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Synthesis, Crystal Structure, and ESR Study of a Novel Phosphorylated Lipophilic Spin Trap

Ying-Kai Xu, Zhong-Wei Chen, Jian Sun, Ke Liu, Wei Chen, Wei Shi, Hong-Mei Wang, and Yang Liu*

State Key Lab for Structural Chemistry of Unstable and Stable Species, Center for Molecular Science, Institute of Chemistry, the Chinese Academy of Sciences, Beijing 100080, China

yliu@infoc3.icas.ac.cn

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In this article, the synthesis of a novel α-phosphorus-containing spin trap DEPPEPO [2-(diethoxyphosphoryl)-2-phenethyl-3,4-dihydro-2*H*-pyrrole-1-oxide] and the evaluation of its ability to spin-trap radicals, especially superoxide and hydroxyl radicals, are described. Single crystal X-ray structure analysis reveals that there exist a lot of intramolecular nonbonded attractive interactions in the molecule. The phenethyl group is located away from the diethoxyphosphoryl group and the nitronyl plane, and only one face of the nitronyl plane is sterically hindered by the oxygen attached to the phosphorus with a double bond. The latter feature is responsible for the stereoselection of the free radical additions on the nitronyl moiety. The ability of DEPPEPO to trap the active superoxide anion radical generated in the HX/XO system and the stability of their spin adduct were investigated with that for DEPMPO. The half-life of DEPPEPO is about 13.4 min, and as a result, the DEPPEPO seems to be a promising lipophilic spin trap, perhaps in both in vitro and in vivo ESR investigation. Because DEPPEPO is a solid compound, it is quite easy to purify by recrystallization and to store the compound even at room temperature. In addition, an obvious increase in lipophilicity for DEPPEPO was found as a contribution of 2-substituted phenethyl.

Introduction

The study of the superoxide anion radical ($O_2^{\bullet -}$) in biological systems recently has received much attention mainly because superoxide and reactive oxygen species (ROS) derived from (HOO•, HO•, and H_2O_2) very likely play an important role in many pathological processes, such as cancer, heart attack, Alzheimer's, ischemia-reperfusion syndrome, aging and DNA damage, etc.[1] However, the superoxide anion radical is too unstable to be detected directly by ESR spectrometry. Fortunately, ESR−spin trapping technique can help solve this problem[2] and consequently has been widely used to trap oxygen-centered radicals in biological milieu.[3,4]

DMPO (2,2-dimethyl-3,4-dihydro-2*H*-pyrrole-1-oxide, **1**, Chart 1) is one of the most popular spin traps to detect oxygen-centered radicals in vitro. However, application of DMPO in probing superoxide radicals, in particular in vivo, is limited by the instability of the DMPO−superoxide spin-adduct (half-life less than 1min). Therefore the development of new spin traps with relatively long-lived superoxide spin adducts is becoming one of the main goals in spin trapping−ESR study. In addition, it is also very important that spin traps have the desired lipophilicities to make the spin trap molecules available to interested bioradicals located in different regions of a living system.

In recent years, several α-phosphorus-containing spin traps have been developed.[5] DEPMPO [2-(diethoxyphosphoryl)-2-methyl-3,4-dihydro-2*H*-pyrrole 1-oxide**, 2**, Chart 1] with its outstanding spin trapping properties and long-lived superoxide spin-adduct (with half-life estimated to be 14.8 min at physiological pH)[6] is gradually replacing DMPO as a widely used spin trap in biological studies.[7] However, these α-phosphorus-containing spin traps have

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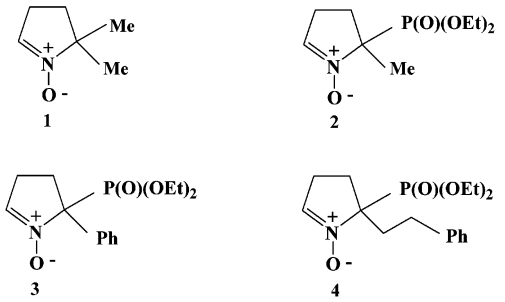
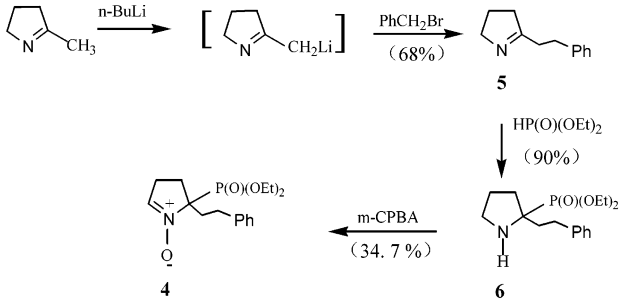
CHART 1



SCHEME 1



their deficiencies. The partition coefficient (K_p) of **2** in the 1-octanol/water system is only 0.06, very similar to that of **1** ($K_p = 0.1$),[8] which limited their applications in lipophilic condition. Another phosphorus-containing spin trap, DEPPPO [2-(diethoxyphosphoryl)-2-phenyl-3,4-dihydro-2*H*-pyrrole 1-oxide**, 3**, Chart 1], is more lipophilic ($K_p = 2.4$)[9] than **2,** while maintaining some good characteristics of **2**. But the radical trapping with **3** is so poorly stereoselective that its ESR spectra become more complicated, which actually limits its usage as a spin trap in many living systems.

More importantly, to understand the key factors of phosphoryl group in stabilizing the superoxide spin-adduct and design some other desirable novel spin traps, we need more information about their geometrical structures. The present paper addresses the synthesis of a new phosphorylated lipophilic spin trap, DEPPEPO [2-(diethoxyphosphoryl)-2-phenethyl-3,4-dilhydro-2*H*-pyrrole 1-oxide**, 4**, Chart 1], and the evaluation of its spin trapping abilities. In addition, authors first demonstrate a crystal structure of cyclic nitrone spin trap and discuss the structural effect on stabilization of the superoxide spin-adduct.

Results

Synthesis. Compound **4** can be synthesized by a three-step route as illustrated in Scheme 1. First, *n*-BuLi and $PhCH_2Br$ were added dropwise to a solution of 2-methyl-1-pyrroline in THF at −78 °C in nitrogen atmosphere to produce 2-phenethyl-1-pyrroline (**5**). Then, according to a method described by Barbati,[5a] diethyl (2-phenethyl-pyrrolidin-2-yl) phosphonate (**6**) was prepared by 1 week of stirring in a flask containing **5** and diethyl phosphite at room temperature. Finally, the compound **6** was oxidized by *m*-CPBA in chloroform at −10 °C producing the final product (**4**). After purified by column chromatography, a crystal of compound **4** was obtained.

X-ray Crystallographic Determination of Structure. A single crystal of **4** suitable for X-ray analysis was grown from the mixed solvent of *n*-hexane and dichloromethane. The different perspective views of the crystal structure of **4** are shown in Figure 1. There exists a chiral carbon in the compound **4**, and without separation two enantiomers are arranged in alternate rows in the unit cell. Inside the bulky diethoxyphosphoryl group, the ethoxy groups extend themselves unsymmetrically away from the phosphorus atom and the nitronyl plane. The five-membered ring is almost in one plane, and the angle between C(6)−C(5)−N(1)−C(8) and C(6)−C(7)−C(8) planes is just 15.9°, which indicates that C(7) is only a little deviated from the plane and up forward to O(1). The angle between the O(1)−P(1)−C(8) plane and the C(6)−C(5)−N(1)−C(8) plane is 104.9°, and the distance between O(1)−N(1) (3.064 Å) is a little shorter than that between O(1)−C(7) (3.347 Å), suggesting that the O(1) leans slightly to the nitrogen atom. The anti-conformational C(8)−C(9)−C(10)−C(11) is entirely located in the same plane that keeps an angle of 101.7° with the phenyl group for the sake of reducing the steric hindrance. The phenethyl group is oriented far away from the nitronyl plane and the diethoxyphosphoryl group. In addition, C(10) atom and O(1) atom seem to be mirror-symmetric about the nitronyl plane.

ESR Studies. (a) Spin Trapping of Hydroxyl Radical. Hydroxyl radical was generated by the Fenton system (H_2O_2–$FeSO_4$) in phosphate buffer of pH 7.0. In the presence of **4**, a strong ESR signal composed of a doublet of quartet was detected and could last for hours (Figure 2). The quartet (1:2:2:1) was attributed to similar hyperfine splitting constants of N and H, and the large phosphorus coupling further split the quartet to octet (Table 1). The signal can be inhibited by catalase and was assigned to [DEPPEPO−OH]•.

(b) Spin Trapping of Superoxide Anion Radical. Superoxide anion radical was trapped at pH 7.0 and pH 7.4 by using DEPPEPO as a spin trap in different superoxide-generating systems. As shown in Figure 3a, the same signal was observed by using either hypoxanthine in the presence of xanthine oxidase (HX/XO) or irradiation of a riboflavin−DTPA combination. A typical ESR signal could be detected when superoxide was generated in the presence of DEPPEPO. The trapped ESR signal was inhibited by addition of SOD in both superoxide-generating systems, which confirmed that the spectra were due to the trapping of $O_2^{\bullet-}$. This signal corresponds to the superimposition of two spectra, which were considered to be two diastereoisomers of the DEPPEPO−superoxide spin adduct by computer simulation. Two diastereoisomers can be unambiguously assigned to the trans (major spectra) and the cis (minor spectrum) diastereoisomers of the DEPPEPO−superoxide (DEPPEPO−OOH) spin adduct. The trans diastereoiso-

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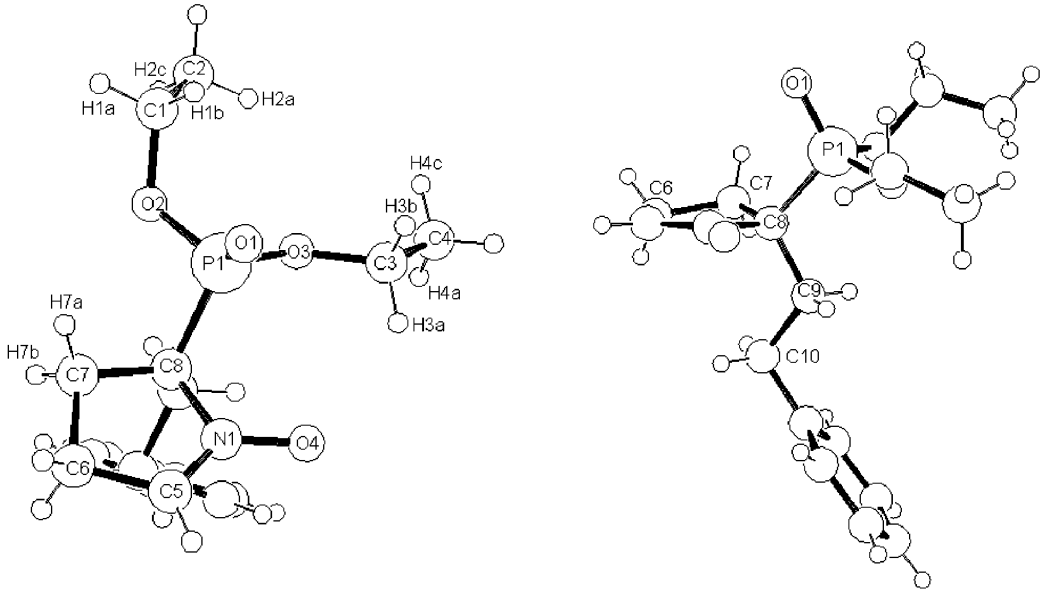

FIGURE 1. Different perspective view of DEPPEPO in the crystal with the atom-numbering scheme (ORTEP plot).

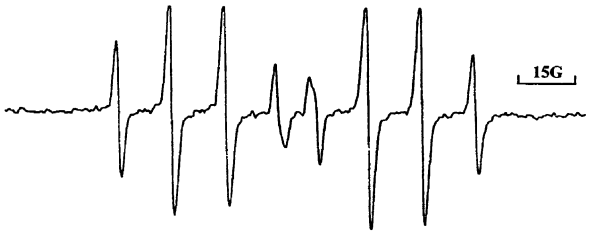

FIGURE 2. ESR spectrum of DEPPEPO−OH obtained by Fenton reaction, incubating H_2O_2 (1 mM), $FeSO_4$ (0.5 mM), and DEPPEPO (50 mM) at room temperature in phoshphate buffer (0.1 M, pH 7.4). Spectrometer settings: microwave power = 12.9 mW; modulation frequency = 100 kHz; modulation amplitude = 0.05 mT; time constant = 0.16 s; sweep time = 100 s; sweep width = 15 mT; and receiver gain = 3.17×10^4.

TABLE 1. ESR Hyperfine Splitting Constants of DEPPEPO−Hydroxyl and DEPPEPO−Superoxide Spin Adducts Obtained by an Automatic Fitting Procedure

radical	a_N (mT)	a_H (mT)	a_P (mT)	population		exchange time/ns
•OH	1.40	1.37	5.11			
O_2•						
cis	1.317	1.036	4.428		0.28	
T1	1.326	1.304	5.407	0.51	0.72	12.4
T2	1.282	0.888	5.038	0.49		

mer was involved in a chemical exchange (Table 1). The computer simulation shows that there exists an alternate line width in the ESR spectrum. The alternate line width induced by the existence of a chemical exchange between two conformers in ESR spectrum has been reported for the DEPMPO−superoxide and DEPPPO−superoxide spin adducts.[6,9]

(c) Kinetic Decay of the Superoxide Adduct. Superoxide radical was generated in a mixture of hypoxanthine (0.4 mM), xanthine oxidase (0.04 U mL^{-1}), spin trap (55 mM), and DTPA (300 μM) at pH 7.4. To terminate any further production of the superoxide spin-adduct, 0.5 mg mL^{-1}superoxide dismutase (SOD) was

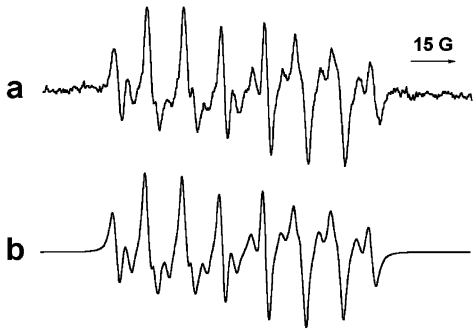

FIGURE 3. Spin trapping of superoxide radical anion. (a) Experimental spectrum obtained upon the reaction of hypoxanthine (0.5 mM) with xanthine oxidase (0.04 U mL^{-1}) in the presence of DEPPEPO (50 m M) and DTPA (1 mM) in phosphate buffer (0.1 M, pH 7.4). Spectrometer settings: microwave power = 12.9 mW; modulation frequency = 100 kHz; modulation amplitude = 0.11 mT; time constant = 0.33 s; sweep time = 100 s; sweep width = 15 mT; and receiver gain = 1.0×10^5. (b) Computer simulation of a.

introduced, and immediately, decay of the superoxide spin-adduct was monitored by measuring the decrease of the corresponding ESR spectrum of the diastereoisomer. The kinetic was simulated as a pseudo-first-order process with $R = 0.979$. As a result, the half-life of the DEPPEPO−superoxide adduct is about 13.4 min.

(d) Spin Trapping of Other Radicals. Hfcs's values of DEPPEPO spin adducts are listed in Table 2. The trapped hydrogen atom exhibited a unique ESR spectrum with a large H-hfsc that is slightly larger than the N-hfsc. This feature is very similar to the spectrum of DMPO−H spin adduct.[10] Figure 4b illustrated a computer simulation of the experimental spectrum of DEPPEPO−H (Figure 4a). *tert*-Butoxyl radical (*t*-BuO•) was generated by UV photolysis of a solution of (Bu^tOOBu^t) in benzene and, then, was simultaneously trapped by DEPPEPO. The experimental spectrum of spin adduct DEPPEPO−

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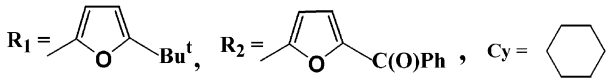
TABLE 2. ESR Hyperfine Splitting Constants of DEPPEPO Spin Adducts in Benzene

adduct[a]	source	a_N (mT)	a_H (mT)	a_p (mT)
CH_3O•	$CH_3OH/Pb(OAc)_4$	1.25	0.65	5.01
EtO•	$EtOH/Pb/(OAc)_4$	1.25	0.64	5.02
i-Pro•	*i*-$PrOH/Pb/(OAc)_4$	1.27	0.70	5.03
n-BuO•	*n*-$BuOH/Pb(OAc)_4$	1.25	0.66	5.01
t-BuO•	$(t\text{-BuO})_2/h\nu$	1.27	0.84	4.96
$PhCH_2S$•	$(PhCH_2S)_3/h\nu$			
	65.2%	1.25	0.93	4.70
	34.8%	1.24	1.12	5.30
H•	$(n\text{-Bu})_3SnH/h\nu$	1.4	1.90	5.03
p-NO_2PhO•	$(p\text{-}NO_2PhO)Sn\ Cy_3/h\nu$	1.26	0.75	5.01
t-Bu•	$t\text{-Bu(O)COSnCy}_3/h\nu$	1.28	0.72	5.00
Ph•	$Ph(O)COSnCy_3/h\nu$	1.27	0.71	5.02
p-MePh•	$p\text{-MePh(O)COSn Cy}_3/h\nu$	1.25	0.78	5.02
p-Iph•	$p\text{-IPh(O)COSnCy}_3/h\nu$	1.27	0.72	5.02
p−ClPh•	$p\text{−ClPh(O)COSnCy}_3$	1.26	0.75	5.02
m−ClPh•	$m\text{−ClPh(O)COSn Cy}_3$	1.26	0.72	5.01
m-FPh•	$m\text{-FPh(O)COSn Cy}_3$	1.26	0.74	5.01
2,4,6--Cl_3PhOCH_2•	$(2,4,6\text{−}Cl_3PHOCH_2)(O)COSnCy_3$	1.27	0.73	5.02
p-BrPh•	$(p\text{-}CrPhOCH_2)(O)\ COSnCy_3$	1.26	0.73	5.02
R_1•	$R_1(O)COSnCy_3$	1.26	0.75	5.00
R_2•	$R_2(O)COSnCy_3$	1.27	0.71	5.01

[a] Note:



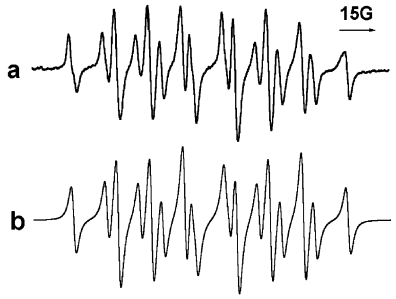


FIGURE 4. (a) ESR spectrum of DEPPEPO−H obtained as a consequence of UV photolysis of a solution of tributyltin hydride (1 M) and DEPPEPO (50 mM) in benzene. Spectrometer settings: microwave power = 12.9 mW; modulation frequency = 100 kHz; modulation amplitude = 0.056 mT; time constant = 0.33 s; sweep time = 80 s; sweep width = 15 mT; and receiver gain = 7.96×10^4. (b) Computer simulation of a.

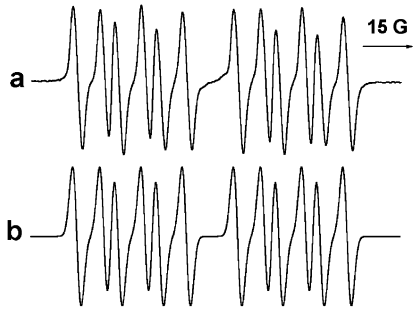


FIGURE 5. ESR spectra of the *tert*-butoxyl adduct of DEPPEPO and its computer simulation. (a) Spectrum obtained by UV photolysis of a solution of di-*tert*-butyl peroxide (1 M) in the presence of DEPPEPO (50 mM) in benzene. Spectrometer settings: microwave power = 12.9 mW; modulation frequency = 100 kHz; modulation amplitude = 0.044 mT; time constant = 0.33 s; sweep time = 80 s; sweep width = 11.4 mT; and receiver gain = 1.0×10^5. (b) Computer simulation of a.

t-BuO can be comparatively identified as one isomer by theoretical simulation (see Figure 5).

(e) Partition Coefficient (K_p). Using the method described by Konorev et al.,[11] the partition coefficient (K_p) of DEPPEPO was evaluated as the ratio between its absorbance (λ_{max} = 238 nm) in 1-octanol to that in water. The K_p value is about 7.6, which indicates that DEPPEPO is much more lipophilic than the spin traps DMPO ($K_p = 0.1$),[8] DEPMPO ($K_p = 0.06$),[6] and DEPPPO ($K_p = 2.4$).[9] It should be noted that the phenethyl group increased its lipophilicity greatly.

Discussion

Metalation of 2-Methyl-1-pyrroline. Hosomi[12] reported that alkylithium-mediated deprotonation of ketimines generally occurs anti to the nitrogen substituent. Houk et al.[13] further suggested, more theoretically, that endocyclic ketimines such as 2-alkyl-1-pyrroline had a thermodynamic bias for the (*E*)-azaallyl anion (which was postulated to result from excessive angle strain of the *(Z)*-azaallyl anion). In other words, by judicious selection of nucleophiles, proton abstraction might be minimal in addition to endocyclic aldimines that are devoid of 2-alkyl substitution, such as 1-pyrroline. Our preparation of 2-phenethyl-1-pyrroline demonstrates an example of the deprotonation of 2-methyl-1-pyrroline by *n*-BuLi occurring selectively in the methyl group (Scheme 2).

Structural Features of DEPPEPO. From the crystal structure analysis of DEPPEPO, we find two remarkable characteristics. **(1)** The diethoxyphosphoryl group ex-

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SCHEME 2

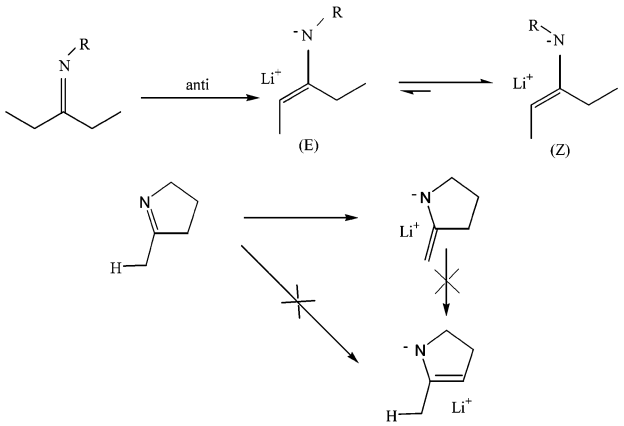

TABLE 3. Selected Nonbonding Distances (Å) and Dihedral Angles (deg) between Atoms of DEPPEPO

O(2)−H(7a)	2.70	O(2)−H(2a)	2.629
O(2)−H(2c)	2.627	O(2)−H(9a)	2.814
O(1)−H(3b)	2.788	O(1)−H(1b)	2.668
O(3)−H(2a)	2.708	O(3)−H(9b)	2.664
O(3)−H(4c)	2.539	O(4)-H(9b)	2.663
O(4)−H(3a)	2.427		
O(3)−C(3)-C(4)−H(4a)	63.4	O(3)−C(3)-C(4)H(4c)	56.6
O(2)−C(1)-C(2)−H(2a)	60.2	O(2)−C(1)-C(2)−H(2c)	59.8
P(1)−C(8)-C(9)−H(9b)	60.0	O(1)−P(1)-C(8)−C(9)	162.7

tends unsymmetrically, although there is no bulky group nearby that can hinder its extension (see Figure 1, left); **(**2**)** the C(7) atom deviated from the five-membered ring plane prefers to lean up toward O(1), rather than down toward C(10), even when the steric hindrances coming from O(1) and C(10) look similar (refer to Figure 1, right).

As a calculation from the above picture, the dihedral angle of P(1)−O(3)−C(3)−C(4) is 174.9°, suggesting a near-trans lower energy conformation. In contrast, the dihedral angle of P(1)−O(2)−C(1)−C(2) is 99.4°, indicating a higher energy conformation between eclipse and stagger. By comparing some nonbonding distances between oxygen and hydrogen (Table 3), the cause of the higher energy conformation could be attributed to the intramolecular nonbonding attractive interaction,[14] e.g. electrostatic force, between the O atom and a neighboring H atom from the same molecule. The oxygen atom is electronegative, and the hydrogen atom is electropositive. Especially, the partial positive charge on the hydrogen is highly concentrated because of its small size. Fortunately, the distances between O and H listed in Table 3 vary from 2.427 to 2.814 Å, which are within the range of nonbonding interaction as illustrated by Tsuzuki[14] (from 2.591 to 2.963 Å). It is nonbonding attractive interactions among the four oxygen atoms with the nearer hydrogen atoms that can optimize the conformation of DEPPEPO with a lower energy and introduce an unsymmetrical extension between two ethoxy groups accordingly.

Furthermore, we successfully explain that the deviation of C(7) up toward O(1) is the result of the attraction between O(2) and H(7a) (2.700 Å) and also the repulsion among H(10a) or H(10b) and H(7b) or H(7c). These are supported by comparison of the spatial angles around P(1). The angle of O(2)−P(1)−C(8) (99.19°) is smaller than those of O(2)−P(1)−O(3) (101.54°) and O(3)−P(1)−C(8) (109.07°), which means O(2) is closest to the nitronyl ring. For the same reason, O(3) keeps away from the nitronyl ring in order to avoid the strong repulsion from O(4) that is almost negatively charged and reserves enough space to let O(4) and H(3a) strongly attract each other (2.427 Å). Obviously, O(1) and O(3) also could stabilize the branch of O(2)−C(1)−C(2) by attracting the corresponding hydrogen atoms therein (O(1)−H(1b), 2.668 Å; O(3)−H(2a), 2.708 Å). These features probably make DEPPEPO a fixed structure and solidify itself at room temperature.

Stereoselection of DEPPEPO Spin Adducts. As viewed from the right picture of Figure 1, the most electronegative O(1) of the diethoxyphosphoryl group is located just above the nitronyl face, and there is an obviously repulsive force to the other approaching electronegative atom, such as radicals, and therefore, the radical addition is stereoselective. As a result, in most cases, only one diastereoisomer can be observed.

Structural Comparison with DEPMPO. The dihedral angle [P(1)−C(8)−C(7)−C(6)] of DEPPEPO is 129.6°, and its calculated value that derived from the $^{13}J_{p-c6}$ coupling (7.56 Hz) of ^{13}C NMR data is 131.5° according to Thiem's method.[15] Comparing the calculated dihedral angle with that from DEPMPO (131° [9]), we are sure that the conformation of the diethoxyphosphoryl group in DEPMPO is quite similar to that in DEPPEPO. A slight structural difference is that the five-membered ring of DEPMPO can be more easily converted from one conformation to another when losing the structural hindrances caused by phenethyl group.

Stabilization Behavior of the Diethoxyphosphryl Group. It has been reported that the diethoxyphosphoryl group plays an important role in stabilizing its superoxide spin adduct.[6,9] Tordo[16] further suggested that the strong electron-withdrawing effect and the large steric hindrance of the diethoxyphosphoryl group were responsible for the stabilization. But the key factor for the stabilization was still ambiguous, at least, partially because of lack of information on their molecular structures. On the basis of our X-ray crystallography and ESR data about the DEPPEPO adduct, the steric hindrance from diethoxyphosphoryl group does not seem to be the main reason for the structural stabilization. In fact, the diethoxyphosphoryl group efficiently interferes with the *trans*-OOH not just by its steric exclusion, since the major bulky parts of the diethoxyphosphoryl group are located away from the ring area except for the O(1).

Structurally comparing some other spin traps, it is obvious that the stability of superoxide spin adducts is not only concerned with electron-withdrawing effect, but also with the microenvironment around the trapped OOH group, especially the substituent group in 5-positions of the ring. For example, both the 5-substituted trifluoromethyl group (CF_3) and the 5-substituted diethoxyphosphoryl group are strongly electron-withdrawing, but half-lives of compound **7** and compound **8** superoxide spin

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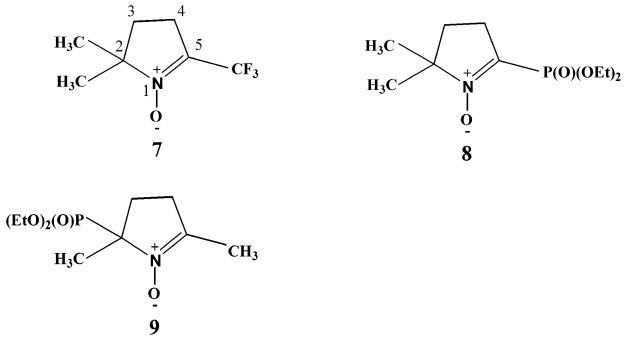
CHART 2



adducts are quite short-lived, as described in the literature[17,18] (see Chart 2, compound **7** and compound **8**). In our previous work[19] (also see Chart 2**,** compound **9**), the authors found 5-methyl substitution had an unstable effect on the spin adducts of superoxide anion radicals. Accordingly, we propose that one of the key effects on the stability of the corresponding cyclic nitrone−OOH spin adduct is the steric hindrance coming from a substituent group in the 5-postion. Perhaps, the neighboring substituents in the 5-position could change the conformation of the −OOH group, reduce the intramolecular nonbonded attractive interaction between the −OOH group and other atoms, and decrease the stability of the spin adducts.

Kinetic Study of the Superoxide Adduct. It was difficult to use the light-riboflavin−DTPA as a superoxide generating system because the persistent carbon-centered radical from the electron donor[20] leads to overestimating the measurement of half-lives. Therefore, we used the hypoxanthine−xanthine oxidase couple to generate superoxide anion radicals.

The half-life of the DEPPEPO−superoxide adduct (13.4 min) is rather close to that of DEPMPO−superoxide spin adduct (14.8 min),[6] which indicates that the phenethyl group does not obviously influence the decay rates. This result is not only consistent with our observation on the crystal structure of DEPPEPO in which the phenethyl group is located away from the nitronyl plane but also supports our proposal of only 5-substitution destabilizing its superoxide spin adducts.

Conclusions

Solid nitrone spin trap DEPPEPO has been prepared for the first time. As the only example of phosphorylated cyclic nitrone spin trap, the crystal structure of DEPPEPO has been determined by X-ray diffraction. Structural analysis reveals that the oxygen atom attached to the phosphorus with a double bond is responsible for the stereoselection of free radical addition on the nitronyl moiety, and there are intramolecular nonbonded attractive interactions between oxygen atoms and the nearest hydrogen atoms. The ability of DEPPEPO to trap free radicals, especially hydroxyl and superoxide, was evaluated. The similarity between half-lives of DEPPEPO−superoxide and that of DEPMPO−superoxide indicates that the 2-substituted phenethyl group does not obviously influence the decay of the spin adducts. Taking into account its solid state that can be easily purified, its higher lipophilicity and its higher stability on the superoxide adducts, DEPPEPO is a promising lipophilic spin trap.

Experimental Section

Synthesis and Characterizations. (a) General Methods. All reactions with moisture- and air-sensitive compounds were carried out under dry nitrogen atmosphere (ultrahigh purity) using standard Schlenk techniques. All chemicals are commercially available and were used upon receipt unless otherwise stated. NMR spectra were obtained at 200 MHz for ^{1}H and ^{31}P and at 300 MHz for ^{13}C.

(b) 2-Phenethyl-1-pyrroline (5). *n*-BuLi (31.7 mmol) was added dropwise to a solution of 2-methyl-1-pyrroline of 3.0 mL (2.6 g, 31.5 mmol) in 50 mL of THF at −78 °C for a half-hour. Then 3.7 mL (5.3 g, 31 mmol) of benzyl bromide was added and stirred for an hour at the same temperature. The mixture was acidified with dilute hydrochloric acid and extracted with ether to remove residual starting materials. The aqueous layer was poured over sodium carbonate and extracted with dichloromethane. Then the dichloromethane layer was washed with a saturated aqueous sodium chloride solution and dried over anhydrous sodium sulfate. The product was purified by column chromatography over silica gel (ethanol:dichloromethane = 5:100) (68%). IR (KBr, cm^{-1}): 2952.36, 2865.55, 1644.28, 1453.96, 751.84, 701.11. ^{1}H NMR ($CDCl_3$): δ 1.849 (2H, m, J = 8 Hz), 2.448 (2H, t, J = 8 Hz), 2.636 (2H, t, J = 8 Hz), 2.946 (2H, t, J = 8 Hz), 3.815 (2H, t, J = 8 Hz), 7.241 (5H, m). ^{13}C NMR($CDCl_3$): δ 22.27, 32.27, 35.05, 37.28, 60.47, 125.71, 127.96, 128.10, 141.24, 177.28. MS (FAB, *m*/*z*): 174 ($M^+ + 1$, 100).

(c) Diethyl (2-Phenethyl-pyrrolidin-2-yl) phosphonate (6). A 3.5 g (20.2 mmol) amount of 2-phenylethyl-1-pyrroline reacts with a small excess of diethyl phosphite (21.7 mmol) at room temperature for 7 days. The mixture was purified by column chromatography over silica gel (ethanol:dichloromethane = 5:100) and gave the product (90%). IR (KBr, cm^{-1}): 3321.37, 2976.82, 2869.03, 1232.47, 1162.93, 1053.93, 1028.01, 755.23, 701.77. ^{1}H NMR ($CDCl_3$): δ 1.336 (6H, t, J = 7 Hz), 1.7−2.4 (7H, m), 2.778 (2H, m), 3.05 (2H, t, J = 7 Hz), 4.184 (4H, m, J = 7 Hz), 7.241 (5H, m). ^{13}C NMR ($CDCl_3$): δ 16.466 (d, J = 5 Hz), 25.823 (d, J = 4 Hz), 30.370 (d, J = 5 Hz), 32.286, 39.336 (d, J = 8 Hz), 62.472 (d, J = 153 Hz), 125.657, 128.228, 142.029. ^{31}P NMR ($CDCl_3$, H_3PO_4, external standard): δ 30.5. ^{31}P NMR ($CDCl_3$, PPh_3, external standard): δ 35.706. MS (FAB, *m*/*z*): 312 ($M^+ + 1$, 10), 174 [$M^+ - P(O)(OEt)_2$, 100].

(d) 2-(Diethoxyphosphoryl)-2-phenethyl-3,4-dihydro-2*H*-pyrrole 1-Oxide (DEPPEPO) (4). A solution of 70% *m*-chloroperbenzoic acid (6.2 g, 25 mmol) in chloroform (200 mL) was added over a period of 1 h to a stirring solution of diethyl (2-phenethyl-pyrrolidin-2-yl)phosphonate (4.05 g, 12.4 mmol) in chloroform (300 mL) at −10 °C. The reaction mixture was then washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over sodium sulfate. The solvent was removed under vacuum. Column chromatography of the residue (silica, 10% ethanol in dichloromethatne) afforded product as a white solid (1.4 g, 34.7%): mp, 73 °C. ^{1}H NMR ($CDCl_3$): δ 1.33 (6H, t, J = 7 Hz), 2−3 (8H, m), 4.25 (4H, m, J = 7 Hz), 7.30 (5H, m). ^{13}C NMR ($CDCl_3$): δ 16.355 (d, J = 5 Hz), 26.182, 26.632, 28.752 (d, CH_2(4), J = 8 Hz), 33.784, 62.836 (d, J = 6 Hz), 64.043(d, J = 6 Hz), 126.178, 128.418, 138.007, 140.389. ^{31}P NMR ($CDCl_3$, H_3PO_4, external standard): δ 22.683. IR (KBr, $\tilde{\nu}$/cm^{-1}): 709.21, 781.62, 1063.58, 1147.35, 1239.55, 1597.81, 1656.00, 3363.14. UV (methanol;

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λ_{max}/nm): 241.8 (ϵ = 7731). MS (FAB, *m/z*): 326 (M^+ + 1, 85), 188 [M^+ − P(O)(OEt)$_2$, 100]. Anal. Calcd for $C_{16}H_{24}NO_4P$ (%): 59.06; H, 7.44; N, 4.30;O, 19.67; P, 9.53. Found (%): C, 59.17; H, 7.42; N, 4.31.

Spin Trapping Studies. (a) General Methods. Unless otherwise noted, all materials were obtained from commercial supplier and used without further purification. All buffers were stirred for 4 h in the presence of a chelating iminodiacetic acid resin (4 g/(100 mL)) to remove traces of metal impurities.

(b) ESR Measurements. ESR spectra were recorded at room temperature by using a computer-controlled X band ESR spectrometer. The spectrometer settings were normally at the modulation amplitude of 0.05 mT, modulation frequency of 100 kHz, microwave power of 12.9 mW, and microwave frequency of 9.5 GHz. Reaction mixtures were prepared in a phosphate buffer (0.1 M, pH 7.4). When ultraviolet (UV) light was utilized to generate free radicals, the UV light beam from a 200 W high-pressure mercury lamp was directly focused on sample solution just for a few seconds. Standard ESR spectra were simulated using ESR software developed by D. Duling from the Laboratory of Molecular Biophysics, NIEHS,[20] and with the ESR software developed by A. Rockenbauer from the Central Research Institute of Chemistry, Hungary.[22]

(c) Superoxide Trapping: Hypoxanthine-Xanthine Oxidase System. Xanthine oxidase (XOD, 0.04U mL^{-1}) was added to a solution of DEPPEPO (50m M), DTPA (1 mM), and hypoxanthine (0.5 mM) in phosphate buffer (0.1 M, pH 7.4). The ESR spectrum was recorded 1 min after the addition of XOD.

(d) Hydroxyl Trapping: Fenton System. The hydroxyl radical was generated with the addition of $FeSO_4$ (0.5 mM) to a solution of DEPPEPO (50 mM) and H_2O_2 (1 mM) in phosphate buffer (0.1 M, pH7.4). The ESR spectrum of the hydroxyl adduct was recorded 1 min after addition of ferrous sulfate. No ESR signal was observed in the presence of catalase (50 U mL^{-1}) in the incubation mixture.

(e) H· Trapping. H· was produced by UV photolysis of a solution of tributyltin hydride (1 M) and DEPPEPO (50 mM) in benzene.

(f) Alkoxyl Radicals Trapping. *t*-BuO· was generated by UV photolysis of a solution of di-*tert*-butyl peroxide (1 M) in the presence of DEPPEPO (50 mM) in benzene. CH_3O•, EtO•, *i*-PrO•, and *n*-BuO• were produced by adding a small amount of $Pb(OAc)_4$ to a solution of DEPPEPO (50 mM) in benzene.

(g) Thiyl Radicals Trapping. $PhCH_2S$• was generated by UV photolysis of dibenzyl disulfide (1.5 M) and DEPPEPO (1 M) in benzene.

(h) Other Radicals Trapping. Other radicals were generated by UV photolysis of a solution of corresponding sources (1 M) in the presence of DEPPEPO (50 mM) in benzene.

(i) Kinetics of Decay of Superoxide Spin Adducts. We used the hypoxanthine−xanthine oxidase system to generate the superoxide radical in phosphate buffer (0.1 M, pH 7.4). The spin trap concentration was 55 mM for DEPPEPO. The superoxide generation was initiated by incubating xanthine oxidase in the reaction mixture for 10 min and suppressed by adding SOD (0.5 mg mL^{-1}). The spin adduct decay was followed by monitoring the decrease of an appropriate line of the spin adduct. Computer simulations were performed by use of Origin 6.0. In these calculations, the monitored ESR peak intensity is related to the actual radical concentration by a scale factor.

Partition Coefficient (K_p) Determination. The lipophilicity of DEPPEPO was evaluated by the method described by Konorev et al. A solution of DEPPEPO was prepared in 1-octanol at a concentration of 0.50 mM. The concentration of spin trap was measured at its optical absorption maximum by using a computer-controlled OOIBase 32 UV/visible spectrometer. Equal volumes (5 mL) of water were vigorously mixed at 37 °C for 1 h, and the two phases were separated by brief centrifugation. K_p was measured as the ratio between the absorbency of the spin trap in 1-octanol to that in water.

Structural Determination. Data of DEPPEPO were collected on a Siemens MSC/AFC diffractometer with a graphite monochromator using Mo Kα radiation. The structure was solved by direct methods and refined by full-matrix least-squares methods on F^2. All calculations were performed by SHELX97.[23,24] Hydrogen atoms were treated as idealized contributions.

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Supporting Information Available: X-ray crystallographic details for compound **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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