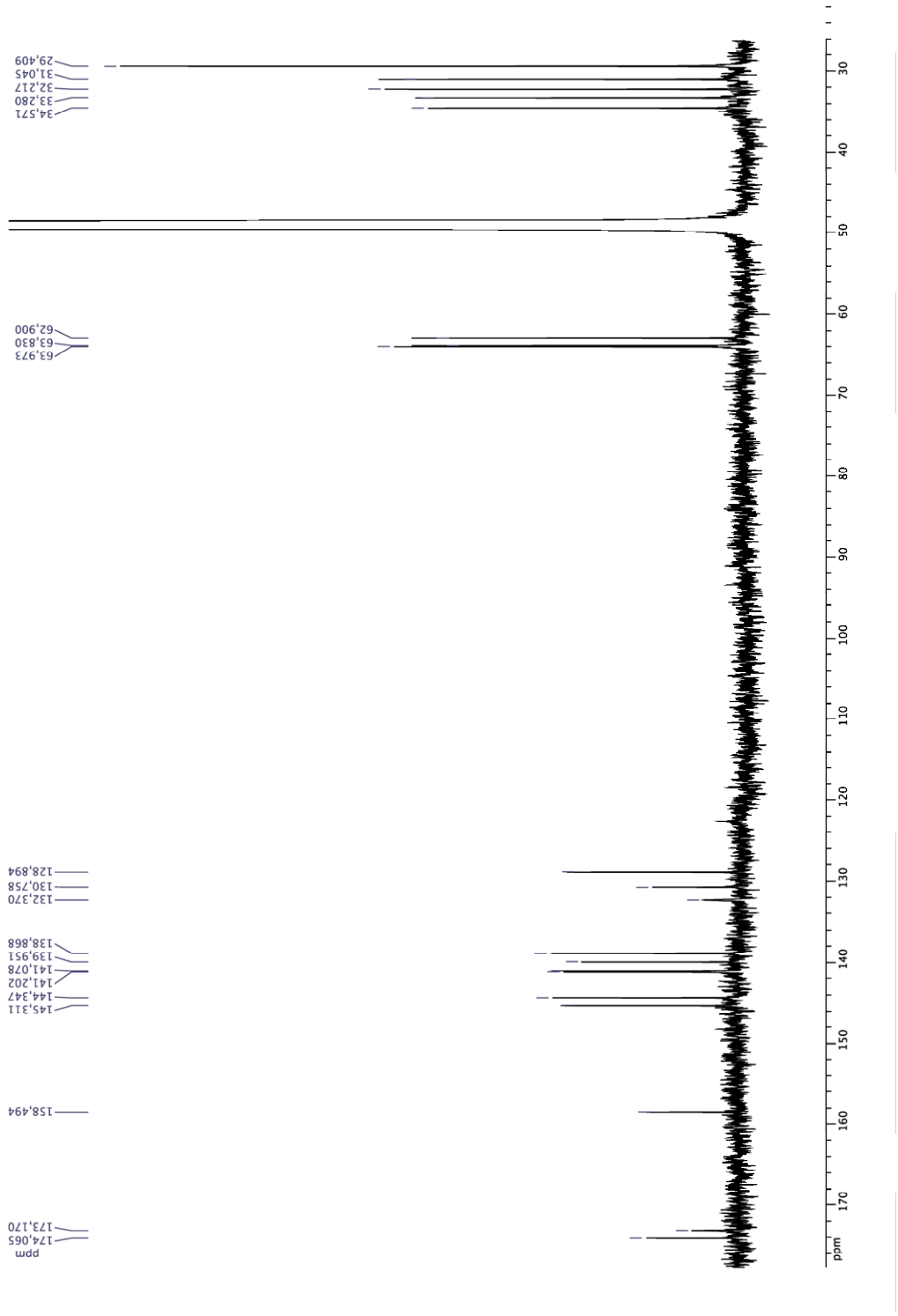

Fig. S6 ^{13}C NMR spectrum of QM **2b** in CD_3OD.

- IR

The IR spectra of **1b** and **2b** (under their COO^- form) both exhibited a broad band around 1585 cm^{-1} corresponding to the COO^- and CO functions, which did not permit to distinguish ν_{CO} for the quinone-type CO.

The acid form (COOH) of **2b** was obtained by treatment of **2b** with 1M HCl and extraction by ether. Its IR spectrum (neat) exhibited a band at 1607 cm^{-1} (in addition to the band at 1702 cm^{-1} corresponding to ν_{CO} of the COOH functions) that did not appear in the IR spectrum of **1b** (acid form) recorded under identical conditions. This band is expected for ν_{CO} of a quinone-type structure.

III- Oxidation product from reaction of 1b with $KHSO_5$ or $NaIO_4$

a- Experimenal procedure

To a solution of 100 µM TAM **1b** in phosphate buffer (0.1 M, pH = 7.4) containing 0.1 mM EDTA, was added 2 eq of $KHSO_5$ or $NaIO_4$. The solution was kept 1 hour at room temperature and then analyzed by HPLC-MS. This analysis showed the appearance of a new product (retention time: 18.0 min) that is presumably the S-oxidized product of **1b** (see ESI-MS below).

b- ESI-MS

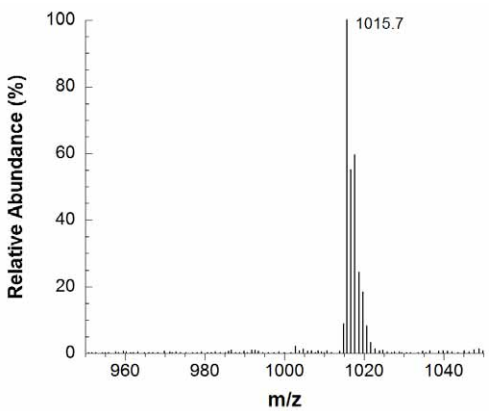

Fig. S7 ESI mass spectrum (positive mode) of the oxidation product of **1b** with $KHSO_5$ or $NaIO_4$ (molecular ion corresponding to M+16, M being the molecular ion of **1b**).

IV- Quinone-methide 2a

a- Experimenal procedure

QM **2a** has been generated from the X-XO system using the same experimental conditions as for QM **2b** (100 µM Oxo63 **1a**, 1 mM X, 0.04 U/mL XO, phosphate buffer (0.1 M, pH = 7.4) containing 0.1

mM EDTA). **2a** has also been prepared from reaction with alkylperoxyl radicals as follows: to a solution of 100 µM Oxo63 **1a** in phosphate buffer (0.1 M, pH = 7.4) containing 0.1 mM EDTA was added AAPH (final concentration 10 mM). The solution was kept under strirring at 37°C for 2 hours. HPLC-MS analysis of the reaction mixture gave the mass spectrum of QM **2a** (see below).

b- ESI-MS

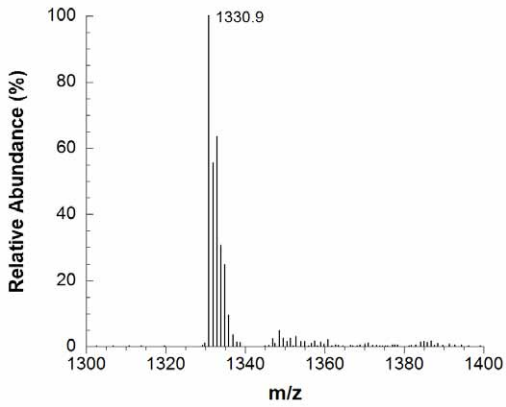

Fig. S8 ESI mass spectrum (positive mode) of **2a** (molecular ion corresponding to [**2a** + 3H]$^+$).