Improvements in the Preparation
of Spin-Labeled Phosphorus Compounds of Potential Cytotoxicity

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Spin Labeled Phosphates, Aziridine Derivatives, Potential Antitumor Agents, Nitroxy Radical, Nitrogen Mustard Derivative

The syntheses of spin-labeled phosphorus compounds containing the ethyleneimine, \(-\text{N}_3\), and nitrogen mustard, \(-\text{N}((\text{CH}_2\text{CH}_2\text{Cl})_2\), moieties were reinvestigated. By a judicious choice of solvents, stoichiometry, and reaction conditions yields of these compounds have been optimised. Thus, monoradical diamidates (5) and (9) were prepared in 95 and 82% yields, respectively, and diradical monoamidates (7) and (12) were prepared in 95 and 45% yields, respectively. The nitrogen mustard derivative (16) was obtained in 92% yield.

\[
\begin{array}{ccc}
X & X & O \\
\text{RO-P(N}_3\text{)}_2 & (\text{RO})_2\text{P-N}_3 & (\text{ClCH}_2\text{CH}_2\text{N-P-OR}) \\
5: X = O & 7: X = O & 12: X = S \\
9: X = S & & 16
\end{array}
\]

Within the last five years there have come several reports from our laboratory\(^1\) on the preparation of spin-labeled phosphorus compounds containing the ethyleneimine (aziridine) and the nitrogen mustard moieties.

\[
\begin{array}{cccc}
X & X & S \\
(\text{N-P-N}) & (\text{N-P-N}) & \\
1 & 2
\end{array}
\]

Both of these functionalities are known\(^1\) to exhibit selective action against tumors. Thus, for example, TEPA (1) and thio-TEPA (2) have been clinically used\(^4\) to a certain extent as antineoplastic drugs, although, to date, for the most part, the chemotherapy of cancer has met with only modest success\(^1\)\(^2\).

Recently, in conjunction with a testing program together with the National Cancer Institute, we were in need of several grams of certain spin-labeled compounds which have shown preliminary activity. It was then realized that the procedures previously reported from our laboratory\(^2\) were inadequate since they were difficult to reproduce with consistent results and often cumbersome due to the extended periods of time required. Therefore, we have reinvestigated the synthesis of certain spin-labeled phosphorus compounds, specifically those containing the ethyleneimine and the nitrogen mustard moieties, with the intent to improve the procedures for their preparation. It was found that, with a judicious choice of solvents, reaction times, and stoichiometry, various phosphoroamidates containing cytotoxic functionalities can be synthesized in yields, in most cases, exceeding 70%.

In 1973, we reported the preparation of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)phosphorodichloridate (3) in four hours from phosphorus oxychloride and 4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (4).
It was now found that this time is indeed sufficient for the preparation of compound 3. Thus, if the dichloridate 3 is prepared in four hours, and subsequently reacted with ethyleneimine in 20 hours, the (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)N,N,N',N'-bis(ethylene)-phosphorodiamidate (5) is obtained in 95% yield in analytically pure form as a crystalline substance. However, if the reaction for the preparation of 3 is allowed to proceed for 20 hours, the product 5 is of inferior quality, and must be purified by column chromatography before a crystalline 5 is obtained in 80% yield.

The replacement of two halogens of phosphorus oxychloride proved to be more difficult. It was previously reported that the preparation of bis-(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-phosphorochloridate (6) requires four hours. Since that time, we have experienced difficulties in consistently reproducing our results. As a possible explanation for the discrepancy we now feel that at the time of our earlier report, we were using phosphorus oxychloride which was not previously distilled and might have contained a trace of an unknown impurity which caused the reaction for the preparation of 6 to proceed in four hours. However, with a higher concentration of reagents, slightly elevated temperature of 30–35 °C, and distilled phosphorus oxychloride, chloridate 6 can be prepared in benzene in 20 hours, and in diethyl ether in four hours with consistently reproducible results. The subsequent reaction of chloridate 6 with ethyleneimine afforded bis(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)N,N-(ethylene)-phosphoroamidate (7) in 95% yield. The total time required for the synthesis of 7 was 20 hours, i.e., considerably shorter than that of six days which was previously reported.

We have earlier reported a failure in the preparation of tri(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)phosphate (8) from phosphorus oxychloride and radical 4 in the presence of triethylamine. In the course of our work on the successive replacement of the halogens of phosphorus oxychloride with the nitroxyl moiety, it was found that although the substitution of each chloride becomes progressively more difficult, nevertheless, triphosphate 8 can be prepared in a one-step procedure, although in only 38% yield. An increase in the reaction time did not appreciably increase the yield of 8.

The halogens of the analogous sulfur compound are much more difficult to react with the weak nucleophile 4 than those of phosphorus oxychloride. A total of twelve days was prescribed for the total synthesis of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-N,N,N',N'-bis(ethylene)-phosphorodiamidothioate (9). This extended time period is undesirable, and, furthermore, in our laboratory, the results could not be consistently reproduced. It is now found that the best method for the consistently reproducible synthesis of diamidate 9 in 82% yield involves a 20 hour reaction of 1 mol of phosphorus thiochloride with, surprisingly, 2 moles of radical 4, followed by a 20 hour reaction with ethyleneimine.
dichloridate 10. The extent of the replacement of the halogens was determined by the amount of triethylamine hydrochloride isolated from the mixture. The difficult step in this reaction is the preparation of 10, and, therefore, we have investigated the effect of different solvents on the yields of 10, and, consequently, 9. In methylene chloride, compound 9 was obtained in 54% yield and in diethyl ether in 22% yield. Compound 9 was also prepared from N,N,N',N'-bis(ethylene)-phosphinothioic chloride 7 (11) and radical 4. This reaction is a difficult and slow process, as are many other reactions of 4 with sulfur-containing phosphorus chlorides. Therefore, two weeks were required for the preparation of 9 in only 42% yield. Similar difficulties were encountered during the synthesis of bis(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)N,N-(ethylene)-phosphorodiamidate (12). The results of the experiments for the preparation of 12 at elevated temperature previously reported 2 were found to be poorly reproducible. However, monomamide 12 can best be prepared using a two-fold excess of radical 4, i.e., the molar ratio of 4 to phosphorus thiochloride is 4:1.

\[
\begin{align*}
\text{Et}_3\text{N} & \rightarrow (\text{RO})_3\text{P} = \text{SCl}_3 \text{P} = \text{S} + 3 \text{ROH} \\
\text{C}_6\text{H}_6 / \text{Et}_3\text{N} & \rightarrow (\text{ClCH}_2 \text{CH}_2)\text{N}-\text{PCl}_2
\end{align*}
\]

The intermediate monochloridate 13 was not isolated. Ether was the preferred solvent for the synthesis of 13.

Attemps to prepare chloridate 13 in various other solvents met with little success. Although phosphorus thiochloride is sluggish to react with the radical 4, it is sensitive to the solvent used. Thus, in dioxan, nitromethane, and ethyl acetate, unwanted side reactions took place involving phosphorus thiochloride, and only unreacted 4 could be isolated from the reaction mixture. No evidence for the formation of chloridate 13 could be found.

The sluggish reactivity of phosphorus thiochloride toward radical 4 was even more clearly evidenced in the preparation of tris(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)thiophosphate (14).

\[
\begin{align*}
\text{Cl}_3\text{P} = \text{S} + 3 \text{ROH} & \rightarrow (\text{RO})_3\text{P} = \text{S} \\
\text{Et}_3\text{N} & \rightarrow (\text{RO})_3\text{P} = \text{S}
\end{align*}
\]

Elevated temperature and/or extended reaction time did not enhance the formation of 14, and only a 14–17% yield of 14 was obtained (see experimental section). Addition of potassium chloride to the reaction mixture, together with the elevated temperature of 50 °C and an extended reaction time of 4 days appeared to facilitate the formation of 14 in 33% yield, possibly because of a stabilization of phosphorus thiochloride by potassium chloride. In this case the reaction mixture did not darken as much as was usually observed with other reaction mixtures utilizing phosphorus thiochloride. The best method for the preparation of thiophosphate 14 in 58% yield involves the reaction of tris(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)phosphite 8 (15) with sulfur in benzene at 50 °C.

\[
\begin{align*}
(\text{RO})_3\text{P} + \text{C}_6\text{H}_6 & \rightarrow (\text{RO})_3\text{P} = \text{S} \\
\text{Et}_3\text{N} & \rightarrow (\text{RO})_3\text{P} = \text{S}
\end{align*}
\]

The synthesis of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-N-bis(2-chloroethyl)-N',N'(ethylene)-phosphorodiamidate (16) was reported 2 to require a total of 13 days duration in order to obtain only a 14% yield of 16. Similarly to phosphorus thiochloride, the phosphorus mustard dichloridate, di-(2-chloroethyl)-phosphoroamidic chloride (17), reacts sluggishly with weak nucleophiles in the presence of triethylamine. However, the reaction is not as sensitive to solvents. It was found that dioxan is by far the best solvent for the reaction of 17 with radical 4. The spin-labeled mustard intermediate 18 was not
isolated. The duration of the reaction for preparation of intermediate 17 is critical. If the time was shortened to one day, the yield of 16 was reduced to 45%.

**Experimental**

**Materials:** All reagents were of the best quality commercially available and were used without further purification. Benzene was distilled from and stored over sodium. Dioxan and nitromethane were stored over calcium chloride. Diethyl ether was stored over lithium aluminium hydride. Triethylamine was stored over potassium hydroxide. Stable radical 4 was prepared by known methods\(^{10,11}\) and di-(2-chloroethyl)-phosphoramidic dichloride (11) and di-(2-chloroethyl)-phosphoric acid chloride (10) were prepared according to Friedmann and coworkers\(^{7,9}\).

**Analytical procedures:** All melting points are uncorrected. Molecular weights were determined isopistically on a Hitachi Perkin-Elmer Model 115 Molecular Weight apparatus. The EPR spectra were obtained on a Varian E3 spectrometer. Solutions approximately 10⁻⁴ molar in concentration were purified with dry nitrogen for a few minutes, then analyzed. Microanalyses were performed on a F & M Scientific Corporation Carbon, Hydrogen, Nitrogen Analyzer, Model 185. Alcolumn chromatography was performed on basic aluminium oxide, 80–200 mesh, Brockman, activity IV. Approximately 1 g of crude reaction mixture was dissolved in a minimal amount of solvent, then eluted from 20 g of Al₂O₃ with the appropriate eluant. The pure product was isolated after removal of the solvent on a rotating evaporator at 23–25 °C (12 torr).

**Preparation of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-N,N,N',N'-bis(ethylene)-phosphorochloridate (6)**

A. In diethyl ether: To a solution of phosphorus oxychloride (0.76 g, 0.0050 mol) in 40 ml benzene was added dropwise at 8–10 °C a solution of 4 (0.86 g, 0.0050 mol) in 50 ml benzene. After the addition, the reaction mixture was stirred at 20–25 °C for 3 h, then filtered. To the filtrate was added as rapidly as possible a solution of triethylamine (0.43 g, 0.010 mol) in 25 ml benzene, followed by ethylenimine (0.43 g, 0.010 mol) in 25 ml benzene at 8–10 °C. After the addition, the reaction mixture was stirred at 8–10 °C for 1 h, at 20–23 °C for 16 h, then filtered. The filtrate was concentrated on a rotating evaporator at 23 °C (12 torr) to give a red oil.

B. In benzene: To a solution of phosphorus oxychloride (0.76 g, 0.0050 mol) in 30 ml benzene was added dropwise at 8–10 °C a solution of 4 (1.72 g, 0.010 mol) and triethylamine (1.22 g, 0.012 mol) in 40 ml benzene. The reaction mixture was stirred at 30–35 °C for 20 h, then filtered to remove triethylamine hydrochloride (1.41 g, 100% of theory). The filtrate was concentrated on a rotating evaporator to obtain oil which was solidified in heptane at 20 °C to give 1.81 g (85%\(^{\circ}\)) of 6, m.p. 88–89 °C (dec). Lit.\(^{2}\) m.p. 88–89 °C (dec).

**Preparation of bis(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)phosphoroamidate (7)**

Bis(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)phosphorochloridate (6) was prepared on a 0.0050 mol scale in benzene and used without isolation. To the filtrate containing 0.0050 mol chloridate (6) in 70 ml benzene was first added rapidly at 8 °C a solution of triethylamine (0.60 g, 0.0090 mol) in 10 ml benzene. Then was added slowly at 8–10 °C a solution of ethylenimine (0.22 g, 0.0050 mol) in 20 ml benzene. 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 give a red oil. This oil was purified by chromatography on Al₂O₃ (basic, activity IV), eluted with a 2:1 (v/v) mixture of benzene and chloroform. There was obtained 2.03 g (95%\(^{\circ}\)) of 7, a red oil.

**EPR:** Five lines; \(\alpha_N = 7.8\) g.
Preparation of tris(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)phosphorus (8) from phosphorus oxychloride

To a solution of phosphorus oxychloride (0.51 g, 0.0033 mol) in 10 ml benzene was added dropwise at 8–10 °C a solution of 4 (1.72 g, 0.010 mol) and triethylamine (1.10 g, 0.010 mol) in 30 ml benzene. Following the addition, the reaction mixture was stirred at 30–35 °C for 20 h, and filtered. The filtrate was concentrated on a rotating evaporator at 25 °C (12 torr) to an oil. The oil was purified by chromatography on Al₂O₃ (basic, activity IV), with a 4:1 (v/v) mixture of benzene and ethyl acetate to give 0.70 g (38%) of 8, m.p. 175–177 °C (dec). Lit. ⁶ m.p. 168–171 °C.

Preparation of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-N,N,N',N'-bis(ethylene)-phosphorodiamidothioate (9)

1. From N,N,N',N'-bis(ethylene)-thiophosphorochloridate (11)

The chloridate 11 was prepared as described by FRIEDMAN ⁷. Thus, to a solution of phosphorus thiochloride (5.07 g, 0.030 mol) in 70 ml 1,2-dimethoxyethane was added dropwise at —20 °C over a period of 20 min a solution of triethylamine (6.06 g, 0.060 mol) in 50 ml 1,2-dimethoxyethane. Then was added, also at —20 °C, over a period of 60 min a solution of ethyleneimine (2.58 g, 0.060 mol) in 50 ml 1,2-dimethoxyethane. After the addition, the reaction mixture was stirred at ambient temperature for 20 h, then filtered. The filtrate was cooled to 8 °C, then treated with a solution of 4 (5.16 g, 0.030 mol) and triethylamine (3.03 g, 0.030 mol) in 40 ml 1,2-dimethoxyethane. Since the triethylamine hydrochloride did not form rapidly, the reaction mixture was stirred at room temperature for 2 weeks, then filtered. The filtrate was concentrated on a rotating evaporator at 23 °C (12 torr) to a red oil. The oil was purified by chromatography on Al₂O₃ (basic, activity IV), eluted with a 4:1 (v/v) benzene:ethyl acetate mixture. There was obtained 3.91 g (42%) of (1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-N,N,N',N'-bis(ethylene)-thiophosphoroamidate (9), m.p. 102–104 °C (dec). Lit. ² m.p. 100–102 °C (dec).

2. From phosphorus thiochloride and (1-oxyl-2,2,6,6-tetramethyl-4-hydroxy)piperidine (4)

a. In methylene chloride. General procedure: To a solution of phosphorus thiochloride (1.69 g, 0.010 mol) in 50 ml methylene chloride was added dropwise at 8–10 °C a solution of 4 (1.72 g, 0.010 mol) and triethylamine (1.01 g, 0.010 mol) in 100 ml methylene chloride. After the addition, the reaction mixture was stirred at room temperature for 20 h, then concentrated on a rotating evaporator at 23 °C (12 torr) to a solid. The solid was treated with 100 ml dry ether, and the suspension was filtered. To this filtrate was first added rapidly at 8 °C a solution of triethylamine (2.02 g, 0.020 mol) in 20 ml benzene, then slowly at 8–10 °C a solution of ethyleneimine (0.86 g, 0.020 mol) in 30 ml benzene. After the addition, the reaction mixture was stirred at room temperature for 20 h, and filtered. The filtrate was concentrated on a rotating evaporator to an oil. The oil was purified by chromatography as described in the preceding experiment. There was obtained 1.71 g (54%*) of 9, m.p. 102–103 °C (dec), m.p. 102–103 °C (dec).

b. In ether: To a solution of phosphorus thiochloride (1.69 g, 0.010 mol) in 30 ml diethyl ether was added dropwise at 8–10 °C a solution of 4 (1.72 g, 0.010 mol) and triethylamine (1.10 g, 0.010 mol) in 30 ml diethyl ether. After the addition, the reaction mixture was stirred at room temperature for 20 h, then filtered. To the filtrate was first added rapidly at 8 °C a solution of triethylamine (2.02 g, 0.020 mol) in 20 ml benzene, then slowly at 8–10 °C a solution of ethyleneimine (0.86 g, 0.020 mol) in 20 ml benzene. Following the addition, the reaction mixture was stirred at ambient temperature for 20 h, then filtered. The filtrate was concentrated to an oil which was purified by chromatography as described in the preceding experiment. In this manner was obtained 0.72 g (22%*) of 9, m.p. 102–103 °C (dec), m.p. 102–103 °C (dec).

c. In benzene: To a solution of phosphorus thiochloride (0.84 g, 0.0050 mol) in 40 ml methylene chloride was added dropwise at 8–10 °C a solution of 4 (1.72 g, 0.010 mol) and triethylamine (1.01 g, 0.010 mol) in 70 ml methylene chloride. After the addition, the reaction mixture was stirred at room temperature for 20 h, then concentrated on a rotating evaporator at 25 °C (12 torr). The solid material was treated with 50 ml diethyl ether and filtered to remove the insoluble triethylamine hydrochloride (0.62 g, 91% of theory for replacement of one halogen). To this filtrate was first added rapidly at 8 °C a solution of triethylamine (1.01 g, 0.010 mol) in 20 ml benzene, then slowly at 8–10 °C a solution of ethyleneimine (0.43 g, 0.010 mol) in 30 ml benzene. After the addition, the reaction mixture was stirred at ambient temperature for 20 h, then filtered, and the filtrate was concentrated at 23 °C on a rotating evaporator (12 torr). The remaining crude oil was purified by chromatography as described in the preceding experiment to give 1.32 g (82%) of 9, m.p. 103–104 °C (dec), m.p. 102–104 °C (dec).

Preparation of bis(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-N,N-(ethylene)-phosphoroamidothioate (12)

To a solution of phosphorus thiochloride (0.84 g, 0.0050 mol) in 20 ml diethyl ether was added dropwise at 8–10 °C a solution of 4 (3.44 g, 0.020 mol) in 30 ml diethyl ether. After the addition, the reaction mixture was stirred at 20–25 °C for 20 h,
then filtered to remove triethylamine hydrochloride (1.36 g, 100% of theory for replacement of two halogens). The filtrate was concentrated on a rotating evaporator at 23 °C (12 torr) to give a red solid. This solid was immediately dissolved in 30 ml benzene. To the solution was first added rapidly triethylamine (0.60 g, 0.0060 mol) in 10 ml benzene, then slowly at 8–10 °C ethyleneimine (0.22 g, 0.0050 mol) in 10 ml benzene. After the addition, the reaction mixture was stirred at room temperature for 20 h, then filtered. The filtrate was concentrated on a rotating evaporator at 23 °C (12 torr) to a red semi-solid material. Purification of this substance on Al₂O₃ (basic, activity IV), eluted with a 4:1 (v/v) mixture of benzene and ethyl acetate, afforded 1.01 g (45%) of 12, a red oil.

EPR: Five lines; aN = 7.8 g.

C₂₀H₃₃N₅O₅PS

Attempted preparation of bis(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-thiophosphorochloride (13)

a. In ethyl acetate: To a solution of phosphorus thiochloride (0.84 g, 0.0050 mol) in 30 ml ethyl acetate was added at 8–10 °C a solution of 4 (1.72 g, 0.010 mol) and triethylamine (1.01 g, 0.010 mol) in 30 ml ethyl acetate. After the addition, the reaction mixture was stirred 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. This solid was immediately dissolved in 30 ml ethyl acetate. After the addition, the reaction mixture was stirred at 23–25 °C for 20 h, then filtered. The filtrate was concentrated on a rotating evaporator at 25 °C (12 torr). The remaining dark red oil was purified by chromatography as described in the preceding experiment to give 0.31 g (17%) of 14, m.p. 167–169 °C (dec).

b. In nitromethane: As described in the preceding experiment, phosphorus thiochloride (0.84 g, 0.0050 mol) in 20 ml nitromethane was reacted with 4 (1.72 g, 0.010 mol) and triethylamine (1.01 g, 0.010 mol) in 30 ml nitromethane. After 20 h at 20–23 °C, the reaction mixture was concentrated on a rotating evaporator to a solid. This solid was worked up as in the preceding experiment to give 1.43 g (83%) of 4, m.p. 66–68 °C. Lit. 10 m.p. 71.5 °C.

c. In dioxan: As described in the preceding experiment, phosphorus thiochloride (0.84 g, 0.0050 mol) in 40 ml dioxan was reacted with 4 (1.72 g, 0.010 mol) and triethylamine (1.10 g, 0.010 mol) in 40 ml dioxan. After 4 h at 20–23 °C, the reaction mixture began to darken, and therefore was filtered. The filtrate was concentrated as in the preceding experiment to give 1.45 g (84%) of 4, m.p. 66–68 °C. Lit. 10 m.p. 71.5 °C.

Preparation of tris(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl)-thiophosphate (14)

A. From the phosphite 15: A solution of tris(1-oxyl-2,2,6,6-tetramethyl-4-piperidyl) phosphite 8 (15, 1.81 g, 0.0033 mol) and sulfur (0.16 g, 0.0050 mol) in 40 ml benzene was stirred at 50 °C for 40 h. The reaction mixture was then concentrated on a rotating evaporator at 23 °C (12 torr). The oily residue was treated with 30 ml diethyl ether, filtered, and concentrated to an oil. Purification of this oil by chromatography on Al₂O₃ (basic, activity IV) afforded 1.11 g (58%) of 14, a pink solid, m.p. 167–169 °C (dec).

EPR: Seven lines; aN = 8.1 g.

C₅₇H₸₅N₅O₅PS
Caled C 56.23 H 8.91 N 7.29 mol wt. 576.76, Found C 56.05 H 9.11 N 7.22 mol wt. 559.

B. At elevated temperature: To a solution of phosphorus thiochloride (0.56 g, 0.0033 mol) in 10 ml benzene was added at 25–27 °C a solution of 4 (1.72 g, 0.010 mol) and triethylamine (1.50 g, 0.015 mol) in 15 ml benzene. After the addition, the reaction mixture was warmed to 56 °C, left to stir at that temperature for 4 days, then filtered. The filtrate was concentrated on a rotating evaporator at 25 °C (12 torr). The remaining dark red oil was purified by chromatography as described in the preceding experiment to give 0.31 g (17%) of 14, m.p. 168–170 °C (dec), m.m.p. 167–169 °C (dec).

C. In diethyl ether: To a solution of phosphorus thiochloride (0.84 g, 0.0050 mol) in 20 ml diethyl ether was added at 0–5 °C a solution of 4 (3.44 g, 0.020 mol) and triethylamine (2.02 g, 0.020 mol) in 30 ml diethyl ether. After the addition, the reaction mixture was stirred at 23–25 °C for 12 days, then filtered. The filtrate was concentrated on a rotating evaporator at 25 °C (12 torr) to give a crude oil which was purified by chromatography as described in the preceding experiment. There was obtained 0.42 g (14%) of 14, m.p. 169–171 °C (dec), m.m.p. 168–169 °C (dec).

D. In benzene at room temperature: To a solution of phosphorus thiochloride (0.56 g, 0.0033 mol) in 30 ml benzene was added at 15 °C a solution of 4 (1.72 g, 0.010 mol) and triethylamine (1.01 g, 0.010 mol) in 35 ml benzene. Following the addition the reaction mixture was stirred at 23–25 °C for 5 days, then filtered. The filtrate was concentrated on a rotating evaporator at 25 °C (12 torr) to give a crude oil which was purified by chromatography as described in the preceding experiment. There was obtained 0.29 g (16%) of 14, m.p. 168–170 °C (dec), m.m.p. 167–169 °C (dec).

E. With added KCl at elevated temperature: To a solution of phosphorus thiochloride (0.50 g, 0.0033 mol) and potassium chloride (0.50 g, 0.0067 mol) in 30 ml benzene was added at 23–25 °C a solution of 4 (1.72 g, 0.010 mol) and triethylamine (1.01 g, 0.010 mol) in 50 ml benzene. After the addition, the reaction mixture was stirred at 50 °C for 4 days, then filtered. The filtrate was concentrated on a rotating evaporator at 25 °C (12 torr) to give a crude oil. Purification of the oil by chromatography as described in the preceding experiment afforded 0.61 g (33%) of 14, m.p. 167–169 °C (dec), m.m.p. 167–169 °C (dec).
Preparation of \((1\text{-oxyl}-2,2,6,6\text{-tetramethyl}-4\text{-piperidyl})\text{-N-bis}\text{-(2-chloroethyl)-N'}\text{'-N'-(ethylene)-phosphorodiamidate}\) (16)

To a solution of di-(2-chloroethyl)-phosphoroamidic dichloride\(^9\) (17; 1.30 g, 0.0050 mol) in 30 ml dioxan was added dropwise at 20–23 °C a solution of 4 (0.86 g, 0.0050 mol) and triethylamine (0.60 g, 0.0060 mol) in 30 ml dioxan. Following the addition, the reaction mixture was stirred at 20–23 °C for 2 days, then filtered to remove triethylamine hydrochloride (0.68 g, 100% of theory). The filtrate was concentrated on a rotating evaporator at 23 °C (12 torr) to a red oil which was immediately dissolved in benzene (30 ml) and cooled to 8 °C. To this filtrate was added as rapidly as possible at 8 °C a solution of triethylamine (0.60 g, 0.0060 mol) in 10 ml benzene. The reaction mixture was stirred at 8 °C for 10 min, and then was added slowly at 8–10 °C a solution of ethyleneimine (0.22 g, 0.0050 mol) in 10 ml benzene. Following the addition, the reaction mixture was stirred at 8 °C for 1 h, at ambient temperature for 20 h, then filtered. The filtrate was concentrated on a rotating evaporator to an oil. The oil was purified by chromatography on \(\text{Al}_2\text{O}_3\) (basic, activity IV) with a 2:1 (v/v) mixture of chloroform and benzene to give 1.80 g (92%) of 16, a red oil.

\[\text{C}_{15}\text{H}_{29}\text{Cl}_{2}\text{N}_{3}\text{O}_{3}\text{P}\]

Caled \[
\text{H} 7.28 \quad \text{N} 10.47 \quad \text{mol. wt.} 401.30,
\]
Found \[
\text{H} 7.13 \quad \text{N} 10.53 \quad \text{mol. wt.} 398.
\]

If the first step of the reaction, i.e., the reaction between 4 and 17, is allowed to proceed for one day instead of two, 16 is obtained in only 45% yield.

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2. G. Sosnovsky and G. Karas, Phosphorus 6, 123 [1976], and references therein.