The synthesis of water soluble isoindoline nitroxides and a pronitroxide hydroxylamine hydrochloride UV–VIS probe for free radicals

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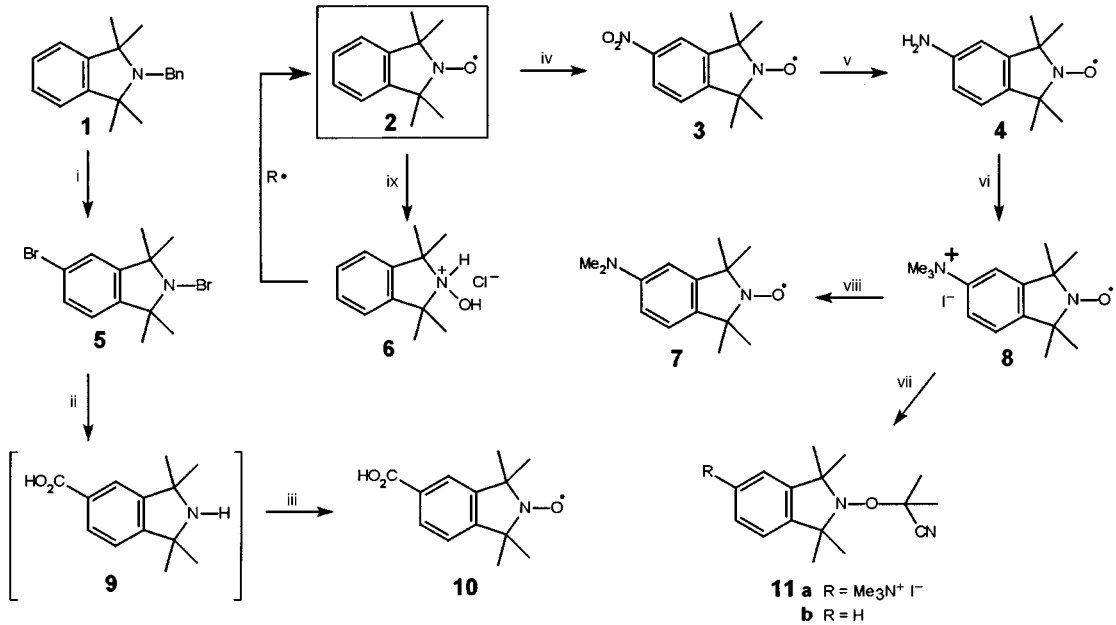
The novel water soluble nitroxides 5-trimethylammonio-1,1,3,3-tetramethylisoindolin-2-yloxyl iodide 8 and 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl 10 are prepared by nitration of the parent nitroxide 2 and bromination of its amine precursor 1; the stable ($t_{1/2}$ > 580 h in MeOH; *ca* 72 h in physiological saline) pronitroxide 2-hydroxy-1,1,3,3-tetramethylisoindoline hydrochloride 6 was synthesised by treatment of 2 with HCl gas in dry EtOH and reacts with radicals to form 2; the transformation can be followed by UV–VIS spectroscopy.

Nitroxide radicals have long been used as spin labels and probes in biological systems, where, in some cases, their presence also affords protection from oxidative stress and radiative damage.[1–4] However, the 1,1,3,3-tetramethylisoindolin-2-yloxyl (TMIO) nitroxides **2** (Scheme 1) have been largely overlooked for these applications, their main use to date being in spin trapping experiments.[5,6] The TMIO system enjoys a number of advantages over the commercially available nitroxides, the most significant being its excellent thermal and chemical stability and superior EPR linewidths.[7] However, its use in biological systems has been limited because of the near absence of any water soluble derivatives. The sulfonate has been reported,[8] but has not been fully characterised. We here report the synthesis of two water soluble TMIO derivatives, 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl **10** and 5-trimethylammonio-1,1,3,3-tetramethylisoindolin-2-yloxyl iodide **8**. We also report an alternative strategy for converting the hydrophobic TMIO into a water soluble derivative, with the synthesis of 2-hydroxy-1,1,3,3-tetramethylisoindoline hydrochloride **6**, the first stable water soluble pronitroxide incorporating the isoindoline system.

Compounds **1** and **2** were prepared according to the literature procedures of Griffiths *et al.*[9] Treatment of **1** with Br_2 (6 equiv.) in the presence of $AlCl_3$ (12 equiv.) resulted in oxidative debenzylation and bromination giving 2,5-dibromo-1,1,3,3-tetramethylisoindoline **5** (40%).[10] The slow addition of Bu^nLi (3.6 equiv.) to a solution of **5** in dry THF and quenching gave 5-carboxy-1,1,3,3-tetramethylisoindoline **9** (not isolated) upon aqueous workup [δ_H([$^2H_6$]DMSO) 1.70 (12H, s, Me), 7.45 (1H, d, ArH), 7.88 (1H, d, ArH), 7.94 (1H, dd, ArH), 9.55 (2H, br, NH_2^+); δ_C([$^2H_6$]DMSO) 28.5 (Me), 68.0 (C1, C3), 122.3 (ArC), 123.3 (ArC), 123.7 (ArC), 130.7 (ArC), 143.0 (ArC), 146.8 (ArC), 166.8 (C=O)]. Tungstate oxidation[9] of **9** gave **10** [23% (based on **5**), mp 214–218 °C; $CHCl_3$, g = 2.00585, a_N = 14.45 G (Found: C, 66.57; H, 6.92; N, 6.28; Calc. for $C_{13}H_{16}NO_3$: C, 66.67; H, 6.84; N, 5.98%); *m/z* (EI) 234, 220, 219, 204, 189].

The synthesis of **3** was achieved *via* the quantitative nitration of **2** according to the method of Bolton *et al.*[11] Hydrogenation of **3** (H_2, Pd/C, 10 psi, 4 h) followed by treatment with PbO_2 (0.5 equiv.) to reoxidise to the nitroxide gave **4** as a yellow solid [98%; mp 195–196 °C (from EtOH) (lit.,[12] 198 °C)]. This material possesses substantial synthetic utility, the amine providing a pathway for coupling to amino acids and proteins as well as incorporating into sugars.[13]

The water soluble ammonium nitroxide **8** was prepared *via* basic methylation of **4** (MeI, 120 equiv.; NaH, 10 equiv.) in a sealed vessel (65 °C, 96 h). The product was isolated *via* extraction into water (**CAUTION**: excess NaH) and recovered



Scheme 1 *Reagents and conditions*: i, Br_2, $AlCl_3$, 0 °C, 40%; ii, Bu^nLi, −78 °C, then CO_2; iii, H_2O_2, Na_2WO_4, 23 (from **5**); iv, HNO_3, H_2SO_4, > 95%; v, H_2, Pd, then PbO_2, > 95%; vi, MeI, NaH, 65 °C, 90%; vii, AIBN, 90 °C, 90%; viii, 170 °C, 0.1 mmHg; ix, HCl, EtOH, > 95%

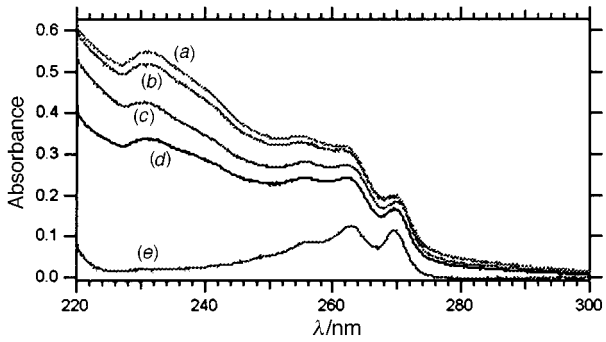


Fig. 1 UV–VIS spectra following the reaction of **6** (2.6×10^{-4} M) with AIBN (0.5 equiv.), 90 °C, in degassed MeOH under N_2 after (*a*) 0.0, (*b*) 1.5, (*c*) 3.0, (*d*) 7.5 and (*e*) 9.0 h. Chromatography confirmed only trace amounts of 2-cyanopropyl adduct **11b**.

by evaporation. The crude solid was taken up in hot EtOH (*ca.* 80 ml) and passed through a short Al_2O_3 column [Al_2O_3 (neutral, act. I), eluent: EtOH–EtOAc (1:4), 700 ml]. Evaporation yielded a yellow crystalline solid **8** [90%; mp 173–174 °C (decomp.) (from H_2O), 175–176 °C (decomp.) (from EtOH, slightly contaminated with inorganics) (Found: C, 46.70; H, 6.58; N, 7.21. Calc. for $(C_{15}H_{24}N_2OI)_2 \cdot H_2O$: C, 46.88; H, 6.56; N, 7.29%); *m/z* (FAB) 248; (EI) 233 ($M - 15$), 142 (MeI^+), 127 (I^+) (accurate to *ca.* 1 ppm)]. The nitroxide **8** has also been characterised by EPR (H_2O, g = 2.00562, a_N = 15.83 G, a_N = 0.204 G), NMR analysis of its 2-cyanopropyl adduct **11a** and by its characteristic thermal decomposition product **7** [mp 126–129 °C (Found: C, 72.10; H, 9.18; N, 11.90. Calc. for $C_{14}H_{21}N_2O$: C, 72.06; H, 9.07; N, 12.01%); *m/z* (EI) 233]. The nitroxide **8** is soluble in water at a concentration of *ca.* 120 mM, exceeding the typical[14,15] solubility requirement for spin probes.

The synthesis of **6** was achieved by bubbling dry HCl into a stirred solution of **2** in EtOH. The reaction was complete when the strong orange colour of the nitroxide had reduced to a faint yellow (*ca.* 0.5 h). Evaporation yielded a white solid **6** [98%; mp 196–198 °C (decomp.) (from MeCN) (Found: C, 63.54; H, 8.05; N, 6.07. Calc. for $C_{12}H_{18}NOCl$: C, 63.29; H, 7.97; N, 6.15%); $\delta_H(CDCl_3)$ 1.79 (12H, s, Me), 7.18 (2H, d, ArH), 7.39 (2H, d, ArH); $\delta_C(CDCl_3)$ 25.5 (Me), 75.9 (C1, C3), 121.9 (ArC), 129.7 (ArC), 139.3(ArC)]. The hydroxylamine hydrochloride **6** is soluble in water at concentrations greater than 1 M.

Hydroxylamines have been used[16] as oxygen sensitive probes in MRI and recently[17] in the determination of reactive oxygen species by EPR analysis with ten-fold greater sensitivities than can be achieved using conventional nitrone spin traps. We have found that the transition from **6** (and its unprotonated equivalent) to **2** can be monitored by UV–VIS spectroscopy and have followed the reaction of **6** with the 2-cyanopropyl radicals generated from the thermolysis of AIBN (see Fig. 1). Blank reactions confirmed this transition did not occur in the absence of AIBN under identical conditions. The ability to observe the formation of a nitroxide spectroscopically, without the use of EPR methods, is quite novel and demonstrates the potential for **6** to act as a UV–VIS probe for free radicals. The hydroxylamine hydrochloride **6** is particulary inert in the solid form and is even stable in solution, having a half life ($t_{1/2}$) > 580 h at 0.26 mM in MeOH exposed to the atmosphere at room temperature (determined by UV–VIS analysis). Treatment with base, however, greatly reduces the stability of **6**, the free hydroxylamine having $t_{1/2}$ < 4 h in MeOH solution. This pronitroxide also possesses significant stability in physiological saline phosphate buffer (pH 6.9) where it has $t_{1/2}$ *ca.* 72 h at 0.26 mM. Notably, when converted to the nitroxide in physiological media this species remains in solution (at this concentration), again demonstrating the potential of **6** as a water soluble radical scavenger.

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Notes and References

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