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4-Dialkylamino-2,5-dihydroimidazol-1-oxyls with Functional Groups at the Position 2 and at the Exocyclic Nitrogen: The pH-Sensitive Spin Labels

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Abstract: Local acidity and electrostatic interactions are associated both with catalytic properties and the adsorption activity of various materials, and with the vital functions of biomolecules. The observation of acid–base equilibria in stable free radicals using EPR spectroscopy represents a convenient method for monitoring pH changes and the investigation of surface electrostatics, the advantages of which are especially evident in opaque and turbid samples and in porous materials such as xerogels. Imidazoline nitroxides are the most commonly used pH-sensitive spin probes and labels due to the high sensitivity of the parameters of the EPR spectra to pH changes, their small size, and their well-developed chemistry. In this work, several new derivatives of 4-(N,N-dialkylamino)-2,5-dihydroimidazol-1-oxyl, with functional groups suitable for specific binding, were synthesized. The dependence of the parameters of their EPR spectra on pH was studied. Several showed a pKₐ close to 7.4, following the pH changes in a normal physiological range, and some demonstrated a monotonous change of the hyperfine coupling constant by 0.14 mT upon pH variation by four units.

Keywords: nitroxide; spin label; spin probe; EPR; local pH; surface electrostatics; near-surface layer

1. Introduction

Interfacial phenomena and local protonation effects play an important role in biophysics, biochemistry, and in the chemistry of heterogeneous systems [1]. Catalytic and sorption properties of various materials are dependent on the local acidity and electrostatic interactions inside the pores [2]. Measurements of the local acidity and electrostatic potential of the inner pore surfaces represent a problem of great practical interest. Several methods have been developed for the characterization of the acid–base properties of different surface locations [3].

EPR spectroscopy of ionizable nitroxides is a convenient method for the investigation of the above-mentioned phenomena [2,4,5], and is fully applicable to opaque or turbid materials [5,6]. Nitroxide spin probes are small enough to penetrate directly into the pores and to be adsorbed onto the surface of the material under study. The protonation of basic centers in specially designed spin probes affects the hyperfine coupling A-tensor and g-factor matrix, as well as the rotational dynamics of the nitroxide molecule in the proximity of charged surfaces, and this is reflected in the EPR spectra [7]. An analysis of these data gives information about the acidic centers in the material and the local surface electrostatic potential. Recently, EPR studies using pH-sensitive spin probes were successfully used for the investigation of binary TiO₂-SiO₂ xerogels [8].

Imidazoline nitroxides are the most commonly used pH-sensitive spin probes and labels due to the high sensitivity of the parameters of the EPR spectra to pH changes, their...
small size, and their well-developed chemistry. A large number of pH-sensitive nitroxides of the imidazoline series have been prepared [1,4,9]. Some of them are highly sensitive to pH changes in physiologically important regions. The development of a convenient method for the synthesis of 4-(N,N-dialkylamino)-2,5-dihydriimidazol-1-oxyls from 4H-imidazole-3-oxides [10] allowed for easy variation of the substituents in position two of the heterocycle to prepare useful spin probes. Examples illustrating the benefits of this strategy include the synthesis of nitroxides with two pKa values showing high sensitivity in a broad range of pH [11,12], e.g., Scheme 1, label 1, and pH-sensitive alkylating spin, labels 2a–c [13–15], which were used to prepare the hydrophilic spin probes from glutathione [13,14], thiol-specific pH-sensitive spin, label 3, for site-directed labeling of proteins and lipids [16,17], and siloxane-derived spin, label 4, capable of binding to silica or alumina surfaces [7]. Despite the significant advances in this area, the broad variety of potential research objects produces a request for new pH-sensitive spin labels capable of specific attachment. Here we describe a new set of pH-sensitive imidazoline nitroxides with various functional groups in the side chain. Some of them may find an application in material science or in biophysics.

Scheme 1. Structure of the nitroxides 1–4.

2. Results and Discussion

The high reactivity of 2 in nucleophilic substitution reactions offers easy access to new functional derivatives. Expectedly, 2b readily reacts with sodium azide to produce 5 with a nearly quantitative yield. Nitroxide azides can be used for the spin labeling of acetylene-modified molecules via Huisgen 1,3-dipolar cycloaddition [18,19]. In analogy to the literature [20], a reaction of 5 with tetraisopropyl but-3-yne-1,1-diylidiphosphonate 6 in the presence of Cu(II) salt and ascorbic acid after subsequent re-oxidation produced 7 (Scheme 2).

The addition of nitroxides with a terminal acetylene group to azide-modified biomolecules, e.g., nucleic acids, is another way to use the Huisgen-click reaction for spin labeling. Terminal acetylenes can also be attached via Pd-catalyzed coupling [21]. A spin label with a terminal acetylene group was prepared from 8 in two steps (Scheme 3). The oxidation of benzyl alcohol 8 with the activated manganese dioxide in methanol smoothly led to the formation of the corresponding aldehyde 9, with the nitroxyl group and the amidine moiety being unaffected. Alternatively, 8 can be oxidized to the aldehyde 9 with 1-oxo-2,2,6,6-tetramethylpiperidinium chloride 10 with similar yield. The aldehyde 9 readily reacts with Bestmann-Ohira reagent to produce 11 with a yield of 84% [22]. The structure of 11 was confirmed with X-ray analysis data (Figure S1).
Scheme 2. Synthesis of the nitroxide 7.

Scheme 3. Synthesis of the nitroxide 11.

Unless the attachment of a pH-sensitive nitroxide to a primary amino group is successfully performed via alkylation with 2b [7], the acylation reaction is more selective, allowing for the binding of a single nitroxide to the site. Carboxylic acids can be easily prepared from 8. Here we used a convenient one-pot process, where the reaction of 8 with an oxoammonium salt 10 was followed by the Lindgren-Kraus-Pinnick procedure [23] (Scheme 4). The carboxylic acid 12 was isolated with a 95% yield. To activate carboxylic group for acylation, the nitroxide 12 was treated with SOCl₂ in the presence of pyridine. The chloroanhydride formed readily reacted with ethanol to give ester 14. The reaction of 13 with N-hydroxysuccinimide (NHS) produced spin label 15.

Another nitroxide with a carboxylic group on a longer spacer was prepared in one step from 8 via acylation with succinic anhydride (Scheme 5).

The EPR spectra of nitroxides 7, 11, 12, 15, and 16 are strongly pH-dependent with ΔνN > 0.1 mT, and a pKₐ between 6 and 6.7 (Table 1). The titration curves demonstrate optimal sensitivity in slightly acidic media [14,15], but the sensitivity is not optimal in the normal physiological range of 7.35–7.45 [24]. Similar structures without aromatic substituents are known to show higher pKₐ values [11].

To prepare 2-functionalized nitroxides with a pKₐ above 7, the reaction of 4H-imidazol-3-oxide 17 with Grignard reagents was used (Scheme 6). The treatment of 17 with alkenylmagnesium bromides produced nitroxides with a terminal ethylene bond 18a,b. The hydroboration of 18a,b with 9-BBN, followed by oxidation with hydrogen peroxide, was performed using the protocol developed by Hideg for 2-allyl pyrrolidine nitroxides [25]. The reaction produced alcohols 19a,b, which were then treated with carbonylidiimidazole (CDI) to give 20a,b. To demonstrate the feasibility of the carbonylimidazole pH-sensitive spin labels for binding to primary amino groups, 20b was allowed to react with N,N-diethyl-1,3-diaminopropane.
Scheme 4. Synthesis and esterification of 12.

Another nitroxide with a carboxylic group on a longer spacer was prepared in one step from 16 via acylation with succinic anhydride (Scheme 5).

Scheme 5. A reaction of 8 with succinic anhydride.

Table 1. Titration data for the pH-sensitive niroxides: HFC constants for protonated and unprotonated forms, changes in hyperfine splitting between these forms, $\Delta N_a$, and $pK_a$ values.

| Nitroxide | R-H$^+$ | $a_{N_a}$, mT | $\Delta a_{N_a}$, mT | $pK_a$ |
|-----------|---------|---------------|---------------------|--------|
| 5         | 1.434   | 1.539         | 0.105               | 6.42   |
| 7         | 1.429   | 1.531         | 0.102               | 6.24   |
| 8         | 1.424   | 1.529         | 0.105               | 6.54   |
| 11        | 1.436   | 1.539         | 0.103               | 6.21   |
| 12        | 1.417   | 1.522         | 0.105               | 6.50   |
| 15        | 1.422   | 1.531         | 0.109               | 6.49   |
| 16        | 1.466   | 1.575         | 0.109               | 6.64   |
| 18a       | 1.479   | 1.586         | 0.107               | 7.14   |
| 18b       | 1.468   | 1.565         | 0.097               | 7.20   |
| 19a       | 1.493   | 1.589         | 0.096               | 7.25   |
| 19b       | 1.472   | 1.567         | 0.095               | 7.28   |
| 20a       | 1.459   | 1.555         | 0.096               | 6.95   |
| 20b       | 1.448   | 1.545         | 0.097               | 7.24 ± 0.05 |
| 21        | 1.472   | 1.565         | 0.093               | 7.19   |
| 22        | 1.468   | 1.562         | 0.094               | 7.64   |
| 23        | 1.471   | 1.569         | 0.098               | 7.09   |
| 24        | 1.434   | 1.529         | 0.095               | 6.81 ± 0.05 |
| 25        | 1.482   | 1.587         | 0.105               | 7.50   |
Scheme 6. Synthesis of spin labels and spin probes from 17.

The nitroxide 21 was isolated with a 60% yield. The oxidative cleavage of the terminal double carbon–carbon bond in 18b with osmium tetraoxide–oxone system yielded carboxylic acid 22.

The addition of 2-(1,3-dioxolan-2-y1)ethylmagnesium bromide to 17 is another convenient way to create 2-functionalized pH-sensitive spin labels. The reaction produced nitroxide 23 with a high yield. The dioxolane protection group in 23 was readily removed under relatively mild conditions to give the corresponding aldehyde 24, which can be either oxidized to carboxylic acid 25 using the Lindgren-Kraus-Pinnick procedure, or reduced with sodium borohydride to 19a. The sequence 17 → 23 → 24 → 19a gives a remarkably higher yield of the target nitroxide than the addition of allylmagnesium bromide with subsequent hydroboration. A titration of the nitroxides 20, 22, and 25 showed that they may be valuable spin labels and probes with high sensitivities to changes of pH within the physiological range (see Table 1, Figure 1 and Supplementary Materials).

Figure 1. Titration curve of nitroxide 25.
An investigation of the surfaces of many inorganic and organo-inorganic materials (catalysts, sorbents, etc.) requires nitroxides with a high sensitivity to acidity changes within a broad range of pH. A good example of such a spin probe is two-pK_{a} nitroxide 1, which was successfully used in numerous studies [8,26–31]. A covalent attachment of (catalysts, sorbents, etc.) requires nitroxides with a high sensitivity to acidity changes.

Scheme 7. Synthesis of N-(4-(1,3-dioxolan-2-yl)benzyl)-N-methylamine (26).

A reaction of the 5-cyano-4H-imidazole-3-oxide 29 with 26 resulted in cyanide substitution with the formation of 30, and the latter was treated with an excess of ethylmagnesium bromide (Scheme 8). The nitroxide 31 was isolated after a quenching of the reaction mixture with water and oxidation. To hydrolyze the dioxolane ring, 31 was heated to reflux in 0.5 M aqueous HCl. The resulting aldehyde 32 was reduced with sodium borohydride to the corresponding alcohol 34, or oxidized with sodium chlorite to carboxylic acid 33 as described above for 23. Similarly to 11, the nitroxide 33 was converted into succinimidyl ester 35 via a reaction of in situ generated chloroanhydride with NHS.

Scheme 8. Synthesis of two-pK_{a} nitroxides. Structure of the nitroxide 36.
Titration of the nitroxides 32–35 showed a gradual monotonous increase of HFC on the nitroxide nitrogen atom by ca. 0.14 mT upon a pH change from 1.5 to 5.5 (see Figure 2, Table 2, and Supplementary Materials). The shape of the titration curve perfectly corresponded to a two-step acid–base equilibrium, and fitting with the Henderson-Hasselbach function (Equation (2), see experimental part) gave two pKₐ values for each nitroxide (Table 1), corresponding to the sequential protonation of the basic centers, amidine group and pyridine nitrogen.

Table 2. Titration data for the pH-sensitive nitroxides: HFC constants for double protonated, mono-protonated, and unprotonated forms, changes in hfi splitting, ΔaN, between these forms, pK values.

| Nitroxide | a₉N, mT  | ΔaN, mT | pK     |
|-----------|----------|---------|--------|
|           | R·H₂⁺    | R·H⁺   | R      |
| 32        | 1.376    | 1.460   | 1.514  | 0.084  | 2.24 ± 0.02 |
|           |          |         |        | 0.054  | 4.73 ± 0.02 |
| 33        | 1.377    | 1.457   | 1.516  | 0.080  | 2.36 ± 0.02 |
|           |          |         |        | 0.059  | 4.86 ± 0.02 |
| 34        | 1.377    | 1.454   | 1.517  | 0.077  | 2.58 ± 0.02 |
|           |          |         |        | 0.063  | 4.85 ± 0.03 |
| 35        | 1.377    | 1.469   | 1.519  | 0.092  | 2.20 ± 0.01 |
|           |          |         |        | 0.050  | 4.89 ± 0.01 |

In accordance with the general concept of basicity, the pKₐ value of the amidine fragment should be higher than that of the pyridine one. However, according to the simulation, the protonation of the center with a more acidic pKₐ is accompanied by a change in the hyperfine constant by 0.077–0.092 mT, which is typical of the amidine group in 4-amino-2,5-dihydroimidazol-1-oxyls, while the higher pKₐ (4.73–4.89) corresponds to a smaller change in the hyperfine constant (0.05–0.063 mT), which may correspond to pyridine moiety protonation. Moreover, the basic pKₐ showed minor dependence on the nature of the substituent at the exocyclic nitrogen, while the acidic pKₐ varies from 2.58 for 34 to 2.19 for 35. Meanwhile, a comparison of 34 and 35 shows that an increase in the electron-withdrawing character of the substituent at the exocyclic nitrogen leads to an increase of ΔaN in the more acidic region, and a decrease of that correspondent to higher pKₐ. A comparison of the titration data for 1 and 36 [11] gives similar results. Data in the literature show that pKₐ values for 4-amino-2,5-dihydroimidazol-1-oxyls are strongly
dependent on substituents at exocyclic nitrogen and can go below four [32,33]. Thus, it is obvious that the pyridine nitrogen and amidine group in 31–35 have similar basicity and the contribution of different monoprotonated forms is varying depending on the electronic effect of the substituents at the exocyclic nitrogen.

3. Conclusions

In this paper feasibility of the approach to synthesis of pH-sensitive spin labels of 4-amino-2,5-dihydriimidazol-1-oxyl series was once again demonstrated. We have showed that various functional groups can be easily placed in the substituents both in position 2 and to exocyclic nitrogen to make the spin probe suitable for a specific purpose. The potential of this synthetic scheme is still far from exhaustion.

4. Materials and Methods

4.1. General Information

The nitroxides and 4H-imidazol-3-oxides 2b, 8, 17 and 29 were prepared according to the literature protocols [10,11,14]. 1,1′-Carbonyldiimidazole and Ohira-Bestmann Reagent (dimethyl (1-diazo-2-oxopropyl)phosphonate, 10% solution in acetonitrile) were purchased from TCI Europe N.V. (Zwijndrecht, Belgium); 2-(2-bromoethyl)-1,3-dioxolan and 9-BBN 0.5 M solution in THF were purchased from Acros Organics B.V.B.A. (Geel, Belgium).

The IR spectra were recorded on a Bruker Vector 22 FT-IR spectrometer (Bruker, Billerica, MA, USA) in KBr pellets (1:150 ratio) or in neat samples (for oily compounds). UV spectra were acquired on a HP Agilent 8453 spectrometer (Agilent Technologies, Santa Clara, CA, USA) in ethanol solutions (concentration ~10^{-4} M). NMR spectra 1H and 13C were recorded on a Bruker AV-300 (300.132 and 75.467 MHz), AV-400 (400.134 and 100.614 MHz). 1H and 13C chemical shifts (δ) were internally referenced to the residual solvent peak. The nitroxides were reduced to diamagnetic compounds with PhSH [34], N2D4 [35], Zn/CF3COOH [36], or Zn/ND4Cl/D2O [37] prior to recording the 1H NMR spectra. HRMS analyses were performed with High Resolution Mass Spectrometer DFS (Thermo Electron, Brehmen, Germany). Reactions were monitored by TLC carried out using UV light 254 nm or 1% aqueous permanganate. Column chromatography was performed on silica gel 60 (70–230 mesh).

The X-ray diffraction experiment was carried out on a Bruker KAPPA APEX II (Bruker, Billerica, MA, USA) diffractometer (graphite-monochromated Mo Kα radiation). Reflection intensities were corrected for absorption by SADAB-2016 program [38]. The structure of compound 11 was solved by direct methods using the SHELXT-2014 program [39] and refined by anisotropic (isotropic for all H atoms) full-matrix least-squares method against F2 of all reflections by SHELXL-2018 [40]. The positions of the hydrogen atoms were calculated geometrically and refined in riding model. One of the geminal ethyl groups is disordered due to thermal motion at approximate ratio 3:2. Crystallographic data for 11 have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 2124865. Copy of the data can be obtained, free of charge, by application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (Fax: +44-122-3336033 or e-mail: deposit@ccdc.cam.ac.uk; internet: www.ccdc.cam.ac.uk (accessed on 29 November 2021)). The details are shown in Supplementary Materials.

4.2. Synthesis

2-(4-(Azidomethyl)phenyl)-2,5,5-triethyl-4-pyrrolidino-2,5-dihydro-1H-imidazol-1-oxyl (5)

A mixture of 2b hydrochloride [15] (320 mg, 0.82 mmol), sodium hydrocarbonate (250 mg, 3 mmol), diethyl ether (30 mL), and water (20 mL) was vigorously stirred until powder of 2b completely dissolved. The ether solution was separated and concentrated in vacuum without heating. The residue was dissolved in DMSO (5 mL), a solution of NaN3 (0.5 g, 7.7 mmol) was added, and the mixture was stirred at 60 °C for 10 h. The mixture was diluted with water (20 mL) and saturated solution of NaCl (50 mL) and extracted with diethyl ether. The extract was washed with saturated solution of NaCl and
dried with Na₂CO₃, concentrated in vacuum, and the residue was separated using column chromatography on silica gel, eluent chloroform, to give 5, yield 290 mg (95%), yellow crystals, m.p. 63–65 °C (hexane). Elemental analysis, found: C, 65.33; H, 8.01; N, 22.47; calcd. for C₁₀H₁₃N₆O: C, 65.01; H, 7.91; N, 22.75%. IR (KBr) νmax: 2091 (N₃), 1597 and 1574 (C≡N, C=O).

**Tetraisopropyl but-3-yne-1,1-diylidiphosphonate (6)**

(In analogy to procedure by C. Li and C. Yuan [41]) Tetraisopropyl methylenediphosphonate (5 g, 14.5 mmol) was added dropwise to a stirred suspension of NaH (0.8 g 50% in mineral oil) in THF (50 mL) and ethanol. The mixture was stirred for 3 h, then the mixture was diluted with water (50 mL) and pH was adjusted to neutral with hydrochloric acid. The mixture was extracted with dichloromethane, the extract was dried with Na₂SO₄ and concentrated in vacuum. The residue was separated using column chromatography on silica gel, eluent chloroform, to give 6, yield 1.4 g (25%), colorless liquid. Elemental analysis, found: C, 50.35; H, 8.49; P, 16.10; calcd. for C₁₀H₁₆O₄P₂: C, 50.26; H, 8.44; P, 16.20%; IR (neat) νmax (cm⁻¹): 2122 (C≡C).

**2-(4-(2,2-Bis(diisopropoxyphosphoryl)ethyl)-1H-1,2,3-triazol-1-yl)methyl)phenyl)-2,5,5-triethyl-4-pyrrolidino-2,5-dihydro-1H-imidazol-1-oxyl (7)**

Ascorbic acid (140 mg, 0.79 mmol) was added to a mixture of 5 (241 mg, 0.65 mmol), 6 (259 mg, 0.67 mmol), EtOH (1.5 mL), and saturated solution of CuSO₄ in water (0.15 mL). The mixture was stirred for 2 h, then PbO₂ (1 g, 4.17 mmol) was added, the mixture was stirred for 1 h, then the precipitate was filtered off and washed with ethanol. The combined solutions were evaporated in vacuum and the residue was separated using column chromatography on silica gel, eluent chloroform, to give 7, yield 320 mg (65%), yellow oil. Elemental analysis, found: C, 57.23; H, 8.30; N, 10.98; P, 8.35; calcd. for C₃₆H₆₁N₆O₂P₂: C, 57.51; H, 8.18; N, 11.18; P, 8.24%; IR (neat) νmax (cm⁻¹): 1595, 1576 (C=O); 1597 and 1574 (C≡N, C=C). IR (neat) νmax (cm⁻¹): 2091 (N₃).
Method B

A powder of 2,2,6,6-tetramethylpiperidinum chloride (0.4 g, 2.03 mmol) was added to a solution of 8 (0.5 g, 1.5 mmol) in chloroform (10 mL) and the solution was stirred for 2 h at room temperature. The solvent was distilled off in vacuum, the residue was separated using column chromatography on silica gel, eluent chloroform, to give 9, yield 440 mg (85%).

2,5,5-Triethyl-2-(4-ethynylphenyl)-4-pyrrolidino-2,5-dihydro-1H-imidazol-1-oxyl (11)

A solution of dimethyl (1-diazo-2-oxopropyl)phosphonate in acetonitrile (10%, 0.7 mL, 0.31 mmol) was added to a mixture of 8 (100 mg, 0.29 mmol), freshly annealed K₂CO₃ (84 mg, 0.61 mmol) and anhydrous methanol (5 mL). The mixture was stirred overnight, methanol was distilled off in vacuum, the residue was triturated with ethyl acetate, the precipitate was filtered off and washed with ethyl acetate, the combined solution was concentrated in vacuum and separated by column chromatography on silica gel, eluent diethyl ether–hexane 1:1 to give 11, yield 82 mg (84%), orange crystals, m.p. 156–158°C (hexane-ethyl acetate). Elemental analysis, found: C, 74.81; H, 7.97; N, 12.50; calcd. for C₂₃H₂₈N₃O: C, 74.52; H, 8.34; N, 12.41%. IR (KBr) νₘₐₓ (cm⁻¹): 3151 (≡C–H), 2094 (C=O), 1593 (C=N), 1571 (C=C).

2-(4-Carboxyphenyl)-2,5,5-triethyl-4-pyrrolidino-2,5-dihydro-1H-imidazol-1-oxyl (12)

A solution of 8 (0.5 g, 1.5 mmol) in CHCl₃ (10 mL) was cooled to 0°C and 2,2,6,6-tetramethyloxyperipiperidinium chloride (10) (0.4 g, 2.0 mmol) was added in one portion. The mixture was stirred for 2 h at 0°C. Then 2-methylbut-2-ene (1.8 mL, 17.4 mmol) was added to reaction mixture followed by addition of a solution of NaClO₂ (0.9 g, 9.9 mmol) and KH₂PO₄ (1.3 g, 9.8 mmol) in H₂O (44 mL). The mixture was stirred for 2 h, the organic layer was separated, washed with saturated aqueous solution of Na₂CO₃ (3 × 20 mL) and concentrated in vacuum. The residue was separated by column chromatography on silica gel using CHCl₃–EtOH mixture (100:16) as an eluent to give light-yellow crystals of 12, yield 463 mg (89%), m.p. 205–207°C (AcOEt–i-PrOH 10:1). Elemental analysis, found: C, 68.60; H, 8.10; N, 10.80; calcd. for C₂₃H₂₈N₃O: C, 66.85; H, 7.87; N, 11.71%. IR (KBr) νₘₐₓ (cm⁻¹): 2974 (C–H), 1693 (C=O), 1591 (C=N), 1571 (C=C). UV (EtOH) λₘₐₓ (log ε): 232 (4.41), 3151 (C–H), 1718 (C=O), 1593 (C=N), 1571 (C=C).

2-(4-(Ethoxycarbonyl)phenyl)-2,5,5-triethyl-4-pyrrolidino-2,5-dihydro-1H-imidazol-1-oxyl (13)

A solution of 11 (0.5 g, 1.4 mmol) in CHCl₃ (10 mL) was cooled to 0°C and 2,2,6,6-tetramethylpiperidinum chloride (0.4 g, 2.03 mmol) was added to a suspension of KH₂PO₄ (3.1 g, 31.4 mmol) in H₂O (44 mL). The mixture was stirred for 2 h, the organic layer was separated, washed with saturated aqueous solution of Na₂CO₃ (3 × 20 mL) and concentrated in vacuum. The residue was separated by column chromatography on silica gel using CHCl₃–EtOH mixture (100:16) as an eluent to give light-yellow crystals of 13, yield 352 mg (65%), yellow crystals, m.p. 85–90°C (hexane).

Elemental analysis, found: C, 67.01; H, 7.87; N, 11.72%. IR (KBr) νₘₐₓ (cm⁻¹): 2974 (C–H), 1693 (C=O), 1591 (C=N), 1571 (C=C). UV (EtOH) λₘₐₓ (log ε): 232 (4.41), 3151 (C–H), 1718 (C=O), 1593 (C=N), 1571 (C=C).

2-(4-(Ethoxycarbonyl)phenyl)-2,5,5-triethyl-4-pyrrolidino-2,5-dihydro-1H-imidazol-1-oxyl (14)

Pyridine (340 μL, 4.2 mmol) was added to a suspension of 12 (0.5 g, 1.4 mmol) acid in dry CHCl₃ (10 mL). The resulting solution was stirred at 0°C, and SOCl₂ (130 μL, 1.8 mmol) was added dropwise. The stirring continued for 3 h, then ethanol (1 mL, 17 mmol) was added in one portion. The mixture was stirred for 2 h, the solvent was removed in vacuum, and the residue was separated using column chromatography on silica gel, eluent CHCl₃–EtOH 200:1, to give 14, yield 352 mg (65%), yellow crystals, m.p. 85–90°C (hexane).

Elemental analysis, found: C, 68.60; H, 8.10; N, 10.80; calcd. for C₂₃H₂₈N₃O₃: C, 68.27; H, 8.35; N, 10.87%. IR (KBr) νₘₐₓ (cm⁻¹): 2970 (C–H), 1718 (C=O), 1593 (C=N), 1571 (C=C). UV (EtOH) λₘₐₓ (log ε): 231 (4.45), 3151 (C–H), 1718 (C=O), 1593 (C=N), 1571 (C=C).

2-(4-(Ethoxycarbonyl)phenyl)-2,5,5-triethyl-4-pyrrolidino-2,5-dihydro-1H-imidazol-1-oxyl (15)

Pyridine (100 μL, 1.2 mmol) was added to a suspension of 12 (0.138 g, 0.39 mmol) in dry CHCl₃ (5 mL), the resulting solution was stirred at 0°C, and SOCl₂ (30 μL, 0.4 mmol) was added dropwise. The reaction mixture was stirred for 3 h, then N-hydroxysuccinimide (44 mg, 0.39 mmol) was added in one portion. The mixture was stirred for 1 h, the solvent was removed in vacuum, and residue was separated using column chromatography on silica gel, eluent CHCl₃–EtOH 100:1, to give 15, yield 102 mg (58%), yellow crystals, m.p.
tert-
107–108 °C (Et₂O—hexane 1:2). Elemental analysis, found: C, 63.09; H, 6.86; N, 11.92; calcd. for C₂₃H₃₄N₄O₂: C, 63.28; H, 6.86; N, 12.30%. IR (KBr) νmax (cm⁻¹): 2974 (C–H), 1774, 1743 (C=O), 1591 (C=N), 1571 (C=C). UV (EtOH) λmax (log ε): 235 (4.41).

2-(4-(3-Carboxypropanoyloxy)methyl)phenyl)-2,5,5-triethyl-4-pyrrolidino-2,5-dihydro-1H-imidazol-1-oxyl (16)

Sucinic anhydride (0.15 g, 1.5 mmol) was added to a solution of 8 (0.2 g, 0.6 mmol) in CHCl₃ (10 mL) and the reaction mixture was heated to reflux for 2 h. The resulting solution was washed with H₂O (10 mL), dried with Na₂SO₄, and the solvent was removed in vacuum. The solid residue was triturated with ether, the crystalline precipitate of 16 was filtered off and washed with diethyl ether, yield 213 mg (80%), yellow crystals, m.p. 168–169 °C dec. (Et₂O). Elemental analysis, found: C, 64.37; H, 7.52; N, 9.48; calcd. for C₂₃H₃₄N₄O₂: C, 64.24; H, 7.71; N, 9.45%. IR (KBr) νmax (cm⁻¹): 2969 (C–H), 1731 (C=O ester), 1587 (C=N), 1569 (C=O carboxy). UV (EtOH) λmax. (log ε): 220 (4.03). ¹H NMR (300 MHz; CDCl₃) δ: 0.85 (3H, t, J = 7, CH₃), 1.00, 1.24 (2H, m, CH₂, Et), 1.56–1.81 (4H, m, CH₂=CH₂, Et₂), 1.89 (4H, m, C–CH₂CH₂–C, Pyrr), 2.44 (4H, m, CH₂CH₂CO₂H), 3.44 (4H, m, CH₂–N–CH₂, Pyrr), 4.9 (2H, m, CH₂O), 7.14 (2H, d, J = 8, CH Ar), 7.31 (2H, d, J = 8, CH Ar).

2-Allyl-2-ethyl-5,5-dimethyl-4-(pyrrolidino)-2,5-dihydroimidazol-1-oxyl (18a)

A solution of allylmagnesium bromide prepared from allyl bromide (1.69 mL, 20 mmol) and Mg (0.5 g, 20.5 mmol) in diethyl ether (15 mL) under argon was added dropwise to a stirred solution of nitrone 17 (0.83 g, 4.0 mmol) in THF (15 mL). The reaction mixture was stirred for 1 h, then water (30 mL) was added dropwise under vigorous stirring. Then manganese dioxide (5 g, 57 mmol) was added and the reaction mixture was stirred for 1 h. The manganese oxides were filtered off and the precipitate was washed with tert-butylmethyl ether. The organic layer was separated, and the aqueous layer was saturated with NaCl and extracted with tert-butylmethyl ether. The combined organic extracts were concentrated in vacuum and the residue was separated using column chromatography on Al₂O₃, eluent tert-butylmethyl ether–hexane (1:1) to give 18a, yield 630 mg (63%), yellow crystals, m.p. 55–57 °C (hexane). Elemental analysis, found: C, 67.22; H, 10.23; N, 16.88; calcd. for C₁₄H₂₄N₃O: C, 67.16; H, 9.66; N, 16.78%. IR (KBr) νmax (cm⁻¹): 2975 (C–H), 1661 (C=C), 1590 (C=N). UV (EtOH) λmax (log ε): 225 (4.17). ¹H NMR (400 MHz; CD₂OD, reduced with Zn/CF₃COOH in CD₂OD, 65 °C, δ): 0.66 (6H, m, CH₆), 0.81 (3H, t, J = 7, CH₃), 1.00, 1.24 (2H, m, CH₂, Et), 1.47 (6H, t, J = 2, CH₂), 1.60–1.83 (4H, m, CH₂, Et), 2.01 (4H, m, CH₂=CH₂ (pyrr)), 2.39–2.63 (2H, m, CH₂–CH=CH₂), 3.56 (4H, s, CH₂–N–CH₂), 5.08 (2H, m, CH₂O), 5.97 (1H, tdd, J = 10.7, J = 10.7, J = 17.2, CH₂=CH₂).

2-Ethyl-5,5-dimethyl-2-(pent-4-enyl)-4-(pyrrolidino)-2,5-dihydroimidazol-1-oxyl (18b)

A solution of pent-4-ethynylmagnesium bromide prepared from 5-bromopentene (1.6 g, 12 mmol) and Mg (335 mg, 14 mmol) in THF (20 mL) under argon. This solution was added dropwise to a stirred solution of 17 (1 g, 4.8 mmol) in THF (20 mL). The reaction mixture was stirred overnight, then water (4 mL) was added dropwise under vigorous stirring. The reaction mixture was vigorously stirred in air for 1 h, then organic layer was separated, and the aqueous layer was extracted with Et₂O–EtOH (100:1). The combined organic extracts were dried with Na₂SO₄, solvents were distilled off in vacuum, and the residue was separated by column chromatography on Al₂O₃ using hexane–CHCl₃ mixture (2:1) as an eluent to give 18b. Yield 931 mg (70%), yellow oil. Elemental analysis, found: C, 68.93; H, 9.80; N, 15.00; calcd. for C₁₆H₂₈N₃O: C, 69.02; H, 10.14; N, 15.09%. IR (KBr) νmax (cm⁻¹): 2973 (C–H), 1639 (C=C), 1594 (C=N). UV (EtOH) λmax (log ε): 225 (4.19). ¹H NMR (300 MHz; CDCl₃–CD₂OD, reduced with Zn/CF₃COOH in CD₂OD, 65 °C, δ): 0.65 (3H, m, CH₃, Et), 1.16 (2H, m, CH₂, Et), 1.23 (2H, S, CH₂), 1.32 (4H, s, CH₂), 1.36–1.64 (4H, m, CH₂=CH₂–Allyl), 1.77 (2H, m, CH₂–C=), 1.85 (4H, m, CH₂=CH₂–CH₂–CH₂), 3.23, 3.43 (4H, m, CH₂–N–CH₂), 4.60–4.76 (2H, m, CH₂=), 5.48 (1H, tdd, J = CH=, J = 7, J = 10.3, J = 17.1). 2-Ethyl-2-(3-hydroxypropyl)-5,5-dimethyl-4-(pyrrolidino)-2,5-dihydroimidazol-1-oxyl (19a)

Method A

A solution of 9-BBN in THF (0.5 M, 8 mL, 4.1 mmol) was added dropwise to a stirred solution of 18a (400 mg, 1.6 mmol) in THF (10 mL) under argon. The reaction mixture
was vigorously stirred for 4 h, then cooled to 0 °C and cold (0 °C) aqueous NaOH (20%, 10 mL) and cold (0 °C) H₂O₂ (30%, 3 mL) were added dropwise successively. The mixture was allowed to warm to room temperature upon stirring (ca. 2 h), organic layer was separated, dried with Na₂CO₃, and the solvent was distilled off in vacuum. The residue was dissolved in CHCl₃ (25 mL), anhydrous Na₂CO₃ (1 g) was added, and mixture was allowed to stand overnight in air. The solution was concentrated in vacuum and separated by column chromatography on silica gel using CHCl₃–EtOH mixture (100:4) as an eluent to give 19a. Yield 150 mg (35%), yellow oil. Elemental analysis, found: C, 62.53; H, 9.49; N, 15.45; calcd. for C₁₈H₂₈N₂O₃: C, 62.65; H, 9.76; N, 15.66%. IR (KBr) ν max (cm⁻¹): 3386 (br, OH), 1592 (C=O), UV (EtOH) λ max (log ε): 225 (4.07). ¹H NMR (400 MHz; CDCl₃), δ: 0.94 (3H, t, J 7.2, CH₃, Et), 1.43 (6H, s, CH₃), 1.53–1.92 (6H, m, CH₂), 1.98 (4H, m, CH₂-CH₂-CH₂-CH₂), 3.52 (4H, s, CH₂-N-CH₂), 3.57 (2H, br. s, CH₂O). 2-Ethyl-2-(5-hydroxypentyl)-5,5-dimethyl-4-(pyrrolidino)-2,5-dihydroimidazol-1-oxyl (19b) was prepared similarly from 18b. Yield 42%, yellow crystals, m.p. 68–73 °C (Et₂O). Elemental analysis found: C, 65.17; H, 10.56; N, 14.08; calcd. for C₁₆H₃₀N₂O₅: C, 64.83; H, 10.20; N, 14.18%. IR (KBr) ν max (cm⁻¹): 3261 (br, OH), 1593 (C=O), UV (EtOH) λ max (log ε): 225 (4.1). ¹H NMR (300 MHz; CDCl₃–CD₂OD, reduced with Zn/CF₃COOH in CD₂OD, 65 °C, δ): 0.62 (3H, m, CH₃, Et), 1.06 (4H, br. m, CH₂–CH₂–(CH₂)₂, 1.21–1.28 (8H, m, 2 × CH₃, CH₂, Et), 1.40 (2H, m, CH₂–CH₂–CH₂), 1.57 (2H, m, >C(=Et)–CH₂), 1.79 (4H, br. m, C–CH₂CH₂–C, Pyrr), 3.20, 3.44 (4H, m, CH₂–N–CH₂, Pyrr), 3.26 (2H, t, J 6.5, CH₂O).

Method B

Sodium borohydride (60 mg, 1.6 mmol) was added portionwise to a stirred solution of 24 (400 mg, 1.5 mmol) in EtOH (10 mL) at 0 °C. The reaction was controlled with TLC, Silutol UV-254, eluent CHCl₃–EtOH (25:1). Inorganic residue was filtered off, the solution was distilled off in vacuum, and the residue separated by column chromatography as described above to give 19a. Yield 309 mg (72%).

2-(3-(1H-Imidazole-1-carbonyloxy)propyl)-2-ethyl-5,5-dimethyl-4-(pyrrolidino)-2,5-dihydroimidazol-1-oxyl (20a)

Carbonyldimidazole (80 mg, 0.49 mmol) was added to a solution of alcohol 19a (14 mg, 0.43 mmol) in dry CHCl₃ (5 mL) and the mixture was allowed to stand for 24 h. The solution was washed with brine, dried with Na₂SO₄, and concentrated in vacuum. The residue was separated by column chromatography on silica gel using CHCl₃–EtOH mixture (100:2) as an eluent, producing 20a as yellow oil. Yield 139 mg (90%). Elemental analysis, found: C, 59.69; H, 7.72; N, 19.45; calcd. for C₁₉H₂₄N₂O₄: C, 59.65; H, 7.79; N, 19.32%. IR (KBr) ν max (cm⁻¹): 1760 (C=O), 1592 (C=O), UV (EtOH) λ max (log ε): 223 (4.17). 2-(5-(1H-Imidazole-1-carbonyloxy)propyl)-2-ethyl-5,5-dimethyl-4-(pyrrolidino)-2,5-dihydroimidazol-1-oxyl (20b) was prepared similarly, yield 80%, yellow oil. Elemental analysis, found: C, 61.30; H, 8.26; N, 17.70; calcd. for C₂₀H₂₂N₂O₅: C, 61.51; H, 8.26; N, 17.93%. IR (KBr) ν max (cm⁻¹): 1762 (C=O), 1593 (C=O), UV (EtOH) λ max (log ε): 226 (3.99).

2-(3-(Diethylamino)propylcarbamoyloxy)propyl)-2-ethyl-5,5-dimethyl-4-(pyrrolidino)-2,5-dihydroimidazol-1-oxyl (21)

N,N-Diethyl-1,3-diaminopropane (50 mg, 0.38 mmol) was added to a solution of 20a (126 mg, 0.35 mmol) in dry Et₂O (5 mL), and mixture was allowed to stay for 24 h. The solution was concentrated in vacuum, and the residue was separated by column chromatography on Al₂O₃ using CHCl₃ as an eluent to give 21 (Figure 3). Yield 82 mg (60%), yellow oil. Elemental analysis, found: C, 62.21; H, 10.01; N, 16.51; calcd. for C₂₂H₂₈N₂O₃: C, 62.23; H, 9.97; N, 16.49%. IR (KBr) ν max (cm⁻¹): 1718 (C=O), 1593 (C=O), UV (EtOH) λ max (log ε): 225 (4.19). ¹H NMR (300 MHz; CDCl₃–CD₂OD, reduced with Zn/CF₃COOH in CD₂OD, 65 °C, δ): 0.75 (3H, t, J 7.2, CH₃), 1.11 (6H, t, J 7.3, 2 × CH₃) 1.33 (6H, br. m, CH₃), 1.40 (6H, br. m, CH₃), 1.43–1.79 (8H, m, CH₂CH₂C, CH₂CH₂, CH₂CH₂, CH₂CH₂), 1.90 (4H, br. m, CH₂), 2.87–3.03 (8H, m, CH₂, CH₂, CH₂, CH₂, CH₂), 3.36, 3.52 (4H, m, CH₂, 12CH₂), 3.84 (2H, m, CH₃O).
Figure 3. The structure of nitroxide 21.

2-(3-Carboxypropyl)-2-ethyl-5,5-dimethyl-4-(pyrrolidino)-2,5-dihydro-1H-imidazol-1-oxyl (22)

Osmium tetroxide (30 mg, 0.4 mmol) and oxone (1.77 g, 5.8 mmol) were added successively to a solution of 18b (400 mg, 1.4 mmol) in DMF (20 mL) and the mixture was stirred for 3 h. A powder of Na2SO3 (10 g, 63 mmol) was added in one portion. Inorganic precipitate was filtered off and washed with EtOH, the combined solution was evaporated to dryness in vacuum and the residue was separated by column chromatography on silica gel using EtOH as an eluent to give 22, yield 90 mg (21%), yellow oil. M+ (calcld./found) 296.1969/296.1972. IR (neat) νmax (cm−1): 2977 (C-H), 1664 (C=O), 1592 (C=N). λmax (EtOH)/nm: 225 (lgε 4.19).

2-(2-(1,3-Dioxolan-2-yl)ethyl)-2-ethyl-5,5-dimethyl-4-(pyrrolidin-1-yl)-2,5-dihydro-1H-imidazol-1-oxyl (23)

A solution of 2-(1,3-dioxolan-2-yl)ethylmagnesium bromide was prepared from 2-(2-bromoethyl)-1,3-dioxolan (4.3 g, 24 mmol) and Mg (670 mg, 28 mmol) in 20 mL THF under a stream of argon. This solution was added dropwise to a stirred solution of nitrene 17 (850 mg, 4 mmol) in 20 mL Et2O and 6 mL THF. The reaction mixture was stirred overnight, then water (5 mL) was added dropwise under vigorous stirring. The reaction mixture was allowed to air for 1 h, then organic layer was separated, inorganic residue was quenched with Et2O–EtOH (100:1). An isolated organic layer was dried over Na2SO4, solvents were removed in vacuum. The residue was separated using column chromatography on Al2O3 using CHCl3 as an eluent to give 23, yield 1.13 g (90%), yellow oil. Elemental analysis, found: C, 62.08; H, 9.21; N, 13.43; calcld for C16H28N3O3: C, 61.91; H, 9.09; N, 13.54%. IR (KBr) νmax (cm−1): 2972 (C-H), 1593 (C=N), 1143 (C=O). λmax (EtOH)/nm: 225 (lgε 3.90).

2-Ethyl-5,5-dimethyl-2-(3-oxopropyl)-4-(pyrrolidino)-2,5-dihydro-1H-imidazol-1-oxyl (24)

A solution of oxalic acid (180 mg, 2 mmol) in water (6 mL) was added to a solution of nitroxide 23 (250 mg, 0.8 mmol) in EtOH (4 mL). The reaction mixture was stirred for 3 h under reflux, then ethanol was removed in vacuum, saturated aqueous KHC2O3 (10 mL) was added to a residue. The product was extracted with CHCl3–i-ProOH mixture (50:1) (3 × 15 mL). An isolated organic layer was dried over Na2SO4, solvents were removed in vacuum, the residue was separated using column chromatography on silica gel using CHCl3–EtOH mixture (50:1) as an eluent to give 24, yield 161 mg (75%), yellow oil. Elemental analysis, found: %: C, 63.08; H, 9.18; N, 15.63; calcld. for C14H24N3O2: C, 63.13; H, 9.08; N, 15.78. IR (neat) νmax (cm−1): 2972 (C-H), 1720 (C=O), 1593 (C=N). λmax (EtOH)/nm: 225 (lgε 4.16).

2-(2-Carboxyethyl)-2-ethyl-5,5-dimethyl-4-(pyrrolidin-1-yl)-2,5-dihydro-1H-imidazol-1-oxyl (25)

Trimethylenethylene (1 mL, 9.0 mmol) was added to a cooled (0 °C) solution of aldehyde 24 (200 mg, 0.8 mmol) in 10 mL CH3CN followed by addition of a solution of NaClO2 (480 mg, 5.3 mmol) and KH2PO4 (710 mg, 5.3 mmol) in H2O (20 mL). Progress of the reaction was monitored by TLC (silica gel, CHCl3–EtOH (50:1), developing with 1% aq KMN04). CH3CN was removed in vacuum, the product was extracted from water by CHCl3–i-ProOH mixture (100:1) (5 × 15 mL). An isolated organic layer was dried over Na2SO4, the solvents were removed in vacuum, the residue was separated using column chromatography on silica gel using CHCl3–EtOH mixture (5:2) as an eluent to give 25, yield 121 mg (57%), yellow oil, M+ (calcld./found) 282.1812/282.1811. IR (neat) νmax (cm−1): 2973 (C-H), 1729 (C=O), 1591 (C=N). λmax (EtOH)/nm: 225 (lgε 4.04). 1H NMR (300 MHz; CDCl3–CD3OD, reduced with Zn/CF3COOH in CD3OD, 65 °C, δ) 0.75 (3H, m, CH3, Et),
0.98–1.14 (2H, m, CH₂, Et), 1.34, 1.40 (6H, m, 2 × CH₃), 1.47–1.61 (2H, m, CH₂CH₂CO₂H), 1.91 (4H, m, CH₂–CH₂–CH₂–CH₂, Pyrr), 2.14–2.27 (2H, m, CH₂CH₂CO₂H), 3.36, 3.55 (4H, m, CH₂–N–CH₂, Pyrr).

1-(4-(1,3-Dioxolan-2-yl)phenyl)-N-methylmethanamine (26)

p-Toluenesulfonic acid monohydrate (0.5 g, 74.6 mmol) was added to a solution of terephthalaldehyde 27 (10 g, 74.6 mmol) in 175 mL PhCH₂. Water was distilled off with Dean-Stark tube. The reaction mixture was then quenched with aqueous NaHCO₃, dried over Na₂CO₃, the solvent was removed in vacuum, and residue was dissolved in methanol saturated with methylamine (20 mL). The resulting solution was then added to the previously maintained under vigorous stirring for 10 min in a solution of Ti(Oi-Pr)₄ (14 mL, 47 mmol) in methanol saturated with methylamine (30 mL). The mixture was stirred for 5 h, then NaBH₄ (1.34 g, 33.6 mmol) was added portionwise, and mixture was stirred for 2 h. Water (7 mL) was added dropwise, solvents were removed in vacuum, brine was added to a residue, and the product was extracted by ether. Organic layer was dried over NaOH. Residue was separated using column chromatography on silica gel using EtOH/Pr.-OEt (100:3) as an eluent, yield 12.24 g (85%), colorless oil. Elemental analysis, found: C, 67.84; H, 7.91; N, 6.94; calcd for C₂₁H₂₆N₄O₅: C, 66.30; H, 6.36; N, 14.73%. ¹H NMR (400 MHz; CDCl₃, δ): 2.38 (3H, s, CH₃), 3.70 (2H, s, N–CH₂), 3.95–4.09 (4H, m, –O–CH₂–CH₂–O–), 5.75 (1H, s, O–CH–O), 7.29, 7.38 (4H, AA’BB’, C₆H₄), 131.44 (Py), 133.86 (Py, Ar), 137.47 (C–CH–C–), 136.32 (C–CH₂NHC₃H₅), 141.10 (C–CH). IR (neat) ν max (cm⁻¹): 3325 (N–H), 1082 (O–C–O), 1507 (C=O), 2960 (C–H). The residue was washed by CHCl₃. KMnO₄ reaction was monitored by TLC (silica gel, CHCl₃/MeOH). The mixture was allowed to stand for 1 h. Then water (3 mL) was added dropwise, solvents were removed in vacuum, residue was triturated with ether and crystallization from CH₂CN to give 30, yield 3.97 g (75%), dirty-yellow crystals, m.p. 160 °C (dec.). Elemental analysis, found: C, 66.30; H, 6.36; N, 14.73%. ¹H NMR (400 MHz; CDCl₃, δ): 1.66 (6H, s, 2 × CH₃), 3.06 (3H, s, N–CH₃), 3.86–4.16 (4H, m, O–CH₂–CH₂–O), 4.77 (2H, br. s, N–CH₂–Ar), 5.75 (1H, s, O–C–O), 7.26, 7.46 (4H, AA’BB’, C₆H₄), 8.46, 8.70 (4H, AA’BB’, Py). ¹C NMR (75 MHz; CDCl₃, δ): 21.50 (2 × Me), 35.22 (N–CH₃), 53.11 (N–CH₃), 65.06 (O–CH₂–CH₂–O), 75.73 (Me₂C), 102.82 (O–CH–O), 121.00 (3,5–Py), 126.77 (br, CH (C₆H₄)), 133.86 (Py, i), 136.36 (C–CH₂NHC₃H₅), 137.47 (C–CH), 144.67 (C–N–O), 149.82 (2,6-Py), 172.33 (C–CH). IR (KBr) ν max (cm⁻¹): 1597 (C=O), 1082 (O–C–O). λ max (EtOH)/nm: 210 (log ε 3.94), 260 (log ε 2.36).

5-(4-(1,3-Dioxolan-2-yl)benzyl)(methyl)amino)-4,4-dimethyl-2-(pyridin-4-yl)-4H-imidazole 3-oxide (30)

1-(4-(1,3-Dioxolan-2-yl)phenyl)-N-methylmethanamine 26 (6.72 g, 34.8 mmol) was added to a solution of 5-cyano-4,4-dimethyl-2-(pyridin-4-yl)-4H-imidazole 3-oxide 29 (2.98 g, 13.9 mmol) in THF (25 mL) and the mixture was allowed to stand at r.t. for 24 h. The solvent was removed in vacuum, residue was triturated with ether and crystallization from CH₂CN to give 30, yield 3.97 g (75%), dirty-yellow crystals, m.p. 160 °C (dec.). Elemental analysis, found: C, 66.30; H, 6.36; N, 14.73%. ¹H NMR (400 MHz; CDCl₃, δ): 1.66 (6H, s, 2 × CH₃), 3.06 (3H, s, N–CH₃), 3.86–4.16 (4H, m, O–CH₂–CH₂–O), 4.77 (2H, br. s, N–CH₂–Ar), 5.75 (1H, s, O–C–O), 7.26, 7.46 (4H, AA’BB’, C₆H₄), 8.46, 8.70 (4H, AA’BB’, Py). ¹C NMR (75 MHz; CDCl₃, δ): 21.50 (2 × Me), 35.22 (N–CH₃), 53.11 (N–CH₃), 65.06 (O–CH₂–CH₂–O), 75.73 (Me₂C), 102.82 (O–CH–O), 121.00 (3,5–Py), 126.77 (br, CH (C₆H₄)), 133.86 (Py, i), 136.36 (C–CH₂NHC₃H₅), 137.47 (C–CH), 144.67 (C–N–O), 149.82 (2,6-Py), 172.33 (C–CH). IR (KBr) ν max (cm⁻¹): 1597 (C=O), 1082 (O–C–O). λ max (EtOH)/nm: 210 (log ε 3.94), 260 (log ε 2.36).

4-(4-(1,3-Dioxolan-2-yl)benzyl)(methyl)amino)-2-ethyl-5,5-dimethyl-2-(pyridin-4-yl)-2,5-dihydro-1H-imidazol-1-oxyl (31)

A solution of ethylmagnesium bromide was prepared from ethyl bromide (2.73 g, 25 mmol) and Mg (630 mg, 26 mmol) in 35 mL Et₂O under a stream of argon. This solution was added dropwise to a stirred solution of nitrore 27 (1 g, 2.6 mmol) in 15 mL THF. The reaction mixture was then added to 1 g, 2.6 mmol) in 15 mL THF. The reaction mixture was allowed to stand for 1 h. Then water (3 mL) was added dropwise under vigorous stirring following by MnO₂ (3 g, 34.5 mmol) addition. Progress of the reaction was monitored by TLC (silica gel, CHCl₃–EtOH (100:3), developing with 1% aq. KMnO₄). The mixture was stirred vigorously for 2 h, the oxidant was filtered off and the residue was washed by CHCl₃ and MeOH. The solvent from filtrate was removed in vacuum and the residue was separated by column chromatography on silica gel using CHCl₃–EtOH (100:3) as an eluent. The product 31 was isolated as a hydrochloride. Yield 797 mg (68%), yellow oil. Elemental analysis, found: C, 62.18; H, 6.83; N, 12.48; Cl, 6.70; calcd. for C₂₃H₃₀ClN₄O₅: C, 61.94; H, 6.78; N, 12.56; Cl, 6.95%. IR (neat) ν max (cm⁻¹): 1593 (C=O), 1082 (O–C–O). λ max (EtOH)/nm: 216 (log ε 4.34).

2-Ethyl-4-(4-formylbenzyl)(methyl)amino)-5,5-dimethyl-2-(pyridin-4-yl)-2,5-dihydro-1H-imidazol-1-oxyl (32)
A solution of nitroxide 30 (1.8 g, 4.4 mmol) in 15 mL 0.5 M aq. HCl was refluxed for 4 h, then Na₂CO₃ added to the end of gas evolution. A product was extracted by mixture of 20 mL CHCl₃ + 1 mL i-PrOH three times, organic layer was dried over Na₂CO₃, the solvents were removed in vacuum, and the nitroxide 29 was isolated from the residue by column chromatography on silica gel using CHCl₃ as an eluent. Yield 308 mg (60%), yellow crystals, compound 34.

\[ \lambda_{\text{max}} (\text{EtOH})/\text{nm}: 239 (\log \varepsilon) \]

Trimethylethylene (1.33 g, 19.2 mmol) was added to a cooled (0 °C) solution of aldehyde 29 (583 mg, 1.6 mmol) in 20 mL CHCl₃ followed by addition of a solution of NaClO₂ (1.02 g, 11.2 mmol) and KH₂PO₄ (1.5 g, 11.2 mmol) in H₂O (50 mL). Progress of the reaction was monitored by TLC (silica gel, CHCl₃–AcOEt as an eluent). The organic layer was separated, the product was extracted from water by CHCl₃–i-PrOH mixture (20:1) (2 × 20 mL). A combined organic extracts were washed with brine, dried over Na₂SO₄, the solvents were removed in vacuum, the residue was separated using column chromatography on silica gel using AcOEt–EtOH mixture (10:1) as an eluent. Yield 285 mg (47%), yellow crystals, compound 33 was isolated as a crystal solvate 3 (33) × 2 EtOH (ether–EtOH 100:2), m.p. 204 °C (dec.). Elemental analysis, found: C, 65.12; H, 6.42; N, 13.12; calcd. for C₆₇H₇₂N₁₂O₁₁: C, 65.08; H, 7.09; N, 13.59%. IR (KBr) \( \nu_{\text{max}} (\text{cm}^{-1}) \): 2474 (O–H), 1708 (C=O), 1597 (C=N).

2-Ethyl-4-((4-(hydroxymethyl)benzyl)(methyl)amino)-5,5-dimethyl-2-(pyridin-4-yl)-2,5-dihydro-1H-imidazol-1-oxyl (33)

\[ \lambda_{\text{max}} (\text{EtOH})/\text{nm}: 2474 (\text{O–H}), 1708 (\text{C}=\text{O}), 1597 (\text{C}=\text{N}), \lambda_{\text{max}} (\text{EtOH})/\text{nm}: 220 (\log \varepsilon 4.30). \]

2-Ethyl-4-((4-hydroxymethyl)benzyl)(methyl)amino)-5,5-dimethyl-2-(pyridin-4-yl)-2,5-dihydro-1H-imidazol-1-oxyl (34)

\[ \lambda_{\text{max}} (\text{EtOH})/\text{nm}: 252 (\log \varepsilon 2.5). \]

2,2-Ethyl-4-((4-Carboxybenzyl)(methyl)amino)-2-ethyl-5,5-dimethyl-2-(pyridin-4-yl)-2,5-dihydro-1H-imidazol-1-oxyl (35)

Pyridine (240 µL, 3 mmol) was added to a cooled (0 °C) solution of acid 33 (228 mg, 0.6 mmol) in 10 mL of dry CHCl₃ followed by addition of SOCl₂ (90 µL, 1.2 mmol). The reaction mixture was vigorously stirred for 3 h, then N-hydroxysuccinimide (138 mg, 1.1 mmol) was added and the mixture was allowed to stand for 24 h. The solvents were then removed in vacuum, residue was separated using column chromatography on silica gel using CHCl₃–EtOH mixture (100:2) as an eluent to give 35, yield 123 mg (40%), yellow crystals, compound 35 was isolated as a hydrochloride (hexane), m.p. 58 °C (dec.). Elemental analysis, found: C, 58.42; H, 5.47; N, 13.25; Cl, 6.56; calcd for C₇₅H₇₂ClN₃O₃: C, 58.31; H, 5.68; N, 13.60; Cl, 6.88%. IR (KBr) \( \nu_{\text{max}} (\text{cm}^{-1}) \): 2976 (C–H), 1770 (O=C–N=O), 1741 (C=O), 3421 (C=O), 1590 (C=N), \lambda_{\text{max}} (\text{EtOH})/\text{nm}: 239 (\log \varepsilon 4.25).

4.3. EPR Experiments

EPR experiments were performed on X-band EPR (9.8 GHz) spectrometer Bruker ER-200D. Titrations of the radicals (~0.2 mM) were performed in a buffer mixture of acetate-phosphate-borate (0.5 mM of each) in a pH range of 2–10 starting from the acidic value. Small aliquots of NaOH solution were used for titration to a higher pH. The observed hfi
constants were measured as a distance between low field and central lines of nitroxide triplet spectra.

Single proton exchange:

\[ R\cdot H^+ \rightleftharpoons R\cdot H^+; \quad pK_a \]

was fitted with single pK\(_a\) titration curve function:

\[ a_N (pH) = \frac{a_N(R\cdot) + a_N(R\cdot H^+) \times 10^{pK_a-pH}}{1 + 10^{pK_a-pH}} \]

Double proton exchange implies the serial protonation,

\[ R\cdot H^+ \rightleftharpoons R\cdot H^+; \quad pK_{a1} \]
\[ R\cdot H^+ + H^+ \rightleftharpoons R\cdot H_2^+; \quad pK_{a2} \]

which results in the double pK\(_a\) titration curve function:

\[ a_N (pH) = \frac{a_N(R\cdot) + a_N(R\cdot H^+) \times 10^{pK_{a1}-pH} + a_N(R\cdot H_2^+) \times 10^{pK_{a2}-pH} \times 10^{pK_{a1}-pH}}{1 + 10^{pK_{a1}-pH} + 10^{pK_{a2}-pH} \times 10^{pK_{a1}-pH}} \]

EPR settings. Microwave power, 5 mW; modulation amplitude, 0.08–0.12 mT.
SD for pK\(_a\), 0.05; for hfc, 0.005 mT.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/gels8010011/s1, Figure S1: “The molecular structure of 2,5,5-triethyl-2-(4-ethynylphenyl)-4-pyrrolidino-2,5-dihydro-1H-imidazol-1-oxyl (11)”, IR and NMR spectra of synthesized compounds, titration curves of pH-sensitive nitroxides.

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