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Nitroxides with two p*K* values—useful spin probes for pH monitoring within a broad range

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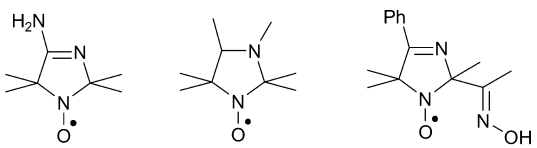
A series of 4-dialkylamino-2,5-dihydroimidazole nitroxides with pyridine-4-yl, 4-dimethylaminophenyl or 4-hydroxyphenyl groups in position 2 of the imidazole ring were prepared using the reaction of RMgBr with corresponding 5-dialkylamino-4,4-dimethyl-4*H*-imidazole 3-oxides. The EPR spectra of the nitroxides were shown to be pH-sensitive due to consecutive protonation of the amidino moiety and the basic group(s) at position 2 of the imidazole ring. The 5,5-dimethyl-4-(dimethylamino)-2-ethyl-2-pyridine-4-yl-2,5-dihydro-1*H*-imidazol-1-oxyl showed a monotonic increase in the isotropic nitrogen hyperfine (hfi) coupling constant a_N of 1 .4 G over a pH range from 2 to 6.5. Such a broad range of pH-sensitivity could be useful for many biophysical and biomedical applications, including pH-monitoring in the stomach.

Introduction

The measurement of pH is probably the most widely performed test in the biochemical laboratory, reflecting its critical role in the physiology and pathophysiology of living organisms. Most non-invasive pH measurements, particularly those conducted *in vivo*, rely on endogenous and/or exogenous molecular probes. The absorption of fluorescent pH-sensitive dyes was found particularly effective for pH study on the cellular and subcellular levels,[1] while magnetic resonance approaches based on EPR and NMR spectroscopies have advantages for *in vivo* applications in animals and humans.[2] EPR has a crucial advantage over NMR in that it is more than three orders of magnitude more sensitive. However, the low depth of microwave penetration into aqueous samples and the absence of endogenous paramagnetic probes significantly limit the application of EPR to biological systems. Despite these formidable problems, recently developed low-field EPR-based techniques, in combination with a wide variety of spin pH probes, offer another unique opportunity for non-invasive pH measurements (for recent reviews see ref. 3 and 4).

Among various pH-sensitive spin probes described to date,[5,6] EPR spectra of perhydroimidazole-derived nitroxides (*e.g.*, HMI, Fig. 1) have the highest sensitivity to pH, Δa_N *ca.* 1.3 G. However, the utility of these nitroxides in biomedical studies is somewhat limited by low p*K* values (*ca.* 4.5). The nitroxides of the 4-amino-2,5-dihydro-1*H*-imidazole series (*e.g.*, ATI, Fig. 1) are considered to be the most promising pH-sensitive spin probes for EPR studies *in vivo* due to the relatively large effect of pH on their EPR spectra (Δa_N varies from 0.7 to 1.0 G) and the appropriate p*K* values in the range from 4.5 to 7.4.[5,7,8]



ATI pK 6.1 Δa_N 0.8 G

HMI pK 4.7 Δa_N 1.27 G

1 pK_1 1.0, Δa_N 0.72 G; pK_2 11.5, Δa_N 0.23 G

Fig. 1

It is well established that nitroxides with ionizable (basic or acidic) groups in the side chain have rather moderate EPR spectral responses to pH changes.[5] However, nitroxides with several basic or acidic groups (intracyclic or exocyclic) may undergo consequent protonation of the two basic centers to produce complementary effects on the hfi splitting constant (a_N) of the nitroxide. Very few examples of such nitroxides are known, *e.g.*, **1** (Fig. 1.). The EPR spectrum of this nitroxide undergoes two consequent transitions at pH 0–2 and 10.5–12. Here we report a further development of this approach to the molecular design of new pH-sensitive spin probes: specifically, we synthesized a series of 4-amino-2,5-dihydro-1*H*-imidazole spin probes with 4-dimethylaminophenyl, 4-hydroxyphenyl and 4-pyridyl groups at the position 2 of the imidazole ring and studied the sensitivity of their EPR spectra to pH.

Results and discussion

Synthesis

The nitroxides **8a**–**e** and **9** were prepared using a recently developed method[7] (Scheme 1), *via* organometallic reagent addition to 4*H*-imidazol-3-oxides.

The 2,5-dihydro-1*H*-imidazoles **3a**–**c** were synthesized by condensation of 3-(hydroxyamino)-3-methylbutan-2-one **2** with ammonia and corresponding aldehydes, in a similar way to the procedure for **3a** described earlier.[9] A mild oxidation of **3a**–**c** with lead dioxide in methylene chloride yielded 4,4,5-trimethyl-4*H*-imidazole 3-oxides **4a**–**c**. The nitrosation of **4b**–**c** was performed using the i-PrONO–i-PrONa system, while for **4a**, a better yield of the 5-hydroximinomethyl derivative **5a** was achieved using the i-PrONO–Et_3N system. Treatment of the oximes **5a**–**c** with TsCl–Et_3N yielded carbonitriles **6a**–**c**, the key compounds which were involved in the reaction with amines to

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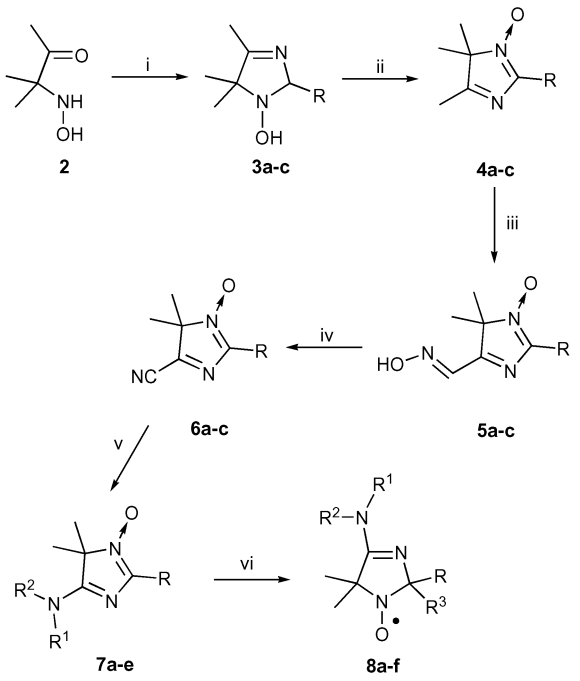
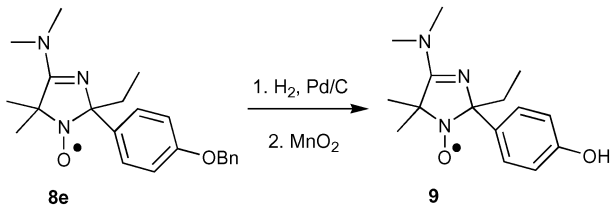


Scheme 1 Reagents: (i) RCHO, NH_3, EtOH–H_2O; (ii) PbO_2–CH_2Cl_2; (iii) i-PrONO, Et_3N–$CHCl_3$ or i-PrONO, i-PrONa–i-PrOH; (iv) TsCl, Et_3N–$CHCl_3$; (v) NHR^1R^2–CH_2Cl_2; (vi) R^3MgBr, THF; then H_2O, MnO_2; for **3**–**6** R = *p*-$Me_2NC_6H_4$ (**a**), 4-Py (**b**) and p-$BnOC_6H_4$ (**c**); for details of R, R^1, R^2 and R^3 for **7** and **8** see Table 1.

give 4*H*-imidazoles **7a**–**e**. The 4*H*-imidazole 3-oxides **7a**–**e** were treated with an excess of organometallic reagent (EtMgBr or *p*-$Me_2NC_6H_4MgBr$). After quenching the reaction mixture with water and then a consequent oxidation with manganese dioxide, the nitroxides **8a**–**f** were isolated. To prepare the nitroxide **9** the benzyl group in **8e** was removed using hydrogenolysis on Pd/C, and the hydroxylamine formed was reoxidized with MnO_2 (Scheme 2).



Scheme 2

Titrations

The EPR spectra of all the nitroxides synthesized were found to be pH-dependent (See Table 1). The hydroxyphenyl derivative **9** showed hfi changes in two distinct pH ranges, apparently corresponding to deprotonation of the phenoxy group (p*K* 9.8) and to protonation of the amidino moiety (p*K* 5.9). The hfi constants a_N of the nitroxides **8a**–**d** and **8f** undergo monotonic changes upon titration due to consecutive protonation of the amidino moiety and the basic groups at position 2 of imidazoline ring with close p*K* values (Fig. 2 and 3). The nitroxides **8b**, **8d** and **8f** showed the highest sensitivity of EPR spectra to pH; these spin probes cover a range over 4 units of pH with anoverall Δa_N of *ca.*1.4 G. It is interesting to note that the introduction of the basic groups, 4-dimethylaminopenyl and pyridine-4-yl, at position 2 of the imidazole ring in **8f** lead neither to an increase in the overall Δa_N nor to an expansion of the range of pH sensitivity in comparison to the nitroxide **8d**. Titration data of this nitroxide may be fitted to three-p*K* base eqn. (3) as well as to eqn. (2), see Fig. 3. It is not clear whether the protonation of both adjacent pyridine and dimethylaminophenyl basic centers occurs within the pH range studied.

Recently we reported on the synthesis of nitroxides **10** and **11** (Fig. 4),[7] which have similar basic groups at position 2 of the imidazole ring. The titration of these nitroxides gave a simple

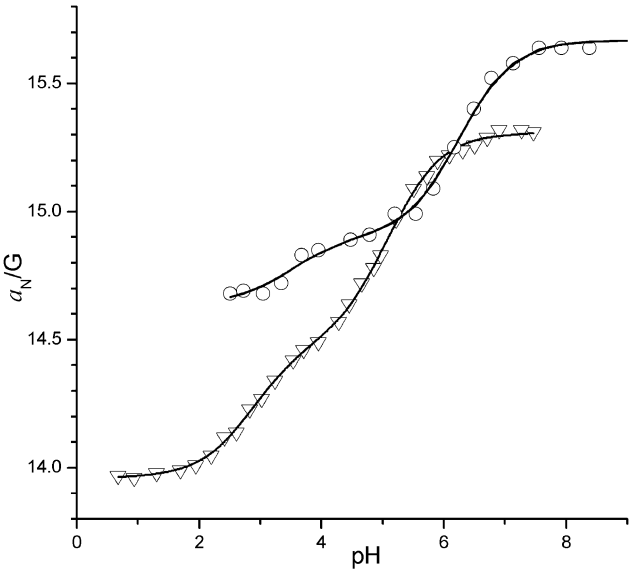


Fig. 2 The pH dependence of nitrogen hyperfine splitting (a_N) measured as a distance between low- and central-field components of the EPR spectra of the nitroxides **8c** (○), and **8d** (▽). The solid line is a nonlinear least-squares fit of the data to eqn. (2), see Experimental.

Table 1 Parameters of the new nitroxide spin probes: p*K* values, changes in hfi splitting (Δa_N) between protonated and unprotonated forms and partition coefficients (K_p) measured in octanol–(0.1 N NaOH) mixtures

	R	R^1	R^2	R^3	p*K*		Δa_N, G		K_p
8a	*p*-$Me_2NC_6H_4$	$(CH_2)_5$		Et	6.1		0.83		>300
					3.4		0.35		
8b	4-Py	$(CH_2)_5$		Et	4.8		0.8		>300
					2.8		0.6		
8c	*p*-$Me_2NC_6H_4$	Me	Me	Et	6.25		0.77		100
					3.50		0.26		
8d	4-Py	Me	Me	Et	5.08		0.81		25
					2.86		0.53		
8f	4-Py	Me	Me	*p*-$Me_2NC_6H_4$	4.76[a]	5.03[b]	0.61[a]	0.44[b]	64
					2.38[a]	3.95[b]	0.68[a]	0.24[b]	
						2.29[b]	0.61[b]		
9	p-HOC_6H_4	Me	Me	Et	5.9		0.82		0.033
					9.8		0.12		

[a] Calculated for two p*K*. [b] Calculated for three p*K*.

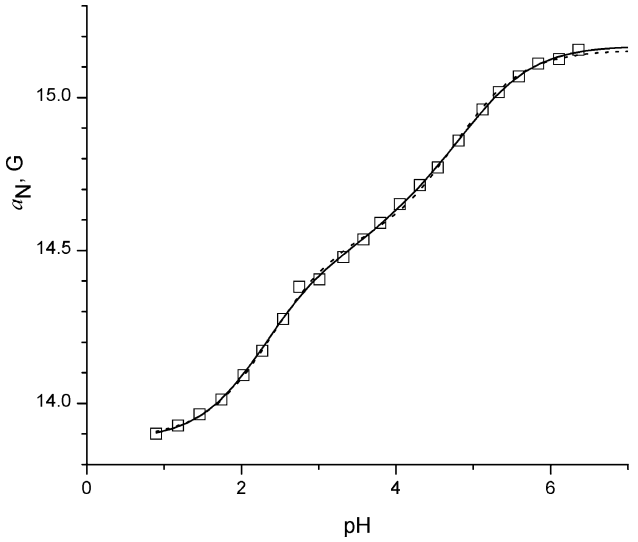


Fig. 3 The pH dependence of hyperfine splitting (a_N) of the EPR spectra of the nitroxide **8f**. The experimental data were fitted to eqn. (3) (3-pK, solid line) and eqn. (2) (2-pK, dashed line). The pK and Δa_N values calculated are listed in Table 1.

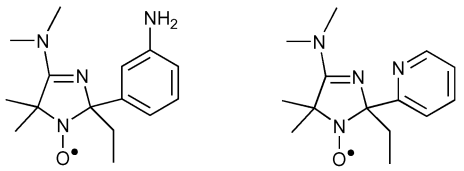


Fig. 4

titration curve with a Δa_N value of 0.8 G, corresponding to protonation of the amidine moiety. The absence of a second pK in the titration curve of **10** could be due inefficiency of the meta-position of amino group in the phenyl ring for the transmission of electronic effect of the substituent. For nitroxide **11**, both the amidine group and the pyridine ring nitrogen are likely to be engaged in coordination with protons. This may also account for the relatively high pK (5.4) of the nitroxide.

The partition coefficients of the nitroxides **8a**, **8c**, **8d**, **8f** and **9** were measured in 0.1 M NaOH–octanol mixtures and represent the relative lipophilicities of the unprotonated forms of the nitroxides. At lower pH values the lipophilicities decrease because of an equilibrium with highly hydrophilic protonated forms. Among all the nitroxides studied, compound **8d** seems to be the most promising spin probe, because of a relatively high solubility in water and a broad range of sensitivity to pH (Fig 2). This probe is particularly suitable for pH monitoring in stomach using non-invasive low-field EPR techniques.[3]

Experimental

The IR spectra were recorded on a Bruker Vector 22 FT-IR spectrometer in KBr pellets (concentration 0.25%, pellet thickness 1 mm). The UV spectra were measured on a HP Agilent 8453 spectrometer in EtOH. The ^{1}H NMR spectra were recorded on a Bruker AC-200 (200.132 MHz) spectrometer for 5–10% solutions using the signal of the solvent as the standard. The ^{13}C NMR spectra were recorded on a Bruker AC-200 (50.323 MHz) and a Bruker AM-400 (100.614 MHz) spectrometers for 5–10% solutions at 300 K using the signal of the solvent as the standard. The assignment of the signals in the ^{13}C NMR spectra was based on analysis of intensities, on the spectra measured in J-modulation mode, and using the data reported previously.[7,8] EPR spectra were recorded with a Bruker ER-200D-SRC spectrometer using a 100 μL quartz capillary. The 3-hydroxyamino-3-methylbutan-2-one **2** was prepared according to the procedure described previously.[10]

Titration of the nitroxides **8** and **9** was performed similarly to the procedure described in.[11] The nitroxides were dissolved in 1 mM phosphate buffer, pH = 6.03, to the final concentration of the nitroxide of *ca.* 0.1 mM. The poorly-soluble nitroxides were first dissolved in 0.1 mL of acetone or DMSO, and then added to the buffer solution. The resulting solutions were titrated with KOH or HCl solutions to the required pH. The pH was measured using a digital pH-meter equipped with a glass electrode. The accuracy of the measurements was estimated to be 0.02 pH units. The hfi splittings were measured as the distance between the low field and the central lines of the nitroxide EPR spectra and are accurate within 0.02 G. To obtain the pK values of the compounds, the experimental dependence of a_N on pH was fitted to one of the conventional titration equations: eqn, (1) for **8e**, eqn. (2) for **8a**–**d** and **9**:

$$a_N(\mathrm{pH}) = \frac{p_1 + p_2 \times 10^{\mathrm{p}K-\mathrm{pH}}}{1 + 10^{\mathrm{p}K-\mathrm{pH}}} \quad (1)$$

$$a_N(\mathrm{pH}) = \frac{(p_1 + p_2 \times 10^{\mathrm{p}K_1-\mathrm{pH}} + p_3 \times 10^{\mathrm{p}K_1+\mathrm{p}K_2-2\times\mathrm{pH}})}{1 + 10^{\mathrm{p}K_1-\mathrm{pH}} + 10^{\mathrm{p}K_1+\mathrm{p}K_2-2\times\mathrm{pH}}} \quad (2)$$

where p_1–p_3 represent nitrogen hfi splittings of the nitroxide in different ionization states. For the nitroxide **8f** both eqn. (2) and (3) were used; the error of pK determination was ±0.1.

$$a_N(\mathrm{pH}) = \frac{(p_1 + p_2 \times 10^{\mathrm{p}K_1-\mathrm{pH}} + p_3 \times 10^{\mathrm{p}K_1+\mathrm{p}K_2-2\times\mathrm{pH}} + p_4 \times 10^{\mathrm{p}K_1+\mathrm{p}K_2+\mathrm{p}K_3-3\times\mathrm{pH}})}{1 + 10^{\mathrm{p}K_1-\mathrm{pH}} + 10^{\mathrm{p}K_1+\mathrm{p}K_2-2\times\mathrm{pH}} + 10^{\mathrm{p}K_1+\mathrm{p}K_2+\mathrm{p}K_3-3\times\mathrm{pH}}} \quad (3)$$

where $p1$–$p4$ represent nitrogen hfi splittings of the nitroxide in different ionization states.

The partition coefficients of the nitroxides **8** and **9** were determined using a previously described procedure.[7] A sample of the nitroxide (*ca.* 2 μmol) was placed in a tube containing octanol (5 mL) and 0.1 M NaOH solution (5 mL). The mixture was shaken vigorously and allowed to stand until separation of the phases was complete. The K_p were determined from the difference in integral intensity of the EPR spectra of the nitroxide in water and in octanol. Accuracy of the measurements was up to 5%.

4,5,5-Trimethyl-2,5-dihydro-1*H*-imidazole-1-ols 3b,c, general procedure

Aldehyde (20 mmol) was added to a stirred solution of 3-hydroxyamino-3-methyl-butan-2-one (**2**) (3 g, 20 mmol) in a mixture of methanol (15 mL) and aqueous ammonia (10 mL). The reaction mixture was stirred for 5 h at 25 °C and allowed to stand overnight at −5 °C. The crystalline precipitate was filtered off and washed with cold EtOH 50% and with cold water to give dihydroimidazoles **3b** and **3c**. Compound **3b**, yield 4.1 g, (70%) colorless crystals, mp 119–122 °C (EtOAc). Found: C, 62.94; H, 7.79; N, 19.56. Calc. for $C_{11}H_{15}N_3O$ 1/2 H_2O: C, 62.54; H, 7.47; N, 19.89. ν_{max}(KBr)/cm^{-1} 1633, 1607, 1568, 1501, 1416, 1384, 1358, 1295, 1250, 1240, 1033, 1022, 1007 and 803; λ_{max}(EtOH)/nm 258 (lg ε 3.21); δ_H(400 MHz; $CDCl_3$-CD_3OD 1 : 1) 1.27 (3 H, s, 2-CH_3), 1.32 (3H, s, 2-CH_3), 2.06 (3 H, d, J 3.0, 4-CH_3), 5.43 (1 H, quartet, J 3.0, CH), 7.55 and 8.52 (4 H, AA′BB′, J 9.0, Py); δ_C(100 MHz; $CDCl_3$-CD_3OD 1 : 1) 16.37, 16.41 (2-CH_3), 24.38 (4-CH_3), 73.11 (C^5), 90.21 (C^2), 124.06 (C^3, Py), 149.56 (C^2, Py), 151.09 (C^i, Py) and 183.13 (C=N). Compound **3c**, yield 5.3 g, (85%) colorless crystals, mp 144–146 °C (EtOAc). Found: C, 73.97; H, 7.27; N, 8.98. Calc. for $C_{19}H_{22}N_2O_2$: C, 73.52; H, 7.14; N, 9.03. ν_{max}(KBr)/cm^{-1} 2965, 2864, 1643, 1612, 1587, 1511, 1461, 1425, 1381, 1300, 1285, 1232, 1171, 1022, 874, 829 and 750; λ_{max}(EtOH)/nm 226 (lg ε 4.20); δ_H(200 MHz; $CDCl_3$) 0.94 (3 H, s, 2-CH_3), 1.05 (3H, s, 2-CH_3), 1.92 (3 H, d, J 3.0, 4-CH_3), 5.07 (2 H, s, CH_2), 5.29 (1 H, quartet, J 3.0, CH), 6.32

(1 H, s, OH), 6.95 and 7.25 (4 H, AA′BB′, *J* 8.5, C_6H_4O), and 7.38 (5 H, m, Ph); δ_C(50 MHz; $CDCl_3$) 15.86, 16.02 (2-CH_3), 23.90 (4-CH_3), 70.04 (CH_2), 71.71 (C^5), 91.01 (C^2), 114.72 (C^m, C_6H_4O), 127.22 (C^o, C_6H_4O), 132.61 (C^i, C_6H_4O), 158.72 (C–O, C_6H_4O), 127.69 (C^p, Ph), 128.38 (C^m, Ph), 128.90 (C^o, Ph), 137.16 (C^i, Ph), and 178.42 (C=N).

4*H*-Imidazole 3-oxides 4a–c, general procedure

A suspension of **3a**–**c** (10 mmol), and PbO_2 (4.78 g, 20 mmol) in CH_2Cl_2 (30 mL) was stirred for 1–3 h. After the reaction was complete (monitored by TLC analysis, Silufol, eluent Et_2O–methanol 25 : 1, development with I_2 vapour) the lead oxides were filtered off and the solvent was removed in vacuum. Compound **4a**, yield 90%, yellow crystals, mp 135–137 °C (EtOAc–hexane 1 : 1), Found: C, 68.69; H, 7.48; N, 17.12. Calc. for $C_{14}H_{19}N_3O$: C, 68.45; H, 7.79; N 17.10. ν_{max}(KBr)/cm^{-1} 1605, 1547, 1508, 1452, 1435, 1390, 1322, 1294, 1218, 1192, 1127, 1106, 1071, 945 and 835; λ_{max}(EtOH)/nm 385 (lg ε 4.25), 295 (4.15); δ_H(200 MHz; $(CD_3)_2CO$) 1.39 (6H,s, 4-Me), 2.30 (3H, s, 5-Me), 3.02 (6H, s, NMe_2), 6.80, 8.58 (2H each, AA′BB′, *J* 9 Hz, Ar); δ_C(50 MHz; $(CD_3)_2SO$) 16.71 (5-Me), 21.52 (4-Me), 36.20 (NMe_2), 80.39 (C^4), 144.78 (C^2), 181.92 (C^5), Ar: 114.81 (C^i), 128.79 (C^o), 111.22 (C^m), 151.29 (C–N). Compound **4b**, yield 90%, yellow crystals, mp 201–204 °C (EtOAc–hexane 1 : 1). Found: C, 65.36; H, 6.38; N, 20.58. Calc. for $C_{11}H_{13}N_3O$: C, 65.01; H, 6.45; N, 20.68. ν_{max}(KBr)/cm^{-1} 1598, 1547, 1523, 1475, 1464, 1428, 1416, 1398, 1376, 1322, 1304, 1233, 1220, 1196, 1066, 990 and 833; λ_{max}(EtOH)/nm 334 (lg ε 3.93), 280 (3.40); δ_H(200 MHz; $CDCl_3$) 1.47 (6H, s, 4-Me), 2.32 (3H, s, 5-Me), 8.41, 8.72 (2H each, AA′BB′, *J* 6.3 Hz, Py); δ_C(50 MHz; $CDCl_3$) 16.48 (5-Me), 21.59 (4-Me), 82.59 (C^4), 144.00 (C^2), 180.52 (C^5), Py: 133.38 (C^i), 120.09 (C^3), 150.09 (C^2). Compound **4c**, yield 90%, yellow crystals, mp 201–204 °C (EtOAc–hexane 1 : 1). Found: C, 74.11; H, 6.64; N, 9.03. Calc. for $C_{19}H_{20}N_2O_2$: C, 74.00; H, 6.54; N 9.08. ν_{max}(KBr)/cm^{-1} 1602, 1587, 1543, 1497, 1421, 1394, 1371, 1302, 1245, 1205, 1174, 1116, 997, 841 and 754; λ_{max}(EtOH)/nm 339 (lg ε 3.97), 268 (4.38); δ_H(200 MHz; $CDCl_3$) 1.43 (6H, s, 4-Me), 2.26 (3H, s, 5-Me), 5.06 (2H, s, CH_2), 7.03, 8.65 (2H each, AA′BB′, *J* 9 Hz, C_6H_4), 7.33 (5H, m, Ph); δ_C(50 MHz; $CDCl_3$) 16.36 (5-Me), 21.59 (4-Me), 69.86 (CH_2), 80.93 (C^4), 144.90 (C^2), 180.52 (C^5), C_6H_4: 136.45 (C^i), 129.43 (C^o), 114.61 (C^m), 160.18 (C–O), Ph: 120.52 (C^i), 127.76 (C^p), 127.18, 128.32 (C^o, C^m).

4,4-Dimethyl-2-(4-dimethylaminophenyl)-4*H*-imidazole-5-carbaldehyde oxime 3-oxide (5a)

Isopropyl nitrite (3 mL, 34 mmol) was added to a solution of 4*H*-imidazole **4a** (2.2 g, 9 mmol) in a mixture of chloroform (5 mL) and triethylamine (2 mL) and the mixture was allowed to stand at 22 °C for 24 h. The crystalline precipitate was filtered off and washed with a mixture tert-butylmethylether–iso-propanol (2 : 1) to yield 1.7 g (70%) of oxime **5a**, red crystals, mp 233–236 °C (dec.) (EtOAc). Found: C, 61.24; H, 6.90; N, 20.39. Calc. for $C_{14}H_{18}N_4O_2$: C, 61.30; H, 6.61; N 20.42. ν_{max}(KBr)/cm^{-1} 1607, 1559, 1539, 1513, 1492, 1439, 1414, 1366, 1345, 1293, 1267, 1201, 1026, 945, 865 and 821; λ_{max}(EtOH)/nm 415 (lg ε 3.82), 336 (4.55); δ_H(200 MHz; $(CD_3)_2SO$) 1.51 (6H, s, 4-Me), 3.06 (6H, s, NMe_2), 8.00 (1H, s, HC=N–O), 6.80, 8.42 (2H each, AA′BB′ *J* 9 Hz, Ar), 12.60 (1H, s, OH); δ_C(50 MHz; $(CD_3)_2SO$ + t-BuOK 5%) 25.00 (4-Me), 39.51 (NMe_2), 78.23 (C^4), 145.45 (C^2), 178.12 (C^5), 143.62 (HC=NO), Ar: 115.81 (C^i), 128.66 (C^o), 110.81 (C^m), 150.80 (C–N).

4,4-Dimethyl-4*H*-imidazole-5-carbaldehyde oxime 3-oxides 5b and 5c, general procedure

Na (1 g, 41 mmol) was dissolved in isopropanol (30 mL); after the reaction slowed down the mixture was heated to 60 °C until all Na was dissolved. The solution was allowed to cool to room temperature to form a suspension of i-PrONa. Isopropyl nitrite (3.5 mL, 39 mmol) and a solution of **4b** or **4c** (16 mmol) in 20 mL of isopropanol were added subsequently to the stirred suspension of i-PrONa in isopropanol and the mixture was allowed to stand for 1–8 h. After the reaction was complete (TLC, Silufol UV-254, eluent EtOAc) the mixture was acidified with AcOH to pH 6–7 and isopropanol was removed in vacuum. A saturated solution of NaCl (20 mL) was added to the residue and the precipitate of **5b** or **5c** was filtered off and recrystallized from EtOAc. Compound **5b**, yield (80%), mp 240–244 °C (EtOAc). Found: C, 57.11; H, 5.29; N, 24.03. Calc. for $C_{11}H_{12}N_4O_2$: C, 56.89; H, 5.21; N 24.12. ν_{max}(KBr)/cm^{-1} 1604, 1559, 1525, 1472, 1414, 1364, 1319, 1204, 1059, 1005, 834 and 812; λ_{max}(EtOH)/nm 356 (lg ε 3.68), 259 (4.11); δ_H(200 MHz; $(CD_3)_2SO$ + t-BuOK 5%) 1.62 (6H, s, 4-Me), 7.96 (1H, s, HC=N–O), 8.39, 8.67 (2H each, AA′BB′ *J* 6 Hz, 4-Py); δ_C(50 MHz; $(CD_3)_2SO$ + t-BuOK 5%) 25.35 (4-Me), 81.04 (C^4), 144.05 (C^2), 177.46 (C^5), 144.19 (HC=NO), Py: 134.40 (C^i), 120.27 (C^3), 150.22 (C^2). Compound **5c**, yield (80%), mp 211–215 °C (EtOAc). Found: C, 67.77; H, 5.71; N, 12.17. Calc. for $C_{19}H_{19}N_3O_3$: C, 67.64; H, 5.68; N 12.46. ν_{max}(KBr)/cm^{-1} 1604, 1537, 1469, 1450, 1429, 1387, 1343, 1315, 1297, 1256, 1183, 1024, 836 and 731; λ_{max}(EtOH)/nm 379 (lg ε 3.63), 291 (4.54); δ_H(200 MHz; $CDCl_3$ + CD_3OD (10%)) 1.66 (6H, s, 4-Me), 5.15 (2H, s, CH_2), 7.99 (1H, s, HC=N–O), 7.11, 8.61 (2H each, AA′BB′, *J* 9 Hz, C_6H_4), 7.40 (5H, m, Ph); δ_C(50 MHz; $CDCl_3$ + CD_3OD (10%)) 23.75 (4-Me), 70.48 (CH_2), 81.70 (C^4), 147.73 (C^2), 174.89 (C^5), 144.06 (HC=NO), C_6H_4: 136.82 (C^i), 130.27 (C^o), 115.25 (C^m), 161.34 (C–O), Ph: 120.31 (C^i), 128.31 (C^p), 127.68, 128.82 (C^o, C^m).

4*H*-Imidazole-5-carbonitrile 3-oxides 6a–c, general procedure

TsCl (9.5 g, 50 mmol) was added portionwise to a stirred solution of oxime **5a**–**c** (50 mmol) of in a mixture of $CHCl_3$ (75 mL) and triethylamine (16 mL, 110 mmol). The resulting solution was stirred for 1 h, washed with water and dried over $MgSO_4$. The $CHCl_3$ was removed in vacuum and the residue was separated by a column chromatography (Kieselgel 60, Merck, eluent-chloroform) to give **6a**–**c**. Compound **6a**, yield 90%, yellow crystals, mp 200–203 °C (hexane) (Found: C, 65.52; H, 6.31; N, 21.99. Calc. for $C_{14}H_{16}N_4O$: C, 65.61; H, 6.29; N, 21.86); ν_{max} (KBr)/cm^{-1} 2982, 2914, 2820, 2223, 1613, 1524, 1488, 1466, 1440, 1391, 1376, 1293, 1235, 1212, 1110, 1066, 946, 820 and 741; λ_{max}(EtOH)/nm 469 (lg ε 3.61), 346 (4.45), 330 (4,38); δ_H(200 MHz; $(CD_3)_2CO$) 1.59 (6H,s, 4-Me), 3.06 (6H, s, NMe_2), 6.84, 8.42 (2H each, AA′BB′, *J* 9 Hz, Ar); δ_C(50 MHz; $CDCl_3$-CCl_4 1 : 1) 21.54 (4-Me), 39.83 (N–Me), 82.14 (C^4), 111.61 (C≡N), 147.20 (C^2), 149.96 (C^5), Ar: 113.80 (C^i), 128.76 (C^o), 111.10 (C^m), 151.78 (C–N). Compound **6b**, yield 80%, yellow crystals, mp 153–156 °C (hexane). Found: C, 61.57; H, 4.46; N, 26.39. Calc. for $C_{11}H_{10}N_4O$: C, 61.67; H, 4.71; N 26.15. ν_{max} (KBr)/cm^{-1} 3044, 2987, 2228, 1598, 1556, 1524, 1508, 1469, 1402, 1386, 1328, 1297, 1201, 993, 829, 789 and 724; λ_{max} (EtOH)/nm 368 (lg ε 3.84), 299 (4.02), 229 (4.26); δ_H(200 MHz; $CDCl_3$) 1.57 (6H, s, 4-Me), 8.22, 8.70 (2H each, AA′BB′ *J* 6 Hz, 4-Py); δ_C(50 MHz; $CDCl_3$) 21.58 (4-Me), 84.88 (C^4), 111.00 (C≡N), 145.37 (C^2), 149.76 (C^5), Py: 132.22 (C^i), 119.48 (C^3), 150.46 (C^2). Compound **6c**, yield 90%, yellow crystals, mp 153–155 °C (hexane). Found: C, 71.40; H, 5.20; N, 13.07. Calc. for $C_{19}H_{17}N_3O_2$: C, 71.46; H, 5.37; N 13.16). ν_{max}(KBr)/cm^{-1} 3089, 3065, 2987, 2924, 2225, 1605, 1521, 1469, 1451, 1427, 1380, 1303, 1294, 1257, 1203, 1176, 1041, 1030, 842 and 745; λ_{max}(EtOH)/nm 269 (lg ε 4.25); δ_H(400 MHz; $CDCl_3$) 1.57 (6H, s, 4-Me), 5.11 (2H, s, CH_2), 7.07, 8.53 (2H each, AA′BB′, *J* 9 Hz, C_6H_4), 7.39 (5H, m, Ph); δ_C(100 MHz; $CDCl_3$) 21.49 (4-Me), 69.87 (CH_2), 83.04 (C^4), 146.59 (C^2), 149.86 (C^5), 111.44 (C≡N), C_6H_4: 136.10 (C^i), 128.96 (C^o), 114.86 (C^m), 160.83 (C–O), Ph: 119.07 (C^i), 127.95 (C^p), 127.25, 128.43 (C^o, C^m).

5-Dialkylamino-4*H*-imidazole 3-oxides 7a–e, general procedure

The amine (piperidine (**7a** or **7b**) or liquid dimethylamine (**7c**–**e**), 3 mmol) was added to a solution of **6a**–**c** (2.5 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was allowed to stand overnight at room temperature. The reaction mixture was diluted with $CHCl_3$ (50 mL), washed with saturated solution of NaCl (10 mL) and dried over K_2CO_3. The solvent was removed in vacuum and the residue was triturated with Et_2O and the precipitate was filtered off to give **7a**–**e**. Compound **7a**, yield 90%, yellow crystals, mp 194–196 °C (THF–t-BuOMe). Found: C, 68.39; H, 8.65; N, 17.82. Calc. for $C_{18}H_{26}N_4O$: C, 68.76; H, 8.33; N, 17.82. ν_{max}(KBr)/cm^{-1} 2939, 2918, 2854, 1592, 1536, 1514, 1458, 1433, 1420, 1392, 1369, 1294, 1239, 1225, 1197, 1185, 1118, 1020, 945, 924, 897, 873, 854 and 738; λ_{max}(EtOH)/nm 329 (lg ε 4.40), 274 (3.47), 239 (3,70); δ_H(200 MHz; $CDCl_3$) 1.63 (6H,s, 4-Me), 1.69 (6H, br. m, C–CH_2–C), 2.99 (6H, s, NMe_2), 3.64 (4H, br. m, N–CH_2), 6.69, 8.57 (2H each, AA′BB′, *J* 9 Hz, Ar); δ_C(50 MHz; $CDCl_3$) 21.88 (4-Me), 39.91 (N–Me), 73.51 (C^4), 147.66 (C^2), 172.41 (C^5), Piperidine: 23.59, 25.37 and 46.21, Ar: 115.73 (C^i), 130.16 (C^o), 110.88 (C^m), 151.44 (C–N). Compound **7b**, yield 90%, yellow crystals, mp 148–158 °C dec. (THF). Found: C, 66.17; H, 7.52; N, 20.79. Calc. for $C_{15}H_{20}N_4O$: C, 66.15; H, 7.40; N, 20.57. ν_{max}(KBr)/cm^{-1} 2986, 2935, 2855, 1601, 1544, 1434, 1361, 1313, 1295, 1235, 1179, 1106, 912, 772 and 703; λ_{max}(EtOH)/nm 394 (lg ε 3.76), 266 (4.29); δ_H(200 MHz; $CDCl_3$) 1.64 (6H, s, 4-Me), 1.69 (6H, br. m, C–CH_2–C), 3.64 (4H, br. m, N–CH_2), 8.44, 8.67 (2H each, AA′BB′ *J* 6 Hz, 4-Py); δ_C(50 MHz; $CDCl_3$) 21.95 (4-Me), 75.49 (C^4), 145.00 (C^2), 171.41 (C^5), Piperidine: 23.83, 25.65 and 46.61, Py: 134.25 (C^i), 121.22 (C^3), 149.84 (C^2). **7c**, yield 90%, yellow crystals, mp 154–156 °C (THF). Found: C, 65.18; H, 8.09; N, 20.37. Calc. for $C_{15}H_{22}N_4O$: C, 65.67; H, 8.08; N, 20.42). ν_{max}(KBr)/cm^{-1} 2986, 2930, 2813, 1599, 1540, 1515, 1472, 1437, 1391, 1367, 1280, 1230, 1200, 1184, 1114, 945 and 833; λ_{max}(EtOH)/nm 332 (lg ε 4.46), 270 (3.77), 238 (4,01); δ_H(400 MHz; $CDCl_3$) 1.63 (6H,s, 4-Me), 3.02 (6H, s, Ar–NMe_2), 3.14 (6H, s, N=C–NMe_2), 6.68, 8.58 (2H each, AA′BB′, *J* 9 Hz, Ar); δ_C(100 MHz; $CDCl_3$) 21.27 (4-Me), 39.88 (N=C–N–Me), 39.88 (Ar–N–Me), 73.74 (C^4), 147.25 (C^2), 173.27 (C^5), Ar: 115.84 (C^i), 130.07 (C^o), 110.92 (C^m), 151.43 (C–N). Compound **7d**, yield 90%, yellow crystals, mp 148–152 °C (THF). Found: C, 62.06; H, 6.96; N, 24.37. Calc. for $C_{12}H_{16}N_4O$: C, 62.05; H, 6.94; N, 24.12. ν_{max}(KBr)/cm^{-1} 3047, 2941, 2898, 1600, 1556, 1514, 1474, 1443, 1406, 1371, 1315, 1226, 1219, 1225, 1197, 1125, 1066, 991, 934, 873, 827, 779 and 714; λ_{max}(EtOH)/nm 390 (lg ε 3.77), 263 (4.30); δ_H(200 MHz; $CDCl_3$) 1.64 (6H, s, 4-Me), 3.17 (6H, s, NMe_2), 8.43, 8.67 (2H each, AA′BB′ *J* 6 Hz, 4-Py); δ_C(50 MHz; $CDCl_3$) 21.34 (4-Me), 37.99 (N–Me), 75.75 (C^4), 144.89 (C^2), 172.43 (C^5), Py: 134.22 (C^i), 121.20 (C^3), 149.91 (C^2). Compound **7e**, yield 90%, yellow crystals, mp 240–245 °C (THF). Found: C, 70.92; H, 6.87; N, 12.48. Calc. for $C_{20}H_{23}N_3O_2$: C, 71.19; H, 6.87; N, 12.45. ν_{max}(KBr)/cm^{-1} 3063, 3003, 2978, 2932, 2885, 1598, 1535, 1502, 1474, 1415, 1391, 1368, 1301, 1279, 1248, 1190, 1170, 1115, 1014, 937, 873, 844, 772 and 746; λ_{max}(EtOH)/nm 361 (lg ε 3.76), 283 (4.51); δ_H(200 MHz; $CDCl_3$-CD_3OD) 1.81 (6H, s, 4-Me), 3.34 (6H, s, NMe_2), 5.26 (2H, s, CH_2), 7.22, 8.81 (2H each, AA′BB′, *J* 9 Hz, C_6H_4), 7.52 (5H, m, Ph); δ_C(100 MHz; $CDCl_3$) 21.07 (4-Me), 38.35 (N–Me), 70.28 (CH_2), 74.52 (C^4), 149.35 (C^2), 174.73 (C^5), C_6H_4: 136.81 (C^i), 131.37 (C^o), 114.77 (C^m), 161.18 (C–O), Ph: 120.74 (C^i), 128.91 (C^p), 127.62, 128.72 (C^o, C^m).

2,5-Dihydroimidazole-1-oxyls 8a–e, general procedure

A solution of EtMgBr (1 M) in THF was added dropwise to a stirred solution or a suspension of **7a**–**e** (2 mmol) in THF (10 mL). The reaction was controlled by TLC (Al_2O_3 Polygram Alox N/UV 254, Macherey-Nagel, eluent $CHCl_3$–methanol 50 : 1–2). Usually 3–5 mL of the organometallic reagent solution was sufficient for the reaction to be complete. The reaction mixture was allowed to stand for 0.5 h. Then water (1–3 mL) was added dropwise under vigorous stirring, the mixture was diluted with t-BuOMe (20 mL) and MnO_2 (3 g, 34.5 mmol) was added. The mixture was stirred vigorously for 2 h, the oxidant was filtered off and the filtrate was dried over Na_2CO_3. The solvent was removed in vacuum and the nitroxides **8a**–**f** were isolated from the residue by column chromatography on Al_2O_3, eluent $CHCl_3$. Compound **8a**, yield 80%, orange crystals, mp 90–92 °C (hexane). Found: C, 70.16; H, 9.15; N, 16.05. Calc. for $C_{20}H_{31}N_4O$: C, 69.93; H, 9.10; N, 16.31. ν_{max}(KBr)/cm^{-1} 2979, 2936, 2849, 2800, 1594, 1561, 1518, 1469, 1443, 1414, 1373, 1346, 1284, 1217, 1189, 1170, 1130, 1025, 946, 923, 894, 860 and 815; λ_{max}(EtOH)/nm 256 (lg ε 4.26), 229 (4.24). Compound **8b**, yield 80%, orange crystals, mp 110–112 °C (hexane). Found: C, 67.89; H, 8.50; N, 18.30. Calc. for $C_{17}H_{25}N_4O$: C, 67.74; H, 8.36; N, 18.59. ν_{max}(KBr)/cm^{-1} 2973, 2936, 2854, 1587, 1478, 1462, 1452, 1429, 1409, 1371, 1325, 1291, 1260, 1233, 1218, 1207, 1172, 1138, 1124, 1073, 1023, 993, 954, 893, 853 and 811; λ_{max}(EtOH)/nm 226 (lg ε 4.24). Compound **8c**, yield 90%, orange crystals, mp 109–111 °C (hexane). Found: C, 67.56; H, 8.83; N, 18.54. Calc. for $C_{17}H_{27}N_4O$: C, 67.29; H, 8.97; N, 18.46. ν_{max}(KBr)/cm^{-1} 2979, 2937, 2920, 2889, 2812, 1601, 1559, 1520, 1479, 1443, 1416, 1401, 1350, 1321, 1227, 1206, 1192, 1165, 1136, 1120, 1062, 960, 941, 932, 908, 826 and 810; λ_{max}(EtOH)/nm 256 (lg ε 4.24). Compound **8d**, yield 70%, orange crystals, mp 94–95 °C (hexane). Found: C, 64.43; H, 7.78; N, 21.45. Calc. for $C_{14}H_{21}N_4O$: C, 64.34; H, 8.10; N, 21.44. ν_{max}(KBr)/cm^{-1} 2966, 2937, 2875, 1600, 1590, 1497, 1467, 1409, 1403, 1366, 1327, 1291, 1274, 1233, 1139, 1120, 1067, 993, 959, 941, 912, 835 and 819; λ_{max}(EtOH)/nm 220 (lg ε 4.19). Compound **8e**, yield 70%, orange crystals, mp 91–93 °C (hexane). Found: C, 71.79; H, 7.72; N, 11.37. Calc. for $C_{22}H_{28}N_3O_2$: C, 72.10; H, 7.70; N, 11.47. ν_{max}(KBr)/cm^{-1} 2972, 2933, 2875, 1603, 1580, 1505, 1497, 1469, 1456, 1400, 1381, 1240, 1172, 1138, 1114, 1012, 827 and 759; λ_{max}(EtOH)/nm 265 (lg ε 3.25), 227 (4.37); pK = 5.7, Δa_N = 0.87 G.

5,5-Dimethyl-4-(dimethylamino)-2-(4-dimethylaminophenyl)-2-pyridine-4-yl-2,5-dihydro-1*H*-imidazol-1-oxyl (8f)

A solution of Grignard reagent was prepared from Mg (0.2 g, 8.3 mmol) and 4-bromo-N,N-dimethylaniline (1.5 g, 7.5 mmol) in THF (10 mL) was added dropwise to a stirred solution of **7c** (0.58 g, 2.5 mmol) in THF (10 mL). The reaction mixture was stirred for 0.5 h. Then water (1–3 mL) was added dropwise under vigorous stirring, the mixture was diluted with t-BuOMe (20 mL) and MnO_2 (3 g, 34.5 mmol) was added. The mixture was stirred vigorously for 2 h, the oxidant was filtered off and the filtrate was dried over Na_2CO_3. The solvent was removed in vacuum and the residue was purified by column chromatography on Al_2O_3, eluent $CHCl_3$ to give nitroxide **8f**, 0.5 g (66%), orange crystals, mp 171–174 °C (hexane–t-BuOMe 1 : 1). Found: C, 68.22; H, 7.85; N, 19.80. Calc. for $C_{20}H_{26}N_5O$: C, 68.15; H, 7.44; N, 19.87. ν_{max}(KBr)/cm^{-1} 3075, 2981, 2936, 2892, 2809, 1592, 1560, 1519, 1490, 1447, 1407, 1366, 1279, 1233, 1179, 1163, 1127, 1067, 951, 919, 852, 829 and 807; λ_{max}(EtOH)/nm 260 (lg ε 4.38); **8f**–H δ_H(200 MHz; D_2O–N_2D_4 1 : 10) 1.33 (3H, s, 5-Me), 1.44 (3H, s, 5-Me), 3.22 (9H, s, NMe_2), 3.34 (3H, s, NMe_2), 7.59, 7.63 (2H each, AA′BB′, *J* 9 Hz, Ar), 8.11, 8.77 (2H each, AA′BB′ *J* 6 Hz, 4-Py).

4-Dimethylamino-2-ethyl-2-(4-hydroxy-phenyl)-5,5-dimethyl-2,5-dihydro-1*H*-imidazol-1-oxyl (9)

Palladium catalyst (Pd/C, 10% Pd, 0.2 g) was added to a solution of **8e** (1 g, 2.7 mmol) in methanol (20 mL), the air in the flask was replaced with H_2 and the suspension was vigorously stirred at 25 °C until absorption of H_2 finished (*ca.* 90 mL, 4.05 mmol, of H_2 absorbed). The catalyst was filtered off, the methanol was removed in vacuum, the residue was dissolved in $CHCl_3$ (20 mL), MnO_2 (1 g, 11 mmol) was added and the mixture was stirred for 1 h. The oxidant was filtered off and $CHCl_3$ was removed under reduced pressure to give yellow crystalline residue of **9**, yield:

90%, mp 85–95 °C (hexane–EtOAc 3 : 1). Found: C, 61.23; H, 8.37; N, 14.07. Calc. for $C_{15}H_{22}N_3O_2$ H_2O: C, 61.20; H, 8.22; N, 14.27. ν_{max}(KBr)/cm^{-1} 1603, 1512, 1455, 1412, 1337, 1279, 1232, 1171, 1140, 1118 and 829; λ_{max}(EtOH)/nm 268 (lg ε 3.40), 226 (4.32).

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References

1 N. F. Sheppard, A. Guiseppi-Elie, in *The Measurement, Instrumentation and Sensors Handbook, Ch. 10: pH Measurement*, ed. J. G. Webster, CRC Press and IEEE Press, Boca Raton, Florida, 1999; G. Miesenbock, D. A. De Angelis and J. E. Rothman, *Nature*, 1998, **394**(6689), 192–5; H. J. Lin, P. Herman and J. R. Lakowicz, *Cytometry*, 2003, **52A**(2), 77–89; C. C. Overly, K. D. Lee, E. Berthiaume and P. J. Hollenbeck, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**(8), 3156–60; D. A. Russell, R. H. Pottier and D. P. Valenzeno, *J. Photochem. Photobiol., B*, 1995, **29**(1), 17–22.

2 R. J. Gillies, N. Raghunand, M. L. Garcia-Martin and R. A. Gatenby, *IEEE Eng. Med. Biol. Mag.*, 2004, **23**(5), 57–64; P. B. Barker, E. J. Butterworth, M. D. Boska, J. Nelson and K. M. Welch, *Magn. Reson. Med.*, 1999, **41**(2), 400–6; J. Zhou, J. F. Payen, D. A. Wilson, R. J. Traystman and P. C. van Zijl, *Nat. Med.*, 2003, **9**(8), 1085–90; N. Raghunand, S. Zhang, A. D. Sherry and R. J. Gillies, *Acad. Radiol.*, 2002, **9**(Suppl 2), S481–3; V. V. Khramtsov, I. A. Grigor'ev, M. A. Foster, D. J. Lurie, J. L. Zweier and P. Kuppusamy, *Spectroscopy*, 2004, **18**(Suppl 2), 213–225; M. A. Foster, I. A. Grigor'ev, D. J. Lurie, V. V. Khramtsov, S. McCallum, I. Panagiotelis, J. M. Hutchison, A. Koptioug and I. Nicholson, *Magn. Reson. Med.*, 2003, **49**(3), 558–67; C. Kroll, W. Hermann, R. Stosser, H. H. Borchert and K. Mader, *Pharm. Res.*, 2001, **18**(4), 525–30.

3 V. V. Khramtsov, I. A. Grigor'ev, M. A. Foster, D. J. Lurie and I. Nicholson, *Cell. Mol. Biol.*, 2000, **46**, 1361–1374.

4 V. V. Khramtsov, I. A. Grigor'ev, M. A. Foster and D. J. Lurie, *Antioxid. Redox Signaling*, 2004, **6**, 667–676.

5 V. V. Khramtsov and L. B. Volodarsky, in *Biological Magnetic Resonance, Volume 14: Spin Labeling, The Next Millennium*, ed. L. J. Berliner, Plenum Press, New York and London, 1998, 109–180.

6 V. V. Khramtsov, and L. M. Weiner, in *Imidazoline Nitroxides, vol. 2*, ed. L. B. Volodarsky, CRC Press, Boca Raton, 1988, p. 37–80.

7 I. A. Kirilyuk, T. G. Shevelev, D. A. Morozov, E. L. Khromovskih, N. G. Skuridin, V. V. Khramtsov and I. A. Grigor'ev, *Synthesis*, 2003, (6), 871–878.

8 I. A. Kirilyuk, A. A. Bobko, I. A. Grigor'ev and V. V. Khramtsov, *Org. Biomol. Chem.*, 2004, **2**, 1025–1030.

9 I. A. Kirilyuk, I. A. Grigor'ev and L. B. Volodarsky, *Izv. Sib. Otd. Akad. Nauk. SSSR, Ser. Khim. Nauk.*, 1989, **2**, 99; I. A. Kirilyuk, I. A. Grigor'ev and L. B. Volodarsky, *Chem. Abs.*, 1990, **111**, 232670.

10 L. B. Volodarskii and T. K. Sevastyanova, *Zh. Org. Khim.*, 1971, **7**(8), 1687–1692; L. B. Volodarskii and T. K. Sevastyanova, *Chem. Abs.*, 1971, **75**(8), 140425–1692.

11 M. Balakirev, V. Khramtsov, T. Berezina, V. Martin and L. Volodarsky, *Synthesis*, 1992, (12), 1223.