Synthesis of Spin Trapping Reagents for Oxygen-Centered Radicals

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Abstract

Huge efforts are still being offer to addressing the role played by reactive oxygen species such as superoxide $O_2^{\bullet -}$ and hydroxyl $OH^{\bullet}$ radicals in mediating a variety of pathological conditions, including toxicity due to several chemicals and ionizing radiation, carcinogenesis, inflammation, and degenerative diseases such as immunodeficiencies, aging, and atherosclerosis. Especially, these oxygen-derived free radicals have been concerned in the so-called ischemia/reperfusion injury,[1] the cytotoxicity occurring when tissues subjected to partial or total oxygen deprivation are reoxygenated. Of the methods available for assessing free radical formation in biological systems, electron spin resonance (ESR) spin trapping appears one of the most appropriate and has been applied to investigate.[2]

Synthesis of spin traps

Nitrones have emerged as the most popular spin traps for biological applications and out of several nitrone spin traps has received the most attention. These nitrone spin traps are divided into two categories. One is a family of pyrroline *N*-oxide represented by 5,5-dimethyl-pyrroline-*N*-oxide (DMPO **1**), and aromatic *tert*-butylnitrones represented by phenyl-*tert*-butylnitrone (PBN). DMPO has good affinity toward hydroxyl radical and superoxide anion radical which were classified active oxygen species. However, these superoxide anion radical adducts of DMPO have short lifetime, poor lipophilicity, and decomposed to give hydroxyl radical adduct. PBN has good lipophilicity, but the affinity of PBN to oxygen centered radical was poor. In order to dissolve these disadvantages, many chemists attempted to synthesize novel spin traps.

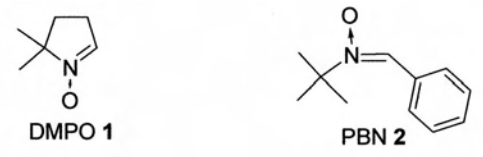
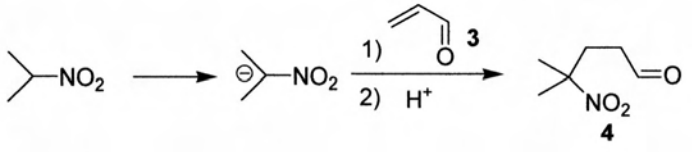


Chart 1

DMPO analogue

The preparation of 5,5-dimetyl-1-pyrroline-*N*-oxide (DMPO **1**) has steps that require care and attention. The initial reaction is a simple Michael addition in which acrolein (**3**) is carefully added to the anion of 2-nitropropane, to give 4-methyl-4-nitro-1-pentanal (**4**). Typical yields of pure aldehyde



Scheme 1

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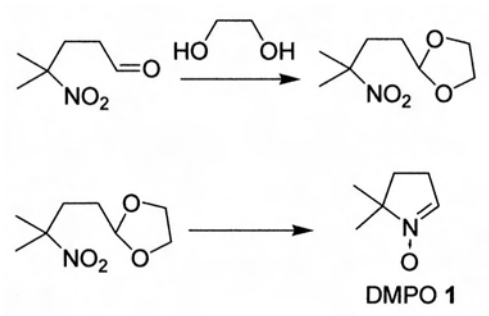
have been reported approximately 45%.[3]

The reduction of γ-nitro-carbonyl compounds by zinc dust has been found to be of general utility in the synthesis of pyrroline-*N*-oxide. In the case of aldehyde, it is advisable to initially protect the aldehyde prior to reduction. The reduction of aqueous solution of 1,3-dioxolane with zinc dust and ammonium chloride, maintaining the reaction at 10-15℃, gives the corresponding hydroxyl amine, which, upon acid hydrolysis to remove the protective group, lead to the desired spin trap, nitrone **1**.

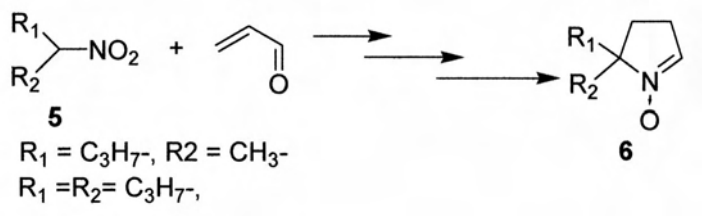


Scheme 2

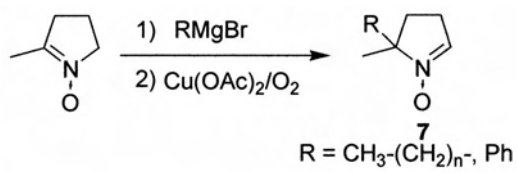
The use of DMPO **1** for oxygen centered radical generation in biological milieu is not without its limitation.[4] Reaction of DMPO **1** with superoxide is rather slow, having a second-order rate constant ranging from $10M^{-1}$ s^{-1} at pH 7.8[5] to $1.2M^{-1}$ s^{-1} at pH 7.4[6]. Its 1-Octanol/water partition coefficient was found to be only 0.02-0.09[7], indicating a preference for water over a lipid environment. On the other hand, the fate of DMPO **1** spin adduct could also be a source of misinterpretation in biological ESR experiments. First, the superoxide adduct undergoes a rapid chemical conversion to hydroxyl radical adduct.[8] Secondary, several cellular components and even superoxide itself are able to reduce DMPO-OOH and DMPO-OH into diamagnetic species.[9,10,11] Finally, in aqueous solution, DMPO is susceptible to metal-ion-catalyzed addition of water, leading *via* a nonradical reaction to variety of unwanted pyrrolidinoxyl radicals, including DMPO-OH.[9,12]

Even though DMPO is considered to be the prototype spin traps for superoxide anion radical $O_2^{\bullet-}$ and hydroxyl radical $OH^{\bullet}$, alkyl groups at either the 4- or 5-position offer the opportunity to vary the lipophilicity of pyrroline-*N*-oxide without altering the selectivity of these cyclic spin traps toward these free radicals. The approach in each of these publications are similar to the general synthetic scheme described in Scheme 3. Several nitroalkenes (**5**) undergo Michael addition to acrolein **3** in the presence of base. This step was followed by reductive cyclization with zinc dust to give appropriate substituted pyrroline-*N*-oxide (**6**).[13,14,15]

Lipophilic nitrones can be prepared by the reaction of various organic magnesium reagents with nitrone. After aerobic oxidation with copper (Ⅱ) acetate, the desired nitrone (**7**) was obtained in a reasonable yield.[16]

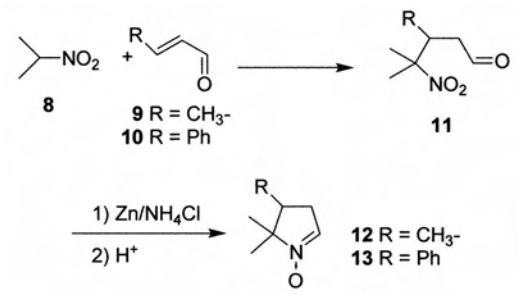


Scheme 3



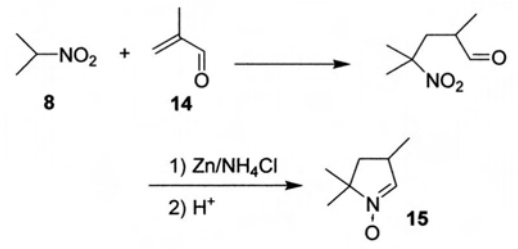
Scheme 4

In the case of placing substituents at 4-position, the base catalyzed reaction of 2-nitropropane (8) with either crotonaldehyde (9) or cinnamaldehyde (10) affords desired γ-nitroaldehyde. The protection of corresponding γ-nitroaldehyde with ethylene glycol followed by zinc reductive cyclization gave in the desired 4-alkyl or 4-aryl-pyrroline-*N*-oxide in reasonable yield (12,13).[13,17,15]



Scheme 5

Pathway that the synthesis of 3-substituted pyrroline *N*-oxide draw below the reaction of 2-nitropropane 8 toward, for example, methacrolein (14) which gave corresponding 3,5,5-trimethyl-1-pyrroline-*N*-oxide (15).[15]



Scheme 6

One of the advantage of using DMPO 1 derivatives, to distinguish between $O_2^{\bullet-}$ and $OH^{\bullet}$, is able to be the corresponding spin trapped adduct of these free radicals. In sometime, however, increasing the conversion of superoxide adduct to hydroxyl radical adduct is too rapid that to verify the production of $O_2^{\bullet-}$.[18] In order to increase a steric hindrance at position 3 of pyrroline ring, 3,3,5,5-tetramethyl-1-pyrroline-*N*-oxide (16) and 3,3-diethyl-5,5-dimethyl-1-pyrroline-*N*-oxide (17) were prepared. It might be possible to prepare nitrones that will not trap $O_2^{\bullet-}$.[19] However, those spin traps were oxidized in aerobic buffers contaminated with metal ion. 4-[(2-Ethoxycarbonyl) ethyl]-3,3,5,5-tetramethyl-1-pyrroline-*N*-oxide (18) is a similar spin trap without explosive potential associated with synthesis of nitrone.[20]

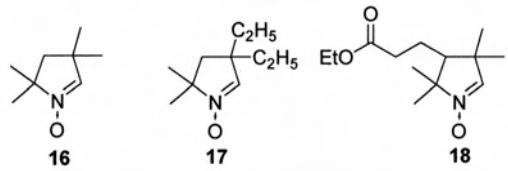
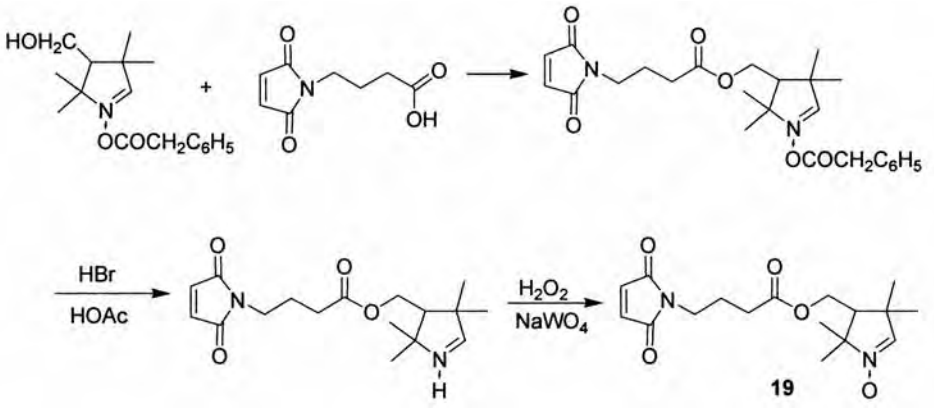


Chart 2

Maleimide-containing nitroxides, covalentry attached functional groups, have been successfully used to study the fluidity of membranes by incorporating the spin labeled maleimide into thiol proteins.[21] By labeling the plasma membrane of intact cells with a maleimide-containing nitroxide (19), it has recently become possible to be investigated the effect of free radical on the integrity of cell.[22]

Based on early *in situ* pharmacokinetic studies with a low-frequency EPR spectrometer, enhanced sensitivity of spin trapping method was found to be essential prior to attempts to spin trap free radicals *in vivo*. Deuterium



Scheme 7

translation increased EPR spectral height of spinadduct. Thus deuterated spintraps (20, 21) was prepared. One approach based on previous studies in which an ^{15}N-^{2}H-containing nitroxide which was shown to exhibit a 10-fold increase in sensitivity as compared with its ^{14}N-^{1}H-analog, is to substitute ^{2}H and ^{15}N in place of ^{1}H and ^{14}N in nitrone 22.[23]

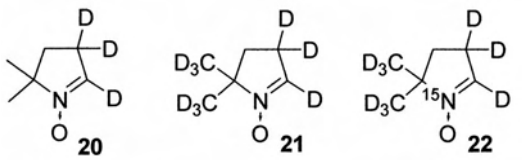


Chart 3

Recently, spin traps (23,24) which have long half-life of superoxide radical adduct and lipophilicity were developed.[24,25] The spin traps contained alchoxycarbonyl group at the 5 position of pyrroline ring. These structures were described in chart 4.

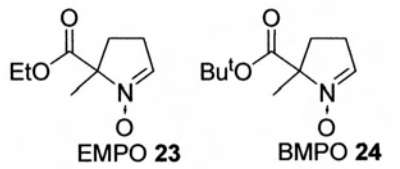
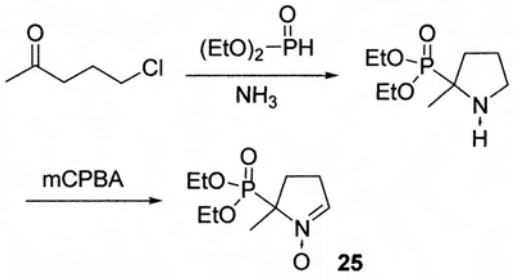


Chart 4

Phosphorus containing nitrone provides an opportunity to incorporate a spin trap at sites where cellular metabolism may generate free radicals. In this case, the phosphonate becomes a carrier for the spin trap.[26] The preparation of this family of pyrroline-*N*-oxides can be obtained from a two step procedure by initially bubbling ammonia into an ethanolic solution of 5-chloropentane-2-one and diethyl phosphite. The resulting diethyl (2-methyl-2-pyrrolidinyl) phosphonate was oxidized by *m*-chloroperbenzoic acid to 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline-*N*-oxide (25). These synthetic methods can lead other phosphorylated DMPO analogues (26-28). The structures are described in Chart 5.[27,28,29]



Scheme 8

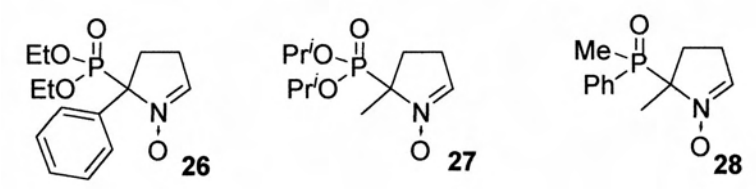


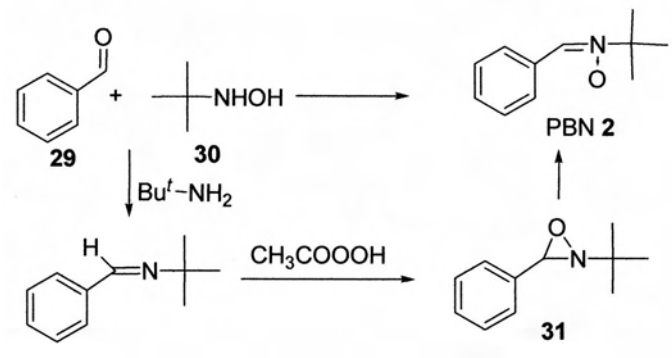
Chart 5

Dialkoxy phosphoryl group enhances hydrophilicity of DMPO analogues (26,27). In contrast, substitution by aromatic ring affects increasing hydrophobicity of spin traps. A phenylated analogue of DMPO (26,28), exhibit a partition coefficient 100 times greater than that of DMPO.[27]

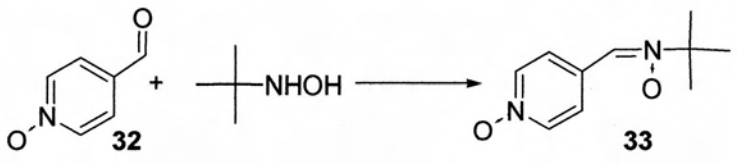
PBN analogue

As with the pyrroline-*N*-oxide family of cyclic nitrones, α-aryl-*tert*-butyl-nitrones (2) have their origin at a time predating the development of the spin trapping technique.[30] The availability of a wide variety of aromatic aldehydes and the ease of preparation make this class of nitrones ideal for exploring xenobiotics-generated free-radicals. There are several synthetic routes for synthesis of α-aryl-*tert*-butyl-nitrones (2). However, they require the gentle heating of benzaldehyde (29) with hydroxylamine (30). Alternative routes to α-aryl-*tert*-butyl-nitrones involve the condensation of *tert*-butylamine with aldehyde. Oxidation of the corresponding imines with peracetic acid gave reasonable yields of 2-*tert*-butyl-3-aryloxaziranes (31). Thermal conversion of these oxaziranes in refluxing acetonitrile gave desired spin trap 2.

Likewise, reacting *N*-(*tert*-butyl) hydroxylamine 30 with 4-pyridinecarboxyal dehyde *N*-oxide 32 affords α-(4-pyridyl 1-oxide)-*N*-*tert*-butylnitrone (POBN) (33). α-Aryl-*tert*-butyl-nitrones are susceptible to hydrolysis, the rate of which is pH dependent. For instance, the half-life of nitrone at pH 2 is 14 min, whereas this nitrone is stable for more than 32 hr at neutral pH.[31] α-(4-pyridyl 1-oxide)-*N*-*tert*-butylnitrone 33 is thousand times more hydrophilic than α-phenyl-*tert*-butyl-nitrones 2.[32]



Scheme 9

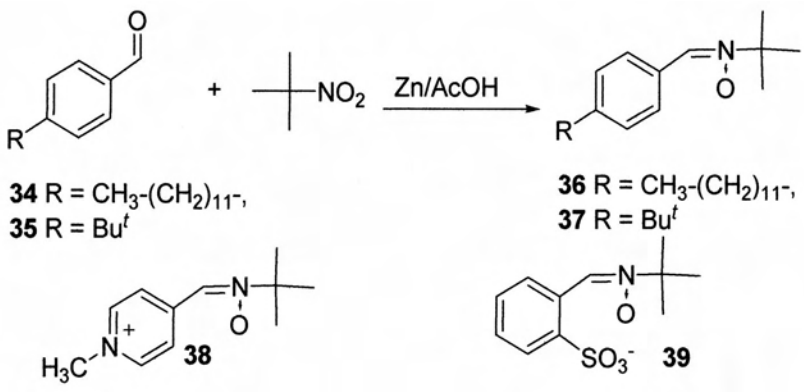


Scheme 10

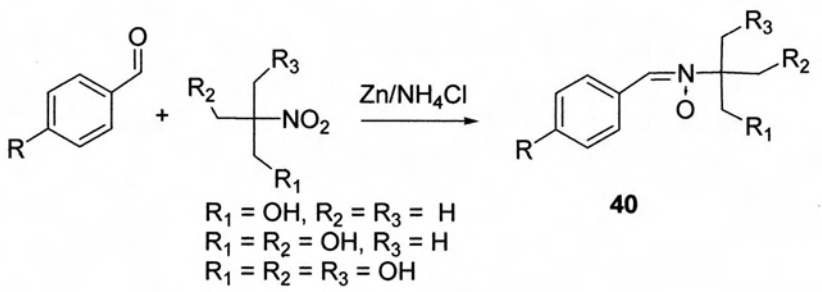
Enhanced hydrophobicity can be easily achieved through specific substitution on aromatic ring.[3] Condensation of either of 4-dodecyloxy (**34**) or 4-*tert*-butyl-benzaldehyde (**35**) with *in situ* produced hydroxyl amine, arising from the zinc reduction of 2-methyl-2-nitropropane results in the desired spin trap.[34] Using similar synthetic routes, hydrophilic spin traps (**38**,**39**) can be prepared.[35]

An alternative approach to enhancing the aqueous solubility of *N*-*tert*-butyl-α-phenyl-nitrone is to replace a methyl group of the *tert*-butyl moiety of this nitrone with hydroxyalkyl group.[31,36]

One of the limitation of acyclic nitrones as spin traps for $O_2^{\bullet -}$ and $OH^{\bullet}$ is poor stability of the corresponding nitroxides.[37] Based on the recent finding that the half-life of the spin adduct of 5-diethoxyphosphoryl-5-methyl-1-pyrroline-*N*-oxide is significantly longer than that of DMPO.[38] The phosphorylated analogs of PBN have been prepared. The enhancement



Scheme 11



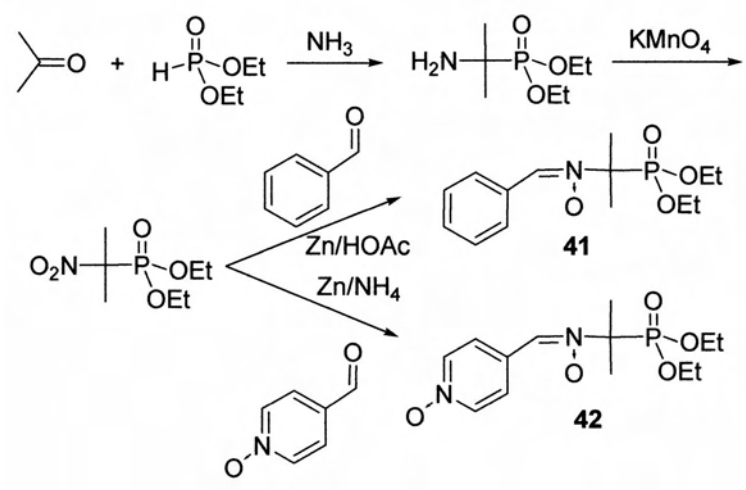
Scheme 12

of half-lives of the resulting spin adduct of phosphorylated analogs of PBN was uncertain.[39] The key step in the synthesis of *N*-benzylidene-1-diethoxyphosphoryl-1-methyl-ethylamine-*N*-oxide (**41**) and 1-diethoxyphosphoryl-1-methyl-*N*-[(oxidopyridin-1-ium-4-yl) methylidene] ethylamine *N*-oxide (**42**) are the oxidation of 1-amino-1-methylethylphosphonate to 1-methyl-1-nitroethylphosphonate. A one-pot reductive condensation with an appropriate aldehyde gave desired nitrone in an acceptable yield.[40]

The synthesis of *N*-2-(2-ethoxycarbonyl-propyl)-α-phenylnitrone (EPPN) (**43**) and its derivatives has been described.[41,42,43] Compared to the structurally related spin traps PBN **2** or POBN **33** ($t_{1/2}$ << 1 min), the half-lives of the superoxide spin adduct of EPPN derivatives **43**,**44** ($t_{1/2}$ = 2-7 min) are similar to the recently reported values for the spin traps EMPO **23**. All of them can be synthesized from commercially available compounds within two or three steps.

Conclusion

Nitrone spin traps have high potential for trapping oxygen-centered radicals. DMPO analogue is relatively stable for addition of oxygen-centered radical. PBN analogue is modified easily. The requirements of spin traps developed hereafter are long lifetime of oxygen-centered adduct, simple purification such as recrystallization, and stability without fridgeration.



Scheme 13

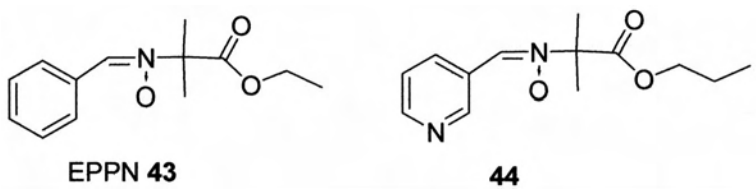


Chart 6

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