Liponitroxides: EPR study and their efficacy as antioxidants in lipid membranes

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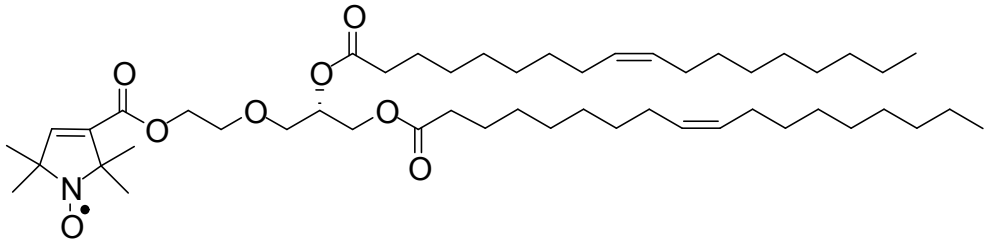
Spectroscopic characterization of liponitroxides

1,2-O-Dioleoyl-3-O-[ethyl (2',2',5',5'-tetramethyl-3'-pyrroline-1'-oxyl-3'-carboxylate)] s*n*-glycerol [DOG(L)-NO]



^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t, *J* = 6.4 Hz, 6H); 1.20-1.45 (m, 52H); 1.50-1.70 (m, 4H); 1.95-2.10 (m, 8H); 2.31 (dd, *J* = 6.8 Hz, *J* = 14.4 Hz, 4H); 3.55-3.80 (m, 4H); 4.10-4.25 (m, 1H); 4.30-4.40 (m, 1H); 4.50-4.70 (m, 2H); 5.18-5.30 (m, 1H); 5.30-5.42 (m, 4H); 6.58 (s, 1H).

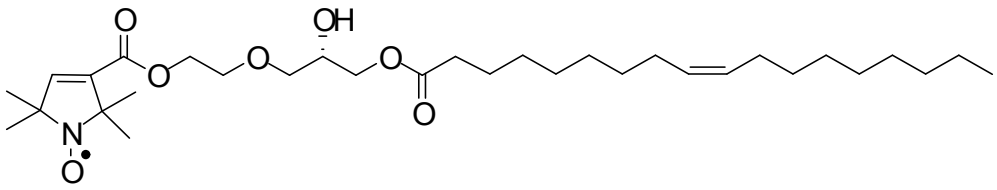
^{13}C NMR ($CDCl_3$, 100 MHz): δ 11.3, 19.8, 22.0, 22.1, 24.3, 24.3, 26.2, 26.3, 26.4, 26.6, 26.8, 29.0, 31.2, 31.6, 59.9, 66.6, 66.7, 66.9, 126.7, 127.1, 170.0, 170.3.

HRMS (TOF ES) Calcd for $C_{50}H_{88}NO_8$ $[M + H]^+$ 831.6589, found 831.6561.

IR (KBr, cm^{-1}) : 2927; 1745; 1727; 1629; 1463.

ESR spectrum ($CHCl_3$): triplet with *g*-factor = 2.0058_3 and a_N = 1.475 mT

1-O-Oleoyl-2-hydroxyl-3-O-[ethyl (2',2',5',5'-tetramethyl-3'-pyrroline-1'-oxyl-3'-carboxylate)] s*n*-glycerol [OG(L)-NO]



^{1}H NMR ($CDCl_3$, 400MHz): δ 0.88 (t, *J* = 6.4 Hz, 3H); 1.18-1.40 (m, 32H); 1.57-1.70 (m, 2H); 1.95-2.05 (m, 4H); 2.32-2.40 (m, 2H); 3.52-3.65 (m, 2H); 3.65-3.80 (m, 3H); 3.96-4.07 (m, 1H); 4.08-4.22 (m, 2H); 4.52-4.68 (m, 1H); 5.29-5.41 (m, 2H); 6.58 (s, 1H).

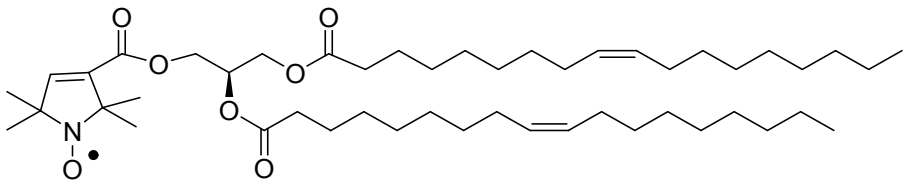
^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.4; 21.9; 24.2; 26.4; 26.4; 28.3; 28.4; 28.5; 28.7; 29.0; 31.1; 33.4; 61.9; 61.9; 64.7; 68.1; 68.9; 69.2; 71.3; 71.9; 128.9; 129.2; 172.7; 173.1.

HRMS (TOF ES) Calcd for $C_{32}H_{57}NO_7$ $[M + Na]^+$ 589.3955, found 589.3952.

IR (KBr, cm^{-1}) : 3445; 2929; 2856; 1723; 1716; 1630; 1463.

ESR spectrum ($CHCl_3$): triplet with *g*-factor = 2.0058_3 and a_N = 1.479 mT

1,2-O-Dioleoyl-3-O-(2',2',5',5'-tetramethyl-3'-pyrroline-1'-oxyl-3'-carboxylate) s*n*-glycerol [DOG-NO]



^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, *J* = 6.4 Hz, 6H); 1.20-1.38 (m, 52H); 1.55-1.68 (m, 4H); 1.92-2.08 (m, 8H); 2.32 (t, *J* = 8 Hz, 4H); 4.17 (dd, *J* = 5.6 Hz, *J* = 12.0 Hz, 1H); 4.25 (dd, *J* = 6 Hz, *J* = 12.0 Hz, 1H); 4.30-4.37 (m, 2H); 5.29-5.40 (m, 5H); 6.59 (s, 1H).

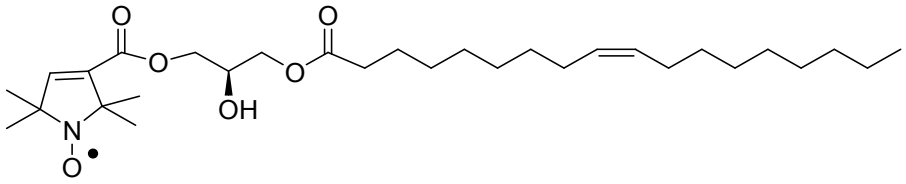
^{13}C NMR ($CDCl_3$, 100 MHz): δ 11.8, 11.8, 20.3, 20.3, 22.4, 22.7, 24.8, 24.8, 24.9, 26.7, 26.9, 26.9, 27.0, 27.0, 27.3, 27.3, 27.4, 29.5, 29.5, 31.6, 32.1, 59.6, 59.7, 66.4, 127.2, 127.6, 127.7, 170.3, 170.8.

HRMS (TOF ES) Calcd for $C_{48}H_{84}NO_7$ $[M + H]^+$ 787.66327, found 787.6346.

IR (KBr, cm^{-1}) : 2927; 2855; 1746; 1728; 1633; 1463.

ESR spectrum ($CHCl_3$): triplet with *g*-factor = 2.0058_4 and a_N = 1.475 mT

1-O-Oleoyl-2-hydroxyl-3-O-(2',2',5',5'-tetramethyl-3'-pyrroline-1'-oxyl-3'-carboxylate) s*n*-glycerol [OG-NO]



^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t, *J* = 6.4 Hz, 3H); 1.20-1.42 (m, 32H); 1.55-1.70 (m, 2H); 1.95-2.05 (m, 4H); 2.30-2.42 (m, 2H); 4.12-4.30 (m, 4H); 4.42-4.62 (m, 1H); 5.30-5.40 (m, 2H); 6.58 (s, 1H).

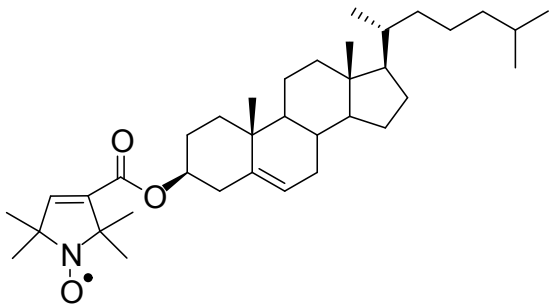
^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.8, 22.3, 24.5, 26.8, 26.9, 28.7, 28.8, 29.0, 29.2, 29.3, 29.4, 31.5, 33.8, 64.3, 64.9, 68.1, 129.3, 129.7, 173.6.

HRMS (TOF ES) Calcd for $C_{30}H_{52}NO_6$ $[M + H]^+$ 523.3874, found 523.3887.

IR (KBr, cm^{-1}) : 3428; 2927; 2855; 1724; 1632; 1463.

ESR spectrum ($CHCl_3$): triplet with *g*-factor = 2.0058_3 and a_N = 1.479 mT

Cholesteryl (2,2,5,5-tetramethyl-3-pyrroline-1-oxyl)-3-carboxylate [Chol-NO]



^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.70 (s, 3H); 0.90-2.06 (m, 40H); 0.90 (d, *J* = 6.4 Hz, 6H); 0.93 (d, *J* = 6.4 Hz, 3H); 1.05 (s, 3H); 1.28 (s, 6H); 1.39 (s, 6H); 2.30-2.60 (m, 2H); 4.80-5.05 (m, 1H); 5.37-5.48 (m, 1H); 6.58 (s, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 12.5; 19.4; 20.0; 21.6; 23.3; 23.5; 24.0; 24.9; 27.7; 28.6; 28.8; 32.4; 32.5; 36.3; 36.7; 37.1; 40.1; 40.3; 42.8; 50.5; 56.7; 57.2; 74.6; 123.6; 139.8.

MS(ESI) m/z: 575.2 $[M + Na^+]^+$. mp = 173-175°C.

IR (KBr, cm^{-1}) : 2934; 2863; 1711; 1634.

Anal $C_{50}H_{88}NO_8$ (575.2) Calc C, 78.21%; H, 10.57%; N, 2.53%. Found: C, 78.35%; H, 10.82%; N, 2.53%.

ESR spectrum ($CHCl_3$): triplet with *g*-factor = 2.0058_7 and a_N = 1.479 mT

Cyclic Voltammetric curves of Chol-NO at low temperature in THF; Background cyclic voltammetric curves for 0.08 M NaBF4 aqueous electrolyte solution with PBS buffer at pH=7.4

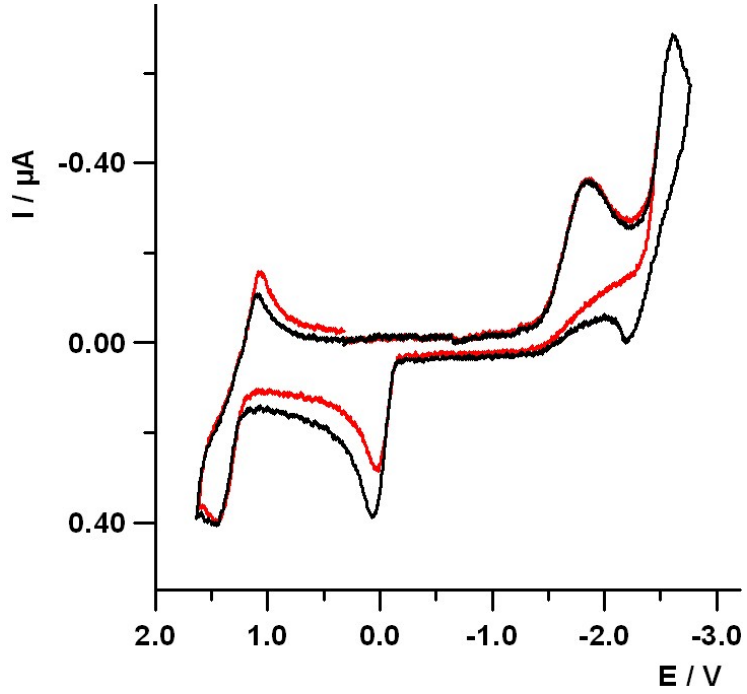


Figure S1. Cyclic voltammetric curves of species Chol-NO 1 mM in 0.08 M TBAH/THF solution. Sweep rate 1 V/s, working electrode: Pt (125 μm, diameter), T=213 K. The red curve has been recorded reversing the potential scan just before the second voltammetric reduction process.

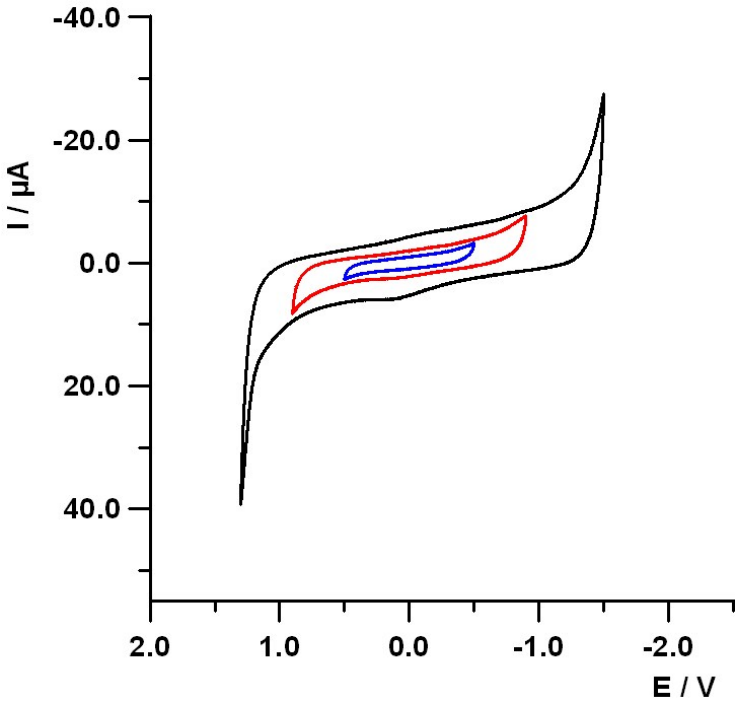


Figure S2. Background cyclic voltammetric curves for 0.08 M NaBF4 aqueous electrolyte solution with PBS buffer at pH=7.4, GC disk electrode (3 mm, diameter), sweep rate 0.2 V/s, T=298 K. The blue, red and black curves have been recorded over three increasing useful potential windows.

Accessibility parameters:

The best-fit $p_{1/2}$ parameters can be used to calculate the dimensionless accessibility parameters Π_i for the two paramagnetic relaxants:

$$\Pi_i = \frac{p_{1/2}^{i} - p_{1/2}^{nitrogen}}{\Delta H_{pp}} \cdot \frac{\Delta H_{pp}^{DPPH}}{p_{1/2}^{DPPH}} = \frac{\Delta p^{i}_{1/2}}{\Delta H_{pp}} \cdot \frac{\Delta H_{pp}^{DPPH}}{p_{1/2}^{DPPH}}$$

The index *i* denotes either oxygen or NiEDDA, ΔH_{pp} is the peak-to-peak linewidth in the absence of saturation. The calculation of the accessibilities requires the calibration of the instrument. For this reason the saturation profile and the saturation parameter were obtained also for DPPH (2,2-diphenyl-1-picrylhydrazyl)[1]: the value for the DPPH calibration factor in our setup is 231 W/T.

Table S1. Oxygen (Π_{O_2}) and NiEDDA accessibility (Π_{NiEDDA}) of the different reference spin labels (**SL**) and of the **NOXs** in POPC small unilamellar vesicles obtained at room temperature. The reported uncertainty is a rough estimate based on repeated experiments

SL	Π_{O2} (±0.02)	Π_{NiEDDA} (±0.02)	**NOX**	Π_{O2} (±0.02)	Π_{NiEDDA} (±0.02)
TempoPC	0.35	0.40	Chol-NO	0.32	0.16
5DPC[a]	0.40	0.10	DOG(L)-NO	0.56	0.14
7DPC[a]	0.31	0.10	DOG-NO	0.57	0.09
10DPC	0.67	0.08	OG(L)-NO	0.63	0.10
12DPC	0.99	0.09	OG-NO	0.76	0.09
14DPC	0.87	0.04			

[a]the apparent inversion of 5DPC and 7DPC is present also in the original paper[2].

[1] C. Altenbach, W. Froncisz, R. Hemker, H. McHaourab and W. L. Hubbell, *Biophys. J.* **2005**, *89*, 2103-2112.
[2] C. Altenbach, D. A. Greenhalgh, H. G. Khorana and W. L. Hubbell, *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 1667-1671.