I), 1550 cm^{-1} (amide II). Anal. ($C_{11}H_{21}NO_2Cl_2$) C, H, N, Cl.

(b) From 16. A 4.0-g portion of **16** (20.4 mmol) was placed in a 200-mL round-bottom flask along with 2.24 mL (2.07 g, 20.4 mmol) of *N*-methylmorpholine, 2.80 g (20.4 mmol) of 1-*N*-hydroxybenzotriazole, 2.66 g (20.4 mmol) of 3-chloropropylamine hydrochloride, and 60 mL of previously dried DMF. The flask was cooled to 0 °C, and 4.6 g (22.4 mmol) of dicyclohexylcarbodiimide was added in one portion with stirring. The yellow solution was allowed to stir at room temperature for 4 days, at which time the DMF was evaporated (35 °C, 2 mmHg) and the residue taken up in 30 mL of ethyl acetate. The ethyl acetate layer was filtered through a 0.45-μm Zetapore filter, washed with three 25-mL portions of 10% HCl, three 25-mL portions of 1.0 M $NaHCO_3$, and 30 mL of saturated aqueous NaCl, and dried over anhydrous $MgSO_4$. Filtration and removal of the solvent in vacuo yielded a pale yellow oil, which was purified by plug filtration (silica gel 60, 7 × 10 cm column) eluted with $CHCl_3$/CH_3OH (9:1) to yield 4.91 g (89%) of **19** (R_f = 0.41) as a clear, colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.44 (3 H, m, CH_2 and OH), 1.7–2.3 (8 H, m, CH_2), 3.1–3.9 (8 H, m, CH_2Cl, CH_2CO, and CH_2N), 4.10 (1 H, m, CH), 7.08 (1 H, m, NH); IR 1635 (amide I), 1535 cm^{-1} (amide II).

***N*-(3-Chloropropyl)-8-chloro-6-hydroxyoctylamine (20).** A dry 50-mL three-necked round-bottom flask equipped with a reflux condenser and rubber septa was charged with 13.3 mL (13.3 mmol) of a 1.0 M solution of diborane in THF under nitrogen, and the solution was cooled to 0 °C. A 1.0-g portion of **19** (3.7 mmol) was dissolved in 10 mL of previously dried THF and added dropwise via syringe to the diborane solution with stirring. The ice bath was removed, and the solution was allowed to stir overnight at room temperature under nitrogen. The solution was again cooled to 0 °C, and the reaction was quenched by the addition of 4 mL of 6.0 N HCl. The THF was removed in vacuo, and the aqueous layer was basified (pH 12) with powdered KOH. The aqueous layer was extracted with three 25-mL portions of chloroform, and the combined organic layers were washed over anhydrous $MgSO_4$. Filtration and removal of the chloroform in vacuo yielded 0.715 g of **20** (75.4%) as a clear oil which solidified to a white solid upon refrigeration. IR spectroscopy indicated complete reduction had occurred (disappearance of the amide bands). This preparation of **20** was used in the subsequent reactions without further purification: ^{1}H NMR ($CDCl_3$) δ 0.93 (2 H, t, CH_2), 1.43 (7 H, m, CH_2 and OH), 1.92 (4 H, pair of q, CH_2–CH–O), 2.6–2.95 (5 H, m, NH and CH_2–N), 3.60 (5 H, m, CH_2–Cl and CH); IR 3700–3020 cm^{-1} (OH and NH).

***N*-(*tert*-Butyloxycarbonyl)-*N*-(3-azidopropyl)-8-azido-6-hydroxyoctylamine (22a).** A solution of *N*-(*tert*-butyloxycarbonyl)-*N*-(3-chloropropyl)-8-chloro-6-hydroxyoctylamine (**21a**) (760 mg, 2.14 mmol), LiN_3 (421 mg, 8.6 mmol), and a catalytic quantity of LiI in DMF (2 mL) was heated at 60 °C under N_2 for 30 h. The DMF was removed at reduced pressure (25 °C, 1 mmHg) and the residue transferred to a separatory funnel with the aid of H_2O and $CHCl_3$. The aqueous phase was extracted with $CHCl_3$ (3 × 40 mL), and the organic phases were pooled, washed with saturated NaCl (50 mL), and dried ($MgSO_4$). Filtration and removal of the solvent at the rotary evaporator (25 °C) gave a yellow oil. Chromatography on silica (CH_2Cl_2/Et_2O, 10:1) provided **22a** as a clear, virtually colorless oil (1.12 g, 93% yield): ^{1}H NMR ($CDCl_3$) δ 3.8–3.6 (quintet, 1 H), 3.6–3.4 (t, 2 H), 3.4–3.0 (m, 6 H), 1.9–1.0 (m, 13 H), 1.5 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 155.7 (C=O), 79.6 ($C(CH_3)_3$), 69.0 (CHOH), 49.2 ($NCH_2CH_2CH_2N_3$), 48.7 ($N_3CH_2CH_2CHOH$), 47.1 (CH_2N), 44.5 ($NCH_2CH_2CH_2N_3$), 37.6 ($CHOHCH_2$), 36.1 ($N_3CH_2CH_2CHOH$), 28.5 ($C(CH_3)_3$), 28.3 ($NCH_2CH_2CH_2N_3$), 28.0 ($CHOHCH_2CH_2$), 26.4 ($CH_2CH_2CH_2N$), 25.1 (CH_2CH_2N); IR 3450 (OH), 2100 (N_3), 1680 cm^{-1} (C=O). Anal. ($C_{16}H_{31}N_7O_3$) C, H, N. Other alkyl azides synthesized by this procedure (method E) are listed in Table I.

***S*-(5′-Deoxy-5′-adenosyl)-*N*-(3-aminopropyl)-8-amino-6-thiooctylamine (1).** A 66-mg portion (0.09 mmol) of **24c** was dissolved in 3 mL of methanol and added to 50 mg of 10% Pd on carbon which had been wetted with 1 mL of ethanol. The resulting mixture was hydrogenated at 50 psi for 24 h, after which time the catalyst was filtered off (Zetapore 0.45 μm) and replaced and the mixture rehydrogenated as above. The catalyst was again replaced, the mixture hydrogenated at 50 psi overnight, and the reaction mixture filtered and concentrated in vacuo to afford 49 mg of a yellow gum. The crude product was dissolved in 1 mL of 88% formic acid and allowed to stir at room temperature for 3 h. A 5-mL portion of water was added, and the aqueous layer was washed with three 5-mL portions of ether. The water was removed by rotary evaporation (25 °C, 2 mmHg) to yield a light brown solid which was plug filtered on silica gel (2.3 × 4.2 cm), eluted with $CHCl_3$/CH_3OH/NH_4OH (2:2:1), to afford 37 mg (68.5%) of **1** (R_f = 0.36) as an off-white, fluffy hygroscopic solid: ^{1}H NMR (CD_3OD) δ 1.23–2.16 (12 H, m, CH_2), 2.82 (2 H, m, H-5′), 2.97–3.16 (9 H, m, CH_2–N, CH–S), 4.22 (1 H, m, H-4′), 4.38 (1 H, m, H-3′), 6.01 (1 H, d, H-1′), 8.24 (1 H, s, H-2), 8.34 (1 H, s, H-8); HPLC (Altex system with Whatman ODS-2 column using the ion-pairing conditions of Wagner et al.[24]) t_R = 28.9 min. HRMS: calcd for $C_{21}H_{38}N_8O_3S$, MH^+ 483.2866; found, 483.2852.

Acknowledgment. This research was supported by grants from the National Institutes of Health, CA37606 to J.K.C. and A.E.P., GM26290 to A.E.P., and CA28097 to J.K.C.

(24) Wagner, J.; Danzin, C.; Mamont, P. *J. Chromatogr.* 1982, 227, 349–368.

Synthesis and Biological Properties of Purine and Pyrimidine 5′-Deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl Analogues of AMP, GMP, IMP, and CMP

Natarajan Raju, Donald F. Smee, Roland K. Robins, and Morteza M. Vaghefi*

Nucleic Acid Research Institute, 3300 Hyland Avenue, Costa Mesa, California. *Received September 16, 1988*

Methyl 2,3-*O*-isopropylidene-D-ribofuranoside (1) was converted to 1-*O*-acetyl-5-bromo-5-deoxy-2,3-di-*O*-benzoyl-D-ribofuranose (6) in five steps with good yield. The Arbuzov condensation of compound 6 with triethyl phosphite resulted in the synthesis of 1-*O*-acetyl-2,3-di-*O*-benzoyl-5-deoxy-5-(diethoxyphosphinyl)-D-ribofuranose (7). Compound 7 was used for direct glycosylation of both purine and pyrimidine bases. The glycosylation was accomplished with the dry silylated heterocyclic base in the presence of trimethylsilyl triflate. Deblocking of the glycosylation products gave exclusively the β anomer of the 5′-phosphonate analogues of 9-[5′-deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]adenine (13), 9-[5′-deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]guanosine (16), 9-[5′-deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]hypoxanthine (17), and 9-[5′-deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]cytosine (15), described here for the first time. The target compounds as well as their intermediates showed no in vitro antiviral or antitumor activity, although phosphorylation of 15 and 16 to di- and triphosphate analogues was demonstrated with use of isolated cellular enzymes.

Although a large number of natural phosphonate derivatives have been discovered in living organisms,[1] the nucleoside phosphonates have not as yet been isolated from biological sources. Analogues of nucleotides, espe-

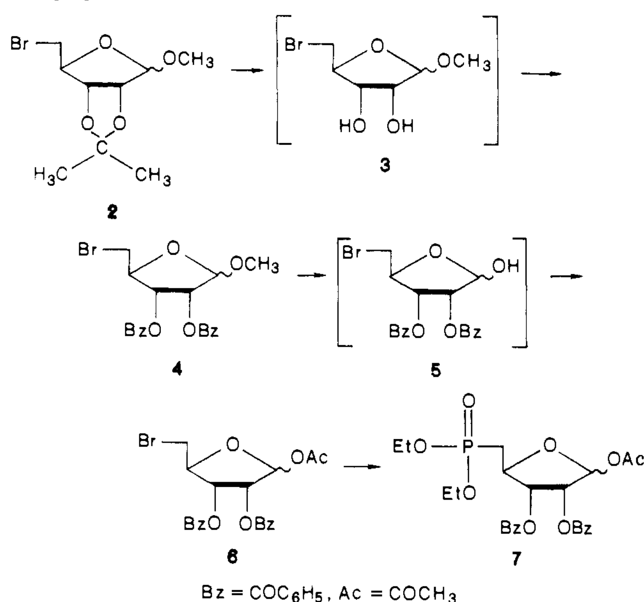
 © 1989 American Chemical Society

cially 5′-deoxynucleoside 5′-phosphonates, are among the most attractive candidates for the development of antitumor and antiviral drugs. The synthesis of 5′,6′-dideoxy-β-D-*ribo*-hexofuranosyl 6′-phosphonate analogues of both purine and pyrimidine nucleosides has been previously reported.[2-4] Such compounds are isosteric phosphonic acid derivatives of nucleotides in which the 5′ –O– is replaced by $-CH_2-$. These nucleotide analogues[5] have shown little biological activity. On the other hand, compounds such as (*S*)-9-[3-hydroxy-2-(phosphonylmethoxy)propyl]adenine, which is related to 5′-deoxyadenosine 5′-phosphonate, has been recently reported by Holy et al.[6] to exhibit anti-DNA viral activity. Other related analogues have also been recently synthesized.[7-9] Such compounds are similar to the nucleotide analogues containing a direct C5′–P bond. The synthesis of 5′-deoxyadenosine 5′-dihydroxyphosphinate and other purine nucleotide analogues is the subject of the present paper as a continuation of the synthesis of phosphonate analogues of nucleotides[10] and nucleoside diphosphate sugars[11,12] previously reported from our laboratory.

The synthesis of 1-[5′-deoxy-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]uracil was first achieved by Banister and Kagan[13] by an Arbuzov condensation of triethyl phosphite with 5′-deoxy-5′-iodouridine. Hydrolysis of this phosphonic ester by these workers could not be successfully achieved because of a base-catalyzed elimination of the uracil. In 1966 Rammler[14] reported the synthesis of 1-[5′-deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]uracil (**14**) using triallyl phosphite which was condensed with 5′-deoxy-5′-iodo-2′,3′-isopropylidene uridine and was deblocked to yield the desired nucleotide **14**. In 1967, Holy[15] reported a similar procedure by condensation of tris[2-(benzyloxy)ethyl] phosphite with 5′-deoxy-5′-iodo-2′,3′-*O*-isopropylideneuridine to give the corresponding 5′-phosphonate diester which was deblocked to provide an overall 16% yield of **14**.

The synthesis of similar purine nucleotide analogues was first attempted by Burger and his co-workers,[16] who reported the synthesis of 7-[2,3,4-tri-*O*-acetyl-6-deoxy-6-(diethoxyphosphinyl)-β-D-glucopyranosyl]theophylline, but the synthesis of ribose analogue by a similar procedure was unsuccessful. In 1959, Wolff and Burger[17] reported the

Scheme I



synthesis of 9-[5′-deoxy-5-(diethoxyphosphinyl)-β-D-ribofuranosyl]-2-methylthioadenine, but the target nucleotide **13** was not achieved due to difficulties of deblocking of the phosphonyl ester. An attempt by Verheyden and Moffatt to synthesize purine nucleotide analogues via the 5′-deoxy-5′-iodo purine nucleoside route resulted in the N^3,5′ cyclization of adenosine, guanosine, and inosine.[18] To date no successful synthesis of the 5′-deoxy-5′-(dihydroxyphosphinyl) analogues of AMP (**13**), GMP (**16**), and IMP (**17**) has been reported.

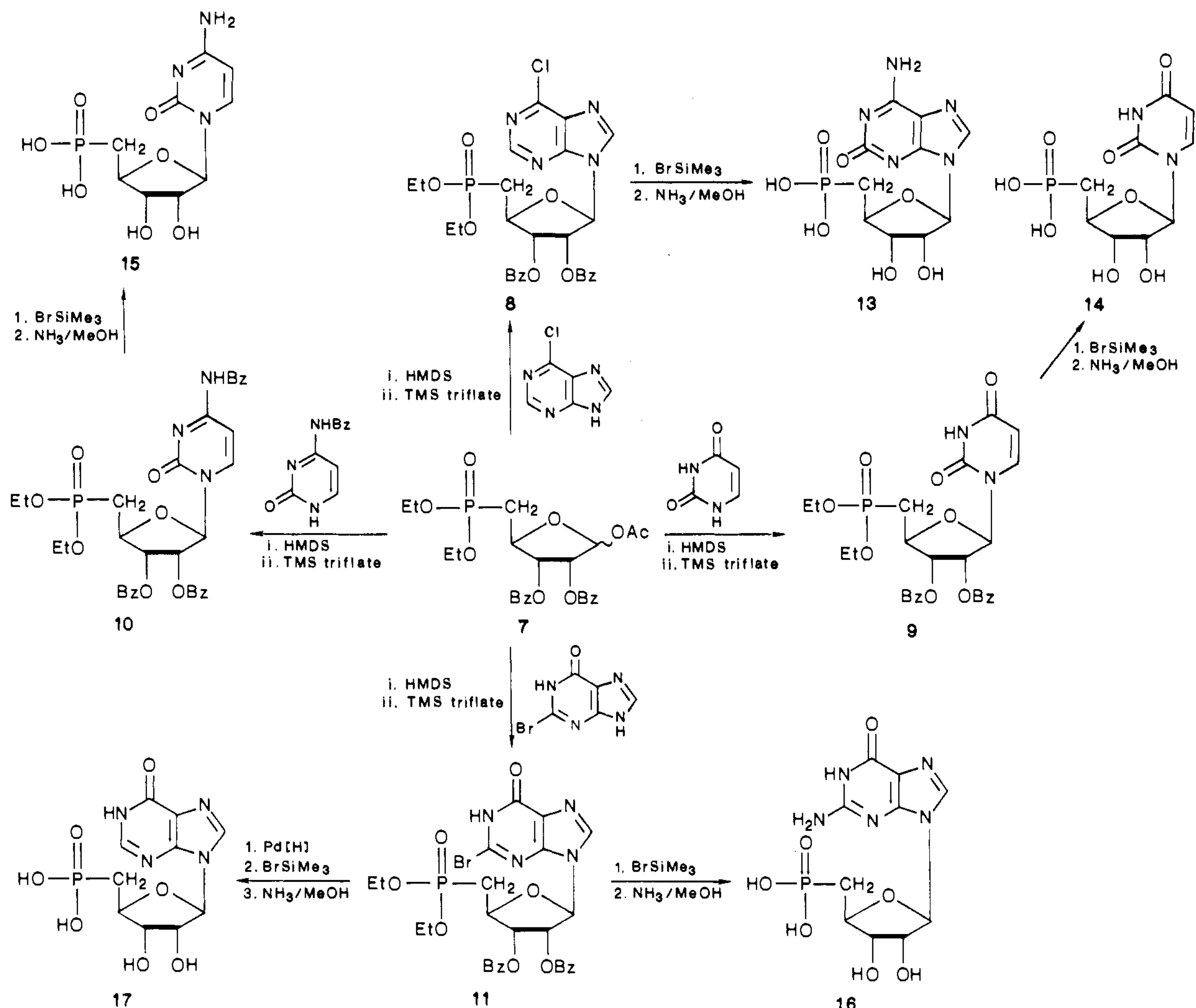
Our strategy for a general procedure for the synthesis of the 5′-deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl analogues of purine and pyrimidine nucleotides is based on the glycosylation of different heterocycles with 1-*O*-acetyl-2,3-di-*O*-benzoyl-5-deoxy-5-(diethoxyphosphinyl)-D-ribofuranose (**7**), which is reported in this paper. Enzymatic phosphorylation of the target compounds to di- and triphosphate analogues were also studied.

Results and Discussion

Methyl 2,3-*O*-isopropylidene-D-ribofuranoside (**1**)[19] was converted to methyl 5-bromo-5-deoxy-2,3-*O*-isopropylidene-D-ribofuranoside (**2**) in over 90% yield according to the procedure of Kiss et al.[20] Compound **2** was refluxed in 0.05 N sulfuric acid and methanol to give methyl k-bromo-5-deoxy-D-ribofuranoside (**3**). Benzoylation of the crude extract of **3** (see Experimental Section) gave methyl 2,3-di-*O*-benzoyl-5-bromo-5-deoxy-D-ribofuranoside (**4**) (Scheme I). The 1-*O*-methyl group was selectively removed by gently refluxing the sugar **4** with 1 N hydrochloric acid in dioxane and the intermediate **5** was acetylated with pyridine/acetic anhydride to yield 1-*O*-acetyl-5-bromo-5-deoxy-2,3-di-*O*-benzoyl-β-D-ribofuranose (**6**) as the major product. Compound 6 was isolated in 51% yield as a mixture containing 5–10% of the α-anomer. The Michaelis–Arbuzov reaction of **6** with triethyl phosphite yielded the desired 1-*O*-acetyl-2,3-di-

(1) Engel, R. *Chem. Rev.* **1977**, *77*, 349.
(2) Jones, G. H.; Moffatt, J. G. *J. Am. Chem. Soc.* **1968**, *90*, 5337.
(3) Pfitzner, K. E.; Moffatt, J. G. *J. Am. Chem. Soc.* **1963**, *85*, 3027.
(4) Montgomery, J. A.; Hewson, K. *Chem. Commun.* **1969**, 15.
(5) Streicher, J. A.; Werner, G.; Rosenwirth, B. *Chem. Scr.* **1986**, *26*, 179.
(6) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. *Nature* **1986**, *323*, 464.
(7) Holy, A.; Rosenberg, I. *Collect. Czech. Chem. Commun.* **1987**, *52*, 2775.
(8) Rosenberg, I.; Holy, A. *Collect. Czech. Chem. Commun.* **1987**, *52*, 2792.
(9) Holy, A.; Rosenberg, I. *Collect. Czech. Chem. Commun.* **1987**, *52*, 2800.
(10) Vaghefi, M. M.; McKernan, P. A.; Robins, R. K. *J. Med. Chem.* **1986**, *29*, 1389.
(11) Vaghefi, M. M.; Bernacki, R. J.; Dalley, N. K.; Wilson, B. E.; Robins, R. K. *J. Med. Chem.* **1987**, *30*, 1383.
(12) Vaghefi, M. M.; Bernacki, R. J.; Hennen, W. J.; Robins, R. K. *J. Med. Chem.* **1987**, *30*, 1391.
(13) Bannister, B.; Kagan, F. *J. Am. Chem. Soc.* **1960**, *82*, 3363.
(14) Yengoyan, L.; Rammler, D. H. *Biochemistry* **1966**, *5*, 3629.
(15) Holy, A. *Tetrahedron Lett.* **1967**, 881.
(16) Parikh, J. R.; Wolff, M. E.; Burger, A. *J. Am. Chem. Soc.* **1957**, *79*, 2778.
(17) Wolff, M. E.; Burger, A. J. *J. Am. Pharm. Assoc.* **1959**, *48*, 56.
(18) Verheyden, J. P. H.; Moffatt, J. G. *J. Org. Chem.* **1970**, *35*, 2319.
(19) Leonard, N. J.; Carraway, K. L. *J. Heterocycl. Chem.* **1966**, 485.
(20) Kiss, Von J.; D'Souza, R.; von Koeveringe, J. A.; Arnold, W. *Helv. Chim. Acta* **1982**, *65*, 1522.

Scheme II



O-benzoyl-5-deoxy-5-(diethoxyphosphinyl)-β-D-ribofuranose (7) in 78% yield as 1:9 mixture of α- and β-anomer. Although the anomeric mixture of 6 and 7 was used without further purification, the β-anomers of either 6 or 7 could be separated on a flash silica gel column for analytical purposes. The distinctive feature of the ^1H NMR of ribose derivative 7 was the upfield shift of H-5′ to 2.35 ppm with an H–P coupling constant of 18.63 Hz. Ribose derivative 7 was used for glycosylation of both purine and pyrimidine bases with the trimethylsilyl triflate catalyst according to the general procedure of Vorbruggen and co-workers.[21] The results of the glycosylation reaction are illustrated in Scheme II.

6-Chloropurine was silylated and then condensed with 7 to yield the blocked nucleotide 9-[5′-deoxy-2′,3′-*O*-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]-6-chloropurine (8). The blocked UMP analogue was synthesized by using silylated uracil which was glycosylated with 7 to give 1-(5′-deoxy-2′,3′-di-*O*-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl)uracil (9). The preparation of the blocked CMP analogue was achieved by using N^4-benzoylcytosine,[22] which was silylated and then condensed with 7 to give 1-[5′-deoxy-2′,3′-di-*O*-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]-4-benzamidopyrimidin-2-one (10). Glycosylation of 2-bromohypoxanthine[23] yielded a 2:1 mixture of N9 and N7 glycosylated products in 80% overall yield. Column chromatography of this mixture gave the desired product 9-[5′-deoxy-2′,3′-di-*O*-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]-2-bromohypoxanthine (11) in 30% yield.

Compound 8 was treated first with bromotrimethylsilane by the procedure of McKenna and Schmidhauser[24] followed by methanolic ammonia to give AMP analogue 9-[5′-deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl)-adenine (13) in overall 31% yield from 6-chloropurine. Compound 9 was similarly deblocked to give 1-[5′-deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]uracil (14) in 36% overall yield from uracil. The yield for the synthesis of 14 by our procedure is considerably higher than the reported procedures by either Yengoyan[14] (8%) or Holy[15] (16%). Compound 10 was similarly treated in succession with bromotrimethylsilane and methanolic ammonia to give 1-[5′-deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]cytosine (15) in overall 37% yield from N^4-benzoylcytosine.

The blocked nucleotide 11 was used as a common intermediate for the preparaton of both GMP and IMP analogues. Treatment of 11 first with bromotrimethylsilane and then with methanolic ammonia gave 9-[5′-deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]-

(21) Vorbruggen, H.; Krolikiewicz, K.; Bennva, B. *Chem. Ber.* **1981**, *144*, 1234.
(22) Brown, D. M.; Todd, S. A.; Varadarajan, S. *J. Chem. Soc.* **1956**, 2384.
(23) Beaman, A. G.; Gerster, J. F.; Robins, R. K. *J. Org. Chem.* **1962**, *27*, 986.
(24) McKenna, C. E.; Schmidhauser, J. *J. Chem. Soc., Chem. Commun.* **1979**, 739.

Table I. NMR Data of Nucleoside 5-Phosphonates

nucleotide (base)	H-1′, ppm	$J_{1',2'}$, Hz	H-5′ (ppm)	$J_{5',P}$, Hz	C-5′, ppm	$J_{C-5',P}$, Hz
13 (adenine)	5.67	4.65	1.91	18.00	33.00	129.86
14 (uracil)	5.41	4.00	1.75	17.75	32.35	131.32
15 (cytosine)	5.45	2.49	1.87	17.83	33.00	127.59
16 (guanine)	5.87	3.12	2.00	18.63	33.63	131.25
17 (hypoxanthine)	5.86	3.30	1.96	17.83	33.00	132.00

guanosine (**16**) in overall 16% yield. 9-[5′-Deoxy-2′,3′-di-*O*-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]-hypoxanthine (**12**) was prepared from **11** by reductive removal of the 2-bromine with Pd/C. The IMP analogue was prepared by deblocking of the phosphonate moiety with bromotrimethylsilane and finally removal of the 2′,3′-benzoyl groups with methanolic ammonia to yield 9-[5′-deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]-hypoxanthine (**17**) in overall 18% yield.

The structural determination of the nucleotide analogues was based on the ^{1}H NMR as well as ^{13}C NMR of both blocked and deblocked compounds. The H-5′ protons in each case coupled with P with a coupling constant between 17.5 and 18.7 Hz. In the case of blocked nucleotide, the H-5′ appeared at around 2.4–2.6 ppm whereas in the deblocked nucleotides they appear at 1.7–2.0 ppm. A summary of the distinctive features of ^{1}H NMR and ^{13}C NMR are given in Table I.

The UV spectrum of compound **14** shows a λ_{max} at 260, which is in agreement with the reported values.[14,15] The stereochemistry of the nucleotides were confirmed by ^{1}H NMR. The anomeric coupling constant ($J_{1',2'}$) for compounds **15–17** are lower than 3.5, which establishes the β-anomeric configuration.[25] The H-1′ protons for compounds **8–12** were observed between 6.2 and 6.4 ppm whereas in the deblocked nucleotides **13–17** the H-1′ proton were observed between 5.4 and 5.8 ppm (Table I), which were in agreement with the reported values for the corresponding natural nucleosides and nucleoside 5′-monophosphates.[18,26] The ^{13}C NMR of the deblocked nucleotides **13–17** shows the upfield shift of C-5′ to around 33 ppm with a large coupling constant of around 130 Hz with P, which is characteristic of the P–C bond.[27] Recently, we were able to obtain a *single-crystal X-ray* of 1-[5′-deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]-cytosine (**15**), which further established the configuration of these nucleotides. The detailed crystallographical data for **15** will be presented elsewhere.

Biological Evaluations. Nucleoside 5′-phosphonates (**13–17**) were evaluated for their in vitro antitumor activity against L1210 leukemia (a murine leukemia), WI-L2 (a human B-lymphoblast), CCRF-CEM (a human T-cell leukemia), and Lovo/L (a human colon carcinoma). None of the above compounds showed antitumor activity at ≤100 μM, determined after 3–5 days in culture. In contrast, active antitumor agents such as tiazofurin or arabinosylcytosine inhibit the replication of these cell lines at 1–3 μm. Compounds **13–17** were also inactive in antiviral screening against parainfluenza virus type 3, herpes simplex virus type 2, adenovirus type 2, rhinovirus type 1-A, influenza A, and visna virus at ≤320 μM. Positive control antiviral agents show activity in this assay against influenza and parainfluenza viruses at 10–30 μM (ribavirin), against adeno- and rhinoviruses at 100 μM (ribavirin), and against herpes simplex and visna viruses at 1–3 μM (5-iodo-2′-deoxyuridine).

Table II. Phosphorylation of Cytidine Monophosphate and Its 5′-Phosphonate Analogue by Nucleoside Monophosphate Kinase

compound	% nucleotide[a] present at 4 h		
	MP	DP	TP
cytidine monophosphate	63	23	14
C-5′ phosphonate **15**	87	13	0

[a] As phosphorylated forms of each compound. MP = monophosphate (starting material for each reaction), DP = diphosphate, TP = triphosphate.

Table III. Phosphorylation of Guanosine Monophosphate and Its 5′-Phosphonate Analogue by Guanylate Kinase

compound	% nucleotide[a] present 2 h			24 h		
	MP	DP	TP	MP	DP	TP
GMP	5	92	3	8	55	37
xylo-GMP	98	0	2	72	0	28
C-5′ phosphonate **16**	76	24	0	16	75	9

[a] See footnote to Table II.

One reason for this lack of biological activity could be due to the inability of the nucleotide analogues to be phosphorylated by cellular enzymes. To explore this possibility, two of the compounds were evaluated in phosphorylation assay, using nucleoside monophosphate (NMP) kinase (a pyrimidine nucleotide kinase) with cytidine analogue (**15**) and guanylate kinase with guanosine analogue (**16**). In the NMP kinase assay, compound **15** was metabolized to di- and triphosphate analogues at 4 h (Table II). Compound **15** was phosphorylated at a much slower rate to the diphosphate and negligibly to the triphosphate when compared to CMP. In this reaction, the extent of phosphorylation did not increase by extending the reaction time to 24 h. In the guanylate kinase assay, the phosphorylation of **16** was compared to GMP as well as *xylo*-GMP (Table III). The phosphonate analogue was phosphorylated at a much slower rate than GMP. With time, however, guanylate kinase converted most of **16** to the diphosphate analogue of GDP, and some triphosphate analogue was also formed in the process. The same enzyme preparation metabolized much of the GDP to GTP by 24 h. The phosphorylation rate of *xylo*-GMP was slower than that of **16**, but by 24 h over 25% of the *xylo*-GMP was converted to the triphosphate.

The above results show that **15** and **16** were indeed phosphorylated by cellular enzymes, although the rates of phosphorylation were less than the natural substrates, and the amounts of the triphosphate analogues formed were less than CTP or GTP. Thus, the lack of biological activity that was manifested in cell culture cannot be attributed to inability to be phosphorylated, at least for the two compounds that were assayed. Since the enzyme assays were performed in a cell-free system, the data may not reflect the degree of phosphorylation achieved inside the intact cells. The ionic strength of **13–17** would decrease their penetration through the cell membrane and into the cells, and subsequently the extent of phosphorylation would be low. The rates of transport of these nucleotide

(25) Townsend, L. B. In *Synthetic Procedures in Nucleic Acid Chemistry*; Vol. 2, Zorbach, W. W., Tipson, R. S., Eds.; Wiley Interscience: New York, 1973; pp 267–398.
(26) Lee, W. W.; Martines, A. P.; Goodman, L. *J. Org. Chem.* **1971**, *36*, 842.
(27) Buchanan, G. W.; Brown, J. H. *Can. J. Chem.* **1977**, *55*, 604.

analogues into cells probably is a major factor to account for biological inactivity.

Experimental Section

General Information. Ultraviolet spectra were recorded on a Beckman DU-50 spectrophotometer. NMR spectra were recorded on an IBM NR/300 FT NMR spectrometer. The chemical shift values are expressed in δ values (parts per million) and referenced with tetramethylsilane (for ^{1}H NMR) and dioxane (for ^{13}C NMR). Melting points were taken on a Haake Buchler melting point apparatus and are uncorrected. EM silica gel (200–400 mesh) was used for flash column chromatography. DEAE-Sephadex A-25 and DEAE-cellulose (DE-52) were used for ion-exchange chromatography. TLC were run on silica gel 60 F-254 (EM Reagent) plates. The components were detected by UV light and with 10% H_2SO_4 in methanol spray followed by heating. The TLC systems were as follows: (A) CH_2Cl_2/acetone (3:2), (B) MeOH/$CHCl_3$ (1:9), (C) *i*-PrOH/NH_4OH/H_2O (7:1:2). HPLC was run on a Rainin Rabbit-HP analytical instrument with reverse phase (C-18) column.

Methyl 2,3-Di-*O*-benzoyl-5-bromo-5-deoxy-D-ribofuranoside (4). A solution of methyl 5-deoxy-5-bromo-2,3-*O*-isopropylidene-D-ribofuranose[20] (100 g, 0.4 mol) in methanol (600 mL) was treated with 0.05 N aqueous sulfuric acid (200 mL) and gently refluxed until the starting material was no longer detectable on TLC (10 h). The reaction mixture was then stirred at room temperature for 12 h. The solution was neutralized with aqueous saturated sodium bicarbonate. Volatiles were removed under reduced pressure to give a paste which was thoroughly extracted with hot ethyl acetate (10 × 100 mL). The combined organic layer was dried with anhydrous sodium sulfate and filtered, and the solvent was removed by evaporation to yield a pale yellow oil (86.0 g, 0.378 mol) which was dissolved in dry pyridine (500 mL) and treated with benzoyl chloride (118 g, 0.84 mol) and stirred at room temperature for 20 h. Excess benzoyl chloride was destroyed by slow addition of water (120 mL) and then the reaction mixture was diluted with water (500 mL). The aqueous portion was thoroughly extracted with ether (5 × 200 mL). The combined organic layer was successively washed with 3 N ice-cold sulfuric acid (5 × 200 mL), water (2 × 200 mL), and then saturated sodium bicarbonate solution (2 × 500 mL). The organic layer was dried and the solvent evaporated to furnish a brown oily residue which was chromatographed on a flash silica gel column. Elution with ethyl acetate/hexanes (2:3) yielded initially the pure β-anomer of 4, which was followed by a mixture of α- and β-anomers (1:9) [yield 127.0 g (0.29 mol) (α and β) (75%)], which was used for the following reaction. A small amount of the β-anomer from the front portion of the elution was recrystallized from hexanes for analytical purposes. β-Anomer: mp 60–61 °C. β-Anomer: ^{1}H NMR ($CDCl_3$) δ 3.53 (s, 3 H, OCH_3), 3.63 (H-5a, dd, $J_{4,5a}$ = 6.7 Hz, J_{5a-5b} = 10.6 Hz), 3.75 (dd, H-5b, $J_{4,5b}$ = 4.89 Hz, J_{5a-5b} = 10.6 Hz), 4.65 (m, 1 H, H-3), 5.19 (s, 1 H, H-1), 5.7 (m, 2 H, H-2 + H-3), 7.4–8.0 (m, 10 H). Anal. ($C_{20}H_{19}O_6Br$) C, H, Br.

2,3-Di-*O*-benzoyl-5-bromo-5-deoxyribofuranosyl Acetate (6). Methyl 2,3-di-*O*-benzoyl-5-bromo-5-deoxy-D-ribofuranoside (4) (127.0 g, 0.29 mol) in dioxane (1300 mL) was gently refluxed with 1 N HCl until the starting material was no longer detectable on TLC (22 h). The reaction mixture was stirred at room temperature for 12 h. The acid was neutralized with saturated sodium bicarbonate solution, and the volatile solvents were removed under reduced pressure at 40 °C. The resulting paste was extracted with hot ethyl acetate (10 × 150 mL). The combined organic layer was dried (Na_2SO_4) and filtered, and the solvent was removed to yield a dark red syrup of 5, which was used in next step without further purification.

Crude 5 (78.0 g, 0.185 mol) was dissolved in dry pyridine (200 mL) and treated with 50 mL of acetic anhydride followed by 1 g of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) and stirred at room temperature for 20 h. The excess acetic anhydride was destroyed with 10 mL of water and the solution stirred for $^1/_2$ h, diluted with water (200 mL), and thoroughly extracted with dichloromethane (5 × 200 mL). The combined organic layer was washed with 3 N ice-cold sulfuric acid (5 × 100 mL), water (2 × 100 mL), and then finally saturated sodium bicarbonate solution (2 × 200 mL). The solvent was removed to furnish a brown syrup, which was chromatographed on a flash silica gel column. Elution with ether/hexanes (2:3) yielded the product as a colorless syrup followed by a mixture of α- and β-anomers of 6 which contained 5–10% of the α-anomer according to the ^{1}H NMR. Combined yield: 68.0 g (50%). For analytical purposes the major β-anomer was fractionally crystallized from hexane as a colorless solid. β-Anomer: mp 68.6–69 °C; ^{1}H NMR ($CDCl_3$) δ 2.18 (s, 3 H, $OCOCH_3$), 3.85 (m, 2 H, H-5), 4.72 (m, 1 H, H-4), 5.76 (d, 1 H, H-2, $J_{H2,H3}$ = 4.9 Hz), 5.83 (dd, 1 H, H-3, $J_{H3,H2}$ = 4.9 Hz, $J_{H3,H4}$ = 6.9 Hz), 6.4 (s, 1 H, H-1), 7.5 and 8.0 (m, 10 H). Anal. ($C_{21}H_{19}O_7Br$) C, H, Br.

1-*O*-Acetyl-2,3-di-*O*-benzoyl-5-deoxy-5-(diethoxyphosphinyl)-D-ribofuranose (7). 2,3-Di-*O*-benzoyl-5-bromo-5-deoxy-D-ribofuranosyl acetate (6) (68.0 g, 0.143 mol) was dissolved in freshly distilled triethyl phosphite (205 mL) and the solution heated under reflux (oil bath temperature 195–200 °C) while a stream of argon was bubbled through the reaction mixture. Every 10 h 25 mL of fresh triethyl phosphite was added to the reaction mixture. At the end of the reaction (30 h), the volatiles were removed under reduced pressure (0.5 mmHg) at 100 °C. The resulting oil was dissolved in ether (780 mL) and the ethereal solution thoroughly washed with ice-cold water (5 × 200 mL) and finally with 10% LiCl solution (200 mL). The ether layer was dried (Na_2SO_4) and evaporation of the solvent left an oily residue. Flash column chromatography of the residue and elution with 4:1 ethyl acetate/hexanes yielded the β- and α-anomers of 7 as a syrup which contained 5–10% of α-anomer: yield 59.4 g (78%); ^{1}H NMR ($CDCl_3$) δ 1.3 (m, 6 H, CH_2CH_3), 2.17 (s, 3 H, $OCOCH_3$), 2.35 (dd, J_{P-H5} = 18.63 Hz, J_{H4-H5} = 6.96 Hz), 4.15 (m, 4 H, CH_2CH_3), 4.83 (m, H-4, 1 H), 5.6 (dd, H-3, $J_{2,3}$ = 4.9 Hz, $J_{3,4}$ = 6.6 Hz), 5.74 (d, 1 H, H-2, $J_{2,3}$ = 4.9 Hz), 6.4 (S, 1 H, H-1), 7.5 and 8 (m, 10 H). Anal. ($C_{25}H_{29}O_{10}P$) C, H, P.

9-[5′-Deoxy-2′,3′-*O*-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]-6-chloropurine (8). 6-Chloropurine[28] (also can be purchased from Aldrich Chemical Co.) (1.75 g, 10 mmol) was mixed with HMDS (50 mL) and refluxed with ammonium sulfate catalyst (0.1 g) for 4 h under argon. Volatiles were distilled off under reduced pressure, and the resulting solid was dried at <0.1 mmHg pressure for 2 h at ambient temperature. The above silylated heterocycle was dissolved in dry acetonitrile (10 mL) and treated with phosphonate 7 in dry acetonitrile (10 mL) followed by the addition of TMS triflate (6.67 g, 30 mmol) under argon. The solution was stirred at room temperature for 24 h and then poured into saturated ice-cold sodium bicarbonate solution. The aqueous portion was saturated with solid sodium chloride and thoroughly extracted with ethyl acetate (10 × 25 mL). The combined organic layer was dried (Na_2SO_4) and solvent was removed to give a gum which was chromatographed on a flash silica gel column. Elution with 9:1 CH_2Cl_2/acetone yielded some decomposed sugar and continued elution with 4:1 CH_2Cl_2/acetone yielded the product 8 as a colorless solid: yield 3.69 g (60%); mp 120–122 °C; ^{1}H NMR ($CDCl_3$) δ 1.33 (m, 6 H, OCH_2CH_3), 2.6 (m, 2 H, H-5′), 4.14 (m, 4 H, OCH_2CH_3), 4.9 (m, 1 H, H-4′), 6.09 (t, 1 H, H-3′), 6.4 (t, 1 H, H-2′), 6.43 (d, 1 H, $J_{1',2'}$ = 4.9 Hz, H-1′), 7.5 and 8.0 (m, 10 H), 8.5 (s, 1 H), 8.85 (s, 1 H); UV (EtOH) λ_{max} 256 (ε 4841). Anal. ($C_{28}H_{28}N_4O_8ClP$) C, H, N, Cl, P.

1-[5′-Deoxy-2′,3′-di-*O*-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]uracil (9). Uracil (1.12 g, 10 mmol) in 50 mL of HMDS was refluxed with 0.1 g of ammonium sulfate under argon until a homogeneous solution was obtained (4 h). Volatiles were removed under reduced pressure, and the resulting oil was kept under vacuum (<0.1 mmHg pressure) for 2 h at ambient temperature. The above crude silylated base was dissolved in dry acetonitrile (10 mL) and treated with 7 (15.2 g, 10 mmol) in dry acetonitrile (10 mL) followed by TMS triflate (6.66 g, 30 mmol) under argon with stirring. The reaction mixture was stirred at ambient temperature for 24 h and then poured into 50 mL of ice-cold saturated sodium bicarbonate solution with vigorous stirring. The aqueous portion was saturated with solid sodium chloride and then extracted with ethyl acetate (10 × 25 mL). The combined organic layer was dried (Na_2SO_4) and solvent was evaporated to give a gummy residue which was chromatographed on a flash silica gel column. The column was eluted with 9:1

(28) Bendich, A.; Russell, R. J.; Fox, J. J. *J. Am. Chem. Soc.* **1954**, *76*, 6073.

CH_2Cl_2/acetone until the less polar materials were eluted. Continued elution with 4:1 CH_2Cl_2/acetone yielded the product as a colorless foam: yield 3.37 g (59%); mp, becomes a glass at 60–70 °C and then melts at 139–141 °C; UV (EtOH) λ_{max} 260 (ϵ 10143); ^1H NMR ($CDCl_3$) δ 1.27 (m, 6 H, OCH_2CH_3), 2.4 (m, H-5′, 2 H), 4.1 (m, 4 H, OCH_2CH_3), 4.55 (m, 1 H, H-4′), 5.69 (m, 2 H, H-3′ and H-2′), 5.76 (d, H-5, $J_{5,6}$ = 8.1 Hz), 6.12 (d, $J_{1',2'}$ = 3.69 Hz, 1 H, H-1′), 7.35 and 7.85 (m, 10 H), 7.6 (d, 1 H, H-6, $J_{6,5}$ = 8.1 Hz), 9.04 (s, 1 H, NH). Anal. ($C_{27}H_{29}N_2O_{10}P$) C, H, N, P.

1-[5′-Deoxy-2′,3′-di-*O*-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]-4-benzamidopyrimidin-2-one (10). N^4-Benzoylcytosine[22] (2–15 g, 10 mmol) was refluxed with HMDS (50 mL) and a catalytic amount of ammonium sulfate. After the solid was dissolved (about 3 h) refluxing was continued for an additional 1 h. After removal of the volatile under reduced pressure, the silylated base was dried under high vacuum for 2 h (<0.1 mmHg pressure). The solid obtained above was dissolved in dry acetonitrile (10 mL) and treated with **7** (5.2 g, 10 mmol) in dry acetonitrile (10 mL) under argon. TMS triflate (6.67 g, 30 mmol) was added and the solution stirred under argon for 24 h at room temperature. The reaction was quenched with 20 mL of saturated sodium bicarbonate solution, and the aqueous portion was saturated with solid sodium chloride and thoroughly extracted with EtOAc (10 × 25 mL). The solvent was removed and the residue was chromatographed on a silica gel column as described for compound **9** above to yield 3.3 g of **10** as a colorless solid (50% yield): ^1H NMR ($CDCl_3$) δ, 1.23 (m, 6 H, OCH_2CH_3), 2.46 (m, 2 H, H-5′), 4.08 (m, 4 H, OCH_2CH_3), 4.65 (m, 1 H, H-4′), 5.75 (t, 1 H, H-3′), 5.8 (t, 1 H, H-2′), 6.19 (d, 1 H, H-1′, $J_{1',2'}$ = 4.4 Hz), 7.21 (d, 1 H, H-5, $J_{5,6}$ = 7.7 Hz), 7.2, 7.4, and 7.9 (m, 15 H), 8.1 (d, 1 H, H-6, $J_{6,5}$ = −7.7 Hz), 8.95 (br s, 1 H, NH); mp 168–170 °C; UV (EtOH) λ_{max} 258 nm (ϵ 21147). Anal. ($C_{34}H_{34}N_3O_{10}P$) C, H, N, P.

9-[5′-Deoxy-2′,3′-di-*O*-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]-2-bromohypoxanthine (11). A suspension of 2-bromohypoxanthine[23] (2.15 g, 10 mmol) and ammonium sulfate (10.1 g) in HMDS (50 mL) was refluxed under argon until the starting material dissolved (4 h). The volatiles were removed under reduced pressure, and the resulting solid was dried under vacuum (<0.1 mmHg pressure) for 2 h at ambient temperature. The above crude silylated base was dissolved in dry acetonitrile (10 mL) and treated with phosphonate **7** (5.2 g, 10 mmol) in dry acetonitrile (10 mL). The solution was cooled to 0 °C and then treated with TMS triflate (6.67 g, 30 mmol) under argon. The reaction mixture was stirred and allowed to come to room temperature and stirring was continued for an additional 48 h. The workup and flash chromatography over silica gel was done according to the procedure described for compound **9**. The column was eluted initially with 2% MeOH in dichloromethane until no more decomposed sugar was observed. The gradient was slowly increased to 4% MeOH in CH_2Cl_2. Fractions containing **11** were combined and concentrated to give a pale yellow solid (yield 5.6 g, 80%) which showed two close moving spots on TLC (5% MeOH in CH_2Cl_2). The solid was rechromatographed on a 100 cm × 5 cm flash silica gel column and eluted with 4% MeOH in CH_2Cl_2. Compound **11** was eluted from the column which was overlapped at the tail with a close moving more polar compound. The pure fractions of compound **11** were combined and evaporated to give a colorless solid: yield 2.03 g (30%); ^1H NMR ($CDCl_3$) δ 1.25 (m, 6 H, OCH_2CH_3), 2.6 (m, 2 H, H-5′), 4.1 (m, 4 H, OCH_2), 4.8 (m, 1 H, H-4′), 5.92 (t, 1 H, H-3′), 6.17 (t, 1 H, H-2′), 6.4 (d, 1 H, H-1′, $J_{1',2'}$ = 5.5 Hz), 7.4 and 7.8 (m, 10 H), 8.29 (s, 1 H); UV λ_{max} (EtOH) 265 nm (ϵ 5444); mp 171–173 °C (becomes glassy at 125–130 °C). Anal. ($C_{28}H_{28}BrN_4O_9P$) C, H, N, Br, P.

9-[5′-Deoxy-2′,3′-di-*O*-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]hypoxanthine (12). 9-[5′-Deoxy-2′,3′-di-*O*-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]-2-bromohypoxanthine (**11**) (2.0 g in 50 mL of ethanol) was hydrogenated with Pd/C (0.4 g) at 60 psi and filtered through a pad of Celite and the Celite was washed with EtOH. The combined filtrate was evaporated to give a solid (1.69 g, 95.5%) which was dissolved in hot dichloromethane. Isopropyl ether was added until the solution became cloudy. After cooling overnight, the product precipitated as oil which resulted in 1.56 g of a colorless foam upon drying (90% yield): mp, becomes glassy at 130–140 °C and melts at 159–161 °C; ^1H NMR ($CDCl_3$) δ 1.26(m, 6 H, OCH_2CH_3), 2.61 (m, 2 H, H-5′), 4.1 (m, 4 H, OCH_2), 4.8 (m, 1 H, H-4′), 6.0 (t, 1 H, H-3′), 6.29 (t, 1 H, H-2′), 6.45 (d, 1 H, H-1′, $J_{1',2'}$ = 5.19 Hz), 7.4 and 7.8 (m, 10 H), 8.2 (s, 1 H), 8.33 (s, 1 H), 12.55 (br s, 1 H, NH); UV (EtOH) λ_{max} 252 nm (ϵ 12073). Anal. ($C_{28}H_{29}N_4O_9P$) C, H, N, P.

9-[5′-Deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]adenine (13). 9-[5′-Deoxy-2′,3′-*O*-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]-6-chloropurine (**8**) (3.07 g, 5 mmol) in dry CH_3CN (10 mL) was treated with bromotrimethylsilane (2.3 g, 15 mmol) and stirred under argon for 6 h. The volatiles were removed under reduced pressure, and the crude product was dissolved in 50 mL of methanolic ammonia and heated in a bomb at 80 °C for 16 h. The solution was concentrated to 10 mL and 10 mL water was added and thoroughly extracted with methylene chloride (3 × 20 mL). The organic layer was washed with water and the combined water solution was chromtographed on a bicarbonate form of DEAE-cellulose column (3 × 50 cm) and eluted first with 500 mL water and then with a linear gradient of 0–0.2 M ammonium bicarbonate (4000 mL). The eluate was fractionally collected, and the fractions containing **13** were pooled and dried on a rotary evaporator. The residue was dissolved in 30 mL of water and freeze-dried to yield the monoammonium salt of **13** as a fluffy solid: yield 0.93 g (51%); UV (pH 1) λ_{max} 261 nm (ϵ 14800); ^1H NMR (D_2O) δ 1.91 (dd, 2 H, H-5′, $J_{P,H5'}$ = 18 Hz, $J_{H4',H5'}$ = 6.78 Hz), 4.05 (t, 1 H), 4.14 (m, 1 H), 4.43 (t, 1 H), 5.67 (d, 1 H, H-1′, $J_{1',2'}$ = 4.65 Hz), 7.71 (s, 1 H), 7.94 (s, 1 H); ^{13}C NMR (D_2O) δ 33.0 (d, $J_{C5',P}$ = 129.86 Hz), 73.64, 74.07 (d, $C_{4'}$, $J_{C4',P}$ = 7.5 Hz), 80.96, 87.6, 118.37, 139.87, 148.34, 152.41, 155.02; ^{31}P NMR (D_2O) δ 19.274. Anal. ($C_{10}H_{17}N_6O_6P\cdot H_2O$) C, H, N, P.

1-[5′-Deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]uracil (14). A solution of 1-[5′-deoxy-2′,3′-di-*O*-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]uracil (**9**) (2.86 g, 5 mmol) in dry acetonitrile (10 mL) was treated with bromotrimethylsilane (2.3 g, 15 mmol) at room temperature and stirred under argon until the starting material disappeared (6 h). All the volatiles were removed under reduced pressure, and the resulting crude material was dissolved in methanolic ammonia (50 mL) and heated in a bomb at 40 °C for 16 h. The solution was concentrated to 10 mL and then diluted with 10 mL of water. The aqueous portion was thoroughly extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was extracted with 10 mL of water. The aqueous portions were combined and concentrated to a smaller volume (5 mL) under reduced pressure at room temperature. The crude product was loaded onto a DEAE-cellulose column (50 cm × 5 cm bicarbonate form) and eluted with a linear gradient of 0–0.2 M ammonium bicarbonate (4000 mL). Fractions containing **14** were collected and lyophilized to yield the monoammonium salt as a colorless fluffy solid: yield 1.05 g (61%). The disodium salt of **14** was prepared by exchanging the cations on a Dowex 50 column: UV (pH 1) λ_{max} 260 nm (ϵ 8540); ^1H NMR (D_2O) δ 1.74 (dd, 2 H, H-5′, $J_{H5',P}$ = 17.75 Hz, $J_{H4',H5'}$ = 6.9 Hz), 3.68 (t, H), 3.86 (m, 1 H), 3.94 (t, 1 H), 5.41 (d, 1 H, H-1′, $J_{1',2'}$ = 4 Hz), 5.45 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 7.3 (d, 1 H, H-6, $J_{6,5}$ = 8.1 Hz); ^{13}C NMR (D_2O) δ 32.35(d, $J_{C5',P}$ = 131.32 Hz), 73.5, 73.83 (d, C-4′, $J_{C4',P}$ = 9.05 Hz), 80.16, 90.02, 102.25, 142.05, 151.69, 168.35; ^{31}P NMR δ 19.68.

1-[5′-Deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]cytosine (15). 1-[5′-Deoxy-2′,3′-di-*O*-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]-4-benzamidopyrimidin-2-one (**10**) (3.37 g, 5 mmol) was treated with bromotrimethylsilane (2.3 g, 15 mmol) and worked up as in the case of **13**. Debenzoylation was effected by heating in a bomb at 40 °C for 20 h with methanolic ammonia. The reaction mixture was dried on a rotary evaporator and the residue was dissolved in 15 mL of water and purified on a DEAE-cellulose column as in the case of 13. After freeze-drying 1.26 g of monoammonium salt of **15** was obtained as a white powder: yield 73.6%; UV (pH 1) λ_{max} 290 nm (ϵ 9710), (pH 7) λ_{max} 269 nm (ϵ 8960); ^1H NMR (D_2O) δ 1.87 (dq, 2 H, H-5′, $J_{H5',P}$ = 18.74 Hz, $J_{H5'',P}$ = 16.92 Hz, $J_{H5',H4'}$ = 6 Hz, $J_{H5'',H4'}$ = 7.9 Hz), 3.76 (t, 1 H), 4.0 (m, 2 H), 5.45 (d, 1 H, H-1′, $J_{H1',H2'}$ = 2.49 Hz), 5.76 (d, 1 H, $J_{H5,H6}$ = 7.5 Hz), 7.42 (d, 1 H, $J_{H6,H5}$ = 7.5 Hz); ^{13}C NMR (D_2O) δ 33.0 (d, $J_{C5',P}$ = 127.59 Hz), 73.96 (d, $J_{C4',P}$ = 2 Hz), 74.32, 79.9, 90.86, 96.1, 141.55, 157.4, 166.11; ^{31}P NMR (D_2O) δ 18.78. Anal. ($C_9H_{17}N_4O_7P\cdot H_2O$) C, H, N, P.

9-[5′-Deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]guanosine (16). 9-[5′-Deoxy-2′,3′-di-O-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl]-2-bromohypoxanthine (11) (3.37 g, 5 mmol) was dealkylated with bromotrimethylsilane (2.37 g, 15 mmol) as described for 15 and debenzoylated and aminated at 140 °C for 16 h with methanolic ammonia. The reaction mixture was dried under reduced pressure and the residue was dissolved in 15 mL of water. The title compound was purified on a DEAE-cellulose column (HCO_3 form) as in the case of 13 to give 0.95 g of the monoammonium salt of 16: yield 50.2%; UV λ_{max} (pH 7) 247 nm (ϵ 13 145), (pH 1) λ_{max} 253 (ϵ = 15 100); ^1H NMR (D_2O) δ 2.0 (dq, 2 H, H-5′, H-5″, $J_{H5',P}$ = 19.12 Hz, $J_{H5'',P}$ = 18.14 Hz, $J_{H5',H4'}$ = 7.2 Hz, $J_{H5'',H4'}$ = 4.5 Hz), 4.04 (d, 1 H), 4.17 (s, 1 H), 4.43 (br s, 1 H), 5.87 (s, 1 H, H-1′), 8.02 (s, 1 H); ^{13}C NMR (D_2O) δ 33.63 (d, $J_{C5',P}$ = 131.25 Hz), 73.8 (d, $J_{C4',P}$ = 9.75 Hz), 74.53, 80.52, 87.52, 90.49, 137.76, 142.59, 155.62, 159.33; ^{31}P NMR (D_2O) δ 19.57. Anal. ($C_{10}H_{17}N_6O_7P \cdot H_2O$) C, H, N, P.

9-[5′-Deoxy-5′-(dihydroxyphosphinyl)-β-D-ribofuranosyl]hypoxanthine (17). 9-(5′-Deoxy-2′,3′-di-O-benzoyl-5′-(diethoxyphosphinyl)-β-D-ribofuranosyl)hypoxanthine (12) (5 mmol, 2.98 g) was treated first with bromotrimethylsilane (15 mmol, 2.37 g) and then with methanolic ammonia exactly as in the case of the adenosine analogue 13. After DEAE-cellulose column chromatography the yield of 17 was 1.0 g (55% as monoammonium salt): (H_2O) λ_{max} 251 nm (ϵ 11 670), (pH 7) 251 nm (ϵ 11 670), (pH 1) 256 nm (ϵ 8040); ^1H NMR (H_2O) δ 1.96 (dd, H-5′ and H-5″, $J_{H5',P}$ = 17.83 Hz, $J_{H5',H4'}$ = 6.4 Hz), 3.93 (t, 1 H), 4.1 (m, 1 H), 4.45 (t, 1 H), 5.86 (d, 1 H, H-1′, $J_{1',2'}$ = 3.3 Hz), 7.79 (s, 1 H), 8.19 (s, 1 H); ^{13}C NMR (D_2O) δ 33.0 (d, C-5′, $J_{C5',P}$ = 132 Hz), 75.11 (d, C-4′, $J_{C4',P}$ = 10.5 Hz), 76.29, 82.16, 89.69, 92.27, 143.87, 147.44, 156.13, 157.64; ^{31}P NMR (D_2O) δ 19.242. Anal. ($C_{10}H_{16}N_5O_7P \cdot H_2O$) C, H, N, P.

In Vitro Anitumor Activity. The cell lines used were L1210 (a murine leukemia), WI-L2 (a human B-lymphoblast), CCRF-CEM (a human T-cell leukemia), and LoVo/L (a human colon carcinoma). Cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 20 mM HEPES, pH 7.4, and 2 mM glutamine. The cytotoxicity determinations were carried out in 96-well microtiter dishes containing a starting number of (5–10) × 10^3 cells per well and 0.1–100 μM concentrations of the compounds in triplicate wells. L1210 and WI-L2 were incubated with the compounds at 37 °C for 3 days, CCRF-CEM was incubated for 4 days, and Lovo/L was incubated for 5 days. After this time period, 25 μL of 4 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added to each well and incubation was continued for 2 h. The formazan product was dissolved in 2-propanol containing 0.04 M HCl and the absorbance was determined with a microtiter plate reader. The absorbance was proportional to the number of cells. The absorbance values were used to calculate the ID_{50} value for each compound, the concentration which inhibited cell growth to 50% of control cell growth.

In Vitro Antiviral Evaluation. Nucleoside 5′-phosphonates 13–17 were evaluated for their ability to inhibit virus-induced cytopathic effect (CPE) produced by six viruses. Viruses used in this evaluation included parainfluenza virus type 3, herpes simplex virus type 2, adenovirus type 2, rhinovirus type 1-A, influenza A virus, and visna virus. The assays were completed when untreated virus-infected cells showed 100% CPE, which took 5 days for visna and influenza A virus and 3 days for other viruses. Antiviral experiments were performed according to our previous procedures.[29]

Enzymatic Reactions. The ability of certain nucleoside monophosphates and their analogues to undergo phosphorylation was assessed with commercially available enzymes. Guanylate kinase (bovine brain) and nucleoside monophosphate kinase (bovine liver) were purchased from Sigma Chemical Co., St. Louis, MO. Enzyme reaction volumes of 300 μL each contained 4 mM ATP, 1 mM test compound, 65 mM Tris·Cl (pH 7.5), 40 mM KCl, 0.5 mM $MgCl_2$, and either guanylate kinase (0.1 unit/mL) or nucleoside monophosphate kinase (0.2 unit/mL). A unit of enzyme activity is defined as 1 μmol of substrate converted per minute at 37 °C. After incubation for 2–24 h, reaction products were analyzed by high-pressure liquid chromatography (HPLC) methods as described previously.[30] The relative amounts of nucleoside mono-, di-, and triphosphates present at the end of the incubation periods were estimated from peak areas printed out for each HPLC run for all compounds.

Registry No. α-2, 120142-51-0; β-2, 38838-05-0; α-4, 120033-27-4; β-4, 120033-28-5; α-5, 120033-29-6; β-5, 120033-30-9; α-6, 120033-31-0; β-6, 120033-32-1; α-7, 120033-34-3; β-7, 120033-33-2; 8, 120033-35-4; 9, 120033-36-5; 10, 120058-47-1; 11, 120033-37-6; 12, 120033-38-7; 13·NH_3, 120033-39-8; 14·NH_3, 120033-40-1; 14·2Na, 30784-89-5; 15·NH_3, 120033-41-2; 16·NH_3, 120033-42-3; 17·NH_3, 120033-43-4; 6-chloropurine, 87-42-3; uracil, 66-22-8; N^4-benzoyl cytosine, 26661-13-2; 2-bromohypoxanthine, 87781-93-9.

(29) Smee, D. F.; McKernan, P. A.; Nord, L. D.; Willis, R. C.; Petrie, C. R.; Riley, T. M.; Revankar, G. R.; Robins, R. K.; Smith, R. A. *Antimicrob. Agents Chemother.* 1987, *31*, 1535.
(30) Smee, D. F.; Matthews, T. R. *Antimicrob. Agent Chemother.* 1986, *30*, 117.

Synthesis of Novel 5-Fluoro Analogues of Norfloxacin and Ciprofloxacin

Daniel B. Moran,* Carl B. Ziegler, Jr., Theresa S. Dunne, Nydia A. Kuck, and Yang-i Lin

American Cyanamid Company, Medical Research Division, Lederle Laboratories, Pearl River, New York 10965.
Received August 19, 1987

A series of polyfluoro-3-quinolonecarboxylic acids have been synthesized and their in vitro antibacterial activity evaluated. The desired 7-(substituted amino) derivatives were prepared from the 5,6,7,8-tetrafluoroquinolone acids. Conversely, amine displacement occurred primarily at the 5-position when the ester was used. Structure–activity studies indicated that the antibacterial activity was greatest when the N-1 substituent was cyclopropyl and the 7-substituent was 4-methyl-1-piperazinyl. All 5-(substituted amino) derivatives showed poor in vitro activity.

In 1963, nalidixic acid (1)[1] was introduced for the treatment of urinary infections. Since that time numerous structurally related derivatives have been synthesized. Gram-negative antibacterials such as pipemidic acid (2), oxolinic acid (3), and rosoxacin (4) were introduced.[2] Recently, more highly effective broad-spectrum antibacterial agents that contain fluorine atoms have been synthesized. Compounds such as ciprofloxacin (5),[3] norflox-

(1) Lesher, G. Y.; Froelich, E. J.; Gruett, M. D.; Bailey, J. H.; Brundage, P. R. *J. Med. Chem.* 1962, *5*, 1063.

(2) (a) Albrecht, R. *Prog. Drug Res.* 1977, *21*, 9. (b) Wolfson, J. S.; Hooper, D. C. *Antimicrob. Agents Chemother.* 1985, *28*, 581.

0022-2623/89/1832-1313$01.50/0 © 1989 American Chemical Society