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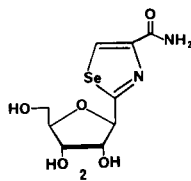
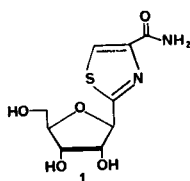
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A new C-glycosyl precursor for C-nucleoside synthesis, 2,5-anhydroallonamidinium hydrochloride (**4**) was prepared and utilized in a Traube type synthesis to prepare 2-(β -D-ribofuranosyl)pyrimidines, a new class of C-nucleosides. The anomeric configuration of **4** was confirmed by single-crystal X-ray analysis. Reaction of **4** with ethyl acetoacetate gave 6-methyl-2-(β -D-ribofuranosyl)pyrimidin-4(1*H*)-one (**5**). Reaction of **4** with diethyl sodio oxaloacetate gave 2-(β -D-ribofuranosyl)pyrimidin-6(1*H*)-oxo-4-carboxylic acid (**6**). Esterification of **6** with ethanolic hydrogen-chloride gave the corresponding ester **7** which when treated with ethanolic ammonia gave 2-(β -D-ribofuranosyl)pyrimidin-6(1*H*)-oxo-4-carboxamide (**8**). Condensation of 2,5-anhydroallonamidinium hydrochloride (**4**) with ethyl 4-(dimethylamino)-2-oxo-3-butenate (**9**), gave ethyl 2-(β -D-ribofuranosyl)pyrimidine-4-carboxylate (**10**). Treatment of **10** with ethanolic ammonia gave 2-(β -D-ribofuranosyl)pyrimidine-4-carboxamide (**11**). Single-crystal X-ray analysis confirmed the β -anomeric configuration of **11**. Acetylation of **11** followed by treatment with phosphorus pentasulfide and subsequent deprotection with sodium methoxide gave 2-(β -D-ribofuranosyl)pyrimidine-4-thiocarboxamide (**14**). Dehydration of the acetylated amide **12** with phosphorous oxychloride provided 2-(β -D-ribofuranosyl)pyrimidine-4-carbonitrile (**15**). Treatment of **15** with sodium ethoxide gave ethyl 2-(β -D-ribofuranosyl)pyrimidine-4-carboximidate (**16**), which was converted to 2-(β -D-ribofuranosyl)pyrimidine-4-carboxamidinium hydrochloride (**17**) by treatment with ethanolic ammonia and ammonium chloride. Treatment of **16** with hydroxylamine yielded 2-(β -D-ribofuranosyl)pyrimidine-4-*N*-hydroxycarboxamidinium (**18**). Treatment of 2-(β -D-ribofuranosyl)pyrimidine-4-carboxamide (**11**) with phosphorus oxychloride gave the corresponding 5'-phosphate, **19**. Coupling of **19** with AMP using the carbonyldiimidazole activation procedure gave the corresponding NAD analog, 2-(β -D-ribofuranosyl)pyrimidine-4-carboxamide-(5' $\rightleftharpoons$ 5')-adenosine pyrophosphate (**20**).

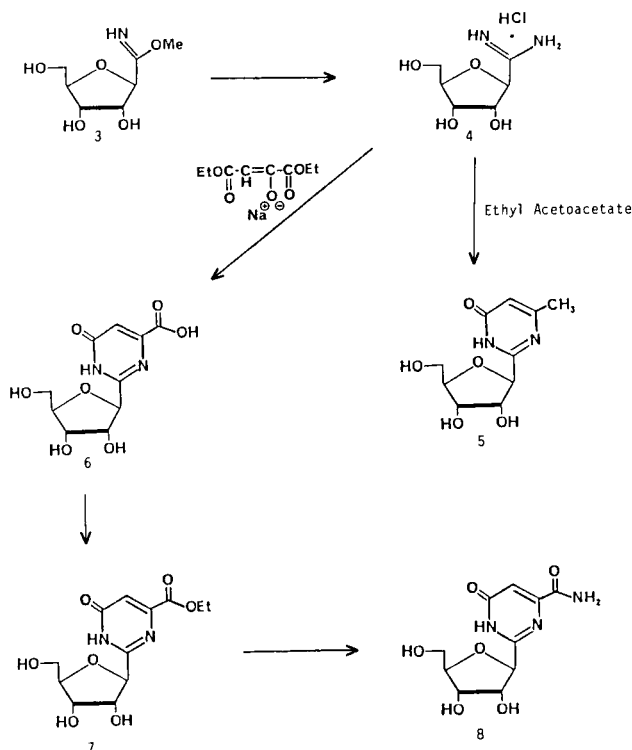
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Introduction.

Two carbon linked nucleosides synthesized by Robins and coworkers, tiazofurin [2] (**1**) and selenazofurin [3] (**2**), have been shown to be effective against L1210 leukemia and Lewis lung carcinoma in mice [4,5,6]. The antitumor activity of **1** and **2** is believed to be due to the *in vivo* conversion of **1** and **2** to the corresponding nicotinamide adenine dinucleotide (NAD) analogs [7,8]. For a review of the biochemistry and mechanism of antitumor action of tiazofurin (**1**) and selenazofurin (**2**), the reader is referred to a recent review [9]. In an effort to prepare compounds with greater antitumor activity, it was decided to synthesize 6-membered *N*-heterocyclic analogs of **1** and **2**.



Relatively few six-membered heterocyclic C-nucleosides have been isolated or synthesized. Whereas Fox and co-



workers have prepared 5-(β -D-ribofuranosyl)pyrimidines [10] and 6-(β -D-ribofuranosyl)pyrimidines [11], to our knowledge no pyrimidine *C*-nucleosides have been prepared with the glycosyl moiety at C-2. We report here the first synthesis of 2-(β -D-ribofuranosyl)pyrimidines that was accomplished by utilizing a new glycosyl amidine, 2,5-anhydro-D-allonamidine hydrochloride (**4**), in a series of ring-closure procedures. This functionalized *C*-glycoside has advantages over those previously used for the synthesis of pyrimidine *C*-nucleosides. Protecting groups were not required for the synthesis of 2,5-anhydro-D-allonamidine hydrochloride (**4**) nor were protecting groups required for direct utilization of **4** in ring-closure procedures.

A ring closure was developed to obtain a carboxamide function at C-4 of the pyrimidine ring *without* substituents at C-5 or C-6. This procedure led to the synthesis of a 6-membered *N*-heterocyclic carbon-linked nucleoside analog of nicotinamide mononucleoside, **11**, as well as the corresponding NAD analog, **20**, which is the first example of replacement of the nicotinamide moiety of NAD with a 6-membered *N*-heterocyclic *C*-nucleoside.

The synthesis of 2-(β -D-ribofuranosyl)pyrimidines required a new, easily obtainable *C*-glycosyl precursor that would allow the use of the traditional Traube synthesis. Poonian and Nowoswiat [12], who synthesized methyl 2,5-anhydro-D-allonimidate (**3**), reported the synthesis of **4**

in a communication [13]. We report the detailed synthesis and characterization of 2,5-anhydro-D-allonamidine hydrochloride (**4**) including its single-crystal X-ray analysis. The amidine **4** was prepared by reacting **3** with methanolic ammonia or ethanolic ammonia in the presence of one equivalent of ammonium chloride to give 2,5-anhydro-D-allonamidine hydrochloride (**4**) in nearly quantitative yield.

Snyder and Foster [14] condensed ethyl acetoacetate with acetamidine to give 2,6-dimethyl-pyrimidin-4(3*H*)-one. 2,5-Anhydro-D-allonamidine hydrochloride (**4**) was condensed with ethyl acetoacetate in ethanolic sodium hydroxide for twenty-four hours at room temperature to give 6-methyl-2-(β -D-ribofuranosyl)pyrimidin-4(2*H*)-one (**5**) as a mixture of the α - and β -anomers in 24% and 48% yields. The anomeric structures were assigned by the coupling constant and shift of the anomeric protons [13]. The α -anomeric proton had a larger coupling constant and was shifted further downfield than the corresponding β -anomer, as is the usual case for α - and β -anomers in the D-ribofura-

Table 1
Crystal and Structure Solution Data for **4** and **11**

	4	11
Formula	[C ₆ H ₁₃ N ₂ O ₄] ⁺ Cl ⁻	C ₁₀ H ₁₃ N ₃ O ₅
M _r	212.63	255.2
F(000)	452	536
Crystal Size (mm)	.64 × .39 × .29	.4 × .1 × .08
Wavelength (Å)	0.71069	1.54178
μ (cm ⁻¹)	3.949	10.0
Crystal System	Orthorhombic	Orthorhombic
Space Group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
<i>a</i> (Å)	6.327(2)	7.806(2)
<i>b</i> (Å)	8.144(3)	7.900(2)
<i>c</i> (Å)	18.126(6)	18.279(5)
<i>V</i> (Å ³)	934.0(5)	1127.2(5)
<i>Z</i>	4	4
<i>d</i> _{meas} (g/cc)	1.511	not measured
<i>d</i> _{calc} (g/cc)	1.512	1.504
Total Reflections	1579	1109
Observed Reflections used	1494 (<i>F</i> ≥ 4 σ_F)	1005 (<i>F</i> ≥ 3 σ_F)
Maximum Sin (θ/λ)	0.70	0.55
<i>R</i>	0.029	0.046
<i>R</i> _w	0.042	0.064
ρ maximum in ΔF map	0.24	0.25
ρ minimum in ΔF map	-0.21	-0.21
2 θ range for cell parameters	22 - 30	9 - 46
Decay correction	1.000 - 1.004	0.996 - 1.019
Weights, <i>w</i> ⁻¹	$\sigma^2 + .0004F^2$	$\sigma^2 + .001F^2$
No. of variables	171	192
<i>S</i> (goodness of fit)	1.588	1.385
Extinction Parameter (× 10 ⁻⁶)	1.89(12)	8(3)
Maximum Δ/σ	0.018	0.20

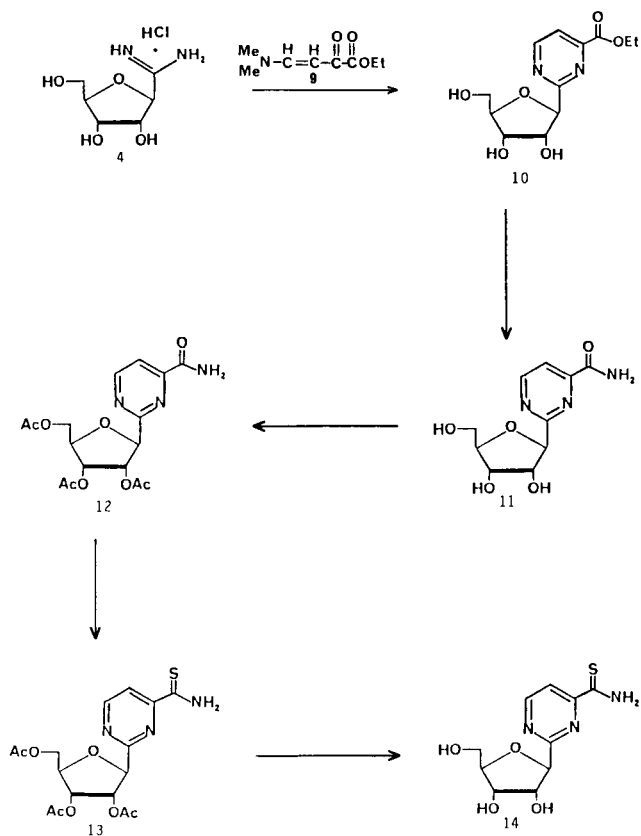


Table 2

Positional Parameters in Fractional Coordinates and U_{eq}/U [a] for **4**

atom	x/a	y/b	z/c	U_{eq}/U
Cl	.51585(8)	.15585(7)	.66733(3)	.04409(14)
C1	1.0307(3)	.3791(2)	.88608(9)	.0298(4)
N1	1.2285(3)	.3449(3)	.89742(11)	.0448(5)
N2	.9671(3)	.5182(2)	.85722(10)	.0389(5)
C1'	.8670(3)	.2486(2)	.90256(9)	.0277(4)
C2'	.8238(3)	.1467(2)	.83299(9)	.0268(4)
C3'	.9727(3)	-.0009(2)	.84447(8)	.0271(4)
C4'	.9680(3)	-.0239(2)	.92799(9)	.0302(4)
C5'	1.1614(4)	-.1006(3)	.96292(11)	.0416(6)
O1'	.9478(2)	.14001(15)	.95734(6)	.0335(3)
O2'	.6106(2)	.0921(2)	.83337(9)	.0370(4)
O3'	.9145(3)	-.1423(2)	.80427(8)	.0362(4)
O5'	1.3443(3)	-.0019(3)	.95273(10)	.0546(6)
HN1A	1.264(5)	.249(3)	.9208(15)	.049(7)
HN1B	1.328(8)	.417(5)	.888(2)	.091(12)
HN2A	1.048(6)	.570(5)	.839(2)	.073(11)
HN2B	.839(5)	.536(3)	.8522(14)	.045(7)
H1'	.733(3)	.305(3)	.9197(11)	.022(4)
H2'	.863(3)	.208(3)	.7887(11)	.025(5)
H3'	1.116(3)	.028(2)	.8303(11)	.023(4)
H4'	.838(4)	-.088(3)	.9415(12)	.033(6)
H5'A	1.142(5)	-.117(3)	1.013(2)	.049(7)
H5'B	1.170(5)	-.219(4)	.9425(15)	.057(8)
HO2'	.567(5)	.101(4)	.797(2)	.051(8)
HO3'	.790(5)	-.156(4)	.811(2)	.058(9)
HO5'	1.396(6)	-.037(5)	.923(2)	.068(11)

[a] Listed thermal parameters for non-hydrogen atoms are U_{eq} defined as $U_{eq} = \frac{1}{3} \sum_i \sum_j U_{ij} a_i^* a_j^* A_{ij}$ where A_{ij} is the dot product of the i^{th} and j^{th} direct-space unit-cell vectors.

nose series [15]. Anomerization probably occurred because of the heat from the Soxhlet extraction with ethanol and the basic nature of the paste that was extracted.

Relatively few reports of pyrimidine C-4 carboxylate formation by a direct synthetic method are in the literature. The ring closure reported by Robins and co-workers [16] employing formamidinium acetate and diethyl sodio oxaloacetate to give pyrimidin-6(1*H*)-oxo-4-carboxylic acid served as a model for the synthesis of 2-(β -D-ribofuranosyl)pyrimidin-6(1*H*)-oxo-4-carboxylic acid (**6**). Reaction of diethyl sodio oxaloacetate and **4** in one equivalent of 1 *N* sodium hydroxide gave **6** in 63% yield. 2-(β -D-ribofuranosyl)pyrimidin-6(1*H*)-oxo-4-carboxylic acid (**6**) was esterified with ethanol saturated with hydrogen chloride gas and triethyl orthoformate to give ethyl 2-(β -D-ribofuranosyl)pyrimidin-6(1*H*)-oxo-4-carboxylate (**7**) in 54% yield. Attempts to form **7** directly by reaction of 2,5-anhydro-D-allonamide hydrochloride (**4**) and diethyl sodio oxaloacetate in absolute ethanol were unsuccessful. Ethyl 2-(β -D-ribofura-

nosyl)pyrimidin-6(1*H*)-oxo-4-carboxylate (**7**) was dissolved in ethanolic ammonia and allowed to stir overnight to give 2-(β -D-ribofuranosyl)pyrimidin-6(1*H*)-oxo-4-carboxamide (**8**) in quantitative yield.

Bredereck and coworkers [17] condensed 4-(dimethylamino)-2-oxo-3-butenal-1-(diethyl acetal) with acetamidine to give 2-methyl-pyrimidine-4-carboxaldehyde diethyl acetal. Following this precedent for the use of enamines in pyrimidine synthesis, ethyl 4-(dimethylamino)-2-oxo-3-butenate (**9**) was condensed with the new C-glycosyl amidine **4** to give the pyrimidine C-4 carboxylate with a glycosyl moiety attached at C-2. Gompper and Sobotta [18] reported the synthesis of ethyl 4-(dimethylamino)-2-oxo-3-butenate (**9**) in a communication. The condensation of dimethylformamide dimethyl acetal and ethyl pyruvate gave ethyl 4-(dimethylamino)-2-oxo-3-butenate (**9**) in 55% yield.

Table 3

Positional Parameters in Fractional Coordinates and U_{eq}/U [a] for **11**

atom	x/a	y/b	z/c	U_{eq}/U
N1	.2694(5)	-.0790(4)	.7468(2)	.050(1)
C2	.2048(5)	-.1968(5)	.7911(2)	.037(1)
N3	.2027(4)	-.3625(4)	.7794(2)	.038(1)
C4	.2726(5)	-.4163(5)	.7168(2)	.039(1)
C	.2733(5)	-.6032(5)	.7012(2)	.042(1)
O	.3288(5)	-.6547(4)	.6420(2)	.063(1)
N	.2129(5)	-.7001(4)	.7535(2)	.050(1)
C5	.3452(7)	-.3054(6)	.6675(2)	.050(1)
C6	.3403(7)	-.1367(5)	.6847(2)	.058(2)
C1'	.1334(5)	-.1418(5)	.8638(2)	.035(1)
C2'	.2581(5)	-.1658(5)	.9269(2)	.036(1)
C3'	.1995(5)	-.0338(5)	.9831(2)	.039(1)
C4'	.0944(6)	.0926(5)	.9384(2)	.040(1)
C5'	.1662(8)	.2683(5)	.9429(2)	.057(2)
O1'	.1013(4)	.0361(3)	.8633(1)	.044(1)
O2'	.2560(5)	-.3321(3)	.9544(1)	.051(1)
O3'	.0905(4)	-.1052(4)	1.0374(1)	.048(1)
O5'	.0652(5)	.3807(4)	.9035(2)	.068(1)
HNA	.187(8)	-.653(7)	.798(3)	.09(2)
HNB	.218(6)	-.820(7)	.753(2)	.066(14)
H5	.399	-.344	.624	.056(13)
H6	.380	-.042	.651	.066(14)
H1'	.030	-.212	.871	.047(12)
H2'	.374	-.143	.906	.030(9)
H3'	.304	.005	1.007	.043(11)
H4'	-.033	.084	.955	.043(11)
H5'A	.270	.291	.920	.009(7)
H5'B	.149	.303	.997	.034(10)
HO2'	.362(17)	-.406(16)	.953(6)	.26(4)
HO3'	.155(12)	-.194(10)	1.056(4)	.15(3)
HO5'	.153(25)	.457(25)	.926(14)	.62(6)

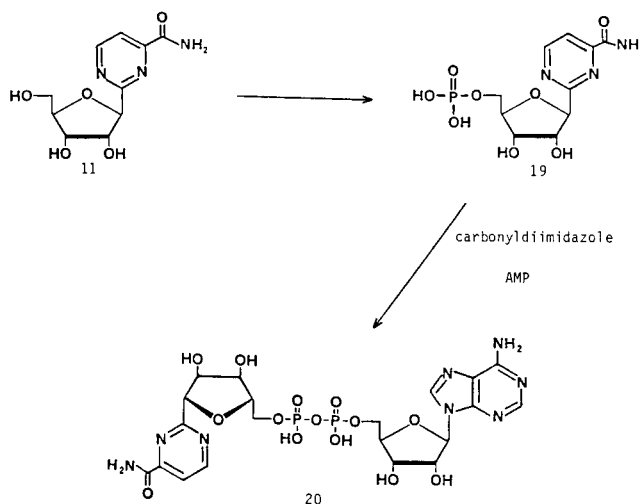
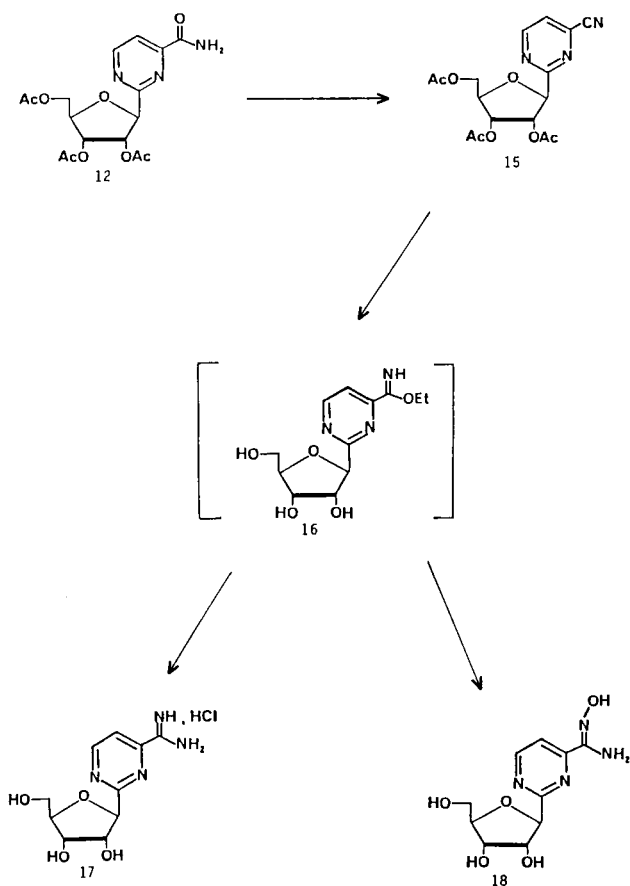
[a] Listed thermal parameters for non-hydrogen atoms are U_{eq} defined as $U_{eq} = \frac{1}{3} \sum_i \sum_j U_{ij} a_i^* a_j^* A_{ij}$ where A_{ij} is the dot product of the i^{th} and j^{th} direct-space unit-cell vectors.

Compound **9** provided the properly functionalized three carbon piece requisite for the synthesis of a pyrimidine-4-carboxylate. Reaction of ethyl 4-(dimethylamino)-2-oxo-3-butenolate (**9**) and 2,5-anhydro-D-allonamidinium hydrochloride (**4**) with one equivalent of sodium ethoxide at 50° gave ethyl 2-(β -D-ribofuranosyl)pyrimidine-4-carboxylate (**10**) in 41% isolated yield and in an overall yield of 40%. By comparison, pseudoisocytidine and 2-(β -D-ribofuranosyl)isocytosine were prepared in 6% and 19% overall yields, respectively [10,11]. We report a direct ring-closure procedure which gives pyrimidine-4-carboxylate that is unsubstituted at C-5 and C-6. By way of comparison Reiner and Eugster [19] reported condensing ethyl 4-ethoxy-2-oxo-3-butenolate with acetamidinium to give ethyl 2-methyl-pyrimidine-4-carboxylates; however, no yield was reported and no analytical data given except a melting point for the corresponding acid.

Reaction of **10** with ethanolic ammonia overnight at room temperature gave 2-(β -D-ribofuranosyl)pyrimidine-4-carboxamide (**11**) in 83% yield. The preparation of 2-(β -D-ribofuranosyl)pyrimidine-4-carboxamide (**11**) from **4** is unique for several reasons. It is a six-membered heterocyclic *C*-nucleoside nicotinamide mononucleoside analog. The preparation of ethyl 2-(β -D-ribofuranosyl)pyrimidine-

4-carboxylate (**10**), which is the precursor to **11**, is the first reported synthesis of a six-membered heterocyclic ring *C*-nucleoside with a carboxylate function. The nucleoside **11** was employed for the synthesis of other nucleoside derivatives. Acetylation of **11** with acetic anhydride, pyridine, and 4-dimethylaminopyridine (DMAP) gave 2',3',5'-tri-*O*-acetyl-2-(β -D-ribofuranosyl)pyrimidine-4-carboxamide (**12**) in 95% yield. Compound **12** was then treated with phosphorus pentasulfide in refluxing dioxane with a catalytic amount of 4-dimethylaminopyridine to give 2',3',5'-tri-*O*-acetyl-2-(β -D-ribofuranosyl)pyrimidine-4-thiocarboxamide (**13**) in 42% yield. The acetylated thioamide **13** was deprotected using sodium methoxide at room temperature to give 2-(β -D-ribofuranosyl)pyrimidine-4-thiocarboxamide (**14**) in 60% yield. 2',3',5'-Tri-*O*-acetyl-2-(β -D-ribofuranosyl)pyrimidine-4-carboxamide (**12**) was dehydrated using phosphorous oxychloride and triethylamine in chloroform to give 2',3',5'-tri-*O*-acetyl-2-(β -D-ribofuranosyl)pyrimidine-4-carbonitrile (**15**) as a syrup in 85% yield. The nitrile band in the ir spectrum was very weak, a phenomenon that has been observed in 9-(β -D-ribofuranosyl)purine-6-carbonitrile [20] and in the pyrazolo[3,4-*d*]pyrimidine series [21]. This syrup was treated with 0.5 equivalents of sodium ethoxide to deacetylate and to form ethyl 2-(β -D-ribofuranosyl)pyrimidine-4-carboximidate (**16**) which was used without further characterization. Treatment of **16** with ethanolic ammonia and one equivalent of ammonium chloride gave 2-(β -D-ribofuranosyl)pyrimidine-4-carboxamide hydrochloride (**17**) in 96% yield. Treatment of **16** with one equivalent of crystalline hydroxylamine [22] in ethanol gave 2-(β -D-ribofuranosyl)pyrimidine-4-*N*-hydroxycarboximidine (**18**) in 78% yield. No anomerization was detected by thin layer chromatography or in the ¹H nmr spectra from these transformations.

The formation of 2-(β -D-ribofuranosyl)pyrimidine-4-carboxamide-5'-phosphate (**19**) was accomplished by treating



2-(β -D-ribofuranosyl)pyrimidine-4-carboxamide (**11**) with phosphorus oxychloride in trimethyl phosphate at 0°. Purification of **19** was accomplished on a charcoal column to give **19** in 32% yield. Coupling of **19** to adenosine monophosphate (AMP) to give 2-(β -D-ribofuranosyl)pyrimidine-4-carboxamide-(5' $\rightleftharpoons$ 5')-adenosine pyrophosphate (**20**) was accomplished using a variation of the general procedure of Marquez and co-workers [23]. Treatment of **19** with carbonyldiimidazole activated the 5'-phosphate. The free acid of adenosine monophosphate treated with tri-*n*-butylamine was added to activated **19** to effect the formation of the dinucleotide. Purification of **20** was accomplished with a strong anion-exchange resin (formate form) using an ammonium formate gradient. Further purification of **20** was accomplished with diethylaminoethyl Sephadex using an

ammonium bicarbonate gradient. The acid form of 2-(β -D-ribofuranosyl)pyrimidine-4-carboxamide-(5' $\rightleftharpoons$ 5')-adenosine pyrophosphate (**20**) was obtained by passing the ammonium salt through a column of strong cation-exchange resin (H⁺ form) in 9.4% isolated yield. This is the first report of an NAD analog with a six-membered *N*-heterocyclic *C*-nucleoside replacing the nicotinamide portion of the NAD molecule.

The molecular conformations of **4** and **11** as determined by X-ray diffraction are illustrated in Figures 1 and 2. The β -anomeric configuration is confirmed for both molecules. Positional and isotropic thermal parameters are found in Tables II and III and bond lengths and bond angles are found in Tables IV and V.

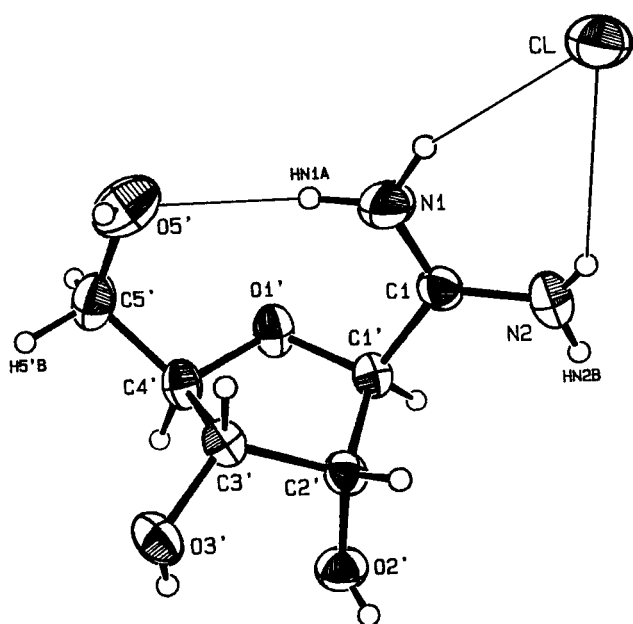


Figure 1. ORTEP [30] drawing of **4** illustrating atom labeling and some hydrogen bonding. Thermal ellipsoids are drawn at the 50% probability level.

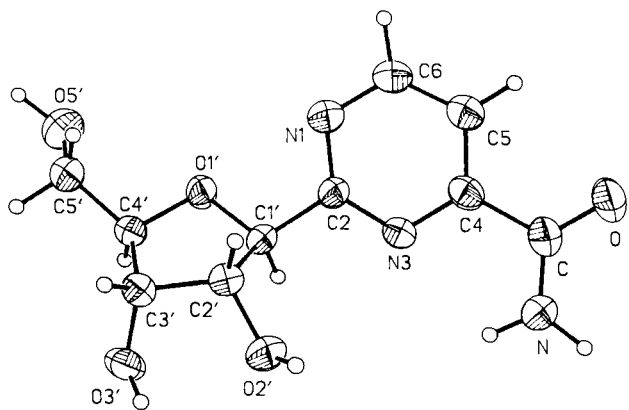


Figure 2. SHELXTL [27] drawing of **11** illustrating atom labels. Thermal ellipsoids are drawn at the 50% probability level.

Table 4

Bond Lengths (Å) and Bond Angles (°) in **4**

1	2	3	1 - 2	1-2-3
N1	C1	N2	1.299(3)	123.0(2)
N2	C1'	C1'	1.311(2)	118.4(2)
C1'	C1	N1	1.514(2)	118.5(2)
C2'	C1'	O1'	1.534(2)	107.54(13)
C2'	C1'	C1		109.83(13)
O1'	C1'	C1	1.424(2)	109.14(14)
C3'	C2'	O2'	1.541(2)	109.59(13)
C3'	C2'	C1'		101.65(13)
O2'	C2'	C1'	1.421(2)	109.54(13)
C4'	C3'	O3'	1.526(2)	114.01(13)
C4'	C3'	C2'		102.58(13)
O3'	C3'	C2'	1.412(2)	114.02(14)
C5'	C4'	O1'	1.513(3)	107.43(14)
C5'	C4'	C3'		116.7(2)
O1'	C4'	C3'	1.442(2)	104.72(12)
O5'	C5'	C4'	1.421(3)	111.7(2)
C1'	O1'	C4'		110.43(12)

The ribose ring in **4** is type N, C_{3'}' endo of form ³T₂. The phase of pseudorotation, P, is 15.5° and the amplitude of pucker, τ_m , is 35.6° [24]. The C5'-side chain is gauche-gauche having torsion angles of -54.5(2)° and 62.6(2)° about the C4'-C5' bond involving O1' and C3', respectively. The torsion angles about the glycosidic bond are 26.0(2)° for O1'-C1'-C1-N1 and -157.67(15)° for O1'-C1'-C1-N2. The C1-N1 bond is 1.299(3) Å whereas the C1-N2 bond is 1.311(2) Å in length. The involvement of N1 in an intramolecular hydrogen bond to O5' may account for this difference. N1, C1, C1', C5' and O5' are planar within 0.01 Å. C1, C1', N1 and N2 are planar within 0.02 Å. Besides the intramolecular hydrogen bond, there appears to be a weak intermolecular hydrogen bond from O5' to O2' [d(O5'...O2')=2.847(2) Å; d(HO5'...O2')=2.36(4) Å] and four fairly strong hydrogen bonding interactions to the chloride ion (H...Cl - distances: HO2', 2.41(3) Å; HN1B, 2.40(4) Å; HN2B, 2.48(3) Å; HO3', 2.50(3) Å).

Table 5
Bond Lengths (Å) and Bond Angles (°) in **11**

1	2	3	1 - 2	1-2-3
N1	C2	N3	1.332(5)	126.7(3)
C1'	C2	N1	1.506(5)	118.3(3)
N3	C2	C1'	1.326(5)	114.9(3)
C2	N3	C4		116.5(3)
N3	C4	C5	1.338(4)	121.7(4)
N3	C4	C		118.4(3)
C	C4	C5	1.504(6)	119.9(4)
C4	C	O		119.4(4)
O	C	N	1.235(5)	124.9(4)
N	C	C4	1.312(5)	115.7(3)
C4	C5	C6	1.379(6)	117.2(4)
C5	C6	N1	1.370(6)	122.4(4)
C6	N1	C2	1.343(5)	115.6(3)
O1'	C1'	C2	1.428(5)	110.1(3)
C2'	C1'	O1'	1.522(5)	103.9(3)
C2'	C1'	C2		113.4(3)
C3'	C2'	O2'	1.533(5)	113.1(3)
C3'	C2'	C1'		103.5(3)
O2'	C2'	C1'	1.407(4)	112.3(3)
C4'	C3'	O3'	1.530(6)	108.1(3)
C4'	C3'	C2'		104.2(3)
O3'	C3'	C2'	1.424(5)	112.1(3)
C5'	C4'	O1'	1.499(6)	109.0(3)
C5'	C4'	C3'		112.0(4)
O1'	C4'	C3'	1.444(4)	106.7(3)
O5'	C5'	C4'	1.389(6)	110.5(4)
C1'	O1'	C4'		107.7(3)

The sugar in **11** is type S, C_{1'} exo of form ¹T°. The P and τ_m values are 122° and 38°, respectively [24]. The side chain is *gauche-trans*; torsion angles are 63.9(5)° and -178.4(3)° involving O1' and C3', respectively. The glycosidic torsion angle, N1-C2-C1'-O1', is 17.5(5)°, resulting in a short N1··O1' contact of 2.661 Å. The base ring and the carboxamide group are planar, the two planes being nearly coplanar. The deviations of the atoms of the carboxamide from the plane of the ring are: C, -0.005 Å; O, -0.081 Å; N, 0.084 Å; H1N, 0.245 Å; and, H2N, 0.97 Å. Resulting from the coplanarity of these planes is a short H1N··N3 distance of 2.320 Å and the formation of a five-membered pseudo-ring containing N3, C4, C, N and H1N. The result is a planar group of atoms which spatially resembles a purine system.

The principal advantage of the current synthetic route is that properly functionalized three carbon pieces could be condensed *via* a Traube synthesis with 2,5-anhydro-D-allonamidine hydrochloride (**4**) to give a variety of 2-(β -D-ribofuranosyl)pyrimidines without the use of blocking groups on the requisite hydroxyl groups. Examples of the versatility of the new C-glycosyl precursor **4** reported here

are the synthesis of 6-methyl-2-(β -D-ribofuranosyl)pyrimidine-4(3*H*)-one (**5**), 2-(β -D-ribofuranosyl)pyrimidin-6(1*H*)-oxo-4-carboxylic acid (**6**) and ethyl 2-(β -D-ribofuranosyl)pyrimidine-4-carboxylate (**10**). The synthesis of the first 6-membered heterocyclic carbon-linked NAD analog **20** is significant since it may prove an effective probe for the elucidation of the mechanism of certain enzymes, such as poly(ADP-ribose)synthetase, which use NAD as a substrate.

EXPERIMENTAL

General Procedures.

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (¹H nmr and ³¹P nmr) spectra were determined at 89.6 MHz with a JEOL FX 90Q spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard for ¹H nmr and phosphoric acid for ³¹P nmr. The presence of solvent, as indicated by elemental analysis, was verified by ¹H nmr. Infrared spectra (ir) were obtained on a Mattson Sirius 100 FT-IR and ultraviolet spectra (uv, sh = shoulder) were recorded on Cary Model 15 or Cary Model 118 spectrophotometers. Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, TN, or Robertson Labs, Florham Park, NJ. Thin layer chromatography (tlc) was run on silic gel 60 F-254 plates (EM Reagents). Flash chromatography techniques were used for preparative-scale chromatography. J. T. Baker silica gel (40 μ m) or Kiesel gel 60 EM Reagents (40-63 μ m) was used for flash chromatography. Solvent E is the saturated upper phase of a 4/1/2 mixture of ethyl acetate/1-propanol/water. Solvent E₂ is a 2/1/1 mixture of ethyl acetate/ethanol/water. 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°.

2,5-Anhydro-D-allonamidine Hydrochloride (**4**).

Methyl 2,5-anhydro-D-allonimidate (**3**) [12] (10.0 g, 52.30 mmoles) and ammonium chloride (2.80 g, 52.30 mmoles) were dissolved in methanolic ammonia (100 ml, saturated at dry ice-acetone temperature for 0.5 hour) and allowed to stir 16 hours in a thick walled, glass, pressure bottle at room temperature. The solution was then evaporated to dryness and the resulting solid dried to yield 11.0 g (98%) of product. An analytical sample was prepared by addition of ether to a methanolic solution of the amidine hydrochloride until the solution turned cloudy, whereupon, crystalline, star-like clusters of **4** slowly formed, mp 176-178°; ir (potassium bromide): ν 1702 (C=NH) cm⁻¹; ¹H nmr (DMSO-d₆): δ 3.6 (m, 2), 3.96 (t, 3, J = 3.6 Hz), 4.48 (d, 1, J = 4.7 Hz, C₁H), 5.2 (br s, 2), 7.60 (br s, 4, (C=NH)NH₂ HCl).

Anal. Calcd. for C₆H₁₃ClN₂O₄: C, 33.89; H, 6.16; N, 13.17; Cl, 16.67. Found: C, 33.83; H, 6.28; N, 12.88; Cl, 16.71.

Preparation of Ethyl 4-(Dimethylamino)-2-oxo-3-butenate (**9**).

Equimolar amounts of dimethyl formamide diethylacetal (31.3 ml, 183 mmoles) and ethyl pyruvate (20.0 ml, 183 mmoles) were added together at room temperature. An exothermic reaction took place which caused the solution to come to reflux within ten minutes, and the solution to turn dark. The dark solution was heated at 80-85° for 24 hours. All the volatile components were removed *in vacuo* and the dark oil was distilled. The fraction collected was colored orange to red and distilled at 180-185° at 0.65 mm Hg to yield 17.2 g (55%) of product. Upon freezing, the distillate formed an orange solid with mp 35-38°, lit [18] mp 38°.

6-Methyl-2-(β -D-ribofuranosyl)pyrimidin-4(3*H*)-one (**5**).

To a solution of 2,5-anhydro-D-allonamidine hydrochloride (**4**) (2.0 g, 9.4 mmoles) and ethyl acetoacetate (1.3 g, 9.99 mmoles) in absolute

ethanol (100 ml) was added sodium hydroxide (0.40 g). The solution was allowed to stir for twenty-four hours after which time the ethanol was removed *in vacuo*. The residue was then thoroughly mixed with sodium carbonate (0.4 g) and sodium bicarbonate (0.4 g) to form a paste. The paste was extracted with absolute ethanol in a Soxhlet extractor. The ethanol was cooled and the salts filtered. To the filtrate was added silica gel (10.0 g, 60-200 mesh) and the suspension was evaporated to dryness. The resultant dry powder was placed on top of a flash column and eluted with solvent E to yield 1.08 g (48%) of the β -anomer and 0.42 g (24%) of the α -anomer. Recrystallization of **5** from absolute ethanol gave an analytical sample, β -anomer, mp 177-179°, α -anomer 115-117°; tlc (solvent E) Rf 0.37; ir (potassium bromide): ν 1690 (C=O); uv: λ max (pH 1) 228 nm (ϵ 2766), 265 nm (sh, ϵ 922), (pH 7) 228 nm (ϵ 2184), 260 nm (sh, ϵ 1262), (pH 11) 218 nm (ϵ 2378), 258 nm (ϵ 1067); ^1H nmr (DMSO- d_6) β -anomer: δ 2.16 (s, 3, CH₃), 3.3-4.2 (m, 8), 4.54 (d, 1, J = 2.69 Hz, C₁H), 6.04 (s, 1, C₅H); ^1H nmr (DMSO- d_6) α -anomer: δ 4.79 (d, 1, J = 5.20 Hz, C₁H).

Anal. Calcd. for C₁₀H₁₄N₂O₅: C, 49.58; H, 5.83; N, 11.56. Found: C, 49.85; H, 6.02; N, 11.28.

2-(β -D-Ribofuranosyl)pyrimidin-6(1*H*)-oxo-4-carboxylic Acid (**6**).

To a solution of 2,5-anhydro-D-allonamide hydrochloride (**4**) (6.3 g, 29.63 mmoles) in water (100 ml) was added 1 *N* sodium hydroxide (30 ml) and ethyl sodium oxaloacetate (6.23 g, 29.63 mmoles). The reaction mixture was allowed to stir 16 hours at room temperature and was subsequently neutralized to pH 2 with H⁺ resin (Dowex 50W-X8). Silica gel (60-200 mesh, 40.0 g) was added and the water removed *in vacuo*. The resultant powder was placed on top of a flash column (silica gel, 200-400 mesh, 40 $\times$ 5 cm) and eluted with 3/1/1 ethyl acetate/acetone/methanol/water until the faster moving components had eluted. The column was then eluted with methanol and the fractions containing the compound were pooled and the methanol removed to yield 5.1 g (63% of crude product). Further purification of the acid **6** was achieved by passing a water solution of the acid through an ion exchange column (H⁺, Dowex 50W-X8) and eluting with water. Fractions containing the product were pooled and the water removed *in vacuo* yielding a tan powder, mp 255-258° dec. Purification was also accomplished by reverse phase (C-18) chromatography using water as the eluant. tlc (solvent E₂) Rf 0.29; ir (potassium bromide): ν 1680, 1620 (C=O); uv: λ max (pH 1) 284 nm (ϵ 1851), (pH 7) 270 nm (ϵ 2449), (pH 11) 275 nm (ϵ 1985); ^1H nmr (DMSO- d_6): δ 3.3 (m, 4), 3.68 (d, 2, J = 7.2 Hz), 3.96 (m, 3), 4.64 (d, 1, J = 3.58 Hz, C₁H), 6.76 (s, 1, C₅H), 12.48 (br s, 1, OH).

Anal. Calcd. for C₁₀H₁₂N₂O₇: C, 44.12; H, 4.44; N, 10.29. Found: C, 43.90; H, 4.47; N, 10.38.

Ethyl 2-(β -D-Ribofuranosyl)pyrimidin-6(1*H*)-oxo-4-carboxylate (**7**).

Through a suspension of the acid **6** (500 mg, 1.84 mmoles) in absolute ethanol (25 ml) cooled in an ice bath, was bubbled dry hydrogen chloride gas for 30 seconds. To this reaction mixture was added triethyl orthoformate (2.0 ml). The mixture was allowed to stir twenty-four hours at room temperature after which silica gel (60-200 mesh, 10.0 g) was added and the ethanol removed *in vacuo*. The resultant dry powder was placed on top of a flash column (silica gel, 200-400 mesh, 27 $\times$ 5 cm) and eluted with 9/1 chloroform/methanol. Pooling of fractions and removal of solvent yielded 300 mg (54%) of a white crystalline material. Recrystallization of **7** from ethyl acetate gave an analytical sample, mp 182-184°; tlc (10% methanol in chloroform) Rf 0.18; ir (potassium bromide): ν 1677, 1737 (C=O) cm⁻¹; uv: λ max (pH 1) 293 nm (ϵ 4598), (pH 7) 293 nm (ϵ 4869), (pH 11) 290 nm (ϵ 3787); ^1H nmr (DMSO- d_6): δ 1.28 (t, 3, J = 7.00 Hz, CH₃), 3.70 (d, 2, J = 9.5 Hz), 4.04 (m, 6), 4.29 (q, 2, J = 6.99 Hz, CH₂), 4.63 (d, 1, J = 2.26 Hz, C₁H), 5.06 (d, 1, J = 5.2 Hz), 5.56 (d, 1, J = 4.74 Hz), 6.77 (s, 1, C₅H).

Anal. Calcd. for C₁₂H₁₆N₂O₇: C, 48.00; H, 5.37; N, 9.33. Found: C, 48.15; H, 5.50; N, 9.21.

2-(β -D-Ribofuranosyl)pyrimidin-6(1*H*)-oxo-4-carboxamide (**8**).

A solution of the ester **7** (100 mg, 0.33 moles) in ethanolic ammonia (50 ml, saturated at dry ice-acetone bath temperature for 0.5 hour) was allowed to stir at room temperature in a thick-walled glass pressure bottle

for 42 hours, by which time a white precipitate had formed. Removal of the ethanolic ammonia yielded 90 mg (100%) of a white crystalline product. Recrystallization of **8** from absolute ethanol gave an analytical sample, mp 226-228°; tlc (solvent E) Rf 0.37; ir (potassium bromide): ν 1677 (C=O) cm⁻¹; uv: λ max (pH 1) 275 nm (ϵ 3905), (pH 7) 283 nm (ϵ 3435), (pH 11) 283 nm (ϵ 3075); ^1H nmr (DMSO- d_6): δ 3.64 (dd, 2, J = 7.2 Hz), 3.76-4.28 (m, 4), 4.63 (d, 1, J = 3.54 Hz, C₁H), 5.04 (d, 1, J = 3.6 Hz), 5.44 (d, 1, J = 3.6 Hz), 6.72 (s, 1, C₅H), 7.88 (br s, 2, NH₂).

Anal. Calcd. for C₁₀H₁₃N₃O₆: C, 44.28; H, 4.83; N, 15.49. Found: C, 44.39; H, 4.95; N, 15.25.

Ethyl 2-(β -D-Ribofuranosyl)pyrimidine-4-carboxylate (**10**).

Finely ground **4** (10.0 g, 47.03 mmoles) was dissolved at reflux in absolute ethanol (500 ml). The solution was then cooled to 50° and sodium ethoxide in ethanol (1 equivalent of a 1 *M* solution) was added. To the resultant suspension was added a solution of the enamine **9** (9.66 g, 56.43 mmoles) in absolute ethanol (20 ml). The orange colored solution was then allowed to stir at 50° for 2 hours after which time the reaction mixture was brought to reflux for 0.5 hour. After cooling the solution, sodium chloride was filtered and silica gel (60-200 mesh, 40 g) was added to the filtrate and the slurry was evaporated to dryness. The resultant powder was loaded on top of a flash column (silica gel, 200-400 mesh, 45 $\times$ 5 cm) and eluted first with chloroform (1.5 l) followed by elution with 95/5 chloroform/methanol (3 l). Pooling of fractions and removal of solvent yielded 5.8 g (42%) of a light tan crystalline powder. Recrystallization of **10** from ethyl acetate yielded an analytical sample, mp 102-104°; tlc (10% methanol in chloroform) Rf 0.29; ir (potassium bromide): ν 1738 (C=O) cm⁻¹; uv: λ max (pH 1) 258 nm (sh, ϵ 2941), (pH 7 and 11) 253 nm (ϵ 4117); ^1H nmr (DMSO- d_6): δ 1.36 (t, 3, J = 7.17 Hz, CH₃), 3.48-3.72 (m, 2), 3.88-4.24 (m, 3), 4.40 (q, 2, J = 7.00 Hz, CH₂), 4.78 (dd, 1, J = 7.2 Hz), 4.90 (d, 1, J = 5.4 Hz, C₁H), 4.98 (d, 1, J = 5.42 Hz), 5.26 (d, 1, J = 5.4 Hz), 7.94 (d, 1, J = 4.96 Hz, C₅H), 9.04 (d, 1, J = 4.96 Hz, C₆H).

Anal. Calcd. for C₁₂H₁₆N₂O₆: C, 50.70; H, 5.67; N, 9.85. Found: C, 50.34; H, 5.73; N, 9.77.

2-(β -D-Ribofuranosyl)pyrimidine-4-carboxamide (**11**).

A solution of the ester **10** (4.2 g, 14.1 mmoles) was stirred in ethanolic ammonia (saturated at dry ice/acetone temperature for 0.5 hour) for 16 hours by which time a white precipitate had formed. The precipitate was filtered and dried. More product was obtained by *in vacuo* removal of the solvent from the filtrate. Total yield was 3.0 g (83%). Recrystallization of **11** from absolute ethanol gave an analytical sample, mp 197-198°; tlc (solvent E) Rf 0.37; ir (potassium bromide): ν 1689 (C=O) cm⁻¹; uv: λ max (pH 1) 268 nm (ϵ 2564), (pH 7 and 11) 260 nm (ϵ 3076); ^1H nmr (DMSO- d_6): δ 3.76 (m, 2), 3.94 (q, 1, J = 3.6 Hz), 4.10 (q, 1, J = 3.6 Hz), 4.30 (q, 1, J = 3.6 Hz), 4.75 (d, 1, J = 5.4 Hz), 4.89 (d, 1, J = 5.4 Hz, C₁H), 4.94 (d, 1, J = 3.6 Hz), 5.13 (d, 1, J = 5.4 Hz), 7.87 (d, 1, J = 5.19 Hz, C₅H), 8.12 (d, 2, J = 21.5 Hz, NH₂), 9.03 (d, 1, J = 4.96 Hz, C₆H).

Anal. Calcd. for C₁₀H₁₃N₃O₅: C, 47.06; H, 5.13; N, 16.46. Found: C, 47.13; H, 5.27; N, 16.41.

2',3',5'-Tri-*O*-acetyl-2-(β -D-ribofuranosyl)pyrimidine-4-carboxamide (**12**).

To a suspension of the amide **11** (2.6 g, 10.2 mmoles) in dry pyridine (25 ml) and acetic anhydride (75 ml) was added dimethylaminopyridine (30 mg). The suspension became a homogeneous solution after 0.5 hour and the solution was stirred for twenty-four hours at room temperature. The solvent was removed *in vacuo* and the resultant syrup dissolved in chloroform and washed with 1 *N* hydrochloric acid, 2 *N* sodium hydroxide and water in that order. The chloroform portion was dried over sodium sulfate and the chloroform removed *in vacuo* to yield 3.75 g (99%) of a pale yellow syrup which crystallized spontaneously overnight. Recrystallization of **12** from anhydrous ether gave an analytical sample, mp 107-109°; tlc (10% methanol in chloroform) Rf 0.50; ir (potassium bromide): ν 3431 (NH₂), 1743 (C=O of acetyls), 1708 (C=O of amide) cm⁻¹; uv: λ max (chloroform): 256 nm (ϵ 4860), 267 (sh, ϵ 3120); ^1H nmr (DMSO- d_6): δ 1.96, 2.04, 2.08 (s, 3, CH₃), 4.04-4.48 (m, 3), 5.10 (d, 1, J = 4.73 Hz, C₁H), 5.48 (t, 1, J = 5.4 Hz), 5.71 (t, 1, J = 5.4 Hz), 8.14 (d, 2, J = 5.19

Hz, C₅H), 9.10 (d, 1, J = 4.96 Hz, C₆H).

Anal. Calcd. for C₁₆H₁₉N₃O₈: C, 50.39; H, 5.02; N, 11.02. Found: C, 50.31; H, 5.14; N, 10.85.

2',3',5'-Tri-O-acetyl-2-(β-D-ribofuranosyl)pyrimidine-4-thiocarboxamide (13).

To a solution of **12** (2.4 g, 6.30 mmoles) in purified 1,4-dioxane (75 ml) was added freshly purified phosphorus pentasulfide (0.7 g, 3.15 mmoles). The suspension was brought to reflux, and it quickly turned dark red. After ten minutes at reflux another portion of phosphorus pentasulfide (0.7 g) was added. After another ten minutes dimethylaminopyridine (20 mg) was added along with another portion of phosphorus pentasulfide (0.7 g). After 45 minutes of total reflux time the dark reaction mixture was cooled and decolorized with Norit A decolorizing carbon. The carbon was removed by filtration and washed with chloroform and the filtrate evaporated to an orange syrup. The syrup was dissolved in fresh chloroform and washed with saturated sodium bicarbonate. To the chloroform solution was added silica gel (60-200 mesh) and the slurry was evaporated to dryness. The resultant powder was then placed on top of a flash column (silica gel, 200-400 mesh, 25 × 5 cm) and eluted with 2:1 hexanes/acetone. Pooling of fractions and *in vacuo* removal of solvent yielded 1.0 g (42%) of a yellow syrup. When the syrup was dissolved in fresh chloroform and the chloroform removed, the resulting syrup crystallized spontaneously, mp 130-133°; tlc (2:1 hexanes/acetone) Rf 0.23; ir (potassium bromide): ν 3379 (NH₂), 1748 (C=O of acetyls), 1577 (C=S); uv: λ max (chloroform) 266 nm (ε 5118), 276 (sh, ε 3914), 332 (ε 6322); ¹H nmr (DMSO-d₆): δ 1.96, 2.04, 2.07 (s, 3, CH₃ of acetyls), 4.02-4.44 (m, 3), 5.09 (d, 1, J = 4.75 Hz, C₁H), 5.40 (t, 1, J = 5.4 Hz), 5.64 (t, 1, J = 5.4 Hz), 8.27 (d, 1, J = 5.42 Hz, C₅H), 9.05 (d, 1, J = 5.09 Hz, C₆H), 10.26 (d, 2, J = 47.49, NH₂).

Anal. Calcd. for C₁₆H₁₉N₃O₇S: C, 48.36; H, 4.82; N, 10.57; S, 8.07. Found: C, 48.31; H, 4.94; N, 10.42; S, 7.95.

2-(β-D-Ribofuranosyl)pyrimidine-4-thiocarboxamide (14).

To a suspension of **13** (1.0 g, 2.52 mmoles) in dry methanol (20 ml) was added sodium methoxide (1.9 ml of a 0.68 M solution, 1.26 mmoles). After ten minutes of stirring at room temperature the suspension had become a yellow homogeneous solution. After 30 minutes the solution was neutralized with H⁺ resin (Dowex 50W-X8). Removal of the solvent yielded 440 mg (65%) of yellow flakes: mp 183-185°. Recrystallization of **14** from absolute ethanol yielded an analytical sample, mp 198-200°; tlc (solvent E) Rf 0.68; ir (potassium bromide): ν 1576 (C=S) cm⁻¹; uv: λ max (pH 1) 265 nm (ε 5420), 325 (ε 5420), (pH 7) 265 nm (ε 5962), 325 (ε 6233), (pH 11) 263 nm (ε 5691), 321 (ε 5420); ¹H nmr (DMSO-d₆): δ 3.84 (q, 1, J = 5.4 Hz), 4.12 (q, 1, J = 5.4 Hz), 4.32 (q, 1, J = 5.4 Hz), 4.80 (d, 1, J = 5.4 Hz), 4.90 (d, 1, J = 5.42 Hz, C₁H), 4.96 (d, 1, J = 5.4 Hz), 5.16 (d, 1, J = 5.4 Hz), 8.19 (d, 1, J = 5.08 Hz, C₅H), 8.98 (d, 1, J = 5.09 Hz, C₆H), 10.26 (d, 2, J = 42.11 Hz, NH₂).

Anal. Calcd. for C₁₀H₁₃N₃O₄S: C, 44.27; H, 4.83; N, 15.49; S, 11.82. Found: C, 44.23; H, 4.98; N, 15.20; S, 12.08.

2',3',5'-Tri-O-acetyl-2-(β-D-ribofuranosyl)pyrimidine-4-carbonitrile (15).

To a solution of **12** (3.1 g, 8.13 mmoles) in chloroform (100 ml) cooled in an ice-salt bath was added triethylamine (17.1 ml, 122.55 mmoles) and freshly distilled phosphorus oxychloride (2.14 ml, 23.34 mmoles). The solution, which quickly turned dark brown, was allowed to warm to room temperature and stir for 4 hours at which time more triethylamine (5.0 ml) and phosphorus oxychloride (1.0 ml) were added and the stirring continued for an additional 2.5 hours. The chloroform was then removed *in vacuo* and the residue redissolved in chloroform and washed with saturated sodium bicarbonate solution (3 × 150 ml). The chloroform portion was then dried over sodium sulfate. Silica gel (60-200 mesh, 20 g) was then added and the chloroform removed *in vacuo*. The resultant powder was then poured on top of a flash column (silica gel, 200-400 mesh, 22 × 5 cm) and eluted with 2:1 hexanes/acetone. Pooling of fractions and removal of solvent yielded 2.52 g (85%) of **15** as a chromatographically pure syrup with respect to 2:1 hexanes/acetone. tlc (2:1 hex-

anes/acetone) Rf 0.20; ir (neat): ν 1743 (C=O of acetyls), 2100 (very weak, CN) cm⁻¹; uv: λ max (chloroform) 261 nm (ε 3944), 270 (sh, ε 2906); ¹H nmr (DMSO-d₆): δ 2.10, 2.11, 2.12 (s, 3, CH₃ of acetyls), 4.12-4.64 (m, 3), 5.21 (d, 1, J = 4.28 Hz, C₁H), 5.44-5.68 (m, 2), 7.62 (d, 1, J = 4.74 Hz, C₅H), 9.01 (d, 1, J = 4.96 Hz, C₆H).

Anal. Calcd. for C₁₆H₁₇N₃O₇: C, 52.89; H, 4.72; N, 11.57. Found: C, 52.60; H, 4.75; N, 11.42.

2-(β-D-Ribofuranosyl)pyrimidine-4-carboxamide Hydrochloride (17).

To a solution of **15** (1.43 g, 3.94 mmoles) in absolute ethanol (20 ml) was added sodium ethoxide (0.7 ml of a 1.09 M solution, 0.763 mmole). After stirring 2.5 hours at room temperature the solution was neutralized with H⁺ resin (Dowex 50W-X8) and evaporated to dryness. The residue was then dissolved in ethanolic ammonia (saturated at 20°, 50 ml). To this pale orange solution ammonium chloride (215 mg, 3.94 mmoles) was added and the solution allowed to stir overnight at room temperature. *In vacuo* removal of the solvent yielded 1.1 g (96% yield) of a pale yellow foam. Recrystallization of **17** from 2-propanol gave an analytical sample, mp 158-160°; tlc (solvent E₂) Rf 0.06; ir (potassium bromide): ν 1713 (C=NH) cm⁻¹; uv: λ max (pH 1) 268 nm (sh, ε 2168), (pH 7) 270 nm (sh, ε 4607), (pH 11) 261 nm (ε 5420), 296 (sh, ε 4607); ¹H nmr (DMSO-d₆): δ 3.42-3.78 (m, 2), 3.78-4.20 (m, 2), 4.30 (q, 1, J = 5.4 Hz), 4.68-5.10 (m, 3), 4.89 (d, 1, J = 5.09 Hz, C₁H), 5.23 (d, 1, J = 5.5 Hz), 8.26 (d, 1, J = 5.42 Hz, C₅H), 9.22 (d, 1, J = 5.09 Hz, C₆H), 9.77 (br s, 4, (C=NH)NH₂·HCl).

Anal. Calcd. for C₁₀H₁₃ClN₃O₄·½ H₂O: C, 40.07; H, 5.38; N, 18.69; Cl, 11.82. Found: C, 40.07; H, 5.34; N, 18.40; Cl, 11.80.

2-(β-D-Ribofuranosyl)pyrimidine-4-N-hydroxycarboxamide (18).

To a solution of **15** (1.47 g, 4.05 mmoles) in absolute ethanol (20 ml) was added sodium ethoxide (1.0 ml of a 0.82 M solution, 0.82 mmole) which was stirred for 2.5 hours at room temperature. The solution was then neutralized with H⁺ resin (Dowex 50W-X8) and evaporated to dryness. The resultant residue was redissolved in absolute ethanol (50 ml) to which was added crystalline hydroxylamine (270 mg, 8.17 mmoles). The clear orange-colored solution was stirred overnight. Silica gel (60-200 mesh, 10.0 g) was added and the slurry evaporated to dryness. The resultant powder was added to the top of a flash column (silica gel, 200-400 mesh, 20 × 5 cm) and eluted with 4:1 chloroform/methanol. Pooling of fractions and *in vacuo* removal of solvent yielded 850 mg (78%) of a white crystalline foam. An analytical sample was obtained by dissolving the foam in water, followed by lyophilization and further drying, mp 131-134° (ethanol); tlc (20% methanol in chloroform) Rf 0.34; ir (potassium bromide): ν 1657 (C=NOH), 1580, 1550 cm⁻¹; uv: λ max (pH 1) 262 nm (ε 4422), (pH 7) 292 nm (ε 5159), (pH 11) 295 nm (ε 5159), 242 (sh, ε 3930); ¹H nmr (DMSO-d₆): δ 3.48-3.66 (m, 2), 3.78-4.38 (m, 3), 4.74-4.98 (m, 3), 5.16 (d, 1, J = 5.08 Hz), 5.95 (br s, 2, NH₂), 7.72 (d, 1, J = 5.01 Hz, C₅H), 8.74 (d, 1, J = 5.42 Hz, C₆H), 10.40 (br s, 1, NOH).

Anal. Calcd. for C₁₀H₁₄N₄O₅: C, 44.44; H, 4.85; N, 20.73. Found: C, 44.52; H, 5.22; N, 20.51.

2-(β-D-Ribofuranosyl)pyrimidine-4-carboxamide-5'-phosphate (19).

To a suspension of the amide **11** (2.61 g, 10.23 mmoles) in freshly distilled trimethyl phosphate (25 ml) cooled in an ice bath was added phosphorus oxychloride (1.2 ml, 13.11 mmoles). The solution became homogeneous upon addition of the phosphorus oxychloride and was allowed to stir for 2 hours after which the reaction mixture was poured into ice water (50 ml) and extracted with ether (3 × 100 ml). The aqueous layer was adjusted to pH 2 using 2 N sodium hydroxide and poured on top of a charcoal column (acid washed, Au-4, 25 × 2.5 cm). The column was washed with copious amounts of distilled water until salt free. The column was then eluted with 10/10/1 ethanol/water/ammonium hydroxide. The fractions containing product were pooled and the eluant removed *in vacuo* to yield 1.2 g (32%). The acid form was obtained by passing the ammonium salt through a strong cation exchange resin (Dowex 50W-X8, H⁺ form) using water as eluant. Fractions which contained **19** were pooled and the eluant removed by lyophilization which gave an analytical sample; tlc (11/7/2 1-propanol/ammonium hydroxide/water) Rf 0.27; ir (potassium

bromide): ν 1693 (C=O); uv: λ max (pH 1) 258 nm (ϵ 2766), (pH 7) 258 nm (ϵ 3111), (pH 11) 258 nm (ϵ 3457); ^1H nmr (deuterium oxide): δ 5.07 (d, 1, J = 6.09 Hz, C₁H), 7.99 (d, 1, J = 4.96 Hz, C₅H), 9.02 (d, 1, J = 4.96 Hz, C₆H).

Anal. Calcd. for C₁₀H₁₄N₃O₈P·H₂O: C, 34.00; H, 4.56; N, 11.90; P, 8.77. Found: C, 33.61; H, 4.67; N, 11.53; P, 8.77.

2-(β -D-Ribofuranosyl)pyrimidine-4-carboxamide-(5' $\Rightarrow$ 5')-adenosine Pyrophosphate (**20**).

To a suspension of 2-(β -D-ribofuranosyl)pyrimidine-4-carboxamide-5'-phosphate (**19**) (1.9 g, 5.67 mmoles) in dimethyl formamide (30 ml) was added carbonyldiimidazole (4.6 g). The reaction mixture became homogeneous within 5 minutes and was allowed to stir 3.5 hours. Methanol (1.84 ml) was then added to solvolyze any excess carbonyldiimidazole, and the solution was allowed to stir 1 hour. The free acid of adenosine monophosphate (AMP) (3.11 g, 8.51 mmoles) dissolved in dimethyl formamide with tributylamine (2.03 ml) was then added and the reaction mixture allowed to stir for 72 hours. Water (100 ml) was then added and the solvent removed *in vacuo*. To the resultant syrup was added a solution of sodium acetate (1.7 g in 290 ml water). The resultant solution was extracted with chloroform (2 $\times$ 150 ml) and ether (2 $\times$ 150 ml). The aqueous layer was treated with triethylamine until pH 10 was reached and allowed to stir for 24 hours. After such time the mixture was lyophilized and the residue chromatographed using a strong anion exchange resin (Amberlite CG-400, 100-200 mesh) and a gradient from water (2 l) to 2 M ammonium bicarbonate (2 l) followed by additional elution with 2 M ammonium bicarbonate (2 l). Passage through a strong cation exchange resin (Dowex 50W-X8, H⁺ form) followed by lyophilization removed the excess ammonium bicarbonate and converted the NAD analog to the acid form to yield 380 mg of **20** (9%). An analytical sample was obtained by chromatography on diethylaminoethyl Sephadex (30 $\times$ 2.5 cm) using a gradient of water (2 l) to 200 mM ammonium bicarbonate (2 l) followed by lyophilization. The fluffy white residue was passed through a strong cation exchange resin (Dowex 50W-X8, H⁺ form) using water as eluant. The fractions containing product were lyophilized to yield a white fluffy solid. tlc (11/7/2 1-propanol/ammonium hydroxide/water) R_f 0.63; ir (potassium bromide): ν 1681 (C=O) cm⁻¹; uv: λ max (pH 1) 255 nm (ϵ 15704), (pH 7) 257 nm (ϵ 16787), (pH 11) 257 nm (ϵ 15163); ^1H nmr (DMSO-d₆): δ 4.83 (d, 1, J = 5.38 Hz, C₁H pyrimidine), 5.88 (d, 1, J = 5.08 Hz, C₁H adenosine), 7.88 (d, 1, J = 5.6 Hz, C₅H pyrimidine), 7.91 (br s, 1, NH amide), 8.25 (br s, 1, NH amide), 8.31 (s, 1, adenosine), 8.56 (s, 1, adenosine), 8.67 (br s, 2, NH₂), 8.99 (d, 1, J = 5.08 Hz, C₆H pyrimidine); ^{31}P nmr (DMSO-d₆): δ -405.27 (s).

Anal. Calcd. for C₂₀H₂₆N₈O₁₄P₂·3H₂O: C, 33.44; H, 4.49; N, 15.60; P, 8.62. Found: C, 33.67; H, 4.57; N, 15.35; P, 8.76.

X-Ray Crystallography.

Crystal and structure solution data for compounds **4** and **11** are listed in Table I. Compound **4** crystallized as long, thick plates. A sample was cut to a suitable size and mounted on an Enraf-Nonius CAD4 diffractometer equipped with graphite monochromated MoK α radiation. Lattice parameters were determined by a least-squares procedure using the setting angles of 25 reflections. The structure was solved with MULTAN82 [25] and a subsequent electron density map. Full-matrix least-squares refinement of all atomic positions, anisotropic thermal parameters for non-hydrogen atoms and isotropic thermal parameters for hydrogens was accomplished with SHELXTL [26].

A suitable crystal of **11** was mounted on a Nicolet R3 diffractometer employing graphite monochromated CuK α radiation. Cell parameters were obtained by a least-squares treatment of the angles of 15 centered reflections. The trial model was obtained by direct methods and refined by blocked cascading least-squares using SHELXTL [27]. Non-hydrogen atoms were refined anisotropically.

It was possible to locate all the hydrogen atoms of the molecule in difference maps. The positional and isotropic thermal parameters for H5'A, H5'B, and the sugar and base-ring hydrogen atoms were refined initially. The values of these parameters converged to values which provided pro-

per geometry and logical thermal motion. Since the positions of these atoms were not crucial to the study, during the final stages of refinement they were allowed to ride on their neighboring carbon atoms with only their isotropic thermal parameters being refined. The positional and isotropic thermal parameters of HNA, NHB, HO2' and HO3' refined normally. However, HO5' refined to a position which made the O5'-HO5' bond unreasonably long (1.35 Å). Therefore in the final refinement this O-H bond length was fixed at a value of 1.00(5) Å. The large thermal motions of the O-H hydrogens are not unexpected for terminal O-H groups and in the case of HO5' it is large enough to suggest disorder. Disorder would explain the inability to properly refine HO5'.

Empirical extinction corrections were applied in both refinements. Scattering factors and anomalous dispersion corrections for non-hydrogen atoms were taken from International Tables for X-ray Crystallography [28]. Hydrogen scattering factors were taken from Stewart, Davidson and Simpson [29]. Figure 1 was drawn with ORTEPII [30] and Figure 2 was drawn with SHELXTL [27]. Data for **4** were processed with SDP-PLUS [31].

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