A One-Step Synthesis of 2-(2-Pyridyl)-3*H*-indol-3-one *N*-Oxide: Is It an Efficient Spin Trap for Hydroxyl Radical?

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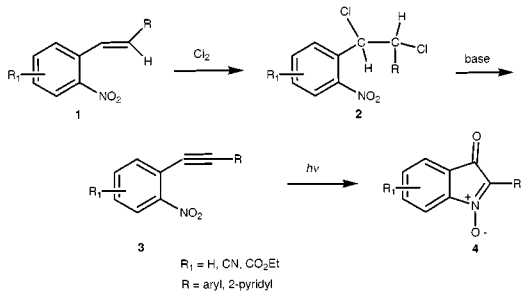
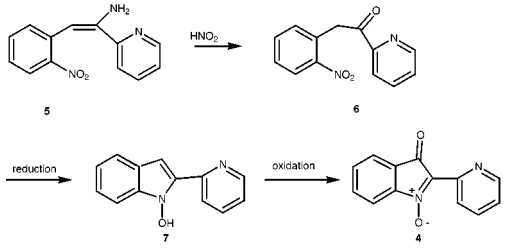
The field of free radicals in biology has its origins in a series of publications in the late 1960s in which the secretion of superoxide ($O_2^{\bullet-}$) during the enzymic cycling of xanthine oxidase was first described.[1] Soon thereafter, a Cu/Zn-containing enzyme was found to disproportionate this free radical into O_2 and H_2O_2.[2] This enzyme, which became known as superoxide dismutase (SOD), has played a pivotal role in defining the ubiquitous nature of $O_2^{\bullet-}$ and other free radicals generated from $O_2^{\bullet-}$.[3]

In the intervening years, a variety of methods have been developed to detect free radicals in biological milieu. Of those, spin trapping/EPR spectroscopy is singular in its ability to characterize specific free radicals, generated in situ, and identified in animal models in real time.[4] Based on our earlier success at identifying HO$^\bullet$ in irradiated leg tumors of mice,[5] we have become particularly interested in syntheses of newer spin traps that would allow the in vivo in situ detection of HO$^\bullet$ under other experimental paradigms. During the course of our investigations, we have studied the specificity of 3-substituted 5,5-dimethyl-1-pyrroline *N*-oxides and a number of imidazoline *N*-oxides toward HO$^\bullet$.[6] Recently, however, a publication caught our fancy[7] in which 2-(2-pyridyl)-3*H*-indol-3-one *N*-oxide **4** was reported to spin trap HO$^\bullet$. The corresponding spin trapped adduct, 2-hydroxy-2-(2-pyridyl)-3*H*-indol-3-on-1-oxyl (**11**), exhibited remarkable stability when compared to the shorter lifetime of 3-hydroxy-5,5-dimethyl-1-pyrrolin-1-oxyl (**13**).[7] However, enthusiasm for such robustness must be tempered by the fact that **4**, by lacking a hydrogen atom at the α-carbon, has lost one of the strengths of spin trapping, additional hyperfine splittings that can aid in the characterization of the parent free radical.[8] Despite this, there are a number of experimental paradigms that would greatly benefit from readily available sources of 2-aryl-3*H*-indol-3-one *N*-oxides.

Scheme 1



Scheme 2



There have been a number of synthetic approaches to 2-aryl-3*H*-indol-3-one *N*-oxides, including 2-(2-pyridyl)-3*H*-indol-3-one *N*-oxide (see, for instance, Schemes 1 and 2). However, multistep pathways, especially those in which intermediates are exposed to sunlight to obtain the desired product, have often resulted in poor yields of the coveted nitrone.[9]

We thought, based on the earlier work of Castro and Stephens[10a] and Sonogashira et al.,[10b] that it might be possible to adapt these methods to the synthesis of the title compounds. To our surprise, we were able to synthesize a family of 2-aryl-3*H*-indol-3-one *N*-oxide in a one-step reaction in excellent yields. Herein, we described our preparative design.

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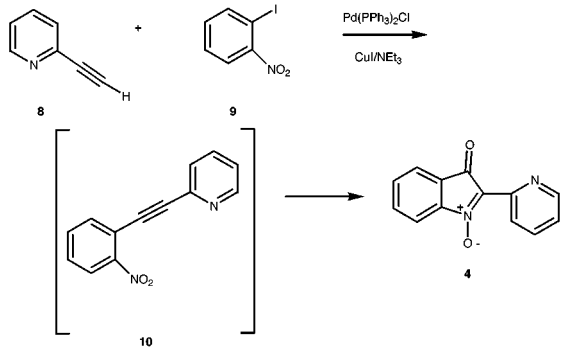
Scheme 3



Our initial approach was to prepare **10** and then, following literature methods,[11] cyclize it to **4** (Scheme 3). In the experiments described by Sonogashira et al.,[10b] the reactions were run in ethyl acetate with dimethylamine added as a catalyst to accelerate the reaction. As the reactants were readily soluble in triethylamine, we felt this amine might serve as both the solvent and promoter of the reaction. We, therefore, combined **8** and **9** in triethylamine and added catalytic amounts of $Pd(PPh_3)_2Cl$ and CuI at room temperature. By following the reaction by TLC, we found that after about 6−8 h a second more polar product appeared, which with time began to be the dominant product. The reaction mixture was allowed to stir for several days to completion, as judged by TLC and filtered. Ethyl acetate was then added to the remaining solid, and again the mixture was filtered. After evaporation, in vacuo, the residual oil was passed through a flash-chromatographic column containing silica gel and eluted with pentane/ethyl acetate mixtures, resulting in the isolation of 2-(2-pyridyl)-3*H*-indol-3-one *N*-oxide **4** in good yields. Using a similar procedure, other analogues of **4** (see, Scheme 1), such as R_1 = H and R = phenyl, were prepared in reasonable yields. Attempts to generalize this reaction to aliphatic alkynes, where R_1 = H and R = methyl, resulted in poor yields of the desired nitrone.

In general, the complete reaction from **8** and **9** to **4** can be accomplished by stirring the mixture at room temperature for 3−4 days. The availability of the catalyst and reagents, the ease of the procedure, and the gentleness of experimental conditions suggest that this one-step preparative scheme to 2-aryl-3*H*-indol-3-one *N*-oxides will become widely used as these compounds have been shown to exhibit significant pharmacological activity.[7]

When nitrone **4** was incubated with H_2O_2 (100 μM), we obtained a small but discernible EPR spectrum corresponding to the nitroxide, 2-hydroxy-2-(2-pyridyl)-3*H*-indol-3-on-1-oxyl (Figure 1A). With the addition of Fe^{2+}, generating $HO^{\bullet}$, the EPR spectrum became considerably large (Figure 1B). Of interest was the finding that at higher concentrations of H_2O_2 (1 mM) in the absence of exogenous Fe^{2+} we observed the same EPR spectrum whose intensity was identical to that found when Fe^{2+} was present (compare Figure 1B,C). In the case of data depicted in Figure 1C, we suggest a direct oxidation of nitrone **4** resulted in the formation of 2-hydroxy-2-(2-pyridyl)-3*H*-indol-3-on-1-oxyl (**11**).[11] A similar reaction

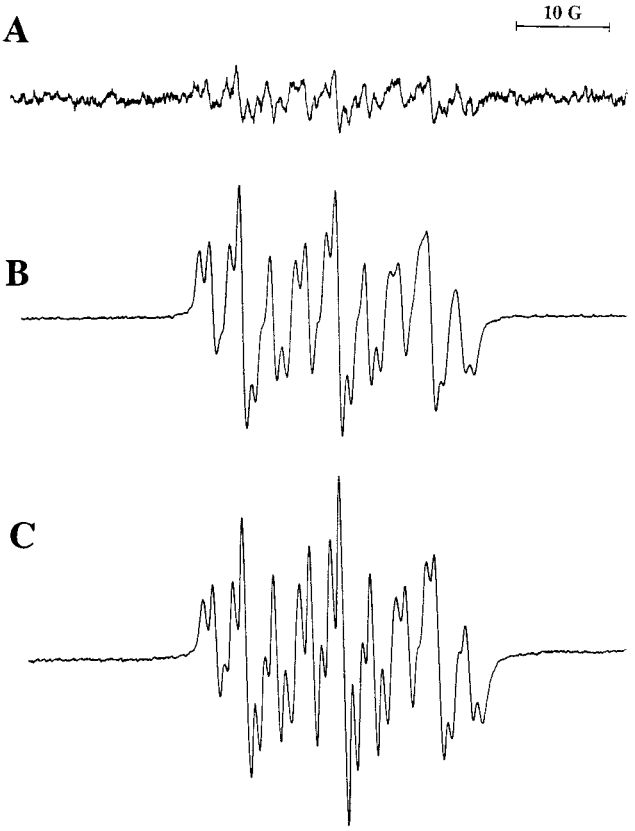


Figure 1. (A) EPR spectrum of **4** (3 mM in H_2O and DMF, 3.5%) in the presence of H_2O_2 (100 μM). The spectrum was recorded 3 min after mixing the reagents. Receiver gain was 1.25×10^4. (B) Same as (A) except Fe^{2+} (100 μM) was added, and the receiver gain was 2.5×10^3. (C) EPR spectrum of **4** (3 mM in 50 mM phosphate buffer, pH 7.4 and DMF, 3.5%) in the presence of H_2O_2 (1 mM). Receiver gain was 2.5×10^3.

has been reported for the oxidation of *N*-hydroxy-2-phenylindole with either lead tetraacetate or *p*-nitroperbenzoic acid, leading to 2-hydroxy-2-phenyl-3*H*-indol-3-on-1-oxyl.[13]

Next, we decided to estimate the efficiency of **4** to spin trap $HO^{\bullet}$ as compared to other nitrones, whose reactions with this free radical are well documented: 5,5-dimethyl-1-pyrroline *N*-oxide (**12**),[14a] 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline *N*-oxide (**14**),[14b] and α-(4-pyridyl 1-oxide)-*N*-*tert*-butylnitrone (**16**) and EtOH.[14c] For these experiments, we used the metal ion-catalyzed Haber−Weiss reaction in the presence of a continued flux of $O_2^{\bullet-}$, at 6 μM/min, as a source of $HO^{\bullet}$.[6e,15] To our surprise, we obtained no EPR spectrum corresponding to nitroxide **11**, whereas under identical conditions, nitrones **12**, **14**, and **16** with EtOH readily spin trapped $HO^{\bullet}$ (Figure 2). In the case of the spin trapping system of **16** and EtOH, $HO^{\bullet}$ reacts with EtOH. This leads to α-hydroxyethyl radical that is spin trapped by nitrone **16**, yielding

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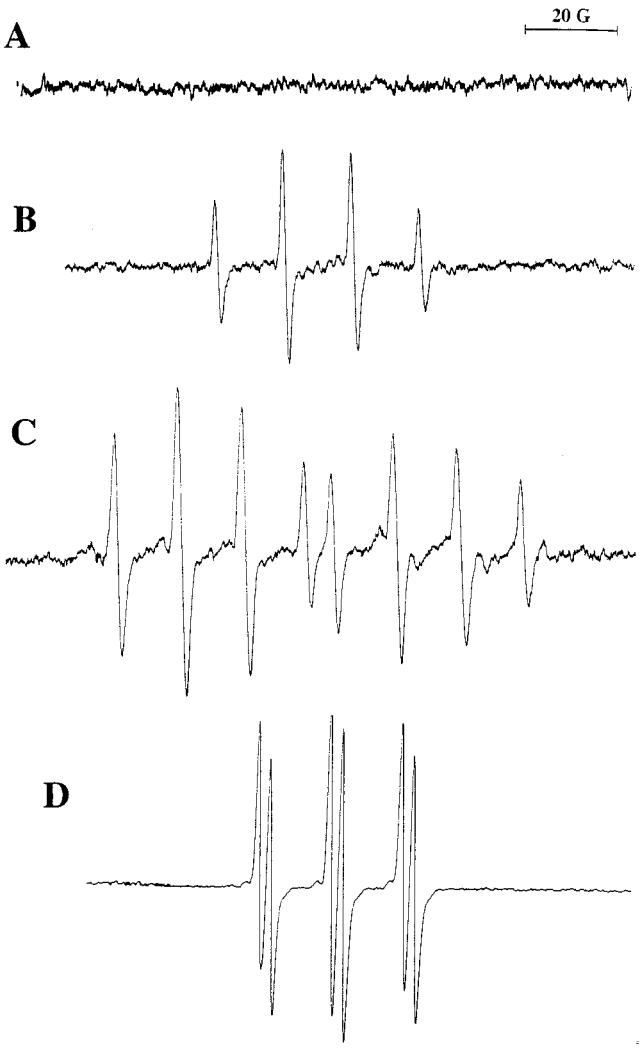
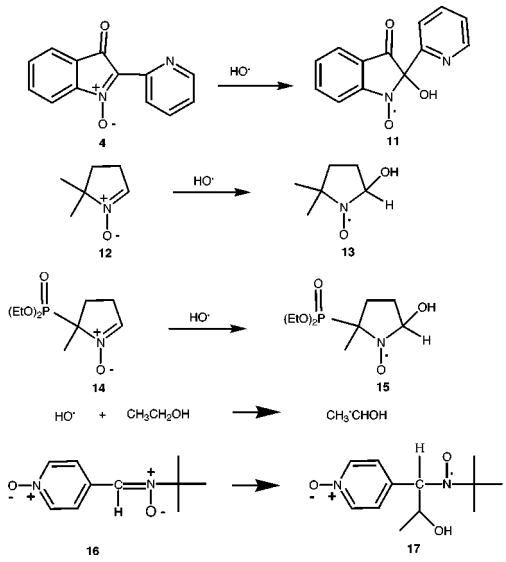


Figure 2. (A) EPR spectrum following the addition of xanthine oxidase to hypoxanthine (400 μM), in the presence of **4** (10 mM, 5% DMF in 50 mM phosphate buffer, pH 7.4, 1 mM DTPA) and Fe^{2+} (400 μM). Superoxide was generated at 6 μM/min. EPR spectra were recorded 2 min after commencing the reaction. Receiver gain was 1.25×10^4. (B) Same as (A) except nitrone **12** (10 mM) was substituted for **4**. (C) Same as (A) except nitrone **14** (10 mM) was used instead of **4**. (D) Same as (A) except nitrone **16** (10 mM) and EtOH (68 mM) were used instead of **4** and the receiver gain was 1.25×10^3.

nitroxide **17** (Scheme 4).[14c] Using γ-radiation as a source of HO$^\bullet$ resulted in poor yields of nitroxide **11**. In fact, by comparing intensities of the corresponding EPR spectra, we determined nitrone **16** and EtOH was 500 times more sensitive at reporting HO$^\bullet$ than was nitrone **4** (data not shown).

We then explored the ability of nitrone **4** to spin trap enzymatically secreted $O_2^{\bullet-}$. To our delight, we were unable to detect an EPR spectrum (data not shown) when nitrone **4** was incubated with the model $O_2^{\bullet-}$ generating system consisting of xanthine and xanthine oxidase, where under identical experimental conditions pyrroline *N*-oxides and some imidazoline *N*-oxides spins trap $O_2^{\bullet-}$.[6] In conclusion, data presented herein demonstrate that the efficiency of nitrone **4** to spin trap HO$^\bullet$ is limited. However, the inability of 2-(2-pyridyl)-3*H*-indol-3-one *N*-oxide to spin trap $O_2^{\bullet-}$ points to the utility of this nitrone to characterize HO$^\bullet$ under specific experimental designs.

Scheme 4



Experimental Section

General Methods. Hypoxanthine and xanthine oxidase were obtained from Sigma Chemical Co. (St. Louis, MO). α-(4-Pyridyl 1-oxide)-*N*-*tert*-butylnitrone (**16**) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Superoxide dismutase (SOD) was purchased from Boehringer Mannheim (Indianapolis, IN). 5,5-Dimethyl-1-pyrroline *N*-oxide (**12**) was synthesized as described in the literature[16] and purified by Kugelrohr distillation. 5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline *N*-oxide (**14**) was obtained from OXIS International (Portalnd, OR). IR spectra were recorded on an FT-IR spectrometer in $CHCl_3$ solution. ^1H NMR spectra were obtained using a GE QE-300/Tecmake NMR spectrometer. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are corrected. The preparation of 2-(2-pyridyl)-3*H*-indol-3-one *N*-oxide (**4**) is illustrative of the general syntheses of 2-substituted 3*H*-indol-3-one *N*-oxides.

Synthesis of 2-(2-Pyridyl)-3*H*-indol-3-one *N*-Oxide (4). 1-Iodo-2-nitrobenzene (2.41 g, 9.67 mmol, Aldrich Chemical Co., Milwaukee, WI) was dissolved in freshly distilled triethylamine (50 mL) to which 2-ethynylpyridine (1 g, 9.69 mmol, Aldrich Chemical Co.) was added. The reaction was stirred at ambient temperature under N_2 for 30 min at which point dichlorobis-(triphenylphosphine)palladium (0.2 g, 0.29 mmol, Aldrich Chemical Co.) and copper(I)iodide (0.185 g, 0.97 mmol, Aldrich Chemical Co.) were added. The mixture was stirred at room temperature for 3−4 days. The reaction mixture was filtered, the remaining solid was washed with ethyl acetate, and the combined solutions were evaporated to dryness, leaving an oil. The residual material was passed through a chromatographic column containing silica gel (Aldrich, mesh 230−400), eluting first with pentane/ethyl acetate (2:1), which removed a small amount of starting 2-ethynylpyridine. Changing to pentane/ethyl acetate (1:1), a second product was isolated to which ether (25 mL) was added. After the ether solution was refluxed for 30 min, the remaining solid was collected, yielding 2-(2-pyridyl)-3*H*-indol-3-one *N*-oxide **4** as a yellow solid (1.58 g, 70%). If desired, this product can be further purified by recrystallization from ethanol: mp 180−182 °C;[9a] IR ($CHCl_3$) 1731, 1714 (C═O), 1180 (N−O) cm^{-1}; NMR ($CDCl_3$) δ 7.30−7.40 (1, m), 7.50−7.90 (5, m), 8.49 (2, d, J = 0.026 Hz), 8.89 (1, s).

In a similar fashion, 2-phenyl-3*H*-indol-3-one *N*-oxide was obtained as a red solid in 65% yield, mp 184−186 °C, from ethanol.[9c] In contrast, poor yields of about 10−15% for 2-methyl-3*H*-indol-3-one *N*-oxide[9e] suggest that the general applicability of this synthetic approach is limited to 2-aryl-3*H*-indol-3-one *N*-oxides.

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EPR Spectral Measurements. Spin-trapped adducts, derived from the reaction of $HO^{\bullet}$ and $O_2^{\bullet-}$ with the nitrones, were recorded using an EPR spectrometer (Varian Associates E-9) at 25 °C. Reaction mixtures were transferred to a flat quartz cell, fitted into the cavity of the EPR spectrometer, and spectra were recorded at room temperature. Instrumentation settings were as follows: microwave power, 20 mW; modulation frequency, 100 kHz; modulation amplitude, 1.0 G; response time, 1 s; and sweep, 12.5 G/min. Receiver gain is indicated in each figure legend.

Superoxide Generation from Hypoxanthine/Xanthine Oxidase. Production of $O_2^{\bullet-}$ was determined by mixing hypoxanthine (400 μM), ferricytochrome *c* (80 μM), and sufficient xanthine oxidase in sodium phosphate buffer (50 mM) containing DTPA (1 mM), at pH 7.4. The rate of $O_2^{\bullet-}$ generation was estimated by measuring the SOD-inhibitable reduction of ferricytochrome *c* (80 μM) at 550 nm using an extinction coefficient of 21 mM^{-1} cm^{-1}.[17]

Spin Trapping of Hydroxyl Radical. The Fenton reaction was used as a source of $HO^{\bullet}$ by mixing H_2O_2 (100 μM), Fe^{2+} (100 μM), and nitrone **4** (3 mM in H_2O and DMF, 3.5%) in either H_2O or phosphate buffer at pH 7.4.

The metal ion-catalyzed Haber−Weiss reaction was used as an alternative source of $HO^{\bullet}$. Xanthine oxidase was added to hypoxanthine (400 μM), in the presence of nitrone **4** (10 mM, 5% DMF in 50 mM phosphate buffer, pH 7.4, 1 mM DTPA) and Fe^{2+} (400 μM). Superoxide was generated at 6 μM/min. Other spin traps were used, and data are presented in Figure 2: 5,5-dimethyl-1-pyrroline *N*-oxide (**12**) (10 mM); 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline *N*-oxide (**14**) (10 mM); α-(4-pyridyl 1-oxide)-N-*tert*-butylnitrone (**16**) (10 mM); and EtOH (68 mM). In each of the experiments, the reaction mixture was transferred to a flat quartz cell and fitted into the cavity of the EPR spectrometer. Spectra were recorded 3 min after commencing the reaction.

Hydroxyl radical was produced from irradiating an aqueous solution of sodium phosphate, 50 mM, pH 7.4 and spin trapped using either nitrone **4** (50 mM) or nitrone **16** (50 mM) and EtOH (150 mM). The source of $HO^{\bullet}$ was from ^{60}Co of H_2O in a Gammacell irradiator at a dose of 3000 Gy/h for 1 h.[18] This yields ~1 mM of $HO^{\bullet}$.

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