Electronic Supplementary Information

Reaction of Ox063 polarizing agent with pyruvic acid under standard sample preparation protocol for dissolution dynamic nuclear polarization

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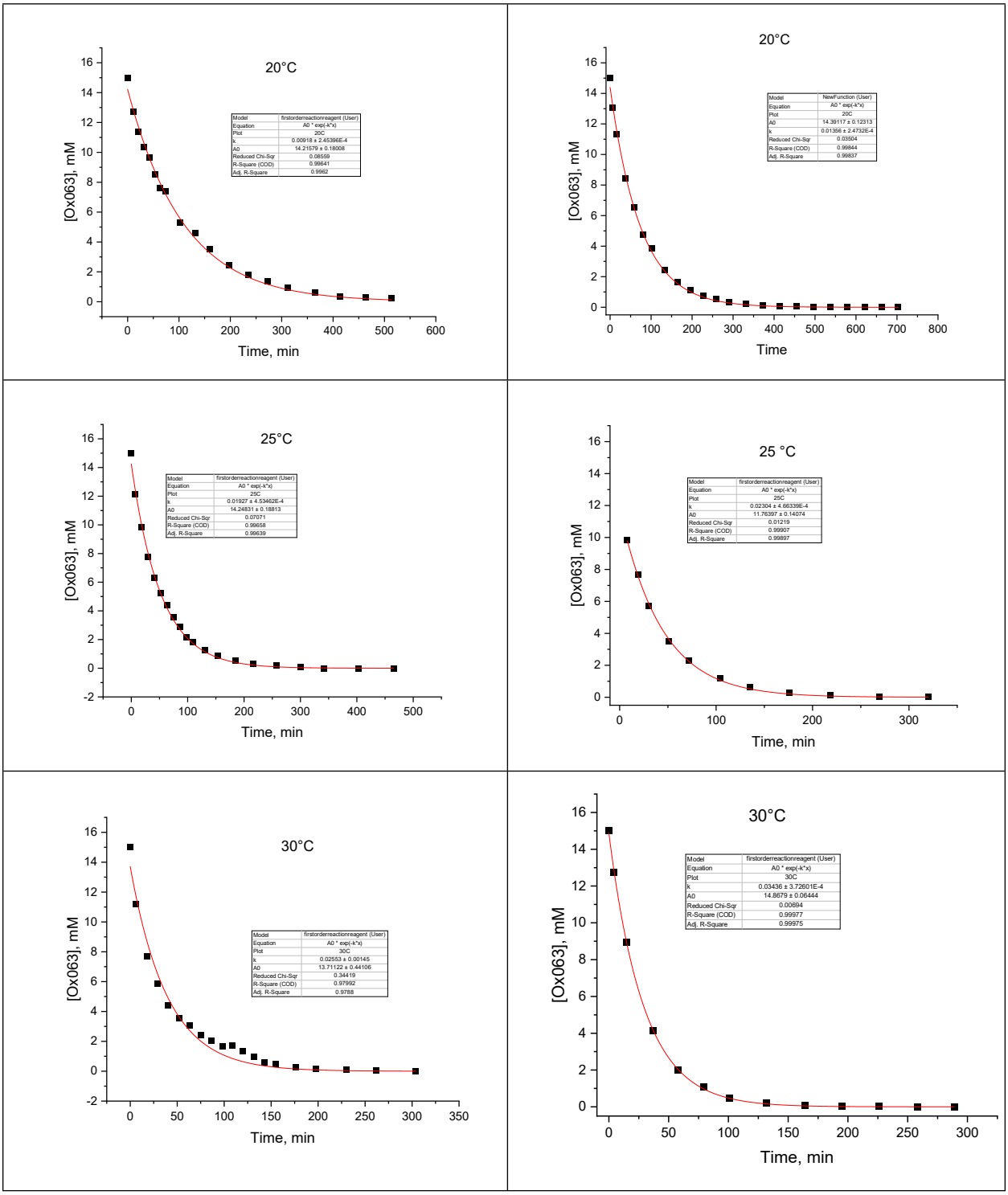
1. Materials and Methods

Ox063 sodium salt (Ox063Na, MW – 1426.7 g/mol) was synthesized as previously described. [1,2] HPLC-MS analyses were carried out using a Water Alliance e2695 separation module, a Water 2998 PDA detector, and a Water SQD2 mass detector; the ESI m/z range was set to 1300-2500. Separations were carried out using a Water XBridge BEH C18 4.6 mm x 50 mm, 2.5 µm. The following gradient conditions were used: Solvent A was acetonitrile, solvent B was water, solvent C was water containing 1 % of trifluoroacetic acid, column temperature: 45°C; UV detection from 210 to 800 nm, flow rate: 1.5 mL/min. Time 0 min: 5 % A, 85% B, 10 % C, time 3 min: 5 % A, 85 % B, 10 % C, time 8 min: 50 % A, 40% B, 10 % C.

For the kinetics with pyruvic acid, 8.6 mg of Ox063Na was placed inside a 500 µL HPLC vial. 400 µL of pyruvic acid (98%, Sigma Aldrich) pre-warmed at 20, 25, 30, or 35°C was added, and the vial was sonicated and vortexed until the solution became fully homogeneous (4-5 min). The vial was placed inside the HPLC sample manager pre-warmed at 20, 25, 30, or 35°C, and the sample was injected over time until >95% of Ox063 peak (RT=1.7 min) was converted. The concentration of Ox063 was calculated from the peak integration at 485 mm.

For the experiment with lactic acid. 8.6 mg of Ox063Na was placed inside a 500 µL HPLC vial. 40 µL of DI water and 500 mg of lactic acid pre-warmed at 30°C were added, and the vial was sonicated and vortexed until the solution became fully homogeneous (~4 min). The vial was placed inside the HPLC sample manager pre-warmed at 30°C, and the sample was injected immediately and after 4, 9, 15, 24, and 48h.

2. Kinetics of Ox063 with pyruvic acid



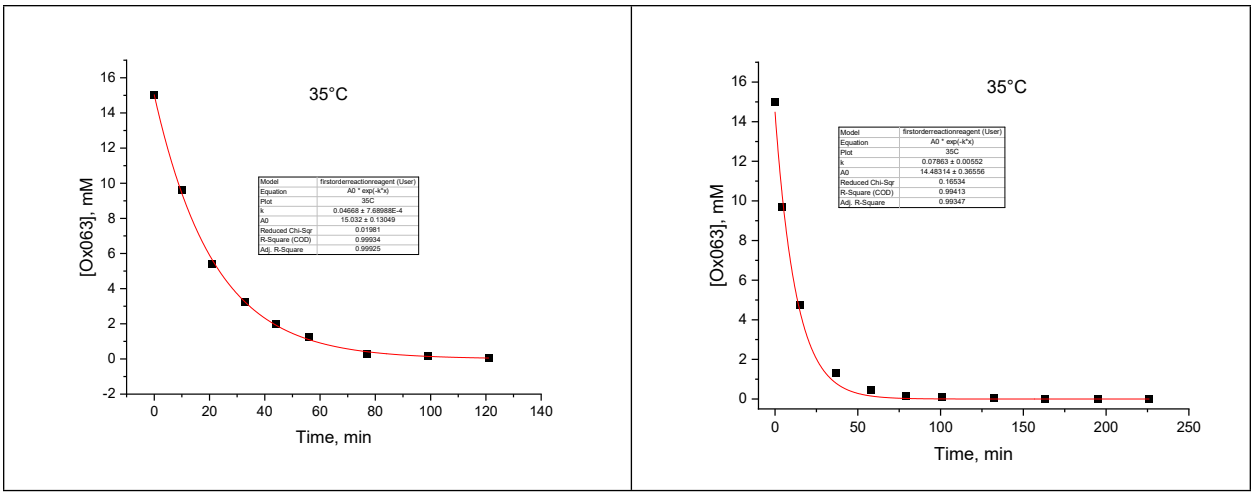


Figure S1. Reaction kinetics of Ox063 in neat pyruvic acid at 20, 25, 30, 35°C followed by HPLC.

Data points:

Ox063 + Pyruvic Acid 20°C

Reaction time (Min)	[Ox063] (mM)
0	15.0000
5	13.0905
16	11.3520
37	8.4345
59	6.5370
80	4.7610
101	3.8520
133	2.4450
164	1.6575
196	1.1055
227	0.7545
258	0.5190
290	0.3540
331	0.2235
372	0.1380
414	0.0660
455	0.0540
497	0.0240
538	0.0195
579	0.0150
621	0.0135
662	0.0000
703	0.0000

Ox063 + Pyruvic Acid 25°C

Reaction time (Min)	[Ox063] (mM)
0	15.0000
5	13.6515
16	11.1195
38	6.6735
59	4.8825
80	3.5700
102	2.4885
133	1.2810
164	0.7545
196	0.4770
227	0.2625
258	0.1695
290	0.1035
331	0.0615
372	0.0390
414	0.0210
455	0.0165
496	0.0090
538	0.0060
579	0.0000

Ox063 + Pyruvic Acid 30°C

Reaction time (Min)	[Ox063] (mM)
0	15.0000
4	12.7590
15	8.9580
37	4.1565
58	1.9830
79	1.0635
101	0.4605
132	0.2040
164	0.0930
195	0.0465
226	0.0195
258	0.0150
289	0.0030
330	0.0000

Ox063 + Pyruvic Acid 35°C

Reaction time (Min)	[Ox063] (mM)
0	15.0000
4	9.7050
15	4.7355
37	1.2990
58	0.4635
79	0.1755
101	0.0780
132	0.0300
163	0.0075
195	0.0045
226	0.0000

Ox063 + Pyruvic Acid 20°C

Reaction time (Min)	[Ox063] (mM)
0	15.0000
11	12.7110
21	11.3655
32	10.3320
42	9.6405
53	8.5065
63	7.5885
74	7.3845
102	5.2785
131	4.5990
160	3.5325
198	2.4345
235	1.8300
273	1.3485
311	0.9240
365	0.6000
413	0.3675
463	0.2940
514	0.2160

Ox063 + Pyruvic Acid 25°C

Reaction time (Min)	[Ox063] (mM)
0	15.0000
6	12.1500
18	9.8550
29	7.7550
41	6.3000
52	5.2500
63	4.4250
75	3.5850
86	2.8950
98	2.1750
109	1.8000
131	1.2600
153	0.8550
185	0.5100
216	0.3300
258	0.1950
300	0.0750
342	0.0000

Ox063 + Pyruvic Acid 30°C

Reaction time (Min)	[Ox063] (mM)
0	15.0000
6	11.2065
18	7.6815
29	5.8485
40	4.4325
52	3.5310
63	3.0840
75	2.4150
86	2.0700
98	1.6665
109	1.7340
120	1.3455
132	0.9795
143	0.5850
155	0.4755
176	0.2760
198	0.1605
230	0.0885
262	0.0510
304	0.0255

Ox063 + Pyruvic Acid 35°C

Reaction time (Min)	[Ox063] (mM)
0	15.0000
10	9.5955
21	5.3850
33	3.2370
44	1.9815
56	1.2750
77	0.3150
99	0.1500
121	0.0735

Table S1. Summary of the kinetic parameters

T (K)	k (obs) min^{-1}	k (obs) s^{-1}	k ($M^{-1}s^{-1}$)	Ln k	1/T (K-1)
293	0.01356	2.26E-04	1.59E-05	-11.0475	0.00341
293	0.00918	1.53E-04	1.08E-05	-11.4376	0.00341
298	0.02300	3.83E-04	2.70E-05	-10.5191	0.00336
298	0.01927	3.21E-04	2.26E-05	-10.6961	0.00336
303	0.03436	5.73E-04	4.04E-05	-10.1177	0.0033
303	0.02553	4.26E-04	3.00E-05	-10.4148	0.0033
308	0.07863	0.00131	9.24E-05	-9.28988	0.00325
308	0.04668	7.78E-04	5.48E-05	-9.81132	0.00325

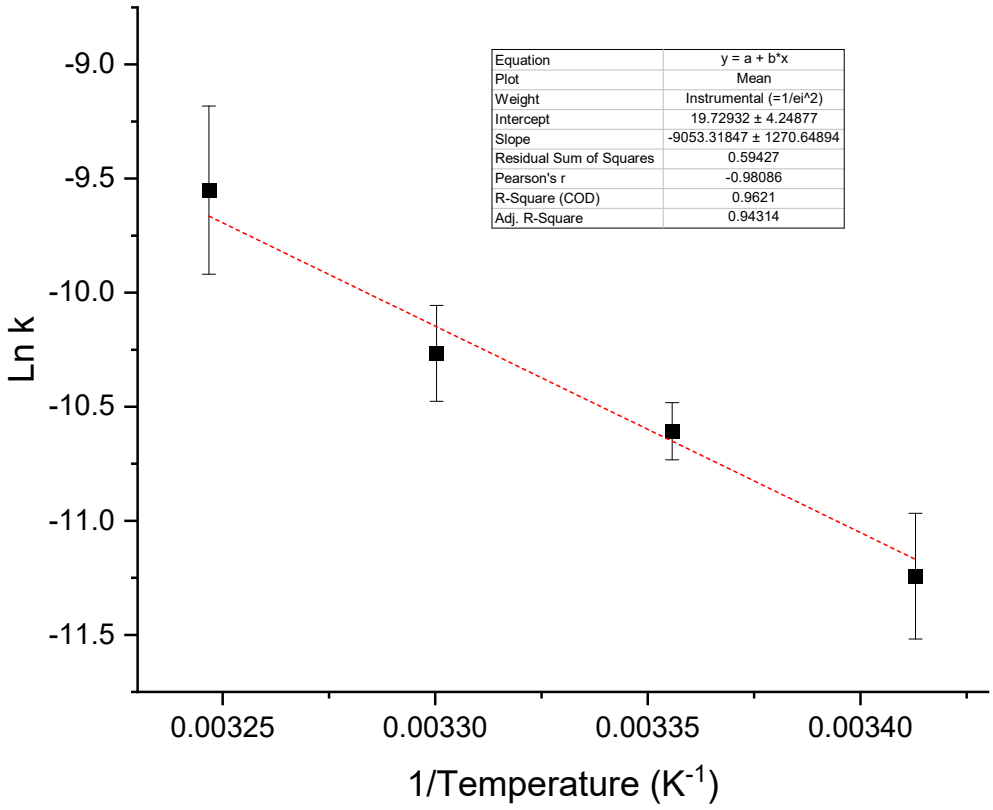


Figure S2. Arrhenius plot for the determination of the activation energy of the esterification of the first alcohol of Ox063 by pyruvic acid.

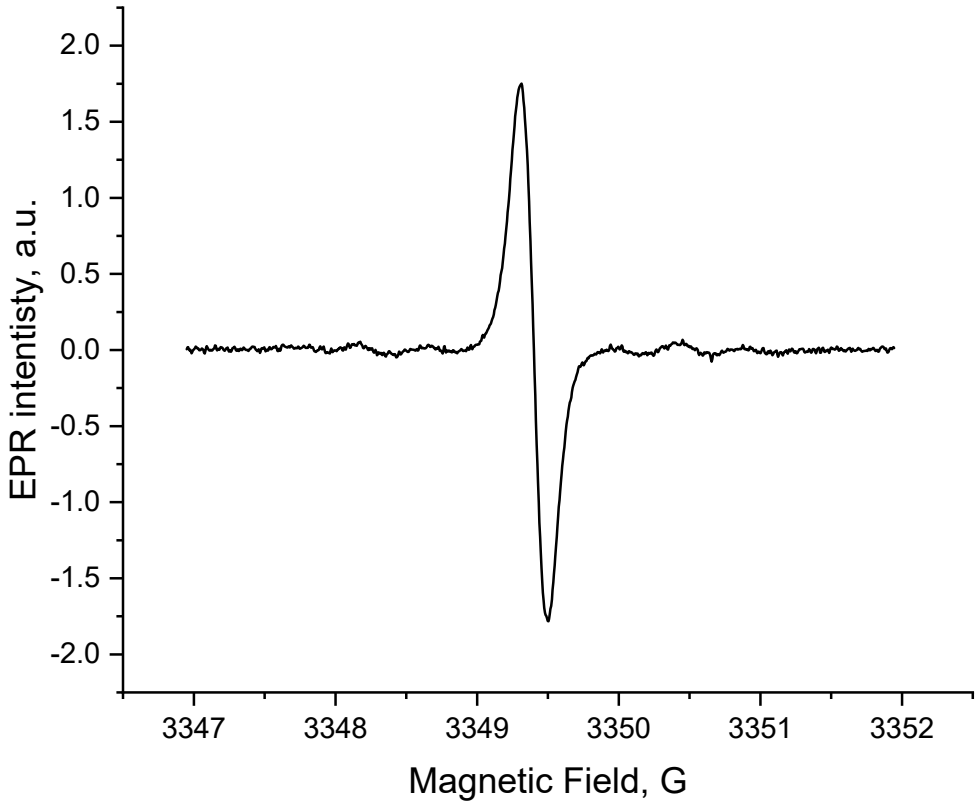


Figure S3. X-Band EPR spectrum of a mixture of 5% Ox063 and 95% Ox063-pyruvic ester mixture (30 µM in deoxygenated PBS, pH = 7).

3. Reaction of Ox063 with lactic acid

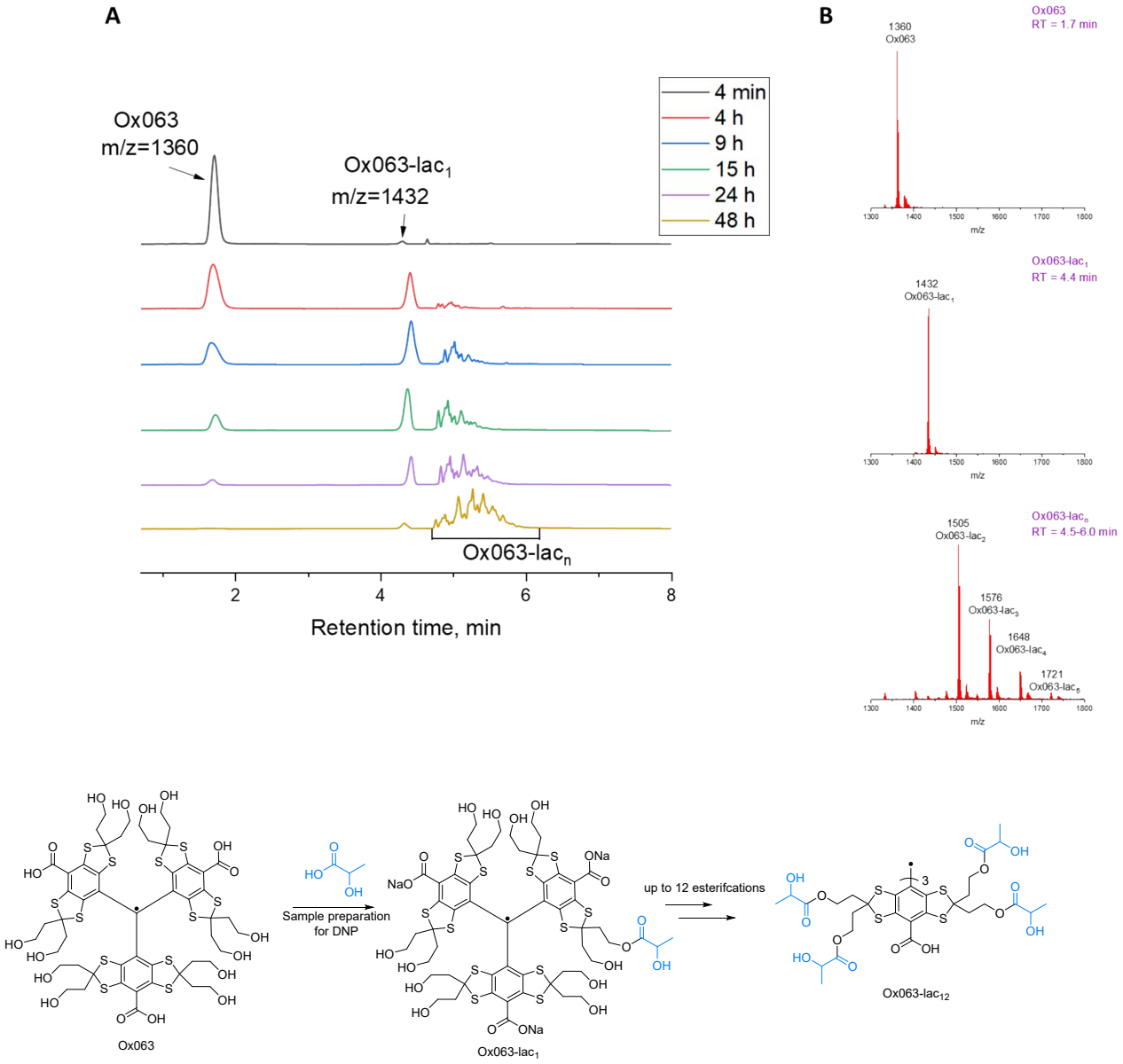
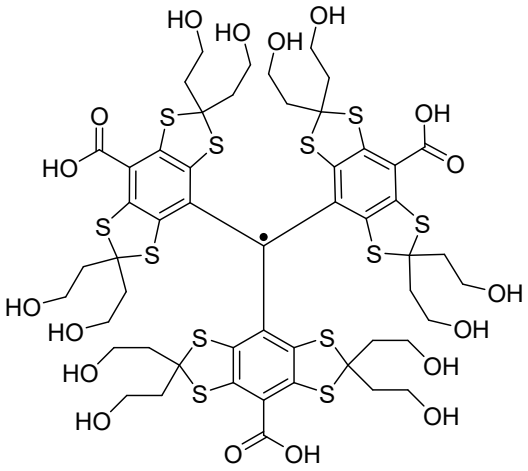
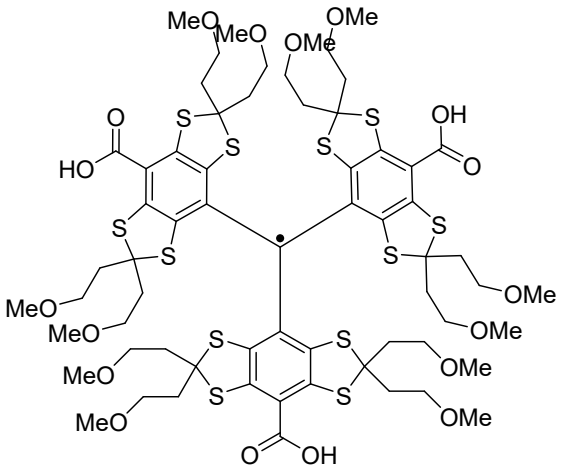


Figure S4. **A.** HPLC chromatograms at 485 nm of Ox063 (15 mM) dissolved in lactic acid at ~30°C after 4 min, 4h, 9h, 15h, 24h and 48h. **B.** MS spectra of the peak at 1.7 min (Ox063) and 4.4 min (Ox063-lac$_1$) and over a retention time window of 4.5-6 min of the reaction after 15h.



Ox063



Ox063Me or AH111501

Figure S5. Molecular structures of Ox063 and Ox063Me or AH111501

1 Poncelet, M., Huffman, J. L., Khramtsov, V. V., Dhimitruka, I. & Driesschaert, B. Synthesis of hydroxyethyl tetrathiatriarylmethyl radicals OX063 and OX071. *RSC Advances* **9**, 35073-35076 (2019). https://doi.org:10.1039/C9RA08633A

2 Poncelet, M. *et al.* Synthesis and characterization of a biocompatible 13C1 isotopologue of trityl radical OX071 for in vivo EPR viscometry. *Analyst* **147**, 5643-5648 (2022). https://doi.org:10.1039/D2AN01527G