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An efficient synthesis of 3-(*N*-piperidinemethyl)-2,2,5,5-tetramethyl-1-oxy-3-pyrroline, a promising radioprotector for cancer radiotherapy

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ABSTRACT

Nitroxides can ameliorate the toxic effects of radiation during cancer therapy. Nitroxides are paramagnetic and can be used in magnetic resonance imaging (MRI) and electron paramagnetic resonance imaging (EPRI) to monitor in vivo oxidative stress status. Compound **5** (3-(*N*-piperidinemethyl)-2,2,5,5-tetramethyl-1-oxy-3-pyrroline) was found to be the most effective nitroxide radioprotector. An efficient synthesis for this promising radioprotector was developed.

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Protection from radiation exposure is important for radiation cancer treatment or a nuclear accident. Cancer is the second leading cause of death in the United States, exceeded only by heart disease. The estimated number of new cancer cases is 1.66 million and about 580,350 people are expected to die in the US alone in 2013.[1] The death rates of cancer did not change much over the past 80 years except for lung and bronchus cancers. It is well known that cancer can be treated with ionizing radiation, but the inevitable normal tissue exposure during cancer radiotherapy or a nuclear accident can promote carcinogenesis and a variety of toxicities.[2,3] Pre-exposure administration of a radiation protective drug can reduce radiation damage by ameliorating the toxic effects of radiation. Preclinical studies in mice have shown that administration of Tempol (1-oxyl-2,2,6,6-tetramethyl-4-hydroxypiperidine) decreases the severity of xerostomia (dry mouth) after salivary gland irradiation and increases the LD50/30 after whole-body irradiation.[4–6] Nitroxides are paramagnetic and their pharmacokinetics can therefore be monitored with magnetic resonance imaging (MRI), electron paramagnetic resonance imaging (EPRI), and their concentration in tissues can be quantified.[5,6] The in vivo redox reactions among nitroxides, reactive oxygen species, and intracellular antioxidants can also be used for redox imaging study. Formation of diamagnetic hydroxylamine from nitroxide redox reaction results in signal loss in T1-weighted MRI scan which can provide an imaging-based assay of tissue redox status.[7] Based on the initial findings on the radioprotective effects of the model compound Tempol[4,8,9] and a systematic in vitro survey of a variety of nitroxide-related radioprotectors,[10] compound 3-(*N*-piperidinemethyl)-2,2,5,5-tetramethyl-1-oxy-3-pyrroline, **5** was found to be the most effective radioprotector against lethal doses of radiation in mice.[11] In addition, the compound readily passes the blood–brain barrier (BBB). A previous synthesis of compound **5** produced about 5% overall yield over a 6-step synthesis (Scheme 1).[10] Following this procedure we noticed a UV invisible byproduct next to product **5** on TLC plate that can be detected by I_2 staining. The separation of the two products was difficult. In addition the product purity cannot be accurately characterized by NMR due to the paramagnetic property of the product or by HPLC due to the weak UV absorption of the byproduct and the similarity of their retention times. The byproduct was found to be hydroxylamine compound **7**. Contamination of the diamagnetic hydroxylamine would adversely affect the MR and EPR imaging quality. The availability of a more convenient and efficient synthesis method of this promising agent would be beneficial.

We found that the Favorskii rearrangement of 3,5-dibromo-4-oxo-2,2,6,6-tetramethylpiperidine **2** could be done in the presence of piperidine to form the piperidine derivative **3** directly in 89% yield (Scheme 2).[12] The conversion of the amine **3** to the nitroxide **4** was carried out in 90% yield by stirring with the H_2O_2 and

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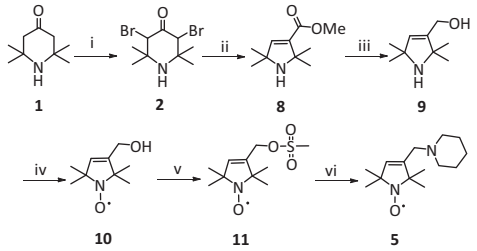
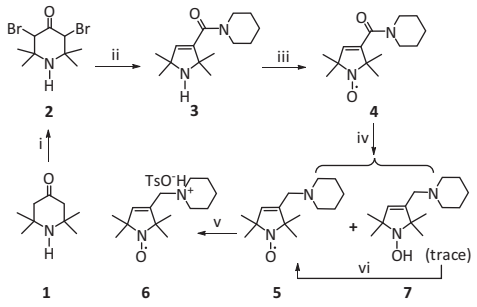


Scheme 1. Reagents and conditions: (i) Br_2, AcOH, 12 h, 90%; (ii) NaOMe in MeOH, rt, 1 h, 40%; (iii) Red-Al in toluene, 40 °C, 1 h, 61%; (iv) H_2O_2, Na_2WO_4 in MeOH, 0 °C to rt, 36 h, 50%; (v) MsCl, TEA in DCM, 0 °C to rt, 3 h, 87%; (vi) piperidine, NaH in DMF, 0 °C to rt, 4 h, 47%.



Scheme 2. Reagents and conditions: (i) Br_2, AcOH, 12 h, 90%; (ii) Piperidine, TEA-H_2O, rt, 6 h and then 50 °C, 6 h, 89%; (iii) H_2O_2, Na_2WO_4 in H_2O, rt, 2d in dark, 90%; (iv) $LiAlH_4$ in THF, rt, 24 h, 92%; (v) *p*-TSA in ether, rt. 96%; (vi) *m*-CPBA, rt, 2 h.

Na_2WO_4 oxidants at room temperature for 2 days. Amide carbonyl reduction of **4** using $LiAlH_4$ instead of Red-Al also gave an improved yield of 92%.[13] However trace amount of hydroxylamine byproduct **7** (<5%) was still present. The byproduct can be completely converted to product **5** by adding *m*-chloroperoxybenzoic acid (*m*-CPBA) oxidant to the reaction mixture and then stirring for 2 h; *m*-CPBA can be easily removed afterward by liquid extraction workup in basic aqueous solution. The improved synthesis route to **5** reduced the total synthesis to four steps and increased the overall yield to 65% while completely eliminating the contamination of hydroxylamine byproduct **7**. The purification procedures were also simplified.

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Supplementary data

Supplementary data (experimental procedures and complete spectral data and the copies of ^1H and ^{13}C NMR spectra of all intermediates and final product) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.08.028.

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