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ISOSTERES OF NATURAL PHOSPHATES. 11. SYNTHESIS OF A PHOSPHONIC ACID ANALOGUE OF AN OLIGONUCLEOTIDE.

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Abstract - The synthesis of an isosteric phosphonic acid analogue of UpUpU has been accomplished. In this analogue each of the normal 3'-phosphate esteric oxygen atoms has been replaced with a methylene group. The synthesis began with diacetone-D-glucose which was converted in a series of reactions to a 3'-deoxy-3'-dihydroxyphosphinylmethyluridine derivative, which bore protection as a 3-benzoylpropionate at the 5'-hydroxyl and as an acetate at the 2'-hydroxyl. This critical intermediate (A) was condensed with 2',3'-O-isopropylidene uridine to generate a protected analogue of a dinucleoside phosphate. Selective removal of the 3-benzoylpropionate followed by condensation with another unit of (A) yielded the protected oligonucleotide. Deprotection was accomplished in two steps.

For some years there has existed significant interest in the application of isosteric phosphonic acid (or phosphonate) analogues of nucleotides for the study of biological processes.[1] Such analogues involve the structural modification of the phosphate esteric linkage, replacing the normal esteric oxygen atom with a methylene group. This modification provides an analogue of the natural material bearing virtually the same size and shape as the natural phosphate but with modified biochemical capabilities at a particular site. At the same time the fundamental possibilities of reactivity at other sites is maintained.

The nucleoside 5'-phosphates have received to date the greater share of attention as regards nucleotide analogue synthesis and use. Several syntheses of the 5'-deoxy-5'-dihydroxyphosphinylmethylnucleosides have been reported.[2-8] There has been reported further a route to produce analogues bearing a polar functionality (hydroxyl, cyano) at the carbon atom present in place of the normal esteric oxygen.[9,10] The compounds synthesized in these efforts have been used in a variety of biochemical investigations and further analogue synthesis.[1]

Less attention has been given to the synthesis of analogues of nucleoside 3'-phosphates. The primary reason for this is that there is relatively little interest in these materials as simple phosphates. However, analogues of

these materials are of greater interest when considered as components of oligonucleotides. An oligonucleotide bearing a methylene group in place of the normal 3'-phosphate esteric oxygens would be anticipated to bear resistance to hydrolysis not present in the natural material and to be of use in mechanistic investigations related to protein biosynthesis. A routine for the preparation of isosteric phosphonic acid analogues of nucleoside 3'-phosphates has been reported by the Syntex group.[11-13] This effort led also to the synthesis of phosphonate analogues of 3',5'-cyclic AMP and dinucleoside phosphates bearing a methylene group in place of the 3'-phosphate esteric oxygen atom.[14]

We here report the synthesis of an oligonucleotide (UpUpU) analogue wherein both of the 3'-phosphate esteric oxygen atoms have been replaced with methylene groups. This represents the first analogue of this type including three nucleoside units, and bears capabilitites for study not available with smaller species. An interesting biological application for this substance involves phosphorylation of the free 5'-hydroxyl to yield a material suitable for investigation of binding of an analogue with Phe-t-RNA. This work is continuing in this laboratory.

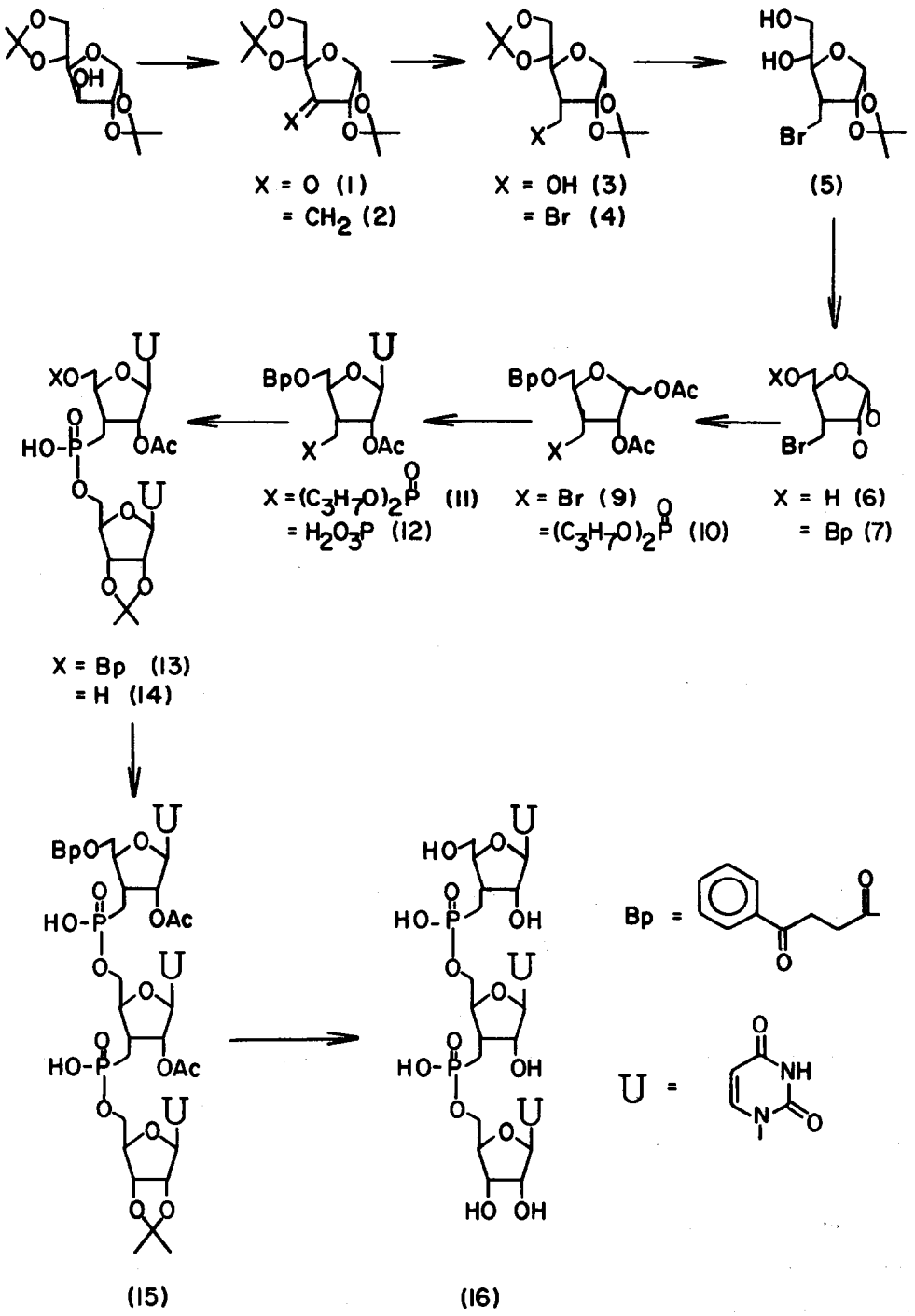
RESULTS AND DISCUSSION

The synthesis of the bis-phosphonate analogue of UpUpU was begun with the readily available diacetone-D-glucose as shown in Scheme 1. Several methods for the oxidation of this material to the ketone (1) have been reported.[15,16] We found that this reaction proceeded in highest yield when the mixture of dimethyl sulfoxide (DMSO) and acetic anhydride was allowed to stand for one hour at room temperature prior to addition of the diacetone-D-glucose. Immediate addition of the alcohol to the oxidizing medium resulted in the formation of a large amount of acetylated material. This suggests that formation of an oxidizing complex is relatively slow, and acetylation of the alcohol competes with it.

The ketone (1) was converted to the olefin (2) *via* a Wittig reaction performed as previously reported.[17] The olefin (2) was then converted stereospecifically to the (alpha) 3-hydroxymethyl-substituted material (3) *via* hydroboration-oxidation using borane-dimethyl sulfide followed by treatment with hydrogen peroxide.[18]

Initial difficulty was encountered in the conversion of alcohol (3) to bromide (4). Only low yields (ca. 10%) were obtained upon treatment of (3) with N-bromosuccinimide (NBS) and triphenylphosphine in dimethylformamide (DMF) according to the reported procedure of Ariatti and Zemlicke.[19] A significant amount of the reactant was diverted to by-product through HBr induced cleavage of the 5,6-O-isopropylidene function. To eliminate this difficulty scrupulously dried DMF was used along with a molar equivalent of dry pyridine. Yields of 75% of the desired (4) could be obtained under these conditions.

The 3-deoxy-3-bromomethyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (4) was hydrolyzed selectively to generate the 5,6-diol (5). A homogeneous solution of chloroform, methanol and 1.6% sulfuric acid was used. The resultant diol was not isolated, but was immediately subjected to cleavage with sodium meta-periodate followed by reduction of the resultant aldehyde with sodium borohydride.[13] The primary bromide



Scheme 1

remained undisturbed under these conditions, yielding 3-deoxy-3-bromomethyl-1,2-O-isopropylidene-α-D-ribofuranose (6).

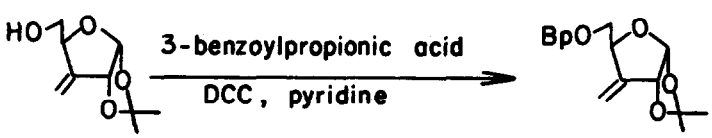
At this stage protecting functions were introduced which would allow the required selective transformations to be performed at several positions. The protecting group chosen for the 5-hydroxyl group was the 3-benzoylpropionate function. This group was expected to be secure to the moderately acidic reaction

conditions necessary for several of the following transformations, but could be removed by the action of either hydrazine or ammonia. Thus (6) was treated with 3-benzoylpropionic acid and dicyclohexylcarbodiimide (DCC) in pyridine to give the protected material (7).[20]

It was noted that the use of an excess of 3-benzoylpropionic acid resulted in the introduction of a second 3-benzoylpropionate function into the molecule, along with a loss of bromide. With the primary bromide present a displacement reaction is occurring under the reaction conditions which precludes the use of an excess of the reagent. In the absence of the primary bromide, such as with the related 3-methylene compound (8), a large excess of 3-benzoylpropionic acid provides no difficulties in the performance of the reaction.

imidine and stannous chloride in acetonitrile according to the procedure of Vorbruggen, et al.[21] This generated an amorphous material which appeared to have undergone polymerization with bromide loss. It was then decided that the phosphonate function should be introduced prior to attachment of the nucleoside base unit.

The phosphonate diester (10) was generated successfully by treatment of (9) with triisopropyl phosphite under standard Arbuzov reaction conditions. Heating at 160-180° for several hours did not disturb other functionalities present. An attempt was made to perform the Arbuzov reaction using tris(trimethylsilyl) phosphite according to the procedure described by Rosenthal, et al.[22] This would have allowed immediate isolation of the free phosphonic acid after treatment with water or alcohol. Unfortunately, the use of tris(trimethylsilyl) phosphite led to the cleavage of the 3-benzoylpropionate linkage without the introduction of phosphorus at the desired site.



The 1,2-diacetate (9) was generated from (7) in two steps. This involved first the hydrolysis of the isopropylidene function using 80% acetic acid followed by acetylation with acetic anhydride in pyridine. The resultant material (9) bears a pattern of hydroxyl protection which allows selective transformations leading to: 1) introduction of the uracil base at C-1, 2) introduction of the phosphonate function in place of the primary bromide, 3) selective deprotection of the 5'-hydroxyl, and 4) coupling to another nucleoside unit.

An initial attempt to introduce the uracil base at this stage failed. The material (9) was treated with 1,2-bis(trimethylsiloxy)pyr-

The nucleoside base could now be introduced without undesired side reaction. The phosphonate (10) was treated with 1,2-bis(trimethylsiloxy) pyrimidine and stannous chloride in dry acetonitrile for 24 hours at room temperature to generate the protected nucleotide (11).

There now remained to be performed the series of selective deprotections and couplings to the remaining nucleoside (nucleotide) units. The first step in this procedure involved the cleavage of the phosphonate esters. This was

accomplished by treatment of (11) with an excess of trimethylbromosilane at room temperature.[23] The bromide is found to be preferable to both the chloride and iodide in this process; it provides a reaction of suitable rate at room temperature without undesirable iodide contamination. The pyridinium salt of the phosphonic acid (12) was isolated upon treatment of the silyl phosphonate ester with a water-pyridine mixture.

The phosphonic acid salt (12) was then condensed with a second nucleoside unit. Treatment of (12) with 2',3'-O-isopropylidene uridine and DCC in dry pyridine in the presence of DOWEX 50 (pyridinium form)[24] yielded the protected dinucleoside phosphate analogue (13).

In order to attach the third nucleoside unit selective cleavage of the 3-benzoylpropionate from (13) was necessary. This was accomplished by treatment with hydrazine hydrate in acetic acid-pyridine buffer. Treatment of the resultant material with another equivalent of (12) under similar coupling conditions (DCC, pyridine) accomplished the generation of the protected oligonucleotide analogue (15).

Final deprotection was accomplished in two stages. Treatment with methanolic ammonia removed both the acetate and 3-benzoylpropionate functions. This was followed by treatment with 80% acetic acid to remove the remaining isopropylidene function and generate the oligonucleotide analogue (16). Overall, (16) was generated from diacetone-D-glucose in fifteen steps of average yield of 73%.

EXPERIMENTAL

General methods - Solvents were purified according to the following procedures: DMSO, tetrahydrofuran and pyridine were distilled over calcium hydride; DMF was mixed with benzene and distilled, the fraction boiling 90-100° being collected and shaken with freshly dried activated alumina. The decanted DMF was then distilled at reduced pressure. Acetonitrile and chloroform were distilled over phosphorus pentoxide.

2,4-Bis(trimethylsilyloxy)pyrimidine was prepared by refluxing dry uracil with an excess of hexamethyldisilazane until all material had dissolved. The desired material was isolated in 91% yield by distillation at 123° (18 Torr).

Thin layer chromatography was performed using KODAK 13179 silica gel plates. Column chromatography was performed using Baker 60-200 mesh silica gel.

NMR spectra were measured using Varian EM-360 and IBM-Bruker WP-200SY instruments. IR spectra were measured using a Perkin-Elmer 598 instrument, and optical rotations were measured at 589 nm using a JASCO DIP-140 instrument.

Elemental analyses were performed by MicAnal of Tucson, AZ.

1,2:5,6-Di-O-isopropylidene-α-D-ribo-hexofuranose-3-ulose (1). A solution of DMSO (620 mL) and acetic anhydride (410 mL) was allowed to stand at room temperature for two hours after which there was dissolved in it 1,2:5,6-di-O-isopropylidene-D-glucofuranose (52.1 g, 0.200 mol) and stirred overnight. The solvent was removed at reduced pressure (0.5 Torr) and the residue distilled at 115° (0.3 Torr) to yield 41.8 g (80.9%) of the desired material which exhibited spectra and analyses in accord with prior reports.

3-Deoxy-3-methylene-1,2:5,6-di-O-isopropylidene-α-D-ribo-hexofuranose (2). Sodium hydride (10.8 g, 0.225 mol) in 50% oil suspension was dissolved in DMSO (415 mL) under a nitrogen atmosphere. The resulting solution was cooled to 20° and methyltriphenylphosphonium bromide (88.5 g, 0.225 mol) was added. After stirring for 30 min, a solution of 1,2:5,6-di-O-isopropylidene-α-D-ribo-hexofuranose-3-ulose (19.4 g, 0.0752 mol) in DMSO (180 mL) was added maintaining the temperature at 20°. After 3 hrs there was added 2.5 L water and the resultant mixture was extracted with ether (5 x 400 mL). The extracts were washed with water (2 x 150 mL), dried over sodium sulfate, and evaporated under reduced pressure to a small volume. The material was filtered and the remainder of the solvent evaporated. The residue was chromatographed on a silica gel column (45 x 3.5 cm) using toluene (500 mL) followed by 3:1 toluene:ethyl acetate (500 mL). The desired product was isolated in the mixed solvent elution in 73.2% yield (13.4 g). NMR ($CDCl_3$, δ) 1.30-1.70 (12H, CH_3x4), 4.00-4.80 (4H, m, C_4H, C_5H, C_6H), 4.90 (1H, d, $J_{1,2}$ = 3Hz, C_2H), 5.80 (1H, d, $J_{1,2}$ = 3Hz, C_1H); IR (CCl_4, cm^{-1}) 2895, 2930, 1450, 1380, 1360, 1210, 1157; Calc. for $C_{13}H_{20}O_5$: C, 60.92; H, 7.86. Found: C, 60.78; H, 7.84%.

3-Deoxy-3-hydroxymethyl-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (3). To a solution of (2) (11.0 g, 0.0430 mol) in dry THF (95 mL) at 0° under a nitrogen atmosphere was added a 10.0 M solution of borane in dimethyl sulfide (13 mL). The resultant solution was stirred for 24 hrs at 0°. Maintaining the cooling, the reaction mixture was treated successively with 1:1 THF:water (45 mL), 2 N NaOH (132 mL) and 30% aqueous hydrogen peroxide (75 mL). The mixture was allowed to warm to room temperature with stirring for a period of 2 hrs. Evaporation of the solvent under reduced pressure afforded a white solid which was dissolved in water (500 mL) and the solution extracted with ether

(5 x 100 mL). The extract was dried with magnesium sulfate and evaporated to yield an oil which was chromatographed on a silica gel column (45 x 3.5 cm) eluting with 3:2 toluene: ethyl axetate to yield 9.14 g (81.1%) of the desired pure product. NMR ($CDCl_3$, δ) 1.40 (12H, m, CH_3 x 4), 3.25 (1H, m, C_4H), 3.60-4.20 (6H, m, $C(OH)H_2$, C_3H, C_5H, C_6H_2), 4.75 (1H, m, C_2H), 5.75 (1H, t, $J_{1,2}$= 3Hz, C_1H); Calc. for $C_{13}H_{22}O_6$: C, 56.92; H, 5.83. Found: C, 57.18; H, 5.83%.

3-Deoxy-3-bromomethyl-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (4). To a solution of N-bromosuccinimide (3.94 g, 0.0210 mol) in dry DMF (55 mL) was added triphenylphosphine (6.36 g, 0.0243 mol). A strongly exothermic reaction ensued and the temperature rose to 65°. The solution was cooled to 0° and there was added dropwise a solution of (3) (5.48 g, 0.200 mol) with pyridine (1.62 mL) and DMF (27 mL). Over a period of one hour the reaction was allowed to warm to room temperature and was then heated at 60° for 3 hrs. The solvent was then evaporated under reduced pressure and the residue was chromatographed on a silica gel column (45 x 3.5 cm) using toluene:ethyl acetate (8:1). Evaporation of the solvent gave the desired product as an oil (5.12 g, 73.8%) which exhibited a single spot on TLC (toluene:ethyl acetate 8:1) of R_f=0.50. NMR ($CDCl_3$, δ) 1.40 (12H, m, CH_3 x 4), 3.30-4.30 (7H, m, C_3H, $CBrH_2$, C_4H, C_5H, C_6H_2), 4.75 (1H, t, $J_{1,2}$=$J_{2,3}$= 3Hz, C_2H), 5.72 (1H, d, $J_{1,2}$= 3Hz, C_1H); Calc. for $C_{13}H_{21}O_5Br$: C, 46.30; H, 6.28. Found: C, 46.18; H, 6.31%.

3-Deoxy-3-bromomethyl-1,2-O-isopropylidene-α-D-allofuranose (5). A solution consisting of (4) (3.37 g, 0.00971 mol), methanol (49 mL), chloroform (16 mL) and 1.6% sulfuric acid (7.8 mL) was stirred at room temperature for 3 days. After this time the solution was treated with sodium bicarbonate (2 g) and evaporated to dryness. The residue was partitioned between water (20 mL) and chloroform (5 x 20 mL). The combined chloroform solutions were dried over sodium sulfate and evaporated to give the desired product as an oil (2.72 g, 91.2%) which exhibited a single spot on TLC (toluene:ethyl acetate 1:1) of R_f= 0.35. NMR ($CDCl_3$, δ) 1.47 (6H, d, CH_3 x 2), 2.50 (1H, m; C_3H), 2.90-4.00 (6H, m, $CBrH_2$, C_4H, C_5H, C_6H_2), 4.80 (1H, t, $J_{1,2}$=$J_{2,3}$= 3Hz, C_2H), 5.80 (1H, d, $J_{1,2}$= 3Hz, C_1H). IR (CCl_4, cm^{-1}) 3500, 2990, 2940, 1455, 1385, 1375, 1240, 1167. $[\alpha]_D^{22}$ = 99.5° ($CHCl_3$, 0.1M). Calc. for $C_{10}H_{17}O_5Br$: C, 40.42; H, 5.77. Found: C, 40.15; H, 5.60%.

3-Deoxy-3-bromomethyl-1,2-O-isopropylidene-α-D-ribofuranose (6). A solution of (5) (2.86 g, 10.0 mmol) in ethanol (113 mL) and water (80 mL) was placed in a flask shielded with light-reflecting foil. To this solution was added a solution of sodium meta-periodate (2.22 g, 10.3 mmol) in water (42 mL) followed by sodium bicarbonate (1.0 g). This reaction mixture was stirred at ambient temperature for 3 hrs. At this time sodium borohydride (1.93 g, 82.4 mmol) was added in small portions while the temperature was maintained below 25°. The mixture was stirred overnight and the excess sodium borohydride was decomposed by the addition of 50% acetic acid until the pH reached 5. The solution was titrated with 10% aqueous sodium bisulfite until colorless and was extracted with chloroform (5 x 50 mL). The extracts were dried over sodium sulfate and the solvent evaporated. The residue was chromatographed on a silica gel column (48 x 2.7 cm) being eluted with toluene:ethyl acetate (55:45). The desired product was isolated from the 100-400 mL eluant as an oil (1.93 g, 74.8%0 which exhibited a single spot on TLC (toluene:ethyl acetate 55:45) of R_f= 0.45. NMR ($CDCl_3$, δ) 1.37 (3H, s, CH_3), 1.53 (3H, s, CH_3), 2.50 (1H, m, C_3H, 2.80-4.10 (7H, m, OH, $CBrH_2$, C_4H, C_5H_2), 4.80 (1H, t, $J_{1,2}$=$J_{2,3}$= 3Hz, C_2H), 5.81 (1H, d, $J_{1,2}$= 3Hz, C_1H). IR (CCl_4, cm^{-1}) 3600, 3500, 3000, 2950, 2890, 1450, 1385, 1375, 1260, 1170. $[\alpha]_D^{22}$= 106.8° (0.1M, $CHCl_3$). Calc. for $C_9H_{15}O_4Br$: C, 40.47; H, 5.66. Found: C, 40.41; H, 5.72%.

5-O-(3-benzoylpropionyl)-3-deoxy-3-bromomethyl-1,2-O-isopropylidene-α-D-ribofuranose (7). A solution of (6) (2.86 g, 10.0 mmol), 3-benzoylpropionic acid (2.18 g, 10.0 mmol) and DCC (10.83 g, 52.0 mmol) in dry pyridine (100 mL) was stored at ambient temperature for 24 hrs. The excess of DCC was destroyed by the addition of water (20 mL) and the precipitate was filtered. The solvent was removed from the filtrate by evaporation with the repeated addition of toluene. The residue was chromatographed on a silica gel column (48 x 2.7 cm) eluting with hexane:ethyl acetate 3:1. The desired material was isolated from the 200-500 mL eluant as an oil (2.29 g, 53.5%) which exhibited a single spot on TLC (hexane:ethyl acetate 3:1) of R_f = 0.40. NMR ($CDCl_3$, δ) 1.31 (3H, s, CH_3), 1.52 (3H, s, CH_3), 2.40 (1H, m, C_3H), 2.80 (2H, t, J = 6Hz, CH_2COO), 3.10-3.40 (4H, m, $COCH_2$, $CBrH_2$), 3.80-4.40 (3H, m, C_4H, C_5H_2), 4.72 (1H, t, $J_{1,2}$=$J_{2,3}$= 4Hz, C_2H), 5.80 (1H, d, $J_{1,2}$= 4Hz, C_1H), 7.80-8.20 (5H, m, ArH). IR (CCl_4, cm^{-1}) 2980, 2930, 2850, 1735, 1685, 1595, 1450, 1385, 1230, 1165. $[\alpha]_D^{22}$= 49.0° (0.1M, $CHCl_3$). Calc. for $C_{19}H_{23}O_6Br$: C, 51.48; H, 5.23. Found: C, 51.79; H, 5.19%.

5-O-(3-Benzoylpropionyl)-3-deoxy-3-bromomethyl-1,2-di-O-acetyl-D-ribofuranose (9). The acetonide (7) (2.14 g, 10.0 mmol) was stirred at 80° in 80% acetic acid (100 mL) for 24 hrs. The solvent was evaporated under reduced pressure with repeated addition of toluene. After drying at 0.01 Torr overnight there was obtained a glassy residue which was dissolved in a mixture of dry pyridine (12 mL) and acetic anhydride (6 mL). The solution was stirred overnight at ambient temperature and the solvent was removed under reduced pressure with repeated addition of toluene. The residue was chromatographed on a silica gel column (45 x 3.5 cm) eluting with hexane:ethyl acetate (7:3). There was isolated from the 200-400 mL eluant the desired material as an oil (3.56 g, 74.0%) which exhibited a single spot on TLC (hexane:ethyl acetate, 7:3) of R_f = 0.60. NMR ($CDCl_3$, δ) 2.05 (3H, s, CH_3), 2.80 (3H, m, CH_2COO, C_3H), 3.10-3.70 (4H, m, $COCH_2$, $BrCH_2$), 4.31 (3H, s, C_4H, C_5H_2), 5.23 (1H, d, $J_{2,3}$= 4Hz, C_2H), 6.00 (1H, s, C_1H), 8.00 (5H, m, ArH). IR (CCl_4, cm^{-1}) 2980, 2955, 2855, 1745, 1690, 1595, 1450, 1375, 1235, 1165. $[\alpha]_D^{22}$= 8.47° (0.1M, $CHCl_3$). Calc. for $C_{20}H_{23}O_8Br$: C, 50.97; H, 4.92. Found: C, 50.71; H, 4.85%.

5-(3-Benzoylpropionyl)-3-deoxy-3-diisopropoxy-phosphinylmethyl-1,2-di-O-acetyl-D-ribofuranose (10). A solution of (9) (2.35 g, 4.89 mmol) in triisopropylphosphite (15 mL) was heated at 160-180° with exclusion of moisture for 3 days. Volatiles were removed under reduced pressure and the residue was purified by chromatography

on a silica gel column (48 x 2.7 cm) eluting with chloroform:ethyl acetate (1:1). From the 150-500 mL eluant there was isolated as an oil (1.73 g, 68.0%) the desired product which exhibited a single spot on TLC (chloroform: ethyl acetate, 1:1) of R_f = 0.50. NMR ($CDCl_3$, δ) 1.30 (12H, d, J = 8Hz, CH_3), 2.05 (3H, s, $COCH_3$), 2.10 (3H, s, $COCH_3$), 2.25 (2H, m, PCH_2), 2.25-3.00 (3H, m, CH_2COO, C_3H), 3.30 (2H, t, J = 6Hz, $COCH_2$), 4.00-5.00 (5H, m, POCH, C_5H_2, C_4H), 5.16 (1H, d, $J_{2',3'}$= 4Hz, C_2H), 7.30-8.00 (5H, m, ArH). IR (CCl_4, cm^{-1}) 2985, 2830, 1745, 1690, 1595, 1450, 1390, 1375, 1235, 1170. $[\alpha]_D^{22}$= 19.6° (0.1M, $CHCl_3$). Calc. for $C_{26}H_{37}O_{11}P$: C, 56.11; H, 6.70. Found: C, 56.45; H, 6.60%.

5'-(3-Benzoylpropionyl)-3'-deoxy-3'-diisopropoxyphosphinylmethyl-2'-O-acetyl uridine (11). To a solution of (10) (2.50 g, 4.81 mmol) in dry acetonitrile (90 mL) at 0° was added bis(trimethylsilyl)uracil (1.25 g, 4.88 mmol) and stannous chloride (0.62 mL, 5.3 mmol). The solution was stirred at ambient temperature for 24 hrs after the addition at which time the reaction mixture was diluted with methylene chloride (125 mL) and extracted with a saturated solution of aqueous sodium bicarbonate (100 mL). The organic layer was dired with sodium sulfate and evaporated under reduced pressure. The residue was recrystallized from ether to yield the desired material (2.20 g, 80.0%) as a solid of R_f = 0.31 (chloroform:ethyl acetate 1:1). NMR ($CDCl_3$, δ) 1.18 (12H, d, J = 8Hz, CH_3), 1.58-2.00 (2H, m, PCH_2), 2.01 (3H, s, $COCH_3$), 2.50-2.90 (3H, m, CH_2COO, $C_3'H$), 3.23 (2H, m, $COCH_2$), 3.80-4.80 (5H, m, POCH, $C_5'H_2$, $C_4'H$), 5.64 (2H, m, C_5H, $C_1'H$), 5.98 (1H, m, $C_3'H$), 7.00-8.00 (6H, m, ArH, C_6H), 9.58 (1H, s, NH). $[\alpha]_D^{22}$ = 55.16° (0.10M, $CHCl_3$). Calc. for $C_{28}H_{37}N_2O_{11}P$: C, 55.26; H, 6.13; N, 4.60. Found: C, 54.91; H, 5.98; N, 4.43%.

5'-(3-Benzoylpropionyl)-3'-deoxy-3'-dihydroxyphosphinylmethyl-2'-O-acetyl uridine (12). To a solution of (11) (2.10 g, 3.45 mmol) in dry chloroform (25 mL) was added trimethylsilylbromide (5.2 mL). After 7 hrs the volatile materials were removed under reduced pressure. The residue was stirred with a mixture of water (35 mL) and pyridine (14 mL) for 1 hr, followed by extraction of the resulting solution with ether (10 mL). The aqueous layer was evaporated under reduced pressure and the residue was crystallized from an ethanol/ether mixture to yield the desired material in the pyridinium salt form (1.85 g, 94.6%) as a solid of R_f = 0.52 (CH_3OH with Anasil RPF). NMR ($CDCl_3$, δ) 1.30-2.25 (5H, m, $COCH_3$, PCH_2), 2.46 (3H, m, CH_2COO, C_3H), 3.17 (2H, d, J= 8Hz, $COCH_2$), 3.52-4.18 (3H, m, $C_4'H$, $C_5'H$), 5.22 (1H, m, $C_2'H$), 5.50 (2H, m, C_5H, $C_1'H$), 7.00-9.00 (10H, m, ArH, pyridine-H, C_6H). $[\alpha]_D^{22}$= 44.5° (0.1M, CH_3OH). Calc. for $C_{27}H_{30}O_{11}N_3P$: C, 53.73; H, 5.01; N, 6.96. Found: C, 53.95; H, 4.97; N, 6.75%.

5'-O-(3-Benzoylpropionyl)-3'-deoxy-3'-dihydroxyphosphinylmethyl-2'-O-acetyl uridylyl-(3'→5')-2',3'-O-isopropylidene uridine (13). A mixture of (12) (0.40 g, 0.66 mmol), 2',3'-O-isopropylidene uridine (0.53 g, 1.9 mmol) and DOWEX 50 (pyridinium form) (1.52 g) was rendered anhydrous by repeated evaporation with anhydrous pyridine and dried in vacuum over phosphorus pentoxide. The residue was dissolved in dry pyridine (8 mL), the flask was covered with a light-reflecting foil, and DCC (1.8 g, 8.7 mmol) was added. The reaction mixture was allowed to stand at ambient temperature for 6 days after which time the excess DCC was destroyed by water addition (8 mL). The resulting precipitate was filtered and the filtrate extracted with ether (10 mL). The aqueous layer was evaporated under reduced pressure and the residue was chromatographed on a silica gel column (30 x 2.7 cm) eluting with chloroform:ethanol (20:1). The desired product was obtained from the 100-300 mL eluant as an amorphous solid (0.45 g, 71%) of R_f = 0.48 (CH_3OH, Anasil RPF). NMR (CD_3OD, δ) -selected signals- 1.58 (3H, s, CH_3), 1.62 (3H, s, CH_3), 2.02 (3H, s, $COCH_3$). $[\alpha]_D^{22}$= 36.9° (0.1M, CH_3OH). Calc. for $C_{39}H_{44}O_{16}N_5P$ (pyridinium salt): C, 51.64; H, 4.98. Found: C, 51.95; H, 5.01%.

3'-Deoxy-3'-dihydroxyphosphinylmethyl-2'-O-acetyl uridylyl-(3'→5')-2',3'-O-isopropylidene uridine (14). A mixture of (13) (0.60 g, 0.82 mmol) with 98% hydrazine hydrate (0.041 mL, 0.84 mmol) in pyridine/acetic acid buffer (4:1) (2.5 mL) was stirred at ambient temperature for 24 hrs. The solution was then evaporated several times with added 95% ethanol and the residue was chromatographed on a silica gel column (30 x 2.7 cm) eluting with chloroform: methanol (7:1). The desired material (0.25 g, 50%) was isolated from the 100-200 mL eluant as an amorphous solid exhibiting a single spot on TLC (chloroform:methanol 7:1, Anasil RPF) of R_f = 0.39. NMR (CD_3OD, δ) -selected signals- 1.25 (3H, s, CH_3), 1.70 (3H, s, CH_3), 2.02 (3H, s, $COCH_3$), 7.55-8.02 (2H, m, C_6H). $[\alpha]_D^{22}$ = 54.1° (0.1M, CH_3OH). Calc. for $C_{29}H_{36}O_{14}N_5P$ (pyridinium salt): C, 45.74; H, 4.92. Found: C, 45.48; H, 4.90%.

5'-O-(3-Benzoylpropionyl)-3'-deoxy-3'-dihydroxyphosphinylmethyl-2'-O-acetyl uridylyl-(3'→5')-3'-deoxy-3'-dihydroxyphosphinylmethyl-2'-O-acetyl uridylyl-(3'→5')-2',3'-O-isopropylidene uridine (15). A mixture of (14) (0.18 g, 0.29 mmol), (12) (0.22 g, 0.36 mmol) and DOWEX 50 (pyridinium form) (0.57 g) was rendered anhydrous by repeated evaporation with anhydrous pyridine under reduced pressure and storage over phosphorus pentoxide. To this mixture was added pyridine (2.5 mL) and DCC (0.60 g). The reaction flask was covered with a light-reflecting foil and kept for 6 days at ambient temperature. The mixture was then stirred for 2 hrs with water (2.5 mL), after which the resultant precipitate was filtered and the filtrate extracted with chloroform (5 mL). The aqueous portion was evaporated under reduced pressure and the residue subjected to chromatography on a silica gel column (20 x 2.7 cm) eluting with chloroform:methanol (10:1). The desired material was isolated from the 80-200 mL eluant (0.20 g, 62%) as an amorphous solid which exhibited a single spot on TLC (CH_3OH, Anasil RPF) of R_f = 0.24. NMR (CD_3OD, δ) -selected signals- 1.27 (3H, s, CH_3), 1.59 (3H, s, CH_3), 2.05 (3H, s, $COCH_3$). $[\alpha]_D^{22}$ = 44.5° (0.1M, CH_3OH). Calc. for $C_{46}H_{54}O_{24}N_6P_2$: C, 48.60; H, 4.79; N, 7.39. Found: C, 48.24; H, 4.69; N, 7.48%.

3'-Deoxy-3'-dihydroxyphosphinylmethyl uridylyl-(3'→5')-3'-deoxy-3'-dihydroxyphosphinylmethyl uridylyl-(3'→5')-uridine (16). A solution of (15) (0.30 g, 0.26 mmol) in methanol saturated with ammonia (10 mL) was stirred at ambient temperature for 24 hrs. The solvent was evaporated under reduced pressure and the residue was dissolved in water (10 mL) and extracted with portions of ethyl acetate (5 mL) until the organic layer became colorless. The aqueous

layer was then evaporated under reduced pressure and the residue was dissolved in 80% acetic acid (10 mL) and stirred at 80° for 24 hrs. The solvents were evaporated under reduced pressure and the residue was crystallized from ethanol to give the desired product as an amorphous solid (0.15 g, 67%) which exhibited a single spot on two-dimensional TLC (AnasilRPF; first - chloroform:methanol, 5:1; second - 2-propanol:ammonia:water, 6:3:1) with R_f: first - 0.05; second - 0.45. The NMR (D_2O) indicated all protecting groups had been cleaved. Calc. for $C_{29}H_{38}O_{20}N_6P_2:(H_2O)_{11}$: C, 33.14; H, 5.75; N, 8.00. Found: C, 33.10; H, 5.78; N, 7.66%. The water content was variable with exposure to air limiting the ability to obtain an accurate optical rotation on this material.

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