1,2,4-Diazaphosphole Nucleosides. Synthesis, Structure, and Antitumor Activity of Nucleosides with a λ^3 Phosphorus Atom

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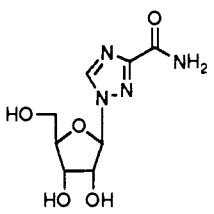
Glycosylation of 1,2,4λ^3-diazaphosphole (**4**) under Lewis acid catalyzed conditions gave 1-α-D-ribofuranosyl-1,2,4λ^3-diazaphosphole (**5**) as the only product. Ethyl 1,2,4λ^3-diazaphosphole-3-carboxylate (**10**) was synthesized by the cyclocondensation of ethyl (chlorophosphinidene)(trimethylsilyl)acetate (**8**) with (trimethylsilyl)diazomethane and subsequent desilylation with tetra-*n*-butylammonium fluoride. Reaction of **10** with methanolic ammonia at 80 °C gave 1,2,4λ^3-diazaphosphole-3-carboxamide. Glycosylation of **10** using trimethylsilyl triflate catalyst followed by ammonolysis gave the ribavirin (**1**) analogue 1-β-D-ribofuranosyl-1,2,4λ^3-diazaphosphole-3-carboxamide (**11**). Acetylation of **11** and subsequent treatment with phosphorus pentasulfide gave 2',3',5'-tri-*O*-acetyl-1-β-D-ribofuranosyl-1,2,4λ^3-diazaphosphole-3-thiocarboxamide (**13**). Deprotection with methanolic ammonia gave 1-β-D-ribofuranosyl-1,2,4λ^3-diazaphosphole-3-thiocarboxamide (**14**). Compound **14** gave a 25% increase in life span (ILS) against L1210 in female BDF_1 mice. The anomeric configuration and site of glycosylation of **5** and **13** were established by single-crystal X-ray crystallography.

Ribavirin, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (**1**), is a broad-spectrum antiviral agent first synthesized by Witkowski[1] and co-workers. One of the most interesting antiviral activities of ribavirin has been reported by McCormick[2] and co-workers, who found that ribavirin inhibits replication of HIV in human T lymphocytes. For a review of the antiviral activity of ribavirin and its clinical activity, the reader is referred to published reviews.[3-5]



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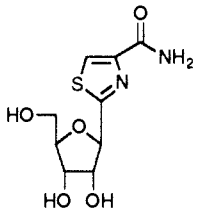

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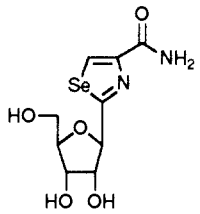

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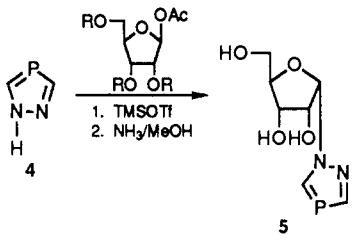
Ribavirin has also shown some antitumor activity in mice against L1210 and adenocarcinoma 755.[6] Ribavirin has been shown to inhibit IMP dehydrogenase isolated from Ehrlich ascites tumor cells.[7,8] The inhibition of IMP dehydrogenase is believed to account for the antitumor activity of ribavirin. Related nucleosides, such as tiazofurin[9] (**2**) and selenazofurin[10] (**3**), are metabolized to NAD analogues which are potent IMP dehydrogenase inhibitors.[11,12] Tricot and co-workers[13] have shown tiazofurin

Scheme I[a]



4 5

[a] R = benzoyl or acetyl.

to be clinically effective against human acute myeloblastic leukemia. Tiazofurin and selenazofurin have also shown antiviral activity in vitro.[14] In keeping with our interest in the discovery of novel inhibitors of IMP dehydrogenase as potential antiviral and antitumor agents, it was decided to synthesize analogues of ribavirin containing a phosphorus atom in place of one of the nitrogens in the triazole base. A review of the literature showed that little work has been done with nucleosides containing phosphorus in the heterocyclic moiety. Bartlett[15] and co-workers synthesized the first nucleosides with bases containing a phosphorus atom; however, these were cyclic phosphoramides and phosphonamides and not heterocycles containing phosphorus atoms in the λ^3 coordination state. We report the synthesis and antitumor activity of the first nucleoside with an aromatic phosphorus atom in the heterocyclic base.

Chemistry. In preliminary studies 1,2,4λ^3-diazaphosphole[16] (**4**) was glycosylated by using the triflate procedure and tetra-*O*-acetyl-D-ribose in 16% yield (Scheme I). Single-crystal X-ray analysis showed the structure to be 1-α-D-ribofuranosyl-1,2,4λ^3-diazaphosphole. No evidence of any β anomer was observed by ^{1}H NMR or TLC. In

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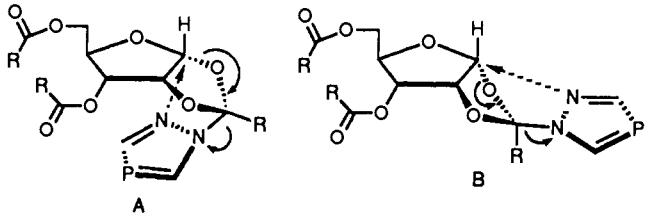
order to see if this novel finding was due to the acetyl protecting groups, the same reaction was carried out by using 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose. The benzoyl protecting group was used to study the effects of a participating group with a better ability to stabilize a carbocation. It was found that the benzoyl groups had no effect on the anomeric configuration and that the reaction only produced the α anomer. By way of comparison, when Witkowski[17] and co-workers glycosylated 1-(trimethylsilyl)-1,2,4-triazole by acid catalysis, they observed only the β anomer. To our knowledge this is the first example of exclusive formation of the α anomer by using D-ribofuranose protected with esters under Lewis acid glycosylation conditions.

One explanation for the observation stated above could involve an intramolecular mechanism. It is well-known that ester groups on the 2-OH of D-ribofuranose act as participating groups to stabilize the carbocation at the anomeric carbon through an ethylidene or benzylidene type intermediate.[18] This intermediate is responsible for the predominance of the β configuration in Lewis acid catalyzed glycosylations. There are reports, however, where "glycosylation" occurs on the carbonyl carbon of the protecting group to form an ortho amide type structure. This occurs with pyrrolo[2,3-*d*]pyrimidines, which are electronically dense ring systems.[19]

In the case of 1,2,4λ^3-diazaphosphole (4), the phosphorus heteroatom seems to contribute to the overall electron density of the heterocycle. This gives the heterocyclic nitrogens nucleophilicity that is probably on the order of that found with pyrrolo[2,3-*d*]pyrimidines or other pyrrole ring systems. Hence, 1,2,4λ^3-diazaphosphole probably forms ortho amide type intermediates similar to the ones shown in structures A and B which may be the kinetically favored products. The reason Witkowski and co-workers[17] observed only the β anomer with 1,2,4-triazole is that the heterocycle is not electron rich enough to form the ortho amide intermediate with the protecting group.

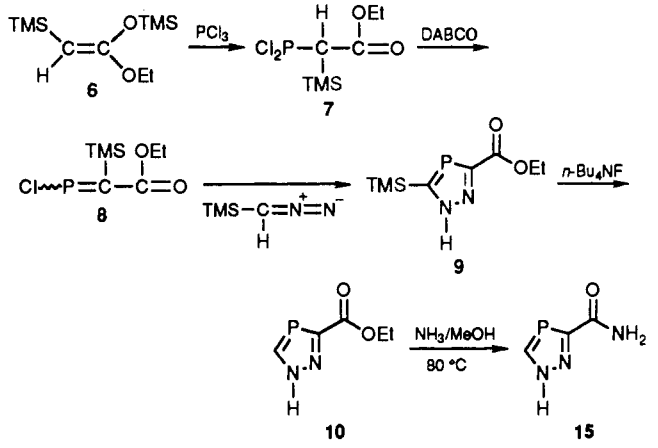


A B

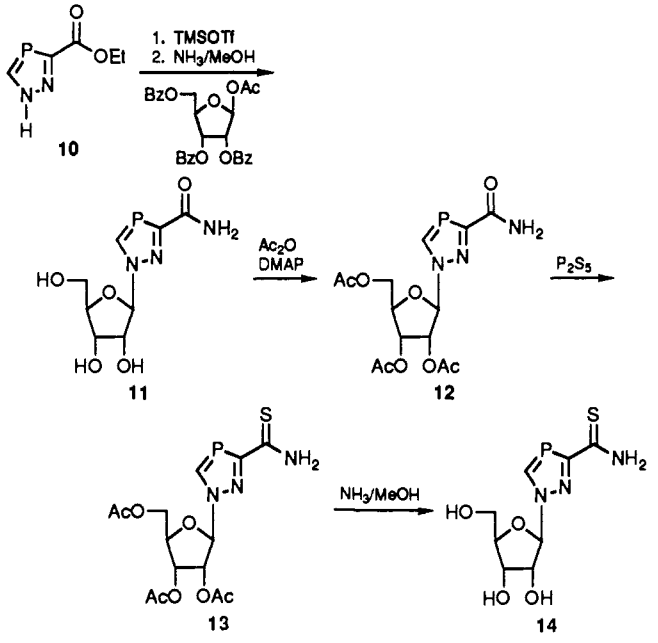
Examination of molecular models of these proposed intermediates shows that a five-membered ring transition state could be postulated, leading to a concerted acid-catalyzed intramolecular rearrangement with attack of N2 of the heterocycle on the anomeric carbon C1′. The reason this heterocycle could rearrange, where the pyrrole heterocycles would not, may be due to the nitrogen atom (N2) ortho to the nitrogen (N1) that makes the initial bond with the carbonyl carbon of the protecting group. Hence, this unexpected result is probably a special case in which the electron density of the heterocycle, due to the phosphorus heteroatom, is sufficient to cause initial attack on the protecting group followed by rearrangement to an ortho nitrogen heteroatom to give only the α anomer.

Ethyl 1,2,4λ^3-diazaphosphole-3-carboxylate was synthesized by a modification of the procedure of Pellon and

Scheme II



Scheme III



Hamelin.[20] [[1-Ethoxy-2-(trimethylsilyl)ethenyl]oxy]-trimethylsilane (6)[21] in an ethereal solution was cooled to between −30 and −40 °C and phosphorus trichloride added to give ethyl (dichlorophosphino)(trimethylsilyl)acetate (7) (Scheme II). Elimination of hydrogen chloride to give ethyl (chlorophosphinidene)(trimethylsilyl)acetate (8) was accomplished with diazabicyclooctane (DABCO) at −78 °C. Others[22-24] have shown that phosphaalkenes with a chlorine atom on the phosphorus and a trimethylsilyl group on the carbon α to the phosphorus atom are in fact phosphaalkyne equivalents which act as efficient 1,3-dipolarophiles and must be trapped in situ. Ethyl (chlorophosphinidene)(trimethylsilyl)acetate (8) was treated with a 10% solution of (trimethylsilyl)diazomethane in hexane.

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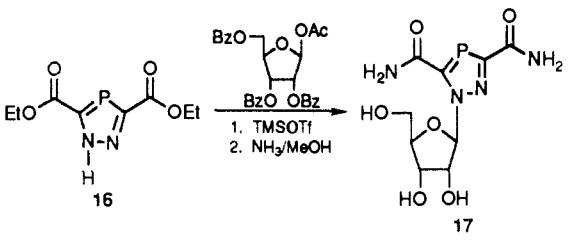
Scheme IV



Subsequent workup with water gave ethyl 5-(trimethylsilyl)-1,2,4λ^3-diazaphosphole-3-carboxylate (**9**). The silyl group was readily removed with tetra-*n*-butylammonium fluoride to give ethyl 1,2,4λ^3-diazaphosphole-3-carboxylate (**10**) in 43% yield with respect to the ketene acetal (**6**). Ethyl 1,2,4λ^3-diazaphosphole-3-carboxylate (**10**) was converted in quantitative yield to 1,2,4λ^3-diazaphosphole-3-carboxamide (**15**) by treating the ester **10** with methanolic ammonia in an autoclave at 80 °C. Ethyl 1,2,4λ^3-diazaphosphole-3-carboxylate (**10**) was glycosylated by using the triflate procedure and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (Scheme III). Treatment of the protected nucleoside with methanolic ammonia simultaneously deprotected the nucleoside and converted the ethyl ester to the amide to give 1-β-D-ribofuranosyl-1,2,4λ^3-diazaphosphole-3-carboxamide (**11**) in 44% yield with respect to ethyl 1,2,4λ^3-diazaphosphole-3-carboxylate (**10**). In contrast with 1,2,4λ^3-diazaphosphole, the glycosylation of **10** resulted in exclusively the β anomer with the glycosidic bond on N1. One can postulate that this is due to the electron-withdrawing effect of the carboxylate group. The reduction of electron density on the heterocyclic nitrogens of **10**, due to the carboxylate group, could preclude an initial attack on the ester protecting group by the heterocycle. Hence, the normal stabilization of the carbocation on the anomeric carbon of the ester-protected D-ribofuranose directed the glycosylation completely to the β anomer.

In order to synthesize 1-β-D-ribofuranosyl-1,2,4λ^3-diazaphosphole-3-thiocarboxamide (**14**) it was necessary to protect the corresponding carboxamide (**11**). Thus, 1-β-D-ribofuranosyl-1,2,4λ^3-diazaphosphole-3-carboxamide (**11**) was acetylated with acetic anhydride and 4-(dimethylamino)pyridine in 90% yield. The resulting triacetate (**12**) was then treated with phosphorus pentasulfide and 4-(dimethylamino)pyridine to give the protected thioamide **13** in 72% yield. Crystallization of 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4λ^3-diazaphosphole-3-thiocarboxamide (**13**) from 2/1 hexane/acetone gave crystals suitable for X-ray diffraction analysis. Although the structure of **11** was verified by single-crystal X-ray diffraction analysis, refinement was discontinued at $R = 0.19$ because of unexplained peaks in the electron density difference map, which were presumed to be randomly oriented solvent in the crystal lattice. Since **13** was derived from **11**, it was decided to use single-crystal X-ray analysis of **13** to indirectly confirm the structure of **11**. The acetyl protecting groups were removed with methanolic ammonia to give 1-β-D-ribofuranosyl-1,2,4λ^3-diazaphosphole-3-thiocarboxamide (**14**) in 62% yield.

1-β-D-Ribofuranosyl-1,2,4λ^3-diazaphosphole-3,5-dicarboxamide (**17**) was synthesized by glycosylating diethyl 1,2,4λ^3-diazaphosphole-3,5-dicarboxylate[20] (**16**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose by using trimethylsilyl trifluoromethanesulfonate as the Lewis acid catalyst (Scheme IV). The protected ester was deblocked with concomitant formation of the diamide **17** by treatment with methanolic ammonia at room temperature.

Table I. Crystal Data for Compounds **5** and **13**

	5	**13**
formula	$C_7H_{11}N_2O_4P$	$C_{14}H_{18}N_3O_7PS$
M_r	218.15	403.35
a, Å	6.0332 (13)	5.2140 (5)
b, Å	7.987 (2)	18.404 (2)
c, Å	20.330 (4)	10.1465 (16)
β, deg	90	100.866 (11)
V, Å^3	979.7 (4)	956.2 (2)
system	orthorhombic	monoclinic
space group	$P2_12_12_1$	$P2_1$
Z	4	2
wavelength, Å	1.54178	1.54178
R	0.0319	0.0390
reflections ($F \geq 4\sigma_F$)	1772	3792
total reflections	2044	3991

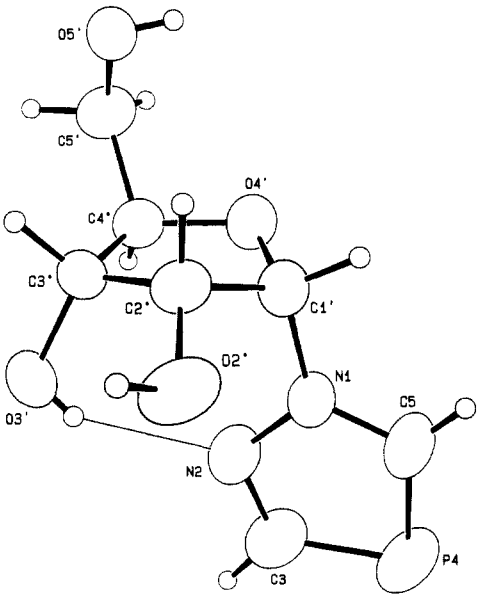


Figure 1. ORTEPII[25] drawing of **5** illustrating atom labeling and some hydrogen bonding. Thermal ellipsoids are drawn at the 50% probability level.

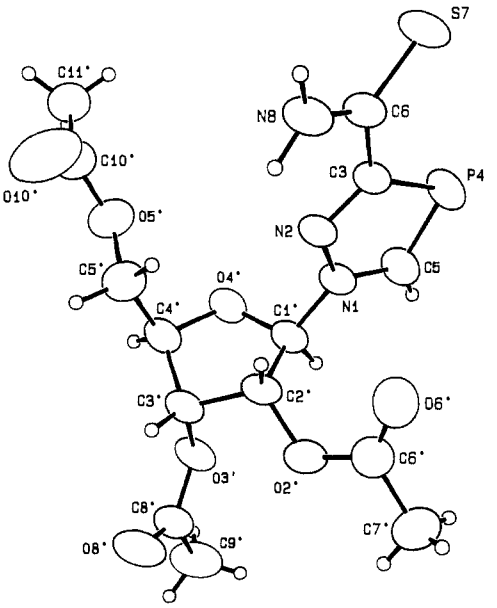


Figure 2. ORTEPII[25] drawing of **13** illustrating atom labeling. Thermal ellipsoids are drawn at the 50% probability level.

X-ray Diffraction Analysis. Crystallization of **5** by slow evaporation of an ethyl acetate solution produced long, rectangular colorless needles. Compound **13** crys-

tallized from a 2/1 hexane/acetone solution as yellow flat elongated plates. A summary of crystal data is given in Table I. ORTEPII[25] plots of **5** and **13** are shown in Figures 1 and 2, respectively.

The anomeric configuration of **5** is α. The sugar conformation is ${}_1T^2$ ($C_{1'}$-exo–$C_{2'}$-endo) with a pseudorotation angle (P) of 138.5° and amplitude of pucker (τ_m) of 32.7°.[26] This conformation combined with the glycosidic torsion angle O4′–C1′–N1–C5 of 113.1 (2)° results in an intramolecular hydrogen bond O3′–HO3′···N2 [d(O3′···N2) = 2.770 (2) Å]. The C4′–C5′ conformation is g^-g^+.

The blocked nucleoside **13** has the β-anomeric configuration. The sugar conformation is ${}_3T^2$ ($C_{3'}$-exo–$C_{2'}$-endo) with P = 192.4° and τ_m = 36.5°.[26] The side chain is gt. The glycosidic torsion angle O4′–C1′–N1–C5 is −111.9 (2)°, placing the base in an apparent syn conformation. Details of the structural studies will be published elsewhere.[27]

Biological Evaluation. In vivo assessments of antileukemic activity and host toxicity were performed as described previously.[28] Briefly, female BDF_1 mice purchased from Jackson Laboratories, Bar Harbor, ME, weighing approximately 20 g each, were housed under conditions of uniform caging, 75 °F temperature, 55% relative humidity, food and water ad libitum, and 12-h light periods starting with the light phase at 8:00 a.m. On day 0, the mice were inoculated ip with 10^6 L1210 ascites tumor cells, and on day 1 they were treated with compound. Control mice were injected with a 0.9% solution of NaCl ip. Solutions of the compounds were made immediately before injection and were delivered ip in volumes of 0.01 mL/g mouse weight.

The end points by which responses to treatment were gauged were the incidence of drug- or leukemia-related deaths and the postinoculation life span of mice that died. Temporal patterns of death and observations at necropsy examination were the major criteria for assigning deaths to leukemia or drug toxicity.

Given once at 480 mg/kg, 1-β-D-ribofuranosyl-1,2,4λ^3-diazaphosphole-3-thiocarboxamide (**14**) gave a 25% increase in life span (ILS) with weight changes of −1.67 and −0.67 g at 24 and 72 h, respectively. When **14** was given three times and two times at 288 mg/kg, all mice died. It was apparent from these data that the drug gave an effect only when pushed to near-lethal levels.

The ribavirin analogue 1-β-D-ribofuranosyl-1,2,4λ^3-diazaphosphole-3-carboxamide (**11**) was lethal in L1210-bearing female BDF_1 mice at 480 mg/kg and showed no activity when a dose-ranging study was done. Compound **11** did not show any in vitro antiviral activity when tested against a number of DNA and RNA viruses.

No in vitro antitumor activity was observed for compounds **11** and **14**. There were no detectable levels of 5′-phosphates observed in vitro. Thus, although their structures are similar to known IMP dehydrogenase inhibitors, compounds **11** and **14** are probably not IMP dehydrogenase inhibitors.

Experimental Section

General Procedures. Melting points were taken on a Haake–Buchler melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR and ^{31}P NMR) spectra were determined at 300 MHz with an IBM NR 300 FT-NMR spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard for ^{1}H NMR and phosphoric acid for ^{31}P NMR. Infrared spectra (IR) were obtained on a Perkin-Elmer 1420 spectrophotometer. Ultraviolet spectra (UV, sh = shoulder) were recorded on a Beckman Model DU-50 spectrophotometer. Elemental analyses were performed by Robertson Labs, Madison, NJ. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (EM reagents). ICN Biomedicals silica gel (32–63 μm, 60 Å) was used for flash chromatography. Detection of components on TLC was by UV light and with 10% sulfuric acid in methanol spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 35 °C.

1-α-D-**Ribofuranosyl-1,2,4λ^3-diazaphosphole (5). Method A.** A suspension of 1,2,4λ^3-diazaphosphole (**4**)[16] (500 mg, 5.81 mmol) in hexamethyldisilazane (100 mL) with a catalytic amount of ammonium sulfate (10 mg) was refluxed for 16 h. The hexamethyldisilazane was removed in vacuo and the residue dissolved in acetonitrile. To this solution were added 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose (1.85 g, 5.81 mmol) and trimethylsilyl trifluoromethanesulfonate (1.86 g, 8.37 mmol). The reaction was allowed to stir at room temperature for 8 h, after which the acetonitrile was removed and the residue dissolved in ethyl acetate and washed with saturated sodium bicarbonate. The organic layer was dried over sodium sulfate and adsorbed onto silica gel. The solvent was removed in vacuo and the resultant powder placed on a flash column and eluted with 1/1 hexane/ether to yield 1.0 g of a clear colorless syrup. This syrup was dissolved in methanol, to which solution sodium methoxide (150 mg) was added. This solution was allowed to stir for 4 h. Silica gel was added to the mixture and the solvent removed. The resultant powder was placed on a flash column and eluted with 95/5 chloroform/methanol to yield 200 mg (16%) of a syrup which crystallized spontaneously upon standing. Recrystallization from ethyl acetate gave X-ray-quality crystals: mp 140–141 °C; ^{1}H NMR (DMSO-d_6) δ 3.50 (m, 1, C_5H), 3.58 (m, 1, C_5H), 4.08 (q, 1, J = 5.45 Hz, C_4H), 4.16 (q, 1, J = 4.06 Hz, C_3H), 4.34 (q, 1, J = 5.2 Hz, C_2H), 4.88 (t, 1, J = 5.2 Hz, 5′-OH), 5.27 (d, 1, J = 5.64 Hz, 3′-OH), 5.33 (d, 1, J = 6.87 Hz, 2′-OH), 6.29 (d, 1, J = 5.28 Hz, C_1H), 8.72 (d, 1, J = 47.63 Hz, C_3H), 9.13 (d, 1, J = 41.51 Hz, C_5H). Anal. ($C_7H_{11}N_2O_4P$) C, H, N, P.

Method B. 1-(2,3,5-Tri-*O*-benzoyl-α-D-ribofuranosyl)-1,2,4λ^3-diazaphosphole was obtained by using identical conditions as above except that 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose was used instead of 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose. Deprotection of the benzoyl groups with sodium methoxide as in method A gave a product identical in all respects with that obtained above.

Ethyl 1,2,4λ^3-Diazaphosphole-3-carboxylate (10). A solution of [[1-ethoxy-2-(trimethylsilyl)ethenyl]oxy]trimethylsilane[21] (**6**) (30.5 g, 131.20 mmol) dissolved in anhydrous ether was cooled to between −30 and −40 °C. To this solution was added freshly distilled PCl_3 (11.44 mL), and the solution was allowed to stir for 3 h at −30 °C. The clear pale yellow reaction mixture was cooled to −78 °C, and diazabicyclooctane (DABCO) (14.8 g, 131 mmol) dissolved in ether was added dropwise. After addition of the DABCO the suspension was allowed to stir for 15 min. To this suspension was added (trimethylsilyl)diazomethane (Petrarch, 10% solution in hexane, 95 g) over a period of 5 min. Stirring was continued at −50 to −60 °C for 2.5 h. The mixture was then warmed to room temperature and allowed to stir for 14 h. Water was added carefully and the organic layer kept and dried over sodium sulfate. The ether was removed in vacuo, yielding 18 g of a yellow solid. Purification of this solid on a flash column using 1/1 hexane/ether would give a white solid with mp 129.5–131.5 °C. This white solid was a mixture of ethyl 5-(trimethylsilyl)-1,2,4λ^3-diazaphosphole-3-carboxylate (**9**) and ethyl 1,2,4λ^3-diazaphosphole-3-carboxylate (**10**). These two compounds could not be separated readily by flash chromatography. The crude solid from the ether extract was normally treated with excess tetrabutylammonium fluoride in tetrahydrofuran. This mixture was stirred for 1.5 h and the clear orange solution evaporated onto silica and loaded on a flash column. The column was eluted with 4/1 hexane/acetone to give 10.8 g of pure ethyl 1,2,4λ^3-diaza-

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phosphole-3-carboxylate (10) (43% yield with respect to the starting ketene acetal), which crystallized spontaneously from the collection tubes: mp 185–187 °C; ^{1}H NMR (DMSO-d_6) δ 2.49 (t, 3, J = 7.67 Hz, $-CH_3$), 4.49 (q, 2, J = 7.12 Hz, CH_2), 9.12 (d, 1, J = 41.06 Hz, C_5H), 9.2 (br s, 1, NH); ^{31}P NMR (DMSO-d_6) δ −25.19. Anal. ($C_5H_7N_2O_2P$) C, H, N, P.

1-β-D-Ribofuranosyl-1,2,4λ^3-diazaphosphole-3-carboxamide (11). Ethyl 1,2,4λ^3-diazaphosphole-3-carboxylate (10) (1.6 g, 8.6 mmol) was suspended in hexamethyldisilazane (HMDS) for 4.5 h with a catalytic amount of ammonium sulfate. The HMDS was removed and the residue dissolved in dry acetonitrile. To this mixture was added 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose (4.34 g, 8.59 mmol) and trimethylsilyl trifluoromethanesulfonate (2.4 mL, 12.4 mmol). The reaction mixture was allowed to stir for 16 h after which the acetonitrile was removed and the residue taken up in chloroform. The chloroform mixture was washed with saturated sodium bicarbonate and the chloroform layer dried over sodium sulfate. Removal of the chloroform gave a golden syrup, which was treated with methanolic ammonia for 24 h. Silica gel was added to the mixture and the solvent removed to give a dry powder. This powder was placed on a silica gel flash column and eluted with 4/1 chloroform/methanol to yield 1.0 g (44%) of 1-β-D-ribofuranosyl-1,2,4λ^3-diazaphosphole-3-carboxamide (11): mp 124–125 °C; UV λ_{max} (pH 1) 238 nm (ϵ 7483), 270 nm (ϵ 3423), 300 nm (sh, ϵ 1751), (pH 7) 244 nm (ϵ 6157), 271 nm (sh, ϵ 3422), pH 11) 244 nm (ϵ 5751), 270 nm (sh, ϵ 3024); ^{1}H NMR (DMSO-d_6) δ 3.56 (m, 1, C_5H), 3.66 (m, 1, C_5H), 3.96 (q, 1, J = 4.45 Hz, C_4H), 4.14 (q, 1, J = 5.13 Hz, C_3H), 4.35 (q, 1, J = 4.74 Hz, C_2H), 5.00 (t, 1, J = 5.42 Hz, 5′-OH), 5.16 (d, 1, J = 5.64 Hz, 3′-OH), 5.57 (d, 1, J = 5.67 Hz, 2′-OH), 5.90 (d, 1, J = 3.81 Hz, C_1H), 7.44 (s, 1, NH_2), 7.62 (s, 1, NH_2), 9.32 (d, 1, J = 39.17 Hz, C_5H); ^{31}P NMR (DMSO-d_6) δ −26.85 (d, J = 39.23 Hz). Anal. ($C_8H_{12}N_3O_5P$) C, H, N, P.

1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4λ^3-diazaphosphole-3-carboxamide (12). To a suspension of the nucleoside 11 (1.2 g, 4.59 mmol) in acetic anhydride was added 4-(dimethylamino)pyridine (DMAP). This was allowed to stir for 16 h, after which time the excess acetic anhydride was removed and the residue taken up in chloroform. The chloroform solution was washed with saturated sodium bicarbonate, 1 N hydrochloric acid, and water. The chloroform was then dried over sodium sulfate. Upon removal of the chloroform the residue crystallized spontaneously to give 1.57 g (88%) of 12: mp 137–139 °C. Anal. ($C_{14}H_{18}N_3O_8P$) C, H, N, P.

1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4λ^3-diazaphosphole-3-thiocarboxamide (13). To a solution of 12 (4.6 g, 11.88 mmol) in freshly purified dioxane (150 mL) were added DMAP (60 mg) and phosphorus pentasulfide (1.2 g). The reaction was refluxed for 1 h after which TLC in 4/1 chloroform/acetone indicated that the reaction was incomplete. More phosphorus pentasulfide (1.1 g) was added and the mixture refluxed for 45 min longer. Decolorizing carbon was added and the mixture filtered. The filter was washed with chloroform, and the combined organic layers were taken to dryness. The resultant orange syrup was taken up in a fresh portion of chloroform and washed with saturated sodium bicarbonate solution. The organic phase was dried over sodium sulfate. The sodium sulfate was filtered, and silica gel was added and the solvent removed. The resultant powder was placed on a flash column and eluted with 2/1 hexane/acetone to give 3.5 g (72%) of 13. The compound crystallized from the eluant: mp 136.5–137.5 °C. Anal. ($C_{14}H_{18}N_3O_7PS$) C, H, N, P, S.

1-β-D-Ribofuranosyl-1,2,4λ^3-diazaphosphole-3-thiocarboxamide (14). 1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4λ^3-diazaphosphole-3-thiocarboxamide (13) (3.5 g, 8.67 mmol) was dissolved in methanolic ammonia (100 mL) and allowed to stir at room temperature for 16 h. Silica gel was added and the solvent removed. The dry powder was placed on a flash column and eluted with 6/1 chloroform/methanol. The fractions containing product were pooled and the solvent removed. The resulting syrup was triturated with ether to give 1.5 g (62%) of a yellow solid: mp 165–166 °C dec; UV λ_{max} (pH 1, 7, 11) 227 nm (ϵ 16 054), 256 nm (ϵ 6527), 290 nm (ϵ 5922), 319 nm (ϵ 5998); ^{1}H NMR (DMSO-d_6) δ 3.53 (m, 1, C_5H), 3.65 (m, 1, C_5H), 3.95 (d, 1, J = 4.29 Hz, C_4H), 4.14 (d, 1, J = 4.80 Hz, C_3H), 4.37 (d, 1, J = 4.32 Hz, C_2H), 4.98 (t, 1, J = 5.04 Hz, 5′-OH), 5.15 (d, 1, J = 5.34 Hz, 3′-OH), 5.55 (d, 1, J = 5.37 Hz, 2′-OH), 5.85 (d, 1, J = 3.54 Hz, C_1H), 9.21 (d, 1, J = 38.12 Hz, C_5H), 9.34 (s, 1, $CSNH_2$), 9.78 (s, 1, $CSNH_2$); ^{31}P NMR (DMSO-d_6) δ −21.93 (d, J = 38.14 Hz). Anal. ($C_8H_{12}N_3O_4PS$) C, H, N, P, S.

1,2,4λ^3-Diazaphosphole-3-carboxamide (15). Ethyl 1,2,4λ^3-diazaphosphole-3-carboxylate (10) (315 mg) was dissolved in methanolic ammonia (50 mL) and placed in an autoclave. The autoclave was heated at 80 °C for 24 h, after which time the conversion was quantitative. The solvent was removed in vacuo and the resulting residue crystallized spontaneously: mp 116–118 °C; UV λ_{max} (pH 1) 242 nm (ϵ 4740), 266 nm (ϵ 2979), (pH 7) 241 nm (ϵ 5026), 266 nm (ϵ 3361), (pH 11) 273 nm (ϵ 5066); ^{1}H NMR (DMSO-d_6) δ 7.36 (s, 1, NH_2), 7.66 (s, 1 NH_2), 9.04 (d, 1, J = 40.22 Hz, C_5H), 14.4 (br s, 1, NH). Anal. ($C_3H_4N_3OP$) C, H, N, P.

1-β-D-Ribofuranosyl-1,2,4λ^3-diazaphosphole-3,5-dicarboxamide (17). A suspension of diethyl 1,2,4λ^3-diazaphosphole-3,5-dicarboxylate[20] (16) (730 mg, 3.17 mmol) in HMDS was refluxed for 3.5 h. The HMDS was removed in vacuo and the residue dissolved in acetonitrile. To this mixture was added 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribose (1.8 g) and trimethylsilyl trifluoromethanesulfonate (0.9 mL). After the reaction mixture was stirred for 24 h, the acetonitrile was removed and the residue dissolved in ethyl acetate. The ethyl acetate was washed with saturated sodium bicarbonate solution. The organic layer was then dried over sodium sulfate, after which the solvent was removed to give a syrup. This syrup was dissolved in methanolic ammonia (100 mL). After the solution was stirred for 16 h, silica gel was added to the reaction mixture and the slurry evaporated to dryness. The dry powder was placed on a flash column and the column eluted with 7/1/1/1 ethyl acetate/acetone/methanol/water. Fractions containing 17 were pooled and the solvent removed. Recrystallization of 17 from ethanol gave a white solid: mp 191–192 °C; ^{1}H NMR (DMSO-d_6) δ 3.48 (m, 1, C_5H), 3.58 (m, 1, C_5H), 3.87 (q, 1, J = 5.26 Hz, C_4H), 4.30 (q, 1, J = 5.48 Hz, C_3H), 4.58 (m, 1, C_2H), 4.71 (t, 1, J = 5.68 Hz, 5′-OH), 5.02 (d, 1, J = 6.24 Hz, 3′-OH), 5.48 (d, 1, J = 5.73 Hz, 2′-OH), 6.36 (d, 1, J = 3.21 Hz, C_1H), 7.55 (s, 1, NH_2), 7.64 (s, 1, NH_2), 7.93 (s, 1, NH_2), 8.29 (s, 1, NH_2). Anal. ($C_9H_{13}N_4O_6P$) C, H, N, P.

Registry No. 4, 42226-36-8; 5, 123486-54-4; 5 tribenzoate, 123486-63-5; 5 triacetate, 123486-53-3; 6, 65946-56-7; 9, 123486-55-5; 10, 123486-56-6; 10 ribofuranosyl tribenzoate derivative, 123486-64-6; 11, 123486-57-7; 12, 123486-58-8; 13, 123486-59-9; 14, 123486-60-2; 15, 123486-61-3; 16, 109541-66-4; 16 ribofuranosyl tribenzoate derivative, 123486-65-7; 17, 123486-62-4; 1,2,3,5-tetra-O-acetyl-D-ribofuranose, 28708-32-9; 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose, 14215-97-5; (trimethylsilyl)diazomethane, 18107-18-1.