2′-*O*,5-Dimethyluridine: A Total Synthesis and Single Crystal X-ray Diffraction Study

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Dedicated to the memory of Dr. Roland K. Robins

A new method for the synthesis of 2′-*O*,5-dimethyluridine (**5**) has provided the title compound in a higher yield. Application of a one-pot ribosylation methodology resulted in an efficient, high yield synthesis of 5-methyluridine (ribothymine, **3b**). An X-ray diffraction analysis of **5** disclosed the conformation of the sugar moiety of this nucleoside as *anti*, *N*(3′-*endo*), g⁺.

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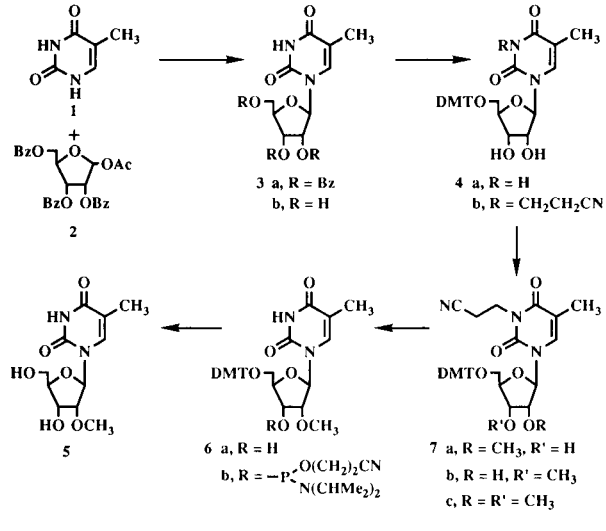
The history of triple helical structures for nucleic acids dates to the mid 1950s [1]; however, the use of triple helix (triplex) formation as a therapeutic mode of action has received great impetus only during the past few years. The history of triplex formation has recently been reviewed [2,3]. Two modes of triplex formation have been defined: a *p*H dependent, parallel binding of the third strand [4-9] and a *p*H independent, antiparallel binding of the third strand to the target duplex [10-12]. Our efforts have been directed to the development of the technology associated with the latter of these two modes of triplex formation. Part of these studies [13,14] has involved the replacement of natural nucleosides in triplex forming oligonucleotides (TFOs) of known binding affinity by modified nucleosides with the goals of improving binding affinity, increasing nuclease resistance, and enhancing cell penetration and/or intracellular transport.

A recent study [15] of triplex formation, in the *p*H dependent, parallel binding mode and using a homopyrimidine oligoribonucleotide, showed that when 2′-*O*-methylribonucleotide TFOs were used, the stability of the formed triplex was increased. It was suggested [15] that both the increased hydrophobicity of the triplex resulting from the methylation of the 2′-hydroxyl of the TFO and the 3′-*endo* sugar conformation of the 2′-*O*-methylated third strand were responsible for the enhanced stability. In this study [15], the triplexes were formed from Hoogsteen-type T·AT and C⁺·GC base triplets with the homopyrimidine, third strand parallel to the purine strand of the duplex. In the alternate approach to triplex formation, reverse-Hoogsteen-type T·AT and G·GC triplets are formed with the third strand (mixed purine-pyrimidine) antiparallel to the purine strand of the duplex. The two types of triplex are structurally distinct, differing in a number of properties, and it was uncertain what effect 2′-*O*-methylribonucleotide substitution would have on the formation of antiparallel triplexes.

For a study of the effect(s) of incorporation of 2′-*O*-methylribonucleotides into antiparallel TFOs it was considered necessary that a true ribonucleoside replacement for thymidine be used. Most of the common 2′-*O*-methylribonucleotide building blocks are commercially available from several sources; however, the in-house synthesis of 2′-*O*,5-dimethyluridine (2′-*O*-methylribothymine, **5**) and the corresponding phosphoramidite **6b** was required. This report describes an alternate, higher yielding synthesis of **5** and **6b** and a study of the conformation of **5** by single

Scheme 1



crystal X-ray diffraction analysis. A discussion of the incorporation of **5** into oligonucleotides and the effect(s) of 2'-*O*-methylation on the formation of antiparallel triple helices will be presented elsewhere.

The presence of 2'-*O*,5-dimethyluridine (**5**) in certain tRNAs has been documented [16,17]; however, the initial report of its synthesis [16] is inconclusive as to the exact site of methylation. The only definitive synthesis of 2'-*O*,5-dimethyluridine was described in a patent by Ohtsuka and Inoue [18]. The low yields reported [18] in several key steps prompted an application of the methodology described by Wagner *et al.* [19], and an outline of this synthesis is shown in Scheme 1. Although this methodology required the separation of isomeric products, it does produce preferentially the 2'-*O*-methyl isomer. Further, this approach requires fewer synthetic steps for the preparation of the protected phosphoramidite **6b** than the Ohtsuka, Inoue method [18], and utilizes inexpensive, easily handled reagents. The later considerations may become of added importance if the larger scale synthesis of **5** or **6b** is contemplated.

A consideration of the cost of commercial 5-methyluridine (ribothymine, **3b**), led to the first time application of a one-pot ribosylation methodology [20] to the synthesis of this compound. Thus, thymine (**1**) was silylated and, without isolation, the persilylated **1** was reacted with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**2**) to produce **3a** which was readily isolated (82%) by crystallization. Using standard methodology, **3a** was debenzoylated to give **3b** (90%). Both **3a** and **3b** were characterized independently as well as by comparison with previously established spectral and physical properties [21-23]. Using this improved ribosylation procedure [20] 5-methyluridine (ribothymine, **3b**) was obtained in high yield and at a small fraction of the current commercial cost.

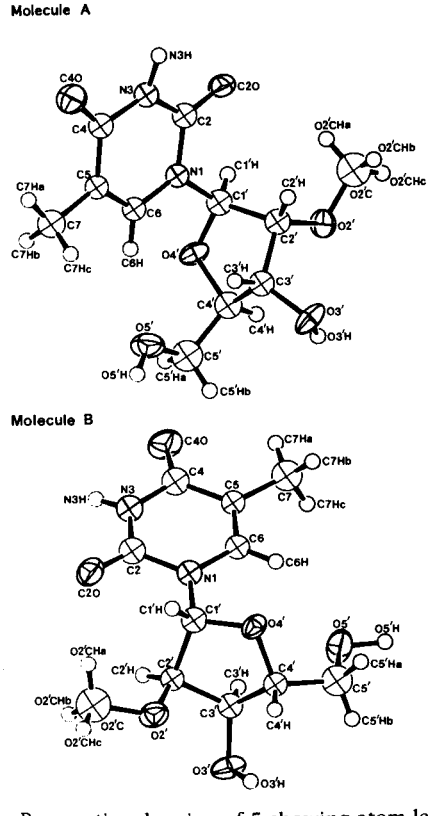


Figure 1. Perspective drawing of 5 showing atom labeling.

Table 1

Crystal and Experimental Data for Compound **5**

A. Crystal Data

Empirical formula	$C_{11}H_{16}N_2O_6$
Formula Weight	272.26
Crystal color, habit	colorless, needle
Crystal dimensions (mm)	0.40 x 0.05 x 0.02
Crystal system	orthorhombic
No. of reflections used for unit cell determination (2θ range)	25 (25.7-49.5°)
Omega scan peak width at half-height	0.41
Lattice parameters:	a = 15.026 (3)Å b = 34.153 (3)Å c = 4.859 (2)Å V = 2493 (1)Å^3
Space group	$P2_12_12_1$
Z value	8
D_{calc}	1.45 g/cm^3
F_{000}	1152 (electrons)
$\mu_{(CuK\alpha)}$	9.71 cm-1

B. Intensity Measurements

Diffractometer	Rigaku AFC6R
Radiation	CuKα (1 = 1.54178Å)
Temperature	23°C
Take-off angle	6.0°
Detector aperture	6.0 mm horizontal 6.0 mm vertical
Crystal to detector distance	40 cm
Scan type	ω–2θ
Scan rate	8.0°/min (in omega) (4 rescans)
Scan width	(0.94 + 0.35 tanθ) °
$2\theta_{max}$	120.2°
No. of reflections measured	Total: 2253
Corrections	Lorentz-polarization Absorption (trans. factor 0.93-1.00) Secondary extinction (coefficient 0.14332E-05)

C. Structure Solution and Refinement

Structure solution	Direct methods
Refinement	Full-matrix least-squares
Function minimized	Σ w (|Fo| - |Fc|)^2
Least-squares weights	$4Fo^2/\sigma^2(Fo^2)$
p-factor	0.025
Anomalous dispersion	All non-hydrogen atoms
No. observations (I>3.00σ(I))	1022
No. variables	214
Reflection/parameter ratio	4.78
Residuals R, R_w	0.053, 0.053
Goodness of fit indicator	1.74
Max. shift/error in final cycle	0.00
Maximum peak in final diff. map	0.26 e$^-$/Å^3
Minimum peak in final diff. map	-0.25 e$^-$/Å^3

The nucleoside **3b** was converted into its 5'-*O*-bis(4-methoxyphenyl)phenylmethyl- [5'-*O*-(4,4'-dimethoxytrityl-, DMT] derivative **4a** (71%) by using the conventional methodology of treating a pyridine solution of **3b** with 4,4'-dimethoxytrityl chloride. To protect the N3 ring nitrogen of the pyrimidine moiety during the alkylation step, a modification [24] of the Michael addition methodology described by Mag and Engels [25] was utilized. Nucleoside **4a**, in refluxing acetonitrile, was treated with one equivalent of acrylonitrile and a catalytic amount of tetrabutylammonium hydroxide. After chromatographic separation from unreacted **4a** (23%), nucleoside **4b** (65%) was obtained and it was characterized by spectral and elemental analyses.

Nucleoside **4b**, in *N*,*N*-dimethylformamide, was treated with one equivalent of sodium hydride and once the sodium salt of **4b** had been formed, an excess of iodomethane was added. From this reaction three nucleosides, in addition to unreacted **4b**, were isolated by column chromatography. Two of the nucleosides were identified by ^{1}H nmr spectral analysis as mono-*O*-methylated derivatives of **4b** and the third was identified as the dimethylated derivative **7c**. The major product from the reaction exhibited an anomeric proton signal ($C_{1'}H$) in its ^{1}H nmr spectrum at δ 5.90, whereas the other mono-*O*-methylnucleoside showed an anomeric proton signal at δ 5.79. The comparative downfield chemical shift for the anomeric proton of the major product confirmed its structure [19,26] as the 2'-*O*-methyl isomer **7a** and thus, the minor mono-*O*-methyl isomer was assigned structure **7b**. The yield of **7a** thus obtained (47%) was significantly higher than that reported (28%) [18] previously wherein no isomers could be obtained in the methylation step.

The N3 protecting group was removed from **7a** by brief treatment with potassium *t*-butoxide and then chromatographic purification yielded compound **6a** (71%). Treatment of **6a** with dilute hydrogen chloride produced the unprotected nucleoside **5** whereas, phosphitylation of **6a** with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite produced the requisite building block **6b** for oligonucleotide synthesis. Nucleotide **6b** was chromatographed to homogeneity and characterized by ^{1}H nmr, ^{31}P nmr, and elemental analyses.

Table 2

Torsion Angles (°) for Compound **5**

Atom (1)	Atom (2)	Atom (3)	Atom (4)	Molecule A		Molecule B	
C20	C2	N3	C4	-180	(1)	177	(1)
C20	C2	N1	C6	-180	(1)	-175	(1)
C20	C2	N1	C1'	-1	(2)	-2	(1)
C40	C4	N3	C2	-179	(1)	178	(1)
C40	C4	C5	C6	-179	(1)	-173	(1)
C40	C4	C5	C7	0	(2)	4	(2)
O4'	C1'	N1	C2	-156.4	(8)	-165.6	(8)
O4'	C1'	N1	C6	22	(1)	8	(1)
O4'	C1'	C2'	O2'	83.8	(9)	85	(1)
O4'	C1'	C2'	C3'	-30	(1)	-29	(1)
O4'	C4'	C3'	O3'	-163.9	(8)	-158.7	(8)
O4'	C4'	C3'	C2'	-38.4	(9)	-33	(1)
O4'	C4'	C5'	O5'	-66	(1)	-67	(1)
O2'	C2'	C1'	N1	-157.2	(7)	-154.8	(8)
O2'	C2'	C3'	O3'	53	(1)	47	(1)
O2'	C2'	C3'	C4'	-72.5	(9)	-79.9	(9)
O3'	C3'	C2'	C1'	166.7	(9)	164.4	(9)
O3'	C3'	C4'	C5'	76	(1)	78	(1)
O5'	C5'	C4'	C3'	51	(1)	53	(1)
N3	C2	N1	C6	5	(1)	7	(1)
N3	C2	N1	C1'	-176.8	(8)	-179.4	(8)
N3	C4	C5	C6	-1	(2)	5	(2)
N3	C4	C5	C7	178.9	(9)	-177.7	(9)
N1	C2	N3	C4	-4	(2)	-5	(2)
N1	C6	C5	C4	2	(2)	-3	(2)
N1	C6	C5	C7	-178	(1)	179	(1)
N1	C1'	O4'	C4'	-113.3	(9)	-113.9	(8)
N1	C1'	C2'	C3'	88.7	(9)	92	(1)
C2	N3	C4	C5	3	(2)	0	(2)
C2	N1	C6	C5	-4	(2)	-3	(2)
C2	N1	C1'	C2'	86	(1)	76	(1)
C5	C6	N1	C1'	177.8	(9)	-176	(1)
C6	N1	C1'	C2'	-95	(1)	-111	(1)
C1'	O4'	C4'	C3'	20	(1)	15	(1)
C1'	O4'	C4'	C5'	146	(1)	143	(1)
C1'	C2'	O2'	O2'C	89.6	(9)	86	(1)
C1'	C2'	C3'	C4'	41	(1)	37	(1)
C2'	C1'	O4'	C4'	7	(1)	9	(1)
C2'	C3'	C4'	C5'	-158.8	(9)	-156.2	(9)
C3'	C2'	O2'	O2'C	-162.1	(9)	164.1	(8)

The sign of the angle is positive if when looking from atom 2 to atom 3 a clockwise motion of atom 1 would superimpose it on atom 4. Estimated standard deviations in the least significant figure are given in parentheses.

Table 3

Intramolecular Bond Angles (°) Involving the Hydrogen Atoms in **5**

Atom	Atom	Atom	Molecule A	Molecule B
C3'	O3'	O3'H	97.58	99.34
C5'	O5'	O5'H	119.75	108.77
C2	N3	N3H	112.89	122.11
C4	N3	N3H	116.80	108.09
N1	C6	C6H	118.79	118.19
C5	C6	C6H	118.79	118.19
C5	C7	C7Hb	109.47	109.47
C5	C7	C7Ha	109.47	109.47
C5	C7	C7Hc	109.47	109.47
C7Hb	C7	C7Ha	109.47	109.47
C7Hb	C7	C7Hc	109.47	109.47
C7Ha	C7	C6Hc	109.47	109.47
O4'	C1'	C1'H	109.95	109.11
N1	C1'	C1'H	109.95	109.11
C2'	C1'	C1'H	109.95	109.11
O2'	C2'	C2'H	112.76	112.10
C1'	C2'	C2'H	112.76	112.10
C3'	C2'	C2'H	112.76	112.10
O3'	C3'	C3'H	108.03	107.55
C2'	C3'	C3'H	108.03	107.55
C4'	C3	C3'H	108.03	107.55
O4'	C4'	C4'H	108.65	107.73
C3'	C4'	C4'H	108.65	107.73
C5'	C4'	C4'H	108.65	107.73
O5'	C5'	C5'Ha	109.17	109.51
O5'	C5'	C5'Hb	109.17	109.51
C4'	C5'	C5'Ha	109.17	109.51
C4'	C5'	C5'Hb	109.17	109.51
C5'Ha	C5'	C5'Hb	109.46	109.46
O2'	O2'C	O2'CHc	109.47	109.47
O2'	O2'C	O2'CHb	109.47	109.47
O2'	O2'C	O2'CHa	109.47	109.47
O2'CHc	O2'C	O2'CHb	109.47	109.47
O2'CHc	O2'C	O2'CHa	109.47	109.47
O2'CHb	O2'C	O2'CHa	109.47	109.47

Table 4
Intramolecular Bond Angles (°) Involving the Nonhydrogen Atoms in **5**

Atom	Atom	Atom	Molecule A		Molecule B	
C1'	O4'	C4'	109.7	(7)	110.4	(7)
C2'	O2'	O2'C	113.6	(8)	112.4	(8)
C2	N3	C4	129.0	(9)	129	(1)
C2	N1	C6	124.1	(9)	119.8	(9)
C2	N1	C1'	115.0	(8)	116.1	(9)
C6	N1	C1'	120.9	(8)	123.8	(8)
C20	C2	N3	124	(1)	126	(1)
C20	C2	N1	124	(1)	120	(1)
N3	C2	N1	111.5	(9)	115	(1)
C40	C4	N3	118	(1)	120	(1)
C40	C4	C5	128	(1)	126	(1)
N3	C4	C5	114	(1)	114	(1)
C4	C5	C6	119.3	(9)	119	(1)
C4	C5	C7	119	(1)	120	(1)
C6	C5	C7	112.1	(9)	121	(1)
N1	C6	C5	122.4	(9)	123.6	(9)
O4'	C1'	N1	109.4	(7)	109.0	(8)
O4'	C1'	C2'	106.4	(7)	106.7	(8)
N1	C1'	C2'	111.2	(9)	113.7	(9)
O2'	C2'	C1'	108.5	(9)	111.8	(9)
O2'	C2'	C3'	109.0	(8)	107.3	(8)
C1'	C2'	C3'	100.3	(8)	100.9	(8)
O3'	C3'	C2'	115.3	(8)	114.5	(7)
O3'	C3'	C4'	115.0	(8)	116.2	(9)
C2'	C3'	C4'	102.0	(9)	103.0	(9)
O4'	C4'	C3'	103.4	(8)	104.7	(8)
O4'	C4'	C5'	109.0	(8)	110.7	(7)
C3'	C4'	C5'	118	(1)	118	(1)
O5'	C5'	C4'	111	(1)	109.3	(9)

Estimated standard deviations in the least significant figure are given in parentheses.

Table 5
Intramolecular Bond Distances (Å)

Atom	Atom	Molecule A	Molecule B
C20	C2	1.20 (1)	1.22 (1)
C40	C4	1.21 (1)	1.21 (1)
O4'	C1'	1.41 (1)	1.41 (1)
O4'	C4'	1.47 (1)	1.46 (1)
O2'	C2'	1.42 (1)	1.40 (1)
O2'	O2'C	1.42 (1)	1.43 (1)
O3'	C3'	1.42 (1)	1.43 (1)
O5'	C5'	1.41 (1)	1.43 (1)
N3	C2	1.39 (1)	1.35 (1)
N3	C4	1.41 (1)	1.39 (1)
N1	C2	1.39 (1)	1.40 (1)
N1	C6	1.37 (1)	1.38 (1)
N1	C1'	1.51 (1)	1.47 (1)
C4	C5	1.44 (1)	1.44 (1)
C5	C6	1.35 (1)	1.34 (1)
C5	C7	1.48 (1)	1.49 (1)
C1'	C2'	1.55 (1)	1.52 (1)
C2'	C3'	1.51 (1)	1.54 (1)
C3'	C4'	1.51 (1)	1.49 (1)
C4'	C5'	1.50 (1)	1.47 (1)
O3'	O3'H	0.936	1.031
O5'	O5'H	0.857	1.071
N3	N3H	1.004	0.887
C6	C6H	0.950	0.950
C7	C7Hb	0.950	0.950
C7	C7Ha	0.950	0.950
C7	C7Hc	0.950	0.950
C1'	C1'H	0.950	0.950
C2'	C2'H	0.950	0.950
C3'	C3'H	0.950	0.950
C4'	C4'H	0.950	0.950
C5'	C5'Ha	0.950	0.950
C5'	C5'Hb	0.950	0.950
O2'C	O2'CHc	0.950	0.950
O2'C	O2CHb	0.950	0.950
O2'C	O2'CHa	0.950	0.950

Estimated standard deviations in the least significant figure are given in parentheses.

The structural assignment of **7a** and **7b** from ^{1}H nmr analysis was based on ample literature precedence [19,26]; however, a more rigorous proof was required and, since it was also important to identify a low-energy conformation for the sugar moiety in **5**, a single crystal X-ray diffraction study of this nucleoside was completed. This analysis confirmed the 2'-oxygen as the site of *O*-methylation. Additionally, the X-ray analysis corroborated the site of ribosylation and the anomeric configuration of all the nucleosides prepared in this study. This analysis further disclosed that in the solid state **5** adopts an *anti*, *N*(3'-*endo*), g$^+$ conformation [27,28].

Crystals (thin needles) of **5** suitable for X-ray diffraction analysis were obtained by the slow evaporation of an aqueous solution of **5**. The structure was solved by direct methods and refined by full-matrix least-squares and difference Fourier methods. Compound **5** crystallized in the chiral space group $P2_12_12_1$. Two unique molecules are present in the asymmetric unit. The two molecules have the same composition and atomic connections but they are oriented differently in three dimensional space in order to form a more compact and thermodynamically stable structure. The same view of the two molecules is shown in the ORTEP drawing, Figure 1. The crystal and experimental data derived from the X-ray diffraction study are summarized in Table 1. Torsion angles are compiled in Table 2, intramolecular bond angles are shown in Tables 3 and 4, and bond distances in compound **5** appear in Table 5.

EXPERIMENTAL

Melting points (mp) were determined with a Thomas-Hoover Unimelt melting point apparatus and are uncorrected. Ultraviolet (uv) spectra were recorded with a Hewlett-Packard 8452 diode array spectrophotometer. Infrared (ir) spectra were recorded with a Perkin-Elmer 1420 ir spectrophotometer. Nuclear magnetic resonance (^{1}H nmr and ^{31}P nmr) spectra were recorded with a Bruker AM400 wide bore nmr spectrometer and the chemical shifts are expressed in δ (parts per million) values relative to tetramethylsilane (internal) for ^{1}H spectra or polyphosphoric acid (external) for ^{31}P spectra (key: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad). Single crystal X-ray diffraction analysis was performed by Molecular Structure Corporation, The Woodlands, TX. Elemental analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ. Thin layer chromatography (tlc) was conducted on aluminum plates coated (0.2 mm) with silica gel 60F_{254} (EM Science) and components were visualized by uv absorbance and/or 10% sulfuric acid in methanol spray followed by heating. Whatman silica gel 60 Å (230-400 mesh) was used for all column chromatographic separations. Evaporations were carried out at a temperature $\leq$35° and

under diminished pressure for solvents with bp $<80°$ or under high vacuum for higher boiling solvents. All chemicals used were reagent grade and were not further dried or purified unless otherwise noted. Tetrabutylammonium hydroxide, 1 *M* in pyridine, was prepared by adding dry (distilled from calcium hydride) pyridine to 1 *M* tetrabutylammonium hydroxide in methanol and then removing the methanol by vacuum distillation.

5-Methyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*,3*H*-pyrimidine-2,4-dione (**3a**).

A mixture of thymine (**1**, 2.52 g, 20 mmoles), acetonitrile (200 ml), 1,1,1,3,3,3-hexamethyldisilazane (8.5 ml, 40.3 mmoles), chlorotrimethylsilane (5.1 ml, 40.2 mmoles), and trimethylsilyl trifluoromethanesulfonate (7.7 ml, 39.8 mmoles) was protected from moisture, stirred, and heated at reflux for 3.5 hours. A solution of trimethylsilyl trifluoromethanesulfonate (4 ml, 20.7 mmoles) in acetonitrile (50 ml) was added and the mixture was stirred and heated at reflux for an additional 0.5 hour. A slurry of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**2**, 11.1 g, 22 mmoles) in acetonitrile (50 ml) was added and the mixture was stirred and heated at reflux for 0.5 hour. The mixture was allowed to cool to below the reflux temperature and then was poured, with stirring, into a solution of sodium hydrogen carbonate (30 g) in water (1 *l*). The aqueous mixture was extracted with ethyl acetate (3 x 100 ml) and then the ethyl acetate extracts were combined and dried (sodium sulfate). The solvent was evaporated and the resulting oil was triturated with diethyl ether (2 x 150 ml). The resulting sticky solid was crystallized from ethanol (100 ml). The crystalline solid was collected by filtration, washed with ethanol (25 ml), then diethyl ether (25 ml), and dried in vacuum, 6.7 g, mp 162-164°. After standing for several hours, a second crop of solid was obtained from the combined diethyl ether triturates, 2.7 g, mp 162-164°. The total yield of **3a** was 9.4 g (16.47 mmoles, 82%). A portion of **3a** (0.9 g) was recrystallized from ethanol (30 ml) and dried in vacuum at 80°, 0.84 g, mp 163-164° (lit mp 164-165° [21], 167-168° [22]); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 1.71 (s, 3 H, C_5CH_3), 4.64-4.81 (m, 3 H, $C_{5'}H_2$ and $C_{4'}H$), 5.93-6.0 (m, 2 H, $C_{3'}H$ and $C_{2'}H$), 6.24 (d, J = 4.1 Hz, 1 H, $C_{1'}H$), 7.44-8.08 (m, 16 H, Ar*H* and C_6H), and 11.60 (s, 1 H, N*H*).

Anal. Calcd. for $C_{31}H_{26}N_2O_9$: C, 65.26; H, 4.59; N, 4.91. Found: C, 65.22; H, 4.57; N, 4.88.

5-Methyl-1-β-D-ribofuranosyl-1*H*,3*H*-pyrimidine-2,4-dione (**3b**).

A mixture of **3a** (42.06 g, 73.7 mmoles), methanol (1.2 *l*), and anhydrous sodium methoxide (12 g, 222 mmoles) was protected from moisture and stirred at ambient temperature for 24 hours. Amberlite IR 120(H$^+$) (105 g, washed with water, then methanol) was added and the mixture was stirred until neutral. The resin was removed by filtration and washed with methanol (2 x 200 ml), adding the washes to the filtrate. The methanol solution was evaporated and the residue was triturated with ethyl acetate (2 x 100 ml). The residue was stirred with warm ethanol (300 ml) until a homogeneous mixture was obtained. After cooling (5°) for 16 hours, the solid was collected by filtration and dried in vacuum over phosphorus pentoxide, 17.11 g (66.3 mmoles, 90%). A sample was crystallized from ethanol and dried in vacuum at 80°, mp 182-184° (lit mp 183-184° [23]); uv (*p*H 1): λ max 210 nm (ε 8,980), 268 nm (ε 9,470); (methanol): λ max 214 nm (ε 3,580), 268 nm (ε 8,590); (*p*H 11): λ max 230 nm (ε 6,620), 266 nm (ε 7,550); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 1.77 (s, 3 H, C_5CH_3), 5.78 (d, J = 5.5 Hz, 1 H, $C_{1'}H$), 7.76 (s, 1 H, C_6H), 11.30 (br s, 1 H, N*H*), and other sugar protons.

Anal. Calcd. for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.85. Found: C, 46.33; H, 5.38; N, 10.77.

1-{5-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-β-D-ribofuranosyl}-5-methyl-1*H*,3*H*-pyrimidine-2,4-dione Hemihydrate (**4a**).

Compound **3b** (2.0 g, 7.75 mmoles) was dried by repeated coevaporation with anhydrous pyridine (2 x 10 ml) and then was dissolved in anhydrous pyridine (30 ml). 4,4'-Dimethoxytrityl chloride (3.15 g, 9.29 mmoles) was added, with stirring, in three portions at 30 minute intervals. The mixture was protected from moisture and stirred at ambient temperature for an additional 1.25 hours. The mixture was diluted with dichloromethane (100 ml) and the solution was washed with a saturated, aqueous, sodium hydrogen carbonate solution (50 ml). The aqueous layer was extracted with dichloromethane (25 ml) and the dichloromethane solutions were combined and dried (sodium sulfate). The solution was evaporated and the residue was coevaporated ($<35°$) with toluene (2 x 100 ml) to remove residual pyridine. The residue was dissolved in dichloromethane (50 ml) and the solution was applied to a silica gel column (2.5 x 29 cm). The column was flash eluted with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in 1): (0, 0.5), (1, 0.5), (2, 1), (3, 0.5). Eluate containing the major homogeneous product was evaporated to obtain a yellow foam. Eluate containing a mixture of products was rechromatographed by an identical procedure. All of the product was combined; 3.14 g (5.51 mmoles, 71%). This material was of sufficient purity for use in subsequent syntheses.

A portion of the product (0.8 g) was again chromatographed on an identical silica gel column using progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in 1): (0, 0.5), (0.5, 1), (1, 1), (2, 1.5) as the eluting solvent. Evaporation of the eluate containing **4a** gave a yellow foam which was dried in vacuum for 16 hours; 0.4 g, mp $>110°$, broad range; uv (methanol): λ max 236 nm (ε 25,300), 270 nm (ε 12,900); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 1.14 (s, 3 H, C_5CH_3), 3.18-3.26 (m, 2 H, $C_{5'}H_2$), 3.73 (s, 6 H, 2 $ArOCH_3$), 3.95-3.98 (m, 1 H, $C_{4'}H$), 4.10-4.13 (m, 1 H, $C_{3'}H$), 4.16-4.21 (m, 1 H, $C_{2'}H$), 5.15 (d, 1 H, $C_{3'}OH$), 5.45 (d, 1 H, $C_{2'}OH$), 5.80 (d, J = 5.2 Hz, 1 H, $C_{1'}H$), 7.22-7.40 (m, 13 H, Ar*H*), 7.50 (s, 1 H, C_6H), and 11.3 (s, 1 H, N*H*).

Anal. Calcd. for $C_{31}H_{32}N_2O_8 \cdot 0.5H_2O$: C, 65.37; H, 5.84; N, 4.92. Found: C, 65.34; H, 5.70; N, 4.74.

3-(2-Cyanoethyl)-1-{5-*O*-[bis(4-methoxyphenyl)phenylmethyl]-β-D-ribofuranosyl}-5-methyl-1*H*,3*H*-pyrimidine-2,4-dione Hemihydrate (**4b**).

Compound **4a** (12.41 g, 21.8 mmoles) was dissolved in acetonitrile (250 ml). Acrylonitrile (1.6 ml, 24.3 mmoles) and a 1 *M* solution of tetrabutylammonium hydroxide in pyridine (0.5 ml) were added. The reaction mixture was protected from moisture, stirred, and heated at reflux for 2 days. The reaction mixture was allowed to cool to ambient temperature and then poured, with stirring, into a mixture of saturated, aqueous, sodium hydrogen carbonate (500 ml), water (500 ml), and dichloromethane (250 ml). The aqueous layer was separated and extracted with additional dichloromethane (100 ml). The dichloromethane solutions were combined, washed with water (500 ml), dried (magnesium sulfate), and evaporated to obtain a yellow foam. The foam was dissolved in dichloromethane (50 ml) and the solution was applied to a silica gel column (5.5 x 30 cm). The column was flash eluted with

progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in 1): (0, 1), (0.5, 5), (1, 2), (2, 3), (5, 2). Eluate containing the product **4b** was evaporated and the residue was dried in vacuum; 8.8 g (14.1 mmoles, 65%). Additionally, 3.11 g of unreacted **4a** was recovered.

A chromatographically and spectroscopically identical sample of **4b** obtained from an exploratory reaction was dried in vacuum for 2 days, mp >95°, broad range; ir (nujol): ν 2255 (CN) cm^{-1}; uv (methanol): λ max 234 nm (ϵ 26,500), 270 nm (ϵ 12,700); ^1H nmr (dimethyl sulfoxide-d_6): δ 1.44 (s, 3 H, C_5CH_3), 2.63 (t, 2 H, CH_2CN), 3.18-3.29 (m, 2 H, $C_{5'}H_2$), 3.74 (s, 6 H, 2 $ArOCH_3$), 3.99-4.01 (m, 1 H, $C_{4'}H$), 4.07 (t, 2 H, NCH_2), 4.11-4.15 (m, 1 H, $C_{3'}H$), 4.17-4.20 (m, 1 H, $C_{2'}H$), 5.19 (d, 1 H, $C_{3'}OH$), 5.51 (d, 1 H, $C_{2'}OH$), 5.83 (d, 1 H, J = 4.8 Hz, $C_{1'}H$), 6.89-7.40 (m, 13 H, Ar*H*), and 7.60 (s, 1 H, C_6H).

Anal. Calcd. for $C_{34}H_{35}N_3O_8 \cdot 0.5H_2O$: C, 65.58; H, 5.83; N, 6.75. Found: C, 65.70; H, 5.79; N, 6.66.

3-(2-Cyanoethyl)-1-{5-*O*-[bis(4-methoxyphenyl)phenylmethyl]-2-*O*-methyl-β-D-ribofuranosyl}-5-methyl-1*H*,3*H*-pyrimidine-2,4-dione Hemihydrate (**7a**).

A mixture of **4b** (6.3 g, 10.1 mmoles) and anhydrous *N,N*-dimethylformamide (40 ml) was protected from moisture, stirred, and cooled to -40°. Sodium hydride (80% dispersion in mineral oil, 0.31 g, 10.3 mmoles) was added and the mixture was maintained at -40° ±5° for 0.75 hour. Iodomethane (1.2 ml, 19.3 mmoles) was added, with stirring, and the temperature was maintained at -30° to -40° for 0.75 hour. The mixture was allowed to warm to ambient temperature and stirred for 4 hours. Ammonium chloride (5 g) was added and, after an additional 5 minutes of stirring, the reaction mixture was poured, with stirring, into a mixture of water (200 ml) and dichloromethane (200 ml). The layers were separated and the aqueous layer was extracted with dichloromethane (200 ml). The dichloromethane solutions were combined, washed with water (100 ml), dried (magnesium sulfate), and evaporated. The oily residue was dissolved in dichloromethane (50 ml) and the solution was applied to a silica gel column (7.6 x 30 cm). The column was eluted (gravity flow) with progressively increasing concentrations of diethyl ether in dichloromethane (% diethyl ether, volume in 1): (0, 2), (1, 1), (2, 3), (3, 3), (5, 3), (10, 2), (20, 1), (30, 1). Eluate containing the homogeneous major product was evaporated and the residue was dried in vacuum for 2 days, 3.03 g (4.76 mmoles, 47%).

A chromatographically and spectroscopically identical sample of **7a** obtained from an exploratory reaction was repeatedly triturated (by sonication) with warm hexanes (2 x 100 ml, 2 x 10 ml) to obtain a white solid free of residual *N,N*-dimethylformamide, mp >75°, broad range; ir (nujol): ν 2255 (CN) cm^{-1}; uv (methanol): λ max 232 nm (ϵ 23,000), 270 nm (ϵ 16,100); ^1H nmr (dimethyl sulfoxide-d_6): δ 1.46 (s, 3 H, C_5CH_3), 2.64 (t, 2 H, CH_2CN), 3.27-3.33 (m, 2 H, $C_{5'}H_2$), 3.44 (s, 3 H, $C_{2'}OCH_3$), 3.74 (s, 6 H, 2 $ArOCH_3$), 3.90-3.93 (m, 1 H, $C_{4'}H$), 4.01-4.03 (m, 1 H, $C_{3'}H$), 4.06-4.11 (m, 2 H, NCH_2), 4.20-4.30 (m, 1 H, $C_{2'}H$), 5.26 (d, 1 H, $C_{3'}OH$), 5.90 (d, J = 3.1 Hz, 1 H, $C_{1'}H$), 6.89-7.42 (m, 13 H, Ar*H*), and 7.61 (s, 1 H, C_6H).

Anal. Calcd. for $C_{35}H_{37}N_3O_8 \cdot 0.5H_2O$: C, 66.03; H, 6.01; N, 6.60. Found: C, 65.87; H, 6.30; N, 6.55.

1-{5-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-2-*O*-methyl-β-D-ribofuranosyl}-5-methyl-1*H*,3*H*-pyrimidine-2,4-dione Monohydrate (**6a**).

Compound **7a** (0.8 g, 1.26 mmoles) was dissolved in dichloromethane (50 ml) and anhydrous potassium *t*-butoxide (0.39 g, 3.48 mmoles) was added. The mixture was protected from moisture and stirred at ambient temperature for 1.5 hours. The reaction mixture was poured, with stirring, into a saturated, aqueous, sodium hydrogen carbonate solution (200 ml). The layers were separated and the aqueous layer was exracted with dichloromethane (50 ml). The dichloromethane solutions were combined, dried (sodium sulfate), and evaporated to obtain a yellow foam. The foam was dissolved in dichloromethane (10 ml) and the solution was applied to a silica gel column (3.5 x 38 cm). The column was flash eluted with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in 1): (0, 0.5), (1, 1.5), (2, 0.5), (3, 1). Eluate containing the major product was evaporated and the residue was dried in vacuum for 1 day, 0.53 g (0.894 mmoles, 71%), mp >105°, broad range; uv (methanol): λ max 234 nm (ϵ 22,100), 270 nm (ϵ 11,300); ^1H nmr (dimethyl sulfoxide-d_6): δ 1.42 (s, 3 H, C_5CH_3), 3.19-3.30 (m, 2 H, $C_{5'}H_2$), 3.35 (s, 3 H, $C_{2'}OCH_3$), 3.74 (s, 6 H, 2 $ArOCH_3$), 3.87-3.89 (m, 1 H, $C_{4'}H$), 3.95-4.00 (m, 1 H, $C_{3'}H$), 4.22-4.25 (m, 1 H, $C_{2'}H$), 5.20 (d, 1 H, $C_{3'}OH$), 5.85 (d, J = 4.8 Hz, 1 H, $C_{1'}H$), 6.88-7.40 (m, 13 H, Ar*H*), 7.44 (s, 1 H, C_6H), and 11.35 (br s, 1 H, N*H*).

Anal. Calcd. for $C_{32}H_{34}N_2O_8 \cdot H_2O$: C, 64.85; H, 6.12; N, 4.73. Found: C, 65.20; H, 6.01; N, 4.59.

1-{5-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-2-*O*-methyl-β-D-ribofuranosyl}-5-methyl-1*H*,3*H*-pyrimidine-2,4-dione 3'-[2-Cyanoethyl Bis(1-methylethyl)]phosphoramidite (**6b**).

Compound **6a** (1 g, 1.69 mmoles) was placed in a septum sealed flask and the flask was flushed with dry argon. Diisopropylethylamine (1.2 ml, distilled from calcium hydride and stored under argon) was added followed by the addition of tetrahydrofuran (20 ml, distilled from lithium aluminum hydride and stored under argon). The mixture was stirred and 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.55 ml, 2.47 mmoles) was added. The course of the reaction was monitored by tlc and additional portions of 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.2 ml, 0.9 mmole; 0.1 ml, 0.45 mmole) were added over a total reaction time of 4.5 hours or until all of **6a** was consumed. The reaction mixture was diluted with ethyl acetate (150 ml) and then washed with a saturated, aqueous, sodium hydrogen carbonate solution (50 ml). The ethyl acetate solution was dried (magnesium sulfate) and evaporated. The residue was dissolved in a dichloromethane:ethyl acetate:triethylamine (70:29:1, v/v/v) mixture (20 ml) and the solution was applied to a silica gel (washed thoroughly with dichloromethane:ethyl acetate:triethylamine, 70:29:1, v/v/v) column (2.5 x 22 cm). The column was eluted (gravity flow) with the same solvent mixture and eluate containing **6b** was evaporated to give a heavy oil. The oil was dissolved in dichloromethane (7 ml) and the solution was added slowly to rapidly stirred, cold (dry ice-acetone bath) pentane (400 ml). The supernatant was decanted from the precipitated solid and the later was dissolved in dichloromethane (50 ml). The dichloromethane solution was evaporated and the residue was dried in vacuum for 2 days to obtain a sticky glass. This material was rechromatographed as described above using a 2.5 x 30 cm column and dichloromethane:ethyl acetate:triethylamine (80:19:1, v/v/v) as the eluting solvent. Eluate containing only **6b** was evaporated and the residue was dried in vacuum for 2 days to obtain a foam; 0.99 g (1.29 mmoles, 75%). ^{31}P nmr and ^1H nmr confirmed structure **6b**; however, the ^1H nmr showed the presence of some

residual organic solvents.

A chromatographically and spectroscopically identical sample of **6b** obtained from a exploratory reaction was continuously dried in vacuum until a hard foam free of residual solvents was obtained; ^{31}P nmr (acetonitrile-d_3): δ 150.99; ^{31}P nmr (acetone-d_6): δ 151.48 and 151.66; ^{1}H nmr (acetonitrile-d_3): δ 1.02-1.43 (m, 12 H, 2 NCH(CH_3)$_2$), 1.41 and 1.43 (2 s, 3 H, C_5CH_3), 2.48 (t, 2 H, CH_2CN), 2.64-2.68 (m, 2 H, 2 NC*H*(CH_3)$_2$), 3.27-3.35 (m, 2 H, $C_{5'}H_2$), 3.47-3.50 (2 s, 3 H, OCH_3), 3.55-3.63 (m, 2 H, OCH_2CH_2-CN), 3.75 and 3.76 (2 s, 6 H, ArOCH_3), 3.99-4.02 (m, 1 H, $C_{4'}H$), 4.12-4.20 (m, 1 H, $C_{3'}H$), 4.43-4.53 (m, 1 H, $C_{2'}H$), 5.89-5.93 (m, 1 H, $C_{1'}H$), 6.85-7.48 (m, 13 H, Ar*H*), 7.516 and 7.519 (2 s, 1 H, C_6H), and 9.10 (s, 1 H, N*H*).

Anal. Calcd. for $C_{41}H_{51}N_4O_9P$: C, 63.55; H, 6.63; N, 7.23; P, 4.00. Found: C, 63.20; H, 6.51; N, 7.13; P, 3.91.

1-(2-*O*-Methyl-β-D-ribofuranosyl)-5-methyl-1*H*,3*H*-pyrimidine-2,4-dione (**5**).

A mixture of **6a** (0.2 g, 0.337 mmole), dichloromethane (10 ml), and 10% hydrogen chloride in methanol (1 ml) was protected from moisture and stirred at ambient temperature for 0.75 hour. A solution of ammonia in methanol (1 ml, saturated at 0°) was added and the mixture was evaporated. The residue was stirred with 5% methanol in dichloromethane (20 ml) and the mixture was applied to a silica gel column (3.5 x 25 cm). The column was flash eluted with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (0, 0.5), (1, 1), (2, 0.5), (3, 0.5), (5, 0.5), (10, 1). Eluate containing pure **5** was evaporated and the residue was dried over phosphorus pentoxide and in vacuum at 56° for 1 day, 37 mg (0.136 mmole, 40%), mp 189-191° (lit mp 197-198° [18]); uv (*p*H 1): 210 nm (ε 8,930), 268 nm (ε 9,520); (methanol): 214 nm (ε 1,230), 268 nm (ε 8,290); (*p*H 11): 232 nm (ε 6,600), 268 nm (ε 7,710); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 1.77 (s, 3 H, C_5CH_3), 3.35 (s, 3 H, $C_{2'}OCH_3$), 3.55-3.68 (m, 2 H, $C_{5'}H_2$), 3.78-3.81 (m, 1 H, $C_{4'}H$), 3.81-3.85 (m, 1 H, $C_{3'}H$), 4.12-4.16 (m, 1 H, $C_{2'}H$), 5.09 (d, 1 H, $C_{3'}OH$), 5.13 (t, 1 H, $C_{5'}OH$), 5.86 (d, J = 5.5 Hz, 1 H, $C_{1'}H$), 7.78 (s, 1 H, C_6H), and 11.24 (s, 1 H, N*H*).

Anal. Calcd. for $C_{11}H_{16}N_2O_6$: C, 48.53; H, 5.92; N, 10.29. Found: C, 48.61; H, 6.03; N, 9.89.

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