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# Synthesis of the tetraethyl substituted pH-sensitive nitroxides of imidazole series with enhanced stability towards reduction

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The synthesis of 2,2,5,5-tetraethylimidazole nitroxides from 3-ethylpent-2-ene is described. The newly synthesized nitroxides, namely 4-methyl-2,2,5,5-tetraethyl-2,5-dihydro-1*H*-imidazol-1-yloxy (**1**), 3,4-dimethyl-2,2,5,5-tetraethylperhydroimidazol-1-yloxy (**2**) and 2,2,5,5-tetraethyl-4-pyrrolidin-1-yl-2,5-dihydro-1*H*-imidazol-1-oxyl (**3**), were found to be pH sensitive spin probes, with p*K* values of 1.2, 4.95 and 7.4, respectively. The most important finding was the fact that these new nitroxides were 20–30 times more stable in the presence of ascorbate and had significantly longer halflifes in rat blood as compared to 2,2,5,5-tetramethyl analogs. The latter observation provides a unique advantage for the application of tetraethyl substituted imidazole nitroxides as functional EPR probes.

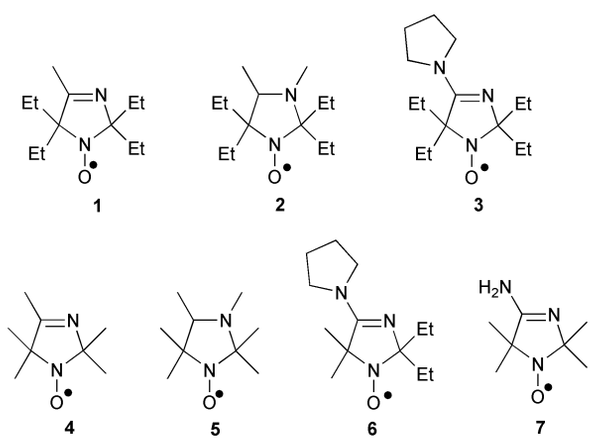
## Introduction

The range of applications of stable nitroxides is immense and involves living polymerisation,[1] magnetic materials,[2] synthetic tools,[3] probes for measurement of oxygen[4] and pH[5,6] in living tissues, structural studies of biological macromolecules,[7] therapeutic agents,[8] radical scavengers,[9] MRI contrast agents,[10,11] and other fields. The unique properties of the nitroxides originate from the presence of an unpaired electron in their structure. However instability of the paramagnetic N–O$^{\bullet}$ fragment towards chemical reduction in many systems is an unavoidable factor that significantly limits the use of nitroxides in many applications, particularly in biological systems.

The reduction rates of nitroxides vary significantly depending on their structure and microenvironment.[10,12–16] The 5-membered nitroxides[14] including imidazoline nitroxides[15] were found to be among the most stable.

Recently it has been shown that 1,1,3,3-tetraethylisoindoline nitroxides have significantly increased stability towards reduction compared to the 1,1,3,3-tetramethyl analogs.[17] Introduction of bulky alkyl substituents into neighboring positions of the nitroxide group might be a useful approach to increase stability of other classes of nitroxides as well. The imidazoline radicals have an additional advantage over other nitroxide classes as functional EPR probes due to their use for measurement of pH and thiols, including *in vivo*.[5,6,18,19] Therefore synthesis of new imidazoline radicals with enhanced stability towards reduction may have a significant impact on the applications of nitroxides as functional EPR probes as well as beyond this field.

In the present work we describe the synthesis of tetraethyl substituted pH-sensitive nitroxides of imidazoline and imidazolidine series, **1**–**3** (Scheme 1). Stability of the new nitroxides towards reduction and sensitivity of their EPR spectra to pH were studied and compared to corresponding data for their analogs **4**–**7**.




**Scheme 1**

## Results and discussion

### Synthesis

Common synthetic routes for obtaining basic structures, **4**, **5**, and **7** of imidazoline and imidazolidine radicals were described earlier.[20,21] Recently we employed organometallic reagent addition to 4*H*-imidazol-3-oxides for the synthesis of 4-amino-3-imidazoline-1-oxyls,[22] *e.g.* the nitroxide **6**. The latter approach significantly increases the number of available structures. In current studies we modified the above approaches to synthesize tetraethyl substituted pH-sensitive nitroxides **1**–**3**. The nitroxides **1**–**3** were prepared from 3-ethylpent-2-ene **8** (Scheme 2). The 3-ethylpent-2-ene nitrosochloride **9** was prepared using the procedure described for preparation of 2-nitroso-3-chloro-3-methylbutane dimer.[20,23] Following treatment with hydroxylamine acetate and hydrolysis (*cf* ref. 24) gave the key compound 3-hydroxyamino-3-ethylpentan-2-one hydrochloride **11**. The condensation of **11** with pentan-3-one and ammonium acetate was performed according to a modified procedure described

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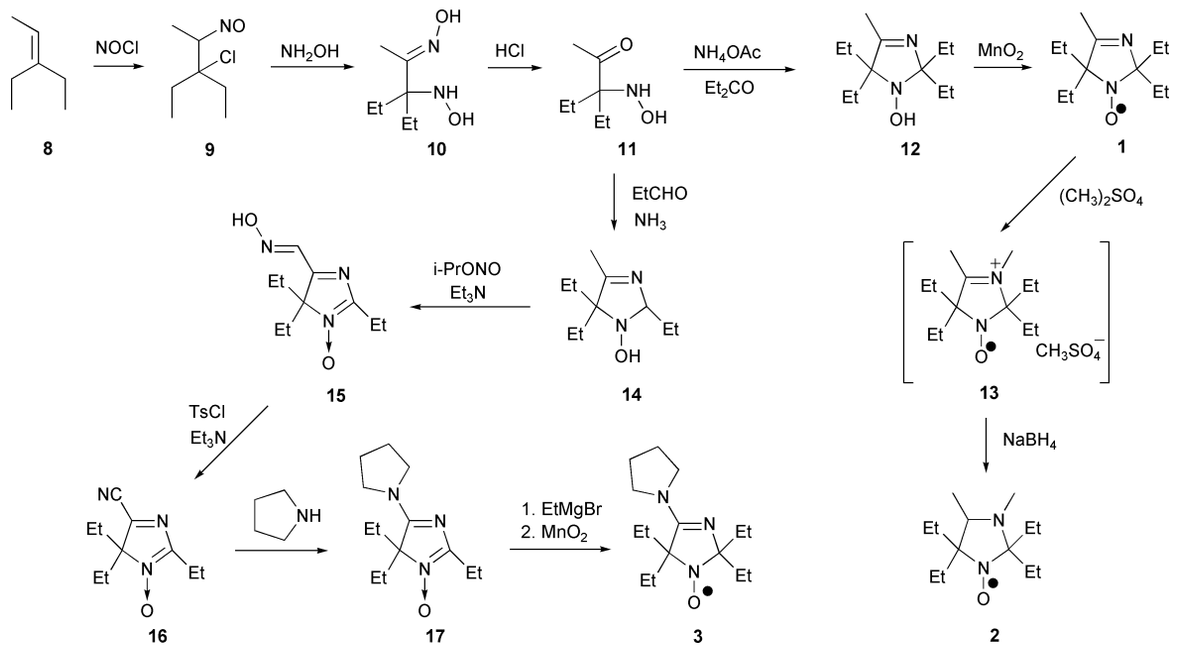


**Scheme 2**

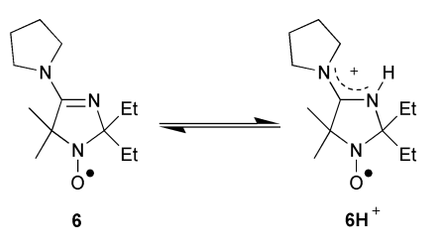
in.[25] A good yield of 4-methyl-2,2,5,5-tetraethyl-2,5-dihydro-1*H*-imidazole-1-ol **12** was obtained when the reaction was carried out under argon to avoid *in situ* oxidation. Pure imidazole **12** was converted into 4-methyl-2,2,5,5-tetraethyl-2,5-dihydro-1*H*-imidazol-1-yloxy, **1**, using careful oxidation with $MnO_2$. Alkylation of **1** with dimethylsulfate gives a highly hygroscopic imidazolinium salt **13** which was immediately, without isolation, converted into nitroxide **2** (*cf* ref. 21).

The nitroxide **3** was prepared from hydroxyaminoketone **11** using the procedures for the synthesis of **6** as described in reference 22.

### Effect of pH on EPR spectra of the nitroxides 1–7

The EPR spectra of imidazoline and imidazolidine nitroxides are known to be highly sensitive to the pH of the medium due to reversible protonation of the N-3 atom of the heterocycle.[26] The range of sensitivity of the EPR spectrum of the particular nitroxide to pH is within about 3 pH units centered on the p*K* value for its protonation. A significant effect of the substitution at carbons C-2 and C-4 of the heterocycle on the p*K* value was found. As a consequence, a wide set of pH sensitive nitroxides, valid for various ranges of pH assessment, was proposed.[18] However a lack of useful spin pH probes with a p*K* value about 7.0–7.4 was also recognized.[5]

Fig. 1 demonstrates the EPR spectra of the nitroxide **6** at various pH values. At pH 7.0 the EPR spectrum shows superposition of the spectra of protonated, $\mathbf{6H^+}$, and unprotonated, **6**, forms (Scheme 3), which differ in their values of hyperfine splitting, $a_N$, and *g*-factor. This is in agreement with $R \leftrightarrows RH^+$ chemical exchange being slow on an EPR timescale.



**Scheme 3**

The dependence of $a_N$ on pH allows its approximation with a conventional titration curve yielding a p*K* value of 7.3 (see Fig. 2). An even higher p*K* value, 7.4, has been found for nitroxide **3** (see Table 1 for spectral parameters). A similar effect of methyl group substitution for the ethyl group on p*K* values was observed for the imidazolidine nitroxides (4.7 and 4.95 for the

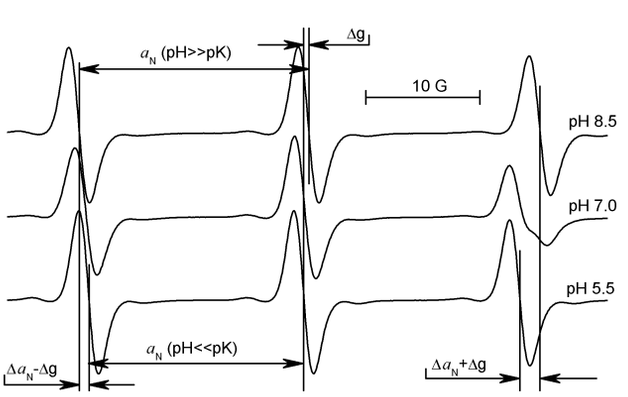



**Fig. 1** EPR spectra of the nitroxide **6** (p*K* = 7.3) at various pH values. The spectrometer settings were as follows: modulation amplitude, 0.5 G; microwave power, 20 mW; time constant, 100 ms; sweep time, 100 s.

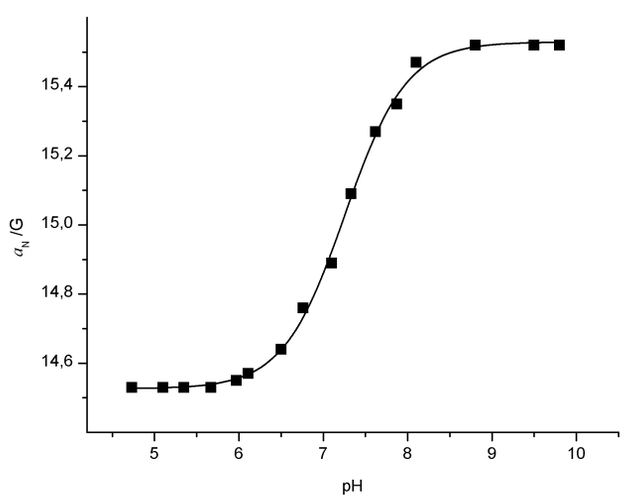



**Fig. 2** The pH dependence of hyperfine splitting, $a_N$, measured as a distance between low- and central-field components of the EPR spectra of the nitroxide **6**. The solid line is a nonlinear least-squares fit of the data to a conventional titration curve (eqn 1, see Experimental) yielding $a_N(\mathbf{6}) = 15.5$ G, $a_N(\mathbf{6H^+}) = 14.5$ G, pK = 7.3.

**Table 1** The differences in hfi splitting constants ($\Delta a_N$) and g-factors ($\Delta g$) between protonated and unprotonated forms of the radicals 1–7[a], their p$K$ values, bimolecular rate constants for the reduction by ascorbic acid ($k$), and initial rates of the reduction in the rat blood ($V_0$)

| | Nitroxides | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| $\Delta a_N$/G | 0.9 | 1.3 | 0.9 | 0.9 | 1.3 | 1.0 | 0.8 |
| $\Delta g$ | 0.0003 | 0.0003 | 0.0003 | 0.0002 | 0.0003 | 0.0003 | 0.0002 |
| p$K$ | 1.2 | 4.95 | 7.4 | 1.3 | 4.7 | 7.3 | 6.1 |
| $k$/M$^{-1}$ s$^{-1}$ | 0.5±0.3 | 0.04±0.003 | 0.74±0.07 | 5.6±0.3 | 0.85±0.05 | 13.4±0.9 | 22.5±1.6 |
| $V_0$/μM min$^{-1}$ | 0.033 | <0.01 | 1.0 | nd | 0.22 | 1.8 | 2.4 |

[a] $\Delta a_N$, $\Delta g$, and p$K$ values for the radicals **4**–**7** are taken from refs. 18, 22.

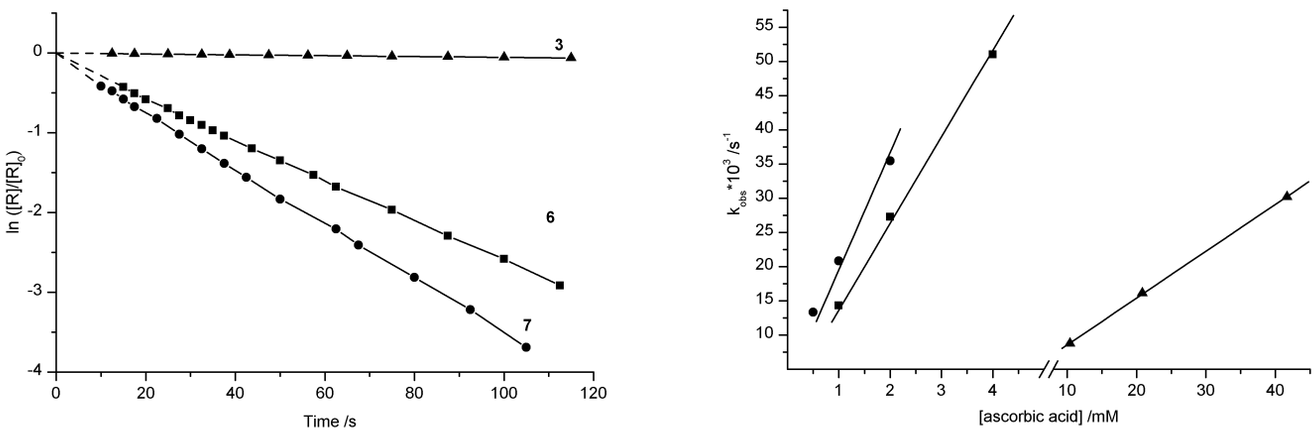



**Fig. 3** **Left.** The ratio of current, [R], and initial, $[R]_0$ concentrations of the imidazoline nitroxides **3**, **6**, and **7** measured by EPR in 0.1 M Na-phosphate buffer, pH 7.5, in the presence of 1 mM ascorbic acid plotted *versus* time. Initial concentration of the nitroxides was 0.1 mM. The experimental data support exponential decay of the EPR signal upon the reduction according to the equation: $[R] = [R]_0\exp(-k_{obs}t)$; **Right.** The dependence of pseudo-first order rate constant of reduction, $k_{obs}$, of nitroxides **3**, **6**, and **7** on the concentration of ascorbate. Solid lines represent linear extrapolation yielding the bimolecular rate constants (see Table 1).

radicals **5** and **2**, correspondingly). The increase of the p$K$ makes the new spin pH probes **2** and **3** more appropriate for application in the physiological pH range.

### The comparative study of the nitroxides reduction with ascorbic acid

Ascorbic acid is a principal reducing agent responsible for the nitroxides reduction in biological fluids.[12] The reduction of the nitroxides **1**–**7** with ascorbic acid in phosphate buffer at pH 7.5 was studied. The typical kinetics of the reduction of the imidazoline nitroxides **3**, **6** and **7** with various numbers of ethyl groups in the vicinity of the radical fragment are shown in Fig. 3. The bimolecular rate constants for the reduction of the nitroxides **1**–**7** with ascorbate are given in the Table 1. Introduction of bulky ethyl groups surrounding the nitroxide moiety, N–O$^{\bullet}$, results in slowing down the reduction of the nitroxide. The 2,2,5,5-tetraethyl substituted nitroxides were found to be 20–30 times more stable towards the reduction by ascorbate as compared to that of their tetramethyl substituted analogs (*cf*. the rate constants for imidazoline nitroxides **3** and **7**, and imidazolidine nitroxides **2** and **5**, Table 1).

Note also that the observed higher resistance against the reduction of the imidazolidine nitroxides compared to that for imidazoline nitroxides is in agreement with earlier reported data.[15]

### The study of the reduction of the nitroxides in the rat blood

The 2,2,5,5-tetraethyl substituted nitroxides **1**–**3** showed excellent stability in rat blood. Typical decay kinetics of the nitroxides **1**–**3** and **5**–**7** are shown in the Fig. 4. Table 1 lists the initial rates of the reduction. The data demonstrate the beneficial effect of the ethyl groups introduced in the vicinity of the N–O$^{\bullet}$ fragment on their stability towards reduction, the most stable being the tetraethyl substituted imidazolidine nitroxide **2**. The

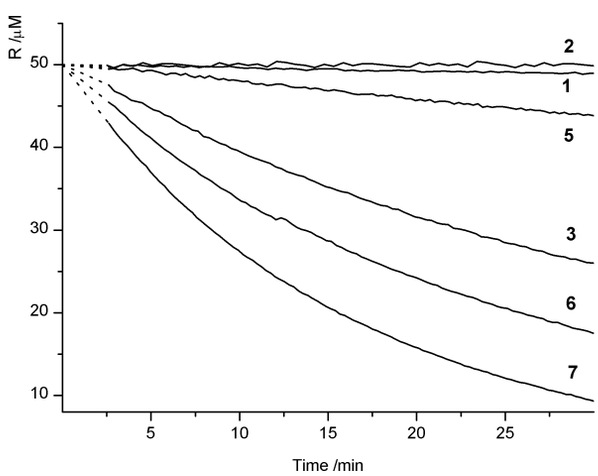



**Fig. 4** The kinetics of the reduction of the radicals **1**–**3**, and **5**–**7** in rat blood measured by EPR.

absence of a quantitative correlation between the reduction of the nitroxides by ascorbate and their reduction by the blood (see Table 1) denotes a complex mechanism for the nitroxides reduction in the blood. This may imply penetration of the nitroxides through the erythrocyte membrane and eventual intracellular reduction by thiols.[12,27]

## Conclusion

Introduction of ethyl groups instead of methyl groups to the α-carbon of the imidazoline and imidazolidine nitroxides results in significant protection of the nitroxyl group against reduction in aqueous solutions of ascorbic acid or in blood samples, presumably due to steric hindrance of the radical fragment. In addition, this substitution shifts the p$K$ values of

the imidazoline spin pH probes to the favorable region of neutral pH, making them particularly useful for biological applications.

## Experimental

The IR spectra were recorded on Bruker Vector 22 FT-IR spectrometer in KBr pellets (the concentration was 0.25%; the pellet thickness was 1 mm). The UV spectra were measured on HP Agilent 8453 spectrometer in EtOH. The $^{1}$H NMR spectra were recorded on a Bruker AC-200 (200.132 MHz) spectrometer for 5% solutions using the signal of the solvent as the standard. The $^{13}$C NMR spectra were recorded on Bruker AC-200 (50.323 MHz) and 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 made based on analysis of intensities, on the spectra measured in J-modulation mode, and using the data reported previously.[22,28] EPR spectra were recorded on a Bruker ER-200D-SRC spectrometer using 100 μl quartz capillary or 200 μl EPR flat cells.

### 3-Chloro-3-ethyl 2-nitrosopentane dimer (9)

A suspension of $NaNO_2$ (105 g, 1.5 mol) in a solution of 3-ethylpent-2-ene **9** (115 ml 84 g, 0.85 mol) in methanol (450 ml) was cooled to −15–−5 °C, and 36% hydrochloric acid (270 ml, 3.2 mol) was added dropwise within 2 h to the stirred suspension keeping the temperature of the reaction mixture below −5 °C. The reaction mixture was stirred for 3 h and then poured into cold water (2 L). Precipitate of nitrosochloride **9** was filtered off, washed with cold water and dried at 25 °C. Yield: 130 g, 93%, colorless crystals, mp 67–71 °C (hexane) (Found: C, 51.24; H, 8.64; N, 8.69, Cl 20.50. Calc. for $C_7H_{14}ClNO$: C, 51.38; H, 8.62; N, 8.56, Cl 21.66%); $\nu_{max}$(KBr)/cm$^{-1}$ 2979, 2947, 1453, 1386, 1373, 1448, 1294, 1197, 1122, 1109, 1009, 938 and 841; $\delta_H$(200 MHz; $CDCl_3$) 1.00 (12 H, t, *J* 7.5, 4 × $CH_3$, Et), 1.44 (6 H, d, *J* 7.0, 2 × $CH_3$, CH–$CH_3$), 2.00 (8 H, m, 4 × $CH_2$, Et) and 6.02 (2 H, quartet, *J* 7.0, 2 × CH, CH–$CH_3$); signals of nitroso monomer (~20%) 0.94 (6 H, t, *J* 7.5, 2 × $CH_3$, Et), 1.41 (3 H, d, *J* 7.0, $CH_3$, CH–$CH_3$), 2.00 (4 H, m, 2 × $CH_2$, Et) and 5.93 (2 H, quartet, *J* 7.0, CH, CH–$CH_3$).

### 3-Hydroxyamino-3-ethylpentan-2-one oxime (10)

A solution of hydroxylamine hydrochloride (120 g, 1.71 mol) in water (235 ml) was added to a solution of NaOAc (140 g, 1.71 mol) in water (235 ml). The resulting solution was diluted with ethanol (1 L) and the precipitate of NaCl was filtered off. Nitrosochloride **9** (130 g) was added to the filtrate and the solution was heated under reflux for 3 h and allowed to stand overnight. The ethanol was removed under reduced pressure, and the residue was shaken with water (0.8 L), saturated NaCl solution (0.5 L), acetic acid (50 ml) and hexane (200 ml). The organic layer was separated, and the water layer was extracted with hexane (200 ml). Combined organic phase was washed with a mixture of saturated NaCl solution (50 ml) and acetic acid (5 ml). Solid $KHCO_3$ was added portionwise to a stirred combined water solution to pH 9 and the precipitate of oxime **10** was filtered off, washed with water and dried at 25 °C. Yield: 77 g, 60%, colorless crystals, mp 115–117 °C (hexane–ethyl acetate 1 : 1) (Found: C, 52.25; H, 9.71; N, 17.39. Calc. for $C_7H_{16}N_2O_2$: C, 52.48; H, 10.07; N, 17.48%); $\nu_{max}$(KBr)/cm$^{-1}$ 3302, 3244, 2978, 2944, 2847, 1489, 1460, 1430, 1385, 1294, 1183, 1083, 1031, 999, 976, 945, 925 and 868; $\delta_H$(200 MHz; $(CD_3)_2SO$) 0.67 (6 H, t, *J* 8, 2 × $CH_3$, Et), 1.44 (4 H, quartet, *J* 8, 2 × $CH_2$, Et), 1.69 (3 H, s, $CH_3$), 4.10 (1 H, br s, NH), 6.80 (1 H, br s, OH) and 10.40 (1 H, br s, C=NOH); $\delta_C$(50 MHz; $(CD_3)_2SO$) 7.61 ($CH_3$, Et), 23.26 ($CH_2$, Et), 9.77 ($CH_3$–C=N), 66.74 ($C(Et)_2$) and 158.58 (C=N).

### 3-Hydroxyamino-3-ethylpentan-2-one hydrochloride (11)

A solution of oxime **10** (50 g, 0.31 mol) in concd. hydrochloric acid (100 ml) was saturated with gaseous HCl and the resulting suspension of hydroxylamine hydrochloride was allowed to stand at 0 °C for 24 h. The hydroxylamine hydrochloride was filtered off and the hydrochloric acid was removed under reduced pressure (40 °C). The crystalline residue was stirred with dry acetonitrile (250 ml), the precipitate of hydroxylamine hydrochloride was filtered off and the solution was diluted with diethyl ether (250 ml) and allowed to stand at −5 °C for 24 h. The hydrochloride **11** (colorless crystals) was filtered off and washed with dry THF. Yield 34 g, 60%, mp 113–117 °C (reprecipitated from acetonitrile with diethyl ether) (Found: C, 46.14; H, 8.54; N, 7.69, Cl 19.20. Calc. for $C_7H_{16}ClNO_2$: C, 46.28; H, 8.88; N, 7.71, Cl 19.52%); $\nu_{max}$(KBr)/cm$^{-1}$ 1711, 3244, 1579, 1466, 1435, 1370, 1297, 1224, 1136, 883 and 762; $\delta_H$(200 MHz; $(CD_3)_2SO$) 0.81 (6 H, t, *J* 8, 2 × $CH_3$, Et), 1.86 (4 H, quartet, *J* 8, 2 × $CH_2$, Et) and 2.25 (3 H, s, $CH_3$); $\delta_C$(50 MHz; $(CD_3)_2SO$) 7.71 ($CH_3$, Et), 23.61 ($CH_2$, Et), 26.08 ($CH_3$–C=O), 76.50 ($C(Et)_2$) and 205.45 (C=O).

### 4-Methyl-2,2,5,5-tetraethyl-2,5-dihydro-1*H*-imidazole-1-ol (12)

A suspension of hydroxyaminoketone **11** (3 g, 16.5 mmol) and ammonium acetate (4.5 g, 60 mmol) in a mixture of pentan-3-one (6 ml, 57 mmol) and methanol (5 ml) was stirred under argon for 3 weeks. The resulting solution was extracted with diethyl ether (3 × 10 ml), the ether extract was thoroughly washed with water (5 × 10 ml) and with saturated $KHCO_3$ solution and dried over $Na_2CO_3$. The ether was removed under reduced pressure and the residue was triturated with hexane, the crystalline precipitate of **12** was filtered off and washed with hexane, yield 2 g, 57%, colorless crystals, mp 119–121 (hexane) (Found: C, 67.93; H, 11.54; N, 13.37. Calc. for $C_{12}H_{24}N_2O$: C, 67.88; H, 11.39; N, 13.19%); $\nu_{max}$(KBr)/cm$^{-1}$ 2966, 2937, 2879, 1660, 1460, 1432, 1378, 1038, 938 and 898; $\delta_H$(200 MHz; $CDCl_3$) 0.90 (6 H, br m, 2 × $CH_3$, Et), 0.96 (6 H, br m, 2 × $CH_3$, Et), 1.70 (8 H, br m, 4 × $CH_2$, Et), 1.91 (3 H, s, $CH_3$C=N) and 4.65 (1 H, br s, OH); $\delta_C$(50 MHz; $CDCl_3$) 7.56, 8.33 ($CH_3$, Et), 26.35, 27.56 ($CH_2$, Et), 15.90 ($CH_3$–C=N), 77.39 ($C^5$), 93.15 ($C^2$) and 171.79 (C=N).

### 4-Methyl-2,2,5,5-tetraethyl-2,5-dihydro-1*H*-imidazol-1-yloxy (1)

Manganese dioxide (2 g) was added to a stirred solution of **12** (1 g, 4.7 mmol) in chloroform (20 ml). The suspension was stirred for 0.5 h, manganese oxides were filtered off and the filtrate was evaporated under reduced pressure to leave an orange oil, which was purified by column chromatography on silica (Kieselgel 60, Merck) using diethyl ether–hexane 1 : 20 mixture as eluent to give nitroxide **1** (1.9 g, 95%) as an orange oil (Found: C, 68.14, H, 10.95; N, 13.28. Calc. for $C_{12}H_{23}N_2O$: C, 68.20; H, 10.97; N, 13.26%); $\nu_{max}$(neat)/cm$^{-1}$ 2971, 2940, 2881, 1640, 1461, 1428, 1383, 1278, 1151, 957, 931, 901 and 831.

### 3,4-Dimethyl-2,2,5,5-tetraethylperhydroimidazol-1-yloxy (2)

Dimethyl sulfate (0.5 g, 4 mmol) was added to a solution of **5** (0.5 g, 2.4 mmol) in dry diethyl ether (3 ml). The solution was allowed to stand for 0.5 h at 25 °C, filtered and diethyl ether was removed under reduced pressure. The residue was heated to 50 °C for 30 min under reduced pressure and then triturated with dry diethyl ether to form a highly hygroscopic yellow crystalline precipitate of quaternary salt **13**. The precipitate was filtered off (without drying), washed with dry diethyl ether and immediately dissolved in ethanol (5 ml). $NaBH_4$ (150 mg, 4 mmol) was added to the solution and the mixture was stirred for 1 h. Ethanol was removed under reduced pressure, the residue was dissolved in water (2 ml) and extracted with diethyl ether (3 × 2 ml). The extract was dried over $Na_2CO_3$, diethyl

ether was removed under reduced pressure to leave a yellow oil, which was purified by column chromatography on silica (Kieselgel 60, Merck), using a mixture of diethyl ether–hexane 1 : 20 as eluent to give the nitroxide **2** (320 mg, 60%) as a yellow oil (mp *ca*. 15 °C) (Found: C, 69.04, H, 11.95; N, 12.78. Calc. for $C_{13}H_{27}N_2O$: C, 68.67; H, 11.97; N, 12.32%); $\nu_{max}$(neat)/cm$^{-1}$ 2971, 2938, 2881, 2792, 1462, 1383, 1337, 1243, 1222, 972, 938 and 830.

### 4-Methyl-2,5,5-triethyl-2,5-dihydro-1*H*-imidazole-1-ol (14)

A mixture of propanal (2.8 mL, 38 mmol), hydroxyaminoketone **11** (6 g, 33 mmol), ethanol (5 mL) and of 25% aqueous ammonia (10 mL) was stirred for 2 h at 25 °C and allowed to stand at −5 °C overnight. The crystalline precipitate was filtered off, washed with cold 50% EtOH to give dihydroimidazole **14** (5.6 g, 90%) as colorless crystals, mp 114–115 °C (hexane) (Found: C, 64.94; H, 11.35; N, 15.11. Calc. for $C_{10}H_{20}N_2O$: C, 65.18; H, 10.94; N, 15.20%); $\nu_{max}$(KBr)/cm$^{-1}$ 2962, 2926, 2868, 1650, 1456, 1430, 1386, 1033, 1017 and 903; $\delta_H$(200 MHz; $(CD_3)_2CO$) 0.78 (3 H, t, *J* 8.0, $CH_3$, Et), 0.84 (3H, t, *J* 8.0, $CH_3$, Et), 0.95 (3 H, t, *J* 8.0, $CH_3$, Et), 1.36 (1 H, m, $CH_2$, Et), 1.49 (2 H, m, $CH_2$, Et), 1,53 (1H, m, $CH_2$, Et), 1.67 (1 H, m, $CH_2$, Et), 2.02 (1 H, m, $CH_2$, Et), 1.82 (3 H, d, *J* 2, $CH_3$C=N), 4.50 (1 H, m, CH) and 7.15 (1 H, s, OH). $\delta_C$(100 MHz; $(CD_3)_2CO$) 8.86, 9.66, 10.04 ($CH_3$, Et), 26.54, 29.15, 30.17 ($CH_2$, Et), 17.03 ($CH_3$–C=N), 79.09 (C$^5$), 94.11 (C$^2$) and 174.86 (C=N).

### 2,4,4-Triethyl-4*H*-imidazole-5-carbaldehyde oxime 3-oxide (15)

Isopropyl nitrite (4 mL, 45 mmol) was added portionwise to a solution of dihydroimidazole **14** (5 g, 27 mmol) in $CH_2Cl_2$ (10 ml), $CCl_4$ (10 ml) and triethylamine (1 mL) during 3 h. Then another portion of isopropyl nitrite (8 ml, 90 mmol) was added and the solution was allowed to stand for 5 h. The mixture was evaporated under reduced pressure to leave an orange oil, which was dissolved in a mixture of diethyl ether (100 ml) and water (100 ml). The mixture was shaken vigorously, the organic layer was separated and extracted with 1% water solution of NaOH, 5 × 100 ml. Combined water extracts were saturated with NaCl, acidified with HOAc to pH 5–6 and oxime **15** was extracted back with $CHCl_3$ (3 × 50 ml). The $CHCl_3$ extract was dried over $MgSO_4$, $CHCl_3$ was removed under reduced pressure and the residue was triturated with EtOAc, the precipitate was filtered off and recrystallized from *t*-BuOMe to yield oxime **15** (4.5 g, 80%) as yellow crystals, mp 113–115 °C (*t*-BuOMe) (Found: C, 56.55; H, 8.45; N, 19.40. Calc. for $C_{10}H_{17}N_3O_2$: C, 56.85; H, 8.11; N, 19.89%); $\nu_{max}$(KBr)/cm$^{-1}$ 1544, 1459, 1365, 1330, 1218, 1025, 1002, 882 and 734; $\lambda_{max}$(EtOH)/nm 367 (log $\varepsilon$ 3.84), 275 (3.44) and 229 (4.07); $\delta_H$(200 MHz; $(CD_3)_2CO$) 0.51 (6 H, t, *J* 7.5, 2 × $CH_3$, 4-Et), 1.28 (3 H, t, *J* 7.5, $CH_3$, 2-Et), 2.15 (4 H, quartet, *J* 7.5, 2 × $CH_2$, 4-Et), 2.83 (3 H, quartet, *J* 7.5, $CH_2$, 2-Et), 7.97 (1 H, s, CH=N) and 12.17 (1 H, s, OH); $\delta_C$(50 MHz; $(CD_3)_2CO$) 6.55 ($CH_3$, 4-Et), 9.02 ($CH_3$, 2-Et), 18.49 ($CH_2$, 2-Et), 29.95 ($CH_2$, 4-Et), 88.19 (C$^4$), 144.12 (CH=N), 155.41 (C$^2$) and 170.05 (C=N).

### 4,4-Trimethyl-4*H*-imidazole-5-carbonitrile 3-oxide (16)

TsCl (9.5 g, 50 mmol) was added portionwise to a stirred solution of oxime **15** (10.55 g 50 mmol) 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 under reduced pressure to leave an orange oil, which was purified by column chromatography (Kieselgel 60, Merck), using dichloromethane as eluent to give nitrile **16** (4.8 g, 85%), yellow crystals, mp 25–30 °C (pentane) (Found: C, 61.19; H, 7.71; N, 21.03. Calc. for $C_{10}H_{15}N_3O$: C, 62.15; H, 7.82; N, 21.74%); $\nu_{max}$(neat, 30 °C)/cm$^{-1}$ 2977, 2939, 2882, 2217, 1532, 1478, 1456, 1399, 1306, 1179, 1057, 854, 799 and 707; $\lambda_{max}$(EtOH)/nm 375 (log $\varepsilon$ 4.08); $\delta_H$(200 MHz; $(CDCl_3)$ 0.62 (6H, t, *J* 7.5, 2 × $CH_3$, 4-Et), 1.25 (3H, t, *J* 7.5, $CH_3$, 2-Et), 2.02 (4H, m, 2 × $CH_2$, 4-Et) and 2.79 (2H, quartet, *J* 7.5, $CH_2$, 2-Et); $\delta_C$(50 MHz; $(CD_3)_2CO$–$CCl_4$ 1 : 5) 4.21 ($CH_3$, 4-Et), 6.87 ($CH_3$, 2-Et), 16.03 ($CH_2$, 2-Et), 25.78 ($CH_2$, 4-Et), 87.66 (C$^4$), 108.91 (C≡N), 143.70 (C$^2$) and 152.33 (C=N).

### 5-Pyrrolidin-1-yl-2,4,4-triethyl-4*H*-imidazol 3-oxide (17)

Pyrrolidine (1 ml, 12 mmol) was added to a solution of nitrile **16** (1 g, 5.2 mmol) in $CH_2Cl_2$ (2 mL) and the mixture was allowed to stand overnight. The solution was evaporated under reduced poressure to leave brown oil, which was dissolved in a mixture of diethyl ether (20 ml) and water (20 ml). The mixture was shaken vigorously, organic layer was separated and extracted with water 5 × 10 ml. The combined water extracts were saturated with NaCl, and basified with $Na_2CO_3$ (1 g), and then pyrrolidinoimidazole **17** was extracted back with $CHCl_3$ (3 × 5 ml). The $CHCl_3$ extract was dried over $Na_2CO_3$, $CHCl_3$ was removed *in vacuo* and the residue was separated by column chromatography on aluminium oxide (neutral) using chloroform as eluent to give pyrrolidinoimidazole **17** (0.7 g, 57%) as an orange oil; $\nu_{max}$(neat)/cm$^{-1}$ 2973, 2937, 2878, 1607, 1558, 1459, 1215, 1177, 1081, 1057, 713 cm$^{-1}$; $\delta_H$(200 MHz; $(CDCl_3)$ 0.65 (6 H, t, *J* 7.5, 2 × $CH_3$, 4-Et), 1.22 (3 H, t, *J* 7.5, $CH_3$, 2-Et), 1.90 (8 H, br m, $CH_2$, 4-Et and 2 × $CH_2$ pyrrolidin-1-yl), 2.68 (2 H, quartet, *J* 7.5, $CH_2$, 2-Et), 3.58 (4 H, br m, 2 × $CH_2$ pyrrolidin-1-yl); the compound was used for preparation of the nitroxide **3** without further purification and characterized as hydrochloride, 2,4,4-triethyl-5-pyrrolidin-1-yl-4*H*-imidazol 3-oxide hydrochloride **17** × HCl. A solution of pyrrolidinoimidazole **17** (0.7 g, 6.8 mmol) in ethanol (10 mL) was gently acidified with concd. hydrochloric acid to pH 3 and evaporated under reduced pressure, the residue was dried in vacuum, dissolved in propan-2-ol (10 ml) and diluted with diethyl ether (20 mL). The crystalline brownish precipitate of hydrochloride **17** × HCl was filtered off, washed with THF and dried at 25 °C, mp 148–151 °C (Found: C, 56.78, H, 8.77, N, 15.08, Cl 12.80. Calc. for $C_{13}H_{24}N_3OCl$: C, 57.03, H, 8.84; N, 15.35, Cl, 12.95%); $\nu_{max}$(KBr)/cm$^{-1}$ 1633, 1572, 1462, 1422, 1383, 1346, 1192, 1173, 1065, 948 and 907; $\lambda_{max}$(EtOH)/nm 312 (log $\varepsilon$ 4.01); $\delta_H$(200 MHz; $(CDCl_3)$ 0.67 (6 H, br t, *J* 6.5, 2 × $CH_3$, 4-Et), 1.20 (3 H, t, *J* 7.5, $CH_3$, 2-Et), 2.02 (8 H, br m, $CH_2$, 4-Et and 2 × $CH_2$ pyrrolidin-1-yl), 2.76 (2 H, quartet, *J* 7.5, $CH_2$, 2-Et), 3.69 (4 H, br m, 2 × $CH_2$ pyrrolidin-1-yl) and 12.44 (1 H, br s, HCl); $\delta_C$(50 MHz; $(CDCl_3)$ 7.66 ($CH_3$, 4-Et), 9.62 ($CH_3$, 2-Et), 20.77 ($CH_2$, 2-Et), 24.61 ($CH_2$, 4-Et), 23.05, 25.94, 47.38, 50.19 (pyrrolidin-1-yl), 80.35 (C$^4$), 176.66 (C$^2$) and 177.63 (C$^5$).

### 2,2,5,5-Tetraethyl-4-pyrrolidin-1-yl-2,5-dihydro-1*H*-imidazol-1-oxyl (3)

A 1 M solution of EtMgBr in THF (3 mL) was added dropwise to a stirred solution of pyrrolidinoimidazole **17** (0.5 g, 2.1 mmol) in THF (5 mL). A drop of the reaction mixture was quenched with water, and absence of the starting compound in the reaction mixture was verified by TLC ($Al_2O_3$ Polygram Alox N/UV 254, Macherey-Nagel) using a $CHCl_3$–methanol mixture 50 : 1–2 as eluent. After the reaction was complete (usually 0.5 h), the reaction mixture was quenched with water (1–3 mL) under vigorous stirring and diluted with *t*-BuOMe (20 mL). Then $MnO_2$ (3 g, 34.5 mmol) was added and 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 under reduced pressure to leave an orange oil, which was purified by column chromatography on aluminium oxide (neutral) using chloroform as eluent to give the nitroxide **3** (250 mg, 45%) as orange oil (Found: C, 67.73; H, 10.76; N, 15.63. Calc. for $C_{15}H_{28}N_3O$: C, 67.63, H, 10.59; N, 15.77%); $\nu_{max}$(neat)/cm$^{-1}$ 2969, 2939, 2877, 1593, 1458, 1417, 1377, 1345, 1320, 1275, 1225, 1191, 1166, 962, 930 and 820.

### Determination of ionization constants for nitroxides 1–3

A solution of the nitroxides (0.5 mM) in a mixture of 2.5 mM sodium phosphate and 2.5 mM sodium citrate buffers containing DTPA (0.1 mM) were titrated with solutions of HCl or NaOH to the required pH. The pH was measured using a pH meter OP-205/1 (Hungary) to ±0.05 pH units. EPR spectra of the samples were recorded in a 200 μl quartz EPR flat cell. Hfi splitting constants ($a_N$) were measured as a distance between low-field and central-field components of the EPR spectra. To obtain the p$K$ values of the compounds, the experimental dependence of $a_N$ on pH was fitted to the conventional titration equation:

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

where $p_1$ and $p_2$ are experimentally measured hfi splittings at pH≫p$K$ and pH≪p$K$, correspondingly.

### Determination of the rate constant of nitroxides reduction by ascorbic acid

A solution of the nitroxides (**1**, **4**, and **6**, 0.05 mM; **2**, **3**, **5**, and **7**, 0.1 mM) in 0.1 M sodium phosphate buffer, pH = 7.5, containing 0.1 mM DTPA, were prepared. Ascorbic acid (Sigma) was dissolved in the same buffer, and the following concenrations were used: 33, 66, and 133 mM for **1**; 250, 375, and 500 mM for **2**; 10.4, 20.8, and 41.7 mM for **3**; 0.83, 1.67, and 2.5 mM for **4**; 4.2, 8.3, 16.7, and 33.3 mM for **5**; 1, 2, and 4 mM for **6**; 0.5, 1, 1.5, and 2 mM for **7**. The solutions of the nitroxides and of ascorbic acid were bubbled with argon for 20 min, and mixed afterwards. Then 0.1 ml aliquots were immediatelly placed into 100 μl quartz capillary tubes, transferred into the cavity of EPR spectrometer, and the spectra were recorded at 23–25 °C. The kinetics of decreasing the peak intensity of the low field spectral component was measured and fitted to exponential decay, yielding the rate constant, $k_{obs}$. The experiment for each concentration of ascorbic acid was repeated three times, and the bimolecular rate constants were calculated.

### EPR measurements of the nitroxides reduction in the rat blood

A WAG rat (AL Bacharach, Glaxo Ltd 1924 from Wistar stock, female, 1 year old) was kindly donated by N. G. Kolosova (Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia) and treated in compliance with Russian Legislation. The rat was decapitated and blood was collected. A 20 μl aliquot of the nitroxides **1**–**3**, and **5**–**7** in 0.1 M sodium phosphate buffer, pH =7.5, containing 0.1 mM DTPA, was mixed with 180 μl of whole rat blood (final concentration of the nitroxide 50 μM). The sample was placed in 100 μl quartz capillary and transferred into the EPR cavity immediately afterwards, and the kinetics of the nitroxide decay was measured by following the decrease in the peak intensity of the low field spectral component.

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