Synthesis and Antiviral Activity of Phosphonoacetic and Phosphonoformic Acid Esters of 5-Bromo-2'-deoxyuridine and Related Pyrimidine Nucleosides and Acyclonucleosides[†]
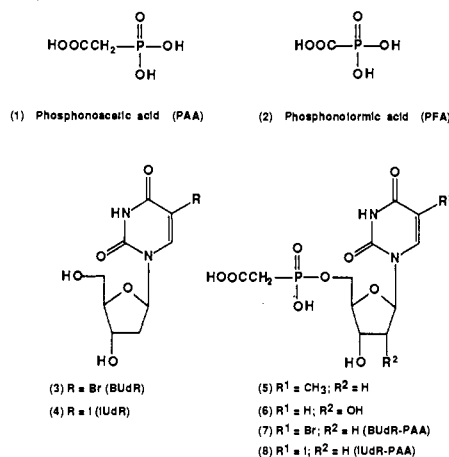
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Phosphonoacetic acid (PAA, 1) was coupled with various acyclonucleosides, 2'-deoxyuridines, cytidines, and arabinosyluracils, with 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) or dicyclohexylcarbodiimide (DCCI) as condensing agents, to give a range of phosphonate esters. The carboxylic ester linkage of PAA to the 5'-position of 5-bromo-2'-deoxyuridine (BUdR, 3) was achieved via the mixed anhydride formed from (diethylphosphono)acetic acid and trifluoroacetic anhydride. Phosphonoformic acid (PFA, 2) was coupled with BUdR by using the DCCI method to give the phosphonate ester (59). Of these compounds only phosphonate esters in the 2'-deoxyuridine series showed significant activity against herpes simplex virus types 1 and 2. The BUdR-PAA derivative (7) and the BUdR-PFA derivative (59) were highly active, especially the latter, which was more active than the parent nucleoside BUdR (3) against the type 2 virus. The active compounds may exert their effects by extracellular or intracellular hydrolysis to the corresponding antiviral agents, but an intrinsic component of antiviral activity may also be involved.

Phosphonoacetic acid (PAA, 1) and phosphonoformic acid (PFA, 2) show good antiviral activity[1,2] against herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2). PAA appears to have a high affinity for bone that may preclude its use in humans[3] whereas PFA (Foscarnet) has been used clinically against HSV-1 and HSV-2.

(1) Phosphonoacetic acid (PAA)

(2) Phosphonoformic acid (PFA)

(9) R' = OH; R^2 = H

(10) R' = R^2 = H

(11) R' = H; R^2 = OH (AraA-PAA)

(12)

(3) R = Br (BUdR)

(4) R = I (IUdR)

(5) R^1 = CH$_3$; R^2 = H

(6) R^1 = H; R^2 = OH

(7) R^1 = Br; R^2 = H (BUdR-PAA)

(8) R^1 = I; R^2 = H (IUdR-PAA)

In an earlier study, PAA had been coupled to the nucleosides adenosine, guanosine, thymidine, uridine, arabinosyladenosine (*ara*-A), 5-bromo-2'-deoxyuridine (BUdR, 3), and 5-iodo-2'-deoxyuridine (IUdR, 4) to give the novel compounds 5–12.[4] Of these compounds, BUdR-PAA (7) and IUdR-PAA (8) were the most active in the protection
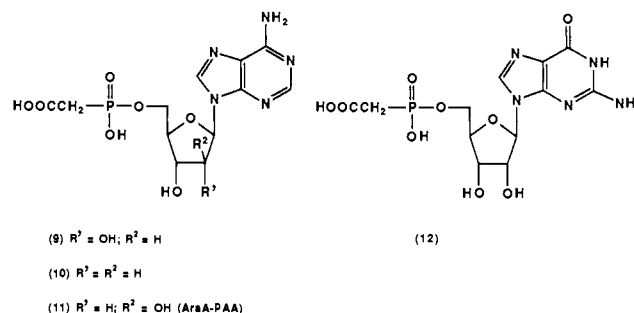
of mice against a systemic infection of HSV-1.[5] These compounds did not inhibit HSV-induced DNA polymerase.[6] In contrast to the naturally occurring nucleoside 5'-monophosphates, these phosphonates were reported[5] to be resistant to the dephosphorylative action of bacterial alkaline and calf intestinal phosphatases. A slow release of nucleoside was observed upon incubation with snake venom 5'-nucleotidase.[5]

We have further investigated the attachment of PAA to BUdR and to the 5'-position of some of the newer antiviral agents that have improved therapeutic ratios. In

[†]Abbreviations used are: PAA = phosphonoacetic acid (1), PFA = phosphonoformic acid (2), BUdR = 5-bromo-2'-deoxyuridine (3), IUdR = 5-iodo-2'-deoxyuridine (4), TPS = 2,4,6-triisopropylbenzenesulfonyl chloride, DCCI = dicyclohexylcarbodiimide, *ara*-A = arabinoadenine, ACG = acycloguanosine, BVDU = (*E*)-5-(2-bromovinyl)-2'-deoxyuridine.
[‡]Chemistry Group, Roche Products Limited.
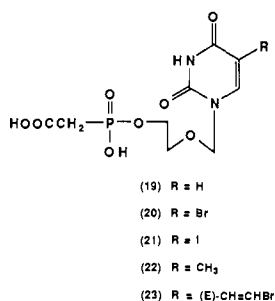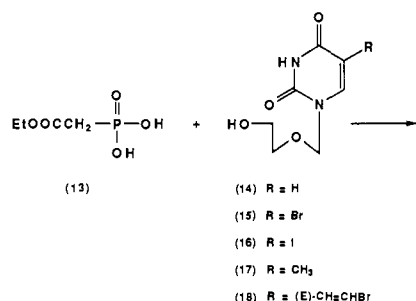[§]Biology Group, Roche Products Limited.
[‖]Hoffmann-La Roche Inc.

(1) Shipkowitz, N. L.; Bower, R. R.; Appell, R. N.; Nordeen, C. W.; Overby, L. R.; Roderick, W. R.; Schleicher, J. B.; Von Esch, A. M. *Appl. Microbiol.* 1973, *27*, 264.
(2) Helgestrand, E.; Eriksson, B. F. H.; Johansson, N. G.; Lannero, B.; Larsson, A.; Misiorny, A.; Noren, J. O.; Sjoberg, B. O. H.; Stenberg, K.; Stening, G.; Stridh, S.; Öberg, B.; Alenius, S.; Philipson, L. *Science (Washington, DC)* 1978, *201*, 819.
(3) Boezei, J. A. *Pharmacol. Ther.* 1979, *4*, 231.
(4) Heimer, E. P.; Nussbaum, A. L. United States Patent 4 056 673, 1977.
(5) Heimer, E. P.; Ahmad, M.; Kramer, M. *Abstracts of Papers*, 175th National Meeting of the American Chemical Society, Anaheim, CA, American Chemical Society: Washington, DC, 1978; MEDI 39.
(6) Personal communication, Dr. E. P. Heimer and Dr. H. Weissbach.

addition, we have coupled PAA to acycloguanosine, to some related acyclonucleosides, and to the C-nucleoside pseudoisocytidine. We now report the synthesis and antiviral activity of these compounds.

Chemistry

The pyrimidine acyclonucleoside-PAA derivatives 19–23 were synthesized from the known compounds 14–18[7] and ethyl phosphonoacetate (13) by using the TPS coupling route[4,5] followed by alkaline hydrolysis. The products were isolated as disodium salts. A similar reaction of acyclo-
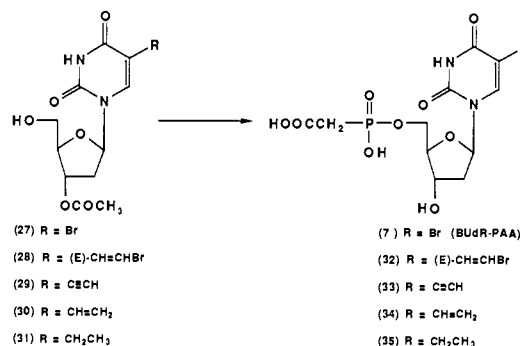

(13)

(14) R = H
(15) R = Br
(16) R = I
(17) R = CH₃
(18) R = (E)-CH=CHBr

(19) R = H
(20) R = Br
(21) R = I
(22) R = CH₃
(23) R = (E)-CH=CHBr

guanosine (ACG) gave the *O,N*-bis-PAA derivative 24, but when *N*-acetylacycloguanosine (25) was used, the ACG-PAA derivative 26 was obtained on hydrolysis.


(24)

(25)   (26) (ACG-PAA)
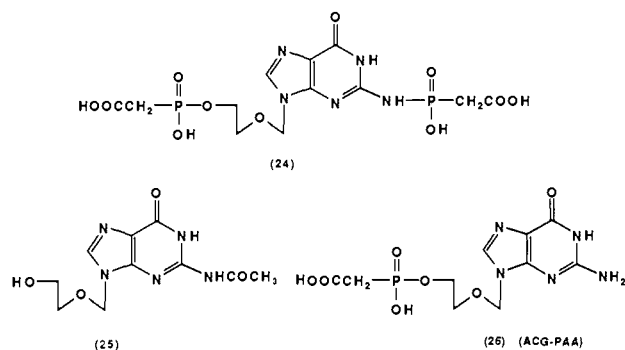
The 3′-*O*-acetyl derivatives 27, 28, and 31 were prepared according to standard procedures.[8–10] 5-Ethynyl-2′-deoxyuridine[11] was selectively reduced to 5-vinyl-2′-deoxyuridine by hydrogenation in the presence of Lindlar catalyst, which represents a more convenient route to this compound than hitherto reported. Coupling of the 3′-*O*-acetyl-2′-deoxyuridines 27, 28, and 31 with 13 using TPS as condensing agent gave the adducts 7, 32, and 35, respectively. For the conversions of 29 to 33 and 30 to 34 dicyclohexylcarbodiimide (DCCI) was the preferred con-
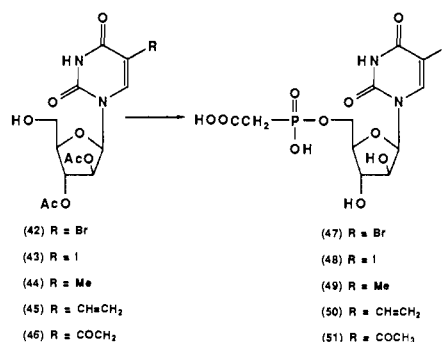
densing agent. This avoided acid-catalyzed hydration of the acetylenic bond and acid-promoted polymerization of the vinyl derivative, respectively.

(27) R = Br
(28) R = (E)-CH=CHBr
(29) R = C≡CH
(30) R = CH=CH₂
(31) R = CH₂CH₃

(7) R = Br (BUdR-PAA)
(32) R = (E)-CH=CHBr
(33) R = C≡CH
(34) R = CH=CH₂
(35) R = CH₂CH₃

Cytidine and 5-bromo-2′-deoxycytidine were protected to give 36 and 37, which were coupled with PAA by using the TPS method and after deprotection gave 38 and 39, respectively. The antiviral C-nucleoside pseudoisocytidine was similary protected to give 40, which was coupled with 13 and deprotected to give 41.

(36)

(38)

(37)

(39)

(40)

(41)
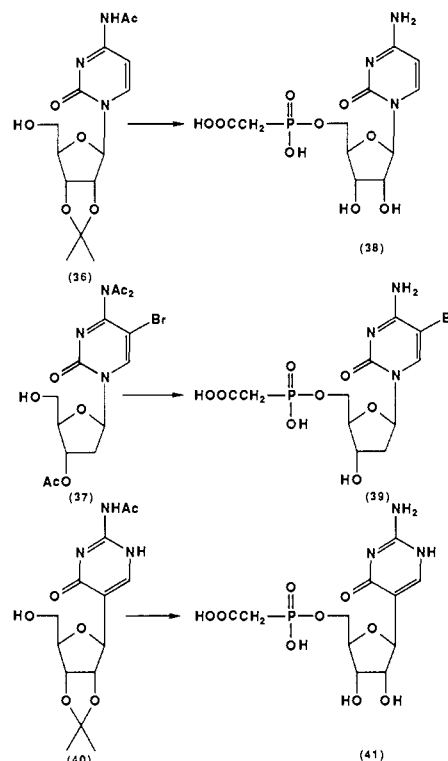
The protected 2′,3′-di-*O*-acetylarabinosides 42–46 similarly gave 47–51. Compound 46 was adventitiously obtained as an impurity (15%) during the protection of 1-β-D-arabinofuranosyl-5-ethynyluracil. The mixture was coupled to PAA, but only the 5-acetyl product (51) was isolated.

(42) R = Br
(43) R = I
(44) R = Me
(45) R = CH=CH₂
(46) R = COCH₃

(47) R = Br
(48) R = I
(49) R = Me
(50) R = CH=CH₂
(51) R = COCH₃

(7) Robins, M. J.; Hatfield, P. W. *Can. J. Chem.* 1982, *60*, 547.
(8) Montgomery, J. A.; Thomas, H. J. *J. Med. Chem.* 1967, *10*, 1163.
(9) European Patent Application 0,061,283.
(10) Keppeler, K.; Kiefer, G.; De Clercq, E. *Arch. Pharm. (Weinheim, Ger.)* 1986, *319*, 154.
(11) Robins, M. J.; Barr, P. J. *J. Org. Chem.* 1983, *48*, 1854.

The coupling of PAA through the carboxylic ester linkage to the 5'-position of (BUdR) was achieved by reaction with the mixed anhydride[12] formed from (diethylphosphono)acetic acid[13] (**52**) and trifluoroacetic anhydride to give **53**. The ester **53**, formed in 50% yield, was

(52) (53)
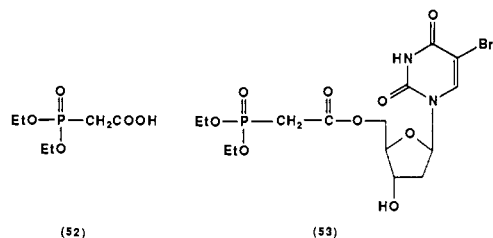
identified by infrared (C=O ester), NMR and fast atom bombardment mass spectroscopy. Deprotection of **53** with trimethylsilyl iodide (prepared in situ) followed by aqueous workup gave a mixture of three major products (38%), which were separated by preparative HPLC and identified as the desired product **54**, the desbromo derivative **55**, and the 3'-PAA derivative **56**.

(54) R = Br
(55) R = H
(56)
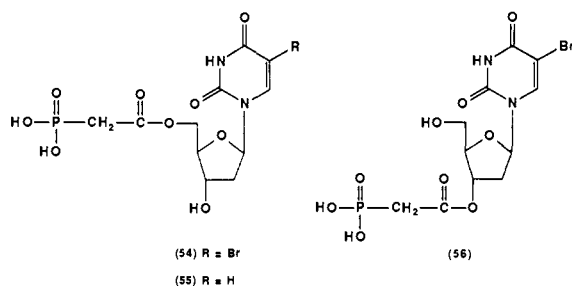
The coupling of ethyl phosphonoformate to the protected BUdR derivative **27** using DCCI as the condensing agent gave a mixture of two compounds (3:1), which were separated by preparative HPLC, and the products were identified as the decarboxylated derivative **57** and desired ester **58**. Hydrolysis of **58** with sodium hydroxide then gave **59**.

(57) (58)

(59) (BUdR-PFA)
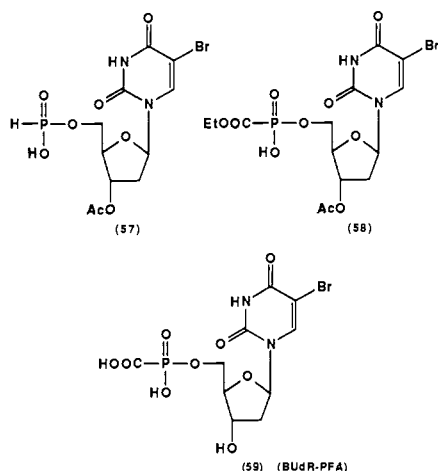
Antiviral Activity

The in vitro antiviral activity of the nucleoside-PAA derivatives previously reported[4,5] was reexamined (Table I). The PAA adducts **10–12** derived from the purines deoxyadenosine, arabinoadenine, and guanosine, respectively, showed no in vitro antiviral activity. However, the

Table I. In Vitro and in Vivo Antiviral Activity of Nucleoside 5'-Phosphonoacetates and a 5'-Phosphonoformate

compd	ID_{50},[a] μM HSV-1[b]	ID_{50},[a] μM HSV-2[c]	PD_{50},[e] mM/kg ip:[4,5] HSV-1
9	580[d]		0.40
12	>2000	>2000	0.36
5	400	160	0.45
6	300[d]		0.31
10	>2000	>2000	1.05
11	>2000	>2000	0.53
8 (IUdR-PAA)	40	10	0.21
7 (BUdR-PAA)	16	1.2	0.20
1 (PAA)	230	240	0.67
2 (PFA)	130	120	
ara-A	11	75	
4 (IUdR)	2.0	2.3	
3 (BUdR)	2.0	0.7	
54	12	7.4	
56	72	14	
59	4.6	0.44	
BVDU[19,20]	0.6	4.2	
32	2.7	1000	
5-ethynyl-2'-deoxyuridine[14,15]	11	11	
33	>200	>200	
5-vinyl-2'-deoxyuridine	0.8	2	
34	14	50	
5-ethyl-2'-deoxyuridine	40	23	
35	300	700	
acycloguanosine	1.3	2.2	
26	1800	1500	
38	1600	1200	
5-bromo-2'-deoxycytidine	1.5	0.15	
39	72	15	
pseudoisocytidine	110	100	
41	780	770	
5-bromoarabinosyluracil	5	5.9	
5-iodoarabinosyluracil	13	60	
arabinosylthymine	10	19	
47–51	>2000	>2000	

[a] ID_{50} = concentration of compound required to reduce the number of viral plaques in Vero cell monolayers by 50%. [b] HSV-1 = herpes simplex virus type 1 (strain HFEM). [c] HSV-2 = herpes simplex virus type 2 (strain 3345). [d] Old result.[4] [e] PD_{50} = concentration of compound required to protect 50% of mice against a systemic infection of HSV-1.

PAA adduct of adenosine **9** showed almost half the activity of PAA. Compounds **5** and **6** derived from the pyrimidines thymidine and uridine, respectively, showed an improved level of activity compared to that of **9**. The antiviral activity was greatly enhanced in compounds **7** and **8** where the nucleoside components BUdR and IUdR have marked antiviral activity. Interestingly, BUdR-PAA (**7**) also had good activity in vitro against varicella-zoster virus (VZV) and human cytomegalovirus (HCMV).[14] The activity of BUdR-PAA (**7**) against HSV-2 was of the same order as that of the parent BUdR, but against HSV-1 the activity decreased 8-fold (Table I). The activities of the compounds in Table I were all somewhat similar in a systemic mouse model[4,5] and did not parallel their in vitro potency. This might imply in vivo hydrolysis to the active nucleosides in the case of **7**, **8**, and **11**, but this mechanism would not account for the activity of the remainder and intrinsic in vivo action must be inferred.

Acyclonucleosides **14–18** and their phosphonoacetate esters **19–23** were all inactive against HSV-1 and HSV-2, but the ACG-PAA adduct **26** had weak activity against HSV-1 and HSV-2 (ID_{50} = 1800 and 1500 μM) compared

(12) Donovan, S. F.; Avery, M. A.; McMurry, J. E. *Tetrahedron Lett.* **1979**, 3287.

(13) Cooke, M. P.; Biciunas, K. P. *Synthesis* **1981**, 283.

(14) Bird, R. M.; Broadhurst, A. V.; Duncan, I. B.; Hall, M. J.; Lambert, R. W.; Wong-Kai-In, P. *J. Antimicrob. Chemother.* **1986**, *18*, Suppl. B, 201.

to the parent nucleoside, ACG (ID_{50} = 1.3 and 2.2 μM).

BVDU has very potent in vitro antiviral activity[15] against HSV-1 and a much improved therapeutic ratio compared to IUdR, but BVDU is less potent against HSV-2.[16] The BVDU-PAA derivative 32 had good activity against HSV-1 (ID_{50} = 2.6 μM) but was almost inactive against HSV-2 (Table I). This represents a 40–240-fold decrease in activity and was much greater than the reduction found with BUdR-PAA compared to BUdR.

The 5-vinyl-2'-deoxyuridine-PAA adduct 34 showed a smaller reduction of activity (18–25-fold) than BVDU-PAA (32), particularly against HSV-2. The PAA adduct 35 of the marketed antiviral agent 5-ethyl-2'-deoxyuridine, Edurid, showed a 7.5–30-fold reduction of antiviral activity.

In the 2'-deoxycytidine series, 39 was about 50–100-fold less active than the parent nucleoside, a reduction that was much greater than for the corresponding compound in the BUdR series. The C-nucleoside pseudoisocytidine suffered only a 7-fold reduction of activity after conversion to 41, but this was from a modest initial activity.

Surprisingly, all the PAA derivatives 47–51 of the arabinosyluracils were totally inactive.

The 5'-linked carboxylic ester derivative of 54 had similar activity against HSV-1 to the phosphonate ester linked derivative 7, but was less active against HSV-2 (Table I). The 3'-linked carboxylic ester 56 showed a further significant decrease in activity against both HSV-1 and HSV-2.

The BUdR-PFA derivative 59 had antiviral activity almost equal to that of the parent nucleoside 3 against HSV-1 (Table I) but was more active against HSV-2 than either BUdR or PFA.

Discussion

Most of the PAA and PFA derivatives tested show in vitro antiviral activity against HSV-1 and HSV-2 that is less than that of the component antiviral agents. One explanation is that these compounds may be acting as prodrugs, thereby releasing the antiviral agent(s) by hydrolysis. In a recent report[17] it has been found that the 5'-phosphonoformate esters of adenosine and guanosine had no in vitro antiviral activity against HSV-1 and HSV-2, whereas the corresponding 2'-deoxyadenosine derivative was active. These results are not consistent with our findings on the corresponding 5'-phosphonoacetate esters 9 and 10 but could be accounted for by different rates of hydrolysis in the two series leading to the release of PFA and PAA, respectively. Our results have also shown that the reduction in activity of the PAA adducts compared to the component pieces is not in a fixed ratio nor is the ratio between HSV-1 and HSV-2 activity. In addition the BUdR-PFA derivative 59 is more active against HSV-2 than either component. Therefore it is possible that some of the PAA and PFA adducts themselves have an intrinsic antiviral activity. Alternatively, the observed effects could arise by synergy. However, delivery seems to be the most likely reason for the observed differences in the in vitro activity. Attempts to assess the extent of breakdown of BUdR-PAA (7) in tissue culture using HPLC were not conclusive. Clearly, more detailed biochemical studies are

necessary before the mode of action of these compounds can be explained.

Experimental Section

Antiviral Testing.[4,14] Compounds were dissolved in water and serial dilutions were made with the same solvent.

In vitro antiviral activity was measured in a plaque-inhibition assay. Strains of HSV-1 and HSV-2 were grown and assayed on monkey kidney (Vero) cells, which were cultured in Dulbecco's modification of Eagles' minimum essential medium (DMEM) supplemented with 10% v/v newborn bovine serum. A standard amount of virus [50 plaque-forming units (pfu) in duplicate] was allowed to adsorb at 4 °C to drained monolayers of the host cells in 24-well plastic culture plates (Costar or similar); serum-free maintenance medium containing (carboxymethyl)cellulose and a dilution series of test compound was then added, and plaques were allowed to develop at 37 °C for 3 days. The plates were fixed with formaldehyde and stained with Giemsa to reveal plaques, and the ID_{50} was then calculated for each compound and target by interpolation from a graphic plot of results.

In vivo activity of compounds against HSV-1 infections in mice was determined by the following procedure.

Swiss albino mice weighing 9–12 g received 0.5 mL intraperitoneally of the test substances 24 h before virus infection, immediately after virus infection (0 h), and again at 24 h after virus infection for a total of three treatments. Control mice received 0.5 mL of water intraperitoneally at the same time intervals (–24, 0, +24 h). Twenty-four hours after the first treatment, drug-treated and water-treated mice were infected intraperitoneally with approximately 10 times the lethal dose (LD_{50}) of herpes simplex virus. Mice were observed for 21 days after virus infection, and the number of animals alive on day 21 was used to calculate the protective dose (PD_{50}) as described by Reed and Muench (*Am. Jour. Hygiene* 1938, *27*, 493).

Chemical Synthesis. Melting points were determined in open capillary tubes on a Büchi apparatus and are uncorrected. Elemental analyses were carried out on a Perkin-Elmer Model 240 instrument. Analyses are reported only by the elemental symbols, and results were within ±0.4% of the theoretical values. Infrared (IR) spectra were determined on a Pye-Unicam SP 1000 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL 100/15 or Bruker WM 30 spectrometer, and chemical shifts are presented in ppm from internal tetramethylsilane as a standard. Mass spectra were obtained with a Kratos MS 902 mass spectrometer in the normal electron impact mode or fitted with a Kratos fast atom bombardment (FAB) source. Positive-ion FABMS were generally recorded, but occasionally the negative ion mode was used. Samples were mixed with glycerol on the probe tip from acetone, dimethylformamide, or dimethyl sulfoxide solutions. The source accelerating voltage was 8 KV and the multiplier gain 10.[5]

Homogeneity of the products was determined by ascending thin-layer chromatography (TLC) on precoated cellulose sheets (Eastman Chromatogram Sheet, 13254 Cellulose) with fluorescent indicator (No. 6065) or on glass-supported silica gel (Kieselgel 60 F_{254} plates) (E. Merck, Darmstadt, West Germany) using principally the solvent system acetonitrile/ammonium acetate (0.1 M), 60:40, and occasionally 1-butanol/acetic acid/water (12:3:5). Products were visualized by successive exposure (where appropriate) to ultraviolet (UV) light, iodine vapor, ninhydrin, and ammonium molybdate spray reagents.

The cation-exchange resin used throughout was British Drug Houses, Zerolit 225, SRC 13, RSO_3H, freshly regenerated in the acid cycle.

The anion-exchange resin used was Bio-Rad Laboratories (Richmond, CA) analytical grade, AG 1-X8, 200–400 mesh, formate form, 3.2 mequiv/dry g.

HPLC apparatus for analytical and for preparative purposes was as described for the individual compounds.

1-[[2-[[(Carboxymethyl)hydroxyphosphinyl]oxy]ethoxy]methyl]-5-iodouracil (21) Disodium Salt. The aniline salt of ethyl phosphonoacetate (13) (6.53 g, 25 mmol) was dissolved in H_2O (25 mL) and passed down a column of Zerolit (H^+, 50 g) and eluted with water to give a total acid eluate of 300 mL which was evaporated in vacuo. The residue was dried by reevaporation with toluene (3 × 50 mL) and dissolved in dry pyridine (50 mL)

(15) De Clercq, E.; Descamps, J.; De Somer, P.; Barr, P. J.; Jones, A. S.; Walker, R. T. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2947.

(16) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. *J. Infect. Dis.* **1980**, *141*, 563.

(17) Vaghefi, M. M.; McKernan, P. A.; Robins, R. K. *J. Med. Chem.* **1986**, *29*, 1389.

Table II. Phosphonoacetate Adducts[a] of Acyclonucleosides and 24

compd	R	yield, %	mp, °C	formula	anal.[b]	MS (FAB) m/e	ion	¹H NMR (D₂O) δ
19	H	30	>360	$C_9H_{11}N_2O_8PNa_2$· 0.25C_2H_5OH	CHN	351	(M – H)⁻	2.71 (2 H, d, CH₂P), 5.29 (2 H, s, OCH₂N), 5.88 (1 H, d, 5-H), 7.78 (1 H, d, 6-H)
20	Br	17	225	$C_9H_{10}BrN_2O_8PNa_2$	CHN	431	(M – H)⁻	2.73 (2 H, d, CH₂P), 5.27 (2 H, s, OCH₂N), 8.22 (1 H, s, 6-H)
22	CH₃	29	300	$C_{10}H_{13}N_2O_8PNa_2$	CHN	367	(M + H)⁺	1.88 (3 H, d, CH₃), 2.68 (2 H, d, CH₂P), 5.26 (2 H, s, OCH₂N), 7.62 (1 H, q, 6-H)
23	(E)-CH=CHBr	9	250–260	$C_{11}H_{12}BrN_2O_8PNa_2$· C_2H_5OH[c]	CHN			2.67 (2 H, d, CH₂P), 5.28 (2 H, s, OCH₂N), 6.86 (1 H, d, CH=CHBr), 7.20 (1 H, d, CH=CHBr), 7.83 (1 H, s, 6-CH)
24		23	ca. 300	$C_{12}H_{13}N_5O_{11}P_2Na_4$	CHN	558	(M + H)⁺	2.55 (2 H, d, CH₂PNH), 2.77 (2 H, d, CH₂PO), 5.45 (2 H, s, OCH₂N), 7.9 (1 H, s, 8-CH)

[a] Obtained by the TPS coupling procedure, as described for **21**. [b] Analyses for C, H, N were within ±0.4%. [c] Recrystallized from ethanol.

and stirred twice, each time for 2 h, with 4A molecular sieves (2 × 20 g). To the filtrate at 25 °C was added 2,4,6-triisopropyl-benzenesulfonyl chloride (9.1 g, 30 mmol) followed by 16[7] (1.56 g, 5 mmol). The mixture was stirred, and after about 10 min a solution was obtained which was allowed to stand overnight at room temperature. TLC on silica gel (CH₂Cl₂/MeOH) (9:1) showed that no starting material (16) remained.

The reaction mixture was evaporated and reevaporated with water (4 × 50 mL). The resulting gum was taken up in 2 M NaOH (75 mL), and the solution was allowed to stand for 3 h at 25 °C to complete the hydrolysis. The solution was neutralized to pH 4 with Zerolit (H⁺) (ca. 55 g). The resin was filtered off and washed well with water. The combined filtrate was evaporated to low bulk, and the sulfonic acid byproduct was filtered off and washed with cold H₂O. The combined filtrate was concentrated to low bulk and passed down a column of AG 1-X8 (formate) resin in formate form (50 g). The column was gradient eluted with aqueous formic acid (1–100%). Fractions of 75 mL were monitored by TLC on cellulose [MeCN/ammonium acetate (0.1 M), 60:40].

Fractions 4 and 5 (R_f 0.62, UV positive) were combined and evaporated. The residue was reevaporated with water (3 × 50 mL), ethanol, and, finally, ether. The resulting gum was triturated with ether, which was decanted. The residue was reevaporated with acetone and then triturated with acetone to give a hygroscopic solid, which was filtered off (80 mg). The filtrate was evaporated to give about 2.2 g of red oil.

The above oil was dissolved in water and passed down to a column of Zerolit (Na⁺, 160 g). The eluate was evaporated and reevaporated with ethanol to give 1.5 g (62%) of crude product, mp 230–265 °C dec. Recrystallization of 0.9 g from a mixture of water (2 mL) and methanol (18 mL) gave 0.27 g of **21**: mp 280–285 °C dec; UV λ_max (H₂O) 214 and 284 nm (ε 9080 and 5750); MS, m/e (FAB) 477 (M – H)⁻; NMR (D₂O) δ 2.70 (2 H, d, CH₂P), 3.80 (2 H, m, OCH₂CH₂O), 4.01 (2 H, m, OCH₂CH₂O), 5.26 (2 H, s, OCH₂N), 8.29 (1 H, s, 6-CH). Anal. ($C_9H_{10}IN_2O_8PNa_2$) C, H, N.

Similar procedures were employed in the synthesis of compounds **19–24** (Table II) and **32, 35, 39, 41, 47–51** (Table III).

5'-O-[(Carboxymethyl)hydroxyphosphinyl]cytidine (38) Disodium Salt Dihydrate. Ethyl phosphonoacetate (from 1.3 g, 5 mmol, of aniline salt) in 25 mL of dry pyridine was treated with 2,4,6-triisopropylbenzenesulfonyl chloride (1.8 g, 6 mmol) followed by 36[18] (0.33 g, 1.0 mmol) as for the synthesis of **21**. The coupled product was hydrolyzed with 2 M NaOH (20 mL) and passed down a column of AG 1-X8 (formate) and gradient eluted with formic acid (0–100%). The product containing fractions (UV) were evaporated to a gum which was dissolved in 5 mL of 80% formic acid and stood 1 h to remove the isopropylidene protecting

group. The solution was evaporated and the residue was evaporated twice with 10 mL of water. The residue was taken up in water and converted to the disodium salt by passage over cation-exchange resin (Na⁺ form). The eluate was concentrated and freeze-dried to give the disodium dihydrate of **38** as a solid: MS, m/e (FAB) [M – H]⁻ 408; NMR (D₂O) δ 2.65 (2 H, d, CH₂P), 4.08 (2 H, m, 5'-CH₂), 4.20 (1 H, m, 4'-CH), 4.25 (1 H, m, 3'-CH or 2'-CH), 4.30 (1 H, m, 2'-CH or 3'-CH), 5.95 (1 H, d, 1'-CH), 6.05 (1 H, d, 5-CH), 7.93 (1 H, d, 6-CH). Anal. ($C_{11}H_{14}N_3O_9PNa_2$· 2H₂O) C, H, N.

9-[[2-[[(Carboxymethyl)hydroxyphosphinyl]oxy]ethoxy]methyl]guanine (26) Disodium Salt. Ethyl phosphonoacetate (**13**) (prepared as above from 1.3 g, 5 mmol, of the aniline salt) was reacted in dry pyridine with 2,4,6-triisopropylbenzenesulfonyl chloride (1.8 g, 6 mmol) followed by N-acetylacycloguanosine (**25**) (0.27 g, 1.0 mmol). The coupled product was deprotected with sodium hydroxide as above. The crude (carboxymethyl)phosphonate **26** was purified by chromatography on a column of Sephadex G-10, eluting with 1% aqueous acetic acid to give the product (195 mg) which was contaminated with sodium acetate. This product (120 mg) was dissolved in water and passed through a column of Zerolit (Na⁺) to ensure complete formation of the disodium salt. The eluate was evaporated and the residue was recrystallized from aqueous ethanol to give 35 mg of **26** (9%): mp 292–295 °C; NMR (D₂O, 300 MHz) δ 2.68 (2 H, d, CH₂P), 3.76 (2 H, m, CH₂), 4.02 (2 H, m, CH₂), 5.55 (2 H, s, NCH₂O), 7.96 (1 H, s, 8-CH); MS, m/e (+FAB) 392 (MH)⁺ accurate mass 392.0281, $C_{10}H_{13}N_5Na_2O_7P$ requires 392.0345.

3'-O-Acetyl-2'-deoxy-5-ethynyluridine (29). To a suspension of 2'-deoxy-5-ethynyluridine[11] (0.26 g, 1.0 mmol) in 3 mL of dry pyridine was added 4,4'-dimethoxytrityl chloride (0.37 g, 1.1 mmol). The mixture was shaken for 2 min and then allowed to stand for 4.5 h. Methanol (0.1 mL) was added and the solution was evaporated to give a gum which was partitioned between 10 mL of CH₂Cl₂ and 5 mL of water. The solvent layer was dried over Na₂SO₄ and evaporated to give an oil which was chromatographed on silica gel (30 g) in ethyl acetate/hexane (3:1) to give 0.5 g (90%) of 2'-deoxy-5-ethynyl-5'-(4,4'-dimethoxytrityl)uridine, mp ca. 135 °C.

To a solution of this intermediate (0.4 g, 0.7 mmol) in 10 mL of dry pyridine was added acetic anhydride (0.15 mL, 1.6 mmol). The mixture was allowed to stand for 24 h and then poured onto ice and extracted with CH₂Cl₂. The CH₂Cl₂ extract was dried over Na₂SO₄ and evaporated to give crude 3'-acetyl-2'-deoxy-5-ethynyl-5'-(4,4'-dimethoxytrityl)uridine, which was taken up in 35 mL of 80% (v/v) acetic acid/H₂O and allowed to stand for 4.5 h. The mixture was evaporated and the residual oil was chromatographed over silica gel in ethyl acetate/hexane (3:1) to give 0.16 g (79%) of **29**: mp 195–198 °C; UV (EtOH) λ_max 225 and 285 nm (ε 8900 and 9400); IR (Nujol) 1715 cm⁻¹ (ester); MS, m/e (E1) 294 (M)⁺; NMR [(CD₃)₂SO] δ 2.07 (3 H, s, OAc), 2.31 (2 H, m, 2'-CH₂), 3.65 (2 H, m, 5'-CH₂), 4.03 (1 H, m, 4'-CH), 4.13

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Table III. Phosphonoacetate Adducts of 2′-Deoxyuridines, Arabinosyluracils, and Cytosine Analogues 39 and 41

compd	R^1	R^2	route[a]	yield, %	mp, °C	formula	anal.[b]	MS(FAB), m/e (ion)	^{1}H NMR (D$_2$O) δ
32	(E)-CH=CHBr	H	A	52	ca. 215 dec	C$_{13}$H$_{14}$BrN$_2$O$_9$PNa$_2$· 0.4(CH$_3$)$_2$CO	CHN	499 (M + H)$^{+c}$	2.56 (2 H, d, CH$_2$P), 3.94 (2 H, m, 5′-CH$_2$), 4.44 (1 H, m, 3′-CH), 6.81 (1 H, d, CH=CHBr), 7.00 (1 H, d, CH=CHBr), 7.78 (1 H, s, 6-CH)
34	CH=CH$_2$	H	B	29^d	d	C$_{13}$H$_{15}$N$_2$O$_9$PNa$_2$	d	421 (M + H)$^+$, 419 (M − H)$^-$	2.84 (2 H, d, CH$_2$P), 4.12 (2 H, m, 5′-CH$_2$), 5.28 (1 H, dd, CCH=CH), 5.92 (1 H, dd, CCH=CH), 6.48 (1 H, dd, CH=CH$_2$), 7.92 (1 H, s, 6-H)
35	CH$_2$CH$_3$	H	A	44	210–250 dec^e	C$_{13}$H$_{17}$N$_2$O$_9$PNa$_2$· 0.5H$_2$O· 0.3C$_2$H$_5$OH	CHN	423 (M + H)$^{+f}$	1.01 (3 H, t, CH$_3$CH$_2$), 2.23 (4 H, m, CH$_3$CH$_2$ and 2′-CH$_2$), 2.65 (2 H, m, CH$_2$P), 4.02 (2 H, m, 5′-CH$_2$), 4.50 (1 H, m, 3′-CH), 7.59 (1 H, s, 6-CH)
39			A	13	g	C$_{11}$H$_{13}$BrN$_3$O$_8$PNa$_2$		474 (M + H) (^{81}Br), 472 (M + H) (^{79}Br)	2.32 (1 H, m, 2′-CH), 2.47 (1 H, ddd, 2′-CH), 2.83 (2 H, d, CH$_2$P), 4.17 (2 H, m, 5′-CH$_2$), 4.22 (1 H, m, 4′-CH), 4.57 (1 H, m, 3′-CH), 6.27 (1 H, t, 1′-CH), 8.23 (1 H, s, 6-CH)
41			A	26^h		C$_{11}$H$_{16}$N$_3$O$_9$P		366 (M + H)$^+$, 364 (M − H)$^-$	2.95 (2 H, d, CH$_2$P), 4.1–4.6 (5 H, m, 2′-CH, 3′-CH, 4′-CH, 5′-CH$_2$), 4.96 (1 H, m, 1′-CH), 7.95 (1 H, s, 6-CH)
47	Br	OH	A	16	i,j	C$_{11}$H$_{12}$BrN$_2$O$_{10}$PNa$_2$· 2H$_2$O	CHN	489 (M − H)$^-$ (^{81}Br), 487 (M − H)$^-$ (^{79}Br)	2.75 (2 H, d, CH$_2$P), 4.08 (1 H, m, 4′-CH), 4.18 (2 H, m, 5′-CH$_2$), 4.24 (1 H, t, 2′-CH or 3′-CH), 4.40 (1 H, t, 2′-CH or 3′-CH), 6.17 (1 H, d, 1′-CH), 8.16 (1 H, s, 6-CH)
48	I	OH	A	9	i,j	C$_{11}$H$_{12}$IN$_2$O$_{10}$PNa$_2$· 2H$_2$O	CHN	537 (M + H)$^+$	2.90 (2 H, d, CH$_2$P), 4.15–4.30 (3 H, m, 2′-CH or 3′-CH and 5′-CH$_2$), 6.14 (1 H, d, 1′-CH), 8.20 (1 H, s, 6-CH)
49	CH$_3$	OH	A	18	217 dec	C$_{12}$H$_{15}$N$_2$O$_{10}$PNa$_2$	CHN	381 (MH − 2Na)$^+$	1.90 (3 H, s, 5-CH$_3$), 2.81 (2 H, d, CH$_2$P), 4.20 (2 H, m, 5′-CH$_2$), 6.19 (1 H, d, 1′-CH), 7.70 (1 H, s, 6-CH)
50	CH=CH$_2$	OH	B	30	ca. 240 dec	C$_{13}$H$_{15}$N$_2$O$_{10}$PNa$_2$· 4H$_2$O^i	CHN (H$_2$O)	437 (M + H)$^{+k}$	2.77 (2 H, d, CH$_2$P), 4.10–4.25 (3 H, m, 2′-CH or 3′-CH and 5′-CH$_2$), 5.28 (1 H, d, CCH=CH), 5.91 (1 H, d, CCH=CH), 6.18 (1 H, d, 1′-CH), 6.48 (1 H, dd, CH=CH$_2$), 7.85 (1 H, s, 6-CH)
51	CH$_3$CO	OH	A	l	180–195	C$_{13}$H$_{15}$N$_2$O$_{11}$PNa$_2$		453 (M + H)$^{+m}$	2.53 (3 H, s, COCH$_3$), 2.92 (2 H, d, CH$_2$P), 6.22 (1 H, d, 1′-CH), 8.52 (1 H, s, 6-H)

^{a} Route A using TPS as for 21 and route B using DCCI as for 33. ^{b} Analyses were within ±0.4% except as noted. ^{c} Measured m/e 498.9551, calcd 498.9491. M/e 477 (M − Na + H)$^+$, measured 476.9692, calcd 476.9672. ^{d} Hygroscopic disodium salt; unstable as free acid. ^{e} Efflorescence at 110 °C. ^{f} Measured m/e 423.0597, calcd 423.0543. M/e 445 (M + Na)$^+$, measured 445.0395, calcd 445.0362. ^{g} Disodium salt as a freeze-dried foam. ^{h} Free acid after additional step to remove the isopropylidene group with HCOOH as for 38. ^{i} Purified by preparative HPLC and converted to disodium salt. ^{j} Obtained as a gum. ^{k} Measured (M) m/e 436.0222, calcd 436.0262. ^{l} From impure (46). ^{m} Measured (M) m/e 452.0210, calcd 452.0212.

(1 H, s, C≡CH), 5.22 (1 H, m, 3′-CH), 5.32 (1 H, br s, 5′-OH), 6.13 (1 H, t, 1′-CH), 8.30 (1 H, s, 6-CH), 11.71 (1 H, br s, NH). Anal. (C$_{13}$H$_{14}$N$_2$O$_6$) C, H, N.
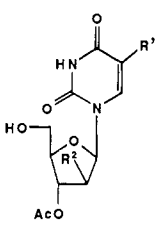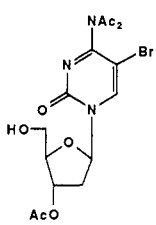
Compounds 30, 37, 42–46 were similarly synthesized (Table IV).

5′-O-[(Carboxymethyl)hydroxyphosphinyl]-2′-deoxy-5-ethynyluridine (33) Disodium Salt. To ethyl phosphonoacetate (from 0.8 g, 3 mmol of aniline salt) in 20 mL of dry pyridine was added 29 (0.29 g, 1.0 mmol) followed by (DCCI)[19] (3.1 g, 15 mmol).

The mixture was stirred for 3 days at room temperature when TLC (silica gel/EtOAc) showed that no 29 remained.

Water (20 mL) was added and the mixture was stirred for 0.5 h to destroy metaphosphates.[19] The mixture was evaporated and 10 mL of water was added to give a solid (dicyclohexylurea) which was filtered off. The filtrate was extracted with ethyl acetate and

(19) Tener, G. M. *J. Am. Chem. Soc.* 1961, 83, 159.

Table IV. Properties of 3'-Acetyl and 3,5-Diacetyl Protected Intermediates[a]

(37)

compd	R[1]	R[2]	yield, %	mp, °C	formula	anal.[b]	MS (FAB), m/e (ion)	^{1}H NMR (D_2O) δ
30[c]	CH=CH$_2$	H	19	d	$C_{13}H_{16}N_2O_6$	CHN	297 (M + H)[+]	2.08 (3 H, s, OAc), 3.64 (2 H, m, 5'-CH$_2$), 5.15 (1 H, dd, CCH=C*H*), 5.24 (1 H, m, 3'-CH), 5.93 (1 H, dd, CCH=C*H*), 6.38 (1 H, dd, C*H*=CH$_2$), 8.13 (1 H, s, 6-CH)[e]
37[f]			96	75–80	$C_{15}H_{18}BrN_3O_7$· 0.1(C_2H_5)$_2$O	CHN	432 (M + H)[+]	2.10 (3 H, s, OAc), 2.35 (6 H, s, NAc$_2$) 4.02 (2 H, m, 5'-CH$_2$), 5.38 (1 H, m, 3'-CH), 8.80 (1 H, s, 6-CH)
42	Br	OAc	61[g]	171–172	$C_{13}H_{15}BrN_2O_8$	CHN	409 (M + H)[+] ([81]Br), 407 (M + H)[+] ([79]Br)	2.04 (3 H, s, OAc), 2.12 (3 H, s, OAc), 6.27 (1 H, d, 1'-CH), 8.16 (1 H, s, 6-CH)[h]
43	I	OAc	16[i]	205–206	$C_{13}H_{15}IN_2O_8$	CHN	453 (M – H)[−]	2.07 (3 H, s, OAc), 2.17 (3 H, s, OAc), 4.05 (2 H, m, 5'-CH$_2$), 6.27 (1 H, d, 1'-CH), 8.12 (1 H, s, 6-CH)
44	Me	OAc	47[j]	168.5–169	$C_{14}H_{18}N_2O_8$	CHN	342 (M[+]) (EI)	1.96 (3 H, d, 5-CH$_3$), 2.00 (3 H, s, OAc), 2.16 (3 H, s, OAc), 6.29 (1 H, d, 1'-CH), 7.46 (1 H, d, 6-CH)
45	CH=CH$_2$	OAc	34[k]	168–170	$C_{15}H_{18}N_2O_8$	CHN	354 (M[+]) (EI)	2.00 (3 H, s, OAc), 2.15 (3 H, s, OAc), 4.00–4.07 (2 H, m, 5'-CH$_2$), 5.23–5.30 (2 H, m, CCH=C*H* and 2'-CH or 3'-CH), 5.98 (1 H, d, CCH=C*H*), 6.31 (1 H, d, 1'-CH), 6.44 (1 H, dd, C*H*=CH$_2$), 7.77 (1 H, s, 6-CH)
46	COCH$_3$	OAc	l		$C_{15}H_{18}N_2O_9$	l	371 (M + H)[+]	2.00 (3 H, s, OAc), 2.63 (3 H, s, OAc), 6.30 (1 H, d, 1'-CH), 8.64 (H, s, 6-CH)

[a] Synthesized by the general method described for **29**. [b] Analyses were within ±0.4% except as noted. [c] 2'-Deoxy-5-vinyluridine starting material was obtained by hydrogenation of 2'-deoxy-5-ethynyluridine[11] over Lindlar catalyst poisoned with 1,2-bis[(2-hydroxyethyl)thio]-ethane. [d] Solid. [e] DMSO. [f] From N-acetyl-5-bromo-2'-deoxycytidine, mp 167–168 °C. [g] From 1-(β-D-2,3-di-O-acetylarabinofuranosyl)uracil[21] and N-bromosuccinimide in DMF. [h] CDCl$_3$ + DMSO. [i] From 1-(β-D-2,3-di-O-acetylribofuranosyl)uracil[21] and iodine monochloride. [j] From 1-β-D-arabinofuranosylthymine.[22] [k] From 1-β-D-arabinofuranosyl-5-vinyluracil.[23] [l] Minor component from acetylation of 1-β-D-arabino-furanosyl-5-ethynyluracil;[24] mixture taken on to **51**.

the aqueous phase was evaporated to give the protected product.

Deprotection was effected with 2 M NaOH (40 mL) for 2 h at room temperature. Zerolit (H^{+}, 50 g) was added to pH 4. The filtrate was evaporated to give 0.8 g of oil which contained (HPLC, see below) 67% of **33** and 13% of 2'-deoxy-5-ethynyluridine. NMR showed the presence of PAA as a major contaminant. This oil (0.2 g) was dissolved in 10 mL of H$_2$O and repeatedly extracted with ethyl acetate to remove the 2'-deoxy-5-ethynyluridine. The aqueous layer was evaporated to give 144 mg of gum which was purified by preparative HPLC on Spherisorb 5-μm ODS with 7% MeOH/0.05% HCOOH w/v as mobile phase. This gave, on freeze-drying, as a fawn solid, 86 mg (72%) of the disodium salt of **33**, which was 99% pure by HPLC: MS, m/e (FAB) 419 [M + H][+], m/e 419.0258 ($C_{13}H_{14}N_2O_9PNa_2$ = 419.0230), m/e 397 [419 – Na + H][+], m/e 397.0393 ($C_{13}H_{15}N_2O_9PNa$ = 397.0410); NMR (D$_2$O) δ 2.38 (2 H, m, 2'-CH$_2$), 2.85 (2 H, d, CH$_2$P), 3.58 (1 H, s, C≡CH), 4.12 (2 H, m, 5'-CH$_2$), 4.20 (1 H, m, 4'-CH), 4.55 (1 H, m, 3'-CH), 6.25 (1 H, t, 1'-CH), 8.26 (1 H, s, 6-CH). Anal. ($C_{13}H_{13}N_2O_9PNa_2$ + 0.3 MeOH (NMR) + 0.1 HCOONa + 2.5 H$_2$O) C, H, N.

Similar procedures were used for **34** and **50**.

2-Acetamido-5-(2,3-*O*-isopropylidene-β-D-ribo-furanosyl)-4(1*H*)-pyrimidinone (40). 2-Amino-5-(2,3-*O*-isopropylidene)-β-D-ribofuranosyl-4(1*H*)-pyrimidinone[20] (0.3 g, 1

mmol) was dissolved in 20 mL of MeOH and acetic anhydride (0.3 g) was added. The solution was heated at reflux, and seven further portions of 0.3 g of Ac$_2$O were added at hourly intervals. The solution was evaporated in vacuo and the residue was purified by column chromatography on silica gel and recrystallization from EtOAc/Et$_2$O to give 0.285 g of **40**: mp 226–227 °C; NMR (CDCl$_3$) δ 1.36 [3 H, S, C*H$_3$*C(CH$_3$)], 1.58 (3 H, s, CH$_3$CC*H$_3$*), 2.30 (3 H, s, OAc), 3.80 (2 H, m, 5'-CH$_2$), 4.30 (1 H, m, 4'-CH), 4.60 (1 H, m, 3'-CH), 4.95 (2 H, m, 2'-CH and 1'-CH), 7.75 (1 H, s, 6-CH). Anal. ($C_{14}H_{19}N_3O_6$) C, H, N.

5-Bromo-2'-deoxy-5'-*O*-[(diethylphosphono)acetyl]uridine (53). (Diethylphosphono)acetic acid[12] (0.79 g, 4.0 mmol) was stirred with trifluoroacetic anhydride (1.1 mL, 8 mmol) at room temperature to generate the mixed anhydride. The mixture was evaporated in vacuo to give an oil. A suspension of **3** [1.2 g, 4 mmol in dry 1,2-dimethoxyethane (20 mL)] was added and the mixture was stirred overnight. A further aliquot (4 mmol) of mixed anhydride was added and the mixture was stirred for 72 h to give a clear solution. The solution was evaporated under vacuum and the residual oil was partitioned between CH$_2$Cl$_2$ and H$_2$O to remove disubstituted product (NMR) in the CH$_2$Cl$_2$ layer. The CH$_2$Cl$_2$ layer was washed with aqueous NaHCO$_3$. The aqueous layers were repeatedly back-extracted with CH$_2$Cl$_2$. The combined CH$_2$Cl$_2$ extracts were evaporated to give 0.98 g (50%) of **53** as an oil: IR (film) 1740, 1715 (ester) cm^{−1}; MS, m/e (FAB) 485 and 487 [M + H][+]. The NMR showed the phosphonate and sugar moieties and that this product was a mixture.

5-Bromo-2'-deoxy-5'-*O*-(phosphonoacetyl)uridine (54) and the 3'-Isomer 56. The crude **53** (0.95 g, 2 mmol), prepared above,

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(24) De Clercq, E.; Descamps, J.; Balzarini, J.; Giziewicz, J.; Barr, P. J.; Robins, M. J. *J. Med. Chem.* **1983**, *26*, 661.

was taken up in 25 mL of dry acetonitrile. Sodium iodide (1.5 g, 10 mmol) and trimethylsilyl chloride (1.3 mL, 10 mmol) were added, and the stirred mixture was heated on a bath at 50 °C for 0.5 h. The resulting precipitate (NaCl) was filtered off and the filtrate was evaporated to give 1.5 g of gum. This silylated intermediate was taken up in 50 mL of water and stirred for 0.5 h. The resulting red-brown solution was extracted with CH_2Cl_2 and the aqueous layer was evaporated and reevaporated with toluene to give 1.3 g of oil. This oil was taken up in 15 mL of Me_2CO and aniline (0.5 mL, 5 mmol)) was added to give a precipitate which was filtered off and washed with Me_2CO to give 0.48 g (46%) of crude product, mp ca. 190 °C. This solid was taken up in H_2O and reprecipitated with Me_2CO to give 0.39 g (38%) of solid, mp ca. 210 °C. The NMR spectrum suggested the presence of three major products which were separated by HPLC on Spherisorb 5-μm ODS using 5% MeOH/0.05% ammonium formate w/v at pH 6.58 as the mobile phase.

The fraction having retention time 7.55 min was freeze-dried to give 97.6% pure 54: NMR (D_2O, 300 MHz) δ 2.46 (2 H, m, 2'-CH_2), 2.91 (2 H, 8 lines (ABX), CH_2P), 4.21 (1 H, m, 4'-CH), 4.37 (2 H, m, 5'-CH_2), 4.53 (1 H, m, 3'-CH), 6.22 (1 H, t, 1'-CH), 8.09 (1 H, s, 6-CH); MS, m/e (FAB) 429 and 431 [M + H]$^+$; accurate mass 428.9707, $C_{11}H_{15}N_2O_9P^{79}Br$ requires 428.9696. An earlier fraction, retention time 3.6 min, was identified as the 99.6% pure 3'-isomer 56 by NMR (D_2O, 300 MHz): δ 2.45 (1 H, ddd, 2'-CH), 2.58 (1 H, ddd, 2'-CH), 2.87 (2 H, d, CH_2P), 3.83 (2 H, m, 5'-CH_2), 4.27 (1 H, m, 4'-CH), 5.31 (1 H, m, 3'-CH), 6.30 (1 H, t, 1'-CH), 8.27 (1 H, s, 6-CH).

3'-Acetyl-5-bromo-2'-deoxy-5'-phosphinouridine (57) Ammonium Salt. Ethyl phosphonoformate[25] (from 3.0 g, 12 mmol, of aniline salt) in dry pyridine (80 mL) was reacted with 27 (1.4 g, 4.0 mmol) with DCCI (12.4 g, 60 mmol) as condensing agent as for 33. The coupled protected product was worked up as for 33 but was not hydrolyzed at this stage. Instead, the protected material was passed down a column of anion-exchange resin (formate form) and gradient eluted with aqueous formic acid (1–50%), collecting 100-mL fractions. Fractions 12–14 (single spot, R_f 0.9 on cellulose with MeCN/NH$_4^+$OAc$^-$; UV and phosphorus positive) were evaporated and reevaporated with H_2O. Ammonia was added to pH 7. The solution was evaporated and reevaporated with methanol, ethanol, and ether to give a solid which was triturated with ether and filtered off to give 0.85 g of solid, mp ca. 90–120 °C. HPLC on Apex 5-μm ODS with 25% MeOH/0.05% ammonium formate w/v as the mobile phase showed two major products, A (63%), retention time 4.6 min and B (25%), retention time 18 min, which were separated by preparative HPLC on Partisil 10 μm ODS.

Fraction A (t_R 2.5 min) (250 mL) was evaporated and reevaporated three times with ethanol to give a solid. This solid was dissolved in 5 mL of H_2O, filtered, and freeze-dried to give 200 mg of hygroscopic solid (57): MS, m/e 413 [M + H]$^+$ (free acid) (^{79}Br), m/e 415 [M + H]$^+$ (^{81}Br); NMR (D_2O, 300 MHz) δ 2.10 (3 H, s, OAc), 2.46 (2 H, m, 2'-CH_2), 4.09 (2 H, m, 5'-CH_2), 4.38 (1 H, m, 4'-CH), 5.33 (1 H, m, 3'-CH), 5.71 (0.5 H, s, PH), 6.28 (1 H, dd, 1'-CH), 7.83 (0.5 H, s, PH), 8.22 (1 H, s, 6-CH).

3'-Acetyl-5-bromo-2'-deoxy-5'-[(ethoxycarbonyl)hydroxyphosphinyl]uridine (58). Fraction B (t_R 6.0 min) (700 mL) from the previous example (57) was evaporated and reevaporated three

(25) Morita, T.; Okamoto, Y.; Sakurai, H. *Bull. Chem. Soc. Jpn* **1981,** *54,* 267.

times with ethanol to give about 300 mg of solid. This solid was taken up in 5 mL of water and freeze-dried to give 150 mg of hygroscopic solid (58): MS, m/e (FAB) 485 [M + H]$^+$; NMR (D_2O) δ 1.38 (3 H, t, CH_3CH_2), 2.24 (3 H, s, OAc), 2.60 (2 H, m, 2'-CH_2), 4.36 (4 H, m, CH_3CH_2 and 5'-CH_2), 4.52 (1 H, m, 4'-CH), 5.50 (1 H, m, 3'-CH), 6.43 (1 H, dd, 1'-CH), 8.35 (1 H, s, 6-CH).

5'-Bromo-5'-O-(carboxyhydroxyphosphinyl)-2'-deoxyuridine (59) Disodium Salt. The above-prepared 58 (150 mg) was hydrolyzed with 10 mL of 2 M NaOH for 2 h at room temperature. Water (10 mL) was added and the solution was concentrated to 10 mL to remove ammonia. The pH was adjusted to 9 with cation-exchange resin. The resin was filtered off and the filtrate was evaporated and reevaporated three times with ethanol. The residue was triturated twice with ethanol (2 × 10 mL) to remove sodium acetate and the residue was dissolved in water and freeze-dried to give about 100 mg of the very hygroscopic disodium salt of 59: NMR (D_2O) δ 2.39 (2 H, m, 2'-CH_2), 4.12 (2 H, m, 5'-CH_2), 4.20 (1 H, m, 4'-CH), 4.61 (1 H, m, 3'-CH), 6.32 (1 H, t, 1'-CH), 8.26 (1 H, s, 6-CH); MS, m/e 454 [M + H]$^+$ (^{79}Br). Accurate mass could not be obtained due to weak mole ion peak.