Pergamon

Tetrahedron 54 (1998) 10973–10986

TETRAHEDRON

Synthesis and Characterization of α-Phosphorylated Ketones: Models for the Molybdopterin Precursor.

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Received 10 April 1998; revised 26 June 1998; accepted 29 June 1998

Abstract

Synthesis of pyridyl substituted α-keto six memebered cyclic phosphates is reported. These compounds are structurally similar to the precursor of molybdopterin (MPT), an important biological cofactor. We demonstrate that these α-phosphorylated ketones can undergo the fundamental transformation required for MPT synthesis.

Keywords: Phosphates, Pyridines, NMR.

Introduction

Molybdopterin (MPT) is the metal ligand of the cofactors found at the active site of 37 molybdenum and tungsten enzymes (Scheme 1).[1-4] These molybdopterin-containing enzymes are ubiquitous, being found in bacteria, archae, fungi, plants, and animals.

Molybdopterin synthase is a low molecular weight multi-subunit protein responsible for the last steps in cofactor synthesis and the subsequent delivery of MPT to the apo-molybdenum and -tungsten enzymes.[5,6] Rajagopolan and coworkers have shown the substrate of molybdopterin synthase (the MPT precursor) to be an α-phosphorylated ketone where the phosphate is part of a six-membered ring (Equation 1).[7,8] It is thought that protein bound thiolaspartic or thiolglutamic acid residues are involved in the conversion of the precursor and the stabilization of MPT.[5]

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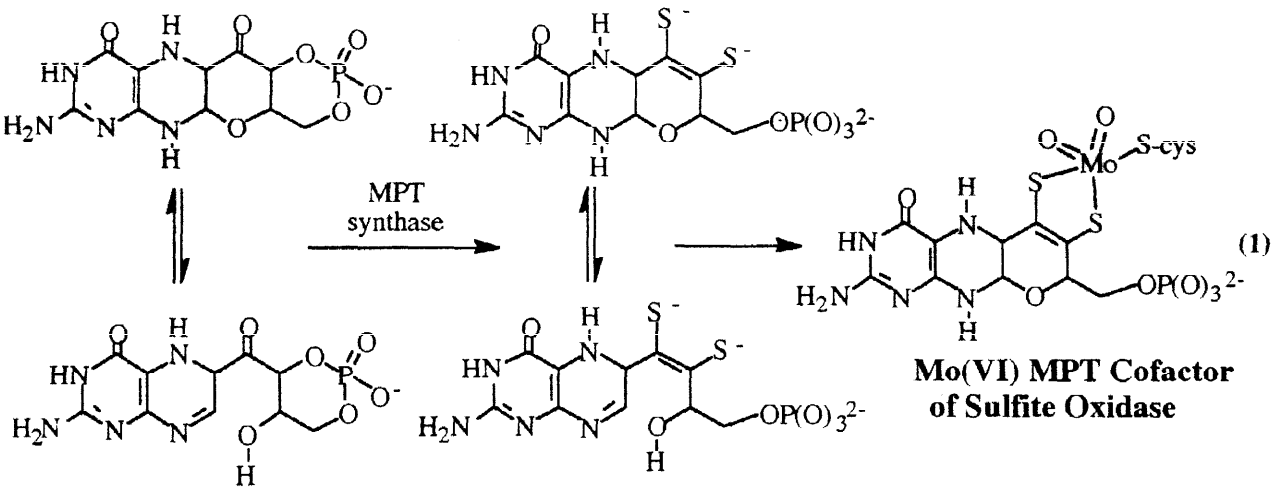
PII: S0040-4020(98)00663-2

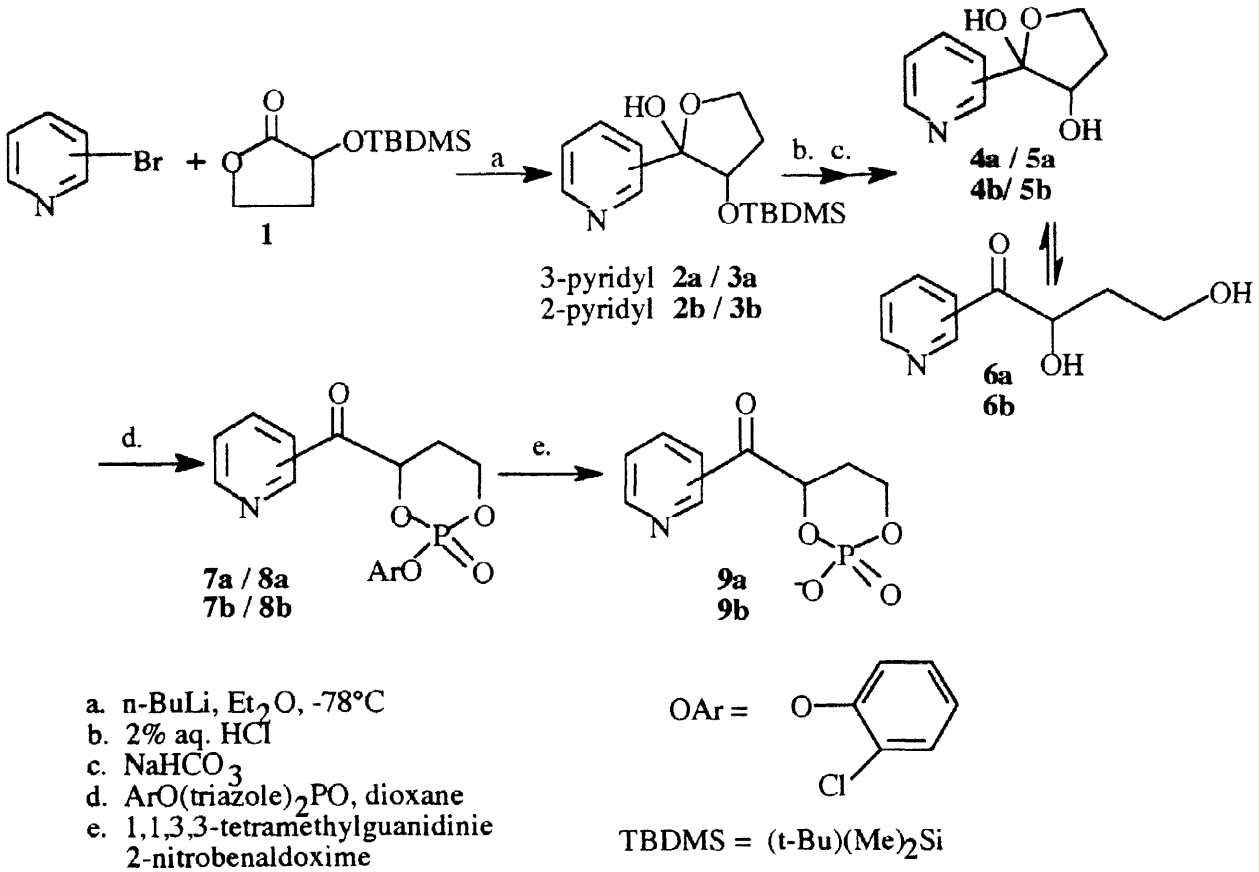


It has been difficult to explore the final steps of MPT synthesis, due to the instability and the limited availability of the molybdopterin precursor. The chemical conversion of the precursor to MPT must involve displacement of the phosphate from the α-carbon, loss of the ketone oxygen, loss of a proton α- to the ketone, and the addition of two thiolate sulfurs. A study of the fundamental reactivity of α-phosphorylated ketones should provide insight into this biological transformation. Such chemical insight is necessary for the rational design of potential inhibitors of molybdopterin synthase, which could have agricultural and medicinal uses. Most studies of α-phosphorylated ketones concentrated on the hydrolytic instability of this moiety.[9,10] This paper reports the first synthesis of α-phopshorylated ketones where the phosphate is contained in a six memebered ring, a moiety similar to that found in the MPT precursor. This paper also describes the reactivity of these α-phopshorylated ketones with organic and inorganic thiolates.

Results and discussion.

Preparation of α-Phosphorylated Ketones. The syntheses of the 2- and 3-pyridyl substituted α-phosphorylated ketones **7-9** is shown in Scheme 1. Addition of either 2- or 3-lithiopyridine (generated from the corresponding bromopyridine) to the TBDMS-protected

Scheme 1



α-hydroxy-substituted butyrolactone, **1**,[11] affords a mixture of inseparable TBDMS-protected hemiketal diastereomers, **2** and **3**, in 88 % and 77 % yield for the corresponding 2- and 3-pyridines, respectively. In the ^{13}C NMR resonances for the hemiketal carbons are observed at δ 102/106 ppm and 103/106 ppm for **2a/3a** and **2b/3b**, respectively. Desilylation of **2** and **3** with 2% aqueous HCl, followed by neutralization afforded an equilibrium mixture of the hemiketals, **4** and **5**, and the 1,3-diol, **6**. The diols, **6a** and **6b**, possess a diagnostic ^1H-NMR resonance assigned to the proton on the carbon α to the ketone centered at ≈ 5.25 ppm. Diastereomeric mixtures of the hemiketals **4aH+/5aH+** and **4bH+/5bH+** are observed exclusively under acidic conditions (10% DCl/ D_2O). The mixture of **4-6** was phosphorylated using 2-chlorophenyl-bis-triazoloyl phosphate.[12,13] This reagent was chosen since it can phosphorylate diols while initially attacking terminal alcohols.[13] This allowed **6** to be selectively phosphorylated in an equilibrium mixture of **4-6**. The diastereomers, **7** and **8**, were generated in >75% spectroscopic yield as measured against an internal ^1H NMR standard (Cp_2Fe). Compounds **7** and **8**, possess a diagnostic ^1H-NMR resonance assigned to the proton on the α-carbon to the ketone centered at ≈ 6.5 ppm. Mixtures of diastereomers, **7** and **8**, where used to prepare **9**, and for reactivity studies. However, **7a** and **8a** were separated for

spectroscopic studies by column chromatography as a 7:3 mixture in a combined yield of 40%.

Diastereomers **7** and **8** were deprotected in >95% yield to afford the cyclic phosphate anions, **9a** and **9b**, as the guanidinium salts.[14,15] The ^{31}P NMR resonances for **9a** and **9b** are shifted downfield of **7** and **8** by approximately 10 ppm, an amount expected for the conversion of a phosphate triesters to the corresponding diester monoanion.[7,16]

Configurational and Conformational Analysis of the α-Phosphorylated Ketones. The cyclic nature of the phosphates in **7a-9a**, and the stereochemical assignments for **7** and **8**, were determined from an analysis of NOE and selective ^{1}H decoupled ^{31}P NMR experiments. In **7a-9a** the phosphorus is more strongly coupled to the equatorial C(4) protons with a $J_{P\text{-}H(equ)} - J_{P\text{-}H(ax)} > 15$ Hz (Figures 1-3). This is consistent with findings for other six-membered phosphates including the MPT precursor.[7,8,16,17]

In diastereomer **7a**, H(4') is strongly coupled to phosphorus ($J_{P\text{-}H(4')} = 23$ Hz), and H(2) and H(4) are weakly coupled to phosphorus ($J_{P\text{-}H(4)}$ and $J_{P\text{-}H(2)} < 2$ Hz) (Figure 1). The couplings support H(4') being equatorial while H(2) and H(4) are axial. The axial position of both H(2) and H(4) is also evident from NOE transfer between these protons. From a

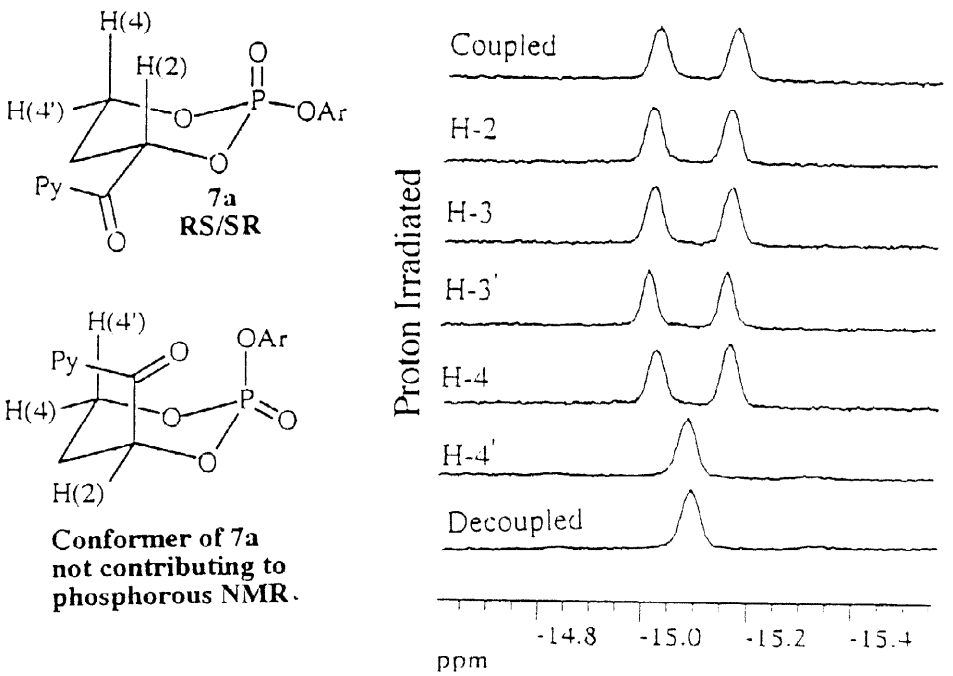


Figure 1. Selectively decoupled ^{1}H decoupled ^{31}P NMR Spectrum for **7a** in $CDCl_3$ at 25 °C.

comparison of these couplings, to those found for other six-member ring phosphates,[16] it is likely that the room temperature $CDCl_3$ ^{31}P NMR spectrum of **7a** is dominated by a single conformer.

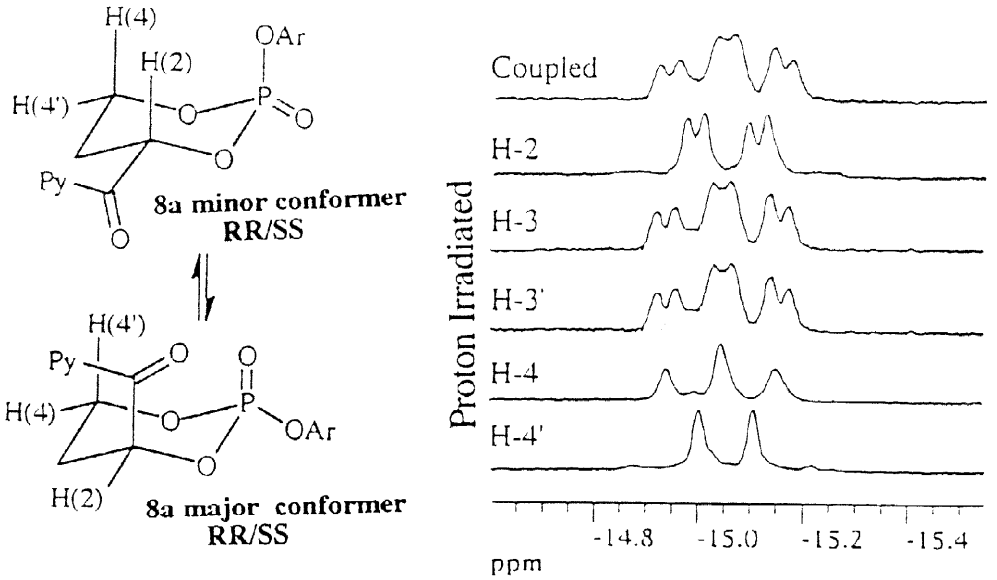


Figure 2. Selectively decoupled ^{1}H decoupled ^{31}P NMR Spectrum for **8a** in $CDCl_3$ at 25 °C.[18]

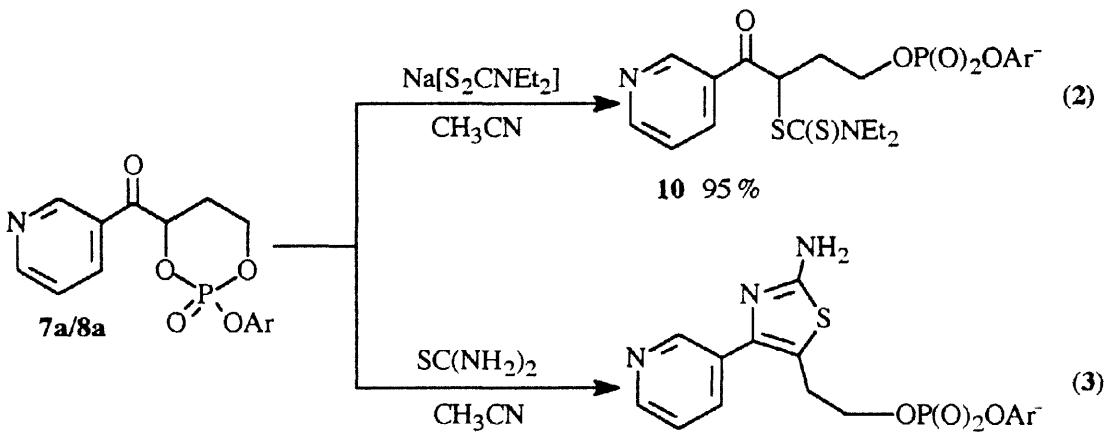
Unlike **7a**, the phosphorus in **8a** is strongly coupled to H(2) ($J_{P\text{-}H(2)}$ = 17 Hz) and H(4) ($J_{P\text{-}H(4)}$ = 19 Hz) while being weakly coupled to H(4') ($J_{P\text{-}H(4')}$= 5 Hz) (Figure 1 and 2). This would suggest a dominant configuration and conformation where both H(2) and H(4) are equatorial while H(4') is axial. However, a comparison of the $J_{P\text{-}H(4)}$ in **7a** (< 2Hz), with $J_{P\text{-}H(4')}$ in **8a** (5 Hz) and $J_{P\text{-}H(4')}$ in **7a** (23 Hz) with $J_{P\text{-}H(4)}$ in **8** (19 Hz) would suggest that **8a** is an ≈ 4:1 mixture of conformers at room temperature in $CDCl_3$. If there were a single conformer contributing to the ^{31}P NMR spectra of **8** then the $J_{P\text{-}H(ax)}$ and $J_{P\text{-}H(equ)}$ would be the same as observed in **7a**.

The large $J_{P\text{-}H(2)}$ and $J_{P\text{-}H(4)}$ coupling in **8a** could arise from either the RR/SS or RS/SR diastereomer. However, for the RS/SR isomer to have both H(2) and H(4) equatorial requires that both the sterically demanding pyridyl substituted ketone and the 2-chlorophenyl group be axial. As such, it seems more likely that **8a** is RR/SS. These isomers require that only one bulky group be axial and changing the axial bulky group accounts for the presence of the two conformers (see Figure 2). The dominant conformer of **8a** has the pyridyl substituted ketone in the axial position which minimizes lone-pair interaction between the phosphate ring oxygens and the ketone oxygen. **7a** is assigned to the SR, RS diastereomer where both sterically demanding groups (the pyridyl substituted ketone and the 2-chlorophenyl) are equatorial. Since the other RS/SR conformer requires that both bulky groups be axial, it does significantly contribute to the $J_{P\text{-}H}$ couplings.

Selective ^{1}H decoupled ^{31}P NMR experiments for **9** (like that of **7a**) show a strong coupling of phosphorus to H(4') (21 Hz) and weaker coupling to H(4) (3 Hz) supporting the cyclic nature of the phosphate.

Reactivity of α-Phosphorylated Ketones with Sulfur Nucleophiles. Since the conversion of the molybdopterin precursor to MPT appears to involve thiolate nucleophiles,[5,6] the reactivity of **7a-9a** with a variety of sulfur nucleophiles was investigated. Reaction of **7a** and **8a** with diethyldithiocarbamate[19] (as well as other thiol and thiol acids, under basic conditions)[20] produce the monoanionic phosphates with addition of the thiolate at the α-position (Equation 2). The ring-opening of the cyclic phosphate was characterized by a downfield shift of ≈ 10 ppm in the ^{31}P NMR and a simplification of the ^{1}H NMR signal due to a loss of the chiral center at phosphorus. The ketone is evident from both ^{13}C NMR and IR.

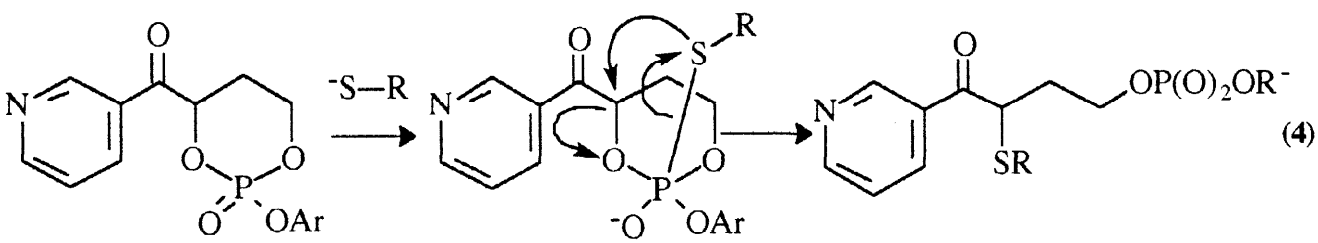
The reaction of **7a** and **8a**, with thiourea[19] (Equation 3) generates the thiazole, **11**, (which was isolated and characterized in its zwitterionic form). This reaction requires attack at the α-carbon, ring opening of the phosphate and elimination of the ketone oxygen, steps required for the conversion of the MPT precursor to MPT.



(2)

(3)

Nucleophilic addition directly to the α-ketocarbon in α-substituted ketones is not expected to be facile.[9,10] Rather, the reactions shown in equations 2 and 3 with thiolate nucleophiles (as well as the addition of 2-nitrobenzyaldoxime, Scheme 1) likely proceed by initial attack at phosphorus (Equation 4). In reactions of oxygen based nucleophiles, such as oximate with



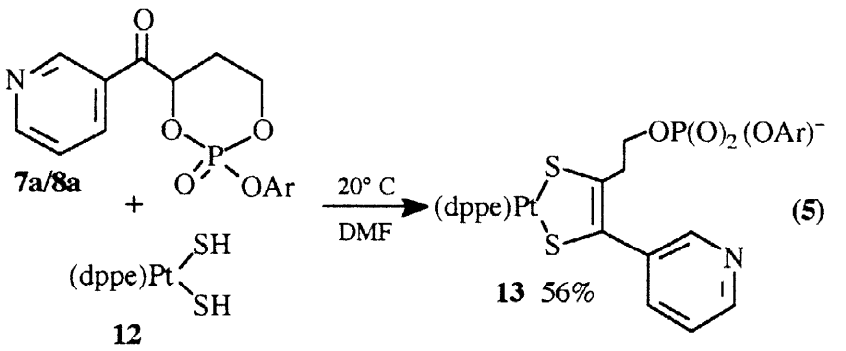
(4)

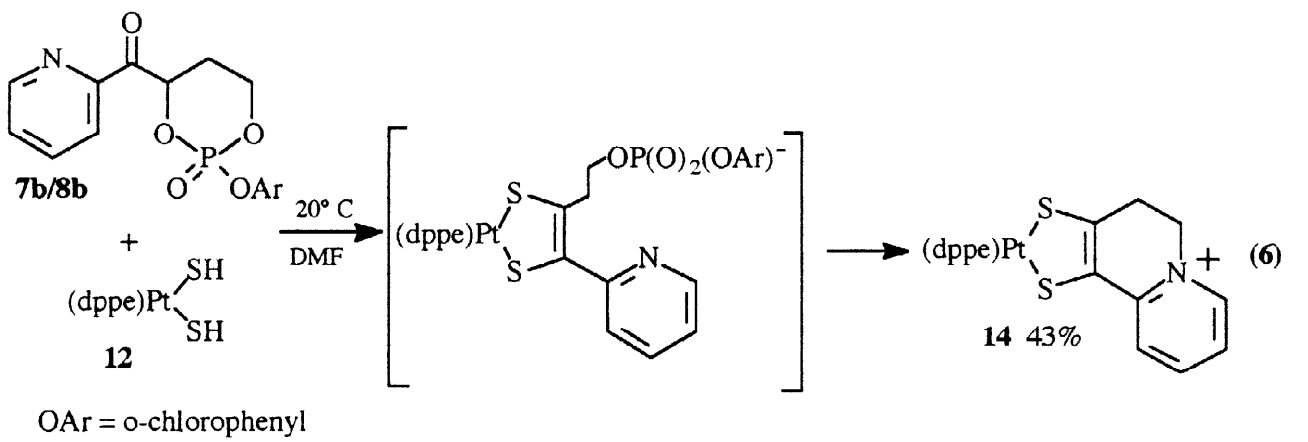
α- keto-phosphotriesters there is competition between exchange of the phosphate ester groups and phosphate elimination.[9,10] Only the later is seen with thiolate nucleophiles and this likely reflects the relative stability of P-O and P-S linkages. However, in the case of oximate, only OAr elimination is observed.

Attempts to convert **7** and **8** to a 1,4-dithiine with ethanedithiol (both in the presence and absence of added Lewis acids)[21,22] lead to either no reaction or the generation of complex mixtures of products. However, work in our laboratory has demonstrated that when reacted with metallo-bishydrosulfido complexes (a metallo-dithiol), α-bromo- and α-tosyl-ketones can be used to prepare metallo-1,2-enedithiolate complexes.[23-25] Given that the molybdenum and tungsten cofactors are metallo-1,2-enedithiolates, attempts to convert the α-phosphorylated ketones **7** and **8** to 1,2-enedithiolates seemed particular pertinent to this study. The mixture of **7a** and **8a** reacts with dppePt$(SH)_2$, where dppe= diphenyldiphoshphinoethane, **12**, to yield the metallo-1,2-enedithiolate complex **13** (equation 5). The reaction of **7b** and **8b** appears to generate a complex that is analogous to **12** but which undergoes nucleophilic attack by the pyridine upon the side chain to yield the pyridinium substituted-1,2-enedithiolate complex, **14**, with loss of 2-chlorophenylphosphate (Equation 6). Compound **14** is identical to the product generated from the reaction of dppePt$\{S_2C_2$(2-pyridine)$(CH_2CH_2OH)\}$ with either p-toluenesulfonyl chloride or 2-chlorophenyl-bis-triazoloyl phosphate and is structurally similar to the corresponding palladium complex which has been crystallographically characterized.[23] A detailed description of the synthesis, characterization and photophysical properties of these complexes are reported elsewhere.[23,26]

Studies of the cyclic phosphate anion **9**, show that it is significantly less reactive with both organic and inorganic sulfur nucleophiles under conditions that ring-opened the cyclic phosphate triesters **7** and **8**. Such a finding with a model that contains the necessary functional groups of the MPT precursor suggests the need for activation of the precursor toward nucleophiles by MPT synthase. However, since the models prepared in this study lack the β-hydroxyl found in the molybdopterin precursor,[8] it is possible that this group participates in phosphate activation.

Conclusion. In this study, methods for the synthesis of a unique family of cyclic phosphates (those α to a ketone) are described. With the exception of the molybdopterin precursor and its oxidation product Form Z[5,6,8], this researcher can find no other examples of α-keto six member cyclic phosphate. The reactivity of these phosphates has been investigated in an attempt to better understand chemical aspect of MPT biosynthesis. Indeed, it has been demonstrated that α-phosphorylated ketones can undergo the fundamental transformation required for MPT synthesis. The anionic phosphate diesters, **9a** and **9b** prepared in this study were surprisingly inert to attack by sulfur nucleophiles. This observation is consistent with both a diminished reactivity at the α-position (due to an appended anion) and the phosphate monoester being a poorer leaving group than the phosphate diester. However, these findings





do suggest that the cyclic phosphate diester of the MPT precursor must undergo activation for conversion to MPT. This activation could be accomplished by the protein, (MPT synthase) or be due to the β- OH group present in the MPT precursor but lacking in the model compounds. Future studies include preparing analogs of **9** which contain the β- OH group as well as the screening of **9a** and **9b** as inhibitors of MPT synthase.

Experimental.

Physical Measurements. NMR spectra were acquired with a Brüker AF 200, AM 400, DRX 400 or a DRX 500. IR spectra were collected either with a Perkin Elmer 1600 or a Nicolet 5 DXL FT-IR Spectrometer. UV-visible spectra were recorded on either a Perkin Elmer Lambda 2S or a Hewlet Packard 8452A spectrometer. EI and FAB mass spectral data were collected on a Magnetic Sector VG 7070E.

Materials. The compounds α-hydroxy-γ-butyrolactone, imidazole, *t*-butyldimethylsilyl chloride, 2-and 3-bromopyridine, *n*-butyllithium, 2-chlorophenyl dichlorophosphate, 1,2,4-triazole, 2-nitrobenzaldoxime, 1,1,3,3-tetramethyl guanidine, diethyldithiocarbamate-sodium salt, and thiourea were purchased from Aldrich or Acros and used without further

purification. dppePt(SH)$_2$[27] and 2-chlorophenyl-bis-triazoloyl phosphate[12,13] were synthesized according to the literature procedure. All chromatographic purifications were done using silica gel, 60-200 mesh, purchased from VWR Scientific on a 20 x 2.5 cm column.

Synthesis.

α-(*t*-Butyl-dimethylsilyoxy)-γ-butyrolactone, 1.[11] To a CH_3CN solution (50 mL) of α-hydroxy-γ-butyrolactone (0.966 g, 6.33 mmol) and imidazole (0.996 g, 6.60 mmol) was added *t*-butyldimethylsilyl chloride (1.00 g, 6.63 mmol) at 25 °C and solution was stirred for 24 h. The CH_3CN was removed under vacuo and the residue was dissloved in CH_2Cl_2 (100 mL), washed with brine (50 mL), and water (2X50mL). The CH_2Cl_2 was removed under vacuo and compound **1** was purified by vacuum distillation (0.1 torr, 82-84°C) to give a clear liquid in 84% yield (1.198 g, 5.54 mmol). ^1H NMR ($CDCl_3$): δ 4.43-4.27 (m, 2H, C*H*$_2$OCO), 4.23-4.08 (m, 1H, COC*H*OSi), 2.50-2.31 (m, 1H, C*H*$_2$CH$_2$OCO), 2.25-2.06 (m, 1H, C*H*$_2$CH$_2$OCO), 0.83 (s, 9H, C(C*H*$_3$)$_3$), 0.09 (s, 3H, C*H*$_3$), 0.07 (s, 3H, C*H*$_3$). Mass Spectrum (CI): *m/z* 217 (M+1).

2-(3-Pyridyl)-3-(*t*-Butyl-dimethylsilyoxy)-tetrahydro-furan-2-ol, 2a/3a. To a -78 °C ether solution (50 mL) of 3-bromopyridine (0.968 g, 6.12 mmol) was added 3.83 mL (6.12 mmol) of 1.6 M *n*-butyllithium, dropwise over 30 min. The solution was stirred for an additional 15 minutes at -78 °C, and the silylated lactone (1.200 g, 5.56 mmol) was added and the mixture was warmed to room temperature over 1 h. Brine (50 mL) was added to the ether and the mixture was extracted with *n*-butanol (3 x 50 mL). The organic layer was washed with brine and the solvent was removed to give the crude hemiketals in 88% yield (1.589 g, 5.39 mmol). This material was sufficiently pure for subsequent reaction but could be purified by column chromatography where **2a** and **3a** eluted with 5% MeOH/CH_2Cl_2 in a 3:2 ratio. **2a/3a**: ^1H NMR ($CDCl_3$): δ 8.70-8.62 (m, 2H, C$_5$*H*$_4$N), 8.49-8.33 (m, 2H, C$_5$*H*$_4$N), 7.82-7.70 (m, 2H, C$_5$*H*$_4$N), 7.22-7.09 (m, 2H, C$_5$*H*$_4$N), 4.29-4.14 (m, 4H, C*H*OSiR$_3$), 4.13-3.99 (m, 2H, OC*H*$_2$), 2.60-2.39 (m, 1H, C*H*$_2$CHOSiR$_3$), 2.29-2.09 (m, 1H, C*H*$_2$CHOSiR$_3$), 2.00-1.79 (m, 2H, C*H*$_2$CHOSiR$_3$), 0.85 (s, 9H, C(C*H*$_3$)$_3$), 0.59 (s, 9H, C(C*H*$_3$)$_3$), 0.00 (s, 3H, C*H*$_3$), -0.05 (s, 3H, C*H*$_3$), -0.18 (s, 3H, C*H*$_3$), -0.48 (s, 3H, C*H*$_3$). ^{13}C NMR ($CDCl_3$): δ 148.9, 148.8, 148.4, 147.7, 138.1, 136.8, 135.5, 133.9, 122.7, 122.1, 106.6, 102.6, 78.6, 78.2, 67.0, 65.7, 34.3, 33.2, 25.5, 25.4, 17.9, 17.6, -5.0, -5.2, -5.3, -5.7. IR (thin film, cm^{-1}): 3181 (s), 2956 (s), 2856 (s), 1706 (m), 1688 (m), 1582 (s). High Resolution Mass Spectrum (FAB) calc. *m/z* = 296.1682 for $C_{15}H_{26}O_3NSi$; found 296.1689.

2-(3-Pyridyl)-3-(hydroxy)-tetrahydro-furan-2-ol, 4a/5a and **1-(3-Pyridyl)-2,4-dihydroxy-butan-1-one, 6a.** A 2 % HCl/H_2O (25 mL) solution of **2a/3a** (0.500 g, 1.69 mmol) was stirred at 25°C for 8h, and then extracted with hexane. The aqueous layer was neutralized with $NaHCO_3$ and the water was removed. The resulting residue was extrated with 10% MeOH/CH_2Cl_2. The solvent was removed to yield a mixture of **4a-6a**, 70%, (0.214 g,

1.18 mmol). While it was generally not necessary in subsequent steps, this mixture could be further purified by column chromatography where the products eluted with 15% MeOH/CH_2Cl_2. **4a-6a** ^{1}H NMR ($CDCl_3$): δ 8.72–8.54 (m, 2H, C_5H_4N), 7.86-7.62 (m, 4H, C_5H_4N), 7.55-7.44 (m, 4H, C_5H_4N), 7.25-7.15 (m, 2H, C_5H_4N), 5.20-5.16 (m, 1H, C(O)*CH*OH of **6a**), 4.15-3.99 (m, 10H, OC*H*OH of **4a/5a**, O*CH*$_2$CH$_2$ of **4a/5a**, C(O)C*H* of **4a/5a**, C*H*$_2$OH of **6a**), 2.43-1.88 (m, 6H, OCH$_2$C*H*$_2$ of **4a/5a**, CH$_2$C*H*$_2$OH of **6a**). Protonation of **4a-6a** produces the protonated hemiketals **4aH+/5aH+**. **4aH+/5aH+**: ^{1}H NMR (10% DCl/ D_2O): δ 8.70 (br s, 1H, C_5H_4N), 8.59-8.50 (m, 2H, C_5H_4N), 7.93-7.89 (m, 2H, C_5H_4N), 4.16-3.93 (m, 3H, OC*H*OH, O*CH*$_2$), 2.49-2.30 (m, 1H, C*H*$_2$CHOH), 2.00-1.81 (m, 1H, C*H*$_2$CHOH). ^{13}C NMR (10 % DCl/ D_2O): δ145.8, 144.7, 142.3, 141.1, 141.0, 140.4, 140.0, 139.0, 127.5, 127.0, 105.2, 100.6 (*O*COH), 76.8, 76.8, 67.6, 66.1, 32.3, 30.8 (*CH*$_2$CHOH). IR (thin film, cm^{-1}) 3310 (s), 2959 (s), 1720 (s), 1588 (s), 1423 (m). High Resolution Mass Spectrum (FAB) calc. *m/z* = 182.1941 for $C_9H_{12}O_3N$; found 182.1936.

2-(o-Chlorophenylphospho)-4-(acetyl-3-pyridyl)-1,3,2-dioxaphophorinane, 7a/8a. To solid **4a-6a**, (0.409 g, 2.26 mmol) was added 13.6 mL of a 0.2 M dioxane solution of 2-chlorophenyl-bistriazoloyl phosphate (2.71 mmol). The solution was stirred for 12 h, and the solvent was removed under *vacuo*. The residue was dissolved in CH_2Cl_2, washed with water (3 x 5 mL), and the solvent was removed to yield a mixture of diasteromers **7a/8a**. **7a** and **8a** were separated by chromatograpy were they eluted with 2% MeOH/CH_2Cl_2 in a combined yield of 40% in a 7:3 ratio (0.320g, 0.904 mmol). **7a**: ^{1}H NMR ($CDCl_3$): δ 9.03 (br s, 1H, C_5H_4N), 8.78 (m, 1H, C_5H_4N), 8.10 (dt, 1H, C_5H_4N, J_{H-H}= 7, 1 Hz), 7.70 (dt, 1H, C_5H_4N, J_{H-H}= 7, 1 Hz), 7.48-7.15 (m, 4H, C_6H_4ClO), 5.79 (dt, 1H, C*H*, line spacings of 11 and 2 Hz), 4.89-4.48 (m, 2H, C*H*$_2$,OP), 2.65-2.39 (m, 1H, C*H*$_2$,CH$_2$OP), 2.33-2.18 (m, 1H, C*H*$_2$,CH$_2$OP). ^{13}C NMR ($CDCl_3$): δ 191.6 (J_{C-P}= 12 Hz), 153.9 150.1, 145.8 (J_{C-P}= 6 Hz), 136.4, 130.5, 129.1, 128.3, 126.1, 124.1 (J_{C-P}= 7 Hz), 123.5, 120.9, 79.7 (J_{C-P}= 8 Hz), 68.3 (J_{C-P}= 7 Hz), 26.8 (J_{C-P}= 5 Hz). ^{31}P NMR ($CDCl_3$): δ -14.7. IR (thin film, cm^{-1}): 3072 (w), 2967 (w), 2920 (w),0 1706 (s) 1586 (s), 1482 (s), 1449 (m), 1422 (m), 1312 (s), 1231 (s), 1074 (s), 1059 (s), 1042 (s), 975 (s), 943. High Resolution Mass Spectrum (FAB) calc. *m/z* = 354.0298 for $C_{15}H_{14}O_5NPCl$; found 354.0294. **8a**: ^{1}H NMR ($CDCl_3$): δ 9.16 (d, 1H, C_5H_4N, J_{H-H}= 2 Hz), 8.70 (m, 1H, C_5H_4N), 8.21 (dt, 1H, C_5H_4N, J_{H-H}= 2, 7 Hz), 7.34-6.98 (m, 5H, C_5H_4N and C_6H_4ClO), 5.73 (second order dt, 1H, C*H*, line spacings of 18 and 8 Hz), 4.89-4.70 (m, 1H, C*H*$_2$,OP), 4.70-4.41 (m, 1H, C*H*$_2$,OP), 2.70-2.38 (m, 2H, C*H*$_2$CH$_2$OP). ^{13}C NMR ($CDCl_3$): δ 192.8 (J_{C-P}= 2 Hz), 153.9, 150.6, 146.2 (J_{C-P}= 5 Hz), 139.2 (J_{C-P}= 5 Hz), 136.7, 130.5, 129.5, 127.8, 125.8, 123.5, 120.6, 79.6 (J_{C-P}= 7 Hz), 67.3 (J_{C-P}= 7 Hz), 25.1 (J_{C-P}= 9 Hz). ^{31}P NMR ($CDCl_3$): δ -14.3. IR (thin film, cm^{-1}): 3065 (m) , 2973 (m),

2931 (m), 1701 (vs), 1587 (vs), 1481 (vs), 1447 (s), 1419 (s), 1312 (vs), 1234 (vs), 1080 (s), 1058 (s), 1042 (s), 970 (s), 942 (vs).

2-Oxo-4-(acetyl-3-pyridyl)-1,3,2-dioxaphophorinane, 9a. To a dioxane solution (4 mL) of **7a/8a** (0.082 g, 0.232 mmol) in dioxane (4 mL) was added a dioxane solution (1 mL) of 1,1,3,3-tetramethylguanidine (58 μL, 0.464 mmol) and 2-nitrobenzaldoxime (0.027 g, 0.232 mmol). The solution was stirred at 25°C for 4 hours. The solvent was removed to yield an orange residue which was dissolved in CH_2Cl_2 (10 mL) and washed with brine (3 x 2 mL). The CH_2Cl_2 was removed to yield the guanidinium salt of **9a** in 95 % yield (0.083 g, 0.232 mmol). ^{1}H NMR ($CDCl_3$): δ 9.18 (d, 1H, C_5H_4N, J_{H-H}= 2 Hz), 8.69 (dd, 1H, C_5H_4N, J_{H-H}= 5, 1 Hz), 8.33 (dt, 1H, C_5H_4N, J_{H-H}= 8, 2 Hz), 7.36 (dd, 1H, C_5H_4N, J_{H-H}= 8, 5 Hz), 5.57 (second order dt, 1H, CH, line spacings of 11 and 3 Hz), 4.54-4.39 (m, 1H, CH_2,OP), 4.27-4.08 (m, 1H, CH_2,OP), 2.25-2.02 (m, 1H, CH_2,CH_2OP), 1.94-1.80 (m, 1H, CH_2,CH_2OP). ^{13}C NMR ($CDCl_3$): δ 195.8 (J_{C-P}= 10 Hz), 161.9, 153.1, 150.5, 136.8, 130.2, 123.3, 77.2 (J_{C-P}= 4 Hz), 64.6 (J_{C-P}= 5 Hz), 39.8, 28.3 (J_{C-P}= 3 Hz). ^{31}P NMR ($CDCl_3$): δ -3.6. IR (thin film, cm^{-1}): 3343 (br, s), 2972 (s), 1672 (s), 1606 (vs), 1571 (vs), 1531 (w), 1470 (m), 1454 (m), 1411 (s), 1346 (w), 1324 (w), 1248 (vs), 1097 (s), 1078 (s), 1045 (m), 1002 (w). High Resolution Mass Spectrum (FAB) calc. *m/z* = 244.0375 for $C_9H_{11}O_5NP$; found 244.0368.

2-(2-Pyridyl)-3-(*t*-Butyl-dimethylsilyoxy)-tetrahydro-furan-2-ol, 2b/3b. Compounds **2b/3b** were prepared and isolated as described for **2a/3a** using 2-bromopyridine (2.81 g, 17.8 mmol), 12.2 mL (19.4 mmol) of 1.6 M *n*-butyllithium, and lactone **1** (3.49 g, 16.2 mmol). Compounds **2b/3b** were isolated as a (7:3) mixture of diasteriomers (7:3 ratio) in 77% yield (4.06 g, 13.7 mmol). ^{1}H NMR ($CDCl_3$): δ 8.57-8.53 (m, 1H, C_5H_4N), 8.49-8.46 (m, 1H, C_5H_4N), 7.70-7.58 (m, 4H, C_5H_4N), 7.27-7.16 (m, 2H, C_5H_4N), 4.32-4.05 (m, 6H, CHOSiR$_3$ and OCH_2), 2.59-1.88 (m, 4H, CH_2CHOSiR$_3$), 0.86 (s, 9H, C(CH_3)$_3$), 0.75 (s, 9H, C(CH_3)$_3$), -0.03 (s, 3H, CH_3), -0.11 (s, 3H, CH_3), -0.18 (s, 3H, CH_3), -4.8 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 159.9, 157.6, 148.5, 146.6, 136.8, 136.3, 124.3, 123.8, 123.4, 123.1, 122.7, 120.8, 105.8, 102.8, 79.1, 79.1, 67.8, 66.0, 34.1, 33.5, 25.9, 25.6, 15.0, 15.0, -5.0, -5.2, -5.4, -5.5. High Resolution Mass Spectrum (FAB) calc. *m/z* = 296.1682 for $C_{15}H_{26}O_3NSi$; found 296.1684.

2-(2-Pyridyl)-3-(hydroxy)-tetrahydro-furan-2-ol, 4b/5b and **1-(2-Pyridyl)-2,4-dihydroxy-butan-1-one, 6b.** Compounds **4b/5b** and **6b** were prepared and isolated as described for compounds **4a-6a** in 90 % yield (1.92 g, 10.5 mmol) using **4b-6b** (3.46 g, 11.7 mmol). **4b-6b** ^{1}H NMR ($CDCl_3$): δ 8.73–8.51 (m, 2H, C_5H_4N), 7.87-7.62 (m, 4H, C_5H_4N), 7.53-7.44 (m, 4H, C_5H_4N), 7.28-7.17 (m, 2H, C_5H_4N), 5.47-5.38 (M, 1H, C(O)*CH*OH of **6b**), 4.43-3.83 (m, 8H, OC*H*OH of **4b/5b**, OCH_2CH_2 of **4b/5b**, C(O)C*H* of **4b/5b**, CH_2OH of **6b**), 2.43-1.88 (m, 6H, CH_2CH_2OH of **4b/5b**, CH_2CH_2OH of **6b**). Protonation of **4b-6b**

produces the protonated hemiketals **4aH+/5aH+**. **4bH+/5bH+**: ^{1}H NMR (10% DCl/ D_2O): δ 8.65-8.49 (m, 2H, C_5H_4N), 8.14-7.92 (m, 2H, C_5H_4N), 4.33-4.09 (m, 3H, OC*H*OH, OCH_2), 2.58-1.90 (m, 2H, CH_2CHOH). ^{13}C NMR (10 % DCl/ D_2O): δ147.1, 146.9, 143.4, 142.7, 141.9, 141.5, 140.6, 140.4, 127.0, 125.9, 104.0, 99.0 (O*C*OH), 77.2, 76.2, 68.1, 66.3, 32.0, 30.6. Resolution Mass Spectrum (FAB) calc. *m/z* = 182.1941 for $C_9H_{12}O_3N$; found 182.1932.

2-(o-Chlorophenylphospho)-4-(acetyl-2-pyridyl)-1,3,2-dioxaphophorinane, 7b/8b. Compounds **7b/8b** were prepared and isolated as described for compounds **7a/8a** using **4b-6b**, (0.839 g, 4.64 mmol) and 34.8 mL of a 0.2 M solution of 2-chlorophenyl-bis-triazole phosphate (6.95 mmol). The crude diastereomers **7b/8b** were isolated in a 4:1 ratio. While it was not possible to obtain analytically pure **8b** by chromatography, **7b** was isolated as a single diastereomer when eluted with 3:1 CH_2Cl_2/hexane in 40 % yield (0.658 g, 1.86 mmol). **7b** ^{1}H NMR ($CDCl_3$): δ 8.57 (d, 1H, C_5H_4N, J_{H-H} = 5 Hz), 8.05 (m, 1H, C_5H_4N), 7.85 (m, 1H, C_5H_4N), 7.61 (m, 1H, C_5H_4N), 7.51 (m, 1H, C_6H_4ClO), 7.36 (m, 1H, C_6H_4ClO), 7.25 (m, 1H, C_6H_4ClO), 7.12 (m, 1H, C_6H_4ClO), 6.54 (m, 1H, C*H*), 4.87 (m, 1H, CH_2,OP), 4.66-4.43 (m, 1H, CH_2,OP), 2.53 (m, 1H, CH_2,CH_2OP), 2.32 (m, 1H, CH_2,CH_2OP). ^{13}C NMR ($CDCl_3$): δ 192.2 (J_{C-P}= 9 Hz), 150.5 149.0, 146.2 (J_{C-P}= 6 Hz), 137.3, 130.4, 128.0, 127.7, 125.7, 124.5 (J_{C-P}= 8 Hz), 123.0, 121.2, 79.4 (J_{C-P}= 6 Hz), 68.5 (J_{C-P}= 7 Hz), 28.6 (J_{C-P}= 7 Hz). ^{31}P NMR ($CDCl_3$): δ -13.6. IR (thin film, cm^{-1}): 3313 (m), 2963 (s), 2931 (s), 2874 (m), 2858 (m), 1723 (s), 1585 (s), 1482 (vs), 1450 (s), 1316 (s), 1297 (s), 1264 (s), 1232 (s), 1110 (vs), 1075 (s), 1061 (vs), 1038 (vs), 1009 (vs), 946.4 (m). High Resolution Mass Spectrum (FAB) calc. *m/z* = 354.0298 for $C_{15}H_{14}O_5NPCl$; found 354.0303.

2-Oxo-4-(acetyl-2-pyridyl)-1,3,2-dioxaphophorinane, 9b. Compound **9b** was prepared and isolated as described for compound **9a** using **7b/8b** (0.068 g, 0.192 mmol), 1,1,3,3-tetramethylguanidine (48 μL, 0.044 mmol) and 2-nitrobenzaldoxime (0.022 g, 0.192 mmol). Compound **9b** was isolated in > 95% yield (0.044 g, 1.82 mmol). ^{1}H NMR ($CDCl_3$): δ 8.59 (m, 1H, C_5H_4N), 7.97 (m, 1H, C_5H_4N), 7.79 (m, 1H, C_5H_4N), 7.42 (m, 1H, C_5H_4N), 6.22 (m, 1H, C*H*, line spacings of 2 and 11 Hz), 4.56 (m, 1H, CH_2,OP), 4.23-4.03 (m, 1H, CH_2,OP), 2.18 (m, 1H, CH_2,CH_2OP), 1.98-1.81 (m, 1H, CH_2,CH_2OP). ^{13}C NMR ($CDCl_3$): δ 196.6 (J_{C-P}= 9 Hz), 162.0, 151.5, 149.0, 136.8, 127.3, 122.6, 77.2 (J_{C-P}= 4 Hz), 65.1 (J_{C-P}= 5 Hz), 40.0, 30.4. ^{31}P NMR ($CDCl_3$): δ -3.5. IR (thin film, cm^{-1}): 3350 (br, s), 2962 (s), 1714 (s), 1666 (s), 1607 (vs), 1573 (vs), 1525 (w), 1465 (m), 1452 (m), 1435 (m), 1411 (s), 1346 (w), 1324 (w), 1261 (vs), 1100 (s), 1074 (s), 1041 (m), 1009 (w). High Resolution Mass Spectrum (FAB) calc. (M+1) *m/z* = 245.04532 for $C_9H_{12}O_5NP$; found 245.04539.

2-(N,N-diethyldithiocarbamoyl)-4-(o-chlorophenylphosphoro)-1-(3-pyridyl)-butan-1-one, 10. To a solution of the cyclic phosphate **7a/8a** (0.050 g, 0.14 mmol) in CH_3CN (20 mL) was added the sodium salt of diethyldithiocarbamic acid (0.031 g, 0.14

mmol). The mixture was stirred for 20 minutes and then ether was added dropwise to precipitate the product. The product was collected by vacuum filtration to give compound **10** as a pale yellow solid in > 95 % yield (0.070 g, 0.14 mmol). **10:** ^{1}H NMR (CD_3CN): δ 9.18 (br s, 1H, C_5H_4N), 8.66 (m, 1H, C_5H_4N), 8.21 (m, 1H, C_5H_4N), 7.56 (m, 1H, C_5H_4N), 7.37 (m, 1H, C_6H_4ClO), 7.18 (m, 1H, C_6H_4ClO), 7.03 (m, 1H, C_6H_4ClO), 6.83 (m 1H, C_6H_4ClO), 5.43 (t, 1H, CHS, J_{H-H}= 12 Hz), 3.97 (m, 2H, CH_2OP), 3.84 (m, 2H, CH_2CH_3), 3.63 (m, 2H, CH_2CH_3,), 2.20 (m, 2H, CH_2CH_2OP), 1.14 (m, 6H, CH_3). ^{13}C NMR (10 % DCl/ $CDCl_3$): δ 192.8, 191.8, 153.0, 147.6 (J_{C-P}= 5 Hz), 144.3 , 144.0, 142.8, 130.2, 128.0, 127.0, 125.2 (J_{C-P}= 7 Hz), 125.0, 64.3 (J_{C-P}= 5 Hz), 52.5, 50.2, 47.7, 31.6 (J_{C-P}= 6 Hz), 12.6, 11.4. ^{31}P NMR (CD_3CN): δ -3.2. IR (KBr, cm^{-1}): 3400 (s), 3256 (s), 2969 (s), 2931 (m), 1701 (s), 1589 (s), 1495 (s), 1482 (vs), 1446 (s), 1422 (vs), 1357 (m), 1302 (m), 1274 (vs), 1262 (s), 1239 (vs), 1205 (s), 1108 (vs), 1098 (vs), 1061 (s), 1040 (s), 1010 (m), 1001 (m), 940.1 (w), 914.8 (s). High Resolution Mass Spectrum (FAB) calc. *m/z* = 503.0651 for $C_{20}H_{25}ClO_5PS_2$; found 503.0656.

4-(3-Pyridyl)-5-(2-o-chlorophenylphosphoethyl)-2-amino-4-thiazole, 11. To a solution of **7a/8a** (0.040 g, 0.11 mmol) in CH_3CN (20 mL) was added thiourea (0.009 g, 0.11 mmol) and the solution was refluxed for 6 h. The solvent was removed and the residue was washed with ether to yield compound **11** in 65 % yield (0.030 g, 0.072 mmol). ^{1}H NMR (10% DCl): δ 9.04 (m, 1H, C_5H_4N), 8.74 (m, 1H, C_5H_4N), 8.33 (m, 1H, C_5H_4N), 7.54 (m, 1H, C_5H_4N), 7.30 (m, 4H, C_6H_4Cl), 4.37 (m, 2H, CH_2OP), 3.18 (m, 2H, CH_2CH_2OP). ^{13}C NMR (DCl): δ 167.5, 145.7, 144.8 (J_{C-P}= 6 Hz), 140.8, 139.3 , 127.2, 127.1, 126.5, 123.4 (J_{C-P}= 6 Hz), 121.6, 119.7, 125.0, 65.6, 25.4 (J_{C-P}= 8 Hz). ^{31}P NMR (DMSO): δ -8.4. IR (KBr, cm^{-1}): 3306 (s), 3181 (s), 3088 (s), 2719 (m), 2544 (m), 2056 (w), 1998 (w), 1944 (w), 1649 (vs), 1625 (s), 1555 (w), 1479 (vs), 1410 (w), 1265 (m), 1236 (s), 1222 (s), 1084 (vs), 1063 (vs), 1024 (s), 936.6 (m), 905.4 (s), 836.4 (w). High Resolution Mass Spectrum (FAB) calc. *m/z* = 412.0288 for $C_{16}H_{16}ClN_3O_4PS$; found 412.0279.

Acknowledgement. We are indebted to the Donors of the Maryland Chapter of the American Heart Association (Grant MDBG695) and the donors of the Petroleum Research Fund, administered by the ACS (32486-AC3) for supporting this research.

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