Synthesis and Properties of 2′-Deoxy-2′-α-*C*-branched Nucleosides and Nucleotides

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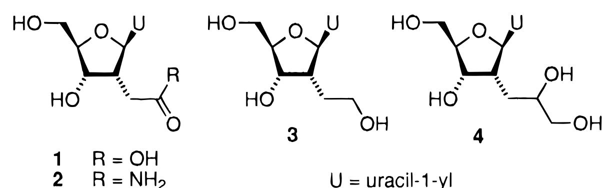
Four functionalized 2′-deoxy-2′-α-*C*-branched nucleosides, namely, 2′-deoxy-2′-α-*C*-(carboxymethyl)-uridine, 2′-deoxy-2′-α-*C*-acetamidouridine, 2′-deoxy-2′-α-*C*-(hydroxyethyl)uridine, and 2′-deoxy-2′-α-*C*-(2,3-dihydroxypropyl)uridine, have been prepared. Conversion of these nucleosides to their appropriately protected phosphoramidites, followed by tetrazole-induced reaction with 2′,3′-di-*O*-acetyluridine, oxidation, and subsequent deprotection furnished the corresponding dinucleoside monophosphates. During the oxidation of the amide-derived phosphite, partial dehydration occurred to give a mixture of the amide- and nitrile-containing dimers. Interestingly, the ratio of amide to nitrile could be largely controlled by choice of oxidant. The hydroxyethyl- and dihydroxypropyl-modified dimers were particularly resistant to snake venom phosphodiesterase-catalyzed hydrolysis (relative half-lives of 129 and 120, respectively, in comparison to UpU). It is anticipated that these nucleoside analogues will ultimately be used in the construction of ribozymes containing enhanced functionality and nuclease resistance.

Introduction

There is increasing circumstantial evidence for the existence of early life forms that were characterized by RNA-catalyzed replication, a situation that has been termed the RNA world.[1] Robertson and Miller have shown that under prebiotic conditions uracil reacts with formaldehyde and simple nucleophiles to give 5-substituted uracils with the side chains of most of the 20 genetically-coded amino acids.[2] The authors proposed that ribozymes in the RNA world would have contained the functional groups that are currently found in proteins and consequently may have possessed greater catalytic activity than those currently in existence. It is certainly true that the variety of reactions that naturally occurring ribozymes are known to catalyze is extremely narrow in comparison to protein enzymes and is restricted to transformations that involve the RNA phosphodiester backbone.[3] It is therefore interesting to speculate as to whether the catalytic activity and substrate diversity of ribozymes might be enhanced by the inclusion of functionality that is known to be important for protein enzymes.

As the first step toward addressing this issue, functionalized 2′-α-*C*-branched nucleosides have been prepared for incorporation into oligoribonucleotides. The 2′-position was chosen as the site of modification for the following reasons. (i) It enables the regular 3′−5′ phosphodiester linkages and the Watson−Crick hydrogen bonding scheme to be maintained. (ii) The same modification can be introduced into both purine and pyrimidine nucleosides. (iii) It has been shown that the introduction of 2′-α-alkyl substituents into oligodeoxyribonucleotides increases their resistance to degradation by nucleases.[4] Modified nucleosides were designed to contain one of the following functional groups: a carboxylic acid (**1**), a primary amide (**2**), a primary alcohol (**3**), or a 1,2-diol (**4**). While the carboxyl, amide, and alcohol groups have a direct parallel in amino acids, the diol function was chosen as a means to introduce functionality that would have the potential to cleave a phosphodiester bond in the presence of metal ions such as lanthanides. Recently, alcohols such as glycerol and gluconate have been shown to accelerate the hydrolysis of DNA by lanthanide cations.[5] Since metal ions are an absolute requirement for efficient ribozyme catalysis, it may prove possible to create metalloribozymes capable of catalyzing new reactions (*e.g.,* redox reactions) by engineering transition metal binding sites into RNA.[6]



In a recent paper we described the synthesis of the 2′-α-*C*-branched nucleosides **1**−**3**.[7] We now report the full details of syntheses of **1**−**3**, the preparation of the dihydroxypropyl nucleoside **4**, the incorporation of these compounds into dinucleoside monophosphates, and studies on the hydrolysis of the dimers. To our knowledge, this is the first systematic approach to the synthesis of nucleic acids containing catalytically useful functional groups, although 2′-deoxy-2′-amino nucleosides,[8] 2′-thiouridine,[9] and 2′-α-*C*-(hydroxymethyl)thymidine[10] have all been incorporated into oligoribo- or oligodeoxyribonucle-

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(1) Joyce, G. J.; Orgel, L. E. In *The RNA World*; Gesteland, R., Atkins, J., Eds.; Cold Spring Harbor Press: Cold Spring Harbor, NY, 1993; pp 1−25.
(2) Robertson, M. P.; Miller, S. L. *Science* **1995**, *268*, 702.
(3) Wilson, C.; Szostak, J. W. *Curr. Opin. Struct. Biol.* **1992**, *2*, 749.
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(6) Pyle, A. M. *Science* **1993**, *261*, 709.
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(10) Schmit, C.; Bévierre, M.-O.; De Mesmaeker, A.; Altman, K.-H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1969.

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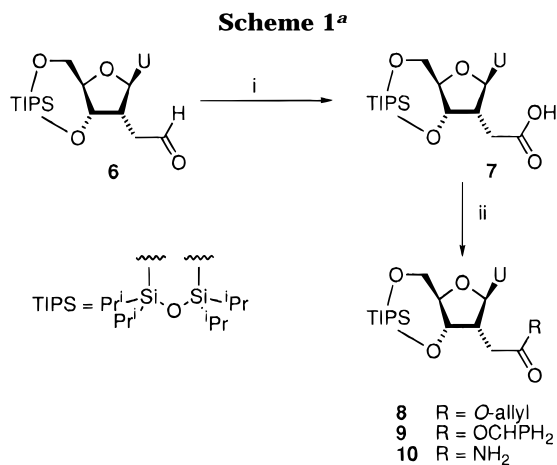
Scheme 1[a]



[a] Key: (i) $NaClO_2$, KH_2PO_4, 2-methyl-2-butene, 2-methyl-2-propanol; (ii) **8**, $H_2C{=}CHCH_2OH$, DCC, DMAP, THF; **9**, Ph_2CN_2, acetone; **10**, (a) *N*-hydroxysuccinimide, DCC, DMAP, 1,4-dioxane, (b) NH_3 (aq).

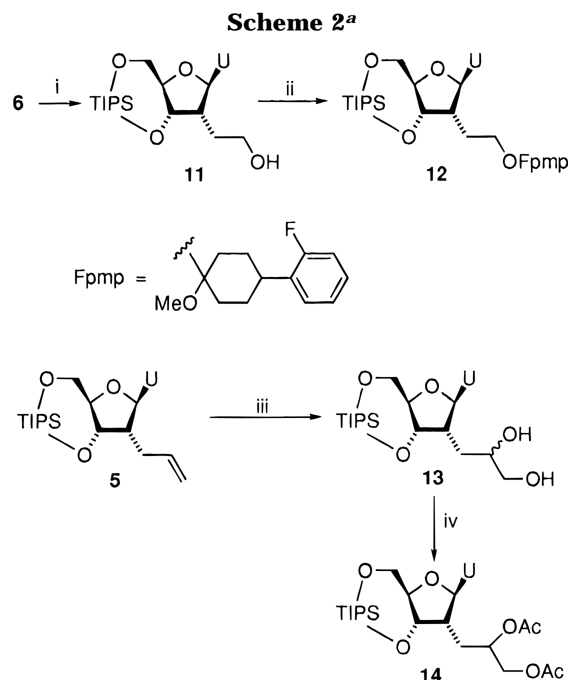
otides, chiefly with the aim of stabilizing oligomers to nuclease digestion.

Results and Discussion

The synthesis of all four 2′-α-*C*-branched nucleosides started from the previously described 2′-α-*C*-allyl nucleoside **5**.[11] Oxidative cleavage of the allylic side chain using OsO_4 in the presence of *N*-methylmorpholine oxide, followed by treatment with $NaIO_4$, gave the 3′,5′-tetraisopropyldisiloxanediyl (TIPS)-protected aldehyde **6**,[11] which served as the starting material for the synthesis of **1**−**3**.

Preparation of 2′-α-*C*-Branched Nucleosides 1−4. For the preparation of the carboxymethyl nucleoside (**1**) the TIPS-protected aldehyde (**6**) was oxidized with $NaClO_2$ in the presence of KH_2PO_4 and 2-methyl-2-butene in aqueous 2-methyl-2-propanol, to give the required carboxylic acid **7** (95%) (Scheme 1). For incorporation of this analogue into oligomers a protective group for the carboxylic acid function was required. There is no precedent for the protection of a comparable carboxylic acid function in oligonucleotide chemistry, but since it is possible that this functionality could assist the hydrolysis of the adjacent phosphodiester bond at extremes of pH, it was considered prudent to use a blocking group that could be removed under neutral conditions. With these considerations in mind we first examined the allyl group, which can be removed under mild conditions using palladium(0).[12] Treatment of the carboxylic acid **7** with allyl alcohol and DCC gave the TIPS-protected allyl ester (**8**) in 80% yield. However, subsequent attempts (see Scheme 3) to remove the TIPS group using $NEt_3{\cdot}3HF$ gave an inseparable mixture of the desilylated allyl ester (**15**) and the γ-butyrolactone (**19**). It seemed likely that lactonization could be reduced by increasing the steric bulk of the protecting group. Diphenylmethyl (benzhydryl) esters are readily prepared from diphenyldiazomethane, and the carboxyl group can be unmasked by either catalytic hydrogenolysis or acid hydrolysis.[13] Thus,

Scheme 2[a]



[a] Key (i) $NaBH_4$, MeOH; (ii) Fpmp vinyl ether, camphorsulfonic acid, CH_2Cl_2; (iii) OsO_4, $(DHQD)_2PYR$, $K_3Fe(CN)_6$, K_2CO_3, *t*-BuOH/water; (iv) Ac_2O, pyridine.

treatment of the carboxylic acid **7** with diphenyldiazomethane in acetone gave the benzhydryl ester **9** (75%).

Conversion of the acid **7** to the primary amide **10** was achieved in a two-step procedure *via* the intermediate *N*-hydroxysuccinimide ester.[14] Treatment of acid **7** with *N*-hydroxysuccinimide and DCC in dioxane, followed by addition of concentrated aqueous ammonia, furnished **10** (79%).

For the preparation of a suitably protected derivative of the hydroxyethyl nucleoside (**3**) the TIPS-protected aldehyde (**6**) was reduced using $NaBH_4$ in MeOH to give the alcohol (**11**, Scheme 2) in quantitative yield. The 1-(2-fluorophenyl)-4-methoxypiperidinyl (Fpmp) group was initially chosen to protect the primary hydroxyl group.[15] This achiral acetal group has been extensively used in RNA synthesis, and unlike the rival *tert*-butyldimethylsilyl group, it is compatible with the removal of the cyclic TIPS ether. Thus, treatment of the hydroxyethyl nucleoside (**11**) with excess 1-(2-fluorophenyl)-4-methoxy-1,2,3,6-tetrahydropyridine in the presence of camphorsulfonic acid gave the Fpmp-protected nucleoside (**12**) in 73% yield.

The TIPS-protected diol (**13**) was accessible through an osmium-catalyzed dihydroxylation of the allyl nucleoside (**5**, Scheme 2). To assist the characterization and isolation of the TIPS-protected diol (**13**), and its subsequent derivatives, it was considered desirable to obtain this compound as a single diastereoisomer. In an initial experiment to assess whether the chirality of the sugar would impose a significant diastereomeric bias on the reaction, a standard dihydroxylation procedure using OsO_4 in the presence of *N*-methylmorpholine oxide was performed and **13** isolated in 81% yield with a slight predominance of one diastereoisomer (ratio 57:43). The diastereomeric composition of **13** could not be determined

(11) De Mesmaeker, A.; Lebreton, J.; Hoffman, P.; Freir, S. M. *Synlett* **1993**, 677.
(12) (a) Wakabyashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691. (b) Kuyl-Yeheskiely, E.; Tromp, C. M.; Lefeber, A. W. M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1988**, *44*, 6515.
(13) Stelakatos, G. C.; Paganou, A.; Zervas, L. *J. Chem. Soc. C* **1966**, 1373.
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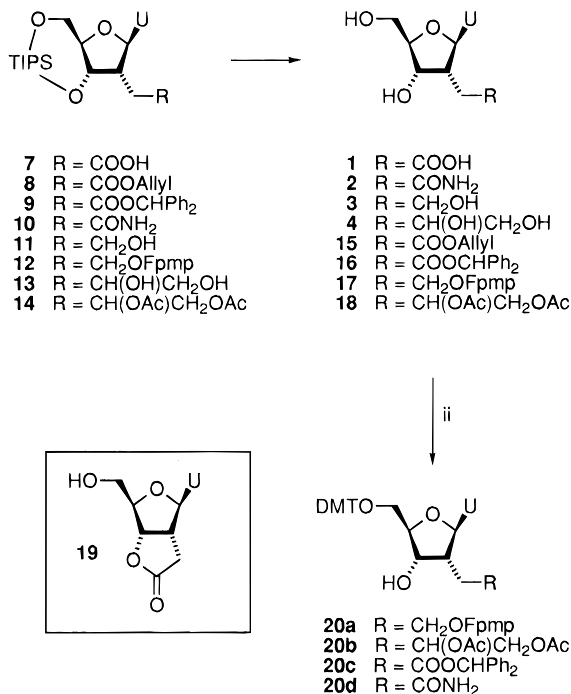
(15) (a) Capaldi, D. C.; Reese, C. B. *Nucleic Acid Res.* **1994**, *22*, 2209. (b) Rao, M. V.; Reese, C. B.; Schehlman, V.; Yu, P. K. *J. Chem. Soc., Perkin Trans. 1* **1993**, 43.

Scheme 3[a]



[a] Key: (i) NEt_3·3HF, THF; (ii) DMTCl, pyridine, CH_2Cl_2.

from analysis of the proton NMR spectrum as the resonances for the two diastereoisomers were almost totally superimposed. However, following removal of the TIPS group the composition could be accurately determined by reversed-phase HPLC. In an attempt to obtain a more heavily biased diastereomeric ratio, Sharpless asymmetric dihydroxylation (ADH) reactions[16] were performed using the commercially available ligands $(DHQD)_2$-PYR and $(DQD)_2$PYR.[17] Literature precedent suggested that these ligands would give the greatest chiral induction in the case of a terminal alkene. The reaction using the former ligand gave an 86% yield of the required diol as an 86:14 mixture of diastereoisomers, whereas the latter gave the diol in 72% yield as a 71:29 mixture with the same diastereoisomer predominating in each case. The fact that each of the matched ligands gave the same predominating diastereoisomer and neither reaction was as selective as examples reported in the literature suggests that the chiral sugar moiety interferes with ligand binding in the reaction complex. The diol mixture from the ADH reaction using $(DHQD)_2$PYR, which was obtained as an oil, was subsequently converted to the crystalline 2,4-dinitrobenzoate mono ester, recrystallized, and treated with methanolic ammonia to give the diol as a single diastereoisomer (de >99%). In preparation for oligonucleotide synthesis, the purified diol was subsequently protected as the bisacetyl derivative (**14**).[18] To our knowledge, this is the first reported instance of the ADH reaction being applied to a nucleoside derivative.

Scheme 3 shows the removal of the cyclic TIPS protecting group to give the nucleosides **1**−**4**, as well as their suitably protected equivalents **16**−**18**. In all cases removal of the TIPS group was accomplished using NEt_3·3HF, and the products were generally isolated in good yield (75−80%). In specific instances this desilylation reaction requires further comment. While the formation of 5-membered ring lactones is extremely favorable the carboxymethyl nucleoside **1** was relatively stable and was readily isolated as the triethylammonium salt following silica gel chromatography. However, under acidic conditions conversion to the γ-butyrolactone (**19**) occurred effortlessly and the carboxymethyl nucleoside was additionally characterized as the lactone.[7] Gratifyingly, the lactonization that accompanied desilylation of the allyl ester (**8**) was not observed with the benzhydryl ester **9**, and **16** was isolated in 87% yield. Compounds **2** and **16**−**18** were subsequently converted to their 5′-*O*-dimethoxytrityl derivatives (**20a**−**d**), in preparation for oligonucleotide synthesis. While there is increasing use of DMAP/NEt_3 as a catalyst for tritylation reactions,[19] we found that reactions performed in pyridine/CH_2Cl_2 were the most rapid and gave the highest yields. The inclusion of DMAP/NEt_3 was particularly deleterious in the preparation of ester **20c** and resulted in partial lactonization.

Preparation of Dinucleoside Monophosphates Containing 1−4. The 5′-*O*-DMT-protected hydroxyethyl (**20a**), dihydroxypropyl (**20b**), carboxymethyl (**20c**), and acetamido (**20d**) monomers were prepared for solid-phase-style coupling by converting them to their 3′-*O*-phosphoramidites **21a**−**d**, respectively, using 2-cyanoethyl *N,N,N′,N*-tetraisopropylphosphorodiamidite under standard conditions (Scheme 4).[20] The phosphoramidites were then coupled with 2′,3′-di-*O*-acetyluridine in acetonitrile in the presence of 1*H*-tetrazole under conditions analogous to those used in solid-phase synthesis.[21] The reactions were followed by ^{31}P NMR, which showed the formation of the intermediate diastereomeric dinucleoside phosphites (**22a**, 144.93/144.53; **22b**, 141.26/141.51; **22c**, 141.27/141.67; and **22d**, 141.70/141.55 ppm). Without isolation, the phosphites were subsequently oxidized to the corresponding phosphates (**23**) by excess addition of a 4% solution (w/v) of I_2 in THF/2,6-lutidine/H_2O (8:1:1). The coupling reaction employing the hydroxyethyl derivative (**21a**) proceeded as expected to give the corresponding fully protected dinucleoside monophosphate (**23a**) in 58% yield. Complete deprotection to furnish **24a** was achieved by sequential treatment with methanolic ammonia to remove the acetyl and cyanoethyl groups, acetate buffer (pH 3·25) to cleave the 1-(2-fluorophenyl)-4-methoxypiperidinyl (Fpmp) group,[15a] and finally AcOH:H_2O (8:2) to completely remove the DMT group. An analogous coupling reaction performed on the dihydroxypropyl monomer (**21b**) gave the fully protected dimer **23b** (87% yield), which was subsequently deprotected using methanolic ammonia followed by aqueous AcOH to yield **24b**. Likewise, the carboxymethyl monomer **21c** was converted to the fully protected dimer **23c** in 33% yield. Following the methanolic ammonia and aqueous AcOH deprotection steps, the benzhydryl blocking group was removed by hydrogenolysis using palladium on charcoal in THF/MeOH. This final deprotection step

(16) Sharpless, K. B.; Hartung, J.; Jeong, K. S.; Kwong, H. L.; Morikawa, K.; Wang, Z. M.; Xu, D.; Zhang, X. L. *J. Org. Chem.* **1992**, *57*, 2768.

(17) Crispino, G. A.; Jeong, K. S.; Kolb, H. C.; Wang, Z. M.; Xu, D.; Sharpless, K. B. *J. Org. Chem.* **1993**, *58*, 3785.

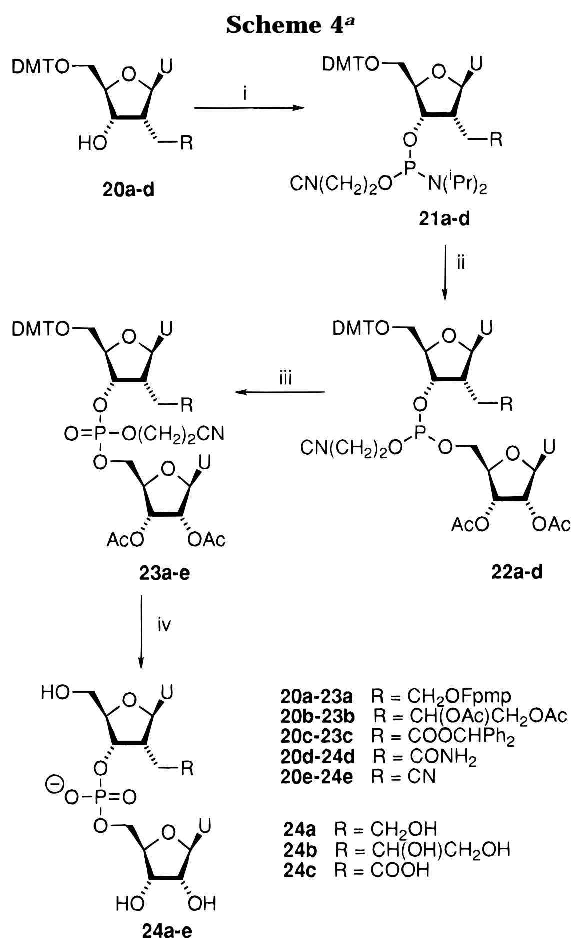
(18) The chronological order in which the work described in this paper was performed was such that studies on the base-catalyzed hydrolysis of the closely related hydroxyethyl-containing dimer (**24a**) had already established that conditions necessary for the removal of the acetyl groups would not be deleterious to the phosphodiester linkage.

(19) Chauhary, S. K.; Hernandez, O. *Tetrahedron Lett.* **1979**, *20*, 95.

(20) (a) Nielsen, J.; Taagaard, Marugg, J. E.; van Boom, J. H.; Dahl, O. *Nucleic Acids Res.* **1986**, *14*, 7391. (b) Kierzek, R.; Caruthers, M. H.; Longfellow, C. E.; Swinton, D.; Turner, D. H.; Frier, S. M. *Biochemistry* **1986**, *25*, 7840.

(21) Sinha, N. D.; Biernat, J.; McManus, J.; Köster, H. *Nucleic Acids Res.* **1984**, *12*, 4539.

Scheme 4[a]

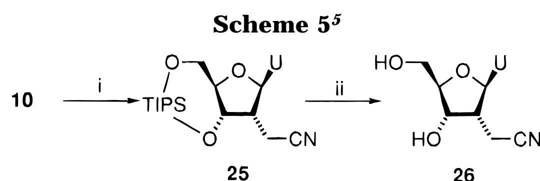


[a] Key: (i) 2-cyanoethyl bis(*N,N*-diisopropylamino)phosphoramidite, diisopropylammonium tetrazolide, CH_2Cl_2; (ii) 2′,3′-di-*O*-acetyluridine, tetrazole, CH_3CN; (iii) I_2, lutidine, water, THF; (iv) **23b**,**d**,**e**, (a) NH_3, MeOH, (b) AcOH/water (4:1); **23a**, (a) NH_3, MeOH, (b) pH 3.5 buffer, (c) AcOH/water (4:1), **23c**, (a) NH_3, MeOH, (b) AcOH/water (4:1), (c) H_2, Pd/C, THF/MeOH (3:1).

could be monitored by reversed-phase HPLC with retention times of 17.03 and 28.10 min, respectively, for **24c** and its benzhydryl derivative. The spectroscopic data obtained on the three dimers **24a**−**c** were absolutely consistent with their proposed structures. Additional characterization was obtained from digestion with SVPD and HPLC analysis of the hydrolysate. In each case only the two expected hydrolysis products were observed, namely uridine 5′-monophosphate (UMP) and either **3**, **4**, or **1**, respectively, for the dimers **23a**−**c**.

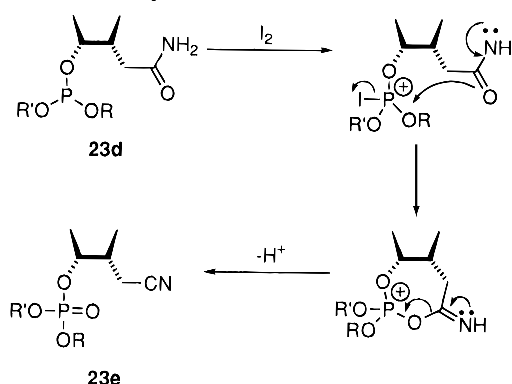
The coupling reaction and subsequent oxidation performed with the amide **21d** yielded two fully protected dinucleoside monophosphates that were isolated by column chromatography. The minor product (∼15% isolated yield) had both a FAB mass spectrum and a ^1H NMR spectrum that were consistent with the anticipated amide dimer **23d**. However, spectroscopic analysis of the major product (∼33% isolated yield) was consistent with the amide undergoing dehydration to yield the nitrile **23e**. In particular, both the FAB and electrospray mass spectra gave pseudomolecular ions that were concordant with the loss of water from **23d**. In order to unequivocally establish the identity of the two products, both **23d** and **23e** were deprotected by treatment with methanolic ammonia followed by 80% aqueous acetic acid. Spectroscopic data on the minor product, derived from **23d**, were absolutely consistent with it being the amide-containing dimer **24d**. In particular, the ^{13}C spectra showed a characteristic amide carbonyl resonance at 176.80 ppm.

Scheme 5[5]



[a] Key: (i) $(CF_3CO)_2O$, pyridine, 1,4-dioxane; (ii) NEt_3·3HF, THF.

Scheme 6. Proposed Mechanism for the Dehydration of Amide 23d



Additionally, hydrolysis of **24d** using SVPD and analysis of the resultant digestion mixture by HPLC showed the presence of the two expected products, UMP and 2′-deoxy-2′-*C*-acetamidouridine (**2**). The ^{13}C NMR spectrum of the major product, derived from deprotection of **23e**, showed no amide carbonyl resonance, but it did show a new resonance with the expected chemical shift for a nitrile carbon (119.71 ppm). Both the FAB mass spectrum and ^1H NMR spectrum were also consistent with **24e** being the nitrile-containing product. Digestion of **24e** with SVPD and HPLC analysis of the hydrolysate showed the formation of two products, UMP and a new, unidentified compound that was presumed to be the nitrile (**26**). To confirm the identity of this product the chemical synthesis of **26** (Scheme 5) was performed. The (TIPS)-protected amide (**10**) was treated with trifluoroacetic anhydride and pyridine[22] to give the protected nitrile-containing nucleoside (**25**) in 78% yield. Subsequent removal of the TIPS protecting group using NEt_3·3HF gave 2′-deoxy-2′-*C*-(cyanomethyl)uridine (**26**), which had an HPLC retention time identical to that of the unidentified product from the SVPD digestion of **24e**.

To explain this dehydration reaction, we propose the mechanism shown in Scheme 6. Involvement of the activated phosphonium species is indirectly supported by the following observations. (i) Treatment of the TIPS-protected amide (**10**) under exactly the same conditions as those involved in the coupling and oxidation procedure gave no dehydration product. (ii) The ^{31}P NMR spectrum of the intermediate dinucleoside phosphite (**22d**) shows the presence of a single diastereomeric product, suggesting that dehydration occurred during the subsequent oxidation step. Additionally, a procedure for the dehydration of amides using triphenylphosphine and CCl_4[23] has been reported previously and involves the formation of a phosphonium salt that acts as the dehydrating agent; it seems probable that a similar species is formed on treatment of the phosphite (**22d**) with iodine. This phosphonium species is then attacked either by the amide functionality, which results in formation of the nitrile

(22) Campagna, F.; Carotti, A.; Casini, G. *Tetrahedron Lett.* **1977**, *21*, 1813.
(23) Yamato, E.; Sugasawa, S. *Tetrahedron Lett.* **1970**, 4383.

Table 1. Oxidation of Intermediate Phosphite 22d under Different Conditions

entry	oxidation condns	amide:nitrile[a]
1	4% I_2 in THF/lutidine/H_2O (8:1:1)	1.5:1
2	4% I_2 in THF/lutidine (9:1)	1:8.7
3	*t*-BuOOH/H_2O (7:3), 10% tetrabutyl-ammonium Oxone	21.9:1
4	in CH_3CN, 10% tetrabutyl-ammonium periodate	20.3:1
5	in CH_3CN	4.7:1

[a] Ratio of amide (**24d**):nitrile (**24e**) was determined after deprotection and analysis by reversed-phase HPLC.

(**23e**), or by water to give the expected amide (**23d**). In order to ascertain whether the oxidation could be manipulated to yield either the amide or nitrile preferentially, several reagents were investigated for this reaction (Table 1). Using I_2 in THF/2,6-lutidine/H_2O (Table 1, entry 1), the amide:nitrile ratio was approximately 1.5:1. As expected, in the absence of H_2O (entry 2) the ratio was heavily biased in favor of the nitrile (amide:nitrile 1:8.7). Interestingly, using either *t*-BuOOH[24] or tetrabutylammonium Oxone[25] (Table 1, entries 3 and 4, respectively) gave amide:nitrile ratios of greater than 20:1. Much poorer selectivity was achieved with tetrabutylammonium periodate[26] (Table 1, entry 5). While these reactions have not been extensively optimized, the results indicate that sufficient bias can be introduced into the oxidation step to enable the preparation of oligomers containing either a single 2′-deoxy-2′-*C*-(cyanomethyl)uridine or a 2′-deoxy-2′-*C*-acetamidouridine residue from the same acetamido phosphoramidite **21d**.

Hydrolysis Studies on the Modified Dimers. To ascertain whether the 2′-substituent conferred nuclease resistance to the dimers, their half-lives, relative to UpU, for hydrolysis with SVPD were measured. Hydrolysis was performed at pH 6.5 (see Experimental Section for conditions) in order to completely eliminate background base-catalyzed hydrolysis of UpU.[27] Under these conditions, UpU was hydrolyzed at a rapid but measurable rate (half-life = 5 min). The relative half-lives were found to be 1, 129, 120, 29, 84, and 18 for UpU and **24a**−**e**, respectively. Hydrolysis by SVPD is known to proceed through a covalent phosphoryl intermediate involving an active site threonine residue.[28] On the basis of this mechanism, it is not unexpected that the introduction of substituents that are bulky, in comparison to the hydroxyl group of the wild-type substrate, result in a reduced rate of hydrolysis. However, the observations that (i) the cyanomethyl group is more than 7-fold less stable than the comparably-sized hydroxyethyl substituent and (ii) the hydroxyethyl substituent confers marginally greater nuclease resistance than the larger dihydroxypropyl group suggest that factors other than steric bulk also require consideration. In particular, it is noted that the two dimers containing the alcoholic substituents show the greatest resistance to SVPD-catalyzed hydrolysis. These observations may be useful in the design of 2′-modified antisense oligomers in which nuclease resistance is introduced on the basis of specific functional groups rather than relying on steric bulk, which results in enhanced hydrolytic stability at the expense of hybridization affinity.[29]

The dimers were also tested as substrates for nuclease P1 and ribonuclease A. Once again, the enzymatic digestions were performed at pH 6.5 to eliminate background hydrolysis.[30] None of the modified dimers were substrates for either nuclease P1 (half-life for UpU = 12 min) or ribonuclease A (half-life for UpU = 29 min). The inability of the hydroxyethyl-containing dimer to act as a substrate for ribonuclease A is of interest in light of previous studies. Schmit *et al.* have attributed the very small (3-fold) increase in stability toward 3′-exonucleases present in fetal calf serum, which is observed for oligonucleotides containing 2′-α-CH_2OH substituents to the "RNA-like structure" of this modification, and hence possible attack by RNA degrading enzymes.[10] However, the susceptibility of these substrates to hydrolysis by specific ribonucleases was not examined. The considerable nuclease stability observed for hydroxyethyl and dihydroxypropyl modifications suggests that these analogs may have potential application to the antisense field; however, previous studies have shown that simple 2′-alkyl-substituted oligonucleotides exhibit reduced binding affinity for complementary RNA and DNA due to their predominant 2′-*endo* sugar conformation.[10] The large $J_{1'-2'}$ coupling constants (range 8.8−9.4 Hz) for nucleosides **1**−**4** are also very indicative of a 2′-*endo* conformation,[10] and on this basis, oligonucleotides containing these modified residues are also likely to have a lower affinity for a complementary strand than the corresponding unmodified oligomer.

The hydroxyethyl-containing dimer, which can be considered as a homologue of UpU, was also shown to be very stable to base-catalyzed hydrolysis and underwent no observable decomposition (less than 1%) when treated with 2 M NaOH at 80 °C for 1 h. In comparison, UpU had a half-life of less than 30 min in 0.1 M NaOH at 20 °C. The base-catalyzed hydrolysis of RNA proceeds through the formation of a strained five-membered cyclic phosphate that results from nucleophilic attack of the vicinal hydroxyl group.[31] The resistance of **24a** to base-catalyzed hydrolysis is similar to that observed for deoxyribonucleotide oligomers and is consistent with the fact that the hydroxyl function on the proximal 2′-ethyl side chain is unable to assist hydrolysis.[32] The ineffectual nature of the hydroxyethyl side chain is probably attributable to two major factors. Firstly, base-catalyzed hydrolysis through the classical mechanism would involve the formation of an entropically less favorable 7-membered cyclic phosphate. Secondly, a diminution in the nucleophilicity of the hydroxyl group on the ethyl side chain of **24a** is expected relative to that of the 2′-hydroxyl group of UpU on the basis of their pK_a's. Christensen *et al.*[33] determined the pK_a of the 2′-hydroxyl group of uridine to be 12.59, whereas that of the hydroxyl group on the ethyl side chain is likely to be closer to that of ethanol (15.9). As expected, none of the other modified

(24) Hayakawa, Y.; Uchiyama, M.; Noyori, R. *Tetrahedron Lett.* **1986**, *27*, 4191.
(25) Cosstick, R.; Vyle, J. S. *Nucleic Acids Res.* **1990**, *18*, 829.
(26) Fourrey, J.-L.; Varenne, J. *Tetrahedron Lett.* **1985**, *26*, 1217.
(27) While the pH optimum for snake venom phosphodiesterases is normally reported as 9.0, it is dependent on the nature of the substrate (Bernardi, A.; Bernardi, G. *The Enzymes*; Boyer, P. D., Ed; Academic Press: New York, 1971; Vol. IV, p 329). In general, the enzyme is active over the pH range 6−9, and some substrates are more rapidly hydrolyzed at pH 6.
(28) Culp, J. S.; Butler, L. G. *Arch. Biochem. Biophys.* **1986**, *246*, 245.
(29) Iribarren, A. M.; Sproat, B. S.; Neuner, P.; Sulston, I.; Lamond, A. I. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7747.
(30) The pH optimum for this enzyme is dependent on the substrate, but it is generally used within the pH range 4.0−7.2 (Shishido, K; Ando, T. *Nucleases*; Lin, S. M., Roberts, R. J., Eds.; Cold Spring Harbor: New York, 1983; p 155).
(31) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70.
(32) Dugas, H. *Bioorganic Chemistry*, 2nd Ed.; Springer-Verlag: New York, 1989; Chapter 3.
(33) Christensen, J. J.; Rytting, J. H. *J. Phys. Chem.* **1967**, *71*, 2700.

dimers underwent phosphodiester bond cleavage when incubated in 2 M NaOH at 25 °C over a period of 5 days. However, under these conditions both the amide (**24d**) and nitrile (**24e**) dimers were quantitatively hydrolyzed to the carboxymethyl dimer (**24c**) within 4 days.

The hydroxyethyl-containing dimer was also resistant to acid-catalyzed hydrolysis. Treatment of **24a** with 1.5 M HCl at 50 °C for 42 h resulted in about 30% hydrolysis to give uridine and uracil as the major products. Under identical conditions UpU was completely hydrolyzed within 18 h to give uridine, a mixture of uridine 2′- and 3′-phosphates, and less than 5% uracil (the uracil peak in the HPLC trace was relatively broad and was not reliably integrated).

The hydrolysis of salicyl phosphates has been extensively investigated by Kirby *et al.*[34] Their results have shown that between pH 3 and 6 the presence of the adjacent carboxyl functionality greatly increases the rate of hydrolysis of the neighboring phosphate by acting as an intramolecular general acid catalyst. We were interested to see if the carboxyl functionalized dimer (**24c**) could catalyze the cleavage of the adjacent phosphodiester bond in a similar fashion, and thus, its hydrolysis was compared to UpU at both pH 3 and pH 4. After 4 days at 25 °C, HPLC analysis revealed that at both pH values neither UpU or **24c** had undergone any measurable cleavage of the phosphodiester bond. Even under more forcing conditions (1.5 M HCl, 50 °C, 24 h) no cleavage of the phosphodiester bond (determined by release of uridine) was observed.

Our present studies show that dinucleoside monophosphates containing 2′-*C*- (hydroxyethyl), (carboxymethyl), -acetamido, and (dihydroxypropyl) groups are more resistant to both enzyme- and acid/base-catalyzed hydroysis than the natural substrates. It is anticipated that these nucleoside analogues will ultimately be used in the construction of ribozymes containing enhanced functionality and nuclease resistance. In order to explore the possibility of using *in vitro* selection techniques[35] to investigate the potential of the 2′-*C*-functionalized nucleosides as components in RNA catalysts and aptamers, work is beginning on the synthesis of the 5′-triphosphates of these analogs to ascertain whether they are substrates for RNA polymerases.

Experimental Section

General Procedures. Most of the general analytical procedures have been decribed in an earlier publication.[36] NMR spectra were recorded at the field strength indicated, and peaks displaying multiplicity due to the presence of two diastereomers are denoted with an asterisk. HPLC was conducted using a Varian Star 9010 liquid chromatograph equipped with a Varian Star 9050 variable-wavelength UV detector recording at 260 nm for analytical puposes and 290 nm for preparative work. Unless stated otherwise, analyses were performed on a Nucleosil C_{18} reversed-phase column, using a gradient of 0 → 10% MeCN in 50 mM triethylammonium acetate (pH 6.5) over 20 min, with a flow rate of 1 mL/min. Data were recorded using a Varian 4400 recording integrator.

Hydroquinine 2,5-diphenyl-4,6-pyrimidinediyl diether [$(DHQ)_2$PYR], hydroquinidine 2,5-diphenyl-4,6-pyrimidinediyl diether [$(DHQD)_2$PYR], and 2-cyanoethyl bis(*N,N*-diisopropylamino)phosphoramidite were obtained from Aldrich. Nuclease P1 (*Penicillium citrium*), ribonuclease A (bovine pancreas), uridine 5′-monophosphate, and uridine 2′/3′-monophosphate were purchased from Sigma. Snake venom phosphodiesterase (*Crotalus durissuss*) was purchased from Boehringer Mannheim. 1-(2-Fluorophenyl)-4,4-dimethoxypiperidine was kindly donated by Cruachem, Glasgow, U.K.

2′-Deoxy-2′-α-*C*-(2-oxoethyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxy)uridine (6). 2′-*C*-(2-Propenyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxy)uridine (**5**) (4.31 g, 8.45 mmol) was dissolved in aqueous acetone (45 mL acetone:12 mL water) along with *N*-methylmorpholine *N*-oxide (1.09 g, 9.39 mmol), and to this was added a 1% (w/v) aqueous solution of OsO_4 (10.78 mL, 0.42 mmol). The mixture was stirred vigorously for 1.5 h and then quenched by the addition of saturated aqueous sodium thiosulfate solution (15 mL) and water (15 mL) and diluted with ethyl acetate (100 mL). The mixture was concentrated *in vacuo* to approximately one-third of the volume and rediluted ethyl acetate (100 mL) before being washed with saturated aqueous $NaHCO_3$ solution (2 × 75 mL) and brine (75 mL). The organic layer was dried ($MgSO_4$) and solvent removed *in vacuo*, affording the crude diol as a white foam (4.58 g, 99.6% crude yield). The crude product (4.58 g, 8.42 mmol) was dissolved in aqueous dioxane (90 mL dioxane: 30 mL water), and to this solution was added $NaIO_4$ (3.58 g, 16.83 mmol). The mixture was stirred vigorously for 10 min, diluted with ethyl acetate (200 mL), washed successively with saturated aqueous $NaHCO_3$ solution (2 × 150 mL) and brine (150 mL), dried ($MgSO_4$), and reduced *in vacuo* to a thick colorless oil. The residue was purified by column chromatography (CH_2Cl_2 containing an increasing gradient of MeOH from 0−3%) yielding the pure product as a white foam (3.72 g, 81% yield, two steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00−1.09 (28H, m), 2.66 (1H, dd, *J* = 9.3, 21.2 Hz), 2.93−2.97 (2H, m), 3.93 (1H, ddd, *J* = 3.1, 4.0, 7.2 Hz), 4.03 (1H, dd, *J* = 3.1, 12.9 Hz), 4.09 (1H, dd, *J* = 4.0, 12.9 Hz), 4.56 (1H, t, *J* = 7.2 Hz), 5.74−5.79 (2H, m), 7.67 (1H, d, *J* = 8.1 Hz), 9.81 (1H, s), 10.18 (1H, br s); ^{13}C NMR (50.4 MHz, $CDCl_3$) δ 12.56−13.26, 16.85−17.31, 40.27, 43.43, 61.03, 69.28, 83.83, 88.31, 102.24, 139.07, 150.52, 163.70, 199.61; FAB HRMS *m/z* found (M + H)$^+$ 513.2448, $C_{23}H_{41}N_2O_7Si_2$ requires (M + H)$^+$ 513.2452. Anal. Calcd for $C_{23}H_{40}N_2O_7Si_2$: C, 53.93; H, 7.87; N, 5.47. Found: C, 53.49; H, 7.84; N, 5.38.

2′-Deoxy-2′-α-*C*-(carboxymethyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxy)uridine (7). To a solution of 2′-*C*-(2-oxoethyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxyl)uridine (**6**) (10.32 g, 20.15 mmol) and 2-methyl-2-butene (35 mL, 330 mmol) in 2-methyl-2-propanol (300 mL) was added an aqueous solution of $NaClO_2$ and KH_2PO_4 (27.45 g, 300 mmol, and 30.18 g, 220 mmol, respectively, in 100 mL of water), and the mixture was stirred vigorously for 1 h. The organic solvents were removed *in vacuo* and the resultant residue diluted with ethyl acetate (250 mL), washed with saturated aqueous $NaHCO_3$ (2 × 200 mL), water (200 mL), and brine (200 mL), dried ($MgSO_4$), and evaporated to yield the carboxylic acid as a pure white foam (10.00 g, 94% yield): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.01−1.08 (28H, m), 2.49 (1H, dd, *J* = 12.1, 19.8 Hz), 2.78−2.84 (2H, m), 3.91 (1H, m), 4.05 (2H, m), 4.53 (1H, t, *J* = 7.2 Hz), 5.77 (1H, d, *J* = 8.8 Hz), 5.90 (1H, d, *J* = 2.5 Hz), 7.66 (1H, d, *J* = 8.8 Hz), 10.69 (1H, br s); ^{13}C NMR (50.4 MHz, $CDCl_3$) δ 12.39−13.17, 16.65−17.24, 30.64, 45.30, 61.08, 69.21, 83.55, 88.30, 102.18, 139.48, 150.78, 164.04, 175.15; FAB HRMS *m/z* found (M + H)$^+$ 529.2393, $C_{23}H_{41}N_2O_8Si_2$ requires (M + H)$^+$ 529.2401.

2′-Deoxy-2′-α-*C*-[[(allyloxy)carbonyl]methyl]-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxy)uridine (8). The acid **7** (0.55 g, 0.97 mmol), dicyclohexylcarbodiimide (0.24 g, 1.16 mmol), and 4-(dimethylamino)pyridine (15 mg, 0.12 mmol) were dissolved in dry THF (20 mL), and to this was added allyl alcohol (0.08 mL, 1.14 mmol). The solution was stirred for 16 h, poured onto ether (100 mL), filtered, washed successively with saturated aqueous $NaHCO_3$ (2 × 50 mL), water (50 mL), brine (50 mL), dried ($MgSO_4$), and evaporated *in vacuo*. The resultant oil was purified by column chromatography (75% petroleum ether/25% ethyl acetate), yielding the allyl ester as a white foam (0.47 g, 80% yield): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.01−1.08 (28H, m), 2.56 (1H, dd, *J* = 9.4, 20.0 Hz), 2.61−2.90 (2H, m), 3.92 (1H, m), 4.03 (2H, d, *J* = 3.9 Hz), 4.57 (3H, m), 5.19−5.37 (2H, m), 5.74 (1H, d, *J* = 8.0 Hz),

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(36) Li, X.; Scott; G. K.; Baxter; A. D.; Taylor, R. J.; Vyle, J. S.; Cosstick, R. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2123.

5.81−6.01 (2H, m), 7.58 (1H, d, $J = 8.3$ Hz); ^{13}C NMR (50.4 MHz, $CDCl_3$) δ 12.51−13.21, 16.69−17.34, 31.84, 44.83, 61.26, 65.46, 69.66, 83.81, 87.91, 102.13, 118.52, 131.82, 139.48, 150.34, 164.03, 171.00; FAB HRMS *m/z* found $(M + H)^+$ 569.2728, $C_{26}H_{45}N_2O_8Si_2$ requires $(M + H)^+$ 569.2714.

2′-Deoxy-2′-α-*C*-[[(diphenylmethyl)oxy]carbonyl]methyl]-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxy)uridine (9). The acid **7** (0.40 g, 0.76 mmol) was dissolved in CH_2Cl_2 (20 mL), washed with 1 N HCl (15 mL), and dried ($MgSO_4$), and the solvent was removed *in vacuo*. Diphenyldiazomethane (0.20 g, 1.03 mmol) was placed into the same flask, and acetone was added dropwise (~0.3 mL) until all the solid had dissolved. The mixture was left until bubbling had ceased (4 h), diluted with ethyl acetate (50 mL), washed with saturated aqueous $NaHCO_3$ (2 × 30 mL) and brine (30 mL), dried ($MgSO_4$), and evaporated *in vacuo* to give a purple oil. The oil was purified by column chromatography (CH_2Cl_2 containing an increasing gradient of MeOH from 0−1%) yielding the required ester as a white foam (0.53 g, 75% yield): ^{1}H NMR (400 MHz, $CDCl_3$) 0.92−1.06 (28H, m), 2.61 (1H, dd, $J = 6.2$, 16.9 Hz), 2.76 (1H, m), 2.95 (1H, dd, $J = 7.7$, 16.8 Hz), 3.91 (1H, m), 3.97 (1H, dd, $J = 5.4$, 12.6 Hz), 4.02 (1H, dd, $J = 3.4$, 12.2 Hz), 4.58 (1H, t, $J = 6.3$ Hz), 5.67 (1H, dd, $J = 2.2$, 8.1 Hz), 5.82 (1H, d, $J = 4.9$ Hz), 6.86 (1H, s), 7.32 (10H, m), 7.43 (1H, d, $J = 8.1$ Hz), 8.27 (1H, bs); ^{13}C NMR (50.4 MHz, $CDCl_3$) δ 12.25−13.09, 16.59−17.29, 31.64, 44.41, 61.72, 70.32, 77.13, 84.20, 87.84, 102.28, 126.87, 127.62, 128.27, 139.08, 139.97, 150.21, 163.34, 170.51; FAB HRMS *m/z* found $(M + Na)^+$ 717.3020, $C_{36}H_{50}N_2O_8Si_2Na$ requires $(M + Na)^+$ 717.3003.

2′-Deoxy-2′-α-*C*-acetamido-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxy)uridine (10). The acid **7** (1.02 g, 1.93 mmol), dicyclohexylcarbodiimide (0.51 g, 2.47 mmol), 4-(dimethylamino)pyridine (70 mg, 0.57 mmol), and *N*-hydroxysuccinimide (0.29 g, 2.52 mmol) were dissolved in dry dioxane (26 mL) and the mixture stirred for 16 h. The solution was filtered and the residue washed with dry dioxane (10 mL). To the combined filtrates was added excess concentrated aqueous NH_3 (2.5 mL, 33% solution) and the solution stirred. After 10 min, the reaction mixture was diluted with CH_2Cl_2 (150 mL), washed with saturated aqueous $NaHCO_3$ (2 × 100 mL) and brine (100 mL), dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by column chromatography (CH_2Cl_2 containing an increasing gradient of MeOH from 0−5%), yielding the required product as a white foam (0.80 g, 78% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02−1.09 (28H, m), 2.48 (1H, dd, $J = 10.4$, 17.0 Hz), 2.66−2.75 (2H, m), 3.91 (1H, dd, $J = 7.3$, 11.6 Hz), 3.99 (1H, ddd, $J = 3.3$, 5.0, 7.3 Hz), 4.08 (1H, dd, $J = 3.3$, 11.6 Hz), 4.52 (1H, dd, $J = 5.0$, 7.1 Hz), 5.75 (1H, d, $J = 8.1$ Hz), 5.97 (1H, d, $J = 6.0$ Hz), 6.30 (1H, br s), 7.08 (1H, br s), 7.48 (1H, d, $J = 8.1$ Hz), 10.66 (1H, br s); ^{13}C NMR (50.4 MHz, $CDCl_3$) δ 13.01−13.33, 16.75−17.40, 31.55, 44.73, 62.71, 72.04, 85.17, 87.84, 103.07, 139.03, 151.51, 163.45, 174.09; FAB HRMS *m/z* found $(M + H)^+$ 528.2562, $C_{23}H_{42}N_3O_7Si_2$ requires $(M + H)^+$ 528.2561.

2′-Deoxy-2′-α-*C*-(2-hydroxyethyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxy)uridine (11). Aldehyde **6** (3.0 g, 5.9 mmol) was dissolved in cold methanol (10 mL) and added, dropwise over 5 min with stirring, to a suspension of sodium borohydride (0.664 g, 17.5 mmol) in methanol (20 mL) at 0 °C. After 20 min the reaction mixture was neutralized with citric acid and the solvent evaporated *in vacuo*. The residue was taken up in CH_2Cl_2 (100 mL) and washed with water (2 × 30 mL), saturated sodium bicarbonate solution (2 × 30 mL) and brine (2 × 20 mL). The organic layer was separated, dried ($MgSO_4$), and filtered and the solvent removed *in vacuo* to afford the required product as a white foam (3.06 g, 100% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26−1.00 (28H), 1.68−1.59 (1H, m), 2.17−2.08 (1H, m), 2.50−2.46 (1H, m), 3.96−3.92 (3H, m), 4.40 (1H, dd, $J = 13.4$, 2.5 Hz), 4.24 (1H, d, $J = 13.4$ Hz), 4.43 (1H, dd, $J = 7.4$, 8.9 Hz), 5.73 (1H, d, $J = 8.1$ Hz), 5.88 (1H, s), 7.98 (1H, d, $J = 8.1$ Hz), 10.38 (1H, s); ^{13}C NMR (50.4 MHz; $CDCl_3$) δ 12.48, 12.85, 13.04, 13.34, 16.82, 16.87, 16.96, 17.03, 17.17, 17.28, 17.36, 17.46, 27.79, 46.26, 59.90, 60.25, 67.47, 82.99, 88.98, 101.81, 139.57, 151.22, 164.11; FAB HRMS *m/z* found $(M + H)^+$ 515.2612, $C_{23}H_{43}N_2O_7Si_2$ requires 515.2609. Anal. Calcd for $C_{23}H_{42}O_7Si_2N_2{\cdot}H_2O$: C, 51.85; H, 8.32; N, 5.26. Found: C, 51.58; H, 7.95; N, 5.06.

2′-Deoxy-2′-α-*C*-[2-[[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]oxy]ethyl]]-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxy)uridine (12). Alcohol **11** (1.5 g, 2.92 mmol) and 1-(2-fluorophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine (Fpmp vinyl ether) [1.0 g, 4.8 mmol, prepared from 1-(2-fluorophenyl)-4,4-dimethoxypiperidine as previously described[37]] were dissolved in 50 mL of CH_2Cl_2. A solution of camphor-10-sulfonic acid (450 mg, 1.46 mmol) in CH_2Cl_2 (15 mL) was added dropwise over 15 min and the resulting solution stirred under nitrogen for 16 h. TLC indicated that some starting material was still present; therefore, a further portion of the Fpmp vinyl ether (0.5 g, 2.4 mmol) and camphor-10-sulfonic acid (225 mg, 1.2 mmol) were added to the reaction mixture. After a further 4 h, the mixture was washed with saturated bicarbonate solution (2 × 30 mL) and brine (2 × 20 mL). The organic layer was separated, dried (Na_2SO_4), and filtered and the solvent removed under reduced pressure. The required product was isolated from the resulting residue by column chromatography, eluting with CH_2Cl_2/MeOH (0−2%) to afford **12** as a pale yellow foam (1.52 g, 73%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08−1.01 (28H, m), 1.71−1.69 (1H, m), 1.93 (4H, br s), 2.31−2.26 (1H, m), 2.36-2.32 (1H, m), 3.06 (4H, br s), 3.21 (3H, s), 3.67 (2H, m), 3.94 (1H, m), 4.00 (1H, d, J=13.1 Hz), 4.13 (1H, d, $J = 13.1$ Hz), 4.45 (1H, t, $J = 7.1$ Hz), 5.69 (1H, d, $J = 8.1$ Hz), 5.83 (1H, s), 7.02−6.95 (4H, m), 7.80 (1H, d, $J = 8.1$ Hz), 10.14 (1H, s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 11.20, 11.72, 12.08, 15.70, 15.62, 15.86, 15.96, 25.13, 32.97, 45.34, 47.38, 47.76, 57.49, 60.40, 68.46, 83.06, 89.07, 97.23, 101.65, 115.85 (d, $J = 21.0$ Hz), 119.33, 122.17, 124.25, 139.60, 140.35, 150.28, 154.85 (d, $J = 245.5$ Hz), 163.93; FAB HRMS *m/z* found M^+ 721.3599, $C_{35}H_{56}N_3O_8FSi_2$ requires M^+ 721.3590.

2′-Deoxy-2′-α-*C*-(2,3-dihydroxypropyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxy)uridine (13) (Mixture of Diastereoisomers). $(DHQD)_2PYR$ (28 mg, 0.032 mmol), K_2CO_3 (1.321 g, 9.6 mmol) and $K_2Fe(CN)_6$ (3.150 g, 9.6 mmol), were dissolved in 2-methyl-2-propanol (20 mL) and distilled water (20 mL) and the mixture cooled to 0 °C. Osmium tetraoxide (8.1 mg, 0.032 mmol, as a 1% solution in water) and 2′-*C*-allyl nucleoside **5** (1.628 g, 3.2 mmol) were added to the mixture with vigorous stirring. After 1.5 h, ethyl acetate (3 × 50 mL) was added to the reaction mixture and the volume of solvent reduced under reduced pressure to give a biphasic mixture. Saturated sodium thiosulfate (2 × 15 mL) was added and the mixture shaken vigorously. The organic layer was separated and washed with saturated sodium bicarbonate (2 × 20 mL) and brine (2 × 20 mL), dried ($MgSO_4$), and filtered and the solvent removed under reduced pressure to give a pale yellow oil. Column chromatography over silica gel eluting with CH_2Cl_2 and methanol (0−4%) afforded the required diol as a white foam 1.50 g (86% yield). Approximately 1 mg of this material was treated with tetrabutylammonium fluoride (1 mL). HPLC of the resulting solution indicated that the reaction gave a 82:18 mixture of diastereoisomers (retention times of 9.56 and 10.01 min for the major and minor diastereoisomers, respectively) ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.22−0.77 (28H, m), 1.57−1.32 (1 H, m), 2.44−2.31 (1 H, m), 2.75−2.60 (1 H, m), 3.75−3.45 (2H, m), 4.14−3.85 (2H, m), 4.32−4.21 (2H, m), 4.39 (1 H, m), 5.71 (1 H, d, $J = 8.1$ Hz), 5.78 (1 H, s), 7.96 (1 H, d, $J = 8.1$ Hz), 9.83 (1 H, s); ^{13}C NMR (50.4 MHz, $CDCl_3$) δ 12.42, 12.88, 13.05, 13.37, 16.84, 16.92, 16.98, 17.06, 17.19, 17.30, 17.38, 17.49, 28.22, 44.77, 59.76, 67.17, 67.39, 68.63, 83.05, 88.93, 102.08, 139.58, 151.57, 164.03; CI HRMS *m/z* found $(M + H)^+$ 545.2713, $C_{24}H_{45}N_2O_8Si_2$ requires $(M + H)^+$ 545.2714.

2′-Deoxy-2′-α-*C*-(2,3-diacetoxypropyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxy)uridine (14). Diol **13** (1.00 g, 1.8 mmol) was dried by coevaporation with distilled pyridine (3 × 30 mL) and dissolved in pyridine (40 mL). The solution was cooled to −5 °C and treated with 3,5-dinitrobenzoyl chloride (0.610 g, 2.6 mol) under an atmosphere of nitrogen. After 2 h the reaction was quenched by addition of methanol (10 mL) and the solvent removed under reduced pressure. The residue was coevaporated with toluene (3 × 15 mL) and dissolved in CH_2Cl_2 (40 mL). The resulting solution was washed with saturated sodium bicarbonate (2 × 20 mL) and brine (2 × 20

(37) Reese, C. B.; Thompson, E. A. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2881.

mL). The organic layer was separated, dried ($MgSO_4$), and filtered and the solvent removed to give an orange oil. The crude product was recrystallized (pentane/ethyl acetate) to afford the 3,5-dinitrobenzoate monoester (713 mg, 64%, based on starting material being only 82% of one diastereoisomer). [Approximately 1 mg of this material was treated with methanolic ammonia (1 mL). After 1 h the solvent was evaporated and the residue treated with tetrabutlyammonium fluoride (1 mL); HPLC analysis of the resulting solution showed the product to be a single diastereoisomer.] The dinitrobenzoate (685 mg, 0.93 mmol) was dissolved in saturated methanolic ammonia (20 mL). After 16 h the solvent was evaporated and the residue coevaporated with dry pyridine (3 × 20 mL). The resulting black residue was dissolved in pyridine (20 mL) and treated with acetic anhydride (1.73 mL, 18.6 mmol). After 20 h the solvent was evaporated, and traces of pyridine were removed by coevaporation with toluene (3 × 30 mL). The residue was taken up in CH_2Cl_2 (30 mL), washed with saturated sodium bicarbonate (2 × 20 mL) and brine (2 × 20 mL), dried ($MgSO_4$), and filtered and the solvent removed under reduced pressure. Column chromatography over silica gel eluting with CH_2Cl_2 and methanol (0–2%) afforded the required product **14** as a white foam (573 mg, 98% yield, 52% from diol **13**): ^1H NMR (400 MHz, $CDCl_3$) δ 1.10–1.03 (28H, m), 1.77–1.70 (1H, m), 2.03 (3H, s), 2.07 (3H, s), 2.23–2.17 (1H, m), 2.35–2.32 (1H, m), 3.93–3.87 (1H, m), 3.99 (1H, dd, *J* = 2.88, 13.2 Hz), 4.13 (1H, dd, *J* = 2.5, 13.2 Hz), 4.18 (1H, dd, *J* = 5.1, 12.0 Hz), 4.29 (1H, dd, *J* = 3.7 Hz, 12.0), 4.46 (1H, t, *J* = 7.7 Hz), 5.34–5.29 (1H, m), 5.69 (1H, dd, *J* = 2.1, 8.2 Hz), 5.77 (1H, d, *J* = 2.1 Hz), 7.74 (1H, d, *J* = 8.1 Hz), 9.08 (1H, s); ^{13}C NMR (50.4 MHz, $CDCl_3$) δ 12.30, 12.68, 12.74, 13.08, 16.56, 16.61, 16.67, 16.72, 16.91, 16.99, 17.02, 17.15, 20.34, 20.60, 26.80, 44.11, 60.34, 64.47, 68.54, 69.29, 82.96, 88.70, 101.64, 139.05, 149.94, 163.34, 169.97, 170.40; CI HRMS *m/z* found (M + H)$^+$ 629.2920, $C_{28}H_{49}N_2O_{10}Si_2$ requires (M + H)$^+$ 629.2926.

2′-Deoxy-2′-α-*C*-(cyanomethyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxy)uridine (25). To a stirred solution of 2′-*C*-acetamido-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxy)uridine (**10**) (130 mg, 0.247 mmol) in dry dioxane (2 mL) at 0 °C was added dry pyridine (0.04 mL, 0.494 mmol) followed by dropwise addition of trifluoroacetic anhydride (0.04 mL, 0.288 mmol) over 10 min. The reaction mixture was left to warm to room temperature over 30 min and then diluted with CH_2Cl_2 (30 mL), washed with water (2 × 20 mL) and brine (20 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Following column chromatography (CH_2Cl_2 containing an increasing gradient of MeOH from 0–3%), the pure product was isolated as a white foam (96 mg, 76% yield): ^1H NMR (400 MHz, $CDCl_3$) δ 1.05–1.09 (28H, m), 2.63 (1H, m), 2.71 (1H, dd, *J* = 5.4, 16.9 Hz), 2.81 (1H, dd, *J* = 7.8, 16.9 Hz), 4.02–4.12 (3H, m), 4.58 (1H, t, *J* = 7.8 Hz), 5.75 (1H, d, *J* = 8.2 Hz), 5.80 (1H, d, *J* = 2.7 Hz), 7.67 (1H, d, *J* = 8.2 Hz), 9.73 (1H, br s); ^{13}C NMR (50.4 MHz, $CDCl_3$) δ 12.52–13.25, 14.65, 16.70–17.32, 45.33, 60.50, 68.54, 83.28, 88.11, 102.28, 117.84, 138.81, 150.35, 163.55; found FAB HRMS *m/z* found (M + H)$^+$ 510.2454, $C_{23}H_{40}N_3O_6Si_2$ requires (M + H)$^+$ 510.2456.

General Procedure for the Removal of the 1,1,3,3-Tetraisopropyldisiloxy Protecting Group. The TIPS-protected nucleoside (0.45 mmol) was dissolved in dry THF (30 mL), and to this was added $NEt_3 \cdot 3HF$ (0.08 mL, 5 mmol). The solution was left for 8–16 h and monitored by TLC. The solution was concentrated *in vacuo*, diluted with methanol (20 mL), and evaporated onto silica gel; the product was subsequently isolated by column chromatography (CH_2Cl_2 containing an increasing gradient of MeOH from 0–10%) as a colorless oil or white foam.

2′-Deoxy-2′-α-*C*-(carboxymethyl)uridine triethylammonium salt (1): 85% yield; ^1H NMR (400 MHz, CD_3OD) δ 1.30 (9H, t, *J* = 7.2 Hz), 2.31 (1H, dd, *J* = 5.8, 15.5 Hz), 2.62 (1H, dd, *J* = 7.9, 15.5 Hz), 2.70 (1H, m), 3.17 (6H, q, *J* = 7.2 Hz), 3.75 (2H, d, *J* = 3.5 Hz), 4.00 (1H, m), 4.35 (1H, m), 5.73 (1H, dd, *J* = 8.1, 2.2 Hz), 6.04 (1H, d, *J* = 8.8 Hz) 7.98 (1H, d, *J* = 8.1 Hz); ^{13}C NMR (50.4 MHz, CD_3OD) δ 9.34, 33.10, 47.34, 47.70, 63.85, 74.62, 89.00, 89.87, 103.27, 143.07, 152.81, 166.24, 178.59; FAB$^+$ *m/z* 287 (M + H$^+$, 3.5), 269 [M + H − H_2O^+ (lactone)]; HPLC retention time 9.41 min. This compound was formally characterized by conversion to **uridine 2′-α-*C*-3′-*O*-γ-butyrolactone (19)**. 2′-Deoxy-2′-α-*C*-carboxymethyluridine (292 mg, 1.09 mmol) was dissolved in acetic acid/methanol (4:1; 29.2 mL) and heated at 60 °C for 3 h. The solution was evaporated *in vacuo*, and the residual acetic acid was coevaporated with water (2 × 30 mL) and dried over P_2O_5 for 48 h. The product was isolated as a pure white crystalline solid (273 mg, 96% yield): mp 221–222 °C. ^1H NMR (400 MHz, d_6-DMSO) 2.64 (1H, dd, *J* = 2.3, 18.2 Hz), 2.92 (1H, dd, *J* = 9.2, 18.2 Hz), 3.17 (1H, m), 3.65 (3H, d, *J* = 3.9 Hz), 4.22 (1H, m), 5.04 (1H, dd, *J* = 2.4, 7.3 Hz), 5.22 (1H, t, *J* = 5.1 Hz), 5.68 (1H, d, *J* = 8.0 Hz), 5.86 (1H, d, *J* = 6.4 Hz), 7.83 (1H, d, *J* = 8.0 Hz), 11.39 (1H, bs); ^{13}C NMR (100.6 MHz, d_6-DMSO) 31.75, 44.76, 61.02, 74.87, 84.08, 84.73, 90.24, 101.96, 140.51, 150.63, 163.16, 175.67; HRMS *m/z* found (M + H)$^+$ 269.0754, $C_{11}H_{13}N_2O_6$ requires (M + H)$^+$ 269.0774; IR ν_{max}/cm^{-1} (KBr) 1780 (C=O lactone). Anal. Calcd for $C_{11}H_{12}N_2O_6$: C, 49.26; H, 4.51; N, 10.44. Found: C, 49.07; H, 4.55; N, 10.39.

2′-Deoxy-2′-α-*C*-acetamidouridine (2): 78% yield; ^1H NMR (400 MHz, d_6-DMSO) δ 2.10 (1H, dd, *J* = 6.1, 15.7 Hz), 2.43 (1H, dd, *J* = 7.7, 15.7 Hz), 2.58 (1H, m), 3.55 (2H, d, *J* = 3.8 Hz), 3.85 (1H, dd, *J* = 3.1, 3.8 Hz), 4.15 (1H, d, *J* = 5.3 Hz), 5.11 (1H, br s), 5.35 (1H, br s), 5.67 (1H, d, *J* = 8.2 Hz), 5.91 (1H, d, *J* = 9.4 Hz), 6.82 (1H, s), 7.34 (1H, s), 7.84 (1H, d, *J* = 8.2 Hz); ^{13}C NMR (100.6 MHz, CD_3OD) δ 33.68, 46.87, 63.79, 74.56, 89.28, 89.68, 103.46, 142.96, 152.95, 166.36, 176.80; FAB HRMS *m/z* found (M + H)$^+$ 286.1031, $C_{11}H_{16}N_3O_6$ requires (M + H)$^+$ 286.1039; HPLC retention time 9.19 min.

2′-Deoxy-2′-α-*C*-(2-hydroxyethyl)uridine (3): 88% yield; ^1H NMR (400 MHz, D_3COD) δ 1.64–1.51 (1H, m), 2.01–1.84 (1H, m), 2.48–2.33 (1H, m), 3.60 (2H, t, *J* = 6.3 Hz), 3.73 (2H, d, *J* = 3.6 Hz), 3.99–3.97 (1H, m), 4.29 (1H, d, *J* = 5.4 Hz), 5.72 (1H, d, *J* = 8.1 Hz), 6.04 (1H, d *J* = 9.2 Hz), 7.97 (1H, d, *J* = 8.1 Hz); ^{13}C NMR (50.4 MHz, D_3COD) δ 28.03, 47.89, 60.86, 63.56, 73.77, 88.89, 89.84, 103.18, 142.59, 152.69, 165.96; FAB HRMS *m/z* found (M + 2H)$^+$ 274.1156, $C_{11}H_{18}N_2O_6$ requires (M + 2H)$^+$ 274.1165; HPLC retention time 10.64 min.

2′-Deoxy-2′-α-*C*-(2,3-dihydroxypropyl)uridine (4): 80% yield; ^1H NMR (200 MHz, CD_3OD) 1.42–1.74 (2H, m), 2.31–2.45 (1H, m), 3.33 (2H, s), 3.44–3.56 (1H, m), 3.62 (1H, s), 3.64 (1H, s), 3.87–3.90 (1H, m), 4.23 (1H, dd, *J* = 1.5 Hz, *J'* = 4.8 Hz), 5.62 (1H, d, *J* = 8.1 Hz), 5.92 (1H, d, *J* = 8.9 Hz), 7.87 (1H, d, *J* = 8.1 Hz); ^{13}C NMR (50.4 MHz, CD_3OD) 28.89, 47.24, 63.53, 67.51, 71.61, 74.58, 88.30, 89.90, 103.18, 142.64, 152.67, 165.96; FAB HRMS *m/z* found (M + Na)$^+$ 325.1018, $C_{12}H_{18}N_2O_7Na$ requires 325.1012; HPLC retention time 10.01 min. Pure samples of **4** were most readily obtained by deprotection of **20b**.

2′-Deoxy-2′-α-*C*-(*O*-diphenylmethyl carboxymethyl)-uridine (16): 87% yield; ^1H NMR (400 MHz, $CDCl_3$) δ 3.09 (1H, dd, *J* = 5.9, 15.9 Hz), 3.26 (1H, m), 3.37 (1H, dd, *J* = 6.9, 15.9 Hz), 4.22 (2H, m), 4.47 (1H, m), 4.82 (1H, dd, *J* = 1.4, 5.5 Hz), 6.10 (1H, d, *J* = 8.1 Hz), 6.53 (1H, d, *J* = 8.5 Hz), 7.24 (1H, s), 7.72 (10H, m), 8.37 (1H, d, *J* = 8.1 Hz); ^{13}C NMR (50.4 MHz, CD_3OD) δ 30.78, 46.03, 63.34, 73.99, 78.71, 88.92, 89.54, 103.13, 127.98, 128.87, 129.49, 141.59, 142.43, 152.59, 165.96, 172.59; FAB HRMS *m/z* found (M + H)$^+$ 453.1630, $C_{24}H_{25}N_2O_7$ requires (M + H)$^+$ 453.1662.

2′-Deoxy-2′-α-*C*-[2-[[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]oxy]ethyl]uridine (17): 76% yield; ^1H NMR (400 MHz, $CDCl_3$) δ 1.60–1.59 (1H, m), 2.05–1.82 (5H, m), 2.47–2.38 (1H, m), 3.10–2.93 (4H, m), 3.11 (3H, s), 3.45–3.43 (1H, m), 3.72–3.61 (2H, m), 3.80 (1H, d, *J* = 11.5 Hz, 3.91 (1H, d, *J* = 11.5 Hz), 4.46 (1H, d, *J* = 5.1 Hz), 5.76 (1H, d, *J* = 8.1 Hz), 5.93 (1H, d, *J* = 9.0 Hz), 7.05–6.91 (4H, m), 7.73 (1H, d, *J* = 8.1 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 24.46, 32.81, 33.42, 47.98, 49.27, 58.88, 63.30, 73.43, 86.34, 90.35, 99.01, 102.92, 116.90 (d, *J* = 21.1 Hz), 119.50, 122.70 (d, *J* = 8.0 Hz), 124.46 (d, *J* = 3.0 Hz), 139.99 (d, *J* = 9.1 Hz), 141.31, 150.78, 155.79 (d, *J* = 245.5 Hz), 163.43; FAB HRMS *m/z* found M$^+$ 479.2036, $C_{23}H_{30}N_3O_7F$ requires M$^+$ 479.2068.

2′-Deoxy-2′-α-*C*-(2,3-diacetoxypropyl)uridine (18): 81% yield; ^1H NMR (400 MHz, CD_3OD) δ 1.84–1.75 (1H, m), 1.97 (3H, s), 2.00 (3H, s), 2.12–2.03 (1H, m), 2.42–2.40 (1H, m), 3.73 (1H, s), 3.74 (1H, s), 4.02–3.97 (3H, m), 4.28–4.24 (2H, m), 5.72 (1H, dd, *J* = 8.1, 0.5 Hz), 6.06 (1H, d, *J* = 9.1 Hz), 7.96 (1H, d, *J* = 8.1 Hz); ^{13}C NMR (50.4 MHz, $CDCl_3$) δ 20.42,

20.78, 26.57, 45.47, 63.31, 65.76, 70.75, 74.50, 88.76, 89.62, 103.45, 142.18, 152.48, 165.77, 171.92, 172.27; CI HRMS *m/z* found $(M + H)^+$ 387.1404, $C_{16}H_{23}N_2O_9$ requires $(M + H)^+$ 387.1403.

2′-Deoxy-2′-α-*C*-(cyanomethyl)uridine (26): 81% yield; ^{1}H NMR (200 MHz, CD_3OD) δ 2.53−2.81 (3H, m), 3.71 (2H, d, *J* = 3.0 Hz), 3.98 (1H, m), 4.29 (1H, dd, *J* = 1.7 Hz, *J* = 3.3 Hz), 5.70 (1H, d, *J* = 8.1 Hz), 6.02 (1H, d, *J* = 8.5 Hz), 7.92 (1H, d, *J* = 8.1 Hz); ^{13}C NMR (50.4 MHz, CD_3OD) δ 13.69, 46.68, 63.12, 73.50, 88.87, 89.03, 103.45, 119.57, 142.08, 152.64, 165.99; FAB HRMS *m/z* found $(M + H)^+$ 268.0933, $C_{11}H_{14}N_3O_5$ requires $(M + H)^+$ 268.0934; HPLC retention time 13.47 min.

General Method for the Preparation of Nucleoside 5′-*O*-dimEthoxytrityl Ethers. The nucleoside (0.30 mmol) was dried by coevaporation with dry pyridine (2 × 3 mL) and dissolved in the same solvent (1.3 mL). Dimethoxytrityl chloride (170 mg, 0.50 mmol) was dissolved in a mixture of dry pyridine and dichloromethane (0.2 and 0.75 mL, respectively) and added to the nucleoside solution dropwise over 30 min, and the mixture was stirred for a further 1 h. Methanol (0.1 mL) was added, and after a further 15 min saturated aqueous $NaHCO_3$ (0.5 mL) was also added; the mixture was then concentrated *in vacuo* to a thick oil. The residue was coevaporated with toluene (2 × 20 mL), diluted with CH_2Cl_2 (5 mL), and washed with saturated aqueous $NaHCO_3$ (2 × 2.5 mL) and brine (2.5 mL), the aqueous layers were combined and back-extracted with CH_2Cl_2 (5 mL), and the organics were dried ($MgSO_4$) and evaporated. The crude product was purified by column chromatography (CH_2Cl_2 containing an increasing gradient of MeOH from 0 to 3%) to yield the required DMT derivative as a white/cream foam.

2′-Deoxy-2′-α-*C*-[2-[[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]oxy]ethyl]-5′-*O*-(dimethoxytrityl)uridine (20a): 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.79−1.89 (1H, m), 1.91−2.18 (5H, m), 2.33−2.45 (1H, m), 3.12−3.02 (4H, m), 3.24 (3H, s), 3.43−3.53 (2H, m), 3.58−3.46 (1H, m), 3.76−3.74 (8H, m), 4.19 (1H, s), 4.58 (1H, d, *J* = 4.9 Hz), 5.43 (1H, d, *J* = 8.1 Hz), 6.13 (1H, d, *J* = 8.8 Hz), 7.02−6.79 (8H, m), 7.19−7.36 (9H, m), 7.75 (1H, d, *J* = 8.1 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 22.65, 24.29, 31.58, 33.03, 33.32, 47.84, 47.93, 50.38, 52.82, 55.20, 58.99, 64.26, 73.71, 85.39, 85.93, 88.27, 98.99, 102.79, 98.99, 102.79, 113.28, 116.03 (d, *J* = 21.1 Hz), 119.38 (d, *J* = 2.0 Hz), 122.48 (d, *J* = 8.0 Hz), 124.45 (d, *J* = 3.0 Hz), 127.13, 127.98, 128.05, 130.05, 135.25, 135.40, 139.95 (d, *J* = 9.1 Hz), 140.30, 144.44, 150.94, 154.48, 157.81 (d, *J* = 245.5 Hz), 163.47; FAB HRMS *m/z* found M^+ 781.3383, $C_{44}H_{48}N_3O_9F$ requires M^+ 781.3375.

2′-Deoxy-2′-α-*C*-(2,3-diacetoxypropyl)-5′-*O*-(dimethoxytrityl)uridine (20b): 94% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89−1.70 (1H, m), 2.20−1.97 (7H, m), 2.49−2.34 (1H, m), 3.41 (2H, s), 3.75 (6H, s), 4.32−4.03 (4H, m), 4.48 (1H, d, *J* = 6.0), 5.07−4.88 (1H, br s), 5.36 (1H, d, *J* = 8.0 Hz), 6.07 (1H, d, *J* = 8.5 Hz), 7.39−6.76 (13H, m), 7.68 (1H, d, *J* = 8.0 Hz); ^{13}C NMR (50.4 MHz, $CDCl_3$) δ 20.39, 20.79, 22.36, 24.92, 45.83, 46.32, 54.98, 63.82, 64.06, 70.56, 73.04, 85.76, 86.89, 87.53, 102.61, 113.09, 126.92, 127.75, 127.89, 129.86, 134.90, 135.06, 139.88, 143.98, 150.67, 158.51, 163.06, 170.48, 170.83; CI HRMS *m/z* found M^+ 688.2614, $C_{37}H_{40}N_2O_{11}$ requires 688.2632.

2′-Deoxy-2′-α-*C*-[[[(diphenylmethyl)oxy]carbonyl]methyl]-5′-*O*-(dimethoxytrityl)uridine (20c): 86% yield: ^{1}H NMR (400 MHz, $CDCl_3$) 2.62−2.77 (2H, m), 2.94 (1H, dd, *J* = 11.5, 17.6 Hz), 3.40 (2H, m), 3.79 (3H, s), 4.10 (1H, m), 4.54 (1H, dd, *J* = 2.2, 5.2 Hz), 5.39 (1H, dd, *J* = 2.2, 8.2 Hz), 6.05 (1H, d, *J* = 8.1 Hz), 6.82 (4H, d, *J* = 8.7 Hz), 6.88 (1H, s), 7.24−7.35 (19H, m), 7.64 (1H, d, *J* = 8.2 Hz), 8.20 (1H, bs); ^{13}C NMR (75.5 MHz, $CDCl_3$) 29.88, 45.96, 55.12, 63.42, 72.98, 77.95, 85.65, 86.96, 87.64, 102.80, 113.31, 126.91, 127.18, 128.01, 128.17, 128.64, 130.11, 134.78, 139.44, 139.63, 144.10, 150.31, 158.51, 162.23, 171.12; FAB HRMS *m*/*z* found M^+ 754.2897, $C_{45}H_{42}N_2O_9$ requires M^+ 754.2890.

2′-Deoxy-2′-α-*C*-acetamido-5′-*O*-(dimethoxytrityl)uridine (20d): 56% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.56 (1H, m), 2.69 (1H, m), 3.37 (2H, br s), 3.74 (3H, s), 4.15 (1H, br s), 4.46 (1H, br s), 5.40 (1H, d, *J* = 8.1 Hz), 6.07 (1H, d, *J* = 7.6 Hz), 6.58 (1H, br s), 6.81 (4H, d, *J* = 8.8 Hz), 6.93 (1H, br s), 7.17−7.39 (9H, m), 7.65 (1H, d, *J* = 8.1 Hz), 8.34 (1H, br s); ^{13}C NMR (100.6 MHz, CD_3OD) δ 31.83, 47.40, 55.89, 64.47, 74.06, 86.59, 87.49, 88.82, 103.57, 112.67, 113.95, 127.71, 128.65, 128.82, 130.76, 135.96, 136.14, 141.02, 144.98, 152.36, 159.26, 164.45, 175.74; FAB HRMS *m/z* found $(M + H)^+$ 588.2330, $C_{32}H_{34}N_3O_8$ requires $(M + H)^+$ 588.2346.

General Method for the Preparation of Nucleoside 3′-[(2-Cyanoethyl)-*N,N*-diisopropylphosphoramidites). The 5′-*O*-tritylated nucleoside (0.613 mmol) and diisopropylammonium tetrazolide (53 mg, 0.307 mmol) were dissolved in dry CH_2Cl_2 and the solution purged with nitrogen. To this was added 2-cyanoethyl bis(*N,N*-diisopropylamino)phosphoramidite (0.22 mL, 0.674 mmol) and the reaction left for 16 h. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous $NaHCO_3$ (3 × 6 mL) and brine (6 mL), the aqueous layers were combined and back-extracted with CH_2Cl_2 (2 × 2 mL), and the organics were dried (Na_2SO_4) and evaporated. The crude residue was purified by column chromatography, the eluent system depending on the polarity of the compound, to yield the product, a mixture of diastereoisomers, as a white foam.

2′-Deoxy-2′-α-*C*-[2-[[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]oxy]ethyl]-5′-*O*-(dimethoxytrityl)uridine 3′-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) (21a): eluent, hexane/CH_2Cl_2/NEt_3 (10:9:1); yield 70%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.23−0.89 (12H, m), 2.20−1.89 (7H, m), 2.34−2.21 (1H, m), 2.71−2.82 (1H, m), 3.15−2.89 (4H, m), 3.18*, 3.20* (3H, s), 3.75−3.27 (9H, m), 3.77 (6H, s), 4.73−4.52 (1H, m), 5.35*, 5.40* (1H, d, *J* = 8.8 Hz, *J′* = 9.0 Hz), 6.10*, 6.15* (1H, d, *J* = 8.8 Hz, *J′* = 9.0 Hz), 7.40−6.79 (17H, m), 7.80*, 7.86* (1H, d, *J* = 8.1 Hz, *J′* = 8.1 Hz); ^{31}P NMR (25 MHz, $CDCl_3$) δ 148.64, 151.47; *m/z* (FAB$^+$) 981 (M^+, 100).

2′-Deoxy-2′-α-*C*-(2,3-diacetoxypropyl)-5′-*O*-(dimethoxytrityl)uridine 3′-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) (21b): eluent, hexane/CH_2Cl_2/NEt_3 (10:9:1); yield 73%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30−1.03 (12H, m), 1.83−1.65 (1H, m), 1.95*, 1.97* (3H, s), 1.99*, 2.01* (3H, s), 2.78−2.27 (4H, m), 3.71−3.35 (6H, m), 3.76 (6H, s), 4.36−3.90 (4H, m), 4.63−4.38 (1H, m), 5.30*, 5.49* (1H, d, *J* = 8.1 Hz, *J′* = 8.1 Hz), 6.06*, 6.10* (1H, d, *J* = 6.0 Hz, *J″* = 6.1 Hz), 7.38−6.76 (13H, m), 7.68*, 7.73* (1H, d, *J* = 8.1 Hz, *J′* = 8.1); ^{31}P NMR (101 MHz, $CDCl_3$) δ 147.00, 149.11; CI HRMS *m/z* found $(M + H)^+$ 889.3756, $C_{46}H_{58}N_4O_{12}P$ requires 889.3789.

2′-Deoxy-2′-α-*C*-[[[(diphenylmethyl)oxy]carbonyl]methyl]-5′-*O*-(dimethoxytrityl)uridine 3′-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) (21c): eluent, hexane/EtOAc/NEt_3 (10:9:1); 94% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.06−1.16 (12H, m), 2.20−2.35 (2H, m), 2.58 (1H, m), 2.87−3.00 (2H, m), 3.40−3.70 (6H, m), 3.78 (6H, s), 4.11−4.34 (1H, m), 4.63*, 4.76* (1H, dd, *J* = 5.0, 9.6, 4.0, 11.6 Hz), 5.35*, 5.39* (1H, d, *J* = 8.4, 6.6 Hz), 6.07*, 6.11* (1H, d, *J* = 8.8, 5.9 Hz), 6.80−6.85 (4H, m), 6.88*, 6.94* (1H, s), 7.24−7.42 (19H, m,), 7.59*, 7.64* (1H, d, *J* = 8.2, 8.2 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 150.01, 151.27; FAB$^+$ *m/z* 977 (M + Na$^+$, 3.3), 955 (M + H$^+$, 9.6).

2′-Deoxy-2′-α-*C*-acetamido-5′-*O*-(dimethoxytrityl)uridine 3′-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) (21d): eluent, EtOAc/CH_2Cl_2/NEt_3 (9:9:2); 29% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.12−1.26 (12H, m), 1.95−2.35 (2H, m), 2.40−3.09 (3H, m), 3.37−3.65 (6H, m), 3.80 (6H, s), 4.26*, 4.39* (1H, m), 4.44*, 4.54* (1H, dd, *J* = 8.2, 14.0, 7.6, 15.7 Hz), 5.45*, 5.46* (1H, d, *J* = 7.9, 6.0 Hz), 5.68*, 5.96* (1H, br s), 6.15*, 6.17* (1H, d, *J* = 9.5, 9.9 Hz), 6.72*, 6.84−6.86 (5H, br s, m), 7.27−7.43 (9H, m), 7.62*, 7.70* (1H, d, *J* = 8.1, 8.2 Hz); ^{31}P NMR (81 MHz, D_2O) δ 152.27, 152.77; *m/z* FAB+ *m*/*z* 826 (M + Na + O$^+$, 6.0), 810 (M + Na$^+$, 1.6).

General Method for the Preparation of the 2′-Modified Dinucleoside Monophosphates. 2′,3′-Di-*O*-acetyluridine[38] (22 mg, 0.067 mmol) and freshly sublimed tetrazole (23 mg, 0.329 mmol) were dissolved in dry CH_3CN and purged with argon, and the mixture was stirred to effect solution. To this an argon-purged solution of the nucleoside phosphoramidite (0.100 mmol) in dry CH_3CN (2 mL) was added dropwise over 10 min. After being stirred for a further 50 min, the reaction mixture was analyzed by ^{31}P NMR. A 4% (w/v) solution of I_2

(38) Kenner, G. W.; Todd, A. R.; Webb, R. F.; Weymouth, F. J. *J. Chem. Soc.* **1954**, 2288.

in 2,6-lutidine/water/THF (1:1:8) was then added dropwise to the mixture until a deep red color persisted. The solution was diluted with CH_2Cl_2 (30 mL) and washed with 10% $Na_2S_2O_4$ solution (2 × 20 mL) and brine (20 mL), the aqueous layers were combined and back-extracted with CH_2Cl_2 (15 mL), and the organics were dried ($MgSO_4$) and evaporated *in vacuo*. The crude product was purified by column chromatography (CH_2Cl_2 containing an increasing gradient of MeOH from 0−3%) to yield the required dinucleoside monophosphate, which was immediately deprotected.

Deprotection of the Dinucleoside Monophosphates: (i) General Procedure for the Removal of Acetyl, 2-Cyanoethyl, and DMT Protecting Groups. The protected dinucleoside monophosphate (∼0.02 mmol) was dissolved in saturated methanolic NH_3 (10 mL) and left overnight. Solvent was removed *in vacuo* and the residue coevaporated twice with methanol (2 × 10 mL). The dimer was subsequently taken up into 80% aqueous acetic acid and left for a further 2 h before solvent was again removed *in vacuo* and the residue coevaporated twice with water (2 × 10 mL). The product was dissolved in water (10 mL), and extracted with EtOAc (3 × 10 mL) and the aqueous layer evaporated *in vacuo* to yield the crude product as a colorless oil.

(ii) Removal of the Fpmp Protecting Group. Following treatment with methanolic ammonia, the dimer was taken up into acetate buffer (1 mL, pH 3.25, 100 mM, supplied by Cruachem, Glasgow, U.K.) and stirred at room temperature for 5 h. After this period, glacial acetic acid (1.5 mL) was added and the suspension stirred for a further 1 h. The solvent was evaporated and the residue coevaporated and extracted as described above.

(iii) Removal of the Benzhydryl Protecting Group. Following treatment with methanolic ammonia and 80% aqueous acetic acid, the residue was taken up into MeOH/THF (3:1; 1 mL), and Pd/C catalyst was suspended in the solution by vigorous stirring. The reaction vessel was evacuated and purged with H_2 three times and the mixture left for 1.5 h. The solution was diluted with MeOH/THF (3:1, 3 mL), filtered through Celite, and evaporated to dryness *in vacuo*.

Purification of the deprotected dinucleoside monophosphates was carried out by HPLC on a Nucleosil C_{18} reversed-phase column, using a gradient of 0 → 10% MeCN in 50 mM triethylammonium bicarbonate (pH 8.0) over 20 min, with a flow rate of 1 mL/min.

2′-Deoxy-2′-α-*C*-(2-hydroxyethyl)uridylyl(3′−5′)uridine (24a). The product was greater than 96% pure as determined by HPLC: ^{1}H NMR (400 MHz, D_2O) δ 1.70−1.61 (1H, m), 2.00−1.93 (1H, m), 3.35−3.29 (1H, m), 3.65−3.59 (2H, m), 3.80 (2H, d, *J* = 4.1 Hz), 4.38−4.14 (7H, m), 5.96−5.92 (3H, m), 6.06 (1H, d, *J* = 9.4 Hz), 7.89 (1H, d, *J* = 8.8 Hz), 7.91 (1H, d, *J* = 8.3 Hz); ^{31}P NMR (60 MHz, D_2O) δ 0.71; *m/z* (FAB$^-$, glycerol matrix) 577 (M$^-$, 5.5), 91 (100); HPLC retention time 17.75 min. HPLC analysis of SVPD hydrolysate gave only two products: 4.52 min (UMP), 10.64 min [2′-deoxy-2′-α-*C*-(2-hydroxyethyl)uridine].

2′-Deoxy-2′-α-*C*-(2,3-dihydroxypropyl)uridylyl(3′−5′)-uridine (24b). The product was greater than 98% pure as determined by HPLC: ^{1}H NMR (400 MHz, D_2O) δ 1.42−1.40 (1H, m), 1.54−1.50 (1H, m), 2.42−2.30 (1H, m), 3.50 (2H, d, *J* = 4.0 Hz), 3.83−4.07 (7H, m), 5.61 (1H, d, *J* = 8.1 Hz), 5.63 (1H, d, *J* = 8.1 Hz), 5.64 (1H, d, *J* = 4.4 Hz), 5.78 (1H, d, *J* = 9.2 Hz), 7.60 (1H, d, *J* = 8.1 Hz), 7.61 (1H, d, *J* = 8.1 Hz); ^{31}P NMR (101 MHz, D_2O) δ −0.59. FAB$^-$ *m/z* (glycerol matrix) 607 (M$^-$, 100); HPLC retention time 17.86 min. HPLC analysis of SVPD hydrolysate gave only two products: 4.05 min (UMP), 9.72 min [2′-deoxy-2′-α-*C*-(2,3-dihydroxypropyl)-uridine].

2′-Deoxy-2′-α-*C*-(carboxymethyl)uridylyl(3′−5′)uridine (24c). The product was greater than 97% pure as determined by HPLC: ^{1}H NMR (400 MHz, D_2O) δ 2.42 (1H, dd, *J* = 7.8, 16.7 Hz), 2.69 (1H, dd, *J* = 6.0, 16.7 Hz), 2.81 (1H, m), 3.76 (2H, d, *J* = 3.9 Hz), 4.05 (1H, m), 4.11−4.23 (3H, m), 4.26 (1H, t, *J* = 5.2 Hz), 4.30−4.33 (2H, m), 5.85−5.89 (3H, m), 6.01 (1H, d, *J* = 9.3 Hz), 7.80 (1H, d, *J* = 8.2 Hz), 7.83 (1H, d, *J* = 8.2 Hz); ^{13}C NMR 100.6 MHz, D_2O) δ 33.86, 46.90, 64.67, 67.83, 72.56, 76.57, 80.53, 85.93, 88.58, 91.32, 92.03, 105.57, 105.82, 144.66, 144.81, 154.74, 168.99, 179.74; ^{31}P NMR (101 MHz, D_2O) δ 1.27; FAB$^-$ *m/z* (glycerol matrix) 591 (M$^-$, 17.4); HPLC retention time 17.03 min. HPLC analysis of SVPD hydrolysate gave only two products: 4.89 min (UMP), 9.41 min (2′-deoxy-2′-α-*C*-(carboxymethyl)uridine).

2′-Deoxy-2′-α-*C*-acetamidouridylyl(3′−5′)uridine (24d). The product was greater than 94% pure as determined by HPLC: ^{1}H NMR (400 MHz, D_2O) δ 2.48 (1H, dd, *J* = 8.7, 15.8 Hz), 2.71 (1H, dd, *J* = 5.5, 15.8 Hz), 2.88 (1H, m), 3.80 (2H, d, *J* = 3.9 Hz), 4.10 (1H, ddd, *J* = 3.9, 5.9, 11.7 Hz), 4.14−4.25 (3H, m), 4.31 (1H, t, *J* = 5.2 Hz), 4.34−4.38 (2H, m), 5.89−5.93 (3H, m), 6.08 (1H, d, *J* = 9.3 Hz), 7.83 (1H, d, *J* = 8.2 Hz), 7.87 (1H, d, *J* = 8.1 Hz); ^{13}C NMR (100.6 MHz, D_2O) δ 33.14, 46.48, 64.42, 67.75, 72.32, 76.39, 80.48, 85.87, 88.38, 90.77, 92.09, 105.32, 105.77, 144.53, 144.58, 154.57, 169.00, 178.42; ^{31}P NMR (101 MHz, D_2O) δ −0.71; FAB$^-$ *m/z* (glycerol matrix) 590 (M$^-$, 100); HPLC retention time 17.41 min. HPLC analysis of SVPD hydrolysate gave only two products: 4.94 min (UMP), 9.19 min (2′-deoxy-2′-α-*C*-(acetamidomethyl)-uridine).

2′-Deoxy-2′-α-*C*-(cyanomethyl)uridylyl(3′−5′)uridine (24e). The product was greater than 96% pure as determined by HPLC: ^{1}H NMR (400 MHz, D_2O) δ 2.78−2.94 (3H, m), 3.80 (2H, d, *J* = 3.2 Hz), 4.09−4.38 (7H, m), 5.89−5.93 (3H, m), 6.14 (1H, d, *J* = 6.1 Hz), 7.86 (2H, d, *J* = 8.1 Hz); ^{13}C NMR (100.6 MHz, D_2O) δ 13.83, 44.40, 62.10, 65.77, 70.24, 74.36, 77.30, 83.52, 86.32, 88.15, 90.18, 103.26, 103.98, 119.71, 142.12, 142.64, 152.56, 152.69, 166.80, 167.01; ^{31}P NMR (101 MHz, D_2O) δ −0.91; FAB$^-$ *m/z* (glycerol matrix) 572 (M$^-$, 100); HPLC retention time 19.17 min. HPLC analysis of SVPD hydrolysate gave only two products: 4.67 min (UMP), 13.47 min (2′-deoxy-2′-α-*C*-(cyanomethyl)uridine).

Enzymatic Digestion of Dinucleoside Monophosphates. (i) SVPD. Digestion mixtures contained dinucleoside monophosphate (8 OD_{260} units/mL), potassium phosphate buffer (40 mM, pH 6.5), and SVPD (*C. durissuss*) (0.12 mg/mL) in a total of 250 μL. Reaction mixtures were incubated at 25 °C and analyzed at appropriate time intervals by HPLC. Digestion products were identified by coelution with authentic samples.

(ii) Nuclease P1 and ribonuclease A. Digestion mixtures contained dinucleoside monophosphate (3.5 OD_{260} units/mL), potassium phosphate buffer (50 mM, pH 6.5), and enzyme (67 μg/mL) in a total of 250 μL. Reaction mixtures were incubated at 25 °C and analyzed at appropriate time intervals by HPLC. Digestion products were identified by coelution with authentic samples.

Acid/base Hydrolysis Reactions. Reaction mixtures contained dinucleoside monophosphate (3.5 OD_{260} units/mL) in the following solutions: 1.5 M HCl; 2.0 M NaOH; 40 mM citrate buffer (pH 3.0); 56 mM citrate buffer (pH 4.0), in a total of 250 μL. Reaction mixtures were incubated at temperatures described in the Results and Discussion and analyzed at appropriate time intervals by HPLC.

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Supporting Information Available: Copies of ^{1}H NMR spectra for compounds **4**, **6−14**, **17**, **18**, **20a−d**, **21a−d**, **25**, and **26** and ^{13}C NMR spectra for compounds **16** and **19** (27 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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