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Synthesis and Biological Evaluation of Phosphonopyrimidine and Phosphonopurine Ribonucleosides[1)]

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Treatment of lithiated 2′,3′,5′-tri-*O*-protected uridine and 6-chloropurine ribonucleoside with diethyl chlorophosphate, followed by deblocking (and amination) and hydrolysis, provided 5- and 6-phosphonouridine (IV and VII), and 8-phosphonoadenosine (Xb), respectively. The Arbuzov reaction of 2′,3′,5′-tri-*O*-protected 4-chloro-2(1*H*)-pyrimidinone ribonucleoside and triethyl phosphite afforded the diethyl 4-phosphonate derivative (XII). Compounds IV, VII and Xb, and their respective diethyl esters (IIb and VIb) and monoethyl ester (Xa) were inactive *in vitro* as antiviral and cytostatic agents, but the diethyl 8-phosphonate derivative (IXb) of 6-chloro-9-(β-D-ribofuranosyl)purine (VIIIa) showed some antiviral and cytostatic activities, which were comparable in all respects to those of VIIIa.

Keywords——phosphonopyrimidine ribonucleoside; phosphonopurine ribonucleoside; Arbuzov reaction; phosphonylation; antiviral activity; cytostatic activity

The potential of pyrimidine and purine nucleoside analogs as chemotherapeutic agents in the treatment of virus infections and cancer has long been recognized.[2)] The biochemistry of phosphono compounds has also been reviewed.[3)] The occurrence of antibiotics (*e.g.*, phosphonomycin and N-1409) and phosphonolipids has raised intriguing questions about the biological role of these compounds in nature. Some phosphono nucleosides have been reported in which the phosphono group is linked to the sugar.[4)] However, no report has ever appeared on phosphono nucleosides which contain a phosphono group attached to the base moiety. This paper deals with the synthesis and biological activity of such novel nucleoside analogs.

Synthesis

In a previous paper,[5)] we reported the synthesis of some phosphono derivatives of pyrimidine and purine bases, based upon a halogen–metal or proton–metal exchange reaction of bromopyrimidine or purine followed by phosphonylation. Thus, the reaction of 5-bromouridine (Ia) with dihydropyran[6)] in dimethylformamide (DMF) in the presence of *p*-toluenesulfonic acid afforded 5-bromo-2′,3′,5′-tri-*O*-(tetrahydro-2-pyranyl)uridine (Ib) in a quantitative yield. Successive treatment of Ib with *n*-butyllithium and with diethyl chlorophosphate in tetrahydrofuran (THF) at −78 °C under argon gas provided, after work-up, two products, which were separated by silica gel column chromatography. The major product was isolated in 38% yield. Its ultraviolet (UV) absorption maximum was shifted by 10 nm to longer wavelength, and the proton nuclear magnetic resonance (^{1}H-NMR) spectrum revealed the absence of the C-5 proton signal and the presence of signals due to methyl and methylene protons in the diethyl phosphonate group. The compound was thus confirmed to be the 5-diethyl phosphonate derivative (IIa).[7)] The minor product, which was isolated in 26% yield,

had a UV absorption spectrum similar to that of uridine. The ^{1}H-NMR spectrum showed the presence of C-5 and C-6 proton signals. The product was identified as 2′,3′,5′-tri-*O*-(tetrahydro-2-pyranyl)uridine (III), which may have been formed by hydrolysis of the lithiouridine derivative.[8] Deblocking of the tetrahydropyranyl group in IIa with pyridinium *p*-toluenesulfonate[9] (PPTS) gave white needles in 51% yield. This product was confirmed to be diethyl 5-uridinylphosphonate (IIb) by elemental analysis and ^{1}H-NMR spectroscopy. Hydrolysis of the ethyl phosphonate in IIb with iodotrimethylsilane[10] in acetonitrile gave, after work-up, a white powder in 76% yield. This product was proved to be pure by high-performance liquid chromatography (HPLC) and migrated similarly to uridine 5′-monophosphate on paper electrophoresis (PE). The ^{1}H-NMR spectrum disclosed the absence of signals due to ethyl protons. The compound was thus identified as 5-phosphonouridine (IV) (Chart 1).

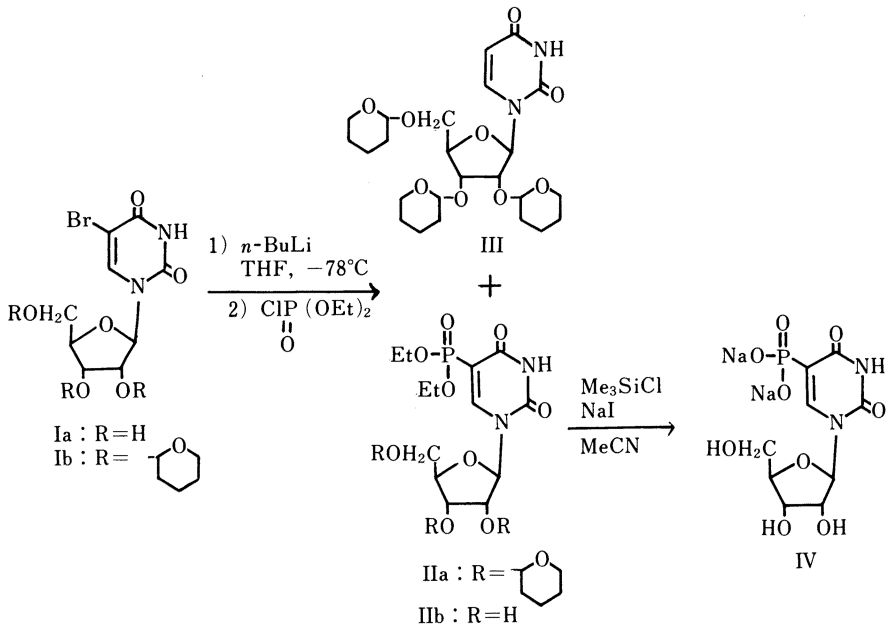


Chart 1

The reaction of 2′,3′-*O*-isopropylideneuridine (Va) with 2,3-dihydrofuran provided the 5′-*O*-(tetrahydro-2-furanyl) derivative (Vb). Successive treatment of Vb with lithium diisopropylamide[11a-c] and with diethyl chlorophosphate, followed by removal of the tetrahydrofuranyl group with PPTS, afforded, after purification by silica gel chromatography, the diethyl phosphonate derivative (VIa) in an overall yield of 51% relative to Va, and recovered Va in 19% yield. Removal of the isopropylidene group of VIa with 80% trifluoroacetic acid provided diethyl 6-uridinylphosphonate (VIb). Hydrolysis of the diethyl phosphonate group in VIb, as described for IIb, resulted in cleavage of the glycosidic bond so as to release 6-phosphonouracil, as proven by ^{1}H-NMR spectroscopy. However, a modified hydrolysis with the addition of a small amount of pyridine provided a white solid in 89% yield. The structure was confirmed to be 6-phosphonouridine (VII) by UV and ^{1}H-NMR spectroscopies as well as PE (Chart 2).

A sequence of reactions starting from 6-chloro-9-(β-D-ribofuranosyl)purine (VIIIa), similar to that starting from Ia, provided diethyl 6-chloro-9-(β-D-ribofuranosyl)-8-purinylphosphonate (IXb)[11d] *via* the 2′,3′,5′-tri-*O*-(tetrahydro-2-pyranyl) derivative (VIIIb)

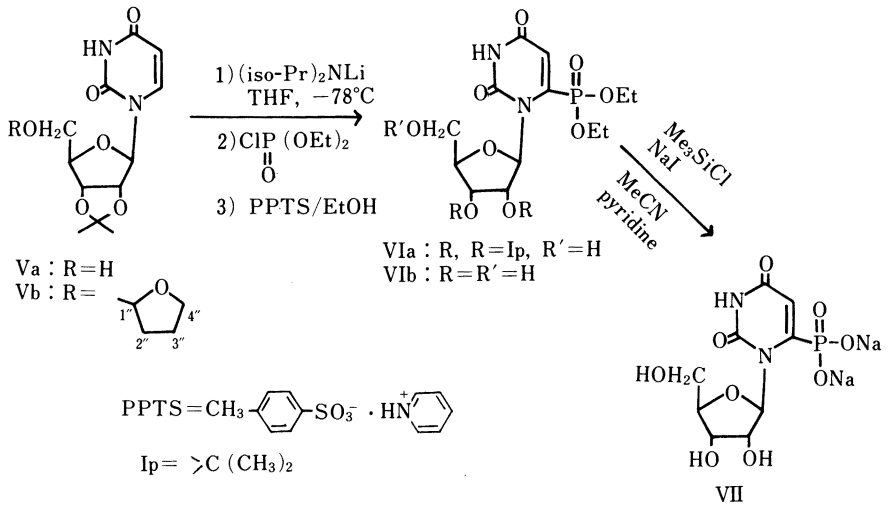


Chart 2

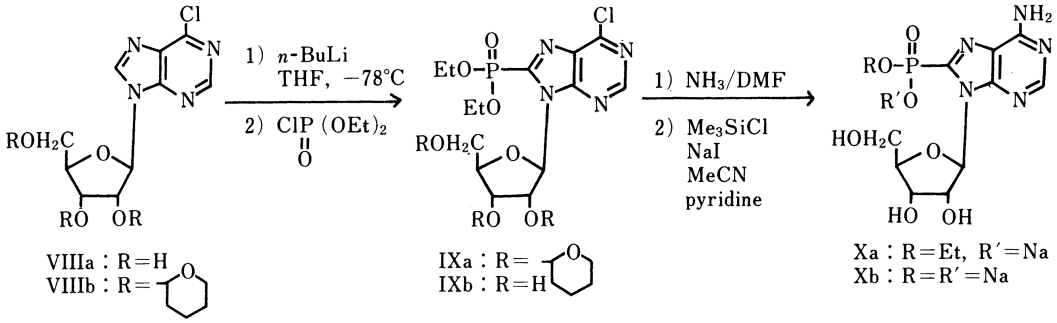


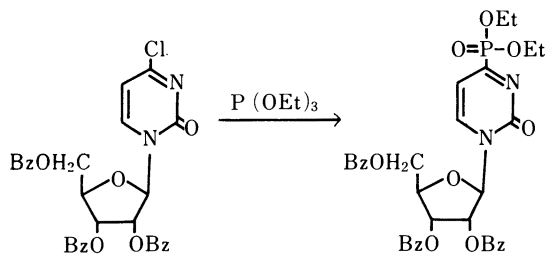
Chart 3

and the diethyl phosphonate derivative (IXa). Treatment of IXb with DMF saturated with ammonia afforded, after work-up including repeated purification by DE 23 column chromatography, a white powder in 82% yield; this product was ethyl 8-adenosinylphosphonate (Xa). It showed a single UV-absorbing spot of $M_{5'\text{-AMP}}$[12]$=0.44$ on PE. Compound IXb was treated with DMF saturated with ammonia and the product was allowed to react, without isolation, with iodotrimethylsilane in acetonitrile containing a small amount of pyridine. The product (a white powder) showed a single UV-absorbing spot of $M_{5'\text{-AMP}}=0.94$ on PE and was assigned the 8-phosphonoadenosine (Xb) structure from the results of UV and ^{1}H-NMR spectroscopies as well as elemental analysis (Chart 3).

Finally, we subjected 1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-4-chloro-2(1*H*)-pyrimidinone (XI) to the Arbuzov reaction[13] (treatment with triethyl phosphite at 125 °C). It gave a white crystalline 4-diethyl phosphonate derivative (XII) in 75% yield. The structure of XII was proven by elemental analysis and ^{1}H-NMR spectroscopy (Chart 4).

Biological Activity

Compounds IIb, IV, VIb, VII, VIIIa, IXb, Xa and Xb were evaluated for biological activity in a variety of antiviral and cytostatic assay systems according to previously established procedures.[14,15] The antiviral assays were run in primary rabbit kidney (PRK) cells (herpes simplex virus type 1 (strain KOS), herpes simplex virus type 2 (strain G), vaccinia virus,



XI XII

Chart 4

TABLE I. Antiviral and Cytostatic Activities of VIIIa and IXb

Assay system	MIC_{50} (μg/ml)[a]	
	VIIIa	IXb
Antiviral activity		
Herpes simplex virus type 1 (KOS)/PRK	>200	>100
Herpes simplex virus type 2 (G)/PRK	≥100	>100
Vaccinia virus/PRK	7	20
Vesicular stomatitis virus/PRK	7	20
Vesicular stomatitis virus/HeLa	2	10
Coxsackie B4 virus/HeLa	70	200
Poliovirus type 1/HeLa	150	200
Reovirus type 1/Vero	40	100
Parainfluenza virus type 3/Vero	150	300
Sindbis virus/Vero	200	>400
Semliki forest virus/Vero	300	>400
Coxsackie B4 virus/Vero	20	10
Cytostatic activity		
Murine leukemia (L1210)	15	27
Murine mammary carcinoma (FM3A)	138	54
Human B-lymphoblast (Raji)	85	38
Human T-lymphoblast (Molt-4F)	286	284

a) Minimum inhibitory concentration required to reduce virus-induced cytopathogenicity or tumor cell count by 50%.

vesicular stomatitis virus), HeLa cells (vesicular stomatitis virus, Coxsackie B4 virus, poliovirus type 1) or Vero (African green monkey kidney) cells (reovirus type 1, parainfluenza virus type 3, Sindbis virus, Semliki forest virus, Coxsackie B4 virus). Cytostatic activity was assessed with murine leukemia (L1210), murine mammary carcinoma (FM3A), human B-lymphoblast (Raji) and human T-lymphoblast (Molt/4F) cells.

Compounds IIb, IV, VIb, VII, Xa and Xb proved inactive as antiviral and cytostatic agents at concentrations up to 400 and 1000 μg/ml, respectively (data not shown). Compound IXb showed some antiviral activity, *i.e.* against vaccinia virus (in PRK cells), vesicular stomatitis virus (in PRK and HeLa cells) and Coxsackie B4 virus (in Vero cells). Compound IXb was also inhibitory to the proliferation of tumor cells (Table I). It is evident, however, that the biological activity of compound IXb is due to its 6-chloro group rather than its 8-diethylphosphonate group, since compound VIIIa showed antiviral and cytostatic activities that were comparable in all respects to those of IXb.

Discussion

Substitution of a phosphonate or diethyl phosphonate group at C-5 or C-6 of the uracil ring did not endow uridine with either antiviral or cytostatic activity. Nor did adenosine acquire any antiviral or cytostatic activity upon substitution of a phosphonate or diethyl phosphonate group at C-8 of the adenine moiety. The only phosphonyl derivative that was found to be biologically active was the 6-chloropurine ribonucleoside in which a diethyl phosphonate group was substituted at C-8. However, the activity of this compound was comparable to that of the 6-chloropurine ribonucleoside itself, suggesting that the chlorine, and not the diethyl phosphonate, group was responsible for its biological effects.

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus (hot stage type) and are uncorrected. HPLC was conducted with a Shimadzu LC-2 apparatus using a column packed with Nucleosil 10DMA (10 μ) and a mobile phase of 10 mM phosphate buffer (pH 5.3). The UV spectra were recorded with a Shimadzu UV-190 digital spectrometer. The ^{1}H-NMR spectra were recorded with a JEOL GX-400 (400 MHz) spectrometer in $CDCl_3$ or dimethyl sulfoxide (DMSO)-d_6 with tetramethylsilane as an internal standard and in D_2O with sodium 3-(trimethylsilyl)propionate as an internal standard, respectively. PE was carried out at 22 V/cm using 0.01 M phosphate buffer (pH 7.5).

5-Bromo-2′,3′,5′-tri-*O*-(tetrahydro-2-pyranyl)uridine (Ib)——*p*-Toluenesulfonic acid (4.0 g) was added to a cooled solution of 5-bromouridine (5.30 g, 16.4 mmol) in a mixture of DMF (28 ml) and dihydropyran (12 ml), and the solution was kept at 4 °C for 15 h. Triethylamine (3.5 ml) was added to the reaction mixture and the solvent was evaporated off *in vacuo* to give a residue, which was partitioned between $CHCl_3$ (200 ml) and H_2O (200 ml). The organic phase was dried over $MgSO_4$, concentrated to a small volume, and chromatographed over a column of Silica gel G (4.0 × 25 cm) using a gradient (2 l) of 0—4% EtOH in $CHCl_3$ to obtain a syrup (8.90 g, 94%), which showed a single UV-absorbing spot on thin layer chromatography (TLC) with $CHCl_3$–EtOH (25 : 1). MS *m/z*: 490, 492 ($M^+ - C_5H_8O$). UV λ_{max}^{MeOH} nm: 278. ^{1}H-NMR ($CDCl_3$) δ: 9.90 (1H, d-like, N^3-H), 8.23 (1H, q-like, H-6), 6.0—6.4 (1H, m, H-1′), 3.3—5.2 (14H, H-2′, H-3′, H-4′, H-5′, –OC<u>H</u>(–O–)CH_2– and –OC<u>H</u>$_2$(CH_2)$_3$–). 1.2—2.2 (18H, –OCH(–O–)C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$–).

Diethyl 2′,3′,5′-Tri-*O*-(tetrahydro-2-pyranyl)uridine-5-phosphonate (IIa)——A solution of Ib (5.75 g, 10 mmol) in THF (120 ml) was cooled at −78 °C under argon, then *n*-butyllithium (11.8 ml of a 1.7 M solution in hexane, 20 mmol) was added dropwise for 5 min. The brownish solution was stirred at −78 °C for 1 h, and then diethyl chlorophosphate (4.14 g, 24 mmol) was added dropwise. The mixture was stirred for 5 h, and warmed to room temperature, then 20% ammonium formate (30 ml) and pyridine (2 ml) were added. The residue obtained after removal of the solvents was partitioned between benzene (100 ml) and H_2O (50 ml). The organic layer was washed twice with water (50 ml), dried over $MgSO_4$, and concentrated to a small volume. Toluene was added, and the azeotropic mixture was distilled off. The residue was taken up in $CHCl_3$ (30 ml) and the solution was chromatographed over a column of Silica gel G (4.0 × 30 cm) with $CHCl_3$–EtOH (50 : 1) to give two main fractions. The first fraction was evaporated to dryness to give a caramel (2.40 g, 38%). UV λ_{max}^{MeOH} nm: 264.5. ^{1}H-NMR ($CDCl_3$) δ: 9.8 (1H, br s, N^3-H), 8.25 (1H, m, H-6), 5.9 (1H, m, H-1′), 4.2 (4H, m, –C<u>H</u>$_2$CH$_3$), 1.28 (6H, t, –CH$_2$C<u>H</u>$_3$).

2′,3′,5′-Tri-*O*-(tetrahydro-2-pyranyl)uridine (III)——Evaporation of the other main fraction in the preceding section gave a caramel (1.28 g, 26%). UV λ_{max}^{MeOH} nm: 260. ^{1}H-NMR ($CDCl_3$) δ: 7.95 (1H, m, H-6), 6.05 (1H, m, H-1′), 5.64 (1H, d-like, H-5), 3.3—5.1 (H-2′, H-5′, –OC<u>H</u>(–O–)(CH_2)$_3$C<u>H</u>$_2$–), 1.3—2.0 (–OCH(–O–)C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$–).

Diethyl 5-Uridinylphosphonate (IIb)——PPTS (244 mg, 0.97 mmol) was added to a solution of IIa (1.60 g, 2.53 mmol) in EtOH (25 ml). The mixture was kept at 50 °C for 5 h and concentrated to dryness. The residue was dissolved in CH_2Cl_2 (15 ml) and the solution was chromatographed over a column of Silica gel G (3.0 × 35 cm) with a gradient (1 l) of 5—25% EtOH in $CHCl_3$. The residue obtained after removal of the solvents was triturated from EtOH (20 ml) to give a white crystalline product (490 mg, 51%). mp 161—163 °C. *Anal.* Calcd for $C_{13}H_{21}N_2O_9P$: C, 41.06; H, 5.57; N, 7.38. Found: C, 41.32; H, 5.69; N, 7.22. UV $\lambda_{max}^{0.1\,N\,HCl}$ nm: (ε): 265.5 (11400), $\lambda_{max}^{H_2O}$ nm (ε): 265 (11600), $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 264 (7500). ^{1}H-NMR (DMSO-d_6) δ: 11.63 (1H, d, J_{HNCCP} = 4.58 Hz, N^3-H), 8.35 (1H, d, J_{HCCP} = 13.19 Hz, H-6), 5.79 (1H, d, $J_{1',2'}$ = 4.58 Hz, H-1′), 5.44 (1H, d, $J_{2'OH,2'}$ = 5.31 Hz, H-2′OH), 5.09 (1H, d, $J_{3'OH,3'}$ = 5.13 Hz, H-3′OH), 5.06 (1H, t, $J_{5'\text{-}OH,5'}$ = 4.68 Hz, H-5′OH), 4.04 (5H, m, –C<u>H</u>$_2$CH$_3$, and H-2′), 3.95 (1H, q, $J_{3',2'} = J_{3',4'}$ = 4.53 Hz, H-3′), 3.90 (1H, m, H-4′), 3.63 (1H, m, $J_{5a',4'}$ = 2.7 Hz, H-5′a), 3.55 (1H, m, $J_{5'b,4'}$ = 3.05 Hz, H-5′b), 1.23 (6H, t, –CH$_2$C<u>H</u>$_3$).

5-Phosphonouridine 2Na (IV)——Chlorotrimethylsilane (0.63 ml, 5.0 mmol) was added to a solution of IIb (150 mg, 0.39 mmol) and NaI (750 mg, 5.0 mmol) in acetonitrile (5 ml). The mixture was stirred at room temperature for 1 h (NaCl precipitated immediately). The supernatant was concentrated and the residue was dissolved in MeOH

(5 ml). This solution was adjusted to pH 7—9 with conc. NH_4OH to afford a white solid (109 mg, 74%), which showed a single UV-absorbing spot and a single peak on PE and HPLC, respectively. mp 204—210 °C. *Anal.* Calcd for $C_9H_{11}N_2Na_2O_9P \cdot H_2O$: C, 27.99; H, 3.39; N, 7.25. Found: C, 27.84; H, 3.39; N, 7.22. UV $\lambda_{max}^{0.1\,N\,HCl}$ nm (ε): 264 (12500), $\lambda_{max}^{H_2O}$ nm (ε): 264 (12100), $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 261.5 (8700). ^{1}H-NMR (D_2O) δ: 7.91 (1H, d, J_{HCCP} = 12.09 Hz, H-6), 5.81 (1H, d, $J_{1',2'}$ = 4.95 Hz, H-1'), 4.23 (1H, t, $J_{2',3'}$ = 5.31 Hz, H-2'), 4.10 (1H, t, $J_{3',4'}$ = 5.14 Hz, H-3'), 3.99 (1H, sestet, H-4'), 3.76 (1H, q, $J_{5'a,4'}$ = 2.93 Hz, $J_{5'a,5'b}$ = 12.87 Hz, H-5'a), 3.66 (1H, q, $J_{5'b,4'}$ = 4.86 Hz, H-5'b).

5'-*O*-(Tetrahydro-2-furanyl)-2',3'-*O*-isopropylideneuridine (Vb)——2,3-Dihydrofuran (1.4 ml) and PPTS (244 mg, 0.97 mmol) were added to a solution of 2',3'-*O*-isopropylideneuridine (Va) (2.77 g, 9.75 mmol) in CH_2Cl_2 (50 ml). After standing at room temperature overnight, the mixture was concentrated and chromatographed over a column of Silica gel G (3.2 × 30 cm) with a gradient (800 ml × 2) of 0—6.5% EtOH in $CHCl_3$. The combined fractions was evaporated to dryness to give a white crystalline product (2.90 g, 84%). mp 94—97 °C. *Anal.* Calcd for $C_{16}H_{22}N_2O_7$: C, 54.23; H, 6.26; N, 7.91. Found: C, 54.21; H, 6.13; N, 7.74. MS *m/z*: 339 ($M^+ - CH_3$), 383 ($M^+ - C_4H_7O$). UV λ_{max}^{MeOH} nm (ε): 259.5 (9500). ^{1}H-NMR ($CDCl_3$) δ: 7.60 (1H, d, $J_{6,5}$ = 8.06 Hz, H-6), 7.59 (1H, d, $J_{6,5}$ = 8.06 Hz, H-6), 5.90 (1H, d, $J_{1',2'}$ = 1.47 Hz, H-1'), 5.87 (1H, d, $J_{1',2'}$ = 2.20 Hz, H-1'), 5.692 (1H, d, H-5), 5.686 (1H, d, H-5), 5.13 (2H, m, H-2'), 4.72—4.78 (4H, m, H-3' and H-1''), 4.44 (1H, m, H-4'), 4.38 (1H, m, H-4'), 3.99 (1H, q, $J_{5'a,4'}$ = 2.93 Hz, $J_{5'a,5'b}$ = 10.99 Hz, H-5'a), 3.86—3.91 (5H, m, H-5'b and H-4''), 3.67 (1H, q, $J_{5'a,4'}$ = 2.20 Hz, $J_{5'a,5'b}$ = 10.99 Hz, H-5'a), 3.55 (1H, q, $J_{5'b,4'}$ = 4.39 Hz, H-5'b), 1.76—2.00 (8H, m, H-2'', H-3''), 1.59, 1.36 (each 6H, s, $>C(CH_3)_2$). These data indicate that Va is a mixture of two diastereoisomers.

Diethyl 2',3'-*O*-Isopropylideneuridine-6-phosphonate (VIa)——A solution of Vb (10.0 g, 28.2 mmol) and diisopropylamine (9.8 ml, 70 mmol) in THF (130 ml) was cooled at −78 °C under argon for 1 h, and *n*-butyllithium (4.1 ml of 1.7 M solution in hexane, 70 mmol) was added. The mixture was stirred for 1 h, then diethyl chlorophosphate (16.2 g, 94 mmol) was added dropwise. The solution was stirred for 6 h and chromatographed in a manner similar to that described in the case of IIa. TLC ($CHCl_3$–EtOH, 20 : 1) of the crude products showed two spots of *Rf* 0.28 and *Rf* 0.44. The product was dissolved in EtOH (50 ml), and PPTS (700 mg, 2.79 mmol) was added. After standing at 40 °C for 6 h, the mixture was evaporated to dryness. The residue was dissolved in a small amount of $CHCl_3$ and chromatographed over a column of Silica gel G (3.2 × 30 cm) with a gradient (2 l) of 5—15% EtOH in $CHCl_3$ to afford the two main fractions. The first fraction was evaporated to dryness to yield a caramel (VIb) (4.91 g, 51%). UV λ_{max}^{MeOH} nm: 268. ^{1}H-NMR ($CDCl_3$) δ: 9.87 (1H, br s, N^3-H), 6.47 (1H, d, J_{HCCP} = 13.92 Hz, H-5), 6.15 (1H, d, $J_{1',2'}$ = 2.56 Hz, H-1'), 5.27 (1H, q, $J_{2',3'}$ = 6.78 Hz, H-2'), 5.06 (1H, q, $J_{3',4'}$ = 4.40 Hz, H-3'), 4.31 (5H, m, H-5'OH and $-C\underline{H}_2CH_3$), 4.22 (1H, m, H-4'), 3.91 (1H, q, $J_{5'a,4'}$ = 2.93 Hz, $J_{5'a,5'b}$ = 12.09 Hz, H-5'a), 3.80 (1H, q, $J_{5'b,4'}$ = 4.03 Hz, H-5'b), 1.56, 1.35 (each 3H, s, $>C(C\underline{H}_3)_2$), 1.42 (6H, sestet, $-CH_2C\underline{H}_3$). The residue obtained from the other main fraction was crystallized from AcOEt, giving colorless prisms (Va) (1.38 g, 19%). mp 161—163 °C (lit.[16] 159—160 °C).

Diethyl 6-Uridinylphosphonate (VIb)——Compound VIa (320 mg, 0.76 mmol) was treated with 80% trifluoroacetic acid (2 ml) at 0 °C for 30 min and the solution was evaporated to dryness. EtOH (5 ml) was added to the residue and the azeotropic mixture was distilled off. The resulting syrup was dissolved in CH_2Cl_2 and the solution was purified by column chromatography (Silica gel G) to give a foam (280 mg). TLC ($CHCl_3$–EtOH, 9 : 1), *Rf* 2.90. UV $\lambda_{max}^{0.05\,N\,HCl}$ nm: 265.5, $\lambda_{max}^{H_2O}$ nm: 265, $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 262. ^{1}H-NMR (DMSO-d_6) δ: 11.63 (1H, d, N^3-H), 6.15 (1H, q, J_{HCCP} = 13.92 Hz, J_{HCCNH} = 1.47 Hz, H-5), 5.69 (1H, d, $J_{1',2'}$ = 2.74 Hz, H-1'), 5.04 (1H, d, $J_{2'OH,2'}$ = 4.95 Hz, H-2'OH), 4.93 (1H, d, $J_{3'OH,3'}$ = 6.77 Hz, H-3'OH), 4.60 (1H, t, $J_{5'\text{-}OH,5'}$ = 5.77 Hz, H-5'OH), 4.50 (1H, m, H-2') 4.21—4.08 (6H, m, H-3', H-4', and $-C\underline{H}_2CH_3$), 3.62 (1H, m, H-5'a), 3.43 (1H, m, H-5'b), 1.31 (6H, sestet, J_{HCCP} = 7.23 Hz, $-CH_2C\underline{H}_3$).

6-Phosphonouridine 2Na (VII)——Chlorotrimethylsilane (1.92 ml, 15.0 mmol) was added to a solution of VIb (350 mg, 0.91 mmol), NaI (2.25 g, 15.0 mmol) and pyridine (0.5 ml) in acetonitrile (15 ml). The mixture was stirred at room temperature for 1 h (NaCl precipitated immediately). The supernatant was treated in a manner similar to that described for IV to provide a white solid, which was passed through a column of Amberlite IR120B (Na^+ form, 1.8 × 10 cm). Evaporation of the eluate gave a caramel (186 mg, 51%), which showed a single UV-absorbing spot at the same position as that of uridine 5'-monophosphate on PE. mp > 300 °C. *Anal.* Calcd for $C_9H_{11}N_2Na_2O_9P \cdot H_2O$: C, 27.99; H, 3.39; N, 7.24. Found: C, 27.66; H, 3.37; N, 7.14. UV $\lambda_{max}^{0.1\,N\,HCl}$ nm (ε): 266.5 (9300), $\lambda_{max}^{H_2O}$ nm (ε): 268 (9500), $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 268 (7600). ^{1}H-NMR (D_2O) δ: 6.02 (1H, d, $J_{1',2'}$ = 2.93 Hz, H-1'), 5.74 (1H, d, J_{HCCP} = 10.63 Hz, H-5), 4.16 (1H, q, $J_{2',3'}$ = 6.60 Hz, H-2'), 3.83 (1H, t, $J_{3',4'}$ = 7.32 Hz, H-3'), 3.43 (1H, sestet, H-4'), 3.34 (1H, q, $J_{5a',4'}$ = 2.94 Hz, $J_{5'a,5'b}$ = 12.09 Hz, H-5'a), 3.20 (1H, q, $J_{5'b,4'}$ = 6.22 Hz, H-5'b).

6-Chloro-9-(2',3',5'-tri-*O*-(tetrahydro-2-pyranyl)-β-D-ribofuranosyl)purine (VIIIb)——*p*-Toluenesulfonic acid (6.0 g, dried over P_2O_5) was added to an ice-cooled solution of 6-chloro-9-(β-D-ribofuranosyl)purine (VIIIa) (4.0 g, 13.9 mmol) in a mixture of DMF (20 ml) and 2,3-dihydropyran (20 ml). The reaction mixture was allowed to stand at 0—5 °C for 6 h and treated in a manner similar to that described for Ib to give a syrup (6.44 g, 86%), which showed a single UV-absorbing spot on TLC (C_6H_6–AcOEt, 2 : 1). UV λ_{max}^{MeOH} nm: 263. ^{1}H-NMR ($CDCl_3$) δ: 8.5—8.8 (2H, m, H-2 and H-8), 6.35 (1H, m, H-1'), 3.30—5.0 (14H, H-2', H-3', H-4', H-5', $-OC\underline{H}(-O-)CH_2-$ and $-OC\underline{H}_2(CH_2)_3-$), 1.2—2.2 (18H, $-OCH(-O-)C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2-$).

Diethyl 6-Chloro-9-(2',3',5'-tri-*O*-(tetrahydro-2-pyranyl)-β-D-ribofuranosyl)purine-8-phosphonate (IXa)——A solution of VIIIb (1.21 g, 2.24 mmol) in THF (25 ml) was cooled at −78 °C under argon and treated with *n*-butyllithium

(5.75 mmol) in a similar manner to that described for VIa. After 1 h, diethyl chlorophosphate (1.0 ml, 6.9 mmol) was added and the mixture was stirred for 30 min and treated in a manner similar to that described for IIa to obtain a foam (1.04 g, 69%), which showed a single UV-absorbing spot on TLC (C_6H_6–AcOEt, 5 : 1). UV λ_{max}^{MeOH} nm: 270, 255 (sh). ^{1}H-NMR ($CDCl_3$) δ: 8.74 (1H, s, H-2), 6.72 (1H, m, H-1′), 5.61 (1H, m, H-2′), 4.30 (4H, m, $-C\underline{H}_2CH_3$), 1.45 (6H sestet, $-CH_2C\underline{H}_3$).

Diethyl 6-Chloro-9-(β-D-ribofuranosyl)purine-8-phosphonate (IXb)——PPTS (800 mg, 3.5 mmol) was added to a solution of IXa (6.95 g, 10.3 mmol) in EtOH (35 ml), and the solution was kept at 50 °C for 6 h. The reaction mixture was worked up to obtain pale yellowish crystals (2.41 g, 55%). mp 119—121.5 °C. *Anal.* Calcd for $C_{14}H_{20}ClN_4O_7P$: C, 39.77; H, 4.77; N, 13.27. Found: C,39.73; H, 4.71; N, 13.38. UV $\lambda_{max}^{0.1\,N\,HCl}$ nm (ε): 270 (13800), $\lambda_{max}^{H_2O}$ nm (ε): 270 (13500). ^{1}H-NMR (DMSO-d_6) δ: 8.95 (1H, s, H-2), 6.42 (1H, d, $J_{1',2'}=5.74$ Hz, H-1′), 5.38 (1H, d, $J_{2'OH,2'}=5.74$ Hz), 5.24 (1H, d, $J_{3'OH,3'}=5.64$ Hz, H-3′OH), 5.12 (1H, q, $J_{2',3'}=5.49$ Hz, H-2′), 4.83 (1H, t, $J_{5'\text{-}OH,5'}=5.74$ Hz, H-5′OH), 4.27 (4H, m, $-C\underline{H}_2CH_3$), 3.95 (1H, q, H-4′), 3.72 (1H, m, $J_{5a',4'}=4.88$ Hz, $J_{5'a,5'b}=11.84$ Hz, H-5′a), 3.56 (1H, m, H-5′b), 1.33 (6H, q, $-CH_2C\underline{H}_3$).

Ethyl Adenosine-8-phosphonate Na (Xa)——A solution of IXb (350 mg, 0.83 mmol) in DMF (20 ml) was saturated with ammonia at 0 °C and the mixture was heated at 100 °C overnight in a stainless steel bomb, and then cooled to 0 °C. The solvent was evaporated off *in vacuo* and the residue was dissolved in water (10 ml). The solution was chromatographed over a column of diethyl amino ethyl (DEAE) cellulose (bicarbonate form, 2.1 × 25 cm) with a gradient (1 l) of 0—0.05 M triethylammonium bicarbonate (TEAB) as the eluent. The required fraction was evaporated to dryness. Water (10 ml) was added to the residue, and the azeotropic mixture was distilled off. The residue was dissolved in water (20 ml) and the solution was passed through an Amberlite IR 120B column (Na^+, 1.6 × 25 cm). The flow-through fraction was evaporated to dryness and the residue was triturated with EtOH, giving a white solid (269 mg, 82%). PE $M_{5'\text{-}AMP}=0.44$. mp > 300 °C. UV $\lambda_{max}^{0.05\,N\,HCl}$ nm: 266, $\lambda_{max}^{H_2O}$ nm: 268, $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 268. ^{1}H-NMR (D_2O) δ: 8.01 (1H, s, H-2), 6.41 (1H, d, $J_{1',2'}=7.08$ Hz, H-1′), 4.91 (1H, t, $J_{2',3'}=4.64$ Hz, H-2′), 4.35 (1H, m, H-3′), 4.15 (1H, s-like, H-4′), 3.84 (2H, m, H-5′), 3.74 (2H, q, $-C\underline{H}_2CH_3$), 1.10 (3H, t-like, $-CH_2C\underline{H}_3$).

8-Phosphonoadenosine 2Na (Xb)——A solution of IXb (250 mg, 0.59 mmol) in DMF (15 ml) was hydrolyzed with ammonia in a manner similar to that described for Xa. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in a mixture of acetonitrile (25 ml) and pyridine (1 ml). Chlorotrimethylsilane (3.25 ml, 25 mmol) and NaI (3.63 g, 24.2 mmol) were added to the solution, and the mixture was stirred for 1 h (NaCl precipitated immediately). The supernatant was treated in a manner similar to that described for IV to give a white solid (106 mg, 43%). mp > 300 °C. *Anal.* Calcd for $C_{10}H_{12}N_5Na_2O_7P\cdot 1.7H_2O$: C, 28.46; H, 3.68; N, 16.60. Found: C, 28.80; H, 3.87; N, 7.12. UV $\lambda_{max}^{0.1\,N\,HCl}$ nm (ε): 265.5 (20300), $\lambda_{max}^{H_2O}$ nm (ε): 267 (15900), $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 267.5 (16900). ^{1}H-NMR (D_2O) δ: 8.02 (1H, s, H-2), 6.58 (1H, d, $J_{1',2'}=6.96$ Hz, H-1′), 4.85 (1H, t, $J_{2',3'}=5.49$ Hz, H-2′), 4.38 (1H, q, $J_{3',4'}=2.20$ Hz, H-3′), 4.19 (1H, d-like, H-4′), 3.82 (1H, q, $J_{5a',4'}=1.47$ Hz, $J_{5'a,5'b}=12.82$ Hz, H-5′a), 3.73 (1H, q, $J_{5'b,4'}=2.93$ Hz, H-5′b).

Diethyl 1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone-4-phosphonate (XII)——A mixture of 1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-4-chloro-2(1*H*)-pyrimidinone (XI) (2.35 g, 4.09 mmol) and triethyl phosphite (5 ml) was heated at 125 °C for 4 h, and then cooled to room temperature. Ether (50 ml) was added to give a white crystalline product (2.08 g, 75%). mp 162—164 °C. *Anal.* Calcd for $C_{34}H_{33}N_2O_{11}P$: C, 60.36; H, 4.92; N, 4.14. Found: C, 60.02; H, 4.67; N, 4.17. UV λ_{max}^{MeOH} nm (ε): 328 (3340), 281 (3090), 274.5 (3420), 234 (15900). ^{1}H-NMR ($CDCl_3$) δ: 8.3—7.2 (16H, m, $C_6\underline{H}_5$–, H6), 6.73 (1H, q, $J_{HCCP}=4$ Hz, $J_{5,6}=11$ Hz, H5), 6.35 (1H, d, $J_{1',2'}=3$ Hz, H1′), 5.88 (2H, m, H2′, H3′), 4.81 (3H, m, H4′, H5′), 4.27 (4H, pentet, $-C\underline{H}_2CH_3$), 1.35 (6H, t-like, $-CH_2C\underline{H}_3$).

References and Notes

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