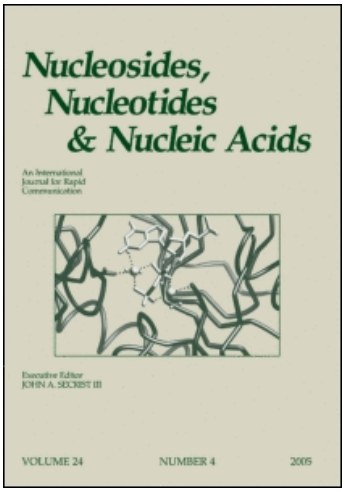


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

New Methods for the Synthesis of Base Sensitive Cyanopyrimidine and Cyanopyridine Nucleosides

Celeste A. Schlieper[a]; James Wemple[a]
[a] SDS Biotech Corporation, Painesville

To cite this Article Schlieper, Celeste A. and Wemple, James(1984) 'New Methods for the Synthesis of Base Sensitive Cyanopyrimidine and Cyanopyridine Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 3: 4, 369 — 388
To link to this Article: DOI: 10.1080/07328318408081276
URL: http://dx.doi.org/10.1080/07328318408081276

NUCLEOSIDES & NUCLEOTIDES, 3(4), 369-388 (1984)

NEW METHODS FOR THE SYNTHESIS OF BASE SENSITIVE CYANOPYRIMIDINE AND CYANOPYRIDINE NUCLEOSIDES

Celeste A. Schlieper, James Wemple* (1)

SDS Biotech Corporation, World Headquarters
7528 Auburn Rd, Painesville, Ohio 44077

Abstract: Four cyanonucleosides, 6, 11, 16 and 21 have been prepared using three synthetic methods that are particularly suited to the preparation of base-sensitive nucleosides.

The attachment of the cyano group to purine and pyrimidine bases has been an important objective for nucleoside chemists due to its small size and unusual chemical properties.(2) The cyano group is considered a bioisostere of the carbonyl oxygen atom.(3) In our work we have become interested in the possibility of replacing the carbonyl oxygen with cyano in pyrimidine as well as purine nucleosides. In this connection analogs of uridine, cytidine, thymidine and guanosine are of interest. A few cyanonucleosides are known such as 5-cyanouridine(4) in which the thymine methyl group is replaced by cyano. The 6-amino group of adenosine has been replaced by cyano in work reported recently involving CuCN induced displacement of the 6-methylsulfonyl group(5a) or in much better yield the 6-iodo group(5b) of purine ribosides.

The major obstacle to preparation of cyanonucleosides is the instability of the cyano group under conditions normally employed in deblocking riboside derivatives. An illustration was encountered by Townsend and co-workers(6) when they attempted to prepare 6-azatoyocamycin by reaction

0732-8311/84/0304-0369$3.50/0

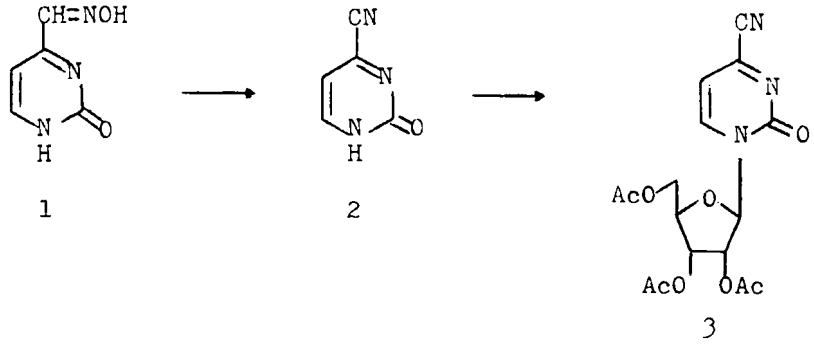
of N-acetyl-2',3',5'-tri-O-acetyl-6-azatoyocamycin with ammonia or sodium methoxide in methanol. In these attempts, the nitrile group was converted to an amidine in the presence of ammonia and to the O-methyl carboximidate in the presence of sodium methoxide. These workers succeeded in preparing 6-azatoyocamycin by conversion of the carboximidate to the corresponding thioamide using NaSH, followed by desulfurization with $HgCl_2$-triethylamine. An interesting result was encountered by Fox and co-workers when they obtained cytidine from the reaction of 2-oxo-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-4-pyrimidinecarbonitrile with ammonia.[7] In this instance apparently cyanide serves as a leaving group in a nucleophilic displacement reaction. Yet another reaction mode was encountered when 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine-6-carbonitrile was treated with ammonia resulting in an unusual rearrangement to N^4-(β-D-ribofuranosyl)pyrimido[4,5-d]pyrimidine-4,8-diamine.[8] However, in certain cases in which a relatively stable nitrile group is involved it is possible to prepare the cyanonucleoside system by deblocking the corresponding riboside triacetate derivative using the usual NaOMe-MeOH[4] or NH_3-MeOH[9] conditions.

In view of the presence of the cyano group in the biologically active nucleoside antibiotic, toyocamycin,[9] as well as in the antibacterially active mesoionic nucleoside recently obtained in our work,[10] we were interested in finding new methods for the preparation of cyano substituted nucleosides. Three such methods have now been developed. The first uses a mild $NaHCO_3$-MeOH procedure for deblocking of cyanonucleoside triacetates.[10] The second method involves $(CF_3CO)_2O$-pyridine induced dehydration of a deblocked nucleoside carboxaldehyde oxime derivative and the last procedure uses a similar $(CF_3CO)_2O$-triethylamine induced dehydration of a deblocked nucleoside carboxamide. The critical feature of these oxime and amide dehydration reactions is the use of the reagent $(CF_3CO)_2O$ for generating a labile tris trifluoroacetate derivative of the ribose moiety which makes possible the use of very mild deblocking

conditions in the final step leading to the free cyanonucleoside. Using these methods, four cyanonucleosides have been prepared. Thus, the cyano group was introduced in place of the carbonyl oxygen atom at the 4-position of the pyrimidine nucleosides uridine and also 3-deaza-5-azauridine. The 4-hydroxy substituent of the antibacterially active 3-deazauridine[11] was replaced by the cyano function. Finally, the 2-deaza isostere of the antibacterially active 4-cyano-3-oxido-1-(β-D-ribofuranosyl)-pyridazinium[10] was synthesized. Of importance as potential starting materials for the preparation of these nucleosides, were the corresponding cyanopyrimidine and cyanopyridine bases. To our surprise, none of these cyano substituted heterocycles had been reported. Methods were developed for the preparation of three of these nitriles, including one cyanopyrimidine and two cyanopyridine bases.

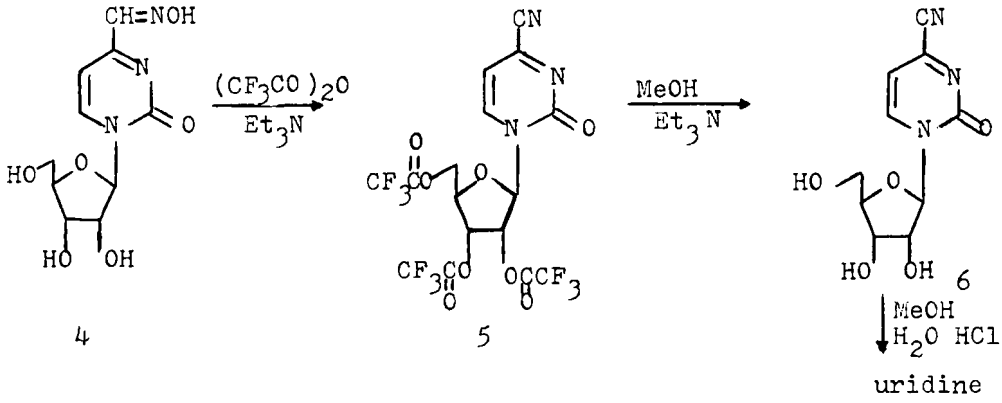
RESULTS AND DISCUSSION

1,2-Dihydro-2-oxo-1-(β-D-ribofuranosyl)pyrimidine-4-carbonitrile (6) serves as a close structural relative of both uridine and cytidine. This compound bears a reactive electrophilic site at the 4 position of the pyrimidine ring and thus has the potential to undergo covalent binding to target macromolecules. The triacetate derivative 3, has been prepared previously in a 4 step sequence starting with 2-hydroxy-4-methylpyrimidine.[7] We considered the possibility of direct synthesis of this triacetate from the cyanopyrimidine base, 2, using the silyl Hilbert-Johnson reaction.[12] The desired nitrile, 2, was readily prepared by dehydration of the known[13] 2-hydroxypyrimidine-4-carboxaldehyde oxime (1) using $(CF_3CO)_2O$-Et_3N. This compound evolves HCN on standing in air at room temperature attesting to the electrophilic reactivity at the 4-position. However, it is sufficiently stable to withstand a rapid recrystallization from aqueous-ethanol which was found to be the most convenient way of purifying this nitrile. In this manner analytically pure 2 was obtained. Ribosylation of 2 using the silyl Hilbert-Johnson procedure went smoothly affording only one detectable isomer which was obtained in 73% purified yield. The 1,2-



dihydro-2-oxo-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-pyrimidine-4-carbonitrile structural assignment, 3, was confirmed by conversion of 3 to uridine upon treatment with HCl and water in methanol.[7] Attempts to deblock the triacetate to give the desired cyanonucleoside, 6, using the $NaHCO_3$-MeOH or other deblocking conditions were unsuccessful.

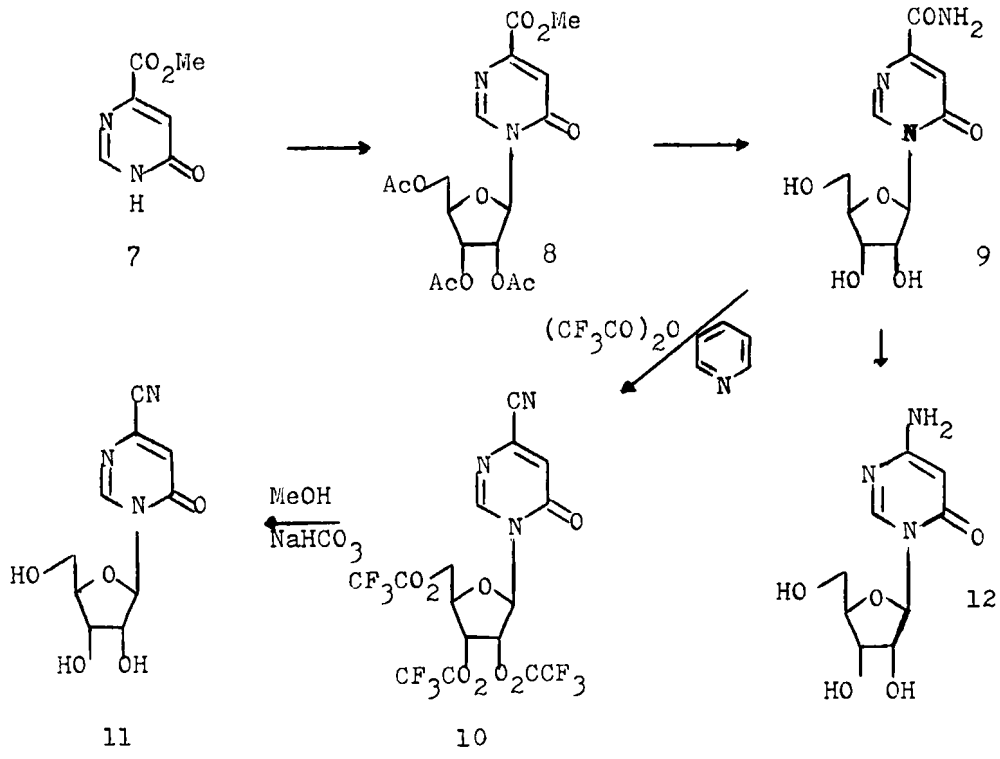
An alternative approach to this cyanonucleoside was considered involving dehydration of the known nucleoside carboxaldehyde oxime, 4.[7] Such a dehydration procedure has previously been reported using acetic anhydride as the dehydrating reagent. However, this method, of course, also results in acetylation of the three ribose alcohol groups leading to the same nucleoside triacetate, 3, that we have now prepared by the silyl Hilbert-Johnson procedure. Use of trifluoroacetic anhydride as the oxime dehydrating reagent,[14][15] would result in formation of the tris trifluoroacetate derivative, 5. The trifluoroacetate groups were easily cleaved under mild conditions - for example by simple treatment with methanol at low temperature. Triethylamine was added to neutralize any acid produced in the reaction. The triethylammonium-trifluoroacetate salt could be removed owing to its greater solubility in chloroform-hexanes. Attempts to separate the salt by column chromatography on silica gel resulted in some decomposition of the cyanonucleoside. The purified cyanonucleoside, 6, as reported[7] earlier for the triacetate derivative, 3, did not give any appreciable nitrile absorption in the IR spectrum. However, the odor of HCN was detected upon treatment of 6 with hydrochloric acid in aqueous methanol and uridine was isolated from this reaction.



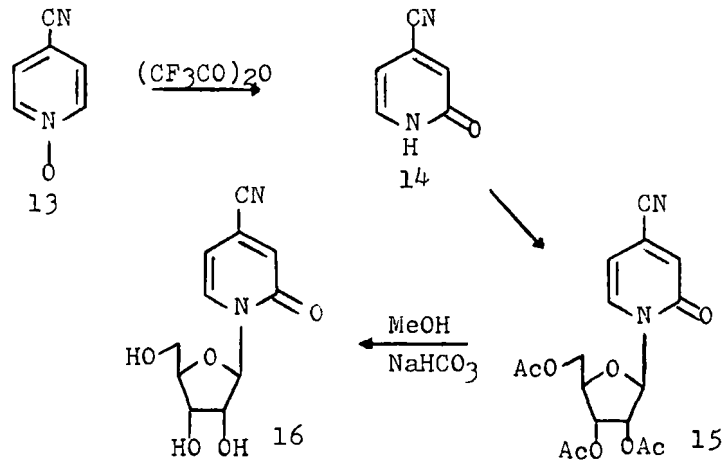
4 5 6

Treatment of methyl 6-hydroxypyrimidine-4-carboxylate (7)[17] with hexamethyldisilazane followed by ribose tetraacetate and $SnCl_4$ gave only one nucleoside derivative in high yield according to NMR and TLC analysis of the crude reaction product. This is believed to be the 4-carbomethoxy-1-(β-D-ribofuranosyl)-6-pyrimidone derivative, 8, rather than the 3-(β-D-ribofuranosyl) isomer based on literature precedent with related silyl Hilbert-Johnson reactions.[18][19] Treatment of this material with methanolic ammonia gave cleanly the deacetylated amide, 9. The structure of this amide was confirmed by subjecting it to the Hofmann rearrangement with sodium hypochlorite resulting in formation of the known[19] 3-deaza-5-azacytidine (12). The required cyanonucleoside, 11, was prepared by treatment of the amide with trifluoroacetic anhydride and pyridine[20] in dioxane followed by methanoylsis of the labile tris(trifluoroacetate) derivative, 10, using methanol-sodium bicarbonate. This cyanonucleoside was sufficiently stable to withstand purification by column chromatography on silica gel in contrast to our experience with 6.

Although examples are known in which NH_3-MeOH or NaOMe-MeOH are effective in deblocking cyanonucleoside triacetates,[4][9] many instances have been observed in which treatment of an acylated cyanonucleoside with NH_3-MeOH or NaOMe-MeOH did not give rise to the corresponding deblocked cyanonucleoside.[6-8][10][21] We have found that the $NaHCO_3$-MeOH approach is preferable to NaOMe-MeOH or NH_3-MeOH in the synthesis of cyano substituted nucleosides[10] and also certain



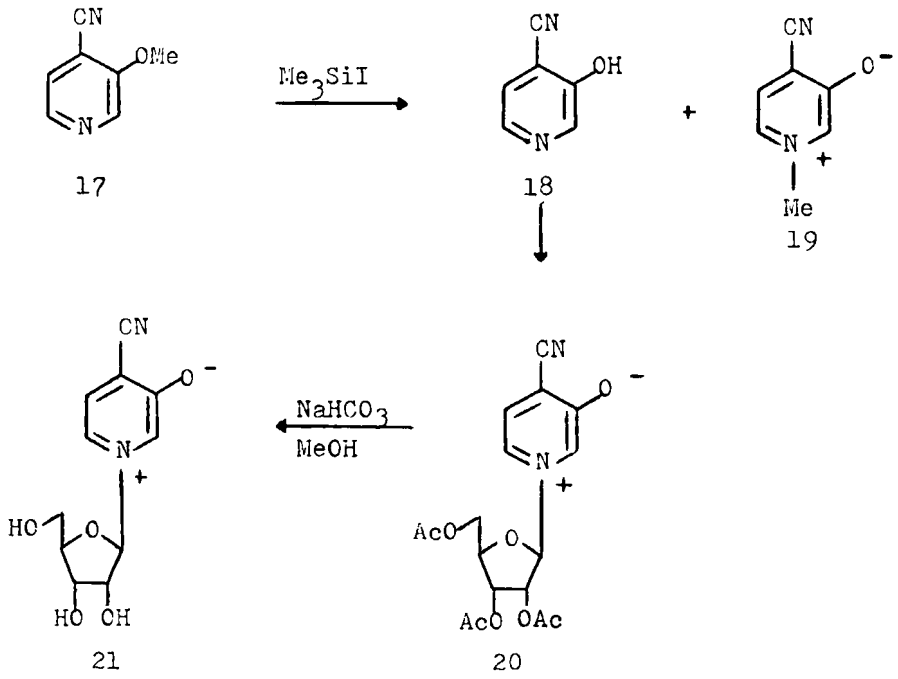
other base sensitive nucleoside systems. Two illustrations of the $NaHCO_3$-MeOH method for deblocking cyanonucleoside triacetates are examined here. 1,2-Dihydro-2-oxo-1-(β-D-ribofuranosyl)pyridine-4-carbonitrile (16) serves as a close structural relative of 3-deazauridine, a potent antibacterial nucleoside.[11] The required cyanopyridine base, 14, was prepared by a trifluroracetic anhydride induced rearrangement of the commercially available and relatively inexpensive 4-cyanopyridine-N-oxide (13). The cyanopyridone, 14, was converted to the riboside triacetate derivative, 15, using the silyl Hilbert-Johnson procedure. Deblocking of this triacetate using $NaHCO_3$-MeOH at room temperature gave a mixture of two compounds according to TLC analysis. The desired cyanonucleoside, 16, was obtained crystalline. The mother liquors from these crystals were subjected to column chromatography on silica gel eluting with $CHCl_3$-MeOH affording additional cyanonucleoside, 16. The lower R_f by-product underwent substantial decomposition during the chromatographic separation. Although this compound was not obtained



analytically pure, NMR and IR evidence points to an O-methyl carboximidate derivative.

We were interested in preparing new mesoionic systems bearing cyano substituents. It appeared that the 4-cyano-3-oxidopyridinium riboside as well as the 4-cyano-5-oxidopyrimidinium riboside triacetates should be accessable using the silyl Hilbert-Johnson approach. Initially we set out to prepare the pyridine system.[10] The required 3-hydroxypyridine-4-carbonitrile (18) starting material was unknown. Recently an efficient synthesis of 3-methoxypyridine-4-carbonitrile (17) has been reported.[22] Treatment of this compound with iodotrimethylsilane[23] gave two compounds of interest including the desired 3-hydroxypyridine-4-carbonitrile (18) and the betaine, 19, which is presumably formed by reaction of 18 with methyl iodide generated <u>in situ</u> during the course of the reaction. UV data obtained for these structures including the N-methyl betaine, 19, was useful in providing an unequivicol structural assignment for the betaine riboside, 21. The silyl Hilbert-Johnson procedure was effective in the synthesis of the triacetate, 20. A high yield (81%) was also achieved in the $NaHCO_3$-MeOH induced deblocking step although some decomposition was noted in this procedure resulting in the formation of a deep red reaction color.

The four nucleosides prepared in this work, 6, 11, 16 and 21 were evaluated for antibacterial activity <u>in vitro</u> against <u>E. coli</u> and <u>in vivo</u> against a systemic <u>E. coli</u> infection in mice and were found to be inactive.



EXPERIMENTAL

Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. Routine NMR spectra were obtained on a Hitachi Perkin-Elmer R-24B spectrophotometer. High resolution 90 MHz NMR spectra were obtained on a Varian EM-390 spectrometer or a Bruker WH90 spectrometer. Infrared spectra were recorded on a Perkin-Elmer 297 spectrophotometer and UV spectra on a Cary 118 UV-VIS spectrophotometer. E. Merck silica gel 60 (70-230 mesh) was used for column chromatography. Reagent grade solvents were obtained from commercial sources and used without further purification. When necessary solvents or reagents were dried by appropriate methods. Methanol was dried by distillation from magnesium. DMF was dried by distillation from calcium hydride. Dioxane was dried by distillation from potassium metal. Pyridine and triethylamine dried by storage over potassium hydroxide, and 1,2-dichloroethane was dried by distillation from phosphorous pentoxide.

1,2-Dihydro-2-oxopyrimidine-4-carbonitrile (2): 1,2-Dihydro-2-hydroxypyrimidine-4-carboxaldehyde oxime (1)[13] (62.5 g) was added to anhydrous dioxane (400 ml) and the mixture cooled in an ice bath. Trifluoroacetic anhydride (134 ml) was added (2 min)and the mixture stirred 15 min at 0-5°. Triethylamine (198ml) was added dropwise (1 h) with cooling. The clear red solution was allowed to stir 3 h at room temperature and then placed in the refrigerator overnight. The dioxane was removed under reduced pressure and the residue dissolved in chloroform (500 ml). Methanol (20 ml) was added. The solution was cooled and filtered to give a pink solid (44 g). Although some decomposition of the product takes place on warming in water, it was possible to improve the purity by recrystallization from aqueous ethanol. The crystals were washed with ethanol and ether and dried under vacuum at 70° for 6 h to give 2 (32 g, 59%): mp: blackens-decomposes at approximately 200°; ^{1}H NMR (DMSOd_6, TMS): δ 12.6 (broad singlet, 1H, D_2O exchangeable) 8.37 (d, 1H, J=6Hz); 7.00 (d, 1H, J=6Hz); IR (KBr): 3300-2600, 2255, 1645, 1620, 1595 cm^{-1}. Anal Calcd for $C_5H_3N_3O$: C, 49.59; H, 2.50; N, 34.70. Found: C, 49.7; H, 2.2; N, 34.4.

1,2-Dihydro-2-oxo-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-pyrimidine-4-carbonitrile (3): 1,2-Dihydro-2-oxo-pyrimidine-4-carbonitrile (2, 0.61 g) and $(NH_4)_2SO_4$ (50 mls) were added to hexamethyldisilazane (10 ml) and the mixture heated at reflux for 2.5 h. The excess HMDS was removed under reduced pressure and the residual oil dissolved in 1,2-dichloroethane (15 ml). Ribose tetraacetate (1.60 g) was added followed by $SnCl_4$ (0.65 ml). The solution became warm. It was stirred 2 h at room temperature and then poured into saturated aqueous $NaHCO_3$ (150 ml) and CH_2Cl_2 (150 ml). The aqueous layer was extracted with CH_2Cl_2 (2x150 ml) and the organic layers combined, dried (Na_2SO_4) and concentrated to an oil which was placed on a silica gel column (3/4" diameter x 12" long) eluting with hexanes-$CHCl_3$-CH_3CN (4:3:3). The fractions containing product were concentrated to an oil which was dried 3 days at 70° to give 3 (1.4 g, 73%) as an amorphous, hygroscopic white solid: ^{1}H NMR ($CDCl_3$, TMS): δ 8.35 (d, J=7 Hz, 1H), 6.74 (d, J=7 Hz, 1H), 6.07 (d, J=3 Hz, 1H), 5.55-5.4 (d,d,

1H, J=6 Hz, 3 Hz), 5.30 (t, J=6 Hz, 1H), 4.65-4.35 (m, 3H), 2.14 (s) and 2.10 (s) (9H). IR (KBr): 1750, 1680; UV (MeOH): λ_{max} 251 nm (ϵ2700), 340 (2000). Anal Calcd for $C_{16}H_{17}N_3O_8$: C, 50.66; H, 4.52; N, 11.08. Found: C, 50.0; H, 4.6; N, 10.8; C, 49.8; H, 4.5; N, 10.6.

1,2-Dihydro-2-oxo-1(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-pyrimidine-4-carbonitrile (3): (25 mg) was dissolved in methanol (1 ml) and 12 NHCl (0.1 ml) added. Hydrogen cyanide gas was detected immediately. The solution was allowed to stand 30 h at room temperature and then concentrated to dryness. Toluene was added to the residue and evaporated (2 x 2 ml). The residue was triturated with ethanol and the resulting solid recrystallized twice from 95% ethanol to give white crystals (10 mg): mp 164-65°. The mixture melting point of this material with uridine is 164-65°. The ^{1}H NMR spectrum was identical to that of an authentic sample of uridine.

1,2-Dihydro-2-oxo-1-(β-D-ribofuranosyl)pyrimidine-4-carbonitrile (6): 1,2-Dihydro-2-oxo-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrimidinecarboxaldehyde oxime[7] (4, 2.70 g) was added to anhydrous, peroxide free dioxane (60 ml). Triethylamine (8.2 ml) was added and the mixture cooled in an ice bath. $(CF_3CO)_2O$, (4 ml) was then added dropwise (30 min). The ice bath was removed, the remainder of the $(CF_3CO)_2O$ (2.3 ml) was added and the solution stirred at room temperature for 1 h. A cold solution of triehtylamine (5 ml) in methanol (50 ml) was added and the whole immediately evaporated on the rotary evaporator below room temperature. The residue was dissolved in chloroform (500 ml) and hexanes (250 ml) were added. Standing in the freezer gave a precipitate which was collected, dried under vacuum and then dissolved in warm acetonitrile (500 ml). This solution was filtered and the filtrate concentrated to 35 ml. The resulting precipitate was redissolved in warm acetonitrile (50 ml) and the warm solution filtered. Cooling gave a precipitate that was collected, washed with chloroform and dried under vacuum at 80° overnight to give 6 (2.3 g, 88%): mp 147-149°; ^{1}H NMR (DMSOd_6, TMS): δ 8.95 (d, 1H, J=7 Hz); 7.02 (d, 1H, J=7 Hz), 5.67 (broad singlet, 1H superimposed on a D_2O exchangeable

multiplet, 1H) 5.28 (t, 1H, J=5 Hz, D_2O exchangeable) 5.0 (m, 1H, D_2O exchangeable), 4.05-3.5 (m, 5H); IR (KBr): 3480, 3350 (s), 3100 (w), 2245 (very weak), 1675 (s), 1655 (s) cm^{-1}; UV (MeOH): λ 250 nm (shl), 341 (4500). Anal Calcd for $C_{10}H_{11}N_3O_5 \cdot 1/2\,H_2O$: C, 45.80; H, 4.61; N, 16.03. Found: C, 45.9; H, 4.3; N, 15.8.

1,2-Dihydro-2-oxo-1-(β-D-ribofuranosyl)pyrimidine-4-carbonitrile (6, 50 mg) was dissolved in methanol (2 ml) and 12 NHCl (0.20 ml) was added. Hydrogen cyanide gas was detected immediately. The solution was allowed to stand 30 h at room temperature and then concentrated on the rotary evaporator. Toluene was added and then evaporated (2 x 2 ml). The residue was triturated in ethanol to give crystals. The solid was collected and recrystallized twice from 95% ethanol to give white crystals (22 mg): mp 164-165. The mixture melting point of this material with uridine is 164-65°. The ^1H NMR spectrum of this material was identical to that of an authentic sample of uridine.

Methyl 1,6-Dihydro-6-oxo-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrimidine-4-carboxylate (8): Methyl 6-hydroxypyrimidine-4-carboxylate[17] (7, 6.1 g) was silylated in the usual way. The resulting oil was dissolved in dichloroethane (75 ml). Ribose tetraacetate (12.0 g) was added and the solution cooled using an ice bath. $SnCl_4$ (5.0 ml) was added dropwise (5 min) and the resulting solution stirred 45 min at room temperature and then poured into saturated aqueous $NaHCO_3$ (300 ml) and methylene chloride (300 ml). The layers were separated and the aqueous layer extracted with CH_2Cl_2 (3 x 300 ml). The combined organic layer was dried (Na_2SO_4) and concentrated to a gum (17 g) that was sufficiently pure to be used in the next step. NMR analysis of this material indicated the presence of 80-90% of the desired nucleoside triacetate, 8, along with some ribose tetraacetate. There was no evidence in this NMR spectrum for more than one nucleoside isomer. A portion of this crude material was purified by column chromatography on silica gel eluting with hexanes-$CHCl_3$-CH_3CN (4:4:2). In this manner the ribose tetraacetate was removed affording 8 as an amorphous, white solid: ^1H NMR ($CDCl_3$, TMS): δ 8.42 (s, 1H), 7.13 (s, 1H), 6.08 (d, 1H, J=4 Hz), 5.6-5.25 (m, 2H), 4.33

(broad singlet, 3H), 3.94 (s, 3H), 2.05-2.1 (m, 9H); IR (KBr): 1750, 1690 cm^{-1}; UV (MeOH): λ_{max} 292 nm (ϵ3000).

1,6-Dihydro-6-oxo-1(β-D-ribofuranosyl)pyrimidine-4-carboxamide (9): Crude methyl 1,6-dihydro-6-oxo-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrimidine-4-carboxylate (8, 1.7 g) was added to cold methanol (85 ml) that had been saturated with ammonia. Stirring was continued (30 min) as the solution was allowed to warm to room temperature and then for another 3.5 h at room temperature. The solution was concentrated to 10 ml. Crystals formed. These were collected, washed with ether and dried under vacuum at 100° to give analytically pure 9 (0.90 g, 81%): mp 188-190°; ^{1}H NMR (DMSOd_6, TMS): δ 8.91 (s, 1H), 8.02 (s) and 7.80 (s) (2H, D_2O exchangeable), 6.85 (s, 1H), 5.96 (d, J=2 Hz, 1H), 5.57 (d, J 4 Hz, 1H, D_2O exchangeable), 5.24 (t, J=4 Hz, 1H, D_2O exchangeable), 5.07 (d, J=5 Hz, 1H, D_2O exchangeable), 4.30-3.50 (m, 5H); IR (KBr): 3430, 3350, 3240, 1690, 1670 cm^{-1}; UV (MeOH): λ_{max} 292 nm (ϵ3000). Anal Calcd for $C_{10}H_{13}N_3O$: C, 44.28; H, 4.83; N, 15.49. Found: C, 44.1; H, 4.6; N, 15.5.

4-Amino-1,6-dihydro-1-(β-D-ribofuranosyl)6-pyrimidone (12): 1,6-Dihydro-6-oxo-1-(β-D-ribofuranosyl)pyrimidine-4-carboxamide (9, 542 mg) was dissolved in warm water (10 ml). The solution was allowed to cool to room temperature and 2.9 ml of a 5.25% solution of sodium hypochlorite in water was added. This solution was stirred 15 min at room temperature and then brought to reflux where it was maintained for 30 min. The solution was concentrated to dryness under reduced pressure and the residue stirred with water and filtered. The resulting crystals were recrystallized from water and then dried overnight under vacuum at 60° to give 12 as white crystals (180 mg): mp 236-38 (literature[19] mp 237-39); ^{1}H NMR (DMSOd_6, DMSOd_5): δ 8.35 (s, 1H), 6.60 (broad singlet, 2H, D_2O exchangeable), 5.90 (d, 1H, J=3 Hz), 5.04 (s, 1H) superimposed on a D_2O exchangeable multiplet from 5.5-4.7 (3H), 4.3-3.5 (m, 5H); IR (KBr): 3375, 3200, 3600-2000, 1635 cm^{-1}; UV (H_2O, pH 4): λmax 216 (ϵ34000), 257 (5800).

1,6-Dihydro-6-oxo-1-(β-D-ribofuranosyl)pyrimidine-4-carbonitrile (11): 1,6-Dihydro-6-oxo-1-(β-D-ribofuranosyl)pyrimidine-4-carboxamide (9, 2.71 g, 10 mmol) was added to anhydrous dioxane (40 ml). Pyridine (4.4 ml) was added and the mixture was cooled in an ice bath. It was then stirred vigorously while $(CF_3CO)_2O$ (4.0 ml) was added (2 min). Vigorous stirring was continued (20 min) with cooling. More $(CF_3CO)_2O$ (2.0 ml) was then added. Stirring was continued another 10 min at 0-5° and then 2 h at room temperature. The solution was poured into a cold mixture of $NaHCO_3$ (20 g) in methanol (250 ml). After stirring 30 min the mixture was filtered and the filtrate treated with Amberlite^R IRC 50 (5 g) with stirring. This was filtered off and the filtrate concentrated to an oil. This entire procedure was repeated on a 20 mmol scale using 5.42 g of the starting nucleoside carboxamide. The oils obtained from the two procedures were combined and chromatographed on a silica gel column (2" diameter x 24" long) eluting initially with $CHCl_3$:MeOH (12:1). The solvent polarity was gradually increased to 5:1 chloroform-methanol. Fractions containing product were combined and concentrated to a gum that was triturated with methanol-ether to give crystals. These were washed with ether and dried under vacuum at 50° to give 11 (4.5 g, 59%): mp 129-131; ^1H NMR (DMSOd_6, TMS): δ9.03 (s, 1H), 7.25 (s, 1H), 5.90 (d, 1H, J=1Hz), 5.60 (d, 1H, J=4 Hz, D_2O exchangeable), 5.30 (t, 1H, J=4 Hz, D_2O exchangeable), 5.10 (d, 1H, J=4 Hz, D_2O exchangeable), 4.2-3.85 (m, 3H), 3.85-3.5 (m, 2H); IR (KBr): 3490, 3460, 3320 (s), 3120, 2250 (w), 1660 cm^{-1} (s); MS: m/e: 254 (1%),253 (M$^+$, 1), 223 (1), 192 (1), 188 (4), 181 (3), 180 (32), 164 (11), 162 (8), 161 (42), 150 (6), 133 (21), 132 (13), 123 (8), 122 (100), 121 (14); UV (MeOH): λ_{max} 294 nm (ε3600). Anal Calcd for $C_{10}H_{11}N_3O_5$: C, 47.43; H, 4.38; N, 16.60. Found: C, 47.3; H, 4.5; N, 16.3.

1,2-Dihydro-2-oxopyridine-4-carbonitrile (14): 4-Cyanopyridine-N-oxide[24] (25 g) was added to trifluoroacetic anhydride (150 ml) and the mixture heated in a sealed tube for 7 days in an oil bath maintained at 110°. After cooling the tube was opened, the contents filtered and the filtrate added dropwise (1 h) with

stirring and ice bath cooling to methanol (300 ml). The resulting mixture was filtered and the solid washed with methanol and ether to give the crude product (10 g). This was recrystallized from aqueous ethanol using a charcoal decolorization to give needles (6.0 g). This material was recrystallized again from aqueous ethanol to give tan needles that were dried overnight under vacuum at 60° (4.3 g, 17%): mp 278-280°; ^{1}H NMR (DMSOd_6, TMS): δ 12.3 (s, 1H, D_2O exchangeable), 7.63 (d, J=7 Hz, 1H), 6.97 (d, J=2 Hz, 1H), 6.49 (d, d, J=7 Hz, 2 Hz, 1H); IR (KBr): 3200-2300, 2240 (w), 1690, 1675 (s), 1625 cm^{-1}; MS:m/e 121 (7%), 120 (M^+, 85), 93 (7), 92 (100), 77 (2), 76 (5), 75 (3), 66 (7), 65 (25), 64 (25), 63 (7); UV (MeOH): λmax 331 nm (ϵ4600). Anal Calcd for $C_6H_4N_2O$: C, 60.00; H, 3.36; N, 23.33. Found: C, 59.9; H, 3.1; N, 23.3.

1,2-Dihydro-2-oxo-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyridine-4-carbonitrile (15): 1,2-Dihydro-2-oxopyridine-4-carbonitrile (14, 4.8 g) was converted to its O-trimethylsilyl derivative in the usual way. The oil was dissolved in dichloroethane (100 ml). Ribose tetraacetate (11.85 g) was added and the solution cooled using an ice bath. $SnCl_4$ (4.95 ml) was then added dropwise (10 min). The solution was stirred 30 min at 0-5° and then 1.5 h at room temperature. It was then poured into saturated aqueous $NaHCO_3$ (300 ml) and CH_2Cl_2 (300 ml). The layers were separated and the aqueous layer extracted with CH_2Cl_2 (3 x 200 ml). The combined organic layer was dried (Na_2SO_4) and concentrated to an oil that was placed on a silica gel column (2" diameter x 30" long) eluting with hexanes-$CHCl_3$-CH_3CN (5:3:2). The product was found in fractions 8-16 (13 g, 86%). Fraction 10 was obtained analytically pure: mp 94-95°; ^{1}H NMR ($CDCl_3$, TMS): δ7.78 (d, J=7 Hz, 1H), 6.92 (d, J=2 Hz, 1H), 6.40 (d, d, J=7 Hz, 2 Hz, 1H), 6.26 (d, J=4 Hz, 1H), 5.55-5.25 (m, 2H), 4.42 (broad singlet, 3H), 2.14 (s), 2.12 (s), 2.10 (s), (9H); IR (KBr): 2240 (w), 1755 (s), 1680 (s), 1605 cm^{-1}; UV (MeOH): λmax 335 nm (ϵ4900). Anal Calcd for $C_{17}H_{18}N_2O_8$: C, 53.97; H, 4.79; N, 7.40. Found: C, 54.0; H, 4.7; N, 7.4.

1,2-Dihydro-2-oxo-1-(β-D-ribofuranosyl)pyridine-4-carbonitrile (16): 1,2-Dihydro-2-oxo-1-(2,3,5-tri-O-acetyl-β-

D-ribofuranosyl)pyridine-4-carbonitrile (15, 10.0 g) was dissolved in methanol (1.5 l) and $NaHCO_3$ (15 g) was added. The mixture was allowed to stand at room temperature for 18 h and then filtered and the filtrate added to Amberlite^R IRC 50 (200 g). After stirring 30 min this mixture was filtered and the filtrate concentrated to an oil. Ethanol (50 ml) was added followed by ether. Standing gave crystals (2.7 g, 40%). This was recrystallized from ethanol-hexanes and the white plates dried under vacuum at 50° over the weekend to give 16 (1.5 g): mp 144-45°; ^1H NMR (DMSOd_6, TMS); δ8.20 (d, J=7 Hz, 1H), 6.97 (d, J=2 Hz, 1H), 6.54 (d, d, J=7 Hz and 2 Hz, 1H), 5.95 (d, J=2 Hz, 1H), 5.55-5.25 (m, 1H, D_2O exchangeable), 5.25-4.9 (m, 2H, D_2O exchangeable), 4.1-3.6 (m, 5H); IR (KBr): 3440 (s), 3340 (s), 2240 (w), 1660 (s); MS: m/e 253 (0.5%), 252 (M^+, 2%), 234 (1), 179 (10), 163 (10), 149 (5), 145 (7), 133 (35), 132 (13), 122 (8), 121 (100), 120 (39), 115 (12), 103 (31), 92 (42); UV (MeOH): λmax 336 nm (ϵ5000). Anal Calcd for $C_{11}H_{12}N_2O_5$: C, 52.38; H, 4.80; N, 11.11. Found: C, 52.5; H, 4.5; N, 11.2. The mother liquors from the above crystallizations were combined and subjected to chromatography on a silica gel column (2" diameter x 30" long) eluting with $CHCl_3$-MeOH (10:1). Fractions containing product were concentrated affording more 16 (1.5 g) isolated as a white solid: mp 141-143.

3-Hydroxypyridine-4-carbonitrile (18) and 4-cyano-3-oxido-1-methylpyridinium (19): Iodotimethylsilane (90 ml) was heated at reflux and 3-methoxypyridine-4-carbonitrile (15 g)[22] (UV (MeOH): λmax 304 nm (ϵ5500)) added. The mixture was stirred at reflux for 3 h and then concentrated to dryness under reduced pressure. Toluene was added and this evaporated (2 x 150 ml). The resulting red solid was dissolved in methanol (50 ml) and the resulting solution concentrated to dryness. Methanol (50 ml) was added to the residue. After stirring the mixture was filtered and the filtrate concentrated to a yellow-orange solid that was then recrystallized from ethanol-ether to give crude product, 11.0 g. The mother liquors were saved and used in the isolation of 3-hydroxypyridine-4-carbonitrile. The 11 grams of crude material were dissolved in methanol (200 ml)

and $NaHCO_3$ added to neutralize the solution. The solution was concentrated to 35 ml, the salt removed by filtration and the filtrate applied to a silica gel column (3" diameter x 12" long) eluting with chloroform-methanol (3:1). Fractions containing product were concentrated to a solid which was recrystallized from ethanol to give 19 as yellow needles that were dried at 40° for 3 days under vacuum (3.6 g, 24%): mp 237-240° (dec); ^1H NMR (DMSOd_6, TMS): δ 7.82 (d, 1H, J=1 Hz), 7.70 (d, 1H, J=7 Hz), 7.33 (d, d, 1H, J=7 Hz and 1 Hz), 4.01 (s, 3H); IR (KBr): 3120, 2220, 1615 cm^{-1}; UV (MeOH) λmax 229 nm (ϵ22,900), 253 (5800), 372 (7600). Anal Calcd for $C_7H_6N_2O$: C, 62.68; H, 4.51; N, 20.89. Found: C, 62.6; H, 4.4; N, 20.7.

The ethanol-ether mother liquors described above were concentrated and the residue placed on a silica gel column (1" diameter x 30" long) eluting with $CHCl_3$: MeOH (6:1). The fractions containing product were concentrated to dryness and the residue dissolved in water and the pH adjusted to 6 with $NaHCO_3$. Crystals were formed which were collected and dried to provide 18 (6.0 g, 45%). This was sufficiently pure for the ribosylation step. A portion was recrystallized from ethyl acetate-hexanes and the crystals dried under vacuum at 80° overnight to give the analytical sample: mp 163-165°; ^1H NMR (DMSOd_6, TMS): δ11.6 (broad singlet, 1H, D_2O exchangeable), 8.48 (s, 1H), 8.22 (d, 1H, J=5 Hz), 7.66 (d, 1H, J=5 Hz); IR (KBr): 3200-2100, 2235 (w), 1605 cm^{-1} (w); UV (MeOH): λmax 306 nm (ϵ5200). Anal Calcd for $C_6H_4N_2O$: C, 60.00; H, 3.36; N, 23.33. Found: C, 60.3; H, 3.1; N, 23.3.

4-Cyano-3-oxido-1(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-pyridinium (20): The O-trimethylsilyl derivative of 3-hydroxypyridine-4-carbonitrile (18, 3.6 g) was prepared and the oil dissolved in dichloroethane (30 ml). Ribose tetraacetate (9.54 g) was added, the solution cooled in an ice bath and $SnCl_4$ (3.9 ml) added dropwise (5 min). The mixture was stirred at 0-5° for 1 h and then poured into saturated aqueous $NaHCO_3$ (500 ml) and methylene chloride (500 ml). The layers were separated and the aqueous layer extracted with methylene chloride (3 x 300 ml). The combined organic layers were dried (Na_2SO_4) and concentrated to an oil which

was redissolved in EtOAc (50 ml). Standing gave crystals: 6.5 g (57%). A portion of this material was recrystallized from chloroform-ethyl acetate and then from methanol to give the analytical sample which was dried overnight at 50°: mp 153-155; ^{1}H NMR (DMSOd_6, TMS): δ 8.05 (d, 1H, J=1 Hz), 7.87 (d, 1H, J=6 Hz), 7.54 (d, d, 1H, J=6 Hz, 1 Hz), 6.25 (d, 1H, J=4 Hz), 5.6-5.25 (m, 2H), 4.8-4.35 (m, 3H), 2.11 (s, 9H). IR (KBr): 2220 (w), 1745 (s), 1610 cm^{-1}; UV (MeOH): λmax 232 nm (ϵ23000), 253 (shl), 376 (7200). Anal Calcd for $C_{17}H_{18}N_2O_8$: C, 53.97; H, 4.79; N, 7.40. Found: C, 53.8; H, 5.1; N, 7.2.

4-Cyano-3-oxido-1(β-D-ribofuranosyl)pyridinium (21): 4-Cyano-3-oxido-1(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyridinium (20, 5.0 g) was dissolved in methanol (500 ml) and $NaHCO_3$ (2.5 g) was added. After standing 44 h at room temperature, the $NaHCO_3$ was filtered off and Amberlite^R IRC 50 (35 g) added. This was stirred 1 h at room temperature and filtered. The filtrate was concentrated to 5 ml and ethanol (10 ml) added. Crystals settled out. These were washed with 1:1 EtOH-ether and dried under vacuum at 80° for 2 days to give 21 (2.7 g, 81%): mp 176-178°; ^{1}H NMR (DMSOd6, TMS): δ 8.10 (d, 1H, J=1 Hz), 7.80 (d, 1H, J=6 Hz), 7.60 (d, d, 1H, J=6 Hz, 1 Hz), 5.66 (d, J=4 Hz, 1H) superimposed on a D_2O exchangeable multiplet at 5.7-5.9 (1H), 5.25-5.5 (m, 2H, D_2O exchangeable), 4.25-4.0 (m, 3H), 3.8-3.6 (m, 2H); IR (KBr): 3350 (s), 3160, 2235 (w), 1610 cm^{-1}; UV (MeOH): λmax 231 nm (ϵ20,600), 254 (shl), 373 (7,100). Anal Calcd for $C_{11}H_{12}N_2O_5$: C, 52.38; H, 4.80; N, 11.11. Found: C, 52.1; H, 4.9; N, 10.9.

REFERENCES AND NOTES

1. Address correspondence to Warner Lambert/Parke Davis, 188 Howard Ave., Holland, Michigan 49423.

2. For recent reviews of **nucleoside derivatives** see: J.A. Montgomery, Med. Res. Rev., 2, 271 (1982); Y.Mizuno, T.Itoh, A.Nomura, Heterocycles, 17, 615 (1982); R.J. Suhadolnik, "Nucleosides as Biological Probes", Wiley-

Interscience, New York, 1980; K.H.Scheit, "Nucleotide Analogs", Wiley-Interscience, New York, 1980; "Nucleoside Analogues: Chemistry, Biology and Medicinal Applications", R.J.Walker, E.DeClerg, E.Eckstern, Eds., Plenum, New York, 1979; R.J.Suhadolnik, Prog. Nucleic Acid Res., 22, 193 (1979); A.Bloch in "Drug Design, vol. IV." E.J.Arien, Ed., Academic Press, New York, 1973, pp. 286-360.

3. C.W.Thornber, Chem., Soc., Rev., 8, 563, (1979); K. Wallenfels, K. Friedrich, J.Rieser, W.Ertel, H.K. Thieme, Angew.Chem.Int.Ed.Eng., 15, 261 (1976) and references therein.

4. a) G.Shaw, R.N.Warrener, M.H.Maquire, R.K.Ralph, J. Chem. Soc., 2294 (1958); (b) N.J.Cusack, B.J.Hildick, D.H.Robinson, P.W.Rugg, G.Shaw, J. Chem. Soc. Perkin. Trans I., 1720 (1973); c) P.F.Torrence, B. Bhooshan, J. Descamps, E.deClercq; J. Med. Chem., 20, 974 (1977).

5. a) A.Yamane, A.Matsuda, T.Ueda, Chem. Pharm. Bull., 28, 150 (1980); b) J.D.Westover, G.R.Revankar, R.K. Robins, R.D.Madsen, J.R.Ogden, J.A.North, R.W.Mancuso, R.J.Rousseau, E.L.Stephen, J. Med. Chem., 24, 941 (1981).

6. R.A.Earl, L.B.Townsend, J. Heterocycl. Chem., 11, 1033 (1974).

7. R.S.Klein, I.Wempen, K.A.Watanabe, J.J.Fox, J. Org. Chem., 35, 2330 (1970).

8. H.M.Berman, R.J.Rousseau, R.W.Mancuso, G.P.Kreishman, R.K.Robins, Tetrahedron Lett., 3099 (1973); P.Narayanan, H.M.Berman, Carbohydr. Res., 44, 169 (1975).

9. R.L.Tolman, R.K.Robins, L.B.Townsend, J. Amer. Chem. Soc., 91, 2102 (1969); B.C.Hinshaw, O.Leonoudakis, K.S.Schram, L.B.Townsend, J.C.S. Perkin Trans I, 1248 (1975).

10. A preliminary report of a portion of this work has been published: R.E.Bambury, D.T.Feeley, G.C.Lawton, J.M. Weaver, J.Wemple, J. Chem. Soc. Chem. Commun., 422 (1984).

11. M.J.Robins, B.L.Currie, R.K.Robins, A.Bloch, Proc.

Am. Assoc. Cancer Res., 10, 73 (1969); A.Bloch, G.Dutschman, B.L.Currie, R.K.Robins, M.J.Robins, J. Med. Chem., 16, 294 (1973).

12. U.Niedballa, H.Vorbrueggen, Angew. Chem. Int. Ed. Eng, 9, 461 (1970); J. Org. Chem., 39, 3654, 3661, 3668 (1974); J. Org. Chem., 41, 2084 (1976).

13. G.D.Daves, D.E.O'Brien, L.R.Lewis, C.C.Cheng, J. Heterocycl. Chem., 1, 130 (1964); A.J.Boulton, D.T.Hurst, J.F.W.McOmie, M.S.Tute, J. Chem. Soc. C., 1202 (1967).

14. F.Carotti, F.Campagna, Synthesis, 56 (1979).

15. In some of our attempts to synthesize this cyano-nucleoside more than the calculated amount of $(CF_3CO)_2O$ and triethylamine were required in order to obtain complete conversion of the oxime to the nitrile according to TLC analysis. We attribute this to formation of the $(CF_3CO)_2$)-triethylamine condensation product recently reported by Schreiber[16] although we did not attempt to isolate and characterize this compound. One proposal for formation of this condensation product involves a one electron transfer process.[16] If this possibility is correct, careful exclusion of peroxides in the dioxane solvent could be important in avoiding this side reaction. We had greatest success in avoiding the side reaction when the dioxane was dried by distillation from potassium metal.

16. S.L.Schreiber, Tetrahedron Lett., 1027 (1980).

17. M.Claesen, H.Vanderhaeghe, Bull Soc. Chim. Belg., 66, 292 (1957).

18. I.A.Mikilaupulo, E.N.Kalinichenke, A.A.Akhrem, J. Carbohydrates Nucleosides Nucleotides, 8, 227 (1981).

19. M.W.Winkley, R.K.Robins, J. Org. Chem., 34, 431 (1969).

20. F.Campagna, A.Carotti, G.Casini, Tetrahedron Lett., 1813 (1977).

21. T.Ueda, H.Inoue, A.Matsuda, Ann. N.Y. Acad. Sci., 255, 121 (1975); J.T.Witkowski, L.F.Christensen, R.K.Robins, J. Carbohydr. Nucleosides and Nucleotides, 5, 363 (1978) and references therein.

22. J.L.LaMattina, R.L.Taylor, J. Org. Chem., 46, 4179 (1981).

23. M.E.Jung, M.A.Lyster, J. Org. Chem., 42, 3761 (1977).

24. Aldrich Chemical Co.

Received July 20, 1984