Electronic supplementary materials *Mendeleev Commun.*, 2014, **24**, 298−300

3-Carboxy-2,2,5,5-tetra($^2H_3$)methyl-[4-^{2}H(^{1}H)]-3-pyrroline-(1-^{15}N)-1-oxyl as a spin probe for *in vivo* L-band electron paramagnetic resonance imaging

Leonid A. Shundrin, Igor A. Kirilyuk and Igor A. Grigor'ev

Table S1 The parameters of EPR[a] spectra of nitroxides **1, 2** in X- and L-band ranges.

Spine probe	Experimental conditions	a_N, mT	a_{13C}, mT	Line width (M_N), mT	g-factor
1a, 1b	X-band/50 mM phosph. buffer	2.2714	0.3358	0.0300 (−1/2) 0.0327 (+1/2)	2.0058
	L-band/*in vivo*[b]	2.2465	—	0.0573 (−1/2) 0.0584 (+1/2)	
2	X-band/50 mM phosph. Buffer	1.6182	0.3360	0.0562(−1) 0.0549 (0) 0.0580 (+1)	2.0058
	L-band/*in vivo*[b]	1.6101	—	0.1505(−1) 0.1460 (0) 0.1603 (+1)	

a M_N – quantum number of line, concentration of **1, 2** for X-band EPR spectroscopy was $2\cdot10^{-4}$ M; L-band *in vivo* EPR spectrum[b] was recorded with FOV 1 [Figure 3 (*a*)] 10 min after tail intravenous injections of 300 μl of 75 mM solutions of spin probes in standard physiological buffer.
b See Fig.S2.

Discussion. Line width and hyperfine coupling with the residual proton in the 4th position of pyrrolin cycle of **1b** and the total EPR spectrum of **1a**, **1b** mixture.

Halpern et al. [1] reported, that the observed hyperfine coupling constant value with ^{1}H nuclei in EPR spectra of 0.2 mM solution of 3-carbamoyl-2,2,5,5-tetra(2H3)methyl-3-(1-15N)pyrrolin-1-oxyl (mHCTPO) is 0.0550 mT and does not depend on oxygen concentration. From the other hand, EPR spectrum of perdeuterated analog of CTPO (structure II in [1]) has no hyperfine splitting from any nuclei, except for nitrogen. The structures of **1a**, **1b** have a carboxylic group instead of carbamoyl and exist in anionic forms in physiological range of pH. In the case of **1a**, **1b** mixture, hyperfine splitting with ^{1}H nucleus in 4th position of the pyrrolin cycle can be observed from **1b** only, the content of which was found to be 27 %. Nevertheless, EPR spectra of **1a, 1b** (73 and 27 % mixture) indicate no observable proton hyperfine coupling constant (Figure S1, low field line), even for solution in oxygen free

buffer*. To clarify this phenomenon, a simulation of the low field line has been done with WINSIM 2002 program [2] using independent set of parameters for **1a** and **1b** species with corresponding content. Optimization of line widths, proton hyperfine coupling constant and line shape was performed with LMB1 algorithm.

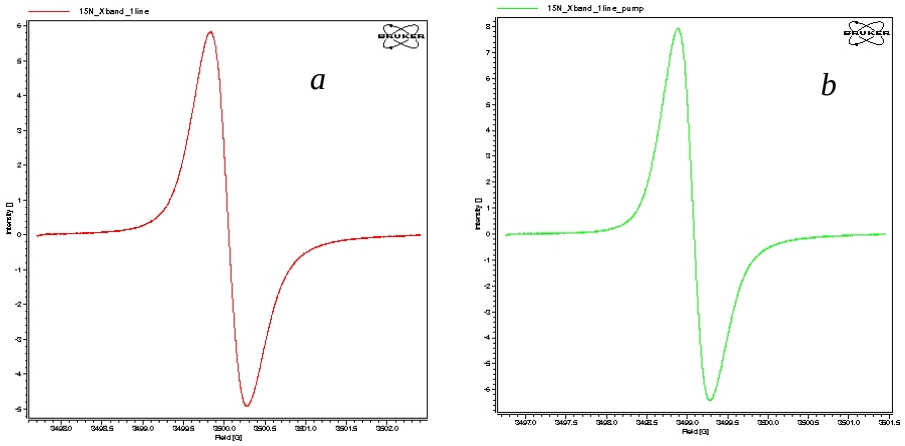

Figure S1 Low field line (M_N= −1/2) in EPR spectrum (X-band) of **1** in 50 mM phosphate buffer with pH=7.4 at 21% of oxygen concentration (total line width is 0.0300 mT) (*a*) and the same line in oxygen free buffer (total line width is 0.0240 mT) (*b*).

The simulation demonstrated, that the observed composite line (Figure S2 (*a*, *b*) consist from two lines from 1a and 1b (Figure S2 (*c*)), and hyperfine coupling constant with ^{1}H nucleus in the 4th position of the cycle is 0.0273 mT in accordance with optimization data. Calculated value of the constant for **1b** is 1.85 times less than the one obtained for mHCTPO [1] and can be caused by influence of negative charge.

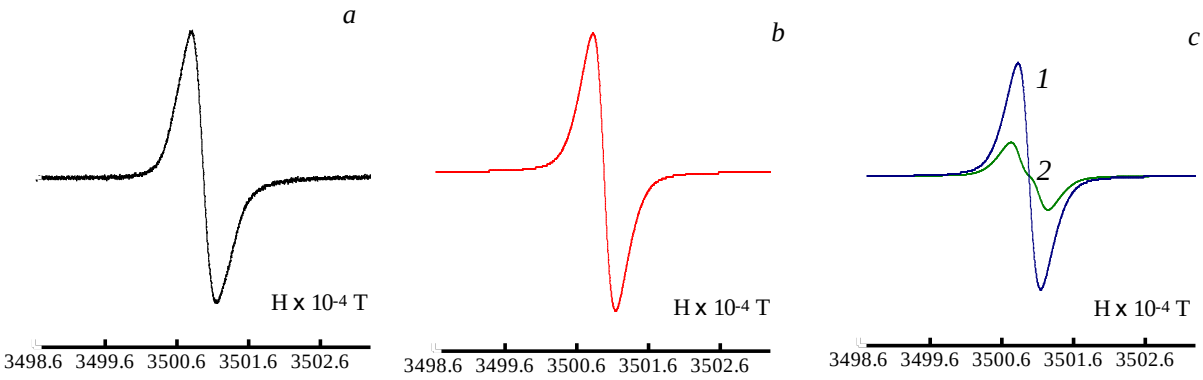

Figure S2 Experimental (*a*) and simulated (*b, c*) low field line of EPR spectrum of **1a**, **1b** mixture: total (b) and separate representation (c) of contributions from **1a** (*1*) and **1b** (*2*).

Optimized parameters are the following: **1a***: LW=0.0231 mT, Lorentz %=73.61;* **1b***: a_H=0.0273 mT, LW=0.0217 mT, Lorentz %=67.73.*

Note, that no hyperfine coupling with the proton in the 4th position of pyrrolin cycle has been observed for **2** [3].

* Oxygen was removed from solution by «freezing-pumping-defrosting» procedure.

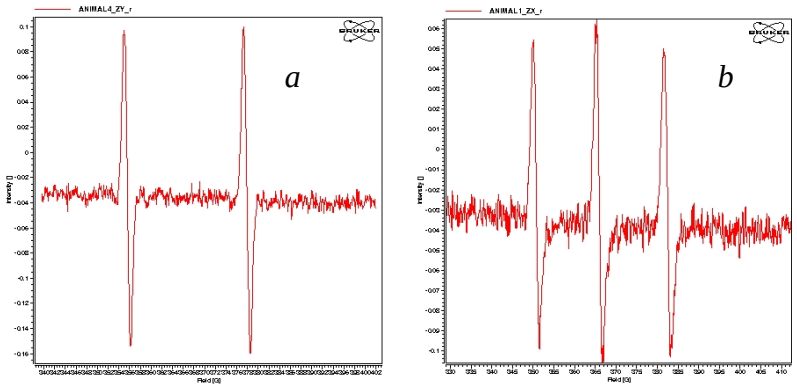

Figure S3 *In vivo* L-band EPR spectra of **1** (*a*) and **2** (*b*) recorded with FOV1.

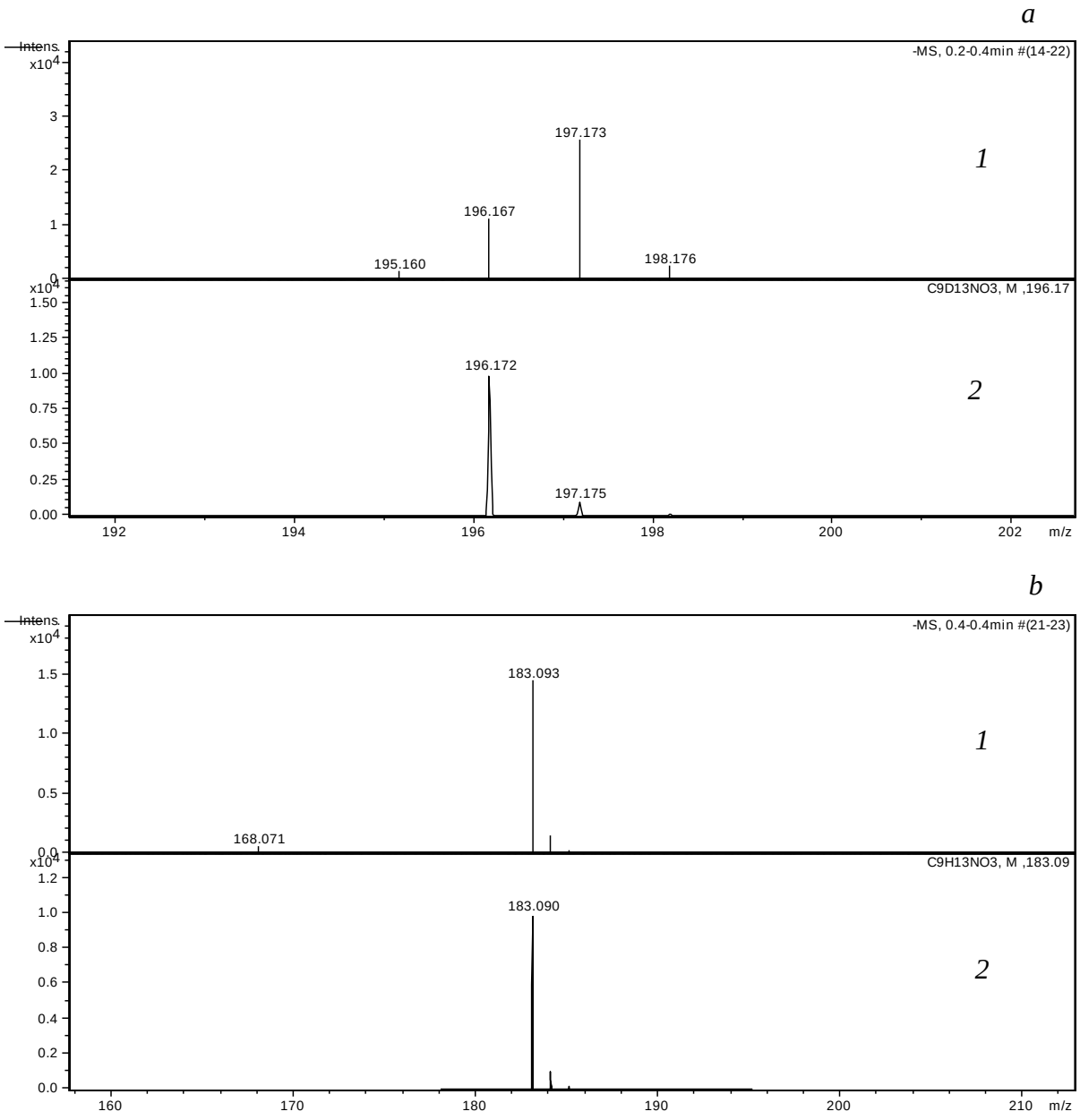

Figure S4 Mass-spectrum of **1a**, **1b** mixture (*a*) (*1* – experiment, *2* - simulation for $C_9HD_{12}NO_3$ (**1b**)) and **2** (*b*) (*1* – experiment, *2*- simulation).

References

1. H. J. Halpern, M. Peric, T.-D. Nguyen, D. P. Spencer, B. A. Teicher and Y. J. Lin, *J. Magn. Reson.*, 1990, **90**, 40.

2. D. R. Duling, *J. Magn. Reson.*, 1994, **104**, 105.

3. I. A. Kirilyuk, Yu. F. Polienko, O. A. Krumkacheva, R. K. Strizhakov, Yu. V. Gatilov, I. A. Grigor'ev and E. G. Bagryanskaya, *J. Org. Chem.*, 2012, **77**, 8016.