SYNTHESIS OF 2-DIETHOXYPHOSPHORYL-2-METHYL-5-PHENYL-3,4-DIHYDRO-2H-PYRROLE-1-OXIDE (DEPMPO-PH): A NEW RADICAL SPIN-TRAP

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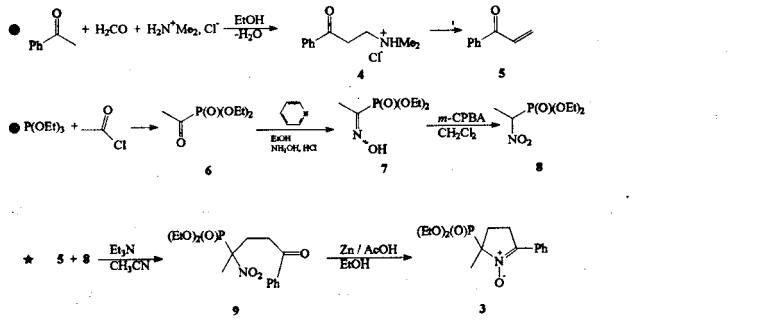
Oxygen-centered radicals are suspected to play an important role in number of pathological processes as inflammatory processes[1], during the ischemia-reperfusion[2]...The ESR spin-trapping method has been extensively used to study their role. Numerous spin-traps have been prepared[3,4], but the most widely used was the 2,2-dimethyl-3,4-dihydro-2*H*-pyrrole-1-oxide (DMPO) **1** (scheme 1). In the search of new nitrones, we previously reported the synthesis and the features of the spin-trap 2-diethoxyphosphoryl-2-methyl-3,4-dihydro-2*H*-pyrrole-1-oxide (DEPMPO) **2** (scheme 1)[5], whose superoxyde adduct was shown to be about 15 times more persistent than that of DMPO in phosphate buffer at pH 7.
The introduction of a phenyl group in position 5 will increase the lipophilicity, which can favour the trapping of radicals generated in lipid-rich locations. Furthermore, the potentialities of lipophilic nitrones as neuroprotective agents[6,7] is largely investigated.

Scheme 1.

We report here, the synthesis of a new spin-trap, the 2-diethoxyphosphoryl-2-methyl-5-phenyl-3,4-dihydro-2*H*-pyrrole-1-oxide (DEPMPO-Ph) **3** (scheme 2).



Scheme 2.

1) McCord, J. M. *Science* **1974**, *185*, 529-531.
2) Halliwell, B.; Gutteridge, J. M. C. *Free radicals in Biology and Medicine*; Clarendon Press: Oxford, 1989.
3) Anderson Evans, C. *Aldrichima Acta* **1979**, *12*, 23-29.
4) Konaka, R.; Kawai, M.; Noda, H.; Kohno, M.; Niwa, R. *Free Rad. Res.* **1995**, *23*, 15-25.
5) Barbati, S.; Clément, J. L.; Olive, G.; Roubaud, V.; Tuccio, B.; Tordo, P. *^{31}P Labeled cyclic nitrones : a new class of spin traps for free radicals in biological mitieu*; Minisci, F., Ed.; Kluwer Academic Publishers: Dordrecht / Boston / London, 1997; Vol. 27 - 3. High Technology, pp 39-47.
6) Floyd, R. A. *Adv. Pharmacol.* **1997**, *38*, 361-378.
7) Hensley, K.; Carney, J. M.; Stewart, C. A.; Tabatabaie, T.; Pye, Q.; Floyd, R. A. *International Review of Neurobiology* **1997**, *40*, 299-317.

SYNTHESIS OF 2-DIETHOXYPHOSPHORYL-2-METHYL-5-PHENYL-3,4-DIHYDRO-2*H*-PYRROLE-1-OXIDE (DEPMPO-Ph): A NEW RADICAL SPIN-TRAP

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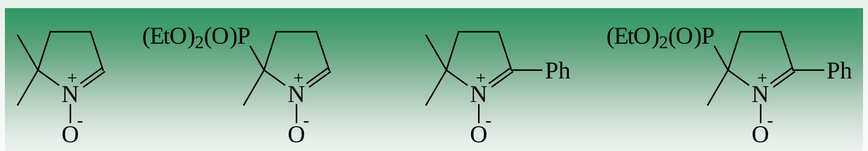
Introduction

Oxygen-centered radicals are suspected to play an important role in number of pathological processes as inflammatory processes, cancers, during the ischemia-reperfusion, *etc* … The ESR spin-trapping method has been extensively used to study their role. Numerous spin traps have been prepared but the most widely used was the 2,2-dimethyl-3,4-dihydro-2*H*-pyrrole-1-oxide (DMPO) **1**. However, **1** is known to present some limitations such as its sensitivity to nucleophilic addition of water, decomposition of the superoxide spin adduct of DMPO-OO(H) to give DMPO-OH and the low persistence of DMPO-OO(H) (60 s at pH 7.0). In the search of new nitrones, we previously reported the synthesis and the features of the spin-trap 2-diethoxyphosphoryl-2-methyl-3,4-dihydro-2*H*-pyrrole-1-oxide (DEPMPO) **2**.[1] With DEPMPO **2**, the persistence of the superoxide adduct DEPMPO-OO(H) was shown to be about 15 times higher than that of DMPO-OO(H) in phosphate buffer at pH 7, and no significant decomposition of DEPMPO-OO(H) into DEPMPO-OH adduct was observed. In 1994, Janzen *et al.* reported the synthesis of 2,2-dimethyl-5-phenyl-3,4-dihydro-2*H*-pyrrole-1-oxide (DMPO-Ph) **3**[2], and he found that the persistence of **3**-OO(H) was improved compared to that of DMPO-OO(H). This could be explained by preventing the disproportionnation reaction of the spin adducts. Furthermore, the replacement of the β hydrogen atom by an alkyl or aryl group leads to a simplification of the ESR signal of spin adducts and can hence improve the signal to noise ratio. Furthermore, the 5- phenyl group will not favour dimerization processes as described for a methyl group in the same position. [3]

The introduction of a phenyl group will also increase the lipophilicity, which can favour the trapping of radicals generated in lipid-rich locations. Furthermore, the potentialities of lipophilic nitrones as neuroprotective agents[4,5] is largely investigated. We report here the synthesis of a new phosphorylated spin-trap, the 2-diethoxyphosphoryl-2-methyl-5-phenyl-3,4-dihydro-2*H*-pyrrole-1-oxide **4**.



1 **2** **3** **4**

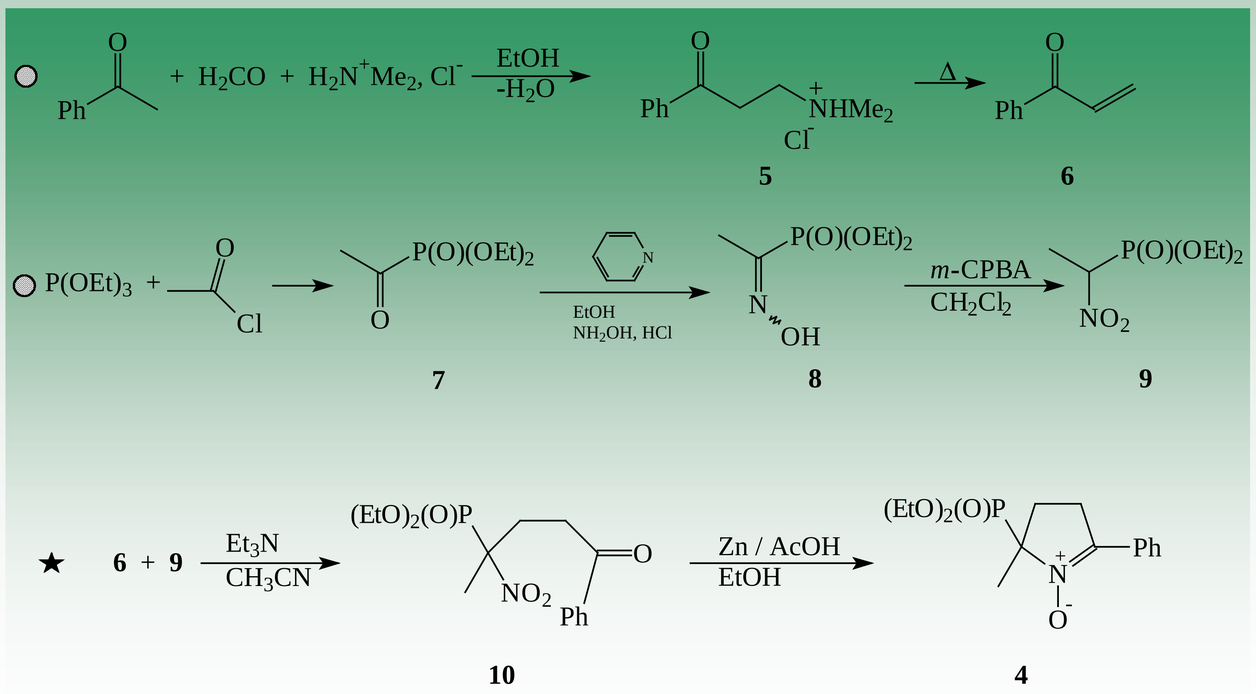
Synthesis

The direct synthesis by the addition of phenylmagnesium[2] bromide over nitrone **2** gave a mixture of 9 products (detected in ^{31}P) in which the intermediate hydroxylamine was not identified. The synthesis of **4** was then carried out using a convergent synthesis, which is summarized in scheme below.

The synthesis of **5** was carried out by a Mannich addition, according to ref. 6, in 65 % yield. Preparation of **6** was achieved by collecting the distillate between 62 and 77 °C from the pyrolysis of **5** (230 °C, 1.5 mmHg).[6] **6** was obtained in 63% yield and was pure by ^{1}H- and ^{13}C-NMR.

The procedure developed by Zòn[7] was followed for the synthesis of **9**. After purification **8** is obtained in 79 % yield. The oxidation of **8** led to **9** (60 % yield, purity about 95 %).

The Michael addition was achieved using a catalytic amount of triethylamine in acetonitrile as solvent .[8] A mixture of **6** and **9** was stirred at room temperature for 3 days, to afford the γ nitro ketone **10** in 71 % yield. Cyclization was achieved by zinc / acetic acid in ethanol at 4 °C during 48 h.[9] After purification by silica gel chromatography (ether as eluent) to give the title coumpound **4** in 22 % yield.



References

(1) Barbati, S.; Clément, J. L.; Olive, G.; Roubaud, V.; Tuccio, B.; Tordo, P. In *Free Radicals in Biology and Environment*; Minisci, F., Ed.; Kluwer Academic Publishers: Dordrecht / Boston / London, **1997**; *Vol. 27 - 3*. High Technology, p 39-47.
(2) Janzen, E. G.; Zhang, Y.-K.; Haire, D. L. *J. Am. Chem. Soc.* **1994**, *116*, 3738-3743.
(3) Janzen, E. G.; Zhang, Y.-K.; Arimura, M. *Chem. Lett.* **1993**, 497-500.
(4) Floyd, R. A. *Adv. Pharmacol.* **1997**, *38*, 361-378.
(5) Hensley, K.; Carney, J. M.; Stewart, C. A.; Tabatabaie, T.; Pye, Q.; Floyd, R. A. *International Review of Neurobiology* **1997**, *40*, 299-317.
(6) Furniss, B. S.; Hannaford, A. J.; Rogers, V.; Smith, P. W. G.; Tatchell, A. R. In *Vogel's Textbook of pratical organic chemistry*; 4th edition ; Longman: London and New-York, **1978**, p 815-816.
(7) Zòn, J. *Synthesis* **1984**, 661-663.
(8) Ono, N.; Kamimura, A.; Miyake, H.; Hamamoto, I.; Kaji, A. *J. Org. Chem.* **1985**, *50*, 3692-3698.
(9) Haire, D. L.; Hilborn, J. W.; Janzen, E. G. *J. Org. Chem.* **1986**, *51*, 4298-4300.