New series of pH-sensitive imidazoline nitroxides with enhanced spectral properties

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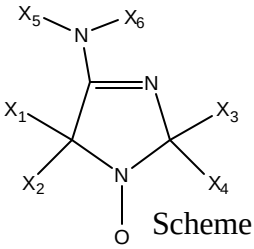
The application of spin pH probes offers a unique opportunity for non-invasive pH assessment in living animals using low-field ESR-based techniques [1]. The imidazoline nitroxide with 4-amino group, R1 (scheme 1) with pK=6.1 was found to be the most useful probe for measurement of pH in physiological range close to 7.0. Here we reported synthesis of series of the derivatives of the R1 with enhanced spectral properties. The isotopic substitution, ^{1}H for ^{2}H and ^{14}N-1 for ^{15}N-1, has been used to enhance spectral resolution and spectral peak intensity. This substitution resulted in narrowing of the peak-to-peak linewidth from ΔH_{pp}=0.76 G for R1 down to ΔH_{pp}=0.33 G for the nitroxide R2 (scheme 1) with corresponding increase in peak intensity up to 5-7 times which is an important factor particularly for application *in vivo* where the fundamental sensitivity is much less. Recently we proposed a new method of synthesis of 4-amino-3-imidazoline nitroxides via organometallic compounds addition to 5-amino-4*H*-imidazol 3-oxides and oxidation [2]. Series of new nitroxides (R3-R6) obtained by this approach demonstrate enhanced spectral properties. The compounds R3-R5 demonstrate much higher pK values (6.9, 7.2 and 7.4, correspondingly) compared with that for R1 (6.1) which makes them useful for applications in physiological range of pH. Moreover, the introduction of two (R3 and R4) or four (R5) bulky ethyl groups in α-position to N-O$^{\bullet}$ makes these compounds more protected against bioreduction. Note that the proposed synthetic scheme [2] allows introducing additional function in the structure of the 4-amino-3-imidazoline nitroxides. In the case of the radical R6 introducing pyridine group at C2 results in appearance of additional pK (2.7) and, as consequence, in extended range of pH sensitivity of its EPR spectrum. Preliminary data shows the usefulness of the R6 for monitoring stomach acidity *in vivo* in rats. *This work was partly supported by INTAS grant 99-1086 and RFBR grant 01-03-32452.*



R1:	$X_1=X_2=X_3=X_4=Me$; $X_5=X_6=H$	**pK=6.1**
R2:	$X_1=X_2=X_3=X_4=CD_3$; N-1=^{15}N; $X_5=X_6=H$	**pK=6.1**
R3:	$X_1=X_2=$ $X_5=X_6=Me$; $X_3=X_4=$ Et;	**pK=6.9**
R4:	$X_1=X_2=Me$; $X_3=X_4=Et$; $X_5+X_6=(CH_2)_4$	**pK=7.2**
R5:	$X_1=X_2=$ $X_3=X_4=$ Et; $X_5+X_6=(CH_2)_4$	**pK=7.4**
R6:	$X_1=X_2=Me$; $X_3=Et$; $X_4=Py$; $X_5+X_6=(CH_2)_5$	**pK_1=2.7; pK_2=4.8**

Scheme 1.

[1] Khramtsov, V.V., Grigor'ev, I.A., Foster, M.A., Lurie, D.J., and Nicholson, I., 2000, Biological applications of spin pH probes, *Cell.Mol.Biol.,* 46 (8), 1361-1374.

[2] Kirilyk, I.A., and Grigor'ev, I.A. 2002, In: 35th Annual Intern. Meeting of Royal Society of Chemistry: Advanced techniques and applications of EPR, p.P16, Aberdeen (UK).