Transphosphorylative Spin-Labeling Utilizing Diimidazolides of Pentavalent Phosphorus

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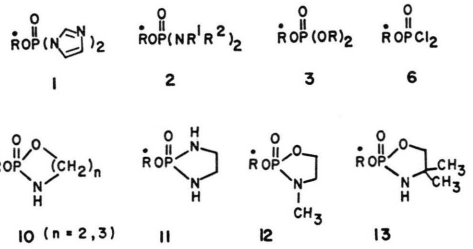
Spin-labeled Imidazolides, Phosphates, Cyclophosphoramidates, Transphosphorylation, Phosphorodiamidates

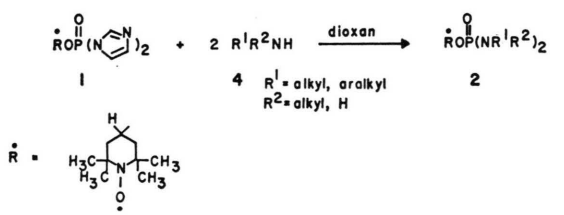
The reaction of the spin-labeled diimidazolide **1** with hydroxy and amino compounds was studied. Diimidazolide **1** was superior to the dichloridate **6** for the preparation of spin-labeled phosphates **3** and phosphorodiamidates **2**. The spin-labeled phosphate **15** was synthesized through hydrolysis of **6**. Spin-labeled cyclophosphoramidates (**10–13**), analogs of the cytotoxic Endoxan, were prepared by the reaction of **1** and **6** with the appropriate difunctional nucleophile. Inductive effects of substituents in the imidazolyl moiety on the transphosphorylation reaction were also studied.



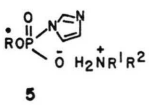
In 1975, we reported[1] preliminary results concerning the reaction of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)diimidazole-1,1′-phosphinate (**1**) with amines and alcohols to give the corresponding phosphorodiamidates **2** and phosphates **3**, respectively. Since that time, we have investigated these reactions more thoroughly, in addition to other transphosphorylation reactions[2–4]. Now we wish to report our findings in detail.

Phosphates and Phosphoramidates

Diimidazolide **1** proved to be a useful reagent for the preparation of N,N′-dialkylphosphorodiamidates (**2**) under mild conditions without the necessity of a condensing agent, such as, triethylamine.



In two of the cases (**4**; *i*-$C_3H_7NH_2$ and *c*-$C_6H_{11}NH_2$) a pink solid, insoluble in dioxan, but soluble in water, was also formed during the reaction. On the basis of microanalyses, solubility characteristics, and behaviour in the presence of aqueous hydrochloric acid and sodium hydroxide (see Experimental Section), the structure **5** is proposed for this compound. The formation of a salt-like byproduct during the transphosphorylation reaction is not without precedent, since BADDILEY and coworkers[5] observed the formation of cyclohexylammonium diphenylphosphate during the preparation of N-cyclohexylphosphoramidate from diphenyl imidazole-1-phosphonate and cyclohexylamine in dioxan.



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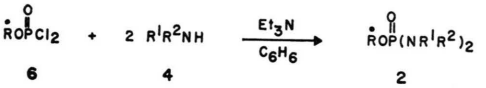
The byproduct **5** appeared shortly after mixing of the reagents. Thorough drying of the reagents prior to the reaction did not diminish the amount of **5** (Table I).

Among the diamidates prepared by the transphosphorylation reaction was (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl) N,N′-di-morpholinylphosphorodiamidate

$$\left(\mathbf{2},\ R^1R^2 = O\begin{matrix}\diagup CH_2CH_2^- \\ \diagdown CH_2CH_2^-\end{matrix}\right).$$

The preparation of this compound from (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)phosphorodibromidate and morpholine was described[6]. Now we have found that this compound has a decomposition point approximately 50 °C below that reported[6]. In order to verify the identity of the morpholine derivative **2**, and similarly of all other amine derivatives **2**, these compounds were prepared from the corresponding dichloridate **6**[7,8] (Table II).

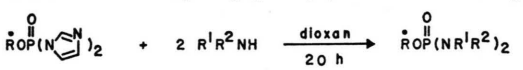


6 **4** **2**

A mixture melting point of compound **2**, prepared

$$R^1R^2 = O\begin{matrix}\diagup CH_2CH_2^- \\ \diagdown CH_2CH_2^-\end{matrix},$$

Table I. Reaction of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)diimidazole-1,1′-phosphinate (**1**) with amines.



1 **4** **2**

Amine (**4**)	Yield [%]	m.p. [°C] (dec)	M. W.[a] Found (Calcd)	EPR No. of lines	a_N [G]
i-Propylamine	77[b]	98–99	330 (334.42)	3	15.0
n-Butylamine	60	47–50	355 (362.48)	3	15.1
s-Butylamine	83	softens 75 melts 81	359 (362.48)	3	15.1
i-Pentylamine	52	39–43	378 (390.53)	3	15.0
Cyclohexylamine	50[c]	softens 85 melts 112	408 (414.63)	3	15.0
Cyclohexylmethylamine	62	oil	438 (442.63)	3	15.2
n-Decylamine	69	softens 10 melts 15[d]	500 (530.80)	3	15.1
Phenethylamine	83	87–90	463 (458.57)	3	15.3
Benzylamine	79	softens 84 melts 90	423 (430.51)	3	15.0
Aziridine[e]	89	63–65			
Morpholine[f]	89	51–52		3	15.3

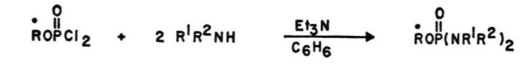
[a] The microanalyses are in agreement with calculated values: C, ±0.3%; H, ±0.3%; N, ±0.3%. The actual values for the analyses were submitted to the editor.

[b] Also isolated was 13% of **5** ($R^1 = H$, $R^2 = i\text{-}C_3H_7$), m.p. 138–141 °C (dec).
Analysis for $C_{15}H_{30}N_4O_4P$:
Calcd C 49.85 H 8.37 N 15.50,
Found C 49.66 H 8.34 N 15.38.
EPR: 3 lines; a_N (aqueous) = 17.2 G.

[c] Also isolated was 40% of **5** ($R^1 = H$, $R^2 = c\text{-}C_6H_{11}$), m.p. 225 °C (dec).
Analysis for $C_{18}H_{34}N_4O_4P$:
Calcd C 53.85 H 8.54 N 13.96,
Found C 53.69 H 8.66 N 14.01.
EPR: 3 lines; a_N (aqueous) = 16.9 G.

[d] A melting point, not a decomposition point. [e] Lit.[15,16] m.p. 63–65 °C (dec). [f] Lit.[6] m.p. 101 °C.

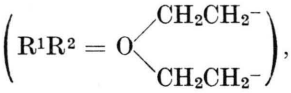
Table II. Preparation of spin-labeled phosphorodiamidates **2** using the spin-labeled chloridate **6**.



Amine (4)	Yield [%]	m.p. [°C] (dec)	M. W.[a] Found (Calcd)	EPR No. of lines	a_N [G]
n-Propylamine	89	oil	328 (334.42)	3	15.7
i-Propylamine	90	98–99	354 (334.42)	3	15.3
n-Butylamine	86	47–50	369 (362.48)	3	14.6
s-Butylamine	83	softens 75 melts 81	353 (362.48)	3	14.8
i-Pentylamine	86	39–43	385 (390.53)	3	15.0
Cyclohexylamine	87	softens 85 melts 112	400 (414.63)	3	15.0
Cyclohexylmethylamine	84	softens 84 melts 90	428 (430.51)	3	15.2
Phenethylamine	89	87–90	450 (458.57)	3	14.9
n-Decylamine	77	softens 10[b] melts 15	551 (530.80)	3	14.7
Morpholine[c]	93	51–52	390 (390.44)	3	15.3

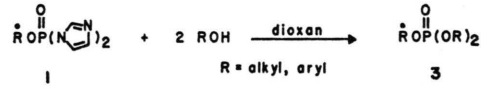
[a] The microanalyses are in agreement with calculated values: C, ±0.3%; H ±0.3%; N ±0.3%. The actual values for the analyses were submitted to the editor.
[b] A melting point, not a decomposition point. [c] Lit.[6] m.p. 101 °C.

by the imidazole and chloridate methods gave no depression. It has been our experience that spin-labeled organophosphorus compounds are sensitive to elevated temperatures even for short periods of time. It is conceivable that **2**

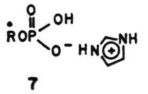


previously reported as the solid melting at 101 °C was, instead, some decomposition product possessing the same empirical formula as morpholide **2**. Furthermore, morpholide **2** was purified by chromatography on silica gel[6]. In contrast, we were unable to obtain crystalline morpholide **2** by any means other than chromatography on basic alumina. In fact, chromatography on alumina is a generally applicable method for the purification of all spin-labeled phosphorus compounds, and was used extensively for obtaining analytically pure products. In the first communication[1], products **2** were described as oils. Now it was possible to obtain crystalline **2**, albeit with difficulties.

The reaction of **1** with hydroxy compounds afforded moderate to good yields of the dialkyl or diaryl phosphates **3** (Table III). In most cases, the



yields were in excess of 50%. Also formed as a byproduct in the reaction were variable amounts (12–52%) of a benzene-, ether-, and dioxan-insoluble but water-soluble salt-like material. The first traces of this substance appeared very shortly after the mixing of the reagents. On the basis of the solubility characteristics and microanalytical information, the structure **7** is proposed for this byproduct. Unlike in the case of the transphos-



phorylation reaction with amines, where the hydrolysis of the second imidazole moiety does not take place because of the immediate salt formation with the primary amine (**4**; *i*-$C_3H_7NH_2$ and *c*-$C_6H_{11}NH_2$), in the case of hydroxy compounds, complete hydrolysis takes place. Furthermore, since the first ionization constant of alkyl phosphates is approximately 2, the strong acid reacts with the weak base imidazole to form a salt. However, the second ionization constant has a pK_a of about 8, therefore, no reaction occurs with imidazole. On the basis of these facts, the structure **7** is plausible.

In order to verify the identity of phosphates **3**, these compounds were also prepared from the dichloridate **6** and the appropriate hydroxy compound in either benzene or dioxan in the presence of triethylamine (Table IV). Even though the transphosphorylation to hydroxy compounds, with imidazolide **1** produces significant amounts of the salt **7**, thereby lowering the yield of phosphates **3**, the transphosphorylation reaction is the superior method as compared to the dichloridate method for the

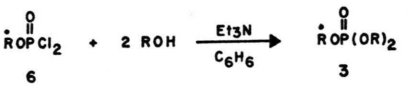


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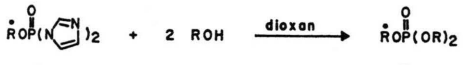
Table III. Reaction of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl) diimidazole-1,1′-phosphinate (**1**) with hydroxy compounds.



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Hydroxy compound	Yield [%]	M. W.[a] Found (Calcd)	EPR No. of lines	a_N [G]
Methanol[b]	43[c]	273 (280.28)	3	15.1
Ethanol[b]	45[c]	300 (308.34)	3	15.0
n-Propanol[b]	55	330 (336.42)	3	15.2
i-Butanol[b]	69[c]	350 (364.43)	3	15.0
n-Butanol[b]	53	374 (364.43)	3	15.0
Phenol[c]	82	402 (404.43)	3	15.4
2-Aziridine ethanol[b]	69	395 (390.44)	3	14.3
4-Methylcyclohexanol[b]	51[c]	420 (444.60)	3	14.9
Cyclohexanol[b]	70[c]	394 (416.53)	3	14.5
Benzyl alcohol[b]	85	439 (432.48)	3	15.0
Propanolamine[e]	89	293 (291.31)	3	14.7
N-Methylaminoethanol[f]	84	300 (291.31)	3	14.8

[a] The microanalyses are in agreement with calculated values: C ±0.3%; H ±0.3%; N ±0.3%. The actual values for the analyses were submitted to the editor.
[b] Product is an oil.
[c] Also isolated was 12–52% of **5**, m.p. 160–162 °C (dec). The microanalyses are in agreement with calculated values for $C_{12}H_{23}N_3O_5P$: C ±0.3%; H ±0.3%; N ±0.3%. EPR: 3 lines; a_N (aqueous) = 17.1 G.
[d] Compound is a solid, m.p. 52–54 °C (dec).
[e] Compound is a solid, softening at 78 °C, melting at 85 °C (dec).
[f] Compound is a solid, softening at 54 °C, melting at 84 °C (dec).

Table IV. Preparation of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl) dialky and diaryl phosphates (**3**) using the chloridate **6**.

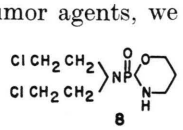
Hydroxy compound	Yield [%]	M. W.[a] Found (Calcd)	EPR No. of lines	a_N [G]
Ethanol[b]	33	312 (308.34)	3	15.2
2-Aziridine ethanol[b]	48	386 (390.44)	3	14.3
n-Propanol[b]	29	328 (336.42)	3	14.8
i-Propanol[b]	33	329 (336.42)	3	15.2
n-Butanol[b]	52	358 (364.45)	3	14.7
s-Butanol[b]	44	369 (364.45)	3	14.8
n-Pentanol[b]	33	389 (392.50)	3	14.7
Cyclohexanol[b]	41	400 (416.53)	3	14.5
n-Heptanol[b]	28	445 (448.61)	3	14.9
4-Methylcyclohexanol[b]	37	440 (444.58)	3	15.0
Phenol[c]	50	390 (390.44)	3	15.4

[a] The microanalyses are in agreement with calculated values: C ± 0.3%; H ± 0.3%; N ± 0.3%. The actual values for the analyses were submitted to the editor.
[b] Compound is an oil. [c] m.p. 52–54 °C (dec). Lit.[8] m.p. 52–54 °C (dec).

preparation of **3** (see Tables III and IV). Furthermore, the need for a strong base, such as triethylamine, is obviated, since a relatively neutral moiety, namely imidazole, is expelled instead of hydrogen chloride, which must be neutralized with triethylamine, since the free acid is capable of destroying the nitroxyl moiety[9]. The triethylamine hydrochloride can potentially also undergo side reactions and hence is undesirable.

Cyclophosphoramidates

Over the years, there have been numerous reports[10–13] concerning the biological activity of Endoxan (N,N-bis-(β-chloroethyl)-N′,O-propylene-phosphoric acid ester diamide) (**8**). In view of our interest in antitumor agents, we prepared a series



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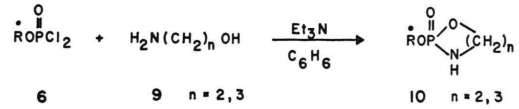
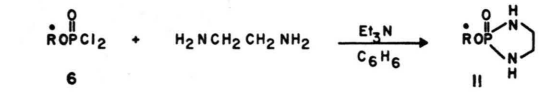
of spin-labeled cyclophosphoramidates **10–13** in nearly quantitative yield both from the spin-labeled dichloridate **6** and imidazolide **1** and the corresponding difunctional nucleophile. Thus, the reaction of ethanolamine (**9**, $n = 2$) with dichloridate **6** in the presence of triethylamine afforded 2-(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-2-oxo-1,3,2-oxazaphospholan (**10**, $n = 2$) in 96% yield. Similarly, (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-N,O-propylene phosphoric acid diester amide (**10**, $n = 3$) was prepared in 96% yield from chloridate **6** and propanolamine (**9**, $n = 3$).



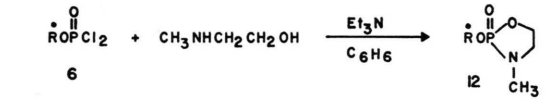
6 **9** n = 2,3 **10** n = 2,3

The analogous reaction of **6** with ethylene diamine afforded 2-(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-2-oxo-1,3,2-diazaphospholan (**11**). Diamidate **11**

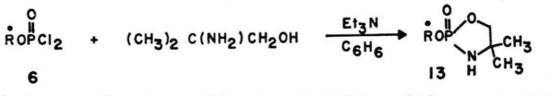


was a red, slightly sticky solid, which transformed to a liquid of unknown composition on storage for several days at room temperature.

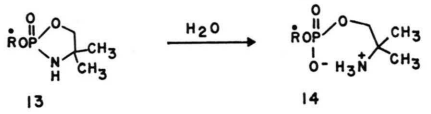
The spin-labeled 2-(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-2-oxo-3-methyl-1,3,2-oxazaphospholan (**12**) was prepared from N-methylethanolamine and



dichloridate **6**, while 2-(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-2-oxo-4,4-dimethyl-1,3,2-oxazaphospholan (**13**) was prepared similarly from 2-amino-2-methyl-1-propanol. Compound **13** transformed slowly, even at —20 °C to a benzene-insoluble

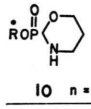
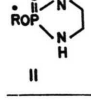
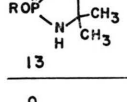
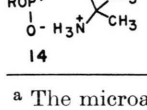


substance. In view of the insolubility of the material in nonpolar solvents, and its microanalysis, it is probably the hydrolysis product of **13**, the spin-labeled salt **14** (Table V). All spin-labeled cyclo-



phosphoramidates, except for compounds **10** ($n = 3$) and **12**, are sensitive to chromatography and/or hydrolytic conditions. Hence, in view of the workup which is required in the case of imidazolide **1** as the transphorylating agent, only compounds **10** ($n = 3$) and **12** could be prepared also by the imidazole method, in 89% and 84% yields, respectively (Table III).

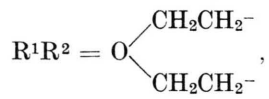
Table V. Preparation of spin-labeled cyclophosphoramidates **10**, **11**, **12**, **13**, and **14**.

Product	Yield [%]	m.p. [°C] (dec)	M. W.[a] Found (Calcd)	EPR No. of lines	a_N [G]
 **10** n = 2	96	softens 96 melts 106	272 (277.28)	3	15.4[b]
 **10** n = 3	96	softens 79 melts 85	296 (291.31)	3	14.7[b]
 **11**	97	softens 37 melts 52	285 (276.30)	3	15.2[a]
 **12**	96	softens 55 melts 84	300 (291.31)	3	14.8[b]
 **13**	97	125	310 (305.35)	3	13.8[b]
 **14**	100	softens 130 melts 163		3	16.8[c]

[a] The microanalyses are in agreement with calculated values: C ±0.3%; H ±0.3%; N ±0.3%. The actual values for the analyses were submitted to the editor.
[b] In benzene. [c] In water.

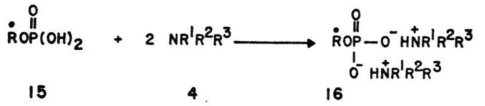
Spin-Labeled Salts

In order to gain insight into the salt-like materials **5** and **7**, which were isolated from the transphosphorylation reactions of diimidazolide **1** with alcohols and amines, we attempted to prepare (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl) phosphate (**15**) from dichloridate **6**. The spin-labeled phosphate **15** was reported previously[6,14], however, it was not completely characterized[14] and prepared from an intermediate, **2**[6],



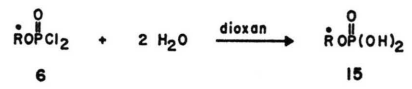
whose identity we were unable to verify.

Now an attempt was made to prepare phosphate **15** from the dichloridate **6** by hydrolysis. It was found that although dichloridate **6** is an air- and moisture-sensitive[8] compound, its rate of hydrolysis is slow, and that 20 h were required for completion of the reaction. Furthermore, although the radical moiety was found to be sensitive to concentrated mineral acids[9], it is not affected by dilute acids. Phosphate **15** is a very hygroscopic orange solid which is insoluble in nonpolar solvents, such as chloroform, benzene, and ether, and soluble in water, methanol, and ethanol. The phosphate **15** was further characterized as the bis-triethylammonium (**16**, $R^1 = R^2 = R^3 = C_2H_5$) and bis-cyclohexylammonium (**16**, $R^1 = c\text{-}C_6H_{11}$, $R^2 = R^3 =$ H) phosphate salts. Compounds **16** are pale pink solids, considerably less sensitive to atmospheric moisture than the parent acid **15**.



Effect of Alkyl Substituents on the Imidazole Ring

The effect of alkyl substituents on the imidazole ring towards the transphosphorylation reaction was also studied. The introduction of one methyl group in the imidazolyl moiety produced a retarding effect on the rate of the transphosphorylation reaction. Thus, the reaction of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)bis[2-methyl-1-imidazolyl]phosphinate (**17**) with alcohols gave 61–85% yield of the phos-



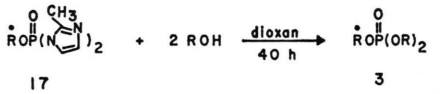
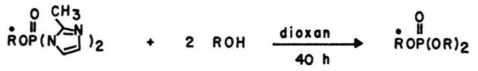


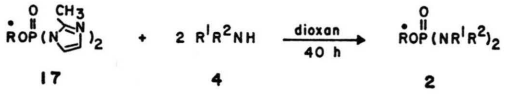
Table VI. Reaction of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)bis[2-methyl-1-imidazolyl]phosphinate (**17**) with alcohols.



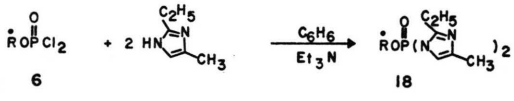
Alcohol	Yield[a] [%]	M. W.[b] Found (Calcd)	EPR No. of lines	a_N [G]
Cyclohexanol	85	409 (416.53)	3	14.5
n-Heptanol	67	450 (448.61)	3	14.8
n-Butanol	61	359 (364.45)	3	15.0
Benzyl alcohol	74	439 (432.48)	3	15.1
4-Methylcyclohexanol	67	425 (444.60)	3	15.3
n-Pentanol	69	399 (392.50)	3	15.1

[a] Products are oils.
[b] The microanalyses are in agreement with calculated values: C ±0.3%; H ±0.3%; N ±0.3%. The actual values for the analyses were submitted to the editor.

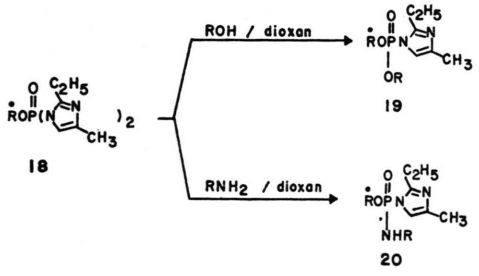
phates **3**, while the reaction of **17** with amines gave the diamidates **2** in 50–74% yields. However, 40 h were needed for the reaction as compared to the transphosphorylation using the unsubstituted imidazole derivative (Tables VI and VII).



Introduction of a further alkyl group into the imidazole nucleus stabilizes the spin-labeled imidazolide to such an extent that (1-oxyl-2,2,6,6-tetramethy-4-piperidyl)-bis[2-ethyl-4-methyl-1-imidazolyl]phosphinate (**18**) survives chromatography on alumina. This increase in the stability of the



imidazolide is also evidenced by the decreased reactivity of phosphinate **18**. Thus, the reaction of imidazolide **18** with either alcohols or amines gave, only after two days at room temperature, the O-alkyl and N-alkyl (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-2-ethyl-4-methyl-1-imidazolyl derivatives of phosphoric acid, **19** and **20**, respectively, in 78–90% yield. Heating of the reaction mixture for 20 h at 36 °C was insufficient in effecting the replacement of the second 2-ethyl-4-methyl-1-imidazolyl moiety (Table VIII).

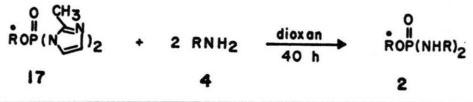


Experimental

Materials: All reagents were of the best quality commercially available and were used without further purification. All amines were stored over sodium hydroxide. Benzene and dioxan were distilled from and stored over sodium. The imidazole and 2-methyl imidazole were generously donated by BASF Corporation of Parsippany, New Jersey.

Analytical procedures: All melting points are uncorrected. Molecular weights were determined isopiestically on a Hitachi Perkin-Elmer Model 115 Molecular Weight apparatus. The EPR spectra were obtained on a Varian E 3 spectrometer. An approximately 10^{-3} M solution of the sample in benzene was purged for a few minutes with a stream

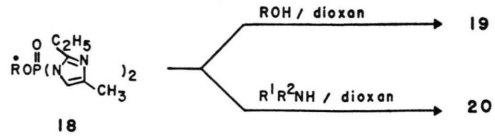
Table VII. Reaction of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)bis[2-methyl-1-imidazolyl]phosphinate (**17**) with amines.



Amine (**4**)	Yield [%]	m.p. [°C] (dec) [m.m.p.]	M. W.[a] Found (Calcd)	EPR No. of lines	a_N [G]
Cyclohexylamine	65	softens 85 melts 112 [s 85, m 111]	403 (414.55)	3	15.1
Phenethylamine	63	87–90 [87–89]	469 (458.57)	3	15.3
Benzylamine	52	softens 84 melts 90 [s 83, m 90]	450 (430.51)	3	15.0
s-Butylamine	74	softens 75 melts 81 [s 76, m 80]	355 (362.48)	3	15.1
n-Decylamine	50	softens 10 melts 15[b] [s 9, m 14]	525 (530.81)	3	14.8

[a] The microanalyses are in agreement with calculated values: C ±0.3%; H ±0.3%; N ±0.3%. The actual values for the analyses were submitted to the editor.
[b] Melting point, not a decomposition point.

Table VIII. Reaction of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)bis[2-ethyl-4-methyl-1-imidazolyl]phosphinate (**18**) with alcohols and amines.



18

Nucleophile	Yield[a] [%]	M. W.[b] Found (Calcd)	EPR No. of lines	a_N [G]
Cyclohexanol	85	429 (426.53)	3	15.0
Cyclohexylamine	82	429 (425.54)	3	14.8
Cyclohexylmethylamine	97	450 (439.56)	3	15.1
Benzylamine	93	459 (433.52)	3	15.1
4-Methylcyclohexanol	89	421 (440.55)	3	15.2

[a] Compounds are oils.
[b] The microanalyses are in agreement with calculated values: C ± 0.3%; H ± 0.3%; N ± 0.3%. The actual values for the analyses were submitted to the editor.

of dry nitrogen, then immediately analyzed. Water-soluble samples were analyzed as 10^{-3} M aqueous solutions with the appropriate accessories. The IR analyses of the radical products for verification of the presence of various functional groups were performed on a Perkin-Elmer Infracord Spectrophotometer Model 137. Microanalyses were performed on a F & M Scientific Corporation Carbon, Hydrogen, Nitrogen Analyzer, Model 185. All column chromatography was performed on basic or neutral aluminium oxide (20:1 w/w) (activities I and IV, according to Brockman). Approximately 1 g of crude reaction mixture was dissolved in a minimal amount of eluant, then chromatogrammed. The pure product was isolated after concentration of the appropriate fraction on a rotating evaporator at 12 torr (25–30 °C).

Reaction of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-diimidazole-1,1'-phosphinate (**1**) *with amines and alcohols*

General procedure: A solution of **1**[8] (1.84 g, 0.005 mol) and the appropriate amine or alcohol (0.01 mol) in dioxan (100 ml) was stirred at 20–23 °C for 15 h. Any solid which formed during the reaction was filtered off. The filtrate was concentrated on a rotating evaporator at 25 °C (12–15 torr), and the concentrate purified by column chromatography on alumina (basic, activity IV according to Brockman) with chloroform as the eluant to give the phosphorodiamidates **2** and the phosphates **3** listed in Tables I and III, respectively. Crystallization of the solid derivatives was induced by trituration of the concentrate with pentane, followed by storage at —20 °C.

Reaction of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-phosphorodichloridate (**6**) *with amines and alcohols*

General procedure: To a solution of chloridate **6**[8] (1.44 g, 0.005 mol) in benzene (50 ml) was added at 8–10 °C a solution of the amine or the alcohol (0.01 mol) and triethylamine (1.10 g, 0.01 mol) in benzene (30 ml). After the addition, the reaction mixture was stirred at 20–25 °C for 20 h, then filtered. The filtrate was concentrated on a rotating evaporator at 20–23 °C (12–15 torr). The crude oil was purified by chromatography on alumina (basic, activity IV according to Brockman) with chloroform as the eluant. Concentration of the eluted fraction afforded the phosphorodiamidates **2** and phosphates **3** listed in Tables II and IV, respectively. Crystallization of the solid derivatives was induced by trituration of the concentrate with pentane, followed by storage at —20 °C.

Reaction of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-diimidazole-1,1'-phosphinate (**1**) *with amino alcohols*

Preparation of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-N,O-propylene phosphoric acid diester amide (**10**, $n = 3$)

A solution of **1**[8] (1.84 g, 0.005 mol) and 3-amino-1-propanol (0.38 g, 0.005 mol) in dioxan (20 ml) was stirred at 20–23 °C for 15 h. The reaction mixture was concentrated on a rotating evaporator at 23 °C (12–15 torr), and the concentrate purified by column chromatography on alumina (basic, activity IV according to Brockman) with chloroform as the eluant to give **10**, $n = 3$ (1.30 g, 89%), a red solid softening at 78 °C, melting at 85 °C (dec); m.m.p. softening at 77 °C, melting at 85 °C (dec).

Preparation of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-2-oxo-3-methyl-1,3,2-oxaphospholan (**12**)

A solution of **1**[8] (1.84 g, 0.005 mol) and N-methylethanolamine (0.38 g, 0.005 mol) in dioxan (20 ml) was stirred at 20–23 °C for 15 h. The reaction mixture was concentrated on a rotating evaporator at 23 °C (12–15 torr). The oil was dissolved in diethyl ether (50 ml), and the ethereal solution shaken with distilled water (5 × 10 ml). The organic layer was dried over sodium sulfate, and the drying agent removed by filtration. The filtrate was concentrated to an oil on a rotating evaporator. The oil solidified upon trituration with pentane. There was obtained **12** (1.21 g, 84%), a solid softening at 54 °C, melting at 84 °C (dec); m.m.p. softening at 55 °C, melting at 84 °C (dec).

Preparation of cyclic phosphorylated spin labels **10–13**

General procedure: To a solution of chloridate **6**[8] (1.44 g, 0.005 mol) in benzene (60 ml) was added dropwise at 8–10 °C a solution of the appropriate amino alcohol or diamine (0.005 mol) and triethylamine (1.10 g, 0.01 mol) in benzene (30 ml). In the case of propanolamine, dioxan (5 ml) was added to aid in solubilization. Following the addition, the reaction mixture was stirred at 8–10 °C for 1 h, at ambient temperature for 20 h, and filtered. The filtrate was concentrated on a rotating evaporator at 23 °C (15 torr) to give the cyclic phosphorylated spin labels **10–13** listed in Table V.

2-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidyl)-2-oxo-4,4-dimethyl-1,3,2-oxazaphospholan (**13**) converts slowly, even at —20 °C, to a benzene-insoluble compound. Thus, after several days, phospholan **13** hydrolyzes to the Zwitterionic compound 2-(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-2-oxo-2,2-dimethyl-2-amino)-phosphoric acid diester (**14**) a red solid softening at 130 °C melting at 163 °C (dec).

EPR: 3 lines; a_N (aqueous) = 16.8 g.

$C_{13}H_{28}N_2O_5P$ (mol. wt. 323.35)

Calcd	C 48.29	H 8.73	N 8.66,
Found	C 48.40	H 8.67	N 8.88.

Preparation of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)phosphate (**15**)

To a solution of phosphorus oxychloride (0.76 g, 0.005 mol) in benzene (30 ml) was added dropwise at 8–10 °C a solution of 4-hydroxy-2,2,6,6-tetramethyl-1-oxyl[9] (0.86 g, 0.005 mol) and triethylamine (0.60 g, 0.006 mol) in benzene (50 ml). Following the addition, the reaction mixture was stirred at 8 °C for 1 h, at room temperature for 3 h, then filtered. The filtrate was concentrated on a rotating evaporator at 23 °C (12–15 torr) to give (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)phosphorodichloridate (**6**, 1.41 g, 98%), m.p. 68–70 °C (dec). This solid was dissolved in dioxan (25 ml), and the solution was rapidly added to distilled water (0.5 ml). The reaction mixture was left undisturbed at room temperature overnight, and then treated with triethylamine (1.0 g, 0.01 mol). After 2 h at ambient temperature, the reaction mixture was concentrated on a rotating evaporator overnight. The resulting solid was treated with chloroform (5 × 10 ml). Each time, the chloroform solution containing triethylamine hydrochloride was decanted from an insoluble oil. This oil was then washed with dioxan (2 × 5 ml), diethyl ether (3 × 5 ml) and kept at 0.3 torr on a rotating evaporator to give **15** (1.22 g, 91%), an orange solid softening at 68 °C melting at 78 °C (dec).

EPR: 3 lines; a_N (aqueous) = 17.3 g.

$C_9H_{19}NO_5P$ (mol. wt. 252.23)

Calcd	C 42.86	H 7.59	N 5.55,
Found	C 42.55	H 7.88	N 5.47.

Preparation of bis-triethylammonium(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)phosphate (**16**; $R^1 = R^2 = R^3 = C_2H_5$)

To a solution of phosphorus oxychloride (0.76 g, 0.005 mol) in benzene (30 ml) was added dropwise at 8–10 °C a solution of 4-hydroxy-2,2,6,6-tetramethyl-1-oxyl[9] (0.86 g, 0.005 mol) and triethylamine (0.60 g, 0.006 mol) in benzene (30 ml). Following the addition, the reaction mixture was stirred at 8 °C for 1 h, at ambient temperature for 3 h, then filtered. The filtrate containing dichloridate **6** was then added slowly at 8–15 °C to a mixture of triethylamine (2.50 g, 0.025 mol) and distilled water (20 ml). The organic layer rapidly lost its red coloration, while the aqueous layer became orange.

After 4 h at ambient temperature, the organic layer was separated and the aqueous layer concentrated to a solid on a rotating evaporator at 23 °C (12–15 torr). Chloroform (25 ml) was added, and the mixture was filtered. The remaining solid was washed again with chloroform (2 × 20 ml) to remove all of the triethylamine hydrochloride, and then air-dried. There was obtained **16** ($R^1 = R^2 = R^3 = C_2H_5$; 2.21 g, 97%), a pale pink powder softening at 98 °C, melting at 132 °C (dec).

EPR: 3 lines; a_N (aqueous) = 17.2 g.

$C_{21}H_{49}N_3O_5P$ (mol. wt. 454.62)

Calcd	C 55.48	H 10.86	N 9.24,
Found	C 55.53	H 10.94	N 9.16.

Preparation of bis-cyclohexylammonium(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)phosphate (**16**; $R^1 = R^2 = H$; $R^3 = c\text{-}C_6H_{11}$)

To a solution of phosphorus oxychloride (0.76 g, 0.005 mol) in benzene (30 ml) was added dropwise at 8–10 °C a solution of 4-hydroxy-2,2,6,6-tetramethyl-1-oxyl[9] (0.86 g, 0.005 mol) and triethylamine (0.60 g, 0.006 mol) in benzene (30 ml). Following the addition, the reaction mixture was stirred at 8 °C for 1 h, at ambient temperature for 3 h, then filtered. Concentration of the filtrate on a rotating evaporator at 23 °C (12 torr) afforded **6**

(1.44 g, 99%), m.p. 68–70 °C (dec)[8]. The solid **6** was dissolved in dioxan (20 ml). This solution was added rapidly to a solution of distilled water (0.20 g, 0.01 mol) in dioxan (50 ml). The reaction mixture was stirred for 1 h at ambient temperature, and then cyclohexylamine (2.10 g, 0.02 mol) was added rapidly. This mixture was left at ambient temperature for 20 h. The solid which was formed was collected by filtration. It was washed with chloroform (4 × 10 ml) to remove cyclohexylammonium chloride, then with dioxan (2 × 5 ml), diethyl ether (2 × 5 ml), and air-dried. There was obtained **16** ($R^1 = R^2 = H$; $R^3 = c\text{-}C_6H_{11}$; 2.21 g, 98%), a pale pink powder, m.p. 148 °C (dec).
EPR: 3 lines; a_N (aqueous) = 17.6 g.

$C_{21}H_{45}N_3O_5P$ (mol. wt. 450.58)
Calcd C 55.98 H 10.07 N 9.33,
Found C 55.87 H 10.22 N 9.24.

Preparation of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)bis[2-methyl-1-imidazolyl]phosphinate (**17**)

To a suspension of 2-methyl imidazole (0.82 g, 0.01 mol) and triethylamine (1.10 g, 0.01 mol) in benzene (30 ml) was added dropwise at 8–10 °C the solution containing chloridate **6**[8] (0.005 mol) in benzene (100 ml). After the addition, the reaction mixture was stirred at 8 °C for 1 h, at 20–23 °C for 20 h, then filtered. The filtrate was concentrated on a rotating evaporator at 23 °C (12 torr) to a solid. The solid was dissolved in diethyl ether, and the solution was filtered. The solution was then concentrated. There was obtained **17** (1.70 g, 90%), a pale pink powder, softening at 76 °C, melting at 104 °C (dec).
EPR: 3 lines; $a_N = 15.6$ g.

$C_{17}H_{27}N_5O_3P$
Calcd C 53.65 H 7.15 N 18.41, mol. wt. 380.41,
Found C 53.45 H 7.32 N 18.14, mol. wt. 378.

Reaction of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-bis[2-methyl-1-imidazolyl]phosphinate (**17**) *with alcohols and amines*

General procedure: A solution of **17** (1.90 g, 0.005 mol) and the appropriate alcohol or amine (0.01 mol) in dioxan (50 ml) was stirred at 20–23 °C for 40 h. The reaction mixture was concentrated on a rotating evaporator at 25 °C (12–15 torr) and the concentrate purified by column chromatography on alumina (basic, activity IV according to Brockman) with chloroform as the eluant, to give the phosphates **3** and phosphorodiamidates **2** listed in Tables VI and VII, respectively.

Preparation of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)bis[2-ethyl-4-methyl-1-imidazolyl]-phosphinate (**18**)

To a solution of 2-ethyl-4-methylimidazole (1.10 g, 0.01 mol) and triethylamine (1.1 g, 0.01 mol) in benzene (50 ml) was added dropwise at 8–10 °C the solution containing chloridate **6**[8] (0.005 mol) in benzene (100 ml). After the addition, the reaction mixture was stirred at 8 °C for 1 h, at 20–23 °C for 20 h, then filtered. The filtrate was concentrated on a rotating evaporator to a red oil. The oil was dissolved in diethyl ether, and the solution was filtered. The solution was then concentrated. There was obtained **18** (1.81 g, 84%), a red oil.
EPR: 3 lines; $a_N = 15.2$ g.

$C_{21}H_{35}N_5O_3P$
Calcd C 57.78 H 8.08 N 16.04, mol. wt. 436.52,
Found C 57.53 H 8.34 N 16.33, mol. wt. 429.

Reaction of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-bis[2-ethyl-4-methyl-1-imidazolyl]phosphinate (**18**) *with alcohols and amines*

General procedure: A solution of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl) bis[2-ethyl-4-methyl-1-imidazolyl]phosphinate (**18**; 2.18 g, 0.005 mol) and the appropriate alcohol or amine was stirred at 20–23 °C for 40 h. The reaction mixture was concentrated on a rotating evaporator at 25 °C (12–15 torr) and the concentrate purified by column chromatography on alumina (basic, activity IV according to Brockman) with chloroform as the eluant to give the O-alkyl and N-alkyl (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-2-ethyl-4-methyl-1-imidazolyl derivatives of phosphoric acid, **19** and **20**, respectively. Heating of the reaction mixture for 20 h at 36 °C was insufficient in effecting the replacement of the second 2-ethyl-4-methyl-1-imidazolyl moiety. The results of these experiments are listed in Table VIII.

Hydrolysis of compound **5** *for elucidation of structure*

A solution of **5** ($R^1 = H$, $R^2 = c\text{-}C_6H_{11}$; 4.01 g, 0.01 mol) in distilled water (30 ml) was treated with sodium hydroxide (1.0 g). The reaction mixture was cooled on an ice bath, then extracted with diethyl ether (3 × 5 ml). The combined ether extracts were dried over potassium hydroxide. The drying agent was removed by filtration and the solvent was removed on a rotating evaporator at 20 °C (12–15 torr). There was obtained cyclohexylamine (0.80 g, 80%), identified by comparison of its infrared spectrum with that of an authentic sample.

The aqueous (alkaline) extract was acidified with concentrated hydrochloric acid with external cooling to a pH of approximately 1. The solution was left to stand at ambient temperature for 20 h, then made basic to a pH of approximately 10 with sodium hydroxide. The reaction mixture was cooled, and extracted with benzene (5 × 10 ml). The combined organic extracts were dried over sodium sulfate. The drying agent was removed by filtration, and the solvent was removed on a rotating evaporator at 30 °C (12–15 torr). There was obtained imidazole (0.64 g, 95%), m.p. 89–90 °C; m.m.p. 89–90 °C.

This investigation was supported by grants from the Public Health Service, U. S. Department of Health, Education, and Welfare, GM 16741, and from the Graduate School of the University of Wisconsin–Milwaukee.

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