Convenient Synthesis of Linear Spin Traps Containing Diphenylphosphoryl Groups

Kosei Shioji,* Masahiko Takao, and Kentaro Okuma
Department of Chemistry, Faculty of Science, Fukuoka University, Jonan-Ku, Fukuoka 814-0180

(Received August 31, 2006; CL-060994; E-mail: shioji@fukuoka-u.ac.jp)

Novel spin traps in which a methyl group of the *tert*-butyl of PBN was substituted with a diphenylphosphoryl group, were successfully synthesized. The nitrone precursors were synthesized from diphenylphosphine oxide, acetone, and appropriate amines under microwave irradiation. The obtained amines were converted to the desired nitrones using Oxone. Spin adducts of oxygen-centered radicals such as the superoxide anion radical and the hydroxyl radical, which could not be detected in adducts using PBN, were observed using these nitrones.

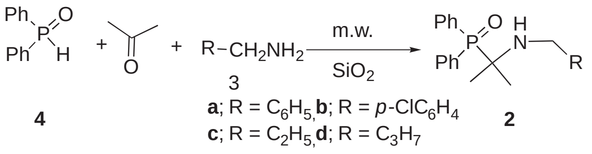
Trapping of active oxygen is one of the most important means of elucidating oxidative stress in vivo.[1] Linear spin traps, for example phenyl *tert*-butyl nitrone (PBN), are widely used for ESR.[2] However, there is a limitation to their use, because the superoxide radical adduct of PBN is unstable and ESR spectra of the hydroxyl radical ($OH^{\bullet}$) adduct (PBN–OH) and the superoxide anion radical ($O_2^{\bullet-}$) adduct (PBN–OOH) are similar.[3] Many spin traps using modified PBN have been developed to solve these problems. Insertion of an electron-withdrawing group, such as diethoxyphosphoryl or ethoxycarbonyl into the *tert*-butyl group, extends the lifetime of an oxygen-centered radical adduct.[4] Mono-ethoxycarbonylated analogues of PBN are efficient at trapping superoxide, whereas additional substitution results in decreased trapping rates of the superoxide.[5]

Previously, we succeeded in synthesizing crystalline spin traps which contained an alkylphenylphosphoryl group at the 5-position of pyrrole-*N*-oxide.[6] The superoxide adducts of these spin traps possess a long lifetime originating from electronic and/or steric effects of the phosphoryl moiety. A diphenylphosphoryl substituent has a further advantage, improvement of the ability for crystallization. These effects led us to synthesize novel linear spin traps containing a diphenylphosphoryl group. We describe herein a simple synthetic method for PBN analogues using microwave irradiation, and verification of their efficiency for trapping active oxygen species.

1 5

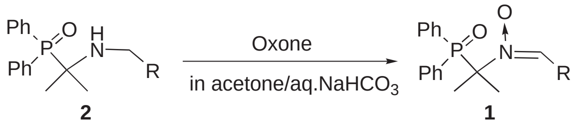
Chart 1.

Preparation of the desired nitrone **1** (Chart 1) was performed by oxidation of *N*-alkyl- or benzyl(1-diphenylphosphorylmethyl)ethylamine (**2**). Attempted synthesis of **2** by the reaction of (1-diphenylphosphorylmethyl)ethylamine with alkyl or benzyl chloride led to only diphenylphosphine oxide. Thus, preparation of amine **2** was carried out by imination of acetone with several amines **3** followed by reaction with phosphine oxide **4**. The reaction was accelerated by microwave irradiation in the presence of silica gel (Scheme 1). The reaction was completed within a few minutes without any solvent. This method is applicable to the addition of both aromatic and aliphatic amines. Our results are summarized in Table 1.[7]



Scheme 1.

Previously, we reported the preparation of 5-methylphenylphosphoryl-5-methylpyrroline-*N*-oxide (**5**).[6] Since the oxidation using *m*-chloroperbenzoic acid was difficult to purify, we used Oxone for conversion of amine **2** to nitrone **1**. The oxidation of **2** with Oxone in acetone-aq. $NaHCO_3$ solution afforded nitrone **1** (Scheme 2). Nitrone **1a**–**1c** was purified by recrystallization. Since nitrone **1d** is in liquid form, column chromatography was employed for purification. Pure nitrone **1** is stable at room temperature, thus it can be stored without refrigeration.



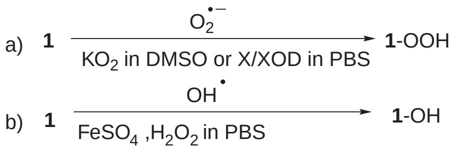
Scheme 2.

The lipophilicities of nitrones **1** were estimated from the partition coefficients (K_p) of **1** between water and 1-octanol (Table 1).[8] Due to its three phenyl moieties, nitrone **1a** has a high lipophilicity, higher than that of PBN ($K_p = 21$).[9] The high lipophilicity was decreased by a substituent on a phenyl group. The aliphatic analogues **1c**, **1d** have lower lipophilicities than **1a**, **1b**. These results suggest that spin traps **1a**, **1b** and **1c**, **1d** can be used for radical trapping in organic and aqueous media, respectively.

We attempted ESR experiments using **1**. Since spin traps **1a**, **1b** retain high lipophilicity, spin trapping of $O_2^{\bullet-}$ generated from KO_2 in DMSO was carried out (Scheme 3a). In a trapping experiment using both **1a** and **1b**, complex signals which changed during the analysis were observed. These results suggest that the $O_2^{\bullet-}$ adducts **1a**, **1b**–OOH were unstable in DMSO (Figure S1).[7]

Table 1. Synthesis of nitrone **1** and partition coefficients of **1**

R	Yield of **2**/%	Yield of **1**/%	K_p
C_6H_5	68	44	57
p-ClC_6H_4	65	47	23
C_2H_5	69	46	7.8
C_3H_7	63	34	9.9



Scheme 3.

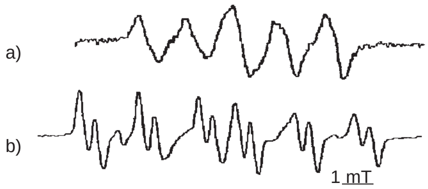


Figure 1. ESR spectra for oxygen-centered radicals of **1c**. a) $O_2^{\bullet-}$ adduct; [**1c**] = 10 mM, 3 mM of phosphate buffer pH = 7.2 b) $OH^{\bullet}$ adduct; [**1c**] = 10 mM, 100 mM of phosphate buffer pH = 7.2.

Table 2. Hyperfine splitting constants (mT) and total band widths (mT) of oxygen centered radical adduct of **1c**, **1d**

Adduct	A_P	A_N	A_H	Total band width
1c–OOH	3.18	1.36	—	6.9
1c–OH	4.66	1.68	0.40	9.2
1d–OOH	3.20	1.36	—	7.0
1d–OH	4.71	1.70	0.45	9.1

Because of the higher hydrophilicities of spin traps **1c**, **1d**, a trapping experiment using **1c**, **1d** can be carried out using a xanthine/xanthine oxidase (X/XOD) system in buffer solution. The spectra of adducts **1c**, **1d**–OOH indicated 5 broad lines, which were split by nitrogen and phosphorus. The signal for **1c**–OOH is shown in Figure 1a. These signals disappeared in the presence of superoxide dismutase (SOD). The half-life of adduct **1c**–OOH was measured in the X/XOD system. The decrease could be fitted to a first order reaction curve with a rate constant of $1.7 \times 10^{-3}\,s^{-1}$, which corresponds to a half-life of 6.8 min (Figure S4).[7] Hyperfine splitting by the β-hydrogen of the spin adduct was not identified. The signals of **1c**, **1d**–OOH in KO_2 in a 90% DMSO system attenuated quickly during analysis, similar to those of **1a**, **1b**. In contrast, when the addition reaction of **1c**, **1d** with $OH^{\bullet}$ generated from a Fenton reaction in phosphate buffer (Scheme 3b) was carried out, the ESR signals of the $OH^{\bullet}$ adduct **1c**, **1d**–OH were 12 lines split by nitrogen, phosphorus, and β-hydrogen (Figure 1b). The hyperfine coupling constants and total band widths are summarized in Table 2.

Hyperfine coupling constants from phosphorus in **1c**, **1d**–OOH were small, compared with that of *N*-benzylidene-1-diethoxyphosphoryl-1-methylethylamine ($A_P = 4.13$, $A_N = 1.35$, and $A_H = 0.21$ mT) reported by Tordo et al.[4c] The difference in hyperfine coupling between **1c**, **1d**–OH and **1c**, **1d**–OOH was larger than that of the diethoxyphosphoryl analogue ($A_P = 4.32$ mT for $OH^{\bullet}$ adduct). The phosphorus coupling was affected by the geometry of adduct.[10] Liu et al. reported that calculation for optimized geometries of superoxide adduct of phosphorylated PBN suggested the interaction between phosphoryl oxygen and hydroperoxyl group through the intramolecular hydrogen bond.[11] The interaction influences the rotations around the carbon–phosphorus bond, which resulted in a significant broadening and decreasing of hfsc in the observed signals.[12]

In summary, we have synthesized PBN analogues containing diphenylphosphoryl groups under microwave irradiation followed by Oxone oxidation. These spin traps form stable hydroxyl radical and superoxide radical adducts. The total width of the ESR signal for the $OH^{\bullet}$ adduct was smaller than that of the $O_2^{\bullet-}$ adduct. The difference caused by the diphenylphosphoryl moiety was useful for distinguishing between these radical species.

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