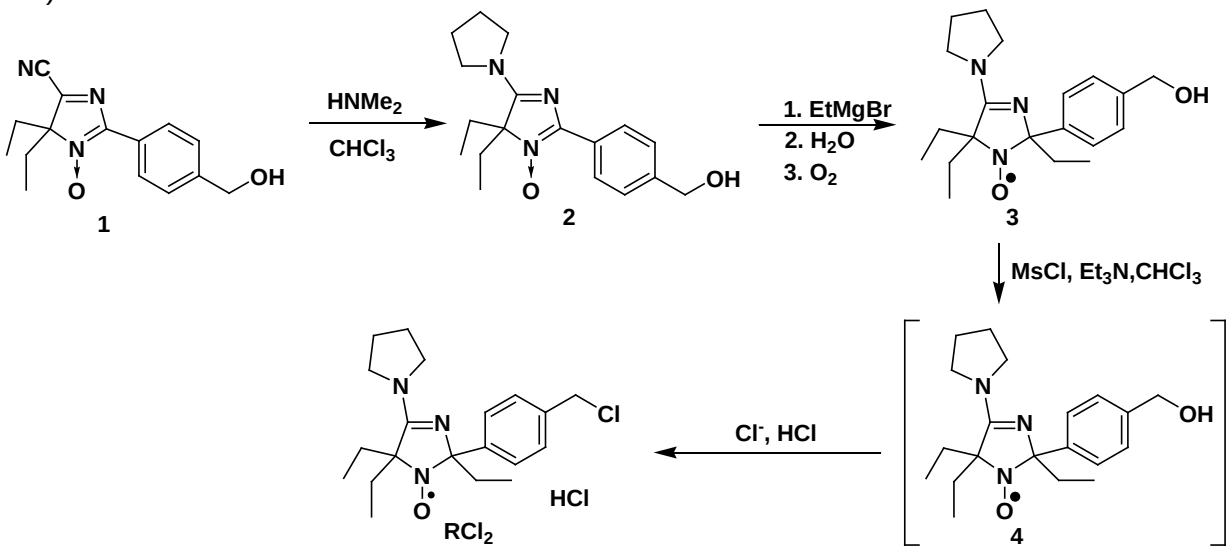
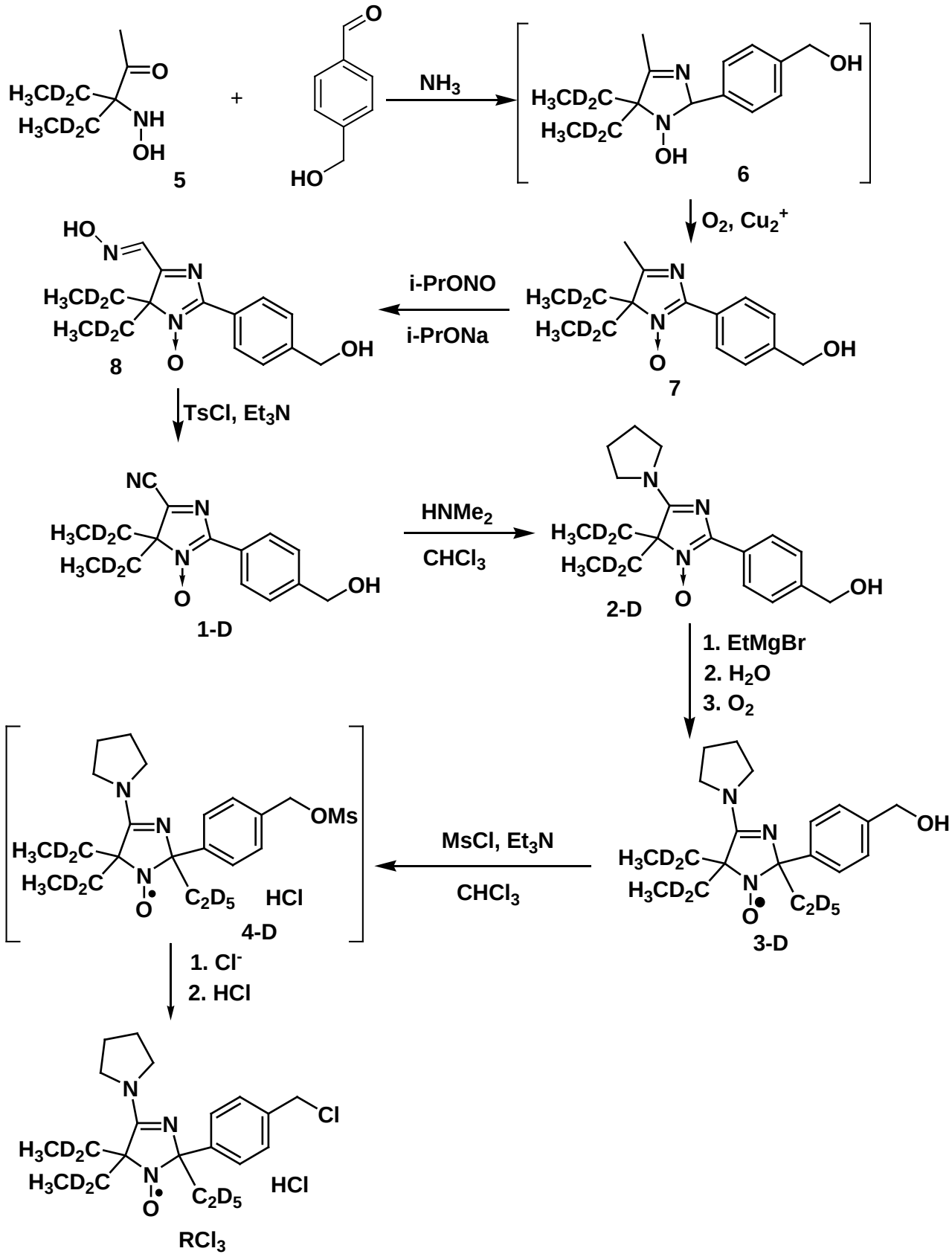
The synthesis of 2-(4-((2-(4-amino-4-carboxybutanamido)-3-(carboxymethylamino)-3-oxoproylthio)methyl)phenyl)-4-pyrrolidino-2,5,5-triethyl-2,5-dihydro-1H-imidazol-1-oxyl (R_2) and its deuterium-enriched analog R_3.

The spin label **RCl_2** was prepared from 2-(4-(Hydroxymethyl)phenyl)-4,4-diethyl-4*H*-imidazole-5-carbonitrile 3-oxide (**1**) in three-step synthesis using the procedures described earlier (Scheme SI1)[1].



Scheme SI1.

The pH-sensitive spin probe **RCl_3** was prepared from deuterium enriched 3-hydroxyamino-3-ethylpentan-2-one hydrochloride-D_6 (**5**) in analogy to previously reported synthesis of **RCl_1** [1] according to the Scheme SI2. The **5** was prepared from 3-pentanone and bromoethane-D_5 according to previously published procedure[2]. The method used implies statistical distribution of CD_3 group, thus **5** contains on average 6 deuterium atoms in Et_2C moiety. The first step of the synthesis was modified in order to obtain maximum yield.


5
NH3
6
O2, Cu2+
7
i-PrONO
i-PrONa
8
TsCl, Et3N
1-D
HNMe2
CHCl3
2-D
1. EtMgBr
2. H2O
3. O2
3-D
MsCl, Et3N
CHCl3
4-D
1. Cl-
2. HCl
RCl3

Scheme SI2.

The pH-sensitive spin probes **R2** and **R3** were synthesized by the procedure similar to that described earlier by Woldman et al[1].

2-(4-Hydroxymethylphenyl)-4,4-diethyl-5-pyrrolidino-4H-imidazole 3-oxide **(2).**

Pyrrolidine (5 ml, 58 mmol) was added to a solution of 2-(4-(hydroxymethyl)phenyl)-4,4-diethyl-4*H*-imidazole-5-carbonitrile 3-oxide (**1**)[1] (2.0 g, 7.4 mmol) in $CHCl_3$ (30 mL). The reaction mixture was allowed to stand for 12 h, washed with brine, dried over Na_2CO_3. The solvent was removed in vacuum and the residue was recrystallized from the mixture $CHCl_3$-CCl_4 3:1 to yield **1** (1.86 g, 80%), yellow crystals, m.p 241-245 ºC (EtOAc – *i*-PrOH 10:1), (Found, %: C, 68.33; H, 8.08; N, 13.20; Calcd for $C_{18}H_{25}N_3O_2$: C, 68.54; H, 7.99; N 13.32); ν_{max}(KBr)/cm^{-1} 2967, 2935, 2876, 2815, 2721, 1596, 1531, 1460, 1421, 1379, 1345, 1226, 1184, 1144, 1050, 1022, 984, 888, 852, 838, 793, 762, 711, 670; λ_{max}(EtOH)/nm 370 (lg ε 3.77), 269 (4.48); δ_H(300 MHz; $CDCl_3$) 0.74 (6 H, t, J 7.2 Hz, 2×CH_3, Et), 1.92 and 2.32 (each 2H, AB q, J_q 7.2 Hz, J_{AB} 14.5 Hz, 2×CH_2, Et), 1.98 and 3.72 (each 4H br m, $(CH_2)_4$), 4.72 (s, 2H, CH_2), 7.39 and 8.62 (AA'BB', 4H, J 8.0 Hz, Ar); δ_C(75 MHz; $CDCl_3$) 7.69 (CH_3, Et), 23.49, 26.11, 45.91 and 48.07 ($(CH_2)_4$), 26.34 (CH_2, Et), 64.04 (CH_2), 83.85 (4-C), 150.34 (C=N-O), 168.46 (C=N), 144.74 (Ar, p-*i*), 125.57 (Ar, *i*), 128.65 (Ar, o), 125.84 (Ar, m).

2-(4-(Hydroxymethyl)phenyl)-5,5-dimethyl-4-dimethylamino-2-ethyl-2,5-dihydro-1H-imidazole-1-oxyl **(3).**

A 1 M solution of EtMgBr in Et_2O (50 ml) was slowly added dropwise under argon to a stirred suspension of **2** (1.3 g, 4.0 mmol) in a mixture of dry THF (20 mL), dry diethyl ether (50 ml) and dry benzene (50 ml). At the beginning of the addition a fine precipitate is formed, which then sticks into a lump and then is dissolved with clear solution formation. The reaction mixture was allowed to stand overnight. Then brine (15 mL) was added dropwise under vigorous stirring. The organic phase was separated and water solution was extracted with EtOAc. The combined organic extracts were dried with anhydrous Na_2CO_3 and then bubbled with air for 24 hr. Then the solvent was removed in vacuum. The nitroxide **3** was isolated from the residue by column chromatography on Al_2O_3, eluent $CHCl_3$. Yield 1.30 g (90%), orange crystals, m.p.149-152 ºC (hexane), (Found, %: C, 69.88; H, 8.85; N, 12.32; Calcd for $C_{20}H_{30}N_3O_2$: C, 69.73; H, 8.78; N, 12.20); ν_{max}(KBr)/cm^{-1} 3272 br, 2970, 2936, 2877, 1586, 1569, 1461, 1402, 1350, 1301, 1285, 1206, 1155, 1050, 1018, 976, 930, 911, 831, 807.

2-(4-(Chloromethyl)phenyl)-2,5,5-triethyl-4-(pyrrolidino)-2,5-dihydro-1H-imidazol-1-oxyl hydrochloride **(RCl_2).**

Methanesulfonyl chloride (0.5 mL, 6.5 mmol) was added dropwise to a solution of nitroxide **3** (1 g, 2.8 mmol) in dry chloroform (5 ml) under stirring. Then triethylamine (0.4 mL, 2.9 mmol) was added dropwise and the reaction mixture was stirred for ca. 2 hr. The TLC analysis of the reaction mixture (Kieselgel 60 F_{254}, eluent $CHCl_3$) shows gradual conversion of **3** into mesylat (R_f ca. 0.8) and then into Rb. (10 mL). Then the solvent was removed under reduced pressure, the residue was shaken with diethyl ether (30 ml), and saturated solution of $NaHCO_3$ (10 ml). Ether extract was separated and a saturated solution of NaCl in 2 % hydrochloric acid was added dropwise to a stirred ether solution until pH of water phase decreased to 1 and colored nitroxide completely moved to water phase. The solution was stirred until the mesylate 4 completely disappeared Water solution was separated, washed with ether and basified with $NaHCO_3$. The basified mixture was again extracted with diethyl ether, the combined extracts were dried with Na_2CO_3, the solvent was removed under reduced pressure and the residue was separated using column chromatography on silicagel, eluent $CHCl_3$. The RCl_2 isolated was converted into chlorohydrate by careful dissolving in 2% HCl and evaporation under reduced pressure. Yield 890 mg (78 %), m.p. 199-202 ºC (precipitated from isopropanol with diethyl ether), (Found, %: C, 60.30; H, 7.49; N, 10.28; Cl, 17.43; Calcd for $C_{20}H_{30}N_3OCl_2$: C, 60.15; H, 7.57; N, 10.52; Cl, 17.75); ν_{max}(KBr)/cm^{-1} 3400 br, 2972, 2934, 2880,

2734 br, 2574 br, 1667, 1455, 1412, 1388, 1345, 1298, 1217, 1172, 1130, 1059, 931, 907, 823, 678.

2-(4-Hydroxymethylphenyl)-4,4-diethyl-5-methyl-4H-imidazole 3-oxide-D_6 ***7*** was prepared using optimized general procedure for 1-hydroxy-2,5-dihydro-1*H*-imidazoles synthesis[3]. A solution of **5** (3 g, 16 mmol) in methanol (15 ml) and a solution of 4-(hydroxymethyl)benzaldehyde (2.3 g, 17 mmol) in methanol (10 ml) were prepared separately and saturated with gaseous ammonia. The solutions were mixed and gaseous ammonia was bubbled through this mixture for 0.5 hr with stirring and then left overnight. Then a solution of copper-ammonium complex was prepared from 25% aqueous ammonia (1 ml), $CuSO_4 \times 5\ H_2O$ (200 mg, 0.77 mmol) and water (2 ml) and poured into the reaction mixture. Air was bubbled through the reaction mixture for approximately 3 hr (until the solution become green due to Cu^{2+} accumulation). The solvents were distilled off under reduced pressure and the residue was triturated with ethyl acetate. The inorganic precipitate was filtered off, the solvent was distilled off under reduced pressure and the residue was separated using column chromatography on slilcagel, eluent ethyl acetate – diethyl ether 1:1 to give **7** (3.8 g, 90%), δ_H(300 MHz; $CDCl_3$) 0.54 4H s (CH_3, Et partly enriched with D), 2.25 3H s (5-CH_3), 2.87 1H br s (OH), 4.71 2H s (CH_2OH), 7.43 and 8.61 both 2H AA'BB' (C_6H_4).

All the other steps of the synthesis were performed in accordance to the procedures described for RCl_1[1] and above.

2-(4-(Hydroxymethyl)phenyl)-5,5-dimethyl-4-dimethylamino-2-ethyl-2,5-dihydro-1H-imidazole-1-oxyl –D_{11} **(3-D)** was synthesized using C_2D_5MgBr solution prepared from C_2D_5Br (4 ml, 53 mmol) and Mg (1.5 g) in 40 ml of diethyl ether, yield 90%. ν_{max}(KBr)/cm^{-1} 3379 br, 3272 br, 2976, 2933, 2873, 2222, 2146, 2119, 2073, 1587, 1568, 1456, 1402, 1348, 1232, 1207, 1144, 1049, 812, 740.

2-(4-(Chloromethyl)phenyl)-2,5,5-triethyl-4-(pyrrolidino)-2,5-dihydro-1H-imidazol-1-oxyl hydrochloride-D_{11} (**Rc**), ν_{max}(KBr)/cm^{-1} 3429 br, 3018, 2966, 2744 br, 2567 br, 2225, 2148, 2119, 2075, 1666, 1475, 1441, 1411, 1344, 1288, 1223, 1163, 1093, 1066, 991, 852, 835, 816, 675.

*2-(4-((2-(4-Amino-4-carboxybutanamido)-3-(carboxymethylamino)-3-oxoproylthio)methyl)phenyl)-4-pyrrolidino-2,5,5-triethyl-2,5-dihydro-1H-imidazol-1-oxyl (**R_2**) and its deuterium-enriched analog **R_3**.* A solution of a spin label **RCl_2** or **RCl_3** (62.5 mg, 156.5 μmol) in methanol (1 ml) was added dropwise under argon to a solution of glutathione (58.7 mg, 191.0 μmol) and NaOH (30.5 mg, 762.5 μmol) in methanol (5 ml). The reaction mixture was stirred overnight under argon, then the solution was neutralized by addition of HCl and evaporated. The residue was dissolved in 3 ml of H_2O and pH was adjusted to 5.2 by addition of HCl or NaOH. The solution was placed on C18 SPE column (HyperSep, Thermo Scientific) and washed with 10 ml H_2O. The product was eluted from the column with 30% methanol. After evaporation 94.2 mg of the spin probe **R_2** or **R_3** was obtained (yield 95%).

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2. Bobko, A. A.; Kirilyuk, I. A.; Gritsan, N. P.; Polovyanenko, D. N.; Grigor'ev, I. A.; Khramtsov, V. V.; Bagryanskaya, E. G., EPR and quantum chemical studies of the pH-sensitive imidazoline and imidazolidine nitroxides with bulky substituents. *Appl. Magn.Reson.* **2010,** 39, 437-451.
3. Kirilyuk, I. A.; Morozov, D. A.; Tabatchikova, Y. S.; Medvedev, V. S.; Lebedev, A. V.; Romanenko, G. V.; Rybalova, T. V.; Grigorr'ev, I. A., Synthesis of 4H-imidazole-5 carbaldoxime 3-oxides and 4H-imidazole-5-carbonitrile 3-oxides. *Russ. Chem. Bull.* **2008,** 57, (7), 1516-1533.