# EPR imaging spin-probe trityl radical OX063: A method for its isolation from animal effluent, redox chemistry of its quinone-methide oxidation product, and *in vivo* application in a mouse

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## SUPPORTING INFORMATION



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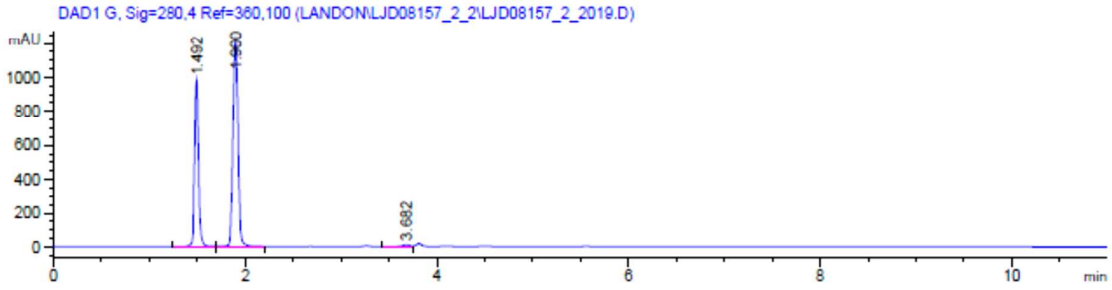
**Figure S1**

RP-HPLC of recycled green fraction **1 (OX063)**.

```
==========================================================================
Acq. Operator   : Maciej                          Seq. Line :  19
Acq. Instrument : Agilent 1290 LC System          Location : P1-D-04
Injection Date  : 6/25/2015 9:35:20 PM                Inj :   1
                                                  Inj Volume : 6.000 µl
Acq. Method     : C:\CHEM32\1\DATA\LANDON\LJD08157_2_2\CZ_GEN1.M
Last changed    : 1/20/2015 1:45:29 PM by Hao
Analysis Method : C:\CHEM32\1\METHODS\JTP_PHILLIPS_LONG.M
Last changed    : 9/17/2015 7:00:22 PM by Mary
                  (modified after loading)
Additional Info : Peak(s) manually integrated
```

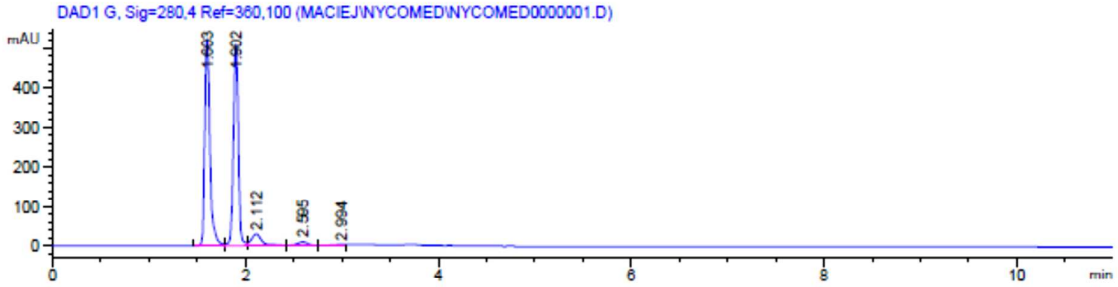



| Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area % |
|---|---|---|---|---|---|---|
| 1 | 1.492 | BB | 0.0459 | 2855.77637 | 982.92749 | 38.5459 |
| 2 | 1.900 | BV | 0.0573 | 4493.37549 | 1209.73108 | 60.6494 |
| 3 | 3.682 | BV | 0.0779 | 59.61863 | 11.60698 | 0.8047 |

**Figure S2**

RP-HPLC of Nycomed's OX063.

```
================================================================
Acq. Operator   : Maciej                        Seq. Line :   1
Acq. Instrument : Agilent 1290 LC System         Location : Vial 1
Injection Date  : 9/15/2015 10:16:37 AM               Inj :   1
                                                Inj Volume : 6.000 µl
Acq. Method     : C:\CHEM32\1\DATA\MACIEJ\NYCOMED\CZ_GEN1.M
Last changed    : 1/20/2015 1:45:29 PM by Hao
Analysis Method : C:\CHEM32\1\METHODS\JTP_PHILLIPS_LONG.M
Last changed    : 9/16/2015 2:18:28 PM by Mary
                  (modified after loading)
Additional Info : Peak(s) manually integrated
```




Signal 4: DAD1 G, Sig=280,4 Ref=360,100

| Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area % |
|---|---|---|---|---|---|---|
| 1 | 1.603 | BV | 0.0603 | 1988.36328 | 523.94324 | 48.5847 |
| 2 | 1.902 | VV | 0.0553 | 1805.85046 | 510.40826 | 44.1250 |
| 3 | 2.112 | VB | 0.1061 | 216.31737 | 30.02617 | 5.2856 |
| 4 | 2.595 | BB | 0.1055 | 62.28352 | 9.14184 | 1.5219 |
| 5 | 2.994 | BV | 0.0999 | 19.76006 | 2.68227 | 0.4828 |

**Figure S3**

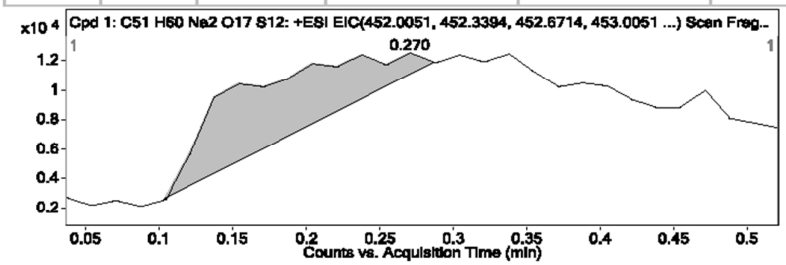
HRMS of quinone methide derived from parent OX063.

| | | | |
|---|---|---|---|
| **Data File** | -140515-QM12.d | **Sample Name** | QM-NONDEUTERATED |
| **Sample Type** | Sample | **Position** | P1-A02 |
| **Instrument Name** | Instrument 1 | **User Name** | Maciej Serda |
| **Acq Method** | FIA_P_M-ES_50-3200_200V.M | **Acquired Time** | 5/14/2015 1:32:35 PM |
| **IRM Calibration Status** | Success | **DA Method** | DA_TARGET_FIA_POSTIVE-REF_FIND BY FORMULA.M |
| **Comment** | purple metabolite\|Easy-Access Method: 'P_ES_50-3200_200V'\|C51H60Na2O17S12 | | |

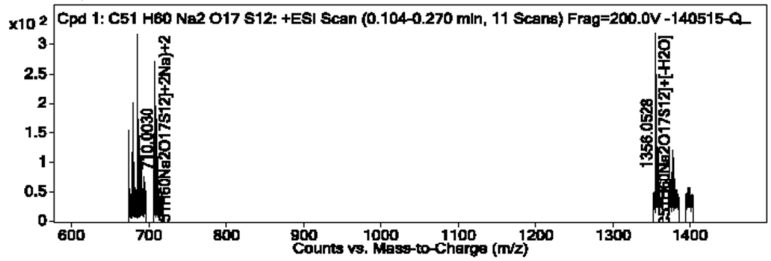
**Compound Table**

| Label | Tgt Score | Mass Error (ppm) | MFG Formula | Tgt Formula | Obs. RT | Ref. Mass | Obs. Mass |
|---|---|---|---|---|---|---|---|
| Cpd 1: C51 H60 Na2 O17 S12 | 86.59 | 9.42 | C51 H60 Na2 O17 S12 | C51 H60 Na2 O17 S12 | 0.27 | 1374.0274 | 1374.0404 |

| Obs. m/z | Obs. RT | Obs. Mass | DB Formula | Tgt Formula | Tgt Mass | Tgt Mass Error | Find Cpds Algorith |
|---|---|---|---|---|---|---|---|
| 710.003 | 0.27 | 1374.0404 | C51 H60 Na2 O17 S12 | C51 H60 Na2 O17 S12 | 1374.0274 | 9.42 | Find By Formula |




MS Zoomed Spectrum




MS Spectrum Peak List

| Obs. m/z | Charge | Abund | Formula | Ion/Isotope | Tgt Mass Error (ppm) |
|---|---|---|---|---|---|
| 678.0293 | 2 | 97.68 | C51H60Na2O17S12 | M+2[-H2O] | -31.55 |
| 679.0278 | 2 | 63.41 | C51H60Na2O17S12 | (M+2H)+2[-H2O] | -17.86 |
| 687.0054 | 2 | 54.52 | C51H60Na2O17S12 | M+2 | 11.26 |
| 688.0203 | 2 | 109.8 | C51H60Na2O17S12 | (M+2H)+2 | 0.99 |
| 710.003 | 2 | 147.56 | C51H60Na2O17S12 | (M+2Na)+2 | -0.13 |
| 1356.0528 | 1 | 131.51 | C51H60Na2O17S12 | M+[-H2O] | -26.93 |
| 1357.0624 | 1 | 116.4 | C51H60Na2O17S12 | M+[-H2O] | -31.86 |
| 1375.0344 | 1 | 124.8 | C51H60Na2O17S12 | (M+H)+ | 0.23 |
| 1377.0359 | 1 | 113.35 | C51H60Na2O17S12 | (M+H)+ | -1.68 |
| 1397.0526 | 1 | 58.31 | C51H60Na2O17S12 | (M+Na)+ | -25.75 |

-- End Of Report ---

## Figure S4

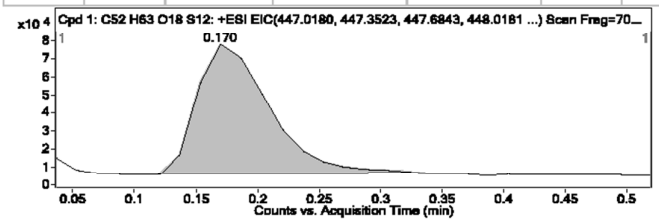
HRMS of recycled **1 (OX063)**.

| | | | |
|---|---|---|---|
| **Data File** | -300615-MS5-OXO631.d | **Sample Name** | MS5-oxo63 |
| **Sample Type** | Sample | **Position** | P1-A06 |
| **Instrument Name** | Instrument 1 | **User Name** | Julius Reyes |
| **Acq Method** | FIA_P_M-ES_50-3200_70V.M | **Acquired Time** | 6/30/2015 3:07:09 PM |
| **IRM Calibration Status** | Success | **DA Method** | DA_TARGET_FIA_POSTIVE-REF_FIND BY FORMULA.M |
| **Comment** | MS5-oxo63\|Easy-Access Method: 'P_ES_50-3200_70V'\|C52H63O18S12, C52H60Na3O18S12 | | |

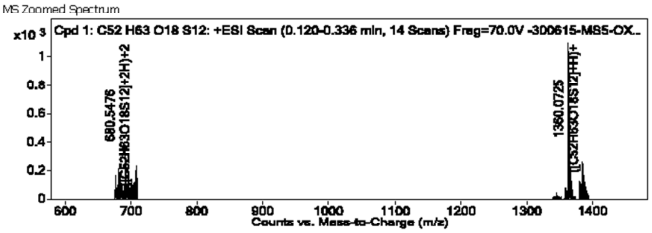
Compound Table

| Label | Tgt Score | Mass Error (ppm) | MFG Formula | Tgt Formula | Obs. RT | Ref. Mass | Obs. Mass |
|---|---|---|---|---|---|---|---|
| Cpd 1: C52 H63 O18 S12 | 94 | 1.04 | C52 H63 O18 S12 | C52 H63 O18 S12 | 0.17 | 1359.0663 | 1359.0677 |

| Obs. m/z | Obs. RT | Obs. Mass | DB Formula | Tgt Formula | Tgt Mass | Tgt Mass Error | Find Cpds Algorith |
|---|---|---|---|---|---|---|---|
| 1360.0725 | 0.17 | 1359.0677 | C52 H63 O18 S12 | C52 H63 O18 S12 | 1359.0663 | 1.04 | Find By Formula |




MS Zoomed Spectrum




MS Spectrum Peak List

| Obs. m/z | Charge | Abund | Formula | Ion/Isotope | Tgt Mass Error (ppm) |
|---|---|---|---|---|---|
| 680.5476 | 2 | 221.93 | C52H63O18S12 | (M+2H)+2 | -10.61 |
| 693.5206 | 2 | 104.06 | C52H63O18S12 | (M+2Na)+2[-H2O] | -5.09 |
| 702.5195 | 2 | 108.8 | C52H63O18S12 | (M+2Na)+2 | 4.01 |
| 1342.0623 | 1 | 52.24 | C52H63O18S12 | (M+H)+[-H2O] | 0.56 |
| 1360.0725 | 1 | 1102.19 | C52H63O18S12 | (M+H)+ | 0.75 |
| 1361.0765 | 1 | 1009.26 | C52H63O18S12 | (M+H)+ | -0.09 |
| 1362.073 | 1 | 1039.12 | C52H63O18S12 | (M+H)+ | -0.39 |
| 1363.0754 | 1 | 654.12 | C52H63O18S12 | (M+H)+ | -1.17 |
| 1364.0719 | 1 | 435.88 | C52H63O18S12 | (M+H)+ | -0.76 |
| 1382.0549 | 1 | 267.08 | C52H63O18S12 | (M+Na)+ | 0.41 |

-- End Of Report --

**Figure S5**

$^{13}$C-NMR spectrum of compound **2**.

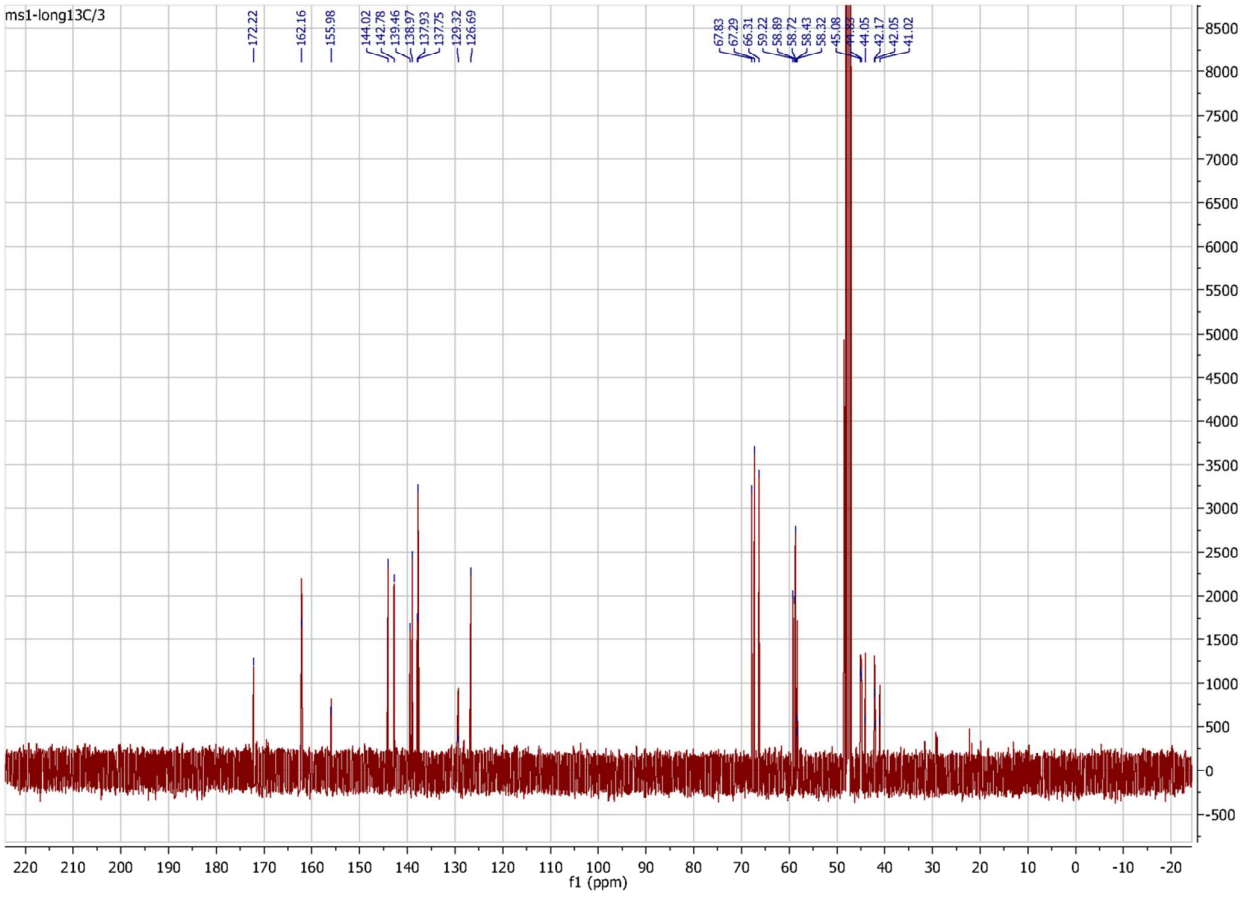


**Figure S6**

Comparison of UV-VIS spectra of recycled OX063 (blue) and QM (red) after column chromatography; (c= 0.025mol/L).

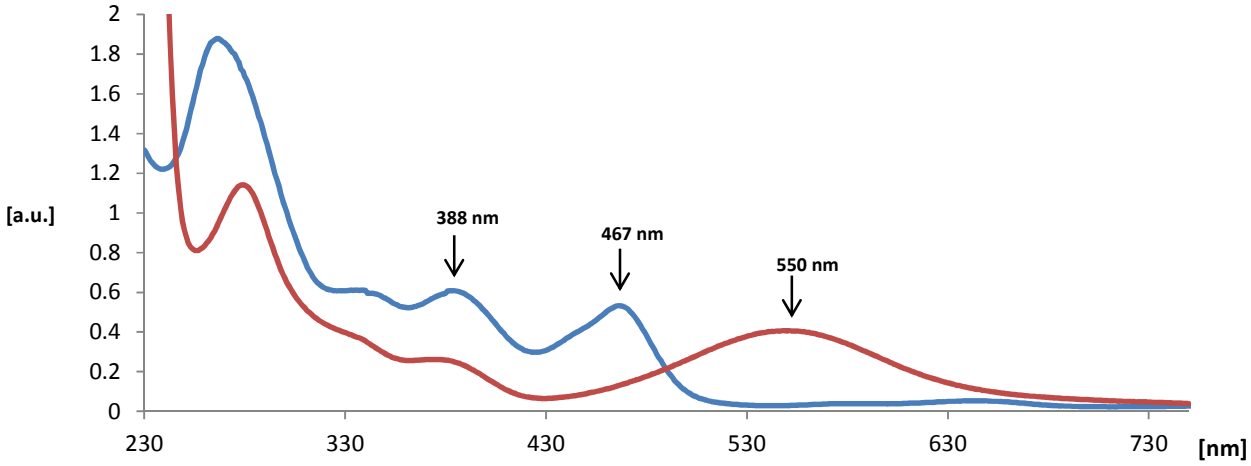
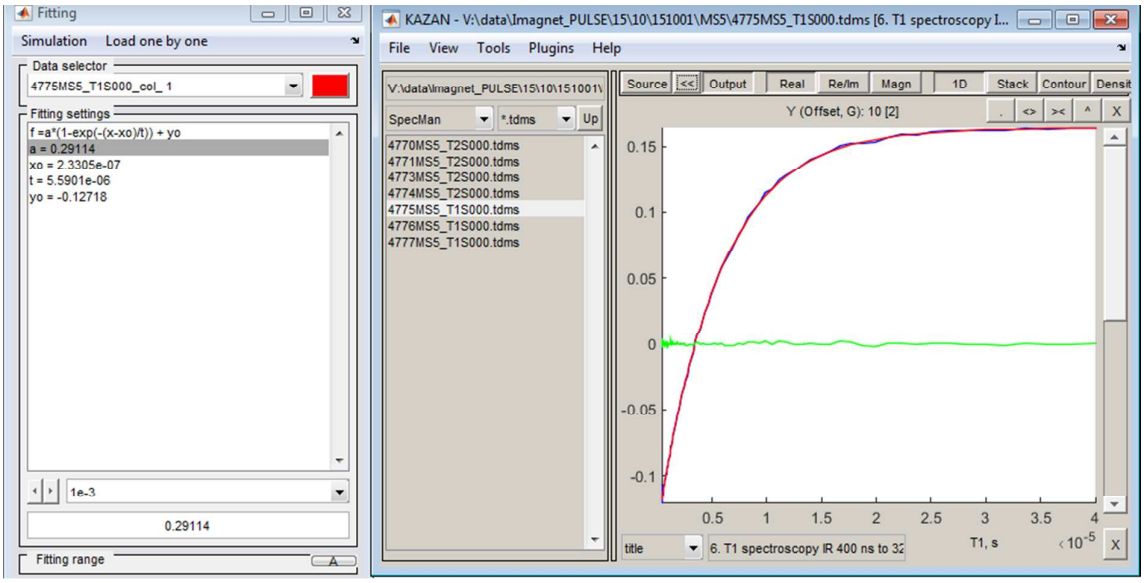


**Figure S7**

Measurements of T1 relaxation time for compound **1** (recycled OX063).




**T1 Relaxation**

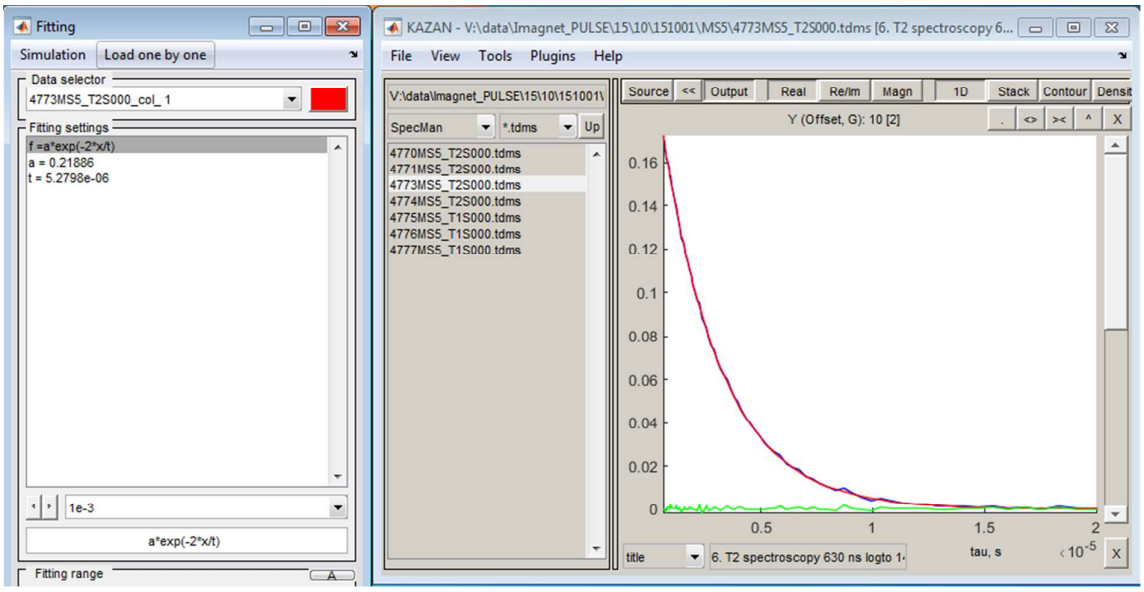
T1 (seconds) = 5.59e-06, 5.60e-06, 5.61e-06

T1 = 5.60 µs +/- 0.01µs (standard deviation of sample, sigma (n-1)), sample size 3.

Representative T1 inversion recovery spectrum fitted to noise level with exponential approach to asymptote from below, 'f=a*(1 – exp(-(x-xo)/t)) + yo'

**Figure S8**

Measurements of T2 relaxation time for compound **1** (recycled OX063).




**T2 Relaxation**

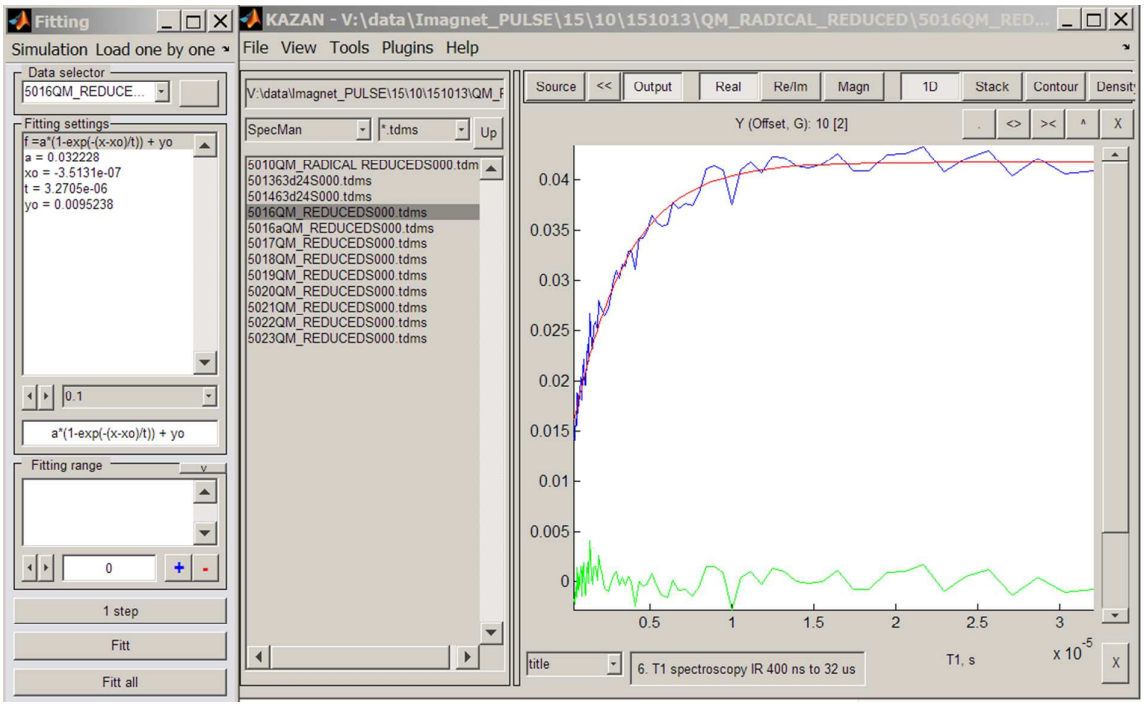
T2 (seconds) = 5.28e-06, 5.29e-06, 5.30e-06

T2 = 5.29 µs +/- 0.01µs (standard deviation of sample, sigma (n-1)), sample size 3.

Representative T2 relaxation spectrum fitted to level noise level with simple exponential decay, 'f = a*exp(-2x/t)'

**Figure S9**

Measurements of T1 relaxation time for compound **3** (air-sensitive radical).




## T1 Relaxation

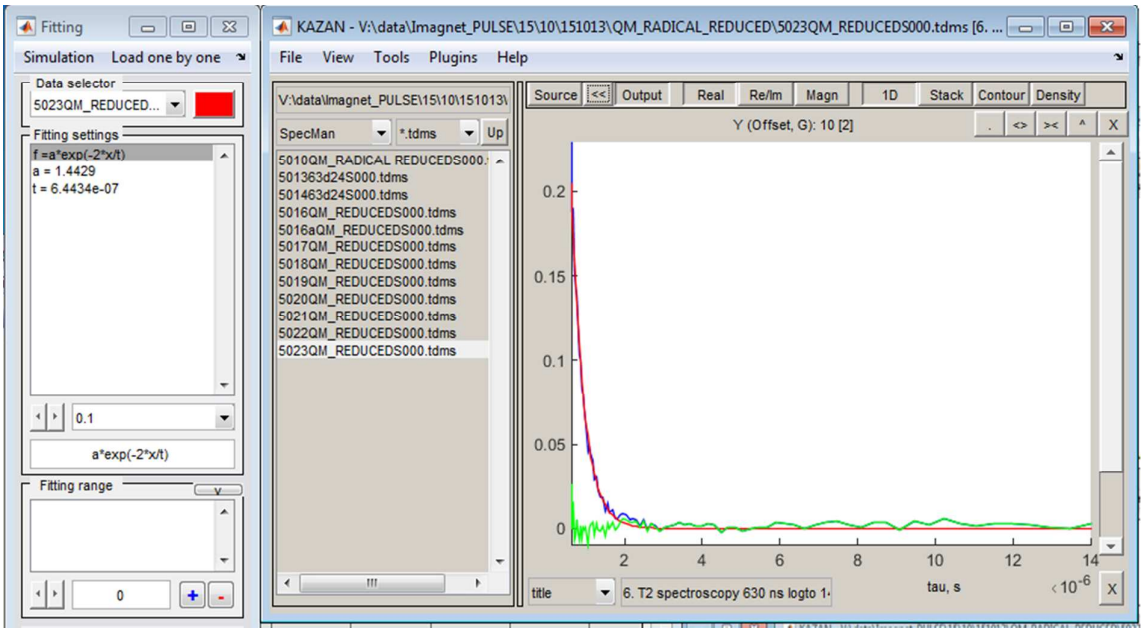
T1 (seconds) = 4.03 e-06; 3.26 e-06; 4.05e-06

T1 = 3.78µs +/- 0.45µs (standard deviation of sample, sigma (n-1)), sample size is 3.

Representative T1 inversion recovery spectrum fitted to noise level with exponential approach to asymptote from below, 'f=a*(1 – exp(-(x-xo)/t)) + yo'

**Figure S10**

Measurements of T2 relaxation time for compound **3** (air-sensitive radical).




**T2 Relaxation**

T2 (seconds) = 6.44e-07s, 6.68e-07s, 6.47e-07s

T2 = 0.653 µs +/- 0.013µs (standard deviation of sample (sigma (n-1), sample size 3).

Representative T2 relaxation spectrum fitted to level noise level with simple exponential decay, 'f = a*exp(-2x/t)'

**Figure S11**

UV-VIS spectrum of air-sensitive radical **3,** formed by treatment quinone methide by $NaBH_4$ (methanolic solution); (c= 0.025mol/L).

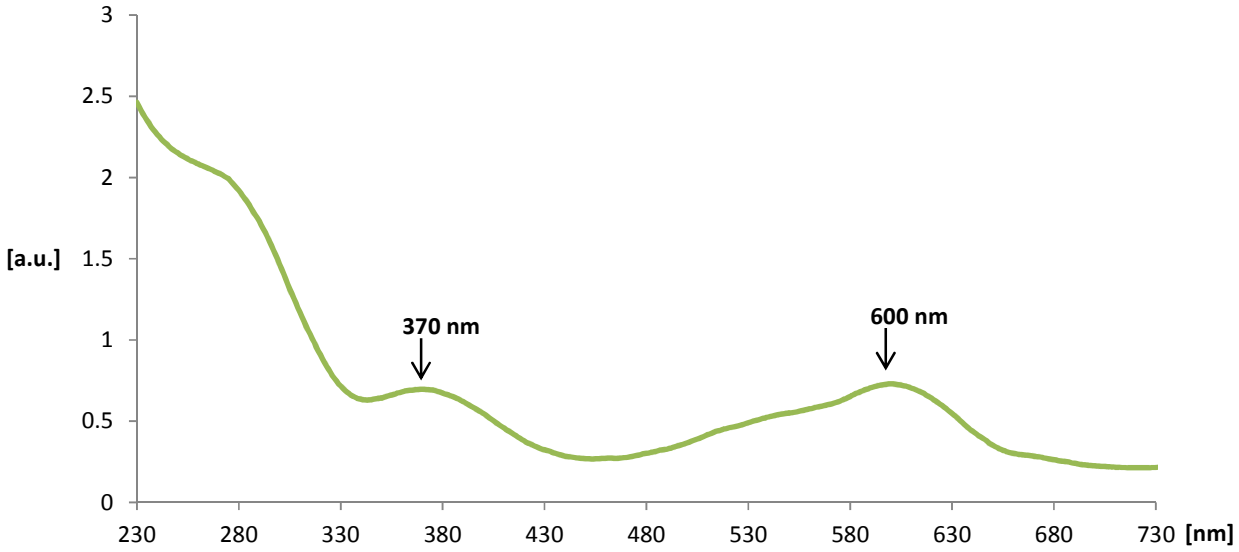


**Figure S12**

CW EPR spectrum of air sensitive radical **3** ($H_2O$).

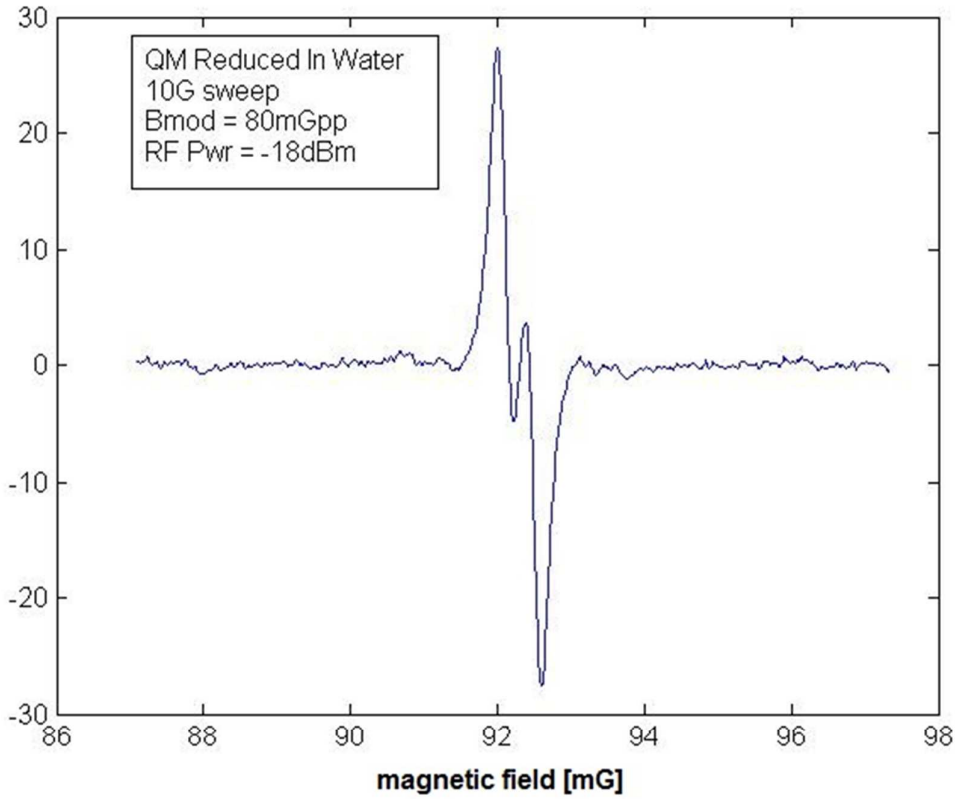


**Figure S13**

CW EPR spectrum of air sensitive radical **3** ($D_2O$).